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Mini Conference

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PRESENTATION NUMBER: MC001

ESSENTIAL FOR THE SYNAPSE: ADNP IS A MAJOR REGULATOR OF DEVELOPMENT AND AGING

MINI CONFERENCE 02: MOLECULAR BASIS FOR SYNAPTIC FUNCTION HIGHLIGHTING DISEASE MECHANISMS. ORGANISED BY EUROPEAN SOCIETY FOR NEUROCHEMISTRY (ESN)

Illana Gozes
Tel Aviv University, Sagol School Of Neuroscience, Faculty Of Medicine, Tel Aviv, Israel

Activity-dependent neuroprotective protein (ADNP) and its smallest active fragment, drug candidate NAP (davunetide) were discovered and first characterized in our laboratory. *De novo* mutations in ADNP cause the autistic intellectual disability (ID) ADNP syndrome. Now, we showed that *ADNP* and related genes are somatically mutated in postmortem aged Alzheimer's disease (AD) brains correlating with increasing AD tau pathology (Ivashko-Pachima, Hadar et al., Molecular Psychiatry, 2021). We then revealed tauopathy in post-mortem 7-year-old ADNP syndrome boy (Grigg et al., Translational Psychiatry, 2020). Using CRISPR-Cas9 genome editing, we developed a mouse model carrying the most abundant ADNP syndrome mutation, showing early tauopathy and reversal by NAP treated (Karmon et al., Biological Psychiatry 2021). Mechanistically, ADNP/NAP fortify microtubules through NAP (amino acid sequence: NAPV**SIP**Q), containing a microtubule end binding proteins (EB1, EB3) domain **S**x**IP** (Ivashko-Pachima et al., J. Mol. Neurosci. 2021). NAP enhances microtubule dynamics, augmenting Tau-microtubule association and protecting against tauopathy. ADNP mutations, disrupt Tau-microtubule interaction, which is ameliorated by NAP treatment. We have further demonstrated NAP enhancement of Tau/sirtuin1(SIRT1)-microtubule interaction in human induced pluripotent stem cell-derived neural cells, with SIRT1 and its partner Forkhead Box O3 (FOXO3, also regulated by ADNP/NAP) being major controllers of healthy aging (Hadar, Kapitansky et al., Molecular Psychiatry, 2021). Lastly, STOP codon mutations in ADNP, implicated in the ADNP syndrome and in AD represent sites of natural caspase cleavage, hallmarking apoptosis (Gozes and Shazman, Frontiers in Endocrinology, 2022). Thus, NAP (davunetide) fortification of ADNP activity is predicted to be beneficial in autism and neurodegenerative diseases.

Plenary Lectures

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PRESENTATION NUMBER: PL001

NEUROBIOLOGY OF SOCIAL AND SICKNESS BEHAVIOURS

PLENARY LECTURE 01: CATHERINE DULAC: NEUROBIOLOGY OF SOCIAL AND SICKNESS BEHAVIOURS (THE FRED KAVLI OPENING LECTURE)

Catherine Dulac
Harvard University, Mcb, Cambridge, United States of America

Social interactions are essential for animals to survive, reproduce, raise their young. Over the years, my lab has attempted to decipher the unique characteristics of social recognition: what are the unique cues that trigger distinct social behaviors, what is the nature and identity of social behavior circuits, how is the function of these circuits different in males and females and how are they modulated by the animal physiological status? In this lecture, I will describe our recent progress in understanding how different parts of the brain participate in the positive and negative control of parental behavior in males and females, providing a new framework to understand the regulation of adult-infant interactions in health and disease. I will also describe how new approaches in in situ single cell transcriptomics have enabled us to uncover specific hypothalamic cell populations involved in distinct social behaviors. Finally, I will describe our most recent work –fit for our current pandemic era—uncovering essential brain circuits underlying sickness symptoms in mice.

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PRESENTATION NUMBER: PL002

FROM MEMORY TO GUT MICROBES: AN UNEXPECTED PATH TO DISCOVERY

PLENARY LECTURE 02: MAURO COSTA-MATTIOLI: FROM MEMORY TO GUT MICROBES: AN UNEXPECTED PATH TO DISCOVERY

Mauro Costa-Mattioli
Baylor College of Medicine, Department of Neuroscience, Houston, TX, United States of America

Before I describe the context of my lecture, I would ask you to consider the following question. What would be the impact on human society of a successful “universal” treatment for memory loss? Memory is essential for animal survival. For humans, memory is the ongoing, real-time narrative of our lives. When our capacity to remember is lost, we are lost to ourselves and lost to those who we love. Even with so much at stake, we still lack a therapy that can effectively reverse the effects of time and disease on human memory.
My presentation, which will focus on mechanism(s) underlying brain function and dysfunction, will be split into two parts. First, I will outline our identification of a protein homeostasis network that is crucially required for normal long-term memory formation. More importantly, I will describe how its dysfunction is emerging as the main causative mechanism underlying the cognitive decline in a wide range of memory and neurodegenerative disorders. While I will briefly describe some of our original findings, I will mainly focus on how we can now leverage this knowledge to identify new therapeutic approaches to promote brain health. Second, in a complementary line of research, I will explain how a serendipitous discovery led us to identify and dissect the causative mechanism(s) by which gut microbes impact brain development & function. I will also discuss our findings supporting an emerging concept: “targeting the brain through the gut”. We discovered that a selective non-invasive, microbial-based approach is beneficial in preclinical animal models or neurological dysfunction and more recently in human patients. Even though the talk is on a Sunday morning (8:30 am), you should consider joining us!

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PRESENTATION NUMBER: PL003

HUNTINGTON'S DISEASE: FROM NEURODEVELOPMENT TO NEURODEGENERATION

PLENARY LECTURE 03: SANDRINE HUMBERT: HUNTINGTON'S DISEASE: FROM NEURODEVELOPMENT TO NEURODEGENERATION

Sandrine Humbert
Inserm U1216, Univ. Grenoble Alpes, Grenoble Institut Neurosciences, Grenoble, France

Huntington disease (HD) is a dominantly inherited neurological disorder characterized by the dysfunction and death of neurons from the cortex and the striatum. Striatal degeneration in HD is due, at least in part, to defective cortical signaling to the striatum. Symptoms in HD typically do not appear until mid-life or later. Yet huntingtin, the protein mutated in HD, and mutant huntingtin are expressed from the very beginning of life and huntingtin is essential for mouse development. Anyway, given the adult onset and dysfunction and death of adult neurons characterizing HD, most studies have focused on the toxic effects elicited by mutant huntingtin in adult post-mitotic neurons and the roles of the wild-type protein during development have been less studied. I will discuss how the huntingtin protein regulates several steps of mouse cortical development. Huntingtin maintains the pool of cycling progenitors, ensures the multipolar-bipolar transition of newborn neurons, their proper migration and maturation. In HD mice, mutant huntingtin causes mitotic spindle misorientation of dividing progenitors and decreases cortical thickness. Mutant huntingtin also interferes with the migration and maturation of post-mitotic neurons. I will also show that, as in HD mouse models, mutant huntingtin reduces the number of proliferating cells and triggers more neural progenitors to enter lineage specification prematurely in human HD mutation carrier fetuses. Finally, I will discuss how early axonal growth defects in the developing cortex contribute to presymptomatic HD signs and consider the viewing of HD with a neurodevelopmental component.

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PRESENTATION NUMBER: PL004

ASSEMBLY AND OPERATION OF THE NEOCORTEX

PLENARY LECTURE 04: SONG-HAI SHI: ASSEMBLY AND OPERATION OF THE NEOCORTEX (THE HERTIE FOUNDATION LECTURE)

Song-Hai Shi
Tsinghua University, School Of Life Sciences；idg/mcgovern Institute For Brain Research, Beijing, China

The ability of the neocortex to command higher-order brain functions depends on the assembly and operation of intricate neural circuitries comprised of a vast number of diverse neurons and glia. Notwithstanding the progress made in our understanding of the initial specification and the general histology and information flow of the neocortex, the principles and mechanisms that instruct the assembly and operation of neocortical circuits remain largely elusive. The research in my laboratory has focused on searching for the common developmental commodities of neocortical circuits at both the structural and functional levels and linking them with animal behaviors under normal and disease conditions. We have analyzed the molecular and cellular mechanisms that control the production and positioning of neocortical excitatory and inhibitory neurons, as well as glia, with the premise that the origin and lineage relationship guide the formation of defined neuronal ensembles or substrates for effective functional circuit assembly. In this presentation, I will discuss our recent findings and ongoing efforts.

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PRESENTATION NUMBER: PL005

THE MOLECULAR LOGIC OF SYNAPSE FORMATION

PLENARY LECTURE 05: THOMAS C. SüDHOF: THE MOLECULAR LOGIC OF SYNAPSE FORMATION (PRESIDENTIAL LECTURE)

Thomas C. Südhof
Stanford, Mcp, Stanford, United States of America

The brain processes information in million of parallel and intersecting neural circuits that compute information by transmitting and processing synaptic signals. Neural circuit computations critically depend on the number, locations and properties of their constituent synapses. We hypothesize that the construction of the neural circuits by formation of defines synapses is based on an overall simple molecular logic that is determined by interactions between pre- and postsynaptic impairments in the molecular logic of neural circuits, such that the input/output relations in affected circuits exhibit a skewed information processing capacity. In my presentation, I will discuss key drivers of the molecular logic of neural circuits, such as neurexins and their multifarious ligans or latrophilin- and BAI-adhesion GPCRs, to illustrate the broader concepts of how trans-cellular signaling drives synapse formation.

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PRESENTATION NUMBER: PL006

HUNGRY BRAINS AND CLEVER GUTS

PLENARY LECTURE 06: IRENE MIGUEL-ALIAGA: HUNGRY BRAINS AND CLEVER GUTS

Irene Miguel-Aliaga
MRC LMS, Imperial College London, Faculty of Medicine, London, United Kingdom

Our research group explores the idiosyncrasies of adult organs: how they differ between the sexes or across life stages, and why they engage in crosstalk with other organs. We were one of the first labs to tackle the study of the brain-gut axis using the powerful genetics of Drosophila: work that we have now extended to mouse and human models. We discovered that the brain-gut axes of males and females are very different, and that these intestinal sex differences impact food intake, gamete production and tumour susceptibility. We have also investigated how the intestine senses nutrients, revealing unexpected roles for metal sensing in the regulation of feeding and growth. I will present some of this work, as well as our ongoing attempts to shed some light on the molecular nature of this brain-gut communication and its spatiotemporal logic.

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PRESENTATION NUMBER: PL007

LEARNING WITH DENDRITES IN BRAINS AND MACHINES

PLENARY LECTURE 07: PANAYIOTA POIRAZI: LEARNING WITH DENDRITES IN BRAINS AND MACHINES (TCCI PLENARY LECTURE)

Panayiota Poirazi
FORTH, Imbb, Heraklion, Crete, Greece

Dendrites are thin processes that extend from the cell body of neurons and receive the vast majority of synaptic input. Their biophysical, anatomical and plasticity properties allow them to shape incoming signals in complex ways and have thus been suggested to serve as key players in learning and memory functions. In my presentation I will discuss how computational modelling has helped us illuminate dendritic function and its role in cognitive processes. I will present the main findings of a number of projects in lab dealing with dendritic nonlinearities in excitatory and inhibitory neurons and their consequences on memory formation, the role of dendrites in solving nonlinear problems in human neurons and recent efforts to adopt dendritic features in order to improve learning in artificial systems. Relevant references: [1] Panayiota Poirazi & Athanasia Papoutsi. Illuminating dendritic function with computational models. Nature Reviews Neuroscience, 11 May 2020 | DOI: 10.1038/s41583-020-0301-7 [2] Tzilivaki A, Kastellakis G, Schmitz D & Poirazi P. GABAergic Interneurons with nonlinear dendrites: from neuronal computations to memory engrams. Neuroscience, Nov 2021 | doi: 10.1016/j.neuroscience.2021.11.033 [3] Gidon A, Zolnik TA, Fidzinski P, Bolduan F, Papoutsi A, Poirazi P, Holtkamp M, Vida I, Larkum ME. Dendritic action potentials and computation in human layer 2/3 cortical neurons. Science. 2020 Jan 3;367(6473):83-87. doi: 10.1126/science.aax6239. [4] Chavlis S, Poirazi P. Drawing inspiration from biological dendrites to empower artificial neural networks. Current Opinion in Neurobiology, Oct 2021. doi: 10.1016/j.conb.2021.04.007

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PRESENTATION NUMBER: PL008

DEVELOPMENTAL COGNITIVE NEUROSCIENCE IN THE ERA OF BIG DATA

PLENARY LECTURE 08: DAMIEN FAIR: DEVELOPMENTAL COGNITIVE NEUROSCIENCE IN THE ERA OF BIG DATA (THE ERA-NET NEURON LECTURE)

Damien Fair
University of Minnesota, Masonic Institute for The Developing Brain, Minneapolis, United States of America

Abstract:
Developmental cognitive neuroscience is being pulled in new directions by network science and big data. Brain imaging (e.g. functional MRI, functional connectivity MRI), analytical advances (e.g. graph theory, machine learning), and access to large computing resources have empowered us to collect and process neuro-behavioral data faster and in larger populations than ever before. The translational potential from these advances is unparallelled, as a better understanding of complex human brain function is best grounded in the onset of these functions during human development. However, the maturation of the developmental cognitive neuroscience has seen the emergence of new challenges and pitfalls, which have significantly slowed progress and need to be overcome to maintain momentum. Here I examine the state of developmental cognitive neuroscience in the era of networks and ‘big data’ and highlight the solid footing we can take forward into the future.

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PRESENTATION NUMBER: PL009

ORGANISATION OF NEURAL ACTIVITY ACROSS THE BRAIN

PLENARY LECTURE 09: MATTEO CARANDINI: ORGANISATION OF NEURAL ACTIVITY ACROSS THE BRAIN (CLOSING PLENARY LECTURE)

Matteo Carandini
University College London, Ucl Institute Of Ophthalmology, London, United Kingdom

Neuroscience textbooks are typically arranged according to function (e.g., vision, movement, or navigation) or anatomy (e.g. visual cortex, spinal cord, or hippocampus), and often assume a relation between the two. However, new techniques that record the activity of thousands of neurons are revealing marked deviations from this view. In this talk I will provide examples of such deviations. First, I will discuss recordings from >30,000 neurons across the brain of mice involved in a simple visual decision task. Neurons that process images were found in restricted areas, including but not limited to the classical visual system; neurons predicting the animal's upcoming choices were rare, and found in a subset of these regions, with lateralized choice correlates particularly found in midbrain structures. In contrast, signals related to task engagement and to impending movements were distributed across the entire brain. Second, I will discuss recordings showing that neurons in the visual cortex carry not only visual signals but also signals encoding the animal's position in a virtual reality environment. These signals are absent in the thalamic afferents to the cortex and are coherent with those measured in the hippocampus. These results, and results from other laboratories, indicate that while some signals are encoded by localized brain circuits, others are much more distributed than one might assume from textbooks. Looking back, this suggests we should reevaluate some previous findings. Looking forward, this poses a new challenge: to understand what advantage is gained by broadcasting certain signals so widely across the brain.

Special Lectures

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PRESENTATION NUMBER: SL001

FENS-EJN AWARD: IMMUNOTHERAPY TO BEAT ALZHEIMER'S DISEASE: A TRANSFORMATIVE UNDERSTANDING OF NEURODEGENERATIVE DISEASES

SPECIAL LECTURES 01: FENS - EJN AWARDS LECTURES

Michal Schwartz
The Weizmann Institute of Science, Brain Science, Rehovot, Israel

Since the time of Medawar and Burnet's Nobel prize in 1960, it has been widely accepted that the CNS cannot tolerate any immune activity under any circumstances. Our team initiated more than two decades ago a change in this dogma, by demonstrating that the brain requires support from innate and adaptive immune cells for its maintenance and repair, with implications to aging and neurodegenerative diseases. Deep understanding that neurodegenerative diseases like Alzheimer's disease (AD) encompass not only the brain but also the immune system, led us to propose that defeating such diseases could be accomplished by promoting the process of repair, by harnessing the immune system that is either exhausted or insufficient. We found that transient blocking the inhibitory PD-1/PD-L1 immune checkpoint pathway, initiates an immune response in the periphery that leads to disease modification within the brain. This approach was found to be effective regardless of disease etiology, and independent of TREM2 activity. In both models of AD and tauopathy, we found that the treatment improved behavior and reduced multiple parameters that contribute to disease escalation, including neural loss, local inflammation and phospho-tau and aggregated tau in tauopathy, soluble oligomers of amyloid beta in amyloidosis. The effect was found to be dependent on bone-marrow derived macrophages, and was also associated with homing of FoxP3 regulatory T cells. A brief intermitted treatment was found to be for long-term effect, with AD-optimized antibody. Together, these studies show that targeting the immune system provides new avenues for understanding and treating neurodegenerative diseases.

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PRESENTATION NUMBER: SL002

FENS-EJN YOUNG INVESTIGATOR PRIZE: UNCOVERING HOW INHIBITORY BRAIN CIRCUITS REGULATE BEHAVIOUR

SPECIAL LECTURES 01: FENS - EJN AWARDS LECTURES

Sara Mederos
University College London, Sainsbury Wellcome Centre For Neural Circuits And Behaviour, London, United Kingdom

To pursue goal-directed behaviours, humans and animals have to select appropriate actions after evaluating all available information. Beyond their well-recognized homeostatic functions, astrocytes have emerged as relevant elements in brain physiology. Through their ability to regulate neuronal and synaptic activity, astrocytes influence neural networks. However, it is largely unknown how GABAergic interneurons and astrocytes interact and contribute to stable performance of complex animal behaviors. By combining optogenetics, gene ablation in astrocytes with electrophysiological recordings in behaving mice and ex vivo electrophysiology we have revealed interactions between astrocytes and cortical inhibitory cells, showing the significance of this pathway for sustained adaptive behaviours. We found that PV interneuron signaling recruits astrocyte networks through GABABRs activation enhancing inhibitory transmission. Additionally, the genetic ablation of GABAB receptors in medial prefrontal cortex astrocytes (GFAP/GABAB ablated mice) altered firing properties of cortical neurons, which affected decision-making. By optogenetic stimulation of astrocytes with melanopsin, a GPCRs optogenetic tool mimicking astrocytic activity, we were able to restore the cognitive disabilities shown by GFAP/GABAB ablated mice, demonstrating the ability of astrocytes to fine-tuning cortical circuits' activity, which underlies animal behavior. Therefore, our work identifies astrocytes as a hub for controlling inhibition in cortical circuits, providing a novel pathway for the behaviorally relevant midrange time-scale regulation of cortical information processing and consistent goal-directed behaviors.

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PRESENTATION NUMBER: SL003

FENS-EJN YOUNG INVESTIGATOR PRIZE: MAPPING NEURONAL AND VASCULAR NETWORKS IN TRANSPARENT BRAINS

SPECIAL LECTURES 01: FENS - EJN AWARDS LECTURES

Nicolas Renier
Paris Brain Institute, Sorbonne Universite, INSERM, CNRS, Laboratory Of Structural Plasticity, Paris, France

Over the past six years, there has been a convergence in the fields of optics, biochemistry, and computing, leading to dramatic improvements in light-sheet microscopy, tissue clearing protocols, and image analysis algorithms. The convergence of these different fields can streamline brain studies by accelerating data acquisition speed and reliability over the current whole-brain analysis pipelines based on serial sectioning methods. We previously developed the iDISCO+ (Renier et al., 2014) protocol for immunostaining and imaging intact adult mouse brains. As a companion tool, we also developed and distributed ClearMap (Renier et al., 2016, Liebmann et al., 2016, Kirst et al. 2020), an open-source environment to segment objects and map them onto reference atlases optimized for large 3D datasets. We used this pipeline as a discovery tool to find brain regions active in correlation with various behaviors by mapping neuronal activity landscapes derived from Fos expression (Renier et al. 2017, Nectow et al., 2017, Schneeberger Pane et al. 2019). Here, I will present how iDISCO+ and ClearMap 2 can help leverage data obtained from intact whole brain preparations. We hope that ongoing developments in light-sheet microscopy and image analysis pipelines will facilitate our understanding of individual variations in brain activity, connectivity, and structure.

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PRESENTATION NUMBER: SL004

FENS-EJN YOUNG INVESTIGATOR PRIZE: SOMATOSENSORY NEUROPROSTHESES: CONNECT THE BRAIN TO BIONIC LIMBS

SPECIAL LECTURES 01: FENS - EJN AWARDS LECTURES

Giacomo Valle
ETH Zurich, D-hest, Zurich, Switzerland

In the recent past, several research groups are studyng the fascinating and futuristic research of connecting the human nervous system with bionic limbs. Striving to close the gap between humans and machines, this research combines the knowledge of neuroscience and medicine, as well as engineering and artificial intelligence, with the fundamentals of psychology. The research is now working to create prosthetic limbs that utilize the body's complex senses and nervous system to restore sensory-motor functions lost after an injury or a disease. Decades of technological developments have populated the field of brain-machine interfaces (BMI) and neuroprosthetics with several replacement strategies, neural modulation treatments, and rehabilitation strategies to improve the quality of life for patients affected by sensory and motor disabilities. Neuroprosthetics are implantable devices designed to replace or improve the function of a disabled part of the nervous system. Such approach has been expanded to many different applications, among which motor prosthetics, sensorimotor prosthetics, and cognitive prosthetics. The approaches for the restoration of sensory functions through neuroprostheses in amputees will be presented. This field is now quickly expanding thanks to advances in neural interfaces, machine learning techniques, and robotics. In the next future, the neurotechnologies will continue to grow thanks also to faster and more advanced computer simulations allowing to test and validate these technologies even faster. The transformation of neuro-technologies blurs the boundaries between human and machine and raises several ethical, social, and cultural questions.

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PRESENTATION NUMBER: SL005

SITE OF ACTION OF DRUGS BLOCKING CGRP SIGNALING IN MIGRAINE

SPECIAL LECTURES 02: THE BRAIN PRIZE LECTURE

Lars Edvinsson
Institute of clinical sciences Lund, Department Of Medicine, Lund, Sweden

Site of action of drugs blocking CGRP signaling in migraine. Lars Edvinsson The trigeminovascular system (TGV) comprises of the trigeminal ganglion with neurons and satelitte glial cells, with sensory unmyelinated C-fibres and myelinated Aδ-fibres picking up information from different parts of the head and sending signals to the brainstem and the central nervous system (CNS). The presentation will discuss aspects of signalling at the distal parts of the sensory fibres, the extrasynaptic signalling between C-fibres and Aδ-fibres, and the contact between the trigeminal fibres at the nerve root entry zone where they transit into the CNS. We will adress the possible role of the neuropeptides calcitonin gene-related peptide (CGRP), the neurokinin family and pituitary adenylyl cyclase-activating polypeptide 38, all found in the TGV system together with their respective receptors. Elucidation of the expression and localization of neuropeptides and their receptors in the TGV system may provide novel ways to understand their roles in migraine pathophysiology and indicate novel ways for treatment of migraine patients.

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PRESENTATION NUMBER: SL006

CALCITONIN GENE-RELATED PEPTIDE IN CLUSTER HEADACHE
SPECIAL LECTURES 02: THE BRAIN PRIZE LECTURE

Peter Goadsby[1,2]
[1]King's College London, ., London, United Kingdom, [2]University of California, ., Los Angeles, United States of America

Cluster headache is a devastating primary headache disorder characterised by attacks of lateralised pain, a sense of restlessness and cranial autonomic features, such as lacrimation, conjunctival injection, nasal or aural symptoms. Cluster headache is one of the trigeminal-autonomic cephalalgias (TACs) (1). Calcitonin gene-related peptide (CGRP) is a neuropeptide that is found in the trigeminal (2) and sphenopalatine ganglia. CGRP is released during acute attacks of cluster headache: spontaneous (3) or nitroglycerin-evoked (4). CGRP pathway blockade with galcanezumab in patients with episodic cluster headache was effective at reducing attack frequency (5). CGRP is involved in the expression of attacks of cluster headache and has a role in some patients as an effective and well tolerated novel therapy.

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2. Edvinsson L, Haanes KA, Warfvinge K, Krause DN. CGRP as the target of new migraine therapies - successful translation from bench to clinic. Nat Rev Neurol. 2018;14(6):338-50.
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PRESENTATION NUMBER: SL007

TRIGEMINOVASCULAR SYSTEM AS A MIGRAINE TARGET: HOW DID IT BEGIN AND WHEN WILL IT END?

SPECIAL LECTURES 02: THE BRAIN PRIZE LECTURE

Michael Moskowitz
MGH/Harvard, Center For Systems Biology/radiology, Charlestown, United States of America

Migraine entered the modern neuroscience era with a Lancet hypothesis in 1979. This hypothesis proposed that trigeminal meningeal afferents and their peptide neuromediators were important to the genesis of migraine headache, and as such, targets for therapy. Subsequent research on the trigeminovascular system (TV) led to mechanisms relevant to actions of ergots, triptans and 5-HT1F receptor agonists. They also identified other therapeutically relevant constituent molecules, receptors and channels that populate TV afferents including CGRP, SP, PACAP, PAR-2, prolactin, NGF and KATP and TRP ion channels, for example. Further research also identified an upstream trigger for headache in migraine with aura; cortical spreading depression (CSD), a slowly propagating intense depolarization of neurons and glia, is proinflammatory and releases noxious molecules that trigger overlying TV afferents and cause unilateral headache. It's also a target for some preventative drugs like anticonvulsants and a cause for the aura. Recently, inflammatory signals within the meninges and skull were found in migraine with aura patients. A second related discovery found CSF within dural perivascular spaces that extend from the subarachnoid space into the bone marrow via bony channels. These newly discovered channels provide a migration route for CSF-containing inflammatory signals from brain via meninges to bone. Hence, skull marrow may act as a reservoir for activated inflammatory cells and signaling molecules as recently shown in other neuroinflammatory disorders (e.g.,MS, stroke and trauma). This new body of information, inspired initially by interrogating the TV system, portends a bright future for novel migraine discoveries and therapeutic targets.

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PRESENTATION NUMBER: SL008

TRANSLATING MIGRAINE MOLECULAR MECHANISMS FROM MAN TO ANIMAL

SPECIAL LECTURES 02: THE BRAIN PRIZE LECTURE

Jes Olesen
University of Copenhagen, Neurology, Copenhagen, Denmark

Translational research usually means that discoveries in basic science are translated to humans. For migraine the opposite has mostly been the case. Animal models of migraine have uncertain validity, but migraine poses the unique possibility that attacks can be provoked in patients in a fully ethical fashion. Provocation experiments revealed that histamine induces migraine attacks, but antihistamines were ineffective in the treatment of migraine. Nitric oxide (NO) donors induced migraine and a non-selective nitric oxide synthase (NOS)-inhibitor was affective in migraine treatment. Selective antagonists of steps in the NO-cGMP cascade have not yet been tested. Calcitonin gene-related peptide (CGRP) induced migraine attacks and this was crucial for the development of modern CGRP antagonistic treatments of migraine that now revolutionize migraine treatment. Pituitary adenylate cyclase activating peptide (PACAP) induced migraine and a monoclonal antibody against PACAP is currently in trial. Prostanoids and several other signaling molecules, most importantly openers of ATP sensitive potassium channels (Katp) also induced migraine. Mechanisms of provoking molecules have been analyzed in detail in rodent models of migraine. They suggest that migraine induction as well as migraine treatment take place outside of the blood-brain-barrier, that CGRP antagonistic drugs and triptans are not additive, that NO mechanisms and CGRP mechanisms are closely interrelated and that PACAP is independent of CGRP. Katp channel blockers were effective in rodent models and may represent a new target for migraine treatment.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: SL009

ORGANIZATION AND COMPUTATION IN NEURAL CIRCUITS DURING DEVELOPMENT

SPECIAL LECTURES 03: ERIC KANDEL PRIZE LECTURE & SCIENTIFIC AWARD CEREMONY

Julijana Gjorgjieva
Technical University of Munich, School Of Life Sciences, Freising, Germany

During early development, neural circuits in the brain generate precisely coordinated output without previous experience. This suggests that powerful developmental mechanisms drive substantial circuit organization and tuning as animals are still in the womb. These mechanisms need to be coordinated and appropriately timed at the molecular, single neuron and network level to enable animals to learn, establish a rich repertoire of computations and generate robust behaviors. I will discuss our recent theoretical and modeling work on understanding how neural circuits are built and organized during development, how they learn and adapt to sensory experience, and how they generate complex network dynamics to implement various computations. I will focus on mechanisms of synaptic plasticity that shape network connectivity and give rise to functional network dynamics. Our models are constrained by experimental data and yield new insights not only into normal brain development but also various developmental disorders.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: SL010

NEUROETHICS AND AI ETHICS: TOWARDS A PRODUCTIVE INTERACTION

SPECIAL LECTURES 04: EDAB / SPECIAL LECTURE ON NEUROETHICS

Arleen Salles
Uppsala University, Center For Research Ethics And Bioethicse, Uppsala, Sweden

Beyond the important insights provided by the development of neuroscience and artificial intelligence and beyond their goal to serve society, each field raises a number of ethical, social, and regulatory issues that are generally explored by neuroethics and the ethics of AI respectively. Neuroethics and AI ethics have been gaining prominence in the last few decades but it is only recently that some neuroethicists have begun to call for their mutual collaboration. But what can neuroethics bring to the ethics of artificial intelligence? How can AI ethics contribute to the neuroethical discourse? I reflect on these questions, providing an overview of some conceptual and practical advantages of their collaboration and of foreseeable challenges.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: SL011

DEVELOPMENT OF MULTICILIATED CELLS

SPECIAL LECTURES 05: HOST SOCIETY SPECIAL LECTURE

Nathalie Spassky
Institut de Biologie de l'Ecole Normale Supérieure, Biology And Neurogenesis, PARIS, France

Adult neural stem cells (NSCs) and post-mitotic multiciliated ependymal cells (E cells) are glial cells that compose the neurogenic niche in the mammalian brain. By clonal analysis of large numbers of radial glial progenitors (RGPs) using the Nucbow strategy or single-cell resolution of progenitor division patterns and fate, we and others recently showed that the vast majority of B1 and E cells are sister cells generated through sequential symmetric and asymmetric divisions. Interestingly, we also discovered that the Geminin family members, initially identified as regulators of DNA replication can modify the relative numbers of B1 and E cells in the resulting clones. More recently, we developed cellular and molecular approaches to decipher how cell cycle regulators drive the fate decision of neural progenitors during embryonic mouse brain development. I will present our current experimental evidences that the final fate decision of these glial cells occurs before their final cell cycle exit.

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PRESENTATION NUMBER: SL012

FROM PRIMATE ANATOMY TO HUMAN NEUROIMAGING: LINKING CIRCUITS TO PSYCHIATRIC DISEASE AND NEUROTHERAPEUTIC TARGETS

SPECIAL LECTURES 06: EDAB / MAX COWAN SPECIAL LECTURE

Suzanne Haber
University of Rochester, Pharmacology And Physiology, Rochester, United States of America

The cortico-basal ganglia networks are central to incentive-based learning and good decision making. There is growing consensus that obsessive-compulsive disorder (OCD), major depressive disorder, and addiction are manifestations of dysfunction of these networks. Deep brain stimulation (DBS), an effective therapeutic approach for several treatment resistant disorders, targets key circuits within these networks. This talk will first review the network connections most associated with psychiatric illnesses. Circuits include the cortico-striatal, cortico-thalamic, the cortico-subthalamic connections, and the ascending midbrain dopaminergic projection. Second, it will introduce the four most commonly targeted pathways; the anterior limb of the internal capsule (descending and ascending cortical fibers), the ventral striatum, the subthalamic nucleus, and midbrain ventral tegmental area (VTA). Here, using animal tracing experiments, I will outline the precise trajectory and location of fibers from different cortical regions through each target using animal tracing data. Combining this anatomy with diffusion magnetic resonance imaging (MRI) animals, I will demonstrate how accurate diffusion MRI reflects the anatomic organization. Using that analysis as a guide, connections through each target will be identified using diffusion MRI in humans. Finally, the circuits captured at the four surgical targets in humans with be compared.

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PRESENTATION NUMBER: SL013

THE EVOLUTION OF COMPUTATION IN THE BRAIN - INSIGHTS FROM STUDYING THE RETINA

SPECIAL LECTURES 07: BOEHRINGER INGELHEIM / FENS RESEARCH AWARD

Tom Baden
University of Sussex, Sussex Neuroscience, School Of Life Sciences, Brighton, United Kingdom

The retina is probably the most accessible part of the vertebrate central nervous system. Its computational logic can be interrogated in a dish, from patterns of lights as the natural input, to spike trains on the optic nerve as the natural output. Consequently, retinal circuits include some of the best understood computational networks in neuroscience. The retina is also ancient, and central to the emergence of neurally complex life on our planet. Alongside new locomotor strategies, the parallel evolution of image forming vision in vertebrate and invertebrate lineages is thought to have driven speciation during the Cambrian. This early investment in sophisticated vision is evident in the fossil record and from comparing the retina's structural make up in extant species. Animals as diverse as eagles and lampreys share the same retinal make up of five classes of neurons, arranged into three nuclear layers flanking two synaptic layers. Some retina neuron types can be linked across the entire vertebrate tree of life. And yet, the functions that homologous neurons serve in diffierent species, and the circuits that they innervate to do so, are often distinct to acknowledge the vast differences in species-specific visuo-behavioural demands. In the lab, we aim to leverage the vertebrate retina as a discovery platform for understanding the evolution of computation in the nervous system. Working on zebrafish alongside birds, frogs and sharks, we ask: How do synapses, neurons and networks enable 'function', and how can they rearrange to meet new sensory and behavioural demands on evolutionary timescales?

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PRESENTATION NUMBER: SL014

HETEROSYNAPTIC PLASTICITY EMERGING FROM MOLECULAR INTERACTIONS IN DENDRITES

SPECIAL LECTURES 07: BOEHRINGER INGELHEIM / FENS RESEARCH AWARD

Tatjana Tchumatchenko
University of Mainz Medical Center, University of Bonn Medical Center, Ag Tchumatchenko, Mainz, Germany

The dendrites of neurons evolved to maximize the amount of exposed surface available for intercellular communication. How do neurons distribute proteins across their dendrites such that all synapses get enough proteins and can respond to short-term changes in demand? Using a novel computational framework we made three key discoveries which we validated using fluorescently labeled proteins in vitro and 3D EM volumetric reconstructions. Our model predictions and in vitro measurements both show that soluble proteins preferentially move back toward the soma at branch points and surface proteins move forward distal sites. This surface protein bias in pyramidal morphologies is approximately 35%. Using 3D volumetric EM reconstructions of pyramidal neurons we show that the ratio of daughter radii in pyramidal neurons is selectively favoring protein diffusion lengths that overlap with the experimental reports of dendritic proteins. This suggests that dendritic morphology is optimized for long-range protein transport. Our results show that daughter radii optimization at branch points can reduce the total protein count a neurons needs to produce to serve all synapses by two orders of magnitude. In the next step, we are exploring how temporal features of the protein distributions impact synaptic plasticity rules.

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PRESENTATION NUMBER: SL015

FROM STEM CELLS TO BRAIN ASSEMBLOIDS: CONSTRUCTING AND DECONSTRUCTING THE HUMAN NERVOUS SYSTEM

SPECIAL LECTURES 09: IBRO – KEMALI PRIZE LECTURE

Sergiu Pasca
Stanford, The Bonnie Uytengsu & Family Director Of Stanford Brain Organogenesis, Stanford, United States of America

A critical challenge in understanding the programs underlying the development, assembly and dysfunction of the human brain is the lack of direct access to intact, functioning human neural tissue for direct investigation and manipulation. In this talk, I will describe efforts in my laboratory to build functional cellular models and to capture previously inaccessible aspects of human brain development and dysfunction. To achieve this, we have been using instructive signals to create, from pluripotent stem cells, self-organizing 3D cellular structures called regionalized brain organoids (or neural spheroids) that resemble domains of the developing central nervous system. We have shown that these cultures, such as the ones resembling the cerebral cortex, recapitulate features of neural development, can be derived with high reliability across dozens of cell lines and experiments, and can be maintained for years in vitro to capture advanced stages of neural and glial maturation and function. Moreover, we demonstrated that regionalized brain organoids can be put together to form functionally-integrated structures we named assembloids, which can be subsequently applied to investigate cell migration and formation of neural circuits. Lastly, I will illustrate how our modular, stem-cell derived 3D system can be used to study the cellular and molecular consequences of mutations or copy number variants associated with neuropsychiatric disorders.

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PRESENTATION NUMBER: SL016

FIRST CLINICAL TRIALS IN VISION RESTORATION

SPECIAL LECTURES 10: CHICA AND HEINZ SCHALLER FOUNDATION AWARD LECTURE

José-Alain Sahel[1,2], Botond Roska[3], Serge Picaud[1,4], Deniz Dalkara[1,5]
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Rod-cone dystrophies, also termed Retinitis Pigmentosa, are a leading cause of heritable blindness. Mutations in photoreceptor cells or Retinal Pigment Epithelium lead to loss of function and eventually irreversible degeneration of photoreceptor cells. The number of known gene defects already identified and the late-stage diagnosis of the condition in many patients form challenges to therapeutic strategies aiming at preserving or restoring useful visual function. As part of a spectrum of therapeutic approaches developed over the past decades (gene therapy, neuroprotection, cell therapy, prosthetic vision), we designed and tested at the preclinical level an optogenetic therapy combining 1) gene-based expression of a red-shifted optogene, ChrimsonR, in Retinal Ganglion Cells, 2) a stimulation device comprising a neuromorphic camera and a projecting mirror in the corresponding amber wavelength, 3) vision rehabilitation protocols. The clinical trial, supported by GenSight Biologics, enrolled nine patients in Paris, London, and Pittsburgh using a dose-escalation protocol and demonstrated the safety of the procedure, gene product, and device as well as partial vision restoration in an initial subject (reported in Sahel et al., Nature Medicine, 2021) and subsequent patients. Multi-EEG recording demonstrated corresponding patterns of activation of the primary visual cortex. Ongoing studies include an extension cohort, refinement in the stimulation goggle design, the stimulation protocol, and the rehabilitation process. This initial demonstration of the potential of optogenetics paves the way to further programs aiming at improving cell selectivity, stimulation accuracy, and patterns. Optogenetics could potentially be applied to other blinding conditions (e.g., macular dystrophies, age-related macular degeneration).

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PRESENTATION NUMBER: SL017

A CIRCUIT MODEL FOR ADDICTION OPENING THE ROAD TO TRANSLATIONAL RESEARCH

SPECIAL LECTURES 10: CHICA AND HEINZ SCHALLER FOUNDATION AWARD LECTURE

Christian Lüscher
University of Geneva, Basic Neurosciences, Geneva, Switzerland

Addiction may be considered a disorder of dysfunctional motivation and decision circuits. We will review the empirical evidence that addiction starts with exposure to drugs that have in common to increase dopamine in the mesolimbic system. Adaptive behavior then arises from dopamine modulation of glutamatergic and GABAergic synaptic transmission, which alters circuit function, eventually leading to compulsive drug taking in vulnerable individuals. A special emphasis will be on the translational implication of the circuit model for addiction and the roadmap to novel therapies.

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PRESENTATION NUMBER: SL018

SLEEP-WAKE CYCLES SHAPE PROTEOME AND PHOSPHOPROTEOME DYNAMICS IN BRAIN

SPECIAL LECTURES 11: EJN SPECIAL FEATURE

Maria Robles
Ludwig Maximilian University of Munich, Institute Of Medical Psychology And Biomedical Center, Munich, Germany

Nearly all physiological processes in the body display circadian (close to 24 hours) oscillations. Brain processes, including memory and learning performance, are also shaped in a daily manner, however little is known about the molecular mechanisms driving this rhythmicity. Mass-spectrometry (MS) based quantitative proteomics is a powerful technique used to study tissue, cellular and sub-cellular proteomes as well as protein post-translational modifications (PTMs). This technology has revolutionized the quantification of protein levels and modifications in many biological fields, amongst them chronobiology. In my talk I will present our work using MS-based label free quantitative proteomics and phosphoproteomics to elucidate sleep- and circadian-driven cycling mechanisms in murine brains and synaptoneurosomes.

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PRESENTATION NUMBER: SL019

CALCIUM-INDEPENDENT ASTROCYTIC LIPID RELEASE MODULATES NEURONAL EXCITABILITY

SPECIAL LECTURES 11: EJN SPECIAL FEATURE

Nathan A. Smith
Unversity of Rochester School of Medicine and Dentistry, Department Of Neuroscience, The Del Monte Institute For Neuroscience, Rochester, United States of America

Accumulating data point to a key role of Ca^{2+}-dependent gliotransmitter release as a modulator of neuronal networks. Here, we tested the hypothesis that astrocytes in response to agonist exposure also release lipid modulators through activation of Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) activity. We found that cultured rat astrocytes treated with selective ATP and glutamatergic agonists released arachidonic acid (AA) and/or its derivatives, including the endogenous cannabinoid 2-arachidonoyl-sn-glycerol (2AG) and prostaglandin E_2 (PGE_2). Surprisingly, buffering of cytosolic Ca^{2+} resulted in a sharp increase in agonist-induced astrocytic lipid release. In addition, astrocytic release of PGE_2 enhanced miniature excitatory post-synaptic potentials (mEPSPs) by inhibiting the opening of neuronal Kv channels in brain slices. This study provides the first evidence for the existence of a Ca^{2+}-independent pathways regulating the release of PGE2 from astrocytes, and furthermore demonstrates a functional role for astrocytic lipid release in the modulation of synaptic activity.

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PRESENTATION NUMBER: SL020

MOLECULAR BIOMARKERS FOR STROKE

SPECIAL LECTURES 12: ERA-NET NEURON/EXCELLENT PAPER IN NEUROSCIENCE AWARD

Steffen Tiedt
LMU Munich, Institute For Stroke And Dementia Research, Munich, Germany

Stroke is a leading cause of death and disability worldwide. Its heterogeneity poses a challenge for assigning patients to optimal treatment strategies and is a major reason for the large number of failed clinical trials, including those on neuroprotection. Current diagnostic algorithms are insufficient to capture the heterogeneity of stroke: stroke etiology remains undetermined in 40 % of patients, thus impeding the allocation of these patients to optimal secondary prevention regimens. Heterogeneity is also seen at the level of neuronal injury, which greatly varies between stroke patients, but can neither be assessed in the pre-hospital setting nor serially in the acute phase to monitor stroke progression. Circulating proteins and metabolites capture pathophysiological events in multiple organs including local and systemic events (e.g. stress) related to acute stroke and might thus inform on neuronal injury and the mechanisms causing stroke. Our work focuses on the discovery of such biomarkers for stroke using omics technologies and ultrasensitive targeted assays. Here, I will first present the awarded project that used a discovery-validation and global metabolomics approach across four independent cohorts to identify, validate, and replicate a set of four circulating metabolites that shows unprecedented utility and outperforms neuroimaging in identifying patients with stroke upon hospital arrival. Further, I will show recent data on how metabolomic data could be leveraged to improve our understanding of human stroke pathophysiology. The talk will end with an outlook on how molecular biomarkers can guide future precision medicine, in stroke, neurology, and beyond.

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PRESENTATION NUMBER: SL021

NEURAL CIRCUITS FOR MULTISENSORY INTEGRATION AND PERCEPTUAL DECISIONS

SPECIAL LECTURES 13: ALBA-ELSEVIER AWARD LECTURE ON BRAIN SCIENCES

Seung-Hee Lee
KAIST, Biological Sciences, Daejeon, Korea, Republic of

Cortical circuits process multiple sensory information and transform them into the motor decision in animals performing perceptual tasks. However, it is still unclear how sensory inputs are integrated and transformed into motor signals in the cortex to initiate goal-directed actions. We recently found cortical circuits that are important for multisensory integration and perceptual decision-making. First, we found that the posterior parietal cortex (PPC) integrates visual and auditory inputs and process multisensory decisions in a state-dependent manner. Parvalbumin-positive inhibitory neurons in the PPC receive strong inputs from the auditory cortex and exert feedforward inhibition, which leads to auditory-dominant decisions in head-fixed mice under audiovisual conflicts. Notably, locomotion suppresses auditory inputs to the PPC, switching mice under the same conflict conditions to make visually dominant decisions more often. Second, we found that visual inputs from the visual cortex to the anterior cingulate cortex (ACC) trigger gated feedforward inhibition to release goal-directed actions in mice performing Go/No-go tasks. Three different types of ACC neuronal activities—visual amplitudes of sensory neurons, suppression times of motor neurons, and network activity from other neurons—predicted response times of the mice. Moreover, optogenetic activation of visual neurons in the ACC prompted task-relevant actions in mice by suppressing ACC motor neurons, which disinhibited downstream striatal neurons. Collectively, our study illustrates how cortical circuits integrate and transform sensory information into flexible motor decisions. Our data demonstrate that feedforward inhibitory circuits in cortico-cortical projections play critical roles in perceptual decision-making.

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PRESENTATION NUMBER: SL022

POST-TRANSCRIPTIONAL REGULATION OF THE MICROTUBULE ASSOCIATED PROTEIN TAU: FUNCTIONAL CONSEQUENCES AND THERAPEUTIC PERSPECTIVES

SPECIAL LECTURES 13: ALBA-ELSEVIER AWARD LECTURE ON BRAIN SCIENCES

Maria Elena Avale, Ana Damianich, Carolina Facal, Javier Muñiz, Sonia Espindola
Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto De Investigaciones En Ingeniería Genética Y Biología Molecular "dr Hector N Torres" (ingebi-conicet), Buenos Aires, Argentina

Tau is a microtubule-associated protein, predominantly expressed in neurons, that regulates a myriad of neuronal processes. Abnormal tau metabolism underly many neurodegenerative diseases, named tauopathies, such as Alzheimer's disease, frontotemporal dementia and progressive supranuclear palsy. The tau primary transcript undergoes highly regulated post-transcriptional modifications in the brain; and failures in such processing leads to disease. Particularly, in the normal adult brain, the alternative splicing of exon 10 (E10) produces equal amounts of tau protein isoforms with three (3R) or four (4R) microtubule binding repeats. Several mutations -and also other non-genetic factors- affecting E10 alternative splicing are associated with tauopathies. Targeting Tau RNA processing is therefore a promising avenue to develop effective therapies. In this talk I will outline our achievements using RNA based strategies to modulate either the tau 3R:4R ratio or total tau contents, in a mouse model of tauopathy (htau mice). We used lentiviral vectors to express molecules that modulate E10 inclusion/exclusion by RNA trans-splicing with the endogenous tau transcript. When delivered into the prefrontal cortex, these molecules improved cognitive deficit, restored neuronal firing patterns and reduced hyperphosphorylated tau contents. Moreover, shifting of 3R to 4R tau into the striatum rescued motor coordination deficits. Furthermore, we tested designed microRNAs to locally reduce tau contents in the adult htau brain. Tau knockdown in the htau prefrontal cortex rescued cognitive impairments and pathological phenotypes, even after phenotypic onset. Together, our results evidence the (dys)functional consequences of abnormal tau post-transcriptional regulation and highlight the potential of using RNA-based therapeutic strategies for tauopathies.

Symposia

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PRESENTATION NUMBER: S001

INTRINSIC MECHANISMS OF HUMAN NEURONAL NEOTENY.

SYMPOSIUM 01 - THE LINKS BETWEEN NEURAL CIRCUIT DEVELOPMENT, HUMAN BRAIN EVOLUTION AND DISEASES' - RYOHEI IWATA, APARNA BHADURI, ALESSANDRO VITRIOLO, EWOUD SCHMIDT

Ryohei Iwata, Pierre Casimir, Emir Erkol, Leïla Boubakar, Pierre Vanderhaeghen
VIB, Center For Brain And Disease Research, Leuven, Belgium

Developmental programs are highly conserved across all vertebrates, although there are significant temporal variations in interspecies developmental processes. Changing the timing and rate of developmental processes can profoundly affect subsequent organogenesis and may also have been a critical factor in evolution. However, despite their potential importance, the cellular and molecular mechanisms that control interspecies differences in developmental timeline remain unclear. Neuronal development is considerably prolonged in the human cerebral cortex compared with other mammals, leading to brain neoteny. Moreover, changes in the maturation timing and rate were proposed to be associated with brain malformation and dysfunction. Here, we explore whether mitochondria influence the species-specific properties of cortical neuron maturation. By comparing human and mouse cortical neuronal maturation at high temporal and cellular resolution, we found a slower pattern of mitochondria development in human cortical neurons compared with the mouse, together with lower mitochondria metabolic activity, particular oxidative phosphorylation. Stimulation of mitochondria-dependent metabolism in human neurons resulted in accelerated maturation, leading to increased excitability and morphological complexity weeks ahead of time. Our data identify mitochondria as important regulators of the rate of neuronal development underlying human-specific features of brain evolution.

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PRESENTATION NUMBER: S002

NEURAL SPECIFICATION IN THE DEVELOPING HUMAN CORTEX

SYMPOSIUM 01 - THE LINKS BETWEEN NEURAL CIRCUIT DEVELOPMENT, HUMAN BRAIN EVOLUTION AND DISEASES' - RYOHEI IWATA, APARNA BHADURI, ALESSANDRO VITRIOLO, EWOUD SCHMIDT

Aparna Bhaduri
University of California, Los Angeles, Biological Chemistry, Los Angeles, United States of America

The human brain is comprised of myriad cell types organized into distinct regions, including the cerebral cortex, the outermost layer of the human brain which enables aspects of cognition and other higher order processes. Compared to other mammals and even primates, the human cortex is substantially expanded. These size differences emerge during developmental timepoints and the cell types and gene expression programs enriched in humans at these developmental timepoints also make humans uniquely susceptible to neurodevelopmental and neuropsychiatric disorders. In order to study the emergence of these functional cortical regions, cell types, and gene expression networks, we have leveraged single-cell RNA-sequencing to identify cell types across developmental time. These data have highlighted the early specification of frontal and occipital poles of the cortex, and suggest that in between regions become further specified at later timepoints. We have also identified extremely dynamic gene expression programs, with the specific gene signatures that define cortical areas varying immensely both across developmental time and between cell types across the differentiation spectrum. In an effort to inventory and compare these gene signatures, we have compiled a meta-atlas of datasets from peak neurogenesis. This compendium of single-cell data enables us to identify biological pathways that driv these cell fate specification events. In turn, these data sets can be compared across species, *in vivo* and *in vitro* model systems, and across developmental epochs. Paired with experiments in primary tissue and cortical organoids, we can begin to decipher the logic of neural specification in the developing human cortex.

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PRESENTATION NUMBER: S003

EMERGING INSIGHTS ON THE MODERN HUMAN BRAIN THROUGH THE PRISM OF NEURODEVELOPMENTAL DISORDERS

SYMPOSIUM 01 - THE LINKS BETWEEN NEURAL CIRCUIT DEVELOPMENT, HUMAN BRAIN EVOLUTION AND DISEASES' - RYOHEI IWATA, APARNA BHADURI, ALESSANDRO VITRIOLO, EWOUD SCHMIDT

Giuseppe Testa[1], Cedric Boeckx[2], Adrianos Skaros[1], Alessandro Vitriolo[1], Oliviero Leonardi[1], Nicolò Caporale[1], Emanuele Villa[1], Sebastiano Trattaro[1], Juan Moriano Palacios[2]
[1]Human Technopole, Neurogenomics, Milan, Italy, [2]ICREA and Universitat de Barcelona, Cognitive Biology Of Language, Barcelona, Spain

Anatomically modern humans have evolved facial and neural features that significantly differ from those of archaic hominins and plausibly underlie their cognitive-behavioral specificities. Their evolution has thus far been inferred from fossil records and through the comparison of modern and archaic genomes. The modern face, with its overall smaller size and retracted features, has been understood as an instance of self-domestication, a process hypothesized to have selected mild neural crest deficits, and for which we recently provided the first experimental evidence. Yet, the modern human brain does not meet the core predictions of the self-domestication hypothesis, either in size (not reduced, unlike with most domesticates) or temporal emergence and shape. It is in fact the growth reconfiguration in specific areas of the braincase (e.g. globularity) that sets us apart from our closest relatives, and this evolved much more recently than the modern face. Here, I discuss our latest inroads to make the evolutionary logic of the modern human brain experimentally tractable, by leveraging selected neurodevelopmental disorders whose neural phenotypes, aligned to novel paleogenomic datasets, illuminate the modern human condition. Specifically, we mobilize the latest advances from human developmental biology at single cell resolution and paleogenomics to enable an empirically tested, systems-level definition of the molecular logic underlying our recent evolution.

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PRESENTATION NUMBER: S004

HUMANIZING THE MOUSE BRAIN: HOW A HUMAN-SPECIFIC MODIFIER OF CORTICAL CONNECTIVITY SHAPES BRAIN DEVELOPMENT, FUNCTION, AND DISEASE.

SYMPOSIUM 01 - THE LINKS BETWEEN NEURAL CIRCUIT DEVELOPMENT, HUMAN BRAIN EVOLUTION AND DISEASES' - RYOHEI IWATA, APARNA BHADURI, ALESSANDRO VITRIOLO, EWOUD SCHMIDT

Ewoud Schmidt
Medical University of South Carolina, Department Of Neuroscience, Charleston, United States of America

Human cortical pyramidal neurons (PNs) are characterized by increased synaptic density and prolonged synaptic maturation. These features are considered critical for the emergence of human cognition by enabling the formation of more densely connected cortical networks. However, we lack insight into how these features of cortical connectivity emerged during evolution and how they contribute to the cognitive capacity of humans. We previously identified a human-specific gene duplication, SRGAP2C, that when expressed in mouse cortical PNs induces traits characterizing human cortical PNs, including increased synapse density and delayed synaptic maturation. When we mapped connectivity of layer 2/3 PNs, we discovered that SRGAP2C selectively increases cortical connectivity, without affecting subcortical inputs. Moreover, *in vivo* 2-photon Ca^{2+} imaging revealed that SRGAP2C expression improves sensory coding of layer 2/3 PNs by increasing stimulus response probability, while reducing overall spontaneous activity. Strikingly, when we examined the impact of these circuit changes on behavior, we found that SRGAP2C mice display an increased ability to learn in a cortex-dependent sensory discrimination task. Our results suggest that the emergence of SRGAP2C in the human genome critically changed cortical circuit connectivity and function and provided a key evolutionary step towards improved cognition. We currently investigate how modified cortical connectivity drives improved learning by studying circuit dynamics at the cellular and cortex-wide level. Furthermore, given how human-specific traits of synaptic development impact the structural and functional makeup of cortical circuits, our work seeks to understand how human-specific genes shape the phenotypic expression of disease-causing mutations implicated in neurodevelopmental disorders.

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PRESENTATION NUMBER: S005

MAPPING THE TRANSCRIPTIONAL DIVERSITY OF CELL TYPES IN THE DEVELOPING HUMAN BRAIN

SYMPOSIUM 02 - THE CEREBRAL CORTEX CHALLENGE: GENERATING MANY CELL TYPES FROM DISTINCT SOURCES IN A RESTRICTED TIME - ARNOLD KRIEGSTEIN, TANJA VOGEL, MATTHEW HOLT, KARINE LOULIER

Arnold Kriegstein
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The human cerebral cortex is more than three times expanded compared to our closest non-human primate relatives. The cortex emerges from an initially pseudostratified neuroepithelium that gives rise to radial glia, the neural stem cells of the cortex. A number of subtypes of radial glia have been identified as the cortex matures, and single cell RNA sequencing (scRNAseq) has added substantially to our knowledge of these processes. However, the first trimester of cortical development has not been described at this level of molecular detail, and important questions remain about the timing of neurogenesis, the presumed uniformity of the neuroepithelium, and the signals that promote the transition to radial glia. We have begun to characterize the molecular populations of cellular subtypes that exist at the onset of neurogenesis across regions of the developing human brain. Our single-cell transcriptomic and *in-situ* data suggest that early cortical areal patterning is strongly defined by the mutual exclusion of strong frontal or occipital gene expression signatures, with the specification of areas between these two poles arising at later developmental timepoints. Thus, we find evidence supporting the existence of a cortical protomap at the extremities, but also support for the protocortex hypothesis to refine spatial identity between the poles. We find that major signaling pathways including Notch, Wnt, and mTOR drive the specification and maintenance of neuroepithelial stem cells and radial glia. Overall, we provide a comprehensive molecular and spatial atlas of early stages of human brain and cortical development at the onset of neurogenesis.

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PRESENTATION NUMBER: S006

TEMPORAL AND SPATIAL REGULATION OF NEURONAL DIFFERENTIATION IN THE DEVELOPING MOUSE NEOCORTEX

SYMPOSIUM 02 - THE CEREBRAL CORTEX CHALLENGE: GENERATING MANY CELL TYPES FROM DISTINCT SOURCES IN A RESTRICTED TIME - ARNOLD KRIEGSTEIN, TANJA VOGEL, MATTHEW HOLT, KARINE LOULIER

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Transcription factors play various roles in the process of neuronal differentiation during neocortical development. Recent studies have described the waves of activation of transcription factors and alterations in chromatin accessibility at the corresponding target loci in differentiating neurons. However, given that most of these studies analyzed a mixture of neuronal types born at different developmental time points from radial glial cells (a.k.a. neural stem-progenitor cells, NPCs) in the neocortex, fine and subtle temporal changes might have been missed in these studies. We therefore selectively labeled neurons born at around E16 in the mouse neocortex and traced their differentiation process for 4 days by performing single cell ATAC-sequencing analysis. A graph-based clustering identified cell clusters with distinct peaks of chromatin accessibility patterns along the differentiation axis (pseudotime). Importantly, a hierarchical clustering of the motif activity score of 884 transcription factors revealed waves of activation of transcription factor sets at specific temporal windows of differentiation. Some of such transcription factors showed a significant delay of motif activation after their gene expression along the differentiation pseudotime. This analysis also unveiled narrow temporal windows of motif activation for some transcription factors which have not so far been described as regulators of neuronal differentiation. These results thus highlight the advantage of pulse-labeling of a certain neuronal lineage, which leads to high temporal resolution of chromatin analysis and identification of putative factors involved in fine differentiation processes.

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PRESENTATION NUMBER: S007

THE EMERGING CONCEPT OF ASTROCYTE HETEROGENEITY: CONSEQUENCES FOR CNS FUNCTION.

SYMPOSIUM 02 - THE CEREBRAL CORTEX CHALLENGE: GENERATING MANY CELL TYPES FROM DISTINCT SOURCES IN A RESTRICTED TIME - ARNOLD KRIEGSTEIN, TANJA VOGEL, MATTHEW HOLT, KARINE LOULIER

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Astrocytes are a numerous cell type in the central nervous system. They perform many important functions in synapse formation and maintenance, control of local homeostasis and modulation of synaptic transmission. However, the degree to which specialist astrocyte subtypes fulfil these specific tasks is currently unclear. In this talk, I will present work using single cell transcriptomic and *in situ* hybridization approaches, which demonstrates that even astrocytes lying within the same region of mouse brain show distinct molecular and spatial profiles. Furthermore, I will provide evidence obtained from morphological analyses and Ca^{2+} imaging experiments that these subtypes also possess both distinct anatomies and physiologies. These findings are evidence for the existence of specialized astrocyte subtypes, residing both within and between brain regions, and strongly suggest that specific CNS functions, such as axon guidance, synaptogenesis and synaptic transmission, are differentially controlled by specialized astrocyte subtypes.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: S008

ASTROCYTE DIVERSITY AND PLASTICITY IN THE DEVELOPING CEREBRAL CORTEX

SYMPOSIUM 02 - THE CEREBRAL CORTEX CHALLENGE: GENERATING MANY CELL TYPES FROM DISTINCT SOURCES IN A RESTRICTED TIME - ARNOLD KRIEGSTEIN, TANJA VOGEL, MATTHEW HOLT, KARINE LOULIER

Laura Dumas[1], Edson Rodrigues[1], Alexandre Pattyn[1], Jason Durand[1], Hassan Boukhaddaoui[1], Solène Clavreul[2], Jean Livet[2], Karine Loulier[1]
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The functions of the mammalian cerebral cortex rely on the cooperation of distinct cell types, including neurons and astrocytes, which must be produced in defined proportions and whose imbalance can lead to severe pathologies. Astrocytes constitute an heterogeneous population and, while distinct molecular signatures of astrocyte subtypes are becoming better known, the developmental origin underlying this diversity in the mouse cerebral cortex and how it translates into specific functions remains unknown. Using combinatorial genetic markers and multicolor imaging techniques, we labeled adjacent cortical progenitors with rare color markers prior to gliogenesis and tracked their descent over long periods of time to characterize astrogliogenesis. We determined that cortical progenitors contribute both pre- and postnatally to the generation of the astrocyte 3D matrix and that cortical astrocyte clones exhibit high variability in terms of structural organization, localization, number and subtype of cells generated. Although we did not identify dedicated subpopulations of cortical progenitors responsible for astrocyte diversity, we nevertheless found an alternative embryonic source for cortical astrocytes. In addition to characterizing the cellular and molecular properties of these progenitors located in a restricted area of the developing brain, we compare the characteristics of cortical astrocytes derived from these two distinct sources and investigate whether they might be differentially affected in the context of neurodevelopmental disorders. Overall, our work highlights the unsuspected complexity of cortical astrocyte genesis and will lead to a better understanding of the critical cellular and molecular components of astrogliogenesis that may be altered in brain pathologies.

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PRESENTATION NUMBER: S009

PSYCHEDELICS IN TINY AMOUNTS TO ENHANCE COGNITIVE AND EMOTIONAL PROCESSES: WHAT DOES SCIENCE SAY?

SYMPOSIUM 03 - PSYCHEDELIC EFFECTS WITHOUT PSYCHEDELIC EXPERIENCE - KIM KUYPERS, SCOTT THOMPSON, LINDSAY CAMERON, RAFAEL MOLINER

Kim Kuypers
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Microdosing with psychedelics (MD) has gained popular and scientific attention in recent years. It is the practice of taking repeatedly small doses of substances like LSD and psilocybin, for a certain period which can be a few weeks, up to months, and years. People who engage in this are most typically motivated to gain cognitive, psychological, emotional, and/or health benefits. Most of the knowledge we have so far comes from questionnaire studies in which users report retrospectively or keep diaries of the effects they experience during MD. According to users, it leads to positive effects on mood, concentration, focus, and productivity. While there is little attention in the media for potential negative or unwanted effects, these also seem to occur including physical discomfort and heightened feelings of fear. The limited number of placebo-controlled studies in healthy people revealed that MD has subtle effects on cognitive processes and brain connectivity. The findings of placebo-controlled studies in combination with reports from users suggest that MD with psychedelics can be effective in controlling certain psychological symptoms. This means that this phenomenon deserves more attention, partly because psychedelics are relatively safe substances. In the future, placebo-controlled studies will provide more clarity for who (age, diagnosis) MD can be effective and for which (cognitive, emotional) processes. The purpose of this presentation will be to give an overview of the knowledge we have so far about MD and to identify which unanswered questions still exist.

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PRESENTATION NUMBER: S010

HARNESSING PSILOCYBIN FOR NEUROPSYCHIATRIC DISORDERS: PRECLINICAL PERSPECTIVES ON PHARMACOLOGICAL AND PHYSIOLOGICAL MECHANISMS

SYMPOSIUM 03 - PSYCHEDELIC EFFECTS WITHOUT PSYCHEDELIC EXPERIENCE - KIM KUYPERS, SCOTT THOMPSON, LINDSAY CAMERON, RAFAEL MOLINER

Scott Thompson
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Psychedelics have the potential to revolutionize psychiatric pharmacotherapy. Dozens of companies are exploring hundreds of compounds as therapeutics. But how do they work and what are the ideal pharmacological properties these compounds should possess? Preclinical studies have the potential to suggest the pharmacology and physiology underlying their therapeutic actions, both during the induction phase, when the compounds are acting on the brain, and in the induction phase, when persistent beneficial changes result in lasting restoration of the normal state. This presentation will consider both aspects of the anti-anhedonic actions of psilocybin in mice subjected to chronic stress. We find that a single administration of psilocybin restored two behavioral measures of hedonic state: the preference of male mice for sucrose solution and the scent of female urine. We next tested the role of 5HT2ARs, known to underlie the mind-altering properties of psychedelics. We found that psilocybin remained effective when co-administered with the 5HT2R antagonist ketanserin. Following completion of behavioral testing, we prepared hippocampal brain slices and observed that the strength of stress-sensitive TA-CA1 synapses was greater in stressed mice administered psilocybin, compared to vehicle, and that this restoration of synaptic strength was also seen in mice co-administered ketanserin. We conclude that 1) the beneficial actions of psilocybin can be studied in mice, 2) restoration of stress-impaired synaptic function may underlie their therapeutic actions, and 3) neither the behavioral nor synaptic actions of psilocybin require 5HT2AR activation, at least in mice.

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PRESENTATION NUMBER: S011

RETHINKING PSYCHEDELICS: NON-HALLUCINOGENIC ANALOGS OF PSYCHEDELICS AS TREATMENTS FOR NEUROPSYCHIATRIC DISORDERS

SYMPOSIUM 03 - PSYCHEDELIC EFFECTS WITHOUT PSYCHEDELIC EXPERIENCE - KIM KUYPERS, SCOTT THOMPSON, LINDSAY CAMERON, RAFAEL MOLINER

Lindsay Cameron
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The prefrontal cortex (PFC) is a critical brain structure that regulates mood, anxiety and reward. Atrophy of neurons in this brain region is implicated in the development of neuropsychiatric disorders, and thus, strategies to regrow these neurons are highly desirable. Classic psychedelics are potent psychoplastogens that have been shown to produce rapid and sustained antidepressant-like effects in both humans and rodents. Psychedelics target the serotonin 2A (5-HT2A) receptor, which is densely expressed in layer V pyramidal neurons of the PFC. By comparing the effects of hallucinogenic and non-hallucinogenic congeners, we demonstrate that both types of 5-HT2A ligands produce long-lasting changes in neuronal structure and function. Specifically, we demonstrate that these non-hallucinogenic analogs increase cortical neuron dendritic arbor complexity and spine density, comparable to psychedelic compounds. The effects of these compounds were further characterized using whole cell patch clamp, calcium imaging, and beta-arrestin recruitment assays. Mouse behavioral studies revealed that both psychedelics and non-hallucinogenic analogs facilitate anti-depressant-like effects in the tail suspension test. Additionally, psychedelics reverse anhedonia-like behaviour as measured in the sucrose preference test. By using both antagonists of the 5-HT2A receptor and 5-HT2A KO animals, we demonstrate that this receptor plays a critical role in the beneficial effects of non-hallucinogenic analogs of psychedelics. Our work suggests that the hallucinogenic effects of psychedelic compounds can be dissociated from their beneficial effects on neuronal growth and behavior. These data provide an important starting point for the design of safer next-generation therapeutics for treating depression and other stress-related disorders.

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PRESENTATION NUMBER: S012

NEUROPLASTICITY AND TRKB: KEY TO THE THERAPEUTIC POTENTIAL OF PSYCHEDELICS?

SYMPOSIUM 03 - PSYCHEDELIC EFFECTS WITHOUT PSYCHEDELIC EXPERIENCE - KIM KUYPERS, SCOTT THOMPSON, LINDSAY CAMERON, RAFAEL MOLINER

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Psychedelics like LSD and psilocybin induce structural and functional neuroplasticity resembling the action of clinically approved antidepressants. They also show promise as fast-acting antidepressants with long-lasting therapeutic effects. Pharmacologically diverse antidepressants including fluoxetine and ketamine promote neuroplasticity through TrkB, the brain-derived neurotrophic factor (BDNF) receptor. BDNF and TrkB are also thought to play a central role in the action of psychedelics as effectors downstream of their canonical target, the serotonin 2A (5-HT_{2A}) receptor. In this presentation, we will discuss the short and long-term effects of psychedelics on receptor activation, signaling, plasticity and behavior. The roles of 5-HT_{2A} and TrkB in the hallucinogenic and plasticity-inducing effects of psychedelics will be addressed. Finally, we will compare the mechanisms of action of psychedelics with those of other conventional and rapid-acting antidepressants, highlighting the differences and commonalities that may underlie their therapeutic value.

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PRESENTATION NUMBER: S013

A TRANSCRIPTIONAL RHEOSTAT IN OLFACTORY SENSORY NEURONS

SYMPOSIUM 04 - SMELL, CELL-BY-CELL - SANDEEP ROBERT DATTA, ALEXANDER FLEISCHMANN, MARCELA LIPOVSEK, KOEN VAN DEN BERGE

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Animals traversing different environments encounter both stable background stimuli and novel cues, which are thought to be detected by primary sensory neurons and then distinguished by downstream brain circuits. Here, we show that each of the ~1,000 olfactory sensory neuron (OSN) subtypes in the mouse harbors a distinct transcriptome whose content is precisely determined by interactions between its odorant receptor and the environment. This transcriptional variation is systematically organized to support sensory adaptation: expression levels of more than 70 genes relevant to transforming odors into spikes continuously vary across OSN subtypes, dynamically adjust to new environments over hours, and accurately predict acute OSN-specific odor responses. The sensory periphery therefore separates salient signals from predictable background via a transcriptional rheostat whose moment-to-moment state reflects the past and constrains the future; these findings suggest a general model in which structured transcriptional variation within a cell type reflects individual experience.

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PRESENTATION NUMBER: S014

MOLECULAR SIGNATURES OF OLFACTORY CIRCUITS REVEALED BY SINGLE CELL MULTIOMICS ANALYSIS

SYMPOSIUM 04 - SMELL, CELL-BY-CELL - SANDEEP ROBERT DATTA, ALEXANDER FLEISCHMANN, MARCELA LIPOVSEK, KOEN VAN DEN BERGE

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The mammalian cortex comprises ancient paleocortical structures, exemplified by the olfactory cortex, as well as more recently evolved neocortical areas. Neurons in the mouse olfactory cortex differ from neurons in neocortex in their developmental origin, morphology, functional properties and molecular identities. However, the mechanisms driving cell type diversification across these distinct cortical traits remain unknown. Here, we characterize the gene regulatory network (GRN) activity that defines cell type identity in the mouse olfactory cortex, and we compare GRN activity across paleo-, peripaleo- and neocortical areas. Using single cell ATAC and RNA sequencing, we reveal enhancer-gene interactions and identify transcription factors cross-repression as a key mechanism for cell type diversification. Finally, we propose semilunar cells of the olfactory cortex as the ancestral neuronal cell type of the mammalian cortex. Our data provide the first comprehensive molecular description of cell types in the mouse olfactory cortex and identify epigenetic mechanisms underlying cell type diversification during evolution.

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PRESENTATION NUMBER: S015

USING PATCH-SEQ TO STUDY DIVERSITY, FUNCTION AND PLASTICITY IN OLFACTORY BULB DOPAMINERGIC NEURONS

SYMPOSIUM 04 - SMELL, CELL-BY-CELL - SANDEEP ROBERT DATTA, ALEXANDER FLEISCHMANN, MARCELA LIPOVSEK, KOEN VAN DEN BERGE

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Dopaminergic (DA) neurons in the olfactory bulb regulate the transmission of information at the earliest stages of sensory processing and are one of the few neuronal types in the mammalian brain continually generated throughout postnatal life. Here, we ask whether this continuous neuronal production results in a gradient of cell states within the resident population. Birthdating in 4-week old DAT-IRES-Cre/Flox-tdT mice revealed that resident DA neurons span an age range of at least 3 weeks. We next collected individual DA neurons by either manual sorting of tdT positive DA neurons, or aspiration after patch-clamp recordings in acute slices (Patch-seq), and performed deep single-cell RNA sequencing. Clustering analysis identified putative subpopulations of DA neurons, while cell trajectory analysis described a single, unbranched, trajectory that closely matched the clusters. Further analysis revealed differentially expressed genes, significantly enriched for GO terms related to neuronal and synaptic function, indicating that the identified trajectory may reflect a transcriptional maturational gradient. Ongoing analysis of electrophysiological properties along the identified trajectory will reveal whether it describes a gradient of functional states. In summary, we are exploring a hitherto unanticipated gradient of cell states within a specific neuronal subtype that could underpin the functional maturation of DA cells in the postnatal brain.

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PRESENTATION NUMBER: S016

STATISTICAL METHODS FOR THE INTERROGATION OF SINGLE-CELL RNA-SEQ DATA ON THE OLFACTORY EPITHELIUM

SYMPOSIUM 04 - SMELL, CELL-BY-CELL - SANDEEP ROBERT DATTA, ALEXANDER FLEISCHMANN, MARCELA LIPOVSEK, KOEN VAN DEN BERGE

Koen Van Den Berge
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The olfactory epithelium (OE) is one of the few sites in the nervous system that supports active neurogenesis throughout life. Since the olfactory neurons are exposed to the environment, cells must continuously be renewed in order to maintain and/or repair the tissue, from a source population of globose basal cells. In case of injury, however, the olfactory epithelium is capable of regeneration from an otherwise resting stem cell population of horizontal basal cells (HBCs). In this talk, we will introduce statistical methodology that allows us to characterize dynamic systems such as the olfactory epithelium. Specifically, we will introduce Slingshot to be able to characterize continuous developmental cell processes such as cell activation, cell cycle and cellular development. These processes can be summarized as a 'trajectory' including one or multiple lineages, each referring to a possible cellular differentiation path. Based on such a trajectory, we introduce tradeSeq to discover differential gene expression within and between such lineages, as demonstrated through an application on the olfactory epithelium. Finally, contemporary single-cell RNA-seq studies may focus on comparing such dynamic developments between experimental or biological conditions, such as treatment groups or genotypes. We introduce condiments, a novel framework to compare global characteristics of the trajectory, such as topology and cell fates, between conditions, as well as detailed differential gene expression analysis between conditions, within each lineage.

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PRESENTATION NUMBER: S017

DOPAMINE SIGNALS FOR CONFIDENCE-DEPENDENT CHOICE UPDATING

SYMPOSIUM 05 - NEUROMODULATION OF PERCEPTUAL DECISION-MAKING - ARMIN LAK, KENNETH KISHIDA, KATHARINA SCHMACK, MATTHEW MCGINLEY

Armin Lak
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Learning from successes and failures often improves the quality of subsequent decisions. Past outcomes, however, should not influence purely perceptual decisions after task acquisition is complete since these are designed so that only sensory evidence determines the correct choice. Yet, numerous studies report that outcomes can bias perceptual decisions, causing spurious changes in choice behaviour without improving accuracy. In this talk, I will show that the effects of reward on perceptual decisions are principled: past rewards bias future choices specifically when previous choice was difficult and hence decision confidence was low. I will identify this phenomenon in various species and sensory modalities. I will demonstrate that this choice updating can be explained by reinforcement learning models incorporating statistical decision confidence into their prediction errors. Lastly, I will provide three pieces of evidence indicating that midbrain dopamine neurons play critical roles in regulating confidence-dependent choice updating. a) Dopamine neural responses during perceptual decisions quantitatively match confidence-dependent prediction errors in both monkeys and mice. b) Dopamine neural responses predict the magnitude of the psychometric choice bias in the subsequent trial. c) Manipulation of dopamine neurons biases psychometric curves in a trial-by-trial fashion. Together, these results provide a framework for studying learning under sensory uncertainty, and indicate that dopamine signals are well-suited to guide this form of learning.

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PRESENTATION NUMBER: S018

COORDINATION OF EXTRACELLULAR DOPAMINE AND SEROTONIN SIGNALS IN HUMAN STRIATUM DURING CONSCIOUS DECISION-MAKING.

SYMPOSIUM 05 - NEUROMODULATION OF PERCEPTUAL DECISION-MAKING - ARMIN LAK, KENNETH KISHIDA, KATHARINA SCHMACK, MATTHEW MCGINLEY

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How the human brain generates conscious experience is a fundamental problem. In particular, it is unknown how highly variable and dynamic changes in subjective experience are driven by interactions with objective phenomena. We hypothesize a 'dynamic *affective* core' that requires not only dynamic representations of the contents and context of conscious experiences but also their value. The latter, 'representations of value', have begun to be well characterized by computational reinforcement learning models and empirical data that support the idea that dopamine neurons signal 'better (or worse) than expected' experiences by encoding 'reward prediction errors' in changes in their firing rate. However, whether first-person subjective experiences align with dopaminergic encoding of better or worse than expected 'reward prediction errors' has, to our knowledge, not been directly tested. Recently developed technology allows, for the first time ever, high temporal resolution (i.e., sub-second) measurements of the neurotransmitters dopamine and serotonin from deep within the human brain during conscious decision-making and behavior. I will present recent work that supports the hypotheses that dopamine and serotonin encode reward and punishment prediction errors, respectively; that these signals coordinate and drive human behaviors; and, that sub-second fluctuations in dopamine and serotonin levels may drive moment-to-moment changes in the valence of self-reported subjective experiences. Together, our results support the idea that dopamine and serotonin provide valuation update signals in the human brain that guide behavior and may also be critical modulators of subjective phenomenal experience.

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PRESENTATION NUMBER: S019

STRIATAL DOPAMINE MEDIATES HALLUCINATION-LIKE PERCEPTION IN MICE

SYMPOSIUM 05 - NEUROMODULATION OF PERCEPTUAL DECISION-MAKING - ARMIN LAK, KENNETH KISHIDA, KATHARINA SCHMACK, MATTHEW MCGINLEY

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Hallucinations, a central symptom of psychotic disorders, are attributed to excessive dopamine in the striatum. However, the neural circuit mechanisms by which dopamine produces hallucinations remain elusive, largely because hallucinations have been challenging to study in model organisms. Here, we aimed to establish a novel readout of hallucinations that easily translates from humans to mice. Our goal was to provide a circuit-level description of the suggested link between dopamine and hallucinations. We reasoned that hallucinations can be operationalized as false perceptions of non-existent signals that are experienced with high confidence. We developed a task to quantify hallucination-like perception in mice and humans alike. We found evidence across species that hallucination-like perceptions were a valid readout of hallucinations: In humans, self-reported hallucinations were correlated with hallucination-like perceptions. In mice, hallucinogenic manipulations using ketamine or prior expectations consistently induced hallucination-like perceptions. Neural circuit investigations in mice showed that elevated baseline dopamine in both ventral and tail of striatum preceded hallucination-like perceptions. Computational modelling suggested that ventral striatal dopamine was consistent with the encoding of reward expectations, whereas the tail of striatum dopamine signalled prior expectation of hearing a signal. In line with this, optogenetic stimulation of dopaminergic activity in the tail of the striatum induced more hallucination-like perceptions, and this was reversed by the antipsychotic haloperidol. Taken together, our findings reveal a causal role for dopamine-dependent striatal circuits in hallucination-like perception and open new avenues to develop circuit-based treatments for psychotic disorders.

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PRESENTATION NUMBER: S020

TOWARDS NEUROMODULATORY MECHANISMS OF AROUSAL AND MOTIVATION IN SUSTAINED ATTENTION

SYMPOSIUM 05 - NEUROMODULATION OF PERCEPTUAL DECISION-MAKING - ARMIN LAK, KENNETH KISHIDA, KATHARINA SCHMACK, MATTHEW MCGINLEY

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The expectation of reward can lead to heightened perceptual sensitivity and improved decision-making. This is sometimes referred to as ‘the arousal effect.’ However, it is not clear how reward expectancy relates to arousal in its underlying circuit mechanisms or precise behavioral impacts. We here developed a challenging auditory feature-based sustained attention task in head-fixed mice, called the ‘attentional intensity’ task. Mice respond to unpredictable emergence of temporal coherence in an otherwise incoherent tone cloud by licking for sugar-water reward, analogous to coherent motion in visual attention. Difficulty is parametrically varied by partially degrading the coherence. The task alternates between blocks of trials with large and small rewards, resulting in shifts in reward expectancy that manifest as shifts in performance. Arousal is monitored via pupil size. We find that mice exhibit >5 attentional shifts/session, tightly time-locked to transitions in reward block. These shifts in attention manifest as increase discriminatory licking, and multiple signatures in a drift diffusion model of improved target stimulus engagement. Contrary to the naive prediction that high reward expectancy increases arousal, baseline pupil size actually decreased on average in high reward blocks, moving towards an optimal middle size, accounting for a fraction of the effect of reward expectation on attentional intensity. In ongoing experiments, we use two-photon calcium imaging to determine the roles of frontal-sensory and neuromodulatory signals in mediating the adaptation of attentional intensity to task utility.

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PRESENTATION NUMBER: S021

CHOROID PLEXUS AND NON-CELL AUTONOMOUS REGULATION

SYMPOSIUM 06 - GO WITH THE FLOW! CHOROID PLEXUS MECHANISMS SHAPE BRAIN FUNCTION IN HEALTH AND DISEASE - ANNARITA PATRIZI, JEAN-FRANCOIS GHERSI-EGEA, FIONA DOETSCH, ROOSMARIJN VANDENBROUCKE

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The Choroid Plexus (ChP), a specialized epithelial membrane located in all ventricles of the brain, produces 60-70% of the total cerebrospinal fluid (CSF) and forms the blood-CSF barrier impacting CSF and brain homoeostasis. In addition, ChP carries out neuro-endocrine, excretory and neuro-immune actions that are most likely involved in multiple neurological diseases. Recently, great attention has been given to the ChP as a key structure contributing to brain-body communication by sensing and reacting to both internal and external stimuli. However, only a few selective stimuli have been identified and very little is known about how the ChP can sense and respond to them. In the last few years our group has been focusing on understanding whether and how the ChP epithelial cell primary cilia, located on the apical cellular membrane and bathed in the CSF, can act as bio-sensors detecting surrounding stimuli. We did, indeed, demonstrate that ChP is characterized by a unique process of multi-ciliation that is spatio-temporally controlled. Furthermore, we found that ChP cilia are highly dynamic organelles that adapt and change throughout lifetime. In fact, ChP primary cilia are formed during embryogenesis but are gradually lost in early postnatal life both in mice and human postmortem samples. Interestingly, the maintenance of the cilium can be modulated by the presence of selective growth factors, suggesting that they can adapt to and reflect selective and different needs of the brain.

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PRESENTATION NUMBER: S022

THE CHOROID PLEXUS-CSF SYSTEM IN PERINATAL ADVERSE CONDITIONS

SYMPOSIUM 06 - GO WITH THE FLOW! CHOROID PLEXUS MECHANISMS SHAPE BRAIN FUNCTION IN HEALTH AND DISEASE - ANNARITA PATRIZI, JEAN-FRANCOIS GHERSI-EGEA, FIONA DOETSCH, ROOSMARIJN VANDENBROUCKE

Jean-Francois Ghersi-Egea
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The choroid-plexus CSF system plays an important role in protecting brain fluid environment during pre-and post-natal periods of development. It also synthetizes or transports important proteins and trophic factors necessary to the development and normal maturation of the brain. These functions can be challenged in the setting of perinatal injuries, which increases the risk of abnormal development. Such injuries include inflammatory/infectious diseases. The choroid plexuses are highly sensitive to cytokines and toll-like receptor ligands. They respond by secreting an array of cytokines and chemokines in the choroidal stroma and CSF, that can be followed by immune cell infiltration into the CSF. Independently of their function as inflammatory modulators, some of these bioactive molecules are present in CSF during the perinatal/postnatal period. Their physiological levels appear finely regulated, and following cytokine-specific developmental patterns, likely reflecting the role that these bioactive molecules play in the development of the neuronal and vascular networks. Changes in CSF cytokines that result from choroid plexus activation or from immune cells invading the CSF through the choroid plexuses may participate in the disorganization of brain development induced by perinatal injuries.

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PRESENTATION NUMBER: S023

CHOROID PLEXUS REGULATION OF ADULT NEURAL STEM CELLS

SYMPOSIUM 06 - GO WITH THE FLOW! CHOROID PLEXUS MECHANISMS SHAPE BRAIN FUNCTION IN HEALTH AND DISEASE - ANNARITA PATRIZI, JEAN-FRANCOIS GHERSI-EGEA, FIONA DOETSCH, ROOSMARIJN VANDENBROUCKE

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Specialized niches support the lifelong maintenance and function of tissue-specific stem cells. Stem cells in the adult ventricular-subventricular zone (V-SVZ) adjacent to the lateral ventricles give rise to olfactory bulb neurons, as well as different types of glia. Adult V-SVZ neural stem cells contact the cerebrospinal fluid, which flows through the brain ventricles, and depending on their spatial location generate different types of cells. They continuously integrate extrinsic signals to either maintain their quiescent state or to become activated and generate progeny.We have found that different physiological states and long-range signals regulate regionally distinct pools of V-SVZ stem cells. A key niche compartment is the choroid plexus, a vascularized epithelial structure in the brain ventricles that forms the blood-CSF barrier. The choroid plexus also secretes diverse factors into the CSF that differentially regulate adult neural stem cells. The choroid plexus is uniquely poised to sense signals in the blood and the CSF and in turn change its secretome in response to different states. Here we show that the choroid plexus transcriptome exhibits circadian changes, sex differences, and changes during the estrous cycle. Females have more macrophages than males, and male and female choroid plexus-secreted factors elicit different effects on adult neural stem cells. Given the multiple roles of the choroid plexus, our findings highlight that dynamic fluctuations in the choroid plexus transcriptome and secretome may have important impact on adult neural stem cells, as well as brain physiology in both health and disease.

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PRESENTATION NUMBER: S024

NORMAL AND ATYPICAL NEURODEVELOPMENTAL TRAJECTORIES OVER CHILDHOOD/ADOLESCENCE

SYMPOSIUM 07 - NEUROIMAGING APPROACHES TO UNDERSTANDING ADOLESCENT MENTAL HEALTH: OPPORTUNITIES FOR INTERVENTION - SOPHIA FRANGOU, HEATHER WHALLEY, MARIEKE BOS, RYAN MUETZEL

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Background: Prior literature has documented multiple biological and social factors that influence both brain development and mental health outcomes in adolescence. The current presentation discusses the role of the biosocial context of adolescent brain development and its association with psychopathology. Methods: A range of computational methods was applied to data pooled from multiple cohorts of adolescents to derive normative measures of regional brain development and to quantify the contribution of environmental exposures to brain morphometry, myelination and synaptic density in healthy adolescents and those with affective psychopathology. Results: The presentation introduces CentileBrain (www.centilebrain.org), an online tool for normative modeling of morphometric data and demonstrates its utility in quantifying the degree of morphometric deviance in adolescents with affective psychopathology. Application of clustering approaches to neuroimaging data and social exposures identified clusters of adolescents with advanced, delayed or atypical maturation. Conclusion: The availability of large datasets and computational tools is opening new avenues in the study of adolescent brain development.

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PRESENTATION NUMBER: S025

PUBERTAL TRANSITIONS, CHANGES IN NEUROBIOLOGY AND RISK FOR ADOLESCENT DEPRESSION

SYMPOSIUM 07 - NEUROIMAGING APPROACHES TO UNDERSTANDING ADOLESCENT MENTAL HEALTH: OPPORTUNITIES FOR INTERVENTION - SOPHIA FRANGOU, HEATHER WHALLEY, MARIEKE BOS, RYAN MUETZEL

Heather Whalley[1], Niamh Macsweeney[1], Xeuyi Shen[1], Breda Cullen[2], Alex Kwong[1], Sophia Frangou[3], Liana Romaniuk[1]
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Adolescence is a period of increased vulnerability to mental health conditions, particularly internalising difficulties such as depression. Earlier-onset is often associated with a more severe illness course and a range of psychosocial and physical difficulties which perpetuate across the lifespan. Given the emergence of depression during the adolescent period, the role that pubertal development may play in this heightened vulnerability has garnered increasing attention. Earlier pubertal timing (relative to same-age, same-sex peers) has been associated with higher rates of depressive disorders, particularly in females. Further, genetic studies have found that earlier age of menarche is implicated in depression. Adolescence is also a time of immense neurobiological change and brain structural differences have been found in both adults and adolescents with depression. However the role that neural mechanisms may play in the relationship between pubertal timing and depression risk remains unknown. Here, we present findings examining whether brain structure mediates the association between early pubertal timing and depressive symptoms in a large sample of early adolescents (aged 9-13 years) from the Adolescent Brain and Cognitive Development (ABCD) Study®.

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PRESENTATION NUMBER: S026

LONGITUDINAL STRUCTURAL BRAIN DEVELOPMENT AND EXTERNALIZING BEHAVIOR IN ADOLESCENCE

SYMPOSIUM 07 - NEUROIMAGING APPROACHES TO UNDERSTANDING ADOLESCENT MENTAL HEALTH: OPPORTUNITIES FOR INTERVENTION - SOPHIA FRANGOU, HEATHER WHALLEY, MARIEKE BOS, RYAN MUETZEL

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BACKGROUND AND AIMS: Most adolescents take part in some kind of antisocial behavior, such as rule-breaking, aggression, curving, or underage drinking. Antisocial behavior is marked by individual differences, not only in extent and severity but also in stability over time. Specifically, the transition between adolescence and young adulthood has long been recognized as a potential turning point for the development of antisocial behavior, due to changes in social contexts and ongoing psychological and neurobiological maturation. To increase our understanding of developmental trajectories of antisocial behavior, longitudinal study designs are pivotal. METHODS: I will present data of two longitudinal studies focusing on neurodevelopment of aggressive behavior. In the first study, we examined the relation between structural brain development and aggressive behavior in a population sample (N=299; age 8-28). In the second study, we examined brain activity in a social aggression task in a high-risk sample of young adults (N=55; age 20 - 28). RESULTS: (1) decrease of hippocampal volume was associated with increase in aggressive behavior (2) individuals with persistent or desistent trajectories of antisocial behavior showed dissociable patterns of neural activity when receiving social feedback in a social aggression task: desisting and persisting trajectory groups showed higher activity in the insula compared to controls, and the desisting trajectory group showed higher activity in dlPFC. CONCLUSION: In this talk, I will emphasize the value of studying different neurobiological mechanisms and using an individual differences approach to better understand the complexity of antisocial behavior and its diverse trajectories.

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PRESENTATION NUMBER: S027

NEUROIMAGING OF NEURODEVELOPMENT AND EMERGING MENTAL ILLNESS: A POPULATION NEUROSCIENCE PERSPECTIVE

SYMPOSIUM 07 - NEUROIMAGING APPROACHES TO UNDERSTANDING ADOLESCENT MENTAL HEALTH: OPPORTUNITIES FOR INTERVENTION - SOPHIA FRANGOU, HEATHER WHALLEY, MARIEKE BOS, RYAN MUETZEL

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Neuroimaging offers a unique window into the developing brain, providing important insights into emergent mental illness. Identification of reproducible neurobiological signatures of psychopathology with strong predictive value remains a priority. However, relatively modest effect sizes from univariate statistical techniques combined with heterogenous symptom presentation may impede the detection of such clinically-relevant imaging biomarkers. Multivariate statistical techniques show promise in identifying complex patterns in high dimensional data and in aggregating across many small effects. For example, techniques such as canonical correlation analysis have demonstrated utility in identifying maximally-correlated features from both neuroimaging and behavioral data. This approach allows for the delineation of brain-based dimensions of psychiatric problems which are not constrained by traditional diagnostic nosology and potentially yield better clinical predictive value. This presentation will cover the application of canonical correlation analysis to identify maximally correlated features of resting-state functional connectivity and dimensional psychiatric symptoms in children 9-to-11 years old. Using data from two large, population-based cohorts (Generation R, ABCD), we present different brain-based dimensional features of psychiatric problems which may offer insights into transdiagnostic neurobiological signatures of mental illness. Importantly, we also highlight several caveats and potential limitations related to the application of canonical correlation analysis in high-dimensional resting-state functional connectivity data.

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PRESENTATION NUMBER: S028

MACHINE LEARNING APPROACHES FOR UNRAVELING HUMAN MECHANISMS OF BRAIN DYSFUNCTION IN MICE.

SYMPOSIUM 08 - NEURONAL NETWORK AND SYNAPTIC DYSFUNCTION IN ALZHEIMER'S DISEASE - JORGE PALOP, SILVIA VIANA DA SILVA, MARTIN FUHRMANN, ANNABELLE SINGER

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New molecular approaches of genome editing are creating a paradigm shift in modeling neurodegenerative disorders by humanizing mouse genes carrying disease-associated genomic changes. These exquisite disease-induced manipulations – often affecting only a few amino acids – mimic closely human disease mechanisms, yet represent a tremendous challenge for assessing their behavioral and functional manifestations that define neurological disorders because standard behavioral approaches often lack the required sensitivity and breadth. We approached this technological barrier by implementing machine learning platforms for assessing diseased-induced behavioral manifestations of humanized models of Alzheimer's disease (AD). We found that machine learning approaches reveal prominent behavioral manifestations and cognitive dysfunction in humanized AD mice with an unprecedented level of quantitative precision and insights into behavioral organization.

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PRESENTATION NUMBER: S029

SUB-CIRCUIT SPECIFIC DEFICITS UNDERLYING SPATIAL MEMORY IMPAIRMENTS IN ALZHEIMER'S DISEASE

SYMPOSIUM 08 - NEURONAL NETWORK AND SYNAPTIC DYSFUNCTION IN ALZHEIMER'S DISEASE - JORGE PALOP, SILVIA VIANA DA SILVA, MARTIN FUHRMANN, ANNABELLE SINGER

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Deficits in spatial navigation are among the early symptoms in Alzheimer's disease (AD) patients, consistent with the hippocampal formation being necessary for spatial computations and with disease onset in the hippocampal formation. Although it is recognized that early symptoms correspond to brain regions that are affected early in the disease, it is not clear whether further cognitive decline is solely caused by a spreading cellular pathology, or whether a focal pathology can by itself cause aberrant neuronal activity in a larger network. These possibilities cannot be distinguished in standard disease models, which broadly express AD-related proteins across brain regions. We therefore generated a mouse model in which the expression of mutant human APP was limited to hippocampal CA3 cells (CA3-APP mice). We first asked whether the limited pathology in CA3 can result in memory deficits and found memory impairments in CA3-APP mice. By recording neuronal activity in the hippocampus, during the memory task, we asked to what extent pathological neuronal activity patterns emerged in different hippocampus sub-regions. We found that early circuit dysfunction did not include differences in the spatial firing patterns of place cells, but were rather encompassed by a reduced theta oscillation frequency and disruption in sequential firing of CA1 place cells during the task. This underlines the influence that altered network function has on the memory deficits associated with AD, indicating that the disease progression can be driven by altered network mechanisms rather than changes at the level of individual cells.

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PRESENTATION NUMBER: S030

MEMORY TRACE DISTURBANCES UNDER AD-LIKE CONDITIONS

SYMPOSIUM 08 - NEURONAL NETWORK AND SYNAPTIC DYSFUNCTION IN ALZHEIMER'S DISEASE - JORGE PALOP, SILVIA VIANA DA SILVA, MARTIN FUHRMANN, ANNABELLE SINGER

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Memory impairment is a characteristic of Alzheimer's disease (AD). The hippocampus is important for memory processes and primarily affected in AD. Ensembles of neurons in the hippocampus are thought to be active during encoding and retrieval of a memory. These reactivated ensembles of neurons are called a memory trace or engram. We hypothesized that memory traces would be disturbed under AD-like conditions in a mouse model. To test this hypothesis, we visualized hippocampal memory traces under AD-like conditions over the course of a hippocampus-dependent memory test – contextual fear conditioning. Surprisingly, we detected intact memory traces even in mice with AD-like pathology. However, these memory traces were superimposed by other active ensembles of neurons that most likely encoded novelty-like information. These results indicated that memory trace interference could be the basis for memory impairment. We used chemogenetic experiments to manipulate novelty-like neuronal ensemble activity, either artificially activating or inhibiting novelty-like ensembles. We found that artificial silencing of novelty-like neuronal activity improved memory under AD-like conditions, suggesting that memory trace interference might be a neuronal network mechanism affecting memory in AD.

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PRESENTATION NUMBER: S031

NEW APPROACHES TO ALZHEIMER'S: FROM NEURAL DEFICITS TO STIMULATION THAT BOOSTS IMMUNE FUNCTION

SYMPOSIUM 08 - NEURONAL NETWORK AND SYNAPTIC DYSFUNCTION IN ALZHEIMER'S DISEASE - JORGE PALOP, SILVIA VIANA DA SILVA, MARTIN FUHRMANN, ANNABELLE SINGER

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In this talk I will describe how discovering deficits in neural activity in Alzheimer's disease (AD) led to the development of new neural stimulation approaches to treat the disease. Spatial navigation deficits are one of the earliest symptoms of AD and the hippocampus is one of the areas first affected by the disease. First, we discovered how neural codes underlying memory-based spatial decisions fail in animal models Alzheimer's disease (AD). Using a virtual reality behavior paradigm to record and manipulate neural activity in transgenic mice, the primary animal model of AD, we found deficits in synaptic efficacy during behavior and in patterns of activity that are required for spatial memory. The neural deficits we found inspired us to stimulate specific frequencies of activity that are lacking in the Alzheimer's model mice. To achieve this, we developed new non-invasive sensory stimulation to drive rhythmic neural activity at different frequencies. Driving gamma frequency (40Hz) activity non-invasively mobilized the immune system and reduced pathogenic proteins in mouse models of Alzheimer's disease. Investigating the biochemical mechanisms involved, we discovered a unique immune signaling cascade by which gamma neural activity recruits neuroimmune function. Interestingly, other frequencies have distinct effects on neuroimmune signaling and cells. Finally in humans with Alzheimer's disease, gamma sensory stimulation altered neuroimmune signaling in the cerebral spinal fluid and stengthened connectivity of neural circuits important for memory. These discoveries could lead to new therapies for Alzheimer's disease by driving specific patterns of neural activity to impact cognitive, cellular, and molecular dysfunction.

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PRESENTATION NUMBER: S032

DEVELOPMENTAL WIRING OF PREFRONTAL CIRCUITS: ACTIVITY DECORRELATION AS RESULT OF EXCITATION/INHIBITION SHIFT

SYMPOSIUM 09 - THE EMERGENCE OF FUNCTION IN DEVELOPING CORTICAL CIRCUITS - ILEANA HANGANU-OPATZ, NATALIA DE MARCO GARCIA, CARLOS PORTERA-CAILLIAU, OSCAR MARIN

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The transition from early stages in which neurons display highly synchronous activity patterns to a mature state in which neural activity is sparse and decorrelated, is poorly understood. This transition has important functional consequences, as the latter state allows for more efficient storage, retrieval and processing of information. Here, we show that in the developing mouse prefrontal cortex (PFC), neural activity decorrelates across timescales spanning more than three orders of magnitude. This developmental phenomenon is accompanied by a concomitant tilting of excitation/inhibition (E-I) ratio towards inhibition. Using optogenetic manipulations and neural network modeling, we provide extensive evidence revealing how the two processes are causally linked, and how, across the first two postnatal weeks, in the mouse PFC, a relative increase of inhibition drives the decorrelation of neural activity. Accordingly, in two mouse model of neurodevelopmental disorders, subtle alterations in E-I ratio are associated with specific impairments in the correlational structure of spike trains. Finally, we show that an analogous transition takes place also in the developing human brain. We conclude that changes in E-I ratio control the sparseness of neural activity, and that developmental imbalances in this process might be relevant for understanding the pathogenesis of neurodevelopmental disorders.

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PRESENTATION NUMBER: S033

GABAERGIC INPUTS AND THE FUNCTIONAL INTEGRATION OF PYRAMIDAL NEURON SUBTYPES IN THE SOMATOSENSORY CORTEX

SYMPOSIUM 09 - THE EMERGENCE OF FUNCTION IN DEVELOPING CORTICAL CIRCUITS - ILEANA HANGANU-OPATZ, NATALIA DE MARCO GARCIA, CARLOS PORTERA-CAILLIAU, OSCAR MARIN

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Dysfunction of gamma-aminobutyric acid (GABA)-mediated inhibitory circuits isstrongly associated with neurodevelopmental disorders. However, it is unclear how genetic predispositions impact circuit assembly. Using in vivo two-photon and widefield calcium imaging in developing mice, we study how early GABAergic dysfunction impacts neuronal development and circuit assembly. Using longitudinal in vivo two-photon and widefield calcium imaging in developing mice, we show that altered interneuron function and $GABA_A$ receptor signaling lead to a long-lasting increase in correlated activity in the somatosensory cortex. This aberrant network pattern impacts interneuron survival and neuronal development including the assembly of long-range circuits.

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PRESENTATION NUMBER: S034

DEVELOPMENTAL HYPOFUNCTION OF CORTICAL PARVALBUMIN INTERNEURONS IN FRAGILE X SYNDROME

SYMPOSIUM 09 - THE EMERGENCE OF FUNCTION IN DEVELOPING CORTICAL CIRCUITS - ILEANA HANGANU-OPATZ, NATALIA DE MARCO GARCIA, CARLOS PORTERA-CAILLIAU, OSCAR MARIN

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Cortical circuit dysfunction is a primary pathophysiology in neurodevelopmental disorders (NDDs). Considering how symptoms in NDDs/autism become apparent in toddlers, circuit changes must emerge very early in cortical development. Over the last decade, developmental differences in cortical synaptic dynamics, neuronal adaptation, and network synchrony have been reported in the *Fmr1* knockout (KO) mouse model of Fragile X Syndrome (FXS), a prototypical NDD. In addition, the density, maturity and activity of parvalbumin cortical interneurons (PV INs) are all reduced in *Fmr1* KO mice and in other autism mouse models. Here, we will present our latest research on PV cells and their precursors from the medial ganglionic eminence (MGE) and how their dysfunction in *Fmr1* KO mice within feedforward circuits in the primary somatosensory cortex (S1) contributes to atypical sensory processing. We find that the density of PV INs in S1 is reduced in both juvenile and adult *Fmr1* KO mice, as well as in post-mortem tissue from human FXS cases. Furthermore, the density and the firing of Nkx2.1-expressing precursors of PV INs are both reduced in *Fmr1* KO mice by postnatal day (P) 6. Remarkably, increasing their activity from P5 to P9 using excitatory DREADDs significantly increases the density of PV cells at P15. Moreover, increasing the activity of PV cells in *Fmr1* KO mice with a novel allosteric modulator of Kv3.1 channels (responsible for fast-spiking characteristics of PV INs) improves deficits in the tuning and the adaptation of excitatory neurons in S1 to repetitive whisker stimulation.

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PRESENTATION NUMBER: S035

MOLECULAR MECHANISMS REGULATING THE MATURATION OF CORTICAL GABAERGIC INTERNEURONS

SYMPOSIUM 09 - THE EMERGENCE OF FUNCTION IN DEVELOPING CORTICAL CIRCUITS - ILEANA HANGANU-OPATZ, NATALIA DE MARCO GARCIA, CARLOS PORTERA-CAILLIAU, OSCAR MARIN

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GABAergic interneurons play crucial roles in the regulation of neural circuit activity in the cerebral cortex. A hallmark of cortical interneurons is their remarkable structural and functional diversity, yet the molecular determinants and the precise timing underlying their diversification remain largely unknown. The search for mechanisms controlling the diversity of GABAergic interneurons has primarily focused on the analysis of transcriptional programs driving the initial specification of different types of interneurons. However, relatively little is known about the mechanisms regulating their terminal differentiation. In this talk, I will describe molecular mechanisms regulating the maturation and plasticity of PV+ interneurons, which allow their functional integration into neuronal assemblies in the neocortex.

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PRESENTATION NUMBER: S036

REGULATION OF ADULT NEURAL STEM CELL CYCLING HETEROGENEITY

SYMPOSIUM 10 - NEURAL STEM CELLS IN THE ADULT VERTEBRATE BRAIN - ISABEL FARIñAS, LUCA BONFANTI, LAURE BALLY-CUIF, DIEGO GARCíA-GONZáLEZ

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Most mammalian tissues harbor long-lived stem cells (SCs), which balance their self-renewing proliferation with the production of differentiated progeny to compensate for physiological cell loss. Subsets of SCs can spend long periods in quiescence, but the molecular regulation of this state as well as the reversible transitions between dormancy and activation have started to be understood only recently. Furthermore, subsets of non-cycling SCs in a particular tissue can be found at varying depths of quiescence. While all these cells share a transcriptional program of quiescence, some of them are more prone to engage proliferation upon mitogen stimulation. This state of shallow quiescence has been described in muscle SCs, hematopoietic SCs, and neural SCs (NSCs). Recent refinements of the procedures to isolate NSCs from the adult mouse subependymal zone (SEZ) by fluorescence-activated cell sorting together with deep sequencing and cycling analysis now allows to recognize NSCs in three states: quiescent (q), quiescent-primed (p), and activated (a). Molecularly defined primed-like and activated-like NSCs dividing at different rates can detected in neurospheres growing under mitogenic stimulation suggesting intrinsic properties. The existence and interchangeability of these states suggest finely tuned mechanisms of cycling regulation, the molecular details of which, however, remain unknown. The combination of the molecular knowledge about these states with ways to label quiescent NSCs since mid-gestation by in utero electroporation is helping unravel physical interactions between quiescent NSCs and their niche with impact on cell transitions.

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PRESENTATION NUMBER: S037

BEYOND STEM CELL-DRIVEN ADULT NEUROGENESIS: THE COMPLEX ISSUE OF “IMMATURE” NEURONS

SYMPOSIUM 10 - NEURAL STEM CELLS IN THE ADULT VERTEBRATE BRAIN - ISABEL FARIñAS, LUCA BONFANTI, LAURE BALLY-CUIF, DIEGO GARCíA-GONZáLEZ

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Research in adult neurogenesis has revealed some general trends: a progressive reduction across the animal lifespan (due to stem cell depletion); remarkable interspecies differences (with reduction from non-mammalian vertebrates to mammals, and from mouse to gyrencephalic species).
In mammals, “canonical” neurogenic processes (stem cell-driven neurogenesis) are restricted to small stem cell niches persisting from embryonic germinal layers. The genesis of new neurons has also been reported in parenchymal brain regions, their outcome being mostly “incomplete”. Whichever the process involved, several populations of “young” neurons can be found at various locations of the brain.
Across the last few years, further complexity emerged: i) molecules of immaturity can also be expressed by pre-natally generated cells, then maintaining immature features later on (“immature” or “dormant” neurons); ii) interspecies differences exist concerning the types, location, amount of undifferentiated neurons; iii) re-expression of immaturity can occur in aging (dematuration). These twists are introducing a somewhat different definition of neurogenesis than normally assumed, in which our knowledge of the “immature” neurons is less sharp. Moreover, a mix of newlyborn and non-newly generated immature cells expressing common markers of immaturity can exist (maybe coexist).
In this emerging complexity, there is a need for complete mapping of the different “types” of young neurons, considering their role in postnatal development, plasticity, functioning, and interspecies differences. Important aspects are at stake: the possible role(s) that these neuronal populations may play in maintaining brain efficiency and in prevention/repair of neurological disorders; nonetheless, the correct translation of results obtained from laboratory rodents.

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PRESENTATION NUMBER: S038

SINGLE-CELL AND POPULATIONAL CONTROL OF ADULT NEURAL STEM CELL MAINTENANCE

SYMPOSIUM 10 - NEURAL STEM CELLS IN THE ADULT VERTEBRATE BRAIN - ISABEL FARIñAS, LUCA BONFANTI, LAURE BALLY-CUIF, DIEGO GARCíA-GONZáLEZ

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We aim to understand how single cell and population events interplay to ensure the maintenance of neural stem cell (NSC) pools in the adult brain. We focus on the dorsal telencephalon (pallium), which hosts NSCs in all adult vertebrates. In the zebrafish, pallial NSCs are a monolayer of tightly juxtaposed radial glia. These cells are mostly quiescent but can transiently activate (ie. enter the cell cycle) to divide and generate other NSCs and/or neurons. The NSC decision to activate, and the fate choices it makes at division, are two key events conditioning NSC maintenance. These events are controlled at both the single-cell and the population levels, and we are taking quantitative and dynamic approaches to understand these processes in time and space. For this, we developed an intra-vital imaging method to record, over weeks and with single cell resolution, the behavior of all NSCs in their niche. With this, we generated a 4D map of NSC activation and division events. Using spatial statistics and mathematical modeling in an NSC lattice, we showed that NSC activation events are spatiotemporally correlated by local and temporally delayed interactions that occur between brain germinal cells and generate self-propagating dynamics. We also observed that NSC apical size is highly predictive of NSC fate decisions at division, and are analyzing the mechanisms involved and their cell- and non-cell-autonomous impact. Together, this work will highlight how NSCs across the germinal sheet coordinate their state and fate decisions for the harmonious and long-lasting maintenance of the NSC pool.

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PRESENTATION NUMBER: S039

MECHANISMS REGULATING INTERNEURON IDENTITY IN THE POSTNATAL BRAIN

SYMPOSIUM 10 - NEURAL STEM CELLS IN THE ADULT VERTEBRATE BRAIN - ISABEL FARIñAS, LUCA BONFANTI, LAURE BALLY-CUIF, DIEGO GARCíA-GONZáLEZ

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An ample range of interneuron subtypes are generated during brain development. In the mammalian brain, specification of interneuron identity is thus a highly controlled process that emerges in the ventricular-subventricular zone (V-SVZ) around the lateral ventricles. There, a population of heterogeneous neural stem cells (NSCs) give rise to intermediate progenitor cells (IPC), which in turn, produce neuroblasts that migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB). Once within the OB, neuroblasts integrate into specific layers where they become distinct subtypes of mature interneurons, which are involved in the processing of olfactory information. The V-SVZ-OB system is thus an excellent model to study interneuron identity specification during brain development. Here we present a comprehensive analysis of V-SVZ-OB interneuron lineages in postnatal mouse brain development with the help of single-cell transcriptomics. We provide further evidence for the existence of different subsets of NSCs, depending on their spatial localisation within the V-SVZ, and we show a deep characterisation of mature OB interneuron supopulations. In addition, we employ virus-mediated gene expression modification to provide functional evidence of how the specific expression of transcription factors modulates the number of OB interneurons.

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PRESENTATION NUMBER: S040

CO-TRANSMISSON OF ACH AND GABA THROUGH DEVELOPMENT

SYMPOSIUM 11- REVISITING DALE'S PRINCIPLE- NEUROTRANSMITTER PLASTICITY AND CO- TRANSMISSION IN HEALTH AND DISEASE - ADAM GRANGER, JING REN, THOMAS STEINKELLNER, SWETHA GODAVARTHI

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The cholinergic system plays a crucial role in a variety of cognitive processes, including maintaining attention, learning and memory, and sensory processing. However, our understanding of how cholinergic neurons influence cortical activity is incomplete. Specifically, we have recently learned that many cholinergic neurons of the basal forebrain, medial septum, and cortex can also release the neurotransmitter GABA. Because acetylcholine is typically excitatory and GABA is inhibitory, co-transmission of both of these molecules appears contradictory. In addition, we found that that while an integrated genetic measure of GABA release indicates that nearly all cholinergic neurons are able to release GABA, *in situ* labeling of GABAergic markers at a single adult time point finds GABA release machinery in only a subset of neurons. To explain this discrepancy, we characterized GABAergic marker expression in cholinergic neurons throughout development, and found that at each stage, a subset of cholinergic neurons are capable of GABA release. These results suggest a model of pulsatile GABA release from cholinergic neurons, whereby different subset of cholinergic neurons are able to release GABA at different time points.

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PRESENTATION NUMBER: S041

CO-TRANSMISSION IN THE MID-BRAIN CHOLINERGIC AND SEROTONERGIC SYSTEMS

SYMPOSIUM 11- REVISITING DALE'S PRINCIPLE- NEUROTRANSMITTER PLASTICITY AND CO- TRANSMISSION IN HEALTH AND DISEASE - ADAM GRANGER, JING REN, THOMAS STEINKELLNER, SWETHA GODAVARTHI

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Cotransmission was proposed in 1976 and found in the peripheral nervous system. More recently it is recognized that, in the mammalian central nervous system (CNS), neurons not only contain multiple transmitter substances like neuropeptides, co-released with small-molecule transmitters; but also can co-release two or more small molecule transmitters. The midbrain contains multiple neuromodulatory systems, and almost all of them possess complex contransmission organization. The habenulo-interpeduncular projection is a major cholinergic pathway in the brain. In 2011, we found that the habenula cholinergic neurons co-release glutamate and acetylcholine. In addition, glutamatergic responses are fast and likely mediated by synaptic ionotropic glutamate receptors, whereas slow cholinergic effects are triggered only by tetanic stimuli and likely produced by the volume transmission of acetylcholine. These results suggest dual modes of signal transmission by cholinergic neurons in the brain. In 2017, we found that the terminals of projection from dorsal raphe (DR) to orbital frontal cortex (OFC) co-release serotonin and glutamate to modulate OFC network activity. Our data further suggested that these neurons are mainly located at the ventral DR, expressing Vglut3. In 2019, we profiled the transcriptomic properties of the serotonin neurons in the midbrain, revealing the extremely complex organization of multiple transmitter substances in the serotonin system. Recently, we also generated the transcriptomic profile of the DR dopamine system to reveal its cotransmission properties. These data confirmed that cotransmission is a core signalling mechanism in the CNS, operating in remarkable diversity.

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PRESENTATION NUMBER: S042

DOPAMINE NEURONS EXHIBIT EMERGENT GLUTAMATERGIC IDENTITY IN PARKINSON'S DISEASE

SYMPOSIUM 11- REVISITING DALE'S PRINCIPLE- NEUROTRANSMITTER PLASTICITY AND CO- TRANSMISSION IN HEALTH AND DISEASE - ADAM GRANGER, JING REN, THOMAS STEINKELLNER, SWETHA GODAVARTHI

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Loss of midbrain dopamine neurons causes the cardinal symptoms of Parkinson's disease. However, not all dopamine neurons are equally vulnerable and a better understanding of the cell-type specific properties relating to selective dopamine neuron degeneration is needed. Most midbrain dopamine neurons express the vesicular glutamate transporter VGLUT2 during development and a subset continue to express low levels of VGLUT2 in adulthood, enabling the co-release of glutamate. Moreover, VGLUT2 expression in dopamine neurons can be neuroprotective since its genetic disruption was shown to sensitize dopamine neurons to neurotoxins. Here, we show that in response to toxic insult, and in two distinct models of alpha-synuclein stress, VGLUT2 dopamine neurons were resilient to degeneration. Dopamine neurons expressing VGLUT2 were enriched whether or not insult induced dopamine neuron loss, suggesting that while VGLUT2 dopamine neurons are more resilient, VGLUT2 expression can also be transcriptionally upregulated by injury. Finally, we observed that VGLUT2 expression was enhanced in surviving DA neurons from postmortem Parkinson's disease subjects. These data indicate that emergence of a glutamatergic identity in dopamine neurons may be part of a neuroprotective response in Parkinson's disease.

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PRESENTATION NUMBER: S043

POSTSYNAPTIC RECEPTORS REGULATE PRESYNAPTIC NEUROTRANSMITTER EXPRESSION

SYMPOSIUM 11- REVISITING DALE'S PRINCIPLE- NEUROTRANSMITTER PLASTICITY AND CO- TRANSMISSION IN HEALTH AND DISEASE - ADAM GRANGER, JING REN, THOMAS STEINKELLNER, SWETHA GODAVARTHI

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Robust communication systems include an acknowledgement signal from the receiver to the sender indicating that the sender's message has been received. We have tested this organization at the synapse, with loss-of-function (LOF) and gain-of-function experiments (GOF). Using the *Xenopus* neuromuscular junction (NMJ), we find that LOF with focal blockade of endogenous acetylcholine receptors by pancuronium results in loss of ChAT in presynaptic motor neurons (MNs). Ectopic expression of GABA receptors ($GABA_AR\alpha\beta\gamma$) in myocytes in a GOF during development leads to expression of GABA in presynaptic cholinergic MNs. GABA expression is seen in innervating axon terminals and in cell bodies in the spinal cord. Co-expression of the MN markers Hb9, ChAT and Lim3 confirms their primary MN identity. Consistent with the appearance of GAD67 and VGAT in these MNs, GABAergic miniature endplate potentials at the NMJs are recorded from the myocytes. Misexpression of a single $GABA_AR\alpha$ subunit, resulting in a non-functional GABAR that is not trafficked to the postsynaptic membrane, does not produce these changes. Expressing the GABA-insensitive mutant $GABA_AR\alpha\beta_{E179Q}\gamma$ reveals that $GABA_AR$ channel activity is not necessary for presynaptic expression of GABA. Because these MNs normally express GABA transiently at earlier stages of development, exogenous $GABA_AR\alpha\beta\gamma$ likely prevents the loss of GABA expression. Our GOF results suggest that ectopically expressed postsynaptic receptors promote presynaptic neurotransmitter stability. Our LOF results suggest that blockade of endogenous receptors destabilizes presynaptic neurotransmitter expression. Receptor-mediated retrograde signaling at the synapse may be an acknowledgment signal for maintaining transmitter expression.

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PRESENTATION NUMBER: S044

PATTERN DETECTION IN SUBCORTICAL AND CORTICAL AUDITORY STRUCTURES

SYMPOSIUM 12 - STATISTICAL LEARNING AND PATTERN RECOGNITION FOR NATURAL BEHAVIOUR IN MICE - LIVIA DE HOZ, ROBERT FROEMKE, JEFFREY GAVORNIK, CATHERINE PERRODIN

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Detecting the spatiotemporal structure that threads external stimuli together is an essential function of the brain. It facilitates subsequent distinction between background and foreground, familiar and novel, or salient and neutral information. This is particularly true for the auditory soundscape, which our brain detects continuously and pre-attentively. The detection and learning of a soundscape's structure begins already in sensory subcortical stations. This processing both feeds and is modulated by cortical structures, through feedforward and feedback projections respectively. Using a combination of behaviour and electrophysiology, we study the emergence of activity patterns that code both neutral and behavioural relevant soundscape patterns across auditory subcortical and cortical processing stations, as well as across mental states.

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PRESENTATION NUMBER: S045

LEARNING SOUND STATISTICS FOR MATERNAL CARE VIA THE CENTRAL OXYTOCIN SYSTEM

SYMPOSIUM 12 - STATISTICAL LEARNING AND PATTERN RECOGNITION FOR NATURAL BEHAVIOUR IN MICE - LIVIA DE HOZ, ROBERT FROEMKE, JEFFREY GAVORNIK, CATHERINE PERRODIN

Robert Froemke
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Oxytocin is important for social interactions and maternal behavior. However, little is known about when, where, and how oxytocin modulates neural circuits to improve social cognition. Here I will discuss recent results and unpublished data from our lab on how oxytocin enables maternal behavior in new mother mice. I will focus on experience-dependent plasticity in auditory cortex related to recognizing the significance and statistical structure of pup distress calls, which are important for mother mice retrieving lost pups back to the nest. Surprisingly, this behavior, neural responses, and oxytocin receptor expression were lateralized to the left side of the auditory cortex, perhaps similar to the lateralization of language abilities in humans. I will also describe a new system we have built to combine neural recordings from the auditory cortex and oxytocin neurons of the hypothalamus in vivo, synchronized with days-to-weeks long continuous video monitoring of homecage behavior to identify when oxytocin release and cortical plasticity might occur during natural social and maternal experience.

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PRESENTATION NUMBER: S046

LEARNING TO ANTICIPATE TEMPORAL RELATIONSHIPS IN VISUAL INFORMATION

SYMPOSIUM 12 - STATISTICAL LEARNING AND PATTERN RECOGNITION FOR NATURAL BEHAVIOUR IN MICE - LIVIA DE HOZ, ROBERT FROEMKE, JEFFREY GAVORNIK, CATHERINE PERRODIN

Jeffrey Gavornik
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The last decade has produced unequivocal evidence that the primary visual cortex (V1) is not simply a simple feature detector. There are cells in V1 that detect edges and other simple features of visual space, but V1 dynamics are also affected by non-visual information associated with movement, auditory activity, hippocampal place fields, and cholinergic projections from the forebrain. Visual experience can also drive local plasticity that allows V1 to represent time, spatial patterns, and to predict expected inputs. In addition to raising interesting questions about V1's precise function in the visual hierarchy, these findings also make clear that V1 is a model system that can be used to study more general aspects of cortical computation and inter-area integration. In this talk, I will focus on what V1 reveals about how cortical circuits learn to encode ordinal and temporal relationships, and how neural circuits use this coding to anticipate expected events. I will focus particularly on the role of muscarinic acetylcholine receptors in selecting plasticity to represent either spatial or temporal information and also how V1 circuits are modified to make predictions at various timescales.

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PRESENTATION NUMBER: S047

BEHAVIOURAL RELEVANCE OF VOCAL TEMPORAL REGULARITY FOR MOUSE COURTSHIP

SYMPOSIUM 12 - STATISTICAL LEARNING AND PATTERN RECOGNITION FOR NATURAL BEHAVIOUR IN MICE - LIVIA DE HOZ, ROBERT FROEMKE, JEFFREY GAVORNIK, CATHERINE PERRODIN

Catherine Perrodin
University College London, Ear Institute, London, United Kingdom

Animals navigating the real world continuously face large amounts of complex sensory information. One of the strategies used by the brain to perceptually compress multidimensional input is to selectively extract the features relevant for behaviour. Like many other animals, mice communicate using acoustically complex vocal sequences. However, little is known about what acoustic information in these sensory streams listeners monitor to guide their social interactions. Here, we utilized C57Bl/6 female mice's natural behavioural response to ultrasonic songs emitted by conspecific males to identify socially-relevant acoustic dimensions in mouse communication. Using competing playbacks of intact and artificial version of male songs, we quantified whether and how different acoustic manipulations affected the females' place preference. This allowed us to evaluate the behavioural relevance of several candidate acoustic features for mouse communication. In this ethologically-relevant laboratory assay, we found that females listeners were highly sensitive to disruptions of song temporal regularity and preferentially approached playbacks of intact male songs over rhythmically irregular versions of the songs. In contrast, female behaviour was invariant to manipulations affecting the global song structure, such as randomization of syllable order and song temporal reversal. Similarly, female approach behaviour was robust to the removal of syllable spectro-temporal dynamics such as phase-scrambling or pure tone approximation. The results highlight temporal structure as a key acoustic cue monitored by female listeners during vocal-driven mate selection, and suggest vocal regularity as a potential acoustic signature of singer fitness. This behavioural work informs ongoing electrophysiological investigations into the neuronal substrates supporting the perception of vocal sequences for mate choice.

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PRESENTATION NUMBER: S048

TIME AND NEURAL POPULATION ACTIVITY IN ENTORHINAL CORTEX

SYMPOSIUM 13 - EPISODIC TIME - EDVARD MOSER, CHRISTIAN DOELLER, CORNELIA MCCORMICK, VIRGINIE VAN WASSENHOVE

Edvard Moser
NTNU, Kavli Institute For Systems Neuroscience And Centre For Neural Computation, Trondheim, Norway

The representation of time is a critical component of episodic memories encoded in the hippocampal formation. We showed four years ago in tetrode recordings in freely foraging rats that temporal information is robustly and specifically encoded in the overall neural population activity of lateral entorhinal cortex (LEC), across time scales from seconds to hours. We have now used Neuropixels recordings from hundreds to more than a thousand simultaneously recorded cells, along with dimensionality reduction approaches, to describe the evolution of neural activity in LEC during performance in tasks with different event structures. In LEC there was a strong tonic drift in population activity during individual behavioral trials, independently of whether the animals were pretrained. Only minimal change was observed in parallel recordings from MEC, CA1 or V1. Discrete events were associated with phasic jumps in the state space, possibly proportional to the novelty of the event, supporting the notion that the representation of episodic time in the LEC is discretized, with progression through state space scaled to the density and salience of events. We hypothesize that these event-driven dynamics of population activity in LEC is stored in hippocampal memory and may be used both during ongoing task performance to estimate how much time has passed and subsequently in order to estimate retrospectively the duration of the entire experience. The correlation with event structure suggests that estimates of duration must be non-linear, not merely reflecting the difference in population state between beginning and end of a block of experience.

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PRESENTATION NUMBER: S049

STRUCTURING TIME IN THE HIPPOCAMPAL-ENTORHINAL SYSTEM

SYMPOSIUM 13 - EPISODIC TIME - EDVARD MOSER, CHRISTIAN DOELLER, CORNELIA MCCORMICK, VIRGINIE VAN WASSENHOVE

Christian Doeller
Max Planck Institute for Human Cognitive and Brain Sciences, Department Of Psychology, Leipzig, Germany

The hippocampal-entorhinal system supports the representation of task regularities. A critical function may be the encoding of temporal context, i.e., forming integrated relational representations of co-occurring events and stimuli. In my talk, I will present data from two fMRI studies. In one study, participants mnemonically constructed times of events from multiple sequences (see Bellmund et al, bioRxiv 2022). Here, temporal relations were generalized across sequences, revealing distinct representational formats in the hippocampus for events from the same or different sequences. Furthermore, structural knowledge about time patterns across different sequences biased the construction of specific event times. In another study (cf. Polti, Nau et al, bioRxiv 2021), we used a time-to-contact estimation task to test whether temporal context modulates grid-like coding in the entorhinal cortex. In addition, we characterized in detail the relationship between trial-wise entorhinal fMRI activity and participants' task performance. Critically, we found that entorhinal activity reflected biases in timing behaviour, and that the strength of grid-like signals depended on the timing errors, consistent with temporal-context encoding. In sum, our findings demonstrate that the human hippocampal-entorhinal system contributes to adapting internal timing mechanisms to the temporal statistics of the environment and supports the generalization of temporal relations across experiences.

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THE NEURAL CONSTRUCTION OF IMAGERY-RICH MENTAL EVENTS

SYMPOSIUM 13 - EPISODIC TIME - EDVARD MOSER, CHRISTIAN DOELLER, CORNELIA MCCORMICK, VIRGINIE VAN WASSENHOVE

Cornelia Mccormick
University Medical School Bonn, Department Of Neurodegenerative Diseases And Geriatric Psychiatry, Bonn, Germany

Most people can voluntarily play out vivid, detail-rich mental events from their past, envision various scenarios set in the future or imagine never-experienced events in a fictitious setting. These imagery-rich events seem ubiquitous in our mental life, underlying autobiographical memory retrieval and episodic future thinking, and spatial navigation. However, there is still a dearth of evidence, whether people actively use imagery-based strategies in these cognitive tasks and which neural substrates support their initiation and elaboration. In this talk, I will synthesize evidence that imagery-rich mental events may be the modus operandi of our minds. Moreover, I suggest that these mental movies are constructed by a functional neural hierarchy spanning from the ventromedial prefrontal cortex (vmPFC), anterior and posterior segments of the hippocampus to the posterior neocortex. The vmPFC represents the apex of this hierarchy, initiating and coordinating extended, movie-like events that are supported by hippocampal-dependent snapshot-scenes and enriched by short-lasting visual-perceptual details of the posterior neocortex. The implications of this neural model of event construction urge to rethink traditional medial temporal lobe memory systems, and open goal-directed questions of why we rely on this system.

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BUILDING MENTAL CHRONOLOGIES IN THE HUMAN BRAIN

SYMPOSIUM 13 - EPISODIC TIME - EDVARD MOSER, CHRISTIAN DOELLER, CORNELIA MCCORMICK, VIRGINIE VAN WASSENHOVE

Virginie Van Wassenhove
CEA DRF-Joliot, INSERM, CNRS, Université Paris-Saclay, Neurospin, Cognitive Neuroimaging Unit, Gif sur Yvette, France

"The representation of a sequence is not a sequence of representations" (Friston & Buzsáki, 2016) captures the non-isomorphism between the serial ordering of world events, neural sequences, and experienced chronologies (e.g. Lashley, 1951; Gallistel, 1990; Kinsbourne & Dennett, 1992). How then can we study mental timelines generated in the human brain? In a series of behavioral, fMRI and MEG experiments (Gauthier et al., 2016a, 2016b, 2017, 2019, 2020), we explored "mental time travel" by manipulating the context in which participants recalled and organized learned historical events. Participants imagined themselves away from the here and now and subsequently reported as correctly and as fast as possible whether a learned historical event (*what*) occurred before or after (*when*), or west or east (*where*) of their mental self-positioning. Behavioral outcomes (reaction times and error rates) were comparable in the temporal and spatial dimensions of the task: participants took longer and made more errors when they were self-projected, and when events were closer to the location of their imagined self. fMRI findings showed that despite behavioral similarities, brain regions mediating self-projection, distance, and ordinality in time and space were partially distinct. Crucially, classifying MEG activity as a function of the imagined location of the self, revealed neural indices of both absolute and signed symbolic distances: how far in past/future or west/east a memory was to the mental self also engaged hippocampal activity. Our results suggest that, temporal cognitive maps, like spatial ones, are flexibly and endogenously generated using allocentric and egocentric coordinate systems.

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PRESENTATION NUMBER: S052

MECHANISMS OF CEREBRAL SMALL VESSEL DYSFUNCTION IN VASCULAR COGNITIVE IMPAIRMENT AND DEMENTIA

SYMPOSIUM 14 - VASCULAR HYPOTHESES FOR UNDERSTANDING AND RESTORING MEMORY IMPAIRMENTS - JOANNA WARDLAW, CECILE DUPLAà, FABRICE DABERTRAND, AXEL MONTAGNE

Joanna Wardlaw
University of Edinburgh and NHS Lothian, Neuroimaging Sciences, Centre For Clinical Brain Sciences And Uk Dementia Research Institute, Edinburgh, United Kingdom

This talk will provide an overview of the evidence from clinical research linking cerebral small vessel disease with cognitive decline and dementia. It will also briefly present information on small vessel disease and vascular dysfunctions, considering not just cerebral blood flow, but cerebral vasoreactivity, pulsatility and vascular stiffness, and briefly blood-brain barrier dysfunction in clinical studies. It will consider whether any of these vascular dysfunctions influence longitudinal lesion change or cognition, and finally also discuss small vessel lesion regression, the potential influences and whether lesion regression might delay or even reverse cognitive decline.

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PRESENTATION NUMBER: S053

TARGETING PDZRN3 MAINTAINS ADULT BLOOD-BRAIN BARRIER AND CENTRAL NERVOUS SYSTEM HOMEOSTASIS

SYMPOSIUM 14 - VASCULAR HYPOTHESES FOR UNDERSTANDING AND RESTORING MEMORY IMPAIRMENTS - JOANNA WARDLAW, CECILE DUPLAà, FABRICE DABERTRAND, AXEL MONTAGNE

Florian Gueniot[1], Sebastien Rubin[1], Pauline Bougaran[1], Alice Abelanet[1], Carole Proust[1], Jean-Luc Morel[2], Bruno Bontempi[2], Thierry Couffinhal[1], Cecile Duplaà[1]
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Blood brain barrier (BBB) disruption is a critical component of the pathophysiology of cognitive impairment of vascular etiology (VCI) and associated with Alzheimer's disease (AD). The Wnt pathway plays a crucial role in BBB maintenance, but there is limited data on its role in cognitive pathologies. The E3 ubiquitin ligase PDZRN3 is a regulator of the Wnt pathway. In a murine model of VCI, overexpressing *Pdzrn3* in endothelial cell (EC) exacerbated BBB hyperpermeability and accelerated cognitive decline. We extended these observations, in both VCI and AD models, showing that EC-specific depletion of *Pdzrn3,* reinforced the BBB, with a decrease in vascular permeability and a subsequent spare in cognitive decline. We found that in cerebral vessels, Pdzrn3 depletion protects against AD-induced Wnt target gene alterations and enhances endothelial tight junctional proteins. Our results provide evidence that Wnt signaling could be a molecular link regulating BBB integrity and cognitive decline under VCI and AD pathologies.

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PRESENTATION NUMBER: S054

RESCUE OF CEREBRAL BLOOD FLOW DEFICITS IN SMALL VESSEL DISEASE, THE CRUCIAL ROLE OF PIP2

SYMPOSIUM 14 - VASCULAR HYPOTHESES FOR UNDERSTANDING AND RESTORING MEMORY IMPAIRMENTS - JOANNA WARDLAW, CECILE DUPLAà, FABRICE DABERTRAND, AXEL MONTAGNE

Fabrice Dabertrand
University of Colorado | Anschutz Medical Campus, Anesthesiology, Aurora, United States of America

Cerebral small vessel diseases (SVDs) are a central link between stroke and dementia—two co-morbidities without specific treatments. Despite the emerging consensus that SVDs are initiated in the endothelium, the early mechanisms remain largely unknown. Deficits in on-demand delivery of blood to active brain regions (functional hyperemia) are early manifestations of the underlying pathogenesis. The capillary endothelial cell strong inward-rectifier K^+ channel Kir2.1, which senses neuronal activity and initiates a propagating electrical signal that dilates upstream arterioles, is a cornerstone of functional hyperemia. Using a genetic SVD mouse model, we show that impaired functional hyperemia is caused by diminished Kir2.1 channel activity. We link Kir2.1 deactivation to depletion of phosphatidylinositol 4,5-bisphosphate (PIP_2), a membrane phospholipid essential for Kir2.1 activity. Systemic injection of soluble PIP_2 rapidly restored functional hyperemia in SVD mice, suggesting a possible strategy for rescuing functional hyperemia in brain disorders in which blood flow is disturbed.

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PRESENTATION NUMBER: S055

DYSFUNCTION OF THE BLOOD-BRAIN BARRIER IN DEMENTIA

SYMPOSIUM 14 - VASCULAR HYPOTHESES FOR UNDERSTANDING AND RESTORING MEMORY IMPAIRMENTS - JOANNA WARDLAW, CECILE DUPLAà, FABRICE DABERTRAND, AXEL MONTAGNE

Axel Montagne
University of Edinburgh, Uk Dementia Research Institute, Centre For Clinical Brain Sciences, Edinburgh, United Kingdom

Our brain is an energy-hungry organ surrounded by a rich network of blood vessels supplying the oxygen and nutrients required to function. It is essential that the microenvironment in the brain is finely controlled, and this is achieved through the specialist blood-brain barrier (BBB) structure. However, dysfunction of the BBB is recognised as one of the earliest events in the progression of brain disorders that cause dementia. We have previously discovered that one type of cell within the BBB, the pericyte, is particularly affected during disease and we aim to fully understand the consequences to the BBB and brain health as a whole. Using a combination of advanced molecular and imaging techniques including MRI, we seek to uncover the disease mechanisms at play and identify therapeutic targets for intervention.

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PRESENTATION NUMBER: S056

FAST CLOSED LOOP OPTICAL BRAIN COMPUTER INTERFACES TO STUDY NEURAL PLASTICITY

SYMPOSIUM 15 - HYBRID AND MULTIFUNCTIONAL APPROACHES TO STUDY NEURALCIRCUITS - BERNARDO SABATINI, DUYGU KUZUM, ILKA DIESTER, FILIPPO PISANO

Bernardo Sabatini, Richard Hakim
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In standard behavioral tasks, mice adapt their action selection in order to achieve positive outcomes, such as access to water and food. Learning to perform these tasks requires adaptations in neural circuits that are driven by action-outcome associations. Brain-machine interfaces (BMI), in which neural activity is read directly from the brain to control devices or set environmental contingencies, allow direct testing of the possible mechanisms of neural plasticity that underly learning. Here we teach mice to move the activity of their primary motor cortex in specific directions in neural state space in order to achieve reward. We control the BMI by rapidly reading and analyzing activity of neurons expressing Ca-sensitive fluorophores with a 2-photon laser scanning microscope. Furthermore, we analyze movements associated with performing the task using a novel method. We examine the ability of the animal to discover and move neural activity along complex trajectories in order to achieve reward and the consequences of this plasticity on the activity of neurons that are not explicitly selected as part of the reward-guided ensemble.

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PRESENTATION NUMBER: S057

TRANSPARENT NEURAL PROBE TECHNOLOGIES FOR MULTIMODAL, MULTI-REGION RECORDINGS

SYMPOSIUM 15 - HYBRID AND MULTIFUNCTIONAL APPROACHES TO STUDY NEURALCIRCUITS - BERNARDO SABATINI, DUYGU KUZUM, ILKA DIESTER, FILIPPO PISANO

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The complexity of neural activities has challenged both neuroscience research and clinical practice for decades. Understanding neuronal dynamics and information processing performed by neural populations requires advanced technologies with high-resolution sensing and stimulation capability. Conventional neural interfaces offering electrical, optical, or chemical signals have greatly advanced our understanding of neural functions, however, most of these technologies are based on a single functionality and limited to recording local activity in specific brain regions. Combining multiple functionalities in a single system has recently been pursued as an integrative approach to achieve high resolution monitoring of neural activity across multiple spatial and temporal scales. Graphene has recently emerged as a neural interface material offering several outstanding properties, such as optical transparency, flexibility, high conductivity, and biocompatibility. In this talk, I will present our recent work on graphene-based neural interfaces, highlight key applications, and demonstrate examples of in vivo multimodal imaging and recording experiments utilizing optical transparency of the microelectrodes. I will also present a Flexible, Insertable, and Transparent Microelectrode array technology (‘Neuro-FITM’) and discuss how this technology can be applied to investigate coordination and interactions between the cortex and subcortical structures.

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PRESENTATION NUMBER: S058

NEURAL PROBES FOR OPTIMIZED IN VIVO OPTOGENETIC MANIPULATIONS AND ELECTROPHYSIOLOGICAL MEASUREMENTS

SYMPOSIUM 15 - HYBRID AND MULTIFUNCTIONAL APPROACHES TO STUDY NEURALCIRCUITS - BERNARDO SABATINI, DUYGU KUZUM, ILKA DIESTER, FILIPPO PISANO

Ilka Diester
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Optogenetics involves delivery of light-sensitive opsins to the target brain region, combined with the introduction of optical and electrical devices to manipulate and record neural activity. Combining these functionalities in a single implantable device is of relevance for a precise investigation of neural networks while minimizing tissue damage. We report on the development, characterization, and in vivo validation of a multifunctional optrode that combines a silicon-based neural probe with an integrated microfluidic channel, and an optical glass fiber in a compact assembly. The size and position of fluidic channels, electrodes, and optical fiber can be precisely tuned according to the in vivo application. With a total system weight of 0.97 g, our multifunctional optrode is suitable for chronic in vivo experiments requiring simultaneous drug delivery, optical stimulation, and neural recording. In comparison to conventional multi-step surgeries, our approach achieves higher spatial specificity while minimizing tissue damage. In a second approach, we developed a technique for simultaneous multi-side, large-scale recordings and optogenetic interventions. The approach is based on unprecedentedly thin Lambertian side-emitting optical fibers combined with silicon probes to achieve high quality recordings and ultrafast multichannel optogenetic inhibition in freely moving animals. Our new framework paves the way for large-scale photo tagging and controlled interrogation of rapid neuronal communication in any combination of brain areas. Lastly, we established a setup for training the required surgical techniques underlying opto- and electrophysiology. It can be adapted to serve in testing and optimizing newly developed tools as the ones described above.

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CAPTURING OPTICAL, ELECTRICAL AND BIOCHEMICAL SIGNALS FROM DEEP BRAIN REGIONS WITH TAPERED FIBERS

SYMPOSIUM 15 - HYBRID AND MULTIFUNCTIONAL APPROACHES TO STUDY NEURALCIRCUITS - BERNARDO SABATINI, DUYGU KUZUM, ILKA DIESTER, FILIPPO PISANO

Filippo Pisano[1], Marco Pisanello[2], Antonio Balena[3], Barbara Spagnolo[1], Marco Bianco[1], Maria Andriani[1], Muhammad Kashif[1,4], Cinzia Montinaro[1,5], Di Zheng[1], Liam Collard[1], Elisa Scarpa[1], Mariam Masmudi-Martìn[6], Niall Mcalinden[7], Nicole Byron[8], Teresa Jurado-Parras[9], Jaeeon Lee[10], Suk-Joon Lee[10], Rui Peixoto[11], Mohammad Mohammadiaria[1], Antonella D'Orazio[4], Francesco De Angelis[12], John Assad[10], Leonardo Sileo[2], Shuzo Sakata[8], Keith Mathieson[7], Liset Menendez De La Prida[9], Manuel Valiente[6], Marco Grande[4], Bernardo Sabatini[10], Massimo De Vittorio[1,5], Ferruccio Pisanello[1]
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Capturing the dynamics of brain activity in its multifaceted components is a longstanding challenge in neuro-technology. To achieve this aim, we propose a new class of multifunctional implants based on Tapered optical fibers (TF). TF have recently emerged as a versatile, minimally invasive tool for depth-resolved optogenetic stimulation and fiber photometry (Pisanello *et al.*, *Neuron* 2014; Pisanello *et al.*, *Nat. Neurosci.*2017; Pisano *et al.*, *Nat. Methods,* 2019). Here, we will present recent efforts to extend this approach to electrical and biochemical monitoring in the deep brain. Taking advantage of the inherently light-coupled tip, we used an unconventional two-photon lithography approach to develop a *fibertrode* that combines multiple optical windows with artifact-free extracellular microelectrodes. As demonstrated *in vivo*, the *fibertrode* allows for simultaneous optical control and electrical readout of custom-designed brain volumes. At the same time, we pushed on the photonic properties of the TFs to achieve implantable label-free biomolecular monitoringe. Using implantable Raman spectroscopy, we observed biomolecular alterations linked with cancer invasion label-free. Finally, we investigated the potential of harnessing nanoscale light-matter interactions in the deep brain by decorating TF implants with plasmonic nanostructures. This lead us to a proof-of-principle of surface enhanced Raman scattering (SERS) detection of neurotransmitters as well as of novel perspectives in spectrally-modulated light delivery and collection through subwavelength apertures (Pisano *et al.*, *Adv. Opt. Mater* 2022). We view multifunctional TF as a promising complement to existing technologies for multimodal brain evaluation.

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PRESENTATION NUMBER: S060

CORTICOSTRIATAL CIRCUITS FOR REWARD LEARNING AND DECISION-MAKING

SYMPOSIUM 16 - CAUSAL INTERACTIONS BETWEEN NEUROMODULATORS AND CORTICOSTRIATAL CIRCUITS IN DECISION MAKING - ILANA WITTEN, DOUGAL TERVO, NIMA KHALIGHINEJAD, HANNEKE DEN OUDEN

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A classic view of the striatum holds that activity in direct and indirect pathways oppositely modulates motor output. Whether this involves direct control of movement, or reflects a cognitive process underlying movement, remains unresolved. We have found that both the demands imposed by a task, as well as the internal state of mice when performing a task, determine whether striatal pathways provide strong and opponent control of behavior. We are in the process of idenfying state-dependent correlates of decision-making.

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PRESENTATION NUMBER: S061

RODENTS PLAYING GAMES: THE NEURAL BASIS OF BEHAVIORAL VARIABILITY

SYMPOSIUM 16 - CAUSAL INTERACTIONS BETWEEN NEUROMODULATORS AND CORTICOSTRIATAL CIRCUITS IN DECISION MAKING - ILANA WITTEN, DOUGAL TERVO, NIMA KHALIGHINEJAD, HANNEKE DEN OUDEN

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The ability to rapidly adjust one's strategy in complex environments depends on computations in frontal cortical circuits. In addition, perturbations of the more slowly changing neuromodulatory tone in frontal cortical regions have detrimental effects on the ability to behave strategically, pointing to the importance of the interaction between the neuromodulatory systems and the cortical circuits. How this interplay is accomplished mechanistically and its connection to decision-making in volatile and adaptive environments remains poorly understood. We had previously established that increasing noradrenergic tone in the ACC decreases commitment to the default strategy in a task that models a game-theoretic interaction. More recently, we tied the balance between persistence with the default strategy and a choice to commit to an alternative one to an interplay between the intratelecenphalic (IT)- and the pyramidal tract (PT)- subcircuits within the ACC. Specifically, we demonstrated that the output of the ACC's PT sub-circuit drives the pursuit of alternative learned strategies but is kept clamped by the IT pathway through local opponent interaction until evidence grows in favor of deviating from the ongoing strategy. Combined, our two studies are intriguing considering the earlier findings that highlight the increased responsiveness of the PT neurons under high noradrenergic tone to long-range inputs. As such, an interesting possibility is that one mechanism by which the more slowly-acting noradrenergic input interacts with the otherwise cortically-computed strategy selection process is through lowering the threshold for deviating from the default strategy typically informed by the often incomplete understanding of the environment's structure.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: S062

NEUROMODULATION OF DECISIONS ABOUT 'IF' AND 'WHEN' TO ACT

SYMPOSIUM 16 - CAUSAL INTERACTIONS BETWEEN NEUROMODULATORS AND CORTICOSTRIATAL CIRCUITS IN DECISION MAKING - ILANA WITTEN, DOUGAL TERVO, NIMA KHALIGHINEJAD, HANNEKE DEN OUDEN

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Decision making not only involves deciding about which action to choose but when and whether to initiate an action in the first place. Here, I present results from three experiments investigating the role of neuromodulatory systems in decisions about when to act. In experiment 1, we recorded blood-oxygen-level-dependent (BOLD) indices of activity from the dorsal raphe nucleus (DRN) and basal forebrain (BF). Brain data from fMRI showed that while the effect of immediate context on action timing was mediated by BF, the broader, general features of the environment – environmental richness -- and its effect on action timing was mediated by activity in DRN. DRN and BF are major sources of serotonin (5-HT) and acetylcholine (ACh) in the brain, respectively. In experiment 2, systemic 5-HT levels were manipulated by protracted administration of a selective serotonin reuptake inhibitor (SSRI). Increasing 5-HT levels prolonged the time animals would wait before making a response. This effect was more evident during blocks with long inter-trial intervals (ITI), where good opportunities were sparse, a similar pattern to the effect of average value of the environment observed in the first study. In experiment 3 we manipulated systemic levels of ACh by protracted administration of a cholinesterase inhibitor and evaluated its effects on decision time to act. Increasing ACh levels invigorated movements so that animals acted faster in response to features in their immediate context. These findings suggest complementary roles for serotonin/DRN and acetylcholine/BF in decisions about when to initiate an action.

FENS Forum 2022
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PRESENTATION NUMBER: S063

DISSOCIATING FRONTAL AND STRIATAL DOPAMINERGIC CONTROL OF MOTIVATION-ACTION COUPLING

SYMPOSIUM 16 - CAUSAL INTERACTIONS BETWEEN NEUROMODULATORS AND CORTICOSTRIATAL CIRCUITS IN DECISION MAKING - ILANA WITTEN, DOUGAL TERVO, NIMA KHALIGHINEJAD, HANNEKE DEN OUDEN

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Motivations shape our behaviour: the promise of reward invigorates, while in the face of punishment, we hold back. Abnormalities of motivational processing are implicated in clinical disorders characterised by excessive habits and loss of top-down control, notably substance and behavioural addictions. Striatal and frontal dopamine have been hypothesised to play complementary roles in the respective generation and control of these motivational or 'Pavlovian' biases. However, while dopaminergic interventions have indeed been found to modulate motivational biases, these previous pharmacological studies used regionally non-selective pharmacological agents. Here, I will present two recent studies, testing the following hypotheses: i) We can overcome maladaptive motivational biases through frontal cognitive control. To test this hypothesis, we use a combination of EEG and computational modelling. ii) Frontal dopamine controls the balance between Pavlovian, bias-driven automated responding and instrumentally learned action values. Specifically, we examined whether selective enhancement of cortical dopamine enables adaptive suppression of Pavlovian control when biases are maladaptive, where we modulate frontal dopamine with COMT inhibitor tolcapone.

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PRESENTATION NUMBER: S063

GABA-MEDIATES THE SENSORY DEPENDENT DEVELOPMENT OF INTERHEMISPHERIC CIRCUITS OF THE CORPUS CALLOSUM

SYMPOSIUM 17 - DEVELOPMENT AND EVOLUTION OF BILATERAL SENSORY CIRCUITS - MARTA NIETO, OLIVIER COLLIGNON, ELOISA HERRERA, FILIPPO DEL BENE

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Bilateral sensory circuits provide complex representations of the external world by computing overlapping information. In this workshop, we will present complementing perspectives of how bilateral sensory circuits built and function during development and evolution, from fish to humans, and from vision to crossmodal perception. In mammals, an ulterior exchange of information occurs through the corpus callosum (CC), which enables the addition of novel brain functions. The developmental and evolutionary mechanisms mediating the appearance of this structure are unknown. According to predominant views, callosal and non-callosal fates are predetermined early after neuronal birth. Certain populations are born pre-programmed as callosal projecting neurons while others, such as cortical layer (L) 4 excitatory neurons of the primary somatosensory (S1) barrel, invariably project ipsilaterally. Using a novel axonal-retrotracing strategy and GFP-targeted visualization of young neurons, we instead demonstrate the opposite in L2/3 and 4 neurons. Virtually all these neurons develop transient interhemispheric axons, and local-projecting fates emerge as a postnatal alternative when exuberant callosal axons refine in an area- and layer-specific under the influence of sensory-specific inputs. This developmental exuberance during CC formation appears a strategy that enables plasticity and robust stereotyped wiring of complex circuits during evolution. Furthermore, I will show that inhibitory interneurons select callosal fates and diversify area-specific circuits through their control of CC refinement.

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PRESENTATION NUMBER: S064

ARTIFICIAL REWIRING OF THE EARLY VISUAL PATHWAY LEADS TO THE EMERGENCE OF OCULAR DOMINANCE COLUMNS IN MICE

SYMPOSIUM 17 - DEVELOPMENT AND EVOLUTION OF BILATERAL SENSORY CIRCUITS - MARTA NIETO, OLIVIER COLLIGNON, ELOISA HERRERA, FILIPPO DEL BENE

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Species with good binocular vision, as cats or primates, have ocular dominance columns and other well-organized cortical features while mice have poorly organized visual cortices and are essentially monocular. Ocular dominance columns are defined in the visual cortex previous to eye-opening, but it remains unknown to what extent cortical circuits are intrinsically imprinted of haphazardly wired by the influence of the early emerging features in the thalamus, such as eye-specific lamination. As a test-bed to investigate the connection between early-emerging retinothalamic features and cortical properties such as ocular dominance columns, we created a mouse line with a rewired retinothalamic pathway that have a number of ipsilateral projections similar to those species with good binocular vision. Strikingly, adding extra numbers of ipsilateral retinal fibers in mice modifies cortical integration and generates ectopic microdomains resembling the columns of higher mammals. The consequences of these results will be discussed in my talk.

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PRESENTATION NUMBER: S065

EVOLUTION AND FUNCTIONAL ORGANISATION OF BILATERAL VISUAL CIRCUITS IN FISH

SYMPOSIUM 17 - DEVELOPMENT AND EVOLUTION OF BILATERAL SENSORY CIRCUITS - MARTA NIETO, OLIVIER COLLIGNON, ELOISA HERRERA, FILIPPO DEL BENE

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Binocular stereopsis requires the convergence of visual information from corresponding points in visual space seen by two different lines of sight. This may be achieved by superposition of retinal input from each eye onto the same downstream neurons via ipsi- and contralaterally projecting optic nerve fibers. Zebrafish larvae can perceive binocular cues during prey hunting but have exclusively contralateral retinotectal projections. We report brain activity in the tectal neuropil ipsilateral to the visually stimulated eye, despite the absence of ipsilateral retinotectal projections. This activity colocalizes with arbors of commissural neurons, termed intertectal neurons (ITNs), that connect the tectal hemispheres. ITNs are GABAergic, establish tectal synapses bilaterally and respond to small moving stimuli. In zebrafish and presumably other teleost an intertectal circuit controls execution of the prey-capture motor program following binocular localization of prey, without requiring ipsilateral retinotectal projections. Conversely, bilateral visual projections exist in non-teleost fishes and that the appearance of ipsilateral projections does not correlate with terrestrial transition or predatory behavior. We have also reported that the developmental program that specifies visual system laterality differs between fishes and mammals, as the Zic2 transcription factor, which specifies ipsilateral retinal ganglion cells in land vertebrates, appears to be absent from the ganglion cells of ray finned fish species. However, overexpression of human ZIC2 is able to induce ipsilateral visual projections in zebrafish. Therefore, we propose that the existence of bilateral visual projections preceded the emergence of binocular vision in land vertebrates.

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PRESENTATION NUMBER: S066

ORGANOID MODELING OF FOREBRAIN DEVELOPMENT AND RELEVANCE TO NEURODEVELOPMENTAL DISORDERS

SYMPOSIUM 18 - CAPTURING HUMAN BRAIN CIRCUIT DEVELOPMENT AND DISEASE MECHANISMS WITH STEM CELL-BASED TECHNOLOGIES - FLORA VACCARINO, MADELINE LANCASTER, SILVIA CAPPELLO, VINCENZO DE PAOLA

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To model developmental disorders of the human brain, we used induced pluripotent stem cell (iPSC)-derived brain organoids derived from a cohort of individuals with idiopathic autism spectrum disorder (ASD) and their unaffected first-degree family members. Our main goal was to overcome the heterogeneity in genetic risk for ASD and identify a convergent pathophysiology. Differences in cellular composition and cell type-specific gene expression were evaluated by single-cell RNA-sequencing (scRNA-seq) over a 3-month time course. The scale of the dataset allowed an investigation of the sources of variation influencing human neurogenesis in the organoid system over multiple genetic backgrounds and culture batches. We found that organoids were patterned into regional and cellular identities similar to those present in human fetal brains. Analyses of gene expression comparing probands and unaffected relatives revealed alterations in genes driving the tempo of cortical excitatory neurogenesis from undifferentiated progenitors. Macrocephalic ASD probands showed a delay in initial excitatory neuron differentiation and an increase in the progenitor pool. This resulted in an increased proportions of excitatory neurons of the dorsal cortex, whereas normocephalic ASD probands showed a diametrically opposite phenotype. This difference in gene expression and imbalance in excitatory neuron subtypes reveals intrinsically different subtypes of ASD, with head circumference as a biomarker, that could be used as potential stratifying factors in clinical or genetic studies of the disorder. Overall, omics studies in organoid allow to understand heterogeneity of human brain development across individuals and delineate altered trajectories in developmental disorders.

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PRESENTATION NUMBER: S067

HUMAN BRAIN DEVELOPMENT IN CEREBRAL ORGANOIDS

SYMPOSIUM 18 - CAPTURING HUMAN BRAIN CIRCUIT DEVELOPMENT AND DISEASE MECHANISMS WITH STEM CELL-BASED TECHNOLOGIES - FLORA VACCARINO, MADELINE LANCASTER, SILVIA CAPPELLO, VINCENZO DE PAOLA

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The human brain sets us apart as a species, with its size and complexity unrivaled in the animal kingdom. Brain size is largely determined during development as vast numbers of neurons and supportive glia are generated, followed by a highly coordinated process of axonal outgrowth to set up the intricate connectome. In an effort to better understand the events that determine human brain cellular makeup, size, and connectivity, we use a human model system in a dish, called cerebral organoids. These 3D tissues are generated from pluripotent stem cells through neural differentiation and a supportive 3D microenvironment to generate organoids with the same tissue architecture as the early human fetal brain. Such organoids are allowing us to tackle questions previously impossible with more traditional approaches. Indeed, our recent findings provide insight into regulation of brain size and neuron number across ape species, identifying key stages of early neural stem cell expansion that set up a larger starting cell number to enable the production of increased numbers of neurons. We are also investigating the roles of extrinsic regulators in determining numbers and types of neurons produced in the human cerebral cortex. Overall, our findings are pointing to key, human-specific aspects of brain development and function, that have important implications for neurological disease.

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PRESENTATION NUMBER: S068

HUMAN CELLULAR MODELS FOR BRAIN INJURY OF PREMATURITY

SYMPOSIUM 18 - CAPTURING HUMAN BRAIN CIRCUIT DEVELOPMENT AND DISEASE MECHANISMS WITH STEM CELL-BASED TECHNOLOGIES - FLORA VACCARINO, MADELINE LANCASTER, SILVIA CAPPELLO, VINCENZO DE PAOLA

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Cellular crosstalk is an essential process during brain development and it is influenced by numerous factors, including the morphology of the cells, their adhesion molecules, the local extracellular matrix and the secreted vesicles. Inspired by mutations associated with neurodevelopmental disorders, we focus on understanding the role of extracellular mechanisms essential for the correct development of the human brain. Hence, we combine the in vivo mouse model and the in vitro human-derived neurons, cerebral organoids, and dorso-ventral assembloids in order to better comprehend the molecular and cellular mechanisms involved in progenitors' proliferation and fate as well as migration and maturation of inhibitory and excitatory neurons during human brain development and tackle the causes of neurodevelopmental disorders. We particularly focus on mutations in genes influencing cell-cell contacts, extracellular matrix, and secretion of vesicles and therefore study intrinsic and extrinsic mechanisms contributing to the formation of the brain. Our data reveal an important contribution of cell non-autonomous mechanisms in the development of neurodevelopmental disorders.

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PRESENTATION NUMBER: S069

IN VIVO MODELLING OF HUMAN AXON DEGENERATION AND REGENERATION

SYMPOSIUM 18 - CAPTURING HUMAN BRAIN CIRCUIT DEVELOPMENT AND DISEASE MECHANISMS WITH STEM CELL-BASED TECHNOLOGIES - FLORA VACCARINO, MADELINE LANCASTER, SILVIA CAPPELLO, VINCENZO DE PAOLA

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Central nervous system (CNS) axons fail to regenerate or recover synaptic connectivity leading to permanent disability in many brain and spinal cord injury patients. Work in model organisms has revealed that CNS axon regeneration is dependent on both the microenvironment and the intrinsic growth state of the damaged neuron itself. Mechanistic insights from these models need to be validated in humans to advance translational therapies. Therefore, there is a pressing need for new preclinical systems that can mimic human-specific features to (i) characterise axon regeneration of major CNS neuronal subclasses e.g. the cortico-spinal tract, and (ii) develop strategies to enhance axon regeneration of mature human neurons. I will present recent work to establish a new *in vivo* system to study human cortical axon degeneration and regeneration using transplanted donor-derived stem cell grafts and 2-photon time-lapse *in vivo* imaging. This model recapitulates many important features of the physiological axon response to injury including fragmentation, the loss of regeneration potential with maturity, progressive die-back, as well as sprouting and branching, and can be used to test strategies to enhance human axon regeneration after nerve damage.

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PRESENTATION NUMBER: S070

FEEDFORWARD AND FEEDBACK PATHWAYS IN VISUAL CORTEX

SYMPOSIUM 19 - INTEGRATION OF TOP-DOWN INFORMATION IN CORTICAL LAYER 1 AND ITS CONTRIBUTION TO BEHAVIOR - ANDREAS KELLER, LEOPOLDO PETREANU, JOHANNES LETZKUS, MATTIA AIME

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We hardly notice when there is a speck on our glasses, the obstructed visual information seems to be magically filled in. The mechanistic basis for this fundamental perceptual phenomenon has, however, remained obscure. What enables neurons in the visual system to respond to context when the stimulus is not available? While feedforward information drives the activity in cortex, feedback information is thought to provide contextual signals that are merely modulatory. We have made the discovery that mouse primary visual cortical neurons are strongly driven by feedback projections from higher visual areas when their feedforward sensory input from the retina is missing. This drive is so strong that it makes visual cortical neurons fire as much as if they were receiving a direct sensory input. These signals are likely used to predict input from the feedforward pathway. Preliminary results show that these feedback projections are strongly influenced by experience and learning.

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PRESENTATION NUMBER: S071

CORTICAL FEEDBACK INPUTS AND HIERARCHICAL COMPUTATION

SYMPOSIUM 19 - INTEGRATION OF TOP-DOWN INFORMATION IN CORTICAL LAYER 1 AND ITS CONTRIBUTION TO BEHAVIOR - ANDREAS KELLER, LEOPOLDO PETREANU, JOHANNES LETZKUS, MATTIA AIME

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The neocortex is organized in a hierarchy of functionally specialized areas. This architecture influences our understanding of sensory, motor and cognitive processes and inspires machine learning algorithms. However, the precise computations that the hierarchical organization of the neocortical network endows us with remain largely unknow. A mayor hub of hierarchical interactions is Layer 1, where descending feedback projections from multiple high order cortical areas contact interneurons and the apical dendrites of pyramidal cells. Thus, understanding hierarchical cortical interactions requires unveiling the organizing rules of feedback inputs in Layer 1. Using optogenetic circuit mapping methods and two-photon imaging we studied how the connectivity of feedback inputs from higher visual areas in primary visual cortex (V1) depends on the projection type and functional properties of its target neurons. Using different visual deprivation paradigms, we also measured how the organization of feedback inputs is shaped by visual experience. We found that feedback inputs to L1 are selectively wired to engage in recurrent inter-area computations between retinotopically aligned neurons. The extent of the retinotopic alignment depends on the laminar position of the source of the inputs. The selectivity of feedback for reciprocating neurons also varies depending on the laminar depth of the innervated neurons. While the overall organization of cortical feedback inputs in Layer 1 does not require vision, their fine scale connectivity, and functional properties reflect experienced spatio-temporal correlations between areas. Our observations shine light on the computations implemented by long-range cortical networks, constraining existing theories of cortical computation.

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PRESENTATION NUMBER: S072

PROCESSING OF TOP-DOWN INFORMATION IN LAYER 1 OF THE AUDITORY CORTEX

SYMPOSIUM 19 - INTEGRATION OF TOP-DOWN INFORMATION IN CORTICAL LAYER 1 AND ITS CONTRIBUTION TO BEHAVIOR - ANDREAS KELLER, LEOPOLDO PETREANU, JOHANNES LETZKUS, MATTIA AIME

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Top-down projections convey a family of signals encoding previous experiences and current aims to sensory neocortex, where they converge with bottom-up information from the environment to produce perception. Whereas several excitatory afferent systems for top-down control of neocortex have been identified, the existence, connectivity and information content of inhibitory top-down projections remains elusive. Here we use a combination of circuit tracing, connectivity mapping, chemogenetics, cortex-dependent learning and synaptic 2-photon calcium imaging to identify GABAergic afferents from the subthalamic zona incerta as a major source of memory-related top-down input to neocortex. Incertocortical projections transmit integrated information from many sources preferentially to auditory cortex layer 1, where they connect selectively to inhibitory interneurons to disinhibit the cortical circuit. This pathway encodes both auditory stimuli and primary reinforcers, and undergoes robust plasticity during learning that improves information transfer and facilitates behavioural memory. Unlike excitatory top-down pathways, incertocortical afferents engage in two distinct plasticity regimes with different temporal dynamics and response properties. A unique hallmark is the rapid *de novo* appearance of negative responses as the major driver of learning-related changes in stimulus representation. Our results therefore uncover the distinctive contribution of long-range (dis)inhibitory afferents to the computational flexibility of neocortical circuits.

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PRESENTATION NUMBER: S073

PARADOXICAL SOMATO-DENDRITIC DECOUPLING SUPPORTS CORTICAL PLASTICITY DURING REM SLEEP

SYMPOSIUM 19 - INTEGRATION OF TOP-DOWN INFORMATION IN CORTICAL LAYER 1 AND ITS CONTRIBUTION TO BEHAVIOR - ANDREAS KELLER, LEOPOLDO PETREANU, JOHANNES LETZKUS, MATTIA AIME

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REM sleep is associated with the consolidation of emotional memories encoded by neuronal circuits from the limbic system and prefrontal cortex in mammals. Yet, the underlying neocortical circuits and synaptic mechanisms remain unclear. Here, we found that REM sleep is associated with a somato-dendritic decoupling in pyramidal neurons of the prefrontal cortex, using simultaneous 2-photon calcium imaging and electrophysiological recordings in sleeping mice. This decoupling reflects a shift of inhibitory balance between PV neurons-mediated somatic inhibition and VIP-mediated dendritic disinhibition, mostly driven by neurons from the central medial thalamus. We further showed that REM-specific optogenetic suppression of dendritic activity led to a loss of danger versus safety discrimination during associative learning and a lack of synaptic plasticity, whereas optogenetic release of somatic inhibition resulted in enhanced discrimination and synaptic potentiation. Collectively, our results demonstrated that somato-dendritic decoupling during REM sleep promotes opposite synaptic plasticity mechanisms that optimize emotional response to future behavioral stressors.

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PRESENTATION NUMBER: S074

NEURON CLASS-SPECIFIC RESPONSES GOVERN ADAPTIVE MYELIN REMODELING IN THE NEOCORTEX

SYMPOSIUM 20 - NOT JUST INSULATION: OLIGODENDROCYTE FUNCTION AND MYELINATION IN BRAIN PLASTICITY AND BEHAVIOR - PAOLA ARLOTTA, IIRIS HOVATTA, HANNELORE EHRENREICH, MARIA-CECILIA ANGULO

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Myelin plasticity is critical for neurological function, including learning and memory. However, it is unknown whether this plasticity reflects uniform changes across all neuronal subtypes, or whether myelin dynamics vary between neuronal classes to enable fine-tuning of adaptive circuit responses. We performed in vivo two-photon imaging of myelin sheaths along single axons of excitatory callosal neurons and inhibitory parvalbumin-expressing interneurons in adult mouse visual cortex. We found that both neuron types show homeostatic myelin remodeling under normal vision. However, monocular deprivation results in adaptive myelin remodeling only in parvalbumin-expressing interneurons. An initial increase in elongation of myelin segments is followed by contraction of a separate cohort of segments. This data indicates that distinct classes of neurons individualize remodeling of their myelination profiles to diversify circuit tuning in response to sensory experience.

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PRESENTATION NUMBER: S075

MYELIN PLASTICITY IN CHRONIC PSYCHOSOCIAL STRESS AND ANXIETY

SYMPOSIUM 20 - NOT JUST INSULATION: OLIGODENDROCYTE FUNCTION AND MYELINATION IN BRAIN PLASTICITY AND BEHAVIOR - PAOLA ARLOTTA, IIRIS HOVATTA, HANNELORE EHRENREICH, MARIA-CECILIA ANGULO

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Chronic stress, a major risk factor for anxiety disorders, has been associated with changes in myelin gene expression and thickness in multiple mouse models. Our unbiased genetic screen of stress resilient, stress susceptible and control mice from two inbred mouse strains (C57BL/6NCrl and DBA/2NCrl) suggested that myelin plasticity is one of the major responses to chronic psychosocial stress in mammals, varies across brain regions, and is genetically controlled. Myelin plasticity also encompasses changes in the nodes of Ranvier, which have not been extensively studied in stress. Our new data indicates mouse strain-dependent alterations in the paranode length after chronic stress. These changes are likely mediated by neuronal activity because chemogenetic activation of the ventral hippocampus to medial prefrontal cortex pathway specifically influenced paranode length of stimulated, but not unstimulated, axons, while also affecting anxiety-like behavior. To investigate whether white matter structural differences are associated with anxiety symptoms in humans, we carried out diffusion tensor imaging in young adults. We found larger mean diffusivity in the forceps minor in individuals with subclinical anxiety symptoms compared to those without, suggesting alterations in myelination. Thus, myelin plasticity may be involved in anxiety disorders via regulation of spatial connectivity. Identification of the mechanisms underlying the myelin response will provide mechanistic insight into the molecular basis of anxiety, a critical step in developing targeted therapy.

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PRESENTATION NUMBER: S076

ISOLATED CATATONIA-EXECUTIVE DYSFUNCTION COMPLEX IN AGED MICE INDUCED BY FOREBRAIN-SPECIFIC LOSS OF MYELIN INTEGRITY

SYMPOSIUM 20 - NOT JUST INSULATION: OLIGODENDROCYTE FUNCTION AND MYELINATION IN BRAIN PLASTICITY AND BEHAVIOR - PAOLA ARLOTTA, IIRIS HOVATTA, HANNELORE EHRENREICH, MARIA-CECILIA ANGULO

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The etiology of catatonia, an executive 'psychomotor' syndrome seen across diseases, has remained mysterious. We studied humans and myelin mutant mice, using microglia depletion as therapeutic strategy. In schizophrenic patients (N>2000), we uncovered >25% of subjects with catatonic signs. *CNP* rs2070106-AA, a *loss-of-function* genotype of a myelin-specific gene, was associated with catatonia in schizophrenia cohorts, and white matter hyperintensities in the general population. Subtle myelination defects in mouse mutants of *Cnp*, but also *Plp* or *Mbp,* led to catatonia and microgliosis. We hypothesized that neuroinflammation of myelinated tracts might cause catatonia and be alleviated by microglia depletion. Indeed, the CSF1 receptor inhibitor, PLX5622, attenuated catatonia in *Cnp* mutants, whereas executive dysfunction and brain atrophy failed to improve. Next, we wondered which brain areas might be causative of catatonic phenotypes. We generated mice lacking *Plp1*, encoding the major integral myelin membrane protein, selectively from ventricular zone stem cells of the forebrain. In contrast to conventional *Plp1* mutants, subtle myelin defects were restricted to the cortex and underlying callosal tracts. Forebrain-specific *Plp1* mutants exhibited no defect of basic motor-sensory performance. Behavioral alterations reported for conventional *Plp1* null mice were absent and even social interactions appeared normal. However, we determined catatonia-like symptoms and isolated executive function defects in both genders at progressed age, suggesting that a gradual loss of myelin integrity affects cortical connectivity and underlies specific defects of executive function, emerging only with increasing age. These observations are likewise relevant for human brain aging and neuropsychiatric conditions.

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PRESENTATION NUMBER: S077

SINGLE-CELL GENOMIC STUDIES OF MAJOR DEPRESSIVE DISORDER POINT TO THE ROLE OF IMMATURE OLIGODENDROCYTES

SYMPOSIUM 20 - NOT JUST INSULATION: OLIGODENDROCYTE FUNCTION AND MYELINATION IN BRAIN PLASTICITY AND BEHAVIOR - PAOLA ARLOTTA, IIRIS HOVATTA, HANNELORE EHRENREICH, MARIA-CECILIA ANGULO

Anjali Chawla[1], Malosree Maitra[1], Matthew Suderman[1], Corina Nagy[1], Gustavo Turecki[1], Maria Cecilia Angulo[2]
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Background: Understanding molecular changes associated with major depression at single-cell resolution is key to better understand the illness and develop new treatments. In this study, we aimed to study single-nucleus transcriptomic and open-chromatin states of individuals affected with major depression. **Methods:** We isolated N~80,000 nuclei from the prefrontal cortex (PFC) of cases and controls for single nucleus RNA sequencing. We used single-cell fluorescence in situ hybridization to validate the gene expression changes in given cell types of interest, as well as a high-throughput fluorescence assisted nuclei sorting to validate changes in broader cell types. We then used a droplet based single nucleus snATAC-seq approach to profile chromatin accessibility in the same samples. **Results:** Our transcriptomic results implicate 96 genes differentially expressed in 16 cells types, particularly lower layer excitatory neurons and immature oligodendrocytes. The addition of a modality—chromatin accessibility, improved and refined the transcript-only based clustering. Our data showed an enrichment of open chromatin at functional non-coding regions, while enhancer regions showed the most cell specific patterning. Differential open chromatin regions between cases and controls showed enrichment for gene regulatory regions and transcription factor binding motifs associated with disease-related biological pathways. We also identified gene regulators with cell-type specificity, and disease-specific epigenetic signatures of differential gene expression patterns. **Conclusion:** Leveraging the power of single cell technologies has allowed us to produce cell-type specific epigenetic signature. Specifically, we have identified cell types mostly affected in depression, as well as chromatin accessibility architecture that partly explains expression patterns associated with this illness.

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PRESENTATION NUMBER: S078

ALTERED SOCIAL LEARNING AND ECONOMIC INVESTMENT BEHAVIOR IN HUMANS WITH BILATERAL BASOLATERAL AMYGDALA DAMAGE AS A RESULT OF URBACH WIETHE DISEASE

SYMPOSIUM 21 - GENETICALLY DISSECTING NEUROBIOLOGY OF SOCIAL COLLABORATION IN HUMANS, MACAQUES AND RODENTS - JACK VAN HONK, CHENGYU LI, RON STOOP, SARA FREDA

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According to the leading computational framework, organisms learn the value of actions and outcomes via punishment and reward algorithmically. A model-free (or action-based) algorithm, rigidly assigns actions based upon immediate intrinsic value (value > action), and a model-based (or outcome-based) algorithm flexibly derives value from a causal model of the environment and is instrumentally guided by action-outcome probabilities (outcome > action). The amygdala conveys a translational obstacle for the cross-species applicability of this framework of value-based learning and choice. That is, the human amygdala is typically researched and discussed as a single unit despite the fact that the mammalian amygdala consists of sub-regions different in structure and function. Most prominently, the basolateral amygdala (BLA) and the central-medial amygdala (CMA), which consist of respectively cortical-type and striatal-type neural structures. Rodent research has determined that by parallel actions on the striatum, the CMA sub-serves habitual (which is action-based) choice behavior, while the BLA sub-serves goal-directed (which is outcome-based) choice behavior. Reconceptualized in terms of the computational framework, the CMA subserves the model-free algorithm, while the BLA subserves the model-based algorithm. At the symposium, research in human subjects with selective bilateral basolateral amygdala damage will be presented. In this research, which focuses on social learning, and social-economic and moral decision-making, it is established that role of the human BLA in value-based learning and choice behaviors translates seamlessly to the rodent BLA. The value-based learning and choice behaviors of our BLA-damaged subjects are entirely action-based and thus model-free.

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PRESENTATION NUMBER: S079

CRITICAL ROLE OF PREFRONTAL-RELATED CIRCUITS IN PROSOCIAL BEHAVIOR REVEALED BY ACUTE GENETIC MANIPULATION IN NON-HUMAN PRIMATES

SYMPOSIUM 21 - GENETICALLY DISSECTING NEUROBIOLOGY OF SOCIAL COLLABORATION IN HUMANS, MACAQUES AND RODENTS - JACK VAN HONK, CHENGYU LI, RON STOOP, SARA FREDA

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Social behavior is one of the most important driving forces of human society evolution. This has allowed us to reach major achievements in which many individuals are involved with each fulfilling a specialized task. An insight in how and why the brain can organize individual efforts at such large scale would therefore represent an important goal for brain research. In the monkey brain a number of regions, including prefrontal cortices and amygdala, have been identified to play important roles in social behavior, mostly through electrophysiological recording, and pharmacology and lesion techniques with limited social interactions. Here we established three groups of cynomolgus macaques, each group with 8-10 female individuals free ranging in a 30 m^2 room. Chronic behavioral monitoring and AI-assisted analysis revealed detailed social-interaction patterns in the groups. We perturbed neuronal activity of dorsolateral prefrontal cortex while observing changes in social behaviors in manipulated monkeys and other group members. In future electrophysiological recordings will also be performed in these free ranging and group housed monkeys with close monitoring of behavioral states. Our results will shed new insights to neural mechanism underlying social organization.

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PRESENTATION NUMBER: S080

ROLE OF OXYTOCIN IN THE CENTRAL AMYGDALA AND MEDIAL PREFRONTAL CORTEX FOR THE BUFFERING OF FEAR AND SOCIAL BEHAVIOR IN RATS

SYMPOSIUM 21 - GENETICALLY DISSECTING NEUROBIOLOGY OF SOCIAL COLLABORATION IN HUMANS, MACAQUES AND RODENTS - JACK VAN HONK, CHENGYU LI, RON STOOP, SARA FREDA

Ron Stoop[1], Chloé Hegoburu[2], Supriya Ghosh[2], Rodrigo Triana Del Rio[2], Isabel Salgado[2], Marios Abatis[2], Ruifang Niu[2], Yan Tang[2], Erwin Van Den Burg[2], Christophe Grundschober[2]
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Social support can help to reduce fear, but much is unknown about the neurobiological mechanisms underlying this "social buffering of fear" (SBF). Here we show, by chemogenetic and optogenetic manipulations, as well as *in vivo* single-unit recordings, that the neuropeptide oxytocin, originating from the hypothalamic paraventricular nucleus (PVN) and acting in the central nucleus of the amygdala (CeA), mediates SBF in fear-conditioned rats. The reduced freezing during fear memory recall in the presence of a companion is strong and immediate and maintained during a second fear recall 24h later without the companion. This is accompanied by acute and long-lasting changes in spiking patterns in oxytocinergic neurons in the PVN and three different neuron types in the CeA. Our findings reveal how acute and long-lasting SBF is mediated by an oxytocinergic signaling pathway between PVN and CeA that is completely subcortical, and thereby different from classical cortical-basolateral amygdala fear extinction pathways.

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PRESENTATION NUMBER: S081

THE FUNCTION OF THE VENTRAL TEGMENTAL AREA AND MEDIAL PREFRONTAL CORTEX IN SOCIAL INTERACTIONS AND AVERSIVE LEARNING IN MICE

SYMPOSIUM 21 - GENETICALLY DISSECTING NEUROBIOLOGY OF SOCIAL COLLABORATION IN HUMANS, MACAQUES AND RODENTS - JACK VAN HONK, CHENGYU LI, RON STOOP, SARA FREDA

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Aims: Central release of oxytocin modulates a wide range of social behaviors including pair bond formation, mating, and parental care. Long-standing research links oxytocin to neural processing of reward, primarily via actions in the ventral striatum. Our recent studies, among others, extend the oxytocin-dopamine interaction landscape to encompass dopamine-synthesizing circuits of the ventral tegmental area and substantia nigra pars compacta, with medial prefrontal cortex emerging as a recent critical element in this distributed network mediating aspects of social interactions and resilience. Methods: Anterograde and retrograde projections, receptor mRNA studies, and electrophysiology combine with wired and wireless opto- and pharmacogenetics with fiber photometry and 2-photon imaging, to characterize the relevant pathways of oxytocin and dopamine system convergence and function. Results and Conclusions: To dissect convergence sites for oxytocinergic system connectivity with reward circuitry, we performed whole-brain anterograde and retrograde tracing of oxytocin neurons in the paraventricular nucleus. We found oxytocin terminals widely distributed in the mesocorticolimbic dopamine system and neighboring hypothalamic and thalamic nuclei. The atlases provide a detailed mapping of cortical projections, revealing unexpected areas of strong connectivity, including medial prefrontal cortex (mPFC). Optogenetically induced release of oxytocin from local neuronal fibers increases tonic activity of ventral tegmental area dopamine neurons, linked to reward processing. Besides controlling the activity of DA neurons, oxytocin changes how they respond to their inputs, by dampening excitatory synaptic transmission onto DA neurons via endogenous cannabinoids. In the mPFC, dopamine mediates neuroplasticity and supports motivational resilience, also potentially facilitating neural synchrony during social interactions.

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PRESENTATION NUMBER: S082

MICROGLIAL CLOCKS: FRIENDS OR FOES OF CIRCADIAN TIMEKEEPING?

SYMPOSIUM 22 - GLIA: CRUCIAL REGULATORS OF CIRCADIAN RHYTHMS AND SLEEP - MARCO BRANCACCIO, OLGA BARCA MAYO, RUNE ENGER, LAUREN HABLITZ

Marco Brancaccio
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Circadian timekeeping regulates daily behaviour and physiology, including sleep/wake cycles, cognition and inflammatory process. The suprachiasmatic nucleus (SCN) is responsible for internally coordinating circadian activities and aligning them to light-dark cycles in mammals. SCN astrocytes can generate circadian patterns of behaviour and neuronal activity in mice genetically ablated of clock function. In revealing an unexpected functional reservoir of circadian resilience in glia, this work has also highlighted an unforeseen vulnerability of timekeeping in brain disease. Astrocyte transformation from a homeostatic to a reactive phenotype may disrupt central timekeeping and drive alterations of circadian function associated with early neurodegeneration. Microglia play a key role in turning astrocyte behaviour from neuroprotective to neurotoxic, but their circadian properties are poorly understood, mainly because limited availability of tools to genetically target and manipulate them. We have developed new intersectional genetic approaches to target microglia in SCN tissue, by using AAV-based genetically-encoded reporters of clock gene expression and microglia activation. Microglial circadian activities were monitored by live imaging, together with neurons and astrocytes, in physiological conditions and upon challenge with pro-inflammatory and proteotoxic stimuli. This has revealed highly dynamic properties of microglial timekeeping in the SCN. While unchallenged microglia show circadian oscillations that are less robust and coherent than astrocytes and neurons, this is rapidly transformed by proteotoxic and proinflammatory challenges, which can either reduce, or enhance, coherence of microglial timekeeping. Further work is underway investigating the role of astrocytes-microglia neuro-immune circadian coupling in physiological and pathological conditions and will be discussed at the meeting.

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PRESENTATION NUMBER: S083

CLOCKS, GLIA, BRAIN AND METABOLISM

SYMPOSIUM 22 - GLIA: CRUCIAL REGULATORS OF CIRCADIAN RHYTHMS AND SLEEP - MARCO BRANCACCIO, OLGA BARCA MAYO, RUNE ENGER, LAUREN HABLITZ

Olga Barca Mayo
Universidade de Santiago de Compostela, Center For Research In Molecular Medicine And Chronic Diseases, Santiago de Compostela, Spain

In mammals, endogenous circadian clocks sense and respond to daily feeding and lighting cues, adjusting internal ~24 h rhythms to resonate with, and anticipate, external cycles of day and night. This ensures a temporal partitioning of catabolic and anabolic reactions synchronizing organism metabolism to the feeding-fasting cycle. The mechanism underlying circadian entrainment to feeding time is critical for understanding why mistimed feeding, as occurs during shift work, disrupts circadian physiology, a state associated with increased incidence of chronic diseases such as type 2 diabetes. Metabolic state sensing pathways can alter the molecular clock in anticipation of the environmental light-dark cycles. During feeding, anabolic processes are triggered by the activation of the Insulin (INS)-AKT-mTOR signaling pathway. On the other hand, during fasting, AMPK activation triggers catabolic processes and inhibits mTOR activity. As astrocytes are at the interface between vessels and neurons, they are in a privileged position to act as metabolic sensors of systemic cues that entrain the peripheral clocks linking the periphery and brain clocks. Here, we will discuss the role of INS and AMPK signaling pathways in the entrainment of astrocyte clocks and their impact in regulating daily rhythms in physiology and behavior.

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PRESENTATION NUMBER: S084

ASTROCYTES AND NEURONS: CORTEX AND SLEEP

SYMPOSIUM 22 - GLIA: CRUCIAL REGULATORS OF CIRCADIAN RHYTHMS AND SLEEP - MARCO BRANCACCIO, OLGA BARCA MAYO, RUNE ENGER, LAUREN HABLITZ

Rune Enger
University of Oslo, Department Of Molecular Medicin, Oslo, Norway

Astrocytes inextricably influence neuronal physiology by providing structural and trophic support, extracellular homeostasis, and by active signaling. With such essential functions astrocytes are consequential to many different behaviors. Sleep is no exception, and astrocytes have been shown to influence sleep state maintenance, sleep drive and waste clearance. Disentangling the roles of astrocytes in sleep is challenging, as it requires subcellular resolution imaging of natural sleep. We have developed a model for two-photon microscopy of naturally sleeping head fixed mice, and have employed this model to describe astrocytic signaling in sleep. We have shown that astrocytic Ca^{2+} signals exhibit distinct features across the sleep-wake cycle and are reduced during sleep compared to wakefulness. Moreover, an increase in astrocytic Ca^{2+} signaling precedes transitions from slow wave sleep to wakefulness, with a peak upon awakening exceeding the levels during whisking and locomotion. Finally, attenuated astrocytic Ca^{2+} signaling impairs slow wave sleep and results in an increased number of microarousals, abnormal brain rhythms, and an increased frequency of slow wave sleep state transitions and sleep spindles. We are currently working on disentangling the relationship between astrocytic Ca^{2+} signals and memory function, by studying sleep spindles and sharp wave ripple dynamics. Moreover, we are investigating the roles of astrocytic endfoot Ca^{2+} signaling and potential effects on blood flow dynamics and perivascular fluid flow in sleep.

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PRESENTATION NUMBER: S085

SLEEP, CIRCADIAN CLOCKS AND THE GLYMPHATIC SYSTEM

SYMPOSIUM 22 - GLIA: CRUCIAL REGULATORS OF CIRCADIAN RHYTHMS AND SLEEP - MARCO BRANCACCIO, OLGA BARCA MAYO, RUNE ENGER, LAUREN HABLITZ

Lauren Hablitz
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The glymphatic system is a network of perivascular spaces through the brain that enables movement of cerebrospinal fluid into, and interstitial fluid through the brain. Glymphatic fluid movement is regulated by the circadian system and increased during sleep, enabling clearance of metabolic waste from the brain parenchyma. This talk will cover the basics of sleep and circadian regulation of the glymphatic system, explore the contribution of astrocytic circadian molecular clocks to glymphatic fluid movement, and present novel findings on the regulation of glymphatic flow.

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PRESENTATION NUMBER: S086

THE ANTERIOR CLAUSTRUM ENCODES MOTOR PLANNING

SYMPOSIUM 23 - THE FUNCTION(S) OF THE CLAUSTRUM: ATTENTION, SALIENCE, SLEEP, ALL OF THE ABOVE, OR SOMETHING ELSE? - SOLANGE BROWN, ALAN CARLETON, MAR REUS-GARCIA, LORENZ AUGUST FENK

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The claustrum, a thin, elongated subcortical nucleus located between the neocortex and striatum, forms extensive reciprocal connections with the neocortex and has been implicated in sensory selection. It has been proposed that claustrum activity evoked by sensory input modulates the neocortex's context-dependent responses to sensory stimuli. Recording from neurons in anterior claustrum while mice performed a crossmodal sensory-selection task, we found that claustrum neurons, including claustrocortical neurons projecting to primary somatosensory cortex, rarely responded to tactile or visual input alone. We found instead that claustrum neurons exhibited direction-tuned motor responses and encoded upcoming movement during intertrial intervals. Chemogenetic inhibition of claustrocortical neurons decreased lick responses to inappropriate sensory stimuli. These data indicate that the claustrum is integrated into higher-order premotor circuits recently implicated in decision-making.

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PRESENTATION NUMBER: S087

THE CLAUSTRUM-MEDIAL PREFRONTAL CORTEX NETWORK CONTROLS COGNITIVE FLEXIBILITY

SYMPOSIUM 23 - THE FUNCTION(S) OF THE CLAUSTRUM: ATTENTION, SALIENCE, SLEEP, ALL OF THE ABOVE, OR SOMETHING ELSE? - SOLANGE BROWN, ALAN CARLETON, MAR REUS-GARCIA, LORENZ AUGUST FENK

Alan Carleton
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In various psychiatric disorders, prefrontal cortex dysfunction is thought to induce cognitive deficits. Here we studied how the claustrum (CLA), a nucleus sharing dense reciprocal connections with the cortex, may contribute to cognitive impairments. We molecularly characterized CLA neurons using single cell RNA sequencing and used a Cre-driver transgenic mouse line to specifically study CLA glutamatergic projection neurons. We show that CLA projection neurons exert a direct excitatory input on medial prefrontal cortex (mPFC) neurons. Furthermore, specific ensembles of CLA and of mPFC neurons are activated during a task requiring cognitive flexibility such as attentional set-shifting (i.e. the ability to shift attention towards newly relevant stimulus-reward associations while disengaging from irrelevant ones. Perturbing the recruitment of specific CLA assemblies through opto/chemogenetic manipulations impairs the activation of mPFC ensembles and alters cognitive flexibility. Our results emphasize a potential role of the CLA-mPFC network in cognitive dysfunctions observed in some mental disorders.

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PRESENTATION NUMBER: S088

IS LEARNING THE FINAL TARGET TO ALL PUTATIVE FUNCTIONS OF THE CLAUSTRUM?

SYMPOSIUM 23 - THE FUNCTION(S) OF THE CLAUSTRUM: ATTENTION, SALIENCE, SLEEP, ALL OF THE ABOVE, OR SOMETHING ELSE? - SOLANGE BROWN, ALAN CARLETON, MAR REUS-GARCIA, LORENZ AUGUST FENK

Maria Del Mar Reus-Garcia
Nanyang Technological University, Lee Kong Chian School Of Medicine, Singapore, Singapore

Finding an unassailable definition for *learning* might be as challenging as describing the function of the claustrum. Nonetheless, most authors will agree on the following: 1. Learning is a mechanism by which organisms acquire behaviours that improve their adaptation to the environment; and 2. Claustral activity supports numerous cognitive-related processes, mainly filtering out irrelevant information to focus attention on significant or salient stimuli. My recent publication examined claustrum neuronal activity during classical conditioning. Claustral neurons, usually silent in awake, resting state, became significantly active while the animals learned the task.Inhibiting claustral activity during the acquisition of conditioned responses disrupted the learning process, while no effect was observed when claustral cells were silenced after learning was achieved. This suggests that claustrum activity is required for the acquisition of learning, but not for the expression of the learned behaviour.Concurrently, several authors have revealed that claustral cells are also active in behavioural experiments in which animals not only learn a cognitive task but also adjust their behaviour constantly to succeed. Furthermore, during slow-wave sleep -when consolidation of learning occurs- claustrum cells are particularly active. Learning is essential for adaptation, survival and evolution; undoubtedly many brain structures are recruited in proper learning. Is the claustrum responsible for identifying information that needs to be stored and generating a suitable brain state for learning-related processes? To answer that, I will revisit what we know so far regarding how the claustrum could support learning. **This research/project is supported by the Ministry of Education, Singapore, under its MOEAcRF Tier 3 Award MOE2017-T3-1-002.**

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PRESENTATION NUMBER: S089

DRAGONS, SLEEP, AND THE CLAUSTRUM

SYMPOSIUM 23 - THE FUNCTION(S) OF THE CLAUSTRUM: ATTENTION, SALIENCE, SLEEP, ALL OF THE ABOVE, OR SOMETHING ELSE? - SOLANGE BROWN, ALAN CARLETON, MAR REUS-GARCIA, LORENZ AUGUST FENK

Lorenz Fenk
Max Planck Institute for Brain Research, Neural Systems, Frankfurt am Main, Germany

This talk will explore the dynamics and coordination of sleep rhythms in the dragon *Pogona vitticeps,* drawing from some of our latest work on the reptilian claustrum.

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PRESENTATION NUMBER: S090

THE ROLE OF ASTROGLIA-NEURON INTERACTIONS IN GENERATION AND SPREAD OF SEIZURES

SYMPOSIUM 24 - NEURAL MECHANISMS OF EPILEPSY - EMRE YAKSI, LAURA EWELL, ESTHER KROOK-MAGNUSON, GABRIELE LIGNANI

Emre Yaksi
Norwegian University of Science and technology, Kavli Institute For Systems Neuroscience, Trondheim, Norway

Astroglia-neuron interactions are involved in multiple processes, regulating development, excitability and connectivity of neural circuits. Accumulating number of evidences highlight a direct connection between aberrant astroglial genetics and physiology in various forms of epilepsies. Using multiple zebrafish seizure models, we showed that neurons and astroglia follow different spatiotemporal dynamics during transitions from pre-ictal to ictal activity. We observed that during pre-ictal period neurons exhibit local synchrony and low level of activity, whereas astroglia exhibit global synchrony and high-level of calcium signals that are anti correlated with neural activity. Instead, generalized seizures are marked by a massive release of astroglial glutamate release as well as a drastic increase of astroglia and neuronal activity and synchrony across the entire brain. Furthermore, using a combination of genetic and pharmacological perturbations, we revealed that astroglial glutamate signalling and astroglial gap junctions plays a major role in generation and spreading of epileptic seizures across the brain. Knocking out astroglial glutamate transporters leads to recurrent spontaneous generalized seizures accompanied with massive astroglial glutamate release. Whereas perturbing astroglial gap junctions leads to more complex alterations of neural activity and excitability, mitigating the impact of PTZ induced seizures. Our results highlight astroglial glutamate transporters and gap junctions as potential targets for developing novel anti-seizure therapies.

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PRESENTATION NUMBER: S091

NETWORK MECHANISMS OF IMPAIRED MEMORY CODING IN EPILEPSY

SYMPOSIUM 24 - NEURAL MECHANISMS OF EPILEPSY - EMRE YAKSI, LAURA EWELL, ESTHER KROOK-MAGNUSON, GABRIELE LIGNANI

Laura Ewell
University of California – Irvine, Department Of Anatomy & Neurobiology, Irvine, United States of America

A common and devastating comorbidity of temporal lobe epilepsy (TLE) is impaired learning and memory. Strikingly, few studies have performed awake neural recordings *during* memory tasks, which would facilitate discovery of the physiological basis of such memory problems. To this end, we tested working memory performance in a mouse model of focal TLE, and found two types of impairment: (1) impaired learning of a constant reference frame, and (2) dynamic, intermittent impairment in working memory depending on real-time pathophysiology. Single unit and LFP recordings from various sub-regions of the hippocampus during the task revealed that mice were experiencing frequent sub-threshold (so called 'clinical') seizures during the task – and that such seizures occurred reliably at particular locations on the maze. Despite showing no overt behavioral phenotype during seizures, we suspect that such synchronized activity is interfering with normal hippocampal coding at key phases of the memory task – such as real-time replay of recent reward visits. The 'place' component of the seizure activity is indicative of heightened attractor dynamics in area CA3 of the hippocampus – and ongoing experiments in the lab are testing this hypothesis.

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PRESENTATION NUMBER: S092

IN VIVO MANIPULATION OF NEURONAL CIRCUITS IN EPILEPSY

SYMPOSIUM 24 - NEURAL MECHANISMS OF EPILEPSY - EMRE YAKSI, LAURA EWELL, ESTHER KROOK-MAGNUSON, GABRIELE LIGNANI

Esther Krook-Magnuson
University of Minnesota, Department Of Neuroscience, Minneapolis, United States of America

In vivo perturbation of neuronal circuits, including selectively during spontaneous seizures, can provide important insight into which circuits and circuit components may contribute to epileptiform activity, and, importantly, which elements may be able to curtail seizures. Using on demand optogenetics, we have explored the key role of circuit elements near the seizure focus in a mouse model of temporal lobe epilepsy, and show that engagement of the dentate gyrus is critical for such seizures. Selective in vivo manipulation techniques also allow exploration of cell-types and circuits remote from the seizure focus. Somewhat surprisingly, we find that strong modulation of hippocampal seizures is achieved by cerebellar-directed interventions. Pathway dissection coupled with selective modulation indicates that cerebellar-mediated inhibition of hippocampal seizures rests on excitation of excitatory cerebellar nuclear neurons which project to a particular nucleus of the thalamus. An in-depth exploration of the parameter space further indicates that robust seizure inhibition can be achieved when targeting this circuitry via more traditional electrical stimulation approaches. These findings highlight how circuit dissection via selective in vivo manipulation can provide new insights into seizure networks and provide new avenues for potential intervention strategies.

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PRESENTATION NUMBER: S093

MANIPULATION OF EPILEPTIC NEURONAL ACTIVITY USING ACTIVITY-DEPENDENT GENE THERAPY

SYMPOSIUM 24 - NEURAL MECHANISMS OF EPILEPSY - EMRE YAKSI, LAURA EWELL, ESTHER KROOK-MAGNUSON, GABRIELE LIGNANI

Gabriele Lignani
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Epilepsy remains one of the commonest serious neurological diseases, affecting 1% of the world's population. 30% of people with epilepsy are refractory to pharmacological treatment and for these patients, surgical resection of the focal brain area where the seizures arise remains the best hope to achieve seizure freedom, but this procedure is often precluded by proximity to eloquent regions. Gene therapy is the most promising candidate replacement for surgical treatment. However, current experimental gene therapies do not discriminate between neurons involved in seizure generation and 'healthy' surrounding neurons. There is an urgent need to develop methods that identify and treat only the neurons involved in the generation of seizures. The possibility to target only the neurons actively involved in seizures by modifying their activity would be an important step towards to a rational treatment of epilepsy. This project is based on the use of activity-dependent promoters to drive a therapeutic transgene that attenuates neuronal excitability. Pathological hyperactivity triggers expression of the transgene in transduced neurons, which then reduces their likelihood to fire and release neurotransmitter. Once seizures resolve, the gene therapy tool automatically turns off, until excessive activity occurs again (if it does). Importantly, transgene expression only occurs in neurons that are recruited to fire in excess, and neighbouring or intermingled neurons not involved in the seizure are unaffected. Self-time-limited expression of the transgene and specificity for over-active neurons argue that the treatment is better tolerated than an indiscriminate and permanent reduction in excitability in the epileptic focus.

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PRESENTATION NUMBER: S094

GENERALIZED NEOHEBBIAN SYNAPTIC PLASTICITY RULES FOR LEARNING CORTICAL REPRESENTATIONS

SYMPOSIUM 25 - ENCODING IN NEURONS AND BEYOND: APPLICATIONS IN MACHINE LEARNING - WULFRAM GERSTNER, ANDREW SAXE, MIGUEL ANGEL NUNEZ, JONATHAN CORNFORD

Wulfram Gerstner, Bernd Illing, Guillaume Bellec
EPFL, School Of Computer And Communication Sciences And School Of Life Sciences, Lausanne, Switzerland

Learning in the brain is based on synaptic plasticity rules, but learning rules that respect biological constraints pose challenges if the aim is to build deep hierarchical representations. Here, we propose a generalized NeoHebbian learning rule that depends on five factors: pre- and postsynaptic activity, a layer-wide broadcast signal of surprise, information about presence or absence of a saccade, and dendritic membrane potential the encodes learned predictions. We apply this rule in a multi-layer neural network where each layer can be interpreted as one cortical area. The learning rule applies contrastive predictive learning from machine learning to a causal, biological setting using saccades (ie rapid shifts in gaze direction). The learning rule is derived by minimizing an area-specific loss function and does not need to back-propagate error signals between cortical areas. We show that with such a local rule, the usefullness of cortical representations increases over several cortical areas.

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PRESENTATION NUMBER: S095

RICH AND LAZY LEARNING OF TASK REPRESENTATIONS IN BRAINS AND NEURAL NETWORKS

SYMPOSIUM 25 - ENCODING IN NEURONS AND BEYOND: APPLICATIONS IN MACHINE LEARNING - WULFRAM GERSTNER, ANDREW SAXE, MIGUEL ANGEL NUNEZ, JONATHAN CORNFORD

Andrew Saxe
UCL, Gatsby Computational Neuroscience Unit & Sainsbury Wellcome Centre, London, United Kingdom

How do neural populations code for multiple, potentially conflicting tasks? We undertake a combination of computational simulations and mathematical analyses involving neural networks to define “lazy” and “rich” coding solutions to context-dependent tasks, which trade off learning speed for robustness. During lazy learning the input dimensionality is expanded by random projections to the network hidden layer, whereas in rich learning hidden units acquire structured representations that privilege relevant over irrelevant features. For context-dependent decision-making, one rich solution is to project task representations onto low-dimensional and orthogonal manifolds. Using behavioral testing and neuroimaging in humans and analysis of neural signals from macaque prefrontal cortex, we report evidence for neural coding patterns in biological brains whose dimensionality and neural geometry are consistent with the rich learning regime.

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PRESENTATION NUMBER: S096

UNDERSTANDING INVARIANT TEXTURE CODING IN MOUSE VISUAL CORTEX THROUGH NEURAL NETWORKS

SYMPOSIUM 25 - ENCODING IN NEURONS AND BEYOND: APPLICATIONS IN MACHINE LEARNING - WULFRAM GERSTNER, ANDREW SAXE, MIGUEL ANGEL NUNEZ, JONATHAN CORNFORD

Carsen Stringer[1], Fengtong Du[2], Marius Pachitariu[2]
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The discrimination of visual textures, irrespective of rotation, scale and viewpoint, can help animals perform a variety of visual tasks such as object recognition and object segmentation. The neural basis of such invariant texture recognition is largely unknown. We recorded ~40,000 neurons simultaneously in mouse visual cortex while presenting ~14,000 visual stimuli sampled from 32 texture classes via random rotation, scaling and cropping. We found that the neural responses encoded the texture class robustly and a linear classifier trained on the neural data achieved test performance on single trials of 81.67% ± 2.74% (stddev, n=4 recordings, chance=0.03). Furthermore, we found that the patterns of errors in the task were highly-consistent across mice, and classification accuracy improved slightly in higher-order visual areas. These computations were supported by a subset of texture-coding neurons which formed 10% of the population and were spread throughout visual areas. Classification accuracy from the texture-coding neurons was similar to that from the entire population. To determine the computational operations leading to texture invariance, we next analyzed the responses of artificial neurons from a pretrained AlexNet model. These artificial neurons performed well in the texture classification task, but their pattern of errors did not match the patterns from the neural data. To better model the neural data, we next fit a CNN encoding model directly to the responses of the neurons. The model explained additional variability of the response patterns in individual images, but it did not fully reproduce the pattern of errors in the texture classification task.

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PRESENTATION NUMBER: S097

THE ROLE OF RECURRENCE IN NOISE-ROBUST VISUAL PROCESSING

SYMPOSIUM 25 - ENCODING IN NEURONS AND BEYOND: APPLICATIONS IN MACHINE LEARNING - WULFRAM GERSTNER, ANDREW SAXE, MIGUEL ANGEL NUNEZ, JONATHAN CORNFORD

There is strong evidence that animals use top-down and local recurrent connections to help resolve ambiguous visual stimuli, but there are also surprisingly few ANN architectures that explicitly take advantage of such recurrent connections to generate robust visual perception. Here, we explore sequential visual tasks designed to require higher-order and sequential information to resolve ambiguities. We show that on these tasks recurrent convolutional networks that are more brain-like in their architecture perform better than networks with more traditional convolutional architectures. This work helps to identify task designs that could be used to examine robust visual processing with non-feedforward circuits, both in experiments on living subjects, and benchmarks for artificial intelligence agents.

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PRESENTATION NUMBER: S098

MATERNAL INFLAMMATION DURING PREGNANCY AND OFFSPRING BRAIN DEVELOPMENT – EVIDENCE FROM PROSPECTIVE LONGITUDINAL STUDIES IN HUMANS

SYMPOSIUM 26 - VULNERABILITIES OF THE DEVELOPING NEOCORTEX TO MATERNAL INFLAMMATION: FROM PROGENITORS TO CIRCUITS - CLAUDIA BUSS, MELISSA BAUMAN, ANTHONY C VERNON, NAVNEET VASISTHA

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The origins of alterations in brain anatomy and connectivity, that may underlie cognitive impairment and mental illness, can often be traced back to the fetal period of life when the developing embryo/fetus responds to suboptimal conditions during critical periods of brain development (“Fetal Programming”). Maternal inflammation is among such conditions that can alter fetal brain development and consequently have an impact on her child’s cognitive function and mental health. Animal models support a key role for cytokines, inflammatory signaling proteins, as *sensors*, *transducers,* and *effectors* of maternal inflammation and other environmental conditions on the developing embryonic and fetal brain. Results from prospective longitudinal studies in mother-fetal/infant dyads will be presented. Concentrations of the pro-inflammatory cytokine Il-6 were measured serially in early, mid and late pregnancy. Multimodal brain MRI scans were acquired in neonates, who were followed up with developmental assessments across infancy. Evidence will be presented in support of higher maternal IL-6 concentrations to be associated with variation in neonatal brain anatomy and connectivity that predict cognitive function and behavioral problems in infancy. Furthermore, variation in maternal inflammatory mediators during pregnancy will be discussed as potential mediators underlying intergenerational transmission of maternal preconceptual and prepartum stress. These findings suggest that the origins of variation in cognitive ability and mental health may, in part, trace back to the intrauterine period of life and support variation in brain anatomy and connectivity related to prenatal inflammation as a putative pathway.

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PRESENTATION NUMBER: S099

FINDINGS FROM A NON-HUMAN PRIMATE MODEL OF MATERNAL IMMUNE ACTIVATION

SYMPOSIUM 26 - VULNERABILITIES OF THE DEVELOPING NEOCORTEX TO MATERNAL INFLAMMATION: FROM PROGENITORS TO CIRCUITS - CLAUDIA BUSS, MELISSA BAUMAN, ANTHONY C VERNON, NAVNEET VASISTHA

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Children born to women who experience infection during pregnancy have an increased risk of neurodevelopmental disorders, including schizophrenia and autism spectrum disorder. Rodent models of maternal immune activation (MIA) have identified the maternal immune response as the critical link between maternal infection and altered neurodevelopment. The nonhuman primate model provides an opportunity to maximize translational utility in a species more closely related to humans. Here we report data from the first longitudinal behavior and neuroimaging study conducted in a rhesus monkey (*Macaca mulatta*) MIA model. A modified form of the viral mimic, Polyinosinic-polycytidylic acid (PolyIC), was delivered to pregnant monkeys (N=14) in the late first trimester to stimulate maternal immune response. Control dams received saline injections (N=10) or were untreated (N=4). The offspring underwent behavioral assessments paired with longitudinal magnetic resonance imaging (MRI) from birth to four years to evaluate the long-lasting consequences of prenatal immune challenge. Although offspring born to control or MIA-treated dams did not differ on measures of physical growth and early developmental milestones, the MIA-treated animals exhibited subtle changes in cognitive development and deviated from species-typical brain growth trajectories. Longitudinal MRI revealed significant gray matter volume reductions in the prefrontal and frontal cortices of MIA-treated offspring at 6 months that persisted through the final time-point at 45 months, along with smaller frontal white matter volumes at 36 and 45 months. Comprehensive assessments of social development paired with an evaluation of the developing immune system will be presented to further explore the impact of prenatal immune challenge.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: S100

INTERFERON-GAMMA SIGNALLING DRIVES MOLECULAR AND CELLULAR PHENOTYPES ASSOCIATED WITH NEURODEVELOPMENTAL DISORDERS IN HUMAN IPSC-DERIVED NEURONS

SYMPOSIUM 26 - VULNERABILITIES OF THE DEVELOPING NEOCORTEX TO MATERNAL INFLAMMATION: FROM PROGENITORS TO CIRCUITS - CLAUDIA BUSS, MELISSA BAUMAN, ANTHONY C VERNON, NAVNEET VASISTHA

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Background: Converging lines of evidence support a link between elevated levels of pro-inflammatory cytokines such as interferon-γ (IFN-γ) and interleukin (IL)-6 during neurodevelopment and increased risk for psychiatric disorders. The cellular basis of these effects in the brain however remains unclear. We tested the hypothesis that exposure to either cytokine would contribute to molecular and cellular phenotypes associated with neurodevelopmental disorders in two different cell types. **Methods:** Neural progenitor cells (NPCs) and microglia-like cells (MGLs) were differentiated from hiPSC collected (n=3 neurotypical male donors, n=3 clones per donor) and exposed to either IFN-γ (25 ng/ml) or IL-6 (100 ng/ml). Molecular phenotypes were assessed by RNA sequencing and cellular phenotypes by cell-specific assays including neurite outgrowth, motility and secretion of cytokines and chemokines. **Results:** At the cellular level, transient exposure of NPCs to IFN-γ increased neurite outgrowth dependent on up-regulation of major histocompatibility class (MHC)-I and promyelocytic leukemia (PML) nuclear bodies (Warre-Cornish et al. Science Advances, 2020). By contrast, transient exposure to IL-6 increased Y705-STAT-3 phosphorylation in MGLs but not in NPCs. In MGLs transient exposure to IL-6 triggered increases in cell/process motility and secretion of pro- and anti-inflammatory cytokines. At the molecular level, we provide evidence that transient exposure of NPCs to IFN-γ and MGLs to IL-6 disproportionately alter the expression of genes associated with either schizophrenia or autism, suggestive of an interaction between genetic and environmental risk factors. **Conclusion:** These data provide evidence for putative cell-specific effects of IFN-γ and IL-6 signalling in the eitiology of neurodevelopmental disorders.

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PRESENTATION NUMBER: S101

TIME AND SUBTYPE-DEPENDENT IMPAIRMENT OF CORTICAL INTERNEURONS BY MATERNAL IMMUNE ACTIVATION

SYMPOSIUM 26 - VULNERABILITIES OF THE DEVELOPING NEOCORTEX TO MATERNAL INFLAMMATION: FROM PROGENITORS TO CIRCUITS - CLAUDIA BUSS, MELISSA BAUMAN, ANTHONY C VERNON, NAVNEET VASISTHA

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Infections during pregnancy are associated with an increased risk for disorders such as autism and schizophrenia. Virus infections induce maternal immune system activation (MIA) and the release of several cytokines that cross the placental barrier and severely impact the development of the cerebral cortex. We have previously shown that MIA has a non-uniform impact on cell types in the developing brain. Specifically, MIA displays a developmental time and neuronal subtype-dependent impairment on progenitors that give rise to inhibitory neurons. Progenitor proliferation in the medial (MGE) and caudal ganglionic eminence (CGE) is affected during early- and mid-neurogenesis stages respectively. Furthermore, despite the acute nature of MIA, the impact on neuronal lineages is enduring and continues even after birth leading to physiological dysfunction. Finally, using high-throughput single-nuclei RNA sequencing (snRNA-seq) technology, we describe the possible mechanisms underlying the differential vulnerability due to MIA. We show that receptors for key cytokines are unequally expressed between MGE and CGE progenitors. Furthermore, non-canonically and canonical Wnt signaling is disrupted in MGE and CGE progenitors respectively. Finally, using RNA dynamics to study cell lineages, we show that MIA induces precocious differentiation of dorsal cortical progenitors to novel cell states. Our study hence emphasizes the fundamental differences between neural progenitor cells and how this shapes the development of the neocortex. Understanding the mechanistic basis behind such differential vulnerability is also crucial for resolving the pathogenesis of neurodevelopmental disorders.

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PRESENTATION NUMBER: S102

NEW FUNCTIONAL PROPERTIES OF HIPPOCAMPAL NEURAL STEM CELLS IN EPILEPSY

SYMPOSIUM 27 - ADULT HIPPOCAMPAL NEUROGENESIS IN PHYSIOLOGY AND PATHOLOGY - JUAN ENCINAS, THOMAS LARRIEU, NORA ABROUS, MARIA LLORENS-MARTíN

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In normal conditions, **neural stem cells (NSCs)** dwelling in the dentate gyrus **of the hippocampus generate neurons and astrocytes**. **After seizures**, however, NSCs enter massively into the cell cycle and change their morphology drastically, **transforming into reactive-NSCs (React-NSCs), which ultimately differentiate into reactive astrocytes.** As a result, very little neurogenesis, and with abnormal properties, remains in the epileptic hippocampus. React-NSCs are distinct form normal NSCs, astrocytes and reactive astrocytes. Importantly, **React-NSCs contribute to reactive gliosis and are pro-inflammatory.** React-NSCs overexpress **interleukin 1β (IL-1β),** one of the major mediators of neuroinflammation in the brain. The seizure-induced **transformation of NSCs into pro-inflammatory React-NSCs is mediated** through the activation of **purinergic 2X receptors (P2XR)** and of **epidermal growth factor receptor (EGFR)**. By blocking these pathways, the induction of React-NSCs can be prevented and thus NSCs and neurogenesis can be preserved.

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PRESENTATION NUMBER: S103

BLOOD CIRCULATING FACTORS REGULATE ADULT NEUROGENESIS IN THE CONTEXT OF ANXIETY

SYMPOSIUM 27 - ADULT HIPPOCAMPAL NEUROGENESIS IN PHYSIOLOGY AND PATHOLOGY - JUAN ENCINAS, THOMAS LARRIEU, NORA ABROUS, MARIA LLORENS-MARTíN

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Adult hippocampal neurogenesis, which consists in the continuous formation of neurons throughout adulthood, confers stress resilience but is also inhibited by stress and anxiety. Thus, mechanisms of regulation of adult neurogenesis are relevant to mood disorders. Using an in vitro assay, we found that blood-circulating molecules regulate adult neural stem cell proliferation and are necessary and sufficient to mediate the stress- or anxiety-mediated reduction of adult neurogenesis in mice. In a cohort of patients at high risk of developing psychiatric diseases, we found that some of these molecules are correlated with levels of anxiety and may participate to disease mechanisms. Together, our results show that blood-circulating molecules regulate adult neurogenesis and may represent biomarkers or drug targets relevant to stress and anxiety-related diseases.

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PRESENTATION NUMBER: S104

THE TEMPORAL ORIGIN OF DENTATE GRANULE NEURONS DICTATES THEIR MORPHO-FUNCTIONAL PROPERTIES

SYMPOSIUM 27 - ADULT HIPPOCAMPAL NEUROGENESIS IN PHYSIOLOGY AND PATHOLOGY - JUAN ENCINAS, THOMAS LARRIEU, NORA ABROUS, MARIA LLORENS-MARTíN

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Neurogenesis was traditionally believed to occur only during embryonic stages in the mammalian brain. However, over the last 30 years, research has firmly established that active neurogenesis persists throughout adult life in discrete areas of the brain such as the hippocampal dentate gyrus (DG). In fact, the DG is predominantly formed after birth, and it exhibits a permanent remodeling through continuous addition of new neurons throughout the life-span. Because of this continuous addition of new cells, the DG appears as a highly heterogeneous structure composed of different generations of granule neurons. I will provide some examples of this heterogeneity and will show that the temporal origin of DGNs dictates their morphology, their properties in response to learning and their role in hippocampal-dependent function. I will conclude on the potential implication of these different cohorts of neurons in establishing resilience/vulnerability to mental disorders.

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PRESENTATION NUMBER: S105

HUMAN ADULT HIPPOCAMPAL NEUROGENESIS AND NEURODEGENERATIVE DISEASES

SYMPOSIUM 27 - ADULT HIPPOCAMPAL NEUROGENESIS IN PHYSIOLOGY AND PATHOLOGY - JUAN ENCINAS, THOMAS LARRIEU, NORA ABROUS, MARIA LLORENS-MARTíN

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The hippocampus hosts one of the most unique phenomena of the adult mammalian brain, namely the addition of new neurons throughout lifetime. While synapse loss and consequent death of mature neurons may be responsible for much of the hippocampal malfunctioning in neurodegenerative diseases, studies in mice suggest that the disease could also target the generation of new neurons – or adult hippocampal neurogenesis (AHN). In my talk, I will provide evidence from our lab that supports the occurrence of continued neurogenesis in the human hippocampus of aged healthy subjects and patients with distinct neurodegenerative diseases, using brain material obtained under tightly controlled conditions and applying state-of-the-art tissue processing methods. Our data evidence that AHN is a robust phenomenon in the human brain, and point to the impairment of adult neurogenesis and the homeostasis of the hippocampal neurogenic niche as a potentially relevant mechanism underlying hippocampal malfunctioning that may be amenable to novel therapeutic strategies.

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PRESENTATION NUMBER: S106

OBJECT VISION TO HAND ACTION IN MACAQUE PARIETAL, PREMOTOR AND MOTOR CORTICES

SYMPOSIUM 28 - MULTISENSORY INTEGRATION AND SPACE REPRESENTATION IN HUMANS AND MONKEYS - STEFAN SCHAFFELHOFER, ROSSELLA BREVEGLIERI, TOBIAS HEED, SULIANN BEN HAMED

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Grasping requires translating object geometries into appropriate hand shapes. How the brain computes these transformations remained widely unclear. We investigated three key areas of the macaque cortical grasping circuit in a delayed grasping task with microelectrode arrays in primates and found cooperative but anatomically separated visual and motor processes. The parietal area AIP operated primarily in a visual mode. Its neuronal population revealed a specialization for shape processing, even for abstract geometries, and processed object features ultimately important for grasping. Premotor area F5 acted as a hub that shared the visual coding of AIP only temporarily and switched to highly dominant motor signals towards movement planning and execution. We visualize these non-discrete premotor signals that drive the primary motor cortex M1 to reflect the movement of the grasping hand. In addition to these active tasks, we collected neural activity during observation tasks in search of mirror neurons in this network. F5 and AIP responded substantially during this observation context, but there was no categorically distinct class of mirror neurons. In fact, even at the population level, there was little evidence for processing of the observed grip *per se*, only of passive visual processing of object features. Our results reveal visual and motor features encoded in the grasping circuit and their communication to achieve transformation for grasping. They also suggest that, whatever the putative mirror mechanism may be, it does not involve the processing of motor features of grasps which are merely observed.

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PRESENTATION NUMBER: S107

MULTISENSORY INTEGRATION FOR MOVEMENT IN THE DORSOMEDIAL VISUAL STREAM OF MONKEY AND HUMANS

SYMPOSIUM 28 - MULTISENSORY INTEGRATION AND SPACE REPRESENTATION IN HUMANS AND MONKEYS - STEFAN SCHAFFELHOFER, ROSSELLA BREVEGLIERI, TOBIAS HEED, SULIANN BEN HAMED

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Accurate movements require correct multisensory integration, a complex function performed by different brain areas including the posterior parietal cortex (PPC). The PPC of the monkeys contains area V6A, which receives different sensory input, namely visual and somatosensory, uses these signals to estimate the state of the arm and communicates with the frontal cortex to perform correct movements. Moreover, V6A is active before and during arm movements because it contains reach-related cells modulated by the depth and by the direction of reaching. In humans, V6A (hV6A) is in the posterior part of Brodmann's area 7 and shares many functions with monkey V6A, such as the processing of sensory stimuli and the activation during reaching. Recently, in our lab, the causal role of hV6A was investigated using transcranial magnetic stimulation (TMS): after TMS during reach planning, impairments in the encoding of depth of reaching were observed, in keeping with the presence of cells modulated by the depth of reaching targets during reach planning in monkey V6A. Furthermore, we have also proved the functional connectivity between hV6A and the frontal cortex at rest using a dual-site paired-pulse TMS protocol. We found inhibitory effects of hV6A on the frontal cortex excitability at longer inter-stimulus intervals (12 and 15ms). All these experiments support a strict homology between monkey V6A and hV6A and suggest that this region subserves crucial sensorimotor integration functions to interact with the external world.

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REFERENCE FRAMES AND TASK REPRESENTATION FOR TACTILE-SPATIAL PROCESSING IN HUMAN POSTERIOR PARIETAL CORTEX

SYMPOSIUM 28 - MULTISENSORY INTEGRATION AND SPACE REPRESENTATION IN HUMANS AND MONKEYS - STEFAN SCHAFFELHOFER, ROSSELLA BREVEGLIERI, TOBIAS HEED, SULIANN BEN HAMED

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When humans have to reach or saccade towards a visual target, spatial information is coded in different reference frames anchored to the eyes, head, and world. When one wants to act towards a tactile stimulus, the target's location on the skin cannot sufficiently inform the movement, because the body's limbs can be flexibly configured, so that posture must be taken into account to aim for a skin location. To elucidate which brain regions contribute to transforming tactile information into a location in space and in planning the respective movement, 16 participants pointed towards, or away from, tactile stimuli presented to uncrossed and crossed feet in a delayed movement paradigm. Multi-voxel pattern decoding revealed that bilateral rostral regions of posterior parietal cortex (PPC) differentiated tactile stimuli based on the anatomical foot on which they had occurred. A caudal PPC region, in contrast, coded whether a stimulus had occurred in left or right space, independent of the anatomical foot on which it had been presented. The transformation rule, i.e. whether the pointing was towards or away from the stimulus, was decodable in caudal PPC, partly overlapping with the regions that coded stimuli anatomically and in space. Thus, the different reference frames relevant to tactile-motor processing appear to be integrated in the context of an overarching task representation.

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MULTISENSORY INTEGRATION AND SPACE REPRESENTATION IN THE VENTRAL INTRAPARIETAL AREA OF MONKEYS AND HUMANS

SYMPOSIUM 28 - MULTISENSORY INTEGRATION AND SPACE REPRESENTATION IN HUMANS AND MONKEYS - STEFAN SCHAFFELHOFER, ROSSELLA BREVEGLIERI, TOBIAS HEED, SULIANN BEN HAMED

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The macaque ventral intraparietal area (VIP) in the fundus of the intraparietal sulcus has been implicated in a diverse range of sensorimotor and cognitive functions such as motion processing, multisensory integration, processing of head peripersonal space, defensive behavior, and numerosity coding. These functional properties have been used in order to identify VIP in the human parietal cortex as well as in other species. I will however show that human VIP research has consistently identified three, rather than one, bilateral parietal areas that each appear to subsume some, but not all, of macaque VIP's functionality. These three human areas are dominated, roughly, by coding the head or self in the environment, visual heading direction, and the peripersonal environment around the head, respectively. Based on cross-species functional comparison, I will suggest that VIP has evolved as a progressive expansion and specialization of a parietal area, from prosimians to new-world and old-world monkeys to humans. Based on this new understanding of human VIP, focusing on the visual and touch modalities, I will present data suggesting that some precursory specialization within macaque VIP, arising from specific functional brain connectivity patterns as well as from inter-individual differences, has been previously overlooked. I will then propose prediction in space and time, linking VIP to state estimation, as a unifying functional principle for the diversity of seemingly unrelated functions associated with area VIP. Last, I will propose that VIP's expansive differentiation of head and self-related processing may have been key in the emergence of human bodily self-consciousness.

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PRESENTATION NUMBER: S110

ELECTROPHYSIOLOGICAL PROPERTIES OF DOPAMINE NEURONS PROJECTING TO TAIL OF THE STRIATUM

SYMPOSIUM 29 - THE BASAL GANGLIA SENSORY DOMAIN THROUGH TAIL OF THE STRIATUM: REWARD, THREAT AND SENSORY FILTERING - ROEPER JOCHEN, EMMANUEL VALJENT, MITSUKO WATABE-UCHIDA, SEBASTIAN KRUETTNER

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The biophysical differences of dopamine midbrain neurons with defined axonal projections have been well characterized by in vitro slice patch-clamp experiments on retrogradely characterized and neurochemically identified cells. In contrast, little is known about those dopamine neurons in the pars lateralis of the substantia nigra (SNpl) that project to the tail of the striatum, a caudal part of the dorsal striatum. Therefore, combining in vivo retrograde tracing and in vitro patch-clamping we set out the characterize this subtype of dopamine neurons involved in fear responses. In contrast to those DA neurons residing in the lateral part of the substantia nigra (i.e. medial of the pars lateralis) that project to the more rostral dorsolateral stiatum, SNpl DA neurons had smaller somato and fired a higher baseline frequencies. The possessed intermediate sag levels and short rebound delays. We currently study these neurons in vivo to better capture their specific electrcophysiological properties.

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PRESENTATION NUMBER: S111

SPATIOMOLECULAR HETEROGENEITY OF D1R AND D2R NEURONS OF THE TAIL OF THE STRIATUM

SYMPOSIUM 29 - THE BASAL GANGLIA SENSORY DOMAIN THROUGH TAIL OF THE STRIATUM: REWARD, THREAT AND SENSORY FILTERING - ROEPER JOCHEN, EMMANUEL VALJENT, MITSUKO WATABE-UCHIDA, SEBASTIAN KRUETTNER

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The striatum is the gateway to the basal ganglia, an ensemble of subcortical structures involved in motor planning, procedural and reinforcement-based behaviors. In mammals, three functional domains have been characterized based on differences spanning across mediolateral and dorsoventral axis. Increasing evidences indicate however that the extreme caudal part of the striatum, also referred to as the tail of striatum (TS), constitutes a fourth functional domain. Here, I will discuss recent findings suggesting that the TS displays heterogeneous cell-type-specific organization and unique input-output connectivity. I will illustrate how the use of transgenic mice expressing reporters driven by specific promoters enables the delimitation of distinct TS domains, which are highly conserved across the *Muridae* family. Finally, I will provide evidence that the spatiomolecular heterogeneity of TS neurons expressing dopamine D1 and D2 receptors, and the peculiarity of TS-projecting DA neurons contribute to make this fourth striatal domain particularly responsive to psychostimulants.

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PRESENTATION NUMBER: S112

BALANCING THE TAIL OF THE STRIATUM PATHWAYS UNDER REWARD-THREAT CONFLICT

SYMPOSIUM 29 - THE BASAL GANGLIA SENSORY DOMAIN THROUGH TAIL OF THE STRIATUM: REWARD, THREAT AND SENSORY FILTERING - ROEPER JOCHEN, EMMANUEL VALJENT, MITSUKO WATABE-UCHIDA, SEBASTIAN KRUETTNER

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Animals adaptively learn to repeat rewarding actions and refrain from dangerous actions. Although our understanding of reward learning and threat learning has advanced tremendously in recent years, these are mostly studied independently of one another. However, in the natural world, it is critical to consider both reward and threat. Especially in case of threat, it is important to utilize information of potential threat, not only the ultimate outcomes such as pain, injury and death. What is the brain mechanism for balancing potential reward and threat? On the other hand, computational models often treat reward and threat as a single dimension. Does the brain map both potential reward and threat onto a single axis for behavioral choice? In this talk, I will introduce a series of our studies using both classical and naturalistic behavioral paradigms where mice must navigate threats to obtain water rewards. This talk will focus on the tail of the striatum (TS, or sensory striatum), which we found receive input from a unique subpopulation of dopamine neurons that encodes threat (but not reward) prediction errors. I will discuss why it is critical for survival that value and threat are represented in separate systems along two axes. Finally, from our preliminary data, I will share our working hypothesis that animals integrate reward and threat using multi-layered competing systems in the brain, both within local basal ganglia circuits and globally across cortico-striatal loops.

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PRESENTATION NUMBER: S113

PREFRONTAL CORTICAL OUTPUTS TO THE TAIL OF STRIATUM TRANSLATE TASK RULES TO CONTROL SENSORY FILTERING

SYMPOSIUM 29 - THE BASAL GANGLIA SENSORY DOMAIN THROUGH TAIL OF THE STRIATUM: REWARD, THREAT AND SENSORY FILTERING - ROEPER JOCHEN, EMMANUEL VALJENT, MITSUKO WATABE-UCHIDA, SEBASTIAN KRUETTNER

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To make adaptive decisions, organisms must appropriately filter sensory inputs, augmenting relevant signals and suppressing noise. It has been known that the prefrontal cortex (PFC) plays a critical role in guiding sensory filtering but how the PFC influences sensory processing remains poorly understood. Using multiple approaches, I find that engagement of the PFC projections to the tail of striatum are critical for the suppression of distracting sensory inputs, enhancing behaviorally relevant signals over noise. In addition, I identified distinct subnetworks of PFC neurons which project to visual or auditory domains of the tail of striatum and control the corresponding sensory modality. These subnetworks acted to decode sensory filtering rules encoded in the PFC, providing outputs capable of differentially controlling the gain of sensory inputs depending on task-demands. The outputs of these subnetworks were anatomically fixed, placing a fundamental constraint on the internal dynamics of the PFC. Overall, my results identify a key interaction between the PFC and the tail of striatum responsible for the control of sensory processing and introduce important principles that provide a framework for understanding how this circuit adaptively controls sensory processing.

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PRESENTATION NUMBER: S114

GLUCOCORTICOID SPINE PLASTICITY IN STRESS

SYMPOSIUM 30 - NOVEL INSIGHTS INTO THE NEUROBIOLOGY OF STRESS VULNERABILITY AND RESILIENCE - FREDDY JEANNETEAU, LAURENCE COUTELLIER, VIRGINIE RAPPENEAU, STEFAN REBER

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In vivo microscopy of dendritic spines in pyramidal neurons of the mouse brain provided imaging correlates for the robust but complex physiological and behavioral effects of glucocorticoids in the context of learning and memory. Glucocorticoids enhance spine formation and elimination within distinct time domains. It takes minutes for glucocorticoids to promote spine formation while it takes hours to elicit spine elimination because it requires new gene products. Consolidation of newly formed spines depends on the mobilization of AMPA receptors through glucocorticoid-induced trafficking and synthesis. Glucocorticoid spine remodeling occurs in clusters physically gathering connectivity from projections neurons encoding the associative contents of the learning experience. Clustering is not random and could reduce the cost of plasticity. Dual imaging of mitochondrial function and spine turnover in the living mouse brain seems to support this model. It is possible that functional connectivity is sustained by the metabolism locally allocated to spine clusters. This adds to the current framework for better understanding the detailed accuracy of memories.

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GABA-MODULATED SEX-SPECIFIC VULNERABILITY TO STRESS

SYMPOSIUM 30 - NOVEL INSIGHTS INTO THE NEUROBIOLOGY OF STRESS VULNERABILITY AND RESILIENCE - FREDDY JEANNETEAU, LAURENCE COUTELLIER, VIRGINIE RAPPENEAU, STEFAN REBER

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Women are twice as likely as men to develop an anxiety disorder after exposure to stress. The underlying mechanisms behind this sex-specific vulnerability to stress remains unknown. Our work in mice revealed that parvalbumin (PV)-expressing neurons of the prefrontal cortex (PFC) are more sensitive to chronic stress in females than in males. Indeed, we observed that female prefrontal PV cells develop a hyperactive phenotype not seen in males after four weeks of unpredictable chronic mild stress (UCMS). We further showed that chemogenetically driving the activity of these cells enhances anxiety in females but not males identifying a novel mechanism that can explain female vulnerability to stress-induced anxiety. We then aimed to understand how female prefrontal PV neurons become more sensitive to stress. These neurons undergo important maturation during adolescence, and the higher rate of diagnosis of stress-related neuropsychiatric disorders in women is observed after puberty. This led us to hypothesize that the surge of hormones during puberty drives the maturational processes that render PV neurons increasingly responsive to stress in adult females. We used a prepubertal ovariectomy approach in mice, followed by adult exposure to UCMS. We observed that ovariectomy prevents not only the development of an anxious phenotype after stress in adulthood, but also the stress-induced increase in detectable PV neurons in the PFC that is seen in control mice and reflects their increased level of activity. Altogether, these findings identified puberty as a critical period for the development of sex-specific vulnerability to stress.

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PRESENTATION NUMBER: S116

STRESS, DIET & DEPRESSION: VULNERABILITY AND RESILIENCE MODELED IN THE 'STRESS REACTIVITY' MOUSE LINES

SYMPOSIUM 30 - NOVEL INSIGHTS INTO THE NEUROBIOLOGY OF STRESS VULNERABILITY AND RESILIENCE - FREDDY JEANNETEAU, LAURENCE COUTELLIER, VIRGINIE RAPPENEAU, STEFAN REBER

Virginie Rappeneau, Lars Wilmes, Dorothea Ziemens, Chadi Touma
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Major depression (MD) has a high rate of comorbidity with metabolic diseases. Both involve alterations in the hypothalamic-pituitary-adrenal (HPA) axis. However, our knowledge of the precise molecular mechanisms by which the HPA axis contributes to their association remains limited. In this study, we used an animal model of MD to determine the behavioural and metabolic consequences of a short-term high-fat diet (HFD) treatment. Juvenile male mice of the 'Stress Reactivity' model, consisting of three independent lines selectively bred for high (HR), intermediate (IR) or low (LR) HPA axis reactivity, were fed for 4 weeks with either a HFD (45% kcal from fat) or a control diet (10% kcal from fat). Our results showed that the HFD treatment had a major impact on morphometric measures in the LR and IR lines, as indicated by marked increase in the body weight gain, overall adiposity and ectopic fat deposition. At the behavioural level, the HFD treatment did not alter locomotion in the open-field test. However, it significantly increased the floating duration of LR mice in the forced swim test, further increasing their passive stress-coping behaviour. Surprisingly, HR mice were seemingly resistant to the diet-induced behavioural and metabolic alterations. In both the adipose tissue and the brain, the HFD treatment produced major changes in gene expression related to glucose homeostasis and insulin signalling. The present work highlights the 'Stress Reactivity' model as a promising tool for improving our understanding of the involvement of HPA axis dysregulation in susceptibility vs. resilience to diet-induced metabolic dysfunction.

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PRESENTATION NUMBER: S117

OLD FRIENDS, IMMUNOREGULATION, AND STRESS RESILIENCE

SYMPOSIUM 30 - NOVEL INSIGHTS INTO THE NEUROBIOLOGY OF STRESS VULNERABILITY AND RESILIENCE - FREDDY JEANNETEAU, LAURENCE COUTELLIER, VIRGINIE RAPPENEAU, STEFAN REBER

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Immunodysregulation and subsequent chronic low-grade inflammation are risk factors for the development of stress-related somatic and psychiatric disorders, including inflammatory bowel disease (IDB) and posttraumatic stress disorder (PTSD). Thus, immunoregulatory approaches that counterbalance basal and/or stress-induced immune activation are expected to be protective in this context. In this talk Prof. Reber will discuss evidence indicating that increases in immune (re-)activity and inflammation, potentially promoted by a reduced exposure to immunoregulatory microorganisms (“Old Friends”) in today’s modern society, may be causal factors in mediating the vulnerability to development and persistence of stress-related pathologies. Moreover, he will discuss bioimmunoregulatory approaches, as for instance facilitating the contact with immunoregulatory Old Friends, as novel and promising strategies for the treatment and/or prevention of stress-related disorders.

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PRESENTATION NUMBER: S118

TARGETING TAU AT PRESYNAPTIC TERMINALS

SYMPOSIUM 31 - INTRACELLULAR DYNAMICS AND NEURODEGENERATION - PATRIK VERSTREKEN, SUSANNE WEGMANN, SUBHOJIT ROY, CLOTILDE LAGIER-TOURENNE

Patrik Verstreken
VIB-KU Leuven Center for Brain and Disease Research, Laboratory For Neuronal Communication, Leuven, Belgium

Synaptic dysfunction is an early pathological feature of neurodegenerative diseases associated with Tau, including Alzheimer's disease. Interfering with early synaptic dysfunction may be therapeutically beneficial to prevent cognitive decline and disease progression, but the mechanisms underlying synaptic defects associated with Tau are unclear. In disease conditions in patients and in mouse and fly Tau-disease-models, the protein mislocalizes into pre- and postsynaptic compartments and we show that presynaptic Tau induces synaptic vesicle trafficking defects. I will present work that shows that interfering with this presynaptic function of Tau, by lowering the levels of the synaptic vesicle associated protein Synaptogyrin-3, rescues Tau-induced synaptic loss and cognitive decline in mice. Our work not only identifies new presynaptic roles for Tau but it also opens a new therapeutic window to tackle early synaptic dysfunction and cognitive decline in neurodegenerative disease.

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PRESENTATION NUMBER: S119

THE MULTIPLE PHASES OF THE MICROTUBULE BINDING PROTEIN TAU

SYMPOSIUM 31 - INTRACELLULAR DYNAMICS AND NEURODEGENERATION - PATRIK VERSTREKEN, SUSANNE WEGMANN, SUBHOJIT ROY, CLOTILDE LAGIER-TOURENNE

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The microtubule associated protein Tau (MAPT) binds and stabilizes microtubules (MTs) in the axons of the central nervous system. It is best known, however, for its role in neurodegenerative brain diseases, such as Alzheimer's disease and forms of Fronntotemporal Dementia. In these diseases, the highly soluble and abundant protein forms amyloid-like intraneuronal aggregates and leads to neurotoxicity. Interestingly, from the protein physicochemical point of view, Tau is an intrinsically disordered protein that can adopt multiple protein conformations and assembly states, such as monomeric, oligomeric, liquid condensed, and aggregated. Since these "phases" of Tau have different biochemical and physical properties, they can encode different functions and misfunctions of Tau. The most recent discovered assembly form of Tau are liquid-like condensates, for which both physiological and pathologcical functions have been suggested, e.g. in MT binding and in aggregation. The concept and role of condensed Tau phases will be introduced in this presentation.

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PRESENTATION NUMBER: S120

THE SECRET LIVES OF SYNUCLEINS

SYMPOSIUM 31 - INTRACELLULAR DYNAMICS AND NEURODEGENERATION - PATRIK VERSTREKEN, SUSANNE WEGMANN, SUBHOJIT ROY, CLOTILDE LAGIER-TOURENNE

Subhojit Roy
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Despite much effort, the physiologic role of alpha-synuclein – a small presynaptic protein with established roles in the pathogenesis of Parkinson's and related disorders – is unclear. Phenotypes in alpha-synuclein knockout mice are mild, precluding definitive conclusions, and though mice lacking all synucleins (alpha/beta/gamma synuclein "triple knockout" mice) have given some insight into function, it has been difficult to parse out the specific effects of alpha-synuclein in this setting. Other studies have made conclusions based on over-expression of alpha-synuclein, but it has been unclear if these phenotypes are due to mis-localization of alpha-synuclein and gain of abnormal function. We will report updates from studies where we used CRISPRs to systematically and selectively delete each synuclein in mouse and human neurons, followed by experiments evaluating synaptic physiology.

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PRESENTATION NUMBER: S121

NEURODEGENERATION IN AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA

SYMPOSIUM 31 - INTRACELLULAR DYNAMICS AND NEURODEGENERATION - PATRIK VERSTREKEN, SUSANNE WEGMANN, SUBHOJIT ROY, CLOTILDE LAGIER-TOURENNE

Clotilde Lagier-Tourenne
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Alteration of RNA metabolism has emerged as a central theme in neurodegenerative diseases with mutations and/or mislocalization of RNA binding proteins, including TDP-43 and FUS, in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 and FUS are involved in fundamental RNA processing activities including RNA transcription, splicing, and transport. Following the recognition of their crucial role in neurodegeneration, we have used genome wide approaches to define their role in regulating expression and splicing of their RNA targets. We recently demonstrated that the human RNA most affected by reduction in TDP-43 is encoding the neuronal growth-associated factor called stathmin-2 (also known as SCG10), an essential component for neuronal regeneration and axonal maintenance. Reduced nuclear TDP-43 results in abnormal usage of cryptic splice and polyadenylation sites in pre-mRNAs from the *STMN2* gene, leading to loss of stathmin-2 protein. Remarkably, although TDP-43 affects the levels or splicing of many RNAs, restoration of stathmin-2 alone was sufficient to rescue regeneration after axotomy of iPSC-derived TDP-43 depleted motor neurons. Reduced levels in stathmin-2 is a hallmark in sporadic and familial ALS/FTD, and restoration of stathmin-2 expression emerges as an attractive therapeutic strategy in the vast majority of patients with ALS/FTD. We developed approaches to block cryptic splicing of stathmin-2 by targeting dCaRx or antisense oligonucleotides (ASOs) to stathmin-2 pre-mRNA leading to rescue of axonal regeneration capacity of human motor neurons with TDP-43 deficiency. Finally, using “humanized” stathmin-2 mice with constitutive misplicing, we establish that ASO injection into cerebral spinal fluid is a therapeutically viable approach to rescue stathmin-2 mRNA levels in TDP-43 proteinopathies.

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PRESENTATION NUMBER: S122

THE HIPPOCAMPUS CONVERTS VOLATILE ENTORHINAL INPUTS INTO STABLE SPATIAL MAPS

SYMPOSIUM 32 - UNDERSTANDING THE FORMATION OF SPATIAL REPRESENTATIONS: FROM SYNAPSES TO CIRCUITS - MARLENE BARTOS, JUDIT MAKARA, MATTHEW NOLAN, CHRISTINE GRIENBERGER

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The medial entorhinal cortex (MEC)-hippocampal network plays a key role in the processing, storage and recall of spatial information. However, how the spatial code provided by MEC inputs relates to spatial representations generated by principal cell assemblies within hippocampal subfields remains enigmatic. To investigate this coding relationship, we employed two-photon calcium imaging in mice navigating through dissimilar virtual environments. Imaging large MEC bouton populations revealed spatially tuned activity patterns. MEC inputs drastically changed their preferred spatial field locations between environments, whereas hippocampal cells showed lower levels of place field reconfiguration. Decoding analysis indicated that higher place field reliability and larger context-dependent activity-rate differences allow low numbers of principal cells, particularly in the DG and CA1, to provide a more rapid and accurate information about location and context than MEC inputs. Thus, conversion of dynamic MEC inputs into stable spatial hippocampal maps may enable fast encoding and efficient recall of spatio-contextual information.

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PRESENTATION NUMBER: S123

COOPERATIVITY AND PLASTICITY OF SYNAPTIC INPUT PATTERNS IN PYRAMIDAL CELL DENDRITES

SYMPOSIUM 32 - UNDERSTANDING THE FORMATION OF SPATIAL REPRESENTATIONS: FROM SYNAPSES TO CIRCUITS - MARLENE BARTOS, JUDIT MAKARA, MATTHEW NOLAN, CHRISTINE GRIENBERGER

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Spatially selective activity of hippocampal pyramidal neurons provides a representation of the environment supporting navigation and memory; however, the cellular mechanisms of the formation of hippocampal representations are incompletely understood. The activity of pyramidal neurons is shaped by the thousands of excitatory synaptic inputs arriving onto their dendritic tree. The spatiotemporal pattern of the active inputs, together with the active and passive properties of the targeted dendrites determine the integration of synaptic voltage signals in dendritic segments and may induce local forms of synaptic plasticity. Although synaptic long term potentiation has been proposed to contribute to the establishment of place coding, the fine-scale input pattern requirements to induce potentiation remained unclear. I will present our recent results demonstrating that local synaptic plasticity rules in thin perisomatic dendrites of CA1 pyramidal neurons depend on several factors, including the location of the input within the dendritic branch, the fine spatial organisation of the active synapses, the capacity of the input pattern to evoke dendritic spikes, and the activity of non-synchronous neighbouring synapses. The diversity of pattern-dependent cooperative synaptic plasticity rules can support fine-scaled discrimination of storage of information carried by input patterns, contributing to the versatility of hippocampal network coding.

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PRESENTATION NUMBER: S124

READ OUT OF SPATIAL MEMORIES BY RAMP-LIKE FIRING RATE TRAJECTORIES

SYMPOSIUM 32 - UNDERSTANDING THE FORMATION OF SPATIAL REPRESENTATIONS: FROM SYNAPSES TO CIRCUITS - MARLENE BARTOS, JUDIT MAKARA, MATTHEW NOLAN, CHRISTINE GRIENBERGER

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Neurons in the retrohippocampal cortices, which include the entorhinal cortex, presubiculum and parasubiculum, play crucial roles in spatial memory. Many retrohippocampal neurons, for example grid cells and border cells, have firing fields associated with specific locations. These representations are thought to be building blocks for spatial localisation and memory. However, they are usually investigated in tasks in which relationships between locations and reward are absent. Whether these discrete codes are the sole strategy for representing spatial memories is therefore unclear. We investigated this using a memory task in which locations on a virtual track are associated with rewards. When mice solve this task we find that neurons in the retrohippocampal cortices encode location through ramping activity within discrete regions of the track. Rewarded locations were represented by offsets or switches in the slope of the ramping activity. These representations were maintained after removal of cues that mark reward locations indicating that they result from recall of the track structure. We find that similar representations emerged in recurrent neural networks trained to navigate to a rewarded location, suggest they may be part of a general computational strategy for spatial memory. We suggest that retrohippocampal ramping activity and its interruption mediates is well suited to readout of learned models for goal-directed navigation.

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PRESENTATION NUMBER: S125

EXPERIENCE-DEPENDENT SHAPING OF HIPPOCAMPAL REPRESENTATIONS

SYMPOSIUM 32 - UNDERSTANDING THE FORMATION OF SPATIAL REPRESENTATIONS: FROM SYNAPSES TO CIRCUITS - MARLENE BARTOS, JUDIT MAKARA, MATTHEW NOLAN, CHRISTINE GRIENBERGER

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A crucial function of the brain is to produce useful representations of the external world. Previous results demonstrate that a non-Hebbian type of synaptic plasticity, behavioral timescale synaptic plasticity (BTSP), has a fundamental role in place field formation in hippocampal area CA1. BTSP has several distinct characteristics, including that it is driven by dendritic plateau potentials (plateaus) instead of APs. As axons from layer 3 entorhinal cortex (EC) impinge onto the site of plateau initiation and their activity regulates plateau probability, we hypothesized that this EC input might shape CA1 representations during learning. To test this hypothesis, we used two-photon calcium imaging in mice exploring a novel linear track. We observed the experience-dependent development of place cell representations in CA1, which featured an increased place field density (2-3x) around the reward location. Several findings implicated BTSP: (1) the abrupt appearance of new place cells; (2) the width of the new fields was correlated with the animal's running speed during induction; (3) new fields were shifted back in space with respect to their induction; (4) the development of the representation was severely attenuated by plasticity and calcium spike blockers. Using two-photon calcium imaging and optogenetic silencing of layer 3 EC axons we found that EC3 activity provides a signal to CA1 capable of increasing plateau probability and BTSP driven place field formation around the reward location. Taken together, our results identify the layer 3 entorhinal cortex input as a key signal that instructs CA1 neurons in how to represent an environment.

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PRESENTATION NUMBER: S126

MAPPING AND PERTURBING THE FUNCTIONAL CONNECTOME IN DROSOPHILA

SYMPOSIUM 33 - PERTURBATION-BASED NEUROCONNECTOMICS: PRINCIPLES AND APPLICATIONS ACROSS SPECIES - THOMAS CLANDININ, ALESSANDRO GOZZI, ANNA WANG ROE, EMILIANO SANTARNECCHI

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Coordinated activity across networks of neurons is a hallmark of both resting and active behavioral states in many species. These global patterns alter energy metabolism in the brain, making oxygen consumption and glucose uptake widely used proxies of neural activity. However, how changes in neural activity are causally related to changes in metabolic flux in intact circuits on sub-second timescales is unclear. We combine two-photon microscopy across the fruit fly brain with sensors that allow simultaneous measurements of neural activity and metabolic flux, across both resting and active behavioral states. We demonstrate that neural activity drives changes in metabolic flux, creating a tight coupling between these signals that can be measured across large-scale brain networks. Further, using local optogenetic perturbation, we demonstrate that even transient increases in neural activity result in rapid and persistent increases in cytosolic ATP, suggesting that neuronal metabolism predictively allocates resources to meet the energy demands of future neural activity. These studies reveal that the initiation of even minimal behavioral movements causes large-scale changes in the pattern of neural activity and energy metabolism, revealing unexpectedly widespread engagement of the brain. Moreover, the availability of the fruit fly connectome allows direct comparisons of functional measurements with anatomical constraints, revealing the extent to which specific functional networks can be predicted. As the relationships between neural activity, energy metabolism and anatomy are likely evolutionarily ancient, these studies provide a critical foundation for using metabolic proxies and connectomes to predict changes in neural activity.

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PRESENTATION NUMBER: S127

DECONSTRUCTING FUNCTIONAL CONNECTIVITY IN THE MOUSE WITH CHEMOGENETIC-FMRI

SYMPOSIUM 33 - PERTURBATION-BASED NEUROCONNECTOMICS: PRINCIPLES AND APPLICATIONS ACROSS SPECIES - THOMAS CLANDININ, ALESSANDRO GOZZI, ANNA WANG ROE, EMILIANO SANTARNECCHI

Alessandro Gozzi
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Resting-state fMRI (rsfMRI) has been widely used to map brain network organization in healthy and pathological states. However, the neural underpinnings and dynamic rules governing brain-wide rsfMRI coupling remain unclear. Filling this knowledge gap is of crucial importance, given our current inability to decipher and relate clinical signatures of aberrant functional connectivity to interpretable neurophysiological events that can help understand or diagnose brain disorders. Towards this goal, my laboratory has recently combined chemogenetics, rsfMRI and *in vivo* electrophysiology to investigate how excitatory and inhibitory perturbations of brain activity causally affect whole-brain patterns of functional connectivity in the mouse. In my talk, will summarize some key results from this recent line of inquiry. Specifically, I will highlight how our approach can be used to identify the fundamental elements of rsfMRI coupling, challenging long-held assumptions regarding the relationship between rsfMRI connectivity and its underlying axonal determinants. I will also illustrate how disease-related signatures such as increased excitatory/inhibitory imbalance can generate clinically-relevant network signatures of dysconnectivity. These examples outline a novel research platform that is poised to shed light on the basis and determinants of macroscale rsfMRI coupling, offering opportunities to physiologically decipher signatures of atypical connectivity in human brain disorders.

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PRESENTATION NUMBER: S128

INFRARED STIMULATION OF THE MESOSCALE PRIMATE NEURO-CONNECTOME

SYMPOSIUM 33 - PERTURBATION-BASED NEUROCONNECTOMICS: PRINCIPLES AND APPLICATIONS ACROSS SPECIES - THOMAS CLANDININ, ALESSANDRO GOZZI, ANNA WANG ROE, EMILIANO SANTARNECCHI

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Connectome projects worldwide have contributed enormously to our understanding of network-based brain function. However, little is known about brain connectivity at the scale of fundamental submillimeter functional domains that characterize human and nonhuman primate brains. This mesoscale understanding is critical as primate brains are functionally organized at this scale. Here, we present a novel method for studying connectivity in the macaque monkey. By combining focal (~0.5mm) near infrared neural stimulation at single brain sites and ultrahigh-field 7T fMRI (INS-fMRI), we map evoked activations at connected sites thereby providing maps of a single-site based mesoscale networks. Data collection is rapid, whole brain scale, and *in vivo*. This novel method uniquely enables comparison of many mesoscale networks within a single individual. Our data show that single site stimulation leads to activation of focal mesoscale nodes in multiple areas across the brain, including sensory, motor, cognitive, and limbic sites. Activated nodes are functionally specific, surprisingly sparse, and topographically organized (reflecting shifting connectivities). We suggest that each mesoscale brainwide network underlies a highly specific multi-dimensional behavior. These findings lead to a conceptual change in our understanding of the organization of behavioral networks in the brain. We are using this approach to develop a functional mesoscale connectome of the primate brain.

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PRESENTATION NUMBER: S129

AXON-DENDRITE POLARITY ESTABLISHMENT IN C. ELEGANS, A MICROTUBULE BALANCING ACT

SYMPOSIUM 34 - CYTOSKELETON DYNAMICS IN NEURONAL DEVELOPMENT - MARTIN HARTERINK, CORALIE FASSIER, ANA RITA COSTA, MONIKA LEISCHNER-BRILL

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Neurons are highly polarized cells that extend axons and dendrites to mediate information flow through the nervous system. The microtubule cytoskeleton is crucial to form these specialized cell extensions. It provides structural support and enables for cargo transport through the neuron. Whereas microtubules are organized plus-end out in axons, dendrites are characterized by minus-end out microtubues. This difference in organization between axons and dendrites allows for selective cargo transport into either neurite to establish neuronal polarity. However, the mechanisms that organize and maintain the neuronal microtubule cytoskeleton are still poorly understood. To study this, we used the in vivo model *C. elegans* in combination with advanced microscopy techniques. We found that during neuron development the establishment of this microtubule organization relies on local microtubule nucleation in dendrites in combination with stabilization by the microtubule minus-end binding CAMSAP protein. In addition, we identified and characterized the Ankyrin/UNC119/CRMP complex that connects the microtubule cytoskeleton to the cortex, to maintain this microtubule organization. In the absence of these mechanisms, neuronal polarity is lost leading to defects in neuron morphology and animal locomotion.

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PRESENTATION NUMBER: S130

LINKING CYTOSKELETON REMODELING TO GUIDANCE SIGNALS: FIDGETIN-LIKE 1 AS AN INTEGRATOR FOR AXON NAVIGATION AT THE MIDLINE

SYMPOSIUM 34 - CYTOSKELETON DYNAMICS IN NEURONAL DEVELOPMENT - MARTIN HARTERINK, CORALIE FASSIER, ANA RITA COSTA, MONIKA LEISCHNER-BRILL

Samya Zerkoune[1], Fiona Roche[2], Camille Cuveillier[3], Yvrick Zagar[2], Juliette Vougny[1], Gaëlle Botton-Amiot[1], Christian Delphin[3], Isabelle Arnal[3], Annie Andrieux[3], Xavier Nicol[2], Coralie Fassier[1]
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The mature nervous system is an intricate network in which the precise connectivity between neurons is critical for the optimal functioning of the system. Neuronal circuit wiring critically relies on the accurate navigation of developing axons towards their appropriate targets. Aberrant connections during development can lead to major neurological disorders. While the repertoire of guidance cues that wire the nervous system is mostly known, our understanding of the intracellular mechanisms that regulate axon responsiveness to these guidance signals is far from being complete. Notably, how these extracellular chemical signals are integrated and translated into cytoskeleton-driven growth cone mechanical behaviours remains largely unknown. We recently identified the microtubule-associated ATPase Fidgetin-like 1 (Fignl1) as a key player in zebrafish motor circuit wiring and larval locomotion. We showed that Fignl1 controls growth cone morphology and steering behaviours at guidance choice points through its versatile regulation of microtubule plus-end dynamics and bidirectional axonal transport. Using a combination of biological systems and cutting-edge imaging techniques, we showed that Fignl1 is also required for zebrafish retinal axon pathfinding at the optic chiasm and identified novel molecular links between this microtubule-depolymerizing enzyme, repulsive guidance pathways and the actin cytoskeleton. Altogether, these preliminary results suggest that Fignl1 acts as a key integrator of repulsive guidance signals coordinating microtubule/actin remodelling to steer developing axons.

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PRESENTATION NUMBER: S131

THE BIOGENESIS AND FUNCTION OF THE AXONAL MEMBRANE PERIODIC SKELETON (MPS)

SYMPOSIUM 34 - CYTOSKELETON DYNAMICS IN NEURONAL DEVELOPMENT - MARTIN HARTERINK, CORALIE FASSIER, ANA RITA COSTA, MONIKA LEISCHNER-BRILL

Ana Rita Costa[1], Sérgio Leite[1], Marko Lampe[2], Monica Sousa[1]
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Neurons present a unique arrangement of the axonal cytoskeleton unveiled by super-resolution microscopy, consisting of periodically distributed actin rings interconnected by spectrin tetramers - the membrane periodic skeleton (MPS). The MPS was initially considered to mechanically support the axons. Our previous data contributed to better understanding the role of the MPS in the regulation of axonal diameter by showing that it is an actomyosin network. Since there is still a lack of understanding regarding the biology of this structure, it is our aim to fully comprehend its biogenesis and function. Currently, actin nucleation and arrangement within the actin rings, as well as how non-muscle myosin II engages with actin rings to provide for axonal expansion and contraction, remains to be fully understood. To explore the role of different actin nucleators in the formation, maintenance and function of MPS actin rings, we are using complementary drugs and genetic approaches, together with single molecule localization microscopy. Our current data indicate that Arp2/3, that is involved in the nucleation of branched actin filaments, is essential for the initial formation but not for the maintenance of the MPS. Conversely, formins that promote the polymerization of linear actin filaments are essential for both the formation and maintenance of the MPS. The emerging picture is that a complementary action of actin nucleators is needed to provide for the timely MPS assembly, as is the case of actin rings that exist in other biological contexts.

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PRESENTATION NUMBER: S132

MICROTUBULE POLYGLUTAMYLATION DRIVES MOTOR AXON REMODELING

SYMPOSIUM 34 - CYTOSKELETON DYNAMICS IN NEURONAL DEVELOPMENT - MARTIN HARTERINK, CORALIE FASSIER, ANA RITA COSTA, MONIKA LEISCHNER-BRILL

Antoneta Gavoci[1], Mengzhe Wang[1], Maria Magiera[2], Carsten Janke[2], Thomas Misgeld[1], Monika Leischner-Brill[1]
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Microtubules are dynamic cytoskeletal structures build from tubulin dimers, which can carry a range of post-translational modifications (PTMs). Together with associated proteins, post-translational modifications establish a ‘code’, endowing the microtubule scaffoldmwith specific and local functionality, such as regulating microtubule length andmdynamics and microtubule-dependent transport. However, how this codemtranslates into shaping cell- or tissue-level events, remains largely unexplored. We investigate how polyglutamylation, a post-translational modification enriched on neuronal microtubules, regulates postnatal motor axon remodelling: a regressive process in which ~90% of terminal branches prune, involving spastin-mediated loss of microtubules. While spastin is known to preferentially sever polyglutamylated microtubules, it remains elusive whether branch-specific polyglutamylation drives pruning.
Consistent with an instructive role of polyglutamylation, motoneurons ablated of deglutamylases CCP1&6 accelerate axon dismantling, while deletion of chainelongating glutamylase TTLL1, delayed axonal remodelling. Further measurements of polyglutamylation, microtubule mass and dynamics corroborate the predicted branch-specific regulation of microtubule stability, hinting at a rheostatic regulation of spastin-mediated severing and hence axonal remodelling. Surprisingly, deleting TTLL7, which ‘seeds’ the first glutamate residue, had no effect on polyglutamylation, suggesting several layers of regulation that are engrained in parallel or consecutive steps of editing the tubulin code. Together, a specific tubulin post-translational modification—polyglutamylation—acts as an instructive signal for spastin-mediated severing, which in turn paces developmental axon pruning. The ‘tubulin code’ could thus control specific morphogenetic events during nervous system development. Future work tries to unravel if synaptic activity coordinates glutamylases and deglutamylases and whether similar mechanisms determine axon stability in central neurons and during disease-related remodelling.

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PRESENTATION NUMBER: S133

AUTOIMMUNE ENCEPHALITIS WITH ANTIBODIES AGAINST NMDA, AMPA OR KAINATE RECEPTORS: DISTINCT MECHANISMS AND PHENOTYPES

SYMPOSIUM 35 - AUTOANTIBODIES IN BRAIN PATHOPHYSIOLOGY - JOSEP DALMAU, ANA LUISA CARVALHO, LONNIE WOLLMUTH, LAURENT GROC

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The autoimmune encephalitides associated with antibodies against glutamate receptors (NMDAr, AMPAr, or GluK2) form part of a new category of diseases in which symptoms are antibody-mediated. Whereas anti-NMDAr (GluN1) encephalitis manifests with predominant psychiatric symptoms accompanied by severe neurological alterations (seizures, dyskinesias, coma, hypoventilation), anti-AMPAr (Glu2A>GluA1) encephalitis predominantly manifests with limbic dysfunction (memory deficits, seizures), and anti-GluK2 encephalitis associates with cerebellar dysfunction. For each of these diseases the pathogenicity of the antibody has been demonstrated in cultured neurons or animal models of passive antibody transfer or active immunization. For all 3 diseases, a fundamental pathogenic effect of the antibodies is the specific binding and internalization of the corresponding targets and the disruption of the surface interaction with synaptic partners (e.g., NMDAr with ephrinB2 or dopamine 1 receptor; AMPAr withTARP) resulting in alterations in synaptic transmission and plasticity. These alterations, which are antibody specific, associate with symptoms and downstream effects similar to those of diseases in which the same targets are affected by other mechanisms (e.g., schizophrenia and hypofunction of NMDAr). In some instances, synaptic scaling-like mechanisms (e.g., enhanced incorporation of inwardly rectifying non-GluA2 containing AMPArs) likely contribute to some of the symptoms. The reversibility of the behavioral and synaptic alterations caused by patients' antibodies is in line with the substantial improvement of patients after immunotherapy. Recent work focused on a better understanding of the underlying mechanisms may lead to treatment strategies that enhance or accelerate this improvement (e.g., positive allosteric modulation of NMDAr in anti-NMDAr encephalitis).

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PRESENTATION NUMBER: S134

DISRUPTED AMPA RECEPTOR FUNCTION UPON AUTOANTIBODY-MEDIATED LOSS OF CASPR2

SYMPOSIUM 35 - AUTOANTIBODIES IN BRAIN PATHOPHYSIOLOGY - JOSEP DALMAU, ANA LUISA CARVALHO, LONNIE WOLLMUTH, LAURENT GROC

Ana Luisa Carvalho[1], Dominique Fernandes[1], Ana Vasconcelos[1], Ester Coutinho[1,2,3], Sarosh Irani[4], Bo Sun[4], Sofija Paneva[4], Jessica Whitt[5], Hey-Kyoung Lee[5], Sandra Santos[1]
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Contactin-associated protein 2 (CASPR2) is a cell adhesion molecule that is expressed both in the peripheral and central nervous system. Autoantibodies targeting surface epitopes of CASPR2 have been associated with neuromyotonia and autoimmune synaptic encephalitis, and patients with anti-CASPR2 autoantibodies (CASPR2-Abs) present a wide clinical spectrum ranging from acquired peripheral nerve hyperexcitability to CNS dysfunction, including cognitive impairments, memory loss and seizures. Notwithstanding the well-established pathogenic potential of CASPR2-Abs, the mechanisms triggered by antibody-mediated perturbations in CASPR2 function are still largely explored. Given the recently-described role for CASPR2 in the regulation of glutamate AMPA receptors (AMPAR) and cortical excitatory synaptic transmission, we explored whether patient CASPR2-Abs disrupt excitatory synapse function. We found that purified IgGs from patients with anti-CASPR2 synaptic encephalitis lead to dose-dependent changes in the dendritic and synaptic expression of Caspr2, and impact AMPAR trafficking by affecting receptor internalization and cell surface diffusion. Both IgGs purified from patients as well as patient-derived monoclonal antibodies targeting different domains in CASPR2 decrease the amplitude of AMPAR-mediated miniature excitatory postsynaptic currents. Moreover, CASPR2-Abs interfere with the synaptic incorporation of AMPARs following chemically-induced long-term potentiation. Finally, we found that excitatory synaptic transmission in the mouse visual cortex is perturbed following *in vivo* incubation with CASPR2-Abs. Collectively, our data show that CASPR2-Abs perturb the function of CASPR2 in regulating AMPAR trafficking and synaptic plasticity, pointing to the glutamatergic system as a target for pathogenesis in CASPR2 synaptic encephalitis.

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PRESENTATION NUMBER: S135

AUTOANTIBODIES TARGETING THE NMDA RECEPTOR IN LUPUS

SYMPOSIUM 35 - AUTOANTIBODIES IN BRAIN PATHOPHYSIOLOGY - JOSEP DALMAU, ANA LUISA CARVALHO, LONNIE WOLLMUTH, LAURENT GROC

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Systemic lupus erythematosus (SLE) or Lupus is an autoimmune disorder characterized by numerous pathologies including brain dysfunction. SLE patients suffer from diverse neuropsychiatric symptoms, ranging from the subtle (spatial memory deficits) to the severe (epilepsy, depression, & psychosis). A subset of SLE autoantibodies, referred to as DNRAbs, cross-react with DNA and the NMDA receptor (NMDAR), a glutamate-gated ion channel central to brain function. In patients, DNRAbs are associated with brain dysfunction, and in murine models with cell death, microglia activation, dendritic remodeling, and brain dysfunction. NMDARs are composed of two obligate GluN1 and typically two GluN2 subunits. DNRAbs bind to a pentapeptide amino acid sequence DWEYS within the GluN2A and GluN2B subunits. We find that DNRAbs act as positive allosteric modulators (PAMs), enhancing glutamate-induced channel opening. This action occurs preferentially at GluN2A-containing NMDARs, even NMDARs containing a single copy of GluN2A. Given this gain-of-function action, GluN2A-specific antagonists provide greater protection from DNRAb-mediated neuronal cell death than GluN2B antagonists. We also find, using transgenic mice to perturb expression of either GluN2A or GluN2B *in vivo*, that GluN2A mediates all DNRAb-induced pathologies. Hence, the PAM action is a key factor driving brain dysfunction. Notably, we find that the extent of this PAM action varies between DNRAbs derived from different SLE patients. We propose that clonal variations in DNRAbs may be a key factor leading to the diversity of clinical symptoms in SLE patients expressing DNRAbs.

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PRESENTATION NUMBER: S136

MOLECULAR INTERPLAY BETWEEN AUTOANTIBODIES AND GLUTAMATE RECEPTOR AT THE PLASMA MEMBRANE

SYMPOSIUM 35 - AUTOANTIBODIES IN BRAIN PATHOPHYSIOLOGY - JOSEP DALMAU, ANA LUISA CARVALHO, LONNIE WOLLMUTH, LAURENT GROC

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The identification of autoimmune encephalitis in which patients express autoantibodies directed against neurotransmitter receptors has generated great hope to shed new light on the molecular mechanisms underpinning neurological and psychiatric conditions. Among these autoimmune encephalitis, the discovery of autoantibodies directed against the glutamatergic NMDA receptor (NMDAR-Ab), in the anti-NMDAR encephalitis, has provided some key information on how clinical symptoms can be caused by altered NMDAR signalling. Here, I will discuss recent advances on the molecular action of NMDAR-Ab on their targets and associated receptors at the single molecule level, unveiling unsuspected effects of such an autoantibody on the functional cross-talk between neurotransmitter systems.

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PRESENTATION NUMBER: S137

NEURAL CIRCUIT MECHANISMS FOR NAVIGATING TO SHELTER DURING INSTINCTIVE ESCAPE

SYMPOSIUM 36 - NEURAL CIRCUITS FOR CONTEXT-DEPENDENT DEFENSIVE BEHAVIORS IN MICE AND FLIES - TIAGO BRANCO, TIHANA JOVANIC, PHILIP TOVOTE, CLARA FERREIRA

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When faced with predatorial threats the most adaptive action is to escape towards a shelter that offers long term protection against the attacker. Achieving this requires knowing where shelter is and using this knowledge to execute shelter-directed escape actions. Here we have investigated how information about shelter location is encoded in the mouse brain, and how this information is transmitted to neural circuits that control escape execution. We find that neurons in the retrosplenial cortex (RSP) and the lateral superior colliculus (lSC) specifically encode the angular distance to shelter in body-centered coordinates - the key variable for orienting and running along a direct vector to the shelter. Neurons in the RSP and lSC are monosynaptically connected and when this synapse is inactivated mice cannot find the shelter - escape is initiated, but the flight action is towards random places in the arena. In constrast, inactivating this synaptic connection does not impair sensory-guided orientation or navigation-to-reward tasks, indicating that the RSP to lSC pathway is specifically recruited during urgent navigation to safety. Recordings from cell type- and projection-specific neural dynamics combined with theoretical modelling sugest that the RSP maps shelter direction onto the lSC network using a centre-surround inhibition mechanism. These results identify a new circuit mechanism for representing instinctive goals and executing goal-directed actions during defensive behavior.

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PRESENTATION NUMBER: S138

STATE-DEPENDENT MODULATION OF ESCAPE DECISIONS

SYMPOSIUM 36 - NEURAL CIRCUITS FOR CONTEXT-DEPENDENT DEFENSIVE BEHAVIORS IN MICE AND FLIES - TIAGO BRANCO, TIHANA JOVANIC, PHILIP TOVOTE, CLARA FERREIRA

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In face of an aversive stimulus animals need to decide whether to avoid and how to avoid them. In addition, they constantly need to balance their decisions to avoid dangers and threats with their drive to pursue food search. These decisions are modulated by animal's internal state and animals lacking nutrient were shown to be more likely to ignore aversive stimuli. However, how feeding states affect esacpe decisions and the neural circuit mechanisms underlying their state-dependent modulation are not well known. In order to adress this question we take advantage of *Drosophila* larva as a model system and combine automated behavioral detection, neuronal manipulation, functional imaging and electron microscopy reconstruction of synaptic connectivity We have previously described a circuit that controls the decision between startle and escape-like behavior following a mechanical stimulus. We hypothesized that integration of other information about the feeding state, might occur through long-range projections that connect to this circuit. We characterize how feeding state affect behavioral responses following a mechanical stimulus. Then, we describe how the activity of the already known neuronal substrates of decision making are differentially modulated by animal's state using 2-photon Calcium imaging in intact larvae. Understanding how the neural circuit mechanisms underlying escape decisions of whether and how these decisions can be modulated by the internal state in *Drosophila* larva where we can study neural circuit mechanisms with single cell and synaptic resolution across the nervous system, might shed light on principles underlying this process across species

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INTEGRATED DEFENSE STATES AND THEIR NEURONAL SUBSTRATES IN THE PERIAQUEDUCTAL GREY

SYMPOSIUM 36 - NEURAL CIRCUITS FOR CONTEXT-DEPENDENT DEFENSIVE BEHAVIORS IN MICE AND FLIES - TIAGO BRANCO, TIHANA JOVANIC, PHILIP TOVOTE, CLARA FERREIRA

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Fear and anxiety are brain states that evolved to mediate defensive responses to threat. Brainstem neuronal circuits including the periaqueductal grey (PAG) play major roles in mediating behavioural and autonomic components of defensive states, yet their integrative nature is poorly understood. In particular, threat has been associated with various cardiac changes, but a clear consensus on their relevance for the integrated defense reaction is missing. This hinders our understanding of the neuronal underpinnings of dynamic defensive states. Novel analyses of concomitant behavioural, HR and thermal measures during various behavioural paradigms in freely moving mice, enable us to define transient microstates, including the notorious fear-induced freezing response and its interaction with longer-lasting macrostates. Applying integrated cardio-behavioural analyses to optogenetic perturbational approaches enable assignment of particular ‘state generator’ roles to individual neural circuit elements within the PAG. Our novel framework also guides descriptive calcium-imaging approaches that reveal PAG neuronal correlates for cardiac interoception under threat conditions. This talk will introduce characterization of integrated cardio-behavioural defensive states as the basis for a comprehensive understanding of complex neuronal mechanisms underlying aversive emotions such as fear and anxiety.

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BEHAVIOURAL AND NEURONAL BASIS OF SAFETY IN NUMBERS

SYMPOSIUM 36 - NEURAL CIRCUITS FOR CONTEXT-DEPENDENT DEFENSIVE BEHAVIORS IN MICE AND FLIES - TIAGO BRANCO, TIHANA JOVANIC, PHILIP TOVOTE, CLARA FERREIRA

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A major benefit of being in a group is the possibility of adding social information to directly-perceived information about the environment to guide behaviour. Across the animal kingdom, social information acquired via specific signals or cues animals produce as they engage in their daily activities are used for decisions in reproduction, foraging and protection against predation. Acute fitness benefits of the usage of this information are flagrant in the context of a response to a potential threat: failure to detect a predator can lead to an animal's immediate demise whereas needless engagement in metabolically costly defence responses can, unnecessarily, negatively impact survival. We previously showed that Drosophila melanogaster display a graded decrease in freezing behaviour, triggered by an inescapable visual threat, with increasing group sizes. Crucially we identified that the movement of others is used as a cue of threat and safety, and that group responses are primarily guided by a safety in numbers effect. We have now further manipulated group composition, titrating the levels of the motion cues of safety, and uncovered that flies show chronometric (freezing bout length) and psychometric (probability of exiting freezing) curves similar to those observed in learned-choice decision-making tasks. Interestingly, simultaneously manipulating group composition and threat imminence, revealed that the social cue of safety has a preponderant role, overriding differences in perceived threat levels. These findings set the stage for uncovering the circuitry underlying the integration of motion cues from others, predators and conspecifics, in the deployment of responses to threat in groups.

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PRESENTATION NUMBER: S141

CIRCUITS AND THE ENGRAMS FOR SYSTEMS CONSOLIDATION OF MEMORY

SYMPOSIUM 37 - ENGRAMS, CIRCUITS AND BIOCHEMICAL REORGANIZATION FOR REMOTE MEMORY CONSOLIDATION - TAKASHI KITAMURA, GISELLA VETERE, MICHEL VAN DEN OEVER, LAURA DENARDO

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Episodic memories initially require rapid synaptic plasticity within the hippocampus for their formation and are gradually consolidated in neocortical networks for permanent storage. However, the engrams and circuits that support neocortical memory consolidation have thus far been unknown.In my talk, I will present that neocortical prefrontal memory engram cells, which are critical for remote contextual fear memory, are rapidly generated during initial learning through inputs from both the hippocampal–entorhinal cortex network and the basolateral amygdala by using contextual fear conditioning as well as observational contextual fear conditioning. After their generation, the prefrontal engram cells, with support from hippocampal memory engram cells, become functionally mature with time. Whereas hippocampal engram cells gradually become silent with time, engram cells in the basolateral amygdala, which are necessary for fear memory, were maintained. Our study provides new insights into the functional reorganization of engrams and circuits underlying systems consolidation of memory.

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THALAMIC INVOLVEMENT IN THE STABILIZATION OF LONG LASTING MEMORIES

SYMPOSIUM 37 - ENGRAMS, CIRCUITS AND BIOCHEMICAL REORGANIZATION FOR REMOTE MEMORY CONSOLIDATION - TAKASHI KITAMURA, GISELLA VETERE, MICHEL VAN DEN OEVER, LAURA DENARDO

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ESPCI, Brain Plasticity Unit, Paris, France

Understanding how memories are created and maintained over time is a fundamental problem in neuroscience. In this talk, I will explore how neural networks of co-active brain regions support memory recall. Within such networks, highly connected hub regions are assumed to disproportionately influence behavioural output. I will focus on the role of 2 thalamic regions, the anterodorsal and laterodorsal thalamus as key regions for fear memory stabilization and I will explore their contribution within the hippocampal-prefrontal cortex network. Ca^{2+} imaging and optogenetic manipulations suggest that the switch between recent to remote memory state requires a different engagement of these thalamic regions through direct control of the hippocampal and cortical ensembles. Finally, the role of head direction cells, present in both anterodorsal and laterodorsal thalamic nuclei, will be identified in representing spatial information as well as in processing emotional long-lasting memories.

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PRESENTATION NUMBER: S143

CORTICAL ENGRAM CELLS DEVELOP TIME- AND MEMORY STRENGTH-DEPENDENT NEUROADAPTATIONS

SYMPOSIUM 37 - ENGRAMS, CIRCUITS AND BIOCHEMICAL REORGANIZATION FOR REMOTE MEMORY CONSOLIDATION - TAKASHI KITAMURA, GISELLA VETERE, MICHEL VAN DEN OEVER, LAURA DENARDO

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Memory storage and retrieval is thought to depend on the consolidation of physical changes by sparsely distributed neurons that are highly activated during an experience, so-called engram cells. Recent data by us and others demonstrate that retrieval of remote (e.g. 1-month-old) contextual fear memory requires time-dependent maturation of engram cells in the medial prefrontal cortex (mPFC). Moreover, we found that the engagement of these neurons depends on memory strength, meaning that learning-activated mPFC neurons are required for remote memory expression after mild, but not strong, contextual fear conditioning (CFC). Based on this, we hypothesized that CFC-activated mPFC neurons undergo a time-dependent maturation process as a function of the intensity of conditioning. To investigate this, we made use of the TRAP2 mouse line to tag and identify mPFC neurons that are activated (*Fos*-expressing) during mild or strong CFC, or during context exposure only (no foot-shock). We compared changes in intrinsic excitability, synaptic and structural properties of tagged versus non-tagged mPFC neurons at a recent (≤ 1 week) and remote (≥ 4 weeks) timepoint after conditioning. Our findings demonstrate that following both mild and strong CFC, tagged mPFC neurons progressively develop adaptations in synaptic properties, however, these are most pronounced after mild CFC. Temporal changes in the structural properties of tagged mPFC neurons developed selectively after mild CFC. These observations support our hypothesis that time- and memory strength-dependent adaptations mirror the engagement of cortical engram cells in remote memory expression.

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THE ORGANIZATION OF CORTICO-CORTICAL CIRCUITS UNDERLYING REMOTE MEMORY RETRIEVAL

SYMPOSIUM 37 - ENGRAMS, CIRCUITS AND BIOCHEMICAL REORGANIZATION FOR REMOTE MEMORY CONSOLIDATION - TAKASHI KITAMURA, GISELLA VETERE, MICHEL VAN DEN OEVER, LAURA DENARDO

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Memories of emotional experiences can last a lifetime, guiding behavior from minutes to years after learning. In the clinic, remote memories of trauma are more treatment resistant than recent ones, linking memory evolution with memory resilience and highlighting the urgent need to understand the neurobiology of remote memory. As memories age, they are reorganized into a distributed cortical network, yet our understanding of this network at the cellular and synaptic level is incomplete. Activity in the prelimbic cortex (PL), part of the dorsomedial prefrontal cortex, is essential for both recent and remote fear memory recall, but accumulating evidence indicates that distinct PL output circuits control memory recall over time. In recent studies, we found that PL neurons involved in remote cued fear memory preferentially activate high order auditory association areas. Of these, the temporal association area (TeA), a major target of PL projections, is specifically required for remote memory recall. Yet the role of PL>TeA projections in memory recall and consolidation is unexplored. Using circuit-specific optogenetics and calcium imaging in freely behaving mice, we are examining the time-dependent functions of the PL-TeA connection. Our ongoing work will uncover the organizing principles and behavioral functions of key cortical networks underlying remote fear memories and can build foundations for new clinical interventions for trauma-based disorders.

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PRESENTATION NUMBER: S145

BINGE DRINKING DURING ADOLESCENCE: MICROBIOME, IMMUNE AND COGNITIVE ALTERATIONS

SYMPOSIUM 38 - UNRAVELLING THE ROLE OF THE GUT MICROBIOME IN ADDICTION - CARINA CARBIA, BENJAMIN BOUTREL, LORENZO LEGGIO, NATHALIE DELZENNE

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Binge drinking (BD) is defined as the consumption of six or more standard drinks in one session. This pattern of consumption is highly prevalent during adolescence, which is considered to last up to 25 years of age. This developmental period involves ongoing neuromaturation that results in greater vulnerability to disruptive events in the brain such as excessive alcohol consumption. BD has been associated with both neuroanatomical impairments and neuropsychological deficits. Accumulating evidence indicates that chronic alcohol consumption induces inflammation, both from a direct interaction with the brain and the periphery, particularly from the gut. Recently, chronic alcoholism has been linked with increased intestinal permeability and alteration of microbial profile that correlated with craving levels. However, no study to date has investigated the gut microbiota in young people with a repeated pattern of alcohol intoxication. The aim of this study is to investigate the potential link between alcohol-induced altered microbial profile, neurocognitive functioning and craving in healthy young BDs. Additionally, blood inflammatory markers will be measured. We will characterize both composition and functionality of the gut microbiome. The Cambridge Neuropsychological Test Automated Battery (CANTAB) will be used to assess neurocognitive performance. We will propose and discuss the results within a neuro-immuno-affective framework to integrate recent evidence on how central and peripheral alterations might affect the still developing young brain.

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PRESENTATION NUMBER: S146

FECAL MICROBIOTA TRANSFER REVERSES EXCESSIVE ALCOHOL SEEKING IN STRESSED RATS

SYMPOSIUM 38 - UNRAVELLING THE ROLE OF THE GUT MICROBIOME IN ADDICTION - CARINA CARBIA, BENJAMIN BOUTREL, LORENZO LEGGIO, NATHALIE DELZENNE

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Although only a small proportion of individuals develop persistent alcohol used disorder, unhealthy alcohol use represents a significant public health concern, accounting for 4.5% of global disease burden. Identifying biological markers predicting vulnerability to develop excessive alcohol consumption may lead to real improvement of prevention strategies and possibly clinical care.
Considering that gut microbiota is capable of influencing immunity, brain and behavior, we investigated gut microbiome and signs of peripheral inflammation in rats exhibiting uncontrolled alcohol seeking behaviors. We monitored 1) the inability to abstain during a signaled period of reward unavailability, 2) the increased motivation assessed in a progressive effortful task and 3) the persistent alcohol seeking despite aversive foot shocks (Jadhav et al., 2017, 2018). After a long-training for alcohol (10%weight/volume) self-administration, all rats were screened according to the 3 criteria defined above and were identified as resilient versus vulnerable to uncontrolled alcohol seeking. All rats were then given access to 2 sources of reward: 0,1mL 10% w/v ethanol and 0.1mL saccharine (0.2 %, 0.00625%, 0%), 2 consecutive sessions for each concentration, during which vulnerable rats exhibited a clear-cut preference for alcohol compared to controls. Strikingly, we identified signs of peripheral inflammation in vulnerable rats, and not only fecal microbiota transfer lowered vulnerable rats' motivation and preference for alcohol but it restored inflammation modulators levels to those observed in controls. In the near future, manipulation of the gut microbiome may represent an original strategy to alleviate the overwhelming urge for compulsive alcohol drinking in human patients.

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PRESENTATION NUMBER: S147

GUT MICROBIOME AND HARMFUL ALCOHOL DRINKING: FINDINGS FROM NON-HUMAN PRIMATE AND HUMAN STUDIES

SYMPOSIUM 38 - UNRAVELLING THE ROLE OF THE GUT MICROBIOME IN ADDICTION - CARINA CARBIA, BENJAMIN BOUTREL, LORENZO LEGGIO, NATHALIE DELZENNE

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Rodent studies report that chronic alcohol use leads to a loss in the diversity of the fecal microbial community, and in changes in the relative abundance of microbial taxa. Human studies also show an association between gut dysbiosis and chronic alcohol use. Non-human primate models provide a degree of experimental control that is not possible in humans, while, compared to rodents, are evolutionarily closer to humans. We conducted a study in a unique baboon model of chronic excessive alcohol drinking (Papio Anubis; N=14 males). Baboons were studied in controlled conditions, with consistent and reliable alcohol self-administration and blood alcohol levels, controlled food/caloric intake, and identical housing and exposure to stimuli. We compared three groups: L = Long-term alcohol drinking (12.1 years), S = Short-term alcohol drinking (2.7 years), and C = Control group, drinking an isocaloric, non-alcoholic reinforcer (8.2 years). We found that fecal microbial α- and β-diversity were significantly lower in the L group, vs. the other two groups. Members of the commensal bacterial families Lachnospiraceae and Prevotellaceae showed a relative decrease, whereas the opportunistic pathogen Streptococcus genus showed a relative increase, in the L group, vs. the other two groups. Microbiota-related metabolites of aromatic amino acids, tricarboxylic acid cycle, and pentose increased in the L group, vs. the other two groups, suggesting high energy metabolism and enhanced glycolysis in the gut lumen in response to alcohol. We are currently analyzing the results of a human gut microbiome and metabolome study.

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THE ROLE OF THE GUT MICROBIOTA IN FOOD AND ALCOHOL ADDICTION

SYMPOSIUM 38 - UNRAVELLING THE ROLE OF THE GUT MICROBIOME IN ADDICTION - CARINA CARBIA, BENJAMIN BOUTREL, LORENZO LEGGIO, NATHALIE DELZENNE

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Our recent data unravelled how the gut microbiome can participate to metabolic and behavioural disorders in obesity and alcohol disorders. In a cohort of obese individuals supplemented with prebiotic dietary fibers (DF), that modulate the gut microbiota, the presence of specific bacteria prior the nutritional intervention influenced both metabolic and behavioral outcomes. The potential relevance of nutritional disorders and intervention have been evaluated in alcohol use disorders (AUD). We have previously shown that depressive and social behavioural alterations of AUD patients was associated with gut microbial dysbiosis (changes in gut microbiota composition and activity). Dysbiotic patients had a lower sociability score and a smaller/less connected social network. Data obtained in mice transferred with the gut microbiome from AUD patients allowed to show that gut microbiota from AUD may drive inflammatory processes and hepatic metabolism, leading to decreased ketogenesis that may affect brain function. More recently, we found that among nutritional disorders that characterize AUD patients, DF intake, which is associated with anxiety and sociability, was lower in AUD than in healthy subjects. However, the supplementation with inulin as prebiotic DF versus placebo during a 3-weeks period of alcohol detoxification program, had only minor effect on behavioral and biological alterations as compared to alcohol withdrawal itself. Those data, obtained on a limited number of patients, could be extended to larger cohorts in order to unravel the multiple systems biology involved in behaviour related to beverage and food addiction, that remains very difficult to treat and has a tremendous consequence on health.

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PRESENTATION NUMBER: S149

HOW ATTENTION AND NEUROMODULATORS CONTROL SYNAPTIC PLASTICITY IN DEEP CORTICAL NETWORKS

SYMPOSIUM 39 - REVISITING SENSORY CORTICES: IMPLICATIONS FOR DIVERSE COGNITIVE PROCESSING - PIETER ROELFSEMA, NATHALIE ROCHEFORT, ALEXANDRA LIBBY, ABHISHEK BANERJEE

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Humans and many other animals have an enormous capacity to learn about sensory stimuli and to master new skills. However, many of the mechanisms that enable us to learn remain to be understood. One of the greatest challenges of systems neuroscience is to explain how synaptic connections change to support maximally adaptive behaviour. I will provide an overview of factors that determine the change in the strength of synapses, with a focus on synaptic plasticity in sensory cortices. I will highlight the influence of neuromodulators and feedback connections in synaptic plasticity and suggest a specific framework in which these factors can interact to improve the functioning of the entire network.

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PRESENTATION NUMBER: S150

HOW DOES FOOD AVAILABILITY IMPACT ENERGY USAGE AND CODING PRECISION IN VISUAL CORTEX?

SYMPOSIUM 39 - REVISITING SENSORY CORTICES: IMPLICATIONS FOR DIVERSE COGNITIVE PROCESSING - PIETER ROELFSEMA, NATHALIE ROCHEFORT, ALEXANDRA LIBBY, ABHISHEK BANERJEE

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Information processing is energetically expensive. In the mammalian brain, it is unclear how information coding and energy use are regulated during food scarcity. Using whole-cell recordings and two-photon imaging in layer 2/3 mouse visual cortex, we found that food restriction reduced AMPA receptor conductance, reducing synaptic ATP use by 29%. Neuronal excitability was nonetheless preserved by a compensatory increase in input resistance and a depolarized resting potential. Consequently, neurons spiked at similar rates as controls but spent less ATP on underlying excitatory currents. This energy-saving strategy had a cost because it amplified the variability of visually-evoked subthreshold responses, leading to a 32% broadening of orientation tuning and impaired fine visual discrimination. This reduction in coding precision was associated with reduced levels of the fat mass-regulated hormone leptin and was restored by exogenous leptin supplementation. Our findings reveal that metabolic state dynamically regulates the energy spent on coding precision in neocortex.

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PRESENTATION NUMBER: S151

ROTATIONAL DYNAMICS REDUCE INTERFERENCE BETWEEN SENSORY AND MEMORY REPRESENTATIONS

SYMPOSIUM 39 - REVISITING SENSORY CORTICES: IMPLICATIONS FOR DIVERSE COGNITIVE PROCESSING - PIETER ROELFSEMA, NATHALIE ROCHEFORT, ALEXANDRA LIBBY, ABHISHEK BANERJEE

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Behavior relies on the ability to make predictions. To form predictions, the brain must associate sensory information with the memory of recent stimuli. Because information is distributed within populations of neurons, interference can occur between these sensory and memory representations. To study the neural dynamics that underlie sensory processing, memory maintenance, and the formation of predictions, we recorded from the auditory cortex of mice, while they experienced sequences with statistical regularities. Exposure to predictable sequences aligned the sensory codes in auditory cortex, such that the first sensory event triggered the representation of later expected events. However, because predictions were embedded in the sensory codes, they could not be used as reliable memory readouts; unexpected trials resulted in overwriting of short-term memories. Interestingly, the memories were not lost; sensory information rotated over time into an orthogonal representation, where memories were accurately maintained. Multiple mechanisms can support this kind of orthogonal neural code. One mechanism simply keeps the sensory and memory representations in two separate populations of neurons. Alternatively, both sensory and memory information could be represented orthogonally in a single neural population. Our results support the latter hypothesis; we found the sensory representation rotated into an orthogonal dimension within the same population, protecting the memory from interference when new stimuli were presented. This rotation was supported by a combination of 'stable' and 'switching' neurons, which either maintained or inverted their selectivity over time. Theoretical modeling showed that such structured rotational dynamics are efficient, compared against alternative mechanisms for generating orthogonal representations.

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PRESENTATION NUMBER: S152

BUILDING A FLEXIBLE SENSORY CORTEX

SYMPOSIUM 39 - REVISITING SENSORY CORTICES: IMPLICATIONS FOR DIVERSE COGNITIVE PROCESSING - PIETER ROELFSEMA, NATHALIE ROCHEFORT, ALEXANDRA LIBBY, ABHISHEK BANERJEE

Abhishek Banerjee
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Animals adapt their behaviour in response to variable changes in reward reinforcement. The prefrontal areas of the mammalian neocortex, especially the orbitofrontal cortex (OFC), play an important role in invoking rule-based strategies to enable flexible learning. However, the neural circuit mechanisms in OFC and its interactions with different hierarchical cortical areas underlying such processes remain elusive. In my talk, I will discuss interactions between orbitofrontal and somatosensory cortices that implement flexible decision-making in a tactile reversal-learning task in mice. The talk will shed light on the circuit mechanisms underlying behavioural flexibility, indicating a crucial role of mouse OFC neurons in encoding predictive ‘teaching signals’ that drive adaptive changes in sensory cortices and in behaviour.

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PRESENTATION NUMBER: S153

SPERM SMALL RNA-MEDIATED INTERGENERATIONAL INHERITANCE OF PATERNAL DIETARY EFFECTS

SYMPOSIUM 40 - TRANSGENERATIONAL EPIGENETIC INHERITANCE: IS THERE A CONNECTION BETWEEN THE LEARNED AND THE INNATE? - UPASNA SHARMA, KEVIN MITCHELL, ISABELLE MANSUY, ODED RECHAVI

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Although there is mounting evidence from worms to humans suggesting that parental environment can influence phenotypes in offspring, the mechanism of such transgenerational inheritance remains deeply mysterious. Male mice fed a low protein diet sire offspring with altered lipid and cholesterol biosynthesis. We examined the mechanistic basis of epigenetic inheritance of paternal dietary effects. We show that protein restriction affects small RNA levels in mature sperm, including specific tRNA-derived small RNAs or tRNA fragments (tRFs). Specific tRFs can regulate early embryonic gene expression. These studies suggest that sperm small RNAs potentially mediate epigenetic inheritance of paternal dietary effects. Intriguingly, our studies revealed surprising dynamics of small RNA biogenesis during sperm maturation in the epididymis. We find that a subset of sperm small RNAs are synthesized in the somatic epididymis epithelial cells and shipped to sperm via extracellular vesicles. These studies revealed a role for soma-to-germline trafficking in shaping the RNA payload of mammalian sperm. Current studies are focused on elucidating the mechanism of this RNA-mediated soma-germline communication and its consequences on offspring development.

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PRESENTATION NUMBER: S154

THE TROUBLE WITH EPIGENETICS

SYMPOSIUM 40 - TRANSGENERATIONAL EPIGENETIC INHERITANCE: IS THERE A CONNECTION BETWEEN THE LEARNED AND THE INNATE? - UPASNA SHARMA, KEVIN MITCHELL, ISABELLE MANSUY, ODED RECHAVI

Kevin Mitchell
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The concept of epigenetics - that gene expression can be modified by experience and that this provides a mechanism of cellular memory - has entered the mainstream of public consciousness. Despite a lack of clarity on usage of the term, it seems to offer a way to escape the perceived threat of genetic determinism, a means of rewriting our own psychological traits through experience. Moreover, the idea that the effects of such experiences might be transmitted across generations has truly gripped the public imagination, with studies of transgenerational psychological effects of famine or trauma – even the Holocaust – making headlines in newspapers across the world. There is strong evidence for transgenerational epigenetic inheritance in plants and simple animals like nematodes, with an understandable rationale for why such phenomena would exist and well worked out mechanisms. Neither the evidence nor the rationale is as convincing in mammals, however. This talk will explore methodological issues in seminal studies in rodents and humans, and examine the principles of genotype-phenotype relations and behavioural adaptation and flexibility in larger, more complex organisms. In particular, it will examine the idea that epigenetic mechanisms can provide a generational bridge between learning and instinct.

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PRESENTATION NUMBER: S155

NEUROEPIGENETICS: HOW CHILDHOOD TRAUMA IS PASSED TO DESCENDANTS VIA THE GERM LINE

SYMPOSIUM 40 - TRANSGENERATIONAL EPIGENETIC INHERITANCE: IS THERE A CONNECTION BETWEEN THE LEARNED AND THE INNATE? - UPASNA SHARMA, KEVIN MITCHELL, ISABELLE MANSUY, ODED RECHAVI

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Behavior and physiology in mammals are strongly influenced by the environment and life experiences, particularly in childhood. While positive factors can favor proper development and good mental and physical health later in life, childhood adversity and traumatic events increase the risk for cancer and psychiatric, metabolic and autoimmune diseases. Such disorders can affect exposed individuals directly and in some cases, impact the offspring across generations. The biological mechanisms underlying transmission are thought to involve epigenetic factors. We developed a transgenerational mouse model of postnatal stress that recapitulates trauma symptoms including increased risk-taking, depressive behaviors, cognitive and social deficits, and metabolic and cardiovascular dysregulation in adulthood. The symptoms persist throughout life and are transmitted to the offspring, in some cases, up to the 5th generation. Comparable symptoms affect traumatized children, indicating conserved effects in mouse and human. Symptoms are associated with molecular changes involving RNA in germ cells, that in mice, are causally linked to symptoms transmission. MiRNAs are also affected in extracellular vesicles in the reproductive tract. Circulating factors were identified as mediators of alterations in germ cells. Chronic injection of serum from trauma-exposed mouse males into controls recapitulates metabolic phenotypes in the offspring, suggesting information transfer from serum to germ cells. Circulating factors involving peroxisome proliferator-activated receptor (PPAR) pathways are causally involved, and pharmacological PPAR activation *in vivo* affects sperm transcriptome and metabolic functions in the offspring and grand-offspring. These results suggest an ensemble of mechanisms from the periphery to germ cells for the inheritance of acquired traits.

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PRESENTATION NUMBER: S156

CHALLENGING THE BASIC DOGMAS OF EVOLUTION: HERITABLE SMALL RNA

SYMPOSIUM 40 - TRANSGENERATIONAL EPIGENETIC INHERITANCE: IS THERE A CONNECTION BETWEEN THE LEARNED AND THE INNATE? - UPASNA SHARMA, KEVIN MITCHELL, ISABELLE MANSUY, ODED RECHAVI

Oded Rechavi
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In *C. elegans* nematodes, small RNAs enable transmission of epigenetic responses across multiple generations, independently of changes to the DNA sequence. Different environmental challenges, including exposure to viruses, starvation, and heat stress generate heritable small RNA responses, that in certain cases could be adaptive. Recently we have shown that neuronal activity can also produce small RNA-mediate heritable responses, and that the decisions that the progeny makes are affected by whether their ancestors' experienced stress or not. Our results indicate that specific processing of small RNAs in the parents leads to marked differences in the physiology of the progeny. I will discuss the underlying mechanisms, and the potential of small RNA inheritance to affect the worm’s fate and perhaps even evolution.

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PRESENTATION NUMBER: S157

STRUCTURAL AND FUNCTIONAL ANNOTATION OF THE MOUSE PREFRONTAL CORTEX

SYMPOSIUM 41 - DECODING THE PREFRONTAL CIRCUITS OF COGNITIVE FLEXIBILITY - MARIE CARLEN, KATHLEEN CHO, JULIO MARTINEZ, DANIEL DURSTEWITZ

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Karolinska Institutet, Neuroscience, Stockholm, Sweden

The structure and function of the prefrontal cortex (PFC) across species remain unresolved. Taking advantage of the technological toolbox available to studies in rodents, our studies aim to outline the mouse PFC through integration of structural, molecular, and functional characteristics. Whole-brain tracing of connectivity and spatial transcriptomics give a novel and unbiased view of the structure of the mouse PFC. The connectivity tracing outlines the mouse PFC as a module with dense intraconnectivity, questioning independent processing in discrete prefrontal subregions. In line with this, higher cognitive functions are considered to be integrative rather than localized. Non-localized functions of the PFC can only be revealed by studies focusing on the PFC as a whole, and high-density Neuropixels recordings have opened up for massive and concurrent sampling of neuronal activities across the subregions of the PFC. I will in my talk present our current work focused on large-scale recording of neuronal activities across the PFC subregions and what our investigations reveal about functional hierarchy and local versus non-local functions in prefrontal subterritories.

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PRESENTATION NUMBER: S158

PREFRONTAL PARVALBUMIN INTERNEURON FUNCTION IN COGNITIVE CONTROL

SYMPOSIUM 41 - DECODING THE PREFRONTAL CIRCUITS OF COGNITIVE FLEXIBILITY - MARIE CARLEN, KATHLEEN CHO, JULIO MARTINEZ, DANIEL DURSTEWITZ

Kathleen Cho
Paris Brain Institute (ICM), INSERM, -, Paris, France

Changes in patterns of activity within the medial prefrontal cortex enable rodents, non-human primates, and humans to update their behavior to adapt to changes in the environment, e.g., during cognitive tasks. Within medial prefrontal cortex, inhibitory neurons expressing parvalbumin are important for updating strategies in a rule shift task. Nevertheless, causal mechanisms through which parvalbumin neurons recruit specific circuits to produce prefrontal network dynamics that switch from maintaining to updating task-related patterns of activity remain unknown. Here, I will discuss the role of gamma synchrony in cognitive flexibility, specifically cross-hemispheric gamma synchrony of prefrontal parvalbumin interneurons, and how long-range GABAergic projections support and affect synchrony in prefrontal cortex-dependent cognitive tasks.

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PRESENTATION NUMBER: S159

MENTAL REPRESENTATIONS FOR COGNITIVE CONTROL IN THE MACAQUE LATERAL PREFRONTAL CORTEX

SYMPOSIUM 41 - DECODING THE PREFRONTAL CIRCUITS OF COGNITIVE FLEXIBILITY - MARIE CARLEN, KATHLEEN CHO, JULIO MARTINEZ, DANIEL DURSTEWITZ

Julio Martinez-Trujillo
Western University, Schulich School of Medicine and Dentistry, Physiology, Pharmacology And Psychiatry, London, Canada

Humans and other anthropoid primates can represent and manipulate complex information in the mind. A brain area that has been deemed critical to such cognitive ability is the lateral prefrontal cortex. We study the neural substrates of mental representations and learning in the lateral prefrontal cortex of non-human primates using naturalistic virtual reality associative learning and working memory tasks. During the tasks the animals are required to maintain and manipulate information about spatial and no spatial features of objects and their associations while navigating through virtual environments using a joystick. We used multielectrode arrays and pharmacological manipulations to record the activity of neurons and neuronal populations in the lateral prefrontal cortex (areas 9/46 of Brodmann). We found that single neurons and populations encode complex combinations of space and features (e.g., context-stimulus response associations) while animals navigate through the virtual environments. When the task contingencies change (e.g., introduction of new associations), neuronal populations become tuned for the new contingencies over a few trials reflecting changes in the animals' behavioral performance. Tuning was robust to changes in visual scenes produced by exploratory saccades during navigation but was disrupted by administration of the NMDA antagonist Ketamine. We conclude that the lateral prefrontal cortex flexibly encodes complex mental representations and their associations during naturalistic behavior providing a neural substrate for primates' remarkable adaptability to changes in the environment.

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PRESENTATION NUMBER: S160

USING RECURRENT NEURAL NETWORKS FOR RESOLVING THE COMPUTATIONAL DYNAMICS UNDERLYING MULTIPLE SINGLE-UNIT RECORDINGS FROM PREFRONTAL CORTEX

SYMPOSIUM 41 - DECODING THE PREFRONTAL CIRCUITS OF COGNITIVE FLEXIBILITY - MARIE CARLEN, KATHLEEN CHO, JULIO MARTINEZ, DANIEL DURSTEWITZ

Daniel Durstewitz
Central Institute of Mental Health (CIMH), Theoretical Neuroscience, Mannheim, Germany

Recurrent Neural Networks (RNNs) are deep learning tools for processing and predicting time series data. They can also be used to identify, or reconstruct, the nonlinear dynamical system that underlies observed data, thus providing mechanistic insight into the system dynamics. In fact, RNNs are known to be computationally and dynamically universal, i.e., in theory they can represent and emulate any other computational or dynamical system, like the brain. By directly training RNNs on neural recordings and behavioral observations we can therefore formally examine the computational processes behind these experimental observations, thus linking cellular ensembles and behavior to computation. In my talk, I will first discuss recent advances in RNN models and training algorithms that were designed for the specific purpose of dynamical systems reconstruction, including the identification of limit sets like attractors and their geometrical or topological structure. Such techniques enable not only out-of-sample, but also out-of-distribution generalization to novel experimental settings. I will particularly focus on methodological approaches for identifying dynamical systems from multiple data modalities simultaneously. This enables, for instance, to link simultaneously recorded neurophysiological and behavioral observations to the same underlying latent dynamical process model, and thereby to elucidate their functional relationships. It will be illustrated on multiple-single-unit recordings from the rodent prefrontal cortex and behavioral responses during rule learning tasks how these techniques can be applied to faithfully reconstruct the underlying system with its observed physiological and behavioral properties, to decipher prefrontal computations, to detect cell assemblies, and to quantify inter-regional information transfer.

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PRESENTATION NUMBER: S161

CE-PUNCTIN SETS RECEPTOR CONTENT AT EXCITATORY AND INHIBITORY SYNAPSES BY DUAL CONTROL OF EXTRA- AND INTRACELLULAR SCAFFOLDS

SYMPOSIUM 42 - SPECIFYING NEURONAL CONNECTIVITY ACROSS SPECIES - JEAN-LUIS BESSEREAU, THOMAS BIEDERER, MICHELA MATTEOLI, CAMIN DEAN

Jean-Louis Bessereau
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The molecular mechanisms involved in the organization and regulation of chemical synapses are central questions in neuroscience. Apart from synaptic adhesion molecules that engage trans-synaptic interactions in the synaptic cleft, some secreted proteins behave as 'synaptic scaffolders', *i. e.* proteins that interact with pre- or postsynaptic components within the synaptic cleft and organize the synapse without necessarily triggering a signal transduction cascade. Some extracellular scaffolders can position neurotransmitter receptors at the synapse by interacting with receptor-associated scaffolds in the synaptic cleft. Others bridge presynaptic adhesion molecules with the extracellular domains of neurotransmitter receptors or with adhesion receptors. Ce-Punctin/MADD-4 was initially identified in the nematode *C. elegans* as an extracellular matrix protein secreted by neuronal boutons and controlling the excitatory vs inhibitory identity of postsynaptic domains (*Nature*, 2014, 511, 466-70). Its vertebrate ortholog, ADAMTSL3/Punctin-2, is expressed in the central nervous system but its function has not been characterized so far. Specific combinations of Ce-Punctin isoforms are expressed by cholinergic and GABAergic motoneurons and trigger the clustering of acetylcholine- or GABA-gated ion channels at *C. elegans* excitatory and inhibitory neuromuscular junctions, respectively. This involves the postsynaptic positioning of transmembrane scaffolding molecules and the activation of the netrin receptor DCC. In turn, these proteins recruit intracellular scaffolding molecules that interact with the receptors. In addition, we recently discovered that Ce-Punctin is processed into discrete proteolytic fragments with distinct localization and function. These results shed light on novel mechanisms controlling the molecular organization of chemical synapses, with potential implications for neuropsychiatric diseases.

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PRESENTATION NUMBER: S162

CONCERTED ROLES OF SYNAPTIC ADHESION MOLECULES IN ORGANIZING PREFRONTAL CORTEX CONNECTIVITY AND COGNITIVE FUNCTIONS

SYMPOSIUM 42 - SPECIFYING NEURONAL CONNECTIVITY ACROSS SPECIES - JEAN-LUIS BESSEREAU, THOMAS BIEDERER, MICHELA MATTEOLI, CAMIN DEAN

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Trans-synaptic adhesion complexes organize synapse development. Their molecular diversity offers a profound cooperative potential to guide neuronal connectivity. Yet, it is only known for a few of these proteins whether they act in concert. We investigated the prefrontal cortex, a region impacted in multiple brain disorders, and analyzed the postsynaptic adhesion molecule LRRTM1 that is associated with schizophrenia and forms a complex with presynaptic Neurexins. Biochemical studies determined that loss of LRRTM1 elevates the synaptic amounts of the immunoglobulin adhesion molecule SynCAM 1, which promotes excitatory synapse number similar to LRRTM1 but does not bind it. To abrogate possible redundancy, we generated double knock-out mice lacking LRRTM1 and SynCAM 1. Their combined KO reduced the number of dendritic spines more than the sum of single KO losses and impacted presynaptic sites, making Neurexin puncta less spatially restricted. On a functional level, we used in vivo recordings and fMRI to determine that LRRTM1 and SynCAM 1 jointly control neuronal firing in the prefrontal cortex and synchronized brain activity. Moreover, behavioral tasks involving the prefrontal cortex were impacted in double but not single KO mice. We obtained no evidence in the hippocampus for a synaptic cooperation of these molecules. Our results provide evidence that LRRTM1 and SynCAM 1 act together in the prefrontal cortex to organize synapses and together modulate neuronal firing and cognition-relevant functions. These findings support that molecularly distinct trans-synaptic complexes can cooperate in a brain region-selective manner.

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PRESENTATION NUMBER: S163

ROLE OF MICROGLIAL TREM2 IN SYNAPSE ELIMINATION AND CIRCUIT FORMATION

SYMPOSIUM 42 - SPECIFYING NEURONAL CONNECTIVITY ACROSS SPECIES - JEAN-LUIS BESSEREAU, THOMAS BIEDERER, MICHELA MATTEOLI, CAMIN DEAN

Raffaella Morini[1], Alessandra Folci[2], Matteo Bizzotto[3], Fabio Perrucci[3], Erica Tagliatti[3], Michela Matteoli[1,2]
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Triggering Receptor Expressed on Myeloid cells 2 (Trem2), is an immunoglobulin superfamily transmembrane receptor, expressed exclusively by microglia in the brain. Trem2 engages with membrane-bound or soluble ligands and interacts with the adaptor proteins DAP10 and DAP12, which recruit the intracellular signal transduction machineries. The known ligands of Trem2 comprise a large spectrum of anionic molecules, such as phospholipids, lipoproteins, DNA, and bacterial products. Further, membrane-bound Trem2 can be cleaved into its soluble form by the α-secretases ADAM10 and ADAM17, leading to the release into the extracellular environment of soluble Trem2 (sTrem2), which maintains its biological activity. We have found that Trem2 controls the process of synapse elimination during development. When this process is defective due to the lack of the trem2 gene, altered functional brain connectivity and defective behavioral phenotype emerge in the adult (Filipello, Morini et al., Immunity 2018). We also showed that externalized phosphatidylserine (PS) represents a neuronal “eat-me” signal recognized by Trem2 (Scott-Hewitt, Perrucci et al., EMBO J 2020). Using a fusion protein between the extracellular domain of Trem2 and the IgG Fc fragment, immobilized onto a solid support, we identified Trem2 ligands from brain synaptosomes. We are in the process of characterizing these receptors and defining whether they can be bound also by sTrem2. These data will shed a light on the mechanisms which control glia-mediated circuit sculpting.

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PRESENTATION NUMBER: S164

REGULATION OF SYNAPSE CONNECTIVITY AND STABILITY

SYMPOSIUM 42 - SPECIFYING NEURONAL CONNECTIVITY ACROSS SPECIES - JEAN-LUIS BESSEREAU, THOMAS BIEDERER, MICHELA MATTEOLI, CAMIN DEAN

Camin Dean
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Synapses form, strengthen, weaken, and dissociate not only for proper development of the nervous system, but also to modulate circuit function. Synaptic dynamics are especially important during learning, remembering, and forgetting, for example, since memories are encoded by adjusting strength of specific synapses. The impairment of these dynamics can lead to neurodegenerative and psychiatric diseases. To follow synapse formation and dissociation from the initial point of contact between a pre and postsynaptic neuron, we are using GRASP (GFP Reconstitution Across Synaptic Partners), initially developed for circuit mapping. GRASP consists of two non-fluorescent parts of GFP, each fused extracellularly to a pre or postsynaptic membrane-targeted molecule. When a cell expressing the presynaptic split GFP forms a synapse with a cell expressing the postsynaptic split GFP, the GFP parts become close enough to reconstitute and produce a fluorescent signal indicating contact. Using this technique, we can detect synapse formation and dissociation in primary neuronal cultures transduced with pre and postsynaptic GRASPs by time-lapse imaging. In addition, synapse stability can be assessed by fluctuations in fluorescence over time. Fluorescence intensity, and magnitude of fluctuations, is increased or decreased by compounds that strengthen or weaken synapses to affect learning or forgetting. In addition, GRASP expression does not affect neuronal health and synaptogenesis, making it a powerful tool to investigate synaptic dynamics. We aim to apply this technique to human induced pluripotent stem cell (hIPSC)-derived neuronal networks to assay synapse formation, stability, and dissociation upon addition of compounds that influence synapse strength and memory.

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PRESENTATION NUMBER: S165

PROCESSING OF FEEDFORWARD AND FEEDBACK SIGNALS IN MOUSE VISUAL THALAMUS

SYMPOSIUM 43 - MORE THAN A RELAY: NEURONAL CIRCUIT DYNAMICS IN THE VISUAL THALAMUS ACROSS INTERNAL STATES - LAURA BUSSE, JUDITH HIRSCH, MARK ANDERMANN, SANTIAGO ROMPANI

Laura Busse
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Traditionally, the dorsolateral geniculate nucleus (dLGN) of the thalamus has been considered a feedforward relay station for retinal signals to reach primary visual cortex. The local and long-range circuits of dLGN, however, have long suggested that this view is not correct. Indeed, besides the thalamo-cortical relay cells, dLGN contains local inhibitory interneurons, and receives not only feedforward input from the retina, but also anatomically massive direct and indirect feedback from primary visual cortex. Furthermore, it is one of the earliest processing stages in the visual system that integrates visual information with neuromodulatory signals.

In my talk, I will present recent findings from the mouse visual system regarding the impact of corticothalamic (CT) feedback on visual processing of synthetic and naturalistic visual stimuli. Here, I will focus on the role and circuits of CT feedback in modulating contextual spatial integration. In addition, I will consider the impact of behavioral state on dLGN activity across several temporal scales. Together, the results will highlight that dLGN is more than just a simple relay, offering opportunities for feedforward convergence of retinal inputs and being modulated by feedback and behavioral state.

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COMPARATIVE ANALYSIS OF INHIBITORY CIRCUITS IN THE VISUAL THALAMUS

SYMPOSIUM 43 - MORE THAN A RELAY: NEURONAL CIRCUIT DYNAMICS IN THE VISUAL THALAMUS ACROSS INTERNAL STATES - LAURA BUSSE, JUDITH HIRSCH, MARK ANDERMANN, SANTIAGO ROMPANI

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The sensory thalamus was once viewed as a relay, serving to communicate information from the periphery to cortex during waking and halting this downstream flow during sleep. It has become increasing clear, however, that thalamus actively integrates sensory information and, most recently, that inhibitory circuits are central to this process. Indeed, inhibitory cells dominate intrinsic circuits in thalamus. Specifically, thalamocortical cells make few local connections but are embedded in two dense inhibitory networks. First, local interneurons supply feedforward inhibition; second, neurons in the adjacent thalamic reticular nucleus provide feedback inhibition. Our work focuses on visual components of the sensory thalamus, including the dorsal lateral geniculate nucleus and the visual compartment of the thalamic reticular nucleus. We use anatomical approaches to explore the structure of inhibitory networks in concert with with physiological, optogenetic, and computational tools to learn how intrathalamic inhibition might influence vision. Moreover, we compare results across species to resolve evolutionarily conserved aspects of thalamic circuitry and function.

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STATE-DEPENDENT CONVERGENCE AND TRANSMISSION OF RETINAL INFORMATION CHANNELS IN THALAMUS

SYMPOSIUM 43 - MORE THAN A RELAY: NEURONAL CIRCUIT DYNAMICS IN THE VISUAL THALAMUS ACROSS INTERNAL STATES - LAURA BUSSE, JUDITH HIRSCH, MARK ANDERMANN, SANTIAGO ROMPANI

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Throughout the brain, the processing of sensory information is modulated to guide adaptive behaviors. Here, we investigate how serotonergic axons may specifically gate visual inputs at the level of retinal ganglion cell (RGC) axon terminals, prior to further integration and processing of sensory information in the thalamus. Bulk recordings of RGC axons showed that baseline and visually evoked calcium activity and glutamate release were suppressed by serotonin axon stimulation. Two-photon calcium imaging revealed that retinal axons preferring fullfield changes in luminance were more suppressed than those driven selectively by spatially localized stimuli, even when accounting for differences in each axon's baseline activity. Slice electrophysiology confirmed that serotonergic axon-evoked suppression of retinal glutamate release depends on presynaptic 5-HT1B receptors. Using single-cell sequencing, whole-mount electrophysiology and immunohistochemistry, we demonstrate differences in 5-HT1B receptor gene expression and axonal protein expression between RGCs that prefer fullfield changes in luminance vs. local stimuli. These data suggest a mechanism by which serotonin axons selectively gate specific visual information channels.

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INTEGRATION AND MODULATION OF VISUAL INFORMATION IN THE THALAMUS

SYMPOSIUM 43 - MORE THAN A RELAY: NEURONAL CIRCUIT DYNAMICS IN THE VISUAL THALAMUS ACROSS INTERNAL STATES - LAURA BUSSE, JUDITH HIRSCH, MARK ANDERMANN, SANTIAGO ROMPANI

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Image-forming visual information is transmitted from the retina to the lateral geniculate nucleus (LGN) of the thalamus, which is the principal driver of the primary visual cortex and thus conscious vision. The retina encodes the visual scene an organism sees into over 40 different features, such as motion in a particular direction or sharp edges. Previously, it was thought that these simple features were relayed by the LGN to the cortex without substantial combination or modulation. In this seminar, I will talk about recent work using single-cell initiated transsynaptic rabies in the mouse showing that LGN cells receive far more types of retinal input than was previously appreciated. Furthermore, I will present the more recent efforts from our lab in understanding the functional and structural convergence of different visual features in the thalamus using a combination of *in vivo* calcium imaging, optogenetics, and 3D electron microscopy, among other tools. Altogether, we hope to elucidate not only the early circuits of vision, but the mechanisms whereby simple neuronal features are combined to form complex representations.

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SOCIAL DECISION-MAKING IN FORAGING CONTEXTS

SYMPOSIUM 44 - SOCIAL COGNITION… BEYOND SOCIAL INTERACTIONS - CRISTINA MARQUEZ, CHRISTIAN KEYSERS, EWELINA KNAPSKA, FRANCESCO PAPALEO

Cristina Marquez
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Prosocial behaviours are actions that benefit others. They are thought to be evolutionary conserved across different mammal species however, the behavioural and neural mechanisms that explain this type of actions are yet poorly understood. In this talk we will focus on how animals perceive rewarding states from others and incorporate these into social decision-making. Using novel behavioural paradigms that allow for deep analysis of social behaviour, calcium imaging, closed-loop optogenetic experiments and computational modelling, we will explore how social hierarchy and the perception of the well-being of others guide the decision to help or not to help others.

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FROM EMOTIONAL CONTAGION TO DECISION MAKING IN THE RODENT CINGULATE CORTEX

SYMPOSIUM 44 - SOCIAL COGNITION… BEYOND SOCIAL INTERACTIONS - CRISTINA MARQUEZ, CHRISTIAN KEYSERS, EWELINA KNAPSKA, FRANCESCO PAPALEO

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In humans, the mid/anterior cingulate cortex (Brodmann Area 24) is one of the most consistently activated in neuroimaging studies while witnessing the distress of others. Many have speculated that this region plays a causal role in empathy and, because it is also recruited during pain experience, that it does so by mapping the pain of others onto the witness' own via mirror neurons. Testing these speculations in humans is difficult, and we therefore developed rat models. Recording from cingulate area 24 in rats, we showed that it indeed contains neurons that respond both while the animal experiences pain and witnessing others receive footshocks, demonstrating the presence of pain mirror neurons. Rats typically freeze while witnessing a conspecific receive footshocks, deactivating area 24 dramatically reduces this sign of emotional contagion and establishes the causal role of this region in emotional contagion. To assess whether it also contributes to social harm aversion, we developed a paradigm in which a rat can choose between two levers delivering food, one of which also delivers a footshock to an adjacent conspecific. Rats were found to avoid using this shock lever, even if the alternative lever provided less food or required more effort, confirming the presence of a prosocial motivation to avoid harming others. Deactivating area 24 abolished that harm aversion. Together, these rodent paradigms therefore allow us to gain insights into the cellular and causal mechanisms linking cingulate area 24 to emotional contagion and harm aversion.

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SOCIAL LEARNING ABOUT REWARDS AND THREATS – HOW INFORMATION FROM OTHERS HELPS TO ADAPT TO CHANGING ENVIRONMENT.

SYMPOSIUM 44 - SOCIAL COGNITION… BEYOND SOCIAL INTERACTIONS - CRISTINA MARQUEZ, CHRISTIAN KEYSERS, EWELINA KNAPSKA, FRANCESCO PAPALEO

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In social species, emotions displayed by others influence the cognition and behavior of the interacting individuals. The capacity to be affected by or share emotional states is observed both in humans and rodents. It is believed that this capacity facilitates building relationships because it fosters emotional synchrony between individuals. However, the utility of sharing emotion surpasses a purely social function. Since to survive, an animal must continuously learn about challenges and opportunities in its environment, the emotions of other individuals can also be a source of valuable information. Thus, our rodent studies focus on the neuronal circuits underlying socially perceived emotions and their role in adaptation to the environment. In particular, we show that social cues convey information about the imminence of threat and that socially triggered responses recruit different neuronal circuits in the central amygdala. Further, we show that rodents socially transfer information about a distant food source through direct interaction with an individual who encountered the food reward or with a scent thereof. We also show that socially acquired knowledge modifies the exploration patterns of familiar and novel environments. Thereby, perceiving affective states of others evoked by a threat or a reward helps the individual adapt its behavior and thus avoid harm and maximize rewards. Perceiving others' emotions carries informational value, which offers a new perspective on the evolutionary origins of socially shared emotions.

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PRESENTATION NUMBER: S172

CIRCUITS OF EMOTION DISCRIMINATION

SYMPOSIUM 44 - SOCIAL COGNITION… BEYOND SOCIAL INTERACTIONS - CRISTINA MARQUEZ, CHRISTIAN KEYSERS, EWELINA KNAPSKA, FRANCESCO PAPALEO

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Emotion recognition and consequent social reaction are important for a wealthy animal life. These socio-cognitive processes are supposed to rely on evolutionary conserved long-range brain networks, regulated by an inhibitory/excitatory balance. We found that inhibitory somatostatin (SOM) neurons within the medial prefrontal cortex (mPFC) are a fundamental biological substrate of emotion discrimination. However, it is unclear how cell-specific long-range circuits might process socially derived information for reliable emotion recognition. Here, we show the specific involvement of atypical long-range SOM projections from the mPFC to the retrosplenial cortex (RSC), and an excitatory feedback loop from the RSC to mPFC in emotion discrimination. Using human imaging and rodent anatomical tracing, we highlight the involvement of the mPFC-RSC network in emotion recognition and the existence of a subpopulation of SOM GABAergic neurons projecting from the mPFC to the RSC. Our findings demonstrate a specific cortico-cortical inhibitory/excitatory circuit subtending emotion recognition.

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PRESENTATION NUMBER: S173

EARLY PATTERNS OF ACTIVITY IN CEREBRAL CORTEX OF NEONATAL RATS

SYMPOSIUM 45 - THE NEWBORN BRAIN: UNRAVELING THE INTERPLAY BETWEEN EARLY ACTIVITY AND NETWORK DEVELOPMENT - ROUSTEM KHAZIPOV, CHARLIE DEMENé, REBECCAH SLATER, PETRA HüPPI

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Development of thalamocortical sensory maps during the neonatal period in rodents is characterized by particular intermittent network activity patterns, which are implicated in the activity-dependent formation of the topographic thalamocortical circuits. Several developmental rules determine cortical functions during these stages. Firstly, thalamocortical maps develop from a crude protomap and refine through the competition between sensory inputs for the cortical territories. Temporal binding of thalamic and cortical neurons by virtue of the early thalamocortical oscillations is instrumental for the enforcement and stabilization of the topographic synapses and elimination of the non-topographic synapses. Secondly, early operation of the immature cortex is fundamentally reflexological and it is largely driven by the thalamic input. Intracortical circuits at these stages are yet poorly developed to support internally organized activities. The ability of cortical networks to generate internally organized activities and a transition to the adult-like mode of cortical function emerges only with acquisition of sustained neuronal firing patterns by cortical neurons and the formation of the intracortical excitatory and inhibitory circuits. Thirdly, natural stimulus to the thalamocortical circuits is provided by spontaneous activity at the sensory periphery (retinal waves in visual system, cochlear bursts in auditory system and sensory feedback from twitches in somatosensory system). Thus, both rodents possess the internal mechanisms driving activity within the developing sensory systems independently on external inputs.

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PRESENTATION NUMBER: S174

DYNAMIC, BEDSIDE ASSESSMENT OF NEONATAL BRAIN CONNECTIVITY USING FUNCTIONAL ULTRASOUND IMAGING

SYMPOSIUM 45 - THE NEWBORN BRAIN: UNRAVELING THE INTERPLAY BETWEEN EARLY ACTIVITY AND NETWORK DEVELOPMENT - ROUSTEM KHAZIPOV, CHARLIE DEMENé, REBECCAH SLATER, PETRA HüPPI

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Neonatologists have long been interested in functional brain monitoring, as reversible functional losses often precedes the observable irreversible structural insults. By characterizing neonatal functional cerebral networks, resting-state functional connectivity is envisioned to provide early markers of cognitive impairments. Here we present a pioneering deep brain resting-state functional connectivity imaging on human neonates using functional ultrasound (fUS). fUS leverages in-depth cerebral blood volume high sensitivity, beside portability, 250µm spatial resolution and <1s temporal resolution, making it unique in the landscape of neonatal brain imaging. Using a micro-motorized and miniaturized probe facing the anterior fontanel, it enables to acquire 3D images of the neonate's brain that can be registered with an MRI neonate atlas. Signal correlations between cerebral regions revealed interhemispheric connectivity at an early stage in very preterm newborns. Furthermore, fUS high spatial resolution enabled to build fine-grain homotopic connectivity maps, based on correlations between mirror pixels, which revealed underlying structures, such as the white/grey matter boundary in the cortex. Finally, resting-state connectivity could be assessed dynamically showing a significant occurrence decrease of thalamo-cortical networks for very preterm neonates (N=6) as compared to control term newborns (N=4), a subtle difference that would have staid concealed with a more classical static connectivity analysis. The same method also showed abnormal patterns in a congenital seizure disorder case compared with the control group. We hope fUS can help to quickly identify and quantify atypical connectivity patterns at bedside and to study the emergence of functional networks in the early days of life.

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PRESENTATION NUMBER: S175

NEONATAL PAIN PERCEPTION: BEHAVIORAL AND BRAIN EXPLORATIONS BY ELECTROENCEPHALOGRAPHY AND MAGNETIC RESONANCE IMAGING

SYMPOSIUM 45 - THE NEWBORN BRAIN: UNRAVELING THE INTERPLAY BETWEEN EARLY ACTIVITY AND NETWORK DEVELOPMENT - ROUSTEM KHAZIPOV, CHARLIE DEMENé, REBECCAH SLATER, PETRA HüPPI

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The human brain has a central role in pain perception, and individual differences in brain structure and function underpin differences in pain sensitivity and pain tolerance. Humans display wide variation in their response to painful events, and even infants of a few days old differ dramatically in their evoked pain-related behavioural, physiological and cerebral activity. In adults, the emergence of pain experience is considered to arise when a set of brain networks, that are not individually unique to pain, become active together, and there is a high degree of correspondence between brain activity patterns observed at rest and patterns of activity evoked by salient events or tasks. Although the newborn infant brain is highly immature, it has many functional properties that are similar to that observed in adults. A key question is how does the structural and functional organisation of the infant brain give rise to the individual variability that is observed between infants during pain experience? I will describe a series of studies conducted in newborn infants that will show how resting-state brain activity recorded in the first few days of life can be used to predict individual differences in pain-related cerebral activity evoked by a controlled experimental noxious stimuli. I will also discuss underlying differences in white matter microstructural complexity that can explain between-subject differences in pain-related brain activity and discuss how responses to noxious stimulation mature during early development. This work provides insight into the structural and functional differences that underpin normal variability in response to noxious input in newborn infants.

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PRESENTATION NUMBER: S176

THE INFLUENCE OF PRETERM BIRTH AND EARLY ENVIRONMENT ON STRUCTURAL AND FUNCTIONAL BRAIN DEVELOPMENT EXPLORED BY MAGNETIC RESONANCE IMAGING

SYMPOSIUM 45 - THE NEWBORN BRAIN: UNRAVELING THE INTERPLAY BETWEEN EARLY ACTIVITY AND NETWORK DEVELOPMENT - ROUSTEM KHAZIPOV, CHARLIE DEMENé, REBECCAH SLATER, PETRA HüPPI

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Preterm birth is one of the leading causes for neurodevelopmental delay in surviving infants, and has been associated with a wide range of behavioural and cognitive problems from childhood to adult life. Studies by us and others have helped to uncover the underlying neural mechanisms of these difficulties, which is paramount to identify potential avenues for interventions to improve outcome. MR imaging studies have allowed to identify altered global brain tissue growth rates in preterm infants and have identified microstructurally altered brain white matter networks in the associative and limbic cortico-basal ganglia-thalamocortical circuits, involving the dorsolateral prefrontal cortex, the orbitofrontal cortex and the amygdala (Sa de Almeida et al., 2021). Recently we we have further evidence for altered salience (anterior insula to anterior cingulate) network functionality already in the newborn period, a network that allows to adapt behavior according to the predictive value of stimuli, positive (reward) or negative (punishment)(Lordier et al., 2019). Predictive relations between stimuli and outcome are learned through experience and preterm infants clearly have very different early life experiences with extreme situations of non-predictability. These findings raise the question of how to induce resilience through more predictable stimuli in the newborn period. Our recent research has engaged in introducing interventions, in the newborn period by maternal voice and music (Adam-Darque et al., 2020; Lordier et al., 2019; Loukas et al., 2021). The combination of fMRI and high density EEG are shown to be valid methods to study early functional competence of the developing brain.

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PRESENTATION NUMBER: S177

GENETIC AND EXPERIENCE DEPENDENT MOLECULAR PATTERNING OF NEURAL DIVERSITY

SYMPOSIUM 46 - BARRIERS AND HOPES FOR FUNCTIONAL RESTORATION AFTER TRAUMATIC CNS INJURY - FRANK BRADKE, KARTHIK SHEKHAR, JESSICA WHITED, AYA TAKEOKA

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Restoration of CNS tissue function and neuronal death following traumatic injury requires the regeneration of hundreds of diverse neuronal types interconnected via orderly and specific synapses. Moreover, different neuronal types can exhibit a wide range of susceptibility to injury, and differ in their capacity to respond to interventions. A systematic investigation of this diversity is necessary to identify viable strategies to promote the functional restoration of disrupted circuits. I will describe our efforts to use single-cell genomic approaches to study the diversity, development and response to injury of the projection neurons of the retina, the retinal ganglion cells (RGCs). Using single-cell transcriptomic profiling, we identified 46 types of RGCs in mice, which collectively parcellate visual input. We have also now unified molecular definitions with morphology and physiology (rgctypes.org). We then used this molecular atlas as a foundation for three lines of investigation: (1) Identifying selectively resilient RGC types following axotomy as a screen to identify molecular factors that may promote neuroprotection/regeneration, (2) Studying the natural developmental process of diversification of RGC types, and (3) Studying the impact of early activity on molecular aspects of RGC development and maturation. Together, these studies are providing us new insights into the developmental processes that guide the establishment and maintenance of a diverse neuronal class in the CNS.

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LOCAL AND SYSTEMIC RESPONSES TO INJURY IN AXOLOTL

SYMPOSIUM 46 - BARRIERS AND HOPES FOR FUNCTIONAL RESTORATION AFTER TRAUMATIC CNS INJURY - FRANK BRADKE, KARTHIK SHEKHAR, JESSICA WHITED, AYA TAKEOKA

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Regenerative abilities vary dramatically across animal lineages. Yet, how systems-level injury responses in individual species shape ultimate regenerative outcomes is not well understood. We recently discovered a body-wide stem cell activation response that occurs following amputation in the highly-regenerative axolotl salamander. Here, we present evidence that this systemic activation primes appendages distant to the original injury for their own future regeneration events. We hypothesize that stem cell priming may have evolved due to cannibalism faced by juvenile salamanders. We investigated the mechanism of systemic activation in axolotls and found the process requires innervation by the peripheral nervous system at distant responding sites. Using transcriptomic studies, we identified gene expression changes in nerve processes and responding tissues underlying systemic activation, which led us to consider adrenergic signaling. We found inhibiting adrenergic signaling is sufficient to block systemic activation. Inspired by previous reports of mTOR activity being required for systemic stem cell activation following injury in both highly-regenerative species and species with modest regenerative abilities, we also tested mTOR signaling in axolotl. Our studies also implicate mTOR signaling as required for axolotl systemic activation following amputation. Together, these results demonstrate a direct link between systemic stem cell responses and localized regeneration of an appendage, and they also highlight roles for peripheral nerves, adrenergic signaling, and the mTOR pathway in systemic injury responses in axolotls. They also suggest a model whereby common initial injury responses are used as a foundation across species, while species-specific life history constraints shape differing downstream responses that ultimately drive the regenerative outcome.

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PRESENTATION NUMBER: S179

NEUROTRANSMITTER SWITCH BY SPINAL EXCITATORY INTERNEURONS DEFINES AGE OF INJURY-DEPENDENT LOCOMOTOR CIRCUIT PLASTICITY AFTER SPINAL CORD INJURY

SYMPOSIUM 46 - BARRIERS AND HOPES FOR FUNCTIONAL RESTORATION AFTER TRAUMATIC CNS INJURY - FRANK BRADKE, KARTHIK SHEKHAR, JESSICA WHITED, AYA TAKEOKA

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Age is one of the defining factors for functional outcomes after a traumatic injury to the central nervous system. Both neuronal intrinsic and extrinsic factors contribute to age of injury-dependent plasticity. Immature neurons are more resilient to trauma and exhibit robust regeneration capabilities than mature neurons. In addition, the scar environment of juvenile animals is more permissive to growth than that of adults, supporting the sprouting and regeneration of neurons to establish novel connections. While these findings highlight the regenerative ability of injured cells themselves, our recent work reveals that circuits located at a distance from the injury site also undergo reorganization depending on the age of injury. Severe spinal cord injury to the mature nervous system leads to irreversible paralysis below the lesion. In contrast, a complete thoracic lesion just after birth leads to proficient hindlimb locomotion without brain input as an adult. How the spinal cord achieves such striking functionality remains unknown. We uncover age of injury-dependent divergent synaptic connectivity from interneurons to motor neurons. Adult injury prompts neurotransmitter switching of spatially defined excitatory interneurons to inhibitory phenotype, promoting inhibition at synapses interfacing motor neurons. In contrast, neonatal injury causes synaptic sprouting of identical populations to facilitate excitation. Furthermore, genetic manipulation to mimic inhibitory phenotype observed after adult injury by excitatory interneurons abrogates autonomous locomotor functionality in neonatally injured mice. In comparison, attenuating inhibitory phenotype improves locomotor recovery after adult injury. Together, our study demonstrates that flexible neurotransmitter phenotype of defined excitatory interneurons steers locomotor capacity after injury.

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PRESENTATION NUMBER: S180

THE INVOLVEMENT OF ASTROCYTE CALCIUM-DEPENDENT SIGNALING IN BEHAVIOR

SYMPOSIUM 47 - ASTROCYTES CONTROL BRAIN CIRCUITS UNDERLYING BEHAVIOR - JOãO FILIPE OLIVEIRA, MARTA NAVARRETE, ALFONSO ARAQUE, INBAL GOSHEN

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Astrocytes are critical players in the regulation of brain development and function. They sense and respond to neuronal activity by elevating intracellular calcium levels, which derive from different sources and display complex spatiotemporal properties. Calcium elevations appear spatially distributed in global (soma and main processes) and focal regions (microdomains). Such astrocytic calcium activity is expected to underlie the astrocyte involvement in synaptic transmission, metabolism, and brain homeostasis. In this work, we studied the IP3 receptor type 2 knockout (IP3R2 KO) mouse model that lacks global calcium elevations in astrocytes to disclose its implications in cognitive function. We found an influence of global astrocyte calcium long-term memory performance. Thus, we performed a structural and molecular analysis of cortico-limbic regions that revealed a shift to immature spines in pyramidal neurons of the dorsal hippocampus that could support the changes in synaptic plasticity underlying our behavioral observations. The characterization of the IP3R2 KO mouse model provided new insights into the importance of astrocytic calcium-dependent signaling in the modulation of neural activity. These findings broaden the scope of astrocytic modulation of brain circuits.

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PRESENTATION NUMBER: S181

ASTROCYTIC NETWORK HETEROGENEITY IN THE NUCLEUS ACCUMBENS: IMPLICATIONS FOR BEHAVIOR

SYMPOSIUM 47 - ASTROCYTES CONTROL BRAIN CIRCUITS UNDERLYING BEHAVIOR - JOãO FILIPE OLIVEIRA, MARTA NAVARRETE, ALFONSO ARAQUE, INBAL GOSHEN

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Unraveling the principles of information processing in complex cellular circuits requires techniques capable of specifically targeting and modulating the activity of the elements involved. Neuro-astrocyte networks display a surprising degree of complexity and state-of-the-art complementary tools are required to understand astrocyte involvement in circuit modulation and behavior. Although the evolution of genetic tools to study and control these circuits has focused mainly on neuronal activity, in this talk, I will show newly developed techniques to specifically dissect the active astrocyte circuits with spatio-temporal precision, i.e. *CaMPARI*$_{GFAP}$ (calcium-modulated photoactivatable ratiometric integrator under GFAP promoter) and *Astro-Light* (calcium- and light-gated switch to induce gene expression in activated astrocytes). Furthermore, I will discuss our recent data about mapping the functional astrocytic-circuitries within the Nucleus Accumbens (NAc) that reveal the existence of specific-astrocyte circuits in the NAc. In short, I will present data, acquired using cutting-edge tools, which supports the idea that NAc astrocytic networks are critical players in understanding the way that the NAc integrates information. Supported by: RYC-2016-20414, MINECO (RTI2018-094887-B-I00)

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PRESENTATION NUMBER: S182

ASTROCYTE REGULATION OF SYNAPTIC AND NETWORK FUNCTION

SYMPOSIUM 47 - ASTROCYTES CONTROL BRAIN CIRCUITS UNDERLYING BEHAVIOR - JOãO FILIPE OLIVEIRA, MARTA NAVARRETE, ALFONSO ARAQUE, INBAL GOSHEN

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Astrocytes, a major type of glial cells, are recognized as key supportive elements in neuronal function, providing structural and metabolic support for neurons, and controlling brain homeostasis mechanisms. Historically, they were ignored as being active players in cellular processes underlying brain function. However, accumulating evidence indicate that astrocytes and neurons establish bidirectional communication. Astrocytes respond to synaptically-released neurotransmitters and, in turn, release gliotransmitters that influence neuronal and synaptic activity. This evidence has led to the establishment of the tripartite synapse concept, a novel view of synaptic physiology in which astrocytes are integral elements involved in synaptic function. I will present and discuss current evidence revealing the specific mechanisms of astrocyte-neuron signaling in different brain areas. I will also present the functional consequences of astrocyte-neuron signaling at different levels of analysis, from cellular and synaptic levels to network and behavioral levels. Finally, I will discuss how this evidence supports a paradigm shift in our understanding of the cellular basis of brain function, which would result not solely from the neuronal activity, but from the coordinated activity of astrocytes and neurons.

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NAVIGATING BY THE STARS

SYMPOSIUM 47 - ASTROCYTES CONTROL BRAIN CIRCUITS UNDERLYING BEHAVIOR - JOãO FILIPE OLIVEIRA, MARTA NAVARRETE, ALFONSO ARAQUE, INBAL GOSHEN

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Recent studies, including ours, have implicated hippocampal astrocytes in memory processes; e.g. they can improve memory when activated, and have a projection specific effect on the acquisition of remote memory. Astrocytic calcium dynamics are involved in sensory information encoding, but their real-time calcium activity in awake mice has not been investigated as of yet. We chronically imaged dozens of CA1 astrocytes using 2-photon microscopy when head-fixed mice run on a linear treadmill and proceed in a virtual environment to obtain water rewards. We find that astrocytic activity persistently ramps towards the reward location in a familiar environment. However, when mice were introduced to a novel context, the ramping was not apparent. Following additional training, as the mice were familiarized with the novel context, the ramping was reestablished, suggesting that spatial modulation of astrocytic activity is experience dependent. Similarly, when mice were tested in the familiar environment, and the reward location was changed (in the same environment), the ramping was no longer apparent. When, after learning, the mice learned the new reward location, the ramping reappeared, suggesting again that astrocytic activity is learning dependent. This is the first indication that astrocytes can encode position related information in learnt spatial contexts, thus broadening their known computational abilities, and their role in cognitive functions.

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PRESENTATION NUMBER: S184

PERINEURONAL NET MODULATION, EXCITATORY/INHIBITORY BALANCE, AND ANTIDEPRESSANT EFFICACY

SYMPOSIUM 48 - HIPPOCAMPAL PERINEURONAL NETS IN SCHIZOPHRENIA AND DEPRESSION: OPPOSING OR COMPLEMENTARY ROLES? - KATHERINE CONANT, ANDREAS FAISSNER, VIVIEN CHEVALEYRE, HANNAH CLARKE

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Emerging evidence suggests that there is a relative reduction in glutamatergic neurotransmission in major depressive disorder (MDD), which afflicts approximately 14-20% of individuals. Pyramidal arborization and dendritic spine abundance are reduced with this disorder. Of interest, functional changes in parvalbumin-expressing interneurons have also been observed in rodent models of MDD. In particular, enhanced deposition of perineuronal net (PNN) components occurs with social defeat-inducing persistent stress. Since parvalbumin-expressing interneurons are the predominant cell population that is enveloped by PNNs, which enhances their ability to release GABA, excess PNN deposition likely increases pyramidal cell inhibition. In the present study we investigate the potential for matrix metalloprotease-9 (MMP-9), an endopeptidase secreted in response to neuronal activity, to contribute to the antidepressant efficacy of venlafaxine, a serotonin/norepinephrine reuptake inhibitor. Herein we observe that PNN expression is increased in a corticosterone-induced stress model of disease and reduced by venlafaxine in wild type but not MMP-9 null animals. Corticosterone treated mice also display reduced *ex vivo* gamma power and impaired working memory, with normalization by venlafaxine. This is a relevant endpoint because gamma power is increased with pyramidal cell disinhibition and with remission from MDD. Consistent with our murine studies, autopsy-derived prefrontal cortex samples show elevated MMP-9 levels in anti-depressant treated MDD patients as compared to controls. These preclinical and postmortem findings highlight a link between extracellular matrix regulation and MDD.

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PRESENTATION NUMBER: S185

REGULATION OF NEURONAL NETWORKS BY PERINEURONAL NETS AND MATERNAL IMMUNE ACTIVATION (MIA)

SYMPOSIUM 48 - HIPPOCAMPAL PERINEURONAL NETS IN SCHIZOPHRENIA AND DEPRESSION: OPPOSING OR COMPLEMENTARY ROLES? - KATHERINE CONANT, ANDREAS FAISSNER, VIVIEN CHEVALEYRE, HANNAH CLARKE

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Synapses are surrounded by extracellular matrix (ECM) molecules composed of glycoproteins of the tenascin gene family and the lectican chondroitinsulfate proteoglycans (CSPGs) aggrecan, neurocan and brevican. Around a subpopulation of inhibitory CNS interneurons the ECM condenses to superstructures termed perineuronal nets (PNNs). PNNs regulate synaptic plasticity, are implicated in several forms of memory formation and modified in the context of addiction and neuropsychiatric disease. We have developed a system that allows to cultivate embryonic hippocampal neurons in the presence of primary astrocytes of distinct genetic backgrounds. We could show that the elimination of tenascins and lecticans shifts the balance of excitatory and inhibitory synapses. Activation of the maternal immune system (MIA) during gestation is linked to neuropsychiatric diseases like schizophrenia. Pregnant mice were treated with polyinosinic-polycytidylic acid (Poly I:C), embryonic hippocampal neurons were cultivated and a significant reduction of PNN area, aggrecan staining intensity and neuronal soma size could be documented. Applying multielectrode array analysis (MEA) a remarkable increase of the spontaneous network activity in neuronal networks was detected. Activated microglia can cause the loss of PNNs. We analyzed the impact of Poly I:C stimulated activated microglia on hippocampal neuronal networks. Immunocytochemistry of the PNN component Aggrecan revealed a clear disruption of PNNs accompanied by an increase of glutamatergic synapse numbers and a significantly increased spontaneous network activity using MEA. In conclusion, we could demonstrate a strong impact of microglial secreted factors on PNN integrity, synaptic plasticity, and electrophysiological properties of cultured hippocampal neurons. Funded by DFG

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PRESENTATION NUMBER: S186

PNN MATURATION IN HIPPOCAMPAL AREA CA2 DURING ADOLESCENCE AND ROLE IN SOCIAL MEMORY

SYMPOSIUM 48 - HIPPOCAMPAL PERINEURONAL NETS IN SCHIZOPHRENIA AND DEPRESSION: OPPOSING OR COMPLEMENTARY ROLES? - KATHERINE CONANT, ANDREAS FAISSNER, VIVIEN CHEVALEYRE, HANNAH CLARKE

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In the hippocampus, the highest density of perineuronal net (PNN) is observed in area CA2. In addition to surrounding PV+ interneurons, as seen in other hippocampal areas, the PNN also encompasses CA2 pyramidal neurons. As reported in other brain regions, maturation of the PNN in CA2 prevents the induction of long-term potentiation at CA3-CA2 excitatory synapses. However, we have found that the PNN is also permissive for the plasticity induction at inhibitory synapses between parvalbumin-expressing (PV+) interneurons and CA2 pyramidal neurons. This plasticity of inhibitory transmission emerges during adolescence in parallel with an increase in PNN density, the control of PV transmission by Neuregulin/ErbB4 signaling and the maturation of social recognition memory. Degradation of the PNN or blockade of PV-CA2 synaptic plasticity in vivo impairs social memory. Interestingly, these changes occur during the same age as PV+ interneuron loss and plasticity reduction in area CA2 in a mouse model of the 22q11 deletion syndrome, suggesting that adolescence is a period of high susceptibility for PV+ interneurons. We also found that the PNN density is reduced and PV+ interneuron physiology is altered in the Tg2576 mouse model of Alzheimer's disease. By injecting neuregulin 1 injection in CA2, we were able to restore PV and PNN expression as well as social memory. These data indicate that area CA2 plays a critical role in the social cognition impairments observed during neurodegenerative and psychiatric diseases and the peculiar PNN expression in this region may be a key component in this process.

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REGULATION OF PREFRONTAL NEUROCHEMISTRY BY HIPPOCAMPAL PNN LOSS IN THE MARMOSET MONKEY, AND THE CONSEQUENCES FOR COGNITION

SYMPOSIUM 48 - HIPPOCAMPAL PERINEURONAL NETS IN SCHIZOPHRENIA AND DEPRESSION: OPPOSING OR COMPLEMENTARY ROLES? - KATHERINE CONANT, ANDREAS FAISSNER, VIVIEN CHEVALEYRE, HANNAH CLARKE

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Schizophrenia is a devastating psychiatric disorder that is associated with three main symptom clusters: positive symptoms that are well treated with antipsychotic drugs, and emotional and cognitive disruptions that are harder to treat, and represent an urgent clinical need (Green and Nuechterlein, 2004). Clinical neuroimaging studies and rodent models of schizophrenia have implicated dysfunction in the prefrontal cortex (PFC), and glutamatergic overactivity within the anterior hippocampus (aHipp) in the mechanisms underlying schizophrenia symptoms (Schobel et al., 2013). In addition, disruption of hippocampal parvalbumin-positive inhibitory GABAergic interneurons, and their surrounding perineuronal net (PNN), induce schizophrenia-like changes in rodent models (Shah and Lodge, 2013). However, while the role of aHipp hyperfunction has been considered in the positive symptoms, aHipp-PFC projections may also underlie the cognitive symptoms. We therefore evaluated how selective PNN degradation within the aHipp of marmoset monkeys altered performance of a probabilistic discrimination learning task known to depend on orbitofrontal-striatal circuitry. We also investigated how aHipp PNN degradation altered stereotypy in a marmoset version of the amphetamine-induced hyperlocomotion test. Probabilistic discrimination learning was impaired by aHipp PNN degradation, with animals more likely to respond to misleading, probabilistic feedback. Compared to pre-aHipp PNN degradation behaviour, amphetamine increased the amount of stereotypical behaviours, while microdialysis indicates these behavioural changes were accompanied by increased tonic catecholamine levels in the orbitofrontal cortex. Thus aHipp PNN degradation not only alters PFC neurochemistry, but also alters behaviours of relevance to both the positive and cognitive symptoms of schizophrenia, and links such deficits to aberrant aHipp-PFC circuitry.

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PRESENTATION NUMBER: S188

DISSECTING THE MOLECULAR CONNECTOME IN DEVELOPING NEURAL CIRCUITS

SYMPOSIUM 49 - BUILDING CORTICAL NETWORKS FROM DEVELOPMENT TO ADULTHOOD - JORIS DE WIT, BEATRIZ RICO, SIMON BUTT, CéCILE CHARRIER

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Neural circuits are composed of distinct neuronal cell types connected in highly specific patterns. Unraveling how neurons form appropriate synaptic connections during development is a key challenge in neuroscience and is essential to understand brain function and disease. Neural circuit formation critically relies on cell-cell recognition and communication mediated by cell-surface ligands and receptors. The complexity of the cell-surface interactions that pattern precise synaptic connectivity is only beginning to emerge. In this talk, I will discuss our recent work dissecting the cell-surface interaction networks that control connectivity, structure and function of specific synapses in the hippocampal circuit.

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PRESENTATION NUMBER: S189

MECHANISMS ORCHESTRATING THE ASSEMBLY OF INTERNEURON-PYRAMIDAL CELL NETWORKS

SYMPOSIUM 49 - BUILDING CORTICAL NETWORKS FROM DEVELOPMENT TO ADULTHOOD - JORIS DE WIT, BEATRIZ RICO, SIMON BUTT, CéCILE CHARRIER

Beatriz Rico
King's College London, Centre For Developmental Neurobiology, Centre For Neurodevelopmental Disorders, London, United Kingdom

The function of neural networks in the mammalian cerebral cortex relies on the interaction between two main classes of neurons, excitatory projection neurons (pyramidal cells) and inhibitory neurons (interneurons). In these circuits, the output of excitatory neurons is fine-tuned and synchronised by the activity of interneurons. Recent work suggests that distinct classes of interneurons preferentially target pyramidal cells with specific projection patterns to gate information flows in cortical circuits, but the mechanisms controlling this sophisticated form of interneuron specialisation are unknown. Furthermore, integration between pyramidal cells and interneurons requires independent control at multiple subcellular compartments. The local translation is ubiquitous in neuronal pre- and postsynaptic compartments. However, to what extent local translation is differentially regulated at the level of specific synapses during wiring or in mature neural circuits is unknown. In my talk, I will discuss our last findings on synaptic specificity, what are the cellular rules and molecular codes that interneurons use to make synapses into pyramidal cells. Our work deciphers a molecular mechanism by which these synapses are formed, segregate into different pyramidal cell types and some have synapse-specific control of local translation.

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PRESENTATION NUMBER: S190

CONTRIBUTION OF TRANSIENT GABAERGIC NETWORKS TO EMERGENT SENSORY PROCESSING ACROSS NEONATAL SENSORY CORTICES

SYMPOSIUM 49 - BUILDING CORTICAL NETWORKS FROM DEVELOPMENT TO ADULTHOOD - JORIS DE WIT, BEATRIZ RICO, SIMON BUTT, CéCILE CHARRIER

Simon Butt
Oxford University, Physiology, Anatomy And Genetics, Oxford, United Kingdom

GABAergic interneurons (INs) are necessary for normal information transfer in both the developing and adult mammalian neocortex. Somatostatin-expressing (SST+) INs are ideally placed to be the main source of GABAergic signalling during postnatal development as they integrate early into cortical networks. In mouse somatosensory barrel cortex (S1BF), thalamo-recipient layer (L)5b SST+ INs establish a transient, translaminar, reciprocal circuit with L4 spiny stellate neurons during the critical period for plasticity. This network plays a role in timing the emergence of sensory-driven, feed-forward architecture in L4. Despite this critical role, the L5b-L4 circuit is not present in primary visual cortex (V1) through postnatal life. Moreover, layer 5 SST+ INs in V1 do not receive monosynaptic thalamic input. Comparison of known markers of layer 5 SST+ interneurons revealed similar transcriptomic subtypes across both areas, with the exception of those defined by expression of *Lpar1-EGFP* transgene which were found in reduced numbers in V1. *In vivo* optogenetic spike-tagging of either SST+ INs alone or *Nkx2-1*-derived INs, that capture both SST+ and parvalbumin-expressing (PV+) IN subtypes, allowed us to investigate the contribution of both SST+ and putative PV+ INs to emergent sensory processing. We observed distinct recruitment of SST+ INs in S1BF versus V1 over early ages. We conclude that local GABAergic circuits differ across these two primary sensory areas and that the function of GABAergic INs – and resultant control of information transfer, is tuned to modality-specific needs early in life, with consequences for understanding of both normal and dysfunctional circuit development.

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PRESENTATION NUMBER: S191

MOLECULAR CROSSROAD IN SYNAPSE DEVELOPMENT AND DEGENERATION

SYMPOSIUM 49 - BUILDING CORTICAL NETWORKS FROM DEVELOPMENT TO ADULTHOOD - JORIS DE WIT, BEATRIZ RICO, SIMON BUTT, CéCILE CHARRIER

Cécile Charrier
Ecole Normale Supérieure, CNRS, INSERM, Université PSL, Institut De Biologie De L'ens (ibens), Paris, France

Synaptic connections of the human neocortex present species-specific features often altered in brain disorders. Yet, little is known about the molecular pathways that link human brain evolution and diseases. Here, we identify catenin delta-2 (CTNND2) as a binding partner of the human-specific protein and synapse regulator SRGAP2C. CTNND2 is a cadherin-binding protein whose mutations cause intellectual disability in the Cri-du-Chat syndrome and severe autism. We demonstrate that CTNND2 deficiency in sparse layer 2/3 cortical pyramidal neurons disrupts excitation/inhibition coordination and increases intrinsic excitability in juvenile mice. These effects are followed by premature loss of dendritic spines in adults. We further show that (1) CTNND2 forms a postsynaptic protein complex that acts as a brake on excitatory activity and synaptic maturation, and (2) human-specific SRGAP2C enhances CTNND2 synaptic accumulation. Thus, while CTNND2 loss of function causes failure of neuronal homeostasis, its regulation by SRGAP2C may contribute to synaptic neoteny and long-term maintenance in humans.

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PRESENTATION NUMBER: S192

CEREBELLAR CONTROL OF LOCOMOTOR COORDINATION

SYMPOSIUM 50 - CIRCUITS FOR MOTOR CONTROL: MOVING FROM THE CORTEX TO THE SPINAL CORD AND BACK - MEGAN CAREY, IAN DUGUID, ARIEL LEVINE, GRAZIANA GATTO

Megan Carey
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Whole-body movements require precise coordination across the body. The cerebellum is critical for coordinating movement; during locomotion it is particularly important for interlimb coordination. Decades of recordings have consistently shown that cerebellar Purkinje cell output is broadly correlated with the locomotor stride cycle. However, much of the firing rate variability has remained unexplained; moreover, previous analyses do not provide a clear model for how Purkinje cell activity could be read out to control coordination. I will describe our recent work demonstrating that a substantial proportion of Purkinje cells simultaneously encode movements of multiple body parts to provide precise representations of temporal coordination across diverse combinations of behavioral events. These findings resolve long-standing controversies surrounding the role of Purkinje cells in locomotor control and could allow for efficient readouts of whole-body coordination by a simple linear decoder.

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PRESENTATION NUMBER: S193

NEURAL CIRCUITS FOR SKILLED BEHAVIOR

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SYMPOSIUM 50 - CIRCUITS FOR MOTOR CONTROL: MOVING FROM THE CORTEX TO THE SPINAL CORD AND BACK - MEGAN CAREY, IAN DUGUID, ARIEL LEVINE, GRAZIANA GATTO

Dexterous movements serve the major functions of the brain, perception and manipulation of the world. Considering the range of possible actions and the complexity of musculoskeletal arrangements, control of the hand is an amazing achievement of the nervous system. Dexterous behavior involves understanding objects in the world, developing appropriate plans, converting those plans into appropriate motor commands, and adaptively reacting to feedback. The myriad of these underlying operations is likely performed by a diverse set of neural circuits. By combining anatomy, physiology, and specific (genetic and temporal) manipulations, we are identifying the neural elements responsible for dexterous motor control. Currently, we focus on the role of the cortico-cerebellar loop in a skilled reach-grab-eat task in the rodent.

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PRESENTATION NUMBER: S194

SPINAL CORD CELLS AND CIRCUITS FOR MOTOR CONTROL

SYMPOSIUM 50 - CIRCUITS FOR MOTOR CONTROL: MOVING FROM THE CORTEX TO THE SPINAL CORD AND BACK - MEGAN CAREY, IAN DUGUID, ARIEL LEVINE, GRAZIANA GATTO

Ariel Levine
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The mammalian spinal cord links the brain and the body to enable voluntary movements and disruption of this critical role through spinal cord injury or neurodegeneration can lead to paralysis and death. My lab seeks to understand how spinal neurons encode simple movements and how they are integrated into larger networks throughout the nervous system to execute, refine, and learn broad motor behaviors. Here, I will focus on the direct descending and ascending pathways that connect the spinal cord and the cerebellum. First, I will present our recent work characterizing the structure and function of the CerebelloSpinal Tract (CeST), which we found is required for aspects of online motor control and motor learning. Second, I will present our new work on Spinocerebellar Tract (SCT) neurons and how they undergo structural plasticity and remodeling after spinal cord injury, raising the hope that these cells could support re-learning of motor skills and recovery. Together, these findings highlight the importance and bidirectional nature of communication between the spinal cord and cerebellum.

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PRESENTATION NUMBER: S195

THE FUNCTIONAL CONTRIBUTION OF SPINAL NEURON HETEROGENEITY TO SENSORIMOTOR BEHAVIORS

SYMPOSIUM 50 - CIRCUITS FOR MOTOR CONTROL: MOVING FROM THE CORTEX TO THE SPINAL CORD AND BACK - MEGAN CAREY, IAN DUGUID, ARIEL LEVINE, GRAZIANA GATTO

Graziana Gatto
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The spinal cord comprises networks of interconnected neuron types, dedicated to the generation of movement and the processing of sensory input. Despite the great advances in identifying the molecular identity of the heterogeneous populations making up the spinal networks, we are still far from understanding the role of the distinct cell types and their synergistic or antagonistic interactions in the execution of sensorimotor behaviors. Therefore, even the spinal neural circuits that underlie simpler movements, like reflex responses, remain poorly characterized. To dissect the neural circuit that patterns the coordination of flexor and extensor muscles, we undertook an intersectional genetic approach to modulate the activity of distinct neuron types during the execution of the scratch reflex. We identified a core circuit composed of excitatory and inhibitory interneurons that synergistically cooperate to titrate the rhythm of scratching and to coordinate antagonistic muscle pairs for the smooth execution of the movement trajectory. Our model, built on the scratch experimental data, is also capable to recapitulate the dynamics of flexor and extensor muscle coordination during locomotion. Taken together, we identified and modeled the synergistic interactions of specialized spinal neuron types, which coordinate the recruitment of flexor and extensor muscles during rhythmic motor behaviors. Interestingly, this specialized network seems to be a convergence point for diverse motor responses, functioning as a malleable substrate under the modulation of sensory and descending inputs.

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PRESENTATION NUMBER: S196

CHILDHOOD ADVERSITY AND BRAIN ADAPTATION: IMPACT ON SOCIAL FUNCTIONING AND MENTAL HEALTH VULNERABILITY

SYMPOSIUM 51 - BRAIN ADAPTATION AND MENTAL HEALTH THROUGH A SOCIAL TRANSACTIONAL LENS - EAMON MCCRORY, CHARLOTTE CECIL, CARMEN SANDI, BERNET ELZINGA

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University College London, Psychology And Language Sciences, London, United Kingdom

Childhood trauma, in the form of maltreatment and neglect, is one of the strongest predictors of later mental health problems across the lifespan. However, the neurobiological mechanisms by which childhood adversity 'gets under the skin' remain poorly understood. Prof McCrory will highlight the complex relationship between the brain, childhood trauma, the social world and mental health. He will argue that mental health and wellbeing are intrinsically relational phenomena, and it is erroneous to think of them as ‘located’ within an individual or their brain. Rather, we need to think of the brain as a socially embedded organ. The domains of threat, reward and autobiographical memory processing will be briefly reviewed. The concepts of Stress Generation and Social Thinning will also be introduced to illustrate this argument, and implications for prevention and intervention will be noted.

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PRESENTATION NUMBER: S197

GENOME WIDE GENE X ENVIRONMENT INTERACTION STUDY OF BRAIN STRUCTURE IN CHILDHOOD AND ITS LINK TO SUBSEQUENT PSYCHOPATHOLOGY

SYMPOSIUM 51 - BRAIN ADAPTATION AND MENTAL HEALTH THROUGH A SOCIAL TRANSACTIONAL LENS - EAMON MCCRORY, CHARLOTTE CECIL, CARMEN SANDI, BERNET ELZINGA

Koen Bolhuis[1], Rosa Mulder[1], Louk De Mol[2], Serena Defina[1], Varun Warrier[3], Tonya White[1], Henning Tiemeier[4], Ryan Muetzel[1], Charlotte Cecil[1]
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Although it is well-established that both genetic and the environmental factors influence brain development, these are typically examined separately. Here, we prospectively investigated the interactive effects of genetic variants – from a genome-wide approach – and early life stress (ELS) on child subcortical brain structures, and their association with subsequent mental health problems. Primary analyses were conducted using data from the Generation R Study (N=2,257), including genotype and cumulative prenatal and postnatal ELS scores. Neuroimaging data were collected at 10 years, including total and subcortical brain volumes (accumbens, amygdala, caudate, hippocampus, pallidum, putamen, thalamus). Genome-wide-by-environment interaction analyses (GWEIS) were conducted, from which polygenic scores (PGS) were calculated for validation in an independent cohort (ABCD Study; N=10,751), in relation to subcortical volumes and mother-reported mental health problems. One GWEIS-prenatal stress locus significantly associated with caudate volume (rs139505895, mapping onto PRSS12 and NDST3) and two GWEIS-postnatal stress loci associated with the accumbens (rs2397823 and rs3130008, mapping onto CUTA, SYNGAP1, and TABP). Functional annotation revealed that these genes play a role in neuronal plasticity and synaptic function, and have been implicated in neurodevelopmental and psychiatric phenotypes such as intellectual disability, autism, and schizophrenia. In the validation sample, PGS_{GxE} associated with several subcortical volumes, including the caudate, and all $PGS_{genotype}$ associated with their respective brain volumes. A minority of PGS scores further associated with child mental health problems. Overall, our study lends novel insights into gene-environment interplay on the developing brain as well as pointing to promising candidate loci for future mechanistic studies.

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PRESENTATION NUMBER: S198

NEUROENDOCRINE AND METABOLIC FACTORS REGULATING THE LONG-TERM IMPACT OF EARLY LIFE ADVERSITY ON SOCIAL BEHAVIOURS

SYMPOSIUM 51 - BRAIN ADAPTATION AND MENTAL HEALTH THROUGH A SOCIAL TRANSACTIONAL LENS - EAMON MCCRORY, CHARLOTTE CECIL, CARMEN SANDI, BERNET ELZINGA

Carmen Sandi
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The period comprising late childhood and puberty (i.e., peripuberty) is a critical time-window for brain development, and chronic stress during this period can lead in some individuals to protracted alterations in sociability and aggressive behavior. Using rodent models, we have underscored a differential regulation of the glucocorticoid responsiveness to stress as a critical factor explaining individual variation in social behaviors and aggression, particularly in individuals formerly exposed to peripubertal stress. In addition, our recent work underscores a link between increased fat mass at adulthood in individuals exposed to peripubertal stress and reduced sociability. We report a fat-to-brain pathway, involving the adipokine nicotinamide phosphoribosyltransferase (Nampt) and the nicotinamide adenine dinucleotide (NAD^+)/SIRT1 pathway in the nucleus accumbens in the mediation of sociability deficits of early life stress origin. Our work highlights nutritional interventions targeting NAD^+ as potential treatments to ameliorate social deficits of early life stress origin.

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PRESENTATION NUMBER: S199

EARLY LIFE ADVERSITY AND DEPRESSION: THE NEURAL NETWORKS IMPLICATED IN ADOLESCENT SOCIAL FEEDBACK AND IDENTITY FORMATION

SYMPOSIUM 51 - BRAIN ADAPTATION AND MENTAL HEALTH THROUGH A SOCIAL TRANSACTIONAL LENS - EAMON MCCRORY, CHARLOTTE CECIL, CARMEN SANDI, BERNET ELZINGA

Bernet Elzinga[1], Lisanne Van Houtum[1], Mirjam Wever[1], Geert-Jan Will[2], Charlotte Van Schie[3], Marieke Tollenaar[1], Loes Janssen[1], Wilma Wentholt[1]
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Adolescence is a key period for the development of a sense of self. Depression, a mental disorder typically related to low self esteem, also frequently emerges during this period. One of the key factors in the formation of adolescents' self-concept is socio-evaluative feedback from parents. How exactly parental feedback relates to adolescent depression is not clear, however. To elucidate the affective and neural responses to parental feedback in depressed (*n*=35) and healthy control adolescents (*n*=63), and how this relates adolescents own self views and to parental criticism in daily life, we developed a social feedback task. In this task, adolescents receive positive and negative feedback, supposedly provided by their mother or father in the form of appraisals about their personality (e.g., 'respectful' or 'lazy') during fMRI scanning. After each feedback word, adolescents reported their mood and adolescents rated whether these words match their self-views. In healthy controls, negative parental feedback worsened adolescents' mood, which was exacerbated when feedback did not match adolescents' self-views. Negative feedback increased activity in the neural 'saliency network' (incl. anterior insula, anterior cingulate cortex and dorsomedial prefrontal cortex), while positive feedback improved mood and increased activity in brain regions supporting social cognition (including TPJ, posterior superior temporal sulcus, and precuneus). Parental warmth (but not criticism in daily life) was associated with adolescent's positive self views and mood. Results in adolescents with depresson (*n*=35) on the role of parental criticism and aberrant affective and neural responses to parental feedback will be presented (analyses currenlty in prep) and clinical implications will be discussed.

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PRESENTATION NUMBER: S200

HIPPOCAMPAL MECHANISMS OF FEAR RELAPSE AFTER EXTINCTION

SYMPOSIUM 52 - HIPPOCAMPAL PLASTICITY ALONG THE DORSOVENTRAL AXIS: IMPLICATIONS FOR PSYCHIATRIC DISEASES - MICHAEL DREW, CHRISTINE DENNY, DENIS DAVID, EERO CASTREN

Michael Drew
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Learned fear often relapses after extinction, suggesting that extinction training generates a new memory that coexists with the original fear memory. Recent work from our lab has identified the hippocampal dentate gyrus (DG) as a region where such fear and extinction memories are generated. Specifically, using activity-dependent neural tagging in mice, we discovered that extinction training suppresses reactivation of contextual fear engram cells in DG while activating a second ensemble, a putative extinction engram. Here we investigate (1) whether these effects of extinction training on fear ensembles are limited to the DG or exist in other regions of the hippocampus and parahippocampal cortex, (2) whether fear and extinction ensembles in DG exhibit distinct patterns of gene expression, and (3) whether fear and extinction activate unique hippocampal output pathways. We used ArcCreErt2-eYFPflx mice injected tag neurons active during fear acquisition. Mice then received extinction training or were left undisturbed in their home cages. After a test of conditioned fear in the training context, we first assessed reactivation of eYFP-tagged fear acquisition neurons using immunohistochemistry against Arc and Fos proteins. Extinction training suppressed reactivation of YFP-tagged fear acquisition neurons in dorsal and ventral DG, dorsal CA1, and dorsal and ventral lateral entorhinal cortex. Next we used single-nuclei RNA Sequencing (snRNA-seq) to characterize gene expression profiles in eYFP+ and eYFP- cells in the dorsal DG of mice from Extinction and No-Extinction groups. We recovered the canonical DG cell types, examined how the transcriptomes of eYFP+, Fos+ and Arc+ fear acquisition neurons changed after extinction training, and identified the transcriptomes of extinction-activated neurons. Finally, we used cholera toxin retrograde tracing and immediate-early gene assays to characterize activity of hippocampal projections pathways during expression of fear and extinction. Our results show that fear recall favors activity in hippocampal projections to basolateral amygdala, whereas extinction recall favors activity in projections to prefrontal cortex. Together our results that extinction training has anatomically and molecularly robust effects on activity of fear acquisition neurons.

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PRESENTATION NUMBER: S201

DRUG-DEPENDENT MODULATION OF STRESSFUL MEMORY TRACES IN HIPPOCAMPAL CA3

SYMPOSIUM 52 - HIPPOCAMPAL PLASTICITY ALONG THE DORSOVENTRAL AXIS: IMPLICATIONS FOR PSYCHIATRIC DISEASES - MICHAEL DREW, CHRISTINE DENNY, DENIS DAVID, EERO CASTREN

Christine Denny
Columbia University Irving Medical Center, Psychiatry, New York, United States of America

A memory trace or engram is a neural ensemble activated during learning (or during an individual experience) and whose reactivation by the original stimuli results in memory retrieval. In order to permanently tag activated neurons that are part of an engram, we previously generated the ArcCreER^{T2} mouse line. This line is unique in that it allows for an indelible label of previously activated neurons and thus, allows for a comparison of the cells activated during memory encoding with those activated during memory expression. Most notably, it allows for a brain-wide tagging of an individual memory with single-cell resolution. These activity-dependent tagging mice can be utilized in a number of studies to determine how external factors such as context, mood, stress, or drugs influence an individual memory trace, resulting in altered behavior. In this talk, I will summarize our recent work elucidating the neural mechanisms of drug administration on fear learning and memory utilizing the ArcCreER^{T2} mice.

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PRESENTATION NUMBER: S202

ADULT HIPPOCAMPAL NEUROGENESIS IS REQUIRED FOR VORTIOXETINE-INDUCED PREVENTION OF ANXIETY/DEPRESSION RELAPSE PHENOTYPE

SYMPOSIUM 52 - HIPPOCAMPAL PLASTICITY ALONG THE DORSOVENTRAL AXIS: IMPLICATIONS FOR PSYCHIATRIC DISEASES - MICHAEL DREW, CHRISTINE DENNY, DENIS DAVID, EERO CASTREN

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Previously, we showed that vortioxetine (VORT) [a serotonin reuptake inhibitor combined with actions at serotonin receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_3, 5-HT_7)] protected against stress reinstatement induced anxiety/depression-like phenotype and decreased adult hippocampal neurogenesis (AHN). In a mouse model of genetic ablation of AHN [glial fibrillary acidic protein (GFAP)-positive neural progenitor cells mouse line], we assessed whether AHN is required for VORT -induced prevention of anxiety/depression. Four weeks before the start of the corticosterone (CORT) treatment to induce depression-like behavior and until the end of the protocol, male GFAP-TK positive mice (TK+) and their littermates (TK-) were administered with valganciclovir in the chow to arrest AHN. After 4 weeks of chronic vehicle (VEH) or CORT, TK+ and TK- mice were administered with saline or VORT (10 mg/kg/day, i.p) treatment for 4 weeks. Behavioral assays (Elevated Plus Maze, EPM; the Novelty Suppressed Feeding, NSF; the Splash Test, ST) were chosen to assay anxiolytic-antidepressant-like activity during VORT treatment and 3 weeks after withdrawal. We confirmed that chronic VORT induced anxiolytic/antidepressant-like effects and protected against CORT reinstatement–induced anxiety/depression-like phenotype in both genotypes. In the EPM and the ST, ablation of AHN in TK+ mice did not alter anxiolytic/antidepressant-like response induced by VORT and prevention of stress reinstatement. However, in the NSF, chronic VORT treatment-induced decrease in latency to feed and prophylactic effects against stress reinstatement in TK- mice, is arrested in TK+ mice. In conclusion, AHN is required not only for VORT -induced antidepressant effects but also for the prevention of relapse.

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PRESENTATION NUMBER: S203

CHOLESTEROL-DEPENDENT HIPPOCAMPAL PLASTICITY IS MEDIATED BY TRKB

SYMPOSIUM 52 - HIPPOCAMPAL PLASTICITY ALONG THE DORSOVENTRAL AXIS: IMPLICATIONS FOR PSYCHIATRIC DISEASES - MICHAEL DREW, CHRISTINE DENNY, DENIS DAVID, EERO CASTREN

Eero Castren, Plinio Casarotto
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Cholesterol is known to be required for the proper maturation and function of cortical and hippocampal synapses, but the molecular mechanisms underlying these effects have remained unclear. We recently found that cholesterol critically regulates the function of TrkB, the receptor for brain-derived neurotrophic factor (BDNF), a central regulator of neuronal connectivity and plasticity. Depletion of cholesterol in primary hippocampal cultures blocks the ability of BDNF to increase TrkB autophosphorylation and neurite sprouting, and, conversely, increase in cholesterol in the culture medium promotes the effects of BDNF in an inverted U-shaped manner. We found that the transmembrane domain (TMD) of TrkB possesses a cholesterol recognition consensus sequence (CRAC) and provided evidence that cholesterol directly binds to this site, as mutation of a central tyrosine (Y433F) within the TrkB TMD blocks cholesterol interaction. Atomistic molecular modeling revealed that the dimer of TrkB TMDs cross each other within the membrane and that the angle between the two domains is dependent on the thickness of plasma membrane, which in turn depends on cholesterol concentration. Our data indicate that the configuration of TrkB TMD dimers is optimal in membranes with moderate concentration of cholesterol, but the dimer structure is instable in very low as well as in high concentrations of cholesterol, such as found in synaptic membranes. Our data suggest that neuronal cholesterol is a critical regulator of TrkB function, which may at least partially explain the requirement for cholesterol in the maturation and plasticity of cortical and hippocampal synapses.

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PRESENTATION NUMBER: S204

SPATIAL MAPS IN POSTERIOR PIRIFORM CORTEX DURING NAVIGATION

SYMPOSIUM 53 - OLFACTORY-DRIVEN BEHAVIORS AND THEIR REPRESENTATIONS IN THE BRAIN - CINDY POO, CARL SCHOONOVER, PRIYANKA GUPTA, VENKATESH MURTHY

Cindy Poo
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Odors are a fundamental part of the sensory environment used by animals to inform behaviors such as foraging and navigation. Primary olfactory (piriform) cortex is thought to be dedicated to encoding odor identity. Using neural ensemble recordings in freely moving rats performing a novel odor-cued spatial choice task, we show that posterior piriform cortex neurons also carry a robust spatial map of the environment. Piriform spatial maps were stable across behavioral contexts independent of olfactory drive or reward availability, and the accuracy of spatial information carried by individual neurons depended on the strength of their functional coupling to the hippocampal theta rhythm. Ensembles of piriform neurons concurrently represented odor identity as well as spatial locations of animals, forming an “olfactory-place map”. Our results reveal a previously unknown function for piriform cortex in spatial cognition and suggest that it is well-suited to form odor-place associations and guide olfactory cued spatial navigation. We

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PRESENTATION NUMBER: S205

LEARNING AND FORGETTING IN PRIMARY OLFACTORY CORTEX

SYMPOSIUM 53 - OLFACTORY-DRIVEN BEHAVIORS AND THEIR REPRESENTATIONS IN THE BRAIN - CINDY POO, CARL SCHOONOVER, PRIYANKA GUPTA, VENKATESH MURTHY

Carl Schoonover
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We have discovered that in the rodent primary olfactory cortex (piriform) the pattern of neural activity evoked by a smell changes with the passage of time. These changes, which unfold absent a task or learning paradigm, accumulate to such an extent that after just a few weeks odor responses bear little resemblance to their original form. The piriform has been traditionally hypothesized to establish the identity of odorants. Our observations have forced us to radically reconsider the role of this vast brain region in olfactory perception. We propose that the piriform operates instead as a flexible learning system, a ‘scratch pad’ that continually learns and continually overwrites itself. This poses the problem of how transient memory traces can subsequently be stored over long timescales.

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PRESENTATION NUMBER: S206

UNDERSTANDING THE NEURONAL SUBSTRATES OF SENSORIMOTOR TRANSFORMATIONS USING A NOVEL CLOSED-LOOP OLFACTORY TASK (SMELLOCATOR) FOR MICE

SYMPOSIUM 53 - OLFACTORY-DRIVEN BEHAVIORS AND THEIR REPRESENTATIONS IN THE BRAIN - CINDY POO, CARL SCHOONOVER, PRIYANKA GUPTA, VENKATESH MURTHY

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Growing evidence suggests that sensory perception is better described as the continuous tally of actual inputs and predictions, than solely as a process of stimulus feature representation. To date, however, little is known about the mechanisms that facilitate the evaluation of sensory inputs against internally generated predictions. To study sensorimotor predictions both at behavioral and circuit-level, we developed a novel closed-loop behavioral task (*Smellocator*), wherein head-fixed mice learn to steer a lever to control the lateral location of an odor source. To obtain rewards, mice need to move the lever so as to bring the odor source, initialized in different trials at different starting locations, in front of their nose. Mice thus learn to link their motor action to well-defined sensory expectations (odor location). To *read-out the learnt sensorimotor predictions in expert mice*, we violate the learnt expectations by decoupling the stimulus from the current action and creating brief sensorimotor errors. Additionally, across longer timescales, we invert the sensorimotor mapping (direction of odor movement), so as to engage sensorimotor adaptation. Strikingly, we find that *expert mice readily counter these sensorimotor errors and display precise corrective movements which provide a behavioral read-out of their individual specific, learnt sensorimotor expectations*. Simultaneous recordings from olfactory cortex show that *odor-driven responses are strongly modulated by movement related expectations* about the stimulus arrival. Further, transient perturbations often drive olfactory cortex neurons stronger than any other variable in our task including odor onset, target entry and rewards.

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PRESENTATION NUMBER: S207

MODIFICATION OF NEURAL REPRESENTATIONS IN THE OLFACTORY CORTEX DURING LEARNING

SYMPOSIUM 53 - OLFACTORY-DRIVEN BEHAVIORS AND THEIR REPRESENTATIONS IN THE BRAIN - CINDY POO, CARL SCHOONOVER, PRIYANKA GUPTA, VENKATESH MURTHY

Venkatesh Murthy[1], Alice Berners-Lee[1], Liz Shtrahman[1], Julien Grimaud[2]
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There has been significant recent progress in understanding how odors are represented in the olfactory cortex, but how these representations are modified during learning is largely unknown. We investigated how learned smells are encoded and evolve in the olfactory cortex of mice while they learn to discriminate a unique target odor blend against hundreds of nontarget blends. A significant proportion of posterior piriform cortex neurons discriminate between target and nontarget odor blends. Neurons that prefer the target odor tend to respond with brief increases in firing rate at odor onset, whereas neurons preferring non-target odors tend to respond with sustained increases or decreases in firing rates. Mice trained on the target/non-target task are initially confused by morphed stimuli that contain a mixture of target and nontarget components, but quickly learn to categorize them as nontarget odors; the cortical representation of morphed stimuli evolves in parallel with learning. Although all non-target odors elicit the same behavioral choice, individual non-target mixtures maintain their identity by developing unique representations upon repeated presentations. These data show that populations of piriform cortex neurons can change their representation of complex stimuli in different ways to enable categorization, while preserving identity coding.

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PRESENTATION NUMBER: S208

THE CONTRIBUTION OF VISCERAL SIGNALS TO BRAIN DYNAMICS AND COGNITION IN HUMANS

SYMPOSIUM 54 - COMMON MECHANISMS OF BRAIN-BODY INTERACTIONS ACROSS HUMANS AND ANIMAL MODELS - CATHERINE TALLON-BAUDRY, MICAH ALLEN, YOAV LIVNEH, MONICA DUS

Catherine Tallon-Baudry
Ecole Normale Supérieure, Inserm, Paris, France

The stomach is not only active when it mixes and grinds food during digestion, but continuously and intrinsically generates a slow electrical rhythm (0.05Hz) which could be relayed up to the central nervous system without giving rise to conscious gastric feelings. We thus asked whether the stomach could act as an electrical pacemaker constraining brain activity. In a series of experiments where we measured the gastric rhythm and brain activity using either fMRI or magneto-encephalography (MEG), we found that spontaneous brain activity is coupled with the stomach rhythm in a large, distributed network. The gastric network cuts across classical resting-state networks, and does not depend on fasting state nor anxiety. Both fMRI and MEG reveal that the gastric network encompasses all sensory cortices (known interoceptive cortices as well as visual, auditory, etc.) and motor and premotor regions, but includes only a very limited set of higher order brain areas. The anatomo-functional distribution of the gastric network holds true whether participants are passive or engaged in a visual task. Altogether, these results suggest a pervasive influence of the gastric rhythm on brain dynamics extending well beyond the circuits of interoceptive feelings or feeding behavior.

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PRESENTATION NUMBER: S209

INTEROCEPTIVE SELF-INFERENCE: COMPUTATIONAL AND PSYCHOPHYSICAL APPROACHES TO MODELLING RESPIRATORY AND CARDIAC INTEROCEPTION

SYMPOSIUM 54 - COMMON MECHANISMS OF BRAIN-BODY INTERACTIONS ACROSS HUMANS AND ANIMAL MODELS - CATHERINE TALLON-BAUDRY, MICAH ALLEN, YOAV LIVNEH, MONICA DUS

Micah Allen
Aarhus University, Institute Of Clinical Medicine, Aarhus, Denmark

Empirical evidence and theoretical models both increasingly emphasize the importance of interoceptive processing in mental health. Indeed, many mood and psychiatric disorders involve disturbed feelings and/or beliefs about the visceral body. However, current methods to measure
interoceptive ability are limited in a number of ways, restricting the utility and interpretation of interoceptive biomarkers in psychiatry. I will present some newly developed measures and models which aim to improve our understanding of disordered brain-body interaction in
psychiatric illnesses.

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PRESENTATION NUMBER: S210

CORTICAL COMPUTATIONS OF CURRENT AND FUTURE INTEROCEPTION WITHIN THE BRAIN-BODY LOOP

SYMPOSIUM 54 - COMMON MECHANISMS OF BRAIN-BODY INTERACTIONS ACROSS HUMANS AND ANIMAL MODELS - CATHERINE TALLON-BAUDRY, MICAH ALLEN, YOAV LIVNEH, MONICA DUS

Yoav Livneh
Weizmann Institute of Science, Dept. Of Brain Sciences, Rehovot, Israel

The brain and body are in a continuous dialog that is essential for our physical and mental health. Little is known about how this dialog is achieved at the neurobiological level. A large corpus of work implicates the insular cortex as a central node for bi-directional brain-body communication. However, there is very little direct evidence for its functional role. We developed a microprism-based cellular imaging approach to monitor insular cortex activity in behaving mice across different physiological need states. We combine this imaging approach with manipulations of peripheral physiology and related circuits to investigate the underlying mechanisms. I will first present our recent data suggesting that insular cortex population activity represents both current bodily states, as well as future predicted ones. I will then describe our current efforts to further our understanding of these predictions, and their potential role in regulating bodily physiology.

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PRESENTATION NUMBER: S211

FOOD FOR THOUGHT: INTERACTIONS BETWEEN DIET, GENES, AND CIRCUITS IN THE NEUROSCIENCE OF NUTRITION

SYMPOSIUM 54 - COMMON MECHANISMS OF BRAIN-BODY INTERACTIONS ACROSS HUMANS AND ANIMAL MODELS - CATHERINE TALLON-BAUDRY, MICAH ALLEN, YOAV LIVNEH, MONICA DUS

Monica Dus, Anoumid Vaziri, Hayeon Sung
The University of Michigan, Department Of Molecular, Cellular, And Developmental Biology, Ann Arbor, United States of America

Diet composition has a profound influence on brain physiology and behavior, but the mechanisms through which nutrient information is transmuted into neural changes remain elusive. Here we uncover how the metabolic enzyme O-GlcNAc Transferase (OGT) transforms information about the dietary environment into taste adaptations. We show that in the fly D. melanogaster, OGT decorates the chromatin of the sweet taste neurons and provides the nutrient context to drive changes in chromatin accessibility in response to high dietary sugar. Specifically, we found that OGT cooperates with the epigenetic silencer Polycomb Repressive Complex 2.1 (PRC2.1) to promote nutrient-sensitive variations in chromatin openness; these chromatin dynamics result in changes in gene expression and taste plasticity that are dependent on the catalytic activity of OGT. Parallel nutrigenomic signatures were also observed in the lingual epithelium of rats exposed to high dietary sugar, suggesting that this conserved metabolic-epigenetic pathway may also underlie diet-dependent taste changes in mammals. Together our findings reveal a novel role for nutriepigenetic signaling in the brain: amplifying nutrient perturbations into robust changes in chromatin accessibility and transcriptional output that shape neural and behavioral plasticity.

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PRESENTATION NUMBER: S212

CONTRACTING EXPANDED CAG/CTG REPEATS USING THE CRISPR-CAS9 NICKASE

SYMPOSIUM 55 - MODULATING REPEAT EXPANSIONS AS A THERAPEUTIC AVENUE IN TANDEM REPEAT DISORDERS - ALVARO MURILLO, KAREN USDIN, IRINA ANTONIJEVIC, NICOLE DEGLON

Laura Heraty[1], Meghan Larin[1], Florence Gidney[1], Alysha Taylor[1], Eleanor Heuchan[1], Emma Randall[1], Alvaro Murillo Bartolome[1], Thomas Massey[2], Lesley Jones[2], Vincent Dion[1]
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Expanded CAG/CTG repeats cause at least 15 different diseases, including Huntington's disease, myotonic dystrophy, and several spinocerebellar ataxias. They all remain without effective treatments. Disease severity scales with the size of the repeat tract and thus contracting them to non-pathogenic lengths is expected to remove the underlying cause of the disease and provide much needed relief. Here we show that the CRISPR-Cas9 nickase targeted to the CAG/CTG repeat itself leads to efficient contractions in multiple cellular systems. It appears to be safe, with undetectable levels of off-target mutations or changes in repeat sizes at other, non-expanded repeat loci. Moreover, sequencing analysis did not reveal rearrangements or mutations, other than the contraction of the repeat tract itself. We further found that contractions are transcription-dependent and they are prevented by FAN1, an endonuclease involved in repeat instability. Together, these results build a case for the translation of the Cas9 nickase approach *in vivo*.

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PRESENTATION NUMBER: S213

RECONCILING DIFFERENCES BETWEEN DIFFERENT TANDEM REPEAT DISEASE MODELS FOR EXPANSION

SYMPOSIUM 55 - MODULATING REPEAT EXPANSIONS AS A THERAPEUTIC AVENUE IN TANDEM REPEAT DISORDERS - ALVARO MURILLO, KAREN USDIN, IRINA ANTONIJEVIC, NICOLE DEGLON

Karen Usdin, Diego Jimenez, Carson Miller, Bruce Hayward, Xiaonan Zhao
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The repeat expansion diseases (REDs) are a group of 40+ disorders that arise from the expansion of a disease-specific short tandem repeat (STR) tract. The expansion mechanism(s) is incompletely understood. Expansions in many of these diseases occur in both the germline and somatic cells. The STRs, which vary in sequence composition and repeat unit length—ranging from 3-12 nucleotides, are situated in different regions of the affected gene. Expansions situated in the coding sequence tend to be relatively small, while expansion of some STRs located outside the primary open reading frame of the gene can involve the gain of hundreds or even thousands of repeats. While some genetic modifiers of expansion risk differ in different mouse models of the REDs, a case can be made that these diseases all share a common expansion mechanism. We suggest that such mechanism likely involves a form of aberrant mismatch repair that proceeds via the production of a double-strand break, with common factors like FAN1 protecting against expansion similarly across all of these diseases. A common expansion mechanism raises the tantalizing possibility that a single treatment strategy could be effective against this entire group of diseases.

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PRESENTATION NUMBER: S214

DEVELOPMENT OF TTX-3360 TO PREVENT ONSET AND/OR PROGRESSION IN HD AND OTHER REPEAT EXPANSION DISORDERS BY HALTING SOMATIC EXPANSION

SYMPOSIUM 55 - MODULATING REPEAT EXPANSIONS AS A THERAPEUTIC AVENUE IN TANDEM REPEAT DISORDERS - ALVARO MURILLO, KAREN USDIN, IRINA ANTONIJEVIC, NICOLE DEGLON

Irina Antonijevic[1], Peter Bialek[2], George Lai[2], Shi-Ying Ding[2], Ming Lee[2], Joseph Hedde[2], Gaurang Patel[2], Deboarh Gouveia[2], Satya Kuchimanchi[2], Jia Tay[2], Brad Snodgrass[2]
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Triplet is harnessing the growing evidence that DNA damage response (DDR) genes are potent modifiers of onset and rate of progression of Repeat Expansion Diseases (REDs), such as Huntington's disease (HD), myotonic dystrophy, and spinocerebellar ataxias. DDR modifier genes act by modulating the rate of somatic expansion at disease causing gene loci, such as mutant Huntingtin (mHTT). The DDR gene *MSH3* has been strongly associated with disease progression in HD, including during premanifest disease. Only about 50% lowering of *MSH3* appears sufficient to halt or markedly slow somatic expansion of mHTT. As no untoward safety signals have been associated with this moderate level of *MSH3* lowering, *MSH3* has emerged as a prime target with the potential to treat HD and multiple other REDs by acting upstream of individual disease genes. Triplet is advancing its *MSH3*-lowering antisense oligonucleotide (ASO) TTX-3360 towards a Phase 1 trial by mid-2022. With a long duration of action in non-human primates (NHPs), TTX-3360 has the potential for infrequent clinical dosing. To support the Phase 1 trial, Triplet has initiated a natural history study, SHIELD HD, in 2020. Premanifest and early manifest people with HD have been enrolled in Europe and North America. SHIELD HD data allow to 1) perform trial simulations to design a robust Proof-of-Concept (PoC) trial, and 2) develop a novel target engagement assay based on measuring *MSH3* messenger RNA (mRNA) in CSF exosomes. Key data from NHPs and SHIELD HD, including the target engagement biomarker assay, will be presented.

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PRESENTATION NUMBER: S215

GENOME EDITING IN THE CENTRAL NERVOUS SYSTEM

SYMPOSIUM 55 - MODULATING REPEAT EXPANSIONS AS A THERAPEUTIC AVENUE IN TANDEM REPEAT DISORDERS - ALVARO MURILLO, KAREN USDIN, IRINA ANTONIJEVIC, NICOLE DEGLON

Nicole Déglon
Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Department Of Clinical Neuroscience, Lausanne, Switzerland

The combined use of gene transfer and editing technologies has pushed the boundaries of precise genome modification and promoted the development of promising strategies to treat genetic diseases, including pathologies affecting the central nervous system. In this presentation, we will describe the different tools available for genome editing and summarize in vivo studies of CNS genome editing with the KamiCas9 self-activating system.

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PRESENTATION NUMBER: S216

THE SUBTHALAMIC NUCLEUS AS A TARGET FOR ADDICTION TREATMENT

SYMPOSIUM 56 - THINKING OUTSIDE THE BOX: ALTERNATIVE TARGETS AND STRATEGIES FOR ADDICTION - MICKAëL DEGOULET, PATRICIA JANAK, SERGE AHMED, MARCO VENNIRO

Mickaël Degoulet
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Transition to addiction does not solely result from the mere consumption of a drug of abuse, but relies on the onset of complex behaviors, such as loss of control over drug intake and perseverance of drug seeking despite its negative consequences. Although critical advances in our understanding of neuronal processes leading to addiction have been made, it still remains a critical problem for the society, affecting both individual and public health. Our recent works indicate that the neuronal activity of the subthalamic nucleus, a small glutamatergic structure of the basal ganglia system, can be used as a proxy for the onset of addiction-like behaviors in rats. Indeed, escalation of cocaine intake, a behavioral model mimicking the loss of control over drug intake, induces changes in the subthalamic nucleus electrical activity, which can further predict which individual would subsequently display compulsive-like seeking behavior. Furthermore, manipulating activity of the subthalamic nucleus with deep brain stimulation can reduce, or worsen, addiction-like behaviors in a frequency-dependent manner. Our results thus emphasize the critical role played by the subthalamic nucleus in the onset and expression of addiction-like behaviors and further advocate for the use of deep brain stimulation in human suffering from addicted disorders.

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PRESENTATION NUMBER: S217

THE VENTRAL PALLIDUM AS A CRITICAL ACTOR IN REWARD PROCESSING

SYMPOSIUM 56 - THINKING OUTSIDE THE BOX: ALTERNATIVE TARGETS AND STRATEGIES FOR ADDICTION - MICKAëL DEGOULET, PATRICIA JANAK, SERGE AHMED, MARCO VENNIRO

Patricia Janak
Johns Hopkins School of Medicine, The Solomon H. Snyder Department Of Neuroscience, Baltimore, United States of America

The governance of reward-seeking behavior requires coordination among multiple brain regions, with the ventral striatopallidal system playing a key role. We have identified an unexpected contribution for the ventral pallidum in reporting a dynamic history-dependent reward preference signal. This signal is sensitive to drive state and largely comports with reward prediction error encoding. Although the ventral pallidum receives a major projection from the nucleus accumbens, our data show that reward-specific neuronal firing in the ventral pallidum is present in a greater proportion of the neural population, and arises sooner following rewarding events, than in the accumbens. These findings challenge the existing model of information flow in the ventral basal ganglia and suggest a reevaluation of the contributions of the ventral pallidum to reward-seeking behavior and reward-based decisions.

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PRESENTATION NUMBER: S218

NON-DRUG REWARDS AS ALTERNATIVE TO DRUG ADDICTION

SYMPOSIUM 56 - THINKING OUTSIDE THE BOX: ALTERNATIVE TARGETS AND STRATEGIES FOR ADDICTION - MICKAëL DEGOULET, PATRICIA JANAK, SERGE AHMED, MARCO VENNIRO

Serge Ahmed
CNRS/Université de Bordeaux, Incia - Umr 5287, Bordeaux, France

Since the first experimental hint for the existence of “an actual desire or striving for the drug” in nonhuman animals by Sidney Spragg in the late 1930s, much effort has been expended by researchers to try to model the key behavioral aspects and signs of addiction in animals, typically in rodents. Despite much advances, there still remains some lingering doubt about the disordered status of drug use in animals. This is mainly because drug use occurs in an artificial setting where animals have access to a drug for self-administration but without access to alternative nondrug reward options that could compete with and divert from continued drug use. I will review evidence showing how offering access to alternative nondrug rewards during drug access can under certain circumstances incite animals to quit using drugs and discuss the possible interpretations of this finding.

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PRESENTATION NUMBER: S219

SOCIAL INTERACTIONS AS ALTERNATIVE REWARDS TO DRUGS

SYMPOSIUM 56 - THINKING OUTSIDE THE BOX: ALTERNATIVE TARGETS AND STRATEGIES FOR ADDICTION - MICKAëL DEGOULET, PATRICIA JANAK, SERGE AHMED, MARCO VENNIRO

Marco Venniro
University of Maryland, School of Medicine, Anatomy And Neurobiology, Baltimore, United States of America

The goal of our research, and of the addiction field in general, has not merely been to identify neuropharmacological and circuit mechanisms of drug addiction and relapse. The goal was to achieve *prospective predictive validity*—the ability to identify new treatments. As I will describe in the talk, the classical relapse model in its traditional form has not appreciably changed the options available to patients in need of relapse prevention. This shortcoming is not unique to relapse, but it is an increasing source of disappointment, and it calls for a regrouping. In our laboratory, we have regrouped by developing a set of approaches that begin with *reverse* translation. As a first step, we have mimicked behavioral treatments that are largely successful in humans such as the community-reinforcement approach where nondrug rewards (e.g., family, friends, employment) are given in exchange for being drug free to maintain abstinence. Our research has revelead three main findings: (1) rats strongly prefer operant social interaction over drugs, (2) social choice-induced abstinence (voluntary abstinence) prevents drug craving, and (3) the protective social-based effect on drug craving is associated with selective brain mechanisms in insula and amygdala. These reverse-translated “treatments” are an ecologically relevant platform from which we can move on to translation itself, using different methods to discover new relapse-related circuits and to identify new medications for relapse prevention in “treated” or post-“treated” rats. In the lecture, I will introduce these animal models, describe our initial pharmacological and circuit results, and discuss implications for treatment.

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Technical Workshops

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PRESENTATION NUMBER: TW001

VOLTAGE IMAGING IN LIVE MICE

WORKSHOP 01 - VOLTAGE IMAGING AND BEYOND, NEXT-GENERATION MOLECULAR AND OPTICAL TOOLS FOR HIGH-SPEED BRAIN IMAGING - HE TIAN, ERIC SCHREITER, ROBERT CAMPBELL, XUE HAN, STéPHANE DIEUDONNE

He Tian
Harvard University, Chemistry And Chemical Biology, Cambridge, United States of America

The brain encodes and processes information through the dynamic membrane voltage of neurons. However, in vivo electrophysiology, i.e., the study of the membrane voltage of individual cells in live animals, has been a major challenge for neuroscience. I will present recent advances in molecular and optical voltage imaging tools to visualize the membrane voltage dynamics of cells in live animals. Imaging of near infrared Archaerhodopsin-derived voltage indicators can be combined with optogenetic stimulation to enable “all-optical electrophysiology”, opening a path for high-throughput characterization of single-cell excitability, synaptic connections, and microcircuit dynamics in live animals. I will discuss how to use voltage imaging and all-optical electrophysiology to understand network dynamics and cellular biophysics in the live mouse brain. Together, these molecular and optical methods greatly expand our ability to decipher mechanisms of brain function.

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PRESENTATION NUMBER: TW002

CHEMIGENETIC INDICATORS OF NEURONAL ACTIVITY

WORKSHOP 01 - VOLTAGE IMAGING AND BEYOND, NEXT-GENERATION MOLECULAR AND OPTICAL TOOLS FOR HIGH-SPEED BRAIN IMAGING - HE TIAN, ERIC SCHREITER, ROBERT CAMPBELL, XUE HAN, STéPHANE DIEUDONNE

Eric Schreiter
Howard Hughes Medical Instituite, Janelia Research Campus, Ashburn, United States of America

Fluorescent indicators of cell physiology have become ubiquitous tools for biology, especially neuroscience, because they allow sensitive monitoring of signals with high spatiotemporal precision over large areas of tissue. Many of the most popular indicators, like the calcium indicator GCaMP, are engineered from fluorescent proteins like GFP. During the last several years, we have collaboratively worked to develop new scaffolds for fluorescent indicators that combine engineered protein sensor domains with small molecule fluorophore reporters that have better photophysical properties than fluorescent proteins, such as higher brightness, improved photostability, and red-shifted emission. We call these hybrid protein-small molecule sensors ‘chemigenetic’ indicators. I will present the development and application of several such chemigenetic indicators, highlighting their advantages over existing sensors.

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PRESENTATION NUMBER: TW003

PUSHING THE WAVELENGTH FRONTIER FOR GENETICALLY ENCODED BIOSENSORS OF NEURAL ACTIVITY

WORKSHOP 01 - VOLTAGE IMAGING AND BEYOND, NEXT-GENERATION MOLECULAR AND OPTICAL TOOLS FOR HIGH-SPEED BRAIN IMAGING - HE TIAN, ERIC SCHREITER, ROBERT CAMPBELL, XUE HAN, STéPHANE DIEUDONNE

Robert Campbell
The University of Tokyo, Department Of Chemistry, Tokyo, Japan

Advances in microscopy and fluorescent probe development are revolutionizing biological research by enabling the normally achromatic world of the cell to be visualized in high resolution and with vivid colours. The Campbell research group uses protein engineering and chemical biology to expand the palette of biosensor colours and specificities, with the overall aim of enabling the visualization of cell signalling and metabolism. Protein engineering, using a combination of rational protein design and directed protein evolution, is the most effective and versatile approach for generating such biosensors. Accordingly, by exploiting structure-guided design, combined with iterative cycles of high-throughput fluorescence image-based screening of bacterial colonies, and lower throughput testing of promising variants in mammalian cells, we are developing a growing selection of fluorescent protein-based biosensors with improved properties. In this seminar I will present some of our most recent efforts to expand the colour and specificity palette of biosensors, focussing on metabolites of relevance to neural metabolism and metal ions other than calcium.

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PRESENTATION NUMBER: TW004

VOLTAGE IMAGING ANALYSIS OF SINGLE NEURON MEMBRANE DYNAMICS DURING BEHAVIOR

WORKSHOP 01 - VOLTAGE IMAGING AND BEYOND, NEXT-GENERATION MOLECULAR AND OPTICAL TOOLS FOR HIGH-SPEED BRAIN IMAGING - HE TIAN, ERIC SCHREITER, ROBERT CAMPBELL, XUE HAN, STéPHANE DIEUDONNE

Xue Han
Boston University, Biomedical Engineering, Boston, United States of America

Recent advances in genetically encoded voltage indicators have enabled the direct measurement of transmembrane voltage changes from individual neurons in the mammalian brain. In this talk, I will present a newly developed genetically encoded voltage indicator SomArchon, which faithfully translates cellular membrane potential dynamics into fluorescence intensity fluctuations. I will describe our recent effort in performing large scale voltage imaging from tens of individual neurons in the brains of behaving mice, and ultrafast ten kilohertz imaging of individual hippocampal neurons. Finally, I will highlight the use of membrane voltage imaging in understanding the relationship of single cell dynamics and circuit states during behavior.

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PRESENTATION NUMBER: TW005

A LIGHT STEERING STRATEGY FOR TWO-PHOTON VOLTAGE IMAGING OF NEURONAL ACTIVITY IN DEPTH

WORKSHOP 01 - VOLTAGE IMAGING AND BEYOND, NEXT-GENERATION MOLECULAR AND OPTICAL TOOLS FOR HIGH-SPEED BRAIN IMAGING - HE TIAN, ERIC SCHREITER, ROBERT CAMPBELL, XUE HAN, STéPHANE DIEUDONNE

Vincent Villette[1], Benjamin Mathieu[2], Jonathan Bradley[1], Walther Akemann[3], Annick Ayon[1], Laurent Bourdieu[3], Stéphane Dieudonne[1]
[1]Ecole Normale Supérieure, PSL University, Ibens, Paris, France, [2]Inserm, Ibens, Ecole Normale Supérieure, Paris, France, [3]Institut de Biologie de l'Ecole Normale Superieure, Cortical Dynamics And Coding Mechanisms, Paris, France

Technologies to record membrane potential *in vivo* in defined neuronal populations with high fidelity will be essential to understand how information is represented, processed, and propagated in the brain. Genetically encoded voltage indicators (GEVIs) are especially promising as they can be expressed in defined cell types and are compatible with long-term chronic imaging *in vivo*. However, optical recording of cellular voltage *in vivo* is constrained by the speed and sensitivity inherent in current indicators and imaging modalities. *In vivo* 2P excitation of membrane proteins has been limited by three main factors: the low number of membrane proteins within a 2P focal volume, the low frame rate of standard 2P imaging and the possible imaging artefacts linked to brain motion in awake behaving mice. To address these three issues, we have developed light patterning strategies based on the acousto-optic technology. These strategies combine fast scanning (resonant rate), fast random-access (100 kHz- 1 MHz), holographic shaping of the focal volume and beam multiplexing. We demonstrate 10 kHz random-access voltage recordings from several neurons in awake behaving mice. Using ASAP3 and JEDI-2P, newly developed fast GEVIs, we report single spike detection with sub-millisecond precision and subthreshold membrane potential recordings with cellular resolution deep in the cortex and in the hippocampus of awake mice. Finally, we present new developments for 3D imaging within a 500x500x500 µm volume at 500 kHz sampling speed.

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PRESENTATION NUMBER: TW006

HIGH RESOLUTION DEEP BRAIN IMAGING USING ADAPTIVE OPTICS THREE-PHOTON MICROSCOPY

WORKSHOP 02 - MULTISCALE OPTICAL TECHNOLOGIES FOR DEEP AND LARGE-VOLUME BRAIN IMAGING - ROBERT PREVEDEL, ELIZABETH HILLMAN, ALIPASHA VAZIRI, ANGUS SILVER, BENJAMIN JUDKEWITZ

Robert Prevedel
European Molecular Biology Laboratory, Cell Biology And Biophysics, Heidelberg, Germany

Multiphoton microscopy has become a powerful tool to visualize the morphology and function of neural cells and circuits in the intact mammalian brain. Yet, tissue scattering, optical aberrations, and motion artifacts degrade the imaging performance at depth. In my talk, I will describe our efforts to overcome these limitations with the aim of advancing deep-tissue imaging in terms of imaging depth and spatial resolution. In particular, I will describe a recently developed minimally invasive intravital imaging methodology based on three-photon excitation, indirect adaptive optics (AO), and active electrocardiogram gating. Here we developed a modal-based, sensorless AO approach which we show to be robust to low signal-to-noise conditions as often encountered in deep in-vivo mouse brain imaging and that is sufficiently fast to be used on samples with slowly varying signal levels, including Ca^{2+} signals in astrocytes. We also found that our approach permits AO correction over large axial fields of view, i.e. AO enhancements in regions far away from the site of AO measurements and optimization. With this technique, we demonstrate near-diffraction-limited imaging of deep cortical spines and (sub)cortical dendrites up to a depth of 1.4 mm in the mouse CA1 hippocampus. In addition, we show applications to deep-layer calcium imaging of astrocytes, including fibrous astrocytes that reside in the highly scattering corpus callosum. Towards the end of my talk, I will discuss complementary strategies that in the future might allow to further push the performance of optical microscopy for recording neural structure and function in-vivo.

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PRESENTATION NUMBER: TW007

LIGHT SHEET MICROSCOPY FOR HIGH-SPEED VOLUMETRIC FUNCTIONAL IMAGING IN THE LIVING BRAIN

WORKSHOP 02 - MULTISCALE OPTICAL TECHNOLOGIES FOR DEEP AND LARGE-VOLUME BRAIN IMAGING - ROBERT PREVEDEL, ELIZABETH HILLMAN, ALIPASHA VAZIRI, ANGUS SILVER, BENJAMIN JUDKEWITZ

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Although point-scanning microscopy can achieve high-resolution imaging deep into the living brain, 3D imaging speeds are fundamentally limited by low per-pixel integration times. Light-sheet microscopy represents a highly efficient form of spatial multiplexing that can overcome these speed limitations, enabling fast, high signal to noise, optically sectioned imaging with minimal photodamage. However, conventional orthogonal light-sheet geometries are unsuitable for high-speed 3D imaging in complex intact samples such as the in-vivo rodent brain. Our single-objective swept confocally-aligned planar excitation (SCAPE) microscopy approach leverages the benefits of light sheet microscopy in a simple form factor that can achieve imaging at between 10-100 volumes per second through a stationary objective lens in a diverse range of living, intact samples. This talk will demonstrate a range of different SCAPE configurations including 2P-SCAPE which uses two-photon light-sheet excitation to capture calcium activity in the living mouse brain to over 400 microns deep at 20 volumes per second with near isotropic 3D sampling over a continuous 300 micron depth range. The unique high-resolution 3D spatiotemporal analysis enabled by this kind of 3D data will also be demonstrated. Meso-scale SCAPE, designed to capture fields-of-view up to 4 mm across will also be demonstrated along with other novel configurations developed for deep brain imaging, as well as pan-neuronal imaging in freely moving C. elegans worms, and brain-wide imaging in awake, behaving Drosophila. This talk will showcase the unqiue and surprising advantages of light-sheet illumination over point-scanning microscopy methods for high-speed 3D functional brain imaging across species.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: TW008

HIGH-SPEED CORTEX-WIDE BRAIN IMAGING WITH MULTIPLEXED MULTI-PHOTON MICROSCOPY

WORKSHOP 02 - MULTISCALE OPTICAL TECHNOLOGIES FOR DEEP AND LARGE-VOLUME BRAIN IMAGING - ROBERT PREVEDEL, ELIZABETH HILLMAN, ALIPASHA VAZIRI, ANGUS SILVER, BENJAMIN JUDKEWITZ

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Understanding how sensory information is represented, processed and leads to generation of complex behavior from the activity of neurons is the major goal of systems neuroscience. However, the ability to detect and manipulate such large-scale functional circuits has been hampered by the lack of appropriate tools and methods that allow parallel and spatiotemporally specific manipulation of neuronal population activity while capturing the dynamic activity of the entire network at high spatial and temporal resolutions. Two-photon microscopy has enabled high-resolution imaging of neuroactivity at depth within scattering brain tissue. However, its various realizations have not overcome the tradeoffs between speed and spatiotemporal sampling that would be necessary to enable mesoscale volumetric recording of neuroactivity at cellular resolution and speed compatible with resolving calcium transients. Light beads microscopy (LBM) is a scalable and spatiotemporally optimal acquisition approach limited only by fluorescence lifetime. In LBM a set of axially separated and temporally distinct foci record the entire axial imaging range near-simultaneously, enabling volumetric recording at 1.41×10^8 voxels per second. Using LBM, we demonstrate mesoscopic and volumetric imaging at multiple scales in the mouse cortex, including cellular-resolution recordings within ~3 × 5 × 0.5 mm volumes containing >200,000 neurons at ~5 Hz and recordings of populations of ~1 million neurons within ~5.4 × 6 × 0.5 mm volumes at ~2 Hz, as well as higher speed (9.6 Hz) subcellular-resolution volumetric recordings. LBM provides an opportunity for discovering the neurocomputations underlying cortex-wide encoding and processing of information in the mammalian brain.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: TW009

MULTISCALE 3D IMAGING OF NEURAL CIRCUITS WITH NONLINEAR ACOUSTO-OPTIC LENS MICROSCOPY

WORKSHOP 02 - MULTISCALE OPTICAL TECHNOLOGIES FOR DEEP AND LARGE-VOLUME BRAIN IMAGING - ROBERT PREVEDEL, ELIZABETH HILLMAN, ALIPASHA VAZIRI, ANGUS SILVER, BENJAMIN JUDKEWITZ

Antoine Valera, Thomas Younts, Victoria Griffiths, Diccon Coyle, Sameer Punde, Km Naga Srinivas Nadella, George Konstantinou, Tomas Fernandez-Alfonso, Paul Kirkby, R. Angus Silver
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Determining how information is represented and transformed in microcircuits is essential for understanding brain function. However, the low density of some cell types, plus their 3D morphological complexity, make them challenging to investigate with conventional two-photon (2P) microscopy. Brain movement in behaving animals also complicates measurements from fine calibre structures like dendrites and axons. To overcome these limitations, we have developed a non-linear acousto-optic lens (nAOL) 2P microscope, which enables ultra-high speed remote focusing and line scanning in any orientation in X,Y, and Z. Inertia-free random-access scanning enables selective imaging of sparsely distributed regions of interest (ROI), such as cell bodies or dendritic trees within the 400x400x400µm volume. Combining 3D scanning with closed-loop FPGA-based processing has enabled real-time tracking and correction for tissue movement in behaving animals. We will present our latest nAOL microscope developments and illustrate its utility for multiscale imaging by showing movement stabilised calcium imaging of the 3D dendritic trees of layer II/III and V pyramidal cells in awake behaving mice. By combining this *Arboreal* scanning with selective imaging of multiple ROIs, we demonstrate multiscale recordings from spines, large fractions of the dendritic tree, and groups of neurons from the surrounding population, simultaneously.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: TW010

WHOLE BRAIN IMAGING IN THE SMALLEST VERTEBRATE BRAIN

WORKSHOP 02 - MULTISCALE OPTICAL TECHNOLOGIES FOR DEEP AND LARGE-VOLUME BRAIN IMAGING - ROBERT PREVEDEL, ELIZABETH HILLMAN, ALIPASHA VAZIRI, ANGUS SILVER, BENJAMIN JUDKEWITZ

Benjamin Judkewitz
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Understanding how distributed neuronal circuits integrate sensory information and generate behavior is a central goal of neuroscience. However, it has been difficult to study neuronal networks at single-cell resolution across the entire adult brain in vertebrates because of their size and opacity. We addressed this challenge by introducing the fish Danionella cerebrum (formerly D. translucida) as a model organism. This teleost remains small and transparent even in adulthood, when neural circuits and behavior have matured. Despite having the smallest known adult vertebrate brain, Danionella displays a rich set of behaviors, including courtship, shoaling, schooling and acoustic communication. In my talk I will present our latest work on oblique plane miscropscopy for large field-of-view whole-brain volumetric imaging in Danionella.

FENS Forum 2022
9-13 July 2022 | Paris, France

PRESENTATION NUMBER: TW011

THE ROLE OF FUNCTIONAL CONNECTIVITY IN THE PROPAGATION OF LESIONS IN ALZHEIMER'S DISEASE

WORKSHOP 03 - NOVEL APPROACHES IN TRANSPECIES WHOLE-BRAIN NETWORK ANALYSIS - ROBIN DE FLORèS, CHRISTOPHE BERNARD, CHRISTINA GRIMM, LAURA HARSAN, MARINA CéLESTINE, DANIEL GUTIERREZ

Robin De Florès, Gaël Chételat
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From a neuropathologic point of view, Alzheimer's Disease (AD) is defined by the presence of neurofibrillary tangles (NFTs) due to hyperphosphorylated tau peptides and extracellular deposits of amyloid β (Aβ). Histopathological observations have consistently reported that these pathologies spread in a stereotypical manner. Nearly 30 years ago, Braak and Braak proposed a hierarchical scheme for tau propagation, where the pathology is first found in the medial temporal lobe before spreading to limbic regions and ultimately to the neocortex. Interestingly, the most sensitive regions to the pathology are strongly interconnected, which has led to the hypothesis that NFTs spread directly from cell to cell through anatomical connections. Technical progresses in neuroimaging methods reinforced this theory decades later, with multimodal studies showing a similar topography between neurodegeneration patterns, as measured using structural MRI or FDG-PET, and specific networks highlighted using resting-state fMRI. Then, the recent development of PET tracers targeting AD molecular alterations (ie NFTs and Aβ) has furthered our understanding of the role of functional connectivity in the propagation of lesions in the course of the disease. For example, studies showed that tau pathology is more strongly and specifically associated to brain networks than Aβ. In addition, tau spreading is not specific to a single network and seems to be partly promoted by the presence of Aβ, although Aβ-independent spreading processes have also been described. Overall, the recent multimodal imaging studies that will be discussed in our presentation play a pivotal role in our understanding of the biological mechanisms underlying AD.

FENS Forum 2022
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PRESENTATION NUMBER: TW012

CONTROLLING BRAIN DYNAMICS: FROM WHOLE BRAIN MODELLING TO EXPERIMENTAL VALIDATION IN INDIVIDUALS

WORKSHOP 03 - NOVEL APPROACHES IN TRANSPECIES WHOLE-BRAIN NETWORK ANALYSIS - ROBIN DE FLORèS, CHRISTOPHE BERNARD, CHRISTINA GRIMM, LAURA HARSAN, MARINA CéLESTINE, DANIEL GUTIERREZ

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Whole-brain modeling offers the possibility to explore mechanisms in silico and provide predictions that can be tested experimentally. As each brain is unique, a personalized approach is necessary. The structural connectome (who is connected to who), which is unique to individuals, enables the construction of a personalized brain avatar. The Virtual Brain (TVB) platform uses individual brain avatars from humans and mice, and generative models to analyze whole-brain dynamics. I will show that individual connectomes from mice contain enough information for a personalized approach of whole-brain dynamics, and thus personalized medicine. I will show how it is possible to control brain dynamics via specific interventions, such as lesions and stimulation, in silico, and verify these predictions experimentally. I will show a concrete application of this approach in patients with drug-resistant epilepsy and who are candidates for neurosurgery.

FENS Forum 2022
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PRESENTATION NUMBER: TW013

CAUSAL INTERPLAY BETWEEN LOCUS COERULEUS AND NETWORK DYNAMICS USING RODENT FMRI COMBINED WITH OPTOGENETICS AND CHEMOGENETICS

WORKSHOP 03 - NOVEL APPROACHES IN TRANSPECIES WHOLE-BRAIN NETWORK ANALYSIS - ROBIN DE FLORèS, CHRISTOPHE BERNARD, CHRISTINA GRIMM, LAURA HARSAN, MARINA CéLESTINE, DANIEL GUTIERREZ

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The Locus Coeruleus (LC) is a brain stem nucleus that provides norepinephrine (NE) to most of the forebrain and contributes to numerous brain functions. Collective behavioural and electrophysiological studies have suggested that the LC/NE system exerts its influence via different neuronal firing modes, which reflect on the amount of terminal NE release. In our previous work, we found that chemogenetic excitation of LC in mice – which mimics strong and sustained LC activation – leads to the recruitment of brain networks like those reported after stressful stimuli in humans. However, the role of the LC/NE system is not limited to stress, and distinct patterns of LC activity can have specific effects on behaviour. In this work, we implemented unilateral optogenetic stimulation of LC noradrenergic neurons at physiologically relevant tonic and burst firing patterns in anaesthetised mice and simultaneously recorded BOLD-fMRI. We show that LC activation evokes changes in whole-brain activity that are specific to both its firing frequency and firing pattern. Furthermore, we demonstrate macroscopic network and energy landscape reconfigurations highly dependent on LC firing frequency and firing pattern by tracking whole-brain BOLD signal fluctuations. Together these observations provide insight into systems-level differences between tonic and burst firing of LC and their impact on brain activity and neural network changes. This project lays the foundation for studying how the LC/NE system modulates whole-brain dynamics in health and disease using fMRI.

FENS Forum 2022
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PRESENTATION NUMBER: TW014

FUNCTIONAL MRI OF LARGE SCALE ACTIVITY IN BEHAVING MICE

WORKSHOP 03 - NOVEL APPROACHES IN TRANSPECIES WHOLE-BRAIN NETWORK ANALYSIS - ROBIN DE FLORèS, CHRISTOPHE BERNARD, CHRISTINA GRIMM, LAURA HARSAN, MARINA CéLESTINE, DANIEL GUTIERREZ

Laura-Adela Harsan[1], Laetitia Degiorgis[1], Marion Sourty[1], Lea Becker[2], Julien Todeschi[1], Vincent Noblet[1], Ipek Yalcin[3]
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Introduction: Maladaptive changes in specific neuromodulatory systems and neural circuits can lead to manifestation of several prominent brain disorders, including anxiety, depression or other pathologies involving cognitive/memory impairments. Resting State Networks mapping and brain diffusion tractography in rodent models are increasingly used to capture such circuitry based underpinnings of pathological traits. **Aims and Methods:** We comparatively present findings from longitudinal mapping of functional and structural brain pathways in rodent models of mood disorders. We use the neuromodulatory approaches to create the depression phenotype in mice, via repetitive optogenetic activation of the pyramidal anterior cingulate cortex (ACC) neurons expressing Channel rhodopsin 2 (ChR). Upon the establishment of the depression phenotype we further use resting state functional MRI (rsfMRI at 7T) as non-invasive read-out of the effects at the level of functional brain connectivity. Seed based analysis and graph theory approaches are used to identify the functional circuitry signatures underlying depression phenotype. Dynamic resting-state functional connectivity (DFC) analyses using a sliding-window method complement the analytic approach. Additionally, we perform opto-fMRI for mapping the dynamics of brain responses upon optogenetic stimulation. **Results and conclusion:** Four consecutive sessions of optogenetic ACC stimulation induce strong depression phenotype and brain-wide modifications of the functional brain networks. Particularly, altered patterns of connectivity between key nodes of the mesocorticolimbc circuitry are observed, suggesting altered cognitive processing of motivation, aversion and reward. Additionally, our data indicate a major impact of optogenetic stimulation on the default mode network (DMN) pattern and on its cross-talk with the rest of the brain.

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PRESENTATION NUMBER: TW015

RESTING-STATE FUNCTIONAL MRI: PROCESSING AND ANALYSIS IN RODENT

WORKSHOP 03 - NOVEL APPROACHES IN TRANSPECIES WHOLE-BRAIN NETWORK ANALYSIS - ROBIN DE FLORèS, CHRISTOPHE BERNARD, CHRISTINA GRIMM, LAURA HARSAN, MARINA CéLESTINE, DANIEL GUTIERREZ

Marina Célestine[1], Jean-Baptiste Perot[2], Nachiket Nadkarni[2], Clément Garin[2], Salma Bougacha[2], Marc Dhenain[2]
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Aims Resting-state functional magnetic resonance imaging (rs-fMRI) provides non-invasive *in vivo* exploration of large-scale brain networks. Yet, whole-brain fMRI imaging in rodents suffers from non-linear geometric and intensity distortions caused by static magnetic field inhomogeneity that worsen at higher field strengths. In the last decade, novel tools have emerged to analyze animal fMRI data including to correct preprocessing, but current software remain less powerful than the available tools that process human brain data. **Methods** We present here an adaptive protocol that perform data preprocessing using flexible and efficient pipelines encompassing in the python library Sammba-MRI. Then, to unveil brain network signatures, we investigated spatial brain signal decomposition using multi-subject dictionary learning. The method was used to investigate Alzheimer's disease (AD) pathology in rodents. Six-month-old APPswe/PS1dE9 mice (n=10) inoculated with AD related amyloid-β (Aβ) seeds were scanned on an 11.7T spectrometer. These mice were compared to their age-matched littermates inoculated with PBS. **Results** First, decomposition of brain signal into 25 components revealed nine bilateral cortical functional components that constitute networks involved in AD. We revealed abnormal brain connectivity through impairment of the default mode and the hippocampal-memory networks in pathological brains. Second, through seed-based analysis and atlas-based functional connectivity analysis, we highlighted functional connectivity disruption of the hippocampus (inoculum site) with other regions. **Conclusions** Our protocol allowed to register rs-fMRI data from numerous subjects and to analyze data using several complementary tools: from network analysis to region-to-region analysis. We were able to highlight functional connectivity differences between animals in pathological condition.

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PRESENTATION NUMBER: TW016

EVOLUTIONARILY CONSERVED FMRI NETWORK DYNAMIC PRINCIPLES IN THE MOUSE, MACAQUE, AND HUMAN BRAIN

WORKSHOP 03 - NOVEL APPROACHES IN TRANSPECIES WHOLE-BRAIN NETWORK ANALYSIS - ROBIN DE FLORèS, CHRISTOPHE BERNARD, CHRISTINA GRIMM, LAURA HARSAN, MARINA CéLESTINE, DANIEL GUTIERREZ

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Networks of synchronized brain activity have been robustly and reproducibly mapped in humans, primates and rodents using resting state fMRI (rsfMRI). Recent research has revealed that fMRI network activity dynamically reconfigures over the time scale of seconds into recurring and reproducible brain states. It is however unclear if the dynamic rules that govern fMRI network activity are species invariant and as such evolutionarily conserved, or if they follow species-specific principles. In my talk, I will illustrate recent research on how rsfMRI network topography and dynamics in the mammalian brain are governed by recurrent transitions between a limited set of fluctuating BOLD Co-Activation Patterns (CAPs), which can be simply obtained by clustering fMRI frames given their spatial similarity. I will explain the rich spatio-temporal features of CAPs and how to compute them. I will next show how this approach is sensitive to disease processes, neuromodulatory input and arousal states, critically shaping the ensuing spatio-temporal patterns of rsfMRI networks activity. I will finally demonstrate how key topographic and dynamic characteristics of CAPs are evolutionarily conserved in mouse, macaque, and human data, providing a novel interpretational framework to comparatively investigate whole-brain dynamics between species.

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Poster Session 01

- Poster Session 01 - Section: Circuitry of Memory and Learning
- Poster Session 01 - Section: Reward Processing and Reinforcement Learning
- Poster Session 01 - Section: Neuroscience Tools, Techniques & Ethics
- Poster Session 01 - Section: Computational Neuroscience of Learning and Memory
- Poster Session 01 - Section: Neuronal Population Coding and Dynamics
- Poster Session 01 - Section: Emergent Dynamics in Neural Networks
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- Poster Session 01 - Section: Stress, Anxiety and Mental Health
- Poster Session 01 - Section: Thermoregulation, Energy Homeostasis and Hormones
- Poster Session 01 - Section: Molecular Neuropharmacology
- Poster Session 01 - Section: Synaptic Plasticity and Cognition
- Poster Session 01 - Section: Synaptic Transmission and Plasticity in Health and Disease
- Poster Session 01 - Section: Subcellular Structure and Mechanisms Involved in Neural Activity
- Poster Session 01 - Section: Brain Inflammation, Neurodevelopment and Disease
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- Poster Session 01 - Section: Pain and Inflammation
- Poster Session 01 - Section: Huntington's Disease
- Poster Session 01 - Section: Optogenetics & Imaging
- Poster Session 01 - Section: Retinal Circuitry and Function

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BOARD NUMBER: S01-001

PERTURBATION OF INHIBITORY ACTIVITY INTERFERES WITH MEMORY REACTIVATION AND PREVENTS LEARNING IN VISUAL ASSOCIATION CORTEX

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

Kristian Kinden Lensjø[1], Ingeborg Nymoen[1], Frederik Rogge[1], Arthur Sugden[2], Inga Shurnayte[2], Sverre Grødem[1], Torkel Hafting[3], Mark Andermann[2], Marianne Fyhn[1]
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Consolidation of salient experiences to form memories rely on precise coupling of high-frequency neural network events between hippocampus and neocortex. Within these events, reactivation of subpopulations of neurons is believed to strengthen connections within and between networks. We have previously shown that reactivation occurs in visual association cortex during learning of a visual association task. Moreover, recent work suggests a role for parvalbumin-expressing (PV) inhibitory interneurons in consolidation by synchronizing hippocampal and cortical activity. We therefore hypothesize that PV interneurons ensure the precise timing between hippocampus and cortex to drive successful reactivations necessary for learning. To investigate this we trained mice in a visual association task, and silenced PV interneurons in visual association cortex after training by chemogenetics on alternating days. In ongoing experiments, we perform two-photon imaging of cell ensembles in visual association cortex, during and after daily training. Activity is estimated by RiboL1-jGCaMP8s fluorescence, and PV+ cells identified (and inhibited) by expressing DIO-DREADD-mScarlet, or DIO-Ruby in control mice. In a separate group we perform simultaneous electrophysiological recordings in visual association cortex and hippocampus area CA1 after training. Our preliminary results indicate that local silencing of PV interneurons strongly attenuates memory consolidation. Notably, the animals' performance improved following training days with saline injections, and reverted to chance levels following training days with PV silencing. Early imaging results suggest that reactivations are present after chemogenetic silencing, but less structured compared to controls. Together our preliminary data indicate that PV silencing during consolidation prevents learning, possibly by interfering with memory reactivations.

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BOARD NUMBER: S01-002

THE ANATOMICAL AND FUNCTIONAL COMPLEXITY OF MEDIAL THALAMUS-PREFRONTAL CORTEX CIRCUIT

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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The medial thalamic regions (MT) play important role in regulation of cognitive processes through the prefrontal cortex (PFC). Previous data suggest a functional dichotomy in the MT-PFC circuit formed by the calretinin (CR+)-expressing and non-expressing (CR-) thalamic cells; however, the exact cellular and network features as well as the effector mechanism(s) of these pathways are poorly understood. Here, by integrating in vivo acute and chronic electrophysiological recordings, anatomical and optogenetic approaches in mice we demonstrate that the CR+ and CR- MT populations have qualitatively and quantitatively distinct cortical and subcortical input/output organization and possess diverse cortical functions. In general, CR+ MT (paraventricular thalamic) neurons have global efferent connections, drive persistent cortical activation and the majority of them show arousal-predicting firing pattern. In contrast, CR- MT (mediodorsal thalamic) cells target much fewer brain regions, provide focal excitations and their activity pattern only follow the behavioral arousal. Within the PFC, the CR+ and CR- MT cells activate qualitatively and quantitatively different PFC networks with laminar and subregional-specificity In addition, the proportions of the CR+ and CR- MT activated principal cells and interneuron are different in the prefrontal cortex. Finally, we also found potential indirect interaction between the CR+ and CR- systems, via the thalamic reticular nucleus and the deep-layers of PFC. These findings indicate that, although the CR+ and CR- MT-PFC networks are anatomically and functionally different, they form sequentially activated ‘inter-loop’ system. Building on each other, they can collectively mediate complex, arousal-dependent cognitive functions.

Pubmed:
32284608: Barsy B, Kocsis K, Magyar A, Babiczky Á, Szabó M, Veres JM, Hillier D, Ulbert I, Yizhar O, Mátyás F
Associative and plastic thalamic signaling to the lateral amygdala controls fear behavior.
Decades of research support the idea that associations between a conditioned stimulus (CS) and an unconditioned stimulus (US) are encoded in the lateral amygdala (LA) during fear learning. However, direct proof for the sources of CS and US information is lacking. Definitive evidence of the LA as the primary site for cue association is also missing. Here, we show that calretinin (Calr)-expressing neurons of the lateral thalamus (CalrLT neurons) convey the association of fast CS (tone) and US (foot shock) signals upstream from the LA in mice. CalrLT input shapes a short-latency sensory-evoked activation pattern of the amygdala via both feedforward excitation and inhibition. Optogenetic silencing of CalrLT input to the LA prevents auditory fear conditioning. Notably, fear conditioning drives plasticity in CalrLT neurons, which is required for appropriate cue and contextual fear memory retrieval. Collectively, our results demonstrate that CalrLT neurons provide integrated CS-US representations to the LA that support the formation of aversive memories.
Nat Neurosci, 2020; 23
30349105: Mátyás F, Komlósi G, Babiczky Á, Kocsis K, Barthó P, Barsy B, Dávid C, Kanti V, Porrero C, Magyar A, Szűcs I, Clasca F, Acsády L
A highly collateralized thalamic cell type with arousal-predicting activity serves as a key hub for graded state transitions in the forebrain.
Sleep cycles consist of rapid alterations between arousal states, including transient perturbation of sleep rhythms, microarousals, and full-blown awake states. Here we demonstrate that the calretinin (CR)-containing neurons in the dorsal

medial thalamus (DMT) constitute a key diencephalic node that mediates distinct levels of forebrain arousal. Cell-type-specific activation of DMT/CR cells elicited active locomotion lasting for minutes, stereotyped microarousals, or transient disruption of sleep rhythms, depending on the parameters of the stimulation. State transitions could be induced in both slow-wave and rapid eye-movement sleep. The DMT/CR cells displayed elevated activity before arousal, received selective subcortical inputs, and innervated several forebrain sites via highly branched axons. Together, these features enable DMT/CR cells to summate subcortical arousal information and effectively transfer it as a rapid, synchronous signal to several forebrain regions to modulate the level of arousal.
Nat Neurosci, 2018; 21

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BOARD NUMBER: S01-003

SYSTEMATIC VARIATION OF SYNAPTIC PLASTICITY MARKERS ACROSS AREAS OF THE TEMPORAL CORTEX IN HUMANS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

Alicia Uceda Heras, Ariadna Sancha Velasco, Carmen Cavada, Miguel Ángel García-Cabezas
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The expression of synaptic plasticity markers varies systematically across cortical areas in primates, being higher in limbic areas, of poor laminar elaboration, and lower in eulaminate areas, with six-well developed layers (Eur J Neurosci 2017; 46: 2392-2405). In the temporal cortex, perirhinal and parahippocampal limbic areas are more vulnerable to epilepsy and Alzheimer's disease, suggesting that synaptic plasticity of cortical areas and selective vulnerability to neurological disorders are linked. In this context, we studied the expression and distribution of markers related to synaptic plasticity across the temporal cortex of neurotypical humans to identify areas with higher synaptic plasticity. Our goal is to stablish baseline distributions of plasticity markers in temporal areas for future comparison with pathological brains. We quantified the intracortical content of myelin and the density of perineuronal nets because both markers are strong inhibitors of synaptic plasticity. We also quantified the expression of GFAP, because it labels activated astrocytes and provides an indirect measurement of metabolic activity. Our data shows increased myelin and perineuronal net density in eulaminate areas compared to limbic areas. The content of myelin also increased across eulaminate areas of progressively better laminar elaboration. In contrast, the expression of GFAP was higher in limbic areas and lower in eulaminate areas. These findings suggest that limbic areas of the human temporal cortex are more plastic and have higher metabolic activity than eulaminate temporal areas. Higher synaptic plasticity of limbic temporal areas may be related to selective vulnerability to neurological disorders showed in perirhinal and parahippocampal areas.

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BOARD NUMBER: S01-004

CONDITIONAL DELETION OF GPRASP2 IN PV-POSITIVE NEURONS DRIVES HIPPOCAMPAL CIRCUIT ALTERATIONS AND COGNITIVE DYSFUNCTION

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

Mariana Laranjo
University of Coimbra, Center For Neuroscience And Cell Biology, Coimbra, Portugal

While no single gene accounts for more than 1% of cases for diagnosed autism spectrum disorder, many neurodevelopmental disorders are connected to functional alterations in synaptic function. We previously showed that global deletion of Gprasp2, a regulator of metabotropic glutamate receptors, leads to cognitive and synaptic communication dysfunction. We found that Gprasp2 is enriched in parvalbumin (PV)-positive inhibitory neurons using in situ mRNA hybridization and single-cell RNA sequencing. Considering the role of PV neurons in brain disorders, we aim to study the consequences of specific loss of Gprasp2 in parvalbumin (PV) positive inhibitory neurons, and if loss of Gprasp2 only in PV-positive neurons is sufficient to drive cognitive and behavioural alterations. To achieve this, we generated a new mouse line via a triple cross of (i) ROSA26 loxP-STOP-loxP-tdTomato- mice with a (ii) PV-Cre driver line and (iii) our conditional Gprasp2 mice, in which Gprasp2 coding exon is flanked with loxP sites. Behavioural assessment and electrophysiological recordings showed that Gprasp2 deletion in PV inhibitory neurons was sufficient to disrupt performance in tasks associated with learning and memory and to alterations in inhibitory currents in the hippocampus of adult mice. These results suggest a functional role for Gprasp2 in PV inhibitory neurons and highlight the relevance of this cell type in the appearance of discrete phenotypes associated with neurodevelopmental disorders.

Pubmed:
30926797: Edfawy M, Guedes JR, Pereira MI, Laranjo M, Carvalho MJ, Gao X, Ferreira PA, Caldeira G, Franco LO, Wang D, Cardoso AL, Feng G, Carvalho AL, Peça J
Abnormal mGluR-mediated synaptic plasticity and autism-like behaviours in Gprasp2 mutant mice.
Autism spectrum disorder (ASD) is characterized by dysfunction in social interactions, stereotypical behaviours and high co-morbidity with intellectual disability. A variety of syndromic and non-syndromic neurodevelopmental disorders have been connected to alterations in metabotropic glutamate receptor (mGluR) signalling. These receptors contribute to synaptic plasticity, spine maturation and circuit development. Here, we investigate the physiological role of Gprasp2, a gene linked to neurodevelopmental disabilities and involved in the postendocytic sorting of G-protein-coupled receptors. We show that Gprasp2 deletion leads to ASD-like behaviour in mice and alterations in synaptic communication. Manipulating the levels of Gprasp2 bidirectionally modulates the surface availability of mGluR and produces alterations in dendritic complexity, spine density and synaptic maturation. Loss of Gprasp2 leads to enhanced hippocampal long-term depression, consistent with facilitated mGluR-dependent activation. These findings demonstrate a role for Gprasp2 in glutamatergic synapses and suggest a possible mechanism by which this gene is linked to neurodevelopmental diseases.
Nat Commun, 2019; 10
30776044: Pinto SMA, Calvete MJF, Ghica ME, Soler S, Gallardo I, Pallier A, Laranjo MB, Cardoso AMS, Castro MMCA, Brett CMA, Pereira MM, Tóth É, Geraldes CFGC
A biocompatible redox MRI probe based on a Mn(ii)/Mn(iii) porphyrin.
For the development of redox responsive MRI probes based on the MnIII/MnII couple, stable complexation of both reduced and oxidized forms of the metal ion and appropriate tuning of the redox potential in the biologically relevant range are key elements. The water soluble fluorinated Mn-porphyrin derivative Mn-3 satisfies both requirements. In aqueous solutions, it can reversibly switch between MnIII/MnII oxidation states. In the presence of ascorbic acid or β-mercaptoethanol, the MnIII form undergoes reduction, which is slowly but fully reversed in the presence of air oxygen. A UV-Vis kinetic study of MnIII/MnII reduction under oxygen-free conditions yielded second-order rate constants, k2, of 46.1 M-1 s-1 and 13.8 M-1 s-1 for the reaction with ascorbic acid and β-mercaptoethanol, respectively. This could correspond, in the absence of oxygen, to a half-life of a few minutes in blood plasma and a few seconds in circulating immune cells where ascorbic acid reaches 20-40 μM and a few mM concentrations, respectively. In contrast to expectations based on the redox potential, reduction with glutathione or cysteine does not occur. It is prevented by the coordination of the glutathione carboxylate group(s) to MnIII in

the axial position, as was evidenced by NMR data. Therefore, MnIII-3 acts as an ascorbate specific turn-on MRI probe, which in turn can be re-oxidized by oxygen. The relaxivity increase from the oxidized to the reduced form is considerably improved at medium frequencies (up to 80 MHz) with respect to the previously studied Mn-TPPS4 analogues; at 20 MHz, it amounts to 150%. No in vitro cytotoxicity is detectable for Mn-3 in the typical MRI concentration range. Finally, 19F NMR resonances of MnIII-3 are relatively sharp which could open further opportunities to exploit such complexes as paramagnetic 19F NMR probes.
Dalton Trans, 2019; 48

FENS Forum 2022
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BOARD NUMBER: S01-005

NEUROMODULATORY LOGIC OF AMYGDALA CIRCUITS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Friedrich Miescher Institute for Biomedical Research, Neurobiology, Basel, Switzerland

Emotional learning and behavior are associated with brain-wide changes in the activity of neuronal circuits. Such global shifts are believed to be implemented by the changes in the neuromodulatory tone. Neuromodulators originate from a few neurons and are released widely throughout the brain to alter the function of target neural circuits. The amygdala plays an essential role in the processing of emotional stimuli, and salience and synaptic alterations in this region are critical for learning and emotional processing. The amygdala is innervated by every major neuromodulator, yet we know little about the dynamics of neuromodulator release under physiological conditions and how neuromodulation regulates amygdala circuit activity during different behavioral states and learning. Here, we characterized the neuromodulatory inputs to the amygdala during reward and aversion. Using novel sensors that report the dynamics of neuromodulator release, paired with multi-site, multi-color fiber photometry, we simultaneously measured the activity of all major neuromodulators in the amygdala. Using this data, we characterized the release patterns during distinct behaviors and identified the interaction between different neuromodulators. Further, using simultaneous optical and electrophysiological recordings from the amygdala, we have identified distinct patterns of neuronal activity that are associated with differential neuromodulatory input during distinct behavioral states. In summary, using this novel approach and multi-modal recordings, we are able to jointly characterize the activity of these two core systems and to generate predictions about the causal influence of simultaneous combinatorial neuromodulation on the activity of downstream circuits and the resulting changes in the behavior.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-006

HIGHLY UNSTABLE HETEROGENEOUS REPRESENTATIONS IN VIP INTERNEURONS OF THE ANTERIOR CINGULATE CORTEX

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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A hallmark of the anterior cingulate cortex (ACC) is its functional heterogeneity. Functional and imaging studies revealed its importance in the encoding of anxiety-related and social stimuli, but it is unknown how microcircuits within the ACC encode these distinct stimuli. One type of inhibitory interneuron, which is positive for vasoactive intestinal peptide (VIP), is known to modulate the activity of pyramidal cells in local microcircuits, but it is unknown whether VIP cells in the ACC (VIP^{ACC}) are engaged by particular contexts or stimuli. Additionally, recent studies demonstrated that neuronal representations in other cortical areas can change over time at the level of the individual neuron. However, it is not known whether stimulus representations in the ACC remain stable over time. Using in vivo Ca^{2+} imaging and miniscopes in freely behaving mice to monitor neuronal activity with cellular resolution, we identified individual VIP^{ACC} that preferentially activated to distinct stimuli across diverse tasks. Importantly, although the population-level activity of the VIP^{ACC} remained stable across trials, the stimulus-selectivity of individual interneurons changed rapidly. These findings demonstrate marked functional heterogeneity and instability within interneuron populations in the ACC. This work contributes to our understanding of how the cortex encodes information across diverse contexts and provides insight into the complexity of neural processes involved in anxiety and social behavior.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-007

REM AND NON-REM SLEEP-DEPENDENT NEURAL DYNAMICS IN THE HIPPOCAMPUS-AMYGDALA NETWORK

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Sleep is a physiological state of reduced vigilance and alertness known to be important for brain homeostasis, memory consolidation, and emotional regulation. During sleep, the brain cycle through two main stages: Non-REM sleep (also called slow-wave sleep) and REM sleep. The neuronal dynamics of REM and Non-REM sleep have been extensively studied, notably in the hippocampus and neocortex, in link with homeostasis and plastic processes related to memory consolidation. The amygdala is a critical brain region for the processing of emotions. However, the sleep dynamics of the amygdala are comparatively understudied, despite a hypothesized role for both REM and Non-REM sleep in emotional memory consolidation. Here, using large-scale recording of LFPs and multiple single-unit activity in freely moving rats, we extensively describe the dynamics of neuronal firing in the basolateral amygdala (BLA) locally and in link with hippocampal sleep rhythms. At transitions from Non-REM to REM sleep, we observe an increase in the activity of BLA interneurons and principal cells. This heightened activity during REM sleep remains stable while firing rates during NREM across extended sleep epochs decrease. Hippocampal theta and local (BLA) theta and gamma oscillations during REM-sleep influence spiking activity in the amygdala. These unique firing patterns in the BLA during REM and Non-REM sleep might underlie the specific roles of these two sleep stages in homeostasis, emotional regulation, and memory.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-008

BRIDGING THE GAP BETWEEN BEHAVIORAL- AND NETWORK-LEVEL PATTERN SEPARATION IN THE DENTATE GYRUS.

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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The large number of granule cells and presence of adult born neurons (ABNs) in the dentate gyrus (DG) have led to the hypothesis that this region is especially sensitive to small changes in input information, making it particularly important when encoding highly similar stimuli, a processed named 'Pattern Separation' (PS). While it is known that ABNs are required for spatial pattern separation at the behavioral level, the contribution of ABNs to network-level computations throughout the hippocampus remains less clear. To address this directly, we have obtained large population recordings from CA1 and CA3 regions of the hippocampus, as well from mature Granule Cells (mGCs) of the DG, before and after a gene-mediated ablation of ABNs as animals perform a spatial PS task. Specifically, we used the UCLA miniscope to obtain these recordings while animals performed on the Spontaneous Location Recognition task (SLR). Here, we report here that ABNs ablation impairs an animals' ability to perform on the SLR-PS task. Preliminary c-fos immunofluorescence experiments indicate an increase in neuronal activation in regions CA3 and CA1 after exposure to a version of the task that requires PS, compared to non-PS versions. We are now analyzing calcium imaging data to identify precisely how ABNs contribute to mGC-DG, CA3 and CA1 population codes that facilitate successful performance on the SLR-PS task.

FENS Forum 2022
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BOARD NUMBER: S01-009

AAV DELIVERY OF SHRNA AGAINST IRS1 IN GABAERGIC NEURONS IN RAT HIPPOCAMPUS ALTERS SYNAPTIC PLASTICITY AND IMPAIRS SPATIAL MEMORY IN FEMALES AND MALE RATS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Brain insulin resistance is a major factor leading to impaired cognitive function. Many studies have revealed how pro-inflammatory cytokines lead to insulin resistance by inhibiting insulin receptor substrate 1 (IRS1) function. Thus, dysfunction of insulin signaling is concomitant with inflammatory biomarkers. However, the effect of IRS1 impaired function in otherwise healthy brain has not been dissected out. Our aim is to study the specific role of IRS1 in the hippocampus, in absence of comorbidities. shRNA against rat and human IRS1 was designed and tested in cultured HEK cells to evaluate mRNA levels and specificity. Best candidate sequence was encapsulated in an AAV vector (strain DJ8). AAV-CMV-shIRS1-EGFP and control AAV-CMV-EGFP were inoculated into dorsal hippocampus of female and male Wistar rats. One month later, animals undertook behavioral paradigms evaluating memory and anxiety. Our results suggest that females displayed increased susceptibility to AAV-shIRS1 in the novel object recognition paradigm; whereas both females and males showed impaired performance in the T-maze when infected with AAV-shIRS1. We observed specific fluorescence within the hilus, in parvalbumin and somatostatin neurons. EGFP fibers were found in fornix, mammillary bodies and medial septum indicating that hippocampal efferent had been efficiently targeted by AAV DJ8 infection. We observed that AAV-shIRS1 alters spine maturation in hilar hippocampal neurons and reduced significantly synaptophysin puncta in hippocampal-septal projections compared to controls. These results support that small alterations in the insulin/IGF1 pathway in specific hippocampal circuitries can underlie modifications in synaptic plasticity and affect behavior, in the absence of inflammatory conditions.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-010

FROM SYNAPSES TO BEHAVIOR: THE IMPACT OF DNA METHYLTRANSFERASE 1 (DNMT1) IN PARVALBUMINERGIC, MURINE INTERNEURONS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

Jenice Linde[1,2], Daniel Pensold[1], Julia Reichard[1,2], Georg Pitschelatow[1,2], Can Yildiz[1,2], Geraldine Zimmer-Bensch[1,2]
[1]RWTH Aachen University, Biology 2 - Functional Epigenetics In The Animal Model, Aachen, Germany, [2]RTG2416 MultiSenses -MultiScales, Biology Ii, Aachen, Germany

The mammalian cerebral cortex, the seat of higher cognitive function, is characterized by a sensitive balance between excitation and inhibition. This balance resembles a key element of the cortex´ function, enabling proper information processing. Although interneurons constitute, in comparison, a minority, their function sets the tone. The input of interneurons is crucial to synchronize surrounding neuronal activity and thus modulate circuit activity. A shift of interneuron abundance and/or function in any direction does usually have severe consequences for the whole organism. For example, this shift can result in neurodevelopmental or neuropsychiatric diseases, such as schizophrenia and autism. Next to genetic causes, environmental stimuli, often in specific phases of brain development, have a great impact on disease onset and severity. Thus, epigenetic mechanisms of transcriptional control were recently moved into the focus of research. Especially, the epigenetic regulation of interneuron development and function represents a disease-relevant topic of interest. Here, we investigated the role of the DNA methyltransferase 1 (DNMT1) in parvalbuminergic (PV) interneurons at different scales. On the cellular level, we found that DNMT1-dependent DNA methylations influence clathrin-mediated endocytosis. Moreover, we show that this change in endocytosis upregulates synaptic vesicle replenishment and GABAergic transmission. At first glance, these results suggest increased inhibition levels of DNMT1-deficient PV-interneurons. To further investigate the functional impacts of the DNMT1-knockout in PV-Cre mice at a behavioral level, we examined their convulsion susceptibility during repeated administrations of pentylenetetrazole as well as their visuo-spatial learning capabilities during navigation in a Morris Water Maze.

Pubmed:

33670788: Bachmann S, Linde J, Bell M, Spehr M, Zempel H, Zimmer-Bensch G
DNA Methyltransferase 1 (DNMT1) Shapes Neuronal Activity of Human iPSC-Derived Glutamatergic Cortical Neurons.
Epigenetic mechanisms are emerging key players for the regulation of brain function, synaptic activity, and the formation of neuronal engrams in health and disease. As one important epigenetic mechanism of transcriptional control, DNA methylation was reported to distinctively modulate synaptic activity in excitatory and inhibitory cortical neurons in mice. Since DNA methylation signatures are responsive to neuronal activity, DNA methylation seems to contribute to the neuron's capacity to adapt to and integrate changing activity patterns, being crucial for the plasticity and functionality of neuronal circuits. Since most studies addressing the role of DNA methylation in the regulation of synaptic function were conducted in mice or murine neurons, we here asked whether this functional implication applies to human neurons as well. To this end, we performed calcium imaging in human induced pluripotent stem cell (iPSC)-derived excitatory cortical neurons forming synaptic contacts and neuronal networks in vitro. Treatment with siRNA that diminishs the expression of the DNA (cytosine-5)-methyltransferase 1 (DNMT1) was conducted to investigate the functional relevance of DNMT1 as one of the main enzymes executing DNA methylations in the context of neuronal activity modulation. We observed a lowered proportion of actively firing neurons upon -knockdown in these iPSC-derived excitatory neurons, pointing to a correlation of DNMT1-activity and synaptic transmission. Thus, our experiments suggest that DNMT1 decreases synaptic activity of human glutamatergic neurons and underline the relevance of epigenetic regulation of synaptic function also in human excitatory neurons.
Int J Mol Sci, 2021; 22
33572758: Pensold D, Gehrmann J, Pitschelatow G, Walberg A, Braunsteffer K, Reichard J, Ravaei A, Linde J, Lampert A, Costa IG, Zimmer-Bensch G
The Expression of the Cancer-Associated lncRNA Is Modulated by EphrinA5-Induced Signaling.
The Eph receptor tyrosine kinases and their respective ephrin-ligands are an important family of membrane receptors, being involved in developmental processes such as proliferation, migration, and in the formation of brain cancer such as glioma. Intracellular signaling pathways, which are activated by Eph receptor signaling, are well characterized. In contrast, it is

unknown so far whether ephrins modulate the expression of lncRNAs, which would enable the transduction of environmental stimuli into our genome through a great gene regulatory spectrum. Applying a combination of functional in vitro assays, RNA sequencing, and qPCR analysis, we found that the proliferation and migration promoting stimulation of mouse cerebellar granule cells (CB) with ephrinA5 diminishes the expression of the cancer-related lncRNA In a human medulloblastoma cell line (DAOY) ephrinA5 stimulation similarly reduced expression. Computational analysis identified triple-helix-mediated DNA-binding sites of in promoters of genes found up-regulated upon ephrinA5 stimulation and known to be involved in tumorigenic processes. Our findings propose a crucial role of downstream of ephrinA5-induced signaling in regulating gene transcription in the nucleus. These findings could be potentially relevant for the regulation of tumorigenic processes in the context of glioma.
Int J Mol Sci, 2021; 22
33041771: Linde J, Zimmer-Bensch G
DNA Methylation-Dependent Dysregulation of GABAergic Interneuron Functionality in Neuropsychiatric Diseases.
Neuropsychiatric diseases, such as mood disorders, schizophrenia, and autism, represent multifactorial disorders, differing in causes, disease onset, severity, and symptoms. A common feature of numerous neuropsychiatric conditions are defects in the cortical inhibitory GABAergic system. The balance of excitation and inhibition is fundamental for proper and efficient information processing in the cerebral cortex. Thus, altered inhibition is suggested to account for pathological symptoms like cognitive impairments and dysfunctional multisensory integration. While it became apparent that most of these diseases have a clear genetic component, environmental influences emerged as an impact of disease manifestation, onset, and severity. Epigenetic mechanisms of transcriptional control, such as DNA methylation, are known to be responsive to external stimuli, and are suspected to be implicated in the functional impairments of GABAergic interneurons, and hence, the pathophysiology of neuropsychiatric diseases. Here, we provide an overview about the multifaceted functional implications of DNA methylation and DNA methyltransferases in cortical interneuron development and function in health and disease. Apart from the regulation of gamma-aminobutyric acid-related genes and genes relevant for interneuron development, we discuss the role of DNA methylation-dependent regulation of synaptic transmission by the modulation of endocytosis-related genes as potential pathophysiological mechanisms underlying neuropsychiatric conditions. Deciphering the hierarchy and mechanisms of changes in epigenetic signatures is crucial to develop effective strategies for treatment and prevention.
Front Neurosci, 2020; 14
32751461: Bayer C, Pitschelatow G, Hannemann N, Linde J, Reichard J, Pensold D, Zimmer-Bensch G
DNA Methyltransferase 1 (DNMT1) Acts on Neurodegeneration by Modulating Proteostasis-Relevant Intracellular Processes.
The limited regenerative capacity of neurons requires a tightly orchestrated cell death and survival regulation in the context of longevity, as well as age-associated and neurodegenerative diseases. Subordinate to genetic networks, epigenetic mechanisms, such as DNA methylation and histone modifications, are involved in the regulation of neuronal functionality and emerge as key contributors to the pathophysiology of neurodegenerative diseases. DNA methylation, a dynamic and reversible process, is executed by DNA methyltransferases (DNMTs). DNMT1 was previously shown to act on neuronal survival in the aged brain, whereby a DNMT1-dependent modulation of processes relevant for protein degradation was proposed as an underlying mechanism. Properly operating proteostasis networks are a mandatory prerequisite for the functionality and long-term survival of neurons. Malfunctioning proteostasis is found, inter alia, in neurodegenerative contexts. Here, we investigated whether DNMT1 affects critical aspects of the proteostasis network by a combination of expression studies, live cell imaging, and protein biochemical analyses. We found that DNMT1 negatively impacts retrograde trafficking and autophagy, with both being involved in the clearance of aggregation-prone proteins by the aggresome-autophagy pathway. In line with this, we found that the transport of GFP-labeled mutant huntingtin (HTT) to perinuclear regions, proposed to be cytoprotective, also depends on DNMT1. Depletion of accelerated perinuclear HTT aggregation and improved the survival of cells transfected with mutant HTT. This suggests that mutant HTT-induced cytotoxicity is at least in part mediated by DNMT1-dependent modulation of degradative pathways.
Int J Mol Sci, 2020; 21
32793592: Hahn A, Pensold D, Bayer C, Tittelmeier J, González-Bermúdez L, Marx-Blümel L, Linde J, Groß J, Salinas-Riester G, Lingner T, von Maltzahn J, Spehr M, Pieler T, Urbach A, Zimmer-Bensch G
DNA Methyltransferase 1 (DNMT1) Function Is Implicated in the Age-Related Loss of Cortical Interneurons.
Increased life expectancy in modern society comes at the cost of age-associated disabilities and diseases. Aged brains not only show reduced excitability and plasticity, but also a decline in inhibition. Age-associated defects in inhibitory circuits likely contribute to cognitive decline and age-related disorders. Molecular mechanisms that exert epigenetic control of gene expression contribute to age-associated neuronal impairments. Both DNA methylation, mediated by DNA methyltransferases (DNMTs), and histone modifications maintain neuronal function throughout lifespan. Here we provide evidence that DNMT1 function is implicated in the age-related loss of cortical inhibitory interneurons. deletion in parvalbumin-positive interneurons attenuates their age-related decline in the cerebral cortex. Moreover, conditional -deficient mice show improved somatomotor

performance and reduced aging-associated transcriptional changes. A decline in the proteostasis network, responsible for the proper degradation and removal of defective proteins, is implicated in age- and disease-related neurodegeneration. Our data suggest that DNMT1 acts indirectly on interneuron survival in aged mice by modulating the proteostasis network during life-time.
Front Cell Dev Biol, 2020; 8
34707847: Joel AC, Linde JRN, Comanns P, Emonts C, Weissbach M, Flecks M, Rödder D
Phylogenetic and morphological influence on habitat choice in moisture-harvesting horned lizards (spp.).
In previous studies, the superhydrophilic skin of moisture-harvesting lizards has been linked to the morphological traits of the lizards' integument, that is, the occurrence of honeycomb-shaped microstructures. Interestingly, these structures can also cover the skin of lizards inhabiting wet habitats. We therefore tested the influence of the microstructures' main features on the habitat choice and wettability in the genus . The genus comprises moisture-harvesting species as well as nonspecialists. Lizards of this genus inhabit large areas of North America with diverse climatic conditions. Remarkably, the differences in the manifestation of microstructures are just as versatile as their surroundings. The phylogeny of the lizards as well as the depth of their ventral microstructures, though independent of each other, correlated with the precipitation in their respective habitat. All other morphological traits, as well as the skin's wettability itself, could not predict the habitat of species. Hence, it is unlikely that the microstructure influences the wettability, at least directly. Hence, we presume an indirect influence for the following reasons: (a) As the ventral side cannot get wet by rain, but the belly could easily interact with a wet surface, the microstructure might facilitate water absorption from wet soil following precipitation. (b) We found the number of dorsal microstructures to be linked to the occurrence of silt in the habitat. In our study, we observed scales being heavily contaminated, most likely with a mixture of dead skin (after shedding) and silt. As many lizards burrow themselves or even shovel sand onto their backs, deploying the substrate might be a mechanism to increase the skin's wettability.
Ecol Evol, 2021; 11

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-011

NEUROPEPTIDERGIC MODULATION OF CORTICAL CIRCUITS FOR FEAR MEMORY

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Neuropeptides throughout the mammalian brain are diverse regulators of circuit excitability and plasticity. All major cell types in the cortex are marked by differential expression of neuropeptide receptors, suggesting highly diverse context- and experience-dependent peptidergic modulation of cortical processing. Our aim is to identify neuropeptides in the cortex that modulate the acquisition and retrieval of fear memories in mice. We found that the bombesin-like neuropeptide gastrin-releasing peptide (GRP) serves as an important regulator of cortical VIP cells. Using in vivo imaging approaches, CRISPR/Cas9-mediated knockout of the GRP receptor and a combination of anterograde and retrograde tracing techniques, we aimed at gaining a detailed understanding of the peptidergic signaling mechanisms and behavioral functions. Our data establish GRP as a regulator of cortical disinhibitory microcircuits that are involved in the modulation of auditory fear memories. We identified several downstream signaling pathways that are putative mediators of this behavioral effect.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-012

DEEP BRAIN IMAGING OF AXON INITIAL SEGMENT DYNAMICS DURING ASSOCIATIVE FEAR LEARNING.

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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The axon initial segment (AIS) is the site of action potential initiation and plays a crucial role in the generation of neuronal activity and the maintenance of network function during sensory processing and learning. Previous studies identified the AIS as a site of homeostatic plasticity. However, whether and how structural AIS plasticity occurs *in vivo* and its potential link to learning remains unknown. Here we established a gradient refractive index (GRIN) lens based two-photon imaging approach to monitor the structure and dynamics of the AIS in the medial prefrontal cortex (mPFC)-amygdala network of mice expressing an intrinsic AIS live-stain. We visualized and tracked AIS structure in the infralimbic subdivision of mPFC across a four-day fear conditioning and extinction paradigm and identified population level changes in AIS length with increasing levels of fear extinction. Importantly, AIS length remained stable during consecutive baseline sessions outside of the learning paradigm. Our results demonstrate that AIS length is dynamic during associative learning *in vivo* and might mediate experience-dependent changes in neuronal excitability and output upon learning.

Pubmed:
33028878: Shifman AR, Sun Y, Benoit CM, Lewis JE
Dynamics of a neuronal pacemaker in the weakly electric fish Apteronotus.
The precise timing of neuronal activity is critical for normal brain function. In weakly electric fish, the medullary pacemaker network (PN) sets the timing for an oscillating electric organ discharge (EOD) used for electric sensing. This network is the most precise biological oscillator known, with sub-microsecond variation in oscillator period. The PN consists of two principle sets of neurons, pacemaker and relay cells, that are connected by gap junctions and normally fire in synchrony, one-to-one with each EOD cycle. However, the degree of gap junctional connectivity between these cells appears insufficient to provide the population averaging required for the observed temporal precision of the EOD. This has led to the hypothesis that individual cells themselves fire with high precision, but little is known about the oscillatory dynamics of these pacemaker cells. As a first step towards testing this hypothesis, we have developed a biophysical model of a pacemaker neuron action potential based on experimental recordings. We validated the model by comparing the changes in oscillatory dynamics produced by different experimental manipulations. Our results suggest that this relatively simple model can capture a large range of channel dynamics exhibited by pacemaker cells, and will thus provide a basis for future work on network synchrony and precision.
Sci Rep, 2020; 10
33903596: Taylor JA, Hasegawa M, Benoit CM, Freire JA, Theodore M, Ganea DA, Innocenti SM, Lu T, Gründemann J
Single cell plasticity and population coding stability in auditory thalamus upon associative learning.
Cortical and limbic brain areas are regarded as centres for learning. However, how thalamic sensory relays participate in plasticity upon associative learning, yet support stable long-term sensory coding remains unknown. Using a miniature microscope imaging approach, we monitor the activity of populations of auditory thalamus (medial geniculate body) neurons in freely moving mice upon fear conditioning. We find that single cells exhibit mixed selectivity and heterogeneous plasticity patterns to auditory and aversive stimuli upon learning, which is conserved in amygdala-projecting medial geniculate body neurons. Activity in auditory thalamus to amygdala-projecting neurons stabilizes single cell plasticity in the total medial geniculate body population and is necessary for fear memory consolidation. In contrast to individual cells, population level encoding of auditory stimuli remained stable across days. Our data identifies auditory thalamus as a site for complex neuronal plasticity in fear learning upstream of the amygdala that is in an ideal position to drive plasticity in cortical and limbic brain areas. These findings suggest that medial geniculate body's role goes beyond a sole relay function by balancing experience-dependent, diverse single cell plasticity with consistent ensemble level representations of the sensory

environment to support stable auditory perception with minimal affective bias.
Nat Commun, 2021; 12

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-013

INTRANASAL DELIVERY OF GALANIN 2 AND NEUROPEPTIDE Y1 AGONISTS ENHANCED SPATIAL MEMORY PERFORMANCE AND ANTIDEPRESSANT EFFECTS THROUGH NEURONAL PRECURSOR CELLS PROLIFERATION IN THE HIPPOCAMPUS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Neuropeptide Y(NPY) Y1 receptor (Y1R) and galanin (GAL) receptor 2 (GALR2) interact in brain regions responsible for learning and memory processes, emphasizing the hippocampus. The current study assesses the sustained memory performance and antidepressive-like effects induced by GALR2 and NPYY1R agonists intranasal coadministration and their neurochemical hippocampal correlates. Object-in-place task and forced swimming test were conducted together with in situ proximity ligation assay (PLA) to manifest the formation of GALR2/Y1R heteroreceptor complexes. We evaluated cell proliferation through a 5-Bromo-2'-deoxyuridine (BrdU) expression study within the hippocampus. The GalR2 agonist M1145 was demonstrated to act with the Y1R agonist to improve memory retrieval and antidepressive-like actions at 24 hours in both tasks, enhancing the cell proliferation in the DG of the hippocampus through BrdU expression and the GALR2/Y1R heteroreceptor complexes upon agonist coactivation. Our results may provide the basis for developing heterobivalent agonist pharmacophores targeting GALR2-Y1R heterocomplexes. It involves especially the neuronal precursor cells of the dentate gyrus in the hippocampus for the novel treatment of Alzheimer's disease or depression. The work was supported by the UMA18-FEDERJA-100 and Proyecto Jovenes Investigadores (B1-2019_04) and Proyecto Puente (B4-2021) UMA , Spain to MN. Special mention to Grupo Vithas.

Pubmed:
33044017: Borroto-Escuela DO, Pita-Rodriguez M, Fores-Pons R, Barbancho MA, Fuxe K, Narváez M
Galanin and neuropeptide Y interactions elicit antidepressant activity linked to neuronal precursor cells of the dentate gyrus in the ventral hippocampus.
A need for new antidepressants is necessary since traditional antidepressants have several flaws. Neuropeptide Y(NPY) Y1 receptor (NPYY1R) and galanin (GAL) receptor 2 (GALR2) interact in several regions of the limbic system, including the hippocampus. The current study assesses the antidepressant effects induced by GALR2 and NPYY1R coactivation, together with the evaluation of cell proliferation through 5-Bromo-2'-deoxyuridine expression within the dentate gyrus of the ventral hippocampus (vDG). We employed in situ proximity ligation assay to manifest GALR2/NPYY1R heteroreceptor complexes. Additionally, the expression pattern of GALR2 and the activation of the extracellular-regulated kinases (ERK) pathway after GALR2 and NPYY1R costimulation in cell cultures were examined. GALR2 and NPYY1R coactivation resulted in sustained antidepressant behaviors in the FST after 24 h, linked to increased cell proliferation in the vDG. Moreover, an increased density of GALR2/NPYY1R heteroreceptor complexes was observed in vDG, on doublecortin-expressing neuroblasts. Recruitment of the GALR2 expression to the plasma membrane was observed upon the coactivation of GALR2 and NPYY1R in cell cultures, presumably associated to the enhanced effects on the activation of ERK pathway. GALR2 may promote the GALR2/NPYY1R heteroreceptor complexes formation in the ventral hippocampus. It may induce a transformation of cell proliferation toward a neuronal lineage by enhancement of ERK pathway. Thus, it may give the mechanism for the antidepressant behavior observed. These results may provide the basis for the development of heterobivalent agonist pharmacophores, targeting GALR2/NPYY1R heteromers, especially in the neuronal precursor cells of the dentate gyrus in the ventral hippocampus for the novel treatment of depression.
J Cell Physiol, 2021; 236
32229140: Narváez M, Andrade-Talavera Y, Valladolid-Acebes I, Fredriksson M, Siegele P, Hernandez-Sosa A, Fisahn A, Fuxe K, Borroto-Escuela DO
Existence of FGFR1-5-HT1AR heteroreceptor complexes in hippocampal astrocytes. Putative link to 5-HT and FGF2 modulation of hippocampal gamma oscillations.

The majority of the fibroblast growth factor receptor 1-serotonin 1 A receptor (FGFR1-5-HT1AR) heterocomplexes in the hippocampus appeared to be located mainly in the neuronal networks and a relevant target for antidepressant drugs. Through a neurochemical and electrophysiological analysis it was therefore tested in the current study if astrocytic FGFR1-5-HT1AR heterocomplexes also exist in hippocampus. They may modulate the structure and function of astroglia in the hippocampus leading to possible changes in the gamma oscillations. Localization of hippocampal FGFR1-5-HT1AR heterocomplexes in astrocytes was found using in situ proximity ligation assay combined with immunohistochemistry using glial fibrillary acidic protein (GFAP) immunoreactivity as a marker for astroglia. Acute i.c.v. treatment with 8-OH-DPAT alone or together with basic fibroblast growth factor (FGF2) significantly increased FGFR1-5-HT1AR heterocomplexes in the GFAP positive cells, especially in the polymorphic layer of the dentate gyrus (PoDG) but also in the CA3 area upon combined treatment. No other hippocampal regions were studied. Also, structural plasticity changes were observed in the astrocytes, especially in the PoDG region, upon these pharmacological treatments. They may also be of relevance for enhancing the astroglial volume transmission with increased modulation of the neuronal networks in the regions studied. The effects of combined FGF2 and 5-HT agonist treatments on gamma oscillations point to a significant antagonistic interaction in astroglial FGFR1-5-HT1AR heterocomplexes that may contribute to counteraction of the 5-HT1AR-mediated decrease of gamma oscillations. This article is part of the special issue entitled 'Serotonin Research: Crossing Scales and Boundaries'.
Neuropharmacology, 2020; 170
22035699: Borroto-Escuela DO, Romero-Fernandez W, Mudó G, Pérez-Alea M, Ciruela F, Tarakanov AO, Narvaez M, Di Liberto V, Agnati LF, Belluardo N, Fuxe K
Fibroblast growth factor receptor 1- 5-hydroxytryptamine 1A heteroreceptor complexes and their enhancement of hippocampal plasticity.
The hippocampus and its 5-hydroxytryptamine transmission plays an important role in depression related to its involvement in limbic circuit plasticity.
Biol Psychiatry, 2012; 71
24841617: Narváez M, Millón C, Borroto-Escuela D, Flores-Burgess A, Santín L, Parrado C, Gago B, Puigcerver A, Fuxe K, Narváez JA, Díaz-Cabiale Z
Galanin receptor 2-neuropeptide Y Y1 receptor interactions in the amygdala lead to increased anxiolytic actions.
Galanin (GAL) and neuropeptide Y (NPY) are neuropeptides involved in behaviors associated with anxiety. Both neuropeptides interact in several central functions. However, the potential behavioral and cellular interactions between them in anxiety are unknown. GAL was found to act through GAL receptor 2 (GALR2) to enhance NPYY1 receptor (NPYY1R)-mediated anxiolytic behaviors in rats. Using receptor autoradiography, c-Fos expression and in situ proximity ligation assay, the medial paracapsular intercalated nuclei of the amygdala were determined to be a key area in the interaction probably involving the formation of GALR2/NPYY1R heteroreceptor complexes. In cell cultures costimulation of GALR2 and NPYY1R induced changes in the functions of these receptors. The changes involved a potentiation of the decrease in the phosphorylation of CREB induced by NPYY1R and a delay in the internalization of NPYY1R. These results indicate that GALR2/NPYY1R interactions can provide a novel integrative amygdaloid mechanism in anxiety.
Brain Struct Funct, 2015; 220
26666529: Narváez M, Borroto-Escuela DO, Millón C, Gago B, Flores-Burgess A, Santín L, Fuxe K, Narváez JA, Díaz-Cabiale Z
Galanin receptor 2-neuropeptide Y Y1 receptor interactions in the dentate gyrus are related with antidepressant-like effects.
Galanin (GAL) and the NPYY1 agonist play a role in mood regulation and both neuropeptides interact in several central functions. The present study examined the interaction between Galanin receptor 2 (GALR2) and Neuropeptide Y Y1 receptor (NPYY1R) in the dentate gyrus (DG) of the Hippocampus in relation to depression-like behavior. Using receptor autoradiography, in situ hybridization and in situ proximity ligation assay an interaction between GALR and NPYY1R was demonstrated in the DG probably involving the formation of GALR2-NPYY1R heteroreceptor complexes. These complexes were specifically observed in the polymorphic and subgranular subregions of the DG, where both receptors were found to colocalize. Moreover, this GALR2/NPYY1R interaction was linked to an enhancement of the antidepressive-like behavior mediated by NPYY1R in the forced swimming test. Specific cells populations within DG subregions may be involved in this behavioral effect since the coactivation of GALR2 and NPYY1R enhances the NPYY1R-mediated reduction in the number of c-Fos immunoreactive nuclei in the polymorphic region. These results indicate that GALR2/NPYY1R interactions can provide a novel integrative mechanism in DG in depression-related behavior and may give the basis for the development of drugs targeting GALR2/NPYY1R heteroreceptor complexes in the DG of the hippocampus for the treatment of depression.
Brain Struct Funct, 2016; 221
26792005: Millón C, Flores-Burgess A, Narváez M, Borroto-Escuela DO, Santín L, Gago B, Narváez JA, Fuxe K, Díaz-Cabiale Z
Galanin (1-15) enhances the antidepressant effects of the 5-HT1A receptor agonist 8-OH-DPAT: involvement of the raphe-

hippocampal 5-HT neuron system.
Galanin N-terminal fragment (1-15) [GAL(1-15)] is associated with depression-related and anxiogenic-like effects in rats. In this study, we analyzed the ability of GAL(1-15) to modulate 5-HT1A receptors (5-HT1AR), a key receptor in depression. GAL(1-15) enhanced the antidepressant effects induced by the 5-HT1AR agonist 8-OH-DPAT in the forced swimming test. These effects were stronger than the ones induced by Galanin (GAL). This action involved interactions at receptor level since GAL(1-15) affected the binding characteristics and the mRNA levels of 5-HT1AR in the dorsal hippocampus and dorsal raphe. The involvement of the GALR2 was demonstrated with the GALR2 antagonist M871. Proximity ligation assay experiments indicated that 5-HT1AR are in close proximity with GALR1 and GALR2 in both regions and in raphe RN33B cells. The current results indicate that GAL(1-15) enhances the antidepressant effects induced by 8-OH-DPAT acting on 5-HT1AR operating as postjunctional or as autoreceptors. These results may give the basis for the development of drugs targeting potential GALR1-GALR2-5-HT1AR heteroreceptor complexes linked to the raphe-hippocampal 5-HT neurons for the treatment of depression.
Brain Struct Funct, 2016; 221
32565059: Di Palma M, Sartini S, Lattanzi D, Cuppini R, Pita-Rodriguez M, Diaz-Carmenate Y, Narvaez M, Fuxe K, Borroto-Escuela DO, Ambrogini P
Evidence for the existence of A2AR-TrkB heteroreceptor complexes in the dorsal hippocampus of the rat brain: Potential implications of A2AR and TrkB interplay upon ageing.
Adenosine A2A receptors (A2AR) are crucial in facilitating the BDNF action on synaptic transmission in the rat hippocampus primarily upon ageing. Furthermore, it has been suggested that A2AR-Tropomyosin related kinase B receptor (TrkB) crosstalk has a pivotal role in adenosine A2AR-mediated modulation of the BDNF action on hippocampal plasticity. Considering the impact of the above receptors interplay on what concerns BDNF-induced enhancement of synaptic transmission, gaining a better insight into the mechanisms behind this powerful crosstalk becomes of primary interest. Using in situ proximity ligation assay (PLA), the existence of a direct physical interaction between adenosine A2AR and TrkB is demonstrated. The A2AR-TrkB heteroreceptor complexes show a heterogeneous distribution within the rat dorsal hippocampus. High densities of the heteroreceptor complexes were observed in the pyramidal cell layers of CA1-CA3 regions and in the polymorphic layer of the dentate gyrus (DG). The stratum radiatum of the CA1-3 regions showed positive PLA signal in contrast to the oriens region. The molecular and granular layers of the DG also lacked significant densities of PLA positive heteroreceptor complexes, but subgranular zone showed some PLA positive cells. Their allosteric receptor-receptor interactions may significantly modulate BDNF signaling impacting on hippocampal plasticity which is impaired upon ageing.
Mech Ageing Dev, 2020; 190
30972626: Borroto-Escuela DO, Narváez M, Romero-Fernández W, Pinton L, Wydra K, Filip M, Beggiato S, Tanganelli S, Ferraro L, Fuxe K
Acute Cocaine Enhances Dopamine DR Recognition and Signaling and Counteracts DR Internalization in Sigma1R-DR Heteroreceptor Complexes.
The current study was performed to establish the actions of nanomolar concentrations of cocaine, not blocking the dopamine transporter, on dopamine D2 receptor (DR)-sigma 1 receptor (δ1R) heteroreceptor complexes and the DR protomer recognition, signaling and internalization in cellular models. We report the existence of DR-δ1R heteroreceptor complexes in subcortical limbic areas as well as the dorsal striatum, with different distribution patterns using the in situ proximity ligation assay. Also, through BRET, these heteromers were demonstrated in HEK293 cells. Furthermore, saturation binding assay demonstrated that in membrane preparations of HEK293 cells coexpressing DR and δ1R, cocaine (1 nM) significantly increased the DR B values over cells singly expressing DR. CREB reporter luc-gene assay indicated that coexpressed δ1R significantly reduced the potency of the DR-like agonist quinpirole to inhibit via DR activation the forskolin induced increase of the CREB signal. In contrast, the addition of 100 nM cocaine was found to markedly increase the quinpirole potency to inhibit the forskolin-induced increase of the CREB signal in the DR-δ1R cells. These events were associated with a marked reduction of cocaine-induced internalization of DR protomers in DR-δ1R heteromer-containing cells vs DR singly expressing cells as studied by means of confocal analysis of DR-δ1R trafficking and internalization. Overall, the formation of DR-δ1R heteromers enhanced the ability of cocaine to increase the DR protomer function associated with a marked reduction of its internalization. The existence of DR-δ1R heteromers opens up a new understanding of the acute actions of cocaine.
Mol Neurobiol, 2019; 56
25522404: Millón C, Flores-Burgess A, Narváez M, Borroto-Escuela DO, Santín L, Parrado C, Narváez JA, Fuxe K, Díaz-Cabiale Z
A role for galanin N-terminal fragment (1-15) in anxiety- and depression-related behaviors in rats.
Galanin (GAL) plays a role in mood regulation. In this study we analyzed the action of the active N-terminal fragment [GAL(1-15)] in anxiety- and depression-related behavioral tests in rats.
Int J Neuropsychopharmacol, 2014; 18

21396946: Díaz-Cabiale Z, Parrado C, Narváez M, Puigcerver A, Millón C, Santín L, Fuxe K, Narváez JA
Galanin receptor/Neuropeptide Y receptor interactions in the dorsal raphe nucleus of the rat.
The aim of this study was to evaluate by quantitative receptor autoradiography the interactions between Neuropeptide Y Y1 (NPY Y1) and Galanin (GAL) receptors in the dorsal raphe nucleus (DRN) where both GAL receptors and NPY Y1 receptors exist. The ability of the GAL receptor antagonist M35 to block the GAL action was also evaluated. Double immunocytochemical staining of 5-hydroxytryptmine and c-Fos and stereology techniques were used to study the specific cell activation in the DRN after the intracerebroventricular coinjections of GAL and the NPY Y1/Y5 agonist [(125)I] Leu(31),Pro(34)PYY. GAL (0.3 nM) decreases [(125)I] Leu(31),Pro(34)PYY binding in the DRN by 48% ($p < 0.01$) as shown by quantitative receptor autoradiography. This effect was reversed with the GAL receptor antagonist M35. Intracerebroventricular coinjections of NPY Y1/Y5 agonist and GAL reduced the c-Fos expression in the serotoninergic cells induced by the NPY Y1/Y5 agonist in DRN. These results indicate the existence of antagonistic interactions between GAL receptors and NPY Y1 receptors in the DRN that may be of relevance in mood disorders.
Neuropharmacology, 2011 Jul-Aug; 61

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-014

CHOLINERGIC MODULATION OF AUDITORY THALAMUS UPON ASSOCIATIVE LEARNING

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Associative learning links predictive sensory stimuli from the environment with their outcomes. The accuracy of this learning will depend on reliable integration of sensory inputs that result in behavioural adaptations to ensure an animal's survival. Several cortical and limbic brain areas have been identified as sites for associative learning. However, the role of thalamic structures that relay sensory information is largely unknown. The medial geniculate body (MGB), or auditory thalamus, is a site of convergence for auditory as well as somatosensory information and is crucial for associative learning (e.g. in fear conditioning). It receives feedforward sensory as well as neuromodulatory inputs. One prominent neuromodulatory cholinergic input to MGB originates in the pedunculopontine tegmental nucleus (PPT). However, the role of brainstem cholinergic inputs to MGB during associative learning remains elusive. Here, we use a combination of deep brain calcium imaging techniques such as fibre photometry and miniaturized microscopes combined with optogenetics to unravel the functional role of cholinergic projections in MGB during associative fear learning in freely moving mice. We found that optogenetic manipulation of cholinergic PPT inputs in MGB during fear acquisition affects learning. Furthermore, we show that cholinergic projections from the brainstem modulate sensory responses of MGB neurons during fear conditioning. This study identifies the role of brainstem cholinergic inputs in multimodal sensory integration during fear conditioning in MGB, which broadens our view on how neuromodulators contribute to associative learning in thalamic areas.

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BOARD NUMBER: S01-015

IMPROVED DISCRIMINATION LEARNING AFTER SPATIO-TEMPORAL DISRUPTION OF PLANAR CELL POLARITY SIGNALLING USING TOUCH-SCREEN-BASED TEST

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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To be able to remember past experiences, we need to use two types of memory: one that will allow the “reconstruction” of the memory from sometimes partial information (pattern completion), one that will allow the discrimination of one memory from a very similar one (pattern separation). Pattern separation (PS) and pattern completion (PC) rely on a key brain structure of episodic memory, the hippocampus. Mechanistically, PS and PC are supported by a hippocampal circuit: the dentate gyrus (DG) and the CA3. Using a conditional knockout, we previously showed that the core Planar Cell Polarity (PCP) protein Vangl2 participate in these processes (Robert et al., 2020). We showed that a specific postnatal deletion of Vangl2 in the DG, impairs the PC and improves the PS processes. In order to bypass the need for Vangl2 deletion and modulate PCP signalling with spatio-temporal resolution, our team developed a molecular construct in order to block PCP signalling, without affecting Vangl2 levels. We injected the virus in the hippocampus of adult animal before testing the mice in PS and PC protocols. For this, we used an innovating and non-aversive technology, called the touchscreen chamber. Our preliminary results show an improvement in the discrimination memory test in the mice model for PCP disruption, supporting our team’s previous results.
Key-words: Hippocampus, PCP, behaviour, touchscreen, pattern separation/completion, DG/CA3 circuit

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BOARD NUMBER: S01-016

DISSOCIATING OBJECT VERSUS SPATIAL PATTERN SEPARATION: FROM BEHAVIOR TO NEURONAL CIRCUIT

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Convergent behavioral, physiological, and anatomical observations have led to the hypothesis that the dentate gyrus (DG) of the hippocampus is especially sensitive to small changes in input, making it particularly important when encoding highly similar stimuli, a process named ‘pattern separation’. The entorhinal cortex (EC) is the main source of cortical inputs to the DG: while its medial segment (MEC) preferentially forwards spatial information, its lateral part (LEC) rather sends object-related information. Reelin-positive cells, located in the layer II EC, send direct projections onto granule cell dendrites, DG’s largest excitatory population. Nevertheless, how the EC-DG circuit defined by these two cell types participates to object and spatial pattern separation remains largely elusive. To address this question, we use comparable behavioral paradigms to assess object and spatial pattern separation in mice combined with immunohistological detection of cfos protein, a proxy of neuronal activation. Preliminary results show greater activation of the DG when spatial - but not object - pattern separation is required. On the other hand, object pattern separation seems to activate layer II cells in the LEC. Thanks to selective viral delivery of inhibitory DREADD receptors in sim1-cre mice – a mouse line that specifically allows manipulating reelin cells in the EC – we will further seek to determine whether inhibition of MEC or LEC reelin-positive neurons is associated with a deficit in spatial (MEC-DG) and object (LEC-DG) pattern separation.

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COGNITIVE DEFICITS IN MOUSE MODEL OF FRAGILE X SYNDROME REFLECT SPECIFIC MEMORY ENSEMBLE RECRUITMENT FAILURE ALONG THE VENTRAL HIPPOCAMPUS-PRELIMBIC CORTEX AXIS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Fragile X Syndrome (FXS) is the most common form of intellectual disability and a leading monogenic cause of autism spectrum disorder (ASD). The protein whose expression is lost in FXS (FMRP) is expressed ubiquitously in the brain, where it has important roles for synaptic function and plasticity. Whether the defects in FXS, and their potential therapeutic alleviation, reflect ubiquitous loss of function or deficits specific to particular brain circuits has remained unclear. Here we show using brain area specific conditional knockout of FMRP (cKO) in principal neurons, that area-specific loss in adult wildtype (WT) mice behaviorally mimics acute silencing of the same area. However, in Fmr1(y/-) mice despite the ubiquitous absence of FMRP, behavioral and immediate early gene (IEG) expression analyses, revealed that cognitive functions of most brain areas were unaffected by the absence of FMRP, with the exception of ventral Hippocampus (vH) and Prelimbic cortex (PreL). Using conditional restoration of FMRP in Fmr1(y/-), we found that the specific behavioral deficits of Fmr1(y/-) mice can entirely be accounted for by absence of FMRP in a vH-PreL axis. Mechanistic investigations revealed that despite of apparently normal re-expression of cFos at recall in vH neurons of Fmr1(y/-) mice, vH cFos+ ensemble recruitment was insufficient for cognitive function.

Pubmed:

31289139: Tripodi M, Bhandari K, Chowdhury A, Mukherjee A, Caroni P
Parvalbumin Interneuron Plasticity for Consolidation of Reinforced Learning.
Parvalbumin (PV) basket cells are widespread local interneurons that inhibit principal neurons and each other through perisomatic boutons. They enhance network function and regulate local ensemble activities, particularly in the γ range. Organized network activity is critically important for long-term memory consolidation during a late time window 11-15 h after acquisition. Here, we discuss the role of learning-related plasticity in PV neurons for long-term memory consolidation. The plasticity can lead to enhanced (high-PV) or reduced (low-PV) expression of PV/GAD67. High-PV plasticity is induced upon definite reinforced learning in early-born PV basket cells, whereas low-PV plasticity is induced upon provisional reinforced learning in late-born PV basket cells. The plasticity is first detectable 6 h after acquisition, at the end of a time window for memory specification through experience, and is critically important 11-15 h after acquisition for enhanced network activity and long-term memory consolidation. High- and low-PV plasticity appear to regulate activity in distinct local networks of principal neurons and PV basket cells. These findings suggest how flexibility and stability in learning and memory might be implemented through parallel circuits and networks.
Cold Spring Harb Symp Quant Biol, 2018; 83

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BOARD NUMBER: S01-018

DO THE REUNIENS/RHOMBOID NUCLEI PARTICIPATE IN ENCODING CONTEXTUAL MEMORY?

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Memory consolidation at systems level refers to the process by which a recently encoded memory trace is transformed into a persistent one. This process requires a hippocampo-cortical dialog. The midline thalamic nuclei, the reuniens and rhomboid (ReRh), and their bidirectional connections with the hippocampus and medial prefrontal cortex holds a key position to regulate these cortico-hippocampal interactions. We previously demonstrated their role in contextuel memory persistence, after permanent fiber-sparing ReRh lesion (Quet et al., 2020). We have also shown that the ReRh nuclei are not required for retrieval process of such memory. Based on the idea that interactions crucial for systemic memory consolidation might also be engaged during information encoding, we asked if the ReRh nuclei are necessary for encoding contextual information. We used a chemogenetic ReRh inhibition (DREADD) performed before encoding a context in a fear-conditioning memory task. We found that pre-encoding ReRh inactivation had no impact on contextual fear conditioning, although DREADD silencing altered a strategy-shifting task sensitive to the disruption of ReRh function. The data suggest that ReRh nuclei do not play a crucial role in processing contextual information, what challenges recent data pointing to their possible contribution to contextual encoding.

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COMMUNICATION BETWEEN THE NUCLEUS INCERTUS AND THE MEDIAL SEPTUM AND THE ENTORHINAL CORTEX

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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The nucleus incertus (NI) in the pontine tegmentum contains the largest population of neurons that produce and release the neuropeptide Relaxin3. The NI is involved in different cognitive processes such as memory and learning, mediated by the medial septum, hippocampus, and entorhinal cortex. A portion of these connections are mediated by Relaxin3 and its receptor RXFP3. This work aims to analyse is collateralization of the projections from the NI to the medial septum and/or the medial entorhinal cortex. Combinations of the retrograde tracers FluoroGold (FG) and cholera toxin subunit B (CTB) injected in the medial septum (MS) or medial entorhinal cortex (MEnt) and lateral entorhinal cortex (LEnt) with immunofluorescence to relaxin3. After surgeries, the number of Relaxin3 positive neurons projecting from the NI to each of these nuclei was recorded. Retrograde tracer injections placed in the medial and lateral entorhinal cortex produced prominent retrograde labelling in the ipsilateral NI and some labelling in the contralateral NI. Moreover, the projections from the NI to the MS were twice as numerous as to the entorhinal. Most of NI neurons projects independently to the medial septum or entorhinal cortex. In addition, we observed that approximately a 21% of the NI-MS, 28% of NI-MEnt and 37% of NI-LEnt projections were Relaxin3 positive. These results show the importance of the Relaxin3-NI projections over the medial septum and entorhinal cortex.

Pubmed:
33867946: Gil-Miravet I, Mañas-Ojeda A, Ros-Bernal F, Castillo-Gómez E, Albert-Gascó H, Gundlach AL, Olucha-Bordonau FE
Involvement of the and Relaxin-3/RXFP3 Signaling System in Explicit and Implicit Memory.
Telencephalic cognitive and emotional circuits/functions are strongly modulated by subcortical inputs. The main focus of past research on the nature of this modulation has been on the widespread monoamine projections to the telencephalon. However, the (NI) of the pontine tegmentum provides a strong GABAergic and peptidergic innervation of the hippocampus, basal forebrain, amygdala, prefrontal cortex, and related regions; and represents a parallel source of ascending modulation of cognitive and emotional domains. NI GABAergic neurons express multiple peptides, including neuromedin-B, cholecystokinin, and relaxin-3, and receptors for stress and arousal transmitters, including corticotrophin-releasing factor and orexins/hypocretins. A functional relationship exists between NI neurons and their associated peptides, relaxin-3 and neuromedin-B, and hippocampal theta rhythm, which in turn, has a key role in the acquisition and extinction of declarative and emotional memories. Furthermore, RXFP3, the cognate receptor for relaxin-3, is a G protein-coupled receptor, and its activation inhibits the cellular accumulation of cAMP and induces phosphorylation of ERK, processes associated with memory formation in the hippocampus and amygdala. Therefore, this review summarizes the role of NI transmitter systems in relaying stress- and arousal-related signals to the higher neural circuits and processes associated with memory formation and retrieval.
Front Neuroanat, 2021; 15

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BOARD NUMBER: S01-020

LEARNING FLEXIBILITY IN PARAHIPPOCAMPAL NETWORKS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Mammals show remarkable flexibility of behaviors, and have the ability to abstract specific rules and learning sets into generalized low dimensional structures that can be applied in new conditions. Recent work suggests that this generalization could take place in parahippocampal regions, in particular in the entorhinal cortex (EC). Here, the medial part (MEC) seems to extract the structure of state space irrespective of modality (e.g. spatial or sound). The lateral part (LEC) receives input about complex features of objects and multimodal sensory information from association cortices, including the postrhinal cortex (POR). This puts the EC in a unique position to function as an integration center for higher order representations. To investigate the role of the entorhinal cortex in abstracting and generalizing task content, we have established a set of simple association tasks for mice that enable both rule switching and change of sensory modality. We train mice in these tasks over multiple sessions and record large neuronal populations in the EC during and after training using two-photon imaging. To this end, we utilize custom right-angle prism assemblies that cover either the MEC, or POR and LEC. We hypothesize that both MEC and LEC will display learning-induced changes in the low dimensional neural representations over time, with consistent structures that are used and transferred across different tasks. Our preliminary data from mice trained in a visual association task indicate that neurons in both regions develop representations of task structure with training.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-021

NEURAL ENSEMBLE DYNAMICS OF AUDITORY THALAMUS UPON SENSORY LEARNING

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Sensory cortices receive and process feedforward uni- and multisensory information from the thalamus to guide perception and behaviours. However, so far, the neural coding strategies of sensory thalamic nuclei that precede cortex remain poorly understood on both, the single cell as well as the population level. Here, we established a gradient refractive index (GRIN) lens-based in vivo two-photon calcium imaging approach to monitor the activity of large populations of individual neurons in auditory thalamus (medial geniculate body, MGB) in awake mice during a sensory learning paradigm. Using a go/no-go task where the animals associated auditory or visual stimuli with a reward, we followed the same cells across weeks and identified distinct ensembles of MGB neurons that differentially encode the reward-contingency of the sensory stimuli as well as task-features. We found distinct classes of auditory thalamus neurons that exhibit plasticity to the go or no-go stimuli, both dependent or independent of the sensory modality. Additionally, we found a class of neurons that predicts the stimulus outcome by ramping their activity during the go stimulus and exhibit persistent calcium responses during the delay period preceding the reward, which may maintain the stimulus information and instruct the reward-related action. Our findings support the view that sensory thalamic nuclei are more than a mere sensory relay and contribute actively to the systemwide computation of cognitive processes in a bottom-up fashion.

Pubmed:
20641164: Hasegawa M, Kida I, Wada H
A volumetric analysis of the brain and hippocampus of rats rendered perinatal hypothyroid.
The thyroid hormone is essential for the proper development of the central nervous system (CNS). Hormone deficiency during CNS development causes neurological abnormalities in the brain. The hippocampus is one of the brain regions vulnerable to hormone deficiency, and the volume of dentate gyrus (DG) and cornu ammonis (CA) are reduced by transient hypothyroidism during CNS development. However, it remains unclear whether transient hypothyroidism specifically reduces the whole hippocampal volume. In the present study, we used magnetic resonance imaging (MRI) to examine the effects of perinatal hypothyroidism on the ratio of hippocampal volume to brain volume as well as brain and hippocampal volumes overall. Perinatal hypothyroidism was induced by adding the anti-thyroid drug, methimazole, to the drinking water of pregnant dams from gestational day 15 to postnatal day 21. The MRI experiment was conducted when the rats were between 7 and 11 months old. The results showed reductions of the hippocampal and brain volume of the treated group. However, the ratio of hippocampal volume to brain volume was comparable between the control and treated groups. These results indicate that perinatal hypothyroidism minimizes the brain as a whole, but does not minimize the hippocampus in particular.
Neurosci Lett, 2010; 479
23474134: Hasegawa M, Wada H
Developmental hypothyroidism disrupts visual signal detection performance in rats.
Thyroid hormones (THs) are essential for proper brain development in mammals. TH insufficiency during early development causes structural and functional abnormalities in brain leading to cognitive dysfunction. The specific effects of developmental hypothyroidism on attention have not been well characterized in animal models. The present study was conducted to characterize the effects of developmental hypothyroidism on attention in rats, and tested the hypothesis that the hypothyroidism has adverse impacts on attention by means of a visual signal detection task. Pregnant rats were exposed to the anti-thyroid drug, methimazole (0.02% w/v) via drinking water from gestational day 15 through postnatal day (PND) 21 to induce maternal and neonatal hypothyroidism. Male offspring served as subjects for the task started on PND 90. A light stimulus (500 ms, 250 ms or 50 ms) was presented in signal trials and not in blank trials. The offspring were required to discriminate these signal events, and subsequently press the correct lever. The correct response for signal and non-signal events was considered as hit and correct rejection, respectively. The hypothyroid offspring exhibited a decreased hit response for short signals (250 ms and 50 ms) which requires the higher attentional demand. The total number of lever

responses during inter-trial interval (ITI) was also increased in the hypothyroid group. The number of lever responses was negatively correlated with a hit response at 50 ms, not at 250 ms. These results suggest that developmental hypothyroidism disrupts signal detection performance via impairment of visual attention and the altered lever response behavior.
Physiol Behav, 2013; 112-113
23732561: Johnstone AF, Gilbert ME, Aydin C, Grace CE, Hasegawa M, Gordon CJ
Thermoregulatory deficits in adult Long Evans rat exposed perinatally to the antithyroidal drug, propylthiouracil.
Developmental exposure to endocrine disrupting drugs and environmental toxicants has been shown to alter a variety of physiological processes in mature offspring. Body (core) temperature (T(c)) is a tightly regulated homeostatic system but is susceptible to disruptors of the hypothalamic pituitary thyroid (HPT) axis. We hypothesized that thermoregulation would be disrupted in adult offspring exposed perinatally to an HPT disruptor. Propylythiouracil (PTU) was used as a prototypical compound because of its well known antithyroidal properties. PTU was added to the drinking water of pregnant rats in concentrations of 0, 1, 2, 3, and 10 ppm from gestational day (GD) 6 through postnatal day (PND) 21. Adult male offspring were implanted with radiotransmitters to monitor Tc and motor activity (MA) and were observed undisturbed at an ambient temperature of 22 °C for 12 consecutive days. Data were averaged into a single 24 hour period to minimize impact of ultradian changes in T(c) and MA. All treatment groups showed a distinct circadian temperature rhythm. Rats exposed to 10 ppm PTU exhibited a marked deviation in their regulated T(c) with a reduction of approximately 0.4 °C below that of controls throughout the daytime period and a smaller reduction at night. Rats exposed to 1 or 2 ppm also had smaller but significant reductions in T(c). MA was unaffected by PTU. Overall, developmental exposure to moderate doses of an antithyroidal drug led to an apparent permanent reduction in T(c) of adult offspring that was independent of changes in MA.
Neurotoxicol Teratol, 2013 Sep-Oct; 39
27693142: Niethard N, Hasegawa M, Itokazu T, Oyanedel CN, Born J, Sato TR
Sleep-Stage-Specific Regulation of Cortical Excitation and Inhibition.
Sleep is characterized by unique patterns of cortical activity alternating between the stages of slow-wave sleep (SWS) and rapid-eye movement (REM) sleep. How these patterns relate to the balanced activity of excitatory pyramidal cells and inhibitory interneurons in cortical circuits is unknown. We investigated cortical network activity during wakefulness, SWS, and REM sleep globally and locally using in vivo calcium imaging in mice. Wide-field imaging revealed a reduction in pyramidal cell activity during SWS compared with wakefulness and, unexpectedly, a further profound reduction in activity during REM sleep. Two-photon imaging on local circuits showed that this suppression of activity during REM sleep was accompanied by activation of parvalbumin (PV)+ interneurons, but not of somatostatin (SOM)+ interneurons. PV+ interneurons most active during wakefulness were also most active during REM sleep. Our results reveal a sleep-stage-specific regulation of the cortical excitation/inhibition balance, with PV+ interneurons conveying maximum inhibition during REM sleep, which might help shape memories in these networks.
Curr Biol, 2016; 26
28297671: Hasegawa M, Majima K, Itokazu T, Maki T, Albrecht UR, Castner N, Izumo M, Sohya K, Sato TK, Kamitani Y, Sato TR
Selective Suppression of Local Circuits during Movement Preparation in the Mouse Motor Cortex.
Prepared movements are more efficient than those that are not prepared for. Although changes in cortical activity have been observed prior to a forthcoming action, the circuits involved in motor preparation remain unclear. Here, we use in vivo two-photon calcium imaging to uncover changes in the motor cortex during variable waiting periods prior to a forepaw reaching task in mice. Consistent with previous reports, we observed a subset of neurons with increased activity during the waiting period; however, these neurons did not account for the degree of preparation as defined by reaction time (RT). Instead, the suppression of activity of distinct neurons in the same cortical area better accounts for RT. This suppression of neural activity resulted in a distinct and reproducible pattern when mice were well prepared. Thus, the selective suppression of network activity in the motor cortex may be a key feature of prepared movements.
Cell Rep, 2017; 18
29362373: Itokazu T, Hasegawa M, Kimura R, Osaki H, Albrecht UR, Sohya K, Chakrabarti S, Itoh H, Ito T, Sato TK, Sato TR
Streamlined sensory motor communication through cortical reciprocal connectivity in a visually guided eye movement task.
Cortical computation is distributed across multiple areas of the cortex by networks of reciprocal connectivity. However, how such connectivity contributes to the communication between the connected areas is not clear. In this study, we examine the communication between sensory and motor cortices. We develop an eye movement task in mice and combine it with optogenetic suppression and two-photon calcium imaging techniques. We identify a small region in the secondary motor cortex (MO) that controls eye movements and reciprocally connects with a rostrolateral part of the higher visual areas (V). These two regions encode both motor signals and visual information; however, the information flow between the regions depends on the direction of the connectivity: motor information is conveyed preferentially from the MO to the V, and sensory information is transferred primarily in the opposite direction. We propose that reciprocal connectivity streamlines information

flow, enhancing the computational capacity of a distributed network.
Nat Commun, 2018; 9
33903596: Taylor JA, Hasegawa M, Benoit CM, Freire JA, Theodore M, Ganea DA, Innocenti SM, Lu T, Gründemann J
Single cell plasticity and population coding stability in auditory thalamus upon associative learning.
Cortical and limbic brain areas are regarded as centres for learning. However, how thalamic sensory relays participate in plasticity upon associative learning, yet support stable long-term sensory coding remains unknown. Using a miniature microscope imaging approach, we monitor the activity of populations of auditory thalamus (medial geniculate body) neurons in freely moving mice upon fear conditioning. We find that single cells exhibit mixed selectivity and heterogeneous plasticity patterns to auditory and aversive stimuli upon learning, which is conserved in amygdala-projecting medial geniculate body neurons. Activity in auditory thalamus to amygdala-projecting neurons stabilizes single cell plasticity in the total medial geniculate body population and is necessary for fear memory consolidation. In contrast to individual cells, population level encoding of auditory stimuli remained stable across days. Our data identifies auditory thalamus as a site for complex neuronal plasticity in fear learning upstream of the amygdala that is in an ideal position to drive plasticity in cortical and limbic brain areas. These findings suggest that medial geniculate body's role goes beyond a sole relay function by balancing experience-dependent, diverse single cell plasticity with consistent ensemble level representations of the sensory environment to support stable auditory perception with minimal affective bias.
Nat Commun, 2021; 12

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BOARD NUMBER: S01-022

PLASTICITY OF AMYGDALA INTERNEURONS IN ASSOCIATIVE LEARNING

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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The basolateral amygdala (BLA) is a cortex-like structure known to be involved in simple forms of emotional learning such as fear conditioning. It is the main entry site for sensory information to the amygdala complex, and local plasticity in the BLA is crucial for associative memory formation. Plastic changes of glutamatergic projection neurons (PNs) induced during learning have been well characterized, while little is known about the contribution of GABAergic interneurons. Although inhibitory interneurons only constitute about 20% of the neuronal population in the BLA, they tightly control PN activity and plasticity. Nonetheless, the behavioral relevance of different interneuron subtypes and their plasticity upon learning remain largely unexplored. Thus, the present study aimed to assess how the activity of local interneurons in the BLA is modulated across fear conditioning and extinction. To address this, we performed deep-brain calcium imaging with miniature microscopes in freely behaving mice during an associative fear learning paradigm. We selectively targeted discrete interneuron subtypes by injecting a cre-dependent GCaMP6 into the BLA of VIP-Cre, SST-Cre, or PV-Cre mice, followed by an implantation of a gradient-index (GRIN) lens. Following the same neuronal populations across days, we found that interneuron subtypes show diverse activity patterns during the conditioning paradigm, with distinct plastic responses upon fear and extinction learning. Overall, our results suggest that BLA inhibitory interneuron plasticity can contribute to the acquisition and expression of associative fear memories.

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BOARD NUMBER: S01-023

NEURAL CORRELATES AND MODULATION OF THREAT PREDICTION IN AUDITORY THALAMUS UPON ASSOCIATE FEAR LEARNING.

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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The medial geniculate body (MGB) is the thalamus' key hub for auditory transmission and processing. Besides feedforward relay of sensory information, it has been shown that MGB neurons exhibit response plasticity to reward- or threat-predicting conditioned tones (CS+) across learning. Here, we use a miniature microscope-based deep brain imaging approach in freely moving mice to track the dynamics of foot shock (US) predicting CS+ tone responses upon auditory fear conditioning. We identified a subtype of CS+ responsive neurons, which exhibit ramping calcium response throughout the shock-predicting 30 second CS+ period. These CS+ ramp-up cells were not present during habituation and their proportion increased with consecutive CS+ - US pairings during conditioning. Vice versa, the proportion of ramp-up cells decreased with advancing reduction of the animals' CS+-driven freezing response during extinction sessions following fear conditioning. Furthermore, the magnitude of the calcium signal of ramp-up cells was correlated with the vigor of the US-driven escape behavior of the animal suggesting that ramp-up cells are predictive of the unconditioned behavioral response. Finally, we found that optogenetic manipulation of brainstem-originating cholinergic inputs to MGB leads to a reduction in the proportion of ramp-up cells, suggesting that the response plasticity of ramp-up cells is controlled by acetylcholine. Overall, this study identified a subtype of MGB neurons that are predictive of the behavioral response to an upcoming threat and may play a key role in associative fear learning.

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BOARD NUMBER: S01-024

POPULATION LEVEL CODING OF SENSORY REPRESENTATIONS AND TASK FEATURES IN AUDITORY THALAMUS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Primary sensory areas are crucial for the computations of sensory representations, learning and cognition, while the role of their bottom-up inputs from thalamic areas remains less well understood. Here, we use a gradient refractive index (GRIN) lens-based in vivo two-photon calcium imaging approach to monitor the activity of large populations of individual neurons in auditory thalamus (medial geniculate body, MGB) in awake mice longitudinally during a sensory mapping and go/no-go learning paradigm. Using a cluster analysis approach, we found that pure tone frequency tuning of groups of MGB neurons is plastic across learning. During learning, we found an increase in trial-by-trial population vector correlations during the pre-reward delay period following the go-stimulus in expert animals, while correlations of the sensory period remained unchanged. This indicates that auditory thalamus encodes task-outcomes during a preparatory period by more consistent population-level representations further supporting thalamus' role in learning upon complex behaviours.

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BOARD NUMBER: S01-025

CELL NUMBERS IN THE REFLECTED BLADE OF CA3 AND THEIR RELATION TO OTHER HIPPOCAMPAL PRINCIPAL CELL POPULATIONS ACROSS NINE SPECIES

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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[1]University of Zürich, Institute of Anatomy, Division Of Functional Neuroanatomy, Zurich, Switzerland, [2]University of Zürich, Institute of Anatomy, Division of Functional Neuroanatomy, Eth Zürich, Department Of Health Sciences And Technology, Zürich, Switzerland, [3]Natural History Museum, Natural History Museum, St.Gallen, Switzerland, [4]Pasteur Institute of Novi Sad, Department Of Microbiology, Novi Sad, Serbia

A reflected blade (RB) of CA3 is present in many species, including humans, but not in laboratory rats or mice. Distinguishing the RB from typical CA3, RB neuron may extend their dendrites into the dentate molecular layer or may not receive entorhinal inputs in some species. Direct evidence for RB function is absent, but, extrapolating from rodent CA3c, they are more likely to function with dentate neurons in pattern separation rather than with CA3 in pattern completion. We have shown before that the size of CA3 and hilar cell populations (including RB neurons) differentiate between taxonomic groups. In this study, we investigated three issues related to the RB in nine species in which RB cells and hilar polymorphic cells could be reliably distinguished: rock mouse, guinea pig, rabbit, hare, jackal, sheep, wild boar, roe deer and red deer. (1) we describe the histoarchitecture of the RB, and (2) the distribution of the Ca-binding proteins. (3) We answer how the assignment of the RB neuron population to CA3 or hilus impacts on taxonomic assessment. Our results show that (1) there are prominent RB septotemporal differences, (2) calretinin does not reliably distinguish between RB and hilar polymorphic cells, but, in some species between CA3 and the RB and (3) that the pooling RB and hilus results in a strong species differentiation, while assigning the RB to CA3 leads to clustering of all species that include humans.

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BOARD NUMBER: S01-026

DENTATE GYRUS OF THE VENTRAL HIPPOCAMPUS UNDER CONTROL OF BRAINSTEM NUCLEUS INCERTUS - ELECTROPHYSIOLOGICAL, ANATOMICAL AND NEUROCHEMICAL STUDIES IN RAT

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

Aleksandra Trenk[1], Kinga Przybylska[1], Anna Gugula[1], Sylwia Drabik[1], Magdalena Walczak[1], Angelika Kaleta[1], Akhter Hossain[2], Andrew Gundlach[2], Anna Blasiak[1]
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The ventral hippocampus (vHPC), a key region involved in processing emotional information, is heavily innervated by the brainstem *nucleus incertus* (NI). NI neurons are the main source of the neuropeptide relaxin-3 (RLN3) and activation of the relaxin-3 receptor, RXFP3, in vHPC promotes anxiety and social avoidance. However, little is known about the neuronal mechanisms underlying NI-vHPC interactions. Therefore, the aims of these studies were to map the distribution of NI originating fibres within vHPC, define the neurochemical nature of vHPC cells expressing RXFP3 mRNA, and verify the influence of the RXFP3 agonist, A2, on vHPC neuronal activity. Viral-based, neural tract-tracing revealed a high density of NI-originating fibres within the polymorph layer of the dentate gyrus (DG) with the majority RLN3-positive. Using fluorescent multiplex *in situ* hybridization we demonstrated that a majority of vGAT1 mRNA-positive cells in vHPC GC express RXFP3-mRNA. Finally, using *ex vivo* multielectrode array recordings we demonstrated that specific RXFP3 activation (A2, 1 µM), exerted both inhibitory and excitatory effects on vHPC network activity, and that D2-receptor agonist sensitive, vHPC mossy cells are both directly and indirectly sensitive to A2. These findings indicate that NI influences information processing in the vHPC by modulation of DG neuronal activity, and this (mostly inhibitory) influence may underlie the observed increase in anxiety behaviour and social avoidance upon chronic RXFP3 activation in vHPC. Further studies are needed to verify the influence of NI/RLN3 signalling in vHPC on specific DG cell types, and its possible impact on DG-dependent pattern separation. Funding: UMO-2018/30/E/NZ4/00687.

Pubmed:
35078925: Trenk A, Walczak M, Szlaga A, Pradel K, Blasiak A, Blasiak T
Bidirectional Communication between the Pontine Nucleus Incertus and the Medial Septum Is Carried Out by Electrophysiologically-Distinct Neuronal Populations.
Theta oscillations are key brain rhythm involved in memory formation, sensorimotor integration, and control of locomotion and behavioral states. Generation and spatiotemporal synchronization of theta oscillations rely on interactions between brain nuclei forming a large neural network, which includes pontine nucleus incertus (NI). Here we identified distinct populations of NI neurons, based on the relationship of their firing to hippocampal waves, with a special focus on theta oscillations, and the direction and type of interaction with the medial septum (MS) in male, urethane-anesthetized rats. By recording NI neuronal firing and hippocampal LFP, we described NI neurons that fire action potentials in a theta phase-independent or theta phase-locked and delta wave-independent or delta wave-locked manner. Among hippocampal activity-independent NI neurons, irregular, slow-firing, and regular, fast-firing cells were observed, while hippocampal oscillation-/wave-locked NI neurons were of a bursting or nonbursting type. By projection-specific optotagging, we revealed that only fast-firing theta phase-independent NI neurons innervate the MS, rarely receiving feedback information. In contrast, the majority of theta-bursting NI neurons were inhibited by MS stimulation, and this effect was mediated by direct GABAergic input. Described NI neuronal populations differ in reciprocal connections with the septohippocampal system, plausibly forming separate neuronal loops. Our results suggest that theta phase-independent NI neurons participate in theta rhythm generation through direct innervation of the MS, while theta-bursting NI neurons further transmit the rhythmic signal received from the MS to stabilize and/or strengthen rhythmic activity in other structures. The generation and spatiotemporal synchronization of theta oscillations rely on interactions between nuclei forming a large neural network, part of which is the pontine nucleus incertus (NI). Here we describe that within NI there are populations of neurons that can be distinguished based on the relationship of their firing to hippocampal theta oscillations and delta waves. We show that these neuronal populations largely do not have reciprocal connections with the septohippocampal system, but form separate neuronal loops. Our results suggest that medial septum (MS)-projecting, fast-firing, theta phase-independent NI neurons may participate in theta rhythm generation through direct

innervation of the MS, while theta-bursting NI neurons may further transmit the rhythmic signal received from the MS to other structures.
J Neurosci, 2022; 42
29463910: Stojakovic A, Walczak M, Cieślak PE, Trenk A, Sköld J, Zajdel J, Mirrasekhian E, Karlsson C, Thorsell A, Heilig M, Parkitna JR, Błasiak T, Engblom D
Several behavioral traits relevant for alcoholism are controlled by γ2 subunit containing GABA receptors on dopamine neurons in mice.
The risk factors for developing alcohol addiction include impulsivity, high sensitivity to the rewarding action of ethanol, and low sensitivity to its sedative and intoxicating effects. Genetic variation in GABA receptor subunits, including the γ2 subunit (Gabrg2), affects the risk for developing alcoholism. Alcohol directly potentiates GABA receptors and activates the mesolimbic dopamine system. Here, we deleted Gabrg2 selectively in dopamine cells of adult mice. The deletion resulted in elevated firing of dopamine neurons and made them less sensitive to drugs acting at GABA receptors. At the behavioral level, the deletion increased exploratory behavior and augmented both correct and incorrect responding in the go/no-go task, a test often used to assay the response inhibition component of impulsivity. In addition, conditioned place preference to alcohol, but not to cocaine or morphine, was increased. Ethanol-induced locomotor activation was enhanced in the mice lacking Gabrg2 on dopaminergic cells, whereas the sedative effect of alcohol was reduced. Finally, the alcohol drinking, but not the alcohol preference, at a high concentration was increased in the mutant mice. In summary, deletion of Gabrg2 on dopamine cells induced several behavioral traits associated with high risk of developing alcoholism. The findings suggest that mice lacking Gabrg2 on dopaminergic cells could be used as models for individuals at high risk for developing alcoholism and that GABA receptors on dopamine cells are protective against the development of excessive alcohol drinking.
Neuropsychopharmacology, 2018; 43
27693266: Danielewicz J, Trenk A, Hess G
Imipramine ameliorates early life stress-induced alterations in synaptic plasticity in the rat lateral amygdala.
Long-term potentiation (LTP) and long-term depression (LTD) are two opposite forms of synaptic plasticity at the cortical and thalamic inputs to the lateral amygdala (LA). It has been demonstrated that maternal separation (MS) of rat pups results in alterations in the potential for both pathways to undergo LTP and LTD in adolescence. Imipramine, a prototypic tricyclic antidepressant, has been shown to counteract some detrimental effects of MS on rat behavior, however it is not known whether MS-induced alterations in the potential for bidirectional synaptic plasticity in the LA could be reversed by imipramine treatment. To this end, rat pups were subjected to MS (3h/day) on postnatal days (PNDs) 1-21. On each of PNDs 29-42, male rats previously subjected to MS were injected subcutaneously with imipramine (10mg/kg). Field potentials were recorded ex vivo from slices containing the LA and saturating levels of LTP and LTD were induced. At the thalamic input to the LA, both the maximum LTP and the maximum LTD were reduced in rats subjected to MS when compared to control animals, confirming earlier results. However, these effects were no longer present in rats subjected to MS and later treated with imipramine. At the cortical input in slices prepared from MS-subjected rats, an impairment of the maximum LTP and an enhancement of the maximum LTD were observed. At the cortical input in rats subjected to MS and receiving imipramine treatment, the level of LTD was comparable to control but imipramine did not restore the potential for LTP at this input. These results demonstrate that imipramine fully reverses the effects of MS in the thalamo-amygdalar pathway, however, in the cortico-amygdalar pathway the reversal of the effects of MS by imipramine is partial.
Behav Brain Res, 2017; 317

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BOARD NUMBER: S01-027

REDUCED PERINEURONAL NET FORMATION IN PV+ INTERNEURONS CAUSES BEHAVIORAL DEFICITS BUT ONLY MINUTE CHANGES TO CELLULAR AND CIRCUIT FUNCTION

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Transition from the juvenile to the adult brain coincides with maturation of parvalbumin expressing (PV+) inhibitory neurons, and assembly of perineuronal nets (PNNs).The PNNs are specialized extracellular matrix structures that reduce neural plasticity. Aggrecan is a major component of PNNs, and adult knock-out of aggrecan reactivates juvenile plasticity, but the mechanisms by which aggrecan regulates PV+ neurons and network function are not understood. To this end, we have generated a PV+ specific germline aggrecan knock-out (AcanKO), by crossing the PV-Cre mice strain with Acan-flex mice. In a separate group of adult Acan-flex mice, we systemically inject a viral vector that expresses Cre under control of a PV enhancer. We show that without expression of aggrecan, PNNs do not assemble. In behavioral testing, AcanKO mice showed lower levels of anxiety-like behavior compared to controls, in both standardized anxiety tests and the Morris water maze. Notably, electrophysiological properties of PV+ interneurons in visual cortex (V1) of adult AcanKO mice were similar to controls *in vitro*, and neither spontaneous nor visually evoked network activity in V1 revealed significant differences between AcanKOs and controls *in vivo*. In ongoing work, we are testing expression levels of PNN components in adult AcanKO mice. In summary, our data shows that PV+ neurons produce aggrecan that is necessary for correct formation of PNNs during the transition from juvenile to adult. Germline knock-out of aggrecan led to minor phenotypic effects compared to adult knock-out, suggesting compensatory mechanisms. The nature of these mechanisms will be the focus of future work.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-028

OPTOGENETIC MAPPING OF NEURONAL INTERACTIONS IN THE MOTOR CORTEX DURING GOAL-DIRECTED BEHAVIOR

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Behavior-related neural dynamics in the frontal cortex is an emergent property of network connectivity. The network structure at the level of individual neurons, and its relationship to neural coding are largely unknown. Here we developed an optical method for *rapid* (500k pairwise connections / 30 minutes) mapping of effective connectivity *in vivo*. This method is based on 2-photon optogenetic stimulation of individual excitatory neurons and simultaneous 2-photon volumetric calcium imaging of evoked responses in non-stimulated neurons ('effective connection'). We applied this method in anterior lateral motor cortex (ALM) in a novel behavioral task in which untrained mice performed multidirectional tongue-reaching for water rewards presented at multiple (up to 16) locations on a grid in front of the mouse face. A majority of ALM neurons were modulated by task variables with a subset of neurons (~25%) exhibited strong tuning to the reward location. Specifically, some neurons showed tuning to direction of the reward location with respect to the mouse face, whereas other neurons were selective for particular reward locations. We then mapped effective connectivity between 10,000,000 pairs of layer 2/3 neurons imaged in this task. Nearby neurons were more strongly connected and shared directional selectivity, revealing a fine-scale columnar architecture. By analyzing connectivity patterns with methods borrowed from network science, we discovered neurons that function as network hubs. Hub neurons had an unexpectedly high number of connections, weak directional tuning, and strong influence on neighboring neurons – suggesting that they may act as local conductors of the neural orchestra.

FENS Forum 2022
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BOARD NUMBER: S01-029

ADAPTIVE CHANGES IN MEMORY-RELATED BRAIN REGIONS UPON CHRONIC CIRCADIAN MANIPULATION

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

Inês Marques-Morgado[1], Marcelo Dias[1], Joana Coelho[1], Joana Ribeiro[1], Miguel Remondes[1], Neil Dawson[2], Luisa Lopes[1]
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There are important circadian rest-activity pattern changes in aging and preclinical Alzheimer's Disease (Musiek et al, Jama Neurology. 2018). Despite these observations, little is known on the manner circadian timing modulates hippocampal-based learning and memory. Whole-brain functional connectivity was modelled by combining ^{14}C-2- deoxyglucose functional brain imaging with Partial Least Squares Regression (as in Dawson et al, Neuropsychopharmacol. 2014) on male Sprague-Dawley rats; either under a normal circadian cycle or after a chronic circadian alteration (Craig et al, Brain Res. Bull. 2008). The anatomical connectivity between SCN and memory-related regions was traced by injecting transsynaptic anterograde [rVSV-(VSV-G)-Venus] and retrograde [rVSV-(RABV-G)-eGFP] viral tracers in the rat SC N and HIPP, using our optimized surgical procedure (Marcelo, Marques-Morgado et al, (2021) ENEURO.0146-21.2021) We found alterations in the metabolic activity of SCN and the HIPP, Medial Entorhinal Cortex, Perirhinal Cortex and Dorsal Raphé. A total of 127 functional interactions were impacted by the circadian shift, with a strong remodelling of the connections between cognitive regions, suggesting theta oscillations as a mechanism for the interaction between circadian rhythms and cognition. Moreover, trans-synaptic antero- and retrograde tracing found anatomical projections between the SCN and the HIPP with a relay in the Septum, that may act as a hub of circadian information onto the hippocampal system. We describe a functional network between circadian clock components and memory-encoding regions, pointing new targets to explore the physiological role of circadian rhythms in memory. Funding: Bial Foundation

FENS Forum 2022
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BOARD NUMBER: S01-030

OLFACTORY CODING IN HIPPOCAMPAL AREA CA2

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Although the hippocampus is crucial for social memory, how social sensory information is incorporated with contextual information to form episodic social memories remains unknown. Here we investigate the mechanisms for social sensory information processing by performing high-resolution calcium imaging from hippocampal neurons in awake head-fixed mice exposed to social and non-social odors. We focus on the CA2 region because of its importance for social memory. We describe for the first time how novel social odors from single conspecifics rapidly evoke unique representations in a subpopulation of CA2 pyramidal cells upon repeated odor exposure. The representations are refined during associative social-odor-reward learning, and CA2 activity is important for this learning. Finally, we find that CA2 firing in response to sets of odors from different categories (i.e, mouse urine, monomolecular odors, or human urine sampoes) enables generalization of representations along categories of social versus non-social odors. Thus, our study provides the first evidence that hippocampus encodes and distinguishes complex social odors and their reward associations, providing a likely substrate for CA2's crucial function in social memory.

FENS Forum 2022
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BOARD NUMBER: S01-031

ORGANISATION OF INPUTS FROM THE BASO-AMYGDALA TO THE MEDIAL AND LATERAL ENTORHINAL CORTEX

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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University of Edinburgh, Centre For Discovery Brain Sciences, Edinburgh, United Kingdom

The baso-amygdala complex (BA) contains highly inter-connected nuclei involved in emotional, contextual and reward learning. It has been proposed that the BA to medial entorhinal cortex (MEC) pathway modulates the consolidation of spatial and contextual information, yet the cell types that the BA innervates within the medial or lateral entorhinal cortex (LEC) have yet to be established. To localise BA inputs to the entorhinal cortex, we injected AAVs expressing mCherry or anterograde dextrans tracers into sub-regions of the BA of C57Bl/6 mice. To trace the origin of BA inputs, we injected retrograde AAVs expressing mCherry or GFP into the EC, or applied a combinatorial approach targeting a retro-AAV expressing Cre to the LEC and a Cre-dependent reporter AAV to the BA. We identified entorhinal cortex neurons that receive BA inputs by injecting AAVs expressing channelrhodopsin-2 into the BA and made patch-clamp recordings from entorhinal cortex neurons in ex-vivo brain slices and activated axons using blue light. We found that BA axons project to all layers of the LEC but are restricted to layer 1 and 5a of the MEC. Separate populations of cells in the BA project to different sublayers of the MEC and LEC. Optogenetic activation of BA axons generated glutamatergic excitatory responses in cells in layer 2, 3 and 5a of the MEC and LEC. The majority of cells that received BA input project to the hippocampus, suggesting distinct emotional signals may be passed onto brain areas involved in episodic and contextual memories.

Pubmed:
34593254: Gerlei KZ, Brown CM, Sürmeli G, Nolan MF
Deep entorhinal cortex: from circuit organization to spatial cognition and memory.
The deep layers of the entorhinal cortex are important for spatial cognition, as well as memory storage, consolidation and retrieval. A long-standing hypothesis is that deep-layer neurons relay spatial and memory-related signals between the hippocampus and telencephalon. We review the implications of recent circuit-level analyses that suggest more complex roles. The organization of deep entorhinal layers is consistent with multi-stage processing by specialized cell populations; in this framework, hippocampal, neocortical, and subcortical inputs are integrated to generate representations for use by targets in the telencephalon and for feedback to the superficial entorhinal cortex and hippocampus. Addressing individual sublayers of the deep entorhinal cortex in future experiments and models will be important for establishing systems-level mechanisms for spatial cognition and episodic memory.
Trends Neurosci, 2021; 44
24808828: Huma Z, Du Beau A, Brown C, Maxwell DJ
Origin and neurochemical properties of bulbospinal neurons projecting to the rat lumbar spinal cord via the medial longitudinal fasciculus and caudal ventrolateral medulla.
Bulbospinal systems (BS) originate from various regions of the brainstem and influence spinal neurons by classical synaptic and modulatory mechanisms. Our aim was to determine the brainstem locations of cells of origin of BS pathways passing through the medial longitudinal fasciculus (MLF) and the caudal ventrolateral medulla (CVLM). We also examined the transmitter content of spinal terminations of the CVLM pathway. Six adult rats received Fluorogold (FG) injections to the right intermediate gray matter of the lumbar cord (L1-L2) and the b-subunit of cholera toxin (CTb) was injected either into the MLF or the right CVLM (3 animals each). Double-labeled cells were identified within brainstem structures with confocal microscopy and mapped onto brainstem diagrams. An additional 3 rats were injected with CTb in the CVLM to label axon terminals in the lumbar spinal cord. Double-labeled cells projecting via the MLF or CVLM were found principally in reticular regions of the medulla and pons but small numbers of cells were also located within the midbrain. CVLM projections to the lumbar cord were almost exclusively ipsilateral and concentrated within the intermediate gray matter. Most (62%) of terminals were immunoreactive for the vesicular glutamate transporter 2 while 23% contained the vesicular GABA transporter. The inhibitory subpopulation was glycinergic, GABAergic or contained both transmitters. The proportions of excitatory and inhibitory axons projecting via the CVLM to the lumbar cord are similar to those projecting via the MLF. Unlike the MLF pathway, CVLM projections are predominantly ipsilateral and concentrated within intermediate gray but do not extend into motor nuclei or

laminia VIII. Terminations of the CVLM pathway are located in a region of the gray matter that is rich in premotor interneurons; thus its primary function may be to coordinate activity of premotor networks.
Front Neural Circuits, 2014; 8

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BOARD NUMBER: S01-032

PRENATAL EXPOSURE TO DEXAMETHASONE IMPAIRS LEARNING AND MEMORY IN A SEX-DEPENDENT MANNER

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Background: The synthetic glucocorticoid (sGC) dexamethasone (DEX) is prescribed to pregnant women at risk of preterm delivery or who are bearing fetuses at risk of developing congenital adrenal hyperplasia. However, prenatal activation of glucocorticoid receptors results in long-lasting deleterious effects on cognitive functions during adulthood. **Aim:** Assess prenatal DEX effects on learning, memory and synaptic proteins expression in the prefrontal cortex (PFC) and dorsal hippocampus (D-HPC). **Methods:** Pregnant rats received daily intra-peritoneal injections of either DEX (0.2 mg/kg) or saline from gestation day (GD)14 until GD21. Protein expression of the pre- (synaptophysin: SynP) and post-synaptic proteins (post synaptic density 95:PSD95) in the PFC & D-HPC of adult male & female offspring were explored using western blot. Adult offspring were subjected to spatial Morris water maze (MWM) and novel object recognition memory (NOR) tests. **Results:** Prenatal exposure to DEX was associated with reduced expression of SynP and PSD95 proteins in the D-HPC and the PFC of adult female but not male rat offspring. Prenatal DEX was associated with a significant decrease in the time spent in the target quadrant and significant increase in the latency to enter the target quadrant in the MWM in females but not males' adult offspring. Maternal exposure to sGC led to reduced time in exploring and roaming around the novel object in the NOR test in both female and male adult offspring. **Conclusion:** Prenatal DEX-induced decrease in synaptic proteins expression is associated with memory impairment in a sex-dependent manner.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-033

KETAMINE REGULATES HOMEOSTATIC FIRING RATE SET POINT IN HIPPOCAMPAL CIRCUITS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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N-methyl-D-aspartate receptors (NMDARs) function as key regulators of synaptic plasticity and thus are one of the few well-known mechanisms of learning and memory. In parallel, NMDAR antagonists present antidepressant effects that are remarkably fast-acting and long-lasting. Still, the effects of NMDAR antagonists on spiking activity at the network level remain elusive. Long-term recordings of hippocampal cultures using multi electrode arrays revealed that chronic application of structurally distinct NMDAR blockers stably (> 24hr) suppressed mean firing rate (MFR) while preserving homeostatic responses to activity perturbations. In the presence of the NMDAR antagonist ketamine, knocking out eukaryotic elongation factor 2 kinase (eEF2K) or chelating brain derived neurotrophic factor (BDNF) restored MFR set point to its baseline, pre-ketamine levels. This renormalization of MFR occurred in the absence of synaptic scaling at excitatory synapses and solely by intrinsic plasticity. *In vivo,* mice implanted with tetrodes and a cannula directed towards CA1 *stratum pyramidale* showed a stable (> 6hr) reduction in MFR of both putative pyramidal cells and interneurons following local administration of ketamine. Notably, LFP oscillations, state-dependent modulation of MFRs, and neuronal firing patterns (e.g. burstiness) were not affected by ketamine. These local and long-term effects of ketamine imply that NMDAR regulate firing rate set points via the eEF2K-BDNF pathway. Further, ketamine selectively reduces MFR in the hippocampus while preserving other temporal features of neuronal activity that are fundamental to cognitive processing. Taken together, our data can help bridge the gap between the synaptic effects of NMDAR and the clinical features of rapid antidepressants.

Pubmed:
35118427: Heim LR, Shoob S, de Marcas L, Zarhin D, Slutsky I
Measuring synaptic transmission and plasticity with fEPSP recordings in behaving mice.
Spontaneous spiking activity depends on intrinsic excitability and synaptic input. Historically, synaptic activity has been mostly studied . Here, we describe a versatile and robust protocol to record field excitatory postsynaptic potentials (fEPSPs) in behaving rodents. The protocol allows estimating the input-output relationship of a specific pathway, short-term and long-term plasticity, and their modulation by pharmacological or pharmacogenetic interventions and behavioral states. However, experimenters must be aware of the protocol's specificity and interpret results with care. For complete details on the use and execution of this profile, please refer to Styr et al. (2019).
STAR Protoc, 2022; 3
35045289: Zarhin D, Atsmon R, Ruggiero A, Baeloha H, Shoob S, Scharf O, Heim LR, Buchbinder N, Shinikamin O, Shapira I, Styr B, Braun G, Harel M, Sheinin A, Geva N, Sela Y, Saito T, Saido T, Geiger T, Nir Y, Ziv Y, Slutsky I
Disrupted neural correlates of anesthesia and sleep reveal early circuit dysfunctions in Alzheimer models.
Dysregulated homeostasis of neural activity has been hypothesized to drive Alzheimer's disease (AD) pathogenesis. AD begins with a decades-long presymptomatic phase, but whether homeostatic mechanisms already begin failing during this silent phase is unknown. We show that before the onset of memory decline and sleep disturbances, familial AD (fAD) model mice display no deficits in CA1 mean firing rate (MFR) during active wakefulness. However, homeostatic down-regulation of CA1 MFR is disrupted during non-rapid eye movement (NREM) sleep and general anesthesia in fAD mouse models. The resultant hyperexcitability is attenuated by the mitochondrial dihydroorotate dehydrogenase (DHODH) enzyme inhibitor, which tunes MFR toward lower set-point values. Ex vivo fAD mutations impair downward MFR homeostasis, resulting in pathological MFR set points in response to anesthetic drug and inhibition blockade. Thus, firing rate dyshomeostasis of hippocampal circuits is masked during active wakefulness but surfaces during low-arousal brain states, representing an early failure of the silent disease stage.
Cell Rep, 2022; 38
31047779: Styr B, Gonen N, Zarhin D, Ruggiero A, Atsmon R, Gazit N, Braun G, Frere S, Vertkin I, Shapira I, Harel M, Heim LR, Katsenelson M, Rechnitz O, Fadila S, Derdikman D, Rubinstein M, Geiger T, Ruppin E, Slutsky I
Mitochondrial Regulation of the Hippocampal Firing Rate Set Point and Seizure Susceptibility.

Maintaining average activity within a set-point range constitutes a fundamental property of central neural circuits. However, whether and how activity set points are regulated remains unknown. Integrating genome-scale metabolic modeling and experimental study of neuronal homeostasis, we identified mitochondrial dihydroorotate dehydrogenase (DHODH) as a regulator of activity set points in hippocampal networks. The DHODH inhibitor teriflunomide stably suppressed mean firing rates via synaptic and intrinsic excitability mechanisms by modulating mitochondrial Ca buffering and spare respiratory capacity. Bi-directional activity perturbations under DHODH blockade triggered firing rate compensation, while stabilizing firing to the lower level, indicating a change in the firing rate set point. In vivo, teriflunomide decreased CA3-CA1 synaptic transmission and CA1 mean firing rate and attenuated susceptibility to seizures, even in the intractable Dravet syndrome epilepsy model. Our results uncover mitochondria as a key regulator of activity set points, demonstrate the differential regulation of set points and compensatory mechanisms, and propose a new strategy to treat epilepsy.
Neuron, 2019; 102
28514188: Heim LR, Bader M, Edut S, Rachmany L, Baratz-Goldstein R, Lin R, Elpaz A, Qubty D, Bikovski L, Rubovitch V, Schreiber S, Pick CG
The Invisibility of Mild Traumatic Brain Injury: Impaired Cognitive Performance as a Silent Symptom.
The present study was designed to tackle two notorious features of mild traumatic brain injury (mTBI)-heterogeneity and invisibility-by characterizing the full scope of mTBI symptoms. Mice were exposed to brain injuries of different intensities utilizing a weight-drop model (10, 30, 50, and 70 g) and subsequently subjected to a comprehensive battery of behavioral tests at different time points and immunohistochemical examination of cortical slices. Whereas the physiological, neurological, emotional, and motor function of mTBI mice (i.e., their well-being) remained largely intact, cognitive deficits were identified by the y-maze and novel object recognition. Results from these two cognitive tests were combined and a dose-response relationship was established between injury intensity and cognitive impairment, ranging from an 85% decline after a 70-g impact ($p < 0.001$) to a 20% decline after a 10-g impact (essentially no effect). In addition, higher intensities of injury were accompanied by decreased expression of axonal and synaptic markers. Thus, our mTBI mice showed a clear discrepancy between performance (poor cognitive function) and appearance (healthy demeanor). This is of major concern given that diagnosis of mTBI is established on the presence of clinical symptoms and emphasizes the need for an alternative diagnostic modality.
J Neurotrauma, 2017; 34
27285176: Baratz-Goldstein R, Deselms H, Heim LR, Khomski L, Hoffer BJ, Atlas D, Pick CG
Thioredoxin-Mimetic-Peptides Protect Cognitive Function after Mild Traumatic Brain Injury (mTBI).
Mild traumatic brain injury (mTBI) is recognized as a common injury among children, sportsmen, and elderly population. mTBI lacks visible objective structural brain damage but patients frequently suffer from long-lasting cognitive, behavioral and emotional difficulties associated with biochemical and cellular changes. Currently there is no effective treatment for patients with mTBI. The thioredoxin reductase/thioredoxin pathway (TrxR/Trx1) has both anti-inflammatory and anti-oxidative properties. If the system is compromised, Trx1 remains oxidized and triggers cell death via an ASK1-Trx1 signal transduction mechanism. We previously showed tri and tetra peptides which were derived from the canonical -CxxC- motif of the Trx1-active site, called thioredoxin mimetic (TXM) peptides, reversed inflammatory and oxidative stress damage mimicking Trx1 activity. Here, TXM-peptides were examined for protecting cognitive function following weight drop closed-head injury in a mouse model of mTBI. TXM-CB3 (AcCys-Pro-CysNH2), TXM-CB13 (DY-70; AcCys-Met-Lys-CysNH2) or AD4 (ACysNH2) were administered at 50 mg/kg, 60 min after injury and cognitive performance was monitored by the novel-object-recognition and Y-maze tests. Behavioral deficits subsequent to mTBI injury were reversed by a single dose of TXM-CB3, TXM-CB13 and, to a lesser extent, by AD4. TXM-CB13 similar to TXM-CB3 and AD4 reversed oxidative stress-induced phosphorylation of mitogen-activated kinases, p38MAPK and c-Jun N-terminal kinase, (JNK) in human neuronal SH-SY5Y cells. We conclude that significantly improved cognitive behavior post mTBI by the TXM-peptides could result from anti-apoptotic, and/or anti-inflammatory activities. Future preclinical studies are required to establish the TXM-peptides as potential therapeutic drugs for brain injuries.
PLoS One, 2016; 11
24906196: Schreiber S, Lin R, Haim L, Baratz-Goldstien R, Rubovitch V, Vaisman N, Pick CG
Enriched environment improves the cognitive effects from traumatic brain injury in mice.
To date, there is yet no established effective treatment (medication or cognitive intervention) for post-traumatic brain injury (TBI) patients with chronic sequelae. Enriched environment (EE) has been recognized of importance in brain regulation, behaviour and physiology. Rodents reared in, or pre-exposed to EE, recovered better from brain insults. Using the concussive head trauma model of minimal TBI in mice, we evaluated the effect of transition to EE following a weight-drop (30g or 50g) induced mTBI on behavioural and cognitive parameters in mice in the Novel Object Recognition task, the Y- and the Elevated Plus mazes. In all assays, both mTBI groups (30g, 50g) housed in normal conditions were equally and significantly impaired 6 weeks post injury in comparison with the no-mTBI ($p<0.001$ and $p<0.03$, respectively) and the

mTBI+EE groups ($p<0.001$ for the 30g, and $p<0.017$ for the 50g). No differences were found between the control and the EE mice. Two separate finding emerge: (1) the significantly positive effects of the placement in EE following mTBI, on the rehabilitative process of the tested behaviours in the affected mice; (2) the lack of difference between the groups of mice affected by 30g or by 50g. Further studies are needed in order to characterize the exact pathways involved in the positive effects of the EE on mice recovery from mTBI. Possible clinical implications indicate the importance of adapting correlates of EE to humans, i.e., prolonged and intensive physical activity - possibly combined with juggling training and intensive cognitive stimulation.

Behav Brain Res, 2014; 271

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BOARD NUMBER: S01-035

DECIPHERING ARC-DEPENDENT CONTROL OF CIRCUIT DYNAMICS UNDERLYING THE CONSOLIDATION OF ASSOCIATIVE MEMORIES

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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[1]University of Tokyo, Department Of Neurochemistry, Bunkyo, Tokyo, Japan, [2]University of Kyoto, Department Of Advanced Imaging, Kyoto, Japan, [3]University of Texas Southwestern Medical Centre, Department Of Psychiatry, Dallas, United States of America, [4]University of Kagoshima, Department Of Biochemistry And Molecular Biology, Kagoshima, Japan

Adapting to changing associations between environmental cues and rewards is necessary for survival. Medial prefrontal cortex (mPFC) neurons mediate these associations and show altered signal processing in response to learning. However, the processes underlying these alterations remain to be elucidated. The protein Arc is expressed in highly stimulated neuronal ensembles following associative learning and is well a recognized marker for persistent plasticity. Yet, how Arc-expressing ensembles acquire dynamic attributes during associative learning is unclear. **Aim:** Thus, our goal is to characterize the dynamics of mPFC Arc-expressing ensembles during associative learning. **Methods:** To this aim, we performed longitudinal in vivo 2-photon imaging of the mPFC in mice as they performed a cue-reward associative learning task. Imaged mice were injected with viral constructs coding for both the calcium indicator XCaMP-G and a red fluorophore driven by the active ensemble-marking E-SARE promoter. This method allowed us to track the temporal and spatial dynamics of mPFC Arc-expressing ensembles and their activity patterns during learning epochs. **Results:** We observed that Arc-expressing ensembles demonstrated increased neuronal activity to task-related cues compared to surrounding control neurons. However, ensembles identified in early learning did not show increased signaling in subsequent sessions. Instead, early learning Arc- expressing ensembles demonstrated reduced neuronal activity during reward-seeking behavior in later sessions. **Conclusion:** This suggests a two-way dynamic interaction between neuronal activity and Arc expression during associative learning. We hypothesize that Arc may be involved in regulating persistence of mPFC circuit strength to enable future flexible behaviors.

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BOARD NUMBER: S01-036

SOCIAL AND SPATIAL CODES IN HIPPOCAMPUS CA2 AND ENTORHINAL CORTEX

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

Sarah Thon[1], Ane Charlotte Christensen[1], Maria Fjeldstad[2], Vemund Schøyen[3], Elise Olsen[1], Mikkel Lepperød[4], Marianne Fyhn[3], Torkel Hafting[1]
[1]University of Oslo, Institute Of Basic Medical Sciences, Oslo, Norway, [2]University of Oslo, Department Of Informatics, Oslo, Norway, [3]University of Oslo, Department Of Biosciences, Oslo, Norway, [4]University of Oslo, Department Of Physics, Oslo, Norway

The hippocampal formation and parahippocampal regions are important for formation, consolidation and retrieval of episodic memories. While the spatial aspect of episodic memories has been studied in great detail, it remains elusive how social information is encoded in the brain. Emerging evidence suggests that the hippocampus region CA2 plays an essential role for social recognition memories, and silencing of neuronal activity in this area impairs social recognition. In contrast to the place cells in hippocampus CA1 and CA3, which show precise location-specific activity, place cells in CA2 change their activity over time and in response to novel objects and conspecifics. Entorhinal cortex is directly connected to CA2, but the contribution to social memories by this route is largely unknown. Here, we recorded from CA2 and entorhinal cortex from rats in a social recognition task to determine the social and spatial coding in the two regions. Social recognition can be defined as the ability to recognize and memorize familiar conspecifics. We measured this as a decrease in time that a rat spends around a previous littermate compared to a novel conspecific in a social recognition task, which was then followed by a similar task with object exploration. Preliminary data analysis confirms that grid cells from entorhinal cortex show a stable spatial code during the recordings, while the CA2 cells contains a more diverse code.

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BOARD NUMBER: S01-037

PROBING SUBTHRESHOLD DYNAMICS OF HIPPOCAMPAL NEURONS BY PULSED OPTOGENETICS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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A basic transformation process in the brain is the conversion of a neuron's excitatory and inhibitory inputs to spikes. Experimentally examining the transformation process requires access to subthreshold membrane dynamics. To date, only intracellular recordings meet this requirement. Here we probed the subthreshold dynamics using optogenetic depolarizing pulses in hippocampal neuronal assemblies in freely moving mice. Using chronically implanted µLED probes (4 shanks with 3 µLED on each shank), we recorded and probed large numbers of neurons simultaneously in freely moving CamKIIα-Cre::Ai32 mice. Light-sensitive neurons responded to one or more µLEDs and the spike numbers were used as a proxy for estimating relative changes of the membrane potential dynamics. Excitability decreased during sharp-wave ripples coupled with increased inhibitory tone. In contrast to this "negative gain," optogenetic probing showed increased within-field excitability in place cells by weakening and unmasked stable place fields in initially non–place cells. Neuronal assemblies active during sharp-wave ripples in the home cage predicted spatial overlap and sequences of place fields of both place cells and unmasked preexisting place fields of non–place cells during track running. We have developed a method for studying subthreshold dynamics of individual cells in chronic recordings using novel high-resolution optical stimulation as a proxy for the membrane polarization. Indirect probing of subthreshold dynamics in neuronal populations permits the disclosing of preexisting assemblies and modes of neuronal operations.

Pubmed:
35113721: Valero M, Zutshi I, Yoon E, Buzsáki G
Probing subthreshold dynamics of hippocampal neurons by pulsed optogenetics.
Understanding how excitatory (E) and inhibitory (I) inputs are integrated by neurons requires monitoring their subthreshold behavior. We probed the subthreshold dynamics using optogenetic depolarizing pulses in hippocampal neuronal assemblies in freely moving mice. Excitability decreased during sharp-wave ripples coupled with increased I. In contrast to this "negative gain," optogenetic probing showed increased within-field excitability in place cells by weakening I and unmasked stable place fields in initially non-place cells. Neuronal assemblies active during sharp-wave ripples in the home cage predicted spatial overlap and sequences of place fields of both place cells and unmasked preexisting place fields of non-place cells during track running. Thus, indirect probing of subthreshold dynamics in neuronal populations permits the disclosing of preexisting assemblies and modes of neuronal operations.
Science, 2022; 375
33619404: Valero M, Viney TJ, Machold R, Mederos S, Zutshi I, Schuman B, Senzai Y, Rudy B, Buzsáki G
Sleep down state-active ID2/Nkx2.1 interneurons in the neocortex.
Pyramidal cells and GABAergic interneurons fire together in balanced cortical networks. In contrast to this general rule, we describe a distinct neuron type in mice and rats whose spiking activity is anti-correlated with all principal cells and interneurons in all brain states but, most prevalently, during the down state of non-REM (NREM) sleep. We identify these down state-active (DSA) neurons as deep-layer neocortical neurogliaform cells that express ID2 and Nkx2.1 and are weakly immunoreactive to neuronal nitric oxide synthase. DSA neurons are weakly excited by deep-layer pyramidal cells and strongly inhibited by several other GABAergic cell types. Spiking of DSA neurons modified the sequential firing order of other neurons at down-up transitions. Optogenetic activation of ID2Nkx2.1 interneurons in the posterior parietal cortex during NREM sleep, but not during waking, interfered with consolidation of cue discrimination memory. Despite their sparsity, DSA neurons perform critical physiological functions.
Nat Neurosci, 2021; 24
29729527: Valero M, de la Prida LM
The hippocampus in depth: a sublayer-specific perspective of entorhinal-hippocampal function.
Understanding how the brain represents events is a fundamental question in neuroscience. The entorhinal-hippocampal system is central to such representations, which are severely compromised in some neurological diseases. In spite of much

progress, a comprehensive, integrated view of spatial, temporal and other aspects of episodic representation remains elusive. Here, we review recent data on the role of cell-type specific entorhinal inputs which excite deep and superficial CA1 pyramidal cells by direct and indirect pathways. We discuss how an entorhinal dialogue with deep-superficial CA1 cells can multiplex neuronal activity along theta phases and how their reactivation may be segregated during sharp-wave ripples. Thus, deep and superficial CA1 sublayers provide substrate for general hippocampal function.
Curr Opin Neurobiol, 2018; 52
28641116: Valero M, Averkin RG, Fernandez-Lamo I, Aguilar J, Lopez-Pigozzi D, Brotons-Mas JR, Cid E, Tamas G, Menendez de la Prida L
Mechanisms for Selective Single-Cell Reactivation during Offline Sharp-Wave Ripples and Their Distortion by Fast Ripples.
Memory traces are reactivated selectively during sharp-wave ripples. The mechanisms of selective reactivation, and how degraded reactivation affects memory, are poorly understood. We evaluated hippocampal single-cell activity during physiological and pathological sharp-wave ripples using juxtacellular and intracellular recordings in normal and epileptic rats with different memory abilities. CA1 pyramidal cells participate selectively during physiological events but fired together during epileptic fast ripples. We found that firing selectivity was dominated by an event- and cell-specific synaptic drive, modulated in single cells by changes in the excitatory/inhibitory ratio measured intracellularly. This mechanism collapses during pathological fast ripples to exacerbate and randomize neuronal firing. Acute administration of a use- and cell-type-dependent sodium channel blocker reduced neuronal collapse and randomness and improved recall in epileptic rats. We propose that cell-specific synaptic inputs govern firing selectivity of CA1 pyramidal cells during sharp-wave ripples.
Neuron, 2017; 94
26214372: Valero M, Cid E, Averkin RG, Aguilar J, Sanchez-Aguilera A, Viney TJ, Gomez-Dominguez D, Bellistri E, de la Prida LM
Determinants of different deep and superficial CA1 pyramidal cell dynamics during sharp-wave ripples.
Sharp-wave ripples represent a prominent synchronous activity pattern in the mammalian hippocampus during sleep and immobility. GABAergic interneuronal types are silenced or fire during these events, but the mechanism of pyramidal cell (PC) participation remains elusive. We found opposite membrane polarization of deep (closer to stratum oriens) and superficial (closer to stratum radiatum) rat CA1 PCs during sharp-wave ripples. Using sharp and multi-site recordings in combination with neurochemical profiling, we observed a predominant inhibitory drive of deep calbindin (CB)-immunonegative PCs that contrasts with a prominent depolarization of superficial CB-immunopositive PCs. Biased contribution of perisomatic GABAergic inputs, together with suppression of CA2 PCs, may explain the selection of CA1 PCs during sharp-wave ripples. A deep-superficial gradient interacted with behavioral and spatial effects to determine cell participation during sleep and awake sharp-wave ripples in freely moving rats. Thus, the firing dynamics of hippocampal PCs are exquisitely controlled at subcellular and microcircuit levels in a cell type-selective manner.
Nat Neurosci, 2015; 18
34890566: Zutshi I, Valero M, Fernández-Ruiz A, Buzsáki G
Extrinsic control and intrinsic computation in the hippocampal CA1 circuit.
In understanding circuit operations, a key problem is the extent to which neuronal spiking reflects local computation or responses to upstream inputs. We addressed this issue in the hippocampus by performing combined optogenetic and pharmacogenetic local and upstream inactivation. Silencing the medial entorhinal cortex (mEC) largely abolished extracellular theta and gamma currents in CA1 while only moderately affecting firing rates. In contrast, CA3 and local CA1 silencing strongly decreased firing of CA1 neurons without affecting theta currents. Each perturbation reconfigured the CA1 spatial map. However, the ability of the CA1 circuit to support place field activity persisted, maintaining the same fraction of spatially tuned place fields and reliable assembly expression as in the intact mouse. Thus, the CA1 network can induce and maintain coordinated cell assemblies with minimal reliance on its inputs, but these inputs can effectively reconfigure and assist in maintaining stability of the CA1 map.
Neuron, 2022; 110
33288910: Mederos S, Sánchez-Puelles C, Esparza J, Valero M, Ponomarenko A, Perea G
GABAergic signaling to astrocytes in the prefrontal cortex sustains goal-directed behaviors.
GABA interneurons play a critical role in higher brain functions. Astrocytic glial cells interact with synapses throughout the whole brain and are recognized as regulatory elements of excitatory synaptic transmission. However, it is largely unknown how GABAergic interneurons and astrocytes interact and contribute to stable performance of complex behaviors. Here, we found that genetic ablation of GABA receptors in medial prefrontal cortex astrocytes altered low-gamma oscillations and firing properties of cortical neurons, which affected goal-directed behaviors. Remarkably, working memory deficits were restored by optogenetic stimulation of astrocytes with melanopsin. Furthermore, melanopsin-activated astrocytes in wild-type mice enhanced the firing rate of cortical neurons and gamma oscillations, as well as improved cognition. Therefore, our work identifies astrocytes as a hub for controlling inhibition in cortical circuits, providing a novel pathway for the behaviorally

relevant midrange time-scale regulation of cortical information processing and consistent goal-directed behaviors.
Nat Neurosci, 2021; 24
32371879: Navas-Olive A, Valero M, Jurado-Parras T, de Salas-Quiroga A, Averkin RG, Gambino G, Cid E, de la Prida LM
Multimodal determinants of phase-locked dynamics across deep-superficial hippocampal sublayers during theta oscillations.
Theta oscillations play a major role in temporarily defining the hippocampal rate code by translating behavioral sequences into neuronal representations. However, mechanisms constraining phase timing and cell-type-specific phase preference are unknown. Here, we employ computational models tuned with evolutionary algorithms to evaluate phase preference of individual CA1 pyramidal cells recorded in mice and rats not engaged in any particular memory task. We applied unbiased and hypothesis-free approaches to identify effects of intrinsic and synaptic factors, as well as cell morphology, in determining phase preference. We found that perisomatic inhibition delivered by complementary populations of basket cells interacts with input pathways to shape phase-locked specificity of deep and superficial pyramidal cells. Somatodendritic integration of fluctuating glutamatergic inputs defined cycle-by-cycle by unsupervised methods demonstrated that firing selection is tuneable across sublayers. Our data identify different mechanisms of phase-locking selectivity that are instrumental for flexible dynamical representations of theta sequences.
Nat Commun, 2020; 11
31400358: Valero M, English DF
Head-mounted approaches for targeting single-cells in freely moving animals.
Neural network processing is usually studied using the spike times of many extracellularly recorded neurons. Elucidating the cellular-synaptic mechanisms underlying these firing patterns requires identifying and controlling single cells and assessing their inputs. Single cell glass electrode techniques (intracellular, patch and juxtacellular) are well suited to filling this gap, in terms of physiology, cell identity and behavior. However, they are typically limited to in vitro and immobilized in vivo experiments, primarily due to the necessity for mechanical stability and steep learning curves. Several approaches have been recently developed to extend these technologies to freely moving animals. Here we summarize the advantages and results for different methods of single neuron glass recordings in vivo. We further review three approaches used to date for single cell recording in freely moving animals: static anchor systems, manual mechanic drives and motorized drives. Finally, we highlight new technologies capable of expanding the utility of single neuron recording in freely moving animals.
J Neurosci Methods, 2019; 326
33372130: Rogers S, Rozman PA, Valero M, Doyle WK, Buzsáki G
Mechanisms and plasticity of chemogenically induced interneuronal suppression of principal cells.
How do firing patterns in a cortical circuit change when inhibitory neurons are excited? We virally expressed an excitatory designer receptor exclusively activated by a designer drug (Gq-DREADD) in all inhibitory interneuron types of the CA1 region of the hippocampus in the rat. While clozapine N-oxide (CNO) activation of interneurons suppressed firing of pyramidal cells, unexpectedly the majority of interneurons also decreased their activity. CNO-induced inhibition decreased over repeated sessions, which we attribute to long-term synaptic plasticity between interneurons and pyramidal cells. Individual interneurons did not display sustained firing but instead transiently enhanced their activity, interleaved with suppression of others. The power of the local fields in the theta band was unaffected, while power at higher frequencies was attenuated, likely reflecting reduced pyramidal neuron spiking. The incidence of sharp wave ripples decreased but the surviving ripples were associated with stronger population firing compared with the control condition. These findings demonstrate that DREADD activation of interneurons brings about both short-term and long-term circuit reorganization, which should be taken into account in the interpretation of chemogenic effects on behavior.
Proc Natl Acad Sci U S A, 2021; 118

FENS Forum 2022
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BOARD NUMBER: S01-038

PHARMACOLOGICAL ENHANCEMENT OF ADULT NEUROGENESIS IMPROVES PATTERN SEPARATION IN YOUNG AND AGED MICE

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Background: In mice, adult hippocampal neurogenesis is elevated by interventions that improve affect and cognition. Here, we test whether chronic treatment with a novel neurogenic compound, RO6871135, can alter behaviors relevant to cognitive, anxiety, mood, and trauma-related disorders. We also test which behavioral effects are neurogenesis-dependent by ablating neurogenesis with irradiation. Methods: Aged and young adult c57BL/6J male mice were administered 7.5 mg/kg of RO6871135 compound daily for 21 days prior to behavioral testing. When applicable, X-irradiation was applied to bilateral hippocampi, prior to initiation of drug treatment. Mice were tested in a contextual fear discrimination task where they explored two similar contexts but received a foot shock in only one context. (*N* = 8-9 mice/group). Results: Aged mice showed a deficit in pattern separation compared to young adult mice. Compared to vehicle, treatment with RO6871135 improved the ability to distinguish between the similar contexts in both age groups of mice when task difficulty was appropriately tuned. RO6871135 did not alter initial contextual fear conditioning. Doublecortin staining in the dentate gyrus was increased after treatment with RO6871135 compared to vehicle as well. RO6871135 effects on context discrimination were blocked by X-irradiation. Conclusions: We demonstrated that RO6871135 improved performance of both aged and young adult mice in a contextual fear discrimination task, a measure of pattern separation. This effect was blocked by ablating neurogenesis in young mice. These findings support the further development of neurogenic compounds for novel therapeutics.

Pubmed:
15846821: Buchsbaum BR, Greer S, Chang WL, Berman KF
Meta-analysis of neuroimaging studies of the Wisconsin card-sorting task and component processes.
A quantitative meta-analysis using the activation likelihood estimation (ALE) method was used to investigate the brain basis of the Wisconsin Card-Sorting Task (WCST) and two hypothesized component processes, task switching and response suppression. All three meta-analyses revealed distributed frontoparietal activation patterns consistent with the status of the WCST as an attention-demanding executive task. The WCST was associated with extensive bilateral clusters of reliable cross-study activity in the lateral prefrontal cortex, anterior cingulate cortex, and inferior parietal lobule. Task switching revealed a similar, although less robust, frontoparietal pattern with additional clusters of activity in the opercular region of the ventral prefrontal cortex, bilaterally. Response-suppression tasks, represented by studies of the go/no-go paradigm, showed a large and highly right-lateralized region of activity in the right prefrontal cortex. The activation patterns are interpreted as reflecting a neural fractionation of the cognitive components that must be integrated during the performance of the WCST.
Hum Brain Mapp, 2005; 25
17827280: Marenco S, Siuta MA, Kippenhan JS, Grodofsky S, Chang WL, Kohn P, Mervis CB, Morris CA, Weinberger DR, Meyer-Lindenberg A, Pierpaoli C, Berman KF
Genetic contributions to white matter architecture revealed by diffusion tensor imaging in Williams syndrome.
Little is known about genetic regulation of the development of white matter. This knowledge is critical in understanding the pathophysiology of neurodevelopmental syndromes associated with altered cognition as well as in elucidating the genetics of normal human cognition. The hemideletion of approximately 25 genes on chromosome 7q11.23 that causes Williams syndrome (WS) includes genes that regulate cytoskeletal dynamics in neurons, especially LIMK1 and CYLN2, and therefore offers the opportunity to investigate the role of these genes in the formation of white matter tracts. We used diffusion tensor imaging to demonstrate alteration in white matter fiber directionality, deviation in posterior fiber tract course, and reduced lateralization of fiber coherence in WS. These abnormalities are consistent with an alteration of the late stages of neuronal migration, define alterations of white matter structures underlying dissociable behavioral phenotypes in WS, and provide human in vivo information about genetic control of white matter tract formation.

Proc Natl Acad Sci U S A, 2007; 104
19245676: Tang B, Chang WL, Lanigan CM, Dean B, Sutcliffe JG, Thomas EA
Normal human aging and early-stage schizophrenia share common molecular profiles.
We examined genome-wide expression datasets from human prefrontal cortex of normal and schizophrenic individuals ranging from 19 to 81 years of age. We found that changes in gene expression that are correlated with aging in normal subjects differ dramatically from those observed with aging in schizophrenic subjects. Only 2.5% of genes were correlated with age in both groups. Surprisingly, we also found a significant overlap (29-34%) between those genes whose expression was correlated with aging in normal subjects and those significantly altered in subjects with early-stage schizophrenia (within 4 years of diagnosis). This suggests that schizophrenia onset anticipates the normal aging process, and further, that some symptoms of aging, i.e. dementia and psychosis, might be explained by these common molecular profiles.
Aging Cell, 2009; 8
20385162: Chang WL, Swerdlow NR, Breier MR, Thangaraj N, Weber M
Parametric approaches towards understanding the effects of the preferential D3 receptor agonist pramipexole on prepulse inhibition in rats.
The preferential dopamine D3 receptor agonist pramipexole (PRA) disrupts prepulse inhibition (PPI) of acoustic startle, an operational measure of sensorimotor gating, in rats. Drug effects on PPI are sensitive to numerous experimental variables; proceeding with in-depth analyses of drug effects without a clear understanding of these variables is inefficient. The present studies characterized the impact on PRA-induced PPI deficits by a range of experimental parameters. As shown previously, PRA reduced both PPI and startle magnitude beginning 5-15 min post-injection; PRA effects on PPI were statistically significant through 35 min post-injection, while those on startle magnitude were still significant 65 min post-injection. PRA-induced PPI deficits were evident under conditions that matched startle magnitude in vehicle and PRA conditions and were independent of PRA-induced changes in prepulse-elicited motor activity. Additionally, PRA-induced PPI deficits did not differ significantly between uni- vs. cross-modal stimuli or between male vs. female rats, with no robust effect of estrous phase in females. These findings demonstrate that PRA effects on PPI are observed across several different experimental conditions and are dissociable from changes in startle magnitude or prepulse-elicited responses. Recommendations are made regarding "optimal" experimental conditions for studying the neurobiology of PRA-induced changes in PPI in rats.
Pharmacol Biochem Behav, 2010; 95
19020413: Weber M, Chang WL, Breier M, Ko D, Swerdlow NR
Heritable strain differences in sensitivity to the startle gating-disruptive effects of D2 but not D3 receptor stimulation.
Prepulse inhibition (PPI) of the startle reflex is an operational measure of sensorimotor gating that is deficient in several brain disorders and is disrupted in rats by dopamine (DA) agonists. Robust heritable strain differences are observed between Sprague-Dawley (SD) and Long-Evans (LE) strains in sensitivity to the PPI-disruptive effects of DA agonists associated with differential gene expression in the nucleus accumbens. Here, we compared the contribution of D2 versus D3 receptors with this heritable difference, using the D3-preferential agonist (pramipexole), the mixed D3/D2 agonist (quinpirole), the mixed D1/D2-like agonist (apomorphine), and the preferential D2 antagonist (L741,626). All DA agonists disrupted PPI in SD and LE rats. Greater sensitivity for this effect was evident with apomorphine and quinpirole in SD than LE rats, but not with pramipexole. The selective D2 antagonist L741,626 preferentially reversed apomorphine-induced PPI deficits at a dose that did not alter pramipexole-induced PPI deficits. We conclude that the heritable pattern of greater PPI 'disruptability' by DA agonists in SD versus LE rats reflects differences in D2 but not D3 receptor-associated mechanisms.
Behav Pharmacol, 2008; 19
22227455: Chang WL, Weber M, Breier MR, Saint Marie RL, Hines SR, Swerdlow NR
Stereochemical and neuroanatomical selectivity of pramipexole effects on sensorimotor gating in rats.
In rats, prepulse inhibition (PPI) of acoustic startle is disrupted by systemic administration of dopaminergic agonists, such as the dopamine D3 receptor (D3R)-preferential agonist pramipexole (PPX). PPX has D3R-active (S) and -inactive (R) stereoisomers. Here, we tested the neuroanatomical and stereochemical selectivity of PPX effects on PPI.
Brain Res, 2012; 1437
21683731: Chang WL, Breier MR, Yang A, Swerdlow NR
Disparate effects of pramipexole on locomotor activity and sensorimotor gating in Sprague-Dawley rats.
Prepulse inhibition (PPI) of acoustic startle and locomotor activity are both widely studied in the preclinical development of dopaminergic agents, including those acting at D3 dopamine receptors. In mice, the dopamine D3 receptor-preferential agonist pramipexole (PPX) alters locomotor activity in a biphasic manner at doses that have no effect on PPI. The present study examined the time-course of PPX effects on locomotion and PPI in rats. In adult male Sprague-Dawley rats, PPX (0, 0.1, 0.3, 1.0mg/kg) was injected prior to measurement of locomotor activity for 90 min in photobeam chambers. Based on disparate early vs. late effects of PPX on locomotion, the effects of PPX (0 vs. 0.3mg/kg) on PPI were tested 20 and 80 min after injection. All doses of PPX decreased locomotor activity for 30 min compared to vehicle, and the higher doses

stimulated hyperlocomotion later in the session; the late hyperlocomotion, but not the early hypolocomotion, was blocked by the D2-selective antagonist, L741626 (1.0mg/kg sc). In contrast to its locomotor effects, PPX caused a similar reduction in PPI at 20 and 80 min after administration. These findings suggest both a temporal and pharmacological dissociation between PPX effects on locomotor activity and PPI; these two behavioral measures contribute non-redundant information to the investigation of D3-related behavioral pharmacology.
Pharmacol Biochem Behav, 2011; 99
20215963: Chang WL, Geyer MA, Buell MR, Weber M, Swerdlow NR
The effects of pramipexole on prepulse inhibition and locomotor activity in C57BL/6J mice.
Pramipexole (PRA) is a preferential D3R agonist that, in rats and humans, modifies prepulse inhibition (PPI) of the acoustic startle reflex, an operational measure of sensorimotor gating. The ability to use similar PPI measures across species, and the relative ease of genetic manipulations in mice, suggests that molecular studies of the D3R regulation of sensorimotor gating might be best pursued in mice. Here, we evaluate the effects of PRA on PPI and locomotion in C57BL/6J mice, the background strain for many gene knockout mouse models. Male C57BL/6J mice were tested for PPI and locomotor activity after injection of PRA. No significant effects of PRA on PPI were observed at any dose (0.1-10.0 mg/kg), but a significant reduction in startle magnitude was observed after 10 mg/kg PRA. In contrast, the D1/2 agonist, apomorphine (5 mg/kg) significantly reduced PPI in these mice. At doses of PRA that did not alter startle magnitude (0.3, 1, 3 mg/kg), significant decreases in the amount of locomotor and investigatory behavior were observed. Distinct from findings in rats and humans, it seems that either: (i) PRA does not activate D3Rs in C57BL/6J mice, or (ii) D3R agonists are not sufficient to alter PPI in this mouse strain.
Behav Pharmacol, 2010; 21
20346635: Weber M, Chang WL, Breier MR, Yang A, Millan MJ, Swerdlow NR
The effects of the dopamine D2 agonist sumanirole on prepulse inhibition in rats.
Dopamine agonists reduce prepulse inhibition (PPI) of startle in rats. While it is used to predict antipsychotic efficacy, the specific receptor subtypes mediating this effect of dopamine agonists remain unclear. We characterized the effects of sumanirole, a highly selective D2 agonist, on PPI in rats. Sumanirole decreased PPI at 60-120 ms prepulse intervals, and increased PPI at 10-20 ms intervals. PPI deficits were antagonized by low doses of the preferential D2 antagonist L741626, supporting a D2 mechanism of action. Sumanirole is a valuable tool for parsing the role of dopamine receptor subtypes in the regulation of PPI.
Eur Neuropsychopharmacol, 2010; 20
19506839: Swerdlow NR, Lelham SA, Sutherland Owens AN, Chang WL, Sassen SD, Talledo JA
Pramipexole effects on startle gating in rats and normal men.
Dopamine D3 receptors regulate sensorimotor gating in rats, as evidenced by changes in prepulse inhibition (PPI) of startle after acute administration of D3 agonists and antagonists. In this study, we tested the effects of the D3-preferential agonist, pramipexole, on PPI in normal men and Sprague-Dawley rats.
Psychopharmacology (Berl), 2009; 205

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-039

IMPROVED LEARNING IN ADULT MICE WITH PTEN GENE DEPLETION IN HIPPOCAMPAL NEURONS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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In hippocampal neuronal circuit, Pten/PI3K opposing expression is crucial for maintaining synaptic strength and plasticity. Pten mutation causes overactivation of mTOR through PI3K-AKT pathway. Active state of this intrinsic pathway promotes long-term synaptic plasticity underlying learning and memory. Pten loss in several brain structures during neurodevelopment at neonatal stage causes morphological changes that affects neuronal connectivity contributing to memory impairment and ASD, ataxia and seizures development. However, Pten function in adult neurons is not fully examined. We investigated whether Pten loss in adult glutaminergic neurons causes improved cognitive function in hippocampal-dependent spatial learning tasks. To knockout Pten gene in hippocampus, we used $Pten^{flox/flox}$ mouse model and AAV-CaMKII*α*-Cre vector (stereotactic injection).
Between 7th and 15th weeks post-AAV treatment, we conducted cognitive tests in the IntelliCage and recorded ultrasonic vocalizations (USV). Additionally, at 7th weeks, we checked neurons excitability from CA1 area and animals' phenotype: activity, anxiety, social memory and skills. Around 7 weeks' time-point, when mTOR hyperactivation occurs (IHC staining), and decreased Pten expression was observed (western blot), Pten KO mice achieved improved learning performance compared to controls in simple and advanced tests. Additionally, mutant mice showed hyperactivity and altered social novelty behavior but normal sociability skills, and normal USV suggesting that Pten KO have not developed ASD phenotype. Whole-cell patch-clamp recordings revealed altered excitability. At 15 weeks post mutation induction, there was no significant differences between groups in learning observed. However, we have detected altered USV and mTOR expression attenuation due to progressive neurodegeneration that occurred in hippocampal neurons.

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BOARD NUMBER: S01-040

STABLE PATTERNS OR DYNAMIC CHANGES: HOW DOES THE PREFRONTAL CORTEX COPE WITH CHANGING BEHAVIORAL DEMANDS?

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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The prefrontal cortex (PFC) plays an important role in cognitive processes such as working memory, but it is also involved in emotional and motivational processes such as anxiety and social interaction. Previous studies using electrophysiological recordings have shown that PFC neurons are modulated by different variables in one specific task, but it remains unclear how the same neurons respond during different tasks. Do PFC neurons show stable coding for similar events across tasks, or do they change their responses? Here we address this question by imaging the same PFC neurons in mice while they perform multiple tasks. We infused an adeno-associated virus to express the calcium indicator GCaMP6f under the CamKII-promoter into the medial PFC (mPFC) of C57BL/6N mice and implanted a GRIN lens to image the activity of mPFC neurons over multiple weeks. Animals were food restricted and imaged during five distinct behavioral tests: A T-maze spatial working memory task, elevated plus-maze exploration, social interaction, novel object recognition and discriminative auditory fear conditioning. We successfully imaged the same cells over multiple sessions and tasks. We used generalized linear models to identify the task variables that mPFC neurons coded for and compared this across multiple tasks. While many task variables were encoded by mPFC neurons, animals' position influenced cell activity most prominently across the different tasks. We also found stable representations of task variables across multiple sessions of the same task and currently investigate how the same neurons' responses are influenced by the changing demands of different tasks.

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BOARD NUMBER: S01-041

THE ROLE OF HIPPOCAMPAL CA1 IN RELATIONAL LEARNING IN MICE

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Learning relationships between cues in our environment enables us to recognise common underlying structures of events and is thought to form the basis of episodic memory. This type of learning - often called relational learning - requires retaining both the stimulus identity as well as its relation to other cues. Despite being essential for many of our everyday decisions, there is limited insight into how relational learning is achieved within the brain. One area implicated in relational learning is the hippocampus. Specifically, neurons in the CA1 area of the hippocampus have been shown to represent variables that are essential for constructing a relational structure of the environment, such as cue configurations, the value of such configurations and their order in space and time. To investigate the neural mechanisms of relational learning, we designed an odour sequence task for mice that requires subjects to learn about both the identity of an odour and its temporal position within a sequence. Importantly, the task design allows for manipulation of the temporal structure and value of cues separately. This allowed us to probe generalisation to novel cues in the same structure, and the ability to update the value associated with learnt relational structure. We found that mice quickly learnt this task and, in line with a key role for hippocampal circuitry, optogenetic inactivation of CA1 impaired task performance. In addition, we found that after initial learning, mice could adapt to manipulations of cue value and identity, suggesting flexible use of previously learnt relational structures.

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BOARD NUMBER: S01-042

COMPUTATIONAL STRATEGIES AND NEURAL CORRELATES OF PROBABILISTIC REVERSAL LEARNING IN MICE

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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When faced with a changing, uncertain environment it is necessary to infer its underlying structure to guide behaviour. This has often been formalised as a value updating problem – where actions are chosen based on the weighted average of past reward. Activity of midbrain dopamine neurons is commonly associated with the error in such value prediction, and is proposed to be crucial for learning and updating of values to inform decision making. However, it has become increasingly apparent that both humans and animals often use an alternative strategy – inferring hidden states to make predictions and guide behaviour. In this study we hypothesised that mice might also use hidden state strategies during decision making, and that this would be reflected in midbrain dopamine activity. To investigate this, we used a probabilistic reversal learning task in mice. In this paradigm, for optimal performance it is necessary to continuously integrate past trial outcomes to predict reward contingencies associated with different actions across reversals. Probing animals' behaviour with computational modelling, we found that it was consistently best fit by models that incorporated hidden states. Furthermore, by recording dopamine release in the nucleus accumbens during the task, we found phasic dopamine was most strongly predicted by error associated with hidden state inference strategies. Overall, we find that mouse behaviour and midbrain dopamine activity during probabilistic reversal learning is best described by a hidden state inference strategy. Ongoing work is investigating the sources of the state prediction that influence dopamine signalling during decision making.

Pubmed:
35039510: Sánchez-Bellot C, AlSubaie R, Mishchanchuk K, Wee RWS, MacAskill AF
Two opposing hippocampus to prefrontal cortex pathways for the control of approach and avoidance behaviour.
The decision to either approach or avoid a potentially threatening environment is thought to rely upon the coordinated activity of heterogeneous neural populations in the hippocampus and prefrontal cortex (PFC). However, how this circuitry is organized to flexibly promote both approach or avoidance at different times has remained elusive. Here, we show that the hippocampal projection to PFC is composed of two parallel circuits located in the superficial or deep pyramidal layers of the CA1/subiculum border. These circuits have unique upstream and downstream connectivity, and are differentially active during approach and avoidance behaviour. The superficial population is preferentially connected to widespread PFC inhibitory interneurons, and its activation promotes exploration; while the deep circuit is connected to PFC pyramidal neurons and fast spiking interneurons, and its activation promotes avoidance. Together this provides a mechanism for regulation of behaviour during approach avoidance conflict: through two specialized, parallel circuits that allow bidirectional hippocampal control of PFC.
Nat Commun, 2022; 13
34845987: AlSubaie R, Wee RW, Ritoux A, Mishchanchuk K, Passlack J, Regester D, MacAskill AF
Control of parallel hippocampal output pathways by amygdalar long-range inhibition.
Projections from the basal amygdala (BA) to the ventral hippocampus (vH) are proposed to provide information about the rewarding or threatening nature of learned associations to support appropriate goal-directed and anxiety-like behaviour. Such behaviour occurs via the differential activity of multiple, parallel populations of pyramidal neurons in vH that project to distinct downstream targets, but the nature of BA input and how it connects with these populations is unclear. Using channelrhodopsin-2-assisted circuit mapping in mice, we show that BA input to vH consists of both excitatory and inhibitory projections. Excitatory input specifically targets BA- and nucleus accumbens-projecting vH neurons and avoids prefrontal cortex-projecting vH neurons, while inhibitory input preferentially targets BA-projecting neurons. Through this specific connectivity, BA inhibitory projections gate place-value associations by controlling the activity of nucleus accumbens-projecting vH neurons. Our results define a parallel excitatory and inhibitory projection from BA to vH that can support goal-directed behaviour.
Elife, 2021; 10
34381241: Grieves RM, Jedidi-Ayoub S, Mishchanchuk K, Liu A, Renaudineau S, Duvelle É, Jeffery KJ

Irregular distribution of grid cell firing fields in rats exploring a 3D volumetric space.
We investigated how entorhinal grid cells encode volumetric space. On a horizontal surface, grid cells usually produce multiple, spatially focal, approximately circular firing fields that are evenly sized and spaced to form a regular, close-packed, hexagonal array. This spatial regularity has been suggested to underlie navigational computations. In three dimensions, theoretically the equivalent firing pattern would be a regular, hexagonal close packing of evenly sized spherical fields. In the present study, we report that, in rats foraging in a cubic lattice, grid cells maintained normal temporal firing characteristics and produced spatially stable firing fields. However, although most grid fields were ellipsoid, they were sparser, larger, more variably sized and irregularly arranged, even when only fields abutting the lower surface (equivalent to the floor) were considered. Thus, grid self-organization is shaped by the environment's structure and/or movement affordances, and grids may not need to be regular to support spatial computations.
Nat Neurosci, 2021; 24
32959344: Jedidi-Ayoub S, Mishchanchuk K, Liu A, Renaudineau S, Duvelle É, Grieves RM
Volumetric spatial behaviour in rats reveals the anisotropic organisation of navigation.
We investigated how access to the vertical dimension influences the natural exploratory and foraging behaviour of rats. Using high-accuracy three-dimensional tracking of position in two- and three-dimensional environments, we sought to determine (i) how rats navigated through the environments with respect to gravity, (ii) where rats chose to form their home bases in volumetric space, and (iii) how they navigated to and from these home bases. To evaluate how horizontal biases may affect these behaviours, we compared a 3D maze where animals preferred to move horizontally to a different 3D configuration where all axes were equally energetically costly to traverse. Additionally, we compared home base formation in two-dimensional arenas with and without walls to the three-dimensional climbing mazes. We report that many behaviours exhibited by rats in horizontal spaces naturally extend to fully volumetric ones, such as home base formation and foraging excursions. We also provide further evidence for the strong differentiation of the horizontal and vertical axes: rats showed a horizontal movement bias, they formed home bases mainly in the bottom layers of both mazes and they generally solved the vertical component of return trajectories before and faster than the horizontal component. We explain the bias towards horizontal movements in terms of energy conservation, while the locations of home bases are explained from an information gathering view as a method for correcting self-localisation.
Anim Cogn, 2021; 24
32034157: Grieves RM, Jedidi-Ayoub S, Mishchanchuk K, Liu A, Renaudineau S, Jeffery KJ
The place-cell representation of volumetric space in rats.
Place cells are spatially modulated neurons found in the hippocampus that underlie spatial memory and navigation: how these neurons represent 3D space is crucial for a full understanding of spatial cognition. We wirelessly recorded place cells in rats as they explored a cubic lattice climbing frame which could be aligned or tilted with respect to gravity. Place cells represented the entire volume of the mazes: their activity tended to be aligned with the maze axes, and when it was more difficult for the animals to move vertically the cells represented space less accurately and less stably. These results demonstrate that even surface-dwelling animals represent 3D space and suggests there is a fundamental relationship between environment structure, gravity, movement and spatial memory.
Nat Commun, 2020; 11

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-043

NEUROPROTECTIVE POTENTIAL OF CHEBULINIC ACID IN STREPTOZOTOCIN-INDUCED DIABETES-ASSOCIATED COGNITIVE DECLINE: BEHAVIORAL AND BIOCHEMICAL EVIDENCES

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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ISF College of Pharmacy Moga India, Neuropharmacology, Moga, India

Previous studies demonstrated that streptozotocin (STZ) induced intracellular glucose following neurochemical and structural abnormalities are the leading causes of neuronal damage consequently learning and memory deficits. Chebulinic acid (ChA), a flavonoid isolated from Terminalia Chebula, has potent protective effects on peripheral and central streptozotocin (STZ)-induced diabetic and diabetic AD rats. Recently, the effects of ChA on learning and memory performances were monitored in many animal models of cognitive impairment. However, to date, no studies have investigated the ameliorative effects of ChA on diabetes associated with cognitive decline (DACD). In this study, we investigated the effects of ChA, using a STZ-treated rat model and explored its potential mechanism. Diabetic rats were treated with ChA (25, 50 and 100 mg/kg/i.p) for 14 days. The learning and memory function were evaluated by Morris water maze test. The oxidative stress markers malondialdehyde (MDA), nitrite (NO) and GSH (glutathione) and inflammatory cytokines (TNF-a, IL-1β, and IL-6) were measured in hippocampus. P38 MPAK was evaluated using Elisa. The results showed that ChA supplement in STZ administered rats amended learning and memory performances compared with the STZ group. Moreover, ChA complement GSH levels, reduced MDA and NO levels, and alleviated TNF-α, IL-1β, and IL-6 compared with the STZ group in the hippocampus. The post-treatment with ChA also significantly decreased p38 MAPK expression. Our results showed that ChA may be a promising satisfying agent for improving cognitive decline in DACD.

Pubmed:

27744573: Arora R, Deshmukh R
Embelin Attenuates Intracerebroventricular Streptozotocin-Induced Behavioral, Biochemical, and Neurochemical Abnormalities in Rats.
Embelin, the main active constituent of Embelia ribes, has been reported to possess various pharmacological actions, including anti-inflammatory, antioxidant, anticonvulsant, and neuroprotective. The present study was designed to investigate neuroprotective mechanisms and therapeutic potential of embelin against intracerebroventricular streptozotocin (ICV-STZ)-induced experimental sporadic dementia in rats. STZ was infused bilaterally at the dose of (3 mg/kg/1 µl/1 min) ICV on day first and third. Spatial and non-spatial memory was evaluated using Morris water maze and object recognition task in rats. Embelin (2.5, 5, and 10 mg/kg, i.p.) was administrated for 14 days from seventh day onwards after first ICV-STZ infusion in rats. On day 22, rats were sacrificed and hippocampal brain regions were used to identify biochemical, neurochemical, and neuroinflammatory alterations. STZ-infused rats showed significant learning and memory deficit which was associated with an increase in oxidative stress (lipid peroxidation and nitrite), compromised antioxidant defense (reduced glutathione), neurotransmitter alterations (AChE, dopamine, noradrenaline, 5-hydroxytryptamine, gama amino butyric acid, and glutamate), and elevation in neuroinflammatory cytokine (IL-1 β, IL-6, and TNF-α) levels. Embelin dose dependently attenuated STZ-induced cognitive deficit and biochemical alterations and restored hippocampal neurochemical levels. The observed protective effect might be attributed to the antioxidant and anti-inflammatory potential of embelin and its ability to restore hippocampal neurochemistry. Thus, the outcomes of the current study suggest therapeutic potential of embelin in cognitive disorders such as sporadic Alzheimer's disease (SAD).
Mol Neurobiol, 2017; 54
32621278: Arora R, Deshmukh R
Correction to: Embelin Attenuates Intracerebroventricular Streptozotocin-Induced Behavioral, Biochemical, and Neurochemical Abnormalities in Rats.
The original version of this article unfortunately missed to include the other affiliation of the first author Rimpi Arora as listed below.
Mol Neurobiol, 2020; 57
26057771: Arora RB, Kumar K, Deshmukh RR

FENS Forum 2022
9-13 July 2022 | Paris, France

FK506 attenuates intracerebroventricular streptozotocin-induced neurotoxicity in rats.

Upregulation in calcineurin (CaN) signaling has been implicated in various neurodegenerative disorders. In the present study, we have investigated the effect of FK506--a CaN inhibitor--on streptozotocin (STZ)-induced experimental dementia of the Alzheimer's type in rats. STZ was administered intracerebroventricularly to induce a cognitive deficit and oxidative stress. Nonimmunosuppressive doses (0.5 and 1 mg/kg postoperatively) of FK506 (tacrolimus) were administered for 21 day in STZ-treated rats. Cognitive functions were assessed using the Morris water maze and passive avoidance tasks. Malondialdehyde and nitrite glutathione levels, as well as acetylcholinesterase activity, were determined to evaluate oxidative stress and cholinergic functions. Lactate dehydrogenase levels were estimated and histological analysis of the dentate gyrus and the CA1 region of the hippocampus was carried out to identify degenerative changes. STZ produced significant deterioration of cognitive functions, oxidative stress, and degenerative changes in the cortical and hippocampal brain regions. FK506 dose-dependently attenuated STZ-induced cognitive deficits, oxidative stress, and degenerative changes in the cortex and hippocampus. These results suggest a potential role of CaN signaling in degenerative processes, and that inhibition of CaN may be useful in the treatment of neurodegenerative disorders such as Alzheimer's disease.

Behav Pharmacol, 2013; 24

FENS Forum 2022
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BOARD NUMBER: S01-044

MOTIVATIONAL PERFORMANCE IN HUMANS AS A FUNCTION OF THE NEUROCHEMICAL COMPOSITION OF ANTERIOR INSULA AND DORSOMEDIAL PREFRONTAL CORTEX/DORSAL ANTERIOR CINGULATE CORTEX.

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Aims: Motivation can be characterized as a series of cost–benefit valuations in which individuals weight the amount of effort expendable in return for a chosen reward. Previous work has revealed that several brain regions, such as the dorsomedial prefrontal cortex/dorsal anterior cingulate cortex (dmPFC/dACC) and the anterior insula (IA), are involved in different aspects of motivated behavior. However, little is known about the neurochemical underpinnings of individual differences in motivation, particularly in these brain regions. Methods: We apply proton magnetic resonance spectroscopy (^{1}H-MRS) at ultra-high field (7T), enabling distinction between glutamine (Gln) and glutamate (Glu), in two different brain regions: the dmPFC/dACC and AI. Specifically, we inquire whether levels of metabolites in each of these brain regions correlate with inter-individual motivational parameters. Using effort-based monetary incentivized tasks involving either physical and mental effort, we extract computational parameters from participants' behaviors allowing to relate brain metabolites to sensitivity to reward, effort, and fatigue. Given the relevant literature comparing win and loss aversion, we also examine participants' differences in loss versus gain sensitivity. Results: Our results reveal relationships between specific metabolites including Gln, γ-aminobutyric acid (GABA), Glu and their ratios with participants' motivational parameters mainly in the AI. Conclusion: Our findings provide insights implicating specific brain metabolites on computational components of motivated performance. This approach and findings can help to develop novel therapeutic strategies based on the targeting of brain metabolism to ameliorate deficits in motivated behavior.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-045

REDUCTION OF ADULT NEUROGENESIS BY TEMOZOLOMIDE INHIBITS INTRINSIC PREFERENCE FOR EXPLORING COMPLEX OBJECTS IN MICE

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Intrinsic exploratory bias is an innate tendency to prefer certain types of stimuli or environments over others. For example, mice would genuinely spent more time exploring perceptually complex objects (i.e. with edges and concavities) than simpler objects without irregularities. Intrinsic exploratory bias are relevant as they may be associated to cognitive, emotional and even personality-like traits. However, their neurobiological basis are scarcely investigated. Adult hippocampal neurogenesis (AHN) is a key neuroplastic phenomenon for the processing of spatial and contextual stimuli in rodents, being involved in novelty recognition, spatial navigation and spatial pattern separation tasks. Therefore, here we studied whether a pharmacological inhibition of AHN influences intrinsic motivation for exploring complex objects. Twenty male young adult C57BL/6J mice (~3 months old) received vehicle or the DNA alkylating agent temozolomide (TMZ) for four weeks. Bromodeoxyuridine (BrdU) was administered weekly, confirming a reduction of AHN-related markers by TMZ. After the pharmacological treatment, mice were tested for behavior. TMZ did not impair mice's health nor their general exploratory and anxiety-like responses. Unlike control mice, the TMZ-treated mice did not prefer exploring a complex (i.e. irregular) object over a simple (i.e. non-irregular) object of similar size presented at once. Nevertheless, they were able to discriminate a novel complex object from a familiar complex object. This suggest that the lack of intrinsic preference for complexity could be explained by motivational and not by cognitive variables. Future studies should investigate a new role of AHN in modulating exploratory bias. **Project PID2020-114374RB-I00 funded by MCIN/AEI/10.13039/501100011033/. University of Malaga.**

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-046

USING THE KNOWLEDGE BASE HIPPOCAMPOME.ORG TO INVESTIGATE HIPPOCAMPAL CIRCUIT DYNAMICS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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AIMS: Understanding brain operation demands linking basic behavioural traits to cell-type specific dynamics of different brain-wide subcircuits. This requires a system to classify the basic operational modes of neurons and circuits. Single-cell phenotyping of firing behaviour during ongoing oscillations in vivo has provided a large body of evidence on hippocampal function, but data are dispersed and diverse. METHODS: We mined literature to search for in vivo single-cell firing patterns and phase-locking values of multiple cell types from the hippocampal formation and entorhinal cortex during spontaneous oscillations. To complement missing and unresolved pieces of knowledge, we combined single-cell sharp and silicon probe recordings in adult rats and mice under urethane and in awake head-fixed conditions. RESULTS: We updated information on oscillatory dynamics of over 100 hippocampal neuronal types defined in Hippocampome.org. We integrated all current knowledge about the morphology, biophysics, genetic identity, connectivity, and firing patterns of a wealth of GABAergic and glutamatergic neurons to provide a comprehensive single-cell map of the hippocampal region. By exploiting this resource, we explored trends of firing dynamics across the hippocampal proximo-distal and deep-superficial axes. Finally, we show how using Hippocampome.org can provide knowledge-based classification of hippocampal cell types when coupled to genetically driven optotagging approaches. CONCLUSIONS: An online resource that is a part of Hippocampome.org has been created that integrates data about which cell activities occur during oscillations across experimentation modalities with single cell resolution.

Pubmed:

33956790: Sanchez-Aguilera A, Wheeler DW, Jurado-Parras T, Valero M, Nokia MS, Cid E, Fernandez-Lamo I, Sutton N, García-Rincón D, de la Prida LM, Ascoli GA
An update to Hippocampome.org by integrating single-cell phenotypes with circuit function in vivo.
Understanding brain operation demands linking basic behavioral traits to cell-type specific dynamics of different brain-wide subcircuits. This requires a system to classify the basic operational modes of neurons and circuits. Single-cell phenotyping of firing behavior during ongoing oscillations in vivo has provided a large body of evidence on entorhinal-hippocampal function, but data are dispersed and diverse. Here, we mined literature to search for information regarding the phase-timing dynamics of over 100 hippocampal/entorhinal neuron types defined in Hippocampome.org. We identified missing and unresolved pieces of knowledge (e.g., the preferred theta phase for a specific neuron type) and complemented the dataset with our own new data. By confronting the effect of brain state and recording methods, we highlight the equivalences and differences across conditions and offer a number of novel observations. We show how a heuristic approach based on oscillatory features of morphologically identified neurons can aid in classifying extracellular recordings of single cells and discuss future opportunities and challenges towards integrating single-cell phenotypes with circuit function.
PLoS Biol, 2021; 19
33597170: Sanchez-Aguilera A, Quintanilla JP
Sharp Wave Ripples in Alzheimer's Disease: In Search of Mechanisms.
J Neurosci, 2021; 41
32634531: Sánchez-Aguilera A, Monedero G, Colino A, Vicente-Torres MÁ
Development of Action Potential Waveform in Hippocampal CA1 Pyramidal Neurons.
CA1 pyramidal neurons undergo intense morphological and electrophysiological changes from the second to third postnatal weeks in rats throughout a critical period associated with the emergence of exploratory behavior. Using whole cell current-clamp recordings in vitro and neurochemical methods, we studied the development of the somatic action potential (AP) waveform and some of the underlying channels in this critical period. At the third postnatal week, APs showed a more

hyperpolarized threshold, higher duration and amplitude. Subthreshold depolarization broadened APs and depolarized their peak overshoots more pronouncedly in immature neurons (2 weeks old). These features were mimicked by pharmacologically blocking the fast-inactivating A-type potassium current (I) and matched well with the higher concentrations of K4.2 and K4.3 and the lower concentrations of BK and K1.2 channels detected by Western blotting. Repetitive stimulation with high frequency trains (50 Hz) reproduced AP broadening associated to inactivation of the A-type current in immature cells. Moreover, repetitive firing showed changes in AP amplitude consistent with the inactivation of both sodium and potassium subthreshold currents, which resulted in higher AP amplitudes in the more immature neurons. We propose that maturation of AP waveform and excitability in this critical developmental period could be related to the onset of exploratory behaviors.
Neuroscience, 2020; 442
30759386: Fernandez-Lamo I, Gomez-Dominguez D, Sanchez-Aguilera A, Oliva A, Morales AV, Valero M, Cid E, Berenyi A, Menendez de la Prida L
Proximodistal Organization of the CA2 Hippocampal Area.
The proximodistal axis is considered a major organizational principle of the hippocampus. At the interface between the hippocampus and other brain structures, CA2 apparently breaks this rule. The region is involved in social, temporal, and contextual memory function, but mechanisms remain elusive. Here, we reveal cell-type heterogeneity and a characteristic expression gradient of the transcription factor Sox5 within CA2 in the rat. Using intracellular and extracellular recordings followed by neurochemical identification of single cells, we find marked proximodistal trends of synaptic activity, subthreshold membrane potentials, and phase-locked firing coupled to theta and gamma oscillations. Phase-shifting membrane potentials and opposite proximodistal correlations with theta sinks and sources at different layers support influences from different current generators. CA2 oscillatory activity and place coding of rats running in a linear maze reflect proximodistal state-dependent trends. We suggest that the structure and function of CA2 are distributed along the proximodistal hippocampal axis.
Cell Rep, 2019; 26
30045968: Sanchez-Aguilera A, Navas-Olive A, Valero M
Feedback and Feedforward Inhibition May Resonate Distinctly in the Ripple Symphony.
J Neurosci, 2018; 38
29300891: Scott R, Sánchez-Aguilera A, van Elst K, Lim L, Dehorter N, Bae SE, Bartolini G, Peles E, Kas MJH, Bruining H, Marín O
Loss of Cntnap2 Causes Axonal Excitability Deficits, Developmental Delay in Cortical Myelination, and Abnormal Stereotyped Motor Behavior.
Contactin-associated protein-like 2 (Caspr2) is found at the nodes of Ranvier and has been associated with physiological properties of white matter conductivity. Genetic variation in CNTNAP2, the gene encoding Caspr2, has been linked to several neurodevelopmental conditions, yet pathophysiological effects of CNTNAP2 mutations on axonal physiology and brain myelination are unknown. Here, we have investigated mouse mutants for Cntnap2 and found profound deficiencies in the clustering of Kv1-family potassium channels in the juxtaparanodes of brain myelinated axons. These deficits are associated with a change in the waveform of axonal action potentials and increases in postsynaptic excitatory responses. We also observed that the normal process of myelination is delayed in Cntnap2 mutant mice. This later phenotype is a likely modulator of the developmental expressivity of the stereotyped motor behaviors that characterize Cntnap2 mutant mice. Altogether, our results reveal a mechanism linked to white matter conductivity through which mutation of CNTNAP2 may affect neurodevelopmental outcomes.
Cereb Cortex, 2019; 29
28712654: Favuzzi E, Marques-Smith A, Deogracias R, Winterflood CM, Sánchez-Aguilera A, Mantoan L, Maeso P, Fernandes C, Ewers H, Rico B
Activity-Dependent Gating of Parvalbumin Interneuron Function by the Perineuronal Net Protein Brevican.
Activity-dependent neuronal plasticity is a fundamental mechanism through which the nervous system adapts to sensory experience. Several lines of evidence suggest that parvalbumin (PV+) interneurons are essential in this process, but the molecular mechanisms underlying the influence of experience on interneuron plasticity remain poorly understood. Perineuronal nets (PNNs) enwrapping PV+ cells are long-standing candidates for playing such a role, yet their precise contribution has remained elusive. We show that the PNN protein Brevican is a critical regulator of interneuron plasticity. We find that Brevican simultaneously controls cellular and synaptic forms of plasticity in PV+ cells by regulating the localization of potassium channels and AMPA receptors, respectively. By modulating Brevican levels, experience introduces precise molecular and cellular modifications in PV+ cells that are required for learning and memory. These findings uncover a molecular program through which a PNN protein facilitates appropriate behavioral responses to experience by dynamically gating PV+ interneuron function.
Neuron, 2017; 95

28039042: Sánchez-Aguilera A, Sánchez-Alonso JL, Vicente-Torres MÁ, Colino A
Role of low-voltage-activated calcium current and extracellular calcium in controlling the firing pattern of developing CA1 pyramidal neurons.
The firing pattern of individual neurons is an important element for information processing and storing. During the first weeks of development, there is a transitional period during which CA1 pyramidal neurons display burst-spiking behavior in contrast to the adult regular-firing pattern. Spike after-depolarizations (ADPs) constitute a major factor underlying burst-spiking behavior. Using current-clamp recordings, we studied ADP waveforms and firing patterns in CA1 pyramidal neurons of Wistar rats from 9 to 19 postnatal days (P9-19). The percentage of burst-spiking neurons increased up to P16, in correlation with the emergence of an active component in the ADP. The application of low-voltage-activated (LVA) calcium channel blockers such as nickel or mibefradil suppressed the generation of the active ADP component and burst-spiking behavior. In agreement with the development of the ADP waveform and burst-spiking behavior, voltage-clamp experiments in dissociated pyramidal neurons showed an increase in the LVA calcium current in P16-19 vs P9-12. Finally, we found that a reduction of extracellular calcium levels decreases the percentage of burst-spiking cells due to a reduction in the active component of the ADP. We conclude that a major contribution of LVA calcium channels to ADP determines the bursting capability of CA1 pyramidal neurons during a transitional postnatal period in contrast to adulthood.
Neuroscience, 2017; 344
26214372: Valero M, Cid E, Averkin RG, Aguilar J, Sanchez-Aguilera A, Viney TJ, Gomez-Dominguez D, Bellistri E, de la Prida LM
Determinants of different deep and superficial CA1 pyramidal cell dynamics during sharp-wave ripples.
Sharp-wave ripples represent a prominent synchronous activity pattern in the mammalian hippocampus during sleep and immobility. GABAergic interneuronal types are silenced or fire during these events, but the mechanism of pyramidal cell (PC) participation remains elusive. We found opposite membrane polarization of deep (closer to stratum oriens) and superficial (closer to stratum radiatum) rat CA1 PCs during sharp-wave ripples. Using sharp and multi-site recordings in combination with neurochemical profiling, we observed a predominant inhibitory drive of deep calbindin (CB)-immunonegative PCs that contrasts with a prominent depolarization of superficial CB-immunopositive PCs. Biased contribution of perisomatic GABAergic inputs, together with suppression of CA2 PCs, may explain the selection of CA1 PCs during sharp-wave ripples. A deep-superficial gradient interacted with behavioral and spatial effects to determine cell participation during sleep and awake sharp-wave ripples in freely moving rats. Thus, the firing dynamics of hippocampal PCs are exquisitely controlled at subcellular and microcircuit levels in a cell type-selective manner.
Nat Neurosci, 2015; 18
24756640: Sánchez-Aguilera A, Sánchez-Alonso JL, Vicente-Torres MA, Colino A
A novel short-term plasticity of intrinsic excitability in the hippocampal CA1 pyramidal cells.
Changes in neuronal activity often trigger compensatory mechanisms aimed at regulating network activity homeostatically. Here we have identified and characterized a novel form of compensatory short-term plasticity of membrane excitability, which develops early after the eye-opening period in rats (P16-19 days) but not before that developmental stage (P9-12 days old). Holding the membrane potential of CA1 neurons right below the firing threshold from 15 s to several minutes induced a potentiation of the repolarizing phase of the action potentials that contributed to a decrease in the firing rate of CA1 pyramidal neurons in vitro. Furthermore, the mechanism for inducing this plasticity required the action of intracellular Ca(2+) entering through T-type Ca(2+) channels. This increase in Ca(2+) subsequently activated the Ca(2+) sensor K(+) channel interacting protein 3, which led to the increase of an A-type K(+) current. These results suggest that Ca(2+) modulation of somatic A-current represents a new form of homeostatic regulation that provides CA1 pyramidal neurons with the ability to preserve their firing abilities in response to membrane potential variations on a scale from tens of seconds to several minutes.
J Physiol, 2014; 592

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-047

ROLE OF A HYPOTHALAMUS-HABENULA CIRCUIT IN ACUPUNCTURE INHIBITION OF COCAINE ADDICTION-LIKE BEHAVIORS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Abstract: Acupuncture has been used to treat drug addiction, although the underlying mechanism is largely unknown. The lateral habenula (LHb), the lateral hypothalamus (LH), and the mesolimbic dopamine (DA) system are not only reciprocally connected, but also involved in drug reward. We explored the role of the LH-LHb circuit in acupuncture inhibition of cocaine seeking-behavior. Acupuncture at HT7, but not LI5, attenuated locomotor activity and 50-kHz USVs and NAc DA release following cocaine injection. The acupuncture effects were ablated by electrolytic lesion or optogenetic inhibition of LHb. Optogenetic activation of LHb suppressed cocaine-enhanced 50 kHz USVs and locomotion. Acupuncture at HT7 reversed cocaine suppression of neuronal activity of LHb. Acupuncture at HT7 inhibited cocaine-primed reinstatement of drug-seeking behavior, which was blocked by chemogenetic inhibition of an LH-LHb circuit. These findings suggest that acupuncture requires LH-LHb pathways to attenuate cocaine-induced psychomotor responses and seeking behaviors. **Key words**: Acupuncture, cocaine, Lateral habenula, locomotor activity, ultrasonic vocalization (USVs), optogenetics Acknowledgment: This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (Nos. 2018R1A5A2025272, 2019R1A2C1002555 and KMDF_PR_20200901_01179991006790).

FENS Forum 2022
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BOARD NUMBER: S01-048

THE EFFECTS OF THE JAGEUMJUNG AND ACUPUNCTURE ON METH'S REINFORCING EFFECTS THROUGH THE CENTRAL AMYGDALA

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Methamphetamine(METH) is a representative widely abused drug, and acupuncture has been used to treat drug addiction. The present study was conducted to investigate the effects of acupuncture(SI5) and Jageumjung(JGJ) on METH's reinforcing effects through the central amygdala(CeA). The CeA plays a critical role in physiological and behavioral responses. JGJ is composed of six herbs and its effect on drug-enhanced treatment is unclear. And there are no studies examining the combined effects of the two treatments, JGJ and SI5. To measure the reinforcing effects of METH, locomotor activity, 50-kHz ultrasonic vocalization(USV), Fast-scan cyclic voltammetry(FSCV) and immunochemistry were used. JGJ, METH, and GABA receptor agonists($GABA_A$, $GABA_B$) were administered through oral, intravenous or intraperitoneal, and intracerebral administration, respectively, and then acupuncture was treated by manual stimulation. The results show that JGJ inhibits METH-induced increases in locomotor activity and USVs. Acupuncture had an inhibitory effect on METH, especially SI5 not HT7. And the group that treated JGJ and SI5 together showed more inhibitory effects than the group that treated JGJ and SI5, respectively. The GABA agonists significantly reversed the inhibition of the METH's effect by JGJ. However, FSCV unlikes the previous experiments. Taken together, our findings indicate that JGJ contributes to the effect of METH inhibition by the GABA receptor in CeA, and that the combination of SI5 and JGJ may be an effective strategy in reducing the reinforcing properties of METH. This research was supported by the National Research Foundation of Korea(NRF) grants funded by the Korea government(MSIT)(No.2018R1A5A2025272) and 2020R1A2C1103154.

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BOARD NUMBER: S01-049

ESTABLISHING AN ASSAY FOR INDIVIDUAL OLFACTORY LEARNING IN DROSOPHILA LARVAE

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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[1]Cambridge University, Department Of Zoology, Cambridge, United Kingdom, [2]Laboratory of molecular biology, Neurobiology, Cambridge, United Kingdom

Inter-individual variation is present in every animal species and reflected in many behavioural aspects, one being the learning speed: while all animals can learn, the speed with which different individuals from the same species learn can vary widely. Very little is known about the circuit and molecular mechanisms underlying inter-individual differences. Investigating such questions requires both a deep understanding of the neural computations and circuit responsible for a chosen task and knowledge of anatomical and functional differences between those circuits in different individuals. Such level of precision remains out of reach for most model organisms, including rodents. However, for some invertebrate models such as the drosophila larva, the relative simplicity and small size of their brains coupled to the development of new high-resolution tools now allow to address these problematics. To assess individual behaviour, we have adapted an experimental set-up previously developed by the Gershow laboratory to be able to test a single drosophila larva in an olfactory associative paradigm. The animal is freely crawling in a Y-maze where it is given the choice between two different odours every time it reaches the 3-arms intersection. Choices are recorded before and after an odour-punishment paired training to assess learning. This fully automated system will allow the behavioural classification of individuals according to their learning speed. Structural and functional differences in the relevant neuronal circuits between the identified subgroups will then be assessed to characterize the neuronal basis of inter-individual variation.

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BOARD NUMBER: S01-050

DIFFERENTIAL VALUE AND OUTCOME PROCESSING BETWEEN DORSAL AND VENTRAL CA1 REGIONS OF THE HIPPOCAMPUS

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Despite tremendous progress, our understanding of the full spectrum of hippocampal functions is still incomplete. This is in large part because of our limited understanding of functional specialization along the longitudinal axis of the hippocampus. To tackle this matter, we examined how value- and outcome-related neural activity varies between the dorsal (dCA1) and ventral CA1 (vCA1) in mice performing probabilistic classical conditioning tasks. Inactivation of either structure disrupted value-dependent anticipatory licking, and value-coding neurons were found in both structures, indicating their involvement in value processing. However, we found several outstanding differences in value- and outcome-related neural activity between the dCA1 and vCA1. The dCA1 neuronal population increased activity monotonically as a function of value, while the vCA1 neuronal population was preferentially responsive to the highest-value sensory cue. Also, signals related to outcome-dependent value learning (value and outcome signals during the outcome period; signals related to the history of past cues and outcomes; and signals related to updated value) were stronger in the dCA1. vCA1 neurons instead showed rapid responses to punishment and strongly biased responses to negative prediction error. These findings suggest that the dCA1 faithfully represents values associated with sensory cues, while the vCA1 preferentially represents behaviorally relevant, salient features of experienced events.

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BOARD NUMBER: S01-051

NEURAL REPRESENTATIONS OF LEARNED CATEGORIES IN MOUSE PREFRONTAL CORTEX

POSTER SESSION 01 - SECTION: CIRCUITRY OF MEMORY AND LEARNING

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Grouping objects and experiences into categories is a fundamental skill for humans and many animals. Particularly, learning and recalling rules for categorization enables us to flexibly adapt to changes in context. Because the neuronal mechanisms underlying this process are not understood, we characterized representations of learned categories in mouse medial prefrontal cortex (mPFC) by chronically recording neuronal activity during visual categorization learning. In a head-fixed go/no-go task, mice were trained to categorize drifting gratings either based on orientation or spatial frequency. All mice successfully learned to group the stimuli into categories and generalized the learned rule to novel stimuli. Upon a rule-switch, mice quickly sorted the stimuli into new categories. Strikingly, the animals also generalized the new rule to stimuli they had previously only experienced under the old rule. Thus, mice can learn rules for categorization. Throughout this learning paradigm, we followed the activity of neurons in layer 2/3 of mPFC using two-photon calcium imaging. We found that a set of neurons acquired category-selective responses. After the rule-switch, category-selective neurons partly remapped and previously non-selective neurons became responsive to the new categories. By requiring mice to change their learned motor response from go/no-go to go-right/go-left, we aimed to disentangle task parameters, like behavioral choice and reward, from category selectivity. Hereby, we identified uniquely category-modulated neurons that represented the category identity of stimuli across tasks. Together, these results demonstrate that mouse mPFC forms representations of learned categories and flexibly encodes changes in rules.

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BOARD NUMBER: S01-052

DOPAMINE REWARD PREDICTION ERRORS REPORT BUT ARE NOT USED TO UPDATE CHOICE BEHAVIOUR IN STRUCTURED ENVIRONMENTS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Aims Dopamine signals are thought to aid flexible behaviour by carrying reward prediction errors (RPEs), used as a teaching signal to update values and future behaviour. However, in real-world situations, environments contain structure, meaning changes have patterns or rules that can be learned, like the day-night cycle. In these situations, instead of continuously updating and changing values, *inferring* that the *state* of the world has changed can allow prior experiences to be reused when situations are reencountered, which can enable more efficient updating. In this study, we explored dopamine's involvement in such structured environments. Methods We i) trained mice to solve a sequential probabilistic decision task in which the reward state (i.e. the location of reward) could be inferred, ii) performed behavioural modelling, iii) recorded calcium activity in midbrain dopamine neuron cell bodies, and axons in ventral and dorsomedial striatum using GCaMP fibre photometry, together with dopamine concentrations using dLight, and iv) optogenetically stimulated and inhibited midbrain dopamine activity during the task. Results Choice behaviour and dopamine signals indicated mice learned the task structure. Dopamine was strongly influenced by the value of state and actions, consistent with RPE signalling, using value information that respected the task structure. Nonetheless, neither activating nor inhibiting dopamine at trial outcome affected subsequent choices, although both manipulations strongly modulated choices in separate task contexts. Conclusion During flexible behaviour in well-learned structured environments, **dopamine RPEs** are strongly influenced by **state value**, but this relationship is not reciprocal, and rewards have a **dopamine-independent influence on behaviour**.

Pubmed:

31343680: Hervig ME, Fiddian L, Piilgaard L, Božič T, Blanco-Pozo M, Knudsen C, Olesen SF, Alsiö J, Robbins TW
Dissociable and Paradoxical Roles of Rat Medial and Lateral Orbitofrontal Cortex in Visual Serial Reversal Learning.
Much evidence suggests that reversal learning is mediated by cortico-striatal circuitries with the orbitofrontal cortex (OFC) playing a prominent role. The OFC is a functionally heterogeneous region, but potential differential roles of lateral (lOFC) and medial (mOFC) portions in visual reversal learning have yet to be determined. We investigated the effects of pharmacological inactivation of mOFC and lOFC on a deterministic serial visual reversal learning task for rats. For reference, we also targeted other areas previously implicated in reversal learning: prelimbic (PrL) and infralimbic (IL) prefrontal cortex, and basolateral amygdala (BLA). Inactivating mOFC and lOFC produced opposite effects; lOFC impairing, and mOFC improving, performance in the early, perseverative phase specifically. Additionally, mOFC inactivation enhanced negative feedback sensitivity, while lOFC inactivation diminished feedback sensitivity in general. mOFC and lOFC inactivation also affected novel visual discrimination learning differently; lOFC inactivation paradoxically improved learning, and mOFC inactivation had no effect. We also observed dissociable roles of the OFC and the IL/PrL. Whereas the OFC inactivation affected only perseveration, IL/PrL inactivation improved learning overall. BLA inactivation did not affect perseveration, but improved the late phase of reversal learning. These results support opponent roles of the rodent mOFC and lOFC in deterministic visual reversal learning.
Cereb Cortex, 2020; 30
31036947: Kostadinov D, Beau M, Blanco-Pozo M, Häusser M
Predictive and reactive reward signals conveyed by climbing fiber inputs to cerebellar Purkinje cells.
There is increasing evidence for a cerebellar contribution to cognitive processing, but the specific input pathways conveying this information remain unclear. We probed the role of climbing fiber inputs to Purkinje cells in generating and evaluating predictions about associations between motor actions, sensory stimuli and reward. We trained mice to perform a visuomotor integration task to receive a reward and interleaved cued and random rewards between task trials. Using two-photon calcium imaging and Neuropixels probe recordings of Purkinje cell activity, we show that climbing fibers signal reward expectation, delivery and omission. These signals map onto cerebellar microzones, with reward delivery activating some microzones and suppressing others, and with reward omission activating both reward-activated and reward-suppressed microzones.

Moreover, responses to predictable rewards are progressively suppressed during learning. Our findings elucidate a specific input pathway for cerebellar contributions to reward signaling and provide a mechanistic link between cerebellar activity and the creation and evaluation of predictions.
Nat Neurosci, 2019; 22

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BOARD NUMBER: S01-053

MULTIPLE STIMULUS-STIMULUS ASSOCIATIONS DURING MULTI-STEP REINFORCEMENT LEARNING IN HUMANS IN SPATIALLY STRUCTURED AND UNSTRUCTURED FRAMES

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Multi-step learning and decision-making paradigm are the most commonly used tools to investigate the trade-off between retrospective, model-free reinforcement learning that requires the representation of state-action-reward and prospective, model-based one that requires to explicitly represent the state transitions. In most paradigms, state transitions are provided by instructions, while reward values have to be learned on the fly. The goal of the present study was to investigate the mechanisms of learning in parallel action-state transitions and reward values and the impact of the spatial structure of the task on the learning. Therefore, we conducted laboratory (N=30) and online (N=200) experiments using new proposed designs of the two-step paradigm. After the learning phase, we administered additional tests to explicitly assess different stimulus-stimulus associations. The results revealed that subjects could retrieve the correct action-state transitions, despite behavior in the learning not showing signs of prospective valuation. We concluded the experiment using Raven's matrices and cognitive reflection tests. Scores in these tests correlated with spatially unstructured learning, spatially structured learning (marginally) and not at all with action-state transition learning, suggesting that fluid intelligence and cognitive control do not play a role in action-state transition learning, but in the deployment of structure learning at the service of decision-making. Finally these results are not affected by the task's spatial framing. Together our results indicate that the reward values and the action-state transitions are concomitantly learned, even if behavior does not provide signs of prospective valuation suggesting an intriguing dissociation between knowledge and performance in reinforcement learning.

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TASK ELICITED CONTEXT-DEPENDENCY AND VALENCE BIAS IN VALUE ENCODING: AN ELUSIVE RELATIONSHIP WITH MENTAL HEALTH PROFILES

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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École Normale Supérieure - PSL, Laboratoire De Neurosciences Cognitives, Paris, France

Poor mental health has frequently been associated with decision-making that deviates from the norm. An integral step of decision-making is value encoding. We aim to explore a relationship between mental health profiles and value encoding, as elicited by features of two tasks; the Hiragana task (Frank et al. 2004) and the Agathodaimon task (Pessiglione et al. 2006). Both have been fundamental in providing firm support for a relationship between dopamine availability in the brain and valence induced bias in value encoding. Thereupon we conducted a focused literature survey on published data from either of the tasks. Informed about the limitations of the current literature, we developed refined research approaches to experimentally address our aim. For the ensuing dimensional approach to mental health, our participants answered a novel combination of standard mental health questionnaires. The analysis supports the emergence of three psychiatric dimensions, including a dimension describing substance abuse profiles. Our online behavioural task features a valanced learning phase and a knowledge transfer phase. The performance data suggests context-dependent value encoding and valence bias. Furthermore, our reinforcement learning model analysis supports interindividual differences in context-dependency and asymmetric learning. Nevertheless, there was no robust evidence for a relationship between behavioural features and mental health profiles. While behavioural features are thoroughly replicated, the interindividual reliability measures are poor. This suggests the behavioural traits this task elicits, and our model characterises, might lack temporal sturdiness. In conclusion, we present a difficulty in relating mental health profiles to a reinforcement learning characterisation of value encoding.

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BOARD NUMBER: S01-055

EMOTIONAL DYSREGULATION AND ALTERED REWARD PROCESSING IN SELF-HARM

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Background: Self-Harm (SH), being “any act of self-injury or poisoning carried out by someone irrespective of motivation”, increases suicide risk, necessitating intervention. People mostly SH to relieve negative affect (NA), with NA precipitating SH. SH participants show reward hypersensitivity. NA may trigger reward hypersensitivity, instigating SH, but whether this interaction exists remains unclear. Aim: Investigate whether SH participants process potentially rewarding SH stimuli differently to healthy controls (HCs) post-NA induction. Hypothesis: Post-NA induction, SH participants will have significantly shorter reaction latencies (RL) and greater reaction accuracies (RA) in the SH condition of the Incentive Delay task (IDT) than HCs. Methods: 16-25-year-old SH (n=35) and HC (n=20) participants recruited using social media completed the Trier Social Stress Test, to induce NA, then the IDT. In the latter, participants were cued to respond to a target as quickly as possible, seeing images of either SH acts, people socializing or money on responding (SH, social and monetary conditions respectively). Control conditions showed a neutral image on responding (SH-neutral, social-neutral, and monetary-neutral conditions respectively). Results: A main effect of condition on RA ($p < 0.05$) but not RL ($p > 0.05$), and no main group or group-by-condition interaction effect on either ($p > 0.05$) was found. Past-week SH frequency and RA positively correlated in social, social-neutral, and monetary conditions ($p < 0.05$). Conclusion: Reward processing non-significantly differed by group post-NA induction. However, past-week SH frequency significantly correlated with RA. Larger longitudinal studies investigating this interaction may help elucidate SH triggers and develop therapeutic strategies.

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FEEDBACK-RELATED NEURAL ACTIVITY OBSERVED IN A DRUMMING TASK PREDICTS SUBSEQUENT TIMING PERFORMANCE

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Aims: Recent studies showed that reward prediction error can bias interval timing, but the direction of impact remains inconclusive (Soares et al., 2016; Toren et al., 2020). Feedback-related negativity (FRN), an EEG signal around 250ms following negative feedback, may reflect a neural prediction error (Holroyd et al., 2011). This study utilises the FRN to test how performance feedback, as a special case of reward, biases subsequent timing. **Methods:** We recorded electroencephalogram (EEG) from 20 participants while they engaged in a drumming task with varying tempo. To evaluate the impact of the neural prediction error on timing, we derived a trial-by-trial proxy of FRN using regression-based EEG analysis, and used it to predict the timing adjustment in the next trial. We compared a series of hierarchical linear models, controlling for tempo, learning effect, drumming pattern, handedness, etc. **Results:** On the participant level, larger FRN was observed for slower tempos, and its amplitude did not differentiate between early and late feedback. This may imply that “early” and “late” feedback elicit a similar negative neural prediction error compared to “on time” feedback. On the trial level, larger trial-by-trial FRN (i.e., more negative residual EEG) predicted greater timing adjustment following late, but not early feedback. This result most likely supports the hypothesis that negative prediction errors temporarily slow down psychological timing.

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BOARD NUMBER: S01-057

CONTEXTUAL INFLUENCE OF REINFORCEMENT LEARNING PERFORMANCE OF DEPRESSION: EVIDENCE FOR A NEGATIVITY BIAS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Aim Value-based decision-making impairment in depression is a complex phenomenon: while some studies did find evidence of blunted reward learning and reward-related signals in the brain, others indicate no effect. Here we test whether such reward sensitivity deficits are dependent on the overall value of the decision problem. **Methods** We used a two-armed bandit task with two different contexts: one 'rich', one 'poor' where both options were associated with an overall positive, negative expected value, respectively. We tested patients (N=30) undergoing a major depressive episode and age, gender and socio-economically matched controls (N=26). To assess whether differences in learning performance were due to a decision or a value-update process, we analysed performance in a transfer phase, where options were extrapolated from their original learning context. **Results** Healthy subjects showed similar learning performance in the 'rich' and the 'poor' contexts, while patients showed reduced learning in the 'poor' context. Analysis of the transfer phase showed that the context-dependent deficit in patients generalized, thus suggesting that the effect of depression has to be traced to the outcome encoding. Computational model-based results showed that patients displayed higher learning rate for negative compared to positive outcomes (the opposite was true in healthy controls). **Conclusions** Our results illustrate that reinforcement learning performances in depression depend on the value of the context. We show that depressive patients have a specific trouble in contexts with an overall negative state value, which in our task is consistent with a negativity bias at the level of learning rates.

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BOARD NUMBER: S01-058

REDUCED ANHEDONIA FOLLOWING INTERNET-BASED COGNITIVE BEHAVIORAL THERAPY FOR DEPRESSION IS MEDIATED BY ENHANCED REWARD CIRCUIT ACTIVATION

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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[1]University of Haifa, School Of Psychological Sciences, Haifa, Israel, [2]McLean Hospital, Center For Depression, Anxiety And Stress Research, Belmont, United States of America, [3]Harvard Medical School, Boston, Department Of Psychiatry, Boston, United States of America, [4]University of Haifa, The Integrated Brain And Behavior Research Center (ibbrc), Haifa, Israel, [5]Harvard Medical School, Department Of Psychiatry, Boston, United States of America, [6]University of Arizona, Department Of Psychiatry, Tucson, United States of America

Background: Major depressive disorder (MDD) is a highly prevalent psychiatric condition, yet many patients do not receive adequate treatment. Novel and highly scalable interventions such as internet-based cognitive-behavioral-therapy (iCBT) may help to address this treatment gap. Anhedonia, a hallmark symptom of MDD that accounts for diminished interest and ability to experience pleasure, has been associated with reduced reactivity in a neural reward circuit that includes medial prefrontal and striatal brain regions. Whether iCBT can reduce anhedonia severity in MDD patients, and whether these therapeutic effects are accompanied by enhanced reward circuit reactivity has yet to be examined. Methods: Fifty-two MDD patients were randomly assigned to either 10-week iCBT (*n*=26) or monitored attention control (MAC, *n*=26) programs. All patients completed pre- and post-treatment assessments of anhedonia (Snaith–Hamilton Pleasure Scale; SHAPS) and reward circuit reactivity (monetary incentive delay; MID) task during functional magnetic resonance imaging (fMRI). Results: Both iCBT and MAC groups exhibited reduction in anhedonia severity post-treatment (P_{iCBT} = 0.001; P_{MAC} = 0.006). Nevertheless, only the iCBT group exhibited enhanced nucleus accumbens (Nacc) (*P* = 0.005) and subgenual anterior cingulate cortex (sgACC) activation (*P* = 0.009) and functional connectivity from pre- to post-treatment in response to reward feedback. Enhanced Nacc and sgACC activations were associated with reduced anhedonia severity following iCBT treatment (*P* = 0.01, *P* = 0.023), with enhanced Nacc activation also mediating the reduction in anhedonia severity post-treatment (*P* = 0.004). Conclusions: These findings suggest that increased reward circuit reactivity may contribute to reduction in anhedonia severity following iCBT treatment for depression.

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BOARD NUMBER: S01-059

THE COMPUTATIONAL RULES OF VALUE NORMALIZATION IN HUMAN REINFORCEMENT LEARNING

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Context-dependent learning has been shown to lead to irrational choices in humans. This is specifically true when the options are extrapolated from their original learning context. In a previous study, we showed that this process was well captured by a dynamical range normalization model, inspired by the range-frequency theory and electrophysiological findings in monkeys. However, the previously used two-armed bandit task is ill-suited to precisely characterize the functional form of context-dependence as range normalization or divisive normalization. To fill this gap, we designed a new online-based learning task simultaneously manipulating the number of options per context (2-armed bandit versus 3-armed bandit) and the range magnitude of the options, by varying their expected values. We also included an explicit valuation phase where participants had to report their estimation of each option. Behavioral and computational analyses seriously challenge divisive normalization, but suggest that simple range normalization cannot account for all behavioral patterns. Together, these results shed new light on the mechanisms of context-dependent learning in humans.

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BOARD NUMBER: S01-060

FRONTAL MIDLINE THETA REFLECTS AN INTEGRATED COST/BENEFIT SIGNAL, BUT NOT DISCOUNTED NET VALUE

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Overcoming effort costs to obtain a reward is critical for successful goal-directed behavior. Dominate theories of effort-based decision-making posit that the ACC plays a critical role in valuing and integrating reward and effort signals. However, it remains unclear if the ACC tracks the subjective value of effort-based rewards or is simply implicated in cognitive control functions that subserve effort-based choice. In this study, we investigated the effect of effort costs and reward magnitude on ACC activity – putatively measured by frontal midline theta (FMT) – at both cue and feedback, and compared this pattern of activity to a well-established index of incentive salience, the P-300 component. 32 participants completed an EEG experiment with a novel task where they obtained low or high rewards by completing a cognitive task with different degrees of difficulty (low or high), but same reward probability rate. Findings indicate that FMT was significantly enhanced when evaluating high compared to low effort cues, but only when rewards were high. This signal was qualitatively different from the pattern of the P-300 component, which tracked reward magnitude during the cue evaluation phase and was enhanced for both high reward and high effort at feedback. These findings suggest that FMT activity does not reflect pure incentive value or a discounted net value signal, but instead an integrated cost/benefit signal that reflects the need to exert cognitive control to facilitate goal attainment.

Pubmed:
34710515: Lopez-Gamundi P, Yao YW, Chong TT, Heekeren HR, Mas-Herrero E, Marco-Pallarés J
The neural basis of effort valuation: A meta-analysis of functional magnetic resonance imaging studies.
Choosing how much effort to expend is critical for everyday decisions. While several neuroimaging studies have examined effort-based decision-making, results have been highly heterogeneous, leaving unclear which brain regions process effort-related costs and integrate them with rewards. We conducted two meta-analyses of functional magnetic resonance imaging data to examine consistent neural correlates of effort demands (23 studies, 15 maps, 549 participants) and net value (15 studies, 11 maps, 428 participants). The pre-supplementary motor area (pre-SMA) scaled positively with pure effort demand, whereas the ventromedial prefrontal cortex (vmPFC) showed the opposite effect. Moreover, regions that have been previously implicated in value integration in other cost domains, such as the vmPFC and ventral striatum, were consistently involved in signaling net value. The opposite response patterns of the pre-SMA and vmPFC imply that they are differentially involved in the representation of effort costs and value integration. These findings provide conclusive evidence that the vmPFC is a central node for net value computation and reveal potential brain targets to treat motivation-related disorders.
Neurosci Biobehav Rev, 2021; 131
33202256: Webber HE, Lopez-Gamundi P, Stamatovich SN, de Wit H, Wardle MC
Using pharmacological manipulations to study the role of dopamine in human reward functioning: A review of studies in healthy adults.
Dopamine (DA) plays a key role in reward processing and is implicated in psychological disorders such as depression, substance use, and schizophrenia. The role of DA in reward processing is an area of highly active research. One approach to this question is drug challenge studies with drugs known to alter DA function. These studies provide good experimental control and can be performed in parallel in laboratory animals and humans. This review aimed to summarize results of studies using pharmacological manipulations of DA in healthy adults. 'Reward' is a complex process, so we separated 'phases' of reward, including anticipation, evaluation of cost and benefits of upcoming reward, execution of actions to obtain reward, pleasure in response to receiving a reward, and reward learning. Results indicated that i) DAergic drugs have different effects on different phases of reward; ii) the relationship between DA and reward functioning appears unlikely to be linear; iii) our ability to detect the effects of DAergic drugs varies depending on whether subjective, behavioral, imaging measures are used.

Neurosci Biobehav Rev, 2021; 120
34137904: Hoots JK, Webber HE, Nunez C, Cooper JA, Lopez-Gamundi P, Lawlor VM, Lane SD, Treadway MT, Wardle MC
Acute drug effects differentially predict desire to take dextroamphetamine again for work and recreation.
Misuse of dextroamphetamine occurs in work and recreational contexts. While acute drug effects broadly predict abuse liability, few studies have considered the relationship between acute effects and context.
Psychopharmacology (Berl), 2021; 238
32722661: Soder HE, Cooper JA, Lopez-Gamundi P, Hoots JK, Nunez C, Lawlor VM, Lane SD, Treadway MT, Wardle MC
Dose-response effects of d-amphetamine on effort-based decision-making and reinforcement learning.
Effort-related decision-making and reward learning are both dopamine-dependent, but preclinical research suggests they depend on different dopamine signaling dynamics. Therefore, the same dose of a dopaminergic medication could have differential effects on effort for reward vs. reward learning. However, no study has tested how effort and reward learning respond to the same dopaminergic medication within subjects. The current study aimed to test the effect of therapeutic doses of d-amphetamine on effort for reward and reward learning in the same healthy volunteers. Participants (n = 30) completed the Effort Expenditure for Reward Task (EEfRT) measure of effort-related decision-making, and the Probabilistic Reward Task (PRT) measure of reward learning, under placebo and two doses of d-amphetamine (10 mg, and 20 mg). Secondarily, we examined whether the individual characteristics of baseline working memory and willingness to exert effort for reward moderated the effects of d-amphetamine. d-Amphetamine increased willingness to exert effort, particularly at low to intermediate expected values of reward. Computational modeling analyses suggested this was due to decreased effort discounting rather than probability discounting or decision consistency. Both baseline effort and working memory emerged as moderators of this effect, such that d-amphetamine increased effort more in individuals with lower working memory and lower baseline effort, also primarily at low to intermediate expected values of reward. In contrast, d-amphetamine had no significant effect on reward learning. These results have implications for treatment of neuropsychiatric disorders, which may be characterized by multiple underlying reward dysfunctions.
Neuropsychopharmacology, 2021; 46
33119414: Stamatovich SN, Lopez-Gamundi P, Suchting R, Colpo GD, Walss-Bass C, Lane SD, Schmitz JM, Wardle MC
Plasma pro- and anti-inflammatory cytokines may relate to cocaine use, cognitive functioning, and depressive symptoms in cocaine use disorder.
Inflammation is implicated in cocaine use and associated problems, including depression and cognitive impairment.
Am J Drug Alcohol Abuse, 2021; 47
29620381: Lopez-Gamundi P, Wardle MC
The cognitive effort expenditure for rewards task (C-EEfRT): A novel measure of willingness to expend cognitive effort.
Research in animals suggests that decisions about physical versus cognitive effort have distinct neural bases, but exploration of this question in humans is hampered by lack of parallel measures of physical and cognitive effort for rewards. We developed a novel measure of willingness to exert cognitive effort for rewards, the C-EEfRT, paralleling the validated physical effort expenditure for rewards task (EEfRT). To validate the C-EEfRT we: (a) tested whether EEfRT and C-EEfRT tasks were equivalently difficult; (b) tested whether decisions on the EEfRT and C-EEfRT were equivalently responsive to changes in reward; (c) examined relationships between the C-EEfRT and anhedonia, intelligence, and working memory. Last, we tested the relationship between willingness to exert physical and cognitive effort for rewards in humans. Sixty healthy adults completed the EEfRT, the C-EEfRT, an anhedonia self-report, an intelligence test, and a working memory task. Overall willingness to exert effort was higher on the C-EEfRT than the EEfRT, particularly when reward probability and amount were low. This was despite participants perceiving the cognitive task as more difficult, and having greater difficulty completing it. Differential effects of physical fatigue may have contributed. Anhedonia was not related to effort on either measure. Working memory, but not intelligence, was associated with cognitive effort. There was a moderate relationship between cognitive and physical effort. These findings suggest the importance of measuring cognitive effort as distinct from physical effort in humans. Future studies should consider calibrating task difficulty for each individual, and exploring cognitive effort in clinical populations. (PsycINFO Database Record
Psychol Assess, 2018; 30
30957525: Brandt CP, Paulus DJ, Lopez-Gamundi P, Green C, Lemaire C, Zvolensky MJ
HIV Anxiety Reduction/Management Program (HAMRT): pilot randomized controlled trial.
Research has indicated that mental health disorders, particularly anxiety, predicts poorer antiretroviral medication adherence among persons living with HIV/AIDS (PLWHA). The present study tests a novel six-session Cognitive-Behavioral Therapy-based integrated treatment/management program for PLWHA with concurrent anxiety delivered in community health clinics Houston, Texas. Twenty-Seven PLWHA (= 48.5, SD = 8.9, 44.4% female) were recruited for a proof-of-concept study and randomized to either an active treatment condition, or a waitlist control condition of equal length. Participants were assessed pre-randomization, at the mid-treatment time point (after three sessions for the active participants and three weeks for the

control participants) and post-treatment (six sessions for active participants, six weeks for control participants). Data were examined used Bayesian multilevel models. Results indicated a reliable (99.87% posterior probability of a moderating effect) interaction between active and control groups for depressive symptoms and reliable (99.65% probability) interaction for anxiety symptoms. Results indicated an unreliable interaction for combined antiretroviral therapy adherence. These findings are discussed in terms of the feasibility and potential utility of administering an anxiety-reduction therapy program designed for PLWHA with HIV medication adherence difficulties.
AIDS Care, 2019; 31
30442329: Buckner JD, Zvolensky MJ, Ecker AH, Schmidt NB, Lewis EM, Paulus DJ, Lopez-Gamundi P, Crapanzano KA, Bakhshaie J
Integrated cognitive behavioral therapy for comorbid cannabis use and anxiety disorders: A pilot randomized controlled trial.
Cannabis use disorder (CUD) is the most common illicit substance use disorder and individuals with CUD have high rates of comorbid anxiety disorders. Comorbidity between CUD and anxiety disorders is of public health relevance given that although motivation enhancement therapy (MET) combined with cognitive-behavioral therapy (CBT) is an efficacious intervention for CUD, outcomes are worse for patients with elevated anxiety. The current study tested the acceptability and efficacy of the integration of a transdiagnostic anxiety CBT (i.e., treatment of patients with any anxiety disorder) with MET-CBT (integrated cannabis and anxiety reduction treatment, or ICART) for CUD compared to MET-CBT alone. Treatment-seeking cannabis users (56.4% male, M = 23.2, 63.3% non-Hispanic White) with CUD and at least one comorbid anxiety disorder were randomly assigned to ICART (n = 27) or MET-CBT (n = 28). Patients in the ICART condition attended significantly more treatment sessions than those in the MET-CBT condition. Patients in the ICART condition were more likely to be abstinent post-treatment than those in MET-CBT. Further, treatment produced decreases in cannabis use and related problems. Notably, therapy type did not moderate the impact of treatment on frequency of use and related problems. Together, these data suggest that ICART may be at least as efficacious as a gold-standard psychosocial CUD treatment, MET-CBT, for a difficult-to-treat subpopulation of cannabis users.
Behav Res Ther, 2019; 115
30017698: Wardle MC, Lopez-Gamundi P, LaVoy EC
Effects of an acute bout of physical exercise on reward functioning in healthy adults.
Exercise has been proposed as a treatment for several psychiatric disorders. Exercise may act in part through beneficial effects on reward functioning, as it alters neurotransmitter levels in reward-related circuits. However, there has been little investigation of the effect of exercise on reward functions in humans. We hypothesized an acute bout of exercise would increase motivation for and pleasurable responses to rewards in healthy humans. In addition, we examined possible moderators of exercise's effects, including demographics, fitness and previous exercise experience. Thirty-five participants completed exercise and sedentary control sessions in randomized, counterbalanced order on separate days. Immediately after each activity, participants completed measures of motivation for and pleasurable responses to rewards, consisting of willingness to exert effort for monetary rewards and subjective responses to emotional pictures. Exercise did not increase motivation or pleasurable responses on average. However, individuals who had been running for more years showed increases in motivation for rewards after exercise, while individuals with less years running showed decreases. Further, individuals with higher resting heart rate variability reported lower arousal in response to all emotional pictures after exercise, while individuals with low heart rate variability reported increased arousal in response to all emotional pictures after exercise. General fitness did not have similar moderating effects. In conclusion, acute exercise improved reward functioning only in individuals accustomed to that type of exercise. This suggests a possible conditioned effect of exercise on reward functioning. Previous experience with the exercise used should be examined as a possible moderator in exercise treatment trials.
Physiol Behav, 2018; 194
29408238: Wardle MC, Lopez-Gamundi P, Flagel SB
Measuring appetitive conditioned responses in humans.
Clinical and preclinical findings suggest that individuals with abnormal responses to reward cues (stimuli associated with reward) may be at risk for maladaptive behaviors including obesity, addiction and depression. Our objective was to develop a new paradigm for producing appetitive conditioning using primary (food) rewards in humans, and investigate the equivalency of several outcomes previously used to measure appetitive responses to conditioned cues. We used an individualized food reward, and multimodal subjective, psychophysiological and behavioral measures of appetitive responses to a conditioned stimulus (CS) that predicted delivery of that food. We tested convergence among these measures of appetitive response, and relationships between these measures and action impulsivity, a putative correlate of appetitive conditioning. 90 healthy young adults participated. Although the paradigm produced robust appetitive conditioning in some measures, particularly psychophysiological ones, there were not strong correlations among measures of appetitive responses to the CS, as would be expected if they indexed a single underlying process. In addition, there was only one measure that related to impulsivity.

These results provide important information for translational researchers interested in appetitive conditioning, suggesting that various measures of appetitive conditioning cannot be treated interchangeably.
Physiol Behav, 2018; 188

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BOARD NUMBER: S01-061

HUMAN AGING INFLUENCES NEURAL REWARD PROCESSING TO MAINTAIN ROBUST REWARD SENSITIVITY

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Aging is associated with an overarching process that does not occur equally in all regions and particularly not in all brain regions. Neural processing of reward is considered as an influential factor in controlling individual's daily behavior. Comparative studies showed age-related neurocognitive changes and it remains unclear whether the aging brain is able to maintain functional integrity of the reward system. To investigate different activation patterns of elderly during prediction and receiving of reward, functional magnetic resonance images (fMRI) were acquired in 23 older (50-80 years) and 35 younger (19-31 years) healthy adults. While undergoing fMRI-measurements participants performed an incentive delay task offering monetary outcome (Monetary Incentive Delay Task). According to our behavioral results, age was associated with slower reaction times. Despite slower reaction times of elderly, reward sensitivity does not differ from that of the younger subsample. Therefore, our results suggest maintaining functional integrity of the aging reward system. Performing region of interest analyses, feedback of monetary reward revealed significant greater activation of reward-related brain structures in older adults compared to the younger group. These structures included left insula, right middle and inferior frontal gyrus as well as right middle cingulate gyrus. In contrast reward prediction led to enhanced activation of bilateral insula, caudate nucleus, right putamen and anterior cingulate cortex in the younger subsample. Our results indicate the older brain to react more sensitive to the receipt of reward. The different neural activation patterns suggest a compensation pattern of the aging brain underlying the preserved reward sensitivity.

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BOARD NUMBER: S01-062

UNDERSTANDING INDIVIDUAL DIFFERENCES IN REWARD-GUIDED LEARNING AS AN EFFICIENT ADAPTATION TO TASK UNCERTAINTY AND COMPUTATIONAL NOISE

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Humans have been shown to be capable of adjusting their learning rates in response to expected and unexpected uncertainty in the environment. Moreover, their inferences have also been shown to be subject to computational noise, driving exploratory decisions. However, it has not been investigated how these two parameters are set in a coordinated manner during reward guided learning. To fill this gap in our understanding, we have recruited 175 human subjects to take part in a decision-making task administered online, in which they had to take reward maximizing actions in an environment that was characterized by both expected and unexpected uncertainty. Their behaviour was modelled with a noisy Kalman filter to estimate individuals' perceived uncertainty, learning rates and computational noise levels. Our results indicate that learning rates and computational noise levels covary in a systematic manner, and that their covariation is guided by the perceived expected and unexpected uncertainty of subjects. Moreover, there is evidence for both state and trait like sources of inter-individual variability.

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BOARD NUMBER: S01-063

CONTRIBUTIONS OF DOPAMINE TO INTEGRATED COGNITIVE FUNCTION: ONLINE ASSESSMENT OF GENOTYPED VOLUNTEERS USING A PROBABILISTIC LEARNING TASK

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Dopamine modulates many component processes of cognition, including reinforcement learning (RL), working memory (WM), and memory consolidation (MC). Pinpointing the roles of dopamine in natural behaviour is therefore challenging, but critical, given its contributions to healthy and disordered cognition. We have begun addressing this challenge using a paradigm designed to assay RL, WM and MC in participants genotyped at rs2514218 and rs1076560, D2-receptor polymorphisms associated with psychiatric risk and altered receptor expression respectively. 125 healthy participants (21.5±3.5Y, 86 Female) performed an online probabilistic selection task (PST) adapted to incorporate different WM loads by varying the number of stimuli, with evening training and morning testing to assay overnight MC. The PST is a two-alternative forced choice task with stimuli presented in fixed pairs. Pairs differ in the reward probability contrast of the two stimuli. After learning to preferentially select higher reward probability stimuli, participants are tested on all permutations of stimulus pairs, with no reward feedback. Participants provided saliva samples for genotyping. Carriers of minor alleles for either rs2514218 or rs1076560 tended to show higher asymptotic learning phase accuracies, though this difference depended on PST version. Logistic regression analyses also indicate differential dependence on WM across PST versions and genotypes. MC manifested as improved performance during morning testing but appeared similar across groups. We are currently adapting a version of the Opponent-Actor Learning model (a dual learning system RL-based model) to relate these task- and genotype-dependent effects to dopaminergic mechanisms in basal-ganglia-thalamo-cortical circuits.

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BOARD NUMBER: S01-064

IDENTIFYING SIGN-TRACKING AND GOAL-TRACKING BEHAVIOURS IN HUMANS – AN EYE-TRACKING TRANSLATIONAL STUDY

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Aims: In Pavlovian conditioning, learnt behaviour varies according to the perceived value of environmental cues. For goal-trackers, the cue merely predicts a reward, whilst for sign-trackers, the cue holds incentive value. Sign-tracking can be maladaptive and is associated with impulsivity and attentional deficits. The sign-tracking-goal-tracking model is well-validated in animals, but more work is needed to explore these individual differences in humans. The study aimed to establish a Pavlovian conditioning paradigm to identify individuals who sign-track or goal-track. **Methods**: Eye movements of a sample of healthy adults (N=71, 18-35 years) were recorded using EyeLink1000 whilst performing a Pavlovian conditioning task, in which participants pressed a button to reveal the outcomes (monetary or neutral) paired with the visual cues shown (CS+ or CS-). Dwell time was measured for two areas of interest: sign (i.e. cue) and goal (i.e. reward). An eye-gaze index was computed based on the dwell time sign-to-goal ratio, with higher values indicating sign-tracking behaviour. **Results:** Sign- and goal-trackers were determined using a median split of the eye-gaze index (median=-.177), with sign-trackers displaying values above the median. Sign-trackers represented 45.1% (N=32) of the sample. The eye-gaze index was significantly higher for sign-trackers compared to goal-trackers in the CS+ trials (t=2.49, df=31, *p*=.019, d=.31, 95% CI [.07,.80]), but not in the CS- trials. **Conclusions:** We demonstrate that sign-tracking is a learnt behaviour associated with CS+ trials only. This holds translational potential for understanding individual differences in reward-learning, implicated in impulse control disorders, such as addictions.

Pubmed:
32967498: Byrom NC, Dinu L, Kirkman A, Hughes G
Predicting stress and mental wellbeing among doctoral researchers.
Although mental health in higher education is increasingly recognised as a public health issue, postgraduate research students are often overlooked. Recent studies indicate a high prevalence of mental distress in this population.
J Ment Health, 2020;

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BOARD NUMBER: S01-065

RELATIVE VALUE LEARNING IS NOT WEIRD: LOSING MONEY AROUND THE WORLD WITH CONTEXT-DEPENDENT REINFORCEMENT LEARNING.

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Recent findings pertaining to value encoding in reinforcement learning show that options that were deemed optimal in a given context will continue to be favored even when extrapolated to a context where they are no longer profitable. This economic version of the Ebbinghaus effect suggests that values are rescaled as a function of the range of available options, even when this adaptation leads to suboptimal choices. However, as with many other allegedly-robust economic behaviors, it is possible that this rescaling may not reflect a general property of decision-making, but rather some unique aspect of choice behavior present only in Western, Educated, Industrialized, Rich and Democratic (WEIRD) societies. Moreover, suboptimal choices could be potentially explained by risk-aversion behavior (e.g. favoring highest outcome probability rather than highest expected value), which is well known to differ between rich and developing countries. In the present work, we administered the 2-arm bandit reinforcement learning task previously shown to demonstrate such contextual effects, across 11 countries of markedly different socioeconomic and cultural makeup (Argentina, Iran, Russia, Japan, China, India, Israel, Chile, Morocco, France and US). Crucially, we added an explicit dimension to the task, where risk behind choices was explicitly declared. Our findings show that despite pronounced sociocultural differences, all participants presented evidence of context-dependent learning. The results was not explained by risk tolerance in the explicit task, which in turn, was moderately different across nations. These findings strengthen the idea that relative value learning is biologically hard coded and not a cultural artefact.

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BOARD NUMBER: S01-066

TEST-RETEST RELIABILITY IN HUMAN REINFORCEMENT LEARNING VERSUS SELF-REPORTED MEASURES

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Aims Recent meta-analysis (Enkavi et al., 2019) showed that test-retest reliability of self-reported surveys is higher than in behavioural tasks. We aimed to test this result in reinforcement learning (RL). **Methods** We defined a behavioural class of measures as the accuracy scores in the learning contexts of a RL task (Palminteri et al., 2015). For self-reported measures, we defined two classes: propensity-based and clinical. The former comprised four behavioural inhibition system (BIS) and behavioural activation system (BAS) scales; the latter measured nicotine consumption, alcohol consumption, anxiety score and depression score. We compared variability within and between these three classes of measures on the same participants (N=169), with a retest six months later. **Results** We defined test-retest reliability as Pearson's correlation between test and retest across participants. Correlations were statistically significant for each variable ($p<0.01$). Reliability was low or very low for behavioural measures, but moderate and strong for both propensity and clinical measures.
Cross-correlation between variables showed that behavioural measures were essentially correlated only among themselves, while propensity and clinical measures were correlated within and between themselves.
Finally, plotting test-retest reliabilities with the associated Coefficient of Variation (CV) revealed a clear clustering of the three classes of measurement. While behavioural and propensity measures differed by reliability, clinical measures reported greater CV. **Conclusions** Altogether, reliability and cross-correlation results not only back-up and align with the evidence that self-reported measures capture more stable traits, but more importantly, they extend it to the field of human RL.

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BOARD NUMBER: S01-067

DISRUPTED FUNCTIONAL CONNECTIVITY IS ASSOCIATED WITH REDUCED REWARD SENSITIVITY AND LEARNING DEFICITS AFTER STROKE

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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With a frequency of one stroke event every 40 seconds in the United States, stroke represents the leading cause of long-term disability worldwide (GBD 2016). To return stroke patients back to independence, a key feature to enhance rehabilitation efficiency is motor learning (Kitago and Krakauer 2013). About one third of stroke survivors suffer from apathy that is associated with reward insensitivity and negatively impacts rehabilitation outcome (Towfighi et al. 2017). One possible explanation is that stroke lesions fundamentally disrupt functional reward-network-interaction (Marsh et al. 2020, Oestreich et al. 2020). To test this hypothesis this study firstly aims to correlate both behavioral reward response and neuronal network connectivity in subacute stroke patients (n=27) compared to age-matched healthy controls (n=26). Reward system activity was analyzed using the Monetary-Incentive-Delay Task (MID, Knutson et al 2010). Corresponding effects on the level of brain-connectivity were analyzed with coherence analyses of magnetoencephalography (MEG) data. As a main result, stroke patients behaviourally show a significantly lower reward sensitivity and learning deficits in MID. They required greater monetary incentives than the healthy controls to increase their reaction speed compared to control conditions. These effects correlate with functional brain network connectivity changes in fronto-temporal regions depending on low reward magnitudes in stroke patients. In the future perspective, the understanding of this large variability in network responses following stroke provides perspectives for individual network readouts to achieve optimal therapeutic strategies. Hence, novel treatments poststroke are needed to reduce stroke-induced morbidity and to increase the patient’s and caregiver’s quality of life.

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BOARD NUMBER: S01-068

IMPAIRED REWARD PROCESSING IN CHRONIC STROKE SURVIVORS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Rehabilitation is essential for stroke patients to try to return to their premorbid functioning. Therefore reward-related learning keeps patients motivated repeating exercises. Based on our previous studies we know that reward sensitivity is reduced in acute state of stroke. Accordingly, we need a more detailed understanding of the reward system in chronic stroke survivors. The hypothesis that chronic stroke survivors show deficits in reward processing and altered reward networks was tested. Chronic stroke survivors (n=24, 100 ± 20.8 days post stroke) and healthy controls (n=23) underwent a functional magnetic resonance imaging (fMRI) scan during which they performed the Monetary Incentive Delay Task (MID). Two resting state acquisitions, one before and one after the paradigm, were acquired for connectivity analyses.
Behaviorally intact reward processing was established in the stroke and control group. Both required greater monetary reward to decrease their reaction time during the MID. However, fMRI data revealed alterations in neuronal function during reward processing. While activity in regions of positive reward decreased during prediction of reward in stroke survivors, they showed increasing activity during receiving of reward compared to healthy controls. E.g., the middle-frontal-gyrus as a key hub of reward decision making revealed significant differences. These data suggest that behaviorally chronic stroke survivors seemed to be recovered in functional reward processing, but different activation patterns may be associated with neuronal deficits. Stronger rewarding stimuli may be necessary to improve rehabilitation. We need further details about network changes, as these can act as potential compensation for reward processing.

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BOARD NUMBER: S01-069

A VIEW-BASED DECISION MECHANISM FOR REWARDS IN PRIMATE AMYGDALA

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Primates forage visually by shifting their view between objects and deciding on the best reward before acting. Here we show that when monkeys made value-guided choices, amygdala neurons encoded their decisions in an abstract, purely internal representation defined by the monkey's view but not by specific object or reward properties. Across amygdala subdivisions, activity patterns evolved gradually from an object-specific value code to a transient, object-independent code in which currently viewed and last-viewed objects competed to form a view-based choice. Neural-network modelling identified a sequence of computations by which amygdala neurons implemented view-based decision-making and eventually recovered the chosen object's identity when the monkeys acted on their choice. These findings reveal a neural mechanism that derives object choices from abstract, view-based computations, suggesting an efficient solution for decision problems with many objects.

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BOARD NUMBER: S01-070

NUTRIENT-SENSITIVE REINFORCEMENT LEARNING IN RHESUS MONKEYS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Animals adapt food choices to regulate nutrient intake for survival. In reinforcement learning (RL), animals choose by assigning values to options and update these values with experience. However, canonical RL models do not address how learning depends on biologically critical constituents of food rewards, such as nutrients. To address these questions, we trained two adult male rhesus monkeys (*Macaca mulatta*) to choose from binary food options with varying reward probabilities (monkey Ya: N = 4,684 trials; monkey Ym: N = 5,321 trials). These options were randomly drawn from four nutrient-defined liquids that differed only in fat or sugar content. All liquids were cued by fixed but novel visual stimuli within each session and were matched in flavor (blackcurrant or peach), temperature, and other ingredients (protein, salt, etc) to isolate the effects of individual nutrient content on learning and decision-making. We found that the monkeys learned faster from preferred nutrient rewards and chose them frequently even when they were associated with lower reward probability. Although more recent experiences generally had a stronger influence on choices, the impact of reward history depended on reward nutrient composition and individual nutrient preferences. Accordingly, we propose a nutrient-sensitive RL model that separately updates the value of fat and sugar to form scalar reward values that explain choices. Our findings suggest that preference for nutrients contributes to subjective valuation, learning, and choice of food rewards. Incorporating subjective nutrient values into RL models enhance their biological validity and help reveal unrecognized nutrient-specific learning and decision computations.

Pubmed:
34155111: Huang FY, Sutcliffe MPF, Grabenhorst F
Preferences for nutrients and sensory food qualities identify biological sources of economic values in monkeys.
Value is a foundational concept in reinforcement learning and economic choice theory. In these frameworks, individuals choose by assigning values to objects and learn by updating values with experience. These theories have been instrumental for revealing influences of probability, risk, and delay on choices. However, they do not explain how values are shaped by intrinsic properties of the choice objects themselves. Here, we investigated how economic value derives from the biologically critical components of foods: their nutrients and sensory qualities. When monkeys chose nutrient-defined liquids, they consistently preferred fat and sugar to low-nutrient alternatives. Rather than maximizing energy indiscriminately, they seemed to assign subjective values to specific nutrients, flexibly trading them against offered reward amounts. Nutrient-value functions accurately modeled these preferences, predicted choices across contexts, and accounted for individual differences. The monkeys' preferences shifted their daily nutrient balance away from dietary reference points, contrary to ecological foraging models but resembling human suboptimal eating in free-choice situations. To identify the sensory basis of nutrient values, we developed engineering tools that measured food textures on biological surfaces, mimicking oral conditions. Subjective valuations of two key texture parameters-viscosity and sliding friction-explained the monkeys' fat preferences, suggesting a texture-sensing mechanism for nutrient values. Extended reinforcement learning and choice models identified candidate neuronal mechanisms for nutrient-sensitive decision-making. These findings indicate that nutrients and food textures constitute critical reward components that shape economic values. Our nutrient-choice paradigm represents a promising tool for studying food-reward mechanisms in primates to better understand human-like eating behavior and obesity.
Proc Natl Acad Sci U S A, 2021; 118

FENS Forum 2022
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BOARD NUMBER: S01-071

RATS ADAPT OPTIMALLY TO CHANGES IN REINFORCEMENT PROBABILITIES, STIMULUS PRESENTATION PROBABILITIES AND DISCRIMINATION DIFFICULTY IN A PERCEPTUAL DECISION MAKING TASK

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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In a world full of uncertainty in which resources are scarce, animals need to categorize objects (e.g., edible vs. non-edible food items), track environmental variables, (e.g., food availability), and flexibly adapt to changing circumstances in order to efficiently guide action. In the lab, this can be studied by means of perceptual decision making tasks with blockwise changes of reinforcement schedules. In these tasks, statistical modelling of the environment allows computation of the optimal response strategy to serve as a benchmark for gauging subjects' performance. Indeed, several previous studies have reported that animals quickly adapt to changing contingencies, often even maximizing long-term reward. Nonetheless, the cognitive algorithm underlying adaptive behaviour in these situations is largely unknown and existing learning algorithms have not been systematically investigated. Here, we aimed at comparing three different learning models: an optimal account as calculated from Signal Detection Theory, a greedy income-based model (that only learns after rewards) and an error-based account (that only learns after errors). Rats were trained on a sound discrimination forced choice task. In several conditions, discrimination difficulty, stimulus presentation probability and reward probability were manipulated. The conditions were constructed such that the three different accounts yielded divergent predictions. We found that, despite rats' steady state performance was well explained by the optimal account, none of the mechanistic models tested here was able to explain how they did it, calling for further investigations into the cognitive algorithm underlying adaptive perceptual choice behaviour.

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BOARD NUMBER: S01-072

DIVERGENT ROLE OF NUCLEUS ACCUMBENS D2-MSN-VENTRAL PALLIDUM PROJECTIONS IN DIFFERENT PHASES OF MOTIVATED BEHAVIOR

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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The nucleus accumbens (NAc) is key in regulating reward-seeking and motivated behaviors. The NAc receives dopamine signals from the ventral tegmental area (VTA), which acts predominantly via D1 or D2 dopamine receptors that are expressed by largely non-overlapping populations of medium spiny neurons (MSNs). These two MSN sub-populations project to different outputs: D1-MSNs project to the VTA, while both D1- and D2-MSNs project to the ventral pallidum (VP). In order to better understand the contribution of D2-MSN-VP projections in reward-related behaviors, we performed optical manipulation of these inputs during different segments of a progressive ratio task for food and in a free choice instrumental task. We show that cue-paired optogenetic activation of D2-MSN-VP neurons is sufficient to increase the breakpoint in a progressive ratio task, indicative of increased motivation. Optical inhibition results in the opposite behavioral effect. Interestingly, optical activation of D2-MSN-VP projections during reward delivery significantly decreases motivation; the opposite is observed for optical inhibition. In agreement, in the free choice instrumental task, animals prefer the lever that originates one pellet in opposition to pellet plus D2-MSN-VP optogenetic activation, and vice versa for optogenetic inhibition. In summary, D2-MS-VP projections bi-directionally modulate motivated behavior, depending on the timing of stimulation: activation of D2-MS-VP inputs during cue exposure increases motivation, while activation at reward delivery decreases motivation in distinct behavioral paradigms.

Pubmed:

34374447: Coimbra B, Domingues AV, Soares-Cunha C, Correia R, Pinto L, Sousa N, Rodrigues AJ
Laterodorsal tegmentum-ventral tegmental area projections encode positive reinforcement signals.
The laterodorsal tegmentum (LDT) is a brainstem nucleus classically involved in REM sleep and attention, and that has recently been associated with reward-related behaviors, as it controls the activity of ventral tegmental area (VTA) dopaminergic neurons, modulating dopamine release in the nucleus accumbens. To further understand the role of LDT-VTA inputs in reinforcement, we optogenetically manipulated these inputs during different behavioral paradigms in male rats. We found that in a two-choice instrumental task, optical activation of LDT-VTA projections shifts and amplifies preference to the laser-paired reward in comparison to an otherwise equal reward; the opposite was observed with inhibition experiments. In a progressive ratio task, LDT-VTA activation boosts motivation, that is, enhances the willingness to work to get the reward associated with LDT-VTA stimulation; and the reverse occurs when inhibiting these inputs. Animals abolished preference if the reward was omitted, suggesting that LDT-VTA stimulation adds/decreases value to the stimulation-paired reward. In addition, we show that LDT-VTA optical activation induces robust preference in the conditioned and real-time place preference tests, while optical inhibition induces aversion. The behavioral findings are supported by electrophysiological recordings and c-fos immunofluorescence correlates in downstream target regions. In LDT-VTA ChR2 animals, we observed an increase in the recruitment of lateral VTA dopamine neurons and D1 neurons from nucleus accumbens core and shell; whereas in LDT-VTA NpHR animals, D2 neurons appear to be preferentially recruited. Collectively, these data show that the LDT-VTA inputs encode positive reinforcement signals and are important for different dimensions of reward-related behaviors.
J Neurosci Res, 2021; 99
31534159: Soares-Cunha C, de Vasconcelos NAP, Coimbra B, Domingues AV, Silva JM, Loureiro-Campos E, Gaspar R, Sotiropoulos I, Sousa N, Rodrigues AJ
Correction: Nucleus accumbens medium spiny neurons subtypes signal both reward and aversion.
A correction to this paper has been published and can be accessed via a link at the top of the paper.
Mol Psychiatry, 2020; 25
31462765: Soares-Cunha C, de Vasconcelos NAP, Coimbra B, Domingues AV, Silva JM, Loureiro-Campos E, Gaspar R, Sotiropoulos I, Sousa N, Rodrigues AJ

Nucleus accumbens medium spiny neurons subtypes signal both reward and aversion.
Deficits in decoding rewarding (and aversive) signals are present in several neuropsychiatric conditions such as depression and addiction, emphasising the importance of studying the underlying neural circuits in detail. One of the key regions of the reward circuit is the nucleus accumbens (NAc). The classical view on the field postulates that NAc dopamine receptor D1-expressing medium spiny neurons (D1-MSNs) convey reward signals, while dopamine receptor D2-expressing MSNs (D2-MSNs) encode aversion. Here, we show that both MSN subpopulations can drive reward and aversion, depending on their neuronal stimulation pattern. Brief D1- or D2-MSN optogenetic stimulation elicited positive reinforcement and enhanced cocaine conditioning. Conversely, prolonged activation induced aversion, and in the case of D2-MSNs, decreased cocaine conditioning. Brief stimulation was associated with increased ventral tegmenta area (VTA) dopaminergic tone either directly (for D1-MSNs) or indirectly via ventral pallidum (VP) (for D1- and D2-MSNs). Importantly, prolonged stimulation of either MSN subpopulation induced remarkably distinct electrophysiological effects in these target regions. We further show that blocking κ-opioid receptors in the VTA (but not in VP) abolishes the behavioral effects induced by D1-MSN prolonged stimulation. In turn, blocking δ-opioid receptors in the VP (but not in VTA) blocks the behavioral effects elicited by D2-MSN prolonged stimulation. Our findings demonstrate that D1- and D2-MSNs can bidirectionally control reward and aversion, explaining the existence of controversial studies in the field, and highlights that the proposed striatal functional opposition needs to be reconsidered.
Mol Psychiatry, 2020; 25
30034328: Soares-Cunha C, Coimbra B, Borges S, Domingues AV, Silva D, Sousa N, Rodrigues AJ
Mild Prenatal Stress Causes Emotional and Brain Structural Modifications in Rats of Both Sexes.
Stress or high levels of glucocorticoids (GCs) during developmental periods is known to induce persistent effects in the neuroendocrine circuits that control stress response, which may underlie individuals' increased risk for developing neuropsychiatric conditions later in life, such as anxiety or depression. We developed a rat model (Wistar han) of mild exposure to unpredictable prenatal stress (PS), which consists in a 4-h stressor administered three times per week on a random basis; stressors include strobe lights, noise and restrain. Pregnant dams subjected to this protocol present disrupted circadian corticosterone secretion and increased corticosterone secretion upon acute stress exposure. Regarding progeny, both young adult (2 months old) male and female rats present increased levels of circulating corticosterone and hyperactivity of the hypothalamus-pituitary-adrenal axis to acute stress exposure. Both sexes present anxious- and depressive-like behaviors, shown by the decreased time spent in the open arms of the elevated plus maze (EPM) and in the light side of the light-dark box (LDB), and by increased immobility time in the forced swim test, respectively. Interestingly, these results were accompanied by structural modifications of the bed nucleus of stria terminalis (BNST) and hippocampus, as well as decreased norepinephrine and dopamine levels in the BNST, and serotonin levels in the hippocampus. In summary, we characterize a new model of mild PS, and show that stressful events during pregnancy can lead to long-lasting structural and neurochemical effects in the offspring, which affect behavior in adulthood.
Front Behav Neurosci, 2018; 12
29780881: Soares-Cunha C, Coimbra B, Domingues AV, Vasconcelos N, Sousa N, Rodrigues AJ
Nucleus Accumbens Microcircuit Underlying D2-MSN-Driven Increase in Motivation.
The nucleus accumbens (NAc) plays a central role in reinforcement and motivation. Around 95% of the NAc neurons are medium spiny neurons (MSNs), divided into those expressing dopamine receptor D1 (D1R) or dopamine receptor D2 (D2R). Optogenetic activation of D2-MSNs increased motivation, whereas inhibition of these neurons produced the opposite effect. Yet, it is still unclear how activation of D2-MSNs affects other local neurons/interneurons or input terminals and how this contributes for motivation enhancement. To answer this question, in this work we combined optogenetic modulation of D2-MSNs with pharmacological delivery of specific neurotransmitter antagonists in rats. First, we showed that optogenetic activation of D2-MSNs increases motivation in a progressive ratio (PR) task. We demonstrated that this behavioral effect relies on cholinergic-dependent modulation of dopaminergic signalling of ventral tegmental area (VTA) terminals, which requires D1R and D2R signalling in the NAc. D2-MSN optogenetic activation decreased ventral pallidum (VP) activity, reducing the inhibitory tone to VTA, leading to increased dopaminergic activity. Importantly, optogenetic activation of D2-MSN terminals in the VP was sufficient to recapitulate the motivation enhancement. In summary, our data suggests that optogenetic stimulation of NAc D2-MSNs indirectly modulates VTA dopaminergic activity, contributing for increased motivation. Moreover, both types of dopamine receptors signalling in the NAc are required in order to produce the positive behavioral effects.
eNeuro, 2018 Mar-Apr; 5
27337658: Soares-Cunha C, Coimbra B, David-Pereira A, Borges S, Pinto L, Costa P, Sousa N, Rodrigues AJ
Activation of D2 dopamine receptor-expressing neurons in the nucleus accumbens increases motivation.
Striatal dopamine receptor D1-expressing neurons have been classically associated with positive reinforcement and reward, whereas D2 neurons are associated with negative reinforcement and aversion. Here we demonstrate that the pattern of

activation of D1 and D2 neurons in the nucleus accumbens (NAc) predicts motivational drive, and that optogenetic activation of either neuronal population enhances motivation in mice. Using a different approach in rats, we further show that activating NAc D2 neurons increases cue-induced motivational drive in control animals and in a model that presents anhedonia and motivational deficits; conversely, optogenetic inhibition of D2 neurons decreases motivation. Our results suggest that the classic view of D1-D2 functional antagonism does not hold true for all dimensions of reward-related behaviours, and that D2 neurons may play a more prominent pro-motivation role than originally anticipated.
Nat Commun, 2016; 7
27235078: Soares-Cunha C, Coimbra B, Sousa N, Rodrigues AJ
Reappraising striatal D1- and D2-neurons in reward and aversion.
The striatum has been involved in complex behaviors such as motor control, learning, decision-making, reward and aversion. The striatum is mainly composed of medium spiny neurons (MSNs), typically divided into those expressing dopamine receptor D1, forming the so-called direct pathway, and those expressing D2 receptor (indirect pathway). For decades it has been proposed that these two populations exhibit opposing control over motor output, and recently, the same dichotomy has been proposed for valenced behaviors. Whereas D1-MSNs mediate reinforcement and reward, D2-MSNs have been associated with punishment and aversion. In this review we will discuss pharmacological, genetic and optogenetic studies that indicate that there is still controversy to what concerns the role of striatal D1- and D2-MSNs in this type of behaviors, highlighting the need to reconsider the early view that they mediate solely opposing aspects of valenced behaviour.
Neurosci Biobehav Rev, 2016; 68
25928947: Soares-Cunha C, Coimbra B, Borges S, Carvalho MM, Rodrigues AJ, Sousa N
The motivational drive to natural rewards is modulated by prenatal glucocorticoid exposure.
Exposure to elevated levels of glucocorticoids (GCs) during neurodevelopment has been identified as a triggering factor for the development of reward-associated disorders in adulthood. Disturbances in the neural networks responsible for the complex processes that assign value to rewards and associated stimuli are critical for disorders such as depression, obsessive–compulsive disorders, obesity and addiction. Essential in the understanding on how cues influence behavior is the Pavlovian–instrumental transfer (PIT), a phenomenon that refers to the capacity of a Pavlovian stimulus that predicts a reward to elicit instrumental responses for that same reward. Here, we demonstrate that in utero exposure to GCs (iuGC) impairs both general and selective versions of the PIT paradigm, suggestive of deficits in motivational drive. The iuGC animals presented impaired neuronal activation pattern upon PIT performance in cortical and limbic regions, as well as morphometric changes and reduced levels of dopamine in prefrontal and orbitofrontal cortices, key regions involved in the integration of Pavlovian and instrumental stimuli. Normalization of dopamine levels rescued this behavior, a process that relied on D2/D3, but not D1, dopamine receptor activation. In summary, iuGC exposure programs the mesocorticolimbic dopaminergic circuitry, leading to a reduction in the attribution of the incentive salience to cues, in a dopamine-D2/D3-dependent manner. Ultimately, these results are important to understand how GCs bias incentive processes, a fact that is particularly relevant for disorders where differential attribution of incentive salience is critical.
Transl Psychiatry, 2014; 4

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BOARD NUMBER: S01-073

PRENATAL GLUCOCORTICOID EXPOSURE ALTERS EFFORT DECISION MAKING AND TRIGGERS NUCLEUS ACCUMBENS AND ANTERIOR CINGULATE CORTEX FUNCTIONAL CHANGES

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Verónica Domingues
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Individuals select actions based on cost-benefit to allocate resources into goal-directed actions. Different brain regions coordinate this complex decision, including the nucleus accumbens (NAc), anterior cingulate cortex (ACC) and ventral tegmental area (VTA). *In utero* exposure to glucocorticoids (iuGC) triggers prominent motivation deficits in adulthood, but the impact of this exposure in the ACC-NAc and/or ACC-VTA circuits is unknown. In this work, we tested adult iuGC-exposed animals on a classical motivation task and on an effort-based decision-making task. We also evaluated basal and evoked neuronal activity of the ACC, NAc and VTA. We show that iuGC exposure causes decreased motivation for food in a progressive ratio task and impaired effort-based decision-making in adulthood. These behavioral deficits were associated with reduced neuronal activation of the NAc and ACC, as evaluated through the number of c-fos+ neurons after task performance. Interestingly, iuGC treatment led to increased NAc and ACC basal neuronal activity. Optogenetic activation of ACC terminals in the NAc triggered a different response in iuGC animals in comparison to control group, while the ACC-VTA circuit seems to be preserved. In sum, these data suggest that iuGC animals present motivational and effort-based decision-making deficits that occur in parallel with ACC-NAc dysfunction.

Pubmed:

34859784: Loureiro-Campos E, Mateus-Pinheiro A, Patrício P, Soares-Cunha C, Silva J, Sardinha VM, Mendes-Pinheiro B, Silveira-Rosa T, Domingues AV, Rodrigues AJ, Oliveira J, Sousa N, Alves ND, Pinto L
Constitutive deficiency of the neurogenic hippocampal modulator AP2γ promotes anxiety-like behavior and cumulative memory deficits in mice from juvenile to adult periods.
The transcription factor activating protein two gamma (AP2γ) is an important regulator of neurogenesis both during embryonic development as well as in the postnatal brain, but its role for neurophysiology and behavior at distinct postnatal periods is still unclear. In this work, we explored the neurogenic, behavioral, and functional impact of a constitutive and heterozygous AP2γ deletion in mice from early postnatal development until adulthood. AP2γ deficiency promotes downregulation of hippocampal glutamatergic neurogenesis, altering the ontogeny of emotional and memory behaviors associated with hippocampus formation. The impairments induced by AP2γ constitutive deletion since early development leads to an anxious-like phenotype and memory impairments as early as the juvenile phase. These behavioral impairments either persist from the juvenile phase to adulthood or emerge in adult mice with deficits in behavioral flexibility and object location recognition. Collectively, we observed a progressive and cumulative impact of constitutive AP2γ deficiency on the hippocampal glutamatergic neurogenic process, as well as alterations on limbic-cortical connectivity, together with functional behavioral impairments. The results herein presented demonstrate the modulatory role exerted by the AP2γ transcription factor and the relevance of hippocampal neurogenesis in the development of emotional states and memory processes.
Elife, 2021; 10
34374447: Coimbra B, Domingues AV, Soares-Cunha C, Correia R, Pinto L, Sousa N, Rodrigues AJ
Laterodorsal tegmentum-ventral tegmental area projections encode positive reinforcement signals.
The laterodorsal tegmentum (LDT) is a brainstem nucleus classically involved in REM sleep and attention, and that has recently been associated with reward-related behaviors, as it controls the activity of ventral tegmental area (VTA) dopaminergic neurons, modulating dopamine release in the nucleus accumbens. To further understand the role of LDT-VTA inputs in reinforcement, we optogenetically manipulated these inputs during different behavioral paradigms in male rats. We found that in a two-choice instrumental task, optical activation of LDT-VTA projections shifts and amplifies preference to the laser-paired reward in comparison to an otherwise equal reward; the opposite was observed with inhibition experiments. In a progressive ratio task, LDT-VTA activation boosts motivation, that is, enhances the willingness to work to get the reward associated with LDT-VTA stimulation; and the reverse occurs when inhibiting these inputs. Animals abolished preference if the reward was omitted, suggesting that LDT-VTA stimulation adds/decreases value to the stimulation-paired reward. In

addition, we show that LDT-VTA optical activation induces robust preference in the conditioned and real-time place preference tests, while optical inhibition induces aversion. The behavioral findings are supported by electrophysiological recordings and c-fos immunofluorescence correlates in downstream target regions. In LDT-VTA ChR2 animals, we observed an increase in the recruitment of lateral VTA dopamine neurons and D1 neurons from nucleus accumbens core and shell; whereas in LDT-VTA NpHR animals, D2 neurons appear to be preferentially recruited. Collectively, these data show that the LDT-VTA inputs encode positive reinforcement signals and are important for different dimensions of reward-related behaviors.
J Neurosci Res, 2021; 99
33614864: Gaspar R, Soares-Cunha C, Domingues AV, Coimbra B, Baptista FI, Pinto L, Ambrósio AF, Rodrigues AJ, Gomes CA
Resilience to stress and sex-specific remodeling of microglia and neuronal morphology in a rat model of anxiety and anhedonia.
Prenatal exposure to stress or glucocorticoids (GC) is associated with the appearance of psychiatric diseases later in life. Microglia, the immune cells of the brain, are altered in stress-related disorders. Synthetic GC such as dexamethasone (DEX) are commonly prescribed in case of preterm risk labour in order to promote fetal lung maturation. Recently, we reported long-lasting differences in microglia morphology in a model of exposure to DEX (iuDEX), that presents an anxious phenotype. However, it is still unclear if stress differentially affects iuDEX males and females. In this work, we evaluated how iuDEX animals of both sexes cope with chronic mild stress for 2 weeks. We evaluated emotional behavior and microglia and neuronal morphology in the dorsal hippocampus (dHIP) and (NAc), two brain regions involved in emotion-related disorders. We report that males and females prenatally exposed to DEX have better performance in anxiety- and depression-related behavioral tests after chronic stress exposure in adulthood than non-exposed animals. Interestingly, iuDEX animals present sex-dependent changes in microglia morphology in the dHIP (hypertrophy in females) and in the NAc (atrophy in females and hypertrophy in males). After chronic stress, these cells undergo sex-specific morphological remodeling. Paralleled to these alterations in cytoarchitecture of microglia, we report inter-regional differences in dendritic morphology in a sex-specific manner. iuDEX females present fewer complex neurons in the NAc, whereas iuDEX males presented less complex neuronal morphology in the dHIP. Interestingly, these alterations were modified by stress exposure. Our work shows that stressful events during pregnancy can exert a preserved sex-specific effect in adulthood. Although the role of the observed cellular remodeling is still unknown, sex-specific differences in microglia plasticity induced by long-term stress exposure may anticipate differences in drug efficacy in the context of stress-induced anxiety- or depression-related behaviors.
Neurobiol Stress, 2021; 14
32617639: Domingues AV, Pereira IM, Vilaça-Faria H, Salgado AJ, Rodrigues AJ, Teixeira FG
Glial cells in Parkinson´s disease: protective or deleterious?
Glial cells have been identified more than 100 years ago, and are known to play a key role in the central nervous system (CNS) function. A recent piece of evidence is emerging showing that in addition to the capacity of CNS modulation and homeostasis, glial cells are also being looked like as a promising cell source not only to study CNS pathologies initiation and progression but also to the establishment and development of new therapeutic strategies. Thus, in the present review, we will discuss the current evidence regarding glial cells' contribution to neurodegenerative diseases as Parkinson's disease, providing cellular, molecular, functional, and behavioral data supporting its active role in disease initiation, progression, and treatment. As so, considering their functional relevance, glial cells may be important to the understanding of the underlying mechanisms regarding neuronal-glial networks in neurodegeneration/regeneration processes, which may open new research opportunities for their future use as a target or treatment in human clinical trials.
Cell Mol Life Sci, 2020; 77
32012897: Teixeira FG, Vilaça-Faria H, Domingues AV, Campos J, Salgado AJ
Preclinical Comparison of Stem Cells Secretome and Levodopa Application in a 6-Hydroxydopamine Rat Model of Parkinson's Disease.
Parkinson's Disease (PD) is characterized by the massive loss of dopaminergic neurons, leading to the appearance of several motor impairments. Current pharmacological treatments, such as the use of levodopa, are yet unable to cure the disease. Therefore, there is a need for novel strategies, particularly those that can combine in an integrated manner neuroprotection and neuroregeneration properties. In vitro and in vivo models have recently revealed that the secretome of mesenchymal stem cells (MSCs) holds a promising potential for treating PD, given its effects on neural survival, proliferation, differentiation. In the present study, we aimed to access the impact of human bone marrow MSCs (hBM-MSCs) secretome in 6-hydroxydopamine (6-OHDA) PD model when compared to levodopa administration, by addressing animals' motor performance, and substantia nigra (SN), and striatum (STR) histological parameters by tyrosine hydroxylase (TH) expression. Results revealed that hBM-MSCs secretome per se appears to be a modulator of the dopaminergic system, enhancing TH-positive cells expression (e.g., dopaminergic neurons) and terminals both in the SN and STR when compared to the

untreated group 6-OHDA. Such finding was positively correlated with a significant amelioration of the motor outcomes of 6-OHDA PD animals (assessed by the staircase test). Thus, the present findings support hBM-MSCs secretome administration as a potential therapeutic tool in treating PD, and although we suggest candidate molecules (Trx1, SEMA7A, UCHL1, PEDF, BDNF, Clusterin, SDF-1, CypA, CypB, Cys C, VEGF, DJ-1, Gal-1, GDNF, CDH2, IL-6, HSP27, PRDX1, UBE3A, MMP-2, and GDN) and possible mechanisms of hBM-MSCs secretome-mediated effects, further detailed studies are needed to carefully and clearly define which players may be responsible for its therapeutic actions. By doing so, it will be reasonable to presume that potential treatments that can, per se, or in combination modulate or slow PD may lead to a rational design of new therapeutic or adjuvant strategies for its functional modeling and repair.
Cells, 2020; 9
31534159: Soares-Cunha C, de Vasconcelos NAP, Coimbra B, Domingues AV, Silva JM, Loureiro-Campos E, Gaspar R, Sotiropoulos I, Sousa N, Rodrigues AJ
Correction: Nucleus accumbens medium spiny neurons subtypes signal both reward and aversion.
A correction to this paper has been published and can be accessed via a link at the top of the paper.
Mol Psychiatry, 2020; 25
31515512: Coimbra B, Soares-Cunha C, Vasconcelos NAP, Domingues AV, Borges S, Sousa N, Rodrigues AJ
Role of laterodorsal tegmentum projections to nucleus accumbens in reward-related behaviors.
The laterodorsal tegmentum (LDT) is associated with reward considering that it modulates VTA neuronal activity, but recent anatomical evidence shows that the LDT also directly projects to nucleus accumbens (NAc). We show that the majority of LDT-NAc inputs are cholinergic, but there is also GABAergic and glutamatergic innervation; activation of LDT induces a predominantly excitatory response in the NAc. Non-selective optogenetic activation of LDT-NAc projections in rats enhances motivational drive and shifts preference to an otherwise equal reward; whereas inhibition of these projections induces the opposite. Activation of these projections also induces robust place preference. In mice, specific activation of LDT-NAc cholinergic inputs (but not glutamatergic or GABAergic) is sufficient to shift preference, increase motivation, and drive positive reinforcement in different behavioral paradigms. These results provide evidence that LDT-NAc projections play an important role in motivated behaviors and positive reinforcement, and that distinct neuronal populations differentially contribute for these behaviors.
Nat Commun, 2019; 10
31462765: Soares-Cunha C, de Vasconcelos NAP, Coimbra B, Domingues AV, Silva JM, Loureiro-Campos E, Gaspar R, Sotiropoulos I, Sousa N, Rodrigues AJ
Nucleus accumbens medium spiny neurons subtypes signal both reward and aversion.
Deficits in decoding rewarding (and aversive) signals are present in several neuropsychiatric conditions such as depression and addiction, emphasising the importance of studying the underlying neural circuits in detail. One of the key regions of the reward circuit is the nucleus accumbens (NAc). The classical view on the field postulates that NAc dopamine receptor D1-expressing medium spiny neurons (D1-MSNs) convey reward signals, while dopamine receptor D2-expressing MSNs (D2-MSNs) encode aversion. Here, we show that both MSN subpopulations can drive reward and aversion, depending on their neuronal stimulation pattern. Brief D1- or D2-MSN optogenetic stimulation elicited positive reinforcement and enhanced cocaine conditioning. Conversely, prolonged activation induced aversion, and in the case of D2-MSNs, decreased cocaine conditioning. Brief stimulation was associated with increased ventral tegmenta area (VTA) dopaminergic tone either directly (for D1-MSNs) or indirectly via ventral pallidum (VP) (for D1- and D2-MSNs). Importantly, prolonged stimulation of either MSN subpopulation induced remarkably distinct electrophysiological effects in these target regions. We further show that blocking κ-opioid receptors in the VTA (but not in VP) abolishes the behavioral effects induced by D1-MSN prolonged stimulation. In turn, blocking δ-opioid receptors in the VP (but not in VTA) blocks the behavioral effects elicited by D2-MSN prolonged stimulation. Our findings demonstrate that D1- and D2-MSNs can bidirectionally control reward and aversion, explaining the existence of controversial studies in the field, and highlights that the proposed striatal functional opposition needs to be reconsidered.
Mol Psychiatry, 2020; 25
30034328: Soares-Cunha C, Coimbra B, Borges S, Domingues AV, Silva D, Sousa N, Rodrigues AJ
Mild Prenatal Stress Causes Emotional and Brain Structural Modifications in Rats of Both Sexes.
Stress or high levels of glucocorticoids (GCs) during developmental periods is known to induce persistent effects in the neuroendocrine circuits that control stress response, which may underlie individuals' increased risk for developing neuropsychiatric conditions later in life, such as anxiety or depression. We developed a rat model (Wistar han) of mild exposure to unpredictable prenatal stress (PS), which consists in a 4-h stressor administered three times per week on a random basis; stressors include strobe lights, noise and restrain. Pregnant dams subjected to this protocol present disrupted circadian corticosterone secretion and increased corticosterone secretion upon acute stress exposure. Regarding progeny, both young adult (2 months old) male and female rats present increased levels of circulating corticosterone and hyperactivity

of the hypothalamus-pituitary-adrenal axis to acute stress exposure. Both sexes present anxious- and depressive-like behaviors, shown by the decreased time spent in the open arms of the elevated plus maze (EPM) and in the light side of the light-dark box (LDB), and by increased immobility time in the forced swim test, respectively. Interestingly, these results were accompanied by structural modifications of the bed nucleus of stria terminalis (BNST) and hippocampus, as well as decreased norepinephrine and dopamine levels in the BNST, and serotonin levels in the hippocampus. In summary, we characterize a new model of mild PS, and show that stressful events during pregnancy can lead to long-lasting structural and neurochemical effects in the offspring, which affect behavior in adulthood.
Front Behav Neurosci, 2018; 12
29780881: Soares-Cunha C, Coimbra B, Domingues AV, Vasconcelos N, Sousa N, Rodrigues AJ
Nucleus Accumbens Microcircuit Underlying D2-MSN-Driven Increase in Motivation.
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eNeuro, 2018 Mar-Apr; 5

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ACTION PREDICTION ERROR, A VALUE FREE DOPAMINERGIC TEACHING SIGNAL, DRIVES SELECTIVE CORTICOSTRIATAL PLASTICITY DURING AN AUDITORY DISCRIMINATION TASK. II: BEHAVIORAL AND CAUSAL EVIDENCE.

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Hernando Vergara[1], Francesca Greenstreet[1], Sthitapranjya Pati[1], Laura Schwarz[1], Matthew Wisdom[1], Fred Marbach[1], Yvonne Johansson[1], Theodore Moskovitz[2], Claudia Clopath[3], Marcus Stephenson-Jones[1]
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Frequency-specific plasticity in the posterior striatum (pStr) during auditory discrimination learning suggests it is the locus for forming and storing task-relevant associations. Dopamine signals, thought to reinforce these associations, have been reported to encode information about threat in the pStr, raising the question of how reward-guided associations are formed in this region. Here we show that pStr dopaminergic input is indeed critical for learning and forming frequency-specific associations. We propose a model where dopaminergic input to the pStr forms the value-free half of a dual value-based/value-free dopaminergic learning system. Chronic lesions of the pStr, or the pStr-projecting dopaminergic cells, result in identical learning deficits affecting later learning stages. This is consistent with our dual controller model where initial learning is driven by a value-based system and then rapidly consolidated in a value-free manner in the pStr. Supporting this, optogenetic inhibition of either the direct or the indirect pathways in pStr disrupt behavioural performance specifically in later stages of learning. In line with pStr-projecting dopamine providing a teaching signal we show using LFP recordings that these dopaminergic inputs are required for frequency-specific plasticity and that temporally-specific optogenetic manipulations of dopamine levels in pStr bias future behaviour. These results, together with our finding of an action-related and value-free dopaminergic signal in the pStr (see part I), suggest a model where stable state-action associations are formed in the pStr in a dopamine-dependent but value-free manner, leading to a more habitual behaviour.

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ACTION PREDICTION ERROR, A VALUE-FREE DOPAMINERGIC TEACHING SIGNAL, DRIVES SELECTIVE CORTICOSTRIATAL PLASTICITY DURING AN AUDITORY DISCRIMINATION TASK. I: DOPAMINE RECORDINGS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Francesca Greenstreet[1], Hernando Vergara[1], Sthitapranjya Pati[1], Laura Schwarz[1], Matthew Wisdom[1], Fred Marbach[1], Yvonne Johansson[1], Theodore Moskovitz[1], Claudia Clopath[2], Marcus Stephenson-Jones[1]
[1]Sainsbury Wellcome Center for neural circuits and behaviour, Ucl, London, United Kingdom, [2]Imperial College London, Bioengineering, London, United Kingdom

Experience dependent changes in corticostriatal plasticity are critical for reinforcement learning. In line with this, in an auditory frequency discrimination task, selective frequency-specific corticostriatal plasticity is observed in the posterior striatum (pStr). Dopamine reward prediction error signals are thought to provide a critical teaching signal for such corticostriatal modifications. However the dopaminergic input to the auditory striatum is reported to be activated by threat and not reward prediction error. How such a threat prediction error could help animals learn to transform sound into actions that will obtain reward is unclear. To address this we used photometry to record dopamine levels in the pStr of mice performing an auditory frequency discrimination task. Here we show that dopamine in the pStr encodes an action prediction error, a value free teaching signal that could be used to reinforce stable sound-action associations. pStr dopamine levels are correlated with the contralateral action, and show no variation to reward, outcome value or predicted value. Consistent with a prediction error the response to action is stimulus specific and decreases over training as the action becomes predicted by the auditory stimulus. The dopamine signal we observed in the pStr could be explained by an action prediction error model but not by other alternative models of dopaminergic activity including, rewards prediction error, movement, novelty or salience. Taking inspiration from computational models of habit formation we propose that this value-free teaching signal works in concert with canonical value-based dopaminergic teaching signals to consolidate stable state-action associations in the sensory striatum.

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BOARD NUMBER: S01-076

STRIATAL DOPAMINE ENCODES THE RELATIONSHIP BETWEEN ACTIONS AND REWARD

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Genevra Hart
University of New South Wales, Psychology, Kensington, Australia

Despite the well described role of dopamine in the striatum for motor learning and movement, its function in encoding actions and their outcomes for goal-directed action is less clear. Here, rats acquired two actions for distinct outcomes while we simultaneously recorded dopamine release in the dorsomedial striatum using dLight1.1 with fiber photometry, as these action-outcome associations were incremented and selectively degraded. Goal-directed actions generated a lateralized dopamine signal that reflected the strength of the action-outcome association, and tracked increments and decrements in the action-outcome contingency. In addition to this lateralized signal, dopamine signalled reward predictions, which were broadcast bilaterally during the action, and updated by a reward prediction error following exposure to the outcome. Striatal dopamine release, therefore, both directly modulates and updates encoding of the instrumental action-outcome association for goal-directed action.

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BOARD NUMBER: S01-077

IS MOTIVATION FOR PHYSICAL EXERCISE ENCODED BY MIDBRAIN DOPAMINERGIC NEURONS?

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Emma Mesguich[1], Maxime Come[2], Bastien Redon[1,3], Imane Hurel[1], Francisca Julio-Kalajzic[1], Philippe Faure[2], Giovanni Marsicano[1], Francis Chaouloff[1]
[1]Neurocentre Magendie, Bordeaux University, Inserm U1215, Bordeaux, France, [2]ESPCI, Brain Plasticity Lab Umr8249 Cnrs, Paris, France, [3]Geneva University, Département Des Neurosciences Fondamentales, Genève, Switzerland

Aims Our studies combining conditioned wheel-running and electrophysiology suggest that exercise motivation requires a cannabinoid type-1 receptor-mediated disinhibition of ventral tegmental area (VTA) dopaminergic (DA) activity (Muguruza et al., 2019; Medrano et al., 2021). However, direct evidence for a link between VTA DA activity and exercise motivation is still lacking. We thus aim at examining how midbrain DA neuronal activity varies with the intensity of the effort required to access a running wheel. ***Methods*** Operant chambers hosted a locked wheel surrounded by nose poke (NP) ports and light cues. In a first step, wheel unlocking was indicated by a light cue (Pavlovian rule). In a second series of experiments using instrumental conditioning, mice had access to the wheel following correct light cue-conditioned NP performance, i.e. under intra-session fixed ratio (FR)1 and FR5 reinforcement schedules. Our *in vivo* electrophysiology technique uses 16-channel extracellular recordings in the VTA of freely-moving mice trained before electrode implantation. ***Results*** Once mice learned the Pavlovian association, VTA DA neuronal activity increased during cue presentation, in keeping with the reward prediction error theory (Schultz et al. 1997). Having set up a conditioning protocol wherein mice learn within several sessions that wheel access is under alternate FR1/FR5 rules, ongoing experiments test whether VTA DA neuronal activity is effort-dependent. ***Conclusions*** We have first observed that VTA DA activity might be involved in the rewarding effect of running. We are now testing whether such an activity varies with the intensity of the effort needed to access that reward.

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TASK-DEPENDENT ENSEMBLE CODING IN RAT MEDIAL PREFRONTAL CORTEX

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Ida V. Rautio[1], Fredrik Nevjen[2], David Klindt[2], Benjamin Dunn[2], Jonathan Whitlock[1]
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The medial prefrontal cortex has been implicated in diverse cognitive processes such as attention, goal-related navigation, action-planning, social information processing, learning and strategy-switching. Previous work has shown that clear behavioural and cognitive correlates in the mPFC are less common at the single cell level, which often necessitates methods to uncover higher-dimensional aspects to give meaningful behavioral- or task-related interpretations of their findings. In an effort to better understand the apparent flexibility of this brain area, we have implemented a multi-task approach where animals repeatedly experience an open field foraging task and a bait-chasing task in the same arena, as well as a social context with a conspecific on a linear track, while we record from hundreds of neurons simultaneously using Neuropixel probes. Additionally, we employed precise 3D tracking to investigate potentially low-dimensional features like pose and movement in addition to the cognitive aspects of each context. Single neuron and population responses have so far shown sparse and unstable tuning to posture and movement, and the same for self-motion and spatial tuning. However, more complex task-specific behaviors, in particular the chasing task, elicit stronger, reliable spiking responses, and exhibit stable correlational structures at the ensemble level. Leveraging the multiple tasks to disentangle behavioral selectivity from the context, we aim to elucidate the extent to which the selectivity is unique and common in these seemingly distinct cognitive processes.

Pubmed:
32948095: Karlsen RH, Saksvik SB, Stenberg J, Lundervold AJ, Olsen A, Rautio I, Folvik L, Håberg AK, Vik A, Karr JE, Iverson GL, Skandsen T
Examining the Subacute Effects of Mild Traumatic Brain Injury Using a Traditional and Computerized Neuropsychological Test Battery.
This study investigates subacute cognitive effects of mild traumatic brain injury (MTBI) in the Trondheim Mild TBI Study, as measured, in part, by the neuropsychological test battery of the Collaborative European NeuroTrauma Effectiveness Research in TBI (CENTER-TBI) program, including computerized tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) and traditional paper-and-pencil tests. We investigated whether cognitive function was associated with injury severity: intracranial traumatic lesions on neuroimaging, witnessed loss of consciousness (LOC), or post-traumatic amnesia (PTA) >1 h. Further, we explored which of the tests in the CENTER-TBI battery might be associated with the largest subacute effects of MTBI (i.e., at 2 weeks post-injury). We recruited 177 patients with MTBI (16-59 years of age) from a regional trauma center and an outpatient clinic,79 trauma control participants, and 81 community control participants. The MTBI group differed from community controls only on one traditional test of processing speed (coding; = 0.009, Cliff's delta [Δ] = 0.20). Patients with intracranial abnormalities performed worse than those without on a traditional test (phonemic verbal fluency; = 0.043, Δ = 0.27), and patients with LOC performed differently on the Attention Switching Task from the CANTAB (= 0.020, Δ = -0.20). Patients with PTA >1 h performed worse than those with <1 h on 10 measures, from traditional tests and the CANTAB (Δ = 0.33-0.20), likely attributable, at least in part, to pre-existing differences in intellectual functioning between groups. In general, those with MTBI had good neuropsychological outcome 2 weeks after injury and no particular CENTER-TBI computerized or traditional tests seemed to be more sensitive to subtle cognitive deficits.
J Neurotrauma, 2021; 38

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BOARD NUMBER: S01-079

DIFFERENTIAL DOPAMINE SIGNALLING IN VENTRAL AND DORSAL STRIATUM IN VIVO

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Striatal dopamine signals encode information related to reward and movement, but the precise patterning of these signals can vary across striatal regions. Links between regional diversity and functional diversity in stratum are often simplified into a framework incorporating two main pathways originating from the dopaminergic midbrain; a ‘reward pathway’ that terminates in ventral striatum (or nucleus accumbens, NAc), and a ‘movement pathway’ terminating in dorsal striatum. To elucidate regional and functional heterogeneity in striatal dopamine signalling, fluorescence emitted from the genetically-encoded dopamine sensor dLight was photometrically recorded from the NAc and dorsal striatum of anaesthetised and awake mice. In anaesthetised mice, electrical stimulation of the dopaminergic midbrain across a wide parameter space was used to systematically investigate regional differences in evoked dLight fluorescence. Awake, head-fixed mice were trained in a treadmill-based go/no-go task and dLight photometry was then used to assess the extent to which each striatal region selectively encodes reward and movement during behavioural performance.

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BOARD NUMBER: S01-080

WHERE DID MY CHEESE MOVE? BEHAVIOURAL AND HIPPOCAMPAL TRACES OF UNCERTAINTIES INDUCED BY CHANGES IN REWARD DISTRIBUTIONS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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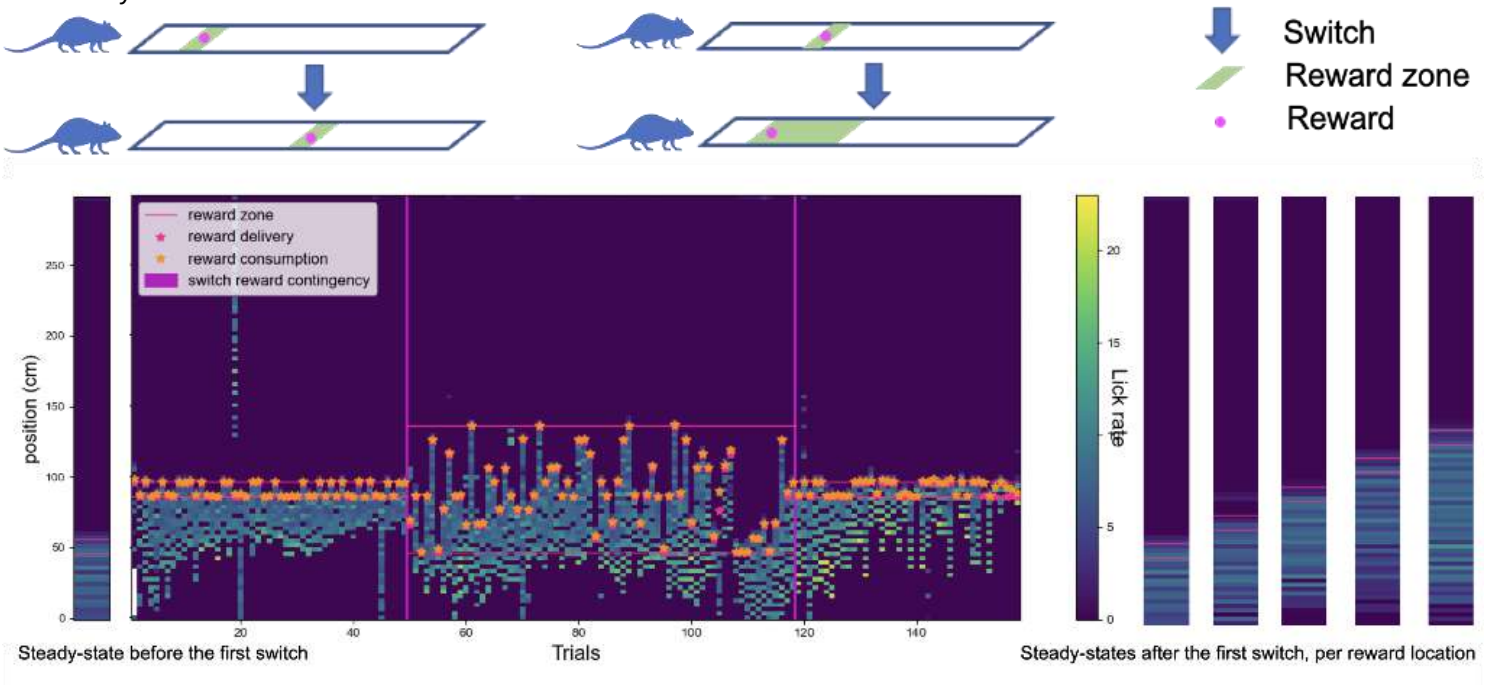
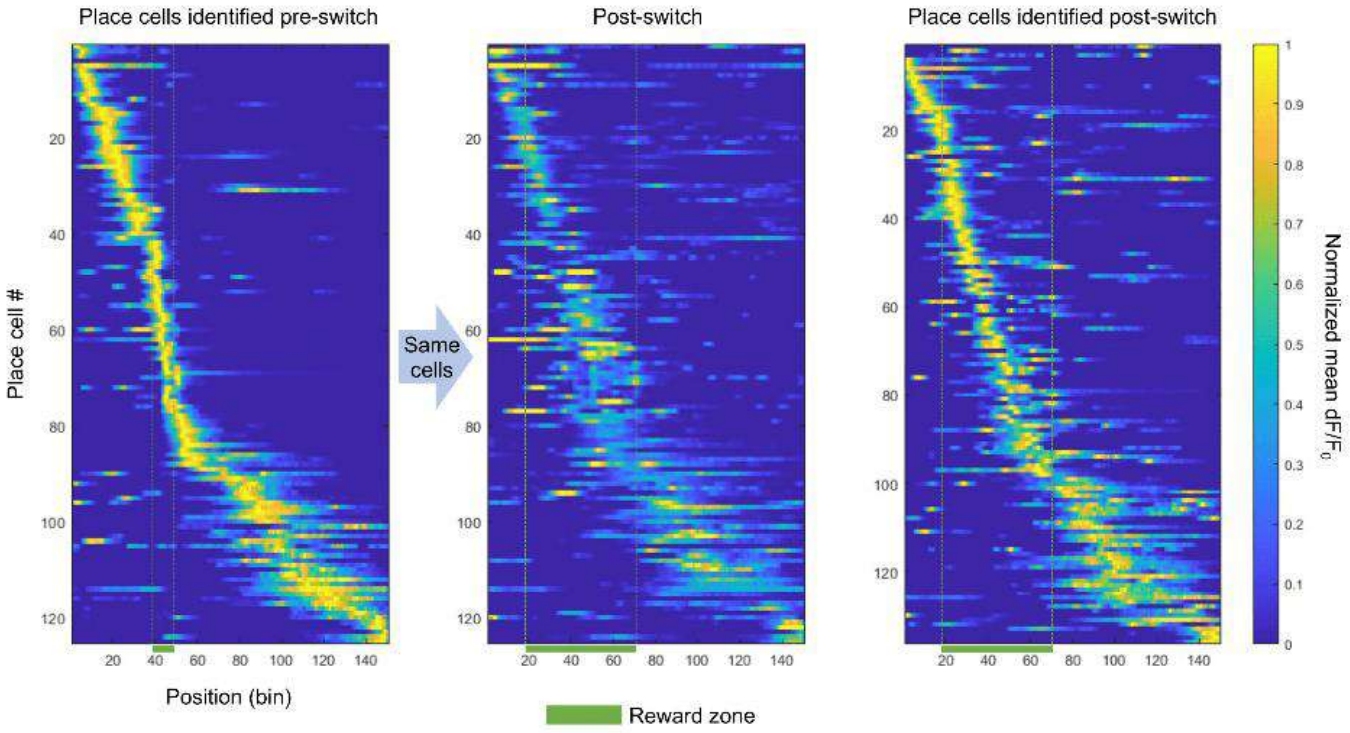
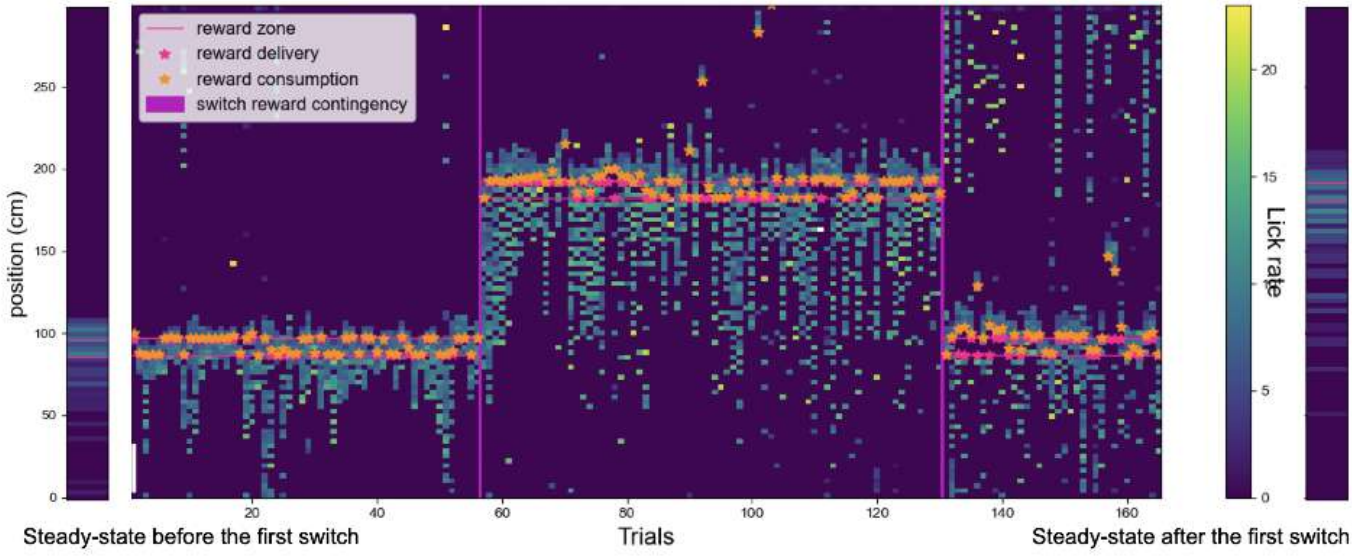
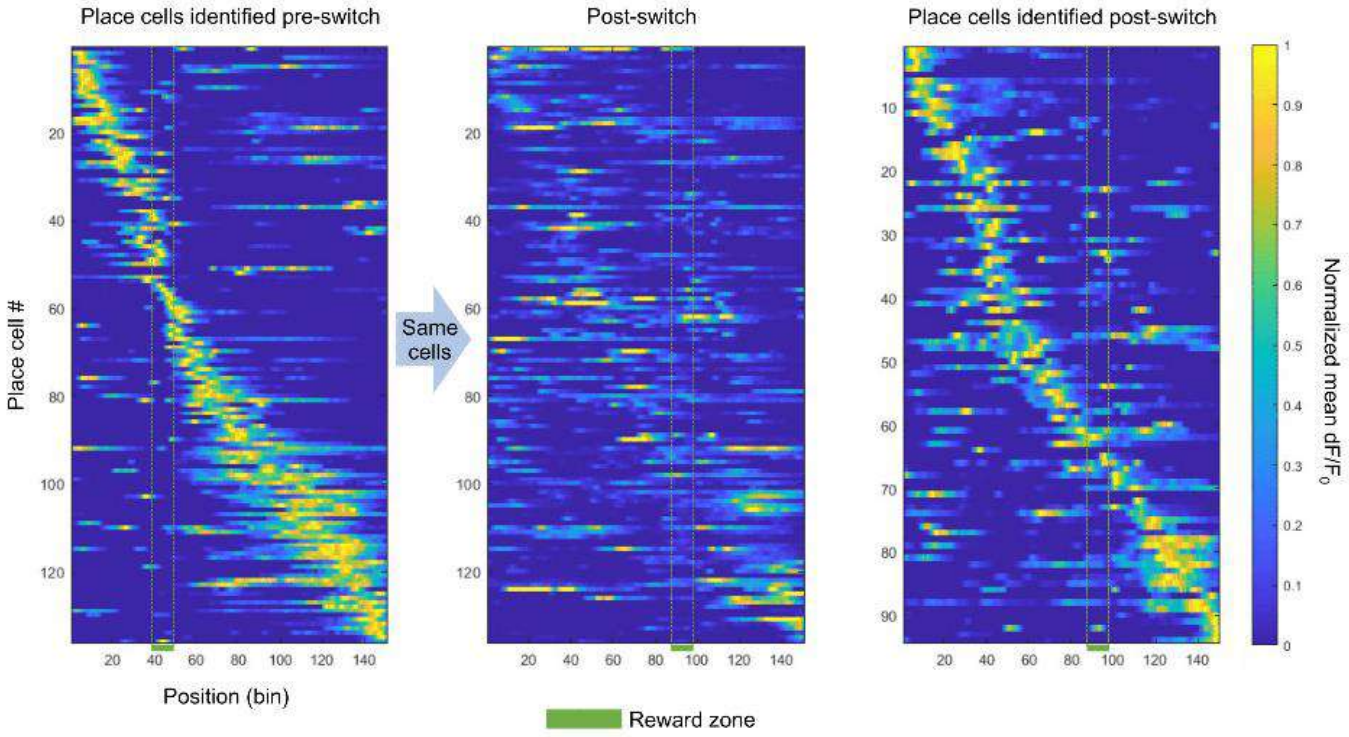
[1]Northwestern University, Department Of Neurobiology, Evanston, United States of America, [2]Max Planck Institute for Biological Cybernetics, Department For Computational Neuroscience, Tübingen, Germany, [3]University of Bristol, School Of Physiology, Pharmacology And Neuroscience, Bristol, United Kingdom, [4]Imperial College London, Department Of Bioengineering, London, United Kingdom, [5]Champalimaud Foundation, Champalimaud Neuroscience Programme, Lisbon, Portugal, [6]University of Oxford, Department Of Experimental Psychology, Oxford, United Kingdom

New, changing, and inherently variable environments force animals to address different sorts of uncertainty. These in turn, are associated with various aspects of behavioural and neural activity. Hippocampal place cells code in a behaviourally-relevant manner for aspects of space and reward. By inducing various forms of uncertainty in these two quantities, we sought to shed light on the ways that mice experience and accommodate the incompletely known. We designed a novel Uncertain Reward Task (URTask) in a virtual reality (VR) apparatus (figure1). Mice (six adults) were trained to run along a VR linear track and lick for a water reward. Different forms of uncertainty were manipulated by varying the distributions of reward locations and changing the virtual surround. Using two-photon calcium imaging, we recorded activities of place cells in CA1 along with mouse behaviour (licking and velocity) as uncertainty was induced and partially resolved by experience. Given inherently variable reward locations, animals tend to slow down and lick continuously within the expected reward zone (figure 2), and the place map within the reward zone becomes less spatially precise (figure 3). From limpid changes in reward locations, the behaviour requires a few trials to adjust to the new task configuration (figure 4), and the place map undergoes a higher degree of remapping (figure 5). Our data demonstrates that the URTask can engender different forms of uncertainty that produce different behavioural and neuronal adaptations. The URTask may therefore be used to investigate how the brain distinguishes and decodes varying forms of uncertainty.




Place cells identified pre-switch
Post-switch
Place cells identified post-switch
Place cell #
Same cells
Position (bin)
Reward zone
Normalized mean dF/F0


reward zone
reward delivery
reward consumption
switch reward contingency
position (cm)
Lick rate
Steady-state before the first switch
Trials
Steady-state after the first switch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Post-switch
Place cells identified post-switch
Place cell #
Same cells
Position (bin)
Reward zone
Normalized mean dF/F0

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BOARD NUMBER: S01-081

DOPAMINE CORRELATES OF HABIT VERSUS GOAL-DIRECTED BEHAVIOR IN THE VENTRAL TEGMENTAL AREA

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Habitual performance is commonly considered in light of dorsolateral striatum and its nigral dopaminergic (DA) input. However, how ventral tegmental area DA neuron activity might also differentially signal habitual *versus* goal-directed performance remains largely unknown. To address this question, we tested rats in two operant procedures, a lever insertion fixed-ratio 5 (LI5) task and a lever retraction fixed-ratio 5 (LR5) task. Here, the timing of lever insertion (LI) and retraction (LR) is varied so that one is a relevant cue signaling reward (sucrose 20%) availability while the other cue is made irrelevant. We then used satiety-induced devaluation to test whether behavior was goal-directed or habitual. Finally, we compared activity of DA neurons *via in vivo* fiber photometry recording after VTA infusion of Cre- dependent GCaMP6f virus in adult TH-Cre rats performing either the LR5 or LI5 task. We found rats quickly developed habitual behavior and chunking in the LR5 task in which the LR cue predicts immediate reward delivery. We further observed rapid shifts in activation of DA VTA neurons from reward retrieval to the earlier LR cue, followed by decreases in cue-related DA signals across repeated trials as performance become automatic and habitual. In contrast, behavior in the LI5 task was goal-directed, and cue-induced DA activation remained relatively constant across trials and sessions, when reward availability was signaled by the LI cue, more distal from reward delivery. These results show manifest task differences in DA signaling that appear consistent with differences in habitual *versus* goal-directed control of behavior.

Pubmed:
30618665: Magnard R, Vachez Y, Carcenac C, Boulet S, Houeto JL, Savasta M, Belin D, Carnicella S
Nigrostriatal Dopaminergic Denervation Does Not Promote Impulsive Choice in the Rat: Implication for Impulse Control Disorders in Parkinson's Disease.
Impulse control disorders (ICDs) are frequent behavioral complications of dopaminergic (DA) replacement therapies (DRTs) in Parkinson's disease (PD). Impulsive choice, which refers to an inability to tolerate delays to reinforcement, has been identified as a core pathophysiological process of ICDs. Although impulsive choices are exacerbated in PD patients with ICDs under DRTs, some clinical and preclinical studies suggest that the DA denervation of the dorsal striatum induced by the neurodegenerative process as well as a pre-existing high impulsivity trait, may both contribute to the emergence of ICDs in PD. We therefore investigated in a preclinical model in rats, specifically designed to study PD-related non-motor symptoms, the effect of nigrostriatal DA denervation on impulsive choice, in relation to pre-existing levels of impulsivity, measured in a Delay Discounting Task (DDT). In this procedure, rats had the choice between responding for a small sucrose reinforcer delivered immediately, or a larger sucrose reinforcer, delivered after a 0, 5, 10 or 15 s delay. In two different versions of the task, the preference for the large reinforcer decreased as the delay increased. However, and in contrast to our initial hypothesis, this discounting effect was neither exacerbated by, or related to, the extent of the substantia nigra pars compacta (SNc) DA lesion, nor it was influenced by pre-existing variability in impulsive choice. These results therefore question the potential implication of the nigrostriatal DA system in impulsive choice, as well as the DA neurodegenerative process as a factor contributing significantly to the development of ICDs in PD.
Front Behav Neurosci, 2018; 12
32562462: Vachez Y, Carcenac C, Magnard R, Goff LK, Salin P, Savasta M, Carnicella S, Boulet S
Reply to: Letter to the Editor by Martínez-Fernández.
Mov Disord, 2020; 35
31930749: Vachez Y, Carcenac C, Magnard R, Kerkerian-Le Goff L, Salin P, Savasta M, Carnicella S, Boulet S
Subthalamic Nucleus Stimulation Impairs Motivation: Implication for Apathy in Parkinson's Disease.
Apathy is one of the most disabling neuropsychiatric symptoms in Parkinson's disease (PD) patients and has a higher prevalence in patients under subthalamic nucleus deep brain stimulation. Indeed, despite its effectiveness for alleviating PD motor symptoms, its neuropsychiatric repercussions have not yet been fully uncovered. Because it can be alleviated by

dopaminergic therapies, especially D and D dopaminergic receptor agonists, the commonest explanation proposed for apathy after subthalamic nucleus deep brain stimulation is a too-strong reduction in dopaminergic treatments. The objective of this study was to determine whether subthalamic nucleus deep brain stimulation can induce apathetic behaviors, which remains an important matter of concern. We aimed to unambiguously address this question of the motivational effects of chronic subthalamic nucleus deep brain stimulation.
Mov Disord, 2020; 35

27303314: Houeto JL, Magnard R, Dalley JW, Belin D, Carnicella S
Trait Impulsivity and Anhedonia: Two Gateways for the Development of Impulse Control Disorders in Parkinson's Disease?
Apathy and impulsivity are two major comorbid syndromes of Parkinson's disease (PD) that may represent two extremes of a behavioral spectrum modulated by dopamine-dependent processes. PD is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta to which are attributed the cardinal motor symptoms of the disorder. Dopamine replacement therapy (DRT), used widely to treat these motor symptoms, is often associated with deficits in hedonic processing and motivation, including apathy and depression, as well as impulse control disorders (ICDs). ICDs comprise pathological gambling, hypersexuality, compulsive shopping, binge eating, compulsive overuse of dopaminergic medication, and punding. More frequently observed in males with early onset PD, ICDs are associated not only with comorbid affective symptoms, such as depression and anxiety, but also with behavioral traits, such as novelty seeking and impulsivity, as well as with personal or familial history of alcohol use. This constellation of associated risk factors highlights the importance of inter-individual differences in the vulnerability to develop comorbid psychiatric disorders in PD patients. Additionally, withdrawal from DRT in patients with ICDs frequently unmasks a severe apathetic state, suggesting that apathy and ICDs may be caused by overlapping neurobiological mechanisms within the cortico-striato-thalamo-cortical networks. We suggest that altered hedonic and impulse control processes represent distinct prodromal substrates for the development of these psychiatric symptoms, the etiopathogenic mechanisms of which remain unknown. Specifically, we argue that deficits in hedonic and motivational states and impulse control are mediated by overlapping, yet dissociable, neural mechanisms that differentially interact with DRT to promote the emergence of ICDs in vulnerable individuals. Thus, we provide a novel heuristic framework for basic and clinical research to better define and treat comorbid ICDs in PD.
Front Psychiatry, 2016; 7

26954980: Magnard R, Vachez Y, Carcenac C, Krack P, David O, Savasta M, Boulet S, Carnicella S
What can rodent models tell us about apathy and associated neuropsychiatric symptoms in Parkinson's disease?
In addition to classical motor symptoms, Parkinson's disease (PD) patients display incapacitating neuropsychiatric manifestations, such as apathy, anhedonia, depression and anxiety. These hitherto generally neglected non-motor symptoms, have gained increasing interest in medical and scientific communities over the last decade because of the extent of their negative impact on PD patients' quality of life. Although recent clinical and functional imaging studies have provided useful information, the pathophysiology of apathy and associated affective impairments remains elusive. Our aim in this review is to summarize and discuss recent advances in the development of rodent models of PD-related neuropsychiatric symptoms using neurotoxin lesion-based approaches. The data collected suggest that bilateral and partial lesions of the nigrostriatal system aimed at inducing reliable neuropsychiatric-like deficits while avoiding severe motor impairments that may interfere with behavioral evaluation, is a more selective and efficient strategy than medial forebrain bundle lesions. Moreover, of all the different classes of pharmacological agents, D2/D3 receptor agonists such as pramipexole appear to be the most efficient treatment for the wide range of behavioral deficits induced by dopaminergic lesions. Lesion-based rodent models, therefore, appear to be relevant tools for studying the pathophysiology of the non-motor symptoms of PD. Data accumulated so far confirm the causative role of dopaminergic depletion, especially in the nigrostriatal system, in the development of behavioral impairments related to apathy, depression and anxiety. They also put forward D2/D3 receptors as potential targets for the treatment of such neuropsychiatric symptoms in PD.
Transl Psychiatry, 2016; 6

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BOARD NUMBER: S01-082

DISTRIBUTED NEURAL CODING OF MULTISENSORY VARIABLES AND TRIAL OUTCOME IN THE CORTICO-HIPPOCAMPAL HIERARCHY

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Julien Fiorilli[1], Pietro Marchesi[1], Thijs Ruikes[1], Gerjan Huis In 'T Veld[1], Rhys Buckton[1], Mariana Quintero[1], Ingrid Reiten[2], Jan Bjaalie[2], Cyriel Pennartz[1]
[1]University of Amsterdam, Cognitive And Systems Neuroscience, Amsterdam, Netherlands, [2]University of Oslo, Institute Of Basic Medical Sciences, Oslo, Norway

Neural circuits are believed to support adaptive behavior by integrating sensory input with reward and error-driven learning signals. How these signals are distributed across different levels of the cortico-hippocampal hierarchy remains poorly understood. We trained four rats on a multisensory object discrimination task and compared sensory and reward-driven responses from simultaneously recorded neurons in barrel cortex (S1BF, N=99), higher visual cortex (V2L, N=287), perirhinal cortex (PER, N=175) and hippocampus (HPC, N=202). We found that V2L neurons discharged after light onset, but not following whisker touch. A significant proportion of neurons was modulated by either of the two modalities in HPC (Touch: 16%; Light:10%), despite insignificant proportions of light or touch-modulated cells in PER. A significant proportion (9%) of V2L neurons differentiated between the objects during visual sampling; this object representation was not found in HPC or PER. Upon reward-poke entry, 16% of PER neurons differentiated between trial outcome (rewarded or unrewarded). In contrast to the other regions, this modulation preceded reward-delivery dependent changes in lick rate. These neurons did not merely react to reward delivery itself, because the majority of these neurons in fact discharged upon an unrewarded poke. Altogether, we report a surprising absence of object-identity representations in PER during sensory sampling. Instead, PER primarily represented information about choice outcome, especially in unrewarded trials. Our results support a distributed neural coding of multisensory variables in the cortico-hippocampal hierarchy during object sampling and reward processing, with a particularly important role for PER in signaling the absence an expected reward.

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BOARD NUMBER: S01-083

PHASIC DOPAMINE BUILDS AND REVEALS LATENT ATTRACTORS.

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Jérémie Naudé[1,2], Matthieu Sarazin[3], Sarah Mondoloni[1,4], Alexandre Mourot[1,5], Philippe Faure[1,5], Bruno Delord[3]
[1]CNRS, Neurosciences Paris Seine, Paris, France, [2]CNRS, Institut De Génomique Fonctionnelle, MONTPELLIER CEDEX , France, [3]CNRS, Institut Des Systèmes Intelligents Et De Robotique, PARIS CEDEX, France, [4]University of Lausanne, Department Of Fundamental Neuroscience, Lausanne, Switzerland, [5]ESPCI, Brain Plasticity Lab Umr8249 Cnrs, Paris, France

Transient dopamine (DA) neuron activity contributes to both future decisions (learning) and ongoing behavior (motivation), but reconciling these two roles remains challenging. The popular reinforcement learning theory interprets fast, phasic DA activity as a reward-related teaching signal. At the neuronal level, such role of DA in learning is thought to correspond to the gating of synaptic plasticity, effectively building neural representations of the value of actions leading to reward. By contrast, the role of phasic DA in motivation relies on modulating synaptic excitability online, but has proven hard to reconcile with reinforcement-learning accounts. We thus sought to characterize the dual role of DA in the framework of dynamical system theory, based on DA biophysical effects, and achieved a specific control of VTA DA with optogenetics to test predictions derived from our model. We considered mice navigating between places reinforced by DA stimulation, modeled by a recurrent neural network model of decision. Mice behavior could be explained by a synergy between plasticity and excitability effects on animals' internal goals. DA-gated synaptic plasticity reinforced synapses onto neurons encoding for the rewarded location, building a latent (i.e. not always expressed) attractor, which could be revealed by DA effects on synaptic excitability. We verified experimentally the model predictions that VTA photostimulation energizes, attracts and directs movements specifically toward a previously-rewarded location, without exerting any motor effects out of reward context. We thus propose that the motivational role of VTA DA is to widen the basin of DA-built, latent attractors; corresponding to DA expressing a potential goal.

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BOARD NUMBER: S01-084

BASAL GANGLIA OUTPUT AND THALAMIC CIRCUITS FOR CONTEXT DEPENDENT ANTICIPATION AND ACTION SIGNALING

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Mauricio Toro, Margarida Sousa, Sofia Castro E Almeida, Tiago Monteiro, Filipe Rodrigues, Margarida Pexirra, Joe Paton
Champalimaud Foundation, Champalimaud Research, Lisboa, Portugal

The expression of behavior is highly context-dependent. Prior knowledge about the environment is required to prepare and modulate action selection and execution. The basal ganglia (BG) are thought to contribute to this process through downstream influence on motor areas. However, little data exists regarding the interaction between BG and downstream targets during context-dependent action execution. Here, we studied the interaction between BG outputs and motor thalamus while rats performed delayed movements for varying reward. We trained rats to withhold cued, lateralized movements for variable delays until delivery of a go cue. Crucially, reward location alternated in blocks of trials, meaning that rightwards and leftwards movements were sometimes rewarded, sometimes not. Rats indicated their knowledge of impending reward by reacting and moving more quickly. Next, we simultaneously recorded neural activity from substantia nigra pars reticulata and VA/VL thalamus, and found that both regions carried signatures of reward-context preceding trial initiation, and reward expectation, movement direction, and their interaction before and during movement execution. We then modeled the development of these neural signals using a recurrent actor-critic reinforcement learning agent trained in the task. Surprisingly, we were only able to fully recapitulate behavior and neural activity using a multi-agent architecture with sparse access to state variables, reminiscent of parallel BG circuitry. More broadly, these results suggest distributed, parallel behavioral control as a possible pre-requisite for the expression of general features of context-dependent behavior.

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BOARD NUMBER: S01-085

MESOLIMBIC DOPAMINE NEURON STIMULATION INFLUENCE'S GOAL DIRECTED ACTION INITIATION AND RESTRAINT.

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

George Jenkins, Hironori Ishii, Emilie Werlen, Juan-Carlos Cerpa, Lauren Burgeno, Mark Walton
University of Oxford, Experimental Psychology, Oxford, United Kingdom

Aims: Previous studies have shown that dopamine release in the nucleus accumbens in response to reward predictive cues differs depending on the action required to obtain upcoming reward. When animals must initiate action, cue-evoked accumbal dopamine follows reward prediction error encoding, but when animals must restrain action to earn reward release is instead suppressed. To determine whether increased release of dopamine in the accumbens causally influences the likelihood of initiating action, we stimulated VTA dopamine neurones whilst animals were engaged in a Go/NoGo task. Methods TH-Cre rats were trained in a Go/NoGo task, where cues indicated whether animals had to either leave a nosepoke and make two lever presses (Go trials) or remain in the nosepoke for 1.7-1.9s (NoGo trials) to receive reward. Following transfection of midbrain dopamine neurons with AAV2-EF1a-ChR2 or AAV2-EF1a-GFP, a 20hz stimulation was delivered either shortly after the cue (50ms after cue) or later in the trial (800ms after cue). Results Stimulation of midbrain dopamine neurones impaired both the ability of animals to restrain action on NoGo trials, and to execute the correct action sequence on Go trials. These effects were only observed in animals expressing ChR2 and were present only when the stimulation was delivered shortly after cue presentation, not later in the trial. Conclusion These experiments indicate that dopamine release is not merely providing a readout of behaviour, but rather acts to shape online behaviour in situations when animals must either initiate or restrain action to obtain reward.

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BOARD NUMBER: S01-086

SELECTIVE ATTENTION AIDS RAPID LEARNING IN COMPLEX ENVIRONMENTS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Hazem Toutounji[1], Tom Merten[2,3], Nico Boehme[2,3], Selina Hermann[2,3], Daniel Durstewitz[4], Florian Bähner[2,3]
[1]University of Nottingham, School Of Psychology, Nottingham, United Kingdom, [2]ZI Mannheim, Rg Behavioral Physiology In Psychiatry, Mannheim, Germany, [3]ZI Mannheim, Department Of Psychiatry And Psychotherapy, Mannheim, Germany, [4]ZI Mannheim, Theoretical Neuroscience, Mannheim, Germany

Cognitive flexibility is the ability to adaptively respond to changes in the environment. Neural processes involved in cognitive flexibility have been identified. However, it is less clear how animals determine the correct rules governing environmental changes and how rule acquisition is neurally implemented. Reinforcement learning (RL) is a powerful theory for understanding how animals choose actions to maximize reward. RL models provide a direct link to neural computations underlying reward-driven behavior. However, they predict learning should be slow and gradual, thus struggling to explain hallmarks of flexible behavior such as rapid rule learning in complex environments. To gain insight into how animals learn in such environments, we trained rats on a novel rule-switching task where correct choices require integrating information from different sensory modalities and memory of past choices. We found that, rather than learning complex, high-dimensional state-action-space mappings, rats follow general, low-dimensional strategies to test environmental features for their relevance to reward. We developed statistical methods to measure strategy-specific attention to task features during choice and learning. We found that rats focus attention on one specific feature at a time, switching attention abruptly to another feature when they accumulate enough evidence that the current strategy is unreliable. Furthermore, we found that an RL model where attention modulates both choice and learning is best at explaining animal behavior. Our findings support the notion that animals work around computational complexity in real-world environments by adapting attentional mechanisms that allow them to reduce task dimensionality, thus providing an explanation for rapid learning.

Pubmed:

33531416: Russo E, Ma T, Spanagel R, Durstewitz D, Toutounji H, Köhr G
Coordinated Prefrontal State Transition Leads Extinction of Reward-Seeking Behaviors.
Extinction learning suppresses conditioned reward responses and is thus fundamental to adapt to changing environmental demands and to control excessive reward seeking. The medial prefrontal cortex (mPFC) monitors and controls conditioned reward responses. Abrupt transitions in mPFC activity anticipate changes in conditioned responses to altered contingencies. It remains, however, unknown whether such transitions are driven by the extinction of old behavioral strategies or by the acquisition of new competing ones. Using multiple single-unit recordings of mPFC in male rats, we studied the relationship between single-unit and population dynamics during extinction learning, using alcohol as a positive reinforcer in an operant conditioning paradigm. To examine the fine temporal relation between neural activity and behavior, we developed a novel behavioral model that allowed us to identify the number, onset, and duration of extinction-learning episodes in the behavior of each animal. We found that single-unit responses to conditioned stimuli changed even under stable experimental conditions and behavior. However, when behavioral responses to task contingencies had to be updated, unit-specific modulations became coordinated across the whole population, pushing the network into a new stable attractor state. Thus, extinction learning is not associated with suppressed mPFC responses to conditioned stimuli, but is anticipated by single-unit coordination into population-wide transitions of the internal state of the animal. The ability to suppress conditioned behaviors when no longer beneficial is fundamental for the survival of any organism. While pharmacological and optogenetic interventions have shown a critical involvement of the mPFC in the suppression of conditioned responses, the neural dynamics underlying such a process are still largely unknown. Combining novel analysis tools to describe behavior, single-neuron response, and population activity, we found that widespread changes in neuronal firing temporally coordinate across the whole mPFC population in anticipation of behavioral extinction. This coordination leads to a global transition in the internal state of the network, driving extinction of conditioned behavior.
J Neurosci, 2021; 41
31433810: Koppe G, Toutounji H, Kirsch P, Lis S, Durstewitz D
Identifying nonlinear dynamical systems via generative recurrent neural networks with applications to fMRI.

A major tenet in theoretical neuroscience is that cognitive and behavioral processes are ultimately implemented in terms of the neural system dynamics. Accordingly, a major aim for the analysis of neurophysiological measurements should lie in the identification of the computational dynamics underlying task processing. Here we advance a state space model (SSM) based on generative piecewise-linear recurrent neural networks (PLRNN) to assess dynamics from neuroimaging data. In contrast to many other nonlinear time series models which have been proposed for reconstructing latent dynamics, our model is easily interpretable in neural terms, amenable to systematic dynamical systems analysis of the resulting set of equations, and can straightforwardly be transformed into an equivalent continuous-time dynamical system. The major contributions of this paper are the introduction of a new observation model suitable for functional magnetic resonance imaging (fMRI) coupled to the latent PLRNN, an efficient stepwise training procedure that forces the latent model to capture the 'true' underlying dynamics rather than just fitting (or predicting) the observations, and of an empirical measure based on the Kullback-Leibler divergence to evaluate from empirical time series how well this goal of approximating the underlying dynamics has been achieved. We validate and illustrate the power of our approach on simulated 'ground-truth' dynamical systems as well as on experimental fMRI time series, and demonstrate that the learnt dynamics harbors task-related nonlinear structure that a linear dynamical model fails to capture. Given that fMRI is one of the most common techniques for measuring brain activity non-invasively in human subjects, this approach may provide a novel step toward analyzing aberrant (nonlinear) dynamics for clinical assessment or neuroscientific research.
PLoS Comput Biol, 2019; 15
30349472: Toutounji H, Durstewitz D
Detecting Multiple Change Points Using Adaptive Regression Splines With Application to Neural Recordings.
Time series, as frequently the case in neuroscience, are rarely stationary, but often exhibit abrupt changes due to attractor transitions or bifurcations in the dynamical systems producing them. A plethora of methods for detecting such in time series statistics have been developed over the years, in addition to test criteria to evaluate their significance. Issues to consider when developing change point analysis methods include computational demands, difficulties arising from either limited amount of data or a large number of covariates, and arriving at statistical tests with sufficient power to detect as many changes as contained in potentially high-dimensional time series. Here, a general method called is developed for detecting multiple change points in the mean of multivariate time series. The method's advantages over alternative approaches are demonstrated through a series of simulation experiments. This is followed by a real data application to neural recordings from rat medial prefrontal cortex during learning. Finally, the method's flexibility to incorporate useful features from state-of-the-art change point detection techniques is discussed, along with potential drawbacks and suggestions to remedy them.
Front Neuroinform, 2018; 12
27783690: Kovac AD, Koall M, Pipa G, Toutounji H
Persistent Memory in Single Node Delay-Coupled Reservoir Computing.
Delays are ubiquitous in biological systems, ranging from genetic regulatory networks and synaptic conductances, to predator/pray population interactions. The evidence is mounting, not only to the presence of delays as physical constraints in signal propagation speed, but also to their functional role in providing dynamical diversity to the systems that comprise them. The latter observation in biological systems inspired the recent development of a computational architecture that harnesses this dynamical diversity, by delay-coupling a single nonlinear element to itself. This architecture is a particular realization of Reservoir Computing, where stimuli are injected into the system in time rather than in space as is the case with classical recurrent neural network realizations. This architecture also exhibits an internal memory which fades in time, an important prerequisite to the functioning of any reservoir computing device. However, fading memory is also a limitation to any computation that requires persistent storage. In order to overcome this limitation, the current work introduces an extended version to the single node Delay-Coupled Reservoir, that is based on trained linear feedback. We show by numerical simulations that adding task-specific linear feedback to the single node Delay-Coupled Reservoir extends the class of solvable tasks to those that require nonfading memory. We demonstrate, through several case studies, the ability of the extended system to carry out complex nonlinear computations that depend on past information, whereas the computational power of the system with fading memory alone quickly deteriorates. Our findings provide the theoretical basis for future physical realizations of a biologically-inspired ultrafast computing device with extended functionality.
PLoS One, 2016; 11
25826022: Toutounji H, Schumacher J, Pipa G
Homeostatic plasticity for single node delay-coupled reservoir computing.
Supplementing a differential equation with delays results in an infinite-dimensional dynamical system. This property provides the basis for a reservoir computing architecture, where the recurrent neural network is replaced by a single nonlinear node, delay-coupled to itself. Instead of the spatial topology of a network, subunits in the delay-coupled reservoir are multiplexed in time along one delay span of the system. The computational power of the reservoir is contingent on this temporal multiplexing. Here, we learn optimal temporal multiplexing by means of a biologically inspired homeostatic plasticity

mechanism. Plasticity acts locally and changes the distances between the subunits along the delay, depending on how responsive these subunits are to the input. After analytically deriving the learning mechanism, we illustrate its role in improving the reservoir's computational power. To this end, we investigate, first, the increase of the reservoir's memory capacity. Second, we predict a NARMA-10 time series, showing that plasticity reduces the normalized root-mean-square error by more than 20%. Third, we discuss plasticity's influence on the reservoir's input-information capacity, the coupling strength between subunits, and the distribution of the readout coefficients.
Neural Comput, 2015; 27

24904403: Toutounji H, Pasemann F
Behavior control in the sensorimotor loop with short-term synaptic dynamics induced by self-regulating neurons.
The behavior and skills of living systems depend on the distributed control provided by specialized and highly recurrent neural networks. Learning and memory in these systems is mediated by a set of adaptation mechanisms, known collectively as neuronal plasticity. Translating principles of recurrent neural control and plasticity to artificial agents has seen major strides, but is usually hampered by the complex interactions between the agent's body and its environment. One of the important standing issues is for the agent to support multiple stable states of behavior, so that its behavioral repertoire matches the requirements imposed by these interactions. The agent also must have the capacity to switch between these states in time scales that are comparable to those by which sensory stimulation varies. Achieving this requires a mechanism of short-term memory that allows the neurocontroller to keep track of the recent history of its input, which finds its biological counterpart in short-term synaptic plasticity. This issue is approached here by deriving synaptic dynamics in recurrent neural networks. Neurons are introduced as self-regulating units with a rich repertoire of dynamics. They exhibit homeostatic properties for certain parameter domains, which result in a set of stable states and the required short-term memory. They can also operate as oscillators, which allow them to surpass the level of activity imposed by their homeostatic operation conditions. Neural systems endowed with the derived synaptic dynamics can be utilized for the neural behavior control of autonomous mobile agents. The resulting behavior depends also on the underlying network structure, which is either engineered or developed by evolutionary techniques. The effectiveness of these self-regulating units is demonstrated by controlling locomotion of a hexapod with 18 degrees of freedom, and obstacle-avoidance of a wheel-driven robot.
Front Neurorobot, 2014; 8

24651447: Toutounji H, Pipa G
Spatiotemporal computations of an excitable and plastic brain: neuronal plasticity leads to noise-robust and noise-constructive computations.
It is a long-established fact that neuronal plasticity occupies the central role in generating neural function and computation. Nevertheless, no unifying account exists of how neurons in a recurrent cortical network learn to compute on temporally and spatially extended stimuli. However, these stimuli constitute the norm, rather than the exception, of the brain's input. Here, we introduce a geometric theory of learning spatiotemporal computations through neuronal plasticity. To that end, we rigorously formulate the problem of neural representations as a relation in space between stimulus-induced neural activity and the asymptotic dynamics of excitable cortical networks. Backed up by computer simulations and numerical analysis, we show that two canonical and widely spread forms of neuronal plasticity, that is, spike-timing-dependent synaptic plasticity and intrinsic plasticity, are both necessary for creating neural representations, such that these computations become realizable. Interestingly, the effects of these forms of plasticity on the emerging neural code relate to properties necessary for both combating and utilizing noise. The neural dynamics also exhibits features of the most likely stimulus in the network's spontaneous activity. These properties of the spatiotemporal neural code resulting from plasticity, having their grounding in nature, further consolidate the biological relevance of our findings.
PLoS Comput Biol, 2014; 10

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BOARD NUMBER: S01-087

CELLULAR DETERMINANTS OF SUBTHALAMIC FUNCTIONAL DIVERSITY

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Michaël Loureiro, Fabrice Chaudun, Nand Mule, Vahid Esmaeili, Anna Ramisch, Sabine Fièvre, Lucile Marion-Poll, Christian Lüscher
University of Geneva, Basic Neurosciences, Geneva, Switzerland

Aims. Deep brain stimulation of the subthalamic nucleus (STN) is used to treat motor symptoms in Parkinsonian patients with motor fluctuations. After prolonged stimulation, undesired side effects (e.g.anhedonia) may appear, which are believed to originate from a non specific stimulation of STN circuits mediating a variety of functions. A comprehensive description of the cellular diversity of the STN is lacking. Here we use complementary approaches to characterize the cellular determinants of STN functions. **Methods.**To determine the spatial organization of projection neurons within the STN, we performed dual retrograde tracings in mice in two established targets. To characterize STN neuronal subtypes, we then used single-nucleus-RNAseq. To study the correlations between neuronal activity and rewarding behaviors, we carried out in vivo recordings coupled with opto-tagging of STN projection neurons in an operant conditioning task where mice had to press a lever to obtain a rewarding solution. **Results.**Anatomical tracings showed that VP-projectors were concentrated ventrally in the STN. Conversely, SNR- and GPE-projector distribution did not show a dorso-ventral gradient. Gene expression analysis revealed 7 STN clusters with specific marker candidates. In vivo recording suggest the existence of at least 3 neuronal clusters based on the waveform and firing parameters. Furthermore, some neurons showed increased activity during rewarding actions. **Conclusion.**Ongoing experiments aim at linking the electrophysiological activity of STN clusters with their gene expression and to further explore their function during motor and rewarding behaviors. Altogether, our results hold promise for a better understanding of the cellular diversity and functional implications of the STN.

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EVALUATION OF A TOUCHSCREEN ADAPTATION OF A RODENT REWARD-INDUCED BIAS ASSAY

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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AIMS: A recent study in an early life adversity model found a specific impairment in reward learning in a novel foraging-based task, independent of changes in reward sensitivity or motivation. In this study, we sought to test if the foraging task could be adapted to run in a touch-screen operant chamber. METHODS: The study used male Lister-hooded rats (n=14). Following initial training to establish the instrumental response, animals were pseudo-randomly presented with two pairs of images within the same session. For each pair of images, one was assigned correct and one incorrect but the value of the reward for the correct response was either high (3 reward pellets) or low (1 reward pellet). The association between the image and reward value was kept constant. Animals were tested over 14–22 sessions until they reached >70% accuracy and were then tested in a choice test protocol where high and low rewarded images were presented together with a randomised reinforcement schedule. RESULTS: Animals showed an increase in accuracy for each pair over time ($F_{7,189}$=10.83, p<0.001) but did not develop a bias towards the higher value image. In the choice test, there was a bias towards the high reward image (t_{13}=2.520, p<0.05). In a subsequent replication study in the same cohort using new images no bias was observed during learning or during the choice test. CONCLUSIONS: Animal's rate of learning in this touchscreen task was much slower than seen in the foraging-based and failed to develop a reliable reward-induced bias.

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RATIONAL INATTENTION IN MICE

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Nikola Grujic[1], Jeroen Brus[2], Denis Burdakov[1], Rafael Polania[2]
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Behaviour exhibited by humans and other organisms is generally inconsistent and biased, and thus is often labelled irrational. However, the origins of this seemingly suboptimal behaviour remain elusive. We developed a behavioural task and normative framework to reveal how organisms should allocate their limited processing resources such that there is an advantage to being imprecise and biased for a given metabolic investment that guarantees maximal utility. We trained mice to perform a novel choice task based on ordinal comparisons of orientation stimuli. Additionally, we introduced trial-to-trial variability in the reliability of sensory stimuli and controlled the prior distribution of the sensory inputs in terms of presented orientations. Mice were trained in three different stimulus-reward association environments. This allowed us to model the allocation of sensory resources across the orientation space in each environment, and check whether it complies with the predictions of rational inattention theory. We found that mice act as rational-inattentive agents by adaptively allocating their sensory resources in a way that maximizes reward consumption in each stimulus-reward association environment. Surprisingly, perception to commonly occurring stimuli was relatively imprecise. However, this apparent statistical fallacy implies “awareness” and efficient adaptation to their neurocognitive limitations. Interestingly, distributional reinforcement learning mechanisms efficiently regulate sensory precision via top-down normalization. Furthermore, we found that pupil size contains information about reward expectancy of orientation stimuli in each sensory-reward association environment. These findings establish a neurobehavioral foundation for how organisms efficiently perceive and adapt to environmental states of the world within the constraints imposed by neurobiology.

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BETA OSCILLATIONS IN THE MONKEY STRIATUM ENCODES REWARD PREDICTION ERROR

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Reward prediction errors (RPEs) reflect the difference between predicted and obtained rewards, and they are a building block of basic forms of reinforcement learning. RPE signals are encoded by the activity of midbrain dopaminergic neurons that innervate the striatum and frontal cortex, suggesting that RPE signals are integrated in cortico-basal ganglia circuits. In the current study, we investigated the participation of the different territories of the striatum in the encoding of RPE. To do so, we recorded local field potentials (LFPs) in the striatum of two rhesus monkeys performing a task involving a choice among options for movement with different reward probabilities. The trial-by-trial evolution of RPE was estimated using a reinforcement learning model fitted on monkeys' choice behavior. We found that changes in beta band oscillations (15-30 Hz) during the outcome period appear consistent with RPE encoding. Moreover, the learning-relevant outcome information contained in beta oscillations increased along a dorsolateral-to-ventromedial gradient. These region-specific changes in LFP activity suggest a relationship between beta oscillations in the striatum and the evaluation of outcome based on reward feedback, highlighting a specific contribution of the ventral striatum to the updating of choice behavior.

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BOARD NUMBER: S01-091

THE VAGUS NERVE SCALES NATURAL AND RECREATIONAL REWARDS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Oriane Onimus[1], Julien Castel[1], Faustine Arrivet[2], Nicolas Heck[3], Serge Luquet[1], Giuseppe Gangarossa[1]
[1]Université de Paris, Unité De Biologie Fonctionnelle Et Adaptative, PARIS, France, [2]Sorbonne Université, Neuroscience Paris Seine, PARIS, France, [3]Sorbonne Université, Neuroscience Paris Seine, Paris, France

Interoception is a fundamental physiological property which allows the brain to be constantly informed about the homeostatic state of peripheral organs. Among a plethora of interoceptive mediators, the vagus nerve represents the main fast neuronal relay, thus ensuring the rapid and dynamic bidirectional communication between the periphery and the brain. Indeed, recent breakthroughs have highlighted the key role of the vagus nerve in governing food reward-associated events and eating disorders. However, the functional underpinnings of this “extended circuit” remain still elusive. Here, we hypothesize that the gut-to-brain vagal axis may serve as an integrative (holistic) lever to gate the hedonic and homeostatic effects of natural (food) and recreational (drugs of abuse) stimuli, thereby playing a permissive role in the development of reward-based dysfunctions such as eating disorders and drug addiction. Using a wide array of approaches (motivated behaviours, *in vivo* Ca^{2+} and dopamine imaging, metabolic efficiency, immunofluorescence, structural neuronal morphology), we have gathered compelling evidence indicating that the vagus nerve plays a pivotal role in scaling the reward processes associated to natural as well as recreational stimuli. In fact, disruption of the vagus nerve led to profound adaptations of the dopamine (DA) system which represents the cornerstone central circuit underlying reward and reinforcement events. In conclusion, our results reveal that the gut-to-brain vagal axis represents an interesting and innovative target which may pave the way to new therapeutic strategies for reward-based disorders.

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CONTEXT-DEPENDENT REWARD AND AVERSIVE MEMORIES IN THE VENTRAL HIPPOCAMPUS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Joana Duarte, Robin Nguyen, Stéphane Ciocchi
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Acquiring and exploiting memories of rewarding and aversive experiences is critical for animal survival. Memory-guided behavior depends on the brain's capability to simultaneously construct salient emotional experiences and internal representations of encountered spatial environments. The ventral hippocampus (vHip) is a high-order cortical area involved in emotional behaviors associated with rewarding and aversive experiences. However, the neuronal dynamics and circuits within the vHip underlying the formation of context-dependent emotional memories remain poorly understood. In this study, using *in vivo* single-unit recordings in freely-moving mice during a contextual reward- and fear conditioning paradigm, we found that neuronal activity in vHip discriminates between emotional contexts with distinct valence. Contextual neuronal activity in vHip did preferentially form in emotion-contingent contexts after learning and a support-vector-machine based classifier was able to predict context identity. We next identified which pre-synaptic input to vHip mediate the formation of emotional contextual memories. To do so, we optogenetically manipulated the locus coeruleus (LC), a brain region known to release noradrenalin and regulate hippocampal plasticity and learning. We observed that suppression of LC activity in vHip during learning impaired contextual reward memory formation, although contextual fear memory was preserved. These findings identify selective neuronal activity patterns in vHip underlying emotional contextual memories and provide evidence that synaptic inputs from the LC to vHip plays a key role in contextual reward memory.

Pubmed:
31454441: Simões-Henriques C, Mateus-Pinheiro M, Gaspar R, Pinheiro H, Mendes Duarte J, Baptista FI, Canas PM, Fontes-Ribeiro CA, Cunha RA, Ambrósio AF, Gomes CA
Microglia cytoarchitecture in the brain of adenosine A receptor knockout mice: Brain region and sex specificities.
Microglia cells exert a critical role in brain development, mainly supported by their immune functions, which predicts an impact on the genesis of psychiatric disorders. In fact, microglia stress during gestation is, for instance, associated with chronic anxiety and cognitive deficits accompanied by long-lasting, region- and sex-specific changes in microglia morphology. We recently reported that the pattern of microglia morphologic plasticity, which is sex-determined, impacts on anxious-like behaviour and cognition. We also reported that the pharmacologic blockade of adenosine A receptors (A R) is able to reshape microglia morphology, in a sex-specific manner and with behavioural sequelae. In order to better understand the role of A R in the sex differentiation of microglia, we now compared their morphology in wild-type and A R knockout male and female C57BL/6 mice in two cardinal brain regions implicated in anxiety-like behaviour and cognition, the prefrontal cortex (PFC) and the dorsal hippocampus (dHIP). We report interregional differences between PFC and dHIP in a sex-specific manner: while males presented more complex microglia in the dHIP, microglia from females had a more complex morphology in the PFC. Surprisingly, the genetic deletion of A R did not alter these sex differences, but promoted the exclusive remodelling (increase in complexity) in PFC microglia from females. These findings further support the existence of a heterogeneous microglial network, distinct between sexes and brain regions, and help characterizing the role of A R in the sex- and brain region-specific morphologic differentiation of microglia.
Eur J Neurosci, 2020; 51
30461068: Duarte JM, Gaspar R, Caetano L, Patrício P, Soares-Cunha C, Mateus-Pinheiro A, Alves ND, Santos AR, Ferreira SG, Sardinha V, Oliveira JF, Fontes-Ribeiro C, Sousa N, Cunha RA, Ambrósio AF, Pinto L, Rodrigues AJ, Gomes CA
Region-specific control of microglia by adenosine A receptors: uncoupling anxiety and associated cognitive deficits in female rats.
Epidemiologic studies have provided compelling evidence that prenatal stress, through excessive maternal glucocorticoids exposure, is associated with psychiatric disorders later in life. We have recently reported that anxiety associated with prenatal exposure to dexamethasone (DEX, a synthetic glucocorticoid) correlates with a gender-specific remodeling of microglia in the medial prefrontal cortex (mPFC), a core brain region in anxiety-related disorders. Gender differences in microglia morphology, the higher prevalence of anxiety in women and the negative impact of anxiety in cognition, led us to specifically

evaluate cognitive behavior and associated circuits (namely mPFC-dorsal hippocampus, dHIP), as well as microglia morphology in female rats prenatally exposed to dexamethasone (in utero DEX, iuDEX). We report that iuDEX impaired recognition memory and deteriorated neuronal synchronization between mPFC and dHIP. These functional deficits are paralleled by microglia hyper-ramification in the dHIP and decreased ramification in the mPFC, showing a heterogeneous remodeling of microglia morphology, both postnatally and at adulthood in different brain regions, that differently affect mood and cognition. The chronic blockade of adenosine A receptors (A R), which are core regulators of microglia morphology and physiology, ameliorated the cognitive deficits, but not the anxiety-like behavior. Notably, A R blockade rectified both microglia morphology in the dHIP and the lack of mPFC-dHIP synchronization, further heralding their role in cognitive function.
Glia, 2019; 67

31028728: Duarte J, Fernandes EC, Kononenko O, Sarkisyan D, Luz LL, Bakalkin G, Safronov BV
Differential suppression of the ipsi- and contralateral nociceptive reflexes in the neonatal rat spinal cord by agonists of μ-, δ- and κ-opioid receptors.
Nociceptive discharges caused by the unilateral tissue damage are processed in the spinal cord by both ipsi- and contralateral neuronal circuits. The mechanisms of the neurotransmitter control of this bilateral excitation spread is poorly understood. Spinally administered opiates are known to suppress nociceptive transmission and nociceptive withdrawal reflexes. Here we investigated whether three major types of opioid receptors are involved in the bilateral control of the spinal nociceptive sensorimotor processing. Effects of the μ-, δ- and κ-opioid receptor agonists on the ipsi- and contralateral nociceptive reflexes were studied by recording slow ventral root potentials in an isolated spinal cord preparation of the new-born rat. Absolute levels of expression of the opioid genes were analyzed by the droplet digital PCR. Ipsi- and contralateral slow ventral root potentials were most strongly suppressed by the μ-opioid receptor agonist DAMGO, by 63% and 85%, followed by the κ-opioid receptor agonist U-50488H, by 44% and 73%, and δ-opioid receptor agonist leucine-enkephalin, by 27% and 49%, respectively. All these agonists suppressed stronger contra- than ipsilateral responses. Naloxone prevented effects of the agonists indicating that they act through opioid receptors, which, as we show, are expressed in the neonatal spinal cord at the levels similar to those in adults. Thus, opioid receptor agonists suppress the segmental nociceptive reflexes. Stronger contralateral effects suggest that the endogenous opioid system regulates sensorimotor processing in the spinal commissural pathways. These effects of opioids may be relevant for treatment of symmetric clinical pain symptoms caused by unilateral tissue injury.
Brain Res, 2019; 1717

28334068: Franquinho F, Nogueira-Rodrigues J, Duarte JM, Esteves SS, Carter-Su C, Monaco AP, Molnár Z, Velayos-Baeza A, Brites P, Sousa MM
The Dyslexia-susceptibility Protein KIAA0319 Inhibits Axon Growth Through Smad2 Signaling.
KIAA0319 is a transmembrane protein associated with dyslexia with a presumed role in neuronal migration. Here we show that KIAA0319 expression is not restricted to the brain but also occurs in sensory and spinal cord neurons, increasing from early postnatal stages to adulthood and being downregulated by injury. This suggested that KIAA0319 participates in functions unrelated to neuronal migration. Supporting this hypothesis, overexpression of KIAA0319 repressed axon growth in hippocampal and dorsal root ganglia neurons; the intracellular domain of KIAA0319 was sufficient to elicit this effect. A similar inhibitory effect was observed in vivo as axon regeneration was impaired after transduction of sensory neurons with KIAA0319. Conversely, the deletion of Kiaa0319 in neurons increased neurite outgrowth in vitro and improved axon regeneration in vivo. At the mechanistic level, KIAA0319 engaged the JAK2-SH2B1 pathway to activate Smad2, which played a central role in KIAA0319-mediated repression of axon growth. In summary, we establish KIAA0319 as a novel player in axon growth and regeneration with the ability to repress the intrinsic growth potential of axons. This study describes a novel regulatory mechanism operating during peripheral nervous system and central nervous system axon growth, and offers novel targets for the development of effective therapies to promote axon regeneration.
Cereb Cortex, 2017; 27

26834566: Rial D, Lemos C, Pinheiro H, Duarte JM, Gonçalves FQ, Real JI, Prediger RD, Gonçalves N, Gomes CA, Canas PM, Agostinho P, Cunha RA
Depression as a Glial-Based Synaptic Dysfunction.
Recent studies combining pharmacological, behavioral, electrophysiological and molecular approaches indicate that depression results from maladaptive neuroplastic processes occurring in defined frontolimbic circuits responsible for emotional processing such as the prefrontal cortex, hippocampus, amygdala and ventral striatum. However, the exact mechanisms controlling synaptic plasticity that are disrupted to trigger depressive conditions have not been elucidated. Since glial cells (astrocytes and microglia) tightly and dynamically interact with synapses, engaging a bi-directional communication critical for the processing of synaptic information, we now revisit the role of glial cells in the etiology of depression focusing on a dysfunction of the "quad-partite" synapse. This interest is supported by the observations that depressive-like conditions are associated with a decreased density and hypofunction of astrocytes and with an increased microglia "activation" in

frontolimbic regions, which is expected to contribute for the synaptic dysfunction present in depression. Furthermore, the traditional culprits of depression (glucocorticoids, biogenic amines, brain-derived neurotrophic factor, BDNF) affect glia functioning, whereas antidepressant treatments (serotonin-selective reuptake inhibitors, SSRIs, electroshocks, deep brain stimulation) recover glia functioning. In this context of a quad-partite synapse, systems modulating glia-synapse bidirectional communication-such as the purinergic neuromodulation system operated by adenosine 5'-triphosphate (ATP) and adenosine-emerge as promising candidates to "re-normalize" synaptic function by combining direct synaptic effects with an ability to also control astrocyte and microglia function. This proposed triple action of purines to control aberrant synaptic function illustrates the rationale to consider the interference with glia dysfunction as a mechanism of action driving the design of future pharmacological tools to manage depression.
Front Cell Neurosci, 2015; 9

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BOARD NUMBER: S01-093

REWARD RANGE NORMALIZATION AS AN EFFICIENT WAY TO BALANCE EXPLORATION AND EXPLOITATION IN REINFORCEMENT LEARNING

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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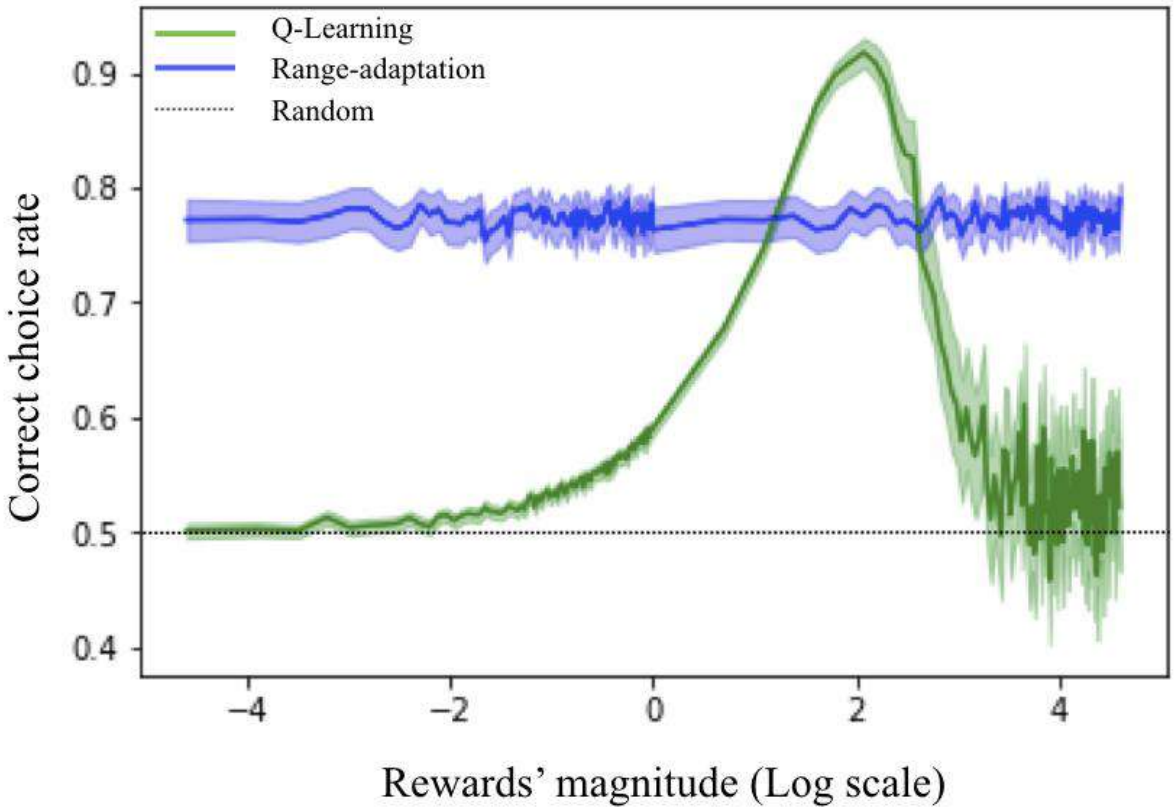
Experimental investigations have suggested that humans perceive received rewards in a context-dependent manner. More precisely, rewards are rescaled as a function of the maximal and minimal values received in the same context, a process referred to as range-adaptation. We here set out to explore what could be the advantage of performing range-adaptation from a computational point of view. We used computational modeling to compare a classical reinforcement learning algorithm (Q-Learning) with a new reinforcement learning algorithm that implements range-adaptation. This revealed that performing range-adaptation allows the algorithm to reach a high performance for rewards of any magnitude; on the contrary, the baseline algorithm reaches a high performance only a certain range of magnitudes. We analytically demonstrate that these results are explained by the incapacity of the baseline model to efficiently address the exploration-exploitation dilemma. Indeed, the optimal value of the parameter that controls the degree of exploration depends on the rewards' magnitude, thus a fixed value cannot be suited for any magnitude. We then show that performing range-adaptation acts as a dynamic update of the exploration parameter, maintaining it close to its optimal value for any magnitude. Finally, we show that these results can be extended to multi-step reinforcement learning tasks, i.e. tasks in which future rewards must be anticipated. We conclude by discussing how these results could contribute to research in artificial intelligence, on top of providing insights as to why humans implement range-

adaptation.


Q-Learning
Range-adaptation
Random
Correct choice rate
0.9
0.8
0.7
0.6
0.5
0.4
−4
−2
0
2
4
Rewards' magnitude (Log scale)

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BOARD NUMBER: S01-094

ALTERED REWARD-MOTIVATION PROCESSING OF THE TALLYHO/JNGJ MOUSE MODEL IN OPERANT LEARNING TASK IS ASSOCIATED WITH INSULIN SIGNALLING MECHANISMS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

Mairead Sullivan[1], Jeffrey Glennon[1], Sarita Dam[2], Charlotte Oomen[2], Ilse Van De Vondervoort[2], Joao Paulo Rodrigues[3]
[1]University College Dublin, Physiology, Dublin, Ireland, [2]RadboundUMC, Cognitive Neuroscience, Nijmegen, Netherlands, [3]Universidade Federal de Juiz de Fora, School Of Medicine, -, Brazil

Behavioural inflexibility is a debilitating feature of numerous psychiatric disorders, impairing ability to change behavioural strategy to match environmental reward. Insulin signalling has been implicated in Obsessive-Compulsive Disorder, with TALLYHO/JngJ mouse models of Type 2 diabetes showing impaired spontaneous alteration and increased anxiety. Here, we assessed extinction learning, the ability to inhibit previously-rewarded behaviour in TALLYHO/JngJ mice and their SWR/J controls. TALLYHO/JngJ mice acquire the task similar to controls (51.77 ± 18.07 vs 50.13 ± 16.37) but extinguish responses more rapidly (27.81 ± 2.30 vs 38.73 ± 2.79), show greater omissions (21.81 ± 0.70 vs 18.38 ± 0.84) and require more time (567.9 ± 3.83 vs 549.6 ± 5.95) for task completion following reward removal. Transcriptomic analysis in cerebellum shows significantly reduced expression in TALLYHO/JngJ of IGF1R (0.41 ± 0.03 vs 0.51 ± 0.03) and KCNQ1 (0.30 ± 0.027 vs 0.41 ± 0.03), two genes implicated in insulin signalling, with a significant positive correlation between expression levels and the extinction response. This suggests a link between reward-motivation processing and insulinergic signalling. Insulin controls not only blood glucose and lipid metabolism but also is involved in inflammation. Ongoing inflammatory proteomics work in blood sera and matching brain regions involved in compulsive and reward-related behaviour (anterior cingulate cortex and nucleus accumbens) is underway to map onto the extinction phenotype.

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CODING AND CONTROL OF GOAL-DIRECTED BEHAVIOR BY AMYGDALA

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Acting to achieve goals depends on the ability to motivate specific behaviors. By definition, instrumental goal-directed actions are oriented toward specific outcomes and are sensitive to variations in outcome value and action-outcome contingency. However, the underlying neuronal mechanisms that encode and maintain such parameters are poorly understood. Here we used calcium imaging and optogenetic manipulations in basolateral amygdala of freely moving mice performing non-cued, self-paced instrumental goal-directed actions to receive and consume rewards. We show that distinct neuronal activity patterns sequentially represent the entire action-consumption behavioral sequence. Whereas action-associated patterns integrate the identity, value and expectancy of pursued goals, consumption-associated patterns reflect the identity and value of experienced outcomes. Thus, the interplay between these patterns allows the maintenance of specific motivational states necessary to adaptively direct behavior toward prospective rewards. Finally, by tracking the neuronal activity of same neurons over a multi-phase behavioral task we show that such motivational states allow the transfer and reinstatement of specific memories.

Pubmed:

34990249: Courtin J, Bitterman Y, Müller S, Hinz J, Hagihara KM, Müller C, Lüthi A
A neuronal mechanism for motivational control of behavior.
Acting to achieve goals depends on the ability to motivate specific behaviors based on their predicted consequences given an individual's internal state. However, the underlying neuronal mechanisms that encode and maintain such specific motivational control of behavior are poorly understood. Here, we used Ca imaging and optogenetic manipulations in the basolateral amygdala of freely moving mice performing noncued, self-paced instrumental goal-directed actions to receive and consume rewards. We found that distinct neuronal activity patterns sequentially represent the entire action-consumption behavioral sequence. Whereas action-associated patterns integrated the identity, value, and expectancy of pursued goals, consumption-associated patterns reflected the identity and value of experienced outcomes. Thus, the interplay between these patterns allows the maintenance of specific motivational states necessary to adaptively direct behavior toward prospective rewards.
Science, 2022; 375
31636447: Krabbe S, Paradiso E, d'Aquin S, Bitterman Y, Courtin J, Xu C, Yonehara K, Markovic M, Müller C, Eichlisberger T, Gründemann J, Ferraguti F, Lüthi A
Adaptive disinhibitory gating by VIP interneurons permits associative learning.
Learning drives behavioral adaptations necessary for survival. While plasticity of excitatory projection neurons during associative learning has been extensively studied, little is known about the contributions of local interneurons. Using fear conditioning as a model for associative learning, we found that behaviorally relevant, salient stimuli cause learning by tapping into a local microcircuit consisting of precisely connected subtypes of inhibitory interneurons. By employing deep-brain calcium imaging and optogenetics, we demonstrate that vasoactive intestinal peptide (VIP)-expressing interneurons in the basolateral amygdala are activated by aversive events and provide a mandatory disinhibitory signal for associative learning. Notably, VIP interneuron responses during learning are strongly modulated by expectations. Our findings indicate that VIP interneurons are a central component of a dynamic circuit motif that mediates adaptive disinhibitory gating to specifically learn about unexpected, salient events, thereby ensuring appropriate behavioral adaptations.
Nat Neurosci, 2019; 22
28117439: Fadok JP, Krabbe S, Markovic M, Courtin J, Xu C, Massi L, Botta P, Bylund K, Müller C, Kovacevic A, Tovote P, Lüthi A
A competitive inhibitory circuit for selection of active and passive fear responses.
When faced with threat, the survival of an organism is contingent upon the selection of appropriate active or passive behavioural responses. Freezing is an evolutionarily conserved passive fear response that has been used extensively to study the neuronal mechanisms of fear and fear conditioning in rodents. However, rodents also exhibit active responses such

as flight under natural conditions. The central amygdala (CEA) is a forebrain structure vital for the acquisition and expression of conditioned fear responses, and the role of specific neuronal sub-populations of the CEA in freezing behaviour is well-established. Whether the CEA is also involved in flight behaviour, and how neuronal circuits for active and passive fear behaviour interact within the CEA, are not yet understood. Here, using in vivo optogenetics and extracellular recordings of identified cell types in a behavioural model in which mice switch between conditioned freezing and flight, we show that active and passive fear responses are mediated by distinct and mutually inhibitory CEA neurons. Cells expressing corticotropin-releasing factor (CRF) mediate conditioned flight, and activation of somatostatin-positive (SOM) neurons initiates passive freezing behaviour. Moreover, we find that the balance between conditioned flight and freezing behaviour is regulated by means of local inhibitory connections between CRF and SOM neurons, indicating that the selection of appropriate behavioural responses to threat is based on competitive interactions between two defined populations of inhibitory neurons, a circuit motif allowing for rapid and flexible action selection.
Nature, 2017; 542
27409809: Dejean C, Courtin J, Karalis N, Chaudun F, Wurtz H, Bienvenu TC, Herry C
Prefrontal neuronal assemblies temporally control fear behaviour.
Precise spike timing through the coordination and synchronization of neuronal assemblies is an efficient and flexible coding mechanism for sensory and cognitive processing. In cortical and subcortical areas, the formation of cell assemblies critically depends on neuronal oscillations, which can precisely control the timing of spiking activity. Whereas this form of coding has been described for sensory processing and spatial learning, its role in encoding emotional behaviour remains unknown. Fear behaviour relies on the activation of distributed structures, among which the dorsal medial prefrontal cortex (dmPFC) is known to be critical for fear memory expression. In the dmPFC, the phasic activation of neurons to threat-predicting cues, a spike-rate coding mechanism, correlates with conditioned fear responses and supports the discrimination between aversive and neutral stimuli. However, this mechanism does not account for freezing observed outside stimuli presentations, and the contribution of a general spike-time coding mechanism for freezing in the dmPFC remains to be established. Here we use a combination of single-unit and local field potential recordings along with optogenetic manipulations to show that, in the dmPFC, expression of conditioned fear is causally related to the organization of neurons into functional assemblies. During fear behaviour, the development of 4 Hz oscillations coincides with the activation of assemblies nested in the ascending phase of the oscillation. The selective optogenetic inhibition of dmPFC neurons during the ascending or descending phases of this oscillation blocks and promotes conditioned fear responses, respectively. These results identify a novel phase-specific coding mechanism, which dynamically regulates the development of dmPFC assemblies to control the precise timing of fear responses.
Nature, 2016; 535
26878674: Karalis N, Dejean C, Chaudun F, Khoder S, Rozeske RR, Wurtz H, Bagur S, Benchenane K, Sirota A, Courtin J, Herry C
4-Hz oscillations synchronize prefrontal-amygdala circuits during fear behavior.
Fear expression relies on the coordinated activity of prefrontal and amygdala circuits, yet the mechanisms allowing long-range network synchronization during fear remain unknown. Using a combination of extracellular recordings, pharmacological and optogenetic manipulations, we found that freezing, a behavioral expression of fear, temporally coincided with the development of sustained, internally generated 4-Hz oscillations in prefrontal-amygdala circuits. 4-Hz oscillations predict freezing onset and offset and synchronize prefrontal-amygdala circuits. Optogenetic induction of prefrontal 4-Hz oscillations coordinates prefrontal-amygdala activity and elicits fear behavior. These results unravel a sustained oscillatory mechanism mediating prefrontal-amygdala coupling during fear behavior.
Nat Neurosci, 2016; 19
24256726: Courtin J, Chaudun F, Rozeske RR, Karalis N, Gonzalez-Campo C, Wurtz H, Abdi A, Baufreton J, Bienvenu TC, Herry C
Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression.
Synchronization of spiking activity in neuronal networks is a fundamental process that enables the precise transmission of information to drive behavioural responses. In cortical areas, synchronization of principal-neuron spiking activity is an effective mechanism for information coding that is regulated by GABA (γ-aminobutyric acid)-ergic interneurons through the generation of neuronal oscillations. Although neuronal synchrony has been demonstrated to be crucial for sensory, motor and cognitive processing, it has not been investigated at the level of defined circuits involved in the control of emotional behaviour. Converging evidence indicates that fear behaviour is regulated by the dorsomedial prefrontal cortex (dmPFC). This control over fear behaviour relies on the activation of specific prefrontal projections to the basolateral complex of the amygdala (BLA), a structure that encodes associative fear memories. However, it remains to be established how the precise temporal control of fear behaviour is achieved at the level of prefrontal circuits. Here we use single-unit recordings and optogenetic manipulations in behaving mice to show that fear expression is causally related to the phasic inhibition of

prefrontal parvalbumin interneurons (PVINs). Inhibition of PVIN activity disinhibits prefrontal projection neurons and synchronizes their firing by resetting local theta oscillations, leading to fear expression. Our results identify two complementary neuronal mechanisms mediated by PVINs that precisely coordinate and enhance the neuronal activity of prefrontal projection neurons to drive fear expression.
Nature, 2014; 505

24091205: Courtin J, Karalis N, Gonzalez-Campo C, Wurtz H, Herry C
Persistence of amygdala gamma oscillations during extinction learning predicts spontaneous fear recovery.
Extinction of auditory fear conditioning induces a temporary inhibition of conditioned fear responses that can spontaneously reappear with the passage of time. Several lines of evidence indicate that extinction learning relies on the recruitment of specific neuronal populations within the basolateral amygdala. In contrast, post-extinction spontaneous fear recovery is thought to result from deficits in the consolidation of extinction memory within prefrontal neuronal circuits. Interestingly, recent data indicates that the strength of gamma oscillations in the basolateral amygdala during auditory fear conditioning correlates with retrieval of conditioned fear responses. In the present manuscript we evaluated the hypothesis that post-extinction spontaneous fear recovery might depend on the maintenance of gamma oscillations within the basolateral amygdala by using single unit and local field potential recordings in behaving mice. Our results indicate that gamma oscillations in the basolateral amygdala were enhanced following fear conditioning, whereas during extinction learning gamma profiles were more heterogeneous despite similar extinction learning rates. Remarkably, variations in the strength of gamma power within the basolateral amygdala between early and late stages of extinction linearly predicted the level of post-extinction spontaneous fear recovery. These data suggest that maintenance of gamma oscillations in the basolateral amygdala during extinction learning is a strong predictive factor of long term spontaneous fear recovery.
Neurobiol Learn Mem, 2014; 113

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BOARD NUMBER: S01-096

REWARD SIGNAL CONTROLS THE SPEED OF EVOLVING POPULATION DYNAMICS IN MOUSE POSTERIOR PARIETAL CORTEX

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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[1]University of Florida, Biomedical Engineering, Gainesville, United States of America, [2]University of Florida, Electrical Eng, Biomedical Eng And Neuroscience, Gainesville, United States of America

When rewards are immediately available, sensory-guided action selection typically occurs with little to no delay. In contrast, memory-guided action selection could be slower when rewards are delayed or unavailable. Individual neurons in association cortex have been shown to exhibit mixed selectivity towards sensory and choice variables, but how reward presence or absence influences the encoding of these variables at the population level remains largely unknown. Here we investigated the role of posterior parietal cortex (PPC) neurons in stimulus categorization and choice selectivity conditioned on reward availability. We trained mice on an auditory categorization task under two contexts in which reward was either immediately available (context 1) or delayed (context 2). We first found that PPC population dynamics encode the reward state (present or absent) independent of stimulus or choice variables. We further found that population dynamics evolve at different speeds in state space depending on whether reward was immediately available (context 1) or delayed (context 2). This difference in speed could not be entirely explained by changes in stimulus only, decision only or stimulus-decision components in the neural activity. Our results suggest a novel mechanism whereby the reward signal controls the mnemonic representation of task variables to maintain economic neural activity states that can be rapidly and reliably readout by other downstream areas at the time of action selection.

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BOARD NUMBER: S01-097

CONVERGENT AND DIVERGENT CIRCUITRY OF CENTRAL AMYGDALA FOR PROCESSING ADDICTIVE AND NATURAL REWARDS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Since few years, it is claimed that drug addiction should be considered as a specific form of learning as it creates changes in neural networks by strengthening or weakening some of neuronal connections - synapses. Similar modifications occur during natural reward learning and it is believed that these are spots in the brain where information- engrams, are encoded. It was deliberated that drugs of abuse hijack these engrams to create extremely durable forms of memories. Thus, in this study we were testing how much similarities shares the initial exposure to addictive substances with natural form of learning. As a mice model of “addictive” learning we chose cocaine intraperitoneal injections, while sucrose self-administration mimicked the natural form of learning. Series of electrophysiology and immunohistochemical experiments were performed on a reward-related brain structure: Central Amygdala with its medial (CeM) and lateral (CeL) parts. Our results indicate that indeed both cocaine IP injection and sugar administration cause rapid (already two hours after the exposure) plastic changes and c-Fos expression in CeM. On the other hand, cocaine additionally triggers plasticity in CeL. Interestingly, electrophysiology experiments unraveled, that neurons in CeL, but not in CeM, diminish their activity upon cocaine exposure. Together, we found common and divergent pathways in central amygdala for natural and addictive rewards processing.

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BOARD NUMBER: S01-098

REDUCED CONTEXT LEARNING IN OLDER ADULTS

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Aging is associated with increased susceptibility to fraud and impairments in financial decision making. To be an effective financial decision maker, in addition to learning how to gain reward, one must also learn how to avoid losses. We use behavioral and computational methods to investigate the computational mechanisms underlying reward and punishment learning in older adults and to study the effect of aging to the ability to correctly represent the choice context. The results of our experiments indicate that, over time, younger adults learn to adjust based on context such that in loss contexts, having a no loss outcome triggers reinforcement-learning processes that allow them to learn as effectively about avoiding losses as gaining rewards. In contrast, our behavioral findings indicate that older adults show selective impairments in learning to avoid losses. This age-related impairment could either be due to impairments in the ability to learn about the decision context (i.e. impaired context learning) or to a more general 'positivity effect' in which they attend relatively less to negative than positive outcomes. We develop and test computational models to identify the contribution of different types of learning mechanisms to these age differences. In particular, we compare the degree to which impairments in learning about the context versus a bias to attend more to positive than negative feedback can account for older adults' learning patterns.

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BOARD NUMBER: S01-099

ELECTROENCEPHALOGRAPHIC CORRELATES OF CONTINUOUS FEEDBACK PROCESSING

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Feedback processing is commonly studied by analyzing the brain's response to discrete events. However, the neural correlates of continuous feedback processing are less understood. Recent animal work suggests that moment-to-moment changes in reward are tracked by slowly ramping midbrain dopaminergic activity. Our goal was to identify a candidate electroencephalographic (EEG) measure of continuous reward tracking in humans. Twenty participants completed a stimulus-response learning task in which reward increased or decreased gradually. Continuous reward elicited EEG activity consistent with a component of the event-related potential (ERP) previously linked to slowly ramping dopaminergic activity. Importantly, reward-related activity depended on outcome expectancy. Consistent with a reward prediction error, activity for expected continuous rewards was reduced compared to activity for unexpected continuous rewards. These results demonstrate the possibility of using human scalp-recorded potentials to track continuous feedback processing.

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BOARD NUMBER: S01-100

ACUPUNCTURE DECREASES BRAIN TEMPERATURE INDUCED BY METHAMPHETAMINE VIA RMTG

POSTER SESSION 01 - SECTION: REWARD PROCESSING AND REINFORCEMENT LEARNING

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Aims: Methamphetamine (METH) is a representative psychostimulant that has been abused worldwide. The reinforcing effects of METH is known to stronger than any other abused drug due to its neuronal action to the dopamine release in the nucleus accumbens (NAc). The present study examined if acupuncture could suppress METH-induced increase of brain temperature and a possible neuronal mechanism was also investigated. **Methods:** Male SD rats were anesthetized with sodium pentobarbital (50 mg/kg) and placed at a stereotaxic apparatus. Rats were subjected for the implantation of thermometer in the NAc of the mesolimbic system. Temperature was recorded for 60 minutes. Acupuncture treatments at each acupoint bilaterally immediately after METH infusion. For the electronic lesion, electrodes were inserted into each target area. **Results:** Acute administration of MTEH increased the temperature in the NAc of mesolimbic system. However, acupuncture inhibited this increase of brain temperature when stimulated at HT7, but not at TE9. In addition, this inhibitory effect of acupuncture at HT7 disappeared when the RMTg was blocked by electronic lesion. But other areas couldn't block HT7 effect. **Conclusion:** Acupuncture at HT7 inhibited the increase of brain temperature in the NAc by MTEH and this inhibition of acupuncture was blocked by the lesion of the RMTg. Results of the present study suggest that acupuncture at HT7 can be a useful therapy for the MTEH addiction. **Key words:** Acupuncture, HT7, Methamphetamine, RMTg, NAc, Brain temperature.

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BOARD NUMBER: S01-101

PEERS — AN OPEN SCIENCE "PLATFORM FOR THE EXCHANGE OF EXPERIMENTAL RESEARCH STANDARDS" IN NEUROSCIENCE AND BIOMEDICAL RESEARCH

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Pavlina Pavlidi[1], Annesha Sil[2], Nikolaos Kokras[1,3], Chantelle Ferland-Beckham4[4], Arnoud Herremans[5], Konstantinos Karantzalos[6], Martien Kas[7], Kostis Pristouris[6], Gernot Riedel[2], Christoph Emmerich[8], Christina Dalla[1]
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Aims Preclinical research has become progressively complex due to diverse and/or conflicting reported results, generating a reproducibility crisis in the field. Without knowing, scientists may dismiss critical factors that influence the planning and outcome of a study. To address this problem, "PEERS", an open-access online platform, was constructed to advise scientists on which experimental factors and variables are most likely to affect the outcome of a test or model and must be considered during design, execution, and reporting stages. Methods "PEERS" was constructed by identifying commonly utilised *in vivo* and *in vitro* protocols in neuroscience followed by the generation of an extensive list of factors deemed critical for their outcome, based on published literature. Furthermore, with "PEERS" we developed a structured and transparent system for rating the strength of evidence related to each identified factor and its relevance for a specific method/model. Results So far, certain protocols were chosen for the working prototype as a proof-of-concept. Here, we describe the Open Field paradigm in rodents and demonstrate the selection of factors specific to the experimental setup and details of the rating system. Lastly, we provide evidence on how "PEERS" supports a community-driven approach to grade evidence and add protocols to the platform. Conclusions Collectively, by aiding scientists to search for specific factors relevant to their experiments constructively and to report results in a standardised and adequate manner, "PEERS" will serve as a collaborative exchange and analysis tool to elevate data robustness and validity, as well as reproducibility of preclinical research.

Pubmed:
34990380: Pavlidi P, Megalokonomou A, Sofron A, Kokras N, Dalla C
[Pharmacology of ketamine and esketamine as rapid-acting antidepressants].
The lack of utter efficacy and fast action of commonly used antidepressants that selectively target the monoaminergic neurotransmission has led to the exploration of ketamine's actions. Ketamine's antidepressant effect was firstly described in 1973 and nowadays its therapeutic value as a fast- and long- lasting antidepressant has been extensively established. Ketamine is an antagonist of the N-Methyl-D-aspartate receptor (NMDAR) and its main mechanism of action via NMDAR inhibition expressed in GABAergic (gamma-Aminobutyric acid, GABA) interneurons may be relayed to its antidepressant effects. This review aims to describe the pharmacokinetic and pharmacodynamic profile of ketamine when used for treatment-resistant depression. Moreover, ketamine is a racemic mixture consisting of two enantiomers, R- and S- ketamine. We describe the pharmacology of esketamine, along with the guidelines for effective and safe intranasal administration of esketamine. Lastly, this review presents sex differences in preclinical and clinical studies of ketamine and esketamine administration.
Psychiatriki, 2021; 32
34744655: Sil A, Bespalov A, Dalla C, Ferland-Beckham C, Herremans A, Karantzalos K, Kas MJ, Kokras N, Parnham MJ, Pavlidi P, Pristouris K, Steckler T, Riedel G, Emmerich CH
PEERS - An Open Science "Platform for the Exchange of Experimental Research Standards" in Biomedicine.
Laboratory workflows and preclinical models have become increasingly diverse and complex. Confronted with the dilemma of a multitude of information with ambiguous relevance for their specific experiments, scientists run the risk of overlooking critical factors that can influence the planning, conduct and results of studies and that should have been considered . To

address this problem, we developed "PEERS" (Platform for the Exchange of Experimental Research Standards), an open-access online platform that is built to aid scientists in determining which experimental factors and variables are most likely to affect the outcome of a specific test, model or assay and therefore ought to be considered during the design, execution and reporting stages. The PEERS database is categorized into and experiments and provides lists of factors derived from scientific literature that have been deemed critical for experimentation. The platform is based on a structured and transparent system for rating the strength of evidence related to each identified factor and its relevance for a specific method/model. In this context, the rating procedure will not solely be limited to the PEERS working group but will also allow for a community-based grading of evidence. We here describe a working prototype using the Open Field paradigm in rodents and present the selection of factors specific to each experimental setup and the rating system. PEERS not only offers users the possibility to search for information to facilitate experimental rigor, but also draws on the engagement of the scientific community to actively expand the information contained within the platform. Collectively, by helping scientists search for specific factors relevant to their experiments, and to share experimental knowledge in a standardized manner, PEERS will serve as a collaborative exchange and analysis tool to enhance data validity and robustness as well as the reproducibility of preclinical research. PEERS offers a vetted, independent tool by which to judge the quality of information available on a certain test or model, identifies knowledge gaps and provides guidance on the key methodological considerations that should be prioritized to ensure that preclinical research is conducted to the highest standards and best practice.
Front Behav Neurosci, 2021; 15

33676942: Pavlidi P, Kokras N, Dalla C
Antidepressants' effects on testosterone and estrogens: What do we know?
Various antidepressants are commonly used to treat depression and anxiety disorders, and sex differences have been identified in their efficacy and side effects. Steroids, such as estrogens and testosterone, both in the periphery and locally in the brain, are regarded as important modulators of these sex differences. This review presents published data from preclinical and clinical studies that measure testosterone and estrogen level changes during and/or after acute or chronic administration of different antidepressants. The majority of studies show an interaction between sex hormones and antidepressants on sexual function and behavior, or in depressive symptom alleviation. However, most of the studies omit to investigate antidepressants' effects on circulating levels of gonadal hormones. From data reviewed herein, it is evident that most antidepressants can influence testosterone and estrogen levels. Still, the evidence is conflicting with some studies showing an increase, others decrease or no effect. Most studies are conducted in male animals or humans, underscoring the importance of considering sex as an important variable in such investigations, especially as depression and anxiety disorders are more common in women than men. Therefore, research is needed to elucidate the extent to which antidepressants can influence both peripheral and brain levels of testosterone and estrogens, in males and females, and whether this impacts the effectiveness or side effects of antidepressants.
Eur J Pharmacol, 2021; 899

33610083: Doostdar N, Airey J, Radulescu CI, Melgosa-Ecenarro L, Zabouri N, Pavlidi P, Kopanitsa M, Saito T, Saido T, Barnes SJ
Multi-scale network imaging in a mouse model of amyloidosis.
The adult neocortex is not hard-wired but instead retains the capacity to reorganise across multiple spatial scales long into adulthood. Plastic reorganisation occurs at the level of mesoscopic sensory maps, functional neuronal assemblies and synaptic ensembles and is thought to be a critical feature of neuronal network function. Here, we describe a series of approaches that use calcium imaging to measure network reorganisation across multiple spatial scales in vivo. At the mesoscopic level, we demonstrate that sensory activity can be measured in animals undergoing longitudinal behavioural assessment involving automated touchscreen tasks. At the cellular level, we show that network dynamics can be longitudinally measured at both stable and transient functional assemblies. At the level of single synapses, we show that functional subcellular calcium imaging approaches can be used to measure synaptic ensembles of dendritic spines in vivo. Finally, we demonstrate that all three levels of imaging can be spatially related to local pathology in a preclinical rodent model of amyloidosis. We propose that multi-scale in vivo calcium imaging can be used to measure parallel plasticity processes operating across multiple spatial scales in both the healthy brain and preclinical models of disease.
Cell Calcium, 2021; 95

32372528: Kopanitsa MV, Lehtimäki KK, Forsman M, Suhonen A, Koponen J, Piiponniemi TO, Kärkkäinen AM, Pavlidi P, Shatillo A, Sweeney PJ, Merenlender-Wagner A, Kaye J, Orbach A, Nurmi A
Cognitive disturbances in the cuprizone model of multiple sclerosis.
Cognitive problems frequently accompany neurological manifestations of multiple sclerosis (MS). However, during screening of preclinical candidates, assessments of behaviour in mouse models of MS typically focus on locomotor activity. In the present study, we analysed cognitive behaviour of 9 to 10-week-old female C57Bl/6J mice orally administered with the toxin cuprizone that induces demyelination, a characteristic feature of MS. Animals received 400 mg/kg cuprizone daily for 2 or

4 weeks, and their performance was compared with that of vehicle-treated mice. Cuprizone-treated animals showed multiple deficits in short touchscreen-based operant tasks: they responded more slowly to visual stimuli, rewards and made more errors in a simple rule-learning task. In contextual/cued fear conditioning experiments, cuprizone-treated mice showed significantly lower levels of contextual freezing than vehicle-treated mice. Diffusion tensor imaging showed treatment-dependent changes in fractional anisotropy as well as in axial and mean diffusivities in different white matter areas. Lower values of fractional anisotropy and axial diffusivity in cuprizone-treated mice indicated developing demyelination and/or axonal damage. Several diffusion tensor imaging measurements correlated with learning parameters. Our results show that translational touchscreen operant tests and fear conditioning paradigms can reliably detect cognitive consequences of cuprizone treatment. The suggested experimental approach enables screening novel MS drug candidates in longitudinal experiments for their ability to improve pathological changes in brain structure and reverse cognitive deficits.
Genes Brain Behav, 2021; 20

28648717: van den Boom BJG, Pavlidi P, Wolf CJH, Mooij AH, Willuhn I
Automated classification of self-grooming in mice using open-source software.
Manual analysis of behavior is labor intensive and subject to inter-rater variability. Although considerable progress in automation of analysis has been made, complex behavior such as grooming still lacks satisfactory automated quantification.
J Neurosci Methods, 2017; 289

FENS Forum 2022
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BOARD NUMBER: S01-102

SMARTKAGE: A FULLY-AUTOMATED SYSTEM FOR LIFE-LONG CONTINUOUS PHENOTYPING OF MOUSE COGNITION AND BEHAVIOUR

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Hinze Ho[1], Nejc Kejzar[2], Bart De Strooper[3,4,5], Marius Bauza[6], Julija Krupic[7]
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Comprehensive and ethologically-relevant cognitive and behavioural phenotyping in rodent experiments is essential to deciphering the neural basis of animal cognition. Existing tests are often labour-intensive, time-consuming and lack specificity in their readouts. Furthermore, the results of these tests usually compare effects between groups and cannot distinguish differences between individual animals. To address these shortcomings, we developed a novel fully automated AI-based mouse phenotyping system ('smartKage'). Our system integrates T-maze spatial alternation, novel-object recognition (NOR) and object-in-place recognition (OPR) cognitive tests that can be run long-term (>12 months) continuously and in parallel without any interference from the Experimenter in an animal's home-cage environment. In addition to cognitive assessment, the smartKage measures other behavioural outcomes such as sleep and activity patterns, exercise and food and water consumption. In line with the results reported in the conventional tests, we showed that T-maze performance declines with inter-trial interval and the spatial working-memory spans up to 10 min. Furthermore, the object exploration time increases with introduction of a 'novel object' or with changing location of a familiar object. Importantly, in double-blind experiments we showed that using the smartKage in combination with a brief testing on a standard T-maze we were able to separate hippocampal-lesioned, entorhinal-lesioned and sham controls with perfect accuracy on the individual animal basis, which has never been previously achieved. Finally, we demonstrate the utility of the smartKage for translational applications by conducting cognitive and behavioural phenotyping in popular Alzheimer's disease mouse models (APP^{NL-G-F} and Tau^{PS19}).

Pubmed:
25981962: Cummings DM, Liu W, Portelius E, Bayram S, Yasvoina M, Ho SH, Smits H, Ali SS, Steinberg R, Pegasiou CM, James OT, Matarin M, Richardson JC, Zetterberg H, Blennow K, Hardy JA, Salih DA, Edwards FA
First effects of rising amyloid-β in transgenic mouse brain: synaptic transmission and gene expression.
Detecting and treating Alzheimer's disease, before cognitive deficits occur, has become the health challenge of our time. The earliest known event in Alzheimer's disease is rising amyloid-β. Previous studies have suggested that effects on synaptic transmission may precede plaque deposition. Here we report how relative levels of different soluble amyloid-β peptides in hippocampus, preceding plaque deposition, relate to synaptic and genomic changes. Immunoprecipitation-mass spectrometry was used to measure the early rise of different amyloid-β peptides in a mouse model of increasing amyloid-β ('TASTPM', transgenic for familial Alzheimer's disease genes APP/PSEN1). In the third postnatal week, several amyloid-β peptides were above the limit of detection, including amyloid-β40, amyloid-β38 and amyloid-β42 with an intensity ratio of 6:3:2, respectively. By 2 months amyloid-β levels had only increased by 50% and although the ratio of the different peptides remained constant, the first changes in synaptic currents, compared to wild-type mice could be detected with patch-clamp recordings. Between 2 and 4 months old, levels of amyloid-β40 rose by ~7-fold, but amyloid-β42 rose by 25-fold, increasing the amyloid-β42:amyloid-β40 ratio to 1:1. Only at 4 months did plaque deposition become detectable and only in some mice; however, synaptic changes were evident in all hippocampal fields. These changes included increased glutamate release probability ($P < 0.001$, n = 7-9; consistent with the proposed physiological effect of amyloid-β) and loss of spontaneous action potential-mediated activity in the cornu ammonis 1 (CA1) and dentate gyrus regions of the hippocampus ($P < 0.001$, n = 7). Hence synaptic changes occur when the amyloid-β levels and amyloid-β42:amyloid-β40 ratio are still low compared to those necessary for plaque deposition. Genome-wide microarray analysis revealed changes in gene expression at 2-4 months including synaptic genes being strongly affected but often showing significant changes only by 4 months. We thus

demonstrate that, in a mouse model of rising amyloid-β, the initial deposition of plaques does not occur until several months after the first amyloid-β becomes detectable but coincides with a rapid acceleration in the rise of amyloid-β levels and the amyloid-β42:amyloid-β40 ratio. Prior to acceleration, however, there is already a pronounced synaptic dysfunction, reflected as changes in synaptic transmission and altered gene expression, indicating that restoring synaptic function early in the disease progression may represent the earliest possible target for intervention in the onset of Alzheimer's disease.
Brain, 2015; 138
28334103: Cummings DM, Benway TA, Ho H, Tedoldi A, Fernandes Freitas MM, Shahab L, Murray CE, Richard-Loendt A, Brandner S, Lashley T, Salih DA, Edwards FA
Neuronal and Peripheral Pentraxins Modify Glutamate Release and may Interact in Blood-Brain Barrier Failure.
Neuronal pentraxin 1 (NPTX1) has been implicated in Alzheimer's disease, being present in and around dystrophic neurons in plaques, affecting glutamatergic transmission postsynaptically and mediating effects of amyloidβ. Here, we confirm the presence of NPTX1 around plaques in postmortem Alzheimer's disease brain and report that acutely applied human NPTX1 increases paired-pulse ratio at mouse CA3-CA1 hippocampal synapses, indicating a decrease in glutamate release. In contrast, chronic exposure to NPTX1, NPTX2, or NPTX receptor decreases paired-pulse ratio, mimicking some of the earliest changes in mice expressing familial Alzheimer's disease genes. The peripheral pentraxin, serum amyloid P component (SAP), causes similar synaptic effects to NPTX1. The presence of SAP on amyloid plaques in Alzheimer's disease confirms that it can enter the brain. We show that SAP and neuronal pentraxins can interact and that SAP can enter the brain if the blood-brain barrier is compromised, suggesting that peripheral pentraxins could affect central synaptic transmission via this interaction, especially in the event of blood-brain barrier breakdown.
Cereb Cortex, 2017; 27
26966189: Herguedas B, García-Nafría J, Cais O, Fernández-Leiro R, Krieger J, Ho H, Greger IH
Structure and organization of heteromeric AMPA-type glutamate receptors.
AMPA-type glutamate receptors (AMPARs), which are central mediators of rapid neurotransmission and synaptic plasticity, predominantly exist as heteromers of the subunits GluA1 to GluA4. Here we report the first AMPAR heteromer structures, which deviate substantially from existing GluA2 homomer structures. Crystal structures of the GluA2/3 and GluA2/4 N-terminal domains reveal a novel compact conformation with an alternating arrangement of the four subunits around a central axis. This organization is confirmed by cysteine cross-linking in full-length receptors, and it permitted us to determine the structure of an intact GluA2/3 receptor by cryogenic electron microscopy. Two models in the ligand-free state, at resolutions of 8.25 and 10.3 angstroms, exhibit substantial vertical compression and close associations between domain layers, reminiscent of N-methyl-D-aspartate receptors. Model 1 resembles a resting state and model 2 a desensitized state, thus providing snapshots of gating transitions in the nominal absence of ligand. Our data reveal organizational features of heteromeric AMPARs and provide a framework to decipher AMPAR architecture and signaling.
Science, 2016; 352
30872532: Herguedas B, Watson JF, Ho H, Cais O, García-Nafría J, Greger IH
Architecture of the heteromeric GluA1/2 AMPA receptor in complex with the auxiliary subunit TARP γ8.
AMPA-type glutamate receptors (AMPARs) mediate excitatory neurotransmission and are central regulators of synaptic plasticity, a molecular mechanism underlying learning and memory. Although AMPARs act predominantly as heteromers, structural studies have focused on homomeric assemblies. Here, we present a cryo-electron microscopy structure of the heteromeric GluA1/2 receptor associated with two transmembrane AMPAR regulatory protein (TARP) γ8 auxiliary subunits, the principal AMPAR complex at hippocampal synapses. Within the receptor, the core subunits arrange to give the GluA2 subunit dominant control of gating. This structure reveals the geometry of the Q/R site that controls calcium flux, suggests association of TARP-stabilized lipids, and demonstrates that the extracellular loop of γ8 modulates gating by selectively interacting with the GluA2 ligand-binding domain. Collectively, this structure provides a blueprint for deciphering the signal transduction mechanisms of synaptic AMPARs.
Science, 2019; 364
34426577: Watson JF, Pinggera A, Ho H, Greger IH
AMPA receptor anchoring at CA1 synapses is determined by N-terminal domain and TARP γ8 interactions.
AMPA receptor (AMPAR) abundance and positioning at excitatory synapses regulates the strength of transmission. Changes in AMPAR localisation can enact synaptic plasticity, allowing long-term information storage, and is therefore tightly controlled. Multiple mechanisms regulating AMPAR synaptic anchoring have been described, but with limited coherence or comparison between reports, our understanding of this process is unclear. Here, combining synaptic recordings from mouse hippocampal slices and super-resolution imaging in dissociated cultures, we compare the contributions of three AMPAR interaction domains controlling transmission at hippocampal CA1 synapses. We show that the AMPAR C-termini play only a modulatory role, whereas the extracellular N-terminal domain (NTD) and PDZ interactions of the auxiliary subunit TARP γ8 are both crucial, and each is sufficient to maintain transmission. Our data support a model in which γ8 accumulates AMPARs at the

postsynaptic density, where the NTD further tunes their positioning. This interplay between cytosolic (TARP γ8) and synaptic cleft (NTD) interactions provides versatility to regulate synaptic transmission and plasticity.
Nat Commun, 2021; 12
28290985: Watson JF, Ho H, Greger IH
Synaptic transmission and plasticity require AMPA receptor anchoring via its N-terminal domain.
AMPA-type glutamate receptors (AMPARs) mediate fast excitatory neurotransmission and are selectively recruited during activity-dependent plasticity to increase synaptic strength. A prerequisite for faithful signal transmission is the positioning and clustering of AMPARs at postsynaptic sites. The mechanisms underlying this positioning have largely been ascribed to the receptor cytoplasmic C-termini and to AMPAR-associated auxiliary subunits, both interacting with the postsynaptic scaffold. Here, using mouse organotypic hippocampal slices, we show that the extracellular AMPAR N-terminal domain (NTD), which projects midway into the synaptic cleft, plays a fundamental role in this process. This highly sequence-diverse domain mediates synaptic anchoring in a subunit-selective manner. Receptors lacking the NTD exhibit increased mobility in synapses, depress synaptic transmission and are unable to sustain long-term potentiation (LTP). Thus, synaptic transmission and the expression of LTP are dependent upon an AMPAR anchoring mechanism that is driven by the NTD.
Elife, 2017; 6
32569783: Ho H, Fowle A, Coetzee M, Greger IH, Watson JF
An inhalation anaesthesia approach for neonatal mice allowing streamlined stereotactic injection in the brain.
Investigating brain function requires tools and techniques to visualise, modify and manipulate neuronal tissue. One powerful and popular method is intracerebral injection of customised viruses, allowing expression of exogenous transgenes. This technique is a standard procedure for adult mice, and is used by laboratories worldwide. Use of neonatal animals in scientific research allows investigation of developing tissues and enables long-term study of cell populations. However, procedures on neonatal mice are more challenging, due to the lack of reliable methods and apparatus for anaesthesia of these animals.
J Neurosci Methods, 2020; 342
35136046: Herguedas B, Kohegyi BK, Dohrke JN, Watson JF, Zhang D, Ho H, Shaikh SA, Lape R, Krieger JM, Greger IH
Mechanisms underlying TARP modulation of the GluA1/2-γ8 AMPA receptor.
AMPA-type glutamate receptors (AMPARs) mediate rapid signal transmission at excitatory synapses in the brain. Glutamate binding to the receptor's ligand-binding domains (LBDs) leads to ion channel activation and desensitization. Gating kinetics shape synaptic transmission and are strongly modulated by transmembrane AMPAR regulatory proteins (TARPs) through currently incompletely resolved mechanisms. Here, electron cryo-microscopy structures of the GluA1/2 TARP-γ8 complex, in both open and desensitized states (at 3.5 Å), reveal state-selective engagement of the LBDs by the large TARP-γ8 loop ('β1'), elucidating how this TARP stabilizes specific gating states. We further show how TARPs alter channel rectification, by interacting with the pore helix of the selectivity filter. Lastly, we reveal that the Q/R-editing site couples the channel constriction at the filter entrance to the gate, and forms the major cation binding site in the conduction path. Our results provide a mechanistic framework of how TARPs modulate AMPAR gating and conductance.
Nat Commun, 2022; 13

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BOARD NUMBER: S01-103

CLOSED-LOOP SYSTEM FOR REAL-TIME COMPULSIVE BEHAVIOR DETECTION AND WIRELESS OPTOGENETIC STIMULATION IN OBSESSIVE-COMPULSIVE DISORDER MOUSE MODEL

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Patricia Silva[1,2,3], Patricia Monteiro[2,3], Luis Jacinto[2,3]
[1]Center for Microelectromechanical Systems (CMEMS), Department Of Industrial Electronics, Guimarães, Portugal, [2]University of Minho, Life And Health Sciences Research Institute (icvs) / School Of Medicine, Braga, Portugal, [3]PT Government Associate Laboratory, Icvs/3b's, Braga/Guimarães, Portugal

Obsessive-Compulsive Disorder (OCD) is a prevalent neuropsychiatric disorder characterized by recurrent intrusive thoughts (obsessions) that can lead to repetitive behaviors (compulsions), which can cause significant disruption of daily activities. To simultaneously study the neuronal circuits involved in OCD and test novel therapies, it is critical to develop methodologies that can automatically detect and/or predict compulsive behavior and allow effective modulation of relevant brain circuits with temporal precision. Thus, we developed a closed-loop system that combines computer vision and machine learning approaches for effective real-time detection of compulsive/repetitive behaviors, which are coupled with wirelessly controlled optogenetic brain stimulation in the *Sapap3* KO mouse model of OCD. Accurate online detection of repetitive behavioral motifs, such as face grooming, was achieved by combining DeepLabCut for real-time animal body part tracking with supervised extraction of kinematic behavioral features and classification of behavioral states with Support Vector Machines (SVM). To reduce/correct body part tracking outlier labels that can skew behavioral classification, different correction and smoothing strategies including average, median and Kalman filters were also tested. Online detection of repetitive behavior triggers a small wireless optical brain stimulator that delivers light to the brain for low-latency optogenetic stimulation. The brain stimulator consists of an implanted microscale inorganic lightemitting diode (μ-ILED) and battery-powered external electronics for Bluetooth Low Energy communication in a lightweight PCB. This new closed-loop system allows a direct assessment of the impact of brain circuit manipulations in compulsive behavior, contributing to the elucidation of the brain circuits involved in OCD and novel therapies developments.

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BOARD NUMBER: S01-104

BLUEBERRY: PROVIDING WIRELESS OPTOGENETIC FEEDBACK IN FREELY MOVING ANIMALS BASED ON REAL-TIME BEHAVIORAL TRACKING

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

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University of Geneva, Department Of Basic Neuroscience, Geneva, Switzerland

Optogenetics has been extensively used by neuroscientists in a quest to manipulate neuronal activity in different brain areas and study their causal link to behavior. Applications widely vary from head-fixed settings, where stable optical access to the brain is necessary, to freely moving scenarios where maintaining natural sensory-motor variables are crucial. Thanks to recent technological advances in wireless communication, optogenetic stimulation devices can be controlled remotely, allowing to extend freely moving studies to multi-animal setups or complex three dimensional environments. However, the relatively high cost and the lack of open source systems has placed constraints on the current wireless optogenetic devices. Here we present the BlueBerry, an open source, low cost (30$) multi-channel optogenetic device controlled and programmed remotely using Bluetooth Low Energy (BLE) protocol. The light and compact design (1.4gr - 11 x 15 x 6 mm) of the BlueBerry makes it an ideal candidate for real-time optogenetic application in freely roaming small animals such as mice or small primates. We illustrate the capability of the BlueBerry system combined with other open source frameworks such as EthoLoop (www.etholoop.org) for freely moving mice in two different experimental settings: 1) stimulation of the ventral tegmental area (VTA) to reinforce execution of specific type of behavior repeatedly in naturalistic 3D environments and 2) stimulation of somatosensory cortex in a “infinity-Y-maze” solving task where the mouse has to transform the artificial sensory feedbacks into navigation decisions (left or right) at every intersection.

Pubmed:
32994566: Nourizonoz A, Zimmermann R, Ho CLA, Pellat S, Ormen Y, Prévost-Solié C, Reymond G, Pifferi F, Aujard F, Herrel A, Huber D
EthoLoop: automated closed-loop neuroethology in naturalistic environments.
Accurate tracking and analysis of animal behavior is crucial for modern systems neuroscience. However, following freely moving animals in naturalistic, three-dimensional (3D) or nocturnal environments remains a major challenge. Here, we present EthoLoop, a framework for studying the neuroethology of freely roaming animals. Combining real-time optical tracking and behavioral analysis with remote-controlled stimulus-reward boxes, this system allows direct interactions with animals in their habitat. EthoLoop continuously provides close-up views of the tracked individuals and thus allows high-resolution behavioral analysis using deep-learning methods. The behaviors detected on the fly can be automatically reinforced either by classical conditioning or by optogenetic stimulation via wirelessly controlled portable devices. Finally, by combining 3D tracking with wireless neurophysiology we demonstrate the existence of place-cell-like activity in the hippocampus of freely moving primates. Taken together, we show that the EthoLoop framework enables interactive, well-controlled and reproducible neuroethological studies in large-field naturalistic settings.
Nat Methods, 2020; 17
34504074: Prsa M, Kilicel D, Nourizonoz A, Lee KS, Huber D
A common computational principle for vibrotactile pitch perception in mouse and human.
We live surrounded by vibrations generated by moving objects. These oscillatory stimuli propagate through solid substrates, are sensed by mechanoreceptors in our body and give rise to perceptual attributes such as vibrotactile pitch (i.e. the perception of how high or low a vibration's frequency is). Here, we establish a mechanistic relationship between vibrotactile pitch perception and the physical properties of vibrations using behavioral tasks, in which vibratory stimuli were delivered to the human fingertip or the mouse forelimb. The resulting perceptual reports were analyzed with a model demonstrating that physically different combinations of vibration frequencies and amplitudes can produce equal pitch perception. We found that the perceptually indistinguishable but physically different stimuli follow a common computational principle in mouse and human. It dictates that vibrotactile pitch perception is shifted with increases in amplitude toward the frequency of highest vibrotactile sensitivity. These findings suggest the existence of a fundamental relationship between the seemingly unrelated

concepts of spectral sensitivity and pitch perception.
Nat Commun, 2021; 12

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BOARD NUMBER: S01-105

A NEW MINI-VR AND DUAL-WHEEL PLATFORM FOR CLOSED-LOOP MOTOR LEARNING AND ADAPTATION IN MICE

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

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We live in environments that are constantly changing, and as a result, our motor commands need to be continually adapted to account for changing sensory inputs. Visual inputs are easy to control and manipulate, making visual-adaptation an ideal way to study the neural mechanisms of motor adaptation. In particular, modern virtual reality platforms paired with tractable models such as mice, are ideally suited. Yet current approaches rely on either open or closed loop approaches where mice run on an air-supported styrofoam ball, limiting the ability to perform perturbations across the body axis. To tackle this, we developed a novel fully closed-loop split-wheel virtual reality (VR) and mini-VR "headset" for mice to study specific types of visuomotor adaptations. The virtual environment is displayed in a "mini-VR" system, and to navigate in the virtual environment the mice must rotate two wheels placed ipsilaterally underneath their limbs. Moving the wheels synchronously during locomotion therefore directly translates in navigating straight in the virtual environment. Thus, an imposed visual perturbation can modulate each wheel independently to shift the overall angle of movement in the VR world. We show that mice can learn to navigate on this split wheel VR system within 14 days and when challenged with a visual perturbation they can rapidly adapt within 100 trials and show classical signatures of adaptive behavior.

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BOARD NUMBER: S01-106

THE HELICO MAZE TO STUDY EARLY LEARNING AND SUBCATEGORIES OF LONG-TERM MEMORY IN MICE

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

François S. Roman[1], Guy Escoffier[2], Martine Migliorati[2], Melinda Rahrah[2], Charles Roman[2], Michel Khrestchatisky[2]
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Aims: The Helico Maze (HM) was developed to study procedural and reference subcategories of long-term memory as early as possible in the mouse. Methods: BALB/c AnNCrl (BALB), C57BL/6JRj (C57) and DBA/2 JRj (DBA) mice were 8 weeks old at the beginning of training on this new apparatus. Results: The three strains learned how to use the HM (procedural memory), and they then learned and remembered four odor-reward associations (reference memory). The three strains differed in the number of correct responses. BALB mice showed better performance than C57 and DBA mice. The results of the first trials of each session revealed that only the BALB and C57 mice remembered the odor-reward associations. DBA mice, well-known having hippocampal circuitry dysfunctioning, needed to relearn the associations each day. Conclusions: With this new maze, the number of olfactory cue-reward associations was increased from 2 to 4 in comparison to a previous olfactory tubing maze. Consequently, a supplementary effort of memory was required, and the chance level was decreased from 50 % to 25 %. Thus, the HM can be considered to measure the hippocampus-dependent behavior of the mouse, allowing to study, as early as possible in young mice, the different subcategories of long-term memory, such as those observed in humans. In addition, using the Helico Maze may open new avenues to validate efficacy of treatments that target early events related neurodegenerative diseases.

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BOARD NUMBER: S01-107

DEVELOPMENT OF A HIGH-THROUGHPUT PHENOTYPIC ASSAY TO SCREEN FOR CHEMICAL ENHANCERS OF PROTEOSTASIS ACTIVITY IN CAENORHABDITIS ELEGANS

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Daniela Vilasboas-Campos[1,2], Joana Lopes[1,2], Jorge Diogo Da Silva[1,2], Bruna Ferreira-Lomba[1,2], Marta Daniela Costa[1,2], Patrícia Maciel[1,2], Andreia Teixeira-Castro[1,2]
[1]University of Minho, Life And Health Sciences Research Institute (icvs), School Of Medicine, Braga, Portugal, [2]PT Government Associate Laboratory, Icvs/3b's, Braga/Guimarães, Portugal

INTRODUCTION: Accumulation of aberrantly processed and misfolded proteins into aggregates causes proteotoxic stress in neuronal cells, resulting in long-term deleterious effects and neurodegeneration, associated to several diseases. **AIM:** This work addresses an unmet medical need: the lack of effective treatment for aging-associated neurodegenerative diseases (ND). **METHODS:** Here, we established an automated assay to screen for small molecules able to act as enhancers of protein folding and homeostasis capacity, that reduce proteotoxic stress in a whole organism, using motor activity after heat-shock (HS) as a readout. For this purpose, we used a transgenic model of a polyglutamine disease, a strain expressing a Firefly-Luciferase folding sensor, and Wild-type animals, evaluating their motor function in the basal situation and during post-HS recovery. The protocol developed consisted in exposing animals to 60 min of HS at 37ºC, after which their motor activity was measured in an automated manner, for 15h, using an automated movement detector. **RESULTS:** The HS of 60 min caused paralysis, however, all *C. elegans* models tested were still able to recover close to baseline levels 15h post-HS. Using this newly established protocol, we found that the pharmacologic and genetic modulation of serotonergic signaling prevented proteotoxic damage and significantly increased motor function recovery. **CONCLUSION:** Currently, we are screening libraries of novel and repurposed compounds, for which some of the molecular targets have been genetically defined. This should allow the identification of novel candidate therapeutics and a deeper understanding of the biological pathways underlying proteotoxic stress, of relevance to many human diseases.

Pubmed:
34871736: Raposo M, Bettencourt C, Melo ARV, Ferreira AF, Alonso I, Silva P, Vasconcelos J, Kay T, Saraiva-Pereira ML, Costa MD, Vilasboas-Campos D, Bettencourt BF, Bruges-Armas J, Houlden H, Heutink P, Jardim LB, Sequeiros J, Maciel P, Lima M
Novel Machado-Joseph disease-modifying genes and pathways identified by whole-exome sequencing.
Machado-Joseph disease (MJD/SCA3) is a neurodegenerative polyglutamine disorder exhibiting a wide spectrum of phenotypes. The abnormal size of the (CAG)n at ATXN3 explains ~55% of the age at onset variance, suggesting the involvement of other factors, namely genetic modifiers, whose identification remains limited. Our aim was to find novel genetic modifiers, analyse their epistatic effects and identify disease-modifying pathways contributing to MJD variable expressivity. We performed whole-exome sequencing in a discovery sample of four age at onset concordant and four discordant first-degree relative pairs of Azorean patients, to identify candidate variants which genotypes differed for each discordant pair but were shared in each concordant pair. Variants identified by this approach were then tested in an independent multi-origin cohort of 282 MJD patients. Whole-exome sequencing identified 233 candidate variants, from which 82 variants in 53 genes were prioritized for downstream analysis. Eighteen disease-modifying pathways were identified; two of the most enriched pathways were relevant for the nervous system, namely the neuregulin signaling and the agrin interactions at neuromuscular junction. Variants at PARD3, NFKB1, CHD5, ACTG1, CFAP57, DLGAP2, ITGB1, DIDO1 and CERS4 modulate age at onset in MJD, with those identified in CFAP57, ACTG1 and DIDO1 showing consistent effects across cohorts of different geographical origins. Network analyses of the nine novel MJD modifiers highlighted several important molecular interactions, including genes/proteins previously related with MJD pathogenesis, namely between ACTG1/APOE and VCP/ITGB1. We describe novel pathways, modifiers, and their interaction partners, providing a broad molecular portrait of age at onset modulation to be further exploited as new disease-modifying targets for MJD and related diseases.
Neurobiol Dis, 2022; 162
33516872: Pereira-Sousa J, Ferreira-Lomba B, Bellver-Sanchis A, Vilasboas-Campos D, Fernandes JH, Costa MD, Varney MA, Newman-Tancredi A, Maciel P, Teixeira-Castro A

Identification of the 5-HT serotonin receptor as a novel therapeutic target in a C. elegans model of Machado-Joseph disease.
Machado-Joseph disease (MJD) or Spinocerebellar ataxia type 3 (SCA3) is a progressive neurodegenerative disorder that affects movement coordination leading to a premature death. Despite several efforts, no disease-modifying treatment is yet available for this disease. Previous studies pinpointed the modulation of serotonergic signaling, through pharmacological inhibition of the serotonin transporter SERT, as a promising therapeutic approach for MJD/SCA3. Here, we describe the 5-HT receptor as a novel therapeutic target in MJD, using a C. elegans model of ATXN3 proteotoxicity. Chronic and acute administration of befiradol (also known as NLX-112), a highly specific 5-HT agonist, rescued motor function and suppressed mutant ATXN3 aggregation. This action required the 5-HT receptor orthologue in the nematode, SER-4. Tandospirone, a clinically tested 5-HT receptor partial agonist, showed a limited impact on animals' motor dysfunction on acute administration and a broader receptor activation profile upon chronic treatment, its effect depending on 5-HT but also on the 5-HT/SER-5 and 5-HT/SER-7 receptors. Our results support high potency and specificity of befiradol for activation of 5-HT/SER-4 receptors and highlight the contribution of the auto- and hetero-receptor function to the therapeutic outcome in this MJD model. Our study deepens the understanding of serotonergic signaling modulation in the suppression of ATXN3 proteotoxicity and suggests that a potent and selective 5-HT receptor agonist such as befiradol could constitute a promising therapeutic agent for MJD.
Neurobiol Dis, 2021; 152
33318982: Vilasboas-Campos D, Costa MD, Teixeira-Castro A, Rios R, Silva FG, Aierken A, Zhang X, Bessa C, Dias ACP, Maciel P
Data on the effects of spp. and spp. plant extracts in models of genetically determined neurodegenerative diseases.
Here, we present the data on the biological effects of spp. and spp. plant extracts in () models of neurodegenerative diseases, which is related to the work presented in the article "Neurotherapeutic effect of spp. leaf extracts in models of tauopathy and polyglutamine disease: role of the glutathione redox cycle" [1]. This dataset was generated to define non-toxic concentrations of these plant extracts and to assess their impact on the motor phenotype and oxidative stress resistance of transgenic models of two genetically defined neurodegenerative diseases: Machado-Joseph disease and Frontotemporal dementia with Parkinsonism associated to the chromosome 17. The impact of the plant extracts on toxicity was assessed using the food-clearance assay, absorbance being measured daily for seven days at 595 nm to quantify () strain OP50 bacteria consumption. Worm length and motor behaviour, including spontaneous and stimulated movement, were analysed using videos acquired with an Olympus SZX7 stereomicroscope with an integrated camera (Olympus SC30) and processed using the Image J® software and the Wrmtrck plugin. The resistance to oxidative stress induced by 240 µM juglone was assessed by determining the percentage of live animals after 1 hour of exposure.
Data Brief, 2020; 33
33096249: Vilasboas-Campos D, Costa MD, Teixeira-Castro A, Rios R, Silva FG, Bessa C, Dias ACP, Maciel P
Neurotherapeutic effect of Hyptis spp. leaf extracts in Caenorhabditis elegans models of tauopathy and polyglutamine disease: Role of the glutathione redox cycle.
Hyptis suaveolens (HS), Hyptis pectinata (HP) and Hyptis marrubioides (HM) are plants used in folk medicine for treatment of several diseases. Here, we tested the in vivo antioxidant and neuroprotective potential of methanolic extracts from these plants, containing several rosmarinic acid derivatives and isoquercetin. In C. elegans, HS, HP and HM leaf extracts enhanced the antioxidant responses through the induction of specific antioxidant enzymes and demonstrated neurotherapeutic potential in transgenic models of genetically determined human neurodegenerative diseases - Frontotemporal dementia with parkinsonism linked to chromosome 17 and Machado-Joseph disease. Chronic treatment of disease models with HS, HP and HM leaf extracts improved the animals' motor function and increased their tolerance to an oxidative insult. The restorative effect of HM extract in motor performance of both disease models required the presence of glutathione reductase (gsr-1), an enzyme that assures the glutathione redox cycle, highlighting the role of this pathway and unveiling a common candidate therapeutic target for these diseases. Our findings strengthen the relevance of plant-derived bioactive compound discovery for neurodegenerative disorders that remain without effective treatment.
Free Radic Biol Med, 2021; 162

FENS Forum 2022
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BOARD NUMBER: S01-108

A VERSATILE OPEN SOURCE 1-PHOTON IMAGING PLATFORM FOR INVESTIGATING THE NEURONAL CORRELATES OF BEHAVIOURAL FLEXIBILITY

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

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In order to survive in a complex and changing environment, animals have to adapt their behaviour. This ability is called behavioural flexibility and is classically evaluated by a reversal learning paradigm. During such a paradigm, the animals adapt their behaviour according to a change in the reward contingencies, allowing an investigation of complex cognitive functions, ranging from outcome expectancies to motor adaptation. Here, we describe FreiBox, a versatile, low-cost (~750 Euros) and open-source platform allowing us to investigate the neuronal correlates of behavioural flexibility with 1-photon Calcium imaging. It is an Arduino-based setup, integrating a new licking sensor for detecting directional licking in a behavioural task, controlled by a custom-built Python interface. We show that the Arduino is able to track mouse licking behaviour in real time and to control task events in a sub-millisecond timescale allowing both discriminative and serial reversal learning tasks. To complete our setup for freely-moving animals, we also developed and validated a cost-effective commutator (<150 Euros), which is crucial for calcium imaging with the Miniscope v4 and measuring electrophysiological signals with Neuropixel probes. Finally, we demonstrate that FreiBox can be used in combination with 1-photon imaging and other open-source initiatives (e.g. openEphys) to form a versatile platform for exploring the neuronal substrates of licking-based behaviour in mice. In summary, FreiBox and our low-cost commutator complement the recently emerging battery of open-source initiatives, opening new avenues to investigate behavioural flexibility with a specific focus on Calcium imaging in mice involved in a reversal learning paradigm.

Pubmed:
24622813: Arlicot N, Tronel C, Bodard S, Garreau L, de la Crompe B, Vandevelde I, Guilloteau D, Antier D, Chalon S
Translocator protein (18 kDa) mapping with [125I]-CLINDE in the quinolinic acid rat model of excitotoxicity: a longitudinal comparison with microglial activation, astrogliosis, and neuronal death.
Excitotoxicity leads to an inflammatory reaction involving an overexpression of: translocator protein 18 kDa (TSPO) in cerebral microglia and astrocytes. Therefore, we performed ex vivo explorations with [125]-CLINDE, a TSPO-specific radioligand, to follow the time course of TSPO expression, in parallel with lesion progression, over 90 days after induction of cerebral excitotoxicity in rats intrastriatally injected with quinolinic acid. Biodistribution data showed a significant increase in CLINDE uptake on the injured side from 1 days postlesion (dpl); the maximal striatal binding values evidenced a plateau between 7 and 30 dpl. [125I]-CLINDE binding was displaced from the lesion by PK11195, suggesting TSPO specificity. These results were confirmed by ex vivo autoradiography. Combined immunohistochemical studies showed a marked increase in microglial expression in the lesion, peaking at 14 dpl, and astrocytic reactivity enhanced at 7 and 14 dpl, whereas a prominent neuronal cell loss was observed. At 90 dpl, CLINDE binding and immunoreactivity targeting activated microglia, astrogliosis, and neuronal cell density returned to a basal level. These results show that both neuroinflammation and neuronal loss profiles occurred concomitantly and appeared to be transitory processes. These findings provide the possibility of a therapeutic temporal window to compare the differential effects of antiinflammatory treatments in slowing down neurodegeneration in this rodent model, with potential applications to humans.
Mol Imaging, 2014; 13
25442003: Bastide MF, de la Crompe B, Doudnikoff E, Fernagut PO, Gross CE, Mallet N, Boraud T, Bézard E
Inhibiting Lateral Habenula Improves L-DOPA-Induced Dyskinesia.
A systematic search of brain nuclei putatively involved in L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia (LID) in Parkinson's disease shed light, notably, upon the lateral habenula (LHb), which displayed an overexpression of the ΔFosB, ARC, and Zif268 immediate-early genes only in rats experiencing abnormal involuntary movements (AIMs). We thus

hypothesized that LHb might play a role in LID.
Biol Psychiatry, 2016; 79
32218441: Crompe B, Aristieta A, Leblois A, Elsherbiny S, Boraud T, Mallet NP
The globus pallidus orchestrates abnormal network dynamics in a model of Parkinsonism.
The dynamical properties of cortico-basal ganglia (CBG) circuits are dramatically altered following the loss of dopamine in Parkinson's disease (PD). The neural circuit dysfunctions associated with PD include spike-rate alteration concomitant with excessive oscillatory spike-synchronization in the beta frequency range (12-30 Hz). Which neuronal circuits orchestrate and propagate these abnormal neural dynamics in CBG remains unknown. In this work, we combine in vivo electrophysiological recordings with advanced optogenetic manipulations in normal and 6-OHDA rats to shed light on the mechanistic principle underlying circuit dysfunction in PD. Our results show that abnormal neural dynamics present in a rat model of PD do not rely on cortical or subthalamic nucleus activity but critically dependent on globus pallidus (GP) integrity. Our findings highlight the pivotal role played by the GP which operates as a hub nucleus capable of orchestrating firing rate and synchronization changes across CBG circuits both in normal and pathological conditions.
Nat Commun, 2020; 11
33306949: Aristieta A, Barresi M, Azizpour Lindi S, Barrière G, Courtand G, de la Crompe B, Guilhemsang L, Gauthier S, Fioramonti S, Baufreton J, Mallet NP
A Disynaptic Circuit in the Globus Pallidus Controls Locomotion Inhibition.
The basal ganglia (BG) inhibit movements through two independent circuits: the striatal neuron-indirect and the subthalamic nucleus-hyperdirect pathways. These pathways exert opposite effects onto external globus pallidus (GPe) neurons, whose functional importance as a relay has changed drastically with the discovery of two distinct cell types, namely the prototypic and the arkypallidal neurons. However, little is known about the synaptic connectivity scheme of different GPe neurons toward both motor-suppressing pathways, as well as how opposite changes in GPe neuronal activity relate to locomotion inhibition. Here, we optogenetically dissect the input organizations of prototypic and arkypallidal neurons and further define the circuit mechanism and behavioral outcome associated with activation of the indirect or hyperdirect pathways. This work reveals that arkypallidal neurons are part of a novel disynaptic feedback loop differentially recruited by the indirect or hyperdirect pathways and that broadcasts inhibitory control onto locomotion only when arkypallidal neurons increase their activity.
Curr Biol, 2021; 31
32795553: De La Crompe B, Coulon P, Diester I
Functional interrogation of neural circuits with virally transmitted optogenetic tools.
The vertebrate brain comprises a plethora of cell types connected by intertwined pathways. Optogenetics enriches the neuroscientific tool set for disentangling these neuronal circuits in a manner which exceeds the spatio-temporal precision of previously existing techniques. Technically, optogenetics can be divided in three types of optical and genetic combinations: (1) it is primarily understood as the manipulation of the activity of genetically modified cells (typically neurons) with light, i.e. optical actuators. (2) A second combination refers to visualizing the activity of genetically modified cells (again typically neurons), i.e. optical sensors. (3) A completely different interpretation of optogenetics refers to the light activated expression of a genetically induced construct. Here, we focus on the first two types of optogenetics, i.e. the optical actuators and sensors in an attempt to give an overview into the topic. We first cover methods to express opsins into neurons and introduce strategies of targeting specific neuronal populations in different animal species. We then summarize combinations of optogenetics with behavioral read out and neuronal imaging. Finally, we give an overview of the current state-of-the-art and an outlook on future perspectives.
J Neurosci Methods, 2020; 345

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BOARD NUMBER: S01-109

ELECTROCHEMICAL APTAMER-BASED BIOSENSORS ENABLE IN-BRAIN DRUG CONCENTRATION-BEHAVIORAL RESPONSE ANALYSES.

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Tod Kippin
University of California, Santa Barbara, Psychological & Brain Sciences, Santa Barabara, United States Minor Outlying Islands

Drugs exert their behavioral effects via concentration-dependent actions upon neural circuits. However, to date, there has been relatively limited attention to the role pharmacokinetics plays in psychopharmacology because, amongst other factors, the temporal resolution with which we can measure these compounds is *orders of magnitude* too slow to capture the concentrations associated with altered physiological processes. For instance, procaine and cocaine are psychoactive compounds that have short half-lives and elicit highly dynamic behavioral effects upon intravenous administration making determination of the relation between in-brain concentration and on-going behavior challenging. Thus motivated, we adopted electrochemical aptamer-based sensors (E-AB sensors) to the task of measuring drugs in-situ and in real-time in awake, freely behaving animals. Here, we report EAB sensors that exhibit sufficient sensitivity, appropriate temporal resolution (~12s), and stable drift characteristics to fully resolve pharmacokinetics of procaine and cocaine in the brains of rats. These data combined with standard locomotor measurements enable detailed analyses of the relations between in-brain concentration and behavioral response for individual male and female subjects. In parallel, we employed EAB-supported feedback-control of drug delivery to produce constant in-brain drug concentrations to remove individual differences in PK as well as examine within-session neurobehavioral adaptations caused by drug exposure. In conclusion, we have developed technology capable of determining individual, in-brain pharmacokinetics of psychoactive drugs in behaving animals that can enable concentration-response analyses.

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BOARD NUMBER: S01-110

MULTI-ANIMAL POSE ESTIMATION, IDENTIFICATION AND TRACKING WITH DEEPLABCUT

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Mu Zhou[1], Jessy Lauer[1], Shaokai Ye[1], William Menegas[2], Steffen Schneider[1], Tanmay Nath[3], Mohammed Mostafizur Rahman[4], Valentina Di Santo[5,6], Daniel Soberanes[3], Guoping Feng[2], Venkatesh Murthy[4], George Lauder[6], Catherine Dulac[4], Mackenzie Mathis[1,3], Alexander Mathis[1,3,4]
[1]EPFL, Brain Mind Institute, Geneva, Switzerland, [2]Massachusetts Institute of Technology, Department Of Brain And Cognitive Sciences And Mcgovern Institute For Brain Research, Cambridge, United States of America, [3]Harvard University, Rowland Institute, Cambridge, United States of America, [4]Harvard University, Department For Molecular Biology And Center For Brain Science, Cambridge, United States of America, [5]Stockholm University, Department Of Zoology, Stockholm, Sweden, [6]Harvard University, Department Of Organismic And Evolutionary Biology, Cambridge, United States of America

Estimating the pose of multiple animals is a challenging computer vision problem: frequent interactions cause occlusions and complicate the association of detected keypoints to the correct individuals, as well as having highly similar looking animals that interact more closely than in typical multi-human benchmarking datasets. To take up this challenge, we build on DeepLabCut, an open source pose estimation toolbox, and provide high-performance animal assembly and tracking—features required for multi-animal scenarios. We propose a multi-task architecture that predicts multiple conditional random fields and therefore can predict keypoints, within-animal body part connections ("limbs"), as well as animal identity. Advantageously, the definition of an animal's skeleton, i.e. the list of limbs that will be used for assembly, is data-driven, thus requiring no user input. Furthermore, we integrate the ability to predict an animal's identity to assist tracking (in case of occlusions). We illustrate the power of this framework with four datasets varying in complexity, which we release to serve as a benchmark for future algorithm development.

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BOARD NUMBER: S01-111

SOURIS-CITY: A MULTI-ENVIRONMENT FOR UNDERSTANDING THE SOCIAL BASIS OF INTER-INDIVIDUAL VARIABILITY AND DRUG VULNERABILITY IN MICE.

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Sophie Fayad[1], Nicolas Torquet[2], Lauren Reynolds[1], Fabio Marti[1], Stefania Tolu[3], Claire Nguyen[4], Sarah Mondoloni[5], Robin Justo[1], Steve Didienne[1], Nicolas Debray[6], Nicolas Renier[7], Alexandre Mourot[8], Philippe Faure[8]
[1]CNRS - ESPCI - PSL, Brain Plasticity Laboratory, Paris, France, [2]IGBMC, Institut Clinique De La Souris, Illkirch-Graffenstaden, France, [3]PARIS DIDEROT - CNRS, Bfa - Unité De Biologie Fonctionnelle Et Adaptative - Cnrs Umr 8251, PARIS, France, [4]CNRS - ESPCI, Umr8249 - Plasticité Du Cerveau, PARIS, France, [5]University of Lausanne, Department Of Fundamental Neuroscience, Lausanne, Switzerland, [6]CNRS - IBPS - Sorbonne Université, Adaptation Biologique Et Vieillissement, Paris, France, [7]Institut du Cerveau et de la Moelle Epinière., Laboratory Of Structural Plasticity, Paris, France, [8]ESPCI, Brain Plasticity Lab Umr8249 Cnrs, Paris, France

Behavioral differences among individuals are ubiquitous in animal studies. When consistent across time and contexts, they define animal personality. In adulthood, environmental factors, such as social context, play a major role in personality adaptation. However, the underlying neurophysiological mechanisms are poorly understood and bring promising leads towards understanding inter-individual differences in vulnerability to drugs. We aim at unraveling the social determinants of inter-individual variability and drug vulnerability, using a semi-natural multi-environment, “Souris City”, where mice are living in large social groups and individually tracked for several weeks. This system combines a compartmentalized social homecage and a T-maze with individual access. It allows us to observe correlations between decision-making strategies in the T-maze, and behavioral traits in the social zone, and thus to describe individual profiles. Using juxtacellular recordings in anaesthetized mice, we found that individuals with distinct behavioral traits in Souris-City also display differences in the spontaneous activity of their Ventral Tegmental Area (VTA) dopaminergic (DA) neurons. We next investigated whether these mice also showed differences in their response to nicotine exposure, by recording VTA DA neurons responses to intravenous nicotine injections. Using c-fos immunoreactivity in cleared whole brains, we further sought for inter-individual differences in global responses of brain structures to intraperitoneal nicotine injections. Finally, we looked into the impact of nicotine intake on individual behavior, either through voluntary consumption in Souris City, or by chronic administration via subcutaneous osmotic pumps. This combinatorial approach allows to address key questions on individual personality and vulnerability to nicotine.

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BOARD NUMBER: S01-112

A LEARNING ASSAY FOR HEAD-FIXED WALKING FLIES

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

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Understanding the function of sleep is hindered by the extended nature of sleep control circuits over many brain areas and by the many different brain functions that are impacted by sleep. In the brain of Drosophila, multiple neural populations have been identified that are important for sleep control. In the central complex, an area in the center of the fly brain, sleep control circuits interact with circuits that have well characterized functions in the context of navigation as well as memory. Based on the fly connectome as well as the dynamics of the relevant neural populations, which are known thanks to electrophysiology and calcium imaging experiments, we developed a computational model of the interaction of sleep control and navigation circuits [Flores-Valle et al., Plos. Comp. Biol., 2021]. How memory circuits interact with sleep or navigation circuits in the fly brain is however only little understood and mostly only through computational modeling. Constraining such models requires experimental paradigms that allow investigating neural dynamics underlying sleep and learning. This necessitates recording neural activity in behaving animals over multiple timescales, including bouts of wakefulness and sleep while the animal is engaged in a learning task. In particular, such an approach needs a reliable learning paradigm that can be implemented in tethered flies and can therefore be combined with neural recordings. Here, we develop a novel learning paradigm for tethered walking flies and use it for analyzing the function of putative learning circuits in the Drosophila brain.

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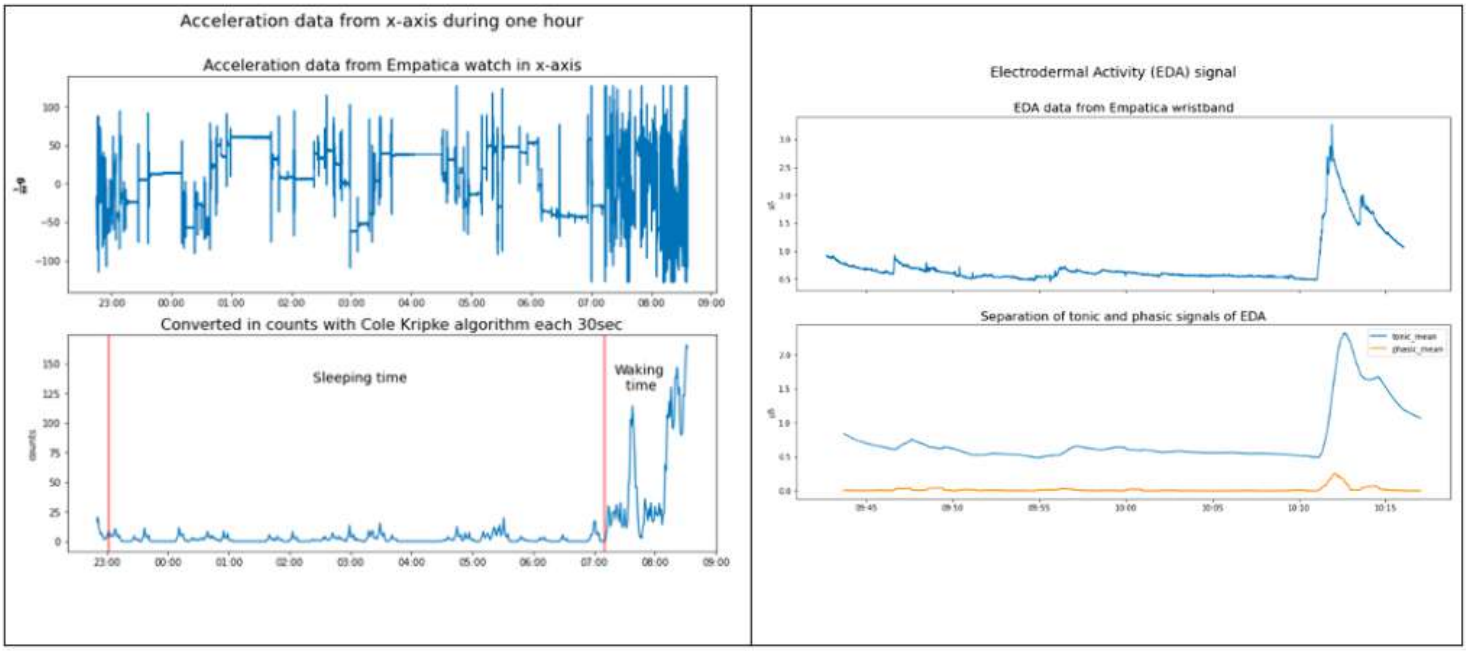
BOARD NUMBER: S01-113

ECOCAPTURE@HOME: DEVELOPMENT OF AN ASSESSMENT METHOD FOR APATHY IN EVERYDAY LIFE CONDITIONS, TARGETED TOWARDS PATIENTS WITH NEURODEGENERATIVE DISEASES AND THEIR CAREGIVERS.

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Idil Sezer, Valérie Godefroy, Mathilde Boucly, Armelle Rametti-Lacroux, Arabella Bouzigues, Raffaella Migliaccio, Richard Lévy, Bénédicte Batrancourt
Sorbonne Université, Paris Brain Institute – Institut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP, Hôpital Pitié Salpêtrière, Paris, France

Aims: Apathy is the most frequent behavioral syndrome in neurological diseases and is associated with high levels of distress both in patients and their caregivers. As the current assessment of apathy is biased by subjectivity, we have developed a research program to objectively assess apathy under ecological conditions. In this program, the ECOCAPTURE@HOME study aims to: 1/ measure behavioral markers of apathy from data collected in patient-caregiver dyads in everyday life conditions, and 2/ predict the psychological status of the patient-caregiver dyad from these markers of apathy. **Methods:** The ECOCAPTURE@HOME method will be applied on 40 patient-caregiver dyads (patients with behavioral variant frontotemporal dementia or Alzheimer's Disease) and 20 healthy control dyads. Each dyad will be monitored for 28 consecutive days, in particular through the remote collection of passive data from the Empatica E4 wristband, a wearable device providing real-time physiological data (acceleration, heart rate, electrodermal activity). Using these sensor data, our first goal is to develop algorithms to extract behavioral metrics reflecting assumed markers of apathy: daytime activity, quality of sleep, emotional arousal. **Results:** Preliminary results of ECOCAPTURE@HOME in pilot subjects (n = 10) will be detailed. Sensor data will be computed to extract behavioral metrics (e.g., activity counts/day, time awake/asleep, tonic and phasic phase of
EDA).



Conclusions: We are developing a method that leverages the capabilities of physiological sensors to detect apathy. This method should contribute to the remote follow-up of patient's apathy and caregiver's status, thus laying the foundation for a personalized therapeutic program.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BOARD NUMBER: S01-114

WILLIAM JAMES' THEORY OF EMOTION: WHAT WAS JAMES'S INITIAL RESPONSE TO LANGE'S THEORY OF EMOTION?

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Toshihiko Sato
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William James (1884), an American philosopher and psychologist, and Carl Lange (1885), a Danish physician, proposed their independent theories of emotion in the late 19th century. Their theories were prominent contributions to affective neuroscience and the psychology of emotion. They emphasized the role of afferent neural input, information transmitted from various peripheral body responses to the brain, to produce subjective experiences of emotion. Although their theories of emotions were similar in content and commonly referred to as the "James–Lange" theory of emotion, the two paradigms had different academic backgrounds. For example, James published his theoretical paper "What is an emotion?" in a philosophical journal, *Mind*. At the same time, Lange's monograph included new theoretical ideas of emotions and clinical observations. In this presentation, the author will elucidate how James responded to Lange's theory of emotion in James's famous book, *The Principles of Psychology*, in 1890 and discuss how Lange's theory influenced later versions of James's theories of emotions. At first, James seemed to be stimulated and encouraged by reading the contents of Lange's monograph. He quoted several sentences from Lange's monograph five times, including a description of expressions of grief, and used part of these phrases to explain the reflex mechanisms of emotions in *Principles*. On the other hand, while James agreed with Lange's theory in general, James did not compromise Lange's key concept regarding the crucial role of the vasomotor center in the brain.

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BOARD NUMBER: S01-115

GENE THERAPY RESEARCH AND THE BRAIN: IS AFRICA ETHICALLY, LEGALLY AND SOCIALLY READY?

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

George Wanderi
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Interventional modalities that include gene therapy and stem cell techniques for treating neurological disorders affecting the central nervous system (CNS) are rapidly growing in developed nations. They are largely driven by well-funded and organized academic and research institution with collaborations across the borders. Gene therapy for example, can result in a stable or inducible expression of transgene(s), and can allow a nearly specific expression in target cells. It has been used successfully in gene knockout approach for Huntington's disease. In this review, we will discuss the social implications of such techniques with respect to socio-cultural norms in many African nations from 2003 to present. We will discuss the legal implications of such advancements in the context of identity, scientific regulation and communication, capacity building and clinical opportunities for collaborative research across the African continent. **Keywords:** gene therapy, central nervous system, viral vector, gene regulation, brain disease, culture, Africa

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BOARD NUMBER: S01-116

RESPONSIBILITY ISSUES RAISED BY NEUROTECHNOLOGIES IN LIGHT OF ETHICS AND PHILOSOPHY.

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Laure Tabouy
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What kind of world do we want? How far can and should we go? Several European and international projects aiming at advancing the knowledge of the brain by combining the expertise of neuroscience research with that of computer science research are making it possible to miniaturize, make more efficient and more effective invasive and non-invasive neurotechnologies. Developed in research laboratories as well as in private companies, and already marketed to the general public in good health, the boundary between medical and non-medical uses is becoming very porous, with different objectives and investments. The acceleration of these innovations makes it essential to reflect on the societal, ethical and legal issues at stake, in particular on the notion of responsibility. The design of interdisciplinary safeguards, evaluation and monitoring systems, and the definition of governance adapted to the sociological, ethical and legal values of the countries involved are currently being developed worldwide. It is around the need to agree on the notion of social responsibility that neuroethics is called for by the OECD Council through its recommendation n°0457 of 2019 on responsible innovation in neurotechnologies. Reflecting on the notion of responsibility in the light of the philosopher will bring a non-negligible lighting to approach responsibility. It is thus by summoning Hans Jonas, Ernst Bloch, Hannah Arendt, Paul Ricoeur and Heather Douglas, but also by using neuroethics and existing laws and recommendations that this work around social responsibility concerning neurotechnologies has taken shape.

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BOARD NUMBER: S01-117

A CRITICAL STUDY OF SOME NEUROSCIENTIFIC STUDIES ON FREEWILL

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Sam (Seyed Amir Mohammad) Moosavi (Moosavi Jashni)
Missing Institution, Missing Department, Tehran, Iran

Since neuroscientific studies have been established, several disciplines have utilized their findings and this has changed our understanding of the world in many ways. But the study of high cortical functions like, judgment, language, reasoning, decision-making, and so on, require a multidisciplinary approach, for these functions have a socio-politico-cultural nature, and, disciplinary approaches (including a neuroscientific one) is not adequate and sufficient to answer complex questions of such a subject-matter. One of the fields that has been seriously affected by neuroscience and also requires multidisciplinary research is the freewill. Some of the logical, epistemological, ethical, social, and political consequences of these studies are so vital that increase the importance of precise and even obsessive verification of their methodology, research design, and implementation or the act of doing the experiment. Ever since science has become disciplinary, some of the related studies have become like separate islands, while taking a stand in any of these fields requires a transdisciplinary approach. Also, in addition to interaction between the disciplines, the findings of different disciplines sometimes suffer from logical opposition, and, at times some findings are in contradiction with the principles, assumptions, rules, and other findings of the same field. In this study, some of the famous neuroscientific studies' methodologies and findings will be critically examined and the logical opposition between their findings and some dogmas of modern thinking (including the scientific method) will be discussed. For this purpose, reflective analysis and review article methods have been used.

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BOARD NUMBER: S01-118

PEER COMMUNITY IN NEUROSCIENCE

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Florent Lebon[1], Mahesh Karnani[2], Marion Mercier[3], Vincent Magloire[3], Thibaut Sesia[4], Ian Greenhouse[5]
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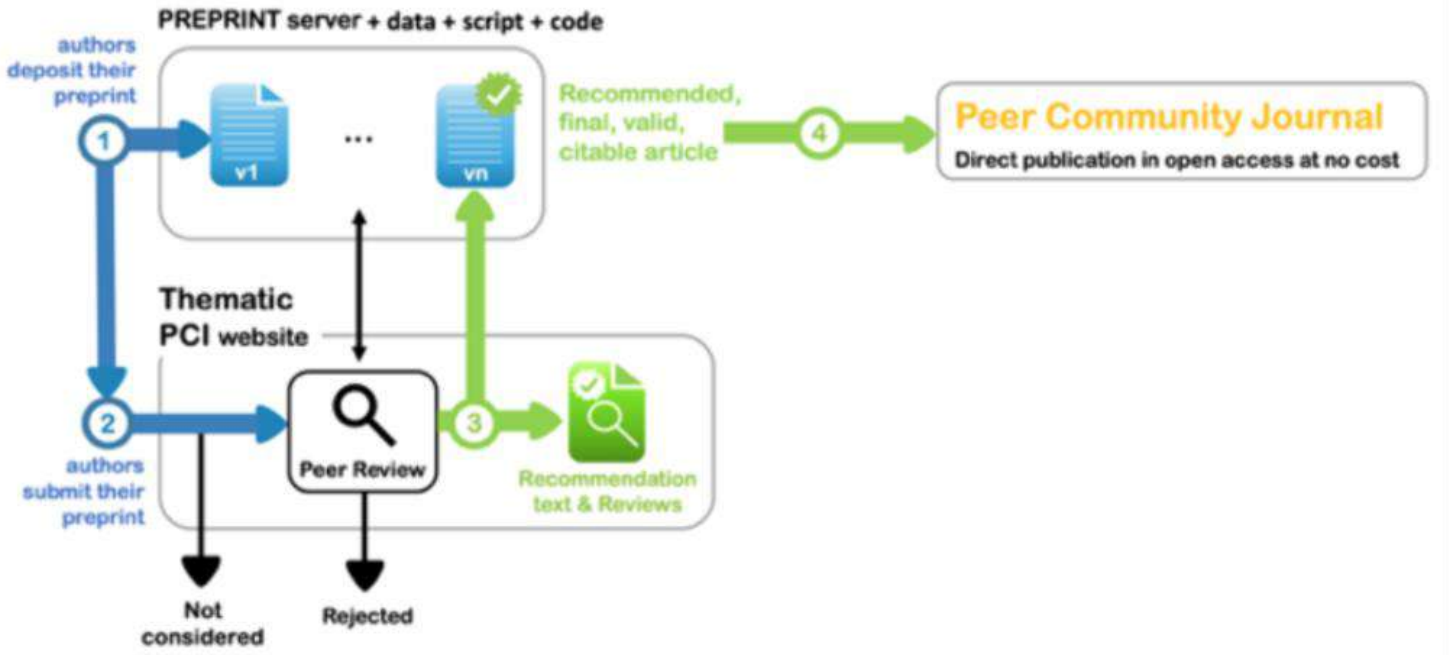
The dissemination of results and new technologies in neuroscience is rapidly evolving from an exclusive and fee-oriented publishing system towards more open, free and independent strategies for sharing knowledge. In this context, preprint servers such as bioRxiv answer a very real scientific need by enabling the rapid, free and easy dissemination of findings, regardless of whether these are novel, replicated, or showcasing negative results. In 2019, we launched Peer Community In (PCI) Circuit Neuroscience, a platform that provides rigorous evaluation and validation of preprints in this subfield of neuroscience, at no cost for authors and readers. As neuroscience is the largest subject category on bioRxiv, and a highly interdisciplinary field, we recently launched PCI Neuroscience (PCI Neuro). It now covers research topics such as neural circuits, synaptic physiology, neuroanatomy but also behavioral neuroscience, cognitive neuroscience, sensorimotor functions, and others, using a variety of techniques (behavioral testing, electrophysiology, neuroimaging, brain stimulation, neuromodulation, computational modeling, etc.). At this poster we discuss PCI Neuro's mission, how it works, and why it is an essential initiative in this new era of open science. To contribute to this grass-roots community effort, you can sign up online at https://neuro.peercommunityin.org/. We want to hear all of your opinions so please stop by to talk to us!



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WHY MEN RAPE: PERSPECTIVES FROM INCARCERATED RAPISTS IN A KWAZULU-NATAL PRISON

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Lihle Qulu[1], Relebohile Relebohile Moletsane[2], Abigail Wilkinson[1,3], Jani Nothling[4], Lindokuhle Ngubane[5]
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South Africa is one of the leading countries in the world that records high rate of crimes against women and children especially on sexual violence. South African government have implemented numerous initiatives to curb the escalating rate of sexual violence, but the effect is minimal. This study aimed to investigate the experiences and social factors influencing the behavior of rape incarcerated men in KwaZulu-Natal prison. An interpretative and exploratory qualitative research design consisted of purposive sampling was used to select eighteen sex offenders. Data was obtained from face-to-face interviews and analyzed using Thematic analysis to identify and describe six themes, (1) the way perpetrators understood rape, (2) childhood trauma and adverse events, (3) substance abuse, (4) perceptions of gender roles, (5) avoiding responsibility, and (6) recidivism. The findings revealed that all sex perpetrators have been exposed to at least one childhood trauma and adversity. Some perpetrators of child rape described the act as consensual and did not consider it as rape. Most participants avoided taking responsibility for their actions and blamed the victim, victim family, alcohol use, traditional gender roles and transactional sex. Recidivism was common to perpetrators with previous conviction for rape, physical assault, theft etc. The findings demonstrated a complex personality dynamic involved in the cycle of abuse and the evolution of criminal behavior, starting as a victim and ending as a perpetrator. This study provides a foundational understanding of the rape perpetration which may be used to further explore the psychology and neurobiology of the rape perpetrators.

Pubmed:
33225279: Mosili P, Maikoo S, Mabandla MV, Qulu L
The Pathogenesis of Fever-Induced Febrile Seizures and Its Current State.
Febrile seizures, commonly in children between the ages of 3 months to 5 years, are a neurological abnormality characterized by neuronal hyper-excitability, that occur as a result of an increased core body temperature during a fever, which was caused by an underlying systemic infection. Such infections cause the immune system to elicit an inflammatory response resulting in the release of cytokines from macrophages. The cytokines such as interleukin (IL)- 1β, IL-6, and tumour necrosis factor-α (TNF-α) combat the infection in the localized area ultimately spilling over into circulation resulting in elevated cytokine levels. The cytokines, along with pathogen-associated molecular patterns (PAMPs) expressed on pathogens for example, lipopolysaccharide (LPS), interact with the blood brain barrier (BBB) causing a 'leaky' BBB which facilitates cytokines and LPS entry into the central nervous system. The cytokines activate the microglia which release their own cytokines, specifically IL1β. IL-β interacts with the brain endothelium resulting in the activation of cyclooxygenase 2 which catalyzes the production of prostaglandin 2 (PGE2). PGE2 enters the hypothalamic region and induces a fever. Abnormally increased IL-1β levels also progressively increases excitatory (glutamatergic) neurotransmission, and decreases inhibitory (GABAergic) neurotransmission, thus mediating the pathogenesis of convulsions. Current treatments for febrile seizures present with side effects that are detrimental to health, which fosters the need for an alternative, more affordable treatment with fewer adverse side effects, and 1 that is easily accessible, especially in low income areas that are also affected by other underlying socio-economic factors, in which febrile seizures are of growing concern.
Neurosci Insights, 2020; 15

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BOARD NUMBER: S01-120

BURNOUT EVALUATION IN THE HEALTHCARE WORKERS DURING THE COVID-19

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Diana Galletta, Vittorio Freda, Roberta Rossi, Alessandro Vellucci, Niccolo' Solini, Maria Stella Vescio, Claudio Caiazza, Ester Picillo
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Background: Healthcare workers burnout is an under-recognized and under-reported problem, characterized by a state of mental exhaustion, detachment, and a decreased sense of personal accomplishment. Methods: A three months observational study will take place in the A.O.U. “Federico II”, Naples. The participants sample will be divided into three groups of healthcare workers: medical doctors (MD), nursing staff (NS) and unlicensed assistive personnel (UAP). Data will be collected both at the baseline (March to May 2022) and at the endpoint (March to May 2023). We will administer an Occupational Anamnesis (OA), the Link Burnout Questionnaire (24-LBQ), the Perceived Stress Scale (10-PSS) and the Work Ability Index (7-WAI), in order to evaluate their work-related stress. Results: Overall 400 workers agreed to participate to our observational study. The overall purpose of the study is to evaluate if there is a significant mean difference between the score of the three groups and if the variations between the baseline and the endpoint scores are significant. The other goal is to verify the correlations between the moderators of the OA and the scores of MD, NS and UAP. Conclusions: We expect to find a significant mean difference between the scores within the three groups and a significant mean difference between the baseline and the endpoint scores among the healthcare workers. We also suppose some of the moderators might have a significant correlation with the results of LBQ, PSS and WAI.

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BOARD NUMBER: S01-121

STRESS AND SLEEP DISORDERS AMONG INTERNATIONAL MEDICAL STUDENTS IN GEORGIA

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Irine Sakhelashvili[1], Karen Spruyt[2]
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Introduction: This study estimated prevalence rates of daytime sleepiness, stress, and pre-sleep arousals among international medical students by gender studying in Georgia. **Methods:** 207 international medical students of the European University (Georgia) participated in the study (November-December, 2018). Participants completed the Epworth Sleepiness Scale (ESS), Pre-Sleep Arousal Scale (PSAS), and Student-Life Stress Inventory (SLSI). **Results:** The average age of the participants was 21.31±2.27 years; 44.4% (92) were women. Most study participants reported higher-than-normal values in all categories of stress and sleep studied. A quarter of students (25.12%) reported excessive daytime sleepiness which was more common in male students. 97.1% of students reported high pre-sleep arousal. Also a high frequency of stress levels has been reported across all stress categories in 78% of participants; 79.9% in men, 87.8% in women. Nearly all (98.06%) participants reported high levels of total SLSI scores, however more women than men experienced a stressful student life. **Conclusion:** Foreign medical students in Georgia experience sleep problems and stress. Further research is needed to assess the relationship between sleep and stress parameters and identify predictors. But it is clear that universities need to implement preventive strategies to help international medical students. **Keywords:** Sleep; stress; daytime sleepiness; pre-sleep arousal, international medical student

Pubmed:
35053761: Basishvili T, Oniani N, Sakhelashvili I, Eliozishvili M, Khizanashvili M, Arabidze M, Tsaava M, Charekishvili T, Tsertsvadze N, Darchia N
Insomnia, Pre-Sleep Arousal, Psychosocial Factors and Changes in Sleep Pattern during the Second Wave Lockdown of the COVID-19 Pandemic in Georgia.
Studies performed across the COVID-19 pandemic waves point to the persistent impact of the pandemic on sleep and mental health. We expand these data by examining insomnia, pre-sleep arousal, psychosocial factors, and retrospective changes in sleep pattern during the COVID-19 second wave lockdown period in Georgia. Data were collected through an online survey (= 1117). The prevalence rate of probable insomnia disorder was 24.2%. Clinically relevant somatic and cognitive pre-sleep arousal was present in 49.8% and 58.0% of participants, and high levels of anxiety, depression and social isolation were found in 47.0%, 37.3%, 47.2% of respondents, respectively. We observed high prevalence rates of worse sleep quality, delayed bedtimes and risetimes, longer sleep latencies, higher awakenings and shorter sleep durations, relative to the pre-pandemic period. COVID-19-infected participants showed more severe sleep and mental problems. Specific predictors differentially affected insomnia, somatic and cognitive pre-sleep arousal. Depression and COVID-19 infection emerged as vulnerability factors for pre-sleep arousal, which, in turn, was associated with a higher predisposition to insomnia disorder. We confirm the strong deteriorating impact of the COVID-19 pandemic on sleep and psychosocial well-being during the second wave lockdown period. The specific association between pre-sleep arousal, insomnia, and psychosocial factors is of clinical relevance for the prevention of severity and persistence of sleep and mental problems across the repeated lockdown/reopening waves. Modulation of pre-sleep arousal may prove beneficial to implement targeted interventions.
Brain Sci, 2021; 12
33601229: Darchia N, Campbell IG, Basishvili T, Eliozishvili M, Tchintcharauli T, Oniani N, Sakhelashvili I, Shetekauri T, Oniani T, Feinberg I
Longitudinal assessment of NREM sleep EEG in typically developing and medication-free ADHD adolescents: first year results.
Clinical observation and structural MRI studies suggest that delayed brain maturation is a major cause of attention deficit hyperactivity disorder (ADHD). Sleep electroencephalogram (EEG) which exhibits major changes across adolescence provides an opportunity to investigate brain electrophysiology evidence for maturational delay. We present data from an ongoing longitudinal study of sleep EEG in medication-free ADHD and typically developing adolescents to investigate brain

electrophysiological evidence for this maturational delay.
Sleep Med, 2021; 80
30049991: Darchia N, Oniani N, Sakhelashvili I, Supatashvili M, Basishvili T, Eliozishvili M, Maisuradze L, Cervena K
Relationship between Sleep Disorders and Health Related Quality of Life-Results from the Georgia SOMNUS Study.
The extent to which sleep disorders are associated with impairment of health-related quality of life (HRQoL) is poorly described in the developing world. We investigated the prevalence and severity of various sleep disorders and their associations with HRQoL in an urban Georgian population. 395 volunteers (20⁻60 years) completed Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, STOP-Bang questionnaire, Insomnia Severity Index, Beck Depression Inventory-Short Form, and Short Form Health Survey (SF-12). Socio-demographic data and body mass index (BMI) were obtained. The prevalence of sleep disorders and their association with HRQoL was considerable. All SF-12 components and physical and mental component summaries (PCS, MCS) were significantly lower in poor sleepers, subjects with daytime sleepiness, apnea risk, or insomnia. Insomnia and apnea severity were also associated with lower scores on most SF-12 dimensions. The effect of insomnia severity was more pronounced on MCS, while apnea severity-on PCS. Hierarchical analyses showed that after controlling for potential confounding factors (demographics, depression, BMI), sleep quality significantly increased model's predictive power with an R^2 change (ΔR^2) by 3.5% for PCS (adjusted $R^2 = 0.27$) and by 2.9% for MCS (adjusted $R^2 = 0.48$); for the other SF-12 components ΔR^2 ranged between 1.4% and 4.6%. ESS, STOP-Bang, ISI scores, all exerted clear effects on PCS and MCS in an individual regression models. Our results confirm and extend the findings of studies from Western societies and strongly support the importance of sleep for HRQoL. Elaboration of intervention programs designed to strengthen sleep-related health care and thereof HRQoL is especially important in the developing world.
Int J Environ Res Public Health, 2018; 15
29982089: Sakhelashvili I, Eliozishvili M, Oniani N, Darchia N, Bruni O
Sleep and psycho-behavioral problems in internally displaced children in Georgia.
The aim of this study was to examine sleep and psycho-behavioral variables in Georgian Internally Displaced (ID) Children and their population-based controls.
Sleep Med, 2018; 50
28123823: Sakhelashvili I, Eliozishvili M, Basishvili T, Datunashvili M, Oniani N, Cervena K, Darchia N
Sleep-wake patterns and sleep quality in urban Georgia.
Sleep problems represent a worldwide health concern but their prevalence and impacts are unknown in most non-European/North American countries. This study aimed to evaluate sleep-wake patterns, sleep quality and potential correlates of poor sleep in a sample of the urban Georgian population.
Transl Neurosci, 2016; 7
26311481: Sakhelashvili I, Eliozishvili M, Lortkipanidze N, Oniani N, Cervena K, Darchia N
Sleep quality among internally displaced Georgian adolescents and population-based controls.
Sleep problems in children and adolescents are a significant public health concern and may be linked to a variety of psychoemotional difficulties. This study aimed to evaluate sleep quality and associated factors in conflict-affected Georgian adolescents after 9 months of forced displacement. Thirty-three internally displaced adolescents (mean age 11.4 years) and 33 adolescents (mean age 10.8 years) from the general population completed the Epworth Sleepiness Scale and the Children's Depression Inventory (CDI). Parents completed the Children's Sleep-Wake Scale and provided information on their socioeconomic status (SES) and the adolescents' sleep behavior, academic performance, and peer social relationships. The groups differed significantly in sleep quality, peer relationships, SES, and CDI scores. In the internally displaced group, the only significant predictor of sleep quality was SES, which increased the predictive capacity of the model (demographic and psychosocial variables) by 20% in the hierarchical analyses. The most significant predictor in the non-internally displaced group was CDI. This research indicates that displacement may affect sleep quality and psychosocial functioning. The importance of family SES as a contributing factor to displaced adolescents' poor sleep quality is highlighted. An integrated approach designed to improve the psychosocial environment of internally displaced adolescents is needed for their protection.
J Child Health Care, 2016; 20

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BOARD NUMBER: S01-122

NEUROETHICS GUIDANCE DOCUMENTS: PRINCIPLES, ANALYSIS, AND IMPLEMENTATION STRATEGIES

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Lucy Tournas[1], Karen Rommelfanger[2], Matthew O'Shaughnessy[3], Walter Johnson[4], Christopher Rozell[5]
[1]Arizona State University, School Of Life Sciences, Tempe, United States of America, [2]Emory University, School Of Medicine Departments Of Neurology And Psychiatry & Behavioral Sciences, Atlanta, United States of America, [3]Georgia Tech, School Of Electrical & Computer Engineering, Atlanta, United States of America, [4]Australian National University, School Of Regulation And Global Governance (regnet), Acton, Australia, [5]Georgia Institute of Technology, School Of Electrical & Computer Engineering, Atlanta, United States of America

Innovations in neurotechnologies have ignited conversations about ethics around the world, with implications for researchers, policymakers, and the private sector. The human rights impacts of neurotechnologies have drawn the attention of United Nations bodies; nearly 40 states are tasked with implementing the Organization for Economic Co-operation and Development's principles for responsible innovation in neurotechnology; and the United States is considering placing export controls on brain-computer interfaces. Against this backdrop, we offer the first review and analysis of neuroethics guidance documents recently issued by prominent government, private, and academic groups, focusing on commonalities and divergences in articulated goals; envisioned roles and responsibilities of different stakeholder groups; and the suggested role of the public. Drawing on lessons from the governance of other emerging technologies, we suggest implementation and evaluation strategies to guide practitioners and policymakers in operationalizing these ethical norms in research, business, and policy settings.

FENS Forum 2022
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BOARD NUMBER: S01-123

BEHAVIORAL TESTS ASSESSING NEUROPSYCHIATRIC ENDOPHENOTYPES IN ADOLESCENT MICE REVEAL STRAIN- AND SEX-SPECIFIC EFFECTS

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Claudia Pitzer[1], Barbara Kurpiers[1], Ahmed Eltokhi[2]
[1]University of Heidelberg, Interdisciplinary Neurobehavioral Core, heidelberg, Germany, [2]University of Washington, Department Of Pharmacology, Seattle, United States of America

Neuropsychiatric disorders including autism spectrum disorder, schizophrenia, attention deficit hyperactivity, and depression can be efficiently modeled and assessed in rodents via different sets of behavioral tests. Although adolescence is known to be a sensitive developmental stage for neuropsychiatric disorders in humans, most behavioral test batteries in the literature were performed in adult mice of different ages, missing valuable phenotypic information related to the effect of synaptic maturation during development and increasing the variabilities of the results between different studies. In a set of multiple studies, we explored the possibility of performing behavioral tests assessing different endophenotypes of neuropsychiatric disorders in very young male and female wild-type mice from three strains, C57BL/6N, DBA/2, and FVB/N. The tested endophenotypes included anxiety, social behaviors, cognitive functions, motor abnormalities and depression, covering the main behavioral impairments that occur in neuropsychiatric disorders. The results of our studies showed mainly that most of these endophenotypes can be assessed reliably in young adolescent mice, even the complex learning and memory functions. Additionally, our analysis revealed that these three strains displayed significant differences under certain behavioral paradigms. Although these tests were performed before puberty, mice revealed some sex-related differences in specific behavioral tests and depending on the tested strain. Our results provide new insights into discrete behaviors during development and emphasize the crucial importance of the genetic background, sex, and experimental settings in the age-dependent regulation of different behaviors related to neuropsychiatric disorders.

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BOARD NUMBER: S01-124

THE NEED FOR PRESERVATION OF MEDICAL DECISION MAKING WITH DIMINISHED COGNITIVE CAPACITY

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Jennifer Jin
Emory University, Bioethics, Atlanta, United States of America

Alzheimer's Disease (AD) and other neurodegenerative dementias are reaching epidemic proportions. Because of the way AD impacts brain pathology and pathways associated with higher level cognitive functions, a diagnosis can often lead to a presumed compromise in independent decision-making. This can call into question an impaired individual's ability to make sound medical decisions. However, decision-making capacity should not be treated as a binary concept, and simply having AD does not mean individuals are completely unable to make their own decisions regarding their medical care. Studies suggest that with assistance from tools and protocols, individuals with cognitive compromise can still demonstrate understanding and make independent decisions. To explore underlying assumptions regarding medical decision-making capacity in individuals with AD, we performed a neuroethical analysis with qualitative interviews, which revealed recurrent themes and knowledge gaps across four broad themes: 1. examination and evaluation of medical agency, 2. assignment of surrogacy, 3. current cognitive exams, 4. communication with various stakeholders about medical agency. We found that people generally need more time with diagnostic information to make lifestyle decisions and implement necessary changes to ensure their desires for the future. Impacted individuals and their families were not being equipped with important information needed to make such big decisions. This can create tension and distrust between the stakeholders involved but can be easily remedied by placing a greater emphasis on notifying patients early enough with the necessary information regarding their diagnosis and prognosis to process through them and discuss with their families on how to move forward.

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BOARD NUMBER: S01-125

UPPSALA UNIVERSITY BEHAVIORAL FACILITY (UUBF), YOUR NEW SCIENTIFIC PARTNER IN BEHAVIORAL STUDIES IN MICE RATS OR FISH

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Åsa Konradsson-Geuken[1], Klas Kullander[2], Stina Lundberg[1,2], Erika Roman[1,3], Svante Winberg[4]
[1]Uppsala University, Pharmaceutical Biosciences, Uppsala, Sweden, [2]Uppsala University, Department Of Immunology, Genetics And Pathology, Uppsala, Sweden, [3]Swedish University of Agricultural Sciences, Department Of Anatomy, Physiology And Biochemistry, Uppsala, Sweden, [4]Uppsala University, Department Of Medical Cell Biology, Uppsala, Sweden

Behavioral core facilities are becoming key features in academic institutes, because they provide expertise and specialized services for researchers within the field of neuroscience. Uppsala University Behavioral Facility (UUBF; https://www.uu.se/en/research/research-platforms/uubf/) is a non-profit core facility supported by the Faculty of Medicine and Pharmacy, Uppsala University, Sweden. With employed personnel, UUBF's main aims are to provide administration- and organization services for internal and external research groups. UUBF is a unique infrastructure, because it includes support and expertise for three of the most common vertebrate model organisms: mouse, rat and fishes (mainly focused on zebrafish), and also involve researchers investigating insect behaviors. UUBF provide equipment and protocols for behavioral experiments and assists with research in the range of species and methodologies used within UUBF. Examples include setting up behavioral tests for new species, behavioral and optogenetic integrations, and welfare studies as well as deep learning (DeepLabCut) approaches and statistical methodologies. UUBF also provides assistance with data analysis, interpretation of results, writing of ethical applications and training and guidance in experimental design. Furthermore, UUBF offers graduate courses on animal behavior e.g. "How to study behavior in vertebrates with focus on fish and rodents", and administers the Swedish Society for Neuroscience. You are welcome to contact UUBF (uubf@farmbio.uu.se) for discussing your future behavioral experiments.

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BOARD NUMBER: S01-126

DEVELOPMENT OF DIGITAL SENSITIVITY SCALE: DIGITAL LITERACY AND DIGITAL EFFICACY SELF-ASSESSMENT

POSTER SESSION 01 - SECTION: NEUROSCIENCE TOOLS, TECHNIQUES & ETHICS

Jin Young Park, Hae In Park, Ji Seon Ahn, Seul Bit Pi, Min Jeong Cho
Yongin Severance Hospital, Yonsei University College Of Medicine, Dept. Of Psychiatry, Yongin-si, Korea, Republic of

Introduction: This study reconceptualizes the definition of digital literacy and derives factors in order to develop a measurement tool for digital literacy, which is suitable for contemporary societies, while presenting self-efficacy as a personal characteristic that is expected to affect the use of digital technology based on related prior research. Method: As a result of an online survey conducted on 475 university students, 3 factors and 13 questions out of 18 preliminary questions for which the suitability index satisfies the standard were selected with exploratory and confirmatory factor analysis on the digital literacy measurement. 2 factors and 7 questions out of 9 preliminary questions for which the suitability index satisfies the standard were selected through exploratory and confirmatory factor analysis on the digital efficacy measurement. Results: 3 factors of digital literacy consisted of 6 questions of general use, 4 questions of digital attitude, and 3 questions of digital apply, and 2 factors of digital efficacy consisted of 4 questions of digital confidence and 3 questions of digital anxiety. A total of 20 questions were selected with the measurement tool of digital sensitivity. The developed measurement tool showed good results in reliability and validity evaluation and was verified as a suitable tool to measure digital literacy and digital efficacy. Conclusion The digital sensitivity measurement developed in this study can be usefully used to measure one's digital literacy in the form of self-diagnosis and to identify the individual's attitude toward digital technology use and resistance factors to the use of digital technology.

Funding: This work was supported by the Korea Medical Device Development Fund grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, the Ministry of Food and Drug Safety) (Project Number: 1711138277, KMDF_PR_20200901_0143).

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SPIKING NEURAL NETWORK SIMULATIONS REVEAL THE ROLE OF PLACE AND GRID CELLS IN SPATIAL LEARNING

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Much is known about the neural representations of space, e.g. place cells (PC) in the hippocampus and grid cells (GC) in the medial entorhinal cortex. However, the functional role of these neural spatial representations in driving behavior remains unclear. We therefore developed a spiking neural network model to study the role of PC and GC representations in spatial learning in a closed-loop simulation. The task was similar to the Morris-Watermaze, i.e., an artificial agent had to find a hidden goal in an open-field environment. The network consisted of either PCs or GCs, which projected to action selection neurons that formed a ring attractor. The location of the activity bump in this network determined the movement directions. We found that smaller place field sizes lead to better learning performance at the cost of requiring more PCs to tile the environment. Similarly, learning with GCs that have smaller grid spacings and smaller fields was superior to learning with large grid spacings. Since GCs have multiple firing fields, their spatial representation is ambiguous. As a result, we expected learning performance based on GC inputs to be lower, as compared to using PC inputs. Surprisingly, this was not the case. This counterintuitive result might be due to the fact that superpositions of multiple periodic grid maps results in unique representations of the spatial locations in the environment. Our work therefore shows that the function of neural representations does not simply follow common intuition and more computational modeling is required.

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BOARD NUMBER: S01-128

COMPUTATIONAL TOOL FOR COMPARING DEVELOPMENT OF CELLULAR-SCALE NETWORK ACTIVITY FROM MICROELECTRODE ARRAY (MEA) RECORDINGS OF 2D NEURONAL CULTURES AND 3D HUMAN CEREBRAL ORGANOIDS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

Susanna Mierau[1,2,3], Timothy Sit[4], Rachael Feord[1], Alexander Dunn[1], Jeremi Chabros[1], Elise Chang[1], Yin Yuan[1], David Oluigbo[2], Leo Nagy[1], Lance Burn[1], Hugo Smith[1], Erik Hemberg[5], Martin Hemberg[2,3], Madeline Lancaster[6], Andras Lakatos[7], Guillaume Hennequin[8], Stephen Eglen[9], Ole Paulsen[1]
[1]University of Cambridge, Physiology, Development And Neuroscience, Cambridge, United Kingdom, [2]Brigham & Women's Hospital, Neurology, Boston, United States of America, [3]Harvard Medical School, Neurology, Boston, United States of America, [4]University College London, Sainsbury Wellcome Centre, London, United Kingdom, [5]Massachusetts Institute of Technology, Csail, Cambridge, United States of America, [6]MRC Laboratory of Molecular Biology, Cell Biology, Cambridge, United Kingdom, [7]University of Cambridge, Cambridge Centre For Brain Repair, Cambridge, United Kingdom, [8]University of Cambridge, Engineering, Cambridge, United Kingdom, [9]University of Cambridge, Applied Mathematics & Theoretical Physics, Cambridge, United Kingdom

Microelectrode array (MEA) recordings are commonly used to compare neuronal activity in cultures. Yet MEA recordings can also reveal cellular-scale network activity in 2D cultures (Downes et al, 2012; Schroeter et al, 2015) and 3D human cerebral organoids (Giandomenico et al, 2019; Szebenyi et al, 2021). Graph theoretical metrics are frequently applied at larger spatial scales to model whole brain networks. However, few computational tools are available to assist neurobiologists in identifying cellular-scale network effects. Our aim is to provide a diagnostic tool for batch analysis of MEA experiments for comparing network function over time and conditions (e.g., genetic mutation). We created a MATLAB analysis pipeline for raw voltage time-series acquired from single- (Multichannel System) or multi-well MEAs (Axion). First, multiple threshold- and/or template-based spike detection methods can be compared, including our novel electrode-specific custom wavelets. Second, spiking and bursting activity are compared at the electrode and well/array level. Third, functional connectivity is inferred using the spike time tiling coefficient (Cutts & Eglen, 2014) and probabilistic thresholding to determine significant connections between activity observed at electrode pairs. Fourth, graph theoretical and other network metrics are compared by age and condition. Our pipeline reveals differences in spike and burst firing upon electrical or optogenetic stimulation. It recapitulates age-related differences in network topology from published data. In sum, the pipeline enables new users to perform MEA analysis beyond measures of activity or correlation alone to identify differences in network topology and roles of individual nodes in network activity.

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BOARD NUMBER: S01-129

INVESTIGATING LONG-TERM CONTEXT OF LANGUAGE MODELS ON BRAIN ACTIVITY DURING NARRATIVES LISTENING IN FMRI

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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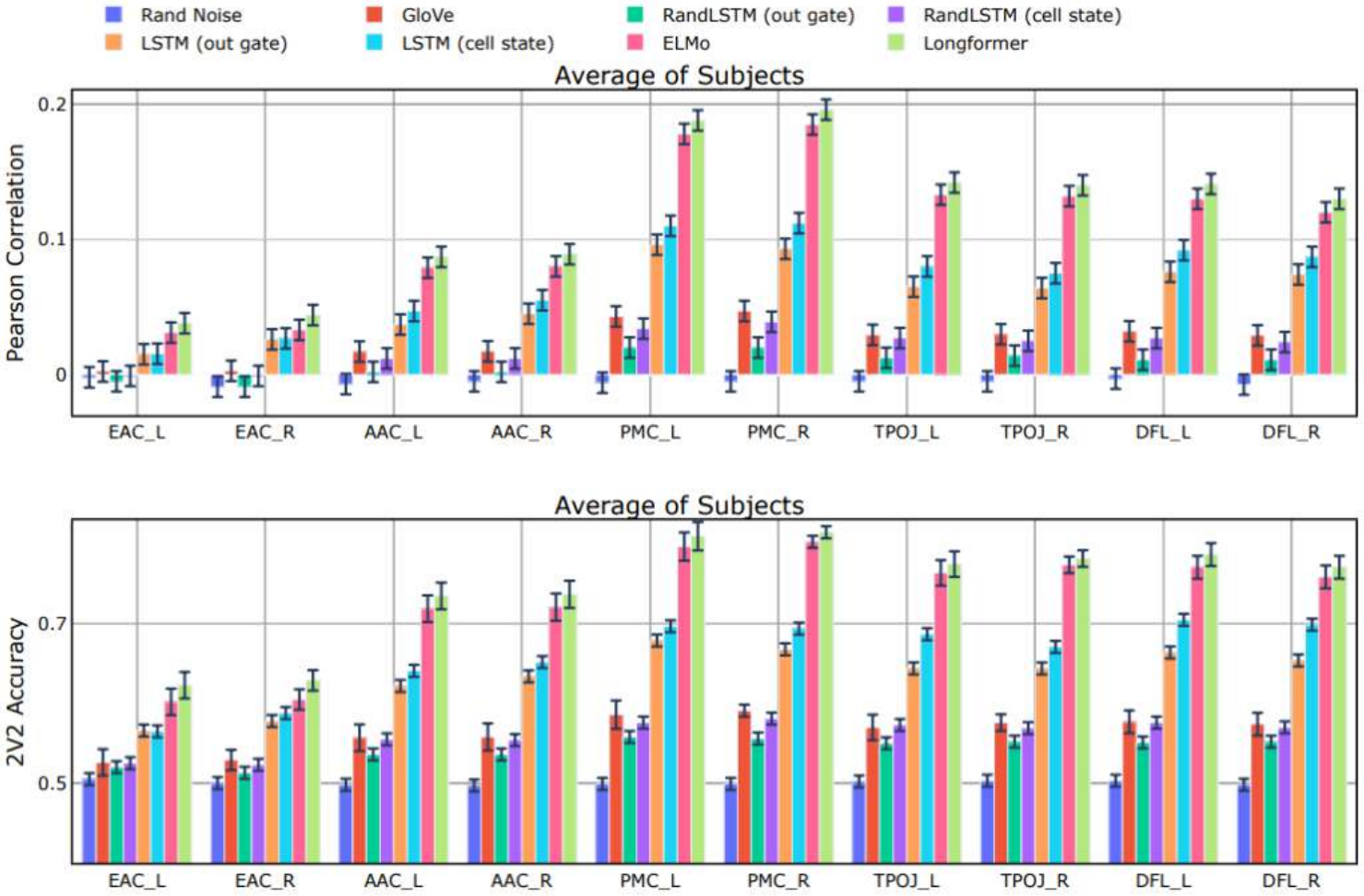
An interesting way to evaluate the representations obtained with machine learning language models is to compared them with human brain recordings. Encoding models have been used to partially predict fMRI recordings of different areas given the features of language models (such as Transformers). However, these models still lack long-term cognitive plausibility as well as insights on the underlying neural substrate mechanisms: e.g. how their representations differ across model layer depth and longer contexts. We study the influence of context representations of different language models such as sequence-based models: Long short-term memory networks (LSTMs), ELMo, and a popular pretrained Transformer language model (Longformer). In particular, we study how the internal hidden representations of such models are aligned with the fMRI brain activity. We use fMRI recordings of subjects listening to narrative stories to interpret word and sequence embedding representations. We further investigate how the representations of language model layers reveal better semantic context during listening. One of the novelties is that we look at several hidden states of LSTMs: cell and output gate states. Our computational experiments provide the following cognitive insights: (i) LSTM cell states are better aligned with brain recordings than LSTM output gate states: the cell state activity can represent more long-term information; (ii) the representations of ELMo and Longformer display a good predictive performance across brain regions for listening stimuli; (iii) Posterior Medial Cortex (PMC), Temporo-Parieto-Occipital junction (TPOJ) and Dorsal Frontal Lobe (DFL) have higher correlation versus Early Auditory (EAC) and Auditory Association Cortex

(AAC).



Pubmed:
21939760: Hinaut X, Dominey PF
A three-layered model of primate prefrontal cortex encodes identity and abstract categorical structure of behavioral sequences.
Categorical encoding is crucial for mastering large bodies of related sensory-motor experiences, but what is its neural substrate? In an effort to respond to this question, recent single-unit recording studies in the macaque lateral prefrontal cortex (LPFC) have demonstrated two characteristic forms of neural encoding of the sequential structure of the animal's sensory-motor experience. One population of neurons encodes the specific behavioral sequences. A second population of neurons encodes the sequence category (e.g. ABAB, AABB or AAAA) and does not differentiate sequences within the category (Shima, K., Isoda, M., Mushiake, H., Tanji, J., 2007. Categorization of behavioural sequences in the prefrontal cortex. Nature 445, 315-318.). Interestingly these neurons are intermingled in the lateral prefrontal cortex, and not topographically segregated. Thus, LPFC may provide a neurophysiological basis for sensorimotor categorization. Here we report on a neural network simulation study that reproduces and explains these results. We model a cortical circuit composed of three layers (infragranular, granular, and supragranular) of 5*5 leaky integrator neurons with a sigmoidal output function, and we examine 1000 such circuits running in parallel. Crucially the three layers are interconnected with recurrent connections, thus producing a dynamical system that is inherently sensitive to the spatiotemporal structure of the sequential inputs. The model is presented with 11 four-element sequences following Shima et al. We isolated one subpopulation of neurons each of whose activity predicts individual sequences, and a second population that predicts category independent of the specific sequence. We argue that a richly interconnected cortical circuit is capable of internally generating a neural representation of category membership, thus significantly extending the scope of recurrent network computation. In order to demonstrate that these representations can be used to create an explicit categorization capability, we introduced an additional neural structure

corresponding to the striatum. We showed that via cortico-striatal plasticity, neurons in the striatum could produce an explicit representation both of the identity of each sequence, and its category membership.
J Physiol Paris, 2011 Jan-Jun; 105
23383296: Hinaut X, Dominey PF
Real-time parallel processing of grammatical structure in the fronto-striatal system: a recurrent network simulation study using reservoir computing.
Sentence processing takes place in real-time. Previous words in the sentence can influence the processing of the current word in the timescale of hundreds of milliseconds. Recent neurophysiological studies in humans suggest that the fronto-striatal system (frontal cortex, and striatum--the major input locus of the basal ganglia) plays a crucial role in this process. The current research provides a possible explanation of how certain aspects of this real-time processing can occur, based on the dynamics of recurrent cortical networks, and plasticity in the cortico-striatal system. We simulate prefrontal area BA47 as a recurrent network that receives on-line input about word categories during sentence processing, with plastic connections between cortex and striatum. We exploit the homology between the cortico-striatal system and reservoir computing, where recurrent frontal cortical networks are the reservoir, and plastic cortico-striatal synapses are the readout. The system is trained on sentence-meaning pairs, where meaning is coded as activation in the striatum corresponding to the roles that different nouns and verbs play in the sentences. The model learns an extended set of grammatical constructions, and demonstrates the ability to generalize to novel constructions. It demonstrates how early in the sentence, a parallel set of predictions are made concerning the meaning, which are then confirmed or updated as the processing of the input sentence proceeds. It demonstrates how on-line responses to words are influenced by previous words in the sentence, and by previous sentences in the discourse, providing new insight into the neurophysiology of the P600 ERP scalp response to grammatical complexity. This demonstrates that a recurrent neural network can decode grammatical structure from sentences in real-time in order to generate a predictive representation of the meaning of the sentences. This can provide insight into the underlying mechanisms of human cortico-striatal function in sentence processing.
PLoS One, 2013; 8
34570710: Pedrelli L, Hinaut X
Hierarchical-Task Reservoir for Online Semantic Analysis From Continuous Speech.
In this article, we propose a novel architecture called hierarchical-task reservoir (HTR) suitable for real-time applications for which different levels of abstraction are available. We apply it to semantic role labeling (SRL) based on continuous speech recognition. Taking inspiration from the brain, this demonstrates the hierarchies of representations from perceptive to integrative areas, and we consider a hierarchy of four subtasks with increasing levels of abstraction (phone, word, part-of-speech (POS), and semantic role tags). These tasks are progressively learned by the layers of the HTR architecture. Interestingly, quantitative and qualitative results show that the hierarchical-task approach provides an advantage to improve the prediction. In particular, the qualitative results show that a shallow or a hierarchical reservoir, considered as baselines, does not produce estimations as good as the HTR model would. Moreover, we show that it is possible to further improve the accuracy of the model by designing skip connections and by considering word embedding (WE) in the internal representations. Overall, the HTR outperformed the other state-of-the-art reservoir-based approaches and it resulted in extremely efficient with respect to typical recurrent neural networks (RNNs) in deep learning (DL) [e.g., long short term memory (LSTMs)]. The HTR architecture is proposed as a step toward the modeling of online and hierarchical processes at work in the brain during language comprehension.
IEEE Trans Neural Netw Learn Syst, 2022; 33
31703171: Strock A, Hinaut X, Rougier NP
A Robust Model of Gated Working Memory.
Gated working memory is defined as the capacity of holding arbitrary information at any time in order to be used at a later time. Based on electrophysiological recordings, several computational models have tackled the problem using dedicated and explicit mechanisms. We propose instead to consider an implicit mechanism based on a random recurrent neural network. We introduce a robust yet simple reservoir model of gated working memory with instantaneous updates. The model is able to store an arbitrary real value at random time over an extended period of time. The dynamics of the model is a line attractor that learns to exploit reentry and a nonlinearity during the training phase using only a few representative values. A deeper study of the model shows that there is actually a large range of hyperparameters for which the results hold (e.g., number of neurons, sparsity, global weight scaling) such that any large enough population, mixing excitatory and inhibitory neurons, can quickly learn to realize such gated working memory. In a nutshell, with a minimal set of hypotheses, we show that we can have a robust model of working memory. This suggests this property could be an implicit property of any random population, that can be acquired through learning. Furthermore, considering working memory to be a physically open but functionally closed system, we give account on some counterintuitive electrophysiological recordings.
Neural Comput, 2020; 32

26335997: Hinaut X, Lance F, Droin C, Petit M, Pointeau G, Dominey PF
Corticostriatal response selection in sentence production: Insights from neural network simulation with reservoir computing.
Language production requires selection of the appropriate sentence structure to accommodate the communication goal of the speaker - the transmission of a particular meaning. Here we consider event meanings, in terms of predicates and thematic roles, and we address the problem that a given event can be described from multiple perspectives, which poses a problem of response selection. We present a model of response selection in sentence production that is inspired by the primate corticostriatal system. The model is implemented in the context of reservoir computing where the reservoir - a recurrent neural network with fixed connections - corresponds to cortex, and the readout corresponds to the striatum. We demonstrate robust learning, and generalization properties of the model, and demonstrate its cross linguistic capabilities in English and Japanese. The results contribute to the argument that the corticostriatal system plays a role in response selection in language production, and to the stance that reservoir computing is a valid potential model of corticostriatal processing.
Brain Lang, 2015; 150
24834050: Hinaut X, Petit M, Pointeau G, Dominey PF
Exploring the acquisition and production of grammatical constructions through human-robot interaction with echo state networks.
One of the principal functions of human language is to allow people to coordinate joint action. This includes the description of events, requests for action, and their organization in time. A crucial component of language acquisition is learning the grammatical structures that allow the expression of such complex meaning related to physical events. The current research investigates the learning of grammatical constructions and their temporal organization in the context of human-robot physical interaction with the embodied sensorimotor humanoid platform, the iCub. We demonstrate three noteworthy phenomena. First, a recurrent network model is used in conjunction with this robotic platform to learn the mappings between grammatical forms and predicate-argument representations of meanings related to events, and the robot's execution of these events in time. Second, this learning mechanism functions in the inverse sense, i.e., in a language production mode, where rather than executing commanded actions, the robot will describe the results of human generated actions. Finally, we collect data from naïve subjects who interact with the robot via spoken language, and demonstrate significant learning and generalization results. This allows us to conclude that such a neural language learning system not only helps to characterize and understand some aspects of human language acquisition, but also that it can be useful in adaptive human-robot interaction.
Front Neurorobot, 2014; 8

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BOARD NUMBER: S01-130

NEURONAL DYNAMICS IN THE CEREBELLUM DURING REACHING

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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The cerebellum is a fundamental structure of the hindbrain, and it is essential for the coordination of movements, balance and motor learning. Cerebellar computations are performed within functional microzones that process information in parallel and form distinct output streams. In the cerebellar cortex, sets of neighboring Purkinje cells belonging to the same microzone are organized in parasagittal bands. How neuronal activity is coordinated within each microzone is currently unknown. In this study, we have recorded simultaneously sets of neighboring cells activated during fast forelimb voluntary movements. Neighboring Purkinje cells exhibit millisecond-timescale synchrony during motor execution. This synchrony is maintained, embedded in high-frequency oscillations, during sleep and active exploration, suggesting that recurrent inhibition continuously shapes the correlations between neighboring cells. Our results indicate that during a fast and complex movement, local assemblies of Purkinje cells, whose projections converge in the cerebellar nuclei, dynamically form at the millisecond time scale and will thus produce very transient episodes of inhibition in the cerebellar nuclei. Thanks to a novel approach based on penalized likelihood fits of the discharge of neurons we were able to discriminate the correlations at different time scales and obtain confidence intervals for the significance of such correlations.

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SENSORY REPRESENTATION VARIABILITY DURING NEURODEVELOPMENT IN FRAGILE X SYNDROME

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Atypical sensory processing, including hypersensitivity to tactile, visual or auditory stimuli, is a common challenge across Autism Spectrum Disorders (ASD). It can affect behavioural performance on sensory discrimination tasks and lead to various other symptoms, such as anxiety, attention deficit, and learning disability. Recent studies in humans have associated this trait with high trial-to-trial variability in responses to sensory stimuli, as recorded by EEG and fMRI; however, the underlying circuit mechanisms are not yet well understood. We studied the *Fmr1* knockout (*Fmr1*$^{-/-}$) mouse model of Fragile-X Syndrome, the most common single-gene (FMR1) cause of ASD, to identify analogous alterations at the level of neural population coding. We performed longitudinal in vivo 2-photon calcium imaging to record spontaneous and whisker-evoked activity in layer 2/3 neurons in primary somatosensory cortex (S1) of lightly anaesthetised wild-type (WT) and *Fmr1*$^{-/-}$ mice. Repeated bouts of whisker stimuli (1 sec-long, every 3 sec) were delivered at physiologically natural frequencies (5 or 10 Hz). Our experimental design enabled the tracking of individual neuron activity across a critical window of S1 development between P14 and P19, which coincides with high expression of *Fmr1*. We found that, despite similar overall statistics, within-animal heterogeneity in neuronal activity was greater in *Fmr1*$^{-/-}$ mice. Although decoding accuracy (how well the L2/3 population could discriminate the stimuli) was similar between WT and *Fmr1*$^{-/-}$ mice at both ages, we observed different dynamics during the inter-stimulus intervals, in the time scale of seconds. We suggest a relation to observed differences in response attenuation and reliability.

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BOARD NUMBER: S01-132

A CHARACTERIZATION OF THE NEURAL REPRESENTATION OF CONFIDENCE DURING PROBABILISTIC LEARNING WITH 7T FMRI

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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In a stochastic and uncertain world, learning is difficult. Nonetheless, humans estimate the probability of future events with remarkable accuracy based on past observations. The Bayesian brain hypothesis posits that the human brain uses uncertainty (or conversely, confidence) to update its beliefs and make predictions. **Aim:** The current study aims to characterize the neural representation of confidence during probabilistic learning in the human brain. **Methods:** We measured the neural activity of 26 human adults during a probability-learning task with 7T fMRI. Participants were shown binary sequences of visual stimuli drawn from a Bernoulli process whose generative probabilities changed abruptly and without notice. Subjects were instructed to learn those probabilities and to occasionally report them along with their estimation confidence. We analyzed the latent variables of learning with a Bayesian ideal observer model that distinguishes confidence from prediction, predictability and surprise. **Results:** Reported probability estimates and confidence levels correlated with the Bayesian solution. fMRI analyses revealed the existence of a frontoparietal network whose activity is 1) sensitive to confidence (linearly correlated), 2) specific to confidence rather than confounded with surprise and predictability (included as covariates, and whose correlates did not overlap with those of confidence at the subject-level), 3) invariant to which item is predicted in the sequence, and 4) functional because it predicts subjective confidence reports. **Conclusions:** By locating neural correlates of confidence that meet four general criteria of neural representations (sensitivity, specificity, invariance and functionality), our findings contribute to the understanding of the neural mechanisms of probabilistic learning.

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THETA CYCLE SKIPPING IN SEPTAL NUCLEI DURING EXECUTION OF SPATIAL ALTERNATION TASK

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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The ability to navigate the environment in search for food and shelter is crucial for the survival of mammals. An internal representation of the environment – or map – allows animals to evaluate multiple routes and adapt their navigation strategy to current needs and future goals. This spatial cognition depends on position-related activity in the hippocampal formation. Septal nuclei receive strong projections from hippocampus, therefore it is a very good candidate to link different aspects of spatial information between hippocampal formation and other brain regions. In this study, we compared the spatial firing properties of cells in different subdivisions of the septal region in rats that performed a spatial alternation task. Using a chronically implanted Neuropixels probe, we recorded from 1561 cells in lateral septum (LS), medial septum (MS) and septohippocampal nuclei (SHi). We observed that during locomotion, intrinsic theta rhythmicity (6-12 Hz) of cells was strongest in the dorsal LS and MS, and weaker in the SHi. We identified cells in dorsal LS and SHi that fired on alternate theta cycles (cycle skipping cells). We found more skipping cells on the outbound journeys, with a vast majority firing on alternate theta cycle while the animal approached the choice point. In contrast, very few cells in MS showed theta cycle skipping behavior. Finally, we observed that cells in dorsal LS convey more spatial information than cells in other septal subdivisions. These results highlight the functional complexity of the septal region and suggest that different subregions have distinct contributions to spatial navigation.

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EMERGENCE OF OPTIMAL STRUCTURED MIXED SELECTIVITY IN THE PRIMATE PREFRONTAL CORTEX DURING LEARNING

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Aims. Primates can represent task rules in an abstract format and re-use these codes in novel contexts to facilitate learning. Although advances have been made in elucidating the neuronal geometry of these codes, how such representations emerge in the prefrontal cortex (PFC) over learning is still poorly understood. Here, we explored learning-induced changes to the dynamics of the macaque PFC using a learning paradigm that encourages abstraction and generalisation of task rules (XOR). *Methods*. We developed new, theoretically principled metrics to measure how the selectivity of cells to task variables changes over learning. Critically, we compared two models of selectivity: (1) *random mixed selectivity*, which assumes that cell selectivity is randomly distributed in the space spanned by both linear and nonlinear mixtures of task variables, and (2) *optimal structured mixed selectivity*, which we derived mathematically and confirmed using simulations, in which linear selectivity is precisely balanced with nonlinear selectivity to optimally perform an XOR task. *Results*. When animals learned a single XOR mapping, we found that selectivity profiles diverged from random towards the optimal model. When an additional XOR mapping was introduced, previously learned cell selectivity remained non-random, and selectivity for the new mapping also converged towards the optimal, non-random, model. *Conclusions.* We found that PFC cells became functionally similar over learning. Our results also indicate that to solve an XOR mapping, non-linear selectivity strikes a balance with linear selectivity. This solution was generalised across different instances of the same rule allowing for an abstract task structure to be utilised.

Pubmed:
33595078: Żochowska A, Nowicka MM, Wójcik MJ, Nowicka A
Self-face and emotional faces-are they alike?
The image of one's own face is a particularly distinctive feature of the self. The self-face differs from other faces not only in respect of its familiarity but also in respect of its subjective emotional significance and saliency. The current study aimed at elucidating similarities/dissimilarities between processing of one's own face and emotional faces: happy faces (based on the self-positive bias) and fearful faces (because of their high perceptual saliency, a feature shared with self-face). Electroencephalogram data were collected in the group of 30 participants who performed a simple detection task. Event-related potential analyses indicated significantly increased P3 and late positive potential amplitudes to the self-face in comparison to all other faces: fearful, happy and neutral. Permutation tests confirmed the differences between the self-face and all three types of other faces for numerous electrode sites and in broad time windows. Representational similarity analysis, in turn, revealed distinct processing of the self-face and did not provide any evidence in favour of similarities between the self-face and emotional (either negative or positive) faces. These findings strongly suggest that the self-face processing do not resemble those of emotional faces, thus implying that prioritized self-referential processing is driven by the subjective relevance of one's own face.
Soc Cogn Affect Neurosci, 2021; 16
32707162: Doradzińska Ł, Wójcik MJ, Paź M, Nowicka MM, Nowicka A, Bola M
Unconscious perception of one's own name modulates amplitude of the P3B ERP component.
The P3 event-related potential has been known for over 50 years, but its function is still a matter of ongoing debate. Current theories interpret P3b either as a correlate of perceptual consciousness, or as reflecting cognitive processes, like working memory and executive functions. Unexpectedly, recent studies indicate that P3b might occur in response to unconsciously

presented stimuli which, if further replicated, will be important for defining its functional role. Therefore, in the present study we investigated the P3b component in response to participants' own name - a stimulus which is subjectively extremely salient and known to evoke a robust P3b response. The self-name and control (other) names were presented supra- and subliminally (backward-masked), in a subjective rating task and in a forced-choice identification task. We found that a consciously perceived self-name evoked a P3b of larger amplitude than the other-name in both tasks, which confirms that the self-name was processed preferentially. When the self-name was presented subliminally it was associated with larger P3b amplitude than the other-name in the identification task, but not in the subjective rating task. This indicates that a salient stimulus can in principle modulate the P3b amplitude even when processed outside of awareness, but also that subliminal processing depends on the task-set and top-down factors. Taken as a whole, our results provide evidence against the interpretation of P3b as a correlate of consciousness (and more generally conflict with the Global Workspace Theory) and will allow a more precise description of the relation between P3b and cognitive processes.
Neuropsychologia, 2020; 147
30785866: Wójcik MJ, Nowicka MM, Bola M, Nowicka A
Unconscious Detection of One's Own Image.
A key mechanism behind preferential processing of self-related information might be an early and automatic capture of attention. Therefore, the present study tested a hypothesis that one's own face will attract bottom-up attention even without conscious identification. To test this, we used a dot-probe paradigm with electrophysiological recordings, in which participants (N = 18) viewed masked and unmasked pairs of faces (other, self) presented laterally. Analysis of the sensitivity measure d ' indicated that faces were not consciously identified in the masked condition. A clear N2 posterior-contralateral (N2pc) component (a neural marker of attention shifts) was found in both the masked and unmasked conditions, revealing that one's own face automatically captures attention when processed unconsciously. Therefore, our study (a) demonstrates that self-related information is boosted at an early (preconscious) stage of processing, (b) identifies further features (beyond simple physical ones) that cause automatic attention capture, and (c) provides further evidence for the dissociative nature of attention and consciousness.
Psychol Sci, 2019; 30
30011309: Nowicka MM, Wójcik MJ, Kotlewska I, Bola M, Nowicka A
The impact of self-esteem on the preferential processing of self-related information: Electrophysiological correlates of explicit self vs. other evaluation.
Preferential processing of self-related information is a well-documented phenomenon on both the behavioral and neural levels. However, the impact of self-esteem on this self-preference has not been studied in a systematic way. Here, the electrophysiological correlates of explicit self-reflection were investigated in individuals with low (LSE) and high self-esteem (HSE). Participants evaluated trait adjectives in reference to the self or to an "other" person (close-other, famous) while EEG was recorded. The analysis of event-related potentials focused on the late positive component (LPC), which exhibits a fronto-central distribution and latency over 500 ms. In both LSE and HSE groups, the amplitudes of LPC were enhanced in the self condition when compared to control conditions (both close-other and famous). Crucially, LPC amplitudes in the HSE group were significantly higher than in the LSE group. Moreover, the self-preference effect, defined as the difference between amplitudes of LPC associated with the evaluation of words in relation to oneself vs. other people, was significantly higher in the HSE group than in the LSE group. Overall, our findings indicate that people with high self-esteem tend to engage in self-referential processing to a higher extent.
PLoS One, 2018; 13
29375456: Wójcik MJ, Nowicka MM, Kotlewska I, Nowicka A
Self-face Captures, Holds, and Biases Attention.
The implicit self-recognition process may take place already in the pre-attentive stages of perception. After a silent stimulus has captured attention, it is passed on to the attentive stage where it can affect decision making and responding. Numerous studies show that the presence of self-referential information affects almost every cognitive level. These effects may share a common and fundamental basis in an attentional mechanism, conceptualized as attentional bias: the exaggerated deployment of attentional resources to a salient stimulus. A gold standard in attentional bias research is the dot-probe paradigm. In this task, a prominent stimulus (cue) and a neutral stimulus are presented in different spatial locations, followed by the presentation of a target. In the current study we aimed at investigating whether the self-face captures, holds and biases attention when presented as a task-irrelevant stimulus. In two dot-probe experiments coupled with the event-related potential (ERP) technique we analyzed the following relevant ERPs components: N2pc and SPCN which reflect attentional shifts and the maintenance of attention, respectively. An inter-stimulus interval separating face-cues and probes (800 ms) was introduced only in the first experiment. In line with our predictions, in Experiment 1 the self-face elicited the N2pc and the SPCN component. In Experiment 2 in addition to N2pc, an attentional bias was observed. Our results indicate that unintentional self-face processing disables the top-down control setting to filter out distractors, thus leading to the

engagement of attentional resources and visual short-term memory.
Front Psychol, 2017; 8

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CORRELATION PATTERNS IN WORKING-MEMORY TASKS: FMRI FRACTAL AND SPECTRAL ANALYSIS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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We study differences in fMRI correlation patterns of short-term memory distortions. We considered resting state (RS) and four experimental tasks: two visual-verbal (V; based on lists of semantically or phonetically associated words) and two non-verbal (NV; based on pictures of similar objects) in memorisation and retrieval phases.
Since functional activations have a non-trivial temporal and spatial correlation structure, we quantified the regional activity (116-region AAL atlas) with the Hurst exponent, detrended fluctuation analysis and non-linear cross-correlations (CC). Collective behaviour of CC was analysed through eigenvalues of their matrices using a novel method of grouping them according to eigenvector similarity across subjects. The signals in specific occipital lobe areas depended both on the type of experimental tasks and on information memorisation or retrieval. A particularly apparent difference was visible between memorisation in V and NV tasks. In the former, for some brain regions in the Visual II RS network, the Hurst exponents are very close to 0.5, indicating a lack of linear temporal correlations; in the latter, we observe persistent behaviour. The reduction of exponents in tasks relative to the spontaneous brain activity in RS is significant in many brain areas. We additionally uncovered regionally coordinated changes by comparing CC eigenvalue distributions. They showed the greatest differences between: RS and other tasks, memorisation and retrieval, V and NV tasks. We confirmed that temporal correlations are a clear regionally dependent discriminant of tasks and that, methodologically, the non-linear correlations are more sensitive to differences than linear ones and grouped eigenvalues to ungrouped.

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BOARD NUMBER: S01-136

EVENT-RELATED VARIABILITY IS MODULATED BY TASK AND DEVELOPMENT

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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In carefully designed experiments, cognitive scientists interpret mean event-related potentials (ERP) in terms of cognitive operations. However, the huge signal variability from one trial to the next, questions the representability of such mean events. Here we explored whether this variability is an unwanted noise, or an informative part of the neural response. We took advantage of the rapid changes in the visual system during human infancy and analyzed the variability of visual responses to central and lateralized faces in 2-to 6-month-old infants and adults using high-density electroencephalography (EEG). We observed that that the effects of stimulus on the spontaneous background fluctuations is only "modulatory" rather than determinant. I.e. Neural trajectories of individual trials always remain very far from ERP components, only moderately bending their direction with a substantial temporal jitter across trials. In infants nonetheless, the temporal structure of these weak modulations depend on the difficulty of the task at hand possibly dependent on the top-down attention at this age. Dynamical systems interpretation of these results allows us to suggest that the Event-Related Potential (ERP) components act as weakly attracting modes of a low-dimensional energy landscape in which neural trajectories are transiently constrained due to stimulus arrival.However, single trial trajectories displayed characteristic patterns of acceleration and deceleration when approaching ERP components, as if they were under the active influence of steering forces causing transient attraction and stabilization. Our approaches to characterize Event Related Variability (ERV) allows more faithful description of ERPs in terms of brain state kinematics of neural trajectories.

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BOARD NUMBER: S01-137

DOES THE BRAIN CARE ABOUT AVERAGES? A SIMPLE TEST

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Trial-averaged metrics, e.g. tuning curves or population response vectors, are a basic and ubiquitous way of characterizing neuronal activity. But how relevant are such trial-averaged responses to neuronal computation itself? Here we present a simple test to estimate whether average responses reflect aspects of neuronal activity that contribute to neuronal processing. The test probes two assumptions inherent in the usage of average neuronal metrics: 1. Reliability: Neuronal responses repeat consistently enough across trials that the averageresponse template remains recognizable to downstream regions. 2. Behavioural relevance: If a single-trial response is more similar to the average template, it will be easier for the animal to respond correctly. We apply this test to a large publicly available data set featuring electrophysiological recordings from 71 brain areas in behaving mice. We show that single-trial responses were less correlated to the average response template than would be expected if they represented discrete versions of the template. Moreover, single-trial responses could not be clearly assigned to the template of one stimulus over another. Crucially, better-matched single-trial responses did not predict correct behaviour. We conclude that in this dataset, average responses do not seem particularly relevant to neuronal computation, and we encourage other researchers to apply similar tests when using trial-averaged neuronal metrics.

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INTRINSIC NEURAL EXCITABILITY INDUCES TIME-DEPENDENT OVERLAP OF MEMORY ENGRAMS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Memories are thought to be stored in neural ensembles known as engrams that are specifically reactivated during memory recall. According to recent studies, memory engrams of two events that happened close in time tend to overlap in the hippocampus and the amygdala, and this overlap has been shown to support memory linking. It has been hypothesised that these overlaps arise from the mechanisms that regulate memory allocation itself, involving neural excitability, but the exact process remains unclear. Indeed, most theoretical studies focus on synaptic plasticity and little is known about the role of intrinsic plasticity, which could be mediated by neural excitability and serve as a complementary mechanism for forming memory engrams. Here, we developed a rate-based model that includes neural excitability as a variable threshold of the firing rate input-output function. We obtained overlapping memory engrams for contexts that are presented close in time, consistent with experimental studies. Moreover, we showed that increasing the initial excitability of a subset of neurons just before presenting a context biases the memory allocation to these neurons. We then explored the role of global inhibition as a way of controlling competition between neurons from two ensembles. These results have identified the possible mechanisms underlying the role of intrinsic excitability in memory allocation and linking, and now allow for predictions regarding the dynamics of memory engrams.

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A DUAL PATHWAY ARCHITECTURE FOR VOCAL LEARNING IN SONGBIRDS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Juvenile songbirds learn to imitate adult vocalizations. This behaviour is governed by a dedicated neural circuitry, comprising a cortical pathway required for vocal production and a parallel basal ganglia-thalamo-cortical (BG) pathway necessary for plasticity. The BG pathway induces variability in production during vocal exploration, receives a performance signal via midbrain dopaminergic projections and drives a motor bias that rectifies errors. This dopamine-modulated change in vocal output is gradually consolidated within the cortical pathway. Reinforcement learning has been hypothesized to underlie such sensorimotor learning. However, pure RL approaches may result in non-optimal solutions under uneven reward contours in a continuous action space. We re-interpret the role of the dual pathway architecture in songbirds to address these limitations. We posit that the BG pathway conducts exploration by inducing large daily jumps in vocal exploration. As the cortical pathway matures, it gradually consolidates BG reward-modulated exploration using Hebbian learning. We demonstrate how this process essentially implements simulated annealing. The dual pathway architecture converges at the global optimum in a number of trials, comparable with the learning period of songbirds (60 days, 1000 trials per day). We further emulate an experimental protocol to induce plasticity in adults by locally modulating the reward profile around the global optimum, post-convergence. As observed empirically, the network adapts to this change in reward contour and modifies its vocal exploration profile. To further test this interpretation, we contrast with ongoing electro-physiological investigations in the output nuclei of these two pathways, in zebra finches subjected to a distorted feedback protocol.

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BOARD NUMBER: S01-140

A REALISTIC SPIKING CEREBELLAR MODEL IN CLOSED-LOOP PREDICTS THE UNDERLYING NEURAL MECHANISMS OF EYEBLINK CONDITIONING IN BEHAVING HEALTHY AND PATHOLOGICAL MICE

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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During Eye-Blink Classical Conditioning (EBCC), the cerebellum learns to associate two time-locked stimuli, such that a conditioned stimulus can elicit a motor response (eyelid closure) anticipating the action of the unconditioned stimulus. Experimental findings suggest that this association results from integration of multiple neuronal and synaptic mechanisms in the cerebellar cortex, which regulate the Deep Cerebellar Nuclei (DCN) output and hence the behavioural response. Here, we used a data-driven Spiking Neural Network (SNN) of the cerebellum in a control loop to test the empirical hypothesis that plasticity in the inhibitory molecular layer is crucial for EBCC. The SNN was reconstructed from neuronal densities in different layers, morphological and geometrical features of the circuit and local connectivity rules (Fig. 1A). Neurons were represented as advanced point models, retaining the main electroresponsive features of cerebellar neurons. By exploiting *ad hoc* Spike-Timing Dependent Plasticity (STDP) rules, parallel fiber-Purkinje cell (pf-PC) and parallel fiber-Molecular Layer Interneuron (pf-MLI) connections underwent long-term plasticity driving learning. The model reproduced the EBCC learning curve with neural activities quite similar to those recorded in mice (Fig. 1B). By switching-off pf-PC STDP and MLI inhibition to reproduce mutant mice alterations, learning was compromised, matching the dysfunctional behavioural curves observed in experiments. Our simulations demonstrate that plasticity in the MLI inhibitory pathway is indeed necessary and sufficient to account for a prominent component of learning in EBCC. Distributed cortical plasticity, feedforward inhibition to PC, and DCN rebound bursting were identified as key elements for learning, eventually compensating for localized lesions.

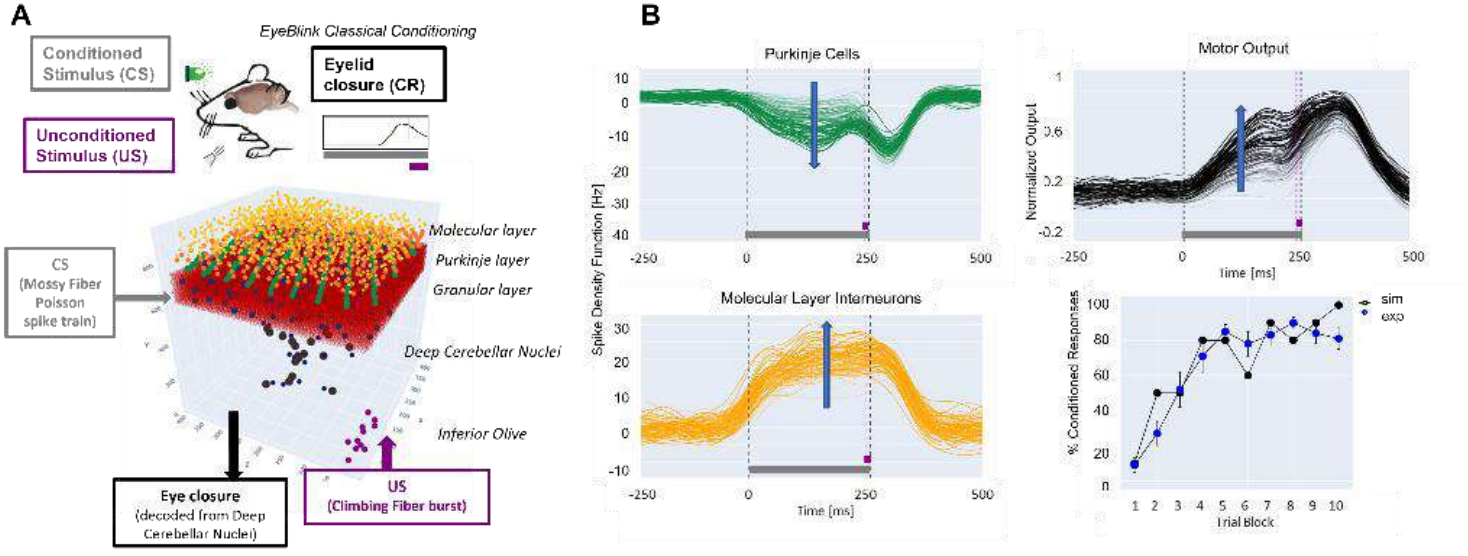

Figure 1. SNN simulations of EyeBlink Conditioning. (A) Schematic representation of EBC, cerebellar SNN with cerebellar cortex, nuclei and Inferior Olive volumes, input/output EBC signals encoding/decoding in the network neural populations. (B) EBC simulations in control conditions, with Spike Density Function of PC and MLI changing throughout learning trials (from light to dark colours), output motor response decoded from DCN activity (from light to dark black lines) and corresponding percentage of conditioned responses throughout trial blocks, comparing simulation and mice experiments.

Pubmed:

28264639: Geminiani A, Casellato C, Antonietti A, D'Angelo E, Pedrocchi A
A Multiple-Plasticity Spiking Neural Network Embedded in a Closed-Loop Control System to Model Cerebellar Pathologies.
The cerebellum plays a crucial role in sensorimotor control and cerebellar disorders compromise adaptation and learning of motor responses. However, the link between alterations at network level and cerebellar dysfunction is still unclear. In principle, this understanding would benefit of the development of an artificial system embedding the salient neuronal and plastic properties of the cerebellum and operating in closed-loop. To this aim, we have exploited a realistic spiking computational model of the cerebellum to analyze the network correlates of cerebellar impairment. The model was modified to reproduce three different damages of the cerebellar cortex: (i) a loss of the main output neurons (Purkinje Cells), (ii) a lesion to the main cerebellar afferents (Mossy Fibers), and (iii) a damage to a major mechanism of synaptic plasticity (Long Term Depression). The modified network models were challenged with an Eye-Blink Classical Conditioning test, a standard learning paradigm used to evaluate cerebellar impairment, in which the outcome was compared to reference results obtained in human or animal experiments. In all cases, the model reproduced the partial and delayed conditioning typical of the pathologies, indicating that an intact cerebellar cortex functionality is required to accelerate learning by transferring acquired information to the cerebellar nuclei. Interestingly, depending on the type of lesion, the redistribution of synaptic plasticity and response timing varied greatly generating specific adaptation patterns. Thus, not only the present work extends the generalization capabilities of the cerebellar spiking model to pathological cases, but also predicts how changes at the neuronal level are distributed across the network, making it usable to infer cerebellar circuit alterations occurring in cerebellar pathologies.
Int J Neural Syst, 2018; 28
26736803: Antonietti A, Casellato C, Geminiani A, D'Angelo E, Pedrocchi A
Healthy and pathological cerebellar Spiking Neural Networks in Vestibulo-Ocular Reflex.
Since the Marr-Albus model, computational neuroscientists have been developing a variety of models of the cerebellum, with different approaches and features. In this work, we developed and tested realistic artificial Spiking Neural Networks inspired to this brain region. We tested in computational simulations of the Vestibulo-Ocular Reflex protocol three different models: a network equipped with a single plasticity site, at the cortical level; a network equipped with a distributed plasticity, at both cortical and nuclear levels; a network with a pathological plasticity mechanism at the cortical level. We analyzed the learning performance of the three different models, highlighting the behavioral differences among them. We proved that the model with a distributed plasticity produces a faster and more accurate cerebellar response, especially during a second session of acquisition, compared with the single plasticity model. Furthermore, the pathological model shows an impaired learning capability in Vestibulo-Ocular Reflex acquisition, as found in neurophysiological studies. The effect of the different plasticity conditions, which change fast and slow dynamics, memory consolidation and, in general, learning capabilities of the cerebellar network, explains differences in the behavioral outcome.
Annu Int Conf IEEE Eng Med Biol Soc, 2015; 2015
30559658: Geminiani A, Casellato C, Locatelli F, Prestori F, Pedrocchi A, D'Angelo E
Complex Dynamics in Simplified Neuronal Models: Reproducing Golgi Cell Electroresponsiveness.
Brain neurons exhibit complex electroresponsive properties - including intrinsic subthreshold oscillations and pacemaking, resonance and phase-reset - which are thought to play a critical role in controlling neural network dynamics. Although these properties emerge from detailed representations of molecular-level mechanisms in "realistic" models, they cannot usually be generated by simplified neuronal models (although these may show spike-frequency adaptation and bursting). We report here that this whole set of properties can be generated by the (E-GLIF) neuron model. E-GLIF derives from the GLIF model family and is therefore mono-compartmental, keeps the limited computational load typical of a linear low-dimensional system, admits analytical solutions and can be tuned through gradient-descent algorithms. Importantly, E-GLIF is designed to maintain a correspondence between model parameters and neuronal membrane mechanisms through a minimum set of equations. In order to test its potential, E-GLIF was used to model a specific neuron showing rich and complex electroresponsiveness, the cerebellar Golgi cell, and was validated against experimental electrophysiological data recorded from Golgi cells in acute cerebellar slices. During simulations, E-GLIF was activated by stimulus patterns, including current steps and synaptic inputs, identical to those used for the experiments. The results demonstrate that E-GLIF can reproduce the whole set of complex neuronal dynamics typical of these neurons - including intensity-frequency curves, spike-frequency adaptation, post-inhibitory rebound bursting, spontaneous subthreshold oscillations, resonance, and phase-reset - providing a new effective tool to investigate brain dynamics in large-scale simulations.
Front Neuroinform, 2018; 12
31244635: Geminiani A, Casellato C, D'Angelo E, Pedrocchi A
Complex Electroresponsive Dynamics in Olivocerebellar Neurons Represented With Extended-Generalized Leaky Integrate and Fire Models.

The neurons of the olivocerebellar circuit exhibit complex electroresponsive dynamics, which are thought to play a fundamental role for network entraining, plasticity induction, signal processing, and noise filtering. In order to reproduce these properties in single-point neuron models, we have optimized the Extended-Generalized Leaky Integrate and Fire (E-GLIF) neuron through a multi-objective gradient-based algorithm targeting the desired input-output relationships. In this way, E-GLIF was tuned toward the unique input-output properties of Golgi cells, granule cells, Purkinje cells, molecular layer interneurons, deep cerebellar nuclei cells, and inferior olivary cells. E-GLIF proved able to simulate the complex cell-specific electroresponsive dynamics of the main olivocerebellar neurons including pacemaking, adaptation, bursting, post-inhibitory rebound excitation, subthreshold oscillations, resonance, and phase reset. The integration of these E-GLIF point-neuron models into olivocerebellar Spiking Neural Networks will allow to evaluate the impact of complex electroresponsive dynamics at the higher scales, up to motor behavior, in closed-loop simulations of sensorimotor tasks.
Front Comput Neurosci, 2019; 13

31632258: Geminiani A, Pedrocchi A, D'Angelo E, Casellato C
Response Dynamics in an Olivocerebellar Spiking Neural Network With Non-linear Neuron Properties.
Sensorimotor signals are integrated and processed by the cerebellar circuit to predict accurate control of actions. In order to investigate how single neuron dynamics and geometrical modular connectivity affect cerebellar processing, we have built an olivocerebellar Spiking Neural Network (SNN) based on a novel simplification algorithm for single point models (Extended Generalized Leaky Integrate and Fire, EGLIF) capturing essential non-linear neuronal dynamics (e.g., pacemaking, bursting, adaptation, oscillation and resonance). EGLIF models specifically tuned for each neuron type were embedded into an olivocerebellar scaffold reproducing realistic spatial organization and physiological convergence and divergence ratios of connections. In order to emulate the circuit involved in an eye blink response to two associated stimuli, we modeled two adjacent olivocerebellar microcomplexes with a common mossy fiber input but different climbing fiber inputs (either on or off). EGLIF-SNN model simulations revealed the emergence of fundamental response properties in Purkinje cells (burst-pause) and deep nuclei cells (pause-burst) similar to those reported . The expression of these properties depended on the specific activation of climbing fibers in the microcomplexes and did not emerge with scaffold models using simplified point neurons. This result supports the importance of embedding SNNs with realistic neuronal dynamics and appropriate connectivity and anticipates the scale-up of EGLIF-SNN and the embedding of plasticity rules required to investigate cerebellar functioning at multiple scales.
Front Comput Neurosci, 2019; 13

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DATA-DRIVEN MODEL OF MULTI-PROTEIN ACTIVITY OUTLINES SYNAPTIC PLASTICITY RULES

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Despite the circuit function that drives memory and learning being an area of intense study over the last decades, the exact mechanisms that underlie the function are not fully understood. Using data-driven modeling techniques, we aim to develop an experimentally testable computational framework that links dendritic molecular dynamics to synaptic plasticity rules, ultimately bridging this knowledge gap. Using data obtained from the mouse model of Tuberous Sclerosis (TSC) Disease, associated with learning disabilities and autistic behavior, we study the disturbed synaptic plasticity and hyperactive mammalian target of rapamycin (mTOR) signaling pathway that regulates protein synthesis. Comparing the difference in synaptic composition between wild type (WT) and mutants (MT), related to mTOR hyperactivity, gives us insight into the crucial characteristics that drive synaptic plasticity and allows us to build an effective computational model. After analyzing both homogenates and synaptosomes from WT and MT mice at discrete timepoints during early development, we discovered a complex temporal dynamic network involving multiple potentially interacting proteins. Thus, we were able to construct a mathematical framework consisting of a system of Ordinary Differential Equations (ODE) that can (1) explain the interaction among synaptic proteins participating in the mTOR pathway, revealing the mechanism underlying the observed protein activity and (2) fill in information about experimentally unobserved timepoints. In conclusion, our work shows how the experimentally observed protein concentrations in WT and MT can be used to gain insight into the underlying protein interaction network and the dynamics across timepoints.

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WORLD STRUCTURE INFERENCE BY HIPPOCAMPAL REPLAY

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

Zhenrui Liao[1,2], Darian Hadjiabadi[3], Satoshi Terada[2], Ivan Soltesz[4], Attila Losonczy[1]
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Memory consolidation assimilates noisy experiences into a cognitive map of the world. This process requires the replay of learned sequences in the hippocampus, though the content of these sequences remains controversial. Recent work has shown that the statistics of replay deviate significantly from those of experience: stimuli which are experientially salient may be either selected or suppressed from replay. We propose a parsimonious novel mechanism for this phenomenon: the use of the symmetric spike-time dependent plasticity rule (sSTDP), previously reported at CA3 excitatory synapses, to remodel inhibitory synapses as well. We show using three levels of modeling--spiking network, detailed biophysical, and abstract normative--that this mechanism enables efficient inference of the latent statistical structure of the world given noisy observations. We develop a mathematical theory of how sSTDP shapes sequence dynamics in a recurrent network, and prove that replay, viewed as a statistical estimator of a latent sequence, converges asymptotically to the true sequence. Finally, we make a number of predictions illustrating the power of inhibitory plasticity as a conceptual advance in our understanding of hippocampal dynamics and memory consolidation, foremost that CA3 replay consolidates "world structure'' rather than specific experience. Our experimental and theoretical work here outlines a potential direct link between the synaptic and cognitive levels of memory consolidation.

Pubmed:

34880499: Terada S, Geiller T, Liao Z, O'Hare J, Vancura B, Losonczy A
Adaptive stimulus selection for consolidation in the hippocampus.
Associative memories guide behavioural adaptation by binding together outcome-predictive sensory stimuli. However, in a feature-rich environment, only a subset of stimuli may predict a desired outcome. How neural circuits enable behavioural adaptation by selectively and durably representing subsets of sensory stimuli that are pertinent to a specific outcome is not known. We investigated this feature selection process in the hippocampus during memory acquisition and subsequent consolidation. Two-photon calcium imaging of CA3 axonal projections to CA1 combined with simultaneous local field potential recordings revealed that CA3 projections that encode behaviourally informative sensory stimuli were selectively recruited during the memory replay events that underlie hippocampal memory consolidation. These axonal projections formed sequential assemblies that conjunctively link sensory features to spatial location and thus reward proximity. By contrast, axons encoding uninformative, peripatetic sensory cues were notably suppressed during memory replay. Thus, while the hippocampus comprehensively encodes the real-time sensory environment, it implements a flexible filtering mechanism to maximize the utility of memories destined for long-term storage. We propose that utility-dependent recruitment of sensory experience during memory consolidation is a general coding principle for associative learning.
Nature, 2022; 601
34648750: Dudok B, Szoboszlay M, Paul A, Klein PM, Liao Z, Hwaun E, Szabo GG, Geiller T, Vancura B, Wang BS, McKenzie S, Homidan J, Klaver LMF, English DF, Huang ZJ, Buzsáki G, Losonczy A, Soltesz I
Recruitment and inhibitory action of hippocampal axo-axonic cells during behavior.
The axon initial segment of hippocampal pyramidal cells is a key subcellular compartment for action potential generation, under GABAergic control by the "chandelier" or axo-axonic cells (AACs). Although AACs are the only cellular source of GABA targeting the initial segment, their in vivo activity patterns and influence over pyramidal cell dynamics are not well understood. We achieved cell-type-specific genetic access to AACs in mice and show that AACs in the hippocampal area CA1 are synchronously activated by episodes of locomotion or whisking during rest. Bidirectional intervention experiments in head-restrained mice performing a random foraging task revealed that AACs inhibit CA1 pyramidal cells, indicating that the effect of GABA on the initial segments in the hippocampus is inhibitory in vivo. Finally, optogenetic inhibition of AACs at specific track locations induced remapping of pyramidal cell place fields. These results demonstrate brain-state-specific dynamics of a

critical inhibitory controller of cortical circuits.
Neuron, 2021; 109
34197732: Hadjiabadi D, Lovett-Barron M, Raikov IG, Sparks FT, Liao Z, Baraban SC, Leskovec J, Losonczy A, Deisseroth K, Soltesz I
Maximally selective single-cell target for circuit control in epilepsy models.
Neurological and psychiatric disorders are associated with pathological neural dynamics. The fundamental connectivity patterns of cell-cell communication networks that enable pathological dynamics to emerge remain unknown. Here, we studied epileptic circuits using a newly developed computational pipeline that leveraged single-cell calcium imaging of larval zebrafish and chronically epileptic mice, biologically constrained effective connectivity modeling, and higher-order motif-focused network analysis. We uncovered a novel functional cell type that preferentially emerged in the preseizure state, the superhub, that was unusually richly connected to the rest of the network through feedforward motifs, critically enhancing downstream excitation. Perturbation simulations indicated that disconnecting superhubs was significantly more effective in stabilizing epileptic circuits than disconnecting hub cells that were defined traditionally by connection count. In the dentate gyrus of chronically epileptic mice, superhubs were predominately modeled adult-born granule cells. Collectively, these results predict a new maximally selective and minimally invasive cellular target for seizure control.
Neuron, 2021; 109
30713030: Turi GF, Li WK, Chavlis S, Pandi I, O'Hare J, Priestley JB, Grosmark AD, Liao Z, Ladow M, Zhang JF, Zemelman BV, Poirazi P, Losonczy A
Vasoactive Intestinal Polypeptide-Expressing Interneurons in the Hippocampus Support Goal-Oriented Spatial Learning.
Diverse computations in the neocortex are aided by specialized GABAergic interneurons (INs), which selectively target other INs. However, much less is known about how these canonical disinhibitory circuit motifs contribute to network operations supporting spatial navigation and learning in the hippocampus. Using chronic two-photon calcium imaging in mice performing random foraging or goal-oriented learning tasks, we found that vasoactive intestinal polypeptide-expressing (VIP), disinhibitory INs in hippocampal area CA1 form functional subpopulations defined by their modulation by behavioral states and task demands. Optogenetic manipulations of VIP INs and computational modeling further showed that VIP disinhibition is necessary for goal-directed learning and related reorganization of hippocampal pyramidal cell population dynamics. Our results demonstrate that disinhibitory circuits in the hippocampus play an active role in supporting spatial learning. VIDEO ABSTRACT.
Neuron, 2019; 101
28869582: Zaremba JD, Diamantopoulou A, Danielson NB, Grosmark AD, Kaifosh PW, Bowler JC, Liao Z, Sparks FT, Gogos JA, Losonczy A
Impaired hippocampal place cell dynamics in a mouse model of the 22q11.2 deletion.
Hippocampal place cells represent the cellular substrate of episodic memory. Place cell ensembles reorganize to support learning but must also maintain stable representations to facilitate memory recall. Despite extensive research, the learning-related role of place cell dynamics in health and disease remains elusive. Using chronic two-photon Ca imaging in hippocampal area CA1 of wild-type and Df(16)A mice, an animal model of 22q11.2 deletion syndrome, one of the most common genetic risk factors for cognitive dysfunction and schizophrenia, we found that goal-oriented learning in wild-type mice was supported by stable spatial maps and robust remapping of place fields toward the goal location. Df(16)A mice showed a significant learning deficit accompanied by reduced spatial map stability and the absence of goal-directed place cell reorganization. These results expand our understanding of the hippocampal ensemble dynamics supporting cognitive flexibility and demonstrate their importance in a model of 22q11.2-associated cognitive dysfunction.
Nat Neurosci, 2017; 20
33262339: Sparks FT, Liao Z, Li W, Grosmark A, Soltesz I, Losonczy A
Hippocampal adult-born granule cells drive network activity in a mouse model of chronic temporal lobe epilepsy.
Temporal lobe epilepsy (TLE) is characterized by recurrent seizures driven by synchronous neuronal activity. The reorganization of the dentate gyrus (DG) in TLE may create pathological conduction pathways for synchronous discharges in the temporal lobe, though critical microcircuit-level detail is missing from this pathophysiological intuition. In particular, the relative contribution of adult-born (abGC) and mature (mGC) granule cells to epileptiform network events remains unknown. We assess dynamics of abGCs and mGCs during interictal epileptiform discharges (IEDs) in mice with TLE as well as sharp-wave ripples (SPW-Rs) in healthy mice, and find that abGCs and mGCs are desynchronized and differentially recruited by IEDs compared to SPW-Rs. We introduce a neural topic model to explain these observations, and find that epileptic DG networks organize into disjoint, cell-type specific pathological ensembles in which abGCs play an outsized role. Our results characterize identified GC subpopulation dynamics in TLE, and reveal a specific contribution of abGCs to IEDs.
Nat Commun, 2020; 11


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CO-TUNED, BALANCED EXCITATION AND INHIBITION IN OLFACTORY MEMORY NETWORKS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Friedrich Miescher Institute for biomedical research, Neurobiology, Basel, Switzerland

Odor memories are exceptionally robust and essential for the survival of many species. In rodents, the olfactory cortex shows features of an autoassociative memory network and plays a key role in the retrieval of olfactory memories. Interestingly, the telencephalic area Dp, the zebrafish homolog of olfactory cortex, transiently enters a state of precise balance during the presentation of an odor. This state is characterized by large synaptic conductances (relative to the resting conductance) and by co-tuning of excitation and inhibition in odor space and in time at the level of individual neurons. Our aim is to understand how this precise balance affects memory function. For this purpose, we build a simplified, yet biologically plausible spiking neural network model of Dp using experimental observations as constraints. Co-tuning of excitation and inhibition is achieved when we structure and increase connectivity among ensembles of both excitatory and inhibitory neurons, which could reflect the formation of olfactory memories. This corroborates recent evidence from other species which suggests that memory traces are not purely encoded by excitatory neurons, as classically described. We then investigate the benefits of co-tuning for olfactory and memory processing. Among others, tuned inhibition stabilizes activity and enhances the retrieval of stored information from partial or degraded sensory inputs, which is relevant in light of the instability of the olfactory environment. These findings provide valuable insights into the computations performed by the olfactory cortex, and into general effects of balanced state dynamics in associative memory networks.

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INTERPERSONAL ALIGNMENT OF SHARED BELIEFS BY DYNAMIC COUPLING OF NEURAL EVIDENCE ACCUMULATION TO SOCIAL EXCHANGE OF CONFIDENCE

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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[1]Ludwig Maximilian University of Munich, Graduate School Of Systemic Neurosciences Gsn-lmu, Munich, Germany, [2]Institute for Research in Fundamental Sciences (IPM), School Of Cognitive Sciences, Tehran, Iran, [3]Ludwig Maximillian University, Department Of General Psychology And Education, Munich, Germany

Private, subjective beliefs about uncertainty have been found to have idiosyncratic computational and neural substrates yet, humans share such beliefs seamlessly and cooperate successfully. Bringing together decision making under uncertainty and interpersonal alignment in communication, we examined the neuro-computational basis of the relationship between privately-held and socially-shared uncertainty. Examining confidence-speed-accuracy tradeoff in uncertainty-ridden perceptual decisions under social vs isolated context, we found that shared (i.e., reported confidence) and subjective (inferred from pupillometry) uncertainty dynamically followed social information. An attractor neural network model incorporating social information as top-down additive input captured the observed behavior and spontaneously demonstrated the emergence of confidence matching in virtual dyadic simulations. Electroencephalography showed that social exchange of confidence modulated the neural signature of evidence accumulation in the central parietal cortex and produced a sustained a top-down flow of information from prefrontal to parietal cortex. Our findings offer a neural population model for interpersonal alignment of shared beliefs.

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MODELING REWARD-DRIVEN DECISION MAKING USING BIOPHYSICALLY REALISTIC ADEX MEAN-FIELD MODELS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

Emre Baspinar[1], Gloria Cecchini[2], Michael Depass[2], Ignasi Cos[2], Ruben Moreno-Bote[3], Alain Destexhe[1]
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The Adaptive Exponential (AdEx) mean-field framework describes the averaged neuronal population behavior modeled by AdEx network. In the case of cerebral cortex, AdEx networks are used to model two cell types: Regular Spiking (RS) neurons, displaying spike-frequency adaptation as observed in excitatory pyramidal neurons, and Fast Spiking (FS) neurons, with no adaptation, as observed in inhibitory interneurons. AdEx networks are high dimensional, complex and difficult to analyze. AdEx mean-field models are low dimensional, simpler and easier to analyze compared to networks, yet they approximate closely the network dynamics, motivating our choice of model. Here, we extend the AdEx mean-field framework to model two networks of excitatory-inhibitory neurons, representing two cortical columns, and interconnected with excitatory connections contacting both RS and FS cells. Thus, this connection scheme introduces bicolumnar competition. Each column represents a pool of neurons making the decision in favor of one of two choices represented by two partially filled bars on a computer screen. Task is based on maximizing total reward provided at the end of each episode consisting of a number of trials. The total reward depends on the coherency between choices of the subject and implemented strategy. A reward-driven online learning mechanism allows the model to capture the implemented strategy, as well as subject exploratory behavior. We compare simulation results to performance data obtained from human subjects. Finally, this model provides a biophysical ground for simpler phenomenological models proposed for similar decision-making tasks and can be applied to neurophysiological data obtained from the monkey brain.

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DON’T STOP THE TRAINING: CONTINUOUSLY-UPDATING SELF-SUPERVISED ALGORITHMS BEST ACCOUNT FOR AUDITORY RESPONSES IN THE CORTEX.

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Cortical responses to sensory inputs are history dependent, taking into accounts a wide context. Frozen deep neural network exhibit sensory representations that do not integrate this wider context. Numerous studies have shown that these representations are similar to those of the mammalian in that their activations linearly map onto cortical responses to the same sensory inputs. However, it is unclear if unfrozen artificial networks integrate historical content like the brain. To address this issue, we analyze the brain responses of two ferret auditory cortices recorded with functional UltraSound imaging (fUS), while the animals were presented with 320 10\,s sounds. We compare these brain responses to the activations of Wav2vec 2.0, a self-supervised neural network pretrained with 960\,h of speech, and input with the same 320 sounds. Critically, we evaluate Wav2vec 2.0 under two distinct modes: (i) "Pretrained", where the same model is used for all sounds, and (ii) "Continuous Update", where the weights of the pretrained model are modified with back-propagation after every sound, presented in the same order as the ferrets. Our results show that the Continuous-Update mode leads Wav2Vec 2.0 to generate activations that are more similar to the brain than a Pretrained Wav2Vec 2.0 or than other control models using different training modes. These results suggest that the trial-by-trial modifications of self-supervised algorithms induced by back-propagation aligns with the corresponding fluctuations of cortical responses to sounds. Our finding thus provides empirical evidence of a common learning mechanism between self-supervised models and the mammalian cortex during sound processing.

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HOW CEREBELLAR ARCHITECTURE FACILITATES RAPID ONLINE LEARNING

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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The cerebellum is critically involved in motor control, refining trajectories as movements are being executed. This requires fast, online learning. What features of cerebellar circuit structure make it particularly suited to online learning? The cerebellum has a distinctive circuit architecture in which each mossy fibre input typically projects to 250 granule cells, a population that comprises more than half of the neurons in the brain. Each granule cell forms ~4 synapses with mossy fibres. The main hypotheses for this sparse input expansion are that it facilitates pattern separation and smooth function approximation. However, we currently lack a theory that explains why this architecture is suited to online motor learning. We show that the large input expansion allows rapid and accurate learning in an online context. We consider a cerebellar-like network with sparse connections that map low-dimensional inputs into a high-dimensional internal, 'granule cell' layer. The network is tasked with simultaneously learning an internal model of a motor system, and using this model to better control motor output. Learning online introduces a narrow time window that severely limits the information available for synaptic plasticity mechanisms to appropriately adjust synaptic weights. We find that the effect of having limited information depends on the spread of the eigenvalues Hessian of the task error. As the input expansion increases, the geometry of the error surface becomes more favourable for online learning, diminishing the effect of information error and allowing for faster learning.

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CONDUCTION BLOCK STIMULATION OPTIMIZATION BY ENVELOPE MODULATION TOWARD THE REDUCTION OF ONSET RESPONSE

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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The electrical stimulation of peripheral nerves is used in various pathological contexts, from functional/sensory rehabilitation, to chronic pains and epilepsy[1]. Among the techniques inducing specific axonal response, the high-frequency (>kHz) conduction-block has been of growing interest[2] and have also been approved for post-amputation pain syndrome. However, this technique generates unwanted onset response at the start of the stimulation[3]. [4] proposed to reduce onset response by controlling the sodium channel closed-states proportions. Our aim was to develop an optimization algorithm that minimises the parasitic effect induced by the onset response. We developed an approach using recognized models[3] enabling correct quantification of the onset-response as observed in-vivo. We minimise the onset-response phenomenologically, based on spike counting. This results in a highly non-linear problem that we treated using Particle Swarm Optimization. Stimuli were coded using sinusoidal high-frequency modulated by an envelope coded with piecewise-splines. Our formulation of the problem enabled the optimization algorithm to converge and find a solution of enveloppe generating no onset-response. Moreover, we obtained several solutions, thus proving the non-uniqueness of such stimuli. We explored the solutions regularity. This opens the perspective of only-blocking stimulation. Our current work is to apply optimization algorithms on large fascicule to explore the minimization of the onset-response at the nerve-level and in-vivo validation of these waveshapes. [1]Slavin et al. Karger Medical and Scientific Publishers, 2015.
[2]Patel, Buttera Journal of neural engineering 15.3 (2018): 031002.
[3]Bhadra et al. Journal of computational neuroscience 22.3 (2007): 313-326.
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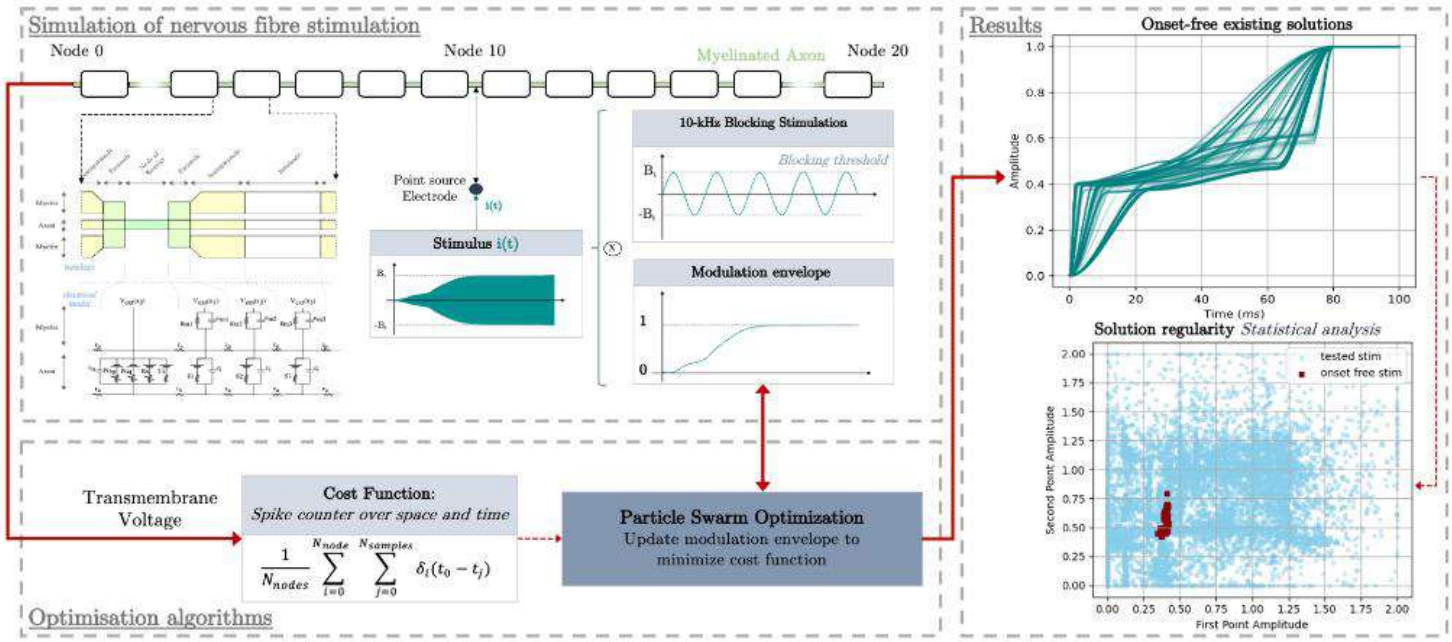

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DIFFERENCES IN POPULATION SPARSITY CAN EXPLAIN DIFFERENT MEMORY CONSOLIDATION SPEEDS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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During systems memory consolidation, memories that were first stored in the hippocampus are transferred to the neocortex where they can survive for much longer time periods. The speed and success of such a learning process in the neocortex can vary [Tse et al., Science (2007)]. Our work aims at explaining the relation between the learning/consolidation speed of a network and its population sparsity. We address this question theoretically by using a feedforward network model to which uncorrelated input patterns are successively presented. Following a Hebbian learning scenario, synapses between active input and output neurons are turned on with a chosen transition probability; a fixed connectivity level is maintained by turning others off. An output sparsity level is enforced by imposing an activation threshold on the output neurons. We compute the storage capacity as the number of subsequent patterns that can be stored before a memory can't be successfully retrieved anymore [e.g. Leibold/Kempter, Cerebral Cortex (2008)]. We find that the optimal transition probability strongly depends on the sparsity level - the sparser the output, the higher the optimal transition probability. Thus, for sparse representations, the system can obtain a high storage capacity while learning/consolidating fast. We conclude that, in the context of systems memory consolidation, the consolidation process could be faster for memories that are related to sparser (than usual) representations in neocortical areas. This could guide us towards a neurophysiological understanding of so-called schemas - supposed knowledge bases in the neocortex which facilitate the consolidation of new schema-consistent memories.

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A COMPUTATIONAL MODEL OF THE ROLE OF HIPPOCAMPAL REPLAY FOR REWARD- AND PUNISHMENT-BASED SPATIAL LEARNING IN MICE

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Exploratory behaviour in a novel environment is altered when rewards or punishments are introduced. Despite appearing as opposites, these conditions cannot be modelled symmetrically. Reward will favour approach and multiple occasions to reinforce this knowledge, while punishment will generate as few visits as possible, thus minimal opportunities to learn from direct exposure. Hippocampal replays are reactivations of neuronal activity in place cells that represent spatially and temporary coherent paths and that play a fundamental role in spatial memory and learning. We suggest that hippocampal replay mechanisms are critical in avoidance learning tasks since they act as compensation mechanisms to overcome the sampling bias towards reward. To assess this hypothesis, we have designed a new experimental protocol where 8 mice are exposed to a negative stimulus, always in the end of the same arm of an U-shape maze, after being able to explore the entire maze freely. Then, we developed a computational behavioural model for the exploration of the mice prior to the stimuli, that also includes a Reinforcement Learning (RL) component that intervenes in the avoidance learning phase. After fitting the model on the data and performing model comparison, we found that standard RL cannot account for mice behaviour in the post-learning phase of the task, but RL with replay can better replicate the behaviour. In future works, the same method will be applied on reward-conditioning data in the same task, to compare the relative contribution of hippocampal replay in learning punishment or reward.

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TEMPORAL CONTINUITY AND LEARNING AUDITORY OBJECTS: IMPLICATIONS FOR SOLVING THE “COCKTAIL PARTY PROBLEM” AND AUDITORY PERCEPTION

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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A fundamental problem animals face is the unsupervised/minimally supervised learning of objects. Auditory objects present special challenges because they superpose, as opposed to occlude, and because of the large variability within auditory categories. A famous example of the challenge of learning and discriminating auditory objects is the “cocktail party problem”', learning and discriminating vocalizations amid the clutter of many similar stimuli. The mechanisms by which the brain solves this problem are not fully elucidated. One solution suggested by efficient coding theory is that neural circuits exploit the statistical regularities of the environment to solve perceptual tasks. Temporal regularities are a defining feature of many ethologically relevant stimuli, such as human speech and animal vocalizations. Accordingly, we hypothesize that efficient learning and discrimination of auditory objects can be achieved by training circuits to extract temporal regularities features. It follows from this hypothesis that unsupervised learning of the temporal regularities in stimuli (e.g., vocalizations), can support learning to discriminate between different vocalizations. To this end, we applied Slow Feature Analysis (SFA) to extract temporally regular features, i.e., those that vary in time as slowly as possible, from macaque vocalizations. Consistent with our hypothesis, we found that a linear classifier could discriminate effectively between vocalizations projected onto only five of the features found by SFA. These results suggest that the ``slow'' temporal features of auditory stimuli may be sufficient for parsing auditory scenes, providing a powerful computational mechanism that the brain utilizes for auditory perception.

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ROLE OF INHIBITION FOR THE FORMATION OF NEURAL ASSEMBLIES IN PLASTIC NEURAL NETWORKS SUBJECT TO SELECTIVE STIMULI

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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We investigate the role of inhibition in structuring new neural assemblies following a learning phase in the brain. Although many studies have been devoted to this subject, few of them have considered the specific role of inhibitory neurons. Therefore, this work aims at studying the impact of inhibition in adaptive neural networks. In particular we focus on the role of synchronized dynamics for the creation and maintenance of structural modules in the brain circuits.
Specifically, we considered an excitatory-inhibitory neural network made of θ-neurons with plastic Hebbian synapses. The learning process is mimicked by stimulating different areas of the network with temporal alternating external stimuli. This stimulation process leads to the emergence of modular structures. Then, the network dynamics is analysed after the learning phase revealing the maintenance of co-active neural assemblies over the long term.
We show that the presence of inhibitory neurons controls the emergence of the modular structures and their maintenance in the resting phase following the learning period. Furthermore, we demonstrate that the number of inhibitory neurons in the network is directly related to the maximal number of neural assemblies that can be maintained.
These results support the idea that inhibition has a direct impact on the memory capacity of a neural network.

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REVISITING THE CEREBELLAR MEMORY CONSOLIDATION MECHANISM FROM AI PERSPECTIVE: THE CEREBELLUM AS A DUAL LEARNING MACHINE

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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The cerebellum is known for the critical site for motor learning, and many studies have been conducted to explore the neural circuits and mechanisms responsible for the memory formation and consolidation. Although they have provided detailed observations in several cerebellum-dependent motor learning paradigms, our knowledge of the underlying processes remains fragmentary. In this study, we employ statistical learning theory in machine learning to propose a novel framework that can explain why and how the cerebellum learns and consolidates memories by transfer mechanism. We model the cerebellar system as dual learning machine which composed of two systems with different dimensions-cerebellar cortex and vestibular nuclei. The cerebellar cortex represents a “complex” system, which can be characterized as complex representation, fast adaptation, but relatively large overheads. The vestibular nuclei represent a “simple” system, which can be characterized as simple representation, slow adaption, but low overheads. Based on modified empirical risk minimization theory, we predicted that adaptive learning occurs first in the cerebellar cortex and simple components are then transferred to the vestibular nuclei, and the extent and timing of the transfer can vary depending on the task difficulty. Predictions were validated with both computer simulation and optomotor behavioral experiments. In this study, we tried to model and interpret the cerebellar system from the machine learning perspective. This framework can provide a comprehensive understanding on the cerebellar learning and contribute to further elucidating the essence of cerebellar computations.

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MODELING NEUROMODULATORY-MEDIATED MODIFICATIONS OF CALCIUM-BASED PLASTICITY RULES THAT PREVENT HOMEOSTATIC RESET DURING SWITCHES IN FIRING ACTIVITY

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Synaptic plasticity refers to the ability of neurons to change their connections based on the correlation level in the activity of neighboring neurons. In parallel, neuron activity is affected by brain states. For example, during wake-sleep cycles, neurons switch from tonic to bursting activity. This firing pattern fluctuation is organized by neuromodulators - signaling molecules that induce reversible changes in functional properties of neurons or synapses. In our previous work, we have shown that synaptic plasticity rules such as triplet [Pfister,2006] and calcium-dependent models [Graupner,2016; Shouval,2002] are *not compatible* with switches in firing patterns. Using a conductance-based model robust to neuromodulation and synaptic plasticity [Jacquerie,2021], we built a cortical network to study the evolution of synaptic weights during switches in firing activity. We reproduced experimental plasticity protocol validated in wakefulness. Then, switching the network from tonic to burst without any modification of the synaptic rule leads to a *homeostatic reset*. All synaptic weights converge towards the same basal value whatever the rule due to neuromodulation of neuronal activity. This reset is overcome in the triplet model thanks to neuromodulators that alter the shape of the spike-time dependent curve as demonstrated in [Gonzalez-Ruedas,2018]. To uncover the biological mechanisms of this change, we translated this modification in calcium-based rules by neuromodulating the calcium thresholds, potentiation and depression levels, as well as the learning rates. Our model is a powerful tool that leads the way to unravel biological explanations of the bursting activity role during sleep and its support on the down-selection mechanism.

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SYNAPSES LEARN TO UTILIZE RELEASE NOISE TO PREDICT POSTSYNAPTIC DYNAMICS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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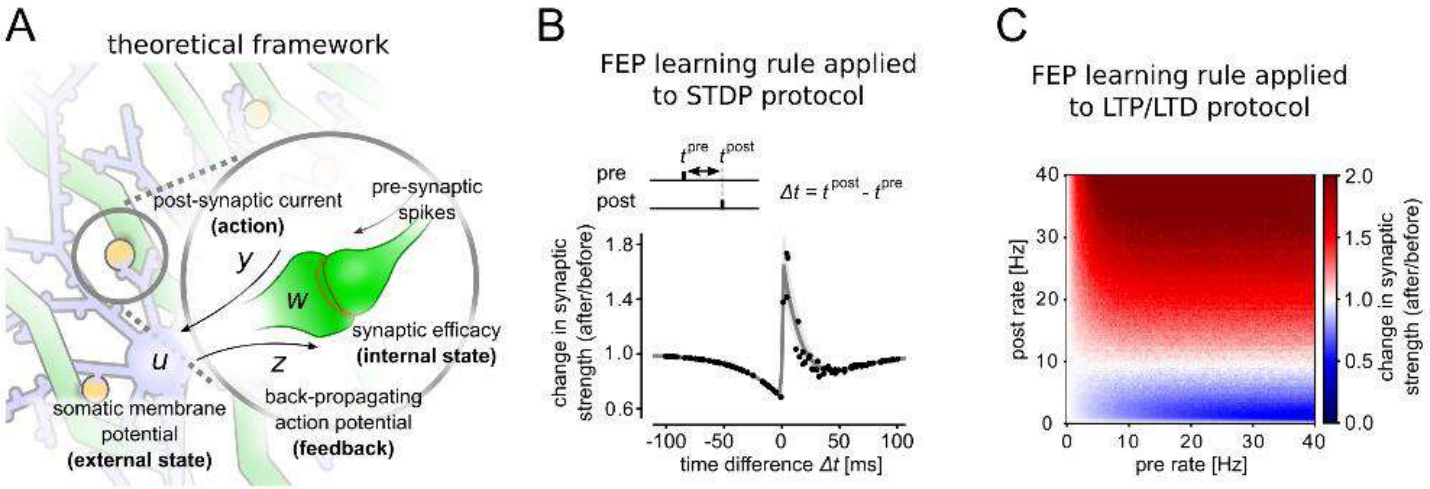

Fig.1: *Illustration of the theoretical framework and predicted synaptic learning rules.* Synapses in the brain are highly noisy, which leads to a large trial-by-trial variability in post-synaptic currents (PSCs). Given how costly synapses are in terms of energy consumption, these high levels of noise are surprising. In this theoretical study we establish the role of the experimentally found high levels of PSC noise to encode uncertainties about the post-synaptic activity and to efficiently use the sparse information provided by back-propagating action potentials. Furthermore, we show how synaptic release noise can be utilized as a computational resource to probe the behavior of the post-synaptic neuron. To arrive at our results we applied the Free Energy Principle (FEP) – a mathematical framework to describe the behavior of organisms and neural networks - for the first time to the level of individual synapses that communicate with the soma of the post-synaptic neuron (Fig.1A). In recent years there has been an increasing number of studies to show that the FEP can explain brain activity on different spatial and temporal scales. In this study we treat arguably the lowest level by considering the interaction of individual synapses with their environment. Using this approach we derive a synaptic learning rule from first principles that resembles experimentally well-established plasticity mechanisms and make precise predictions for future experiments (see Fig.1B,C). In addition, our synaptic FEP model allows us to show how neuronal systems utilize the unreliability of synaptic release to master behaviorally ambiguous situations.

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FUNCTIONAL CONSEQUENCES OF TWO-PHASE SYNAPTIC PLASTICITY FOR CODING, ORGANIZATION, AND PRIMING OF LONG-TERM MEMORY REPRESENTATIONS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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In the brain, groups of neurons with particularly strong synaptic connections are linked to memory representations. These so-called Hebbian cell assemblies are formed by synaptic plasticity and consolidated by synaptic tagging and capture (STC), influenced by diverse neuromodulatory signals. In order to characterize the impact of these mechanisms on long-term memory, their functional role at the level of neuronal networks needs to be understood. To this end, we employed a biologically detailed model of a recurrent network of spiking neurons featuring synaptic plasticity and neuromodulator-dependent STC. Thereby, we modeled and investigated the learning, consolidation, and recall of long-term memory representations. First, we found that the amount of an abstract neuromodulator present during consolidation can retroactively control the degree to which rate-based and spike timing-based information is stored in the network. Second, we found that the topological organization of multiple memory representations, determined by learning and consolidation, has diverse effects on memory functionality. Third, we identified a mechanism that enables the priming of a memory representation on a timescale of minutes to hours. We quantified these effects by using measures derived from the neuronal spiking activity in the network. Our findings demonstrate how STC along with other synaptic and neuronal mechanisms enables rich cognitive functionality. This may provide an explanatory basis for known long-term memory effects such as switching between the storing of semantic and episodic information, recency in free recall tasks, retroactive interference and enhanced recall caused by similarity of memories, and priming.

Pubmed:
33615093: Luboeinski J, Tchumatchenko T
Nonlinear response characteristics of neural networks and single neurons undergoing optogenetic excitation.
Optogenetic stimulation has become the method of choice for investigating neural computation in populations of neurons. Optogenetic experiments often aim to elicit a network response by stimulating specific groups of neurons. However, this is complicated by the fact that optogenetic stimulation is nonlinear, more light does not always equal to more spikes, and neurons that are not directly but indirectly stimulated could have a major impact on how networks respond to optogenetic stimulation. To clarify how optogenetic excitation of some neurons alters the network dynamics, we studied the temporal and spatial response of individual neurons and recurrent neural networks. In individual neurons, we find that neurons show a monotonic, saturating rate response to increasing light intensity and a nonmonotonic rate response to increasing pulse frequency. At the network level, we find that Gaussian light beams elicit spatial firing rate responses that are substantially broader than the stimulus profile. In summary, our analysis and our network simulation code allow us to predict the outcome of an optogenetic experiment and to assess whether the observed effects can be attributed to direct or indirect stimulation of neurons.
Netw Neurosci, 2020; 4
33658641: Luboeinski J, Tetzlaff C
Memory consolidation and improvement by synaptic tagging and capture in recurrent neural networks.
The synaptic-tagging-and-capture (STC) hypothesis formulates that at each synapse the concurrence of a tag with protein synthesis yields the maintenance of changes induced by synaptic plasticity. This hypothesis provides a biological principle underlying the synaptic consolidation of memories that is not verified for recurrent neural circuits. We developed a theoretical model integrating the mechanisms underlying the STC hypothesis with calcium-based synaptic plasticity in a recurrent spiking neural network. In the model, calcium-based synaptic plasticity yields the formation of strongly interconnected cell assemblies encoding memories, followed by consolidation through the STC mechanisms. Furthermore, we show for the first time that STC mechanisms modify the storage of memories such that after several hours memory recall is significantly improved. We

identify two contributing processes: a merely time-dependent passive improvement, and an active improvement during recall. The described characteristics can provide a new principle for storing information in biological and artificial neural circuits. Commun Biol, 2021; 4

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CHOLINERGIC-MEDIATED ADAPTIVE LEARNING IN CORTICAL MICROCIRCUITS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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With frequent exposure to novelty, it is imperative that the brain is capable of rapidly adapting in order to execute appropriate responses to new situations. In deep artificial neural networks, adaptive learning rules are used to provide fast learning but whether the brain employs similar strategies remains unknown. Here, we propose a model of the cholinergic system as an adaptive learning system that modulates long-term synaptic plasticity in cortical microcircuits across multiple brain areas. In this model, the cholinergic system integrates backprop-like local prediction error signals to modulate credit assignment across a multi-layered cortical network of pyramidal cells and feedback somatostatin interneurons. We incorporated different adaptive methods (Adagrad and RMSprop) from machine learning into our cholinergic model and tested diffuse cholinergic modulation at the neuron, layer and network levels on benchmark pattern recognition tasks. Our results show that modulation at the level of neurons and layers achieves similar learning performance compared to standard adaptive methods which require synapse-specific modulation. This demonstrates that the relative diffuse nature of cholinergic neuromodulation may be sufficient for effective adaptive learning. Furthermore, our model predicts that such adaptive credit assignment can only be achieved if cholinergic modulation jointly controls excitatory and inhibitory synapses in cortical networks. Experimental testing of this prediction using in vitro slice electrophysiology indicated that acetylcholine reduces the output of optogenetically identified feedback somatostatin interneurons highlighting its control of feedback inhibition. Overall, we demonstrate how incorporation of neuromodulatory signals into credit assignment processes can lead to efficient adaptive learning in the brain.

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NEUROPHYSIOLOGICALLY-INSPIRED COMPUTATIONAL MODEL OF THE VISUAL RECOGNITION OF SOCIAL BEHAVIOR AND INTENT.

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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AIMS: Humans recognize social interactions and intentions from videos of moving abstract stimuli, including simple geometric figures (Heider & Simmel, 1944). The neural machinery supporting such social interaction perception is completely unclear. Here, we present a physiologically plausible neural model of social interaction recognition that identifies social interactions in videos of simple geometric figures and fully articulating animal avatars, moving in naturalistic environments. METHODS: We generated the trajectories for both geometric and animal avatars using an algorithm based on a dynamical model of human navigation (Hovaidi-Ardestani, et al., 2018, Warren, 2006). Our neural recognition model combines a Deep Neural Network, realizing a shape-recognition pathway (VGG16), with a top-level neural network that integrates RBFs, motion energy detectors, and dynamic neural fields. The model implements robust tracking of interacting agents based on interaction-specific visual features (relative position, speed, acceleration, and orientation). RESULTS: A simple neural classifier, trained to predict social interaction categories from the features extracted by our neural recognition model, makes predictions that resemble those observed in previous psychophysical experiments on social interaction recognition from abstract (Salatiello, et al. 2021) and naturalistic videos. CONCLUSION: The model demonstrates that recognition of social interactions can be achieved by simple physiologically plausible neural mechanisms and makes testable predictions about single-cell and population activity patterns in relevant brain areas. Acknowledgments: ERC 2019-SyG-RELEVANCE-856495, HFSP RGP0036/2016, BMBF FKZ 01GQ1704, SSTeP-KiZ BMG: ZMWI1-2520DAT700, and NVIDIA Corporation.

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ROBUST DECISION-MAKING: NON-LINEAR NEURAL RESPONSIVENESS CAN ENHANCE STIMULUS INFORMATION

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Sensory decision-making typically involves a choice between multiple alternatives and the evidence supporting each alternative may comprise some number of independent signals (*e.g.*, from different receptive fields). To make an optimal choice, all signals supporting each alternative must be integrated before the results can be compared. In the brain, sensory signals are integrated and represented by downstream neural responses that perform a non-linear transformation of sensory input. Here we report that choice performance can be more accurate when it relies on integrated (averaged) neural responses rather than on integrated (averaged) noisy sensory signals, at odds with the common-sense expectation that any neural processing should degrade sensory information. The improvement is obtained when sensory signals *s* are drawn from heavy-tailed (non-Gaussian) distributions *P(s)* and when non-linear neural response functions $\Psi(s)$ broadly match $\delta_s \ln P(s)$. As proof-of-principle, we devised a decision-making toy-model in which a separate population of spiking neurons represented evidence for each alternative. Specifically, we simulated responses of integrate-and-fire neurons to uncorrelated synaptic inputs, with variance encoding an independent 'sensory signal'. In this toy framework, integrating neural responses rather than sensory signals more than doubled choice performance, depending on assumptions. The intuitive reason for this improvement is that compressive (saturating) responsiveness reduces the influence of outlier signals. Although not widely known in neuroscience, this effect is exploited routinely in engineering and statistics by preprocessing samples with non-linear "influence functions" [*e.g.*, Huber, Ronchetti (2009) *Robust Statistics*, John Wiley & Sons]. An intriguing question for further work is whether neural response functions *in vivo* match or adapt to heavy-tailed input distributions.

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THE ROLE OF THE SUPERIOR COLLICULUS IN ATTENTION DEMANDING TASKS: A COMPUTATIONAL MODEL

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Experimental work has revealed "*attention deficits without cortical neuronal deficits"* in experiments in which animals performed a motion detection task and 'decided' to or not to press a button (Alexandre Zénon and Richard J. Krauzlis, Nature 2012). Transient inactivation in the superior colliculus (SC) disturbed the performance whilst cortical attentional signals remained unaffected. The question of how the SC affects the performance is still open. To advance our understanding of the temporal structure of involved brain areas' signals and the unknown critical role of the SC in accomplishing the task we aimed at a system-level modeling of the behavioral task dynamics. The complexity of both the task and the connections between involved cortical and subcortical areas is approached by a simplistic modular modeling paradigm using neuron-like units enabling the inclusion of the SC, the basal ganglia, the visual cortex and the motor cortex. We hypothesize a SC-role as a critical component in a spatial working memory system biasing the decision making process. With the aid of our hypothesized model we discuss this possibility and show simulation results supporting this idea. The presented model helps sketching the *temporal structure* of the trial and serves as a *computational template* that — due to its modularity — can be flexibly adapted and extended.

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VOXELWISE ENCODING MODEL REVEALS 2D KEY POINTS LIKE REPRESENTATION IN EXTRASTRIATE BODY AREA.

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Aim: Currently, the extrastriate body area (EBA) is considered to be an object category area that represents body stimuli. However, our understanding of body posture shows a gap between processing of low-level features and high-level semantic categories. This mid-level gap might be filled by the role played by EBA in the processing of body postures. In this study, we used voxelwise encoding model to investigate the role of EBA in body processing. **Methods:** Twenty participants viewed body stimuli while 7T fMRI responses were recorded. The stimuli were generated using a variational autoencoder (VAE), via random sampling from the latent space parameters from 3 different viewpoints. The fMRI response was modeled using several features extracted from the stimuli, such as VAE representation (encoding/decoding layers, latent space), 2D/3D coordinates of key joints (kp2d/kp3d), pixel space (Gabor like representation). The fMRI predicted responses from each model were generated via banded ridge regression using crossvalidation. **Results:** Results show a pattern of responses across visual cortex with Gabor and kp2d model which best predict responses to our stimuli. Specifically, the Gabor representation shows higher prediction accuracy in early visual occipital area as opposed to the kp2d representation which shows higher prediction accuracy in high-level temporal areas such as EBA. Furthermore, kp2d model (viewpoint variant) shows higher accuracy in EBA than kp3d model (viewpoint invariant). **Conclusion:** These findings suggest that EBA codes for specific features of the body, which in the case of kp2d model, are the joints position, and that this representation is viewpoint variant.

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A CEREBELLO-CORTICAL RECURRENT NEURAL NETWORK FOR PROBING THE CEREBELLUM ROLE IN MOTOR LEARNING

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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The motor cortex and the cerebellum are key regions involved in motor learning and adaptation. Based on its excitatory feedforward connectivity, classic theories of cerebellar function posit that it performs sensorimotor pattern separation and predicts the sensory consequences of movement. The motor cortex, on the other hand, has been hypothesised to use its recurrent connectivity to generate rich dynamics that can control complex movements. While these two regions have traditionally been studied in isolation, recent technological advances have made it now possible to access large populations of neurons in both regions simultaneously. Recent studies based on these techniques have demonstrated that coordinated cerebello-cortical activity is critical for motor control. As a result, the development of models that incorporate both cerebellar and neocortical dynamics is crucial. Towards this end, we leverage recurrent neural networks (RNNs), a machine learning model recently used to study neocortical engagement in complex tasks. We extend traditional RNNs to additionally incorporate a cerebellar module with feedforward divergent-convergent architecture, and train these networks to perform motor and cognitive tasks. The resulting RNN may be used as a model to study the structural pathways and functional interactions linking these regions, and can help to understand how the neocortex and the cerebellum coordinate during behaviour.

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A MODEL OF HIPPOCAMPAL REPLAY DRIVEN BY EXPERIENCE AND ENVIRONMENTAL STRUCTURE FOR NEAR-OPTIMAL LEARNING

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Hippocampal replay during awake resting states and sleep is thought to play an important role in learning and consolidation. Consistent with this function, tentative evidence suggests that replay sequences adapt to changing spatial constraints (Widloski & Foster, 2022). However, other studies suggest that replay does not always reflect the animal's previous behavior. For instance, the statistics of replay was found to resemble random walks (Stella et al., 2019), represent shortcuts that the animals had never taken (Gupta et al., 2010), and represent trajectories through regions that the animals had seen, but never explored (Ólafsdóttir et al., 2015). It is unclear how these different types of sequences are generated and what functions they serve. We address these questions in a computational study using reinforcement learning (RL). We propose a mechanism that reactivates experiences stochastically according to their priority ratings based on three variables: 1. Experience strength, 2. experience similarity and 3. inhibition of return. The relative importance of these variables differs depending on the statistics of experience and the environmental structure. This replay mechanism, together with the stochasticity of replay generates the diverse types of replay mentioned above. Importantly, this mechanism facilitates learning in spatial navigation tasks. Training an RL agent with replay sequences generated by our model outperforms training with random replay, and performs close to the state-of-the-art, but biologically unrealistic, model by Mattar & Daw (2018). In conclusion, different types of replay can be generated by a unified mechanism, which appears to be optimized to drive spatial learning.

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PLACE AND GRID CELL NAVIGATION IN MULTIPLE ENVIRONMENTS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Recently, artificial recurrent neural networks (RNNs) trained to do path integration have been shown to emergently produce putative grid cells. Place and grid cells are known to remap in response to new environments. Specifically, place cells globally remap (randomly) while grid cells with the same spacing and scale appear to randomly translate and rotate in concert. We address how the emergent grid cells in the RNN model respond to changes in the environment, modelled by place cell global remapping. In particular we address, (i) whether the model produces grid cell patterns when required to learn multiple environments, (ii) how these patterns respond to different environments and if they reproduce biologically plausible grid cell remapping behaviour. Using the same methodology, we also unpack the generalisation capabilities of the grid cell network. For example, if the grid cells form a general path integrator, then the grid cell network should be invariant to arbitrary environments. In this work, we describe whether the model can effectively perform path integration in arbitrarily many environments, and how the dynamics that form grid cell patterns are impacted by an increasing number of environments. This work investigates emergent general computational modules which is of interest to AI research in areas such as transfer learning. Moreover, extending the model to include remapping will reveal either (i) an incomplete model of how place and grid cells can navigate in a seemingly arbitrary number of environments, or (ii) give theoretical insights into the functional role of remapping.

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USING DEEP NEURAL NETWORKS TO MEASURE MULTI-DAY DYNAMICS OF DISTRIBUTED LIMBIC-CORTICAL SPATIAL REPRESENTATIONS IN LOCAL FIELD POTENTIALS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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AIMS We propose a machine learning approach to quantifying multi-day stability of neural representations in local field potential (LFP) data. Our test case focuses on spatial information encoding in adult rat hippocampus (CA1), prelimbic (PrL) and parietal (PC) cortex. METHODS We trained deep neural networks (DNN) to decode position from wavelet decomposition images of LFP simultaneously recorded from CA1, PrL and PC of four rats. Data were collected while each rat learned a maze-based memory and decision-making task over >20 days, with different DNN trained using data from separate days. By calculating the decoding accuracy of a DNN trained on data from different days, we quantified the representational stability of spatial encoding in these brain regions. RESULTS Our analysis yielded three main results; first, that decoding error in all brain regions increases monotonically as time between training and testing day is increased. Second, that stability of same-day location representations increases over learning. Lastly, that stability differs significantly between brain regions, with the most stable location representations emerging in parietal cortex. CONCLUSION This method enables a holistic, unbiased analysis of neural codes, requiring only minimally-processed LFP input and negating sampling or sorting bias inherent in spike-based metrics. Tracking neural representations over multi-day timescales reveals differential stability across hippocampus and neocortex, and quantifying representational drift for distinct frequency bands has potential to yield mechanistic insights into the nature of distributed information coding.

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DIMENSIONAL CAUSALITY ANALYSIS METHOD ON EVOKED EPILEPTIC ACTIVITY IN VITRO

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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In recent years, we have developed a new causality analysis method named Dimensional Causality (DC), which is capable to detect and distinguish all basic forms of causal connections between two dynamical systems, based on observed time series: DC can detect directed causal connections, circular or bidirectional connection and can distinguish them from the two unconnected systems driven by a hidden common cause, or confounder. While the efficacy of the method has been demonstrated on time series from simulated dynamical systems here we validate our method on in vitro measurements, where the form of the causal connection is more or less well known. LFP and Intrinsic Optical Signal (IOS) were recorded during evoked epileptic activity in vitro, evoked by magnesium-free Ringer-solution. In our earlier study, we have shown that Sugihara's cross-convergent mapping (CCM) method detects directed causal relationship from the LFP towards the IOS activity without detectable signs of a feedback effect. However, CCM can not distinguish the directed connection from the effect of a confounder. Furthermore, the outcome of the CCM was ambiguous, one had to consider the time delay of the effect to get to a clear conclusion. DC analysis showed a clear unidirectional causal connection from the LFP to the IOS in most of the cases, especially in the first period of the evoked seizure-like activity. Note, that the direction of the causal connection was inferred without considering any observable time delay of the effect.

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BOARD NUMBER: S01-167

A STATISTICAL PHYSICS THEORY OF HUMAN ADAPTIVE BEHAVIOR

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Human exhibit impressive adaptive behavior in various environments. However, a unifying theory accounting for basic principles underlying human adaptive behavior is lacking. We propose a statistical physics theory describing adaptive behavior based on the following postulate: actions are chosen to maximize knowledge acquisition within internal world models about the environment under energetic resources constraints. Through differential calculus and Dirichlet series, we derived a unique choice distribution optimally balancing exploration and exploitation behavior. We applied this model to multi-armed bandits and investigated the dynamics of behaviors over time. We will experimentally test model predictions through online experiments.

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BOARD NUMBER: S01-168

PROBABILISTIC PATH INTEGRATION, LOCALIZATION AND PLANNING IN THE HIPPOCAMPUS THROUGH DISTRIBUTED DISTRIBUTIONAL CODING

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Aim: Positional information is available from many sensory modalities, but each is imperfect, and accurate navigation depends on integrating, representing and reasoning with the resultant positional uncertainty. We suggest that posterior probabilities over location are encoded by distributed distributional codes (DDC), and show that probabilistic path integration, localization and planning can be performed using the DDC representation. The modeled DDC neurons capture the dynamics of hippocampal CA1 and CA3 neurons. Methods: Distributed distributional coding (DDC) proposes that probability distributions are represented by the expected values of a set of encoding functions. We model navigation by probabilistic inference in a generative model, and encode the posterior over the current location by “filtering DDC” neurons, and the posterior over the next location by “predictive DDC” neurons. We implement probabilistic localisation, prediction, and structural learning through the activity of these DDC neurons. Results: Filtering DDC neurons show rapid spatial tuning in a new environment. In contrast, formation of place fields in predictive DDC neurons is gradual; fields emerge only after learning the structure of the environment. Furthermore, the tuning functions of both filtering and predictive DDC neurons are skewed backwards by further exploration, with larger skewness in predictive DDC neurons. These results are juxtaposed with the gradual formation and high skewness of place fields in CA3, and rapid formation and lower skewness of fields in CA1. Conclusions: DDC representation of positional uncertainty provides a framework for inference and learning in hippocampal navigation and explains some of the dynamics of CA1 and CA3 neurons.

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BOARD NUMBER: S01-169

ENCODING OF BEHAVIOUR BY PAIRWISE NEURONAL INTERACTIONS CORRELATES WITH REPRESENTATIONAL DRIFT

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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In some brain areas, populations of neurons can change their behavioural tuning gradually over days. At the single cell level, the rate of this drift varies considerably, raising the question: Does the amount of information contained in interactions between cells predict how much they drift? We reanalysed data from Driscoll et al. (2017), in which the activity of mouse posterior parietal cortex (PPC) neurons was tracked over weeks as mice performed a learned navigation task.

We quantified the informativeness of pairwise interactions by measuring the mutual information between behaviour and pairwise neuronal responses, relative to a conditionally shuffled baseline. This conditional shuffling preserves the tuning of single cells while destroying noise correlations or higher-order dependencies in a pair. The amount of pairwise information relative to this baseline can be synergistic (positive) or redundant (negative).

After thresholding these measures, we found that neurons with high average pairwise redundancy typically also showed greater average pairwise synergy. Furthermore, the average redundancy was positively correlated with tuning stability, more strongly than was single-cell informativeness. This suggests that redundant subpopulations of neurons form a more stable 'backbone' that is less susceptible to drift and more informative about behaviour on average. Thus, redundant coupling is more predictive of stable tuning than the information conveyed about behaviour at the single cell level.

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BOARD NUMBER: S01-170

EMBODIED SPIKING MUSHROOM BODY MODEL FOR THE INVESTIGATION OF ANT NAVIGATION

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Introduction: Ants are efficient navigators; they have small brains but can forage over large distances. Replicating the neural circuitry that underlies this, will increase our knowledge on how brains can represent the environment, while giving inspiration for energy-efficient robot navigation systems. **Aims:** Produce a spiking neural network (SNN) model of the mushroom body (MB), a region of Hymenoptera brains vital in spatial learning. Train and test the MB model on images from foraging routes of wild ants and compare to the performance of the model on a robot. **Methods:** To build the MB model we used the GPU-accelerated SNN library PyGeNN. To train it, we presented it with many route lengths as different series of images. To test the model, we presented it with rotations of images from the training routes and found which angles the model categorised as familiar to get a heading direction. **Results:** We found our model acted similarly to a rotational image difference function, where unfamiliarity produces increased responses. On the natural dataset, we found the MB model could only learn short routes of approximately 25 images. However, on the robot, it was able to learn a 100-image route. **Conclusions:** Our real-time model is sufficient to navigate routes of reasonable length. The discrepancy between simulation and robot performance may be due to differences in input image statistics. This inspires our current work to build ant navigation experiments that can be easily replicated in a simulated environment, for improved comparison between real-world and simulated experiments.

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BOARD NUMBER: S01-171

NEUROMODULATION VIA IONIC ACTUATION OF POTASSIUM: AN IN SILICO STUDY

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Aims. The concentration of potassium K^+ in the extracellular environment is a crucial modulator of neuronal excitability. Recent advances in the technology of ionic actuators are enabling spatiotemporal resolution at the scale of neuronal cells and action potentials, thereby holding the promise of making the control of K^+ concentration a viable neuromodulation modality. In this regard, *in silico* studies are pivotal to understand the possibilities offered by such technology. *Methods.* To this aim, we first introduce a theoretical framework based on the Hodgkin-Huxley formalism and bifurcation analysis. We then propose a computational approach to couple the circuit-level description of neural membranes with the finite element modeling (FEM) of ionic transport in cellular fluids and ionic actuators. As case studies, we assume cortical neurons as target cells and PEDOT:PSS-coated electrodes as iontronic transducers. *Results.* Bifurcation analysis suggests a differential K^+-induced modulation of neural excitability, depending on the targeted cell. Remarkably, K^+-induced tonic spiking is not for granted. Simulations enable to assess if neural activity is elicited or inhibited by the ionic actuator as a function of the extracellular geometry, the profile of ionic actuation, and the physical parameters of the actuator. Examples are shown considering a 3D model of a neuronal soma and a planar electrode. *Conclusion.* These efforts may shed light on how neural activity can be shaped by targeting extracellular K^+ and represent a first step towards design-oriented studies of ionic actuators.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-172

ACTIVE DENDRITES CAN SUPPORT RELIABLE SPIKING COMPUTATIONS

POSTER SESSION 01 - SECTION: COMPUTATIONAL NEUROSCIENCE OF LEARNING AND MEMORY

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Dendritic action potentials exhibit long plateaus of many milliseconds, lasting much longer than axonal spikes. On the other hand, the nervous system often has to be fast to facilitate rapid actions and decision making. Furthermore, cortical neurons are often in a high conductance state in vivo, shortening the membrane timeconstant and leading to very short integration windows of a few milliseconds. This raises the question of why slow dendritic plateaus exist in a system that with very short timeconstants elsewhere. Previous work has hypothesized that this timescale separation could facilitate the detection of temporal spiking patterns. Here we propose an alternative role for active dendrites in enabling the reliable integration of asynchronous spikes. We develop a physiologically grounded model in which the extended duration of dendritic spikes equips each dendrite with a memory of incoming signals, effectively buffering temporal jitter in the spike timings of incoming spikes. With our abstract model of a neuron whose dendrites exhibit plateau potentials, we demonstrate that this allows a neuron to integrate asynchronous input spikes. We show that the qualitative behaviour of this model maps onto a detailed biophysical model, where dendritic spikes plateaus are mediated by NMDA receptors. We then use the simplified model to show how active dendrites can make network-level spiking computations reliable and robust to noise and parameter uncertainty. Lastly, we demonstrate nontrivial computations via simulation of a network solving a classification task, in which decisions occur quickly, reliably, and with a low number of spikes.

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BOARD NUMBER: S01-173

NETWORK DIMENSIONALITY OF HIPPOCAMPAL POPULATION ACTIVITY DURING CONTINUOUS NOVELTY DETECTION

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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The structured firing activity of hippocampal neurons allows to process and integrate new information in memory. Notably, during active exploration, neural coordination in the theta-band (5-12Hz) is sparse, suggesting a high degree of dimensionality in information representation. On the other hand, the neural population fires in a synchronised manner during sharp wave/ripple (SWR) (150-250Hz) events during post-exploration sleep/rest, suggesting that mnemonic information is defined within a lower dimensional space. However, the extent to which changes in the dimensionality of population-level firing preserves structural information across behavioural states remains unknown. Here, we investigate how continuous integration of place-object information alters the dimensionality of the hippocampal population activity. We first report preliminary results showing that short timescale neuronal co-firing increases in post-task sleep, with the population activity space becoming lower dimensional. Furthermore, we investigated the offline reactivation of waking firing patterns during post-task individual SWR events. We found that as more information is integrated, spatial reactivation maps increase their selectivity and their dimensionality. We discuss how future work will assess how continual information integration affects the geometry of the network across behavioural states.

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BOARD NUMBER: S01-174

MODULATION OF IN VITRO CORTICAL NETWORKS BY MECHANICAL PERTURBATION OF INDIVIDUAL NEURONS

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Recent investigations of network responses to a stimulus point to the ability to modulate a network at the single neuron level. Different perturbation modalities, ranging from single-cell electrical stimulation to focal damage, modulate the local network's firing dynamics and functional topology. With a growing body of evidence revealing that the target neurons respond to mechanical stimulation, we examined the neuronal network behavior upon mechanical stimulus. Specifically, we investigated to what extent a patch-clamp-induced mechanical perturbation influences the activity in the local network of dissociated rat cortical neurons. Simultaneous patch-clamp and calcium imaging experiments demonstrated that patch-pipette mediated mechanical stimulus induces calcium plateaus in targeted neurons, and on average in about 30% of neighbors in 0.185 mm^2. Interestingly, the initiation and propagation of these responses are independent of the neuron's spiking, as the calcium plateaus persisted after the pharmacological block of action potentials and synapses. However, combined electrophysiology experiments demonstrated a bursting activity during the mechanical perturbation, suggesting that the underlying mechanisms induce action potentials. To investigate the impact of patch-clamp-induced perturbation on the network's functional topology, we assessed the effective network connectivity before and after the mechanical stimulus. Here we show that a single mechanical perturbation reduces the network's ability to integrate information, with the recovery time of around 10 minutes. These findings indicate the potential use of mechanostimulation in network manipulation, while concurrently emphasizing the restrictions in the application of patch-clamp for network investigations.

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BOARD NUMBER: S01-175

HIPPOCAMPAL-NEOCORTICAL CIRCUITS INVOLVED IN INFERENTIAL LEARNING

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Coordination between the hippocampus and the neocortex during sleep has been shown to promote memory consolidation and may play a crucial role in connecting memories which were not directly experienced together. To investigate this role, we used multi-unit electrophysiology and calcium imaging in freely moving mice during a multi-day inference task. We show that reinstatement in cortical and hippocampal areas supports inferential reasoning and, using immunohistochemistry, highlight the inhibitory and disinhibitory circuits which may control this process.

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BOARD NUMBER: S01-176

ABNORMAL FUNCTIONAL CONNECTIVITY IN TECTAL CIRCUIT IMPAIRS DECODING AND BEHAVIOR IN MEPC2 MUTANT ZEBRAFISH

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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The zebrafish larva (danio rerio) optic tectum detects and processes visual stimuli, and generates appropriate motor behavior. Its spontaneous dynamics show a spatiotemporal structure organized in distinct functional neuronal assemblies (groups of highly correlated, co-firing neurons). Recent studies have shown that retinal inputs are dispensable for the development of these assemblies. Hence, to understand the functional role of these assemblies, it is necessary to disrupt their functional connectivity (FC). Here, we use an *mecp2*-null fish which has abnormal tectal activity to address this question. We used two-photon calcium imaging to simultaneously record hundreds of neurons of the periventricular layer of the optic tectum of both wild-type and *mepc2*-null larvae. We detected the assemblies by combining principal component analysis (PCA) and factor analysis for categorization of correlated neurons. To study the tectal FC, we measured the neural response to light spots at four different positions. Finally, we developed a behavioral setup to study prey capture of both fish. The results show that the number of neuronal assemblies of the wild type is higher than that of the *mecp2*-null. In contrast, the average number of neurons per assembly were higher in the *mecp2*-null. By using a linear decoder, we found that *mecp2*-null neural population decoding was at chance level. The spatiotemporal structure of the spontaneous activity is degraded in the *mecp2*-null larvae. This fact has not only implications in the processing of visual stimuli; it strongly affects the ability of the larvae to distinguish large objects that may represent predators.

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BOARD NUMBER: S01-177

RAPID ODOR PROCESSING BY LAYER 2 SUBCIRCUITS IN LATERAL ENTORHINAL CORTEX

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Olfactory information is encoded in lateral entorhinal cortex (LEC) by two classes of layer 2 (L2) principal neurons: fan and pyramidal cells. However, the functional properties of L2 cells and how they contribute to odor coding are unclear. Here, we show in awake mice that L2 cells respond to odors early during single sniffs and that LEC is essential for rapid discrimination of both odor identity and intensity. Population analyses of L2 ensembles reveals that rate coding distinguishes odor identity, but firing rates are only weakly concentration-dependent and changes in spike timing can represent odor intensity. L2 principal cells differ in afferent olfactory input and connectivity with inhibitory circuits and the relative timing of pyramidal and fan cell spikes provides a temporal code for odor intensity. Downstream, intensity is encoded purely by spike timing in hippocampal CA1. Together, these results reveal the unique processing of odor information by LEC subcircuits and highlight the importance of temporal coding in higher olfactory areas.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-178

ELUCIDATING THE NEURAL CORRELATES OF COST-BENEFIT DECISIONS IN A RAT CORTICO-BASAL GANGLIA NETWORK

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Aims Adaptive value-guided decision-making often requires weighing-up of costs and benefits of pursuing an available opportunity. While several brain areas have been detected that are important for this behaviour, little is currently known regarding how the decision variables are represented and evolve on the single cell and population levels *across* medial prefrontal cortex and the associated basal ganglia circuitry within the confines of a single behavioural paradigm. **Methods** We developed a novel cost-benefit "accept-reject" task for rats, in which rats choose whether or not to run to the end of a corridor to collect reward based on the prospective reward (4 different levels, varied trial-by-trial, signalled by auditory cues) and effort cost (3 different levels, varied over blocks of trials, implemented with barriers placed in the corridor that needed to be scaled to reach the reward zone). Rats (n=5) were implanted with a driveable micro-electrode array targeting medial orbitofrontal cortex, anterior cingulate cortex, dorsomedial striatum, ventral pallidum, and subthalamic nucleus and recordings were performed as rats performed the task. **Results** Behavioural data (n=13) demonstrated a positive effect of reward and a negative influence of effort on run likelihood, without an interaction between the two. Outcome devaluation strongly reduced preference for any offer. Preliminary analysis of electrophysiology data (n=2; 164 cells per region on average) suggests heterogenous reward and effort coding on run and omit trials distributed across the network. **Conclusions** We have successfully established a pipeline that allows us to probe the neural correlates of cost-benefit decisions across brain networks.

Pubmed:
34762147: Härmson O, Grima LL, Panayi MC, Husain M, Walton ME
5-HT receptor perturbation has bidirectional influence over instrumental vigour and restraint.
The serotonin (5-HT) system, particularly the 5-HT receptor, has consistently been implicated in behavioural control. However, while some studies have focused on the role 5-HT receptors play in regulating motivation to work for reward, others have highlighted its importance in response restraint. To date, it is unclear how 5-HT transmission at this receptor regulates the balance of response invigoration and restraint in anticipation of future reward. In addition, it remains to be established how 5-HT receptors gate the influence of internal versus cue-driven processes over reward-guided actions. To elucidate these issues, we investigated the effects of administering the 5-HT receptor antagonist SB242084, both systemically and directly into the nucleus accumbens core (NAcC), in rats performing a Go/No-Go task for small or large rewards. The results were compared to the administration of d-amphetamine into the NAcC, which has previously been shown to promote behavioural activation. Systemic perturbation of 5-HT receptors-but crucially not intra-NAcC infusions-consistently boosted rats' performance and instrumental vigour on Go trials when they were required to act. Concomitantly, systemic administration also reduced their ability to withhold responding for rewards on No-Go trials, particularly late in the holding period. Notably, these effects were often apparent only when the reward on offer was small. By contrast, inducing a hyperdopaminergic state in the NAcC with d-amphetamine strongly impaired response restraint on No-Go trials both early and late in the holding period, as well as speeding action initiation. Together, these findings suggest that 5-HT receptor transmission, outside the NAcC, shapes the vigour of ongoing goal-directed action as well as the likelihood of responding as a function of expected reward.
Psychopharmacology (Berl), 2022; 239
24773038: Kilk K, Meitern R, Härmson O, Soomets U, Hõrak P
Assessment of oxidative stress in serum by d-ROMs test.
Assessment of oxidative stress is an important but technically challenging procedure in medical and biological research. The

reactive oxygen metabolites (d-ROMs) test is a simple assay marketed for analyzing the total amount of hydroperoxides in serum via the Fenton's reaction. Earlier reports have raised a suspicion that a part of the signal detected in the assay comes from sources other than metabolites generated by oxidative stress. The aim of this study was to identify which serum components interfere with the d-ROMs signal. By application of sodium azide, ethylenediaminetetraacetic acid, sodium dodecylsulphate, varying temperature, and spiking endogenous substances we demonstrate that in the case of mammalian sera the assay determines ceruloplasmin (CP) activity with potential interferences from hydroperoxides, iron level, thiols, and albumin. In sera of avian species hydroperoxides contribute more to the test outcome, but the CP part is insensitive to inhibition by azide. In conclusion, this assay has deficiencies in terms of detecting realistic concentrations of hydroperoxides, is mostly measuring CP and is also interfered with other serum components, making it very difficult to interpret in most biological systems.
Free Radic Res, 2014; 48

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-179

CONTEXTUAL OVER-EATING INDUCES TOPOLOGICAL DISTORTION OF THE HIPPOCAMPAL NETWORK

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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New cognitive challenges require overcoming old routines that memories of salient past experiences invigorate. However, the network-level neuronal operations that underlie cross-memory interaction are unclear. To address this, here we monitor hippocampal CA1 ensembles in mice that continually process new stimuli (objects) within an environment previously associated with palatable food intake. We found that robust contextual over-eating proactively interferes with subsequent object-place memory, decreasing selectivity of object-place population representation. By analysing the structure of population activity, we found that robust food-context memory induces a network state of heightened neuronal co-activity. This topological alteration developed through repeated contextual feeding, preventing further integration of mnemonic patterns for continued object discrimination. These findings suggest a functional principle of memory circuits where sparse population firing structure permits flexible integration and interaction of memories, which contextual over-eating can compromise.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-180

SPATIAL CODING BY SOMATOSTATIN AND NEUROTENSIN NEURONS IN THE LATERAL SEPTUM

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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The animal's ability to remember the location of rewarding and/or aversive stimuli is crucial for survival. The lateral septum (LS) integrates information about environmental stimuli and internal states signals to weigh the expression of different behavioral outcomes, like food-seeking, social recognition, locomotion, and anxiety-like behaviors. Here we used single cell, deep-brain calcium imaging in freely moving mice to investigate two GABAergic neuronal populations identified in the LS, neurotensin (Nts) and somatostatin (Sst) neurons. Mice explored an enclosure containing rewarding stimuli, or a non-rewarded enclosure. We found that a subset of Sst and Nts cells shows spatial, location-restricted activity. Sst neurons exhibited a higher percentage of place cells, a higher mutual information and a higher place field stability than Nts neurons. Further, Sst neurons showed a higher percentage of reward-associated cells and a higher reward-type selectivity. Our results suggest the involvement of a subset of LS neurons in the spatial and reward-related coding. We gratefully acknowledge support by the ERC Consolidator Grant (772994, FeedHypNet, to T.K.) and DFG (SFB1089 and EXC2030-CECAD, to T.K., 233886668-GRK1960, to F.S.).

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-181

GLUTAMATERGIC AFFERENTS TO THE NUCLEUS ACCUMBENS INTEGRATE OUTCOMES IN REWARD-LEARNING.

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Aims. Alterations in reward learning are associated with psychopathologies such as depression. The nucleus accumbens (NAc) is implicated in motivation and reward as well as depression, integrating input from a range of afferents including the ventral hippocampus (vHip) and the medial prefrontal cortex (mPFC). However, the role of these glutamatergic inputs to the NAc in reward learning remains relatively unexplored. **Methods.** Using frame-projected independent-fiber photometry, we recorded population-level activity of NAc afferents from the mPFC and the vHip in male and female mice in a two-armed bandit task. We estimated reward expectation and prediction error by fitting a dual learning rate reinforcement learning model to each animal's observed behavior and probed relationships between neural activity and behavior. **Results.** Both neural projections dynamically encode information about trial outcome and outcome history, with notable pathway-way specific time-dependent trajectories. Rewarded vs non-rewarded outcomes are associated with suppression in the mPFC and vHIP following a choice, with vHIP suppression emerging at a longer delay following choice. Additionally, we found a relationship between neural activity preceding a choice and an animal's modeled expectation of reward on a given trial as well as between post-choice neural activity and reward prediction error, further implicating these projections in tracking outcomes over time. **Conclusions**. Together, these findings demonstrate that mPFC and vHip projections to the NAc integrate outcomes over time in reward learning. Given evidence implicating these pathways in depression-like states, our results may suggest that these neural circuits could contribute to alterations in reward learning observed in psychopathologies.

Pubmed:
34516164: Iyer ES, Weinberg A, Bagot RC
Ambiguity and conflict: Dissecting uncertainty in decision-making.
Making decisions is fundamental to how we navigate, survive, and thrive in our environment. The quality of information used to support decisions is rarely perfect. Many decisions are made under conditions of uncertainty, arising from ambiguous or conflicting information. Conflict and ambiguity, though conceptually distinct, both generate uncertainty, a commonality that has led to overlapping and inconsistent terminology in the literature. Evidence from human and animal research suggests a behavioral dissociation in responding to conflict and ambiguity. This dissociation can be studied through the implementation of spatial or operant tasks in rodents which find close parallels in gambling tasks in humans. Pharmacological manipulations in rodents and fMRI studies in humans further suggest a dissociation in the neural processing of conflict and ambiguity such that fronto-striato-parietal circuits may be most important for interpreting ambiguous information, while the ventral striatum and ventral hippocampus are critical for resolving conflicting information. Overall, the neural representation and resolution of conflict and ambiguity remain relatively understudied despite the fundamental importance of these processes to understanding decision-making. We highlight the need for further research to differentiate these related yet distinct processes through implementation of carefully designed behavioral tasks with neural circuit-dissection techniques and the potential to pursue translational research between rodents and humans. (PsycInfo Database Record (c) 2022 APA, all rights reserved).
Behav Neurosci, 2022; 136
32682566: Muir J, Tse YC, Iyer ES, Biris J, Cvetkovska V, Lopez J, Bagot RC
Ventral Hippocampal Afferents to Nucleus Accumbens Encode Both Latent Vulnerability and Stress-Induced Susceptibility.
Stress is a major risk factor for depression, but not everyone responds to stress in the same way. Identifying why certain individuals are more susceptible is essential for targeted treatment and prevention. In rodents, nucleus accumbens (NAc) afferents from the ventral hippocampus (vHIP) are implicated in stress-induced susceptibility, but little is known about how this pathway might encode future vulnerability or specific behavioral phenotypes.
Biol Psychiatry, 2020; 88
32417532: Iyer ES, Kairiss MA, Liu A, Otto AR, Bagot RC

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Probing relationships between reinforcement learning and simple behavioral strategies to understand probabilistic reward learning.

Reinforcement learning (RL) and win stay/lose shift model accounts of decision making are both widely used to describe how individuals learn about and interact with rewarding environments. Though mutually informative, these accounts are often conceptualized as independent processes and so the potential relationships between win stay/lose shift tendencies and RL parameters have not been explored.

J Neurosci Methods, 2020; 341

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BOARD NUMBER: S01-182

NEURAL POPULATION REPRESENTATION OF PLACE-ITEM EXPERIENCES IN THE HIPPOCAMPAL NETWORK

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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A critical feature to thrive in a complex environment is the ability to form strong representations of salient locations important to satisfy basic needs. The hippocampus is a brain region crucial to learning and memory, however the network-level mechanisms that support the co-existence of multiple memories and their relation to saliency are unclear. To address these questions, here we monitor over multiple days large-scale ensembles of hippocampal CA1 neurons simultaneously in mice exploring a star-shaped maze where basic needs (e.g., eat, drink, sleep, run and socially interact) are met. Using a combination of network science and population dynamics manifold analyses, we investigate the dynamics of the network-level representation of discrete locations and items featuring such a complex environment. We observe that network representations become sparser and more resilient as the animal gains across-days experience of stable place-item relationships. These preliminary findings allow discussing the complexity of the multivariate hippocampal network-level representation and their underpinning mechanisms by which heterogeneous environmental features are simultaneously encoded.

Pubmed:
33603228: Gava GP, McHugh SB, Lefèvre L, Lopes-Dos-Santos V, Trouche S, El-Gaby M, Schultz SR, Dupret D
Integrating new memories into the hippocampal network activity space.
By investigating the topology of neuronal co-activity, we found that mnemonic information spans multiple operational axes in the mouse hippocampus network. High-activity principal cells form the core of each memory along a first axis, segregating spatial contexts and novelty. Low-activity cells join co-activity motifs across behavioral events and enable their crosstalk along two other axes. This reveals an organizational principle for continuous integration and interaction of hippocampal memories.
Nat Neurosci, 2021; 24
33642996: Go MA, Rogers J, Gava GP, Davey CE, Prado S, Liu Y, Schultz SR
Place Cells in Head-Fixed Mice Navigating a Floating Real-World Environment.
The hippocampal place cell system in rodents has provided a major paradigm for the scientific investigation of memory function and dysfunction. Place cells have been observed in area CA1 of the hippocampus of both freely moving animals, and of head-fixed animals navigating in virtual reality environments. However, spatial coding in virtual reality preparations has been observed to be impaired. Here we show that the use of a real-world environment system for head-fixed mice, consisting of an air-floating track with proximal cues, provides some advantages over virtual reality systems for the study of spatial memory. We imaged the hippocampus of head-fixed mice injected with the genetically encoded calcium indicator GCaMP6s while they navigated circularly constrained or open environments on the floating platform. We observed consistent place tuning in a substantial fraction of cells despite the absence of distal visual cues. Place fields remapped when animals entered a different environment. When animals re-entered the same environment, place fields typically remapped over a time period of multiple days, faster than in freely moving preparations, but comparable with virtual reality. Spatial information rates were within the range observed in freely moving mice. Manifold analysis indicated that spatial information could be extracted from a low-dimensional subspace of the neural population dynamics. This is the first demonstration of place cells in head-fixed mice navigating on an air-lifted real-world platform, validating its use for the study of brain circuits involved in memory and affected by neurodegenerative disorders.
Front Cell Neurosci, 2021; 15
31775929: Schultz SR, Gava GP
Neural codes - Necessary but not sufficient for understanding brain function.
Brains are information processing systems whose operational principles ultimately cannot be understood without resource to information theory. We suggest that understanding how external signals are represented in the brain is a necessary step

towards employing further engineering tools (such as control theory) to understand the information processing performed by brain circuits during behaviour.
Behav Brain Sci, 2019; 42
30212563: Muzzu T, Mitolo S, Gava GP, Schultz SR
Encoding of locomotion kinematics in the mouse cerebellum.
The cerebellum is involved in coordinating motor behaviour, but how the cerebellar network regulates locomotion is still not well understood. We characterised the activity of putative cerebellar Purkinje cells, Golgi cells and mossy fibres in awake mice engaged in an active locomotion task, using high-density silicon electrode arrays. Analysis of the activity of over 300 neurons in response to locomotion revealed that the majority of cells (53%) were significantly modulated by phase of the stepping cycle. However, in contrast to studies involving passive locomotion on a treadmill, we found that a high proportion of cells (45%) were tuned to the speed of locomotion, and 19% were tuned to yaw movements. The activity of neurons in the cerebellar vermis provided more information about future speed of locomotion than about past or present speed, suggesting a motor, rather than purely sensory, role. We were able to accurately decode the speed of locomotion with a simple linear algorithm, with only a relatively small number of well-chosen cells needed, irrespective of cell class. Our observations suggest that behavioural state modulates cerebellar sensorimotor integration, and advocate a role for the cerebellar vermis in control of high-level locomotor kinematic parameters such as speed and yaw.
PLoS One, 2018; 13

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NEURAL MANIFOLDS IN HUMAN SUPERIOR TEMPORAL GYRUS SCAFFOLD SPEECH PHONOLOGICAL PROCESSING

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Recent work in early sensory and motor cortices has shown that functionally-relevant population activity can be represented as a set of trajectories confined to a low-dimensional neural manifold, a subspace spanned by the patterns of correlated neuronal activity. However, it remains unknown whether the same encoding principles apply to speech comprehension, a multi-level hierarchical neural processing that integrates several cognitive functions. We analyzed population activity recorded with a microelectrode Utah array implanted in the anterior superior temporal gyrus (aSTG) of a patient with pharmacoresistant epilepsy, and sought after a speech-associated neural manifold. We applied dimensionality reduction methods to the single and multiunit activity collected while the patient performed several auditory tasks encompassing a rich repertoire of stimuli. Our preliminary results show that distinct linguistic units (e.g., words, syllables, and phonemes) could be represented as distinct trajectories on a low-dimensional neural manifold. Moreover, our analysis suggests that differences in the dynamics of the trajectories spanned on the aSTG manifold preferably account for the variability present in the phonetic features of the stimuli. These findings extend previous work on single-neuron to neuronal population response to auditory stimuli in the aSTG, and shed new light on neuronal dynamics during speech processing by uncovering phoneme-specific trajectories confined to a speech-associated neural manifold.

Pubmed:
34649280: Iannotti GR, Orepic P, Brunet D, Koenig T, Alcoba-Banqueri S, Garin DFA, Schaller K, Blanke O, Michel CM
EEG Spatiotemporal Patterns Underlying Self-other Voice Discrimination.
There is growing evidence showing that the representation of the human "self" recruits special systems across different functions and modalities. Compared to self-face and self-body representations, few studies have investigated neural underpinnings specific to self-voice. Moreover, self-voice stimuli in those studies were consistently presented through air and lacking bone conduction, rendering the sound of self-voice stimuli different to the self-voice heard during natural speech. Here, we combined psychophysics, voice-morphing technology, and high-density EEG in order to identify the spatiotemporal patterns underlying self-other voice discrimination (SOVD) in a population of 26 healthy participants, both with air- and bone-conducted stimuli. We identified a self-voice-specific EEG topographic map occurring around 345 ms post-stimulus and activating a network involving insula, cingulate cortex, and medial temporal lobe structures. Occurrence of this map was modulated both with SOVD task performance and bone conduction. Specifically, the better participants performed at SOVD task, the less frequently they activated this network. In addition, the same network was recruited less frequently with bone conduction, which, accordingly, increased the SOVD task performance. This work could have an important clinical impact. Indeed, it reveals neural correlates of SOVD impairments, believed to account for auditory-verbal hallucinations, a common and highly distressing psychiatric symptom.
Cereb Cortex, 2022; 32
35150452: Orepic P, Park HD, Rognini G, Faivre N, Blanke O
Breathing affects self-other voice discrimination in a bodily state associated with somatic passivity.
A growing number of studies have focused on identifying cognitive processes that are modulated by interoceptive signals, particularly in relation to the respiratory or cardiac cycle. Considering the fundamental role of interoception in bodily self-consciousness, we here investigated whether interoceptive signals also impact self-voice perception. We applied an interactive, robotic paradigm associated with somatic passivity (a bodily state characterized by illusory misattribution of self-generated touches to someone else) to investigate whether somatic passivity impacts self-voice perception as a function of concurrent interoceptive signals. Participants' breathing and heartbeat signals were recorded while they performed two self-voice tasks (self-other voice discrimination and loudness perception) and while simultaneously experiencing two robotic conditions (somatic passivity condition; control condition). Our data reveal that respiration, but not cardiac activity, affects

self-voice perception: participants were better at discriminating self-voice from another person's voice during the inspiration phase of the respiration cycle. Moreover, breathing effects were prominent in participants experiencing somatic passivity and a different task with the same stimuli (i.e., judging the loudness and not identity of the voices) was unaffected by breathing. Combining interoception and voice perception with self-monitoring framework, these data extend findings on breathing-dependent changes in perception and cognition to self-related processing.
Psychophysiology, 2022; 59
35046522: Serino A, Bockbrader M, Bertoni T, Colachis Iv S, Solcà M, Dunlap C, Eipel K, Ganzer P, Annetta N, Sharma G, Orepic P, Friedenberg D, Sederberg P, Faivre N, Rezai A, Blanke O
Sense of agency for intracortical brain-machine interfaces.
Intracortical brain-machine interfaces decode motor commands from neural signals and translate them into actions, enabling movement for paralysed individuals. The subjective sense of agency associated with actions generated via intracortical brain-machine interfaces, the neural mechanisms involved and its clinical relevance are currently unknown. By experimentally manipulating the coherence between decoded motor commands and sensory feedback in a tetraplegic individual using a brain-machine interface, we provide evidence that primary motor cortex processes sensory feedback, sensorimotor conflicts and subjective states of actions generated via the brain-machine interface. Neural signals processing the sense of agency affected the proficiency of the brain-machine interface, underlining the clinical potential of the present approach. These findings show that primary motor cortex encodes information related to action and sensing, but also sensorimotor and subjective agency signals, which in turn are relevant for clinical applications of brain-machine interfaces.
Nat Hum Behav, 2022; 6
33866262: Orepic P, Rognini G, Kannape OA, Faivre N, Blanke O
Sensorimotor conflicts induce somatic passivity and louden quiet voices in healthy listeners.
Sensorimotor conflicts are known to alter the perception of accompanying sensory signals, and deficits in sensory attenuation have been observed in schizophrenia. In the auditory domain, self-generated tones or voices (compared to tones or voices presented passively or with temporal delays) have been associated with changes in loudness perception and attenuated neural responses. It has been argued that for sensory signals to be attenuated, predicted and sensory consequences must have a consistent spatiotemporal relationship, between button presses and reafferent signals, via predictive sensory signaling, a process altered in schizophrenia. Here, we investigated auditory sensory attenuation for a series of morphed voices while healthy participants applied sensorimotor stimulations that had no spatiotemporal relationship to the voice stimuli and that have been shown to induce mild psychosis-like phenomena. In two independent groups of participants, we report a loudening of silent voices and found this effect only during maximal sensorimotor conflicts (versus several control conditions). Importantly, conflicting sensorimotor stimulation also induced a mild psychosis-like state in the form of somatic passivity and participants who experienced stronger passivity lacked the sensorimotor loudening effect. We argue that this conflict-related sensorimotor loudness amplification may represent a reduction of auditory self-attenuation that is lacking in participants experiencing a concomitant mild psychosis-like state. We interpret our results within the framework of the comparator model of sensorimotor control, and discuss the implications of our findings regarding passivity experiences and hallucinations in schizophrenia.
Schizophr Res, 2021; 231
33686522: Schaller K, Iannotti GR, Orepic P, Betka S, Haemmerli J, Boex C, Alcoba-Banqueri S, Garin DFA, Herbelin B, Park HD, Michel CM, Blanke O
The perspectives of mapping and monitoring of the sense of self in neurosurgical patients.
Surgical treatment of tumors, epileptic foci or of vascular origin, requires a detailed individual pre-surgical workup and intra-operative surveillance of brain functions to minimize the risk of post-surgical neurological deficits and decline of quality of life. Most attention is attributed to language, motor functions, and perception. However, higher cognitive functions such as social cognition, personality, and the sense of self may be affected by brain surgery. To date, the precise localization and the network patterns of brain regions involved in such functions are not yet fully understood, making the assessment of risks of related post-surgical deficits difficult. It is in the interest of neurosurgeons to understand with which neural systems related to selfhood and personality they are interfering during surgery. Recent neuroscience research using virtual reality and clinical observations suggest that the insular cortex, medial prefrontal cortex, and temporo-parietal junction are important components of a neural system dedicated to self-consciousness based on multisensory bodily processing, including exteroceptive and interoceptive cues (bodily self-consciousness (BSC)). Here, we argue that combined extra- and intra-operative approaches using targeted cognitive testing, functional imaging and EEG, virtual reality, combined with multisensory stimulations, may contribute to the assessment of the BSC and related cognitive aspects. Although the usefulness of particular biomarkers, such as cardiac and respiratory signals linked to virtual reality, and of heartbeat evoked potentials as a surrogate marker for intactness of multisensory integration for intra-operative monitoring has to be proved, systemic and automatized testing of BSC in neurosurgical patients will improve future surgical outcome.

Acta Neurochir (Wien), 2021; 163
33259460: Solcà M, Krishna V, Young N, Deogaonkar M, Herbelin B, Orepic P, Mange R, Rognini G, Serino A, Rezai A, Blanke O
Enhancing analgesic spinal cord stimulation for chronic pain with personalized immersive virtual reality.
Spinal cord stimulation (SCS) is an approved treatment for truncal and limb neuropathic pain. However, pain relief is often suboptimal and SCS efficacy may reduce over time, requiring sometimes the addition of other pain therapies, stimulator revision, or even explantation. We designed and tested a new procedure by combining SCS with immersive virtual reality (VR) to enable analgesia in patients with chronic leg pain. We coupled SCS and VR by linking SCS-induced paresthesia with personalized visual bodily feedback that was provided by VR and matched to the spatiotemporal patterns of SCS-induced paresthesia. In this cross-sectional prospective interventional study, 15 patients with severe chronic pain and an SCS implant underwent congruent SCS-VR (personalized visual feedback of the perceived SCS-induced paresthesia displayed on the patient's virtual body) and 2 control conditions (incongruent SCS-VR and VR alone). We demonstrate the efficacy of neuromodulation-enhanced VR for the treatment of chronic pain by showing that congruent SCS-VR reduced pain ratings on average by 44%. Spinal cord stimulation-VR analgesia was stronger than that in both control conditions (enabling stronger analgesic effects than incongruent SCS-VR analgesia or VR alone) and kept increasing over successive stimulations, revealing the selectivity and consistency of the observed effects. We also show that analgesia persists after congruent SCS-VR had stopped, indicating carry over effects and underlining its therapeutic potential. Linking latest VR technology with recent insights from the neuroscience of body perception and SCS neuromodulation, our personalized new SCS-VR platform highlights the impact of immersive digiceutical therapies for chronic pain.Registration: clinicaltrials.gov, Identifier: NCT02970006.
Pain, 2021; 162

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PREDICTIVE ODOR REINSTATEMENT IN PRIMARY OLFACTORY CORTEX

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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While primary sensory areas are robustly activated by sensory input signals, both theory and experiment suggest that the same cortices are modulated by internally generated activity. Predictive coding theories propose that sensory cortical responses incorporate comparisons between incoming sensory signals and expectations generated by internal models of the sensory scene. Yet several aspects of how sensory expectation is expressed in sensory circuits remain unclear. A fundamental question is whether distinct prediction effects accompany functional and anatomical differences between sensory modalities. The primary olfactory cortex (OC) receives unpatterned sensory inputs via the main olfactory bulb, and OC responses to odors reflect local associative dynamics more so than input activity. How sensory predictions are implemented in this system is not well understood. To address this, we chronically recorded neural populations in the mouse OC during presentation of odor pairs without reinforcement. 12 odors were systematically paired to establish stimulus-specific sensory predictions. In a subset of trials, the second odor was either presented alone (unpredicted) or omitted. After odor pairing, we observed changes to odor responses in both single cells and on the population level. Prediction-matched responses were bidirectionally modulated across the neural population, and became decodable from neural activity prior to odor onset. Surprisingly, after just one session, we observed reinstatement of the predicted odor representation instead of a mismatch response in trials where the predicted odor was omitted. These data indicate that prediction generates activity in the primary olfactory cortex that differs from what has been observed in other sensory modalities.

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BOARD NUMBER: S01-185

INFERENCE OF HIPPOCAMPAL REPRESENTATIONS WITH NEURAL MANIFOLDS

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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AIMS: A major question in neuroscience is inferring neural representations without relying on external information. A dominating approach has been looking for external inputs that maximally explain firing rate and timing variability. Coupling large-scale single-cell recordings with unsupervised machine learning techniques provides unique opportunities to challenge this perspective. **METHODS:** Here, we combine miniscope calcium imaging of CA1 neurons and dimensionality reduction methods to investigate the representational dynamics of the hippocampus, using spatial navigation as the ground truth. **RESULTS:** We show that during a forced-alternation task in a linear track, neural population activity in the mouse hippocampus can be constrained to a low-dimensional subspace (i.e. a neural manifold) in which both continuous (position) and discrete (direction) task-specific variables are jointly mapped. Moreover, when mice perform the alternation task on consecutive days, the manifold topology remains stable, even when recording from different sets of neurons. To gain interpretability, we trained various decoders to predict the animal's position during the task, using either the original neural population firing rates or the low-dimensional manifold activity as inputs. A comparative analysis of different dimensionality reduction methods highlighted their varying performance when inferring space representations from neural manifolds. Moreover, when adding complexity to the behavioural task (e.g. rotation of the maze in the room), this approach facilitates the evaluation of the stability and adaptation of the inferred internal representations. **CONCLUSION:** Our results reveal the more promising strategies for unbiased inference of internal hippocampal neural representations, offering new insights into the representational dynamics of the hippocampus.

Pubmed:

33288910: Mederos S, Sánchez-Puelles C, Esparza J, Valero M, Ponomarenko A, Perea G
GABAergic signaling to astrocytes in the prefrontal cortex sustains goal-directed behaviors.
GABA interneurons play a critical role in higher brain functions. Astrocytic glial cells interact with synapses throughout the whole brain and are recognized as regulatory elements of excitatory synaptic transmission. However, it is largely unknown how GABAergic interneurons and astrocytes interact and contribute to stable performance of complex behaviors. Here, we found that genetic ablation of GABA receptors in medial prefrontal cortex astrocytes altered low-gamma oscillations and firing properties of cortical neurons, which affected goal-directed behaviors. Remarkably, working memory deficits were restored by optogenetic stimulation of astrocytes with melanopsin. Furthermore, melanopsin-activated astrocytes in wild-type mice enhanced the firing rate of cortical neurons and gamma oscillations, as well as improved cognition. Therefore, our work identifies astrocytes as a hub for controlling inhibition in cortical circuits, providing a novel pathway for the behaviorally relevant midrange time-scale regulation of cortical information processing and consistent goal-directed behaviors.
Nat Neurosci, 2021; 24
34539376: Zegarra-Valdivia JA, Chaves-Coira I, Fernandez de Sevilla ME, Martinez-Rachadell L, Esparza J, Torres-Aleman I, Nuñez A
Reduced Insulin-Like Growth Factor-I Effects in the Basal Forebrain of Aging Mouse.
It is known that aging is frequently accompanied by a decline in cognition. Furthermore, aging is associated with lower serum IGF-I levels that may contribute to this deterioration. We studied the effect of IGF-I in neurons of the horizontal diagonal band of Broca (HDB) of young (≤6 months old) and old (≥20-month-old) mice to determine if changes in the response of these neurons to IGF-I occur along with aging. Local injection of IGF-I in the HDB nucleus increased their neuronal activity and induced fast oscillatory activity in the electrocorticogram (ECoG). Furthermore, IGF-I facilitated tactile responses in the primary somatosensory cortex elicited by air-puffs delivered in the whiskers. These excitatory effects decreased in old mice. Immunohistochemistry showed that cholinergic HDB neurons express IGF-I receptors and that IGF-I injection increased the expression of c-fos in young, but not in old animals. IGF-I increased the activity of optogenetically-identified cholinergic

neurons in young animals, suggesting that most of the IGF-I-induced excitatory effects were mediated by activation of these neurons. Effects of aging were partially ameliorated by chronic IGF-I treatment in old mice. The present findings suggest that reduced IGF-I activity in old animals participates in age-associated changes in cortical activity.
Front Aging Neurosci, 2021; 13

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PREMOTOR AND MOTOR CORTICAL DYNAMICS DURING SKILLED MOTOR LEARNING

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Many regions of the central nervous system contribute to motor control, but skilled motor learning is dependent on the motor cortex. Spiking in motor cortical circuits is reorganised during learning to coordinate different movements into a single action guided by the outcome of previous actions. It has been shown that, as motor performance improves, more neurons become recruited, and that the activity of these neurons becomes increasingly correlated. It is not yet clear to what extent such spatiotemporal refinement of cortical population dynamics results from changes in long-range inputs or emerge at the local microcircuit level. We performed high-density electrophysiological recordings across multiple motor regions in head-fixed mice during learning of a skilled forelimb reaching task. Mice showed increased task performance over consecutive days and movement kinematics are being analysed using automated pose estimation. We observed extensive recruitment of neurons in premotor and motor cortex during the reaching task. These data allowed detailed characterisation of the evolution of coordinated activity patterns within and across motor cortices as skilled movement was refined. This will help to disentangle the contribution of local and long-range connections during cortical learning.

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A LATENT VARIABLE MODEL USING FEATURE SHARING, FOR NEURAL STATE SPACE DISCOVERY

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Due to the recent advances in neural recording techniques, the number of simultaneously recordable neurons available is at an all-time high. Abundant amounts of data has generated a demand for models that are able to recover interpretable underlying latent structures from large neural populations, so-called Latent Variable Models. However, many of these models are prone to finding only locally optimal solutions, with a heavy dependency on initialisation and neuron selection. In our work, we propose novel priors, constraining the model to assume a common shape for the tuning curves, referred to as feature sharing in related work, which acts a strong but reasonable prior. Said priors are easily enforced using splines to model the neural response functions, which we combine with a variational autoencoder framework for latent variable encoding. This allows us to infer latent variables and tuning curves using less data, as well as providing a more stable inference procedure, with the feature sharing prior lessening the model's dependency on the initialisation. Furthermore, our model also enables the detection of multiple ensembles, allowing for multiple latent variables with parametric, selective neuron inclusion. Finally, we show that where feature sharing and ensemble detection are applicable, such as in the cases of tuning to head direction and spatial position (grid cells), we are able to recover latent trajectories on various manifolds from both simulated and real data.

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NEURONAL ACTIVITY IN THE MEDIAL ENTORHINAL CORTEX IS COORDINATED WITH THALAMIC HEAD-DIRECTION CELLS DURING SLEEP.

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Aims: Flexible navigation requires signals that are updated irrespective of the external conditions. The demonstration that pairwise coordination of both grid and head-direction (HD) cells is maintained during sleep when external inputs are largely reduced, has provided experimental evidence for attractor dynamics in these systems. Specifically, this was shown separately for the HD cells of the anterodorsal nucleus (ADN) of the thalamus and for HD and grid cells of the medial entorhinal cortex (MEC). Inactivation studies previously showed that ADN HD cells are necessary for normal grid cell activity, begging the question of the exact role of the thalamic HD cells in MEC population activity. To address this problem, we performed simultaneous recording of thalamic HD cells and the MEC. **Methods:** We implanted 32-channel and 32 or 64-channel probes into ADn and MEC respectively. The recordings consisted of a 2h sleep session, 30 mins exploration, followed by another 2h sleep session. **Results:** We analyzed 35 pairs of ADN-MEC HD cells. The coordination of ADN-MEC HD cell pairs was preserved during all phases of sleep, NREM and REM, the angular offset of the preferred direction during wake predicting the correlation during sleep, **Conclusions:** Our findings suggest that the HD signal is coherent across multiple areas of the brain's navigation system across all brain states. As ADN certainly exerts more influence on its cortical targets than it is itself influenced by cortical feedback, it is thus possible that the attractor dynamics observed in the MEC are inherited from upstream coherent activity.

Pubmed:
31516943: Narvaez-Delgado O, Rojas-Vite G, Coronado-Leija R, Ramírez-Manzanares A, Marroquín JL, Noguez-Imm R, Aranda ML, Scherrer B, Larriva-Sahd J, Concha L
Histological and diffusion-weighted magnetic resonance imaging data from normal and degenerated optic nerve and chiasm of the rat.
Diffusion-weighted magnetic resonance imaging (dMRI) is widely used to infer microstructural characteristics of tissue, particularly in cerebral white matter. Histological validation of the metrics derived from dMRI methods are needed to fully characterize their ability to capture biologically-relevant histological features non-invasively. The data described here were used to correlate metrics derived from dMRI and quantitative histology in an animal model of axonal degeneration ("Histological validation of per-bundle water diffusion metrics within a region of fiber crossing following axonal degeneration" [1]). Unilateral retinal ischemia/reperfusion was induced in 10 rats, by the elevation of pressure of the anterior chamber of the eye for 90 min. Five rats were used as controls. After five weeks, injured animals were intracardially perfused to analyze the optic nerves and chiasm with dMRI and histology. This resulted in 15 brain scans, each with 80 diffusion-sensitizing gradient directions with b = 2000 and 2500 s/mm and 20 non-diffusion-weighted images (b = 0 s/mm), with isometric voxel resolution of 125 μm. Histological sections were obtained after dMRI. Optical microscopy photomicrographs of the optic nerves (stained with toluidine blue) are available, as well as their corresponding automatic segmentations of axons and myelin.
Data Brief, 2019; 26
31326575: Rojas-Vite G, Coronado-Leija R, Narvaez-Delgado O, Ramírez-Manzanares A, Marroquín JL, Noguez-Imm R, Aranda ML, Scherrer B, Larriva-Sahd J, Concha L
Histological validation of per-bundle water diffusion metrics within a region of fiber crossing following axonal degeneration.
Micro-architectural characteristics of white matter can be inferred through analysis of diffusion-weighted magnetic resonance imaging (dMRI). The diffusion-dependent signal can be analyzed through several methods, with the tensor model being the most frequently used due to its straightforward interpretation and low requirements for acquisition parameters. While valuable information can be gained from the tensor-derived metrics in regions of homogeneous tissue organization, this model does not provide reliable microstructural information at crossing fiber regions, which are pervasive throughout human white matter. Several multiple fiber models have been proposed that seem to overcome the limitations of the tensor, with few providing per-bundle dMRI-derived metrics. However, biological interpretations of such metrics are limited by the lack of histological

confirmation. To this end, we developed a straightforward biological validation framework. Unilateral retinal ischemia was induced in ten rats, which resulted in axonal (Wallerian) degeneration of the corresponding optic nerve, while the contralateral was left intact; the intact and injured axonal populations meet at the optic chiasm as they cross the midline, generating a fiber crossing region in which each population has different diffusion properties. Five rats served as controls. High-resolution ex vivo dMRI was acquired five weeks after experimental procedures. We correlated and compared histology to per-bundle descriptors derived from three methodologies for dMRI analysis (constrained spherical deconvolution and two multi-tensor representations). We found a tight correlation between axonal density (as evaluated through automatic segmentation of histological sections) with per-bundle apparent fiber density and fractional anisotropy (derived from dMRI). The multi-fiber methods explored were able to correctly identify the damaged fiber populations in a region of fiber crossings (chiasm). Our results provide validation of metrics that bring substantial and clinically useful information about white-matter tissue at crossing fiber regions. Our proposed framework is useful to validate other current and future dMRI methods.
Neuroimage, 2019; 201

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SPARSE CHAOS AND LOCALIZATION IN THE DYNAMICS OF NEURONAL CIRCUITS

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Nerve impulses, the currency of information flows in the brain, are generated by an instability of the neuronal membrane potential dynamics. Neuronal circuits exhibit collective chaos that appears essential for learning, memory, sensory processing, and motor control. What controls the nature and intensity of collective chaos in neuronal circuits, however, is not well understood. Here we use computational ergodic theory to demonstrate that basic features of nerve impulse generation profoundly affect collective chaos in neuronal circuits. Numerically exact calculations of Lyapunov spectra, Kolmogorov-Sinai-entropy, and upper and lower bounds on attractor dimension show that changes in nerve impulse generation in individual neurons only moderately modify rate of information encoding but qualitatively transform phase space structure, reducing the number of unstable manifolds, Kolmogorov-Sinai-entropy, and attractor dimension by orders of magnitude. Beyond a critical point, marked by a localization transition of the leading covariant Lyapunov vector, the network exhibits sparse chaos: extended periods of near stable dynamics interrupted by short bursts of intense chaos. Analysis of large networks with more realistic structure indicate the generality of these findings. In cortical circuits biophysics appears tuned to this regime of episodic chaos. Our results demonstrate a tight link between fundamental features of single neuron biophysics and the collective dynamics of cortical circuits and suggest that the machinery of nerve impulse generation is tailored to enhance circuit controllability and information flow. References
E. Lazarov et al., Sci Adv 4 (2018)
M. Puelma-Touzel F. Wolf, Phys. Rev. E. (2019)
R.M. Merino et al., PNAS (2021)
C. Zhang et al., PLoS Comput Biol (2022)

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BOARD NUMBER: S01-190

PERCEPTION AND PROPAGATION OF ACTIVITY THROUGH THE CORTICAL HIERARCHY IS DETERMINED BY NEURAL VARIABILITY

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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The brains of higher organisms are composed of anatomically and functionally distinct regions performing specialised tasks; but regions do not operate in isolation. Orchestration of complex behaviours requires communication between brain regions, but how neural activity dynamics are organised to facilitate reliable transmission is not well understood. We studied this process directly by generating neural activity that propagates between brain regions and drives behaviour, allowing us to assess how populations of neurons in sensory cortex cooperate to transmit information. We achieved this by imaging two hierarchically organised and densely interconnected regions, the primary and secondary somatosensory cortex (S1 and S2) in mice while performing two-photon photostimulation of S1 neurons and assigning behavioural salience to the photostimulation. We found that the probability of perception is determined not only by the strength of the photostimulation signal, but also by the variability of S1 neural activity. Therefore, maximising the signal-to-noise ratio of the stimulus representation in cortex is critical to its continued propagation downstream. Further, we show that propagated, behaviourally salient activity elicits balanced, persistent, and generalised activation of the downstream region. Hence, our work adds to existing understanding of cortical function by identifying how population activity is formatted to ensure robust transmission of information, allowing specialised brain regions to communicate and coordinate behaviour.

Pubmed:
33452267: Contreras S, Dehning J, Loidolt M, Zierenberg J, Spitzner FP, Urrea-Quintero JH, Mohr SB, Wilczek M, Wibral M, Priesemann V
The challenges of containing SARS-CoV-2 via test-trace-and-isolate.
Without a cure, vaccine, or proven long-term immunity against SARS-CoV-2, test-trace-and-isolate (TTI) strategies present a promising tool to contain its spread. For any TTI strategy, however, mitigation is challenged by pre- and asymptomatic transmission, TTI-avoiders, and undetected spreaders, which strongly contribute to "hidden" infection chains. Here, we study a semi-analytical model and identify two tipping points between controlled and uncontrolled spread: (1) the behavior-driven reproduction number [Formula: see text] of the hidden chains becomes too large to be compensated by the TTI capabilities, and (2) the number of new infections exceeds the tracing capacity. Both trigger a self-accelerating spread. We investigate how these tipping points depend on challenges like limited cooperation, missing contacts, and imperfect isolation. Our results suggest that TTI alone is insufficient to contain an otherwise unhindered spread of SARS-CoV-2, implying that complementary measures like social distancing and improved hygiene remain necessary.
Nat Commun, 2021; 12

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POPULATION COUPLING ACROSS SENSORY AND MEMORY-RELATED CORTICAL AND HIPPOCAMPAL AREAS

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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In the interconnected mammalian cortex, the dimensionality of firing patterns is confined to a lower dimensional space. Generally, neurons in a population are constrained by the local circuit and display strong correlations. Thus, local spiking activity tends to be highly coupled. This behaviour has been assessed inside a single cortical area, but not between different cortical areas. Since neurons are likewise constrained by meso- and macro-scale connectivity, we further investigate interareal coupling. We ask if the population coupling strength is correlated to any functional relation between primary visual cortex, somatosensory cortex, perirhinal cortex and hippocampal CA1 region. Next, we ask if internal coupling is predictive for external coupling. We analyse the level of population coupling with the spike-triggered population rate: a convolution of a single unit's firing-rate with a target population. We assess the level of population coupling between different areas. We see different dynamics in population coupling within-areas versus between-areas. Generally, interareal coupling strength is low, but a small subset of units per area shows significant coupling between areas. We find correlations between within and between-area coupling, thought these are weak. Furthermore, we find divergent coupling between areas in comparison to anatomical connections. In hippocampus, spatial selectivity is negatively correlated with coupling. Different behavioural states affect coupling in divergent ways, with decreased coupling within al areas during sleeping conditions, and only the sleeping hippocampus shows significantly decrease coupling to other areas. Overall, SNPC offers an important window on cell-to-population synchronization in multi-area networks.

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MOSSY CELLS ORTHOGONALLY COMPRESS HIPPOCAMPAL INFORMATION

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Neuronal activity in the hippocampal CA3 region is disynaptically transmitted to the dentate gyrus via hilar mossy cells, which are extremely fewer in number than CA3 pyramidal cells and dentate granule cells and constitute a bottleneck middle layer. How this small layer relays hippocampal information remains unknown. We previously demonstrated that the subthreshold membrane potentials (*V*ms) of mossy cells reflect the activity of hippocampal sharp waves (SWs)/ ripples initiated in the CA3 subregion in acute hippocampal slices. Moreover, we observed the weak correlation between Δ*V*ms and the SW amplitudes which implies that the mossy cell ensembles may collectively encode information of SWs. We thus hypothesized that the SW waveforms were predictable from a set of the *V*m responses in mossy cells. In this study, we simultaneously performed *in vitro* whole-cell recordings from up to five mossy cells in hippocampal slices and *in vivo* whole-cell recordings from a mossy cell from anesthetized mice. Using machine learning analysis, we succeeded in predicting SW waveforms from the *V*m dynamics of 1-5 mossy cells more precisely than was expected by chance, and found that the use of more mossy cells was correlated with a better SW prediction. Remarkably, spatial entropy analysis revealed the orthogonal coding by mossy cell populations, which are analogous to data compression in information engineering. Therefore, we conclude that SW activity is densely compressed in the MC layer and that the MC layer may serve as a biological bottleneck layer in hippocampal computations.

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BOARD NUMBER: S01-193

PRINCIPLES UNDERLYING INFORMATION FLOW ACROSS THE ENTIRE BRAIN OF THE ZEBRAFISH

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Our knowledge of how the brain works, although growing, has always been constrained by our limited capability of measuring it. Previously, through work on individual brain regions, it has been shown that information can be stored through functional clusters of neurons (assemblies). However, it remains unclear how the brain represents information as a whole and how information flows through it. To study neuronal assemblies and their sequences, we recorded spontaneous and stimuli triggered neuronal activity in the whole brain of zebrafish larvae, while simultaneously recording free tail movement. We found a large variability in the characteristics of the functional assemblies. Although we found evidence of compact and local assemblies, most assemblies were sparse and spanned the entire brain. As a measure of information flow, we will present a Markov model of assembly transitions to create a functional graph network allowing topological analysis. The functional network shows distribution degree compatible with scale free. From this graph we identified the role of individual assemblies, some acting as information hubs connecting different brain regions. We additionally carried out and exhaustive characterization of the activation sequences triggered spontaneously, from visual and auditory stimuli and during different behavioral motives. In sum we will present for the first time a rich description of whole brain assemblies and assemblies' sequences.

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REVEALING LATENT KNOWLEDGE IN CORTICAL NETWORKS DURING GOAL-DIRECTED LEARNING

POSTER SESSION 01 - SECTION: NEURONAL POPULATION CODING AND DYNAMICS

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Behavioral performance during goal-directed learning is typically measured in the presence of reinforcement. In this context, learning has been described as a slow process with high inter-subject variability. Exploration of the neural mechanisms, therefore, has focused on identifying dynamics concomitant with these slow performance improvements. Recent work, however, has shown that task acquisition is much faster and more stereotyped than previously thought. Performance was evaluated daily in reinforced and non-reinforced ('probe') trials. These probe trials revealed a rapid and stereotyped acquisition of task contingencies early in learning which was only expressed much later in reinforced trials. Here we ask whether and how sensory cortical networks encode and control the acquisition of this latent knowledge. We used longitudinal, two-photon calcium imaging of the same large population of excitatory neurons in layer II/III of the auditory cortex (AC) while mice learned an auditory Go/No-Go task. We used unsupervised low-rank tensor decomposition to uncover low-dimensional network dynamics at different timescales. We identified a subset of neurons that were initially S+-driven but then shifted to firing at the time of reward, suggesting a role in reward learning. Another subset of S−-responsive neurons exhibited a rapid enhancement of their stimulus-driven response, suggesting a role in behavioral inhibition. Optogenetic silencing of the AC on reinforced trials led to a striking delay in the acquisition of stimulus-action associations. Overall, our work argues that latent task knowledge emerges rapidly in the AC and is crucial for goal-directed learning.

Pubmed:

30409880: Drieu C, Todorova R, Zugaro M
Nested sequences of hippocampal assemblies during behavior support subsequent sleep replay.
Consolidation of spatial and episodic memories is thought to rely on replay of neuronal activity sequences during sleep. However, the network dynamics underlying the initial storage of memories during wakefulness have never been tested. Although slow, behavioral time scale sequences have been claimed to sustain sequential memory formation, fast ("theta") time scale sequences, nested within slow sequences, could be instrumental. We found that in rats traveling passively on a model train, place cells formed behavioral time scale sequences but theta sequences were degraded, resulting in impaired subsequent sleep replay. In contrast, when the rats actively ran on a treadmill while being transported on the train, place cells generated clear theta sequences and accurate trajectory replay during sleep. Our results support the view that nested sequences underlie the initial formation of memory traces subsequently consolidated during sleep.
Science, 2018; 362
31263399: Drieu C, Zugaro M
Hippocampal Sequences During Exploration: Mechanisms and Functions.
Although the hippocampus plays a critical role in spatial and episodic memories, the mechanisms underlying memory formation, stabilization, and recall for adaptive behavior remain relatively unknown. During exploration, within single cycles of the ongoing theta rhythm that dominates hippocampal local field potentials, place cells form precisely ordered sequences of activity. These neural sequences result from the integration of both external inputs conveying sensory-motor information, and intrinsic network dynamics possibly related to memory processes. Their endogenous replay during subsequent sleep is critical for memory consolidation. The present review discusses possible mechanisms and functions of hippocampal theta sequences during exploration. We present several lines of evidence suggesting that these neural sequences play a key role in information processing and support the formation of initial memory traces, and discuss potential functional distinctions between neural sequences emerging during theta vs. awake sharp-wave ripples.
Front Cell Neurosci, 2019; 13
24667574: Cei A, Girardeau G, Drieu C, Kanbi KE, Zugaro M
Reversed theta sequences of hippocampal cell assemblies during backward travel.
Hippocampal cell assemblies coding for past, present and future events form theta-timescale (~100 ms) sequences that

represent spatio-temporal episodes. However, the underlying mechanisms remain largely unknown. We recorded hippocampal and entorhinal cortical activity as rats experienced backward travel on a model train. Although the firing fields of place cells remained stable, the order in which they were activated in the theta sequence was reversed during backward travel. Thus, hippocampal cell assemblies coordinated their relative timing to correctly predict the sequential traversal of place fields in reverse order. At the single-cell level, theta phase represented distance traveled through the field, even though the head of the rat was oriented opposite to travel direction and entorhinal head-direction cells maintained their preferred firing direction. Our results challenge most theoretical models of theta sequence generation in the hippocampus.
Nat Neurosci, 2014; 17

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PREDICTING SPATIAL BEHAVIOR FROM COMPLEX HIPPOCAMPAL OSCILLATORY CODES

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Theta and gamma oscillations are believed to organise hippocampal activity and function. The current dominant view posits the existence of two gamma frequency sub-bands, occurring at different theta phases in CA1, produced by different generators and sub-serving exclusive cognitive operations. However, this view relies on averaging over many oscillatory events, possibly overshadowing recently suggested manifold theta-nested gamma contents. We thus explored agnostically the diversity and informational capacity of individual theta-gamma bouts in LFPs recorded in the mouse dorsal hippocampus (n=5) during the learning of a spatial reference memory task. We found gamma bouts with nearly every combination of frequency, amplitude and theta-phase whichever the hippocampal layer. Importantly, although their average can resemble the two classic sub-bands pattern, they mainly reflect few strong power events. Furthermore, this diversity would not depend entirely on external generators: a spiking computational model of local recurrent circuitry involving one excitatory and one inhibitory (fast-spiking-like) populations can generate similarly complex oscillations fluctuating over the entire gamma spectrum without fine-tuning for most parameter combinations. Finally, gamma diversity is functional: a machine learning approach could accurately decode mice running speed and coarse maze position from individual theta-gamma oscillatory events, for all layers. The theta-gamma “code” nonetheless differed across layers and learning stages. Altogether, our findings suggest that hippocampal LFP oscillatory diversity is not mere noise but carries an actual encoding of context and behavior. These population codes are complex in nature and not reducible to simpler descriptions relying on few reference bands.

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A CROSS-NETWORK OSCILLATORY MOTIF UNDERPINNING COCAINE-PAIRED MEMORY RETRIEVAL

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

Charlie Clarke-Williams[1], Vitor Lopes-Dos-Santos[1], Laura Lefèvre[1], Katja Hartwich[1], Pavel Perestenko[1], Robert Toth[1], Colin Mcnamara[1], David Bannerman[2], Andrew Sharott[1], David Dupret[1]
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Memories of drug experience invigorate behavioural actions biased towards drug-paired stimuli. These maladaptive memories engage many brain regions; however, the patterns of inter-region coordination that relate to cocaine-paired memory retrieval remain elusive. Here, we uncover a brain-distributed motif of cooperative network oscillations that underlies dynamic retrieval of cocaine-paired memory. By simultaneously recording oscillatory activity in the prefrontal cortex, nucleus accumbens, amygdala, hippocampus and ventral tegmental area of the mouse brain, we show that a higher-order, cross-network pattern of beta-band (15–25 Hz) oscillatory activities reports initial recall of cocaine-paired memory, and its subsequent renewal following extinction. We further provide evidence that such beta-specific patterns of oscillatory coordination are organised by ventral tegmental area 4-Hz oscillations. Binding together a set of distributed brain networks in this manner may underlie the robustness of drug-paired memories, and hence the resilient nature of drug seeking behaviour.

Pubmed:
29617358: Lazic SE, Clarke-Williams CJ, Munafò MR
What exactly is 'N' in cell culture and animal experiments?
Biologists determine experimental effects by perturbing biological entities or units. When done appropriately, independent replication of the entity-intervention pair contributes to the sample size (N) and forms the basis of statistical inference. If the wrong entity-intervention pair is chosen, an experiment cannot address the question of interest. We surveyed a random sample of published animal experiments from 2011 to 2016 where interventions were applied to parents and effects examined in the offspring, as regulatory authorities provide clear guidelines on replication with such designs. We found that only 22% of studies (95% CI = 17%-29%) replicated the correct entity-intervention pair and thus made valid statistical inferences. Nearly half of the studies (46%, 95% CI = 38%-53%) had pseudoreplication while 32% (95% CI = 26%-39%) provided insufficient information to make a judgement. Pseudoreplication artificially inflates the sample size, and thus the evidence for a scientific claim, resulting in false positives. We argue that distinguishing between biological units, experimental units, and observational units clarifies where replication should occur, describe the criteria for genuine replication, and provide concrete examples of in vitro, ex vivo, and in vivo experimental designs.
PLoS Biol, 2018; 16

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SLOW AND FAST OSCILLATORY DYNAMICS OF NEURAL NETWORKS DURING LEARNING OF AN OLFACTORY DISCRIMINATION TASK IN RAT

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Neural oscillations are thought to provide a temporal structure facilitating information processing and inter-area communication. These oscillations coexist in different frequency bands involving neural networks at different spatial scales and are related to different aspects of behavior. We questioned the effect of learning on the dynamics of neural oscillations and the evolution of the associated neural networks. We tackled this question during an olfactory discrimination task in rats. Local field potentials were recorded in a large set of brain areas (including sensory, motor and limbic areas). We focused our analysis on the neural networks defined by rhythm synchronization in two frequency bands: (i) slow rhythms (1-10Hz), including animal respiration frequency and hippocampal theta rhythm, and (ii) beta band (15-30Hz), known to be modulated by odor learning. For the slow rhythms, preliminary analyses during odor sampling showed that hippocampal and respiratory rhythms were in a close frequency range only during a short window allowing for phase synchronization (1 or 2 cycles). Functional connectivity showed that these slow rhythms were supported by two distinct but overlapping networks which evolved during initial learning but stabilized as soon as the task rules were acquired. In the beta band, a large oscillation emerged at the end of odor sampling as the rat was learning the task. This oscillation was coherent across all recorded areas where it was expressed and when the context learning was acquired. In addition, rat behavior seemed no longer plastic when beta was strongly expressed.

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UNSUPERVISED ANALYSIS OF A DIVERSITY OF SHARP-WAVE RIPPLES

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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AIMS: Sharp-wave ripples (SWR) are high frequency hippocampal events, which presumably play different cognitive roles in memory consolidation and planning. Understanding how SWR waveform variability relates to the underlying microcircuits remains elusive but is essential to dissect cognitive function. METHODS: Here, we use topological data analysis to estimate the intrinsic structure of a wealth of SWR events recorded from awake head-fixed mice. We focus the analysis in the SWR waveform using filtered and unfiltered data centered on the SWR peak. We then apply dimensionality reduction and visualization methods to facilitate discovery of different SWR features. RESULTS: First, we show that a low number of intrinsic dimensions can explain waveform variability using a set of methods (Maximum Likelihood; Isomap; Expected Simplex Skewness; Principal Components Analysis) tested against a ground-truth. Next, we reduce dimensionality of SWR events to visualize how different potential features (e.g. frequency, amplitude, slopes, etc…) accounted for waveform variability. Using cluster measures, we evaluate the distribution of these different features over the low dimensional projection. We find different contribution of frequency, amplitude, slope and spectral entropy to the global variance of SWR, and confirmed some of these trends in synthetic datasets. CONCLUSIONS: Our study shows how topological analysis can be applied for unsupervised examination of SWR events. Moreover, by projecting physiologically relevant measures over the low dimensional SWR representation, we identify potential mechanisms associated to the expression of different features.

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NMDA AND SIGMA-1 RECEPTOR MODULATION OF RODENT NETWORK OSCILLATIONS AND NEUROINFLAMMATION

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Aims: Aberrant beta and gamma frequency oscillations are reported in patients with schizophrenia, alongside impaired working memory and attention. Additionally, increased neuroinflammation is reported in *post-mortem* tissue. Beta (20-30Hz) and gamma (30-80Hz) oscillations can be recorded from the rat anterior cingulate cortex (ACC) and hippocampus (HPC) in vitro. Cognitive and network dysfunction can be modelled in rodents using the NMDA receptor antagonist phencyclidine (PCP) and in vivo studies suggest sigma-1 receptor (σ1R) activation may reverse these deficits. However, the mechanism by which PCP causes disruption of oscillations, and the effects of PCP and σ1R modulation on network activation and neuroinflammation are unknown. **Methods:** Rat ACC/HPC slices were prepared by standard methods and transferred to an interface chamber for electrophysiological recording. Network oscillations were evoked using bath-applied kainate. PCP and/or σ1R agonist PRE-084, were bath applied with or without kainate. After recording, slices were re-sectioned and immunostained for reactive astrocyte marker GFAP. **Results:** PCP significantly increased the power of stable beta/gamma oscillations and slowed the oscillation frequency. However, the σ1R agonist PRE-084 had no effect on network oscillations. Exposing slices to PCP, versus kainate alone, showed a marked increase in reactive astrocytes. Our preliminary data suggests this PCP-evoked neuroinflammatory response was reduced by pre-application of PRE-084. **Conclusions:** Our data suggests that the NMDA-R antagonist PCP induces an abnormally large oscillation and increases neuroinflammation, whilst the σ1R agonist PRE-084 reduces PCP's neuroinflammatory effect. Further investigation is required to better understand the interplay between these two receptors and their effects on cognitive impairment.

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HUMAN VISUAL GAMMA FOR COLOR STIMULI: WHEN LGN DRIVE IS EQUALIZED, RED IS NOT SPECIAL

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Strong gamma-band oscillations in primate early visual cortex can be induced by homogeneous color surfaces. Compared to other hues, particularly strong gamma oscillations have been reported for red stimuli. However, precortical color processing and the resultant strength of input to V1 has often not been fully controlled for. Therefore, stronger responses to red might be due to differences in V1 input strength. We presented stimuli that had equal luminance and color contrast levels in a color coordinate system based on responses of the lateral geniculate nucleus, the main input source for area V1. With these stimuli, we recorded magnetoencephalography in 30 human participants. We found gamma oscillations in early visual cortex which, contrary to previous reports, did not differ between red and green stimuli of equal L-M cone contrast. Notably, blue stimuli with contrast exclusively on the S-cone axis induced very weak gamma responses, as well as smaller event-related fields and poorer change-detection performance. The strength of human color gamma responses could be well explained by the strength of thalamic input induced by each hue and does not show a clear red bias when this input strength is properly equalized.

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COMP360 PSILOCYBIN INCREASES HIGH GAMMA AND DECREASES LOW THETA AND DELTA POWER AND COHERENCE WITHIN AND BETWEEN PREFRONTAL CORTEX AND DORSAL HIPPOCAMPUS OF URETHANE-ANAESTHETISED RATS

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Background and Aims Psilocybin is a serotonergic psychedelic compound with great psychotherapeutic potential, however, its neural effects are not well understood. Psilocybin's profound cognition-, perception- and emotion-altering effects are associated with fMRI signal changes in human prefrontal cortex (PFC) and hippocampus, key structures in self-referential information processing. As these regions' functions rely on their orchestrated electrical oscillatory activity, our study aimed to evaluate psilocybin's effect on neural oscillations within and between these regions. **Methods** Multi-electrode arrays were inserted into the PFC (prelimbic cortex: PrL) and dorsal hippocampus (CA1) of urethane-anaesthetised adult male Lister Hooded rats. Local field potentials were recorded continuously during 20 min baseline, 60 min post-saline and 90 min post-dose with COMP360 (COMPASS Pathways' proprietary formulation of psilocybin; 3mg/kg i.p.) to evaluate power spectral and coherence changes. **Results** COMP360 induced a 10- and 32-fold increase in high frequency gamma (51-75 Hz) power in the PrL and CA1, respectively, and a 28-fold increase in PrL–CA1 high gamma coherence. These changes co-occurred with a decrease in low theta (3-5 Hz) and delta (0.5-3 Hz) power within each region. **Conclusion** COMP360 alters the activity within, and communication between, prelimbic cortex and hippocampal area CA1 in the rat, two brain regions whose functional equivalents in humans are strongly implicated in the pathophysiology of psychiatric disease. It remains to be seen to what extent these drug-induced changes in an anaesthetised rodent model can be related to COMP360's therapeutic mechanism of action in humans.

Pubmed:
31580184: Gigg J, McEwan F, Smausz R, Neill J, Harte MK
Synaptic biomarker reduction and impaired cognition in the sub-chronic PCP mouse model for schizophrenia.
Sub-chronic phencyclidine treatment (scPCP) provides a translational rat model for cognitive impairments associated with schizophrenia (CIAS). CIAS genetic risk factors may be more easily studied in mice; however, CIAS associated biomarker changes are relatively unstudied in the scPCP mouse.
J Psychopharmacol, 2020; 34

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BOARD NUMBER: S01-202

SHARP WAVE RIPPLES MODULATION BY THE RAPHE TO HIPPOCAMPUS GLUTAMATERGIC PATHWAY

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Aims: Sharp wave ripples (SWR), brief, high frequency oscillations (~50 ms, 100-250 Hz) in the hippocampus are essential for memory consolidation. The median raphe is known to modulate SWR activity, but the mechanism behind this modulation remains poorly understood. We are interested in the hippocampus-projecting glutamatergic neuronal population of the median raphe, characterized by the expression of the vesicular glutamate transporter type 3 (VGLUT3). We aim in this study to investigate more specifically their activity in relation to SWR and their contribution to SWR modulation. *Methods:* To target hippocampus-projecting VGLUT3-positive neurons we injected Cre-dependent retrograde viral vectors in VGLUT3-Cre mice allowing specific expression of our optogenetics tools in our population of interest. Optogenetic light stimulations are delivered through an optic fiber over the median raphe region while the hippocampal SWR are recorded in the dorsal hippocampus in the freely behaving animal. *Results:* During, non-rapid-eye-movement (non-REM) sleep, optogenetic activation of hippocampus-projecting glutamatergic neurons led to strong inhibition of SWR activity. Online detection of SWR followed by activation of the glutamatergic neurons led to reduction in length of the SWR. Control experiments in mice receiving a viral vector without an opsin showed no effect of light delivery in the median raphe on SWR activity. *Conclusion:* We reveal a powerful inhibitory control of SWR through median raphe glutamatergic neurons, suggesting a strong impact of this pathway on memory consolidation.

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BOARD NUMBER: S01-203

HIGHER COGNITION IN CROWS AND MONKEYS SHARES A NEURONAL FOUNDATION

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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The mammalian neocortex produces coordinated neuronal dynamics that result in distinct local field potentials (LFP). Models of higher cognition often implicitly assume a layered neocortical organization. Higher associative regions of bird brains do not have cortical layering, yet these regions exhibit neuronal single-cell correlates of higher cognition that are very similar to those found in mammals.
We investigated the activity of single neurons, neuronal populations, and of LFP in the avian equivalent of prefrontal cortex, while crows performed a highly controlled and cognitively demanding working memory task, adapted from monkeys.
We found that neuronal encoding and maintenance of information were affected by item load, in a way that is virtually identical to results obtained from monkey prefrontal cortex. Further, the neuronal population of crows showed divisive normalization, a computational mechanism also observed in macaque monkeys. Moreover, signatures of LFP in a narrow gamma (30-59 Hz) and the beta frequency bands (13-19 Hz) contained information about the location of the target items on the screen and were modulated by working memory load. These LFP dynamics also included bursts in beta and gamma frequencies, similar to those observed in monkeys.
Our results demonstrate that computational principles underlying higher cognition have evolved convergently, originating from different neural organizations in birds and mammals. Furthermore, similar neural oscillations support the notion that oscillations are a key component of computations underlying cognition. Therefore, our ‘bird’s eye view’ on higher cognition lends support to many modern cognitive theories that so far are entirely based on mammalian data.

Pubmed:
34859781: Hahn LA, Balakhonov D, Fongaro E, Nieder A, Rose J
Working memory capacity of crows and monkeys arises from similar neuronal computations.
Complex cognition relies on flexible working memory, which is severely limited in its capacity. The neuronal computations underlying these capacity limits have been extensively studied in humans and in monkeys, resulting in competing theoretical models. We probed the working memory capacity of crows () in a change detection task, developed for monkeys (), while we performed extracellular recordings of the prefrontal-like area nidopallium caudolaterale. We found that neuronal encoding and maintenance of information were affected by item load, in a way that is virtually identical to results obtained from monkey prefrontal cortex. Contemporary neurophysiological models of working memory employ divisive normalization as an important mechanism that may result in the capacity limitation. As these models are usually conceptualized and tested in an exclusively mammalian context, it remains unclear if they fully capture a general concept of working memory or if they are restricted to the mammalian neocortex. Here, we report that carrion crows and macaque monkeys share divisive normalization as a neuronal computation that is in line with mammalian models. This indicates that computational models of working memory developed in the mammalian cortex can also apply to non-cortical associative brain regions of birds.
Elife, 2021; 10
32849144: Hahn LA, Rose J
Working Memory as an Indicator for Comparative Cognition - Detecting Qualitative and Quantitative Differences.
Working memory (WM), the representation of information held accessible for manipulation over time, is an essential component of all higher cognitive abilities. It allows for complex behaviors that go beyond simple stimulus-response associations and inflexible behavioral patterns. WM capacity determines how many different pieces of information (items) can be used for these cognitive processes, and in humans, it correlates with fluid intelligence. As such, WM might be a useful tool for comparison of cognition across species. WM can be tested using comparatively simple behavioral protocols, based on operant conditioning, in a multitude of different species. Species-specific contextual variables that influence an animal's performance on a non-cognitive level are controlled by adapting the WM paradigm. The neuronal mechanisms by which WM

emerges in the brain, as sustained neuronal activity, are comparable between the different species studied (mammals and birds), as are the areas of the brain in which WM activity can be measured. Thus WM is comparable between vastly different species within their respective niches, accounting for specific contextual variables and unique adaptations. By approaching the question of "general cognitive abilities" or "intelligence" within the animal kingdom from the perspective of WM, the complexity of the core question at hand is reduced to a fundamental memory system required to allow for complex cognitive abilities. This article argues that measuring WM can be a suitable addition to the toolkit of comparative cognition. By measuring WM on a behavioral level and going beyond behavior to the underlying physiological processes, qualitative and quantitative differences in cognition between different animal species can be identified, free of contextual restraints.
Front Psychol, 2020; 11

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BOARD NUMBER: S01-204

NEURAL OSCILLATIONS IN STRIATUM-HIPPOCAMPUS-AMYGDALA NETWORK DURING A DOUBLE PAVLOVIAN CONDITIONING IN RATS

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Pavlovian conditioning pairing a conditioned stimulus (CS) with an unconditioned stimulus (US) leads to a conditioned response. With training, a temporal pattern emerges, reflecting a timed expectancy of the US arrival. The literature suggests an anticipated expectancy (peak time) with an aversive US, but a better accuracy with an appetitive US. Different brain structures have been highlighted to be involved in temporal behavior. The difference in temporal response between appetitive and aversive conditions raises the question of whether the implication of these structures may differ between conditions. In the present study, we compared the temporal response of rats submitted to both appetitive and aversive Pavlovian conditioning, and we explored the implication of the striatum-hippocampus-amygdala network in this response through local field potentials (LFP) recording. Rats were trained to a double Pavlovian conditioning (sound1-shock and sound2-chocolate), with the US moment at 20 seconds in both conditions. After training, we recorded LFP in three structures (dorsal striatum, hippocampus, and basolateral amygdala) during unreinforced probe trials. Time-frequency analyses highlighted the engagement of the striatum and the hippocampus in both conditions around the moment of the expected reinforcement, but with opposite modulations in the power spectrum density of oscillatory activity at 8Hz. Concerning the engagement of the amygdala, the aversive, but not appetitive, CS triggered onset-evoked modulations. Whether the differential modulations reflect different behavior/motor-related correlates and/or some competition in neuronal mechanism between appetitive and aversive conditions is an open question to be addressed in future studies. Funding: ANR TimeMemory

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BOARD NUMBER: S01-205

SHIFT OF PREFERRED THETA PHASE OF SLOW GAMMA IN HIPPOCAMPAL CA1 IS DEPENDENT ON THE LEARNING PHASE

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Aims Oscillatory brain activity reflects brain states associated with learning and memory. In the hippocampus, slow gamma (25-55 Hz) and fast gamma (60-100 Hz) oscillations are related to the encoding and retrieval of memories, respectively. Theta oscillations (4-12 Hz) are associated with information encoding. Interestingly, slow and fast gamma appear to be nested within different theta phases, and therefore, associated with its cycles. Although such interactions are likely to be related to mnemonic functions, the relationship between theta phase of gamma oscillations and memory demands remains unclear. **Methods** We recorded from the hippocampal CA1region while animals were learning an appetitive task in a T-maze over a three day period. We assessed the theta-phase preference of gamma oscillations in the starting zone, and the junction zone of the maze i.e. maze location where animals must choose a right or left turn. **Results** We found a ~180 degree shift in theta-phase preference of slow gamma when animals learned the task and evolved their performance from novice to skilled. This phase-shift occurred in the starting zone and not in the junction zone, suggesting that either a recall or a decision process took place at the starting zone. **Conclusions** Our findings support the idea of a double role of slow gamma oscillations in encoding and retrieval of memories which is dependent on the theta-phase at which it occurs. **Acknowledgements** This work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) to D.M.-V. and S.C (SFB 1280/A04 & F01, project number: 316803389).

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-206

MMP-9 INHIBITION REDUCES SHARP WAVE RIPPLE ABUNDANCE AND WORKING MEMORY IN ZEBRAFISH

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Major depressive disorder (MDD) affects over 300 million people worldwide. Currently 30-50% of patients are treatment resistant necessitating novel therapeutic targets. Previously, our lab showed that the antidepressant Venlafaxine rescues working memory in a stress mouse model combined with increased gamma power and sharp wave ripple (SWR) abundance, which are the neural correlates of learning and memory. The increase in gamma power and SWRs were dependent on Matrix Metalloproteinase-9 (MMP-9), altered in many psychiatric disorders including MDD, suggesting MMP-9 modulation as a novel mechanism for treating MDD. To investigate how modulation of MMP-9 impairs learning and memory, my research uses varied cellular and molecular techniques to answer the question: How does MMP-9 inhibition impair learning and memory in the zebrafish model? Behaviorally, we have corroborated our hypothesis that MMP-9 inhibition impairs working memory. At the cellular level MMP-9 inhibition changes the excitation to inhibition (E/I) balance in the brain as evidenced by a decrease in SWR abundance. Molecularly, the change in E/I can be partially explained by a decrease in GluA4 and Kv3.1 receptors, both of which are predominantly expressed by Parvalbumin positive (PV) neurons – a neuronal population critical for both gamma oscillations and SWR events. Though future studies will examine MMP-9 associated changes in pyramidal neurons, molecular changes in PV neurons could contribute to impaired gamma oscillations and working memory in this model organism. The implications of this research lie in the potential of MMP-9 to directly modulate E/I balance and learning and memory in zebrafish.

Pubmed:
29426953: Kahl A, Blanco I, Jackman K, Baskar J, Milaganur Mohan H, Rodney-Sandy R, Zhang S, Iadecola C, Hochrainer K
Cerebral ischemia induces the aggregation of proteins linked to neurodegenerative diseases.
Protein aggregation critically affects cell viability in neurodegenerative diseases, but whether this also occurs in ischemic brain injury remains elusive. Prior studies report the post-ischemic aggregation of ubiquitin, small ubiquitin-related modifier (SUMO) and ribosomes, however whether other proteins are also affected is unknown. Here we employed a proteomic approach to identify the insoluble, aggregated proteome after cerebral ischemia. Mice underwent transient middle cerebral artery occlusion or sham-surgery. After 1-hour reperfusion, prior to apparent brain injury, mice were sacrificed and detergent-insoluble proteins were obtained and identified by nanoLC-MS/MS. Naturally existing insoluble proteins were determined in sham controls and aggregated proteins after cerebral ischemia/reperfusion were identified. Selected aggregated proteins found by proteomics were biochemically verified and aggregation propensities were studied during ischemia with or without reperfusion. We found that ischemia/reperfusion induces the aggregation of RNA-binding and heat-shock proteins, ubiquitin, SUMO and other proteins involved in cell signalling. RNA-binding proteins constitute the largest group of aggregating proteins in ischemia. These include TDP43, FUS, hnRNPA1, PSF/SFPQ and p54/NONO, all of which have been linked to neurodegeneration associated with amyotrophic lateral sclerosis and frontotemporal dementia. The aggregation of neurodegeneration-related disease proteins in cerebral ischemia unveils a previously unappreciated molecular overlap between neurodegenerative diseases and ischemic stroke.
Sci Rep, 2018; 8

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-207

PULVINAR INACTIVATION INCREASES THE GAMMA BAND CONTRAST RESPONSE IN AREA 21A.

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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A recent study from our group has described the effect of pulvinar inactivation on the contrast response function (CRF) of neurons in areas 17 and 21a of the cat visual cortex (de Souza et al., 2019). It revealed that pulvinar signals modulate the activity of area 17 neurons, while modulatory and driver influences are observed in area 21a. These findings were based on the linear change of the CRF position (driver effect) and nonlinear variation in the CRF dynamic range (Rmax; modulatory effect). To study whether oscillatory cortical signals depend on the activity of the pulvinar, we investigated the contrast response of the gamma band in areas 17 and 21a during thalamic inactivation. Extracellular responses to full-field gratings of varying contrast were recorded in both cortical areas in anesthetized cats using linear probes before, during, and after the GABA inactivation of the pulvinar. Local field potentials (LFPs), from low-pass filtering of raw recordings, were analyzed with Wavelet to assess oscillatory gamma waves. The gamma-band contrast response was different across areas and cortical layers during inactivation. In area 17, the inactivation showed no significant changes across cortical layers. In area 21a, the inactivation yielded an increase in Rmax in most layers but mainly in layer IV. On average, for layer IV in area 21a, the coefficient of variation for Rmax increased by ~25%. These findings indicate that pulvinar signals modulate gamma rhythms in area 21a but not in the primary visual cortex. *Funding* : CIHR to CC

FENS Forum 2022
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BOARD NUMBER: S01-208

DEVELOPMENT OF A REAL-TIME, OPEN-SOURCE SHARP WAVE-RIPPLE DETECTOR PLUGIN FOR THE OPEN EPHYS PLATFORM

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Sharp wave-ripples (SWRs) are high-frequency oscillatory events involved in the dialogue between the hippocampus and cortical regions to promote memory consolidation during sleep. Many studies aiming to investigate the role of SWRs in mnemonic processes employ closed-loop strategies to detect those events and manipulate different brain areas in real-time. However, the codes and schematics necessary to replicate the detection system are not always available, which contributes negatively to the reproducibility of experiments among different research groups. Furthermore, information about the performance is not usually reported. We present the development and validation of an open-source, real-time SWR detection plugin integrated to the Open Ephys acquisition system. It contains a built-in movement detector based on accelerometer or electromyogram data that prevents false ripple events (due to chewing, grooming or moving, for instance) from triggering the stimulation/manipulation device. To determine the accuracy of the detection algorithm we first carried out simulations in Matlab with synthetic and real ripple recordings. Using a specific combination of detection parameters, we obtained 97.9% of true positive rate and 2.29 false positives per minute on the real data. Next, an Open Ephys plugin based on the same detection algorithm was developed and a closed-loop system was set up to evaluate the round trip (ripple onset-to-stimulation) latency over synthetic data. The lowest latencies obtained were around 35 ms. Besides contributing to increased reproducibility, we anticipate that the developed SWR detector plugin will be useful for a number of closed-loop applications in the field of Systems Neuroscience.

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BOARD NUMBER: S01-209

MODULATION OF SHARP-WAVE RIPPLES BY DIFFERENT COGNITIVE DEMANDS

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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AIMS: Hippocampal activity is crucial for navigation and memory abilities. However, understanding how hippocampal representations are influenced by different cognitive demands remains challenging. One example is sharp-wave ripple (SWR) events underlying memory retrieval and consolidation. Yet, whether changes in SWR follow specific trends in response to learning, novelty and memory remains unclear. METHODS: We recorded freely moving mice exposed to different types of tasks using either tetrodes or multi-site silicon probes. They explored familiar or novel open fields, and learnt running for water reward in an automatized maze that allows implementation of different navigational strategies. SWR were recorded before, during and after these several tasks, both in awake and sleep condition. SWR features were studied using standard spectral analysis and unsupervised classification algorithms. RESULTS: We identify specific trends of SWR features influenced by novelty, habituation and learning of different tasks. Novelty and learning had a significant impact in modulating SWR frequency, amplitude and spectral entropy during wake conditions. These changes however tended to habituate upon repeated expositions to the task. After the first learning session of a linear maze, SWR dynamic was strongly influenced by sleep and exhibited consistent segregation of frequency but neither of amplitude nor entropy. Unsupervised evaluation of SWR topological features suggests specific modulatory trends depending on task demands. CONCLUSION: SWR dynamics evolve distinctly under the influences of different cognitive demands.

Pubmed:
33597170: Sanchez-Aguilera A, Quintanilla JP
Sharp Wave Ripples in Alzheimer's Disease: In Search of Mechanisms.
J Neurosci, 2021; 41

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-210

ALTERATIONS OF VISUAL CORTICAL ACTIVITY IN A GENETIC MOUSE MODEL OF MIGRAINE

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Aims: Migraine is a complex disorder characterized by altered responsivity and abnormal processing of visual information. How the known synaptic alterations characterizing migraine reverberate into these network-level dysfunctions is however still unclear. **Methods**: We investigated the primary visual cortex (V1) extracellular potentials of wild-type (WT) and of a genetic rodent model of type-1-familial-hemiplegic-migraine (FHM1). **Results:** We found reduced multi-unit-activity in FHM1 mice in response to high visual contrasts. The local-field-potential γ narrow band (NB, peaking at ~60 Hz) induced by low contrasts was similar in WT and in FHM1 mice. High contrasts elicited activity in the low γ ([20-55] Hz) range for WT mice as previously observed. We observed instead that such activity induced by high visual contrasts was shifted towards higher frequencies (high γ, [70-90] Hz) in FHM1 mice. However, visual information transmission was preserved.
We investigated then with a computational model the relationship between migraine-driven synaptic alterations and the experimentally observed γ-band frequency shift. Embedding the pathological synaptic alterations characterizing the model in a spiking network model of mice V1, we replicated the aforementioned experimental results. Specifically, we found the γ band frequency shift to be linked to the increase of glutamatergic cortico-cortical signaling in FHM1. Interestingly, FHM1 alterations of thalamocortical afferents compensated for the increase in cortical recurrent excitation leading to an overall decrease of the excitation/inhibition ratio. **Conclusions:** These results shed light on the etiology of migraine-associated hypersensitivity to visual stimuli and might help properly tackling V1 circuitry to prevent this dysfunction.

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BOARD NUMBER: S01-211

USING DEEP CONVOLUTIONAL NEURAL NETWORKS TO DETECT AND INTERPRET SHARP-WAVE RIPPLES

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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AIMS: Sharp-wave ripples (SWR) are high frequency events recorded in the local field potential (LFP) of the hippocampus of rodents and humans. During SWR, the sequential firing of ensembles of neurons acts to reactivate memory traces of previously encoded experience. SWR-related interventions can influence hippocampal-dependent cognitive function, making their real-time detection crucial to understand underlying mechanisms. Moreover, with the advent of ultra-dense recordings the need to automatic detection is pressing. METHODS: Here, we introduce a 1D convolutional neural network (CNN) operating over high-density LFP recordings to detect hippocampal SWR both offline and on-line. The adapted architecture included seven convolutional deep layers composed of different filters to process 8-channel LFP inputs in increasing hierarchical complexity and one output layer delivering the probability of an occurring SWR. RESULTS: We report offline performance on several types of recordings (e.g. linear arrays, high-density probes, ultradense Neuropixels) as well as on open databases that were not used for training. By saturating the operation of different filters, we examine and interpret their optimal behaviour associated to the ground truth versus a random selection. We then use dimensionality reduction techniques to visualize how the network evolve across learning. Finally, we show how by building a plug-in for a widely used open system such Open Ephys, our method detects SWRs in real time. CONCLUSIONS: We conclude this approach can be used as a discovery tool for better understanding the dynamics of SWR.

Pubmed:
30045968: Sanchez-Aguilera A, Navas-Olive A, Valero M
Feedback and Feedforward Inhibition May Resonate Distinctly in the Ripple Symphony.
J Neurosci, 2018; 38
32371879: Navas-Olive A, Valero M, Jurado-Parras T, de Salas-Quiroga A, Averkin RG, Gambino G, Cid E, de la Prida LM
Multimodal determinants of phase-locked dynamics across deep-superficial hippocampal sublayers during theta oscillations.
Theta oscillations play a major role in temporarily defining the hippocampal rate code by translating behavioral sequences into neuronal representations. However, mechanisms constraining phase timing and cell-type-specific phase preference are unknown. Here, we employ computational models tuned with evolutionary algorithms to evaluate phase preference of individual CA1 pyramidal cells recorded in mice and rats not engaged in any particular memory task. We applied unbiased and hypothesis-free approaches to identify effects of intrinsic and synaptic factors, as well as cell morphology, in determining phase preference. We found that perisomatic inhibition delivered by complementary populations of basket cells interacts with input pathways to shape phase-locked specificity of deep and superficial pyramidal cells. Somatodendritic integration of fluctuating glutamatergic inputs defined cycle-by-cycle by unsupervised methods demonstrated that firing selection is tuneable across sublayers. Our data identify different mechanisms of phase-locking selectivity that are instrumental for flexible dynamical representations of theta sequences.
Nat Commun, 2020; 11

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-212

PROGRESSIVE ELECTROPHYSIOLOGICAL CHANGES IN A MOUSE PRION MODEL OF TERMINAL NEURODEGENERATIVE DISEASE

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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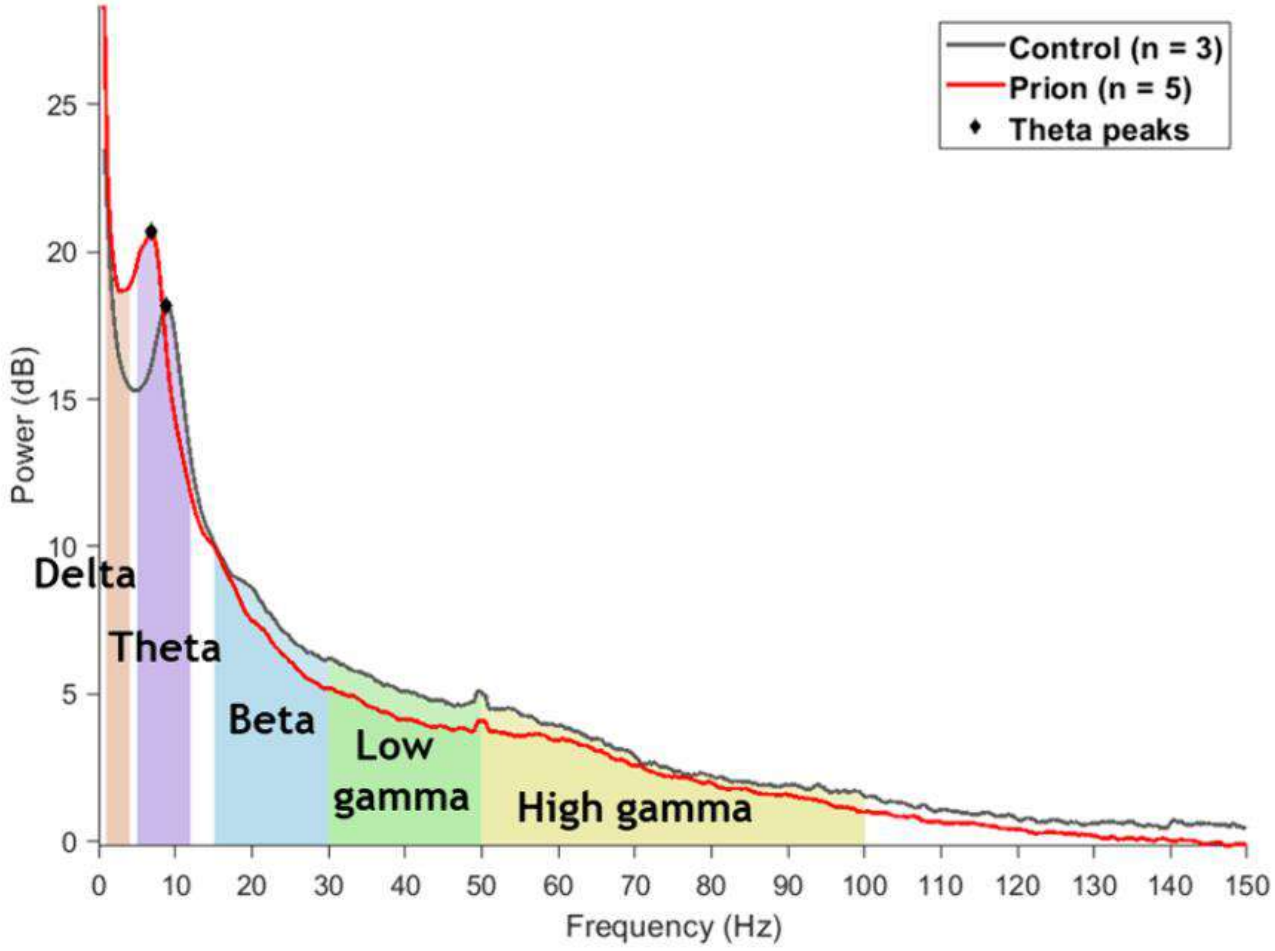
Injection of prion brain homogenate into mice induces memory deficits, progressive neuronal loss and terminal disease. Pharmacological interventions can extend survival and preserve species-specific behaviour, but it is unclear whether neural activity is preserved. Here, we define oscillatory changes in the murine prion model in order to inform future studies of the effects of interventions on neural activity. Prion and control-inoculated mice implanted with seven surface electrodes underwent weekly wireless recording from 4 weeks post-inoculation. Recordings were made until prion mice exhibited clinical signs of disease, 9–12 weeks post-inoculation. At this point, mice were humanely killed and brains retained for histological analysis. Decibel power spectra were generated from recordings using a 2.5 s sliding window with 50% overlap. Peak theta frequency and area under the curve measures were extracted (Figure 1). Effects of prion and week post-inoculation were determined using mixed effects analyses. Prion mice exhibited a progressive reduction in peak theta frequency, which differed from control mice at 8- and 9-weeks post-inoculation (Figure 1). Prion mice with peak theta frequency >7.0 Hz at 9 weeks post-inoculation survived 10–25 days longer than mice <6.5 Hz. Area under the curve measures were similar between groups except for delta power, which showed a week by inoculation interaction. The murine prion model exhibits a slowing of theta oscillatory activity and altered delta power, which are common to human neurodegenerative diseases including Alzheimer's disease. This validates the use of the prion model to investigate potential disease modifying effects of interventions aimed at treating Alzheimer's disease and related

dementias.

Figure 1. Reduced peak theta frequency in prion mice at 9 weeks post-inoculation.



FENS Forum 2022
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BOARD NUMBER: S01-213

ALTERED MARKERS OF NEURAL OSCILLATORY ACTIVITY ARE LINKED TO IMPAIRED TACTILE TEMPORAL PERCEPTION IN HEPATIC ENCEPHALOPATHY

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

Thomas Baumgarten[1], Moritz Lazar[1], Nur-Deniz Fuellenbach[2], Markus Joerdens[2], Dieter Haeussinger[2], Alfons Schnitzler[1], Joachim Lange[1]
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The perceptual separation of tactile stimuli presented in quick succession into temporally distinct sensations is linked to low-frequency prestimulus neural oscillations. Hepatic encephalopathy (HE) is a clinical complication in patients with liver cirrhosis, featuring prominent visual and tactile perceptual deficits. Here, we investigated if the relationship between tactile temporal resolution and low-frequency prestimulus neural oscillations is likewise present in HE patients demonstrating tactile perceptual impairments. 17 controls (12 male, 63 ± 2.8 y) and 17 HE patients (13 male, 59 ± 2.3 y) performed a tactile temporal discrimination task. Two suprathreshold electrical stimuli with varying stimulus-onset-asynchrony (SOA; 0-400 ms) were presented to the left index finger. Participants reported if they perceived the stimulation as one single or two temporally separate sensations, yielding an individual measure of tactile temporal resolution. Whole-head neuromagnetic activity recorded during the task was used to compute source-level peak frequency estimates in the low frequency range (5-30 Hz), which were compared across groups and correlated with metrics of tactile temporal resolution. HE patients perceived a single stimulus more often than controls for SOAs between 125-400 ms, indicating impaired tactile temporal resolution. Patients demonstrated lower alpha- and beta- band peak frequencies in posterior and frontal cortex areas, respectively. Across groups, peak frequencies were negatively correlated with metrics of individual tactile temporal resolution. Perceptual impairments in HE extend to the tactile domain. Established oscillatory parameters known to underlie temporal perception in healthy subjects are decreased in HE patients and remain connected to metrics of impaired tactile temporal sampling.

Pubmed:

34454259: Nikolov P, Baumgarten TJ, Hassan SS, Meissner SN, Füllenbach ND, Kircheis G, Häussinger D, Jördens MS, Butz M, Schnitzler A, Groiss SJ
Altered motor cortical plasticity in patients with hepatic encephalopathy: A paired associative stimulation study.
Hepatic encephalopathy (HE) is a potentially reversible brain dysfunction caused by liver failure. Altered synaptic plasticity is supposed to play a major role in the pathophysiology of HE. Here, we used paired associative stimulation with an inter-stimulus interval of 25 ms (PAS25), a transcranial magnetic stimulation (TMS) protocol, to test synaptic plasticity of the motor cortex in patients with manifest HE.
Clin Neurophysiol, 2021; 132
33976118: Baumgarten TJ, Maniscalco B, Lee JL, Flounders MW, Abry P, He BJ
Neural integration underlying naturalistic prediction flexibly adapts to varying sensory input rate.
Prediction of future sensory input based on past sensory information is essential for organisms to effectively adapt their behavior in dynamic environments. Humans successfully predict future stimuli in various natural settings. Yet, it remains elusive how the brain achieves effective prediction despite enormous variations in sensory input rate, which directly affect how fast sensory information can accumulate. We presented participants with acoustic sequences capturing temporal statistical regularities prevalent in nature and investigated neural mechanisms underlying predictive computation using MEG. By parametrically manipulating sequence presentation speed, we tested two hypotheses: neural prediction relies on integrating past sensory information over fixed time periods or fixed amounts of information. We demonstrate that across halved and doubled presentation speeds, predictive information in neural activity stems from integration over fixed amounts of information. Our findings reveal the neural mechanisms enabling humans to robustly predict dynamic stimuli in natural environments despite large sensory input rate variations.
Nat Commun, 2021; 12
30425672: Lazar M, Butz M, Baumgarten TJ, Füllenbach ND, Jördens MS, Häussinger D, Schnitzler A, Lange J

Impaired Tactile Temporal Discrimination in Patients With Hepatic Encephalopathy.
The sensory system constantly receives stimuli from the external world. To discriminate two stimuli correctly as two temporally distinct events, the temporal distance or stimulus onset asynchrony (SOA) between the two stimuli has to exceed a specific threshold. If the SOA between two stimuli is shorter than this specific threshold, the two stimuli will be perceptually fused and perceived as one single stimulus. Patients with hepatic encephalopathy (HE) are known to show manifold perceptual impairments, including slowed visual temporal discrimination abilities as measured by the critical flicker frequency (CFF). Here, we hypothesized that HE patients are also impaired in their tactile temporal discrimination abilities and, thus, require a longer SOA between two tactile stimuli to perceive the stimuli as two temporally distinct events. To test this hypothesis, patients with varying grades of HE and age-matched healthy individuals performed a tactile temporal discrimination task. All participants received two tactile stimuli with varying SOA applied to their left index finger and reported how many distinct stimuli they perceived ("1" vs. "2"). HE patients needed a significantly longer SOA (138.0 ± 11.3 ms) between two tactile stimuli to perceive the stimuli as two temporally distinct events than healthy controls (78.6 ± 13.1 ms; < 0.01). In addition, we found that the temporal discrimination ability in the tactile modality correlated positively with the temporal discrimination ability in the visual domain across all participants (i.e., negative correlation between tactile SOA and visual CFF: = -0.37, = 0.033). Our findings provide evidence that temporal tactile perception is substantially impaired in HE patients. In addition, the results suggest that tactile and visual discrimination abilities are affected in HE in parallel. This finding might argue for a common underlying pathophysiological mechanism. We argue that the known global slowing of neuronal oscillations in HE might represent such a common mechanism.
Front Psychol, 2018; 9
30109194: Baumgarten TJ, Neugebauer J, Oeltzschner G, Füllenbach ND, Kircheis G, Häussinger D, Lange J, Wittsack HJ, Butz M, Schnitzler A
Connecting occipital alpha band peak frequency, visual temporal resolution, and occipital GABA levels in healthy participants and hepatic encephalopathy patients.
Recent studies have proposed a connection between the individual alpha band peak frequency and the temporal resolution of visual perception in healthy human participants. This connection rests on animal studies describing oscillations in the alpha band as a mode of phasic thalamocortical information transfer for low-level visual stimuli, which critically relies on GABAergic interneurons. Here, we investigated the interplay of these parameters by measuring occipital alpha band peak frequency by means of magnetoencephalography, visual temporal resolution by means of behavioral testing, and occipital GABA levels by means of magnetic resonance spectroscopy. Importantly, we investigated a sample of healthy participants and patients with varying grades of hepatic encephalopathy, which are known to exhibit decreases in the investigated parameters, thus providing an increased parameter space. We found that occipital alpha band peak frequency and visual temporal resolution were positively correlated, i.e., higher occipital alpha band peak frequencies were on average related to a higher temporal resolution. Likewise, occipital alpha band peak frequency correlated positively with occipital GABA levels. However, correlations were significant only when both healthy participants and patients were included in the analysis, thereby indicating a connection of the measures on group level (instead of the individual level). These findings provide new insights into neurophysiological and neurochemical underpinnings of visual perception.
Neuroimage Clin, 2018; 20
28382013: Baumgarten TJ, Schnitzler A, Lange J
Beyond the Peak - Tactile Temporal Discrimination Does Not Correlate with Individual Peak Frequencies in Somatosensory Cortex.
The human sensory systems constantly receive input from different stimuli. Whether these stimuli are integrated into a coherent percept or segregated and perceived as separate events, is critically determined by the temporal distance of the stimuli. This temporal distance has prompted the concept of temporal integration windows or perceptual cycles. Although this concept has gained considerable support, the neuronal correlates are still discussed. Studies suggested that neuronal oscillations might provide a neuronal basis for such perceptual cycles, i.e., the cycle lengths of alpha oscillations in visual cortex and beta oscillations in somatosensory cortex might determine the length of perceptual cycles. Specifically, recent studies reported that the peak frequency (the frequency with the highest spectral power) of alpha oscillations in visual cortex correlates with subjects' ability to discriminate two visual stimuli. In the present study, we investigated whether peak frequencies in somatosensory cortex might serve as the correlate of perceptual cycles in tactile discrimination. Despite several different approaches, we were unable to find a significant correlation between individual peak frequencies in the alpha- and beta-band and individual discrimination abilities. In addition, analysis of Bayes factor provided evidence that peak frequencies and discrimination thresholds are unrelated. The results suggest that perceptual cycles in the somatosensory domain are not necessarily to be found in the peak frequency, but in other frequencies. We argue that studies based solely on analysis of peak frequencies might thus miss relevant information.
Front Psychol, 2017; 8

28276493: Baumgarten TJ, Königs S, Schnitzler A, Lange J
Subliminal stimuli modulate somatosensory perception rhythmically and provide evidence for discrete perception.
Despite being experienced as continuous, there is an ongoing debate if perception is an intrinsically discrete process, with incoming sensory information treated as a succession of single perceptual cycles. Here, we provide causal evidence that somatosensory perception is composed of discrete perceptual cycles. We used in humans an electrotactile temporal discrimination task preceded by a subliminal (i.e., below perceptual threshold) stimulus. Although not consciously perceived, subliminal stimuli are known to elicit neuronal activity in early sensory areas and modulate the phase of ongoing neuronal oscillations. We hypothesized that the subliminal stimulus indirectly, but systematically modulates the ongoing oscillatory phase in S1, thereby rhythmically shaping perception. The present results confirm that, without being consciously perceived, the subliminal stimulus critically influenced perception in the discrimination task. Importantly, perception was modulated rhythmically, in cycles corresponding to the beta-band (13-18 Hz). This can be compellingly explained by a model of discrete perceptual cycles.
Sci Rep, 2017; 7
26324922: Baumgarten TJ, Schnitzler A, Lange J
Beta oscillations define discrete perceptual cycles in the somatosensory domain.
Whether seeing a movie, listening to a song, or feeling a breeze on the skin, we coherently experience these stimuli as continuous, seamless percepts. However, there are rare perceptual phenomena that argue against continuous perception but, instead, suggest discrete processing of sensory input. Empirical evidence supporting such a discrete mechanism, however, remains scarce and comes entirely from the visual domain. Here, we demonstrate compelling evidence for discrete perceptual sampling in the somatosensory domain. Using magnetoencephalography (MEG) and a tactile temporal discrimination task in humans, we find that oscillatory alpha- and low beta-band (8-20 Hz) cycles in primary somatosensory cortex represent neurophysiological correlates of discrete perceptual cycles. Our results agree with several theoretical concepts of discrete perceptual sampling and empirical evidence of perceptual cycles in the visual domain. Critically, these results show that discrete perceptual cycles are not domain-specific, and thus restricted to the visual domain, but extend to the somatosensory domain.
Proc Natl Acad Sci U S A, 2015; 112
25331603: Baumgarten TJ, Schnitzler A, Lange J
Prestimulus Alpha Power Influences Tactile Temporal Perceptual Discrimination and Confidence in Decisions.
Recent studies have demonstrated that prestimulus alpha-band activity substantially influences perception of near-threshold stimuli. Here, we studied the influence of prestimulus alpha power fluctuations on temporal perceptual discrimination of suprathreshold tactile stimuli and subjects' confidence regarding their perceptual decisions. We investigated how prestimulus alpha-band power influences poststimulus decision-making variables. We presented electrical stimuli with different stimulus onset asynchronies (SOAs) to human subjects, and determined the SOA for which temporal perceptual discrimination varied on a trial-by-trial basis between perceiving 1 or 2 stimuli, prior to recording brain activity with magnetoencephalography. We found that low prestimulus alpha power in contralateral somatosensory and occipital areas predicts the veridical temporal perceptual discrimination of 2 stimuli. Additionally, prestimulus alpha power was negatively correlated with confidence ratings in correctly perceived trials, but positively correlated for incorrectly perceived trials. Finally, poststimulus event-related fields (ERFs) were modulated by prestimulus alpha power and reflect the result of a decisional process rather than physical stimulus parameters around ~150 ms. These findings provide new insights into the link between spontaneous prestimulus alpha power fluctuations, temporal perceptual discrimination, decision making, and decisional confidence. The results suggest that prestimulus alpha power modulates perception and decisions on a continuous scale, as reflected in confidence ratings.
Cereb Cortex, 2016; 26

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BOARD NUMBER: S01-214

NEURAL NETWORK DYNAMICS OF ADULT MICE IN AFFILIATIVE SOCIAL CONTEXTS

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Social interactions between mammalian conspecifics involve the dynamic coupling of multiple brain regions to modulate and control behavioral decisions (Chen & Hong, 2018). Oscillatory neural activity in the theta (4-12 Hz) and gamma (30-80 Hz) bands, coordinates communication within neuronal ensembles dispersed over multiple brain regions (Buzsáki & Draguhn, 2004). These coordinated processes underlie the demanding cognitive function of social behavior (Tendler & Wagner, 2015; Uhlhaas & Singer, 2006). Further, modified theta (Geschwind & Levitt, 2007; Rippon et al., 2007; Wass, 2011) and gamma rhythms (Lazaro et al., 2019) have been reported in autism spectrum disorders. We recorded extracellular neural activity from 19 brain regions of behaving CD-1 male mice in two social contexts to reveal the neural dynamics governing affiliative social interactions. The mice displayed similar social preferences but variable theta power between the contexts. Further, the various brain regions displayed altered coherence of theta rhythmicity during the investigation of the same stimuli while encountering them in distinct contexts, indicating the role of theta coherence in the neural representation of social context. Overall, our results suggest that a specific sub-network of brain regions exhibits synchronous activity in a given context.

Pubmed:
31333400: Kumar S, Mohapatra AN, Sharma HP, Singh UA, Kambi NA, Velpandian T, Rajan R, Iyengar S
Altering Opioid Neuromodulation in the Songbird Basal Ganglia Modulates Vocalizations.
Although the interplay between endogenous opioids and dopamine (DA) in the basal ganglia (BG) is known to underlie diverse motor functions, few studies exist on their role in modulating speech and vocalization. Vocal impairment is a common symptom of Parkinson's disease (PD), wherein DA depletion affects striosomes rich in μ-opioid receptors (μ-ORs). Symptoms of opioid addiction also include deficiencies in verbal functions and speech. To understand the interplay between the opioid system and BG in vocalization, we used adult male songbirds wherein high levels of μ-ORs are expressed in Area X, a BG region which is part of a circuit similar to the mammalian thalamocortical-basal ganglia loop. Changes in DA, glutamate and GABA levels were analyzed during the infusion of different doses of the μ-OR antagonist naloxone (50 and 100 ng/ml) specifically in Area X. Blocking μ-ORs in Area X with 100 ng/ml naloxone led to increased levels of DA in this region without altering the number of songs directed toward females (FD). Interestingly, this manipulation also led to changes in the spectro-temporal properties of FD songs, suggesting that altered opioid modulation in the thalamocortical-basal ganglia circuit can affect vocalization. Our study suggests that songbirds are excellent model systems to explore how the interplay between μ-ORs and DA modulation in the BG affects speech/vocalization.
Front Neurosci, 2019; 13
32839617: Yuste R, Hawrylycz M, Aalling N, Aguilar-Valles A, Arendt D, Armañanzas R, Ascoli GA, Bielza C, Bokharaie V, Bergmann TB, Bystron I, Capogna M, Chang Y, Clemens A, de Kock CPJ, DeFelipe J, Dos Santos SE, Dunville K, Feldmeyer D, Fiáth R, Fishell GJ, Foggetti A, Gao X, Ghaderi P, Goriounova NA, Güntürkün O, Hagihara K, Hall VJ, Helmstaedter M, Herculano-Houzel S, Hilscher MM, Hirase H, Hjerling-Leffler J, Hodge R, Huang J, Huda R, Khodosevich K, Kiehn O, Koch H, Kuebler ES, Kühnemund M, Larrañaga P, Lelieveldt B, Louth EL, Lui JH, Mansvelder HD, Marin O, Martinez-Trujillo J, Chameh HM, Mohapatra AN, Munguba H, Nedergaard M, Němec P, Ofer N, Pfisterer UG, Pontes S, Redmond W, Rossier J, Sanes JR, Scheuermann RH, Serrano-Saiz E, Staiger JF, Somogyi P, Tamás G, Tolias AS, Tosches MA, García MT, Wozny C, Wuttke TV, Liu Y, Yuan J, Zeng H, Lein E
A community-based transcriptomics classification and nomenclature of neocortical cell types.
To understand the function of cortical circuits, it is necessary to catalog their cellular diversity. Past attempts to do so using anatomical, physiological or molecular features of cortical cells have not resulted in a unified taxonomy of neuronal or glial cell types, partly due to limited data. Single-cell transcriptomics is enabling, for the first time, systematic high-throughput measurements of cortical cells and generation of datasets that hold the promise of being complete, accurate and permanent. Statistical analyses of these data reveal clusters that often correspond to cell types previously defined by morphological or physiological criteria and that appear conserved across cortical areas and species. To capitalize on these new methods, we

propose the adoption of a transcriptome-based taxonomy of cell types for mammalian neocortex. This classification should be hierarchical and use a standardized nomenclature. It should be based on a probabilistic definition of a cell type and incorporate data from different approaches, developmental stages and species. A community-based classification and data aggregation model, such as a knowledge graph, could provide a common foundation for the study of cortical circuits. This community-based classification, nomenclature and data aggregation could serve as an example for cell type atlases in other parts of the body.
Nat Neurosci, 2020; 23
26039701: Michael FM, Mohapatra AN, Venkitasamy L, Chandrasekar K, Seldon T, Venkatachalam S
Contusive spinal cord injury up regulates mu-opioid receptor (mor) gene expression in the brain and down regulates its expression in the spinal cord: possible implications in spinal cord injury research.
Traumatic spinal cord injury (SCI) is one of the dreaded neurological conditions and finding a cure for it has been a hot area of research. Naloxone - a mu-opiate receptor (mor) antagonist was considered for SCI treatment based on its positive effects under shock conditions. In contrary to animal studies based reports about the potential benefits of naloxone in treating SCI, a large scale clinical trial [National Acute Spinal Cord Injury Study II (NASCIS II)] conducted in USA failed to witness any effectiveness. The inconsistency noticed was intriguing. Therefore, the objective of the present study was to re-examine the role of naloxone in treating SCI using a highly standardised Multicenter Animal Spinal Cord Injury Study (MASCIS) animal model of contusive SCI. Results indicated that naloxone produced negligible and insignificant neuroprotection. In an attempt to understand the cause for the failure, it was found that mu-opioid receptor (mor) gene expression was upregulated in the brain but was down regulated in the spinal cord after contusive SCI. Given that the beneficial effects of naloxone are through its action on the mor, the results indicate that unlike the brain, spinal cord might not be bracing to utilise the opiate system in the repair process. This could possibly explain the failure of naloxone treatment in NASCIS II. To conclude, opiate antagonists like naloxone may be neuroprotective for treating traumatic brain injuries, but not for traumatic/contusive spinal cord injuries.
Neurol Res, 2015; 37
33071736: Kumar S, Mohapatra AN, Pundir AS, Kumari M, Din U, Sharma S, Datta A, Arora V, Iyengar S
Blocking Opioid Receptors in a Songbird Cortical Region Modulates the Acoustic Features and Levels of Female-Directed Singing.
The organization of the anterior forebrain pathway (AFP) of songbirds important for context-dependent singing is similar to that of cortical basal ganglia loops (CBG) in mammals, which underlie motor behaviors including vocalization. Since different components of the AFP express high levels of μ-opioid receptors (μ-ORs) as do CBG loops, songbirds act as model systems to study the role of opioid modulation on vocalization and the motivation to sing. The AFP in songbirds includes the cortical/pallial region LMAN (lateral magnocellular nucleus of the anterior nidopallium) which projects to Area X, a nucleus of the avian basal ganglia. In the present study, microdialysis was used to infuse different doses of the opioid antagonist naloxone in LMAN of adult male zebra finches. Whereas all doses of naloxone led to significant decreases in the number of FD (female-directed) songs, only 100 and 200 ng/ml of naloxone affected their acoustic properties. The decrease in FD song was not accompanied by changes in levels of attention toward females or those of neurotransmitters (dopamine, glutamate, and GABA) in LMAN. An earlier study had shown that similar manipulations in Area X did not lead to alterations in the number of FD songs but had significantly greater effects on their acoustic properties. Taken together, our results suggest that there are reciprocal effects of OR modulation on cortical and basal ganglia components of the AFP in songbirds.
Front Neurosci, 2020; 14
33935888: Parishar P, Mohapatra AN, Iyengar S
Investigating Behavioral Responses to Mirrors and the Mark Test in Adult Male Zebra Finches and House Crows.
Earlier evidence suggests that besides humans, some species of mammals and birds demonstrate visual self-recognition, assessed by the controversial "mark" test. Whereas, there are high levels of inter-individual differences amongst a single species, some species such as macaques and pigeons which do not spontaneously demonstrate mirror self-recognition (MSR) can be trained to do so. We were surprised to discover that despite being widely used as a model system for avian research, the performance of zebra finches () on the mark test had not been studied earlier. Additionally, we studied the behavioral responses of another species of passerine songbirds (Indian house crows;) to a mirror and the MSR mark test. Although a small number of adult male zebra finches appeared to display heightened responses toward the mark while observing their reflections, we could not rule out the possibility that these were a part of general grooming rather than specific to the mark. Furthermore, none of the house crows demonstrated mark-directed behavior or increased self-exploratory behaviors when facing mirrors. Our study suggests that self-directed behaviors need to be tested more rigorously in adult male zebra finches while facing their reflections and these findings need to be replicated in a larger population, given the high degree of variability in mirror-directed behaviors.
Front Psychol, 2021; 12


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BOARD NUMBER: S01-215

EVOLUTION OF HIPPOCAMPAL AND NEOCORTICAL SLEEP OSCILLATIONS DURING GRADUAL LEARNING OF A Y-MAZE ALLOCENTRIC TASK IN MICE.

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Learning to navigate in space toward a goal (escape plateforme, reward, etc) is a gradual process. Some behavioural tasks for mice are designed so that gradual learning spans over few days. Here, we hypothesise that during sleep in between training sessions, coordination of sleep oscillations evolve with the learning stages. We ask whether some sleep oscillation parameters could predict mice performance on the following training session. To do so, we use an allocentric Y-maze spatial navigation task in which mice have to use visual clues to navigate toward a reward. Mice were allowed to run 10 sessions a day and took 3-4 days to learn to find reliably the reward. After each training session, we recorded hippocampal and neocortical local field potentials during sleep for 1-2 hours. We observe in our preliminary data that hippocampal sharp wave ripple density increases throughout learning, while thalamocortical spindle density increases in the initial phases of learning but decreases after mice reach learning plateau. Thus spindle density might predict next day task performance.

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BOARD NUMBER: S01-216

CHAOTIC DYNAMICS OF LARGE CORTICAL ENSEMBLES: THEORY VS EXPERIMENT

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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The variability of cortical single unit responses to external stimuli is well investigated. However, variability of the collective activity of large ensembles of cortical neurons is not understood. During the slow wave sleep (SWS) or immobility, large blocks of cortical tissue show synchronous slow oscillations. Here we show that the amplitude and duration of the slow oscillations are highly variable. Further, a minimal mean-field, deterministic model, with only two free parameters, without any noise, can capture this in vivo dynamics. In fact, we were able to obtain a quantitative match between the model and about ten independent experimental measurements, such as the duration and amplitude of the individual cycles and their fluctuations from cycle to cycle. The quantitative, theory-experiment match was accurate across several sessions and rats. Although this was a minimal, mean field theory model, we were able to explain several spiking characteristics of individual neurons. This close match between theory and experiment is unprecedented. It allowed us to interrogate the model to determine the mechanisms of collective variability of neural ensemble. The model revealed that the cortical ensemble cycles between a more active states that is near a dynamical critical point and a less active states that is subcritical. These predictions too match in a quantitative fashion between theory and experiment. In sum, the collective dynamics of large cortical ensembles is highly variable and it rapidly fluctuates between critical and subcritical states. These features are the emergent phenomena of a simple deterministic network without any noise.

Pubmed:
34425227: Azimi A, Alizadeh Z, Ghorbani M
The essential role of hippocampo-cortical connections in temporal coordination of spindles and ripples.
The predominant activity of slow wave sleep is cortical slow oscillations (SOs), thalamic spindles and hippocampal sharp wave ripples. While the precise temporal nesting of these rhythms was shown to be essential for memory consolidation, the coordination mechanism is poorly understood. Here we develop a minimal hippocampo-cortico-thalamic network that can explain the mechanism underlying the SO-spindle-ripple coupling indicating of the succession of regional neuronal interactions. Further we verify the model predictions experimentally in naturally sleeping rodents showing our simple model provides a quantitative match to several experimental observations including the nesting of ripples in the spindle troughs and larger duration but lower amplitude of the ripples co-occurring with spindles or SOs compared to the isolated ripples. The model also predicts that the coupling of ripples to SOs and spindles monotonically enhances by increasing the strength of hippocampo-cortical connections while it is stronger at intermediate values of the cortico-hippocampal projections.
Neuroimage, 2021; 243
34003291: Dehnavi F, Koo-Poeggel PC, Ghorbani M, Marshall L
Spontaneous slow oscillation-slow spindle features predict induced overnight memory retention.
Synchronization of neural activity within local networks and between brain regions is a major contributor to rhythmic field potentials such as the EEG. On the other hand, dynamic changes in microstructure and activity are reflected in the EEG, for instance slow oscillation (SO) slope can reflect synaptic strength. SO-spindle coupling is a measure for neural communication. It was previously associated with memory consolidation, but also shown to reveal strong interindividual differences. In studies, weak electric current stimulation has modulated brain rhythms and memory retention. Here, we investigate whether SO-spindle coupling and SO slope during baseline sleep are associated with (predictive of) stimulation efficacy on retention performance.
Sleep, 2021; 44
33463873: Moghimi S, Shadkam A, Mahmoudzadeh M, Calipe O, Panzani M, Edalati M, Ghorbani M, Routier L, Wallois F

The intimate relationship between coalescent generators in very premature human newborn brains: Quantifying the coupling of nested endogenous oscillations.
Temporal theta slow-wave activity (TTA-SW) in premature infants is a specific neurobiomarker of the early neurodevelopment of perisylvian networks observed as early as 24 weeks of gestational age (wGA). It is present at the turning point between non-sensory driven spontaneous networks and cortical network functioning. Despite its clinical importance, the underlying mechanisms responsible for this spontaneous nested activity and its functional role have not yet been determined. The coupling between neural oscillations at different timescales is a key feature of ongoing neural activity, the characteristics of which are determined by the network structure and dynamics. The underlying mechanisms of cross-frequency coupling (CFC) are associated with several putative functions in adults. In order to show that this generic mechanism is already in place early in the course of development, we analyzed electroencephalography recordings from sleeping preterm newborns (24-27 wGA). Employing cross-frequency phase-amplitude coupling analyses, we found that TTAs were orchestrated by the SWs defined by a precise temporal relationship. Notably, TTAs were synchronized to the SW trough, and were suppressed during the SW peak. Spontaneous endogenous TTA-SWs constitute one of the very early signatures of the developing temporal neural networks with key functions, such as language and communication. The presence of a fine-tuned relationship between the slow activity and the TTA in premature neonates emphasizes the complexity and relative maturity of the intimate mechanisms that shape the CFC, the disruption of which can have severe neurodevelopmental consequences.
Hum Brain Mapp, 2020; 41
31881184: Haghighi SS, Ghorbani M, Dehnavi F, Safaie M, Moghimi S
Motivated forgetting increases the recall time of learnt items: Behavioral and event related potential evidence.
We investigated modulation of the recall time in a motivated forgetting (MF) paradigm and the neural manifestation of it through event related potential (ERP) analysis. We studied whether compared to failed attempts in suppression, partial success can potentiate control mechanisms and this might manifest, neurally as modulation of ERP components related to conscious recollection, and behaviorally as delayed recall of learnt items. We employed a modified version of the Think\No-Think paradigm with dominant number of No-Think words (cued to forget). We defined a forgetting index as FI = Final Recall Time-Initial Recall Time. The MF trials were separated into three conditions according to their corresponding FI; Forget, Delayed Recall, and Recall conditions. The findings revealed significant late ERP effects in terms of a late parietal positivity (LPP), modulated by the item condition, that appeared to reflect the consequence of conscious suppression on actual retrieval of stored memory. Over the same topographic location, FI was negatively correlated with the LPP amplitude, demonstrating the consequence of inhibition processing during MF in modulating the recall time. The negative correlation between LPP and FI provides evidence that increased recall time due to MF is also related to reduced activity, probably in the hippocampal-parietal network, corresponding to recollection of suppressed memories.
Brain Res, 2020; 1729
30946463: Dehnavi F, Moghimi S, Sadrabadi Haghighi S, Safaie M, Ghorbani M
Opposite effect of motivated forgetting on sleep spindles during stage 2 and slow wave sleep.
Memories selectively benefit from sleep. In addition to the importance of the consolidation of relevant memories, the capacity to forget unwanted memories is also crucial. We investigated the effect of suppressing unwanted memories on electroencephalography activity of subsequent sleep using a motivated forgetting (MF) paradigm as compared with a control non-forgetting task. Subjects were randomly assigned to nap or no-nap groups. We used a modified version of the think/no-think paradigm with dominant number of no-think words cued to be forgotten and included only subjects capable of suppressing unwanted memories by performing an initial subject inclusion experiment. In both groups and conditions, the performance of the subjects in recalling the word pairs learned in the beginning of the day was evaluated in a final recall test. We found that both nap and no-nap groups recalled significantly less no-think words in the MF condition compared to the control condition. Moreover, for the nap group, in the MF compared to the control condition, spindle power and density increased during stage 2 (S2) whereas they decreased during slow wave sleep (SWS). Interestingly, recall performance of no-think words was negatively correlated with spindle power during S2 whereas it was positively correlated with spindle power during SWS. These results indicate that sleep spindles are sensitive to the previous MF experiences and suggest a differential role of sleep spindles during S2 and SWS in memory processing during sleep.
Sleep, 2019; 42
30639838: Hashemi NS, Dehnavi F, Moghimi S, Ghorbani M
Slow spindles are associated with cortical high frequency activity.
Thalamocortical network shows self-sustained oscillations in a broad frequency range especially during slow wave sleep when cortical neurons show synchronized transitions between a quiescent down state and an active up state with beta and gamma oscillations. Inconsistent with previous models, thalamocortical spindles are separated into slow spindles (8_12 Hz) and fast spindles (13_17 Hz) with differential properties. We proposed that cortical high frequency (~ 25 Hz) activity during up states is the key ingredient for the generation of slow spindles. In fact, the nonlinear interaction between cortical high

frequency and thalamic oscillations at fast spindle frequency reproduces oscillations in the range of the difference between the two frequencies that lies into the range of slow spindle. The developed simple deterministic thalamocortical model is able to reproduce up and down states with stochastic high-frequency up-state activity as well as both fast and slow spindles. In agreement with the previous experimental observations, the fast and slow spindles are generated at opposing phases of the up state. To further confirm the causal relationship between slow spindles and cortical high frequency oscillations, we next showed that externally applied high frequency stimulation enhanced the slow spindle activity. Moreover, the prediction of the model was validated experimentally by recording EEG from subjects during nap. Both model and experimental results show increase in high frequency activity before slow spindles. Our findings suggest the important role of cortical high frequency activity in the generation of slow spindles.
Neuroimage, 2019; 189
22463245: Ghorbani M, Mehta M, Bruinsma R, Levine AJ
Nonlinear-dynamics theory of up-down transitions in neocortical neural networks.
The neurons of the neocortex show ~1-Hz synchronized transitions between an active up state and a quiescent down state. The up-down state transitions are highly coherent over large sections of the cortex, yet they are accompanied by pronounced, incoherent noise. We propose a simple model for the up-down state oscillations that allows analysis by straightforward dynamical systems theory. An essential feature is a nonuniform network geometry composed of groups of excitatory and inhibitory neurons with strong coupling inside a group and weak coupling between groups. The enhanced deterministic noise of the up state appears as the natural result of the proximity of a partial synchronization transition. The synchronization transition takes place as a function of the long-range synaptic strength linking different groups of neurons.
Phys Rev E Stat Nonlin Soft Matter Phys, 2012; 85
20972223: Ghorbani M, Mohammad-Rafiee F
Geometrical correlations in the nucleosomal DNA conformation and the role of the covalent bonds rigidity.
We develop a simple elastic model to study the conformation of DNA in the nucleosome core particle. In this model, the changes in the energy of the covalent bonds that connect the base pairs of each strand of the DNA double helix, as well as the lateral displacements and the rotation of adjacent base pairs are considered. We show that because of the rigidity of the covalent bonds in the sugar-phosphate backbones, the base pair parameters are highly correlated, especially, strong twist-roll-slide correlation in the conformation of the nucleosomal DNA is vividly observed in the calculated results. This simple model succeeds to account for the detailed features of the structure of the nucleosomal DNA, particularly, its more important base pair parameters, roll and slide, in good agreement with the experimental results.
Nucleic Acids Res, 2011; 39
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Twist-stretch correlation of DNA.
We present an elastic model for B-form DNA with variable radius to study the elastic twist-stretch coupling of stretched DNA. In this model, only two strain variables as well as the changes in the energy of the hydrogen and covalent bonds of DNA during the deformation are considered. It is found that, depending on the elastic constants, the correlation between twisting and stretching of a helical molecule can be positive or negative. It is shown that for the suitable elastic constants in the model, the twist-stretch coupling of DNA behaves nonmonotonically, and contrary to intuition, the DNA twisting and stretching are positively correlated for small distortions. This result is entirely consistent with recent experimental results.
Phys Rev E Stat Nonlin Soft Matter Phys, 2008; 78

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LOW FREQUENCY FLUCTUATIONS OF BRAIN ACTIVITY DURING PROLONGED COGNITIVE PERFORMANCE: TOWARDS A PROACTIVE MODEL OF RESSOURCE CONTROL OF ATTENTION

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Sustained attention refers to the capacity to focus on a particular task for a long period of time. Sustaining such cognitive activity at an optimal regime comes at a cost, and a consequent decrease of cognitive resources accompanied by a decrement in performance during long-lasting cognitive effort has been largely theorized. However, recent evidence from monkey research show that, during the execution of an attentional task for one hour and more, behavioural performance consistently fluctuates between phases of optimal and suboptimal performance at a very slow rhythm of circa 5 cycles per second, concomitant with fluctuations in the prefrontal spatial attention information, challenging the classical view that cognitive effort wanes executive control. Here, we provide evidence that this is also at play in humans and we describe the underlying neural markers. Specifically, we recorded EEG activity and pupil size diameter from 19 human participants while they performed a vigilance task for 90 minutes. We found that both detection times and pupil size fluctuated in the frequency rage around 5 cycles per hour. In addition, frequency analysis on the EEG signal along the whole recording session revealed significant low-frequency fluctuations in the same frequency range as detection time and pupil size, that were independent from response times. A topographical analysis highlights the involvement of prefrontal and parietal regions. These results suggest a proactive resource control mechanism, in which the system copes with resource depletion by imposing a fluctuation in cognitive resources, possibly mediated by the noradrenergic system and the locus coeruleus.

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BOARD NUMBER: S01-218

RESPIRATORY MODULATION IN THE BRAIN: DEEP IN THE CELLS

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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The brain is a place of intense rhythmic activity, each brain area can express one or more rhythms. A central question in neuroscience is to understand how these rhythmic activities can coordinate very distant brain areas. In recent years, several studies have showed the respiratory rhythm can drive the LFP from numerous brain areas. Hence, beyond its primary vital function, the respiratory rhythm could be used by the brain as a master clock promoting communication between distant brain areas. However, outside the olfactory system it is not known if respiration-related oscillation (RRo) could exist in the membrane potential (MP) of neurons neither if it can structure spiking discharge. To fill this gap, we co-recorded MP and LFP activities in different non-olfactory brain areas: median prefrontal cortex (mPFC), primary somatosensory cortex (S1), primary visual cortex (V1), and hippocampus (HPC), in urethane-anesthetized rats. Using respiratory cycle by respiratory cycle analysis, we observed that respiration could modulate both MP oscillations and spiking discharges in all recorded areas. Further quantifications revealed RRo episodes were transient in most neurons (5 consecutive cycles in average). RRo development in MP was largely influenced by the presence of respiratory modulation in the LFP. By showing the respiratory rhythm influenced brain activity deep to the MP of non-olfactory neurons, our data support the idea that respiratory rhythm could mediate long-range communication. As a conclusion, these findings reinforce the idea that the respiratory rhythm is a fundamental component of the neuronal network dynamics, and should not be underestimated.

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ZOOMING IN ON HUMAN CORTEX DYNAMICS: AN IN VITRO SPATIOTEMPORAL STUDY

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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The use of *in vitro* brain slices from animal models has largely been the one studied in both physiological and pathological research. However, from a translational perspective many fundamental neurobiological issues remain open. Here we undertake research on human brain tissue obtained from surgery for the treatment of epilepsy and brain tumors. We studied the dynamics of the human cerebral cortex network in vitro under spontaneous slow-wave activity and modulation of the excitation/inhibition balance. We recorded extracellular local field potentials using 16 and 32 multielectrode arrays and performed histology for an anatomical and physiological correlation. The average frequency of spontaneous slow oscillations was 0.36±0.31Hz (equivalent to that reported for other mammals). Interestingly, we found differences between slices from peritumoral tissue (0.38±0.31Hz) and those from epilepsy surgery (0.13±0.16Hz) that were characterized by shorter Up states (0.19±0.39sec) with higher firing and longer Down states (25±29.18sec). These findings are coherent with those reported before for $GABA_A$ blockade (Barbero-Castillo et al, 2021), suggesting a mechanistic base for the hyperexcitability in epileptic tissue. Indeed, applying $GABA_A$-Rs blockers, these changes were still enhanced, making peritumoral tissue fire similarly to the epileptic one. Finally, we found a layer-specific dynamics consistent with the one described for other species, showing higher firing rate and longer Up states in infragranular layers (infra: 0.17±0.07s, supra: 0.12±0.08s). This dynamical characterization broadens our understanding of the mechanistic organization of the human cortical network at the microscale.
Funded by European Union’s Horizon 2020 No. 945539 (Human Brain Project SGA3).

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CONTROL OF CORTICAL SLOW OSCILLATIONS AND EPILEPTIFORM DISCHARGES BY PHOTOSWITCHABLE TYPE 1 MUSCARINIC LIGANDS

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Neuromodulation techniques aim to precisely control neural activity, and their development is relevant for both basic neuroscience and functional repair in neurological disorders. A promising neuromodulation tool is photopharmacology, the control by light of drug activation in the tissue following chemical drug manipulation to be photoswitchable. Here, we investigated the effects of two such novel drugs, a photoswitchable type-1 muscarinic agonist, Benzylquinolone-carboxylic acid–Azo-Iperoxo (BAI, Agnetta et al, 2017), and a photoswitchable type-1 muscarinic antagonist, Cryptozepine-2 (Riefolo et al, 2021), to determine their effects over synchronized network activity in the cerebral cortex. We first studied *in vitro* photoresponses in HEK cells and demonstrated that light-controlled BAI and Cryptozepine-2 were M1 specific. Next, evaluated them over a complex neuronal network, the cerebral cortex. Slow oscillatory activity in slices was transformed into a significantly faster oscillation following white light illumination of the BAI-containing tissue. The modulation of activity *in vivo* with BAI was consistent with *in vitro* results. In the case of M_1-antagonist Cryptozepine-2, not only slow oscillatory activity was modulated into a slower rhythm, but also seizure activity evoked by the muscarinic super-agonist, Iperoxo, was transformed into an oscillatory-like activity after ultra-violet light illumination of Cryptozepine-2. These results shed light on the contribution of the M1-acetylcholine receptor to cortical dynamics and validate the use of photoswitchable drugs for spatiotemporal modulation of brain networks by light. Funded by CORTICOMOD PID2020-112947RB-I00 by MCIN/ AEI /10.13039/501100011033, CECH(AGAUR IU16-011508) and EU grant No.945539 (HBP, SGA3). JMS is supported by FPI Spanish Ministry of Science PRE2018-086203.

Pubmed:
34018704: Barbero-Castillo A, Riefolo F, Matera C, Caldas-Martínez S, Mateos-Aparicio P, Weinert JF, Garrido-Charles A, Claro E, Sanchez-Vives MV, Gorostiza P
Control of Brain State Transitions with a Photoswitchable Muscarinic Agonist.
The ability to control neural activity is essential for research not only in basic neuroscience, as spatiotemporal control of activity is a fundamental experimental tool, but also in clinical neurology for therapeutic brain interventions. Transcranial-magnetic, ultrasound, and alternating/direct current (AC/DC) stimulation are some available means of spatiotemporal controlled neuromodulation. There is also light-mediated control, such as optogenetics, which has revolutionized neuroscience research, yet its clinical translation is hampered by the need for gene manipulation. As a drug-based light-mediated control, the effect of a photoswitchable muscarinic agonist (Phthalimide-Azo-Iper (PAI)) on a brain network is evaluated in this study. First, the conditions to manipulate M2 muscarinic receptors with light in the experimental setup are determined. Next, physiological synchronous emergent cortical activity consisting of slow oscillations-as in slow wave sleep-is transformed into a higher frequency pattern in the cerebral cortex, both in vitro and in vivo, as a consequence of PAI activation with light. These results open the way to study cholinergic neuromodulation and to control spatiotemporal patterns of activity in different brain states, their transitions, and their links to cognition and behavior. The approach can be applied to different organisms and does not require genetic manipulation, which would make it translational to humans.
Adv Sci (Weinh), 2021; 8

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QUANTIFICATION OF INFRA-SLOW BRAIN SIGNALS USING GRAPHENE MICROTRANSISTORS (GSGFETS)

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Conventional methods for the acquisition and recording of cortical signals are limited by their filtering out of infra-slow signals (<0.1 hz). Traditional AC amplifiers filter out frequencies below 0.1-0.2Hz to avoid voltage drifts and signal attenuation due to electrode vs amplifier impedance. Graphene microtransistors (gSGFETs) allow the quantification of the brain's full band signal, hence they can record with high fidelity the infra-slow activity (DC potentials) simultaneously with the rest of higher (>0.1 Hz) frequencies (AC). Here, we used this capability of gSGFETs to measure full band activity, including infra-slow one, during spontaneous and evoked cerebral cortex activity in vitro. To address this objective, we recorded from slices of the cerebral cortex in animal models as well as in human cortical slices with arrays of 16 gSGFETs. The excitability levels as well as the emergent activity patterns were systematically controlled by varying potassium levels, electric fields, excitatory / inhibitory balance, $GABA_B$-Rs activation or cholinergic agonists. The DC components of the slow oscillations (Up and Down states), pre-epileptic activity and epileptiform discharges, was quantified. The use of graphene transistors allows the detailed investigation of the physiological properties of the infra-slow component of brain signals in space and time. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 881603 (Graphene Flagship).

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PERTURBATIONAL CORTICAL COMPLEXITY ACROSS CORTICAL AREAS, BRAIN STATES AND STIMULUS LOCATION

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Perturbational complexity index (PCI) is a measure of evoked cortical complexity used in the clinic to quantify awareness levels in patients with disorders of consciousness. Following stimulation, the causal interactions between cortical areas determine the spatiotemporal profile of the response and the evoked cortical complexity. In Dasilva et al 2021, we adapted this measure to micro-ECoG recordings in the mouse brain. There, we determined that PCI varied with anaesthesia levels, being lower for deeper versus lighter anaesthesia. Here, we explored the recently introduced PCI state-transition (PCI^{st}, Comolatti et al 2019) evoked by stimulation in two different areas: frontal versus occipital. We found that frontal-evoked PCI^{st} was larger than the one evoked by stimulation of the occipital cortex. This difference was consistent at all levels of anaesthesia. Interestingly, occipital-evoked PCI^{st} showed larger sensitivity to anaesthesia levels, while frontal-evoked PCI^{st} maintained higher values across all of them. We also evaluated PCI^{st} evoked by either cortical or thalamic stimulation. No differences were detected between both stimulus under isoflurane anaesthesia. Instead, under light ketamine, thalamic stimulation evoked significantly lower complexity than cortical stimulation. We include a computational model of PCI in the rodent's brain consisting of mean field models from different cortical areas integrated through a full brain connectome. We investigate the properties of complexity depending on brain states and stimulation location, providing some potential mechanisms underlying the observed features. Funded by the European Union’s Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3)

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DESCRIBING THE GRADUAL TRANSFORMATION OF PHYSIOLOGICAL CORTICAL NETWORK ACTIVITY (UP/DOWN STATES) INTO PAROXYSMAL DISCHARGES (INTERICTAL EVENTS, SWDS, SLES)

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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AIMS: Epilepsy is a neurological disorder affecting millions worldwide. Up to 30% of the patients remain refractive to medications and many more experience severe side effects. It is therefore important to determine the factors that either promote, or prevent the emergence, propagation and maintenance of the paroxysmal discharges that characterize the epileptic brain. In the present study we explore the transition from normal/physiological network activity (in the form of spontaneously occurring Up/Down states), through recurrent interictal spikes to the establishment of full blown seizure-like events (SLEs).
METHODS: We performed LFP recordings from young and adult mouse brain slices, with up to 4 electrodes in cortical layers II/III and V, or in hippocampal CA3 area. Epileptiform activity was induced with the zero-Mg2+ model.
RESULTS: Upstate duration exhibits a bimodal distribution in layer V, but not in layers II/III. Under epileptogenic conditions, Upstate activity is accelerated until eventually eliminated and replaced by epileptiform events, beginning (interictal spikes) and stabilizing (SLEs) simultaneously in the different layers and columns. Compared to the hippocampus, the neocortex exhibits a reduced threshold for epileptiform activity, which is strongly dependent on the presence or absence of Upstate activity.
CONCLUSIONS: Our data reveal age-dependent, as well as region and layer-specific differences both in the pattern of endogenous activity and in the progression to epileptiform discharges. Ongoing analysis aims to characterize the patterns of synchronized activity in the different states, through dynamical systems analysis and data-driven comparative analysis of various network features evolution, both in time and in time-frequency domains.

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TRAVELING UP STATES IN THE POST-SUBICULUM REVEAL AN ANATOMICAL GRADIENT OF INTRINSIC PROPERTIES

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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The post-subiculum (PoSub) is the primary cortical stage of the head-direction (HD) system. PoSub's main downstream target, the medial entorhinal cortex (MEC), is organized along its dorsoventral (DV) axis with increasing scale of spatial tuning from dorsal to ventral regions accompanied by a decrease in rectifying currents. To investigate if a similar organization exists in PoSub, we recorded populations of PoSub neurons with linear silicon probes. In contrast to MEC, HD tuning was uniform across the PoSub DV axis. However, we found that a gradient of spiking properties emerged during non-Rapid Eye Movement (NREM) sleep. Neuronal firing rates decreased along the DV axis, corresponding to a gradient in the duration of population-wide periods of inactivity (DOWN states). Interestingly, DOWN-to-UP but not UP-to-DOWN transitions traveled from dorsal to ventral PoSub, resulting in the sequential activation of neurons along the DV axis. Because DOWN-to-UP sequences are thought to be important for the functional properties of NREM sleep, we sought to understand the mechanism underlying this gradient. We built a computational model with a linear array of recurrently connected units and compared the spatiotemporal properties of DOWN states generated by various biophysical gradients. The model uniquely matched experimental observations with a gradient in the strength of a rectifying current, suggesting that the sleep-related gradient in PoSub has a common anatomical origin to the functional gradient in MEC. These results reveal that MEC receives spatiotemporally structured input along the DV axis during NREM sleep, and shed light on the mechanisms of DOWN-to-UP spike sequences.

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CHARACTERIZATION OF CONSCIOUS AND UNCONSCIOUS BRAIN PATTERNS IN RATS

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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When going from unconscious to awake states, a wide range of spatio-temporal brain patterns are observed. The description and analysis of these dynamics is critical to assess the subject's level of consciousness. Here, we quantify the dynamics and properties of brain patterns occurring in the transition from unconsciousness towards wakefulness in rats. Local Field Potentials were recorded from six different cortical areas in freely moving rats under anesthesia and natural sleep conditions. Brain patterns were first manually classified as deep anesthesia (DEEP), light anesthesia (LIGHT), slow wave sleep (SWS), microarousals (MA), REM and awake (AW). We applied a set of computational metrics to find a unique identification of these patterns: spectral power, synchronization between different areas and the characteristics of the slow oscillations in DEEP, LIGHT and SWS (Torao-Angosto et al, 2021) were studied. A separation of the different brain patterns was obtained by analyzing and clustering their spectral properties, with the best separation being in the delta (1-4 Hz)-gamma (30-100 Hz) plane. Further, we identified sub-states within the bistable regime of unconsciousness (DEEP, LIGHT and SWS) by including the duration of Up/Down states in the feature space. The synchronicity between different brain areas was found to be higher in unconscious than in aroused states. The characterization of brain dynamics allowed us to identify and describe the different paths the brain takes to recover consciousness. Funded by the EU 2020 Framework Programme for Research and Innovation No. 945539 (Human Brain Project SGA3).

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BOARD NUMBER: S01-226

SOUND-EVOKED MULTIAREA CORTICAL RESPONSES IN DIFFERENT BRAIN STATES IN RATS

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Not only the properties of stimulation, but also the functional state of the cortical network determine the amplitude, gain, or spatiotemporal patterns of cortical responses to stimuli. In a previous study we described that during a brain state dominated by slow waves, the gain of the system varied between Up/Down states in a relationship that depended on stimulus intensity (Reig et al., 2015). Here, we explored different brain states exhibiting distinct activity patterns, including both physiological (slow wave sleep, rapid-eye movement sleep and wakefulness) and pharmacologically induced (deep, medium and light anesthesia levels), characterized as in (Manasanch et al., FENS-2022). Sound-evoked responses had maximum amplitude in those states of higher network synchronization, namely SWS and deep anesthesia. Such responses propagated to auditory related (A1, A2) and non-related primary cortices (M1, V1), associative (PtA) and higher-order cortical areas (OFC) in all stages of the sleep-wake cycle. Responses to identical stimuli, though, had smaller amplitude during REM and awake states than in SWS or deep anesthesia. Interestingly, such modulation of amplitude followed similar patterns as to those during Up (for awake or even REM) and Down states (as in SWS), suggesting that Up states share features with wakefulness. Instead, under different levels of anesthesia, sound-evoked responses were rather circumscribed to A1 and A2 but hardly propagated to other non-auditory cortical areas. We quantify the dialogue across areas to capture brain state dynamics and how incoming information flows across the network in different states. Funded by EU 2020 No 945539 (Human Brain Project, SGA3).

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SPATIOTEMPORAL DYNAMICS OF ANOXIC DEPOLARIZATION IN THE RAT SOMATOSENSORY CORTICES

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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To investigate the neocortical spatiotemporal dynamics induced by brain anoxia, we recorded electrocorticographic activities, multi-layer local field potential (LFP) using linear silicon probes and intracellular activities of single pyramidal neurons from the primary somatosensory cortex (S1) in an asphyxia rodent model. Oxygen deprivation resulted first in an increase in high-frequency activity (> 10 Hz), simultaneously present at all recording sites. This was followed by a short period of gradually fading slow oscillations that ended, about one minute after anoxia onset, in a continuous isoelectric profile. The isoelectric state was then transiently interrupted by a large amplitude tri-phasic wave - named ‘Wave of Anoxic Depolarization’ (WAD) -, which correlated with an anoxic-dependent large membrane depolarization of cortical neurons. LFP recordings showed that the anoxic depolarization was initiated in the deep layers of S1, from two potential cortical sites, and then propagated bidirectionally through the cortical column. Reinstating oxygen supply to the brain interrupted the depolarization-block and engaged a slow membrane repolarization in cortical neurons, captured extracellularly as a ‘Wave of post-Anoxic Repolarization’ (WpAR). Neuronal repolarization also started in the deep layers, although from a less circumscribed region than the onset depolarization process. The WpAR was systematically followed by a progressive return of cellular and network activities, suggesting that it could represent a valuable marker of brain functions recovery and may serve clinical research on interventional strategies to optimize resuscitation procedures.

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STRUCTURE OR DYNAMICS? ON THE ROLE OF THE CANONIC CIRCUIT IN THE EMERGENCE OF CORTICAL MULTI-FREQUENCY OSCILLATIONS

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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Bottom-up and top-down functional connectivity (FC) *between* cortical regions are mediated by faster and slower frequency oscillations. Eventually, different cortical layers also oscillate at different frequencies. Therefore, it has been proposed that FC in different directions operates at different frequencies because of the distinct resonance frequencies of the source and target layers. Based on a systematic modelling analysis, we propose here on the contrary that the frequency specialization of directed FC stems as an emergent by-product of self-organized collective dynamics rather than of hardwired anatomy and interneuronal diversity. Specifically, we analyze rate models of coupled cortical regions embedding a realistic multi-layer anatomical organization. Every layer includes an excitatory and a unique inhibitory population of the fast-spiking type, resonating at a fast gamma-range frequency. Despite this intrinsic resonance, we can obtain a great diversity of possible dynamical states as a function of contextual inputs and the relative strength of excitation and inhibition. Regimes in which faster and slower frequency oscillations predominate respectively in superficial or deeper layers arise spontaneously and robustly due to non-linear inter-layer interactions. However, we also find that the strength and the dominant frequencies of directed FC can be flexibly adjusted by modulating the dynamical regime of the network, without the need of structural changes. We furthermore show that dynamical regimes allowing such a multi-frequency adaptivity of directed functional interactions are extremely unlikely to arise in randomized networks. We thus speculate that the canonic circuit wiring may be optimized to favour the frequency multiplexing of inter-regional information routing.

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NON-NECESSARY NEURAL ACTIVITY IN THE PRIMATE CORTEX

POSTER SESSION 01 - SECTION: EMERGENT DYNAMICS IN NEURAL NETWORKS

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When neurophysiologists record neural activity from the brain, they often conclude that neural tuning to task variables suggests a functional role of the brain area studied in task performance. However, it remains unknown how reliably such correlations indicate a functional role. To answer this question, we chronically recorded neural activity in the prefrontal cortex of monkeys during the performance of four cognitive tasks. Previous studies had demonstrated that only one of those tasks causally depends on the recorded area; the other three tasks are not impaired by lesions in the relevant area. Surprisingly, we found that the prevalence and strength of single neuron and ensemble tuning were equivalent across all four tasks. This suggests that non-necessary cognitive signals are prevalent in the cerebral cortex of primates, challenging one of the fundamental assumptions of cognitive neurophysiology.

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3D POSE ESTIMATION ENABLES VIRTUAL HEAD-FIXATION IN FREELY MOVING RATS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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When studying movement control, questions are often tackled in a task designed to cover specific aspects of movement e.g., initiation or direction. However, such constrained behaviors might impact our ability to understand the continuous movement control in a natural setting. Additionally, spontaneous movements outside the task-related movements impact the ongoing neural activity. While movements of interest can be controlled, a wide range of out-of-context movements are still executed by the animal. This complicates the analysis, since neural activity is “contaminated” with out-of-context movements. We employed FreiPose, a versatile framework to capture 3D motion during freely moving behavior. FreiPose enabled us to reconstruct body poses and individual paw movements, detect behavioral classes, and optogenetic stimulation effects. Using GAMs we were able to describe the ongoing activity of recorded neurons in rat's motor cortex as a combination of simultaneous, multiplexed coding of body posture and paw movement parameters. Thus, the activity in the motor cortex was strongly influenced by movements of not just individual body parts, but the whole rat's body. To separate the individual influences, we developed “virtual head-fixation” approach which enabled us to remove the contribution of body posture-related neuronal activity. This revealed a much clearer representation of paw-related activity. We unveiled a large fraction of neurons with tuning to the paw movements, which was previously masked by body posture tuning. Together, measuring behavior using FreiPose and applying virtual head-fixation will enable a much better understanding of the single-trial neural activity in freely moving as well as constrained behavioral tasks.

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PROSPECTIVE AND RETROSPECTIVE INFLUENCES OF HEARING ONE'S VOICE IN THE SENSE OF AGENCY OVER SPEECH

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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The subjective experience of causing an action is known as the sense of agency (SoA). It has been suggested that an auditory hallucination results from dysfunction in the SoA over speech. However, the SoA over speech has not been well investigated so far. The current study sought to characterize the SoA over speech using implicit and explicit measures. We first examined temporal compression of a perceived interval between an action and its outcome, termed intentional binding, in a speech paradigm. We distorted the pitch of feedback voice to manipulate self-voice identity. As a result, a perceived interval was more compressed when participants heard their natural voice than the distorted voice, suggesting that the SoA over speech is enhanced by hearing one's own voice. Next, we switched the action for intentional binding from vocalization to button press to investigate the significance of hearing one's voice in the SoA. In consequence, we did not find a significant difference in perceived intervals between the different pitch conditions. This result suggests that the retrospective effect of hearing one's voice is less important for the SoA over speech than the prospective effect of predicting an outcome caused by one's speech act. Finally, we examined agency judgment as an explicit measure. We found a top-down effect of self-voice identity, causing the agency judgment to be resistant regarding low-level sensorimotor inconsistency. Our findings highlight the mechanism of how one experiences the SoA in speech and contribute to understanding aberrant experience in auditory hallucinations.

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BOARD NUMBER: S01-232

THE RELATIONSHIP BETWEEN THE VISUAL PREDICTION ERROR AND THE AUTOMATIC ONLINE CONTROL

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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The online control system allows for rapid corrective response to unexpected perturbation. The mechanism of the automatic motor correction is thought to be based on the sensory prediction error between the efference copy and the actual sensory feedback signals. However, it is still uncertain whether the amount of the prediction error affects the corrective response. Therefore, the purpose of the current study was to clarify the relationship between the prediction error and the automatic corrective response. Participants wore an electrode cap for EEG measurement and were required to control a cursor on a monitor synchronized with a stylus. They were asked to reach the center of a Gaussian blob target as accurate as possible for small and large Gaussian blob conditions. In addition, the cursor was displaced 1 cm to the left or right from the actual cursor position in half of the trials as a perturbation. The automatic corrective response was detected by the arm acceleration in the lateral direction, and the amount of prediction error was estimated by the N1 event-related potential (ERP). Our results showed that the amplitude of the automatic response and the N1 ERP to the cursor displacement was larger in the small Gaussian blob conditions than in the large one. In addition, the amplitude of the automatic response was correlated with the N1 ERP. Therefore, we suggested that the automatic corrective response is dependent on the prediction error manipulated by the size of the Gaussian blob.

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BOARD NUMBER: S01-233

THE COMMON DESCENDING NEURAL DRIVE TO AGONIST AND ANTAGONIST MUSCLES IS HIGHER IN STROKE PATIENTS COMPARED TO CONTROLS.

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Aims: The mechanisms underlying the disruption of motor control after stroke are still misunderstood. The study of the oscillatory link between electromyographic activity of synergistic muscles, i.e. intermuscular coherence (IMC), can provide information on the alteration of the neural control mechanisms underpinning muscular activation. To date, no study has investigated the effects of stroke on IMC in muscle pairs whose muscles have either similar or opposite functional roles, especially during an ecologic task with clinical implications. ***Methods***: Twenty-five chronic post-stroke subjects and twenty-four controls were included. Kinematic and electromyographic data were recorded during twenty active elbow extensions at spontaneous speed. IMC was quantified in the beta band between the biceps brachii and the brachioradialis (BB-BR) as antagonist muscle pair and between the triceps brachii and the brachioradialis (TB-BR) as agonist-antagonist muscle pair. ***Results***: A two-factor ANOVA revealed an overall Group effect, where patients had higher IMC than controls for both muscle pairs ($F(1,47)=5.23$, $p=0.02$, $\eta^2_p=0.05$ [0.003:0.13]) (**Fig. 1**).

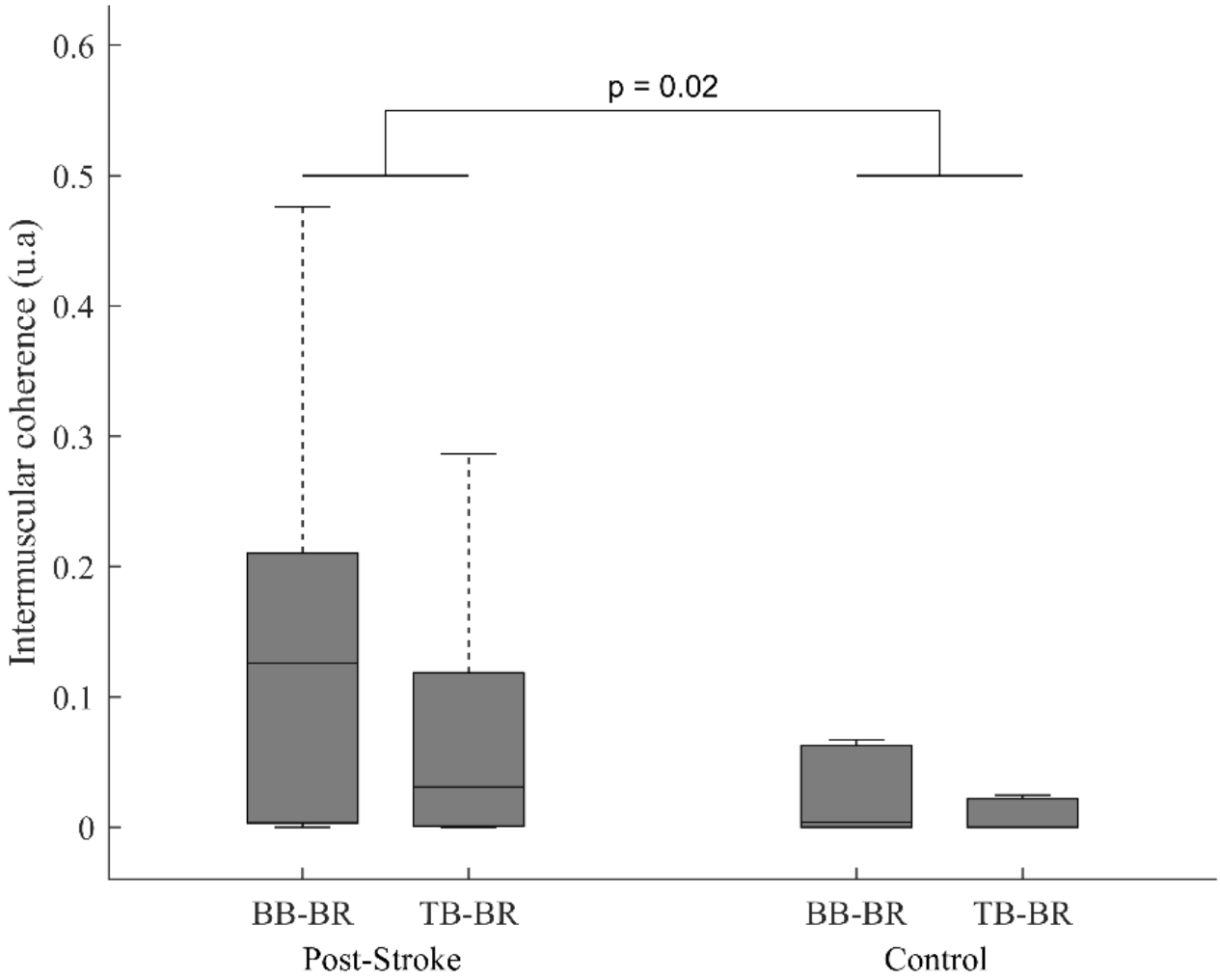

Figure 1: Intermuscular coherence of post-stroke patients and control subjects for the antagonist-antagonist (BB-BR) and agonist-antagonist (TB-BR) muscle pairs. ***Conclusions***: This work revealed higher IMC in post-stroke subjects compared to controls during active elbow extension. Whether the muscle pairs are composed of muscles with similar or opposite functional roles, there is no interaction effect that would allow to further specify this main result. We propose that these findings could reflect a simplification of the motor control mechanisms in post-stroke subjects, through a more common descending neural drive to the paretic upper-limb muscles.

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MOLECULAR DELINEATION OF CORTICO-BRAINSTEM VERSUS CORTICO-SPINAL PROJECTION NEURONS DURING DEVELOPMENT

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Skilled motor control requires precise connections between subcerebral projection neurons (SCPN) in the cerebral cortex with their appropriate targets, e.g., in brainstem or spinal cord. Corticospinal neurons (CSN) extend axons under tight molecular control to distinct spinal levels; however, the molecular mechanisms that limit axons of cortico-brainstem neurons (CBN) to supra-spinal levels are yet unknown. Here, we show that the brainstem-cervical transition zone distinguishes CBN from CSN from the earliest times of axon extension, and that CBN preferentially reside in lateral cortex. Using high throughput single cell RNA sequencing of retrogradely labeled, FACS purified CBN and CSN at critical times of white matter axon extension, we identified molecularly defined subpopulations within the broader SCPN population. SCPN can be further delineated based on their axonal projection specificity (brainstem vs. spinal) as well as their location in lateral vs. medial cortex. We establish that *Neuropeptide Y* (*Npy*) is specifically enriched in CBN in the lateral cortex. Our results highlight a molecular delineation of SCPN axon targeting at developmental times well before their axons reach their appropriate segmental target. This work will have direct impact on investigations into the development of circuitry required for skilled motor control and can potentially inform treatment options to enhance functional recovery after adult CNS injury.

Pubmed:
29605090: Holm MM, Kaiser J, Schwab ME
Extracellular Vesicles: Multimodal Envoys in Neural Maintenance and Repair.
The physiology of the central nervous system (CNS) is built on a foundation of connection, integration, and the exchange of complex information among brain cells. Emerging evidence indicates that extracellular vesicles (EVs) are key players in the intercellular communication that underlies physiological processes such as synaptic plasticity and the maintenance of myelination. Furthermore, upon injury to the CNS, EVs may propagate inflammation across the blood-brain barrier and beyond, and also appear to mediate neuroprotection and modulate regenerative processes. In neurodegenerative diseases, EVs may play roles in the formation, spreading, and clearance of toxic protein aggregates. Here, we discuss the physiological roles of EVs in the healthy and the diseased CNS, with a focus on recent findings and emerging concepts.
Trends Neurosci, 2018; 41
32887692: Kaiser J, Maibach M, Piovesana E, Salpeter I, Escher N, Ormen Y, Schwab ME
TGFβ1 Induces Axonal Outgrowth via ALK5/PKA/SMURF1-Mediated Degradation of RhoA and Stabilization of PAR6.
Transforming growth factor (TGF)β1 has repeatedly been associated with axonal regeneration and recovery after injury to the CNS. We found TGFβ1 upregulated in the stroke-denervated mouse spinal cord after ischemic injury to the motor cortex as early as 4 d postinjury (dpi) and persisting up to 28 dpi. Given the potential role of TGFβ1 in structural plasticity and functional recovery after stroke highlighted in several published studies, we investigated its downstream signaling in an model of neurite outgrowth. We found that in this model, TGFβ1 rescues neurite outgrowth under growth inhibitory conditions via the canonical TGFβR2/ALK5 signaling axis. Thereby, protein kinase A (PKA)-mediated phosphorylation of the E3 ubiquitin ligase SMURF1 induces a switch of its substrate preference from PAR6 to the Ras homolog A (RhoA), in this way enhancing outgrowth on the level of the cytoskeleton. This proposed mechanism of TGFβ1 signaling could underly the observed increase in structural plasticity after stroke as suggested by the temporal and spatial expression of TGFβ1. In accordance with previous publications, this study corroborates the potential of TGFβ1 and associated signaling cascades as a target for future therapeutic interventions to enhance structural plasticity and functional recovery for stroke patients.
eNeuro, 2020 Sep/Oct; 7
29768943: Wahl AS, Erlebach E, Brattoli B, Büchler U, Kaiser J, Ineichen BV, Mosberger AC, Schneeberger S, Imobersteg S, Wieckhorst M, Stirn M, Schroeter A, Ommer B, Schwab ME
Early reduced behavioral activity induced by large strokes affects the efficiency of enriched environment in rats.
The majority of stroke patients develop post-stroke fatigue, a symptom which impairs motivation and diminishes the success

of rehabilitative interventions. We show that large cortical strokes acutely reduce activity levels in rats for 1-2 weeks as a physiological response paralleled by signs of systemic inflammation. Rats were exposed early (1-2 weeks) or late (3-4 weeks after stroke) to an individually monitored enriched environment to stimulate self-controlled high-intensity sensorimotor training. A group of animals received Anti-Nogo antibodies for the first two weeks after stroke, a neuronal growth promoting immunotherapy already in clinical trials. Early exposure to the enriched environment resulted in poor outcome: Training intensity was correlated to enhanced systemic inflammation and functional impairment. In contrast, animals starting intense sensorimotor training two weeks after stroke preceded by the immunotherapy revealed better recovery with functional outcome positively correlated to the training intensity and the extent of re-innervation of the stroke denervated cervical hemi-cord. Our results suggest stroke-induced fatigue as a biological purposeful reaction of the organism during neuronal remodeling, enabling new circuit formation which will then be stabilized or pruned in the subsequent rehabilitative training phase. However, intense training too early may lead to wrong connections and is thus less effective.
J Cereb Blood Flow Metab, 2019; 39
27977023: Ineichen BV, Schnell L, Gullo M, Kaiser J, Schneider MP, Mosberger AC, Good N, Linnebank M, Schwab ME
Direct, long-term intrathecal application of therapeutics to the rodent CNS.
Systemic application of therapeutics to the CNS tissue often results in subtherapeutic drug levels, because of restricted and selective penetration through the blood-brain barrier (BBB). Here, we give a detailed description of a standardized technique for intrathecal drug delivery in rodents, analogous to the technique used in humans. The intrathecal drug delivery method bypasses the BBB and thereby offers key advantages over oral or intravenous administration, such as maximized local drug doses with minimal systemic side effects. We describe how to deliver antibodies or drugs over several days or weeks from a s.c. minipump and a fine catheter inserted into the subdural space over the spinal cord (20 min operative time) or into the cisterna magna (10 min operative time). Drug levels can be sampled by quick and minimally invasive cerebrospinal fluid (CSF) collection from the cisterna magna (5 min procedure time). These techniques enable targeted application of any compound to the CNS for therapeutic studies in a wide range of CNS disease rodent models. Basic surgery skills are helpful for carrying out the procedures described in this protocol.
Nat Protoc, 2017; 12
32378027: Wahl AS, Correa D, Imobersteg S, Maurer MA, Kaiser J, Augath MA, Schwab ME
Targeting Therapeutic Antibodies to the CNS: a Comparative Study of Intrathecal, Intravenous, and Subcutaneous Anti-Nogo A Antibody Treatment after Stroke in Rats.
Antibody-based therapeutics targeting CNS antigens emerge as promising treatments in neurology. However, access to the CNS is limited by the blood-brain barrier. We examined the effects of a neurite growth-enhancing anti-Nogo A antibody therapy following 3 routes of administration-intrathecal (i.t.), intravenous (i.v.), and subcutaneous (s.c.)-after large photothrombotic strokes in adult rats. Intrathecal treatment of full-length IgG anti-Nogo A antibodies enhanced recovery of the grasping function, but intravenous or subcutaneous administration had no detectable effect in spite of large amounts of antibodies in the peripheral circulation. Thus, in contrast to intravenous and subcutaneous delivery, intrathecal administration is an effective and reliable way to target CNS antigens. Our data reveal that antibody delivery to the CNS is far from trivial. While intrathecal application is feasible and guarantees defined antibody doses in the effective range for a biological function, the identification and establishment of easier routes of administration remains an important task to facilitate antibody-based future therapies of CNS disorders.
Neurotherapeutics, 2020; 17
30962276: Kaiser J, Maibach M, Salpeter I, Hagenbuch N, de Souza VBC, Robinson MD, Schwab ME
The Spinal Transcriptome after Cortical Stroke: In Search of Molecular Factors Regulating Spontaneous Recovery in the Spinal Cord.
In response to cortical stroke and unilateral corticospinal tract degeneration, compensatory sprouting of spared corticospinal fibers is associated with recovery of skilled movement in rodents. To date, little is known about the molecular mechanisms orchestrating this spontaneous rewiring. In this study, we provide insights into the molecular changes in the spinal cord tissue after large ischemic cortical injury in adult female mice, with a focus on factors that might influence the reinnervation process by contralesional corticospinal neurons. We mapped the area of cervical gray matter reinnervation by sprouting contralesional corticospinal axons after unilateral photothrombotic stroke of the motor cortex in mice using anterograde tracing. The mRNA profile of this reinnervation area was analyzed using whole-genome sequencing to identify differentially expressed genes at selected time points during the recovery process. Bioinformatic analysis revealed two phases of processes: early after stroke (4-7 d post-injury), the spinal transcriptome is characterized by inflammatory processes, including phagocytic processes as well as complement cascade activation. Microglia are specifically activated in the denervated corticospinal projection fields in this early phase. In a later phase (28-42 d post-injury), biological processes include tissue repair pathways with upregulated genes related to neurite outgrowth. Thus, the stroke-denervated spinal gray matter, in particular its intermediate laminae, represents a growth-promoting environment for sprouting corticospinal fibers originating from the contralesional motor cortex.

This dataset provides a solid starting point for future studies addressing key elements of the post-stroke recovery process, with the goal to improve neuroregenerative treatment options for stroke patients. We show that the molecular changes in the spinal cord target tissue of the stroke-affected corticospinal tract are mainly defined by two phases: an early inflammatory phase during which microglia are specifically activated in the target area of reinnervating corticospinal motor neurons; and a late phase during which growth-promoting factors are upregulated which can influence the sprouting response, arborization, and synapse formation. By defining for the first time the endogenous molecular machinery in the stroke-denervated cervical spinal gray matter with a focus on promotors of axon growth through the growth-inhibitory adult CNS, this study will serve as a basis to address novel neuroregenerative treatment options for chronic stroke patients.
J Neurosci, 2019; 39

28646336: Ineichen BV, Kapitza S, Bleul C, Good N, Plattner PS, Seyedsadr MS, Kaiser J, Schneider MP, Zörner B, Martin R, Linnebank M, Schwab ME
Nogo-A antibodies enhance axonal repair and remyelination in neuro-inflammatory and demyelinating pathology.
Two hallmarks of chronic multiple sclerosis lesions are the absence of significant spontaneous remyelination and primary as well as secondary neurodegeneration. Both characteristics may be influenced by the presence of inhibitory factors preventing myelin and neuronal repair. We investigated the potential of antibodies against Nogo-A, a well-known inhibitory protein for neuronal growth and plasticity, to enhance neuronal regeneration and remyelination in two animal models of multiple sclerosis. We induced a targeted experimental autoimmune encephalomyelitis (EAE) lesion in the dorsal funiculus of the cervical spinal cord of adult rats resulting in a large drop of skilled forelimb motor functions. We subsequently observed improved recovery of forelimb function after anti-Nogo-A treatment. Anterograde tracing of the corticospinal tract revealed enhanced axonal sprouting and arborisation within the spinal cord gray matter preferentially targeting pre-motor and motor spinal cord laminae on lesion level and above in the anti-Nogo-A-treated animals. An important additional effect of Nogo-A-neutralization was enhanced remyelination observed after lysolecithin-induced demyelination of spinal tracts. Whereas remyelinated fiber numbers in the lesion site were increased several fold, no effect of Nogo-A-inhibition was observed on oligodendrocyte precursor proliferation, migration, or differentiation. Enhancing remyelination and promoting axonal regeneration and plasticity represent important unmet medical needs in multiple sclerosis. Anti-Nogo-A antibodies hold promise as a potential new therapy for multiple sclerosis, in particular during the chronic phase of the disease when neurodegeneration and remyelination failure determine disability evolution.
Acta Neuropathol, 2017; 134
28469010: Rust R, Kaiser J
Insights into the Dual Role of Inflammation after Spinal Cord Injury.
J Neurosci, 2017; 37

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BOARD NUMBER: S01-235

HIP NEUROMUSCULAR CONTROL IN ATHLETES WITH ADDUCTOR-RELATED GROIN PAIN

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Background: Injury and pain have significant influence on athlete's neuromuscular control and performance. Hip adductor-related groin pain (ARGP) is a common injury in athletes requiring excessive hip mobility during sports. Functional movement test is commonly used clinically to determine functional performance. Here we investigated how ARGP affects functional performance, muscle activation, as well as functional connectivity between motor cortex and muscles during a functional movement screening test. **Method:** 15 for each healthy and ARGP athletes were recruited. Subjects were asked to perform Y-balance test (YBT), while electromyography (EMG) of the hip and trunk muscles and electroencephalography (EEG) were collected. Y-balance test scores, EMG amplitude, and corticomuscular coherence were calculated. We used independent t-test to determine if there were differences between healthy adults and ARGP athletes with α level at 0.05. **Results:** We have collected data on 10 healthy athletes so far. The reaching distance of the YBT were normalized with leg length, which results were 65.7±8.8% anteriorly, 106.4±12.8% posterolaterally, and 100.5±13.2% posteromedially[LC1] . The averaged muscles activation ratio of hip abductor/ adductor were 145.04±68.28%, 296.96±261.70%, and 159.43±129.95% in these three directions of YBT respectively. We observed dominant β and γ band CMC between the cortex and each of 3 muscles during the YBT. **Conclusion:** We plan to complete the whole data collection and present the overall results at the FENS 2022 conference.

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MOTOR ADAPTATION AND ANTICIPATORY POSTURAL ADJUSTMENTS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Transitioning between different voluntary movements has the potential to destabilise balance and posture through disturbance of the body's centre of mass. To prevent this, the nervous system makes pre-emptive adjustments to body posture prior to limb movements, termed anticipatory postural adjustments (APAs). These APAs are a form of motor adaptation. Through experience, the nervous system learns and updates how destabilising upcoming movements will disrupt postural equilibrium and adjusts the body posture accordingly. However, it is not clear how APAs are adapted during dynamic movements and we have little understanding of the neural circuitry that underlies these adjustments. Inspired by visuomotor transformation experiments in humans, we have developed a novel dual-belt travellator paradigm to induce adaptive APAs in freely moving mice. This behavioural assay allows a mismatch to be created between the expected (visual cue) and actual (belt) speeds of two moving belts as mice locomote between them, giving us the ability to flexibly manipulate the postural requirements of the task within the same experimental context. This provides an exciting avenue to probe the neural circuits involved in performing APAs.

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BOARD NUMBER: S01-237

CORRELATION BETWEEN RESPIRATORY MOTION DURING SPONTANEOUS BREATHING AND VISUOMOTOR REACTION TIME IN WOMEN

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Aims: There is experimental evidence that abdominal breathing is able to improve mental function and reduce exercise-induced oxidative stress. Visuomotor reaction time (VMRT) represents both mental and physical performance. Do people with more abdominal motion during spontaneous breathing have shorter VMRT? The present study aimed to make a preliminary investigation to answer this question. **Methods:** 434 healthy women (age: 20-59) living in the urban area in Beijing were enrolled by convenience sampling, received VMRT test on a simple visual reaction time tester (Xindonghuateng, Beijing, China) and respiration test using two respiration belts (Vernier, Beaverton, OR, USA). Two belts were tied at the height of the xiphoid and navel separately and ten continuous stable breathing cycles were selected for calculating the abdominal motion (AM), thoracic motion (TM), and thoracoabdominal ratio (TA ratio, the ratio of thoracic motion to abdominal motion). 393 subjects, whose data met the inclusion criteria, were divided into four age groups for analysis (20-29 years, n=40; 30-39 years, n=108; 40-49 years, n=146; 50-59 years, n=99). Both linear and non-linear correlation tests were conducted on SPSS and R-language. **Results:** The VMRT increased gradually from 550ms to 620ms with aging. The variability of TM was consistently higher compared to AM through all age groups. No correlation was found between VMRT and AM, TM, TA ratio. **Conclusions:** Women's visuomotor reaction time is not associated with the respiratory motion from the chest and abdomen recorded during spontaneous breathing.

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DIRECTIONAL ACCURACY OF POINT-TO-POINT ARM MOVEMENTS IN DIFFERENT BODY ORIENTATIONS WITH RESPECT TO GRAVITY

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Aims: On Earth, gravity plays a primordial role during motor planning and control, both as a reference axis for proprioception and as a driving force for motor optimization. Previous studies have reported a progressive re-optimization of arm kinematics during exposure to microgravity, while discrete movements directed towards a target position get less accurate in the absence of visual feedback in microgravity compared to normal gravity. Here, we study the influence of body posture relative to gravity on the accuracy and kinematics trajectory of discrete point-to-point arm movements. Methods: Twelve participants performed discrete point-to-point arm movements parallel to the longitudinal body axis, with eyes open or with eyes closed, in three positions relative to gravity (0° Upright, 45° Intermediate and 90° Supine). A motion-tracking system was used to measure hand kinematics and a manipulandum was held between the thumb and the index finger during the tasks. Results: Body position had a significant impact on movement accuracy, and in particular on movement direction, when eyes were closed. More precisely, in the Intermediate and Supine positions movement direction drifted across trials in the absence of visual feedback and this drift was larger in the Supine position. Conclusion: Our results show that, in the absence of visual feedback, the direction of discrete point-to-point arm movements drifts across trials when the body axis is not aligned with the upright position.

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CELL TYPE-SPECIFIC VISUAL INFORMATION ROUTING VIA THE SUPERIOR COLLICULUS IS INDISPENSABLE FOR GOAL-DIRECTED FORELIMB REACHING MOVEMENTS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Integration of visual information providing an object's location, shape, and size is essential for executing forelimb reaching and grasping. However, the primary visual cortex is not required to learn or execute the behavior. Using an intersectional approach of mouse genetics, virus-mediated circuit manipulation and high-resolution kinematic analysis, we uncover that the ablation of narrow field neurons residing in the superficial layer of the superior colliculus (SC) deteriorates the refinement and execution of forelimb reaching. Optical tagging of narrow field neurons reveals activity tuning to a specific kinematic phase during reaching. They preferentially form synaptic connections to glutamatergic neurons in the intermediate SC (iSC) projecting to the pontine reticular nuclei (PRN) in the rostral brainstem. Selective ablation of the iSC-PRN projection neurons impairs the consistency of reaching kinematics, but not digit movements for grasping, indicating that the superior colliculi encode visual information necessary for the end-point refinement during the reaching phase. Moreover, the projection neurons receive input from diverse motor and sensory processing-related structures. Together, our study reveals cell type-specific dissemination of visual information provides essential input to the superior colliculus to act as a sensorimotor integration node to regulate forelimb reaching behavior.

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BOARD NUMBER: S01-240

MULTIVARIATE PATTERN ANALYSIS FOR CHARACTERIZATION OF CORTICAL NETWORKS OF BIMANUAL CONTROL

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Background and Aim: Bimanual coordination is central to many manual skills but our knowledge on how sensorimotor networks represent different bimanual movement contexts is fragmented. The aim of this study was to use multi-voxel pattern analysis (MVPA) to explore how unimanual as well as symmetric and asymmetric bimanual movements are represented in cortical motor areas. **Methods:** 27 young (mean age 26 y/o) healthy participants (12 females) performed a visually guided pinch-force task that required quick shifts between movement contexts, i.e. experimental conditions varying in force direction (increase/decrease) and in bimanual context (symmetric/asymmetric/unimanual). We estimated task-related brain responses and trained a multivariate logistic regression classification algorithm, to discriminate response patterns based on BOLD signal within regions-of-interest (ROI). Based on the task nature (visually guided pinch force) one ROI covered cortical sensory-motor areas (M1, S1, SMA, PMd) while a second ROI covered visual areas (V1,V2, cuneus). **Results:** In the visual ROI, classification robustly discriminated all visual cues. In the motor ROI, bimanual tasks could be discriminated based on movement context but not force direction, e.g. symmetry vs. asymmetry, left hand stronger vs. right hand stronger. Voxels in the right dorsal premotor cortex contributed most to discrimination based on bimanual context. **Discussion:** Our results indicate that bimanual movements are represented in the motor cortices by unique activation patterns based on the bimanual coordination context and not as a simple summation of two unimanual movements – consistent with the notion that bimanual coordination requires complex and nonlinear interactions between representations in the motor cortex.

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BEHAVIOURAL STRATEGIES AND BRAIN-WIDE NEURAL CIRCUITS DRIVING POSTURAL CONTROL IN LARVAL ZEBRAFISH

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Loss of balance requires motor actions to stabilise posture. However, the behavioural strategies used and neural circuits involved in stabilisation are not well-characterised. We rolled head-mounted larval zebrafish around the rostrocaudal axis at various angles away from their preferred dorsal-up posture and characterised the tail response as a function of angle. We found different behavioural strategies at small and large angles respectively. At *small angles*, the fish responded by bending its tail to the contraversive side in a continuous manner proportional to the stimulus. The response saturated at a roll angle of about 12º. At *larger angles*, the fish executed, with increasing probability, additional discrete tail bouts of three types - swims, flips and flicks. The roll angle did not have any effect on the proportion of each type of motor response, tail-flip amplitude, or tail-frequency. However, at larger roll amplitudes, tail-flips became increasingly biased toward the contraversal side. This suggests that contraversal tail-flips are central discrete motor movements executed to regain stable posture. To identify the neuronal circuits involved in implementing these behavioural strategies, we recorded brain-wide neuronal activity during the responses using a rotating light-sheet microscope. A regression analysis identified neurons whose activity correlated with these different motor behaviours. We also found neuronal assemblies that displayed decreased activity during the triggering of discrete motor responses, suggesting that these assemblies are involved in motor output selection. Further studies will reveal whether these assemblies are part of a general circuit for motion selection or are specific to the vestibular response.

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BOARD NUMBER: S01-242

FAST-SPIKING INTERNEURONS OF THE PREMOTOR CORTEX CONTRIBUTE TO ACTION INITIATION

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Planning and execution of voluntary movement depend on the contribution of distinct classes of neurons in primary motor and premotor areas. However, the specific functional role of GABAergic cells remains only partly understood. Here, electrophysiological and computational analyses are employed to compare directly the response properties of putative pyramidal (PNs) and fast-spiking, GABAergic neurons (FSNs) during licking and forelimb retraction in mice. Recordings from anterolateral motor cortex and rostral forelimb area, reveal that FSNs fire earlier and for a longer duration than PNs, with the exception of a subset of early-modulated PNs in deep layers. Computational analysis reveals that FSNs carry vastly more information than PNs about the onset of movement. While PNs differently modulate their discharge during distinct motor acts, most FSNs respond with a stereotyped increase in firing rate. Accordingly, the informational redundancy was greater among FSNs than PNs. These data suggest that a global rise of inhibition contributes to action initiation.

Pubmed:
35028271: Del Grosso A, Parlanti G, Angella L, Giordano N, Tonazzini I, Ottalagana E, Carpi S, Pellegrino RM, Alabed HBR, Emiliani C, Caleo M, Cecchini M
Chronic lithium administration in a mouse model for Krabbe disease.
Krabbe disease (KD; or globoid cell leukodystrophy) is an autosomal recessive lysosomal storage disorder caused by deficiency of the galactosylceramidase (GALC) enzyme. No cure is currently available for KD. Clinical applied treatments are supportive only. Recently, we demonstrated that two differently acting autophagy inducers (lithium and rapamycin) can improve some KD hallmarks in-vitro, laying the foundation for their in-vivo pre-clinical testing. Here, we test lithium carbonate in-vivo, in the spontaneous mouse model for KD, the Twitcher (TWI) mouse. The drug is administered ad libitum via drinking water (600 mg/L) starting from post natal day 20. We longitudinally monitor the mouse motor performance through the grip strength, the hanging wire and the rotarod tests, and a set of biochemical parameters related to the KD pathogenesis [i.e., GALC enzymatic activity, psychosine (PSY) accumulation and astrogliosis]. Additionally, we investigate the expression of some crucial markers related to the two pathways that could be altered by lithium: the autophagy and the β-catenin-dependent pathways. Results demonstrate that lithium has not a significant rescue effect on the TWI phenotype, although it can slightly and transiently improves muscle strength. We also show that lithium, with this administration protocol, is unable to stimulate autophagy in the TWI mice central nervous system, whereas results suggest that it can restore the β-catenin activation status in the TWI sciatic nerve. Overall, these data provide intriguing inputs for further evaluations of lithium treatment in TWI mice.
JIMD Rep, 2022; 63
34466801: Jurkute N, Bertacchi M, Arno G, Tocco C, Kim US, Kruszewski AM, Avery RA, Bedoukian EC, Han J, Ahn SJ, Pontikos N, Acheson J, Davagnanam I, Bowman R, Kaliakatsos M, Gardham A, Wakeling E, Oluonye N, Reddy MA, Clark E, Rosser E, Amati-Bonneau P, Charif M, Lenaers G, Meunier I, Defoort S, Vincent-Delorme C, Robson AG, Holder GE, Jeanjean L, Martinez-Monseny A, Vidal-Santacana M, Dominici C, Gaggioli C, Giordano N, Caleo M, Liu GT, , Webster AR, Studer M, Yu-Wai-Man P
Pathogenic variants cause a developmental ocular phenotype recapitulated in a mutant mouse model.
Pathogenic variants cause a rare autosomal dominant neurodevelopmental disorder referred to as the Bosch-Boonstra-Schaaf Optic Atrophy Syndrome. Although visual loss is a prominent feature seen in affected individuals, the molecular and cellular mechanisms contributing to visual impairment are still poorly characterized. We conducted a deep phenotyping study on a cohort of 22 individuals carrying pathogenic variants to document the neurodevelopmental and ophthalmological manifestations, in particular the structural and functional changes within the retina and the optic nerve, which have not been detailed previously. The visual impairment became apparent in early childhood with small and/or tilted hypoplastic optic

nerves observed in 10 cases. High-resolution optical coherence tomography imaging confirmed significant loss of retinal ganglion cells with thinning of the ganglion cell layer, consistent with electrophysiological evidence of retinal ganglion cells dysfunction. Interestingly, for those individuals with available longitudinal ophthalmological data, there was no significant deterioration in visual function during the period of follow-up. Diffusion tensor imaging tractography studies showed defective connections and disorganization of the extracortical visual pathways. To further investigate how pathogenic variants impact on retinal and optic nerve development, we took advantage of an mutant mouse disease model. Abnormal retinogenesis in early stages of development was observed in mutant mice with decreased retinal ganglion cell density and disruption of retinal ganglion cell axonal guidance from the neural retina into the optic stalk, accounting for the development of optic nerve hypoplasia. The mutant mice showed significantly reduced visual acuity based on electrophysiological parameters with marked conduction delay and decreased amplitude of the recordings in the superficial layers of the visual cortex. The clinical observations in our study cohort, supported by the mouse data, suggest an early neurodevelopmental origin for the retinal and optic nerve head defects caused by pathogenic variants, resulting in congenital vision loss that seems to be non-progressive. We propose as a major gene that orchestrates early retinal and optic nerve head development, playing a key role in the maturation of the visual system.
Brain Commun, 2021; 3
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Dysregulated autophagy as a new aspect of the molecular pathogenesis of Krabbe disease.
Krabbe disease (KD) is a childhood leukodystrophy with no cure currently available. KD is due to a deficiency of a lysosomal enzyme called galactosyl-ceramidase (GALC) and is characterized by the accumulation in the nervous system of the sphingolipid psychosine (PSY), whose cytotoxic molecular mechanism is not fully known yet. Here, we study the expression of some fundamental autophagy markers (LC3, p62, and Beclin-1) in a KD murine model [the twitcher (TWI) mouse] by immunohistochemistry and Western blot. Moreover, the autophagy molecular process is also shown in primary fibroblasts from TWI and WT mice, with and without PSY treatment. Data demonstrate that large p62 cytoplasmic aggregates are present in the brain of both early and late symptomatic TWI mice. p62 expression is also upregulated in TWI sciatic nerves compared to that measured for WT nerves. In vitro data suggest that this effect might not be fully PSY-driven. Finally, we investigate in vitro the capability of autophagy inducers (Rapamycin, RAP and Resveratrol, RESV) to reinstate the WT phenotype in TWI cells. We show that RAP administration can partially restore the autophagy markers levels, while RESV cannot, indicating a line along which new therapeutic approaches can be developed.
Neurobiol Dis, 2019; 129
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Quantitative Microproteomics Based Characterization of the Central and Peripheral Nervous System of a Mouse Model of Krabbe Disease.
Krabbe disease is a rare, childhood lysosomal storage disorder caused by a deficiency of galactosylceramide beta-galactosidase (GALC). The major effect of GALC deficiency is the accumulation of psychosine in the nervous system and widespread degeneration of oligodendrocytes and Schwann cells, causing rapid demyelination. The molecular mechanisms of Krabbe disease are not yet fully elucidated and a definite cure is still missing. Here we report the first in-depth characterization of the proteome of the Twitcher mouse, a spontaneous mouse model of Krabbe disease, to investigate the proteome changes in the Central and Peripheral Nervous System. We applied a TMT-based workflow to compare the proteomes of the corpus callosum, motor cortex and sciatic nerves of littermate homozygous Twitcher and wild-type mice. More than 400 protein groups exhibited differences in expression and included proteins involved in pathways that can be linked to Krabbe disease, such as inflammatory and defense response, lysosomal proteins accumulation, demyelination, reduced nervous system development and cell adhesion. These findings provide new insights on the molecular mechanisms of Krabbe disease, representing a starting point for future functional experiments to study the molecular pathogenesis of Krabbe disease. Data are available via ProteomeXchange with identifier PXD010594.
Mol Cell Proteomics, 2019; 18
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Motor learning and metaplasticity in striatal neurons: relevance for Parkinson's disease.
Nigro-striatal dopamine transmission is central to a wide range of neuronal functions, including skill learning, which is disrupted in several pathologies such as Parkinson's disease. The synaptic plasticity mechanisms, by which initial motor learning is stored for long time periods in striatal neurons, to then be gradually optimized upon subsequent training, remain unexplored. Addressing this issue is crucial to identify the synaptic and molecular mechanisms involved in striatal-dependent learning impairment in Parkinson's disease. In this study, we took advantage of interindividual differences between outbred rodents in reaching plateau performance in the rotarod incremental motor learning protocol, to study striatal synaptic plasticity ex vivo. We then assessed how this process is modulated by dopamine receptors and the dopamine active transporter, and

whether it is impaired by overexpression of human α-synuclein in the mesencephalon; the latter is a progressive animal model of Parkinson's disease. We found that the initial acquisition of motor learning induced a dopamine active transporter and D1 receptors mediated long-term potentiation, under a protocol of long-term depression in striatal medium spiny neurons. This effect disappeared in animals reaching performance plateau. Overexpression of human α-synuclein reduced striatal dopamine active transporter levels, impaired motor learning, and prevented the learning-induced long-term potentiation, before the appearance of dopamine neuronal loss. Our findings provide evidence of a reorganization of cellular plasticity within the dorsolateral striatum that is mediated by dopamine receptors and dopamine active transporter during the acquisition of a skill. This newly identified mechanism of cellular memory is a form of metaplasticity that is disrupted in the early stage of synucleinopathies, such as Parkinson's disease, and that might be relevant for other striatal pathologies, such as drug abuse.
Brain, 2018; 141

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Intravenous infusion of human bone marrow mesenchymal stromal cells promotes functional recovery and neuroplasticity after ischemic stroke in mice.
Transplantation of human bone marrow mesenchymal stromal cells (hBM-MSC) promotes functional recovery after stroke in animal models, but the mechanisms underlying these effects remain incompletely understood. We tested the efficacy of Good Manufacturing Practices (GMP) compliant hBM-MSC, injected intravenously 3.5 hours after injury in mice subjected to transient middle cerebral artery occlusion (tMCAo). We addressed whether hBM-MSC are efficacious and if this efficacy is associated with cortical circuit reorganization using neuroanatomical analysis of GABAergic neurons (parvalbumin; PV-positive cells) and perineuronal nets (PNN), a specialized extracellular matrix structure which acts as an inhibitor of neural plasticity. tMCAo mice receiving hBM-MSC, showed early and lasting improvement of sensorimotor and cognitive functions compared to control tMCAo mice. Furthermore, 5 weeks post-tMCAo, hBM-MSC induced a significant rescue of ipsilateral cortical neurons; an increased proportion of PV-positive neurons in the perilesional cortex, suggesting GABAergic interneurons preservation; and a lower percentage of PV-positive cells surrounded by PNN, indicating an enhanced plastic potential of the perilesional cortex. These results show that hBM-MSC improve functional recovery and stimulate neuroprotection after stroke. Moreover, the downregulation of "plasticity brakes" such as PNN suggests that hBM-MSC treatment stimulates plasticity and formation of new connections in the perilesional cortex.
Sci Rep, 2017; 7

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Combining robotics with enhanced serotonin-driven cortical plasticity improves post-stroke motor recovery.
Despite recent progresses in robotic rehabilitation technologies, their efficacy for post-stroke motor recovery is still limited. Such limitations might stem from the insufficient enhancement of plasticity mechanisms, crucial for functional recovery. Here, we designed a clinically relevant strategy that combines robotic rehabilitation with chemogenetic stimulation of serotonin release to boost plasticity. These two approaches acted synergistically to enhance post-stroke motor performance. Indeed, mice treated with our combined therapy showed substantial functional gains that persisted beyond the treatment period and generalized to non-trained tasks. Motor recovery was associated with a reduction in electrophysiological and neuroanatomical markers of GABAergic neurotransmission, suggesting disinhibition in perilesional areas. To unveil the translational potentialities of our approach, we specifically targeted the serotonin 1A receptor by delivering Buspirone, a clinically approved drug, in stroke mice undergoing robotic rehabilitation. Administration of Buspirone restored motor impairments similarly to what observed with chemogenetic stimulation, showing the immediate translational potential of this combined approach to significantly improve motor recovery after stroke.
Prog Neurobiol, 2021; 203

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MOTOR CORTEX POPULATION DYNAMICS ASSOCIATED TO FACIAL MOVEMENT DIVERSITY IN THE MOUSE

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Voluntary face movements are key behavioral features across animal species. These movements can be directly linked to survival, for example in nutrition, or have other relevant roles, such as communication through face expressions or the generation of sounds. Yet, to date, the relationship between neuronal activity in the primary motor cortex (M1) and face movement is not fully understood. The aim of this project is to characterize the link between M1 population dynamics and the generation of a diversity of facial movements. To this end we record the simultaneous neuronal population activity from a facial area of M1 while monitoring the associated face motor behavior in head-fixed awake mice.
By using a cutting-edge fast-volumetric calcium imaging method based on scanned temporal focusing two-photon microscopy, we recorded the simultaneous activity of thousands of neurons across a facial area of M1 and captured the associated face movements through videography. We implemented an unsupervised algorithm to classify the diversity of the captured behaviors, i.e. different whisking and sniffing movements, which allowed to compare the dynamics of the simultaneously recorded M1 population activity during the execution of these movements, and in comparison to resting states. The established approach opens new possibilities for the study of the integration of sensory feedback to M1 population dynamics in mice performing spontaneous or evoked voluntary face movements.

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NEURAL CORRELATES OF SYMMETRIC AND ASYMMETRIC BIMANUAL CONTROL OF PINCH FORCE IN HUMANS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Background: Although bimanual activities require quick shifts between symmetric and asymmetric coordination, previous functional neuroimaging research has mainly focused on the continuous performance of symmetric or asymmetric bimanual movements. Here we used functional magnetic resonance imaging (fMRI) to probe the bimanual motor network in a task context with rapid shifts between symmetric and asymmetric control . **Methods:** 25 healthy, right-handed volunteers performed a novel, visually guided pinch-force task that required quick shifts between bimanual symmetric, bimanual asymmetric and unimanual changes in pinch force. Task related changes in brain activity and functional coupling were analyzed with General Linear Models (GLM) and Dynamic Causal Modelling (DCM). **Results:** During asymmetric bimanual modulation of pinch force, the dorsal premotor cortex showed a bilateral increase in activity and effective coupling with the ipsilateral supplementary motor area (SMA). Coupling from the SMA to the ipsilateral M was high during all bimanual pinch force conditions. In left SMA, task related activity scaled positively with the degree of synchronous movement initiation, irrespectively of the bimanual context. Additionally, activity in a left-dominant network including the insula, cerebellum and M1 showed a linear increase with overall task accuracy. **Discussion:** Together, the results indicate an increasing engagement of the dorsal premotor cortex with the complexity of bimanual coordination. The relationship between left-hemispheric task-related activity and task measures of bimanual synchronicity and accuracy suggests some degree of hemispheric dominance during bimanual motor control.

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MAPPING BRAINSTEM AND SPINAL CIRCUITS FOR LOCOMOTION AND ORIENTATION: DIVERSITY AND SPECIALIZATION OF V2A RETICULAR NEURONS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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The brainstem reticular formation (RF) is key for controlling motor actions. Indeed, reticular neurons interface multiple sensory and cognitive modalities upstream, with executory motor circuits downstream. However, how the RF selects, initiates and combines multiple motor actions is largely unknown. We previously showed that the glutamatergic V2a neurons in the gigantocellular reticular nucleus (Gi) encompass a cervical-projecting subset inducing a head rotation and a change of locomotor trajectory, and a lumbar-projecting subset that, surprisingly, arrests locomotion (*Usseglio et al., 2020*). Here, we reveal that V2a reticular neurons also control other motor components of orienting whose executive circuits are located in the brainstem, including snout and eye. Importantly, these functions are supported by brainstem-projecting V2a subsets rather than by collaterals of spinally-projecting ones. Secondly, we question the possibility that the divergent outcomes across muscular groups (e.g., ipsilateral excitation for the head and snout, bilateral inhibition for the hindlimbs) might reflect differences in V2a efferent connectivity at each executory module. Hence, using a rabies-based transsynaptic strategy on newborn mice, we document the identity of the postsynaptic cells of the V2a neurons at different brainstem and spinal cord levels. Our work highlights that V2a Gi neurons orchestrate orienting motor actions and can be subdivided in at least two macro-categories, i) one controlling ocular and orofacial movements through supra-spinal projections, and ii) one controlling trunk and limb movements through spinal projections. Within each, a further specialization by projection site and postsynaptic target supports the control of individual motor components of a coherent multi-faceted behavior.

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MULTIPLE BETA OSCILLATIONS BANDS UNDERLIE COMPLEMENTARY COGNITIVE AND SENSORIMOTOR ROLES IN THE MACAQUE FRONTAL MOTOR AREAS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Oscillations at specific frequencies might facilitate neuronal interactions. Particularly, sensorimotor beta oscillations were linked to postural control, movement preparation, but also sensorimotor anticipation and integration. Here, we investigated the relation of local field potential (LFP) beta amplitude in primary motor (M1) and dorsal premotor cortex (PMd) with these processes, while macaque monkeys were engaged in a rule-based cue-selection task requiring reaching movements. The timing of the valid spatial cue varied and was pre-cued in each trial. Therefore, temporal attention and movement preparation epochs depended on the task condition. Two spectral peaks in the beta range were visible in the LFPs. A high band centered on 26Hz was dominant in PMd. It decreased temporarily in trial-averaged amplitude slightly in anticipation of, and during the presentation of each visual stimulus, but somewhat more for the valid spatial cue. A low band centered on 16Hz was dominant in M1. It decreased in amplitude after the valid cue, when movement preparation started, remaining lower until the GO signal. Single-trial analysis revealed that both bands were modulated by small ‘fixational’ hand movements and exploratory eye movements. Whereas eye velocity was mainly negatively correlated with amplitude of both bands, hand velocity was mainly negatively correlated with low beta and positively correlated with high beta amplitude. These results might reconcile the somewhat disparate roles proposed for sensorimotor beta, with distinct, co-existing bands related differently to movement preparation and postural control on the one hand, but also anticipation and sensorimotor integration on the other hand.

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TWO BRAINS IN ACTION: JOINT-ACTION CODING IN PARIETAL CORTEX OF MACAQUES

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Often our goals cannot be accomplished by acting alone but for a successful outcome rather require motor interaction among individuals. The neural mechanisms subtending inter-individual coordination remain largely unknown. In a previous study of the dorsal premotor cortex (PMd) in macaques engaged in a joint-action (JA) task, we found a class of JA-neurons discharging preferentially when a given action was performed jointly with another partner, relative to when the identical action was performed in a solo fashion (Solo task). Here, we report the results obtained in posterior parietal cortex (PPC), where single cell activity was recorded simultaneously from areas PFG of two interacting monkeys. The animals of the dyad were each trained to guide a visual cursor on a screen, in a Solo or in JA context, by exerting isometric hand force on a joystick. As in PMd, in PPC, beyond the canonical action-related cells, whose activity did not change depending on task conditions, we found a population of JA-neurons modulated by the dyadic context, despite the fact that identical actions were performed in the two conditions (Solo vs JA). However, JA parietal cells differed from PMd ones in their onset of activation, since the latter lead the former in time. Furthermore, parietal JA-neurons discriminate task conditions during action execution time only, contrary to PMd cells which showed high discrimination power mostly during action planning. Our findings provide evidence for a fronto-parietal shared representation of joint behavior, that might constitute a neural substrate for successful visuo-motor coordination between individuals.

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POSTACTIVATION POTENTIATION EFFECTS ON SQUAT PERFORMANCE AND EMG IN RESISTANCE TRAINED MEN OF DIFFERENT LEVELS OF EXPERTISE

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Postactivation potentiation (PAP) is defined as an increased muscle contractile response following a stimulation called conditioning activity (CA) characterized by intense voluntary contraction. The generation of PAP has been speculated to be caused by the enhancement of the neuromuscular structure following CA. Indeed CA is shown to affect the peak and rate of a muscle contraction. This study aims to evaluate the effectiveness of PAP following CA in squat performance in experienced (EXP) and intermediate (INT) resistance trained men, by measuring the correlation between the increase in strength performance on the total tonnage lifted in the test and the electrical activity of the specific involved muscles. Following an initial evaluation of their one repetition maximum (1RM) of squat, after 4 days subjects were instructed to performed a protocol with CA, which involved performing 1 set of 2 repetitions at 90% of 1RM, after 7 minutes of rest 4 sets at 70% were proposed until concentric exhaustion with 3 minutes rest between sets. The EMG analysis used root mean square amplitude parameter normalized with maximum voluntary contraction and was evaluated on the vastus medialis, vastus lateralis, gluteus maximus and hamstring muscles. From the data analysis we can hypothesize that the subjects could show greater EMG activity in both groups, but the EXP group may present greater EMG activity than the INT group. We also assume that the total volume of work in the EXP group will be greater than the INT group.

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SHARED NEURAL POPULATION DYNAMICS ACROSS ANIMALS PERFORMING THE SAME BEHAVIOUR

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Animals of the same species share a characteristic set of behaviours. This species-typical behavioural repertoire is shaped under selection pressures over evolutionary timescales. However, it is unclear how individual animals produce these similar behaviours despite the large differences in their brain circuitry. Here, we hypothesised that preserved neural population dynamics are the basis of species-specific behaviours: animals from the same species possess unique brains that are constrained so they can generate the 'appropriate' latent dynamics needed to produce their typical behaviours. Using motor cortical population recordings from monkeys and mice, we show that individuals from the same species share remarkably similar neural population latent dynamics when they perform the same behaviour. Furthermore, shared latent dynamics extend beyond cortical regions to the dorsal striatum, an evolutionarily older structure, and are also present during a cognitive task in which animals covertly plan future movements. These shared dynamics capture a large fraction of the variance of single-neuron activity and have behavioural relevance: they can be used to predict both ongoing movements and future actions. While neural population dynamics have been proposed as the first-level explainers of behavioural and cognitive phenomena, here we extend recent works to show that they are shared across different individuals engaged in the same behaviour, thus providing a novel framework for the implementation of behavioural adaptations in the brain. Our results also have strong implications for the development of brain-controlled devices, such as neuroprosthetics, that can better generalise across individuals to restore motor, affective, or even cognitive functions.

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BOARD NUMBER: S01-250

MOVEMENT-RELATED ELECTROPHYSIOLOGICAL ALTERATIONS AS BIOMARKERS TO PREDICT SPONTANEOUS RECOVERY AFTER MCAO IN MICE

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Stroke is a devastating pathology and the main cause of long-term disability, allowing currently less than 15% of the stroke survivors to fully recover and get back to normal life. Many cognitive domains result affected by strokes and motor functions are impaired in 80-85% of subjects. Efforts in improving rehabilitative therapy remain unsatisfactory, leaving many stroke survivors significantly impaired and with a need of continuous assistance from health care providers. Therefore, nowadays it is not possible to predict the final degree of recovery, making difficult to stratify stroke survivors and address them towards their optimal therapeutic protocol in order to maximize its own restoration potentiality. Here, we longitudinally collected Local Field Potentials (LFP) from stroke and healthy mice to define acute post-stroke alterations to be used as biomarkers to predict the final degree of recovery for each subject. Mice were implanted in their left and right Caudal Forelimb Areas (CFA) and electrophysiological signals were acquired during a forelimb retraction movement, before and after Middle Cerebral Artery Occlusion (MCAO). In particular, we analysed post-stroke alterations in the Event Related Potential (ERP), in the Perievent Spectograms and in the Event Related Synchronization/Desynchronization (ERS/ERD). These measures were also correlated with the individual degree of spontaneous recovery. Findings from this study will contribute to the Neurorehabilitation quest in identifying early, reliable neurophysiological biomarkers of recovery, which would allow a better tailoring of rehabilitation pathways and allocation of resources in an ageing world destined to a sharp increase in stroke disability.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-251

INTEGRITY OF MOTOR PATHWAY AND ACUTE DEFICIT CONTRIBUTE TO POST-STROKE SPONTANEOUS RECOVERY IN MICE

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Stroke is a disabling condition, despite many patients show a spontaneous recovery, due to a reorganization of spared areas and connections. Currently, there are no ways to determine either the extent or time-course of recovery in stroke patients, thus its prediction represents an urgent need for personalization of treatments. In our study we used a mouse model of middle cerebral artery occlusion (MCAO) to investigate novel prognostic tools in preclinical models. To determine spontaneous recovery, we conducted a battery of sensori-motor tests and a forelimb retraction task at different time points. To mimic clinical scales used in stroke patients, we implemented a novel "Global Motor Score" (GMS) comprehensive of the sensorimotor performance assessed in all single tests. GMS was able to detect a global deficit after MCAO but showing a partial overall recovery 4 weeks post-stroke. However, going deeper in individual performances, we found a large variability among stroke mice, allowing a distinction between poor and good recoverers. We found that chronic deficit can be explained by acute performance (as in humans) and by the structural integrity of the descending motor system. In particular, the CFA involvement and the anterior extension of the lesion, together with the Internal Capsule integrity showed to play a role in the extent of spotaneous recovery. These results confirm the MCAO model as a good animal model for studying stroke recovery and could be important to find reliable biomarkers with high translational potential to human stroke patients.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-252

NEUROPHYSIOLOGICAL CORRELATES OF PERIPERSONAL SPACE REPRESENTATION IN HUMAN AND NON-HUMAN PRIMATES

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Sensory events occurring close to the body have particular behavioural relevance. Perhaps unsurprisingly, these events elicit enhanced behavioural or neurophysiological responses. Such “peripersonal space” (PPS) responses, traditionally interpreted as reflecting the position of the stimulus in egocentric coordinates, may in fact play a role in creating or avoiding contact with objects near the body. Here we provide a comprehensive characterisation of PPS responses across humans and non-human primates (NHP), with the aim of facilitating the assessment of their role in contact-related actions. We recorded high-density EEG in humans and intracranial neuronal signals in NHPs during the same task. Visual and tactile stimuli were presented along a mediolateral axis centred on the right hand (distance from the hand: ±24, ±12, 0cm [visual]; 0cm [tactile]). Crucially, eye and hand positions were manipulated to disentangle eye- versus hand-centered encoding. Both scalp and intracortical local field potentials were analysed in the frequency domain by computing power spectral densities. Preliminary results (24 humans, 1 NHP) revealed gaze-independent coding of stimulus position across species. In humans, alpha, beta, and gamma band power in posterior, predominantly lateralized regions was modulated by stimulus position. A qualitatively comparable positional coding was observed in NHP ventral premotor cortex. Further, hand position affected alpha and beta power in left-lateralised frontal and posterior electrodes, though this effect was rather independent of stimulus distance. These results provide the foundation for testing whether PPS responses reflect the value of contact-related actions. Additional computational modelling analyses will assess whether these responses represent action intention.

Pubmed:
30342238: Fitzpatrick AM, Dundon NM, Valyear KF
The neural basis of hand choice: An fMRI investigation of the Posterior Parietal Interhemispheric Competition model.
The current study investigates a new neurobiological model of human hand choice: The Posterior Parietal Interhemispheric Competition (PPIC) model. The model specifies that neural populations in bilateral posterior intraparietal and superior parietal cortex (pIP-SPC) encode actions in hand-specific terms, and compete for selection across and within hemispheres. Actions with both hands are encoded bilaterally, but the contralateral hand is overrepresented. We use a novel fMRI paradigm to test the PPIC model. Participants reach to visible targets while in the scanner, and conditions involving free choice of which hand to use (Choice) are compared with when hand-use is instructed. Consistent with the PPIC model, bilateral pIP-SPC is preferentially responsive for the Choice condition, and for actions made with the contralateral hand. In the right pIP-SPC, these effects include anterior intraparietal and superior parieto-occipital cortex. Left dorsal premotor cortex, and an area in the right lateral occipitotemporal cortex show the same response pattern, while the left inferior parietal lobule is preferentially responsive for the Choice condition and when using the ipsilateral hand. Behaviourally, hand choice is biased by target location - for targets near the left/right edges of the display, the hand in ipsilateral hemispace is favoured. Moreover, consistent with a competitive process, response times are prolonged for choices to more ambiguous targets, where hand choice is relatively unbiased, and fMRI responses in bilateral pIP-SPC parallel this pattern. Our data provide support for the PPIC model, and reveal a selective network of brain areas involved in free hand choice, including bilateral posterior parietal cortex, left-lateralized inferior parietal and dorsal premotor cortices, and the right lateral occipitotemporal cortex.
Neuroimage, 2019; 185
30039397: Valyear KF, Fitzpatrick AM, Dundon NM
Now and then: Hand choice is influenced by recent action history.
Action choices are influenced by recent past and predicted future action states. Here, we demonstrate that recent hand-choice history affects both current hand choices and response times to initiate actions. Participants reach to contact visible

targets using one hand. Hand choice is biased in favour of which hand was used recently, in particular, when the biomechanical costs of responding with either hand are similar, and repeated choices lead to reduced response times. These effects are also found to positively correlate. Participants who show strong effects of recent history on hand choice also tend to show strong effects of recent history on response times. The data are consistent with a computational efficiency interpretation whereby repeated action choices confer computational gains in the efficiency of underpinning processes. We discuss our results within the framework of this model, and with respect to balancing predicted gains and losses, and speculate about the possible underlying mechanisms in neural terms.
Psychon Bull Rev, 2019; 26

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BOARD NUMBER: S01-253

A CASE-REPORT OF AN ALIEN HAND SYNDROME

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Alien hand syndrome (AHS) is a rare movement disorder. We describe a case-report. A 46-years-old male patient was admitted to the clinic with complaints of weakness and impaired movement in his left hand, slowing down of the rate of speech and fuzziness. Describing the symptoms, the patient used the formulations "the hand does not obey", "the hand has its own head." Anamnesis morbi: the patient felt ill 5 weeks before the admission: on the stress background his left leg “failed”, speech became slurred. An urgently performed MRI of the brain revealed areas of cytotoxic edema in the frontal, parietal and occipital lobes and an extensive areas of paraventricular edema in the right hemisphere and in the structure of the corpus callosum. On neurological examination: Apraxia in the left hand: kinesthetic according to the visual and kinesthetic pattern, including transferring poses, kinetic, apraxia tests trying to perform only on the verbal model.
Clinical diagnosis: ischemic stroke in the right MCA circulation. Left-sided upper monoparesis, monohypalgesia, AHS, central prosoparesis, glossoparesis. Dysarthria.
To control the “alien” hand the patient put on a cloth glove at home, held a cane in this hand or held his wife by the elbow outside. Also he was prescripted clonazepam 0.25 mg/day. Completed a course of rehabilitation: mirror therapy, techniques of fine motor skills and interhemispheric interaction, classes with a speech therapist. After 2 months the patient fully controls the function of hand. This is a case-report of rare syndrome that inspires fear to patients and confuses doctors.

Pubmed:
29798976: Manysheva KB, Abusueva BA, Alieva AD, Ismail-Zade EN
[Clinical/epidemiological characteristics of ischemic stroke in Dagestan].
To analyze risk factors and course of acute ischemic stroke (IS) based on hospital register data.
Zh Nevrol Psikhiatr Im S S Korsakova, 2018; 118
30251987: Abusueva BA, Manyshev SB, Manysheva KB
[Mikhail Sergeyevich Dobrohotov (To the 140th anniversary of his birth)].
Zh Nevrol Psikhiatr Im S S Korsakova, 2018; 118
35175711: Abusueva BA, Manyshev SB, Manysheva KB
[Formation of neurology in the Daghestan (to the 85th anniversary of the Department of Nervous Diseases, Medical Genetics and Neurosurgery, Daghestan State Medical University)].
The article describes the history of the Department of Nervous Diseases, Medical Genetics and Neurosurgery of the Daghestan State Medical University on the basis of literature data and archival sources introduced into scientific circulation for the first time. The department, created in 1936, has gone through a difficult path in its formation and development. The authors reconstruct the history of the formation of this department of the university, dwelling on the problems faced by its first employees. Special attention is paid to the work of the department during the Great Patriotic War, when its staff was reduced to a minimum. The authors describe the appearance, stages of development and successful functioning of the scientific school for the study of thermoregulation under the guidance of Associate Professor V.A. Liechtenstein. The formation of the teaching of neurosurgery, as well as the work of the department in recent years, is described.
Zh Nevrol Psikhiatr Im S S Korsakova, 2022; 122

FENS Forum 2022
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BOARD NUMBER: S01-254

PHYSIOLOGY AND PHYSIOPATHOLOGY OF CEREBELLO-THALAMIC PATHWAYS IN MOTOR SKILL LEARNING

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Cerebellum is known to exert a key role in regulating fine locomotion. However, its broad connectivity with various cortical and subcortical structures supports its involvement in higher brain functions, such as executive functions, cognitive functions, and motor skill learning. A murine paradigm of motor skill learning, the accelerating rotarod, was shown to sequentially involve the primary motor cortex and the different compartments of the striatum, but little was known about an involvement of the cerebellum in this learning. Previous work from the team (Varani et al 2020, bioRxiv) demonstrated that the cerebellar output pathways differentially contribute to phases of accelerating rotarod learning. Indeed, cerebellar projections to motor thalamus (VAL) were involved in the offline consolidation of the motor skill, while its projection to striatal-projecting thalamus (CL) contributed to the expression of previously-consolidated motor skill. These cerebello-thalamic pathways can be altered in the context of brain disorders such as primary dystonia. Notably, primary forms of dystonia are associated to incomplete penetrance and endophenotypes in non-manifesting individuals, suggesting the presence of network alterations in both presymptomatic and symptomatic state. We used a combination of extracellular recordings and optogenetic stimulations to reveal an abnormal plasticity in a cerebello-thalamic tract (DN-CL) in a mouse model of DYT25, a genetic form of primary dystonia. We also observed an altered expression of accelerating rotarod learning in presymptomatic mice, consistent with the abnormalities in DN-CL pathway. All together, our results indicate that alteration of cerebello-thalamic tracts may explain the presence of endophenotypes in dystonia.

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BOARD NUMBER: S01-255

ACTING ALONE OR TOGETHER? EVALUATING THE COST OF INTER-INDIVIDUAL MOTOR COORDINATION IN MACAQUES

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Acting with others can be advantageous to achieve goals unattainable by a single agent, however joint action (JA) requires costly inter-individual motor coordination. In macaque monkeys, we studied the decision-making processes subtending the free choice to act in a SOLO or joint fashion (JA), under different payoff contexts. Our aim was to quantitatively evaluate in lab settings the cost of dyadic motor coordination. Two animals, each guiding a visual cursor through an isometric joystick, were trained to choose between two alternative targets to gain a cued amount of reward to be obtained through individual or joint behaviour. In the JA option, animals had to coordinate their force to jointly bring their cursors to the final target location. The results indicate that for low Δ differences in reward amount associated to the two action types, both animals preferred the SOLO action regardless of the offer. By increasing the differences (Δ) in reward amounts, monkeys opted always in favour of JA. The indifference points of psychometric curves linking choice probability for JA vs SOLO found at Δ≠0 provided an estimate of the perceived cost of their collective behaviour. In conclusion, in macaques the economic evaluation of acting alone or together is not merely dictated by the payoff value but is subdued to the cost of their reciprocal motor coordination. The animals can accurately estimate this cost and use it to decide whether to act alone or with others. The neural mechanisms underlying these cognitive processes are under investigation in prefrontal cortex.

Pubmed:
34732524: Quarta E, Scaglione A, Lucchesi J, Sacconi L, Allegra Mascaro AL, Pavone FS
Distributed and Localized Dynamics Emerge in the Mouse Neocortex during Reach-to-Grasp Behavior.
A long-standing question in systems neuroscience is to what extent task-relevant features of neocortical processing are localized or distributed. Coordinated activity across the neocortex has been recently shown to drive complex behavior in the mouse, while activity in selected areas is canonically associated with specific functions (e.g., movements in the case of the motor cortex). Reach-to-grasp (RtG) movements are known to be dependent on motor circuits of the neocortex; however, the global activity of the neocortex during these movements has been largely unexplored in the mouse. Here, we characterized, using wide-field calcium imaging, these neocortex-wide dynamics in mice of either sex engaging in an RtG task. We demonstrate that, beyond motor regions, several areas, such as the visual and the retrosplenial cortices, also increase their activity levels during successful RtGs, and homologous regions across the ipsilateral hemisphere are also involved. Functional connectivity among neocortical areas increases transiently around movement onset and decreases during movement. Despite this global phenomenon, neural activity levels correlate with kinematics measures of successful RtGs in sensorimotor areas only. Our findings establish that distributed and localized neocortical dynamics co-orchestrate efficient control of complex movements. Mammals rely on reaching and grasping movements for fine-scale interactions with the physical world. In the mouse, the motor cortex is critical for the execution of such behavior, yet little is known about the activity patterns across neocortical areas. Using the mesoscale-level networks as a model of cortical processing, we investigated the hypothesis that areas beyond the motor regions could participate in RtG planning and execution, and indeed a large network of areas is involved while performing RtGs. Movement kinematics correlates mostly with neural activity in sensorimotor areas. By demonstrating that distributed and localized neocortical dynamics for the execution of fine movements coexist in the mouse neocortex during RtG, we offer an unprecedented view on the neocortical correlates of mammalian motor control.
J Neurosci, 2022; 42
33537380: Quarta E, Bravi R, Minciacchi D, Cohen EJ
Circle drawing and tracing dataset for evaluation of fine motor control.
We introduce a motion dataset from healthy human subjects (= 125) performing two fine motor control tasks on a graphic tablet, namely circle drawing and circle tracing. The article reports the methods and materials used to capture the motion data. The method for data acquisition is the same as the one used to investigate some aspects of fine motor control in

healthy subjects in the paper by Cohen et al. (2018) "Precision in drawing and tracing tasks: Different measures for different aspects of fine motor control" (https://doi.org/10.1016/j.humov.2018.08.004) [1]. The dataset shared here contains new raw files of the two-dimensional motion data, as well information on the participants (gender, age, laterality index). These data could be instrumental for assessing other aspects of fine motor control, such as speed-accuracy tradeoff, speed-curvature power law, etc., and/or test machine learning algorithms for e.g., task classification.
Data Brief, 2021; 35
33281568: Quarta E, Cohen EJ, Bravi R, Minciacchi D
Future Portrait of the Athletic Brain: Mechanistic Understanding of Human Sport Performance Animal Neurophysiology of Motor Behavior.
Sport performances are often showcases of skilled motor control. Efforts to understand the neural processes subserving such movements may teach us about general principles of behavior, similarly to how studies on neurological patients have guided early work in cognitive neuroscience. While investigations on non-human animal models offer valuable information on the neural dynamics of skilled motor control that is still difficult to obtain from humans, sport sciences have paid relatively little attention to these mechanisms. Similarly, knowledge emerging from the study of sport performance could inspire innovative experiments in animal neurophysiology, but the latter has been only partially applied. Here, we advocate that fostering interactions between these two seemingly distant fields, i.e., animal neurophysiology and sport sciences, may lead to mutual benefits. For instance, recording and manipulating the activity from neurons of behaving animals offer a unique viewpoint on the computations for motor control, with potentially untapped relevance for motor skills development in athletes. To stimulate such transdisciplinary dialog, in the present article, we also discuss steps for the reverse translation of sport sciences findings to animal models and the evaluation of comparability between animal models of a given sport and athletes. In the final section of the article, we envision that some approaches developed for animal neurophysiology could translate to sport sciences anytime soon (e.g., advanced tracking methods) or in the future (e.g., novel brain stimulation techniques) and could be used to monitor and manipulate motor skills, with implications for human performance extending well beyond sport.
Front Syst Neurosci, 2020; 14
29580900: Quarta E, Fulgenzi G, Bravi R, Cohen EJ, Yanpallewar S, Tessarollo L, Minciacchi D
Deletion of the endogenous TrkB.T1 receptor isoform restores the number of hippocampal CA1 parvalbumin-positive neurons and rescues long-term potentiation in pre-symptomatic mSOD1(G93A) ALS mice.
Amyotrophic lateral sclerosis (ALS) causes rapidly progressive paralysis and death within 5 years from diagnosis due to degeneration of the motor circuits. However, a significant population of ALS patients also shows cognitive impairments and progressive hippocampal pathology. Likewise, the mutant SOD1(G93A) mouse model of ALS (mSOD1), in addition to loss of spinal motor neurons, displays altered spatial behavior and hippocampal abnormalities including loss of parvalbumin-positive interneurons (PVi) and enhanced long-term potentiation (LTP). However, the cellular and molecular mechanisms underlying these morpho-functional features are not well understood. Since removal of TrkB.T1, a receptor isoform of the brain-derived neurotrophic factor, can partially rescue the phenotype of the mSOD1 mice, here we tested whether removal of TrkB.T1 can normalize the number of PVi and the LTP in this model. Stereological analysis of hippocampal PVi in control, TrkB.T1, mSOD1, and mSOD1 mice deficient for TrkB.T1 (mSOD1/T1) showed that deletion of TrkB.T1 restored the number of PVi to physiological level in the mSOD1 hippocampus. The rescue of PVi neuron number is paralleled by a normalization of high-frequency stimulation-induced LTP in the pre-symptomatic mSOD1/T1 mice. Our experiments identified TrkB.T1 as a cellular player involved in the homeostasis of parvalbumin expressing interneurons and, in the context of murine ALS, show that TrkB.T1 is involved in the mechanism underlying structural and functional hippocampal degeneration. These findings have potential implications for hippocampal degeneration and cognitive impairments reported in ALS patients at early stages of the disease.
Mol Cell Neurosci, 2018; 89
28069532: Cohen EJ, Quarta E, Bravi R, Granato A, Minciacchi D
Neural plasticity and network remodeling: From concepts to pathology.
Neuroplasticity has been subject to a great deal of research in the last century. Recently, significant emphasis has been placed on the global effect of localized plastic changes throughout the central nervous system, and on how these changes integrate in a pathological context. Specifically, alterations of network functionality have been described in various pathological contexts to which corresponding structural alterations have been proposed. However, considering the amount of literature and the different pathological contexts, an integration of this information is still lacking. In this paper we will review the concepts of neural plasticity as well as their repercussions on network remodeling and provide a possible explanation to how these two concepts relate to each other. We will further examine how alterations in different pathological contexts may relate to each other and will discuss the concept of plasticity diseases, its models and implications.
Neuroscience, 2017; 344
27604420: Yanpallewar S, Wang T, Koh DC, Quarta E, Fulgenzi G, Tessarollo L

Nedd4-2 haploinsufficiency causes hyperactivity and increased sensitivity to inflammatory stimuli.
Nedd4-2 (NEDD4L in humans) is a ubiquitin protein ligase best known for its role in regulating ion channel internalization and turnover. Nedd4-2 deletion in mice causes perinatal lethality associated with increased epithelial sodium channel (ENaC) expression in lung and kidney. Abundant data suggest that Nedd4-2 plays a role in neuronal functions and may be linked to epilepsy and dyslexia in humans. We used a mouse model of Nedd4-2 haploinsufficiency to investigate whether an alteration in Nedd4-2 levels of expression affects general nervous system functions. We found that Nedd4-2 heterozygous mice are hyperactive, have increased basal synaptic transmission and have enhanced sensitivity to inflammatory pain. Thus, Nedd4-2 heterozygous mice provide a new genetic model to study inflammatory pain. These data also suggest that in human, SNPs affecting NEDD4L levels may be involved in the development of neuropsychological deficits and peripheral neuropathies and may help unveil the genetic basis of comorbidities.
Sci Rep, 2016; 6
25837726: Bravi R, Quarta E, Del Tongo C, Carbonaro N, Tognetti A, Minciacchi D
Music, clicks, and their imaginations favor differently the event-based timing component for rhythmic movements.
The involvement or noninvolvement of a clock-like neural process, an effector-independent representation of the time intervals to produce, is described as the essential difference between event-based and emergent timing. In a previous work (Bravi et al. in Exp Brain Res 232:1663-1675, 2014a. doi: 10.1007/

BOARD NUMBER: S00221-014-3845-9), we studied repetitive isochronous wrist's flexion-extensions (IWFEs), performed while minimizing visual and tactile information, to clarify whether non-temporal and temporal characteristics of paced auditory stimuli affect the precision and accuracy of the rhythmic motor performance. Here, with the inclusion of new recordings, we expand the examination of the dataset described in our previous study to investigate whether simple and complex paced auditory stimuli (clicks and music) and their imaginations influence in a different way the timing mechanisms for repetitive IWFEs. Sets of IWFEs were analyzed by the windowed (lag one) autocorrelation-wγ(1), a statistical method recently introduced for the distinction between event-based and emergent timing. Our findings provide evidence that paced auditory information and its imagination favor the engagement of a clock-like neural process, and specifically that music, unlike clicks, lacks the power to elicit event-based timing, not counteracting the natural shift of wγ(1) toward positive values as frequency of movements increase.
Exp Brain Res, 2015; 233
25684566: Quarta E, Bravi R, Scambi I, Mariotti R, Minciacchi D
Increased anxiety-like behavior and selective learning impairments are concomitant to loss of hippocampal interneurons in the presymptomatic SOD1(G93A) ALS mouse model.
Amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease primarily characterized by motor neuron death, causes damages beyond motor-related areas. In particular, cognitive impairments and hippocampal damage have been reported in ALS patients. We investigated spatial navigation learning and hippocampal interneurons in a mutant SOD1(G93A) mouse (mSOD1) model of ALS. Behavioral tests were performed by using presymptomatic mSOD1 mice. General motor activity was comparable to that of wild-type mice in the open-field test, in which, however mSOD1 exhibited increased anxiety-like behavior. In the Barnes maze test, mSOD1 mice displayed a delay in learning, outperformed wild-type mice during the first probe trial, and exhibited impaired long-term memory. Stereological counts of parvalbumin-positive interneurons, which are crucial for hippocampal physiology and known to be altered in other central nervous system regions of mSOD1 mice, were also performed. At postnatal day (P) 56, the population of parvalbumin-positive interneurons in mSOD1 mice was already reduced in CA1 and in CA3, and at P90 the reduction extended to the dentate gyrus. Loss of parvalbumin-positive hippocampal interneurons occurred mostly during the presymptomatic stage. Western blot analysis showed that hippocampal parvalbumin expression levels were already reduced in mSOD1 mice at P56. The hippocampal alterations in mSOD1 mice could at least partly account for the increased anxiety-like behavior and deficits in spatial navigation learning. Our study provides evidence for cognitive alterations and damage to the γ-aminobutyric acid (GABA)ergic system in the hippocampus of murine ALS, thereby revealing selective deficits antecedent to the onset of motor symptoms.
J Comp Neurol, 2015; 523
25445612: Cohen EJ, Quarta E, Fulgenzi G, Minciacchi D
Acetylcholine, GABA and neuronal networks: a working hypothesis for compensations in the dystrophic brain.
Duchenne muscular dystrophy (DMD), a genetic disease arising from a mutation in the dystrophin gene, is characterized by muscle failure and is often associated with cognitive deficits. Studies of the dystrophic brain on the murine mdx model of DMD provide evidence of morphological and functional alterations in the central nervous system (CNS) possibly compatible

with the cognitive impairment seen in DMD. However, while some of the alterations reported are a direct consequence of the absence of dystrophin, others seem to be associated only indirectly. In this review we reevaluate the literature in order to formulate a possible explanation for the cognitive impairments associated with DMD. We present a working hypothesis, demonstrated as an integrated neuronal network model, according to which within the cascade of events leading to cognitive impairments there are compensatory mechanisms aimed to maintain functional stability via perpetual adjustments of excitatory and inhibitory components. Such ongoing compensatory response creates continuous perturbations that disrupt neuronal functionality in terms of network efficiency. We have theorized that in this process acetylcholine and network oscillations play a central role. A better understating of these mechanisms could provide a useful diagnostic index of the disease's progression and, perhaps, the correct counterbalance of this process might help to prevent deterioration of the CNS in DMD. Furthermore, the involvement of compensatory mechanisms in the CNS could be extended beyond DMD and possibly help to clarify other physio-pathological processes of the CNS.
Brain Res Bull, 2015; 110

25309355: Bravi R, Quarta E, Cohen EJ, Gottard A, Minciacchi D

A little elastic for a better performance: kinesiotaping of the motor effector modulates neural mechanisms for rhythmic movements.

A rhythmic motor performance is brought about by an integration of timing information with movements. Investigations on the millisecond time scale distinguish two forms of time control, event-based timing and emergent timing. While event-based timing asserts the existence of a central internal timekeeper for the control of repetitive movements, the emergent timing perspective claims that timing emerges from dynamic control of nontemporal movements parameters. We have recently demonstrated that the precision of an isochronous performance, defined as performance of repeated movements having a uniform duration, was insensible to auditory stimuli of various characteristics (Bravi et al., 2014). Such finding has led us to investigate whether the application of an elastic therapeutic tape (Kinesio® Tex taping; KTT) used for treating athletic injuries and a variety of physical disorders, is able to reduce the timing variability of repetitive rhythmic movement. Young healthy subjects, tested with and without KTT, have participated in sessions in which sets of repeated isochronous wrist's flexion-extensions (IWFEs) were performed under various auditory conditions and during their recall. Kinematics was recorded and temporal parameters were extracted and analyzed. Our results show that the application of KTT decreases the variability of rhythmic movements by a 2-fold effect: on the one hand KTT provides extra proprioceptive information activating cutaneous mechanoreceptors, on the other KTT biases toward the emergent timing thus modulating the processes for rhythmic movements. Therefore, KTT appears able to render movements less audio dependent by relieving, at least partially, the central structures from time control and making available more resources for an augmented performance.
Front Syst Neurosci, 2014; 8

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BOARD NUMBER: S01-256

EFFECTS OF REPETITIVE VIBRATION ON FORCE CONTROL, PROPRIOCEPTION, AND NEURAL ACTIVITIES IN THE CNS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Background: Sensory afferent inputs play an important role in sensorimotor control and plasticity in the neuromuscular system. Here we investigate how repetitive vibration input affects sensorimotor control and neural activities in the CNS. **Methods:** Healthy adults received sensory level vibration (frequency range = 125 – 300 Hz) applied on the muscle belly of right wrist/finger flexors. We recorded and analyzed grip force control and proprioception, EEG and EMG in the following 6 time points: before vibration, during vibration, 10-min vibration, 20-min vibration, 10-post vibration, and 20-min post vibration. Principle Component Analysis was used to identify major EEG signals in alpha, beta and gamma frequency bands. **Results:** Our preliminary results indicated that the accuracy and stability of force control were significantly improved after 20-min vibration ($p < 0.05$). During vibration, moderate correlation was observed between grip force steadiness and alpha band EEG power ($r=0.71$, $p < 0.05$). Also, grip force steadiness was highly correlated with alpha band EEG power with 10-min of vibration ($r=0.85$, $p < 0.05$), and 20 minutes post vibration ($r=0.70$, $p < 0.05$). On the other hand, improvement in force accuracy was highly correlated with changes in EEG beta band power during vibration ($r=0.69$, $p < 0.05$). **Conclusion:** 20-min of repetitive vibration improves grip force accuracy and stability. Improvement in force control performance was associated with changes in brain activity, suggesting central mechanisms for vibration induced improvement in sensorimotor performance.

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PRE-MOVEMENT SPINAL CORD ACTIVITY IN HUMANS: A SIMULTANEOUS BRAIN-SPINAL CORD FMRI STUDY

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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A corticospinal circuit is an important role in the voluntary control of limb movements. Previous non-human primate studies demonstrated that spinal interneurons show pre-movement preparatory activity. However, it remains unknown pre-movement preparatory activity in the human spinal cord. Here, we aimed to illustrate pre-movement preparatory activity in the human corticospinal circuit by using simultaneous brain-spinal cord fMRI. Seventeen healthy volunteers were scanned with simultaneous brain-spinal cord fMRI while they performed the ready-set-go task in a 3T-MRI scanner. In this behavioral task, participants were asked to prepare to respond at the display of ready cue and to grip the force device as quickly as possible at the display of go cue. The averaged pre-movement activity was observed in the contralateral primary motor cortex (M1) and supplementary motor area (SMA) at the ready phase, but not in the spinal cord. On the other hand, the degree of preparatory activity at the C6-C7 segments, where the motor nuclei for forearm muscles are located, was positively correlated with subsequent reaction time and peak-grip force measured at the following go phase. In addition, the activity at C4 segment, where the propriospinal neurons which relay the signal to forearm motoneurons are located, was positively correlated only with subsequent peak force. Taken together, the present findings firstly reveal pre-movement preparatory activity in the human spinal cord, and that this pre-movement activity of cervical enlargement encodes future motor outputs of the handgrip.

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BOARD NUMBER: S01-258

IMPACT OF VISUOSPATIAL ATTENTION ON MOTOR CONTROL AND HAND FUNCTION AFTER STROKE

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Introduction: Impaired manual dexterity is common after stroke, reducing autonomy and quality of life. Dexterity, defined as the fine control of finger movements, requires interplay between sensorimotor and cognitive functions. Impairments in divided attention, involved in allocating resources during dual-task exercises, may contribute to dexterity impairment and impact rehabilitation. However, studies relating visuospatial and dexterity impairments after stroke are lacking. **Aim**: To compare visuospatial attention in chronic stroke patients and controls and to establish the relationship between visuospatial attention and manual dexterity. **Methods**: A visuomotor grip-force tracking task is combined with oculomotor recordings to quantify sensorimotor precision during (i) simple force tracking, (ii) dual-task condition with visual distractors where the subject is instructed to inhibit ocular saccades and (iii) dual-task condition with addition of numbers displayed on the screen (subject is instructed to perform saccades). Two measures inform on visuospatial divided attention capacity: the % of saccades inhibited in the distractor task and the dual-task cost on precision of grip force tracking. Chronic stroke patients with mild-moderate hemiparesis are compared to age-comparable healthy controls. Visuospatial attention will be correlated with clinical motor and cognitive assessments and with dexterity data obtained using novel force sensor technology and tablet-based applications. **Results**: Data collection is ongoing and results will be presented. **Conclusion**: The data will provide insights on visuospatial attention in stroke and its impact on manual dexterity.

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BOARD NUMBER: S01-259

STUDY OF THE CORTICOSPINAL ACTIVITY IN SENSORIMOTOR INTEGRATION

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Motion is an essential function for animals. The motor command is generated in the motor cortex and relayed to different targets involved in muscle contraction and its fine control. One of them is the spinal cord, receiving direct cortical input through the corticospinal tract (CST). The cortex is also involved in the modulation of sensory information, which is essential for the performance of adapted and coordinated movements. We have recently demonstrated that the CST projecting to the lumbar cord is mainly involved in sensory modulation while the muscle contraction command is relayed more indirectly. Yet the exact functional role of the lumbar CST during behavior is still imperfectly described. Since motor cortex involvement in locomotion is debated, our aim is to elucidate the importance of the lumbar CST in different locomotor behaviors, and to characterize the function of the corticospinal (CS) neurons during these behaviors. To do so, we first set out to identify behavioral paradigms in which the lumbar CST is critically involved. This is performed by selectively perturbing the lumbar CS neurons through optogenetics, while precisely recording hindlimb movements using a motion capture apparatus during 'skilled locomotion tasks' such as crossing obstacles, a round beam, and a ladder. We are also setting up extracellular in vivo recordings in the cortex on behaving animals, with optotagging used to identify CS neurons. The activity of this population will inform us about the dynamics of the information targeting the spinal sensorimotor circuits during the different phases of the movement.

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PLANNING OF MULTIPLE ACTIONS IN MOTOR CORTEX

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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It has been argued that the motor system has the capacity to plan multiple movements simultaneously. The leading model based on neurophysiological data proposes that parallel motor plans are formed and compete for movement execution. Here we provide experimental evidence for an alternative model grounded in the dynamical system's theory of motor cortex. We recorded motor cortical activity in non-human primates performing an instructed two-grip grasping task. We found that, contrary to what the existing model predicts, motor plans associated with the two possible movements were not simultaneously formed. Rather, a single motor plan emerged reflecting a tradeoff between the two possible motor plans, consistent with a model where movement preparation is optimized for both movements. This model predicts a direct relationship between the optimization process and behavioral performance, which holds true empirically. Together, our results challenge current views on multiple-movement preparation and further point to motor planning as a controlled dynamical process.

Pubmed:
34534456: Meirhaeghe N, Sohn H, Jazayeri M
A precise and adaptive neural mechanism for predictive temporal processing in the frontal cortex.
The theory of predictive processing posits that the brain computes expectations to process information predictively. Empirical evidence in support of this theory, however, is scarce and largely limited to sensory areas. Here, we report a precise and adaptive mechanism in the frontal cortex of non-human primates consistent with predictive processing of temporal events. We found that the speed of neural dynamics is precisely adjusted according to the average time of an expected stimulus. This speed adjustment, in turn, enables neurons to encode stimuli in terms of deviations from expectation. This lawful relationship was evident across multiple experiments and held true during learning: when temporal statistics underwent covert changes, neural responses underwent predictable changes that reflected the new mean. Together, these results highlight a precise mathematical relationship between temporal statistics in the environment and neural activity in the frontal cortex that may serve as a mechanism for predictive temporal processing.
Neuron, 2021; 109
31320220: Sohn H, Narain D, Meirhaeghe N, Jazayeri M
Bayesian Computation through Cortical Latent Dynamics.
Statistical regularities in the environment create prior beliefs that we rely on to optimize our behavior when sensory information is uncertain. Bayesian theory formalizes how prior beliefs can be leveraged and has had a major impact on models of perception, sensorimotor function, and cognition. However, it is not known how recurrent interactions among neurons mediate Bayesian integration. By using a time-interval reproduction task in monkeys, we found that prior statistics warp neural representations in the frontal cortex, allowing the mapping of sensory inputs to motor outputs to incorporate prior statistics in accordance with Bayesian inference. Analysis of recurrent neural network models performing the task revealed that this warping was enabled by a low-dimensional curved manifold and allowed us to further probe the potential causal underpinnings of this computational strategy. These results uncover a simple and general principle whereby prior beliefs exert their influence on behavior by sculpting cortical latent dynamics.
Neuron, 2019; 103
33258769: Wang J, Hosseini E, Meirhaeghe N, Akkad A, Jazayeri M
Reinforcement regulates timing variability in thalamus.
Learning reduces variability but variability can facilitate learning. This paradoxical relationship has made it challenging to tease apart sources of variability that degrade performance from those that improve it. We tackled this question in a context-dependent timing task requiring humans and monkeys to flexibly produce different time intervals with different effectors. We identified two opposing factors contributing to timing variability: slow memory fluctuation that degrades performance and reward-dependent exploratory behavior that improves performance. Signatures of these opposing factors were evident

across populations of neurons in the dorsomedial frontal cortex (DMFC), DMFC-projecting neurons in the ventrolateral thalamus, and putative target of DMFC in the caudate. However, only in the thalamus were the performance-optimizing regulation of variability aligned to the slow performance-degrading memory fluctuations. These findings reveal how variability caused by exploratory behavior might help to mitigate other undesirable sources of variability and highlight a potential role for thalamocortical projections in this process.
Elife, 2020; 9
33349476: Sohn H, Meirhaeghe N, Rajalingham R, Jazayeri M
A Network Perspective on Sensorimotor Learning.
What happens in the brain when we learn? Ever since the foundational work of Cajal, the field has made numerous discoveries as to how experience could change the structure and function of individual synapses. However, more recent advances have highlighted the need for understanding learning in terms of complex interactions between populations of neurons and synapses. How should one think about learning at such a macroscopic level? Here, we develop a conceptual framework to bridge the gap between the different scales at which learning operates, from synapses to neurons to behavior. Using this framework, we explore the principles that guide sensorimotor learning across these scales, and set the stage for future experimental and theoretical work in the field.
Trends Neurosci, 2021; 44

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CHARACTERIZATION OF IMPAIRED MOTOR MOVEMENTS IN A MOUSE MODEL OF FREEZING OF GAIT

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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The ability to voluntarily initiate goal-directed motor movements is crucial for all animals. When suffering from movement disorders such as Parkinson's disease, fluent, continuous movements are often impaired. A common motor deficit experienced by Parkinson patients is freezing of gait (FoG), a form of akinesia described as sudden short periods of inability to start walking or failure to continue movement. FoG symptoms have been shown to be particularly difficult to treat with traditional methods, therefore, more dedicated models to investigate the underlying dynamics of FoG are necessary. Here we use open-source software packages to characterize motor movements in head-fixed mice with and without haloperidol-induced cataleptic periods to establish if this is an effective model to recapitulate FoG. Using DeepLabCut (Mathis et al. 2018) we track individual body parts and further utilize VAME (Luxem et al. bioRxiv), a probabilistic machine learning framework, to define motor repertoires in healthy motor states and haloperidol induced 'FoG' periods. We found dose-dependent changes in movement repertoires, with haloperidol injected mice showing less movement. The ability to map body movements reliably across individuals, with emphasis on deciphering specific motor states such as freezing, and then further linking these behavioral states to neuronal activity is vital to facilitate the dissection of neuronal mechanisms involved in these movement disorders.
Mathis, A. et al. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. Nat Neurosci (2018)
Luxem K. et al. Identifying Behavioral Structure from Deep Variational Embeddings of Animal Motion (bioRxiv, 2022)

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A BAYESIAN HIDDEN MARKOV MODEL FOR TRACKING LONG-TERM DYNAMIC CHANGES IN NEURONAL POPULATION ACTIVITY OF THE MOTOR CORTEX OF MONKEYS CHRONIC MULTI-ELECTRODE ARRAYS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

Kirchherr[1], Sebastián Mildiner Moraga[2], Coudé[1], Emmeke Aarts[2], Pier Francesco Ferrari[1], Bonaiuto[1]
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Plasticity induced by long-term learning, for example in motor control, is challenging to analyze at the single cell level due to difficulty in tracking single units over long periods of time. We demonstrate the application of Bayesian hidden Markov models (HMMs) to analyze the dynamics of neural population collected from chronic multi-electrode arrays from the primary and premotor cortex of two macaque monkeys during a reach-and-grasp task. This approach uses a parametric HMMs, which includes time-varying condition covariates and can be extended to have a multi-level structure including continuously distributed random effects. Our preliminary results suggest stable population dynamics over several weeks of recording for a well-learned reach and grasp task. Although the model trained only on multi-unit spiking activity and had no information about task-related or behavioral events, the onset or offset of each state is associated with a particular behavioral event, and this remains unchanged throughout the recording duration. This HMM framework is therefore suitable for analysis of longitudinal electrophysiological data, and potentially of experimentally induced plasticity at the neural population level during motor learning.

Pubmed:

30796121: Auriacombe M, Roux P, Briand Madrid L, Kirchherr S, Kervran C, Chauvin C, Gutowski M, Denis C, Carrieri MP, Lalanne L, Jauffret-Roustide M,
Impact of drug consumption rooms on risk practices and access to care in people who inject drugs in France: the COSINUS prospective cohort study protocol.
The high prevalence of hepatitis C and the persistence of HIV and hepatitis C virus (HCV) risk practices in people who inject drugs (PWID) in France underlines the need for innovative prevention interventions. The main objective of this article is to describe the design of the COSINUS cohort study and outline the issues it will explore to evaluate the impact of drug consumption rooms (DCR) on PWID outcomes. Secondary objectives are to assess how DCR (a) influence other drug-related practices, such as the transition from intravenous to less risky modes of use, (b) reduce drug use frequency/quantity, (c) increase access to treatment for addiction and comorbidities (infectious, psychiatric and other), (d) improve social conditions and (e) reduce levels of violence experienced and drug-related offences. COSINUS will also give us the opportunity to investigate the impact of other harm reduction tools in France and their combined effect with DCR on reducing HIV-HCV risk practices. Furthermore, we will be better able to identify PWID needs.
BMJ Open, 2019; 9
29031837: Lalanne L, Ferrand-Devouge E, Kirchherr S, Rauch L, Koning E, Speeg C, Laprevote V, Giersch A
Impaired contrast sensitivity at low spatial frequency in cannabis users with early onset.
The regular use of cannabis generates pronounced cognitive disorders, especially in users who begin before the age of 15-16. However, less is known about the impact of regular cannabis on visual function, especially in the case of early onset. Cannabinoid receptors (CB1) are expressed in areas of the visual system, like the thalamus and primary cortex, which might originate sensory disorders. Hence, we measured contrast sensitivity (CS) in three groups, i.e. cannabis users with late onset of cannabis use (after 16 years old), cannabis users with early onset". We used a constant method which allowed us to control for biased responses. Stimuli were presented at high and low spatial frequencies and in both static and dynamic conditions (8Hz). As contrast sensitivity is measured behaviorally based on an explicit response and could thus be impacted by attentional or vigilance disorders, participants' attention and vigilance were carefully monitored by means of the D2 test, CPT-AX for attention and pupillography for vigilance. Cannabis users with early onset were significantly impaired only at low spatial frequency. This effect was independent of response bias, vigilance and attention. These results show for the first time that early cannabis use impacts contrast sensitivity at low spatial frequency.
Eur Neuropsychopharmacol, 2017; 27


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DISSECTING THE ROLE OF THE EXTERNAL GLOBUS PALLIDUS SUBPOPULATIONS IN AN INSTRUMENTAL, NON-LOCOMOTOR TASK

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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The external globus pallidus (GPe) constitute the middle node of the basal ganglia's (BG) indirect pathway, receiving inputs mainly from D2R-expressing striatal neurons and projecting to BG outputs, finally regulating behaviour. Within it, two main and non-overlapping populations have been described as prototypic (Lhx6+, PV+/-) and arkypallidal (FoxP2+) neurons. They possess different projection patterns, developmental origins, and are differently affected by the loss of dopamine in Parkinson's. Despite early evidences pointing at different roles for these subpopulations in locomotor control in health and disease, little is known on how dopamine release dynamically tune the different GPe populations, and how these, in turn, influence non-locomotor movements and action selection. We developed a reaching-and-grasping instrumental version of a "Go-NoGo" paradigm were mice were instructed to initiate a ballistic movement in order to obtain a sucrose reward on a "Go" cue, whereas they should remain still for the whole duration of a "NoGo" cue, to avoid a mild air-puff. Dopamine concentration in the dorsal striatum was monitored using fiber photometry and prototypic or arkypallidal neurons where suppressed at specific epochs within the task by the delivery of light to the GPe of transgenic mice expressing a soma-targeted opsin. Our preliminary observations suggests that contralateral prototypic inhibition preferentially decreases reaching (Go) performance, especially when initiated before movement onset. These early results suggest that prototypic neurons could be particularly involved during motor preparation and/or initiation. They will be complemented by large-scale electrophysiological recordings to examine the physiological dynamics of these subpopulations during our task.

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CEREBELLAR GRANULE CELL TEMPORAL REPRESENTATIONS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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The cerebellum processes contextual information with an internal model (Wolpert et al, 1998) and generates delayed temporal associations between conditioned sensory and unconditioned stimuli (Medina et al, 2000) to fine-tune motor and cognitive behaviors. Each granule cell (GC) receives diverse sensory information from mossy fibers (MFs) to represent rich contextual information within the population (Chabrol et al, 2015; Ishikawa et al, 2015). Information from multiple modalities is integrated, expanded and recoded to maximize the number of sensory patterns that can be detected and finally control body muscles (Marr, 1969; Albus, 1971). The GC layer is thought to generate a diverse temporal representation that enables learning temporally precise behaviors (Medina et al. 2000). A network model from the lab suggests that input specific diversity of MF-GC synaptic dynamics are sufficient to generate diverse GC firing patterns that act as a temporal basis for cerebellar learning. We expressed fast GCaMP8f in GCs and measured the diversity of sensory responses in vivo using two-photon laser-scanning microscopy. We observed a temporal distribution of calcium response times and durations across the population, which could not be accounted for by trial to trial variability and was consistent with a diverse temporal representation. By sparse expression of glutamate reporter SF-iGluSnfr (Marvin et al., 2017) we simultaneously recorded from all MF-GC synapses of a given GC. Specific MF-GC synapses responded to selective sensory stimuli or combinations and with differences in firing frequencies and delays. Hence it is possible to explore the synaptic basis of GC temporal representations in vivo.

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ONGOING CLINICAL, ELECTROPHYSIOLOGICAL AND BIOPHYSICAL EVIDENCE OF HIGH-DENSITY FRONTO-CEREBELLAR TRANSCRANIAL DIRECT CURRENT STIMULATION IN MOTOR STROKE

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Transcranial direct current stimulation has shown promise to boost and guide neural activity towards a facilitatory state for the recovery of motor deficits after large-scale networks dysfunctions following storkes. However, large heterogenic results have been reported mainly influenced by stroke lesion features and the large variety of tDCS protocols highlighting the need to move towards the use of multi-site set-ups targeting networks rather than focusing on local isolated effects. A first step to address such ambitious goals is explore the accumulative potential of neuromodulation to face new paradigms such a “large-scale modulation”, guiding the hole brain towards a facilitatory state for motor recovery reversing a previously identified large-scale neural signature linking attentional with motor networks. We here designed a double-blind, sham-controlled, randomized pilot experimental study probing a new tDCS protocol targeting ipsilesional prefrontal and contralesional cerebellar areas aiming to recruit a cohort of 60 stroke chronic patients which will be randomized in 4 groups according to stimulation site: (I): Prefrontal ipsilesional (DLPFC) tDCS, (II): Contralesional cerebellar tDCS, (III): Combined prefrontal and cerebellar tDCS, (IIII): Sham tDCS, delivering respectively 1.7mA, 2.0mA, 3.7mA or 0mA in a 10 daily sessions intervention of 21 min. each. To date, we have designed the clinical trial and successfully employed a MRI based biophysical computational model tDCS optimization in a pilot cohort of n=8 stroke patients, allowing us to proof the feasibility and tolerability of this protocol and extract the electroencephalographic and behavioral response to this new intervention aiming to became a new tDCS treatment.

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DYNAMIC MOTOR PRACTICE IMPROVES MOVEMENT ACCURACY, FORCE CONTROL AND LEADS TO INCREASED CORTICOSPINAL EXCITABILITY COMPARED TO ISOMETRIC MOTOR PRACTICE

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Different studies of human motor learning use behavioral models involving isometric contractions or dynamic motor practice (MP). To our knowledge, it has however not been investigated how dynamic motor practice influences force control and vice versa. Furthermore, it remains unexplored whether different types of MP are accompanied by differential effects on corticospinal excitability (CE). We tested effects of motor practice in a force or position control task involving discrete wrist flexions in the absence of online visual feedback. Motor performance quantified as errors (pixels) between the displayed target and the movement endpoint. In the main experiment, fourty-eight young adults were randomized to; position control (PC), force control (FC) motor practice or a resting control group (CON). Motor performance was assessed in both tasks before and after four blocks of motor practice with augmented visual feedback on performance. In the supplementary experiment, measures of CE was obtained in 20 additional participants by application of transcranial magnetic stimulation to the contralateral primary motor cortex prior to and following either PC or FC motor practice. The results demonstrate that performance in the force and position tasks improved from baseline to post tests, particularly for the task practiced. Additionally, position control practice also lead to improvements in force control. Motor practice with emphasis on position control was accompanied by increased CE compared to the changes observed following force control motor practice. In conclusion, dynamic motor practice improves movement accuracy, force control and leads to increased corticospinal excitability compared to isometric motor practice.

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HEBBIAN PRIMING OF SPINAL MECHANISMS INVOLVED IN MOTOR LEARNING

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Improvements in motor control with practice rely on experience-dependent plasticity in sensorimotor networks involving spike-timing- and frequency-dependent changes in synaptic efficacy. Spike-timing-dependent bidirectional changes in corticospinal transmission can be induced by repeatedly pairing cortical transcranial magnetic stimulation (TMS) and motoneuronal electrical stimulation (i.e. paired corticomotoneuronal stimulation, PCMS) timed to arrive at the corticomotoneuronal synapses in close temporal proximity. It remains to be investigated if PCMS-induced Hebbian plasticity at the corticomotoneuronal level can prime subsequent motor learning. In a within-subject, cross-over design, eighteen neurologically intact participants practiced a ballistic finger flexion task following PCMS with inter-arrival intervals adjusted to promote ($PCMS_{-2}$) or inhibit ($PCMS_{+15}$) corticomotoneuronal transmission and following a control protocol ($PCMS_{+100}$). Superior learning was observed following $PCMS_{-2}$ compared to $PCMS_{+15}$ and the control protocol. As baseline performance did not differ between the three PCMS protocols, spinal mechanisms induced by $PCMS_{-2}$ likely interacted with training-induced effects to enhance learning. Follow-up experiments comparing $PCMS_{-2}$ to training alone confirmed the enhancing effect on learning. Amplitudes of motor evoked potentials (MEP) elicited in the resting FDI muscle by use of primary motor cortex TMS increased following PCMS. Only the $PCMS_{-2}$ protocol maintained increased corticospinal excitability after motor practice. Taken together, the results provide proof-of-principle that spinal plasticity accompanying motor learning can be primed by non-invasively induced plasticity governed by Hebbian learning rules. This offers a mechanistic rationale for priming of spinal mechanisms involved in sensorimotor training with individualized PCMS to enhance effects of motor practice e.g., in neurorehabilitation and sports.

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MOVEMENT DIRECTION AND JOINT KINEMATICS DEFINE TRAJECTORY VARIABILITY PATTERNS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Comprehension of Movement Variability is essential for exploring motor performance and motor skill learning. Even very trivial movements have considerable variability when repeated across trials. Earlier studies in humans have shown that movement variability is at the core of our rich motor repertoire and its adaptability. The current study aims at understanding the kinematic and neuronal sources of movement variability in monkeys. The kinematic data for this study has been recorded from two rhesus macaques during their performance of a visuo-motor (sequential landing) task using an exoskeleton robotic arm (Kinarm, Bkin). In parallel, extracellular multi-electrode recordings were obtained from multiple cortical areas along the visuo-motor pathway (motor areas M1/PMd, parietal areas DP, 7A and visual areas V1, V2). The monkeys were over-trained and performed the same task over months under the same protocols. We first found that the variability of hand trajectories is modulated by movement amplitude and direction, but for each given movement it is highly consistent across sessions and animals. Furthermore, movements with similar directions tend to show very similar trajectory variabilities. Trajectory variability show distinct levels of correlation with elbow and shoulder angular velocities and with their degrees of coordination that is specific to each monkey. The specific trends and patterns thus observed reveal the complex origin of hand movement variability. Cortical recordings will be further used to investigate the contribution of neuronal variability in different areas to hand movement variabilities.

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BOARD NUMBER: S01-269

BEHAVIORAL CORRELATES OF LONG-TERM MOTOR SKILL LEARNING IN MACAQUE MONKEYS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Learning a motor task involves two distinct processes: the selection of a motor strategy that meets the objectives of the task and the optimisation of movement execution within that strategy. Previous studies have highlighted the key role of the basal ganglia, cerebellum and sensorimotor cortical areas in each of these two processes. However, the nature of the interactions between these processes during long-term learning and their neural correlates is not yet determined (*Penhune et al.,* 2011; *Diedrichsen et al.,* 2019). In order to address this issue, our work aims to characterise the behavioural correlates of long-term learning of a complex visual-motor task in macaque monkeys. In a second step, we will analyse the neural activity of several visual-motor cortical areas during this learning. In our task, six visual targets appear simultaneously on a screen, always at the same location. The monkey has to touch each target to obtain a reward. No instructions were given on the sequence in which the targets had to be reached. The performance of the two monkeys during the learning of this task was analysed over several months. Interestingly, both animals adopted the same evolution of the strategy used to reach the targets and of the motor performance to execute the movement. Our results demonstrate the close coupling between these two processes and clarifies the contribution of the visual-motor cortical network to their implementation.

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BOARD NUMBER: S01-270

USING EEG, EMG, AND OP-MEG TO STUDY FUNCTIONAL OSCILLATORY CONNECTIVITY DURING HUMAN STEPPING

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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The brain and spinal mechanisms underlying the act of taking a step - including planning, execution, and modification - are not well understood. We hypothesized that oscillatory communication in a parieto-frontal and cortico-muscular network is involved in the neural control of visually guided steps. We addressed this hypothesis using source reconstruction and directed functional connectivity analysis of electroencephalographic (EEG) and electromyographic (EMG) recordings during visually guided stepping (n=24). We also report preliminary data from optically-pumped magnetometry-based magnetoencephalography (OP-MEG) during the same task (n=1). Steps were divided into planning, initiation, and execution phases. The EEG analysis demonstrated that taking a step was characterized by an up-regulation of beta/gamma coherence within the parieto-frontal network during planning followed by a down-regulation of alpha and beta/gamma coherence during initiation and execution. Step modification based on visual information involved bi-directional alpha and beta/gamma coherence modulations in the parieto-frontal network during the phases leading up to step execution. The OP-MEG analysis was able to replicate features of cortico-muscular coherence demonstrated previously with other modalities during walking. Results from an exploratory analysis also suggest that OP-MEG may be able to capture functional cerebellar-spinal and cerebello-cortical connectivity during human movement. The results suggest that the central nervous system modulates lagged synchronization of neural oscillations in the context of large-scale movements, suggesting a process of flexibly fine-tuning inter-regional communication by coherence to achieve precision control during human stepping. The OP-MEG results support the feasibility of applying this new technique to explore the spatio-temporal brain dynamics underlying natural movement.

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BOARD NUMBER: S01-271

CORTICOSPINAL NEURONS IN THE SELECTIVE EXECUTION OF LEARNED ACTIONS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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When interacting with their environment animals must select actions that result in favourable outcomes, whilst avoiding inappropriate actions. Primate and rodent studies have demonstrated strong correlations between the activity of corticospinal neurons (CSNs) and sensory-evoked motor responses, but how their spatiotemporal activity patterns relate to the selective execution of appropriate actions, remains unresolved. To address this, we used two-photon population calcium imaging of CSNs in head-restrained mice trained to execute a Go/NoGo forelimb lever push task. During Go trials, mice learned to execute highly stereotyped lever pushes; during NoGo trials mice responded with a rapid lever release-and-regrasp, followed by a long period of quiescence. From a population of 1051 CSNs, approximately half exhibited cue-evoked activity changes, the majority (91%) of which were trial-type specific. In upper layer 5B, we found that trial-type-specific responses and the direction of change (i.e., increase or decrease) were depth dependent, with superficial CSNs encoding either Go or NoGo trials via an increase in activity, while deeper CSNs almost exclusively encoded Go trials with a reduction in activity. To investigate whether dendritic activity patterns reflected somatic trial-type-specific output, we performed near-simultaneous imaging of the apical and distal dendrites of identified CSNs during task execution. We found that large-scale global changes in proximal and distal dendritic activity mirrored somatic trial-type specificity, suggesting a high degree of somato-dendritic coupling in motor cortical layer 5B neurons. Together, our data provide new insights into the spatiotemporal and depth-dependent organisation of movement-specific signalling in mouse CSNs.

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BOARD NUMBER: S01-272

NEURAL BASIS OF ANTICIPATION AND PREMATURE IMPULSIVE ACTION IN THE FRONTAL CORTEX

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Planning and anticipating motor actions can increase behavioral performance[1], however, it can also lead to premature impulsive actions[2]. Although the anterior lateral motor cortex (ALM) is known to contribute to correct motor planning[3], it is currently unknown whether it is also involved in premature motor output. Here, this was addressed by using whole-cell patch clamp recordings from layer 2/3 (L2/3) pyramidal neurons within the ALM while mice were performing a cued-sensory association task. During both correct and premature performance in the task, a robust voltage response was evoked in L2/3 pyramidal neurons during the auditory cue. This cue-evoked voltage response was context dependent and greater during premature behavior. Optogenetically suppressing ALM during the cued-sensory association task lead to enhanced behavior, with fewer, and more delayed, premature responses and faster correct responses. Taken together, our findings suggest that ALM plays a critical role in anticipation by not only contributing to motor planning but by also driving premature actions. [1] Nobre AC, van Ede F (2018) Anticipated moments: temporal structure in attention. *Nat Rev Neurosci* 19, 34-48. [2] Kim S, Lee D (2011) Prefrontal cortex and impulsive decision making. *Biol Psychiatry* 69, 1140-1146. [3] Li N, Chen TW, Guo ZV, Gerfen CR, Svoboda K (2015) A motor cortex circuit for motor planning and movement. *Nature* 519, 51-56. Funding: NHMRC (APP1086082 and APP1063533, L.M.P), the Sylvia and Charles Viertel Charitable Foundation (L.M.P) and the Australian Research Council (L.M.P), IBRO (Return Home Fellowship and Early Career Award; R.G.) and MJJ fellowship (R.G.).

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BOARD NUMBER: S01-273

ACTIVITY IN MOUSE MOTOR CORTEX REFLECTS ACTION AND ITS LEARNED AUDITORY CONSEQUENCES

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Movements make sounds. It is essential to retain sensitivity to environmental sounds during behavior, amidst all the sounds produced by one's own actions. Consistent with this perceptual need, neural responses to self-generated sounds are suppressed in the auditory cortex (A1), which is thought to involve motor cortical inputs that engage local A1 inhibitory circuits. However, it remains unknown what signals are sent from motor cortex to A1 during sound-generating behaviors, nor is it known whether motor-cortical activity reflects expectations regarding the acoustic outcome of an action. To answer these questions, we trained mice to push a lever that makes a highly predictable, experimenter-controlled sound. We then made extracellular recordings from the secondary motor cortex (M2), paired with optogenetic identification of A1-projecting M2 cells. A majority of M2 neurons are active during lever presses and a large fraction of cells have peak firing rates tightly aligned to the self-generated tone. Compared to the entire M2 population, A1-projecting M2 cells are more likely to be active just prior to the tone and a sub-population of M2 cells also respond to passively played tones. Interestingly, during sound-generating movements, the activity of many M2 cells are not well predicted as a linear sum of the same cell's activity during silent movements and in response to passive tones. These data indicate that M2 neurons integrate signals related to movement and sound, that specific movement-related information is selectively routed to A1, and that aspects of M2 timing are shaped by motor-auditory experience.

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BOARD NUMBER: S01-274

SEVEN YEARS OF BAD LUCK: CRACKS IN THE LONGSTANDING MIRROR NEURON HYPOTHESIS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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The primate parietofrontal grasping network, which comprises the anterior intraparietal area (AIP), rostroventral premotor cortex (F5), and primary motor cortex (M1), canonically performs visuomotor transformations necessary for the planning and execution of dexterous hand movements. Embedded in this circuit is also thought to be an action observation system wherein mirror neurons process observed movements as though they were executed. To address substantial controversy surrounding this putative mirror neuron system, we leveraged a large object set and population analyses enabled by multi-array neuronal recordings. We find substantial activity in F5 and AIP during action observation, but fail to find evidence for distinct clusters of mirror and nonmirror neurons. Nonetheless, we identify a latent subspace which processes visual features of objects even in the absence of the intention to move, and another orthogonal subspace which captures patterns of movement-related activity that are identical across action execution and observation contexts. However, within all cortical areas spanned by the grasping network, this latter subspace lacks grip-specific patterns of activity. Moreover, the observation context *per se* lacks grip-specific information interpretable by a linear grip classifier. We conclude that existing hypotheses about the neural processing of observed action are in need of revisions which dispense with a privileged subset of mirror neurons and which unequivocally identify the constraints on the action specificity of observation-related activity.

Pubmed:
33200745: Suresh AK, Goodman JM, Okorokova EV, Kaufman M, Hatsopoulos NG, Bensmaia SJ
Neural population dynamics in motor cortex are different for reach and grasp.
Low-dimensional linear dynamics are observed in neuronal population activity in primary motor cortex (M1) when monkeys make reaching movements. This population-level behavior is consistent with a role for M1 as an autonomous pattern generator that drives muscles to give rise to movement. In the present study, we examine whether similar dynamics are also observed during grasping movements, which involve fundamentally different patterns of kinematics and muscle activations. Using a variety of analytical approaches, we show that M1 does not exhibit such dynamics during grasping movements. Rather, the grasp-related neuronal dynamics in M1 are similar to their counterparts in somatosensory cortex, whose activity is driven primarily by afferent inputs rather than by intrinsic dynamics. The basic structure of the neuronal activity underlying hand control is thus fundamentally different from that underlying arm control.
Elife, 2020; 9
31668844: Goodman JM, Tabot GA, Lee AS, Suresh AK, Rajan AT, Hatsopoulos NG, Bensmaia S
Postural Representations of the Hand in the Primate Sensorimotor Cortex.
Manual dexterity requires proprioceptive feedback about the state of the hand. To date, study of the neural basis of proprioception in the cortex has focused primarily on reaching movements to the exclusion of hand-specific behaviors such as grasping. To fill this gap, we record both time-varying hand kinematics and neural activity evoked in somatosensory and motor cortices as monkeys grasp a variety of objects. We find that neurons in the somatosensory cortex, as well as in the motor cortex, preferentially track time-varying postures of multi-joint combinations spanning the entire hand. This contrasts with neural responses during reaching movements, which preferentially track time-varying movement kinematics of the arm, such as velocity and speed of the limb, rather than its time-varying postural configuration. These results suggest different representations of arm and hand movements suited to the different functional roles of these two effectors.
Neuron, 2019; 104
28440308: Goodman JM, Bensmaia SJ
A Variation Code Accounts for the Perceived Roughness of Coarsely Textured Surfaces.
For decades, the dominant theory of roughness coding in the somatosensory nerves posited that perceived roughness was determined by the spatial pattern of activation in one population of tactile nerve fibers, namely slowly adapting type 1 (SA1) afferents. Indeed, the perceived roughness of coarsely textured surfaces tracks the spatial variation in SA1 responses - the

degree to which response strength varies across SA1 afferents. However, in a later study, the roughness of a different set of dot patterns was found to be a monotonic function of dot spacing, a result interpreted as evidence that roughness was determined by the strength of SA1 responses - the population firing rate - rather than their spatial layout. Then again, the spatial variation hypothesis was not tested directly as afferent responses to the conflicting patterns were not measured. To fill this gap, we simulated afferent responses to the dot patterns used in these roughness coding experiments using a model of skin mechanics. We then implemented the spatial variation and firing rate models of roughness based on these simulated responses to generate predictions of perceived roughness. We found that the spatial variation model accounts for perceived roughness under all tested conditions whereas the firing rate model does not.
Sci Rep, 2017; 7
22717526: Dougherty JB, Goodman JM, Knudsen EB, Moxon KA
Controlled unilateral isometric force generated by epidural spinal cord stimulation in the rat hindlimb.
Epidural electrical stimulation (EES) has often been used to restore stereotypic locomotor movements after spinal cord injury (SCI). However, restoring freeform movement requires specific force generation and independently controlled limbs for changing environments. Therefore, a second stimulus location would be advantageous, controlling force separately from locomotor movements. In normal and transected rats treated with mineral oil or saline, EES was performed at L1-L6 vertebral levels, caudal to spinal segments typical for locomotion, identifying secondary sites capable of activating hindlimb musculature, producing unilateral force at the paw. Threshold for generating force was identified and stimulation amplitude and duration varied to assess effects on evoked forces. Stimulation at L2 and L3 vertebral levels elicited negative vertical forces from extensor musculature while stimulation at L4 and L5 elicited positive vertical forces from flexion musculature. Thresholds were unchanged with transection or hydration method. Peak force magnitude was significantly correlated to stimulus amplitude, and response duration significantly correlated to stimulus duration in all animals. No differences were found in correlation coefficients or slopes of the regression for force or duration analyses with spinal condition or hydration method. This model demonstrates the ability to induce controlled forces with EES after SCI.
IEEE Trans Neural Syst Rehabil Eng, 2012; 20
32442987: Okorokova EV, Goodman JM, Hatsopoulos NG, Bensmaia SJ
Decoding hand kinematics from population responses in sensorimotor cortex during grasping.
The hand-a complex effector comprising dozens of degrees of freedom of movement-endows us with the ability to flexibly, precisely, and effortlessly interact with objects. The neural signals associated with dexterous hand movements in primary motor cortex (M1) and somatosensory cortex (SC) have received comparatively less attention than have those associated with proximal upper limb control.
J Neural Eng, 2020; 17
32678102: Yan Y, Goodman JM, Moore DD, Solla SA, Bensmaia SJ
Unexpected complexity of everyday manual behaviors.
How does the brain control an effector as complex and versatile as the hand? One possibility is that neural control is simplified by limiting the space of hand movements. Indeed, hand kinematics can be largely described within 8 to 10 dimensions. This oft replicated finding has been construed as evidence that hand postures are confined to this subspace. A prediction from this hypothesis is that dimensions outside of this subspace reflect noise. To address this question, we track the hand of human participants as they perform two tasks-grasping and signing in American Sign Language. We apply multiple dimension reduction techniques and replicate the finding that most postural variance falls within a reduced subspace. However, we show that dimensions outside of this subspace are highly structured and task dependent, suggesting they too are under volitional control. We propose that hand control occupies a higher dimensional space than previously considered.
Nat Commun, 2020; 11
31636297: Prendergast B, Brooks J, Goodman JM, Boyarinova M, Winberry JE, Bensmaia SJ
Finger Posture and Finger Load are Perceived Independently.
The ability to track the time-varying postures of our hands and the forces they exert plays a key role in our ability to dexterously interact with objects. However, how precisely and accurately we sense hand kinematics and kinetics has not been completely characterized. Furthermore, the dominant source of information about hand postures stems from muscle spindles, whose responses can also signal isometric force and are modulated by fusimotor input. As such, one might expect that changing the state of the muscles - for example, by applying a load - would influence perceived finger posture. To address these questions, we measure the acuity of human hand proprioception, investigate the interplay between kinematic and kinetic signals, and determine the extent to which actively and passively achieved postures are perceived differently. We find that angle and torque perception are highly precise; that loads imposed on the finger do not affect perceived joint angle; that joint angle does not affect perceived load; and that hand postures are perceived similarly whether they are achieved actively or passively. The independence of finger posture and load perception contrasts with their interdependence in the

upper arm, likely reflecting the special functional importance of the hand.
Sci Rep, 2019; 9

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THE LIMIT OF CROSSING FINGERS: WHEN SPATIAL REPRESENTATION FAILS TO FOLLOW BODY POSTURE

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Aristotle's illusion, a well-known tactile illusion, corresponds to the perception of two objects when crossed index and middle fingers are in contact with only one object. In this case, perceiving two objects rather than one involves spatial coding of skin parts not updated by the posture of the crossed fingers. This failure to take the postural configuration into account, when processing the two tactile sensations arising from the contact with the object, has been supposed to be caused by the unusual finger posture or the unusual simultaneous contact area (external part of the two fingers). However, these two potential explanations suggest different mechanisms: the first relies on failed updating of spatial body representation and the second on the failure of the spatial association between two tactile sensations. The aim of this study is to identify if Aristotle's illusion originates from a failure of body representation or tactile object processing. To do so, we assessed the spatial representation of both the skin and fingers using tactile and verbal instructions, respectively. Participants with eyes closed had to indicate where he/she localizes different targets (knuckles and nails) in three different hand configuration conditions: fingers apart, fingers together, crossed fingers. As there is no object perception in the task, a failure to update body representation -and accordingly individual tactile stimuli- in the finger crossed condition, would mean that a spatial body representation mechanism is the origin of the illusion. Indeed, preliminary data indicate that the Aristotle's illusion has not a pure somatosensory origin.

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MOTOR RESPONSES INDUCED BY INTRACORTICAL MICROSTIMULATION OF VENTRAL PREMOTOR MIRROR NEURONS

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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The premotor cortex is known to play a pivotal role in the processing of visuomotor actions. Previous studies have shown that it is possible to evoke mouth, hand and arm movements using intracortical microstimulation in premotor cortex. However, it is unknown to what extent motor responses can be evoked by microstimulation of mirror neurons in ventral premotor cortex (PMv). These neurons, which respond to both action execution and action observation, were discovered in area F5c of PMv. To investigate the causal role of PMv mirror neurons, we implanted 96-channel Utah arrays in area F5c in two macaque monkeys that were trained to perform a visually guided grasping task and to fixate videos of actions. We tested mirror activity during passive fixation of videos of a human or a monkey hand performing the same grasping task. In 33 sessions, we tested the motor responses when stimulating both mirror and non-mirror sites. Stimulation trains of biphasic pulses (duration 1 ms) were delivered at 100 Hz for 1000 ms at rest. We could evoke simple to complex movements of hand, arm and/or mouth with moderate currents, ranging from 60 to 130µA, in the large majority of both mirror and non-mirror sites. Furthermore, subthreshold microstimulation of F5c while the monkeys performed the visually guided delayed grasping task induced increased grasping times ($p < 0.05$), both in mirror (47%) and non-mirror (43%) sites. Together, these results have important implications for the interpretation of mirror neuron activity in PMv.

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MOVEMENT EXECUTION ERROR AND TASK-PERFORMANCE FEEDBACK INDUCE OPPOSITE PATTERNS OF BETA BAND ACTIVITY

POSTER SESSION 01 - SECTION: VOLUNTARY CONTROL OF MOVEMENT

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Beta-band modulations following reward, task outcome feedback and error have been observed in cognitive and motor tasks. Observations from different studies are, however, difficult to conciliate. Among studies using cognitive response selection task, several reported an increase in beta-band activity following reward, whereas others on the contrary observed increased beta-power after negative outcome feedback (i.e., task error). On the other hand, in motor adaptation tasks, movement-execution errors are followed by an attenuation of the post-movement beta-rebound. Considering that, in motor tasks, kinematic error typically leads to and thus is associated with task error (e.g., target missed), one may wonder how opposite patterns (decrease for movement error vs increase for task error) may coexist. In the present study, with the same healthy participants and within the same task, we separate beta-activity in medial parietal (precuneus) and medial frontal (SMA) regions using independent component analysis. Consistent with our previous results, we find that medial-parietal low-beta power is significantly decreased by kinematic errors but is not sensitive to task error. Whereas medial-frontal high-beta activity is significantly modulated by task error, but not significantly altered by kinematic error. In addition, we find that medial-frontal high-beta band activity is significantly modulated during two trial epochs. Shortly after (~250ms) task performance feedback, high-beta is increased in successful trials, whereas at a longer latency (~800ms) high-beta power increase is associated with task failure. Our findings allow conciliating seemingly contradictory observations reported in separate studies. We discuss complementary hypotheses that may explain the functionally distinct observed beta-band activities.

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EFFECTS OF EARLY LIFE STRESS IN AMYGDALA MICROCIRCUITRY AND SOCIO-AFFECTIVE BEHAVIOR: WHY SEX AND AGE MATTER

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

Aroa Mañas-Ojeda, Clara García Mompó, José Francisco Hidalgo-Cortés, Mónica Navarro Sánchez, Isis Gil-Miravet, Esther Castillo-Gómez
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Neglect is the most common form of child abuse. It has been related to serious socio-affective disorders that can emerge either immediately or later in life. Although the underlying neural mechanisms remain poorly understood, it has been demonstrated that the amygdala has an important role in mediating these behavioral effects. Moreover, little attention has been paid to whether males and females respond differently to similar situations of child social neglect and whether these differences can be observed at the microcircuitry level (excitatory and inhibitory synapses). We hypothesize that early life stress in mice will affect socio-affective behavior and the balance between excitatory and inhibitory (E/I) synapses in MeA, BLA and/or CeA. These effects might be observed in the short and long term and also be dependent on sex. Therefore, our main objective will be to demonstrate our hypothesis in an animal model of child social neglect (maternal separation with early weaning, MSEW). Pups from the MSEW group were daily separated from their mothers for 4-8h from P2-P16 and were definitely weaned at P17. Socio-affective behavior was evaluated by the Open Field Test, Forced Swimming Test, Three Chamber (sociability and novelty), and Tube Dominance test. Then, E/I balance was measured by immunofluorescence of VGLUT1, VGLUT2 and VGAT markers. The long-term consequences of early life stress on socio-affective behavior are sex-dependent and are correlated with imbalances between excitatory and inhibitory synapses in different nuclei of the amygdala. However, in the short term, only behavioral alterations were observed in both males and females.

Pubmed:

32823723: Mañas-Ojeda A, Ros-Bernal F, Olucha-Bordonau FE, Castillo-Gómez E
Becoming Stressed: Does the Age Matter? Reviewing the Neurobiological and Socio-Affective Effects of Stress throughout the Lifespan.
Social and affective relations occur at every stage of our lives. Impairments in the quality of this "social world" can be exceptionally detrimental and lead to psychopathology or pathological behavior, including schizophrenia, autism spectrum disorder, affective disorders, social phobia or violence, among other things. Exposure to highly stressful or traumatic events, depending on the stage of life in which stress exposure occurs, could severely affect limbic structures, including the amygdala, and lead to alterations in social and affective behaviors. This review summarizes recent findings from stress research and provides an overview of its age-dependent effects on the structure and function of the amygdala, which includes molecular and cellular changes, and how they can trigger deviant social and affective behaviors. It is important to highlight that discoveries in this field may represent a breakthrough both for medical science and for society, as they may help in the development of new therapeutic approaches and prevention strategies in neuropsychiatric disorders and pathological behaviors.
Int J Mol Sci, 2020; 21
34239421: García-Díaz C, Gil-Miravet I, Albert-Gasco H, Mañas-Ojeda A, Ros-Bernal F, Castillo-Gómez E, Gundlach AL, Olucha-Bordonau FE
Relaxin-3 Innervation From the Nucleus Incertus to the Parahippocampal Cortex of the Rat.
Spatial learning and memory processes depend on anatomical and functional interactions between the hippocampus and the entorhinal cortex. A key neurophysiological component of these processes is hippocampal theta rhythm, which can be driven from subcortical areas including the pontine (NI). The NI contains the largest population of neurons that produce and presumably release the neuropeptide, relaxin-3, which acts via the G -protein-coupled receptor, relaxin-family peptide 3 receptor (RXFP3). NI activation induces general arousal including hippocampal theta, and inactivation induces impairment of spatial memory acquisition or retrieval. The primary aim of this study was to map the NI/relaxin-3 innervation of the parahippocampal cortex (PHC), including the medial and lateral entorhinal cortex, endopiriform cortex, perirhinal, postrhinal, and ectorhinal cortex, the amygdalohippocampal transition area and posteromedial cortical amygdala. Retrograde tracer

injections were placed in different parts of the medial and lateral entorhinal cortex, which produced prominent retrograde labeling in the ipsilateral NI and some labeling in the contralateral NI. Anterograde tracer injections into the NI and immunostaining for relaxin-3 produced fiber labeling in deep layers of all parahippocampal areas and some dispersed fibers in superficial layers. Double-labeling studies revealed that both hippocampal projecting and calcium-binding protein-positive (presumed GABAergic) neurons received a relaxin-3 NI innervation. Some of these fibers also displayed synaptophysin (Syn) immunoreactivity, consistent with the presence of the peptide at synapses; and relaxin-3-positive fibers containing Syn bouton-like staining were frequently observed in contact with hippocampal-projecting or calcium-binding protein-positive neuronal somata and more distal elements. Finally, hybridization studies revealed that entorhinal neurons in the superficial layers, and to a lesser extent in deep layers, contain RXFP3 mRNA. Together, our data support functional actions of the NI/relaxin-3-parahippocampal innervation on processes related to memory, spatial navigation and contextual analysis.
Front Neuroanat, 2021; 15

33867946: Gil-Miravet I, Mañas-Ojeda A, Ros-Bernal F, Castillo-Gómez E, Albert-Gascó H, Gundlach AL, Olucha-Bordonau FE

Involvement of the and Relaxin-3/RXFP3 Signaling System in Explicit and Implicit Memory.

Telencephalic cognitive and emotional circuits/functions are strongly modulated by subcortical inputs. The main focus of past research on the nature of this modulation has been on the widespread monoamine projections to the telencephalon. However, the (NI) of the pontine tegmentum provides a strong GABAergic and peptidergic innervation of the hippocampus, basal forebrain, amygdala, prefrontal cortex, and related regions; and represents a parallel source of ascending modulation of cognitive and emotional domains. NI GABAergic neurons express multiple peptides, including neuromedin-B, cholecystokinin, and relaxin-3, and receptors for stress and arousal transmitters, including corticotrophin-releasing factor and orexins/hypocretins. A functional relationship exists between NI neurons and their associated peptides, relaxin-3 and neuromedin-B, and hippocampal theta rhythm, which in turn, has a key role in the acquisition and extinction of declarative and emotional memories. Furthermore, RXFP3, the cognate receptor for relaxin-3, is a G protein-coupled receptor, and its activation inhibits the cellular accumulation of cAMP and induces phosphorylation of ERK, processes associated with memory formation in the hippocampus and amygdala. Therefore, this review summarizes the role of NI transmitter systems in relaying stress- and arousal-related signals to the higher neural circuits and processes associated with memory formation and retrieval.
Front Neuroanat, 2021; 15

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BOARD NUMBER: S01-279

COMPARING EFFICACY OF SSRIS TO AMELIORATE ANXIETY-LIKE BEHAVIOUR INDUCED EITHER BY VENTROMEDIAL PREFRONTAL OVERACTIVATION OR OCCURRING NATURALLY IN TRAIT-ANXIOUS MARMOSETS

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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Extensive individual variability in pharmacological treatment efficacy remains a persistent obstacle in ameliorating clinical anxiety. The most common first-line treatments are selective serotonin reuptake inhibitors (SSRIs), but these typically show anxiolytic action only following weeks of treatment. More recently though, individual differences in the efficacy of acute SSRIs on aversive processing associated with anxiety has been demonstrated (Bhagwagar et al., 2004; Di Simplicio et al., 2014). Moreover, in marmoset monkeys (*Callithrix jacchus)* we have shown anxiolytic effects of an acute dose of the SSRI citalopram, that are genotype dependent (Santangelo et al., 2016). Here, we investigated further the contexts in which an acute SSRI may have anxiolytic effects, by comparing their efficacy in state-anxiety, induced by overactivation of area 14 in ventromedial (vm)PFC, and in naturally occurring high trait-anxiety. The human-intruder test measures the behavioural reactivity to an unfamiliar human; the human in this context being an uncertain stimulus provoking an anxiety-like response. Overactivation of area 14 via intracerebral infusion of dihydrokainic acid, a glutamate transporter (GLT-1) inhibitor has been shown to exacerbate anxiety within this paradigm (Stawicka et al., 2020). The efficacy of an acute, systemic dose of 10 mg/kg citalopram to reduce anxiety-like behaviour was compared in state and trait anxiety. Although the precise behavioural expression of anxiety differed between the two contexts, both were ameliorated by acute citalopram. Preliminary comparisons with alternative anxiolytics, including ketamine and a kappa-opioid antagonist, support the notion of a unique anxiolytic profile of citalopram on anxiety behaviour induced by uncertainty.

*Research funded by Medical Research Council (MRC-MR/V033492/1) of the United Kingdom.

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BOARD NUMBER: S01-280

CHARACTERIZATION OF DISEASE-ASSOCIATED MICROGLIA IN SOCIAL DEFICITS LINKED TO EARLY LIFE ADVERSITY

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

A. Catarina Rodrigues-Neves[1], Rita Gaspar[1], Patrícia Patrício[2], Luísa Pinto[2], João Bessa[2], António F. Ambrósio[1], Catarina A. Gomes[1]
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Background: Synaptogenesis during brain development, which extends until adolescence, implicates microglia, innate immune cells able to screen and select newborn synapses to be kept or eliminated. The screening ability is mainly supported by dynamic morphologic alterations at the level of microglia cellular processes, that contact synapses, sense (via receptor activation) their functioning level and eventually phagocyte abnormal synaptic elements. A disturbance of microglia morphology during critical periods of development, as occurs in early life adversity (ELA) may compromise brain circuits and behavior (e.g. social impairment). Here, we hypothesize that social deficits associated with ELA are correlated with particular disease-associated microglia (DAM) phenotypes yet unidentified. **Methods:** Neurodevelopmental (infancy), social behavioral tests (adolescence) and microglia morphometry (in the *nucleus accumbens*, brain region involved in social behavior) were conducted in the offspring of pregnant rats administered with methylazoxymethanol acetate (MAM). **Results:** In postnatal period, MAM offspring presented alterations in neurodevelopmental milestones and, at adolescence, social deficits. DAM arise as early as postnatal phase, although following an age-dependent pattern of evolution characterized by a postnatal increase in complexity and a shift towards a microglia atrophy. **Conclusion:** Atrophic DAM observed at adolescence lead us to anticipate that a possible dysfunctional phagocytosis might contribute for the social deficits, a hypothesis that needs to be clarified. Foundation for Science and Technology (FCT), Portugal (Strategic Projects UID/NEU/04539/2019, UIDB/04539/2020 and UIDP/04539/2020), and COMPETE-FEDER (POCI-01-0145-FEDER-007440). Centro2020 Regional Operational Programme (CENTRO-01-0145-FEDER-000008: BrainHealth 2020). Faculty of Medicine of the University of Coimbra and Santander Totta Bank (FMUC-BST-2019).

Pubmed:
28981089: Boia R, Elvas F, Madeira MH, Aires ID, Rodrigues-Neves AC, Tralhão P, Szabó EC, Baqi Y, Müller CE, Tomé ÂR, Cunha RA, Ambrósio AF, Santiago AR
Treatment with A receptor antagonist KW6002 and caffeine intake regulate microglia reactivity and protect retina against transient ischemic damage.
Transient retinal ischemia is a major complication of retinal degenerative diseases and contributes to visual impairment and blindness. Evidences indicate that microglia-mediated neuroinflammation has a key role in the neurodegenerative process, prompting the hypothesis that the control of microglia reactivity may afford neuroprotection to the retina against the damage induced by ischemia-reperfusion (I-R). The available therapeutic strategies for retinal degenerative diseases have limited potential, but the blockade of adenosine A receptor (AR) emerges as candidate strategy. Therefore, we evaluated the therapeutic potential of a selective AR antagonist (KW6002) against the damage elicited by I-R. The administration of KW6002 after I-R injury reduced microglia reactivity and inflammatory response and afforded protection to the retina. Moreover, we tested the ability of caffeine, an adenosine receptor antagonist, in mediating protection to the retina in the I-R injury model. We demonstrated that caffeine administration dually regulated microglia reactivity and cell death in the transient retinal ischemic model, depending on the reperfusion time. At 24 h of reperfusion, caffeine increased microglial reactivity, inflammatory response and cell death elicited by I-R. However, at 7 days of reperfusion, caffeine administration decreased microglia reactivity and reduced the levels of proinflammatory cytokines and cell death. Together, these results provide a novel evidence for the use of adenosine AR antagonists as potential therapy for retinal ischemic diseases and demonstrate the effect of caffeine on the regulation of microglia-mediated neuroinflammation in the transient ischemic model.
Cell Death Dis, 2017; 8
29416510: Rodrigues-Neves AC, Aires ID, Vindeirinho J, Boia R, Madeira MH, Gonçalves FQ, Cunha RA, Santos PF, Ambrósio AF, Santiago AR

Elevated Pressure Changes the Purinergic System of Microglial Cells.
Glaucoma is the second cause of blindness worldwide and is characterized by the degeneration of retinal ganglion cells (RGCs) and optic nerve atrophy. Increased microglia reactivity is an early event in glaucoma that may precede the loss of RGCs, suggesting that microglia and neuroinflammation are involved in the pathophysiology of this disease. Although global changes of the purinergic system have been reported in experimental and human glaucoma, it is not known if this is due to alterations of the purinergic system of microglial cells, the resident immune cells of the central nervous system. We now studied if elevated hydrostatic pressure (EHP), mimicking ocular hypertension, changed the extracellular levels of ATP and adenosine and the expression, density and activity of enzymes, transporters and receptors defining the purinergic system. The exposure of the murine microglial BV-2 cell line to EHP increased the extracellular levels of ATP and adenosine, increased the density of ecto-nucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1, CD39) and decreased the density of the equilibrative nucleotide transporter 2 as well as the activity of adenosine deaminase. The expression of adenosine A receptor also decreased, but the adenosine A receptor was not affected. Notably, ATP and adenosine selectively control migration rather than phagocytosis, both bolstered by EHP. The results show that the purinergic system is altered in microglia in conditions of elevated pressure. Understanding the impact of elevated pressure on the purinergic system will help to unravel the mechanisms underlying inflammation and neurodegeneration associated with glaucoma.
Front Pharmacol, 2018; 9
30612332: Chiquita S, Rodrigues-Neves AC, Baptista FI, Carecho R, Moreira PI, Castelo-Branco M, Ambrósio AF
The Retina as a Window or Mirror of the Brain Changes Detected in Alzheimer's Disease: Critical Aspects to Unravel.
Alzheimer's disease is the most frequent cause of dementia worldwide, representing a global health challenge, with a massive impact on the quality of life of Alzheimer's disease patients and their relatives. The diagnosis of Alzheimer's disease constitutes a real challenge, because the symptoms manifest years after the first degenerative changes occurring in the brain and the diagnosis is based on invasive and/or expensive techniques. Therefore, there is an urgent need to identify new reliable biomarkers to detect Alzheimer's disease at an early stage. Taking into account the evidence for visual deficits in Alzheimer's disease patients, sometimes even before the appearance of the first disease symptoms, and that the retina is an extension of the brain, the concept of the retina as a window to look into the brain or a mirror of the brain has received increasing interest in recent years. However, only a few studies have assessed the changes occurring in the retina and the brain at the same time points. Unlike previous reviews on this subject, which are mainly focused on brain changes, we organized this review by comprehensively summarizing findings related with structural, functional, cellular, and molecular parameters in the retina reported in both Alzheimer's disease patients and animal models. Moreover, we separated the studies that assessed only the retina, and those that assessed both the retina and brain, which are few but allow establishing correlations between the retina and brain. This review also highlights some inconsistent results in the literature as well as relevant missing gaps in this field.
Mol Neurobiol, 2019; 56
30667095: Aires ID, Boia R, Rodrigues-Neves AC, Madeira MH, Marques C, Ambrósio AF, Santiago AR
Blockade of microglial adenosine A receptor suppresses elevated pressure-induced inflammation, oxidative stress, and cell death in retinal cells.
Glaucoma is a retinal degenerative disease characterized by the loss of retinal ganglion cells and damage of the optic nerve. Recently, we demonstrated that antagonists of adenosine A receptor (A R) control retinal inflammation and afford protection to rat retinal cells in glaucoma models. However, the precise contribution of microglia to retinal injury was not addressed, as well as the effect of A R blockade directly in microglia. Here we show that blocking microglial A R prevents microglial cell response to elevated pressure and it is sufficient to protect retinal cells from elevated pressure-induced death. The A R antagonist SCH 58261 or the knockdown of A R expression with siRNA in microglial cells prevented the increase in microglia response to elevated hydrostatic pressure. Furthermore, in retinal neural cell cultures, the A R antagonist decreased microglia proliferation, as well as the expression and release of pro-inflammatory mediators. Microglia ablation prevented neural cell death triggered by elevated pressure. The A R blockade recapitulated the effects of microglia depletion, suggesting that blocking A R in microglia is able to control neurodegeneration in glaucoma-like conditions. Importantly, in human organotypic retinal cultures, A R blockade prevented the increase in reactive oxygen species and the morphological alterations in microglia triggered by elevated pressure. These findings place microglia as the main contributors for retinal cell death during elevated pressure and identify microglial A R as a therapeutic target to control retinal neuroinflammation and prevent neural apoptosis elicited by elevated pressure.
Glia, 2019; 67
30816415: Chiquita S, Ribeiro M, Castelhano J, Oliveira F, Sereno J, Batista M, Abrunhosa A, Rodrigues-Neves AC, Carecho R, Baptista F, Gomes C, Moreira PI, Ambrósio AF, Castelo-Branco M
A longitudinal multimodal in vivo molecular imaging study of the 3xTg-AD mouse model shows progressive early hippocampal and taurine loss.

The understanding of the natural history of Alzheimer's disease (AD) and temporal trajectories of in vivo molecular mechanisms requires longitudinal approaches. A behavioral and multimodal imaging study was performed at 4/8/12 and 16 months of age in a triple transgenic mouse model of AD (3xTg-AD). Behavioral assessment included the open field and novel object recognition tests. Molecular characterization evaluated hippocampal levels of amyloid β (Aβ) and hyperphosphorylated tau. Magnetic resonance imaging (MRI) included assessment of hippocampal structural integrity, blood-brain barrier (BBB) permeability and neurospectroscopy to determine levels of the endogenous neuroprotector taurine. Longitudinal brain amyloid accumulation was assessed using 11C Pittsburgh compound B positron emission tomography (PET), and neuroinflammation/microglia activation was investigated using 11C-PK1195. We found altered locomotor activity at months 4/8 and 16 months and recognition memory impairment at all time points. Substantial early reduction of hippocampal volume started at month 4 and progressed over 8/12 and 16 months. Hippocampal taurine levels were significantly decreased in the hippocampus at months 4/8 and 16. No differences were found for amyloid and neuroinflammation with PET, and BBB was disrupted only at month 16. In summary, 3xTg-AD mice showed exploratory and recognition memory impairments, early hippocampal structural loss, increased Aβ and hyperphosphorylated tau and decreased levels of taurine. In sum, the 3xTg-AD animal model mimics pathological and neurobehavioral features of AD, with early-onset recognition memory loss and MRI-documented hippocampal damage. The early-onset profile suggests temporal windows and opportunities for therapeutic intervention, targeting endogenous neuroprotectors such as taurine.
Hum Mol Genet, 2019; 28

31748653: Aires ID, Madeira MH, Boia R, Rodrigues-Neves AC, Martins JM, Ambrósio AF, Santiago AR
Intravitreal injection of adenosine A receptor antagonist reduces neuroinflammation, vascular leakage and cell death in the retina of diabetic mice.
Diabetic retinopathy is a major complication of diabetes mellitus and a leading cause of blindness. The pathogenesis of diabetic retinopathy is accompanied by chronic low-grade inflammation. Evidence shows that the blockade of adenosine A receptors (AR) affords protection to the retina through the control of microglia-mediated neuroinflammation. Herein, we investigated the therapeutic potential of an antagonist of AR in a model of diabetic retinopathy. Type 1 diabetes was induced in 4-5 months old C57BL/6 J mice with a single intraperitoneal injection streptozotocin. Animals were treated one month after the onset of diabetes. The AR antagonist was delivered by intravitreal injection once a week for 4 weeks. Microglia reactivity and inflammatory mediators were increased in the retinas of diabetic animals. The treatment with the AR antagonist was able to control microglial reactivity and halt neuroinflammation. Furthermore, the AR antagonist rescued retinal vascular leakage, attenuated alterations in retinal thickness, decreased retinal cell death and the loss of retinal ganglion cells induced by diabetes. These results demonstrate that intravitreal injection of the AR antagonist controls inflammation, affords protection against cell loss and reduces vascular leakage associated with diabetes, which could be envisaged as a therapeutic approach for the early complications of diabetes in the retina.
Sci Rep, 2019; 9

32012676: Salobrar-García E, Rodrigues-Neves AC, Ramírez AI, de Hoz R, Fernández-Albarral JA, López-Cuenca I, Ramírez JM, Ambrósio AF, Salazar JJ
Microglial Activation in the Retina of a Triple-Transgenic Alzheimer's Disease Mouse Model (3xTg-AD).
Alzheimer's disease (AD) is the most common type of dementia in the world. The main biomarkers associated with AD are protein amyloid-β (Aβ) plaques and protein tau neurofibrillary tangles, which are responsible for brain neuroinflammation mediated by microglial cells. Increasing evidence has shown that the retina can also be affected in AD, presenting some molecular and cellular changes in the brain, such as microglia activation. However, there are only a few studies assessing such changes in the retinal microglia in animal models of AD. These studies use retinal sections, which have some limitations. In this study, we performed, for the first time in a triple-transgenic AD mouse model (3xTg-AD), a quantitative morphometric analysis of microglia activation (using the anti-Iba-1 antibody) in retinal whole-mounts, allowing visualization of the entire microglial cell, as well as its localization along the extension of the retina in different layers. Compared to age-matched animals, the retina of 3xTg-AD mice presents a higher number of microglial cells and a thicker microglial cell body area. Moreover, the microglia migrate, reorient, and retract their processes, changing their localization from a parallel to a perpendicular position relative to the retinal surface. These findings demonstrate clear microglia remodeling in the retina of 3xTg-AD mice.
Int J Mol Sci, 2020; 21

32109489: Santiago AR, Madeira MH, Boia R, Aires ID, Rodrigues-Neves AC, Santos PF, Ambrósio AF
Keep an eye on adenosine: Its role in retinal inflammation.
Adenosine is an endogenous purine nucleoside ubiquitously distributed throughout the body that interacts with G protein-coupled receptors, classified in four subtypes: AR, AR, AR and AR. Among the plethora of functions of adenosine, it has been increasingly recognized as a key mediator of the immune response. Neuroinflammation is a feature of chronic neurodegenerative diseases and contributes to the pathophysiology of several retinal degenerative diseases. Animal models

of retinal diseases are helping to elucidate the regulatory roles of adenosine receptors in the development and progression of those diseases. Mounting evidence demonstrates that the adenosinergic system is altered in the retina during pathological conditions, compromising retinal physiology. This review focuses on the roles played by adenosine and the elements of the adenosinergic system (receptors, enzymes, transporters) in the neuroinflammatory processes occurring in the retina. An improved understanding of the molecular and cellular mechanisms of the signalling pathways mediated by adenosine underlying the onset and progression of retinal diseases will pave the way towards the identification of new therapeutic approaches.
Pharmacol Ther, 2020; 210
33606195: Rodrigues-Neves AC, Carecho R, Correia SC, Carvalho C, Campos EJ, Baptista FI, Moreira PI, Ambrósio AF
Retina and Brain Display Early and Differential Molecular and Cellular Changes in the 3xTg-AD Mouse Model of Alzheimer's Disease.
The concept 'the retina as a window to the brain' has been increasingly explored in Alzheimer´s disease (AD) in recent years, since some patients present visual alterations before the first symptoms of dementia. The retina is an extension of the brain and can be assessed by noninvasive methods. However, assessing the retina for AD diagnosis is still a matter of debate. Using the triple transgenic mouse model of AD (3xTg-AD; males), this study was undertaken to investigate whether the retina and brain (hippocampus and cortex) undergo similar molecular and cellular changes during the early stages (4 and 8 months) of the pathology, and if the retina can anticipate the alterations occurring in the brain. We assessed amyloid-beta (Aβ) and hyperphosphorylated tau (p-tau) levels, barrier integrity, cell death, neurotransmitter levels, and glial changes. Overall, the retina, hippocampus, and cortex of 3xTg-AD are not significantly affected at these early stages. However, we detected a few differential changes in the retina and brain regions, and particularly a different profile in microglia branching in the retina and hippocampus, only at 4 months, where the number and length of the processes decreased in the retina and increased in the hippocampus. In summary, at the early stages of pathology, the retina, hippocampus, and cortex are not significantly affected but already present some molecular and cellular alterations. The retina did not mirror the changes detected in the brain, and these observations should be taking into account when using the retina as a potential diagnostic tool for AD.
Mol Neurobiol, 2021; 58
34120349: Ferreira AS, Galvão S, Gaspar R, Rodrigues-Neves AC, Ambrósio AF, Matafome P, Gomes CA, Baptista FI
Sex-specific changes in peripheral metabolism in a model of chronic anxiety induced by prenatal stress.
Prenatal stress is associated with increased susceptibility to psychiatric and metabolic disorders later in life. Prenatal exposure to stress mediators may have sex-dependent effects on offspring brain and metabolic function, promoting a sex-specific vulnerability to psychopathology and metabolic alterations at adulthood. In this work, the impact of prenatal stress on glucose homeostasis and peripheral metabolism of male and female offspring was investigated in a chronic anxiety animal model.
Eur J Clin Invest, 2021; 51

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BASAL AMYGDALA-NUCLEUS ACCUMBENS GLUTAMATE NEURONS ARE IMPORTANT FOR REWARD BEHAVIOUR AND BOTH ARE DYSREGULATED BY CHRONIC SOCIAL STRESS IN MICE

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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[1]University of Zürich, Psychiatry, Psychotherapy, Psychosomatics, Zürich, Switzerland, [2]University of Zurich, Brain Research Institute, Zürich, Switzerland, [3]University of Zürich, Institute Of Pharmacology And Toxicology, Zürich, Switzerland, [4]Boerhinger Ingelheim, Cns Diseases, Ingelheim am Rhein, Germany, [5]Boerhinger Ingelheim, Global Computational Biology And Digital Sciences, Ingelheim am Rhein, Germany, [6]Boehringer Ingelheim Pharma GmbH & Co. KG, Cns Diseases, Biberach an der Riss, Germany

Reduced responsiveness to rewarding stimuli is a common and major symptom in stress-related neuropsychiatric disorders such as depression and schizophrenia. The amygdala is a major region in neural circuitries of aversion and reward processing. The basal amygdala (BA) contains both reward- and aversion-responsive glutamate pyramidal neurons, some of which project to the nucleus accumbens. In young-adult male mice, chronic social stress (CSS) resulted in attenuated discriminative reward learning (DRLM) and reward-to-effort valuation (REV) compared to control mice (CON). Chronic tetanus toxin-mediated inhibition of BA-NAc glutamate pyramidal neurons led to similar deficits in DRLM and REV behaviour. Using fibre photometry of GCaMP6-expressing BA-NAc neurons, the CSS-induced deficits in the DRL and REV tests co-occurred with increased BA-NAc neuron activity at reward reinforcement. Using laser capture microdissection and RNA-Sequencing to assess CSS effects on the transcriptome of BA-NAc neuron samples, dysregulated genes were over-expressed in CSS compared to CON mice and a number of these were enriched in neurotransmitter signal transduction pathways. Based on the behavioural, fibre photometry and transcriptome evidence for CSS effects, mice underwent chronic chemogenetic activation of BA-NAc neurons and reward behaviour testing: chronic BA-NAc neuron activation recapitulated the effects of CSS in the DRLM and REV tests. These iterative findings identify the importance of the homeostatic functioning of BA-NAc glutamate pyramidal neurons for typical reward-directed behaviour and the restoration of such functioning as a therapeutic target for stress-induced deficits in reward responsiveness.

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IDENTIFICATION OF A CORE IMMUNE SIGNALLING-ASSOCIATED DYSREGULATION IN AN ISOLATION REARING MODEL OF NEUROPSYCHIATRIC ILLNESS.

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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Aims: Treatment and diagnosis of neuropsychiatric illnesses, such as schizophrenia, are complicated by the fact that each patient experiences a different combination of symptoms along with considerable differences in response to therapeutics. This creates a unique unmet need for novel treatment options beyond current strategies. This study aimed to identify potential targets for future therapeutic exploitation using an established model of neuropsychiatric disease. *Methods:* We utilised transcriptomic analysis of mRNA expression change in the isolation reared rodent model along with *in-vitro* investigation of identified gene clusters using a co-culture model of neurons and microglia. *Results:* We have correlated the emergence of behavioural, neurochemical and synapse ultrastructure deficits to transcriptional dysregulation in both the medial prefrontal cortex and dentate gyrus of the hippocampus of Wistar rats reared in isolation. A temporal map of sequential dysregulation across key life stages in both brain regions has been established, allowing a unique insight into the potential molecular cascade underlying disease emergence. The temporally altered genes were from a wide range of functional domains including transcriptional regulation, synaptic structure and function and, strikingly, immune-related signalling. Our *in-vitro* characterisation of members of this latter gene cluster suggests that altered expression of such immune-related genes may contribute to aberrant synaptic pruning, thought to be a key deficit underpinning the symptoms of schizophrenia. *Conclusions:* Further dissection of immune-related gene expression alterations, identified to be common to both brain regions, may reveal key insights into neuronal circuitry dysregulation and subsequent emergence of neurocognitive and psychotic symptoms of schizophrenia.

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NICOTINIC RECEPTORS PROMOTE SUSCEPTIBILITY TO SOCIAL STRESS IN FEMALE MICE LINKED WITH NEUROADAPTATIONS WITHIN VTA DOPAMINE NEURONS

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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There are about twice as many women as men who experience depression during their lifetime. Although life circumstances and especially exposure to stressful situations constitute a major risk factor to develop depression, the underlying mechanisms have yet to be unraveled. We employed the chronic social defeat procedure to elicit depressive-like symptoms in females and ketamine to validate the model. We performed *ex-vivo* patch clamp recordings to assess cellular adaptations and used pharmacological agents to dissect these deregulations. Chronic social defeat exposure triggers a hyperactivity of VTA dopamine (DA) neurons, in females susceptible to stress but not resilient ones. This hyperactivity was fully reversed by a single administration of ketamine. In virally-identified brain circuits of both susceptible and resilient females, we found a hypercholinergic tone to the VTA arising from the laterodorsal tegmentum. Application of puffs of nicotine revealed a decreased sensitivity of DA neurons in resilient mice when compared to naive or susceptible ones. The *in vivo* acute administration of the positive allosteric modulator for a7 nicotinic acetylcholine receptors (nAChRs) not only increased susceptibility to stress by enhancing activity of VTA DA neurons, but also triggered a switch in phenotype from resilient to susceptible. Our data unravel dysregulation of VTA DA neurons activity exclusively in females exhibiting depressive-like symptoms and identify VTA nAChRs mediates susceptibility vs. resilience to social stress in female mice.

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EFFECTS OF SOCIAL ISOLATION STRESS AND KETOGENIC DIET ON MICE BEHAVIOR AND METABOLISM

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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While current antidepressants do not help up to 40% of patients with depression, other approaches are developing, including distinct diets. Here we estimated beneficial effect of ketogenic diet in a mice model of depression. Adult male and female mice were fed with one of the three diets during 8 weeks: normal diet (energy profile – 55% carbohydrates : 32% proteins : 13% fats), medium chain fatty acid-based ketogenic high-fat low-carb diet (KD, 3% C : 7% P : 90% F), non-ketogenic high-fat low-carb diet (LCD, 17% C : 23% P : 60% F). Half of each group underwent social isolation stress model of depression along with diet to explore effect of nutrition profile on stress-induced disturbances. During weeks 5-6 set of behavioral tests was performed to estimate depression-like behavior (sucrose preference test, tail suspension), anxiety (elevated plus maze, light-dark box), communication (social interaction test) and spatial memory (Barnes maze). After sacrifice blood, brain and fat tissue were extracted for histological and biochemical studies. KD decreased body weight only in males regardless of stress, while no changes were found for LCD and in all female groups. Behavioral tests showed that social isolation model is not severe enough to induce significant anxiety, depressive-like behavior and spatial memory disturbances in mice. However, in males but not in females, stress resulted in suppressed communication which in turn was restored by KD. Adult neurogenesis in hippocampus and metabolic factors in fat and blood samples and are planned to be analyzed further.

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EFFECT OF EARLY-LIFE STRESS ON THE FUNCTIONAL DEVELOPMENT OF RAPHE-PREFRONTAL NETWORKS

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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Exposure to early-life stress (ELS), such as inadequate maternal care, has been shown to increase anxiety and depression-like behavior in adult rodents. There are indications that these adverse experiences elicit developmental dysregulation of the dorsal raphe (DR) 5-HT system, resulting in elevated 5-HT levels in limbic projection areas, including the medial prefrontal cortex (mPFC). However, it is not yet known how ELS affects the activity of developing 5-HTergic neurons in the DR and the maturation of functional interactions within raphe-prefrontal networks. **Aims:** To investigate how ELS affects the firing activity of neonatal putative 5-HT neurons in the DR and the development of functional interactions between DR and mPFC. **Methods:** We induced stress between postnatal day (P) 4 and P9 in mice with a paradigm that limits the amount of bedding and nesting (LBN) and has been shown to cause fragmented and unpredictable maternal behavior. We performed multi-unit activity and local-field potential recordings simultaneously in DR and mPFC after LBN at P10/P11. We analyzed the firing activity in DR after ELS compared to controls as well as functional interactions within raphe-prefrontal networks. **Results:** Preliminary results indicate that LBN alters the firing activity of putative 5-HTergic neurons in DR and affects functional interactions between DR and mPFC. **Conclusions:** Our results highlight the importance of the 5-HT system in the functional development of networks underlying emotional behavior.

Pubmed:
31555093: Teppola H, Aćimović J, Linne ML
Unique Features of Network Bursts Emerge From the Complex Interplay of Excitatory and Inhibitory Receptors in Rat Neocortical Networks.
Spontaneous network activity plays a fundamental role in the formation of functional networks during early development. The landmark of this activity is the recurrent emergence of intensive time-limited network bursts (NBs) rapidly spreading across the entire dissociated culture . The main excitatory mediators of NBs are glutamatergic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and -Methyl-D-aspartic-acid receptors (NMDARs) that express fast and slow ion channel kinetics, respectively. The fast inhibition of the activity is mediated through gamma-aminobutyric acid type A receptors (GABARs). Although the AMPAR, NMDAR and GABAR kinetics have been biophysically characterized in detail at the monosynaptic level in a variety of brain areas, the unique features of NBs emerging from the kinetics and the complex interplay of these receptors are not well understood. The goal of this study is to analyze the contribution of fast GABARs on AMPAR- and NMDAR- mediated spontaneous NB activity in dissociated neonatal rat cortical cultures at 3 weeks The networks were probed by both acute and gradual application of each excitatory receptor antagonist and combinations of acute excitatory and inhibitory receptor antagonists. At the same time, the extracellular network-wide activity was recorded with microelectrode arrays (MEAs). We analyzed the characteristic NB measures extracted from NB rate profiles and the distributions of interspike intervals, interburst intervals, and electrode recruitment time as well as the similarity of spatio-temporal patterns of network activity under different receptor antagonists. We show that NBs were rapidly initiated and recruited as well as diversely propagated by AMPARs and temporally and spatially maintained by NMDARs. GABARs reduced the spiking frequency in AMPAR-mediated networks and dampened the termination of NBs in NMDAR-mediated networks as well as slowed down the recruitment of activity in all networks. Finally, we show characteristic super bursts composed of slow NBs with highly repetitive spatio-temporal patterns in gradually AMPAR blocked networks. To the best of our knowledge, this study is the first to unravel in detail how the three main mediators of synaptic transmission uniquely shape the NB characteristics, such as the initiation, maintenance, recruitment and termination of NBs in cortical cell cultures .
Front Cell Neurosci, 2019; 13
29765315: Manninen T, Aćimović J, Havela R, Teppola H, Linne ML
Challenges in Reproducibility, Replicability, and Comparability of Computational Models and Tools for Neuronal and Glial Networks, Cells, and Subcellular Structures.
The possibility to replicate and reproduce published research results is one of the biggest challenges in all areas of science.

In computational neuroscience, there are thousands of models available. However, it is rarely possible to reimplement the models based on the information in the original publication, let alone rerun the models just because the model implementations have not been made publicly available. We evaluate and discuss the comparability of a versatile choice of simulation tools: tools for biochemical reactions and spiking neuronal networks, and relatively new tools for growth in cell cultures. The replicability and reproducibility issues are considered for computational models that are equally diverse, including the models for intracellular signal transduction of neurons and glial cells, in addition to single glial cells, neuron-glia interactions, and selected examples of spiking neuronal networks. We also address the comparability of the simulation results with one another to comprehend if the studied models can be used to answer similar research questions. In addition to presenting the challenges in reproducibility and replicability of published results in computational neuroscience, we highlight the need for developing recommendations and good practices for publishing simulation tools and computational models. Model validation and flexible model description must be an integral part of the tool used to simulate and develop computational models. Constant improvement on experimental techniques and recording protocols leads to increasing knowledge about the biophysical mechanisms in neural systems. This poses new challenges for computational neuroscience: extended or completely new computational methods and models may be required. Careful evaluation and categorization of the existing models and tools provide a foundation for these future needs, for constructing multiscale models or extending the models to incorporate additional or more detailed biophysical mechanisms. Improving the quality of publications in computational neuroscience, enabling progressive building of advanced computational models and tools, can be achieved only through adopting publishing standards which underline replicability and reproducibility of research results.
Front Neuroinform, 2018; 12

26518675: Teppola H, Sarkanen JR, Jalonen TO, Linne ML
Morphological Differentiation Towards Neuronal Phenotype of SH-SY5Y Neuroblastoma Cells by Estradiol, Retinoic Acid and Cholesterol.
Human SH-SY5Y neuroblastoma cells maintain their potential for differentiation and regression in culture conditions. The induction of differentiation could serve as a strategy to inhibit cell proliferation and tumor growth. Previous studies have shown that differentiation of SH-SY5Y cells can be induced by all-trans-retinoic-acid (RA) and cholesterol (CHOL). However, signaling pathways that lead to terminal differentiation of SH-SY5Y cells are still largely unknown. The goal of this study was to examine in the RA and CHOL treated SH-SY5Y cells the additive impacts of estradiol (E2) and brain-derived neurotrophic factor (BDNF) on cell morphology, cell population growth, synaptic vesicle recycling and presence of neurofilaments. The above features indicate a higher level of neuronal differentiation. Our data show that treatment for 10 days in vitro (DIV) with RA alone or when combined with E2 (RE) or CHOL (RC), but not when combined with BDNF (RB), significantly ($p < 0.01$) inhibited the cell population growth. Synaptic vesicle recycling, induced by high-K(+) depolarization, was significantly increased in all treatments where RA was included (RE, RC, RB, RCB), and when all agents were added together (RCBE). Specifically, our results show for the first time that E2 treatment can alone increase synaptic vesicle recycling in SH-SY5Y cells. This work contributes to the understanding of the ways to improve suppression of neuroblastoma cells' population growth by inducing maturation and differentiation.
Neurochem Res, 2016; 41

21436988: Aćimović J, Mäki-Marttunen T, Havela R, Teppola H, Linne ML
Modeling of Neuronal Growth In Vitro: Comparison of Simulation Tools NETMORPH and CX3D.
We simulate the growth of neuronal networks using the two recently published tools, NETMORPH and CX3D. The goals of the work are (1) to examine and compare the simulation tools, (2) to construct a model of growth of neocortical cultures, and (3) to characterize the changes in network connectivity during growth, using standard graph theoretic methods. Parameters for the neocortical culture are chosen after consulting both the experimental and the computational work presented in the literature. The first (three) weeks in culture are known to be a time of development of extensive dendritic and axonal arbors and establishment of synaptic connections between the neurons. We simulate the growth of networks from day 1 to day 21. It is shown that for the properly selected parameters, the simulators can reproduce the experimentally obtained connectivity. The selected graph theoretic methods can capture the structural changes during growth.
EURASIP J Bioinform Syst Biol, 2011; 2011

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BOARD NUMBER: S01-286

STRESSING BUT RELAXIN' THE BRAIN: HOW EARLY LIFE STRESS AFFECTS RLN-3 CIRCUITRY DEVELOPMENT AND AFFECTIVE BEHAVIOR

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

Esther Castillo-Gómez, Aroa Mañas-Ojeda, Zineb Bouargane, Clara García-Mompó, José Francisco Hidalgo-Cortés, Isis Gil-Miravet, Mónica Navarro Sánchez, Francisco Ros-Bernal, Francisco Olucha-Bordonau
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Early postnatal life is a critical period during which the brain undergo important changes to reach maturation. Indeed, the exposure to aversive experiences during this period influences brain development and leads to altered behaviour and impairment of neural structures that can persist through lifespan. The nucleus incertus (NI) has been described as a small cluster of neurons in the brainstem, containing predominately GABAergic neurons that express the neuropeptide Relaxin-3 (RLN-3). Those neurons participate into the modulation of brain circuits related to socio-affective behavior and, in fact, recent evidence suggests the role of NI in stress responsiveness. Here, we hypothetized that early postnatal stress is able to induce changes in the development of RLN3 networks that will result in higher emotional vulnerability, dependent on sex. To address this question, early life stress was induced by exposing both male and female mice mice pups to maternal separation from PND2-PND16. Our results show that maternal separation-induced stress generates depressive-like behavior in female but not male pups. However, the alterations in the number of RLN-3 positive neurons in the NI and the density of its projections to the Medial Septum (MS) are not dependent on sex, since they increased both in male and female mice. In sum, we demonstrated that early life stress alters the normal development of RLN-3 projections to the MS and the NI functioning and that in females, this neuroanatomical changes are correlated with disturbances in affective behavior.

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BOARD NUMBER: S01-287

UNDERSTANDING THE EMOTIONAL CONSEQUENCES OF CHRONIC PAIN: INSIGHT FROM ACC-LHB PATHWAY

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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Depression and anxiety are some of the leading causes of disability worldwide, contributing to the global burden of diseases. Mood disorders often arise consequently to chronic pain. Indeed, the comorbidity between chronic pain and mood disorders is frequently observed with 50% of chronic pain patients developing mood or anxiety disorders during their lifespan. Compelling evidence from human studies and animal models suggest an important role of the anterior cingulate cortex (ACC) and the lateral habenula (LHb) in the development and maintenance of this comorbidity. In addition, track tracing analyses performed in our team showed a connection from the ACC towards the LHb and our fMRI study displayed altered ACC-LHb functional connectivity in our chronic pain induced depression (CPID) mice model. Our aim was then to decipher the functional role of the ACC-LHb pathway in CPID by combining optogenetic approaches with behavioural analysis. Our results showed that chronic activation of the ACC-LHb pathway induces anxiodepressive-like behaviours in naive animals while inhibiting this pathway in CPID mice alleviates chronic pain induced depressive-like behavior. We then also sought to understand whether this pathway is only implicated in CPID or also in other depression models, such as chronic variable stress (CVS). Our preliminary results suggest that inhibiting this pathway does not affect depressive-like behaviours induced by CVS. Altogether, these results highlight the importance of the ACC-LHb pathway specifically in chronic pain induced anxio-depressive like behaviours.

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BOARD NUMBER: S01-288

SOCIAL ISOLATION STRESS IN AGED MICE: WHAT ABOUT AFFECTIVE BEHAVIOR AND INHIBITORY CIRCUITS.

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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While healthy aging has been associated with a stable emotional state and weakened brain responses to negative stimuli, senescence has long been viewed as a period of decreased adaptiveness to stress, in part because depressive symptoms are very common in older people. However, despite the importance and high prevalence of socio-affective-related disorders among elderly people, and the apparent role of the basolateral amygdala (BLA) in these disorders, preclinical studies addressing these questions are still scarce. Moreover, little attention has been paid to whether males and females respond differently to similar stressful situations and whether these differences can be observed at the microcircuitry level. In the present study we investigated the impact of chronic isolation stress (CIS) and a subsequent resocialization period in aged (18-24 months) male and female mice. Interestingly CIS demonstrated sex-dependent effects in both behavior and inhibitory circuitry (parvalbumin expressing cells surrounded by perineuronal nets). At the behavioral level, CIS induced depressive-like behavior only in females that was effectively rescued by resocialization. Remarkably, these changes were correlated with modulatory effects on inhibitory circuitry. Therefore, the present study suggests that social isolation stress in aging induces high emotional response and modulates BLA inhibitory circuits.

Pubmed:
28466069: Castillo-Gómez E, Pérez-Rando M, Bellés M, Gilabert-Juan J, Llorens JV, Carceller H, Bueno-Fernández C, García-Mompó C, Ripoll-Martínez B, Curto Y, Sebastiá-Ortega N, Moltó MD, Sanjuan J, Nacher J
Early Social Isolation Stress and Perinatal NMDA Receptor Antagonist Treatment Induce Changes in the Structure and Neurochemistry of Inhibitory Neurons of the Adult Amygdala and Prefrontal Cortex.
The exposure to aversive experiences during early life influences brain development and leads to altered behavior. Moreover, the combination of these experiences with subtle alterations in neurodevelopment may contribute to the emergence of psychiatric disorders, such as schizophrenia. Recent hypotheses suggest that imbalances between excitatory and inhibitory (E/I) neurotransmission, especially in the prefrontal cortex and the amygdala, may underlie their etiopathology. In order to understand better the neurobiological bases of these alterations, we studied the impact of altered neurodevelopment and chronic early-life stress on these two brain regions. Transgenic mice displaying fluorescent excitatory and inhibitory neurons, received a single injection of MK801 (NMDAR antagonist) or vehicle solution at postnatal day 7 and/or were socially isolated from the age of weaning until adulthood (3 months old). We found that anxiety-related behavior, brain volume, neuronal structure, and the expression of molecules related to plasticity and E/I neurotransmission in adult mice were importantly affected by early-life stress. Interestingly, many of these effects were potentiated when the stress paradigm was applied to mice perinatally injected with MK801 ("double-hit" model). These results clearly show the impact of early-life stress on the adult brain, especially on the structure and plasticity of inhibitory networks, and highlight the double-hit model as a valuable tool to study the contribution of early-life stress in the emergence of neurodevelopmental psychiatric disorders, such as schizophrenia.
eNeuro, 2017 Mar-Apr; 4
24736324: Tzanoulinou S, García-Mompó C, Castillo-Gómez E, Veenit V, Nacher J, Sandi C
Long-term behavioral programming induced by peripuberty stress in rats is accompanied by GABAergic-related alterations in the Amygdala.
Stress during childhood and adolescence is a risk factor for psychopathology. Alterations in γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the brain, have been found following stress exposure and fear experiences and are often implicated in anxiety and mood disorders. Abnormal amygdala functioning has also been detected following stress exposure and is also implicated in anxiety and social disorders. However, the amygdala is not a unitary structure; it includes several nuclei with different functions and little is known on the potential differences the impact of early life stress may have on this system within different amygdaloid nuclei. We aimed here to evaluate potential regional differences in the expression of

GABAergic-related markers across several amygdaloid nuclei in adult rats subjected to a peripuberty stress protocol that leads to enhanced basal amygdala activity and psychopathological behaviors. More specifically, we investigated the protein expression levels of glutamic acid decarboxylase (GAD; the principal synthesizing enzyme of GABA) and of GABA-A receptor subunits α2 and α3. We found reduced GAD and GABA-A α3, but not α2, subunit protein levels throughout all the amygdala nuclei examined (lateral, basolateral, basomedial, medial and central) and increased anxiety-like behaviors and reduced sociability in peripubertally stressed animals. Our results identify an enduring inhibition of the GABAergic system across the amygdala following exposure to early adversity. They also highlight the suitability of the peripuberty stress model to investigate the link between treatments targeting the dysfunctional GABAergic system in specific amygdala nuclei and recovery of specific stress-induced behavioral dysfunctions.
PLoS One, 2014; 9
20843898: Gómez-Climent MÁ, Guirado R, Castillo-Gómez E, Varea E, Gutierrez-Mecinas M, Gilabert-Juan J, García-Mompó C, Vidueira S, Sanchez-Mataredona D, Hernández S, Blasco-Ibáñez JM, Crespo C, Rutishauser U, Schachner M, Nacher J
The polysialylated form of the neural cell adhesion molecule (PSA-NCAM) is expressed in a subpopulation of mature cortical interneurons characterized by reduced structural features and connectivity.
Principal neurons in the adult cerebral cortex undergo synaptic, dendritic, and spine remodeling in response to different stimuli, and several reports have demonstrated that the polysialylated form of the neural cell adhesion molecule (PSA-NCAM) participates in these plastic processes. However, there is only limited information on the expression of this molecule on interneurons and on its role in the structural plasticity of these cells. We have found that PSA-NCAM is expressed in mature interneurons widely distributed in all the extension of the cerebral cortex and have excluded the expression of this molecule in most principal cells. Although PSA-NCAM expression is generally considered a marker of immature neurons, birth-dating analyses reveal that these interneurons do not have an adult or perinatal origin and that they are generated during embryonic development. PSA-NCAM expressing interneurons show reduced density of perisomatic and peridendritic puncta expressing different synaptic markers and receive less perisomatic synapses, when compared with interneurons lacking this molecule. Moreover, they have reduced dendritic arborization and spine density. These data indicate that PSA-NCAM expression is important for the connectivity of interneurons in the adult cerebral cortex and that its regulation may play an important role in the structural plasticity of inhibitory networks.
Cereb Cortex, 2011; 21

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HIPPOCAMPUS PROTEOMICS PROFILING OF MAJOR DEPRESSION AND ANTIDEPRESSANT TREATMENT REVEALS PATHWAYS INVOLVED IN CELL PROLIFERATION, DIFFERENTIATION AND CONNECTIVITY

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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[1]Columbia University, Department Of Psychiatry, New York, United States of America, [2]CUIMC - NYSPI - NYPH, Psychiatry, New York, United States of America, [3]Columbia University, Quantitative Proteomics Center, New York, United States of America

Major Depressive Disorder (MDD) involves deficits in memory and emotion regulation that are dependent on hippocampus cell viability and connectivity, for which molecular regulators remain largely unknown. We performed shotgun proteomics and advanced data mining strategies, which revealed clusters of differentially expressed proteins (DEPs) in untreated MDD (uMDD) compared with non-psychiatric controls (CONT) and antidepressant-treated MDD subjects (MDDT, n=12/group). Some DEPs showed group-specific positive and negative correlations with numbers of cells at different stages of maturation in the dentate gyrus (DG). Our data indicates that DEPs with lower expression in uMDD *vs.* CONT are involved in cell cycle, mitosis, differentiation, and negative regulation of apoptosis; proteins with higher expression in uMDD control cell adhesion, neurite outgrowth, DNA damage repair and telomere maintenance, repress transcription, and inhibit cell cycle progression. DEPs with lower expression in uMDD *vs.*CONT and higher expression in MDDT *vs.* uMDD ("normalized") regulate Ca^{++}-dependent exocytosis, and supply choline to cells; proteins lower in MDDT *vs.* uMDD negatively regulate cell proliferation; a protein higher in uMDD and MDDT *vs.* CONT is a suppressor of mitosis. DEPs altered in uMDD and not "reversed" in MDDT may become new testable treatment targets. DEPs negatively correlated with number of NPCs and DCX+ cells in uMDD only are involved in cell cycle, adhesion, differentiation, neurite outgrowth and migration. DEPs positively correlated with NPC and DCX+ and GN cells number in CONT and/or MDDT and not uMDD regulate cell division and migration, differentiation, mitochondrial electron transport and negatively regulate apoptosis.

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INVESTIGATION OF NMDA RECEPTOR FUNCTION IN A RODENT MODEL OF EARLY LIFE STRESS

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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Aims: Early life stress (ELS) is an important risk factor in the aetiology of depression. Excess developmental glucocorticoid exposure impacts multiple brain regions, with the hippocampus being particularly vulnerable. Hippocampal-dependent behavioural adaptation, often impaired in depression, is dependent on NMDA receptor (NMDAR) mediated synaptic plasticity. In this study we investigated the effect of ELS upon hippocampal NMDAR function. **Methods:** Long-Evans rat pups (n=52) were either undisturbed or maternally separated for 180 minutes per day (MS180) between Post Natal Day 1 (PND1) and PND14. Animals were tested as adults for well-characterised markers of ELS: sucrose preference (n=18) and novelty supressed feeding (NSFT, n=34) alongside assessment of CORT and PVN cFos reactivity to stress and hippocampal neurogenesis (all n=18). To assess NMDAR function in CA1 pyramidal neurones, AMPA/NMDA ratios (n=19) were measured using whole-cell patch clamp alongside separate miniEPSC recordings (n=19). **Results:** MS180 animals showed increased feeding latency in the NSFT alongside increased overall CORT in the restraint stress experiment and increased PVN cFos expression in males but no changes in neurogenesis or sucrose preference. MS180 was associated with a lower AMPA/NMDA ratio with no change in miniEPSC amplitude but decreased frequency. There were no changes in input resistance or paired-pulse ratio between conditions. **Conclusions:** The MS180 model showed a behavioural phenotype consistent with previous work (Bonapersona et al.,2019,Neurosci-Biobehav-Rev,102,299–307). MS180 animals showed evidence supporting increased NMDAR function whereas changes in miniEPSC frequency may reflect decreased pre-synaptic release probability. Work is needed to confirm these findings and assess impact upon synaptic plasticity.

Pubmed:

34890390: Wilkinson MP, Slaney CL, Mellor JR, Robinson ESJ
Investigation of reward learning and feedback sensitivity in non-clinical participants with a history of early life stress.
Early life stress (ELS) is an important risk factor for the development of depression. Impairments in reward learning and feedback sensitivity are suggested to be an intermediate phenotype in depression aetiology therefore we hypothesised that healthy adults with a history of ELS would exhibit reward processing deficits independent of any current depressive symptoms. We recruited 64 adults with high levels of ELS and no diagnosis of a current mental health disorder and 65 controls. Participants completed the probabilistic reversal learning task and probabilistic reward task followed by depression, anhedonia, social status, and stress scales. Participants with high levels of ELS showed decreased positive feedback sensitivity in the probabilistic reversal learning task compared to controls. High ELS participants also trended towards possessing a decreased model-free learning rate. This was coupled with a decreased learning ability in the acquisition phase of block 1 following the practice session. Neither group showed a reward induced response bias in the probabilistic reward task however high ELS participants exhibited decreased stimuli discrimination. Overall, these data suggest that healthy participants without a current mental health diagnosis but with high levels of ELS show deficits in positive feedback sensitivity and reward learning in the probabilistic reversal learning task that are distinct from depressed patients. These deficits may be relevant to increased depression vulnerability.
PLoS One, 2021; 16
32219179: Wilkinson MP, Grogan JP, Mellor JR, Robinson ESJ
Comparison of conventional and rapid-acting antidepressants in a rodent probabilistic reversal learning task.
Deficits in reward processing are a central feature of major depressive disorder with patients exhibiting decreased reward learning and altered feedback sensitivity in probabilistic reversal learning tasks. Methods to quantify probabilistic learning in both rodents and humans have been developed, providing translational paradigms for depression research. We have utilised a probabilistic reversal learning task to investigate potential differences between conventional and rapid-acting antidepressants on reward learning and feedback sensitivity. We trained 12 rats in a touchscreen probabilistic reversal learning task before investigating the effect of acute administration of citalopram, venlafaxine, reboxetine, ketamine or

scopolamine. Data were also analysed using a Q-learning reinforcement learning model to understand the effects of antidepressant treatment on underlying reward processing parameters. Citalopram administration decreased trials taken to learn the first rule and increased win-stay probability. Reboxetine decreased win-stay behaviour while also decreasing the number of rule changes animals performed in a session. Venlafaxine had no effect. Ketamine and scopolamine both decreased win-stay probability, number of rule changes performed and motivation in the task. Insights from the reinforcement learning model suggested that reboxetine led animals to choose a less optimal strategy, while ketamine decreased the model-free learning rate. These results suggest that reward learning and feedback sensitivity are not differentially modulated by conventional and rapid-acting antidepressant treatment in the probabilistic reversal learning task.
Brain Neurosci Adv, 2020 Jan-Dec; 4

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BOARD NUMBER: S01-291

NODES OF RANVIER ARE MODULATED BY CHRONIC PSYCHOSOCIAL STRESS IN MICE AND UNDERGO AXON-SPECIFIC STRUCTURAL REMODELING IN RESPONSE TO CHRONIC NEURONAL ACTIVATION

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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[1]University of Helsinki, Sleepwell Research Program, Faculty Of Medicine, Helsinki, Finland, [2]University of Eastern Finland, A.i. Virtanen Institute For Molecular Sciences, Kuopio, Finland, [3]University of Helsinki, Department Of Pharmacology, Faculty Of Medicine, Helsinki, Finland

Differential expression of myelin-related genes and changes in myelin thickness have been demonstrated in mice after chronic psychosocial stress, a risk factor for anxiety disorders. Here, we developed a 3D reconstruction analysis to investigate whether and how chronic stress affects nodes of Ranvier structural remodeling, another form of myelin plasticity, in male mice from two genetic backgrounds, C57BL/6NCrl (B6) and DBA/2NCrl (D2). We report strain-dependent effects of chronic stress on nodes of Ranvier morphology, including elongation of paranodes in the medial prefrontal cortex (mPFC) gray matter in D2 mice. Furthermore, chronic chemogenetic activation of the ventral hippocampus (vHPC)-to-mPFC pathway resulted in increased risk assessment behavior and shortening of paranodes specifically in stimulated axons, providing a direct link between anxiety-like behavior and remodeling of the nodes of Ranvier. Overall, our data demonstrate the involvement of nodes of Ranvier remodeling in the stress response and suggest an activity-dependent regulation of paranodes in anxiety-related circuits. Nodal remodeling may thus contribute to aberrant circuit function associated with anxiety disorders.

Pubmed:
33057053: Koskinen MK, van Mourik Y, Smit AB, Riga D, Spijker S
From stress to depression: development of extracellular matrix-dependent cognitive impairment following social stress.
Stress can predispose to depressive episodes, yet the molecular mechanisms regulating the transition from the initial stress response to a persistent pathological depressive state remain poorly understood. We profiled the development of an enduring depressive-like state by assessing affective behavior and hippocampal function during the 2 months following social-defeat stress. We measured remodeling of hippocampal extracellular matrix (ECM) during this period, as we recently identified ECM changes to mediate cognitive impairment during the sustained depressive-like state. Affective disturbance and cognitive impairments develop disparately after social stress, with gradual appearance of affective deficits. In contrast, spatial memory was impaired both early after stress and during the late-emerging chronic depressive-like state, while intact in-between. Similarly, we observed a biphasic regulation of the hippocampal ECM coinciding with hippocampus-dependent memory deficits. Together our data (1) reveal a dichotomy between affective and cognitive impairments similar to that observed in patients, (2) indicate different molecular processes taking place during early stress and the chronic depressive-like state, and (3) support a role of the ECM in mediating long-lasting effects on memory. From a translational point of view, it is important to prioritize on temporal phenotypic aspects in animal models to elucidate the underlying mechanisms of depression.
Sci Rep, 2020; 10
32687884: Spijker S, Koskinen MK, Riga D
Incubation of depression: ECM assembly and parvalbumin interneurons after stress.
The extracellular space is occupied by a complex network of proteins creating a mesh-like assembly known as the extracellular matrix (ECM). ECM assembles into dense net-like structures, perineuronal nets (PNNs), that envelope cell somas and proximal neurites of predominantly parvalbumin-(PV) interneurons. ECM regulates cell-to-cell communication, thereby modulating neuronal network function. Accumulating evidence points to the importance of network dysfunction in the pathophysiology of psychiatric diseases, in which stress acts as a major predisposing factor. Here we review stress-induced changes in ECM/PNNs and PV-interneurons in preclinical models of (or for) depression, with a special focus on social stress. We argue that the direction of these alterations largely depends on stress recency, as well as on stress timing and the brain region under investigation. A biphasic temporal regulation of ECM/PNNs and PV-interneuron function is typically observed after stress. Understanding the complex mechanisms underlying ECM organization in relation to stress-induced molecular,

cellular and network changes is crucial to further decipher the implications of ECM remodeling in the incubation of depressive symptoms.
Neurosci Biobehav Rev, 2020; 118
29263233: Riga D, Kramvis I, Koskinen MK, van Bokhoven P, van der Harst JE, Heistek TS, Jaap Timmerman A, van Nierop P, van der Schors RC, Pieneman AW, de Weger A, van Mourik Y, Schoffelmeer ANM, Mansvelder HD, Meredith RM, Hoogendijk WJG, Smit AB, Spijker S
Hippocampal extracellular matrix alterations contribute to cognitive impairment associated with a chronic depressive-like state in rats.
Patients with depression often suffer from cognitive impairments that contribute to disease burden. We used social defeat-induced persistent stress (SDPS) to induce a depressive-like state in rats and then studied long-lasting memory deficits in the absence of acute stressors in these animals. The SDPS rat model showed reduced short-term object location memory and maintenance of long-term potentiation (LTP) in CA1 pyramidal neurons of the dorsal hippocampus. SDPS animals displayed increased expression of synaptic chondroitin sulfate proteoglycans in the dorsal hippocampus. These effects were abrogated by a 3-week treatment with the antidepressant imipramine starting 8 weeks after the last defeat encounter. Next, we observed an increase in the number of perineuronal nets (PNNs) surrounding parvalbumin-expressing interneurons and a decrease in the frequency of inhibitory postsynaptic currents (IPSCs) in the hippocampal CA1 region in SDPS animals. In vivo breakdown of the hippocampus CA1 extracellular matrix by the enzyme chondroitinase ABC administered intracranially restored the number of PNNs, LTP maintenance, hippocampal inhibitory tone, and memory performance on the object place recognition test. Our data reveal a causal link between increased hippocampal extracellular matrix and the cognitive deficits associated with a chronic depressive-like state in rats exposed to SDPS.
Sci Transl Med, 2017; 9

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BOARD NUMBER: S01-292

EFFECTS OF EARLY LIFE STRESS ON THE EXCITABILITY OF PARVALBUMIN-EXPRESSING GABAERGIC INTERNEURONS IN THE HIPPOCAMPUS AND AMYGDALA

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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Early life stress (ELS) is a known cause of numerous mental health disorders. Limited bedding and nesting (LBN) model of ELS in rodents has now been extensively described from the behavioral point of view, but the associated functional changes remain less well characterized. Aims: To elucidate the effects of the LBN on physiological functions of GABAergic parvalbumin-positive (PV+) interneurons in the amygdala and hippocampus. Methods: WT and PV-CrexAi14 reporter mouse line was used. LBN conditions were kept during P4-P14, with exposure to maternal separation (1h) at P8, P10 and P12. Behavior was assessed at P60-P80 via a battery of tests: elevated plus maze, open field, light-dark box, and fear conditioning. Electrophysiological recordings were performed in acute slices at P60-P80 with the whole-cell patch clamp method. Results: Male mice exposed to LBN displayed increased anxiety-like behavior and both males and females showed impaired fear learning. We found a significant reduction in sEPSC frequency in PV+ neurons in the basolateral amygdala of male but not female mice, and an increase in the intrinsic excitability of PV+ neurons in both sexes. In PV+ cells of ventral hippocampus CA3 we observed no changes in sEPSC characteristics, while the intrinsic excitability was upregulated in females and downregulated in males. Conclusions: ELS produces gender-specific functional changes in PV+ neurons in the hippocampus and amygdala in adult animals. Given the important role PV+ cells play in circuit regulation, observed alterations of their functional properties likely contribute to the behavioral changes induced by the ELS.

Pubmed:

34663781: Englund J, Haikonen J, Shteinikov V, Amarilla SP, Atanasova T, Shintyapina A, Ryazantseva M, Partanen J, Voikar V, Lauri SE
Downregulation of kainate receptors regulating GABAergic transmission in amygdala after early life stress is associated with anxiety-like behavior in rodents.
Early life stress (ELS) is a well-characterized risk factor for mood and anxiety disorders. GABAergic microcircuits in the amygdala are critically implicated in anxiety; however, whether their function is altered after ELS is not known. Here we identify a novel mechanism by which kainate receptors (KARs) modulate feedforward inhibition in the lateral amygdala (LA) and show that this mechanism is downregulated after ELS induced by maternal separation (MS). Specifically, we show that in control rats but not after MS, endogenous activity of GluK1 subunit containing KARs disinhibit LA principal neurons during activation of cortical afferents. GluK1 antagonism attenuated excitability of parvalbumin (PV)-expressing interneurons, resulting in loss of PV-dependent inhibitory control and an increase in firing of somatostatin-expressing interneurons. Inactivation of Grik1 expression locally in the adult amygdala reduced ongoing GABAergic transmission and was sufficient to produce a mild anxiety-like behavioral phenotype. Interestingly, MS and GluK1-dependent phenotypes showed similar gender specificity, being detectable in male but not female rodents. Our data identify a novel KAR-dependent mechanism for cell-type and projection-specific functional modulation of the LA GABAergic microcircuit and suggest that the loss of GluK1 KAR function contributes to anxiogenesis after ELS.
Transl Psychiatry, 2021; 11
32202495: Ryazantseva M, Englund J, Shintyapina A, Huupponen J, Shteinikov V, Pitkänen A, Partanen JM, Lauri SE
Kainate receptors regulate development of glutamatergic synaptic circuitry in the rodent amygdala.
Perturbed information processing in the amygdala has been implicated in developmentally originating neuropsychiatric disorders. However, little is known on the mechanisms that guide formation and refinement of intrinsic connections between amygdaloid nuclei. We demonstrate that in rodents the glutamatergic connection from basolateral to central amygdala (BLA-CeA) develops rapidly during the first 10 postnatal days, before external inputs underlying amygdala-dependent behaviors emerge. During this restricted period of synaptic development, kainate-type of ionotropic glutamate receptors (KARs) are highly expressed in the BLA and tonically activated to regulate glutamate release via a G-protein-dependent mechanism.

Genetic manipulation of this endogenous KAR activity locally in the newborn LA perturbed development of glutamatergic input to CeA, identifying KARs as a physiological mechanism regulating formation of the glutamatergic circuitry in the amygdala.
Elife, 2020; 9
30959896: Shteinikov VY, Potapieva NN, Gmiro VE, Tikhonov DB
Hydrophobic Amines and Their Guanidine Analogues Modulate Activation and Desensitization of ASIC3.
Acid-sensing ion channel 3 (ASIC3) is an important member of the acid-sensing ion channels family, which is widely expressed in the peripheral nervous system and contributes to pain sensation. ASICs are targeted by various drugs and toxins. However, mechanisms and structural determinants of ligands' action on ASIC3 are not completely understood. In the present work we studied ASIC3 modulation by a series of "hydrophobic monoamines" and their guanidine analogs, which were previously characterized to affect other ASIC channels via multiple mechanisms. Electrophysiological analysis of action via whole-cell patch clamp method was performed using rat ASIC3 expressed in Chinese hamster ovary (CHO) cells. We found that the compounds studied inhibited ASIC3 activation by inducing acidic shift of proton sensitivity and slowed channel desensitization, which was accompanied by a decrease of the equilibrium desensitization level. The total effect of the drugs on the sustained ASIC3-mediated currents was the sum of these opposite effects. It is demonstrated that drugs' action on activation and desensitization differed in their structural requirements, kinetics of action, and concentration and state dependencies. Taken together, these findings suggest that effects on activation and desensitization are independent and are likely mediated by drugs binding to distinct sites in ASIC3.
Int J Mol Sci, 2019; 20
30557561: Shteinikov VY, Barygin OI, Gmiro VE, Tikhonov DB
Multiple modes of action of hydrophobic amines and their guanidine analogues on ASIC1a.
Hydrophobic monoamines containing only a hydrophobic/aromatic moiety and protonated amino group are a recently described class of acid-sensing ion channel (ASIC) modulators. Intensive studies have revealed a number of active compounds including endogenous amines and pharmacological agents and shown that these compounds potentiate and inhibit ASICs depending on their specific structure and on subunit composition of the target channel. The action of monoamines also depends on the application protocol, membrane voltage, conditioning and activating pH, suggesting complex mechanism(s) of the ligand-receptor interaction. Without understanding of these mechanisms analysis of structure-function relationships and predictive search for new potent and selective drugs are hardly possible. To this end, we investigated the modes of action for a representative series of amine and guanidine derivatives of adamantane and phenylcyclohexyl. The study was performed on transfected Chinese hamster ovary (CHO) cells and rat hippocampal interneurons using whole-cell patch clamp recording. We found that complex picture of monoamine action can be rationalized assuming four modes of action: (1) voltage-dependent pore block, (2) acidic shift of activation, (3) alkaline shift of activation and (4) acidic shift of steady-state desensitization. Structure-activity relationships are discussed in the light of this framework. The experiments on native heteromeric ASICs have shown that some of these mechanisms are shared between them and recombinant ASIC1a, implying that our results could also be relevant for amine action in physiological and pathological conditions.
Eur J Pharmacol, 2019; 844
29058095: Shteinikov VY, Tikhonova TB, Korkosh VS, Tikhonov DB
Potentiation and Block of ASIC1a by Memantine.
Acid-sensing ion channels (ASICs) are modulated by various classes of ligands, including the recently described hydrophobic monoamines, which inhibit and potentiate ASICs in a subunit-specific manner. In particular, memantine inhibits ASIC1a and potentiates ASIC2a homomers. The aim of the present work was to characterize action mechanism of memantine on recombinant ASIC1a expressed in CHO (Chinese hamster ovary) cells. We have demonstrated that effect of memantine on ASIC1a strongly depends on membrane voltage, conditioning pH value and application protocol. When applied simultaneously with activating acidification at hyperpolarized voltages, memantine caused the strongest inhibition. Surprisingly, application of memantine between ASIC1a activations at zero voltage caused significant potentiation. Analysis of the data suggests that memantine produces two separate effects, voltage-dependent open-channel block and shift of steady-state desensitization curve to more acidic values. Putative binding sites are discussed based on the computer docking of memantine to the acidic pocket and the pore region.
Cell Mol Neurobiol, 2018; 38
28688766: Shteinikov VY, Korosteleva AS, Tikhonova TB, Potapieva NN, Tikhonov DB
Ligands of histamine receptors modulate acid-sensing ion channels.
Recently we found that synthetic compounds containing amino group linked to hydrophobic or aromatic moiety are potent modulators of the proton-gated channels (ASICs). These structures have clear similarity with ligands of histamine receptors. We have also demonstrated that histamine potentiates homomeric ASIC1a by shifting its activation dependence to less acidic

conditions. In the present work the action of a series of histamine receptors ligands on recombinant ASIC1a and ASIC2a was characterized. Two types of action were found for ASIC1a. 1-methylhistamine, N-alpha-methylhistamine, dimaprit and thioperamide caused significant potentiation, which was pH-dependent and voltage-independent. The H4R antagonist A943931 caused inhibition, which is likely due to voltage-dependent pore block. ASIC2a were virtually insensitive to the drugs tested. We conclude that ligands of histamine receptors should also be considered as ASIC modulators.
Biochem Biophys Res Commun, 2017; 490

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BOARD NUMBER: S01-293

THE PSYCHOTOMIMETIC KETAMINE DISRUPTS THE TRANSFER OF SENSORY INFORMATION IN THE CORTICOTHALAMIC NETWORK

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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In prodromal and early schizophrenia, disorders of attention and perception are associated with structural and chemical brain abnormalities, and with dysfunctional, highly distributed corticothalamic networks exhibiting disturbed brain rhythms. The underlying mechanisms are elusive. The non-competitive NMDA receptor antagonist ketamine simulates the symptoms of prodromal and early psychotic disorders, including the disturbances in ongoing and task/sensory-related broadband gamma frequency (30-80 Hz) oscillations in corticothalamic networks. In normal healthy subjects and rodents, complex integration processes, like sensory perception, induce transient, large-scale synchronized gamma oscillations in a time window of a few hundreds of ms (200-700 ms) after the presentation of the object of attention (e.g., sensory stimulation). AIM: Our goal was to investigate the effects of a single psychotomimetic low-dose (2.5 mg/kg, subcutaneous) of ketamine on sensory-induced gamma oscillations in the frontoparietal somatosensory system in an attempt to understand the effect of NMDA receptor antagonism on the functional interactions between the cortex and thalamus. METHODS: Electrophysiological multisite cortical and thalamic EEG and extracellular recordings were performed in lightly anesthetized rats. Induced gamma oscillations occurred 200-700 ms after electrical stimulation of the vibrissae teguments. Spectral analysis, multiscale entropy, and coherence connectivity were computed. RESULTS: Ketamine transiently increased the power of baseline gamma oscillations and decreased sensory-induced gamma oscillations. In addition, it disrupted information transferability in both the somatosensory thalamus and the related cortex and decreased the stimulus-induced gamma-frequency band thalamocortical connectivity. CONCLUSION: The present findings support the hypothesis that NMDA receptor antagonism disrupts the transfer of perception-related information in the cortico-thalamo-cortical system.

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BOARD NUMBER: S01-294

ROLE OF GHRELIN ISOFORMS ON FOOD ANTICIPATORY ACTIVITY AND NEURONAL ACTIVATION IN A MOUSE MODEL OF CHRONIC FOOD RESTRICTION

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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Aims. Anorexia nervosa is a psychiatric disorder characterized by food restriction, intense physical activity, and elevated plasma ghrelin concentrations. Ghrelin is a gastrointestinal signal of undernutrition and exists under an acylated peptide (AG) that targets GHS-R-expressing neurons in both the hypothalamus and mesolimbic structures, and a desacylated peptide (DAG), which exact function remains unknown. We hypothesized that during states of chronic undernutrition, elevated ghrelin participates in the rewarding effect of physical activity. We measured running wheel activity during food anticipation (FAA), a period of intense physical activity in food restricted animals. Furthermore, c-Fos activation was assessed in hypothalamic and mesolimbic neurons following peripheral injections of AG and/or DAG. **Methods.** WT or GHS-R KO female mice were placed in a cage containing a running wheel with access to food (ALW) then exposed to 50% food restriction (FRW). Physical activity was measured using automated running wheels. AG, DAG (10 nmol/10 g BW) or saline were injected subcutaneously in WT mice under FRW conditions. Physical activity and c-Fos immunoreactivity were evaluated in hypothalamic, VTA and nucleus accumbens neurons 2 hours following the injection. **Results.** In FRW conditions, FAA was reduced in GHS-R KO mice. In the absence of food, AG, but not DAG, induced c-Fos activation in hypothalamic AgRP neurons known to convey negative valence to reward neurons. We also explored the rewarding effect of activity in a conditioned place preference. **Conclusions.** These results suggest that, when food is not available, ghrelin signalling is important for food anticipatory activity by targeting hypothalamic-mesolimbic circuits.

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BOARD NUMBER: S01-295

EARLY-LIFE STRESS ALTERS THE DEVELOPMENT OF FUNCTIONAL INTERACTIONS WITHIN PREFRONTAL-AMYGDALA NETWORKS

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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Early-life stress (ELS) is known to affect the development of the brain, with its consequences extending into adult life. Although many effects of ELS have been described, its impact on the functional maturation of networks underlying emotional behavior is still poorly understood. **Aims:** To describe the effect of ELS onto the development of functional interactions between the prefrontal cortex (PFC) and basolateral amygdala (BLA) in male and female mice. **Methods:** ELS was induced in mice with the established model of limited bedding and nesting (LBN) implemented between P4-P14 and combined with maternal separation for one hour at P8, P10, and P12. *In vivo* multi-unit activity and local-field potential (LFP) recordings were performed simultaneously in the prelimbic subdivision of PFC and BLA of urethane anaesthetized pups at P18-P20. **Results:** We found that male mice exposed to ELS showed a markedly lower spectral power in the theta and low-gamma bands in both layers II/III of PFC and BLA. Functional interactions between the BLA and PFC, as measured with LFP-LFP coherence, showed a decrease between 1-10 Hz. Interestingly, this alteration did not extend to females, nor to the deep layers of PFC. **Conclusions:** These data highlight a previously unknown effect of ELS onto brain development, appearing both as an impairment within the BLA and the PFC but also indicating an altered functional connectivity between them. These changes in the functional development of prefrontal-amygdala networks may underlie the increased susceptibility to neuropsychiatric disorders caused by ELS.

Pubmed:

33684513: La Barbera L, Vedele F, Nobili A, Krashia P, Spoleti E, Latagliata EC, Cutuli D, Cauzzi E, Marino R, Viscomi MT, Petrosini L, Puglisi-Allegra S, Melone M, Keller F, Mercuri NB, Conti F, D'Amelio M
Nilotinib restores memory function by preventing dopaminergic neuron degeneration in a mouse model of Alzheimer's Disease.
What happens precociously to the brain destined to develop Alzheimer's Disease (AD) still remains to be elucidated and this is one reason why effective AD treatments are missing. Recent experimental and clinical studies indicate that the degeneration of the dopaminergic (DA) neurons in the Ventral Tegmental Area (VTA) could be one of the first events occurring in AD. However, the causes of the increased vulnerability of DA neurons in AD are missing. Here, we deeply investigate the physiology of DA neurons in the VTA before, at the onset, and after onset of VTA neurodegeneration. We use the Tg2576 mouse model of AD, overexpressing a mutated form of the human APP, to identify molecular targets that can be manipulated pharmacologically. We show that in Tg2576 mice, DA neurons of the VTA at the onset of degeneration undergo slight but functionally relevant changes in their electrophysiological properties and cell morphology. Importantly, these changes are associated with accumulation of autophagosomes, suggestive of a dysfunctional autophagy, and with enhanced activation of c-Abl, a tyrosine kinase previously implicated in the pathogenesis of neurodegenerative diseases. Chronic treatment of Tg2576 mice with Nilotinib, a validated c-Abl inhibitor, reduces c-Abl phosphorylation, improves autophagy, reduces Aβ levels and - more importantly - prevents degeneration as well as functional and morphological alterations in DA neurons of the VTA. Interestingly, the drug prevents the reduction of DA outflow to the hippocampus and ameliorates hippocampal-related cognitive functions. Our results strive to identify early pathological brain changes in AD, to provide a rational basis for new therapeutic interventions able to slow down the disease progression.
Prog Neurobiol, 2021; 202
31611555: Krashia P, Cordella A, Nobili A, La Barbera L, Federici M, Leuti A, Campanelli F, Natale G, Marino G, Calabrese V, Vedele F, Ghiglieri V, Picconi B, Di Lazzaro G, Schirinzi T, Sancesario G, Casadei N, Riess O, Bernardini S, Pisani A, Calabresi P, Viscomi MT, Serhan CN, Chiurchiù V, D'Amelio M, Mercuri NB
Author Correction: Blunting neuroinflammation with resolvin D1 prevents early pathology in a rat model of Parkinson's disease.
An amendment to this paper has been published and can be accessed via a link at the top of the paper.

Nat Commun, 2019; 10
31477726: Krashia P, Cordella A, Nobili A, La Barbera L, Federici M, Leuti A, Campanelli F, Natale G, Marino G, Calabrese V, Vedele F, Ghiglieri V, Picconi B, Di Lazzaro G, Schirinzi T, Sancesario G, Casadei N, Riess O, Bernardini S, Pisani A, Calabresi P, Viscomi MT, Serhan CN, Chiurchiù V, D'Amelio M, Mercuri NB
Blunting neuroinflammation with resolvin D1 prevents early pathology in a rat model of Parkinson's disease.
Neuroinflammation is one of the hallmarks of Parkinson's disease (PD) and may contribute to midbrain dopamine (DA) neuron degeneration. Recent studies link chronic inflammation with failure to resolve early inflammation, a process operated by specialized pro-resolving mediators, including resolvins. However, the effects of stimulating the resolution of inflammation in PD - to modulate disease progression - still remain unexplored. Here we show that rats overexpressing human α-synuclein (Syn) display altered DA neuron properties, reduced striatal DA outflow and motor deficits prior to nigral degeneration. These early alterations are coupled with microglia activation and perturbations of inflammatory and pro-resolving mediators, namely IFN-γ and resolvin D1 (RvD1). Chronic and early RvD1 administration in Syn rats prevents central and peripheral inflammation, as well as neuronal dysfunction and motor deficits. We also show that endogenous RvD1 is decreased in human patients with early-PD. Our results suggest there is an imbalance between neuroinflammatory and pro-resolving processes in PD.
Nat Commun, 2019; 10
30915711: La Barbera L, Vedele F, Nobili A, D'Amelio M, Krashia P
Neurodevelopmental Disorders: Functional Role of Ambra1 in Autism and Schizophrenia.
The activating molecule in Beclin-1-regulated autophagy (Ambra1) is a highly intrinsically disordered protein best known for its role as a mediator in autophagy, by favoring the formation of autophagosomes. Additional studies have revealed that Ambra1 is able to coordinate cell responses to stress conditions such as starvation, and it actively participates in cell proliferation, cytoskeletal modification, apoptosis, mitochondria removal, and cell cycle downregulation. All these functions highlight the importance of Ambra1 in crucial physiological events, including metabolism, cell death, and cell division. Importantly, Ambra1 is also crucial for proper embryonic development, and its complete absence in knock-out animal models leads to severe brain morphology defects. In line with this, it has recently been implicated in neurodevelopmental disorders affecting humans, particularly autism spectrum disorders and schizophrenia. Here, we discuss the recent links between Ambra1 and neurodevelopment, particularly focusing on its role during the maturation of hippocampal parvalbumin interneurons and its importance for maintaining a proper excitation/inhibition balance in the brain.
Mol Neurobiol, 2019; 56

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BOARD NUMBER: S01-296

THE ROLE OF THE NUCLEUS ACCUMBENS SHELL IN ALCOHOL USE DESPITE NEGATIVE CONSEQUENCES

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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[1]Amsterdam UMC, Location VUmc, Anatomy & Neurosciences, Amsterdam, Netherlands, [2]Vrije Universiteit, Neuroscience Master's Program, Amsterdam, Netherlands

Alcohol use is widespread across most societies. While most people can control their alcohol consumption, a vulnerable sub-population may develop alcohol use disorder (AUD), characterized by continued alcohol use despite negative consequences. We use a rodent model of alcohol use despite negative consequences, to identify neurobiological differences that may underlie addiction-like vulnerability. This model reliably identifies two sub-populations of rats: Some substantially decrease alcohol use in the face of punishment (punishment-sensitive, controlled use) while others continue alcohol use despite negative consequences (punishment-resistant, addictive-like behavior). Here, we trained Long-Evans outbred rats (n=92, m/f) to self-administer alcohol, and then introduced punishment of alcohol self-administration with response-contingent foot-shock. Interestingly, we found that more females developed punishment-resistant alcohol use compared to males. Using immunohistochemical detection of c-Fos (neural activity marker), we found that lower c-Fos expression in nucleus accumbens shell (NAcSh) was associated with punishment-resistant alcohol use, compared to punishment-sensitive use or unpunished consumption (n=24). To test for a causal role of NAcSh activity, in another group (n=68), we applied chemogenetic inhibition of NAcSh throughout each phase of the experiment. We found that chemogenetic NAcSh inhibition increased alcohol consumption during unpunished alcohol self-administration. In punished sessions, however, NAcSh inhibition did not alter alcohol consumption. These results imply that NAcSh may be involved in the drive to consume alcohol, but not when alcohol use involves the possibility of negative consequences. Identifying the contributions of NAcSh, and associated neural circuits, in alcohol use despite negative consequences will improve our understanding of the neurobiological underpinnings of AUD.

Pubmed:
34744653: McDonald AJ, Alonso-Lozares I, Rauh V, van Mourik Y, Schetters D, De Vries TJ, Marchant NJ
Alcohol Seeking Under Risk of Punishment Is Associated With Activation of Cortical and Subcortical Brain Regions.
In humans, stimuli associated with alcohol availability can provoke relapse during abstinence. In this study, we investigated the role of discriminative stimuli (DS) in the control of alcohol seeking in two types of behavioral tests. The first test examined the ability of an alcohol-associated DS to promote alcohol seeking (relapse) after punishment-imposed abstinence in the presence of a different DS. Following this, we tested whether the differentially associated DS can promote and suppress alcohol self-administration in a within-session discrimination task. During the within-session discrimination task, we also tested the rate of alcohol self-administration when two DS are presented in a compound. We first trained Long-Evans male rats (= 24) to self-administer alcohol in the presence of one DS (reward-associated discriminative stimulus, rewDS) and then punished that behavior in the presence of a different DS (punishment-associated discriminative stimulus, punDS). On the test, we found that rats tested with the rewDS showed higher alcohol seeking than rats tested with the punDS. This result shows that a single Cue DS can promote alcohol seeking in a manner comparable to contexts. Subsequently, we trained 16 of these rats in a within-session trial-based discrimination task, comprised of intervening 2-min trials of rewDS, punDS, or conflict with rewDS and punDS in compound and a reduced probability of punishment. We found that alcohol self-administration is bi-directionally regulated by the rewDS and punDS. In conflict trials, alcohol self-administration was at a rate that was intermediate between the rewDS and punDS trials. In a final test, rats were presented with one of the three trial conditions and perfused for Fos immunohistochemistry. We found Fos expression was higher in the rats tested in the conflict condition in three interconnected sub-cortical brain regions. This study demonstrated the important role that alcohol-associated DS plays an important role in promoting relapse to alcohol seeking after punishment-imposed abstinence. We also implemented a within-session discrimination task that allows for the study of alcohol seeking under motivational conflict, which may be relevant for alcohol use despite negative consequences. The results from the Fos data suggest that higher alcohol seeking in approach-avoidance motivational conflict is associated with activation of sub-cortical regions but not

cortical regions.
Front Behav Neurosci, 2021; 15

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BOARD NUMBER: S01-297

CONSISTENT METABOLIC AND MIRNA SIGNATURES OF CHILDHOOD TRAUMA ACROSS DIFFERENT BODY FLUIDS IN HUMAN

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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[1]Nencki Institute of Experimental Biology, PAS, Laboratory For Translational Research In Neuropsychiatric Disorders (trend), Warsaw, Poland, [2]Nencki Institute of Experimental Biology, PAS, Laboratory Of Molecular Neurobiology, Warsaw, Poland, [3]Institute of Zoology and Biomedical Research, Jagiellonian University, Department Of Anthropology, Cracow, Poland

Aims: The main goal of this project is to systematically examine miRNA changes in different human body fluids, i.e., serum, sperm and milk that are relevant to long-term sequelae of childhood trauma including its intergenerational transmission. **Methods:** Small RNA sequencing followed by quantitative reverse transcriptase polymerase chain reaction assays were performed to identify and validate differentially regulated miRNAs in the serum of children with recent trauma in the form of paternal loss and maternal separation (PLMS), sperm of adult men with a history of complex trauma up to the age of 17 years, and milk from lactating mothers with prior history of childhood trauma. Pathway analysis for altered miRNAs was performed based on the Tarbase database. **Results:** Small RNA sequencing analysis revealed 48 miRNAs to be differentially expressed in the serum of PLMS children vs. control; whereas 29 miRNAs were differentially expressed in the sperm of adult men with a history of complex childhood trauma. Several differentially regulated miRNAs overlapped between the analyses. Furthermore, the pathway analysis and functional relevance of the altered miRNAs suggest a potential implication of lipids-associated miRNA carriers in the observed changes. **Conclusions:** This study found overlapping miRNA changes in a range of human body fluids after childhood trauma that seem to be conserved across diverse cohorts and age groups. The close relevance of these miRNAs to lipid-derived carriers is the premise of our current investigations.

Pubmed:
34908372: Wszoła M, Klak M, Tymicki G, Gomółka M, Kowalska P, Bryniarski T, Berman A
P.127: The Influence of Temperature Conditions and Reconstruction of the Extracellular Matrix Environment on the Viability of Pancreatic Islets.
Transplantation, 2021; 105
34207441: Wszoła M, Nitarska D, Cywoniuk P, Gomółka M, Klak M
Stem Cells as a Source of Pancreatic Cells for Production of 3D Bioprinted Bionic Pancreas in the Treatment of Type 1 Diabetes.
Type 1 diabetes (T1D) is the third most common autoimmune disease which develops due to genetic and environmental risk factors. Often, intensive insulin therapy is insufficient, and patients require a pancreas or pancreatic islets transplant. However, both solutions are associated with many possible complications, including graft rejection. The best approach seems to be a donor-independent T1D treatment strategy based on human stem cells cultured in vitro and differentiated into insulin and glucagon-producing cells (β and α cells, respectively). Both types of cells can then be incorporated into the bio-ink used for 3D printing of the bionic pancreas, which can be transplanted into T1D patients to restore glucose homeostasis. The aim of this review is to summarize current knowledge about stem cells sources and their transformation into key pancreatic cells. Last, but not least, we comment on possible solutions of post-transplant immune response triggered stem cell-derived pancreatic cells and their potential control mechanisms.
Cells, 2021; 10
33799490: Klak M, Kowalska P, Dobrzański T, Tymicki G, Cywoniuk P, Gomółka M, Kosowska K, Bryniarski T, Berman A, Dobrzyń A, Sadowski W, Górecki B, Wszoła M
Bionic Organs: Shear Forces Reduce Pancreatic Islet and Mammalian Cell Viability during the Process of 3D Bioprinting.
3D bioprinting is the future of constructing functional organs. Creating a bioactive scaffold with pancreatic islets presents many challenges. The aim of this paper is to assess how the 3D bioprinting process affects islet viability.
Micromachines (Basel), 2021; 12

32629779: Klak M, Bryniarski T, Kowalska P, Gomolka M, Tymicki G, Kosowska K, Cywoniuk P, Dobrzanski T, Turowski P, Wszola M
Novel Strategies in Artificial Organ Development: What Is the Future of Medicine?
The technology of tissue engineering is a rapidly evolving interdisciplinary field of science that elevates cell-based research from 2D cultures through organoids to whole bionic organs. 3D bioprinting and organ-on-a-chip approaches through generation of three-dimensional cultures at different scales, applied separately or combined, are widely used in basic studies, drug screening and regenerative medicine. They enable analyses of tissue-like conditions that yield much more reliable results than monolayer cell cultures. Annually, millions of animals worldwide are used for preclinical research. Therefore, the rapid assessment of drug efficacy and toxicity in the early stages of preclinical testing can significantly reduce the number of animals, bringing great ethical and financial benefits. In this review, we describe 3D bioprinting techniques and first examples of printed bionic organs. We also present the possibilities of microfluidic systems, based on the latest reports. We demonstrate the pros and cons of both technologies and indicate their use in the future of Medicine.
Micromachines (Basel), 2020; 11
32584858: Klak M, Gomółka M, Dobrzański T, Tymicki G, Cywoniuk P, Kowalska P, Kosowska K, Bryniarski T, Berman A, Dobrzyń A, Idaszek J, Święszkowski W, Wszoła M
Irradiation with 365 nm and 405 nm wavelength shows differences in DNA damage of swine pancreatic islets.
3D printing is being used more extensively in modern biomedicine. One of the problems is selecting a proper crosslinking method of bioprinted material. Amongst currently used techniques we can distinguish: physical crosslinking (e.g. Ca2+ and Sr2+) and chemical crosslinking-the UV light crosslinking causing the biggest discussion. UV radiation is selectively absorbed by DNA, mainly in the UV-B region but also (to some extent) in UV-A and UV-C regions. DNA excitement results in typical photoproducts. The amount of strand breaks may vary depending on the period of exposition, it can also differ when cells undergo incubation after radiation.
PLoS One, 2020; 15
33658892: Klak M, Gomółka M, Kowalska P, Cichoń J, Ambrożkiewicz F, Serwańska-Świętek M, Berman A, Wszoła M
Type 1 diabetes: genes associated with disease development.
Type 1 diabetes (T1D) is the third most common autoimmune disease which develops due to genetic and environmental risk factors. Based on the World Health Organization (WHO) report from 2014 the number of people suffering from all types of diabetes ascended to 422 million, compared to 108 million in 1980. It was calculated that this number will double by the end of 2030. In 2015 American Diabetes Association (ADA) announced that 30.3 million Americans (that is 9.4% of the overall population) had diabetes of which only approximately 1.25 million had T1D. Nowadays, T1D represents roughly 10% of adult diabetes cases total. Multiple genetic abnormalities at different loci have been found to contribute to type 1 diabetes development. The analysis of genome-wide association studies (GWAS) of T1D has identified over 50 susceptible regions (and genes within these regions). Many of these regions are defined by single nucleotide polymorphisms (SNPs) but molecular mechanisms through which they increase or lower the risk of diabetes remain unknown. Genetic factors (in existence since birth) can be detected long before the emergence of immunological or clinical markers. Therefore, a comprehensive understanding of the multiple genetic factors underlying T1D is extremely important for further clinical trials and development of personalized medicine for diabetic patients. We present an overview of current studies and information about regions in the human genome associated with T1D. Moreover, we also put forward information about epigenetic modifications, non-coding RNAs and environmental factors involved in T1D development and onset.
Cent Eur J Immunol, 2020; 45
31445766: Klak M, Urban S, Gomółka M, Cichoń J, Ambrożkiewicz F, Berman A, Serwańska-Świętek M, Wszoła M
Changes in Gene Expression of Selected Genes in Patients with Type 1 Diabetes and Pancreas Transplant in Peripheral Blood.
Diabetes is an autoimmunologic disease that may have a different background. The aim of our study was to show that type 1 diabetes is accompanied by changes in gene expression in peripheral blood mononuclear cells. We analyzed the genes characteristic of pancreatic islet cells and genes playing a big part in autoimmune diseases and cancer.
Transplant Proc, 2019; 51

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BOARD NUMBER: S01-298

STUDIES ON HIPPOCAMPAL HISTOARCHITECTURE AND NEUROCHEMISTRY IN PSYCHOLOGICALLY STRESSED RATS GIVEN GUT MICROBIOME SUPPLEMENTATION

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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University of Lagos, Anatomy, Lagos, Nigeria

Introduction: Life-threatening psychological stress (PS) disrupts the gut microbiome and accelerates decline in cognitive and memory functions and may predispose to certain neurodegenerative diseases. It is not known if the administration of probiotics (probio) can mitigate the detrimental effect of psychological stress on hippocampal histoarchitecture and neurochemistry. **Aim:** This study, was, therefore, designed to test the hypothesize that the administration of probiotics has beneficial effects on the neurohistology and neurochemistry of the hippocampus following exposure to psychological stress. **Methods**: Thirty-five adult male Wistar rats were assigned into seven groups (n=5) comprising the control, acute PS, acute probio, acute PS+probio, chronic PS, chronic probio, chronic PS+probio. Each animal in the probio groups was fed 10×10^6 colony-forming units of *lactobacillus acidophilus* every other day while the PS groups were exposed to predator stress for one hour between 7-10 am daily. The treatments lasted for 21days. Animals were sacrificed, the hippocampus was carefully harvested for histology and assay of dopamine, serotonin, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and reduced glutathione, (GHS). **Results**: Data analyses reveal that both acute and chronic PS significantly ($p< 0.05$) depress hippocampal serotonin and dopamine levels, cause increased lipid peroxidation, and impaired antioxidant parameters. The probiotics groups exhibited statistically significant better results on all parameters assessed and compared. There were no obvious histoarchitectural differences seen between any two groups. **Discussion**: Overall, data analyses suggest that the gut microbiome might play a significant role in hippocampal function because supplementing it mitigate stress-induced perturbations of hippocampal neurochemistry and redox status

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EXPOSURE TO CHRONIC SOCIAL STRESS OF IMMATURE ANIMALS EXPERIENCED EARLY-LIFE SEIZURES: BEHAVIORAL PHENOTYPING

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

Anna Mikulecka[1], Martin Kucera[2], Hana Kubova[2]
[1]Institute of Physiology The Czech Academy of Sciences, Developmental Epileptology, Praha , Czech Republic, [2]Institute of Physiology The Czech Academy of Sciences, Developmental Epileptology, Praha, Czech Republic

This study aimed to determine whether unpredictable chronic social stress induces long-lasting effects on the behavioral responsiveness of immature animals experienced status epilepticus We exposed Wistar rats with early-life seizures to chronic stress using a resident–intruder paradigm, starting at adolescence. The animals were transferred to PhenoTyper cages (Noldus Information Technology), where their behavior was automatically monitored for 24 hours. The animals were tested three times with one-, two-and three-month delays. Phenotypic markers of locomotor, emotional, and reward behavior were evaluated. PhenoTyper testing has revealed an increase in the distance moved during the day and a decrease in that moved during the night in both groups, suggesting that stress has affected chronobiologic rhythms in both controls and SE animals. In addition, SE animals exposed to stress had a higher sucrose preference than controls, indicating that social stress triggered a higher level of anxiety and not hedonic behavior as a marker of depressive-related behavior. In conclusion, our results have shown that chronic social stress leads to higher sensitivity to anxiety and emotive behavior in animals experiencing early status epilepticus. This study was supported by grant Czech Science Foundation (19-11931S), European Regional Development Fund project PHARMABRAIN, CZ.02.1.01/0.0/0.0/16_025/000744 (co-supported by EU).

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BOARD NUMBER: S01-300

NEWLY FORMED SYNAPSES BETWEEN VTA PROJECTIONS AND ACC PYRAMIDAL NEURONS IN RESPONSE TO CHRONIC SOCIAL DEFEAT STRESS DIFFERENTIATE STRESS SUSCEPTIBLE FROM STRESS RESILIENT MICE

POSTER SESSION 01 - SECTION: STRESS, ANXIETY AND MENTAL HEALTH

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Hacettepe University, Institute Of Neurological Sciences And Psychiatry, Ankara, Turkey

Aims Identify differences in new VTA synapses on ACC pyramidal neurons formed during stress exposure in stress resilient and stress susceptible mice. **Methods** Pre-eGRASP and post-eGRASP expressing AAV's are used to label newly formed synapses formed by projections from ventral tegmental area (VTA), ventral hippocampus (VH), basolateral amygdala (BLA) onto anterior cingulate cortex (ACC) in cyan, green, yellow, respectively. TagRFP-T is used to label ACC dendritic tree. Three C57BL/6 male mice were exposed to chronic social defeat stress for 10 days and separated into stress susceptible and resilient groups by social interaction test. 60 µm-thick sections were imaged by acquiring 30 µm z-stacks under 405,488,552 and 635 nm excitation. Acquisition was done in multiple spectral emission channels with different bandwidths (445-800 nm) to differentiate between cyan, green, yellow-eGRASP, TagRFP-T and autofluorescence. Pixels were spectrally unmixed using LUMoS algorithm. Unmixed images were deconvolved and median-filtered to increase signal-to-noise ratio. Fluorescent signals corresponding to newly formed VTA-ACC synapses in layer 2/3 were manually counted in 3D Z-stacks. **Results** Number of new synapses formed between VTA projections and L2/3 ACC neurons was significantly higher in stress susceptible mice than in resilient mice (10.6±5.16 and 5.44±2.91, respectively, $p = 0.047$, 14 z-stacks, mean ± SD). Evaluation of newly formed synapses between VH-ACC and BLA -ACC is in progress. **Conclusions** Not only dopaminergic but also glutamatergic VTA neuronal projections onto ACC determine stress susceptibility. This work was supported by Hacettepe University Scientific Research Projects Coordination Unit (TSA-2020-18753)

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BOARD NUMBER: S01-301

HYPOTHALAMIC UROCORTIN3 EXPRESSING NEURONS PROJECT TO THE PITUITARY GLAND AND SIGNAL TO THE PERIPHERY

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

Ilaria Carta
Albert Einstein College of Medicine, Neuroscience, Bronx, United States of America

Urocortin3 (Ucn3) expressing neurons located in the perifornical area of the hypothalamus (PeFA) are important for many functions, including social behavior and stress. Previous work from our lab revealed that these neurons send projections to the median eminence (ME), the fiber bundle that connects the brain and the pituitary gland, suggesting that Ucn3+ neurons might be implicated in neuroendocrine signaling. First, we sought to find out whether Ucn3+ axons terminate in the pituitary and to which subdivision/s. We found that Ucn3+ puncta can be observed in the posterior pituitary in close proximity to blood vessels, although they are not very abundant. We also observed a population of cells, not previously described, that expresses Ucn3 and densely populates the anterior pituitary. Optogenetic stimulation of $PeFA^{Ucn3}$ neurons results in increased plasma Ucn3, suggesting that Ucn3 is either released in the blood from hypothalamic axons, or from the anterior pituitary upon hypothalamic activation. Interestingly, acute stress also results in increased levels of plasma Ucn3, with peak levels 2 hours after stress and recovery to baseline levels at 6 hours. Taken together, our preliminary findings suggest that $PeFA^{Ucn3}$ neurons may regulate the stress response by signaling to peripheral organs, either by interacting with the HPA (hypothalamo-pituitary-adrenal) axis or via an independent neuroendocrine circuit. Our lab is currently trying to further dissect the contribution of $PeFA^{Ucn3}$ projections to regulating circulating Ucn3 levels, via the pituitary, using molecular and in vivo approaches.

Pubmed:
30655412: Carta I, Chen CH, Schott AL, Dorizan S, Khodakhah K
Cerebellar modulation of the reward circuitry and social behavior.
The cerebellum has been implicated in a number of nonmotor mental disorders such as autism spectrum disorder, schizophrenia, and addiction. However, its contribution to these disorders is not well understood. In mice, we found that the cerebellum sends direct excitatory projections to the ventral tegmental area (VTA), one of the brain regions that processes and encodes reward. Optogenetic activation of the cerebello-VTA projections was rewarding and, in a three-chamber social task, these projections were more active when the animal explored the social chamber. Intriguingly, activity in the cerebello-VTA pathway was required for the mice to show social preference in this task. Our data delineate a major, previously unappreciated role for the cerebellum in controlling the reward circuitry and social behavior.
Science, 2019; 363
34423776: Autry AE, Wu Z, Kapoor V, Kohl J, Bambah-Mukku D, Rubinstein ND, Marin-Rodriguez B, Carta I, Sedwick V, Tang M, Dulac C
neurons in the mouse perifornical area promote infant-directed neglect and aggression.
While recent studies have uncovered dedicated neural pathways mediating the positive control of parenting, the regulation of infant-directed aggression and how it relates to adult-adult aggression is poorly understood. Here we show that ()-expressing neurons in the hypothalamic perifornical area (PeFA) are activated during infant-directed attacks in males and females, but not other behaviors. Functional manipulations of PeFA neurons demonstrate the role of this population in the negative control of parenting in both sexes. PeFA neurons receive input from areas associated with vomeronasal sensing, stress, and parenting, and send projections to hypothalamic and limbic areas. Optogenetic activation of PeFA axon terminals in these regions triggers various aspects of infant-directed agonistic responses, such as neglect, repulsion, and aggression. Thus, PeFA neurons emerge as a dedicated circuit component controlling infant-directed neglect and aggression, providing a new framework to understand the positive and negative regulation of parenting in health and disease.
Elife, 2021; 10

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BOARD NUMBER: S01-302

PARADOXICAL ROLES OF TRPV1 AND TRPM2 IN WARM TEMPERATURE DETECTION

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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TRPV1 and TRPM2 are temperature sensitive cation channels belonging to the family of transient receptor potential (TRP) channels. Recent findings implicate a role for both TRPV1 and TRPM2 in warm-temperature detection. Cellular data from animals lacking TRPV1 and TRPM2, as well as pharmacological inhibition experiments, support this idea. However, in-vivo assessment of TRPV1- and TRPM2-involvement in temperature detection yields seemingly contradictory results. In this study, the role of TRPV1 and TRPM2 in warmth detection was evaluated in-vivo and in-vitro under comparable conditions. We developed a behavioral paradigm that assesses the thermal preference of animals to ambient and floor temperature. Animals lacking TRPM2 showed deficits in warm-temperature detection. TRPV1-KO animals, however, did not present a marked phenotype. Calcium imaging of DRG neurons from TRPV1- or TRPM2-KO animals cultured for three days showed a significant reduction in the proportion of warmth-sensitive neurons (WSN) compared to wildtype cultures. These results confirm the involvement of TRPM2 in warm temperature detection, while presenting a disconnect between the in-vitro and in-vivo observations for TRPV1. Intriguingly, animals over-expressing TRPV1 in TRPV1-positive sensory neurons showed a faster aversion to warm temperatures compared to wildtype animals in the behavioral paradigm. This is reflected in DRG cultures as an increase in the proportion of WSN, compared to wildtype cultures. This suggests that TRPV1 is capable of modulating warm temperature responses, in-vitro and in-vivo. In summary, we show that both TRPV1 and TRPM2 participate in the detection of warmth in-vitro and in-vivo, albeit with different and seemingly paradoxical contributions.

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A SODIUM-PERMEABLE CONDUCTANCE IS CRITICAL FOR THE EXCITABILITY OF MOUSE ADRENAL CHROMAFFIN CELLS IN SITU

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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In response to stress, catecholamines are the first hormones to be released into the blood. Catecholamine secretion is allowed by a tightly coordinated sequence of cellular and tissular mechanisms, in which the adrenal neuroendocrine chromaffin cells play a crucial role. A finely-tuned regulation of chromaffin cell electrical activity is required for appropriate hormone secretion in response to the whole-organism demand. While the repertoire of ion channels involved in generating the spiking activity is well known, how this spiking pattern is regulated by ion conductances operating nearby the resting membrane potential (RMP) is much less investigated. We show in mouse acute adrenal slices that at RMP chromaffin cells display a composite firing pattern, alternating between active periods composed of action potentials firing with a regular or a bursting mode, and silent periods. RMP is sensitive to changes in extracellular sodium (Na^+) concentration, as such a low Na^+-containing saline hyperpolarizes the membrane. This RMP drive reflects the contribution of a depolarizing conductance, which is i) not blocked by tetrodotoxin or cesium, ii) displays a linear I/V relationship between -110 to -40 mV and iii) is carried by cations with $g_{Na}>g_K>g_{Cs}$. These biophysical attributes, together with the expression of the sodium-leak channel *Nalcn* transcript in chromaffin cells, argue for a possible contribution of NALCN to chromaffin cell excitability. These results are the first description of a regulatory action of a background Na^+ conductance on chromaffin cell excitability. They also extend the inventory of tissues in which NALCN is expressed to neuroendocrine glands.

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INVESTIGATING THE EFFECTS OF HEDGEHOG SIGNALING ACTIVATION IN ASTROCYTES ON ENERGY METABOLISM AND INFLAMMATION.

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

Mariagiovanna Russo[1], Linda Tirou[1], Giuliana Pellegrino[1], Helene Faure[1], Ariane Sharif[2], Martial Ruat[1]
[1]CNRS, Paris-Saclay University, UMR-9197, Neuroscience Paris-saclay Institute, Saclay, France, [2]Univ. Lille, Inserm, CHU Lille, U1172 - Lilncog (jparc) - Lille Neurosciences & Cognition, Lille, France

Astrocytes are glial cells proposed as the main Sonic Hedgehog (Shh)-responsive cells in the adult brain. In the hypothalamus, astrocytes support neuronal circuits implicated in the regulation of energy metabolism. We have identified the distribution of gene transcripts of the Shh pathway including the Shh-receptor Patched (Ptc) and Gli1-3 in astrocytes using single molecule fluorescence *in situ* hybridization in the adult mouse hypothalamus. Further phenotyping of Shh mRNA expressing cells in the hypothalamus revealed that almost 30% of neurons were positive for Shh transcripts in the dorsomedial hypothalamic nucleus (DMH), 40% in the Arcuate nucleus (ARC), and 70% in the ventromedial hypothalamic nucleus (VMH). We recently reported that the activation of Shh signaling in Glast^{+} astrocytes induced by Ptc deletion enhanced insulin responsiveness (Tirou et al, Mol Metab, 2021). We have further characterized Glast-Cre^{ERT2}-YFP-Ptc$^{-/-}$ (YFP-Ptc$^{-/-}$) mice and their controls (YFP-Ptc$^{+/+}$) to investigate the potential effects of conditional astrocytic deletion of Ptc on hypothalamic inflammation. Interestingly, we observed that the increase over time of Iba1^{+} microglial cells identified in the DMH, the ARC and the VMH, was not observed in YFP-Ptc$^{-/-}$ compared to controls. Furthermore, Iba1^{+} microglial cells displayed a reactive morphology 7 days post-tamoxifen in YFP-Ptc$^{-/-}$ compared to controls while inflammation related gene CD11b was downregulated 7 months post-tamoxifen in Ptc-deleted mice. Experiments are in progress to further characterize hypothalamic Shh action from neurons on glial cells during aging and obesity-related metabolic disorders.

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ADAPTABILITY OF TUBEROINFUNDIBULAR DOPAMINE (TIDA) NEURON ELECTRICAL ACTIVITY IN FEMALE MICE: THE ROLE OF ESTRADIOL IN THE NEUROENDOCRINE CONTROL OF PROLACTIN.

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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Aim: Hypothalamic tuberoinfundibular dopamine neurons (TIDA) control prolactin secretion. Male mouse TIDA neurons exhibit asynchronous membrane potential oscillations associated with tonic low serum prolactin, but prolactin levels in females fluctuate along the oestrous cycle in an oestradiol-dependent manner. As sex-hormone sensitive tissues change daily over the oestrous cycle, we aimed to determine if direct oestradiol-mediated effects on TIDA membrane properties could explain this rapid adaptive control. Methods: Whole-cell patch-clamp recordings in acute hypothalamic slices from postpubertal female mice expressing tdTomato under control of the dopamine transporter promoter. Recordings were performed during a) natural sex hormone variations (oestrous cycle), b) chronic changes in hormone levels (ovariectomy) and c) local acute application of oestradiol and progesterone. Results: TIDA neurons in female mice exhibited either oscillatory or non-oscillatory firing patterns, which could not be interconverted by current injection or synaptic blockade. The oscillatory:non-oscillatory ratio was cycle phase-dependent, and significantly altered after ovariectomy. Oestradiol powerfully converted TIDA electrical state in a phenotype-dependent pattern, inducing hyperpolarization of non-oscillatory, and depolarization of oscillatory, TIDA cells. Both of these opposite effects – mediated by different membrane conductances - appeared, however, to both yield silencing of TIDA neurons, as the hormone-induced depolarization block of oscillatory cells. Application of progesterone did not affect the firing of TIDA neurons. Conclusions: The membrane properties of TIDA neurons may be subject to both acute and long-term regulation by gonadal steroids. This modulation may be involved in shaping the prolactin serum profile, by contributing to oestradiol-mediated stimulation of prolactin release.

Pubmed:

32671919: Orrillo SJ, de Dios N, Asad AS, De Fino F, Imsen M, Romero AC, Zárate S, Ferraris J, Pisera D
Anterior pituitary gland synthesises dopamine from l-3,4-dihydroxyphenylalanine (l-dopa).
Prolactin (PRL) is a hormone principally secreted by lactotrophs of the anterior pituitary gland. Although the synthesis and exocytosis of this hormone are mainly under the regulation of hypothalamic dopamine (DA), the possibility that the anterior pituitary synthesises this catecholamine remains unclear. The present study aimed to determine if the anterior pituitary produces DA from the precursor l-3,4-dihydroxyphenylalanine (l-dopa). Accordingly, we investigated the expression of aromatic l-amino acid decarboxylase (AADC) enzyme and the transporter vesicular monoamine transporter 2 (VMAT2) in the anterior pituitary, AtT20 and GH3 cells by immunofluorescence and western blotting. Moreover, we investigated the production of DA from l-dopa and its release in vitro. Then, we explored the effects of l-dopa with respect to the secretion of PRL from anterior pituitary fragments. We observed that the anterior pituitary, AtT20 and GH3 cells express both AADC and VMAT2. Next, we detected an increase in DA content after anterior pituitary fragments were incubated with l-dopa. Also, the presence of l-dopa increased DA levels in incubation media and reduced PRL secretion. Likewise, the content of cellular DA increased after AtT20 cells were incubated with l-dopa. In addition, l-dopa reduced corticotrophin-releasing hormone-stimulated adrenocorticotrophic hormone release from these cells after AADC activity was inhibited by NSD-1015. Moreover, DA formation from l-dopa increased apoptosis and decreased proliferation. However, in the presence of NSD-1015, l-dopa decreased apoptosis and increased proliferation rates. These results suggest that the anterior pituitary synthesises DA from l-dopa by AADC and this catecholamine can be released from this gland contributing to the control of PRL secretion. In addition, our results suggest that l-dopa exerts direct actions independently from its metabolisation to DA.
J Neuroendocrinol, 2020; 32
32209609: Thörn Pérez C, Ferraris J, van Lunteren JA, Hellysaz A, Iglesias MJ, Broberger C
Adaptive Resetting of Tuberoinfundibular Dopamine (TIDA) Network Activity during Lactation in Mice.
Giving birth triggers a wide repertoire of physiological and behavioral changes in the mother to enable her to feed and care for her offspring. These changes require coordination and are often orchestrated from the CNS, through as of yet poorly

understood mechanisms. A neuronal population with a central role in puerperal changes is the tuberoinfundibular dopamine (TIDA) neurons that control release of the pituitary hormone, prolactin, which triggers key maternal adaptations, including lactation and maternal care. Here, we used Ca imaging on mice from both sexes and whole-cell recordings on female mouse TIDA neurons to examine whether they adapt their cellular and network activity according to reproductive state. In the high-prolactin state of lactation, TIDA neurons shift to faster membrane potential oscillations, a reconfiguration that reverses upon weaning. During the estrous cycle, however, which includes a brief, but pronounced, prolactin peak, oscillation frequency remains stable. An increase in the hyperpolarization-activated mixed cation current, I, possibly through unmasking as dopamine release drops during nursing, may partially explain the reconfiguration of TIDA rhythms. These findings identify a reversible plasticity in hypothalamic network activity that can serve to adapt the dam for motherhood. Motherhood requires profound behavioral and physiological adaptations to enable caring for offspring, but the underlying CNS changes are poorly understood. Here, we show that, during lactation, neuroendocrine dopamine neurons, the "TIDA" cells that control prolactin secretion, reorganize their trademark oscillations to discharge in faster frequencies. Unlike previous studies, which typically have focused on structural and transcriptional changes during pregnancy and lactation, we demonstrate a functional switch in activity and one that, distinct from previously described puerperal modifications, reverses fully on weaning. We further provide evidence that a specific conductance (I) contributes to the altered network rhythm. These findings identify a new facet of maternal brain plasticity at the level of membrane properties and consequent ensemble activity.
J Neurosci, 2020; 40

30856609: Camilletti MA, Abeledo-Machado A, Perez PA, Faraoni EY, De Fino F, Rulli SB, Ferraris J, Pisera D, Gutierrez S, Thomas P, Díaz-Torga G

mPRs represent a novel target for PRL inhibition in experimental prolactinomas.

Membrane progesterone receptors are known to mediate rapid nongenomic progesterone effects in different cell types. Recent evidence revealed that mPRα is highly expressed in the rat pituitary, being primarily localized in lactotrophs, acting as an intermediary of P4-inhibitory actions on prolactin secretion. The role of mPRs in prolactinoma development remains unclear. We hypothesize that mPR agonists represent a novel tool for hyperprolactinemia treatment. To this end, pituitary expression of mPRs was studied in three animal models of prolactinoma. Expression of mPRs and nuclear receptor was significantly decreased in tumoral pituitaries compared to normal ones. However, the relative proportion of mPRα and mPRβ was highly increased in prolactinomas. Interestingly, the selective mPR agonist (Org OD 02-0) significantly inhibited PRL release in both normal and tumoral pituitary explants, displaying a more pronounced effect in tumoral tissues. As P4 also regulates PRL secretion indirectly, by acting on dopaminergic neurons, we studied mPR involvement in this effect. We found that the hypothalamus has a high expression of mPRs. Interestingly, both P4 and OrgOD 02-0 increased dopamine release in hypothalamus explants. Moreover, in an in vivo treatment, that allows both, pituitary and hypothalamus actions, the mPR agonist strongly reduced the hyperprolactinemia in transgenic females carrying prolactinoma. Finally, we also found and interesting gender difference: males express higher levels of pituitary mPRα/β, a sex that does not develop prolactinoma in these mice models. Taken together, these findings suggest mPRs activation could represent a novel tool for hyperprolactinemic patients, especially those that present resistance to dopaminergic drugs.
Endocr Relat Cancer, 2019; 26

30400046: Camilletti MA, Abeledo-Machado A, Ferraris J, Pérez PA, Faraoni EY, Pisera D, Gutierrez S, Díaz-Torga G

Role of GPER in the anterior pituitary gland focusing on lactotroph function.

Ovarian steroids control a variety of physiological functions. They exert actions through classical nuclear steroid receptors, but rapid non-genomic actions through specific membrane steroid receptors have been also described. In this study, we demonstrate that the G-protein-coupled estrogen receptor (GPER) is expressed in the rat pituitary gland and, at a high level, in the lactotroph population. Our results revealed that ~40% of the anterior pituitary cells are GPER positive and ~35% of the lactotrophs are GPER positive. By immunocytochemical and immuno-electron-microscopy studies, we demonstrated that GPER is localized in the plasmatic membrane but is also associated to the endoplasmic reticulum in rat lactotrophs. Moreover, we found that local Gper expression is regulated negatively by 17β-estradiol (E2) and progesterone (P4) and fluctuates during the estrus cycle, being minimal in proestrus. Interestingly, lack of ovarian steroids after an ovariectomy (OVX) significantly increased pituitary GPER expression specifically in the three morphologically different subtypes of lactotrophs. We found a rapid estradiol stimulatory effect on PRL secretion mediated by GPER, both in vitro and ex vivo, using a GPER agonist G1, and this effect was prevented by the GPER antagonist G36, demonstrating a novel role for this receptor. Then, the increased pituitary GPER expression after OVX could lead to alterations in the pituitary function as all three lactotroph subtypes are target of GPER ligand and could be involved in the PRL secretion mediated by GPER. Therefore, it should be taken into consideration in the response of the gland to an eventual hormone replacement therapy.
J Endocrinol, 2019; 240

30376668: de Dios N, Orrillo S, Irizarri M, Theas MS, Boutillon F, Candolfi M, Seilicovich A, Goffin V, Pisera D, Ferraris J

JAK2/STAT5 Pathway Mediates Prolactin-Induced Apoptosis of Lactotropes.

Prolactinomas are increasingly viewed as a "problem of signal transduction." Consequently, the identification of factors and signaling pathways that control lactotrope cell turnover is needed in order to encourage new therapeutic developments. We have previously shown that prolactin (PRL) acts as a proapoptotic and antiproliferative factor on lactotropes, maintaining anterior pituitary cell homeostasis, which contrasts with the classical antiapoptotic and/or proliferative actions exerted by PRL in most other target tissues. We aimed to investigate the PRLR-triggered signaling pathways mediating these nonclassical effects of PRL in the pituitary. Our results suggest that (i) the PRLR/Jak2/STAT5 pathway is constitutively active in GH3 cells and contributes to PRL-induced apoptosis by increasing the Bax/Bcl-2 ratio, (ii) PRL inhibits ERK1/2 and Akt phosphorylation, thereby contributing to its proapoptotic effect, and (iii) the PI3K/Akt pathway participates in the PRL-mediated control of lactotrope proliferation. We hypothesize that the alteration of PRL actions in lactotrope homeostasis due to the dysregulation of any of the mechanisms of actions described above may contribute to the pathogenesis of prolactinomas.
Neuroendocrinology, 2019; 108

29869822: Camilletti MA, Ferraris J, Abeledo-Machado A, Converse A, Faraoni EY, Pisera D, Gutierrez S, Thomas P, Díaz-Torga G
Participation of membrane progesterone receptor α in the inhibitory effect of progesterone on prolactin secretion.
The membrane progesterone receptors (mPRα, mPRβ, mPRγ, mPRδ and mPRε) are known to mediate rapid nongenomic progesterone functions in different cell types. However, the functions of these receptors in the pituitary have not been reported to date. In the present study, we show that the expression of mPRα was the highest among the mPRs in the rat anterior pituitary gland. Immunostaining of mPRα was detected in somatotrophs, gonadotrophs and lactotrophs. Interestingly, 63% of mPRα-positive cells within the pituitary were lactotrophs, suggesting that mPRα is involved in controlling prolactin (PRL) secretion in the pituitary. To test this hypothesis, rat pituitaries were incubated (1 hour) with either progesterone (P4) or the mPRα-specific agonist Org OD 02-0. PRL secretion was then measured by radioimmunoassay. The results of this experiment revealed that both P4 and Org OD 02-0 decreased PRL secretion. Moreover, the results from the GH3 cell line (CCL-82.1) showed that P4 and Org OD 02-0 inhibited PRL release, although the nuclear PR agonist R5020 was ineffective. Our investigation of the cellular mechanisms behind mPRα activity indicated that both P4 and Org OD 02-0 decreased cAMP accumulation, whereas R5020 was ineffective. In addition, the Org OD 02-0-effect on PRL release was blocked by pretreatment with pertussis toxin, an inhibitor of Go/Gi proteins. Because transforming growth factor (TGF)β1 is a potent inhibitor of PRL secretion in lactotrophs, we lastly evaluated whether TGFβ1 was activated by progesterone and whether this effect was mediated by mPRα. Our results showed that P4 and Org OD 02-0, but not R5020, increased active TGFβ1 levels. This effect was not observed when cells were transfected with mPRα-small interfering RNA. Taken together, these data provide new evidence suggesting that mPRα mediates the progesterone inhibitory effect on PRL secretion through both decreases in cAMP levels and activation of TGFβ1 in the lactotroph population.
J Neuroendocrinol, 2018; 30
24859278: Ferraris J, Zárate S, Jaita G, Boutillon F, Bernadet M, Auffret J, Seilicovich A, Binart N, Goffin V, Pisera D
Prolactin induces apoptosis of lactotropes in female rodents.
Anterior pituitary cell turnover occurring during female sexual cycle is a poorly understood process that involves complex regulation of cell proliferation and apoptosis by multiple hormones. In rats, the prolactin (PRL) surge that occurs at proestrus coincides with the highest apoptotic rate. Since anterior pituitary cells express the prolactin receptor (PRLR), we aimed to address the actual role of PRL in the regulation of pituitary cell turnover in cycling females. We showed that acute hyperprolactinemia induced in ovariectomized rats using PRL injection or dopamine antagonist treatment rapidly increased apoptosis and decreased proliferation specifically of PRL producing cells (lactotropes), suggesting a direct regulation of these cell responses by PRL. To demonstrate that apoptosis naturally occurring at proestrus was regulated by transient elevation of endogenous PRL levels, we used PRLR-deficient female mice (PRLRKO) in which PRL signaling is totally abolished. According to our hypothesis, no increase in lactotrope apoptotic rate was observed at proestrus, which likely contributes to pituitary tumorigenesis observed in these animals. To decipher the molecular mechanisms underlying PRL effects, we explored the isoform-specific pattern of PRLR expression in cycling wild type females. This analysis revealed dramatic changes of long versus short PRLR ratio during the estrous cycle, which is particularly relevant since these isoforms exhibit distinct signaling properties. This pattern was markedly altered in a model of chronic PRLR signaling blockade involving transgenic mice expressing a pure PRLR antagonist (TGΔ1-9-G129R-hPRL), providing evidence that PRL regulates the expression of its own receptor in an isoform-specific manner. Taken together, these results demonstrate that i) the PRL surge occurring during proestrus is a major proapoptotic signal for lactotropes, and ii) partial or total deficiencies in PRLR signaling in the anterior pituitary may result in pituitary hyperplasia and eventual prolactinoma development, as observed in TGΔ1-9-G129R-hPRL and PRLRKO mice, respectively.
PLoS One, 2014; 9
23969780: Ferraris J, Bernichtein S, Pisera D, Goffin V
Use of prolactin receptor antagonist to better understand prolactin regulation of pituitary homeostasis.

The anterior pituitary is permanently regulated by processes of apoptosis and proliferation in order to maintain tissue homeostasis. Several factors have been implicated in this regulation and lately, prolactin (PRL) has been included into that list. However, since PRL is secreted by anterior pituitary lactotropes, the actual outcome of its autocrine/paracrine actions on pituitary cells has remained difficult to assess. The availability of the pure PRL receptor antagonist Del1-9-G129R-hPRL has been helpful to circumvent this problem. While PRL has been traditionally associated with increased cell proliferation, recent studies revealed that this hormone actually induces apoptosis and decreases proliferation of anterior pituitary cells, by mechanisms involving the PRL receptor. The aim of this short review is to overview our current understanding of the regulation of pituitary homeostasis by PRL. Moreover, studies involving Del1-9-G129R-hPRL have helped anticipate to what extent future treatments involving PRL receptor inhibitors may interfere with processes regulated by PRL at the central level.
Neuroendocrinology, 2013; 98

22094470: Ferraris J, Boutillon F, Bernadet M, Seilicovich A, Goffin V, Pisera D
Prolactin receptor antagonism in mouse anterior pituitary: effects on cell turnover and prolactin receptor expression.
Since anterior pituitary expresses prolactin receptors, prolactin secreted by lactotropes could exert autocrine or paracrine actions on anterior pituitary cells. In fact, it has been observed that prolactin inhibits its own expression by lactotropes. Our hypothesis is that prolactin participates in the control of anterior pituitary cell turnover. In the present study, we explored the action of prolactin on proliferation and apoptosis of anterior pituitary cells and its effect on the expression of the prolactin receptor. To determine the activity of endogenous prolactin, we evaluated the effect of the competitive prolactin receptor antagonist Δ1-9-G129R-hPRL in vivo, using transgenic mice that constitutively and systemically express this antagonist. The weight of the pituitary gland and the anterior pituitary proliferation index, determined by BrdU incorporation, were higher in transgenic mice expressing the antagonist than in wild-type littermates. In addition, blockade of prolactin receptor in vitro by Δ1-9-G129R-hPRL increased proliferation and inhibited apoptosis of somatolactotrope GH3 cells and of primary cultures of male rat anterior pituitary cells, including lactotropes. These results suggest that prolactin acts as an autocrine/paracrine antiproliferative and proapoptotic factor in the anterior pituitary gland. In addition, anterior pituitary expression of the long isoform of the prolactin receptor, measured by real-time PCR, increased about 10-fold in transgenic mice expressing the prolactin receptor antagonist, whereas only a modest increase in the S3 short-isoform expression was observed. These results suggest that endogenous prolactin may regulate its own biological actions in the anterior pituitary by inhibiting the expression of the long isoform of the prolactin receptor. In conclusion, our observations suggest that prolactin is involved in the maintenance of physiological cell renewal in the anterior pituitary. Alterations in this physiological role of prolactin could contribute to pituitary tumor development.
Am J Physiol Endocrinol Metab, 2012; 302

21760910: Ferraris J, Radl DB, Zárate S, Jaita G, Eijo G, Zaldivar V, Clapp C, Seilicovich A, Pisera D
N-terminal prolactin-derived fragments, vasoinhibins, are proapoptoptic and antiproliferative in the anterior pituitary.
The anterior pituitary is under a constant cell turnover modulated by gonadal steroids. In the rat, an increase in the rate of apoptosis occurs at proestrus whereas a peak of proliferation takes place at estrus. At proestrus, concomitant with the maximum rate of apoptosis, a peak in circulating levels of prolactin is observed. Prolactin can be cleaved to different N-terminal fragments, vasoinhibins, which are proapoptotic and antiproliferative factors for endothelial cells. It was reported that a 16 kDa vasoinhibin is produced in the rat anterior pituitary by cathepsin D. In the present study we investigated the anterior pituitary production of N-terminal prolactin-derived fragments along the estrous cycle and the involvement of estrogens in this process. In addition, we studied the effects of a recombinant vasoinhibin, 16 kDa prolactin, on anterior pituitary apoptosis and proliferation. We observed by Western Blot that N-terminal prolactin-derived fragments production in the anterior pituitary was higher at proestrus with respect to diestrus and that the content and release of these prolactin forms from anterior pituitary cells in culture were increased by estradiol. A recombinant preparation of 16 kDa prolactin induced apoptosis (determined by TUNEL assay and flow cytometry) of cultured anterior pituitary cells and lactotropes from ovariectomized rats only in the presence of estradiol, as previously reported for other proapoptotic factors in the anterior pituitary. In addition, 16 kDa prolactin decreased forskolin-induced proliferation (evaluated by BrdU incorporation) of rat total anterior pituitary cells and lactotropes in culture and decreased the proportion of cells in S-phase of the cell cycle (determined by flow cytometry). In conclusion, our study indicates that the anterior pituitary production of 16 kDa prolactin is variable along the estrous cycle and increased by estrogens. The antiproliferative and estradiol-dependent proapoptotic actions of this vasoinhibin may be involved in the control of anterior pituitary cell renewal.
PLoS One, 2011; 6

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BOARD NUMBER: S01-306

RNA-SEQUENCING REVEALS TREATMENT AND SEX DIFFERENCES IN THE BRAINS OF LETROZOLE-TREATED COMMON MARMOSETS (C. JACCHUS)

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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Aromatase inhibitors (AIs) are drugs commonly given to patients with estrogen receptor (ER)-dependent breast cancers to reduce estrogenic stimulation. However, AIs like Letrozole are associated with negative side effects such as cognitive deficits, sleep disturbances and hot flashes. We have previously shown that these negative effects can be recapitulated in common marmosets (*Callithrix jacchus*) treated with Letrozole (20 µg daily) for 4 weeks and that Letrozole-treated marmosets show increased levels of estradiol in the hippocampus (Gervais et al., 2019). In order to better understand the mechanisms through which AIs affect cognitive function and increase steroid levels in the hippocampus, we used bulk, paired-end RNA-sequencing to examine gene expression differences between Letrozole-treated (LET; n=8) and vehicle-treated (VEH; n=8) male and female animals. Gene ontology results show significant reduction in LET animals across hundreds of categories ($P < 0.05$), some of the most significant being inflammatory response, stress response, MHC Class II protein complex binding, T-cell activation, carbohydrate binding and signaling receptor binding ($P < 5.0E-5$). GSEA results indicate only LET females show enrichment for hormonal gene sets (FDR = 0.1), an effect not observed in LET males (FDR = 0.67). Based on the transcriptional changes observed, we conclude that AIs may differentially affect the sexes in part due to processes mediated by the CYP-450 superfamily. Ongoing studies will further investigate the longitudinal effects of AIs on behavior and whether AIs increase the risk of stress-induced neurodegeneration.

Pubmed:

34018622: Rothwell ES, Freire-Cobo C, Varghese M, Edwards M, Janssen WGM, Hof PR, Lacreuse A
The marmoset as an important primate model for longitudinal studies of neurocognitive aging.
Age-related cognitive decline has been extensively studied in humans, but the majority of research designs are cross-sectional and compare across younger and older adults. Longitudinal studies are necessary to capture variability in cognitive aging trajectories but are difficult to carry out in humans and long-lived nonhuman primates. Marmosets are an ideal primate model for neurocognitive aging as their naturally short lifespan facilitates longitudinal designs. In a longitudinal study of marmosets tested on reversal learning starting in middle-age, we found that, on average, the group of marmosets declined in cognitive performance around 8 years of age. However, we found highly variable patterns of cognitive aging trajectories across individuals. Preliminary analyses of brain tissues from this cohort also show highly variable degrees of neuropathology. Future work will tie together behavioral trajectories with brain pathology and provide a window into the factors that predict age-related cognitive decline.
Am J Primatol, 2021; 83
34768597: Anekonda VT, Thompson BW, Ho JM, Roberts ZS, Edwards MM, Nguyen HK, Dodson AD, Wolden-Hanson T, Chukri DW, Herbertson AJ, Graham JL, Havel PJ, Wietecha TA, O'Brien KD, Blevins JE
Hindbrain Administration of Oxytocin Reduces Food Intake, Weight Gain and Activates Catecholamine Neurons in the Hindbrain Nucleus of the Solitary Tract in Rats.
Existing studies show that CNS oxytocin (OT) signaling is important in the control of energy balance, but it is unclear which neurons may contribute to these effects. Our goals were to examine (1) the dose-response effects of acute OT administration into the third (3V; forebrain) and fourth (4V; hindbrain) ventricles to assess sensitivity to OT in forebrain and hindbrain sites, (2) the extent to which chronic 4V administration of OT reduces weight gain associated with the progression of diet-induced obesity, and (3) whether nucleus tractus solitarius (NTS) catecholamine neurons are downstream targets of 4V OT. Initially, we examined the dose-response effects of 3V and 4V OT (0.04, 0.2, 1, or 5 µg). 3V and 4V OT (5 µg) suppressed 0.5-h food intake by 71.7 ± 6.0% and 60 ± 12.9%, respectively. 4V OT (0.04, 0.2, 1 µg) reduced food intake by 30.9 ± 12.9, 42.1 ± 9.4, and 56.4 ± 9.0%, respectively, whereas 3V administration of OT (1 µg) was only effective at reducing 0.5-h food intake by

38.3 ± 10.9%. We subsequently found that chronic 4V OT infusion, as with chronic 3V infusion, reduced body weight gain (specific to fat mass) and tended to reduce plasma leptin in high-fat diet (HFD)-fed rats, in part, through a reduction in energy intake. Lastly, we determined that 4V OT increased the number of hindbrain caudal NTS Fos (+) neurons (156 ± 25) relative to vehicle (12 ± 3). The 4V OT also induced Fos in tyrosine hydroxylase (TH; marker of catecholamine neurons) (+) neurons (25 ± 7%) relative to vehicle (0.8 ± 0.3%). Collectively, these findings support the hypothesis that OT within the hindbrain is effective at reducing food intake, weight gain, and adiposity and that NTS catecholamine neurons in addition to non-catecholaminergic neurons are downstream targets of CNS OT.
J Clin Med, 2021; 10
34566687: Edwards MM, Nguyen HK, Dodson AD, Herbertson AJ, Wietecha TA, Wolden-Hanson T, Graham JL, Honeycutt MK, Slattery JD, O'Brien KD, Havel PJ, Blevins JE
Effects of Combined Oxytocin and Beta-3 Receptor Agonist (CL 316243) Treatment on Body Weight and Adiposity in Male Diet-Induced Obese Rats.
Previous studies have indicated that oxytocin (OT) reduces body weight in diet-induced obese (DIO) rodents through reductions in energy intake and increases in energy expenditure. We recently demonstrated that hindbrain [fourth ventricular (4V)] administration of OT evokes weight loss and elevates interscapular brown adipose tissue temperature (T) in DIO rats. What remains unclear is whether OT can be used as an adjunct with other drugs that directly target beta-3 receptors in IBAT to promote BAT thermogenesis and reduce body weight in DIO rats. We hypothesized that the combined treatment of OT and the beta-3 agonist, CL 316243, would produce an additive effect to decrease body weight and adiposity in DIO rats by reducing energy intake and increasing BAT thermogenesis. We assessed the effects of 4V infusions of OT (16 nmol/day) or vehicle (VEH) in combination with daily intraperitoneal injections of CL 316243 (0.5 mg/kg) or VEH on food intake, T , body weight and body composition. OT and CL 316243 alone reduced body weight by 7.8 ± 1.3% (< 0.05) and 9.1 ± 2.1% (< 0.05), respectively, but the combined treatment produced more pronounced weight loss (15.5 ± 1.2%; < 0.05) than either treatment alone. These effects were associated with decreased adiposity, adipocyte size, energy intake and increased uncoupling protein 1 (UCP-1) content in epididymal white adipose tissue (EWAT) (< 0.05). In addition, CL 316243 alone (< 0.05) and in combination with OT (< 0.05) elevated T and IBAT UCP-1 content and IBAT thermogenic gene expression. These findings are consistent with the hypothesis that the combined treatment of OT and the beta-3 agonist, CL 316243, produces an additive effect to decrease body weight. The findings from the current study suggest that the effects of the combined treatment on energy intake, fat mass, adipocyte size and browning of EWAT were not additive and appear to be driven, in part, by transient changes in energy intake in response to OT or CL 316243 alone as well as CL 316243-elicited reduction of fat mass and adipocyte size and induction of browning of EWAT.
Front Physiol, 2021; 12
33470901: Edwards MM, Nguyen HK, Herbertson AJ, Dodson AD, Wietecha T, Wolden-Hanson T, Graham JL, O'Brien KD, Havel PJ, Blevins JE
Chronic hindbrain administration of oxytocin elicits weight loss in male diet-induced obese mice.
Previous studies indicate that oxytocin (OT) administration reduces body weight in high-fat diet (HFD)-induced obese (DIO) rodents through both reductions in food intake and increases in energy expenditure. We recently demonstrated that chronic hindbrain [fourth ventricular (4V)] infusions of OT evoke weight loss in DIO rats. Based on these findings, we hypothesized that chronic 4V OT would elicit weight loss in DIO mice. We assessed the effects of 4V infusions of OT (16 nmol/day) or vehicle over 28 days on body weight, food intake, and body composition. OT reduced body weight by approximately 4.5% ± 1.4% in DIO mice relative to OT pretreatment body weight (< 0.05). These effects were associated with reduced adiposity and adipocyte size [inguinal white adipose tissue (IWAT)] (< 0.05) and attributed, in part, to reduced energy intake (< 0.05) at a dose that did not increase kaolin intake (= NS). OT tended to increase uncoupling protein-1 expression in IWAT (0.05 < < 0.1) suggesting that OT stimulates browning of WAT. To assess OT-elicited changes in brown adipose tissue (BAT) thermogenesis, we examined the effects of 4V OT on interscapular BAT temperature (). 4V OT (1 µg) elevated at 0.75 (= 0.08), 1, and 1.25 h (< 0.05) postinjection; a higher dose (5 µg) elevated at 0.75-, 1-, 1.25-, 1.5-, 1.75- (< 0.05), and 2-h (0.05 < < 0.1) postinjection. Together, these findings support the hypothesis that chronic hindbrain OT treatment evokes sustained weight loss in DIO mice by reducing energy intake and increasing BAT thermogenesis at a dose that is not associated with evidence of visceral illness.
Am J Physiol Regul Integr Comp Physiol, 2021; 320
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Mentoring to Foster a Diverse Future.
Cell, 2020; 183
31844315: de Vries SEJ, Lecoq JA, Buice MA, Groblewski PA, Ocker GK, Oliver M, Feng D, Cain N, Ledochowitsch P, Millman D, Roll K, Garrett M, Keenan T, Kuan L, Mihalas S, Olsen S, Thompson C, Wakeman W, Waters J, Williams D, Barber C, Berbesque N, Blanchard B, Bowles N, Caldejon SD, Casal L, Cho A, Cross S, Dang C, Dolbeare T, Edwards M,

Galbraith J, Gaudreault N, Gilbert TL, Griffin F, Hargrave P, Howard R, Huang L, Jewell S, Keller N, Knoblich U, Larkin JD, Larsen R, Lau C, Lee E, Lee F, Leon A, Li L, Long F, Luviano J, Mace K, Nguyen T, Perkins J, Robertson M, Seid S, Shea-Brown E, Shi J, Sjoquist N, Slaughterbeck C, Sullivan D, Valenza R, White C, Williford A, Witten DM, Zhuang J, Zeng H, Farrell C, Ng L, Bernard A, Phillips JW, Reid RC, Koch C

A large-scale standardized physiological survey reveals functional organization of the mouse visual cortex.

To understand how the brain processes sensory information to guide behavior, we must know how stimulus representations are transformed throughout the visual cortex. Here we report an open, large-scale physiological survey of activity in the awake mouse visual cortex: the Allen Brain Observatory Visual Coding dataset. This publicly available dataset includes the cortical activity of nearly 60,000 neurons from six visual areas, four layers, and 12 transgenic mouse lines in a total of 243 adult mice, in response to a systematic set of visual stimuli. We classify neurons on the basis of joint reliabilities to multiple stimuli and validate this functional classification with models of visual responses. While most classes are characterized by responses to specific subsets of the stimuli, the largest class is not reliably responsive to any of the stimuli and becomes progressively larger in higher visual areas. These classes reveal a functional organization wherein putative dorsal areas show specialization for visual motion signals.

Nat Neurosci, 2020; 23

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INVESTIGATING SEX DIFFERENCES IN THE DEVELOPING BRAIN OF MICE USING THE SEX CHROMOSOME TRISOMY (SCT) MOUSE MODEL.

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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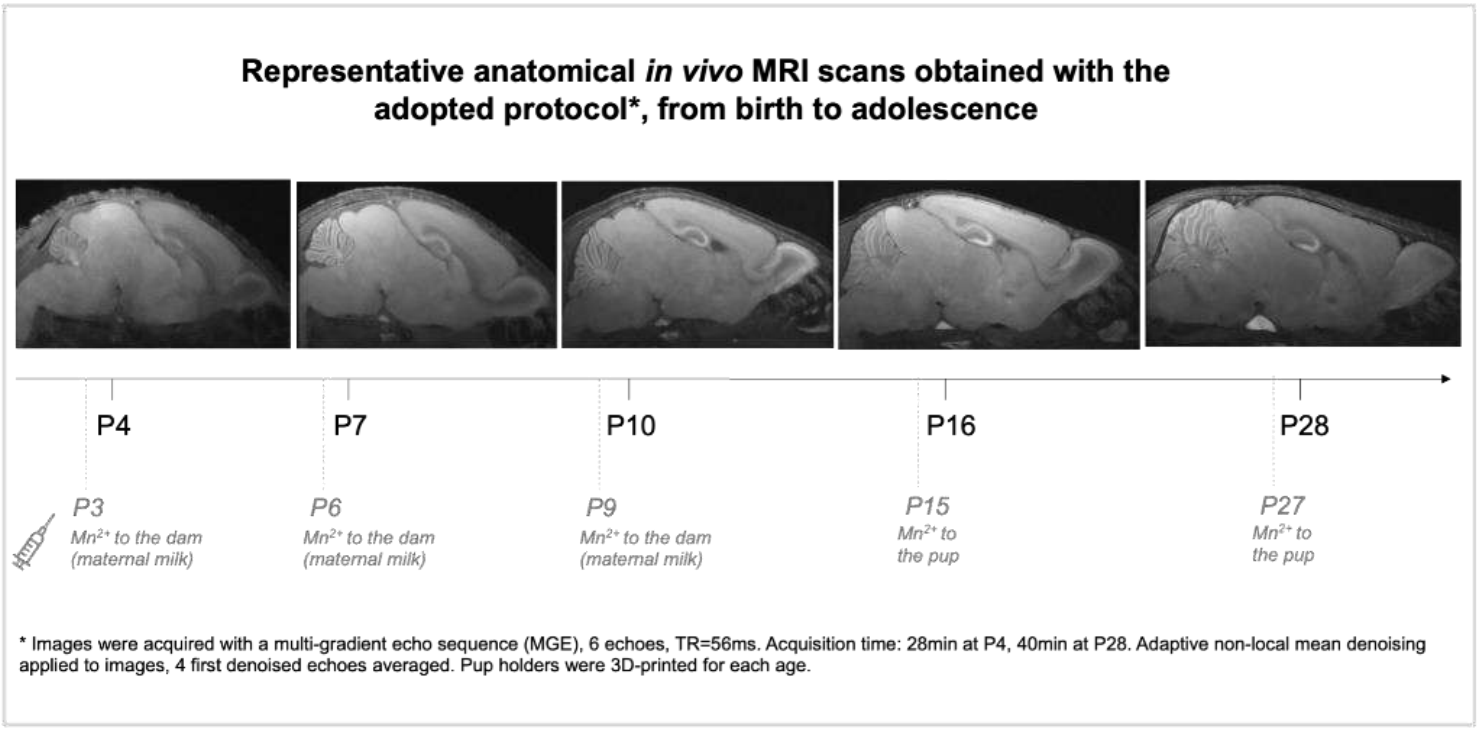
Background and aims How sex chromosomes and steroids interact to shape the sexually dimorphic brain during development has, to date, only been studied in a few brain areas. We have shown that high-field MRI can map the development of sex dimorphisms across the entire mouse brain (*Qiu et al., 2018*). We have also investigated sex differences in adult brain structure with MRI using the Four Core Genotype (FCG) model, separating out the effects of base XX vs XY chromosomes from gonadal factors (*Corre et al., 2016*). Here we propose to establish the impact of gonadal hormones and sex chromosome dosage on brain development from early postnatal days (P) using anatomical imaging. To this end we will image the Sex Chromosome Trisomy (SCT) mouse model, which allows for the investigation of the effects of sex chromosome dosage, comparing gonadal male and female mice with different combinations of sex chromosomes (XX, XY, XXY, XYY). **Methods** The 8 genotypes of the SCT mice (n=10 mice/genotype) will be imaged in 7 developmental timepoints (P4, P7, P10, P17, P23, P33 and P65) with high-resolution MRI at 7T (MGE, 6 echoes, 60μm isotropic). To optimise contrast, Mn^{2+}will be administered to pups 24h prior imaging. **Results/Conclusions** To date, a scanning protocol has been successfully optimised, while the experimental setup for neonatal studies has been established. By the end of the study and following the longitudinal MRI analysis, structural changes in the basis of the age, gonadal sex and sex chromosome dosage will be
established.

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NEUROENDOCRINE MECHANISMS GOVERNING SEX DIFFERENCES IN ARCUATE NUCLEUS NEURONS SIGNALING FOR PROLACTIN RELEASE CONTROL

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

Stanislav Cherepanov[1], Pierre Fontanaud[2], Mari Aoki[3], Ulrich Boehm[3], Dave Grattan[4], Patrice Mollard[1], Agnès Martin Martin[1,2]
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Prolactin (PRL) secretion is involved in the regulation of numerous functions depending on the target it reaches. A number of physiological functions involve feedback of the hormone in the brain. We investigate the impact of PRL on the best-known central target of PRL, the arcuate nucleus. This hypothalamic nucleus contains several populations of PRL-sensitive neurons and in particular the dopaminergic neurons of the arcuate nucleus (TIDA) that are the major regulator of PRL secretion via inhibition of its release by lactotroph cells of the pituitary. By in vivo imaging, we showed that both TIDA cells display sexually dimorphic spontaneous and PRL-induced activities, with a robust spontaneous activity in males, significantly reduced in females. This sexual dimorphism is absent in acute slices. To investigate the sensitivity of TIDA to PRL, PRL was bath applied on slices. It induced reorganization of calcium activity with the recruitment of coactive cells displaying persisting rhythmic oscillations both in males and females. Single PRL i.p injections, in vivo, induced a similar reorganization of the functional network in males and a temporary increase in activity of female TIDA neurons. Long-term modifications of the levels of circulating PRL by pharmacology in vivo, suggest that the in vivo sexual dimorphism of PRL concentration in the blood orchestrates the TIDA network properties. Altogether our results show that TIDA neurons behave as a PRL sensitive network and that the origin of the sexual dimorphism of PRL secretion is probably not encoded by TIDA neurons of the arcuate themselves.

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TWO ENDOPHENOTYPES OF ANOREXIA NERVOSA BASED ON CLINICAL FACTORS RELATED TO PHYSICAL ACTIVITY AND LEPTIN LEVELS.

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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A frequent symptom of patients with anorexia nervosa (AN) is increased physical activity and excessive exercises. In recent years, this phenomenon has been investigated in rats in Activity Based Anorexia model. In this study we have focused on the relationship between activity, leptin, ghrelin, hunger and anxiety. In 30 patients with AN (age 23, BMI = 14.93 $kg.m^{-2}$), we measured the physical activity using Actiwatch Score, which the patients wore for non-dominant hand for three days. The patient's subjective intensity of hunger and anxiety was recorded in the watch at 2-hour intervals from 8 am to 8 pm. In the whole group, only leptin correlated with physical activity ($r = 0.52$, $p = 0.003$), but this relationship was rather parabolic, suggesting a negative correlation in patients with lower activity (LA) and positive correlation in patients with higher activity (HA). After dividing the group according to the median of physical activity, it has been shown that in the LA group the amount of activity correlated negatively with hunger and positively with anxiety. In the HA group, the amount of activity correlated with BMI. Activity in LA patients may be more biologically determined for reduction of emotion-driven anxiety contrary to the HA patients, whose positive association between activity and leptin and BMI may be more under cognitive control with the intention of deliberately limiting weight gain within the therapeutic program. Supported by COOPERATIO 38

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OXYTOCIN AND LACTATIONALLY-TRIGGERED EMBRYONIC DIAPAUSE IN MICE

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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Embryonic diapause is a state in which the development of the pre-implantation embryo is paused under conditions of maternal duress, such as while nursing a previous litter. Embryonic development is halted at the blastocyst stage and implantation into the uterine endometrium is temporarily prevented, as the required estrogen surge for uterine receptivity is blocked due to an unknown mechanism. Development resumes as normal upon a return to maternal homeostasis (Renfree and Shaw, 2000). We aim to examine whether oxytocin initiates lactationally-triggered facultative diapause in mice. To model low estrogen levels and to test how this loss affects oxytocin neurons, we treated the hypothalamic cell line GT1-7 with the estrogen receptor antagonist 4-hydroxytamoxifen and saw that oxytocin mRNA levels were increased relative to saline treatment. This suggests that maternal oxytocin levels are raised in the diapause state, and that this maternally-sourced oxytocin may function as a direct cue for the embryo to halt its development. We have documented oxytocin receptor expression in mouse blastocysts using reporter mice and tested the effect of oxytocin directly on mouse blastocysts ex vivo. We found that embryo implantation in the presence of oxytocin is delayed in comparison to saline using an in vitro model of implantation (Bedzhov et al., 2014). Our findings suggest that if pregnancy occurs coincident with nursing, maternal oxytocin levels rise and may initiate the pausing of embryonic development. Understanding the role that the hypothalamic pituitary axis plays in altering the timing of embryo implantation has major implications for reproductive medicine.

Pubmed:
34381215: Carcea I, Caraballo NL, Marlin BJ, Ooyama R, Riceberg JS, Mendoza Navarro JM, Opendak M, Diaz VE, Schuster L, Alvarado Torres MI, Lethin H, Ramos D, Minder J, Mendoza SL, Bair-Marshall CJ, Samadjopoulos GH, Hidema S, Falkner A, Lin D, Mar A, Wadghiri YZ, Nishimori K, Kikusui T, Mogi K, Sullivan RM, Froemke RC
Oxytocin neurons enable social transmission of maternal behaviour.
Maternal care, including by non-biological parents, is important for offspring survival. Oxytocin, which is released by the hypothalamic paraventricular nucleus (PVN), is a critical maternal hormone. In mice, oxytocin enables neuroplasticity in the auditory cortex for maternal recognition of pup distress. However, it is unclear how initial parental experience promotes hypothalamic signalling and cortical plasticity for reliable maternal care. Here we continuously monitored the behaviour of female virgin mice co-housed with an experienced mother and litter. This documentary approach was synchronized with neural recordings from the virgin PVN, including oxytocin neurons. These cells were activated as virgins were enlisted in maternal care by experienced mothers, who shepherded virgins into the nest and demonstrated pup retrieval. Virgins visually observed maternal retrieval, which activated PVN oxytocin neurons and promoted alloparenting. Thus rodents can acquire maternal behaviour by social transmission, providing a mechanism for adapting the brains of adult caregivers to infant needs via endogenous oxytocin.
Nature, 2021; 596
32855428: Zheng Q, Jones FK, Leavitt SV, Ung L, Labrique AB, Peters DH, Lee EC, Azman AS,
HIT-COVID, a global database tracking public health interventions to COVID-19.
The COVID-19 pandemic has sparked unprecedented public health and social measures (PHSM) by national and local governments, including border restrictions, school closures, mandatory facemask use and stay at home orders. Quantifying the effectiveness of these interventions in reducing disease transmission is key to rational policy making in response to the current and future pandemics. In order to estimate the effectiveness of these interventions, detailed descriptions of their timelines, scale and scope are needed. The Health Intervention Tracking for COVID-19 (HIT-COVID) is a curated and standardized global database that catalogues the implementation and relaxation of COVID-19 related PHSM. With a team of over 200 volunteer contributors, we assembled policy timelines for a range of key PHSM aimed at reducing COVID-19 risk for the national and first administrative levels (e.g. provinces and states) globally, including details such as the degree of

implementation and targeted populations. We continue to maintain and adapt this database to the changing COVID-19 landscape so it can serve as a resource for researchers and policymakers alike.
Sci Data, 2020; 7
28864972: Mitre M, Minder J, Morina EX, Chao MV, Froemke RC
Oxytocin Modulation of Neural Circuits.
Oxytocin is a hypothalamic neuropeptide first recognized as a regulator of parturition and lactation which has recently gained attention for its ability to modulate social behaviors. In this chapter, we review several aspects of the oxytocinergic system, focusing on evidence for release of oxytocin and its receptor distribution in the cortex as the foundation for important networks that control social behavior. We examine the developmental timeline of the cortical oxytocin system as demonstrated by RNA, autoradiographic binding, and protein immunohistochemical studies, and describe how that might shape brain development and behavior. Many recent studies have implicated oxytocin in cognitive processes such as processing of sensory stimuli, social recognition, social memory, and fear. We review these studies and discuss the function of oxytocin in the young and adult cortex as a neuromodulator of central synaptic transmission and mediator of plasticity.
Curr Top Behav Neurosci, 2018; 35
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Sensitivity and engineered resistance of myeloid leukemia cells to BRD9 inhibition.
Here we show that acute myeloid leukemia (AML) cells require the BRD9 subunit of the SWI-SNF chromatin-remodeling complex to sustain MYC transcription, rapid cell proliferation and a block in differentiation. Based on these observations, we derived small-molecule inhibitors of the BRD9 bromodomain that selectively suppress the proliferation of mouse and human AML cell lines. To establish these effects as on-target, we engineered a bromodomain-swap allele of BRD9 that retains functionality despite a radically altered bromodomain pocket. Expression of this allele in AML cells confers resistance to the antiproliferative effects of our compound series, thus establishing BRD9 as the relevant cellular target. Furthermore, we used an analogous domain-swap strategy to generate an inhibitor-resistant allele of EZH2. To our knowledge, our study provides the first evidence for a role of BRD9 in cancer and reveals a simple genetic strategy for constructing resistance alleles to demonstrate on-target activity of chemical probes in cells.
Nat Chem Biol, 2016; 12
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Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation.
Cancer cells frequently depend on chromatin regulatory activities to maintain a malignant phenotype. Here, we show that leukemia cells require the mammalian SWI/SNF chromatin remodeling complex for their survival and aberrant self-renewal potential. While Brg1, an ATPase subunit of SWI/SNF, is known to suppress tumor formation in several cell types, we found that leukemia cells instead rely on Brg1 to support their oncogenic transcriptional program, which includes Myc as one of its key targets. To account for this context-specific function, we identify a cluster of lineage-specific enhancers located 1.7 Mb downstream from Myc that are occupied by SWI/SNF as well as the BET protein Brd4. Brg1 is required at these distal elements to maintain transcription factor occupancy and for long-range chromatin looping interactions with the Myc promoter. Notably, these distal Myc enhancers coincide with a region that is focally amplified in ~3% of acute myeloid leukemias. Together, these findings define a leukemia maintenance function for SWI/SNF that is linked to enhancer-mediated gene regulation, providing general insights into how cancer cells exploit transcriptional coactivators to maintain oncogenic gene expression programs.
Genes Dev, 2013; 27
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Sex-Specific Differences in Oxytocin Receptor Expression and Function for Parental Behavior.
Parental care is among the most profound behavior expressed by humans and other animals. Despite intense interest in understanding the biological basis of parental behaviors, it remains unknown how much of parenting is encoded by the genome and which abilities instead are learned or can be refined by experience. One critical factor at the intersection between innate behaviors and experience-dependent learning is oxytocin, a neurohormone important for maternal physiology and neuroplasticity. Oxytocin acts throughout the body and brain to promote prosocial and maternal behaviors and modulates synaptic transmission to affect neural circuit dynamics. Recently we developed specific antibodies to mouse oxytocin receptors, found that oxytocin receptors are left lateralized in female auditory cortex, and examined how oxytocin enables maternal behavior by sensitizing the cortex to infant distress sounds. In this study we compare oxytocin receptor expression and function in male and female mice. Receptor expression is higher in adult female left auditory cortex than in right auditory

cortex or males. Developmental profiles and mRNA expression were comparable between males and females. Behaviorally, male and female mice began expressing parental behavior similarly after cohousing with experienced females; however, oxytocin enhanced parental behavior onset in females but not males. This suggests that left lateralization of oxytocin receptor expression in females provides a mechanism for accelerating maternal behavior onset, although male mice can also effectively co-parent after experience with infants. The sex-specific pattern of oxytocin receptor expression might genetically predispose female cortex to respond to infant cues, which both males and females can also rapidly learn.
Gend Genome, 2017; 1

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MPGES-1 DEFICIENT RATS LACK LPS-INDUCIBLE SUPPRESSION OF PULSATILE SECRETION OF LUTEINIZING HORMONE

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

Takashi Matsuwaki, Keitaro Yamanouchi
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The function of the mammalian reproductive system is controlled by hypothalamus-pituitary-gonadal (HPG) axis, which is suppressed under infectious stress conditions. We have previously demonstrated the possible role of prostaglandin E_2 (PGE_2) in the CNS to mediate the suppressive effect of immune signals. On the other hand, PGE_2 is known to enhance the activity of the HPG axis, inducing the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus and luteinizing hormone (LH) from the pituitary, when injected intracerebroventricularly. Accordingly, we have established a line of knockout (KO) rats lacking the inducible PGE_2 synthase, microsomal prostaglandin E synthase-1 (mPGES-1) for further investigation of the role of endogenous PGE_2 in the maintenance of the reproductive function. As an infectious stressor, lipopolysaccharide (LPS, 0.1 mg/kg) was injected into the animals. Three hours after LPS injection, PGE_2 concentration in the CSF has significantly increased in the wild-type (WT) rats but not in the KO rats. Subsequently, a blood sample was withdrawn every 5 minutes through the indwelling jugular cannula for 3 hours and LPS was administered 1 hour after starting sampling through the cannula. In the WT rats, LPS strongly suppressed the pulsatile secretion of LH, which is correspondent with the previous studies. In contrast, LPS did not affect the LH pulsatility of the KO rats. Those results suggest that PGE_2 is the key mediator of infectious stress to suppress reproductive function.

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TRH NEURONS IN ENERGY HOMEOSTASIS AND REGULATION OF BROWN ADIPOSE TISSUE

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

Andreea Constantinescu
University of Lübeck, Institute Of Experimental And Clinical Pharmacology And Toxicology, Lübeck, Germany

Neurons containing thyrotropin releasing hormone (TRH) in the paraventricular hypothalamus (PVN) are a key component of the hypothalamic-pituitary-thyroid (HPT) axis and have been well characterized along with their projections to the median eminence (ME). Despite the existence of TRH neuron populations throughout the brain, these remain little described. We focus on the effect of TRH neurons in the dorsomedial hypothalamus (DMH) and medial preoptic area (MPA) on brown adipose tissue (BAT) thermogenesis, food intake, and energy expenditure, by using a strategy involving transgenic mice and AAV-based chemogenetic tools in combination with calorimetric measurements.
Stimulation of TRH neurons in the PVN, DMH, and MPA increased energy expenditure, respiratory exchange rate and feeding behavior. However, only stimulation of TRH neurons in the PVN led to increased T3 and T4 levels along with BAT activation, while DMH stimulation failed to increase T4 levels and MPA stimulation showed no effect on these parameters. BAT activation depended on beta-adrenergic signaling as shown by administering a β_3 adrenergic receptor (β_3-AR) blocker. We show functional differences of TRH neurons in the PVN, DMH and MPA regarding the stimulation of BAT thermogenesis, in a β_3-AR-dependent manner. We confirm that TRH signaling plays a pivotal role in the regulation of food intake and subsequent energy expenditure. Furthermore, our findings show that these effects are separable from the action of TRH on the HPT axis through projections to the median eminence.

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HYPOTHALAMIC PACEMAKING MECHANISM THAT DRIVES ACCLIMATION-INDUCED HEAT RESILIENCE.

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

Wojciech Ambroziak, Sara Nencini, Joerg Pohle, Kristina Zuza, Jan Siemens
University of Heidelberg, Pharmacology, Heidelberg, Germany

Thermal acclimation is an important adaptive process allowing homeothermic organisms to adapt to long-lasting changes in ambient temperature. Due to increasing environmental temperatures world-wide, heat acclimation is an especially interesting aspect of such adaptation. While the biology of heat acclimation is not well understood, the present study aims to elucidate central neuronal components of adaptation to heat in a mouse model. We have recently identified a discreet population of hypothalamic preoptic area (POA) neurons, marked by the expression of the Leptin receptor, that fundamentally change their electrophysiological profile over the course of heat acclimation to eventually become intrinsically-driven “pacemaker”-like neurons. We found this phenotype shift to be vital for mice to become heat-resilient and therefore we refer to this neuronal population as acclimation-activated neurons (AANs). POA is known to contain the so-called warm-responsive neurons (WRNs) that express the immediate-early gene Fos upon acute warm stimuli. While we hypothesized that AANs could to a large extent comprise of WRNs, our results suggest that AAN and WRN populations do not overlap significantly, and AANs likely do not receive extensive direct synaptic input from the spinoparabrachial tract. We then used electrophysiology to disentangle the ionic mechanism behind the intrinsic tonic firing in AANs; we found that a combination of sodium “leak”-driven depolarization bias and enhanced subthreshold NaV currents largely passed by the NaV1.3 ion channel drive the pacemaker-like cellular phenotype. In conclusion, we identified a functionally novel neuronal population that plastically changes its electrophysiological properties upon long-lasting heat exposure to promote heat resilience**.**

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ROLE OF TRPM8 IN THERMOREGULATION AND THERMOGENESIS IN NAKED MOLE-RAT

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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The subterranean dwelling naked mole-rat (*Heterocephalus glaber*, NM-R) has received much attention for their extreme and unique physiology. One unique feature of amongst mammals is the fact that NM-Rs fail to maintain their body temperature when exposed to temperatures outside their thermoneutral zone, and are therefore defined as poikilotherms. In mammals, temperature-sensitive primary sensory neurons detect environmental temperature and transmit thermal information from the skin and peripheral organs to the central nervous system. The transient receptor potential melastatin 8 (TRPM8) ion channel is a major sensor of environmental cold temperatures in mammals. So far little is known about TRPM8 functions in NM-Rs. To clarify whether TRPM8 plays a role in thermoreception and thermoregulation in NM-Rs, we applied RNAscope, high-resolution fluorescence imaging and molecular biology approaches. As predicted by protein sequence alignment we found that NM-R TRPM8 cDNA has an extended N-terminal part, not found in other rodents or mammals. RNA sequencing and RNAscope data showed a broad expression pattern of TrpM8 mRNA in NM-Rs in particular in the grey matter of the spinal cord. In contrast, TrpM8 expression in mice is largely restricted to sensory ganglia. This preliminary data suggests that TRPM8 channel might play a different role in thermoreception and thermoregulation in NM-Rs compared to other rodents.

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ASTROCYTIC-BMAL1 CONTROLS METABOLIC HOMEOSTASIS AND CIRCADIAN BEHAVIOR IN A SEX AND DIET-DEPENDENT MANNER

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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Energy expenditure, glucose homeostasis, and thermogenesis has been strongly associated with circadian clock gene expression in mammals. Astrocytes autonomously function as a central pacemaker regulating molecular and behavioral circadian rhythms. Remarkably, the effects of deletion of astrocytic BMAL1 on circadian locomotor activity, cognition, and its metabolic disturbances including body weight and glucose homeostasis were just reported in male mice. Here we show that selective ablation of BMAL1 from astrocytes induces sexual dimorphic alterations in circadian behavior, energy homeostasis, glucose tolerance, and thermogenesis in mice. Specifically, while male mutants displayed altered glucose homeostasis and increased body weight with age, female mice showed decreased body weight despite increased food intake and normal spontaneous locomotor activity. Moreover, female mutants had increased energy expenditure and brown adipose tissue thermogenesis. Remarkably, in a high-fat diet, this metabolic phenotype of the females was reverted and showed increased body weight and respiratory quotient, altered glucose homeostasis, and normal thermogenesis. In sum, our results indicate that the astrocyte clock is required to optimize energetic resources and thermogenesis in a sex- and diet-specific manner in mice.

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LINKING MITOCHONDRIAL G-PROTEIN SIGNALING TO CANNABINOIDS-INDUCED AMNESIA: A NEW MITOCHONDRIA-SPECIFIC CHEMOGENETIC TOOL…

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

Antonio Pagano Zottola[1], Rebeca Martin-Jimenez[2], Gianluca Lavanco[1], Yamuna Mariani[1], Astrid Cannich[3], Francisca Julio-Kalajzic[4], Doriane Gisquet[1], Giovanni Marsicano[4], Etienne Hebert Chatelain[2], Luigi Bellocchio[4]
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Direct bioenergetic effects of cannabinoids (via CB1 receptor present on mitochondrial membranes) play a central role on brain mitochondrial activity and memory. The activation of intra-mitochondrial Gαi proteins by exogenous agonists of CB1 leads to a decrease of brain mitochondrial activity, which is responsible for cannabinoids-induced amnesia. However, the direct evidence on how the modulation of mitochondrial G-protein signaling can affect the mitochondrial bioenergetic and cognitive processes is still missing. To this aim, we generated and characterized a novel chemogenetic tool (MLS-DREADD-Gs) which allow us to manipulate mitochondrial Gs activity in vitro an in vivo. This tool was able to increase mitochondrial activity and counteract the inhibitory effects of CB1 activation on mitochondrial respiration. Thus, given the relevance of mitochondrial G-protein signaling and bioenergetic processes in behavioral cognitive aspects, we evaluated how in vivo MLS-DREADD-Gs activation can interfere with the effects of cannabinoids on memory consolidation. This study extends our knowledge on direct link between the impact of cannabinoids on brain mitochondrial signaling and some of their most important intracellular and behavioral effects, such as cellular respiration and amnesia respectively. In particular, it introduces an innovative pharmaco-genetic tool which can be used to manipulate intra-mitochondrial GPCRs signaling with relevant impacts on mitochondrial bioenergetics processes and relevant brain functions as memory.

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ACUTE ENDURANCE EXERCISE MODULATES CEREBROSPINAL FLUID AND PLASMA METABOLOME IN RELATION TO COGNITIVE FUNCTIONS IN HEALTHY YOUNG INDIVIDUALS.

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

Barbara Ukropcova[1,2], Kefeng Li[3], Martin Schon[2], Jane Naviaux[3], Jonathan Monk[3], Nikoleta Alchus Laiferova[1,2], Lin Wang[3], Igor Straka[4], Peter Matejicka[4], Peter Valkovic[4], Mark Tarnopolsky[5], Robert Naviaux[3], Jozef Ukropec[2]
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Background: Physical activity enhances brain plasticity and improves cognition. A complex exercise-induced adaptive response is coordinated by network of molecules, including metabolites, which represent both energy substrates and signaling molecules. **The aim** of our study was to identify changes in metabolome before & after 90-minute run in plasma and cerebrospinal fluid (CSF), and to explore relationships between CSF & plasma metabolome and cognitive functions. **Methods:** Study population: 19 healthy young adults (M/F 13/6; median age 25(IQR 22-31)yrs, BMI 23.2(IQR 21.7-24.5)kg/m^2 &VO_2max 47(IQR 38.1-51.2)mL/kg/min). Sampling was performed before (CSF/plasma), immediately after (plasma) and 1-hour after (CSF/plasma) 90-minute run. Targeted broad-spectrum metabolomics was performed (HPLC-TMS). Cognitive functions were assessed by (MemTrax, Cogstate) before & after run. **Results:** In CSF, purines & pyrimidines changes represented 32% and neurotransmiter changes 14% of the metabolic impact. Around 25% of top regulated metabolites in CSF decreased and 75% increased after run. In plasma, metabolites of fatty acids accounted for 46%-60% of the metabolic impact immediately & 1-hour after run, respectively. There was a limited number of associations between metabolites in CSF and plasma, indicating a narrow window of communication between brain and periphery. Specific CSF metabolites were correlated to cognitive functions and their running-induced change. **Conclusions:** An acute bout of physical exercise distinctly regulated metabolite profile in cerebrospinal fluid. Running-induced changes of specific metabolites and cognitive functions were related, indirectly indicating the role of metabolites & neurotransmitters in the adaptive response to endurance exercise. Funding: SAS-MOST-JRC2018/10, APVV-20-0466, COST-CA19101, UCSD_ChristiniFund, Lennox-Foundation, JMS-Fund, Malone-FF, Westreich-Foundation, Daniel&Kelly-White-Family, UCSD-MRF

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MIRNA-186-5P – A NEW CULPRIT OF CHRONIC STRESS-INDUCED SYNAPTIC DYSFUNCTION

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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Exposure to chronic stress (CS) represents a major risk for mental illnesses. In the prefrontal cortex (PFC), CS leads to synaptic alterations, which consequently disrupt PFC-dependent functions. However, the molecular targets of CS ensuing synaptic dysfunctions remain largely unknown. miRNAs are implicated in brain (dys)function, including the stress response, and are ideally suited to trigger the complex changes in gene expression underlying the synaptic and behavioural effects of CS. miR-186-5p is increased in the brain of stressed animals and our data show both a stress hormone-induced increase in miR-186-5p levels and miR-186-5p-dependent silencing of synaptic glutamate receptors and transmission in cultured neurons. Hence, we hypothesize that, by silencing critical transcripts for synaptic function, upregulated miR-186-5p orchestrates essential features of the synaptic and behavioural responses to CS. By assessing the effects of prolonged glucocorticoid receptor (GR) activation on cortical cultures, we found a reduction on excitatory synapse number, alongside with a decrease on GluA2-containing AMPAR clusters and AMPAR-mediated currents, which correlates with the miR-186-5p-induced switch on AMPAR composition. Furthermore, miR-186-5p inhibition during GR activation reverted the maladaptive response on AMPAR levels and synaptic transmission. Since functional alteration of excitatory transmission are closely related with anxiety and cognitive impairments, we are currently assessing, in chronically stressed mice, a relationship between anxious behaviours, PFC-dependent cognition and miR-186-5p levels and synaptic function. This work will provide new mechanistic insights into CS-induced changes in PFC neurotransmission and related behavioural outputs, identifying key molecular players in maladaptive brain response to CS, pointing to future therapeutic targets.

Pubmed:
30808806: Silva MM, Rodrigues B, Fernandes J, Santos SD, Carreto L, Santos MAS, Pinheiro P, Carvalho AL
MicroRNA-186-5p controls GluA2 surface expression and synaptic scaling in hippocampal neurons.
Homeostatic synaptic scaling is a negative feedback response to fluctuations in synaptic strength induced by developmental or learning-related processes, which maintains neuronal activity stable. Although several components of the synaptic scaling apparatus have been characterized, the intrinsic regulatory mechanisms promoting scaling remain largely unknown. MicroRNAs may contribute to posttranscriptional control of mRNAs implicated in different stages of synaptic scaling, but their role in these mechanisms is still undervalued. Here, we report that chronic blockade of glutamate receptors of the AMPA and NMDA types in hippocampal neurons in culture induces changes in the neuronal mRNA and miRNA transcriptomes, leading to synaptic upscaling. Specifically, we show that synaptic activity blockade persistently down-regulates miR-186-5p. Moreover, we describe a conserved miR-186-5p-binding site within the 3'UTR of the mRNA encoding the AMPA receptor GluA2 subunit, and demonstrate that GluA2 is a direct target of miR-186-5p. Overexpression of miR-186 decreased GluA2 surface levels, increased synaptic expression of GluA2-lacking AMPA receptors, and blocked synaptic scaling, whereas inhibition of miR-186-5p increased GluA2 surface levels and the amplitude and frequency of AMPA receptor-mediated currents, and mimicked excitatory synaptic scaling induced by synaptic inactivity. Our findings elucidate an activity-dependent miRNA-mediated mechanism for regulation of AMPA receptor expression.
Proc Natl Acad Sci U S A, 2019; 116

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SEXUALLY-DIMORPHIC CELL AND GENE REGULATORY NETWORKS IN THE MEDIAN-EMINENCE AND PERIVENTRICULAR ZONE OF THE TUBERAL REGION OF THE HYPOTHALAMUS

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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Despite our knowledge on diet-induced plasticity at the blood-hypothalamus barrier, much remains to be uncovered in terms of transcriptional activity and sexual-dimorphism, a critical but rarely studied parameter. In this light, we sought to determine how the brain integrates and responds to sexually-dimorphic metabolic signals at the cellular and molecular level,and understand marked sex-specific differences under metabolic perturbations such as obesity. Using single-cell RNA sequencing, we studied cell and gene regulatory networks in the median-eminence and the periventricular (ME-PV) zone of the tuberal hypothalamus, the key brain region influencing food-intake and energy homeostasis, in wild-type C57BL/6J adult male and female mice fed with high-fat diet (HFD,60%) and standard-diet for ten-weeks. The body-weight composition and glucose-tolerance were measured for both male and female mice, based on which HFD-sensitive and resistant mice were grouped. Estrous stage of female mice was recorded daily to determine their cyclicity. Single-cell RNA sequencing of microdissected ME-PV region (from chow and HFD-fed male and female mice including chow-fed female mice at different stages of estrous cyclicity) and subsequent analysis resulted in 35 cell clusters comprising of 46,161 cells that were annotated based on the top marker genes, with astrocytes, tanycytes, endothelial cells and oligodendrocytes forming major cell-populations, and revealing distinct and yet unidentified sexually-dimorphic and HFD-induced gene expression profiles. Together, these results represent a comprehensive resource demonstrating ME-PV cell types and sex-specific gene expression dynamics in response to ovarian cycle in standard-diet fed female mice as well as sex-specific long term HFD-fed male and female mice.

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THE KISSPEPTIN AND NNOS INTERPLAY IN THE RHYTHMICAL SHAPING OF GNRH RELEASE: THE KING OF REPRODUCTION.

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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Normal reproductive function requires the precise temporal and quantitative regulation of gonadotropin-releasing hormone (GnRH) secretion at all levels of the hypothalamic–pituitary–gonadal (HPG) axis. GnRH control depends on a multidimensional cellular network involving among others, kisspeptin, the main GnRH stimulator, and NO, the only known negative regulator of GnRH. We hypothesize that kisspeptin and NO-releasing neurons (nNOS; NOS1) may form a neuronal microcircuit responsible for rhythmically shaping GnRH release. RNAscope is used to identify the expression pattern of *Kiss1*, Kiss1 receptor and *Nos1* mRNAs in female mouse hypothalamus. Chemogenetics are used to selectively manipulate kisspeptin and nNOS afferences *in vivo*. The concentration of NO being released is measured *in vivo* and *ex vivo* using a newly-designed cGMP biosensor. Patch-clamp approach is applied on $Nos1^{KO}$::$GnRH^{gfp}$ mouse model in order to decipher the dynamics of the KiNG network. nNOS and kisspeptin are distinct neuronal populations, found to anatomically associate and interact with GnRH neurons in the mediobasal hypothalamus (MBH) and preoptic area (POA). *Kiss1R* is found expressed by nNOS neurons and kisspeptin-induced nNOS activation is observed in both MBH and POA. Kisspeptin is able to stimulate the production of NO in a dose dependent manner, through nNOS functioning. Kisspeptin and nNOS interplay leads to the generation of GnRH pulses and surges, crucial for the proper function of reproduction. We postulate that kisspeptin and nNOS create a dynamic network, where kisspeptin provides the “ON” signal, promoting GnRH release, while NO mediates the “OFF” signal, acting as a tonic brake on GnRH secretion.

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EFFECTS OF CHEMOGENETIC MODULATION OF HYPOTHALAMIC ARCUATE NUCLEUS KISSPEPTIN NEURONS ON FOLLICULOGENESIS IN KISS-CRE POLYCYSTIC OVARIAN SYNDROME MOUSE MODEL

POSTER SESSION 01 - SECTION: THERMOREGULATION, ENERGY HOMEOSTASIS AND HORMONES

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Aims: Polycystic Ovarian Syndrome (PCOS) is a complex endocrine disease and characterized with oligo/amenorrhea, hyperandrogenemia, hirsutism, chronic anovulation and cystic structures. We aimed to investigate effects of chemogenetic manipulations of kisspeptin neurons in the hypothalamic arcuate nucleus (ARC) on folliculogenesis in Kiss-Cre PCOS mouse model.
Methods: Female Kiss-Cre mice were used. For chemogenetic manipulations, hM3D receptor genes were injected intracranially into the ARC using adeno-associated virus. Pre-pubertal Kiss-cre mice were daily injected subcutaneous with DHEA (6mg/100g body weight for 20 consecutive days). Chronic stimulation was performed by administration of CNO (160µg/5ml) to the drinking water of the mice for four days. After chronic activation, ovaries were isolated. Hematoxylin-eosin staining was performed for morphological evaluation. Alterations in the electrical activity of neurons were measured by electrophysiology patch clamp technique. Data was analyzed by using Student's t-test.
Results: Follicular morphology was impaired in the ovaries of kisspeptin-activated mice. Atretic follicles increased and non-ovulated follicular cysts were detected, and corpus luteum (CL) structure was not observed in the PCOS group. In the chronic activation group, atretic follicles were also present in the ovaries, but follicular cysts were not observed. However, the CL structure was not determined in the activation group. The firing frequency of kisspeptin neurons did not significantly change between the groups.
Conclusions: Our results suggest that the cystic structures formed in the PCOS model regressed after chronic activation of ARC-kisspeptin neurons. However, it was determined that the absence of CL in the ARC-kisspeptin group was not sufficient to induce ovulation.
Acknowledgement: This study was supported by TUBITAK (Project # 219S554).
Keywords: Kisspeptin, Arcuate Nucleus, PCOS, Chemogenetics, Electrophysiology, DHEA.

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BOARD NUMBER: S01-322

CELL PLASTICITY OF NEUROPEPTIDERGIC SYSTEMS IN THE MOUSE HYPOTHALAMUS

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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The neuropeptides oxytocin (OXT) and arginine vasopressin (AVP) regulate brain-body homeostasis and complex behaviors, such as maternal and social behavior. Both neuropeptides are synthetized at specific hypothalamic nuclei, mainly at paraventricular and supraoptic nucleus (PVN and SON). Our previous work (Madrigal & Jurado, 2021) revealed a significant number of neurons co-expressing OXT and AVP during early postnatal stages coinciding with a critical period for social interaction. This mixed population drastically declines in the adult brain suggesting that a switch in neuropeptide expression is required for neuronal maturation. Here, we have analyzed the plastic properties of OXT circuits in the adult brain using tissue clearing techniques (iDISCO$^+$) and 3D imaging. Our study has revealed region-specific cell plasticity in several hypothalamic nuclei in response to sexual experience, motherhood, and aging. Our data indicate higher OXT level in females, which seemed significantly increased after parturition in several hypothalamic nuclei, particularly the SON, with some regions exhibiting the expression of additional neuromodulators, suggesting changes in the internal program of a distinct population of OXT neurons. Furthermore, natural aging also induced plastic changes in the oxytocinergic system by reducing the number of OXT-expressing cells in rostral hypothalamic areas. Our findings revealed new information relevant to understand some of the key properties and temporal dynamics of neuropeptidergic circuits´ adaptations during development, motherhood, and aging.

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BOARD NUMBER: S01-323

EVALUATION OF THE CIRCADIAN EXPRESSION OF OREXIN RECEPTORS IN THE MOUSE BRAIN BY RNASCOPE®

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Orexin A and B are wake-promoting neuropeptides that originate from hypothalamic neurons and project to diverse brain areas widespread throughout the central nervous system. There, they modulate various physiological functions via their orexin 1 (OXR1) and 2 (OXR2) receptors, including sleep-wake rhythm but also cognitive function. The expression of orexins varies over the course of a day, peaking during the active awake phase. To investigate now whether orexin receptors show circadian and region-specific expression differences as well, RNAscope® as a novel multiplex *in situ* hybridization technique was used. OXR1 and OXR2 mRNA was analyzed in subareas of the dorsal hippocampus and medial prefrontal cortex at four different timepoints over the course of 24 hours. The percentage of orexin receptor mRNA expressing cells was constant over time within brain areas, but significant expression differences between brain regions and subareas were evident. The highest percentage of OXR1 mRNA-positive cells was observed in the hilus of the dentate gyrus and the stratum pyramidale of the CA3 region, while the highest percentage of OXR2 mRNA-positive cells was seen in the stratum pyramidale of the CA1 and CA3 regions of the dorsal hippocampus. In subareas of the PFC expression of both receptor subtypes was lower. Detecting orexin receptor mRNA expression with RNAscope® provides high selectivity and great spatial resolution. The distinct expression profiles of both receptor subtypes within hippocampal subareas provide an interesting basis for future interventional studies of orexin receptor function in spatial and emotional memory.

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BOARD NUMBER: S01-324

OPIOID-INDUCED SYNAPTIC PLASTICITY IN THE LATERAL PARABRACHIAL NUCLEUS

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Background Withdrawal from opioids can induce complex neuroadaptations that underlie severe side effects, including an aversive dysphoric state. A key site for aversive behaviour is the lateral parabrachial nucleus (LPBN). μ-Opioid receptors (MORs) activation has been shown to acutely modulate LPBN synaptic transmission; however, long lasting effects are still unexplored. In this study, we aim to understand whether the LPBN is a site of opioid-induced plasticity. **Methods** We performed patch-clamp recordings in acute slices containing the LPBN from rats and evoked excitatory post synaptic currents by electrical or optogenetic stimulation. Channelrhodopsin-2 was expressed in either spinal or periaqueductal grey neurons using a viral vector. The MOR agonist DAMGO was bath applied at a low or high dose for 15 minutes, followed by abrupt withdrawal via application of the MOR antagonist CTOP. **Results** Withdrawal from low dose DAMGO induced a long term depression (LTD), while withdrawal from high dose DAMGO induced a long term potentiation (LTP). Glia inhibitor fluoroacetate and NMDA receptor antagonist D-AP5 blocked the induction of high-dose LTP, but not low-dose LTD. Low-dose LTD was widely expressed at all synapses, while high-dose LTP was induced only at spino-parabrachial synapses. **Conclusion** Here, we demonstrate a bimodal effect of opioid withdrawal on excitatory transmission in the LPBN. Low-dose LTD involves a neuronal, NMDAR-independent mechanism; high-dose LTP requires glia and NMDAR activation. These synaptic adaptations are differentially expressed in a synapse-specific manner. These novel forms of opioid-induced plasticity in the LPBN possibly contribute to the development of aversive symptoms during opioid withdrawal.

Pubmed:
31159727: Raffaelli B, Mussetto V, Israel H, Neeb L, Reuter U
Erenumab and galcanezumab in chronic migraine prevention: effects after treatment termination.
Monoclonal antibodies (mAbs) targeting the CGRP pathway are safe and efficacious therapies for the prevention of migraine. In this study we assessed the effects of discontinuation of preventive erenumab and galcanezumab treatment in patients with chronic migraine.
J Headache Pain, 2019; 20

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DIFFERENTIAL REGULATION OF CREB AND AKT BY CYCLIC AMP IN THE DEVELOPING RETINA: THE ROLE OF A1 ADENOSINE RECEPTORS

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Aims Neurotransmitters activate distinct signaling pathways and regulate specific functions during development such as survival and differentiation of neurons. We are currently studying the development of important transcription factors and signaling pathways in the developing chick retina, where previous work showed that cyclic AMP levels are regulated by dopamine and adenosine. Here we studied the effects of activation or inhibition of adenylyl cyclase on CREB and AKT phosphorylation in diferent stages of retina development. **Methods** Retinas from 10-day-old chick embryos (E10), an early stage of development, and E16, a more developed stage, were dissected and stimulated for 30 minutes in Hanks saline with forskolin (10 µM) or the adenosine A1 receptor agonist cyclohexyladenosine (CHA, 100 nM) to respectively activate or inhibit adenylyl cyclase. After the incubation period, retinas were lysed and analysed by western blotting using antibodies against phosho-CREB or phospho AKT, or the respective total proteins. **Results** Forskolin strongly stimulates CREB phosphorylation in E10 retinas, but a much smaller stimulation was observed in E16 retinas. However, in an interesting way, forskolin promotes a decrease of AKT phosphorylation in E10 or even with a higher degree in E16 retinas. Moreover, although A1 adenosine receptors are negatively coupled to adenylyl cyclase, CHA stimulates CREB and to a smaller extent Akt in E10, but not in E16 retinas, in a Src kinase, PKC and Erk-dependent way. **Conclusions** Our results indicate that cyclic AMP and its regulation by A1 adenosine receptors differentially controls diferent signaling pathways during retinal development.

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BOARD NUMBER: S01-326

TRANSIENT DEVELOPMENTAL WINDOW FOR OREXIN ACTION ON THE VISUAL THALAMUS

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

Lukasz Chrobok[1], Anna Sanetra[2], Jagoda Jeczmien-Lazur[2], Jasmin Klich[3], Katarzyna Palus-Chramiec[2], Kamil Pradel[2], Marcelina Janik[4], Mino Belle[5], Marian Lewandowski[2]
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The orexinergic system of the lateral hypothalamus is classically considered to regulate arousal, sleep/wake cycle, and feeding behaviour. New evidence suggests that it may also play a role during the development of nervous system, with orexin concentration in the brain peaking in early infancy. The aim of our study was to therefore assess the role of orexins in the developing rodent visual system. Specifically, we focused on the mouse and rat dorsolateral geniculate nucleus (DLG), as its retinorecipient thalamocortical neurons are directly responsible for image-forming vision. Here, using RT-qPCR and fluorescent *in situ* hybridisation, we showed that orexin receptor 2 (*Hcrtr2*) is transiently expressed in the DLG during development. *Hcrtr2* expression was high at postnatal day 1 (P1) but linearly declined until P19. Patch-clamp and multi-electrode array recordings in DLG slices *ex vivo* revealed that orexin A robustly activate DLG neurons at P10-16 (few days prior to and following eye opening around P13). However, both the amplitude and frequency of this response were significantly reduced after P16. This early developmental period was also accompanied by dramatic changes in the basic electrophysiology of DLG neurons (e.g., membrane potential, resistance, capacitance, and excitability), stabilising at P14-20. Together, our results revealed a possible role for orexin in early visual system development. The confined developmental window of the orexinergic effects on DLG neurons suggests that this neuropeptide may play an important role in retinothalamic synaptic maturation, at a time when robust activation of thalamocortical cells is critical for appropriate visual function in adulthood.

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NOAEL DOSE INTEREST ASSESSMENT AS AN OPTIMIZED DOSE OF OXIME IN THE TREATMENT OF ORGANOPHOSPHORUS COMPOUNDS EXPOSURE

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

Marilène Trancart, Anne-Sophie Hanak, Méliati Madi, André-Guilhem Calas
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Despite the international convention on the prohibition of chemical weapons signed in 1997, the threat of conflicts and terrorist attacks involving such weapons still exists. Among these, organophosphorus-nerve agents (NOPs) inhibit cholinesterases causing cholinergic syndrome. The reactivation of these enzymes is therefore essential to protect the intoxicated individual. However, these reactivating molecules called oximes have major drawbacks with an efficacy spectrum limited against some NOPs and a non-negligible cholinesterase inhibitor potential if administered to an inadequate dose, an effect which they are precisely supposed to mitigate. As a result, this project focused, in mice, on the assessment of HI-6 therapeutic efficacy to its NOAEL dose, the maximum dose of oxime which does not induce any observable toxic effect. After determining the HI-6 NOAEL dose by double-chamber plethysmography, the protective index against 2 NOPs were established using survival tests. A pharmacokinetic and pharmacodynamic study completed the evaluation of HI-6. The HI-6 NOAEL dose does not show any better protection compared to the dose of 100 µmol/kg conventionally used by the laboratory. On the contrary, increasing doses of HI-6 improved the protection correlated with more favorable pharmacokinetics, but by inducing a respiratory function impairment. This alteration was not explained by cholinesterase inhibition as expected.

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BOARD NUMBER: S01-328

DOWNSTREAM SIGNALING OF MUSCARINIC M4 RECEPTORS IS INFLUENCED BY RECEPTOR DENSITY AND CELLULAR ENVIRONMENT

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

Madeleine Merz[1], Charlotte Playle[1], Pia Saegmueller[1], Monika Palchaudhuri[1], Ulrike Gross[2], Daniel Ursu[1]
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Multiple muscarinic M4 receptors modulators are currently advancing in clinical development for treatment of positive symptoms in schizophrenia: agonists (KarXT by Karuna-Ph3, Heptares/Neurocrine-Ph2) or positive allosteric modulators (Emraclidine by Cerevel-Ph2). The aim of this work was to investigate M_4 receptor signaling pathways (Gi/o, Gs and Gq coupling) upon activation by a structurally diverse set of muscarinic agonists. We looked into the role receptor expression levels and cellular environment have on downstream signaling. We have used HEK-293 cells and rat primary neurons expressing human M4 receptors and measured kinetics of cAMP levels and effects on neuronal network activity. Receptor expression levels were controlled by a TET-ON system and quantified by using a radioactive binding assay. Most agonists caused a concentration dependent reduction of cAMP levels (Gi/o) at low concentrations, while inducing increase of cAMP at higher concentrations (Gs). When receptor density in HEK-293 cells was reduced we observed a less prominent coupling via Gs. In the neuronal assay most compounds showed a consistent inhibition of neuronal activity, in line with the cAMP data. A distinct group of agonists showed a specific profile, with no Gs coupling at high receptor density, partial activation at low receptor density and low to no effects in the neuronal assay. This study provides a side-by-side comparison of activity of structurally diverse M4 agonists and points to compound specific activation of GPCR intracellular signaling pathways. The data bring new insights into M4 receptor pharmacology that might lead to development of novel therapies for treatment of psychiatric diseases.

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BOARD NUMBER: S01-329

DISTINCT ROLES OF DOPAMINE AND DIRECT- AND INDIRECT-PATHWAY NEURONS IN THE TAIL OF THE STRIATUM IN THREAT MANAGEMENT IN A SEMI-NATURALISTIC FORAGING TASK

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Harvard University, Molecular And Cellular Biology, Cambridge, United States of America

In natural environments, it is critical to properly assess potential threats for appropriate behavioral choices. Here we used a semi-naturalistic foraging paradigm to model avoidance of potential threats. In this paradigm, a thirsty mouse freely goes out of a homing area and enters a foraging arena to obtain a water reward. In test sessions, a predator-like object ("monster") surges when a mouse approaches a reward. In the presence of the monster, mice failed to retrieve reward and fled into the homing area. Across trials, mice returned home earlier and earlier before the monster charged, indicating that mice learned to predict a potential threat. Over multiple monster sessions, mice gradually succeeded in acquiring reward. This task, thus, allows us to study various aspects of threat managements under threat-reward conflicts, such as threat avoidance, threat prediction and eventual overcoming. We found that ablation of dopamine neurons that project to the tail of the striatum impaired threat avoidance and prediction. Mice with ablation of direct-pathway medium spiny neurons (dMSNs) decreased avoidance of the monster, while mice with ablation of indirect-pathway medium spiny neurons (iMSNs) exhibited intact avoidance but failed to improve success rate. dMSNs were more activated in avoidance trials (failure of reward acquisition) while iMSNs were more activated in successful trials, especially in later sessions. These results indicate differential roles of dMSNs and iMSNs in threat management: avoidance and overcoming, respectively. These findings provide a basic principle of how MSNs dynamically regulate different aspects of behavior under the control of dopamine.

Pubmed:

33345774: Tsutsui-Kimura I, Matsumoto H, Akiti K, Yamada MM, Uchida N, Watabe-Uchida M
Distinct temporal difference error signals in dopamine axons in three regions of the striatum in a decision-making task.
Different regions of the striatum regulate different types of behavior. However, how dopamine signals differ across striatal regions and how dopamine regulates different behaviors remain unclear. Here, we compared dopamine axon activity in the ventral, dorsomedial, and dorsolateral striatum, while mice performed a perceptual and value-based decision task. Surprisingly, dopamine axon activity was similar across all three areas. At a glance, the activity multiplexed different variables such as stimulus-associated values, confidence, and reward feedback at different phases of the task. Our modeling demonstrates, however, that these modulations can be inclusively explained by moment-by-moment in the expected reward, that is the temporal difference error. A major difference between areas was the overall activity level of reward responses: reward responses in dorsolateral striatum were positively shifted, lacking inhibitory responses to negative prediction errors. The differences in dopamine signals put specific constraints on the properties of behaviors controlled by dopamine in these regions.
Elife, 2020; 9
28966085: Tsutsui-Kimura I, Natsubori A, Mori M, Kobayashi K, Drew MR, de Kerchove d'Exaerde A, Mimura M, Tanaka KF
Distinct Roles of Ventromedial versus Ventrolateral Striatal Medium Spiny Neurons in Reward-Oriented Behavior.
The ventral striatum (VS) is a key brain center regulating reward-oriented behavior [1-4]. The VS can be anatomically divided into medial (VMS) and lateral (VLS) portions based on cortical input patterns. The VMS receives inputs from medial pallium-originated limbic structures (e.g., the medial prefrontal cortex [mPFC]), and the VLS receives inputs from the lateral pallium-originated areas (e.g., the insula) [5, 6]. This anatomical feature led us to hypothesize a functional segregation within the VS in terms of the regulation of reward-oriented behavior. Here, we engineered a fiber photometry system [4] and monitored population-level Ca activities of dopamine D2-receptor-expressing medium spiny neurons (D2-MSNs), one of the major cell types in the striatum, during a food-seeking discrimination task. We found that VLS D2-MSNs were activated at the time of cue presentation. In stark contrast, VMS D2-MSNs were inhibited at this time point. Optogenetic counteraction of those changes in the VLS and VMS impaired action initiation and increased responding toward non-rewarded cues, respectively. During lever-press reversal training, VMS inhibition at the time of cue presentation temporarily ceased and optogenetic activation of VMS D2-MSNs facilitated acquisition of the new contingency. These data indicate that the opposing inhibition

and excitation in VMS and VLS are important for selecting and initiating a proper action in a reward-oriented behavior. We propose distinct subregional roles within the VS in the execution of successful reward-oriented behavior.
Curr Biol, 2017; 27
28694090: Tsutsui-Kimura I, Ohmura Y, Yoshida T, Yoshioka M
Milnacipran affects mouse impulsive, aggressive, and depressive-like behaviors in a distinct dose-dependent manner.
Serotonin/noradrenaline reuptake inhibitors (SNRIs) are widely used for the treatment for major depressive disorder, but these drugs induce several side effects including increased aggression and impulsivity, which are risk factors for substance abuse, criminal involvement, and suicide. To address this issue, milnacipran (0, 3, 10, or 30 mg/kg), an SNRI and antidepressant, was intraperitoneally administered to mice prior to the 3-choice serial reaction time task, resident-intruder test, and forced swimming test to measure impulsive, aggressive, and depressive-like behaviors, respectively. A milnacipran dose of 10 mg/kg suppressed all behaviors, which was accompanied by increased dopamine and serotonin levels in the medial prefrontal cortex (mPFC) but not in the nucleus accumbens (NAc). Although the most effective dose for depressive-like behavior was 30 mg/kg, the highest dose increased aggressive behavior and unaffected impulsive behavior. Increased dopamine levels in the NAc could be responsible for the effects. In addition, the mice basal impulsivity was negatively correlated with the latency to the first agonistic behavior. Thus, the optimal dose range of milnacipran is narrower than previously thought. Finding drugs that increase serotonin and dopamine levels in the mPFC without affecting dopamine levels in the NAc is a potential strategy for developing novel antidepressants.
J Pharmacol Sci, 2017; 134
28482015: Tsutsui-Kimura I, Bouchekioua Y, Mimura M, Tanaka KF
A New Paradigm for Evaluating Avoidance/Escape Motivation.
Organisms have evolved to approach pleasurable opportunities and to avoid or escape from aversive experiences. These 2 distinct motivations are referred to as approach and avoidance/escape motivations and are both considered vital for survival. Despite several recent advances in understanding the neurobiology of motivation, most studies addressed approach but not avoidance/escape motivation. Here we develop a new experimental paradigm to quantify avoidance/escape motivation and examine the pharmacological validity.
Int J Neuropsychopharmacol, 2017; 20
28167674: Natsubori A, Tsutsui-Kimura I, Nishida H, Bouchekioua Y, Sekiya H, Uchigashima M, Watanabe M, de Kerchove d'Exaerde A, Mimura M, Takata N, Tanaka KF
Ventrolateral Striatal Medium Spiny Neurons Positively Regulate Food-Incentive, Goal-Directed Behavior Independently of D1 and D2 Selectivity.
The ventral striatum is involved in motivated behavior. Akin to the dorsal striatum, the ventral striatum contains two parallel pathways: the striatomesencephalic pathway consisting of dopamine receptor Type 1-expressing medium spiny neurons (D1-MSNs) and the striatopallidal pathway consisting of D2-MSNs. These two genetically identified pathways are thought to encode opposing functions in motivated behavior. It has also been reported that D1/D2 genetic selectivity is not attributed to the anatomical discrimination of two pathways. We wanted to determine whether D1- and D2-MSNs in the ventral striatum functioned in an opposing manner as previous observations claimed, and whether D1/D2 selectivity corresponded to a functional segregation in motivated behavior of mice. To address this question, we focused on the lateral portion of ventral striatum as a region implicated in food-incentive, goal-directed behavior, and recorded D1 or D2-MSN activity by using a gene-encoded ratiometric Ca indicator and by constructing a fiberphotometry system, and manipulated their activities via optogenetic inhibition during ongoing behaviors. We observed concurrent event-related compound Ca elevations in ventrolateral D1- and D2-MSNs, especially at trial start cue-related and first lever press-related times. D1 or D2 selective optogenetic inhibition just after the trial start cue resulted in a reduction of goal-directed behavior, indicating a shared coding of motivated behavior by both populations at this time. Only D1-selective inhibition just after the first lever press resulted in the reduction of behavior, indicating D1-MSN-specific coding at that specific time. Our data did not support opposing encoding by both populations in food-incentive, goal-directed behavior. An opposing role of dopamine receptor Type 1 or Type 2-expressing medium spiny neurons (D1-MSNs or D2-MSNs) on striatum-mediated behaviors has been widely accepted. However, this idea has been questioned by recent reports. In the present study, we measured concurrent Ca activity patterns of D1- and D2-MSNs in the ventrolateral striatum during food-incentive, goal-directed behavior in mice. According to Ca activity patterns, we conducted timing-specific optogenetic inhibition of each type of MSN. We demonstrated that both D1- and D2-MSNs in the ventrolateral striatum commonly and positively encoded action initiation, whereas only D1-MSNs positively encoded sustained motivated behavior. These findings led us to reconsider the prevailing notion of a functional segregation of MSN activity in the ventral striatum.
J Neurosci, 2017; 37
28145402: Tsutsui-Kimura I, Takiue H, Yoshida K, Xu M, Yano R, Ohta H, Nishida H, Bouchekioua Y, Okano H, Uchigashima M, Watanabe M, Takata N, Drew MR, Sano H, Mimura M, Tanaka KF

Dysfunction of ventrolateral striatal dopamine receptor type 2-expressing medium spiny neurons impairs instrumental motivation.
Impaired motivation is present in a variety of neurological disorders, suggesting that decreased motivation is caused by broad dysfunction of the nervous system across a variety of circuits. Based on evidence that impaired motivation is a major symptom in the early stages of Huntington's disease, when dopamine receptor type 2-expressing striatal medium spiny neurons (D2-MSNs) are particularly affected, we hypothesize that degeneration of these neurons would be a key node regulating motivational status. Using a progressive, time-controllable, diphtheria toxin-mediated cell ablation/dysfunction technique, we find that loss-of-function of D2-MSNs within ventrolateral striatum (VLS) is sufficient to reduce goal-directed behaviours without impairing reward preference or spontaneous behaviour. Moreover, optogenetic inhibition and ablation of VLS D2-MSNs causes, respectively, transient and chronic reductions of goal-directed behaviours. Our data demonstrate that the circuitry containing VLS D2-MSNs control motivated behaviours and that VLS D2-MSN loss-of-function is a possible cause of motivation deficits in neurodegenerative diseases.
Nat Commun, 2017; 8
26341319: Tsutsui-Kimura I, Ohmura Y, Izumi T, Matsushima T, Amita H, Yamaguchi T, Yoshida T, Yoshioka M
Neuronal codes for the inhibitory control of impulsive actions in the rat infralimbic cortex.
Poor impulse control is a debilitating condition observed in various psychiatric disorders and could be a risk factor for drug addiction, criminal involvement, and suicide. The rat infralimbic cortex (IL), located in the ventral portion of the medial prefrontal cortex, has been implicated in impulse control. To elucidate the neurophysiological basis of impulse control, we recorded single unit activity in the IL of a rat performing a 3-choiceserial reaction time task (3-CSRTT) and 2-choice task (2-CT), which are animal models for impulsivity. The inactivation of IL neuronal activity with an injection of muscimol (0.1 μg /side) disrupted impulse control in the 3-CSRTT. More than 60% (38/56) of isolated IL units were linked to impulse control, while approximately 30% of all units were linked to attentional function in the 3-CSRTT. To avoid confounding motor-related units with the impulse control-related units, we further conducted the 2-CT in which the animals' motor activities were restricted during recording window. More than 30% (14/44) of recorded IL units were linked to impulse control in the 2-CT. Several types of impulse control-related units were identified. Only 16% of all units were compatible with the results of the muscimol experiment, which showed a transient decline in the firing rate immediately before the release of behavioral inhibition. This is the first study to elucidate the neurophysiological basis of impulse control in the IL and to propose that IL neurons control impulsive actions in a more complex manner than previously considered.
Behav Brain Res, 2016; 296
25522418: Tsutsui-Kimura I, Yoshida T, Ohmura Y, Izumi T, Yoshioka M
Milnacipran remediates impulsive deficits in rats with lesions of the ventromedial prefrontal cortex.
Deficits in impulse control are often observed in psychiatric disorders in which abnormalities of the prefrontal cortex are observed, including attention-deficit/hyperactivity disorder and bipolar disorder. We recently found that milnacipran, a serotonin/noradrenaline reuptake inhibitor, could suppress impulsive action in normal rats. However, whether milnacipran could suppress elevated impulsive action in rats with lesions of the ventromedial prefrontal cortex, which is functionally comparable with the human prefrontal cortex, remains unknown.
Int J Neuropsychopharmacol, 2014; 18
22892727: Tsutsui-Kimura I, Ohmura Y, Izumi T, Kumamoto H, Yamaguchi T, Yoshida T, Yoshioka M
Milnacipran enhances the control of impulsive action by activating D_1-like receptors in the infralimbic cortex.
Elevated impulsivity is often observed in patients with depression. We recently found that milnacipran, an antidepressant and a serotonin/noradrenaline reuptake inhibitor, could enhance impulse control in rats. However, the neural mechanisms underlying the effects of milnacipran on impulsive action remain unclear. Milnacipran increases not only extracellular serotonin and noradrenaline but also dopamine specifically in the medial prefrontal cortex, which is one of the brain regions responsible for impulsive action.
Psychopharmacology (Berl), 2013; 225
19730368: Tsutsui-Kimura I, Ohmura Y, Izumi T, Yamaguchi T, Yoshida T, Yoshioka M
The effects of serotonin and/or noradrenaline reuptake inhibitors on impulsive-like action assessed by the three-choice serial reaction time task: a simple and valid model of impulsive action using rats.
Impulsivity is a pathological symptom in several psychiatric disorders, underscoring the need for animal models of impulsive action to develop a brief screening method for novel therapeutic agents of impulsive action. The aims of this study were (i) to evaluate whether the three-choice serial reaction time task (3-CSRTT), a simple version of the five-choice serial reaction time task (5-CSRTT), is appropriate for brief assessment of impulsive-like action and (ii) to examine the effects of fluvoxamine, a selective serotonin reuptake inhibitor, and milnacipran, a serotonin/noradrenaline reuptake inhibitor, on impulsive-like action using the 3-CSRTT. After training in the 3-CSRTT, rats were administered nicotine (0, 0.1, 0.2, and 0.4 mg/kg, salt, subcutaneously), atomoxetine [0, 0.01, 0.1, and, 1.0 mg/kg, intraperitoneally (i.p.)], fluvoxamine (0, 2, 4, and 8 mg/kg, i.p.), or

milnacipran (0, 3, and 10 mg/kg, i.p.). The training time for the 3-CSRTT was significantly shorter than that for the 5-CSRTT. Nicotine increased, whereas atomoxetine decreased the number of premature responses, an index of impulsive-like action, which is consistent with earlier studies. Milnacipran, but not fluvoxamine, dose-dependently decreased premature responses. These results indicate that the 3-CSRTT could provide an appropriate and simpler rodent model of impulsive-like action and that milnacipran could have some beneficial effects on impulsivity-related disorders.
Behav Pharmacol, 2009; 20

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BOARD NUMBER: S01-330

ROLE OF RAP1GAP2 IN SEROTONIN AUTOREGULATION IN MOUSE BRAIN

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Serotonin (5-HT) is a major monoamine neurotransmitter, its dysfunction causes psychiatric disorders, such as depression. Selective serotonin reuptake inhibitors (SSRIs) are a class of antidepressants that block the serotonin reuptakes . Chronic SSRI administration leads first to a decrease in 5-HT levels by a negative feedback exerted by the 5-HT1A receptor on serotonin producing cells Further treatment induces a long-lasting 5-HT1A desensitization and increase of extracellular 5-HT levels. To uncover the molecular mechanisms involved in serotonin autoregulation and 5-HT1A desensitization, we used Tph2-KO mice which completely lack serotonin in the brain, and SERT-KO mice which have high extracellular serotonin levels, as models of two extreme states of extracellular serotonin. First, we collected the dorsal raphe area in which serotonergic neurons are enriched and run high-throughput proteomics and phosphoproteomics to investigate the effects of high and low serotonin. The results showed 11 proteins and 8 phosphorylated proteins (FDR=0.1) differentially expressed among different genotypes. Among the selected potential factors, the most interesting one is RAP1GAP2, which activates a GTPase for the regulatory protein RAP-1A, converting it into the inactive GDP-bound state. RAP1GAP2 and A0A17EBK8, an isoform of RAP1GAP2 with a longer alternative N-terminus, were down-regulated in the SERT-KO group. The phosphorylation site at serine 53 of A0A17EBK8 was also down-regulated in the SERT-KO group. RAP1 activity analysis showed that SERT-KO raphe cells with lower RAP1GAP2 level have a significantly higher RAP1 activity. These data provide evidence, that RAP1GAP2 and RAP1 are involved in 5-HT1A mediated autoregulation of serotonin in the brain.

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BOARD NUMBER: S01-331

ISOLATION OF DOPAMINERGIC INPUTS TO THE STRIATUM REVEALS DOPAMINE HUB SYNAPSES WITH THEIR PROTEOME.

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

Vincent Paget-Blanc[1], Marlene Pfeffer[2], Marie Pronot[1], Paul Lapios[1], Maria-Florencia Angelo[1], Roman Walle[3], Fabrice Cordelières[4], Florian Levet[1], Stéphane Claverol[5], Sabrina Lacomme[6], Melina Petrel[6], Christelle Martin[1], Vincent Pitard[7], Véronique De Smedt Peyrusse[3], Thomas Biederer[8], David Perrais[1], Pierre Trifilieff[9], Etienne Herzog[10]
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Dopamine transmission exerts a central neuromodulatory control over neuronal networks involved in reward processing and motor control. Its impairment plays an important role in numerous neurological disorders. Despite its pathophysiological relevance, the molecular and structural organization of the dopaminergic synapse largely remains to be established. We used targeted labeling and fluorescence-activated sorting to purify striatal dopaminergic synaptosomes. This methodology, coupled with mass spectrometry, allowed us to explore the proteome of dopaminergic synapses. Beyond canonical markers of dopamine neurotransmission, we found 57 proteins specifically enriched and further validated 6 of them that were not previously identified at these synapses (Cpne7, Apba1/Mint1, Cadps2, Cadm2/SynCAM 2, Stx4, Mgll). Moreover, our data reveal a complex organization of the dopaminergic synapse with the adhesion of dopaminergic varicosities to glutamatergic, GABAergic, or cholinergic synapses in multipartite structures we named « hub synapses ». Indeed, our dopaminergic synaptosomes proteomic dataset exhibits different pre-and post-synaptic markers from glutamatergic, GABAergic, and cholinergic synapses that are maintained after purification. We confirmed these results by performing wide field and super-resolution immunofluorescence of sorted striatal dopaminergic synaptosomes. With those findings and methodology, we provide a new framework not only for the molecular exploration of dopaminergic transmission but also for the characterization of projection-specific synaptomes.

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BOARD NUMBER: S01-332

INFLUENCE OF GLYPHOSATE INTOXICATION ON GALANIN-LIKE IMMUNOREACTIVE ENTERIC NEURONS IN THE PORCINE SMALL INTESTINE

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Glyphosate is the main active component of Roundup, one of the most widely used herbicide in the world. The present study was created to establish the effect of glyphosate supplementation on population of galanin- like immunoreactive (GAL-LI) intramural neurons in the porcine small intestine. Fifteen sexually immature gilts divided into 3 groups were used: control - animals receiving empty gelatine capsules; G1 - animals receiving a low dose of glyphosate - corresponding to the theoretical maximum daily intake (TMDI) - 0.05 mg/kg bw/day; G2 - animals receiving a higher dose of glyphosate - corresponding to the acceptable daily intake (ADI) - 0.5 mg/kg/day) in gelatine capsules orally for 28 days. After this time, the animals were euthanized and small intestine samples were collected. Frozen sections were then subjected to the procedure of double immunofluorescent staining. Glyphosate supplementation increased the number of GAL-LI neurons. The most remarkable changes were observed in the ileum, where an increase was noted in both experimental groups and all types of intramural plexuses (myenteric plexus (MP), outer submucous plexus (OSP), inner submucous plexus (ISP)). In the jejunum, the greatest increase was recorded in the G2 group and the OSP. Similarly in the duodenum, the higher dose of glyphosate increased the number of GAL-LI neurons in all type of plexus, whereas in the G1 group changes was observed only in the ISP. The obtained results suggest that GAL may play an important role in the protection of enteric nervous system neurons against the harmful effects of glyphosate.

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BOARD NUMBER: S01-333

AUTOCRINE ACTION OF SOMATOSTATIN RELEASED BY O-LM INTERNEURONS

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Somatostatin (SST) is a neuropeptide whose expression characterises a subpopulation of GABAergic interneurons. SST is of great interest in the pathophysiological context as a deficit of SST is observed in many neurological and psychiatric diseases. SST exerts its physiological role through five G protein-coupled receptors. In the hippocampus, the effects of SST on the principal cells are well known. It inhibits excitatory synaptic transmission by decreasing glutamate release from pyramidal neurons and reduces their intrinsic excitability. This decrease in excitability is achieved by facilitating currents carried by Kv7 channels. Within the hippocampus, O-LM interneurons contain SST and express Kv7.2/3 channels. However, there is no data on the presence of SST auto-receptors on O-LM interneurons, on its mode of release and on the effect it might have on the intrinsic excitability of these interneurons as well as on the synaptic transmission they have with neighbouring pyramidal neurons. We therefore studied how SST is released by O-LM interneurons in the hippocampal CA1 and defined its potential inhibitory action by autocrine (on O-LM) pathway. The preliminary results obtained suggest that SST transiently decreases the excitability of O-LM interneurons as well as the synaptic transmission that takes place between the pyramidal cells and the latter and that there is indeed an autocrine effect of SST that the O-LM interneurons release only following stimulation at a frequency > 20 Hz. In conclusion, the SST neuropeptide similarly to the GABA neurotransmitter modulates neuronal activity by controlling synaptic excitation and excitability onto O-LM cells.

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THE MODULATION OF THALAMIC RETICULAR NUCLEUS NEURONS BY CORTICOTROPIN-RELEASING HORMONE

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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We aim to investigate the effect of the stress- and anxiety-related mediator corticotropin-releasing hormone (CRH) on the thalamic reticular nucleus (TRN), a main modulator of thalamic function that contributes to sleep rhythmogenesis. Previous literature and our data show that the CRH receptor 1 (CRHR1) is highly expressed in TRN neurons. However, how CRH affects TRN function, in particular for sleep regulation, has not been explored. Using *ex-vivo* electrophysiology with bath-application of CRH or photoactivation of CRH-releasing fibers, we found that CRHR1 activation decreases the propensity of TRN neurons to fire low-threshold calcium bursts through a negative modulation of the afterhyperpolarizing potentials. Furthermore, synaptic inputs of cortical origin were increased in amplitude upon CRHR1 activation. We mapped through viral tracing the endogenous sources of CRH to the TRN. Our data indicate diverse origins, such as the basolateral-amygdala, zona incerta, and other candidate regions, projecting to different subsectors of TRN. Overall, we found that CRH modulates TRN excitability, potentially impacting the generation and maintenance of thalamocortical rhythms. We are investigating the functional implications of these findings for sleep regulation by combining *in-vivo* polysomnographic recordings with optogenetic manipulations, along with monitoring of the endogenous CRH fluctuations using fiberphotometry. Revealing the mechanisms of interaction between the CRH system and sleep regulation at the TRN level may help advance our understanding of how stress affects sleep stability and sleep-related functions, such as memory consolidation, processes that are often impaired and exacerbated by stress in neuropsychiatric conditions.

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BOARD NUMBER: S01-335

PACAP-VGLUT1 EXPRESSING SUBPOPULATION IN HINDBRAIN PARABRACHIAL COMPLEX FORMS SYNAPSE IN EXTENDED AMYGDALA: MOLECULAR AND ULTRASTRUCTURAL SIMILARITIES AND PARTICULARITIES COMPARING WITH CALYX-OF-HELD IN BRAINSTEM AUDITORY SYSTEMS

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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The hindbrain parabrachial complex (PBc) is a sensory relay in the CNS. The forebrain extended amygdala (EA) orchestrates adaptive responses to emotional events. While PBc-EA connection has been extensively studied, cell type specificity and types of synapses remained unclear. Here, using systematic anatomical analysis (IHC and DISH) of neuropeptides PACAP, CGRP and neurotensin-NT distribution in rat and mouse brains, we observed perisomatic ring-like structures containing the three peptides as well as VGLUT1/VGLUT2 in the EA. The origin of those structures was studied using in vivo juxtacellular labeling and tracing study using Adcyap1-Cre for Cre-dependent expression of fluorescent marker fused with artificial channelrhodopsin. PACAP/CGRP/NT/VGLUT1/VGLUT2 expression was a molecular signature of the pre-synaptic neurons from the posterior-ventral division of PBc. This signature was found in the Calyx-of-Held synapse in brainstem' medial nucleus of the trapezoid body (MNTB), which receive globular bushy neurons in the ventral cochlear nucleus. Calyx-of-Held is a rare perisomatic large glutamatergic synapse in the MNTB. PKCdelta-GABAergic neurons in the EA, co-expressing Adcyap1r1 and Vipr1 mRNA were identified as the post-synaptic cells – the same molecular signature was found in the MNTB, except co-expressing PKCdelta. Using TEM and FIBSEM in combination with immunohistochemistry, we demonstrated that the ring-structures in EA are highly similar to the Calyx-of-Held observed in the MNTB. Taken together, our results suggest a common molecular basis for calyceal synaptogenesis both in hindbrain and forebrain. The PBc-->EA Calyx-of-Held synapse may represent a previously unappreciated morphological substrate for high fidelity sensory alert to the forebrain center for adaptive response.

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GAP43 IS A NEW CB1R-INTERACTING PROTEIN THAT DECREASES RECEPTOR FUNCTION AT MOSSY CELL - GRANULAR CELL SYNAPSE IN HIPPOCAMPUS.

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Cannabinoid CB1 receptor (CB_1R) located at both excitatory and inhibitory synapses strongly influences the brain-transmission balance showing a complex and biphasic fine-tuning of several brain functions. This complexity can be sustained by the striking differences in the signaling events triggered by CB_1R among different cell types. Previous evidence had shown that CB_1R can be associated to certain intracellular proteins that modulate its action. By using a high-throughput proteomic approach, complemented with a wide array of *in vitro* and *in vivo* assays, we unveil that CB1R interacts with growth-associated protein 43 (GAP-43)/neuromodulin, a presynaptic cytoskeleton-associated protein, mostly in its S41-phosphorylated form. In brain tissue, we detected CB_1R-GAP43 complexes selectively in glutamatergic axon terminals of the MCs in the dentate gyrus (DG), as shown by in situ PLA in brain sections from conditional knockout and rescue mouse models. MCs establish an associative excitatory circuit with granular cells (GCs) in the DG involved in epileptic seizures. By adeno-associated virus (AAV)-mediated intracranial delivery of GAP43-S41D or GAP43-S41A, we could determine that the phosphorylated form of GAP43 decreases DSE and WIN-55,212-2-mediated suppression of neurotransmission at MC-GC synapses, strongly suggesting that GAP43 inhibits CB_1R at those synapses. In addition, we generated conditional GAP43-knockout mice, specifically in glutamatergic and GABAergic neurons. We tested kainate-induced epilepsy on these mouse lines and found that GAP43 deletion from glutamatergic neurons facilitates CB_1R anti-seizure activity. Taken together, these findings unveil a new protein that can interact with CB_1R in a synapse-specific manner, hampering CB_1R action on the MC-GC recurrent excitatory circuit.

Pubmed:
34353897: Costas-Insua C, Moreno E, Maroto IB, Ruiz-Calvo A, Bajo-Grañeras R, Martín-Gutiérrez D, Diez-Alarcia R, Vilaró MT, Cortés R, García-Font N, Martín R, Espina M, Botta J, Ginés S, McCormick PJ, Sánchez-Prieto J, Galve-Roperh I, Mengod G, Urigüen L, Marsicano G, Bellocchio L, Canela EI, Casadó V, Rodríguez-Crespo I, Guzmán M
Identification of BiP as a CB Receptor-Interacting Protein That Fine-Tunes Cannabinoid Signaling in the Mouse Brain.
Cannabinoids, the bioactive constituents of cannabis, exert a wide array of effects on the brain by engaging Type 1 cannabinoid receptor (CBR). Accruing evidence supports that cannabinoid action relies on context-dependent factors, such as the biological characteristics of the target cell, suggesting that cell population-intrinsic molecular cues modulate CBR-dependent signaling. Here, by using a yeast two-hybrid-based high-throughput screening, we identified BiP as a potential CBR-interacting protein. We next found that CBR and BiP interact specifically , and mapped the interaction site within the CBR -terminal (intracellular) domain and the BiP -terminal (substrate-binding) domain-α. BiP selectively shaped agonist-evoked CBR signaling by blocking an "alternative" G protein-dependent signaling module while leaving the "classical" G protein-dependent inhibition of the cAMP pathway unaffected. proximity ligation assays conducted on brain samples from various genetic mouse models of conditional loss or gain of CBR expression allowed to map CBR-BiP complexes selectively

on terminals of GABAergic neurons. Behavioral studies using cannabinoid-treated male BiP mice supported that CBR-BiP complexes modulate cannabinoid-evoked anxiety, one of the most frequent undesired effects of cannabis. Together, by identifying BiP as a CBR-interacting protein that controls receptor function in a signaling pathway- and neuron population-selective manner, our findings may help to understand the striking context-dependent actions of cannabis in the brain.
Cannabis use is increasing worldwide, so innovative studies aimed to understand its complex mechanism of neurobiological action are warranted. Here, we found that cannabinoid CB receptor (CBR), the primary molecular target of the bioactive constituents of cannabis, interacts specifically with an intracellular protein called BiP. The interaction between CBR and BiP occurs selectively on terminals of GABAergic (inhibitory) neurons, and induces a remarkable shift in the CBR-associated signaling profile. Behavioral studies conducted in mice support that CBR-BiP complexes act as fine-tuners of anxiety, one of the most frequent undesired effects of cannabis use. Our findings open a new conceptual framework to understand the striking context-dependent pharmacological actions of cannabis in the brain.
J Neurosci, 2021; 41
33792539: Maglio LE, Noriega-Prieto JA, Maroto IB, Martin-Cortecero J, Muñoz-Callejas A, Callejo-Móstoles M, Fernández de Sevilla D
IGF-1 facilitates extinction of conditioned fear.
Insulin-like growth factor-1 (IGF-1) plays a key role in synaptic plasticity, spatial learning, and anxiety-like behavioral processes. While IGF-1 regulates neuronal firing and synaptic transmission in many areas of the central nervous system, its signaling and consequences on excitability, synaptic plasticity, and animal behavior dependent on the prefrontal cortex remain unexplored. Here, we show that IGF-1 induces a long-lasting depression of the medium and slow post-spike afterhyperpolarization (mAHP and sAHP), increasing the excitability of layer 5 pyramidal neurons of the rat infralimbic cortex. Besides, IGF-1 mediates a presynaptic long-term depression of both inhibitory and excitatory synaptic transmission in these neurons. The net effect of this IGF-1-mediated synaptic plasticity is a long-term potentiation of the postsynaptic potentials. Moreover, we demonstrate that IGF-1 favors the fear extinction memory. These results show novel functional consequences of IGF-1 signaling, revealing IGF-1 as a key element in the control of the fear extinction memory.
Elife, 2021; 10
30914306: Ruiz-Calvo A, Bajo-Grañeras R, Maroto IB, Zian D, Grabner GF, García-Taboada E, Resel E, Zechner R, Zimmermann R, Ortega-Gutiérrez S, Galve-Roperh I, Bellocchio L, Guzmán M
Astroglial monoacylglycerol lipase controls mutant huntingtin-induced damage of striatal neurons.
Cannabinoids exert neuroprotection in a wide array of preclinical models. A number of these studies has focused on cannabinoid CB receptors in striatal medium spiny neurons (MSNs) and the most characteristic MSN-degenerative disease, Huntington's disease (HD). Accruing evidence supports that astrocytes contribute to drive HD progression, and that they express CB receptors, degrade endocannabinoids, and modulate endocannabinergic transmission. However, the possible role of the astroglial endocannabinoid system in controlling MSN integrity remains unknown. Here, we show that JZL-184, a selective inhibitor of monoacylglycerol lipase (MGL), the key enzyme that deactivates the endocannabinoid 2-arachidonoylglycerol, prevented the mutant huntingtin-induced up-regulation of the pro-inflammatory cytokine tumor necrosis factor-α in primary mouse striatal astrocytes via CB receptors. To study the role of astroglial MGL in vivo, we injected stereotactically into the mouse dorsal striatum viral vectors that encode mutant or normal huntingtin under the control of the glial fibrillary acidic protein promoter. We observed that, in wild-type mice, pharmacological blockade of MGL with JZL-184 (8 mg/kg/day, i.p.) conferred neuroprotection against mutant huntingtin-induced striatal damage, as evidenced by the prevention of MSN loss, astrogliosis, and motor coordination impairment. We next found that conditional mutant mice bearing a genetic deletion of MGL selectively in astroglial cells (MGL mice) were resistant to mutant huntingtin-induced MSN loss, astrogliosis, and motor coordination impairment. Taken together, these data support that astroglial MGL controls the availability of a 2-arachidonoylglycerol pool that ensues protection of MSNs in the mouse striatum in vivo, thus providing a potential druggable target for reducing striatal neurodegeneration.
Neuropharmacology, 2019; 150
29121220: Ruiz-Calvo A, Maroto IB, Bajo-Grañeras R, Chiarlone A, Gaudioso Á, Ferrero JJ, Resel E, Sánchez-Prieto J, Rodríguez-Navarro JA, Marsicano G, Galve-Roperh I, Bellocchio L, Guzmán M
Pathway-Specific Control of Striatal Neuron Vulnerability by Corticostriatal Cannabinoid CB1 Receptors.
The vast majority of neurons within the striatum are GABAergic medium spiny neurons (MSNs), which receive glutamatergic input from the cortex and thalamus, and form two major efferent pathways: the direct pathway, expressing dopamine D1 receptor (D1R-MSNs), and the indirect pathway, expressing dopamine D2 receptor (D2R-MSNs). While molecular mechanisms of MSN degeneration have been identified in animal models of striatal damage, the molecular factors that dictate a selective vulnerability of D1R-MSNs or D2R-MSNs remain unknown. Here, we combined genetic, chemogenetic, and pharmacological strategies with behavioral and neurochemical analyses, and show that the pool of cannabinoid CB1 receptor (CB1R) located on corticostriatal terminals efficiently safeguards D1R-MSNs, but not D2R-MSNs, from different

insults. This cell-specific response relies on the regulation of glutamatergic signaling, and is independent from the CB1R-dependent control of astroglial activity in the striatum. These findings define cortical CB1R as a pivotal synaptic player in dictating a differential vulnerability of D1R-MSNs versus D2R-MSNs, and increase our understanding of the role of coordinated cannabinergic-glutamatergic signaling in establishing corticostriatal circuits and its dysregulation in neurodegenerative diseases.
Cereb Cortex, 2018; 28

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BOARD NUMBER: S01-337

NANOSCALE ADAPTATIONS IN THE STRIATAL ENDOCANNABINOID MACROMOLECULAR COMPLEX SHAPE BEHAVIORAL FLEXIBILITY

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Across life span, the brain learns to choose adaptively between different behavioral options to reach specific goals. This process requires behavioral flexibility, and is supported by frontostriatal circuits and their optimal modulation by ascending neuromodulatory systems (i.e. dopamine) and local neuromodulatory signals (i.e. endocannabinoids). In the striatum, the endocannabinoid (eCB) system, formed by synthetizing enzymes (i.e. DAG lipase alpha, DGL) and receptors (cannabinoid receptor type 1, CB_1R), acts as a highly-organized neuromodulatory signaling complex that controls the release of glutamate at cortical afferents to principal striatal projection neurons (SPNs). We investigated whether behavioral flexibility is supported by nanoscale adaptations in the eCB signaling complex. To this purpose, we subjected mice to different training regimes of instrumental conditioning of nose poke for food reward, which promote either flexible goal-directed (short-training) or inflexible behavior (over-training). Each training was followed by an omission procedure in which mice have to learn a new causal A-O association. By using ex-vivo brain slices electrophysiology and super resolution microscopy, we find that dynamic regulation of molecular components of striatal eCB signaling complex represents a key molecular substrate for behavioral flexibility.

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HIV-1 TAT PROTEIN EXACERBATES METHAMPHETAMINE-DYSREGULATED DOPAMINE UPTAKE INTO VESICULAR MONOAMINE TRANSPORTER-2 AND POTENTIATES METHAMPHETAMINE CONDITIONED PLACE PREFERENCE IN HIV-1 TAT TRANSGENIC MICE

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Methamphetamine (METH) abuse increases the incidence of HIV-1 associated neurocognitive disorders. We have demonstrated that the HIV-1 regulatory protein, transactivator of transcription (Tat), decreases dopamine (DA) transport via the DA transporter (DAT) and vesicular monoamine transporter-2 (VMAT-2). This study examined the synergistic effects of Tat and METH on DA uptake through VMAT-2 and associated METH-induced conditioned place preference (CPP). *In vitro* incubation of isolated mouse whole brain vesicles with recombinant Tat_{1-86} or METH displayed a concentration-dependent inhibition of vesicular [^{3}H]DA uptake, in which a combination of Tat and METH induced an additive reduction of DA uptake via VMAT-2 compared to Tat or METH alone. 21-day doxycycline (Dox, 100 mg/kg/day, i.p.)-induced Tat expression caused a 57% decrease in the maximal velocity (V_{max}) value in inducible Tat transgenic (iTat-tg) mice, which was attenuated in control G-tg (Tat null) mice. 14-day administration of METH (3 mg/kg, i.p.) alone induced a 35% and 52% reduction of V_{max} in iTat-tg and G-tg mice, respectively, whereas a greater decrease (66%) in V_{max} was observed in iTat-tg mice of Dox-METH group. Following 14-day treatment of Dox, the iTat-tg mice potentiated METH-CPP 6-fold over saline-treated mice, suggesting that Tat expression potentiates the rewarding effect of METH. Considering that both Tat and METH interact with DAT and VMAT-2, these results provide evidence that Tat and METH could disrupt DA transmission by inhibiting both DAT and VMAT-2 function. This study raises the exciting possibility of a potential therapeutic strategy by targeting VMAT-2 for METH abuse in HIV infected individuals.

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BOARD NUMBER: S01-339

LATERAL AXO-AXONAL NEUROMODULATION IS REQUIRED FOR NOISE FREE AVERSIVE OLFACTORY CONDITIONING IN ADULT DROSOPHILA MELANOGASTER

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Objective: In order to survive animals must learn to modify their behavior according to environmental cues. Such learning often occurs by reinforcing signals that modulate connection strengths between neurons. Importantly, such neuromodulation can occur via direct synaptic release onto postsynaptic structures located at all target neurons or even by volume transmission. How then does the neuromodulatory signal affects only the relevant subset of target neurons? It is generally agreed that coupling between the release of the modulatory signal and activity of the target neurons is required. However, it was demonstrated that neuromodulation occurs even without coupling. Thus, a question arises whether learning and memory resides on a background of noisy neuromodulation or whether mechanisms exist which reduce this noisy background modulation. To address this question, we focused on the *Drosophila* cholinergic Kenyon cells (KCs) where olfactory learning and memory occurs following Dopaminergic neuromodulation. **Results:** We show using a combination of behavioral experiments, 2-photon functional imaging, genetics, and connectomics analysis, that KCs have numerous axo-axonal connections mediated by the muscarinic type B receptor (mAChR-B). These axo-axonal connections have a dual function: they reduce Ca^{2+} elevation in KCs presynaptic terminals that is required for dopaminergic neuromodulation, and they induce a neuromodulatory signal that counters the dopaminergic neuromodulation. As a result, this lateral neuromodulation limits dopaminergic neuromodulation only to relevant KCs and reduces unspecific learning. **Conclusion**: Cholinergic activation of mAChR-B contrasts the noisy background modulation that occurs during the learning process, resulting in sharpening and enhancement of the learning process.

Pubmed:
34528727: Weiss S, Clamon LC, Manoim JE, Ormerod KG, Parnas M, Littleton JT
Glial ER and GAP junction mediated Ca waves are crucial to maintain normal brain excitability.
Astrocytes play key roles in regulating multiple aspects of neuronal function from invertebrates to humans and display Ca fluctuations that are heterogeneously distributed throughout different cellular microdomains. Changes in Ca dynamics represent a key mechanism for how astrocytes modulate neuronal activity. An unresolved issue is the origin and contribution of specific glial Ca signaling components at distinct astrocytic domains to neuronal physiology and brain function. The Drosophila model system offers a simple nervous system that is highly amenable to cell-specific genetic manipulations to characterize the role of glial Ca signaling. Here we identify a role for ER store-operated Ca entry (SOCE) pathway in perineurial glia (PG), a glial population that contributes to the Drosophila blood-brain barrier. We show that PG cells display diverse Ca activity that varies based on their locale within the brain. Ca signaling in PG cells does not require extracellular Ca and is blocked by inhibition of SOCE, Ryanodine receptors, or gap junctions. Disruption of these components triggers stimuli-induced seizure-like episodes. These findings indicate that Ca release from internal stores and its propagation between neighboring glial cells via gap junctions are essential for maintaining normal nervous system function.
Glia, 2022; 70

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BOARD NUMBER: S01-340

INTERACTION OF BDNF-TRKB AND CORTICOSTEROIDS SIGNALING PATHWAYS

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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The rewiring of neuronal networks that allows brains to learn and to adapt to changing environments is called neuroplasticity, and impaired neuroplasticiy is involved in the pathophysiology of a wide range of brain disorders. This raises the need to understand the molecular pathways underneath in order to design effective treatments to restore physiological neuroplasticity. One of the central players maintaining it is the Brain derived neurotrophic factor (BDNF) signaling through its cognate Tropomyosin receptor kinase B (TRKB), ultimately responsible for synaptic development and maintenence. However, other molecules, such as corticosteroid hormones, physiologically promote neuroplasticity, suggesting an interplay between different pathways. In this study, we investigate in vitro the relationship between glucocorticoids, such as corticosterone and dexamethasone, and mineralocorticoids, such as aldosterone, on TRKB signaling. We developed a protein-fragment complementation assay (PCA) to monitor TRKB homodimerization and interaction with other cellular proteins to examine its activity. We found that corticosteroids affect TRKB signaling in a non-genomic way, indicating a possible synergistic effect of the two pathways.

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REGULATION OF NMDA RECEPTOR DYNAMICS BY BRAIN-DERIVED NEUROTROPHIC FACTOR IN HIPPOCAMPAL NEURONS

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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The neurotrophin brain-derived neurotrophic factor (BDNF) is an important mediator of long-term synaptic potentiation (LTP) induced by high-frequency presynaptic stimulation, in the hippocampus and in other brain regions. The early effects of BDNF are mediated by posttranslational modification of synaptic components, while the delayed responses require transcription activity and de novo protein synthesis. However, the BDNF-induced alterations in the synaptic proteome coupled to synaptic strengthening are poorly understood. Studies performed in cultured hippocampal neurons showed that BDNF induces synaptic accumulation of NMDA receptors and increases the amplitude of NMDAR-mediated mEPSCs, by a mechanism dependent on the activation of the tyrosine kinase Pyk2 and dependent of protein synthesis. The BDNF-induced upregulation in synaptic NMDAR was correlated with an increase in their stability in this compartment, as determined by single particle quantum dot imaging. The effects of BDNF on local protein synthesis at the synapse depends on the delivery of transcripts along dendrites, within RNA granules. The RNA binding protein hnRNP K was found to play an important role in the delivery of Pyk2 mRNA to the synapse. Activation of BDNF-TrkB signaling at the synapse leads to the release of the latter transcripts from the RNA binding protein, allowing the synthesis of the kinase. Together, the results show a key role for Pyk2 synthesis at the synapse as a mediator of the effects of BDNF on the local distribution of NMDAR, which may have an impact on LTP.
(Supported by FCT, Portugal)

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BOARD NUMBER: S01-342

PHARMACOLOGICAL ACTIVATION OF HISTAMINE RECEPTOR TYPE 2 ENHANCES EVOKED FIRING IN MEDIUM SPINY NEURONS OF THE NUCLEUS ACCUMBENS THROUGH DOWNREGULATION OF KV4.2 CHANNELS.

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

Giuseppe Aceto[1,2], Luca Nardella[1], Claudia Colussi[3], Valeria Pecci[4], Alessia Bertozzi[3], Simona Nanni[4], Claudio Grassi[1,2], Marcello D'Ascenzo[1,2]
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The nucleus accumbens (NAc), a brain area that integrates diverse monoaminergic inputs to coordinate motivated behaviours, are innervated by histaminergic fibers originating in neurons located in the hypothalamic tuberomammillary nucleus.
Although the NAc expresses different subtypes of histamine receptors, the mechanisms by which histamine may affect NAc activity have not been identified yet. By performing whole-cell patch-clamp recordings, we found that activation of histamine 2 (H2) receptors elevates evoked firing of NAc medium spiny neurons (MSNs). The evoked firing of MSNs increased after seconds of local H2 agonist administration and remained elevated for minutes. H2 receptor (H2R) activation quickened subthreshold depolarization in response to current injection, increased the action potential half-width, diminished action potential afterhyperpolarization and reduced the latency to fire. The increased excitability was PKA-dependent and associated with decreased A-type K+ currents. Indeed, selective pharmacological inhibition of the Kv4.2 channel, the main molecular determinant of A-type K+ currents in MSNs, occluded the increased excitability induced by H2R activation. Overall, these findings indicate that histaminergic transmission in the NAc increases the excitability of MSN through H2R-dependent modulation of Kv4.2 channels and extend the current conceptual framework of HA signaling in the brain.

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BOARD NUMBER: S01-343

PHARMACOLOGICAL INHIBITION OF THE AGGREGATION-PROMOTING ATXN1-MED15 PROTEIN INTERACTION

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

Ioannis Gkekas[1], Stelios Mylonas[2], Sotiris Katsamakas[3], Apostolos Axenopoulos[2], Petros Daras[2], Spyros Petrakis[1]
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Aim: Spinocerebellar ataxia type 1 (SCA1) is caused by a polyQ expansion in the ataxin-1 protein. MED15, a key component of Mediator Complex interacts with ataxin-1 and strongly enhances its aggregation, suggesting that this interaction (PPI) may affect the progression of SCA1. The aim of our work is the identification of compounds that would inhibit ATXN1-MED15 protein interaction. **Methods:** Here, we present a computational and experimental workflow enabling the discovery of compounds that block ATXN1-MED15 PPI. We used I-TASSER software for prediction of ATXN1 and MED15 protein structures and simulation of their interaction. Protein docking results were validated using the LuTHy assay for ATXN1-MED15 PPI. Next, we employed an in-silico screening for inhibitors that bind to the PPI interaction site and hamper ATXN1-MED15 interaction. Selected compounds were tested in high-throughput ATXN1-MED15 LuTHy assays and a MED15-induced protein aggregation primary cell model. **Results:** First, we predicted the structures of target proteins using the I-TASSER software and computationally simulated their interaction. Predicted interaction sites between ATXN1 and MED15 were validated using LuTHy, a mammalian cell-based assay which enables the detection and quantification of PPIs. Next, we performed an *in-silico* screening and identified 30 compounds that bind to the ATXN1 interaction site. These compounds were tested whether they inhibit this interaction in high-throughput LuTHy assays. 7 hit compounds were further tested whether they reduce MED15-induced ataxin-1 protein aggregation in a novel inducible cell-based model. **Conclusions:** Our work may lead to the discovery of novel compounds that would suppress SCA1 disease progression.

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BOARD NUMBER: S01-344

BRAIN DIFFUSION PARAMETERS ARE CHANGED BY DISRUPTION OF EXTRACELLULAR MATRIX AFTER ORAL TREATMENT OF 4-METHYLUMBELLIFERONE.

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Transient changes of extracellular matrix (ECM) and perineuronal nets (PNN) have been observed to change extracellular space (ECS) diffusion parameters during brain pathologies or in knock-out animals. We investigated the effect of oral treatment of rats with 4-methylumbelliferone (4-MU), inhibitor of hyaluronan synthesis, on ECM and brain diffusion parameters. We measured the level of hyaluronan (using hyaluronan binding protein) and chondroitin sulfates (using CS56 antibody) by densitometry measurement on brain sections. We found that 2 months of 4-MU diet have led to 42.3% ± 11.2% and 38.7% ± 9.1% reduction of hyaluronan and chondroitin sulfates, respectively. There was a significant down-regulation of PNN on neuronal surface, labelled by *Wisteria floribunda* agglutinin. We used *in vivo* diffusion-weighted magnetic resonance imaging (DW-MRI) and found that exposure to 4-MU diet resulted in cortex, thalamus, hippocampus and pallidum in a significant decrease of the apparent diffusion coefficient of water from 967 ± 8 to 903 ± 12 mm^2/s, the fractional anisotropy from 0.21 ± 0.02 to 0.16 ± 0.01 and perfusion detected by arterial spin labelling from 82 ± 9 to 52 ± 4 ml/100g/min (data from cortex). We have not found changes in brain metabolites as measured by MR proton spectroscopy. After the animals were fed again with a standard diet, the parameters returned to control values. We suggest that DW-MRI can be used for *in vivo* detection of the changes in ECM which can affect synaptic plasticity, extrasynaptic transmission and contribute to observed functional effects of 4-MU. Supported by LM2018129 and APVV-20-0331.

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BOARD NUMBER: S01-345

IDENTIFICATION THROUGH HIGH CONTENT SCREENING OF CALCIUM CHANNELS ANTAGONIST AS NOVEL NEUROPROTECTIVE INHIBITORS

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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There is increasing evidence for influence of Alzheimer´s proteins and neuropathology on ischemic brain injury. Production and deposition of β-amyloid protein is an important key player involved in mechanism in Alzheimer´s disease as well as ischemic neurodegeneration. To date, efficient therapeutics drugs are still insuficient, making necessary to find relevant compounds amongst masses of potential candidates. A first cell-based screening model for secretase inhibitors using MCDK cells followed by a second assay in cortical neurons where cell parameters associated with membrane damage, cell viability and neurite outgrowth were measured using HCS analysis. In the primary screen we identified 57 inhibitors of β-secretase in the 1200 compounds of Prestwich library. These initial hits were further analyzed for their capacity to protect primary cortical neurons against the neurotoxicity induced by oxygen glucose deprivation (OGD). In this neuron-screen, just only 4 and of these 57 compound (metergoline, bepridil, carbetapentane and raloxifene), all antagonist of calcium channels and inhibitors of amyloid production, displayed as highly neuroprotective in a pre-treatment toxicity assay but only metergoline compound remained active when tested in a post-treatment protocol administered six hours post OGD damage. Our work stablishes the feasibility of identifying molecule inhibitors of neuronal damage using both screening models and provides novel insights into the mechanisms of neuroprotection by calcium channels antagonists.

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BOARD NUMBER: S01-346

EXPLORING P2X4 PURINERGIC RECEPTOR MODULATION BY ALLOPREGNANOLONE.

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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Aim: allopregnanolone (alloP) positively modulates P2X4 receptor (P2X4R) current. We explored the possibility that alloP modulation might vary depending on ATP concentration. Also, we compared a naïve (BV2 microglia cells) and a heterologous model (HEK-293 cells transfected with rat P2X4R-YFP) to identify eventual differences. Methods: patch-clamp recordings were performed in both cell lines. Cells were stimulated for 5s with ATP for a stable current, exposed to alloP for 25s, followed by a co-stimulation with ATP for 5s. Pre-exposure to alloP is necessary to observe current potentiation, suggesting its distribution on the plasma membrane. Current obtained with alloP was normalized to corresponding basal ATP current. One-way ANOVA test was performed. Results: we determined the ATP concentration-response curves (HEK-293 cells, EC_{50} 13.3 μM, Hill slope 0.649; BV2 cells, EC_{50} 5 μM, Hill slope 0.172). We compared two different alloP concentrations (0.1 and 10 μM) on P2X4 current elicited by ATP 5 and 50 μM. In HEK-293, current potentiation was observed with alloP 0.1 μM using ATP 5 μM (1.95±0.06), while alloP 10 μM potentiated at both ATP concentrations (1.73±0.06; 1.18±0.03). In BV2 cells, alloP 0.1 and 10 μM potentiated current with ATP 5 μM (1.63±0.04; 1.19±0.03, respectively), while at ATP 50 μM, only alloP 10 μM showed potentiation (1.29±0.04). Conclusions: P2X4 current is potentiated by alloP but the effect is less pronounced at increasing ATP concentrations, in both cell lines. Such outcome suggests that alloP influences the pore opening features, because its plasma membrane redistribution is essential to observe P2X4 current potentiation.

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BOARD NUMBER: S01-347

ANALYSIS OF RAT DOPAMINERGIC NEURON NMDA RECEPTOR DOSE-RESPONSE RELATION AND POTENTIATION EFFECTS OF THE POSITIVE ALLOSTERIC MODULATOR, PTC-174.

POSTER SESSION 01 - SECTION: MOLECULAR NEUROPHARMACOLOGY

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NMDA receptor (NMDAR) dysfunction is involved in a variety of neurological disorders including NMDA receptor hypofunction in schizophrenia and there is growing interest in NMDARs as therapeutic targets and in development of subtype-specific allosteric modulator drugs. NMDARs are tetrameric proteins mainly composed of two different types of subunits with two glycine-binding GluN1 and two glutamate-binding GluN2 subunits (4 types, A, B, C and D). Varying expression of each subunit type in different brain regions is the main determinant of functional diversity of NMDARs. NMDARs expressed by dopaminergic neurons of the substantia nigra pars compacta (SNc) are likely to be GluN1/2B/2D triheteromers, which have some characteristics of both diheteromeric GluN1/2B and GluN1/2D receptors. In our experiments, seven day old neonatal rats were humanely killed followed by rapid brain removal to obtain acute brain slices, and patch-clamp whole cell recordings from dopaminergic neurons in substantia nigra compacta (SNc) region were used as a model system to study drug effects on the electrophysiological properties of SNc NMDARs, as well as the pharmacological character of a recently-developed NMDAR positive allosteric modulator, PTC-174 (10.1016/j.neuropharm.2020.107971). NMDAR activation was potentiated 1.7-fold (n=20) by PTC-174 (10 μM), at near maximal NMDAR activation by 10 μM glycine and 200 μM NMDA which suggests the open probability of NMDARs might have been increased by PTC-174 providing insights into the mechanism of drug action. Modelling the NMDAR response suggests the action of PTC can be described by a combination of increased channel open probability combined with a moderate decrease in agonist affinity.

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BOARD NUMBER: S01-348

KIAA1217 AS A NOVEL SYNAPTIC PROTEIN THAT INTERACTS WITH PSD95 AND SHANK3 AND CONTROLS DENDRITIC SPINE PLASTICITY

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Altered synaptic functions have been associated with neurological disabilities and cognitive impairments. In particular, the normal synaptic function requires a tight organization of the postsynaptic density, including receptors and structural and signaling proteins. Here we analyze the role of the adaptor protein KIAA1217 (the human homolog of murine sickle tail gene, hSKT), in regulating synaptic plasticity. A total Skt-/- mouse was characterized for behavioral analysis, in vivo and in vitro dendritic spines (DDs) morphology, electrophysiological properties through Microelectrode Array (MEA), and biochemical interaction of SKT with different synaptic proteins. Finally, we generated the SKT interactomics profile by mass spectrometry (MS) analysis. Mouse brain slices and primary cultured neurons from Skt-/- mice showed a decreased number in total DSs, with a reduction of mushroom DSs and an increase in immature filopodia spine structures. The Puzzle Box Test revealed that Skt-/- mice show an impaired cortical function compared to WT controls. The MEA in primary hippocampal cultures exhibited a substantial delay in neuronal synchronization and maturation. Moreover, to assess the functional molecular interactions of hSKT with the principal PSD components, we found that hSKT interacts with PSD-95 and Shank 3. Finally, MS showed that SKT interacts with proteins involved in cytoskeleton regulation, vesicles cycle, and ionotropic glutamate receptor modulation. All together, these data pave the way to hSKT as a new relevant player in the structural and functional organization of the PSD, suggesting that its absence or alteration could lead to impairment of cognitive processes, memory establishment typical of neurological disorders

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HILAR MOSSY CELLS REGULATE THE ACTIVITY OF HIPPOCAMPAL DENTATE GYRUS CIRCUITRY IN A FREQUENCY-DEPENDENT MANNER

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Mossy cells (MCs) are glutamatergic neurons within the hippocampal dentate gyrus (hDG). They are implicated in key roles of the hDG, such as context discrimination and spatial memory. Our aim was to stimulate the hDG at physiological frequencies associated with spatial memory and determine how MCs regulate this circuitry. We recorded excitatory postsynaptic potentials (EPSPs) induced in dentate granule cells (GCs) by medial perforant pathway (MPP) stimulation in acute hippocampal slices from wildtype (WT) mice and genetically-modified mice that lack MCs. We found the absence of MCs increased the excitability of GCs to MPP stimulation at 20Hz and 50Hz but not at 5Hz. These results were recapitulated in WT slices by the application of type 1 cannabinoid receptor agonist WIN 55,212-2 that selectively blocks glutamate release at MC-GC synapses. These results suggest that MCs regulate GC responses to MPP stimulation at frequencies relevant to spatial memory processing in the hDG.

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BOARD NUMBER: S01-350

SYNAPTIC BASIS OF CEREBELLAR GRANULE CELL POPULATION DYNAMICS

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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As the input layer of the cerebellar cortex, the granule cell layer integrates information from multiple pathways to execute complex sensory-motor behaviors reliably. Sensations and motor actions are temporally extended events that require a representation of the passage of time. Temporal sequences of neural activity have been implicated for reading out time during behavior, but the neural mechanisms underlying such population dynamics are not known. Recently our lab has implemented a simple cerebellar cortex network model that predicts that heterogeneity in short-term synaptic plasticity generates the rich diversity of cerebellar granule cell firing patterns. Synaptic recordings in the lateral cerebellum (Crus I) where, when compared vestibular cerebellum, indicate that synaptic strength and plasticity can vary between regions responsible for different tasks. With the recent development of a fast calcium indicator (GCaMP8f), we reasoned that characterization of the diversity of temporal activity patterns in GCs *in situ* might indeed be possible for the first time. We therefore directly stimulated many MFs with extracellular stimulation and then imaged the activity responses in GCs using two-photon microscopy. Our initial results indicate the existence of a temporal basis generated by the diverse firing patterns of the granule cells. We are currently exploring differences in the population activity patterns between Lobe X and Crus I, as would be suggested by differences in synaptic strength and dynamics between the two regions. Our results begin to provide experimental evidence that short-term synaptic plasticity is a substrate for diverse activity patterns within neural circuits underlying temporal learning.

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MOTOR SEQUENCE LEARNING INDUCES RAPID MICROSTRUCTURAL REORGANIZATION, A DWI-STUDY

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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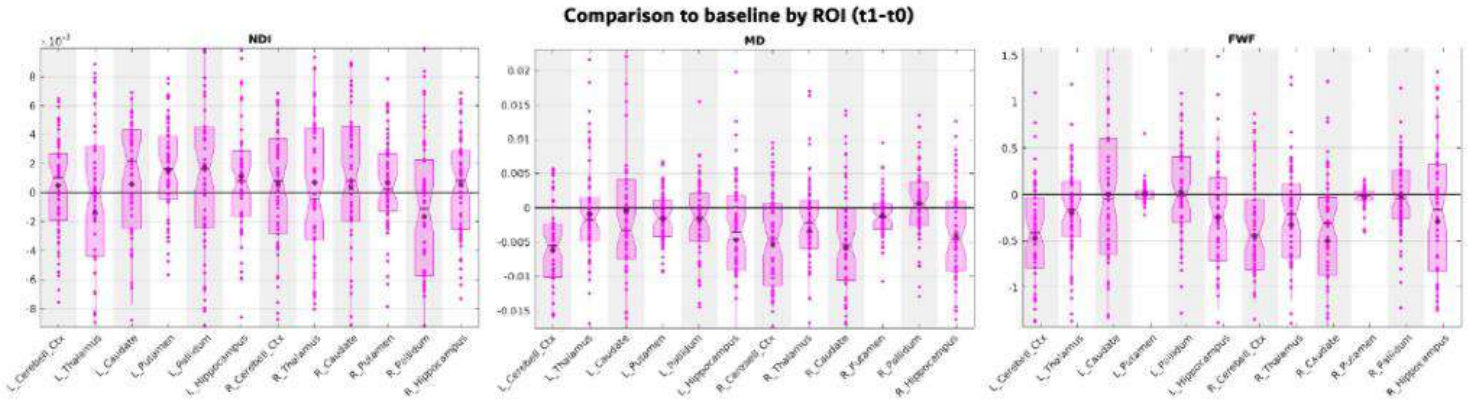
Aims: Diffusion-weighted imaging (DWI) allows observing rapid (micro)structural brain remodeling in cortical and subcortical regions after restricted motor learning time. Even if sensitive, diffusion tensor imaging (DTI) is inherently non-specific, limiting information about underlying cellular processes. Multicompartment diffusion imaging (Neurite Orientation Dispersion and Density Imaging – NODDI) that estimates the microstructural complexity of dendrites and axons would allow tracking neural density changes that dynamically develop. **Methods:** Sixty young (18-30y), healthy adults underwent two DWI MRI sessions before and after 1h-Serial Reaction Time Task learning (30blocks – 96 trials/block) followed by 30min break. DTI and NODDI analyses were conducted on subcortical regions of interest (ROIs). **Results:** A multivariate analysis on DTI/NODDI parameters revealed microstructural modulation in the left thalamus ($p = 0.006$) and putamen ($p = 0.005$), right caudate ($p = 0.006$), and bilateral hippocampus ($ps < 0.007$) and cerebellum ($ps < 0.001$). Univariate post-hoc tests disclosed (a) decreased mean diffusivity (MD) in left putamen, right caudate, bilateral hippocampus and cerebellum; (b) decreased free water fraction (FWF) in bilateral cerebellum and right caudate; (c) increased neurite dispersion index (NDI) in left putamen. Fractional anisotropy (FA) and orientation dispersion index (ODI) were unaltered. **Conclusions:** MD reductions in subcortical regions confirm previously reported motor learning-related microstructural changes. Reduced FWF suggests tissue proportion increased following learning whereas enhanced left putamen NDI suggests a motor learning-related reorganization in brain tissue microstructure and neurite density, potentially reflecting structural brain plasticity changes.



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SEX-SPECIFIC REGULATION OF NMDAR-INDEPENDENT LONG-TERM DEPRESSION BY THE MICROTUBULE-ASSOCIATED PROTEIN TAU

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Dysfunction of the tau protein is the main driver in a group of sex-dependent learning and memory disorders called tauopathies (PMID:33632820). A major factor underlying the low efficacy of past tauopathy therapeutics in humans is the lack of consideration for sex and species differences. Tauopathies impact males and females at different severities and *mice* do not develop tauopathy signs as observed in humans. In comparison to *mice* models, *rat* models better resemble human tau isoform composition (PMID:19141083), tauopathy signs, and overall physiological, genetic and morphologic traits and behaviors of humans (PMID:24161192). For these reasons, we developed a novel CRISPR-Cas9 microtubule-associated protein tau homozygous knock-out (*Mapt*$^{-/-}$) rat model to study sex-specific functions of tau that may be lost in tauopathies. We began by investigating differences in synaptic plasticity in both sexes of P14 – 17 *Mapt*$^{-/-}$ rats by performing electrophysiological recordings of hippocampal Schaffer-collateral to CA1 synapses. Unlike findings in male *Mapt*$^{-/-}$ *mice,* which exhibit no long-term depression (LTD) (PMID:24298146), our findings inversely show that male and female *Mapt*$^{-/-}$*rats*, exhibit increased and no change in LTD, respectively. This is while synaptic transmission, probability-of-neurotransmitter release, and long-term potentiation are unchanged in both sexes of *Mapt*$^{-/-}$ rats. We have further established that this increased LTD in male but not female *Mapt*$^{-/-}$ rats occurs independently of *N*-methyl-$_D$-aspartate receptors (NMDARs). Taken together, our results suggest that sex, and species differences in rodent models may be critical for informing the development of efficacious therapeutics for tauopathies.

Pubmed:
35063495: Klonarakis M, De Vos M, Woo EK, Ralph LT, Thacker JS, Gil-Mohapel J
The three sisters of fate: Genetics, pathophysiology and outcomes of animal models of neurodegenerative diseases. Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) are neurodegenerative disorders characterized by progressive structural and functional loss of specific neuronal populations, protein aggregation, an insidious adult onset, and chronic progression. Modeling AD, PD, and HD in animal models is useful for studying the relationship between neuronal dysfunction and abnormal behaviours. Animal models are also excellent tools to test therapeutic approaches. Numerous genetic and toxin-induced models have been generated to replicate these neurodegenerative disorders. These differ in the genetic manipulation employed or the toxin used and the brain region lesioned, and in the extent to which they mimic the neuropathological and behavioral deficits seen in the corresponding human condition. Each model exhibits unique advantages and drawbacks. Here we present a comprehensive overview of the numerous AD, PD, and HD animal models currently available, with a focus on their utilities and limitations. Differences among models might underlie some of the discrepancies encountered in the literature and should be taken into consideration when designing new studies and testing putative therapies.
Neurosci Biobehav Rev, 2022; 135

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-353

ACTIVITY-INDUCED POLYADENYLATION OF MRNAS IN THE BRAIN

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Local translation of numerous synaptic proteins is essential to the maintenance and plasticity of neuronal synapses. One of the major determinants of mRNA stability and translation is the poly(A) tail. Poly(A) tail is deposited during mRNA maturation in the nucleus, still for some RNAs, its extension may occur in the cytoplasm, allowing for a context-specific regulation of translation. It has been proposed that some dendritically localized mRNAs may undergo activity-induced polyadenylation. However, the mechanism and functional impact of neuronal cytoplasmic mRNA polyadenylation are largely unknown. We applied Oxford Nanopore direct RNA sequencing to measure the genome-wide mRNA poly(A) tail dynamics upon neuronal activity. We analyzed mRNA poly(A) tail length in synaptoneurosomes stimulated *in vitro* for 3, 6 and 12 minutes. Our preliminary data show that indeed some mRNAs display lengthening of poly(A) tails in response to *in vitro* stimulation. Furthermore, we used a model of LTP (long-term synaptic plasticity) induced *in vivo* in rats. LTP was induced by high-frequency stimulation of the medial perforant path input to the dentate gyrus on one side of the rat brain (contralateral non-stimulated side served as control). As a result of these experiments we obtained a dataset of genome-wide poly(A) tail dynamics induced by LTP in the dentate gyrus. We will show and discuss the population of mRNAs that dynamically change their poy(A) tail lengths upon the stimulation.

Pubmed:
32558077: Kuzniewska B, Cysewski D, Wasilewski M, Sakowska P, Milek J, Kulinski TM, Winiarski M, Kozielewicz P, Knapska E, Dadlez M, Chacinska A, Dziembowski A, Dziembowska M
Mitochondrial protein biogenesis in the synapse is supported by local translation.
Synapses are the regions of the neuron that enable the transmission and propagation of action potentials on the cost of high energy consumption and elevated demand for mitochondrial ATP production. The rapid changes in local energetic requirements at dendritic spines imply the role of mitochondria in the maintenance of their homeostasis. Using global proteomic analysis supported with complementary experimental approaches, we show that an essential pool of mitochondrial proteins is locally produced at the synapse indicating that mitochondrial protein biogenesis takes place locally to maintain functional mitochondria in axons and dendrites. Furthermore, we show that stimulation of synaptoneurosomes induces the local synthesis of mitochondrial proteins that are transported to the mitochondria and incorporated into the protein supercomplexes of the respiratory chain. Importantly, in a mouse model of fragile X syndrome, Fmr1 KO mice, a common disease associated with dysregulation of synaptic protein synthesis, we observed altered morphology and respiration rates of synaptic mitochondria. That indicates that the local production of mitochondrial proteins plays an essential role in synaptic functions.
EMBO Rep, 2020; 21
31375980: Nader K, Krysiak A, Beroun A, Pekala M, Szymanska M, Kuzniewska B, Radwanska K, Kaczmarek L, Kalita K
Loss of serum response factor in mature neurons in the dentate gyrus alters the morphology of dendritic spines and hippocampus-dependent behavioral tasks.
Serum response factor (SRF) is a major transcription factor that regulates the expression of several plasticity-associated genes in the brain. Although the developmental expression of SRF in excitatory neurons is crucial for establishing proper hippocampal circuitry, no substantial evidence of its role in unstimulated mature neurons has been provided. The present study used time-controlled, conditional SRF knockout mice and found that the lack of SRF in adult neurons led to decreased actin levels and inactivation of the actin-severing protein cofilin 1 through its increase in phosphorylation at Ser3. The augmentation of cofilin 1 phosphorylation correlated with an alteration of dendritic spine morphology in the dentate gyrus, which was reflected by an increase in the number of spines that clustered into the long-spine category. The changes in spine

morphology coincided with a lower amplitude and frequency of miniature excitatory postsynaptic currents. Moreover, SRF knockout animals were hyperactive and exhibited impairments in hippocampus-dependent behaviors, such as digging, marble burying, and nesting. Altogether, our data indicate that the adult deletion of neuronal SRF leads to alterations of spine morphology and function and hippocampus-dependent behaviors. Thus, SRF deletion in adult neurons recapitulates some aspects of morphological, electrophysiological, and behavioral changes that are observed in such psychiatric disorders as schizophrenia and autism spectrum disorders.
Brain Struct Funct, 2019; 224
30056576: Chmielewska JJ, Kuzniewska B, Milek J, Urbanska K, Dziembowska M
Neuroligin 1, 2, and 3 Regulation at the Synapse: FMRP-Dependent Translation and Activity-Induced Proteolytic Cleavage.
Neuroligins (NLGNs) are cell adhesion molecules located on the postsynaptic side of the synapse that interact with their presynaptic partners neurexins to maintain trans-synaptic connection. Fragile X syndrome (FXS) is a common neurodevelopmental disease that often co-occurs with autism and is caused by the lack of fragile X mental retardation protein (FMRP) expression. To gain an insight into the molecular interactions between the autism-related genes, we sought to determine whether FMRP controls the synaptic levels of NLGNs. We show evidences that FMRP associates with Nlgn1, Nlgn2, and Nlgn3 mRNAs in vitro in both synaptoneurosomes and neuronal cultures. Next, we confirm local translation of Nlgn1, Nlgn2, and Nlgn3 mRNAs to be synaptically regulated by FMRP. As a consequence of elevated Nlgns mRNA translation Fmr1 KO mice exhibit increased incorporation of NLGN1 and NLGN3 into the postsynaptic membrane. Finally, we show that neuroligins synaptic level is precisely and dynamically regulated by their rapid proteolytic cleavage upon NMDA receptor stimulation in both wild type and Fmr1 KO mice. In aggregate, our study provides a novel approach to understand the molecular basis of FXS by linking the dysregulated synaptic expression of NLGNs with FMRP.
Mol Neurobiol, 2019; 56
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Novel calcineurin A (PPP3CA) variant associated with epilepsy, constitutive enzyme activation and downregulation of protein expression.
PPP3CA encodes calmodulin-binding catalytic subunit of calcineurin, a ubiquitously expressed calcium/calmodulin-regulated protein phosphatase. Recently de novo PPP3CA variants were reported as a cause of disease in 12 subjects presenting with epileptic encephalopathy and dysmorphic features. We describe a boy with similar phenotype and severe early onset epileptic encephalopathy in whom a novel de novo c.1324C>T (p.(Gln442Ter)) PPP3CA variant was found by whole exome sequencing. Western blot experiments in patient's cells (EBV transformed lymphocytes and neuronal cells derived through reprogramming) indicate that despite normal mRNA abundance the protein expression level is strongly reduced both for the mutated and wild-type protein. By in vitro studies with recombinant protein expressed in E. coli we show that c.1324C>T (p.(Gln442Ter)) results in constitutive activation of the enzyme. Our results confirm the role of PPP3CA defects in pathogenesis of a distinct neurodevelopmental disorder including severe epilepsy and dysmorphism and provide further functional clues regarding the pathogenic mechanism.
Eur J Hum Genet, 2019; 27
29880715: Jones KJ, Templet S, Zemoura K, Kuzniewska B, Pena FX, Hwang H, Lei DJ, Haensgen H, Nguyen S, Saenz C, Lewis M, Dziembowska M, Xu W
Rapid, experience-dependent translation of neurogranin enables memory encoding.
Experience induces de novo protein synthesis in the brain and protein synthesis is required for long-term memory. It is important to define the critical temporal window of protein synthesis and identify newly synthesized proteins required for memory formation. Using a behavioral paradigm that temporally separates the contextual exposure from the association with fear, we found that protein synthesis during the transient window of context exposure is required for contextual memory formation. Among an array of putative activity-dependent translational neuronal targets tested, we identified one candidate, a schizophrenia-associated candidate mRNA, neurogranin (Ng, encoded by the gene) responding to novel-context exposure. The Ng mRNA was recruited to the actively translating mRNA pool upon novel-context exposure, and its protein levels were rapidly increased in the hippocampus. By specifically blocking activity-dependent translation of Ng using virus-mediated molecular perturbation, we show that experience-dependent translation of Ng in the hippocampus is required for contextual memory formation. We further interrogated the molecular mechanism underlying the experience-dependent translation of Ng, and found that fragile-X mental retardation protein (FMRP) interacts with the 3'UTR of the mRNA and is required for activity-dependent translation of Ng in the synaptic compartment and contextual memory formation. Our results reveal that FMRP-mediated, experience-dependent, rapid enhancement of Ng translation in the hippocampus during the memory acquisition enables durable context memory encoding.

Proc Natl Acad Sci U S A, 2018; 115
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Preparation of polysomal fractions from mouse brain synaptoneurosomes and analysis of polysomal-bound mRNAs.
Here we describe a detailed, reliable protocol for isolation of polysomal fractions from mouse brain synaptoneurosomes. This method is an important tool to study local protein synthesis in neurons.
J Neurosci Methods, 2018; 293
25636686: Kuzniewska B, Nader K, Dabrowski M, Kaczmarek L, Kalita K
Adult Deletion of SRF Increases Epileptogenesis and Decreases Activity-Induced Gene Expression.
Although the transcription factor serum response factor (SRF) has been suggested to play a role in activity-dependent gene expression and mediate plasticity-associated structural changes in the hippocampus, no unequivocal evidence has been provided for its role in brain pathology, such as epilepsy. A genome-wide program of activity-induced genes that are regulated by SRF also remains unknown. In the present study, we show that the inducible and conditional deletion of SRF in the adult mouse hippocampus increases the epileptic phenotype in the kainic acid model of epilepsy, reflected by more severe and frequent seizures. Moreover, we observe a robust decrease in activity-induced gene transcription in SRF knockout mice. We characterize the genetic program controlled by SRF in neurons and using functional annotation, we find that SRF target genes are associated with synaptic plasticity and epilepsy. Several of these SRF targets function as regulators of inhibitory or excitatory balance and the structural plasticity of neurons. Interestingly, mutations in those SRF targets have found to be associated with such human neuropsychiatric disorders, as autism and intellectual disability. We also identify novel direct SRF targets in hippocampus: Npas4, Gadd45g, and Zfp36. Altogether, our data indicate that proteins that are highly upregulated by neuronal stimulation, identified in the present study as SRF targets, may function as endogenous protectors against overactivation. Thus, the lack of these effector proteins in SRF knockout animals may lead to uncontrolled excitation and eventually epilepsy.
Mol Neurobiol, 2016; 53
23508111: Kuzniewska B, Rejmak E, Malik AR, Jaworski J, Kaczmarek L, Kalita K
Brain-derived neurotrophic factor induces matrix metalloproteinase 9 expression in neurons via the serum response factor/c-Fos pathway.
Brain-derived neurotrophic factor (BDNF) plays a pivotal role in the regulation of the transcription of genes that encode proplasticity proteins. In the present study, we provide evidence that stimulation of rat primary cortical neurons with BDNF upregulates matrix metalloproteinase 9 (MMP-9) mRNA and protein levels and increases enzymatic activity. The BDNF-induced MMP-9 transcription was dependent on extracellular signal-regulated kinase 1/2 (ERK1/2) pathway and c-Fos expression. Overexpression of AP-1 dimers in neurons led to MMP-9 promoter activation, with the most potent being those that contained c-Fos, whereas knockdown of endogenous c-Fos by small hairpin RNA (shRNA) reduced BDNF-mediated MMP-9 transcription. Additionally, mutation of the proximal AP-1 binding site in the MMP-9 promoter inhibited the activation of MMP-9 transcription. BDNF stimulation of neurons induced binding of endogenous c-Fos to the proximal MMP-9 promoter region. Furthermore, as the c-Fos gene is a known target of serum response factor (SRF), we investigated whether SRF contributes to MMP-9 transcription. Inhibition of SRF and its cofactors by either overexpression of dominant negative mutants or shRNA decreased MMP-9 promoter activation. In contrast, MMP-9 transcription was not dependent on CREB activity. Finally, we showed that neuronal activity stimulates MMP-9 transcription in a tyrosine kinase receptor B (TrkB)-dependent manner.
Mol Cell Biol, 2013; 33
22810102: Bialopiotrowicz E, Szybinska A, Kuzniewska B, Buizza L, Uberti D, Kuznicki J, Wojda U
Highly pathogenic Alzheimer's disease presenilin 1 P117R mutation causes a specific increase in p53 and p21 protein levels and cell cycle dysregulation in human lymphocytes.
Cell cycle (CC) reentry in neurons precedes the formation of amyloid-β (Aβ) plaques in Alzheimer's disease (AD). CC alterations were also detected in lymphocytes from sporadic AD patients. In the present study, we investigated the influence of nine presenilin 1 (PS1) mutations (P117R, M139V, L153V, H163R, S170F, F177L, I213F, L226F, E318G) on CC and Aβ production in immortalized B-lymphocytes from familial AD (FAD) patients and in stably transfected human embryonic kidney cells. In both cell types, only the P117R mutation increased levels of key G1/S phase regulatory proteins, p53, and its effector p21, causing G1 phase prolongation with simultaneous S phase shortening, and lowering basal apoptosis. The CC changes were rescued by inhibition of p53, but not of γ-secretase. Moreover, the investigated PS1 mutants showed differences in the increased levels of secreted Aβ40 and Aβ42 and in Aβ42/Aβ40 ratios, but these differences did not correlate with CC patterns. Altogether, we found that both CC regulation and Aβ production differentiate PS1 mutations, and that CC PS1 activity is mediated by p53/p21 signaling but not by γ-secretase activity. The identified CC dysregulation linked with increased p53 and p21 protein levels distinguishes the highly pathogenic PS1 P117R mutation and may contribute to the specific severity of the clinical progression of FAD associated with the mutation in the PS1 117 site. These findings suggest that

impairment in lymphocyte CC might play a pathogenic function in AD and are relevant to the development of new diagnostic approaches and personalized therapeutic strategies.
J Alzheimers Dis, 2012; 32
20541838: Bialopiotrowicz E, Kuzniewska B, Kachamakova-Trojanowska N, Barcikowska M, Kuznicki J, Wojda U
Cell cycle regulation distinguishes lymphocytes from sporadic and familial Alzheimer's disease patients.
Cell cycle (CC) reactivation in neurons seems to underlie the development of Alzheimer's disease (AD). We analyzed whether CC alterations can be detected in immortalized lymphocytes from patients with the sporadic and the familial form of AD (SAD and FAD). Real-time polymerase chain reaction (PCR)-arrays, immunoblotting, and flow cytometry demonstrated differences in the regulation of G1/S phases between SAD lymphocytes and cells from nondemented subjects, as well as between SAD and FAD cells. SAD compared to FAD lymphocytes showed differences in expression profiles of the 90 CC genes, and a marked increase in the level of the p21 protein, which promotes G1-arrest. Accordingly, SAD but not FAD cells had a prolonged G1-phase. γ-secretase inhibition did not change the CC profiles of the cell lines. These data show that SAD involves a prolongation of the G1 phase driven by p21 pathway, which is not activated in FAD cells. Thus, the mechanism in SAD differs from FAD. Moreover, disturbances of the CC in lymphocytes have a potential diagnostic value.
Neurobiol Aging, 2011; 32

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BOARD NUMBER: S01-354

ABERRANT LTP IN HTAU P301S MICE RESCUED BY ACUTE APPLICATION OF RECOMBINANT APPSα

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Alzheimer's disease (AD) is a common neurodegenerative disease in the aging population. One of its hallmarks, in addition to Aβ plaques, is the accumulation of hyperphosphorylated Tau. Focusing on the tauopathy with the transgenic mouse model hTau.P301S we could show a progressive phenotype in hippocampal synaptic plasticity of these mice, including an aberrant long-term potentiation (LTP) and reduced basal synaptic transmission after stimulation at the Schaffer collaterals in aged mice while the long-term depression (LTD) was unaltered at the investigated ages (10-13/16-18 weeks). Previously, we could demonstrate the neuroprotective effect of the neurotrophic peptide APPsα, which is produced by the processing of the Amyloid precursor protein (APP) via α-secretases in the non-amyloidogenic pathway. In contrast to the neurodegenerative effect of Aβ, the APPsα molecule was shown to rescue phenotypes of various AD pathology models. In our approach the treatment of P301S hippocampal acute slices with a nanomolar concentration of recombinant APPsα was able to restore the increased LTP to control level. To further investigate the altered synaptic plasticity in P301S mice and its possibly missing inhibitory input, as well as to learn more about the neuro- and synaptoprotective effect of APPsα, we used a GABA receptor agonist treatment before stimulation and applied recAPPsα to wildtype acute slices. Taken together we show the potential of APPsα as a therapeutic for tauopathies in addition to its previously shown positive effect on Aβ-dependent AD phenotypes, as well as the disturbed neuronal circuits resulting in altered synaptic plasticity in the P301S model (DFG-supported, MU1457/14-1).

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LINKING TEMPORALLY PROXIMAL MEMORIES: A PROCESS UNDERLAIN BY SYNAPTIC TAGGING AND CAPTURE MECHANISM

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

Yuki Sakai[1], Bruno Brizard[1], Jonathan Zapata[2], Vincent Camus[1], Catherine Belzung[1], Alexandre Surget[1]
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Introduction: How is it that trivial events are sometimes remembered, which would otherwise be forgotten, when they occur temporally close to a memorable event? We investigate whether linking temporally proximal memories depends on the synaptic and tagging capture (STC) hypothesis, in which weakly stimulated cells benefit from plasticity-related proteins from temporally-close strong stimulations. **Methods:** We monitored cell ensemble activities in hippocampal CA1 using *in-vivo* Ca+ imaging while mice were allowed to freely explore to two different environments (A and B), with protein synthesis inhibition (anisomycin, 150mg/kg) introduced before environment B on day 1. The following day, mice were tested in both environments without inhibition. **Results**: Cells that were only activated in room B on day 1 displayed a significantly lower rate of reactivation on day 2 compared to cells activated in room A, suggesting that protein synthesis is critical for consolidating and retrieving cell ensembles recruited during encoding. Cells that were coactivated in both rooms on day 1 displayed a significantly higher reactivation on day 2 in room B compared to cells uniquely activated in room B on day 1. This suggests that cells coactive on day 1 benefited from the protein synthesis induced by room A, rescuing their incorporation into the cell ensemble associated to room B on day 2. **Conclusion:** These results demonstrate the existence of a STC-like mechanism in freely-exploring animals when they encode and consolidate two temporally-proximal memory traces. These findings suggest a functional role of this mechanism in temporal association of two memory traces.

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ABNORMAL CHANGES IN HIPPOCAMPAL SYNAPTIC PLASTICITY ARE ACCOMPANIED BY PARVALBUMIN REDUCTIONS IN THE TGF344 RAT MODEL FOR ALZHEIMER'S DISEASE

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Introduction: Hippocampal hyper-excitability is a causative factor in memory impairment and has been documented in many animal models for Alzheimer's disease (AD). Here, we sought to track AD-related prodromal changes in hippocampal synaptic strength and plasticity in the TgF344-AD rat model, which exhibits age-dependent beta-amyloid (Aß) aggregation, tauopathy and neuroinflammation. Methods: Electrophysiology: Evoked responses in dorsal CA1 were recorded following CA3 Schaffer stimulation in urethane-anaesthetised 6- and 9-month-old male TgF344-AD (TG) and wild-type (WT) rats, to determine the induction properties for short- and long-term synaptic plasticity and baseline connectivity. Post-mortem: Parvalbumin (PV) levels were measured in dorsal hippocampus by Simple Western analysis and immunohistochemistry of age-matched rats. Results: In contrast to 6 month-old TG rats, the 9-month-old TGs showed significantly smaller paired-pulse facilitation, and this cohort of TG animals also displayed an upwardly-shifted baseline input/output curve. Long-term potentiation induced by high-frequency stimulation was significantly reduced in CA1 of 6- and 9-month old TG rats. LTP depotentiation tested in 6-month old rats was much stronger in TG compared to WT. Immunohistochemical analysis revealed significantly lower PV levels in CA2/3 region, but not CA1 or dentate gyrus of the hippocampus in 9 month old TG rats. Work is underway to determine PV changes at 6 months old. Conclusions: These results suggest that reduced inhibition upstream of the CA3->CA1 synapse leads to abnormal changes in synaptic plasticity, which might imply a possible mechanism for disease progression at pre-symptomatic stage of AD.

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CHRONIC IN VIVO ALTERATIONS OF AETA-ALPHA PEPTIDE LEVELS PERTURB SYNAPTIC PLASTICITY AND IMPACT SPATIAL MEMORY.

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

Jade Dunot[1], Maria Mensch[1], Ana Rita Slagueiro-Pereira[1], Sandy Ma[1], Paula Pousinha[1], Ingrid Bethus[1], Carine Gandin[1], Celeste Ferraguto[2], Camilla Giudici[3], Benedikt Weffers[4], Ronald Naumann[5], Christian Haass[3], Michael Willem[3], Hélène Marie[6]
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Aims: Physiopathological cleavage of the amyloid precursor protein (APP) allows the secretion of Aβ but also of other peptides, including peptides secreted from the η-secretase pathway, named Aη peptides, described in Willem et al., Nature, 2015. These peptides acutely impair neuron function even at low nanomolar concentrations (Mensch et al. Alzheimers Res Ther., 2021). Here we analysed the consequences of chronic in vivo alterations of Aη peptide levels on hippocampal function. Methods: To modulate Aη levels in vivo two new mouse models were created. The first model, named MISEPA2, harbours a transgene expressing a secreted form of Aη-α allowing for a chronic increase of human Aη-α levels in the brain. The second model, named APPΔη, harbours a deletion of a portion of endogenous APP that prevents η-secretase processing. We characterized these models by analysing APP peptides levels by immunoblotting, synaptic plasticity by electrophysiology and spatial memory by submitting mice to the Morris water maze. Results: MISEPA2 mice exhibit normal levels of Aβ in the hippocampus. Long-term potentiation (LTP), long-term depression (LTD) and spatial memory are altered in these mice. APPΔη mice exhibit normal levels of Aβ in the hippocampus. LTP is normal, but LTD is absent and rescued upon acute application of synthetic Aη-α peptide. Spatial memory is impaired in these mice. Conclusions: These results show that a chronic increase of Aη-α is detrimental to hippocampal function. We also validate the hypothesis that the peptides secreted from the η-secretase pathway are necessary for endogenous modulation of synaptic communication and memory processing.

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BOARD NUMBER: S01-358

AREA AND LAYER-SPECIFIC ENCODING OF OBJECT DIMENSIONS IN THE PERIRHINAL CORTEX

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Aims: The perirhinal cortex (PRC) critically engages in sensory information processing and object recognition, supported by its diverse efferent and afferent connections with other brain structures. Here, we investigated to what extent the cortical layers of areas 35 and 36 of the PRC are involved in the encoding of object dimensions. Methods: Rats explored either microscale (add item dimensions and partially concealed) or macroscale (add item dimensions and highly visible) objects for 5 min (Arc mRNA assessment). We then employed fluorescence in situ hybridization to examine the expression of nuclear Arc mRNA in the sublayers of the PRC along its rostro-caudal axis triggered by object exploration. Results: Whereas layers III and V-VI of the middle and caudal parts of area 35 showed a significant increase in nuclear Arc mRNA expression in response to both novel microscale and macroscale object exposure, only layer III of area 36 was activated when animals explored microscale objects. Conclusions: Our observations suggest a functional differentiation of areas 35 and 36 with regard to the encoding of object dimensions: Area 36 is only activated by information about smaller and less visible objects, whereas area 35 engages in object learning regardless of object dimension. Acknowledgements: This work was funded by a German Research Foundation grant to DMV (SFB874/B1; project no.: 122679504).

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BENEFICIAL EFFETCS OF 5-HT4RS AGONIST ON MEMORY PERFORMANCES ARE INTIMATELY LINKED TO CHANGES IN HIPPOCAMPAL FUNCTION.

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Type 4 serotonin receptors (5-HT_4R) have earned a place in the sun as a promising therapeutic target for the treatment of memory dysfunctions. Being highly expressed in the hippocampus, they are a target of choice to treat cognitive disorders. Beneficial effects have been described in rodents following their activation. Thus, pro-memory and anti-amnesic effects were observed in adult and aged animals, in healthy condition or in experimental models of memory deficits. To explore the underlying mechanisms, several approaches combining behavioral, optogenetic, electrophysiological and biochemical analyses, were used. We first confirmed that 5-HT_4R activation (RS67333, 1 mg/kg) before acquisition of an hippocampus-dependent memory test (object recognition), exerts beneficial effect. Besides, RS67333 showed efficacy to improve the location discrimination performances in a pattern separation task. Thereafter, electrophysiological investigations on hippocampal slices, revealed changes in long-term potentiation (LTP) magnitude following RS67333 treatment. Meanwhile, various hippocampal neurotransmitters involved in LTP were quantified by liquid chromatography coupled to tandem mass spectrometry. A significant increase in glutamate levels was measured after RS67333 administration. Overall, these observations support that the beneficial effects of 5-HT_4R activation on memory are intimately linked to changes in hippocampal synaptic plasticity. The latter are likely due to the observed variations in neurotransmitter levels. Although further experiments are needed, we here outline promising leads in the understanding of the molecular pathways involved in the beneficial effects of 5-HT_4R activation on memory.

Pubmed:
32935845: Lecouflet P, Roux CM, Potier B, Leger M, Brunet E, Billard JM, Schumann-Bard P, Freret T
Interplay between 5-HT4 Receptors and GABAergic System within CA1 Hippocampal Synaptic Plasticity.
The type 4 serotonin receptor (5-HT4R) is highly involved in cognitive processes such as learning and memory. Behavioral studies have shown a beneficial effect of its activation and conversely reported memory impairments by its blockade. However, how modulation of 5HT4R enables modifications of hippocampal synaptic plasticity remains elusive. To shed light on the mechanisms at work, we investigated the effects of the 5-HT4R agonist RS67333 on long-term potentiation (LTP) within the hippocampal CA1 area. Although high-frequency stimulation-induced LTP remained unaffected by RS67333, the magnitude of LTP induced by theta-burst stimulation was significantly decreased. This effect was blocked by the selective 5-HT4R antagonist RS39604. Further, 5-HT4R-induced decrease in LTP magnitude was fully abolished in the presence of bicuculline, a GABAAR antagonist; hence, demonstrating involvement of GABA neurotransmission. In addition, we showed that the application of a GABABR antagonist, CGP55845, mimicked the effect of 5-HT4R activation, whereas concurrent application of CGP55845 and RS67333 did not elicit an additive inhibition effect on LTP. To conclude, through investigation of theta burst induced functional plasticity, we demonstrated an interplay between 5-HT4R activation and GABAergic neurotransmission within the hippocampal CA1 area.
Cereb Cortex, 2021; 31
34769511: Roux CM, Leger M, Freret T
Memory Disorders Related to Hippocampal Function: The Interest of 5-HTRs Targeting.
The hippocampus has long been considered as a key structure for memory processes. Multilevel alterations of hippocampal function have been identified as a common denominator of memory impairments in a number of psychiatric and neurodegenerative diseases. For many years, the glutamatergic and cholinergic systems have been the main targets of therapeutic treatments against these symptoms. However, the high rate of drug development failures has left memory impairments on the sideline of current therapeutic strategies. This underscores the urgent need to focus on new therapeutic targets for memory disorders, such as type 4 serotonin receptors (5-HTRs). Ever since the discovery of their expression in the hippocampus, 5-HTRs have gained growing interest for potential use in the treatment of learning and memory

impairments. To date, much of the researched information gathered by scientists from both animal models and humans converge on pro-mnesic and anti-amnesic properties of 5-HTRs activation, although the mechanisms at work require more work to be fully understood. This review addresses a fundamental, yet poorly understood set of evidence of the potential of 5-HTRs to re-establish or limit hippocampal alterations related to neurological diseases. Most importantly, the potential of 5-HTRs is translated by refining hypotheses regarding the benefits of their activation in memory disorders at the hippocampal level.
Int J Mol Sci, 2021; 22

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POST-WEANING N-3 PUFA IMPROVES MEMORY AND SYNAPTIC PLASTICITY ALTERATIONS INDUCED BY DEVELOPMENTAL N-3 PUFA DECREASE ACCORDING TO SEX

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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The brain contains n-3 and n-6 polyunsaturated fatty acids (PUFAs), which have distinct biological activities. PUFAs are provided through the diet and aggregate in the brain since the perinatal period. Our previous data show that perinatal dietary n-3 PUFAs deficiency changes amounts of n-3 PUFAs in the hippocampus, alters spatial memory and impairs hippocampal neuronal plasticity in male mice at weaning (P21). Here, we aim at deciphering whether a n-3 PUFAs-sufficient diet reverses the effect of perinatal dietary n-3 PUFAs deficiency taking into account sex. Male and female mice were fed with n-3 PUFAs-deficient or sufficient diets until P21. Then, half of the n-3 PUFAs-deficient mice were fed with a n-3 PUFAs-sufficient diet until adulthood. We assessed spatial memory abilities, fatty acids and their metabolites in the hippocampus and plasticity. In addition, a genetically modified mouse model expressing iFat1, a C Elegans gene allowing to convert n-6 into n-3 PUFAs into Camk2-cre positive cells, was created to study whether a specific increase of n-3 PUFAs in glutamatergic neurons counteract the deleterious effect of n-3 PUFAs-deficient diet. A n-3 PUFAs-sufficient diet given at weaning restores plasticity in both male and female adult mice, while it partially restores hippocampal fatty acids levels and memory. Perinatal exposition to a deficient diet impairs hippocampal PUFAs levels, memory and plasticity. Interestingly, exposition to a sufficient diet from weaning reversed plasticity alterations and cognition as well as fatty acid levels in the brain but in a different way between the two sexes.

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PHASE SEPARATING PROPERTY OF NARGBP2 COORDINATES ITS FUNCTIONALITY IN MATURE NEURONS

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Ablation of nArgBP2, a candidate gene of intellectual disability (ID), caused defects in spine maturation and excitatory synapse formation in developing neurons. Surprisingly, nArgBP2 knockdown in mature neurons did not cause defects in spine morphogenesis, and its inhibitory effect was evident only when challenged with chemically-induced long-term potentiation (cLTP), raising the question of how it functions in mature neurons. Here, we found that nArgBP2 itself assembles into liquid condensates via intermolecular interaction between SH3 domains and proline-rich domains *in vitro* and in living cells. We further showed that condensate formation was counteracted by WAVE1 interaction, and regulated by calcium/calmodulin-dependent protein kinase IIα (CaMKIIα)-dependent phosphorylation. Finally, we showed that nArgBP2 also forms liquid droplets in the dendritic spines of living neurons, and these droplets were dispersed by cLTP, which coincides with spine enlargement. Blocking its interaction with WAVE abrogated spine enlargement during cLTP. Our results suggest that sequestered nArgBP2 by phase-separation at rest is dissolved by CaMKIIα-dependent phosphorylation during cLTP, which in turn interacts with WAVE1 to manifest spine enlargement. In addition to revealing the underlying mechanism of nArgBP2 functionality in mature neurons, our results also suggest its defect may contribute to the pathogenesis of ID.

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A PROTEIN-DRIVEN HETEROSYNAPTIC RULE FOR SPIKING NEURAL NETWORKS

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Aims: Conditions for synaptic plasticity have been extensively studied throughout the last decades to gain insight into superior cognitive functions like memory or learning and their functional drift during pathological conditions. However, a complete mechanistic model of their complex behavior has not yet been fully developed, and many state of the art modelisations still partially rely on purely heuristic rules.Therefore, our goal in this work is to investigate the molecular machinery that underlies the spatio-temporal long-term heterosynaptic plasticity evolving across hours. **Methods:** Inspired by results and experimental data presented in a related FENS abstract of our group, we will explore spatial dynamics of peri-synaptic calcium and the primary synaptic plasticity driving molecules (calmodulin, calcineurin, CaMKII). Thus, we can expand the diffusion-degradation formalism into a more complex spatio-temporal reaction-diffusion model and account for inter-protein catalytic events, spine-dendrite exchange and resource-bounded interplay. Linking this model to the spine growth rate - considered a synaptic strength proxy - will allow us to define a mathematically tractable, biologically plausible plasticity framework that will then be implemented in a feed-forward spiking neural network. **Results and conclusions:** Finally, the results obtained via the simulation will be illustrated in relation to the current literature, focusing on the direct connection between molecular species, circuit behaviour and eventual pathologic phenotype.

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NEURAL EXTRACELLULAR MATRIX REMODELING AS A POTENTIAL TARGET FOR COGNITIVE ENHANCEMENT IN THE AGING BRAIN

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Aims: Age-dependent accumulation of extracellular matrix (ECM) molecules is implicated in the age-associated decline in synaptic and cognitive functions, which are common phenotypes of both normal or pathological aging. Therefore, we aimed to study age-associated changes in the expression of neural ECM-related genes and to validate new targets for rejuvenation of the aging brain and restoration of synaptic plasticity and cognition. **Methods:** In this study, we performed the qPCR analysis of major ECM-degrading and modifying enzymes in the hippocampus, behavioral analysis, electrophysiology, and immunohistochemistry. Three groups of C57BL6/J mice were studied 2- to 3-month-old (2-3M), 22- to 26-month-old (22-26M), and more than 30-month-old (>30M). **Results:** Using qPCR, we discovered a downregulation of ECM-degrading enzyme, a disintegrin, and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) in hippocampal neurons and astrocytes of 24-26M and >30M mice. Therefore, we used AAV delivery to rejuvenate the hippocampus of 24-26M mice by overexpressing ADAMTS5, which indeed resulted in a loss of aggrecan and other lecticans in the perineuronal nets and improvements of the hippocampus-dependent cognitive functions. Electrophysiological studies demonstrated an enhanced long-term potentiation in the rejuvenated hippocampus of 24-26M mice. Further analysis revealed that ECM degradation in the aged brain inhibits signaling through the extrasynaptic GluN2B-NMDA receptors, which activation is known to impair LTP in aged brains. **Conclusion:** Altogether, we show that age-dependent dysregulation of the ECM proteolytic remodeling is one of the factors responsible for the accumulation of neural ECM, while stimulation of ECM remodeling by overexpression of ADAMTS5 improves hippocampal plasticity and cognitive function.

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NMDAR-LTD IS STAT3 INDEPENDENT IN ADULT MICE

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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NMDA receptor-dependent long-term depression (NMDAR-LTD), a form of synaptic plasticity, has been found to be involved in processes including behavioural flexibility, reversal learning, and synaptic pruning. Despite recent advances in the understanding of this form of plasticity, its mechanisms haven't been fully elucidated. Our lab previously studied the novel role of Signal Transducer and Activator of Transcription 3 (STAT3) in NMDAR-LTD in rats. STAT3 was found to be necessary for LTD expression in the CA1 region of the hippocampus, though not in a transcription dependent manner, as determined through drug inhibition and shRNA treatment. The current study aimed to further this work by investigating the role of STAT3 in NMDAR-LTD specifically in mice using a genetic deletion approach. Mice harbouring a genetic deletion of STAT3 under the CamKII promoter were generated and confirmed through biochemical techniques. Due to the temporal expression of CamKII in CA1, 10-18-week-old male mice were used for experiments. To study the effect of STAT3 deletion on NMDAR-LTD, field recordings from stratum radiatum were performed on hippocampal slices from adult STAT3 control and knockout mice. Following treatment with 20 μM NMDA for three minutes, we found no decrease in the amount of LTD in knockout mice compared to controls. Western blotting of hippocampal slices treated with NMDA from control and knockout animals showed comparable dephosphorylation of the GluA1 subunit on S845, corroborating our electrophysiological findings. Together, these results suggest that STAT3 does not play a necessary role in NMDAR-LTD in CA1 in adult mice.

Pubmed:
33277577: Demmings MD, Tennyson EC, Petroff GN, Tarnowski-Garner HE, Cregan SP
Activating transcription factor-4 promotes neuronal death induced by Parkinson's disease neurotoxins and α-synuclein aggregates.
Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra resulting in severe and progressive motor impairments. However, the mechanisms underlying this neuronal loss remain largely unknown. Oxidative stress and ER stress have been implicated in PD and these factors are known to activate the integrated stress response (ISR). Activating transcription factor 4 (ATF4), a key mediator of the ISR, and has been reported to induce the expression of genes involved in cellular homeostasis. However, during prolonged activation ATF4 can also induce the expression of pro-death target genes. Therefore, in the present study, we investigated the role of ATF4 in neuronal cell death in models of PD. We demonstrate that PD neurotoxins (MPP+ and 6-OHDA) and α-synuclein aggregation induced by pre-formed human alpha-synuclein fibrils (PFFs) cause sustained upregulation of ATF4 expression in mouse cortical and mesencephalic dopaminergic neurons. Furthermore, we demonstrate that PD neurotoxins induce the expression of the pro-apoptotic factors Chop, Trb3, and Puma in dopaminergic neurons in an ATF4-dependent manner. Importantly, we have determined that PD neurotoxin and α-synuclein PFF induced neuronal death is attenuated in ATF4-deficient dopaminergic neurons. Furthermore, ectopic expression of ATF4 but not transcriptionally defective ATF4ΔRK restores sensitivity of ATF4-deficient neurons to PD neurotoxins. Finally, we demonstrate that the eIF2α kinase inhibitor C16 suppresses MPP+ and 6-OHDA induced ATF4 activation and protects against PD neurotoxin induced dopaminergic neuronal death. Taken together these results indicate that ATF4 promotes dopaminergic cell death induced by PD neurotoxins and pathogenic α-synuclein aggregates and highlight the ISR factor ATF4 as a potential therapeutic target in PD.
Cell Death Differ, 2021; 28

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ENVIRONMENTAL ENRICHMENT INCREASES SPARSE CODING IN ADULT HIPPOCAMPUS

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Sparse activity and sparse coding in dentate gyrus (DG) of the hippocampus has been considered to play a key role in memory processing. Environmental enrichment, which includes larger housing and the presence of enrichment toys and running wheels, has been shown to improve hippocampus-dependent spatial learning and memory. However, how different housing conditions influence hippocampal network activity remains still largely unclear. Here we used cFos labeling to study the effect of prolonged environmental enrichment onto hippocampal network activity during exploration of a novel environment. First, we showed that exploration leads to increased activity in DG and CA1 in animals housed under both standard and enrichment conditions. However, cFos activity during exploration was lower in enriched animals in both, granule cells and CA1 pyramidal cells. Remarkably, the number of cFos-labeled cell assemblies in home cage was even more strongly decreased with enriched housing. This indicates that continuous enrichment does not increase overall hippocampal activity but improves sparse coding in DG as well as in CA1. To understand the underlying mechanisms, we explored the possibilities that inhibitory interneurons are involved. Previous data indicate that interneurons contribute to sparse coding partially via α5-subunit-containing $GABA_A$ receptors (Lodge et al. 2021, Cell Reports 37:109768). After we have blocked these receptors by applying a highly selective negative allosteric modulator α5-NAM, the number of active cells was strongly increased in enriched animals. Taken together, environmental enrichment decreases the size of hippocampal cell assemblies during spatial exploration. This might be partially mediated by increased inhibition via dendrite-targeting interneurons.

Pubmed:
27455410: Verdiyan EE, Allakhverdiev ES, Maksimov GV
Study of the Peripheral Nerve Fibers Myelin Structure Changes during Activation of Schwann Cell Acetylcholine Receptors. In the present paper we consider a new type of mechanism by which neurotransmitter acetylcholine (ACh) regulates the properties of peripheral nerve fibers myelin. Our data show the importance of the relationship between the changes in the number of Schwann cell (SC) acetylcholine receptors (AChRs) and the axon excitation (different intervals between action potentials (APs)). Using Raman spectroscopy, an effect of activation of SC AChRs on the myelin membrane fluidity was investigated. It was found, that ACh stimulates an increase in lipid ordering degree of the myelin lipids, thus providing evidence for specific role of the "axon-SC" interactions at the axon excitation. It was proposed, that during the axon excitation, the SC membrane K+- depolarization and the Ca2+-influx led to phospholipase activation or exocytosis of intracellular membrane vesicles and myelin structure reorganization.
PLoS One, 2016; 11

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ARC INTERACTS WITH SPLICING FACTORS AND REGULATES ACTIVITY-DEPENDENT FORMATION OF PABP2 NUCLEAR SPECKLES

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

Tambudzai Kanhema[1], Kamil Parobczak[2], Dagmara Holm[2], Sudarshan Patil[1], Francois Pauzin[1], Adriana Magalska[2], Grzegorz Wilczynski[2], Clive Bramham[1]
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Activity-regulated cytoskeleton associated protein (Arc/Arg3.1) is an immediate early gene which required for long-term memory formation and multiple forms of activity-dependent synaptic plasticity. Arc protein has been extensively studied in the cytoplasm and dendrites but, its role in the nucleus is little understood. In this study we performed structural, functional, and biochemical analysis to identify Arc`s nuclear interactome using rat dentate gyrus post high frequency stimulation, seize and using treated hippocampal neurons. Confocal microscopy showed that Arc occupies internal parts of the nucleus, closely associated with hnRNPs. Electron microscopy further revealed labeling at the peripheral areas of chromatin. Arc pulldown experiments performed in nuclear fractions suggest that Arc interacts with component of the splicing machinery. Collectively, our data suggest that nuclear Arc is involved in pre-mRNA processing. These data underscore multiple roles for Arc protein carried out within distinct subcellular domains.

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A LOCAL DENDRITIC ROLE FOR MIR-218 IN THE REGULATION OF HOMEOSTATIC SYNAPTIC DOWNSCALING

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

David Colameo, Michael Soutschek, Sara Maley, Carlotta Gilardi, Lukas Von Ziegler, Johannes Bohacek, Jochen Winterer, Pierre-Luc Germain, Gerhard Schratt
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Homeostatic synaptic downscaling (HSD) is a negative feedback mechanism by which neurons reduce the overall strength of excitatory synapses in response to chronically elevated neuronal activity to keep their firing rates within a physiological range. Physiologically, HSD is involved during sleep in a process that promotes memory consolidation. Moreover, impairments in HSD have been associated with epilepsy and neurodevelopmental disorders. MicroRNAs (miRNAs) play an essential role in post-transcriptional gene regulation, mostly by inhibiting mRNA translation and inducing mRNA decay through the RISC complex. Among others, two activity-regulated miRNAs, miR-134 and miR-129, have previously been shown to mediate HSD. However, the relative contribution of local and cell-wide mechanisms of miRNA-dependent post-transcriptional regulation to HSD is still elusive. In this project, we performed a comprehensive multi-omics characterization of the somatic and process compartments of primary rat hippocampal neurons during HSD. By analyzing the enrichment of miRNA-binding motifs in genes displaying a compartment-specific regulation based on transcriptomics, we identified miR-218 as possible novel local regulator of HSD within neuronal dendrites. miR-218 is upregulated by PTX specifically in dendrites and required for PTX-dependent reduction in dendritic spine size. Currently, we are establishing local translation assay approaches which will allow us to investigate dendrite-specific regulation of miR-218 targets involved in HSD. Furthermore, we will assess the physiological role of miR-218 and its targets during sleep-regulated synaptic homeostasis and memory consolidation in mice. In conclusion, this project will provide novel insight into the role of local mechanisms in HSD and their relevance for cognition.

Pubmed:

34396684: Colameo D, Rajman M, Soutschek M, Bicker S, von Ziegler L, Bohacek J, Winterer J, Germain PL, Dieterich C, Schratt G
Pervasive compartment-specific regulation of gene expression during homeostatic synaptic scaling.
Synaptic scaling is a form of homeostatic plasticity which allows neurons to adjust their action potential firing rate in response to chronic alterations in neural activity. Synaptic scaling requires profound changes in gene expression, but the relative contribution of local and cell-wide mechanisms is controversial. Here we perform a comprehensive multi-omics characterization of the somatic and process compartments of primary rat hippocampal neurons during synaptic scaling. We uncover both highly compartment-specific and correlating changes in the neuronal transcriptome and proteome. Whereas downregulation of crucial regulators of neuronal excitability occurs primarily in the somatic compartment, structural components of excitatory postsynapses are mostly downregulated in processes. Local inhibition of protein synthesis in processes during scaling is confirmed for candidate synaptic proteins. Motif analysis further suggests an important role for trans-acting post-transcriptional regulators, including RNA-binding proteins and microRNAs, in the local regulation of the corresponding mRNAs. Altogether, our study indicates that, during synaptic scaling, compartmentalized gene expression changes might co-exist with neuron-wide mechanisms to allow synaptic computation and homeostasis.
EMBO Rep, 2021; 22
34545145: Rafique D, Heggli U, Bron D, Colameo D, Schweinhardt P, Swanenburg J
Effects of increasing axial load on cervical motor control.
To investigate the effects of increasing axial load on cervical motor control. Surrogates of cervical motor control were active cervical range of motion (C-ROM) and joint position error (JPE) assessed in flexion, extension, lateroflexion and rotation directions in 49 healthy young men (mean age: 20.2 years). All measurements were executed with 0-, 1-, 2-, and 3-kg axial loads. Linear mixed models were used to assess the effects of axial loading and cervical movement-direction on C-ROM and JPE. Post-hoc analysis was performed to compare load levels. Axial loading ($p = 0.045$) and movement direction ($p < 0.001$) showed significant main effects on C-ROM as well as an interaction ($p < 0.001$). C-ROM significantly changed with 3-kg axial load by decreaseing extension (- 13.6%) and increasing lateroflexion (+ 9.9%). No significant main effect was observed of axial loading on JPE ($p = 0.139$). Cervical motor control is influenced by axial loading, which results in decreased

C-ROM in extension and increased C-ROM lateroflexion direction.
Sci Rep, 2021; 11
31601739: Noya SB, Colameo D, Brüning F, Spinnler A, Mircsof D, Opitz L, Mann M, Tyagarajan SK, Robles MS, Brown SA
The forebrain synaptic transcriptome is organized by clocks but its proteome is driven by sleep.
Neurons have adapted mechanisms to traffic RNA and protein into distant dendritic and axonal arbors. Taking a biochemical approach, we reveal that forebrain synaptic transcript accumulation shows overwhelmingly daily rhythms, with two-thirds of synaptic transcripts showing time-of-day-dependent abundance independent of oscillations in the soma. These transcripts formed two sharp temporal and functional clusters, with transcripts preceding dawn related to metabolism and translation and those anticipating dusk related to synaptic transmission. Characterization of the synaptic proteome around the clock demonstrates the functional relevance of temporal gating for synaptic processes and energy homeostasis. Unexpectedly, sleep deprivation completely abolished proteome but not transcript oscillations. Altogether, the emerging picture is one of a circadian anticipation of messenger RNA needs in the synapse followed by translation as demanded by sleep-wake cycles.
Science, 2019; 366

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THE ROLE OF BRAIN EXTRACELLULAR MATRIX SULFATION EPITOPES IN AGING

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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The extracellular matrix (ECM) structure perineuronal net (PNN) is playing crucial roles in neuronal plasticity. Its composition undergoes regular modifications during the life-time, with two major periods noted: the critical period and the aging. These are associated with changes in sulfation composition of chondroitin sulphate proteoglycans. During aging, there is a significant decrease of chondroitin 6-sulfate (C6S) in the PNNs, shiftin the ratio of chondroitin 4-sulfate (C4S):C6S, concomitant with the reduction in neuronal plasticity and cognition. Using a conditional knockout model of *Chst11* (the gene coding the key enzyme for C4S synthesis, *Chst11KO)*, we have investigated the impact of decreased C4S/C6S ratio on learning, memory and general behaviour at 6- and 12-months using Morris water maze (MWM), spontaneous alternation, spontaneous novel object recognition (SOR) task, three chamber task and zero-maze. Immunohistochemically, synaptic connectivity on parvalbumin interneurons (vGLUT1/PSD95 vs vGAT/Gephyrin) and density of PNNs (WFA, aggrecan) were evaluated. Morever, the hippocampal fEPSP in acute slice was measured. In *Chst11KO* mice, a general increase of both short- and long-term memory was observed in all tasks, accompanied by preserved sociability and social novelty preference. In addition, a significant reduction of PNNs, alteration of synaptic connectivity of parvalbumin interneurons and increased fEPSPs were observed. Our results support the hypothesis that C4S restricts neuronal plasticity and provide a potential therapeutic avenue for cognitive decline in late aging and neurodegenerative disorders such as Alzheimer's disease.
Supported by NEURORECON, CZ.02.1.01/0.0/0.0/15_003/0000419.

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SENSORY-INDUCED TRANSCRIPTION MAINTAINS VISUAL PROCESSING BY NORMALIZING E/I-RATIO EVERY DAY

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Consistent and reliable encoding of sensory information is essential for an animal's survival. However, sensory input in an animal's environment is constantly changing, potentially altering the brain's molecules, synapses, and cellular circuitry. Thus, key questions concern the molecular and cellular mechanisms that dynamically maintain the functional stability of neural circuits in the brain. To address this question, we focused on the visual cortex of adult mice and took advantage of the daily sensory transitions from the dark of night to daylight and back to darkness during a single day. By using RNA-seq, patch clamp electrophysiology in acute slices, and *in vivo* longitudinal calcium imaging in awake mice, we monitor the light driven changes in molecules, synapses, and cells across a single day. At each of these levels (molecular, synaptic, and cellular), we find rapid sensory driven increases shortly after transition from darkness to light which are then normalized later in the day. Together with additional experiments in which we apply a novel genetic approach to knock-out genes overnight, our findings indicate that sensory-driven genetic changes maintain functional stability of neural circuits by regulating E/I ratio in excitatory neurons every day.

Pubmed:

34038743: Cohen-Kashi Malina K, Tsivourakis E, Kushinsky D, Apelblat D, Shtiglitz S, Zohar E, Sokoletsky M, Tasaka GI, Mizrahi A, Lampl I, Spiegel I
NDNF interneurons in layer 1 gain-modulate whole cortical columns according to an animal's behavioral state.
Processing of sensory information in neural circuits is modulated by an animal's behavioral state, but the underlying cellular mechanisms are not well understood. Focusing on the mouse visual cortex, here we analyze the role of GABAergic interneurons that are located in layer 1 and express Ndnf (L1 NDNF INs) in the state-dependent control over sensory processing. We find that the ongoing and sensory-evoked activity of L1 NDNF INs is strongly enhanced when an animal is aroused and that L1 NDNF INs gain-modulate local excitatory neurons selectively during high-arousal states by inhibiting their apical dendrites while disinhibiting their somata via Parvalbumin-expressing interneurons. Because active NDNF INs are evenly spread in L1 and can affect excitatory neurons across all cortical layers, this indicates that the state-dependent activation of L1 NDNF INs and the subsequent shift of inhibition in excitatory neurons toward their apical dendrites gain-modulate sensory processing in whole cortical columns.
Neuron, 2021; 109
30630966: Kushinsky D, Morozova EO, Marder E
effects of temperature on the heart and pyloric rhythms in the crab .
The heart and pyloric rhythms of crustaceans have been studied separately and extensively over many years. Local and hormonal neuromodulation and sensory inputs into these central pattern generator circuits play a significant role in an animal's response to perturbations, but are usually lost or removed during studies. To examine simultaneously the motor output of the crustacean heart and pyloric rhythms, we used photoplethysmography. In the population measured (=49), the heart rhythm frequency ranged from 0.3 to 2.3 Hz. The pyloric rhythm varied from 0.2 to 1.6 Hz. We observed a weak correlation between the frequencies of the heart and pyloric rhythms. During multiple hour-long recordings, many animals held at a controlled temperature showed strong inhibitory bouts in which the heart decreased in frequency or become quiescent and the pyloric rhythm decreased in frequency. We measured the simultaneous responses of the rhythms to temperature ramps by heating or cooling the saline bath while recording both the heart and pyloric muscle movements. , critical temperature (temperature at which muscle function is compromised) and changes in frequency were calculated for each of the rhythms tested. The heart rhythm was more robust to high temperature than the pyloric rhythm.
J Exp Biol, 2019; 222
30269988: Abs E, Poorthuis RB, Apelblat D, Muhammad K, Pardi MB, Enke L, Kushinsky D, Pu DL, Eizinger MF,

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Conzelmann KK, Spiegel I, Letzkus JJ

Learning-Related Plasticity in Dendrite-Targeting Layer 1 Interneurons.

A wealth of data has elucidated the mechanisms by which sensory inputs are encoded in the neocortex, but how these processes are regulated by the behavioral relevance of sensory information is less understood. Here, we focus on neocortical layer 1 (L1), a key location for processing of such top-down information. Using Neuron-Derived Neurotrophic Factor (NDNF) as a selective marker of L1 interneurons (INs) and in vivo 2-photon calcium imaging, electrophysiology, viral tracing, optogenetics, and associative memory, we find that L1 NDNF-INs mediate a prolonged form of inhibition in distal pyramidal neuron dendrites that correlates with the strength of the memory trace. Conversely, inhibition from Martinotti cells remains unchanged after conditioning but in turn tightly controls sensory responses in NDNF-INs. These results define a genetically addressable form of dendritic inhibition that is highly experience dependent and indicate that in addition to disinhibition, salient stimuli are encoded at elevated levels of distal dendritic inhibition. VIDEO ABSTRACT.

Neuron, 2018; 100

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MOLECULAR MECHANISMS OF STRUCTURAL MAINTENANCE AND PLASTICITY IN NEURONS

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Pathological changes of the dendrite architecture are hallmarks of many neurological disorders. Despite the fact that dendrites are mostly stable in adult neurons, little is known on the molecular mechanisms of dendrite maintenance and even less on its relation to structural plasticity. Previously, we identified Vascular Endothelial Growth Factor D (VEGFD) – an angio- and lymphangiogenic factor- as a crucial factor for the maintenance of dendritic morphology and the ability to form long-term memories. In neurodegeneration, such as stroke or in the excitotoxic retina, VEGFD expression is reduced and nasal or intravitreal delivery of VEGFD protects against stroke-induced damage or retinal ganglion cell death, respectively. The mechanisms of VEGFD-mediated dendrite stabilization are, however, not known. We now found that VEGFD acts like a molecular brake on neuronal morphology: normal expression of VEGFD maintains the dendritic architecture while VEGFD downregulation allows dendritic remodeling. We revealed that VEGFD stabilizes both actin and microtubules by performing atomic force microscopy as well as time-lapse live imaging in hippocampal neurons. Moreover, a phosphoproteomic screen identified several potentially VEGFD-regulated cytoskeleton-associated proteins. We functionally characterized the identified targets using gain of function and loss of function approaches. In addition, we monitored the VEGFD-regulated dynamics of dendrite structure using a machine-learning based algorithm for automatic dendrite segmentation in time-lapse series. Our study revealed the mechanisms of VEGFD-mediated dendrite maintenance and thereby contributes to our understanding of pathological dendrite aberrations.

Pubmed:
33094279: Pelucchi S, Vandermeulen L, Pizzamiglio L, Aksan B, Yan J, Konietzny A, Bonomi E, Borroni B, Padovani A, Rust MB, Di Marino D, Mikhaylova M, Mauceri D, Antonucci F, Edefonti V, Gardoni F, Di Luca M, Marcello E
Cyclase-associated protein 2 dimerization regulates cofilin in synaptic plasticity and Alzheimer's disease.
Regulation of actin cytoskeleton dynamics in dendritic spines is crucial for learning and memory formation. Hence, defects in the actin cytoskeleton pathways are a biological trait of several brain diseases, including Alzheimer's disease. Here, we describe a novel synaptic mechanism governed by the cyclase-associated protein 2, which is required for structural plasticity phenomena and completely disrupted in Alzheimer's disease. We report that the formation of cyclase-associated protein 2 dimers through its Cys is important for cyclase-associated protein 2 binding to cofilin and for actin turnover. The Cys-dependent cyclase-associated protein 2 homodimerization and association to cofilin are triggered by long-term potentiation and are required for long-term potentiation-induced cofilin translocation into spines, spine remodelling and the potentiation of synaptic transmission. This mechanism is specifically affected in the hippocampus, but not in the superior frontal gyrus, of both Alzheimer's disease patients and APP/PS1 mice, where cyclase-associated protein 2 is down-regulated and cyclase-associated protein 2 dimer synaptic levels are reduced. Notably, cyclase-associated protein 2 levels in the cerebrospinal fluid are significantly increased in Alzheimer's disease patients but not in subjects affected by frontotemporal dementia. In Alzheimer's disease hippocampi, cofilin association to cyclase-associated protein 2 dimer/monomer is altered and cofilin is aberrantly localized in spines. Taken together, these results provide novel insights into structural plasticity mechanisms that are defective in Alzheimer's disease.
Brain Commun, 2020; 2
32055648: Schlüter A, Aksan B, Diem R, Fairless R, Mauceri D
VEGFD Protects Retinal Ganglion Cells and, consequently, Capillaries against Excitotoxic Injury.
In the central nervous system, neurons and the vasculature influence each other. While it is well described that a functional vascular system is trophic to neurons and that vascular damage contributes to neurodegeneration, the opposite scenario in which neural damage might impact the microvasculature is less defined. In this study, using an excitotoxic approach in adult mice as a tool to cause specific damage to retinal ganglion cells, we detected subsequent damage to endothelial cells in

retinal capillaries. Furthermore, we detected decreased expression of vascular endothelial growth factor D (VEGFD) in retinal ganglion cells. VEGFD supplementation via neuronal-specific viral-mediated expression or acute intravitreal delivery of the mature protein preserved the structural and functional integrity of retinal ganglion cells against excitotoxicity and, additionally, spared endothelial cells from degeneration. Viral-mediated suppression of expression of the VEGFD-binding receptor VEGFR3 in retinal ganglion cells revealed that VEGFD exerts its protective capacity directly on retinal ganglion cells, while protection of endothelial cells is the result of upheld neuronal integrity. These findings suggest that VEGFD supplementation might be a novel, clinically applicable approach for neuronal and vascular protection.
Mol Ther Methods Clin Dev, 2020; 17

31161423: Schlüter A, Aksan B, Fioravanti R, Valente S, Mai A, Mauceri D
Histone Deacetylases Contribute to Excitotoxicity-Triggered Degeneration of Retinal Ganglion Cells In Vivo.
Excitotoxicity is known to modulate the nuclear accumulation, and thus activity state, of histone deacetylases (HDACs) in pyramidal neurons. In the retina, deregulation in activity and expression of different HDACs has been linked to pathological conditions such as retinitis pigmentosa, retinal ischemia, glaucoma, and acute optic nerve injury. Up to now, however, the effects of in vivo excitotoxicity on the different HDACs in retinal ganglion cells (RGCs) have not been thoroughly investigated. Here, we injected adult mice intravitreally with N-methyl-D-aspartate (NMDA) as a mean to trigger excitotoxicity-mediated RGC degeneration and we detected time-dependent loss of RGCs at 1 and 7 days after the insult. Further, we characterized the subcellular localization of HDACs belonging to class I (HDAC1, HDAC3), IIa (HDAC4, HDAC5, HDAC7, HDAC9), IIb (HDAC6, HDAC10), and IV (HDAC11) in RGCs. Our analyses revealed a differential pattern of HDACs nuclear distribution in RGCs following excitotoxicity. After 1 day, HDAC3, HDAC5, HDAC6, HDAC7, and HDAC11 showed altered subcellular localization in RGCs while 7 days after the excitotoxic insult, HDAC4 and HDAC9 were the only HDACs displaying changes in their subcellular distribution. Moreover, we found that in vivo selective inhibition of HDAC1/3 or HDAC4/5 via MS-275 (entinostat) or LMK-235, respectively, could prevent ongoing RGC degeneration. In conclusion, our results point towards a role of HDACs in RGC degeneration and identify HDAC1/3 and HDAC4/5 as potential therapeutic targets to treat degenerative retinal diseases.
Mol Neurobiol, 2019; 56

29306704: Chandrasekar A, Aksan B, Heuvel FO, Förstner P, Sinske D, Rehman R, Palmer A, Ludolph A, Huber-Lang M, Böckers T, Mauceri D, Knöll B, Roselli F
Neuroprotective effect of acute ethanol intoxication in TBI is associated to the hierarchical modulation of early transcriptional responses.
Ethanol intoxication is a risk factor for traumatic brain injury (TBI) but clinical evidence suggests that it may actually improve the prognosis of intoxicated TBI patients. We have employed a closed, weight-drop TBI model of different severity (2cm or 3cm falling height), preceded (-30min) or followed (+20min) by ethanol administration (5g/Kg). This protocol allows us to study the interaction of binge ethanol intoxication in TBI, monitoring behavioral changes, histological responses and the transcriptional regulation of a series of activity-regulated genes (immediate early genes, IEGs). We demonstrate that ethanol pretreatment before moderate TBI (2cm) significantly reduces neurological impairment and accelerates recovery. In addition, better preservation of neuronal numbers and cFos+cells was observed 7days after TBI. At transcriptional level, ethanol reduced the upregulation of a subset of IEGs encoding for transcription factors such as Atf3, c-Fos, FosB, Egr1, Egr3 and Npas4 but did not affect the upregulation of others (e.g. Gadd45b and Gadd45c). While a subset of IEGs encoding for effector proteins (such as Bdnf, InhbA and Dusp5) were downregulated by ethanol, others (such as Il-6) were unaffected. Notably, the majority of genes were sensitive to ethanol only when administered before TBI and not afterwards (the exceptions being c-Fos, Egr1 and Dusp5). Furthermore, while severe TBI (3cm) induced a qualitatively similar (but quantitatively larger) transcriptional response to moderate TBI, it was no longer sensitive to ethanol pretreatment. Thus, we have shown that a subset of the TBI-induced transcriptional responses were sensitive to ethanol intoxication at the instance of trauma (ultimately resulting in beneficial outcomes) and that the effect of ethanol was restricted to a certain time window (pre TBI treatment) and to TBI severity (moderate). This information could be critical for the translational value of ethanol in TBI and for the design of clinical studies aimed at disentangling the role of ethanol intoxication in TBI.
Exp Neurol, 2018; 302

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BOARD NUMBER: S01-371

STRUCTURAL PLASTICITY DURING VISION-DEPENDENT LEARNING IN MOUSE VISUAL CORTEX

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Brandeis University, Biology, Waltham, United States of America

Plasticity is a core feature of neurons and neuronal networks. While functionally distinct forms of plasticity have been intensively studied in isolation, much remains elusive about how they cooperate to refine circuit function. For instance, it is still poorly understood how Hebbian and homeostatic plasticity cooperate to ensure memory encoding and consolidation during and after learning – i.e., how connectivity can change without destabilizing network activity and function. Furthermore, while Hebbian learning is implemented by morphological changes embodying the new memory, the structural correlates of homeostatic plasticity remain unclear. Here, we investigate structural plasticity *in vivo* during cricket hunting, a vision-dependent, intrinsically rewarding and ethologically relevant learning paradigm. Cricket hunting induces a dramatic perturbation to network activity followed by a slow return to baseline, consistent with sequential Hebbian learning and homeostatic scaling down. We show that during the critical period, when experience-dependent plasticity is at its peak, mice learn to hunt crickets in a few consecutive sessions and retain that skill after a break. Using chronic two-photon imaging in awake critical-period animals, we assess changes in spine density and morphology of pyramidal neurons in the primary visual cortex of mice as they encode and memorize their new hunting skill. These experiments allow us to tease apart the respective contributions of Hebbian and homeostatic mechanisms to structural plasticity and memory encoding and consolidation in a natural and self-rewarding learning paradigm.

Pubmed:
30937469: Bissen D, Foss F, Acker-Palmer A
AMPA receptors and their minions: auxiliary proteins in AMPA receptor trafficking.
To correctly transfer information, neuronal networks need to continuously adjust their synaptic strength to extrinsic stimuli. This ability, termed synaptic plasticity, is at the heart of their function and is, thus, tightly regulated. In glutamatergic neurons, synaptic strength is controlled by the number and function of AMPA receptors at the postsynapse, which mediate most of the fast excitatory transmission in the central nervous system. Their trafficking to, at, and from the synapse, is, therefore, a key mechanism underlying synaptic plasticity. Intensive research over the last 20 years has revealed the increasing importance of interacting proteins, which accompany AMPA receptors throughout their lifetime and help to refine the temporal and spatial modulation of their trafficking and function. In this review, we discuss the current knowledge about the roles of key partners in regulating AMPA receptor trafficking and focus especially on the movement between the intracellular, extrasynaptic, and synaptic pools. We examine their involvement not only in basal synaptic function, but also in Hebbian and homeostatic plasticity. Included in our review are well-established AMPA receptor interactants such as GRIP1 and PICK1, the classical auxiliary subunits TARP and CNIH, and the newest additions to AMPA receptor native complexes.
Cell Mol Life Sci, 2019; 76
33789115: Bissen D, Kracht MK, Foss F, Hofmann J, Acker-Palmer A
EphrinB2 and GRIP1 stabilize mushroom spines during denervation-induced homeostatic plasticity.
Despite decades of work, much remains elusive about molecular events at the interplay between physiological and structural changes underlying neuronal plasticity. Here, we combined repetitive live imaging and expansion microscopy in organotypic brain slice cultures to quantitatively characterize the dynamic changes of the intracellular versus surface pools of GluA2-containing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) across the different dendritic spine types and the shaft during hippocampal homeostatic plasticity. Mechanistically, we identify ephrinB2 and glutamate receptor interacting protein (GRIP) 1 as mediating AMPAR relocation to the mushroom spine surface following lesion-induced denervation. Moreover, stimulation with the ephrinB2 specific receptor EphB4 not only prevents the lesion-induced disappearance of mushroom spines but is also sufficient to shift AMPARs to the surface and rescue spine recovery in a GRIP1 dominant-negative background. Thus, our results unravel a crucial role for ephrinB2 during homeostatic plasticity and identify a potential pharmacological target to improve dendritic spine plasticity upon injury.
Cell Rep, 2021; 34

28978486: Pfennig S, Foss F, Bissen D, Harde E, Treeck JC, Segarra M, Acker-Palmer A
GRIP1 Binds to ApoER2 and EphrinB2 to Induce Activity-Dependent AMPA Receptor Insertion at the Synapse.
Regulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking in response to neuronal activity is critical for synaptic function and plasticity. Here, we show that neuronal activity induces the binding of ephrinB2 and ApoER2 receptors at the postsynapse to regulate de novo insertion of AMPA receptors. Mechanistically, the multi-PDZ adaptor glutamate-receptor-interacting protein 1 (GRIP1) binds ApoER2 and bridges a complex including ApoER2, ephrinB2, and AMPA receptors. Phosphorylation of ephrinB2 in a serine residue (Ser-9) is essential for the stability of such a complex. In vivo, a mutation on ephrinB2 Ser-9 in mice results in a complete disruption of the complex, absence of ApoER2 downstream signaling, and impaired activity-induced and ApoER2-mediated AMPA receptor insertion. Using compound genetics, we show the requirement of this complex for long-term potentiation (LTP). Together, our findings uncover a cooperative ephrinB2 and ApoER2 signaling at the synapse, which serves to modulate activity-dependent AMPA receptor dynamic changes during synaptic plasticity.
Cell Rep, 2017; 21
31868584: Harde E, Nicholson L, Furones Cuadrado B, Bissen D, Wigge S, Urban S, Segarra M, Ruiz de Almodóvar C, Acker-Palmer A
EphrinB2 regulates VEGFR2 during dendritogenesis and hippocampal circuitry development.
Vascular endothelial growth factor (VEGF) is an angiogenic factor that play important roles in the nervous system, although it is still unclear which receptors transduce those signals in neurons. Here, we show that in the developing hippocampus VEGFR2 (also known as KDR or FLK1) is expressed specifically in the CA3 region and it is required for dendritic arborization and spine morphogenesis in hippocampal neurons. Mice lacking VEGFR2 in neurons () show decreased dendritic arbors and spines as well as a reduction in long-term potentiation (LTP) at the associational-commissural - CA3 synapses. Mechanistically, VEGFR2 internalization is required for VEGF-induced spine maturation. In analogy to endothelial cells, ephrinB2 controls VEGFR2 internalization in neurons. VEGFR2-ephrinB2 compound mice () show reduced dendritic branching, reduced spine head size and impaired LTP. Our results demonstrate the functional crosstalk of VEGFR2 and ephrinB2 in vivo to control dendritic arborization, spine morphogenesis and hippocampal circuitry development.
Elife, 2019; 8
27650319: Lizen B, Hutlet B, Bissen D, Sauvegarde D, Hermant M, Ahn MT, Gofflot F
HOXA5 localization in postnatal and adult mouse brain is suggestive of regulatory roles in postmitotic neurons.
Hoxa5 is a member of the Hox gene family, which plays critical roles in successive steps of the central nervous system formation during embryonic and fetal development. Hoxa5 expression in the adult mouse brain has been reported, suggesting that this gene may be functionally required in the brain after birth. To provide further insight into the Hoxa5 expression pattern and potential functions in the brain, we have characterized its neuroanatomical profile from embryonic stages to adulthood. While most Hox mapping studies have been based solely on transcript analysis, we extended our analysis to HOXA5 protein localization in adulthood using specific antibodies. Our results show that Hoxa5 expression appears in the most caudal part of the hindbrain at fetal stages, where it is maintained until adulthood. In the medulla oblongata and pons, we detected Hoxa5 expression in many precerebellar neurons and in several nuclei implicated in the control of autonomic functions. In these territories, the HOXA5 protein is present solely in neurons, specifically in γ-aminobutyric acid (GABA)ergic, glutamatergic, and catecholaminergic neurons. Finally, we also detected Hoxa5 transcripts, but not the HOXA5 protein, in the thalamus and the cortex, from postnatal stages to adult stages, and in the cerebellum at adulthood. We provide evidence that some larger variants of Hoxa5 transcripts are present in these territories. Our mapping analysis allowed us to build hypotheses regarding HOXA5 functions in the nervous system after birth, such as a potential role in the establishment and refinement/plasticity of precerebellar circuits during postnatal and adult life. J. Comp. Neurol. 525:1155-1175, 2017. © 2016 Wiley Periodicals, Inc.
J Comp Neurol, 2017; 525

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BOARD NUMBER: S01-372

THE TEMPORAL CONNECTIVITY OF MF AND CA3 PYRAMIDAL NEURONS DURING DEVELOPMENT THAT DETERMINED REFERENCE MEMORY REPRESENTATIONS IS CONTROLLED BY THE PLANAR CELL POLARITY PROTEIN VANGL2

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

Noémie Depret[1], Marie Gleizes[1], Maïté Moreau[1], Gaël Barthet[2], Anne Quiedeville[1], Steve Dos-Santos Carvalho[1], Benjamin Robert[1], Shaam Al Abed[1], Christophe Mulle[2], Aline Desmedt[1], Claudia Racca[3], Aline Mariguetto[1], Mireille Montcouquiol[1], Nathalie Sans[1]
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Vangl2, a core protein of the Wnt/PCP pathway, is a known key player in morphogenesis. One of our recent studies has also linked it to the regulation of cognitive function. However, we lack information on the precise role of Vangl2 in different types of synapses associated to specific cognitive processes. Here, we found that Vangl2 is essential to the development and function of a specific hippocampal connexion, the MFB/TE synapse. We show that Vangl2 is enriched in the postnatal hippocampus, specifically in the DG and CA3. Using 3D reconstruction of both confocal and SBFSEM acquisitions, we show that the early genetic ablation of *vangl2* in mice leads to aberrant morphogenesis of the MFB synapse with long lasting consequences on its structural plasticity. This morphological defect is accompanied by molecular modifications in both pre- and post-synaptic compartments, as well as basal transmission deficits. Lastly, we show that the early loss of Vangl2 leads to specific reference memory deficits in adult animals. Altogether, we show that Vangl2-dependent mechanisms are critical for the correct postnatal morphogenesis and function of the MFB/TE synapse, and altering those mechanisms have long lasting consequences on the flexibility of reference memory. Our data uncover the importance of the PCP pathway in the establishment of hippocampal synaptic connexions and in long term memory.

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BOARD NUMBER: S01-373

GRANULE CELLS OF TUMOR NECROSIS FACTOR (TNF)-DEFICIENT MICE SHOW ALTERATIONS IN SPINE DENSITY AND SIZE

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

Dinko Smilović[1], Michael Rietsche[2], Alexander Drakew[2], Thomas Deller[2], Mario Vukšić[1]
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Aims: Spines are sites of synaptic transmission and synaptic plasticity. Recent findings suggest that the spine population should be viewed as a continuum. Furthermore, the size of the spine head is tightly correlated with AMPA-R density and the strength of synaptic transmission. Since TNF is important in synaptic transmission, we wondered how a lack of constitutive levels of TNF affects spine head size and spine density of dentate granule cells. We extended our investigations to the actin-modulating protein Synaptopodin (SP), which labels the spine apparatus organelle and is a component of stable and strong spines. Methods: Perfusion fixed hippocampal sections of adult male wildtype and TNF-/- mice were used. Single granule cells were injected with fluorescent dye, immunolabeled for SP, imaged in a confocal microscope, and single dendritic segments were analyzed by an investigator blind to genotype. Results: Compared to WTs, TNF-/- segments had ~20% fewer spines. Furthermore, the distribution of spines was changed: The fractions of large and small spines were increased, while the fraction of medium-sized spines was decreased. Large spines also contained larger SP clusters, whereas small spines were almost devoid of SP. Conclusions: TNF-/- granule cells exhibit fewer spines and an increase in the population of large spines. This change is similar to the response of granule cells to entorhinal denervation. In both settings, granule cells may increase the size of their large spines (and the size of SP clusters) to homeostatically compensate for spine loss.

Pubmed:
34498735: Smilovic D, Rietsche M, Drakew A, Vuksic M, Deller T
Constitutive tumor necrosis factor (TNF)-deficiency causes a reduction in spine density in mouse dentate granule cells accompanied by homeostatic adaptations of spine head size.
The majority of excitatory synapses terminating on cortical neurons are found on dendritic spines. The geometry of spines, in particular the size of the spine head, tightly correlates with the strength of the excitatory synapse formed with the spine. Under conditions of synaptic plasticity, spine geometry may change, reflecting functional adaptations. Since the cytokine tumor necrosis factor (TNF) has been shown to influence synaptic transmission as well as Hebbian and homeostatic forms of synaptic plasticity, we speculated that TNF-deficiency may cause concomitant structural changes at the level of dendritic spines. To address this question, we analyzed spine density and spine head area of Alexa568-filled granule cells in the dentate gyrus of adult C57BL/6J and TNF-deficient (TNF-KO) mice. Tissue sections were double-stained for the actin-modulating and plasticity-related protein synaptopodin (SP), a molecular marker for strong and stable spines. Dendritic segments of TNF-deficient granule cells exhibited ~20% fewer spines in the outer molecular layer of the dentate gyrus compared to controls, indicating a reduced afferent innervation. Of note, these segments also had larger spines containing larger SP-clusters. This pattern of changes is strikingly similar to the one seen after denervation-associated spine loss following experimental entorhinal denervation of granule cells: Denervated granule cells increase the SP-content and strength of their remaining spines to homeostatically compensate for those that were lost. Our data suggest a similar compensatory mechanism in TNF-deficient granule cells in response to a reduction in their afferent innervation.
J Comp Neurol, 2022; 530
34255828: Žunić Išasegi I, Kopić J, Smilović D, Krsnik Ž, Kostović I
Transient Subplate Sublayer Forms Unique Corridor for Differential Ingrowth of Associative Pulvinar and Primary Visual Projection in the Prospective Visual Cortical Areas of the Human Fetal Occipital Lobe.
Cytoarchitectonical parcellation of the visual cortex into the striate and extrastriate cortex requires complex histogenetic events within a precise spatio-temporal frame to attain the specification of areal domains and associated thalamocortical connections during the fetal brain development. We analyzed a deep subplate cellular monolayer (subplate "corridor" cells) present during a restricted period of 13-15 postconceptional weeks, showing the 3D caudo-ventro-medial position in the human fetal occipital lobe, corresponding to the segregation point of pulvinocortical and geniculocortical fibers at the

prospective area 17/18 border. Immunofluorescence stainings revealed subplate "corridor" cells as the specific class of the deepest subplate neurons (NeuN+, Tbr1+, Cplx3+) expressing axon guidance molecules (Sema-3A+, EphA6+), presumably for the attraction of pulvinocortical axons and the repulsion of geniculocortical axons growing at that time (SNAP25+, Syn+, FN+). Furthermore, quantitative analysis of the subplate "corridor" region of interest, considering cell number, immunofluorescence signal intensity per cell and per region, revealed significant differences to other regions across the tangential circumference of the developing cerebral wall. Thus, our study sheds new light on the deepest subplate sublayer, strategically aligned along the growing axon systems in the prospective visual system, suggesting the establishment of the area 17/18 border by differential thalamocortical input during the fetal brain development.
Cereb Cortex, 2021; 32

33275099: Yap K, Drakew A, Smilovic D, Rietsche M, Paul MH, Vuksic M, Del Turco D, Deller T
The actin-modulating protein synaptopodin mediates long-term survival of dendritic spines.
Large spines are stable and important for memory trace formation. The majority of large spines also contains synaptopodin (SP), an actin-modulating and plasticity-related protein. Since SP stabilizes F-actin, we speculated that the presence of SP within large spines could explain their long lifetime. Indeed, using 2-photon time-lapse imaging of SP-transgenic granule cells in mouse organotypic tissue cultures we found that spines containing SP survived considerably longer than spines of equal size without SP. Of note, SP-positive (SP+) spines that underwent pruning first lost SP before disappearing. Whereas the survival time courses of SP+ spines followed conditional two-stage decay functions, SP-negative (SP-) spines and all spines of SP-deficient animals showed single-phase exponential decays. This was also the case following afferent denervation. These results implicate SP as a major regulator of long-term spine stability: SP clusters stabilize spines, and the presence of SP indicates spines of high stability.
Elife, 2020; 9

30774737: Babić Leko M, Župunski V, Kirincich J, Smilović D, Hortobágyi T, Hof PR, Šimić G
Molecular Mechanisms of Neurodegeneration Related to Hexanucleotide Repeat Expansion.
Two clinically distinct diseases, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), have recently been classified as two extremes of the FTD/ALS spectrum. The neuropathological correlate of FTD is frontotemporal lobar degeneration (FTLD), characterized by tau-, TDP-43-, and FUS-immunoreactive neuronal inclusions. An earlier discovery that a hexanucleotide repeat expansion mutation in chromosome 9 open reading frame 72 () gene causes ALS and FTD established a special subtype of ALS and FTLD with TDP-43 pathology (C9FTD/ALS). Normal individuals carry 2-10 hexanucleotide GGGGCC repeats in the gene, while more than a few hundred repeats represent a risk for ALS and FTD. The proposed molecular mechanisms by which repeat expansions induce neurodegenerative changes are C9orf72 loss-of-function through haploinsufficiency, RNA toxic gain-of-function, and gain-of-function through the accumulation of toxic dipeptide repeat proteins. However, many more cellular processes are affected by pathological processes in C9FTD/ALS, including nucleocytoplasmic transport, RNA processing, normal function of nucleolus, formation of membraneless organelles, translation, ubiquitin proteasome system, Notch signalling pathway, granule transport, and normal function of TAR DNA-binding protein 43 (TDP-43). Although the exact molecular mechanisms through which repeat expansions account for neurodegeneration have not been elucidated, some potential therapeutics, such as antisense oligonucleotides targeting hexanucleotide GGGGCC repeats in mRNA, were successful in preclinical trials and are awaiting phase 1 clinical trials. In this review, we critically discuss each proposed mechanism and provide insight into the most recent studies aiming to elucidate the molecular underpinnings of C9FTD/ALS.
Behav Neurol, 2019; 2019

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BOARD NUMBER: S01-374

REORGANIZATION OF FOREBRAIN POPULATIONS IN A MODEL OF CHRONIC EPILEPSY INDUCED BY STATUS EPILEPTICUS.

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Aim: Temporal lobe epilepsy (TLE) is the most common form of symptomatic epilepsy where more than 80% of patients develop pharmacoresistance. Although hippocampal sclerosis is one of the morphological hallmarks in TLE, forebrain nuclei are also an important target of epileptogenic mechanisms. The aim of this study was to evaluate the epilepsy-related plasticity in basal forebrain nuclei based on a possible functional interaction between the GABAergic and cholinergic systems. **Methods:** Adult Wistar rats were treated with kainic acid (KA) to induce status epilepticus, leading to the development of chronic epilepsy. Sections were analyzed for parvalbumin-immunostained cells (number and density) and cholinergic varicosity density on the medial septum. MANOVA was used for statistical comparison, with subsequent ANOVAs for significant results, at a significance level of .05. **Results:** A total of 6 control and 5 epileptic animals were analyzed. A reduction in parvalbumin cell number (12849±2715 vs. 9372±1336) and density (16.2±2.62 vs. 10.5±1.00) was observed in KA-group, opposing to an increase in cholinergic varicosities (47.9±11.1 vs. 69.4±17.8 per 30000μm^2). MANOVA showed a significant effect of group on the three dependent variables (Wilks' lambda=.19, $F(3,7)=9.71$, $p=.007$, $\eta_p^2=.81$). ANOVA revealed significant differences between groups on parvalbumin cell number ($F(1,9)=6.74$, $p=.029$, $\eta_p^2=.43$) and density ($F(1,9)=21.2$, $p=.001$, $\eta_p^2=.70$), and cholinergic varicosities ($F(1,9)=6.03$, $p=.036$, $\eta_p^2=.40$). **Conclusion:** In epileptic rats, basal forebrain plasticity appears to be a key mechanism during epileptogenesis. The reduction in GABAergic population and the increased density of cholinergic fibers in this region can partly account for the enhanced excitability of hippocampal circuits in epilepsy.

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BOARD NUMBER: S01-375

MORPHOLOGICAL CHARACTERIZATION OF PYRAMIDAL NEURONS IN THE PREFRONTAL CORTEX OF CYLD KNOCKOUT MOUSE MODEL.

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Cylindromatosis lysine 63 deubiquitinase (CYLD) is highly expressed in the brain, particularly at the postsynaptic density (PSD). CYLD plays a key role in the brain by modulating the mammalian target of rapamycin (mTOR) signaling and autophagy at the synapse. CYLD deletion in mice causes a reduction in hippocampal network excitability and long-term potentiation together with a decrease of dendrite total length and in the total number of basal spines CA1 pyramidal neurons. Furthermore, CYLD Knockout (KO) mice display autism-like phenotypes including impaired social communication, increased repetitive behavior, and cognitive dysfunction.
To identify possible morphological correlates of the observed abnormalities of behavior and cognition and since cortical areas in CYLD KO mice have not been investigated yet, the morphology of pyramidal neurons in cortical layer 2/3 of the medial prefrontal cortex (mPFC) was analyzed.
A double transgenic mouse line CYLD KO Thy1-GFP line M was used to characterize the excitatory cortical neurons in the mPFC.
Since synapse elimination has been shown to be altered in the cortex of mouse models of autism spectrum disorder, mice at postnatal day 42, who are characterized by a period of extensive synaptic pruning, were employed to determine if CYLD deletion influences dendritic branches and spine morphology changes of pyramidal neurons.
This study will shed light on the role of the CYLD gene in prefrontal cortex synaptic plasticity in adolescent mice that, if altered, may contribute to the already observed autism-like phenotypes.

Pubmed:
34791077: Balasco L, Pagani M, Pangrazzi L, Chelini G, Ciancone Chama AG, Shlosman E, Mattioni L, Galbusera A, Iurilli G, Provenzano G, Gozzi A, Bozzi Y
Abnormal Whisker-Dependent Behaviors and Altered Cortico-Hippocampal Connectivity in Shank3b-/- Mice.
Abnormal tactile response is an integral feature of Autism Spectrum Disorders (ASDs), and hypo-responsiveness to tactile stimuli is often associated with the severity of ASDs core symptoms. Patients with Phelan-McDermid syndrome (PMS), caused by mutations in the SHANK3 gene, show ASD-like symptoms associated with aberrant tactile responses. The neural underpinnings of these abnormalities are still poorly understood. Here we investigated, in Shank3b-/- adult mice, the neural substrates of whisker-guided behaviors, a key component of rodents' interaction with the surrounding environment. We assessed whisker-dependent behaviors in Shank3b-/- adult mice and age-matched controls, using the textured novel object recognition (tNORT) and whisker nuisance (WN) test. Shank3b-/- mice showed deficits in whisker-dependent texture discrimination in tNORT and behavioral hypo-responsiveness to repetitive whisker stimulation in WN. Sensory hypo-responsiveness was accompanied by a significantly reduced activation of the primary somatosensory cortex (S1) and hippocampus, as measured by c-fos mRNA induction, a proxy of neuronal activity following whisker stimulation. Moreover, resting-state fMRI showed a significantly reduced S1-hippocampal connectivity in Shank3b mutants, in the absence of altered connectivity between S1 and other somatosensory areas. Impaired crosstalk between hippocampus and S1 might underlie Shank3b-/- hypo-reactivity to whisker-dependent cues, highlighting a potentially generalizable somatosensory dysfunction in ASD.
Cereb Cortex, 2021;

FENS Forum 2022
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BOARD NUMBER: S01-376

OPTOGENETIC NEURAL PLASTICITY IN SOMATOSTATIN-EXPRESSING INTERNEURONS TO SUPPRESS COCAINE-SEEKING BEHAVIOUR

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Addiction is a highly debilitating condition and one of its most challenging features is the high rate of relapse, even after a long time from the last substance use.
Research showed that both the plasticity-related trophic factor BDNF and its receptor TrkB are needed for the development of a reward-seeking behaviour in animal models, and that plasticity in the Ventro-Medial Pre-Frontal Cortex (vmPFC) is involved in the extinction of the cocaine conditioning and in the reduction of relapse in cocaine-conditioned animals.
Somatostatin (SST) expressing interneurons have a role in modulating the cocaine-seeking behaviour in mice, and they are involved in neuronal plasticity and connection wiring. The role plasticity in SST neurons in the extinction and relapse of the cocaine conditioning, however, has still not been investigated.
Using a Conditional Place Preference (CPP) paradigm, with mice in which TrkB has been knocked-down in SST neurons, we demonstrated that blocking plasticity in SST neurons impairs the cocaine-conditioning extinction normally seen in extinction-trained mice.
After this observation we asked whether by boosting plasticity in SST neurons in the vmPFC we could not only facilitate the extinction of the cocaine-conditioning but consolidate it and make it long-lasting.
With a new type of optogenetic tool that allows for a light-induced activation of neural plasticity in SST neurons during the cocaine-conditioning extinction training, we aim to consolidate the “extinction” network, making it long-lasting and reduce the rate of relapse, one of the main challenges when treating addictive behaviors.

Pubmed:
34321594: Winkel F, Ryazantseva M, Voigt MB, Didio G, Lilja A, Llach Pou M, Steinzeig A, Harkki J, Englund J, Khirug S, Rivera C, Palva S, Taira T, Lauri SE, Umemori J, Castrén E
Pharmacological and optical activation of TrkB in Parvalbumin interneurons regulate intrinsic states to orchestrate cortical plasticity.
Elevated states of brain plasticity typical for critical periods of early postnatal life can be reinstated in the adult brain through interventions, such as antidepressant treatment and environmental enrichment, and induced plasticity may be critical for the antidepressant action. Parvalbumin-positive (PV) interneurons regulate the closure of developmental critical periods and can alternate between high and low plasticity states in response to experience in adulthood. We now show that PV plasticity states and cortical networks are regulated through the activation of TrkB neurotrophin receptors. Visual cortical plasticity induced by fluoxetine, a widely prescribed selective serotonin reuptake inhibitor (SSRI) antidepressant, was lost in mice with reduced expression of TrkB in PV interneurons. Conversely, optogenetic gain-of-function studies revealed that activation of an optically activatable TrkB (optoTrkB) specifically in PV interneurons switches adult cortical networks into a state of elevated plasticity within minutes by decreasing the intrinsic excitability of PV interneurons, recapitulating the effects of fluoxetine. TrkB activation shifted cortical networks towards a low PV configuration, promoting oscillatory synchrony, increased excitatory-inhibitory balance, and ocular dominance plasticity. OptoTrkB activation promotes the phosphorylation of Kv3.1 channels and reduces the expression of Kv3.2 mRNA providing a mechanism for the lower excitability. In addition, decreased expression and puncta of Synaptotagmin2 (Syt2), a presynaptic marker of PV interneurons involved in Ca-dependent neurotransmitter release, suggests lower inputs onto pyramidal neurons suppressing feed-forward inhibition. Together, the results provide mechanistic insights into how TrkB activation in PV interneurons orchestrates the activity of cortical networks and mediating antidepressant responses in the adult brain.
Mol Psychiatry, 2021; 26
29802758: Umemori J, Winkel F, Didio G, Llach Pou M, Castrén E
iPlasticity: Induced juvenile-like plasticity in the adult brain as a mechanism of antidepressants.

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The network hypothesis of depression proposes that mood disorders reflect problems in information processing within particular neural networks. Antidepressants (AD), including selective serotonin reuptake inhibitors (SSRI), function by gradually improving information processing within these networks. AD have been shown to induce a state of juvenile-like plasticity comparable to that observed during developmental critical periods: Such critical-period-like plasticity allows brain networks to better adapt to extrinsic and intrinsic signals. We have coined this drug-induced state of juvenile-like plasticity 'iPlasticity.' A combination of iPlasticity induced by chronic SSRI treatment together with training, rehabilitation, or psychotherapy improves symptoms of neuropsychiatric disorders and issues underlying the developmentally or genetically malfunctioning networks. We have proposed that iPlasticity might be a critical component of AD action. We have demonstrated that iPlasticity occurs in the visual cortex, fear erasure network, extinction of aggression caused by social isolation, and spatial reversal memory in rodent models. Chronic SSRI treatment is known to promote neurogenesis and to cause dematuration of granule cells in the dentate gyrus and of interneurons, especially parvalbumin interneurons enwrapped by perineuronal nets in the prefrontal cortex, visual cortex, and amygdala. Brain-derived neurotrophic factor (BDNF), via its receptor tropomyosin kinase receptor B, is involved in the processes of synaptic plasticity, including neurogenesis, neuronal differentiation, weight of synapses, and gene regulation of synaptic formation. BDNF can be activated by both chronic SSRI treatment and neuronal activity. Accordingly, the BDNF/tropomyosin kinase receptor B pathway is critical for iPlasticity, but further analyses will be needed to provide mechanical insight into the processes of iPlasticity.
Psychiatry Clin Neurosci, 2018; 72

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BOARD NUMBER: S01-377

SERUM RESPONSE FACTOR AS A NOVEL MOLECULAR TARGET LINKING DEVELOPMENTAL SYNAPTIC MATURATION IN THE HIPPOCAMPUS AND CHANGES IN SOCIAL BEHAVIOR

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Until the process synaptogenesis, brain development is largely controlled at the transcriptional level. Mutations in transcription factors may cause dysregulation of gene expression programs and disrupt the structure and/or function of spiny synapses, which may be an important substrate in the pathogenesis of neurodevelopmental disorders such as autism or schizophrenia. Thus, disentangling the mechanisms underlying developmental dendritic spine maturation is crucial to better understand neuropsychiatric diseases. Here, we demonstrate the novel role of the major transcriptional regulator - **Serum Response Factor (SRF)** in the regulation of molecular, structural, and functional aspects of developmental glutamatergic synapse maturation. We used *in vivo* and *in vitro* models of SRF deletion enabling specific, time-controlled, neuronal SRF depletion in order to precede the stage of intense spinogenesis and study long-term consequences of SRF-deficiency for neuronal plasticity. By implementing molecular, imaging, and behavioral techniques we show that early, postnatal SRF deletion in the hippocampus increased the percentage of immature dendritic spines, downregulated the level of AMPA receptors – GluA1 and GluA2, and decreased the number of functional synapses. Additionally, our preliminary multichannel recording data suggest that SRF knock-down may impair general neuronal network activity. Moreover, the time-controlled SRF loss had a profound impact on animals' social behavior. Molecular and behavioral phenotype described in SRF-deficient mice shares similar characteristics with ASD mice models. Altogether, developmental SRF-dependent modulation of excitatory synapse structure and function exerts long-term consequences on animals social behavior later in life.

Pubmed:
34200797: Zaręba-Kozioł M, Bartkowiak-Kaczmarek A, Roszkowska M, Bijata K, Figiel I, Halder AK, Kamińska P, Müller FE, Basu S, Zhang W, Ponimaskin E, Włodarczyk J
S-Palmitoylation of Synaptic Proteins as a Novel Mechanism Underlying Sex-Dependent Differences in Neuronal Plasticity. Although sex differences in the brain are prevalent, the knowledge about mechanisms underlying sex-related effects on normal and pathological brain functioning is rather poor. It is known that female and male brains differ in size and connectivity. Moreover, those differences are related to neuronal morphology, synaptic plasticity, and molecular signaling pathways. Among different processes assuring proper synapse functions are posttranslational modifications, and among them, S-palmitoylation (S-PALM) emerges as a crucial mechanism regulating synaptic integrity. Protein S-PALM is governed by a family of palmitoyl acyltransferases, also known as DHHC proteins. Here we focused on the sex-related functional importance of DHHC7 acyltransferase because of its S-PALM action over different synaptic proteins as well as sex steroid receptors. Using the mass spectrometry-based PANIMoni method, we identified sex-dependent differences in the S-PALM of synaptic proteins potentially involved in the regulation of membrane excitability and synaptic transmission as well as in the signaling of proteins involved in the structural plasticity of dendritic spines. To determine a mechanistic source for obtained sex-dependent changes in protein S-PALM, we analyzed synaptoneurosomes isolated from DHHC7-/- (DHHC7KO) female and male mice. Our data showed sex-dependent action of DHHC7 acyltransferase. Furthermore, we revealed that different S-PALM proteins control the same biological processes in male and female synapses.
Int J Mol Sci, 2021; 22
30965559: Krzystyniak A, Baczynska E, Magnowska M, Antoniuk S, Roszkowska M, Zareba-Koziol M, Das N, Basu S, Pikula M, Wlodarczyk J

Prophylactic Ketamine Treatment Promotes Resilience to Chronic Stress and Accelerates Recovery: Correlation with Changes in Synaptic Plasticity in the CA3 Subregion of the Hippocampus.
Ketamine is an -methyl-d-aspartate receptor antagonist that has gained wide attention as a potent antidepressant. It has also been recently reported to have prophylactic effects in animal models of depression and anxiety. Alterations of neuroplasticity in different brain regions; such as the hippocampus; prefrontal cortex; and amygdala; are a hallmark of stress-related disorders; and such changes may endure beyond the treatment of symptoms. The present study investigated whether a prophylactic injection of ketamine has effects on structural plasticity in the brain in mice that are subjected to chronic unpredictable stress followed by an 8-day recovery period. Ketamine administration (3 mg/kg body weight) 1 h before stress exposure increased the number of resilient animals immediately after the cessation of stress exposure and positively influenced the recovery of susceptible animals to hedonic deficits. At the end of the recovery period; ketamine-treated animals exhibited significant differences in dendritic spine density and dendritic spine morphology in brain regions associated with depression compared with saline-treated animals. These results confirm previous findings of the prophylactic effects of ketamine and provide further evidence of an association between the antidepressant-like effect of ketamine and alterations of structural plasticity in the brain.
Int J Mol Sci, 2019; 20
30442964: Basu S, Saha PK, Roszkowska M, Magnowska M, Baczynska E, Das N, Plewczynski D, Wlodarczyk J
Author Correction: Quantitative 3-D morphometric analysis of individual dendritic spines.
A correction to this article has been published and is linked from the HTML and PDF versions of this paper. The error has been fixed in the paper.
Sci Rep, 2018; 8
30291144: Yap CC, Digilio L, Kruczek K, Roszkowska M, Fu XQ, Liu JS, Winckler B
A dominant dendrite phenotype caused by the disease-associated G253D mutation in doublecortin (DCX) is not due to its endocytosis defect.
Doublecortin (DCX) is a protein needed for cortical development, and mutations cause cortical malformations in humans. The microtubule-binding activity of DCX is well-described and is important for its function, such as supporting neuronal migration and dendrite growth during development. Previous work showed that microtubule binding is not sufficient for DCX-mediated promotion of dendrite growth and that domains in DCX's C terminus are also required. The more C-terminal regions of DCX bind several other proteins, including the adhesion receptor neurofascin and clathrin adaptors. We recently identified a role for DCX in endocytosis of neurofascin. The disease-associated DCX-G253D mutant protein is known to be deficient in binding neurofascin, and we now asked if disruption of neurofascin endocytosis underlies the DCX-G253D-associated pathology. We first demonstrated that DCX functions in endocytosis as a complex with both the clathrin adaptor AP-2 and neurofascin: disrupting either clathrin adaptor binding (DCX-ALPA) or neurofascin binding (DCX-G253D) decreased neurofascin endocytosis in primary neurons. We then investigated a known function for DCX, namely, increasing dendrite growth in cultured neurons. Surprisingly, we found that the DCX-ALPA and DCX-G253D mutants yield distinct dendrite phenotypes. Unlike DCX-ALPA, DCX-G253D caused a dominant-negative dendrite growth phenotype. The endocytosis defect of DCX-G253D thus was separable from its detrimental effects on dendrite growth. We recently identified as a dominant allele and can now classify as a second allele that acts dominantly to cause pathology, but does so via a different mechanism.
J Biol Chem, 2018; 293
29476060: Basu S, Saha PK, Roszkowska M, Magnowska M, Baczynska E, Das N, Plewczynski D, Wlodarczyk J
Quantitative 3-D morphometric analysis of individual dendritic spines.
The observation and analysis of dendritic spines morphological changes poses a major challenge in neuroscience studies. The alterations of their density and/or morphology are indicators of the cellular processes involved in neural plasticity underlying learning and memory, and are symptomatic in neuropsychiatric disorders. Despite ongoing intense investigations in imaging approaches, the relationship between changes in spine morphology and synaptic function is still unknown. The existing quantitative analyses are difficult to perform and require extensive user intervention. Here, we propose a new method for (1) the three-dimensional (3-D) segmentation of dendritic spines using a multi-scale opening approach and (2) define 3-D morphological attributes of individual spines for the effective assessment of their structural plasticity. The method was validated using confocal light microscopy images of dendritic spines from dissociated hippocampal cultures and brain slices (1) to evaluate accuracy relative to manually labeled ground-truth annotations and relative to the state-of-the-art Imaris tool, (2) to analyze reproducibility of user-independence of the segmentation method, and (3) to quantitatively analyze morphological changes in individual spines before and after chemically induced long-term potentiation. The method was monitored and used to precisely describe the morphology of individual spines in real-time using consecutive images of the same dendritic fragment.
Sci Rep, 2018; 8

28066226: Bokota G, Magnowska M, Kuśmierczyk T, Łukasik M, Roszkowska M, Plewczynski D
Computational Approach to Dendritic Spine Taxonomy and Shape Transition Analysis.
The common approach in morphological analysis of dendritic spines of mammalian neuronal cells is to categorize spines into subpopulations based on whether they are stubby, mushroom, thin, or filopodia shaped. The corresponding cellular models of synaptic plasticity, long-term potentiation, and long-term depression associate the synaptic strength with either spine enlargement or spine shrinkage. Although a variety of automatic spine segmentation and feature extraction methods were developed recently, no approaches allowing for an automatic and unbiased distinction between dendritic spine subpopulations and detailed computational models of spine behavior exist. We propose an automatic and statistically based method for the unsupervised construction of spine shape taxonomy based on arbitrary features. The taxonomy is then utilized in the newly introduced computational model of behavior, which relies on transitions between shapes. Models of different populations are compared using supplied bootstrap-based statistical tests. We compared two populations of spines at two time points. The first population was stimulated with long-term potentiation, and the other in the resting state was used as a control. The comparison of shape transition characteristics allowed us to identify the differences between population behaviors. Although some extreme changes were observed in the stimulated population, statistically significant differences were found only when whole models were compared. The source code of our software is freely available for non-commercial use.
Front Comput Neurosci, 2016; 10
27799303: Yap CC, Digilio L, McMahon L, Roszkowska M, Bott CJ, Kruczek K, Winckler B
Different Doublecortin (DCX) Patient Alleles Show Distinct Phenotypes in Cultured Neurons: EVIDENCE FOR DIVERGENT LOSS-OF-FUNCTION AND "OFF-PATHWAY" CELLULAR MECHANISMS.
Doublecortin on the X-chromosome (DCX) is a neuronal microtubule-binding protein with a multitude of roles in neurodevelopment. In humans, DCX is a major genetic locus for X-linked lissencephaly. The best studied defects are in neuronal migration during corticogenesis and in the hippocampus, as well as axon and dendrite growth defects. Much effort has been directed at understanding the molecular and cellular bases of DCX-linked lissencephaly. The focus has been in particular on defects in microtubule assembly and bundling, using knock-out mice and expression of WT and mutant Dcx in non-neuronal cells. Dcx also binds other proteins besides microtubules, such as spinophilin (abbreviated spn; gene name Ppp1r9b protein phosphatase 1 regulatory subunit 9b) and the clathrin adaptors AP-1 and AP-2. Even though many non-sense and missense mutations of Dcx are known, their molecular and cellular defects are still only incompletely understood. It is also largely unknown how neurons are affected by expression of DCX patient alleles. We have now characterized several patient DCX alleles (DCX-R89G, DCX-R59H, DCX-246X, DCX-272X, and DCX-303X) using a gain-of-function dendrite growth assay in cultured rat neurons in combination with the determination of molecular binding activities and subcellular localization in non-neuronal and neuronal cells. First, we find that several mutants (Dcx-R89G and Dcx-272X) were loss-of-function alleles (as had been postulated) but surprisingly acted via different cellular mechanisms. Second, one allele (Dcx-R59H) formed cytoplasmic aggregates, which contained Hspa1B (heat shock protein 1B hsp70) and ubiquitinated proteins, trapped other cytoskeletal proteins, including spinophilin, and led to increased autophagy. This allele could thus be categorized as "off-pathway"/possibly neomorph. Our findings thus suggested that distinct DCX alleles caused dysfunction by different mechanisms.
J Biol Chem, 2016; 291
27798233: Roszkowska M, Skupien A, Wójtowicz T, Konopka A, Gorlewicz A, Kisiel M, Bekisz M, Ruszczycki B, Dolezyczek H, Rejmak E, Knapska E, Mozrzymas JW, Wlodarczyk J, Wilczynski GM, Dzwonek J
CD44: a novel synaptic cell adhesion molecule regulating structural and functional plasticity of dendritic spines.
Synaptic cell adhesion molecules regulate signal transduction, synaptic function, and plasticity. However, their role in neuronal interactions with the extracellular matrix (ECM) is not well understood. Here we report that the CD44, a transmembrane receptor for hyaluronan, modulates synaptic plasticity. High-resolution ultrastructural analysis showed that CD44 was localized at mature synapses in the adult brain. The reduced expression of CD44 affected the synaptic excitatory transmission of primary hippocampal neurons, simultaneously modifying dendritic spine shape. The frequency of miniature excitatory postsynaptic currents decreased, accompanied by dendritic spine elongation and thinning. These structural and functional alterations went along with a decrease in the number of presynaptic Bassoon puncta, together with a reduction of PSD-95 levels at dendritic spines, suggesting a reduced number of functional synapses. Lack of CD44 also abrogated spine head enlargement upon neuronal stimulation. Moreover, our results indicate that CD44 contributes to proper dendritic spine shape and function by modulating the activity of actin cytoskeleton regulators, that is, Rho GTPases (RhoA, Rac1, and Cdc42). Thus CD44 appears to be a novel molecular player regulating functional and structural plasticity of dendritic spines.
Mol Biol Cell, 2016; 27
27153678: Basu S, Plewczynski D, Saha S, Roszkowska M, Magnowska M, Baczynska E, Wlodarczyk J
2dSpAn: semiautomated 2-d segmentation, classification and analysis of hippocampal dendritic spine plasticity.

Accurate and effective dendritic spine segmentation from the dendrites remains as a challenge for current neuroimaging research community. In this article, we present a new method (2dSpAn) for 2-d segmentation, classification and analysis of structural/plastic changes of hippocampal dendritic spines. A user interactive segmentation method with convolution kernels is designed to segment the spines from the dendrites. Formal morphological definitions are presented to describe key attributes related to the shape of segmented spines. Spines are automatically classified into one of four classes: Stubby, Filopodia, Mushroom and Spine-head Protrusions.
Bioinformatics, 2016; 32

25300795: Skupien A, Konopka A, Trzaskoma P, Labus J, Gorlewicz A, Swiech L, Babraj M, Dolezyczek H, Figiel I, Ponimaskin E, Wlodarczyk J, Jaworski J, Wilczynski GM, Dzwonek J
CD44 regulates dendrite morphogenesis through Src tyrosine kinase-dependent positioning of the Golgi.
The acquisition of proper dendrite morphology is a crucial aspect of neuronal development towards the formation of a functional network. The role of the extracellular matrix and its cellular receptors in this process has remained enigmatic. We report that the CD44 adhesion molecule, the main hyaluronan receptor, is localized in dendrites and plays a crucial inhibitory role in dendritic tree arborization in vitro and in vivo. This novel function is exerted by the activation of Src tyrosine kinase, leading to the alteration of Golgi morphology. The mechanism operates during normal brain development, but its inhibition might have a protective influence on dendritic trees under toxic conditions, during which the silencing of CD44 expression prevents dendritic shortening induced by glutamate exposure. Overall, our results indicate a novel role for CD44 as an essential regulator of dendritic arbor complexity in both health and disease.
J Cell Sci, 2014; 127

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BOARD NUMBER: S01-378

ABERRANT CORTICAL SPINE DYNAMICS AFTER CONCUSSIVE INJURY ARE REVERSED BY INTEGRATED STRESS RESPONSE INHIBITION

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Aim: Traumatic brain injury (TBI) remains a leading cause of long-term neurological disability in the world and the strongest environmental risk factor for the development of dementia. Mild TBI makes up at least 75% of these injuries and is associated with a >2-fold increase in the risk of dementia diagnosis. The pathophysiology of TBI is complex, resulting in chronic behavioral and cognitive impairments that diminish quality of life. To date, no treatments have been identified to prevent, reverse, or mitigate the long-term consequences resulting from TBI. We previously demonstrated that the integrated stress response (ISR), a conserved pathway involved in cellular response to stress, is activated after TBI and inhibition of the ISR can reverse cognitive deficits. However, the cellular mechanisms by which cognitive functions are affected after TBI and ISR inhibition are unknown.
Methods: Here we used Thy1-YFP-H transgenic mice for longitudinal two-photon imaging *in vivo* after concussive injury to study spine dynamics in the parietal cortex, a brain region involved in working memory, specifically short-term working memory.
Results: Concussive injury altered spine dynamics and density measured up to a month after injury. Strikingly, brief pharmacological treatment with the drug-like small-molecule ISR inhibitor ISRIB reversed the neuronal structural changes measured in vivo in the parietal cortex and restored short-term memory functions.
Conclusion: These findings demonstrate that the short-term working memory deficits resulting from concussive injury are paralleled by altered spine dynamics in the parietal cortex. Importantly, ISR inhibition can rapidly and persistently reverse these effects.

Pubmed:
332584551:
31951566: Rosi S, Frias ES
The Integrated Stress Response: A Central Memory Switch in Down Syndrome.
Genetic and pharmacological evidence causally demonstrate that the integrated stress response (ISR) is a central molecular switch for long-term memory formation across different species. Zhu et al. (2019) recently demonstrated that persistent activation of the ISR could explain the long-term memory and synaptic plasticity deficits in a mouse model of Down syndrome, the most common genetic cause of intellectual disability.
Cell Metab, 2020; 31
31884883: Krukowski K, Nolan A, Frias ES, Grue K, Becker M, Ureta G, Delgado L, Bernales S, Sohal VS, Walter P, Rosi S
Integrated Stress Response Inhibitor Reverses Sex-Dependent Behavioral and Cell-Specific Deficits after Mild Repetitive Head Trauma.
Mild repetitive traumatic brain injury (rTBI) induces chronic behavioral and cognitive alterations and increases the risk for dementia. Currently, there are no therapeutic strategies to prevent or mitigate chronic deficits associated with rTBI. Previously we developed an animal model of rTBI that recapitulates the cognitive and behavioral deficits observed in humans. We now report that rTBI results in an increase in risk-taking behavior in male but not female mice. This behavioral phenotype is associated with chronic activation of the integrated stress response and cell-specific synaptic alterations in the type A subtype of layer V pyramidal neurons in the medial prefrontal cortex. Strikingly, by briefly treating animals weeks after injury with ISRIB, a selective inhibitor of the integrated stress response (ISR), we (1) relieve ISR activation, (2) reverse the increased risk-taking behavioral phenotype and maintain this reversal, and (3) restore cell-specific synaptic function in the affected mice. Our results indicate that targeting the ISR even at late time points after injury can permanently reverse behavioral

changes. As such, pharmacological inhibition of the ISR emerges as a promising avenue to combat rTBI-induced behavioral dysfunction.
J Neurotrauma, 2020; 37
30107198: Krukowski K, Grue K, Frias ES, Pietrykowski J, Jones T, Nelson G, Rosi S
Female mice are protected from space radiation-induced maladaptive responses.
Interplanetary exploration will be humankind's most ambitious expedition and the journey required to do so, is as intimidating as it is intrepid. One major obstacle for successful deep space travel is the possible negative effects of galactic cosmic radiation (GCR) exposure. Here, we investigate for the first time how combined GCR impacts long-term behavioral and cellular responses in male and female mice. We find that a single exposure to simulated GCR induces long-term cognitive and behavioral deficits only in the male cohorts. GCR exposed male animals have diminished social interaction, increased anxiety-like phenotype and impaired recognition memory. Remarkably, we find that the female cohorts did not display any cognitive or behavioral deficits after GCR exposure. Mechanistically, the maladaptive behavioral responses observed only in the male cohorts correspond with microglia activation and synaptic loss in the hippocampus, a brain region involved in the cognitive domains reported here. Furthermore, we measured reductions in AMPA expressing synaptic terminals in the hippocampus. No changes in any of the molecular markers measured here are observed in the females. Taken together these findings suggest that GCR exposure can regulate microglia activity and alter synaptic architecture, which in turn leads to a range of cognitive alterations in a sex dependent manner. These results identify sex-dependent differences in behavioral and cognitive domains revealing promising cellular and molecular intervention targets to reduce GCR-induced chronic cognitive deficits thereby boosting chances of success for humans in deep space missions such as the upcoming Mars voyage.
Brain Behav Immun, 2018; 74
29219210: Ghosh S, Castillo E, Frias ES, Swanson RA
Bioenergetic regulation of microglia.
Microglia have diverse actions, ranging from synapse pruning in development to cytotoxic effects in disease. Brain energy metabolism and substrate availability vary under normal and disease states, but how these variations influence microglial function is relatively unknown. Microglia, like most other cell types, express the full complement of gene products required for both glycolytic and oxidative metabolism. Evidence suggests that microglia increase aerobic glycolysis and decrease respiration when activated by various stimuli. Mitochondrial function, glucose availability, and glycolytic rate influence pro-inflammatory gene expression at both transcriptional and post-translational levels. These effects are mediated through CtBP, an NADH-sensitive transcriptional co-repressor; through effects on NLRP3 inflammasome assembly and caspase-1 activation; through formation of advanced glycation end-products; and by less well-defined mechanisms. In addition to these transcriptional effects, microglial glucose metabolism is also required for superoxide production by NADPH oxidase, as glucose is the obligate substrate for regenerating NADPH in the hexose monophosphate shunt. Microglia also metabolize acetoacetate and β-hydroxybutyrate, which are generated during fasting or ketogenic diet, and respond to these ketones as metabolic signals. β-Hydroxybutyrate inhibits histone de-acetylases and activates microglial GRP109A receptors. These actions suppress microglia activation after brain injury and promote neuroprotective microglia phenotypes. As our understanding of microglial activation matures, additional links between energy metabolism and microglial function are likely to be identified.
Glia, 2018; 66
27281462: David CN, Frias ES, Szu JI, Vieira PA, Hubbard JA, Lovelace J, Michael M, Worth D, McGovern KE, Ethell IM, Stanley BG, Korzus E, Fiacco TA, Binder DK, Wilson EH
GLT-1-Dependent Disruption of CNS Glutamate Homeostasis and Neuronal Function by the Protozoan Parasite Toxoplasma gondii.
The immune privileged nature of the CNS can make it vulnerable to chronic and latent infections. Little is known about the effects of lifelong brain infections, and thus inflammation, on the neurological health of the host. Toxoplasma gondii is a parasite that can infect any mammalian nucleated cell with average worldwide seroprevalence rates of 30%. Infection by Toxoplasma is characterized by the lifelong presence of parasitic cysts within neurons in the brain, requiring a competent immune system to prevent parasite reactivation and encephalitis. In the immunocompetent individual, Toxoplasma infection is largely asymptomatic, however many recent studies suggest a strong correlation with certain neurodegenerative and psychiatric disorders. Here, we demonstrate a significant reduction in the primary astrocytic glutamate transporter, GLT-1, following infection with Toxoplasma. Using microdialysis of the murine frontal cortex over the course of infection, a significant increase in extracellular concentrations of glutamate is observed. Consistent with glutamate dysregulation, analysis of neurons reveal changes in morphology including a reduction in dendritic spines, VGlut1 and NeuN immunoreactivity. Furthermore, behavioral testing and EEG recordings point to significant changes in neuronal output. Finally, these changes in neuronal connectivity are dependent on infection-induced downregulation of GLT-1 as treatment with the ß-lactam antibiotic

ceftriaxone, rescues extracellular glutamate concentrations, neuronal pathology and function. Altogether, these data demonstrate that following an infection with T. gondii, the delicate regulation of glutamate by astrocytes is disrupted and accounts for a range of deficits observed in chronic infection.
PLoS Pathog, 2016; 12
25379358: Tosonian S, Ruiz CJ, Rios A, Frias E, Eichler JF
Synthesis, characterization, and stability of iron (III) complex ions possessing phenanthroline-based ligands.
It has previously been demonstrated that phenanthroline-based ligands used to make gold metallotherapuetics have the ability to exhibit cytotoxicity when not coordinated to the metal center. In an effort to help assess the mechanism by which these ligands may cause tumor cell death, iron binding and removal experiments have been considered. The close linkage between cell proliferation and intracellular iron concentrations suggest that iron deprivation strategies may be a mechanism involved in inhibiting tumor cell growth. With the creation of iron (III) phen complexes, the iron binding abilities of three polypyridal ligands [1,10-phenanthroline (phen), 2,9-dimethyl-1, 10-phenanthroline (phen), and 2,9-di-butyl-1, 10-phenanthroline (phen)] can be tested via a competition reaction with a known iron chelator. Therefore, iron (III) complexes possessing all three ligands were synthesized. Initial mass spectrometric and infrared absorption data indicate that iron (III) tetrachloride complex ions with protonated phen ligands (phenH) were formed: [phenH][FeCl], [phenH][FeCl], [phenH][FeCl]. UV-Vis spectroscopy was used to monitor the stability of the complex ions, and it was found that the pheniron complex was more stable than the phen and phen analogues. This was based on the observation that free ligand was observed immediately upon the addition of EDTA to the [phenH][FeCl] and [phenH] [FeCl] complex ions.
Open J Inorg Chem, 2013; 3
25732707: David CN, Frias ES, Elix CC, McGovern KE, Walker AM, Eichler JF, Wilson EH
Antitumor activity of a polypyridyl chelating ligand: in vitro and in vivo inhibition of glioma.
Glioblastoma multiforme is an extremely aggressive and invasive form of central nervous system tumor commonly treated with the chemotherapeutic drug Temozolomide. Unfortunately, even with treatment, the median survival time is less than 12 months. 2,9-Di-sec-butyl-1,10-phenanthroline (SBP), a phenanthroline-based ligand originally developed to deliver gold-based anticancer drugs, has recently been shown to have significant antitumor activity in its own right. SBP is hypothesized to initiate tumor cell death via interaction with non-DNA targets, and considering most glioblastoma drugs kill tumors through DNA damage processes, SBP was tested as a potential novel drug candidate against glial-based tumors. In vitro studies demonstrated that SBP significantly inhibited the growth of rodent GL-26 and C6 glioma cells, as well as human U-87, and SW1088 glioblastomas/astrocytomas. Furthermore, using a syngeneic glioma model in mice, in vivo administration of SBP significantly reduced tumor volume and increased survival time. There was no significant toxicity toward nontumorigenic primary murine and human astrocytes in vitro, and limited toxicity was observed in ex vivo tissues obtained from noncancerous mice. Terminal deoxynucleotidyl transferase dUTP nick end labeling staining and recovery assays suggest that SBP induces apoptosis in gliomas. This exploratory study suggests SBP is effective in slowing the growth of tumorigenic cells in the brain while exhibiting limited toxicity to normal cells and tissues and should therefore be further investigated for its potential in glioblastoma treatment.
ASN Neuro, 2015 Jan-Feb; 7
34654458: Feng X, Frias ES, Paladini MS, Chen D, Boosalis Z, Becker M, Gupta S, Liu S, Gupta N, Rosi S
Functional role of brain-engrafted macrophages against brain injuries.
Brain-resident microglia have a distinct origin compared to macrophages in other organs. Under physiological conditions, microglia are maintained by self-renewal from the local pool, independent of hematopoietic progenitors. Pharmacological depletion of microglia during whole-brain radiotherapy prevents synaptic loss and long-term recognition memory deficits. However, the origin or repopulated cells and the mechanisms behind these protective effects are unknown.
J Neuroinflammation, 2021; 18
34652936: Krukowski K, Grue K, Becker M, Elizarraras E, Frias ES, Halvorsen A, Koenig-Zanoff M, Frattini V, Nimmagadda H, Feng X, Jones T, Nelson G, Ferguson AR, Rosi S
The impact of deep space radiation on cognitive performance: From biological sex to biomarkers to countermeasures.
In the coming decade, astronauts will travel back to the moon in preparation for future Mars missions. Exposure to galactic cosmic radiation (GCR) is a major obstacle for deep space travel. Using multivariate principal components analysis, we found sex-dimorphic responses in mice exposed to accelerated charged particles to simulate GCR (GCRsim); males displayed impaired spatial learning, whereas females did not. Mechanistically, these GCRsim-induced learning impairments corresponded with chronic microglia activation and synaptic alterations in the hippocampus. Temporary microglia depletion shortly after GCRsim exposure mitigated GCRsim-induced deficits measured months after the radiation exposure. Furthermore, blood monocyte levels measured early after GCRsim exposure were predictive of the late learning deficits and microglia activation measured in the male mice. Our findings (i) advance our understanding of charged particle–induced cognitive challenges, (ii) provide evidence for early peripheral biomarkers for identifying late cognitive deficits, and (iii) offer

potential therapeutic strategies for mitigating GCR-induced cognitive loss.
Sci Adv, 2021; 7

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STRUCTURAL PLASTICITY OF DENDRITIC SPINES WITHIN COCAINE-SEEKING NEURONAL ENSEMBLES

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Background: Effective substance use disorder treatment hinges on successful relapse prevention, which is impeded by our limited knowledge of drug-induced pathology in brain circuits and synaptic physiology (Kuhn et al. 2019). Previous research indicates that relapse and seeking may be driven by morphological changes in nucleus accumbens (NAc) neuronal ensembles, i.e., clusters of neurons coactivated in the reward-seeking and motivation center of the brain (Shen et al. 2011, Gipson et al. 2014). Aim: The proposed study compares the efficacy of spine analysis software SPINEJ to IMARIS and evaluates the structural plasticity of cocaine-seeking ensemble cells in the NAc core. Methods: A short access cocaine self-administration and relapse model combined with genetically modified Ai14xFosTRAP mice was used to induce cocaine-seeking and tag NAc neuronal ensembles with TdTomato. Images of NAc dendritic spines were acquired with confocal microscopy, followed by structural analysis in IMARIS and SPINEJ. Spine plasticity of cocaine-ensemble cells in actively seeking animals was compared to cells of non-seeking animals, as well as non-ensemble cells tagged with an unspecific GFP virus. Results: IMARIS is more accurate and reliable for morphological spine analysis than SPINEJ. Preliminary data on spine plasticity suggests that spine density, but not spine head diameter, is specifically altered in the cocaine-seeking ensemble during seeking. These changes correlate with the strength of cocaine seeking. Conclusion: Characterizing spine morphology in cocaine-seeking ensembles will further our understanding of how the brain processes drug seeking and potentially lead to relapse prevention developments.

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ENHANCING DENDRITIC SPINE PLASTICITY BY COUPLING PHYSICAL ACTIVITY WITH NON-INVASIVE BRAIN STIMULATION

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Aim. There is a huge interest in coupling transcranial direct current stimulation (tDCS) and physical activity as an effective strategy to further enhance cortical excitability in physiological and pathological conditions. Nevertheless, the mechanisms underlying this phenomenon are not well known. Animal studies revealed that tDCS affects the motor cortical plasticity by modulating dendritic spines, similarly to voluntary physical exercise. Thus, in this study we investigated the effects of combining tDCS and physical activity in healthy mice. *Methods.*For this purpose, we studied the effects of coupling anodal tDCS and physical activity on the morphological plasticity in primary motor cortex (M1) layer II/III and layer V in young (2-3 months) and middle-aged (14-16 months) mice. *Results.* At both ages, the joining of stimulation and physical activity results in an increased number of activated cells and a higher density in basal and apical dendrites of both hemispheres, compared to single interventions only. Young mice displayed a higher number of mushrooms spines, while middle-aged mice showed an increase of thin spines. *Conclusions.*Altogether, the coupling between tDCS and physical activity results in a significant inter-hemispheric plasticity enhancement in physiological conditions, maintained with aging. However, the spine morphology is differently displayed in young and middle-aged mice, probably indicating a different effect of the combination in the aging.

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THE CELLULAR ARCHITECTURE OF MEMORY MODULES IN DROSOPHILA SUPPORTS STOCHASTIC INPUT INTEGRATION

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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The ability to associate neutral stimuli with valence information and to store these associations as memories forms the basis for decision making. To determine the underlying computational principles, we build a realistic computational model of a central decision module within the *Drosophila* mushroom body (MB), the fly's center for learning and memory. Our model combines the electron microscopy-based architecture of one MB output neuron (MBON-α3), the synaptic connectivity of its 948 presynaptic Kenyon cells (KCs), and its *in vivo* membrane properties obtained from patch-clamp recordings. We show that this neuron is electrotonically compact and that synaptic input corresponding to simulated odor input robustly drives its spiking behavior. Therefore, sparse innervation by KCs can efficiently control and modulate MBON activity in response to learning with minimal requirements on the specificity of synaptic localization. This architecture allows efficient storage of large numbers of memories using the flexible stochastic connectivity of the circuit.

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AUTOMATED HOME-CAGE SYSTEM FOR AUDITORY TRAINING AND PSYCHOPHYSICS IN COMMON MARMOSETS

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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The common marmoset (*Callithrix jacchus*) has become an important model for auditory neuroscience. Their large vocal repertoire together with strong similarities of auditory perception with humans opens up the possibility to explore several aspects of the auditory system in non-human primates. In order to assess cognitive aspects of hearing it is crucial to develop and refine experimental and training procedures to improve their reliability, and replicability, while also contributing to animal welfare. In this study, we describe a novel, automated home-cage, operant conditioning device which has been successfully employed for training and testing of audio-visual-cued behaviors in socially housed common marmosets. Through a sequence of four different tasks naïve animals autonomously learned: first, to operate a touchscreen proficiently (14 out of 14 animals); second, to discriminate between a conspecific vocalization from a pure tone (9 out of 11 animals); third, to flexibly transfer learned discrimination behaviour to a set of novel stimuli (4 out of 4 animals); and fourth, to detect the presence or absence of a conspecific vocalization (3 out of 3 animals). Marmosets showed an stable engagement over sessions regardless of: the session duration (1 to 5 hours); the level of difficulty; and the task type - performing on average 120 self-initiated trials per session (SD: 40 trials) - without dietary restriction or social separation. Combined with 3R compatible training principles, our device represents a significant step towards to the development of high-throughput protocols for integrating advanced cognitive and behavioral assessments of common marmosets in the auditory domain.

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BEHAVIORAL AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF DIFFERENT FUNCTIONAL REGIONS OF THE SUBTHALAMIC NUCLEUS IN HEALTHY NON-HUMAN PRIMATES

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Aims Behavior consists of motor activity influenced by cognitive and emotional context. The subthalamic nucleus (STN) is thought to play a central role in behavior and current hypotheses, based on anatomy and tracing technics, suggest a tripartite overlapping division of this nucleus. However, functional characterization of these regions needs to be further studied. Here, we investigated the electrophysiological activity and the impact of directional stimulation of the STN during a behavioral task. Methods Two non-human primates (NHPs) were trained to perform a switching task to evaluate: the motor domain through the response time; the cognitive domain by a decision to either do the default task or to check the proximity of a large reward delivery; allowing the evaluation of the emotional domain. The NHPs were then implanted with a segmented lead into the STN, enabling directional recordings and stimulations, at 4Hz or 130Hz, of different regions during the task. Results We found higher beta oscillations in the dorso-lateral region and specific 130Hz stimulation decreased the motor response time. Alpha oscillations increased in the more ventral and medial regions before making the decision to work. Specific stimulation at 4Hz applied to the ventro-medial part decreased both motor and cognitive response times. Finally, gamma oscillations increased at the large reward delivery. 4Hz stimulation of all the STN, except the dorso-medial region, decreased the motivation to check for the large reward. Conclusion Our results suggest overlapping in the functional regions of the STN and specific behavioral performances can be modulated with directional stimulation.

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NOT YOUR FATHER'S SYNAPSE: REVISING THE HEBB SYNAPSE FOR THE 21ST CENTURY

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Aims: Neurobiologists have studied synaptic communication and plasticity for over a century. In 1949, Donald O. Hebb developed his neuropsychological postulate, which proposed that synaptic plasticity was the basis of cognitive function. This postulate involved three neural processes: synaptic modifications (i.e., the Hebb Synapse), the cell assembly, and the phase sequence. The Hebb synapse describes how activity at pre- and post-synaptic neurons modulates the strength of a synapse (and synaptic networks). The discovery of long-term potentiation and long-term depression provided evidence for the existence of the Hebb synapse and guided our understanding of the neurobiology of learning and memory. However, new findings suggest that the concept of the Hebb synapse needs revision to incorporate the role of glial cells, the extracellular matrix (ECM), and the neurovascular unit (NVU). **Methods:** Previous researchers proposed the concepts of the 'tripartite synapse', 'quad-partite synapse', 'tetra-partite synapse' and 'penta-partite synapse' as well as the 'active milieu' to describe synaptic activity. We reviewed existing literature that described the roles of neuronal and non-neuronal cells and cellular components at the synapse. **Results:** We propose a 'hepta-partite' model of the synapse to account for the role of astrocytes, oligodendrocytes, microglia, the ECM, and NVU in the regulation of synaptogenesis and modulation of synaptic activity/plasticity. **Conclusions:** Based on this new information about the synapse, we revise Hebbian theories of synaptic plasticity, the cell assembly, and phase sequence underlying learning and memory to reflect our current understanding of synaptic communication and plasticity, while upholding the legacy of Donald Hebb.

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LOW-FREQUENCY PLASTICITY OF OLFACTORY BULB INPUTS MEDIATED BY KV4.2 CHANNELS IN RODENT PIRIFORM CORTEX

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Voltage-gated A-type K^+ channel Kv4.2 sub-units are highly expressed in the dendrites of Layer II pyramidal neurons of piriform cortex. They are known to regulate back-propagation of action potentials into the dendrites of pyramidal neurons and thus limiting the Spike-timing dependent plasticity (STDP) of distal LOT inputs. Using slice electrophysiology, Ca^{+2} imaging and optogenetic techniques, we describe a novel form of plasticity in pyramidal neurons in which unpaired low-frequency (0.1 Hz) stimulation of LOT inputs resulted in large and robust potentiation of EPSPs and enhanced local excitability. It also resulted in more efficient propagation of somatic action potentials specifically into the activated dendritic segment. This form of plasticity was exclusive to LOT inputs and did not occur in the more proximal IC inputs. Induction of this plasticity was dependent on activation of Kv4.2 potassium and NMDAR channels, resulted in insertion of AMPAR to postsynaptic membrane of activated dendrites and required internalization of membrane proteins. Upon activation of acetylcholine by bath application of CarbaChol, the potentiation frequency shifted from 0.1 Hz to 1 Hz. This phenomenon was also limited to LOT inputs and was abolished by Nicotine-receptor blockers. This form of plasticity enhances the proximal-distal electrical coupling of activated dendritic segment in a branch-specific manner.

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SYNAPSE-SPECIFIC HOMO- AND HETERO-SYNAPTIC LTP-INDUCED MEMORY CONSOLIDATION IN THE AMYGDALA

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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The discovery of experience-dependent plasticity at the cortical (C) and thalamic (T) inputs onto the lateral amygdala (LA) induced by cued Pavlovian aversive conditioning (PAC), is a landmark in elucidating the mechanisms of associative learning. Anatomically, C and T inputs converge onto the LA (C-LA and T-LA) neurons, and functionally the neutral conditioned stimulus (CS) and the aversive unconditioned stimulus (US) also converge in the LA. We have investigated the modes of plasticity of the two inputs independently with optogenetics and tested the behavioral effects by using an optimized optical PAC. We showed for the first time that pairing the optical activation T-LA (OCS) with foot-shocks, which served as the US, is sufficient to support PAC, whereby OCS elicits robust freezing as a conditioned response (CR) 24-hours later. We optimized a milder optical PAC protocol that yields virtually no freezing to study the effect of homosynaptic long-term potentiation (Ho-LTP), whereby high-frequency optical stimulation is applied to the T-LA inputs. Ho-LTP applied immediately, or 24-hours after mild PAC yields a higher CR compared to the baseline and the control group. Next, we asked whether T-LA inputs could undergo heterosynaptic LTP (He-LTP). He-LTP has the same effect as Ho-LTP only if applied immediately after conditioning. While the mild optical PAC protocol doesn't involve activation of the C-LA inputs, the potentiated C-LA inputs consistently yield high freezing levels when tested by applying optical activation of C-LA inputs. This finding was confirmed with freely moving in-vivo electrophysiology in combination with behavioral testing.

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NMDA RECEPTOR-RELATED MECHANISMS OF DOPAMINERGIC MODULATION OF TDCS-INDUCED NEUROPLASTICITY

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Dopamine is a key neuromodulator of neuroplasticity, and an important neuronal substrate of learning, and memory formation, which critically involves glutamatergic N-Methyl-D-aspartate (NMDA) receptors. Dopamine modulates NMDA receptor activity via dopamine D1 and D2 receptor subtypes. It is hypothesized that dopamine focuses long term potentiation (LTP)-like plasticity, i.e. reduces diffuse widespread but enhances locally restricted plasticity via a D2 receptor-dependent NMDA receptor activity reduction. Here, we explored NMDA receptor-dependent mechanisms underlying Dopaminergic modulation of LTP-like plasticity induced by transcranial direct current stimulation (tDCS). Eleven healthy, right-handed volunteers received anodal tDCS (1 mA, 13 min) over the left motor cortex combined with Dopaminergic agents (the D2 receptor agonist bromocriptine, levodopa for general dopamine enhancement, or placebo), and the partial NMDA receptor agonist D-cycloserine (dosages of 50, 100 and 200 mg, or placebo). Cortical excitability was monitored by transcranial magnetic stimulation-induced motor-evoked potentials. We found that LTP-like plasticity was abolished or converted into LTD-like plasticity via dopaminergic activation, but re-established under medium-dose D-cycloserine. These results suggest that diffuse LTP-like plasticity is counteracted via D2 receptor-dependent reduction of NMDA receptor activity.

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LACK OF THE SEZ6 PROTEIN, OR INHIBITION OF ECTODOMAIN SHEDDING, ATTENUATES COCAINE RELAPSE

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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A major problem of cocaine use disorder is the propensity to relapse, even after extended periods of abstinence. The Seizure-related 6 (Sez6) protein, a neuron-specific protein regulating dendritic branching, dendritic spine formation and excitatory synaptic transmission, potentially regulates structural and functional plasticity of nucleus accumbens (NAc) excitatory synapses after repeated cocaine exposure. The Sez6 ectodomain, which is shed by the protease BACE1, may mediate these effects. Aim: To determine Sez6 involvement in cocaine relapse and the effect of BACE inhibition (BACEi). Methods: Sez6 was conditionally deleted in CaMKIIα-expressing neurons (Sez6 cKO). Two models of relapse were utilized – cocaine-primed reinstatement of conditioned place preference (CPP) and cue-induced cocaine seeking following abstinence in a self-administration paradigm. Sez6 cKO and control mice received in-diet BACEi prior to patch-clamp electrophysiology of NAc slices or relapse tests/Rapid Golgi staining. Results: Sez6 cKO mice displayed reduced relapse behaviour compared to controls in both models. After cocaine-primed reinstatement or abstinence from cocaine IVSA, the density of NAc core medium spiny neuron (MSN) dendritic spines was significantly reduced in Sez6 cKO mice (cf. controls). BACEi significantly reduced: i) drug-primed or cue-induced cocaine-seeking, compared to vehicle, in a Sez6-dependent manner; ii) cocaine-induced excitatory synaptic plasticity following extended withdrawal, and iii) mature dendritic spine density on NAc core MSNs after relapse testing. Conclusion: Lack of Sez6 is protective against cocaine relapse and BACE inhibition mitigates excitatory synaptic strengthening in these models. BACE inhibitors, being trialled as Alzheimer's disease therapeutics, are promising therapeutics for relapse prevention in cocaine use disorder.

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ROLE OF POSTSYNAPTIC β2-CONTAINING NICOTINIC ACETYLCHOLINE RECEPTORS IN STRIATAL CIRCUITS AND RELATED BEHAVIOR.

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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The striatum possesses the highest density of acetylcholine (ACh) markers in the basal ganglia underlying the importance of ACh in this structure. Striatal cholinergic interneurons (CINs) are tonically active and have an essential role in cognitive flexibility and goal-directed behaviors. While the effects of ACh in the striatum through muscarinic receptors have received a particular attention, nicotinic receptors (NR) function has been less studied. Our main goal is to better understand the role of striatal cholinergic transmission via NR selectively expressed postsynaptically by striatal GABAergic interneurons. Indeed, there is now evidence that CINs are not simply neuromodulatory elements but are integrated in a fast bidirectional synaptic circuitry with several classes of GABAergic interneurons via NR activation. It was demonstrated that optogenetic activation of CINs elicits very large disynaptic GABAergic IPSP/Cs in striatal projection neurons (SPNs) that are secondary to β2-NR activation. Using a double transgenic/double optogenetic strategies, we demonstrated that NR activation of populations of striatal GABAergic interneurons was responsible for the disynaptic inhibition of SPNs. Further, using transgenic β2-floxed mice we are able to delete β2-NR conditionally and specifically contingent on Cre-expression. We demonstrate that the removal of striatal β2-NR, after localized striatal injections, abolishes the disynaptic inhibition of SPNs. Finally, our preliminary behavioral data demonstrate that removing β2-NR in striatal GABAergic interneurons induces significant impairment in cognitive flexibility measured in a reversal learning paradigm when task contingencies are switched. These results demonstrate the importance of striatal NRs, which are selectively expressed postsynaptically by interneurons, in striatal circuits and related behaviors.

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NOGO-A IS A MELATONIN-DRIVEN REGULATOR OF CIRCADIAN MEMORY DYNAMICS AND LEARNING

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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Synaptic plasticity and subsequent memory formation are time-of-day-dependent processes, with molecular mechanisms unclear. We identified in mouse hippocampus by immunochemistry a circadian oscillation of the neurite-outgrowth-inhibitor Nogo-A, with lowest levels occurring at Zeitgeber Time 2 (ZT2) and highest abundance at ZT14. Low Nogo-A levels at ZT2 coincided with better learning in a food-rewarded spatial memory test, while at highest Nogo-A levels at ZT14 learning was diminished. As in both, melatonin-deficient C57BL mice and in melatonin receptor deficient $MT^{-/-}$ mice hippocampal Nogo-A levels were constantly elevated, we suggested a role of melatonin for the hippocampal Nogo-A rhythm. We extirpated the superior cervical ganglia (SCG) in mice, rendering a knockdown of rhythmic melatonin synthesis. Indeed, in SCGX mice Nogo-A was constantly elevated, and learning showed no day-night differences. When we substituted melatonin at nighttime only in WT-SCGX mice the circadian Nogo-A rhythm re-appeared and the day-night differences in learning was re-established. Our results grant Nogo-A a fundamental and novel function in regulating daily homeostasis of mouse hippocampal signaling, with the rhythm determined by melatonin that temporarily removes constraints on neuronal plasticity, merging into shaping time-of-day-dependent cognitive performance.

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DIVERSE GABAERGIC MODULATION OF STDP IN MOUSE CA1 PYRAMIDAL NEURONS ALONG THE LONGITUDINAL AXIS OF THE HIPPOCAMPUS

POSTER SESSION 01 - SECTION: SYNAPTIC PLASTICITY AND COGNITION

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The hippocampus and its associated medial temporal lobe structures develop as a complex micro-network of excitatory and inhibitory synapses to process learning and memory formation. The longitudinal hippocampal axis spans from dorsal to ventral poles, which are differentially involved in spatial and emotional learning. Along this axis GABA, glutamate, and neuromodulatory receptors are differentially expressed, providing diverse synaptic regulation mechanisms. GABAergic inhibition balances excitatory responses and neuromodulatory transmitter release. Due to the non-uniformi expression of $GABA_A$ and $GABA_B$ receptors along the longitudinal axis synaptic plasticity might be differently modulated by this diverse GABAergic inhibition.
STDP is a paradigm based on precise ms delays of action potentials (APs) in pre- and postsynaptic neurons. Patch clamp recorded CA1 neurons were subjected to canonical (1 presynaptic : 1 postsynaptic AP) or burst t-LTP protocols (1:4), repeated 6 times @0.5 Hz in acute mouse hippocampal slices taken from dorsal (DH), intermediate (IH), or ventral (VH) hippocampus to test timing-dependent LTP (t-LTP) induction under diverse settings for GABAergic inhibition. We used either intact GABAergic inhibition, fully blocked inhibition using co-applied $GABA_AR$ (100µM picrotoxin) or $GABA_BR$ antagonists (10µM CGP55845), or recorded in the presence of only picrotoxin.
We found a complex association of excitatory and inhibitory responses depending on stimulation protocols (canonical or burst) and studied regions (DH or VH). While the 6x 1:1 protocol lost its dependency on GABAergic signaling to induce robust t-LTP from DH to VH pole, our 6x 1:4 protocol mainly depended on active $GABA_BR$ signaling during t-LTP induction.

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EARLY IMPAIRED CA3-CA1 SYNAPSES IN AN APP KNOCK-IN MICE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Synaptic plasticity has been the subject of an intense investigation in the past decades, as it is a mechanism for memory and increased Long Term Potentiation (LTP) at the CA3-CA1 hippocampal synapses is associated with increased cognitive capability. Alzheimer's disease (AD) is the most common neurodegenerative disease with decreased cognition as the main symptom, mostly initiated in the hippocampus. Recently, very early neuronal and synaptic impairments have been unveiled long before the formation of Aβ plaques. The astrocytic contribution at this stage remain yet to be clarified. **AIM**: Our study aims at study neuronal and astrocytic dysfunctions at early stages of AD. **METHODS**: Electrophysiological recordings were used to study synaptic activity and plasticity in hippocampal slices from an APP knock-in mice (APP^{NL-F}) model of AD. Western-blot and immunohistochemistry were used to understand morphological and molecular changes. **RESULTS**: Electrophysiological recordings revealed an overall altered synaptic activity in APP^{NL-F} mice. Whilst neurons look healthy, pre-synaptic activity is reduced and field recordings reveal a facilitated LTP, associated with altered expression with MAPK/ERK signaling pathway. In the meantime, astrocytes seems to express less GFAP and morphological analysis revealed a reduced complexity. **CONCLUSIONS**: We show here that neuronal synaptic transmission as well as astrocytic morphology are altered in the hippocampus at early phases of AD. Interestingly these results contrast with earlier reports showing opposite impairment in older animals. Further functional and molecular studies are therefore needed to better understand the cause of such neuronal and astrocytic early changes.

Pubmed:
34717959: Ygberg S, Akkuratov EE, Howard RJ, Taylan F, Jans DC, Mahato DR, Katz A, Kinoshita PF, Portal B, Nennesmo I, Lindskog M, Karlish SJD, Andersson M, Lindstrand A, Brismar H, Aperia A
A missense mutation converts the Na,K-ATPase into an ion channel and causes therapy-resistant epilepsy.
The ion pump Na,K-ATPase is a critical determinant of neuronal excitability; however, its role in the etiology of diseases of the central nervous system (CNS) is largely unknown. We describe here the molecular phenotype of a Trp931Arg mutation of the Na,K-ATPase catalytic α1 subunit in an infant diagnosed with therapy-resistant lethal epilepsy. In addition to the pathological CNS phenotype, we also detected renal wasting of Mg. We found that membrane expression of the mutant α1 protein was low, and ion pumping activity was lost. Arginine insertion into membrane proteins can generate water-filled pores in the plasma membrane, and our molecular dynamic (MD) simulations of the principle states of Na,K-ATPase transport demonstrated massive water inflow into mutant α1 and destabilization of the ion-binding sites. MD simulations also indicated that a water pathway was created between the mutant arginine residue and the cytoplasm, and analysis of oocytes expressing mutant α1 detected a nonspecific cation current. Finally, neurons expressing mutant α1 were observed to be depolarized compared with neurons expressing wild-type protein, compatible with a lowered threshold for epileptic seizures. The results imply that Na,K-ATPase should be considered a neuronal locus minoris resistentia in diseases associated with epilepsy and with loss of plasma membrane integrity.
J Biol Chem, 2021; 297
33357364: Portal B, Guiard BP
[Role of astrocytic connexins in the regulation of extracellular glutamate levels: implication for the treatment of major depressive episodes].
Major depression is a psychiatric disorder relying on different neurobiological mechanisms. In particular, a hypersensitivity of the hypothalamic-pituitary-adrenal axis leading to an excess of cortisol in blood and a deficit in monoaminergic neurotransmission have been associated with mood disorders. In keeping with these mechanisms, currently available antidepressant drugs act by increasing the extracellular levels of monoamines in the synaptic cleft. Since the discovery of the

rapid and long-lasting antidepressant effects of ketamine, an NMDA receptor antagonist, a growing attention in psychiatry is paid to the pharmacological tools able to attenuate glutamatergic neurotransmission. Astrocytes play an important role in the excitatory/inhibitory balance of the central nervous system through the regulation of glutamate reuptake and secretion. Interestingly, the release of this excitatory amino acid is controlled, at least in part, by plasma membrane proteins (i.e. connexins) that cluster together to form gap junctions or hemichannels. Preclinical evidence suggests that these functional entities play a critical role in emotional behaviour. After a brief overview of the literature on mood disorders and related treatments, this review describes the role of astrocytes and connexins in glutamatergic neurotransmission and major depression. Moreover, we highlight the arguments supporting the therapeutic potential of connexins blockers but also the practical difficulties to target the hemichannels while maintaining gap junctions intact.
Biol Aujourdhui, 2020; 214
32612499: Droguerre M, Duchêne A, Picoli C, Portal B, Lejards C, Guiard BP, Meunier J, Villard V, Déglon N, Hamon M, Mouthon F, Charvériat M
Efficacy of THN201, a Combination of Donepezil and Mefloquine, to Reverse Neurocognitive Deficits in Alzheimer's Disease.
Donepezil (DPZ) is an acetylcholinesterase inhibitor used in Alzheimer's disease to restore cognitive functions but is endowed with limited efficacy. Recent studies pointed out the implication of astroglial networks in cognitive processes, notably via astrocyte connexins (Cxs), proteins involved in gap junction intercellular communications. Hence, we investigated the impact on cognition of pharmacological or genetic modulations of those astrocyte Cxs during DPZ challenge in two rodent models of Alzheimer's disease-like memory deficits. We demonstrated that the Cx modulator mefloquine (MEF) significantly enhanced the procognitive effect of DPZ in both models. In parallel, we determined that MEF potentiated DPZ-induced release of acetylcholine in hippocampus. Finally, local genetic silencing of astrocyte Cxs in the hippocampus was also found to enhance the procognitive effect of DPZ, pointing out the importance of Cx-dependent astrocyte networks in memory processes.
Front Neurosci, 2020; 14
31925934: Portal B, Delcourte S, Rovera R, Lejards C, Bullich S, Malnou CE, Haddjeri N, Déglon N, Guiard BP
Genetic and pharmacological inactivation of astroglial connexin 43 differentially influences the acute response of antidepressant and anxiolytic drugs.
Astroglial connexins (Cxs) 30 and 43 are engaged in gap junction and hemichannel activities. Evidence suggests that these functional entities contribute to regulating neurotransmission, thereby influencing brain functions. In particular, preclinical and clinical findings highlight a role of Cx43 in animal models of depression. However, the role of these proteins in response to currently available psychotropic drugs is still unknown.
Acta Physiol (Oxf), 2020; 229
31797899: Droguerre M, Tsurugizawa T, Duchêne A, Portal B, Guiard BP, Déglon N, Rouach N, Hamon M, Mouthon F, Ciobanu L, Charvériat M
A New Tool for In Vivo Study of Astrocyte Connexin 43 in Brain.
Astrocytes are glial cells organized in dynamic and structured networks in the brain. These plastic networks, involving key proteins such as connexin 43 (Cx43), are engaged in fine neuronal tuning and have recently been considered as emerging therapeutic targets in central nervous system disorders. We developed and validated a new application of the manganese-enhanced magnetic resonance imaging (MEMRI) technique allowing in vivo investigations of astrocyte-neuron interactions through quantification of brain Cx43 functional activity. The proof of concept has been achieved by quantification of MEMRI signals in brain after either local astrocyte-specific Cx43 knockdown with shRNA or systemic administration of Cx43 blockers. Unilateral hippocampal Cx43 genetical silencing was associated with an ipsilateral local increase of MEMRI signal. Furthermore, Cx43 blockers also enhanced MEMRI signal responses in hippocampus. Altogether, these data reveal the MEMRI technique as a tool for quantitative imaging of in vivo Cx43-dependent function in astrocytes under physiological and pathological conditions.
Sci Rep, 2019; 9
26733815: Quesseveur G, Portal B, Basile JA, Ezan P, Mathou A, Halley H, Leloup C, Fioramonti X, Déglon N, Giaume C, Rampon C, Guiard BP
Attenuated Levels of Hippocampal Connexin 43 and its Phosphorylation Correlate with Antidepressant- and Anxiolytic-Like Activities in Mice.
Clinical and preclinical studies have implicated glial anomalies in major depression. Conversely, evidence suggests that the activity of antidepressant drugs is based, at least in part, on their ability to stimulate density and/or activity of astrocytes, a major glial cell population. Despite this recent evidence, little is known about the mechanism(s) by which astrocytes regulate emotionality. Glial cells communicate with each other through gap junction channels (GJCs), while they can also directly interact with neurons by releasing gliotransmitters in the extracellular compartment via an hemichannels (HCs)-dependent process. Both GJCs and HCs are formed by two main protein subunits: connexins (Cx) 30 and 43 (Cx30 and Cx43). Here we

investigate the role of hippocampal Cx43 in the regulation of depression-like symptoms using genetic and pharmacological approaches. The first aim of this study was to evaluate the impact of the constitutive knock-down of Cx43 on a set of behaviors known to be affected in depression. Conversely, the expression of Cx43 was assessed in the hippocampus of mice subjected to prolonged corticosterone (CORT) exposure, given either alone or in combination with an antidepressant drug, the selective serotonin reuptake inhibitor fluoxetine. Our results indicate that the constitutive deficiency of Cx43 resulted in the expression of some characteristic hallmarks of antidepressant-/anxiolytic-like behavioral activities along with an improvement of cognitive performances. Moreover, in a new cohort of wild-type mice, we showed that CORT exposure elicited anxiety and depression-like abnormalities that were reversed by chronic administration of fluoxetine. Remarkably, CORT also increased hippocampal amounts of phosphorylated form of Cx43 whereas fluoxetine treatment normalized this parameter. From these results, we envision that antidepressant drugs may exert their therapeutic activity by decreasing the expression and/or activity of Cx43 resulting from a lower level of phosphorylation in the hippocampus.
Front Cell Neurosci, 2015; 9

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AMYLOID-BETA OLIGOMERS INCREASES THE AMPLITUDE AND CHANGES THE FIRING PATTERN OF SPONTANEOUS EXCITATORY POSTSYNAPTIC CURRENTS OF HIPPOCAMPAL NEURONS IN A MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the deterioration of memory and learning. Synapses are fundamental to the transfer, coding, and storage of information, and the latest findings indicate that the deterioration of synaptic transmission is caused by the accumulation of amyloid-beta (Aβ), dysregulated Ca2+ homeostasis, increased oxidative stress, and mitochondrial dysfunction. The cellular mechanisms that occur at the presynaptic and postsynaptic levels in hippocampal neurons in the early stages of AD have not been fully characterized, nor have their effects on the encoding of neuronal information by these cells. Spontaneous activity, which is the result of the spontaneous fusion of individual vesicles and the release of quanta/unique neurotransmitter packages, allows us to analyze the presynaptic and postsynaptic elements separately by evaluating their spontaneous firing frequency and amplitude. To characterize the spontaneous electrical activity of hippocampal neurons during the early stages of the neurodegeneration process associated with AD, we studied, through the patch-clamp whole-cell technique, the effect of the presence of Aβ oligomers (Aβo) on the amplitude, firing frequency, and firing pattern of spontaneous excitatory postsynaptic currents (sEPSCs) in hippocampal primary cultures from Wistar rat embryos. We found that exposure to Aβo increased the amplitude of sEPSCs, which indicated alterations at the postsynaptic level, and modified the firing pattern of the cultured hippocampal cells, which indicated changes in the coding of the information. These data expand the basic knowledge on the cellular basis of the early deterioration of synaptic transmission and your implications on the processes of synaptic plasticity

Pubmed:
32250284: Cuestas Torres DM, Cardenas FP
Synaptic plasticity in Alzheimer's disease and healthy aging.
The strength and efficiency of synaptic connections are affected by the environment or the experience of the individual. This property, called synaptic plasticity, is directly related to memory and learning processes and has been modeled at the cellular level. These types of cellular memory and learning models include specific stimulation protocols that generate a long-term strengthening of the synapses, called long-term potentiation, or a weakening of the said long-term synapses, called long-term depression. Although, for decades, researchers have believed that the main cause of the cognitive deficit that characterizes Alzheimer's disease (AD) and aging was the loss of neurons, the hypothesis of an imbalance in the cellular and molecular mechanisms of synaptic plasticity underlying this deficit is currently widely accepted. An understanding of the molecular and cellular changes underlying the process of synaptic plasticity during the development of AD and aging will direct future studies to specific targets, resulting in the development of much more efficient and specific therapeutic strategies. In this review, we classify, discuss, and describe the main findings related to changes in the neurophysiological mechanisms of synaptic plasticity in excitatory synapses underlying AD and aging. In addition, we suggest possible mechanisms in which aging can become a high-risk factor for the development of AD and how its development could be prevented or slowed.
Rev Neurosci, 2020; 31

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AIR POLLUTION EFFECTS ON SYNAPTIC TRANSMISSION.

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Air pollution (AP) effects are traditionally associated with increased risks for development of pulmonary and cardiovascular disease (Block & Calderón-Garcidueñas, 2009). In recent years, human epidemiological and animal studies show how the Central Nervous System (CNS) is emerging as an important target for adverse health effects of airborne pollutants (Costa et al., 2017; Genc et al., 2012). Moreover, different studies demonstrate how exposure to environmental air pollutants induces synaptic plasticity impairment, both directly and indirectly (Hajipour S. et al., 2020; Bello-Medina P.C. et al., 2019; Woodward N.C. et al., 2017; Davis D.A. et al., 2013; Morgan T.E. et al., 2011). General aim of our experimental project is to investigate how AP modify and invalidate delicate and complex mechanisms on which synaptic plasticity depends, including LTP and multiple neurotransmission signals of hippocampal and cortical neuronal cells, whose function is at the base of the learning and memory process. For this purpose, we use CD-1 mouse models (P8/20), whose brain slices are exposed to Diesel Exhaust Particles (DEP) (Reference Material, 10µg/mL, 2h exposure), which constitute an important component of AP. Electrophysiological recordings experiments are performed using the *Whole-Cell Patch Clamp* technique both in *Voltage* and in *Current Clamp* on pyramidal neurons. The final aim of this project is to achieve a better understanding of the overall effects of AP on physiological mechanisms of cerebral neuro-communication.

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LOCALIZATION AND FUNCTION OF ENDOCYTIC ZONES IN NEURONAL DENDRITES IN SITU: RELEVANCE FOR SYNAPTIC PLASTICITY

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Post-synaptic receptor trafficking plays an important role in synaptic transmission and plasticity. Receptor internalization occurs at endocytic zones (EZs) marked by clathrin. In neurons in culture, EZs are positioned near post-synaptic densities (PSDs) marking excitatory synapses in dendritic spines as well as in uncharacterized locations on the dendritic shaft. We have recently shown that EZs are stable but produce endocytic vesicles to internalize post-synaptic receptors (Rosendale et al. Cell Reports 2017). We determined the spatial organization and dynamics of EZs in hippocampal brain slices. We expressed by single cell electroporation in CA1 pyramidal neurons CLC-GFP to mark EZs and Homer1c-tdTomato, a marker of PSDs. We imaged these neurons at 15 days in vitro with lattice light sheet microscopy every 4 s for 5-10 minutes and tracked EZs. We show that, in neurons in situ, EZs keep the same general organization as in culture: EZs are distributed in the dendritic shaft and in spines, next to PSDs. However, EZs are much more dynamic, appearing and disappearing within minutes. EZs close to PSDs have longer lifetimes (2.6min) than in the dendritic shaft (1min). We now explore the possible mechanisms for a difference in the dynamics of EZs. In particular, we test the hypothesis that substrate rigidity plays a role by culturing neurons on polyacrylamide gels of controlled stiffness.

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AN ANTISEIZURE ADENOSINE A1R AGONIST INHIBITS HIPPOCAMPAL SYNAPTIC TRANSMISSION IN EPILEPTIC RATS BUT NOT IN CONTROL ONES.

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Resistance to pharmacotherapy requires the development of novel antiepileptic drugs (AEDs). An adenosine A_1 receptor (A_1R) agonist, MRS5474, possesses anticonvulsant activity (Tosh et al., 2012, J Med Chem, 55:8075), without the cardiac side effects of other A_1R agonists. Hypothesizing that it operates via a novel mechanism, we assessed its influence upon hippocampal excitatory transmission and GABA uptake in control and in status-epilepticus (SE)-induced conditions. Excitatory postsynaptic potentials (fEPSPs), Population Spikes (PS), or GAT-1 mediated [^{3}H]GABA uptake was recorded from hippocampal slices. Status epilepticus (SE) was induced by kainate (10mg/kg, i.p) 4 weeks before hippocampal slice preparation, as approved by the ethics committee. In control slices MRS5474 (120-500nM) was devoid of effect in fEPSPs (n=7, mouse; n=2, rat) or PS (n=2, rat). In contrast, the well-known A1R agonists, CPA (30nM, n=2), or CHA (30nM, n=2), caused the expected marked inhibition (>60%) of fEPSPs. In SE rat slices, MRS5474 (250nM) inhibited (30±6.0%, n=7, $p<0.05$) fEPSP slope and PS amplitude (40±9.6%, n=4, $p<0.05$). MRS5474 (50nM) inhibited GAT-1 mediated [^{3}H]GABA uptake by 52±8.4% (n=6, $p<0.05$) in control slices, but this effect is likely not mediated by A1R since it was not blocked by the A1R antagonist, DPCPX (50nM, % inhibition 46±6.2, n=4, $p>0.05$ vs no DPCPX). In conclusion, MRS5474 does not share properties with canonical A_1R agonists, suggesting that this putative AED may have fewer side effects than other A1R agonists and currently available AEDs. Project funded by FCT, Portugal (PTDC/MED- FAR/30933/2017) and by EU Horizon 2020 research and innovation programme (GA 952455, EpiEpiNet).

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EFFECT OF A HIGH-FAT DIET ON HIPPOCAMPAL AREA CA2 AND SOCIAL RECOGNITION MEMORY

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Obesity levels during adolescence is growing at an alarming rate and obese adolescents perform worse in cognitive tasks leading to detrimental academic performance. In mice, the consumption of a diet high in saturated fat (High-Fat Diet: HFD) during adolescence also results in strong deficits in several hippocampal-dependent memory tasks including social recognition memory (SRM). Area CA2 of the hippocampus plays a critical role in SRM. CA2 neuronal activity is enhanced during social interactions and decreasing CA2 pyramidal neuron activity or blocking CA2 transmission onto its targets prevents SRM. Our aim here is to **understand if and how area CA2 activity is altered in HFD-fed mice and whether this underlies their impairments in SRM.** After exposing the mice to 12 weeks of HFD, electrophysiology, chemogenetics and behavioral experiments are used to study the alterations induced by HFD in hippocampal area CA2, and the contribution of these changes on SRM. Decreasing hippocampal pyramidal cell activity throughout the dorsal hippocampus of HFD-fed mice rescued their SRM deficits. In line with this result, we also discovered that CA2 pyramidal neurons are hyper excitable in HFD-fed animals, allowing for CA3 inputs to drive action potentials in CA2 of HFD-fed mice. This hyperexcitability seems to be due to increased presynaptic glutamate release from Schaffer collaterals. HFD during adolescence alters CA2 pyramidal neuron activity possibly underlying SRM deficits. We are currently studying the precise mechanisms underlying the dysfunction as well as whether modulatory signaling such as oxytocin effects are altered in CA2 by HFD consumption.

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OPTOGENETIC MAPPING OF THE CELLULAR SPECIFICITY OF THE AFFERENT INPUTS TO THE RETROSPLENIAL CORTEX

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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One of the defining characteristics of the retrosplenial cortex (RSC) is the interconnectivity it shares with a range of brain regions. This connectivity could be critical to its role in learning and memory, as reciprocal connections have been anatomically identified with other memory-associated regions such as the anterior cingulate cortex (ACC), anterior thalamic nuclei (ATN) and dorsal subiculum (dSub). However, the connection probability and strength of these synapses are still relatively unknown; therefore we aimed to characterise and quantify the properties of the afferent pathways to the RSC from these regions. To achieve this, afferent terminals in *ex vivo* C57BL6/J mouse RSC brain slices were optogenetically stimulated following viral expression of channelrhodopsin2(H134R) in the presynaptic regions, and whole-cell patch clamp recordings were undertaken in pyramidal neurons (PYRs) to measure the synaptic properties of these connections. Our findings indicate projection-specific differences in both afferent strength and specificity. Firstly, projections from the ACC elicited responses in a much sparser subset of PYRs than from the ATN and dSub. Secondly, projections from the ATN elicited significantly larger EPSCs than from the other presynaptic regions; although this effect was limited to cells situated in the dysgranular superficial layers. These findings demonstrate that not all RSC afferent pathways are equal in strength and provides an important addition to standard neuroanatomical tracing to provide insights into the distinct roles that these pathways may play in behaviour. Furthermore, characterisation of these pathways provides a physiological baseline for future comparisons of afferent inputs to RSC in different models of disease.

Pubmed:

33565633: Margetts-Smith G, Macnaghten AI, Brebner LS, Ziminski JJ, Sieburg MC, Grimm JW, Crombag HS, Koya E
Acute, but not longer-term, exposure to environmental enrichment attenuates Pavlovian cue-evoked conditioned approach and Fos expression in the prefrontal cortex in mice.
Exposure to environmental enrichment can modify the impact of motivationally relevant stimuli. For instance, previous studies in rats have found that even a brief, acute (~1 day), but not chronic, exposure to environmentally enriched (EE) housing attenuates instrumental lever pressing for sucrose-associated cues in a conditioned reinforcement setup. Moreover, acute EE reduces corticoaccumbens activity, as measured by decreases in expression of the neuronal activity marker "Fos." Currently, it is not known whether acute EE also reduces sucrose seeking and corticoaccumbens activity elicited by non-contingent or "forced" exposure to sucrose cues, which more closely resembles cue exposure encountered in daily life. We therefore measured the effects of acute/intermittent (1 day or 6 day of EE prior to test day) versus chronic (EE throughout conditioning lasting until test day) EE on the ability of a Pavlovian sucrose cue to elicit sucrose seeking (conditioned approach) and Fos expression in the medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), and nucleus accumbens (NAc) in mice. One day, but not 6 day or chronic EE , reduced sucrose seeking and Fos in the deep layers of the dorsal mPFC. By contrast, 1 day, 6 day, and chronic EE all reduced Fos in the shallow layers of the OFC. None of the EE manipulations modulated NAc Fos expression. We reveal how EE reduces behavioral reactivity to sucrose cues by reducing activity in select prefrontal cortical brain areas. Our work further demonstrates the robustness of EE in its ability to modulate various forms of reward-seeking across species.
Eur J Neurosci, 2021; 53
32307758: Brebner LS, Ziminski JJ, Margetts-Smith G, Sieburg MC, Hall CN, Heintz TG, Lagnado L, Hirrlinger J, Crombag HS, Koya E
Extinction of cue-evoked food-seeking recruits a GABAergic interneuron ensemble in the dorsal medial prefrontal cortex of mice.
Animals must quickly adapt food-seeking strategies to locate nutrient sources in dynamically changing environments.

Learned associations between food and environmental cues that predict its availability promote food-seeking behaviors. However, when such cues cease to predict food availability, animals undergo "extinction" learning, resulting in the inhibition of food-seeking responses. Repeatedly activated sets of neurons, or "neuronal ensembles," in the dorsal medial prefrontal cortex (dmPFC) are recruited following appetitive conditioning and undergo physiological adaptations thought to encode cue-reward associations. However, little is known about how the recruitment and intrinsic excitability of such dmPFC ensembles are modulated by extinction learning. Here, we used in vivo 2-Photon imaging in male Fos-GFP mice that express green fluorescent protein (GFP) in recently behaviorally activated neurons to determine the recruitment of activated pyramidal and GABAergic interneuron dmPFC ensembles during extinction. During extinction, we revealed a persistent activation of a subset of interneurons which emerged from a wider population of interneurons activated during the initial extinction session. This activation pattern was not observed in pyramidal cells, and extinction learning did not modulate the excitability properties of activated pyramidal cells. Moreover, extinction learning reduced the likelihood of reactivation of pyramidal cells activated during the initial extinction session. Our findings illuminate novel neuronal activation patterns in the dmPFC underlying extinction of food-seeking, and in particular, highlight an important role for interneuron ensembles in this inhibitory form of learning.
Eur J Neurosci, 2020; 52

31727794: Brebner LS, Ziminski JJ, Margetts-Smith G, Sieburg MC, Reeve HM, Nowotny T, Hirrlinger J, Heintz TG, Lagnado L, Kato S, Kobayashi K, Ramsey LA, Hall CN, Crombag HS, Koya E

The Emergence of a Stable Neuronal Ensemble from a Wider Pool of Activated Neurons in the Dorsal Medial Prefrontal Cortex during Appetitive Learning in Mice.

Animals selectively respond to environmental cues associated with food reward to optimize nutrient intake. Such appetitive conditioned stimulus-unconditioned stimulus (CS-US) associations are thought to be encoded in select, stable neuronal populations or neuronal ensembles, which undergo physiological modifications during appetitive conditioning. These ensembles in the medial prefrontal cortex (mPFC) control well-established, cue-evoked food seeking, but the mechanisms involved in the genesis of these ensembles are unclear. Here, we used male mice that express green fluorescent protein (GFP) in recently behaviorally activated neurons, to reveal how dorsal mPFC neurons are recruited and modified to encode CS-US memory representations using an appetitive conditioning task. In the initial conditioning session, animals did not exhibit discriminated, cue-selective food seeking, but did so in later sessions indicating that a CS-US association was established. Using microprism-based 2-Photon imaging, we revealed that only a minority of neurons activated during the initial session was consistently activated throughout subsequent conditioning sessions and during cue-evoked memory recall. Notably, using electrophysiology, we found that neurons activated following the initial session exhibited transient hyperexcitability. Chemogenetically enhancing the excitability of these neurons throughout subsequent conditioning sessions interfered with the development of reliable cue-selective food seeking, indicated by persistent, nondiscriminated performance. We demonstrate how appetitive learning consistently activates a subset of neurons to form a stable neuronal ensemble during the formation of a CS-US association. This ensemble may arise from a pool of hyperexcitable neurons activated during the initial conditioning session. Appetitive conditioning endows cues associated with food with the ability to guide food-seeking, through the formation of a food-cue association. Neuronal ensembles in the mPFC control established cue-evoked food-seeking. However, how neurons undergo physiological modifications and become part of an ensemble during conditioning remain unclear. We found that only a minority of dorsal mPFC neurons activated on the initial conditioning session became consistently activated during conditioning and memory recall. These initially activated neurons were also transiently hyperexcitable. We demonstrate the following: (1) how stable neuronal ensemble formation in the dorsal mPFC underlies appetitive conditioning; and (2) how this ensemble may arise from hyperexcitable neurons activated before the establishment of cue-evoked food seeking.
J Neurosci, 2020; 40

31699890: Sieburg MC, Ziminski JJ, Margetts-Smith G, Reeve HM, Brebner LS, Crombag HS, Koya E

Reward Devaluation Attenuates Cue-Evoked Sucrose Seeking and Is Associated with the Elimination of Excitability Differences between Ensemble and Non-ensemble Neurons in the Nucleus Accumbens.

Animals must learn relationships between foods and the environmental cues that predict their availability for survival. Such cue-food associations are encoded in sparse sets of neurons or "neuronal ensembles" in the nucleus accumbens (NAc). For these ensemble-encoded, cue-controlled appetitive responses to remain adaptive, they must allow for their dynamic updating depending on acute changes in internal states such as physiological hunger or the perceived desirability of food. However, how these neuronal ensembles are recruited and physiologically modified following the update of such learned associations is unclear. To investigate this, we examined the effects of devaluation on ensemble plasticity at the levels of recruitment, intrinsic excitability, and synaptic physiology in sucrose-conditioned mice that express green fluorescent protein (GFP) in recently activated neurons. Neuronal ensemble activation patterns and their physiology were examined using immunohistochemistry and slice electrophysiology, respectively. Reward-specific devaluation following 4 d of sucrose

consumption, but not general caloric devaluation, attenuated cue-evoked sucrose seeking. This suggests that changes in the hedonic and/or incentive value of sucrose, and not caloric need, drove this behavior. Moreover, devaluation attenuated the size of the neuronal ensemble recruited by the cue in the NAc shell. Finally, it eliminated the relative enhanced excitability of ensemble (GFP) neurons against non-ensemble (GFP) neurons observed under non-devalued conditions, and did not induce any ensemble-specific changes in excitatory synaptic physiology. Our findings provide new insights into neuronal ensemble mechanisms that underlie the changes in the incentive and/or hedonic impact of cues that support adaptive food seeking.
eNeuro, 2019 Nov/Dec; 6
28540927: Ziminski JJ, Sieburg MC, Margetts-Smith G, Crombag HS, Koya E
Regional Differences in Striatal Neuronal Ensemble Excitability Following Cocaine and Extinction Memory Retrieval in Fos-GFP Mice.
Learned associations between drugs of abuse and the drug administration environment have an important role in addiction. In rodents, exposure to a drug-associated environment elicits conditioned psychomotor activation, which may be weakened following extinction (EXT) learning. Although widespread drug-induced changes in neuronal excitability have been observed, little is known about specific changes within neuronal ensembles activated during the recall of drug-environment associations. Using a cocaine-conditioned locomotion (CL) procedure, the present study assessed the excitability of neuronal ensembles in the nucleus accumbens core and shell (NAc and NAc), and dorsal striatum (DS) following cocaine conditioning and EXT in Fos-GFP mice that express green fluorescent protein (GFP) in activated neurons (GFP+). During conditioning, mice received repeated cocaine injections (20 mg/kg) paired with a locomotor activity chamber (Paired) or home cage (Unpaired). Seven to 13 days later, both groups were re-exposed to the activity chamber under drug-free conditions and Paired, but not Unpaired, mice exhibited CL. In a separate group of mice, CL was extinguished by repeatedly exposing mice to the activity chamber under drug-free conditions. Following the expression and EXT of CL, GFP+ neurons in the NAc (but not NAc and DS) displayed greater firing capacity compared to surrounding GFP- neurons. This difference in excitability was due to a generalized decrease in GFP- excitability following CL and a selective increase in GFP+ excitability following its EXT. These results suggest a role for both widespread and ensemble-specific changes in neuronal excitability following recall of drug-environment associations.
Neuropsychopharmacology, 2018; 43
28213443: Ziminski JJ, Hessler S, Margetts-Smith G, Sieburg MC, Crombag HS, Koya E
Changes in Appetitive Associative Strength Modulates Nucleus Accumbens, But Not Orbitofrontal Cortex Neuronal Ensemble Excitability.
Cues that predict the availability of food rewards influence motivational states and elicit food-seeking behaviors. If a cue no longer predicts food availability, then animals may adapt accordingly by inhibiting food-seeking responses. Sparsely activated sets of neurons, coined "neuronal ensembles," have been shown to encode the strength of reward-cue associations. Although alterations in intrinsic excitability have been shown to underlie many learning and memory processes, little is known about these properties specifically on cue-activated neuronal ensembles. We examined the activation patterns of cue-activated orbitofrontal cortex (OFC) and nucleus accumbens (NAc) shell ensembles using wild-type and mice, which express green fluorescent protein (GFP) in activated neurons, after appetitive conditioning with sucrose and extinction learning. We also investigated the neuronal excitability of recently activated, GFP+ neurons in these brain areas using whole-cell electrophysiology in brain slices. Exposure to a sucrose cue elicited activation of neurons in both the NAc shell and OFC. In the NAc shell, but not the OFC, these activated GFP+ neurons were more excitable than surrounding GFP- neurons. After extinction, the number of neurons activated in both areas was reduced and activated ensembles in neither area exhibited altered excitability. These data suggest that learning-induced alterations in the intrinsic excitability of neuronal ensembles is regulated dynamically across different brain areas. Furthermore, we show that changes in associative strength modulate the excitability profile of activated ensembles in the NAc shell. Sparsely distributed sets of neurons called "neuronal ensembles" encode learned associations about food and cues predictive of its availability. Widespread changes in neuronal excitability have been observed in limbic brain areas after associative learning, but little is known about the excitability changes that occur specifically on neuronal ensembles that encode appetitive associations. Here, we reveal that sucrose cue exposure recruited a more excitable ensemble in the nucleus accumbens, but not orbitofrontal cortex, compared with their surrounding neurons. This excitability difference was not observed when the cue's salience was diminished after extinction learning. These novel data provide evidence that the intrinsic excitability of appetitive memory-encoding ensembles is regulated differentially across brain areas and adapts dynamically to changes in associative strength.
J Neurosci, 2017; 37
27367964: Koya E, Margetts-Smith G, Hope BT
Daun02 Inactivation of Behaviorally Activated Fos-Expressing Neuronal Ensembles.
Learned associations about salient experiences (e.g., drug exposure, stress) and their associated environmental stimuli are mediated by a minority of sparsely distributed, behaviorally activated neurons coined 'neuronal ensembles.' For many years,

it was not known whether these neuronal ensembles played causal roles in mediating learned behaviors. However, in the last several years the 'Daun02 inactivation technique' in Fos-lacZ transgenic rats has proved very useful in establishing causal links between neuronal ensembles that express the activity-regulated protein Fos and learned behaviors. Fos-expressing neurons in these rats also express the bacterial protein β-galactosidase (β-gal) in strongly activated neurons. When the prodrug Daun02 is injected into the brains of these rats 90 min after a behavior (e.g., drug-seeking) or cue exposure, then Daun02 is converted into daunorubicin by β-gal, which selectively inactivates Fos- and β-gal-expressing neurons that were activated 90 min before the Daun02 injection. This unit presents protocols for breeding the Fos-lacZ rats and conducting appropriate Daun02 inactivation experiments.
Curr Protoc Neurosci, 2016; 76

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BOARD NUMBER: S01-398

SUBCOMMISSURAL ORGAN-SPONDIN-DERIVED PEPTIDE (NX210C) PROMOTES GLUTAMATERGIC RECEPTOR-RELATED SYNAPTIC TRANSMISSION AND SIGNALING IN THE MOUSE CNS

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Aim NX210c is a disease-modifying dodecapeptide, derived from the subcommissural organ-spondin, under clinical development for the treatment of neurological disorders. Here, we have evaluated the effect of NX210c on synaptic function/dysfunction. **Methods** NX210c (250 µg/mL) was superfused on mouse brain slices to measure (1) NMDAR- and AMPAR-mediated excitatory postsynaptic currents (EPSCs) at hippocampal CA3-CA1 synapses, and (2) extracellular field excitatory postsynaptic potentials (fEPSPs) recordings at hippocampal and thalamocortical synapses. NX210c (5 mg/kg) was injected intraperitoneally in mice with cortical synaptic dysfunctions induced by chronic administrations of the NMDAR antagonist phencyclidine (PCP) to evaluate working memory (% of alternations in the T-maze) and GluN2A-NMDAR and phosphorylated CREB levels in cortical protein extracts (western-blot). **Results** NX210c increased EPSC amplitudes mediated by NMDAR (+79.2%, p=0.0469 *vs* baseline), more specifically GluN2A-NMDAR, and AMPAR (+16.7%, p=0.0191 *vs* baseline), as well as fEPSP slopes (p=0.0356 and p=0.0027 *vs* control for hippocampal and thalamocortical synapses), consistent with the *in silico* docking of NX210c to glutamatergic receptors. Accordingly, a single acute administration of NX210c in PCP-injected mice restored working memory (-35.7 and -13.5% of alternations for vehicle- and NX210c-treated PCP mice compared with control mice). Further, repeated daily administrations of the peptide induced a two-fold increase in GluN2A-NMDAR protein levels and reversed PCP-induced decrease in pCREB, which also restored memory (-4.3% of alternations for NX210c-treated PCP mice compared with control mice). **Conclusions** The action of NX210c on GluN2A-NMDAR and AMPAR represents an innovative therapeutic opportunity to ameliorate outcomes in patients suffering from CNS disorders with crippling synaptic dysfunctions.

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ROLE OF PRE- AND POSTSYNAPTIC PIRB FOR HIPPOCAMPAL ASYMMETRY FORMATION

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Left-right asymmetry is a fundamental feature of higher-order brain structure and function. In the mouse hippocampus, properties of synapses between pyramidal cells depend on the hemispheric location of presynaptic CA3 neurons. In *stratum radiatum*, right-input synapses consist of larger postsynaptic density (PSD) with higher ratio of perforations and lower density NR2B subunits compared with left-input synapses. The major histocompatibility complex class I (MHCI) and immunoglobulin-like receptor B (PirB) are essential for the formation of this input-side dependent asymmetry, implying a potential trans-synaptic signalling via MHCI and PirB. We aimed to investigate if the PirB is necessary for signalling on the input or target side. To this end, we conducted electrophysiological and morphological analysis. NMDA EPSCs at the PirB-deficient mice (KO) pyramidal cell synapses showed no asymmetry in sensitivity to a NR2B-selective antagonist. Postsynaptic, but not presynaptic AAV-mediated PirB expression rescued this asymmetry. However, ultrastructural analysis revealed that conditional KO of PirB in either pre- or postsynaptic neurons abolished the asymmetry. Both left- and right-input synapses showed a low perforation ratio similar to that of wild-type left-input synapses. This suggests that PirB is necessary on both sides of the synapse for the asymmetry formation. Our results indicate that PirB expression in target side is critical for the asymmetry formation. The discrepant results of presynaptic PirB manipulation might be due to its indirect effects on postsynaptic PirB expression. Further work is needed to elucidate how the PirB in the pre and postsynaptic sites work in concert for the generation of hippocampal asymmetry.

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GLUCAGON-LIKE PEPTIDE 1 RECEPTOR AGONIST EXENDIN-4 HAS DIVERSE EFFECTS ON NEURONAL ACTIVITY

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Glucagon-like peptide-1 receptor (GLP-1R) is an important G-protein coupled receptor in the periphery, which when activated stimulates the glucose-dependent release of insulin by the pancreatic β-cells to lower blood glucose. As such drugs that are agonists of this receptor are an approved class of drugs used for Type 2 diabetes mellitus (T2DM). T2DM and the neurodegenerative disease Alzheimer's disease (AD) share common pathophysiological characteristics such as insulin resistance and decreased glucose metabolism. The GLP-R agonist liraglutide is under a Phase IIb clinical trial (NCT01843075) in patients with mild AD dementia to examine the drug's effect on the hallmarks of the progression of the disease. Other agonists of this receptor have also shown improved spatial learning and memory in animal models of AD. However, to date the details of the effects this receptor system has at a neural circuit level in the brain is not well known. GLP-1R is expressed in many brain structures but it is most highly expressed in the lateral septum. Therefore, we aim to explore the effects of GLP-1R activation on neuronal activity in the lateral septum. We have used a multi-electrode array approach to show the diverse effects of GLP-1R agonist Exendin-4 on neuronal activity within the lateral septum. Consequently, our work will provide a basis for future works to study whether the effects of GLP-1R activation on neuronal activity is a result of direct or modulatory mechanism, and also how the regulation of neural circuits by this receptor system guides behaviour.

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HOW THE HYPOTHALAMIC SUPRAMAMMILLARY-HIPPOCAMPAL NETWORK IS ALTERED IN A MOUSE MODEL OF THE 22Q11.2 DELETION SYNDROME

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Hippocampal area CA2 plays a central role in the encoding of social information. This small region is targeted by long-range glutamatergic axons from the hypothalamic supramammillary nucleus (SuM) that enable the detection of social novelty. In area CA2, these glutamatergic inputs form synapses with parvalbumin-expressing (PV+) interneurons (INs) and pyramidal neurons (PNs). Using a mouse model of the 22q11.2 deletion syndrome (DS), we have previously described a reduction in PV+ IN density in area CA2 that is similar to observations made in postmortem studies from schizophrenic and bi-polar patients. Furthermore, social memory of these mice is strongly impaired. In this study, we aim to examine the local circuitry between the SuM and area CA2. Specifically, how excitatory and feedforward inhibitory (FFI) transmission from the SuM to CA2 PNs are changed in a 22q11.2 DS model. To answer this question, we have crossed SuM-cre mice with 22q11.2 DS mice (LgDel) and used viral vectors to selectively express channelrhodopsin-2 (ChR2) in SuM neurons. We found that FFI from SuM axons onto CA2 PNs is decreased in both female and male LgDel mice while the direct excitation to PNs is unchanged. Furthermore, we have found a decrease PV+ IN density in the deep pyramidal layer of CA2, where SuM axons are localized. Thus, the ability of SuM inputs to convey social novelty information to the hippocampus is likely compromised by the change in PV+ IN function, leading to compromised social memory.

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BOARD NUMBER: S01-402

DUAL ROLE OF C-TERMINAL BINDING PROTEIN IN NEURODEVELOPMENT AND SYNAPTIC PLASTICITY

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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C-terminal binding protein (CtBP1) is a ubiquitously expressed, NAD+/NADH-dependent, transcriptional corepressor protein that shuttles between nucleus and pre-synapse in neurons. CtBP1 regulates gene expression in the nucleus, while it regulates synaptic recycling in the presynaptic region. It has been shown that CtBP1 also regulates cell cycle progression and cellular survival. Specifically, its deficiency causes sensitivity to neurotoxicity in hippocampal and cortical neurons due to increased cell apoptosis and its absence causes a decrease in pluripotency in human embryonic stem cells (hESCs). However, the molecular phenomenon behind this functional dualism remains unclear. Previously a human *de novo* CtBP1^{R331W} mutation has been linked to HADDTS syndrome characterized by mental retardation, ataxia, and hypotonia. To elucidate the role of CtBP1 in the regulation of neurodevelopment and synaptic plasticity, we established hESCs carrying the CtBP1^{R331W} mutation using CRISPR-Cas9 mediated gene editing. Using these cells, we model the disease phenotype in parallel to CtBP1 KO mice studies. In the disease model, we analyze the transcriptional changes in development- and plasticity-related molecular pathways, neuronal architecture, and synaptic plasticity. Besides, studies including extracellular electrophysiology and transcriptional analysis are being done in hippocampus from CtBP1 KO. Our preliminary data show that CtBP1^{R331W} mutation causes an aberrancy in the transcriptional profile in hESC-derived neurons throughout the development, and loss of CtBP1 causes parallel changes in mice hippocampus together with an impaired LTP phenotype and loss of resilience to stress.

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MATERNAL HIGH-FAT DIET CONSUMPTION DURING PREGNANCY AND LACTATION IMPAIRS THE INHIBITORY SYNAPTIC TRANSMISSION IN HIPPOCAMPAL PYRAMIDAL NEURONS OF THE YOUNG MOUSE OFFSPRING.

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Obesity has become a health problem worldwide. In general, the prevalence of overweight and obesity is higher in women than in men. The correlation between the increase in female obesity, especially at childbearing ages, and the increase in the incidence of metabolic and neurological diseases in both children and adolescents, shows the relevance of maternal nutrition and obesity on neurodevelopment and its cognitive effects in youth and adulthood. The hippocampus is important for learning and memory, and its development is sensitive to the metabolic environment in utero. Recently, it has been suggested that fetal exposure to maternal obesity causes decreased neurogenesis and impaired hippocampal learning, but the implications at the level of synaptic physiology have not been examined in detail. Our main aim was to study whether this adverse prenatal environment impairs hippocampal inhibitory synaptic transmission using a model of maternal obesity induced by a high-fat diet (HFD, 60% Kcal from fat) consumption. We performed electrophysiological recordings in the hippocampal CA1 region of juvenile mouse offspring. We observed that maternal HFD consumption increases the amplitude of inhibitory postsynaptic currents, but not the frequency or release probability. We also found that maternal HFD does not change the membrane passive properties of the CA1 pyramidal neurons. These data suggest that maternal obesity can increase the hippocampal inhibitory efficacy, and this could have important consequences in the excitation/inhibition balance, being able to modify the hippocampal cognitive function in offspring mice.

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NMDA RECEPTOR HYPOFUNCTION DURING ADOLESCENCE REDUCES GABAERGIC EFFICACY AND ADULT NEUROGENESIS IN THE DORSAL DENTATE GYRUS OF ADULT MICE.

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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During adolescence, the maturational processes occurring in the brain are highly sensitive to environmental factors, making it a high-risk stage for the onset of neuropsychiatric diseases such as schizophrenia (SZ). The pathophysiology of SZ has been associated with alterations related to GABAergic interneurons, mainly those expressing parvalbumin protein (PV-INs). In our laboratory, using an animal model such as SZ by ketamine treatment in adolescents, we observed a strong decrease in the number of PV-INs and GABA transmission in the prefrontal cortex, but not in the ventral hippocampus. Considering that hippocampal function varies along its dorsal-ventral axis and that PV-INs in the dentate gyrus (DG) are important in adult neurogenesis, the main objective of this study was to evaluate whether ketamine treatment in adolescence alters GABAergic transmission and adult neurogenesis in the dorsal DG (dDG) of mice. To test this possibility, we used an immunofluorescent and electrophysiological approach in the dDG of adult mice. Our results showed that adolescent ketamine treatment induced a morphofunctional alteration of PV-INs and reduced GABAergic transmission in the dentate granule cell (DGC), which induces a change in the excitation-inhibition balance in the dDG. Furthermore, we found that this treatment reduced the rate of adult neurogenesis in the DG. These results suggest that GABAergic transmission plays an important role in synaptic and structural plasticity and that the hypofunction of PV-INs in adult dDG could be key to understanding cognitive impairment associated with SZ.

Pubmed:

31481877: Pérez MÁ, Morales C, Santander O, García F, Gómez I, Peñaloza-Sancho V, Fuentealba P, Dagnino-Subiabre A, Moya PR, Fuenzalida M
Ketamine-Treatment During Late Adolescence Impairs Inhibitory Synaptic Transmission in the Prefrontal Cortex and Working Memory in Adult Rats.
Schizophrenia (SZ) is associated with changes in the structure and function of several brain areas. Several findings suggest that these impairments are related to a dysfunction in γ-aminobutyric acid (GABA) neurotransmission in brain areas such as the medial prefrontal cortex (mPFC), the hippocampus (HPC) and the primary auditory cortex (A1); however, it is still unclear how the GABAergic system is disrupted in these brain areas. Here, we examined the effect of ketamine (Ket) administration during late adolescence in rats on inhibition in the mPFC-, ventral HPC (vHPC), and A1. We observe that Ket treatment reduced the expression of the calcium-binding protein parvalbumin (PV) and the GABA-producing enzyme glutamic acid decarboxylase 67 (GAD67) as well as decreased inhibitory synaptic efficacy in the mPFC. In addition, Ket-treated rats performed worse in executive tasks that depend on the integrity and proper functioning of the mPFC. Conversely, we do not find such changes in vHPC or A1. Together, our results provide strong experimental support for the hypothesis that during adolescence, the function of the mPFC is more susceptible than that of HPC or A1 to NMDAR hypofunction, showing apparent structure specificity. Thus, the impairment of inhibitory circuitry in mPFC could be a convergent primary site of SZ-like behavior during the adulthood.
Front Cell Neurosci, 2019; 13
31252027: Valencia M, Illanes J, Santander O, Saavedra D, Adaros M, Ibarra A, Saavedra G, Pascual R
Environmental enrichment restores the reduced expression of cerebellar synaptophysin and the motor coordination impairment in rats prenatally treated with betamethasone.
Preterm babies treated with synthetic glucocorticoids in utero exhibit behavioural alterations and disturbances in brain maturation during postnatal life. Accordingly, it has been shown in preclinical studies that SGC exposure at a clinical dose

alters the presynaptic and postsynaptic structures and results in synaptic impairments. However, the precise mechanism by which SGC exposure impairs synaptic protein expression and its implications are not fully elucidated. Therefore, the purpose of this study was to investigate the effect of prenatal exposure to a clinical dose of betamethasone on the pre- and postsynaptic proteins expression in the developing rat cerebellum and prefrontal cortex, whose synchronized synaptic activity is crucial for motor control and learning. Consequently, the first objective of the present study was to determine whether prenatal betamethasone -equivalent to the clinically used dose- alters cerebellar vermal and cortical expression of synaptophysin, synaptotagmin I, post-synaptic density protein 95 and gephyrin - four important pre- and post-synaptic proteins, respectively- at a relevant adolescent stage. In addition, our second objective was to assess whether prenatal betamethasone administration induced coordination impairment using a rotarod test. On the other hand, it has been shown that the environmental enrichment is capable of improving synaptic transmission and recovering various behavioural impairments. Nevertheless, there is not enough information about the effect of this non-pharmacological preclinical approach on the regulation of this cerebellar and cortical synaptic proteins. Therefore, the third objective of this study was to examine whether environmental enrichment exposure could recover the possible molecular and behavioural impairments in the offspring at the same developmental stage. The principal data showed that adolescent rats prenatally treated with betamethasone exhibited underexpression of synaptophysin in the vermal cerebellum, but not change in levels of synaptotagmin I, post-synaptic density protein 95 and gephyrin. Analysis of the same pre- and post-synaptic proteins no showed differences in the frontal cortex of the same rats. These results were accompanied by an increase in the number of falls in the rotarod test, when the speed of rotation was fixed and when it was in acceleration, which means motor coordination impairments. Importantly, we found that environmental enrichment restores the betamethasone-induced reduction in the cerebellar synaptophysin together with a recover in the motor coordination impairments in prenatally betamethasone-exposed adolescent rats.
Physiol Behav, 2019; 209

28523300: Pascual R, Santander O, Cuevas I, Valencia M

Prenatal glucocorticoid administration persistently increased the immunohistochemical expression of type-1 metabotropic glutamate receptor and Purkinje cell dendritic growth in the cerebellar cortex of the rat.
Several studies have indicated that abnormal prenatal changes in the circulating glucocorticoids (GCs), induced by either maternal stress or exogenous GC administration, significantly alter the development of Purkinje cells (PCs). Among the suggested mechanisms that could mediate this GC-dependent PC susceptibility are changes in the expression of type-1 metabotropic glutamate receptors (mGluR1). In the current study, we analyzed whether a single course of prenatally administered betamethasone phosphate (BET) in pregnant rats increased the immunohistochemical expression of mGluR1 in PCs and decreased PC dendritic growth. The data obtained showed that in utero BET exposure resulted in a significant immunohistochemical overexpression of mGluR1 and a significant reduction in Purkinje cell dendritic outgrowth during postnatal life.
Rom J Morphol Embryol, 2017; 58

28203043: Pascual R, Cuevas I, Santander O, Valencia M

Influence of antenatal synthetic glucocorticoid administration on pyramidal cell morphology and microtubule-associated protein type 2 (MAP2) in rat cerebrocortical neurons.
Previous animal studies have indicated that excessive prenatal circulating glucocorticoid (GC) levels induced by the antenatal administration of synthetic GC (sGC) significantly alter neuronal development in the cerebellar and hippocampal neurons of the offspring. However, it is unknown whether antenatal sGC administration results in long-term neocortical pyramidal cell impairment. In the current study, we examined whether an equivalent therapeutic dose of antenatal betamethasone phosphate (BET) in pregnant rats alters the Golgi-stained basilar dendritic length and histochemical expression of dendritic microtubule-associated protein 2 (MAP2) of neocortical pyramidal cells in infant, adolescent, and young adult offspring. The results obtained showed that BET exposure resulted in a significant reduction in the basilar dendritic length per neuron and a transient reduction in histochemical MAP2 immunoreactivity. Consistent with previous hippocampal and cerebellar data, the present findings suggest that prenatal BET administration alters the dendritic growth of cerebrocortical pyramidal cells.
Clin Pediatr Endocrinol, 2017; 26

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BOARD NUMBER: S01-405

BRAIN ESTROGEN SYNTHESIS BY AROMATASE REGULATES SYNAPTIC INHIBITION IN THE FEMALE HIPPOCAMPUS

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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In addition to ovarian production, estrogens are synthesized within the brain by neurons that express the enzyme aromatase. In the hippocampus, neuron-derived estrogen regulates excitatory synaptic function and plasticity. In addition to excitatory neurons, the hippocampus is populated by a diverse array of inhibitory neurons (INs). INs regulate synaptic plasticity and organize several forma of hippocampal network activity. Despite their critical role for hippocampal function, the impact of brain-derived estrogen on INs physiology is unknown. Here, we present evidence supporting estrogen (17ß-estradiol) synthesis in female CA1 parvalbumin (PV) expressing neurons, a mayor class of hippocampal INs. Moreover, our *patch-clamp* experiments revealed that brain aromatase regulates CA1 synaptic inhibition through a mechanism that involves modification of perineuronal nets enwrapping PV INs. Interestingly, this regulation of CA1 inhibition by estrogen synthesis is female-specific, determined by the perinatal gonadal hormone milieu and independent of the genetic sex of the brain. *In vivo* fiber photometry experiments revealed that estrogen modulates the activity of PV INs and their coupling to animal behavior. Our results reveal sex differences in hippocampal inhibition that may be related to the female-specific regulation of PV INs by brain synthesized estrogen. Since aromatase inhibitors are widely used in clinics, our results have implications for understanding their effects on cognitive functions in humans.

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BOARD NUMBER: S01-406

SYNAPTIC COMMUNICATION WITHIN THE MICROCIRCUITS OF PYRAMIDAL NEURONS AND BASKET CELLS IN THE MOUSE PREFRONTAL CORTEX

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Parvalbumin- and cholecystokinin-containing basket cells (PVBCs and CCKBCs) are GABAergic interneurons that are able to efficiently control the spiking of their postsynaptic partners. Here, we investigated the intrinsic electrophysiological features and synaptic properties within the networks of basket cells and pyramidal neurons (PNs) in the mouse medial prefrontal cortex (mPFC) to determine the organizational principles of their microcircuits. *In vitro* whole-cell recordings were performed in brain slices prepared from transgenic mice that allowed us to target basket cells. Single-cell features of these interneurons showed major differences regarding both their active and passive membrane properties. By performing paired recordings we uncovered that PVBCs receive larger and faster unitary excitatory postsynaptic currents from PNs than CCKBCs and that PVBCs provide stronger and more reliable synaptic inputs onto PNs than the other basket cell type. In addition, the amplitude of unitary postsynaptic responses between PVBCs were found to be larger than those observed between CCKBCs. Short term-dynamics of connections were also characteristic for the cell type of synaptic partners. Finally, our anatomical data together with electrophysiological recordings combined with pharmacology and optogenetics revealed that there is synaptic communication between the two types of basket cells. Our data provide the first detailed description of connectivity within the networks of pyramidal neurons and basket cells in the mPFC. These results imply that PVBCs may have a larger impact at the microcircuit level than CCKBCs, thus, smaller changes in PVBC function can substantially contribute to altered circuit operation in the mPFC, often linked to psychiatric diseases.

Pubmed:
33837051: Vereczki VK, Müller K, Krizsán É, Máté Z, Fekete Z, Rovira-Esteban L, Veres JM, Erdélyi F, Hájos N
Total Number and Ratio of GABAergic Neuron Types in the Mouse Lateral and Basal Amygdala.
GABAergic neurons are key circuit elements in cortical networks. Despite growing evidence showing that inhibitory cells play a critical role in the lateral (LA) and basal (BA) amygdala functions, neither the number of GABAergic neurons nor the ratio of their distinct types has been determined in these amygdalar nuclei. Using unbiased stereology, we found that the ratio of GABAergic neurons in the BA (22%) is significantly higher than in the LA (16%) in both male and female mice. No difference was observed between the right and left hemispheres in either sex. In addition, we assessed the ratio of the major inhibitory cell types in both amygdalar nuclei. Using transgenic mice and a viral strategy for visualizing inhibitory cells combined with immunocytochemistry, we estimated that the following cell types together compose the vast majority of GABAergic cells in the LA and BA: axo-axonic cells (5.5%-6%), basket cells expressing parvalbumin (17%-20%) or cholecystokinin (7%-9%), dendrite-targeting inhibitory cells expressing somatostatin (10%-16%), NPY-containing neurogliaform cells (14%-15%), VIP and/or calretinin-expressing interneuron-selective interneurons (29%-38%), and GABAergic projection neurons expressing somatostatin and neuronal nitric oxide synthase (5.5%-8%). Our results show that these amygdalar nuclei contain all major GABAergic neuron types as found in other cortical regions. Furthermore, our data offer an essential reference for future studies aiming to reveal changes in GABAergic cell number and in inhibitory cell types typically observed under different pathologic conditions, and to model functioning amygdalar networks in health and disease. GABAergic cells in cortical structures, as in the lateral and basal nucleus of the amygdala, have a determinant role in controlling circuit operation. In this study, we provide the first estimate for the total number of inhibitory cells in these two amygdalar nuclei. In addition, our study is the first to define the ratio of the major GABAergic cell types present in these cortical networks. Taking into account that hyperexcitability in the amygdala, arising from the imbalance between excitation and inhibition typifies many altered brain functions, including anxiety, post-traumatic stress disorder, schizophrenia, and autism, uncovering the number and ratio of distinct amygdalar inhibitory cell types offers a solid base for comparing the changes in inhibition in pathologic brain states.
J Neurosci, 2021; 41


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BOARD NUMBER: S01-407

HOW INHIBITION OF CHOLINERGIC INTERNEURONS IMPACTS CORTICOSTRIATAL TRANSMISSION AND MOTOR LEARNING IN PARKINSONIAN CONDITON

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Striatal cholinergic interneurons (CINs) respond to salient or reward prediction-related stimuli after conditioning with brief pauses in their activity, implicating them in learning and action selection. This pause is lost in animal models of Parkinson's disease. How this signal regulates the functioning of the striatum remains an open question. To address this issue, we examined the impact of CIN firing inhibition on glutamatergic transmission between the cortex and the medium-sized spiny projection neurons expressing dopamine D1 receptors (D1 MSNs). Brief interruption of CIN activity had no effect in control condition whereas it increased glutamatergic responses in D1 MSNs after nigrostriatal dopamine denervation. This potentiation was dependent upon M4 muscarinic receptor and protein kinase A. Decreasing CIN firing by opto/chemogenetic strategies *in vivo* rescued long-term potentiation in some MSNs and alleviated motor learning deficits in parkinsonian mice. Taken together, our findings demonstrate that the control exerted by CINs on corticostriatal transmission and striatal-dependent motor-skill learning depends on the integrity of dopaminergic inputs.

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CONTROL OF NEOCORTICAL MEMORY BY LONG-RANGE INHIBITION IN LAYER 1

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Mounting evidence identifies layer 1 (L1) as a central site of memory in sensory neocortex. While this work revealed plasticity in several excitatory brain-wide afferent systems, the existence, connectivity and memory-related signaling of long-range inhibitory input to L1 remains elusive. We report that inhibitory afferents from zona incerta project specifically to auditory cortex L1, where they connect selectively to interneurons to disinhibit the cortical circuit and facilitate behavioral memory. Chronic calcium imaging of these synapses identifies a balanced form of plasticity that develops rapidly during threat learning and is characterized by the *de novo* appearance of negative stimulus responses which transmit most information. Our results therefore pinpoint malleability of long-range (dis)inhibitory afferents to L1 as a key factor for the exquisite computational flexibility of this unique layer.

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BOARD NUMBER: S01-409

MOLECULAR MECHANISMS OF DRUG-INDUCED SYNAPTOGENESIS

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Relapse is a major challenge to the treatment of substance use disorder, mainly triggered by drug-associated memories, which contrary to physiological memories, exhibit an exceedingly rigid and long-lasting nature as a product of maladaptive plasticity. Repetitive cocaine exposure generates *de novo* silent synapses in the nucleus accumbens, whose maturation underlies drug-associated behaviors. As silent synapses provide novel synaptic opportunities, we hypothesize that drug-induced synaptogenesis reorganizes neural networks to change the connection patterns morphologically and functionally, and as a result encode drug-associated memories with rigid and long-lasting features. Using the electrophysiology procedure of minimal stimulation, we describe repetitive cocaine exposure has more generalized implications than previously reported, as we found that silent synapses are generated in additional brain areas, including the dorsal striatum. Obtaining a blueprint of the molecular mechanisms underlying silent synapse generation will help to unveil the consequences of silent synapse-based network reorganization in substance use disorders. We report that viral vector-mediated knockdown of α2δ1 and integrin-β3 prevented the synaptogenic effect of cocaine in the dorsal striatum, suggesting a glial-neuron signaling pathway for the generation of silent synapses.

Pubmed:
31896673: Diaz-Aparicio I, Paris I, Sierra-Torre V, Plaza-Zabala A, Rodríguez-Iglesias N, Márquez-Ropero M, Beccari S, Huguet P, Abiega O, Alberdi E, Matute C, Bernales I, Schulz A, Otrokocsi L, Sperlagh B, Happonen KE, Lemke G, Maletic-Savatic M, Valero J, Sierra A
Microglia Actively Remodel Adult Hippocampal Neurogenesis through the Phagocytosis Secretome.
During adult hippocampal neurogenesis, most newborn cells undergo apoptosis and are rapidly phagocytosed by resident microglia to prevent the spillover of intracellular contents. Here, we propose that phagocytosis is not merely passive corpse removal but has an active role in maintaining neurogenesis. First, we found that neurogenesis was disrupted in male and female mice chronically deficient for two phagocytosis pathways: the purinergic receptor P2Y12, and the tyrosine kinases of the TAM family Mer tyrosine kinase (MerTK)/Axl. In contrast, neurogenesis was transiently increased in mice in which MerTK expression was conditionally downregulated. Next, we performed a transcriptomic analysis of the changes induced by phagocytosis in microglia and identified genes involved in metabolism, chromatin remodeling, and neurogenesis-related functions. Finally, we discovered that the secretome of phagocytic microglia limits the production of new neurons both and Our data suggest that microglia act as a sensor of local cell death, modulating the balance between proliferation and survival in the neurogenic niche through the phagocytosis secretome, thereby supporting the long-term maintenance of adult hippocampal neurogenesis. Microglia are the brain professional phagocytes and, in the adult hippocampal neurogenic niche, they remove newborn cells naturally undergoing apoptosis. Here we show that phagocytosis of apoptotic cells triggers a coordinated transcriptional program that alters their secretome, limiting neurogenesis both and In addition, chronic phagocytosis disruption in mice deficient for receptors P2Y12 and MerTK/Axl reduces adult hippocampal neurogenesis. In contrast, inducible MerTK downregulation transiently increases neurogenesis, suggesting that microglial phagocytosis provides a negative feedback loop that is necessary for the long-term maintenance of adult hippocampal neurogenesis. Therefore, we speculate that the effects of promoting engulfment/degradation of cell debris may go beyond merely removing corpses to actively promoting regeneration in development, aging, and neurodegenerative diseases.
J Neurosci, 2020; 40

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STRAIN-DEPENDENT EFFECTS OF PHYSICAL EXERCISE ON MITOCHONDRIAL, NEUROTROPHIC AND NEUROGENIC MEASURES IN THE BRAIN IN C57BL/6J AND C57BL/6N MICE

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Physical exercise evokes neurotrophic and neurogenic changes in specific parts of the central nervous system and evokes bioenergetic changes involving mitochondria. These changes have been reported in diverse rodent models but it is unclear whether genetic background plays a role in modulating the nature, pattern, and magnitude of effects evoked by exercise. In the present study, we look at two widely used strains of C57BL/6 mice namely C57BL/6J (B6J) and C57BL/6N (B6N), with the former carrying a null mutation in the *NNT* gene (nicotinamide nucleotide transhydrogenase). NNT is an essential mitochondrial redox regulatory protein and its absence in B6J mice leads to a metabolic profile distinct from B6N mice. Hence, we investigated whether the effects of seven days of voluntary wheel running on mitochondrial, neurotrophic, and neurogenic measures in limbic brain circuits are different, based on the strain of C57BL/6 mice used. We find that while both B6J and B6N mice show similar wheel running activity and weight profile changes, there are multiple distinct strain-specific effects. In the hippocampus, we see an improvement in mitochondrial biogenesis and function in B6J but not in B6N, as assessed by mitochondrial-DNA copy number, ATP levels, and gene expression changes. Further, we see a differential extent of upregulation in growth factor expression and adult neurogenesis in the hippocampus, between B6J and B6N in response to exercise. These results highlight the importance of being watchful of strain differences in animal models while interpreting data across studies and to keep in mind the genetic background of knockout mouse lines, several of which have been backcrossed onto C57BL/6J.

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BOARD NUMBER: S01-411

PLACE-FIELD DYNAMICS AS A WINDOW ON SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Despite decades of research, the rules of synaptic plasticity governing hippocampal representations during memory formation and familiarization remain unclear. We considered the trial-by-trial dynamics of these representations as an indirect indicator of ongoing plasticity. Using 2-photon calcium imaging in mice navigating through virtual environments, we recorded CA1 and CA3 pyramidal neurons and measured how the spatial modulation of their activity (place fields) evolved lap-by-lap[1]. During exploration of a novel environment, a majority of CA1 place fields (less in CA3) continuously shifted, mostly backwards. The proportion of shifting cells and the shift speeds decreased with the level of familiarity to the environment. To determine the mechanisms supporting these shifting dynamics, we designed computational models of spiking neurons with synapses following different plasticity rules. In contrast to early models, we found that classic Hebbian spike-timing-dependent-plasticity is too weak and has too short of a timescale to produce strong backward shifting, especially when realistic firing rates are maintained. The recently discovered Behavioral Timescale Synaptic Plasticity (BTSP), with its large amplitude and long time-constants, is an alternative candidate mechanism. Our model of BTSP combined with homeostatic synaptic normalization produces both forward and backward shifting dynamics that match our experimental data. Overall, our study suggests BTSP as the main plasticity mechanism underlying the dynamics of spatial representations in the hippocampus. Exploration of our model's parameter space and mining of our experimental data for BTSP signatures will refine our understanding of the phenomenology of BTSP during memory formation and familiarization. 1-Dong, Madar & Sheffield, (2021) https://doi.org/10.1038/s41467-021-23260-3

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BOARD NUMBER: S01-412

MOLECULAR SIGNATURES OF THE INTERACTION OF KARS WITH G-PROTEINS

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Kainate receptors (KARs) are found ubiquitously in the CNS and are present presynaptically and postsynaptically regulating synaptic transmission and excitability. Functional studies have proven that KARs act as ion channels as well as potentially activating G-proteins, thus indicating the existance of a dual signaling system for KARs. Previous studies in our laboratory demonstrated a direct interaction of the $GluK1_{2b}$ subunit of KARs with the Gαo-protein, showing that GluK1 and Go proteins are natural partners, accounting for the metabotropic effects of KARs. In this context we have focused on studying which part of the carboxy-terminal domain (CTD) of the $GluK1_{2b}$ subunit is important in this interaction. The CTD of the $GluK1_{2b}$ subunit has a region enriched in Lysine (K) and Arginine (R), similar to that identified as critical for the coupling of muscarinic receptors to G-proteins. Therefore, we generated several $GluK1_{2b}$ subunit mutants, which either lack a part of the CTD (Δ930, Δ914, Δ900) o have the positively charged K an R residues replaced by Alanines (A) and assessed whether the interaction with Gαo was prevented using Biomolecular fluorescence complementation (BiFC). We observed that all mutant forms lacking the positively-charged-poly K-R region (($GluK1_{2b}$, Δ914, Δ900) have reduced interaction with Gαo. Further, the complete substitution of K's and R's in the 15-amino acids-stretch ($GluK1_{2b}$ 914-928K-A), results in a reduction of this interaction to a similar extent as when the entire CTD was removed, supporting the critical role of these residues in the GluK1 to Gαo coupling.

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CONFORMATIONAL-DEPENDENT NANOCLUSTERING OF FYN KINASE CONTROLS INTRACELLULAR SIGNALLING EVENTS IMPLICATED IN FRONTOTEMPORAL DEMENTIA AND ALZHEIMER'S DISEASE

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Fyn is a Src family kinase implicated in learning and memory and is responsible for controlling signalling cascades in neurons. The postsynaptic enrichment of Fyn underpins synaptotoxicity in Alzheimer's Disease (AD) and frontotemporal lobar degeneration (FTLD). Expression of pathogenic FTLD tau promotes nanoclustering of Fyn in the dendrites and spines of hippocampal neurons (Padmanabhan et al., 2019). However, the mechanism underpinning such clustering and the link to altered signalling is unknown. Here, we used single particle tracking photoactivated localization microscopy (sptPALM) to demonstrate that the transition of Fyn into an open, primed conformation promotes nanoclustering in dendritic spines of live hippocampal neurons. Entry into an open conformation was achieved by disrupting the auto-inhibitory, closed conformation of Fyn through phospho-inhibition (Y531F) and perturbing Fyn's SH3 domain. We demonstrate that the resulting entry into an open conformation is required to initiate the Fyn/ERK/S6 signalling cascade, a pathway implicated in AD-related neurodegeneration and synaptotoxicity (Li et al., 2017). In isolation, alteration of Fyn's catalytic SH1 domain through pharmacological and phospho-inhibition (Y420F) did not affect Fyn mobility. Together these findings demonstrate that Fyn fluctuates between a closed and an open conformation, the latter being enzymatically active and conducive to nanoclustering. Our results highlight a tantalizing role of nanoclustering in promoting intracellular signalling and suggest that long term alterations of Fyn clustering and ensued intracellular signalling could contribute to the development of AD and FTLD.
Padmanabhan et al., 2019 - ELife Li & Götz., 2017 - Embo J

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BOARD NUMBER: S01-414

THE ROLE OF ENDOPLASMIC RETICULUM IN DENDRITIC SPINE INDIVIDUALIZATION

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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The ability of dendritic spines to individualize from the rest of the neuron is a key feature in synapse-specific functions, but the underlying mechanisms are unclear. The endoplasmic reticulum (ER) is the largest cellular organelle with key functions in protein and calcium homeostasis as well as compartmentalization of biochemical reactions. In neurons, ER forms a continuous network that has been reported to localize to a subset of dendritic spines, but its functions in spines remain poorly understood. Here we combined diverse imaging methods to study the potential compartmentalization of ER in dendritic spines and its role in spine individualization. Interestingly, our fluorescence loss in photobleaching measurements show that in a subset of spines, the ER is compartmentalized. Approximately 30% of the ER-containing spines displayed restricted diffusion of ER-bound proteins between the spine and dendritic ER, while in 70% of the ER-containing spines, diffusion was rapid and non-restricted (average half-lives 480 s and 6 s, respectively). Importantly, preliminary results indicate that the compartmentalization of spine ER potentiates synaptic calcium signaling. Spines with restricted diffusion of ER-bound proteins showed larger increase in synaptic calcium levels after glutamate uncaging than spines with non-compartmentalized ER or spines without ER. Together, these results suggest that the diffusion of ER-bound molecules at the synapses can be constricted, which may underlie synaptic individualization and plasticity in synaptic function.

Pubmed:
30795565: Kontturi LS, van den Dikkenberg J, Urtti A, Hennink WE, Mastrobattista E
Light-Triggered Cellular Delivery of Oligonucleotides.
The major challenge in the therapeutic applicability of oligonucleotide-based drugs is the development of efficient and safe delivery systems. The carriers should be non-toxic and stable in vivo, but interact with the target cells and release the loaded oligonucleotides intracellularly. We approached this challenge by developing a light-triggered liposomal delivery system for oligonucleotides based on a non-cationic and thermosensitive liposome with indocyanine green (ICG) as photosensitizer. The liposomes had efficient release properties, as 90% of the encapsulated oligonucleotides were released after 1-minute light exposure. Cell studies using an enhanced green fluorescent protein (EGFP)-based splicing assay with HeLa cells showed light-activated transfection with up to 70%⁻80% efficacy. Moreover, free ICG and oligonucleotides in solution transfected cells upon light induction with similar efficacy as the liposomal system. The light-triggered delivery induced moderate cytotoxicity (25%⁻35% reduction in cell viability) 1⁻2 days after transfection, but the cell growth returned to control levels in 4 days. In conclusion, the ICG-based light-triggered delivery is a promising method for oligonucleotides, and it can be used as a platform for further optimization and development.
Pharmaceutics, 2019; 11
25460143: Kontturi LS, Collin EC, Murtomäki L, Pandit AS, Yliperttula M, Urtti A
Encapsulated cells for long-term secretion of soluble VEGF receptor 1: Material optimization and simulation of ocular drug response.
Anti-angiogenic therapies with vascular endothelial growth factor (VEGF) inhibiting factors are effective treatment options for neovascular diseases of the retina, but these proteins can only be delivered as intravitreal (IVT) injections. To sustain a therapeutic drug level in the retina, VEGF inhibitors have to be delivered frequently, every 4-8weeks, causing inconvenience for the patients and expenses for the healthcare system. The aim of this study was to investigate cell encapsulation as a delivery system for prolonged anti-angiogenic treatment of retinal neovascularization. Genetically engineered ARPE-19 cells secreting soluble vascular endothelial growth factor receptor 1 (sVEGFR1) were encapsulated in a hydrogel of cross-linked collagen and interpenetrating hyaluronic acid (HA). The system was optimized in terms of matrix composition and cell density, and long-term cell viability and protein secretion measurements were performed. sVEGFR1 ARPE-19 cells in the optimized hydrogel remained viable and secreted sVEGFR1 at a constant rate for at least 50days. Based on pharmacokinetic/pharmacodynamic (PK/PD) modeling, delivery of sVEGFR1 from this cell encapsulation system is expected to lead only to modest VEGF inhibition, but improvements of the protein structure and/or secretion rate should result in strong

and prolonged therapeutic effect. In conclusion, the hydrogel matrix herein supported the survival and protein secretion from the encapsulated cells. The PK/PD simulation is a convenient approach to predict the efficiency of the cell encapsulation system before in vivo experiments.
Eur J Pharm Biopharm, 2015; 95
25786729: Kontturi LS, Järvinen E, Muhonen V, Collin EC, Pandit AS, Kiviranta I, Yliperttula M, Urtti A
An injectable, in situ forming type II collagen/hyaluronic acid hydrogel vehicle for chondrocyte delivery in cartilage tissue engineering.
In this study, chondrocytes were encapsulated into an injectable, in situ forming type II collagen/hyaluronic acid (HA) hydrogel cross-linked with poly(ethylene glycol) ether tetrasuccinimidyl glutarate (4SPEG) and supplemented with the transforming growth factor β1 (TGFβ1). The chondrocyte-hydrogel constructs were cultured in vitro for 7 days and studied for cell viability and proliferation, morphology, glycosaminoglycan production, and gene expression. Type II collagen/HA/4SPEG formed a strong and stable hydrogel, and the chondrocytes remained viable during the encapsulation process and for the 7-day culture period. In addition, the encapsulated cells showed spherical morphology characteristic for chondrocytic phenotype. The cells were able to produce glycosaminoglycans into their extracellular matrix, and the gene expression of type II collagen and aggrecan, genes specific for differentiated chondrocytes, increased over time. The results indicate that the studied composite hydrogel with incorporated chondrogenic growth factor TGFβ1 is able to maintain chondrocyte viability and characteristics, and thus, it can be regarded as potential injectable cell delivery vehicle for cartilage tissue engineering.
Drug Deliv Transl Res, 2014; 4
21397645: Kontturi LS, Yliperttula M, Toivanen P, Määttä A, Määttä AM, Urtti A
A laboratory-scale device for the straightforward production of uniform, small sized cell microcapsules with long-term cell viability.
Microencapsulated and genetically engineered cells may be used for prolonged delivery of therapeutically active proteins. The objective of this study was to develop a simple, inexpensive and flexible laboratory-scale device for the production of cell microcapsules, especially capsules of small diameter (<300 μm). Many microencapsulation devices are expensive, difficult to assemble and to use, and often more suitable for large-scale experiments. However, the simplicity and low price of the encapsulation system should not limit the quality of capsules and reproducibility of the process: for successful in vitro and in vivo experiments it is important to be able to produce uniform, spherical microcapsules without deformities with high reproducibility. In addition, an advantage of the present procedure compared to other similar, co-axial laminar gas flow systems is the possibility to produce also small microcapsules, less than 200 μm in diameter, with narrow size distribution. First, design, optimization and reproducibility testing of this custom-built device were carried out. Second, microencapsulated retinal pigment epithelial cells (ARPE-19) capable of secreting soluble vascular endothelial growth factor receptor 1 (sVEGFR1) were engineered. The cells remained viable in alginate-poly-L-lysine-alginate microcapsules and secreted sVEGFR1 for prolonged periods.
J Control Release, 2011; 152
21163615: Kontturi LS, Aalto AJ, Wallner M, Uusi-Oukari M
The cerebellar GABAAR α6-R100Q polymorphism alters ligand binding in outbred Sprague-Dawley rats in a similar manner as in selectively bred AT and ANT rats.
The alcohol-tolerant AT and alcohol-nontolerant ANT rat lines have been selectively bred for innate sensitivity to ethanol-induced motor impairment. The cerebellar GABAA receptor (GABAAR) α6 subunit alleles α6-100R and α6-100Q are segregated in the AT and ANT rats, respectively. This α6 polymorphism might explain various differences in pharmacological properties and density of GABAARs between the rat lines. In the present study, we have used nonselected outbred Sprague-Dawley rats homozygous for the α6-100RR (RR) and α6-100QQ (QQ) genotypes to show that these RR and QQ rats display similar differences between genotypes as AT and ANT rat lines. The genotypes differed in their affinity for [3H]Ro 15-4513 and classic benzodiazepines (BZs) to cerebellar "diazepam-insensitive" (DZ-IS) binding sites, in density of cerebellar [3H]muscimol binding and in the antagonizing effect of furosemide on GABA-induced inhibition of [3H]EBOB binding. The results suggest the involvement of α6-R100Q polymorphism in these line differences and in the differences previously found between AT and ANT rats. In addition, the α6-R100Q polymorphism induces striking differences in [3H]Ro 15-4513 binding kinetics to recombinant α6β3γ2s receptors and cerebellar DZ-IS sites. Association of [3H]Ro 15-4513 binding was ~10-fold faster and dissociation was ~3-4-fold faster in DZ-IS α6βγ2 receptors containing the α6-100Q allele, with a resulting change of ~2.5-fold in equilibrium dissociation constant (KD). The results indicate that in addition to the central role of the homologous α6-100R/Q (α1-101H) residue in BZ binding and efficacy, this critical BZ binding site residue has a major impact on BZ binding kinetics.
Alcohol, 2011; 45
31552691: Salonius E, Kontturi L, Laitinen A, Haaparanta AM, Korhonen M, Nystedt J, Kiviranta I, Muhonen V
Chondrogenic differentiation of human bone marrow-derived mesenchymal stromal cells in a three-dimensional environment.

Cell therapy combined with biomaterial scaffolds is used to treat cartilage defects. We hypothesized that chondrogenic differentiation bone marrow-derived mesenchymal stem cells (BM-MSCs) in three-dimensional biomaterial scaffolds would initiate cartilaginous matrix deposition and prepare the construct for cartilage regeneration in situ. The chondrogenic capability of human BM-MSCs was first verified in a pellet culture. The BM-MSCs were then either seeded onto a composite scaffold rhCo-PLA combining polylactide and collagen type II (C2) or type III (C3), or commercial collagen type I/III membrane (CG). The BM-MSCs were either cultured in a proliferation medium or chondrogenic culture medium. Adult human chondrocytes (ACs) served as controls. After 3, 14, and 28 days, the constructs were analyzed with quantitative polymerase chain reaction and confocal microscopy and sulfated glycosaminoglycans (GAGs) were measured. The differentiated BM-MSCs entered a hypertrophic state by Day 14 of culture. The ACs showed dedifferentiation with no expression of chondrogenic genes and low amount of GAG. The CG membrane induced the highest expression levels of hypertrophic genes. The two different collagen types in composite scaffolds yielded similar results. Regardless of the biomaterial scaffold, culturing BM-MSCs in chondrogenic differentiation medium resulted in chondrocyte hypertrophy. Thus, caution for cell fate is required when designing cell-biomaterial constructs for cartilage regeneration.
J Cell Physiol, 2020; 235
30841717: Galli E, Lindholm P, Kontturi LS, Saarma M, Urtti A, Yliperttula M
Characterization of CDNF-Secreting ARPE-19 Cell Clones for Encapsulated Cell Therapy.
Cerebral dopamine neurotrophic factor (CDNF) shows beneficial effects in rodent models of Parkinson's and Alzheimer's disease. The brain is a challenging target for protein therapy due to its exclusive blood-brain barrier. Hence, the therapeutic protein should be delivered directly to the brain parenchyma. Implantation of encapsulated mammalian cells that constantly secrete CDNF is a potential approach for targeted and long-term protein delivery to the brain. In this study, we generated several CDNF-secreting cell clones derived from human retinal pigment epithelial cell line ARPE-19, and studied CDNF secretion from the clones maintained as monolayers and in polymeric microcapsules. The secretion of wild type (wt) CDNF transgene was low and the majority of the produced protein remained intracellular, locating mainly to the endoplasmic reticulum (ER). The secretion of wtCDNF decreased to even lower levels when the clones were in a non-dividing state, as in the microcapsules. Both codon optimization and deletion of the putative ER-retrieval signal (four last amino acids: KTEL) improved CDNF secretion. More importantly, the secretion of KTEL-deleted CDNF remained constant in the non-dividing clones. Thus, cells expressing KTEL-deleted CDNF, in contrast to wtCDNF, can be considered for cell encapsulation applications if the KTEL-deleted CDNF is proven to be biologically active .
Cell Transplant, 2019; 28
27565215: Lajunen T, Nurmi R, Kontturi L, Viitala L, Yliperttula M, Murtomäki L, Urtti A
Light activated liposomes: Functionality and prospects in ocular drug delivery.
Ocular drug delivery, especially to the retina and choroid, is a major challenge in drug development. Liposome technology may be useful in ophthalmology in enabling new routes of delivery, prolongation of drug action and intracellular drug delivery, but drug release from the liposomes should be controlled. For that purpose, light activation may be an approach to release drug at specified time and site in the eye. Technical advances have been made in the field of light activated drug release, particularly indocyanine green loaded liposomes are a promising approach with safe materials and effective light triggered release of small and large molecules. This review discusses the liposomal drug delivery with light activated systems in the context of ophthalmic drug delivery challenges.
J Control Release, 2016; 244
27097108: Lajunen T, Kontturi LS, Viitala L, Manna M, Cramariuc O, Róg T, Bunker A, Laaksonen T, Viitala T, Murtomäki L, Urtti A
Indocyanine Green-Loaded Liposomes for Light-Triggered Drug Release.
Light-triggered drug delivery systems enable site-specific and time-controlled drug release. In previous work, we have achieved this with liposomes containing gold nanoparticles in the aqueous core. Gold nanoparticles absorb near-infrared light and release the energy as heat that increases the permeability of the liposomal bilayer, thus releasing the contents of the liposome. In this work, we replaced the gold nanoparticles with the clinically approved imaging agent indocyanine green (ICG). The ICG liposomes were stable at storage conditions (4-22 °C) and at body temperature, and fast near-infrared (IR) light-triggered drug release was achieved with optimized phospholipid composition and a 1:50 ICG-to-lipid molar ratio. Encapsulated small molecular calcein and FITC-dextran (up to 20 kDa) were completely released from the liposomes after light exposure for 15 s. Location of ICG in the PEG layer of the liposomes was simulated with molecular dynamics. ICG has important benefits as a light-triggering agent in liposomes: fast content release, improved stability, improved possibility of liposomal size control, regulatory approval to use in humans, and the possibility of imaging the in vivo location of the liposomes based on the fluorescence of ICG. Near-infrared light used as a triggering mechanism has good tissue penetration and safety. Thus, ICG liposomes are an attractive option for light-controlled and efficient delivery of small and large drug molecules.

Mol Pharm, 2016; 13
27089512: Viitala L, Pajari S, Lajunen T, Kontturi LS, Laaksonen T, Kuosmanen P, Viitala T, Urtti A, Murtomäki L
Photothermally Triggered Lipid Bilayer Phase Transition and Drug Release from Gold Nanorod and Indocyanine Green Encapsulated Liposomes.
In light-activated liposomal drug delivery systems (DDSs), the light sensitivity can be obtained by a photothermal agent that converts light energy into heat. Excess heat increases the drug permeability of the lipid bilayer, and drug is released as a result. In this work, two near-IR responsive photothermal agents in a model drug delivery system are studied: either gold nanorods (GNRs) encapsulated inside the liposomes or indocyanine green (ICG) embedded into the lipid bilayer. The liposome system is exposed to light, and the heating effect is studied with fluorescent thermometers: laurdan and CdSe quantum dots (QDs). Both photothermal agents are shown to convert light into heat in an extent to cause a phase transition in the surrounding lipid bilayer. This phase transition is also proven with laurdan generalized polarization (GP). In addition to the heating results, we show that the model drug (calcein) is released from the liposomal cavity with both photothermal agents when the light power is sufficient to cause a phase transition in the lipid bilayer.
Langmuir, 2016; 32

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BOARD NUMBER: S01-415

ROLE OF NEURON-DERIVED EXTRACELLULAR VESICLES IN SYNAPTIC PLASTICITY

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Neurodegenerative diseases induce an impairment in synaptic plasticity which eventually leads to cognitive symptoms in patients. Extracellular vesicles, which are involved in intercellular communication, have been suggested to be involved in synaptic processes, as they are carriers of bioactive miRNAs, proteins and lipids that can influence firing rate in recipient neurons. The aim of this study is to investigate whether neuronal EVs have a direct role in the regulation of synaptic plasticity. Extracellular vesicles were isolated from rat cortical neurons culture media, by ultracentrifugation, and used to treat sister cultures for 24h. Samples were subjected to immunohistochemistry, Western blotting and calcium imaging analysis. We found that EVs are taken up by neurons both in the soma and in dendrites, and even in synaptic spines. We found that neuronal EVs carry synaptic proteins and enhance the consolidation of glutamatergic synapses in recipient neurons. We also observed a mild effect of EVs over neural network dynamics. Moreover, EVs had a trophic effect in neurons under nutrient deprivation conditions. All these data put neuronal EVs in the spotlight to understand synaptic plasticity impairment in neurodegenerative conditions and use them as a possible therapeutic approach.

Pubmed:
35053183: Pérez-Sisqués L, Solana-Balaguer J, Campoy-Campos G, Martín-Flores N, Sancho-Balsells A, Vives-Isern M, Soler-Palazón F, Garcia-Forn M, Masana M, Alberch J, Pérez-Navarro E, Giralt A, Malagelada C
RTP801/REDD1 Is Involved in Neuroinflammation and Modulates Cognitive Dysfunction in Huntington's Disease.
RTP801/REDD1 is a stress-regulated protein whose levels are increased in several neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's diseases (HD). RTP801 downregulation ameliorates behavioral abnormalities in several mouse models of these disorders. In HD, RTP801 mediates mutant huntingtin (mhtt) toxicity in in vitro models and its levels are increased in human iPSCs, human postmortem putamen samples, and in striatal synaptosomes from mouse models of the disease. Here, we investigated the role of RTP801 in the hippocampal pathophysiology of HD. We found that RTP801 levels are increased in the hippocampus of HD patients in correlation with gliosis markers. Although RTP801 expression is not altered in the hippocampus of the R6/1 mouse model of HD, neuronal RTP801 silencing in the dorsal hippocampus with shRNA containing AAV particles ameliorates cognitive alterations. This recovery is associated with a partial rescue of synaptic markers and with a reduction in inflammatory events, especially microgliosis. Altogether, our results indicate that RTP801 could be a marker of hippocampal neuroinflammation in HD patients and a promising therapeutic target of the disease.
Biomolecules, 2021; 12
34131105: Pérez-Sisqués L, Sancho-Balsells A, Solana-Balaguer J, Campoy-Campos G, Vives-Isern M, Soler-Palazón F, Anglada-Huguet M, López-Toledano MÁ, Mandelkow EM, Alberch J, Giralt A, Malagelada C
RTP801/REDD1 contributes to neuroinflammation severity and memory impairments in Alzheimer's disease.
RTP801/REDD1 is a stress-regulated protein whose upregulation is necessary and sufficient to trigger neuronal death. Its downregulation in Parkinson's and Huntington's disease models ameliorates the pathological phenotypes. In the context of Alzheimer's disease (AD), the coding gene for RTP801, DDIT4, is responsive to Aβ and modulates its cytotoxicity in vitro. Also, RTP801 mRNA levels are increased in AD patients' lymphocytes. However, the involvement of RTP801 in the pathophysiology of AD has not been yet tested. Here, we demonstrate that RTP801 levels are increased in postmortem hippocampal samples from AD patients. Interestingly, RTP801 protein levels correlated with both Braak and Thal stages of

the disease and with GFAP expression. RTP801 levels are also upregulated in hippocampal synaptosomal fractions obtained from murine 5xFAD and rTg4510 mice models of the disease. A local RTP801 knockdown in the 5xFAD hippocampal neurons with shRNA-containing AAV particles ameliorates cognitive deficits in 7-month-old animals. Upon RTP801 silencing in the 5xFAD mice, no major changes were detected in hippocampal synaptic markers or spine density. Importantly, we found an unanticipated recovery of several gliosis hallmarks and inflammasome key proteins upon neuronal RTP801 downregulation in the 5xFAD mice. Altogether our results suggest that RTP801 could be a potential future target for theranostic studies since it could be a biomarker of neuroinflammation and neurotoxicity severity of the disease and, at the same time, a promising therapeutic target in the treatment of AD.
Cell Death Dis, 2021; 12
33984337: Pérez-Sisqués L, Martín-Flores N, Masana M, Solana-Balaguer J, Llobet A, Romaní-Aumedes J, Canal M, Campoy-Campos G, García-García E, Sánchez-Fernández N, Fernández-García S, Gilbert JP, Rodríguez MJ, Man HY, Feinstein E, Williamson DL, Soto D, Gasull X, Alberch J, Malagelada C
RTP801 regulates motor cortex synaptic transmission and learning.
RTP801/REDD1 is a stress-regulated protein whose upregulation is necessary and sufficient to trigger neuronal death in in vitro and in vivo models of Parkinson's and Huntington's diseases and is up regulated in compromised neurons in human postmortem brains of both neurodegenerative disorders. Indeed, in both Parkinson's and Huntington's disease mouse models, RTP801 knockdown alleviates motor-learning deficits.
Exp Neurol, 2021; 342

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STUDYING FUNDAMENTAL CONNECTIVITY PROPERTIES WITH LOW-DENSITY, NODE-BASED, IN VITRO NEURONAL NETWORKS

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Understanding information storage and processing in the brain remains one of the grand challenges in neuroscience. Reducing the complexity in the study of neuronal systems could help understanding fundamental properties of information processing in the brain. Low-density cultures of neurons with controlled topology presents a simple yet robust experimental platform for studying neuronal communication. The bottom-up approach has proved fruitful in investigating network's electrical properties wtih high spatiotemporal resolution [1], [2], [3]. To study mechanisms of spike-timing dependent plasticity in particular, we designed a PDMS microstructure consisting of two input nodes with axons guided symmetrically around the output node. To control number and location of synaptic connections, the nodes are interconnected with nanochannels, too narrow for axons but allowing for synapse formation [4]. We place the microstructures on top of microelectrode arrays (MEAs) and use a combination of stimulation and recording to analyze how the input-node activity affects the output node. Additionally, we use functional imaging to achieve a high spatial resolution for analyzing signal propagation. In parallel, we develop an *in silico* equivalent that would enable efficient testing of various stimulation patterns. Based on the experimental activity we tune the model parameters and use it to give predictions we can directly test *in vitro*. [1] Duru, et al., *Frontiers*, 2022
[2] Girardin, et al., 2022, https://doi.org/10.1101/2021.12.10.472063
[3] Ihle, et al., *Biosensors and Bioelectronics,* 2022
[4] Mateus et al., 2022, htttps://doi.org/10.1101/2021.12.16.472887

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BOARD NUMBER: S01-417

PRE-GESTATIONAL STRESS AND PERINATAL TREATMENT WITH ANTIDEPRESSANTS IN RATS AFFECT LEVELS OF SYNAPSE-RELATED PROTEINS IN HIPPOCAMPUS OF ADULT OFFSPRING IN A SEX-DEPENDENT MANNER

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Aim: Prenatal maternal stress has been linked to impaired cognitive development of offspring. Mirtazapine and bupropion are new generation antidepressants with different mechanism of action. We aimed to identify possible sex differences in markers of hippocampal synaptic plasticity, i.e., BDNF, PSD-95 and AMPAR during adulthood in offspring of mothers exposed to pre-gestational stress and/or antidepressant treatment. Method: Female Wistar rats were subjected to chronic unpredictable stress for three weeks to evoke a depressive-like condition and were subsequently mated. Bupropion (30 mg/kg/day) or mirtazapine (10 mg/kg/day) were administered from gestation day 10 until weaning on day 21 post-partum. Hippocampal tissues were collected from both sexes of adult offspring for Western-blot analysis to assess levels of BDNF, PSD-95 and AMPA proteins. Results: We observed significant effect of both pre-gestational stress and/or mirtazapine and bupropion treatment on the levels of BDNF protein in the hippocampus of adult offspring. Levels of PSD-95 were increased in both treatments and stress only in male offspring. Levels of AMPAR were affected only by stress, showing higher mean values in males compared to females, however bupropion treatment counteracted this effect. Conclusions: We found that pre-gestational stress and/or treatment with selected antidepressants altered hippocampal levels of synaptic plasticity markers BDNF, PSD-95 and AMPAR in a sex-dependent manner. These changes may relate to neurobehavioral and cognitive dysfunctions in adulthood. Study was supported by the grants VEGA 2/0124/19 and APVV-19-0435.

Pubmed:

33017639: Belovičová K, Šimončičová E, Noguera MV, Dubovický M, Bögi E
Long-term effects of pre-gestational stress and perinatal venlafaxine treatment on neurobehavioral development of female offspring.
Preclinical studies suggest that stress-related disorders even prior gestation can cause long-term changes at the level of neurobehavioral adaptations. Therefore, it is critical to consider undergoing antidepressant therapy which could reverse the negative consequences in the offspring. Venlafaxine is widely used in clinical practice; however insufficient amount of well-controlled studies verified the safety of venlafaxine therapy during gestation and lactation. The aim of this work was to investigate the effects of perinatal venlafaxine therapy on selected neurobehavioral variables in mothers and their female offspring using a model of maternal adversity. Pre-gestational stressed and non-stressed Wistar rat dams were treated with either venlafaxine (10 mg/kg/day) or vehicle during pregnancy and lactation. We have shown that pre-gestational stress decreased the number of pups with a significant reduction in the number of males but not females. Furthermore, we found that offspring of stressed and treated mothers exhibited anxiogenic behavior in juvenile and adolescent age. However, during adulthood pre-gestational stress significantly increased anxiety-like behavior of female, with venlafaxine treatment normalizing the state to control levels. Additionally, we found that even maternal stress prior gestation can have long-term impact on adult number of hippocampal immature neurons of the female offspring. A number of questions related to the best treatment options for maternal depression still remains, however present data may provide greater insight into the possible outcomes associated with perinatal venlafaxine therapy.
Behav Brain Res, 2021; 398
35113878: Viñas-Noguera M, Csatlósová K, Šimončičová E, Bögi E, Ujházy E, Dubovický M, Belovičová K
Sex- and age- dependent effect of pre-gestational chronic stress and mirtazapine treatment on neurobehavioral development of Wistar rat offspring.
Hormonal fluctuations, such as the perinatal period, may increase susceptibility of women to depression, which in turn exert a negative impact on child's neurodevelopment, becoming a risk factor in development of neuropsychiatric disorders. Moreover, the use of antidepressants during this critical period presents a serious health concern for both the mother and the child, due to the consequences of treatment in terms of the reliability and safety for the proper neurodevelopment of the organism being

not well known. Atypical antidepressants, such as mirtazapine, that targets both serotonergic and noradrenergic systems in the central nervous system (CNS), represent a novel focus of research due to its unique pharmacological profile. The aim of this work was to study the effects of maternal depression and/or perinatal antidepressant mirtazapine treatment on the neurobehavioral development of the offspring. Pre-gestationally chronically stressed or non-stressed Wistar rat dams were treated with either mirtazapine (10 mg/kg/day) or vehicle during pregnancy and lactation followed by analysis of offspring's behavior at juvenile and adolescent age. We found mirtazapine induced significant alterations of nursing behavior. In offspring, pregestational stress (PS) had an anxiogenic effect on adolescent males ($p \leq 0.05$) and increased their active behavior in forced swim test ($p \leq 0.01$). Interaction between pregestational stress and mirtazapine treatment variously induced anxiolytic changes of juvenile ($p \leq 0.05$) and adolescent ($p \leq 0.05$) females and impairment of spatial memory ($p \leq 0.01$) in adolescent females as well. Hippocampal density of synaptophysin, pre-synaptic protein marker, was decreased mainly by mirtazapine treatment. In conclusion, our results show mirtazapine induced significant alterations in maternal behavior and several sex- and age-dependent changes in neurobehavioral development of offspring caused by both prenatal mirtazapine treatment and/or chronic pregestational stress.
PLoS One, 2022; 17

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RHYTHMIC WHISKER STIMULATION CHANGES THE ACTIVITY OF NEOCORTICAL PYRAMIDAL NEURONS

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Sensory experience and learning are thought to be associated with brain plasticity. Previously, we have demonstrated in anesthetized mice that a brief period (1min) of passive rhythmic whisker stimulation (RWS, 8Hz) induces long-term potentiation (LTP) of L2/3 Pyramidal Neuron (PN) synapses in the barrel cortex (BC). The potentiated amplitude of sensory-evoked post synaptic potentials (PSPs) in PNs remained mostly below spiking threshold, which is consistent with previous findings that PNs in supragranular layers of the BC rarely spike, but it may also be the consequence of anesthesia. To assess whether L2/3 PN activity is modulated as a consequence of LTP, we monitored whisker-evoked calcium responses in GCaMP6s-expressing L2/3 PNs in awake mice before, during and after RWS (10min - hours). We found that RWS induced a strong, 2 hour-lasting increase in whisker-evoked L2/3 PN population activity. This effect was specific for RWS of the PNs' principal whisker and was not observed for whiskers that functionally project onto distant barrel columns. Previously, we showed that RWS-evoked plasticity is dependent upon activation of vasoactive intestinal peptide-expressing (VIP) interneurons. Therefore, in a separate set of mice, we monitored the activity of GCaMP6s-expressing VIP interneurons and found that VIP interneurons increased their firing for a sustained period during RWS, which suggests that disinhibition-facilitated plasticity may have triggered the transient increase in L2/3 PN activity. Altogether, this work indicates that the cortical representation of sensory input is dynamic and can be modulated over hours by repetitive sensory stimulation, via mechanisms that may involve cortical disinhibitory circuit motifs.

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IF HYPERACTIVE, RELAX WITH AGE? AGING MICE AND LACK OF MITF

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Age-related decline occurs in many brain structures and sensory systems. One example is the olfactory bulb (OB), the first brain structure that mediates olfactory information, which deteriorates with age leading to reduced olfactory ability. A decrease in olfactory ability is also an early symptom of neuropathologies such as Alzheimer´s disease (AD) and Parkinson´s disease (PD). However, the pathophysiology underlying olfactory dysfunction is often unclear. Microphthalmia-associated transcription factor (Mitf), the master regulator of melanocyte development and an important oncogene in melanoma, has distinct expression in the projection neurons, mitral and tufted (M/T) cells, of the OB. We have shown that mutant primary M/T cells are hyperactive and that the expression of key potassium channel subunit, Kv4.3 is reduced in M/T neurons of Mitf mutant mice. This suggest a role for Mitf in homestatic intrinsic plasticity. Neuronal hyperactivity is a frequent first step towards neurodegeneration and the goal of the current study is to determine whether lack of Mitf during aging leads to detrimental effects. OB from aged mice were analysed with regards to neuronal and glia morphology using the Golgi-COX method and immunohistochemistry, as well as gene expression and olfactory ability. Olfaction was affected, however, changes in protein markers and gene expression changes showed no evidence of inflammation or neurodegeneration in aged Mitf mutant mice. Importantly, there was an increased expression of neuronal genes which play an inhibitive role in OB´s neuronal network. This suggest that neuronal hyperactivity in younger animals is buffered or equilized through compensatory mechanisms in aging animals.

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OPTIMIZED PROTOCOL FOR THE REGION-SPECIFIC EXTRACTION OF MRNA FROM MURINE BRAIN MICROGLIA USING THE RIBOTAG APPROACH

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Aim: Microglia are the resident macrophages of the central nervous system (CNS) and are involved in brain homeostasis, synapse formation, plasticity, cognition and learning. Several studies have implicated microglia as important players in regulating experience-dependent synaptic plasticity. In order to better understand the mechanisms through which microglia respond to experience-dependent plasticity, we aim to analyse the microglial transcriptome in the visual cortex after induction of experience-dependent plasticity in adult murine brain. For this, we developed a protocol for RNA extraction from microglia using the RiboTag approach. Method: To characterize the microglia transcriptome from the visual cortex , we first optimized the RiboTag protocol for isolation of mRNA from small brain tissue sections using the microglia-specific RiboTag-based mouse line (Cx3cr1Cre$^{ERT2/+}$;Rpl22$^{HA/+}$), RT-PCR, Western blot and immunostaining. We determined the effect of various tamoxifen-mediated Cre-recombinase activation times and tissue amounts on the RNA-extraction yield. In addition, we evaluated the impact of Qiazol and the RNA precipitant LPA (linear polyacrylamide) on the yield and integrity of the immunoprecipitated mRNA using Agilent-based analyses. Result: an extended period of 30 d after tamoxifen delivery in combination with Qiazol/LPA-mediated extraction and immunoprecipitation led to an increased yield of mRNA from microglia as indicated by qubit measurement and enrichment of microglia genes. The mRNA obtained was of good quality as indicated by RIN values between 7.0-9.0. Conclusion: Our results indicate that the RiboTag approach can be used to selectively obtain mRNA from microglia in specific murine brain regions for downstream applications including RNA-seq

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VULNERABILITY TO COCAINE ADDICTION INDUCED BY CHRONIC STRESS: IMPACT OF VAL66MET POLYMORPHISM IN THE BDNF GENE

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Brain-derived neurotrophic factor (BDNF) is a neurotrophin widely expressed in the brain and it has a primary role in the development, differentiation, maturation and plasticity of the developing nervous system. BDNF is involved in synaptic plasticity, learning and it has a critical role in the neuronal plasticity leading to drug abuse. In the last decades, a single nucleotide polymorfism (SNP) in BDNF gene which results in the substitution of the amino acid Valine by Methionine at codon 66 (Val66Met) in the BDNF prodomain region, has been widely studied. This polymorphism affects intracellular processing and secretion of BDNF. The aim of the present study is evaluate the involvement of Met-prodomain BDNF (Met-pBDNF) in the impact of stress on vulnerability to cocaine addiction. Through an intracranial injection of lentiviral (LV) particles expressing the Val or Met variant of pBDNF in the nucleus accumbens core (NAc), we were able to observe an enhancement of locomotor activity in stressed animals microinjected with Met-pBDNF after a cocaine injection. Moreover, this enhancement is significantly more pronounced in stress Met-pBDNF compared with stressed Val-pBDNF animals. These results suggest that Met-pBDNF could induce an enhancement in the vulnerability to cocaine abuse and play an important role in the development of substance use disorders induced by chronic stress.

Pubmed:
34169122: Rigoni D, Avalos MP, Boezio MJ, Guzmán AS, Calfa GD, Perassi EM, Pierotti SM, Bisbal M, Garcia-Keller C, Cancela LM, Bollati F
Stress-induced vulnerability to develop cocaine addiction depends on cofilin modulation.
Actin dynamics in dendritic spines can be associated with the neurobiological mechanisms supporting the comorbidity between stress exposure and cocaine increase rewards. The actin cytoskeleton remodeling in the nucleus accumbens (NA) has been implicated in the expression of stress-induced cross-sensitization with cocaine. The present study evaluates the involvement of cofilin, a direct regulator of actin dynamics, in the impact of stress on vulnerability to cocaine addiction. We assess whether the neurobiological mechanisms that modulate repeated-cocaine administration also occur in a chronic restraint stress-induced cocaine self-administration model. We also determine if chronic stress induces alterations in dendritic spines through dysregulation of cofilin activity in the NA core. Here, we show that the inhibition of cofilin expression in the NA core using viral short-hairpin RNA is sufficient to prevent the cocaine sensitization induced by chronic stress. The reduced cofilin levels also impede a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor surface expression enhancement and promote the reduction of head diameter in animals pre-exposed to stress after a cocaine challenge in the NA core. Moreover, downregulation of cofilin expression prevents facilitation of the acquisition of cocaine self-administration (SA) in male rats pre-exposed to chronic stress without modifying performance in sucrose SA. These findings reveal a novel, crucial role for cofilin in the neurobiological mechanisms underpinning the comorbidity between stress exposure and addiction-related disorders.
Neurobiol Stress, 2021; 15

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EB3 PROTEIN POTENTIATES ENDOPLASMIC RETICULUM LOCALIZATION IN HIPPOCAMPAL DENDRITIC SPINES AND MAKES THEM RESILIENT TO BETA-AMYLOID TOXICITY

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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EB3 protein is expressed abundantly in the nervous system and transiently enters the dendritic spines at the tip of the growing microtubule. Nevertheless, the role of dynamic microtubules, and particularly EB3 protein, in synapse function is still elusive. We have shown, that this protein is important for normal dendritogenesis – both EB3 overexpression and knockout reduces hippocampal neurons dendritic branching and total dendritic length. Some of the spines contain endoplasmic reticulum (ER) inside it, which is able to form spine apparatus. EB3 overexpression leads to a dramatic increase in dendritic spines head area, number of ER containing spines and ER area inside the spine. EB3 knockout oppositely reduces spine head area and increases spine neck length and spine neck/spine length ratio, alters ER presence in spines. The same effect is observed in conditions of amyloid-beta toxicity, modelling Alzheimer`s disease. Neck elongation is supposed to be a common detrimental effect on the spine's shape, which makes them biochemically and electrically less connected to the dendrite. According to preliminary results EB3 potentates ER movement in dendritic spines through associating with ER resident calcium sensor protein STIM2. EB3 also potentiates the formation of presynaptic protein Synapsin clusters and CaMKII-alpha preferential localization in spines rather than in dendrites of hippocampal neurons, while its downregulation has an opposite effect and reduces the size of Synapsin and PSD95 protein clusters. EB3 overexpression makes dendritic spines resilient to amyloid-beta toxicity and restores its detrimental effects on spines. Supported by RNF № 21-74-00028.

Pubmed:

26424877: Zhang H, Wu L, Pchitskaya E, Zakharova O, Saito T, Saido T, Bezprozvanny I
Neuronal Store-Operated Calcium Entry and Mushroom Spine Loss in Amyloid Precursor Protein Knock-In Mouse Model of Alzheimer's Disease.
Alzheimer's disease (AD) is the most common reason for elderly dementia in the world. We proposed that memory loss in AD is related to destabilization of mushroom postsynaptic spines involved in long-term memory storage. We demonstrated previously that stromal interaction molecule 2 (STIM2)-regulated neuronal store-operated calcium entry (nSOC) in postsynaptic spines play a key role in stability of mushroom spines by maintaining activity of synaptic Ca(2+)/calmodulin kinase II (CaMKII). Furthermore, we demonstrated previously that the STIM2-nSOC-CaMKII pathway is downregulated in presenilin 1 M146V knock-in (PS1-M146V KI) mouse model of AD, leading to loss of hippocampal mushroom spines in this model. In the present study, we demonstrate that hippocampal mushroom postsynaptic spines are also lost in amyloid precursor protein knock-in (APPKI) mouse model of AD. We demonstrated that loss of mushroom spines occurs as a result of accumulation of extracellular β-amyloid 42 in APPKI culture media. Our results indicate that extracellular Aβ42 acts by overactivating mGluR5 receptor in APPKI neurons, leading to elevated Ca(2+) levels in endoplasmic reticulum, compensatory downregulation of STIM2 expression, impaired synaptic nSOC, and reduced CaMKII activity. Pharmacological inhibition of mGluR5 or overexpression of STIM2 rescued synaptic nSOC and prevented mushroom spine loss in APPKI hippocampal neurons. Our results indicate that downregulation of synaptic STIM2-nSOC-CaMKII pathway causes loss of mushroom synaptic spines in both presenilin and APPKI mouse models of AD. We propose that modulators/activators of this pathway may have a potential therapeutic value for treatment of memory loss in AD. Significance statement: A direct connection between amyloid-induced synaptic mushroom spine loss and neuronal store-operated calcium entry pathway is shown. These results provide strong support for the calcium hypothesis of neurodegeneration and further validate the synaptic store-operated calcium entry pathway as a potential therapeutic target for Alzheimer's disease.
J Neurosci, 2015; 35
27881772: Zhang H, Sun S, Wu L, Pchitskaya E, Zakharova O, Fon Tacer K, Bezprozvanny I
Store-Operated Calcium Channel Complex in Postsynaptic Spines: A New Therapeutic Target for Alzheimer's Disease

Treatment.
Mushroom dendritic spine structures are essential for memory storage and the loss of mushroom spines may explain memory defects in aging and Alzheimer's disease (AD). The stability of mushroom spines depends on stromal interaction molecule 2 (STIM2)-mediated neuronal-store-operated Ca influx (nSOC) pathway, which is compromised in AD mouse models, in aging neurons, and in sporadic AD patients. Here, we demonstrate that the Transient Receptor Potential Canonical 6 (TRPC6) and Orai2 channels form a STIM2-regulated nSOC Ca channel complex in hippocampal mushroom spines. We further demonstrate that a known TRPC6 activator, hyperforin, and a novel nSOC positive modulator, NSN21778 (NSN), can stimulate activity of nSOC pathway in the spines and rescue mushroom spine loss in both presenilin and APP knock-in mouse models of AD. We further show that NSN rescues hippocampal long-term potentiation impairment in APP knock-in mouse model. We conclude that the STIM2-regulated TRPC6/Orai2 nSOC channel complex in dendritic mushroom spines is a new therapeutic target for the treatment of memory loss in aging and AD and that NSN is a potential candidate molecule for therapeutic intervention in brain aging and AD.
J Neurosci, 2016; 36
25589721: Zhang H, Liu J, Sun S, Pchitskaya E, Popugaeva E, Bezprozvanny I
Calcium signaling, excitability, and synaptic plasticity defects in a mouse model of Alzheimer's disease.
Alzheimer's disease (AD) and aging result in impaired ability to store memories, but the cellular mechanisms responsible for these defects are poorly understood. Presenilin 1 (PS1) mutations are responsible for many early-onset familial AD (FAD) cases. The phenomenon of hippocampal long-term potentiation (LTP) is widely used in studies of memory formation and storage. Recent data revealed long-term LTP maintenance (L-LTP) is impaired in PS1-M146V knock-in (KI) FAD mice. To understand the basis for this phenomenon, in the present study we analyzed structural synaptic plasticity in hippocampal cultures from wild type (WT) and KI mice. We discovered that exposure to picrotoxin induces formation of mushroom spines in both WT and KI cultures, but the maintenance of mushroom spines is impaired in KI neurons. This maintenance defect can be explained by an abnormal firing pattern during the consolidation phase of structural plasticity in KI neurons. Reduced frequency of neuronal firing in KI neurons is caused by enhanced calcium-induced calcium release (CICR), enhanced activity of calcium-activated potassium channels, and increased afterhyperpolarization. As a result, "consolidation" pattern of neuronal activity converted to "depotentiation" pattern of neuronal activity in KI neurons. Consistent with this model, we demonstrated that pharmacological inhibitors of CICR (dantrolene), of calcium-activated potassium channels (apamin), and of calcium-dependent phosphatase calcineurin (FK506) are able to rescue structural plasticity defects in KI neurons. Furthermore, we demonstrate that incubation with dantrolene or apamin also rescued L-LTP defects in KI hippocampal slices, suggesting a role for a similar mechanism. This proposed mechanism may be responsible for memory defects in AD but also for age-related memory decline.
J Alzheimers Dis, 2015; 45
29247211: Pchitskaya E, Kraskovskaya N, Chernyuk D, Popugaeva E, Zhang H, Vlasova O, Bezprozvanny I
Stim2-Eb3 Association and Morphology of Dendritic Spines in Hippocampal Neurons.
Mushroom spines form strong synaptic contacts and are essential for memory storage. We have previously demonstrated that neuronal store-operated calcium entry (nSOC) in hippocampal neurons is regulated by STIM2 protein. This pathway plays a key role in stability of mushroom spines and is compromised in different mice models of Alzheimer's disease (AD). Actin was thought to be the sole cytoskeleton compartment presented in dendritic spines, however, recent studies demonstrated that dynamic microtubules with EB3 capped plus-ends transiently enter spines. We showed that STIM2 forms an endoplasmic reticulum (ER) Ca -dependent complex with EB3 via Ser-x-Ile-Pro aminoacid motif and that disruption of STIM2-EB3 interaction resulted in loss of mushroom spines in hippocampal neurons. Overexpression of EB3 causes increase of mushroom spines fraction and is able to restore their deficiency in hippocampal neurons obtained from PS1-M146V-KI AD mouse model. STIM2 overexpression failed to restore mushroom dendritic spines after EB3 knockdown, while in contrast EB3 overexpression rescued loss of mushroom spines resulting from STIM2 depletion. We propose that EB3 is involved in regulation of dendritic spines morphology, in part due to its association with STIM2, and that modulation of EB3 expression is a potential way to overcome synaptic loss during AD.
Sci Rep, 2017; 7
26275606: Popugaeva E, Pchitskaya E, Speshilova A, Alexandrov S, Zhang H, Vlasova O, Bezprozvanny I
STIM2 protects hippocampal mushroom spines from amyloid synaptotoxicity.
Alzheimer disease (AD) is a disease of lost memories. Mushroom postsynaptic spines play a key role in memory storage, and loss of mushroom spines has been proposed to be linked to memory loss in AD. Generation of amyloidogenic peptides and accumulation of amyloid plaques is one of the pathological hallmarks of AD. It is important to evaluate effects of amyloid on stability of mushroom spines.
Mol Neurodegener, 2015; 10
33117142: Pchitskaya E, Bezprozvanny I

Dendritic Spines Shape Analysis-Classification or Clusterization? Perspective.
Dendritic spines are small protrusions from the dendrite membrane, where contact with neighboring axons is formed in order to receive synaptic input. Changes in size, shape, and density of synaptic spines are associated with learning and memory, and observed after drug abuse in a variety of neurodegenerative, neurodevelopmental, and psychiatric disorders. Due to the preeminent importance of synaptic spines, there have been major efforts into developing techniques that enable visualization and analysis of dendritic spines in cultured neurons, in fixed slices and in intact brain tissue. The classification of synaptic spines into predefined morphological groups is a standard approach in neuroscience research, where spines are divided into fixed categories such as thin, mushroom, and stubby subclasses. This study examines accumulated evidence that supports the existence of dendritic spine shapes as a continuum rather than separated classes. Using new approaches and software tools we reflect on complex dendritic spine shapes, positing that understanding of their highly dynamic nature is required to perform analysis of their morphology. The study discusses and compares recently developed algorithms that rely on clusterization rather than classification, therefore enabling new levels of spine shape analysis. We reason that improved methods of analysis may help to investigate a link between dendritic spine shape and its function, facilitating future studies of learning and memory as well as studies of brain disorders.
Front Synaptic Neurosci, 2020; 12
27641664: Popugaeva E, Pchitskaya E, Bezprozvanny I
Dysregulation of neuronal calcium homeostasis in Alzheimer's disease - A therapeutic opportunity?
Alzheimer's disease (AD) is the disease of lost memories. Synaptic loss is a major reason for memory defects in AD. Signaling pathways involved in memory loss in AD are under intense investigation. The role of deranged neuronal calcium (Ca) signaling in synaptic loss in AD is described in this review. Familial AD (FAD) mutations in presenilins are linked directly with synaptic Ca signaling abnormalities, most likely by affecting endoplasmic reticulum (ER) Ca leak function of presenilins. Excessive ER Ca release via type 2 ryanodine receptors (RyanR2) is observed in AD spines due to increase in expression and function of RyanR2. Store-operated Ca entry (nSOC) pathway is disrupted in AD spines due to downregulation of STIM2 protein. Because of these Ca signaling abnormalities, a balance in activities of Ca-calmodulin-dependent kinase II (CaMKII) and Ca-dependent phosphatase calcineurin (CaN) is shifted at the synapse, tilting a balance between long-term potentiation (LTP) and long-term depression (LTD) synaptic mechanisms. As a result, synapses are weakened and eliminated in AD brains by LTD mechanism, causing memory loss. Targeting synaptic calcium signaling pathways offers opportunity for development of AD therapeutic agents.
Biochem Biophys Res Commun, 2017; 483
30472945: Pchitskaya EI, Zhemkov VA, Bezprozvanny IB
Dynamic Microtubules in Alzheimer's Disease: Association with Dendritic Spine Pathology.
Alzheimer's disease (AD) is the most common incurable neurodegenerative disorder that affects the processes of memory formation and storage. The loss of dendritic spines and alteration in their morphology in AD correlate with the extent of patient's cognitive decline. Tubulin had been believed to be restricted to dendritic shafts, until recent studies demonstrated that dynamically growing tubulin microtubules enter dendritic spines and promote their maturation. Abnormalities of tubulin cytoskeleton may contribute to the process of dendritic spine shape alteration and their subsequent loss in AD. In this review, association between tubulin cytoskeleton dynamics and dendritic spine morphology is discussed in the context of dendritic spine alterations in AD. Potential implications of these findings for the development of AD therapy are proposed.
Biochemistry (Mosc), 2018; 83
29890840: Popugaeva E, Pchitskaya E, Bezprozvanny I
Dysregulation of Intracellular Calcium Signaling in Alzheimer's Disease.
Calcium (Ca) hypothesis of Alzheimer's disease (AD) gains popularity. It points to new signaling pathways that may underlie AD pathogenesis. Based on calcium hypothesis, novel targets for the development of potential AD therapies are identified. Recent Advances: Recently, the key role of neuronal store-operated calcium entry (nSOCE) in the development of AD has been described. Correct regulation of nSOCE is necessary for the stability of postsynaptic contacts to preserve the memory formation. Molecular identity of hippocampal nSOCE is defined. Perspective nSOCE-activating molecule, prototype of future anti-AD drugs, is described.
Antioxid Redox Signal, 2018; 29
28728834: Pchitskaya E, Popugaeva E, Bezprozvanny I
Calcium signaling and molecular mechanisms underlying neurodegenerative diseases.
Calcium (Ca) is a ubiquitous second messenger that regulates various activities in eukaryotic cells. Especially important role calcium plays in excitable cells. Neurons require extremely precise spatial-temporal control of calcium-dependent processes because they regulate such vital functions as synaptic plasticity. Recent evidence indicates that neuronal calcium signaling is abnormal in many of neurodegenerative disorders such as Alzheimer's disease (AD), Huntington's disease (HD) and Parkinson's disease (PD). These diseases represent a major medical, social, financial and scientific problem, but despite

enormous research efforts, they are still incurable and only symptomatic relief drugs are available. Thus, new approaches and targets are needed. This review highlight neuronal calcium-signaling abnormalities in these diseases, with particular emphasis on the role of neuronal store-operated Ca entry (SOCE) pathway and its potential relevance as a therapeutic target for treatment of neurodegeneration.
Cell Calcium, 2018; 70

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BOARD NUMBER: S01-423

MITF REGULATES GENES WHICH DEFINE MIDDLE TUFTED NEURONS OR REDUCE NEURONAL ACTIVITY

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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[1]University of Iceland, Faculty of Medicine, School of Health Sciences, Biomedical Center, Department Of Anatomy, Reykjavík, Iceland, [2]University of Iceland, Faculty of Medicine, Biomedical Center, School of Health Sciences, Department Of Biochemistry And Molecular Biology, Reykjavík, Iceland

The olfactory bulb (OB) is the first relay center in the brain that mediates olfactory information. Its distinct organization and defined neuronal subtypes make the OB attractive to study neuroplasticity. Micropthalmia-associated transcription factory (MITF) is regarded as a master regulator of the melanocyte lineage. It has a broad variety of target genes involved in various cellular processes in melanocytes and melanoma. MITF is also expressed in the projection neurons, mitral and tufted (M/T) cells, of the OB suggesting a putative role in olfaction. However, little is known regarding target genes of MITF in the central nervous system (CNS). Here, we aim to identify potential MITF target genes in the projection neurons of the OB using bulk RNA-sequencing and RNA in situ hybridization. Electrophysiological analysis of primary M/T cells from *Mitf* null mice (*Mitf* $^{mi\text{-}vga9/mi\text{-}vga9}$) showed hyperexcitability. This has been linked to reduced expression of a key potassium voltage-gated channel subunit, Kv4.3. The current transcriptomic data shows reduction in gene expression of genes which are specifically expressed in middle tufted cells of the OB, as well as genes which are known or likely to reduce neuronal activity. RNA in situ hybridization confirmed the expression changes of selected genes in different OB layers of mice. The presence of MITF binding sites in a gene underpins this direct interaction. MITF thus appears to regulate genes involved in neuronal acitivity through multiple mechanisms, subsequent hyperactivity in mutant mice likely leads to changes in expression of other genes sensitive towards increased activity.

Pubmed:
30471646: Ingason AB, Mechmet F, Atacho DAM, Steingrímsson E, Petersen PH
Distribution of mast cells within the mouse heart and its dependency on Mitf.
Although mast cell distribution has been described in both human and canine hearts, cardiac mast cells in mice have yet to be categorically localized. We therefore sought to describe mast cell distribution within the mouse heart and characterize their dependence on the Microphthalmia-associated transcription factor (Mitf). Cardiac mast cells were visualized using Toluidine Blue and avidin staining, and their distribution within the heart described. Cardiac mast cells were most prevalent in the epicardium (50%) or myocardium (45%). Less frequently, mast cells were noted in the endocardium (5%). Within the myocardium, 31% of the mast cells had perivascular location. By studying two different Mitf mutant strains, Mitf and Mitf, we demonstrated that these mutations led to near-complete deficiency of cardiac mast cells. Accordingly, expression of the mMCP-4 and mMCP-5 genes was lost and chymase enzyme activity was severely reduced. Additionally, hearts from mice heterozygous for these Mitf mutations contained significantly fewer mast cells compared to wild-type mice. Our results demonstrated that the distribution of cardiac mast cells in mice is different from humans and dogs. Cardiac mast cells are dependent on Mitf expression, with loss-of-function mutation in the Mitf gene leading to near-complete lack of cardiac mast cells. Loss of a single Mitf allele is sufficient for relative mast cell deficiency.
Mol Immunol, 2019; 105
31197505: Nóbrega C, Mendonça L, Marcelo A, Lamazière A, Tomé S, Despres G, Matos CA, Mechmet F, Langui D, den Dunnen W, de Almeida LP, Cartier N, Alves S
Restoring brain cholesterol turnover improves autophagy and has therapeutic potential in mouse models of spinocerebellar ataxia.
Spinocerebellar ataxias (SCAs) are devastating neurodegenerative disorders for which no curative or preventive therapies are available. Deregulation of brain cholesterol metabolism and impaired brain cholesterol turnover have been associated with several neurodegenerative diseases. SCA3 or Machado-Joseph disease (MJD) is the most prevalent ataxia worldwide. We show that cholesterol 24-hydroxylase (CYP46A1), the key enzyme allowing efflux of brain cholesterol and activating brain cholesterol turnover, is decreased in cerebellar extracts from SCA3 patients and SCA3 mice. We investigated whether reinstating CYP46A1 expression would improve the disease phenotype of SCA3 mouse models. We show that administration

of adeno-associated viral vectors encoding CYP46A1 to a lentiviral-based SCA3 mouse model reduces mutant ataxin-3 accumulation, which is a hallmark of SCA3, and preserves neuronal markers. In a transgenic SCA3 model with a severe motor phenotype we confirm that cerebellar delivery of AAVrh10-CYP46A1 is strongly neuroprotective in adult mice with established pathology. CYP46A1 significantly decreases ataxin-3 protein aggregation, alleviates motor impairments and improves SCA3-associated neuropathology. In particular, improvement in Purkinje cell number and reduction of cerebellar atrophy are observed in AAVrh10-CYP46A1-treated mice. Conversely, we show that knocking-down CYP46A1 in normal mouse brain impairs cholesterol metabolism, induces motor deficits and produces strong neurodegeneration with impairment of the endosomal-lysosomal pathway, a phenotype closely resembling that of SCA3. Remarkably, we demonstrate for the first time both in vitro, in a SCA3 cellular model, and in vivo, in mouse brain, that CYP46A1 activates autophagy, which is impaired in SCA3, leading to decreased mutant ataxin-3 deposition. More broadly, we show that the beneficial effect of CYP46A1 is also observed with mutant ataxin-2 aggregates. Altogether, our results confirm a pivotal role for CYP46A1 and brain cholesterol metabolism in neuronal function, pointing to a key contribution of the neuronal cholesterol pathway in mechanisms mediating clearance of aggregate-prone proteins. This study identifies CYP46A1 as a relevant therapeutic target not only for SCA3 but also for other SCAs.
Acta Neuropathol, 2019; 138

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INTRACELLULAR ACTIVITY OF CORTICAL PYRAMIDAL NEURONS ACROSS THE WAKE-SLEEP CYCLE

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Sleep is a rapid reversible behavioural state and an essential biological need for higher vertebrates. Amassing experimental evidence implicate sleep-wake promoting and oscillatory networks in the control of sleep-wake states and ultimately sleep-dependent functions, including synaptic plasticity and memory refinement. Yet, the precise synaptic and subcellular mechanisms occurring during sleep, in particular rapid-eye movement sleep (REMS), remains unclear. Here, we recorded cortical neurons across the wake-sleep cycle using in vivo whole-cell patch-clamp recordings in head-fixed and naturally sleeping mice. We found that the intracellular activity of L2/3 pyramidal neurons from prefrontal (PFC) and retrosplenial (RSP) cortices remarkably differed between wakefulness, REMS and non-REMS (NREMS), likely resulting from alterations in intrinsic excitability, synaptic activities and local excitation/inhibition balance. Indeed, the membrane potential (Vm) recorded during NREMS showed a biphasic slow oscillation (~2 Hz) between DOWN (~ -65 mV) and UP (~ 45 mV) states and was similar between PFC and RSP. On the other hand, the recorded Vm during REMS was remarkably different between cortical regions, with PFC being defined by a fast and low amplitude fluctuation and RSP by a long-lasting somatic depolarization. Collectively, our results are consistent with previous studies and suggest that the excitability of cortical neurons during NREMS and REMS at either postsynaptic and somatic level might provide a mechanism for sleep-dependent synaptic plasticity essential to the optimization of behaviour.

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HUMAN CPEB3, A FUNCTIONAL PRIONOID WITH A REMARKABLE CONFORMATIONAL POLYMORPHISM

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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The Cytoplasmic Polyadenylation Element Binding protein 3, CPEB3, is a functional amyloid that plays a crucial role in memory consolidation. The starting point and a key determinant of the aggregation cascade is the conformational change that occurs on the monomeric protein. Here we analyse the mechanical properties of the amyloid region of human CPEB3 (hCPEB3) by atomic force microscopy-based single molecule force spectroscopy (AFM-SMFS) to monitor its conformational polymorphism at monomer level. We have analysed the first 200 residues of the intrinsically disordered region (IDR) of hCPEB3 since this region has been reported to be the amyloid-forming domain. For our unequivocal nanomechanical analysis we use a protein-engineering mechanical-protection strategy. We first validate this strategy using far-UV circular dichroism, immunoreactivity against conformational antibodies and fibrillation studies by Thioflavin-T binding, corroborating the amyloidogenic properties of $hCPEB3_{1-200}$. The AFM-SMFS results indicated that $hCPEB3_{1-200}$ displayed a rich conformational polymorphism at the monomer level with 29.3% non-mechanically resistant events and 68.9% mechanostable conformers. $hCPEB3_{1-200}$ has showed the highest number of mechanostable events out of the fifteen amyloidogenic IDRs analysed so far in our laboratory by AFM-SMFS, including its CPEB orthologs from *Aplysia* (42.1%) and *Drosophila* (37.7%). Considering that an elevated mechanostability is related with an increased acquisition of secondary structure within the intrinsically disorder region, the higher structuring of hCPEB3 may be related to an evolutionary step to fulfil its biological function.

Pubmed:

26743653: Vaquero ME, Barriuso J, Martínez MJ, Prieto A
Properties, structure, and applications of microbial sterol esterases.
According to their substrate preferences, carboxylic ester hydrolases are organized in smaller clusters. Among them, sterol esterases (EC 3.1.1.13), also known as cholesterol esterases, act on fatty acid esters of cholesterol and other sterols in aqueous media, and are also able to catalyze synthesis by esterification or transesterification in the presence of organic solvents. Mammalian cholesterol esterases are intracellular enzymes that have been extensively studied since they are essential in lipid metabolism and cholesterol absorption, and the natural role of some microbial sterol esterases is supposed to be similar. However, besides these intracellular enzymes, a number of microbes produce extracellular sterol esterases, which show broad stability, selectivity, or wide substrate specificity, making them interesting for the industry. In spite of this, there is little information about microbial sterol esterases, and only a small amount of them have been characterized. Some of the most commercially exploited cholesterol esterases are produced by Pseudomonas species and by Candida rugosa, although in the last case they are usually described and named as "high substrate versatility lipases." From a structural point of view, most of them belong to the α/β-hydrolase superfamily and have a conserved "catalytic triad" formed by His, an acidic amino acid and a Ser residue that is located in a highly conserved GXSXG sequence. In this review, the information available on microbial sterol esterases has been gathered, taking into account their origin, production and purification, heterologous expression, structure, stability, or substrate specificity, which are the main properties that make them attractive for different applications. Moreover, a comprehensive phylogenetic analysis on available sequences of cholesterol esterases has been done, including putative sequences deduced from public genomes.
Appl Microbiol Biotechnol, 2016; 100
27188926: Barriuso J, Vaquero ME, Prieto A, Martínez MJ
Structural traits and catalytic versatility of the lipases from the Candida rugosa-like family: A review.
Lipases and sterol esterases are enzymes with broad biotechnological applications, which catalyze the hydrolysis or synthesis of long-chain acylglycerols and sterol esters, respectively. In this paper, we review the current knowledge on the so-called Candida rugosa-like family of enzymes, whose members display in most cases affinity against the two substrates mentioned above. The family includes proteins with the α/β-hydrolase folding, sharing conserved motifs in their sequences, and common structural features. We will go through their production and purification, relate their described structures and

catalytic activity, and discuss the influence of the hydrophobic character of these lipases on their aggregation state and activity. On the basis of the few crystal structures available, the role of each of the functional areas in catalysis will be analyzed. Considering the particular characteristics of this group, we propose their classification as "Versatile Lipases" (EC 3.1.1.x).
Biotechnol Adv, 2016 Sep-Oct; 34
25898146: Vaquero ME, de Eugenio LI, Martínez MJ, Barriuso J
A novel calb-type lipase discovered by fungal genomes mining.
The fungus Pseudozyma antarctica produces a lipase (CalB) with broad substrate specificity, stability, high regio- and enantio-selectivity. It is active in non-aqueous organic solvents and at elevated temperatures. Hence, CalB is a robust biocatalyst for chemical conversions on an industrial scale. Here we report the in silico mining of public metagenomes and fungal genomes to discover novel lipases with high homology to CalB. The candidates were selected taking into account homology and conserved motifs criteria, as well as, phylogeny and 3D model analyses. The most promising candidate (PlicB) presented interesting structural properties. PlicB was expressed in a heterologous host, purified and partially characterized. Further experiments will allow finding novel catalytic properties with biotechnological interest.
PLoS One, 2015; 10
26272094: Vaquero ME, Prieto A, Barriuso J, Martínez MJ
Expression and properties of three novel fungal lipases/sterol esterases predicted in silico: comparison with other enzymes of the Candida rugosa-like family.
Lipases from the Candida rugosa-like family are enzymes with great biotechnological interest. In a previous work, several enzymes from this family were identified by in silico mining of fungal genomes. Here, we describe the cloning, expression, and characterization of putative lipases from the genomes of Nectria haematococca, Trichoderma reesei, and Aspergillus niger and compared their catalytic properties with those of OPE, a well-characterized sterol esterase/lipase from Ophiostoma piceae. All of them hydrolyzed p-nitrophenol esters and triglycerides with different efficiency, but their activity against sterol esters was dissimilar, and the enzyme from A. niger was unable of hydrolyzing these substrates while OPE showed the best k cat values, which in general leads to an improved catalytic efficiency. Similarly, OPE was the best catalyst in the synthesis of β-sitostanyl oleate, followed by the commercial CRL from C. rugosa, while the A. niger enzyme was unable to produce this compound. When the enzymes were evaluated for caprolactone oligomerization, the A. niger enzyme gave similar results than CRL, being OPE slightly more efficient. The expression of the putative selected proteins allowed their functional validation, suggesting that the hydrophobicity of the lid region may be an important factor, although the enzymatic efficiency is also influenced by other parameters, as the aggregation state and the size and morphology of the tunnel, where substrate recognition and catalysis takes place.
Appl Microbiol Biotechnol, 2015; 99
25939548: Vaquero ME, Barriuso J, Medrano FJ, Prieto A, Martínez MJ
Heterologous expression of a fungal sterol esterase/lipase in different hosts: Effect on solubility, glycosylation and production.
Ophiostoma piceae secretes a versatile sterol-esterase (OPE) that shows high efficiency in both hydrolysis and synthesis of triglycerides and sterol esters. This enzyme produces aggregates in aqueous solutions, but the recombinant protein, expressed in Komagataella (synonym Pichia) pastoris, showed higher catalytic efficiency because of its higher solubility. This fact owes to a modification in the N-terminal sequence of the protein expressed in Pichia pastoris, which incorporated 4-8 additional amino acids, affecting its aggregation behavior. In this study we present a newly engineered P. pastoris strain with improved protein production. We also produced the recombinant protein in the yeast Saccharomyces cerevisiae and in the prokaryotic host Escherichia coli, corroborating that the presence of these N-terminal extra amino acids affected the protein's solubility. The OPE produced in the new P. pastoris strain presented the same physicochemical properties than the old one. An inactive form of the enzyme was produced by the bacterium, but the recombinant esterase from both yeasts was active even after its enzymatic deglycosylation, suggesting that the presence of N-linked carbohydrates in the mature protein is not essential for enzyme activity. Although the yield in S. cerevisiae was lower than that obtained in P. pastoris, this work demonstrates the importance of the choice of the heterologous host for successful production of soluble and active recombinant protein. In addition, S. cerevisiae constitutes a good engineering platform for improving the properties of this biocatalyst.
J Biosci Bioeng, 2015; 120
25108239: Gutiérrez-Fernández J, Vaquero ME, Prieto A, Barriuso J, Martínez MJ, Hermoso JA
Crystal structures of Ophiostoma piceae sterol esterase: structural insights into activation mechanism and product release.
Sterol esterases are able to efficiently hydrolyze both sterol esters and triglycerides and to carry out synthesis reactions in the presence of organic solvents. Their high versatility makes them excellent candidates for biotechnological purposes. Sterol esterase from fungus Ophiostoma piceae (OPE) belongs to the family abH03.01 of the Candida rugosa lipase-like proteins. Crystal structures of OPE were solved in this study for the closed and open conformations. Enzyme activation involves a

large displacement of the conserved lid, structural rearrangements of loop α16-α17, and formation of a dimer with a large opening. Three PEG molecules are placed in the active site, mimicking chains of the triglyceride substrate, demonstrating the position of the oxyanion hole and the three pockets that accommodate the sn-1, sn-2 and sn-3 fatty acids chains. One of them is an internal tunnel, connecting the active center with the outer surface of the enzyme 30 Å far from the catalytic Ser220. Based on our structural and biochemical results we propose a mechanism by which a great variety of different substrates can be hydrolyzed in OPE paving the way for the construction of new variants to improve the catalytic properties of these enzymes and their biotechnological applications.
J Struct Biol, 2014; 187

23347930: Salvachúa D, Prieto A, Vaquero ME, Martínez ÁT, Martínez MJ
Sugar recoveries from wheat straw following treatments with the fungus Irpex lacteus.
Irpex lacteus is a white-rot fungus capable of increasing sugar recovery from wheat straw; however, in order to incorporate biopretreatment in bioethanol production, some process specifications need to be optimized. With this objective, I. lacteus was grown on different liquid culture media for use as inoculums. Additionally, the effect of wheat straw particle size, moisture content, organic and inorganic supplementations, and mild alkali washing during solid-state fermentation (SSF) on sugar yield were investigated. Wheat thin stillage was the best medium for producing inoculums. Supplementation of wheat straw with 0.3mM Mn(II) during SSF resulted in glucose yields of 68% as compared to yields of 62% and 33% for cultures grown without supplementation or on untreated raw material, respectively after 21 days. Lignin loss, wheat straw digestibility, peroxidase activity, and fungal biomass were also correlated with sugar yields in the search for biopretreatment efficiency indicators.
Bioresour Technol, 2013; 131

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BOARD NUMBER: S01-426

COMPARATIVE STUDY ON EFFECTS OF MATERNAL DEPRESSION AND PERINATAL BUPROPION AND MIRTAZAPINE TREATMENT ON LEVELS OF SYNAPSE-RELATED PROTEINS IN ADOLESCENT RAT OFFSPRING

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Aims Our aim was to study the adverse effect of maternal depression and perinatal treatment with atypical antidepressant bupropion and mirtazapine on the levels of selected synapse-related proteins in adolescent rats. Methods Wistar rat dams were randomly assigned to control or stress group. The stress group was subjected to chronic unpredictable stress schedule for 3 weeks. Dams were mated with males and from gestational day 10 until day 21 post-partum were treated by bupropion (30 mg/kg/day) or mirtazapine (10 mg/kg/day), respectively. Adolescent offspring was sacrificed, hippocampi were extracted, and subsequently Western-blot analysis was carried-out for selected synapse-related protein level detection. Results In adolescent malesthe BDNF levels in the hippocampus was negatively effected by maternal depression as well as by both antidepressant treatments alone, while treatment after stress with both bupropion and mirtazapine lead to restoration of the BDNF to control levels.PSD-95 levels were lowered in males of stressed dams and restored by both treatment options. In adolescent females highest PSD-95 levels were manifested in control groups with either antidepressant treatment.In both males and females the negative effect of maternal depression on levels of AMPAR Glu1 subunit was compensated by perinatal bupropion and mirtazapine treatment. Conclusions Our results showed negative effect of maternal depression on synaptic related proteins in the hippocampus of adolescent rats. Perinatal antidepressant treatment seemed to mitigate and even compensate the negative effects on neural plasticity and neurogenesis, although the effects appeared to be sex specific. Study was supported by the grants VEGA 2/0124/19 and APVV-19-0435.

Pubmed:

35113878: Viñas-Noguera M, Csatlósová K, Šimončičová E, Bögi E, Ujházy E, Dubovický M, Belovičová K
Sex- and age- dependent effect of pre-gestational chronic stress and mirtazapine treatment on neurobehavioral development of Wistar rat offspring.
Hormonal fluctuations, such as the perinatal period, may increase susceptibility of women to depression, which in turn exert a negative impact on child's neurodevelopment, becoming a risk factor in development of neuropsychiatric disorders. Moreover, the use of antidepressants during this critical period presents a serious health concern for both the mother and the child, due to the consequences of treatment in terms of the reliability and safety for the proper neurodevelopment of the organism being not well known. Atypical antidepressants, such as mirtazapine, that targets both serotonergic and noradrenergic systems in the central nervous system (CNS), represent a novel focus of research due to its unique pharmacological profile. The aim of this work was to study the effects of maternal depression and/or perinatal antidepressant mirtazapine treatment on the neurobehavioral development of the offspring. Pre-gestationally chronically stressed or non-stressed Wistar rat dams were treated with either mirtazapine (10 mg/kg/day) or vehicle during pregnancy and lactation followed by analysis of offspring's behavior at juvenile and adolescent age. We found mirtazapine induced significant alterations of nursing behavior. In offspring, pregestational stress (PS) had an anxiogenic effect on adolescent males ($p \leq 0.05$) and increased their active behavior in forced swim test ($p \leq 0.01$). Interaction between pregestational stress and mirtazapine treatment variously induced anxiolytic changes of juvenile ($p \leq 0.05$) and adolescent ($p \leq 0.05$) females and impairment of spatial memory ($p \leq 0.01$) in adolescent females as well. Hippocampal density of synaptophysin, pre-synaptic protein marker, was decreased mainly by mirtazapine treatment. In conclusion, our results show mirtazapine induced significant alterations in maternal behavior and several sex- and age-dependent changes in neurobehavioral development of offspring caused by both prenatal mirtazapine treatment and/or chronic pregestational stress.
PLoS One, 2022; 17
33341344: Csatlosova K, Bogi E, Durisova B, Grinchii D, Paliokha R, Moravcikova L, Lacinova L, Jezova D, Dremencov E
Maternal immune activation in rats attenuates the excitability of monoamine-secreting neurons in adult offspring in a sex-

specific way.
Higher risk of depression and schizophrenia in descendants of mothers experienced acute infection during the pregnancy has been reported. Since monoamines are fundamental in mentioned psychopathologies, it is possible that maternal immune activation leads to impaired functioning of serotonin (5-HT), noradrenaline, and dopamine neurons in offspring. To test this hypothesis, we examined the effect of maternal immune activation by lipopolysaccharide (LPS) in rats on the excitability of monoamine-secreting neurons in the offspring. LPS was administered during days 15-19 of the gestation in the rising doses of 20-80 µg/kg; control dams received vehicle. During days 53-63 postpartum, rats were anesthetized and electrodes were inserted into the dorsal raphe nucleus, locus coeruleus, and ventral tegmental area for in vivo excitability assessment of 5-HT, noradrenaline, and dopamine neurons. Maternal immune activation suppressed the firing rate of 5-HT neurons in both sexes and stimulated the firing rate of dopamine neurons in males. Decrease in the firing rate of 5-HT neurons was accompanied with an increase, and increase in the firing rate of dopamine neurons with a decrease, in the density of spontaneously active cells. Maternal immune activation also decreased the variability of interspike intervals in 5-HT and dopamine neurons. It is possible that the alteration of excitability of 5-HT and dopamine neurons by maternal immune activation is involved in the psychopathologies induced by infectious disease during the pregnancy. Stimulation of dopamine excitability in males might be a compensatory mechanism secondary to the maternal immune challenge-induced suppression of 5-HT neurons.
Eur Neuropsychopharmacol, 2021; 43
31757051: Koprdova R, Csatlosova K, Durisova B, Bogi E, Majekova M, Dremencov E, Mach M
Electrophysiology and Behavioral Assessment of the New Molecule SMe1EC2M3 as a Representative of the Future Class of Triple Reuptake Inhibitors.
SMe1EC2M3 is a pyridoindole derivative related to the neuroleptic drug carbidine. Based on the structural similarities of SMe1EC2M3 and known serotonin (5-HT), norepinephrine, and dopamine reuptake inhibitors, we hypothesized that this compound may also have triple reuptake inhibition efficacy and an antidepressant-like effect. PreADMET and Dragon software was used for in silico prediction of pharmacokinetics and pharmacodynamics of SMe1EC2M3. Forced swim test was used to evaluate its antidepressant-like effects. Extracellular in vivo electrophysiology was used to assess 5-HT, norepinephrine, and dopamine reuptake inhibition efficacy of SMe1EC2M3. PreADMET predicted reasonable intestinal absorption, plasma protein binding, and blood-brain permeability for SMe1EC2M3. Dragon forecasted its efficiency as an antidepressant. Using behavioral measurements, it was found that SMe1EC2M3 decreased immobility time and increase swimming time during the forced swim test (FST). Electrophysiological investigations showed that SMe1EC2M3 dose-dependently suppressed the excitability of 5-HT neurons of the dorsal raphe nucleus (DRN), norepinephrine neurons of the locus coeruleus (LC), and dopamine neurons of the ventral tegmental area (VTA). The SMe1EC2M3-induced suppression of 5-HT, norepinephrine, and dopamine neurons was reversed by the antagonists of serotonin-1A (5-HT; WAY100135), α-2 adrenergic (α, yohimbine), and dopamine-2 receptors (D, haloperidol), respectively. We conclude that SMe1EC2M3 is prospective triple 5-HT, norepinephrine, and dopamine reuptake inhibitor with antidepressant-like properties, however future studies should be performed to complete the pharmacological profiling of this compound.
Molecules, 2019; 24
31377253: Bögi E, Belovičová K, Moravčíková L, Csatlósová K, Dremencov E, Lacinova L, Dubovicky M
Pre-gestational stress impacts excitability of hippocampal cells in vitro and is associated with neurobehavioral alterations during adulthood.
Chronic stress during pregnancy or even prior to gestation can negatively affect offspring´s neurobehavioural development. Several studies have shown, that offspring who had experienced excessive stress during gestation had higher rates of cognitive and mood disorders later during adolescence or in adulthood. Hippocampal neurons play a crucial role in the regulation of behavior, mainly in anxiety-related behaviors and spatial learning and memory. Recently, it has been shown, that excessive stress even prior to gestation could interfere with sensitive developmental processes in the brain and may affect hippocampal functioning with severe neurobehavioural consequences in later life. The aim of this work was to investigate the effects of pre-gestational stress of the rat dams on the hippocampal excitability of the pups right after the birth. Neurobehavioural consequences of pre-gestational stress were analyzed during adolescence (35-40 postnatal days) and in early adulthood (75-80 postnatal days). We have shown that even pre-gestational chronic maternal stress increased resting membrane potential, suppressed depolarization-activated action potential firing, and increased spontaneous activity of hippocampal cells from newborn offspring. Altered function of hippocampus was reflected at the behavioural level. Adolescent male offspring of dams exposed stress prior to conception showed hyperactivity-like behaviour in a new stressful environment and increased anxiety-like behaviour during adulthood compared to adult males from non-stress group. Together, this work suggests, that chronic stress even prior to gestation can interfere with functional brain development of the offspring and can cause long-term behavioural changes at the level of neurobehavioural adaptations.
Behav Brain Res, 2019; 375

30123035: Belovicova K, Bogi E, Csatlosova K, Dubovicky M
Animal tests for anxiety-like and depression-like behavior in rats.
An animal model of human behavior represents a complex of cognitive and/or emotional processess, which are translated from animals to humans. A behavioral test is developed primarily and specifically to verify and support a theory of cognition or emotion; it can also be used to verify a theory of a psychopathology, but it is not developed for a particular type of psychopathology. The paper reviews tests commonly used in novel drug discovery research. Focus is especially on tests which can evaluate anxiety-like (openfield test, novelty suppressed feeding, elevated plus maze, light/dark box, stressinduced hyperthermia) and depression-like behaviors (forced swim test, tail suspension test, sucrose preference test) as they represent an important methodological tool in pre-clinical as well as in behavioral toxicology studies.
Interdiscip Toxicol, 2017; 10
30123034: Bogi E, Belovicova K, Csatlosova K, Dubovicky M
Animal models of maternal depression for monitoring neurodevelopmental changes occurring in dams and offspring.
Depression is one of the most prevalent and life-threatening forms of mental illness affecting about 20% of the population. Depressive disorder as a biochemical phenomenon, was first recognized in the mid-20 century of research, however the etiology of this disease is still not well understood. Although the need to investigate depressive disorders has emerged from the needs of clinical practice, there are many preclinical studies, which brought new insights into this field of research. During experimental work it was crucial to develop appropriate animal models, where the neurohumoral mechanism was similar to humans. In the past decades, several animal models of maternal depression have been developed. We describe the three most popular rodent models of maternal depression which are based on 1. stress prior to gestation, 2. prenatal stress and 3. early life stress. The above-mentioned animal models appear to fulfill many criteria for a relevant animal model of depression; they alter the regulation of the HPA, induce signs of depression-like behavior and several antidepressant treatments can reverse the state induced by maternal stress. Although, they are not able to model all aspects of maternal depression, they are useful models for monitoring neurodevelopmental changes occurring in dams and offspring.
Interdiscip Toxicol, 2017; 10
30123033: Dubovicky M, Belovicova K, Csatlosova K, Bogi E
Risks of using SSRI / SNRI antidepressants during pregnancy and lactation.
At present, affective disorders are among the most commonly diagnosed mental diseases. In pregnancy, they can occur as pre-delivery depression, recurrent depressive disorder or postnatal depression. The estimated prevalence of depressive disorders in pregnancy is approximately 9-16%, with some statistics reporting up to 20%. Approximately 2-3% of pregnant women take antidepressants during pregnancy, and the number of mothers treated increases by birth to 5-7%. Treatment of depression during pregnancy and breastfeeding is a controversial issue, as antidepressants can negatively affect the developing fetus. According to epidemiological studies, the effects of treated depression in pregnancy are related to premature birth, decreased body weight of the child, intrauterine growth retardation, neonatal adaptive syndrome, and persistent pulmonary hypertension. However, untreated depression can adversely affect maternal health and increase the risk of preeclampsia and eclampsia, as well as of subsequent postnatal depression, which can lead to disruption of the mother-child relationship. Based on the above mentioned facts, the basic question arises as to whether or not to treat depression during pregnancy and lactation.
Interdiscip Toxicol, 2017; 10
28430979: Dremencov E, Csatlósová K, Durišová B, Moravcíková L, Lacinová L, Ježová D
Effect of Physical Exercise and Acute Escitalopram on the Excitability of Brain Monoamine Neurons: In Vivo Electrophysiological Study in Rats.
The antidepressant effect of physical exercise has been reported in several clinical and animal studies. Since serotonin, norepinephrine, and dopamine play a central role in depression, it is possible that the beneficial effects of physical exercise are mediated via monoamine pathways. This study investigates the effects of voluntary wheel running on the excitability of monoamine neurons.
Int J Neuropsychopharmacol, 2017; 20

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BOARD NUMBER: S01-427

THE EFFECT OF HIGH INTENSITY WHITE NOISE ON THE ULTRASTRUCTURE OF LIMBIC AREAS ON IN RATS. ELECTRON MICROSCOPE STUDY

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Noise pollution is a major growing concern, negatively impacting public health. People are continually and increasingly exposed to harmful noise levels, originating from traffic, loud music, home appliances, etc. Impaired cognitive functions, such as language acquisition, memory and other functions are greatly compromised. In the present study, we assessed the ultrastructural alterations in selective auditory pathways of the rat brain following high intensity white noise exposure. In addition, learning, anxiety-like behavior and locomotor activity were assessed. Adult male rats were exposed to 100 dB noise, one hour daily, for 10 consecutive days. Exposure to noise did not affect learning or the components of locomotor activity. However, it induced anxietylike behavior as evidenced by time spent in the closed arm of elevated-plus maze. Concomitantly, ultrastructural changes in medial geniculate body, as well as in the hippocampus and basolateral amygdala. Specifically, noise resulted in neuronal apoptosis, chromatolysis, cytoplasmic organelle destruction, and glial activation in medial geniculate body and hippocampus, as well as mild alterations in amygdala. Such results suggest that due to continuous transmission, the majority of vesicles are unable to replenish their cargo via transporters. Evaluation of synaptic vesicles size undertaken in the current electron microscopic study has advanced the understanding of the pathophysiology of white noise exposure on auditory brain processing regions, in addition to our understanding of fractional neurotransmitter release at the nerve terminal and on overall brain function. This work was supported: Shota Rustaveli National Science Foundation of Georgia DP2016_17 and I. Beritashvili Center of Experimental Biomedicine.

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BOARD NUMBER: S01-428

CHRONIC TOLUENE INHALATION: ELECTRON MICROSCOPE STUDIES

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Toluene addiction is a worldwide public health problem. The use of toluene-containing volatile inhalants is widespread among adolescents, young people and adults. Although the relationships between brain structure and emotions may alter across the life span, this relationship is of particular importance during aging when significant alterations in emotions may be manifested. Understanding the structural-behavioral relationship could not only provide a neurobiological basis of these changes, but could also suggest potential intervention. We undertook this study to determine the extent of associated ultra-structural changes in the amygdala. The goal of the study is using high resolution transmission electron microscope(TEM), to evaluate immediate and persisting effect of toluene chronic exposure on the ultrastructure/presynaptic architecture of the the central nucleus of amygdala (CNA) in adolescent and male Wistar rats; EM revealed the age-dependent effect of toluene misuse on hippocampal and amygdala structure. Specifically, in adolescent rats significant cell loss was observed only immediately after the end of inhalation. Therefore, in adolescents the alterations do not progress during 90 days period of withdrawal. In opposite, in adult rats more numerous alterations were observed after 90 days of withdrawal. Therefore, in these animals the alterations progressed during 90 days period of withdrawal. The most prominent alterations were detected in the CA1 area. Toluene chronic exposure affects the structure/ultrastructure of the amygdala–brain area involved in learning and memory. Persisting and long-term effects were almost the same. We suggest that in the case of withdrawal the e ultrastructure of regions can be restored.

Pubmed:

34213447: Pochkhidze N, Gogokhia N, Japaridze N, Lazrishvili I, Bikashvili T, Zhvania MG
Electron microscopy demonstrating noise exposure alters synaptic vesicle size in the inferior colliculus of cat.
White noise is known to have detrimental effects on different brain regions, especially auditory regions, including inferior colliculus. Although the basis for such alterations has been hypothesized to result from abnormalities in neurotransmitter release, the mechanism is unclear. The final step in neurotransmission is the docking and transient fusion of synaptic vesicles at the base of cup-shaped lipoprotein structures called porosomes at the presynaptic membrane and the consequent release of neurotransmitters. Earlier studies in cat brain document altered morphology of the secretory portal the porosome at nerve terminals in the inferior colliculus following white noise exposure. The current study was performed to test the hypothesis of possible changes to synaptic vesicle size in the colliculus, following white noise exposure.
Noise Health, 2021 Apr-Jun; 23
32224224: Zhvania M, Gogokhia N, Tizabi Y, Japaridze N, Pochkidze N, Lomidze N, Rzayev F, Gasimov E
Behavioral and neuroanatomical effects on exposure to White noise in rats.
Noise pollution is a severe public health problem as continuous exposure to even moderate noise levels between 55-65 dB can lead to various pathologies, including neurological states. In the present study, we assessed the ultrastructural alterations in selective auditory pathways of the rat brain following high intensity white noise exposure. In addition, learning, anxiety-like behavior and locomotor activity were assessed. Adult male rats were exposed to 100 dB noise, one hour daily, for 10 consecutive days. The evaluations were performed on day 11. Exposure to noise did not affect learning or the components of locomotor activity. However, it induced anxiety-like behavior as evidenced by time spent in the closed arm of elevated-plus maze. Concomitantly, ultrastructural changes in medial geniculate body, considered an integral component of classical auditory pathway, as well as in the hippocampus and basolateral amygdala, considered important structures of non-classical auditory pathway were noted. Specifically, noise resulted in neuronal apoptosis, chromatolysis, cytoplasmic organelle destruction, and glial activation in medial geniculate body and hippocampus, as well as mild alterations in amygdala. These results provide further evidence of detrimental consequences following exposure to loud noise.
Neurosci Lett, 2020; 728
33098201: Lomidze N, Zhvania MG, Tizabi Y, Japaridze N, Pochkhidze N, Rzayev F, Gasimov E

Age-related behavioral and ultrastructural changes in the rat amygdala.
Although the relationships between brain structure and emotions may alter across the life span, this relationship is of particular importance during aging when significant alterations in emotions may be manifested. Understanding the structural-behavioral relationship could not only provide a neurobiological basis of these changes, but could also suggest potential intervention. Since anxiety is commonly observed in aging population, we undertook this study to determine the extent of this behavioral manifestations as well as the associated ultrastructural changes in the amygdala. Rats of various age groups, adolescent, adult, and aged were tested for anxiety-like behavior and the ultrastructure/presynaptic architecture of the central nucleus of amygdala (CNA) were evaluated using transmission electron microscopy (EM). Aged rats were consistently more anxious than the other groups as evidenced by their scores in the elevated plus maze. Morphometric EM analysis of axodendritic synapses revealed that the aged rats had a lower presynaptic area as well as number of synapses, but unexpectedly a higher number of presynaptic mitochondria in CNA. Since presynaptic mitochondria are known to provide the energy for neurotransmission, it may be concluded that compensatory mechanisms are still operational during aging, and hence, may be a target for therapeutic intervention at this stage of life span.
Dev Neurobiol, 2020; 80
31437571: Lobzhanidze G, Lordkipanidze T, Zhvania M, Japaridze N, MacFabe DF, Pochkidze N, Gasimov E, Rzaev F
Effect of propionic acid on the morphology of the amygdala in adolescent male rats and their behavior.
Autism spectrum disorder is a group of life-long developmental syndromes, characterized by stereotypic behavior, restricted, communication deficits, cognitive and social impairments. Autism spectrum disorder is heritable state, provided by the mutations of well-conserved genes; however, it has been increasingly accepted, that most of such states are the result of complex interaction between individual's genetic profile and the environment that he/she is exposed to. Gut microbiota plays one of the central roles in the etiology of autism. Propionic acid is one of the most abundant short-chain fatty acids, made by enteric bacteria. Propionic acid has many positive functions and acts as the main mediator between nutrition, gut microbiota and brain physiology. However, increased level of propionic acid is associated with various neurological pathologies, including autism. It is proposed that some types of autism might be partially related with alterations in propionic acid metabolism. The amygdala, the main component of social brain, via its large interconnections with fronto-limbic neural system, plays one of the key roles in social communications, emotional memory and emotional processing. Social behavior is a hot topic in autism research. As to anxiety, it is not the main characteristics of ASD, but represents one of the most common its co morbidities. Several theoretical reasons compatible with amygdala dysfunction have been suggested to account for socio-emotional disturbances in autism. In the present study, using adolescent male Wistar rats, the effect of acute administration of low dose of propionic acid on social behavior, anxiety-like behavior and the structure/ultrastructure of central nucleus of amygdale was described. In addition to qualitative analysis, on electron microscopic level the quantitative analysis of some parameters of synapses was performed. Behavior was assessed 2, 24 and 48 hours after treatment. The results revealed that even single and relatively low dose of propionic acid is sufficient to produce fast and relatively long lasting (48 h after treatment) decrease of social motivation, whereas asocial motivation and emotional sphere remain unaffected. Morphological analyses of propionic acid-treated brain revealed the reduced neuron number and the increase of the number of glial cells. Electron microscopically, in some neurons the signs of apoptosis and chromatolysis were detected. Glial alterations were more common. Particularly, the activation of astrocytes and microglia were often observed. Pericapillary glia was the most changed. Neuronal, glial and presynaptic mitochondria showed substantial structural diversities, mainly in terms of size and form. Total number of the area of presynaptic profile was significantly decreased. Some axons were moderately demyelinated. In general, the data indicate that even low dose of propionic acid produces in adolescent rodents immediate changes in social behavior, and structural/ultrastructural alterations in amygdala. Ultrastructural alterations may reflect moderate modifications in functional networks of social brain.
Micron, 2019; 125

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BOARD NUMBER: S01-429

DEVELOPMENTAL AND ADULT MEMORY CAPACITY CONTROL VIA INTERPLAY BETWEEN NON-CONVENTIONAL GLUN3A-NMDA RECEPTORS AND MTOR SIGNALING

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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GluN3A-containing NMDA receptors ensure appropriate synapse selection during the postnatal refinement of neural circuits by limiting synapse plasticity and stabilization and promoting pruning. While massively down-regulated in most brain areas after postnatal stages, significant GluN3A expression is retained in selected areas of the adult brain where it seems to play roles in controlling the types of memories that are persistently stored (Murillo et al 2021; Conde-Dusman et al 2021). In line with this work, humans with low levels of GRIN3A (human gene encoding GluN3A) perform better in cognitive tasks. Using constitutive and inducible knockout mice, we investigated how GluN3A expression influences the emergence of memory capacities throughout adolescence into adulthood and explored the underlying mechanisms in vivo. We found that genetic removal of GluN3A facilitates long-term associative memory formation in postnatal (P20-P25) and adult (3-6 month-old) mice. Deletion of GluN3A from adult excitatory neurons using CamKII-Cre-ERT2 mice was sufficient for the memory enhancement, whereas deletion in somatostatin interneurons did not recapitulate the phenotype. The enhancement correlated with longer-lasting long-term potentiation (up to 72 hours in GluN3A knockout mice, but only a few hours in wild-types) measured in vivo and di novo protein synthesis measured in vivo, and was blocked by the mTOR inhibitor rapamycin and the general protein synthesis blocker anisomycin. These findings identify GluN3A as regulator of synaptic translational control during memory encoding, and offer a potentially selective target for cognitive modulation. Further work is directed to determine whether memory domains such as memory discrimination or flexibility are compromised.

Pubmed:
34787081: Conde-Dusman MJ, Dey PN, Elía-Zudaire Ó, Rabaneda LG, García-Lira C, Grand T, Briz V, Velasco ER, Andero R, Niñerola S, Barco A, Paoletti P, Wesseling JF, Gardoni F, Tavalin SJ, Perez-Otaño I
Control of protein synthesis and memory by GluN3A-NMDA receptors through inhibition of GIT1/mTORC1 assembly.
De novo protein synthesis is required for synapse modifications underlying stable memory encoding. Yet neurons are highly compartmentalized cells and how protein synthesis can be regulated at the synapse level is unknown. Here, we characterize neuronal signaling complexes formed by the postsynaptic scaffold GIT1, the mechanistic target of rapamycin (mTOR) kinase, and Raptor that couple synaptic stimuli to mTOR-dependent protein synthesis; and identify NMDA receptors containing GluN3A subunits as key negative regulators of GIT1 binding to mTOR. Disruption of GIT1/mTOR complexes by enhancing GluN3A expression or silencing GIT1 inhibits synaptic mTOR activation and restricts the mTOR-dependent translation of specific activity-regulated mRNAs. Conversely, GluN3A removal enables complex formation, potentiates mTOR-dependent protein synthesis, and facilitates the consolidation of associative and spatial memories in mice. The memory enhancement becomes evident with light or spaced training, can be achieved by selectively deleting GluN3A from excitatory neurons during adulthood, and does not compromise other aspects of cognition such as memory flexibility or extinction. Our findings provide mechanistic insight into synaptic translational control and reveal a potentially selective target for cognitive enhancement.
Elife, 2021; 10

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BOARD NUMBER: S01-430

TRANSIENT MOLECULAR CHANGES AND LASTING SYNAPTIC EFFECTS IN THE CEREBELLUM OF THE NEONATAL PHENCYCLIDINE MOUSE MODEL OF SCHIZOPHRENIA

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Environmental perturbations during the first years of life are a major factor in psychiatric diseases. Phencyclidine (PCP), a drug of abuse, has psychomimetic effects, and neonatal subchronic administration of PCP in rodents leads to long-term behavioral changes relevant for schizophrenia. The cerebellum is increasingly recognized for its role in diverse cognitive functions. However, little is known about potential cerebellar changes in models of schizophrenia. Here, we analyzed the characteristics of the cerebellum in the neonatal subchronic PCP model. We found that, while the global cerebellar cytoarchitecture and Purkinje cell spontaneous spiking properties are unchanged, climbing fiber/Purkinje cell synaptic connectivity is increased in juvenile mice. Neonatal subchronic administration of PCP is accompanied by increased cFos expression, a marker of neuronal activity, and transient modification of the neuronal surfaceome in the cerebellum. The largest change is the increased expression of *Ctgf*, a gene previously suggested as a biomarker for schizophrenia. However, *Ctgf* overexpression driven by transient increased neuronal activity in the cerebellum using chemogenetics does not lead to increased climbing fiber/Purkinje cell connectivity. Thus other molecular signaling pathways modulated by PCP lead to the long-term modification of Purkinje cell innervation. Overall, our study shows that administration of the drug of abuse PCP during the developmental period of intense synaptogenesis and circuit remodeling has long-term and specific effects on Purkinje cell connectivity, and warrants the search for this type of synaptic changes in psychiatric diseases.

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TARGETED GENE THERAPY WITH FGF22 INTO SUBSETS OF SPINAL INTERNEURONS IMPROVES CIRCUIT PLASTICITY AND FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Functional recovery following incomplete spinal cord injury depends, among others, on circuit plasticity of supraspinal connections onto spinal relay neurons. Which molecules can promote synaptogenesis and circuit plasticity following spinal cord injury is still unclear. FGF22 is a presynaptic organizer that mediates synaptogenesis in the developing and mature nervous system. Its absence is associated with decreased synapse formation and maturation during post-injury circuit rewiring. Here we identify FGF22 as a powerful enhancer of synaptogenesis during post-injury circuit rewiring and demonstrate that targeted gene therapy with FGF22 to specific subsets of spinal interneurons allows for precise synaptogenesis, synapse maturation and functional recovery following injury. We also demonstrate that therapeutic treatment with FGF22 restores functional recovery with a critical therapeutic window of 1 day. This study highlights the use of pre-synaptic organizers as therapeutic agents following spinal cord injury.

Pubmed:
35169263: Aljovic A, Zhao S, Chahin M, de la Rosa C, Van Steenbergen V, Kerschensteiner M, Bareyre FM
A deep learning-based toolbox for Automated Limb Motion Analysis (ALMA) in murine models of neurological disorders.
In neuroscience research, the refined analysis of rodent locomotion is complex and cumbersome, and access to the technique is limited because of the necessity for expensive equipment. In this study, we implemented a new deep learning-based open-source toolbox for Automated Limb Motion Analysis (ALMA) that requires only basic behavioral equipment and an inexpensive camera. The ALMA toolbox enables the consistent and comprehensive analyses of locomotor kinematics and paw placement and can be applied to neurological conditions affecting the brain and spinal cord. We demonstrated that the ALMA toolbox can (1) robustly track the evolution of locomotor deficits after spinal cord injury, (2) sensitively detect locomotor abnormalities after traumatic brain injury, and (3) correctly predict disease onset in a multiple sclerosis model. We, therefore, established a broadly applicable automated and standardized approach that requires minimal financial and time commitments to facilitate the comprehensive analysis of locomotion in rodent disease models.
Commun Biol, 2022; 5
31391209: Bradley PM, Denecke CK, Aljovic A, Schmalz A, Kerschensteiner M, Bareyre FM
Corticospinal circuit remodeling after central nervous system injury is dependent on neuronal activity.
The remodeling of supraspinal axonal circuits mediates functional recovery after spinal cord injury. This process critically depends on the selection of appropriate synaptic connections between cortical projection and spinal relay neurons. To unravel the principles that guide this target selection, we used genetic and chemogenetic tools to modulate NMDA receptor (NMDAR) integrity and function, CREB-mediated transcription, and neuronal firing of relay neurons during injury-induced corticospinal remodeling. We show that NMDAR signaling and CREB-mediated transcription maintain nascent corticospinal tract (CST)-relay neuron contacts. These activity-dependent signals act during a defined period of circuit remodeling and do not affect mature or uninjured circuits. Furthermore, chemogenetic modulation of relay neuron activity reveals that the regrowing CST axons select their postsynaptic partners in a competitive manner and that preventing such activity-dependent shaping of corticospinal circuits limits motor recovery after spinal cord injury.
J Exp Med, 2019; 216
30991037: Denecke CK, Aljović A, Bareyre FM
Combining molecular intervention with in vivo imaging to untangle mechanisms of axon pathology and outgrowth following spinal cord injury.
In vivo imaging of the spinal cord has allowed the observation of single axons over relatively long periods in the living mouse.

After spinal cord injury, this methodology has helped to differentiate several pathological stages and tissue processes which impact axon morphology. In addition, the combination of in vivo imaging techniques with particular molecular intervention has shown that specific pathological axon changes can respond to distinct treatments. Combining in vivo imaging with molecular interventions is, hence, a powerful approach to extend our knowledge of the pathological processes leading to axonal loss. It also allows testing possible treatment options to, for example, increase axonal outgrowth. This review will provide a detailed description and critical examination of several studies that have combined the two methodologies in spinal cord injury research and pinpoints the specificities of the approach.
Exp Neurol, 2019; 318

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BOARD NUMBER: S01-432

HYDROGEN PEROXIDE DIFFUSIVITY IN VIVO IN THE BRAIN EXTRACELLULAR SPACE SUPPORTS VOLUME NEUROTRANSMISSION

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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A role for hydrogen peroxide (H_2O_2) as an intercellular signaling molecule and neuromodulator in the brain has become increasingly apparent, with evidence showing this biological oxidant to regulate neuronal polarity, connectivity, synaptic transmission, and tuning of neuronal networks. This notion is rooted in its ability to diffuse in the extracellular space, from the source of production to target. It is crucial to understand extracellular H_2O_2 concentration dynamics in the living brain and which intra and extracellular factors shape its diffusion pattern and half-life. We have achieved this by using a novel microsensor to measure H_2O_2 diffusivity in the brain extracellular matrix, as well as uptake and removal kinetics governed by the main antioxidant enzymatic systems. Extracellular H_2O_2 is rapidly removed and degraded intracellularly by glutathione peroxidase and peroxiredoxins in neural cells, and also by catalase in erythrocytes. This rapid uptake and removal results in an average half-life in the extracellular space of $t_{1/2}$ = 2.19 s. We determined the effective diffusion coefficient of H_2O_2 to be D^* = 2.49 x 10^{-5} cm^2 s^{-1}. This allows it to diffuse over 100 µm in the extracellular space within its half-life. Considering this, we can tentatively place H_2O_2 within the class of volume transmitters connecting all cell types with the complex network of brain tissue, regardless of whether the cells are physically connected. These quantitative details allow us to interpret the physiology of the redox signal and lay the pavement to address dysregulation in redox homeostasis associated with disease processes in the brain.

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BOARD NUMBER: S01-433

FUNCTIONAL ANALYSIS OF MICRORNAS IN AUDITORY BRAINSTEM OF MICE

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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MicroRNAs are small non-coding RNAs that posttranscriptionally regulate gene expression. They repress the translation of mRNAs into proteins via complementary base pairing to their target mRNAs.The microRNA 183 cluster, comprising of miRs-183, -96 and -182, plays an important role in the development and function of sensory systems. To gain insights into its function within the central auditory system we previously investigated a *Mir-183/96dko* mouse model in which miR-183 and miR-96 are deleted. Our data showed that *Mir-183/96* play a key role for proper development of auditory brainstem structures and synaptic transmission in an excitatory synapse of the auditory brainstem. Immunohistochemical analysis of AMPA receptor subunit GluA1, which is responsible for excitatory synaptic transmission and is also an established target of the miR-96, revealed significantly increased expression levels of GluaA1 at the calyx of Held, a central synapse in the auditory brainstem, of *Mir-183/96dko*. Here we set out to investigate the effects of *Mir-183/96* deficiency on inhibitory neurotransmission in the auditory brainstem, as miR-96 also targets many mRNAs important for inhibitory neurotransmission. To do so, we focused on synapses in the lateral superior olive (LSO) of the brainstem, which receives inhibitory input. By means of immunohistochemistry, we quantified the expression of miR-96 targeted genes important for inhibitory neurotransmission in the *Mir-183/96dko* mice. So far, our data revealed no significant differences in the expression of *KCC2* which is a critical mediator of synaptic inhibitory neurotransmission speculating there might be additional elements playing a role in its regulation besides microRNAs.

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NOVEL METHOD FOR RELIABLY MEASURING MINIATURE AND SPONTANEOUS POSTSYNAPTIC POTENTIALS/CURRENTS IN WHOLE-CELL PATCH CLAMP RECORDINGS

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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Aims: Measurements of amplitudes of miniature postsynaptic currents (mPSCs) or potentials (mPSPs) (minis), recorded with action potentials blocked, are commonly used to investigate synaptic transmission. Combined with measurements of cell membrane potential noise fluctuations, somatic minis' amplitudes offer a technically straightforward way to estimate distributions of quantal sizes, a key synaptic transmission parameter, as generally each mini is thought to be elicited by the spontaneous release of a single neurotransmitter vesicle. As the first step in devising such a method, we developed and described a novel mPSP/mPSC detection algorithm as part of our quantal analysis software called 'minis'. **Methods:** We tested the performance of the algorithm in detecting real minis and simulated minis added to real noise, using data from rat cortical slice whole-cell recordings, and compared it to two the most commonly-used minis detection algorithms in the field of synaptic function research: Mini Analysis (Blue Cell) and Clampfit (Molecular Devices). **Results:** This benchmarking analysis revealed superior detection performance by our algorithm. The release version of our software also offers great flexibility as it can be controlled through graphical and programming interfaces making it suitable for the needs of most individual researchers studying the synapse function. **Conclusions:** By highlighting common methodological pitfalls surrounding spontaneous synaptic signalling research and offering a transparent and objectively evaluated algorithm to minimise their impact, we hope to set an important methodological standard for the comparative assessment of other algorithms in the field of synaptic function research and contribute to increased openness and reproducibility in science.

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IL-1β TRIGGERS SYNAPTIC AND MEMORY DEFICITS IN HERPES SIMPLEX VIRUS TYPE-1-INFECTED MICE

POSTER SESSION 01 - SECTION: SYNAPTIC TRANSMISSION AND PLASTICITY IN HEALTH AND DISEASE

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[1]IRCCS, Fondazione Policlinico Universitario A. Gemelli, Rome, Italy, [2]Università Cattolica del Sacro Cuore, Department Of Neuroscience, Rome, Italy, [3]National Research Council (CNR), Institute Of Translational Pharmacology, Rome, Italy, [4]Istituto Superiore di Sanità, Department Of Infectious Diseases, Rome, Italy, [5]Laboratory affiliated to Istituto Pasteur Italia - Cenci Bolognetti Foundation, Department Of Public Health And Infectious Diseases, Sapienza University Of Rome, Rome, Italy

Aims: We recently developed a mouse model of multiple Herpes Simplex Virus Type-1 (HSV-1) replications within the brain exhibiting typical AD hallmarks, including amyloid-β and pTau accumulation, neuroinflammation. Here, we investigated the role of the interleukin 1β (IL-1β) to the HSV-1-induced synaptic dysfunction and cognitive decline. **Methods**: HSV-1 was inoculated via lip scarification in 1-month-old male C57BL/6 mice. After thermal stresses (TS) inducing virus reactivation, the AD-like phenotype was studied by behavioral tests, electrophysiology, Golgi staining and molecular analyses. **Results**: Mice undergone 2TS exhibited synaptic dysfunction assessed by deficits of: i) memory and learning; ii) long-term potentiation (LTP) at CA3-CA1 synapses; iii) pre- and post-synaptic proteins; iv) dendritic spine density; v) expression of plasticity-related genes. These effects were associated with increased levels of IL-1β (20.9±1.1 *vs*. 5.8±2.1 pg/mg in controls, $p<0.05$) that may negatively impinge on plasticity-related genes expression by modulating transcriptional repressors. In HSV-1-infected mice we found significant increases of MeCP2 and REST at both protein (+59% and +95%, respectively *vs*. mock, $p<0.05$) and mRNA (+4.8 and +3.8 folds, respectively *vs*. mock, $p<0.05$) levels. The pathogenic role of IL-1β on these effects was assessed by blocking IL-1 receptor with Anakinra. This treatment: i) counteracted the HSV-1-induced increases in MeCP2 and REST levels; ii) rescued plasticity-related genes expression; iii) ameliorated LTP (87.2±10.5% *vs*. 58.2±7.9%, in HSV-1; $p<0.05$) and memory performance (assessed by NOR test: 63.0±2.7% *vs*. 52.2±.1.8% in HSV-1; $p<0.05$). **Conclusions**: Our results suggest that IL-1β-activated pathways contributing to an AD-like phenotype in HSV-1-infected mice probably via MeCP2 and REST upregulation.

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ANTEROGRADE DELIVERY OF RAB10-ORGANELLES REGULATES THE SORTING OF INTERNALISED TRKB FOR RETROGRADE AXONAL TRANSPORT.

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Neurons process real-time information coming from axon terminals to coordinate complex cellular responses, including gene expression, growth and plasticity. Input from distal axons is encoded as a stream of endocytic organelles termed signalling endosomes, which are targeted to the soma. Formation of these organelles depends on target-derived molecules, such as brain-derived neurotrophic factor (BDNF), which is recognised by TrkB receptors on the plasma membrane, endocytosed and transported to the soma along the microtubules network. Notwithstanding its physiological and neuropathological importance, the mechanism controlling the sorting of TrkB to signalling endosomes is currently unknown. In this work, we used primary mouse brain neurons and advanced confocal microscopy to uncover the small GTPase Rab10 as critical for TrkB sorting and propagation of BDNF signalling from axon terminals to the soma. We manipulated the expression and activity of Rab10 in neurons cultured in microfluidic devices, and study axonal transport, sorting of receptors and recruitment of motor proteins. Our data demonstrate that Rab10 defines a class of axonal organelles, which are rapidly mobilised towards the axon terminal upon BDNF stimulation, enabling the axon to fine-tune retrograde signalling depending on BDNF availability at the synapse. These results help to clarify the neuroprotective phenotype recently associated to Rab10 polymorphisms in Alzheimer's disease, providing novel therapeutic targets for neurodegenerative conditions.

Pubmed:

32928890: González-Gutiérrez A, Lazo OM, Bronfman FC
The Rab5-Rab11 Endosomal Pathway is Required for BDNF-Induced CREB Transcriptional Regulation in Hippocampal Neurons.
Brain-derived neurotrophic factor (BDNF) is a key regulator of the morphology and connectivity of central neurons. We have previously shown that BDNF/TrkB signaling regulates the activity and mobility of the GTPases Rab5 and Rab11, which in turn determine the postendocytic sorting of signaling TrkB receptors. Moreover, decreased Rab5 or Rab11 activity inhibits BDNF-induced dendritic branching. Whether Rab5 or Rab11 activity is important for local events only or for regulating nuclear signaling and gene expression is unknown. Here, we investigated, in rat hippocampal neuronal cultures derived from embryos of unknown sex, whether BDNF-induced signaling cascades are altered when early and recycling endosomes are disrupted by the expression of dominant-negative mutants of Rab5 and Rab11. The activity of both Rab5 and Rab11 was required for sustained activity of Erk1/2 and nuclear CREB phosphorylation, and increased transcription of a BDNF-dependent program of gene expression containing CRE binding sites, which includes activity-regulated genes such as , , , , and , and growth and survival genes such as and Based on our results, we propose that early and recycling endosomes provide a platform for the integration of neurotrophic signaling from the plasma membrane to the nucleus in neurons, and that this mechanism is likely to regulate neuronal plasticity and survival. BDNF is a neurotrophic factor that regulates plastic changes in the brain, including dendritic growth. The cellular and molecular mechanisms underlying this process are not completely understood. Our results uncover the cellular requirements that central neurons possess to integrate the plasma membrane into nuclear signaling in neurons. Our results indicate that the endosomal pathway is required for the signaling cascade initiated by BDNF and its receptors at the plasma membrane to modulate BDNF-dependent gene expression and neuronal dendritic growth mediated by the CREB transcription factor. CREB is a key transcription factor regulating circuit development and learning and memory.
J Neurosci, 2020; 40
31670447: Surana S, Villarroel-Campos D, Lazo OM, Moretto E, Tosolini AP, Rhymes ER, Richter S, Sleigh JN, Schiavo G
The evolution of the axonal transport toolkit.
Neurons are highly polarized cells that critically depend on long-range, bidirectional transport between the cell body and

synapse for their function. This continual and highly coordinated trafficking process, which takes place via the axon, has fascinated researchers since the early 20th century. Ramon y Cajal first proposed the existence of axonal trafficking of biological material after observing that dissociation of the axon from the cell body led to neuronal degeneration. Since these first indirect observations, the field has come a long way in its understanding of this fundamental process. However, these advances in our knowledge have been aided by breakthroughs in other scientific disciplines, as well as the parallel development of novel tools, techniques and model systems. In this review, we summarize the evolution of tools used to study axonal transport and discuss how their deployment has refined our understanding of this process. We also highlight innovative tools currently being developed and how their addition to the available axonal transport toolkit might help to address key outstanding questions.
Traffic, 2020; 21
30176054: Villarroel-Campos D, Schiavo G, Lazo OM
The many disguises of the signalling endosome.
Neurons are highly complex and polarised cells that must overcome a series of logistic challenges to maintain homeostasis across their morphological domains. A very clear example is the propagation of neurotrophic signalling from distal axons, where target-released neurotrophins bind to their receptors and initiate signalling, towards the cell body, where nuclear and cytosolic responses are integrated. The mechanisms of propagation of neurotrophic signalling have been extensively studied and, eventually, the model of a 'signalling endosome', transporting activated receptors and associated complexes, has emerged. Nevertheless, the exact nature of this organelle remains elusive. In this Review, we examine the evidence for the retrograde transport of neurotrophins and their receptors in endosomes, outline some of their diverse physiological and pathological roles, and discuss the main interactors, morphological features and trafficking destinations of a highly flexible endosomal signalling organelle with multiple molecular signatures.
FEBS Lett, 2018; 592
26965219: Zepeda R, Contreras V, Pissani C, Stack K, Vargas M, Owen GI, Lazo OM, Bronfman FC
Venlafaxine treatment after endothelin-1-induced cortical stroke modulates growth factor expression and reduces tissue damage in rats.
Neuromodulators, such as antidepressants, may contribute to neuroprotection by modulating growth factor expression to exert anti-inflammatory effects and to support neuronal plasticity after stroke. Our objective was to study whether early treatment with venlafaxine, a serotonin-norepinephrine reuptake inhibitor, modulates growth factor expression and positively contributes to reducing the volume of infarcted brain tissue resulting in increased functional recovery. We studied the expression of BDNF, FGF2 and TGF-β1 by examining their mRNA and protein levels and cellular distribution using quantitative confocal microscopy at 5 days after venlafaxine treatment in control and infarcted brains. Venlafaxine treatment did not change the expression of these growth factors in sham rats. In infarcted rats, BDNF mRNA and protein levels were reduced, while the mRNA and protein levels of FGF2 and TGF-β1 were increased. Venlafaxine treatment potentiated all of the changes that were induced by cortical stroke alone. In particular, increased levels of FGF2 and TGF-β1 were observed in astrocytes at 5 days after stroke induction, and these increases were correlated with decreased astrogliosis (measured by GFAP) and increased synaptophysin immunostaining at twenty-one days after stroke in venlafaxine-treated rats. Finally, we show that venlafaxine reduced infarct volume after stroke resulting in increased functional recovery, which was measured using ladder rung motor tests, at 21 days after stroke. Our results indicate that the early oral administration of venlafaxine positively contributes to neuroprotection during the acute and late events that follow stroke.
Neuropharmacology, 2016; 107
24569882: Escudero CA, Lazo OM, Galleguillos C, Parraguez JI, Lopez-Verrilli MA, Cabeza C, Leon L, Saeed U, Retamal C, Gonzalez A, Marzolo MP, Carter BD, Court FA, Bronfman FC
The p75 neurotrophin receptor evades the endolysosomal route in neuronal cells, favouring multivesicular bodies specialised for exosomal release.
The p75 neurotrophin receptor (p75, also known as NGFR) is a multifaceted signalling receptor that regulates neuronal physiology, including neurite outgrowth, and survival and death decisions. A key cellular aspect regulating neurotrophin signalling is the intracellular trafficking of their receptors; however, the post-endocytic trafficking of p75 is poorly defined. We used sympathetic neurons and rat PC12 cells to study the mechanism of internalisation and post-endocytic trafficking of p75. We found that p75 internalisation depended on the clathrin adaptor protein AP2 and on dynamin. More surprisingly, p75 evaded the lysosomal route at the level of the early endosome, instead accumulating in two different types of endosomes, Rab11-positive endosomes and multivesicular bodies (MVBs) positive for CD63, a marker of the exosomal pathway. Consistently, depolarisation by KCl induced the liberation of previously endocytosed full-length p75 into the extracellular medium in exosomes. Thus, p75 defines a subpopulation of MVBs that does not mature to lysosomes and is available for exosomal release by neuronal cells.
J Cell Sci, 2014; 127

24668469: Bronfman FC, Lazo OM, Flores C, Escudero CA
Spatiotemporal intracellular dynamics of neurotrophin and its receptors. Implications for neurotrophin signaling and neuronal function.
Neurons possess a polarized morphology specialized to contribute to neuronal networks, and this morphology imposes an important challenge for neuronal signaling and communication. The physiology of the network is regulated by neurotrophic factors that are secreted in an activity-dependent manner modulating neuronal connectivity. Neurotrophins are a well-known family of neurotrophic factors that, together with their cognate receptors, the Trks and the p75 neurotrophin receptor, regulate neuronal plasticity and survival and determine the neuronal phenotype in healthy and regenerating neurons. Is it now becoming clear that neurotrophin signaling and vesicular transport are coordinated to modify neuronal function because disturbances of vesicular transport mechanisms lead to disturbed neurotrophin signaling and to diseases of the nervous system. This chapter summarizes our current understanding of how the regulated secretion of neurotrophin, the distribution of neurotrophin receptors in different locations of neurons, and the intracellular transport of neurotrophin-induced signaling in distal processes are achieved to allow coordinated neurotrophin signaling in the cell body and axons.
Handb Exp Pharmacol, 2014; 220
23554492: Lazo OM, Gonzalez A, Ascaño M, Kuruvilla R, Couve A, Bronfman FC
BDNF regulates Rab11-mediated recycling endosome dynamics to induce dendritic branching.
Dendritic arborization of neurons is regulated by brain-derived neurotrophic factor (BDNF) together with its receptor, TrkB. Endocytosis is required for dendritic branching and regulates TrkB signaling, but how postendocytic trafficking determines the neuronal response to BDNF is not well understood. The monomeric GTPase Rab11 regulates the dynamics of recycling endosomes and local delivery of receptors to specific dendritic compartments. We investigated whether Rab11-dependent trafficking of TrkB in dendrites regulates BDNF-induced dendritic branching in rat hippocampal neurons. We report that TrkB in dendrites is a cargo for Rab11 endosomes and that both Rab11 and its effector, MyoVb, are required for BDNF/TrkB-induced dendritic branching. In addition, BDNF induces the accumulation of Rab11-positive endosomes and GTP-bound Rab11 in dendrites and the expression of a constitutively active mutant of Rab11 is sufficient to increase dendritic branching by increasing TrkB localization in dendrites and enhancing sensitization to endogenous BDNF. We propose that Rab11-dependent dendritic recycling provides a mechanism to retain TrkB in dendrites and to increase local signaling to regulate arborization.
J Neurosci, 2013; 33
22458984: Cabeza C, Figueroa A, Lazo OM, Galleguillos C, Pissani C, Klein A, Gonzalez-Billault C, Inestrosa NC, Alvarez AR, Zanlungo S, Bronfman FC
Cholinergic abnormalities, endosomal alterations and up-regulation of nerve growth factor signaling in Niemann-Pick type C disease.
Neurotrophins and their receptors regulate several aspects of the developing and mature nervous system, including neuronal morphology and survival. Neurotrophin receptors are active in signaling endosomes, which are organelles that propagate neurotrophin signaling along neuronal processes. Defects in the Npc1 gene are associated with the accumulation of cholesterol and lipids in late endosomes and lysosomes, leading to neurodegeneration and Niemann-Pick type C (NPC) disease. The aim of this work was to assess whether the endosomal and lysosomal alterations observed in NPC disease disrupt neurotrophin signaling. As models, we used i) NPC1-deficient mice to evaluate the central cholinergic septo-hippocampal pathway and its response to nerve growth factor (NGF) after axotomy and ii) PC12 cells treated with U18666A, a pharmacological cellular model of NPC, stimulated with NGF.
Mol Neurodegener, 2012; 7
19953569: Guillemard V, Ivanisevic L, Garcia AG, Scholten V, Lazo OM, Bronfman FC, Saragovi HU
An agonistic mAb directed to the TrkC receptor juxtamembrane region defines a trophic hot spot and interactions with p75 coreceptors.
The D5 domain of TrkC receptors is a docking site for Neurotrophin-3 (NT-3), but other domains may be relevant for function or harmonizing signals with p75(NTR) coreceptors. We report a monoclonal antibody (mAb) 2B7 targeting the juxtamembrane domain of TrkC. mAb 2B7 binds to murine and human TrkC receptors and is a functional agonist that affords activation of TrkC, AKT, and MAPK. These signals result in cell survival but not in cellular differentiation. Monomeric 2B7 Fabs also affords cell survival. Binding of 2B7 mAb and 2B7 Fabs to TrkC are blocked by NT-3 in a dose-dependent manner but not by pro-NT-3. Expression of p75(NTR) coreceptors on the cell surface block the binding and function of mAb 2B7, whereas NT-3 binding and function are enhanced. mAb 2B7 defines a previously unknown neurotrophin receptor functional hot spot; that exclusively generates survival signals; that can be activated by non-dimeric ligands; and potentially unmasks a site for p75-TrkC interactions.
Dev Neurobiol, 2010; 70
20205865: Lazo OM, Mauna JC, Pissani CA, Inestrosa NC, Bronfman FC

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Axotomy-induced neurotrophic withdrawal causes the loss of phenotypic differentiation and downregulation of NGF signalling, but not death of septal cholinergic neurons.

Septal cholinergic neurons account for most of the cholinergic innervations of the hippocampus, playing a key role in the regulation of hippocampal synaptic activity. Disruption of the septo-hippocampal pathway by an experimental transection of the fimbria-fornix drastically reduces the target-derived trophic support received by cholinergic septal neurons, mainly nerve growth factor (NGF) from the hippocampus. Axotomy of cholinergic neurons induces a reduction in the number of neurons positive for cholinergic markers in the medial septum. In several studies, the reduction of cholinergic markers has been interpreted as analogous to the neurodegeneration of cholinergic cells, ruling out the possibility that neurons lose their cholinergic phenotype without dying. Understanding the mechanism of cholinergic neurodegeneration after axotomy is relevant, since this paradigm has been extensively explored as an animal model of the cholinergic impairment observed in neuropathologies such as Alzheimer's disease.The principal aim of this study was to evaluate, using modern quantitative confocal microscopy, neurodegenerative changes in septal cholinergic neurons after axotomy and to assess their response to delayed infusion of NGF in rats.

Mol Neurodegener, 2010; 5

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BOARD NUMBER: S01-437

SUCELLULAR NETWORKS OF SECOND MESSENGERS SHAPE THE CONNECTIVITY OF THE VISUAL SYSTEM.

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Retinal ganglion cell (RGC) wiring into the brain requires axon pathfinding molecules and their downstream signaling pathways actors including cGMP, cAMP and calcium. These second messengers are also involved in a wide range of cellular processes. How they achieve specificity for each of their downstream pathway remains elusive. The spatial regulation of second messengers appeared as a flexible strategy to specifically activate downstream effectors. Using cell compartment-restricted biosensors and genetically-encoded scavengers to probe and manipulate second messengers with subcellular resolution, we identified the spatio-temporal features of cGMP, cAMP and calcium signals shaping RGC axon connectivity. Focusing on two axon repellents, slit1 and ephrinA5, and investigating lipid rafts, a microdomain of the plasma membrane, we demonstrated that these guidance molecules control second messengers in distinct compartments. Whereas the repellent activity of ephrinA5 requires the modulation of cGMP, cAMP and calcium concentration in the vicinity of lipid rafts, the signals critical for slit1-dependent axon repulsion sit in the membrane excluded from this domain. In addition, combining local biosensors and scavengers enabled to demonstrate that seconds messengers interactions differ in and outside lipid rafts. Finally, recent data suggest that altering lipid raft-restricted calcium and cGMP signaling *in vivo* affects the terminal arbors of RGC axons, in agreement with a role in ephrinA signaling. Overall, these results pave the way to understand how a limited set of signaling molecules specifically regulate their downstream cellular processes. It also provides a description of the signals involved in axonal response to repellent cues, together with their interactions.

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PYK2 AND MBD2 NUCLEAR TRANSLOCATION AND INTERACTION IN HIPPOCAMPAL NEURONS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Pyk2 is a non-receptor calcium-dependent protein-tyrosine kinase, highly expressed in the hippocampus and able to translocate to the nucleus upon neuronal activity. MBD2 is a protein from the methyl-CpG binding domain (MBD) family, which can indirectly repress transcription and was shown to interact with Pyk2 in osteocytes. Here we aimed to assess the impact of glutamate stimulation of hippocampal neurons on (i) the nuclear translocation of Pyk2 and MBD2, (ii) their interaction, and (iii) to dissect the signaling pathway(s) involved. We observed that Pyk2 accumulates in the nucleus of hippocampal neurons in culture following stimulation of glutamate receptors, in a NMDA receptors- and calcineurin-dependent manner. MBD2 showed a similar translocation pattern. We assessed the interaction between Pyk2 and MBD2 with a proximity ligation assay (PLA) and found it was increased by glutamate stimulation. Translocation and increased interaction between Pyk2 and MBD2, may be a mechanism by which synaptic activity leads to changes in gene transcription and long-lasting alterations of neuronal properties.

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ENHANCING POTASSIUM-CHLORIDE CO-TRANSPORTER-2 (KCC2) FUNCTION IN NEURONS BY TARGETING PROTEIN-PROTEIN INTERACTIONS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Fast synaptic inhibition in the adult brain is mediated by the neurotransmitter γ-aminobutyric acid (GABA). The hyperpolarizing action of GABA requires low intracellular chloride (Cl^-) which is maintained by the potassium-chloride co-transporter 2 (KCC2) in mature neurons. KCC2 protein expression and/or function can be regulated by its interactome, which can affect its ability to extrude Cl^- ions. Altered Cl^- homeostasis is associated with various neurological disorders including autism spectrum disorder (ASD). Using two different approaches, we are investigating strategies to promote KCC2 function by targeting its interaction with novel interacting partners, namely Protein kinase C and casein kinase substrate in neurons protein 1 (PACSIN1) and 14-3-3. Our first approach used peptide-based protein-protein interaction inhibitors (PPI inhibitors) which prevent KCC2-PACSIN1 interaction. PACSIN1 is a neuron-specific protein that has been identified as a negative regulator of KCC2. Therefore, inhibiting KCC2-PACSIN1 interaction using PPI inhibitors can provide a neuron-specific therapeutic strategy to rescue KCC2. I have identified and validated two PPI inhibitors that result in hyperpolarized E_{GABA} in primary neurons indicating enhanced KCC2 function. The second approach used genetic manipulation of the ε, γ, and θ isoforms of 14-3-3 which interact with KCC2 in co-immunoprecipitation assay. Overexpression of 14-3-3 γ isoform resulted in reduced KCC2 expression in primary neurons, revealing potential targets for the development of new PPI inhibitors. The remainder of this study will examine the mechanisms underlying regulation of KCC2 by these interacting partners and further development of PPI inhibitors to treat disrupted KCC2 function in neurological disorders.

Pubmed:
33097285: Raveendran VA, Pressey JC, Woodin MA
A Novel Small Molecule Targets NKCC1 To Restore Synaptic Inhibition.
Finely tuned excitation-inhibition balance is essential for proper brain function, and loss of balance resulting from reduced synaptic inhibition is associated with neurological disorders. Savardi and colleagues have discovered a novel inhibitor of a cation-chloride transporter that is required for synaptic inhibition, and which restores behaviors associated with Down syndrome (DS) and autism spectrum disorder (ASD).
Trends Pharmacol Sci, 2020; 41
29934347: Lizama BN, Palubinsky AM, Raveendran VA, Moore AM, Federspiel JD, Codreanu SG, Liebler DC, McLaughlin B
Neuronal Preconditioning Requires the Mitophagic Activity of C-terminus of HSC70-Interacting Protein.
The C terminus of HSC70-interacting protein (CHIP,) is a ubiquitously expressed cytosolic E3-ubiquitin ligase. CHIP-deficient mice exhibit cardiovascular stress and motor dysfunction before premature death. This phenotype is more consistent with animal models in which master regulators of autophagy are affected rather than with the mild phenotype of classic E3-ubiquitin ligase mutants. The cellular and biochemical events that contribute to neurodegeneration and premature aging in CHIP KO models remain poorly understood. Electron and fluorescent microscopy demonstrates that CHIP deficiency is associated with greater numbers of mitochondria, but these organelles are swollen and misshapen. Acute bioenergetic stress triggers CHIP induction and relocalization to mitochondria, where it plays a role in the removal of damaged organelles. This mitochondrial clearance is required for protection following low-level bioenergetic stress in neurons. CHIP expression overlaps with stabilization of the redox stress sensor PTEN-inducible kinase 1 (PINK1) and is associated with increased LC3-mediated mitophagy. Introducing human promoter-driven vectors with mutations in either the E3 ligase or tetracopeptide repeat domains of CHIP in primary neurons derived from CHIP-null animals enhances CHIP accumulation at mitochondria. Exposure to autophagy inhibitors suggests that the increase in mitochondrial CHIP is likely due to diminished clearance of these CHIP-tagged organelles. Proteomic analysis of WT and CHIP KO mouse brains (four male, four female per genotype)

reveals proteins essential for maintaining energetic, redox, and mitochondrial homeostasis undergo significant genotype-dependent expression changes. Together, these data support the use of CHIP-deficient animals as a predictive model of age-related degeneration with selective neuronal proteotoxicity and mitochondrial failure. Mitochondria are recognized as central determinants of neuronal function and survival. We demonstrate that C terminus of HSC70-Interacting Protein (CHIP) is critical for neuronal responses to stress. CHIP upregulation and localization to mitochondria is required for mitochondrial autophagy (mitophagy). Unlike other disease-associated E3 ligases such as Parkin and Mahogunin, CHIP controls homeostatic and stress-induced removal of mitochondria. Although CHIP deletion results in greater numbers of mitochondria, these organelles have distorted inner membranes without clear cristae. Neuronal cultures derived from animals lacking CHIP are more vulnerable to acute injuries and transient loss of CHIP renders neurons incapable of mounting a protective response after low-level stress. Together, these data suggest that CHIP is an essential regulator of mitochondrial number, cell signaling, and survival.
J Neurosci, 2018; 38

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ESTABLISHMENT OF FUNCTIONAL INTERNALLY TAGGED SORTING RECEPTORS OF THE VPS10P DOMAIN RECEPTOR FAMILY AS A NOVEL TOOL TO INVESTIGATE INTRACELLULAR NEURONAL SORTING MECHANISMS IN VIVO

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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The Vps10p domain receptor family consists of Sortilin, SorLA, SorCS1, SorCS2 and SorCS3, which all convey intracellular sorting and internalization of specific cargo proteins in somatodendritic compartments of neurons. They interact with their N-terminal Vps10p domains with several ligands, such as neurotrophic factors and neuropeptides, and bind different cytosolic adaptor proteins with their cytoplasmic moiety. Malfunctioning of Vps10p domain receptors has been associated with a number of neurological and neuropsychological diseases including Alzheimer's disease, Huntington's disease and epilepsy. The investigation of specific cellular functions of these receptors is crucial to understand neuronal intracellular sorting mechanisms and their impact on brain function and dysfunction. To study these receptors *in vivo* e.g. in live cell imaging experiments, and without the need of specific antibodies, we established fully functional fluorophore tagged recombinant versions of all family members. Accordingly, tag positioning had to be selected carefully in order to not interfere with any function of the receptors domains. Since simple N- or C-terminal tags are not applicable due to the functional interaction sites of the receptors at either terminus an internal non-interfering insertion site for the fluorophore tag was chosen. To ensure correct protein folding the structures of the recombinant receptors were predicted computationally, expression constructs generated and exit of tagged receptors from the endoplasmic reticulum was shown in transfected cells. With these recombinant proteins we then demonstrated the subcellular localizations of the different receptors and their ability to internalize the brain derived neurotrophic factor (BDNF) independent of the tropomyosin receptor kinase B (TrkB).

Pubmed:

35183222: Binkle L, Klein M, Borgmeyer U, Kuhl D, Hermey G
The adaptor protein PICK1 targets the sorting receptor SorLA.
SorLA is a member of the Vps10p-domain (Vps10p-D) receptor family of type-I transmembrane proteins conveying neuronal endosomal sorting. The extracellular/luminal moiety of SorLA has a unique mosaic domain composition and interacts with a large number of different and partially unrelated ligands, including the amyloid precursor protein as well as amyloid-β. Several studies support a strong association of SorLA with sporadic and familial forms of Alzheimer's disease (AD). Although SorLA seems to be an important factor in AD, the large number of different ligands suggests a role as a neuronal multifunctional receptor with additional intracellular sorting capacities. Therefore, understanding the determinants of SorLA's subcellular targeting might be pertinent for understanding neuronal endosomal sorting mechanisms in general. A number of cytosolic adaptor proteins have already been demonstrated to determine intracellular trafficking of SorLA. Most of these adaptors and several ligands of the extracellular/luminal moiety are shared with the Vps10p-D receptor Sortilin. Although SorLA and Sortilin show both a predominant intracellular and endosomal localization, they are targeted to different endosomal compartments. Thus, independent adaptor proteins may convey their differential endosomal targeting. Here, we hypothesized that Sortilin and SorLA interact with the cytosolic adaptors PSD95 and PICK1 which have been shown to bind the Vps10p-D receptor SorCS3. We observed only an interaction for SorLA and PICK1 in mammalian-two-hybrid, pull-down and cellular recruitment experiments. We demonstrate by mutational analysis that the C-terminal minimal PDZ domain binding motif VIA of SorLA mediates the interaction. Moreover, we show co-localization of SorLA and PICK1 at vesicular structures in primary neurons. Although the physiological role of the interaction between PICK1 and SorLA remains unsolved, our study suggests that PICK1 partakes in regulating SorLA's intracellular itinerary.
Mol Brain, 2022; 15
34964690: Klein M, Kaleem A, Oetjen S, Wünkhaus D, Binkle L, Schilling S, Gjorgjieva M, Scholz R, Gruber-Schoffnegger D, Storch S, Kins S, Drewes G, Hoffmeister-Ullerich S, Kuhl D, Hermey G

Converging roles of PSENEN/PEN2 and CLN3 in the autophagy-lysosome system.
PSENEN/PEN2 is the smallest subunit of the γ-secretase complex, an intramembrane protease that cleaves proteins within their transmembrane domains. Mutations in components of the γ-secretase underlie familial Alzheimer disease. In addition to its proteolytic activity, supplementary, γ-secretase independent, functions in the macroautophagy/autophagy-lysosome system have been proposed. Here, we screened for PSENEN-interacting proteins and identified CLN3. Mutations in are causative for juvenile neuronal ceroid lipofuscinosis, a rare lysosomal storage disorder considered the most common neurodegenerative disease in children. As mutations in the and genes cause different neurodegenerative diseases, understanding shared cellular functions of both proteins might be pertinent for understanding general cellular mechanisms underlying neurodegeneration. We hypothesized that CLN3 modulates γ-secretase activity and that PSENEN and CLN3 play associated roles in the autophagy-lysosome system. We applied CRISPR gene-editing and obtained independent isogenic HeLa knockout cell lines for and . Following previous studies, we demonstrate that PSENEN is essential for forming a functional γ-secretase complex and is indispensable for γ-secretase activity. In contrast, CLN3 does not modulate γ-secretase activity to a significant degree. We observed in - and -knockout cells corresponding alterations in the autophagy-lysosome system. These include reduced activity of lysosomal enzymes and lysosome number, an increased number of autophagosomes, increased lysosome-autophagosome fusion, and elevated levels of TFEB (transcription factor EB). Our study strongly suggests converging roles of PSENEN and CLN3 in the autophagy-lysosome system in a γ-secretase activity-independent manner, supporting the idea of common cytopathological processes underlying different neurodegenerative diseases.
Autophagy, 2021;
32581775: Klein M, Lohr C, Droste D
Age-Dependent Heterogeneity of Murine Olfactory Bulb Astrocytes.
Astrocytes have a high impact on the structure of the central nervous system, as they control neural activity, development, and plasticity. Heterogeneity of astrocytes has been shown before, but so far only a few studies have demonstrated heterogeneous morphology of astrocytes concerning aging. In this study, we examined morphologic differences of astrocyte subpopulations in adult mice and the progression of these differences with age. We surveyed astrocytes in olfactory bulb slices of mice aged 3 months, 1 year and 2 years (three animals each age group), based on their appearance in anti-GFAP immunostaining. Based on this data we established three different types of astrocytes: type I (stellate), type II (elliptic), and type III (squid-like). We found that with the advanced age of the mice, astrocytes grow in size and complexity. Major changes occurred between the ages of 3 months and 1 year, while between 1 and 2 years no significant development in cell size and complexity could be detected. Our results show that astrocytes in the olfactory bulb are heterogeneous and undergo morphological transformation until late adolescence but not upon senescence. Structural plasticity is further substantiated by the expression of vimentin in some astrocyte processes in all age groups.
Front Aging Neurosci, 2020; 12

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LATERAL SODIUM DIFFUSION IN SPINY DENDRITES

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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During glutamatergic synaptic transmission, influx of Na^+ through voltage- and ligand gated ion channels drives the depolarization of the postsynaptic site. A fast clearance of Na^+ from its point of entry is crucial for the cell, as prolonged increased intracellular Na^+ concentrations lead to persistent depolarizations. Previous work from our laboratory has established that efficient recovery from global Na^+ increases requires activity of the Na^+/K^+-ATPase, whereas local Na^+ increases are mainly cleared via lateral diffusion (Mondragão *et al.,* J Physiol 2016*).* Based on these findings, the goal of the present study was to determine the apparent diffusion constant of Na^+ along dendrites. To this end, we performed local glutamate iontophoresis coupled with whole cell patch-clamp and two-photon Na^+ imaging in the line-scan mode of CA1 pyramidal neurons in organotypic slices of the mouse brain. Combining Na^+ imaging with 3D-reconstructions of dendrites allowed us to analyze the influence of morphological properties on Na^+ dynamics. We found that Na^+ diffuses with an apparent diffusion coefficient (D_{Na+}) of 200-400 µm²/s along spiny dendrites, which is considerably less than values reported from muscle cells (600 µm²/s; Kushmerick and Podolsky 1969) or along large lizard axons (1300 µm²/s; David et al. 1997). Furthermore, our data indicate that D_{Na+} is dependent on spine density. Taken together, our study demonstrates that Na^+ diffusion along dendrites is much slower than previously thought. Moreover, it provides evidence that the presence and density of spines shape the spatiotemporal dynamics of activity-induced Na^+ signals in dendrites.

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SATELLITE GLIAL CELL-PROPRIOCEPTOR INTERACTIONS IN DORSAL ROOT GANGLIA

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Proprioception is a sensory modality that informs the central nervous system about the precise body and limb position in order to estimate the state of movements. Thus, proprioceptive neurons play a major role in locomotion and posture. Their cell bodies are located in the dorsal root ganglia (DRG) and are tightly enwrapped by satellite glial cells (SGCs). SGCs can sense neuronal activity through their Gq protein coupled receptors (GqGPCRs) located at their plasma membrane. Under physiological conditions, SGCs maintain neuronal extracellular homeostasis *via* their hemichannels, channels, transporters. Our laboratory has found that *ex vivo* activation of GqGPCR Ca^{2+} signaling in SGCs leads to activation of proprioceptors *via* a purinergic system. To examine whether such SGC-induced Ca^{2+} elevations in proprioceptors could impact proprioceptive function in a more physiologically-relevant *in vivo* context, we used a transgenic mouse line expressing a DREADD receptor under the control of the GFAP promoter. We observed several abnormal limb positioning in DREADD mice as compared to controls, suggesting that SGC GqGPCR Ca^{2+} signaling alters sensorimotor function *in vivo*. However, a contribution of astrocytes on spinal interneuron and/or motor neuron functions or on brain cannot be excluded. To overcome this technical limitation, we have developed an approach based on Adeno-Associated Viral delivery of transgene selectively in SGCs of single DRG *in vivo*. This approach will allow us to avoid possible confounding results due to DREADD expression in glial cells elswhere than in DRG. Our results have implications in diseases with DRG SGC impairments and sensorimotor disorders.

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BIOPHYSICAL PROPERTIES OF HIPPOCALCIN SIGNALING IN DIFFERENT SUB-CELLULAR COMPARTMENTS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Hippocalcin (HPCA) is a neuronal calcium sensor protein controlling neuronal functions in many types of cells. Ca^{2+} binding of HPCA leads to its conformational changes and highly heterogeneous translocation from a cytosol to different sub-cellular compartments. In spite of the importance of HPCA signaling in neuronal functioning, biophysical properties of HPCA translocation in a physiological range of Ca^{2+} concentrations in the cytosol ($[Ca^{2+}]_i$) have not been studied yet. In this work, high-resolution confocal microscopy was used to follow and quantify spatio-temporal patterns of both $[Ca^{2+}]_i$ and translocation of fluorescently-labeled HPCA in HEK 293 cells. Laser-induced Ca^{2+} uncaging from NP-EGTA was used to control $[Ca^{2+}]_i$. Fast homogeneous increase in $[Ca^{2+}]_i$, induced by the uncaging, resulted in heterogeneous HPCA translocation to certain sites in the plasma membrane and trans-Golgi network indicating diverse affinity of different membranous loci to Ca^{2+}-bound HPCA. Slow decay of $[Ca^{2+}]_i$. after uncaging allowed to obtain a Ca^{2+}-dependency of HPCA translocation to different plasma membrane regions and trans-Golgi network. Half-maximal translocation for all regions was observed in a physiological range of $[Ca^{2+}]_i$ with different parameters obtained by fitting the Ca^{2+}-dependency by Hill equation. We also demonstrated a substantially stronger translocation to the plasma membrane compared to the trans-Golgi network with virtually no translocation to other intracellular membranes. We have concluded that in a physiological range of Ca^{2+} concentrations in the cytosol HPCA can function as a compartment-specific Ca^{2+} sensor having a wide dynamic range for a precise decoding of complex spatio-temporal patterns of $[Ca^{2+}]_i$ changes.

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MODULATING AGE-DEPENDENT CHANGES OF NEURAL MECHANICAL PROPERTIES AFFECTS MECHANOSIGNALING AND SYNAPTIC INTEGRITY IN NEURON-GLIA COCULTURES

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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The ability to sense mechanical stimuli and confer this information into adaptive cellular responses is relevant for many biological processes. In neurons, changes of extracellular matrix composition, ion homeostasis or cytoskeletal rearrangements within cells also generate mechanical forces. Disease features of trauma, inflammation or neurodegenerative diseases include alterations in mechanical properties of the brain and also pharmacological treatment or natural aging processes change membrane lipid composition and brain tissue rigidity but little is known about resulting consequences for cellular signaling and function. Here, we use several strategies to model alterations in brain tissue rigidity and mechanical properties of neuronal membranes to elucidate possible impacts on neuronal function. Neuron-glia cocultures were either grown on hydrogels with adjustable stiffness grades to model different tissue mechanical properties or pharmacological treatment was applied to reduce the cholesterol content of neuronal membranes in order to mimic age-related changes of neuronal membrane properties. We characterized neuron-glia cocultures based on morphological features, protein synthesis rates and key players in mechanosignaling cascades with cells either grown on altered substrate rigidity or with reduced cholesterol content. Indeed, we can confirm the dependency of synaptic integrity on membrane cholesterol and we identify alteration in the activation of key players in mechanosignaling cascades. These results further support the notion that mechanosignaling is relevant for age- and disease-induced changes in the central nervous system cells contributing to changes in neuronal function and survival in disease conditions and in the aging brain.

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EXPERIMENTAL AND COMPUTATIONAL ANALYSIS OF BIASED AGONISM ON FULL-LENGTH AND A C-TERMINALLY TRUNCATED ADENOSINE A2A RECEPTOR

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Biased agonism, the ability of agonists to differentially activate downstream signaling pathways by stabilizing specific receptor conformations, is a key issue for G protein-coupled receptor (GPCR) signaling. The C-terminal domain might influence this functional selectivity of GPCRs as it engages G proteins, GPCR kinases, b-arrestins, and several other proteins. Thus, the aim of this paper is to compare the agonist-dependent selectivity for intracellular pathways in a heterologous system expressing the fulllength (A2AR) and a C-tail truncated (A2A D40R lacking the last 40 amino acids) adenosine A2A receptor, a GPCR that is already targeted in Parkinson's disease using a first-in-class drug. Experimental data such as ligand binding, cAMP production, b-arrestin recruitment, ERK1/2 phosphorylation and dynamic mass redistribution assays, which correspond to different aspects of signal transduction, were measured upon the action of structurally diverse compounds (the agonists adenosine, NECA, CGS-21680, PSB-0777 and LUF-5834 and the SCH-58261 antagonist) in cells expressing A2AR and A2A D40R. The results show that taking cAMP levels and the endogenous adenosine agonist as references, the main difference in bias was obtained with PSB-0777 and LUF-5834. The C-terminus is dispensable for both G-protein and barrestin recruitment and also for MAPK activation. Unrestrained molecular dynamics simulations, at the ls timescale, were used to understand the structural arrangements of the binding cavity, triggered by these chemically different agonists, facilitating G protein binding with different efficacy.

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INTERACTIONS BETWEEN SATELLITE GLIAL CELLS AND PROPRIOCEPTIVE NEURONS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Proprioception is mediated by proprioceptive neurons and is crucial for important motor functions such as standing and walking. Proprioceptor cell bodies reside within the peripheral dorsal root ganglia and are tightly enveloped by satellite glial cells (SGCs). SCGs express a number of G_q protein-coupled receptors (G_q GPCRs), but their functional consequences on proprioceptor activity is unknown. We hypothesized that activation of SGC G_q GPCR-mediated Ca^{2+} signaling induces the release of neuroactive molecules from SGCs to modulate proprioceptor functions. Using 2-photon microscopy, chemogenetics, and novel genetic tools, we have discovered that SGC GPCR signaling drives proprioceptor Ca^{2+} activity through a purinergic pathway, which might alter sensorimotor functions. These findings suggest a new role for SGCs in shaping sensory information processing.

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COLOCALIZATION OF CONNEXIN AND SYNAPTOPODIN AT THE AXON INITIAL SEGMENT IN ADULT MAMMALIAN PROJECTION NEURONS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Connexins are the building blocks of electrical synapses formed between neurons and/or neurons and glial cells. This coupling is suggested to serve as a fundamental circuit element for neuronal communication and has important functional implications for network oscillations. However, most of the evidence pointing towards the formation of bona fide axonal gap junctions is based on recordings and only limited structural evidence has been presented so far. A recent study showed connexin clusters at pyramidal axon initial segments (AIS), however, their subcellular composition, potential contact points and developmental profile remain unknown. Here we provide evidence that connexin puncta indeed appear at the AIS of a vast majority of M1 pyramidal neurons. Surprisingly, they colocalize with synaptopodin, an actin-binding protein essential for the formation of the cisternal organelle, an intra- axonal Ca2+ store. Using multi-channel immunofluorescence, confocal microscopy and 3D reconstruction, we show that in 86% of all pyramidal neurons investigated, connexin43 (Cx43) is expressed at the AIS in direct proximity to synaptopodin-positive intra-axonal structures. In an attempt to better understand the subcellular distribution of connexins, protein expansion microscopy (ProExM) and 3D-PAINT superresolution microscopy were used to visualize nanoscale structural interactions between synaptopodin-positive cisternal organelles and axonal structures immunoreactive for Cx43, Cx36 and Cx45. Our future directive will be to elucidate whether the observed Cx43 staining refers to actual gap junctions with robust intercellular connections and whether or not they are of functional relevance for action potential initiation at the AIS.

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MODULATION OF POINT CONTACT SIGNALING BY SUBCELLULARLY-RESTRICTED CAMP SIGNALS IN RETINAL AXONS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Retinal ganglion cells (RGCs) exit the retina and project into the brain in a highly stereotypic manner. The establishment of these connections involves axon pathfinding and terminal arbor refinement, two processes relying on guidance cues. Intracellular integration of guidance molecules requires several molecular mechanisms, such as local second messenger signaling and cellular adhesion modulation. However, the way those processes interact with each other in axon pathfinding remains elusive. In this regard, we identified a cAMP signal in the vicinity of point contact (PC) in response to ephrin-A5, a repulsive cue. PCs are macromolecular complexes representing one of the main modalities of cellular adhesion in developing axons. Using genetically-encoded chelator, named cAMP-Sponge, we managed to buffer cAMP specifically in PCs. Hence, buffering PC-restricted cAMP signals reduces axonal retraction induced by ephrin-A5. Moreover, we provide evidence that this reduction is linked to a decrease of focal adhesion kinase (FAK) phosphorylation on its tyrosine 925. FAK is key actor of PC dynamic, and its phosphorylation on the tyrosine 925 is linked to PC disassembly. Further investigation will explore if this type of PC disassembly could explain our reduced-axonal retraction phenotype. Taken together, those results give new insights into the fine mechanisms driving RGCs axon pathfinding.

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MEMBRANE FUSION IN E. COLI UPON EXPRESSION OF SYNAPTIC SNARES AND CAVEOLIN: A SYNTHETIC BIOLOGY APPROACH TO STUDYING SNARE PROTEIN FUNCTION IN A BACTERIAL HOST

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Aim: The role of synaptic SNAREs VAMP2, Syntaxin 1 and SNAP-25 in membrane fusion has been established by a large set of *in vivo* and *in vitro* experiments, particularly reconstitution assays using artificial membranes. **Method**: Here we took advantage of the generation of membrane cisternae by caveolin expression in *E. coli* to develop an assay based on co-expression of SNAREs via a polycistronic vector. We show that Syntaxin 1 formed protein complexes with VAMP2 and SNAP-25 which were regulated by co-expression of Munc-18a. Syntaxin1 interacted with caveolin and Syntaxin 1/SNAP-25/VAMP2 complexes were observed and regulated by Munc-18a in the presence of caveolin. Expression of Syntaxin1/SNAP-25/VAMP2 led to increased bacterial cell elongation in the presence of caveolin, but not in its absence. SNAP-25 was required for cell growth and the light chain of tetanus neurotoxin inhibited this process. DAPI staining further showed that increased bacterial cell elongation was not due to a defect of cell fission. Electron microscopy and super-resolution light microscopy showed that synaptic SNARE expression together with caveolin led to the partial loss of the cisternae suggesting their efficient fusion with the cytoplasmic membrane. **Result**: We propose that this assay reconstitutes membrane fusion in a simple organism with an easy-to-observe phenotype amenable to structure-function studies of SNAREs.

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THE ENDOPLASMIC RETICULUM IN FINE ASTROCYTIC PROCESSES: PRESENCE, SHAPE, DISTRIBUTION AND EFFECT ON CALCIUM ACTIVITY

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Neurotransmission at tripartite synapses results in Ca^{2+} signals in astrocytes, that are essential to brain function and impaired in brain disorders. The majority of astrocytic Ca^{2+} signals result from Ca^{2+} fluxes from the endoplasmic reticulum. As most perisynaptic astrocytic processes (PAPs) are below the diffraction limit, the presence of ER in PAPs and its involvement in local Ca^{2+} activity is unclear and highly debated. Here, we reconstruct 3D meshes of hippocampal tripartite synapses from electron microscopy. We find that 75% of PAPs contain some ER, which can be as close as 72 nm from the synapse. We further quantify the geometrical properties of the ER in PAPs, highlighting its diversity. Reaction-diffusion simulations in the realistic 3D PAP meshes were then performed to test the effect of ER shape on Ca^{2+} activity in PAPs. To discern the effect of ER distribution from ER shape, we provide an algorithm that automatically creates realistic 3D PAP meshes with various ER distributions and constant shape. Simulations in those realistic 3D meshes reveal the complex interplay between the clustering of Ca^{2+} channels, the ER surface-volume ratio, Ca^{2+} buffering and the size of ER-PM contact sites that shapes the spatio-temporal properties of Ca^{2+} signals in PAPs. Overall, this study provides key insights into the mechanisms that regulate astrocyte activity at tripartite synapses.

Pubmed:
31425510: Denizot A, Arizono M, Nägerl UV, Soula H, Berry H
Simulation of calcium signaling in fine astrocytic processes: Effect of spatial properties on spontaneous activity.
Astrocytes, a glial cell type of the central nervous system, have emerged as detectors and regulators of neuronal information processing. Astrocyte excitability resides in transient variations of free cytosolic calcium concentration over a range of temporal and spatial scales, from sub-microdomains to waves propagating throughout the cell. Despite extensive experimental approaches, it is not clear how these signals are transmitted to and integrated within an astrocyte. The localization of the main molecular actors and the geometry of the system, including the spatial organization of calcium channels IP3R, are deemed essential. However, as most calcium signals occur in astrocytic ramifications that are too fine to be resolved by conventional light microscopy, most of those spatial data are unknown and computational modeling remains the only methodology to study this issue. Here, we propose an IP3R-mediated calcium signaling model for dynamics in such small sub-cellular volumes. To account for the expected stochasticity and low copy numbers, our model is both spatially explicit and particle-based. Extensive simulations show that spontaneous calcium signals arise in the model via the interplay between excitability and stochasticity. The model reproduces the main forms of calcium signals and indicates that their frequency crucially depends on the spatial organization of the IP3R channels. Importantly, we show that two processes expressing exactly the same calcium channels can display different types of calcium signals depending on the spatial organization of the channels. Our model with realistic process volume and calcium concentrations successfully reproduces spontaneous calcium signals that we measured in calcium micro-domains with confocal microscopy and predicts that local variations of calcium indicators might contribute to the diversity of calcium signals observed in astrocytes. To our knowledge, this model is the first model suited to investigate calcium dynamics in fine astrocytic processes and to propose plausible mechanisms responsible for their variability.
PLoS Comput Biol, 2019; 15
30322407: Ceyzériat K, Ben Haim L, Denizot A, Pommier D, Matos M, Guillemaud O, Palomares MA, Abjean L, Petit F, Gipchtein P, Gaillard MC, Guillermier M, Bernier S, Gaudin M, Aurégan G, Joséphine C, Déchamps N, Veran J, Langlais V,

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Cambon K, Bemelmans AP, Baijer J, Bonvento G, Dhenain M, Deleuze JF, Oliet SHR, Brouillet E, Hantraye P, Carrillo-de Sauvage MA, Olaso R, Panatier A, Escartin C

Modulation of astrocyte reactivity improves functional deficits in mouse models of Alzheimer's disease.

Astrocyte reactivity and neuroinflammation are hallmarks of CNS pathological conditions such as Alzheimer's disease. However, the specific role of reactive astrocytes is still debated. This controversy may stem from the fact that most strategies used to modulate astrocyte reactivity and explore its contribution to disease outcomes have only limited specificity. Moreover, reactive astrocytes are now emerging as heterogeneous cells and all types of astrocyte reactivity may not be controlled efficiently by such strategies.Here, we used cell type-specific approaches in vivo and identified the JAK2-STAT3 pathway, as necessary and sufficient for the induction and maintenance of astrocyte reactivity. Modulation of this cascade by viral gene transfer in mouse astrocytes efficiently controlled several morphological and molecular features of reactivity. Inhibition of this pathway in mouse models of Alzheimer's disease improved three key pathological hallmarks by reducing amyloid deposition, improving spatial learning and restoring synaptic deficits.In conclusion, the JAK2-STAT3 cascade operates as a master regulator of astrocyte reactivity in vivo. Its inhibition offers new therapeutic opportunities for Alzheimer's disease.

Acta Neuropathol Commun, 2018; 6

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BOARD NUMBER: S01-451

UNRAVELING THE MOLECULAR CONTROL OF INTRAORGANELLAR CA2+ FLUXES USING NOVEL CA2+ INTEGRATORS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Although the endoplasmic reticulum (ER) extends throughout all locations in neurons and neuronal ER dysfunction is implicated in numerous neurological diseases, the molecular control of ER Ca^{2+} fluxes remains poorly understood. Sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is constitutively active and transports Ca^{2+} continuosly inside the ER, counteracting the action of an unknown mechanism that leaks Ca^{2+} from the ER into the cytosol. Blocking SERCA pharmacologically, causes a quick depletion of the ER Ca^{2+}, resulting in a state in which the lack of ER Ca^{2+} severely affects neurotransmission. Despite the vital importance of the ER Ca^{2+} leak pathway for neurons, however, the molecular identity of the protein driving this process remains a mistery. We developed a new optical tool that can generate a temporally-precise "ER Ca^{2+} content snapshot". This tool, named ER-CaMPARI, undergoes efficient and irreversible green-to-red conversion only when ER Ca^{2+} is at normal levels and UV illumination coincide at the same time. Conversely, such tool does not convert to red after thapsigargin-induced depletion, which allows to isolate cells that are resistant to ER Ca^{2+} depletion. Combining this sensor with a genome-wide CRISPR-Cas9 knockout screen, we aim to identify the driving mechanisms of ER Ca^{2+} depletion, as we can match the lack of a particular gene with the resistance to ER Ca^{2+} depletion. The use of organelle-specific CaMPARI variants should prove useful in identifying the molecular basis of neuronal disease states associated with organelle Ca^{2+} dyshomeostasis, and as an example we have also generated Mito-CaMPARIs, optimized to explore mitochondrial Ca^{2+} biology.

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VIRUS-MEDIATED ASTROCYTE CAMP QUANTIFICATION

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Astrocytes are the most abundant glial cell type in the central nervous system. They can express many different receptors, such as G-Protein Coupled Receptors (GPCRs). The activation of Gi and Gs GPCRs leads to a decrease or increase of cAMP concentration, respectively. cAMP-dependant pathways are known to modulate synaptic plasticity and memory, and thus constitute crucial components of the glia-neuron relationship. Dysregulations of the aforementioned astrocytic pathways might be implicated in the aetiology of many psychiatric disorders. Investigating astrocyte cAMP dynamics *in* and *ex vivo* is thus critical. So far, only a few tools are available to quantify specifically astrocytic changes in cAMP. Here, we present the characterisation of an adeno-associated viral tool that can be stereotaxically injected and allows *in/ex vivo* visualisation and quantification of astrocyte cAMP dynamics.

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BOARD NUMBER: S01-453

MECHANISTIC INSIGHTS INTO VAMP7-DEPENDENT UNCONVENTIONAL SECRETION IN NEURON-GLIOBLASTOMA CELL COMMUNICATION

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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[1]Université Paris Cité, Institut de Psychiatrie et Neurosciences de Paris, INSERM U1266, Membrane Trafficking In Healthy And Diseased Brain, Paris, France, [2]Inserm U1242, Université de Rennes, Centre De Lutte Contre Le Cancer Eugène Marquis, Rennes, France, [3]GHU PARIS psychiatrie & neurosciences, Hospital Centre Sainte Anne, Paris, France

Aim: To study VAMP7-dependent unconventional secretion in neuron-glioblastoma cell communication Methods: Secretome analysis, Proteomics, genetic alterations, super-resolution microscopy Description and results: Communication between cells, particularly neuronal and non-neuronal cells is important for proper development and function. Conventional secretion which is ER-Golgi mediated relies on secretory vesicles. In the brain, that involves synaptic vesicle and secretory granule mediated release of classical neurotransmitters. However, the molecules released by the vesicular late-endosome mediated unconventional secretion in the brain and their effects are largely unidentified. Recent work by our lab has characterized a new secretory autophagy-like pathway dependent on VAMP7, a tetanus neurotoxin insensitive v-SNARE. We show that ER and mitochondrial elements can be incorporated into late endosomes and can later be released by a secretory mechanism. This process is more efficient in the absence of degradative autophagy. We further observed that in this scenario, neurites grow longer and the neuronal polarity is altered. Conclusions: We combine genetic alterations in the rat, lipidomics, proteomics, metabolomics, and super-resolution microscopy to dissect the mechanisms regulating the VAMP7 dependent secretion. Furthermore, VAMP7 has also recently been linked to various cancers. As VAMP7 is a bridge connecting secretion, cancer migration and invasion and neuronal growth, we are studying the effects of VAMP7 dependent secretion on glioblastoma particularly towards understanding the effect of neuronal VAMP7 dependent secretion on glioblastoma and vice versa.

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SELENOT IS A GUARDIAN OF ER HOMEOSTASIS IN POMC NEURONS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

Isabelle Lihrmann[1], Dorthe Cartier[1], Christine Bucharles[1], Carole Burel[2], Marie-Laure Wallet-Balieu[3], Philippe Chan Tchi Song[3], Muriel Bardor[2], Youssef Anouar[1], Cédric Jehan[1]
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POMC neurons in the hypothalamus play an anorexigenic role in the regulation of food intake via the release of α-MSH derived from post-translational cleavage of POMC in response to various metabolic signals such as lipids, hormones or carbohydrates. In a mouse model of obesity induced by a high-fat diet, ER stress is observed in these neurons, as well as an accumulation of POMC in the ER leading to a defect in α-MSH secretion. In this study, we examined the role of a selenoprotein, SELENOT, in POMC neurons physiology, given its essential role in ER homeostasis and its neuroprotective effect in some models of neurodegenerative diseases. To this end, SELENOT was silenced and ER homeostasis, neuronal differentiation and secretion were examined. We found major defects in SELENOT KO neurons compared to wild-type neurons, including increased ER and cell volume, altered disulfide isomerase distribution, loss of ER/plasma membrane contacts, defects in adhesion, vesicle trafficking, neuritogenesis, and increased senescence. To go further, a thiol trapping approach was performed to identify SELENOT partners. Immunoprecipitation of SELENOT followed by mass spectrometry analysis revealed significant enrichment of partners involved in ER protein folding and N-glycosylation. Comparison of N-glycomes between wild-type and KO cells revealed the species-specific loss of sialylated polysaccharides. Several potential redox partners have been identified and ongoing studies are aimed at determining the nature and role of their interaction with SELENOT in the secretory pathway.

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LOSS OF KINESIN KIF2A CAUSES PREMATURE NEURODEGENERATION.

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

Nuria Ruiz Reig[1], Georges Chehade[1], Janne Hakanen[1], Keimpe Wierda[2], Joris De Wit[3], Laurent Nguyen[4], Philippe Gailly[5], Fadel Tissir[1,6]
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KIF2A is a microtubule-dependent Kinesin that has the capacity to depolymerize microtubules. Patients with dysfunctional KIF2A present microcephaly and intellectual disability, and this has been ascribed to its role in mitotic progression, progenitors fate determination, and neuron production. In this study, we report that KIF2A is necessary for postnatal stages to assemble and maintain cortical circuitries; and that KIF2A-related microcephaly results from connectivity defects and premature neurodegeneration rather than impaired neurogenesis. Cortical neurons, with specific ablation of KIF2A, displayed abnormalities in polarity, neuritogenesis, and synaptogenesis. These neurons exhibited aberrant electrophysiological properties, failed to connect properly, and eventually died. Mechanistically, we show that lack of KIF2A alters the intracellular transport of lysosomes and distribution of insulin-like growth factor-1 receptor, which compromises the PI3K/Akt/mTOR and Erk survival pathways and leads to premature loss of neurons.

Pubmed:
34582949: Hakanen J, Parmentier N, Sommacal L, Garcia-Sanchez D, Aittaleb M, Vertommen D, Zhou L, Ruiz-Reig N, Tissir F
The Celsr3-Kif2a axis directs neuronal migration in the postnatal brain.
The tangential migration of immature neurons in the postnatal brain involves consecutive migration cycles and depends on constant remodeling of the cell cytoskeleton, particularly in the leading process (LP). Despite the identification of several proteins with permissive and empowering functions, the mechanisms that specify the direction of migration remain largely unknown. Here, we report that planar cell polarity protein Celsr3 orients neuroblasts migration from the subventricular zone (SVZ) to olfactory bulb (OB). In Celsr3-forebrain conditional knockout mice, neuroblasts loose directionality and few can reach the OB. Celsr3-deficient neuroblasts exhibit aberrant branching of LP, de novo LP formation, and decreased growth rate of microtubules (MT). Mechanistically, we show that Celsr3 interacts physically with Kif2a, a MT depolymerizing protein and that conditional inactivation of Kif2a in the forebrain recapitulates the Celsr3 knockout phenotype. Our findings provide evidence that Celsr3 and Kif2a cooperatively specify the directionality of neuroblasts tangential migration in the postnatal brain.
Prog Neurobiol, 2022; 208
33899739: Lau EO, Damiani D, Chehade G, Ruiz-Reig N, Saade R, Jossin Y, Aittaleb M, Schakman O, Tajeddine N, Gailly P, Tissir F
DIAPH3 deficiency links microtubules to mitotic errors, defective neurogenesis, and brain dysfunction.
Diaphanous (DIAPH) three (DIAPH3) is a member of the formin proteins that have the capacity to nucleate and elongate actin filaments and, therefore, to remodel the cytoskeleton. DIAPH3 is essential for cytokinesis as its dysfunction impairs the contractile ring and produces multinucleated cells. Here, we report that DIAPH3 localizes at the centrosome during mitosis and regulates the assembly and bipolarity of the mitotic spindle. DIAPH3-deficient cells display disorganized cytoskeleton and multipolar spindles. DIAPH3 deficiency disrupts the expression and/or stability of several proteins including the kinetochore-associated protein SPAG5. DIAPH3 and SPAG5 have similar expression patterns in the developing brain and overlapping subcellular localization during mitosis. Knockdown of SPAG5 phenocopies DIAPH3 deficiency, whereas its overexpression rescues the DIAHP3 knockdown phenotype. Conditional inactivation of in mouse cerebral cortex profoundly disrupts

neurogenesis, depleting cortical progenitors and neurons, leading to cortical malformation and autistic-like behavior. Our data uncover the uncharacterized functions of DIAPH3 and provide evidence that this protein belongs to a molecular toolbox that links microtubule dynamics during mitosis to aneuploidy, cell death, fate determination defects, and cortical malformation.
Elife, 2021; 10
31213986: Hakanen J, Ruiz-Reig N, Tissir F
Linking Cell Polarity to Cortical Development and Malformations.
Cell polarity refers to the asymmetric distribution of signaling molecules, cellular organelles, and cytoskeleton in a cell. Neural progenitors and neurons are highly polarized cells in which the cell membrane and cytoplasmic components are compartmentalized into distinct functional domains in response to internal and external cues that coordinate polarity and behavior during development and disease. In neural progenitor cells, polarity has a prominent impact on cell shape and coordinate several processes such as adhesion, division, and fate determination. Polarity also accompanies a neuron from the beginning until the end of its life. It is essential for development and later functionality of neuronal circuitries. During development, polarity governs transitions between multipolar and bipolar during migration of postmitotic neurons, and directs the specification and directional growth of axons. Once reaching final positions in cortical layers, neurons form dendrites which become compartmentalized to ensure proper establishment of neuronal connections and signaling. Changes in neuronal polarity induce signaling cascades that regulate cytoskeletal changes, as well as mRNA, protein, and vesicle trafficking, required for synapses to form and function. Hence, defects in establishing and maintaining cell polarity are associated with several neural disorders such as microcephaly, lissencephaly, schizophrenia, autism, and epilepsy. In this review we summarize the role of polarity genes in cortical development and emphasize the relationship between polarity dysfunctions and cortical malformations.
Front Cell Neurosci, 2019; 13
30593496: Ruiz-Reig N, Rakotobe M, Bethus I, Le Menn G, Huditz HI, Marie H, Lamonerie T, D'Autréaux F
Developmental Requirement of Homeoprotein Otx2 for Specific Habenulo-Interpeduncular Subcircuits.
The habenulo-interpeduncular system (HIPS) is now recognized as a critical circuit modulating aversion, reward, and social behavior. There is evidence that dysfunction of this circuit leads to psychiatric disorders. Because psychiatric diseases may originate in developmental abnormalities, it is crucial to investigate the developmental mechanisms controlling the formation of the HIPS. Thus far, this issue has been the focus of limited studies. Here, we explored the developmental processes underlying the formation of the medial habenula (MHb) and its unique output, the interpeduncular nucleus (IPN), in mice independently of their gender. We report that the homeobox gene is essential for the proper development of both structures. We show that MHb and IPN neurons require at different developmental stages and, in both cases, deletion leads to disruption of HIPS subcircuits. Finally, we show that Otx2 neurons tend to be preferentially interconnected. This study reveals that synaptically connected components of the HIPS, despite radically different developmental strategies, share high sensitivity to expression. Brain reward circuits are highly complex and still poorly understood. In particular, it is important to understand how these circuits form as many psychiatric diseases may arise from their abnormal development. This work shows that , a critical evolutionary conserved gene implicated in brain development and a predisposing factor for psychiatric diseases, is required for the formation of the habenulo-interpeduncular system (HIPS), an important component of the reward circuit. Otx2 deletion affects multiple processes such as proliferation and migration of HIPS neurons. Furthermore, neurons expressing are preferentially interconnected. Therefore, expression may represent a code that specifies the connectivity of functional subunits of the HIPS. Importantly, the conditional knock-out animals used in this study might represent a new genetic model of psychiatric diseases.
J Neurosci, 2019; 39
29869132: Ruiz-Reig N, Andres B, Lamonerie T, Theil T, Fairén A, Studer M
The caudo-ventral pallium is a novel pallial domain expressing Gdf10 and generating Ebf3-positive neurons of the medial amygdala.
In rodents, the medial nucleus of the amygdala receives direct inputs from the accessory olfactory bulbs and is mainly implicated in pheromone-mediated reproductive and defensive behaviors. The principal neurons of the medial amygdala are GABAergic neurons generated principally in the caudo-ventral medial ganglionic eminence and preoptic area. Beside GABAergic neurons, the medial amygdala also contains glutamatergic Otp-expressing neurons cells generated in the lateral hypothalamic neuroepithelium and a non-well characterized Pax6-positive population. In the present work, we describe a novel glutamatergic Ebf3-expressing neuronal subpopulation distributed within the periphery of the postero-ventral medial amygdala. These neurons are generated in a pallial domain characterized by high expression of Gdf10. This territory is topologically the most caudal tier of the ventral pallium and accordingly, we named it Caudo-Ventral Pallium (CVP). In the absence of Pax6, the CVP is disrupted and Ebf3-expressing neurons fail to be generated. Overall, this work proposes a novel model of the neuronal composition of the medial amygdala and unravels for the first time a new novel pallial subpopulation originating from the CVP and expressing the transcription factor Ebf3.

Brain Struct Funct, 2018; 223
29311773: Ruiz-Reig N, Studer M
Rostro-Caudal and Caudo-Rostral Migrations in the Telencephalon: Going Forward or Backward?
The generation and differentiation of an appropriate number of neurons, as well as its distribution in different parts of the brain, is crucial for the proper establishment, maintenance and plasticity of neural circuitries. Newborn neurons travel along the brain in a process known as neuronal migration, to finalize their correct position in the nervous system. Defects in neuronal migration produce abnormalities in the brain that can generate neurodevelopmental pathologies, such as autism, schizophrenia and intellectual disability. In this review, we present an overview of the developmental origin of the different telencephalic subdivisions and a description of migratory pathways taken by distinct neural populations traveling long distances before reaching their target position in the brain. In addition, we discuss some of the molecules implicated in the guidance of these migratory paths and transcription factors that contribute to the correct migration and integration of these neurons.
Front Neurosci, 2017; 11
27178193: Ruiz-Reig N, Andrés B, Huilgol D, Grove EA, Tissir F, Tole S, Theil T, Herrera E, Fairén A
Lateral Thalamic Eminence: A Novel Origin for mGluR1/Lot Cells.
A unique population of cells, called "lot cells," circumscribes the path of the lateral olfactory tract (LOT) in the rodent brain and acts to restrict its position at the lateral margin of the telencephalon. Lot cells were believed to originate in the dorsal pallium (DP). We show that Lhx2 null mice that lack a DP show a significant increase in the number of mGluR1/lot cells in the piriform cortex, indicating a non-DP origin of these cells. Since lot cells present common developmental features with Cajal-Retzius (CR) cells, we analyzed Wnt3a- and Dbx1-reporter mouse lines and found that mGluR1/lot cells are not generated in the cortical hem, ventral pallium, or septum, the best characterized sources of CR cells. Finally, we identified a novel origin for the lot cells by combining in utero electroporation assays and histochemical characterization. We show that mGluR1/lot cells are specifically generated in the lateral thalamic eminence and that they express mitral cell markers, although a minority of them express ΔNp73 instead. We conclude that most mGluR1/lot cells are prospective mitral cells migrating to the accessory olfactory bulb (OB), whereas mGluR1+, ΔNp73+ cells are CR cells that migrate through the LOT to the piriform cortex and the OB.
Cereb Cortex, 2017; 27
27034423: Touzot A, Ruiz-Reig N, Vitalis T, Studer M
Molecular control of two novel migratory paths for CGE-derived interneurons in the developing mouse brain.
GABAergic interneurons are highly heterogeneous and originate in the subpallium mainly from the medial (MGE) and caudal (CGE) ganglionic eminences according to a precise temporal sequence. MGE-derived cells disperse dorsally and migrate towards all regions of the cortex, but little is known about how CGE-derived cells reach their targets during development. Here, we unravel the existence of two novel CGE caudo-rostral migratory streams, one located laterally (LMS) and the other one more medially (MMS), that, together with the well-known caudal migratory stream (CMS), contribute to populate the neocortex, hippocampus and amygdala. These paths appear in a precise temporal sequence and express a distinct combination of transcription factors, such as SP8, PROX1, COUP-TFI and COUP-TFII. By inactivating COUP-TFI in developing interneurons, the lateral and medial streams are perturbed and expression of SP8 and COUP-TFII affected. As a consequence, adult mutant neocortices have laminar-specific alterations of distinct cortical interneuron subtypes. Overall, we propose that the existence of spatially and temporally regulated migratory paths in the subpallium contributes to the laminar distribution and specification of distinct interneuron subpopulations in the adult brain.
Development, 2016; 143
26269635: Murillo B, Ruiz-Reig N, Herrera M, Fairén A, Herrera E
Zic2 Controls the Migration of Specific Neuronal Populations in the Developing Forebrain.
Human mutations in ZIC2 have been identified in patients with holoprosencephaly and schizophrenia. Similarly, Zic2 mutant mice exhibit holoprosencephaly in homozygosis and behavioral and morphological schizophrenic phenotypes associated with forebrain defects in heterozygosis. Despite the devastating effects of mutations in Zic2, the cellular and molecular mechanisms that provoke Zic2-deficiency phenotypes are yet unclear. Here, we report a novel role for this transcription factor in the migration of three different types of forebrain neurons: the Cajal-Retzius cells that populate the surface of the telencephalic vesicles, an amygdaloid group of cells originated in the caudal pole of the telencephalic pallium, and a cell population that travels from the prethalamic neuroepithelium to the ventral lateral geniculate nucleus. Our results also suggest that the receptor EphB1, previously identified as a Zic2 target, may mediate, at least partially, Zic2-dependent migratory events. According to these results, we propose that deficiencies in cell motility and guidance contribute to most of the forebrain pathologies associated with Zic2 mutations.
J Neurosci, 2015; 35
24759572: Molina-Cimadevila MJ, Segura S, Merino C, Ruiz-Reig N, Andrés B, de Madaria E

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Oral self-administration of buprenorphine in the diet for analgesia in mice.

Postsurgical oral self-administration of analgesics in rodents is an interesting technique of providing analgesia, avoiding the negative effects of manipulation. Several strategies, using gelatin or nutella, have already been described. However, rodents require some habituation period to reach a good intake because of their neophobic behavior. The current study aimed to explore whether buprenorphine when mixed with an extruded diet offers a potential treatment option in the pain management of mice using a triple approach: by measuring the spontaneous intake in healthy animals; by using the hot-plate test; and finally by assessing the drug's ability to provide postoperative analgesia in a surgical intervention of moderate severity (intra-utero electroporation). Mice consumed during 20 hours, similar amounts of extruded diet alone, mixed with glucosaline, and mixed with buprenorphine (0.03 mg per pellet) or meloxicam (0.25 mg per pellet) both of which were diluted in glucosaline, showing that no neophobia was associated with these administrations. Relative increase from baseline latency (% maximal possible effect) in the hot-plate test at 20 h of administration was significantly higher for oral buprenorphine in diet 0.03 mg/pellet, and diet 0.15 mg/pellet, compared with placebo and no differences were found between those oral administrations and subcutaneous buprenorphine 0.1 mg/kg measured 3 h later. The treatment was also effective in attenuating the reductions in food consumption and body weight that occur after surgery. These data suggest that providing buprenorphine with the diet is a feasible and effective way of self-administration of analgesia in mice and does not cause neophobia and may easily contribute to the refinement of surgical procedures.
Lab Anim, 2014; 48

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BOARD NUMBER: S01-456

AUTOPHAGY AND MICROTUBULES-MEDIATED CYTOSKELETON DYNAMICS IN UBIQUITIN LIGASE E3A (UBE3A)-DEFICIENT NEURONS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Ubiquitin E3A ligase (**UBE3A**) has crucial functions in the brain and changes in its expression levels lead to neurodevelopmental disorders, to Angelman Syndrome (**AS**) or 15duplication-autisms. We investigated neuronal guidance *in vitro* in UBE3A-deficient neurons, model of AS, by using micro-grooved substrates (GRs) that can induce specific directional stimuli to cells. We found deficits in topographical contact guidance in AS neurons, linked to a dysregulated focal adhesions' sensing and cytoskeleton polarization, with an aberrant axonal branching on GRs. Recently, it emerged that cytoskeleton and autophagy processes are connected, and of pivotal importance for neuronal homeostasis and development. The cytoskeleton has an important role in autophagy, the process by which cellular waste is isolated inside specialized vesicles called autophagosomes for recycling and degradation. Here, we investigate the trafficking of cytoskeletal cargos along microtubules and the autophagy process in primary AS neurons, under control and stimulated conditions. The migration behaviour of wild-type and AS neurons, under control or stimulated conditions, is also shown, with the aim to clarify UBE3A role during neurodevelopment. We show that the loss of axonal guidance in AS neurons is related to an impairment in the microtubules-mediated process and that the autophagy flux is dysregulated in AS neurons. Overall, cytoskeleton dynamics and autophagy response emerge as players in UBE3A-mediated pathogenesis. These results support the view that UBE3A-related deficits in early neuronal morphogenesis and homeostasis may lead to defective neuronal connectivity and plasticity. This work was supported by MSCA-IF-2017 grant Neuroguide-795948.

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BOARD NUMBER: S01-457

FIDGETIN-LIKE 1, A NOVEL REGULATOR OF THE MICROTUBULE/ACTIN CROSSTALK REQUIRED FOR AXON OUTGROWTH AND NAVIGATION

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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A crucial step in the assembly of neuronal circuits lies in the accurate growth and navigation of developing axons toward their appropriate targets. These axon outgrowth and pathfinding processes involve fine tuned and synchronized modifications of actin filament (F-actin) and microtubule dynamics/structure inside the growth cone. However, the numerous players that coordinate the remodelling of both cytoskeletons to drive growth cone steering remain largely unknown. We previously uncovered the microtubule-associated ATPase fidgetin-like1 (fignl1) as a key regulator of microtubule plus-end dynamics required for zebrafish spinal motor axon navigation. We here aim to investigate the role of this ATPase in the microtubule/actin crosstalk underlying axon guidance in the mammalian central nervous system, using the visual system as a model. We showed that fignl1 is highly expressed in retinal axons at the pick phase of axon navigation at the optic chiasm (E15). Using primary cultures of mouse retinal explants, we further showed that fignl1 overexpression drastically impairs retinal axon outgrowth and leads to ectopic branching while its knockdown reduces axon extension. Moreover, we demonstrated that Fignl1-GFP localizes to F-actin-rich regions in retinal growth cones where it moves with the actin retrograde flow. Consistently, recombinant Fignl1 binds F-actin in *in vitro* co-peletting assays. To further dissect the roles of this microtubule- and F-actin-binding protein in visual circuit wiring, we generated a retinal-specific fignl1 conditional mutant. Extensive characterization of the physiological, cellular and molecular phenotypes of fignl1-deficient embryos should shed new lights on the basic mechanisms governing the microtubule/F-actin crosstalk underlying neuronal connectivity.

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VISUALIZATION OF MAP6-POSITIVE MICROTUBULES THROUGHOUT THE NEURONAL MT NETWORK: INPUT OF EXPANSION MICROSCOPY

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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The shaft of neuron extensions contains bundles of microtubules allowing the continuity of cellular space to reach a length of one meter in humans. In neurons, microtubules properties and functions are regulated by a myriad of accessory proteins including microtubules associated proteins (MAPs) such as Tau, MAP1B or MAP6. Microtubule stability relies on 1/post-translational modifications of tubulin with tyrosinated and detyrosinated/acetylated tubulin isotypes associated with dynamic and stable microtubules, respectively 2/the binding of MAPs. Here we focus on the neuronal protein MAP6 able to protect microtubules against drug- and cold-induced depolymerization and recently shown to localize in microtubule lumen. We charted dynamic and stable microtubules as well as MAP6-positive microtubules throughout the neuronal microtubule network. To reach this objective we have combined 8X expansion microscopy and super-resolution light imaging allowing to visualize individual microtubules in neuronal shafts of cultured hippocampal neurons. We observed tyrosinated microtubules at neurites borders whereas detyrosinated/acetylated microtubules are present in the central part of neurites. MAP6-positive microtubules preferentially co-localized with detyrosinated/acetylated microtubules. Thanks to MAP6 deficient neurons, we investigate possible causal roles of MAP6 in the segregation of dynamic and stable microtubules populations. Also, in rescue experiments with a MAP6 mutant unable to enter microtubule lumen, we are investigating if MAP6 luminal localization dictate its presence on stable microtubules. Deciphering the sub-cellular localisation and organisation of various kinds of microtubules within the whole neuronal network should establish the respective and combined contributions of tubulin isotypes and MAPs to microtubule identity and stability.

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IN VIVO IIIG9 INHIBITION IN EPENDYMAL CELLS INDUCES ADHERENS JUNCTIONS DISASSEMBLING, CELLULAR DETACHMENT AND VENTRICULOMEGALY.

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Ependymal cells have multiple apical cilia that line the ventricular surfaces and the central canal of spinal cord. In cancer, the loss of ependymal cell polarity promotes the formation of different types of tumors, such as supratentorial anaplastic ependymomas, which are highly aggressive in children. IIIG9 (PPP1R32) is a protein restricted to adult ependymal cells located in cilia and in the apical cytoplasm and has unknown function. In this work, we studied the expression and localization of IIIG9 in the adherens junctions (cadherin/β-catenin-positive junctions) of adult brain ependymal cells using confocal and transmission electron microscopy. Through in vivo loss-of-function studies, ependymal denudation (single-dose injection experiments of inhibitory adenovirus) was observed, inducing the formation of ependymal cells with a “balloon-like” morphology. These cells had reduced cadherin expression (and/or delocalization) and cleavage of the cell death marker caspase-3, with “cilia rigidity” morphology (probably vibrational beating activity) and ventriculomegaly occurring prior to these events. Finally, after performing continuous infusions of adenovirus for 14 days, we observed total cell denudation and reactive parenchymal astrogliosis. Our data confirmed that IIIG9 is essential for the maintenance of adherens junctions of polarized ependymal cells. Eventually, altered levels of this protein in ependymal cell differentiation may increase ventricular pathologies, such as hydrocephalus or neoplastic transformation.

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DIFFERENTIAL AXONAL TRAFFICKING OF NEUROPEPTIDE Y-, LAMP1- AND RAB7-TAGGED ORGANELLES IN VIVO

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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A variety of neuronal organelles with diverse functions are transported throughout the axons. The precise delivery of cargo is crucial for neurons to function. The mechanism of organelle trafficking and positioning has been the target of recent studies. Organelle trafficking is typically studied in in vitro model systems, which lack the full complexity of intact brain tissue. We utilized simultaneous dual colour 2-photon microscopy to visualize the trafficking of Neuropeptide Y-, Lamp1- and Rab7-tagged organelles in thalamocortical axons imaged in mouse cortex in vivo. The parallel imaging revealed that Lamp1- and Rab7-tagged organelles move faster than NPY tagged organelles. NPY is also trafficked more selectively in anterograde direction. By the use of a synapse marker and a calcium sensor we further investigated the transport dynamics of NPY-tagged organelles. We found that NPY-tagged organelles, which are transported in anterograde direction slow down at synapses. In contrast to previous in vitro studies, no significant change of transport speed was observed during spontaneously occurring activity and elevated calcium levels in vivo as well as electrically stimulated activity in acute brain slices.

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ELUCIDATING THE ROLE OF STIM PROTEINS IN NEURONAL ER-PM CONTACTS AND THEIR ROLE IN SYNAPTIC PLASTICITY AND ARCHITECTURE.

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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The physical proximity of the endoplasmic reticulum (ER) and the plasma membrane (PM) at contact sites, i.e., ER-PM junctions, enables ER and PM proteins to interact directly. These junctions perform vital functions like store-operated Ca2+ entry (SOCE) and lipid transfer. The functional and structural roles of proteins present in neuronal ER-PM junctions are not well understood. One of these proteins are STIMs (Stromal Interaction Molecule), sensors of Ca2+ in the ER that activate in response to a drop in ER Ca2+ concentration. Activated STIMs open CRAC and TRPC channels in the PM to allow Ca2+ influx from the extracellular space. STIMs were also shown to interact with several other targets at the PM. We aim to understand the role of STIMs in regulating the activity-dependent structural architecture at the ER-PM junctions using TIRF microscopy. We also perform Ca2+ imaging to elucidate the role of STIMs in both pre-and postsynaptic compartments. We perform single-particle tracking experiments to uncover individual STIM molecules' spatial and temporal mobility patterns. Our preliminary data indicate differential mobility patterns for STIM1 and STIM2. STIM2, the more Ca2+ sensitive and dominating STIM homolog in hippocampal neurons, appears to be more mobile in axons and less so in dendrites. The pattern of STIM1 mobility was more uniform within the whole neuron. Both STIM1 and STIM2 seems to change their mobility and localization in response to activity. We expand these findings to understand the events in which STIMs enter the pre or postsynaptic compartment.

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NEURONAL MAP6 COORDINATES ACTIN AND MICROTUBULE NUCLEATION IN VITRO.

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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The neuronal cytoskeleton, composed of actin filaments (F-actin), microtubules (MTs) and neurofilaments, is crucial for neuronal polarization, growth cone steering or dendritic branching that take place during neuronal differentiation and function. These processes rely on a very precise balance between the dynamicity and the stability of the cytoskeleton and are under control of associated proteins that bind directly to these filaments. One of this protein is the Microtubule-Associated-Protein 6 (MAP6) which is known for its strong ability to stabilize MTs in neurons. Recent work from the team has also shown that MAP6 is involved in synaptic plasticity through its ability to stabilize actin dynamics in dendritic spines. Using cell-free assays, we analyzed the role of MAP6 in the regulation of actin and MTs dynamics and identify MAP6 as a nucleator and bundling factor of F-actin. Moreover, we found that MAP6 is also able to nucleate MTs. Finally, we showed that MAP6 can simultaneously nucleate and crosslink together F-actin and MTs. Such ability to nucleate F-actin and MTs at the same time has never been describe so far and could play a role in neuronal branching or spine formation. MAP6, that has been observed in growth cone and dendritic spines, could be involved in these structures by promoting the invasion of MTs along actin filaments. Experiments are ongoing to assign a cellular function of our in vitro findings.

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FORMIN-MEDIATED ACTIN FILAMENT REGULATION IN THE AXON INITIAL SEGMENT OF HIPPOCAMPAL NEURONS

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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The location of the axon initial segment (AIS) at the junction between the soma and axon of neurons makes it instrumental in maintaining neural polarity and serving as the site for action potential generation. Recent research suggests actin structures are involved in AIS activity dependent structural plasticity, maintenance, and vesicle sorting. Thus, our aim is to elucidate how actin is regulated in the AIS mainly, but also how this might affect processes throughout the axon. For this, we have been utilizing pharmacological treatments to modify expression or activity of actin-binding proteins. We are also using super-resolution microscopy, as the specific organization of proteins at the nanoscale is crucial to understanding how the AIS performs its physiological functions. We have examined the stability of the AIS by testing how treatment with the formin inhibiting drug, small molecule inhibitor of formin homology domain 2 (SMIFH2), affected the AIS actin cytoskeleton. From this we learned that some components of the AIS, like Ankyrin-G and actin rings, are unaffected by formin inhibition. We also showed that other, longitudinal actin filaments are lost with this same treatment and that actin patches appear in greater numbers, potentially as a compensatory mechanism. The exact role of these longitudinal filaments is unknown, though they have been described before. To further understand the regulation of these filaments, we have more recently been examining how they respond to changes in neuronal excitability.

Pubmed:

35225255: Micinski D, Lahti L, Abouelezz A
Measuring Properties of the Membrane Periodic Skeleton of the Axon Initial Segment using 3D-Structured Illumination Microscopy (3D-SIM).
The axon initial segment (AIS) is the site at which action potentials initiate and constitutes a transport filter and diffusion barrier that contribute to the maintenance of neuronal polarity by sorting somato-dendritic cargo. A membrane periodic skeleton (MPS) comprising periodic actin rings provides a scaffold for anchoring various AIS proteins, including structural proteins and different ion channels. Although recent proteomic approaches have identified a considerable number of novel AIS components, details of the structure of the MPS and the roles of its individual components are lacking. The distance between individual actin rings in the MPS (~190 nm) necessitates the employment of super-resolution microscopy techniques to resolve the structural details of the MPS. This protocol describes a method for using cultured rat hippocampal neurons to examine the precise localization of an AIS protein in the MPS relative to sub-membranous actin rings using 3D-structured illumination microscopy (3D-SIM). In addition, an analytical approach to quantitively assess the periodicity of individual components and their position relative to actin rings is also described.
J Vis Exp, 2022;
32344377: Abouelezz A, Stefen H, Segerstråle M, Micinski D, Minkeviciene R, Lahti L, Hardeman EC, Gunning PW, Hoogenraad CC, Taira T, Fath T, Hotulainen P
Tropomyosin Tpm3.1 Is Required to Maintain the Structure and Function of the Axon Initial Segment.
The axon initial segment (AIS) is the site of action potential initiation and serves as a cargo transport filter and diffusion barrier that helps maintain neuronal polarity. The AIS actin cytoskeleton comprises actin patches and periodic sub-membranous actin rings. We demonstrate that tropomyosin isoform Tpm3.1 co-localizes with actin patches and that the inhibition of Tpm3.1 led to a reduction in the density of actin patches. Furthermore, Tpm3.1 showed a periodic distribution similar to sub-membranous actin rings but Tpm3.1 was only partially congruent with sub-membranous actin rings. Nevertheless, the inhibition of Tpm3.1 affected the uniformity of the periodicity of actin rings. Furthermore, Tpm3.1 inhibition led to reduced accumulation of AIS structural and functional proteins, disruption in sorting somatodendritic and axonal proteins, and a reduction in firing frequency. These results show that Tpm3.1 is necessary for the structural and functional maintenance of the AIS.
iScience, 2020; 23

30951495: Abouelezz A, Micinski D, Lipponen A, Hotulainen P
Sub-membranous actin rings in the axon initial segment are resistant to the action of latrunculin.
The axon initial segment (AIS) comprises a sub-membranous lattice containing periodic actin rings. The overall AIS structure is insensitive to actin-disrupting drugs, but the effects of actin-disrupting drugs on actin rings lack consensus. We examined the effect of latrunculin A and B on the actin cytoskeleton of neurons in culture and actin rings in the AIS. Both latrunculin A and B markedly reduced the overall amount of F-actin in treated neurons in a dose-dependent manner, but the periodicity of actin rings remained unaffected. The insensitivity of AIS actin rings to latrunculin suggests they are relatively stable.
Biol Chem, 2019; 400
26778482: Jain SK, Kanikarla-Marie P, Warden C, Micinski D
L-cysteine supplementation upregulates glutathione (GSH) and vitamin D binding protein (VDBP) in hepatocytes cultured in high glucose and in vivo in liver, and increases blood levels of GSH, VDBP, and 25-hydroxy-vitamin D in Zucker diabetic fatty rats.
Vitamin D binding protein (VDBP) status has an effect on and can potentially improve the status of 25(OH) vitamin D and increase the metabolic actions of 25(OH) vitamin D under physiological and pathological conditions. Diabetes is associated with lower levels of glutathione (GSH) and 25(OH) vitamin D. This study examined the hypothesis that upregulation of GSH will also upregulate blood levels of VDBP and 25(OH) vitamin D in type 2 diabetic rats.
Mol Nutr Food Res, 2016; 60
24961547: Jain SK, Micinski D, Huning L, Kahlon G, Bass PF, Levine SN
Vitamin D and L-cysteine levels correlate positively with GSH and negatively with insulin resistance levels in the blood of type 2 diabetic patients.
Vitamin D, L-cysteine (LC) and glutathione (GSH) levels are lower in the blood of diabetic patients. This study examined the hypothesis that the levels of vitamin D and LC correlate with those of GSH in the blood of type 2 diabetic patients (T2D), and that vitamin D and LC upregulate glutamate-cysteine ligase (GCLC), which catalyzes GSH biosynthesis, in cultured monocytes.
Eur J Clin Nutr, 2014; 68
22989474: Jain SK, Micinski D, Lieblong BJ, Stapleton T
Relationship between hydrogen sulfide levels and HDL-cholesterol, adiponectin, and potassium levels in the blood of healthy subjects.
Hydrogen sulfide (H(2)S) is an important signaling molecule whose blood levels have been shown to be lower in certain disease states. Increasing evidence indicates that H(2)S plays a potentially significant role in many biological processes and that malfunctioning of H(2)S homeostasis may contribute to the pathogenesis of vascular inflammation and atherosclerosis. This study examined the fasting blood levels of H(2)S, HDL-cholesterol, LDL-cholesterol, triglycerides, adiponectin, resistin, and potassium in 36 healthy adult volunteers. There was a significant positive correlation between blood levels of H(2)S and HDL-cholesterol ($r = 0.49$, $p = 0.003$), adiponectin ($r = 0.36$, $p = 0.04$), and potassium ($r = 0.34$, $p = 0.047$), as well as a significant negative correlation with LDL/HDL levels ($r = -0.39$, $p = 0.02$). This is the first demonstration of an association of circulating levels of H(2)S with the HDL, LDL, and adiponectin homeostasis in the blood of healthy humans.
Atherosclerosis, 2012; 225
23770363: Jain SK, Micinski D
Vitamin D upregulates glutamate cysteine ligase and glutathione reductase, and GSH formation, and decreases ROS and MCP-1 and IL-8 secretion in high-glucose exposed U937 monocytes.
Glutathione is a major endogenous antioxidant and its deficiency is implicated in the etiology and progression of a number of human diseases. Vitamin D is important for the prevention of osteoporosis, cardiovascular disease, diabetes, autoimmune diseases, and some cancers. Using a monocyte cell model, this study examined the hypothesis that vitamin D upregulate glutamate cysteine ligase (GCLC) and glutathione reductase (GR), which catalyzes GSH biosynthesis.
Biochem Biophys Res Commun, 2013; 437
31172369: Kanikarla-Marie P, Micinski D, Jain SK
Hyperglycemia (high-glucose) decreases L-cysteine and glutathione levels in cultured monocytes and blood of Zucker diabetic rats.
L-Cysteine (LC) is an essential precursor of GSH biosynthesis. GSH is a major physiological antioxidant, and its depletion increases oxidative stress. Diabetes is associated with lower blood levels of LC and GSH. The mechanisms leading to a decrease in LC in diabetes are not entirely known. This study reports a significant decrease in LC in human monocytes exposed to high glucose (HG) concentrations as well as in the blood of type 2 diabetic rats. Thus, a significant decrease in the level of LC in response to exposure to HG supports the assertion that uncontrolled hyperglycemia contributes to a reduction of blood levels of LC and GSH seen in diabetic patients. Increased requirement of LC to replace GSH needed to scavenge excess ROS generated by hyperglycemia can result in lower levels of LC and GSH. Animal and human studies

report that LC supplementation improves GSH biosynthesis and is beneficial in lowering oxidative stress and insulin resistance. This suggests that hyperglycemia has a direct role in the impairment of LC and GSH homeostasis in diabetes.
Mol Cell Biochem, 2019; 459
22852873: Jain SK, Manna P, Micinski D, Lieblong BJ, Kahlon G, Morehead L, Hoeldtke R, Bass PF, Levine SN
In African American type 2 diabetic patients, is vitamin D deficiency associated with lower blood levels of hydrogen sulfide and cyclic adenosine monophosphate, and elevated oxidative stress?
African Americans (AA) have a higher incidence of cardiovascular disease and vitamin D (VD) deficiency compared with Caucasians. Hydrogen sulfide (H(2)S) is an important signaling molecule. This study examined the hypothesis that blood levels of H(2)S are lower in AA type 2 diabetic patients (T2D). Fasting blood was obtained from T2D and healthy controls. Results showed a significant decrease in plasma levels of cyclic adenosine monophosphate (cAMP) and H(2)S in AA T2D but not in Caucasian T2D when compared with those of respective age- and race-matched healthy controls. Plasma VD levels were significantly lower in AA T2D compared with Caucasian T2D. Cell culture studies demonstrate that 1,25(OH)(2)-VD supplementation significantly increased expression of cystathionine-γ-lyase (CSE), H(2)S formation, and cAMP secretion, but decreased reactive oxygen species in high glucose-treated U937 monocytes. This suggests that VD supplementation upregulates CSE and H(2)S formation and decreases oxidative stress, and that VD deficiency may contribute to the malfunctioning of H(2)S signaling and thus a higher incidence of vascular inflammation in AA. These results lead to the hypothesis that VD supplementation can replenish blood concentrations of H(2)S and cAMP and lower oxidative stress and cardiovascular disease in AA T2D.
Antioxid Redox Signal, 2013; 18
24665821: Jain SK, Huning L, Micinski D
Hydrogen sulfide upregulates glutamate-cysteine ligase catalytic subunit, glutamate-cysteine ligase modifier subunit, and glutathione and inhibits interleukin-1β secretion in monocytes exposed to high glucose levels.
Glutathione (GSH) deficiency and interleukin-1β (IL-1β) upregulation are linked to the progression of vascular inflammation and atherosclerosis. The consumption of sulfide-rich vegetables is known to lower the risk of atherosclerosis. This study examined the hypothesis that hydrogen sulfide (H2S) upregulates the glutamate-cysteine ligase catalytic subunit (GCLC) and GSH and inhibits IL-1β in a monocyte cell model. U937 monocytes were supplemented with H2S (0-12.5 μM) for 2 hr and then exposed to a control or high glucose (HG, 25 mM) for 22 hr. Levels of GCLC and glutamate-cysteine ligase modifier subunit (GCLM) expression were determined by western blotting and GSH using high-performance liquid chromatography (HPLC), and IL-1β using enzyme-linked immunoassay (ELISA). H2S significantly ($P<0.05$) upregulated expression of GCLC and GCLM, and formation of GSH, and inhibited IL-1β secretion in controls and HG-treated monocytes. This is the first demonstration of H2S upregulation of GCLC and GSH and inhibition of IL-1β levels, which may be what mediates the beneficial effects of H2S-rich compounds in mitigating the pathogenesis of metabolic syndrome and atherosclerosis.
Metab Syndr Relat Disord, 2014; 12

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BOARD NUMBER: S01-464

POLYGLUTAMYLATION OF MICROTUBULES DRIVES MOTOR AXON REMODELLING

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Microtubules are dynamic cytoskeletal structures build from tubulin dimers, which can carry a range of post-translational modifications (PTMs). Together with associated proteins, PTMs establish a ‘code’, endowing the microtubule scaffold with specific and local functionality, such as regulating microtubule length and dynamics and microtubule-dependent transport. However, how this code translates into shaping cell- or tissue-level events, remains largely unexplored.
We investigate how polyglutamylation, a PTM enriched on neuronal microtubules, regulates postnatal motor axon remodelling: a regressive process in which ~90% of terminal branches prune, involving spastin-mediated loss of microtubules. While spastin is known to preferentially sever polyglutamylated microtubules, it remains elusive whether branch-specific polyglutamylation drives pruning.
Consistent with an instructive role of polyglutamylation, motoneurons ablated of deglutamylases CCP1&6 accelerate axon dismantling, while deletion of chain-elongating glutamylase TTLL1, delayed axonal remodelling. Further measurements of polyglutamylation, microtubule mass and dynamics corroborate the predicted branch-specific regulation of microtubule stability, hinting at a rheostatic regulation of spastin-mediated severing and hence axonal remodelling. Surprisingly, deleting TTLL7, which ‘seeds’ the first glutamate residue, had no effect on polyglutamylation, suggesting several layers of regulation that are engrained in parallel or consecutive steps of editing the tubulin code.
Together, a specific tubulin PTM—polyglutamylation—acts as an instructive signal for spastin-mediated severing, which in turn paces developmental axon pruning. The ‘tubulin code’ could thus control specific morphogenetic events during nervous system development. Future work tries to unravel if synaptic activity coordinates glutamylases and deglutamylases and whether similar mechanisms determine axon stability in central neurons and during disease-related remodelling.

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BOARD NUMBER: S01-465

UNRAVELING THE ROLES OF AN ALTERNATIVELY SPLICED MICROEXON IN DAAM1 IN NERVOUS SYSTEM DEVELOPMENT AND FUNCTION

POSTER SESSION 01 - SECTION: SUBCELLULAR STRUCTURE AND MECHANISMS INVOLVED IN NEURAL ACTIVITY

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Microexons are an unusual form of alternative splicing characterized by small size and high evolutionary conservation, however, their functions are still far from being understood. In this work, we focused on a neuronal-specific microexon that modulates the structure of the FH2 domain of DAAM1, a protein important for actin dynamics. Using TIRF microscopy approaches we demonstrated that microexon inclusion directly impacts actin nucleation and polymerization capabilities of the FH2 domain, suggesting a modulatory effect on DAAM1´s function. Actin dynamics is an important factor in neuronal differentiation and function. Our results demonstrate higher neuronal activity upon microexon removal, both in an in vitro system of differentiated glutamatergic neurons and in vivo, in a microexon KO mouse model. This presumably synaptic-driven phenotype translated into further developmental imbalances in young mice, and later on, motor and learning impairments in adulthood. These results thus reveal a highly conserved and splicing-driven control mechanism of neuronal functioning, involved in higher cognitive abilities.

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ALTERED MICROGLIA SIGNALING IN MOUSE MODELS OF SCHIZOPHRENIA

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Microglia, the resident macrophages of the brain, play crucial roles in neuronal development. They directly modulate neuronal connectivity and synaptic density by synaptic pruning, relying on highly specific signaling cascades on the neuronal and on the microglial side. In the context of neurodevelopmental disorders (NDDs), specific elements of microglial signaling cascades have been shown to contribute to network disruption. However, it remains unclear how specific signaling molecules might be involved in the miswiring of neuronal networks in early development of NDDs, specifically on the level of individual synapses. Here, we combined analysis on the RNA level and protein level with computational analysis of morphology and microglia reactivity to assess the role of microglia in early development. We specifically focused on the prefrontal cortex, an area that is involved in cognitive processes which are particularly sensitive to disruption in NDDs. High-throughput quantitative PCR revealed a subset of microglial signaling molecules, which were expressed in a particularly differential manner in early development. Advanced immunohistochemistry confirmed distinct expression patterns on the level of individual synapses, revealing a complex interplay between microglial and neuronal signaling in the context of synaptic maintenance and pruning. Furthermore, we could show disrupted expression of specific signaling molecules in a mouse model of schizophrenia, where morphological analysis revealed a clear redistribution of microglia reactive states, specifically when comparing layer 2/3 and layer 5/6 of the PFC. Taken together, our data provide insights regarding the mechanisms that might underlie the neuronal miswiring characteristic to models of neurodevelopmental disorders.

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BOARD NUMBER: S01-467

COMPLEMENT RECEPTORS C3AR AND CR3 MEDIATE LOSS OF SYNAPTIC INPUTS AND NMDAR HYPOFUNCTION IN A MOUSE MODEL OF SCHIZOPHRENIA-ASSOCIATED HIGH C4 EXPRESSION

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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The complement system is a set of proteins contributing to normal immune responses. Over the last two decades studies have revealed key functions of these components in synaptic pruning, a process by which synapses in excess are eliminated during brain maturation. Interestingly, convergent studies point to a loss of dendritic spines in neurons in the cortex of schizophrenic patients, which could reflect disturbances of the normal process of synaptic pruning. High-expression variants of complement component 4 (C4) gene constitute a major risk factor of schizophrenia. We and others have found that over-expressing C4 in the mouse cortex recapitulates several schizophrenia-associated phenotypes, including synapse loss.
To assess the role of complement pathways on brain circuit development during elevated C4 expression, we over-expressed C4 in the prefrontal cortex using in utero electroporation of a C4-encoding plasmid in two different transgenic mouse lines lacking complement receptors CR3 or C3aR, which are both expressed in microglial cells. Using patch-clamp in layer III pyramidal cells of the PFC, we observed that the absence of CR3, but not C3aR, leads to alterations in functional excitatory transmission. Furthermore, C4-induced changes in synaptic transmission, such as decreased glutamatergic input and altered AMPA/NMDA ratio, were normalized in mice lacking either CR3 or C3aR. Given the known role of CR3 and C3aR in microglial cells, these results suggest that the effects of C4 overexpression may involve both recognition of opsonized synaptic material by CR3 and microglial activation by C3aR.

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HUMAN MICROGLIA ENHANCE NEURONAL MATURATION AND SYNAPTIC CONNECTIVITY

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Background Microglia support neurons during postnatal development and in homeostatic conditions and this is altered in pathological conditions. Microglia invade and colonize the developing brain before the start of corticogenesis and accumulate when synapses start to form in the foetal cortex, however the role of immune cells in prenatal development is not well understood. Clinical data from first and second trimester maternal infections indicate changes in brain development and function by the increased risk of manifestation of neuropsychiatric conditions. Moreover, patients with mutated CSF1R show highly reduced microglia survival and severe developmental brain malformations. Aims We hypothesized that microglia could support neuronal maturation and network formation during prenatal development before the generation of astrocytes and oligodendrocytes. Results We established a highly enriched TBR1 positive human cortical neuron culture system where we can control cell density and neuronal birthdates and tested the role of microglia in neuronal maturation. We found that microglia promote neuronal maturation in multiple ways. First by augment voltage gated sodium and potassium channel densities and thus the generation of action potentials. Second microglia also increase synapse formation and activity before the generation of astrocytes. Conclusion Our results demonstrate that microglia can enhance neuronal maturation and synaptic connectivity during prenatal development.

Pubmed:
34711959: de Faria O, Pivonkova H, Varga B, Timmler S, Evans KA, Káradóttir RT
Periods of synchronized myelin changes shape brain function and plasticity.
Myelin, a lipid membrane that wraps axons, enabling fast neurotransmission and metabolic support to axons, is conventionally thought of as a static structure that is set early in development. However, recent evidence indicates that in the central nervous system (CNS), myelination is a protracted and plastic process, ongoing throughout adulthood. Importantly, myelin is emerging as a potential modulator of neuronal networks, and evidence from human studies has highlighted myelin as a major player in shaping human behavior and learning. Here we review how myelin changes throughout life and with learning. We discuss potential mechanisms of myelination at different life stages, explore whether myelin plasticity provides the regenerative potential of the CNS white matter, and question whether changes in myelin may underlie neurological disorders.
Nat Neurosci, 2021; 24

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BOARD NUMBER: S01-469

MICROGLIA ONTOGENY AND EARLY-LIFE STRESS: MICROGLIAL AND BEHAVIORAL SEX-SPECIFIC RESPONSES IN PREPUBESCENT MICE TO MATERNAL SEPARATION DURING INFANCY

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Early-life stress is etiologically linked to many neuropsychiatric disorders, and several emerge during adolescence, another sensitive developmental window. One common feature of both periods is neuronal circuit-remodeling in response to environmental inputs. Microglia is highly responsive and involved in developmental processes, and its malfunctioning is seen in neurodevelopmental disorders. Aim: To evaluate the effects of maternal separation (MS) on microglia and behavior of prepubescent male and female CX3CR1:GFP+/- mice. Pups were subjected to 3 hours of MS (P2-P14), and tested for anxiety- and depressive-like behaviors at P30; other subgroups were euthanized at P15 or P30, and hippocampal tissue was collected. Microglial numbers and morphology were analyzed by confocal microscopy. Data were analyzed using Student's *t-test* or Two-Way ANOVA and Tukey test. Results: Decreased microglial numbers from P15 to P30 in males; females do not follow this pattern and have fewer microglia already at P15. Female microglia had longer dendrites than male's at P30. They also responded differently to MS: males had smaller cells, whereas females had a decrease in cell complexity. We also see anxiety- and depressive-like behavioral phenotypes in MS-males, whereas MS-females were less responsive and exhibited only an increase in exploratory-motor behavior. Conclusions: We observed sex-specific ontogeny pathways in microglial morphology, and sex-specific responses to MS, with a decrease in cell complexity only in females; we also identified sex-specific behavioral effects after MS (stronger effect in males). Further investigations of the mechanisms behind sex- and stress-related differences are needed to understand how microglial physiology interferes in developmental pathways.

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LACK OF LIPOCALIN 2 EXPRESSION DURING PRENATAL INFECTION AFFECTS BRAIN DEVELOPMENT

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Aims Epidemiological studies indicate that maternal infection during pregnancy is a risk factor for neurodevelopmental disorders. However, the mechanisms underlying this phenomenon remain unclear. One of the highly expressed proteins in the adult brain in response to infection is lipocalin 2 (Lcn2), an innate immune response protein. Our studies aim to characterize the role of Lcn2 in the regulation of neuronal circuitry development during prenatal infection. **Methods** To mimic maternal infection we used a model of maternal immune activation where the pregnant mice received i.p. injections of lipopolysaccharide on E16-18. To evaluate Lcn2 mRNA expression we performed qRT-PCR on fetal (E19) and adolescent (P14) hippocampus. To address how lack of Lcn2 during prenatal infection may influence electrophysiological properties of neurons in the adult brain, we performed excitability and miniature excitatory postsynaptic currents (mEPSC) recordings from hippocampal CA1 pyramidal cells on acute brain slices from Lcn2 WT and KO offspring after the LPS challenge. **Results and Conclusions** Our results indicate that Lcn2 mRNA is significantly upregulated in the hippocampus in response to prenatal infection in the fetal and adolescent brain. We also observed that the absence of Lcn2 in the developing brain leads to higher intrinsic excitability of hippocampal pyramidal neurons in the adult brain, but only in mice exposed to prenatal infection. Moreover, lack of Lcn2 resulted in decreased synaptic strength of hippocampal CA1 neurons manifested by decreased mEPSC amplitude. These findings suggest that lipocalin 2 could be a promising link between the immune response and brain development.

Pubmed:

33375279: Frączek K, Kowalczyk A, Pekala M, Kasarello K, Sygitowicz G, Sulejczak D, Zaremba M, Konop M, Frankowska M, Filip M, Bujalska-Zadrozny M, Kleczkowska P
The Positive and Negative Outcome of Morphine and Disulfiram Subacute Co-Administration in Rats in the Absence of Ethanol Challenge.
Recently, a well-known anti-alcohol agent, disulfiram (DSF), has gain much interest, as it was found to be effective in the treatment of cocaine abusers, thus also giving hope for patients addicted to opioids and other illicit drugs. Therefore, this study was aimed to investigate the possible outcome that might occur within the subacute co-administration of both morphine (MRF) and DSF in rats, but in the absence of ethanol challenge. As observed, intraperitoneal DSF dose-dependently enhanced MRF-mediated analgesia with the maximal efficacy at a dose of 100 mg/kg. Furthermore, MRF-induced tolerance and aggressive behavior were significantly reduced by DSF (100 mg/kg, i.p.) in comparison to MRF solely. Nonetheless, significant blood biochemical markers of hepatotoxicity were found (i.e., alteration in the levels of glutathione, blood urea nitrogen, etc.), following a combination of both drugs. Likewise, histological analysis of liver tissue revealed severe changes in the group of DSF + MRF, which includes swelling, cell death, damage to certain vessels, and hemorrhages into the liver parenchyma. Our findings indicate that DSF should be used with extreme caution, especially within the course of subacute concomitant use with MRF, as several possible side effects may take place.
Pharmaceutics, 2020; 13
33382515: Pekala M, Doliwa M, Kalita K
Impact of maternal immune activation on dendritic spine development.
Dendritic spines are small dendritic protrusions that harbor most excitatory synapses in the brain. The proper generation and maturation of dendritic spines are crucial for the regulation of synaptic transmission and formation of neuronal circuits. Abnormalities in dendritic spine density and morphology are common pathologies in autism and schizophrenia. According to epidemiological studies, one risk factor for these neurodevelopmental disorders is maternal infection during pregnancy. This review discusses spine alterations in animal models of maternal immune activation in the context of neurodevelopmental disorders. We describe potential mechanisms that might be responsible for prenatal infection-induced changes in the dendritic spine phenotype and behavior in offspring.

Dev Neurobiol, 2021; 81
31375980: Nader K, Krysiak A, Beroun A, Pekala M, Szymanska M, Kuzniewska B, Radwanska K, Kaczmarek L, Kalita K
Loss of serum response factor in mature neurons in the dentate gyrus alters the morphology of dendritic spines and hippocampus-dependent behavioral tasks.
Serum response factor (SRF) is a major transcription factor that regulates the expression of several plasticity-associated genes in the brain. Although the developmental expression of SRF in excitatory neurons is crucial for establishing proper hippocampal circuitry, no substantial evidence of its role in unstimulated mature neurons has been provided. The present study used time-controlled, conditional SRF knockout mice and found that the lack of SRF in adult neurons led to decreased actin levels and inactivation of the actin-severing protein cofilin 1 through its increase in phosphorylation at Ser3. The augmentation of cofilin 1 phosphorylation correlated with an alteration of dendritic spine morphology in the dentate gyrus, which was reflected by an increase in the number of spines that clustered into the long-spine category. The changes in spine morphology coincided with a lower amplitude and frequency of miniature excitatory postsynaptic currents. Moreover, SRF knockout animals were hyperactive and exhibited impairments in hippocampus-dependent behaviors, such as digging, marble burying, and nesting. Altogether, our data indicate that the adult deletion of neuronal SRF leads to alterations of spine morphology and function and hippocampus-dependent behaviors. Thus, SRF deletion in adult neurons recapitulates some aspects of morphological, electrophysiological, and behavioral changes that are observed in such psychiatric disorders as schizophrenia and autism spectrum disorders.
Brain Struct Funct, 2019; 224

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BOARD NUMBER: S01-471

DYNAMIC NEURONAL IL-1R1 EXPRESSION IN NEURODEVELOPMENT

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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The signaling of the inflammatory cytokine, interleukin-1 (Il-1), through its receptor interleukin-1 receptor-1 (IL-1R1), plays diverse and critical roles in the central nervous system including memory consolidation, neuroinflammation, and mood control. The role of this signaling system has been mainly studied in adults, whereas its influences on brain development and the relevance of such influences to developmentally related neuropsychiatric disorders are unknown. Recently, we discovered IL-1R1 expression in neurons during development. We hypothesize that activities mediated by neuronal IL-1R1 (nIL-1R1) contribute to behavioral abnormalities of neurodevelopmental disorders. In this study, we investigated the spatiotemporal patterns of nIL-1r1 expression in order to lay a foundation for understanding the role of nIL-1R1 in neurodevelopment. To this end, we used our global IL-1R1 reporter mouse line as well as lines in which we restricted the expression of IL-1R1 to specific neuronal or non-neuronal cell types. We found that nIL-1R1 expression is very dynamic during brain development and changes dramatically in various brain regions (dentate gyrus-DG, dorsal raphe nucleus-DRN, thalamic nuclei) in a short time period. nIL-1R1 expression is not detected until P7 in DG while is detectable as early as P4 in DRN. The expression starts in the inner layer of the granular cell layer and progressively increases throughout of mouse life course. These changes are associated with the development of the memory, sensory, and mood-related circuitry of the brain. Moreover, we found that endothelial cells and T cells contribute to nIL-1R1 expression. These findings suggest that nIL-1R1 is tightly regulated during brain development.

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BOARD NUMBER: S01-472

IN VIVO EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF ALGINATE IN THE TEMPORAL LOBE EPILEPTIC RAT BRAIN

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Aim Temporal Lobe Epilepsy (TLE) is the most common type of drug-resistant epilepsy in adults, and mesial TLE, characterized by hippocampal sclerosis, is the most severe TLE form. Neural stem cell (NSC) therapy has been considered a promising intervention to treat seizures. Since inflammation is a hallmark of epileptic brain, **targeting inflammation represents an important tool to generate a microenvironment suitable for brain regeneration**. The vegetal polysaccharide alginate has been used *in vivo* in central nervous system regeneration approaches as NSC delivery system as well as scaffold providing both structural support and neuroprotective cues to promote axonal regrowth and functional recovery. In this study, we aim to investigate the *in vivo* anti-inflammatory activity of the alginate hydrogel when co-injected with NSCs in a lesioned epileptic brain. **Methods** Adult pilocarpine-treated Sprague-Dawley male rats were transplanted with NSCs with or without alginate in the ventral CA3 area. To avoid an increase in intracranial pressure, rats were previously lesioned with the injection of the cytotoxic agent ibotenic acid. Nine weeks after transplantation, immunofluorescence analyses were performed to investigate astrocytic and microglial activation. **Results and Conclusions** Rat brain tissue transplanted with NSCs and alginate show a thinner glial scar and smaller microglial cell body size compared to brain tissue injected with NSCs alone. These results indicate an anti-inflammatory effect of alginate in the lesioned epileptic brain.

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BOARD NUMBER: S01-473

ANTIBIOTICS ADMINISTRATION DURING GESTATION MAY AFFECT MEMORY AND BRAIN STRUCTURE IN YOUNG OFFSPRING MICE

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

Dmytro Shepilov[1], Dmytro Ilienko[2], Iryna Osadchenko[1], Tetiana Kovalenko[1], Kateryna Smozhanyk[1], Galyna Skibo[1]
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Aims. The present study aimed to evaluate whether antibiotic treatment during gestation can trigger memory decline and brain structural alterations in mouse offspring. **Methods.** C57BL/6J female mice were divided into two groups (n=3/group): 1) *Control* – drank sterile water over the entire gestation, and 2) *Antibiotics* – consumed amoxicillin (205 mg/kg bw/day) and azithromycin (51 mg/kg bw/day) in sterile water during the 3rd week of gestation. 4-week-old mice born from those dams were conducted behavioral tests, immunohistochemistry of the hippocampus, and electron microscopy of the corpus callosum. **Results.** We revealed that the Antibiotics group had significantly higher latency to find a hidden platform on the 2nd and 3rd days of acquisition and less time spent in the target quadrant during probe test in Morris water maze. Novel object recognition test, in turn, did not show a significant difference in associative memory between groups. At the morphological level, mice of the Antibiotics group were characterized by a decrease in the density of CA1 pyramidal neurons and astrocytes in the hippocampus, as well as a thinning of myelin sheaths and a tendency to reduce the number of myelinated axons in the corpus callosum, compared to the Control animals. **Conclusions.** Antibiotic treatment during gestation was demonstrated to impact the developing brain which resulted in spatial reference memory impairments, changed hippocampal cellular structure, and signs of demyelination in young offspring mice.

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BOARD NUMBER: S01-474

ABERRANT INFLAMMATORY ACTIVITY OF MICROGLIA INFLUENCES NEURONAL CONNECTIVITY AND ACTIVITY IN SCHIZOPHRENIA IN VITRO

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Schizophrenia is a complex neuropsychiatric disorder often signified by impairments of synaptic transmission. One hypothesis assigns a role for activated microglia to mediate aberrant synaptic pruning. However, interaction between human neurons and microglia in a co-culture exclusively composed of iPSC-derived cells has not been studied so far. Here, we demonstrate increased inflammatory activation in a neuron-microglia co-culture model derived from patients with schizophrenia. A reduction of the presynaptic marker synapsin is accompanied by increased uptake of synapsin-positive terminals by microglia. Interestingly, pre-treatment of microglia with the pro-inflammatory agent LPS replicated the decreased synaptic densities in neuron-glia cultures whilst stimulation with the anti-inflammatory drug Minocycline rescued the observed synaptic pruning deficits of schizophrenia microglia. Neuronal activity was studied after additional inclusion of iPSC-derived GABAergic interneurons in the cultures, leading to robustly observable single-cell activity. When schizophrenia microglia were co- cultured with healthy neurons, we observed a strong reduction in neuronal peak frequency during calcium imaging. Concurrent with our previous results, a reduction of excitatory presynapses was observed in co-cultures of healthy neurons and patient microglia. In conclusion, we generated a 2D *in vitro* human cellular test system to study neuron-microglia interactions in schizophrenia. We observed an inflammatory phenotype in patient microglia, potentially resulting in aberrant synaptic pruning and decreased neuronal activity in neuron-glia cultures. The observed anti-inflammatory effect of the drug Minocycline provides evidence for the usability of our model system to study mechanisms of disease candidate drugs.

Pubmed:
34638595: Heider J, Vogel S, Volkmer H, Breitmeyer R
Human iPSC-Derived Glia as a Tool for Neuropsychiatric Research and Drug Development.
Neuropsychiatric disorders such as schizophrenia or autism spectrum disorder represent a leading and growing burden on worldwide mental health. Fundamental lack in understanding the underlying pathobiology compromises efficient drug development despite the immense medical need. So far, antipsychotic drugs reduce symptom severity and enhance quality of life, but there is no cure available. On the molecular level, schizophrenia and autism spectrum disorders correlate with compromised neuronal phenotypes. There is increasing evidence that aberrant neuroinflammatory responses of glial cells account for synaptic pathologies through deregulated communication and reciprocal modulation. Consequently, microglia and astrocytes emerge as central targets for anti-inflammatory treatment to preserve organization and homeostasis of the central nervous system. Studying the impact of neuroinflammation in the context of neuropsychiatric disorders is, however, limited by the lack of relevant human cellular test systems that are able to represent the dynamic cellular processes and molecular changes observed in human tissue. Today, patient-derived induced pluripotent stem cells offer the opportunity to study neuroinflammatory mechanisms in vitro that comprise the genetic background of affected patients. In this review, we summarize the major findings of iPSC-based microglia and astrocyte research in the context of neuropsychiatric diseases and highlight the benefit of 2D and 3D co-culture models for the generation of efficient in vitro models for target screening and drug development.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S01-475

INFLAMMATORY PAIN INDUCES MICROGLIAL DEREGULATION WITHIN THE MESOCORTICOLIMBIC SYSTEM: IMPACT ON MU-OPIOID RECEPTOR INTERNALISATION AND ACTIVATION

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

Javier Cuitavi[1], Paula Andrés-Herrera[1], David Meseguer[1], Duart-Abadia[2], Julie Sanchez[3], Jesús David Lorente[1], Isabel Fariñas[2], Meritxell Canals[3], Lucía Hipólito[1]
[1]University of Valencia, Pharmacy And Pharmaceutical Technology And Parasitology, Burjassot, Spain, [2]University of Valencia (CIBERNED and BIOTECMED), Cellular Biology, Functional Biology And Physical Anthropology, Burjassot, Spain, [3]University of Nottingham (Queen's Medical Centre), Physiology, Pharmacology And Neuroscience (compare), Nottingham, United Kingdom

Background and aims Chronic pain is a complex health burden that is increasingly affecting patients worldwide. Interestingly, microglia play a pivotal role in pain management in the spinal cord through neuron-glial interactions. Nevertheless, little is known of this interaction within the mesocorticolimbic system (MCLS) which modulates motivation, reward, and aversion. Pain is usually associated with other pathologies such as depression, anxiety, and addiction, so studying how pain modulates microglial activity in the MCLS could clarify the biochemical substrate of such comorbidities. The present work studies how inflammatory pain regulates microglial activation in the MCLS and observes if microglial activation modulates Mu-Opioid Receptor (MOR), one of the key receptors in pain management and addiction, activation and internalisation. Methods Complete Freund Adjuvant (CFA) inflammatory pain animal model was used in Sprague Dawley rats by subcutaneously injecting CFA and saline in the hind paw. A week after the injection animals were sacrificed and IBA1 Immunohistochemistry was used to assess microglial activation in the areas of the MCLS. Bioluminescence Resonance Energy Transfer (BRET) was used to study changes in neuronal MORs internalisation and activation induced by microglial activation in cultured cells. Results Inflammatory pain alters microglial activation in the MCLS and MORs activation and internalisation is enhanced by microglia in presence of an agonist. Conclusions Although further research is necessary to clarify the mechanisms that underlie pain-induced microglial modulation of the neuronal MORs, understanding these events in the MCLS could be crucial to create new therapeutical strategies to approach pain comorbidities.

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CHONDROITIN SULPHATE PROTEOGLYCANS POTENTIALLY INDUCES REACTIVE ASTROGLIOSIS

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Astrocytes are the essential glial cells important for the maintenance of homeostasis and functions, including energy homeostasis, neurotransmitter recycling and synaptic activity, of the central nervous system. In addition, astrocytes regulate the synthesis and degradation of extracellular matrix (ECM) structures, and with ECM, astrocytes build up tetrapartite structure to maintain pre and post synaptic processes. After spinal cord injury, astrocytes undergo morphological and functional changes called reactive astrogliosis, and migrate to injury area to form the glial scar. During this process, expression, and secretion of chondroitin sulphate proteoglycans (CSPGs) are increased by astrocytes. CSPGs are ECM molecules and regulate axonal pathfinding and circuitry consolidation. CSPGs synthesis during glial scar formation are mostly inhibitory and studies showed that degradation of CSPGs by chondroitinase ABC after spinal cord injury enhances axonal regeneration and functional recovery. However, the effect of secreted CSPGs on astrocytes themselves is unclear. In our study, we have investigated the effect of aggrecan, one of the key CSPGs in CNS, on primary cortical astrocytes *in vitro*. Our results reveal that aggrecan induces cellular migration and inhibits cellular adhesion. Also, our preliminary quantification analysis showed that expression of GLAST and GFAP increases under the soluble aggrecan treatment and causes morphological changes. These results suggest that CSPGs may induces the reactive astrogliosis and we propose that CSPGs may induce expression of CSPGs in an autocrine loop. Further experiment and analysis are ongoing to prove this hypothesis.

Pubmed:
30240706: Ates T, Oncul M, Dilsiz P, Topcu IC, Civas CC, Alp MI, Aklan I, Ates Oz E, Yavuz Y, Yilmaz B, Sayar Atasoy N, Atasoy D
Inactivation of Magel2 suppresses oxytocin neurons through synaptic excitation-inhibition imbalance.
Prader-Willi and the related Schaaf-Yang Syndromes (PWS/SYS) are rare neurodevelopmental disorders characterized by overlapping phenotypes of high incidence of autism spectrum disorders (ASD) and neonatal feeding difficulties. Based on clinical and basic studies, oxytocin pathway defects are suggested to contribute disease pathogenesis but the mechanism has been poorly understood. Specifically, whether the impairment in oxytocin system is limited to neuropeptide levels and how the functional properties of broader oxytocin neuron circuits affected in PWS/SYS have not been addressed. Using cell type specific electrophysiology, we investigated basic synaptic and cell autonomous properties of oxytocin neurons in the absence of MAGEL2; a hypothalamus enriched ubiquitin ligase regulator that is inactivated in both syndromes. We observed significant suppression of overall ex vivo oxytocin neuron activity, which was largely contributed by altered synaptic input profile; with reduced excitatory and increased inhibitory currents. Our results suggest that dysregulation of oxytocin system goes beyond altered neuropeptide expression and synaptic excitation inhibition imbalance impairs overall oxytocin pathway function.
Neurobiol Dis, 2019; 121

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BOARD NUMBER: S01-477

INFLAMMATORY EXOSOMES TRANSFER DANGER SIGNALS AND INDUCE GLIAL DYSFUNCTIONAL CALCIUM DYNAMICS IN NAïVE SPINAL CULTURED EXPLANTS

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Neuroinflammation is a shared hallmark of almost every pathology in the central nervous system (CNS), from ischemic or traumatic injury to neurodegenerative diseases. Despite the increasing knowledge of the molecular and cellular mechanisms contributing to neuroinflammatory pathways, the understanding of shared mediators underlying neuroinflammation for the development of novel therapeutic strategies is still lacking. Extracellular vesicles released by astrocytes, the CNS prevalent cells, are key vectorized systems able to spread and actively transfer signalling molecules, modulating target cell functions. We isolated exosomes from LPS-treated (inflamed) organotypic spinal cord slices to investigate vesicle ability to induce glial cell reactivity and inflammation in naïve organotypic spinal slices. The slices were obtained from E13 mouse embryos, cultured *in vitro* for two weeks and treated for 24 h with lipopolysaccharide (LPS 1 µg/ml), to induce spinal neuroinflammation. Upon medium collection and centrifugation, we isolated for the first time exosomes from cultured slices which were used to treat naive slices. LPS-induced neuroinflammation was documented by immunofluorescence and confocal microscopy quantifying glial reactivity by GFAP and Iba1 staining. Exosomes were characterised by Nanoparticle Tracking Analysis (NTA), Atomic Force Microscopy and western-blot analysis and their impact on healthy spinal tissue was assessed with similar approaches while dysfunctional Ca^{2+} signalling in reactive astrocytes was monitored by AAV5. gfaABC1D-cyto-GCaMP6f, a genetically encoded calcium indicator. Confocal microscopy and live imaging documented the ability of exosomes to activate reactive responses in healthy astrocytes. The results obtained strongly indicate that exosomes enable inflammatory danger signalling transfer from treated to naïve tissue.

Pubmed:

26640075: De Paola M, Sestito SE, Mariani A, Memo C, Fanelli R, Freschi M, Bendotti C, Calabrese V, Peri F
Synthetic and natural small molecule TLR4 antagonists inhibit motoneuron death in cultures from ALS mouse model.
Increasing evidence indicates that inflammatory responses could play a critical role in the pathogenesis of motor neuron injury in amyotrophic lateral sclerosis (ALS). Recent findings have underlined the role of Toll-like receptors (TLRs) and the involvement of both the innate and adaptive immune responses in ALS pathogenesis. In particular, abnormal TLR4 signaling in pro-inflammatory microglia cells has been related to motoneuron degeneration leading to ALS. In this study the effect of small molecule TLR4 antagonists on in vitro ALS models has been investigated. Two different types of synthetic glycolipids and the phenol fraction extracted from commercial extra-virgin olive oil (EVOO) were selected since they efficiently inhibit TLR4 stimulus in HEK cells by interacting with the TLR4·MD-2 complex and CD14 co-receptor. Here, TLR4 antagonists efficiently protected motoneurons from LPS-induced lethality in spinal cord cultures, and inhibited the interleukine-1β production by LPS-stimulated microglia. In motoneurons/glia cocultures obtained from wild type or SOD1 G93A mice, motoneuron death induced by SOD1mut glia was counteracted by TLR4 antagonists. The release of nitric oxide by LPS treatment or SOD1mut glia was also inhibited by EVOO, suggesting that the action of this natural extract could be mainly related to the modulation of this inflammatory mediator.
Pharmacol Res, 2016; 103
34696792: Panattoni G, Amoriello R, Memo C, Thalhammer A, Ballerini C, Ballerini L
Diverse inflammatory threats modulate astrocytes Ca signaling via connexin43 hemichannels in organotypic spinal slices.
Neuroinflammation is an escalation factor shared by a vast range of central nervous system (CNS) pathologies, from neurodegenerative diseases to neuropsychiatric disorders. CNS immune status emerges by the integration of the responses of resident and not resident cells, leading to alterations in neural circuits functions. To explore spinal cord astrocyte reactivity to inflammatory threats we focused our study on the effects of local inflammation in a controlled micro-environment, the organotypic spinal slices, developed from the spinal cord of mouse embryos. These organ cultures represent a complex in

vitro model where sensory-motor cytoarchitecture, synaptic properties and spinal cord resident cells, are retained in a 3D fashion and we recently exploit these cultures to model two diverse immune conditions in the CNS, involving different inflammatory networks and products. Here, we specifically focus on the tuning of calcium signaling in astrocytes by these diverse types of inflammation and we investigate the mechanisms which modulate intracellular calcium release and its spreading among astrocytes in the inflamed environment. Organotypic spinal cord slices are cultured for two or three weeks in vitro (WIV) and exposed for 6 h to a cocktail of cytokines (CKs), composed by tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1 β) and granulocyte macrophage-colony stimulating factor (GM-CSF), or to lipopolysaccharide (LPS). By live calcium imaging of the ventral horn, we document an increase in active astrocytes and in the occurrence of spontaneous calcium oscillations displayed by these cells when exposed to each inflammatory threat. Through several pharmacological treatments, we demonstrate that intracellular calcium sources and the activation of connexin 43 (Cx43) hemichannels have a pivotal role in increasing calcium intercellular communication in both CKs and LPS conditions, while the Cx43 gap junction communication is apparently reduced by the inflammatory treatments.
Mol Brain, 2021; 14
33439088: Memo C
Running with the devil: race against COVID-19.
J Wound Care, 2021; 30

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-478

THE ROLE OF ADF/COFILIN1 IN MICROGLIA MORPHOLOGY AND FUNCTION

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Microglia are the resident immune cells of the central nervous system in which they play a crucial role in homeostasis and disease. Moreover, they are implicated in brain development, neuronal interactions, and higher brain functions such as learning mechanisms. Microglia are highly motile cells that constantly scan the brain parenchyma with their protrusions. The cellular mechanisms underlying these dynamic changes in shape remain to be further elucidated. The cytoskeleton proteins actin depolymerizing factor and cofilin 1 (ADF/Cfl1) have been shown to be crucially involved in neuronal development, function and cell cycle control by actin filament organization. However, their role in microglia remains unknown. We generated a mouse line that conditionally lacks ADF/Cfl1 in microglia in the adult brain. Chronic *in vivo* two photon imaging of ADF/Cfl1-KO microglia in the cortex revealed altered microglia morphology and motility. Additionally, long-term monitoring of microglia migration towards a lesion site was affected in absence of ADF/Cfl1 in microglia. Furthermore, an associative learning task indicated a role of microglial ADF/Cfl1 in learning and memory. Our results assign a crucial role of ADF/Cfl1 to the diverse microglia functions in health and disease. Moreover, our data suggest that microglial integrity in presence of ADF/Cfl1 is important for higher brain network processes that impact cognition.

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BOARD NUMBER: S01-479

AQP4 EXPRESSION LEVEL AND AGGREGATION STATE AFFECT ASTROCYTE MIGRATION IN AN IN VITRO MODEL OF REACTIVE GLIOSIS

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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The water channel Aquaporin-4 (AQP4) is physiologically assembled in Central Nervous System (CNS) astrocytes into large molecular aggregates known as Orthogonal Arrays of Particles (OAPs). Astrocytes are resident cells but can migrate under inflammatory conditions, where it has been reported that AQP4 is upregulated. The aim of this study is to investigate the physiological impact of AQP4 and its supra-molecular level of aggregation in astrocytes migration in an *in vitro* model of reactive gliosis. To this end, we promoted an inflammatory phenotype by co-stimulating primary cultured astrocytes obtained from WT and OAP-null mice with TNFα and IL1β. Astrocyte activation was assessed by morphometric analysis, RT-qPCR and Western Blot of pro-inflammatory markers, while water transport and wound healing assays were used to correlate astrocyte water permeability to migratory ability. We found that, independently from the genotype, reactive astrocytes undergo hypertrophy of intermediate filaments, increase the cell area and display pro-inflammatory gene profile. However, they unexpectedly exhibit low levels of AQP4, as well as Cx43 and GFAP, compared to controls. Wound healing assays of non-reactive astrocytes showed that OAP-null migrate at a higher speed than WT, but both cell types remain almost immotile after 24 hours. Moreover, they both exhibit slow water transport rates under inflammatory conditions, as predicted by the expression levels of AQP4. Altogether these findings show that AQP4 aggregation state drives cell migration, which is impaired when AQP4 is downregulated, and provide new insights on astrocyte role in health and disease.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-480

DECIPHERING THE ROLE OF MECHANICAL CUES ON ACTIVATION OF MICROGLIAL CELLS

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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In brain tissues, mechanical stresses can lead to complex neuroinflammation events. Both microglia and astrocytes play a significant role in mediating the progression of a mechanical damage. Classic ways of microglia activation can be triggered by antigen presentation like chemokines, damaged-associated molecular patterns (DAMPs) or pathogen particles. However, while the impact of chemical signaling on astrocytes and microglia function has been studied in much detail, the current understanding of mechanical signaling, induced by brain injuries, is very limited. To address this challenge, we studied in vitro the role of mechanically injured microglia. Microglial cells were cultivated on elastic membranes and mechanically activated by a rapid single stretch (< 1 sec) in order to mimic *in vivo* condition as we found during a traumatic brain injury (TBI). All experiments were conducted 24 hours post-injury. Our findings indicate that 20% stretch of microglial cells does induce an activation through the increase of IBA1 protein level as well as an increase of actin fluorescence signal. This activation state is found to be simultaneous with the stiffening of BV2 cells as we measure it using a ferule-top nanoindenter. In addition, preliminary results of migration show a change in cell behavior suggesting that immune glial cells are mechanosensitive and can adopt an activated state in response to a single mechanical stretch. Additional experiments have been conducted to decipher the role of Piezo1 mechanosensitive ion channel on the mechano-activation of microglial cells.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-481

MICROGLIA REGULATE LEARNING AND MEMORY THROUGH NF-κB

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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[1]Instituto de Neurociencias, Universidad Miguel Hernández-consejo Superior De Investigaciones Científicas, Sant Joan d'Alacant, Alicante, Spain, [2]Pablo de Olavide University, Division Of Neurosciences, Sevilla, Spain

Microglia, the resident immune cells of the CNS, have been implicated in brain plasticity and function. However, the underlying mechanisms remain largely unknown. Here, we show that Cre-dependent removal of the RelA subunit of the NF-κB transcription factor from adult microglia results in impaired learning and long-term potentiation. Depletion of RelA elicits changes in chromatin accessibility and transcriptome landscapes of microglia associated with specific gene regulatory programs driving the activation of specific microglia phenotypes. Our findings suggest that NF-κB drive specific microglia phenotypes modulating neuronal circuits for learning and memory.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-482

EXPOSURE TO EARLY-LIFE STRESS PROMOTES SEX-DEPENDENT CHANGES IN MICROGLIA MORPHOLOGY AND PHAGOCYTIC ACTIVITY IN THE PREFRONTAL CORTEX

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

Ana Luísa Cardoso[1,2], Jéssica Costa[1,2], Solange Martins[1], Pedro Ferreira[1,3], Carolina Lebre[1,3], Joana Guedes[1,2], João Peça[1,3]
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Exposure to early life stress (ELS) induces maladaptive behaviors and increases the vulnerability to mental illness later in life. ELS can trigger changes in immune effectors, such as microglia cells, contributing to the disruption of microglia's homeostatic functions and interfering with the shaping of neuronal circuits. In this work, we have employed a maternal separation and unpredictable stress model (MSUS) to study the impact of ELS in microglia morphology and phagocytic activity in the pre-frontal cortex (PFC), a brain region implicated in decision making and impulse control. Exposure to the MSUS protocol between P2 and P14 drives sex-dependent changes in microglia morphology and number across the PFC. While an increase in microglia ramification and total number was apparent in P10 female MSUS mice, male MSUS mice failed to present any microglia adaptation at this age, but show significant alterations at P40, including a hyper-ramified phenotype. In addition, a decrease in the volume of CD68 positive structures in PFC microglia of P10 MSUS mice was also observed. In males, this result could be further correlated with a decrease in the number of microglia exhibiting one or more phagocytic cups, particularly in the internal layers of the medial orbital cortex, suggesting a region and layer-specific decrease in microglia phagocytic activity. This reduction in phagocytosis can, in turn, jeopardize the elimination of key cellular populations within the PFC, such as PV+ inhibitory neurons. Overall, this work points towards a differential response of male microglia to ELS, ultimately contributing to shed light on the higher susceptibility of males to this type of stress.

FENS Forum 2022
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BOARD NUMBER: S01-483

INVESTIGATING THE ROLE OF MICROGLIAL TDP-43 IN BRAIN DEVELOPMENT

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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[1]University of Lausanne, Department Of Biomedical Sciences, Lausanne, Switzerland, [2]Lausanne University Hospital, Department Of Radiology, Lausanne, Switzerland, [3]EPFL, Institute Of Bioengineering (ibi), Lausanne, Switzerland, [4]ETH Zurich, Department Of Health Sciences And Technology, Zurich, Switzerland

Microglia, the innate immune cells of the brain, contribute to neural circuits refinement by removing unnecessary synapses during brain development. Recent studies indicate that microglia are also responsible for pathological synapse elimination in neurodegeneration. TDP-43, encoded by the *TARDBP* gene, is a highly conserved DNA/RNA binding protein that shuttles between the nucleus and the cytoplasm. Its aberrant cytoplasmic aggregation, commonly associated with depletion of nuclear TDP-43, is a hallmark of Amyotrophic Lateral Sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). We previously showed that microglial TDP-43 regulates phagocytosis and that conditional knockout (cKO) mice selectively lacking TDP-43 in microglia display pathological synapse loss and defective motor behavior. Here, we hypothesize that microglial TDP-43 plays important roles in shaping neuronal circuitries during early brain development, thus providing susceptibility for developing neurological disorders later in life. To investigate possible developmental defects we used $Cx3cr1^{CREert2}$;$Tardbp^{floxed/floxed}$ (cKO)and $Cx3cr1^{CREert2}$;$Tardbp^{wt/wt}$ (control) mice at post-natal day 15 (P15). Unbiased diffusion MRI analysis of the whole brain revealed sex-dependent microstructure alterations specifically in the somatosensory and motor cortex of cKO mice. Such structural changes were associated with decreased density in microglia and in Olig2-positive cells with myelin abnormalities in the somatosensory-motor area. Further analyses are ongoing to elucidate the mechanisms underlying the specific cortical defects mediated by microglial TDP-43 depletion. Overall, these findings suggest that microglia dysfunction induced by the lack of TDP-43 can influence the maturation of the postnatal motor-somatosensory cortex, and be causally implicated in the pathogenesis of brain disorders.

Pubmed:
30006609: Maatouk L, Yi C, Carrillo-de Sauvage MA, Compagnion AC, Hunot S, Ezan P, Hirsch EC, Koulakoff A, Pfrieger FW, Tronche F, Leybaert L, Giaume C, Vyas S
Glucocorticoid receptor in astrocytes regulates midbrain dopamine neurodegeneration through connexin hemichannel activity.
The precise contribution of astrocytes in neuroinflammatory process occurring in Parkinson's disease (PD) is not well characterized. In this study, using GR mice that are conditionally inactivated for glucocorticoid receptor (GR) in astrocytes, we have examined the actions of astrocytic GR during dopamine neuron (DN) degeneration triggered by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The results show significantly augmented DN loss in GR mutant mice in substantia nigra (SN) compared to controls. Hypertrophy of microglia but not of astrocytes was greatly enhanced in SN of these astrocytic GR mutants intoxicated with MPTP, indicating heightened microglial reactivity compared to similarly-treated control mice. In the SN of GR astrocyte mutants, specific inflammation-associated transcripts ICAM-1, TNF-α and Il-1β as well as TNF-α protein levels were significantly elevated after MPTP neurotoxicity compared to controls. Interestingly, this paralleled increased connexin hemichannel activity and elevated intracellular calcium levels in astrocytes examined in acute midbrain slices from control and mutant mice treated with MPP+ . The increased connexin-43 hemichannel activity was found in vivo in MPTP-intoxicated mice. Importantly, treatment of MPTP-injected GR mutant mice with TAT-Gap19 peptide, a specific connexin-43 hemichannel blocker, reverted both DN loss and microglial activation; in wild-type mice there was partial but significant survival effect. In the SN of post-mortem PD patients, a significant decrease in the number of astrocytes expressing nuclear GR was observed, suggesting the participation of astrocytic GR deregulation of inflammatory process in PD. Overall, these data provide mechanistic insights into GR-modulated processes in vivo, specifically in astrocytes, that contribute to a pro-inflammatory state and dopamine neurodegeneration in PD pathology.
Cell Death Differ, 2019; 26
29934589: Maatouk L, Compagnion AC, Sauvage MC, Bemelmans AP, Leclere-Turbant S, Cirotteau V, Tohme M, Beke A, Trichet M, Bazin V, Trawick BN, Ransohoff RM, Tronche F, Manoury B, Vyas S

TLR9 activation via microglial glucocorticoid receptors contributes to degeneration of midbrain dopamine neurons.
Inflammation is a characteristic feature of Parkinson's disease (PD). We examined the role of TLR9 and its regulation by glucocorticoid receptors (GRs) in degeneration of substantia nigra dopamine neurons (DNs). TLR9 agonist, CpG-ODN, induced DN degeneration in mice lacking GR in microglia but not in controls. TLR9 deletion reduced DN loss in neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. GR regulates TLR9 activation during MPTP neurotoxicity as TLR9 antagonist suppressed increased DN loss in microglia/macrophage GR mutant mice. GR absence in microglia enhanced TLR9 translocation to endolysosomes and facilitated its cleavage leading to pro-inflammatory gene expression. GR-dependent TLR9 activation also triggered DN loss following intranigral injection of mitochondrial DNA. Finally, microglial GR sensitivity to A53T-alpha-synuclein induced DN degeneration as well as decreased microglial GR expression observed in SN of PD brain samples, all suggest that reduced microglial GR activity in SN can stimulate TLR9 activation and DN loss in PD pathology.
Nat Commun, 2018; 9

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-484

EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS REDUCE NEUROINFLAMMATION IN HIPPOCAMPUS AND RESTORE COGNITIVE FUNCTION IN HYPERAMMONEMIC RATS BY REDUCING NF-κB ACTIVATION VIA TGFβ RECEPTOR ACTIVATION

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Background: Chronic hyperammonemia, a main contributor to hepatic encephalopathy, leads to neuroinflammation which alters neurotransmission leading to cognitive impairment. Currently there are no specific treatments for the neurological alterations in hepatic encephalopathy. Extracellular vesicles (EVs) from mesenchymal stem cells (MSCs) reduce neuroinflammation in some pathological conditions. **Aims:** The aim of this work was to assess if treatment of hyperammonemic rats with EVs from MSCs reduces neuroinflammation, improves neurotransmission in hippocampus and restores cognitive function and to study the mechanisms involved. **Methods:** Treatment of EVs from MSCs was performed *in vivo* by i.v. injection and *ex vivo* in hippocampal slices from hyperammonemic or control rats. Effects on neuroinflammation (microglia and astrocytes activation and content of inflammatory markers in hippocampus) were assessed by immunohistochemistry, immunofluorescence and western blot. Learning and memory were assessed using the following tests: object location, object recognition, Y maze and radial maze. **Results:** The EVs injected reached the hippocampus. Hyperammonemia induced neuroinflammation in hippocampus and impaired learning and memory in the tests performed. Treatment with EVs reduced microglia and astrocytes activation, the content of IL-1β and NF-kB activation and restored performance of hyperammonemic rats in all the behavioral tests. Studies adding EVs to hippocampal slices ex vivo showed that these beneficial effects were dependent on TGFβ contained in the EVs, which reduced NF-kB activation and the subsequent neuroinflammation. **Conclusions:** EVs from MSCs reduce neuroinflammation in hippocampus and restore cognitive function in hyperammonemic rats. EVs from MSCs may be useful to improve cognitive function in patients with MHE.

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BOARD NUMBER: S01-485

CHLAMYDIA PNEUMONIAE CAN INFECT THE CENTRAL NERVOUS SYSTEM VIA THE OLFACTORY AND TRIGEMINAL NERVES AND CONTRIBUTES TO ALZHEIMER'S DISEASE RISK

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Chlamydia pneumoniae is a respiratory tract pathogen but can also infect the central nervous system (CNS). Recently, the link between C. pneumoniae CNS infection and late-onset dementia has become increasingly evident. In mice, CNS infection has been shown to occur weeks to months after intranasal inoculation. By isolating live C. pneumoniae from tissues and using immunohistochemistry, we show that C. pneumoniae can infect the olfactory and trigeminal nerves, olfactory bulb and brain within 72 hours in mice. C. pneumoniae infection also resulted in dysregulation of key pathways involved in Alzheimer's disease pathogenesis at 7 and 28 days after inoculation. Interestingly, amyloid beta accumulations were also detected adjacent to the C. pneumoniae inclusions in the olfactory system. Furthermore, injury to the nasal epithelium resulted in increased peripheral, but decreased CNS infection. In vitro, C. pneumoniae was able to infect peripheral nerve and CNS glia. In summary, the nerves extending between the nasal cavity and the brain constitute invasion paths by which C. pneumoniae can rapidly invade the CNS likely by surviving in glia and leading to Aβ deposition.

Pubmed:
35177758: Chacko A, Delbaz A, Walkden H, Basu S, Armitage CW, Eindorf T, Trim LK, Miller E, West NP, St John JA, Beagley KW, Ekberg JAK
Chlamydia pneumoniae can infect the central nervous system via the olfactory and trigeminal nerves and contributes to Alzheimer's disease risk.
Chlamydia pneumoniae is a respiratory tract pathogen but can also infect the central nervous system (CNS). Recently, the link between C. pneumoniae CNS infection and late-onset dementia has become increasingly evident. In mice, CNS infection has been shown to occur weeks to months after intranasal inoculation. By isolating live C. pneumoniae from tissues and using immunohistochemistry, we show that C. pneumoniae can infect the olfactory and trigeminal nerves, olfactory bulb and brain within 72 h in mice. C. pneumoniae infection also resulted in dysregulation of key pathways involved in Alzheimer's disease pathogenesis at 7 and 28 days after inoculation. Interestingly, amyloid beta accumulations were also detected adjacent to the C. pneumoniae inclusions in the olfactory system. Furthermore, injury to the nasal epithelium resulted in increased peripheral nerve and olfactory bulb infection, but did not alter general CNS infection. In vitro, C. pneumoniae was able to infect peripheral nerve and CNS glia. In summary, the nerves extending between the nasal cavity and the brain constitute invasion paths by which C. pneumoniae can rapidly invade the CNS likely by surviving in glia and leading to Aβ deposition.
Sci Rep, 2022; 12
35027585: Basu S, Choudhury IN, Nazareth L, Chacko A, Shelper T, Vial ML, Ekberg JAK, St John JA
In vitro modulation of Schwann cell behavior by VEGF and PDGF in an inflammatory environment.
Peripheral glial cell transplantation with Schwann cells (SCs) is a promising approach for treating spinal cord injury (SCI). However, improvements are needed and one avenue to enhance regenerative functional outcomes is to combine growth factors with cell transplantation. Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are neuroprotective, and a combination of these factors has improved outcomes in rat SCI models. Thus, transplantation of SCs combined with VEGF and PDGF may further improve regenerative outcomes. First, however, we must understand how the two factors modulate SCs. In this in vitro study, we show that an inflammatory environment decreased the rate of SC-mediated phagocytosis of myelin debris but the addition of VEGF and PDGF (alone and combined) improved phagocytosis. Cytokine expression by SCs in the inflammatory environment revealed that addition of PDGF led to significantly lower level of

pro-inflammatory cytokine, TNF-α, but IL-6 and anti-inflammatory cytokines (TGF-β and IL-10), remained unaltered. Further, PDGF was able to decrease the expression of myelination associated gene Oct6 in the presence of inflammatory environment. Overall, these results suggest that the use of VEGF and/or PDGF combined with SC transplantation may be beneficial in SCI therapy.
Sci Rep, 2022; 12
34021227: Choudhury IN, Chacko A, Delbaz A, Chen M, Basu S, St John JA, Huygens F, Ekberg JAK
Antimicrobial responses of peripheral and central nervous system glia against Staphylococcus aureus.
Staphylococcus aureus infections of the central nervous system are serious and can be fatal. S. aureus is commonly present in the nasal cavity, and after injury to the nasal epithelium it can rapidly invade the brain via the olfactory nerve. The trigeminal nerve constitutes another potential route of brain infection. The glia of these nerves, olfactory ensheathing cells (OECs) and trigeminal nerve Schwann cells (TgSCs), as well as astrocytes populating the glia limitans layer, can phagocytose bacteria. Whilst some glial responses to S. aureus have been studied, the specific responses of different glial types are unknown. Here, we compared how primary mouse OECs, TgSCs, astrocytes and microglia responded to S. aureus. All glial types internalized the bacteria within phagolysosomes, and S. aureus-conjugated BioParticles could be tracked with subtle but significant differences in time-course of phagocytosis between glial types. Live bacteria could be isolated from all glia after 24 h in culture, and microglia, OECs and TgSCs exhibited better protection against intracellular S. aureus survival than astrocytes. All glial types responded to the bacteria by cytokine secretion. Overall, OECs secreted the lowest level of cytokines, suggesting that these cells, despite showing strong capacity for phagocytosis, have immunomodulatory functions that can be relevant for neural repair.
Sci Rep, 2021; 11
33489937: Nazareth L, Walkden H, Chacko A, Delbaz A, Shelper T, Armitage CW, Reshamwala R, Trim LK, St John JA, Beagley KW, Ekberg JAK
Can Invade the Central Nervous System the Olfactory and Trigeminal Nerves and Infect Peripheral Nerve Glial Cells.
can infect the brain and has been linked to late-onset dementia. , which infects mice, is often used to model human chlamydial infections. While it has been suggested to be also important for modelling brain infection, nervous system infection by has not been reported in the literature. has been shown to infect the olfactory bulb in mice after intranasal inoculation, and has therefore been suggested to invade the brain the olfactory nerve; however, nerve infection has not been shown to date. Another path by which certain bacteria can reach the brain is the trigeminal nerve, but it remains unknown whether species can infect this nerve. Other bacteria that can invade the brain the olfactory and/or trigeminal nerve can do so rapidly, however, whether spp. can reach the brain earlier than one-week post inoculation remains unknown. In the current study, we showed that can within 48 h invade the brain the olfactory nerve, in addition to infecting the trigeminal nerve. We also cultured the glial cells of the olfactory and trigeminal nerves and showed that readily infected the cells, constituting a possible cellular mechanism explaining how the bacteria can invade the nerves without being eliminated by glial immune functions. Further, we demonstrated that olfactory and trigeminal glia differed in their responses to , with olfactory glia showing less infection and stronger immune response than trigeminal glia.
Front Cell Infect Microbiol, 2020; 10
31978058: Walkden H, Delbaz A, Nazareth L, Batzloff M, Shelper T, Beacham IR, Chacko A, Shah M, Beagley KW, Tello Velasquez J, St John JA, Ekberg JAK
Burkholderia pseudomallei invades the olfactory nerve and bulb after epithelial injury in mice and causes the formation of multinucleated giant glial cells in vitro.
The infectious disease melioidosis is caused by the bacterium Burkholderia pseudomallei. Melioidosis is characterised by high mortality and morbidity and can involve the central nervous system (CNS). We have previously discovered that B. pseudomallei can infect the CNS via the olfactory and trigeminal nerves in mice. We have shown that the nerve path is dependent on mouse strain, with outbred mice showing resistance to olfactory nerve infection. Damage to the nasal epithelium by environmental factors is common, and we hypothesised that injury to the olfactory epithelium may increase the vulnerability of the olfactory nerve to microbial insult. We therefore investigated this, using outbred mice that were intranasally inoculated with B. pseudomallei, with or without methimazole-induced injury to the olfactory neuroepithelium. Methimazole-mediated injury resulted in increased B. pseudomallei invasion of the olfactory epithelium, and only in pre-injured animals were bacteria found in the olfactory nerve and bulb. In vitro assays demonstrated that B. pseudomallei readily infected glial cells isolated from the olfactory and trigeminal nerves (olfactory ensheathing cells and trigeminal Schwann cells, respectively). Bacteria were degraded by some cells but persisted in other cells, which led to the formation of multinucleated giant cells (MNGCs), with olfactory ensheathing cells less likely to form MNGCs than Schwann cells. Double Cap mutant bacteria, lacking the protein BimA, did not form MNGCs. These data suggest that injuries to the olfactory epithelium expose the primary olfactory nervous system to bacterial invasion, which can then result in CNS infection with potential pathogenic consequences for the glial cells.

PLoS Negl Trop Dis, 2020; 14
27834303: Marshall-Gradisnik S, Johnston S, Chacko A, Nguyen T, Smith P, Staines D
Single nucleotide polymorphisms and genotypes of transient receptor potential ion channel and acetylcholine receptor genes from isolated B lymphocytes in myalgic encephalomyelitis/chronic fatigue syndrome patients.
Objective The pathomechanism of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is unknown; however, a small subgroup of patients has shown muscarinic antibody positivity and reduced symptom presentation following anti-CD20 intervention. Given the important roles of calcium (Ca) and acetylcholine (ACh) signalling in B cell activation and potential antibody development, we aimed to identify relevant single nucleotide polymorphisms (SNPs) and genotypes in isolated B cells from CFS/ME patients. Methods A total of 11 CFS/ME patients (aged 31.82 ± 5.50 years) and 11 non-fatigued controls (aged 33.91 ± 5.06 years) were included. Flow cytometric protocols were used to determine B cell purity, followed by SNP and genotype analysis for 21 mammalian TRP ion channel genes and nine mammalian ACh receptor genes. SNP association and genotyping analysis were performed using ANOVA and PLINK analysis software. Results Seventy-eight SNPs were identified in nicotinic and muscarinic acetylcholine receptor genes in the CFS/ME group, of which 35 were in mAChM3. The remaining SNPs were identified in nAChR delta (n = 12), nAChR alpha 9 (n = 5), TRPV2 (n = 7), TRPM3 (n = 4), TRPM4 (n = 1) mAChRM3 2 (n = 2), and mAChRM5 (n = 3) genes. Nine genotypes were identified from SNPs in TRPM3 (n = 1), TRPC6 (n = 1), mAChRM3 (n = 2), nAChR alpha 4 (n = 1), and nAChR beta 1 (n = 4) genes, and were located in introns and 3' untranslated regions. Odds ratios for these specific genotypes ranged between 7.11 and 26.67 for CFS/ME compared with the non-fatigued control group. Conclusion This preliminary investigation identified a number of SNPs and genotypes in genes encoding TRP ion channels and AChRs from B cells in patients with CFS/ME. These may be involved in B cell functional changes, and suggest a role for Ca dysregulation in AChR and TRP ion channel signalling in the pathomechanism of CFS/ME.
J Int Med Res, 2016; 44
27594784: Chacko A, Staines DR, Johnston SC, Marshall-Gradisnik SM
Dysregulation of Protein Kinase Gene Expression in NK Cells from Chronic Fatigue Syndrome/Myalgic Encephalomyelitis Patients.
The etiology and pathomechanism of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) are unknown. However, natural killer (NK) cell dysfunction, in particular reduced NK cytotoxic activity, is a consistent finding in CFS/ME patients. Previous research has reported significant changes in intracellular mitogen-activated protein kinase pathways from isolated NK cells. The purpose of this present investigation was to examine whether protein kinase genes have a role in abnormal NK cell intracellular signaling in CFS/ME.
Gene Regul Syst Bio, 2016; 10
25663156: Chacko A, Beagley KW, Timms P, Huston WM
Human Chlamydia pneumoniae isolates demonstrate ability to recover infectivity following penicillin treatment whereas animal isolates do not.
Chlamydia pneumoniae strains have recently been demonstrated to have substantially different capacities to enter and recover from IFN-γ-induced persistence, depending on whether they are from human or animal host sources. Here, we examined the ability of two human and two animal strains to enter and be rescued from penicillin-induced persistence. The ability to form inclusions after the addition of penicillin was much reduced in the two animal isolates (koala LPCoLN, bandicoot B21) compared to the two human isolates (respiratory AR39 and heart A03). The penicillin treatment resulted in a dose-dependent loss of infectious progeny for all isolates, with the human strains failing to produce infectious progeny at lower doses of penicillin than the animal strains. The most remarkable finding however was the contrasting ability of the isolates to recover infectious progeny production after rescue by removal of the penicillin (at 72 h) and continued culture. The animal isolates both showed virtually no recovery from the penicillin treatment conditions. In contrast, the human isolates showed a significant ability to recovery infectivity, with the heart isolate (A03) showing the most marked recovery. Combined, these data further support the hypothesis that the ability to establish and recover from persistence appears to be enhanced in human C. pneumoniae strains compared to animal strains.
FEMS Microbiol Lett, 2015; 362
24989637: Chacko A, Barker CJ, Beagley KW, Hodson MP, Plan MR, Timms P, Huston WM
Increased sensitivity to tryptophan bioavailability is a positive adaptation by the human strains of Chlamydia pneumoniae. One of the most significant activities induced by interferon-gamma against intracellular pathogens is the induction of IDO (indoleamine 2,3-dioxygenase) expression, which subsequently results in the depletion of tryptophan. We tested the hypothesis that human strains of Chlamydia pneumoniae are more sensitive to tryptophan limitation than animal C. pneumoniae strains. The human strains were significantly more sensitive to IFN-γ than the animal strains in a lung epithelia cell model (BEAS-2B), with exposure to 1 U ml(-1) IFN-γ resulting in complete loss of infectious yield of human strains, compared to the animal strains where reductions in infectious progeny were around 3.5-4.0 log. Strikingly, the IFN-γ induced

loss of ability to form infectious progeny production was completely rescued by removal of the IFN-γ and addition of exogenous tryptophan for the human strains, but not the animal strains. In fact, a human heart strain was more capable of entering a non-infectious, viable persistent stage when exposed to IFN-γ and was also more effectively rescued, compared to a human respiratory strain. Exquisite susceptibility to IFN-γ, specifically due to tryptophan availability appears to be a core adaptation of the human C. pneumoniae strains, which may reflect the chronic nature of their infections in this host.
Mol Microbiol, 2014; 93
24682324: Huston WM, Barker CJ, Chacko A, Timms P
Evolution to a chronic disease niche correlates with increased sensitivity to tryptophan availability for the obligate intracellular bacterium Chlamydia pneumoniae.
The chlamydiae are obligate intracellular parasites that have evolved specific interactions with their various hosts and host cell types to ensure their successful survival and consequential pathogenesis. The species Chlamydia pneumoniae is ubiquitous, with serological studies showing that most humans are infected at some stage in their lifetime. While most human infections are asymptomatic, C. pneumoniae can cause more-severe respiratory disease and pneumonia and has been linked to chronic diseases such as asthma, atherosclerosis, and even Alzheimer's disease. The widely dispersed animal-adapted C. pneumoniae strains cause an equally wide range of diseases in their hosts. It is emerging that the ability of C. pneumoniae to survive inside its target cells, including evasion of the host's immune attack mechanisms, is linked to the acquisition of key metabolites. Tryptophan and arginine are key checkpoint compounds in this host-parasite battle. Interestingly, the animal strains of C. pneumoniae have a slightly larger genome, enabling them to cope better with metabolite restrictions. It therefore appears that as the evolutionarily more ancient animal strains have evolved to infect humans, they have selectively become more "susceptible" to the levels of key metabolites, such as tryptophan. While this might initially appear to be a weakness, it allows these human C. pneumoniae strains to exquisitely sense host immune attack and respond by rapidly reverting to a persistent phase. During persistence, they reduce their metabolic levels, halting progression of their developmental cycle, waiting until the hostile external conditions have passed before they reemerge.
J Bacteriol, 2014; 196

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BOARD NUMBER: S01-486

CHARACTERIZATION OF MICROGLIAL CELLS IN A MODEL OF SELECTIVE NEURONAL LOSS: THE PURKINJE CELL DEGENERATION MOUSE

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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[1]University of Salamanca, Cellular Biology And Pathology Department, Faculty Of Medicine, Salamanca, Spain, [2]Institute for Neuroscience of Castilla y León, Laboratory Of Neuronal Plasticity And Neurorepair, Salamanca, Spain, [3]Institute of Biomedical Research of Salamanca, Tgyc-03, Laboratory Of Neuronal Plasticity And Neurorepair, Salamanca, Spain, [4]Cajal Institute-CSIC, Department Of Functional And Systems Neurobiology, Laboratory Of Neuroactive Steroids, Madrid, Spain

The Purkinje Cell Degeneration (PCD) mouse presents a mutation in the *Ccp1* gene that produces the selective post-natal death of both Purkinje cells in the cerebellum and mitral cells in the olfactory bulb (OB). Along with this neuronal loss, a strong microgliosis takes place in the cerebellum whereas a moderate microgliosis occurs in the OB. Therefore, the PCD is an excellent animal model to analyze microglial response, because it has two different neurodegenerative scenarios at different ages and with different degenerative time-courses. Therefore, we analyze the evolution of microglia in these regions at different ages with several techniques. For this purpose, microglial cells and tissue slices were obtained from the cerebellum and OB of both wild-type and PCD mice. Subsequently, qPCR analyses and immunofluorescence techniques were performed to characterize microglia by studying gene and protein expression. Likewise, the morphology, motility and phagocytosis of microglia were analyzed in both cell cultures and in cerebellar and OB slices. The results obtained suggested that the microglia of PCD mice presents a differential gene expression for the analyzed genes. Besides, a higher inflammatory protein expression was observed in PCD mice. Finally, the motility and phagocytosis were more evident in PCD than in wild-type microglia in cultured cells as well as in cerebellar and OB slices. It can be concluded that both the mutation of *Ccp1* gene and the neurodegenerative environment affects microglial features related to gene and protein expression, morphology, motility and phagocytosis cell. **Support:** MICINN, JCyL, USAL, Banco Santander **E-mail:** dpb@usal.es, ddiaz@usal.es, ewp@usal.es

FENS Forum 2022
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BOARD NUMBER: S01-487

CEREBELLAR INFLAMMATION SUPPORTS AUTISM-RELATED BEHAVIORS IN THE CNTNAP2 MOUSE MODEL OF AUTISM SPECTRUM DISORDERS

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

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Autism spectrum disorders (ASDs) are a heterogeneous group of neurodevelopmental disorders associated to social communication deficits and repetitive sensory–motor behaviors. These symptoms affect children from the early childhood and produce clinically significant developmental impairments. Immune dysfunction has recently emerged as major contributor to the neurodevelopmental deficits observed in people with ASD. This condition is often linked with a strong inflammatory state, which contributes to neurodegeneration and impairments in synaptic plasticity. Cntnap2$^{-/-}$ mice have widely been considered a robust animal model of ASD. In the current study, we analyzed the expression of classical pro-inflammatory molecules in the cerebral cortex, hippocampus and cerebellum of mutant mice. mRNA and protein expression of IL-6, TNF, IFNγ and IL-1β were increased in the cerebellum of Cntnap2$^{-/-}$ mice, in comparison with their WT littermates. In addition, increased levels of the same molecules were found in the blood of both mutant mice. Finally, a link could be identified between inflammation within cerebellum and impaired social and motor behaviors (common ASD-related features) in these mice. Taken together, these results suggest that cerebellar inflammation may support ASD-like behaviors in autism.

Pubmed:

32092501: Pangrazzi L, Weinberger B
T cells, aging and senescence.
The T cell compartment undergoes characteristic changes with age, which contribute to increased incidence and severity of infections and reduced immunogenicity and efficacy of many vaccines in the older population. Production of naïve T cells is severely impaired due to a decreased output of lymphoid cells from the bone marrow and the involution of the thymus. At the same time, antigen-experienced, highly differentiated T cells accumulate resulting in a diminished T cell receptor repertoire. These cells show some similarities with senescent cells, such as shorter telomers, accumulated DNA damage and metabolic changes. Latent infection with Cytomegalovirus also impacts the T cell compartment and aggravates several of its age-associated changes. Loss of CD28 expression is one hallmark of T cells after repeated antigenic stimulation, but CD28 T cells cannot be considered truly senescent as e.g. they are still able to proliferate upon adequate stimulation. Several additional markers have been suggested in order to define a potential fully senescent T cell population, but no consensus definition has been reached so far. It has been postulated that highly differentiated senescent-like T cells are unable to eliminate other senescent cell types. Removal of senescent non-immune cells has been shown to be beneficial for the organism and a reliable definition of senescent T cells is essential for an extension of this concept to T cells.
Exp Gerontol, 2020; 134
33256243: Pangrazzi L, Balasco L, Bozzi Y
Natural Antioxidants: A Novel Therapeutic Approach to Autism Spectrum Disorders?
Autism spectrum disorders (ASD) are a group of neurodevelopmental syndromes with both genetic and environmental origins. Several recent studies have shown that inflammation and oxidative stress may play a key role in supporting the pathogenesis and the severity of ASD. Thus, the administration of anti-inflammatory and antioxidant molecules may represent a promising strategy to counteract pathological behaviors in ASD patients. In the current review, results from recent literature showing how natural antioxidants may be beneficial in the context of ASD will be discussed. Interestingly, many antioxidant molecules available in nature show anti-inflammatory activity. Thus, after introducing ASD and the role of the vitamin E/vitamin C/glutathione network in scavenging intracellular reactive oxygen species (ROS) and the impairments observed with ASD, we discuss the concept of functional food and nutraceutical compounds. Furthermore, the effects of well-known nutraceutical compounds on ASD individuals and animal models of ASD are summarized. Finally, the importance of nutraceutical compounds as support therapy useful in reducing the symptoms in autistic people is discussed.
Antioxidants (Basel), 2020; 9
32384730: Pangrazzi L, Balasco L, Bozzi Y

Oxidative Stress and Immune System Dysfunction in Autism Spectrum Disorders.
Autism Spectrum Disorders (ASDs) represent a group of neurodevelopmental disorders associated with social and behavioral impairments. Although dysfunctions in several signaling pathways have been associated with ASDs, very few molecules have been identified as potentially effective drug targets in the clinic. Classically, research in the ASD field has focused on the characterization of pathways involved in neural development and synaptic plasticity, which support the pathogenesis of this group of diseases. More recently, immune system dysfunctions have been observed in ASD. In addition, high levels of reactive oxygen species (ROS), which cause oxidative stress, are present in ASD patients. In this review, we will describe the major alterations in the expression of genes coding for enzymes involved in the ROS scavenging system, in both ASD patients and ASD mouse models. In addition, we will discuss, in the context of the most recent literature, the possibility that oxidative stress, inflammation and immune system dysfunction may be connected to, and altogether support, the pathogenesis and/or severity of ASD. Finally, we will discuss the possibility of novel treatments aimed at counteracting the interplay between ROS and inflammation in people with ASD.
Int J Mol Sci, 2020; 21
31018996: Naismith E, Pangrazzi L
The impact of oxidative stress, inflammation, and senescence on the maintenance of immunological memory in the bone marrow in old age.
The bone marrow (BM) provides a preferential survival environment for the long-term maintenance of antigen-experienced adaptive immune cells. After the contact with antigens, effector/memory T cells and plasma cell precursors migrate to the BM, in which they can survive within survival niches in an antigen-independent manner. Despite this, the phenotype of adaptive immune cells changes with aging, and BM niches themselves are affected, leading to impaired long-term maintenance of immunological memory in the elderly as a result. Oxidative stress, age-related inflammation (inflammaging), and cellular senescence appear to play a major role in this process. This review will summarize the age-related changes in T and B cell phenotype, and in the BM niches, discussing the possibility that the accumulation of highly differentiated, senescent-like T cells in the BM during aging may cause inflammation in the BM and promote oxidative stress and senescence. In addition, senescent-like T cells may compete for space with other immune cells within the marrow, partially excluding effector/memory T cells and long-lived plasma cells from the niches.
Biosci Rep, 2019; 39
34791077: Balasco L, Pagani M, Pangrazzi L, Chelini G, Ciancone Chama AG, Shlosman E, Mattioni L, Galbusera A, Iurilli G, Provenzano G, Gozzi A, Bozzi Y
Abnormal Whisker-Dependent Behaviors and Altered Cortico-Hippocampal Connectivity in Shank3b-/- Mice.
Abnormal tactile response is an integral feature of Autism Spectrum Disorders (ASDs), and hypo-responsiveness to tactile stimuli is often associated with the severity of ASDs core symptoms. Patients with Phelan-McDermid syndrome (PMS), caused by mutations in the SHANK3 gene, show ASD-like symptoms associated with aberrant tactile responses. The neural underpinnings of these abnormalities are still poorly understood. Here we investigated, in Shank3b-/- adult mice, the neural substrates of whisker-guided behaviors, a key component of rodents' interaction with the surrounding environment. We assessed whisker-dependent behaviors in Shank3b-/- adult mice and age-matched controls, using the textured novel object recognition (tNORT) and whisker nuisance (WN) test. Shank3b-/- mice showed deficits in whisker-dependent texture discrimination in tNORT and behavioral hypo-responsiveness to repetitive whisker stimulation in WN. Sensory hypo-responsiveness was accompanied by a significantly reduced activation of the primary somatosensory cortex (S1) and hippocampus, as measured by c-fos mRNA induction, a proxy of neuronal activity following whisker stimulation. Moreover, resting-state fMRI showed a significantly reduced S1-hippocampal connectivity in Shank3b mutants, in the absence of altered connectivity between S1 and other somatosensory areas. Impaired crosstalk between hippocampus and S1 might underlie Shank3b-/- hypo-reactivity to whisker-dependent cues, highlighting a potentially generalizable somatosensory dysfunction in ASD.
Cereb Cortex, 2021;
30840890: Meryk A, Pangrazzi L, Hagen M, Hatzmann F, Jenewein B, Jakic B, Hermann-Kleiter N, Baier G, Jylhävä J, Hurme M, Trieb K, Grubeck-Loebenstein B
Fcμ receptor as a Costimulatory Molecule for T Cells.
Fc receptor for IgM (FcμR)-deficient mice display dysregulated function of neutrophils, dendritic cells, and B cells. The relevance of FcμR to human T cells is still unknown. We show that FcμR is mostly stored inside the cell and that surface expression is tightly regulated. Decreased surface expression on T cells from elderly individuals is associated with alterations in the methylation pattern of the FCMR gene. Binding and internalization of IgM stimulate transport of FcμR to the cell surface to ensure sustained IgM uptake. Concurrently, IgM accumulates within the cell, and the surface expression of other receptors increases, among them the T cell receptor (TCR) and costimulatory molecules. This leads to enhanced TCR signaling, proliferation, and cytokine release, in response to low, but not high, doses of antigen. Our findings indicate that FcμR is an

important regulator of T cell function and reveal an additional mode of interaction between B and T cells.
Cell Rep, 2019; 26
31327694: Miggitsch C, Meryk A, Naismith E, Pangrazzi L, Ejaz A, Jenewein B, Wagner S, Nägele F, Fenkart G, Trieb K, Zwerschke W, Grubeck-Loebenstein B
Human bone marrow adipocytes display distinct immune regulatory properties.
The bone marrow (BM) is a major reservoir of resting memory T cells and long-lived plasma cells, capable of providing protection against recurrent infections. Whether the age-related accumulation of adipose tissue in the BM affects the functionality and maintenance of memory cells is not well understood.
EBioMedicine, 2019; 46
31755098: Pangrazzi L, Reidla J, Carmona Arana JA, Naismith E, Miggitsch C, Meryk A, Keller M, Krause AAN, Melzer FL, Trieb K, Schirmer M, Grubeck-Loebenstein B, Weinberger B
CD28 and CD57 define four populations with distinct phenotypic properties within human CD8 T cells.
After repeated antigen exposure, both memory and terminally differentiated cells can be generated within CD8 T cells. Although, during their differentiation, activated CD8 T cells may first lose CD28, and CD28 cells may eventually express CD57 as a subsequent step, a population of CD28 CD57 (DP) CD8 T cells can be identified in the peripheral blood. How this population is distinct from CD28 CD57 (DN) CD8 T cells, and from the better characterized non-activated/early-activated CD28 CD57 and senescent-like CD28 CD57 CD8 T cell subsets is currently unknown. Here, RNA expression of the four CD8 T cell subsets isolated from human PBMCs was analyzed using microarrays. DN cells were more similar to "early" highly differentiated cells, with decreased TNF and IFN-γ production, impaired DNA damage response and apoptosis. Conversely, increased apoptosis and expression of cytokines, co-inhibitory, and chemokine receptors were found in DP cells. Higher levels of DP CD8 T cells were observed 7 days after Hepatitis B vaccination, and decreased levels of DP cells were found in rheumatoid arthritis patients. More DP and DN CD8 T cells were present in the bone marrow, in comparison with PBMCs. In summary, our results indicate that DP and DN cells are distinct CD8 T cell subsets displaying defined properties.
Eur J Immunol, 2020; 50
32514279: Pangrazzi L, Naismith E, Miggitsch C, Carmona Arana JA, Keller M, Grubeck-Loebenstein B, Weinberger B
The impact of body mass index on adaptive immune cells in the human bone marrow.
Obesity has been associated with chronic inflammation and oxidative stress. Both conditions play a determinant role in the pathogenesis of age-related diseases, such as immunosenescence. Adipose tissue can modulate the function of the immune system with the secretion of molecules influencing the phenotype of immune cells. The importance of the bone marrow (BM) in the maintenance of antigen-experienced adaptive immune cells has been documented in mice. Recently, some groups have investigated the survival of effector/memory T cells in the human BM. Despite this, whether high body mass index (BMI) may affect immune cells in the BM and the production of molecules supporting the maintenance of these cells it is unknown.
Immun Ageing, 2020; 17
27995612: Pangrazzi L, Meryk A, Naismith E, Koziel R, Lair J, Krismer M, Trieb K, Grubeck-Loebenstein B
"Inflamm-aging" influences immune cell survival factors in human bone marrow.
The bone marrow (BM) plays a key role in the long-term maintenance of immunological memory. However, the impact of aging on the production of survival factors for effector/memory T cells and plasma cells in the human BM has not been studied. We now show that the expression of molecules involved in the maintenance of immunological memory in the human BM changes with age. While IL-15, which protects potentially harmful CD8 CD28 senescent T cells, increases, IL-7 decreases. IL-6, which may synergize with IL-15, is also overexpressed. In contrast, a proliferation-inducing ligand, a plasma cell survival factor, is reduced. IFN-y, TNF, and ROS accumulate in the BM in old age. IL-15 and IL-6 expression are stimulated by IFN-y and correlate with ROS levels in BM mononuclear cells. Both cytokines are reduced by incubation with the ROS scavengers N-acetylcysteine and vitamin C. IL-15 and IL-6 are also overexpressed in the BM of superoxide dismutase 1 knockout mice compared to their WT counterparts. In summary, our results demonstrate the role of inflammation and oxidative stress in age-related changes of immune cell survival factors in the BM, suggesting that antioxidants may be beneficial in counteracting immunosenescence by improving immunological memory in old age.
Eur J Immunol, 2017; 47

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BOARD NUMBER: S01-488

PREDICTIVE BIOMARKERS OF ALTERED NEUROLOGICAL TRAJECTORIES CONSEQUENT TO PRENATAL INFLAMMATORY INSULTS

POSTER SESSION 01 - SECTION: BRAIN INFLAMMATION, NEURODEVELOPMENT AND DISEASE

Genni Desiato[1], Filippo Mirabella[1], Irene Corradini[1,2], Davide Pozzi[1], Michela Matteoli[3]
[1]IRCCS Humanitas Research Center, Pharmacology And Brain Pathology Laboratory, MILANO, Italy, [2]CNR, Institute Of Neuroscience, MILANO, Italy, [3]IRCCS, Humanitas Clinical and Research Center and CNR, Neuroscience Institute, Neuroscience, Rozzano (Milano}, Italy

Prenatal exposure to infectious or inflammatory insults can increase the risk of developing neuropsychiatric disorder of neurodevelopmental etiology. A tight association between elevated IL-6 during pregnancy, IL6 elevation in the offspring and altered behavioral tasks has been widely reported. Taking advantage of a very well-established animal model of Maternal Immune Activation (MIA), we demonstrated that a single IL6 or Poly I:C administration in pregnant dams at gestational days (gd) 15 induces a significant excitation/inhibition (E/I) imbalance in the offspring, accompanied by an abnormal connectivity, as well as behavioral deficits. Furthermore, we found out that the long-lasting effects mediated by IL6 were causally linked to an early genetic signature involving the genes STAT3 and RGS4 (Mirabella et al., Immunity 2021). In the attempt to define putative prognostic biomarkers for altered neurodevelopmental disease as a consequence of MIA, we are analyzing in the Poly I:C treated dams and offspring, the profile of pro-inflammatory biomarkers, to be correlated with connectomic and behavioral alterations. Also, we are performing in humans a characterization of the inflammatory biomarkers generated during infection in pregnancy. The correlation between maternal and newborn inflammatory molecule levels is investigated by comparing the levels of biomarkers in the blood of each mother and her baby (funicular blood). Results from this study will allow to correlate the maternal clinical features and circulating immune biomarkers with neonatal brain signatures, in order to predict high risk of abnormal neurodevelopment in newborns. This work is supported by bando Ricerca Finalizzata RF-2019-12370972

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BOARD NUMBER: S01-489

INVESTIGATION OF MITOCHONDRIAL PROTEIN-IMPORT STRESS INDUCED NEURONAL DEGENERATION

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

Johannes Ebding[1], Marlene Barth[1], Adrian Gackstatter[1], Johannes Herrmann[2], Jan Pielage[1]
[1]University of Kaiserslautern, Department Of Neurobiology And Zoology, Kaiserslautern, Germany, [2]University of Kaiserslautern, Department Of Cell Biology, Kaiserslautern, Germany

The maintenance of organelle and membrane compartment identity and function under stress conditions is essential for all complex organisms. Neurons are particularly well-suited for the investigation of stress responses as they represent one of the most complex cell types in animals and individual neurons must be maintained over the lifetime of an organism. Neurons have long axons separating soma and synapses with synaptic terminals showing one of the highest cellular rates of mitochondrial based energy consumption. Mitochondrial dysfunction is associated with multiple neurodegenerative diseases, however the precise molecular and cellular links between mitochondrial defects and neuronal degeneration remain largely elusive. To address the relationship between mitochondrial stress and synaptic integrity, we established a mitochondrial protein import (MPI)-stress model in Drosophila motoneurons. We used cellular, immunohistochemical, and transcriptomic approaches to identify the cellular mechanisms underlying the synaptic degeneration caused by impaired mitochondrial protein import. Here, we will present first insights into the transcriptional and cellular programs potentially contributing to progressive neurodegeneration in these conditions.

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THE POST-DEVELOPMENTAL ROLES OF THE NETRIN RECEPTOR UNC-40/DCC IN HEALTH AND DISEASE

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

Sapir Sela[1], Meital Oren-Suissa[2]
[1]Weizmann, Brain Science, Rehovot, Israel, [2]Weizmann Institute of Science, Brain Sciences, Rehovot, Israel

Synaptogenesis and maintenance are crucial for the formation of proper brain circuits, and for brain stability and health. The netrin-1 receptor DCC is involved not only in axon guidance and synapse formation in the developing brain, but also in synaptic maintenance, as it is expressed in certain types of cells in the adult mammalian brain. In recent years, polymorphisms in the DCC loci have been shown to associate with multiple neurodegenerative disorders, yet DCCs roles in the mature nervous system are still not completely understood. DCC is expressed in the Substentia nigra of the adult mammalian brain, in Dopamine neurons (DA) which are sensitive to degeneration in early onset of Parkinson's disease (PD). However, it is unclear whether and how DCC in involved in the disease. We utilize *C. elegans* to investigate the role of DCC in neurodegeneration. Surprisingly, we found that the expression of the worm homolog of DCC, UNC-40 in DA neurons is associated with a worse outcome for these neurons in a *C. elegans* model for Parkinson's disease (6-OHDA treatment). Conversely, in UNC-40-null animals, DA neurons survived more under 6-OHDA and showed almost no degeneration phenotype. Remarkably, UNC-40 stabilization using CRISPR was sufficient to significantly enhance DA cell degradation even without 6-OHDA treatment. We have also found that UNC-40 stabilization induces parthanatos, a novel cell-death pathway that has been implicated in the pathology of PD. Taken together, our results reveal new roles for UNC-40/DCC in the mature nervous system, and shed light on its involvement in neuronal health.

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STUDY OF MECHANOTRANSDUCTION AND MIGRATION BEHAVIOR IN A KRABBE DISEASE CELL MODEL.

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Istituto Nanoscienze Consiglio Nazionale delle Ricerche (CNR) and Scuola Normale Superiore, National Enterprise For Nanoscience And Nanotechnology (nest), Pisa, Italy

Aims Krabbe disease (KD) is a genetic rare lysosomal storage pathology causing progressive neurodegeneration. The involved gene in KD codifies for the galactosylceramidase (GALC) enzyme. GALC loss causes the accumulation of toxic lipids, disruption of autophagy and degradation processes, and leads to devastating myelin loss: several molecular pathways are involved and a clear pathogenetic signalling picture is not present to date. Therefore, our research focuses on the identification of molecular linkers in the KD pathogenesis. **Methods** We study mechanotransduction and migration processes in primary fibroblasts collected from the twitcher (TWI) mouse, a KD murine model. By time-lapse microscopy, we investigate single-cell and collective migration. We evaluate the development and the expression of focal adhesions (FAs) components, by immunocytochemistry and western blot. We look at the crosstalk between the mechanotransduction and the autophagy process. We study the presence and localization of NBR1, the protein needed for FAs recycling and turnover, and we test TWI fibroblasts' response to rapamycin (i.e. autophagy activator) treatment. **Results** We discover an impairment in TWI fibroblasts' mechanotransduction. These cells show single cell and collective migration delay, increased FAs number per cell, reduced adhesion junction proteins, aggregated autophagy effector NBR1. We show that rapamycin administration rescues the migration behaviour and partially normalise FAs and NBR1 levels. **Conclusions** Overall, the mechanosensing impairment in TWI fibroblasts correlates with autophagy. We finally suggest that mechanotransduction impairment in TWI fibro may be linked to the lipid raft disorganization, induced by GALC-deficiency in cell membranes, and the consequent disruption of intracellular signal transduction.

Pubmed:
34360664: Scaccini L, Mezzena R, De Masi A, Gagliardi M, Gambarotta G, Cecchini M, Tonazzini I
Chitosan Micro-Grooved Membranes with Increased Asymmetry for the Improvement of the Schwann Cell Response in Nerve Regeneration.
Peripheral nerve injuries are a common condition in which a nerve is damaged, affecting more than one million people every year. There are still no efficient therapeutic treatments for these injuries. Artificial scaffolds can offer new opportunities for nerve regeneration applications; in this framework, chitosan is emerging as a promising biomaterial. Here, we set up a simple and effective method for the production of micro-structured chitosan films by solvent casting, with high fidelity in the micro-pattern reproducibility. Three types of chitosan directional micro-grooved patterns, presenting different levels of symmetricity, were developed for application in nerve regenerative medicine: gratings (GR), isosceles triangles (ISO) and scalene triangles (SCA). The directional patterns were tested with a Schwann cell line. The most asymmetric topography (SCA), although it polarized the cell shaping less efficiently, promoted higher cell proliferation and a faster cell migration, both individually and collectively, with a higher directional persistence of motion. Overall, the use of micro-structured asymmetrical directional topographies may be exploited to enhance the nerve regeneration process mediated by chitosan scaffolds.
Int J Mol Sci, 2021; 22
33055385: Mezzena R, Masciullo C, Antonini S, Cremisi F, Scheffner M, Cecchini M, Tonazzini I
Study of adhesion and migration dynamics in ubiquitin E3A ligase (UBE3A)-silenced SYSH5Y neuroblastoma cells by micro-structured surfaces.
During neuronal development, neuronal cells read extracellular stimuli from the micro/nano-environment within which they exist, retrieving essential directionality and wiring information. Here, focal adhesions (FAs-protein clusters anchoring integrins to cytoskeleton) act as sensors, by integrating signals from both the extracellular matrix environment and chemotactic factors, contributing to the final neuronal pathfinding and migration. In the processes that orchestrate neuronal development, the important function of ubiquitin E3A ligase (UBE3A) is emerging. UBE3A has crucial functions in the brain and changes in its expression levels lead to neurodevelopmental disorders: the lack of UBE3A leads to Angelman syndrome (AS, OMIN 105830), while its increase causes autisms (Dup15q-autism). By using nano/micro-structured anisotropic substrates we

previously showed that UBE3A-deficient neurons have deficits in contact guidance (Tonazzini et al, Mol Autism 2019). Here, we investigate the adhesion and migration dynamics of UBE3A-silenced SH-SY5Y neuroblastoma cells in vitro by exploiting nano/micro-grooved substrates. We analyze the molecular processes regulating the development of FAs by transfection with EGFP-vector encoding for paxillin, a protein of FA clusters, and by live-cell total-internal-reflection-fluorescence microscopy. We show that UBE3A-silenced SH-SY5Y cells have impaired FA morphological development and pathway activation, which lead to a delayed adhesion and also explain the defective contact guidance in response to directional topographical stimuli. However, UBE3A-silenced SH-SY5Y cells show an overall normal migration behavior, in terms of speed and ability to follow the GRs directional stimulus. Only the collective cell migration upon cell gaps was slightly delayed for UBE3Ash SHs. Overall, the deficits of UBE3Ash SHS-SY5Y cells in FA maturation/sensing and in collective migration may have patho-physiological implications, in AS condition, considering the much more complex stimuli that neurons find in vivo during the neurodevelopment.
Nanotechnology, 2021; 32
31529358: De Masi A, Tonazzini I, Masciullo C, Mezzena R, Chiellini F, Puppi D, Cecchini M
Chitosan films for regenerative medicine: fabrication methods and mechanical characterization of nanostructured chitosan films.
Regenerative medicine is continuously facing new challenges and it is searching for new biocompatible, green/natural polymer materials, possibly biodegradable and non-immunogenic. Moreover, the critical importance of the nano/microstructuring of surfaces is overall accepted for their full biocompatibility and in vitro/in vivo performances. Chitosan is emerging as a promising biopolymer for tissue engineering and its application can be further improved by exploiting its nano/microstructuration. Here, we report the state of the art of chitosan films and scaffolds nano/micro-structuration. We show that it is possible to obtain, by solvent casting, chitosan thin films with good mechanical properties and to structure them at the microscale and even nanoscale level, with resolutions down to 100 nm.
Biophys Rev, 2019; 11

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BOARD NUMBER: S01-492

NOCTURNIN'S ROLE IN OXIDATIVE STRESS MEDIATED NEURODEGENERATION

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Oxidative stress, an imbalance of reactive oxygen species (ROS) and antioxidants, has become a central focus in neurodegenerative disorders such as Parkinson's Disease (PD) due to its strong correlation with neuronal cell death. Our lab has identified Nocturnin, an NADPH phosphatase, as a putative regulator of oxidative stress. NADPH is a powerful reductant for regenerating antioxidants, such as glutathione (GSH), to lower ROS. Increases in Nocturnin expression results in a significant decrease in cellular viability following ROS challenge. In addition, Nocturnin is increased in Parkinson's patients and increases dyskinesia in L-DOPA treated patients. However, we do not understand how Nocturnin's role as an NADPH phosphatase is disrupting oxidative homeostasis and causing cell death, and whether this plays a role in development of PD. I hypothesize Nocturnin has a role in neurodegeneration in PD by lowering NADPH levels which exacerbates oxidative stress conditions and therefore a loss of Nocturnin will be protective. To characterize how changes in Nocturnin expression affect cellular health, I induced oxidative stress using H_2O_2, and measured cell viability in WT, Nocturnin knockdown, and Nocturnin overexpressed CAD neurons. Cell viability was significantly decreased in Nocturnin overexpressed cells while knockdown of Nocturnin reversed the phenotype. Interestingly, measurement of GSH in HEK293 cells showed a significant increase in GSH levels in Nocturnin knockout cells compared to WT cells at basal and oxidative stress conditions. This supports the hypothesis that a loss of Nocturnin protects the cell from cellular stress by indirectly increasing antioxidant levels.

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LIGHT-INDUCED STRESS RESPONSE IS IMPAIRED IN THE RETINITIS PIGMENTOSA MOUSE MODEL CERKLKD/KO

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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The retina, the specialized region of the central nervous system that transduces light into neural signals, is endowed with an active metabolism. Therefore, the retina is particularly vulnerable to genetic and environmental alterations that generate reactive oxygen species (ROS), which eventually leads photoreceptors and retinal neurons to cell death. *CERKL* (*CERamide Kinase-Like*) mutations cause Retinitis Pigmentosa, a visual disorder characterized by photoreceptor degeneration and progressive vision loss. *CERKL* has been described as a resilience gene against oxidative stress, as its overexpression protects cells against oxidative stress-induced apoptosis. Moreover, preliminary evidence indicates that CERKL contributes to the formation of RNA stress granules in the retina. Using *Cerkl*$^{KD/KO}$ mouse models, we aimed to study the impact of *Cerkl* depletion on stress response and photoreceptor synaptic connectivity in response to luminic oxidative stress. To this end, we assessed the alteration of retinas from *Cerkl*$^{KD/KO}$ albino mice to acute light stress, as an immediate (early) or after two weeks (late) response using RNA-Seq and immunostaining. Our results show that the depletion of *Cerkl* causes an aberrant early response to stress and increased levels of ROS. In addition, as a late response, we found decreased photoreceptor synaptic connectivity in light exposed *Cerkl*$^{KD/KO}$ retinas. Overall, our studies indicate that *Cerkl* is a novel player in regulating light-challenged retinal homeostasis, by controlling multiple molecular pathways in neurons.

Pubmed:
34943121: García-Arroyo R, Gavaldà-Navarro A, Villarroya F, Marfany G, Mirra S
Overexpression of CERKL Protects Retinal Pigment Epithelium Mitochondria from Oxidative Stress Effects.
The precise function of , a Retinitis Pigmentosa (RP) causative gene, is not yet fully understood. There is evidence that is involved in the regulation of autophagy, stress granules, and mitochondrial metabolism, and it is considered a gene that is resilient against oxidative stress in the retina. Mutations in most RP genes affect photoreceptors, but retinal pigment epithelium (RPE) cells may be also altered. Here, we aimed to analyze the effect of overexpression and depletion in vivo and in vitro, focusing on the state of the mitochondrial network under oxidative stress conditions. Our work indicates that the depletion of increases the vulnerability of RPE mitochondria, which show a shorter size and altered shape, particularly upon sodium arsenite treatment. -depleted cells have dysfunctional mitochondrial respiration particularly upon oxidative stress conditions. The overexpression of two human CERKL isoforms (558 aa and 419 aa), which display different protein domains, shows that a pool of CERKL localizes at mitochondria in RPE cells and that CERKL protects the mitochondrial network-both in size and shape-against oxidative stress. Our results support being a resilient gene that regulates the mitochondrial network in RPE as in retinal neurons and suggest that RPE cell alteration contributes to particular phenotypic traits in patients carrying mutations.
Antioxidants (Basel), 2021; 10
34048907: Mirra S, García-Arroyo R, B Domènech E, Gavaldà-Navarro A, Herrera-Úbeda C, Oliva C, Garcia-Fernàndez J, Artuch R, Villarroya F, Marfany G
CERKL, a retinal dystrophy gene, regulates mitochondrial function and dynamics in the mammalian retina.
The retina is a highly active metabolic organ that displays a particular vulnerability to genetic and environmental factors causing stress and homeostatic imbalance. Mitochondria constitute a bioenergetic hub that coordinates stress response and cellular homeostasis, therefore structural and functional regulation of the mitochondrial dynamic network is essential for the mammalian retina. CERKL (ceramide kinase like) is a retinal degeneration gene whose mutations cause Retinitis Pigmentosa in humans, a visual disorder characterized by photoreceptors neurodegeneration and progressive vision loss. CERKL produces multiple isoforms with a dynamic subcellular localization. Here we show that a pool of CERKL isoforms localizes at mitochondria in mouse retinal ganglion cells. The depletion of CERKL levels in Cerkl(knockdown/knockout) mouse retinas

cause increase of autophagy, mitochondrial fragmentation, alteration of mitochondrial distribution, and dysfunction of mitochondrial-dependent bioenergetics and metabolism. Our results support CERKL as a regulator of autophagy and mitochondrial biology in the mammalian retina.
Neurobiol Dis, 2021; 156

33673358: Aísa-Marín I, García-Arroyo R, Mirra S, Marfany G
The Alter Retina: Alternative Splicing of Retinal Genes in Health and Disease.
Alternative splicing of mRNA is an essential mechanism to regulate and increase the diversity of the transcriptome and proteome. Alternative splicing frequently occurs in a tissue- or time-specific manner, contributing to differential gene expression between cell types during development. Neural tissues present extremely complex splicing programs and display the highest number of alternative splicing events. As an extension of the central nervous system, the retina constitutes an excellent system to illustrate the high diversity of neural transcripts. The retina expresses retinal specific splicing factors and produces a large number of alternative transcripts, including exclusive tissue-specific exons, which require an exquisite regulation. In fact, a current challenge in the genetic diagnosis of inherited retinal diseases stems from the lack of information regarding alternative splicing of retinal genes, as a considerable percentage of mutations alter splicing or the relative production of alternative transcripts. Modulation of alternative splicing in the retina is also instrumental in the design of novel therapeutic approaches for retinal dystrophies, since it enables precision medicine for specific mutations.
Int J Mol Sci, 2021; 22

32658961: Domènech EB, Andrés R, López-Iniesta MJ, Mirra S, García-Arroyo R, Milla S, Sava F, Andilla J, Loza-Álvarez P, de la Villa P, Gonzàlez-Duarte R, Marfany G
A New Cerkl Mouse Model Generated by CRISPR-Cas9 Shows Progressive Retinal Degeneration and Altered Morphological and Electrophysiological Phenotype.
Close to 100 genes cause retinitis pigmentosa, a Mendelian rare disease that affects 1 out of 4000 people worldwide. Mutations in the ceramide kinase-like gene (CERKL) are a prevalent cause of autosomal recessive cause retinitis pigmentosa and cone-rod dystrophy, but the functional role of this gene in the retina has yet to be fully determined. We aimed to generate a mouse model that resembles the phenotypic traits of patients carrying CERKL mutations to undertake functional studies and assay therapeutic approaches.
Invest Ophthalmol Vis Sci, 2020; 61

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THE NMDA RECEPTOR TRIGGERS NEURONAL AUTOPHAGY DURING OXYGEN AND GLUCOSE DEPRIVATION

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Aims: Ischemic stroke induces multiple signaling pathways that contribute to neuronal death. Among them, we previously demonstrated the importance of neuronal autophagy in oxygen and glucose deprivation (OGD, an in vitro stroke model. Thiebaut et al Autophagy 2021). Here, we highlight the importance of the NMDA receptor in the induction of autophagy-mediated neuronal death. Methods: Pure mouse cortical neurons were subjected to OGD (in a hypoxic chamber) or to NMDA stimulations. Necrosis and apoptosis were evaluated by LDH release and caspase 3 cleavage + TUNEL; and autophagy was evaluated by both western blot and IHC. Results: OGD/R induces a rise in neuronal autophagic flux showed by an autophagosome accumulation (increased levels of LC3-II) and enhanced degradation of the autophagy receptor SQSTM1/p62. OGD/R also promotes a necrotic neuronal death as observed with elevated levels of LDH and the absence of cleaved caspase 3 or TUNEL staining. Interestingly, these effects are completely reversed by the NMDA receptor antagonist MK-801. Conclusions: These results indicate a strong link between the activation of the NMDA receptor and the induction of neuronal autophagy during hypoxia.

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BOARD NUMBER: S01-495

UNRAVELLING THE ROLE OF VPS13A IN NEURONS THROUGH ITS PROTEIN INTERACTING PARTNERS

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Chorea-acanthocytosis (ChAc) is caused by VPS13A gene mutations leading to marked reduction or absence of VPS13A protein. ChAc patients show progressive movement disorders such as chorea and dystonia. The main neuropathological feature in ChAc is a selective degeneration of the striatum. However, the study of the VPS13A function in neurons has been poorly addressed. In addition, there are no models available to study the molecular mechanism affected in neurons by the lack of VPS13A. The objective of this work is to understand the VPS13A function in neurons. First, we assessed the VPS13A interacting partners in wild-type mouse brain through a specific protein immunoprecipitation followed by mass spectrometry to dissect its possible functions in neurons. We found that VPS13A interacts predominantly with both Mre11a and Rad50, two proteins that play important roles in double-strand brake repair. Then, we aim to evaluate how the lack of VPS13A and its interacting partners are involved in ChAc pathology. For this, we first generated a cellular ChAc model by miR30-Based shRNA knockdown system. We found a significant reduction of the vps13a mRNA by qPCR, of the VPS13A protein by western blot and a tendency to decrease VPS13A staining intensity by immunocytochemistry in STHdh$^{Q7/Q7}$ cells. In the meeting, we will present our analysis of differences in Mre11a and Rad50 concentration and subcellular localization between wild-type and VPS13A knockdown neurons. The study of VPS13A protein interacting partners can provide novel insights toward VPS13A function in neurons that will contribute to further knowledge of ChAc pathophysiology.

Pubmed:
34884823: García-García E, Chaparro-Cabanillas N, Coll-Manzano A, Carreras-Caballé M, Giralt A, Del Toro D, Alberch J, Masana M, Rodríguez MJ
Unraveling the Spatiotemporal Distribution of VPS13A in the Mouse Brain.
Loss-of-function mutations in the human vacuolar protein sorting the 13 homolog A (VPS13A) gene cause Chorea-acanthocytosis (ChAc), with selective degeneration of the striatum as the main neuropathologic feature. Very little is known about the VPS13A expression in the brain. The main objective of this work was to assess, for the first time, the spatiotemporal distribution of VPS13A in the mouse brain. We found VPS13A expression present in neurons already in the embryonic stage, with stable levels until adulthood. VPS13A mRNA and protein distributions were similar in the adult mouse brain. We found a widespread VPS13A distribution, with the strongest expression profiles in the pons, hippocampus, and cerebellum. Interestingly, expression was weak in the basal ganglia. VPS13A staining was positive in glutamatergic, GABAergic, and cholinergic neurons, but rarely in glial cells. At the cellular level, VPS13A was mainly located in the soma and neurites, co-localizing with both the endoplasmic reticulum and mitochondria. However, it was not enriched in dendritic spines or the synaptosomal fraction of cortical neurons. In vivo pharmacological modulation of the glutamatergic, dopaminergic or cholinergic systems did not modulate VPS13A concentration in the hippocampus, cerebral cortex, or striatum. These results indicate that VPS13A has remarkable stability in neuronal cells. Understanding the distinct expression pattern of VPS13A can provide relevant information to unravel pathophysiological hallmarks of ChAc.
Int J Mol Sci, 2021; 22
33984337: Pérez-Sisqués L, Martín-Flores N, Masana M, Solana-Balaguer J, Llobet A, Romaní-Aumedes J, Canal M, Campoy-Campos G, García-García E, Sánchez-Fernández N, Fernández-García S, Gilbert JP, Rodríguez MJ, Man HY, Feinstein E, Williamson DL, Soto D, Gasull X, Alberch J, Malagelada C
RTP801 regulates motor cortex synaptic transmission and learning.
RTP801/REDD1 is a stress-regulated protein whose upregulation is necessary and sufficient to trigger neuronal death in in vitro and in vivo models of Parkinson's and Huntington's diseases and is up regulated in compromised neurons in human postmortem brains of both neurodegenerative disorders. Indeed, in both Parkinson's and Huntington's disease mouse

models, RTP801 knockdown alleviates motor-learning deficits.
Exp Neurol, 2021; 342
33339845: Izquierdo-Garcia JL, Comella-Del-Barrio P, Campos-Olivas R, Villar-Hernández R, Prat-Aymerich C, De Souza-Galvão ML, Jiménez-Fuentes MA, Ruiz-Manzano J, Stojanovic Z, González A, Serra-Vidal M, García-García E, Muriel-Moreno B, Millet JP, Molina-Pinargote I, Casas X, Santiago J, Sabriá F, Martos C, Herzmann C, Ruiz-Cabello J, Domínguez J
Discovery and validation of an NMR-based metabolomic profile in urine as TB biomarker.
Despite efforts to improve tuberculosis (TB) detection, limitations in access, quality and timeliness of diagnostic services in low- and middle-income countries are challenging for current TB diagnostics. This study aimed to identify and characterise a metabolic profile of TB in urine by high-field nuclear magnetic resonance (NMR) spectrometry and assess whether the TB metabolic profile is also detected by a low-field benchtop NMR spectrometer. We included 189 patients with tuberculosis, 42 patients with pneumococcal pneumonia, 61 individuals infected with latent tuberculosis and 40 uninfected individuals. We acquired the urine spectra from high and low-field NMR. We characterised a TB metabolic fingerprint from the Principal Component Analysis. We developed a classification model from the Partial Least Squares-Discriminant Analysis and evaluated its performance. We identified a metabolic fingerprint of 31 chemical shift regions assigned to eight metabolites (aminoadipic acid, citrate, creatine, creatinine, glucose, mannitol, phenylalanine, and hippurate). The model developed using low-field NMR urine spectra correctly classified 87.32%, 85.21% and 100% of the TB patients compared to pneumococcal pneumonia patients, LTBI and uninfected individuals, respectively. The model validation correctly classified 84.10% of the TB patients. We have identified and characterised a metabolic profile of TB in urine from a high-field NMR spectrometer and have also detected it using a low-field benchtop NMR spectrometer. The models developed from the metabolic profile of TB identified by both NMR technologies were able to discriminate TB patients from the rest of the study groups and the results were not influenced by anti-TB treatment or TB location. This provides a new approach in the search for possible biomarkers for the diagnosis of TB.
Sci Rep, 2020; 10
33016873: Fernández-García S, Conde-Berriozabal S, García-García E, Gort-Paniello C, Bernal-Casas D, García-Díaz Barriga G, López-Gil J, Muñoz-Moreno E, Soria G, Campa L, Artigas F, Rodríguez MJ, Alberch J, Masana M
M2 cortex-dorsolateral striatum stimulation reverses motor symptoms and synaptic deficits in Huntington's disease.
Huntington's disease (HD) is a neurological disorder characterized by motor disturbances. HD pathology is most prominent in the striatum, the central hub of the basal ganglia. The cerebral cortex is the main striatal afferent, and progressive cortico-striatal disconnection characterizes HD. We mapped striatal network dysfunction in HD mice to ultimately modulate the activity of a specific cortico-striatal circuit to ameliorate motor symptoms and recover synaptic plasticity. Multimodal MRI in vivo indicates cortico-striatal and thalamo-striatal functional network deficits and reduced glutamate/glutamine ratio in the striatum of HD mice. Moreover, optogenetically-induced glutamate release from M2 cortex terminals in the dorsolateral striatum (DLS) was undetectable in HD mice and striatal neurons show blunted electrophysiological responses. Remarkably, repeated M2-DLS optogenetic stimulation normalized motor behavior in HD mice and evoked a sustained increase of synaptic plasticity. Overall, these results reveal that selective stimulation of the M2-DLS pathway can become an effective therapeutic strategy in HD.
Elife, 2020; 9
32719030: Blauenfeldt T, Villar-Hernández R, García-García E, Latorre I, Holm LL, Muriel-Moreno B, De Souza-Galvão ML, Millet JP, Sabriá F, Sánchez-Montalva A, Ruiz-Manzano J, Pilarte J, Jiménez MA, Centeno C, Torres C, Molina-Pinargote I, González-Díaz YD, Santiago J, Cantos A, Prat C, Andersen P, Domínguez J, Ruhwald M
Diagnostic Accuracy of Interferon Gamma-Induced Protein 10 mRNA Release Assay for Tuberculosis.
Interferon gamma (IFN-γ) release assays (IGRAs) are increasingly used to test for latent tuberculosis (TB) infection. Although highly specific, IGRAs have a relatively high false-negative rate in active TB patients. A more sensitive assay is needed. IFN-γ-induced protein 10 (IP-10) is an alternative biomarker with a 100-fold-higher expression level than IFN-γ, allowing for different analysis platforms, including molecular detection. The PCR technique is already an integrated tool in most TB laboratories and, thus, an obvious platform to turn to. In this case-control study, we investigated the diagnostic sensitivity and specificity of a molecular assay detecting IP-10 mRNA expression following antigen stimulation of a blood sample. We included 89 TB patients and 99 healthy controls. Blood was drawn in QuantiFeron-TB gold in-tube (QFT) assay tubes. Eight hours poststimulation, IP-10 mRNA expression was analyzed, and 20 h poststimulation, IP-10 and IFN-γ protein plasma levels were analyzed using an in-house IP-10 enzyme-linked immunosorbent assay (ELISA) and the official QFT ELISA, respectively. The IP-10 mRNA assay provided high specificity (98%), sensitivity (80%), and area under the concentration-time curve (AUC) (0.97); however, the QFT assay provided a higher overall diagnostic potential, with specificity of 100%, sensitivity of 90%, and AUC of 0.99. The IP-10 protein assay performed on par with the QFT assay, with specificity of 98%, sensitivity of 87%, and AUC of 0.98. We have provided proof of high technical performance of a molecular assay detecting

IP-10 mRNA expression. As a diagnostic tool, this assay would gain from further optimization, especially on the kinetics of IP-10 mRNA expression.
J Clin Microbiol, 2020; 58
32764560: Villar-Hernández R, Blauenfeldt T, García-García E, Muriel-Moreno B, De Souza-Galvão ML, Millet JP, Sabriá F, Sánchez-Montalvá A, Ruiz-Manzano J, Pilarte J, Jiménez MA, Centeno C, Martos C, Molina-Pinargote I, González-Díaz YD, Santiago J, Cantos A, Casas I, Guerola RM, Prat C, Andersen P, Latorre I, Ruhwald M, Domínguez J
Diagnostic benefits of adding EspC, EspF and Rv2348-B to the QuantiFERON Gold In-tube antigen combination.
Interferon (IFN)-γ release assays (IGRAs) are used to diagnose latent tuberculosis (TB) infection (LTBI). To improve the accuracy of these tests, different approaches, such as alternative cytokine detection and using different antigens, are considered. Following this purpose, this study aims to evaluate the addition of EspC, EspF and Rv2348-B to those present in the QuantiFERON-TB Gold In-Tube (QFN-G-IT). We included 115 subjects: 74 active TB patients, 17 LTBI individuals and 24 healthy controls. Whole blood samples were collected in QFN-G-IT and in-house tubes containing different combinations of EspC, EspF and Rv2348-B, together with ESAT-6, CFP-10, and TB7.7. After overnight incubation at 37 ºC, plasma was harvested and IFN-γ quantified. IFN-γ levels in the QFN-G-IT and in-house tubes correlated very good (Spearman Rho(r) > 0.86). In-house antigen combinations distinguished healthy individuals from those with active TB and LTBI (specificities and sensitivities higher than 87.5% and 96.3%, respectively [AUC > 0.938]). Adding EspC, EspF and Rv2348-B, increased the sensitivity of the test, being the addition of EspC and Rv2348-B the combination that yielded a higher sensitivity with no specificity loss. Addition of these antigens could improve diagnosis in patients with impaired or immature immune response who are at high risk of developing TB.
Sci Rep, 2020; 10
32625064: Kim A, García-García E, Straccia M, Comella-Bolla A, Miguez A, Masana M, Alberch J, Canals JM, Rodríguez MJ
Reduced Fractalkine Levels Lead to Striatal Synaptic Plasticity Deficits in Huntington's Disease.
Huntington's disease (HD) is an inherited neurodegenerative disorder in which the striatum is the most affected brain region. Although a chronic inflammatory microglial reaction that amplifies disease progression has been described in HD patients, some murine models develop symptoms without inflammatory microglial activation. Thus, dysfunction of non-inflammatory microglial activity could also contribute to the early HD pathological process. Here, we show the involvement of microglia and particularly fractalkine signaling in the striatal synaptic dysfunction of R6/1 mice. We found reduced fractalkine gene expression and protein concentration in R6/1 striata from 8 to 20 weeks of age. Consistently, we also observed a down-regulation of fractalkine levels in the putamen of HD patients and in HD patient hiPSC-derived neurons. Automated cell morphology analysis showed a non-inflammatory ramified microglia in the striatum of R6/1 mice. However, we found increased PSD-95-positive puncta inside microglia, indicative of synaptic pruning, before HD motor symptoms start to manifest. Indeed, microglia appeared to be essential for striatal synaptic function, as the inhibition of microglial activity with minocycline impaired the induction of corticostriatal long-term depression (LTD) in wild-type mice. Notably, fractalkine administration restored impaired corticostriatal LTD in R6/1 mice. Our results unveil a role for fractalkine-dependent neuron-microglia interactions in the early striatal synaptic dysfunction characteristic of HD.
Front Cell Neurosci, 2020; 14
32370602: Lacoma A, Usón L, Mendoza G, Sebastián V, Garcia-Garcia E, Muriel-Moreno B, Domínguez J, Arruebo M, Prat C
Novel intracellular antibiotic delivery system against : cloxacillin-loaded poly(d,l-lactide-co-glycolide) acid nanoparticles.
First, to compare minimum inhibitory concentrations (MIC) of free cloxacillin and cloxacillin-containing nanoparticles (NP) against methicillin-susceptible (MSSA) and resistant (MRSA) and second, to assess NP antimicrobial activity against intracellular . Poly(d,l-lactide-co-glycolide) acid (PLGA)-NP were loaded with cloxacillin and physico-chemically characterized. MICs were determined for reference strains Newman-(MSSA) and USA300-(MRSA). Murine alveolar macrophages were infected, and bacterial intracellular survival was assessed after incubating with free-cloxacillin or PLGA-cloxacillin-NP. For both isolates, MICs for antibiotic-loaded-NP were lower than those obtained with free cloxacillin, indicating that the drug encapsulation improves antimicrobial activity. A sustained antibiotic release was demonstrated when using the PLGA-cloxacillin-NP. When considering the lowest concentrations, the use of drug-loaded NP enabled a higher reduction of intracellular bacterial load.
Nanomedicine (Lond), 2020; 15
32117257: Coppola M, Villar-Hernández R, van Meijgaarden KE, Latorre I, Muriel Moreno B, Garcia-Garcia E, Franken KLMC, Prat C, Stojanovic Z, De Souza Galvão ML, Millet JP, Sabriá J, Sánchez-Montalva A, Noguera-Julian A, Geluk A, Domínguez J, Ottenhoff THM
Cell-Mediated Immune Responses to -Expressed and Stage-Specific Antigens in Latent and Active Tuberculosis Across Different Age Groups.
A quarter of the global human population is estimated to be latently infected by (), the causative agent of tuberculosis (TB).

TB remains the global leading cause of death by a single pathogen and ranks among the top-10 causes of overall global mortality. Current immunodiagnostic tests cannot discriminate between latent, active and past TB, nor predict progression of latent infection to active disease. The only registered TB vaccine, Bacillus Calmette-Guérin (BCG), does not adequately prevent pulmonary TB in adolescents and adults, thus permitting continued TB-transmission. Several proteins, mostly discovered through IFN-γ centered approaches, have been proposed as targets for new TB-diagnostic tests or -vaccines. Recently, however, we identified novel antigens capable of eliciting multiple cytokines, including antigens that did not induce IFN-γ but several other cytokines. These antigens had been selected based on high gene-expression in the lung , and have been termed expressed (IVE-TB) antigens. Here, we extend and validate our previous findings in an independent Southern European cohort, consisting of adults and adolescents with either LTBI or TB. Our results confirm that responses to IVE-TB antigens, and also DosR-regulon and Rpf stage-specific antigens are marked by multiple cytokines, including strong responses, such as for TNF-α, in the absence of detectable IFN-γ production. Except for TNF-α, the magnitude of those responses were significantly higher in LTBI subjects. Additional unbiased analyses of high dimensional flow-cytometry data revealed that TNF-α+ cells responding to antigens comprised 17 highly heterogeneous cell types. Among these 17 TNF-α+ cells clusters identified, those with CD8+TEMRA or CD8+CD4+ phenotypes, defined by the expression of multiple intracellular markers, were the most prominent in adult LTBI, while CD14+ TNF-α+ myeloid-like clusters were mostly abundant in adolescent LTBI. Our findings, although limited to a small cohort, stress the importance of assessing broader immune responses than IFN-γ alone in antigen discovery as well as the importance of screening individuals of different age groups. In addition, our results provide proof of concept showing how unbiased multidimensional multiparametric cell subset analysis can identify unanticipated blood cell subsets that could play a role in the immune response against .
Front Immunol, 2020; 11

30850687: Villar-Hernández R, Latorre I, De Souza-Galvão ML, Jiménez MA, Ruiz-Manzano J, Pilarte J, García-García E, Muriel-Moreno B, Cantos A, Altet N, Millet JP, González-Díaz Y, Molina-Pinargote I, Prat C, Ruhwald M, Domínguez J
Use of IP-10 detection in dried plasma spots for latent tuberculosis infection diagnosis in contacts via mail.
The aim of this study was to test the use of IP-10 detection in dried plasma from contact studies individuals (contacts of smear positive patients), by comparing it with IP-10 and IFN-γ detection in direct plasma, to establish IP-10 detection in DPS as a useful assay for LTBI diagnosis. Whole blood samples were collected from 80 subjects: 12 with active tuberculosis (TB), and 68 from contact studies. The amount of IFN-γ produced by sensitized T cells was determined in direct plasma by QuantiFERON Gold In-Tube test. IP-10 levels were determined in direct and dried plasma by an in-house ELISA. For dried plasma IP-10 determination, two 25 µl plasma drops were dried in Whatman903 filter paper and sent by mail to the laboratory. Regarding TB patients, 100.0%, 91.7% and 75.0% were positive for IFN-γ detection and IP-10 detection in direct and dried plasma, respectively. In contacts, 69.1%, 60.3% and 48.5% had positive results after IFN-γ and IP-10 in direct and dried plasma, respectively. The agreement among in vitro tests was substantial and IP-10 levels in direct and dried plasma were strongly correlated (r = 0.897). In conclusion, IP-10 detection in dried plasma is a simple and safe method that would help improve LTBI management.
Sci Rep, 2019; 9

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METABOLOMIC PROFILE OF ASTROCYTES EXPOSED TO PRO-INFLAMMATORY CONDITIONS

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Metabolomic profile of astrocytes exposed to pro-inflammatory conditions O.Alzahrani, H. Fiumelli and P.J. Magistretti King Abdullah University of Science and Technology (KAUST) Saudi Arabia Astrocytes are critical for neuronal function and survival. Astrocytes ensure metabolic support to the neurons, in particular by providing on-demand lactate derived from aerobic glycolysis, through a mechanism known as the Astrocyte Neuron Lactate Shuttle (ANLS). Previous results from our laboratory showed that exposure of astrocytes to the pathological form of amyloid-beta A-beta 25-35 induced marked changes in their metabolic phenotype rendering them less apt to ensure neuronal survival (Allaman et al, J. Neurosci, 2010). In the present study, we exposed cortical astrocyte cultures to pro-inflammatory cytokines such as IL1-beta and TNF-alpha. The cultures were then analyzed for their metabolomic profile in control and cytokine-induced inflammation. Several metabolic pathways were affected by the cytokine treatment, such as an increased glycolytic flux at the expense of mitochondrial oxidation associated with an increase in glutaminolysis. Markers of oxidative stress and nitric oxide synthase activation were also observed. Finally, amino acid oxidation to support the increased metabolic needs was also apparent. Work is now in progress to analyze the metabolomic profile in astrocytes exposed to a-beta 42 in various degrees of aggregation.

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AβPP-INDUCED UPR TRANSCRIPTOMIC SIGNATURE OF GLIAL CELLS TO OXIDATIVE STRESS AS AN ADAPTIVE MECHANISM TO PRESERVE CELL FUNCTION AND SURVIVAL

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Background: Alzheimer's disease (AD) and age-related macular degeneration (AMD) present similarities, particularly with respect to oxidative stress, including production of 4-Hydroxy-2- nonenal (HNE). AMD has been named the AD in the eye. The Müller cells (MC) function as a principal glia of the retina and maintain water/potassium, glutamate homeostasis and redox status. Any MC dysfunction results in retinal neurodegeneration. **Objectives:** We investigated the effects of HNE in human MC. **Results:** HNE induced an increase of the reactive oxygen species associated with mitochondrial dysfunction and apoptosis. HNE induced endoplasmic reticulum (ER) stress (upregulation of GRP78/Bip, and the proapoptotic factor, CHOP). HNE also impaired expression of genes controlling potassium homeostasis (KCNJ10), glutamate detoxification (GS), and the visual cycle (RLBP1). MC adaptive response to HNE included upregulation of amyloid-β protein precursor (AβPP). To determine the role of AβPP, we overexpressed AβPP in MC. Overexpression of AβPP induced strong antioxidant and anti-ER stress (PERK downregulation and GADD34 upregulation) responses accompanied by activation of the prosurvival branch of the unfolded protein response. It was also associated with upregulation of major genes involved in MC-controlled retinal homeostasis (KCNJ10, GS, and RLBP1) and protection against HNE-induced apoptosis. Therefore, AβPP is an ER and oxidative stress responsive molecule, and is able to stimulate the transcription of major genes involved in MC functions impaired by HNE. **Conclusion:** Our study suggests that targeting oxidative and ER stress might be a potential therapeutic strategy against glia impairment in AMD and AD, in light of the common features between the two pathologies

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A QUANTITATIVE MODEL OF SPORADIC AXONAL DEGENERATION IN THE DROSOPHILA VISUAL SYSTEM

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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In human neurodegenerative diseases, neurons undergo axonal degeneration months to years before they die. Here, we developed a system modelling early degenerative events in *Drosophila* adult photoreceptor cells. Thanks to the stereotypy of their axonal projections, this system delivers quantitative data on sporadic and progressive axonal degeneration of photoreceptor cells. Using this method, we show that exposure of adult flies to a constant light stimulation for several days overcomes the intrinsic resilience of R7 photoreceptors and leads to progressive axonal degeneration. This was not associated with apoptosis. We furthermore provide evidence that loss of synaptic integrity between R7 and a postsynaptic partner preceded axonal degeneration, thus recapitulating features of human neurodegenerative diseases. Finally, our experiments uncovered that neurotransmission to postsynaptic partners of R7 and their response are required to initiate degeneration, suggesting that postsynaptic cells signal back to the photoreceptor to maintain axonal structure. This model can be used to dissect cellular circuit mechanisms involved in the early events of axonal degeneration, allowing for a better understanding of how neurons cope with stress and lose their resilience capacities.

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ENHANCED NEURONAL GLYCOLYSIS CAUSES COGNITIVE IMPAIRMENT AND METABOLIC SYNDROME IN MOUSE

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Whether organismal fitness is coordinated by the metabolism of neurons is unknown. In contrast to the predominantly glycolytic nature of astrocytes, neurons use very little glucose through glycolysis to obtain energy (1). This metabolic difference is dictated by the protein stability of the pro-glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), which is highly expressed in astrocytes, but continuously degraded in neurons by the E3-ubiquitin ligase anaphase-promoting complex/cyclosome-Cdh1 (2). However, the *in vivo* physiological significance of such a limited glycolytic activity of neurons is intriguing. To address this matter, we generated a genetically-engineered mouse that persistently expresses PFKFB3 in neurons *in vivo* (CaMKII-PFKFB3). We found that PFKFB3 enhancement in neurons shifted glucose metabolism towards glycolysis causing NAD depletion, dysfunctional mitochondria, redox stress and impairment of the autophagic flux in several brain regions, including the hippocampus and the medio-basal hypothalamus. Phenotypic characterization of these CaMKII-PFKFB3 mice revealed cognitive deterioration, motor discoordination, glucose intolerance and obesity. Interestingly, these biochemical and phenotypic alterations were rescued by abolishing redox stress *via* co-expression of a mitochondrially-tagged isoform of catalase (mCAT) in neurons. Mechanistically, the increase in glycolysis shifted down the pentose-phosphate pathway, causing redox stress that impaired mitochondrial respiration, triggering a signaling pathway leading to an aberrant positive loop of glycolytic activation. These data strongly suggest that the occurrence of a low glycolytic activity in neurons is a natural mechanism aimed to sustain organismal welfare. [Funded by the Agencia Estatal de Investigación (PID2019-105699RB-I00/ AEI / 10.13039/501100011033)]. References: (1) Nat. Cell Biol. 6:45-51, 2004; (2) Nat. Cell Biol. 11:747-752, 2009.

Pubmed:
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Abrogating mitochondrial ROS in neurons or astrocytes reveals cell-specific impact on mouse behaviour.
Cells naturally produce mitochondrial reactive oxygen species (mROS), but the in vivo pathophysiological significance has long remained controversial. Within the brain, astrocyte-derived mROS physiologically regulate behaviour and are produced at one order of magnitude faster than in neurons. However, whether neuronal mROS abundance differentially impacts on behaviour is unknown. To address this, we engineered genetically modified mice to down modulate mROS levels in neurons in vivo. Whilst no alterations in motor coordination were observed by down modulating mROS in neurons under healthy conditions, it prevented the motor discoordination caused by the pro-oxidant neurotoxin, 3-nitropropionic acid (3-NP). In contrast, abrogation of mROS in astrocytes showed no beneficial effect against the 3-NP insult. These data indicate that the impact of modifying mROS production on mouse behaviour critically depends on the specific cell-type where they are generated.
Redox Biol, 2021; 41
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Glucose metabolism links astroglial mitochondria to cannabinoid effects.
Astrocytes take up glucose from the bloodstream to provide energy to the brain, thereby allowing neuronal activity and behavioural responses. By contrast, astrocytes are under neuronal control through specific neurotransmitter receptors. However, whether the activation of astroglial receptors can directly regulate cellular glucose metabolism to eventually

modulate behavioural responses is unclear. Here we show that activation of mouse astroglial type-1 cannabinoid receptors associated with mitochondrial membranes (mtCB) hampers the metabolism of glucose and the production of lactate in the brain, resulting in altered neuronal functions and, in turn, impaired behavioural responses in social interaction assays. Specifically, activation of astroglial mtCB receptors reduces the phosphorylation of the mitochondrial complex I subunit NDUFS4, which decreases the stability and activity of complex I. This leads to a reduction in the generation of reactive oxygen species by astrocytes and affects the glycolytic production of lactate through the hypoxia-inducible factor 1 pathway, eventually resulting in neuronal redox stress and impairment of behavioural responses in social interaction assays. Genetic and pharmacological correction of each of these effects abolishes the effect of cannabinoid treatment on the observed behaviour. These findings suggest that mtCB receptor signalling can directly regulate astroglial glucose metabolism to fine-tune neuronal activity and behaviour in mice.
Nature, 2020; 583
31406177: Burmistrova O, Olias-Arjona A, Lapresa R, Jimenez-Blasco D, Eremeeva T, Shishov D, Romanov S, Zakurdaeva K, Almeida A, Fedichev PO, Bolaños JP
Targeting PFKFB3 alleviates cerebral ischemia-reperfusion injury in mice.
The glycolytic rate in neurons is low in order to allow glucose to be metabolized through the pentose-phosphate pathway (PPP), which regenerates NADPH to preserve the glutathione redox status and survival. This is controlled by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3), the pro-glycolytic enzyme that forms fructose-2,6-bisphosphate, a powerful allosteric activator of 6-phosphofructo-1-kinase. In neurons, PFKFB3 protein is physiologically inactive due to its proteasomal degradation. However, upon an excitotoxic stimuli, PFKFB3 becomes stabilized to activate glycolysis, thus hampering PPP mediated protection of redox status leading to neurodegeneration. Here, we show that selective inhibition of PFKFB3 activity by the small molecule AZ67 prevents the NADPH oxidation, redox stress and apoptotic cell death caused by the activation of glycolysis triggered upon excitotoxic and oxygen-glucose deprivation/reoxygenation models in mouse primary neurons. Furthermore, in vivo administration of AZ67 to mice significantly alleviated the motor discoordination and brain infarct injury in the middle carotid artery occlusion ischemia/reperfusion model. These results show that pharmacological inhibition of PFKFB3 is a suitable neuroprotective therapeutic strategy in excitotoxic-related disorders such as stroke.
Sci Rep, 2019; 9
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Astrocytic mitochondrial ROS modulate brain metabolism and mouse behaviour.
To satisfy its high energetic demand, the brain depends on the metabolic cooperation of various cell types. For example, astrocytic-derived lactate sustains memory consolidation by serving both as an oxidizable energetic substrate for neurons and as a signalling molecule. Astrocytes and neurons also differ in the regulation of glycolytic enzymes and in the organization of their mitochondrial respiratory chain. Unlike neurons, astrocytes rely on glycolysis for energy generation and, as a consequence, have a loosely assembled mitochondrial respiratory chain that is associated with a higher generation of mitochondrial reactive oxygen species (ROS). However, whether this abundant natural source of mitochondrial ROS in astrocytes fulfils a specific physiological role is unknown. Here we show that astrocytic mitochondrial ROS are physiological regulators of brain metabolism and neuronal function. We generated mice that inducibly overexpress mitochondrial-tagged catalase in astrocytes and show that this overexpression decreases mitochondrial ROS production in these cells during adulthood. Transcriptomic, metabolomic, biochemical, immunohistochemical and behavioural analysis of these mice revealed alterations in brain redox, carbohydrate, lipid and amino acid metabolic pathways associated with altered neuronal function and mouse behaviour. We found that astrocytic mitochondrial ROS regulate glucose utilization via the pentose-phosphate pathway and glutathione metabolism, which modulates the redox status and potentially the survival of neurons. Our data provide further molecular insight into the metabolic cooperation between astrocytes and neurons and demonstrate that mitochondrial ROS are important regulators of organismal physiology in vivo.
Nat Metab, 2019; 1
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Melatonin induces the expression of Nrf2-regulated antioxidant enzymes via PKC and Ca2+ influx activation in mouse pancreatic acinar cells.
The goal of this study was to evaluate the potential activation of the nuclear factor erythroid 2-related factor and the antioxidant-responsive element (Nrf2-ARE) signaling pathway in response to melatonin in isolated mouse pancreatic acinar cells. Changes in intracellular free Ca(2+) concentration were followed by fluorimetric analysis of fura-2-loaded cells. The activations of PKC and JNK were measured by Western blot analysis. Quantitative reverse transcription-polymerase chain reaction was employed to detect the expression of Nrf2-regulated antioxidant enzymes. Immunocytochemistry was employed

to determine nuclear location of phosphorylated Nrf2, and the cellular redox state was monitored following MitoSOX Red-derived fluorescence. Our results show that stimulation of fura-2-loaded cells with melatonin (1 µM to 1 mM), in the presence of Ca(2+) in the extracellular medium, induced a slow and progressive increase of [Ca(2+)](c) toward a stable level. Melatonin did not inhibit the typical Ca(2+) response induced by CCK-8 (1 nM). When the cells were challenged with indoleamine in the absence of Ca(2+) in the extracellular solution (medium containing 0.5 mM EGTA) or in the presence of 1 mM LaCl(3), to inhibit Ca(2+) entry, we could not detect any change in [Ca(2+)](c). Nevertheless, CCK-8 (1 nM) was able to induce the typical mobilization of Ca(2+). When the cells were incubated with the PKC activator PMA (1 µM) in the presence of Ca(2+) in the extracellular medium, we observed a response similar to that noted when the cells were challenged with melatonin 100 µM. However, in the presence of Ro31-8220 (3 µM), a PKC inhibitor, stimulation of cells with melatonin failed to evoke changes in [Ca(2+)]c. Immunoblots, using an antibody specific for phospho-PKC, revealed that melatonin induces PKCα activation, either in the presence or in the absence of external Ca(2+). Melatonin induced the phosphorylation and nuclear translocation of the transcription factor Nrf2, and evoked a concentration-dependent increase in the expression of the antioxidant enzymes NAD(P)H-quinone oxidoreductase 1, catalytic subunit of glutamate-cysteine ligase, and heme oxygenase-1. Incubation of MitoSOX Red-loaded pancreatic acinar cells in the presence of 1 nM CCK-8 induced a statistically significant increase in dye-derived fluorescence, reflecting an increase in oxidation, that was abolished by pretreatment of cells with melatonin (100 µM) or PMA (1 µM). On the contrary, pretreatment with Ro31-8220 (3 µM) blocked the effect of melatonin on CCK-8-induced increase in oxidation. Finally, phosphorylation of JNK in the presence of CCK-8 or melatonin was also observed. We conclude that melatonin, via modulation of PKC and Ca(2+) signaling, could potentially stimulate the Nrf2-mediated antioxidant response in mouse pancreatic acinar cells.
Free Radic Biol Med, 2015; 87

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Aberrant upregulation of the glycolytic enzyme PFKFB3 in CLN7 neuronal ceroid lipofuscinosis.
CLN7 neuronal ceroid lipofuscinosis is an inherited lysosomal storage neurodegenerative disease highly prevalent in children. CLN7/MFSD8 gene encodes a lysosomal membrane glycoprotein, but the biochemical processes affected by CLN7-loss of function are unexplored thus preventing development of potential treatments. Here, we found, in the Cln7 mouse model of CLN7 disease, that failure in autophagy causes accumulation of structurally and bioenergetically impaired neuronal mitochondria. In vivo genetic approach reveals elevated mitochondrial reactive oxygen species (mROS) in Cln7 neurons that mediates glycolytic enzyme PFKFB3 activation and contributes to CLN7 pathogenesis. Mechanistically, mROS sustains a signaling cascade leading to protein stabilization of PFKFB3, normally unstable in healthy neurons. Administration of the highly selective PFKFB3 inhibitor AZ67 in Cln7 mouse brain in vivo and in CLN7 patients-derived cells rectifies key disease hallmarks. Thus, aberrant upregulation of the glycolytic enzyme PFKFB3 in neurons may contribute to CLN7 pathogenesis and targeting PFKFB3 could alleviate this and other lysosomal storage diseases.
Nat Commun, 2022; 13

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Cell identity and nucleo-mitochondrial genetic context modulate OXPHOS performance and determine somatic heteroplasmy dynamics.
Heteroplasmy, multiple variants of mitochondrial DNA (mtDNA) in the same cytoplasm, may be naturally generated by mutations but is counteracted by a genetic mtDNA bottleneck during oocyte development. Engineered heteroplasmic mice with nonpathological mtDNA variants reveal a nonrandom tissue-specific mtDNA segregation pattern, with few tissues that do not show segregation. The driving force for this dynamic complex pattern has remained unexplained for decades, challenging our understanding of this fundamental biological problem and hindering clinical planning for inherited diseases. Here, we demonstrate that the nonrandom mtDNA segregation is an intracellular process based on organelle selection. This cell type-specific decision arises jointly from the impact of mtDNA haplotypes on the oxidative phosphorylation (OXPHOS) system and the cell metabolic requirements and is strongly sensitive to the nuclear context and to environmental cues.
Sci Adv, 2020; 6

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Regulation of Bcl-xL-ATP Synthase Interaction by Mitochondrial Cyclin B1-Cyclin-Dependent Kinase-1 Determines Neuronal Survival.
The survival of postmitotic neurons needs continuous degradation of cyclin B1, a mitotic protein accumulated aberrantly in

the damaged brain areas of Alzheimer's disease and stroked patients. Degradation of cyclin B1 takes place in the proteasome after ubiquitylation by the anaphase-promoting complex/cyclosome (APC/C)-cadherin 1 (Cdh1), an E3 ubiquitin ligase that is highly active in neurons. However, during excitotoxic damage-a hallmark of neurological disorders-APC/C-Cdh1 is inactivated, causing cyclin B1 stabilization and neuronal death through an unknown mechanism. Here, we show that an excitotoxic stimulus in rat cortical neurons in primary culture promotes cyclin B1 accumulation in the mitochondria, in which it binds to, and activates, cyclin-dependent kinase-1 (Cdk1). The cyclin B1-Cdk1 complex in the mitochondria phosphorylates the anti-apoptotic protein B-cell lymphoma extra-large (Bcl-xL), leading to its dissociation from the β subunit of F1Fo-ATP synthase. The subsequent inhibition of ATP synthase activity causes complex I oxidative damage, mitochondrial inner membrane depolarization, and apoptotic neuronal death. These results unveil a previously unrecognized role for mitochondrial cyclin B1 in the oxidative damage associated with neurological disorders.
J Neurosci, 2015; 35

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Astrocyte NMDA receptors' activity sustains neuronal survival through a Cdk5-Nrf2 pathway.
Neurotransmission unavoidably increases mitochondrial reactive oxygen species. However, the intrinsic antioxidant defense of neurons is weak and hence the mechanism whereby these cells are physiologically protected against oxidative damage is unknown. Here we found that the antioxidant defense of neurons is repressed owing to the continuous protein destabilization of the master antioxidant transcriptional activator, nuclear factor-erythroid 2-related factor-2 (Nrf2). By contrast, Nrf2 is highly stable in neighbor astrocytes explaining their robust antioxidant defense and resistance against oxidative stress. We also show that subtle and persistent stimulation of N-methyl-d-aspartate receptors (NMDAR) in astrocytes, through a mechanism not requiring extracellular Ca^{2+} influx, upregulates a signal transduction pathway involving phospholipase C-mediated endoplasmic reticulum release of Ca^{2+} and protein kinase Cδ activation. Active protein kinase Cδ promotes, by phosphorylation, the stabilization of p35, a cyclin-dependent kinase-5 (Cdk5) cofactor. Active p35/Cdk5 complex in the cytosol phosphorylates Nrf2 at Thr(395), Ser(433) and Thr(439) that is sufficient to promote Nrf2 translocation to the nucleus and induce the expression of antioxidant genes. Furthermore, this Cdk5-Nrf2 transduction pathway boosts glutathione metabolism in astrocytes efficiently protecting closely spaced neurons against oxidative damage. Thus, intercellular communication through NMDAR couples neurotransmission with neuronal survival.
Cell Death Differ, 2015; 22

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IS RTP801/REDD1 INVOLVED IN TRNA PROCESSING?

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Introduction: RTP801/REDD1 is a stress responsive protein overexpressed in neurons of patients with neurodegenerative disorders such as Parkinson's and Huntington's diseases. Its main function is to inhibit the mTOR pathway, but, if this inactivation is sustained in time, it has a pro-apoptotic effect in differentiated cells like neurons. Nevertheless, RTP801 might have other functions not yet elucidated. In preliminary proteomic studies from our laboratory, RTP801 was found to interact with HSPC117 and DDX1, two proteins that are part of the *tRNA splicing ligase complex*, which performs the ligation of the tRNA fragments generated during splicing. Aims: Since alterations in tRNA metabolism have recently been associated to the development of some neurodegenerative diseases, we aimed to deeper study the relationship between RTP801 and these tRNA-processing enzymes. Results: Here, we confirm by immunoprecipitation that endogenous RTP801 interacts with the *tRNA splicing ligase complex,* concretely with DDX1, HSPC117 and CGI-99. We also observe changes in the intensity and cellular distribution of these tRNA-processing enzymes when we modify the levels of RTP801. Additionally, the maturation of intron-containing tRNAs is altered in the cortex of 2-month-old RTP801 knockout mice compared to wild-type. Finally, we also observe related alterations in hippocampal and striatal postmortem samples from patients with Alzheimer's and Huntington's diseases, where RTP801 is involved in the pathogenesis. Conclusions: These results suggest a novel role of RTP801 in tRNA processing, which must be further studied, as RTP801 could be a potential target to prevent altered tRNA metabolism in neurodegenerative diseases.

Pubmed:
34131105: Pérez-Sisqués L, Sancho-Balsells A, Solana-Balaguer J, Campoy-Campos G, Vives-Isern M, Soler-Palazón F, Anglada-Huguet M, López-Toledano MÁ, Mandelkow EM, Alberch J, Giralt A, Malagelada C
RTP801/REDD1 contributes to neuroinflammation severity and memory impairments in Alzheimer's disease.
RTP801/REDD1 is a stress-regulated protein whose upregulation is necessary and sufficient to trigger neuronal death. Its downregulation in Parkinson's and Huntington's disease models ameliorates the pathological phenotypes. In the context of Alzheimer's disease (AD), the coding gene for RTP801, DDIT4, is responsive to Aβ and modulates its cytotoxicity in vitro. Also, RTP801 mRNA levels are increased in AD patients' lymphocytes. However, the involvement of RTP801 in the pathophysiology of AD has not been yet tested. Here, we demonstrate that RTP801 levels are increased in postmortem hippocampal samples from AD patients. Interestingly, RTP801 protein levels correlated with both Braak and Thal stages of the disease and with GFAP expression. RTP801 levels are also upregulated in hippocampal synaptosomal fractions obtained from murine 5xFAD and rTg4510 mice models of the disease. A local RTP801 knockdown in the 5xFAD hippocampal neurons with shRNA-containing AAV particles ameliorates cognitive deficits in 7-month-old animals. Upon RTP801 silencing in the 5xFAD mice, no major changes were detected in hippocampal synaptic markers or spine density. Importantly, we found an unanticipated recovery of several gliosis hallmarks and inflammasome key proteins upon neuronal RTP801 downregulation in the 5xFAD mice. Altogether our results suggest that RTP801 could be a potential future target for theranostic studies since it could be a biomarker of neuroinflammation and neurotoxicity severity of the disease and, at the same time, a promising therapeutic target in the treatment of AD.
Cell Death Dis, 2021; 12
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Feinstein E, Williamson DL, Soto D, Gasull X, Alberch J, Malagelada C
RTP801 regulates motor cortex synaptic transmission and learning.
RTP801/REDD1 is a stress-regulated protein whose upregulation is necessary and sufficient to trigger neuronal death in in vitro and in vivo models of Parkinson's and Huntington's diseases and is up regulated in compromised neurons in human postmortem brains of both neurodegenerative disorders. Indeed, in both Parkinson's and Huntington's disease mouse models, RTP801 knockdown alleviates motor-learning deficits.
Exp Neurol, 2021; 342
35053183: Pérez-Sisqués L, Solana-Balaguer J, Campoy-Campos G, Martín-Flores N, Sancho-Balsells A, Vives-Isern M, Soler-Palazón F, Garcia-Forn M, Masana M, Alberch J, Pérez-Navarro E, Giralt A, Malagelada C
RTP801/REDD1 Is Involved in Neuroinflammation and Modulates Cognitive Dysfunction in Huntington's Disease.
RTP801/REDD1 is a stress-regulated protein whose levels are increased in several neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's diseases (HD). RTP801 downregulation ameliorates behavioral abnormalities in several mouse models of these disorders. In HD, RTP801 mediates mutant huntingtin (mhtt) toxicity in in vitro models and its levels are increased in human iPSCs, human postmortem putamen samples, and in striatal synaptosomes from mouse models of the disease. Here, we investigated the role of RTP801 in the hippocampal pathophysiology of HD. We found that RTP801 levels are increased in the hippocampus of HD patients in correlation with gliosis markers. Although RTP801 expression is not altered in the hippocampus of the R6/1 mouse model of HD, neuronal RTP801 silencing in the dorsal hippocampus with shRNA containing AAV particles ameliorates cognitive alterations. This recovery is associated with a partial rescue of synaptic markers and with a reduction in inflammatory events, especially microgliosis. Altogether, our results indicate that RTP801 could be a marker of hippocampal neuroinflammation in HD patients and a promising therapeutic target of the disease.
Biomolecules, 2021; 12

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ER MORPHOLOGY AND CA2+ HANDLING: UNRAVELING THEIR ROLE IN NEURODEGENERATION IN DROSOPHILA MODELS OF HSP

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Hereditary spastic paraplegias (HSPs) are axonopathies characterized by progressive lower limbs spasticity. Despite their genetic heterogeneity, morphological alterations of the Endoplasmic Reticulum (ER) appear as critical causative factors, as over 60% of patients carry mutations in genes encoding proteins influencing ER morphology (e.g., Atlastin-1, Reticulon-2, Spastin). Among ER functions, previous studies suggest that ER morphology alterations could affect Ca^{2+} homeostasis. Furthermore, there is increasing evidence of the involvement of ER-shaping proteins in axonal elongation. **Aims**. Our aim is to investigate the correlation between HSP-linked ER shaping defects and Ca^{2+} homeostasis, and to unravel its potential impact on neuronal development and degeneration. **Methods**. To elude genetic redundancy, we focused on *Drosophila* HSP orthologues. Noteworthy, the molecular Ca^{2+} toolkit is conserved in *Drosophila*, enabling imaging of Ca^{2+} dynamics in fly neurons. Previously generated fly lines were exploited to downregulate Rtnl1 (*Drosophila* orthologue of Reticulon-2) or atlastin (orthologue of Atlastin-1), and to express HSP-linked dominant-negative spastin mutation K467R selectively in neurons. **Results and Conclusions**. Confocal and EM analysis of larval brains confirmed characteristic alterations in ER morphology in the three models, i.e. an increase in long profiles ascribable to ER cisternae upon Rtnl1 downregulation or spastin (K467R) expression and ER fragmentation upon atlastin downregulation. Similarly, Ca^{2+} imaging experiments in dissociated larval neurons showed specific defects in ER Ca^{2+} handling. The results obtained suggest a defective pathway potentially relevant for HSP pathogenesis. Experiments aimed at assessing Ca^{2+} dynamics and neuronal morphology in live tissues/animals are ongoing.

Pubmed:

34440902: Redolfi N, García-Casas P, Fornetto C, Sonda S, Pizzo P, Pendin D
Lighting Up Ca Dynamics in Animal Models.
Calcium (Ca) signaling coordinates are crucial processes in brain physiology. Particularly, fundamental aspects of neuronal function such as synaptic transmission and neuronal plasticity are regulated by Ca, and neuronal survival itself relies on Ca-dependent cascades. Indeed, impaired Ca homeostasis has been reported in aging as well as in the onset and progression of neurodegeneration. Understanding the physiology of brain function and the key processes leading to its derangement is a core challenge for neuroscience. In this context, Ca imaging represents a powerful tool, effectively fostered by the continuous amelioration of Ca sensors in parallel with the improvement of imaging instrumentation. In this review, we explore the potentiality of the most used animal models employed for Ca imaging, highlighting their application in brain research to explore the pathogenesis of neurodegenerative diseases.
Cells, 2021; 10
34831093: Sonda S, Pendin D, Daga A
ER Morphology in the Pathogenesis of Hereditary Spastic Paraplegia.
The endoplasmic reticulum (ER) is the most abundant and widespread organelle in cells. Its peculiar membrane architecture, formed by an intricate network of tubules and cisternae, is critical to its multifaceted function. Regulation of ER morphology is coordinated by a few ER-specific membrane proteins and is thought to be particularly important in neurons, where organized ER membranes are found even in the most distant neurite terminals. Mutation of ER-shaping proteins has been implicated in the neurodegenerative disease hereditary spastic paraplegia (HSP). In this review we discuss the involvement of these proteins in the pathogenesis of HSP, focusing on the experimental evidence linking their molecular function to disease onset. Although the precise biochemical activity of some ER-related HSP proteins has been elucidated, the pathological mechanism underlying ER-linked HSP is still undetermined and needs to be further investigated.
Cells, 2021; 10


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BOARD NUMBER: S01-502

BEHIND SPORADIC ALS: A BIOPHYSICAL CHARACTERIZATION OF MOTOR NEURONS IN HEALTH AND DISEASE

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Rationale. Sporadic amyotrophic lateral sclerosis (sALS) is characterized by death of motor neurons due to accumulation of aggregates and perturbation of intracellular transport. The underlying causes of these changes remain yet mysterious. Although a fraction of cases are caused by inherited mutations, the majority are sporadic, and the risk increases with age. The cell interior is extremely crowded with organelles and macromolecular complexes. This intracellular molecular crowding crucially impacts molecular assembly and transport. We hypothesized that age-associated changes could perturb macromolecular crowding and participate in the pathogenesis of sALS. *Methods*. For the first time, we leverage genetically encoded multimeric nanoparticles (GEMs) to quantify macromolecular crowding in human iPSC-derived motor neurons. The diffusivity of tracer particles like GEMs is generally decreased by molecular crowding and increased by active cellular processes. *Results*. GEMs' diffusivity was different depending on the subcellular localization. We observed that GEMs' diffusivity along neurites is regulated by active processes such as microtubules dynamics, while it seems to sensitively depend on the mechanical compression state of the cell in the somata. Microtubule dynamics and compression state are perturbed in ALS. Notably, motor neurons from ALS patients did not show modified basal molecular crowding, but might be more sensitive to perturbations. We are now studying how molecular crowding impacts aggregation and transport of TDP-43, a protein found in ALS aggregates. *Conclusion*. We are performing the first high-throughput characterization of the biophysical properties of motor neurons to decipher the relationship between intracellular biophysical changes and initiation of sALS.

Pubmed:
32581239: Bonucci M, Kuperwasser N, Barbe S, Koka V, de Villeneuve D, Zhang C, Srivastava N, Jia X, Stokes MP, Bienaimé F, Verkarre V, Lopez JB, Jaulin F, Pontoglio M, Terzi F, Delaval B, Piel M, Pende M
mTOR and S6K1 drive polycystic kidney by the control of Afadin-dependent oriented cell division.
mTOR activation is essential and sufficient to cause polycystic kidneys in Tuberous Sclerosis Complex (TSC) and other genetic disorders. In disease models, a sharp increase of proliferation and cyst formation correlates with a dramatic loss of oriented cell division (OCD). We find that OCD distortion is intrinsically due to S6 kinase 1 (S6K1) activation. The concomitant loss of S6K1 in Tsc1-mutant mice restores OCD but does not decrease hyperproliferation, leading to non-cystic harmonious hyper growth of kidneys. Mass spectrometry-based phosphoproteomics for S6K1 substrates revealed Afadin, a known component of cell-cell junctions required to couple intercellular adhesions and cortical cues to spindle orientation. Afadin is directly phosphorylated by S6K1 and abnormally decorates the apical surface of Tsc1-mutant cells with E-cadherin and α-catenin. Our data reveal that S6K1 hyperactivity alters centrosome positioning in mitotic cells, affecting oriented cell division and promoting kidney cysts in conditions of mTOR hyperactivity.
Nat Commun, 2020; 11

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BOARD NUMBER: S01-503

CHARACTERIZATION OF THE MOLECULAR MECHANISMS UNDERLYING NEUROMUSCULAR JUNCTION DEFECTS AND CELL DEATH IN FUS AND SPORADIC ALS

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neurons (MNs) death in the spinal cord and brain, leading the loss of skeletal muscle mass. Previous data collected in my lab highlight an interesting aberrant crosstalk between the ALS-linked FUS protein and the RNA-binding protein HuD, leading to upregulation of HuD levels. As a consequence, some HuD targets such as the axonal proteins GAP43 and NRN1 are upregulated in FUS mutant MNs. Moreover, we have found NRN1-dependent aberrant increase in neurite branching and axonal outgrowth in these cells. The general aim of my PhD project is to assess whether such altered molecular circuitry, besides neurite alteration, can lead to neuromuscular junction (NMJ) disruption, with a particular focus on the involvement of HuD and GAP43. To recapitulate the NMJ circuit *in vitro* I am taking advantage of human induced pluripotent stem cells (hiPSCs) to obtain a neural-muscle model system by 2D co-cultures. We found that FUS-mutant MNs appear less able to establish NMJs with FUS-WT muscle despite the presence of a significant increase of the neurite network. Moreover, mutant MNs co-cultures show degeneration of both cellular components suggesting that this extensive neurite sprouting, as a part of a compensatory reinnervation process, could have a detrimental effect on neuromuscular endplate maturation, leading the axonal and motor unit degeneration. Interestingly, we observed similar phenotypes with FUS-WT, HuD overexpressing MNs, with implications for sporadic ALS.

Pubmed:
33958580: Tiago T, Hummel B, Morelli FF, Basile V, Vinet J, Galli V, Mediani L, Antoniani F, Pomella S, Cassandri M, Garone MG, Silvestri B, Cimino M, Cenacchi G, Costa R, Mouly V, Poser I, Yeger-Lotem E, Rosa A, Alberti S, Rota R, Ben-Zvi A, Sawarkar R, Carra S
Small heat-shock protein HSPB3 promotes myogenesis by regulating the lamin B receptor.
One of the critical events that regulates muscle cell differentiation is the replacement of the lamin B receptor (LBR)-tether with the lamin A/C (LMNA)-tether to remodel transcription and induce differentiation-specific genes. Here, we report that localization and activity of the LBR-tether are crucially dependent on the muscle-specific chaperone HSPB3 and that depletion of HSPB3 prevents muscle cell differentiation. We further show that HSPB3 binds to LBR in the nucleoplasm and maintains it in a dynamic state, thus promoting the transcription of myogenic genes, including the genes to remodel the extracellular matrix. Remarkably, HSPB3 overexpression alone is sufficient to induce the differentiation of two human muscle cell lines, LHCNM2 cells, and rhabdomyosarcoma cells. We also show that mutant R116P-HSPB3 from a myopathy patient with chromatin alterations and muscle fiber disorganization, forms nuclear aggregates that immobilize LBR. We find that R116P-HSPB3 is unable to induce myoblast differentiation and instead activates the unfolded protein response. We propose that HSPB3 is a specialized chaperone engaged in muscle cell differentiation and that dysfunctional HSPB3 causes neuromuscular disease by deregulating LBR.
Cell Death Dis, 2021; 12
34944548: Grassmann G, Miotto M, Di Rienzo L, Salaris F, Silvestri B, Zacco E, Rosa A, Tartaglia GG, Ruocco G, Milanetti E
A Computational Approach to Investigate TDP-43 RNA-Recognition Motif 2 C-Terminal Fragments Aggregation in Amyotrophic Lateral Sclerosis.
Many of the molecular mechanisms underlying the pathological aggregation of proteins observed in neurodegenerative diseases are still not fully understood. Among the aggregate-associated diseases, Amyotrophic Lateral Sclerosis (ALS) is of relevant importance. In fact, although understanding the processes that cause the disease is still an open challenge, its relationship with protein aggregation is widely known. In particular, human TDP-43, an RNA/DNA binding protein, is a major

component of the pathological cytoplasmic inclusions observed in ALS patients. Indeed, the deposition of the phosphorylated full-length TDP-43 in spinal cord cells has been widely studied. Moreover, it has also been shown that the brain cortex presents an accumulation of phosphorylated C-terminal fragments (CTFs). Even if it is debated whether the aggregation of CTFs represents a primary cause of ALS, it is a hallmark of TDP-43 related neurodegeneration in the brain. Here, we investigate the CTFs aggregation process, providing a computational model of interaction based on the evaluation of shape complementarity at the molecular interfaces. To this end, extensive Molecular Dynamics (MD) simulations were conducted for different types of protein fragments, with the aim of exploring the equilibrium conformations. Adopting a newly developed approach based on Zernike polynomials, able to find complementary regions in the molecular surface, we sampled a large set of solvent-exposed portions of CTFs structures as obtained from MD simulations. Our analysis proposes and assesses a set of possible association mechanisms between the CTFs, which could drive the aggregation process of the CTFs. To further evaluate the structural details of such associations, we perform molecular docking and additional MD simulations to propose possible complexes and assess their stability, focusing on complexes whose interacting regions are both characterized by a high shape complementarity and involve β3 and β5 strands at their interfaces.
Biomolecules, 2021; 11

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IDENTIFICATION OF THE GHRELIN AND CANNABINOID CB2 RECEPTOR HETEROMER FUNCTIONALITY AND MARKED UPREGULATION IN STRIATAL NEURONS FROM OFFSPRING OF MICE UNDER A HIGH-FAT DIET.

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Cannabinoids have been reported as orexigenic, i.e. as promoting food intake that, among others, is controlled by the so-called “hunger” hormone, ghrelin. The aim of this project focus in looking for functional and/or molecular interactions between ghrelin GHS-R1a and cannabinoid CB_2 receptors at the central nervous system (CNS) level. In a heterologous system we identified CB_2-GHS-R1a receptor complexes with a particular heteromer print consisting of impairment blockade of CB_2 receptor/Gi-mediated signaling. The blockade was due to allosteric interactions within the heteromeric complex as it was reverted by antagonists of the GHS-R1a receptor. On the other hand, cannabinoids acting on the CB_2 receptor did not affect cytosolic increases of calcium ion induced by ghrelin acting on the GHS-R1a receptor. Finally, in situ proximity ligation imaging assays confirmed the expression of CB_2-GHS-R1a receptor complexes in both heterologous cells and primary striatal neurons. Interestingly, there was a marked upregulation of those complexes in striatal neurons from siblings of pregnant female mice under a high-fat diet.

Pubmed:
34445634: Lillo J, Lillo A, Zafra DA, Miralpeix C, Rivas-Santisteban R, Casals N, Navarro G, Franco R
Identification of the Ghrelin and Cannabinoid CB Receptor Heteromer Functionality and Marked Upregulation in Striatal Neurons from Offspring of Mice under a High-Fat Diet.
Cannabinoids have been reported as orexigenic, i.e., as promoting food intake that, among others, is controlled by the so-called "hunger" hormone, ghrelin. The aim of this paper was to look for functional and/or molecular interactions between ghrelin GHSR1a and cannabinoid CB receptors at the central nervous system (CNS) level. In a heterologous system we identified CB-GHSR1a receptor complexes with a particular heteromer print consisting of impairment of CB receptor/G-mediated signaling. The blockade was due to allosteric interactions within the heteromeric complex as it was reverted by antagonists of the GHSR1a receptor. Cannabinoids acting on the CB receptor did not affect cytosolic increases of calcium ions induced by ghrelin acting on the GHSR1a receptor. In situ proximity ligation imaging assays confirmed the expression of CB-GHSR1a receptor complexes in both heterologous cells and primary striatal neurons. We tested heteromer expression in neurons from offspring of high-fat-diet mouse mothers as they have more risk to be obese. Interestingly, there was a marked upregulation of those complexes in striatal neurons from siblings of pregnant female mice under a high-fat diet.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S01-505

BRAIN DYSFUNCTION INDUCED BY CHRONIC KIDNEY DISEASE

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Chronic kidney disease (CKD) is defined by a reduced filtration of the kidney. 20% of CKD patients develop a mild cognitive impairment (MCI) via unclear mechanisms that are investigated here. Brains of 5/6 nephrectomized mice (CKD model) and controls (n=8 per group) were stained for Nissl, Cytochrome Oxidase, Acetylcholine Esterase (AChE), Tyrosine-hydroxylase (TH), and DARPP32. The cerebral cortex, caudate-putamen, hippocampus, and cerebellum showed a normal cytoarchitecture and minimal changes in brain metabolic activity. The striatum demonstrated modified AChE activity and altered TH and DARPP32 expression. The extracellular spaces surrounding cortical interneurons (WFA staining) were reduced in CKD animals. To translate these data to patients, we analyzed retrospectively 313 patients (controls:139, CKD:79, kidney transplant (Tx):35, hemodialysis (HD):60). The Cognitive function (MoCA test) was reduced in CKD, Tx, and HD groups, inversely correlated with proteinuria, and directly correlated with uric acid levels and inflammatory scores in kidney biopsies. Sleepiness (ESS score) was increased in the HD group, whereas the chronotype was not significantly different among groups. The frequency spectrum of the hand tremor (accelerometer-measure) was altered in Tx and HD groups compared to controls and correlated with age and with BUN.
The extracellular space analyzed on MR-DTI sequences was reduced in patients with MCI compared to controls. In summary, reduced kidney function leads to functional and morphological changes in the brain of animals and patients. The altered water balance and the inflammatory state present in CKD are also relevant for brain dysfunction. This project was developed within the COST ACTION CA19127 "CONNECT".

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BOARD NUMBER: S01-506

THE IN VIVO ROLE OF M6A RNA MODIFICATION IN THE DROSOPHILA BRAIN DURING CELLULAR STRESS AND AGING

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Emerging evidence suggests that *N*^{6}-methyladenosine (m^{6}A), the most prevalent internal modification on eukaryotic mRNA, plays an essential role in various stress responses by regulating RNA processing. The brain is uniquely vulnerable to cellular stress, thus defining how m^{6}A sculpts the brain's stress susceptibility may provide insight into the vulnerability of the brain to age- and disease-related stress. The catalytic component of the methyltransferase complex that adds m^{6}A is *Mettl3*. Here we investigated the regulation and impact of *Mettl3*-dependent m^{6}A mRNA methylation in the adult *Drosophila* brain *in vivo* under normal conditions and stress, age, and in various situations of brain deterioration. Our data suggest a complex brain enriched response, and dynamic changes in m^{6}A levels, that are dependent on the methyltransferase complex component *Mettl3*. Through m^{6}A-immunoprecipitation sequencing, we show that 5'UTR *Mettl3*-dependent m^{6}A is enriched in transcripts of neuronal processes and dynamic signaling pathways, and marks a previously unrecognized class of genes that increase upon stress in the brain. *Mettl3* knockdown results in a brain-specific increase in protein and mRNA levels of *Mettl3*-dependent m^{6}A targets and, surprisingly, confers resilience to stress. We find that knockdown of the nuclear m^{6}A reader protein *Ythdc1* in the brain also results in increased resilience to stress. This work highlights that m^{6}A modification in the fly brain serves to dampen the acute stress response yet confers dynamic and brain-specific regulation on brain enriched genes, fine-tuning RNA levels and translation of critical transcripts involved in cell signaling, neuronal homeostasis, and the response to cellular stress.

Pubmed:
34077532: McGurk L, Rifai OM, Shcherbakova O, Perlegos AE, Byrns CN, Carranza FR, Zhou HW, Kim HJ, Zhu Y, Bonini NM
Toxicity of pathogenic ataxin-2 in Drosophila shows dependence on a pure CAG repeat sequence.
Spinocerebellar ataxia type 2 is a polyglutamine (polyQ) disease associated with an expanded polyQ domain within the protein product of the ATXN2 gene. Interestingly, polyQ repeat expansions in ATXN2 are also associated with amyotrophic lateral sclerosis (ALS) and parkinsonism depending upon the length of the polyQ repeat expansion. The sequence encoding the polyQ repeat also varies with disease presentation: a pure CAG repeat is associated with SCA2, whereas the CAG repeat in ALS and parkinsonism is typically interrupted with the glutamine encoding CAA codon. Here, we asked if the purity of the CAG sequence encoding the polyQ repeat in ATXN2 could impact the toxicity of the ataxin-2 protein in vivo in Drosophila. We found that ataxin-2 encoded by a pure CAG repeat conferred toxicity in the retina and nervous system, whereas ataxin-2 encoded by a CAA-interrupted repeat or CAA-only repeat failed to confer toxicity, despite expression of the protein at similar levels. Furthermore, the CAG-encoded ataxin-2 protein aggregated in the fly eye, while ataxin-2 encoded by either a CAA/G or CAA repeat remained diffuse. The toxicity of the CAG-encoded ataxin-2 protein was also sensitive to the translation factor eIF4H, a known modifier of the toxic GGGGCC repeat in flies. These data indicate that ataxin-2 encoded by a pure CAG versus interrupted CAA/G polyQ repeat domain is associated with differential toxicity, indicating that mechanisms associated with the purity of the sequence of the polyQ domain contribute to disease.
Hum Mol Genet, 2021; 30

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BOARD NUMBER: S01-507

UNEXPECTED LANDSCAPES OF MITOCHONDRIAL DESTRUCTION IN THE AGING BRAIN

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Anna Rappe, Homa Ehsan, Fumi Suomi and Thomas G. McWilliams **Aims:** Mitochondrial damage is neutralised by mitophagy, thereby preventing cellular dysfunction and apoptosis. The selective autophagy of mitochondria (mitophagy) has long been linked to age-dependent neurodegenerative disorders such as Parkinson's and Alzheimer's disease. A prodigious body of work has studied mitophagy in cell-based systems and short-lived model organisms, yet how basal mitophagy proceeds in the aging mammalian brain remains unexplored. Our aim was to profile *in vivo* mitophagy in the aging mammalian brain. **Methods:** We analysed physiological mitophagy in neural tissues from distinct longitudinal cohorts of mitophagy reporter mice, using high-resolution confocal microscopy and quantitative cell biology. **Results:** We report widespread and surprising changes in mitophagy in the mammalian nervous system during progressive aging. Longitudinal quantitative profiling of mitophagy reporter mice cohorts from young (3 months) to geriatric (>26 months) revealed striking cell and tissue-specific alterations in mitochondrial turnover between distinct populations of neurons and non-neuronal cells. **Conclusions:** Our provocative findings establish a new and unexpected landscape of *in vivo* mitophagy through neural space and time. This comprehensive resource will refine therapeutic efforts to target age-mitochondrial dysfunction in the mammalian brain.

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ENERGETIC FAILURE IS RESPONSIBLE FOR HEREDITARY NEURODEGENERATIVE DISORDERS DUE TO MUTATIONS IN THE HEME EXPORTER FLVCR1

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Feline Leukemia Virus subgroup C Receptor 1 (FLVCR1) is a ubiquitously expressed heme exporter. FLVCR1 mutations cause Posterior Column Ataxia and Retinitis Pigmentosa (PCARP), non-syndromic retinitis pigmentosa (RP) and Hereditary Sensory and Autonomic Neuropathy (HSAN). These disorders are characterized by the progressive degeneration of photoreceptors and/or sensory neurons, resulting in progressive vision loss, sensory ataxia and/or pain insensitivity. The reason why photoreceptors and sensory neurons are particularly sensitive to the disruption of heme export is still unknown. We recently reported that heme export through FLVCR1 is required to sustain endogenous heme synthesis. Heme is a crucial cofactor of several proteins, Electron Transport Chain (ETC) complexes included. To investigate whether *FLVCR1* mutations affect energetic metabolism, we analyzed patient-derived primary fibroblasts. We showed that *FLVCR1* mutations result in impaired TCA cycle flux, ETC activity and ATP production resulting in oxidative stress and lipid peroxidation. The observed alterations were completely rescued by restoring the heme synthesis-export axis. Indeed, the treatment of patients' fibroblasts with ALA, to stimulate endogenous heme synthesis, and hemopexin, to promote heme export, completely restored energetic metabolism and lipid peroxidation in patients' fibroblasts. These data suggest that energetic failure is responsible for diseases due to *FLVCR1* mutations. Furthermore, these results also demonstrate the possibility to improve the function of the mutated transporter, at least *in vitro*. Further studies in animal models of the disease are required to fully understand the contribution of energetic metabolism to the progressive degeneration of the retina and sensory neurons.

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BOARD NUMBER: S01-509

MORPHO-FUNCTIONAL ALTERATIONS IN EPITHELIAL CELLS OF THE CHOROID PLEXUS DURING AGING

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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The choroid plexus (ChP), a specialized vascular-epithelial structure located in the brain ventricular system, makes a selective interface between blood and cerebrospinal fluid (CSF), thus regulating brain homeostasis. It has been postulated that age-associated neurodegenerative diseases can be correlated with alterations of ChP function. Here we examined the ultrastructure of the ChP in adult (P60) and aging (P600) mice using electron microscopy. We identified different aging-related changes, including a reduction of epithelial cell height, a decrease in the area covered by apical microvilli, and a structural modification of tight junctions. We also found a redistribution of mitochondria along the basal-apical cell axis and a significant increase in the density of elongated mitochondria in old mouse ChP. Interestingly, 2-photon (2PM) imaging of whole ChP living explants and mitochondria tracking analysis revealed that mitochondria mainly show a wiggling movement, a feature that was rather constant throughout lifetime. Our data demonstrate that the choroid epithelium undergoes multiple structural alterations during aging. In particular, changes in the shape of mitochondria could correlate with increased vulnerability to metabolic stress.

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BOARD NUMBER: S01-510

SMALL MOLECULE FTO INHIBITOR AMELIORATES NEURONAL SENESCENCE

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

Denise Greco[1], Barbora Černá[1], Petra Pohanová[1], Petra Tabáková[1], Zuzana Čočková[1], Karel Jezek[2], Jitka Kuncová[2], Jan Jedlička[2], Stephanie Proskauer-Peña[2], Annu Kala[2], Mark J. Olsen[3], Jiří Novotný[1], Petr Telenský[1,4]
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Fat mass and obesity associated protein (FTO) is an epitranscriptomic eraser that demethylases N6-methyladenosine (m6A), the most abundant mRNA modification in eukaryotes. FTO is upregulated in brains of aged mice and aberrant m6A enrichment patterns can be present in senescence-associated transcripts. However, the role of FTO in neuronal senescence remains elusive. Here we used tert-butyl hydroperoxidase (TBHP) to induce senescence-like state in retinoic acid-differentiated SH-SY5Y neuronal cells. The role of FTO was studied using the novel small molecule inhibitor MO-I-500. We investigated cellular respiration (SeaHorse XF Mito Stress Test), ATP (ATP Bioluminescence Assay kit, Roche) and ROS (MitoSOX™ Red mitochondrial superoxide indicator) levels, β-galactosidase activity at pH 6 (Senescence Cells Histochemical Staining kit, Sigma-Aldrich) and markers of senescence-associated secretory phenotype (Western blotting). We show that FTO inhibition ameliorated the senescence-like phenotype induced by TBHP treatment. To assess whether FTO inhibition may ameliorate neural senescence in animal model, we administered 5 daily doses of 10 mg/kg MO-I-500 to 1 year old F344 rats. Treated rats displayed improved spatial memory acquisition in Active Allothetic Place Avoidance compared to aged-matched vehicle controls. High-resolution respirometry (OxyGraph, Oroboros) from ex vivo hippocampal samples demonstrated moderately increased rate of cellular respiration in the treated group. Overall, our data demonstrate that FTO inhibition might constitute a -novel therapy in the treatment of neuronal senescence in aging and age-related disease. This work was supported by Czech Science Foundation (GAČR) grant 16–12420Y, PRIMUS grant PRIMUS/SCI/33 and the European Regional Development Fund –Project ENOCH (No. CZ. 02. 1.01/0.0./0.0/16_019/0000868).

FENS Forum 2022
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BOARD NUMBER: S01-511

ALTERATIONS IN PACEMAKER CHANNEL'S MODULATIONS OF THALAMIC RELAY NEURONS BY DEMYELINATION DUE TO CPZ TREATMENT AND NEUROPROTECTIVE EFFECTS OF DRF

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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De- and remyelination processes caused by cuprizone (CPZ) treatment in animals mimic the relapsing forms of multiple sclerosis (MS) patients. In the CPZ model, progressive loss of oligodendrocytes and myelin in the brain is heavily associated with thalamic dysfunction – expressed by changes in burst activity in thalamic neurons. Since CPZ feeding alters both distribution and expression of different ion channels along the neuronal somata, we aimed to investigate the effects of CPZ treatment on the pacemaker of thalamic burst activity, the hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels in acute brain slices both during de- and remyelination periods. In addition, we investigated whether the alterations in HCN channel properties may be reversed by diroximel fumarate (DRF), an approved oral drug for the treatment of relapsing forms of MS. C57BL/6 mice received 0.2% CPZ diet for 35 days, after which CPZ was substituted with standard rodent chow. Whole-cell patch clamp recording of thalamic relay neurons were performed after Day1, Day7 and Day25 starting from CPZ removal, corresponding to different states of axonal myelination. Measured I_h properties showed that the alterations of HCN channels depend on the state of the remyelination processes. In thalamic neurons, full demyelination on Day1 was associated with a decrease in I_h current density. On Day7, we found a transient maximum in current density before gradually reaching control values over the course of complete remyelination on Day25. Application of physiological relevant concentrations of DRF reversed alterations of I_h properties back to control values, indicating the neuroprotective effect of DRF.

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BOARD NUMBER: S01-512

ACTIVATION OF THE HEPCIDIN-FERROPORTIN1 PATHWAY IN THE BRAIN AND ASTROCYTIC-NEURONAL CROSSTALK TO COUNTERACT IRON DYSHOMEOSTASIS DURING AGING

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Aims: During aging, iron accumulates in brain regions particularly vulnerable to neurodegeneration: the cerebral cortex and the hippocampus. However, knowledge on the mechanisms of iron regulation, together with its cellular distribution within neuronal tissue, remains scarce. Methods: We studied brain iron metabolism in C57BL/6 mice by Prussian blue Perl's staining for nonheme iron content, by Real-time quantitative PCR and Immunoblotting for genes and proteins related to iron storage and transport. Moreover, we performed Immunofluorescence in order to localize iron transporters and deposits within the nervous tissues and neuroinflammation. Results: We observed an age-dependent accumulation of iron in the cerebral cortex and hippocampus, that triggers neuroinflammation and oxidative stress measured by genes and markers of activated astrocytes and microglia. In old mice brain we found also alteration of the BBB integrity and we demonstrated the role of the Hepcidin/Ferroportin1 pathway during aging. Hepcidin, the major regulator of iron content and availability, interacts with the iron exporter Ferroportin1, causing its degradation and iron retention within the cell. Moreover, we observed NCOA4-dependent ferritinophagy of ferritin heavy-chain isoforms determining the increase of light-chain enriched ferritin heteropolymers that are more efficient as iron chelators. Furthermore, we demonstrated that both in the cerebral cortex and hippocampus Ferroportin1 colocalizes with astrocytes, while iron deposits with neurons. Conclusions: These data highlight the involvement of the Hepcidin/Ferroportin1 axis during mice aging as a response to a higher brain iron influx. This, represents the starting point to clarify iron metabolism in the brain, a typical feature of several neurodegenerative disorders.

Pubmed:
33551720: Contino S, Suelves N, Vrancx C, Vadukul DM, Payen VL, Stanga S, Bertrand L, Kienlen-Campard P
Presenilin-Deficient Neurons and Astrocytes Display Normal Mitochondrial Phenotypes.
Presenilin 1 (PS1) and Presenilin 2 (PS2) are predominantly known as the catalytic subunits of the γ-secretase complex that generates the amyloid-β (Aβ) peptide, the major constituent of the senile plaques found in the brain of Alzheimer's disease (AD) patients. Apart from their role in γ-secretase activity, a growing number of cellular functions have been recently attributed to PSs. Notably, PSs were found to be enriched in mitochondria-associated membranes (MAMs) where mitochondria and endoplasmic reticulum (ER) interact. PS2 was more specifically reported to regulate calcium shuttling between these two organelles by controlling the formation of functional MAMs. We have previously demonstrated in mouse embryonic fibroblasts (MEF) an altered mitochondrial morphology along with reduced mitochondrial respiration and increased glycolysis in PS2-deficient cells (PS2KO). This phenotype was restored by the stable re-expression of human PS2. Still, all these results were obtained in immortalized cells, and one bottom-line question is to know whether these observations hold true in central nervous system (CNS) cells. To that end, we carried out primary cultures of PS1 knockdown (KD), PS2KO, and PS1KD/PS2KO (PSdKO) neurons and astrocytes. They were obtained from the same litter by crossing PS2 heterozygous; PS1 floxed (PS2; PS1) animals. Genetic downregulation of PS1 was achieved by lentiviral expression of the Cre recombinase in primary cultures. Strikingly, we did not observe any mitochondrial phenotype in PS1KD, PS2KO, or PSdKO primary cultures in basal conditions. Mitochondrial respiration and membrane potential were similar in all models, as were the glycolytic flux and NAD/NADH ratio. Likewise, mitochondrial morphology and content was unaltered by PS expression. We further investigated the differences between results we obtained here in primary nerve cells and those previously reported in MEF cell lines by analyzing PS2KO primary fibroblasts. We found no mitochondrial dysfunction in this model, in line with observations in PS2KO primary neurons and astrocytes. Together, our results indicate that the mitochondrial phenotype observed in immortalized PS2-deficient cell lines cannot be extrapolated to primary neurons, astrocytes, and even to primary fibroblasts. The PS-dependent mitochondrial phenotype reported so far might therefore be the consequence of a cell immortalization process and should be critically reconsidered regarding its relevance to AD.
Front Neurosci, 2020; 14

33374485: Stanga S, Boido M, Kienlen-Campard P
How to Build and to Protect the Neuromuscular Junction: The Role of the Glial Cell Line-Derived Neurotrophic Factor.
The neuromuscular junction (NMJ) is at the crossroad between the nervous system (NS) and the muscle. Following neurotransmitter release from the motor neurons (MNs), muscle contraction occurs and movement is generated. Besides eliciting muscle contraction, the NMJ represents a site of chemical bidirectional interplay between nerve and muscle with the active participation of Schwann cells. Indeed, signals originating from the muscle play an important role in synapse formation, stabilization, maintenance and function, both in development and adulthood. We focus here on the contribution of the Glial cell line-Derived Neurotrophic Factor (GDNF) to these processes and to its potential role in the protection of the NMJ during neurodegeneration. Historically related to the maintenance and survival of dopaminergic neurons of the , GDNF also plays a fundamental role in the peripheral NS (PNS). At this level, it promotes muscle trophism and it participates to the functionality of synapses. Moreover, compared to the other neurotrophic factors, GDNF shows unique peculiarities, which make its contribution essential in neurodegenerative disorders. While describing the known structural and functional changes occurring at the NMJ during neurodegeneration, we highlight the role of GDNF in the NMJ-muscle cross-talk and we review its therapeutic potential in counteracting the degenerative process occurring in the PNS in progressive and severe diseases such as Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA). We also describe functional 3D neuromuscular co-culture systems that have been recently developed as a model for studying both NMJ formation in vitro and its involvement in neuromuscular disorders.
Int J Mol Sci, 2020; 22
32466216: Stanga S, Caretto A, Boido M, Vercelli A
Mitochondrial Dysfunctions: A Red Thread across Neurodegenerative Diseases.
Mitochondria play a central role in a plethora of processes related to the maintenance of cellular homeostasis and genomic integrity. They contribute to preserving the optimal functioning of cells and protecting them from potential DNA damage which could result in mutations and disease. However, perturbations of the system due to senescence or environmental factors induce alterations of the physiological balance and lead to the impairment of mitochondrial functions. After the description of the crucial roles of mitochondria for cell survival and activity, the core of this review focuses on the "mitochondrial switch" which occurs at the onset of neuronal degeneration. We dissect the pathways related to mitochondrial dysfunctions which are shared among the most frequent or disabling neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's, Amyotrophic Lateral Sclerosis, and Spinal Muscular Atrophy. Can mitochondrial dysfunctions (affecting their morphology and activities) represent the early event eliciting the shift towards pathological neurobiological processes? Can mitochondria represent a common target against neurodegeneration? We also review here the drugs that target mitochondria in neurodegenerative diseases.
Int J Mol Sci, 2020; 21
32327470: Opsomer R, Contino S, Perrin F, Gualdani R, Tasiaux B, Doyen P, Vergouts M, Vrancx C, Doshina A, Pierrot N, Octave JN, Gailly P, Stanga S, Kienlen-Campard P
Amyloid Precursor Protein (APP) Controls the Expression of the Transcriptional Activator Neuronal PAS Domain Protein 4 (NPAS4) and Synaptic GABA Release.
The amyloid precursor protein (APP) has been extensively studied as the precursor of the β-amyloid (Aβ) peptide, the major component of the senile plaques found in the brain of Alzheimer's disease (AD) patients. However, the function of APP per se in neuronal physiology remains to be fully elucidated. APP is expressed at high levels in the brain. It resembles a cell adhesion molecule or a membrane receptor, suggesting that its function relies on cell-cell interaction and/or activation of intracellular signaling pathways. In this respect, the APP intracellular domain (AICD) was reported to act as a transcriptional regulator. Here, we used a transcriptome-based approach to identify the genes transcriptionally regulated by APP in the rodent embryonic cortex and on maturation of primary cortical neurons. Surprisingly, the overall transcriptional changes were subtle, but a more detailed analysis pointed to genes clustered in neuronal-activity dependent pathways. In particular, we observed a decreased transcription of neuronal PAS domain protein 4 (NPAS4) in APP-/- neurons. NPAS4 is an inducible transcription factor (ITF) regulated by neuronal depolarization. The downregulation of NPAS4 co-occurs with an increased production of the inhibitory neurotransmitter GABA and a reduced expression of the GABA receptors α1. CRISPR-Cas-mediated silencing of NPAS4 in neurons led to similar observations. Patch-clamp investigation did not reveal any functional decrease of GABA receptors activity, but long-term potentiation (LTP) measurement supported an increased GABA component in synaptic transmission of APP-/- mice. Together, NPAS4 appears to be a downstream target involved in APP-dependent regulation of inhibitory synaptic transmission.
eNeuro, 2020 May/Jun; 7
29899726: Stanga S, Brambilla L, Tasiaux B, Dang AH, Ivanoiu A, Octave JN, Rossi D, van Pesch V, Kienlen-Campard P
A Role for GDNF and Soluble APP as Biomarkers of Amyotrophic Lateral Sclerosis Pathophysiology.
The current inability of clinical criteria to accurately identify the "at-risk group" for Amyotrophic Lateral Sclerosis (ALS)

development as well as its unknown etiology are fueling the interest in biomarkers aimed at completing clinical approaches for the diagnosis. The Glial cell line-derived neurotrophic factor (GDNF) is a diffusible peptide critically involved in neuronal differentiation and survival. GDNF is largely studied in various neurological and neuromuscular diseases, with a great interest in the peripheral nervous system (PNS). The recent discovery of Amyloid Precursor Protein (APP)-dependent GDNF regulation driving neuro-muscular junctions' formation in APP null transgenic mice, prompts to study whether neurodegeneration relies on loss or gain of APP function and suggests that it could affect peripheral processes. Here, we explored a brand-new aspect of the loss of trophic support in ALS by measuring GDNF, APP, soluble APP fragments and Aβ peptides levels in SOD1 or SOD1 transgenic mouse models of ALS and in human biological fluids [i.e. serum and cerebrospinal fluid (CSF)] from ALS patients and control subjects. Our results show that both GDNF and soluble APP fragments levels are altered at the onset of motor deficits in mice and that their levels are also modified in patient samples. This study indicates that both GDNF and soluble APPα represent possible biomarkers for ALS.
Front Neurol, 2018; 9

29085303: Contino S, Porporato PE, Bird M, Marinangeli C, Opsomer R, Sonveaux P, Bontemps F, Dewachter I, Octave JN, Bertrand L, Stanga S, Kienlen-Campard P
Presenilin 2-Dependent Maintenance of Mitochondrial Oxidative Capacity and Morphology.
Mitochondrial dysfunction plays a pivotal role in the progression of Alzheimer's disease (AD), and yet the mechanisms underlying the impairment of mitochondrial function in AD remain elusive. Recent evidence suggested a role for Presenilins (PS1 or PS2) in mitochondrial function. Mutations of PSs, the catalytic subunits of the γ-secretase complex, are responsible for the majority of inherited AD cases (FAD). PSs were shown to be present in mitochondria and particularly enriched in mitochondria-associated membranes (MAM), where PS2 is involved in the calcium shuttling between mitochondria and the endoplasmic reticulum (ER). We investigated the precise contribution of PS1 and PS2 to the bioenergetics of the cell and to mitochondrial morphology in cell lines derived from wild type (PS+/+), PS1/2 double knock-out (PSdKO), PS2KO and PS1KO embryos. Our results showed a significant impairment in the respiratory capacity of PSdKO and PS2KO cells with reduction of basal oxygen consumption, oxygen utilization dedicated to ATP production and spare respiratory capacity. In line with these functional defects, we found a decrease in the expression of subunits responsible for mitochondrial oxidative phosphorylation (OXPHOS) associated with an altered morphology of the mitochondrial cristae. This OXPHOS disruption was accompanied by a reduction of the NAD/NADH ratio. Still, neither ADP/ATP ratio nor mitochondrial membrane potential (ΔΨ) were affected, suggesting the existence of a compensatory mechanism for energetic balance. We observed indeed an increase in glycolytic flux in PSdKO and PS2KO cells. All these effects were truly dependent on PS2 since its stable re-expression in a PS2KO background led to a complete restoration of the parameters impaired in the absence of PS2. Our data clearly demonstrate here the crucial role of PS2 in mitochondrial function and cellular bioenergetics, pointing toward its peculiar role in the formation and integrity of the electron transport chain.
Front Physiol, 2017; 8

28994238: Stanga S, Vrancx C, Tasiaux B, Marinangeli C, Karlström H, Kienlen-Campard P
Specificity of presenilin-1- and presenilin-2-dependent γ-secretases towards substrate processing.
The two presenilin-1 (PS1) and presenilin-2 (PS2) homologs are the catalytic core of the γ-secretase complex, which has a major role in cell fate decision and Alzheimer's disease (AD) progression. Understanding the precise contribution of PS1- and PS2-dependent γ-secretases to the production of β-amyloid peptide (Aβ) from amyloid precursor protein (APP) remains an important challenge to design molecules efficiently modulating Aβ release without affecting the processing of other γ-secretase substrates. To that end, we studied PS1- and PS2-dependent substrate processing in murine cells lacking presenilins (PSs) (PS1KO, PS2KO or PS1-PS2 double-KO noted PSdKO) or stably re-expressing human PS1 or PS2 in an endogenous PS-null (PSdKO) background. We characterized the processing of APP and Notch on both endogenous and exogenous substrates, and we investigated the effect of pharmacological inhibitors targeting the PSs activity (DAPT and L-685,458). We found that murine PS1 γ-secretase plays a predominant role in APP and Notch processing when compared to murine PS2 γ-secretase. The inhibitors blocked more efficiently murine PS2- than murine PS1-dependent processing. Human PSs, especially human PS1, expression in a PS-null background efficiently restored APP and Notch processing. Strikingly, and contrary to the results obtained on murine PSs, pharmacological inhibitors appear to preferentially target human PS1- than human PS2-dependent γ-secretase activity.
J Cell Mol Med, 2018; 22

26718890: Stanga S, Zanou N, Audouard E, Tasiaux B, Contino S, Vandermeulen G, René F, Loeffler JP, Clotman F, Gailly P, Dewachter I, Octave JN, Kienlen-Campard P
APP-dependent glial cell line-derived neurotrophic factor gene expression drives neuromuscular junction formation.
Besides its crucial role in the pathogenesis of Alzheimer's disease, the knowledge of amyloid precursor protein (APP) physiologic functions remains surprisingly scarce. Here, we show that APP regulates the transcription of the glial cell line-derived neurotrophic factor (GDNF). APP-dependent regulation of GDNF expression affects muscle strength, muscular

trophy, and both neuronal and muscular differentiation fundamental for neuromuscular junction (NMJ) maturation in vivo In a nerve-muscle coculture model set up to modelize NMJ formation in vitro, silencing of muscular APP induces a 30% decrease in secreted GDNF levels and a 40% decrease in the total number of NMJs together with a significant reduction in the density of acetylcholine vesicles at the presynaptic site and in neuronal maturation. These defects are rescued by GDNF expression in muscle cells in the conditions where muscular APP has been previously silenced. Expression of GDNF in muscles of amyloid precursor protein null mice corrected the aberrant synaptic morphology of NMJs. Our findings highlight for the first time that APP-dependent GDNF expression drives the process of NMJ formation, providing new insights into the link between APP gene regulatory network and physiologic functions.-Stanga, S., Zanou, N., Audouard, E., Tasiaux, B., Contino, S., Vandermeulen, G., René, F., Loeffler, J.-P., Clotman, F., Gailly, P., Dewachter, I., Octave, J.-N., Kienlen-Campard, P. APP-dependent glial cell line-derived neurotrophic factor gene expression drives neuromuscular junction formation.
FASEB J, 2016; 30

22746246: Stanga S, Lanni C, Sinforiani E, Mazzini G, Racchi M
Searching for predictive blood biomarkers: misfolded p53 in mild cognitive impairment.
The identification and validation of biomarkers for preclinical patients with mild cognitive impairment (MCI) at-risk for Alzheimer's disease (AD) development is increasingly important. We used the cytofluorimetric analysis of unfolded p53 to determine the prognostic ability of the protein as predictive signature from MCI to AD in a longitudinal study of a population of presymptomatic patients with the clinical diagnosis of MCI. Venous blood samples from 24 healthy subjects, 28 MCI and 15 AD were analyzed with the cytofluorimetric method for unfolded p53 protein detection. Twenty-four MCI patients had clinical follow-up subsequent to the analysis for unfolded p53. Elevated levels of the conformationally altered protein were able to discriminate both MCI and AD patients comparing with healthy subjects. Longitudinal follow-up revealed that 7/24 MCI patients progressed to AD. All converters (100%) were predicted by elevated levels of unfolded p53, with a positive predictive value of 87.5%. These data support and extend our previous observation that the cytofluorimetric approach for unfolded p53 protein was able to discriminate AD patients from healthy subjects and to predict the progression from MCI to AD in presymptomatic patients before clinical diagnosis for AD was evident.
Curr Alzheimer Res, 2012; 9

20876941: Stanga S, Lanni C, Govoni S, Uberti D, D'Orazi G, Racchi M
Unfolded p53 in the pathogenesis of Alzheimer's disease: is HIPK2 the link?
p53 transcriptional activity depends mainly on posttranslational modifications and protein/protein interaction. Another important mechanism that controls p53 function is its conformational stability since p53 is an intrinsically unstable protein. An altered conformational state of p53, independent from point mutations, has been reported in tissues from patients with Alzheimer's disease (AD), leading to an impaired and dysfunctional response to stressors. Recent evidence shows that one of the activators that induces p53 posttranslational modification and wild-type conformational stability is homeodomain interacting protein kinase 2 (HIPK2). Hence, conditions that induce HIPK2 deregulation would result in a dysfunctional response to stressors by affecting p53 activity. Discovering the mechanisms of HIPK2 activation/inhibition and the ways to manipulate HIPK2 activity are an interesting option to affect several biological pathways, including those underlying AD. Soluble beta-amyloid peptides have recently been involved in HIPK2 degradation, in turn regulating the p53 conformational state and vulnerability to a noxious stimulus, before triggering the amyloidogenic cascade. Here we discuss about these findings and the potential relevance of HIPK2 as a target for AD and highlight the existence of a novel amyloid-based mechanism in AD potentially leading to the survival of injured dysfunctional cells.
Aging (Albany NY), 2010; 2

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REGULATION OF NEURONAL AUTOPHAGY BY ENDOCYTIC KINASE AAK1

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Neuronal autophagy is regulated by a variety of cues, including the clathrin-mediated endocytosis (CME). Previously, we have shown that the CME adaptor protein complex 2 (AP-2) promotes autophagosome transport in neurons. How precisely AP-2 is recruited to autophagosomes is currently unknown. Association of AP-2 with membranes is regulated by the phosphorylation of its µ2 subunit by the Ser/Thr kinase adaptor-associated kinase-1 (AAK1). Here we identify AAK1 as a regulator of autophagic flux in neurons. By combining mouse genetics and biochemical approaches, we reveal that AAK1 is not essential for neuronal survival. Full-body AAK1 knockout mice are born normal but characterized by severe dwarfism and hyperactivity. Strikingly, the AAK1 is required to stabilize the AP-2 complex, since AAK1 deficient brains reveal significantly less of the AP-2 µ2 protein level when compared to the wildtype. Characterization of AAK1 binding partners in the brain reveals tectonin β-propeller containing protein 2 (TECPR2) as a novel AAK1 binding partner. TECPR2 is a hereditary spastic paraplegia–associated protein, required for autophagosome formation at the ER exit sites. We find that AAK1 interacts and co-traffics with TECPR2 in neurons, which raised a question if AP-2 is required for membrane trafficking at the ER exit sites and whether this function is regulated by the AAK1. Taken together, our data highlight AAK1 as a novel player of neuronal autophagy and propose that the function of AP-2 in autophagosome trafficking might be regulated by the AAK1.

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A NEW INTRABODY BASED OPTOGENETIC TOOL TO DEGRADE THE AGGREGATION PRONE PROTEINS.

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Neurodegenerative diseases, such as Parkinson's disease, are characterized by the presence of misfolded protein that produce toxic aggregates. Since the mechanism of the aggregation remains unclear, one strategy could be to find a way to induce the degradation of the aggregates. The ability of intracellular antibodies to bind a pathological protein within the cell environment is not enough to reduce the toxic phenotype. The degradation of the complex, composed by intrabody and toxic target, can only be achieved by introducing a degron domain which is able to activate the ubiquitin proteasome system. The selection of an appropriate degron is a key aspect to develop a tool for the modulation of the proteasome activity, but it may not be sufficient to control the timing of the degradation pathway. In this work, we create a target specific photosensitive degron domain fused to a camelid intrabody directed against alpha-Synuclein protein to modulate the degradation of alpha synuclein protein by using a blue light stimulation protocol on yeast cell cultures. Yeast cells are widely used as eukaryotic model organism to study protein aggregation. Our data show that the photosensitive degron, made by photoreceptor light oxygen voltage (LOV) domain and ornithine decarboxylase enzyme fused with a nanobody directed against alpha synuclein protein, is a powerful tool to detect and reduce the protein level. We provide the proof of concept for the optimization of the illumination protocol in a yeast cellular model using alpha synuclein as target for degradation.

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BOARD NUMBER: S01-515

INVOLVEMENT OF OLIGODENDROCYTES IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) LINKED TO FUSED IN SARCOMA PROTEIN

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Aims : ALS is a neurodegenerative disorder leading to death after 3 to 5 years**.** A mutation in the nuclear localisation signal (NLS) of the Fused in Sarcoma protein (FUS) leads to FUS cytoplasmic mislocalisation and early-onset ALS. FUS is a DNA/RNA binding protein which regulates gene expression and mRNA splicing. Increasing evidence suggests that not only motor neurons but also glial cells are affected in the disease. The aim of my PhD is to understand the contribution of oligodendrocytes in *FUS*-ALS. **Methods :** To conduct my project, we first used a murine model of *FUS*-ALS carrying a truncation of the NLS signal in the FUS protein to characterize the oligodendrocytes deficiencies. Then, we developed two new mice models in which we can either induce the FUS mutation only in oligodendrocytes, or rescue the mutation only in oligodendrocytes using a Cre-Lox recombination technology under the control of an oligodendrocyte promoter. We are currently investigating the pathological hallmarks and behavioural outcomes in those two models to determine whether this *Fus* mutation in oligodendrocytes is sufficient and/or necessary to induce ALS symptoms. **Results and discussion:** The ultrastructure of myelin sheath is altered in *FUS*-ALS mouse model, as well as myelin production. This could be explained by the fact that the FUS protein binds to several myelin mRNAs. Also, cuprizone-induced demyelination leads to increased motor impairements in *FUS*-ALS mice compared to their control.

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BOARD NUMBER: S01-516

MERCURY CONTAMINATION EFFECTS ON RATS SENSORY GANGLION

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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The effects of xenobiotics, such as mercury, primarily affect the nervous system functions. Therefore, the aim was to study the mercury chloride small doses effect on the sensory ganglions structure at different exposures time: 4 and 12 weeks. The morphological study examined the rats sensory ganglion microscopic and electron microscopic features with using a solution of mercuric chloride (II). During examination after 4 weeks of daily exposure, sensory ganglion structural changes in neurons and neuroglia are observed. The cytoplasm of some neurons in the perinuclear zone is enlightened, the granular endoplasmic reticulum and mitochondria are structurally altered. The nuclei of such neurons are swollen, the number of nuclear pores is reduced. Swollen mitochondria and their cristae reduction is also found in some glial cells; as well as widening and swelling of the Golgi complex and endoplasmic reticulum, ribosomes loss; the number of lysosomes increase. Some of the nerve fibers that are part of the sensory ganglion have a myelin structure destruction and swollen mitochondria with partial cristae reduction. Endotheliocytes are functionally active, have cytoplasmic outgrowths on the lumenal surface and vacuoles in the cytoplasm. After 12 weeks of daily exposure, the number of structurally altered neurons and gliocytes increased and endothelial cells were less functionally active. Thus, changes in neurons, neuroglia, and endothelial cells indicate a nerve tissue response according to the mercuric chloride action duration.

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COENZYME Q10 MODULATES Aβ-INDUCED DISRUPTION OF CELLULAR PROTEOSTASIS AND MITOCHONDRIAL DAMAGE IN NEURO2A CELLS

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Oxidative stress (OS) appears as a major component of the pathophysiologic process in Alzheimer´s disease (AD) and contributes to endoplasmic reticulum (ER) stress, mitochondrial dysfunction and ultimately, leads to proteostasis imbalance. Our previous results have demonstrated that the natural antioxidant coenzyme Q10 (CoQ10) protects human endothelial cells against Aβ-induced oxidative injury and cell death. Therefore, we hypothesize that early regulation of OS in AD could prevent ER stress, mitochondrial damage and normalize cellular proteostasis, which would delay the progression of the disease. Methodology: To explore the impact of CoQ10 on Aβ internalization and incorporation to endoplasmic reticulum (ER), mitochondria and lysosomes, Neuro2A cells were incubated with CoQ10 or vehicle for 24 h, stained with Mitotracker and ERtracker or Lysotracker, and then exposed to $A\beta_{25-35}$ conjugated with green HiLyte Fluor. To evaluate autophagy, cells were transfected with the expression vector GFP-LC3. Image acquisition was performed for 24 h using a confocal microscope. Results: CoQ10 reduced both, Aβ uptake and its trafficking/accumulation into mitochondria and ER, whilst enhanced its incorporation into lysosomes. CoQ10 was also efficacious in protecting against Aβ-induced mitochondrial dysfunction and impaired autophagy. Conclusions: CoQ10 could prevent Aβ-associated mitochondrial damage and proteostasis imbalance, thereby delaying the progression of the pathophysiology associated to this disease.

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THE ROLE OF AUTOPHAGY IN PARVALBUMIN-EXPRESSING NEURONS

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Macroautophagy is a highly conserved cellular recycling pathway that sequesters cellular constituents in autophagic vesicles (AVs) and delivers them to the lysosome for degradation. Previous studies demonstrated that ablation of core autophagy genes, such as *atg5* or *atg7*, in pallial progenitors compromises the survival of their glutamatergic neuron progeny, suggesting that autophagy may be required for neuronal survival. Here, we examine the effects of *atg5* conditional ablation in neurons expressing parvalbumin (PV-*atg5*KO), which in the forebrain mainly represent fast-spiking inhibitory neurons that provide peri-somatic inhibition onto the principal pyramidal cells. Contrary to the prevailing view, we show that autophagy-deficient PV neurons survive throughout the brain, with the exception of Purkinje neurons in the cerebellum, which rapidly degenerate. However, proteomic analysis of forebrain PV neurons indicated that autophagy deficiency leads to aberrant proteostasis of synaptic and other proteins. Consistently, in the hippocampus, PV-interneurons show altered synaptic properties and the PV-*atg5*KO animals exhibit memory deficits. Our findings reveal a neuronal type-specific vulnerability to autophagy deficiency while indicating a role of autophagy for the proper function of PV interneurons.

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SIMILARITIES AND DIFFERENCES UPON BINDING OF NATURALLY OCCURRING Δ9-TETRAHYDROCANNABINOL-DERIVATIVES TO CANNABINOID RECEPTORS, POSSIBLE NEW TERAPEUTIC TARGETS

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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We have here assessed, using Δ9 -tetrahydrocannabinol (Δ9 -THC) for comparison, the effect of Δ9 -tetrahydrocannabinolic acid (Δ9 -THCA) and of Δ9 -tetrahydrocannabivarin (Δ9-THCV) that is mediated by human versions of CB1, CB2, and CB1-CB2 receptor functional units, expressed in a heterologous system. CB1 is the most abundant receptor in SNC and CB2 has an important roll as a neuroprotector component in neuroimflamation conditions. Binding to the CB1 and CB2 receptors was addressed in living cells by means of a homogeneous assay. Signaling studies included cAMP level determination, activation of the mitogen-activated protein kinase pathway and ß-arrestin recruitment were performed. The signaling triggered by Δ9 -THCA and Δ9 -THCV via individual receptors or receptor heteromers disclosed differential bias, i.e. the bias observed using a given phytocannabinoid depended on the receptor and on the compound used as reference to calculate the bias factor. These results are consistent with different binding modes leading to differential functional selectivity depending on the agonist structure, and the state (monomeric or heteromeric) of the cannabinoid receptor. In addition, on studying Gi-coupling we showed that Δ9 -THCV and Δ9-THCA and Δ9-THCV were able to revert the effect of a selective CB2 receptor agonist, but only Δ9-THCV, and not Δ9-THCA, reverted the effect of arachidonyl-2′ -chloroethylamide (ACEA 100 nM) a selective agonist of the CB1 receptor. Overall, these results indicate that cannabinoids may have a variety of binding modes that results in qualitatively different effects depending on the signaling pathway that is engaged upon cannabinoid receptor activation.

Pubmed:

34758399: Raïch I, Rivas-Santisteban R, Lillo A, Lillo J, Reyes-Resina I, Nadal X, Ferreiro-Vera C, de Medina VS, Majellaro M, Sotelo E, Navarro G, Franco R
Similarities and differences upon binding of naturally occurring Δ-tetrahydrocannabinol-derivatives to cannabinoid CB and CB receptors.
We have here assessed, using Δ-tetrahydrocannabinol (Δ-THC) for comparison, the effect of Δ-tetrahydrocannabinolic acid (Δ-THCA) and of Δ-tetrahydrocannabivarin (Δ9-THCV) that is mediated by human versions of CB, CB, and CB-CB receptor functional units, expressed in a heterologous system. Binding to the CB and CB receptors was addressed in living cells by means of a homogeneous assay. A biphasic competition curve for the binding to the CB receptor, was obtained for Δ-THCV in cells expressing the two receptors. Signaling studies included cAMP level determination, activation of the mitogen-activated protein kinase pathway and ß-arrestin recruitment were performed. The signaling triggered by Δ-THCA and Δ-THCV via individual receptors or receptor heteromers disclosed differential bias, i.e. the bias observed using a given phytocannabinoid depended on the receptor (CB, CB or CB-CB) and on the compound used as reference to calculate the bias factor (Δ-THC, a selective agonist or a non-selective agonist). These results are consistent with different binding modes leading to differential functional selectivity depending on the agonist structure, and the state (monomeric or heteromeric) of the cannabinoid receptor. In addition, on studying Gi-coupling we showed that Δ-THCV and Δ-THCA and Δ-THCV were able to revert the effect of a selective CB receptor agonist, but only Δ9-THCV, and not Δ9-THCA, reverted the effect of arachidonyl-2'-chloroethylamide (ACEA 100 nM) a selective agonist of the CB receptor. Overall, these results indicate that cannabinoids may have a variety of binding modes that results in qualitatively different effects depending on the signaling pathway that is engaged upon cannabinoid receptor activation.
Pharmacol Res, 2021; 174
31429130: Franco R, Reyes-Resina I, Aguinaga D, Lillo A, Jiménez J, Raïch I, Borroto-Escuela DO, Ferreiro-Vera C, Canela EI, Sánchez de Medina V, Del Ser-Badia A, Fuxe K, Saura CA, Navarro G
Potentiation of cannabinoid signaling in microglia by adenosine A receptor antagonists.
Neuroprotective M2-skewed microglia appear as promising to alter the course of neurodegenerative diseases and G protein-coupled receptors (GPCRs) are potential targets to achieve such microglial polarization. A common feature of adenosine A (A

R) and cannabinoid CB (CB R) GPCRs in microglia is that their expression is upregulated in Alzheimer's disease (AD). On the one hand, CB R seems a target for neuroprotection, delaying neurodegenerative processes like those associated to AD or Parkinson's diseases. A R antagonists reduce amyloid burden and improve cognitive performance and memory in AD animal models. We here show a close interrelationship between these two receptors in microglia; they are able to physically interact and affect the signaling of each other, likely due to conformational changes within the A -CB receptor heteromer (A -CB Het). Particularly relevant is the upregulation of A -CB Het expression in samples from the APP , AD transgenic mice model. The most relevant finding, confirmed in both heterologous cells and in primary cultures of microglia, was that blockade of A receptors results in increased CB R-mediated signaling. This heteromer-specific feature suggests that A R antagonists would potentiate, via microglia, the neuroprotective action of endocannabinoids with implications for AD therapy.
Glia, 2019; 67
35203424: Lillo A, Raïch I, Lillo J, Pérez-Olives C, Navarro G, Franco R
Expression of the Adenosine A-A Receptor Heteromer in Different Brain Regions and Marked Upregulation in the Microglia of the Transgenic APP Alzheimer's Disease Model.
Adenosine (Ado) receptors have been instrumental in the detection of heteromers and other higher-order receptor structures, mainly via interactions with other cell surface G-protein-coupled receptors. Apart from the first report of the A Ado receptor interacting with the A Ado receptor, there has been more recent data on the possibility that every Ado receptor type, A, A, A, and A, may interact with each other. The aim of this paper was to look for the expression and function of the A/A receptor heteromer (AAHet) in neurons and microglia. In situ proximity ligation assays (PLA), performed in primary cells, showed that AAHet expression was markedly higher in striatal than in cortical and hippocampal neurons, whereas it was similar in resting and activated microglia. Signaling assays demonstrated that the effect of the AR agonist, PSB 777, was reduced in the presence of the AR agonist, 2-Cl-IB-MECA, whereas the effect of the AR agonist was potentiated by the AR antagonist, SCH 58261. Interestingly, the expression of the heteromer was markedly enhanced in microglia from the APP model of Alzheimer's disease. The functionality of the heteromer in primary microglia from APP mice was more similar to that found in resting microglia from control mice.
Biomedicines, 2022; 10
34955755: Lillo A, Lillo J, Raïch I, Miralpeix C, Dosrius F, Franco R, Navarro G
Ghrelin and Cannabinoid Functional Interactions Mediated by Ghrelin/CB Receptor Heteromers That Are Upregulated in the Striatum From Offspring of Mice Under a High-Fat Diet.
There is evidence of ghrelinergic-cannabinoidergic interactions in the central nervous system (CNS) that may impact on the plasticity of reward circuits. The aim of this article was to look for molecular and/or functional interactions between cannabinoid CB and ghrelin GHS-R1a receptors. In a heterologous system and using the bioluminescence resonance energy transfer technique we show that human versions of cannabinoid CB and ghrelin GHS-R1a receptors may form macromolecular complexes. Such receptor heteromers have particular properties in terms of CB/G-mediated signaling and in terms of GHS-R1a-G-mediated signaling. On the one hand, just co-expression of CBR and GHS-R1a led to impairment of cannabinoid signaling. On the other hand, cannabinoids led to an increase in ghrelin-derived calcium mobilization that was stronger at low concentrations of the CB receptor agonist, arachidonyl-2'-chloroethylamide (ACEA). The expression of CB-GHS-R1a receptor complexes in striatal neurons was confirmed by proximity ligation imaging assays. Upregulation of CB-GHS-R1a- receptor complexes was found in striatal neurons from siblings of pregnant female mice on a high-fat diet. Surprisingly, the expression was upregulated after treatment of neurons with ghrelin (200 nM) or with ACEA (100 nM). These results help to better understand the complexities underlying the functional interactions of neuromodulators in the reward areas of the brain.
Front Cell Neurosci, 2021; 15
32470563: Navarro G, Varani K, Lillo A, Vincenzi F, Rivas-Santisteban R, Raïch I, Reyes-Resina I, Ferreiro-Vera C, Borea PA, Sánchez de Medina V, Nadal X, Franco R
Pharmacological data of cannabidiol- and cannabigerol-type phytocannabinoids acting on cannabinoid CB, CB and CB/CB heteromer receptors.
Recent approved medicines whose active principles are ΔTetrahidrocannabinol (Δ-THC) and/or cannabidiol (CBD) open novel perspectives for other phytocannabinoids also present in Cannabis sativa L. varieties. Furthermore, solid data on the potential benefits of acidic and varinic phytocannabinoids in a variety of diseases are already available. Mode of action of cannabigerol (CBG), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), cannabidivarin (CBDV) and cannabigerivarin (CBGV) is, to the very least, partial.
Pharmacol Res, 2020; 159
33864900: Osorio-Barrios F, Navarro G, Campos J, Ugalde V, Prado C, Raïch I, Contreras F, López E, Espinoza A, Lladser A, Franco R, Pacheco R
The Heteromeric Complex Formed by Dopamine Receptor D and CCR9 Leads the Gut Homing of CD4 T Cells Upon

Inflammation.
CD4 T cells constitute central players in inflammatory bowel diseases (IBDs), driving inflammation in the gut mucosa. Current evidence indicates that CCR9 and the integrin α4β7 are necessary and sufficient to imprint colonic homing on CD4 T cells upon inflammation. Interestingly, dopaminergic signaling has been previously involved in leukocyte homing. Despite dopamine levels are strongly reduced in the inflamed gut mucosa, the role of dopamine in the gut homing of T cells remains unknown. Here, we study how dopaminergic signaling affects T cells upon gut inflammation.
Cell Mol Gastroenterol Hepatol, 2021; 12

FENS Forum 2022
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BOARD NUMBER: S01-520

DECIPHERING MINIMAL REGIONS IN THE TAU REPEAT DOMAIN THAT ACTS AS THE LOCI FOR AGGREGATION, SEEDING AND PROPAGATION

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Background: Neurofibrillary tangles are pathological hallmarks of Alzheimer's disease (AD), but the prion-like propagation of tau may underlie disease progression. Our study aimed to identify the minimal regions in the tau repeat domain that define seeding and its impact on intracellular tau phosphorylation and aggregation. **Methods:** Peptides of repeat 2 (R2) and repeat 3 (R3) of the tau repeat domain were used to generate different seed types. The effect of seeds on intracellular seeding was examined in cells expressing P301S and P301L tau. **Results:** R2, like R3, forms seed-competent fibrils (seeds) when assembled in the presence of heparin. However, R3, but not R2, forms seed-competent seeds when assembled without heparin, even though both R2 and R3 have identical N-terminal hexapeptide and cysteine residue sequences. Cysteine to alanine substitution in R3 abrogates its self-aggregation and seeding potency. R2 and R3 seeds induce tau phosphorylation at pathological sites and oligomerisation of native tau in tau expressing cells (seeded cells). When introduced in a fresh set of cells, protein fractions of seeded cells reseed endogenous tau aggregation. **Conclusion:** We show that R3 in tau is the minimal region for pathological seed generation under physiological conditions, whereas R2 might need polyanionic cofactors to generate pathogenic seeds. **Acknowledgement**: European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-521

ALTERED POLYUNSATURATED SPHINGOLIPIDS CORRELATE WITH α-SYNUCLEIN IN MULTIPLE SYSTEM ATROPHY CEREBELLAR SUBTYPE

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Background & Aims: Multiple System Atrophy (MSA) is a rapidly progressive neurodegenerative disease that is classified into two subtypes - MSA-Cerebellar (MSA-C) and MSA-Parkinsonian (MSA-P). Both subtypes are pathologically characterised by oligodendroglial α-synuclein inclusions and demyelination. Lipids constitute a large component of myelin and are diminished in disease-affected brain regions. Furthermore, polyunsaturated fatty acids are susceptible to peroxidation and are associated with α-synuclein aggregation. Despite the clear importance of lipids in MSA pathology, little is known about the lipid oxidative state of MSA brain. The aim of this study was to determine the relationship between unsaturated lipids and α-synuclein in MSA brain. **Methods:** Total lipids were extracted from tissue samples collected from MSA-C (n = 5), MSA-P (n = 6) and control (n = 13) putamen, motor cortex white matter, cerebellum and occipital cortex, and analysed by mass spectrometry. The abundance of unsaturated lipids was analysed by multivariate analysis covarying for age and sex. α-Synuclein protein in the same tissue samples was measured by western blotting. **Results:** Abundance of several unsaturated sphingolipids was significantly altered in MSA compared to controls across all four regions. Specifically, in MSA-C cerebellum, polyunsaturated hexosylceramide was reduced ($p = 0.01$), whilst polyunsaturated sphingosine was increased ($p = 0.01$). Polyunsaturated hexosylceramide ($r = -0.421$, $p = 0.04$) and sphingosine ($r = 0.419$, $p = 0.04$) correlated with α-synuclein levels. **Conclusion**: These results indicate that specific unsaturated lipids are altered in disease-affected regions of MSA brain, and this could relate to a regional vulnerability to synucleinopathy.

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BOARD NUMBER: S01-522

IRON CHELATION AND RYANODINE RECEPTOR INHIBITION PROTECT PRIMARY HIPPOCAMPAL NEURONS AGAINST FERROPTOSIS

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Introduction: Ferroptosis, a recently described cell death pathway, is characterized by iron-mediated lipid peroxidation, altered mitochondrial morphology and decreased levels of the intracellular antioxidant glutathione. Inhibition of glutathione peroxidase 4 (Gpx4), a key antioxidant regulator, promotes ferroptosis in different cell types. However, scant information is available on Gpx4-induced ferroptosis in primary hippocampal neurons. Moreover, the role of Ca^{2+}, a universal second messenger, in Gpx4-induced ferroptosis in neuronal cells remains elusive. **Hypothesis:** We propose that Gpx4 inhibition induces ferroptosis in primary hippocampal neurons, and that the ensuing dysregulation of the cellular redox state dysregulates Ca^{2+} signaling through excessive activation of ryanodine receptor (RyR) channels. **Material and Methods:** Primary hippocampal neurons were treated with RSL3, a selective Gpx4 inhibitor, and ferroptosis was characterized by analyzing cell viability, cell morphology and lipid peroxidation. The effects of iron chelation by deferoxamine (DFO), and RyR inhibition by 20 uM ryanodine were evaluated. **Results:** Incubation of cultures with RSL3 (15 µM) for 24 hours induced 50% cell death, dendritic damage, and lipid peroxidation, which was reduced by previous incubation with DFO. Suppression of RyR activity offered partial protection in RSL3-treated hippocampal neurons. **Conclusion:** Gpx4 inhibition induced ferroptosis in primary hippocampal cultures, as evidenced by the protection offered by the iron chelator DFO. Suppression of RyR activity mitigated the effects of Gpx4 inhibition, indicating that Ca^{2+} contributes to ferroptosis in hippocampal neuronal cells, a result with possible implications for neurodegenerative diseases. **Acknowledgements**: CONICYT-PFCHA/Doctorado Nacional/2020-21200346, BNI ICM09_015, BMBF 180051.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-523

ROLES OF GROWTH FACTORS ON USP14 MEDIATED CONTROL OF NEURONAL PROTEOSTASIS

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Ubiquitin specific protease-14 (USP14) is a deubiquitinating enzyme reversibly associated with the proteasome 19S regulatory particle (RP), regulating its degradation activity. Proteasome independent pool of USP14 could also influence other pathways like autophagy and endoplasmic reticulum (ER) signaling. We have identified that proteasome inhibition or USP14 mutant (W58A-USP14) disrupted the association of USP14 with PSMD2, a component of the proteasome 19S RP. Furthermore, we identified a dynamic interaction of USP14 with molecular chaperone, HSC70 coordinating the crosstalk between proteasome, autophagy and ER signaling. By employing the W58A-USP14 mutant, we showed an increase in the formation of GABARAP positive autophagosomes and their clearance via lysosomal degradation in mutant huntingtin (Htt) expressing striatal neurons. We have also identified potential post-translational modifications in USP14, which could play a role in its underlying regulatory mechanisms. In this respect, we show here that stimulation with the growth factor, IGF1 can inhibit autophagy and induce accumulation of K48-linked Polyubiquitin chains indicating proteasomal inhibition. IGF1 further increases the catalytic activity of USP14 in SH-SY5Y neuroblastoma cells and in primary neurons. The upstream mechanisms regulating these dynamics of USP14 warrants further studies.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-524

IMPAIRMENT OF BACH-1/NRF-2 AXIS IN DOWN SYNDROME

POSTER SESSION 01 - SECTION: CELLULAR AND MOLECULAR MECHANISMS OF NEURODEGENERATION

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Oxidative Stress (OS) is a chronic condition that contributes to phenotypical alterations of Down Syndrome (DS) individuals, representing a strong risk factor for neurodegeneration. A number of triplicated genes are involved in the over-production of ROS as well as in defects of the antioxidant response in DS individuals and animal models. Among candidate genes, BACH-1 levels have been found to be elevated in DS and we hypothesize that dysregulation of Keap-1-Nrf-2-ARE/BACH-1 axis may be involved in the alteration of redox homeostasis in DS tissues. Protein oxidation (total HNE adduct), gene expression and protein levels of BACH-1, Nrf-2 and its targets (HO-1 and NQO1) were evaluated in different models of DS: (i) hippocampus of Ts2cje mice, Tg mouse model of DS and primary neurons/astrocytes, and (ii) human DS lymphoblastoid cell lines (LCLs) from pediatric patients. Further, we tested the ability of Caffeic Acid Phenethyl Ester and its synthetic analogue VP961 to modulate Nrf-2 activity. Ts2cje mice and DS LCLs showed the impairment of BACH-1/Nrf-2 pathway: increased protein oxidation; increased gene expression and protein levels iii) reduced Nrf-2 nuclear translocation and subsequently decreased Nrf-2-mediated anti-oxidant response were observed. Both compounds, at different concentrations, 10 mM and 5 mM respectively, were administered to DS LCLs and were able to promote Nrf-2 nuclear translocation, which resulted in the improvement of the antioxidant response. Collected results demonstrated the impairment of BACH-1/Nrf-2 axis as a pathological feature of DS that negatively modulate the anti-oxidant response. The treatment restored BACH1/Nrf-2 axis and rescued OS-phenotype in DS LCLs.

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BOARD NUMBER: S01-525

PHARMACOLOGICAL MODULATION OF NEURONAL ACTIVITY FOR THE TREATMENT OF RETT SYNDROME

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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AIMS: Rett syndrome (RTT) is a neurodevelopmental disorder, representing the most common genetic cause of severe intellectual disability in females. RTT is caused by mutations in the X-linked *MECP2* gene. Given its role as a master regulator of gene expression, transcriptional maturation is affected in null neurons, as well as the ability to respond to external *stimuli*. Neuronal activity plays a key role during brain development, thus any variation from physiological ranges lead to severe consequences. We tested the possible causative link between immaturity and reduced neuronal activity by pharmacologically stimulating *in vitro* and *in vivo Mecp2* null neurons within different time windows of differentiation. **METHODS:** To enhance activity and rescue maturation *in vitro,* we used Ampakine CX546, a positive AMPA receptor modulator. The efficacy of an early treatment with CX546 *in vivo* was tested by evaluating the general well-being of mice, and by performing motor and cognitive behavioral tests. **RESULTS:** By treating cortical neurons with CX546 we ameliorated null neurons transcription and activity, highlighting the contribution of defective mechanisms of development to typical RTT phenotypes. Although the early time window of treatment *in vivo* suggested a prolonged benefic effect on knock-out mice, it was devoid of translational value. We thus tested later timepoints and different Ampakines. **CONCLUSIONS:** Our results support the value of an early therapeutic approach acting on neuronal activity as a strategy for Rett syndrome therapy. More studies are needed to pinpoint the correct time window and to identify the molecular pathways involved in any observed benefits.

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HIPPOCAMPAL GABAERGIC DYSFUNCTION IN MECP2 -/Y MICE

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Rett syndrome (RTT) is a neurodevelopmental disorder caused by a mutation in the gene that encodes for the transcriptional regulator, MeCP2. In RTT, patients exhibit severe synaptic dysfunction in neurons found in layers II and III of the entorhinal cortex (EC), resulting in severely impaired memory and learning. The temporoammonic (TA) pathway provides input directly from layer III of the EC to distal dendrites of CA1 neurons in the stratum lacunosum-moleculare (SLM). Despite the importance of the TA pathway on the firing of the CA1 neurons, spatial navigation, learning, and memory, this pathway has not been fully characterized in the context of Rett syndrome. To characterize the effect of the MeCP2 mutation on synaptic and network connectivity of TA-CA1 and Schaffer collateral (SC)-CA1 pathways, we performed in vitro whole-cell recordings, local field potential recordings, and behavioral assays in a MeCP2-mouse model of RTT. Our results show that the eEPSCs amplitude were significantly larger in MeCP2 -/Y relative to wild-type mice following10 Hz stimulation trains with no change in eIPSCs amplitude. We also found both LTP induction and paired-pulse ratio were unaffected. Additionally, training did not improve rotarod performance in MeCP2-/Y mice, unlike wild-types. Furthermore, GABA reversal potential was depolarized in both SLM and CA1 neurons measured using a high chloride concentration (30 mM) internal solution and KCC2 expression was reduced, suggesting impairments in GABAergic inhibition. Taken together, this data demonstrates that altered GABAergic signaling may underlie synaptic dysfunction in MeCP2 deficient mice and may contribute to disease progression in RTT.

Pubmed:
31697763: Ghafouri S, Fathollahi Y, Semnanian S, Shojaei A, Asgari A, Ebrahim Amini A, Mirnajafi-Zadeh J
Deep brain stimulation restores the glutamatergic and GABAergic synaptic transmission and plasticity to normal levels in kindled rats.
The precise effect of low frequency stimulation (LFS) as a newly postulated, anticonvulsant therapeutic approach on seizure-induced changes in synaptic transmission has not been completely determined.
PLoS One, 2019; 14
27423017: Ghafouri S, Fathollahi Y, Javan M, Shojaei A, Asgari A, Mirnajafi-Zadeh J
Effect of low frequency stimulation on impaired spontaneous alternation behavior of kindled rats in Y-maze test.
Epileptic seizures are characterized with cognitive disorders. In this study we investigated the effect of electrical low frequency stimulation (LFS), as a potential anticonvulsant agent, on kindled seizure-induced cognitive impairments. Animals were kindled through electrical stimulation of hippocampal CA1 area in a semi-rapid manner (12 stimulations/day). One group of animals received LFS 4 times at 0.5, 6.5, 24 and 30h following the last kindling stimulation. Applied LFS was consisted of 4 packages at 5min intervals. Each package contained 200 monophasic square wave pulses of 0.1ms duration at 1Hz. The Y-maze test was performed in all animals to measure the spontaneous alternation behavior. Kindled animals showed significant impairment in spontaneous alternation behavior compared to the control group. Application of LFS improved the observed impairment in spontaneous alternation behavior in kindled animals, so that there was no significant difference between kindled+LFS and control group. The observed improving effect of LFS was accompanied with a significant increase in calcineurin gene expression within the hippocampal area. Therefore, it may be postulated that application of LFS in kindled animals, which resulted in increment of calcineurin gene expression, can improve the seizure-induced impairment in spontaneous alternation behavior in Y-maze test.
Epilepsy Res, 2016; 126
27235746: Asgari A, Semnanian S, Atapour N, Shojaei A, Moradi-Chameh H, Ghafouri S, Sheibani V, Mirnajafi-Zadeh J
Low-frequency electrical stimulation enhances the effectiveness of phenobarbital on GABAergic currents in hippocampal slices of kindled rats.
Low frequency stimulation (LFS) has been proposed as a new approach in the treatment of epilepsy. The anticonvulsant mechanism of LFS may be through its effect on GABAA receptors, which are the main target of phenobarbital anticonvulsant

action. We supposed that co-application of LFS and phenobarbital may increase the efficacy of phenobarbital. Therefore, the interaction of LFS and phenobarbital on GABAergic inhibitory post-synaptic currents (IPSCs) in kindled and control rats was investigated. Animals were kindled by electrical stimulation of basolateral amygdala in a semi rapid manner (12 stimulations/day). The effect of phenobarbital, LFS and phenobarbital+LFS was investigated on GABAA-mediated evoked and miniature IPSCs in the hippocampal brain slices in control and fully kindled animals. Phenobarbital and LFS had positive interaction on GABAergic currents. In vitro co-application of an ineffective pattern of LFS (100 pulses at afterdischarge threshold intensity) and a sub-threshold dose of phenobarbital (100μM) which had no significant effect on GABAergic currents alone, increased the amplitude and area under curve of GABAergic currents in CA1 pyramidal neurons of hippocampal slices significantly. Interestingly, the sub-threshold dose of phenobarbital potentiated the GABAergic currents when applied on the hippocampal slices of kindled animals which received LFS in vivo. Post-synaptic mechanisms may be involved in observed interactions. Obtained results implied a positive interaction between LFS and phenobarbital through GABAA currents. It may be suggested that a combined therapy of phenobarbital and LFS may be a useful manner for reinforcing the anticonvulsant action of phenobarbital.
Neuroscience, 2016; 330
24609823: Asgari A, Semnanian S, Atapour N, Shojaei A, Moradi H, Mirnajafi-Zadeh J
Combined sub-threshold dosages of phenobarbital and low-frequency stimulation effectively reduce seizures in amygdala-kindled rats.
Low-frequency stimulation (LFS) is a potential therapy utilized in patients who do not achieve satisfactory control of seizures with pharmacological treatments. Here, we investigated the interaction between anticonvulsant effects of LFS and phenobarbital (a commonly used medicine) on amygdala-kindled seizures in rats. Animals were kindled by electrical stimulation of basolateral amygdala in a rapid manner (12 stimulations/day). Fully kindled animals randomly received one of the three treatment choices: phenobarbital (1, 2, 3, 4 and 8 mg/kg; i.p.; 30 min before kindling stimulation), LFS (one or 4 packages contained 100 or 200 monophasic square wave pulses, 0.1-ms pulse duration at 1 Hz, immediately before kindling stimulation) or a combination of both (phenobarbital at 3 mg/kg and LFS). Phenobarbital alone at the doses of 1, 2 and 3 mg/kg had no significant effect on the main seizure parameters. LFS application always produced anticonvulsant effects unless applied with the pattern of one package of 100 pulses, which is considered as non-effective. All the seizure parameters were significantly reduced when phenobarbital (3 mg/kg) was administered prior to the application of the non-effective pattern of LFS. Phenobarbital (3 mg/kg) also increased the anticonvulsant actions of the effective LFS pattern. Our results provide an evidence of a positive cumulative anticonvulsant effect of LFS and phenobarbital, suggesting a potential combination therapy at sub-threshold dosages of phenobarbital and LFS to achieve a satisfactory clinical effect.
Neurol Sci, 2014; 35
28197450: Asgari A
Herbal medicines and kidney; friends or foes?
J Nephropharmacol, 2014; 3
27418911: Jafarzadeh L, Rafieian-Kopaei M, Samani RA, Asgari A
The effect of hydroalcoholic extract of Stachys lavandulifolia vahl on pregnant mice.
Stachys lavandulifolia is commonly used for many health problems including anxiety. A couple of reports indicate that this plant might have an abortifacient effect on pregnant women. Here we examined this effect on pregnant mice.
EXCLI J, 2012; 11
33029011: Sharma V, Sood R, Khlaifia A, Eslamizade MJ, Hung TY, Lou D, Asgarihafshejani A, Lalzar M, Kiniry SJ, Stokes MP, Cohen N, Nelson AJ, Abell K, Possemato AP, Gal-Ben-Ari S, Truong VT, Wang P, Yiannakas A, Saffarzadeh F, Cuello AC, Nader K, Kaufman RJ, Costa-Mattioli M, Baranov PV, Quintana A, Sanz E, Khoutorsky A, Lacaille JC, Rosenblum K, Sonenberg N
eIF2α controls memory consolidation via excitatory and somatostatin neurons.
An important tenet of learning and memory is the notion of a molecular switch that promotes the formation of long-term memory. The regulation of proteostasis is a critical and rate-limiting step in the consolidation of new memories. One of the most effective and prevalent ways to enhance memory is by regulating the synthesis of proteins controlled by the translation initiation factor eIF2. Phosphorylation of the α-subunit of eIF2 (p-eIF2α), the central component of the integrated stress response (ISR), impairs long-term memory formation in rodents and birds. By contrast, inhibiting the ISR by mutating the eIF2α phosphorylation site, genetically and pharmacologically inhibiting the ISR kinases, or mimicking reduced p-eIF2α with the ISR inhibitor ISRIB, enhances long-term memory in health and disease. Here we used molecular genetics to dissect the neuronal circuits by which the ISR gates cognitive processing. We found that learning reduces eIF2α phosphorylation in hippocampal excitatory neurons and a subset of hippocampal inhibitory neurons (those that express somatostatin, but not parvalbumin). Moreover, ablation of p-eIF2α in either excitatory or somatostatin-expressing (but not parvalbumin-expressing) inhibitory neurons increased general mRNA translation, bolstered synaptic plasticity and enhanced long-term memory. Thus,

eIF2α-dependent mRNA translation controls memory consolidation via autonomous mechanisms in excitatory and somatostatin-expressing inhibitory neurons.
Nature, 2020; 586
31299345: Asgarihafshejani A, Nashmi R, Delaney KR
Cell-Genotype Specific Effects of Mecp2 Mutation on Spontaneous and Nicotinic Acetylcholine Receptor-Evoked Currents in Medial Prefrontal Cortical Pyramidal Neurons in Female Rett Model Mice.
Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutation in the X-linked MECP2 gene. Random X-inactivation produces a mosaic of mutant (MT) and wild-type (WT) neurons in female Mecp2+/- (het) mice. Many RTT symptoms are alleviated by increasing activity in medial prefrontal cortex (mPFC) in RTT model mice (Howell et al., 2017). Using a GFP-MeCP2 fusion protein to distinguish WT from MT pyramidal neurons in mPFC we found cell autonomous (cell genotype specific) and non-autonomous effects of MeCP2 deficiency on spontaneous excitatory/inhibitory balance, nicotinic acetylcholine receptor (nAChR) currents and evoked activity. MT Layer 5 and 6 (L5, L6) neurons of male nulls, and MT L6 of het mice had reduced spontaneous excitatory synaptic input compared to WT in wild-type male (WTm), female (WTf) and het mice. Inhibitory synaptic charge in MT L6 equaled WT in 2-4-month hets. At 6-7 months inhibitory charge in WT in het slices was increased compared to both MT in het and WT in WTf; however, in hets the excitatory/inhibitory charge ratio was still greater in WT compared to MT. nAChR currents were reduced in L6 of nulls and MT L6 in het slices compared to WT neurons of het, WTm and WTf. At 2-4 months, ACh perfusion increased frequency of inhibitory currents to L6 neurons equally in all genotypes but increased excitatory inputs to MT and WT in hets less than WT in WTfs. Unexpectedly ACh perfusion evoked greater sustained IPSC and EPSC input to L5 neurons of nulls compared to WTm.
Neuroscience, 2019; 414

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BOARD NUMBER: S01-527

MITOCHONDRIAL DYSFUNCTION IN RETT SYNDROME MICE MODELS: STUDYING A NEUROLOGICAL DISORDER FROM SYNAPTIC METABOLISM PERSPECTIVE TO FIND NEW TREATMENT OPTIONS

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Rett syndrome is a neurodevelopmental disease affecting 1:10,000 girls, usually due to *MECP2* mutations. It is characterized by a regression in the neuronal development, resulting in the loss of acquired capabilities and arousal of epileptic crisis. While it is a neurotransmission and neuronal maturation disorder, attention towards bioenergetics has been brought, and can be explored to find therapeutic options. AIMS | We have focused our research on the analysis of mitochondrial homeostasis in different brain areas of Rett mice models and whether it can be modulated in a therapeutic purpose. RESULTS | Resembling the patients, MeCP2 female mice go through an asymptomatic phase to later develop the symptomatology. We observed mitochondrial dysfunction already in pre-symptomatic mice (altered dynamics and antioxidant protein expression), suggesting that the mitochondrial malfunction plays a role in the phenotype development and progression. Treatment of symptomatic mice with a PPARγ agonist resulted in behavioral improvement (in terms of explorative activity and motor coordination) through mitochondrial dysfunction amelioration (especially regarding ATP production in cerebellum, closely related with motor coordination improvement). Neuronal maturation and activation markers were also altered and corrected by the treatment. CONCLUSION | Our results reaffirm mitochondria as an effective target for the treatment of an archetypical neurodevelopment disease as Rett syndrome, and endorse a clinical trial with the mentioned PPARγ agonist. Moreover, we highlight the mitochondrial dysfunction even before the symptoms' onset, setting mitochondria as a highly relevant target to modify the natural history of Rett syndrome, and reassuring the necessity of early diagnosis.

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BOARD NUMBER: S01-528

COMPLEX INTERACTION BETWEEN POSTNATAL ACUTE MTOR INHIBITION AND IN UTERO VALPROIC ACID EXPOSURE ON THE MORPHOLOGICAL, FUNCTIONAL AND MOLECULAR FEATURES OF ACCUMBAL MEDIUM SPINY NEURONS

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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It has been proposed that altered processing of social rewards contributes to defective sociability in Autism Spectrum Disorder (ASD). Exposure to the anticonvulsant drug valproic acid (VPA) during gestation is a recognized environmental risk factor for ASD in humans and is used experimentally to induce ASD features in rodents. We previously described a neurophysiological endophenotype of Nucleus Accumbens (NAc) medium spiny neurons (MSNs) from VPA-exposed rats, consisting of profound alterations in basic electrical properties, likely attributable to inward rectifying potassium currents, which lead to abnormal intrinsic excitability. It was recently reported that the mTOR pathway inhibitor rapamycin (RAPA), administered acutely during adolescence, improves ASD-like behavioral defects induced by prenatal VPA exposure. We decided to study the somatic excitability, synaptic function and neuronal morphology of accumbal MSNs in brain slices obtained from rats exposed to prenatal VPA, postnatal RAPA, or both. On NAc tissue homogenates, we also measured the levels of pre- and post-synaptic markers of excitatory and inhibitory synapses and changes in gene expression pattern. We report that postnatal acute administration of RAPA normalizes the VPA-induced increase of intrinsic excitability in accumbal MSNs, although not affecting inward rectifying potassium currents. No significant differences were found in excitatory or inhibitory synaptic drive across treatments. Accordingly, no major alterations in the expression level and molecular composition of main pre- and post-synaptic proteins were detected. Finally, gene expression analysis revealed that postnatal mTOR inhibition interacts with VPA-induced disruption of neurodevelopmental pathways at multiple levels, an in a complex manner.

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NRG1 SIGNALING PROMOTES AXONAL DEVELOPMENT OF CORTICAL NEURONS

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Angela Rodriguez-Prieto[1], Yaiza Domínguez Canterla[1], Isabel Mateos White[2], Cristina Gil Sanz[2], Mar Anibal-Martinez[3], Guillermina López-Bendito[3], Ana González Manteiga[1], Tomás Armenteros Barron[1], Pietro Fazzari[1]
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Schizophrenia is a developmental disorder characterized by atrophy of neuronal processes and alterations in connections between cortical neurons. Neuregulin 1 (NRG1) was identified as a major schizophrenia risk gene and we and others showed that NRG1 intracellular signaling is required for dendritic elongation, excitatory transmission and synaptic plasticity. Here, we studied the role of NRG1 intracellular signaling in the axonal development *in vitro* and *in vivo* taking advantage of multiple approaches, including culture of primary cortical neurons, *in utero* electroporation and neuronal tracing with DiI in newborn NRG1 *knockout* mice. Our study suggests that NRG1 expression in cortical neurons regulates de neurites growth *in vitro* and *in vivo*, being necessary and sufficient for the formation of the axonal projections. From a molecular point of view, we are currently studying the NRG1 signaling pathways underlying the neurites growth that characterizes our phenotype. These results expand the knowledge about NRG1 signaling, hopefully contributing to the understanding of its molecular role in schizophrenia.

Pubmed:
34369923: Rodríguez-Prieto Á, González-Manteiga A, Domínguez-Canterla Y, Navarro-González C, Fazzari P
A Scalable Method to Study Neuronal Survival in Primary Neuronal Culture with Single-cell and Real-Time Resolution.
Neuronal loss is at the core of many neuropathologies, including stroke, Alzheimer's disease, and Parkinson's disease. Different methods were developed to study the process of neuronal survival upon cytotoxic stress. Most methods are based on biochemical approaches that do not allow single-cell resolution or involve complex and costly methodologies. Presented here is a versatile, inexpensive, and effective experimental paradigm to study neuronal survival. This method takes advantage of sparse fluorescent labeling of the neurons followed by live imaging and automated quantification. To this aim, the neurons are electroporated to express fluorescent markers and co-cultured with non-electroporated neurons to easily regulate cell density and increase survival. Sparse labeling by electroporation allows a simple and robust automated quantification. In addition, fluorescent labeling can be combined with the co-expression of a gene of interest to study specific molecular pathways. Here, we present a model of stroke as a neurotoxic model, namely, the oxygen-glucose deprivation (OGD) assay, which was performed in an affordable and robust homemade hypoxic chamber. Finally, two different workflows are described using IN Cell Analyzer 2200 or the open-source ImageJ for image analysis for semi-automatic data processing. This workflow can be easily adapted to different experimental models of toxicity and scaled up for high-throughput screening. In conclusion, the described protocol provides an approachable, affordable, and effective in vitro model of neurotoxicity, which can be suitable for testing the roles of specific genes and pathways in live imaging and for high-throughput drug screening.
J Vis Exp, 2021;
34246770: Navarro-Gonzalez C, Carceller H, Benito Vicente M, Serra I, Navarrete M, Domínguez-Canterla Y, Rodríguez-Prieto Á, González-Manteiga A, Fazzari P
Nrg1 haploinsufficiency alters inhibitory cortical circuits.
Neuregulin 1 (NRG1) and its receptor ERBB4 are schizophrenia (SZ) risk genes that control the development of both excitatory and inhibitory cortical circuits. Most studies focused on the characterization ErbB4 deficient mice. However, ErbB4 deletion concurrently perturbs the signaling of Nrg1 and Neuregulin 3 (Nrg3), another ligand expressed in the cortex. In addition, NRG1 polymorphisms linked to SZ locate mainly in non-coding regions and they may partially reduce Nrg1 expression. Here, to study the relevance of Nrg1 partial loss-of-function in cortical circuits we characterized a recently developed haploinsufficient mouse model of Nrg1 (Nrg1). These mice display SZ-like behavioral deficits. The cellular and molecular underpinnings of the behavioral deficits in Nrg1 mice remain to be established. With multiple approaches including

Magnetic Resonance Spectroscopy (MRS), electrophysiology, quantitative imaging and molecular analysis we found that Nrg1 haploinsufficiency impairs the inhibitory cortical circuits. We observed changes in the expression of molecules involved in GABAergic neurotransmission, decreased density of Vglut1 excitatory buttons onto Parvalbumin interneurons and decreased frequency of spontaneous inhibitory postsynaptic currents. Moreover, we found a decreased number of Parvalbumin positive interneurons in the cortex and altered expression of Calretinin. Interestingly, we failed to detect other alterations in excitatory neurons that were previously reported in ErbB4 null mice suggesting that the Nrg1 haploinsufficiency does not entirely phenocopies ErbB4 deletions. Altogether, this study suggests that Nrg1 haploinsufficiency primarily affects the cortical inhibitory circuits in the cortex and provides new insights into the structural and molecular synaptic impairment caused by NRG1 hypofunction in a preclinical model of SZ.
Neurobiol Dis, 2021; 157

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BOARD NUMBER: S01-530

ROLE OF USP9X IN REGULATING PREFRONTAL CORTEX EXCITABILITY AND ITS POTENTIAL IMPLICATIONS FOR ANGELMAN SYNDROME

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Aims: The lack of functional ubiquitin E3 ligase UBE3A in the brain leads to the rare neurodevelopmental disorder Angelman Syndrome (AS), associated with alterations in prefrontal cortex (PFC) function and cognitive impairment. Protein ubiquitination, however, is not only modulated by E3 ligases, but also by deubiquitinating (DUB) enzymes. Identifying and characterizing DUBs responsible of counteracting UBE3A could lead to therapeutic targets that could ameliorate AS symptoms. A previous study from our lab demonstrated that USP9X –a DUB associated with X-linked intellectual disability– counteracts UBE3A mediated ubiquitination. We aim to characterize the role of USP9X in regulating cortical excitability, found to be altered in AS mouse models, and shed light on the potential therapeutic value of USP9X inhibitors. **Methods**: We performed *in vitro* patch clamp recordings in cortical slices of C57BL/6 mice focusing on pyramidal cells of layer V in the medial PFC (mPFC), one of the main output neuron types of the PFC. USP9X activity was blocked using the two specific inhibitors. **Results**: Blocking USP9X led to changes in several parameters of neuronal excitability, the most prominent being an increase in the sag ratio and in the medium after hyperpolarization (mAHP) of mPFC layer V pyramidal neurons. **Conclusions**: Our results suggest that UPS9X regulates the activity or expression of different ion channels that contribute to intrinsic properties regulating basic membrane properties and synaptic integration. Following these results, we will examine whether blocking USP9X activity ameliorates changes observed in AS mouse models, to further investigate the therapeutic potential of USP9X inhibitors.

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BOARD NUMBER: S01-531

DEVELOPMENT OF A NEW DRUG SCREENING SYSTEM FOR RETT SYNDROME THERAPY

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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AIMS: Rett syndrome is a severe neurodevelopmental disorder caused by mutations in the X-linked *MECP2* gene, primarily acting as transcriptional repressor. Although Rett proved to be reversible in mice, no cure is available. Animal-based drug screenings are widely used to evaluate drug efficacy, though requiring a large number of animals and elevated costs. New drug screening approaches have emerged *in vitro*, based on the analysis of neuronal defective morphology. However, we demonstrated that the amelioration of the transcriptional profile in *Mecp2* null neurons appears as a better indicator of functional rescue compared to morphological restoration. For this reason, we aim at developing a cell-based drug screening system for Rett syndrome therapy, based on customized high-throughput 96x96 qRT-PCR Arrays. **METHODS**: To identify a Rett-neuronal transcriptional profile, a longitudinal RNASeq was performed in *Mecp2* null neurons differentiated from neuronal precursor cells (NPCs) at three timepoints along maturation (DIV7-14-18). Differentially expressed genes (DEGs) were validated using 96x96 qRT-PCR Microfluidic Cards (Fluidigm). **RESULTS**: Bioinformatics analysis identified 1469 DEGs (p-adj<0,1) and gene ontology well-recapitulated previous results *in vivo*. By using different prioritization criteria and testing selected neuronal DEGs on 96x96 microfluidic cards, we identified 80 reproducible DEGs which we are validating on primary cortical neurons either treated or untreated with specific drugs. **CONCLUSIONS**: Obtained data suggest that our transcriptional platform could represent a new drug screening system to pre-select drugs with a higher chance of success in pre-clinical studies. In this communication we will discuss *pro* and *cons* of the proposed approach for drug screening.

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DECIPHERING THE ROLE OF THE KINASE CK2 IN A NOVEL MOUSE MODEL OF THE OKUR-CHUNG AUTISM-LIKE DISORDER

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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In 2016, WES analysis led to the identification of mutations in the gene coding for the catalytic subunit of the kinase CK2 in the Okur-Chung neurodevelopmental syndrome (OCNDS). It is a rare condition within autism spectrum disorders (ASD), characterised by developmental delay, intellectual disability, behavioral problems, hypotonia, epilepsy and verbalization deficits. CK2 is ubiquitously expressed and consists of 2 α catalytic and 2 β regulatory subunits. CK2 is highly expressed in the brain and has multitude of substrates that regulate homeostasis and neurons signalling. Several different missense variants are known to be linked to OCNDS, however the K198R variant is the most common comprising approximately 30% of the cases. Aim: Our aim is to characterize these mice at behavioral, cellular and proteomic levels in order to understand how and through which mechanisms mutant CK2 can mediate OCNDS symptoms. Methods: We evaluated the behavioral phenotype and analysed the changes in activity and in the phospho-proteome by Western Blotting and Mass Spectrometry. Results: We found differences in learning and memory paradigms (Barnes test and Y-maze) in heterozygote mice compared to wildtype littermates. We show that K198R$^{+/-}$ mice exhibit reduced expression levels of CK2α compared to wild type, and that CK2 activity is reduced in striatal tissue. Total and phospho-proteome pointed towards involvement of pre and postsynaptic proteins. These findings help us to start elucidating CK2α mechanisms underlying OCNDS phenotype and to establish the K198R mice line as a model for the disease and also delivering valid information on ASD in general.

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BOARD NUMBER: S01-533

NO LOSS OF GLUTAMATERGIC NEURONS OR INTERNEURONS IN MICE LACKING THE AUTISM-ASSOCIATED GENE GLRA2 ENCODING THE GLYCINE RECEPTOR ALPHA-2 SUBUNIT

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder with a heterogeneous genetic aetiology. Mutations of the *GLRA2* gene, which encodes the glycine receptor alpha-2 subunit, were implicated in ASD, unveiling a previously undescribed mechanism for the physiopathology of autism. Our group showed that *Glra2*-deficient male (*Glra2*$^{Y/-}$) mice present with cognitive deficits and reduced synaptic plasticity (Pilorge et al. 2016). Developmental studies in mice expressing the same *Glra2* mutation in a different genetic background reported deficits in cortical migration and a global loss of cortical neurons with microcephaly (Avila et al. 2013; Morelli et al. 2017). Since our *Glra2*$^{Y/-}$ mice bred on a C57BL/6JRj background are not microcephalic, we decided to quantify projection neurons and interneurons in the prefrontal and somatosensory cortices of adult and postnatal day 14 *Glra2*$^{Y/-}$ mice using fluorescent *in situ* hybridization and immunofluorescence. No loss of glutamatergic neurons or interneurons (parvalbumin, calretinin and cholecystokinin) was observed at either age with either technique. There was however a significant increase in somatostatin-positive cells in *Glra2*$^{Y/-}$ mice. These findings contrast with those reported by other groups, suggesting a possible interaction of the *Glra2* mutation with genetic background. Our results obtained in the C57BL/6 background suggest *Glra2* plays a more subtle role in neocortical development and assembly, whilst implicating *Glra2* in the development of somatostatin-expressing interneurons. Funded by an EU Horizon 2020 Marie Sklodowska-Curie grant.

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BOARD NUMBER: S01-534

THE DEVELOPMENT OF THE INHIBITORY SYSTEM IN THE NEWBORN MICE BRAIN, IS REGULATED BY THE METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) GENE

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Background: Higher frequency of Mthfr's polymorphism, 677C>T, was found among ASD patients and their mothers. In mice, maternal and offspring Mthfr+/- genotypes lead to ASD like-behavior and alternation in the GABA pathway. Here, we investigate the developmental origin of interneurons deficiency in Mthfr deficient mice. **Experimental design:** Mthfr+/+(WT) and Mthfr+/-(HT) female mice were mated with WT male to generate 3 groups of offspring representing maternal:offspring genotype: 1. WT:WT 2.HT:WT 3.HT:HT. Parental mice carried the GAD65-tdTomato for interneurons identification. Immunofluorescent was used to examine cerebral cortex of postnatal-day 7 pups. **Results:** An interaction between offspring Mthfr+/- genotype and the anterior-posterior axis altered cortical laminar distribution of interneurons: In layer 1 (L1), HT:HT presented higher interneurons density in the anterior cortex (p=0.01), while decreased density was observed in the sub-ventricular zone (p=0.001). Similar results were obtained in the medial and lateral regions in L1. Evaluation of the chloride transporter NKCC1 density has presented a strong interaction between offspring Mthfr+/- genotype and the anterior-posterior axis; while NKCC1 density in L1 was suppressed in the anterior cortex, elevation in the posterior cortex was observed (p=0.001). **Conclusions**: These findings suggest that Mthfr changes in the early postnatal stage, perhaps induced in the in-utero environment, were sufficient to induce de-synchronization of interneuron's development, and may be a part of the mechanisms leading to behavioral changes in a parallel age. Our results point to an essential role of folate-cycle in the normal course of interneurons cortical localization and the transition of GABAergic potential nature to fully inhibitory.

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IMPROVEMENT OF SENSORY DEFICITS IN FRAGILE X MICE BY BOOSTING CORTICAL INTERNEURON ACTIVITY AFTER THE CRITICAL PERIOD.

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Inhibitory interneurons (INs) play a critical role in shaping the activity of excitatory neurons in the mature brain. Although recent studies have begun to shed light onto the origin of different IN subclasses and how they functionally integrate into circuits, less is known about how INs modulate excitatory neurons in the developing brain. This is a critical question because INs have been implicated in the pathophysiology of various neurodevelopmental disorders (NDDs). A reduced density and/or activity of parvalbumin (PV) INs has been reported in the adult brain of various mouse models of autism and intellectual disability, including Fragile X Syndrome (FXS), which is the leading inherited cause of autism and intellectual disability. Here, we sought to identify when IN hypofunction is first apparent in the developing neocortex of Fmr1 knockout (*Fmr1*$^{-/-}$) mice, the best studied animal model of FXS. We find that PV-INs in *Fmr1*$^{-/-}$ mice are already hypoactive in the primary somatosensory cortex (S1) in the first postnatal week and are decoupled from neighboring excitatory neurons. This results in excessive IN apoptosis during a critical early postnatal cell death period which explains the reduced density of PV-INs in *Fmr1*$^{-/-}$ mice, and in humans with FXS. Importantly, boosting the activity of PV-INs after this critical period ameliorates both circuit and behavioral sensory phenotypes of *Fmr1*$^{-/-}$ mice. These findings suggest that circuit changes and sensory symptoms in FXS and other NDDs could be ameliorated with interventions that target PV-INs.

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BRAINCILS: EXPLORING THE MISSING LINK BETWEEN NEURONAL PRIMARY CILIA DYSFUNCTION AND NEURODEVELOPMENTAL DISORDERS - HINTS FROM DENTAL STEM CELL-DERIVED BRAIN ORGANOIDS

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Neurodevelopmental disorders (NDDs) affect over 3% of children worldwide. Recent evidence associates chromatin-related gene mutations in NDDs with neuronal primary cilia dysfunction. These include MBD5, the causal locus for 2q23.1 microdeletion syndrome and MECP2, responsible for Rett Syndrome. Despite evidence linking these mutations to primary cilia, little is known on the mechanisms that lead to NDDs. Our goal is to generate 3D brain organoids, derived non-invasively from patients' dental stem cells, to explore the impact of gene mutations such as MBD5 on ciliary function during neurodevelopment. We established the first biobank of dental stem cells in Portugal, and our dental stem cell-derived brain organoids were characterized at specific timepoints for synaptic and neuronal network activity, using electrophysiology and calcium imaging. We are assessing primary cilia distribution and colocalization with specialized neurons and glia and using CRISPR/Cas9 to correct patient mutations to effectively pinpoint the contribution of those mutations in NDDs. Funding: MSCA-IF (799164), FCT-CEEC 3rd Edition, FCT Exploratory Grant (EXPL/MED-OUT/1470/2021), UC-Santander Seed Grant 2021, Syn2Psy H2020-MSCA-ITN-2018 (813986), Pfizer Award 2019

Pubmed:
32503625: Seabra CM, Aneichyk T, Erdin S, Tai DJC, De Esch CEF, Razaz P, An Y, Manavalan P, Ragavendran A, Stortchevoi A, Abad C, Young JI, Maciel P, Talkowski ME, Gusella JF
Transcriptional consequences of MBD5 disruption in mouse brain and CRISPR-derived neurons.
MBD5, encoding the methyl-CpG-binding domain 5 protein, has been proposed as a necessary and sufficient driver of the 2q23.1 microdeletion syndrome. De novo missense and protein-truncating variants from exome sequencing studies have directly implicated MBD5 in the etiology of autism spectrum disorder (ASD) and related neurodevelopmental disorders (NDDs). However, little is known concerning the specific function(s) of MBD5.
Mol Autism, 2020; 11
30558359: Sequeira DB, Seabra CM, Palma PJ, Cardoso AL, Peça J, Santos JM
Effects of a New Bioceramic Material on Human Apical Papilla Cells.
The development of materials with bioregenerative properties is critically important for vital pulp therapies and regenerative endodontic procedures. The aim of this study was to evaluate the cytocompatibility and cytotoxicity of a new endodontic biomaterial, PulpGuard, in comparison with two other biomaterials widely used in endodontic procedures, ProRoot Mineral Trioxide Aggregate (MTA) and Biodentine.
J Funct Biomater, 2018; 9
28691782: Seabra CM, Szoko N, Erdin S, Ragavendran A, Stortchevoi A, Maciel P, Lundberg K, Schlatzer D, Smith J, Talkowski ME, Gusella JF, Natowicz MR
A novel microduplication of ARID1B: Clinical, genetic, and proteomic findings.
Genetic alterations of ARID1B have been recently recognized as one of the most common mendelian causes of intellectual disability and are associated with both syndromic and non-syndromic phenotypes. The ARID1B protein, a subunit of the chromatin remodeling complex SWI/SNF-A, is involved in the regulation of transcription and multiple downstream cellular

processes. We report here the clinical, genetic, and proteomic phenotypes of an individual with a unique apparent de novo mutation of ARID1B due to an intragenic duplication. His neurodevelopmental phenotype includes a severe speech/language disorder with full scale IQ scores 78-98 and scattered academic skill levels, expanding the phenotypic spectrum of ARID1B mutations. Haploinsufficiency of ARID1B was determined both by RNA sequencing and quantitative RT-PCR. Fluorescence in situ hybridization analysis supported an intragenic localization of the ARID1B copy number gain. Principal component analysis revealed marked differentiation of the subject's lymphoblast proteome from that of controls. Of 3426 proteins quantified, 1014 were significantly up- or down-regulated compared to controls ($q < 0.01$). Pathway analysis revealed highly significant enrichment for canonical pathways of EIF2 and EIF4 signaling, protein ubiquitination, tRNA charging and chromosomal replication, among others. Network analyses revealed down-regulation of: (1) intracellular components involved in organization of membranes, organelles, and vesicles; (2) aspects of cell cycle control, signal transduction, and nuclear protein export; (3) ubiquitination and proteosomal function; and (4) aspects of mRNA synthesis/splicing. Further studies are needed to determine the detailed molecular and cellular mechanisms by which constitutional haploinsufficiency of ARID1B causes syndromic and non-syndromic developmental disabilities.
Am J Med Genet A, 2017; 173
28260531: Collins RL, Brand H, Redin CE, Hanscom C, Antolik C, Stone MR, Glessner JT, Mason T, Pregno G, Dorrani N, Mandrile G, Giachino D, Perrin D, Walsh C, Cipicchio M, Costello M, Stortchevoi A, An JY, Currall BB, Seabra CM, Ragavendran A, Margolin L, Martinez-Agosto JA, Lucente D, Levy B, Sanders SJ, Wapner RJ, Quintero-Rivera F, Kloosterman W, Talkowski ME
Defining the diverse spectrum of inversions, complex structural variation, and chromothripsis in the morbid human genome.
Structural variation (SV) influences genome organization and contributes to human disease. However, the complete mutational spectrum of SV has not been routinely captured in disease association studies.
Genome Biol, 2017; 18
28067909: Shaw ND, Brand H, Kupchinsky ZA, Bengani H, Plummer L, Jones TI, Erdin S, Williamson KA, Rainger J, Stortchevoi A, Samocha K, Currall BB, Dunican DS, Collins RL, Willer JR, Lek A, Lek M, Nassan M, Pereira S, Kammin T, Lucente D, Silva A, Seabra CM, Chiang C, An Y, Ansari M, Rainger JK, Joss S, Smith JC, Lippincott MF, Singh SS, Patel N, Jing JW, Law JR, Ferraro N, Verloes A, Rauch A, Steindl K, Zweier M, Scheer I, Sato D, Okamoto N, Jacobsen C, Tryggestad J, Chernausek S, Schimmenti LA, Brasseur B, Cesaretti C, García-Ortiz JE, Buitrago TP, Silva OP, Hoffman JD, Mühlbauer W, Ruprecht KW, Loeys BL, Shino M, Kaindl AM, Cho CH, Morton CC, Meehan RR, van Heyningen V, Liao EC, Balasubramanian R, Hall JE, Seminara SB, Macarthur D, Moore SA, Yoshiura KI, Gusella JF, Marsh JA, Graham JM, Lin AE, Katsanis N, Jones PL, Crowley WF, Davis EE, FitzPatrick DR, Talkowski ME
SMCHD1 mutations associated with a rare muscular dystrophy can also cause isolated arhinia and Bosma arhinia microphthalmia syndrome.
Arhinia, or absence of the nose, is a rare malformation of unknown etiology that is often accompanied by ocular and reproductive defects. Sequencing of 40 people with arhinia revealed that 84% of probands harbor a missense mutation localized to a constrained region of SMCHD1 encompassing the ATPase domain. SMCHD1 mutations cause facioscapulohumeral muscular dystrophy type 2 (FSHD2) via a trans-acting loss-of-function epigenetic mechanism. We discovered shared mutations and comparable DNA hypomethylation patterning between these distinct disorders. CRISPR/Cas9-mediated alteration of smchd1 in zebrafish yielded arhinia-relevant phenotypes. Transcriptome and protein analyses in arhinia probands and controls showed no differences in SMCHD1 mRNA or protein abundance but revealed regulatory changes in genes and pathways associated with craniofacial patterning. Mutations in SMCHD1 thus contribute to distinct phenotypic spectra, from craniofacial malformation and reproductive disorders to muscular dystrophy, which we speculate to be consistent with oligogenic mechanisms resulting in pleiotropic outcomes.
Nat Genet, 2017; 49
27841880: Redin C, Brand H, Collins RL, Kammin T, Mitchell E, Hodge JC, Hanscom C, Pillalamarri V, Seabra CM, Abbott MA, Abdul-Rahman OA, Aberg E, Adley R, Alcaraz-Estrada SL, Alkuraya FS, An Y, Anderson MA, Antolik C, Anyane-Yeboa K, Atkin JF, Bartell T, Bernstein JA, Beyer E, Blumenthal I, Bongers EM, Brilstra EH, Brown CW, Brüggenwirth HT, Callewaert B, Chiang C, Corning K, Cox H, Cuppen E, Currall BB, Cushing T, David D, Deardorff MA, Dheedene A, D'Hooghe M, de Vries BB, Earl DL, Ferguson HL, Fisher H, FitzPatrick DR, Gerrol P, Giachino D, Glessner JT, Gliem T, Grady M, Graham BH, Griffis C, Gripp KW, Gropman AL, Hanson-Kahn A, Harris DJ, Hayden MA, Hill R, Hochstenbach R, Hoffman JD, Hopkin RJ, Hubshman MW, Innes AM, Irons M, Irving M, Jacobsen JC, Janssens S, Jewett T, Johnson JP, Jongmans MC, Kahler SG, Koolen DA, Korzelius J, Kroisel PM, Lacassie Y, Lawless W, Lemyre E, Leppig K, Levin AV, Li H, Li H, Liao EC, Lim C, Lose EJ, Lucente D, Macera MJ, Manavalan P, Mandrile G, Marcelis CL, Margolin L, Mason T, Masser-Frye D, McClellan MW, Mendoza CJ, Menten B, Middelkamp S, Mikami LR, Moe E, Mohammed S, Mononen T, Mortenson ME, Moya G, Nieuwint AW, Ordulu Z, Parkash S, Pauker SP, Pereira S, Perrin D, Phelan K, Aguilar RE, Poddighe PJ, Pregno G, Raskin S, Reis L, Rhead W, Rita D, Renkens I, Roelens F, Ruliera J, Rump P, Schilit SL, Shaheen R, Sparkes R,

Spiegel E, Stevens B, Stone MR, Tagoe J, Thakuria JV, van Bon BW, van de Kamp J, van Der Burgt I, van Essen T, van Ravenswaaij-Arts CM, van Roosmalen MJ, Vergult S, Volker-Touw CM, Warburton DP, Waterman MJ, Wiley S, Wilson A, Yerena-de Vega MC, Zori RT, Levy B, Brunner HG, de Leeuw N, Kloosterman WP, Thorland EC, Morton CC, Gusella JF, Talkowski ME
The genomic landscape of balanced cytogenetic abnormalities associated with human congenital anomalies.
Despite the clinical significance of balanced chromosomal abnormalities (BCAs), their characterization has largely been restricted to cytogenetic resolution. We explored the landscape of BCAs at nucleotide resolution in 273 subjects with a spectrum of congenital anomalies. Whole-genome sequencing revised 93% of karyotypes and demonstrated complexity that was cryptic to karyotyping in 21% of BCAs, highlighting the limitations of conventional cytogenetic approaches. At least 33.9% of BCAs resulted in gene disruption that likely contributed to the developmental phenotype, 5.2% were associated with pathogenic genomic imbalances, and 7.3% disrupted topologically associated domains (TADs) encompassing known syndromic loci. Remarkably, BCA breakpoints in eight subjects altered a single TAD encompassing MEF2C, a known driver of 5q14.3 microdeletion syndrome, resulting in decreased MEF2C expression. We propose that sequence-level resolution dramatically improves prediction of clinical outcomes for balanced rearrangements and provides insight into new pathogenic mechanisms, such as altered regulation due to changes in chromosome topology.
Nat Genet, 2017; 49
26829649: Tai DJ, Ragavendran A, Manavalan P, Stortchevoi A, Seabra CM, Erdin S, Collins RL, Blumenthal I, Chen X, Shen Y, Sahin M, Zhang C, Lee C, Gusella JF, Talkowski ME
Engineering microdeletions and microduplications by targeting segmental duplications with CRISPR.
Recurrent, reciprocal genomic disorders resulting from non-allelic homologous recombination (NAHR) between near-identical segmental duplications (SDs) are a major cause of human disease, often producing phenotypically distinct syndromes. The genomic architecture of flanking SDs presents a challenge for modeling these syndromes; however, the capability to efficiently generate reciprocal copy number variants (CNVs) that mimic NAHR would represent a valuable modeling tool. We describe here a CRISPR/Cas9 genome engineering method, single-guide CRISPR/Cas targeting of repetitive elements (SCORE), to model reciprocal genomic disorders and demonstrate its capabilities by generating reciprocal CNVs of 16p11.2 and 15q13.3, including alteration of one copy-equivalent of the SDs that mediate NAHR in vivo. The method is reproducible, and RNA sequencing reliably clusters transcriptional signatures from human subjects with in vivo CNVs and their corresponding in vitro models. This new approach will provide broad applicability for the study of genomic disorders and, with further development, may also permit efficient correction of these defects.
Nat Neurosci, 2016; 19
25451826: Seabra CM, Quental S, Lima AC, Carvalho F, Gonçalves J, Fernandes S, Pereira I, Silva J, Marques PI, Sousa M, Barros A, Seixas S, Amorim A, Lopes AM
The mutational spectrum of WT1 in male infertility.
We evaluated the impact of WT1 mutations in isolated severe spermatogenic impairment in a population of European ancestry. WT1 was first identified as the gene responsible for Wilms tumor. It was later associated with a plethora of clinical phenotypes often accompanied by urogenital defects and male infertility. The recent finding of WT1 missense mutations in Chinese azoospermic males without major gonadal malformations broadened the phenotypic spectrum of WT1 defects and motivated this study.
J Urol, 2015; 193
24912414: Seabra CM, Quental S, Neto AP, Carvalho F, Gonçalves J, Oliveira JP, Fernandes S, Sousa M, Barros A, Amorim A, Lopes AM
A novel Alu-mediated microdeletion at 11p13 removes WT1 in a patient with cryptorchidism and azoospermia.
This article describes a patient with cryptorchidism and nonobstructive azoospermia presenting a novel microdeletion of approximately 1 Mb at 11p13. It was confirmed by multiplex ligation-dependent probe amplification that this heterozygous deletion spanned nine genes (WT1, EIF3M, CCDC73, PRRG4, QSER1, DEPDC7, TCP11L1, CSTF3 and HIPK3) and positioned the breakpoints within highly homologous repetitive elements. As far as is known, this is the smallest deletion as-yet described encompassing the WT1 gene and was detected only once in a total of 32 Portuguese patients with isolated uni- or bilateral cryptorchidism. These findings suggest that molecular analysis in patients with genitourinary features suggestive of WT1 impairment, namely cryptorchidism and renal abnormalities, may reveal cryptic genetic defects.
Reprod Biomed Online, 2014; 29
33630165: Sequeira DB, Oliveira AR, Seabra CM, Palma PJ, Ramos C, Figueiredo MH, Santos AC, Cardoso AL, Peça J, Santos JM
Regeneration of pulp-dentin complex using human stem cells of the apical papilla: in vivo interaction with two bioactive materials.
To compare the regenerative properties of human stem cells of the apical papilla (SCAPs) embedded in a platelet-rich

plasma (PRP) scaffold, when implanted in vivo using an organotypic model composed of human root segments, with or without the presence of the bioactive cements - ProRoot MTA or Biodentine.
Clin Oral Investig, 2021; 25

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LEDA-1/PIANP INFLUENCES CEREBELLAR HISTOARCHITECTURE AND CELLULAR COMPOSITION

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Moritz Küchler[1], Manuel Winkler[2], Cyrill Géraud[2], Oliver Von Bohlen Und Halbach[1]
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PIANP is a primarily neuronally localised protein which binds to the N-terminal domain of GABA-B receptors as well as the inhibitory immunoglobuline-like type 2 receptor PILRa [1]. In humans, a homozygous loss-of-function mutation of PIANP is associated with features of intellectual disability and global development delay whilst PIANP knockout mice display autism spectrum disorder (ASD)-like behaviour as well as heightened anxiety [2]. As we previously reported, PIANP knockout mice present with a different composition of telencephalic cell layers and markedly altered hippocampal neurogenesis [2, 3]. To shed further light on the specific cellular processes behind the PIANP-associated ASD-like phenotype, we analysed male homozygous knockout mice (PIANPcre) and compared them to both mutant mice floxed at the PIANP locus (PIANPflp) and wildtype C57Bl6/N mice. PIANP knockouts showed altered gross cerebellar morphology compared to wildtype mice, which seems to be influenced by both structural and cellular alterations. Interestingly enough, we observed changes in all layers of the cerebellum with specific alterations for each individual layer present. These results unveil additional factors which seem to be involved in the observed ASD-like phenotype of PIANP knockout mice. Further research is necessary to map the precise influences of these individual changes and their interplay in effecting the above-mentioned behaviour observed in both humans and mice. Bibliography 1. Kogure A et al. Biochem Biophys Res Commun. 2011;405:428–433. 2. Winkler M et al. Mol Psychiatry. 2020;25:2979–2993. 3. Winkler M et al. Mol Psychiatry. 2020;25:2645–2645.

Pubmed:
31511635: Winkler M, Biswas S, Berger SM, Küchler M, Preisendörfer L, Choo M, Früh S, Rem PD, Enkel T, Arnold B, Komljenovic D, Sticht C, Goerdt S, Bettler B, von Bohlen Und Halbach O, Bartsch D, Géraud C
Pianp deficiency links GABA receptor signaling and hippocampal and cerebellar neuronal cell composition to autism-like behavior.
Pianp (also known as Leda-1) is a type I transmembrane protein with preferential expression in the mammalian CNS. Its processing is characterized by proteolytic cleavage by a range of proteases including Adam10, Adam17, MMPs, and the γ-secretase complex. Pianp can interact with Pilrα and the GB1a subunit of the GABA receptor (GBR) complex. A recent case description of a boy with global developmental delay and homozygous nonsense variant in PIANP supports the hypothesis that PIANP is involved in the control of behavioral traits in mammals. To investigate the physiological functions of Pianp, constitutive, global knockout mice were generated and comprehensively analyzed. Broad assessment did not indicate malformation or malfunction of internal organs. In the brain, however, decreased sizes and altered cellular compositions of the dentate gyrus as well as the cerebellum, including a lower number of cerebellar Purkinje cells, were identified. Functionally, loss of Pianp led to impaired presynaptic GBR-mediated inhibition of glutamate release and altered gene expression in the cortex, hippocampus, amygdala, and hypothalamus including downregulation of Erdr1, a gene linked to autism-like behavior. Behavioral phenotyping revealed that Pianp deficiency leads to context-dependent enhanced anxiety and spatial learning deficits, an altered stress response, severely impaired social interaction, and enhanced repetitive behavior, which all represent characteristic features of an autism spectrum disorder-like phenotype. Altogether, Pianp represents a novel candidate gene involved in autism-like behavior, cerebellar and hippocampal pathology, and GBR signaling.
Mol Psychiatry, 2020; 25
33087874:

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BOARD NUMBER: S01-538

ALTERED ULTRASTRUCTURE OF SYNAPTIC MITOCHONDRIA IN A NOVEL MOUSE MODEL OF AUTISM-ASSOCIATED NEURODEVELOPMENTAL DISORDER, TRAP-1 MUTANT MICE

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Marta Magnowska[1], Bozena Kuzniewska[1], Ewelina Knapska[2], Andrzej Dziembowski[3], Magdalena Dziembowska[1]
[1]University of Warsaw, Centre Of New Technologies, Warsaw, Poland, [2]Nencki Institute of Experimental Biology, Centre Of Excellence For Neural Plasticity And Brain Disorders (braincity), Warsaw, Poland, [3]International Institute of Molecular and Cell Biology, Laboratory Of Rna Biology, Warsaw, Poland

Neurons critically depend on mitochondrial function to establish membrane excitability and to execute the complex processes of neurotransmission and plasticity. Recently it was shown, that synapses are the regions of the neuron with the highest energy consumption, thus they have the highest demand for mitochondrial ATP production. Additionally, dysregulated synthesis of mitochondrial proteins may contribute to the pathomechanism of neurodevelopmental disorders. We created a new mouse model of autism-associated neurodevelopmental disorder, based on the human mutation in TRAP-1 gene. TRAP-1 mutant mice display behaviors characteristic for ASD such as social behavior deficits. TRAP1 is a mitochondrial chaperone from HSP-90 family engaged in the regulation of mitochondrial stress response. Mitochondrial functions are intrinsically linked to their morphology and membrane ultrastructure. For a detailed analysis of mitochondria morphology and organization in the hippocampi of TRAP1 mutant mice, heterozygous and wild-types, we used Serial Block Face-Scanning Electron Microscopy (SBF-SEM). Mitochondria shape and volume reconstruction was performed in RECONSTRUCT software. We compared the morphology and number of synaptic mitochondria in TRAP-1 mutant, heterozygous and wt mice and found significant changes between the analyzed genotypes.

Pubmed:
35169214: Figiel I, Bączyńska E, Wójtowicz T, Magnowska M, Buszka A, Bijata M, Włodarczyk J
The cell adhesion protein dystroglycan affects the structural remodeling of dendritic spines.
Dystroglycan (DG) is a cell membrane protein that binds to the extracellular matrix in various mammalian tissues. The function of DG has been well defined in embryonic development as well as in the proper migration of differentiated neuroblasts in the central nervous system (CNS). Although DG is known to be a target for matrix metalloproteinase-9 (MMP-9), cleaved in response to enhanced synaptic activity, the role of DG in the structural remodeling of dendritic spines is still unknown. Here, we report for the first time that the deletion of DG in rat hippocampal cell cultures causes pronounced changes in the density and morphology of dendritic spines. Furthermore, we noted a decrease in laminin, one of the major extracellular partners of DG. We have also observed that the lack of DG evokes alterations in the morphological complexity of astrocytes accompanied by a decrease in the level of aquaporin 4 (AQP4), a protein located within astrocyte endfeet surrounding neuronal dendrites and synapses. Regardless of all of these changes, we did not observe any effect of DG silencing on either excitatory or inhibitory synaptic transmission. Likewise, the knockdown of DG had no effect on Psd-95 protein expression. Our results indicate that DG is involved in dendritic spine remodeling that is not functionally reflected. This may suggest the existence of unknown mechanisms that maintain proper synaptic signaling despite impaired structure of dendritic spines. Presumably, astrocytes are involved in these processes.
Sci Rep, 2022; 12
31481881: Ruszczycki B, Pels KK, Walczak A, Zamłyńska K, Such M, Szczepankiewicz AA, Hall MH, Magalska A, Magnowska M, Wolny A, Bokota G, Basu S, Pal A, Plewczynski D, Wilczyński GM
Three-Dimensional Segmentation and Reconstruction of Neuronal Nuclei in Confocal Microscopic Images.
The detailed architectural examination of the neuronal nuclei in any brain region, using confocal microscopy, requires quantification of fluorescent signals in three-dimensional stacks of confocal images. An essential prerequisite to any quantification is the segmentation of the nuclei which are typically tightly packed in the tissue, the extreme being the hippocampal dentate gyrus (DG), in which nuclei frequently appear to overlap due to limitations in microscope resolution. Segmentation in DG is a challenging task due to the presence of a significant amount of image artifacts and densely packed nuclei. Accordingly, we established an algorithm based on continuous boundary tracing criterion aiming to reconstruct the nucleus surface and to separate the adjacent nuclei. The presented algorithm neither uses a pre-built nucleus model, nor

performs image thresholding, which makes it robust against variations in image intensity and poor contrast. Further, the reconstructed surface is used to study morphology and spatial arrangement of the nuclear interior. The presented method is generally dedicated to segmentation of crowded, overlapping objects in 3D space. In particular, it allows us to study quantitatively the architecture of the neuronal nucleus using confocal-microscopic approach.
Front Neuroanat, 2019; 13
30965559: Krzystyniak A, Baczynska E, Magnowska M, Antoniuk S, Roszkowska M, Zareba-Koziol M, Das N, Basu S, Pikula M, Wlodarczyk J
Prophylactic Ketamine Treatment Promotes Resilience to Chronic Stress and Accelerates Recovery: Correlation with Changes in Synaptic Plasticity in the CA3 Subregion of the Hippocampus.
Ketamine is an -methyl-d-aspartate receptor antagonist that has gained wide attention as a potent antidepressant. It has also been recently reported to have prophylactic effects in animal models of depression and anxiety. Alterations of neuroplasticity in different brain regions; such as the hippocampus; prefrontal cortex; and amygdala; are a hallmark of stress-related disorders; and such changes may endure beyond the treatment of symptoms. The present study investigated whether a prophylactic injection of ketamine has effects on structural plasticity in the brain in mice that are subjected to chronic unpredictable stress followed by an 8-day recovery period. Ketamine administration (3 mg/kg body weight) 1 h before stress exposure increased the number of resilient animals immediately after the cessation of stress exposure and positively influenced the recovery of susceptible animals to hedonic deficits. At the end of the recovery period; ketamine-treated animals exhibited significant differences in dendritic spine density and dendritic spine morphology in brain regions associated with depression compared with saline-treated animals. These results confirm previous findings of the prophylactic effects of ketamine and provide further evidence of an association between the antidepressant-like effect of ketamine and alterations of structural plasticity in the brain.
Int J Mol Sci, 2019; 20
30442964: Basu S, Saha PK, Roszkowska M, Magnowska M, Baczynska E, Das N, Plewczynski D, Wlodarczyk J
Author Correction: Quantitative 3-D morphometric analysis of individual dendritic spines.
A correction to this article has been published and is linked from the HTML and PDF versions of this paper. The error has been fixed in the paper.
Sci Rep, 2018; 8
29476060: Basu S, Saha PK, Roszkowska M, Magnowska M, Baczynska E, Das N, Plewczynski D, Wlodarczyk J
Quantitative 3-D morphometric analysis of individual dendritic spines.
The observation and analysis of dendritic spines morphological changes poses a major challenge in neuroscience studies. The alterations of their density and/or morphology are indicators of the cellular processes involved in neural plasticity underlying learning and memory, and are symptomatic in neuropsychiatric disorders. Despite ongoing intense investigations in imaging approaches, the relationship between changes in spine morphology and synaptic function is still unknown. The existing quantitative analyses are difficult to perform and require extensive user intervention. Here, we propose a new method for (1) the three-dimensional (3-D) segmentation of dendritic spines using a multi-scale opening approach and (2) define 3-D morphological attributes of individual spines for the effective assessment of their structural plasticity. The method was validated using confocal light microscopy images of dendritic spines from dissociated hippocampal cultures and brain slices (1) to evaluate accuracy relative to manually labeled ground-truth annotations and relative to the state-of-the-art Imaris tool, (2) to analyze reproducibility of user-independence of the segmentation method, and (3) to quantitatively analyze morphological changes in individual spines before and after chemically induced long-term potentiation. The method was monitored and used to precisely describe the morphology of individual spines in real-time using consecutive images of the same dendritic fragment.
Sci Rep, 2018; 8
28066226: Bokota G, Magnowska M, Kuśmierczyk T, Łukasik M, Roszkowska M, Plewczynski D
Computational Approach to Dendritic Spine Taxonomy and Shape Transition Analysis.
The common approach in morphological analysis of dendritic spines of mammalian neuronal cells is to categorize spines into subpopulations based on whether they are stubby, mushroom, thin, or filopodia shaped. The corresponding cellular models of synaptic plasticity, long-term potentiation, and long-term depression associate the synaptic strength with either spine enlargement or spine shrinkage. Although a variety of automatic spine segmentation and feature extraction methods were developed recently, no approaches allowing for an automatic and unbiased distinction between dendritic spine subpopulations and detailed computational models of spine behavior exist. We propose an automatic and statistically based method for the unsupervised construction of spine shape taxonomy based on arbitrary features. The taxonomy is then utilized in the newly introduced computational model of behavior, which relies on transitions between shapes. Models of different populations are compared using supplied bootstrap-based statistical tests. We compared two populations of spines at two time points. The first population was stimulated with long-term potentiation, and the other in the resting state was used as a

control. The comparison of shape transition characteristics allowed us to identify the differences between population behaviors. Although some extreme changes were observed in the stimulated population, statistically significant differences were found only when whole models were compared. The source code of our software is freely available for non-commercial use.
Front Comput Neurosci, 2016; 10
27282248: Magnowska M, Gorkiewicz T, Suska A, Wawrzyniak M, Rutkowska-Wlodarczyk I, Kaczmarek L, Wlodarczyk J
Transient ECM protease activity promotes synaptic plasticity.
Activity-dependent proteolysis at a synapse has been recognized as a pivotal factor in controlling dynamic changes in dendritic spine shape and function; however, excessive proteolytic activity is detrimental to the cells. The exact mechanism of control of these seemingly contradictory outcomes of protease activity remains unknown. Here, we reveal that dendritic spine maturation is strictly controlled by the proteolytic activity, and its inhibition by the endogenous inhibitor (Tissue inhibitor of matrix metalloproteinases-1 - TIMP-1). Excessive proteolytic activity impairs long-term potentiation of the synaptic efficacy (LTP), and this impairment could be rescued by inhibition of protease activity. Moreover LTP is altered persistently when the ability of TIMP-1 to inhibit protease activity is abrogated, further demonstrating the role of such inhibition in the promotion of synaptic plasticity under well-defined conditions. We also show that dendritic spine maturation involves an intermediate formation of elongated spines, followed by their conversion into mushroom shape. The formation of mushroom-shaped spines is accompanied by increase in AMPA/NMDA ratio of glutamate receptors. Altogether, our results identify inhibition of protease activity as a critical regulatory mechanism for dendritic spines maturation.
Sci Rep, 2016; 6
27153678: Basu S, Plewczynski D, Saha S, Roszkowska M, Magnowska M, Baczynska E, Wlodarczyk J
2dSpAn: semiautomated 2-d segmentation, classification and analysis of hippocampal dendritic spine plasticity.
Accurate and effective dendritic spine segmentation from the dendrites remains as a challenge for current neuroimaging research community. In this article, we present a new method (2dSpAn) for 2-d segmentation, classification and analysis of structural/plastic changes of hippocampal dendritic spines. A user interactive segmentation method with convolution kernels is designed to segment the spines from the dendrites. Formal morphological definitions are presented to describe key attributes related to the shape of segmented spines. Spines are automatically classified into one of four classes: Stubby, Filopodia, Mushroom and Spine-head Protrusions.
Bioinformatics, 2016; 32
24853857: Szepesi Z, Hosy E, Ruszczycki B, Bijata M, Pyskaty M, Bikbaev A, Heine M, Choquet D, Kaczmarek L, Wlodarczyk J
Synaptically released matrix metalloproteinase activity in control of structural plasticity and the cell surface distribution of GluA1-AMPA receptors.
Synapses are particularly prone to dynamic alterations and thus play a major role in neuronal plasticity. Dynamic excitatory synapses are located at the membranous neuronal protrusions called dendritic spines. The ability to change synaptic connections involves both alterations at the morphological level and changes in postsynaptic receptor composition. We report that endogenous matrix metalloproteinase (MMP) activity promotes the structural and functional plasticity of local synapses by its effect on glutamate receptor mobility and content. We used live imaging of cultured hippocampal neurons and quantitative morphological analysis to show that chemical long-term potentiation (cLTP) induces the permanent enlargement of a subset of small dendritic spines in an MMP-dependent manner. We also used a superresolution microscopy approach and found that spine expansion induced by cLTP was accompanied by MMP-dependent immobilization and synaptic accumulation as well as the clustering of GluA1-containing AMPA receptors. Altogether, our results reveal novel molecular and cellular mechanisms of synaptic plasticity.
PLoS One, 2014; 9
23392678: Walczak A, Szczepankiewicz AA, Ruszczycki B, Magalska A, Zamlynska K, Dzwonek J, Wilczek E, Zybura-Broda K, Rylski M, Malinowska M, Dabrowski M, Szczepinska T, Pawlowski K, Pyskaty M, Wlodarczyk J, Szczerbal I, Switonski M, Cremer M, Wilczynski GM
Novel higher-order epigenetic regulation of the Bdnf gene upon seizures.
Studies in cultured cells have demonstrated the existence of higher-order epigenetic mechanisms, determining the relationship between expression of the gene and its position within the cell nucleus. It is unknown, whether such mechanisms operate in postmitotic, highly differentiated cell types, such as neurons in vivo. Accordingly, we examined whether the intranuclear positions of Bdnf and Trkb genes, encoding the major neurotrophin and its receptor respectively, change as a result of neuronal activity, and what functional consequences such movements may have. In a rat model of massive neuronal activation upon kainate-induced seizures we found that elevated neuronal expression of Bdnf is associated with its detachment from the nuclear lamina, and translocation toward the nucleus center. In contrast, the position of stably expressed Trkb remains unchanged after seizures. Our study demonstrates that activation-dependent architectural

remodeling of the neuronal cell nucleus in vivo contributes to activity-dependent changes in gene expression in the brain. J Neurosci, 2013; 33

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DEXAMETHASONE IMPROVES CELL SURFACE TRAFFICKING OF R451C NEUROLIGIN3, AN AUTISM GENE RISK

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Tamara Diamanti[1], Federica Serafini[1], Camilla Perfetto[1], Lorenza Mautone[2], Clotilde Lauro[2], Tommaso Seri[1], Andrea Setini[1], Laura Trobiani[3], Davide Comoletti[3], Arianna Rinaldi[1], Silvia Di Angelantonio[2], Emanuele Cacci[1], Antonella De Jaco[1]
[1]La Sapienza University of Rome, Biology And Biotechnologies C. Darwin, Rome, Italy, [2]La sapienza University of Rome, Department Of Physiology And Pharmacology "v. Erspamer", Rome, Italy, [3]Victoria University of Wellington, School Of Biological Sciences, Wellington, New Zealand

Aims Autism spectrum disorders (ASDs) are neurodevelopmental syndromes also characterized by alterations in neurotransmission. Among the risk genes are synaptic molecules such as the Neuroligins (NLGNs). The autism-linked substitution, R451C in NLGN3, induces a local misfolding of the extracellular domain, causing defective trafficking and partial retention of the mutant protein in the Endoplasmic Reticulum (ER) and the activation of the Unfolded Protein Response. The knock-in (KI) R451C mouse model presents reduced NLGN3 levels in the brain and autistic-like behaviors. Compounds belonging to the family of glucocorticoids, such as dexamethasone (DEX), were selected for increasing the secretion of a soluble form of R451C NLGN3, expressed in HEK293 cells. **Methods** The effect of DEX was studied in HEK-293 cells stably expressing full-length NLGN3, either WT or R451C, and in adult neural progenitor cells from the hippocampus of either WT or R451C NLGN3 KI mice, endogenously expressing mutant NLGN3. **Results** DEX treatment rescues R451C NLGN3 mutant protein levels, promotes its trafficking to the cell surface from the ER and mitigates ER stress, both in over-expression and in physiological conditions. The formation of artificial synapses from co-cultured hippocampal neurons and HEK-293 cells expressing R451C NLGN3 will be evaluated in the presence or absence of DEX. **Conclusions** Our data show a strategy to rescue, *in vitro,* NLGN3 R451C trafficking. The effect of the DEX in R451C NLGN3 mouse model will be tested to ascertain whether improving trafficking of mutant NLGN3 R451C, *in vivo*, could rescue behavioral and functional defects of the R451C NLGN3 KI model.

Pubmed:

32991906: Trobiani L, Meringolo M, Diamanti T, Bourne Y, Marchot P, Martella G, Dini L, Pisani A, De Jaco A, Bonsi P
The neuroligins and the synaptic pathway in Autism Spectrum Disorder.
The genetics underlying autism spectrum disorder (ASD) is complex and heterogeneous, and de novo variants are found in genes converging in functional biological processes. Neuronal communication, including trans-synaptic signaling involving two families of cell-adhesion proteins, the presynaptic neurexins and the postsynaptic neuroligins, is one of the most recurrently affected pathways in ASD. Given the role of these proteins in determining synaptic function, abnormal synaptic plasticity and failure to establish proper synaptic contacts might represent mechanisms underlying risk of ASD. More than 30 mutations have been found in the neuroligin genes. Most of the resulting residue substitutions map in the extracellular, cholinesterase-like domain of the protein, and impair protein folding and trafficking. Conversely, the stalk and intracellular domains are less affected. Accordingly, several genetic animal models of ASD have been generated, showing behavioral and synaptic alterations. The aim of this review is to discuss the current knowledge on ASD-linked mutations in the neuroligin proteins and their effect on synaptic function, in various brain areas and circuits.
Neurosci Biobehav Rev, 2020; 119

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BOARD NUMBER: S01-540

THE DISEASE-ASSOCIATED PROTEIN CYFIP1 REGULATES AXONAL DEVELOPMENT AND BRANCHING

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Aims: Copy-number variants of the CYFIP1 gene in humans have been associated to Autism and Schizophrenia, two neuropsychiatric disorders characterized by defects in brain connectivity. CYFIP1 has a dual role regulating protein synthesis and actin remodeling, both molecular events being crucial for correct post-synaptic functions. Consistent with this, *Cyfip1* deficient mice present an immature spine morphology and synaptic plasticity defects. However, a possible role of CYFIP1 in brain connectivity and presynaptic function was largely unknown. *Methods:* Here we use state of the art imaging, cellular and molecular biology techniques to investigate the role of CYFIP1 in axonal development. *Results:* Here, we show that CYFIP1 plays an important role in brain functional connectivity, callosal axonal development and function. We find that adult *Cyfip1* heterozygous (*Cyfip1*$^{+/-}$) mice have reduced bilateral functional connectivity and defects in white matter architecture, deficits in the callosal axons, namely reduced myelination. Reduced CYFIP1 levels during development result in a delayed growth and arborization of callosal axons. We have identified a series of molecular targets of CYFIP1 that might explain the axonal phenotype observed in the adult and provide a therapeutical approach to ameliorate those observed deficits. *Conclusions:* Altogether, our results show that *Cyfip1* haploinsufficiency compromises callosal axons and connectivity in various ways, which not only might explain the genetic association of this gene to neuropsychiatric disorders but also provide further insights into its function as key molecular player in the development of callosal projections.

FENS Forum 2022
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BOARD NUMBER: S01-541

MIRTAZAPINE RESCUES NEURONAL ATROPHY IN RETT SYNDROME THROUGH TRKB TRANSACTIVATION VIA LPA-RECEPTOR

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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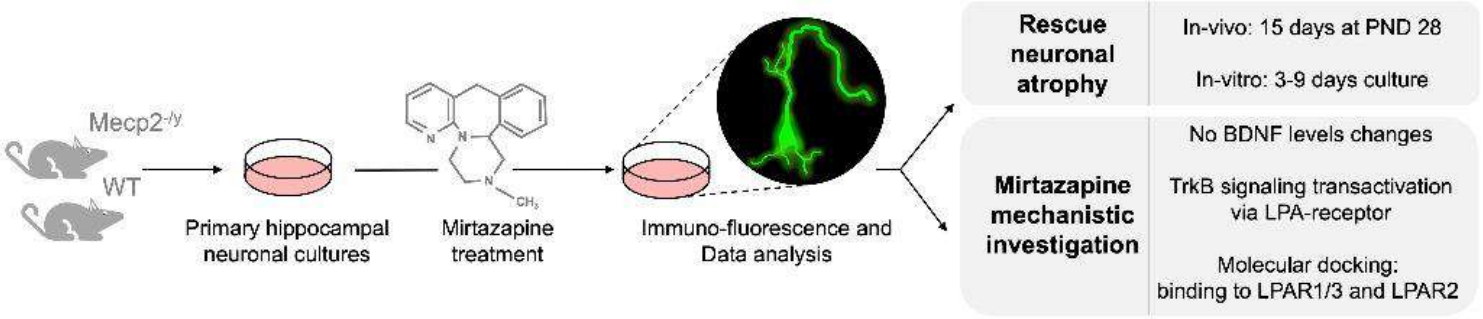
Aims. Rett Syndrome (RTT) is a neurodevelopment disorder mainly caused by Methyl CpG binding protein 2 (*MeCP2*) gene mutations causing neuronal atrophy which is substantially rescued in adult MeCP2-null mice by chronic treatment with the antidepressant mirtazapine (MTZ). However, the mechanisms of action of mirtazapine in RTT remain unclear. **Methods**. MeCP2-null mice were daily-treated with 50 mg/kg MTZ treatment from postnatal-day 28 for 15 days and soma and dendritic size of hippocampal neurons were measured. Cultured hippocampal neurons were treated with 10 µM MTZ for 9 or 3 days. Time course of BDNF mRNA and protein expression and of TrkB phosphorylation were investigated MTZ treatment. Molecular docking of mirtazapine was performed on crystal structure of LPAR1 and homology models of LPAR2 and LPAR3 with AutoDock Vina. **Results**. MTZ treatment in vivo and in vitro completely restored dendritic arborization and soma size of hippocampal neurons. Although BDNF levels in vivo and in vitro were unaffected by MTZ treatment, the TrkB signaling was activated by MTZ by transactivation through the lysophosphaditic acid receptor and src-fyn kinases. Molecular docking revealed that MTZ may bind to LPAR1/3 and weakly to LPAR2. **Conclusions.** Mirtazapine is able to elicit trophic effects through TrkB receptor transactivation leading to the complete rescue of dendritic atrophy in RTT. These results reveal a novel mechanism of action of MTZ and support its use as a novel treatment for Rett syndrome.



Pubmed:
32051524: Nerli E, Roggero OM, Baj G, Tongiorgi E
In vitro modeling of dendritic atrophy in Rett syndrome: determinants for phenotypic drug screening in neurodevelopmental disorders.
Dendritic atrophy, defined as the reduction in complexity of the neuronal arborization, is a hallmark of several neurodevelopmental disorders, including Rett Syndrome (RTT). RTT, affecting 1:10,000 girls worldwide, is mainly caused by mutations in the MECP2 gene and has no cure. We describe here an in vitro model of dendritic atrophy in Mecp2 mouse hippocampal primary cultures, suitable for phenotypic drug-screening. Using High-Content Imaging techniques, we systematically investigated the impact of culturing determinants on several parameters such as neuronal survival, total dendritic length, dendritic endpoints, soma size, cell clusterization, spontaneous activity. Determinants included cell-seeding density, glass or polystyrene substrates, coating with poly-Ornithine with/without Matrigel and miniaturization from 24 to 96-half surface multiwell plates. We show that in all plate-sizes at densities below 320 cells/mm, morphological parameters remained constant while spontaneous network activity decreased according to the cell-density. Mecp2 neurons cultured at

160 cells/mm density in 96 multiwell plates, displayed significant dendritic atrophy and showed a marked increase in dendritic length following treatment with Brain-derived neurotrophic factor (BDNF) or Mirtazapine. In conclusion, we have established a phenotypic assay suitable for fast screening of hundreds of compounds, which may be extended to other neurodevelopmental diseases with dendritic atrophy.
Sci Rep, 2020; 10

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BOARD NUMBER: S01-542

THE AUTISM RISK GENE DDX3X IN SHAPING NEURONAL MORPHOGENESIS AND SYNAPTOGENESIS IN A SEX-SPECIFIC MANNER

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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This project aims at unraveling the cellular and molecular functions of the autism risk gene *DDX3X* during neuronal development. *DDX3X* is X-linked but escapes X chromosome inactivation in human and mice, resulting in higher expression of DDX3X in female brains when compared with male brains. Mutations in *DDX3X* affect primarily females. DDX3X regulates mRNA translation, but the mechanisms of action in neurons, the impact of clinical mutations and the influence of sex have not been studied yet. Understanding the role of DDX3X in the regulation of local mRNA translation in female and male neurons might offer a new key to decipher ASD. We generated a mouse with loxP sites around exon 2 of *Ddx3x* (*Ddx3x*^{flox} mice). Using this model, we can generate *Ddx3x*-haploinsufficient female neurons or *Ddx3x*-null male neurons, upon transfection of male (*Ddx3x*$^{flox/y}$) and female (*Ddx3x*$^{flox/+}$) cortical neurons with a mCherry-Cre construct. We also introduced sex-specific mutations in female and male neurons, after manipulating *Ddx3x* dosage. We then examined morphogenesis, synaptogenesis, and mRNA translation. DDX3X contributes to sex differences in neuronal morphogenesis and synaptogenesis. Female-pathogenic mutations completely disrupt neuronal morphology, while male pathogenic mutations cause a milder phenotype. These findings support a sex-specific role for DDX3X in regulating neuronal development. Our data lay the bases to understand the sex biases in the prevalence and severity of DDX3X syndrome. A deeper insight on the molecular mechanisms underlying this sex difference will also shed the light for more personalized treatment for girls and boys.

Pubmed:

33402706: Mossa A, Pagano J, Ponzoni L, Tozzi A, Vezzoli E, Sciaccaluga M, Costa C, Beretta S, Francolini M, Sala M, Calabresi P, Boeckers TM, Sala C, Verpelli C
Developmental impaired Akt signaling in the Shank1 and Shank3 double knock-out mice.
Human mutations and haploinsufficiency of the SHANK family genes are associated with autism spectrum disorders (ASD) and intellectual disability (ID). Complex phenotypes have been also described in all mouse models of Shank mutations and deletions, consistent with the heterogeneity of the human phenotypes. However, the specific role of Shank proteins in synapse and neuronal functions remain to be elucidated. Here, we generated a new mouse model to investigate how simultaneously deletion of Shank1 and Shank3 affects brain development and behavior in mice. Shank1-Shank3 DKO mice showed a low survival rate, a developmental strong reduction in the activation of intracellular signaling pathways involving Akt, S6, ERK1/2, and eEF2 during development and a severe behavioral impairments. Our study suggests that Shank1 and Shank3 proteins are essential to developmentally regulate the activation of Akt and correlated intracellular pathways crucial for mammalian postnatal brain development and synaptic plasticity. Therefore, Akt function might represent a new therapeutic target for enhancing cognitive abilities of syndromic ASD patients.
Mol Psychiatry, 2021; 26
31872500: Mossa A, Manzini MC
Molecular causes of sex-specific deficits in rodent models of neurodevelopmental disorders.
Neurodevelopmental disorders (NDDs) such as intellectual disability and autism spectrum disorder consistently show a male bias in prevalence, but it remains unclear why males and females are affected with different frequency. While many behavioral studies of transgenic NDD models have focused only on males, the requirement by the National Institutes of Health to consider sex as a biological variable has promoted the comparison of male and female performance in wild-type and mutant animals. Here, we review examples of rodent models of NDDs in which sex-specific deficits were identified in molecular, physiological, and/or behavioral responses, showing sex differences in susceptibility to disruption of genes mutated in NDDs. Haploinsufficiency in genes involved in mechanisms such as synaptic function (GABRB3 and NRXN1), chromatin remodeling (CHD8, EMHT1, and ADNP), and intracellular signaling (CC2D1A and ERK1) lead to more severe

behavioral outcomes in males. However, in the absence of behavioral deficits, females can still present with cellular and electrophysiological changes that could be due to compensatory mechanisms or differential allocation of molecular and cellular functions in the two sexes. By contrasting these findings with mouse models where females are more severely affected (MTHFR and AMBRA1), we propose a framework to approach the study of sex-specific deficits possibly leading to sex bias in NDDs.
J Neurosci Res, 2021; 99
30732858: Zamarbide M, Mossa A, Muñoz-Llancao P, Wilkinson MK, Pond HL, Oaks AW, Manzini MC
Male-Specific cAMP Signaling in the Hippocampus Controls Spatial Memory Deficits in a Mouse Model of Autism and Intellectual Disability.
The prevalence of neurodevelopmental disorders is biased toward male individuals, with male-to-female ratios of 2:1 in intellectual disability and 4:1 in autism spectrum disorder. However, the molecular mechanisms of such bias remain unknown. While characterizing a mouse model for loss of the signaling scaffold coiled-coil and C2 domain-containing protein 1A (CC2D1A), which is mutated in intellectual disability and autism spectrum disorder, we identified biochemical and behavioral differences between male and female mice, and explored whether CC2D1A controls male-specific intracellular signaling.
Biol Psychiatry, 2019; 85
29175319: Mossa A, Giona F, Pagano J, Sala C, Verpelli C
SHANK genes in autism: Defining therapeutic targets.
Prog Neuropsychopharmacol Biol Psychiatry, 2018; 84
27005990: Heise C, Taha E, Murru L, Ponzoni L, Cattaneo A, Guarnieri FC, Montani C, Mossa A, Vezzoli E, Ippolito G, Zapata J, Barrera I, Ryazanov AG, Cook J, Poe M, Stephen MR, Kopanitsa M, Benfante R, Rusconi F, Braida D, Francolini M, Proud CG, Valtorta F, Passafaro M, Sala M, Bachi A, Verpelli C, Rosenblum K, Sala C
eEF2K/eEF2 Pathway Controls the Excitation/Inhibition Balance and Susceptibility to Epileptic Seizures.
Alterations in the balance of inhibitory and excitatory synaptic transmission have been implicated in the pathogenesis of neurological disorders such as epilepsy. Eukaryotic elongation factor 2 kinase (eEF2K) is a highly regulated, ubiquitous kinase involved in the control of protein translation. Here, we show that eEF2K activity negatively regulates GABAergic synaptic transmission. Indeed, loss of eEF2K increases GABAergic synaptic transmission by upregulating the presynaptic protein Synapsin 2b and α5-containing GABAA receptors and thus interferes with the excitation/inhibition balance. This cellular phenotype is accompanied by an increased resistance to epilepsy and an impairment of only a specific hippocampal-dependent fear conditioning. From a clinical perspective, our results identify eEF2K as a potential novel target for antiepileptic drugs, since pharmacological and genetic inhibition of eEF2K can revert the epileptic phenotype in a mouse model of human epilepsy.
Cereb Cortex, 2017; 27
26338675: Sala C, Vicidomini C, Bigi I, Mossa A, Verpelli C
Shank synaptic scaffold proteins: keys to understanding the pathogenesis of autism and other synaptic disorders.
Shank/ProSAP proteins are essential to synaptic formation, development, and function. Mutations in the family of SHANK genes are strongly associated with autism spectrum disorders (ASD) and other neurodevelopmental and neuropsychiatric disorders, such as intellectual disability (ID), and schizophrenia. Thus, the term 'Shankopathies' identifies a number of neuronal diseases caused by alteration of Shank protein expression leading to abnormal synaptic development. With this review we want to summarize the major genetic, molecular, behavior and electrophysiological studies that provide new clues into the function of Shanks and pave the way for the discovery of new therapeutic drugs targeted to treat patients with SHANK mutations and also patients affected by other neurodevelopmental and neuropsychiatric disorders. Shank/ProSAP proteins are essential to synaptic formation, development, and function. Mutations in the family of SHANK genes are strongly associated with autism spectrum disorders (ASD) and other neurodevelopmental and neuropsychiatric disorders, such as intellectual disability (ID), and schizophrenia (SCZ). With this review we want to summarize the major genetic, molecular, behavior and electrophysiological studies that provide new clues into the function of Shanks and pave the way for the discovery of new therapeutic drugs targeted to treat patients with SHANK mutations.
J Neurochem, 2015; 135
24005771: Vasco C, Canazza A, Rizzo A, Mossa A, Corsini E, Silvani A, Fariselli L, Salmaggi A, Ciusani E
Circulating T regulatory cells migration and phenotype in glioblastoma patients: an in vitro study.
Glioblastoma multiforme (GBM) is the most aggressive primary human brain tumor. The relatively high amount of T regulatory lymphocytes present in the tumor, contributes to the establishment of an immunosuppressive microenvironment. Samples of peripheral blood were collected from GBM patients and healthy controls and a purified population of Treg (CD4(+)/CD25(bright)) was isolated using flow cytometric cell sorting. Treg migrating capacities toward human glioma cell line conditioned medium were evaluated through an in vitro migration test. Our data show that supernatants collected from GBM cell lines were more attractant to Treg when compared to complete standard medium. The addition of an anti-CCL2 antibody

to conditioned medium decreased conditioned medium-depending Treg migration, suggesting that CCL2 (also known as Monocyte Chemoattractant Protein, MCP-1) is implicated in the process. The number of circulating CD4(+)/µL or Treg/µL was similar in GBM patients and controls. Specific Treg markers (FOXP3; CD127; Helios; GITR; CTLA4; CD95; CCR2, CCR4; CCR7) were screened in peripheral blood and no differences could be detected between the two populations. These data confirm that the tumor microenvironment is attractive to Treg, which tend to migrate toward the tumor region changing the immunological response. Though we provide evidence that CCL2 is implicated in Treg migration, other factors are needed as well to provide such effect.
J Neurooncol, 2013; 115

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BOARD NUMBER: S01-543

A NOVEL ROLE FOR JANUS KINASE AND MICROTUBULE-INTERACTING PROTEIN 1 (JAKMIP1) IN MODULATING SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION-3 (STAT3)-MEDIATED CYTOKINE SIGNALLING IN NEURONAL CELLS

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Autism spectrum disorder (ASD) is characterised by impaired social interaction, language delay and repetitive or restrictive behaviours. Despite increasing in prevalence, its aetiology remains elusive. *JAKMIP1* was identified as an ASD candidate gene due to its dysregulation in both syndromic and idiopathic ASD. *JAKMIP1* is highly expressed in developing neurons and interacts with a wide array of binding partners. The N-terminus stabilises microtubules, promoting neuronal migration and neurite extension. Conversely, the C-terminus interacts with JAK1, a receptor-associated tyrosine kinase that initiates Signal Transducer and Activator of Transcription (STAT) signalling downstream of cytokine receptors. We demonstrate that JAKMIP1 forms a component of the Interleukin-6 Receptor (IL-6R) complex in neurons, co-localising with IL-6R subunits and associated JAK1 in neuronal cells. This suggests that JAKMIP1 may regulate JAK1/STAT3 signalling downstream of the IL-6R. To investigate this potential role in cytokine signalling, we generated a *JAKMIP1*-knockout human neuroblastoma SH-SY5Y cell line using CRISPR-Cas9 genome-editing. JAKMIP1-deficiency was observed to reduce *STAT3* expression and impair IL-6-induced STAT3 activity, which was validated through measuring the expression of STAT3-target genes. Importantly, IL-6 is reported to promote neuronal differentiation and neuritogenesis. To determine whether JAKMIP1-modulated STAT3 activity may play a role in IL-6-induced neuritogenesis, we assessed the morphology of differentiated SH-SY5Y cells through immunofluorescent staining. Agreeing with previous studies, JAKMIP1-deficiency results in reduced neurite extension and neuritogenesis. Interestingly though, JAKMIP1 deficiency also impairs IL-6-induced neuritogenesis in SH-SY5Y cells. Altogether, these findings suggest that JAKMIP1 may regulate neuronal responses to IL-6 during nervous system development via modulating STAT3 activity.

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BOARD NUMBER: S01-544

NEUROANATOMICAL MAPPING OF THE LIGAND BINDING PROFILE IN VARIOUS AUTISM SPECTRUM DISORDER (ASD) MODELS AT ADULTHOOD

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Leonardo Nardi[1], Stuti Chhabra[1], Petra Leukel[2], Clemens Sommer[2], Michael Schmeisser[1]
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Aims. ASD comprises multifactorial neurodevelopmental disorders characterized by impaired social interaction and communication and repetitive behavior, whose molecular underpinnings are not fully understood. Several lines of evidence highlighted the crucial balance between excitatory and inhibitory transmission in ASD pathogenesis. Aim of the study was to determine the binding densities of NMDA, AMPA and GABA-A receptors in main regions implicated in ASD such as dorsal hippocampus (DH) and prefrontal cortex (PFC) of adult BTBR (idiopathic ASD model) and FMR1 knockout (genetic ASD model) animals. We hypothesize that brain region-specific imbalances between excitatory and inhibitory inputs might account for at least some of the alterations typical of ASD. **Methods.** *In vitro* receptor autoradiography was performed by incubating brain slices containing the regions of interest with tritium-labeled ligands specific for AMPA, NMDA and GABA-A receptors. Hematoxylin-eosin staining supported us in localizing and analyzing the regions of interest on the autoradiograms. The ratio between ligand binding to excitatory and inhibitory receptors (ligand binding E/I ratio) was also calculated. **Results.** Both in BTBR and FMR1 mice, GABA binding density was higher in the DH, also leading to a reduced ligand binding E/I ratio. Interestingly, the ligand binding E/I ratio in the PFC of the FMR1 mice was higher than in controls, while in the BTBR it was reduced. **Conclusions.** Our preliminary results confirm a major involvement of excitatory and inhibitory ligand binding to main ionotropic membrane receptors and clearly outline brain region-specific effects. We are running quantitative western blots and electrophysiology to strengthen our findings.

Pubmed:
33720793: Serena G, Nardi L, Schmeisser MJ, Angus LDG
Carl Toldt Centennial, Surgeon and Anatomist.
Carl Florian Toldt was an Austrian anatomist who made meaningful contributions worldwide and defined what is one of the most important surgical landmarks in abdominal surgery. Through his research studies, the embryologic dissection plane known as the "White Line of Toldt" represents an important anatomical landmark that helps to mobilize either the ascending or descending colon. His career spanned over 45 years, beginning in Verona and continuing to Prague and Vienna. He was an author of several innovative books and scientific articles regarding micro- and macroscopic anatomy. In addition, he received numerous recognitions and prizes for his work, making him an essential figure in the medical scientific community. Even a street in Vienna, Karl-Toldt-Weg, is named in his honor. The purpose of this historical article is to celebrate and honor Toldt 100 years following his death, remembering his scientific contributions to the medical and surgical fields and giving thanks for his numerous accomplishments. This article brings light to the man behind the eponym.
Am Surg, 2021; 87
34478569: Bicker F, Nardi L, Maier J, Vasic V, Schmeisser MJ
Criss-crossing autism spectrum disorder and adult neurogenesis.
Autism spectrum disorder (ASD) comprises a group of multifactorial neurodevelopmental disorders primarily characterized by deficits in social interaction and repetitive behavior. Although the onset is typically in early childhood, ASD poses a lifelong challenge for both patients and caretakers. Adult neurogenesis (AN) is the process by which new functional neurons are created from neural stem cells existing in the post-natal brain. The entire event is based on a sequence of cellular processes, such as proliferation, specification of cell fate, maturation, and ultimately, synaptic integration into the existing neural circuits. Hence, AN is implicated in structural and functional brain plasticity throughout life. Accumulating evidence shows that impaired AN may underlie some of the abnormal behavioral phenotypes seen in ASD. In this review, we approach the interconnections between the molecular pathways related to AN and ASD. We also discuss existing therapeutic approaches targeting such pathways both in preclinical and clinical studies. A deeper understanding of how ASD and AN reciprocally

affect one another could reveal important converging pathways leading to the emergence of psychiatric disorders.
J Neurochem, 2021; 159
33340060: Rojas-Charry L, Nardi L, Methner A, Schmeisser MJ
Abnormalities of synaptic mitochondria in autism spectrum disorder and related neurodevelopmental disorders.
Autism spectrum disorder (ASD) is a neurodevelopmental condition primarily characterized by an impairment of social interaction combined with the occurrence of repetitive behaviors. ASD starts in childhood and prevails across the lifespan. The variability of its clinical presentation renders early diagnosis difficult. Mutations in synaptic genes and alterations of mitochondrial functions are considered important underlying pathogenic factors, but it is obvious that we are far from a comprehensive understanding of ASD pathophysiology. At the synapse, mitochondria perform diverse functions, which are clearly not limited to their classical role as energy providers. Here, we review the current knowledge about mitochondria at the synapse and summarize the mitochondrial disturbances found in mouse models of ASD and other ASD-related neurodevelopmental disorders, like DiGeorge syndrome, Rett syndrome, Tuberous sclerosis complex, and Down syndrome.
J Mol Med (Berl), 2021; 99

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BOARD NUMBER: S01-545

SPECIFIC ANATOMICAL MAPPING OF RECEPTOR DENSITY ALTERATIONS IN VARIOUS MOUSE MODELS OF AUTISM USING QUANTITATIVE IN-VITRO RECEPTOR AUTORADIOGRAPHY

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Stuti Chhabra
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Aims: Tight balance between excitatory and inhibitory synaptic transmission mainly at neural circuits is crucial for normal brain development and function. Accordingly, shifts in balances, particularly glutamatergic and GABAergic synapses are undertaken as one of the key underlying mechanisms in understanding the pathophysiology of ASD. To this axis, we decided to undertake a comparative study to demonstrate the alteration of receptor densities of ionotropic glutamate (iGluRs) and GABAA receptors in two typical mouse lines, Fmr1 (genetic) and BTBR (idiopathic) characterised to study the ASD features in an age and region dependent fashion. **Methods:** *In vitro* receptor autoradiography was performed by incubating brain slices containing the regions of interest with tritium-labeled ligands specific for AMPA, NMDA and GABA-A receptors. Hematoxylin-eosin staining supported us in localizing and analyzing the regions of interest on the autoradiograms. The ratio between ligand binding to excitatory and inhibitory receptors (ligand binding E/I ratio) was also calculated. **Results:** In BTBR mice it is observed that $GABA_A$ receptor density is significantly upregulated in dorsal hippocampus and prefrontal cortex. Interestingly, changes in AMPA receptor densities are particularly seen in striatum as compared to the other regions. Furthermore, in Fmr1 KO mice changes in AMPA and NMDA receptor densities were more profound in dorsal hippocampus of as compared to GABAA receptor density. Significant changes in GABAA receptor density in cerebellum are also highlighted. **Conclusion:** The aforementioned changes thus designate the regional variations of different receptor densities at an early stage, 4week, of postnatal brain development in BTBR and Fmr1 mice.

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BOARD NUMBER: S01-546

MITOCHONDRIAL MORPHOLOGY AND DYNAMIC ABNORMALITIES IN AN IN VITRO MODEL OF RETT SYNDROME

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Rett syndrome (RTT) is a rare neurodevelopmental disorder that affects predominantly females, with a prevalence of 1 in every 10,000 female births. The disorder manifests early in life and to this date there is no cure. It has been reported that oxidative stress in mitochondria contributes to RTT pathogenesis. **Aims**: We aimed to characterize mitochondria morphologies and dynamics to identify new therapeutical targets for RTT. **Methods:** We used a well-established *in vitro* model of dendritic atrophy of Mecp2$^{-/y}$ mouse hippocampal primary neurons to evaluate mitochondria morphology, membrane potential, aging and dynamics. We used specific mitochondrial markers (Mito-Dendra2 and Mito-Timer constructs and JC-1 dye) to label the organelles and evaluate which parameters were affected in RTT using confocal and super-resolution lattice SIM microscopy. **Results:** We detected significant abnormalities in area, perimeter, circularity and maximum Feret diameter of the mitochondria localized in neuronal dendrites. We detect early aging mitochondria in RTT neurons and a significant redox unbalance thanks to JC-1 imaging analysis. Mitochondria in the apical dendrites of Mecp2$^{-/y}$ hippocampal neurons displayed a range of dynamic alterations as we recorded speed, direction, fission/fusion activities. **Conclusions:** In this study we show that mitochondria of Mecp2$^{-/y}$ mouse hippocampal primary neurons are smaller and round-shaped compared with healthy ones. Moreover, we detect an early aging and the impairment of the oxidative/activity status. In addition, we determined a set of measures of mitochondria dynamics. Taken together these analyses could be a powerful tool to investigate RTT syndrome dysfunctions and to identify new possible therapeutic targets.

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BOARD NUMBER: S01-547

CRISPR-MEDIATED ACTIVATION OF AUTISM GENE ITGB3 RESTORES CORTICAL NETWORK EXCITABILITY VIA MGLUR5 SIGNALING

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Many mutations in autism spectrum disorder (ASD) affect a single allele, indicating a key role for gene dosage in ASD susceptibility. Recently, haplo-insufficiency of *ITGB3*, the gene encoding the extracellular matrix receptor β3 integrin, was associated with ASD. Accordingly, *Itgb3* KO mice exhibit autism-*like phenotypes. T*he pathophysiological mechanisms of *Itgb3* remain however unknown, and the potential of targeting this gene for developing ASD therapies uninvestigated. By combining molecular, biochemical, imaging and pharmacological analyses, we establish that *Itgb3* haplo-insufficiency impairs cortical network excitability by promoting extra-synaptic over synaptic signaling of the metabotropic glutamate receptor mGluR5, which is similarly dysregulated in fragile X syndrome, the most frequent monogenic form of ASD. To assess the therapeutic potential of regulating *Itgb3* gene dosage, we implemented CRISPR activation and compared its efficacy with that of a pharmacological rescue strategy for fragile X syndrome. Correction of neuronal *Itgb3* haplo-insufficiency by CRISPR activation rebalanced network excitability as effectively as blockade of mGluR5 with the selective antagonist MPEP. Our findings reveal an unexpected functional interaction between two ASD genes, thereby validating the pathogenicity of *ITGB3* haplo-insufficiency. Further, they pave the way for exploiting CRISPR activation as gene therapy for normalizing gene dosage and network excitability in ASD.

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BOARD NUMBER: S01-548

MNK1/2 KINASES REGULATE MEMORY AND AUTISM-RELATED BEHAVIOURS VIA SYNGAP1

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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[1]Foundation for Research and Technology (FORTH), Biomedical Research Institute (bri), Ioannina, Greece, [2]McGill University, Department Of Anesthesia And Alan Edwards Centre For Research On Pain, Montreal, Canada, [3]The Queen's University of Belfast,, Pathrick G. Johnston Centre For Cancer Research,, Belfast, Ireland

MAPK (mitogen-activated protein kinase) interacting protein kinases 1 and 2 (Mnk1/2) regulate a plethora of functions, presumably via phosphorylation of their best characterised substrate, eukaryotic translation initiation factor 4E (eIF4E) on Ser209. Here, we show that whereas deletion of Mnk1/2 (Mnk DKO) impairs synaptic plasticity and memory in mice, ablation of phopsho-eIF4E (Ser209) does not affect these processes, suggesting that Mnk1/2 possess additional downstream effectors in the brain. Translational profiling revealed only a small overlap between Mnk1/2- and phospho-eIF4E(Ser209)-regulated translatome. We identified the synaptic Ras GTPase activating protein 1 (Syngap1), encoded by a syndromic autism gene, as a downstream target of Mnk1 since Syngap1 immunoprecipitated with Mnk1 and showed reduced phosphorylation (S788) in Mnk DKO mice. Knock-down of Syngap1 reversed memory deficits in Mnk DKO mice, and pharmacological inhibition of Mnks rescued autism-related phenotypes in Syngap1 +/- mice. Thus, Syngap1 is a downstream effector of Mnk1, and the Mnks-Syngap1 axis regulates memory formation and autism-related behaviours.

Pubmed:
34659781: Chalkiadaki K, Statoulla E, Markou M, Bellou S, Bagli E, Fotsis T, Murphy C, Gkogkas CG
Translational control in neurovascular brain development.
The human brain carries out complex tasks and higher functions and is crucial for organismal survival, as it senses both intrinsic and extrinsic environments. Proper brain development relies on the orchestrated development of different precursor cells, which will give rise to the plethora of mature brain cell-types. Within this process, neuronal cells develop closely to and in coordination with vascular cells (endothelial cells (ECs), pericytes) in a bilateral communication process that relies on neuronal activity, attractive or repulsive guidance cues for both cell types and on tight-regulation of gene expression. Translational control is a master regulator of the gene-expression pathway and in particular for neuronal and ECs, it can be localized in developmentally relevant (axon growth cone, endothelial tip cell) and mature compartments (synapses, axons). Herein, we will review mechanisms of translational control relevant to brain development in neurons and ECs in health and disease.
R Soc Open Sci, 2021; 8
34624487: Statoulla E, Chalkiadaki K, Karozis D, Gkogkas CG
Regulation of mRNA translation in stem cells; links to brain disorders.
Translational control of gene expression is emerging as a cardinal step in the regulation of protein abundance. Especially for embryonic (ESC) and neuronal stem cells (NSC), regulation of mRNA translation is involved in the maintenance of pluripotency but also differentiation. For neuronal stem cells this regulation is linked to the various neuronal subtypes that arise in the developing brain and is linked to numerous brain disorders. Herein, we review translational control mechanisms in ESCs and NSCs during development and differentiation, and briefly discuss their link to brain disorders.
Cell Signal, 2021; 88

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BOARD NUMBER: S01-549

COMPARATIVE ANALYSIS OF THE αV AND β3 INTEGRIN KO MOUSE MODELS OF EPILEPSY AND AUTISM

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Lucia Celora[1], Caterina Michetti[2], Riccardo Ruggeri[1], Agnes Thalhammer[1,2], Carmela Vitale[2], Eduardo Morais[2], Fanny Jaudon[1,2], Lorenzo Cingolani[1,2]
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Integrins are biomechanical receptors for extracellular matrix proteins and counter-receptors on adjacent cells. Loss of αV and β3 integrin subunits have been implicated in the onset of epilepsy and autism, respectively, two comorbid brain disorders. Whether this is because of deficits in αvβ3 integrin receptor signaling at synapses is not known. Here, we performed comparative behavioral and electrophysiological analyses on two mouse models: a conditional KO for αV integrin and a constitutive KO for β3 integrin. We find that both mouse models exhibit increased seizure susceptibility and defects in social interactions. To investigate synaptic defects triggered by loss of αVβ3 integrin, we analyzed dendritic spines and excitatory synaptic currents in cortical neurons. We find selective alterations in dendritic spine morphology and synaptic plasticity in cortical pyramidal neurons of the medial prefrontal cortex. We concluded that αvβ3 integrin is important for regulating cortical excitability.

Pubmed:
34525372: Napoletano F, Ferrari Bravo G, Voto IAP, Santin A, Celora L, Campaner E, Dezi C, Bertossi A, Valentino E, Santorsola M, Rustighi A, Fajner V, Maspero E, Ansaloni F, Cancila V, Valenti CF, Santo M, Artimagnella OB, Finaurini S, Gioia U, Polo S, Sanges R, Tripodo C, Mallamaci A, Gustincich S, d'Adda di Fagagna F, Mantovani F, Specchia V, Del Sal G
The prolyl-isomerase PIN1 is essential for nuclear Lamin-B structure and function and protects heterochromatin under mechanical stress.
Chromatin organization plays a crucial role in tissue homeostasis. Heterochromatin relaxation and consequent unscheduled mobilization of transposable elements (TEs) are emerging as key contributors of aging and aging-related pathologies, including Alzheimer's disease (AD) and cancer. However, the mechanisms governing heterochromatin maintenance or its relaxation in pathological conditions remain poorly understood. Here we show that PIN1, the only phosphorylation-specific cis/trans prolyl isomerase, whose loss is associated with premature aging and AD, is essential to preserve heterochromatin. We demonstrate that this PIN1 function is conserved from Drosophila to humans and prevents TE mobilization-dependent neurodegeneration and cognitive defects. Mechanistically, PIN1 maintains nuclear type-B Lamin structure and anchoring function for heterochromatin protein 1α (HP1α). This mechanism prevents nuclear envelope alterations and heterochromatin relaxation under mechanical stress, which is a key contributor to aging-related pathologies.
Cell Rep, 2021; 36

FENS Forum 2022
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BOARD NUMBER: S01-550

IPSC-DERIVED CORTICAL NEURONS AND PATTERNED CORTICAL ORGANOIDS TO DISSECT THE NEURODEVELOPMENTAL ROOTS OF FRAGILE X SYNDROME.

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Fragile X syndrome (FXS) is a human form of mental retardation, caused by expansion of CGG repeat in the *FMR1* gene. The resulting epigenetic silencing causes the loss of the fragile X mental retardation protein with defects in dendritic spine morphology and synaptogenesis. Growing evidence demonstrates how human development and disease can be modeled *in vitro* using human induced pluripotent stem cells (hiPSCs). In this regard, using hiPSC-derived 2D and 3D cultures could give an improvement to better understand this neurodevelopmental disorder. We generated human cortical organoids (hCOs) using both wild type (WT) and FXS-patient derived iPSC. Preliminary data show that day 30 FMR1-WT hCOs, in contrast to FXS hCOs, revealed areas resembling the ventricular zone and the subventricular zone where N-CADHERIN and PAX6 positive neural progenitors were able to form neuroepithelial regions and start cortical neurogenesis. Day 100 FMR1-WT hCOs show a cortical stratification containing the first deep-layer cortical neurons. Moreover, an increased gene expression of *GFAP*, a radial glia and astrocytes marker, in FXS organoids was present at day 50. This evidence is consistence with our previous study where an aberrant presence of astrocytes is detected in FXS whole brain organoids (Brighi et al., 2021). In addition, 2D cultures allows us to investigate the impact of FXS on spine formation and synaptic transmission. In conclusion, combining both model systems, we could investigate dysregulation at cellular and synapses level and analyze the effect of the mutation, in a more physiological context, looking at cytoarchitecture and networking level.

Pubmed:
33993189: Brighi C, Salaris F, Soloperto A, Cordella F, Ghirga S, de Turris V, Rosito M, Porceddu PF, D'Antoni C, Reggiani A, Rosa A, Di Angelantonio S
Novel fragile X syndrome 2D and 3D brain models based on human isogenic FMRP-KO iPSCs.
Fragile X syndrome (FXS) is a neurodevelopmental disorder, characterized by intellectual disability and sensory deficits, caused by epigenetic silencing of the FMR1 gene and subsequent loss of its protein product, fragile X mental retardation protein (FMRP). Delays in synaptic and neuronal development in the cortex have been reported in FXS mouse models; however, the main goal of translating lab research into pharmacological treatments in clinical trials has been so far largely unsuccessful, leaving FXS a still incurable disease. Here, we generated 2D and 3D in vitro human FXS model systems based on isogenic FMR1 knock-out mutant and wild-type human induced pluripotent stem cell (hiPSC) lines. Phenotypical and functional characterization of cortical neurons derived from FMRP-deficient hiPSCs display altered gene expression and impaired differentiation when compared with the healthy counterpart. FXS cortical cultures show an increased number of GFAP positive cells, likely astrocytes, increased spontaneous network activity, and depolarizing GABAergic transmission. Cortical brain organoid models show an increased number of glial cells, and bigger organoid size. Our findings demonstrate that FMRP is required to correctly support neuronal and glial cell proliferation, and to set the correct excitation/inhibition ratio in human brain development.
Cell Death Dis, 2021; 12

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BOARD NUMBER: S01-551

THE CAV1.3 CHANNEL MUTATION A749G DETECTED IN AN AUTISTIC PATIENT AFFECTS DENDRITIC SPINE MORPHOLOGY

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Background: Voltage-gated L-type calcium Cav1.3 channels compose an important calcium entry pathway involved in synapse formation, dendritic refinement, and specifically in the regulation of spine morphology. We and others showed that de novo missense mutations in the Cav1.3 channel α1-subunit (*CACNA1D*) induce a gain-of-function phenotype compatible with increased calcium influx and therefore confer a high risk for neurological disorders. Nonetheless, the influence of these pathogenic mutations on synapse structure is not known.
Aims: Here we studied the effect of the A749G gain-of-function mutation in Cav1.3, associated with autism spectrum disorder and intellectual disability, on the dendritic spine morphology and density.
Methods: We analyzed spine morphology and channel surface expression in cultured hippocampal neurons transfected with untagged and HA-tagged wild-type and mutant Cav1.3 channels. Additionally, by employing a knock-in mouse model carrying the A749G mutation, we currently test the effect of the mutation on spine morphology in Golgi-Cox-stained brain sections.
Results: Expression of the HA-tagged and untagged A749G α1-subunits resulted in a significant increase in dendritic spine length but had no effect on spine density. Furthermore, the spine shape factor was reduced in A749G transfected neurons indicating an overall elongation of spines. Compared to wild-type Cav1.3, the abundance of filopodia-like and thin spines was higher in A749G transfected neurons.
Conclusions: Our results demonstrate an aberrant spine morphology caused by the autism-linked Cav1.3 mutation A749G. We hypothesize that altered local calcium signaling of Cav1.3 gain-of-function mutations can lead to changes in spine stability ultimately triggering neurodevelopmental deficits.

Pubmed:
34647648: Nikonishyna YV, Ortner NJ, Kaserer T, Hoffmann J, Biskup S, Dafotakis M, Reetz K, Schulz JB, Striessnig J, Dohrn MF
Novel CACNA1A Variant p.Cys256Phe Disrupts Disulfide Bonds and Causes Spinocerebellar Ataxia.
Spinocerebellar ataxia (SCA) is a progressive, autosomal dominant neurodegenerative disorder typically associated with CAG repeat expansions.
Mov Disord, 2022; 37
33380256: Hofer NT, Pinggera A, Nikonishyna YV, Tuluc P, Fritz EM, Obermair GJ, Striessnig J
Stabilization of negative activation voltages of Cav1.3 L-Type Ca-channels by alternative splicing.
-->Low voltage-activated Cav1.3 L-type Ca-channels are key regulators of neuronal excitability controlling neuronal development and different types of learning and memory. Their physiological functions are enabled by their negative activation voltage-range, which allows Cav1.3 to be active at subthreshold voltages. Alternative splicing in the C-terminus of their pore-forming α1-subunits gives rise to C-terminal long (Cav1.3) and short (Cav1.3) splice variants allowing Cav1.3 to activate at even more negative voltages than Cav1.3. We discovered that inclusion of exons 8b, 11, and 32 in Cav1.3 further shifts activation (-3 to -4 mV) and inactivation (-4 to -6 mV) to more negative voltages as revealed by functional characterization in tsA-201 cells. We found transcripts of these exons in mouse chromaffin cells, the cochlea, and the brain. Our data further suggest that Cav1.3-containing exons 11 and 32 constitute a significant part of native channels in the brain. We therefore investigated the effect of these splice variants on human disease variants. Splicing did not prevent the gating defects of the previously reported human pathogenic variant S652L, which further shifted the voltage-dependence of activation of exon 11-containing channels by more than -12 mV. In contrast, we found no evidence for gating changes of the missense variant R498L, located in exon 11, which has recently been identified in a patient with an epileptic syndrome. Our

data demonstrate that alternative splicing outside the C-terminus involving exons 11 and 32 contributes to channel fine-tuning by stabilizing negative activation and inactivation gating properties of wild-type and mutant Cav1.3 channels.
Channels (Austin), 2021; 15

31921405: Hofer NT, Tuluc P, Ortner NJ, Nikonishyna YV, Fernándes-Quintero ML, Liedl KR, Flucher BE, Cox H, Striessnig J

Biophysical classification of a de novo mutation as a high-risk mutation for a severe neurodevelopmental disorder.
There is increasing evidence that de novo missense mutations inducing increased Cav1.3 L-type Ca-channel-function confer a high risk for neurodevelopmental disorders (autism spectrum disorder with and without neurological and endocrine symptoms). Electrophysiological studies demonstrating the presence or absence of typical gain-of-function gating changes could therefore serve as a tool to distinguish likely disease-causing from non-pathogenic de novo variants in affected individuals. We tested this hypothesis for mutation S652L, which has previously been reported in twins with a severe neurodevelopmental disorder in the Deciphering Developmental Disorder Study, but has not been classified as a novel disease mutation.
Mol Autism, 2020; 11

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INTEGRATIVE MULTI-OMICS ANALYSES REVEAL MULTI-MODAL FOXG1 FUNCTIONS ACTING ON EPIGENETIC PROCESSES AND IN CONCERT WITH NEUROD1 TO REGULATE SYNAPTOGENESIS IN THE MOUSE HIPPOCAMPUS

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Development of the central nervous system (CNS) depends on spatiotemporal control of signalling pathways and transcription programs. Forkhead Box G1 (FOXG1) is one of the master regulators that plays fundamental roles in forebrain development, from timing of neurogenesis to patterning of the cerebral cortex. Mutations in *FOXG1* cause a neurodevelopmental disorder, FOXG1 syndrome, and patients manifest a spectrum of phenotypes ranging from severe cognitive dysfunction and microcephaly to social withdrawal with varying severities. Despite steady progress towards understanding the role of FOXG1 its multifaceted functions and changes at the molecular level underlying functional abnormalities upon *FOXG1* haploinsufficiency remain largely unexplored. Using multi-omics data (RNA-, ChIP-, and ATAC-seq), we explored the influence of FOXG1 on neuronal maturation at the chromatin level in the adult mouse hippocampus. We revealed that FOXG1 both repressed and activated transcription, binding mainly to enhancer regions, and affecting the epigenetic landscape bidirectionally as displayed by the levels of H3K27ac, H3K4me3 and chromatin accessibility. Alterations in the chromatin affected synaptogenesis and axonogenesis, signifying the role of FOXG1 in the regulation of genes required for proper neuronal function. Notably, FOXG1 interacted with histone deacetylases (HDACs) and inhibition of HDACs partially rescued transcriptional alterations observed upon FOXG1 reduction. We have also identified NEUROD1 as a novel interaction partner of FOXG1, which acted in coordination with FOXG1 to control neuronal differentiation. Together, our integrative approach uncovered that FOXG1 acts through different epigenetic mechanisms and in concert with other TFs, one of which is NEUROD1, emphasising the multimodality of FOXG1 at the chromatin level.

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SCN2A VARIANT S1758R CAUSES A LOSS OF FUNCTION WITH NO EFFECT ON INTRINSIC NEURON EXCITABILITY

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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SCN2A, encoding voltage gated sodium channel 1.2 ($Na_V1.2$), is one of the most significant single-gene contributors to neurodevelopmental disorders. Genetic variants resulting in autism spectrum disorder (ASD) typically confer a severe loss-of-function (LOF) of $Na_V1.2$, however the pathomechanisms of disease, such as associated intellectual disability and developmental delay, are unknown, mostly due to the lack of impressive or consistent behavioural and electrophysiological phenotypes in mouse models of *SCN2A* LOF. We recruited one candidate patient with a novel *SCN2A* ASD genetic variant (S1758R) for phenotypic characterisation and induced-pluripotent stem cell (iPSC) generation. We investigated the pathomechanisms of *SCN2A* in ASD using neurons differentiated from patient iPSCs, in the first phenotypic characterisation of an *SCN2A* ASD variant in a neuron model derived from a patient's own cells. We find that the S1758R variant has no effect on the electrophysiological properties of neurons differentiated by the Ngn2 rapid neuronal differentiation protocol when compared to the isogenic control, suggesting mechanisms of redundancy or homeostatic plasticity in the model that are able to compensate for *SCN2A* LOF. To investigate these processes, we performed RNA-sequencing analysis of neuron cultures and discovered dysregulated potassium channel genes as a possible mechanism for compensation. The findings from this study have motivated the design of a 3D organoid project, investigating *SCN2A* LOF in the context of a more native and complex model of the developing human brain.

Pubmed:

35031483: Heighway J, Sedo A, Garg A, Eldershaw L, Perreau V, Berecki G, Reid CA, Petrou S, Maljevic S
Sodium channel expression and transcript variation in the developing brain of human, Rhesus monkey, and mouse.
Genetic variation in voltage-gated sodium (Na) channels is a significant contributor to neurodevelopmental disorders. Na channel alpha subunits are encoded by the SCNxA family and four are predominately expressed in the brain: SCN1A, SCN2A, SCN3A, and SCN8A. Gene expression is developmentally regulated, and they are known to express functionally distinct transcript variants. Precision therapies targeting these genes and their transcript variants are currently in preclinical development, yet the developmental expression of these transcripts in the human brain is yet to be fully understood. Additionally, the functional consequences of some mutations differ depending on the studied channel isoform, suggesting differential transcript variant expression can affect disease prognoses. We characterise the expression of the four SCNxAs and their transcript variants in human, Rhesus monkey and mouse brain using publicly available RNA-sequencing data and analysis tools, demonstrating that this approach can be used to answer important biological questions of gene and transcript developmental regulation. We find that gene expression and transcript variant regulation are conserved across species at similar developmental stages and determine the developmental milestones for transcript variant expression. Our study provides a guide to researchers testing therapies and clinicians advising prognoses based on the expression of channel isoforms.
Neurobiol Dis, 2022; 164
30149017: Ma L, Chin YKY, Dekan Z, Herzig V, Chow CY, Heighway J, Lam SW, Guillemin GJ, Alewood PF, King GF
Novel venom-derived inhibitors of the human EAG channel, a putative antiepileptic drug target.
Recently, we and other groups revealed that gain-of-function mutations in the human ether à go-go voltage-gated potassium channel hEAG1 (K10.1) lead to developmental disorders with associated infantile-onset epilepsy. However, the physiological role of hEAG1 in the central nervous system remains elusive. Potent and selective antagonists of hEAG1 are therefore much sought after, both as pharmacological tools for studying the (patho)physiological functions of this enigmatic channel and as potential leads for development of anti-epileptic drugs. Since animal venoms are a rich source of potent ion channel modifiers

that have been finely tuned by millions of year of evolution, we screened 108 arachnid venoms for hEAG1 inhibitors using electrophysiology. Two hit peptides (Aa1a and Ap1a) were isolated, sequenced, and chemically synthesised for structure-function studies. Both of these hEAG1 inhibitors are C-terminally amidated peptides containing an inhibitor cystine knot motif, which provides them with exceptional stability in both plasma and cerebrospinal fluid. Aa1a and Ap1a are the most potent peptidic inhibitors of hEAG1 reported to date, and they present a novel mode of action by targeting both the activation and inactivation gating of the channel. These peptides should be useful pharmacological tools for probing hEAG1 function as well as informative leads for the development of novel anti-epileptic drugs.
Biochem Pharmacol, 2018; 158

34850743: Li M, Jancovski N, Jafar-Nejad P, Burbano LE, Rollo B, Richards K, Drew L, Sedo A, Heighway J, Pachernegg S, Soriano A, Jia L, Blackburn T, Roberts B, Nemiroff A, Dalby K, Maljevic S, Reid CA, Rigo F, Petrou S
Antisense oligonucleotide therapy reduces seizures and extends life span in an SCN2A gain-of-function epilepsy model.
De novo variation in SCN2A can give rise to severe childhood disorders. Biophysical gain of function in SCN2A is seen in some patients with early seizure onset developmental and epileptic encephalopathy (DEE). In these cases, targeted reduction in SCN2A expression could substantially improve clinical outcomes. We tested this theory by central administration of a gapmer antisense oligonucleotide (ASO) targeting Scn2a mRNA in a mouse model of Scn2a early seizure onset DEE (Q/+ mice). Untreated Q/+ mice presented with spontaneous seizures at P1 and did not survive beyond P30. Administration of the ASO to Q/+ mice reduced spontaneous seizures and significantly extended life span. Across a range of behavioral tests, Scn2a ASO-treated Q/+ mice were largely indistinguishable from WT mice, suggesting treatment is well tolerated. A human SCN2A gapmer ASO could likewise impact the lives of patients with SCN2A gain-of-function DEE.
J Clin Invest, 2021; 131

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BOARD NUMBER: S01-554

HIGH-THROUGHPUT ANALYSIS IN A FRAGILE X SYNDROME MOUSE MODEL AFTER CB1 RECEPTOR TARGETING REVEALS SPECIFIC TRANSCRIPTOMIC SIGNATURE SENSITIVE TO TREATMENT.

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Lucía De Los Reyes-Ramírez[1], Araceli Bergadà-Martínez[1], Marina Reixachs-Solé[2,3], Sara Martínez-Torres[1], Alba Navarro-Romero[1], Rafael Maldonado[1,4], Eduardo Eyras[2,3], Andrés Ozaita[1]
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Aims: Fragile X syndrome (FXS) is the most common monogenic cause of inherited intellectual disability and autism caused by the silencing of the *FMR1* gene and the subsequent loss of the fragile X mental retardation protein (FMRP). The fragile X mental retardation 1 (*Fmr1*) knockout mouse shows cognitive impairment and some of the synaptic alterations observed in subjects with FXS. These alterations in *Fmr1* KO were significantly prevented by blocking the cannabinoid type-1 receptor (CB1R) with low doses of the systemic antagonist/inverse agonist rimonabant. The molecular hallmarks underlying the neurological and behavioural improvements of CB1R blockade in *Fmr1* KO mice are not yet understood. **Methods**: We performed high-throughput RNA sequencing focused on synaptic relevant transcripts to analyse differential expression patterns between *Fmr1* KO mice and WT after rimonabant (0.1 mg/kg, i.p., 7 days) or vehicle administration. **Results**: Differential expression analysis in *Fmr1* KO samples revealed the up-regulation of transcripts related with synapse structure and the down regulation of transcripts involved in synapse organization and mRNA processing. Notably, rimonabant treatment in *Fmr1* KO samples induced a marked up-regulation of transcripts related with mRNA splicing. Furthermore, alternative splicing analysis identified a relevant number of splicing events in *Fmr1* KO mice that were reverted after rimonabant treatment in terms of Percent Splice In measurements. **Conclusions**: Together, we identified a synaptic transcriptomic signature that may contribute to central deficits in the FXS mouse model, a signature that was sensitive to CB1R inhibition under conditions that improve neurological and behavioural traits.

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NEURAL PRECURSOR/STEM CELL-BASED THERAPY FOR RETT SYNDROME

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Rett syndrome (RTT) is a severe neurodevelopmental disorder that afflicts mostly females and is mainly caused by mutations in the *MECP2* gene. Unfortunately, a cure is still lacking. Neural Precursor/Stem Cells (NPCs) transplantation was proved safe and beneficial in many neurological disorders, including autism. We thus decided to investigate the therapeutic potential of NPCs in the *Mecp2* null mouse modeling RTT. NPCs exert their beneficial effect mainly through the “bystander” mechanism: by sensing the pathological environment, they secrete neurotrophic and immunomodulatory factors that promote neuroprotection and brain plasticity. Using an *in vitro* transwell-based co-culture system, we demonstrated that NPCs promote morphological and synaptic rescues in *Mecp2* null neurons by paracrine secreted factors. I*n vivo*, we collected several data demonstrating that NPCs can prolong lifespan and restore memory and motor functions of *Mecp2* null mice. To identify the molecular mechanisms set in motion by NPCs transplantation, we performed bulk RNA sequencing analyses of wild-type and knock out cerebral tissues transplanted or not with NPCs. Obtained results revealed many transcriptional alterations directly or indirectly caused by the lack of *Mecp2* and by NPCs treatment. Among all the deregulated pathways we focused on Interferon gamma (IFNγ) one, which is upregulated in *Mecp2 null* + NPCs mice vs *Mecp2* null control mice. Assuming that this cytokine could be one of the candidate factors responsible for the benefic effects exerted by NPCs transplant, we collected *in vivo* and *in vitro* evidence in favor of its therapeutic potential for *Mecp2* null models.

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BOARD NUMBER: S01-556

EPIGENETIC MECHANISMS OF HOMEOSTATIC PLASTICITY IN A HUMAN NEURONAL MODEL SYSTEM

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Neurodevelopmental disorders (NDDs) are conditions of impaired cognitive, behavioral, and/or motor functions stemming from an atypical development of the central nervous system. Impairments found in NDDs have been associated with disrupted homeostatic plasticity, a process known to stabilize neuronal network activity by balancing neuronal excitation and inhibition. Several studies have shown that homeostatic plasticity is regulated by epigenetic mechanisms, such as DNA-methylation. However, the epigenetic profile involved in homeostatic plasticity in NDDs is currently insufficiently defined. This study aims to explore the role of epigenetics in altered homeostatic plasticity relevant to NDDs. As a first step, we established a model of homeostatic plasticity in human induced pluripotent stem cell (hiPSC)-derived neurons, grown on micro-electrode arrays (MEAs). In hiPSC-derived excitatory neurons, we demonstrate that chronic deprivation of neuronal activity through the inhibition of sodium channels with tetrodotoxin (TTX) elicits a time-dependent increase in neuronal network activity after TTX removal, and this increase is restored to baseline after 48 hours. In accordance with previous reports, we show that the early stage of TTX-induced homeostatic plasticity is mediated by GluA2-lacking AMPA receptors. We further show that the changes in network properties are caused by an upregulation of postsynaptic AMPA receptors expression and changes in intrinsic properties. Having validated this model as described above, it is now being used to test whether hiPSC-derived neurons with mutations in genes linked to NDD through epigenetic mechanisms display altered homeostatic plasticity.

FENS Forum 2022
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BOARD NUMBER: S01-557

ARID1B-HAPLOINSUFFICIENCY LEADS TO DELAYED NEURONAL NETWORK DEVELOPMENT OF IPSC-DERIVED EXCITATORY NEURONS

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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ARID1B is one of the most commonly mutated genes in patients with intellectual disabilities (ID) ranging from Coffin-Siris syndrome (CSS) to syndromic forms of autism spectrum disorders (ASD). The mechanism by which deficiency of ARID1B leads to the disease phenotypes seen in patients remains unclear. We generated excitatory glutamatergic neurons from induced pluripotent stem cells (iPSCs) derived from fibroblasts of CSS patients with loss-of-function mutations in *ARID1B*, as well as *ARID1B*$^{+/-}$ CRISPR line originated from healthy control iPSCs. Using Micro-Electrode Arrays (MEAs) to investigate the spontaneous neuronal network activity of the neurons, we found that *ARID1B*$^{+/-}$ neurons showed delayed developmental trajectory in comparison to control neurons. In support of this phenotype, mature network activity of ARID1B-deficient neurons showed increased response to blockage of GluA2-lacking AMPA receptors, reminiscent of the response of immature control neuronal network. Single-cell electrophysiological recording and immunofluorescence data suggest altered GluA2-expression in *ARID1B*$^{+/-}$ neuronal synapses. These results give insight into the molecular mechanism by which haploinsufficiency of ARID1B leads to phenotypes seen in CSS, ID , and ASD patients carrying *ARID1B* mutations.

Pubmed:
33771987: Inak G, Rybak-Wolf A, Lisowski P, Pentimalli TM, Jüttner R, Glažar P, Uppal K, Bottani E, Brunetti D, Secker C, Zink A, Meierhofer D, Henke MT, Dey M, Ciptasari U, Mlody B, Hahn T, Berruezo-Llacuna M, Karaiskos N, Di Virgilio M, Mayr JA, Wortmann SB, Priller J, Gotthardt M, Jones DP, Mayatepek E, Stenzel W, Diecke S, Kühn R, Wanker EE, Rajewsky N, Schuelke M, Prigione A
Defective metabolic programming impairs early neuronal morphogenesis in neural cultures and an organoid model of Leigh syndrome.
Leigh syndrome (LS) is a severe manifestation of mitochondrial disease in children and is currently incurable. The lack of effective models hampers our understanding of the mechanisms underlying the neuronal pathology of LS. Using patient-derived induced pluripotent stem cells and CRISPR/Cas9 engineering, we developed a human model of LS caused by mutations in the complex IV assembly gene SURF1. Single-cell RNA-sequencing and multi-omics analysis revealed compromised neuronal morphogenesis in mutant neural cultures and brain organoids. The defects emerged at the level of neural progenitor cells (NPCs), which retained a glycolytic proliferative state that failed to instruct neuronal morphogenesis. LS NPCs carrying mutations in the complex I gene NDUFS4 recapitulated morphogenesis defects. SURF1 gene augmentation and PGC1A induction via bezafibrate treatment supported the metabolic programming of LS NPCs, leading to restored neuronal morphogenesis. Our findings provide mechanistic insights and suggest potential interventional strategies for a rare mitochondrial disease.
Nat Commun, 2021; 12
32766754: Ciptasari U, van Bokhoven H
The phenomenal epigenome in neurodevelopmental disorders.
Disruption of chromatin structure due to epimutations is a leading genetic etiology of neurodevelopmental disorders, collectively known as chromatinopathies. We show that there is an increasing level of convergence from the high diversity of genes that are affected by mutations to the molecular networks and pathways involving the respective proteins, the disrupted cellular and subcellular processes, and their consequence for higher order cellular network function. This convergence is ultimately reflected by specific phenotypic features shared across the various chromatinopathies. Based on these observations, we propose that the commonly disrupted molecular and cellular anomalies might provide a rational target for the development of symptomatic interventions for defined groups of genetically distinct neurodevelopmental disorders.

Hum Mol Genet, 2020; 29
28316757: Živković L, Borozan S, Čabarkapa A, Topalović D, Ciptasari U, Bajić V, Spremo-Potparević B
Antigenotoxic Properties of against Hydrogen Peroxide in Human Peripheral Blood Cells.
The ability of mushroom in its dried and powdered mycelial form was evaluated for its antigenotoxic properties for the first time. Antigenotoxic effects in human peripheral blood cells against HO-induced DNA damage were examined in pretreatment and posttreatment protocol by comet assay. The results showed better antigenotoxic properties of on the interventional level, respectively, after treatment. in concentration of 250 g/mL after treatment was most efficient in regard to its action against DNA damage. The evaluation of repair kinetics showed decrease in HO induced DNA damage 15 min after the application of , reaching the maximum potency after 30 min. Analysis of antioxidant properties of revealed strong OH scavenging properties and moderate reducing power, while its DPPH scavenging ability was weak. In regard to our findings, we can conclude that our preliminary results demonstrated antigenotoxic properties of and its strong OH scavenging ability. Mechanisms underlying its properties should be further evaluated in in vivo studies.
Oxid Med Cell Longev, 2017; 2017

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BOARD NUMBER: S01-558

THE CELL-TYPE SPECIFIC CONTRIBUTION OF EHMT1 TO THE EXCITATORY/INHIBITORY BALANCE IN IN VITRO HUMAN NEURONAL NETWORKS

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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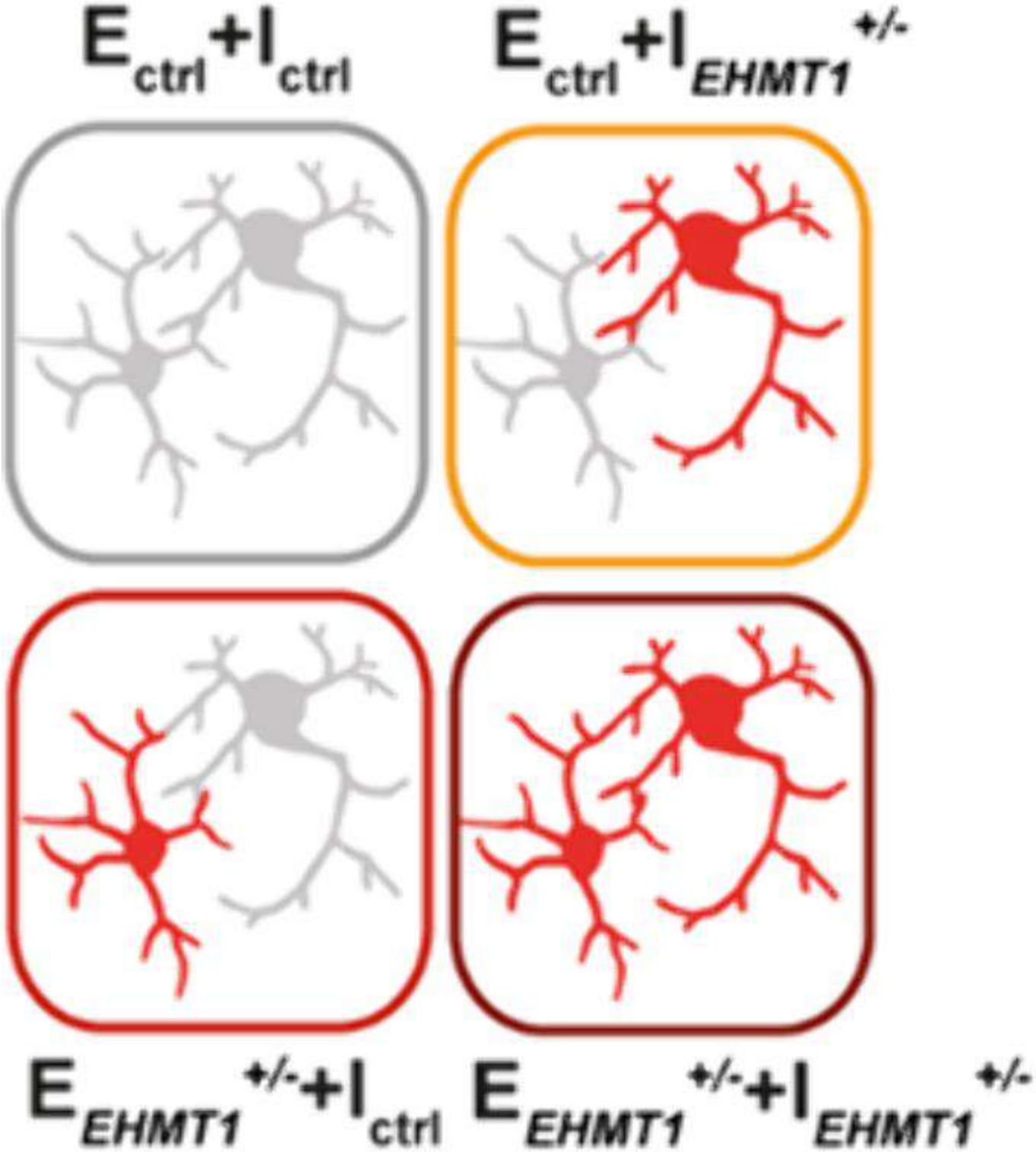
Kleefstra syndrome is a neurodevelopmental disorder caused by mutations in the histone methyltransferase EHMT1. EHMT1 has recently been identified to be a key player in the maintenance of excitatory/inhibitory (E/I) balance. However, little is known about its function in human GABAergic neurons, as well as how EHMT1 controls the function of glutamatergic or GABAergic neurons in maintaining E/I balance. To disentangle the cell-type specific contribution of EHMT1 to E/I balance, we generated four network compositions containing human induced pluripotent stem-cell (hiPSC) derived glutamatergic- and GABAergic neurons from either control or EHMT1-deficient hiPSCs and recorded their electrophysiological network activity on a multi-electrode array (MEA). We found that selective EHMT1-deficieny in glutamatergic or GABAergic neurons results in cell-type specific alterations in neuronal network activity. The largest contribution to the affected network phenotype is attributed to EHMT1-deficient glutamatergic neuron, of which some affected neuronal network parameters are exacerbated when also in the presence of EHMT1-deficient GABAergic neurons. A discriminant analysis of all MEA parameters resulted in four clusters, with the large majority of MEA recordings correctly classified within their own culture composition cluster. Taken together, our results suggest that EHMT1 controls both glutamatergic and GABAergic neuron function during the

maintenance of E/I



balance.

Pubmed:
32975655: Negwer M, Piera K, Hesen R, Lütje L, Aarts L, Schubert D, Nadif Kasri N
EHMT1 regulates Parvalbumin-positive interneuron development and GABAergic input in sensory cortical areas.

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Mutations in the Euchromatic Histone Methyltransferase 1 (EHMT1) gene cause Kleefstra syndrome, a rare form of intellectual disability (ID) with strong autistic traits and sensory processing deficits. Proper development of inhibitory interneurons is crucial for sensory function. Here we report a timeline of Parvalbumin-positive (PV) interneuron development in the three most important sensory cortical areas in the Ehmt1 mouse. We find a hitherto unreported delay of PV neuron maturation early in sensory development, with layer- and region-specific variability later in development. The delayed PV maturation is also reflected in a delayed maturation of GABAergic transmission in Ehmt1 auditory cortex, where we find a reduced GABA release probability specifically in putative PV synapses. Together with earlier reports of excitatory impairments in Ehmt1 neurons, we propose a shift in excitatory-inhibitory balance towards overexcitability in Ehmt1 sensory cortices as a consequence of early deficits in inhibitory maturation.
Brain Struct Funct, 2020; 225

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UNDERSTANDING THE ROLE OF SYNGAP1 IN PARVALBUMIN-EXPRESSING GABAERGIC CIRCUIT DEVELOPMENT AND FUNCTION.

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Haploinsufficiency of *Syngap1* gene encoding the Synaptic Ras-GTPase Activating protein is associated with intellectual disability, autism spectrum disorder and epilepsy. Mouse models of *Syngap1* haploinsufficiency show alterations in synaptic plasticity, behavioural abnormalities and cognitive deficits. Several studies have shown that *Syngap1* regulates the developmental trajectory and function of excitatory neurons; in contrast, the role of *Syngap1* in inhibitory GABAergic neurons is less well understood. GABAergic neurons are a diverse class of neurons with different morphology, connectivity and physiological properties. Parvalbumin (PV)-expressing interneurons, one of the major classes of cortical GABAergic interneurons, form synapses onto the soma and proximal dendrites of pyramidal cells and play an important role in neural circuit development and plasticity. We aim to understand the role of *Syngap1* expressed by PV cells in sensory processing and cognition. We used both a) Nkx2.1 Cre conditional mice to specifically delete *Syngap1* embryonically in interneurons derived from the medial ganglionic eminence (where PV and somatostatin-expressing interneurons originate), and b) PV Cre conditional mice to specifically delete *Syngap1* postnatally in PV cells, respectively. Our results suggest altered social behavior and fear extinction, specifically in *Nkx2.1Cre:Syngap1^{lox}* but not in *PVCre:Syngap1^{lox}* mutant mice. Further we found that *Nkx2.1Cre:Syngap1^{lox}* mice show specific alterations in auditory processing. Haploinsufficiency of *Syngap1* in interneurons can thus contribute to cognitive alterations caused by *Syngap1* mutations during development.

Pubmed:
34135323: Amegandjin CA, Choudhury M, Jadhav V, Carriço JN, Quintal A, Berryer M, Snapyan M, Chattopadhyaya B, Saghatelyan A, Di Cristo G
Sensitive period for rescuing parvalbumin interneurons connectivity and social behavior deficits caused by TSC1 loss.
The Mechanistic Target Of Rapamycin Complex 1 (mTORC1) pathway controls several aspects of neuronal development. Mutations in regulators of mTORC1, such as Tsc1 and Tsc2, lead to neurodevelopmental disorders associated with autism, intellectual disabilities and epilepsy. The correct development of inhibitory interneurons is crucial for functional circuits. In particular, the axonal arborisation and synapse density of parvalbumin (PV)-positive GABAergic interneurons change in the postnatal brain. How and whether mTORC1 signaling affects PV cell development is unknown. Here, we show that Tsc1 haploinsufficiency causes a premature increase in terminal axonal branching and bouton density formed by mutant PV cells, followed by a loss of perisomatic innervation in adult mice. PV cell-restricted Tsc1 haploinsufficient and knockout mice show deficits in social behavior. Finally, we identify a sensitive period during the third postnatal week during which treatment with the mTOR inhibitor Rapamycin rescues deficits in both PV cell innervation and social behavior in adult conditional haploinsufficient mice. Our findings reveal a role of mTORC1 signaling in the regulation of the developmental time course and maintenance of cortical PV cell connectivity and support a mechanistic basis for the targeted rescue of autism-related behaviors in disorders associated with deregulated mTORC1 signaling.
Nat Commun, 2021; 12
31744366: Ammanathan V, Mishra P, Chavalmane AK, Muthusamy S, Jadhav V, Siddamadappa C, Manjithaya R
Restriction of intracellular replication by restoring TFEB-mediated xenophagy.
Macroautophagy/autophagy functions as a part of the innate immune system in clearing intracellular pathogens. Although this process is well known, the mechanisms that control antibacterial autophagy are not clear. In this study we show that during intracellular infection, the activity of TFEB (transcription factor EB), a master regulator of autophagy and lysosome biogenesis, is suppressed by maintaining it in a phosphorylated state on the lysosomes. Furthermore, we have identified a novel, antibacterial small molecule autophagy (xenophagy) modulator, acacetin. The xenophagy effect exerted by acacetin occurs in an MTOR (mechanistic target of rapamycin kinase)-independent, TFEB-dependent manner. Acacetin treatment results in persistently maintaining active TFEB in the nucleus and also in TFEB mediated induction of functional lysosomes

that target -containing vacuoles (SCVs). The enhanced proteolytic activity due to deployment of lysosomes results in clamping down replication in SCVs. Acacetin is effective as a xenophagy compound in an mouse model of infection and reduces intracellular burden.
Autophagy, 2020; 16

FENS Forum 2022
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BOARD NUMBER: S01-560

INSIGHT INTO THE ROLE OF THE PRIMARY CILIUM IN HIPSC-DERIVED NEURONAL NETWORKS

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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In the mammalian cerebral cortex, up to 90% of neurons are ciliated, yet at present, little is known about the role of the primary cilium in postmitotic neurons. Recent work indicates that there is a role of ciliary proteins with the intrinsic excitability of single cells but the role the cilium plays in network dynamics at present is poorly characterised. In addition, findings in mouse models of seizure suggest hippocampal neurons display shortened cilium following excitotoxic events. We sought to discern the effect of pharmacological manipulation of the network on neuronal cilia morphology in human induced pluripotent stem cell derived neuronal networks. We present evidence that the primary cilium dynamically changes (bidirectionally, shortened and longer) when network function is altered . In this system, the cilium shows responsiveness to an array of molecules, including tetrodotoxin and potassium chloride, that alter the excitable state of the neurons. To our knowledge, this work is the first finding in human induced neuronal model that shows that the neuronal primary cilium is reactive to neuromodulation in vitro, and adds novel insight into the role that primary cilia, and ciliary proteins, play in excitable transmission.

Pubmed:
31447328: Fjodorova M, Louessard M, Li Z, De La Fuente DC, Dyke E, Brooks SP, Perrier AL, Li M
CTIP2-Regulated Reduction in PKA-Dependent DARPP32 Phosphorylation in Human Medium Spiny Neurons: Implications for Huntington Disease.
The mechanisms underlying the selective degeneration of medium spiny neurons (MSNs) in Huntington disease (HD) remain largely unknown. CTIP2, a transcription factor expressed by all MSNs, is implicated in HD pathogenesis because of its interactions with mutant huntingtin. Here, we report a key role for CTIP2 in protein phosphorylation via governing protein kinase A (PKA) signaling in human striatal neurons. Transcriptomic analysis of CTIP2-deficient MSNs implicates CTIP2 target genes at the heart of cAMP-Ca signal integration in the PKA pathway. These findings are further supported by experimental evidence of a substantial reduction in phosphorylation of DARPP32 and GLUR1, two PKA targets in CTIP2-deficient MSNs. Moreover, we show that CTIP2-dependent dysregulation of protein phosphorylation is shared by HD hPSC-derived MSNs and striatal tissues of two HD mouse models. This study therefore establishes an essential role for CTIP2 in human MSN homeostasis and provides mechanistic and potential therapeutic insight into striatal neurodegeneration.
Stem Cell Reports, 2019; 13

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-561

ETHANOL-INDUCED MIRNA 137 AND 501-3P MODULATE AMPA NEUROTRANSMISSION IN DEVELOPING HIPPOCAMPAL SLICES IN VITRO

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Lorenzo Curti[1], Lucia Caffino[2], Elisabetta Bigagli[1], Fernando Castillo Díaz[2], Francesca Mottarlini[2], Antonino Iurato La Rocca[1], Fabio Fumagalli[2], Alessio Masi[1], Guido Mannaioni[1], Elisabetta Gerace[1]
[1]Università degli Studi di Firenze, Dipartimento Di Neuroscienze, Psicologia, Area Del Farmaco E Salute Del Bambino – Neurofarba., Firenze, Italy, [2]University of Milan, Department Of Pharmacological And Biomolecular Sciences, Milan, Italy

Background: Drinking during pregnancy can lead to physical, learning and behavioral disorders in newborns, a pathological condition referred as Fetal Alcohol Spectrum Disorder (FASD). Unfortunately, there is no cure for FASD and the molecular mechanisms underlying this pathology are still unknown. We have recently demonstrated that chronic EtOH exposure followed by withdrawal induced a significant decrease in AMPA receptor (AMPAR) expression and function and an incorrect formation of neuronal circuits in developing hippocampus *in vitro* [Gerace et al., 2016]. Here, we hypothesized a role for microRNA (137 and 501-3p) in the regulation of AMPAR-mediated neurotransmission, by translational repression of AMPAR subunit GluA1 via metabotropic glutamate receptor 5 (mGlu5) at CA1 hippocampal synapse. **Methods:** Organotypic hippocampal slices (2 days in cultures) were exposed to EtOH (150 mM) for 7 days followed by 24 h EtOH withdrawal. Then, slices were analyzed by RT-PCR for miRNA content, by western blotting for expression of AMPA related-synaptic proteins in postsynaptic compartment (PSD) and by electrophysiology to record electrical properties from CA1 pyramidal neurons. **Results:** We found that EtOH induces miRNA 137 and 501-3p upregulation that is prevented by application of the selective mGlu5 antagonist MPEP during EtOH withdrawal. Simultaneously, we observed a significant downregulation of postsynaptic AMPA subunits and relative scaffold proteins expression and accordingly, a decrease of AMPA-mediated neurotransmission. **Conclusions:** Our data indicate miRNA137 and 501-3p as key factors in the regulation of AMPAergic neurotransmission, contributing to the pathogenesis of FASD.

Pubmed:
34785165: Ilari A, Curti L, Petrella M, Cannella N, La Rocca A, Ranieri G, Gerace E, Iezzi D, Silvestri L, Mannaioni G, Ciccocioppo R, Masi A
Moderate ethanol drinking is sufficient to alter Ventral Tegmental Area dopamine neurons activity via functional and structural remodeling of GABAergic transmission.
Earlier studies have shown a major involvement of Ventral Tegmental Area (VTA) dopamine (DA) neurons in mediating the rewarding effects of ethanol (EtOH). Much less is known on the role of this system in mediating the transition from moderate to excessive drinking and abuse. Here we sought to explore the hypothesis that early stage drinking in rodents, resembling recreational EtOH use in humans, is sufficient to dysregulate VTA DA transmission thus increasing the propensity to use over time. To this purpose, midbrain slice recordings in mice previously exposed to an escalating (3, 6 and 12%) 18-day voluntary EtOH drinking paradigm was used. By recording from DA and γ-aminobutyric acid (GABA) VTA neurons in midbrain slices, we found that moderate EtOH drinking leads to a significant suppression of the spontaneous activity of VTA DA neurons, while increasing their response to acute EtOH application. We also found that chronic EtOH leads to the enhancement of GABA input frequency onto a subset of DA neurons. Structurally, chronic EtOH induced a significant increase in the number of GABA axonal boutons contacting DA neurons, suggesting deep rewiring of the GABA network. This scenario is consistent with a downmodulation of the reward DA system induced by moderate EtOH drinking, a neurochemical state defined as "hypodopaminergic" and previously associated with advanced stages of drug use in humans. In this context, increased sensitivity of DA neurons towards acute EtOH may represent the neurophysiological correlate of increased unitary rewarding value, possibly driving progression to addiction.
Neuropharmacology, 2022; 203

FENS Forum 2022
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BOARD NUMBER: S01-562

REVERSAL OF NEUROLOGICAL DEFICITS BY PAINLESS NERVE GROWTH FACTOR IN A MOUSE MODEL OF RETT SYNDROME

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Rett syndrome is a rare genetic neurodevelopmental disease, affecting 1 over 10,000 females born worldwide, caused by sporadic mutations in the X-chromosome-located methyl-CpG-binding protein 2 (MeCP2) gene. Despite the great effort put forth by the scientific community, a therapy for this devastating disease is still needed. Here, we tested the therapeutic potential of a painless mutein of the Nerve Growth Factor, called human NGF painless (hNGFp), via a non-invasive intranasal delivery in female MeCP2+/- mice. We report that treatment with hNGFp (1) increased the chance of survival while (2) greatly improving behavioral parameters in MeCP2+/- mice. Furthermore, we observed (3) the rescue of a known target population of NGF, cholinergic neurons in the medial septum. We also reveal (4) a deficit in microglial morphology in MeCP2+/- mice, completely reversed in treated animals. To understand the immunomodulatory activity of hNGFp, we analyzed (5) the cytokine profile after hNGFp treatment in MeCP2+/- mice, to discover that our treatment rescued the expression of key neuroimmune-communication molecules such as Fractalkine and C-C Motif Chemokine Ligand 1 (CCL1). The overall conclusion is that hNGFp delivered intranasally can ameliorate symptoms in the MeCP2+/- model of Rett syndrome via its pleiotropic activity on both neurons and glia.

Pubmed:
29260466: Ardura-Fabregat A, Boddeke EWGM, Boza-Serrano A, Brioschi S, Castro-Gomez S, Ceyzériat K, Dansokho C, Dierkes T, Gelders G, Heneka MT, Hoeijmakers L, Hoffmann A, Iaccarino L, Jahnert S, Kuhbandner K, Landreth G, Lonnemann N, Löschmann PA, McManus RM, Paulus A, Reemst K, Sanchez-Caro JM, Tiberi A, Van der Perren A, Vautheny A, Venegas C, Webers A, Weydt P, Wijasa TS, Xiang X, Yang Y
Targeting Neuroinflammation to Treat Alzheimer's Disease.
Over the past few decades, research on Alzheimer's disease (AD) has focused on pathomechanisms linked to two of the major pathological hallmarks of extracellular deposition of beta-amyloid peptides and intra-neuronal formation of neurofibrils. Recently, a third disease component, the neuroinflammatory reaction mediated by cerebral innate immune cells, has entered the spotlight, prompted by findings from genetic, pre-clinical, and clinical studies. Various proteins that arise during neurodegeneration, including beta-amyloid, tau, heat shock proteins, and chromogranin, among others, act as danger-associated molecular patterns, that-upon engagement of pattern recognition receptors-induce inflammatory signaling pathways and ultimately lead to the production and release of immune mediators. These may have beneficial effects but ultimately compromise neuronal function and cause cell death. The current review, assembled by participants of the Chiclana Summer School on Neuroinflammation 2016, provides an overview of our current understanding of AD-related immune processes. We describe the principal cellular and molecular players in inflammation as they pertain to AD, examine modifying factors, and discuss potential future therapeutic targets.
CNS Drugs, 2017; 31
29473218: Rizzi C, Tiberi A, Giustizieri M, Marrone MC, Gobbo F, Carucci NM, Meli G, Arisi I, D'Onofrio M, Marinelli S, Capsoni S, Cattaneo A
NGF steers microglia toward a neuroprotective phenotype.
Microglia are the sentinels of the brain but a clear understanding of the factors that modulate their activation in physiological and pathological conditions is still lacking. Here we demonstrate that Nerve Growth Factor (NGF) acts on microglia by steering them toward a neuroprotective and anti-inflammatory phenotype. We show that microglial cells express functional NGF receptors in vitro and ex vivo. Our transcriptomic analysis reveals how, in primary microglia, NGF treatment leads to a modulation of motility, phagocytosis and degradation pathways. At the functional level, NGF induces an increase in membrane dynamics and macropinocytosis and, in vivo, it activates an outward rectifying current that appears to modulate glutamatergic neurotransmission in nearby neurons. Since microglia are supposed to be a major player in Aβ peptide

clearance in the brain, we tested the effects of NGF on its phagocytosis. NGF was shown to promote TrkA-mediated engulfment of Aβ by microglia, and to enhance its degradation. Additionally, the proinflammatory activation induced by Aβ treatment is counteracted by the concomitant administration of NGF. Moreover, by acting specifically on microglia, NGF protects neurons from the Aβ-induced loss of dendritic spines and inhibition of long term potentiation. Finally, in an ex-vivo setup of acute brain slices, we observed a similar increase in Aβ engulfment by microglial cells under the influence of NGF. Our work substantiates a role for NGF in the regulation of microglial homeostatic activities and points toward this neurotrophin as a neuroprotective agent in Aβ accumulation pathologies, via its anti-inflammatory activity on microglia.
Glia, 2018; 66

31422108: Saadipour K, Tiberi A, Lombardo S, Grajales E, Montroull L, Mañucat-Tan NB, LaFrancois J, Cammer M, Mathews PM, Scharfman HE, Liao FF, Friedman WJ, Zhou XF, Tesco G, Chao MV
Regulation of BACE1 expression after injury is linked to the p75 neurotrophin receptor.
BACE1 is a transmembrane aspartic protease that cleaves various substrates and it is required for normal brain function. BACE1 expression is high during early development, but it is reduced in adulthood. Under conditions of stress and injury, BACE1 levels are increased; however, the underlying mechanisms that drive BACE1 elevation are not well understood. One mechanism associated with brain injury is the activation of injurious p75 neurotrophin receptor (p75), which can trigger pathological signals. Here we report that within 72 h after controlled cortical impact (CCI) or laser injury, BACE1 and p75 are increased and tightly co-expressed in cortical neurons of mouse brain. Additionally, BACE1 is not up-regulated in p75 null mice in response to focal cortical injury, while p75 over-expression results in BACE1 augmentation in HEK-293 and SY5Y cell lines. A luciferase assay conducted in SY5Y cell line revealed that BACE1 expression is regulated at the transcriptional level in response to p75 transfection. Interestingly, this effect does not appear to be dependent upon p75 ligands including mature and pro-neurotrophins. In addition, BACE1 activity on amyloid precursor protein (APP) is enhanced in SY5Y-APP cells transfected with a p75 construct. Lastly, we found that the activation of c-jun n-terminal kinase (JNK) by p75 contributes to BACE1 up-regulation. This study explores how two injury-induced molecules are intimately connected and suggests a potential link between p75 signaling and the expression of BACE1 after brain injury.
Mol Cell Neurosci, 2019; 99

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KDM1A ENABLES POLYCOMB-MEDIATED SILENCING OF NON-NEURONAL GENES INTO NEURONAL EUCHROMATIN

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Although Kdm1a is the most expressed histone demethylases in adult neuronal cells, its molecular function is unknown. Here, we generated inducible and forebrain-restricted knockout (ifKO) mice in which Kdm1a is specifically eliminated in forebrain excitatory neurons during adulthood. The mice showed a prominent transcriptional and epigenomic dysregulation signature characterized by the expression of non-neuronal genes in excitatory neurons. By combining RNA-seq, ChIP-seq, ChIA-PET, Hi-C and Super-resolution microscopy experiments in adult excitatory neuronal we show that Kdm1a exerts its repressive function in the euchromatin compartment in CTCF-encapsulated micro-domains enriched in H3K27me3. Specifically, Kdm1a loss led to the destabilization of CTCF-encapsulated H3K27me3-microdomains, and weakened the boundaries between active and silent chromatin. These results underscore the role of Kdm1a safeguarding chromatin organization and silencing non neuronal genes in adult neurons.

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IMPACT OF ALTERED UBE3A DOSAGE ON SYNAPSE DEVELOPMENT: THE CASE OF THE ANGELMAN SYNDROME

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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The UBE3A gene codes for an E3 ubiquitin ligase and is critical to ensure a proper brain function. Indeed, perturbations of UBE3A dosage or function result in pathological phenotypes. Loss of UBE3A causes the Angelman Syndrome, a severe neurodevelopmental disorder characterized by intellectual disability, motor delay and seizures, while increased UBE3A copy number or activity are strongly associated with Autism. Importantly, the molecular underpinnings of UBE3A-associated pathogenic mechanisms of neurodevelopmental disorders are still poorly understood. In this project, we study the effects of UBE3A loss (mimicking the genetic alterations of the Angelman syndrome) on the regulation of synaptic development at single cell level in vivo. To this aim, we combine cortex-directed in utero electroporation to inactivate UBE3A in sparse layer 2/3 pyramidal neurons with confocal and super-resolution Stimulated Emission Depletion (STED) microscopy to investigate the consequences of UBE3A loss on the functional organization of excitatory and inhibitory postsynaptic compartments at synaptic and sub-synaptic resolution. As already suggested by other groups, our results indicate that UBE3A critically regulates the formation of excitatory synapses. Strikingly, our data also suggests that UBE3A controls the assembly and the maturation of specific subtypes of inhibitory synapses, namely those located in the perisomatic region and in the axon initial segment. Together, our preliminary results suggest for the first time that the UBE3A gene may be critical to set the number of excitatory and inhibitory synaptic connections at the single-cell level, thus contributing to regulate the ratio between excitation and inhibition through cell-autonomous mechanisms.

Pubmed:
32435178: Piano I, D'Antongiovanni V, Novelli E, Biagioni M, Dei Cas M, Paroni RC, Ghidoni R, Strettoi E, Gargini C
Myriocin Effect on Tvrm4 Retina, an Autosomal Dominant Pattern of Retinitis Pigmentosa.
Tvrm4 mice, a model of autosomal dominant retinitis pigmentosa (RP), carry a mutation of Rhodopsin gene that can be activated by brief exposure to very intense light. Here, we test the possibility of an anatomical, metabolic, and functional recovery by delivering to degenerating Tvrm4 animals, Myriocin, an inhibitor of ceramide synthesis previously shown to effectively slow down retinal degeneration in rd10 mutants (Strettoi et al., 2010; Piano et al., 2013). Different routes and durations of Myriocin administration were attempted by using either single intravitreal (i.v.) or long-term, repeated intraperitoneal (i.p.) injections. The retinal function of treated and control animals was tested by ERG recordings. Retinas from ERG-recorded animals were studied histologically to reveal the extent of photoreceptor death. A correlation was observed between Myriocin administration, lowering of retinal ceramides, and preservation of ERG responses in i.v. injected cases. Noticeably, the i.p. treatment with Myriocin decreased the extension of the retinal-degenerating area, preserved the ERG response, and correlated with decreased levels of biochemical indicators of retinal oxidative damage. The results obtained in this study confirm the efficacy of Myriocin in slowing down retinal degeneration in genetic models of RP independently of the underlying mutation responsible for the disease, likely targeting ceramide-dependent, downstream pathways. Alleviation of retinal oxidative stress upon Myriocin treatment suggests that this molecule, or yet unidentified metabolites, act on cellular detoxification systems supporting cell survival. Altogether, the pharmacological approach chosen here meets the necessary pre-requisites for translation into human therapy to slow down RP.
Front Neurosci, 2020; 14
31607844: Falasconi A, Biagioni M, Novelli E, Piano I, Gargini C, Strettoi E
Retinal Phenotype in the rd9 Mutant Mouse, a Model of X-Linked RP.

Retinal degeneration 9 (rd9) mice carry a mutation in the retina specific "Retinitis Pigmentosa GTPase Regulator (RPGR)" Open Reading Frame (ORF) 15 gene, located on the X chromosome and represent a rare model of X-linked Retinitis Pigmentosa (XLRP), a common and severe form of retinal degeneration (Wright et al., 2010; Tsang and Sharma, 2018). The rd9 RPGR-ORF15 mutation in mice causes lack of the protein in photoreceptors and a slow degeneration of these cells with consequent decrease in Outer Nuclear Layer (ONL) thickness and amplitude of ERG responses, as previously described (Thompson et al., 2012). However, relative rates of rod and cone photoreceptor loss, as well as secondary alterations occurring in neuronal and non-neuronal retinal cell types of rd9 mutants remain to be assessed. Aim of this study is to extend phenotype analysis of the rd9 mouse retina focusing on changes occurring in cells directly interacting with photoreceptors. To this purpose, first we estimated rod and cone survival and its degree of intraretinal variation over time; then, we studied the morphology of horizontal and bipolar cells and of the retinal pigment epithelium (RPE), extending our observations to glial cell reactivity. We found that in rd9 retinas rod (but not cone) death is the main cause of decrease in ONL thickness and that degeneration shows a high degree of intraretinal variation. Rod loss drives remodeling in the outer retina, with sprouting of second-order neurons of the rod-pathway and relative sparing of cone pathway elements. Remarkably, despite cone survival, functional defects can be clearly detected in ERG recordings in both scotopic and photopic conditions. Moderate levels of Muller cells and microglial reactivity are sided by striking attenuation of staining for RPE tight junctions, suggesting altered integrity of the outer Blood Retina Barrier (BRB). Because of many features resembling slowly progressing photoreceptor degeneration paradigms or early stages of more aggressive forms of RP, the rd9 mouse model can be considered a rare and useful tool to investigate retinal changes associated to a process of photoreceptor death sustained throughout life and to reveal disease biomarkers (e.g., BRB alterations) of human XLRP.
Front Neurosci, 2019; 13

31199887: Guadagni V, Biagioni M, Novelli E, Aretini P, Mazzanti CM, Strettoi E
Rescuing cones and daylight vision in retinitis pigmentosa mice.
Hallmark of retinitis pigmentosa (RP) is the primary, genetic degeneration of rods followed by secondary loss of cones, caused by still elusive biologic mechanisms. We previously shown that exposure of rd10 mutant mice, modeling autosomal recessive RP, to environmental enrichment (EE), with enhanced motor, sensorial and social stimuli, results into a sensible delay of retinal degeneration and vision loss. Searching for effectors of EE-mediated retinal protection, we performed transcriptome analysis of the retina of rd10 enriched and control mice and found that gene expression at the peaks of rod and cone degeneration is characterized by a strong inflammatory/immune response, which is however measurably lower in enrichment conditions. Treating rd10 mice with dexamethasone during the period of maximum photoreceptors death lowered retinal inflammation and caused a preservation of cones and cone-mediated vision. Our findings indicate a link between retinal inflammation and bystander cone degeneration, reinforcing the notion that cone vision in RP can be preserved using anti-inflammatory approaches.-Guadagni, V., Biagioni, M., Novelli, E., Aretini, P., Mazzanti, C. M., Strettoi, E. Rescuing cones and daylight vision in retinitis pigmentosa mice.
FASEB J, 2019; 33

28720880: Gargini C, Novelli E, Piano I, Biagioni M, Strettoi E
Pattern of retinal morphological and functional decay in a light-inducible, rhodopsin mutant mouse.
Hallmarks of Retinitis Pigmentosa (RP), a family of genetic diseases, are a typical rod-cone-degeneration with initial night blindness and loss of peripheral vision, followed by decreased daylight sight and progressive visual acuity loss up to legal blindness. Great heterogeneity in nature and function of mutated genes, variety of mutations for each of them, variability in phenotypic appearance and transmission modality contribute to make RP a still incurable disease. Translational research relies on appropriate animal models mimicking the genetic and phenotypic diversity of the human pathology. Here, we provide a systematic, morphological and functional analysis of Rho/Rho rhodopsin mutant mice, originally described in 2010 and portraying several features of common forms of autosomal dominant RP caused by gain-of-function mutations. These mice undergo photoreceptor degeneration only when exposed briefly to strong, white light and allow controlled timing of induction of rod and cone death, which therefore can be elicited in adult animals, as observed in human RP. The option to control severity and retinal extent of the phenotype by regulating intensity and duration of the inducing light opens possibilities to exploit this model for multiple experimental purposes. Altogether, the unique features of this mutant make it an excellent resource for retinal degeneration research.
Sci Rep, 2017; 7

27001178: Boggio EM, Pancrazi L, Gennaro M, Lo Rizzo C, Mari F, Meloni I, Ariani F, Panighini A, Novelli E, Biagioni M, Strettoi E, Hayek J, Rufa A, Pizzorusso T, Renieri A, Costa M
Visual impairment in FOXG1-mutated individuals and mice.
The Forkead Box G1 (FOXG1 in humans, Foxg1 in mice) gene encodes for a DNA-binding transcription factor, essential for the development of the telencephalon in mammalian forebrain. Mutations in FOXG1 have been reported to be involved in the onset of Rett Syndrome, for which sequence alterations of MECP2 and CDKL5 are known. While visual alterations are not

classical hallmarks of Rett syndrome, an increasing body of evidence shows visual impairment in patients and in MeCP2 and CDKL5 animal models. Herein we focused on the functional role of FOXG1 in the visual system of animal models (Foxg1(+/Cre) mice) and of a cohort of subjects carrying FOXG1 mutations or deletions. Visual physiology of Foxg1(+/Cre) mice was assessed by visually evoked potentials, which revealed a significant reduction in response amplitude and visual acuity with respect to wild-type littermates. Morphological investigation showed abnormalities in the organization of excitatory/inhibitory circuits in the visual cortex. No alterations were observed in retinal structure. By examining a cohort of FOXG1-mutated individuals with a panel of neuro-ophthalmological assessments, we found that all of them exhibited visual alterations compatible with high-level visual dysfunctions. In conclusion our data show that Foxg1 haploinsufficiency results in an impairment of mouse and human visual cortical function.
Neuroscience, 2016; 324
27286364: Amato R, Biagioni M, Cammalleri M, Dal Monte M, Casini G
VEGF as a Survival Factor in Ex Vivo Models of Early Diabetic Retinopathy.
Growing evidence indicates neuroprotection as a therapeutic target in diabetic retinopathy (DR). We tested the hypothesis that VEGF is released and acts as a survival factor in the retina in early DR.
Invest Ophthalmol Vis Sci, 2016; 57

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EXCESSIVE DENDRITIC INHIBITION IN THE PREFRONTAL CORTEX OF A MOUSE MODEL OF DOWN SYNDROME PERSISTS THROUGHOUT DEVELOPMENT INTO ADULTHOOD

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Down syndrome (DS) is a condition characterized by various physical and neurological features including mild to severe intellectual disability. Individuals with DS present important deficits in cognitive tasks known to depend on the anatomical and functional integrity of the prefrontal cortex (PFC). Recovery of behavioral and neurophysiological deficits using $GABA_AR$ antagonists in DS mouse models led to hypothesize an excessive activity of inhibitory cortical circuits in this condition. Nonetheless, the rich diversity of inhibitory neurons prompts the question of potential GABAergic circuit-specific alterations in DS. We previously reported that dendritic inhibition, mediated by α5-containing $GABA_ARs$, is enhanced in juvenile (P18-25) Ts65Dn mice, and that parvalbumin-expressing interneurons (PV-INs) exhibit abnormalities of intrinsic excitability leading to the loss of typical fast spiking behavior and precocious firing. Here we show that in adult Ts65Dn mice, PV-INs recovered their excitability deficits. In contrast, α5-mediated dendritic inhibition remains enhanced in adult mice suggesting that over-inhibition of principal neurons' dendrites is a major pathophysiological mechanism in DS that persists throughout development. In order to assess the impact of dendritic over-inhibition on PFC network activity, we implemented *in vivo* electrophysiological recordings using fully integrated, high density Neuropixels probes. Preliminary results suggest that acute treatment with specific α5 negative allosteric modulator (α5IA) drastically enhances neuronal population activity patterns of PFC deep layers, likely enhancing cortical output. Our data indicate dendritic inhibition as a prominent mechanism underlying over-inhibition of cortical circuits in DS mice, and support potential therapeutic strategies based on the modulation of α5-containing $GABA_ARs$.

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DIETARY LOW-LEVEL GLYPHOSATE AND GENETIC PREDISPOSITION: A DOUBLE-HIT IN AUTISM SPECTRUM DISORDERS?

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

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Mutations in genes coding for synaptic proteins are among the most well-characterized disease-associated elements in individuals with autism spectrum disorders (ASDs). The postsynaptic scaffolding protein SHANK3 is a critical orchestrator of the signaling complex at glutamatergic synapses. Hemi-deletion of SHANK3 occurs in neurodevelopmental disorders characterized by ASD-like behaviors, including intellectual disability. Furthermore, epidemiological studies suggest that pesticides may represent an add-on risk factor for ASD. Here, we examined whether dietary exposure to non-observed adverse effect levels of glyphosate exacerbates the neurological phenotype driven by SHANK3 deletions, revealing the interaction between genome and exposome. We used wild-type, heterozygous or homozygous mice for SHANK3 deleted after exon 21 and compared functional sequel of glyphosate dietary exposure from weaning to adulthood. We analyzed cognitive performances, molecular and cellular modifications related to neurological adaptations. Results showed an increased neuro-inflammation signature with no significant impact on cognition. To further mimic developmental exposure, mice of the three genotypes were continuously exposed to glyphosate, first mothers during gestation and lactation, then pups from weaning until adulthood. We found increased mortality of embryos during the gestation period of WT and heterozygous mice fed with glyphosate. The mortality analysis at the weaning reveals an increase in the mortality rate in heterozygous SHANK3 mice exposed to glyphosate. We are now assessing cognitive performance and neuro-inflammation biomarkers. Our results outline links between environmental risks and genetic factors of ASD, depending on the exposure period.

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EXCITATORY SYNAPSES AND GAP JUNCTIONS COOPERATE TO IMPROVE PV NEURONAL BURST FIRING AND CORTICAL SOCIAL COGNITION IN SHANK2-MUTANT MICE.

POSTER SESSION 01 - SECTION: PRECLINICAL MODELS OF AUTISM AND NEURODEVELOPMENTAL DISORDERS

Seungjoon Lee[1], Eunee Lee[2], Jae Jin Shin[1], Woochul Choi[3], Joo Min Park[4], Min Whan Jung[1], Se-Bum Paik[3], Eunjoon Kim[1]
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NMDA receptor (NMDAR) and GABA neuronal dysfunctions are observed in animal models of autism spectrum disorders, but how these dysfunctions impair social cognition and behavior remains unclear. We report here that NMDARs in cortical parvalbumin (Pv)-positive interneurons cooperate with gap junctions to promote high-frequency (>80 Hz) Pv neuronal burst firing and social cognition. *Shank2*$^{-/-}$ mice, displaying improved sociability upon NMDAR activation, show impaired cortical social representation and inhibitory neuronal burst firing. Cortical *Shank2*$^{-/-}$ Pv neurons show decreased NMDAR activity, which suppresses the cooperation between NMDARs and gap junctions (GJs) for normal burst firing. *Shank2*$^{-/-}$ Pv neurons show compensatory increases in GJ activity that are not sufficient for social rescue. However, optogenetic boosting of Pv neuronal bursts, requiring GJs, rescues cortical social cognition in *Shank2*$^{-/-}$ mice, similar to the NMDAR-dependent social rescue. Therefore, NMDARs and gap junctions cooperate to promote cortical Pv neuronal bursts and social cognition.

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THE ROLE OF HIPPOCAMPAL VIP-EXPRESSING INTERNEURONS IN THE PATHOPHYSIOLOGY OF TEMPORAL LOBE EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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In the hippocampus, two groups of VIP-expressing interneurons, containing CCK-expressing basket cells and/or interneuron-selective interneurons (ISIs) control GABAergic transmission and pyramidal cell activity. The aim of the current study is to assess the possible role of VIP-expressing interneurons in the pathophysiology of epilepsy. We permanently inhibited GABA release selectively from VIP interneurons of the ventral subiculum by injecting a viral vector expressing tetanus toxin light chain (TeLC) in male epileptic as well as nonepileptic VIP-cre mice. Mice were then subjected to telemetric EEG recording for 4 weeks. In addition, spontaneous alternation Y-maze and novel location recognition tests were conducted to evaluate spatial memory and learning, respectively. In non-epileptic mice, injection of TeLC and GFP did not cause development of seizures in both groups. In addition, behavioral tests addressing anxiety, memory, and navigation showed no differences between groups. In epileptic animals, the average number of spontaneous seizures per day as well as the average time spent in seizure per day showed a significant reduction in TeLC in comparison to GFP group. Surprisingly, we observed a clear connection between gender and the severity of status epilepticus and the animal's response to vector injection. ISIs mainly target other interneurons; therefore, silencing them would increase inhibition in pyramidal cells. However, the outcome of the silencing of VIP-interneurons in epileptic mice is highly dependent on the status of the network before silencing. To prove, we need further assessment by triple immunohistochemistry labeling for GFP, CCK, and VIP, which is currently ongoing in our lab.

Pubmed:

35167940: Drexel M, Rahimi S, Sperk G
Silencing of Hippocampal Somatostatin Interneurons Induces Recurrent Spontaneous Limbic Seizures in Mice.
The hippocampus proper and the subiculum contain two major populations of somatostatin (SST)-containing interneurons, oriens-lacunosum moleculare (O-LM) cells projecting from the stratum oriens to the stratum lacunosum moleculare and bistratified cells with their cell bodies close to the pyramidal cell layer and axons terminating in the strata radiatum and oriens. Both types of interneurons innervate pyramidal cell dendrites and exert prominent feedback inhibition. We now investigated whether impairing this type of feed-back inhibition by selectively inhibiting GABA release from SST expressing interneurons in hippocampal sector CA1 and subiculum may be sufficient to induce spontaneous recurrent seizures. We injected transgenic mice expressing Cre-recombinase on the SST promoter unilaterally into the ventral CA1 sector and subiculum with an adeno-associated viral (AAV) vector expressing tetanus toxin light chain (TeLC) with its reading frame inverted in a flip-excision (FLEX) cassette. This treatment resulted in specific expression of TeLC and silencing of SST-containing interneurons. We continuously monitored the EEG and behavior of the mice for six weeks. Nine out of eleven mice within 10 days developed series of pre- or interictal spikes (IS, 21.4 ± 6.83 per week) and four mice exposed recurrent spontaneous seizures (SRS, 1.5 ± 0.29 per week). All 23 SRS observed were preceded by IS series. Our data demonstrate a critical role of feed-forward inhibition mediated by SST-containing interneurons suggesting that their sustained malfunctioning can be causatively involved in the development of TLE.
Neuroscience, 2022; 487
33524378: Jafarian M, Modarres Mousavi SM, Rahimi S, Ghaderi Pakdel F, Lotfinia AA, Lotfinia M, Gorji A
The effect of GABAergic neurotransmission on the seizure-related activity of the laterodorsal thalamic nuclei and the somatosensory cortex in a genetic model of absence epilepsy.
The present study aimed to investigate the alterations of the GABAergic system in the laterodorsal nucleus (LDN) of the thalamus and the somatosensory cortex (SC) in an experimental model of absence seizure. The effects of pharmacological manipulation of both GABA and GABA receptor subunits in the LDN on the generation of spike-wave discharges (SWD) were evaluated. The experiments were carried out in four groups of both WAG/Rij and Wistar rats with 2 and 6 months of age. The expressions of various GABA receptor subunits were studied in the LDN and SC. Furthermore, recordings of unit activity from

the LDN and electrocorticography were simultaneously monitored before, during, and after the application of GABA and GABA antagonists in the LDN. The generation of SWD in the older WAG/Rij rats was associated with significant alterations in the expression of GABARα1, GABARβ3, and GABAR2 subunits in the LDN as well as GABARα1, GABARβ3, GABARγ2, and GABAR2 subunits in the SC. Furthermore, the occurrence of SWD was associated with a significant reduction of gene expression of GABAARα1 and increase of GABAARβ3 in the LDN as well as reduction of GABAARα1, GABAARβ3, GABAARγ2, and GABABR2 in the SC. The microionthophoretic application of the GABA antagonist bicuculline resulted in a significant increase in the population firing rate of LDN neurons as well as the mean number and duration of SWD. The application of the GABA antagonist CGP35348 significantly increased the population firing rate of LDN neurons but decreased the mean number of SWD. Our data indicate the regulatory effect of the GABAergic system of the LDN and SC in absence seizures.
Brain Res, 2021; 1757
33350986: Rahimi S, Ragerdikashani M, Beheshti F, Baghishani F, Hosseini M, Saeedi N, Mirdoosti M, Sahab Negah S
Alteration of the neurogenesis and long term potential of olfactory bulb in an animal model of PTSD.
Failure of extinction of fear-conditioned traumatic memory is the main pathology behind post-traumatic stress disorder (PTSD). Functional and structural dysfunctions in the olfactory system are implicated by studies in PTSD patients. However, little is known regarding the neurobiological networks of trauma-related odor sensitivity in PTSD. Male Wistar rats were exposed with a female cat for 10 min and long-term stress was evaluated by behavioral tests, containing open field (OF) and elevated plus maze (EPM). To prove the PTSD model, the serum level of cortisol was evaluated and compared with the control group. Local field potential (LFP) was applied to compare the electrophysiology of the OB in two groups. To assess neurogenesis, the expression of nestin, and doublecortin were evaluated. Data from EPM revealed a significant increase in spent time in the closed arms in PTSD group. We observed a significant reduction in OF parameters in terms of the total distance traveled, the time spent in the central zone, and the number of crossing the central zone in PTSD group compared to the control group. The mean serum cortisol level was significantly higher in the PTSD group than the control group. In LFP recording, the slope and the amplitude of field excitatory postsynaptic potential (fEPSP) in the PTSD group were significantly higher than that of the control group. Our results also showed that the mRNA expression level of nestin as a neural progenitor marker and doublecortin, as an immature neuron marker, significantly decreased in the PTSD group compared to the control group. This study has shown that PTSD can disrupt the OB function through decreasing neurogenesis. More information on PTSD and OB would help us to establish a greater degree of accuracy on this matter.
Acta Neurobiol Exp (Wars), 2020; 80
32219080: Asadpour H, Naghibi SM, Rahimi S, Sharafkhaneh A, Afshari Saleh L, Rezaee Talab F, Amini M, Nikzad F
Prolonged Sleep Apnea in Two Patients with a History of Opium Abuse -A Case Report.
Obstructive sleep apnea (OSA) is a highly prevalent sleep-disordered breathing (SDB).
Iran J Otorhinolaryngol, 2020; 32
25138625: Marschollek C, Karimzadeh F, Jafarian M, Ahmadi M, Mohajeri SM, Rahimi S, Speckmann EJ, Gorji A
Effects of garlic extract on spreading depression: In vitro and in vivo investigations.
The potential use of garlic for prevention and treatment of different types of headaches has been suggested by several medieval literatures. Cortical spreading depression (CSD), a propagating wave of neuroglial depolarization, was established as a target for anti-migraine drugs. This study was designed to investigate the effect of garlic extract on CSD in adult rats.
Nutr Neurosci, 2017; 20
20438812: Jafarian M, Rahimi S, Behnam F, Hosseini M, Haghir H, Sadeghzadeh B, Gorji A
The effect of repetitive spreading depression on neuronal damage in juvenile rat brain.
Spreading depression (SD) is pronounced depolarization of neurons and glia that travels slowly across brain tissue followed by massive redistribution of ions between intra- and extracellular compartments. There is a relationship between SD and some neurological disorders. In the present study the effects of repetitive SD on neuronal damage in cortical and subcortical regions of juvenile rat brain were investigated. The animals were anesthetized and the electrodes as well as cannula were implanted over the brain. SD-like event was induced by KCl injection. The brains were removed after 2 or 4 weeks after induction of 2 or 4 SD-like waves (with interval of 1 week), respectively. Normal saline was injected instead of KCl in sham group. For stereological study, paraffin-embedded brains were cut in 5 microm sections. The sections were stained with Toluidine Blue to measure the volume-weighted mean volume of normal neurons and the numerical density of dark neurons. The volume-weighted mean volume of normal neurons in the granular layer of the dentate gyrus and layer V of the temporal cortex in SD group were significantly decreased after four repetitive SD. Furthermore, densities of dark neurons in the granular layer of the dentate gyrus (after 2 weeks), the caudate-putamen, and layer V of the temporal cortex (after 4 weeks) were significantly increased in SD group. Repetitive cortical SD in juvenile rats may cause neuronal damage in cortical and subcortical areas of the brain. This may important in pathophysiology of SD-related neurological disorders.
Neuroscience, 2010; 169


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ROLE OF HIPPOCAMPAL FOCAL RHYTHMIC DISCHARGES (FRDS) IN IMPAIRED SPATIAL WORKING MEMORY IN EPILEPTIC MICE.

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Memory impairment is a common cognitive deficit in temporal lobe epilepsy (TLE). The hippocampus is severely altered in TLE exhibiting multiple anatomical changes that lead to a hyperexcitable network capable of generating frequent epileptic discharges and seizures. In this study we investigated whether hippocampal involvement in epileptic activity drives working memory deficits. We employed the supra-hippocampal kainic acid (KA) mouse model of TLE. Furthermore, mice experienced frequent focal seizures and less frequent behavioural seizures (stage IV, Racine scale). We observed a working memory deficit in epileptic mice in a delayed-alternation spatial working memory task (Ctrl, 73.54% correct; n = 11; KA ,62.07 % correct, n =13). We further investigated the neural mechanisms of the working memory deficit using bilateral LFP recordings from CA1 during task performance and during long sleep sessions pre and post memory task. We discovered that epileptic mice experienced focal rhythmic discharges (FRDs) while they performed the spatial working memory task (n=1686 on the maze, n=7 mice). Spatial correlation analysis revealed that FRDs were often spatially stable on the maze (7/8 mice and 55/124 sessions) – and were most common around reward zones (25 %) and delay zones (50 %). Preliminary results showed that FRDs shifted to the new reward zones following the change of the reward locations – suggesting that reward-related dynamics such as sharp wave ripple, may drive FRDs. Memory performance was correlated with stability of FRDs ($p \leq .001$, $R=0.56$), suggesting that spatially unstable FRDs interfere with working memory codes in real time.

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CB2, A RECEPTOR TO TARGET DURING EPILEPTOGENESIS: EVIDENCE FROM STUDIES IN RAT PUPS SUBJECTED TO STATUS EPILEPTICUS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Epileptogenesis is the neurobiological process by which epilepsy develops. It most often affects a healthy brain and occurs following severe brain damage. One of the key mechanisms underlying this pathological conversion is the subsequent neuroinflammation, mainly supported by microglial cell activation and extravasation of circulating monocytes. Agonists of the cannabinoid receptor type 2 (CB2), massively expressed in vivo by monocytes and presumably by microglia, are known to have potent anti-inflammatory and neuroprotective effects. Here, we evaluated whether GP1a, a CB2-specific ligand administered following pilocarpine-induced *status epilepticus* (Pilo-SE) in 21-old-day male rat pups, counteracted epileptogenesis and underlying inflammatory processes and reduced the severity of cognitive impairments. GP1a was administered at the dose of 3 mg/kg i.p. every other day for 2 weeks, starting 2 hours after SE onset. We provide evidence that the establishment of memory impairments and their underlying cellular mechanisms can be thwarted by GP1a given during the early phase of epileptogenesis. We also show that the development of handling-induced seizures can be delayed and their number lessened by GP1a. Interestingly, these beneficial effects do not appear to be underpinned by a decrease in the inflammatory peak following Pilo-SE. Contrary to what has been previously described in other neuroinflammatory situations, GP1a did not inhibit but rather potentiated the extravasation of monocytes into the brain. Ongoing investigations at the cellular level using flow cytometry will help to determine the phenotype of CB2-expressing cells and their polarization. Overall, our study highlights the promising therapeutic potential of drugs targeting CB2 during epileptogenesis.

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BOARD NUMBER: S01-571

EVALUATION OF DENDRITE MORPHOLOGY IN WISTAR AND GENETIC ABSENCE EPILEPTIC (GAERS) RATS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Aims The aim of the study is to examine the morphological features of dendrites and dendritic spines of pyramidal neurons in somatosensory cortex and hippocampus of Wistar and GAERS (Genetic Absence Epilepsy Rat from Strasbourg) rats. **Methods** GAERS (n=5) and Wistar (n=5) rats were sacrificed by transcardial perfusion method. Brain tissues were stained using the FD Rapid GolgiStain Kit. Coronal sections of 200 µm thickness were obtained with cryostat. Pyramidal neurons in deep layers of the somatosensory cortex and CA1 region of the hippocampus were examined using light microscope and Neurolucida 360 software. Branching and length of apical dendrite and basal dendrites, and types and densities of dendritic spines were analyzed. **Results** Statistically the total number of dendrite nodes (p=0.0053, p=0,0047), the total number of dendrite segments (dendritic arborization) (p=0.0036, p=0,0036), the total number of dendrite terminations (p=0.0033, p=0,0029), the total dendrite length (µm) (p=0.0002, p=0,0007) and the dendritic spine density (1/µm) (p=0.0168, p=0,0120) of the somatosensory cortex and the hippocampus were significantly higher in GAERS rats, respectively. When dendritic spine types were evaluated separately, stubby type dendritic spines in the hippocampus were found to be significantly higher in GAERS rats compared to Wistar rats (p=0.0204). **Conclusions** It was concluded that intense synaptic activation seen in postsynaptic cells in the somatosensory cortex and hippocampus in GAERS causes changes in the dendrite morphology of pyramidal neurons. This study was supported by Marmara University Scientifical Research Projects Commission (TYL-2021-10244). **Keywords** Absence epilepsy, GAERS, dendrite, dendritic spine

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-572

SPATIO-TEMPORAL DYNAMICS OF SEIZURE INITIATION IN HUMAN PERIGLIOMA CORTEX EX VIVO

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Aims: Our aim is to describe the spatio-temporal dynamics of extracellular neuronal activities during the transition to an ictal-like event (ILE), by assessing multiscale electrophysiological data including field potentials (FP), multiunit activity (MUA) and high frequency oscillations (HFO), in human postoperative neocortical slices. **Methods:** Fourty four peritumoral neocortical slices from 27 glioma patients were included in this study. *Ex vivo* extracellular recordings were performed using a 120 contacts MultiElectrode Array, both at a basal state during which spontaneous interictal discharges (IIDs) are generated and during the transition to ILE, induced by bathing slices with a proexcitatory milieu. **Results:** The transition from the interictal basal state to the ILE was characterized by the emergence of PIDs presenting larger and progressively increasing amplitudes, time to peak, rising slopes, decay and frequency, compared to spontaneous IIDs that remained stable. PIDs then presented a temporal evolution with another increase in amplitude, time to peak, rising slope, decay and frequency just before ILE onset. HFO also presented a progressive increase in their power, duration and a temporal shift in their phase coupling with FP. A spatial recruitment was observed, with larger territories recruited over time. **Conclusion**: During the transition to an ILE, neuronal synchronized activities evolve in their temporal and spatial dynamics, suggesting an increased and wider neuronal synchronization. This pattern peaks during the seconds before the ILE, suggesting a maximal network recruitment. Further, HFO's phase-coupling to FP also evolves, reinforcing such dynamical pattern of recruitment and stressing neuronal interaction complexity in seizure generation.

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THE UNTOLD IMPLICATION OF MICROGLIA ON THE PROTECTIVE EFFECT OF CANNABIDIOL IN EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Cannabinoids (CBs) are implicated in a number of physiological and pathological mechanisms in the central nervous system. Over the past ten years the number of scientific studies demonstrating the efficacy of some of the most abundant non-psychoactive compounds present in Cannabis sativa has massively increased. In particular, cannabidiol (CBD) has been shown to be effective in the treatment of several types of neurological disorders and neurodegenerative diseases. Aims: The present study aimed at exploring the mechanism by which cannabidiol reduces neuronal damage induces by kainite in organotypic hippocampal slices, an *in vitro* model of epilepsy. Methods: Rat organotypic hippocampal slices were exposed to 5 µM kainic acid for 24 hours in presence or absence of cannabinoids. The cell death in the CA3 subregion of slices was quantified by propidium iodide fluorescence. Microglia activation and polarization was evaluated using Flow Cytometry and Morphology Analysis. Results: When present in the incubation medium, cannabidiol and natural compounds reduced CA3 injury induced by kainic acid. Conversely, incubation with Delta-9-tetrahydrocannabinol (Delta-9-THC) exacerbated hippocampal damage. The neuroprotective effects of cannabidiol was blocked by TRPV1, TRPV2, 5-HT1A, and PPARγ antagonists. Cannabidiol incubation significantly reverse microglia activation and transition from the M2 to M1 phenotype observed in epileptic model. Conclusions: Our study suggests that cannabidiol mitigate neuronal death by inhibiting microglial activation and promoting transformation from an M1 to an M2 phenotype.

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LOCAL THERMAL MODULATION OF OPTOGENETICALLY INDUCED EPILEPTIC ACTIVITY BY INFRARED LASER LIGHT

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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New treatments of epilepsy are required since almost 30% of the millions of epileptic patients suffer from a drug-resistant form of it. One possible approach is the local heating of brain tissue, as it has been shown to influence the general network excitability and influence the frequency of neural oscillations. This project aims to reveal the influence of local temperature increase on generalized tonic-clonic epileptic seizures. We used a recently developed optogenetic epilepsy model, in which we can reliably induce tonic-clonic seizures by rhythmic stimulation of channelrhodopsin expressing layer 6 corticothalamic cells of NTSR1 double transgenic mice. Generalized tonic-clonic seizures were provoked by 447 nm blue light stimulation of corticothalamic pathway, under freely moving conditions. The local field potential of epileptiform activity was recorded from the cortex and from the hippocampus. Local heating was induced by 1550 nm infrared stimulation applied at the site of epileptogenesis. Each day only one seizure was evoked from each animal, alternating the heating and non-heating epochs. Local heating of the site of epileptogenesis was unable to inhibit the development of the epileptiform activity, however, it was able to change the pattern of seizure events. A significant decrease in the duration and so far, a non-significant decrease in the prevalence of seizures were found in the heated sessions of seizure induction. The power of spectral components also showed a prominent rearrangement due to the rise of local temperature, but there was no change in spreading patterns or latencies of synchronized activity among distant cortical regions.

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BOARD NUMBER: S01-575

GENETIC SUSCEPTIBILITY TO ACQUIRED EPILEPSY AFFECTS SEIZURE PROGRESSION AFTER AMYGDALA KINDLING: VALIDATION OF THE FAST AND SLOW RAT MODELS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Introduction: Animal models are valuable tools to study how genetic predisposition influences acquired epileptogenesis. Previously, Racine and colleagues (1999) have developed selectively-bred rat strains with different susceptibility to evoked seizures using the amygdala kindling model, designated as FAST (seizure-prone) or SLOW (seizure-resistant). **Aims:** To validate the phenotypes of our current FAST & SLOW rat colony to experimental amygdala kindling, and to evaluate the seizure susceptibility of their F2 generation progeny. **Methods:** A stimulating bipolar electrode was inserted into the left amygdala of 11-week-old male rats—FAST (n= 10), SLOW (n= 14) and their F2 generation (n= 68). One week post-surgery, the after-discharge threshold to generate an electrographic seizure of >5 sec was determined. Rats then either received a maximum of 30 kindling stimulations (2/day, 5 days/week) or until 5 class V seizures were observed (i.e., fully kindled). **Results:** FAST rats required fewer stimulations to fully kindle compared to SLOW rats ($p < 0.001$), with all animals fully kindled within 30 stimulations. The F2 generation required fewer stimulations to fully kindle compared to SLOW rats ($p < 0.01$) but were comparable to FAST rats. Total seizure duration was also significantly shorter for SLOW rats compared to FAST and F2 rats for the first 12-15 stimulations. **Conclusion:** Differences in kindling profiles between FAST, SLOW rats and their F2 generation suggest that differences in susceptibility to acquired epileptogenesis are genetically inherited. This finding supports further investigation of candidate genes and proteins in this model which may explain inherent susceptibility to acquired epilepsy in humans.

Pubmed:
31468516: Leung WL, Casillas-Espinosa P, Sharma P, Perucca P, Powell K, O'Brien TJ, Semple BD
An animal model of genetic predisposition to develop acquired epileptogenesis: The FAST and SLOW rats.
Epidemiological data and gene association studies suggest a genetic predisposition to developing epilepsy after an acquired brain insult, such as traumatic brain injury. An improved understanding of genetic determinants of vulnerability is imperative for early disease diagnosis and prognosis prediction, with flow-on benefits for the development of targeted antiepileptogenic treatments as well as optimal clinical trial design. In the laboratory, one approach to investigate why some individuals are more vulnerable to acquired epilepsy than others is to examine unique rodent models exhibiting either vulnerability or resistance to epileptogenesis. This review focuses on the most well-characterized of these models, the FAST (seizure-prone) and SLOW (seizure-resistant) rat strains, which were derived by selective breeding for differential amygdala electrical kindling rates. We describe how these strains differ in their seizure profiles, neuroanatomy, and neurobehavioral phenotypes, both at baseline and after a brain insult, with this knowledge proving fruitful to identify common pathological abnormalities associated with seizure susceptibility and psychiatric comorbidities. It is important to note that accruing data on strain differences in multiple biological processes provides insight into why some individuals may be more vulnerable to epileptogenesis, although future studies are evidently needed to identify the precise molecular and genetic risk factors. Together, the FAST and SLOW rat strains, and other similar experimental models, are invaluable neurobiological tools to investigate the effect of genetic background on acquired epilepsy risk, as well as the poorly understood relationship between epilepsy development and associated comorbidities.
Epilepsia, 2019; 60
31717556: Sharma R, Leung WL, Zamani A, O'Brien TJ, Casillas Espinosa PM, Semple BD
Neuroinflammation in Post-Traumatic Epilepsy: Pathophysiology and Tractable Therapeutic Targets.
Epilepsy is a common chronic consequence of traumatic brain injury (TBI), contributing to increased morbidity and mortality for survivors. As post-traumatic epilepsy (PTE) is drug-resistant in at least one-third of patients, there is a clear need for novel

therapeutic strategies to prevent epilepsy from developing after TBI, or to mitigate its severity. It has long been recognized that seizure activity is associated with a local immune response, characterized by the activation of microglia and astrocytes and the release of a plethora of pro-inflammatory cytokines and chemokines. More recently, increasing evidence also supports a causal role for neuroinflammation in seizure induction and propagation, acting both directly and indirectly on neurons to promote regional hyperexcitability. In this narrative review, we focus on key aspects of the neuroinflammatory response that have been implicated in epilepsy, with a particular focus on PTE. The contributions of glial cells, blood-derived leukocytes, and the blood-brain barrier will be explored, as well as pro- and anti-inflammatory mediators. While the neuroinflammatory response to TBI appears to be largely pro-epileptogenic, further research is needed to clearly demonstrate causal relationships. This research has the potential to unveil new drug targets for PTE, and identify immune-based biomarkers for improved epilepsy prediction.
Brain Sci, 2019; 9

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BOARD NUMBER: S01-576

CHARACTERIZATION OF THE ANTIAPOPTOTIC AND NEUROPROTECTIVE EFFECT OF DAPSONE IN A MODEL OF STATUS EPILEPTICUS INDUCED WITH KAINIC ACID IN RATS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Status epilepticus (SE) is a serious condition with long-term consequences, including neuronal death. The main pathophysiological mechanism of damage is excitotoxicity, a mechanism characterized by an increased excitatory neurotransmission by glutamate causing neuronal damage. Dapsone (DDS), is a drug currently in use for leprosy, however, its anticonvulsant effects have been reported, both in models and human patients. The objective of this study was to characterize the anticonvulsant, antiapoptotic and neuroprotective effects of dapsone (DDS) treatment in a model of SE induced by kainic acid (KA). Methods: SE was induced in Wistar male rats (250 to 280 g), using KA (5 mg/kg, ip). If a rat approached the SE, half doses (2.5 mg/Kg) were administered at intervals of 1 h, until behavioral SE. 25 h after KA administration, caspases 8 and 3 activities were measured, while degenerated neurons in the hippocampus were counted, using Fluoro Jade-B staining. Results: Activities of caspases 8 and 3 were increased by KA, as compared to the control group. This effect was decreased by DDS doses of 12.5 and 25 mg/kg, showing its ability to inhibit apoptosis markers. (*$p<0.05$). A lower number of degenerated neurons in the hippocampus was also observed, by effect of DDS at both doses employed. Conclusions: The results demonstrate a relevant therapeutic potential of DDS since, currently, most of the drugs prescribed in patients with epilepsy are anticonvulsants, but not neuroprotective. Given its safety, therapeutic success, antiapoptotic and neuroprotective actions, DDS is an excellent candidate for the treatment of patients with SE.

Pubmed:
30724852: Ruíz-Díaz A, Manjarrez J, Nava-Ruíz C, Zaga-Clavellina V, Flores-Espinosa P, Díaz-Ruíz A, Yescas-Gómez P, Méndez-Armenta M
Expression of nuclear factor-erythroid 2-related factor 2 in rat brain following the administration of kainic acid and pentylenetetrazole.
Epilepsy is a neurological disorder of the central nervous system characterized by hypersynchronized neuronal activity and has been associated with oxidative stress. Oxidative stress interferes with the expression of genes as well as transcriptional factors such as nuclear factor-erythroid 2-related factor 2 (Nrf2). We evaluated the expression of Nrf2 in the rat brain in treated with kainic acid (KA) and pentylenetetrazole (PTZ). Nrf2 immunoreactivity was observed in astrocytes of the hippocampal region in rats exposed at KA. Nrf2 expression was increased significantly in rats with KA and PTZ. These results provide evidence that the increased expression of Nrf2 is part of the mechanism against KA and PTZ toxicity.
Neuroreport, 2019; 30
31019649: Munguía-Martínez MF, Nava-Ruíz C, Ruíz-Díaz A, Díaz-Ruíz A, Yescas-Gómez P, Méndez-Armenta M
Immunohistochemical Study of Antioxidant Enzymes Regulated by Nrf2 in the Models of Epileptic Seizures (KA and PTZ).
Epilepsy is a neurological disorder characterized by recurrent spontaneous seizures due to an imbalance between cerebral excitability and inhibition, with a tendency towards uncontrolled excitability. Epilepsy has been associated with oxidative and nitrosative stress due to prolonged neuronal hyperexcitation and loss neurons during seizures. The experimental animal models report level of ATP diminished and increase in lipid peroxidation, catalase, and glutathione altered activity in the brain. We studied the immunohistochemical expression and localization of antioxidant enzymes GPx, SOD, and CAT in the rat brains treated with KA and PTZ. A significant decrease was observed in the number of immunoreactive cells to GPx, without significant changes for SOD and CAT in KA-treated rats, and decrease in the number of immunoreactive cells to SOD, without significant changes for GPx and only CAT in PTZ-treated rats. Evident immunoreactivity of GPx, SOD, and CAT was observed mainly in astrocytes and neurons of the hippocampal brain region in rats exposed at KA; similar results were observed in rats treated with PTZ at the first hours. These results provide evidence supporting the role of activation of the

Nrf2 antioxidant system pathway against oxidative stress effects in the experimental models of epileptic seizures.
Oxid Med Cell Longev, 2019; 2019
30571982: Ríos C, Farfán-Briseño AC, Manjarrez-Marmolejo J, Franco-Pérez J, Méndez-Armenta M, Nava-Ruiz C, Caballero-Chacón S, Ruiz-Diaz A, Baron-Flores V, Díaz-Ruiz A
Efficacy of dapsone administered alone or in combination with diazepam to inhibit status epilepticus in rats.
Status epilepticus (SE) is a serious medical condition, as it may trigger epileptogenesis. SE produces continuous generalized seizures resulting in irreversible brain damage. Therefore, the use of neuroprotective agents to prevent cell damage, may reduce the impact of SE. The use of diazepam (DZP), has shown limited neuroprotective effect in SE patients. According to previous reports, dapsone (DDS) is able to reduce both cell damage and seizures, when administered 30 min before the onset of seizures. This study is aimed to evaluate the ability of DDS, alone or in combination with DZP starting their administration once the SE is onset to evaluate the control of seizures in rats. Results showed a reduced convulsive electrical activity after 30 min, 1 and 2 h after SE induced by kainic acid (KA) administration, in the animals treated with DZP alone or in combination with DDS. At 24 h, we observed electrical activity similar to baseline in all groups receiving treatment. The animals treated with DDS and DZP alone or in combination showed an increase in the number of viable pyramidal cells but only the combination showed a lower number of damaged pyramidal neurons of hippocampal CA3. In conclusion, DDS plus DZP was able to control SE and to prevent SE-induced damage, when administered in combination with DZP. As DDS is already in use for patients with leprosy, that combination may be a safe, good option for human cases of SE.
Brain Res, 2019; 1708

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BOARD NUMBER: S01-577

THE ROLE OF ENKEPHALIN IN HYPOXIC PRECONDITIONING

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Background: Hypoxic preconditioning (HPC) is the application of mild transient hypoxia which protects the brain against a following more severe hypoxic insult as it occurs during epileptic seizures. HPC decreases seizure susceptibility and severity as well as neuronal damage in the hippocampus. The delta opioid receptor (DOR) and its primary endogenous ligands, the neuropeptides met- and leu-enkephalin (Enk), are thought to be involved in the neuroprotective actions of HPC. Recently, we showed that Enk influences mitochondrial respiration that may contribute to the neuroprotective effects of the Enk/DOR system. The present study aims at investigating the effects of the Enk/DOR system on structural and functional alterations of mitochondria in HPC. Methods: Wild type (WT) and met-Enk-knockout (met-Enk-KO) mice were exposed to hypoxia. Subsequently, we determined the seizure threshold, analyzed mitochondrial function and dynamics. Results: In WT mice after HPC we observed an elevated seizure threshold, improved mitochondrial reserve capacity and increased mitochondrial fusion. In addition, our results suggest mitochondrial biogenesis after HPC in WT mice. Naïve met-Enk-KO mice had an increased seizure threshold and increased mitochondrial fusion but no changes upon HPC. Conclusion: The observed mitochondrial alterations after HPC in WT mice could explain improved neuronal survival and increased seizure threshold. Enhanced mitochondrial reserve capacity improves energy supply in stress situations and increased mitochondrial fusion is associated with neuronal survival and elevated Ca^{2+} storage capacities. However, the precise role of met-Enk in HPC is unclear but we observed adaptive mechanisms in WT mice upon hypoxia which are absent in met-Enk-KO mice.

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BOARD NUMBER: S01-578

THERMAL-BASED NEUROMODULATION USING PULSED INFRARED ILLUMINATION IN A PENICILLIN-INDUCED ACUTE EPILEPSY MODEL: PRELIMINARY RESULTS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Background and aims: Thermal-based neuromodulation of neural activity could be achieved depending on thermal-induced changes in the transmembrane capacitance and non-uniform changes in the functionality of the ionic channels. Infrared neuromodulation (INM) is a promising neuromodulation tool relying on the use of pulsed or continuous-wave near-infrared (NIR) laser light (of wavelength range between 1400 and 2100 nm) to induce heat within the neural tissue and modulate the neuronal activity. This research aims to study the impact of infrared-based heat irradiation on the morphological shape and propagation of interictal spikes using penicillin-induced epileptic rat model. **Methods**: This study consists of recording the ECoG signals, pre-processing, inter-ictal spikes detection and classification, and finally, studying the relation between interictal spikes types and features during different frequencies of infrared light. Optrode device is used to induce heat irradiation within the neocortex and multi-modal micro-probe is used to record the cerebral cortex activities of anesthetized rats. The detected interictal spikes are classified into two main categories: (1) Blunt, Double, and Triple (2) Biphasic and Sharp spikes. On other hand, the extracted features of those spikes are tracked in real-time to investigate more the real impact of thermal-based neuromodulation on the morphological shape and inducing rate of interictal spikes. **Results and conclusions**: The results show legible mitigation in the induced interictal spikes during neuromodulation phases compared with baseline (pure epilepsy case), and lower synchrony of firing neuronal networks in the focal epilepsy area. The results provide also a comprehensive insight into the neocortex activities during neuromodulation.

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BOARD NUMBER: S01-579

GABAERGIC MODULATION OF CORTICAL EXCITABILITY AND RESILIENCE TO SEIZURES

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Background: The limbic circuit is particularly prone to seizures, even in non-epileptic brains. Short stimulation of the entorhinal cortex can evoke self-terminating responses of varying magnitude in the hippocampus. Beyond the circuit's resilience (its capacity to absorb perturbation), more sustained stimulation can result in self-sustained seizures. How the limbic circuit transitions between these regimes is unknown. **Aim:** To quantify GABAergic modulation of excitability and resilience in the limbic system in healthy mice. **Methods:** We compared states of relatively low and high cortical excitability in freely-moving mice by administering a control vehicle (NaCl) intraperitoneally or low doses of GABAergic agonist (Diazepam (DZ)) or antagonist (Pentylenetetrazol (PTZ) or Picrotoxin (PTX)), respectively. To probe excitability, we stimulated pyramidal cells in the entorhinal cortex, using optogenetics single-pulses and measured evoked responses in the hippocampus. To probe resilience of this circuit, we administered trains of stimulation of increasing duration until a seizure was triggered. **Results:** We found that the magnitude of evoked hippocampal responses was reduced by 29.3% [95%CI, 27.6-31.2] with DZ and increased with PTZ (5.1% [3.0-7.1]) or PTX (9.3% [7.1-11.3]). Additionally, the amount of stimulation needed to induce seizures increased by 78.1% [54.5-113.0] with DZ and decreased by 19.6% [3.0-10.8] and 10.6% [-4.0-18.5] with PTZ or PTX, respectively, corroborating the changes observed in cortical excitability. **Conclusion:** We provide experimental evidence *in vivo* for a direct relevance of using minute perturbations of ongoing activity as markers of cortical excitability and show their correlation with resilience to epileptic seizures.

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BOARD NUMBER: S01-580

A NEW MECHANISM IMPLICATED IN KAINATE-INDUCED RAT EPILEPTOGENESIS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Alterations in brain-derived neurotrophic factor (BDNF) and its TrkB- FL receptor have been suggested to contribute to epilepsy. Under excitotoxic conditions, TrkB-FL receptor is cleaved, forming an intracellular fragment (TrkB-ICD). In an *in vitro* model of *status epilepticus* (SE) a putative TrkB-FL cleavage was also suggested. Therefore, we studied whether the TrkB-FL cleavage occurs in an *in vivo* model of SE and chronic epilepsy and if it is related with seizure severity, neurogenesis and memory. A rat model of epilepsy was induced with kainate (KA, intraperitoneal) and two groups were obtained: SE group; and chronic group. Seizure severity was classified using the modified Racine's Scale. In the SE group, animals with the highest seizure score showed a significant decrease of TrkB-FL protein levels and increased levels in TrkB-ICD/TrkB-FL ratio. Moreover, a positive significant effect of TrkB-ICD levels on the number of seizures was observed, suggesting that animals with more seizures have higher levels of TrkB-ICD. Nevertheless, no differences were found in $Ki67^+$ proliferative cells comparing with saline-treated animals. Regarding the chronic group, although no evidence of TrkB-FL cleavage was observed, a positive significant effect of proliferative $Ki67^+$ cells in the number of spontaneous seizures per hour was found. Also, a preliminary novel object location test revealed a loss of preference for the displaced object in the epileptic animals, indicating memory impairment. Taken together, our results suggest that TrkB-FL is cleaved in an *in vivo* KA-induced epilepsy model, a mechanism that appears to occur during *SE*, affecting later neurogenesis and memory.

Pubmed:
33022963: Lourenço DM, Ribeiro-Rodrigues L, Sebastião AM, Diógenes MJ, Xapelli S
Neural Stem Cells and Cannabinoids in the Spotlight as Potential Therapy for Epilepsy.
Epilepsy is one of the most common brain diseases worldwide, having a huge burden in society. The main hallmark of epilepsy is the occurrence of spontaneous recurrent seizures, having a tremendous impact on the lives of the patients and of their relatives. Currently, the therapeutic strategies are mostly based on the use of antiepileptic drugs, and because several types of epilepsies are of unknown origin, a high percentage of patients are resistant to the available pharmacotherapy, continuing to experience seizures overtime. Therefore, the search for new drugs and therapeutic targets is highly important. One key aspect to be targeted is the aberrant adult hippocampal neurogenesis (AHN) derived from Neural Stem Cells (NSCs). Indeed, targeting seizure-induced AHN may reduce recurrent seizures and shed some light on the mechanisms of disease. The endocannabinoid system is a known modulator of AHN, and due to the known endogenous antiepileptic properties, it is an interesting candidate for the generation of new antiepileptic drugs. However, further studies and clinical trials are required to investigate the putative mechanisms by which cannabinoids can be used to treat epilepsy. In this manuscript, we will review how cannabinoid-induced modulation of NSCs may promote neural plasticity and whether these drugs can be used as putative antiepileptic treatment.
Int J Mol Sci, 2020; 21
33161136: Miranda-Lourenço C, Ribeiro-Rodrigues L, Fonseca-Gomes J, Tanqueiro SR, Belo RF, Ferreira CB, Rei N, Ferreira-Manso M, de Almeida-Borlido C, Costa-Coelho T, Freitas CF, Zavalko S, Mouro FM, Sebastião AM, Xapelli S, Rodrigues TM, Diógenes MJ
Challenges of BDNF-based therapies: From common to rare diseases.
Neurotrophins are a well-known family of neurotrophic factors that play an important role both in the central and peripheral nervous systems, where they modulate neuronal survival, development, function and plasticity. Brain-derived neurotrophic factor (BDNF) possesses diverse biological functions which are mediated by the activation of two main classes of receptors, the tropomyosin-related kinase (Trk) B and the p75 neurotrophin receptor (p75). The therapeutic potential of BDNF has

drawn attention since dysregulation of its signalling cascades has been suggested to underlie the pathogenesis of both common and rare diseases. Multiple strategies targeting this neurotrophin have been tested; most have found obstacles that ultimately hampered their effectiveness. This review focuses on the involvement of BDNF and its receptors in the pathophysiology of Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS) and Rett Syndrome (RTT). We describe the known mechanisms leading to the impairment of BDNF/TrkB signalling in these disorders. Such mechanistic insight highlights how BDNF signalling compromise can take various shapes, nearly disease-specific. Therefore, BDNF-based therapeutic strategies must be specifically tailored and are more likely to succeed if a combination of resources is employed.
Pharmacol Res, 2020; 162

34151790: Paulo SL, Ribeiro-Rodrigues L, Rodrigues RS, Mateus JM, Fonseca-Gomes J, Soares R, Diógenes MJ, Solá S, Sebastião AM, Ribeiro FF, Xapelli S

Sustained Hippocampal Neural Plasticity Questions the Reproducibility of an Amyloid-β-Induced Alzheimer's Disease Model.
The use of Alzheimer's disease (AD) models obtained by intracerebral infusion of amyloid-β (Aβ) has been increasingly reported in recent years. Nonetheless, these models may present important challenges.
J Alzheimers Dis, 2021; 82

FENS Forum 2022
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BOARD NUMBER: S01-581

INFLUENCE OF ADRENOAGONIST CLONIDINE AND ADRENOBLOCKER PROPRANOLOL ON NEOCORTICAL AND HIPPOCAMPAL EPILEPTIFORM DISCHARGES

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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The goal of the work was investigation of clonidine and propranolol influences on the neocortical and hipocampal epileptiform discharges.
Materials and Methods.
In vivo experiments were carried out on chronic Wistar rats. Under ketalar anesthesia Stimulating (bipolar) and recording (unipolar) electrodes were implanted in the neocortex and dorsal hippocampus (DH) according to stereotaxic coordinates by Paxinos and Watson atlas.epileptiform discharges (EDs) were evoked with electrical stimulation (30Hz) either of neocortex or dorsal hippocampus. Intraperitoneal injection of clonidine, β-adrenoreceptor stimulant (1mg/kg) and propranolol β-adrenoblocker (0,5mg/kg) was performed after establishing a minimal threshold stimulation sufficient for elicitation stable epileptiform discharges either in neocortex or in hippocampus. The brain electrical activity was recorded with electroencephalograph.
Results.
Propranolol injection decreased epileptiform discharges elicited by cortical stimulation, while hippocampally-induced discharges, after propranolol injection – increased. propranolol injection deacreased epileptogenic threshold of hippocampus and increased of neocortex.This result may be due to suppressed inhibitory capacity of β-adrenoreceptors. Influence of clonidine injection was just an opposite to that of clonidine – cortically-induced Epileptiform discharges increased, while the hippocampally-induced ones- decreased.clonidine injection decreased epileptogenic threshold of neocortex and increased epileptogenic threshold of hippocampus.

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BOARD NUMBER: S01-582

CIRCADIAN TIMING OF LIMBIC SEIZURES IN THE EPILEPTIC MOUSE

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Aims: In epilepsy, seizures often display circadian clustering. This clustering may result from recent active-rest states (so-called process S) and/or from the circadian rhythm (so-called process C). The aim of this study is to disentangle the individual contributions of process S and C to seizure timing in the Kainic Acid mouse model of temporal lobe epilepsy. **Methods:** We recorded spontaneous seizures in epileptic mice (n=9) over months. These animals were kept in a 12:12 light-dark cycling environment (LD), before subjecting them to different experimental schedules: 1) constant dim red light (DD), 2) constant light (LL), 3) 10:10 light-dark cycling (T20). We identified the underlying circadian and active-rest cycle based on core body temperature (CBT) and actimetry, respectively, and extracted the preferred phase at which seizures occurred. **Results:** We observed a circadian clustering of seizures between the peak and the falling phase of 24-hour activity in LD (PLV= 0.29+/-0.18). While the circadian clustering persisted at the same phase during DD (PLV= 0.27+/-0.01) it was attenuated in LL (PLV= 0.16+/-0.07), when the strength of the underlying circadian cycle was also weakened. Under T20, we observed a periodic uncoupling of the active rest cycle and CBT cycle (about every third day). The clustering of seizures was higher during the period when both cycles were aligned (PLV= 0.54+/-0.14) and lower when they were misaligned (PLV= 0.32+/-0.13). **Conclusions:** The temporal clustering of seizures depends on the strength and alignment of Process S and Process C, which may help guide chronotherapeutic interventions in the future.

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BOARD NUMBER: S01-583

HARNESSING NEUROGLIAFORM INTERNEURONS TO CONTROL CORTICAL SEIZURES IN AWAKE MICE

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Focal seizures are widely considered to arise from a disturbance of the excitation/inhibition balance, and in particular a failure of the GABAergic inhibitory system. Recent work focusing on parvalbumin-positive and somatostatin-positive interneurons has shown that inhibition mediated by these populations is too weak to suppress seizures effectively. In contrast, neurogliaform (NGF) cells, if appropriately recruited, could be much more effective, due to their high density of GABA release sites and signalling via “volume transmission”. *Here, we therefore investigate the role of NGF cells in seizure generation and maintenance*. For this, we use a mouse line (Ndnf-Cre) that enables targeting of NGF cells and a combination of calcium imaging, electrocorticography and closed-loop optogenetic stimulation in models of acute, focal cortical epilepsy. *In vivo* calcium imaging revealed that Ndnf+ NGF cells are recruited during interictal activity. We also observed a delayed activation of these cells after the onset of ictal discharges. These findings suggests that Ndnf+ NGF neurons are involved in seizure activity but whether they promote or prevent the spread of overexcitation remains unknown. To answer this question, we are using optogenetic activation of NGF cells together with local chemoconvulsant application. Our preliminary data indicate a 30% reduction in interictal spikes during Ndnf+ cell photo-activation and indicate a ~50% reduction in seizure duration during light stimulation. Together, this is the first evidence that Ndnf+ cell photo-activation can have strong anti-epileptic effects. We are currently testing whether a general increase of Ndnf+ NGF neuron excitability using chemogenetics can prevent seizure initiation.

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BOARD NUMBER: S01-584

ROLE OF CA++-PERMEABLE AMPA RECEPTORS IN INTERNEURONS AND PYRAMIDAL CELLS IN SEIZURE ONSET AND PROPAGATION IN HUMAN NEOCORTEX.

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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AMPA receptors play a major role in excitatory signalling in neocortex. Within the plethora of AMPA receptor compositions, GluR2 subunit lacking receptors are of particular importance as they mediate calcium ion permeability. GluR2 lacking AMPA receptors are expressed both in pyramidal cells and interneurons. Their role in the synchronization of neuronal activity in neocortex, specifically their contribution to inhibition/excitation balance and imbalance during seizures is not known. By using human brain slices derived from surgical resections, we aimed to investigate the role of calcium permeable AMPA receptors in the onset, maintenance and propagation of seizure like events using specific blockers such as 1-naphthyl acetyl spermine (NASPM) and IEM-1460. We found that in an acute in vitro seizure model using 0 Mg and bicuculline (10 µM), the application of NASPM (100 µM) leads to the complete block of ictal activity, while IEM-1460 (100-150 µM) changes the shape and duration of seizure like events but does completely block ictal activity. Since in the 0 Mg/bicuculline model the activity of interneurons is blocked, we hypothesize that the block of calcium permeable AMPA receptors in pyramidal cells is sufficient to block ictal activity in this specific model. Next, we aim to apply other models in which interneurons are not blocked and to engage single cell recordings to investigate the interplay between inhibitory and excitatory signalling during ictal activity and the involvement of calcium permeable AMPA receptors in human tissue in more detail

Pubmed:
33724729: Moschetta M, Lee JY, Rodrigues J, Podestà A, Varvicchio O, Son J, Lee Y, Kim K, Lee GH, Benfenati F, Bramini M, Capasso A
Hydrogenated Graphene Improves Neuronal Network Maturation and Excitatory Transmission.
Graphene is regarded as a viable bio-interface for neuroscience due to its biocompatibility and electrical conductivity, which would contribute to efficient neuronal network signaling. Here, monolayer graphene grown via chemical vapor deposition is treated with remote hydrogen plasma to demonstrate that hydrogenated graphene (HGr) fosters improved cell-to-cell communication with respect to pristine graphene in primary cortical neurons. When transferred to polyethylene terephthalate, HGr exhibits higher wettability than graphene (water contact angle of 83.7° vs 40.7°), while preserving electrical conductivity (≈3 kΩ □). A rich and mature network is observed to develop onto HGr. The intrinsic excitability and firing properties of neurons plated onto HGr appears unaltered, while the basic passive and active membrane properties are fully preserved. The formation of excitatory synaptic connections increases in HGr with respect to pristine graphene, leading to a doubled miniature excitatory postsynaptic current frequency. This study supports the use of hydrogenation for tailoring graphene into an improved neuronal interface, indicating that wettability, more than electrical conductivity, is the key parameter to be controlled. The use of HGr can bring about a deeper understanding of neuronal behavior on artificial bio-interfaces and provide new insight for graphene-based biomedical applications.
Adv Biol (Weinh), 2021; 5

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-585

DAPSONE PREVENTS HYPERMETABOLIC EFFECT OF KAINIC ACID IN RATS: AN 18FDG-PET STUDY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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[1]Universidad Autonoma Metropolitana-Xochimilco, Sistemas Biologicos Laboratorio De Neurofarmacologia Molecular, Ciudad de México, Mexico, [2]Instituto Nacional de Neurologia y Neurocirugia, Neuroquimica, Mexico City, Mexico, [3]Facultad de Medicina, Universidad Nacional Autónoma de Mexico, Unidad Radiofarmacia-ciclotron, Mexico City, Mexico, [4]Instituto Nacional de Neurologia y Neurocirugia, Formacion Reticular, Mexico City, Mexico

It is known that approximately 30% of epilepsy cases are drug-resistant, thus, there is a need to develop safe and effective therapies. On the other hand, dapsone (DDS) is a drug used to treat leprosy, with anticonvulsant effects. The aim of this work is to employ a ^{18}FDG-PET study to characterize its anticonvulsant effect in rats. Methods: Male Wistar rats (250 to 280 g) were used. They received a single dose of 10 mg/Kg of kainic acid (KA), as a model of temporal lobe epilepsy. Prior to KA administration, ^{18}FDG-PET studies were performed as basal activity. Electroencephalography (EEG) was also recorded. DDS or vehicle were administered 30 min before KA, while ^{18}FDG uptake and brain EEG activity were evaluated at 2 and 25 h. Results: The PET study showed increased ^{18}FDG uptake in the KA plus vehicle group during the ictal stage, which was decreased by DDS treatment at 2 h. At 25 h after KA, a decreased ^{18}FDG uptake was observed as compared to KA group. ^{18}FDG uptake values increased to basal levels by DDS. EEG study showed an increased activity 2 h after KA administration, which is reversed by effect of DDS, that correlates with changes in ^{18}FDG-PET uptake. (* $p<0.05$). The results of this study demonstrate the therapeutic potential of DDS since, currently, most of the drugs prescribed in patients with epilepsy are anticonvulsants, but not neuroprotective. Given its safety, therapeutic success and neuroprotective effect, DDS may be an excellent candidate to treat epilepsy patients.

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BOARD NUMBER: S01-586

IMPAIRED DENTATE GYRUS PATTERN SEPARATION AND MNEMONIC DISCRIMINATION IN TEMPORAL LOBE EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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In temporal lobe epilepsy (TLE), the ability of the dentate gyrus (DG) to limit excitatory cortical input to the hippocampus breaks down, leading to seizures. DG is also thought to help discriminate between similar memories by performing pattern separation, but whether epilepsy leads to a breakdown in this neural computation, and thus to mnemonic discrimination impairments, remains unclear[1]. First, using the DG-dependent Mnemonic Similarity Task, we found that mnemonic discrimination is specifically impaired in patients with TLE. In mice too, TLE (low-dose systemic kainate model) leads to discrimination impairments on an object-location novelty-preference task. Using our patch-clamp assay in brain slices of the same mice, we measured different forms of pattern separation (PS): On average, the epileptogenic treatment caused a PS deficit. Interestingly, only a subset of granule cells from TLE mice had pathologically low levels of PS, with normal and abnormal neurons coexisting. Low PS was explained by high firing rates and abnormal bursting, likely due to decreased inhibition. Finally, cross-referencing EEG, behavior and patch-clamp records of the same mice revealed that PS levels correlate with individual mnemonic discrimination abilities, thus supporting a long-hypothesized tenet of episodic memory theories. This analysis also suggests that memory deficits and abnormal PS can develop before traditional EEG signs of epilepsy. Overall, our results could help design new diagnostic tests and point to a biological mechanism of early memory problems in people later diagnosed with TLE. [1]Madar et al. Deficits in behavioral and neuronal pattern separation in temporal lobe epilepsy. J. Neurosci. (2021)41:9669-9686

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BOARD NUMBER: S01-587

BEHAVIORAL, CANNABINOID CB1 RECEPTOR AND ASTROCYTIC CHANGES IN A KAINIC ACID MODEL OF TEMPORAL LOBE EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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People with temporal lobe epilepsy (TLE) often endure memory impairments and they are more likely to suffer from psychiatric comorbidities such as anxiety and depression. On top of that, TLE frequently fails to be controlled by antiseizure drugs. Thus, it is crucial to better understand all mechanisms underlying its pathophysiology. The endocannabinoid system has been in the spotlight of pharmacological interest in epilepsy as the cannabinoid CB1 receptor regulates neuronal excitability. Nonetheless, the existence of a long-term correlation between disturbed hippocampal-related behaviors, CB1 receptor expression and glial cells in TLE is still unclear. To investigate this, kainic acid (KA, 25mg/kg) was intraperitoneally administered in 8-week-old C57BL/6J male mice. Controls, mice with mild (Racine scale: RS ≤ 3) and severe seizures (RS > 3) were compared. Then, spatial memory (Barnes-maze), nesting ability and anxiety were assessed. Furthermore, 4 weeks after KA injection, immunohistochemistry was carried out to study hippocampal CB1 expression and astrocytic morphology for light and electron microscopy. RS > 3 mice exhibited impaired spatial memory, harmed nesting ability and anxiolysis. They also showed a drastic reduction in CB1 optical density, especially in the CA1 hippocampus. Moreover, the astrocytic processes were bigger, covered a larger area and displayed an increased number of CB1 immunoparticles. Altogether, these findings suggest that severe epileptic seizures impair long-term hippocampal-dependent behaviors, and cause reactive astrogliosis associated with the increase in astroglial CB1 receptor in the hippocampus.

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BOARD NUMBER: S01-588

INSIGHTS INTO THE ROLE OF THE CANNABINOID CB2 RECEPTOR IN A MOUSE MODEL OF TEMPORAL LOBE EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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The cannabinoid receptor type 2 (CB2) is expressed primarily in peripheral tissues. Nevertheless, it is also present in microglia where it is rapidly upregulated during inflammation. Numerous studies have evidenced the correlation between inflammation and epileptic susceptibility in temporal lobe epilepsy (TLE). However, the role of CB2 receptor in TLE is still unknown. To address this, status epilepticus was induced in CB2-EGFP (CB2-Enhanced Green Fluorescent Protein) and CB2-KO mice by intraperitoneal kainic acid administration (KA, 25 mg/kg). Seizures' severity was assessed by a modified Racine's scale. Afterwards, overall well-being and memory were analyzed by several tests: marble burying, nest building behavior and novel object recognition. During the first 1.5h of the Racine's assessment, CB2-KO mice exhibited higher susceptibility to KA insults, but recovered faster than CB2-EGFP. Moreover, the absence of CB2 together with KA injection seems to impact on marble burying and nesting ability, but not on recognition memory. In order to elucidate whether synaptic dysfunction may be underlying these behavioral changes, in the future, electrophysiological recordings will be performed in this KA model mice. Nevertheless, as the role of CB2 is not fully characterized yet, it has been first performed extracellular field recordings on the hippocampal dentate medial perforant path (MPP) in untreated CB2-KO mice. Preliminary results indicate that in CB2-KO mice low frequency stimulation elicits MPP-LTP instead of the endocannabinoid-dependent LTD reported by our laboratory in WT mice.

Pubmed:
33368252: Bonilla-Del Río I, Puente N, Mimenza A, Ramos A, Serrano M, Lekunberri L, Gerrikagoitia I, Christie BR, Nahirney PC, Grandes P
Acute Δ9-tetrahydrocannabinol prompts rapid changes in cannabinoid CB receptor immunolabeling and subcellular structure in CA1 hippocampus of young adult male mice.
The use and abuse of cannabis can be associated with significant pathophysiology, however, it remains unclear whether (1) acute administration of Δ-9-tetrahydrocannabinol (THC) during early adulthood alters the cannabinoid type 1 (CB) receptor localization and expression in cells of the brain, and (2) THC produces structural brain changes. Here we use electron microscopy and a highly sensitive pre-embedding immunogold method to examine CB receptors in the hippocampus cornu ammonis subfield 1 (CA1) 30 min after male mice were exposed to a single THC injection (5 mg/kg). The findings show that acute exposure to THC can significantly decrease the percentage of CB receptor immunopositive terminals making symmetric synapses, mitochondria, and astrocytes. The percentage of CB receptor-labeled terminals forming asymmetric synapses was unaffected. Lastly, CB receptor expression was significantly lower at terminals of symmetric and asymmetric synapses as well as in mitochondria. Structurally, CA1 dendrites were significantly larger, and contained more spines and mitochondria following acute THC administration. The area of the dendritic spines, synaptic terminals, mitochondria, and astrocytes decreased significantly following acute THC exposure. Altogether, these results indicate that even a single THC exposure can have a significant impact on CB receptor expression, and can alter CA1 ultrastructure, within 30 min of drug exposure. These changes may contribute to the behavioral alterations experienced by young individuals shortly after cannabis intoxication.
J Comp Neurol, 2021; 529
34356889: Rico-Barrio I, Peñasco S, Lekunberri L, Serrano M, Egaña-Huguet J, Mimenza A, Soria-Gomez E, Ramos A,

Buceta I, Gerrikagoitia I, Mendizabal-Zubiaga J, Elezgarai I, Puente N, Grandes P
Environmental Enrichment Rescues Endocannabinoid-Dependent Synaptic Plasticity Lost in Young Adult Male Mice after Ethanol Exposure during Adolescence.
Binge drinking (BD) is a serious health concern in adolescents as high ethanol (EtOH) consumption can have cognitive sequelae later in life. Remarkably, an enriched environment (EE) in adulthood significantly recovers memory in mice after adolescent BD, and the endocannabinoid, 2-arachydonoyl-glycerol (2-AG), rescues synaptic plasticity and memory impaired in adult rodents upon adolescent EtOH intake. However, the mechanisms by which EE improves memory are unknown. We investigated this in adolescent male C57BL/6J mice exposed to a drinking in the dark (DID) procedure four days per week for a duration of 4 weeks. After DID, the mice were nurtured under an EE for 2 weeks and were subjected to the Barnes Maze Test performed the last 5 days of withdrawal. The EE rescued memory and restored the EtOH-disrupted endocannabinoid (eCB)-dependent excitatory long-term depression at the dentate medial perforant path synapses (MPP-LTD). This recovery was dependent on both the cannabinoid CB1 receptor and group I metabotropic glutamate receptors (mGluRs) and required 2-AG. Also, the EE had a positive effect on mice exposed to water through the transient receptor potential vanilloid 1 (TRPV1) and anandamide (AEA)-dependent MPP long-term potentiation (MPP-LTP). Taken together, EE positively impacts different forms of excitatory synaptic plasticity in water- and EtOH-exposed brains.
Biomedicines, 2021; 9
33692673: Egaña-Huguet J, Bonilla-Del Río I, Gómez-Urquijo SM, Mimenza A, Saumell-Esnaola M, Borrega-Roman L, García Del Caño G, Sallés J, Puente N, Gerrikagoitia I, Elezgarai I, Grandes P
The Absence of the Transient Receptor Potential Vanilloid 1 Directly Impacts on the Expression and Localization of the Endocannabinoid System in the Mouse Hippocampus.
The transient receptor potential vanilloid 1 (TRPV1) is a non-selective ligand-gated cation channel involved in synaptic transmission, plasticity, and brain pathology. In the hippocampal dentate gyrus, TRPV1 localizes to dendritic spines and dendrites postsynaptic to excitatory synapses in the molecular layer (ML). At these same synapses, the cannabinoid CB receptor (CBR) activated by exogenous and endogenous cannabinoids localizes to the presynaptic terminals. Hence, as both receptors are activated by endogenous anandamide, co-localize, and mediate long-term depression of the excitatory synaptic transmission at the medial perforant path (MPP) excitatory synapses though by different mechanisms, it is plausible that they might be exerting a reciprocal influence from their opposite synaptic sites. In this anatomical scenario, we tested whether the absence of TRPV1 affects the endocannabinoid system. The results obtained using biochemical techniques and immunoelectron microscopy in a mouse with the genetic deletion of TRPV1 show that the expression and localization of components of the endocannabinoid system, included CBR, change upon the constitutive absence of TRPV1. Thus, the expression of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) drastically increased in TRPV1 whole homogenates. Furthermore, CBR and MAGL decreased and the cannabinoid receptor interacting protein 1a (CRIP1a) increased in TRPV1 synaptosomes. Also, CBR positive excitatory terminals increased, the number of excitatory terminals decreased, and CBR particles dropped significantly in inhibitory terminals in the dentate ML of TRPV1 mice. In the outer 2/3 ML of the TRPV1 mutants, the proportion of CBR particles decreased in dendrites, and increased in excitatory terminals and astrocytes. In the inner 1/3 ML, the proportion of labeling increased in excitatory terminals, neuronal mitochondria, and dendrites. Altogether, these observations indicate the existence of compensatory changes in the endocannabinoid system upon TRPV1 removal, and endorse the importance of the potential functional adaptations derived from the lack of TRPV1 in the mouse brain.
Front Neuroanat, 2021; 15

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-589

ANTIEPILEPTOGENIC EFFECTS OF TRILOSTANE IN THE KAINIC ACID MODEL OF TEMPORAL LOBE EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Background Trilostane selectively inhibits the enzyme 3β-hydroxysteroid dehydrogenase in the adrenal cortex and leads to an increase in neurosteroid levels in the brain. Previously, it was found that epileptogenesis could be anticipated by reducing the synthesis of allopregnanolone in a model of temporal lobe epilepsy. Thus, we hypothesized that epileptogenesis could instead be delayed by increasing the brain availability of neurosteroids with trilostane. **Methods** We designed an experiment in which trilostane (50 mg/kg) was administered once daily for six consecutive days, starting 10 minutes after intraperitoneal administration of kainic acid (15 mg/kg) to induce status epilepticus (SE). Rats were euthanized 1 or 10 weeks after SE induction. **Results** In comparison to the control group, the total duration of convulsive seizures during SE was not significantly reduced in trilostane-treated rats. The latency to develop the first convulsive and nonconvulsive seizures of the SE was unchanged. Additionally, the treatment with trilostane did not affect the neuronal cell density and the area of lesion in the hippocampus. However, the mean duration of convulsive seizures during SE was markedly decreased by trilostane. Notably, the repeated administration of trilostane significantly increased the latency to develop both the first electrocorticographic and convulsive tonic-clonic spontaneous seizures. In subiculum, a significant change was observed in activated microglia morphology by comparing rats repeatedly treated with trilostane with those treated with the vehicle. **Conclusion** Overall, our results indicate that the repeated administration of trilostane delays the onset of epileptogenesis, an effect related to enhanced availability of neurosteroids in the brain.

Pubmed:
31531064: Ghorbanian D, Ghasemi-Kasman M, Hashemian M, Gorji E, Gol M, Feizi F, Kazemi S, Ashrafpour M, Moghadamnia AA
Houtt Extract Attenuates Neuronal Loss and Glial Activation in Pentylenetetrazol-Induced Kindling Model.
Inflammatory reactions are closely associated with the development and progression of epilepsy. It has been shown that inhibition of pro-inflammatory cytokines, which are released from activated astrocytes and microglia, are considered to be an effective therapeutic approach for the treatment of epileptic disorders. Regarding the anti-inflammatory effects of nutmeg (Houtt), the present study was designed to investigate whether the nutmeg ethanolic extract could exert anticonvulsant and inhibitory effects on glial activation in pentylenetetrazol (PTZ)-induced mice model of kindling. Ethanolic extract of nutmeg was administrated intraperitoneally (i.p.) 1 hour before PTZ injection or one week before PTZ as a separate group, to become fully-kindled. The chemical components of nutmeg extract were analyzed by gas chromatography mass spectrometry (GC-MS). Immunostaining against neuronal and glial markers was performed on hippocampus sections. GC-MS data indicated that the main components of nutmeg extract are myristic acid (39.93%), elemicin (22.16%) and myristicin (11.17%). Behavioral studies showed that pre-treatment of nutmeg extract effectively reduced seizures behavior, decreased cell death, and ameliorated glial activation that is followed by PTZ administration. In conclusion, nutmeg extract might be regarded as a useful supplementary agent in epilepsy treatment through its attenuation of neuronal loss and glial activation.
Iran J Pharm Res, 2019; 18
31251091: Dastan Z, Pouramir M, Ghasemi-Kasman M, Ghasemzadeh Z, Dadgar M, Gol M, Ashrafpour M, Pourghasem M, Moghadamnia AA, Khafri S
Arbutin reduces cognitive deficit and oxidative stress in animal model of Alzheimer's disease.
Recent evidences have shown the beneficial effects of natural products for treating of Alzheimer's disease (AD). Arbutin is derived from and exerts a wide range of pharmacological activities including anti-inflammatory and anti-oxidant effects. The present study was designed to examine the protective effects of arbutin on streptozotocin (STZ)-induced neurotoxicity in rats. The spatial memory impairment was induced by intracerebroventricular (i.c.v) microinjection of STZ (3 mg/kg, 10 µL). Animals received the pretreatment of arbutin (50 mg/kg) for 21 days before STZ injection. The Morris Water maze (MWM) task was used to study the spatial learning and memory. The levels of oxidative stress markers including malondialdehyde (MDA), nitrite and carbonyl were measured in serum and hippocampus samples. In addition, antioxidant level was assessed by ferric

reducing antioxidant power (FRAP) test. The obtained result indicated that administration of STZ is led to memory impairment and increases the levels of oxidative stress markers in the hippocampus tissues. Conversely, arbutin improves spatial memory and reduces oxidative and nitrosative stress, as evidenced by a significant decrease in the amount of MDA and nitrite in the serum and hippocampus. In addition, an increase in FRAP levels of hippocampus was observed in arbutin receiving animals. The protein carbonyl content was not reduced in arbutin receiving animals. It could be concluded that arbutin protects the brain against STZ-induced memory impairment and oxidative damage in the hippocampus. The neuroprotective effect of arbutin might be mediated through its antioxidant and free radical scavenging effects.
Int J Neurosci, 2019; 129
30639049: Mousavi Majd A, Ebrahim Tabar F, Afghani A, Ashrafpour S, Dehghan S, Gol M, Ashrafpour M, Pourabdolhossein F
Corrigendum to "Inhibition of GABA a receptor improved special memory impairment in the local model of demyelination in rat hippocampus" [Behav. Brain Res. 336 (2018) 111-121].
Behav Brain Res, 2019; 361
29174617: Ghasemi-Kasman M, Shojaei A, Gol M, Moghadamnia AA, Baharvand H, Javan M
miR-302/367-induced neurons reduce behavioral impairment in an experimental model of Alzheimer's disease.
In vivo reprogramming of reactive glial cells to neurons has opened a new horizon in regenerative medicine. Our previous study showed that astrocytes could be converted to neurons by the microRNA-302/367 (miR-302/367) cluster in adult brains. In this study, we investigated the possible contribution of miR-302/367-induced neurons in behavioral improvement and neural repair in an Alzheimer's disease (AD) animal model. The AD model was induced by an intracerebroventricular (i.c.v) injection of streptozotocin (STZ). GFP-only or miR-302/367+GFP expressing lentiviral particles were injected into the dentate gyrus of the hippocampus along with intraperitoneal (i.p) valproate (VPA) injection, 3weeks after the STZ administration. We assessed short-term and spatial memories by the Y-maze and Morris water maze (MWM) tasks, respectively. Electrophysiological activities of induced neuron-like cells were investigated using a whole-cell patch clamp technique, 6months after injection of miR-302/367. Behavioral analysis showed that the STZ injection significantly impaired short-term memory and increased escape latency parameter in the MWM task. Compared to STZ and STZ+VPA groups, miR-302/367 combined with VPA significantly improved the spontaneous alternation and spatial memory. Immunostaining against NeuN, as a mature neuronal marker, and its quantification indicated that co-labeled GFP and NeuN significantly increased in the miR-302/367+VPA group. Induced neurons were detected 6months after the miR-302/367 injection. The patch-clamp recording suggested that induced neurons could fire repetitive action potential like endogenous neurons. In conclusion, our results indicated that in vivo reprogramming of reactive astrocytes to neurons by the miR-302/367 cluster might be considered as a novel strategy to restore learning and memory in AD patients.
Mol Cell Neurosci, 2018; 86
28939403: Khalili-Fomeshi M, Azizi MG, Esmaeili MR, Gol M, Kazemi S, Ashrafpour M, Moghadamnia AA, Hosseinzadeh S
Piperine restores streptozotocin-induced cognitive impairments: Insights into oxidative balance in cerebrospinal fluid and hippocampus.
Piperine has been shown to have antioxidant activity and a cognitive-enhancing effect following long-term oral administration. In a comparative study of memantine, the current investigation threw light on the cognitive benefits of piperine. Lipid peroxidation and the ferric reducing antioxidant power (FRAP) of cerebrospinal fluid (CSF) and hippocampus in streptozotocin (STZ)-induced experimental dementia of the Alzheimer's type was measured. After reaching a criterion in a memory test, STZ-induced rats received piperine [2.5, 5, and 10mg/kg, intraperitoneally (i.p.)], vehicle, and memantine (10mg/kg, i.p.) for two weeks after the first STZ administration, or two weeks before and one week after, as a preventive approach. After the behavioral studies, samples were taken for biochemical and histological assays. An appropriate concentration of piperine (2.5mg/kg), on a daily basis, effectively increased the number of correct (non-repeated) arm entries and repressed reentry to a previously visited arm, in terms of reference errors as well as memantine (10mg/kg, i.p.), irrespective of the dose administered. The cognitive-enhancing effect induced by piperine at a relevant dose was simultaneous with CSF and hippocampal malonaldehyde decrement, and the redox balance was established to some extent by maintaining the FRAP levels of CSF near to those of the control. Similarly, the neuroprotective properties of piperine are in accordance with histopathological outcomes, which have shown an increased number of live cresyl violet (CV)-positive neurons in a dentate gyrus (DG) subregion. Therefore, the effects of piperine on the redox balance of CSF and hippocampal neurons may certainly contribute to the cognitive-enhancing activity of the drug.
Behav Brain Res, 2018; 337
28866129: Mousavi Majd A, Ebrahim Tabar F, Afghani A, Ashrafpour S, Dehghan S, Gol M, Ashrafpour M, Pourabdolhossein F
Inhibition of GABA A receptor improved spatial memory impairment in the local model of demyelination in rat hippocampus.
Cognitive impairment and memory deficit are common features in multiple Sclerosis patients. The mechanism of memory

impairment in MS is unknown, but neuroimaging studies suggest that hippocampal demyelination is involved. Here, we investigate the role of GABA A receptor on spatial memory in the local model of hippocampal demyelination. Demyelination was induced in male Wistar rats by bilaterally injection of lysophosphatidylcholine (LPC) 1% into the CA1 region of the hippocampus. The treatment groups were received daily intraventricular injection of bicuculline (0.025, 0.05µg/2µl/animal) or muscimol (0.1, 0.2µg/2µl/animal) 5days after LPC injection. Morris Water Maze was used to evaluate learning and memory in rats. We used Luxol fast blue staining and qPCR to assess demyelination extention and MBP expression level respectively. Immunohistochemistry (IHC) for CD45 and H&E staining were performed to assess inflammatory cells infiltration. Behavioral study revealed that LPC injection in the hippocampus impaired learning and memory function. Animals treated with both doses of bicuculline improved spatial learning and memory function; however, muscimol treatment had no effect. Histological and MBP expression studies confirmed that demylination in LPC group was maximal. Bicuculline treatment significantly reduced demyelination extension and increased the level of MBP expression. H&E and IHC results showed that bicuculline reduced inflammatory cell infiltration in the lesion site. Bicuculline improved learning and memory and decreased demyelination extention in the LPC-induced hippocampal demyelination model. We conclude that disruption of GABAergic homeostasis in hippocampal demyelination context may be involved in memory impairment with the implications for both pathophysiology and therapy.
Behav Brain Res, 2018; 336

28812474: Gol M, Ghorbanian D, Soltanpour N, Faraji J, Pourghasem M
Protective effect of raisin (currant) against spatial memory impairment and oxidative stress in Alzheimer disease model.
Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive pathological changes of the brain. A number of studies demonstrated compelling evidence of the importance of oxidative processes in AD pathogenesis. Raisin contains polyphenol, phenolic acid, and tannin compounds, which have antioxidant and anti-inflammatory properties. The present study was aimed to evaluate the protective effect of raisin on neurobehavioral and histological changes in rats with Alzheimer. Animal model of AD was induced by intraperitoneal injection of aluminium chloride for 60 days (100 mg/kg body weight). During these 60 days both Alzheimer's and control rats were given 6 g of raisin per rat. At the end of the treatment, blood was collected for biochemical assessment. We used a Morris water task and passive avoidance test to assess spatial memory. Our results showed that aluminium exposure significantly decreased the memory in the MWT and passive avoidance test, but in the raisin + AlCl group, it significantly increased spatial memory in both tests. Also, Aluminium exposure significantly increased malondialdehyde (MDA) and decreased ferric reducing ability of plasma (ferric reducing/antioxidant power (FRAP)), while treatment with raisin significantly decreased MDA and increased FRAP in plasma of blood. Our findings showed that raisin has a neuroprotective effect and improves the spatial memory in AD animal models.
Nutr Neurosci, 2019; 22

27634580: Gol M, Ghorbanian D, Hassanzadeh S, Javan M, Mirnajafi-Zadeh J, Ghasemi-Kasman M
Fingolimod enhances myelin repair of hippocampus in pentylenetetrazol-induced kindling model.
Recent evidence indicates that demyelination occurs in epilepsy patients and kindling animal models. Regarding the well-known literature on anti-inflammatory and myelin protective effects of fingolimod (FTY720) in multiple sclerosis patients and animal models, we hypostatized whether FTY720 administration could exert myelin protective effects in pentylenetetrazol (PTZ)-induced kindling model. To end this, animals received 0.3 or 1mg/kg dosage of FTY720, 1h before PTZ injections. In another approach, after achieving fully kindling stage, FTY720 was administrated i.p. once daily for 7 consecutive days. Treatment with FTY720 (especially lower dose) reduced the frequency of seizures and epileptiform discharges in both approaches. We found that FTY720 administration decreases cell death and glial activation in CA1 and CA3 regions of hippocampus. Myelin protection effect was shown by increasing myelin levels. Furthermore, post-treatment of FTY720 enhanced endogenous remyelination and the number of oligodendrocyte precursor cells in fully kindled animals. Together, these results demonstrate that FTY720 behind the anti-inflammatory and neuroprotection effects has beneficial role in myelin protection and remyelination enhancement in PTZ kindling model of seizure and it may be provide a new therapeutic option for demyelination associated with epilepsy.
Eur J Pharm Sci, 2017; 96

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BOARD NUMBER: S01-590

INTRACEREBRAL LONGITUDINAL CHARACTERIZATION OF LARGE-SCALE EPILEPTOGENESIS IN THE KAINATE MOUSE MODEL OF FOCAL TEMPORAL LOBE EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

Fabien Friscourt[1,2], Laurent Sheybani[1,3], Guru Padmasola[1], Maëlle Hernot[1], Karl Schaller[3], Christoph Michel[1,3], Charles Quairiaux[1]
[1]Functional Brain Mapping Laboratory, Department Of Fundamental Neurosciences, Geneva, Switzerland, [2]Department of Fundamental Neurosciences, Faculty Of Medicine, Geneva, Switzerland, [3]Neurology Clinic, Department Of Clinical Neurosciences, Geneva, Switzerland

Temporal lobe epilepsy (TLE) is the most common type of focal epilepsies. While TLE is characterized by the presence of an epileptic focus (EF) that is held to be responsible for triggering seizures and driving interictal activities, recent studies show that epileptogenic networks (EN) and brain alterations are widely distributed. In this context, a fundamental question is yet to be answered: is the EN responsible for the emergence of epileptic activities, with the EF as one of its major outputs, or is the EF upstream in the cascade that leads to the formation of the EN? Using multisite chronic mSEEG recordings and chemogenetic tools in the status epilepticus (SE) induced intra hippocampal kainate mouse model of TLE, we propose to characterize in detail the emergence of epileptic activities in the EF and in remote regions and evaluate the role of the primary epileptogenic region for the development of the large-scale EN. We found that different epileptiform signatures, with ictal, ictal-like and interictal patterns, appear in the very early stage of the latent phase (LP) (as early as 72h after SE) in both the EF and distant regions and then evolve along the LP to become typical epileptic events characterizing the chronic phase. We investigate these network dynamics with high spatial and temporal precision using systematic unbiased detection of pathological activities. Our work aims at identifying key mechanisms involved in the development of epileptic neuronal networks and clarify the pathogenic stream of events at play between the EF and the EN.

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SYNAPTIC NETWORK DYSFUNCTION AND INCREASED INTRINSIC NEURONAL EXCITABILITY IN GLUA2 AUTOIMMUNE ENCEPHALITIS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

Yang Yuan, Christian Geis, Holger Haselmann
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Title of the Poster: Synaptic network dysfunction and increased intrinsic neuronal excitability in GluA2 autoimmune encephalitis Authors: Yang Yuan, Christian Geis, Holger Haselmann Abstract Aims Autoimmune encephalitis (AE) is a new group of neurological disorders induced by autoantibodies (aAB) against distinct neuronal surface antigens such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR). As epilepsy is a characteristic feature of AMPA receptor encephalitis, we aim to investigate the effect of anti-GluA2 (AMPAR subunit) aABs on the neuronal network and the balance of excitation and inhibition as a prerequisite for increased susceptibility to seizures. Methods We performed intra-parenchymal injections of a-GluA2 aABs into the hippocampus area of mice and prepared acute brain slices after 24 hours incubation. We hypothesized a dysbalance of the excitatory to inhibitory ratio (I/E ratio) and performed stimulation of Schaffer-collateral pathway to evaluate glutamatergic and bi-synaptic GABAergic signaling on the single-cell level in CA1 pyramidal neurons. Moreover, we investigated the influence of anti-GluA2 aABs on intrinsic neuronal excitability. Results The I/E ratio of evoked postsynaptic currents was changed. The intrinsic excitability of neurons was increased due to anti-GluA2 autoantibodies. In addition, anti-GluA2 antibodies increased significantly the action potentials firing rate after Schaffer-collateral stimulation. Conclusions Anti-GluA2 autoantibodies affect hippocampal networks and the intrinsic excitability of neurons. They may increase synaptic transmission. Our results contribute to the understanding of the epileptogenic potential of anti-GluA2 aABs and may help to identify optimized treatment strategies in controlling epilepsy in AMPAR AE.

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CHLORIDE DYNAMICS IN SPECIFIC NEURONAL SUBTYPES DURING EPILEPTIFORM ACTIVITY IN VITRO

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

Alexandru Călin[1], Tatiana Waseem[1], Joseph Raimondo[2], Sarah Newey[1], Colin Akerman[1]
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Aims: We set out to investigate the intracellular chloride resting levels and activity-dependent chloride dynamics in specific neuronal subtypes of the mouse hippocampus during epileptiform activity. Methods: We developed a floxed version of the genetically-encoded chloride indicator, ClopHensorN, to perform fluorescence chloride imaging in defined neuronal populations. Floxed ClophensorN was successfully delivered to mice expressing Cre recombinase in specific cell types. This enabled us to measure the resting state absolute intracellular chloride concentration in pyramidal neurons, and parvalbumin (PV), somatostatin (SST) and vasoactive intestinal polypeptide (VIP) expressing interneurons. Simultaneous confocal microscopy and electrophysiological recordings were used to quantify the relationship between the dynamics of intracellular chloride and epileptiform activity in organotypic hippocampal brain slices. Results: Intracellular chloride steady states varied significantly across cell types. Average resting somatic chloride was 5.8±2.9 mM in pyramidal neurons, 32.5±6.6 mM in PV interneurons, 12.9±2.3 mM in SST interneurons, and 22.6±4 mM in VIP interneurons. Interestingly, epileptiform activity resulted in a convergence in intracellular chloride concentrations across neuronal subtypes, with PV interneurons maintaining relatively stable somatic chloride levels, and other neuronal subtypes exhibiting activity-dependent increases in intracellular chloride. Conclusions: Using novel optical methods, we reveal distinct chloride steady states and activity-dependent dynamics in defined GABAergic interneuron subtypes. Since intracellular chloride determines the ionic driving force for fast synaptic inhibition, these findings advance our understanding of the interplay between excitatory and inhibitory signalling during epileptic seizures.

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TYPICAL ABSENCE EPILEPSY SIMULATED IN A FULL HUMAN VIRTUAL BRAIN MODEL SUGGEST THAT SEIZURES ARE NOT GENERALIZED AND INDUCE A HYPER-INHIBITION OF THE ASSOCIATIVE CORTEX

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

Pascal Benquet[1,2], Maxime Yochum[2], Elif Koksal Ersoz[1], Paul Berraute[1], Patrick Van Bogaert[3], Anna Kaminska[4], Mathieu Kuchenbuch[4], Rima Nabbout[5], Fabrice Wendling[1]
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Conscious perception results from an interplay between two processes interacting with each other: *awareness* and *wakefulness* supported respectively by large-scale cortico-cortical functional connectivity whereas *wakefulness* depends critically on thalamocortical connectivity[1].
We have developed a full virtual human brain named COALIA able to simulate realistic EEG, as compared to real EEG recorded in humans at two levels of consciousness[2]. We used COALIA to understand the neurobiological mechanisms of typical absence seizures. Accounting for brain characteristics present in the immature brain and the human structural connectivity, we were able to generate realistic absence seizures activity patterns in simulated high-density EEG with our human computational full brain model. Results suggest that a focal initiation in the frontal cortex triggers a switch in the firing mode of the cognitive/associative nuclei of the thalamus from tonic to “UP and down” discharge mode due to and excessive feed-forward cortico-thalamic inhibition. This thalamic bursting mode initiates 3Hz spike-wave discharge onto the associative area of the cortex through an exaggerated thalamo-cortical feed-forward inhibition (hyperexcitation of cortical SST GABAergic subtype onto pyramidal cells) that explain the large wave of the SWD. This large cortical inhibition transiently disrupts the cortico-cortical connectivity by suppression of the PV-PC loop. Simulated TMS-evoked potential confirmed a dramatic drop of the consciousness index during absence seizures. Finally, high-density EEG-based source location during typical absence seizure recorded in child confirm that SWD are not generalized but mainly affect the associative part of the cortex. 1: PMID: 32537530 2:PMID: 31798421 ***Grants****: INCR predilepsy project, ERC Syn Galvani*

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SELECTIVE LOSS OF INTERNEURONS IN THE SUBICULUM IN A MOUSE MODEL FOR MESIAL TEMPORAL LOBE EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Mesial temporal lobe epilepsy (MTLE) is characterized by a loss of principal cells in the hilus, CA1 and CA3, as well as interneurons throughout the hippocampus. Whereas the hippocampus has been intensely investigated, data on structural changes in the subiculum, which is the main output region of the hippocampal formation, are rare. Yet, its connectivity and cellular characteristics render the subiculum an important part of the epileptic network. Based on our previous work showing differential vulnerability of interneurons in the hippocampus, we now performed a detailed study of epilepsy-related changes in the subiculum. Using the intrahippocampal kainate (KA) mouse model for MTLE we analyzed neuronal loss after *status epilepticus*. Furthermore, we performed quantitative immunocytochemistry for different interneuron populations [parvalbumin, calbindin, calretinin and Neuropeptide Y (NPY)] across all layers along the dorsoventral axis of the subiculum and determined GAD67 expression with fluorescent *in situ* hybridization in the chronic stage of MTLE (21 days after KA). Finally, we confirmed the occurrence of epileptic activity by local field potential recordings in the subiculum. We found prominent cell loss in the subiculum and a consistent reduction of parvalbumin-expressing interneurons along its entire dorsoventral axis. The density of CB-positive cells was increased, whereas CR-expressing neurons remained unaltered. Interestingly, the number of NPY-expressing cells was increased, yet NPY- and GAD67-doublelabeled cells were reduced, indicating interneuron loss but NPY upregulation in principal cells. In summary, our results indicate that a selective loss of parvalbumin- and NPY-positive interneurons contributes to epileptogenicity in the subiculum after KA injection.

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THE CONSTITUTIVE ACTIVITY OF THE HISTAMINE H1 RECEPTOR, INTERACTION WITH THE NMDA RECEPTOR: CONSEQUENCES IN EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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We have explored the epileptogenic effects of several drugs specific to the antihistaminic H1 receptor on NMDA and GABA responses. With patch-clamp whole-cell recordings of hippocampal neurons of rats, we observe the effects of several histaminergic agonists and antagonists on the NMDA and GABA currents. During the NMDA rundown and GABA rundown, we applied the drugs and observe the effect on the responses, and compare them to control conditions. **Results** Mepyramine with nanomolar concentrations increases significantly the NMDA responses about 35 %, this effect is mimicked by another Anti H1 drug Triprolidine. Histamine alone has no effect on NMDA rundown but 100 µM Histamine can reduce partially the effect of Mepyramine. An H1 receptor agonist 2,3 Bromophenyl Histamine also shows the same properties as Histamine. The H1 receptor of Histamine seems to be crucial during these experiments, blockade of its constitutive activity by Mepyramine induces a significant increase of the NMDA responses. On the GABA response, the same nanomolar dose of Mepyramine has no effect on the rundown and we also observed no effect of Histamine on the GABA rundown. An interaction between Histamine H1 receptor and the NMDA is revelated by using Mepyramine in nanomolar concentration, Histamine has no effect on these responses also in these experiments Mepyramine acts as an inverse agonist blocking the constitutive activity of the H1 receptor. The constitutive activity of the H1 receptor seems to be crucial in the regulation of NMDA receptor activities.

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ROLE OF THE HYDROXYCARBOXYLIC ACID RECEPTOR 1 (HCAR1) IN HIPPOCAMPAL NETWORK REGULATION DURING EPILEPTIC SEIZURES

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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The goal of this research is to investigate the role of the lactate receptor hydroxycarboxylic acid receptor 1 (HCAR1) in the regulation of the hippocampal network in the pathology of epilepsy and its ability to modulate seizures. HCAR1 is especially enriched in the hippocampal glutamatergic neurons in the CA fields and in the dentate gyrus. Investigations first using mouse primary neuronal cultures then acute brain slices led us to unravel the mechanisms of action of HCAR1 as a modulator of neuronal activity and its interactions with other G_i-protein coupled receptors involving $G_{\beta\gamma}$ subunits. By its ability to reduce neuronal activity and its expression by glutamatergic hippocampal neurons, HCAR1 appears as a potential target for the modulation of seizures. In this study, we focused on the pathophysiology of acute seizures. We elicited seizures on freely behaving mice using the intrahippocampal kainate injection model in the presence or absence of the non-metabolized HCAR1 agonist 3-chloro-5-hydroxybenzoic acid (3Cl-HBA) and investigated the potential modulatory effect of HCAR1 activation on seizures using electroencephalography (EEG). We quantified the frequency bands power of delta, theta, alpha, beta and gamma waves. Contrasting to ex vivo observation by us and other groups, activating HCAR1 during seizures had no significant effect on the recorded signals. We are currently using *in vitro* models of epilepsy to determine if and under which conditions HCAR1 activation can modulate seizures, with particular attention to potential interference with other Gi-coupled receptors.

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TRANSCRIPTOMIC ANALYSIS OF HIPPOCAMPAL SUBREGIONS AFTER INDUCTION OF ACUTE SEIZURES BY ELECTRIC STIMULATION OF THE PERFORANT PATHWAY IN RATS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

Andre Vieira[1], Gabriel Zanetti[1], Elayne Dias[1], Beatriz Aoyama[1], Maria Carolina Pedro Athié[2], Iscia Lopes-Cendes[2]
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Preconditioning is a mechanism in which injuries induced by non-lethal hypoxia or seizures trigger cellular resistance to subsequent events. Norwood et al., in a 2010 study, showed that an 8-hour-long period of electrical stimulation of the perforant pathway in rats is required for the induction of hippocampal sclerosis. However, in order to avoid generalized seizures, status epilepticus (SE), and death, a state of resistance to seizures must be induced in the hippocampus by a preconditioning paradigm consisting of 2 daily 30-minute stimulation periods. The present study aims to investigate differential gene expression patterns in different hippocampal subregions using RNA-sequencing, after induction of a preconditioning protocol by electrical stimulation of the perforant pathway. The dorsal and ventral dentate gyrus (dDG, vDG), CA3 (dCA3, vCA3) and subiculum (dSub, vSub) regions were collected by laser-microdissection 24 hours after preconditioning protocol induction in rats. RNA sequencing was performed in a Hiseq 4000 platform, reads were aligned using the STAR and DESEq2 statistics package was used to estimate gene expression. When comparing control to preconditioned samples, we found the differential expression of 1436 genes in dDG, 987 in vDG, 744 in dCA3, 662 genes in vCA3, 204 in dSub and 972 in vSub. Our results indicate that preconditioning by perforant pathway electrical stimulation may induce a coordinated increase in GABAergic inhibition in both DG regions but not in CA3. Furthermore, we observed changes in synaptic reorganization, increased cholesterol metabolism, and astrogliosis in the subiculum.

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BOARD NUMBER: S01-598

ELEVATED CO2 IS A MAJOR BRAIN-SPARING MECHANISM IN BIRTH ASPHYXIA

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Aims: Birth asphyxia (BA) is a period of severe systemic O_2 deprivation (hypoxia) and build-up of CO_2 (hypercapnia). Most animal models of BA neglect the fundamental hypercapnia component. Here, we have studied the multiple protective roles of CO_2 during oxygen deprivation. Methods: 11-day-old rat pups were exposed to pure hypoxia, pure hypercapnia, or their combination (i.e. asphyxia) by altering the O_2 (from 21% to 5-9%) and CO_2 (up to 20%) concentrations of the inhaled gas. Brain and body pH and PO_2 levels were measured using microsensors. Results: An increase in ambient PCO_2 strongly suppressed the fall in brain PO_2 during asphyxia, obviously reflecting a brain-sparing vasomotor response. Moreover, extending the hypercarbia exposure beyond the asphyxia strongly suppressed post-asphyxia EEG and behavioural seizures. The effects of hypercarbia were mimicked by intraperitoneal or intravenous application of both highly membrane-permeant and impermeant inhibitors of carbonic anhydrase (CAIs). Conclusions: The increase in CO_2 which is always associated with birth asphyxia has brain-sparing effects (elevated oxygenation; suppression of the ictogenic action of hypoxia). These effects can be enhanced and prolonged by elevation of the ambient CO_2 level or by application of CAIs during the post-asphyxia recovery.

Pubmed:
27916957: Kerkkamp HM, Kini RM, Pospelov AS, Vonk FJ, Henkel CV, Richardson MK
Snake Genome Sequencing: Results and Future Prospects.
Snake genome sequencing is in its infancy-very much behind the progress made in sequencing the genomes of humans, model organisms and pathogens relevant to biomedical research, and agricultural species. We provide here an overview of some of the snake genome projects in progress, and discuss the biological findings, with special emphasis on toxinology, from the small number of draft snake genomes already published. We discuss the future of snake genomics, pointing out that new sequencing technologies will help overcome the problem of repetitive sequences in assembling snake genomes. Genome sequences are also likely to be valuable in examining the clustering of toxin genes on the chromosomes, in designing recombinant antivenoms and in studying the epigenetic regulation of toxin gene expression.
Toxins (Basel), 2016; 8
33338272: Ala-Kurikka T, Pospelov A, Summanen M, Alafuzoff A, Kurki S, Voipio J, Kaila K
A physiologically validated rat model of term birth asphyxia with seizure generation after, not during, brain hypoxia.
Birth asphyxia (BA) is often associated with seizures that may exacerbate the ensuing hypoxic-ischemic encephalopathy. In rodent models of BA, exposure to hypoxia is used to evoke seizures, that commence already during the insult. This is in stark contrast to clinical BA, in which seizures are typically seen upon recovery. Here, we introduce a term-equivalent rat model of BA, in which seizures are triggered after exposure to asphyxia.
Epilepsia, 2021; 62
34180051: Pospelov AS, Ala-Kurikka T, Kurki S, Voipio J, Kaila K
Carbonic anhydrase inhibitors suppress seizures in a rat model of birth asphyxia.
Seizures are common in neonates recovering from birth asphyxia but there is general consensus that current pharmacotherapy is suboptimal and that novel antiseizure drugs are needed. We recently showed in a rat model of birth asphyxia that seizures are triggered by the post-asphyxia recovery of brain pH. Here our aim was to investigate whether carbonic anhydrase inhibitors (CAIs), which induce systemic acidosis, block the post-asphyxia seizures.
Epilepsia, 2021; 62
26547277: Pospelov AS, Yukin AY, Blumberg MS, Puskarjov M, Kaila K
Forebrain-independent generation of hyperthermic convulsions in infant rats.
Febrile seizures are the most common type of convulsive events in children. It is generally assumed that the generalization of these seizures is a result of brainstem invasion by the initial limbic seizure activity. Using precollicular transection in 13-day-

old rats to isolate the forebrain from the brainstem, we demonstrate that the forebrain is not required for generation of tonic-clonic convulsions induced by hyperthermia or kainate. Compared with sham-operated littermate controls, latency to onset of convulsions in both models was significantly shorter in pups that had undergone precollicular transection, indicating suppression of the brainstem seizure network by the forebrain in the intact animal. We have shown previously that febrile seizures are precipitated by hyperthermia-induced respiratory alkalosis. Here, we show that triggering of hyperthermia-induced hyperventilation and consequent convulsions in transected animals are blocked by diazepam. The present data suggest that the role of endogenous brainstem activity in triggering tonic-clonic seizures should be re-evaluated in standard experimental models of limbic seizures. Our work sheds new light on the mechanisms that generate febrile seizures in children and, therefore, on how they might be treated.
Epilepsia, 2016; 57
32174009: Pospelov AS, Puskarjov M, Kaila K, Voipio J
Endogenous brain-sparing responses in brain pH and PO in a rodent model of birth asphyxia.
To study brain-sparing physiological responses in a rodent model of birth asphyxia which reproduces the asphyxia-defining systemic hypoxia and hypercapnia.
Acta Physiol (Oxf), 2020; 229

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BOARD NUMBER: S01-599

SYNEDRELLA NODIFLORA EXTRACT DEPRESSES EXCITATORY SYNAPTIC TRANSMISSION AND CHEMICALLY-INDUCED IN VITRO SEIZURES IN THE RAT HIPPOCAMPUS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Extracts of the tropical Cinderella plant *Synedrella nodiflora* are used traditionally to manage convulsive conditions in the West African sub-region. This study sought to determine the neuronal basis of the effectiveness of these plant extracts to suppress seizure activity. Using the hippocampal slice preparation from rats, the ability of the extract to depress excitatory synaptic transmission and *in vitro* seizure activity were investigated. Bath perfusion of the hydro-ethanolic extract of *Synedrella nodiflora* (SNE) caused a concentration-dependent depression of evoked field excitatory postsynaptic potentials (fEPSPs) recorded extracellularly in the CA1 region of the hippocampus with maximal depression of about 80% and an estimated IC_{50} of 0.06 mg/ml. The SNE-induced fEPSP depression was accompanied by an increase in paired pulse facilitation. The fEPSP depression only recovered partially after 20 min washing out. The effect of SNE was not stimulus dependent as it was present even in the absence of synaptic stimulation. Furthermore, it did not show desensitization as repeat application after 10 min washout produced the same level of fEPSP depression as the first application. The SNE effect on fEPSPs was not via adenosine release as it was neither blocked nor reversed by 8-CPT, an adenosine A_1 receptor antagonist. In addition, SNE depressed *in vitro* seizures induced by zero Mg^{2+} and high K^+ -containing artificial cerebrospinal fluid (aCSF) in a concentration-dependent manner. The results show that SNE depresses fEPSPs and spontaneous bursting activity in hippocampal neurons that may underlie its ability to abort convulsive activity in persons with epilepsy.

Pubmed:
33762938: Amoateng P, Tagoe TA, Karikari TK, Kukuia KKE, Osei-Safo D, Woode E, Frenguelli BG, Kombian SB
Extract Depresses Excitatory Synaptic Transmission and Chemically-Induced Seizures in the Rat Hippocampus.
Extracts of the tropical Cinderella plant are used traditionally to manage convulsive conditions in the West African sub-region. This study sought to determine the neuronal basis of the effectiveness of these plant extracts to suppress seizure activity. Using the hippocampal slice preparation from rats, the ability of the extract to depress excitatory synaptic transmission and seizure activity were investigated. Bath perfusion of the hydro-ethanolic extract of (SNE) caused a concentration-dependent depression of evoked field excitatory postsynaptic potentials (fEPSPs) recorded extracellularly in the CA1 region of the hippocampus with maximal depression of about 80% and an estimated IC of 0.06 mg/ml. The SNE-induced fEPSP depression was accompanied by an increase in paired pulse facilitation. The fEPSP depression only recovered partially after 20 min washing out. The effect of SNE was not stimulus dependent as it was present even in the absence of synaptic stimulation. Furthermore, it did not show desensitization as repeat application after 10 min washout produced the same level of fEPSP depression as the first application. The SNE effect on fEPSPs was not via adenosine release as it was neither blocked nor reversed by 8-CPT, an adenosine A receptor antagonist. In addition, SNE depressed seizures induced by zero Mg and high K -containing artificial cerebrospinal fluid (aCSF) in a concentration-dependent manner. The results show that SNE depresses fEPSPs and spontaneous bursting activity in hippocampal neurons that may underlie its ability to abort convulsive activity in persons with epilepsy.
Front Pharmacol, 2021; 12
34939065: Logosu D, Tagoe TA, Adjei P
Transcutaneous electrical nerve stimulation in the management of calf muscle spasticity in cerebral palsy: A pilot study.
This study sets out to evaluate the effectiveness of transcutaneous electrical nerve stimulation (TENS) in the management of

calf muscle spasticity in children with cerebral palsy. The study follows a one group pre-test-post-test design involving fifteen children with spastic cerebral palsy, presenting with calf muscle spasticity. Spasticity was assessed before and after a 30 min application of TENS to the bilateral calf muscles. The H-reflex (electromyography) of the calf muscles and Modified Ashworth Scale (MAS) served as a measure of spasticity. A goniometer was used to measure the range of motion (ROM) angles for ankle dorsiflexion. We report here no significant difference ($p > 0.05$) between the left and right H-reflex responses, MAS scores, and ROM scores recorded at baseline (pre-test). Correlation analysis show no correlation ($p > 0.05$) between the pre-test HA Max (maximum H-reflex amplitude)/MA Max (maximum M-Wave Amplitude) ratio and MAS scores of both the left and right calf muscles. However, TENS significantly reduced ($p < 0.05$) the HA of the left calf muscle and MAS scores of the left and right calf muscles. Additionally, TENS significantly increased the ROM scores of the left and right calf muscles. Our findings lend support to existing evidence that TENS is effective in reducing spasticity. The potential mechanism underlying this effect is a reduction in neuron excitability.
IBRO Neurosci Rep, 2021; 11

24523557: Tagoe T, Barker M, Jones A, Allcock N, Hamann M
Auditory nerve perinodal dysmyelination in noise-induced hearing loss.
Exposure to loud sound (acoustic overexposure; AOE) induces hearing loss and damages cellular structures at multiple locations in the auditory pathway. Whether AOE can also induce changes in myelin sheaths of the auditory nerve (AN) is an important issue particularly because these changes can be responsible for impaired action potential propagation along the AN. Here we investigate the effects of AOE on morphological and electrophysiological features of the centrally directed part of the rat AN projecting from the cochlear spiral ganglion to brainstem cochlear nuclei. Using electron microscopy and immunocytochemistry, we show that AOE elongates the AN nodes of Ranvier and triggers notable perinodal morphological changes. Compound action potential recordings of the AN coupled to biophysical modeling demonstrated that these nodal and perinodal structural changes were associated with decreased conduction velocity and conduction block. Furthermore, AOE decreased the number of release sites in the cochlear nuclei associated with the reduced amplitudes of EPSCs evoked by AN stimulation. In conclusion, AN dysmyelination may be of fundamental importance in auditory impairment following exposure to loud sound.
J Neurosci, 2014; 34

22570693: Barker M, Solinski HJ, Hashimoto H, Tagoe T, Pilati N, Hamann M
Acoustic overexposure increases the expression of VGLUT-2 mediated projections from the lateral vestibular nucleus to the dorsal cochlear nucleus.
The dorsal cochlear nucleus (DCN) is a first relay of the central auditory system as well as a site for integration of multimodal information. Vesicular glutamate transporters VGLUT-1 and VGLUT-2 selectively package glutamate into synaptic vesicles and are found to have different patterns of organization in the DCN. Whereas auditory nerve fibers predominantly co-label with VGLUT-1, somatosensory inputs predominantly co-label with VGLUT-2. Here, we used retrograde and anterograde transport of fluorescent conjugated dextran amine (DA) to demonstrate that the lateral vestibular nucleus (LVN) exhibits ipsilateral projections to both fusiform and deep layers of the rat DCN. Stimulating the LVN induced glutamatergic synaptic currents in fusiform cells and granule cell interneurones. We combined the dextran amine neuronal tracing method with immunohistochemistry and showed that labeled projections from the LVN are co-labeled with VGLUT-2 by contrast to VGLUT-1. Wistar rats were exposed to a loud single tone (15 kHz, 110 dB SPL) for 6 hours. Five days after acoustic overexposure, the level of expression of VGLUT-1 in the DCN was decreased whereas the level of expression of VGLUT-2 in the DCN was increased including terminals originating from the LVN. VGLUT-2 mediated projections from the LVN to the DCN are likely to play a role in the head position in response to sound. Amplification of VGLUT-2 expression after acoustic overexposure could be a compensatory mechanism from vestibular inputs in response to hearing loss and to a decrease of VGLUT-1 expression from auditory nerve fibers.
PLoS One, 2012; 7

28214516: Tagoe T, Deeping D, Hamann M
Saturation of long-term potentiation in the dorsal cochlear nucleus and its pharmacological reversal in an experimental model of tinnitus.
Animal models have demonstrated that tinnitus is a pathology of dysfunctional excitability in the central auditory system, in particular in the dorsal cochlear nucleus (DCN) of the brainstem. We used a murine model and studied whether acoustic over-exposure leading to hearing loss and tinnitus, affects long-term potentiation (LTP) at DCN multisensory synapses. Whole cell and field potential recordings were used to study the effects on release probability and synaptic plasticity, respectively in brainstem slices. Shifts in hearing threshold were quantified by auditory brainstem recordings, and gap-induced prepulse inhibition of the acoustic startle reflex was used as an index for tinnitus. An increased release probability that saturated LTP and thereby induced metaplasticity at DCN multisensory synapses, was observed 4-5days following acoustic over-exposure. Perfusion of an NMDA receptor antagonist or decreasing extracellular calcium concentration,

decreased the release probability and restored LTP following acoustic over-exposure. In vivo administration of magnesium-threonate following acoustic over-exposure restored LTP at DCN multisensory synapses, and reduced gap detection deficits observed four months following acoustic over-exposure. These observations suggest that consequences of noise-induced metaplasticity could underlie the gap detection deficits that follow acoustic over-exposure, and that early therapeutic intervention could target metaplasticity and alleviate tinnitus.
Exp Neurol, 2017; 292

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PROBING CORTICAL EXCITABILITY UNDER GABAERGIC MODULATION IN HUMANS WITH EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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Background In focal epilepsy, seizures result from subnetworks of abnormally high cortical excitability (CE) within a relatively normal brain. Practical means of monitoring CE in the human brain, for example to assess the effects of anti-seizure medications are currently lacking. We asked whether directly probing the brain with minute electrical pulses and recording cortico-cortical evoked potentials (CCEPs) may help quantify CE before and after administration of a benzodiazepine (BZD). Methods In seven epilepsy patients undergoing cortical recordings for diagnostic reasons (median number of electrode contacts: 68 [48, 111]), we probed CE with repeated single pulses (median N= 168 [151, 204]) systematically varying in intensity (0.2 – 12mA). All brain responses, before and after administration of clonazepam (0.75-1mg) were clustered into subnetworks using NMF (Non-negativity matrix factorization) based on the dynamics of CCEPs. CE in each subnetwork was quantified by the magnitude of the NMF activation coefficients. Results Across patients, NMF delineated a total of 20 subnetworks that encompassed a collection of brain areas with shorter- and longer-range connections responding conjointly to the administered probing stimulations with CCEPs. Across these subnetworks, CE was significantly decreased after BZD administration (Wilcoxon signed-rank test: p=0.0003). Conclusion Combining probing neurostimulations with unsupervised pattern recognition algorithms, we introduce a novel method to quantify CE in the human brain. As a proof-of-principle, we apply this method to uncover the network effects of a well-known GABA agonist on CE. Applying such methods over longer durations, may help monitor non-linear dynamics in the human cortex, including in epilepsy.

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BOARD NUMBER: S01-601

THE GAIN OF FUNCTION SCN1A DISORDER SPECTRUM: NOVEL EPILEPSY PHENOTYPES AND THERAPEUTIC IMPLICATIONS

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

Massimo Mantegazza[1], Andreas Brunklaus[2], Tobias Brünger[3], Tony Feng[2], Carmen Fons[4], Anni Lehikoinen[5], Eleni Panagiotakaki[6], Mihaela Vintan[7], Joseph Symonds[2], James Andrew[2], Alexis Arzimanoglou[6], Julie Gallois[8], Sarah Delima[9], Donncha Hanrahan[8], Gaetan Lesca[10], Steward Macleod[2], Dragan Marjanovic[11], Amy Mctague[12], Noemi Nuñez-Enamorado[13], Eduardo Perez-Palma[14], Scott Perry[15], Karen Pysden[16], Sophie Russ-Hall[17], Ingrid Scheffer[17], Krystal Sully[18], Steffen Syrbe[19], Ulvi Vaher[20], Murugan Velayutham[21], Julie Vogt[21], Shelly Weiss[22], Elaine Wirrell[23], Sameer Zuberi[2], Dennis Lal[24], Rikke Møller[11], Sandrine Cestèle[1]
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Voltage-gated sodium channel $Na_V1.1$ (*SCN1A*) loss of function variants cause the severe epilepsy Dravet syndrome (DS), as well as milder genetic epilepsy with febrile seizures plus (GEFS+). Gain of function (GOF) *SCN1A* variants are associated with familial hemiplegic migraine (FHM3). Other phenotypes have been described for *SCN1A* variants, including early infantile developmental and epileptic encephalopathy (EIDEE). We describe the clinical spectrum, genetic and functional evaluation of patients with early onset. We included 35 patients via an international collaborative network and from the literature, and identified three distinct clinical presentations. 1) The most severe phenotype (13 patients) was neonatal developmental and epileptic encephalopathy with movement disorder and arthrogryposis (NDEEMA) 2) 21 patients presented later (2 weeks-3 months) with a severe EIDEE and a movement disorder. 3) One patient presented after 3 months with DEE only. Functional studies of representative variants revealed GOF, in keeping with neuronal hyperexcitability. Notably, GOF epilepsy variants show a moderate effect, leading to a moderate increase in action current amplitude, much smaller compared to that of FHM3 variants, which lead to a larger increase of action current amplitude, consistent with stronger GOF. Differently than for DS, treatment with sodium channel blockers reduced seizure frequency in most of the patients, without evidence of symptom exacerbation. Our study expands the spectrum of gain of function *SCN1A*-related epilepsy phenotypes, defines clinical features, provides novel insights into disease mechanisms between *SCN1A*-related epilepsy and FHM3, and identifies sodium channel blockers as potentially efficacious therapies.

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BOARD NUMBER: S01-602

A NEW RELIABLE PILOCARPINE-CAFFEINE MOUSE MODEL OF TEMPORAL LOBE EPILEPSY

POSTER SESSION 01 - SECTION: EPILEPSY & SEIZURES

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INMED (Inserm), Umr1249, Marseille, France

Aims: Our work aims to understand the cellular alterations occurring in Temporal Lobe Epilepsy (TLE), a disabling and often drug-resistant disease, that represent 1% of adult neurological disorder worldwide. Systemic pilocarpine treatment is one of the most reliable means of inducing temporal lobe epilepsy (TLE) in rodent models. However, the traditional pilocarpine injection protocol using mice was associated with a high death rate, possibly because of cardiorespiratory collapse following status epilepticus (SE). To prevent this, we developed a modified procedure of pilocarpine SE induction. Methods: The modified procedure of pilocarpine SE induction included a single injection of a moderate dose of caffeine during the induction phase. That new protocol was also based on the use of young male mice, as well as on a refined Racine's scale. Results: Using the modified procedure and the refined Racine's scale, we reported a substantially increased survival rate, thus enabling the generation of a large cohort of epileptic mice. Moreover, those mice display the cardinal features that are found in TLE human patients (i) hippocampal sclerosis, (ii) ictal/interictal events, (iii) mossy fiber sprouting and (iiii) behavioural impairment. Conclusion: Our refined caffeine- and pilocarpine-based protocol substantially improves the outcome of the reliable pilocarpine mouse model of TLE.

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BOARD NUMBER: S01-603

GLIAL ACTIVATION CONTRIBUTES TO INCREASED SENSITIVITY OF SPINAL TRPV1 RECEPTORS IN PACLITAXEL INDUCED NEUROPATHIC PAIN.

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Paclitaxel induced peripheral neuropathy (PIPN) is often accompanied with neuropathic pain. Its analgesic treatment is unsatisfactory and brings serious side effects. In previous publications, we showed that the mechanical hyperalgesia in rodents is partially mediated by sensitization of presynaptic TRPV1 receptors in the spinal cord dorsal horn (SCDH)[1] and this effect is dependent on activation of Toll-like 4 receptors (TLR4)[2]. TLR4 are expressed also in glial cells. In the current experiments, we have studied whether glial cells activation is involved in the TRPV1 sensitization. Male rats were used to prepare spinal cord slices for whole-cell patch-clamp recordings of miniature excitatory postsynaptic currents (mEPSC) from superficial SCDH neurons. Minocycline was used as a glial cell activation inhibitor. Capsaicin (200 nM) application resulted in a potent increase of mEPSC frequency that was significantly reduced upon repetition. This was attenuated by acute paclitaxel (50 nM) application[1,2]. *In vitro* (90 min) incubation with minocycline (100 µM) reduced the paclitaxel effect on the TRPV1 mediated response to capsaicin, while acute minocycline application did not have a significant effect. Our results suggest that glial activation plays an important role in the paclitaxel-induced enhancement of TRPV1 responsiveness in the SCDH. Further experiments will evaluate this neuron-glia interaction also in the Bortezomib induced neuropathy. Supported by GAUK 251898, GACR 20-19136S. [1]Adamek et al. Neuropharmacology, 146: 163-174, 2019. [2]Li et al. J Neurosci. 35:13487-13500, 2015.

Pubmed:
35042769: Adamek P, Heles M, Bhattacharyya A, Pontearso M, Slepicka J, Palecek J
Dual PI3Kδ/γ Inhibitor Duvelisib Prevents Development of Neuropathic Pain in Model of Paclitaxel-Induced Peripheral Neuropathy.
The development of painful paclitaxel-induced peripheral neuropathy (PIPN) represents a major dose-limiting side effect of paclitaxel chemotherapy. Here we report a promising effect of duvelisib (Copiktra), a novel FDA-approved PI3Kδ/γ isoform-specific inhibitor, in preventing paclitaxel-induced pain-like behavior and pronociceptive signaling in DRGs and spinal cord dorsal horn (SCDH) in rat and mouse model of PIPN. Duvelisib blocked the development of mechanical hyperalgesia in both males and females. Moreover, duvelisib prevented paclitaxel-induced sensitization of TRPV1 receptors, and increased PI3K/Akt signaling in small-diameter DRG neurons and an increase of CD68 cells within DRGs. Specific optogenetic stimulation of inhibitory neurons combined with patch-clamp recording revealed that duvelisib inhibited paclitaxel-induced weakening of inhibitory, mainly glycinergic control on SCDH excitatory neurons. Enhanced excitatory and reduced inhibitory neurotransmission in the SCDH following PIPN was also alleviated by duvelisib application. In summary, duvelisib showed a promising ability to prevent neuropathic pain in PIPN. The potential use of our findings in human medicine may be augmented by the fact that duvelisib is an FDA-approved drug with known side effects. We show that duvelisib, a novel FDA-approved PI3Kδ/γ isoform-specific inhibitor, prevents the development of paclitaxel-induced pain-like behavior in males and females and prevents pronociceptive signaling in DRGs and spinal cord dorsal horn in rat and mouse model of paclitaxel-induced peripheral neuropathy.
J Neurosci, 2022; 42

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-604

CUTANEOUS C-FIBER SUBTYPES BASED ON NERVE TERMINAL RESPONSIVENESS

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Sensory neurons in the dorsal root ganglia are diverse cells with transcriptional profiling identifying 6-14 subtypes. Transcriptional studies inform nerve phenotypes, but they are at best cautiously translated to nerve function. Moreover, these studies have focused on the whole ganglia comprising neurons innervating different organs. We evaluated sensory C-fibre phenotypes based on the responsiveness of nerve terminals specifically innervating the dorsal skin of the mouse. Using an *ex vivo* mouse skin-spinal nerve preparation we invested the responses of 193 individual C-fibres to chemical (chloroquine (CQ), BAM8-22, histamine, bradykinin, ß-alanine, serotonin, ATP, lysophosphatidic acid and TRPV1, V3, V4, A1, C3/6, M2) agonists and mechanical stimulation. The C-fibres in mouse skin can be subcategorized into low mechanical threshold and high mechanical threshold C-fibres (C-LTMs (8%) and C-HTMs (92%), respectively). C-LTMs were unresponsive to all chemical mediators except the TRPV4 agonist GSK1016790A. The C-HTMs could be subdivided broadly into two subgroups, one responding strongly to pruritogens such as chloroquine, histamine or BAM8-22 (n=58, 30%), but not other mediators; the other failing to respond to pruritic stimuli, but respond strongly to ATP, serotonin or lysophosphatidic acid. Of the latter, about 50% also respond to the MrgprD stimulant ß-alanine; these ß-alanine responsive C-fibres were the only subtype that responded to the TRPV3 channel agonist farnesyl pyrophosphate (10µM). We hypothesize that activating the chloroquine/histamine sensitive C-HTMs likely evokes pruritic sensations; the more promiscuous, CQ/histamine insensitive C-fibres likely subserve more varied sensations including cutaneous pain. This work was funded by VEGA 1/0306/18.

Pubmed:
31047975: Jurcakova D, Ru F, Undem BJ
Allergen-induced histaminergic and non-histaminergic activation of itch C-fiber nerve terminals in mouse skin.
Acute cutaneous exposure to allergen often leads to itch, but seldom pain. The effect of mast cell activation on cutaneous C-fibers was studied using innervated isolated mouse skin preparation that allows for intra-arterial delivery of chemicals to the nerve terminals in the skin. Allergen (ovalbumin) injection into the isolated skin of actively sensitized mice strongly stimulated chloroquine (CQ)-sensitive C-fibers (also referred to as "itch" nerves); on the other hand, CQ-insensitive C-fibers were activated only modestly, if at all. The histamine H1 receptor antagonist pyrilamine abolished itch C-fibers response to histamine, but failed to significantly reduce the response to ovalbumin. Ovalbumin also strongly activated itch C-fibers in skin isolated from Mrgpr-cluster Δ mice. When pyrilamine was studied in the Mrgpr-cluster Δ mice thereby eliminating the influence of both histamine H1 and Mrgpr receptors (MrgprA3 and C11 are selectively expressed by itch nerves), the ovalbumin response was very nearly eliminated. The data indicate that the acute activation of itch C-fibers in mouse skin is largely secondary to the combined effect of activation of histamine H1 and Mrpgr receptors.
Neuroscience, 2019; 410
29941667: Jurcakova D, Ru F, Kollarik M, Sun H, Krajewski J, Undem BJ
Voltage-Gated Sodium Channels Regulating Action Potential Generation in Itch-, Nociceptive-, and Low-Threshold Mechanosensitive Cutaneous C-Fibers.
We evaluated the effect of voltage-gated sodium channel 1 (Na1) blockers in three nonoverlapping C-fiber subtypes in the mouse skin: chloroquine (CQ)-sensitive C-fibers with high mechanical thresholds- second, CQ-insensitive, capsaicin-sensitive C-fibers with high mechanical thresholds- and CQ and capsaicin-insensitive C-fibers with a very low mechanical threshold-C-LTMs. Na1-blocking drugs were applied to the nerve terminal receptive fields using an innervated isolated dorsal mouse skin-nerve preparation where the drugs are delivered into the skin intra-arterially. We combined these studies with an analysis of the mRNA expression of the -subunits of Na1 in individual dorsal root ganglia neurons labeled from the same region of the skin. Our results show that virtually all nociceptors and itch C-fibers expressed the tetrodotoxin (TTX)-resistant channels Na1.8 and Na1.9. However, TTX applied selectively into the skin abolished the action potential firing in response to

mechanical stimulation in 75% of the itch C-fibers, 100% of the nociceptors, and 100% of C-LTMs. Na1.7 was the most commonly expressed TTX-sensitive Na1 in all three C-fiber subtypes innervating the dorsal skin. Selectively blocking Na1.7 abolished responses in about 40% of itch C-fibers, 65% of nociceptors, but only 20% of C-LTMs. Blocking Na1.8 alone had no affect on the firing sensitivity of the C-fibers. However, in itch and nociceptive C-fibers where the activation was not inhibited with a Na1.7 blocker, adding the Na1.8 blocker silenced action potential discharge.
Mol Pharmacol, 2018; 94
28217875: Ru F, Sun H, Jurcakova D, Herbstsomer RA, Meixong J, Dong X, Undem BJ
Mechanisms of pruritogen-induced activation of itch nerves in isolated mouse skin.
Chloroquine (CQ) stimulates itch nerves and causes intense scratching in mice by activating the G-protein coupled receptor (GPCR) MrgprA3; it is not known how stimulation of MrgprA3 (or other GPCRs) leads to activation of the itch nerve terminals in the skin, but previous studies have found that transient receptor potential A1 (TRPA1) gene deletion blocks CQ-induced scratching. In the present study we used a novel dorsal skin-nerve preparation to evaluate mechanisms underlying CQ- and histamine-induced action potential discharge in itch nerve terminals. We found that CQ activation of the nerves requires the beta3 isoform of phospholipase C, but TRPA1 or other TRP channel are not required. Evidence is provided for a role for calcium-activated chloride channels such as TMEM16a in GPCR-activation of itch nerve terminals. The mechanism by which TRP channels participate in pruritogen-induced scratching may involve sites of action other than the primary afferent terminals.
J Physiol, 2017; 595

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BOARD NUMBER: S01-605

CONTRIBUTION OF TTX-RESISTANT NAV CHANNELS TO THE GENERATION OF MEMBRANE POTENTIAL INSTABILITIES IN MOUSE TRIGEMINAL NEURONS

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

Enrique Velasco[1,2], Julio Álvarez[2], Víctor Meseguer[1,3], Juana Gallar[1,3], Karel Talavera[2]
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Introduction. Peripheral sensory neurons are fundamental for stimulus detection, but many of their properties remain poorly understood, as the display of membrane potential instabilities (MPIs). MPIs are enhanced in pain states, but their functional significance and molecular bases remain unknown. We aimed at determining MPIs voltage modulation, relationship to action potential firing during stimulation and their molecular determinants. **Methods.** We used whole-cell current-clamp recordings on primary cultured mouse trigeminal neurons to record MPIs (144 WT neurons, 22 *$Na_V1.8$*-KO and 22 from *$Na_V1.9$*-KO). We used current injection, temperature, TTX, capsaicin and menthol to stimulate the neurons. Finally, we developed scripts in Python3.0 to analyze individual MPIs. **Results.** All MPIs characteristic showed dependencies with membrane voltage ($p < 0.0001$). The modelling of the relationship between MPIs and action potential generation indicated that MPIs are at the core of ongoing activity generation. Amplitude and maximal first derivative of the MPI determine if an action potential is triggered or not ($R^2 = 0.81$, $p < 0.0001$). The blockade of TTX-Sensitive channels did not affect MPIs ($p > 0.1$) while blocking TTX-Resistant channels ($Na_V1.8$-1.9) disrupted every MPIs characteristic ($p < 0.001$). *$Na_V1.8$* and *$Na_V1.9$-KO* mice neurons displayed MPIs with smaller amplitude and maximal first derivative ($p < 0.0001$). In addition, the frequency of MPIs of *$Na_V1.9$* KO neurons was significantly reduced ($p < 0.0001$). **Conclusion.** MPIs are a TTX-R channels dependent phenomenon, which determines ongoing action potential generation, a crucial feature in the sensory encoding of slow-ramp stimulus like cold, heat and chemical compounds

Pubmed:

34086629: Velasco E, Alvarez JL, Meseguer VM, Gallar J, Talavera K
Membrane potential instabilities in sensory neurons: mechanisms and pathophysiological relevance.
Peripheral sensory neurons transduce physicochemical stimuli affecting somatic tissues into the firing of action potentials that are conveyed to the central nervous system. This results in conscious perception, adaptation, and survival, but alterations of the firing patterns can result in pain and hypersensitivity conditions. Thus, understanding the molecular mechanisms underlying action potential firing in peripheral sensory neurons is essential in sensory biology and pathophysiology. Over the past 30 years, it has been consistently reported that these cells can display membrane potential instabilities (MPIs), in the form of subthreshold membrane potential oscillations or depolarizing spontaneous fluctuations. However, research on this subject remains sparse, without a clear conductive thread to be followed. To address this, we here provide a synthesis of the description, molecular bases, mathematical models, physiological roles, and pathophysiological implications of MPIs in peripheral sensory neurons. Membrane potential instabilities have been reported in trigeminal, dorsal root, and Mes-V ganglia, where they are believed to support repetitive firing. They are proposed to have roles also in intercellular communication, ectopic firing, and responses to tonic and slow natural stimuli. We highlight how MPIs are of great interest for the study of sensory transduction physiology and how they may represent therapeutic targets for many pathological conditions, such as acute and chronic pain, itch, and altered sensory perceptions. We identify future research directions, including the elucidation of the underlying molecular determinants and modulation mechanisms, their relation to the encoding of natural stimuli and their implication in pain and hypersensitivity conditions.
Pain, 2022; 163
34765614: Delicado-Miralles M, Velasco E, Díaz-Tahoces A, Gallar J, Acosta MC, Aracil-Marco A
Deciphering the Action of Perfluorohexyloctane Eye Drops to Reduce Ocular Discomfort and Pain.
Perfluorohexyloctane (F6H8) eyedrops have been recently introduced in Europe as a product to treat dry eye disease, based on its ability to reduce tear film instability in Meibomian gland dysfunction and evaporative dry eye disease, although its mechanism of action is still unknown. In the present pilot study, we evaluated the effects of the ocular instillation of a single

drop of commercial F6H8 eyedrops in 20 healthy humans (9 women/11 men), measuring: (a) Corneal surface temperature (CST) from infrared video images; (b) tear volume using phenol red threads; (c) blinking frequency; and (d) ocular surface sensations (cold, dryness, pricking, foreign body, burning, itching, gritty, eye fatigue, watering eyes, and light-evoked discomfort sensations; scored using 10 cm Visual Analog Scales), before and 5-60 min after F6H8 or saline treatment. CST decreased and tearing and blinking frequency increased significantly after F6H8 but not after saline solution. When applied unilaterally, CST decreased only in the F6H8-treated eye. No sensations were evoked after F6H8 or saline. The corneal surface temperature reduction produced by topical F6H8 does not evoke conscious ocular sensations but is sufficient to increase the activity of corneal cold thermoreceptors, leading to an increased reflex lacrimation and blinking that may relieve dry eye condition thus reducing ocular discomfort and pain.
Front Med (Lausanne), 2021; 8
33675004: Viudes-Sarrion N, Velasco E, Delicado-Miralles M, Lillo-Navarro C
Static magnetic stimulation in the central nervous system: a systematic review.
To systematically review the literature on the use of the transcranial static magnetic stimulation (tSMS) technique in humans and animals, its effects on different areas of the central nervous system (CNS), its influence on neural excitability and on the subject's behavior, and its biological effects and future possibilities. All static magnetic field applications that can be considered to have a physiologically similar effect have been reviewed.
Neurol Sci, 2021; 42
34204415: Varela-Rodríguez S, Sánchez-González JL, Sánchez-Sánchez JL, Delicado-Miralles M, Velasco E, Fernández-de-Las-Peñas C, Calderón-Díez L
Effects of Percutaneous Electrolysis on Endogenous Pain Modulation: A Randomized Controlled Trial Study Protocol.
Percutaneous electrolysis consists of the application of a galvanic electrical current throughout an acupuncture needle. It has been previously hypothesized that needling procedures' neurophysiological effects may be related to endogenous pain modulation (EPM). This protocol study describes the design of a double-blind (participant, assessor) randomized controlled trial with the aim to investigate whether percutaneous electrolysis is able to enhance EPM and whether the effect is different between two applications depending on the dosage of the galvanic electrical current. Seventy-two asymptomatic subjects not reporting the presence of pain symptoms the previous 6 months before the study, aged 18-40 years, are randomized into one of four groups: a control group who does not receive any intervention, a needling group who receives a needling intervention without electrical current, a low-intensity percutaneous electrolysis group (0.3 mA × 90 s), and a high-intensity percutaneous electrolysis group (three bouts of 3 mA × 3 s). Needling intervention consists of ultrasound-guided insertion of the needle on the common extensor tendon of the lateral epicondyle. The primary outcome is conditioned pain modulation (CPM), and secondary outcomes include widespread pressure pain sensitivity (pressure pain thresholds (PPT) over the lateral epicondyle, the cervical spine, and the tibialis anterior muscle) and temporal summation (TS). We expected that percutaneous electrolysis would have a greater influence on CPM than an isolated needling procedure and no intervention. In addition, we also postulated that there might be differences in outcome measures depending on the intensity of the electrical current during the percutaneous electrolysis application. This study makes a new contribution to the field of neurophysiological effects of percutaneous electrolysis and needling interventions.
Brain Sci, 2021; 11
34070256: Flix-Díez L, Delicado-Miralles M, Gurdiel-Álvarez F, Velasco E, Galán-Calle M, Lerma Lara S
Reversed Polarity bi-tDCS over M1 during a Five Days Motor Task Training Did Not Influence Motor Learning. A Triple-Blind Clinical Trial.
Transcranial direct current stimulation (tDCS) has been investigated as a way of improving motor learning. Our purpose was to explore the reversal bilateral tDCS effects on manual dexterity training, during five days, with the retention component measured after 5 days to determine whether somatosensory effects were produced. In this randomized, triple-blind clinical trial, 28 healthy subjects (14 women) were recruited and randomized into tDCS and placebo groups, although only 23 participants (13 women) finished the complete protocol. Participants received the real or placebo treatment during five consecutive days, while performing a motor dexterity training program of 20 min. The motor dexterity and the sensitivity of the hand were assessed pre- and post-day 1, post 5 days of training, and 5 days after training concluded. Training improved motor dexterity, but tDCS only produced a tendency to improve retention. The intervention did not produce changes in the somatosensory variables assessed. Thus, reversal bi-tDCS had no effects during motor learning on healthy subjects, but it could favor the retention of the motor skills acquired. These results do not support the cooperative inter-hemispheric model.
Brain Sci, 2021; 11
35076395: Velasco-Aviles S, Patel N, Casillas-Bajo A, Frutos-Rincón L, Velasco E, Gallar J, Arthur-Farraj P, Gomez-Sanchez JA, Cabedo H
A genetic compensatory mechanism regulated by and modulates the expression of distinct class IIa to ensure peripheral nerve myelination and repair.

The class IIa histone deacetylases (HDACs) have pivotal roles in the development of different tissues. Of this family, Schwann cells express , , and but not . Here, we show that a transcription factor regulated genetic compensatory mechanism within this family of proteins, blocks negative regulators of myelination ensuring peripheral nerve developmental myelination and remyelination after injury. Thus, when and are knocked-out from Schwann cells in mice, a JUN-dependent mechanism induces the compensatory overexpression of permitting, although with a delay, the formation of the myelin sheath. When , , and are simultaneously removed, the myocyte-specific enhancer-factor d (MEF2D) binds to the promoter and induces the de novo expression of , and although several melanocytic lineage genes are misexpressed and Remak bundle structure is disrupted, myelination proceeds after a long delay. Thus, our data unveil a finely tuned compensatory mechanism within the class IIa family, coordinated by distinct transcription factors, that guarantees the ability of Schwann cells to myelinate during development and remyelinate after nerve injury.
Elife, 2022; 11

35174893: Velasco E, Delicado-Miralles M, Hellings PW, Gallar J, Van Gerven L, Talavera K
Epithelial and sensory mechanisms of nasal hyperreactivity.
"Nasal hyperreactivity" is a key feature in various phenotypes of upper airway diseases, whereby reactions of the nasal epithelium to diverse chemical and physical stimuli are exacerbated. In this review, we illustrate how nasal hyperreactivity can result from at least three types of mechanisms: (1) impaired barrier function, (2) hypersensitivity to external and endogenous stimuli, and (3) potentiation of efferent systems. We describe the known molecular basis of hyperreactivity related to the functional impairment of epithelial cells and somatosensory innervation, and indicate that the thermal, chemical, and mechanical sensors determining hyperreactivity in humans remain to be identified. We delineate research directions that may provide new insights into nasal hyperreactivity associated with rhinitis/rhinosinusitis pathophysiology and therapeutics. The elucidation of the molecular mechanisms underlying nasal hyperreactivity is essential for the treatment of rhinitis according to the precepts of precision medicine.
Allergy, 2022; 77

35191131: Beltrá P, Ruiz-Del-Portal I, Ortega FJ, Valdesuso R, Delicado-Miralles M, Velasco E
Sensorimotor effects of plasticity-inducing percutaneous peripheral nerve stimulation protocols: a blinded, randomized clinical trial.
Electrical stimulation of skin afferents can induce somatosensory plasticity in humans. Nevertheless, it is unknown if this is possible to do through percutaneous stimulation of a peripheral nerve, which will allow for regional anaesthesia interventions. Furthermore, potentiation protocols applied over mainly non-nociceptive fibres inhibit nociception in rodents, but this has not been tested in humans.
Eur J Pain, 2022; 26

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TARGETING MU OPIOID RECEPTOR TO ALLEVIATE DRY EYE DISEASE-ASSOCIATED CHRONIC CORNEAL ALLODYNIA IN MICE

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

Adrian Guerrero-Moreno[1], Elodie Reboussin[1], Christophe Baudouin[1,2,3], Stéphane Melik Parsadaniantz[1], Annabelle Réaux-Le Goazigo[1]
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Aims: Chronic corneal pain associated with dry eye disease (DED) is very disabling and difficult to treat. Analgesic effects of topical mu-opioid receptor (MOR) agonist have been reported in mice developing acute inflammatory corneal pain. This work evaluates the use of these agonists in a model of chronic corneal allodynia in mice. **Methods:** DED-associated corneal allodynia was induced by unilateral excision of the harderian and extraorbital lacrimal glands of adult male mice. Controls were performed by sham surgery. Topical ocular instillation with the MOR-selective ligand DAMGO ([D-Ala^2,N-Me-Phe^4,Gly^5-ol] enkephalin, 50 µM) or phosphate-buffered saline were performed twice/day. Ocular discomfort, mechanical and chemical corneal sensitivities were quantified by aperture ratio, von Frey test and NaCl 2M and capsaicin 10 µM challenge test, respectively. Those behavioural tests were performed before and every week until 21 day (D21) post-surgery. At D21, mice were perfused and MOR expression was investigated on corneal nerves and trigeminal ganglion (TG) by immunohistochemistry and *in situ* hybridization (RNAScope). **Results:** We detected MOR immunoreactivity in subbasal corneal nerves. MOR RNA expression was significantly higher in the ophthalmic branch of the TG from DED-associated allodynia mice compared with sham animals. RNAscope experiments revealed a high co-expression between MOR and TRPV1$^+$ and TRPM8$^+$ in the trigeminal primary sensory neurons from both DED-associated allodynia and sham mice. Finally, we found that chronic topical DAMGO reduced significantly ocular discomfort, mechanical and chemical allodynia in DED mice compared with controls. **Conclusions:** MOR agonist may constitute a therapeutic target for the treatment of DED-associated chronic corneal allodynia.

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EVALUATION OF TACAN AS A NEW TARGET FOR TREATING OSTEOARTHRITIS PAIN

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

Lise Rabiller
McGill, Physiology, Montreal, Canada

Joint pain is the most prominent symptom of osteoarthritis (OA). Patients suffering from OA experience mechanical allodynia which is due in large part to a dysfunction in pain-sensing nerves (nociceptors). We demonstrated that during OA, mechanosensitive ion channels (MSIC) become sensitized and are important contributors of the mechanical allodynia. The central component of this apparatus is a high-threshold mechanosensitive ion channel that converts high-intensity mechanical forces into electrical signals. We showed that blocking MSICs can be analgesic in a preclinical model of OA pain. However, the molecular identity of these channels remains unknown and prevents progress toward improved OA pain management. Our group recently identified an ion channel expressed in mouse nociceptors called TACAN, essential to the sensation of mechanical pain. In this project, we hypothesize that TACAN contributes to OA pain. We used behavioural tests for assessing mechanical allodynia and pain during OA in mice and histological analyses of the knee joint. We generated a mouse in which TACAN deletion can be induced in nociceptors specifically by tamoxifen injection. Our experiment indicate that TACAN plays an important role in the mechanical allodynia observed in OA pain. Further investigation is being conducted at the cellular and molecular levels in mice deleted for TACAN to obtain a mechanistic readout of these effects.

Pubmed:
34650070: Rabiller L, Labit E, Guissard C, Gilardi S, Guiard BP, Moulédous L, Silva M, Mithieux G, Pénicaud L, Lorsignol A, Casteilla L, Dromard C
Pain sensing neurons promote tissue regeneration in adult mice.
Tissue repair after injury in adult mammals, usually results in scarring and loss of function in contrast to lower vertebrates such as the newt and zebrafish that regenerate. Understanding the regulatory processes that guide the outcome of tissue repair is therefore a concerning challenge for regenerative medicine. In multiple regenerative animal species, the nerve dependence of regeneration is well established, but the nature of the innervation required for tissue regeneration remains largely undefined. Using our model of induced adipose tissue regeneration in adult mice, we demonstrate here that nociceptive nerves promote regeneration and their removal impairs tissue regeneration. We also show that blocking the receptor for the nociceptive neuropeptide calcitonin gene-related peptide (CGRP) inhibits regeneration, whereas CGRP administration induces regeneration. These findings reveal that peptidergic nociceptive neurons are required for adult mice tissue regeneration.
NPJ Regen Med, 2021; 6
34344890: Rabiller L, Robert V, Arlat A, Labit E, Ousset M, Salon M, Coste A, Da Costa-Fernandes L, Monsarrat P, Ségui B, André M, Guissard C, Renoud ML, Silva M, Mithieux G, Raymond-Letron I, Pénicaud L, Lorsignol A, Casteilla L, Dromard Berthézène C, Cousin B
Driving regeneration, instead of healing, in adult mammals: the decisive role of resident macrophages through efferocytosis.
Tissue repair after lesion usually leads to scar healing and thus loss of function in adult mammals. In contrast, other adult vertebrates such as amphibians have the ability to regenerate and restore tissue homeostasis after lesion. Understanding the control of the repair outcome is thus a concerning challenge for regenerative medicine. We recently developed a model of induced tissue regeneration in adult mice allowing the comparison of the early steps of regenerative and scar healing processes. By using studies of gain and loss of function, specific cell depletion approaches, and hematopoietic chimeras we demonstrate here that tissue regeneration in adult mammals depends on an early and transient peak of granulocyte producing reactive oxygen species and an efficient efferocytosis specifically by tissue-resident macrophages. These findings highlight key and early cellular pathways able to drive tissue repair towards regeneration in adult mammals.
NPJ Regen Med, 2021; 6
34298954: Berthézène CD, Rabiller L, Jourdan G, Cousin B, Pénicaud L, Casteilla L, Lorsignol A
Tissue Regeneration: The Dark Side of Opioids.
Opioids are regarded as among the most effective analgesic drugs and their use for the management of pain is considered

standard of care. Despite their systematic administration in the peri-operative period, their impact on tissue repair has been studied mainly in the context of scar healing and is only beginning to be documented in the context of true tissue regeneration. Indeed, in mammals, growing evidence shows that opioids direct tissue repair towards scar healing, with a loss of tissue function, instead of the regenerative process that allows for recovery of both the morphology and function of tissue. Here, we review recent studies that highlight how opioids may prevent a regenerative process by silencing nociceptive nerve activity and a powerful anti-inflammatory effect. These data open up new perspectives for inducing tissue regeneration and argue for opioid-restricted strategies for managing pain associated with tissue injury.
Int J Mol Sci, 2021; 22

30111876: Labit E, Rabiller L, Rampon C, Guissard C, André M, Barreau C, Cousin B, Carrière A, Eddine MA, Pipy B, Pénicaud L, Lorsignol A, Vriz S, Dromard C, Casteilla L
Opioids prevent regeneration in adult mammals through inhibition of ROS production.
Inhibition of regeneration and induction of tissue fibrosis are classic outcomes of tissue repair in adult mammals. Here, using a newly developed model of regeneration in adult mammals i.e. regeneration after massive resection of an inguinal fat pad, we demonstrate that both endogenous and exogenous opioids prevent tissue regeneration in adults, by inhibiting the early production of reactive oxygen species (ROS) that generally occurs after lesion and is required for regeneration. These effects can be overcome and regeneration induced by the use of an opioid antagonist. The results obtained in both our new model and the gold standard adult zebrafish demonstrate that this mechanism can be considered as a general paradigm in vertebrates. This work clearly demonstrates that ROS is required for tissue regeneration in adult mammals and shows the deleterious effect of opioids on tissue regeneration through the control of this ROS production. It thus raises questions about opioid-based analgesia in perioperative care.
Sci Rep, 2018; 8

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ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE ANTI-INFLAMMATORY BENZYDAMINE COMPOUND IN RAT DRG NEURONS

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

Matteo Vergassola[1], Magdalena Nikolaeva-Koleva[2], Ana Espinoza[2], Giorgina Mangano[3], Lorenzo Polenzani[4], Antonio Ferrer-Montiel[5], Sara Zucchi[1], Isabel Devesa[2], Lorella Ragni[1]
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Benzydamine is a well-known anti-inflammatory active principle with an associated and recognized local anaesthetic/analgesic activity. It is widely used in oral affections due to its high efficacy and robust safety profile by local application. Although, several studies showed the multi target action of benzydamine on inflammatory system, its role as neuronal excitability modulator has been less explored. The aim of this project is to evaluate the capacity of benzydamine to decrease neuronal activity in an inflammatory sensitized DRG model by electrophysiological approach, supporting the local pain signal suppression action of the compound. Neurons isolated from dorsal root ganglia of Wistar rats were incubated for 24-hours with or without a specific inflammatory cocktail. The action potential firing was recorded by Multi Electrode Assay after acute exposure to the same inflammatory cocktail, acid pH, or potassium chloride. A dose-related inhibition of neuronal excitability by benzydamine triggered by either the inflammatory cocktail or the acidic pH was shown in sensitized nociceptors and in basal conditions in MEA recording. Notably, a higher potency over basal conditions, was shown under inflammatory sensitized situation, with a significant shift of the dose response curve of benzydamine. To confirm these results, electrophysiological studies based on single cells assay are currently performed on DRG neurons in the same experimental conditions and with longer time of exposure to benzydamine. The results obtained should add insights on the molecular mechanism of benzydamine with regards to the inhibition of neuronal excitability, suggesting complementary effects with the well-known anti-inflammatory activity.

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IDENTIFYING THE MOLECULAR MECHANISM FOR THE PAIN CAUSED BY LIONFISH VENOM

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

Stephanie Mouchbahani-Constance[1,2], Claire Saito[3,4], Shigeru Saito[3,4,5], Makoto Tominaga[3,6,7], Reza Sharif-Naeini[1,2]
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The lionfish (*Pterois volitans*) is a venomous species endemic to the Indo-Pacific, but that has now invaded the Northwestern Atlantic as well as the Caribbean and Mediterranean Seas. It poses a growing health problem due to the increase in frequency of painful stings that it delivers, for which no treatment or antidote exists. A prior collaborative study by our group characterized the pain and inflammation caused by the lionfish venom and provided the first insight into the venom's cellular target in the peripheral nervous system – nonpeptidergic nociceptors. The aim of this research project is to isolate the algogenic toxin in the venom and identify its receptor target to understand the molecular mechanism for the pain caused by lionfish venom. The toxin was isolated using high pressure liquid chromatography and mass spectrometry in order to identify its sequence. Preliminary results point to the venom's receptor target, which has been verified using calcium imaging, behaviour and patch-clamp electrophysiology experiments. We have cloned the venom's target from *Gymnothorax favagineus* (Honeycomb moray eel), a predator of the lionfish, as well as from the lionfish itself to evaluate these targets' sensitivity to lionfish venom using calcium imaging and electrophysiology. These results will 1) identify novel modulators of the pain pathway and 2) provide insights into the parallel evolution of the algogenic toxin in lionfish venom and its target in predators.

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PERIPHERAL VOLTAGE-GATED CALCIUM CHANNELS IN SKIN ARE ESSENTIAL FOR TRANSIENT HEAT HYPERSENSITIVITY IN MICE

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

E. Javier Lopez Soto[1], Daniel Dubreuil[2], Simon Daste[3], Remy Meir[3], Alexander Fleischmann[3], Diane Lipscombe[3]
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$Ca_V2.2$ voltage-gated calcium channels are the dominant Ca_V channel expressed in nociceptors. $Ca_V2.2$ channel inhibitors applied to the spinal cord relieve pain in humans and rodents, especially during pathological pain. $Ca_V2.2$ channels are expressed at nociceptor pre-synaptic terminals, soma, and peripheral axons, but a biological function of nociceptor $Ca_V2.2$ channels outside pre-synaptic terminals is rarely considered. Here, we demonstrate that $Ca_V2.2$ channels at nociceptors free nerve endings in skin are required for heat hypersensitivity. We used Hargreaves and automatic von Frey assays to evaluate heat and mechanical sensitivities in wild-type and in a new $Ca_V2.2$-null mice. We assessed the role of peripheral $Ca_V2.2$ channels in skin, in particular in capsaicin-induced hypersensitivity. Intraplantar injection of capsaicin, a TRPV1 channels agonist, induces transient hypersensitivity to mechanical and heat stimuli at 15 mins with returning to baseline responses within 30 mins. Capsaicin-induced heat, but not mechanical, hypersensitivity was 60% reduced in $Ca_V2.2$-null as compared to wild-type mice. To assess the role of peripheral $Ca_V2.2$ channels in skin we used intraplantar ω-conotoxin MVIIA. In wild-type mice, capsaicin-induced heat hypersensitivity was consistently reduced by co-injecting conotoxin with capsaicin, compared to capsaicin alone. We then directly assessed the contribution of $Ca_V2.2$ gating to the Ca^{2+} signal triggered by capsaicin in the skin by 2-photon microscopy live imaging. We found that capsaicin-induced Ca^{2+} signal in nociceptor projections was reduced ~60% by conotoxin. Our data reveal that local activation of peripheral $Ca_V2.2$ channels in nociceptor termini in skin *in vivo* are required for capsaicin-induced heat hypersensitivity, but not mechanical hypersensitivity.

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BEHAVIORAL RESPONSES EVOKED BY OPTOGENETIC ACTIVATION OF CACNA1H-EXPRESSING LOW THRESHOLD MECHANORECEPTORS IN MICE

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Voltage-gated calcium (Ca_V) channels play a critical role in transmitting information about various sensory modalities including thermal and mechanical stimuli. We recently discovered that $Ca_V2.2$ channels (*Cacna1b*) in peripheral nerve endings of *Trpv1* nociceptors are critical for capsaicin-induced transient hypersensitivity to heat but not mechanical stimuli (DuBreuil et al., 2021). Here we explore the role of $Ca_V3.2$ channels (*Cacna1h*), which are expressed in C and Aδ low threshold mechanoreceptors (LTMRs), in regulating mechanosensitivity. $Ca_V3.2$ channels are implicated in regulating mechanosensitivity thresholds in a pro-excitatory manner, but their precise mechanism of action is not fully elucidated. We generated a *Cacna1h*$^{Cre\ +/+}$ knock-in mouse model to express Cre-dependent reporters, including ChannelRhodopsin (ChR2), in C and Aδ LTMRs. LED light (465 nm) directed at glabrous skin of *Cacna1h*$^{ChR2\text{-}YFP+/-}$ hindpaws triggered robust, intensity dependent plantar paw withdrawal responses, consistent with tactile-free activation of Aδ LTMRs. Intraplantar capsaicin induced robust mechanical hypersensitivity within 15 mins but, interestingly, the sensitivity of optogenetically evoked responses were unaltered. Intraplantar Complete Freund's Adjuvant (CFA) triggers longer lasting hypersensitivity to tactile stimuli within 1 day of treatment and, in contrast to capsaicin, this model exhibited 8.6-fold greater paw withdrawal sensitivity to LED stimulation (75 mA) ipsilateral compared to contralateral. Our findings show that optogenetic activation of *Cacna1h*-expressing Aδ LTMRs can model CFA-enhanced behavioral responses, but not capsaicin-induced transient mechanical hypersensitivity.

Pubmed:
34353899: DuBreuil DM, Lopez Soto EJ, Daste S, Meir R, Li D, Wainger B, Fleischmann A, Lipscombe D
Heat But Not Mechanical Hypersensitivity Depends on Voltage-Gated Ca2.2 Calcium Channel Activity in Peripheral Axon Terminals Innervating Skin.
Voltage-gated Ca2.2 calcium channels are expressed in nociceptors at presynaptic terminals, soma, and axons. Ca2.2 channel inhibitors applied to the spinal cord relieve pain in humans and rodents, especially during pathologic pain, but a biological function of nociceptor Ca2.2 channels in processing of nociception, outside presynaptic terminals in the spinal cord, is underappreciated. Here, we demonstrate that functional Ca2.2 channels in peripheral axons innervating skin are required for capsaicin-induced heat hypersensitivity in male and female mice. We show that Ca2.2 channels in TRPV1-nociceptor endings are activated by capsaicin-induced depolarization and contribute to increased intracellular calcium. Capsaicin induces hypersensitivity of both thermal nociceptors and mechanoreceptors, but only heat hypersensitivity depends on peripheral Ca2.2 channel activity, and especially a cell-type-specific Ca2.2 splice isoform. Ca2.2 channels at peripheral nerve endings might be important therapeutic targets to mitigate certain forms of chronic pain. It is generally assumed that nociceptor termini in the spinal cord dorsal horn are the functionally significant sites of Ca2.2 channel in control of transmitter release and the transmission of sensory information from the periphery to central sites. We show that peripheral Ca2.2 channels are essential for the classic heat hypersensitivity response to develop in skin following capsaicin exposure. This function of Ca2.2 is highly selective for heat, but not mechanical hypersensitivity induced by capsaicin exposure, and is not a property of closely related Ca2.1 channels. Our findings suggest that interrupting Ca2.2-dependent calcium entry in skin might reduce heat hypersensitivity that develops after noxious heat exposure and may limit the degree of heat hypersensitivity associated with certain other forms of pain.
J Neurosci, 2021; 41
33204899: Totsch SK, Kemp KM, Lopez SA, Quinn TL, Meir RY, Gower BA, Sorge RE
The sad weekend: A perilous North American tradition.
Obesity is a global concern and affects millions of Americans who consume poor-quality diets. Diets directly affect the gut microbiota, which can have subsequent effects on inflammation and contribute to other chronic states. Previously we have

shown that a Standard American Diet (SAD) increased immune cell activation and prolonged recovery and that a beneficial diet could reduce these negative effects. Here, male and female mice were given access to regular chow (REG), SAD, our Anti-Inflammatory Diet (AID) or a combination of SAD and AID. This latter group was modeled on the commonplace dietary pattern of healthy eating during the week (AID: Monday-Friday) and relaxed eating patterns on the weekend (SAD: Saturday-Sunday). After 14 weeks of diet consumption and an inflammatory injury, we found that the SAD prolonged and the AID promoted recovery. However, recovery was significantly delayed in those mice consuming the AID-SAD, regardless of weekly healthy diet access. In addition, fecal samples taken during the study revealed dramatic differences in microbial community composition, relative abundance of abundant bacterial phyla and alpha diversity. These data confirm the impact of diet on gut microbiota and suggest a relation between abundance of specific bacterial taxa and susceptibility to prolonged recovery from injury.
Neurobiol Pain, 2020 Aug-Dec; 8
32789515: Trøstheim M, Eikemo M, Meir R, Hansen I, Paul E, Kroll SL, Garland EL, Leknes S
Assessment of Anhedonia in Adults With and Without Mental Illness: A Systematic Review and Meta-analysis.
Anhedonia, a reduced capacity for pleasure, is described for many psychiatric and neurologic conditions. However, a decade after the Research Domain Criteria launch, whether anhedonia severity differs between diagnoses is still unclear. Reference values for hedonic capacity in healthy humans are also needed.
JAMA Netw Open, 2020; 3
30189182: Totsch SK, Meir RY, Orlandella RM, Norian LA, Sorge RE
Effects of diet on immune cells within the central nervous system.
Physiol Behav, 2018; 196
29436058: Totsch SK, Meir RY, Quinn TL, Lopez SA, Gower BA, Sorge RE
Effects of a Standard American Diet and an anti-inflammatory diet in male and female mice.
Obesity and chronic pain are prevalent concerns. Pain is frequently experienced in weight-bearing joints, but is common in other areas of the body as well, suggesting other factors. Poor diet often contributes to obesity and can directly influence the immune system. We have shown that poor diet prolongs recovery from inflammatory injury. Therefore, our goal was to determine whether poor-quality diet-induced consequences could be prevented or reversed by an anti-inflammatory diet (AID).
Eur J Pain, 2018; 22

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BOARD NUMBER: S01-612

PKA-II ACTIVATION IN NOCICEPTIVE NEURONS BY CAPSAICIN AND AITC BUT NOT BY IONOMYCIN

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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We identified KCl- or Veratridine-depolarization to activate PKA-II in nociceptive neurons. This was calcium and L-type voltage-gated-calcium-channel Cav1.2-dependent and resulted in a feed forward manner in sensitization of Cav1.2 and prolonged mechanical hyperalgesia. If calcium entry also through ligand gated ion channels such as TRPV1 and TRA1 results in PKA-II activation and if this is negatively regulated by the phosphatase calcineurin (CaN) is not known. Using High Content Screening (HCS) microscopy, we found that also the TRPV1 agonist, capsaicin, and the TRPA1 agonist, allyl isothiocyanate (AITC), induced PKA-II activity, whereas the calcium ionophore ionomycin was ineffective. CaN inhibitors increased PKA-II activity and phosphorylation of downstream targets upon capsaicin and KCl depolarization, but not upon AITC or ionomycin treatment. In particular, CaN inhibitors increased the phosphorylation of CaV1.2 upon KCl and capsaicin, but not upon AITC or ionomycin treatment. Our findings demonstrates that also ligand gated ion channels initiate the novel process of nociceptor-depolarization-induced PKA-II activation. Interestingly, while converging onto the same kinase, the regulatory mechanisms were strictly stimulus-specific.

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BOARD NUMBER: S01-613

EFFECTS OF PROSTACYCLIN RECEPTOR ACTIVATION ON TRPM8 ACTIVITY

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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TRPM8 (*Transient Receptor Potential Melastatin member 8*) is an ionic channel activated both by cool temperatures, below 25°C, and by an array of natural and synthetic agonists, endogenous compounds and intracellular acidic pH values. This channel plays a role in thermoregulation, cold analgesia and, paradoxically, in cold hypersensitivity and cold pain. Although modulation of TRPM8 by inflammatory mediators is not yet completely understood, it has been shown that mediators which activate $G\alpha_q$-coupled receptors, potently inhibit TRPM8 activation. This inhibition is reported to happen independently of PLCβ (*Phospholipase C beta*) activation, via direct interaction between the activated $G\alpha_q$ and the ionic channel. The prostacyclin receptor IP is a GPCR (*G-protein coupled receptor*), preferentially activated by prostaglandin I_2 (PGI_2), involved in acute inflammation, inflammatory pain and a series of severe cardiovascular and pulmonary diseases. The IP receptor couples both with $G\alpha_s$, in platelets and smooth muscle cells, and $G\alpha_q$, in DRG (*Dorsal Root Ganglia*). Our research aim was to investigate the signaling pathways involved in the modulation of the TRPM8 channel by a series of selective IP receptor agonists. For this purpose, we co-transfected HEK 293T cells with TRPM8 and the IP receptor and treated them with cicaprost and iloprost, two synthetic analogs of prostacyclin. Then, using non-ratiometric calcium microfluorimetry, we monitored how the activation of the IP receptor affects the activation of TRPM8. Our results demonstrate that the signaling pathway triggered by the IP receptor depends on the specific agonist and this effect leads to differential modulation of TRPM8.

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BOARD NUMBER: S01-614

MU-OPIOID RECEPTOR DESENSITIZATION IN THE SPINAL CORD DORSAL HORN IS REDUCED BY THE ENDOGENOUS TRPV1 AGONIST N-OLEOYLDOPAMINE.

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Opioid receptors are expressed throughout the afferent pain pathway, including central terminals of primary afferent neurons in the spinal cord dorsal horn (SCDH), where opioids exert inhibitory control of the nociceptive transmission. At these presynaptic endings opioid receptors co-express at the first nociceptive synapse with the transient receptor potential vanilloid type 1 (TRPV1) channel, known for its role in the development of thermal and mechanical hyperalgesia during pathological states. Our study focused on the interaction of TRPV1 and μ-opioid receptor (MOR) in agonist-induced desensitization of MOR. We used whole-cell patch-clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) from SCDH neurons in rat spinal cord slices. Short application of MOR agonist DAMGO (1 μM, 3 min) depressed mEPSC frequency to 62.3 ± 3.6 %. To induce robust MOR desensitization, we incubated slices with 1 μM DAMGO for 2 hours; acute application of DAMGO after the incubation failed to depress the mEPSC frequency. Addition of endogenous TRPV1 agonist N-oleoyldopamine (OLDA) to DAMGO during incubation prevented the MOR desensitization. Short DAMGO application in slices incubated with DAMGO+OLDA evoked a decrease of mEPSC frequency to 66.94 ± 9.6 %. In slices from animals with chronic constriction injury (CCI) model of neuropathic pain, acute DAMGO application induced only reduced inhibition compared to the controls (74.71 ± 11 %). Our data reveal how endogenous TRPV1-mediated pathways may interact with MOR function, reduce MOR desensitization and thus promote the efficacy of opioids. Further study of the underlying mechanisms could contribute to improved opioid-mediated analgesia. Supported by GACR20-19136S.

Pubmed:

34857006: Heles M, Mrozkova P, Sulcova D, Adamek P, Spicarova D, Palecek J
Chemokine CCL2 prevents opioid-induced inhibition of nociceptive synaptic transmission in spinal cord dorsal horn.
Opioid analgesics remain widely used for pain treatment despite the related serious side effects. Some of those, such as opioid tolerance and opioid-induced hyperalgesia may be at least partially due to modulation of opioid receptors (OR) function at nociceptive synapses in the spinal cord dorsal horn. It was suggested that increased release of different chemokines under pathological conditions may play a role in this process. The goal of this study was to investigate the crosstalk between the μOR, transient receptor potential vanilloid 1 (TRPV1) receptor and C-C motif ligand 2 (CCL2) chemokine and the involvement of spinal microglia in the modulation of opioid analgesia.
J Neuroinflammation, 2021; 18
35042769: Adamek P, Heles M, Bhattacharyya A, Pontearso M, Slepicka J, Palecek J
Dual PI3Kδ/γ Inhibitor Duvelisib Prevents Development of Neuropathic Pain in Model of Paclitaxel-Induced Peripheral Neuropathy.
The development of painful paclitaxel-induced peripheral neuropathy (PIPN) represents a major dose-limiting side effect of paclitaxel chemotherapy. Here we report a promising effect of duvelisib (Copiktra), a novel FDA-approved PI3Kδ/γ isoform-specific inhibitor, in preventing paclitaxel-induced pain-like behavior and pronociceptive signaling in DRGs and spinal cord dorsal horn (SCDH) in rat and mouse model of PIPN. Duvelisib blocked the development of mechanical hyperalgesia in both males and females. Moreover, duvelisib prevented paclitaxel-induced sensitization of TRPV1 receptors, and increased PI3K/Akt signaling in small-diameter DRG neurons and an increase of CD68 cells within DRGs. Specific optogenetic stimulation of inhibitory neurons combined with patch-clamp recording revealed that duvelisib inhibited paclitaxel-induced weakening of inhibitory, mainly glycinergic control on SCDH excitatory neurons. Enhanced excitatory and reduced inhibitory neurotransmission in the SCDH following PIPN was also alleviated by duvelisib application. In summary, duvelisib showed a promising ability to prevent neuropathic pain in PIPN. The potential use of our findings in human medicine may be augmented by the fact that duvelisib is an FDA-approved drug with known side effects. We show that duvelisib, a novel FDA-approved PI3Kδ/γ isoform-specific inhibitor, prevents the development of paclitaxel-induced pain-like behavior in males and females and prevents pronociceptive signaling in DRGs and spinal cord dorsal horn in rat and mouse model of paclitaxel-

induced peripheral neuropathy.
J Neurosci, 2022; 42
30471295: Adamek P, Heles M, Palecek J
Mechanical allodynia and enhanced responses to capsaicin are mediated by PI3K in a paclitaxel model of peripheral neuropathy.
Paclitaxel chemotherapy treatment often leads to neuropathic pain resistant to available analgesic treatments. Recently spinal Toll-like receptor 4 (TLR4) and the transient receptor potential cation channel subfamily V member 1 (TRPV1) were identified to be involved in the pro-nociceptive effect of paclitaxel. The aim of this study was to investigate the role of phosphatidylinositol 3-kinase (PI3K) and serine/threonine kinases in this process, with the use of their antagonists (wortmannin, LY-294002, and staurosporine). The single paclitaxel administration (8 mg/kg i.p.) in mice induced robust mechanical allodynia measured as a reduced threshold to von Frey filament stimulation and generated reduced tachyphylaxis of capsaicin-evoked responses, recorded as changes in mEPSC frequency in patch-clamp recordings of dorsal horn neurons activity in vitro, for up to eight days. Paclitaxel application also induced increased Akt kinase phosphorylation in rat DRG neurons. All these paclitaxel-induced changes were prevented by the wortmannin in vivo pretreatment. Acute co-application of wortmannin or LY-294002 with paclitaxel in spinal cord slices also attenuated the paclitaxel effect on capsaicin-evoked responses. Staurosporine was effective in the acute in vitro experiments and on the first day after the paclitaxel treatment in vivo, but in contrast to wortmannin, it did not have a significant impact later. Our data suggest that the inhibition of PI3K signaling may help alleviate pathological pain syndromes in the paclitaxel-induced neuropathy.
Neuropharmacology, 2019; 146

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BOARD NUMBER: S01-615

ROLE OF DOPAMINE D4 RECEPTOR IN THE DEVELOPMENT OF MORPHINE-INDUCED ANALGESIC TOLERANCE

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Morphine is one of the most effective drugs used for pain management. However, prolonged exposition to morphine produces effects such as tolerance to analgesia and addiction. Downregulation of the mu opioid receptor (MOR) and its uncoupling to G-proteins in the dorsal horn are likely to contribute to the development of morphine tolerance. Previous studies demonstrated that dopamine D_4 receptor (D_4R) activation prevents morphine addiction by modulating dopamine signaling from nigral dopamine cells. This effect seems to be the result of an antagonistic receptor-receptor interaction involving a D_4R-MOR heteroreceptor which could exist in the dorsal striatum. As D_4R is expressed in dorsal horn neurons, we hypothesize that D_4R could interfere in the development of morphine-induced tolerance to its analgesic effects. Here, using a chronic paradigms of combined treatment of morphine with the D_4R agonist PD168.077, we investigated the nociceptive response to three noxious stimuli: thermal (tail-flick test), mechanical (von Frey test) and chemical (formalin test). Moreover, using immunohistochemical techniques, we have evaluated alterations in the primary circuitry of pain (peptidergic and non-peptidergic C fibers and spinal projections neurons NK1-R) and the balance between glutamate and GABA within dorsal horn. Results from the evaluation of analgesic activity of chronic combined treatment of morphine with PD168,077 showed that D_4R prevents the development of morphine-induced analgesic tolerance. This results give support for the existence of antagonistic functional D_4R-MOR interaction in the dorsal horn that could help to the development of a new pharmacology strategy for treatment of pain. Support: CTS161 and UMA20-FEDERJA-122 (Junta de Andalucía, Spain)

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-616

ORGANIC ANION TRANSPORTER 1 IS AN HDAC4-REGULATED MEDIATOR OF PERSISTENT INFLAMMATORY PAIN

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

Christian Litke[1], Anna Hagenston[1], Ann-Kristin Kenkel[1], Eszter Paldy[2], Jianning Lu[2], Rohini Kuner[2], Daniela Mauceri[1]
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Persistent pain is sustained by maladaptive changes in gene transcription resulting in altered function of the relevant circuits; therapies are still unsatisfactory. The epigenetic mechanisms and affected genes linking nociceptive activity to transcriptional changes and pathological sensitivity are unclear. Here, we found that, among several histone deacetylases (HDACs), synaptic activity specifically affects HDAC4 in spinal cord dorsal horn neurons: stimuli that induce long-lasting inflammatory pain cause nuclear export and inactivation of HDAC4. The development of inflammation-associated mechanical hypersensitivity, but neither acute nor basal sensitivity, is impaired by the expression of a constitutively nuclear localized HDAC4 mutant. Next generation RNA-sequencing revealed an inflammatory pain-dependent HDAC4-regulated gene program comprising known and novel mediators of sensitization including the organic anion transporter OAT1, known for its renal transport function. Using pharmacological and molecular tools to modulate OAT1 activity or expression, we causally link OAT1 to persistent inflammatory pain. Thus, HDAC4 is a key epigenetic regulator of pain that translates nociceptive activity into sensitization by regulating OAT1, a novel and appealing target for pain-relieving therapies.

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BOARD NUMBER: S01-617

LOCKED NUCLEIC ACID ANTISENSE OLIGONUCLEOTIDES: A NEW GENETIC TOOL TO KNOCKDOWN THE GENE ENCODING NAV1.7 IN HUMAN INDUCED-PLURIPOTENT STEM CELL-DERIVED PERIPHERAL SENSORY NEURONS

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

Kim Le Cann[1], Yi Liu[1], Frederic Knoflach[2], Marius Hoener[2], Angelika Lampert[1]
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Aims
Chronic pain affects millions of people worldwide. The voltage-gated sodium channel (Nav) 1.7 is predominantly expressed in nociceptors and plays a key role in pain signaling. Nav1.7 gain-of-function mutations lead to chronic pain. Here, we aimed to knockdown the *SCN9a* gene encoding Nav1.7 in human induced-pluripotent stem (hiPS) cell-derived peripheral sensory neurons with the aim to decrease their excitability *in vitro*. Methods
The Chambers differentiation protocol was used to generate peripheral sensory neurons from two hiPS cell lines: a control cell line and a cell line carrying the Nav1.7/I848T mutation responsible for inherited erythromelalgia (IEM). The neurons were treated with locked nucleic acid antisense oligonucleotides (LNA ASO) targeting *SCN9a* or scrambled LNA ASO. The *SCN9a* mRNA expression and the excitability of the neurons were investigated through RT-qPCR and whole-cell patch-clamp experiments at DIV52-58. Results
Although mRNA of *SCN9a* was significantly down-regulated in both control- and IEM-neurons, we did not detect changes in Nav gating properties nor action potential (AP) properties of the control neurons. However, IEM-neurons treated with *SCN9a*–specific LNA ASO displayed a more depolarized resting membrane potential than the scrambled LNA ASO-treated IEM-neurons. They had a smaller AP amplitude and they generated less APs following high current injections. Conclusion
The *SCN9a*–specific LNA ASO treatment did not alter the excitability of the control neurons but decreased firing of the IEM-neurons. These results suggest a more specific role of Nav1.7 downregulation in neurons carrying a Nav1.7 mutation, which is currently subject of further studies.

Pubmed:
33767215: Le Cann K, Foerster A, Rösseler C, Erickson A, Hautvast P, Giesselmann S, Pensold D, Kurth I, Rothermel M, Mattis VB, Zimmer-Bensch G, von Hörsten S, Denecke B, Clarner T, Meents J, Lampert A
The difficulty to model Huntington's disease in vitro using striatal medium spiny neurons differentiated from human induced pluripotent stem cells.
Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded polyglutamine repeat in the huntingtin gene. The neuropathology of HD is characterized by the decline of a specific neuronal population within the brain, the striatal medium spiny neurons (MSNs). The origins of this extreme vulnerability remain unknown. Human induced pluripotent stem cell (hiPS cell)-derived MSNs represent a powerful tool to study this genetic disease. However, the differentiation protocols published so far show a high heterogeneity of neuronal populations in vitro. Here, we compared two previously published protocols to obtain hiPS cell-derived striatal neurons from both healthy donors and HD patients. Patch-clamp experiments, immunostaining and RT-qPCR were performed to characterize the neurons in culture. While the neurons were mature enough to fire action potentials, a majority failed to express markers typical for MSNs. Voltage-clamp experiments on voltage-gated sodium (Nav) channels revealed a large variability between the two differentiation protocols. Action potential analysis did not reveal changes induced by the HD mutation. This study attempts to demonstrate the current challenges in reproducing data of previously published differentiation protocols and in generating hiPS cell-derived striatal MSNs to model a genetic neurodegenerative disorder in vitro.
Sci Rep, 2021; 11
33487118: Le Cann K, Meents JE, Sudha Bhagavath Eswaran V, Dohrn MF, Bott R, Maier A, Bialer M, Hautvast P, Erickson A, Rolke R, Rothermel M, Körner J, Kurth I, Lampert A
Assessing the impact of pain-linked Nav1.7 variants: An example of two variants with no biophysical effect.

Mutations in the voltage-gated sodium channel Nav1.7 are linked to human pain. The Nav1.7/N1245S variant was described before in several patients suffering from primary erythromelalgia and/or olfactory hypersensitivity. We have identified this variant in a pain patient and a patient suffering from severe and life-threatening orthostatic hypotension. In addition, we report a female patient suffering from muscle pain and carrying the Nav1.7/E1139K variant. We tested both Nav1.7 variants by whole-cell voltage-clamp recordings in HEK293 cells, revealing a slightly enhanced current density for the N1245S variant when co-expressed with the β1 subunit. This effect was counteracted by an enhanced slow inactivation. Both variants showed similar voltage dependence of activation and steady-state fast inactivation, as well as kinetics of fast inactivation, deactivation, and use-dependency compared to WT Nav1.7. Finally, homology modeling revealed that the N1245S substitution results in different intramolecular interaction partners. Taken together, these experiments do not point to a clear pathogenic effect of either the N1245S or E1139K variant and suggest they may not be solely responsible for the patients' pain symptoms. As discussed previously for other variants, investigations in heterologous expression systems may not sufficiently mimic the pathophysiological situation in pain patients, and single nucleotide variants in other genes or modulatory proteins are necessary for these specific variants to show their effect. Our findings stress that biophysical investigations of ion channel mutations need to be evaluated with care and should preferably be supplemented with studies investigating the mutations in their context, ideally in human sensory neurons.
Channels (Austin), 2021; 15

30720580: Meents JE, Bressan E, Sontag S, Foerster A, Hautvast P, Rösseler C, Hampl M, Schüler H, Goetzke R, Le TKC, Kleggetveit IP, Le Cann K, Kerth C, Rush AM, Rogers M, Kohl Z, Schmelz M, Wagner W, Jørum E, Namer B, Winner B, Zenke M, Lampert A
The role of Nav1.7 in human nociceptors: insights from human induced pluripotent stem cell-derived sensory neurons of erythromelalgia patients.
The chronic pain syndrome inherited erythromelalgia (IEM) is attributed to mutations in the voltage-gated sodium channel (NaV) 1.7. Still, recent studies targeting NaV1.7 in clinical trials have provided conflicting results. Here, we differentiated induced pluripotent stem cells from IEM patients with the NaV1.7/I848T mutation into sensory nociceptors. Action potentials in these IEM nociceptors displayed a decreased firing threshold, an enhanced upstroke, and afterhyperpolarization, all of which may explain the increased pain experienced by patients. Subsequently, we investigated the voltage dependence of the tetrodotoxin-sensitive NaV activation in these human sensory neurons using a specific prepulse voltage protocol. The IEM mutation induced a hyperpolarizing shift of NaV activation, which leads to activation of NaV1.7 at more negative potentials. Our results indicate that NaV1.7 is not active during subthreshold depolarizations, but that its activity defines the action potential threshold and contributes significantly to the action potential upstroke. Thus, our model system with induced pluripotent stem cell-derived sensory neurons provides a new rationale for NaV1.7 function and promises to be valuable as a translational tool to profile and develop more efficacious clinical analgesics.
Pain, 2019; 160

29253101: Habib AM, Matsuyama A, Okorokov AL, Santana-Varela S, Bras JT, Aloisi AM, Emery EC, Bogdanov YD, Follenfant M, Gossage SJ, Gras M, Humphrey J, Kolesnikov A, Le Cann K, Li S, Minett MS, Pereira V, Ponsolles C, Sikandar S, Torres JM, Yamaoka K, Zhao J, Komine Y, Yamamori T, Maniatis N, Panov KI, Houlden H, Ramirez JD, Bennett DLH, Marsili L, Bachiocco V, Wood JN, Cox JJ
A novel human pain insensitivity disorder caused by a point mutation in ZFHX2.
Chronic pain is a major global public health issue causing a severe impact on both the quality of life for sufferers and the wider economy. Despite the significant clinical burden, little progress has been made in terms of therapeutic development. A unique approach to identifying new human-validated analgesic drug targets is to study rare families with inherited pain insensitivity. Here we have analysed an otherwise normal family where six affected individuals display a pain insensitive phenotype that is characterized by hyposensitivity to noxious heat and painless bone fractures. This autosomal dominant disorder is found in three generations and is not associated with a peripheral neuropathy. A novel point mutation in ZFHX2, encoding a putative transcription factor expressed in small diameter sensory neurons, was identified by whole exome sequencing that segregates with the pain insensitivity. The mutation is predicted to change an evolutionarily highly conserved arginine residue 1913 to a lysine within a homeodomain. Bacterial artificial chromosome (BAC) transgenic mice bearing the orthologous murine p.R1907K mutation, as well as Zfhx2 null mutant mice, have significant deficits in pain sensitivity. Gene expression analyses in dorsal root ganglia from mutant and wild-type mice show altered expression of genes implicated in peripheral pain mechanisms. The ZFHX2 variant and downstream regulated genes associated with a human pain-insensitive phenotype are therefore potential novel targets for the development of new analgesic drugs.awx326media15680039660001.
Brain, 2018; 141

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BOARD NUMBER: S01-618

CAV1.2-DEPENDENT EXCITATION-TRANSCRIPTION COUPLING IN NOCICEPTORS

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Repetitive firing of peripheral nociceptors induces the chronification of pain due to synaptic plasticity within the central nervous system, but associated changes within nociceptors remain largely enigmatic. We have recently discovered that depolarization of nociceptors activates type-II protein kinase A (PKA-II) by calcium influx through CaV1.2 channels leading to mechanical hyperalgesia. In other neurons, however, CaV1 associated signaling translates electrical activity into transcriptional regulation known as excitation-transcription (E-T) coupling. If CaV1.2 also mediates E-T coupling in nociceptors is currently unknown. Using High Content Screening microscopy, we found that depolarization of nociceptors with potassium chloride (KCl) leads to phosphorylation of the transcriptional regulating signaling components CREB and ERK1/2. Accordingly, CREB localization to the nucleus increased. This was inhibited by blocking CaV1 or PKA and reinforced by calcineurin inhibition. RNA-Seq revealed a dose-dependent regulation of up to 1563 genes ($q < 0.05$, >1.5-fold) 6 hours after depolarization. To address the impact of calcium influx through CaV1, we included combinations of low [KCl] (10 mM) and the CaV1 agonist BayK8644 as well as high [KCl] (40 mM) and the CaV1 blocker verapamil. Supporting a CaV1-dependent regulation, multiple genes showed opposing regulation by BayK8644 and verapamil including known activity-regulated transcripts such as Fosb, Npas4, and Rasd1. The cellular basis is currently investigated using fluorescent *in-situ* hybridization. In conclusion, our data suggest a CaV1-dependent E-T coupling mechanism in nociceptors. Further studies need to address which of the regulated genes alters which nociceptive function in short-term activation as well as in the context of chronic pain.

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BOARD NUMBER: S01-619

ANALGESIC EFFECTS OF KB-R7943 IN A STREPTOZOTOCIN-INDUCED RAT'S DIABETIC NEUROPATHY MODEL

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Diabetic neuropathy (DN) is one of the most common chronic complications of poorly treated diabetes mellitus. Amitriptyline (ATL) is a tricyclic antidepressant and often the first-choice option for DN treatment. This study aimed to evaluate the potential analgesic effect of KB-R7943, an inhibitor of NCX, in a model of diabetic neuropathy. Diabetic neuropathic pain was induced by a single intraperitoneal injection (i.p.) of streptozotocin at a dose of 55 mg/kg. After the development of neuropathy, KB-R7943 was administered by oral gavage at two doses, 5 mg/kg and 10 mg/kg, for ten days. Amitriptyline 10 mg/kg was used as a positive control. The following groups were assigned: controls treated with vehicle (C-Veh), rats with DN treated with vehicle (DN-veh), rats with DN and treated with KB-R7943 at 5 mg/kg (DN-KB-R7943-5 mg), rats with DN and treated with KB-R7943 at 10 mg/kg (DN-KB-R7943-10 mg), and rats with DN and ATL 10 mg/kg (DN-ATL-10 mg/kg) (n=7 in each group). A blockade of the reverse-mode sodium-calcium exchanger (NCX) and desensitization of N-methyl-D-aspartate receptor (NMDAR) was demonstrated among its multiple molecular targets. Analgesic effect was assessed by the threshold from Randle-Stellito paw pressure test, cold plate test, and duration of the antinociceptive behavior after intraplantar injection of 0.5% formalin. Both ATL and KB-R7943 at the dose of 10 mg/kg increased the threshold of the Randle-Stellito and decreased the second phase of the formalin test. The present data suggest that developing pain therapy based on selective NCX blockers in DN is promising. Acknowledgments: Supported by BNSF КП-06-Rusia 25/16.12.2020.

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BOARD NUMBER: S01-620

TARGETING THE SEROTONIN 5-HT7 RECEPTOR TO ATTENUATE PAIN-RELATED BEHAVIORS

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Serotonin mediates its physiological functions by activating serotonin receptors. Among them, the serotonin 5-HT_7R receptor (5-HT_7R) belongs to the superfamily of the G proteins coupled receptors (GPCRs) and is coupled to the heterotrimeric G protein Gs which in turn leads to adenylate cyclase activation and initiates specific signaling cascade including activation of MAP/ERK kinases. In the last decade, 5-HT_7 receptor has become a promising target for the treatment of neuropsychiatric and neurologic disorders. In this study, we characterized the pharmacological profile of new 5-HT_7R ligands derived from pharmacomodulation studies. For that purpose, we studied their capacity to activate specific signaling pathways and defined their agonist, inverse agonist or antagonist activity by measurement of cAMP levels in HEK cells stably expressing the 5-HT_7R. We also investigated their ability to activate others signaling pathways using BRET, TR-FRET or Alphascreen methods. In particular BRET studies allow us to follow the recruitment of intracellular partners in real time and in living cells. Our results demonstrate that 5-HT_7R can engage a signaling pathway not described for this receptor until now. Considering the unique pharmacological profile of our molecules, we also investigated their therapeutically potential. By using WT and 5-HT7 KO mice, we demonstrated their specificity of action. Then, we performed various behavioral assays allowing us to demonstrate their therapeutic potential. In conclusion, by using various functional cellular assays and behavioral tests in mice, we characterized an original 5-HT_7R- ligand able to stabilize specific active conformation of 5-HT7R and to reduce sensory dysfunction.

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BOARD NUMBER: S01-621

GREEN LIGHT-INDUCED ANTINOCICEPTION INVOLVES DESCENDING MODULATION OF MECHANICAL SENSITIVITY.

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Green light therapy (GLED) has been shown to be a novel, efficacious, nonpharmacologic pain therapeutic in humans for chronic pain conditions including migraine and fibromyalgia. Previous animal studies suggest that GLED acts on central descending pain modulation areas such as the rostral ventromedial medulla (RVM). Additionally, the involvement of the endogenous opioid (EO) system has been demonstrated. However, the role of EO in the RVM in GLED-induced antinociception has not been confirmed. Therefore, we hypothesized that GLED causes antinociception via opioid receptor agonist stimulation by EO in the RVM. We performed microinjections of naltrexone in the RVM of rats with gp120-induced HIV-related neuropathic pain that had received 6 days of GLED exposure for 8 hours/day. At 1 hour after RVM naltrexone microinjections, mechanical antinociception induced by GLED was reversed, whereas no changes in thermal sensitivity were observed. These results prompted investigation of opioid receptor expression on GABAergic cells, as recent reports indicated that RVM GABAergic neurons are responsible for facilitation of mechanical, but not thermal, pain. In-situ RNA hybridization identified co-localization of Slc31a1, Oprm1, and Oprd1 signals in RVM sections, indicating the presence of opioid-sensitive GABAergic neurons in the RVM. Overall, these results suggest that opioid receptor agonist stimulation in the RVM due to GLED exposure is required for GLED-induced mechanical, but not thermal, antinociception. Future gene editing experiments selectively in GABAergic neurons may confirm the hypothesis that inhibition of pronociceptive GABAergic neurons by endogenous opioids is responsible for GLED-induced mechanical antinociception.

Pubmed:
33910453: Cheng VK, Hasegawa M, Hattori T, Ito N, Linn E, Cheng K, Hughes-Austin J, Masuda K, Sudo A
Prevalence of radiographic hip dysplasia in Japanese population-based study.
The purpose of this study was to measure the indices of radiographic developmental dysplasia of the hip (DDH) in a cross-sectional study of an elderly Japanese population.
Mod Rheumatol, 2022; 32
34157406: Martin LF, Moutal A, Cheng K, Washington SM, Calligaro H, Goel V, Kranz T, Largent-Milnes TM, Khanna R, Patwardhan A, Ibrahim MM
Green Light Antinociceptive and Reversal of Thermal and Mechanical Hypersensitivity Effects Rely on Endogenous Opioid System Stimulation.
Benefits of phototherapy were characterized in multiple diseases including depression, circadian rhythm disruptions, and neurodegeneration. Studies on migraine and fibromyalgia patients revealed that green light-emitting diodes (GLED) exposure provides a pragmatic and safe therapy to manage chronic pain. In rodents, GLED reversed hypersensitivity related to neuropathic pain. However, little is known about the underlying mechanisms of GLED efficacy. Here, we sought to understand how green light modulates the endogenous opioid system. We first characterized how exposure to GLED stimulates release of β-endorphin and proenkephalin in the central nervous system of male rats. Moreover, by individually editing each of the receptors, we found that μ- and δ-opioid receptors are required for green light's antinociceptive effect in naïve rats and a model of HIV-induced peripheral neuropathy. We investigated how GLED could increase pain thresholds, and explored its potential in reversing hypersensitivity in a model of HIV-related neuropathy. Through behavioral and gene editing approaches, we identified that green light provides antinociception via modulation of the endogenous opioid system in the spinal cord. This work identifies a previously unknown mechanism by which GLED can improve pain management. Clinical translation of these results will advance the development of an innovative therapy devoid of adverse effects. PERSPECTIVE: Development of new pain management therapies, especially for HIV patients, is crucial as long-term opioid prescription is not recommended due to adverse side effects. Green light addresses this necessity. Characterizing the underlying mechanisms of this potentially groundbreaking and safe antinociceptive therapy will advance its clinical

translation.
J Pain, 2021; 22
33636371: Cheng K, Martin LF, Slepian MJ, Patwardhan AM, Ibrahim MM
Mechanisms and Pathways of Pain Photobiomodulation: A Narrative Review.
A growing body of evidence supports the modulation of pain by light exposure. As such, phototherapy is being increasingly utilized for the management of a variety of pain conditions. The modes of delivery, and hence applications of phototherapy, vary by wavelength, intensity, and route of exposure. As such, differing mechanisms of action exist depending upon those parameters. Cutaneous application of red light (660 nm) has been shown to reduce pain in neuropathies and complex regional pain syndrome-I, whereas visual application of the same wavelength of red light has been reported to exacerbate migraine headache in patients and lead to the development of functional pain in animal models. Interestingly visual exposure to green light can result in reduction in pain in variety of pain conditions such as migraine and fibromyalgia. Cutaneous application typically requires exposure on the order of minutes, whereas visual application requires exposure on the order of hours. Both routes of exposure elicit changes centrally in the brainstem and spinal cord, and peripherally in the dorsal root ganglia and nociceptors. The mechanisms of photobiomodulation of pain presented in this review provide a foundation in furtherance of exploration of the utility of phototherapy as a tool in the management of pain. PERSPECTIVE: This review synopsizes the pathways and mechanisms through which light modulates pain and the therapeutic utility of different colors and exposure modalities of light on pain. Recent advances in photobiomodulation provide a foundation for understanding this novel treatment for pain on which future translational and clinical studies can build upon.
J Pain, 2021; 22
33651180: Akeda K, Cheng K, Abarado E, Takegami N, Yamada J, Inoue N, Masuda K, Sudo A
Three-dimensional computed tomographic evaluation of lateral lumbar interbody fusion: morphometric change of intervertebral structure.
Two-dimensional (2D) analyses of intervertebral disc (IVD) height and foramen measurements following lateral lumbar interbody fusion (LLIF) have been reported. However, three-dimensional (3D) morphometric analysis of intervertebral structure using 3D computed tomography (3D CT) provides increased precision for measuring morphological changes. The purpose of this study was to evaluate 3D changes of lumbar IVD height and foramen diameter in degenerative lumbar disease patients following LLIF.
Eur Spine J, 2021; 30
30157962: Mwale F, Masuda K, Grant MP, Epure LM, Kato K, Miyazaki S, Cheng K, Yamada J, Bae WC, Muehleman C, Roughley PJ, Antoniou J
Short Link N promotes disc repair in a rabbit model of disc degeneration.
The degeneration of the intervertebral disc (IVD) is characterized by proteolytic degradation of the extracellular matrix, and its repair requires the production of an extracellular matrix with a high proteoglycan-to-collagen ratio characteristic of a nucleus pulposus (NP)-like phenotype in vivo. At the moment, there is no medical treatment to reverse or even retard disc degeneration. The purpose of the present study was to determine if a low dose of short link N (sLN), a recently discovered fragment of the link N peptide, could behave in a manner similar to that of link N in restoring the proteoglycan content and proteoglycan-to-collagen ratio of the disc in a rabbit model of IVD degeneration, as an indication of its potential therapeutic benefit in reversing disc degeneration.
Arthritis Res Ther, 2018; 20
29793459: Murata K, Akeda K, Takegami N, Cheng K, Masuda K, Sudo A
Morphology of intervertebral disc ruptures evaluated by vacuum phenomenon using multi-detector computed tomography: association with lumbar disc degeneration and canal stenosis.
The progression of intervertebral disc (IVD) degeneration leads to rupture within IVD tissues. The location and appearance of areas of gaseous radiolucency in the IVD, known as vacuum phenomena (VPs), are considered to indirectly indicate the position and extent of IVD rupture. The clinical significance of VPs in degenerated IVDs is not fully understood. The purpose of this study is to assess and classify the morphology of IVD ruptures by the presence of intradiscal VPs, and to examine the association between morphological VP-positive IVD ruptures and degenerative lumbar diseases.
BMC Musculoskelet Disord, 2018; 19
29460012: Miyazaki S, Diwan AD, Kato K, Cheng K, Bae WC, Sun Y, Yamada J, Muehleman C, Lenz ME, Inoue N, Sah RL, Kawakami M, Masuda K
ISSLS PRIZE IN BASIC SCIENCE 2018: Growth differentiation factor-6 attenuated pro-inflammatory molecular changes in the rabbit anular-puncture model and degenerated disc-induced pain generation in the rat xenograft radiculopathy model.
To elucidate the effects of growth differentiation factor-6 (GDF6) on: (i) gene expression of inflammatory/pain-related molecules and structural integrity in the rabbit intervertebral disc (IVD) degeneration model, and (ii) sensory dysfunction and changes in pain-marker expression in dorsal nerve ganglia (DRGs) in the rat xenograft radiculopathy model.

Eur Spine J, 2018; 27
28012080: Fukui D, Kawakami M, Cheng K, Murata K, Yamada K, Sato R, Yoshida M, Yamada H, Inoue N, Masuda K
Three-dimensional micro-computed tomography analysis for spinal instability after lumbar facetectomy in the rat.
Intervertebral disc degeneration is thought to contribute to low back pain. However, the pathophysiological mechanisms remain controversial. In a previous study, we developed an animal model that showed delayed gait disturbance after lumbar facetectomy in the rat. We believe that this gait disturbance was caused by low back pain, although the mechanisms of this gait abnormality remain unknown. The purpose of this study was to evaluate structural changes of the lumbar spine after facetectomy in the rat utilizing three-dimensional micro-computed tomography (3DμCT) compared to histology.
Eur Spine J, 2017; 26

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BOARD NUMBER: S01-622

DISTINCT ACTIVITY OF ENDOCANNABINOID-HYDROLYZING ENZYMES MAGL AND FAAH IN KEY REGIONS OF PERIPHERAL AND CENTRAL NERVOUS SYSTEM IMPLICATED IN MIGRAINE

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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In migraine pain, cannabis has a promising analgesic action, which, however, is associated with side psychotropic effects. To overcome these adverse effects of exogenous cannabinoids, we propose migraine pain relief via activation of the endogenous cannabinoid system (ECS) by inhibiting enzymes degrading endocannabinoids. To provide a functional platform for such purpose in the peripheral and central parts of the rat nociceptive system relevant to migraine, we measured by activity-based protein profiling (ABPP) the activity of the main endocannabinoid-hydrolases, mon-oacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH). We found that in trigeminal ganglia, the MAGL activity was nine-fold higher than that of FAAH. MAGL activity exceeded FAAH activity also in DRG, spinal cord and brainstem. However, activities of MAGL and FAAH were comparably high in the cerebellum and cerebral cortex implicated in migraine aura. MAGL and FAAH activities were identified and blocked by the selective and potent inhibitors JJKK-048/KML29 and JZP327A, respectively. The high MAGL activity in trigeminal ganglia implicated in the generation of nociceptive signals suggests this part of ECS as a priority target for blocking peripheral mechanisms of migraine pain. In the CNS, both MAGL and FAAH represent potential targets for attenuation of migraine-related enhanced cortical excitability and pain transmission.

Pubmed:

31973098: Della Pietra A, Mikhailov N, Giniatullin R
The Emerging Role of Mechanosensitive Piezo Channels in Migraine Pain.
Recently discovered mechanosensitive Piezo channels emerged as the main molecular detectors of mechanical forces. The functions of Piezo channels range from detection of touch and pain, to control of the plastic changes in different organs. Recent studies suggested the role of Piezo channels in migraine pain, which is supposed to originate from the trigeminovascular nociceptive system in meninges. Interestingly, migraine pain is associated with such phenomenon as mechanical hypersensitivity, suggesting enhanced mechanotransduction. In the current review, we present the data that propose the implication of Piezo channels in migraine pain, which has a distinctive pulsatile character. These data include: (i) distribution of Piezo channels in the key elements of the trigeminovascular nociceptive system; (ii) the prolonged functional activity of Piezo channels in meningeal afferents providing a mechanistical basis for mechanotransduction in nociceptive nerve terminals; (iii) potential activation of Piezo channels by shear stress and pulsating blood flow; and (iv) modulation of these channels by emerging chemical agonists and modulators, including pro-nociceptive compounds. Achievements in this quickly expanding field should open a new road for efficient control of Piezo-related diseases including migraine and chronic pain.
Int J Mol Sci, 2020; 21
33530477: Della Pietra A, Giniatullin R, Savinainen JR
Distinct Activity of Endocannabinoid-Hydrolyzing Enzymes MAGL and FAAH in Key Regions of Peripheral and Central Nervous System Implicated in Migraine.
In migraine pain, cannabis has a promising analgesic action, which, however, is associated with side psychotropic effects. To overcome these adverse effects of exogenous cannabinoids, we propose migraine pain relief via activation of the endogenous cannabinoid system (ECS) by inhibiting enzymes degrading endocannabinoids. To provide a functional platform for such purpose in the peripheral and central parts of the rat nociceptive system relevant to migraine, we measured by activity-based protein profiling (ABPP) the activity of the main endocannabinoid-hydrolases, monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH). We found that in trigeminal ganglia, the MAGL activity was nine-fold higher than that of FAAH. MAGL activity exceeded FAAH activity also in DRG, spinal cord and brainstem. However, activities of MAGL and FAAH were comparably high in the cerebellum and cerebral cortex implicated in migraine aura. MAGL and FAAH activities were identified and blocked by the selective and potent inhibitors JJKK-048/KML29 and JZP327A, respectively. The high

MAGL activity in trigeminal ganglia implicated in the generation of nociceptive signals suggests this part of ECS as a priority target for blocking peripheral mechanisms of migraine pain. In the CNS, both MAGL and FAAH represent potential targets for attenuation of migraine-related enhanced cortical excitability and pain transmission.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S01-623

CHEMICALLY-INDUCED NOCICEPTION IN PLANARIA AND ITS REGULATION BY MORPHINE AND OTHER ANTINOCICEPTIVE COMPOUNDS.

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Aim : In addition to their regeneration abilities, planarians have been extensively studied in the context of behavior, pharmacology and toxicology during the past 200 years. Most of these assays only looked at basic locomotor behavior such as speed and distance while only a few implemented a full behavior analysis in reaction to different stimuli, with a special interest on 'gliding' vs. 'scrunching' displacement modes. Among these stimuli, some have been linked to nociception, especially due to the involvement of well-known nociceptive ion channels such as TRPV1 and TRPA1. However, nociception in planarians remains poorly investigated and in particular its regulation by common antinociceptive compounds. Methods : We developed a nociceptive assay in the planarian *Girardia dorotocephala* by placing the animal in an arena with various concentrations of allyl isothiocyanate (AITC), a TRPA1 agonist. As a measure of nociception, the 'scrunching' behavior of the animal was assessed. Results : Worms exposed to 0% of AITC displayed normal locomotor behavior (81.87±3.14% of the total time) whereas animals subjected to 100µM of AITC displayed the nociceptive 'scrunching' behavior (74.44±6.43% of the total time). Pre-incubating the worms with morphine 1 µM during 2 hours shifted the dose response curve. For instance at 50 µM AITC, the 'scrunching' behavior went from 61.49±6.41% without morphine to 16.93±4.05% with morphine pre-incubation. Conclusion : Using the same experimental paradigm we aim to investigate the effects of NSAIDs on the planarian nociceptive response thus validating this animal model for antinociceptive drug screening.

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BOARD NUMBER: S01-625

PHYSIOLOGY AND MORPHOLOGY OF LAYER 5 NEURON SUBTYPES OF ANTERIOR CINGULATE CORTEX IN INFLAMMATORY PAIN

POSTER SESSION 01 - SECTION: PAIN AND INFLAMMATION

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Chronic pain is a common and complex problem that has a profound impact on individuals and society. The involvement of different brain regions like the anterior cingulate cortex (ACC) in processing pains' affective component has been established; however, a characterization of its main output neurons in pain has not been performed. To characterize neuron subtypes, we performed whole-cell patch clamp in pyramidal neurons from layer 5 of ACC, followed by anatomical reconstruction. As a model of chronic pain, we injected Complete Freund's Adjuvant (CFA) in the hindpaw of male mice generating inflammation. To investigate how the peripheral injury changes the general neuronal activity, we recorded in brain slices from CFA and saline mice. Cluster analysis of physiological and morphological parameters revealed two subtypes among the sampled neurons: contralateral projecting and subcortical projecting (SC). CFA mice developed mechanical and thermal hyperalgesia, as compared to the saline. At the cellular level, we observed significant changes in intrinsic electrical properties between CFA and saline mice. Changes occur only in a subpopulation of neurons projecting to subcortical areas. Dissecting the underlying circuit mechanisms, we determined the role of ACC-SC neurons projecting to the periaqueductal gray (PAG), subcortical structure with crucial role in the descending modulation of pain. Chemogenetic manipulation of ACC inputs to PAG differentially influence pain sensitivity in different pain conditions. The current study is therefore the first to systematically dissect the effects of inflammatory pain on pyramidal neurons of layers 5 in the ACC, providing evidence for intralayer-specific alterations of electrophysiological properties.

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REPAIRING DEFECTIVE NEURONAL TRANSLATIONAL CONTROL AMELIORATES BEHAVIORAL PHENOTYPE IN R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

Carla Castany Pladevall[1,2,3], Marta Garcia-Forn[1,2,3], Jordi Creus-Muncunill[1,2,3], Arantxa Golbano[1,2,3], Uxía Fraga-Bouzas[1,2,3], Elisa Marin[1,2,3], Esther Perez-Navarro[1,2,3]
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Erroneous protein synthesis disrupts cellular fitness causing a variety of disease phenotypes. Neurons are especially sensitive to translational dysregulation owning to the fact that it is essential for synaptic plasticity and neuronal survival. Hence, a precise regulation of translation initiation plays a critical role in learning and memory. Defects in this process have been linked to numerous cognitive disorders including addiction, fragile X syndrome and autism and has also been related to several neurodegenerative disorders like Parkinson's disease. In this line, this group recently stated that loss of translational control in the striatum leading to an aberrantly increased global cap-dependent protein synthesis is a relevant mechanism in Huntington's disease (HD) pathophysiology. Additionally, its normalization by intraventricular injection of 4EGI-1, an inhibitor of the eIF4E/eIF4G complex assembly, prevented the development of motor deficits in the R6/1 mouse model of HD. In this present work, we explore the potential of a set of repurposed drug candidates, shown to decrease protein synthesis in cellular models and mouse brain, for HD treatment through the normalization of protein synthesis in the striatum. Furthermore, we show that alterations involving translational control extend to other brain regions such as the hippocampus suggesting its involvement in, not only in motor deficits, but also in cognitive and memory impairment also occurring in HD pathology.

Pubmed:
33369245: Alcalá-Vida R, Garcia-Forn M, Castany-Pladevall C, Creus-Muncunill J, Ito Y, Blanco E, Golbano A, Crespí-Vázquez K, Parry A, Slater G, Samarajiwa S, Peiró S, Di Croce L, Narita M, Pérez-Navarro E
Neuron type-specific increase in lamin B1 contributes to nuclear dysfunction in Huntington's disease.
Lamins are crucial proteins for nuclear functionality. Here, we provide new evidence showing that increased lamin B1 levels contribute to the pathophysiology of Huntington's disease (HD), a CAG repeat-associated neurodegenerative disorder. Through fluorescence-activated nuclear suspension imaging, we show that nucleus from striatal medium-sized spiny and CA1 hippocampal neurons display increased lamin B1 levels, in correlation with altered nuclear morphology and nucleocytoplasmic transport disruption. Moreover, ChIP-sequencing analysis shows an alteration of lamin-associated chromatin domains in hippocampal nuclei, accompanied by changes in chromatin accessibility and transcriptional dysregulation. Supporting lamin B1 alterations as a causal role in mutant huntingtin-mediated neurodegeneration, pharmacological normalization of lamin B1 levels in the hippocampus of the R6/1 mouse model of HD by betulinic acid administration restored nuclear homeostasis and prevented motor and cognitive dysfunction. Collectively, our work points increased lamin B1 levels as a new pathogenic mechanism in HD and provides a novel target for its intervention.
EMBO Mol Med, 2021; 13
34518368: Nurm K, Sepp M, Castany-Pladevall C, Creus-Muncunill J, Tuvikene J, Sirp A, Vihma H, Blake DJ, Perez-Navarro E, Timmusk T
Isoform-Specific Reduction of the Basic Helix-Loop-Helix Transcription Factor TCF4 Levels in Huntington's Disease.
Huntington's disease (HD) is an inherited neurodegenerative disorder with onset of characteristic motor symptoms at midlife, preceded by subtle cognitive and behavioral disturbances. Transcriptional dysregulation emerges early in the disease course and is considered central to HD pathogenesis. Using wild-type (wt) and HD knock-in mouse striatal cell lines we observed a HD genotype-dependent reduction in the protein levels of transcription factor 4 (TCF4), a member of the basic helix-loop-helix (bHLH) family with critical roles in brain development and function. We characterized mouse gene structure and expression of alternative mRNAs and protein isoforms in cell-based models of HD, and in four different brain regions of male transgenic HD mice (R6/1) from young to mature adulthood. The largest decrease in the levels of TCF4 at mRNA and specific protein isoforms were detected in the R6/1 mouse hippocampus. Translating this finding to human disease, we found reduced

expression of long TCF4 isoforms in the postmortem hippocampal CA1 area and in the cerebral cortex of HD patients. Additionally, TCF4 protein isoforms showed differential synergism with the proneural transcription factor ASCL1 in activating reporter gene transcription in hippocampal and cortical cultured neurons. Induction of neuronal activity increased these synergistic effects in hippocampal but not in cortical neurons, suggesting brain region-dependent differences in TCF4 functions. Collectively, this study demonstrates isoform-specific changes in TCF4 expression in HD that could contribute to the progressive impairment of transcriptional regulation and neuronal function in this disease.
eNeuro, 2021 Sep-Oct; 8

33345999: Meneses-Salas E, Garcia-Forn M, Castany-Pladevall C, Lu A, Fajardo A, Jose J, Wahba M, Bosch M, Pol A, Tebar F, Klein AD, Zanlungo S, Pérez-Navarro E, Grewal T, Enrich C, Rentero C

Lack of Annexin A6 Exacerbates Liver Dysfunction and Reduces Lifespan of Niemann-Pick Type C Protein-Deficient Mice. Niemann-Pick type C (NPC) disease is a lysosomal storage disorder characterized by cholesterol accumulation caused by loss-of-function mutations in the Npc1 gene. NPC disease primarily affects the brain, causing neuronal damage and affecting motor coordination. In addition, considerable liver malfunction in NPC disease is common. Recently, we found that the depletion of annexin A6 (ANXA6), which is most abundant in the liver and involved in cholesterol transport, ameliorated cholesterol accumulation in Npc1 mutant cells. To evaluate the potential contribution of ANXA6 in the progression of NPC disease, double-knockout mice (Npc1/Anxa6) were generated and examined for lifespan, neurologic and hepatic functions, as well as liver histology and ultrastructure. Interestingly, lack of ANXA6 in NPC1-deficient animals did not prevent the cerebellar degeneration phenotype, but further deteriorated their compromised hepatic functions and reduced their lifespan. Moreover, livers of Npc1/Anxa6 mice contained a significantly elevated number of foam cells congesting the sinusoidal space, a feature commonly associated with inflammation. We hypothesize that ANXA6 deficiency in Npc1 mice not only does not reverse neurologic and motor dysfunction, but further worsens overall liver function, exacerbating hepatic failure in NPC disease.
Am J Pathol, 2021; 191

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BOARD NUMBER: S01-627

CPEB ALTERATION AND ABERRANT TRANSCRIPTOME-POLYADENYLATION UNVEIL A TREATABLE VITAMIN B1 DEFICIENCY IN HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Aims: Although promising gene-silencing therapies are being tested for Huntington's disease (HD), no disease-modifying treatments are available. Thus, study of molecular mechanisms underneath Htt-mutation must continue to identify easily druggable targets. Cytoplasmic polyadenylation element binding proteins 1–4 (CPEB1–4) are RNA-binding proteins that repress or activate translation of CPE-containing transcripts by shortening or elongating their poly(A) tail. Our aim was to analyze CPEBs and polyadenylation in HD. Identify easily druggable targets among genes mis-expressed due to altered CPEB-dependent polyadenylation, to assay them in HD mice. Methods: a) Western blot and immunostaining of CPEBs in brains of HD patients and mouse models. b) Genome-wide poly(A)-tail analysis through poly(U) chromatography+gene chip. c) status of CPEB targets and related metabolites by western blot and HPLC. d) radiological, neuropathological and behavioural analysis of HD mice receiving target-related treatment. Results: There is a CPEB1/4 imbalance in HD striatum with concomitant altered transcriptome polyadenylation affecting many neurodegeneration-linked genes like PSEN1, MAPT, SNCA, LRRK2, PINK1, DJ1, SOD1, TARDBP, FUS and HTT. Among top deadenylated genes was SLC19A3 (ThTr2 thiamine transporter) whose mutation causes biotin+thiamine responsive basal ganglia disease (BTBGD). Decreased ThTr2 in HD and HD mice led us to discover that HD is in part a BTBG-like thiamine deficiency. Remarkably, high dose biotin+thiamine treatment prevented the thiamine deficiency of HD mice and attenuated their radiological, neuropathological and motor phenotypes. Conclusions: This study unveils altered polyadenylation as a new molecular mechanism in neurodegeneration uncovering HD as a thiamine deficiency and, therefore, an easy to implement therapy.

Pubmed:
34586830: Picó S, Parras A, Santos-Galindo M, Pose-Utrilla J, Castro M, Fraga E, Hernández IH, Elorza A, Anta H, Wang N, Martí-Sánchez L, Belloc E, Garcia-Esparcia P, Garrido JJ, Ferrer I, Macías-García D, Mir P, Artuch R, Pérez B, Hernández F, Navarro P, López-Sendón JL, Iglesias T, Yang XW, Méndez R, Lucas JJ
CPEB alteration and aberrant transcriptome-polyadenylation lead to a treatable SLC19A3 deficiency in Huntington's disease. Huntington's disease (HD) is a hereditary neurodegenerative disorder of the basal ganglia for which disease-modifying

treatments are not yet available. Although gene-silencing therapies are currently being tested, further molecular mechanisms must be explored to identify druggable targets for HD. Cytoplasmic polyadenylation element binding proteins 1 to 4 (CPEB1 to CPEB4) are RNA binding proteins that repress or activate translation of CPE-containing transcripts by shortening or elongating their poly(A) tail. Here, we found increased CPEB1 and decreased CPEB4 protein in the striatum of patients and mouse models with HD. This correlated with a reprogramming of polyadenylation in 17.3% of the transcriptome, markedly affecting neurodegeneration-associated genes including , , , , , , , , and and suggesting a new molecular mechanism in neurodegenerative disease etiology. We found decreased protein content of top deadenylated transcripts, including striatal atrophy–linked genes not previously related to HD, such as and the easily druggable (the ThTr2 thiamine transporter). Mutations in cause biotin-thiamine–responsive basal ganglia disease (BTBGD), a striatal disorder that can be treated with a combination of biotin and thiamine. Similar to patients with BTBGD, patients with HD demonstrated decreased thiamine in the cerebrospinal fluid. Furthermore, patients and mice with HD showed decreased striatal concentrations of thiamine pyrophosphate (TPP), the metabolically active form of thiamine. High-dose biotin and thiamine treatment prevented TPP deficiency in HD mice and attenuated the radiological, neuropathological, and motor HD-like phenotypes, revealing an easily implementable therapy that might benefit patients with HD.
Sci Transl Med, 2021; 13

33725094: Elorza A, Márquez Y, Cabrera JR, Sánchez-Trincado JL, Santos-Galindo M, Hernández IH, Picó S, Díaz-Hernández JI, García-Escudero R, Irimia M, Lucas JJ
Huntington's disease-specific mis-splicing unveils key effector genes and altered splicing factors.
Correction of mis-splicing events is a growing therapeutic approach for neurological diseases such as spinal muscular atrophy or neuronal ceroid lipofuscinosis 7, which are caused by splicing-affecting mutations. Mis-spliced effector genes that do not harbour mutations are also good candidate therapeutic targets in diseases with more complex aetiologies such as cancer, autism, muscular dystrophies or neurodegenerative diseases. Next-generation RNA sequencing (RNA-seq) has boosted investigation of global mis-splicing in diseased tissue to identify such key pathogenic mis-spliced genes. Nevertheless, while analysis of tumour or dystrophic muscle biopsies can be informative on early stage pathogenic mis-splicing, for neurodegenerative diseases, these analyses are intrinsically hampered by neuronal loss and neuroinflammation in post-mortem brains. To infer splicing alterations relevant to Huntington's disease pathogenesis, here we performed intersect-RNA-seq analyses of human post-mortem striatal tissue and of an early symptomatic mouse model in which neuronal loss and gliosis are not yet present. Together with a human/mouse parallel motif scan analysis, this approach allowed us to identify the shared mis-splicing signature triggered by the Huntington's disease-causing mutation in both species and to infer upstream deregulated splicing factors. Moreover, we identified a plethora of downstream neurodegeneration-linked mis-spliced effector genes that-together with the deregulated splicing factors-become new possible therapeutic targets. In summary, here we report pathogenic global mis-splicing in Huntington's disease striatum captured by our new intersect-RNA-seq approach that can be readily applied to other neurodegenerative diseases for which bona fide animal models are available.
Brain, 2021; 144

30111840: Parras A, Anta H, Santos-Galindo M, Swarup V, Elorza A, Nieto-González JL, Picó S, Hernández IH, Díaz-Hernández JI, Belloc E, Rodolosse A, Parikshak NN, Peñagarikano O, Fernández-Chacón R, Irimia M, Navarro P, Geschwind DH, Méndez R, Lucas JJ
Autism-like phenotype and risk gene mRNA deadenylation by CPEB4 mis-splicing.
Common genetic contributions to autism spectrum disorder (ASD) reside in risk gene variants that individually have minimal effect sizes. As environmental factors that perturb neurodevelopment also underlie idiopathic ASD, it is crucial to identify altered regulators that can orchestrate multiple ASD risk genes during neurodevelopment. Cytoplasmic polyadenylation element binding proteins 1-4 (CPEB1-4) regulate the translation of specific mRNAs by modulating their poly(A)-tails and thereby participate in embryonic development and synaptic plasticity. Here we find that CPEB4 binds transcripts of most high-confidence ASD risk genes. The brains of individuals with idiopathic ASD show imbalances in CPEB4 transcript isoforms that result from decreased inclusion of a neuron-specific microexon. In addition, 9% of the transcriptome shows reduced poly(A)-tail length. Notably, this percentage is much higher for high-confidence ASD risk genes, correlating with reduced expression of the protein products of ASD risk genes. An equivalent imbalance in CPEB4 transcript isoforms in mice mimics the changes in mRNA polyadenylation and protein expression of ASD risk genes and induces ASD-like neuroanatomical, electrophysiological and behavioural phenotypes. Together, these data identify CPEB4 as a regulator of ASD risk genes.
Nature, 2018; 560

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-628

ALTERED ACTIVITY-REGULATED STRIATAL EPIGENOME CONTRIBUTES TO EGOCENTRIC SPATIAL MEMORY DEFICIT IN HUNTINGTON'S DISEASE MICE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Aims: Molecular mechanisms underlying cognitive deficits in Huntington's disease (HD), a striatal polyglutamine-related neurodegenerative disorder, remain relatively unexplored. Here, we aim to interrogate transcriptional and epigenetic mechanisms interconnection with striatal-dependent cognitive deficits in HD. **Methods:** We challenged the transgenic HD R6/1 mice in a striatum-dependent egocentric spatial memory task, the double-H test, and investigated chromatin and transcriptomic signatures in behaving vs resting control HD R6/1 and wild-type mice by using RNA-seq, ChIP-seq and 4C-seq. **Results:** Our data, including integrated analysis with cell-type specific striatal databases, showed a prominent alteration of activity-regulated transcriptomic and epigenetic regulatory mechanisms in HD mice, involving both neuronal and glial genes implicated in striatal plasticity, accompanying egocentric memory deficits. During memory acquisition, spatial chromatin remodeling and transcriptional induction of BDNF-related effectors of neuronal plasticity were compromised. In addition, we demonstrated that while both, histone H3 acetylation at lysine 27 and 9 (H3K27ac and H3K9ac, respectively) were strongly impaired in HD R6/1 mice, only H3K9ac was modulated during memory processing. Specifically, H3K9ac-mediated epigenetic memory was established during memory consolidation/recall at glia-enriched TGF-beta-associated genes, regulating extracellular matrix and myelination, and this mechanism was compromised in HD mice. These alterations affected protein regulation of key striatal markers of neural plasticity, including the immediate early gene Egr1, myelination and extracellular-matrix-based perineuronal networks, suggesting a disruption of activity-induced re-organization of striatal circuitry required memory process. **Conclusions:** Altogether, our study identified H3K9 acetylation and TGF-beta signaling as new targets of striatal plasticity, which might offer innovative leads to improve cognition in HD.

Pubmed:
35032317: De Sa Nogueira D, Bourdy R, Alcala-Vida R, Filliol D, Andry V, Goumon Y, Zwiller J, Romieu P, Merienne K, Olmstead MC, Befort K
Hippocampal Cannabinoid 1 Receptors Are Modulated Following Cocaine Self-administration in Male Rats.
Cocaine addiction is a complex pathology inducing long-term neuroplastic changes that, in turn, contribute to maladaptive behaviors. This behavioral dysregulation is associated with transcriptional reprogramming in brain reward circuitry, although the mechanisms underlying this modulation remain poorly understood. The endogenous cannabinoid system may play a role in this process in that cannabinoid mechanisms modulate drug reward and contribute to cocaine-induced neural adaptations. In this study, we investigated whether cocaine self-administration induces long-term adaptations, including transcriptional modifications and associated epigenetic processes. We first examined endocannabinoid gene expression in reward-related brain regions of the rat following self-administered (0.33 mg/kg intravenous, FR1, 10 days) cocaine injections. Interestingly, we found increased Cnr1 expression in several structures, including prefrontal cortex, nucleus accumbens, dorsal striatum, hippocampus, habenula, amygdala, lateral hypothalamus, ventral tegmental area, and rostromedial tegmental nucleus, with most pronounced effects in the hippocampus. Endocannabinoid levels, measured by mass spectrometry, were also altered in this structure. Chromatin immunoprecipitation followed by qPCR in the hippocampus revealed that two activating histone marks, H3K4Me3 and H3K27Ac, were enriched at specific endocannabinoid genes following cocaine intake. Targeting CB1 receptors using chromosome conformation capture, we highlighted spatial chromatin re-organization in the hippocampus, as well as in the nucleus accumbens, suggesting that destabilization of the chromatin may contribute to neuronal responses to cocaine. Overall, our results highlight a key role for the hippocampus in cocaine-induced plasticity and broaden the understanding of neuronal alterations associated with endocannabinoid signaling. The latter suggests that epigenetic

modifications contribute to maladaptive behaviors associated with chronic drug use.
Mol Neurobiol, 2022; 59
33441541: Alcalá-Vida R, Seguin J, Lotz C, Molitor AM, Irastorza-Azcarate I, Awada A, Karasu N, Bombardier A, Cosquer B, Skarmeta JLG, Cassel JC, Boutillier AL, Sexton T, Merienne K
Age-related and disease locus-specific mechanisms contribute to early remodelling of chromatin structure in Huntington's disease mice.
Temporal dynamics and mechanisms underlying epigenetic changes in Huntington's disease (HD), a neurodegenerative disease primarily affecting the striatum, remain unclear. Using a slowly progressing knockin mouse model, we profile the HD striatal chromatin landscape at two early disease stages. Data integration with cell type-specific striatal enhancer and transcriptomic databases demonstrates acceleration of age-related epigenetic remodelling and transcriptional changes at neuronal- and glial-specific genes from prodromal stage, before the onset of motor deficits. We also find that 3D chromatin architecture, while generally preserved at neuronal enhancers, is altered at the disease locus. Specifically, we find that the HD mutation, a CAG expansion in the Htt gene, locally impairs the spatial chromatin organization and proximal gene regulation. Thus, our data provide evidence for two early and distinct mechanisms underlying chromatin structure changes in the HD striatum, correlating with transcriptional changes: the HD mutation globally accelerates age-dependent epigenetic and transcriptional reprogramming of brain cell identities, and locally affects 3D chromatin organization.
Nat Commun, 2021; 12
33369245: Alcalá-Vida R, Garcia-Forn M, Castany-Pladevall C, Creus-Muncunill J, Ito Y, Blanco E, Golbano A, Crespí-Vázquez K, Parry A, Slater G, Samarajiwa S, Peiró S, Di Croce L, Narita M, Pérez-Navarro E
Neuron type-specific increase in lamin B1 contributes to nuclear dysfunction in Huntington's disease.
Lamins are crucial proteins for nuclear functionality. Here, we provide new evidence showing that increased lamin B1 levels contribute to the pathophysiology of Huntington's disease (HD), a CAG repeat-associated neurodegenerative disorder. Through fluorescence-activated nuclear suspension imaging, we show that nucleus from striatal medium-sized spiny and CA1 hippocampal neurons display increased lamin B1 levels, in correlation with altered nuclear morphology and nucleocytoplasmic transport disruption. Moreover, ChIP-sequencing analysis shows an alteration of lamin-associated chromatin domains in hippocampal nuclei, accompanied by changes in chromatin accessibility and transcriptional dysregulation. Supporting lamin B1 alterations as a causal role in mutant huntingtin-mediated neurodegeneration, pharmacological normalization of lamin B1 levels in the hippocampus of the R6/1 mouse model of HD by betulinic acid administration restored nuclear homeostasis and prevented motor and cognitive dysfunction. Collectively, our work points increased lamin B1 levels as a new pathogenic mechanism in HD and provides a novel target for its intervention.
EMBO Mol Med, 2021; 13
33127472: Alcalà-Vida R, Awada A, Boutillier AL, Merienne K
Epigenetic mechanisms underlying enhancer modulation of neuronal identity, neuronal activity and neurodegeneration.
Neurodegenerative diseases, including Huntington's disease (HD) and Alzheimer's disease (AD), are progressive conditions characterized by selective, disease-dependent loss of neuronal regions and/or subpopulations. Neuronal loss is preceded by a long period of neuronal dysfunction, during which glial cells also undergo major changes, including neuroinflammatory response. Those dramatic changes affecting both neuronal and glial cells associate with epigenetic and transcriptional dysregulations, characterized by defined cell-type-specific signatures. Notably, increasing studies support the view that altered regulation of transcriptional enhancers, which are distal regulatory regions of the genome capable of modulating the activity of promoters through chromatin looping, play a critical role in transcriptional dysregulation in HD and AD. We review current knowledge on enhancers in HD and AD, and highlight challenging issues to better decipher the epigenetic code of neurodegenerative diseases.
Neurobiol Dis, 2021; 147
29460266: Creus-Muncunill J, Rué L, Alcalá-Vida R, Badillos-Rodríguez R, Romaní-Aumedes J, Marco S, Alberch J, Perez-Otaño I, Malagelada C, Pérez-Navarro E
Increased Levels of Rictor Prevent Mutant Huntingtin-Induced Neuronal Degeneration.
Rictor associates with mTOR to form the mTORC2 complex, which activity regulates neuronal function and survival. Neurodegenerative diseases are characterized by the presence of neuronal dysfunction and cell death in specific brain regions such as for example Huntington's disease (HD), which is characterized by the loss of striatal projection neurons leading to motor dysfunction. Although HD is caused by the expression of mutant huntingtin, cell death occurs gradually suggesting that neurons have the capability to activate compensatory mechanisms to deal with neuronal dysfunction and later cell death. Here, we analyzed whether mTORC2 activity could be altered by the presence of mutant huntingtin. We observed that Rictor levels are specifically increased in the striatum of HD mouse models and in the putamen of HD patients. Rictor-mTOR interaction and the phosphorylation levels of Akt, one of the targets of the mTORC2 complex, were increased in the striatum of the R6/1 mouse model of HD suggesting increased mTORC2 signaling. Interestingly, acute downregulation of

Rictor in striatal cells in vitro reduced mTORC2 activity, as shown by reduced levels of phospho-Akt, and increased mutant huntingtin-induced cell death. Accordingly, overexpression of Rictor increased mTORC2 activity counteracting cell death. Furthermore, normalization of endogenous Rictor levels in the striatum of R6/1 mouse worsened motor symptoms suggesting an induction of neuronal dysfunction. In conclusion, our results suggest that increased Rictor striatal levels could counteract neuronal dysfunction induced by mutant huntingtin.
Mol Neurobiol, 2018; 55
28130160: Saavedra A, Fernández-García S, Cases S, Puigdellívol M, Alcalá-Vida R, Martín-Flores N, Alberch J, Ginés S, Malagelada C, Pérez-Navarro E
Chelerythrine promotes Ca-dependent calpain activation in neuronal cells in a PKC-independent manner.
Chelerythrine is widely used as a broad range protein kinase C (PKC) inhibitor, but there is controversy about its inhibitory effect. Moreover, it has been shown to exert PKC-independent effects on non-neuronal cells.
Biochim Biophys Acta Gen Subj, 2017; 1861
27721240: Rué L, Bañez-Coronel M, Creus-Muncunill J, Giralt A, Alcalá-Vida R, Mentxaka G, Kagerbauer B, Zomeño-Abellán MT, Aranda Z, Venturi V, Pérez-Navarro E, Estivill X, Martí E
Targeting CAG repeat RNAs reduces Huntington's disease phenotype independently of huntingtin levels.
Huntington's disease (HD) is a polyglutamine disorder caused by a CAG expansion in the Huntingtin (HTT) gene exon 1. This expansion encodes a mutant protein whose abnormal function is traditionally associated with HD pathogenesis; however, recent evidence has also linked HD pathogenesis to RNA stable hairpins formed by the mutant HTT expansion. Here, we have shown that a locked nucleic acid-modified antisense oligonucleotide complementary to the CAG repeat (LNA-CTG) preferentially binds to mutant HTT without affecting HTT mRNA or protein levels. LNA-CTGs produced rapid and sustained improvement of motor deficits in an R6/2 mouse HD model that was paralleled by persistent binding of LNA-CTG to the expanded HTT exon 1 transgene. Motor improvement was accompanied by a pronounced recovery in the levels of several striatal neuronal markers severely impaired in R6/2 mice. Furthermore, in R6/2 mice, LNA-CTG blocked several pathogenic mechanisms caused by expanded CAG RNA, including small RNA toxicity and decreased Rn45s expression levels. These results suggest that LNA-CTGs promote neuroprotection by blocking the detrimental activity of CAG repeats within HTT mRNA. The present data emphasize the relevance of expanded CAG RNA to HD pathogenesis, indicate that inhibition of HTT expression is not required to reverse motor deficits, and further suggest a therapeutic potential for LNA-CTG in polyglutamine disorders.
J Clin Invest, 2016; 126
26784526: Bosch M, Fajardo A, Alcalá-Vida R, Fernández-Vidal A, Tebar F, Enrich C, Cardellach F, Pérez-Navarro E, Pol A
Hepatic Primary and Secondary Cholesterol Deposition and Damage in Niemann-Pick Disease.
Niemann-Pick C disease is a neurovisceral disorder caused by mutations in the NPC gene that result in systemic accumulation of intracellular cholesterol. Although neurodegeneration defines the disease's severity, in most patients it is preceded by hepatic complications such as cholestatic jaundice or hepatomegaly. To analyze the contribution of the hepatic disease in Niemann-Pick C disease progression and to evaluate the degree of primary and secondary hepatic damage, we generated a transgenic mouse with liver-selective expression of NPC1 from embryonic stages. Hepatic NPC1 re-expression did not ameliorate the onset and progression of neurodegeneration of the NPC1-null animal. However, the mice showed reduced hepatomegalia and dramatic, although not complete, reduction of hepatic cholesterol and serum bile salts, bilirubin, and transaminase levels. Therefore, hepatic primary and secondary cholesterol deposition and damage occur simultaneously during Niemann-Pick C disease progression.
Am J Pathol, 2016; 186
23896721: Rué L, Alcalá-Vida R, López-Soop G, Creus-Muncunill J, Alberch J, Pérez-Navarro E
Early down-regulation of PKCδ as a pro-survival mechanism in Huntington's disease.
A balance between cell survival and apoptosis is crucial to avoid neurodegeneration. Here, we analyzed whether the pro-apoptotic protein PKCδ, and the pro-survival PKCα and βII, were dysregulated in the brain of R6/1 mouse model of Huntington's disease (HD). Protein levels of the three PKCs examined were reduced in all the brain regions analyzed being PKCδ the most affected isoform. Interestingly, PKCδ protein levels were also decreased in the striatum and cortex of R6/2 and Hdh(Q111/Q111) mice, and in the putamen of HD patients. Nuclear PKCδ induces apoptosis, but we detected reduced PKCδ in both cytoplasmic and nuclear enriched fractions from R6/1 mouse striatum, cortex and hippocampus. In addition, we show that phosphorylation and ubiquitination of PKCδ are increased in 30-week-old R6/1 mouse brain. All together these results suggest a pro-survival role of reduced PKCδ levels in response to mutant huntingtin-induced toxicity. In fact, we show that over-expression of PKCδ increases mutant huntingtin-induced cell death in vitro, whereas over-expression of a PKCδ dominant negative form or silencing of endogenous PKCδ partially blocks mutant huntingtin-induced cell death. Finally, we show that the analysis of lamin B protein levels could be a good marker of PKCδ activity, but it is not involved in PKCδ-mediated cell death in mutant huntingtin-expressing cells. In conclusion, our results suggest that neurons increase the

degradation of PKCδ as a compensatory pro-survival mechanism in response to mutant huntingtin-induced toxicity that can help to understand why cell death appears late in the disease.
Neuromolecular Med, 2014; 16
28603025: Giralt A, Gómez-Climent MÁ, Alcalá R, Bretin S, Bertrand D, María Delgado-García J, Pérez-Navarro E, Alberch J, Gruart A
The AMPA receptor positive allosteric modulator S 47445 rescues in vivo CA3-CA1 long-term potentiation and structural synaptic changes in old mice.
Positive allosteric modulators of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) are small molecules that decrease deactivation of AMPARs via an allosteric site. These molecules keep the receptor in an active state. Interestingly, this type of modulator has been proposed for treating cognitive decline in ageing, dementias, and Alzheimer's disease (AD). S 47445 (8-cyclopropyl-3-[2-(3-fluorophenyl)ethyl]-7,8-dihydro-3H-[1,3]oxazino[6,5-g][1,2,3]benzotriazine-4,9-dione) is a novel AMPAR positive allosteric modulator (AMPA-PAM). Here, the mechanisms by which S 47445 could improve synaptic strength and connectivity were studied and compared between young and old mice. A single oral administration of S 47445 at 10 mg/kg significantly increased long-term potentiation (LTP) in CA3-CA1 hippocampal synapses in alert young mice in comparison to control mice. Moreover, chronic treatment with S 47445 at 10 mg/kg in old alert animals significantly counteracted the deficit of LTP due to age. Accordingly, chronic treatment with S 47445 at 10 mg/kg seems to preserve synaptic cytoarchitecture in old mice as compared with young control mice. It was shown that the significant decreases in number and size of pre-synaptic buttons stained for VGlut1, and post-synaptic dendritic spines stained for spinophilin, observed in old mice were significantly prevented after chronic treatment with 10 mg/kg of S 47445. Altogether, by its different effects on LTP, VGlut1-positive particles, and spinophilin, S 47445 is able to modulate both the structure and function of hippocampal excitatory synapses known to be involved in learning and memory processes. These results open a new window for the treatment of specific age-dependent cognitive decline and dementias such as AD.
Neuropharmacology, 2017; 123

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-629

MHTT AGGREGATES AND NEUROINFLAMMATION IN THE HUNTINGTON'S DISEASE MIDCINGULATE CORTEX

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Aims: Huntington's disease (HD) is a neurodegenerative disorder that can result in motor, mood, and cognitive symptoms. HD mood symptomatology correlates with neuronal death in the cingulate cortex. Neuroinflammation, involving reactive glial cells and inflammatory mediators in the brain parenchyma, may influence HD pathophysiology. Accumulation of mutant huntingtin (mHTT) aggregates has also been linked to neuroinflammation and neuronal loss. Importantly, the degree to which these neuroinflammatory changes are detrimental to neurons and contribute to HD pathology progression is not well understood. **Methods:** Using fluorescent immunohistochemistry, we labeled HD and control post-mortem human midcingulate cortex tissue with HLA DP/DQ/DR, an inflammatory marker, and Iba-1, labeling microglia. We qualitatively and quantitatively assessed activation and morphology changes, indicating neuroinflammation, and mHTT levels - linking neuroinflammation and mHTT burden. **Results:** We found increased activated microglial morphologies across all HD cases (53.82%), and increased ramified microglia in control cases (67.41%). HD cases showed a decreased number of ramified and ameboid microglia. Activated microglia were localized close to neurons containing mHTT aggregates in HD cases, which positively correlated with mHTT burden. The total microglia number did not increase in HD cases. **Conclusions:** Total microglia number remaining constant between HD and control cases suggests ramified microglia change to activated states in HD, increasing neuroinflammation. This data indicates an association between mHTT burden and neuroinflammation in HD.

Pubmed:
34728681: Ethiraj J, Palpagama TH, Turner C, van der Werf B, Waldvogel HJ, Faull RLM, Kwakowsky A
The effect of age and sex on the expression of GABA signaling components in the human hippocampus and entorhinal cortex.
Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the nervous system. The GABA signaling system in the brain is comprised of GABA synthesizing enzymes, transporters, GABAA and GABAB receptors (GABAR and GABAR). Alterations in the expression of these signaling components have been observed in several brain regions throughout aging and between sexes in various animal models. The hippocampus is the memory centre of the brain and is impaired in several age-related disorders. It is composed of two main regions: the Cornu Ammonis (CA1-4) and the Dentate Gyrus (DG), which are interconnected with the Entorhinal Cortex (ECx). The age- and sex-specific changes of GABA signaling components in these regions of the human brain have not been examined. This study is the first to determine the effect of age and sex on the expression of GABA signaling components-GABAR α1,2,3,5, β1-3, γ2, GABAR R1 and R2 subunits and the GABA synthesizing enzymes GAD 65/67-in the ECx, and the CA1 and DG regions of the human hippocampus using Western blotting. No significant differences were found in GABAR α1,2,3,5, β1-3, γ2, GABAR R1 and R2 subunit and GAD65/76 expression levels in the ECx, CA1 and DG regions between the younger and older age groups for both sexes. However, we observed a significant negative correlation between age and GABAR α1subunit level in the CA1 region for females; significant negative correlation between age and GABAR β1, β3 and γ2 subunit expression in the DG region for males. In females a significant positive correlation was found between age and GABAR γ2 subunit expression in the ECx and GABAR R2 subunit expression in the CA1 region. The results indicate that age and sex do not affect the expression of GAD 65/67. In conclusion, our results show age- and sex-related GABAR subunit alterations in the ECx and hippocampus that might significantly influence GABAergic neurotransmission and underlie disease susceptibility and progression.
Sci Rep, 2021; 11
34588956: Yeung JHY, Palpagama TH, Wood OWG, Turner C, Waldvogel HJ, Faull RLM, Kwakowsky A
EAAT2 Expression in the Hippocampus, Subiculum, Entorhinal Cortex and Superior Temporal Gyrus in Alzheimer's Disease.

Alzheimer's disease (AD) is a neuropathological disorder characterized by the presence and accumulation of amyloid-beta plaques and neurofibrillary tangles. Glutamate dysregulation and the concept of glutamatergic excitotoxicity have been frequently described in the pathogenesis of a variety of neurodegenerative disorders and are postulated to play a major role in the progression of AD. In particular, alterations in homeostatic mechanisms, such as glutamate uptake, have been implicated in AD. An association with excitatory amino acid transporter 2 (EAAT2), the main glutamate uptake transporter, dysfunction has also been described. Several animal and few human studies examined EAAT2 expression in multiple brain regions in AD but studies of the hippocampus, the most severely affected brain region, are scarce. Therefore, this study aims to assess alterations in the expression of EAAT2 qualitatively and quantitatively through DAB immunohistochemistry (IHC) and immunofluorescence within the hippocampus, subiculum, entorhinal cortex, and superior temporal gyrus (STG) regions, between human AD and control cases. Although no significant EAAT2 density changes were observed between control and AD cases, there appeared to be increased transporter expression most likely localized to fine astrocytic branches in the neuropil as seen on both DAB IHC and immunofluorescence. Therefore, individual astrocytes are not outlined by EAAT2 staining and are not easily recognizable in the CA1-3 and dentate gyrus regions of AD cases, but the altered expression patterns observed between AD and control hippocampal cases could indicate alterations in glutamate recycling and potentially disturbed glutamatergic homeostasis. In conclusion, no significant EAAT2 density changes were found between control and AD cases, but the observed spatial differences in transporter expression and their functional significance will have to be further explored.
Front Cell Neurosci, 2021; 15
34563056: Chong J, Cheeseman JF, Pawley MDM, Kwakowsky A, Warman GR
The Effects of General Anaesthesia and Light on Behavioural Rhythms and GABA Receptor Subunit Expression in the Mouse SCN.
General anaesthesia (GA) is known to affect the circadian clock. However, the mechanisms that underlie GA-induced shifting of the clock are less well understood. Activation of γ-aminobutyric acid (GABA)type A receptors (GABAR) in the suprachiasmatic nucleus (SCN) can phase shift the clock and thus GABA and its receptors represent a putative pathway via which GA exerts its effect on the clock. Here, we investigated the concurrent effects of the inhalational anaesthetic, isoflurane, and light, on mouse behavioural locomotor rhythms and on α1, β3, and γ2 GABAR subunit expression in the SCN of the mouse brain. Behavioural phase shifts elicited by exposure of mice to four hours of GA (2% isoflurane) and light (400 lux) (n = 60) were determined by recording running wheel activity rhythms in constant conditions (DD). Full phase response curves for the effects of GA + light on behavioural rhythms show that phase shifts persist in anaesthetized mice exposed to light. Daily variation was detected in all three GABAR subunits in LD 12:12. The γ2 subunit expression was significantly increased following GA in DD (compared to light alone) at times of large behavioural phase delays. We conclude that the phase shifting effect of light on the mouse clock is not blocked by GA administration, and that γ2 may potentially be involved in the phase shifting effect of GA on the clock. Further analysis of GABAR subunit expression in the SCN will be necessary to confirm its role.
Clocks Sleep, 2021; 3
34269494: Yeung JHY, Walby JL, Palpagama TH, Turner C, Waldvogel HJ, Faull RLM, Kwakowsky A
Glutamatergic receptor expression changes in the Alzheimer's disease hippocampus and entorhinal cortex.
Alzheimer's Disease (AD) is the leading form of dementia worldwide. Currently, the pathological mechanisms underlying AD are not well understood. Although the glutamatergic system is extensively implicated in its pathophysiology, there is a gap in knowledge regarding the expression of glutamate receptors in the AD brain. This study aimed to characterize the expression of specific glutamate receptor subunits in post-mortem human brain tissue using immunohistochemistry and confocal microscopy. Free-floating immunohistochemistry and confocal laser scanning microscopy were used to quantify the density of glutamate receptor subunits GluA2, GluN1, and GluN2A in specific cell layers of the hippocampal sub-regions, subiculum, entorhinal cortex, and superior temporal gyrus. Quantification of GluA2 expression in human post-mortem hippocampus revealed a significant increase in the stratum (str.) moleculare of the dentate gyrus (DG) in AD compared with control. Increased GluN1 receptor expression was found in the str. moleculare and hilus of the DG, str. oriens of the CA2 and CA3, str. pyramidale of the CA2, and str. radiatum of the CA1, CA2, and CA3 subregions and the entorhinal cortex. GluN2A expression was significantly increased in AD compared with control in the str. oriens, str. pyramidale, and str. radiatum of the CA1 subregion. These findings indicate that the expression of glutamatergic receptor subunits shows brain region-specific changes in AD, suggesting possible pathological receptor functioning. These results provide evidence of specific glutamatergic receptor subunit changes in the AD hippocampus and entorhinal cortex, indicating the requirement for further research to elucidate the pathophysiological mechanisms it entails, and further highlight the potential of glutamatergic receptor subunits as therapeutic targets.
Brain Pathol, 2021; 31
33433477: Kwakowsky A, Waldvogel HJ, Faull RLM

Therapeutic potential of alpha 5 subunit containing GABA receptors in Alzheimer's disease.
Neural Regen Res, 2021; 16
33318426: Kwakowsky A, Waldvogel HJ, Faull RL
The effects of amyloid-beta on hippocampal glutamatergic receptor and transporter expression.
Neural Regen Res, 2021; 16
33224025: Calvo-Flores Guzmán B, Elizabeth Chaffey T, Hansika Palpagama T, Waters S, Boix J, Tate WP, Peppercorn K, Dragunow M, Waldvogel HJ, Faull RLM, Kwakowsky A
The Interplay Between Beta-Amyloid 1-42 (Aβ)-Induced Hippocampal Inflammatory Response, p-tau, Vascular Pathology, and Their Synergistic Contributions to Neuronal Death and Behavioral Deficits.
Alzheimer's disease (AD), the most common chronic neurodegenerative disorder, has complex neuropathology. The principal neuropathological hallmarks of the disease are the deposition of extracellular β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) comprised of hyperphosphorylated tau (p-tau) protein. These changes occur with neuroinflammation, a compromised blood-brain barrier (BBB) integrity, and neuronal synaptic dysfunction, all of which ultimately lead to neuronal cell loss and cognitive deficits in AD. Aβ was stereotaxically administered bilaterally into the CA1 region of the hippocampi of 18-month-old male C57BL/6 mice. This study aimed to characterize, utilizing immunohistochemistry and behavioral testing, the spatial and temporal effects of Aβ on a broad set of parameters characteristic of AD: p-tau, neuroinflammation, vascular pathology, pyramidal cell survival, and behavior. Three days after Aβ injection and before significant neuronal cell loss was detected, acute neuroinflammatory and vascular responses were observed. These responses included the up-regulation of glial fibrillary acidic protein (GFAP), cell adhesion molecule-1 (PECAM-1, also known as CD31), fibrinogen labeling, and an increased number of activated astrocytes and microglia in the CA1 region of the hippocampus. From day 7, there was significant pyramidal cell loss in the CA1 region of the hippocampus, and by 30 days, significant localized up-regulation of p-tau, GFAP, Iba-1, CD31, and alpha-smooth muscle actin (α-SMA) in the Aβ-injected mice compared with controls. These molecular changes in Aβ-injected mice were accompanied by cognitive deterioration, as demonstrated by long-term spatial memory impairment. This study is reporting a comprehensive examination of a complex set of parameters associated with intrahippocampal administration of Aβ in mice, their spatiotemporal interactions and combined contribution to the disease progression. We show that a single Aβ injection can reproduce aspects of the inflammatory, vascular, and p-tau induced pathology occurring in the AD human brain that lead to cognitive deficits.
Front Mol Neurosci, 2020; 13
33218044: Govindpani K, Turner C, Waldvogel HJ, Faull RLM, Kwakowsky A
Impaired Expression of GABA Signaling Components in the Alzheimer's Disease Middle Temporal Gyrus.
γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter, playing a central role in the regulation of cortical excitability and the maintenance of the excitatory/inhibitory (E/I) balance. Several lines of evidence point to a remodeling of the cerebral GABAergic system in Alzheimer's disease (AD), with past studies demonstrating alterations in GABA receptor and transporter expression, GABA synthesizing enzyme activity and focal GABA concentrations in post-mortem tissue. AD is a chronic neurodegenerative disorder with a poorly understood etiology and the temporal cortex is one of the earliest regions in the brain to be affected by AD neurodegeneration. Utilizing NanoString nCounter analysis, we demonstrate here the transcriptional downregulation of several GABA signaling components in the post-mortem human middle temporal gyrus (MTG) in AD, including the GABA receptor α, α, α, α, β, β, β, δ, γ, γ, and θ subunits and the GABA receptor 2 (GABAR2) subunit. In addition to this, we note the transcriptional upregulation of the betaine-GABA transporter (BGT1) and GABA transporter 2 (GAT2), and the downregulation of the 67 kDa isoform of glutamate decarboxylase (GAD), the primary GABA synthesizing enzyme. The functional consequences of these changes require further investigation, but such alterations may underlie disruptions to the E/I balance that are believed to contribute to cognitive decline in AD.
Int J Mol Sci, 2020; 21
32491248: Yeung JHY, Calvo-Flores Guzmán B, Palpagama TH, Ethiraj J, Zhai Y, Tate WP, Peppercorn K, Waldvogel HJ, Faull RLM, Kwakowsky A
Amyloid-beta induced glutamatergic receptor and transporter expression changes in the mouse hippocampus.
Alzheimer's disease (AD) is the leading type of dementia worldwide. With an increasing burden of an aging population coupled with the lack of any foreseeable cure, AD warrants the current intense research effort on the toxic effects of an increased concentration of beta-amyloid (Aβ) in the brain. Glutamate is the main excitatory brain neurotransmitter and it plays an essential role in the function and health of neurons and neuronal excitability. While previous studies have shown alterations in expression of glutamatergic signaling components in AD, the underlying mechanisms of these changes are not well understood. This is the first comprehensive anatomical study to characterize the subregion- and cell layer-specific long-term effect of Aβ on the expression of specific glutamate receptors and transporters in the mouse hippocampus, using immunohistochemistry with confocal microscopy. Outcomes are examined 30 days after Aβ stereotactic injection in aged male C57BL/6 mice. We report significant decreases in density of the glutamate receptor subunit GluA1 and the

vesicular glutamate transporter (VGluT) 1 in the conus ammonis 1 region of the hippocampus in the Aβ injected mice compared with artificial cerebrospinal fluid injected and naïve controls, notably in the stratum oriens and stratum radiatum. GluA1 subunit density also decreased within the dentate gyrus dorsal stratum moleculare in Aβ injected mice compared with artificial cerebrospinal fluid injected controls. These changes are consistent with findings previously reported in the human AD hippocampus. By contrast, glutamate receptor subunits GluA2, GluN1, GluN2A, and VGluT2 showed no changes in expression. These findings indicate that Aβ induces brain region and layer specific expression changes of the glutamatergic receptors and transporters, suggesting complex and spatial vulnerability of this pathway during development of AD neuropathology. Read the Editorial Highlight for this article on page 7. Cover Image for this issue: https://doi.org/10.1111/jnc.14763.
J Neurochem, 2020; 155
32384683: Vinnakota C, Govindpani K, Tate WP, Peppercorn K, Anekal PV, Waldvogel HJ, Faull RLM, Kwakowsky A
An 5 GABAA Receptor Inverse Agonist, 5IA, Attenuates Amyloid Beta-Induced Neuronal Death in Mouse Hippocampal Cultures.
Alzheimer's disease (AD) is a progressive neurodegenerative disorder for which no cognition-restoring therapies exist. Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the brain. Increasing evidence suggests a remodeling of the GABAergic system in AD, which might represent an important therapeutic target. An inverse agonist of 5 subunit-containing GABAA receptors (α5GABAARs), 3-(5-Methylisoxazol-3-yl)-6-[(1-methyl-1,2,3-triazol-4-yl)methyloxy]-1,2,4-triazolo[3-]phthalazine (5IA) has cognition-enhancing properties. This study aimed to characterize the effects of 5IA on amyloid beta (A)-induced molecular and cellular changes. Mouse primary hippocampal cultures were exposed to either A alone, or 5IA alone, 5IA with A or vehicle alone, and changes in cell viability and mRNA expression of several GABAergic signaling components were assessed. Treatment with 100 nM of 5IA reduced A-induced cell loss by 23.8% (< 0.0001) after 6 h and by 17.3% after 5 days of treatment (< 0.0001). Furthermore, we observed an A-induced increase in ambient GABA levels, as well as upregulated mRNA expression of the GABAAR α2,α5,2/3 subunits and the GABABR R1 and R2 subunits. Such changes in GABARs expression could potentially disrupt inhibitory neurotransmission and normal network activity. Treatment with 5IA restored A-induced changes in the expression of α5GABAARs. In summary, this compound might hold neuroprotective potential and represent a new therapeutic avenue for AD.
Int J Mol Sci, 2020; 21

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BOARD NUMBER: S01-630

PREMATURE AGING OF NEURAL STEM CELLS IN HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Neural stem cells (NSC) in the adult Rodent subventricular zone (SVZ) generate neurons throughout life. Neurogenesis persists in the adult Human brain with new interneurons, probably born in the nearby SVZ, adding to the striatum. Interestingly, striatal neurogenesis decreases in Huntington's disease (HD) patients. HD is a fatal neurodegenerative disorder with an adult onset caused by an autosomal dominant mutation of the huntingtin gene. The mutation is an abnormal expansion of a polymorphic CAG repeat leading to an abnormal polyglutamine stretch at the N-terminus of the huntingtin protein (HTT). We showed that mutant HTT (mHTT) decreases SVZ neurogenesis by altering NSC properties. In vitro NSC demonstrate restricted potency and limited capacity to self-renew and proliferate, suggesting a decreased NSC pool. We further hypothesize that this reduction may be due to accelerated aging features observed in HD, triggering stem cells entry in senescence. Preliminary experiments indeed show that HD stem cells express high levels of p16 mRNA as compared to WT counterparts. We will perform further genetic and epigenetic analysis to screen changes in gene expression associated with stemness and aging. Understanding the regulation of NSC in the SVZ may help to design new therapies to sustain neurogenesis in the old and diseased brain.

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BOARD NUMBER: S01-631

BRAIN CONNECTIVITY IN HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Huntington’s disease (HD) is a neurodegenerative disorder characterized by the loss of neurons predominantly in the striatum and the cortex. It is caused by an abnormal CAG expansion in the first exon of the Huntingtin gene, which results in a polyglutamine expansion in the N-terminal part of the huntingtin protein (HTT). HD manifestations appear in adults, but growing evidence suggests a developmental contribution to HD. Neuronal tracts are affected in early-stage HD patients such as the corticospinal tract or the fornix, but no evidence has been found yet in mice models of HD. Using brain clearing approaches, we are investigating if these defects are established during development in the CAG140 mouse model of HD. We observed that tracts such as the anterior commissure and the post-commissural fornix may be altered during postnatal development. As the establishment of neuronal connectivity relies on growth cone dynamics, we are studying this process in cortical neurons using microfluidics devices. Overall, our project will describe the mechanisms underlying early alterations in circuit connectivity in the HD brain.

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BOARD NUMBER: S01-632

KCC2 FUNCTION IS ALTERED IN THE INDIRECT PATHWAY OF THE BASAL GANGLIA IN HUNTINGTON'S DISEASE.

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an inherited neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in Htt gene. The primary sites of degeneration are a group of nuclei called the basal ganglia which are essential for motor planning and voluntary movement. The basal ganglia consists of two major pathways: the direct and indirect, which promote and inhibit unwanted movement, respectively. The indirect pathway is known to degenerate earlier in HD, though the cause of this enhanced susceptibility remains unclear. Accumulating evidence suggests that impaired synaptic inhibition within the basal ganglia circuitry may contribute to this specific pattern of degeneration. Synaptic inhibition in the mature brain is largely mediated through GABA, which exerts fast hyperpolarizing inhibition by binding to Cl- -permeable GABAA receptors. GABAergic inhibition requires low intracellular Cl - concentration ([Cl-]i), which is maintained by the potassium-chloride cotransporter, KCC2. However, when KCC2 function is reduced, [Cl-]i increases, consequently weakening synaptic inhibition. Using electrophysiology and behavioural assays, we determined that KCC2 function was reduced in the indirect pathway of HD mice. In addition, GABAergic signaling was reversed in the output structure of the indirect pathway of early symptomatic HD mice. In addition, pharmacological reduction of intracellular Cl- with bumetanide delayed the onset of motor impairments in HD. This work demonstrates that the impaired GABAergic inhibition in the indirect pathway may be a key mechanism underlying circuitry and motor defects in HD, whereby KCC2 may serve as a potential therapeutic target in the treatment of HD.

Pubmed:

35095429: Serranilla M, Woodin MA
Striatal Chloride Dysregulation and Impaired GABAergic Signaling Due to Cation-Chloride Cotransporter Dysfunction in Huntington's Disease.
Intracellular chloride (Cl) levels in mature neurons must be tightly regulated for the maintenance of fast synaptic inhibition. In the mature central nervous system (CNS), synaptic inhibition is primarily mediated by gamma-amino butyric acid (GABA), which binds to Cl permeable GABA receptors (GABARs). The intracellular Cl concentration is primarily maintained by the antagonistic actions of two cation-chloride cotransporters (CCCs): Cl-importing Na-K-Cl co-transporter-1 (NKCC1) and Cl -exporting K-Cl co-transporter-2 (KCC2). In mature neurons in the healthy brain, KCC2 expression is higher than NKCC1, leading to lower levels of intracellular Cl, and Cl influx upon GABAR activation. However, in neurons of the immature brain or in neurological disorders such as epilepsy and traumatic brain injury, impaired KCC2 function and/or enhanced NKCC1 expression lead to intracellular Cl accumulation and GABA-mediated excitation. In Huntington's disease (HD), KCC2- and NKCC1-mediated Cl-regulation are also altered, which leads to GABA-mediated excitation and contributes to the development of cognitive and motor impairments. This review summarizes the role of Cl (dys)regulation in the healthy and HD brain, with a focus on the basal ganglia (BG) circuitry and CCCs as potential therapeutic targets in the treatment of HD.
Front Cell Neurosci, 2021; 15
30797895: Dargaei Z, Liang X, Serranilla M, Santos J, Woodin MA
Alterations in Hippocampal Inhibitory Synaptic Transmission in the R6/2 Mouse Model of Huntington's Disease.
Huntington's disease (HD) is a genetic neurodegenerative disorder of the central nervous system characterized by choreatic movements, behavioral and psychiatric disturbances and cognitive impairments. Deficits in learning and memory are often the first signs of disease onset in both HD patients and mouse models of HD and are in part regulated by the hippocampus. In the R6/2 mouse model of HD, GABAergic transmission can be excitatory in the hippocampus and restoring inhibition can rescue the associated memory deficits. In the present study we determine that hippocampal GABAergic neurotransmission in the R6/2 mouse is disrupted as early as 4 weeks of age and is accompanied by alterations in the expression of key inhibitory proteins. Specifically, spontaneous inhibitory postsynaptic currents were initially increased in frequency at 4 postnatal weeks and subsequently decreased after the mice displayed the typical R6/2 behavioral phenotype at 10 weeks of age. Symptomatic mice also exhibited a change in the probability of GABA release and changes in the basic membrane properties

including neuronal excitability and input resistance. These electrophysiological changes in presymptomatic and symptomatic R6/2 mice were further accompanied by alterations in the protein expression level of pre- and postsynaptic inhibitory markers. Taken together, the present findings demonstrate profound alterations in the inhibitory neurotransmission in the hippocampus across the lifespan of the disease, including prior to neuronal degeneration, which suggests that the inhibitory hippocampal synapses may prove useful as a target for future therapeutic design.
Neuroscience, 2019; 404

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FUNCTIONAL AND TRANSCRIPTOMIC ANALYSIS OF INDUCED PLURIPOTENT STEM CELL MICROGLIA IN HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

Nina Stöberl[1], Jasmine Donaldson[2], Thomas Massey[2], Hazel Hall-Roberts[3], Lesley Jones[2], Nicholas Allen[1]
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Huntington's disease (HD) is a neurodegenerative disorder caused by a dominantly inherited CAG repeat expansion in the huntingtin gene (*HTT*). Recent findings indicate an involvement of neuroinflammation in HD pathology, including several PET studies demonstrating that microglial activation correlates with disease severity in HD patients. Nonetheless, an open question is whether mutant *HTT* expression leads to cell-autonomous transcriptional and functional changes in microglia, which potentially contribute to disease onset and progression. We used a patient-derived induced pluripotent stem cell (iPSC) model of HD microglia with 109 CAG repeats, and isogenic controls with a corrected wild-type length of 22 repeats. Differentiated iPSC-derived myeloid precursors and microglia were characterized by flow cytometry and immunohistochemistry. Fundamental microglia phenotypes were investigated, including morphology, attachment to fibronectin, secreted cytokines, and phagocytosis of pHrodo-labelled *E. coli* beads. HD and control microglia underwent bulk RNA sequencing under basal conditions and after pro-inflammatory stimulation with IFNγ/LPS. We found that HD myeloid precursors exhibit an impaired attachment to fibronectin and differentiated HD microglia developed a less complex morphology compared to the controls. HD microglia showed an increased secretion of inflammatory-related cytokines, and additionally an impairment in phagocytosis, one of microglia's key functions. The transcriptome analysis revealed significant differences between HD and control microglia in the unstimulated state, and a divergent transcriptional response to pro-inflammatory stimulation. These observations indicate a cell-autonomous altered basal activation state of HD microglia independent of the HD brain environment and might highlight a role of microglia in HD onset and progression.

Pubmed:

34366778: Ruigrok SR, Stöberl N, Yam KY, de Lucia C, Lucassen PJ, Thuret S, Korosi A
Modulation of the Hypothalamic Nutrient Sensing Pathways by Sex and Early-Life Stress.
There are sex differences in metabolic disease risk, and early-life stress (ES) increases the risk to develop such diseases, potentially in a sex-specific manner. It remains to be understood, however, how sex and ES affect such metabolic vulnerability. The hypothalamus regulates food intake and energy expenditure by sensing the organism's energy state via metabolic hormones (leptin, insulin, ghrelin) and nutrients (glucose, fatty acids). Here, we investigated if and how sex and ES alter hypothalamic nutrient sensing short and long-term. ES was induced in mice by limiting the bedding and nesting material from postnatal day (P)2-P9, and the expression of genes critical for hypothalamic nutrient sensing were studied in male and female offspring, both at P9 and in adulthood (P180). At P9, we observed a sex difference in both and expression, while the latter was also increased in ES-exposed animals relative to controls. In adulthood, we found sex differences in , , and expression, whereas ES did not affect the expression of genes involved in hypothalamic nutrient sensing. Thus, we observe a pervasive sex difference in nutrient sensing pathways and a targeted modulation of this pathway by ES early in life. Future research is needed to address if the modulation of these pathways by sex and ES is involved in the differential vulnerability to metabolic diseases.
Front Neurosci, 2021; 15
33498469: Ruigrok SR, Abbink MR, Geertsema J, Kuindersma JE, Stöberl N, van der Beek EM, Lucassen PJ, Schipper L, Korosi A
Effects of Early-Life Stress, Postnatal Diet Modulation and Long-Term Western-Style Diet on Peripheral and Central Inflammatory Markers.
Early-life stress (ES) exposure increases the risk of developing obesity. Breastfeeding can markedly decrease this risk, and it is thought that the physical properties of the lipid droplets in human milk contribute to this benefit. A concept infant milk formula (IMF) has been developed that mimics these physical properties of human milk (Nuturis, N-IMF). Previously, we have

shown that N-IMF reduces, while ES increases, western-style diet (WSD)-induced fat accumulation in mice. Peripheral and central inflammation are considered to be important for obesity development. We therefore set out to test the effects of ES, Nuturis and WSD on adipose tissue inflammatory gene expression and microglia in the arcuate nucleus of the hypothalamus. ES was induced in mice by limiting the nesting and bedding material from postnatal day (P) 2 to P9. Mice were fed a standard IMF (S-IMF) or N-IMF from P16 to P42, followed by a standard diet (STD) or WSD until P230. ES modulated adipose tissue inflammatory gene expression early in life, while N-IMF had lasting effects into adulthood. Centrally, ES led to a higher microglia density and more amoeboid microglia at P9. In adulthood, WSD increased the number of amoeboid microglia, and while ES exposure increased microglia coverage, Nuturis reduced the numbers of amoeboid microglia upon the WSD challenge. These results highlight the impact of the early environment on central and peripheral inflammatory profiles, which may be key in the vulnerability to develop metabolic derangements later in life.
Nutrients, 2021; 13

31201266: Galino J, Cervellini I, Zhu N, Stöberl N, Hütte M, Fricker FR, Lee G, McDermott L, Lalli G, Bennett DLH
RalGTPases contribute to Schwann cell repair after nerve injury via regulation of process formation.
RalA and RalB are small GTPases that are involved in cell migration and membrane dynamics. We used transgenic mice in which one or both GTPases were genetically ablated to investigate the role of RalGTPases in the Schwann cell (SC) response to nerve injury and repair. RalGTPases were dispensable for SC function in the naive uninjured state. Ablation of both RalA and RalB (but not individually) in SCs resulted in impaired axon remyelination and target reinnervation following nerve injury, which resulted in slowed recovery of motor function. Ral GTPases were localized to the leading lamellipodia in SCs and were required for the formation and extension of both axial and radial processes of SCs. These effects were dependent on interaction with the exocyst complex and impacted on the rate of SC migration and myelination. Our results show that RalGTPases are required for efficient nerve repair by regulating SC process formation, migration, and myelination, therefore uncovering a novel role for these GTPases.
J Cell Biol, 2019; 218

34274734: Ruigrok SR, Yim K, Emmerzaal TL, Geenen B, Stöberl N, den Blaauwen JL, Abbink MR, Kiliaan AJ, van Schothorst EM, Kozicz T, Korosi A
Effects of early-life stress on peripheral and central mitochondria in male mice across ages.
Exposure to early-life stress (ES) increases the vulnerability to develop metabolic diseases as well as cognitive dysfunction, but the specific biological underpinning of the ES-induced programming is unknown. Metabolic and cognitive disorders are often comorbid, suggesting possible converging underlying pathways. Mitochondrial dysfunction is implicated in both metabolic diseases and cognitive dysfunction and chronic stress impairs mitochondrial functioning. However, if and how mitochondria are impacted by ES and whether they are implicated in the ES-induced programming remains to be determined. ES was applied by providing mice with limited nesting and bedding material from postnatal day (P)2-P9, and metabolic parameters, cognitive functions and multiple aspects of mitochondria biology (i.e. mitochondrial electron transport chain (ETC) complex activity, mitochondrial DNA copy number, expression of genes relevant for mitochondrial function, and the antioxidant capacity) were studied in muscle, hypothalamus and hippocampus at P9 and late adulthood (10-12 months of age). We show that ES altered bodyweight (gain), adiposity and glucose levels at P9, but not in late adulthood. At this age, however, ES exposure led to cognitive impairments. ES affected peripheral and central mitochondria in an age-dependent manner. At P9, both muscle and hypothalamic ETC activity were affected by ES, while in hippocampus, ES altered the expression of genes involved in fission and antioxidant defence. In adulthood, alterations in ETC complex activity were observed in the hypothalamus specifically, whereas in muscle and hippocampus ES affected the expression of genes involved in mitophagy and fission, respectively. Our study demonstrates that ES affects peripheral and central mitochondria biology throughout life, thereby uncovering a converging mechanism that might contribute to the ES-induced vulnerability for both metabolic diseases and cognitive dysfunction, which could serve as a novel target for intervention.
Psychoneuroendocrinology, 2021; 132

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AXONAL VESICLES ARE PENTOSE PHOSPHATE PATHWAY MOBILE PLATFORMS CRUCIAL FOR ROS DETOXIFICATION AND NEURONAL SURVIVAL

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a rare hereditary neurodegenerative pathology inducing motor, cognitive and/or psychiatric disorders. This disease is caused by an abnormal polyQ expansion in the N-terminal part of the huntingtin protein. We have demonstrated that the cortico-striatal connection is affected in HD, especially the fast axonal transport (FAT) of BDNF vesicles. Recent studies of the team emphasized that glycolysis is crucial for FAT in neurons. The team has observed by quantitative proteomic analysis that not only glycolysis is present on vesicles but also the pentose phosphate pathway (PPP). This metabolic pathway is derived from glycolysis and allows ROS detoxification through NADPH production. This is why it is mostly used by neurons upon oxidative stress. However, nothing is known for the moment about the presence and the role of these PPP enzymes on vesicles. Using biochemical approaches and super-resolution microscopy on microfluidic brain-on-a-chip devices, we observe that PPP enzymes like glucose-6 phosphate dehydrogenase (G6PD) are selectively associated with vesicles. Their vesicular localization is increased upon oxidative stress and their enzymatic activity is essential for their recruitment on vesicles. We have also shown that G6PD regulates the FAT of vesicles and maintains the antioxidant status inside the axons. Oxidative stress plays a central role in the pathophysiology of HD. Exploring the antioxidant role of vesicular PPP enzymes in HD is currently under progress. Altogether, these findings highlight the novel and crucial role of PPP enzymes during FAT and could provide key information on new therapeutic strategies in HD.

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A PLASMA SMALL RNA BIOSIGNATURE IDENTIFIES PREMANIFEST HUNTINGTON´S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Despite the advances in the understanding of Huntington's disease (HD), no disease-modifying treatments exist, and therapeutic development and HD-clinical trials continue to fail. Major efforts are being invested in the assessment of measurable outcomes in early diagnosis and prognosis for optimal therapeutic response. Recent insights on Huntington's disease have pointed to a profound role of RNA in the neuropathogenesis of the disorder. Specifically, growing evidence indicates that small non-coding RNA (sRNA) are key players in the disease. The profiling of extracellular sRNAs (exRNA), found in body fluids as freely circulating, associated to protein-complexes, and/or encapsulated in extracellular vesicles (EVs), supposes a promising approach for defining non-invasive biomarkers that reflect disease status. Using an optimal method for plasma sub-fractionation and EVs purification by Size-exclusion chromatography (SEC) and Ultrafiltration (UF), we explored sRNA content in EVs and Non-EVs compartments, providing a deep exRNA analysis and offering a complementary source of valuable information. Characterization of plasma-EVs from three different cohorts, including healthy controls, premanifest HD, and manifest HD, revealed no differences in size and morphology of EVs. Using SeqCluster bioinformatic tool for sRNA annotation and quantification, we highlighted that most differentially expressed sRNAs in HD-EVs are downregulated in comparison to Control-EVs, with many changes occurring at premanifest stages. Those sRNAs showing the most differential profile between groups were validated as potential future biomarkers for HD. These findings suggest that alterations in circulating exRNAs may reflect early clinical and pathological changes in HD patients.

Pubmed:
33171576: Gámez-Valero A, Guisado-Corcoll A, Herrero-Lorenzo M, Solaguren-Beascoa M, Martí E
Non-Coding RNAs as Sensors of Oxidative Stress in Neurodegenerative Diseases.
Oxidative stress (OS) results from an imbalance between the production of reactive oxygen species and the cellular antioxidant capacity. OS plays a central role in neurodegenerative diseases, where the progressive accumulation of reactive oxygen species induces mitochondrial dysfunction, protein aggregation and inflammation. Regulatory non-protein-coding RNAs (ncRNAs) are essential transcriptional and post-transcriptional gene expression controllers, showing a highly regulated expression in space (cell types), time (developmental and ageing processes) and response to specific stimuli. These dynamic changes shape signaling pathways that are critical for the developmental processes of the nervous system and brain cell homeostasis. Diverse classes of ncRNAs have been involved in the cell response to OS and have been targeted in therapeutic designs. The perturbed expression of ncRNAs has been shown in human neurodegenerative diseases, with these changes contributing to pathogenic mechanisms, including OS and associated toxicity. In the present review, we summarize existing literature linking OS, neurodegeneration and ncRNA function. We provide evidences for the central role of OS in age-related neurodegenerative conditions, recapitulating the main types of regulatory ncRNAs with roles in the normal function of the nervous system and summarizing up-to-date information on ncRNA deregulation with a direct impact on OS associated with major neurodegenerative conditions.
Antioxidants (Basel), 2020; 9

FENS Forum 2022
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BOARD NUMBER: S01-636

CONNECTING METABOLICS AND EPIGENETICS DYSREGULATIONS IN HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an incurable, genetic, neurodegenerative disease that damages primarily the striatum and leads to motor, cognitive and psychiatric symptoms. The HD striatum undergoes early specific epigenetic gene reprograming, progressively leading to impairment of striatal neuron identity and function. Notably, the mechanism implicates decreased H3K27 acetylation (H3K27ac) at neuronal identity genes. Energy metabolism is also early impaired in the HD striatum. Since histone acetylation is strongly connected to energy metabolism through Acetyl-CoA (ACoA), the only donor of acetyl group for histone acetylation, altered energy metabolism might underlie H3K27ac dysregulation in HD striatal neurons. The aim of the project is to explore the hypothesis that deficient energy metabolism interplays with histone acetylation changes in the HD striatum, impairing ACoA regulation. Using HD mouse models, we further characterize HD striatal histone acetylome, implementing ChIPseq/CUT&Tag approach at cell-type-specific level (in sorted striatal neurons). Our preliminary data indicate that H3K9ac and H3K18ac are also dysregulated in HD striatal neurons, supporting broad impairment of histone acetylome. Furthermore, our preliminary proteomic analyses on histones extracted from HD mouse striatum suggest that additional acylation marks may be modified by the HD mutation. Finally, our molecular and histological analyses indicate that key regulators of nuclear ACoA production, *e.g.* ATP citrate lyase (Acly), Acyl-CoA synthetase (Acss2) and pyruvate-dehydrogenase complex (PDC), are dysregulated in HD mouse striatum. Together, this supports the hypothesis of interplay between altered energy metabolism and histone acylome dysregulation in the HD striatum.

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BOARD NUMBER: S01-637

NOVEL OPTOGENETIC TOOLS TO MODULATE CAMP IN NEURONS: EFFECTS ON HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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In neurons, cAMP modulate metabotropic responses and induce many intracellular signalling pathways, including synaptic plasticity. Phytochrome photoreceptors can act as adenylate cyclase and produce cAMP in cells upon photoactivation, allowing spatio-temporal modulation of cAMP, which can be particularly relevant in neurodegenerative diseases such as Huntington's disease (HD). HD is characterized by motor disturbances, associated to a progressive disconnection of the cortico-striatal circuitry. Therefore, our main goal is to evaluate the potential of phytochromes as a novel tool to induce long-term neuronal plasticity in HD's specific brain circuits. We first explored cAMP levels at distinct brain regions and along HD progression by ELISA. Then, we evaluated how cAMP modulates neuronal activity dynamics by analysing Fluo4 calcium fluorescence intensity changes, as well as individual and collective spontaneous neuronal activity by Forskolin application, in WT and R6/1 mice primary cortical cultures at 14 DIV, using the NETCAL software. We observed that Forskolin increases the number of neurons firing collectively, while single neuronal activity (number of spikes, ISI, IBI) remains unaltered. Moreover, this effect was not observed in HD cultures. Accordingly, primary cultures from WT and HD were infected at 7 DIV with an AAV expressing Phytochromes (AAV9-CamKII-DdPAC-Flag-tag) and calcium dynamics induced by phytochrome activation are currently being analysed. We are also implementing fiber photometry tools to study phytochrome effects in vivo by using the calcium sensor GCamp6f and/or cAMP sensor Pink Flamindo. Altogether, these results contribute to the development of new approaches towards modulating brain activity and uncover circuit dynamics in Huntington's disease.

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BOARD NUMBER: S01-638

INVOLVEMENT OF TRNA FRAGMENTATION IN THE PATHOPHYSIOLOGY OF HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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[1]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Biomedicine, Barcelona, Spain, [2]Spanish Ministry of Science and Innovation, Centro De Investigación Biomédica En Red De Enfermedades Neurodegenerativas (ciberned), Madrid, Spain, [3]Institute of Neurosciences, University of Barcelona, Department Of Biomedicine, Barcelona, Spain, [4]Icahn School of Medicine at Mount Sinai, Department Of Neurology, New York, United States of America, [5]Spanish Ministry of Science and Innovation, Biomedical Research Networking Center For Epidemiology And Public Health (ciberesp), Madrid, Spain

Progressive motor alterations and selective death of medium-sized spiny neurons in the caudate and putamen are key pathological hallmarks of Huntington's disease (HD), a neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the coding region of the huntingtin (*HTT*) gene. Growing evidence indicates that expanded CAG repeats within mutant *HTT* mRNA and derived small CAG repeat RNAs (sCAG) participate in HD pathophysiology. However, the role of other classes of small RNAs (sRNA) that are strongly perturbed in HD is uncertain. Our most recent data indicate that sRNA produced in the putamen of HD patients are sufficient to induce HD pathology *in vivo*. This observation prompted us to deeply characterize the sRNA transcriptome and identify which sRNA species are enriched in HD putamen and show neurotoxic potential. Specifically, we have observed a massive increase in tRNA fragments (tRFs). tRFs are bioactive molecules that regulate gene expression at multiple levels, whose biogenesis is linked with cellular stress and regulated by post-transcriptional modifications. Our analyses suggest that many of the tRFs over-represented in HD are dependent on the methylation status of the precursor, mature tRNAs. Validating these results, we have detected altered expression of enzymes regulating tRNA post-transcriptional modifications in HD human and mouse brains. We have also studied the potential modifications in the tRNAs, which provides an additional source of pathogenic alterations in HD. These results highlight that multiple sRNA species are contributing to striatal neuropathology, favouring therapeutic strategies based on the blockage of sRNA biogenesis and/or toxic activity.

Pubmed:
31365052: Creus-Muncunill J, Badillos-Rodríguez R, Garcia-Forn M, Masana M, Garcia-Díaz Barriga G, Guisado-Corcoll A, Alberch J, Malagelada C, Delgado-García JM, Gruart A, Pérez-Navarro E
Increased translation as a novel pathogenic mechanism in Huntington's disease.
Huntington's disease is a neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the huntingtin gene. Striatal projection neurons are mainly affected, leading to motor symptoms, but molecular mechanisms involved in their vulnerability are not fully characterized. Here, we show that eIF4E binding protein (4E-BP), a protein that inhibits translation, is inactivated in Huntington's disease striatum by increased phosphorylation. Accordingly, we detected aberrant de novo protein synthesis. Proteomic characterization indicates that translation specifically affects sets of proteins as we observed upregulation of ribosomal and oxidative phosphorylation proteins and downregulation of proteins related to neuronal structure and function. Interestingly, treatment with the translation inhibitor 4EGI-1 prevented R6/1 mice motor deficits, although corticostriatal long-term depression was not markedly changed in behaving animals. At the molecular level, injection of 4EGI-1 normalized protein synthesis and ribosomal content in R6/1 mouse striatum. In conclusion, our results indicate that dysregulation of protein synthesis is involved in mutant huntingtin-induced striatal neuron dysfunction.
Brain, 2019; 142
33171576: Gámez-Valero A, Guisado-Corcoll A, Herrero-Lorenzo M, Solaguren-Beascoa M, Martí E
Non-Coding RNAs as Sensors of Oxidative Stress in Neurodegenerative Diseases.
Oxidative stress (OS) results from an imbalance between the production of reactive oxygen species and the cellular antioxidant capacity. OS plays a central role in neurodegenerative diseases, where the progressive accumulation of reactive oxygen species induces mitochondrial dysfunction, protein aggregation and inflammation. Regulatory non-protein-coding RNAs (ncRNAs) are essential transcriptional and post-transcriptional gene expression controllers, showing a highly regulated

expression in space (cell types), time (developmental and ageing processes) and response to specific stimuli. These dynamic changes shape signaling pathways that are critical for the developmental processes of the nervous system and brain cell homeostasis. Diverse classes of ncRNAs have been involved in the cell response to OS and have been targeted in therapeutic designs. The perturbed expression of ncRNAs has been shown in human neurodegenerative diseases, with these changes contributing to pathogenic mechanisms, including OS and associated toxicity. In the present review, we summarize existing literature linking OS, neurodegeneration and ncRNA function. We provide evidences for the central role of OS in age-related neurodegenerative conditions, recapitulating the main types of regulatory ncRNAs with roles in the normal function of the nervous system and summarizing up-to-date information on ncRNA deregulation with a direct impact on OS associated with major neurodegenerative conditions.
Antioxidants (Basel), 2020; 9
33547932: Creus-Muncunill J, Guisado-Corcoll A, Venturi V, Pantano L, Escaramís G, García de Herreros M, Solaguren-Beascoa M, Gámez-Valero A, Navarrete C, Masana M, Llorens F, Diaz-Lucena D, Pérez-Navarro E, Martí E
Huntington's disease brain-derived small RNAs recapitulate associated neuropathology in mice.
Progressive motor alterations and selective death of striatal medium spiny neurons (MSNs) are key pathological hallmarks of Huntington's disease (HD), a neurodegenerative condition caused by a CAG trinucleotide repeat expansion in the coding region of the huntingtin (HTT) gene. Most research has focused on the pathogenic effects of the resultant protein product(s); however, growing evidence indicates that expanded CAG repeats within mutant HTT mRNA and derived small CAG repeat RNAs (sCAG) participate in HD pathophysiology. The individual contribution of protein versus RNA toxicity to HD pathophysiology remains largely uncharacterized and the role of other classes of small RNAs (sRNA) that are strongly perturbed in HD is uncertain. Here, we demonstrate that sRNA produced in the putamen of HD patients (HD-sRNA-PT) are sufficient to induce HD pathology in vivo. Mice injected with HD-sRNA-PT show motor abnormalities, decreased levels of striatal HD-related proteins, disruption of the indirect pathway, and strong transcriptional abnormalities, paralleling human HD pathology. Importantly, we show that the specific blockage of sCAG mitigates HD-sRNA-PT neurotoxicity only to a limited extent. This observation prompted us to identify other sRNA species enriched in HD putamen with neurotoxic potential. We detected high levels of tRNA fragments (tRFs) in HD putamen, and we validated the neurotoxic potential of an Alanine derived tRF in vitro. These results highlight that HD-sRNA-PT are neurotoxic, and suggest that multiple sRNA species contribute to striatal dysfunction and general transcriptomic changes, favoring therapeutic strategies based on the blockage of sRNA-mediated toxicity.
Acta Neuropathol, 2021; 141

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BOARD NUMBER: S01-639

DISTINCT INVOLVEMENT OF DIRECT AND INDIRECT PATHWAYS FROM THE DORSOLATERAL AND DORSOMEDIAL STRIATUM IN THE PATHOPHYSIOLOGY OF HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

Sara Conde-Berriozabal[1,2,3], Laia Sitjà-Roqueta[1,2,3], Esther García-García[1,2,3], Lia García-Gilabert[1,2,3], Sara García-Fernández[1,2,3], Ened Rodríguez-Urgellés[1,2,3], Guadalupe Soria[1,4,5], Manuel José Rodríguez[1,2,3], Jordi Alberch[1,2,3], Mercè Masana[1,2,3]
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Disruption of basal ganglia (BG) circuits underlie several movement disorders, such as Huntington's disease (HD). Recent functional studies reveal that distinct BG sub-circuits modulate different behavioral domains, i.e dorso-medial striatum (DMS) seems more involved in goal-directed action control while dorso-lateral striatum (DLS) in habit and skill learning. Here, we unravel functional implications of DLS and DMS and its main striatal output pathways: direct and indirect (expressing D1 or A2a receptors respectively), in HD pathophysiology. First, we analyzed functional connectivity alterations of DLS and DMS using rs-fMRI in the R6/1 mouse model of HD. DLS showed reduced functional connectivity with all cortical subregions and thalamus, while DMS connectivity was less affected. Then, we bilaterally injected AAV-DIO-ChR2 in the DLS or DMS of Drd1-Cre and A2A-Cre mouse lines crossed with R6/1 mice. In WT animals, optogenetic stimulation of DLS direct pathway increases locomotion, whereas DLS indirect pathway activation improves motor learning, assessed in an accelerating rotarod. These effects were not observed in symptomatic HD mice, or when DMS selective pathways were stimulated. Then, we expressed the GCaMP6f calcium sensor in Drd1-neurons in the DLS or DMS during the accelerated rotarod task, and recorded fluorescence changes using fiber photometry. We found an abnormal neuronal engagement of the direct pathway in the DLS but not the DMS during all phases of skill learning. We are currently analyzing the contribution of the indirect pathway in this task. Altogether, our data suggests a major involvement of the DLS in HD pathophysiology.

Pubmed:
33016873: Fernández-García S, Conde-Berriozabal S, García-García E, Gort-Paniello C, Bernal-Casas D, García-Díaz Barriga G, López-Gil J, Muñoz-Moreno E, Soria G, Campa L, Artigas F, Rodríguez MJ, Alberch J, Masana M
M2 cortex-dorsolateral striatum stimulation reverses motor symptoms and synaptic deficits in Huntington's disease.
Huntington's disease (HD) is a neurological disorder characterized by motor disturbances. HD pathology is most prominent in the striatum, the central hub of the basal ganglia. The cerebral cortex is the main striatal afferent, and progressive cortico-striatal disconnection characterizes HD. We mapped striatal network dysfunction in HD mice to ultimately modulate the activity of a specific cortico-striatal circuit to ameliorate motor symptoms and recover synaptic plasticity. Multimodal MRI in vivo indicates cortico-striatal and thalamo-striatal functional network deficits and reduced glutamate/glutamine ratio in the striatum of HD mice. Moreover, optogenetically-induced glutamate release from M2 cortex terminals in the dorsolateral striatum (DLS) was undetectable in HD mice and striatal neurons show blunted electrophysiological responses. Remarkably, repeated M2-DLS optogenetic stimulation normalized motor behavior in HD mice and evoked a sustained increase of synaptic plasticity. Overall, these results reveal that selective stimulation of the M2-DLS pathway can become an effective therapeutic strategy in HD.
Elife, 2020; 9

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IN VIVO STRIATAL ACTIVITY DURING MOTOR SKILL LEARNING AND SPONTANEOUS BEHAVIOUR IN HUNTINGTON'S DISEASE MICE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

Ellen Koch, Judy Cheng, Marja Sepers, Ryan Yeung, Lynn Raymond
UBC Djavad Mowafaghian Center for Brain Health, Psychiatry, Vancouver, Canada

Huntington's disease (HD) is a neurodegenerative disorder characterized by motor, cognitive and psychiatric deficits. The dorsal striatum is the major site of neurodegeneration. Studies have shown aberrant cortico-striatal signalling in HD mice, including changes to the activity of D1- and D2-SPNs, aberrant neurotransmitter signalling, and deficits in cortico-striatal plasticity. We were interested in how changes to cortico-striatal signalling *in vitro* correlate with behaviour *in vivo*. We combined the accelerating rotarod and open field with calcium imaging using fiber photometry in the striatum of HD mouse models (YAC128 and Q175). This includes imaging of overall striatal activity with GCaMP7f, as well as population specific imaging of D1-SPNs and D2-SPNs using green and red calcium sensors. When imaging activity of all striatal neurons, we found that both WT and YAC128 mice showed increased striatal activity when they performed the rotarod, which reduced over training. 2-3 month YAC128 mice showed no deficit in latency to fall from the rotarod, however, the correlation between striatal activity and behaviour on the rotarod and open field in YAC128 was significantly weaker. Interestingly, YAC128 showed deficits in paw kinematics, including increased paw slips below the rotarod, which was associated with aberrant striatal activity. At 6-7 months, YAC128 mice were severely impaired on the rotarod and had significantly increased striatal activity. YAC128 mice also showed elevated striatal activity at rest in the open field. This work begins to bridge the gap between changes to striatal signalling determined from in vitro studies and the behavioural deficits observed in vivo.

Pubmed:
31304622: Koch ET, Raymond LA
Dysfunctional striatal dopamine signaling in Huntington's disease.
Dopamine signaling in the striatum is critical for a variety of behaviors including movement, behavioral flexibility, response to reward and many forms of learning. Alterations to dopamine transmission contribute to pathological features of many neurological diseases, including Huntington's disease (HD). HD is an autosomal dominant genetic disorder caused by a CAG repeat expansion in the Huntingtin gene. The striatum is preferentially degenerated in HD, and this region receives dopaminergic input from the substantia nigra. Studies of HD patients and genetic rodent models have shown changes to levels of dopamine and its receptors in the striatum, and alterations in dopamine receptor signaling and modulation of other neurotransmitters, notably glutamate. Throughout his career, Dr. Michael Levine's research has furthered our understanding of dopamine signaling in the striatum of healthy rodents and HD mouse models. This review will focus on the work of his group and others in elucidating alterations to striatal dopamine signaling that contribute to pathophysiology in HD mouse models, and how these findings relate to human HD studies. We will also discuss current and potential therapeutic interventions for HD that target the dopamine system, and future research directions for this field.
J Neurosci Res, 2019; 97
30332323: Koch ET, Woodard CL, Raymond LA
Direct assessment of presynaptic modulation of cortico-striatal glutamate release in a Huntington's disease mouse model.
Glutamate is the main excitatory neurotransmitter in the brain, and impairments in its signaling are associated with many neurological disorders, including Huntington's disease (HD). Previous studies in HD mouse models demonstrate altered glutamate receptor distribution and signaling at cortico-striatal synapses, and some studies suggest that glutamate release is altered; however, traditional methods to study synaptic glutamate release are indirect or have poor temporal resolution. Here we utilize iGluSnFR, a modified green fluorescent protein reporter for real-time imaging of glutamate transmission, to study presynaptic modulation of cortical glutamate release in the striatum of the YAC128 HD mouse model. We determined that iGluSnFR can be used to accurately measure short- and long-term changes in glutamate release caused by modulation of extracellular Ca levels, activation of presynaptic receptors, and high-frequency stimulation (HFS) protocols. We also confirmed a difference in the expression of HFS-induced long-term depression in YAC128. Together, this research demonstrates the utility of iGluSnFR in studying presynaptic modulation of glutamate release in healthy mice and disease

models that display impairments in glutamate signaling. NEW & NOTEWORTHY We use iGluSnFR to directly assess presynaptic modulation of cortico-striatal glutamate release in brain slice and compare changes in glutamate release between wild type and a Huntington's disease mouse model, YAC128. We observed reductions in glutamate release after low extracellular Ca and activation of various presynaptic receptors. We also demonstrate a presynaptic mechanism of reduced glutamate release in high-frequency stimulation-induced long-term depression and show this to be altered in YAC128.
J Neurophysiol, 2018; 120

27720624: LeDue EE, Mann K, Koch E, Chu B, Dakin R, Gordon MD
Starvation-Induced Depotentiation of Bitter Taste in Drosophila.
Nutrient deprivation can lead to dramatic changes in feeding behavior, including acceptance of foods that are normally rejected. In flies, this behavioral shift depends in part on reciprocal sensitization and desensitization of sweet and bitter taste, respectively. However, the mechanisms for bitter taste modulation remain unclear. Here, we identify a set of octopaminergic/tyraminergic neurons, named OA-VLs, that directly modulate bitter sensory neuron output in response to starvation. OA-VLs are in close proximity to bitter sensory neuron axon terminals and show reduced tonic firing following starvation. We find that octopamine and tyramine potentiate bitter sensory neuron responses, suggesting that starvation-induced reduction in OA-VL activity depotentiates bitter taste. Consistent with this model, artificial silencing of OA-VL activity induces a starvation-like reduction in bitter sensory neuron output. These results demonstrate that OA-VLs mediate a critical step in starvation-dependent bitter taste modulation, allowing flies to dynamically balance the risks associated with bitter food consumption against the threat of severe starvation.
Curr Biol, 2016; 26

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BOARD NUMBER: S01-641

HEPATOMA-DERIVED GROWTH FACTOR IS NEUROPROTECTIVE IN MODELS OF HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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[1]Max Planck Institute for Biological Intelligence, i.f., Molecular Neurodegeneration Group, Martinsried, Germany, [2]Max Planck Institute for Biological Intelligence, i.f., Department Of Molecules – Signaling – Development, Martinsried, Germany

Huntington's disease (HD) is a fatal hereditary disorder with severe neurodegeneration in the striatum and cortex. Despite the monogenic nature of HD, disease pathogenesis is insufficiently understood and effective disease-modifying treatments are lacking to date. Here, we uncover hepatoma-derived growth factor (HDGF) as a neuroprotective protein in the context of HD. HDGF counteracts mutant huntingtin aggregation and cell death in murine primary cortical neurons. Expression levels of HDGF in mouse brain are inversely correlated with vulnerability of brain regions and individual neuronal populations to HD. In R6/2 HD model mice, HDGF deficiency exacerbates motor defects and reduces life span, while viral HDGF delivery into the nervous system significantly improves locomotor activity. Mechanistic studies in cell culture models of HD demonstrate that the neuroprotective activity of HDGF is independent of its subcellular localization: Nuclear and cytoplasmic HDGF are equally effective in rescuing mutant huntingtin toxicity. Moreover, extracellular application of recombinant HDGF is sufficient to improve viability of mutant huntingtin-expressing primary neurons via a mechanism distinct from canonical growth factor signaling. Taken together, this study provides new insights into HDGF function in the diseased brain and unravels the therapeutic potential of HDGF in HD.

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BOARD NUMBER: S01-642

EARLY TRANSCRIPTIONAL MODIFICATIONS OF THE DEVELOPING BRAIN IN HUNTINGTON DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

Radhia Kacher[1], Julie Six[1], Rania Hilab[1], Justine Guegan[2], Alexandra Durr[1], Sandrine Humbert[3]
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Huntington disease (HD) is an inherited neurological disease caused by a pathological CAG repeat expansion in the *HTT* gene encoding an abnormal glutamine repeat in the huntingtin protein (HTT). Given the adult-onset of this disease, the neurological symptoms, and the neuronal cell death, many studies focused on describing the effects of the mutant protein in adult neurons. However, HTT is expressed very early and plays a fundamental role in brain development. Specifically, HTT is required for mitotic spindle orientation, for maintaining the pool of cortical progenitors and at later stages for multipolar-bipolar transition and migration of newborn neurons in the mouse cortex. Mutant HTT impairs these mechanisms, resulting in thinner cortex in HD mice. In presymptomatic mouse models and patients, brain MRI studies have revealed cortical and striatal abnormalities decades before overt clinical signs. These defects could arise from an abnormal brain development in HD. For these reasons, we initiated a program to explore human brain development in HD and found that mutant HTT reduces the number of proliferating cells and triggers more neural progenitors to prematurely enter lineage specification. Yet, a lot remains unknown on the underlying molecular mechanisms. Here, we performed an RNAseq analysis on the developing cortex of fetuses carrying an HD-causing mutation compared to control fetuses at the same age. We show that mutant HTT impairs transcriptional regulations of human neurodevelopment and postulate that this may affect disease progression or onset.

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BOARD NUMBER: S01-643

LXR SIGNALING IN THE STRIATUM AND NEUROPROTECTION IN HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Sorbonne University, Neuroscience Paris Seine, Paris, France

Huntington's disease (HD) is a rare neurodegenerative disease associated to several cellular dysfunctions, including cholesterol metabolism deregulation. Brain cholesterol is catabolized by the neuronal enzyme CYP46A1 into 24S-hydroxycholesterol (24S-OHC), a ligand of Liver X Receptor (LXR). Restoration of CYP46A1 in HD mice induces a neuroprotection with an upregulation of LXR target genes; therefore, we hypothesized their involvement in CYP46A1 neuroprotection in HD. There are two LXR isoforms, LXRβ enriched in the brain for cholesterol metabolism regulation and LXRα mainly expressed in the liver which activation can induces side effects on lipogenesis. The aim of the project is to investigate the role of LXR activation in HD, with the use of new LXRβ agonists. LXR agonists bioactivity were validated in primary cultures of striatal neurons and astrocytes, and their neuroprotective role was studied in a HD cellular model. A treatment protocol with LXR agonist was validated in Wild Type mice. In neurons and astrocytes culture, LXR agonists induce an increase of mRNA level of LXR target genes, involved in cholesterol metabolism and known to be downregulated in HD. The agonists induce a neuroprotection in HD striatal neurons in culture, with a decrease of mHTT aggregates and an increase of cell survival, dependent of proteasome and autophagy mechanisms. ***These results support the biological efficacy of these new LXR compounds and their neuroprotective role in HD striatal neurons. The mechanisms involved in LXR effect in vitro*** **are now investigated, and** ***their role is studied on motor and cognitive symptoms in HD mouse model.***

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BOARD NUMBER: S01-644

EXPLORING GENETIC INTERACTIONS OF THE MUTANT HUNTINGTIN PROTEIN USING DROSOPHILA AND MOUSE MODELS OF HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Medium Spiny Neuron (MSN) degeneration is a major pathological hallmark in the dominantly inherited condition Huntington's Disease (HD) for which there is currently no disease-modifying treatment. Understanding pathogenic mechanisms underlying neuronal degeneration is key to developing therapeutic strategies. The causal mutation is in the Huntingtin (HTT) gene. Our aim is to analyse interactions of mutant HTT (mHTT) with other genes and proteins implicated in the networks that control MSN differentiation and which are dysregulated in HD. One example is FoxP1, a transcription factor that can interact with mHTT and is required for MSN differentiation in mice. Our objective is to determine whether FoxP1 has a role in HD pathogenesis. In a shuttle approach between two model systems, we use the Drosophila eye as an in vivo 'test tube' to analyse the effect of manipulating genes of interest on mHTT-induced neurodegeneration, and then explore interesting results in the R6/1 and knock-in Q150 mouse models of HD. We use Drosophila eye phenotype and rhabdomere counts and mouse histological analysis and stereology to investigate the effect of manipulating FoxP1 expression. Reduction of FoxP1-positive neuronal cell number and levels of protein expression were early events in the course of striatal degeneration in both HD mouse models, consistent with a role for FoxP1 in mHTT-induced neurodegeneration. Overexpression of a FoxP1 fragment rescued mHTT-induced eye degeneration in the Drosophila model. Viral vector mediated overexpression of FoxP1 via stereotactic injection into the striatum of HD mice will be used to extend these findings into the mammalian brain.

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BOARD NUMBER: S01-645

THE DEDICATOR OF CYTOKINESIS 7 (DOCK7) AFFECTS INTRACELLULAR TRAFFICKING OF ENDOLYSOSOMES

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Proteostasis networks are essential for neuronal integrity and homeostasis, being disturbed in various neurodegenerative diseases. Proteostasis involves the bidirectional intracellular trafficking of endosomes and lysosomes along microtubules. Therefore, microtubule stability is a critical parameter. The microtubule stability is fine-tuned by specific proteins, such as the dedicator of cytokinesis 7 (DOCK7). DOCK7 stabilizes microtubules via its guanine nucleotide exchange activity and the following phosphorylation cascades of microtubule destabilizer proteins, such as stathmin/Op18. However, whether DOCK7 affects intracellular trafficking of degradative organelles, especially in the context of neurodegenerative diseases, is not clear yet. To this end, we monitored intracellular trafficking of endolysosomal compartments in live cell imaging experiments in murine CB (Cerebellar granule cells) and N2a (Neuroblastoma cells) cells that were co-transfected with *Dock7*-siRNA and a CD63-GFP-DNA-plasmid construct, labeling late endosomes and lysosomes. We found that siRNA-mediated depletion of *Dock7* caused a significant acceleration of the intracellular trafficking of endolysosomal organelles. As degradative networks modulate neuronal degeneration, we next investigated whether DOCK7 has an effect on mutant Huntingtin (HTT)-induced neurotoxicity. Indeed, we found an improved survival of mutant HTT expressing cells upon *Dock7* depletion, which revealed to improve intracellular trafficking. We hypothesize that DOCK7-mediated regulation of intracellular trafficking affects aggresome formation of mutant HTT, shown to be cytoprotective. In sum, we conclude that DOCK7 affects the intracellular trafficking of degradative vesicles in the proteostasis network, putatively through its microtubule stabilizer property.

Pubmed:
32751461: Bayer C, Pitschelatow G, Hannemann N, Linde J, Reichard J, Pensold D, Zimmer-Bensch G
DNA Methyltransferase 1 (DNMT1) Acts on Neurodegeneration by Modulating Proteostasis-Relevant Intracellular Processes.
The limited regenerative capacity of neurons requires a tightly orchestrated cell death and survival regulation in the context of longevity, as well as age-associated and neurodegenerative diseases. Subordinate to genetic networks, epigenetic mechanisms, such as DNA methylation and histone modifications, are involved in the regulation of neuronal functionality and emerge as key contributors to the pathophysiology of neurodegenerative diseases. DNA methylation, a dynamic and reversible process, is executed by DNA methyltransferases (DNMTs). DNMT1 was previously shown to act on neuronal survival in the aged brain, whereby a DNMT1-dependent modulation of processes relevant for protein degradation was proposed as an underlying mechanism. Properly operating proteostasis networks are a mandatory prerequisite for the functionality and long-term survival of neurons. Malfunctioning proteostasis is found, inter alia, in neurodegenerative contexts. Here, we investigated whether DNMT1 affects critical aspects of the proteostasis network by a combination of expression studies, live cell imaging, and protein biochemical analyses. We found that DNMT1 negatively impacts retrograde trafficking and autophagy, with both being involved in the clearance of aggregation-prone proteins by the aggresome-autophagy pathway. In line with this, we found that the transport of GFP-labeled mutant huntingtin (HTT) to perinuclear regions, proposed to be cytoprotective, also depends on DNMT1. Depletion of accelerated perinuclear HTT aggregation and improved the survival of cells transfected with mutant HTT. This suggests that mutant HTT-induced cytotoxicity is at least in part mediated by DNMT1-dependent modulation of degradative pathways.
Int J Mol Sci, 2020; 21
33572758: Pensold D, Gehrmann J, Pitschelatow G, Walberg A, Braunsteffer K, Reichard J, Ravaei A, Linde J, Lampert A, Costa IG, Zimmer-Bensch G
The Expression of the Cancer-Associated lncRNA Is Modulated by EphrinA5-Induced Signaling.
The Eph receptor tyrosine kinases and their respective ephrin-ligands are an important family of membrane receptors, being involved in developmental processes such as proliferation, migration, and in the formation of brain cancer such as glioma. Intracellular signaling pathways, which are activated by Eph receptor signaling, are well characterized. In contrast, it is unknown so far whether ephrins modulate the expression of lncRNAs, which would enable the transduction of environmental stimuli into our genome through a great gene regulatory spectrum. Applying a combination of functional in vitro assays, RNA

sequencing, and qPCR analysis, we found that the proliferation and migration promoting stimulation of mouse cerebellar granule cells (CB) with ephrinA5 diminishes the expression of the cancer-related lncRNA In a human medulloblastoma cell line (DAOY) ephrinA5 stimulation similarly reduced expression. Computational analysis identified triple-helix-mediated DNA-binding sites of in promoters of genes found up-regulated upon ephrinA5 stimulation and known to be involved in tumorigenic processes. Our findings propose a crucial role of downstream of ephrinA5-induced signaling in regulating gene transcription in the nucleus. These findings could be potentially relevant for the regulation of tumorigenic processes in the context of glioma.
Int J Mol Sci, 2021; 22

32890576: Njila Tchoufack EJ, Hahnfeld L, Pitschelatow G, Bennink S, Pradel G

The endoplasmic reticulum-resident serpentine receptor SR10 has important functions for asexual and sexual blood stage development of Plasmodium falciparum.

Serpentine receptors (SRs) are transmembrane proteins generally acting as mediators to facilitate the communication between a cell and its environment. At least six putative SR-like proteins are encoded in the genome of the malaria parasite Plasmodium falciparum. For two of them, roles in cell stress control were reported; however, for most of the SR-like proteins the functions are not yet known. In this study, we provide a first phenotypic analysis of the plasmodial SR10. The transmembrane protein is expressed in the asexual and sexual blood stages of P. falciparum. Co-localization and co-immunoprecipitation assays demonstrated an association of SR10 with the endoplasmic reticulum protein ERC. Gene disruption of SR10 leads to impaired intraerythrocytic replication and strongly reduces gametocyte numbers. We thus propose that SR10 is a protein associated with the endoplasmic reticulum that has important functions for asexual and sexual blood stage development.
Mol Biochem Parasitol, 2020; 239

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CREATINE KINASE B PROVIDES AN ALTERNATIVE ENERGY SOURCE FOR FAST AXONAL TRANSPORT: ROLE IN HEALTH AND HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Aims: Fast axonal transport (FAT) is ensured by molecular motors that requires energy to carry a wide range of organelles such as the brain-derived neurotrophic factor (BDNF) along microtubules. We have established that the glycolytic machinery is on-board on vesicles generating ATP that is then used by the motors. Using proteomic approaches, we identified the brain specific isoform of the creatine kinase (CKB) as being an additional resident on vesicles. CKB is an essential player of energy homeostasis. We hypothesized that CKB may provide an additional source of ATP to vesicular motors by dephosphorylating phosphocreatine into creatine. **Methods**: We are using microfluidic devices developed in the lab and reconstituting mature neuronal circuits (Virlogeux et al. 2018) to investigate axonal transport, especially of BDNF. **Results**: We confirmed by biochemical and immunostaining approaches that CKB resides on BDNF vesicles. Secondly, we found that CKB participates to FAT by recording BDNF trafficking using spinning disk videomicroscopy. Silencing CKB or expressing an inactive mutant in neurons decreased the number of motile vesicles. Conversely, activation of CKB by its substrate, creatine, induced the movement of vesicles. **Conclusions**: Altogether, we propose that CKB may play a role in the initiation of vesicular motility. Huntingtin protein (HTT) scaffolds glycolytic enzymes on vesicles, and when mutated in Huntington disease (HD), it leads to the decrease of BDNF trafficking. We are now investigating whether CKB might be altered in HD neurons.

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BUILDING A HUNTINGTON DISEASE CORTICO-CORTICAL NEURONAL NETWORK-ON-CHIP FOR DRUG INVESTIGATION

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Huntington Disease is a genetic neurodegenerative disorder caused by the abnormal expansion of a glutamine-encoding CAG repeat in the huntingtin (HTT) gene. This pathology is characterized by the dysfunction and death of neurons in the brain, particularly in the cortex and the striatum. HTT, a scaffolding protein, is involved in the axonal transport of vesicles containing Brain-Derived Neurotrophic Factor (BDNF). In HD, mutated HTT impaired the BDNF trafficking and leads to a reduction in the amounts of BDNF provided to the striatum, which in turn leads to death of both striatal and cortical neurons. The laboratory has developed microfluidic devices to reconstitute a mature neuronal network, in which presynaptic, synaptic and postsynaptic events are compartmentalized. Culturing primary neurons from HD mouse model already provides us a “disease-on-a-chip” platform ideal to fully decipher presynaptic dynamics, synaptic morphology and transmission, postsynaptic trafficking and signaling, as well as global network dynamics in healthy and pathogenic conditions. Here, we developed an human HD brain-on-a-chip by using human cortical neurons derived from induced pluripotent stem cells (iPSCs) to reconstruct HD functional cortico-cortical networks in our microfluidic devices. We used neurons derived from HD patient-specific induced pluripotent stem cells that contained both wild type and mutated *HTT* gene. We are currently characterizing this network by different methods (immunocytochemistry, functional tests, network connectivity) in order to propose a functional model of human HD neuronal network, thus providing a predictive model for drug testing and for the investigation of disease mechanisms.

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DIRECTLY REPROGRAMMED MEDIUM SPINY NEURONS FOR STUDYING PATHOLOGY AND SYNAPTIC DYSFUNCTION IN HUNTINGTON'S DISEASE IN VITRO MODEL

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Many pathological features of neurodegenerative diseases, such as the development of synaptic dysfunction, appear with age. Direct reprogramming preserves the age of induced neurons. However, the low efficiency of direct reprogramming methods limits their utility as a tool for neurodegenerative diseases modeling. In the present study, we modified a direct reprogramming protocol-based microRNAs, transcription factors, and small molecules and obtain a homogenous population of induced medium spiny neurons (iMSN), the most vulnerable cell type in Huntington's disease (HD) from dermal fibroblasts with an efficiency more than 80 percent. At the end of the reprogramming procedure, iMSNs are positively stained for canonical neuronal markers and respond to potassium chloride and glutamate stimulation. Induced excitatory neurons (iEN) can also be obtained from primary fibroblasts to study the pathogenesis of cognitive impairments observed in the case of HD. Moreover, iMSN and iEN are capable to form synaptic connections which makes it possible to study defects in synaptic transmission during the development of HD pathology. Thus the modified protocol makes it possible to obtain a homogeneous population of iEN and iMSN. This protocol might be useful in both studying the molecular and cellular basis of HD pathogenesis and drug discovery as well as in the development of a personalized approach for therapy.

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INDIRECT PATHWAY LINAGE-SPECIFIC ALTERATIONS FROM EARLY EMBRYONIC DEVELOPMENT IN HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a currently incurable neurodegenerative disorder that manifests through motor and cognitive symptoms due to a predominant loss of striatal Medium Spiny Neurons (MSNs). Growing evidence show that developmental alterations could be a key factor on the disease progression, determining the future vulnerability of certain cell types, such as striatal MSNs. Here we show an early HD-related expression pattern elucidated by bulk and 10X single cell RNA-seq performed on wt and HD isolated striatal primordia at different developmental stages. Histological analyses were used to validate alterations of crucial developmental events occurring in HD. We found that early alterations in cell cycle progression and cell fate determination in HD embryos give rise to an imbalance between specific neural precursors and mature neurons, affecting specifically the indirect MSNs (iMSNs) linage generation. Moreover, iMSNs undergo into specific apoptosis postnatally in HD. Development finally ends up with a reduction of the iMSNs population in the adult striatum in HD. In conclusion, we show that iMSNs embryonic developmental alterations set the stage in HD, altering the basal ganglia cell homeostasis. Thus, targeting specific neural populations during development could constitute a therapeutic approach for HD.
This study was supported by grants from the Ministerio de Ciencia, Innovación y Universidades (RTI2018-099001-B-I00, CV fellow); Instituto de Salud Carlos III and European Regional Development Fund (ERDF) (Red de Terapia Celular, RD16/0011/0012); Generalitat de Catalunya (2017SGR-1408), Spain; "la Caixa" Foundation under the grant agreement LCF/PR/HR21-00622"; European Commission (Grant Agreement: 813851); and the CHDI Foundation (A16887 to J. M. C.), USA.

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SUPPRESSION OF MUTANT HUNTINGTIN IMPROVES COGNITIVE SYMPTOMS IN THE R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Introduction: Huntington's disease (HD) is a monogenic neurodegenerative disorder caused by a mutation in the huntingtin (*HTT*) gene. Although HD is typically considered a motor disorder, cognitive symptoms often appear first and are the primary cause of functional decline. **Aim:** To explore the effect of HTT-lowering on memory formation in an acute mouse model of HD. **Methods:** R6/1 transgenic mice were treated with antisense oligonucleotide (ASO) or vehicle by injecting directly into the right lateral ventricle. Allocentric spatial memory and recognition memory were assessed using the Barnes maze and novel object tests respectively. Wild-type (WT) C57BL6/J mice were used as non-transgenic controls. **Results:** On a test of allocentric spatial memory, ASO-treated transgenics and non-transgenic controls employed the optimal spatial search strategy significantly more than the chance level would predict to escape the Barnes maze. Conversely, vehicle-treated HD mice did not learn to use this strategy. HTT suppression also improved memory on novel object tests, where ASO-treated mice spent significantly more time exploring novel contexts compared to vehicle-treated transgenics. This preference for novelty was also seen in non-transgenic controls. Taken together, our data suggests that suppression of mutant HTT in early-symptomatic R6/1 mice partially improves cognitive impairments. **Conclusions:** HTT-lowering ameliorates cognitive symptoms in R6/1 mice. Single ASO injection improved allocentric navigation and object recognition memory. ASO-mediated gene suppression is promising as a treatment for HD, but additional experiments are required to reveal the optimal approach for therapeutic efficacy.

Pubmed:
33578855: Gavin C, Geerts N, Cavanagh B, Haynes M, Reynolds CP, Loessner D, Ewald AJ, Piskareva O
Neuroblastoma Invasion Strategies Are Regulated by the Extracellular Matrix.
Neuroblastoma is a paediatric malignancy of the developing sympathetic nervous system. About half of the patients have metastatic disease at the time of diagnosis and a survival rate of less than 50%. Our understanding of the cellular processes promoting neuroblastoma metastases will be facilitated by the development of appropriate experimental models. In this study, we aimed to explore the invasion of neuroblastoma cells and organoids from patient-derived xenografts (PDXs) grown embedded in 3D extracellular matrix (ECM) hydrogels by time-lapse microscopy and quantitative image analysis. We found that the ECM composition influenced the growth, viability and local invasion of organoids. The ECM compositions induced distinct cell behaviours, with Matrigel being the preferred substratum for local organoid invasion. Organoid invasion was cell line- and PDX-dependent. We identified six distinct phenotypes in PDX-derived organoids. In contrast, NB cell lines were more phenotypically restricted in their invasion strategies, as organoids isolated from cell line-derived xenografts displayed a broader range of phenotypes compared to clonal cell line clusters. The addition of FBS and bFGF induced more aggressive cell behaviour and a broader range of phenotypes. In contrast, the repression of the prognostic neuroblastoma marker, , resulted in less aggressive cell behaviour. The combination of PDX organoids, real-time imaging and the novel 3D culture assays developed herein will enable rapid progress in elucidating the molecular mechanisms that control neuroblastoma invasion.
Cancers (Basel), 2021; 13

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CONTRIBUTION OF ASTROCYTIC MITOCHONDRIA TO STRIATAL VULNERABILITY IN HUNTINGTON'S DISEASE

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Huntington's Disease (HD) is a neurodegenerative disorder characterized by the selective loss of striatal medium spiny neurons. Although the molecular mechanisms of this striatal vulnerability remain unclear, compelling evidence points out that mitochondrial dysfunction could play a major role. However, much of what we know regarding the mechanisms underlying mitochondrial disturbances in HD has been gained from studies conducted in neurons. Less is known about the role and regulation of mitochondrial dynamics in astrocytes, the most abundant cell population in the striatum after neurons. Indeed, relatively unexplored is whether mitochondrial dysfunction in astrocytes may also contribute to neuron disturbances and therefore, to pathology in HD. Hence, we aimed to define the specific contribution of astrocytic mitochondria as well as the communication between neurons and astrocytes mediated by mitochondria in HD. In this work, we used the transgenic R6/1 mouse model of HD to either isolate adult astrocytes at different stages of the disease and to culture primary striatal astrocytes. Since astrocytes is a complex and heterogeneous population, we evaluated several astrocyte markers at the striatum of R6/1 mouse throughout the disease using biochemical and immunofluorescence assays. Then, we assessed mitochondrial functionality and metabolism in WT and R6/1 astrocytes. Finally, we investigated the mechanisms mediating mitochondrial communication from astrocytes to neurons. Overall, we hypothesized that alterations in mitochondrial function in striatal astrocytes could contribute to the striatal neuronal vulnerability that occurs in HD.

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A SYNTHETIC ANALOGUE OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPETIDE (PACAP) IMPOVES MOTOR AND COGNITIVE FUNCTION IN R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE.

POSTER SESSION 01 - SECTION: HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a neurodegenerative disorder caused by the expression of the mutant huntingtin (mHtt). Motor dysfunction and cognitive impairment are two characteristic symptoms of HD and they are associated to the degeneration of striatum and the dysfunction of hippocampus, respectively. Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a multifunctional neuropeptide that acts through three receptors named PAC1R, VPAC1R, and VPAC2R. Recently, we found PACAP improves cognitive and motor symptoms in HD mice mainly through PAC1R. Unfortunately, therapeutic use of PACAP is hindered because its poor metabolic stability and because the activation of VPAC2R is associated with peripheral side effects. Here, we study the therapeutic potential of the Acetyl- [Ala15, Ala20] PACAP-38-propylamide (PACAP-alg), a PACAP analogue showing greater biostability and higher affinity to PAC1R. We found that PACAP-alg (30ug/Kg/day) administrated intranasally for 12 days in R6/1 mice improves motor function evaluated using Rotarod and Balance beam tests, and cognitive impairment analyzed by T-MAZE test. In the striatum, PACAP-alg induces the increase of number and size of DARPP-32 positive neurons and reduces the number of mHtt aggregates, determined by immunofluorescence. In the hippocampus, PACAP-alg also reduced the number of mHtt aggregates. Moreover R6/1 mice treated with PACAP-alg showed an increase in number of dendritic spines in CA1 measured by Golgi stain. In conclusion, PACAP-alg administration improves motor and cognitive deficits of R6/1 mice improving neuronal function and enhancing the synaptic plasticity. Thus, the use of this analogue could be considered as a good therapeutic strategy to fight the symptomatology of HD.

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DECIPHERING NEURONAL AND SYNAPTIC ARCHITECTURE USING NEW METHODS FOR LABELING, IMAGING AND SEGMENTING NEURONAL CELLS VIA STANDARD AND SUPER RESOLUTION MICROSCOPY

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Synaptic composition and morphogenesis of dendritic tree and spines is prone to constant remodeling during development and learning. We address how synaptic molecular arrangement can be revealed and analysed with super-resolution microscopy. **SENSEI** project (Segmentation of neurons using Standard & super-resolution microscopy) objectives are to deliver novel image processing tools along with innovative microscopy-based imaging modalities dedicated to advancing breakthroughs in 3D neuronal segmentation and morphometrics. Specifically, SENSEI aims at accurately quantifying neuronal morphology at tissue and molecular levels through the development of intelligent segmentation-based image processing algorithms and at improving the quality of neuronal imaging using new membrane probes for conventional and super-resolution imaging technologies. The proposed tools are tested for accuracy and reproducibility using different imaging techniques and protocols to deal with data at different spatial scales (from tissue to molecular range) on mouse neurons both *in vitro* for 2D (hippocampal, cortical neurons) and in situ for 3D (tissue slices and clarified brains) in cortex, cerebellum and hippocampus. Once optimized on rodents, new imaging modalities and segmentation protocols will be tested on human brain tissues coming from surgical resections. Samples will be imaged both with confocal microscopy, accounting for 80 % of user needs, and advanced imaging modalities, thus bringing either high resolution details on fixed dendritic spines (e.g., STED, STORM) or on live neurons (i.e. 3D SOFI). In addition to the sub-diffraction resolution, an optical device encoding probe information in the microscope point-spread function will be constructed, allowing the fast acquisition of cellular nanostructuring in full-3D.

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NON-INVASIVE IN VIVO BRAIN INFLAMMATION QUANTIFICATION USING A FAR-RED FLUORESCENT REPORTER MOUSE

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Despite the adaptation of major clinical imaging modalities for small animals, optical bioluminescence imaging technology is the main approach readily reporting gene activity. Yet, in vivo bioluminescence monitoring requires the administration and diffusion of a substrate to the tissues of interest, resulting in experimental variability, high reagent cost, long acquisition time, and stress to the animal. In our study, we avoid such issues upon generating a new transgenic mouse (GFAP-E2crimson) expressing the far-red fluorescent protein E2-Crimson under the control of the glial fibrillary acidic protein (GFAP) promoter. Using microscopy, we validated the selective expression of the reporter in the astrocyte cell population and by non-invasive in vivo fluorescence imaging its detection through the scalps and skulls of live animals. In addition, we performed a longitudinal study validating by in vivo imaging that the E2-crimson fluorescence signal is up-regulated, in pups during astrogenesis and in adult mice during astrogliosis upon kainic acid administration. Furthermore, upon crossing GFAP-E2crimson transgenic with 5XFAD Alzheimer's disease mice model, we were able to quantify the chronic inflammation triggered by amyloid deposit and ageing over 18 months. As many diseases and conditions can trigger neuroinflammation, we believe that the GFAP-E2crimson reporter mice model delivers tremendous value for the non-invasive quantification of astrogliosis responses in living animals.

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MINIMAL TAGS FOR SITE-SPECIFIC FLUORESCENT LABELING OF NF186 AND NAV1.6 IN LIVING NEURONS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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The axon initial segment (AIS) is a unique neuronal compartment responsible for the generation of action potentials. To fulfil this important role, AIS has a distinctive molecular organization that is characterized by high-density clusters of the voltage-gated sodium channels (Na_V), cell adhesion molecules, such as 186 kDa neurofascin isoform (NF186), and scaffolding and cytoskeletal proteins. Due to the highly complex AIS organization, the number of methods that allow live imaging studies of its components is limited. To overcome this limitation, we established a minimally invasive approach for direct fluorescent labeling of two large AIS components, NF186 (186 kDa) and $Na_V1.6$ (260 kDa). Our approach is based on the site-specific incorporation of unnatural amino acids (UAAs) into target proteins via genetic code expansion. Subsequently, UAAs are labeled by biorthogonal click chemistry. The minimal size of the UAA tags and the possibility to introduce them virtually anywhere into target proteins make our approach particularly convenient for labeling of complex and densely packed proteins, such as NF186 and $Na_V1.6$. This approach allowed us to perform widefield and confocal microscopy of click-labeled NF186 and $Na_V1.6$ in fixed and living neurons. We also used click labeling to study localization of two epilepsy causing $Na_V1.6$ variants with loss-of-function effect. To further improve UAA incorporation and click labeling efficiency, we developed adeno-associated viral (AAV) vectors that provided delivery of orthogonal translational machinery to large number of neurons. This will facilitate applications of click labeling in more complex biological systems, such as slice cultures, organoids, and animal models.

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HIGH RESOLUTION, TWO-PHOTON, ACOUSTO-OPTICS BASED SIMULTANEOUS IMAGING AND OPTOGENETICS THROUGH ALL CORTICAL LAYERS FROM UP TO 250 CELLS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Investigating the network dynamics of neural populations in cortical areas involved in vision with photostimulation is one of the hot topics of the past few years. However, measurement protocols, visual paradigms, and experimental scenarios were all limited by the technological limitations of conventional microscope systems. We applied the advantages of AO scanning to 3D photostimulation from a volume of 500x500x500 μm^3. We demonstrate that we can effectively stimulate and measure up to 250 cells simultaneously while maintaining subcellular spatial resolution to avoid off-target stimulation. Next, we developed novel analytic software modules to automatically detect responding neurons and to define, among the acquired cell-level activity patterns, those that are involved in the perception of specific visual stimuli (i.e. representing a latent task variable of a task). Stimulation of perception-specific cellular patterns became possible by the combination of these developments.

Pubmed:
33963220: Kaszas A, Szalay G, Slézia A, Bojdán A, Vanzetta I, Hangya B, Rózsa B, O'Connor R, Moreau D
Two-photon GCaMP6f imaging of infrared neural stimulation evoked calcium signals in mouse cortical neurons in vivo.
Infrared neural stimulation is a promising tool for stimulating the brain because it can be used to excite with high spatial precision without the need of delivering or inserting any exogenous agent into the tissue. Very few studies have explored its use in the brain, as most investigations have focused on sensory or motor nerve stimulation. Using intravital calcium imaging with the genetically encoded calcium indicator GCaMP6f, here we show that the application of infrared neural stimulation induces intracellular calcium signals in Layer 2/3 neurons in mouse cortex in vivo. The number of neurons exhibiting infrared-induced calcium response as well as the amplitude of those signals are shown to be both increasing with the energy density applied. By studying as well the spatial extent of the stimulation, we show that reproducibility of the stimulation is achieved mainly in the central part of the infrared beam path. Stimulating in vivo at such a degree of precision and without any exogenous chromophores enables multiple applications, from mapping the brain's connectome to applications in systems neuroscience and the development of new therapeutic tools for investigating the pathological brain.
Sci Rep, 2021; 11
34151084: Chiovini B, Pálfi D, Majoros M, Juhász G, Szalay G, Katona G, Szőri M, Frigyesi O, Lukácsné Haveland C, Szabó G, Erdélyi F, Máté Z, Szadai Z, Madarász M, Dékány M, Csizmadia IG, Kovács E, Rózsa B, Mucsi Z
Theoretical Design, Synthesis, and In Vitro Neurobiological Applications of a Highly Efficient Two-Photon Caged GABA Validated on an Epileptic Case.
In this paper, we present an additional, new cage-GABA compound, called 4-amino-1-(4'-dimethylaminoisopropoxy-5',7'-dinitro-2',3'-dihydro-indol-1-yl)-1-oxobutane-γ-aminobutyric acid (iDMPO-DNI-GABA), and currently, this compound is the only photoreagent, which can be applied for GABA uncaging without experimental compromises. By a systematic theoretical design and successful synthesis of several compounds, the best reagent exhibits a high two-photon efficiency within the 700-760 nm range with excellent pharmacological behavior, which proved to be suitable for a complex epileptic study. Quantum chemical design showed that the optimal length of the cationic side chain enhances the two-photon absorption by 1 order of magnitude due to the cooperating internal hydrogen bonding to the extra nitro group on the core. This feature increased solubility while suppressing membrane permeability. The efficiency was demonstrated in a systematic, wide range of in vitro single-cell neurophysiological experiments by electrophysiological as well as calcium imaging techniques. Scalable inhibitory

ion currents were elicited by iDMPO-DNI-GABA with appropriate spatial-temporal precision, blocking both spontaneous and evoked cell activity with excellent efficiency. Additionally, to demonstrate its applicability in a real neurobiological study, we could smoothly and selectively modulate neuronal activities during artificial epileptic rhythms first time in a neural network of GCaMP6f transgenic mouse brain slices.
ACS Omega, 2021; 6
26138975: Wertz A, Trenholm S, Yonehara K, Hillier D, Raics Z, Leinweber M, Szalay G, Ghanem A, Keller G, Rózsa B, Conzelmann KK, Roska B
PRESYNAPTIC NETWORKS. Single-cell-initiated monosynaptic tracing reveals layer-specific cortical network modules. Individual cortical neurons can selectively respond to specific environmental features, such as visual motion or faces. How this relates to the selectivity of the presynaptic network across cortical layers remains unclear. We used single-cell-initiated, monosynaptically restricted retrograde transsynaptic tracing with rabies viruses expressing GCaMP6s to image, in vivo, the visual motion-evoked activity of individual layer 2/3 pyramidal neurons and their presynaptic networks across layers in mouse primary visual cortex. Neurons within each layer exhibited similar motion direction preferences, forming layer-specific functional modules. In one-third of the networks, the layer modules were locked to the direction preference of the postsynaptic neuron, whereas for other networks the direction preference varied by layer. Thus, there exist feature-locked and feature-variant cortical networks.
Science, 2015; 349
27773582: Szalay G, Judák L, Katona G, Ócsai K, Juhász G, Veress M, Szadai Z, Fehér A, Tompa T, Chiovini B, Maák P, Rózsa B
Fast 3D Imaging of Spine, Dendritic, and Neuronal Assemblies in Behaving Animals.
Understanding neural computation requires methods such as 3D acousto-optical (AO) scanning that can simultaneously read out neural activity on both the somatic and dendritic scales. AO point scanning can increase measurement speed and signal-to-noise ratio (SNR) by several orders of magnitude, but high optical resolution requires long point-to-point switching time, which limits imaging capability. Here we present a novel technology, 3D DRIFT AO scanning, which can extend each scanning point to small 3D lines, surfaces, or volume elements for flexible and fast imaging of complex structures simultaneously in multiple locations. Our method was demonstrated by fast 3D recording of over 150 dendritic spines with 3D lines, over 100 somata with squares and cubes, or multiple spiny dendritic segments with surface and volume elements, including in behaving animals. Finally, a 4-fold improvement in total excitation efficiency resulted in about 500 × 500 × 650 μm scanning volume with genetically encoded calcium indicators (GECIs).
Neuron, 2016; 92
22231641: Katona G, Szalay G, Maák P, Kaszás A, Veress M, Hillier D, Chiovini B, Vizi ES, Roska B, Rózsa B
Fast two-photon in vivo imaging with three-dimensional random-access scanning in large tissue volumes.
The understanding of brain computations requires methods that read out neural activity on different spatial and temporal scales. Following signal propagation and integration across a neuron and recording the concerted activity of hundreds of neurons pose distinct challenges, and the design of imaging systems has been mostly focused on tackling one of the two operations. We developed a high-resolution, acousto-optic two-photon microscope with continuous three-dimensional (3D) trajectory and random-access scanning modes that reaches near-cubic-millimeter scan range and can be adapted to imaging different spatial scales. We performed 3D calcium imaging of action potential backpropagation and dendritic spike forward propagation at sub-millisecond temporal resolution in mouse brain slices. We also performed volumetric random-access scanning calcium imaging of spontaneous and visual stimulation-evoked activity in hundreds of neurons of the mouse visual cortex in vivo. These experiments demonstrate the subcellular and network-scale imaging capabilities of our system.
Nat Methods, 2012; 9

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NEW TISSUE CLEARING DEVELOPMENT

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Despite recent advances in tissue cleraring methods, the challenge of reproducibility and simplification remains difficult due to the complexity and cost limitations of the method. We have developed a new tissue cleaing method to overcome current problems by simplifying the tissue removal process, increasing reproducibility and reducing costs. The new tissue clearing method was able to protect against protein loss without the use of nanoporous hydrogel hybrid foam. This method also made undamaged tissue clear without the use of organic solvents or harsh detergents or conditions. Finally, the new tissue cleaing method preserved the fluorescent protein for several months, overcoming the working distance of the microscope and reducing the size of clear tissue or organoids. Using thenew tissue cleaing method, the entire clear brain is imaged under a lightsheet microscope to show that there are significant differences in the distribution of excitatory and inhibitory neurons in the hippocampal region. In addition, we were able to quickly discover the extent of drug diffusion and side effects in the brain in a drug delivery system. Therefore, this method can be applied to read the latest research on the development of diseases and therapeutic agents.

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IMAGING THE BRAIN IN ACTION: AN ACTIVE OPTICAL ROTARY JOINT FOR WIDE FIELD FIBROSCOPY IN FREELY MOVING ANIMALS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Our understanding of the neuronal processes governing behaviour is boosted by the constant development of technological strategies allowing the optical recording of large neuronal assemblies in awake behaving mice. Nowadays, the most common approaches consist in using either miniaturized microscopes directly mounted on animals' head, or sophisticated closed-loop virtual reality systems operating around mice accustomed to be firmly fixed by the head below cutting-edge optical imaging systems. The former limits the quality of the recorded signals due to the constrains in size and weight of the optics required for their portability, the later suffers from the restriction of the

movement repertoire of the animal and the quality of the virtual environment, which hardly reproduces the complexity of natural multi-sensory scenes. Another strategy which takes advantage from both approaches consists in the use of a fiber-bundle interface to carry optical signals from a moving animal to a conventional imaging system. However, as the bundle is usually fixed below the optics, its torsion resulting from rotations of the animal inevitably constrains the behavior over long recordings. To overcome this major limitation of fiberscopic imaging, we developed a motorized optical rotary joint. Here we show its principle of operation, demonstrate its efficacy, and propose several modes of operation for a wide range of experimental designs.

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3D IMAGING OF CLEARED HUMAN TISSUES AND TUMORS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Hans-Ulrich Dodt[1,2], Sabina Kirnbauer[2], James Oakes[2], Christoph Fuchssteiner[2], Massih Foroughipour[1], Meraaj Foroughipour[1], Klaus Becker[1], Saiedeh Saghafi[1]
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Light sheet microscopy has been applied very successfully to the investigation of chemically cleared mouse brains. However its application to human tissue has been hampered by difficulties to obtain specific cellular staining. The use of endogenous fluorescent marker is not possible in humans and staining with antibodies is cumbersome due to diffusion problems. Furthermore standard clearing protocols like 3DISCO take weeks to months to clear human tissue. Notoriously difficult to clear are tumors due to their high cell density. We therefore developed in the last years a new clearing protocol, pathoDISCO, which allows clearing of tumor tissue in the centimetre range within days[1]. This method used boosted autofluorescence for cellular visualization but did not yet provide specific subcellular staining. We now searched for specific fluorescent stainings for cytoplasm and nuclei which also survive our harsh tumor clearing. Applying suitable image processing we were able to generate Hematoxilin/Eosin (HE) stained like images of various human tissues. With our ultramicroscope volume recordings of pathology cassette sized tumor pieces of 4 mm thickness could be obtained with subcellular resolution. Our single optical sections provide a resolution and appearance equivalent to histological slides but now for whole tissue volumes. We applied our technology to various human tissues like brain, mamma and colon. We are confident that this approach will open up completely new ways for histological diagnostics in pathology. [1]Sabdyusheva-Litschauer I et al. (2020) 3D histopathology of human tumours by fast clearing and ultramicroscopy, Sci Rep.10:17619

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VINE-SEG: SELF-IMPROVING VISIBLE NEURON SEGMENTATION FROM CALCIUM IMAGING DATA IN AN USER-INTEGRATING INTERFACE

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Aims: Segmentation of neuronal somata is a crucial and typically the most time-consuming step of the analysis of optical microcircuit imaging experiments. Therefore, recently a variety of auto-segmentation tools, typically using deep learning approaches, emerged to make the segmentation process faster and more consistent. As autosegmentation tools still show problems with false positive and false negative segmentation results, especially when applied to data with a high signal-to-noise ratio and as they are mainly applicable to detect active neuronal somata, we developed ViNe-Seg: A self-improving segmentation tool for all visible neurons that incorporates the user's supervision, offers several segmentation models and can learn from the manual corrections made by the user. **Methods:** We used publicly available labeled Ca-Imaging data from the Allen Brain Obervatory, as well as inhouse data for training our deep learning model. For the neural network we chose the widely used U-Net architecture with two input channels, applying different preprocessing steps, namely the Contrast Limiting Adaptive Histogram Equalization and the non-local means preprocessing on the original data. **Results & Conclusion:** We here present ViNe-Seg, a segmentation tool, embedded in a graphical user interface to ensure easy human supervision and refinement of the segmentation result, with the possibility to add those refined data for enhancing the underlying deep learning model making the whole analysis become more consistent and faster. Accordingly, the semi-automatic approach of ViNe-Seg provides a user-integrating approach for segmentation, that does no longer feel like a black box.

Pubmed:
34601559: Ruffini N, Müller M, Schmitt U, Gerber S
IntelliPy: A GUI for analyzing IntelliCage data.
The IntelliCage systems offer the possibility to conduct long-term behavioral experiments on mice in social groups without human intervention. Although this setup provides new findings, only about 150 studies with the IntelliCage system have been published in the last two decades, which is also caused by the challenging problems of processing and handling the large and heterogeneous amounts of captured data. This application note introduces the Python-GUI IntelliPy, especially designed for users not very experienced in using programming languages. IntelliPy allows users to quickly analyze the IntelliCage output in a user-friendly way, thus making the systems more accessible to a broader audience.
Bioinformatics, 2021;
33758244: Rosales Jubal E, Schwalm M, Dos Santos Guilherme M, Schuck F, Reinhardt S, Tose A, Barger Z, Roesler MK, Ruffini N, Wierczeiko A, Schmeisser MJ, Schmitt U, Endres K, Stroh A
Acitretin reverses early functional network degradation in a mouse model of familial Alzheimer's disease.
Aberrant activity of local functional networks underlies memory and cognition deficits in Alzheimer's disease (AD). Hyperactivity was observed in microcircuits of mice AD-models showing plaques, and also recently in early stage AD mutants prior to amyloid deposition. However, early functional effects of AD on cortical microcircuits remain unresolved. Using two-photon calcium imaging, we found altered temporal distributions (burstiness) in the spontaneous activity of layer II/III visual cortex neurons, in a mouse model of familial Alzheimer's disease (5xFAD), before plaque formation. Graph theory (GT) measures revealed a distinct network topology of 5xFAD microcircuits, as compared to healthy controls, suggesting degradation of parameters related to network robustness. After treatment with acitretin, we observed a re-balancing of those network measures in 5xFAD mice; particularly in the mean degree distribution, related to network development and resilience, and post-treatment values resembled those of age-matched controls. Further, behavioral deficits, and the increase of excitatory synapse numbers in layer II/III were reversed after treatment. GT is widely applied for whole-brain network analysis in human neuroimaging, we here demonstrate the translational value of GT as a multi-level tool, to probe networks at

different levels in order to assess treatments, explore mechanisms, and contribute to early diagnosis.
Sci Rep, 2021; 11
33302607: Ruffini N, Klingenberg S, Schweiger S, Gerber S
Common Factors in Neurodegeneration: A Meta-Study Revealing Shared Patterns on a Multi-Omics Scale.
Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) are heterogeneous, progressive diseases with frequently overlapping symptoms characterized by a loss of neurons. Studies have suggested relations between neurodegenerative diseases for many years (e.g., regarding the aggregation of toxic proteins or triggering endogenous cell death pathways). We gathered publicly available genomic, transcriptomic, and proteomic data from 177 studies and more than one million patients to detect shared genetic patterns between the neurodegenerative diseases on three analyzed omics-layers. The results show a remarkably high number of shared differentially expressed genes between the transcriptomic and proteomic levels for all conditions, while showing a significant relation between genomic and proteomic data between AD and PD and AD and ALS. We identified a set of 139 genes being differentially expressed in several transcriptomic experiments of all four diseases. These 139 genes showed overrepresented gene ontology (GO) Terms involved in the development of neurodegeneration, such as response to heat and hypoxia, positive regulation of cytokines and angiogenesis, and RNA catabolic process. Furthermore, the four analyzed neurodegenerative diseases (NDDs) were clustered by their mean direction of regulation throughout all transcriptomic studies for this set of 139 genes, with the closest relation regarding this common gene set seen between AD and HD. GO-Term and pathway analysis of the proteomic overlap led to biological processes (BPs), related to protein folding and humoral immune response. Taken together, we could confirm the existence of many relations between Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis on transcriptomic and proteomic levels by analyzing the pathways and GO-Terms arising in these intersections. The significance of the connection and the striking relation of the results to processes leading to neurodegeneration between the transcriptomic and proteomic data for all four analyzed neurodegenerative diseases showed that exploring many studies simultaneously, including multiple omics-layers of different neurodegenerative diseases simultaneously, holds new relevant insights that do not emerge from analyzing these data separately. Furthermore, the results shed light on processes like the humoral immune response that have previously been described only for certain diseases. Our data therefore suggest human patients with neurodegenerative diseases should be addressed as complex biological systems by integrating multiple underlying data sources.
Cells, 2020; 9

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TOOLS FOR ACQUISITION AND ANALYSIS OF SIMULTANEOUS NEURAL ACTIVITY AND BLOODFLOW IN AWAKE FREELY BEHAVING ANIMALS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Neurovascular coupling, the intricate link between neural activity and local changes in cerebral blood flow, is critical for brain function and prone to disruption in pathological conditions. Studying neurovascular coupling requires precise measurements of the spatial and temporal relationships of neuronal activity and changes in blood flow. We have developed a dual color miniscope, the nVue system, that allows simultaneous imaging of neural activity and blood flow in freely behaving animals. The nVue system acquires dual color data by multiplexing two LEDs at up to 100 Hz, enabling direct visualization of both blood flow and calcium activity. To facilitate analysis, we have extended the Inscopix Data Processing Software package to include a module that provides time-varying estimates of both vascular diameter and red blood cell (RBC) velocity in vessels of interest. Diameter is measured as the full-width at half max of a Lorentzian function fit to a vessel cross section. RBC velocity is measured by calculating time-lagged cross correlations between a seed pixel at each location of interest and all pixels in a neighboring region. The distances between correlation peaks are then tracked as a function of time. The algorithms were benchmarked against ground truth manual annotation data. To our knowledge this represents the first demonstration of simultaneous acquisition of high resolution blood flow and neural circuit activity signals in freely moving animals which, coupled with with out of the box analysis solutions, opens new avenues of study for both basic and translational neuroscience areas.

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SECOND-HARMONIC GENERATION IMAGING OF AXON-LIKE NEURITES IN THE UNFIXED, UNLABELED RETINA

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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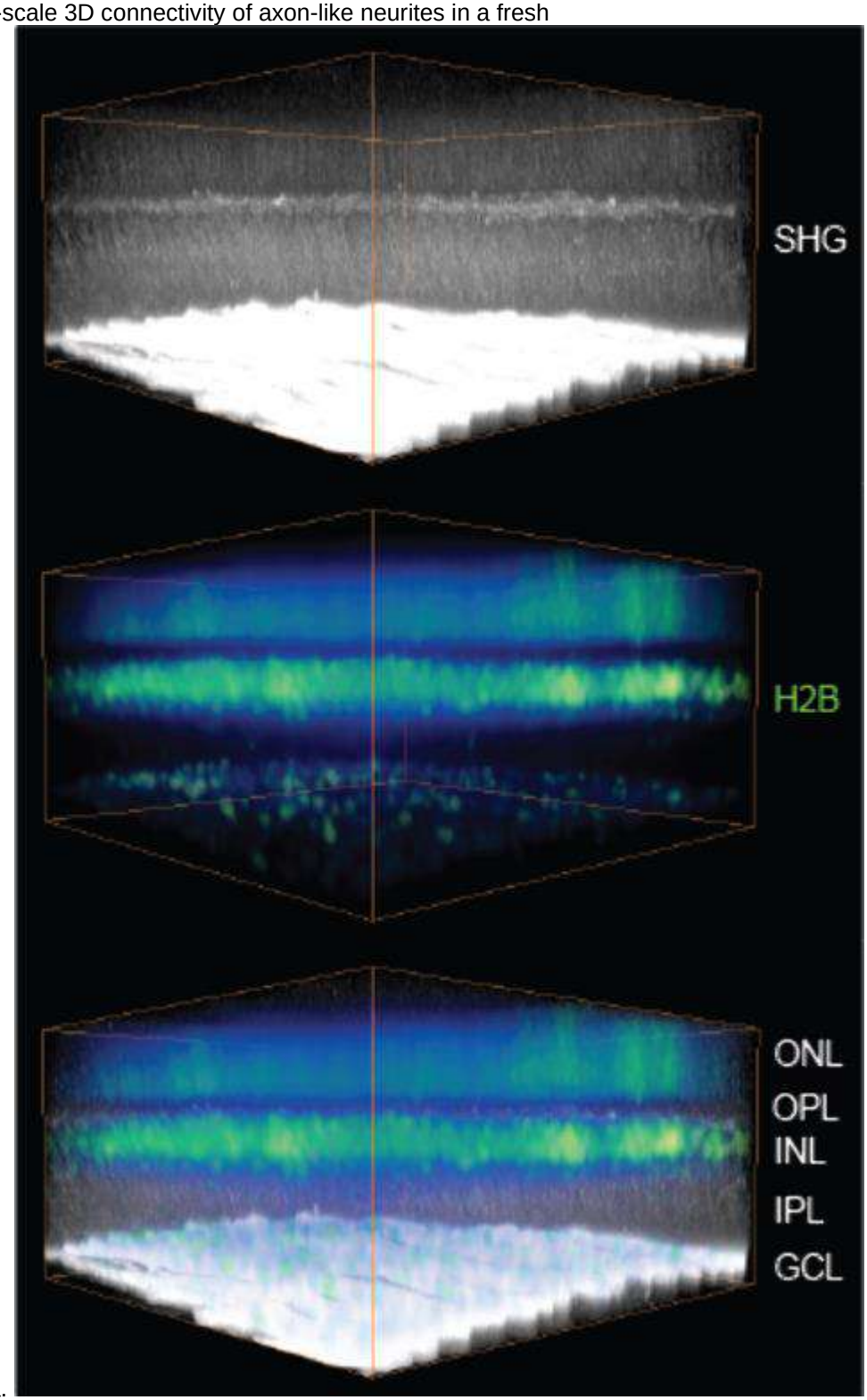
We demonstrate a novel microscopy method for studying the axons in the unfixed, wholemount retina. Second-harmonic generation (SHG) from uniformly polarized microtubules was utilized for visualizing the single axon-like neurites of major retinal neurons, including photoreceptors, horizontal cells, bipolar cells, amacrine cells, as well as the retinal ganglion cells. To confirm the molecular and cellular origin, the nonlinear optical signal was co-registered with GFP/YFP in the retinas of transgenic strains, expressed under the control of GUS, Thy1, ChAT, and GFAP. The axons of visual integration were reconstructed using SHG. In the vertical pathways, the bipolar cell axons terminated in the specific sublayers of inner plexiform layer (IPL) in a cell-type-specific manner. The lateral fibers within the IPL neuropil were also visualized by SHG originating from a specific subset of amacrine cells, i.e., axon-bearing types. To validate the utility of retinal SHG imaging, the pathological degeneration in glaucoma was investigated using a mouse model, DBA/2, and the non-glaucomatous control, DBA/2-*Gpnmb*+. Our results show that there is no loss of compartments of the inner retina pre-synaptic to the RGCs during pathogenesis. In conclusion, retinal SHG imaging provides a valuable tool for interrogating, without exogenous labeling, the

large-scale 3D connectivity of axon-like neurites in a fresh



retina.

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DUAL COLOR IMAGING IN FREELY-BEHAVING RODENTS USING HEAD-MOUNTABLE ONE PHOTON MINISCOPE

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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We have developed a miniscope system that enables dual color imaging in freely-behaving rodents, thus greatly expanding the current range of *in vivo* imaging applications. The nVue™ system enables numerous applications, including simultaneous imaging of Ca^{2+} activity in two distinct cell populations, imaging Ca^{2+} activity with static markers identifying neurons based on projection, activity, or genetics and imaging Ca^{2+} activity alongside neurotransmitter release or blood flow. To enable dual color imaging, we have integrated two separate LEDs to image green and red indicators without optical and biological crosstalk. These LEDs are multiplexed rapidly during imaging, enabling simultaneous visualization of green and red fluorescent signals. To correct for chromatic shifts in green and red focal planes, we have developed novel GRIN lenses that minimize axial chromatic aberrations. Furthermore, we use electronic focusing capabilities of our miniscope to automatically correct for residual color aberration. Here, we present two *in vivo* applications to demonstrate use cases of the nVue system. To demonstrate static + dynamic imaging, we imaged mPFC neurons expressing GCaMP6m and contralaterally-projecting neurons labeled with TdTomato. To demonstrate dual dynamic imaging, we imaged in the dorsal striatum the release of dopamine, using RDA1m, alongside neuronal activity, using GCaMP6m. Demonstration of blood flow imaging, using a rhodamine-dextran dye, alongside neuronal activity, using GCaMP, is presented in a separate poster. These applications demonstrate the utility of the nVue system for exploring the intricacies of how two distinct brain signals interact during free behavior, enabling deeper insights into central nervous system functions.

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HETEROGENOUS DISTRIBUTION OF VACHT AND VGLUT3 IN STRIATAL CHOLINERGIC VARICOSITIES REVEALED BY A NANOSCOPIC ANALYSIS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Mazarine Desplanque[1], Paola Cristofari[1], Odile Poirel[1], Alison Hebert[1], Sylvie Dumas[2], Etienne Herzog[3], Lydia Danglot[4,5,6], David Geny[7], Jean-François Gilles[8], Audrey Geeverding[9], Susanne Bolte[8], Alexis Canette[9], Michael Trichet[9], Véronique Fabre[10], Stephanie Daumas[11], Nicolas Pietrancosta[8,12], Salah El Mestikawy[13], Véronique Bernard[10]
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Aim Striatal cholinergic interneurons (CINs) use both acetylcholine (ACh) and glutamate (Glut) to regulate the striatal network. CINs express vesicular transporters for acetylcholine (VAChT) and Glut (VGLUT3) with a high degree of coexpression in cholinergic varicosities. Whether VAChT and VGLUT3 are present on the same or on different pools of synaptic vesicles (SVs) remains an open question. This is an important issue since the vesicular sorting of both transporters could lead to simultaneous and/or independent release of ACh and/or Glut from CINs. **Methods** We used super-resolution STimulated Emission Depletion microscopy to characterize and quantify the distribution of VAChT and VGLUT3 in CINs SVs. **Results** Nearest-neighbor distances analysis between VAChT and VGLUT3-immunofluorescent spots revealed that 34% of cholinergic SVs contain both VAChT and VGLUT3. 40% of CINS SVs express only VAChT, while 26% contain only VGLUT3. Thus, we propose that CINs terminals have the potential to store and release simultaneously or independently ACh and/or Glut. We also determined the distribution in VAChT and/or VGLUT3 expressing SVs, of vesicular associated membrane proteins (VAMPs), proteins of the SNARE complex involved in neurotransmitter release. Fluorescent in situ hybridization demonstrated that only VAMP2 and VAMP7 are expressed by CINS. We observed that the canonical VAMP2 is expressed in a large part of VAChT and VGLUT3 striatal SVs (63% and 86%). Interestingly, in the shell part of nucleus accumbens, 25% of VAChT and VGLUT3-immunopositive SVs are also VAMP7-positive. **Conclusion** Our results advocate for an unexpected level of anatomical and functional heterogeneity of SVs in CINs varicosities.

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SHAPE MEMORY POLYMER BASED TRANSPARENT ELECTRODE ARRAY FOR LONG-TERM MULTIMODAL NEUROIMAGING

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Zsófia Lantos[1,2], Ágnes Szabó[1,2], Flóra Zsófia Fedor[1,3,4], Anita Zatonyi[1], Miklós Madarász[5,6], Tibor Lőrincz[5], Vindhya Danda[7], Lisa Spurgin[7], Connie Manz[7], Balázs Rózsa[5], Zoltan Fekete[1]
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To map the brain with high temporal and spatial resolution, multimodal neuroimaging methods provide a solution. Combining two-photon microscopy with electrophysiological signal recording is feasible with using transparent electrode materials. We aimed to demonstrate a multimodal neuroimaging scheme using a thiol-ene acrylate based cortical implant. The micro-electrocorticography (μECoG) device's feasibility of measuring intracranial EEG and fluorescent GCamP6 signals using two-photon imaging through the device is presented in mice. The stability of electrode yield was presented with *in vivo* impedance measurement over 75 days. The recording quality was shown by monitoring the signal-to-noise ratio and theta power of the spectrum throughout the experiment. The chronic immune response was characterized by Glial Fibrillary Acidic Protein (GFAP) staining of astrocytes and fluorescent Nissl (NeuroTrace) staining of neurons. To determine the effect of the device on optical distortion and resolution, the sizes of different objects were determined without and under the transparent device placed in the light path of the two-photon microscope. In addition, the change in the relative intensity of fluorescent signals was determined in *in vivo* images under the long-term (22 weeks) implanted device. During the impedance measurement, no sign of delamination or material degradation appeared. After 80 days of implantation, the histological analysis revealed only a modest foreign body response, and no significant difference was shown between implanted and control cortices. During the 22 weeks *in vivo* measurements, the fluorescent activity remained stable and Ca^{2+}signals were captured. Based on the results our device is suitable for multimodal imaging.

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BOARD NUMBER: S01-666

SENPAI: SEGMENTATION OF NEURONS USING PARTIAL DERIVATIVES INFORMATION. A TOOL FOR NEURONAL SEGMENTATION FROM CONFOCAL MICROSCOPY USING K-MEANS AND TOPOLOGICAL INFORMATION

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Simone Cauzzo[1], Miriam Basile[1], Ester Bruno[1], Alejandro Callara[1], Lydia Danglot[2], Arti Ahluwalia[1], Chiara Magliaro[1], Nicola Vanello[1]
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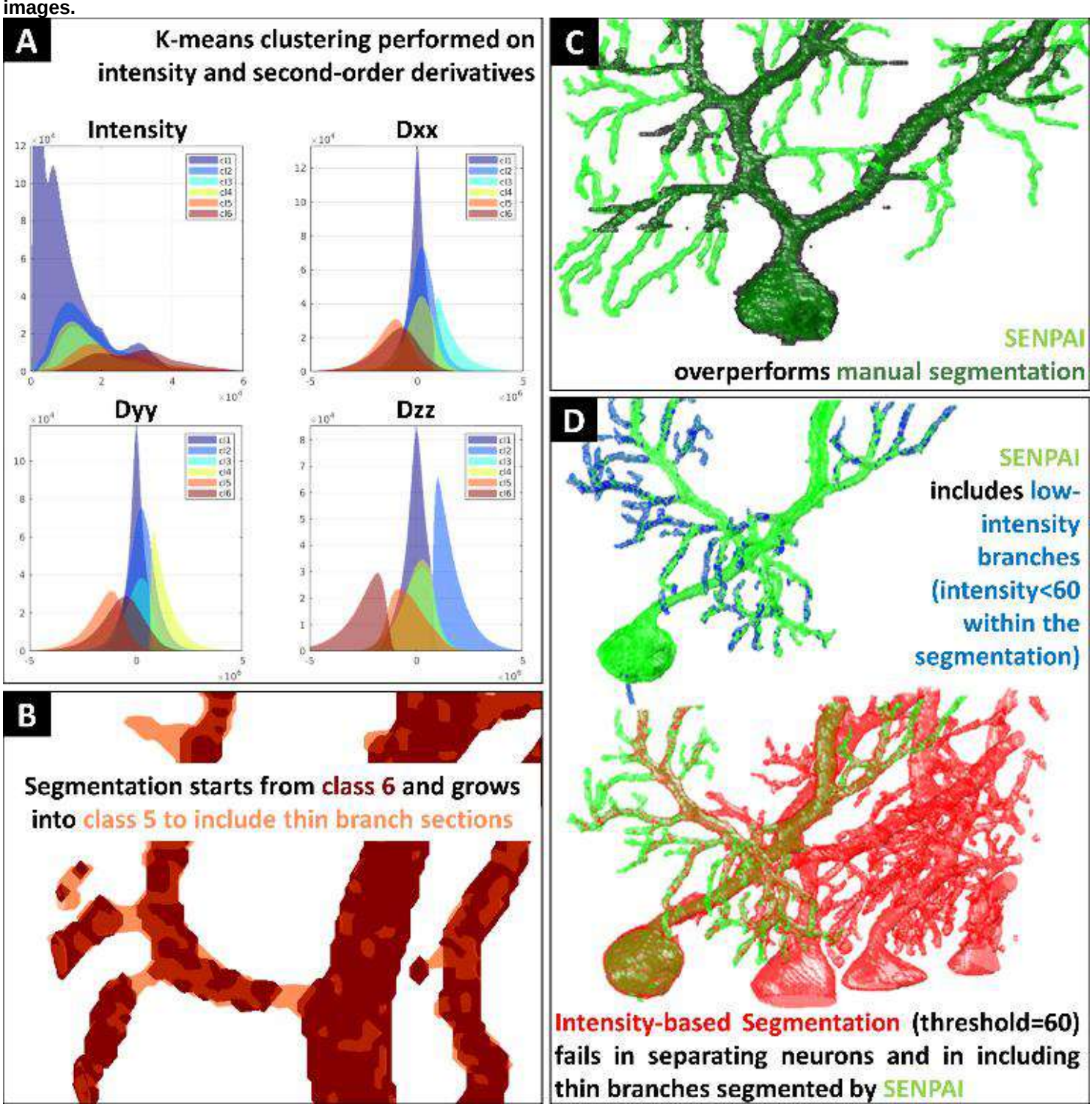
AIMS We introduce a novel approach for segmenting neurons from confocal datasets representing clarified samples. This approach deals with several well-known issues concerning the segmentation of neurons, such as their dense packing and the low contrast affecting small dendrite identification. We propose to improve neuronal segmentation by including volumetric and topological information. Functions of second order derivatives have been used in literature to extract filament-like structures from 2d images. Nonetheless, soma and branch surfaces present irregularities of interest such as those given by dendritic spines. The possibility to build a model-free, topologically-informed approach to 3d neuron segmentation is explored. METHODS An intensity-based k-means clustering approach is coupled with second order spatial derivatives computed along the three main axes. The algorithm is tested on 40x confocal microscopy datasets and compared with manual segmentation and intensity-based clustering. Among the 6 classes, two of them, showing high mean intensity and negative mean second order derivatives, are observed to capture the neural structures (Fig1A-1B). The sixth class, providing the core of each neuron, is dilated into the fifth to include thinner, low-intensity branches (Fig1B). RESULTS Our approach enables us to distinguish and isolate single neurons. The algorithm overperforms manual segmentation (Fig1C) and intensity-based clustering (Fig1D), and captures low-intensity branches. CONCLUSIONS We provide an algorithm that segments the single neurons in 3d image stacks within a single iteration. In future advances, it could be adapted to the segmentation of multi-scale structures (soma, dendrites and dendritic spines) in high-resolution

images.



Pubmed:
34380051: Cauzzo S, Callara AL, Morelli MS, Hartwig V, Esposito F, Montanaro D, Passino C, Emdin M, Giannoni A, Vanello N
Mapping dependencies of BOLD signal change to end-tidal CO: Linear and nonlinear modeling, and effect of physiological noise correction.

Disentangling physiological noise and signal of interest is a major issue when evaluating BOLD-signal changes in response to breath holding. Currently-adopted approaches for retrospective noise correction are general-purpose, and have non-negligible effects in studies on hypercapnic challenges.
J Neurosci Methods, 2021; 362
35031472: Singh K, Cauzzo S, García-Gomar MG, Stauder M, Vanello N, Passino C, Bianciardi M
Functional connectome of arousal and motor brainstem nuclei in living humans by 7 Tesla resting-state fMRI.
Brainstem nuclei play a pivotal role in many functions, such as arousal and motor control. Nevertheless, the connectivity of arousal and motor brainstem nuclei is understudied in living humans due to the limited sensitivity and spatial resolution of conventional imaging, and to the lack of atlases of these deep tiny regions of the brain. For a holistic comprehension of sleep, arousal and associated motor processes, we investigated in 20 healthy subjects the resting-state functional connectivity of 18 arousal and motor brainstem nuclei in living humans. To do so, we used high spatial-resolution 7 Tesla resting-state fMRI, as well as a recently developed in-vivo probabilistic atlas of these nuclei in stereotactic space. Further, we verified the translatability of our brainstem connectome approach to conventional (e.g. 3 Tesla) fMRI. Arousal brainstem nuclei displayed high interconnectivity, as well as connectivity to the thalamus, hypothalamus, basal forebrain and frontal cortex, in line with animal studies and as expected for arousal regions. Motor brainstem nuclei showed expected connectivity to the cerebellum, basal ganglia and motor cortex, as well as high interconnectivity. Comparison of 3 Tesla to 7 Tesla connectivity results indicated good translatability of our brainstem connectome approach to conventional fMRI, especially for cortical and subcortical (non-brainstem) targets and to a lesser extent for brainstem targets. The functional connectome of 18 arousal and motor brainstem nuclei with the rest of the brain might provide a better understanding of arousal, sleep and accompanying motor functions in living humans in health and disease.
Neuroimage, 2022; 249
35074504: Cauzzo S, Singh K, Stauder M, García-Gomar MG, Vanello N, Passino C, Staab J, Indovina I, Bianciardi M
Functional connectome of brainstem nuclei involved in autonomic, limbic, pain and sensory processing in living humans from 7 Tesla resting state fMRI.
Despite remarkable advances in mapping the functional connectivity of the cortex, the functional connectivity of subcortical regions is understudied in living humans. This is the case for brainstem nuclei that control vital processes, such as autonomic, limbic, nociceptive and sensory functions. This is because of the lack of precise brainstem nuclei localization, of adequate sensitivity and resolution in the deepest brain regions, as well as of optimized processing for the brainstem. To close the gap between the cortex and the brainstem, on 20 healthy subjects, we computed a correlation-based functional connectome of 15 brainstem nuclei involved in autonomic, limbic, nociceptive, and sensory function (superior and inferior colliculi, ventral tegmental area-parabrachial pigmented nucleus complex, microcellular tegmental nucleus-prabigeminal nucleus complex, lateral and medial parabrachial nuclei, vestibular and superior olivary complex, superior and inferior medullary reticular formation, viscerosensory motor nucleus, raphe magnus, pallidus, and obscurus, and parvicellular reticular nucleus - alpha part) with the rest of the brain. Specifically, we exploited 1.1mm isotropic resolution 7 Tesla resting-state fMRI, ad-hoc coregistration and physiological noise correction strategies, and a recently developed probabilistic template of brainstem nuclei. Further, we used 2.5mm isotropic resolution resting-state fMRI data acquired on a 3 Tesla scanner to assess the translatability of our results to conventional datasets. We report highly consistent correlation coefficients across subjects, confirming available literature on autonomic, limbic, nociceptive and sensory pathways, as well as high interconnectivity within the central autonomic network and the vestibular network. Interestingly, our results showed evidence of vestibulo-autonomic interactions in line with previous work. Comparison of 7 Tesla and 3 Tesla findings showed high translatability of results to conventional settings for brainstem-cortical connectivity and good yet weaker translatability for brainstem-brainstem connectivity. The brainstem functional connectome might bring new insight in the understanding of autonomic, limbic, nociceptive and sensory function in health and disease.
Neuroimage, 2022; 250
31946934: Callara AL, Vanello N, Sole Morelli M, Cauzzo S, Giannoni A, Hartwig V, Montanaro D, Landini L, Passino C, Emdin M
Exploring the supra linear relationship between PetCO2 and fMRI signal change with ICA.
The relationships between brain functions and the respiratory system are complex. Disentangling brain activity related to CO changes from nonspecific vasoreactivity is a challenge when studying brain activity involved in the control of breathing with fMRI. In this work, we analyzed a dose dependent relationship between arterial CO levels and brain response. To accomplish this goal, we developed a gas administration protocol, together with multi-subject ICA and specific nonlinear post-processing analysis. Our results highlighted a supra-linear response to CO challenges in brainstem, thalamus and putamen. Results were discussed in the light of current knowledge about the central respiratory network.
Annu Int Conf IEEE Eng Med Biol Soc, 2019; 2019
31946018: Cauzzo S, Callara AL, Sole Morelli M, Hartwig V, Montanaro D, Passino C, Emdin M, Giannoni A, Vanello N

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On the Use of Linear-Modelling-based Algorithms for Physiological Noise Correction in fMRI Studies of the Central Breathing Control.

A full characterization of the physiological behavior of human central chemoreceptors through fMRI is crucial to understand the pathophysiology of central abnormal breathing patterns. In this scenario, physiological noise and activity of interest may be naturally correlated. Here, we examined the adequacy of linear-modelling-based retrospective physiological noise correction for studies of the central breathing control. We focused on the relationship between a nonlinear model of BOLD response, hypothesized to describe neuronal specific activity, and noise modelled by correction algorithms. Analyses were performed on fMRI acquisitions from healthy subjects during a breath hold task. A general linear model including static nonlinearities in the response to end-tidal CO was applied to data preprocessed both with and without physiological noise correction. Relations between physiological noise and PETCO were explored both with linear and nonlinear measures. Lastly, parametric maps of noise spatial distribution were extracted. Our results evidenced that correction algorithms based on linear modelling remove components that are both linearly and nonlinearly related to end-tidal CO, whereas uncorrected data showed spurious activations in regions outside gray matter. Thus, despite a correction step is fundamental, these algorithms are shown to be over-conservative approaches to noise correction and need to be adapted to the specific purpose.

Annu Int Conf IEEE Eng Med Biol Soc, 2019; 2019

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BOARD NUMBER: S01-667

IMPROVED OPTICAL RECORDING OF IN-VIVO NEURONAL ACTIVITY IN 3D CORTICAL NEURON POPULATIONS WITH PATTERNED EXCITATION OF SOMA-RESTRICTED CALCIUM PROBES

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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3D Custom Access Serial Holography (3D-CASH) is an advanced acquisition method for *in-vivo* 3D multiphoton excitation microscopy improving upon the intrinsic slowness of volumetric multi-plane imaging of neuronal activity. 3D-CASH combines serial 3D random-access of neuronal targets with holographically patterned excitation to increase the photon yield per target and the number of sampled targets per second[1]. However, besides shaping the focal volume, holographic modulation increases background emission compared to an unmodulated Gaussian beam due to enhanced excitation of fluorescent objects outside of the focal volume, resulting in an effective sensitivity loss of the probe and manifesting, for instance, as diminishment of the untreated response amplitude. For a modulated beam generating a 5x5 focus grid of 16x16 μm^2 size covering the somatic and peri-somatic space of a targeted cell, we measured a sensitivity loss of up to 70 % for GCaMP6f expressed in layer 2/3 neurons of mouse visual cortex at 100 to 200 µm focal depth, in agreement with the loss calculated from the modeled 3D distribution of the light field in a scattering medium resembling brain tissue. We hypothesized that this loss can be partially rescued by suppressing the neuropil background through soma-restricted expression of the probe. We first tested this hypothesis computationally by removing the neuropil component from the modeled medium, which reduced the loss to 10 to 20 % depending on focal depth. Finally, we tested the prediction experimentally in visual cortex neurons expressing ribosome-targeted GCaMPs. [1] Akemann et al. Nature Methods 19, 100-110, 2022

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BOARD NUMBER: S01-668

COMPARING CLEARING METHODS AND IMAGING PROCEDURES IN LIGHT SHEET MICROSCOPY

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Marie Habart[1], Antoine Ameloot[1], Amelie Soumier[2], Guillaume Lio[2], Caroline Demily[2], Angela Sirigu[2]
[1]Centre National de la Recherche Scientifique, Institut Des Sciences Cognitives - Marc Jeannerod Umr5229, VILLEURBANNE, France, [2]iMIND Center of Excellence for Autism, Vinatier Psychiatric Hospital, Lyon, France, Bron, France

Recently, there has been a considerable development of clearing techniques associated with the use of light-sheet-microscopy. The clearing method makes a tissue sample transparent while allowing proteins labelling to be imaged in a 3D representation without the need to slice the tissue. Many clearing procedures are available but it is difficult to choose the most appropriate one for a given biochemical tissue (proteins, lipids, and glucids). Biological samples such as embryos, brains, hearts or bones have a different structure and, therefore, the choice of the right clearing procedure requires a careful consideration. Here we employed several clearing methods in rodents and non-human primates and compared their performance for transparency and staining quality on brain tissues but also lungs, gut, and whole pup samples. We found that the iDISCO+ protocol is most suitable for clearing the mouse brain. However, when performed on a thick bloc of monkey brain tissue, it produces a transparent dark sample with low antibody penetration. The PEGASOS method gives the best clearing result for the gut although it is disappointing when applied to brain tissue. Finally, we use the light-sheet-microscopy to image our 3D samples. We observed that the signal intensity depends on parameters such as the laser power and wavelength. The 568nm laser power is the strongest available on the light sheet, but it should be employed when expecting weak staining. Our findings provide some heuristics to aid decisions on the best match between clearing, immunostaining and 3D-light-sheet imaging.

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BOARD NUMBER: S01-669

SIMULTANEOUS TWO PHOTON IMAGING ON MULTIPLE NEURONS IN THREE DIMENSIONS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Paolo Pozzi[1], Miriam Cavagnini[1], Gabriele Losi[2], Michele Zoli[1], Jonathan Mapelli[1]
[1]University of Modena and Reggio Emilia, Dept. Of Biomedical, Metabolic And Neural Sciences, Modena, Italy, [2]Centro Nazionale delle Ricerche, Nanoscienze, Modena, Italy

AIM: Two photon microscopy (2PM) is considered the state of the art method for the detection of calcium activity from neurons in three dimensional brain tissues and in vivo. However, it is generally limited by its laser scanning nature to imaging of bidimensional planes, severely limiting the information content of the acquired dataset. We present a method, based on computer generated holography, for the simultaneous imaging of multiple neurons axially displaced up to 100 µm. **Methods:** 3D-2PM images area acquired through a conventional raster scanning procedure. A spatial light modulator is then employed to split light from a Ti:Sa laser in multiple diffraction limited foci each positioned on neurons location either automatically identified through a custom-developed algorithm or selected by the user. All laser foci are scanned simultaneously in a raster pattern, and the fluorescence emitted is recorded by a high speed camera. Nonnegative matrix factorization methods are used to unmix the fluorescence signals from different ROIs, allowing to reconstruct multiple raster scanning image sequences. **Results:** The proposed method has been tested in brain slices stained with Fura-2, and in-vivo in mice expressing GCaMP proteins. 10x10 pixel images reporting calcium signals could be acquired simultaneously from tens of neurons within a 400x400x100 μm^3 volume at 10 Hz, demonstrating the that the proposed experimental and software pipeline allows to monitor the functional activity of 3D neuronal circuits with single cells resolution.

Pubmed:
32630905: Pozzi P, Quintavalla M, Wong AB, Borst JGG, Bonora S, Verhaegen M
Plug-and-play adaptive optics for commercial laser scanning fluorescence microscopes based on an adaptive lens.
In this Letter, we present a solution for simple implementation of adaptive optics in any existing laser scanning fluorescence microscope. Adaptive optics are implemented by the introduction of a multiactuator adaptive lens between the microscope body and the objective lens. Correction is performed with a sensorless method by optimizing the quality of the images presented on screen by the microscope software. We present the results acquired on both a commercial linear excitation confocal microscope and a custom-made multiphoton excitation microscope.
Opt Lett, 2020; 45
33716671: Pozzi P, Mapelli J
Real Time Generation of Three Dimensional Patterns for Multiphoton Stimulation.
The advent of optogenetics has revolutionized experimental research in the field of Neuroscience and the possibility to selectively stimulate neurons in 3D volumes has opened new routes in the understanding of brain dynamics and functions. The combination of multiphoton excitation and optogenetic methods allows to identify and excite specific neuronal targets by means of the generation of cloud of excitation points. The most widely employed approach to produce the points cloud is through a spatial light modulation (SLM) which works with a refresh rate of tens of . However, the computational time requested to calculate 3D patterns ranges between a few seconds and a few minutes, strongly limiting the overall performance of the system. The maximum speed of SLM can in fact be employed either with high quality patterns embedded into pre-calculated sequences or with low quality patterns for real time update. Here, we propose the implementation of a recently developed compressed sensing Gerchberg-Saxton algorithm on a consumer graphical processor unit allowing the generation of high quality patterns at video rate. This, would in turn dramatically reduce dead times in the experimental sessions, and could enable applications previously impossible, such as the control of neuronal network activity driven by the feedback from single neurons functional signals detected through calcium or voltage imaging or the real time compensation of motion artifacts.
Front Cell Neurosci, 2021; 15
32403465: Pozzi P, Smith C, Carroll E, Wilding D, Soloviev O, Booth M, Vdovin G, Verhaegen M
Anisoplanatic adaptive optics in parallelized laser scanning microscopy.
Inhomogeneities in the refractive index of a biological microscopy sample can introduce phase aberrations, severely impairing

the quality of images. Adaptive optics can be employed to correct for phase aberrations and improve image quality. However, conventional adaptive optics can only correct a single phase aberration for the whole field of view (isoplanatic correction) while, due to the highly heterogeneous nature of biological tissues, the sample induced aberrations in microscopy often vary throughout the field of view (anisoplanatic aberration), limiting significantly the effectiveness of adaptive optics. This paper reports on a new approach for aberration correction in laser scanning confocal microscopy, in which a spatial light modulator is used to generate multiple excitation points in the sample to simultaneously scan different portions of the field of view with completely independent correction, achieving anisoplanatic compensation of sample induced aberrations, in a significantly shorter time compared to sequential isoplanatic correction of multiple image subregions. The method was tested in whole Drosophila brains and in larval Zebrafish, each showing a dramatic improvement in resolution and sharpness when compared to conventional isoplanatic adaptive optics.
Opt Express, 2020; 28
34808830: Talone B, Pozzi P, Cavagnini M, Polli D, Pozzi G, Mapelli J
Experimental determination of shift-less aberration bases for sensorless adaptive optics in nonlinear microscopy.
Adaptive optics can improve the performance of optical systems and devices by correcting phase aberrations. While in most applications wavefront sensing is employed to drive the adaptive optics correction, some microscopy methods may require sensorless optimization of the wavefront. In these cases, the correction is performed by describing the aberration as a linear combination of a base of influence functions, optimizing an image quality metric as a function of the coefficients. The influence functions base is generally chosen to either efficiently represent the adaptive device used or to describe generic wavefronts in an orthogonal fashion. A rarely discussed problem is that most correction bases have elements which introduce, together with a correction of the aberration, a shift of the imaging field of view in three dimensions. While simple methods to solve the problem are available for linear microscopy methods, nonlinear microscopy techniques such as multiphoton or second harmonic generation microscopy require non-trivial base determination. In this paper, we discuss the problem, and we present a method for calibrating a shift-less base on a spatial light modulator for two-photon microscopy.
Opt Express, 2021; 29
31164587: Pozzi P, Maddalena L, Ceffa N, Soloviev O, Vdovin G, Carroll E, Verhaegen M
Fast Calculation of Computer Generated Holograms for 3D Photostimulation through Compressive-Sensing Gerchberg-Saxton Algorithm.
The use of spatial light modulators to project computer generated holograms is a common strategy for optogenetic stimulation of multiple structures of interest within a three-dimensional volume. A common requirement when addressing multiple targets sparsely distributed in three dimensions is the generation of a points cloud, focusing excitation light in multiple diffraction-limited locations throughout the sample. Calculation of this type of holograms is most commonly performed with either the high-speed, low-performance random superposition algorithm, or the low-speed, high performance Gerchberg-Saxton algorithm. This paper presents a variation of the Gerchberg-Saxton algorithm that, by only performing iterations on a subset of the data, according to compressive sensing principles, is rendered significantly faster while maintaining high quality outputs. The algorithm is presented in high-efficiency and high-uniformity variants. All source code for the method implementation is available as Supplementary Materials and as open-source software. The method was tested computationally against existing algorithms, and the results were confirmed experimentally on a custom setup for in-vivo multiphoton optogenetics. The results clearly show that the proposed method can achieve computational speed performances close to the random superposition algorithm, while retaining the high performance of the Gerchberg-Saxton algorithm, with a minimal hologram quality loss.
Methods Protoc, 2018; 2
26157984: Pozzi P, Gandolfi D, Tognolina M, Chirico G, Mapelli J, D'Angelo E
High-throughput spatial light modulation two-photon microscopy for fast functional imaging.
The optical monitoring of multiple single neuron activities requires high-throughput parallel acquisition of signals at millisecond temporal resolution. To this aim, holographic two-photon microscopy (2PM) based on spatial light modulators (SLMs) has been developed in combination with standard laser scanning microscopes. This requires complex coordinate transformations for the generation of holographic patterns illuminating the points of interest. We present a simpler and fully digital setup (SLM-2PM) which collects three-dimensional two-photon images by only exploiting the SLM. This configuration leads to an accurate placement of laser beamlets over small focal volumes, eliminating mechanically moving parts and making the system stable over long acquisition times. Fluorescence signals are diffraction limited and are acquired through a pixelated detector, setting the actual limit to the acquisition rate. High-resolution structural images were acquired by raster-scanning the sample with a regular grid of excitation focal volumes. These images allowed the selection of the structures to be further investigated through an interactive operator-guided selection process. Functional signals were collected by illuminating all the preselected points with a single hologram. This process is exemplified for high-speed (up to 1 kHz) two-photon calcium imaging on acute cerebellar slices.

Neurophotonics, 2015; 2
24782707: Gandolfi D, Pozzi P, Tognolina M, Chirico G, Mapelli J, D'Angelo E
The spatiotemporal organization of cerebellar network activity resolved by two-photon imaging of multiple single neurons. In order to investigate the spatiotemporal organization of neuronal activity in local microcircuits, techniques allowing the simultaneous recording from multiple single neurons are required. To this end, we implemented an advanced spatial-light modulator two-photon microscope (SLM-2PM). A critical issue for cerebellar theory is the organization of granular layer activity in the cerebellum, which has been predicted by single-cell recordings and computational models. With SLM-2PM, calcium signals could be recorded from different network elements in acute cerebellar slices including granule cells (GrCs), Purkinje cells (PCs) and molecular layer interneurons. By combining WCRs with SLM-2PM, the spike/calcium relationship in GrCs and PCs could be extrapolated toward the detection of single spikes. The SLM-2PM technique made it possible to monitor activity of over tens to hundreds neurons simultaneously. GrC activity depended on the number of spikes in the input mossy fiber bursts. PC and molecular layer interneuron activity paralleled that in the underlying GrC population revealing the spread of activity through the cerebellar cortical network. Moreover, circuit activity was increased by the GABA-A receptor blocker, gabazine, and reduced by the AMPA and NMDA receptor blockers, NBQX and APV. The SLM-2PM analysis of spatiotemporal patterns lent experimental support to the time-window and center-surround organizing principles of the granular layer.
Front Cell Neurosci, 2014; 8

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BOARD NUMBER: S01-670

LARGE-SCALE TWO-PHOTON CALCIUM IMAGING IN FREELY MOVING MICE

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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[1]Kavli Institute for Systems Neuroscience, Ntnu, Trondheim, Norway, [2]NTNU, Kavli Institute For Systems Neuroscience And Centre For Neural Computation, Trondheim, Norway

Due to the unsuitability of benchtop two-photon (2P) imaging for behavior that requires voluntary movement, investigators have tried, for almost two decades, to develop miniature 2P microscopes – 2P miniscopes – that can be carried on the head of freely moving animals. Early 2P miniscopes faced major challenges, however, including temporal dispersion in the excitation fiber, image distortion due to slow scanning, heavy weight and inflexible optical cables. These constraints motivated the development of a new generation of 2P miniscopes (Zong, et al.,2017, Zong, et al.,2021) with 2P benchtop-comparable resolution, fast speed, z-scanning capability, and large field of view (FOV). However, while these 2P miniscopes represented major advances in imaging quality, their applicability in tasks that require movement remains limited, due to heavy weight, stiff optical cable, and low cell yield. Here we present a new generation of 2P miniscopes, MINI2P, that overcomes the limits of previous versions by both meeting requirements for fatigue-free exploratory behavior during extended recording periods and satisfying demands for further increasing the cell yield by an order of magnitude, to thousands of neurons. MINI2P is open source and can be built in most imaging labs. The performance and reliability of MINI2P are validated by recordings of spatially tuned neurons in three brain regions and in three behavioral assays. Imaging quality remained stable across regions and behaviors, and there was no significant difference in behavioral performance between trials with and without the MINI2P miniscope mounted on the animal's head.

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BOARD NUMBER: S01-671

SATURATED RECONSTRUCTION OF LIVING MAMMALIAN BRAIN TISSUE

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Philipp Velicky[1], Eder Miguel[1], Julia Michalska[1], Yoav Ben-Simon[2], Jakob Troidl[3], Jake Watson[2], Zudi Lin[3], Donglai Wei[3], Johanna Beyer[3], Alban Cenameri[1], Christoph Sommer[4], Wiebke Jahr[1], Johannes Broichhagen[5], Seth Grant[6], Gaia Novarino[2], Peter Jonas[2], Hanspeter Pfister[3], Bernd Bickel[1], Johann Danzl[1]
[1]Institute of Science and Technology, Information And Systems Science, Klosterneuburg, Austria, [2]Institute of Science and Technology, Life Sciences, Klosterneuburg, Austria, [3]Harvard University, School Of Engineering And Applied Sciences, Boston, United States of America, [4]Institute of Science and Technology, Imaging And Optics Facility, Klosterneuburg, Austria, [5]Leibniz-FMP, Chemical Biology, Berlin, Germany, [6]University of Edinburgh, Centre For Clinical Brain Sciences, Edinburgh, United Kingdom

Complex wiring between neurons underlies the information processing network enabling brain function, including thought and memory. Electron microscopy delivers nanometre-scale spatial resolution to reconstruct densely packed brain tissue in-silico at synaptic connectivity level but is limited to fixed specimens, delivering static snapshots of tissue architecture. Light microscopy allows observation of living systems and excels at highlighting specific molecules but lacks resolution for brain reconstruction due to light diffraction. Super-resolution optical imaging approaches overcome this resolution barrier but factors including photobleaching, tissue photo-burden, insufficient 3D-resolution and signal-to-noise ratio (SNR) have impeded nanoscale 3D modeling of nervous tissue. Here we demonstrate saturated reconstruction of living brain tissue. We developed an integrated imaging and analysis technology by adapting stimulated emission depletion (STED) microscopy for high-SNR and isotropic resolution in tissue and drastically reducing photo-burden by incorporating previous information on sample structure. This enables volumetric 3D-super-resolved imaging amenable to deep-learning based, saturated tissue reconstruction down to the level of an individual synapse. Moreover, it alllows combination with the full live-imaging toolbox including molecular labelling, electrophysiological recording, chemogenetic activation of specific neurons with simultaneous Ca^{2+} imaging and morphodynamic measurements of neurons and glia cells. This technology will provide yet unknown insight into live brain tissue (nano-)architecture in health and disease.

Pubmed:
29679053: Velicky P, Windsperger K, Petroczi K, Pils S, Reiter B, Weiss T, Vondra S, Ristl R, Dekan S, Fiala C, Cantonwine DE, McElrath TF, Jilma B, Knöfler M, Boehm T, Pollheimer J
Pregnancy-associated diamine oxidase originates from extravillous trophoblasts and is decreased in early-onset preeclampsia.
Human extravillous trophoblast (EVT) invasion of the pregnant uterus constitutes a pivotal event for the establishment of the maternal-fetal interface. Compromised EVT function manifesting in inadequate arterial remodeling is associated with the severe pregnancy disorder early-onset preeclampsia (eoPE). Recent studies suggest that EVTs invade the entire uterine vasculature including arteries, veins and lymphatics in the first trimester of pregnancy. We therefore hypothesized that EVT-derived factors accumulate in the circulation of pregnant women early in gestation and may serve to predict eoPE. In contrast to published literature, we demonstrate that placenta-associated diamine oxidase (DAO) is not expressed by maternal decidual cells but solely by EVTs, especially when in close proximity to decidual vessels. Cultures of primary EVTs express and secret large amounts of bioactive DAO. ELISA measurements indicate a pregnancy-specific rise in maternal DAO plasma levels around gestational week (GW) 7 coinciding with vascular invasion of EVTs. Strikingly, DAO levels from eoPE cases were significantly lower (40%) compared to controls in the first trimester of pregnancy but revealed no difference at mid gestation. Furthermore, DAO-containing pregnancy plasma rapidly inactivates pathophysiologically relevant histamine levels. This study represents the first proof of concept suggesting EVT-specific signatures as diagnostic targets for the prediction of eoPE.
Sci Rep, 2018; 8
26418186: Velicky P, Knöfler M, Pollheimer J
Function and control of human invasive trophoblast subtypes: Intrinsic vs. maternal control.
The establishment of a functional placenta is pivotal for normal fetal development and the maintenance of pregnancy. In the

course of early placentation, trophoblast precursors differentiate into highly invasive trophoblast subtypes. These cells, referred to as extravillous trophoblasts (EVTs), penetrate the maternal uterus reaching as far as the inner third of the myometrium. One of the most fundamental functions of EVTs is the transformation of spiral arteries to establish the uteroplacental blood circulation assuring an adequate nutrient and gas supply to the developing fetus. To achieve this, specialized EVT subpopulations interact with maternal immune cells, provoke elastolysis in the arterial wall and replace the endothelial cells lining the spiral arteries to induce intraluminal vascular remodeling. These and other trophoblast-mediated processes are tightly controlled by paracrine signals from the maternal decidua and furthermore underlie an intrinsic cell-type specific program. Various severe pregnancy complications such as preeclampsia or intrauterine growth retardation are associated with abnormal EVT function, shallow invasion, and decreased blood flow to the placenta. Hence a better understanding of human trophoblast invasion seems mandatory to improve therapeutic intervention. This approach, however, requires a profound knowledge of the human placenta, its various trophoblast subtypes and in particular a better understanding of the regulatory network that controls the invasive phenotype of EVTs.

Cell Adh Migr, 2016; 10

24850908: Velicky P, Haider S, Otti GR, Fiala C, Pollheimer J, Knöfler M

Notch-dependent RBPJκ inhibits proliferation of human cytotrophoblasts and their differentiation into extravillous trophoblasts.

Abnormal development of invasive trophoblasts has been implicated in the pathogenesis of human pregnancy diseases such as pre-eclampsia. However, critical signalling pathways controlling formation and differentiation of these cells have been poorly elucidated. Here, we provide evidence that the canonical Notch pathway, operating through Notch-dependent activation of its key regulatory transcription factor RBPJκ, controls proliferation and differentiation in villous explant cultures and primary trophoblasts of early pregnancy. Immunofluorescence of first trimester placental tissue revealed expression of RBPJκ and its co-activators, the MAML proteins, in nuclei of proliferative cell column trophoblasts (CCT) and differentiated, extravillous trophoblasts (EVTs). However, RBPJκ expression, transcript levels of the Notch target gene HES1 and activity of a Notch/RBPJκ-dependent luciferase reporter decreased during in vitro differentiation of primary cytotrophoblasts on fibronectin. Silencing of RBPJκ using silencing RNAs (siRNAs) increased proliferation of CCTs in floating villous explant cultures analysed by outgrowth and BrdU labelling. Similarly, down-regulation of the transcription factor enhanced BrdU incorporation in isolated primary cultures. However, motility of these cells was not affected. In addition, gene silencing of RBPJκ increased cyclin D1 expression in the two trophoblast model systems as well as markers of the differentiated, EVT, i.e. integrin α1, ADAM12 and T-cell factor 4. In summary, the data suggest that Notch-dependent RBPJκ activity could be required for balanced rates of trophoblast proliferation and differentiation in human placental anchoring villi preventing exaggerated trophoblast overgrowth as well as premature formation of EVTs.

Mol Hum Reprod, 2014; 20

30312291: Velicky P, Meinhardt G, Plessl K, Vondra S, Weiss T, Haslinger P, Lendl T, Aumayr K, Mairhofer M, Zhu X, Schütz B, Hannibal RL, Lindau R, Weil B, Ernerudh J, Neesen J, Egger G, Mikula M, Röhrl C, Urban AE, Baker J, Knöfler M, Pollheimer J

Genome amplification and cellular senescence are hallmarks of human placenta development.

Genome amplification and cellular senescence are commonly associated with pathological processes. While physiological roles for polyploidization and senescence have been described in mouse development, controversy exists over their significance in humans. Here, we describe tetraploidization and senescence as phenomena of normal human placenta development. During pregnancy, placental extravillous trophoblasts (EVTs) invade the pregnant endometrium, termed decidua, to establish an adapted microenvironment required for the developing embryo. This process is critically dependent on continuous cell proliferation and differentiation, which is thought to follow the classical model of cell cycle arrest prior to terminal differentiation. Strikingly, flow cytometry and DNAseq revealed that EVT formation is accompanied with a genome-wide polyploidization, independent of mitotic cycles. DNA replication in these cells was analysed by a fluorescent cell-cycle indicator reporter system, cell cycle marker expression and EdU incorporation. Upon invasion into the decidua, EVTs widely lose their replicative potential and enter a senescent state characterized by high senescence-associated (SA) β-galactosidase activity, induction of a SA secretory phenotype as well as typical metabolic alterations. Furthermore, we show that the shift from endocycle-dependent genome amplification to growth arrest is disturbed in androgenic complete hydatidiform moles (CHM), a hyperplastic pregnancy disorder associated with increased risk of developing choriocarinoma. Senescence is decreased in CHM-EVTs, accompanied by exacerbated endoreduplication and hyperploidy. We propose induction of cellular senescence as a ploidy-limiting mechanism during normal human placentation and unravel a link between excessive polyploidization and reduced senescence in CHM.

PLoS Genet, 2018; 14

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BOARD NUMBER: S01-672

INFLUENCE OF LOCAL BRAIN ANATOMY ON THE SPATIAL EXTENT OF FIBER PHOTOMETRY SIGNAL

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Cinzia Montinaro[1,2], Marco Pisanello[1], Marco Bianco[1,3], Barbara Spagnolo[1], Filippo Pisano[1], Antonio Balena[4], Francesco De Nuccio[2], Dario Lofrumento[2], Tiziano Verri[2], Massimo De Vittorio[1,3], Ferruccio Pisanello[1]
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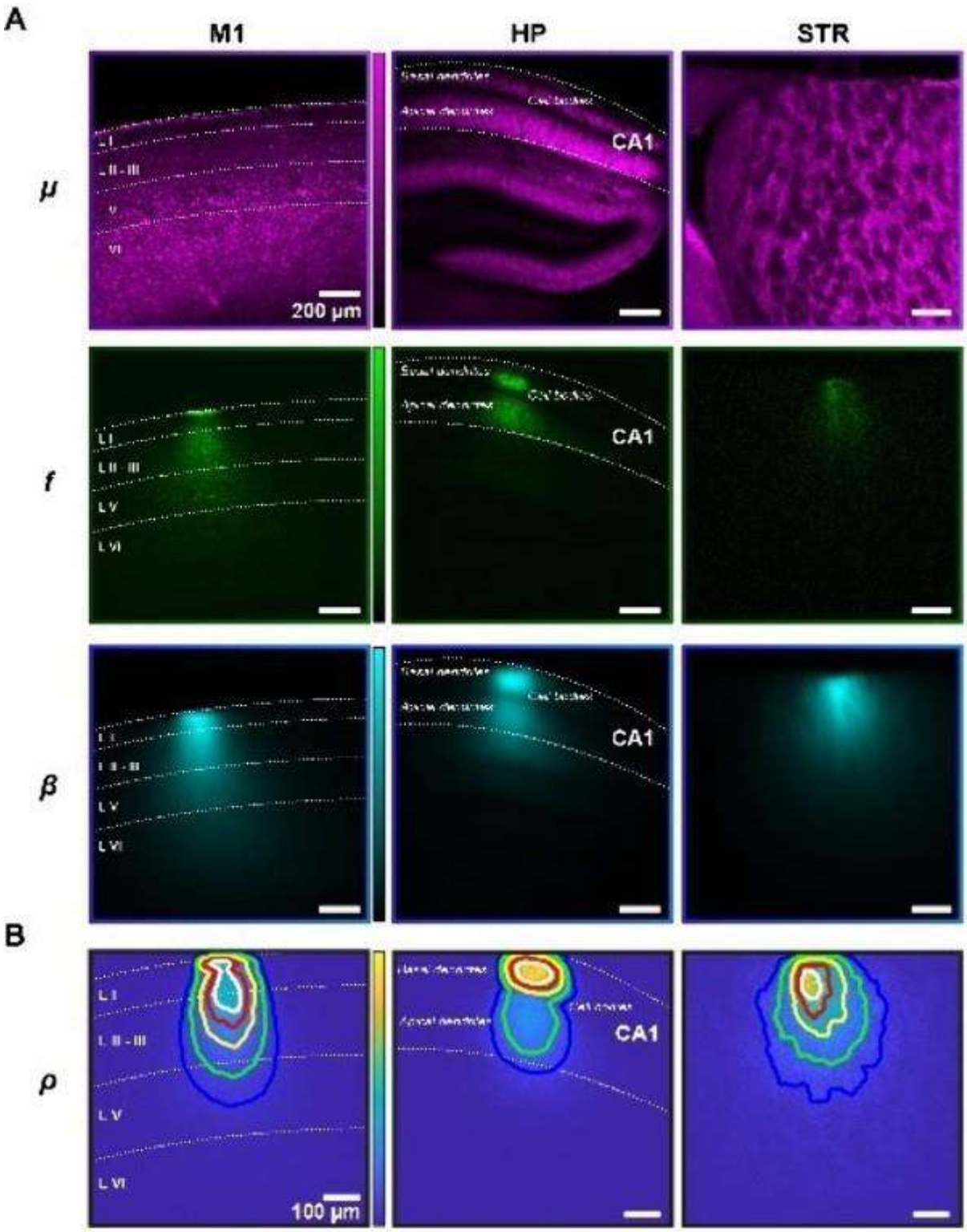
Fiber photometry (FP) is employed to probe in vivo time-dependent functional fluorescence variations related to several physiological phenomena (Piatkevich et al. Nature 2019; Patriarchi et al. Science 2018; Lee et al. Front. Neurosci. 2019). In its implementation, FP exploits an optical fiber (OF) placed next to the brain region of interest both to excite the genetically-encoded optical indicators of neural activity and to collect the resulting fluorescence. The optical properties of the brain are however uneven, suggesting that the same fiber employed in different regions of the brain could behave differently. In light of this, to identify the influence of anatomical structures on the spatial localization of FP signals, we used a two-photon microscope to measure the spatial distribution of excitation (β) and collection (η) collection photons of an OF positioned with the facet attached to the brain region of interest. The combination of η and β was then used to retrieve the photometry efficiency field (ρ) (Pisanello et al., Front. Neurosci 2019; Maglie et al. Opt. Lett. 2020), a direct measurement of the spatial sensitivity of the measurement. We compared light collection volume in the motor cerebral cortex (M1), the hippocampus (HP) and the striatum (STR), highlighting how shape and size of ρ is influenced by the anatomy across different brain regions (Fig.1) and within the same region (Montinaro et al., Biomed. Opt. Express



2021). Fig.1
Reproduced in Ref [Montinaro *et al*., *Biomed. Opt. Express* 2021].

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IMPROVED TWO-PHOTON IMAGING OF GPCR-BASED OPTOGENETIC NEUROTRANSMITTER SENSORS USING ORTHOGONALLY POLARIZED EXCITATION

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Mauro Pulin[1], Kilian Stockhausen[2], Olivia Masseck[3], Martin Kubitschke[3], Björn Busse[2], J. Simon Wiegert[1], Thomas Oertner[1]
[1]University Medical Center Hamburg-Eppendorf, Center For Molecular Neurobiology Hamburg, Hamburg, Germany, [2]University Medical Center Hamburg-Eppendorf, Department Of Osteology And Biomechanics, Hamburg, Germany, [3]University of Bremen, Synthetic Biology, Bremen, Germany

Fluorescent proteins are excited by light that is polarized parallel to the dipole axis of the chromophore. In two-photon microscopy, polarized light is used for excitation. Here we reveal surprisingly strong polarization sensitivity in a class of genetically encoded, GPCR-based neurotransmitter sensors. In tubular structures such as dendrites, this effect led to a complete loss of membrane signal in dendrites running parallel to the polarization direction of the excitation beam. To reduce the sensitivity to dendritic orientation, we designed an optical device that generates interleaved pulse trains of orthogonal polarization. The passive device, which we inserted in the beam path of an existing two-photon microscope, removed the strong direction bias from fluorescence and second-harmonic (SHG) images. We conclude that for optical measurements of transmitter concentration with GPCR-based sensors, orthogonally polarized excitation is essential.

Pubmed:

33979634: Mahn M, Saraf-Sinik I, Patil P, Pulin M, Bitton E, Karalis N, Bruentgens F, Palgi S, Gat A, Dine J, Wietek J, Davidi I, Levy R, Litvin A, Zhou F, Sauter K, Soba P, Schmitz D, Lüthi A, Rost BR, Wiegert JS, Yizhar O
Efficient optogenetic silencing of neurotransmitter release with a mosquito rhodopsin.
Information is carried between brain regions through neurotransmitter release from axonal presynaptic terminals. Understanding the functional roles of defined neuronal projection pathways requires temporally precise manipulation of their activity. However, existing inhibitory optogenetic tools have low efficacy and off-target effects when applied to presynaptic terminals, while chemogenetic tools are difficult to control in space and time. Here, we show that a targeting-enhanced mosquito homolog of the vertebrate encephalopsin (eOPN3) can effectively suppress synaptic transmission through the G signaling pathway. Brief illumination of presynaptic terminals expressing eOPN3 triggers a lasting suppression of synaptic output that recovers spontaneously within minutes in vitro and in vivo. In freely moving mice, eOPN3-mediated suppression of dopaminergic nigrostriatal afferents induces a reversible ipsiversive rotational bias. We conclude that eOPN3 can be used to selectively suppress neurotransmitter release at presynaptic terminals with high spatiotemporal precision, opening new avenues for functional interrogation of long-range neuronal circuits in vivo.
Neuron, 2021; 109
30311904: Wiegert JS, Pulin M, Gee CE, Oertner TG
The fate of hippocampal synapses depends on the sequence of plasticity-inducing events.
Synapses change their strength in response to specific activity patterns. This functional plasticity is assumed to be the brain's primary mechanism for information storage. We used optogenetic stimulation of rat hippocampal slice cultures to induce long-term potentiation (LTP), long-term depression (LTD), or both forms of plasticity in sequence. Two-photon imaging of spine calcium signals allowed us to identify stimulated synapses and to follow their fate for the next 7 days. We found that plasticity-inducing protocols affected the synapse's chance for survival: LTP increased synaptic stability, LTD destabilized synapses, and the effect of the last stimulation protocol was dominant over earlier stimulations. Interestingly, most potentiated synapses were resistant to depression-inducing protocols delivered 24 hr later. Our findings suggest that activity-dependent changes in the transmission strength of individual synapses are transient, but have long-lasting consequences for synaptic lifetime.
Elife, 2018; 7

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DRUG-INDUCIBLE GCAMP REDUCES THE DETRIMENTAL EFFECTS OF EARLY EXPRESSION OF GCAMP IN NEURONS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Changes in intracellular calcium concentration are correlated to neuronal activity and thus they have been extensively studied as proxy for neuronal status. GCaMP is the most widely used class of genetically encoded calcium indicators, but emerging evidence suggest that early or prolonged GCaMP expression is detrimental to neurons and to brain activity. In this study, we created a destabilized version of GCaMP (ddGCaMP) by adding a degradation domain to the sequence of GCaMP6f, so that it is constitutively degraded by the proteasome. Degradation can be suppressed by pharmacological means, causing a rapid rise in ddGCaMP concentration within hours. This represents a fast, reversible expression system, which minimizes the off-target effects of chronic GCaMP expression; this way, ddGCaMP expression is only induced immediately prior to the imaging session. Our tool is designed for studies of neurodevelopmental diseases, for which GCaMP needs to be transfected early during development. We show that mice transfected with intracerebroventricular injections at postnatal day 0 with an adeno-associated virus encoding ddGCaMP do not show the electrophysiological signs of neuronal hyperexcitability found in mice transfected with GCaMP6f. Interestingly, ddGCaMP exhibits a fluorescence decay twice as fast as that of GCaMP6f in acute cortical slices. We also show a modified reporter for Cre recombinase activity that allows the generation of tunable expression mosaics *in vivo*, also allowing to follow Ca activity in neurons of different genotype. We show the simultaneous calcium recording of wild type and PTEN knock-out neurons from a $PTEN^{flox/flox}$ mouse model of cortical dysplasia type II.

Pubmed:
33273479: Trovato F, Parra R, Pracucci E, Landi S, Cozzolino O, Nardi G, Cruciani F, Pillai V, Mosti L, Cwetsch AW, Cancedda L, Gritti L, Sala C, Verpelli C, Maset A, Lodovichi C, Ratto GM
Modelling genetic mosaicism of neurodevelopmental disorders in vivo by a Cre-amplifying fluorescent reporter.
Genetic mosaicism, a condition in which an organ includes cells with different genotypes, is frequently present in monogenic diseases of the central nervous system caused by the random inactivation of the X-chromosome, in the case of X-linked pathologies, or by somatic mutations affecting a subset of neurons. The comprehension of the mechanisms of these diseases and of the cell-autonomous effects of specific mutations requires the generation of sparse mosaic models, in which the genotype of each neuron is univocally identified by the expression of a fluorescent protein in vivo. Here, we show a dual-color reporter system that, when expressed in a floxed mouse line for a target gene, leads to the creation of mosaics with tunable degree. We demonstrate the generation of a knockout mosaic of the autism/epilepsy related gene PTEN in which the genotype of each neuron is reliably identified, and the neuronal phenotype is accurately characterized by two-photon microscopy.
Nat Commun, 2020; 11

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BOARD NUMBER: S01-675

A PORCINE CRANIAL WINDOW MODEL TO STUDY THE GLYMPHATIC SYSTEM

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Lund University, Emv, Lund, Sweden

The glymphatic system acts during sleep to remove waste products from the brain that are built up during the day. While the mainstay of glymphatic research has been carried out in rodents, some work has been done in humans using MRI, but the spatial resolution is not sufficient to explore more microscopic phenomena such as perivascular (PVS) flow. To this end we aimed to set up a large animal model to study the glymphatic system at high resolution both in vivo and ex vivo. First, fluorescent tracer was introduced into the cerebrospinal fluid (CSF) of adult male pigs via cisterna magna cannulation. Following tracer infusion a cranial window was created in the dorsal skull and CSF dynamics were captured using a customised large animal macroscope and real time video recordings, which were sufficient to capture PVS pulses both through the dura mater and after dural excision. After in vivo recordings, whole brains were extracted and processed using several advanced imaging techniques including confocal, light-sheet and electron microscopy which uncovered a glymphatic microarchitecture of tracer inflow into deep brain structures via PVS channels bounded by astrocytic endfeet. Furthermore the folded architecture of the gyrencephalic pig brain acted to enhance global tracer distribution. Taken together these data validate a new model to study the glymphatic system and bring us closer to understanding the nature of human glymphatics.

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BOARD NUMBER: S01-676

DATA PROCESSING TOOL FOR STUDYING SURFACE MOBILITY OF NMDARS.

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Stepan Kortus[1], Marharyta Kolcheva[2,3], Kristyna Rehakova[3], Dominika Chalupska[4], Martin Klima[4], Martin Horak[3]
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Aim: Specialized tools are available for tracking, drift correction, Mean Squared Displacement (MSD) analysis or visualization of single particle-based data. Our aim was to develop complex tool covering all needed steps for systematic research of surface mobility of NMDA receptors. **Methods:** We adapted approach to detecting and tracking the motion of the GluN1 subunit of the NMDA receptors on the neuronal cell surface, using quantum dots (QDs). **Results:** We developed software able to cover processing of the mobility data. The tool works in three steps: Tracking - Characterisation – Visualisation and allows to perform tracking within region of interest, correct for drift or detect Homer1 labelled synaptic areas. Characterisation helps to distinguish between synaptic and extra-synaptic trajectories based on the proximity of QDs to the identified synapses. Multiple descriptive parameters are calculated for each trajectory such as MSD, distance to synapse, mean velocity, active transport etc. Data are stored in database-like format and the visualization tool enables to easily compare multiple parameters and generate high quality charts. We identified the most critical steps in processing to obtain reliable measures.: drift correction and correct assignment of trajectories into different pools (synaptic, extrasynaptic, active transport). **Conclusion:** Mobility is a challenging technique requiring cyclic optimization with time consuming and operator dependent data processing. Proper analysing tool is essential to process and validate results in reasonable time and could be easily adapted for other microscopy-based tracking techniques. *Supported by the Czech Science Foundation (20-12420S) and Grant Agency of Charles University (GAUK: 320521).*

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BOARD NUMBER: S01-677

CALCIUM IMAGING TO DETERMINE THE PATHOGENIC EFFECTS OF NMDAR ANTIBODIES IN AUTOIMMUNE ENCEPHALITIS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Marina Cunquero Navarro[1], Esther Aguilar[2], Pablo Loza-Alvarez[1], Jesús Planagumà[1,2]
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Over the last 15 years a new category of antibody-mediated diseases of the central nervous system has been characterized and are now defined as “autoimmune encephalitis” (AE). There are currently 16 known autoimmune encephalitis syndromes and all are associated with antibodies against neuronal cell surface or synaptic proteins. The clinical syndromes are complex and vary according to the type of associated antibody. The best-known of these diseases is anti-N-methyl D-aspartate receptor (NMDAR) encephalitis which is a severe neuropsychiatric disorder that associates with prominent memory and behavioral deficits. The associated antibodies react with the N-terminal domain of the GluN1 subunit of the NMDAR. The approach most frequently used for the discovery and characterization of AE antibodies includes the culture of dissociated, fetal, rodent hippocampal neurons. During the process of antibody characterization, live neurons in culture are exposed to patients’ serum or CSF, and the detection of reactivity indicates that the serum or CSF samples of the patient contain antibodies against neuronal surface antigens. Hippocampal cultures can also be used to determine whether patients’ antibodies are potentially pathogenic by examining if they cause structural or functional alterations of the neurons. Here, we demonstrate how to determine the potential pathogenic effects of NMDAR antibodies with calcium imaging. Cellular activity studies in combination with techniques of identification of new autoantibodies open the possibility to initiate specific immunotherapies to improve patient’s outcome.

Pubmed:
32649790: Oró-Solé J, Fina I, Frontera C, Gàzquez J, Ritter C, Cunquero M, Loza-Alvarez P, Conejeros S, Alemany P, Canadell E, Fontcuberta J, Fuertes A
Engineering Polar Oxynitrides: Hexagonal Perovskite BaWON.
Non-centrosymmetric polar compounds have important technological properties. Reported perovskite oxynitrides show centrosymmetric structures, and for some of them high permittivities have been observed and ascribed to local dipoles induced by partial order of nitride and oxide. Reported here is the first hexagonal perovskite oxynitride BaWON , which shows a polar 6H polytype. Synchrotron X-ray and neutron powder diffraction, and annular bright-field in scanning transmission electron microscopy indicate that it crystalizes in the non-centrosymmetric space group P6 mc, with a total order of nitride and oxide at two distinct coordination environments in cubic and hexagonal packed BaX layers. A synergetic second-order Jahn-Teller effect, supported by first principle calculations, anion order, and electrostatic repulsions between W cations, induce large distortions at two inequivalent face-sharing octahedra that lead to long-range ordered dipoles and spontaneous polarization along the c axis. The new oxynitride is a semiconductor with a band gap of 1.1 eV and a large permittivity.
Angew Chem Int Ed Engl, 2020; 59
32765537: Ghahremani Z, Escudero N, Beltrán-Anadón D, Saus E, Cunquero M, Andilla J, Loza-Alvarez P, Gabaldón T, Sorribas FJ
Strain I-1582, a Nematode Antagonist by Itself and Through the Plant.
I-1582 is approved in Europe for the management of on vegetable crops. However, little information about its modes of action and temperature requirements is available, despite the effect of these parameters in its efficacy. The cardinal temperatures for bacterial growth and biofilm formation were determined. The bacteria was transformed with GFP to study its effect on nematode eggs and root colonization of tomato () and cucumber () by laser-scanning confocal microscopy. Induction of plant resistance was determined in split-root experiments and the dynamic regulation of genes related to jasmonic acid (JA) and salicylic acid (SA) by RT-qPCR at three different times after nematode inoculation. The bacteria was able to grow and form biofilms between 15 and 45°C; it degraded egg-shells and colonized eggs; it colonized tomato roots more extensively than cucumber roots; it induced systemic resistance in tomato, but not in cucumber; SA and JA related genes were primed at different times after nematode inoculation in tomato, but only the SA-related gene was up-regulated at 7 days after nematode

inoculation in cucumber. In conclusion, I-1582 is active at a wide range of temperatures; its optimal growth temperature is 35°C; it is able to degrade eggs, and to colonize plant roots, inducing systemic resistance in a plant dependent species manner.
Front Plant Sci, 2020; 11
33224158: Expósito A, Pujolà M, Achaerandio I, Giné A, Escudero N, Fullana AM, Cunquero M, Loza-Alvarez P, Sorribas FJ
Tomato and Melon Resistant Rootstocks Improve Crop Yield but Melon Fruit Quality Is Influenced by the Cropping Season. Four rotation sequences consisting of ungrafted tomato cv. Durinta - melon cv. Paloma or tomato grafted onto the resistant rootstock 'Aligator' - melon grafted onto the resistant accession BGV11135, and in reverse order, were conducted from 2015 to 2017 in a plastic greenhouse infested or not with to determine the plant tolerance (), the minimum relative crop yield () and fruit quality. The relationship between densities in soil at transplanting () of each crop and the crop yield was assessed and and were estimated by the Seinhorst's damage model. In addition, the volume and the number of nuclei of single giant cells and the number of giant cells, its volume and the number of nuclei per feeding site in susceptible tomato and melon were compared to those in the resistant tomato and 15 days after nematode inoculation in pot test. The relationship between the and the relative crop yield fitted the Seinhorst's damage model in both ungrafted and grafted tomato and melon, but not for all years and cropping seasons. The estimated for ungrafted and grafted tomato did not differ but was lower in the former (34%) than the latter (67%). Sodium concentration in fruits from ungrafted but not from grafted tomato increased with nematode densities in spring 2015 and 2016. The estimated ungrafted melon did not differ from the grafted melon cultivated in spring, but it did when it was cultivated in summer. The relative crop yield of ungrafted melon was lower (2%) than the grafted cultivated in spring (62%) and summer (20%). Sodium concentration in melon fruits from ungrafted plants increased with nematode densities. No variations in fruit quality from grafted melon cultivated in spring were found, although less dry matter and soluble solid content at highest nematode densities were registered when it was cultivated in summer. Lower number of giant cells per feeding site was observed in both susceptible tomato germplasms compared to the resistant ones but they were more voluminous and held higher number of nuclei per giant cell and per feeding site.
Front Plant Sci, 2020; 11

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MULTICOLOR LARGE-SCALE BRAIN IMAGING WITH CHROMATIC MULTIPHOTON SERIAL MICROSCOPY

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Hugo Blanc[1], Pierre Mahou[1], Gabriel Kaddour[2], Seongbin Lim[1], Lamiae Abdeladim[1], Dragos Niculescu[2], Katherine Matho[3], Solène Clavreul[2], Jean-Marc Sintes[1], Xavier Solinas[1], Ignacio Arganda-Carreras[4], Anatole Chessel[1], Karine Loulier[5], Rob Campbell[6], Willy Supatto[1], Jean Livet[2], Emmanuel Beaurepaire[1]
[1]Ecole polytechnique, Laboratoire D'optique Et Biosciences (umr7645), PALAISEAU cedex, France, [2]INSERM, Sorbonne University, Institut De La Vision, Paris, France, [3]Cold Spring Harbor Laboratory, -, Cold Spring Harbor, United States of America, [4]Facultad de Informática, Universidad del Pais Vasco, Computer Science And Artificial Intelligence Department, Donostia-San Sebastian, Spain, [5]INSERM, Université de Montpellier, Institut Des Neurosciences De Montpellier, Montpellier, France, [6]Sainsbury Wellcome Center, Advanced Microscopy Core, London, United Kingdom

Aims: Large-scale microscopy approaches relying on clearing or serial sectioning are increasingly used to study brain tissue organization and map circuitry in animal models. However, available imaging techniques often lack efficient multicolor and multicontrast modalities and often sacrifice either the spatial resolution, imaging contrast or acquisition speed. *Methods*: To address this issue, we recently introduced chromatic multiphoton serial (ChroMS) microscopy, a method combining multicolor two-photon excitation through wavelength mixing and serial block-face acquisition. This approach enables organ-scale imaging of spectrally distinct fluorescent proteins with intrinsic submicron channel registration and diffraction-limited resolution without slowing down the acquisition. *Results*: ChroMS microscopy is ideal to map mouse brain tissue expressing Brainbow transgenic markers and viral- or electroporation-based multicolor labels. We obtained continuous 3D color imaging over cubic millimeters as well as brain-wide serial 2D multichannel imaging. We present a series of improvements to the ChroMS microscopy workflow, with aiming at increasing the overall speed and robustness of the imaging process. We illustrate these advances in acquisitions of fixed mouse brains at different development states. *Conclusions*: These progress further expand the applicability of ChroMS microscopy for tissue-scale 3D color imaging with diffraction-limited resolution. *References*: Abdeladim et al, Nat Commun (2019). Mahou et al, Nat Meth (2012).

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BOARD NUMBER: S01-679

PHOTOACTIVATION OF INDIVIDUAL SYNAPSES IN VIVO WITH COVALENT PHOTOSWITCHES TARGETING ENDOGENOUS GLUTAMATE RECEPTORS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Miquel Bosch[1,2], Aida Garrido[2,3], Hyojung Lee[2], Xavier Rovira[2,4], Silvia Pittolo[2,5], Artur Llobet[6], Hovy Wong[7], Ana Trapero[2,4], Carlo Matera[2,8], Claudio Papotto[2,8], Carme Serra[4], Amadeu Llebaria[4], Eduardo Soriano[9,10], Maria V. Sanchez-Vives[11,12], Christine Holt[13], Pau Gorostiza[2,14,15]
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Glutamate receptors play key roles in neurotransmission at excitatory synapses and in the regulation of synaptic plasticity. We have recently developed a targeted covalently-attached photoswitch (TCP, Izquierdo-Serra et al., 2016) that allows the remote control of endogenous ionotropic glutamate receptors using light. We combined this photopharmacological effector with genetic and chemical calcium sensors to demonstrate all-optical reversible control of glutamate receptors at multiple levels of spatial resolution in the brain: we achieved the photoactivation of multiple neurons, individual neurons, and single synapses in rat hippocampal slices and in intact *Xenopus laevis* brain *in vivo*, which is challenging using other methods. We show that this compound selectively targets AMPA and kainate receptors. Labeled receptors remained functional for long periods of time (>8 hours). This allowed us to longitudinally track endogenous receptor physiology during events of synaptic plasticity, such as long-term depression (LTD). We could monitor the loss of functionality of AMPA/kainate receptors during NMDAR-dependent LTD in hippocampal neurons. TCPs are therefore a unique optical tool to label, photo-control and functionally track endogenous receptors in brain tissue without genetic manipulation.

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ENGINEERING AND CHARACTERIZATION OF RHODOPSIN-BASED GENETICALLY-ENCODED TOOLS FOR THE ALL-OPTICAL DISSECTION OF NEURAL CIRCUITS USING 2-PHOTON ILLUMINATION

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Christiane Grimm, Aysha Mohamed Lafirdeen, Ruth Sims, Imane Bendifallah, Eirini Papagiakoumou, Dimitrii Tanese, Valentina Emiliani
Institut de la Vision, Photonics Department, Paris, France

Establishing causal links between animal behaviuor and patterns of neuronal activity is a long-standing goal in neuroscience. Additionally, functionally mapping the synaptic connections in this neuron population is crucial for unraveling neural circuitry and ultimately understanding how the brain computes. The low-throughput nature of patch-clamp recordings from pre- and post-synaptic cells can be overcome by performing activity monitoring and manipulation with optical tools instead of electrodes. For such an all-optical experiment, channelrhodopsins under holographic 2-photon stimulation are used to modify the activity of individual neurons complemented by expression of calcium indicators which are employed to monitor activity of the neuron population. In a different line of experiments, presynaptically expressed channelrhodopsins and 2-photon parallel illumination techniques can be used to photoinduce spiking in targeted neurons and monitor post-synaptic sub-threshold responses with a voltage indicator.
Here, we characterise and develop optogenetic tools based on microbial rhodopsins for the use with 2-photon parallel illumination techniques in all-optical experiments. Our efforts entail exploring 2-photon activation of new optogenetic actuators for activity manipulation as well as engineering improved voltage sensors for activity interrogation. Initial characterisation in cultured cells is followed by viral expression in organotypic slices of mouse hippocampus and an in-depth analysis of the 2-photon usability. This *in-vitro* characterisation is fundamental to obtain the next generation of genetically encoded tools for the optical dissection of brain circuits.

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SCULPTED TWO-PHOTON EXCITATION OF CHANNELRHODOPSINS AND GENETICALLY ENCODED VOLTAGE INDICATORS FOR ALL-OPTICAL NEUROPHYSIOLOGY

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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One of the central goals of neuroscience is to decode the neural mechanisms of sensory perception and more specifically, to decipher the specific contributions of each coding dimension. Achieving this goal requires tools capable of precisely and non-invasively perturbing and monitoring the neural activity of selected neurons. Due to recent advances in neurophotonics, all-optical experiments based on the two-photon photostimulation of opsin expressing neurons and the simultaneous readout of neural activity using calcium indicators are now performed routinely. However, inferring neural activity using calcium indicators is lacking information regarding sub-threshold dynamics and complex spike waveforms, which can be overcome using voltage indicators. In combination with temporal focusing, parallel light sculpting methods such as Computer-Generated Holography, Generalized Phase Contrast and low numerical aperture Gaussian beams have been used to perform scanless two-photon photostimulation of multiple neurons. Here, by viral expression of JEDI-2P in organotypic slices of mouse hippocampus, we demonstrate that each of the different used light sculpting approaches developed for parallel two-photon optogenetic manipulation are also suitable for high-resolution two-photon voltage imaging of neural activity, and characterize the optimal acquisition parameters in each case. We show that high frequency spiking and subthreshold depolarizations can be recorded from multiple cells at kilohertz acquisition rates by combining targeted, parallel illumination with a widefield detection axis. Finally, we highlight the utility of performing simultaneous two-photon voltage imaging and photostimulation (by co-expression of the opsin ChroME) to validate the precise number, amplitude and timing of evoked action potentials in-situ during all-optical experiments.

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OPTOGENETIC INHIBITION OF PRESYNAPTIC TRANSMISSION WITH GENETICALLY ENCODED OPSIN-BASED GPCRS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Jonas Wietek[1], Adrianna Nozownik[2], Rivka Levy[1], Asaf Gat[1], Julien Dine[1], Mauro Pulin[3], Inbar Saraf-Sinik[1], Eyal Bitton[1], Dietmar Schmitz[4,5,6], Peter Soba[3], Meital Oren-Suissa[7], Benjamin Rost[4], J. Simon Wiegert[8], Ofer Yizhar[1]
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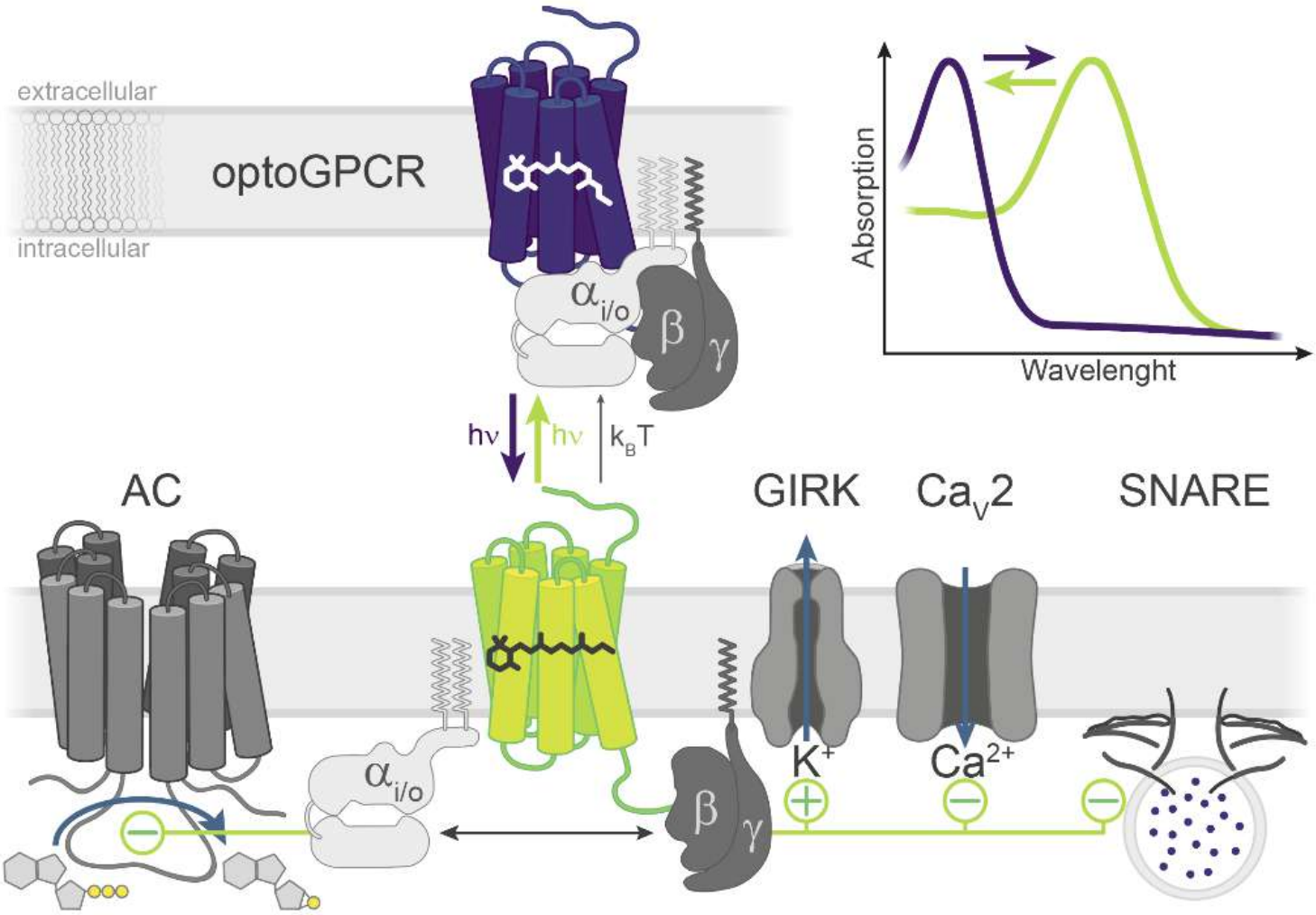

Optogenetic suppression of presynaptic neurotransmitter release has been a long-standing goal in systems neuroscience.

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We recently developed optogenetic tools based on G-protein coupled rhodopsins (optoGPCRs) that allow effective presynaptic silencing. To expand optoGPCRs palette with advantageous properties, we chose a literature mining approach to identify 50+ candidates for silencing synaptic transmission. To validate their applicability, we benchmarked 11 promising optoGPCRs against each other by comparing their expression, membrane targeting, GIRK-channel coupling efficiency, ability to suppress synaptic transmission and their G-protein coupling specificity. While only two of the new optoGPCRs studied exhibited the desired properties with regard to presynaptic inhibition, we were able to identify one new optoGPCR which can be reliably switched between active/inactive states with blue/green light, respectively, enabling multiplexing with other red-shifted tools. While previously utilized optoGPCRs (eOPN3/parapinopsin) couple to G_i- and G_o-proteins, our new candidate allows highly effective silencing of synaptic transmission, but does not couple to G_i-signaling and therefore does not perturb the activity of adenylate cyclases and leaves the cAMP-signaling pathway unchanged. The newly developed optoGPCR doesn't require UV-light for activation, is highly light-sensitive and allows high-efficacy silencing of CA3 to CA1 projections in organotypic slices. Validation of this new optogenetic tool in mice is still ongoing and further demonstrated synaptic silencing and behavioral effects in *C.elegans*. We believe that the new optoGPCRs will serve as highly potent tools for functionally dissecting neuronal circuits via silencing of long-range axonal projections. Fully reversible, the new range of inhibitory optoGPCRs provides temporally-precise control over synaptic transmission.

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A FLEXIBLE TWO-PHOTON ENDOSCOPE FOR FAST ACTIVITY IMAGING AND CELL-PRECISE OPTOGENETIC PHOTO-STIMULATION OF NEURONS IN FREELY MOVING ANIMALS.

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Francois Blot[1], Nicolo Accanto[1], Antonio Lorca Camara[1], Valeria Zampini[1], Vincent De Sars[1], Florence Bui[1], Christophe Tourain[1], Valentina Emiliani[1], Ori Katz[2], Noam Badt[2]
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We developed a flexible two-photon fiber-based microendoscope (2P-FENDO) capable of all-optical brain interrogation at cellular resolution in freely moving mice. The system performs two-photon (2P) fast functional imaging and 2P holographic photostimulation of single and multiple cells using axially confined extended spots. Proof-of-principle experiments were performed on head restrained and freely moving mice co-expressing jGcaMP7s and the opsin ChRmine in visual or barrel cortex. On a field of view of 250 µm in diameter, we demonstrated functional imaging at a frame rate of up to 100 Hz and precise photostimulation of single and multiple cells. With the capability to simultaneously image and control neuronal activity at single-cell resolution in freely moving animals, 2P-FENDO will enable to precisely define the functions of neurons in the brain and their interactions during naturalistic behaviours.

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EFFICIENT OPTOGENETIC SILENCING OF SYNAPTIC TRANSMISSION USING THE BISTABLE OPTO-GPCR PDCO

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Optogenetics enables the perturbation of neuronal activity with superior spatiotemporal precision. While exciting neurons is relatively straightforward, silencing neuronal activity reliably for prolonged time is more challenging, especially when it comes to synaptic transmission. A recent strategy employs GPCR rhodopsins (opto-GPCRs), which activate endogenous Gi/o-pathways, similar to the chemogenetic silencing tool hM4Di. PdCO, a blue light-activated and green light-inactivated bistable GPCR from *Platynereis dumerilii*, is a promising new candidate for reversible optical synaptic inhibition. We used organotypic hippocampal slice cultures (OSC) to investigate light-mediated silencing at Schaffer collateral synapses in detail. PdCO was expressed in CA3 pyramidal cells via single-cell electroporation or local rAAV injections. We recorded Gi/o-protein coupled inwardly rectifying K^+ (GIRK) currents to estimate the optimal activation and inactivation light parameters. Compared to PPO, a switchable opto-GPCR from *Lethenteron camtschaticum/japonicum*, PdCO has a red-shifted activation spectrum and higher light-sensitivity. We further used GIRK current recordings to assess the compatibility of PdCO with two-photon imaging. Finally, by combining PdCO or PPO with a soma-localized red-shifted excitatory tool embedded in a tandem construct with a blue-light activated anion channel (somBiPOLES), we demonstrate all-optical control of synaptic transmission. Both opto-GPCRs efficiently decreased red light-evoked postsynaptic currents in a reversible manner. However, PdCO showed higher potency for synaptic silencing and approximately 10x higher light sensitivity than PPO. In summary, PdCO is a new, bistable opto-GPCR suitable for efficient, reversible synaptic silencing using light in the visible spectrum.

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OPTOGENETIC STIMULATION REDUCES SPECTRAL SPREAD OF COCHLEAR IMPLANTS – A MODELING STUDY

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Electrical cochlear implants (eCIs) are the most successful neuroprosthetic devices, having partially restored hearing in about a million people worldwide. The eCI users, though obtain good speech perception in quiet environments, struggle to understand speech-in-noise and to appreciate music. This limitation is majorly caused by the wide spread of current in the cochlea which leads to broad neural excitation and limits the number of perceptually different channels. A possibility to reduce the spectral spread and to increase the number of independent channels is to advance towards optogenetic cochlear implants (oCIs). This project aims to assist the development of clinical oCIs by predicting the light spread in a human cochlea using an optical ray-tracing model. A reconstructed 3D cochlea model was imported to an optical engineering software to do an *in silico* investigation of the light spread. Ten light emitters were placed in the scala tympani, and simulated with different radiation profiles. The spectral spread was approximated from irradiance profiles in the Rosenthal's canal housing the somata of spiral ganglion neurons. The results showed that emitters having Gaussian profile (LASER-coupled waveguides) provide higher irradiance and lower spectral spread than those having Lambertian profile (LEDs). The spectral spread using optical stimulation was found to be lower than that with electrical stimulation. The emitter-to-neurons distance and orientation, and formation of scar tissue, impacted the irradiance, highlighting directions for development of oCIs and of soft surgery maintaining cochlear health. The modeling results strengthen the idea that oCIs would offer lower spectral spread than eCIs.

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THE BIG, THE FAST AND THE BLUE: TOWARDS THE OPTIMAL CHANNELRHODOPSIN FOR THE FUTURE OPTICAL COCHLEAR IMPLANT

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Around 5% of whole world's population can be affected by hearing impairment. The design of optical cochlear implants (oCIs) aims to improve hearing quality compared to the widely used electric cochlear implant. To provide light sensitivity to the auditory system we employ Channelrhodopsins (ChRs), naturally occurring photosensitive proteins. Optogenetic manipulation of spiral ganglion neurons (SGNs) has been reported, but efforts to improve the performance of ChRs for clinical translation are ongoing. We designed and characterized blue light activated ChRs that meet the needs of hearing restoration by oCIs: high light sensitivity and temporal fidelity. Both aspects are interconnected, and speed is usually in detriment of light sensitivity. Low light sensitivity can be compensated by higher membrane expression, achieved with membrane exporting sequences from Kir2.1 channel. Less desensitizing ChRs provide reproducible and sustained photoresponses over time for high frequency stimulation of SGNs. All these parameters were systematically compared in transiently transfected neuroblastomaglioma cells and in hippocampal neuron primary cultures. Neuronal activation was studied regarding light pulse length, frequency, and light intensity. Selected candidates optimized *in vitro*, were tested *in vivo* by direct injection of AAVs through the round window in early postnatal mice. *In vitro* measurements in cochlear nucleus show that light stimulation of SGN fibers evoke postsynaptic activity. Cochlear nucleus recordings help us to evaluate the effects of optogenetic manipulation in the auditory system. In conclusion, here we show new refined gain of function mutant ChRs with great potential to be tested in established animal models for future clinical translation.

Pubmed:
30796228: Cabré G, Garrido-Charles A, Moreno M, Bosch M, Porta-de-la-Riva M, Krieg M, Gascón-Moya M, Camarero N, Gelabert R, Lluch JM, Busqué F, Hernando J, Gorostiza P, Alibés R
Rationally designed azobenzene photoswitches for efficient two-photon neuronal excitation.
Manipulation of neuronal activity using two-photon excitation of azobenzene photoswitches with near-infrared light has been recently demonstrated, but their practical use in neuronal tissue to photostimulate individual neurons with three-dimensional precision has been hampered by firstly, the low efficacy and reliability of NIR-induced azobenzene photoisomerization compared to one-photon excitation, and secondly, the short cis state lifetime of the two-photon responsive azo switches. Here we report the rational design based on theoretical calculations and the synthesis of azobenzene photoswitches endowed with both high two-photon absorption cross section and slow thermal back-isomerization. These compounds provide optimized and sustained two-photon neuronal stimulation both in light-scattering brain tissue and in Caenorhabditis elegans nematodes, displaying photoresponse intensities that are comparable to those achieved under one-photon excitation. This finding opens the way to use both genetically targeted and pharmacologically selective azobenzene photoswitches to dissect intact neuronal circuits in three dimensions.
Nat Commun, 2019; 10
34018704: Barbero-Castillo A, Riefolo F, Matera C, Caldas-Martínez S, Mateos-Aparicio P, Weinert JF, Garrido-Charles A, Claro E, Sanchez-Vives MV, Gorostiza P
Control of Brain State Transitions with a Photoswitchable Muscarinic Agonist.

The ability to control neural activity is essential for research not only in basic neuroscience, as spatiotemporal control of activity is a fundamental experimental tool, but also in clinical neurology for therapeutic brain interventions. Transcranial-magnetic, ultrasound, and alternating/direct current (AC/DC) stimulation are some available means of spatiotemporal controlled neuromodulation. There is also light-mediated control, such as optogenetics, which has revolutionized neuroscience research, yet its clinical translation is hampered by the need for gene manipulation. As a drug-based light-mediated control, the effect of a photoswitchable muscarinic agonist (Phthalimide-Azo-Iper (PAI)) on a brain network is evaluated in this study. First, the conditions to manipulate M2 muscarinic receptors with light in the experimental setup are determined. Next, physiological synchronous emergent cortical activity consisting of slow oscillations-as in slow wave sleep-is transformed into a higher frequency pattern in the cerebral cortex, both in vitro and in vivo, as a consequence of PAI activation with light. These results open the way to study cholinergic neuromodulation and to control spatiotemporal patterns of activity in different brain states, their transitions, and their links to cognition and behavior. The approach can be applied to different organisms and does not require genetic manipulation, which would make it translational to humans.
Adv Sci (Weinh), 2021; 8
31070376: Cabré G, Garrido-Charles A, González-Lafont À, Moormann W, Langbehn D, Egea D, Lluch JM, Herges R, Alibés R, Busqué F, Gorostiza P, Hernando J
Synthetic Photoswitchable Neurotransmitters Based on Bridged Azobenzenes.
Photoswitchable neurotransmitters of ionotropic kainate receptors were synthesized by tethering a glutamate moiety to disubstituted C2-bridged azobenzenes, which were prepared through a novel methodology that allows access to diazocines with higher yields and versatility. Because of the singular properties of these photochromes, photoisomerizable compounds were obtained with larger thermal stability for their inert cis isomer than for their biologically activity trans state. This enabled selective neuronal firing upon irradiation without background activity in the dark.
Org Lett, 2019; 21
31010281: Riefolo F, Matera C, Garrido-Charles A, Gomila AMJ, Sortino R, Agnetta L, Claro E, Masgrau R, Holzgrabe U, Batlle M, Decker M, Guasch E, Gorostiza P
Optical Control of Cardiac Function with a Photoswitchable Muscarinic Agonist.
Light-triggered reversible modulation of physiological functions offers the promise of enabling on-demand spatiotemporally controlled therapeutic interventions. Optogenetics has been successfully implemented in the heart, but significant barriers to its use in the clinic remain, such as the need for genetic transfection. Herein, we present a method to modulate cardiac function with light through a photoswitchable compound and without genetic manipulation. The molecule, named PAI, was designed by introduction of a photoswitch into the molecular structure of an M2 mAChR agonist. In vitro assays revealed that PAI enables light-dependent activation of M2 mAChRs. To validate the method, we show that PAI photoisomers display different cardiac effects in a mammalian animal model, and demonstrate reversible, real-time photocontrol of cardiac function in translucent wildtype tadpoles. PAI can also effectively activate M2 receptors using two-photon excitation with near-infrared light, which overcomes the scattering and low penetration of short-wavelength illumination, and offers new opportunities for intravital imaging and control of cardiac function.
J Am Chem Soc, 2019; 141
27436051: Izquierdo-Serra M, Bautista-Barrufet A, Trapero A, Garrido-Charles A, Díaz-Tahoces A, Camarero N, Pittolo S, Valbuena S, Pérez-Jiménez A, Gay M, García-Moll A, Rodríguez-Escrich C, Lerma J, de la Villa P, Fernández E, Pericàs MÀ, Llebaria A, Gorostiza P
Optical control of endogenous receptors and cellular excitability using targeted covalent photoswitches.
Light-regulated drugs allow remotely photoswitching biological activity and enable plausible therapies based on small molecules. However, only freely diffusible photochromic ligands have been shown to work directly in endogenous receptors and methods for covalent attachment depend on genetic manipulation. Here we introduce a chemical strategy to covalently conjugate and photoswitch the activity of endogenous proteins and demonstrate its application to the kainate receptor channel GluK1. The approach is based on photoswitchable ligands containing a short-lived, highly reactive anchoring group that is targeted at the protein of interest by ligand affinity. These targeted covalent photoswitches (TCPs) constitute a new class of light-regulated drugs and act as prosthetic molecules that photocontrol the activity of GluK1-expressing neurons, and restore photoresponses in degenerated retina. The modularity of TCPs enables the application to different ligands and opens the way to new therapeutic opportunities.
Nat Commun, 2016; 7

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IN VIVO HIGH-THROUGHPUT PROBING OF SYNAPTIC CONNECTIVITY USING TWO-PHOTON HOLOGRAPHIC OPTOGENETIC STIMULATION AND COMPRESSED SENSING STRATEGIES

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

I-Wen Chen[1], Chung Yuen Chan[1], Vincent De Sars[1], Emiliano Ronzitti[1], Phillip Navarro[2], Karim Oweiss[2], Dimitrii Tanese[1], Valentina Emiliani[1]
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A comprehensive description of patterns and properties of synaptic connections between a population of neurons provides the most relevant insights into the neuronal circuits underlying brain functions. Here we investigated synaptic connectivity between putative excitatory neurons at layer 2/3 of mouse visual cortex in vivo by combining two-photon (2P) holographic optogenetic stimulation and whole-cell recording. Fast, temporally precise, and spatially selective action potential (AP) was induced in each of potential presynaptic neurons by using 2P holographic illumination onto opsin-expressing cell soma and the current activity in a postsynaptic neuron was monitored by inserting one patch pipette for voltage-clamp recording. Spatially-confined 2P holographic light-spot of 12-µm diameter was generated by using computer-generated holography plus temporal focusing and focused into the brain tissue in lightly anesthetized animals. By using soma-illumination of 2P holographic light of 0.15-0.3 mW/µm^2, AP of peak latency <10 ms and jitter <1 ms was induced in a potential presynaptic cell expressing the soma-restricted opsin ChroME. An optimized pipeline for fast sequential photostimulation of single cells enabled probing ~100 potential presynaptic cells in ~5 minutes. Connectivity mapping was further accelerated by a parallel multi-cell photostimulation strategy combined with a compressive sensing algorithm to retrieve the connections. Taken together, our approach allows high-throughput mapping of local synaptic connections to a postsynaptic neuron in vivo with unprecedented speed, precision, and accuracy.

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ULTRAFAST LIGHT TARGETING FOR HIGH-THROUGHPUT PRECISE CONTROL OF NEURONAL NETWORKS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Faini, Molinier, Tanese, Telliez, Hamdani, Tourain, Del Bene, Forget, Ronzitti, Emiliani
Sorbonne Université, INSERM, CNRS, Institut De La Vision, Paris, France

Understanding how specific sets of neurons fire and wire together during cognitive-relevant activity is one of the most pressing questions in neuroscience. Two-photon, single-cell resolution optogenetics based on holographic light-targeting approaches enables generation of precise spatiotemporal activity patterns and has enabled a broad range of experimental configurations including high throughput connectivity mapping, probing how many neurons are sufficient for perception and investigation of functional connectivity. Yet, intrinsic limitations of the technology which currently enables holographic light patterning limit today the resolution for tuning the relative spiking time of distinct cells or cell ensemble to a few milliseconds and the achievable number of targets to several tens. To overcome these limitations and expand the capabilities of single-cell optogenetics, we introduce an optical configuration capable of ultrafast light targeting based on switching temporally focused beams between holograms at kHz rates. We used FLiT to demonstrate two new illumination methods, termed *hybrid-* and *cyclic-illumination* and achieve sub-millisecond control of sequential neuronal activation and high throughput multicell illumination both in vitro and in vivo while minimizing light-induced thermal rises, respectively. These approaches will be important for experiments that require rapid and precise cell stimulation with defined spatio-temporal activity patterns and optical control of large neuronal ensembles.

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DEVELOPMENT OF A SOUND PROCESSOR DRIVING OPTICAL COCHLEAR IMPLANT FOR BEHAVIOURAL EXPERIMENTS IN ANIMALS

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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In case of deafness, cochlear implants (CIs) bypass dysfunctional or lost hair cells by direct stimulation of tonotopically organised spiral ganglion neurons (SGNs) which convey auditory inputs to the brain. The state-of-the-art implants, electrical CIs (eCIs), enables speech understanding in the quiet in most of the approximately 1 million users and hence are considered the most successful neuroprostheses. However, due to wide spread of SGN activation from each electrode, coding of spectral information is very limited resulting in poor speech understanding in background noise as well as reduced music appreciation. Thanks to the ability to confine light in space, optical CIs (oCIs) combined with optogenetics promise to overcome this shortcoming of eCIs by enabling more independent stimulation channels. This requires fast and power-efficient real-time sound analysis and control of multiple microscale light emitters. Here, we present a low-weight (8 g), battery-powered, and wireless-controlled sound processor for driving multichannel oCIs and its sister eCI system. These systems support behavioural experiments in freely moving rodents (e.g. rats) or non-human primates (e.g. marmosets) for assessing auditory percepts of the optogenetic stimulation and comparison off efficacy with electrical stimulation. This proof-of-concept multichannel oCI system paves the way for the future development of medical devices for human patients.

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NOVEL CHEMOGENETIC TOOLS FOR MANIPULATING NICOTINIC NEUROTRANSMISSION WITH HIGH SPATIO-TEMPORAL AND PHARMACOLOGICAL PRECISION IN MICE

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Aims: Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels made of a combination of α and β subunits. In the brain, nAChRs are activated by acetylcholine (ACh), a neuromodulator that broadcasts a diffuse signal. They are also activated by nicotine, the main active substance of tobacco. These receptors are widely expressed throughout the brain, notably in two circuits implicated in nicotine addiction : the mesolimbic dopamine system and the habenulo-interpeduncular pathway. Nicotine hijacks the normal functioning of these circuits to trigger reinforcement and aversion, respectively. Understanding the effect of ACh and nicotine on neural circuits and behaviors requires strategies for manipulating specific nAChR subtypes in targeted pathways. To this end, we have developped new chemogenetic tools for the manipulation of endogenous nAChR subtypes. **Method:** We have generated two transgenic mouse lines that contain a single amino acid substitution E61C on either the β2 or β4 nAChR subunit. The cysteine mutation does not alter receptor functioning, but is used for the covalent attachment of cysteine-reactive ligands. We have engineered different tethered ligands, notably an irreversible antagonist ($MPEG_4Ch$), and a photoswitchable antagonist (MAHoCh). **Results:** Using electrophysiology, we demonstrate the pharmacological specificity of these tools. While MAHoCh enables rapid on-off switching of receptors, $MPEG_4Ch$ can inhibit nAChRs for several days after a single injection in specific brain areas. **Conclusions:** By enabling the rapid *vs.* prolonged interruption of nicotinic neurotransmission in targeted circuits, these versatile tools will help dissect the various roles of ACh, and the many facets of nicotine, in behaving mice.

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TOWARDS BEHAVIORAL EVALUATION OF A MULTICHANNEL OPTOGENETIC COCHLEAR IMPLANT SYSTEM

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Bettina Wolf[1,2], Lukasz Jablonski[1,2], Tamas Harczos[1,2], Alexander Dieter[3,4,5], Gerhard Hoch[1], Christian Dullin[6,7], Patrick Ruther[8,9], Tobias Moser[1,2,10,11]
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Optogenetic cochlear implants (oCI) promise to overcome the issue of current spread of electrical cochlear implants (eCI). However, proof on behavioral level has not been drawn yet. Here, we established a framework using wireless multichannel e/oCI-systems to train freely moving rats on CI-cued behavioral tasks. Rats (n=20) were trained in the ShuttleBox, a negative reinforcement paradigm encouraging avoidance behavior. Animals were implanted either with a multichannel-eCI (array from MED-El with 5 electrodes) or a multichannel-oCI (up to 10 LEDs). Position of the implant was confirmed via CT. Animals for oCI implantation had been postnatally injected with AAV carrying channelrhodopsin-2 variant *CatCh*. Using CI-system, animals were subjected to detection tasks via wireless communication to evaluate perceptual threshold. Secondly, animals were subjected to channel-discrimination paradigms. Functional expression of *CatCh* was confirmed in 87.5% of virus-injected rats by optically evoked auditory brainstem responses. In all animals, avoidance behavior could be evoked in response to stimulation of CI-system (eCI, n=8; oCI, n=6). This was also true when acoustic clicks were transformed to stimuli using CI-system in real time, rather than using pre-programmed stimuli. Using single stimulation channels, mean behavioral thresholds were ~75 µA for eCI and ~0.8 mW for oCIs. Frequency discrimination experiments with acoustic stimulation identified mean frequency difference limen of 0.08 Weber fraction. We established framework based on CI-systems handling multichannel e/oCIs and demonstrated that single LED-driven optogenetic stimulation evokes percept strong enough to elicit behavior. From here we aim to probe spatial selectivity of optogenetic vs. electrical stimulation on behavior level.

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IN-VIVO FAST NON-LINEAR MICROSCOPY REVEALS INTRANEURONAL TRANSPORT IMPAIRMENT INDUCED BY SLIGHT MOLECULAR MOTOR IMBALANCES IN THE BRAIN OF ZEBRAFISH LARVAE

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Baptiste Grimaud[1], Maxence Frétaud[2], Feriel Terras[1], Antoine Bénassy[1], Karine Duroure[3], Valérie Bercier[4], Gaëlle Allard[1], Élodie Chaudan[5], Thierry Gacoin[5], Filippo Del Bene[3], François Marquier[1], Christelle Langevin[2], François Treussart[1]
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Intracellular transport is a vital process, especially in neurons. Axonal transport deficit is found in neurodegenerative diseases. Conventional methods used to measure intraneuronal transport are limited by moderate spatiotemporal resolutions. We developed a method using photostable optically active nanocrystals (NC) tracers spontaneously internalized in endosomes[1,2]. Here we demonstrate its application to zebrafish (Zf) larvae. We used NC exhibiting large second-order non-linear optical properties, injected in Zf brain. We harnessed these properties combined with fast raster scanning of the infrared laser beam to achieve 20 frames/s rate, allowing us to detect short pausing duration underpinning complex molecular mechanisms otherwise smeared out by low temporal resolution. Using this method in axons of neurons with known polarization, we were able to separate the retrograde from the anterograde phases of motion. Our pipeline of video dataset analysis extracts statistical distributions of transport metrics in normal and perturbed situations. To test the sensitivity of our assay, we modulated active motors concentration, either by applying dynapyrazole, a retrograde motor dynein inhibitor, or by using transgenic Zf engineered with loss-of-function alleles of the anterograde motor Kif5aa. Dynapyrazole induces a 32% reduction of mobile NC, with a 37% reduction of their retrograde run length. In *kif5aa* mutants the retrograde run length is increased by 46% compared to wildtype. The high sensitivity of our assay opens prospects in investigating in vivo endosomal transport molecular mechanism in depth. S. Haziza, et al., *Nat. Nanotechnol.*, 2017, **12**, 322–328. Q.-L. Chou et al, *eNeuro*, 2022, ENEURO.0227-21.2022

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MOLECULAR IMAGING OF EXTRACELLULAR GLUTAMATE IN THE RAT AND MARMOSET BRAIN

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Miriam Schwalm[1], Robert Ohlendorf[1], Sarah Bricault[1], Jitendra Sharma[2], Alan Jasanoff[1,2]
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Monitoring extracellular glutamate dynamics provides a way to discern the engagement of excitatory activity that conducts the bulk of long-distance signaling in the mammalian brain. We recently introduced a molecular imaging approach that could permit noninvasive imaging of neurochemicals like glutamate using vasoactive sensors called AVATars. By coupling analyte detection to relaxation of vascular smooth muscle cells, these sensors induce artificial hemodynamic signals detectable by functional magnetic resonance imaging (fMRI) or functional ultrasound. Here we describe the design and *in vivo* validation of Glu-AVATar, a novel protein-based AVATar for selective hemodynamic imaging of extracellular glutamate in the brain. We demonstrate that this probe produces robust hemodynamic responses in the presence of exogenous glutamate, while control experiments performed with vehicle stimulation or control proteins do not result in signal changes. Glu-AVATar functionality is exhibited in both rodent and primate brains, indicating potential translatability of the technology. We further demonstrate that Glu-AVATar reveals correlations between probe-infused regions of the dorsal rat hippocampus and distal regions such as the ventral tegmental area and ventral hippocampus. These measurements constitute a neurochemically specific form of functional connectivity analysis that can be combined with conventional fMRI techniques to reveal correlates of excitatory signaling between different brain regions. Future AVATar-based molecular fMRI approaches will allow for more precise and less invasive methods for imaging extracellular glutamate dynamics over wide fields of view in intact brains.

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CUSTOM LIGHT-SHEET MICROSCOPY SETUPS FOR LARGE-SCALE HUMAN AND MOUSE BRAIN MAPPING

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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We describe two custom-designed Light Sheet Fluorescence Microscopy setups with different geometries for different types of samples: a dual view, inverted, dual color LSFM for large tissue slabs (up to 0.5mm in thickness, several cm in lateral size) and a single view, dual color LSFM for whole organs (e.g. whole mouse brains). These instruments have been used extensively for large acquisition campaigns: within the HBP to image a whole human hippocampus, within the NIH's Brain Initiative Cell Census Network to image large sections of the human Broca's area, and to image a large number of whole mouse brains for cfos-based activation mapping. The custom design of these devices concerns not only the hardware (custom sample holder, dual channel acquisition, autofocus), but also the software needed to efficiently run the whole pipeline on a daily basis. Indeed, LSFM poses significant challenges with data handling and processing, since these instruments easily produce large datasets, in the range of several TB each. For data acquisition, the system must be able to sustain high data rates, especially with dual-view imaging. To this aim, we have developed an open source set of hardware libraries and a dedicated data acquisition software, written in C++, that are able to control and synchronize multiple cameras and to automate the acquisition of big samples. Concerning image processing, we have developed an open source tool for stitching TB-sized datasets named ZetaStitcher. The tool is written in Python and features a handy API for virtual volumetric access of the fused tomography.

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LONGITUDINAL IMAGING OF ELECTRICAL SYNAPSES IN THE AWAKE MOUSE BRAIN USING A NOVEL, CUSTOMIZABLE, 3D-PRINTED HOLDING SYSTEM

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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In vivo 2-photon imaging of the mouse brain has developed into a versatile tool for the analysis of single organelles and neuronal networks. Here, we present an open-source, fully customizable head stage with a running disc, which is easily produced at low cost using a commercial 3D printer. The lightweight head-fixation provides a remarkably high stability even during animal movement, which is partially superior to commercially available systems. Furthermore, the system offers the ability to influence the running speed of the mouse and switch between resting, freely and externally induced moving states. Using our newly developed system, we performed *in vivo* 2-photon imaging in the motor and visual cortex of mice expressing EGFP-tagged Connexin36 (Cx36-EGFP), allowing us to monitor the location of identified electrical synapses over time. We observed a striking positional stability of Cx36-EGFP punctae over months. 2-photon fluorescence recovery after photobleaching (2P-FRAP) of individual punctae revealed an estimated turnover half-life of 3-6 weeks. In contrast to this high stability, visual deprivation induced decoupling of electrical synapses of fast spiking interneurons in V1 as evidenced by paired patch-clamp recordings. In addition, confocal microscopy indicated a decrease in the size and intensity of Cx36-EGFP punctae after 24h of visual deprivation. *In vivo* verification of these plastic changes using two-photon awake imaging is ongoing. In summary, we present a novel, highly stable head-fixation and running system for imaging of awake mice that enabled us to investigate the stability and plasticity of electrical synapses in the mammalian brain for the first time.

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A TOOLSET OF MINIMALLY INVASIVE BINDERS (NANOBODIES) TO STUDY THE SYNAPTIC VESICLE LIFE CYCLE

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

Ronja Rehm[1,2], Kristina Jevdokimenko[1,2], Nikolaos Mougios[1,2], Rashi Goel[3], Shama Sograte-Idrissi[1], Laszlo Albert[1,2], Momchil Ninov[4], Jeong Rhee[5], Svenja Bolz[6], Vladimir Khayenko[7], Natalie Kaempf[8], Carles Calatayud Aristoy[8], Daniela Ivanova[9], Natalí Chanaday Ricagni[10], Felix Lange[11], Ali Shaib[1,2], Viveka Nand Malviya[3], Martina Damenti[12], Francesca Pennacchietti[12], Nazar Oleksiievets[13], Roman Tsukanov[13], Jörg Enderlein[13], Pål Stenmark[14], Markel Martínez-Carranza[14], Stefan Jakobs[11], Hans Michael Maric[7], Henning Urlaub[4], Ege T. Kavalali[10], Frederic Meunier[15], Michael Cousin[9], Patrik Verstreken[8], Volker Haucke[6], Nils Brose[5], Ilaria Testa[12], Merja Joensuu[15], Benjamin H. Cooper[5], Reinhard Jahn[3], Silvio Rizzoli[1,2,16], Felipe Opazo[1,2,16], Eugenio Fornasiero[1,2,16]
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The synaptic vesicle (SV) life cycle encompasses several steps that allow the formation, trafficking, recycling and eventually degradation of these pivotal organelles in the biology of neurons. While their roles in neurotransmission are extensively studied, several aspects that orchestrate their function and define their identity are still poorly understood. Nanobodies (Nbs) offer several advantages when compared to conventional larger binders such as antibodies. As an example, due to their reduced size Nbs minimize linkage errors, decrease molecular crowding while increasing the efficiency of target binding. Moreover, since their sequences can be easily isolated, they can be functionalized in different manners by fusing them to the required protein-based tags or sensors. Here, we present a toolset of Nbs against SV proteins, which have been engineered by fusing them to several reporters. With these, we performed local measures of pH, calcium transients and carried on high resolution imaging experiments in different modalities, including electron microscopy, uPAINT and MoNaLISA. By expressing these Nbs as intrabodies, we also modulated the trafficking of SVs and studied the composition of synapses at the nanoscale providing novel quantitative information about the SV life cycle.

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DEVELOPING A SINGLE-MOLECULE PULL-DOWN (SIMPULL) ASSAY FOR CHARACTERISING PATHOLOGICAL TAU AGGREGATES

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Aims: Hyper-phosphorylation and aggregation of tau protein is a common neuropathological hallmark of various neurological disorders such as Alzheimer's disease. However, the tau aggregates' morphological characteristics as well as their role in the pathogenesis and pathophysiology of these disorders remain a topical area of research. The aim of this study is to develop biophysical tools to study this. **Methods:** We adapted and optimised a method utilising an antibody-based, single-molecule pull-down (SiMPull) assay in order to detect and characterise tau aggregates in biological samples with high sensitivity and specificity. Similar to a sandwich ELISA, SiMPull uses a PEG-passivated surface along with biotin-neutravidin interactions to capture and detect tau oligomers and fibrils using single-molecule fluorescence microscopy. We then combined this method with super-resolution microscopy to perform size and shape measurements of the tau aggregates. **Results:** We first validated this method using Alzheimer's Braak VI brain homogenate samples, achieving a sensitivity of 750 pg/mL for total tau. Specificity for tau aggregates was determined using non-tau antibodies and samples containing aggregates of non-tau proteins. Super-resolution experiments demonstrated morphological differences between Alzheimer's and healthy control samples. After these validation steps, we demonstrated the applicability of this method to other biological and clinically relevant samples such as serum and cerebrospinal fluid. **Conclusions:** Our results provide a proof-of concept for a reliable, sensitive, specific, and efficient method for performing diffraction-limited and super-resolution imaging and characterisation of tau aggregates in biological samples, enabling us to further study the role of tau in the pathophysiology of different neurodegenerative diseases.

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A STATISTICALLY BASED REGION GROWING APPROACH FOR SEGMENTING SUB-NEURONAL STRUCTURES FROM CONFOCAL AND SUPER-RESOLUTION IMAGES

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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Spines are neuronal protrusions implied in signal transmission and their identification is a challenging task. In this work, we customized our smart region growing (SmRG) algorithm for their segmentation. We exploited a statistical approach to find the best (adaptative) threshold to segment the data. SmRG fits a mixture model on the image to describe the background noise, the fluorescence diffusion and the neuronal fluorescence with a gaussian and two negative-binomial distributions, respectively. Region growing schemes assume a connected structure to segment. However, even in high-magnification datasets (e.g. 63x, 93x), nonconnected structures belonging to the neuron, e.g. spines with thin necks, can be found. This may be due to the image noise, which prevents us from clearly detecting the edges of the neuron and thus the spines’ shape. To deal with this aspect, the algorithm was designed to include regions close (but not structurally-connected) to the neuron body by means of a dilation on its borders. As such, SmRG is enabled to identify the spines potentially belonging to the neuron we are segmenting. We tested our approach on 93x STED and 63x confocal images. We were able to segment the neuron and the spines in STED images. On the other hand, the higher amount of noise of confocal images may have limited the efficacy of our algorithm. Our results suggest that the statistical modeling of signal and noise of confocal and STED images may be a strategy for segmenting dendritic spines. Future developments will consider a generalization to different imaging techniques.

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PARCELLATION OF BINARY SEGMENTATIONS IN MICROSCOPY IMAGES OF EX-VIVO CLARIFIED NEURONS VIA MORPHOLOGICAL RECONSTRUCTION AND WATERSHED TRANSFORM

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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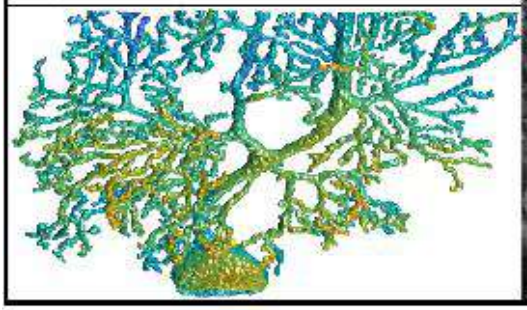
AIMS The growing interest on the morphology of whole neurons and dendritic spines demands for voxel-level precise segmentations. In images from ex-vivo clarified samples, the limited resolution of optical microscopy impairs the detection of gaps between close-by neurons in standard resolution and of spine necks in super-resolution images. This necessitates to post-process binary segmentations by splitting or grouping clusters, i.e. to define catchment basins for single neurons. We face this issue exploiting morphological reconstruction and topographic distance-based algorithms. METHODS We analyzed Purkinjie cells from L7GFP mouse cerebellum, processed with CLARITY protocol. We study two applications of the watershed transform: 1) to split the segmentation of dense 40x images into parcellations of separate whole-neuron structures; 2) to group clusters in a binary segmentation of branches and disconnected dendritic spines in 93x STED images. We employ Matlab routines to 1) morphologically reconstruct the image by imposing minima only within masks of disconnected neural cores; 2) apply a topographic distance-based watershed transform on the complement of the resulting image, to define the catchment basins. Final parcels are compared against manual segmentations. RESULTS On 40x images, we identified single neurons from densely connected segmentations (Fig1A). On 93x images, we obtained meaningful assignations of dendritic spines, with good scores when compared to manual gold-standard (Fig1B). CONCLUSIONS Topographic distance measures can be exploited to improve the usability of binary masks provided by neural segmentation tools that fails in isolating single neurons from dense structures. Moreover, we observed improvements in the characterization of spine

populations.

Figure 1A parcellations on 40x confocal images

Example of sectioned segmentation on 40x confocal



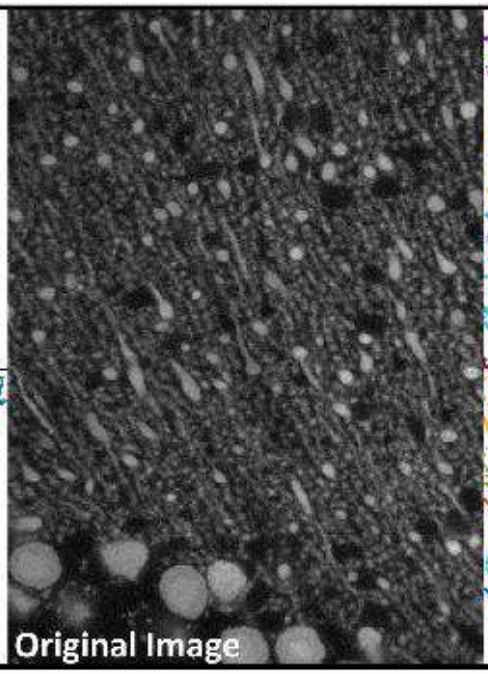
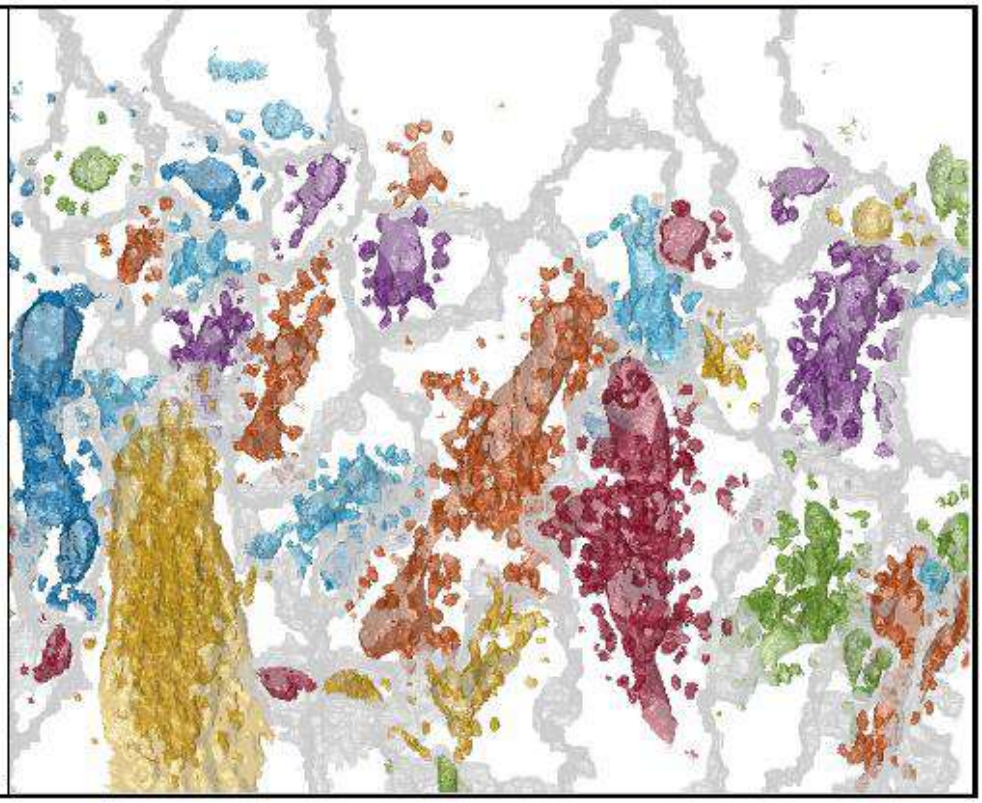


Figure 1B parcellations on 93x STED images (basins edges in gray)

Neuron	Spines number	% correctly assigned spines
A	119	94
B	52	90
C	31	96
D	17	100
E	17	100



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TWO PHOTON UNCAGING OF GLYCINE IN VITRO

POSTER SESSION 01 - SECTION: OPTOGENETICS & IMAGING

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In 1999-2000 I developed the first caged glutamate that was explicitly design for two-photon photolysis (Society for Neuroscience Annual Conference, 2000, 426.12). Electron-rich nitro-indolinyl caged acids such as MNI-glutamate and dinitro derivatives MDNI-Glu (aka DNI) and CNDI-Glu/GABA have proved extremely efficacious for photochemical probing of glutamate and GABA receptors in vitro. Here I describe the first example of a caged glycine designed for two-photon photolysis in complex brain tissue preparations in vitro. The caged glycine probe, called "CNI-Gly", was synthesized in 10 synthetic steps, and purified by preparative HPLC as its TFA salt. Comparative photolysis of CNI-Gly and MNI-Glu in cuvette revealed a quantum yield of photolysis of about 0.1. CNI-Gly was applied to interneurons from the dorsal horn of the spinal cord; irradiation with 1-ms pulses of 720 nm light evoked rapid, hyperpolaring currents. These were blocked by strychnine, indicating the expected inhibitory currents from classical ionotropic glycine receptors in this part of the CNS. We used CNI-Gly to probe the distribution of excitatory glycine receptors on pyramidal neurons in the basolateral amygdala. These currents arise from "unconventional" NMDA receptors consisting of GluN1 and GluN3a subunits. These currents were blocked by DKCA and enhanced by CGP-78608. No currents were detected in GluN3a knockout animals. Finally, unlike conventional NMDA receptors, no differential expression on spines versus dendritic shafts could be detected. Taken together, these data show CNI-Gly is a useful new photochemical probe for slice physiology using two-photon uncaging in vitro.

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BOARD NUMBER: S01-701

VISUAL ENCODING BY RETINAL GANGLION CELLS IN OPTOGENETIC MODELS FOR VISION RESTORATION

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Retinal degeneration is one of the leading causes of blindness and optogenetics as a potential therapeutic measure has garnered much attention. Light-sensitive molecules like Channelrhodopsin (ChR2) and Opto-mGluR6, a chimeric GPCR consisting of mGluR6 and melanopsin (designed by van Wyk et al.,2015) are inserted into the neurons in the inner retina to play the role of photoreceptors after their loss. Previous studies showed responses of retinal ganglion cells (RGCs) to simple light stimuli in blind animal models with optogenetically modified retinas. Our study aims is to go beyond simple light stimuli and investigate encoding by RGCs responding to spatiotemporally complex stimuli in optogenetically modified retinas. Preliminary experiments using multielectrode array recordings with retinas expressing Opto-mGluR6 showed light-dependent construct-driven responses in the RGCs. On comparing the photoreceptor-evoked and construct-evoked responses, a sign inversion in the response polarity (ON to OFF) as reported in the original work was also seen here. However, weaker responses were seen to high frequency contrast changes on activation of the construct as compared to photoreceptor-evoked responses. This indicates that construct-evoked responses cannot drive temporal integration in the cells like those in a normal retina due to the kinetics of Opto-mGluR6 activation. Further detailed experiments are required to estimate how best the construct can be stimulated to mimic the responses of a normal retina and thus improve the methods to be the optimal candidate for vision restoration. Reference: van Wyk et al.,2015. Restoring the ON Switch in Blind Retinas: Opto-mGluR6, a Next-Generation, Cell-Tailored Optogenetic Tool. *PLOS Biology*

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-702

NEURORETINAL CHANGES IN DIABETIC RETINOPATHY: COMPARISON BETWEEN HUMAN AND ZEBRAFISH MODEL

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Diabetic retinopathy (DR) is a frequent complication of diabetes, which comprises a complex interplay of microvascular changes and neurodegeneration. Retinal degeneration in DR is often irreversible and is closely attributed as a cause of blindness. Neuronal regeneration is usually impaired in diabetes, but its precise mechanisms have not been elucidated. Hence, this study aims to examine: 1) The changes of the retinal layer in patients with DR in comparison to the zebrafish model; 2) retina regeneration after insults in the zebrafish model of diabetes. 193 diabetic (51 NPDR and 142 PDR) and 83 healthy eyes were included. All retinal layers were significantly decreased in DR, but this observation was not found in the OPL. No significant changes of the retinal layers between NPDR and PDR. The findings indicated that the RNFL was potentially useful to identify the early development of DR in diabetic patients. In contrast to human findings, only the photoreceptor layer was decreased in the zebrafish model. The mechanical injury was performed to stimulate retina regeneration in zebrafish. Retina regeneration was suppressed in the injured retina exposed to 3% glucose, this plausibly due to the imbalance between cell death and proliferation mediated by the inhibition of Müller glia and brain aromatase activities. Interestingly, the addition of estradiol reversed the effects caused by glucose. Altogether, the results indicate that retinal thinning observed in DR may be associated with the impairment of retina regeneration and could be attributed to the dysfunction of Müller glia and aromatase.

Pubmed:

29867763: Ulhaq ZS, Kishida M
Brain Aromatase Modulates Serotonergic Neuron by Regulating Serotonin Levels in Zebrafish Embryos and Larvae.
Teleost fish are known to express two isoforms of P450 aromatase, a key enzyme for estrogen synthesis. One of the isoforms, brain aromatase (AroB), , is highly expressed during early development of zebrafish, thereby suggesting its role in brain development. On the other hand, early development of serotonergic neuron, one of the major monoamine neurons, is considered to play an important role in neurogenesis. Therefore, in this study, we investigated the role of AroB in development of serotonergic neuron by testing the effects of (1) estradiol (E) exposure and (2) morpholino (MO)-mediated AroB knockdown. When embryos were exposed to E, the effects were biphasic. The low dose of E (0.005 μM) significantly increased serotonin (5-HT) positive area at 48 hour post-fertilization (hpf) detected by immunohistochemistry and relative mRNA levels of tryptophan hydroxylase isoforms (, and) at 96 hpf measured by semi-quantitative PCR. To test the effects on serotonin transmission, heart rate and thigmotaxis, an indicator of anxiety, were analyzed. The low dose also significantly increased heart rate at 48 hpf and decreased thigmotaxis. The high dose of E (1 μM) exhibited opposite effects in all parameters. The effects of both low and high doses were reversed by addition of estrogen receptor (ER) blocker, ICI 182,780, thereby suggesting that the effects were mediated through ER. When AroB MO was injected to fertilized eggs, 5-HT-positive area was significantly decreased, while the significant decrease in relative mRNA levels was found only with but not with two other isoforms. AroB MO also decreased heart rate and increased thigmotaxis. All the effects were rescued by co-injection with AroB mRNA and by exposure to E. Taken together, this study demonstrates the role of brain aromatase in development of serotonergic neuron in zebrafish embryos and larvae, implying that brain-formed estrogen is an important factor to sustain early development of serotonergic neuron.
Front Endocrinol (Lausanne), 2018; 9
31692872: Ulhaq ZS
Brain aromatase modulates cardiac functions in embryonic zebrafish.
Oestradiol (E) is known as a female reproductive hormone with pleiotropic effects on the cardiovascular system. Local E biosynthesis such as in the brain and myocardial cells have important physiological and pathophysiological roles. E production is catalysed by aromatase (Aro) enzyme. In teleost, two Aro isoforms are distinctly expressed in the ovary and

brain. In this study, the role of brain Aro (AroB) in modulating cardiovascular system is investigated. AroB MO-mediated knockdown decreased ventricular functions. Moreover, embryos injected with AroB MO displays a sign in developing heart failure. All the effects caused by AroB MO were partially reversed by exposure to E. Taken together, this study demonstrates the role of AroB in modulating normal cardiovascular function in zebrafish embryos.
Int J Vet Sci Med, 2019; 7
32328758: Ulhaq ZS, Soraya GV
The prevalence of ophthalmic manifestations in COVID-19 and the diagnostic value of ocular tissue/fluid.
Graefes Arch Clin Exp Ophthalmol, 2020; 258
32414512: Ulhaq ZS, Soraya GV
Aqueous humor interleukin-6 levels in primary open-angle glaucoma (POAG): A systematic review and meta-analysis.
To determine the change in aqueous humor interleukin-6 (IL-6) levels among primary open-angle glaucoma (GPAA) patients.
Arch Soc Esp Oftalmol (Engl Ed), 2020; 95
33004198: Ulhaq ZS
Vitamin D and its receptor polymorphisms are associated with glaucoma.
To clarify the association between serum vitamin D levels and its receptor polymorphisms with glaucoma risk.
J Fr Ophtalmol, 2020; 43
33060644: Ulhaq ZS, Soraya GV, Budu , Wulandari LR
The role of IL-6-174 G/C polymorphism and intraocular IL-6 levels in the pathogenesis of ocular diseases: a systematic review and meta-analysis.
Interleukin-6 (IL-6) is one of the key regulators behind the inflammatory and pathological process associated with ophthalmic diseases. The role of IL-6-174 G/C polymorphism as well as intraocular IL-6 levels among various eye disease patients differ across studies and has not been systematically reviewed. Thus, this study aims to provide a summary to understand the relationship between IL-6 and ophthalmic disease. In total, 8,252 and 11,014 subjects for IL-6-174 G/C and intraocular levels of IL-6, respectively, were retrieved from PubMed, Scopus and Web of Science. No association was found between IL-6-174 G/C polymorphisms with ocular diseases. Subgroup analyses revealed a suggestive association between the GC genotype of IL-6-174 G/C with proliferative diabetic retinopathy (PDR). Further, the level of intraocular IL-6 among ocular disease patients in general was found to be higher than the control group [standardized mean difference (SMD) = 1.41, 95% confidence interval (CI) 1.24-1.58, $P < 0.00001$]. Closer examination through subgroup analyses yielded similar results in several ocular diseases. This study thus indicates that the IL-6-174 G/C polymorphism does not predispose patients to ocular disease, although the GC genotype is likely to be a genetic biomarker for PDR. Moreover, intraocular IL-6 concentrations are related to the specific manifestations of the ophthalmic diseases. Further studies with larger sample sizes are warranted to confirm this conclusion.
Sci Rep, 2020; 10
34387111: Ulhaq ZS, Soraya GV, Hasan YTN, Rachma LN, Rachmawati E, Shodry S, Kusuma MAS
Serum IL-6/IL-10 ratio as a biomarker for the diagnosis and severity assessment of primary-open angle glaucoma.
To assess the performance of serum cytokine IL-6 and IL-6/IL-10 ratio as biomarkers for the diagnosis of primary open-angle glaucoma (POAG) and for determining its progression.
Eur J Ophthalmol, 2021;
35053182: Ulhaq ZS, Tse WKF
A Brief Analysis of Proteomic Profile Changes during Zebrafish Regeneration.
Unlike mammals, zebrafish are capable to regenerate many of their organs, however, the response of tissue damage varies across tissues. Understanding the molecular mechanism behind the robust regenerative capacity in a model organism may help to identify and develop novel treatment strategies for mammals (including humans). Hence, we systematically analyzed the current literature on the proteome profile collected from different regenerated zebrafish tissues. Our analyses underlining that several proteins and protein families responsible as a component of cytoskeleton and structure, protein synthesis and degradation, cell cycle control, and energy metabolism were frequently identified. Moreover, target proteins responsible for the initiation of the regeneration process, such as inflammation and immune response were less frequently detected. This highlights the limitation of previous proteomic analysis and suggested a more sensitive modern proteomics analysis is needed to unfold the mechanism. This brief report provides a list of target proteins with predicted functions that could be useful for further biological studies.
Biomolecules, 2021; 12
31955999: Syambani Ulhaq Z
Dopamine D2 receptor influences eye development and function in Zebrafish.
Dopamine is synthesized by tyrosine hydroxylase and is considered as a major catecholamine in the vertebrate retina, including zebrafish. However, little is known about the role of dopamine D2 receptor (DRD2) in retinal physiology. Therefore,

to elucidate the role of DRD2 in the eye development and function in zebrafish, fish were exposed to fluphenazine, quinpirole, or combination of both. Subsequently, the eye size, optic nerve diameter (ONd), and visual background adaptation were evaluated. The results showed that fluphenazine (fluphenazine, DRD2 antagonist) decreased eye size and optic nerve diameter followed by disruption of visual function. The addition of Quinpirole (quinpirole, DRD2 agonist) reversed the effects caused by fluphenazine, implying that DRD2 is necessary for normal eye development and function in zebrafish. Considering the role of dopaminergic neurons in retinal development and function, dysfunction of dopaminergic neuron signaling pathways in the retina may cause visual abnormalities, particularly in the involvement of dopamine in regulating light response.

Arch Soc Esp Oftalmol (Engl Ed), 2020; 95
34647630: Ulhaq ZS, Soraya GV, Indriana K, Devitasari R, Pradiptha IPY, Zulfikar DB, Uxiana V, Zulkarnain , Rachma LN, Arisanti D
The level of Ig anti-RBD SARS-CoV-2 after two doses of CoronaVac vaccine.
J Med Virol, 2022; 94

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BOARD NUMBER: S01-703

TRANS-RESVERATROL EFFECTS ON DIABETES-INDUCED APOPTOSIS IN RETINAL PIGMENT EPITHELIUM IN WISTAR RATS

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Aim Diabetes-induced oxidative stress mediates retinal cell apoptosis leading to the onset of pathological changes related to diabetic retinopathy. This study investigated the modulatory effects of *trans*-resveratrol, a natural stilbene antioxidant, on type 1 diabetes-induced retinal pigment epithelial (RPE) cell apoptosis in Wistar rats. **Methods** Adult male rats (12-14-week-old) were segregated into 1) control, 2) control + *trans*-resveratrol (p.o., 5mg/kg/d for 90 days), 3) single dose Streptozotocin (55mg/kg, ip, glucose level >300 mg/kg considered diabetic) induced diabetic group, and 4) the diabetic group supplemented with *trans*-resveratrol. The eyeballs were dissected, the neural retina was separated from the RPE, and the eye cup containing the RPE was flat-mounted for immunofluorescence for caspases-9 and -3, and cytochrome C. The number and location of protein-labelled RPE cells were counted and mapped. The data were statistically analysed by the Mann-Whitney U test, and $p<0.05$ was considered significant. **Results** Immunofluorescence showed the clustering of apoptotic RPE cells, mainly at the centre of the RPE layer in the diabetic group. Diabetes increased the number of caspases-9 and -3 labelled RPE cells, but more caspase-3 positive cells, and showed a trend to increase the number of cytochrome C labelled cells. *Trans*-resveratrol supplementation showed a trend to reduce the positively labelled cells, but there were no statistically significant effects of the drug on induced apoptosis. **Conclusions** Prolonged type 1 diabetes increases the RPE cells to enter apoptotic phase, however, *trans*-resveratrol supplementation only shows a tendency to reduce the diabetes-induced apoptotic process in the RPE.

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BOARD NUMBER: S01-704

A NOVEL APPROACH TO ANALYZE WHITE NOISE ERGS IN MICE

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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University Hospital Erlangen, Eyeclinic, Erlangen, Germany

ERG measurements are a valuable tool to study retinal physiology. Normally they are recorded to bright flashes which are not physiological. We measured ERGs to temporal white noise (TWN) stimuli in mice, which are more natural. White noise ERGs (wnERGs) were recorded to luminance and opsin isolating TWN. Measurements were performed at different mean luminances (MLs); each recording was performed twice. To quantify the reproducibility, the ERG potentials of the two recordings were plotted against each other and the correlation coefficient (r^2_{repr}) of the linear regression was extracted. This was repeated for all MLs. For luminance stimuli r^2_{repr} values were minimal at -0.3 log cd/m² ML. r^2_{repr} values decreased with increasing MLs for rod isolating stimuli, whereas for cone isolating stimuli they increased with increasing ML. To study if underlying ERG mechanisms depend on ML, the ERG potentials at all MLs were plotted against those obtained at the highest ML for cones and at the lowest ML for rods. The r^2-coefficients (r^2_{ML}) of the linear regression were used to quantify the correlations between responses of different MLs. R^2_{ML} values for luminance, L- and S-opsin stimuli increased with increasing ML, whereas they decreased for rod stimuli. In conclusion rod signals drive the ERGs at low MLs and cones at high MLs.

Pubmed:
33811381: Ryl M, Urbasik A, Gierke K, Babai N, Joachimsthaler A, Feigenspan A, Frischknecht R, Stallwitz N, Fejtová A, Kremers J, von Wittgenstein J, Brandstätter JH
Genetic disruption of bassoon in two mutant mouse lines causes divergent retinal phenotypes.
Bassoon (BSN) is a presynaptic cytomatrix protein ubiquitously present at chemical synapses of the central nervous system, where it regulates synaptic vesicle replenishment and organizes voltage-gated Ca channels. In sensory photoreceptor synapses, BSN additionally plays a decisive role in anchoring the synaptic ribbon, a presynaptic organelle and functional extension of the active zone, to the presynaptic membrane. In this study, we functionally and structurally analyzed two mutant mouse lines with a genetic disruption of Bsn-Bsn and Bsn -using electrophysiology and high-resolution microscopy. In both Bsn mutant mouse lines, full-length BSN was abolished, and photoreceptor synaptic function was similarly impaired, yet synapse structure was more severely affected in Bsn than in Bsn photoreceptors. The synaptic defects in Bsn retina coincide with remodeling of the outer retina-rod bipolar and horizontal cell sprouting, formation of ectopic ribbon synaptic sites-and death of cone photoreceptors, processes that did not occur in Bsn retina. An analysis of Bsn hybrid mice revealed that the divergent retinal phenotypes of Bsn and Bsn mice can be attributed to the expression of the Bsn allele, which triggers cone photoreceptor death and neurite sprouting in the outer retina. These findings shed new light on the existing Bsn mutant mouse models and might help to understand mechanisms that drive photoreceptor death.
FASEB J, 2021; 35

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-705

RESTORING VISION BY CONJUGATED POLYMER NANOPARTICLES IN A MODEL OF RETINITIS PIGMENTOSA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Simona Francia[1], Dmytro Shmal[1,2], Stefano Di Marco[1,3], Giovanni Manfredi[4], Anna Rocchi[1], Greta Chiaravalli[5], José Fernando Maya-Vetencourt[1,6], Caterina Michetti[1,2], Giulia Mantero[1,2], Sara Perotto[5], Mattia Lorenzo Difrancesco[1], Marcella Attanasio[7], Sara Cupini[1,2], Riccardo Sacco[8], Silvia Bisti[1], Maurizio Mete[7], Grazia Pertile[7], Guglielmo Lanzani[5,9], Elisabetta Colombo[1,3], Fabio Benfenati[1,2,3]
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Aims. Retinal dystrophies such as *Retinitis Pigmentosa* (*RP*) are the most common inherited progressive cause of blindness in developed countries affecting some 5.5 million patients, for which treatments are in demand. **Methods.** Retinal prostheses have been developed to stimulate the inner retinal network; however, the inner retinal circuits, although initially spared by neurodegeneration, undergo complex rearrangements and deteriorate in the advanced stages of the disease. We recently reported that conjugated polymer nanoparticles, by virtue of their intrinsic light-induced charge generation, persistently rescue visual activities when subretinally injected in the Royal College of Surgeons (RCS) rat model of *RP.* This rat strain bears a null mutation in the *Mertk* gene that impairs the function of the retinal pigment epithelium (RPE) causing a cone-rod degeneration. **Results.** Here, we demonstrate that conjugated polymer nanoparticles, when microinjected in 10-months-old RCS rats bearing fully light-insensitive and intensely remodeled retinas, reinstate visually driven activities at both subcortical and cortical levels. The efficacy of these nanoparticles in restoring vision is indicated by absence of any recovery in visual activities in sham-injected rats. **Conclusions.** These results highlight the potential clinical relevance of conjugated polymer nanoparticles in restoring visual functions in fully degenerate retinas, completely devoid of residual photoreceptors, and in the presence of a severe inner retinas rewiring, an advanced stage of the disease in which patients affected by *RP* are subjected to prosthetic interventions.

Pubmed:
25539725: Francia S, Silvotti L, Ghirardi F, Catzeflis F, Percudani R, Tirindelli R
Evolution of spatially coexpressed families of type-2 vomeronasal receptors in rodents.
The vomeronasal organ (VNO) is an olfactory structure for the detection of pheromones. VNO neurons express three groups of unrelated G-protein-coupled receptors. Type-2 vomeronasal receptors (V2Rs) are specifically localized in the basal neurons of the VNO and are believed to sense protein pheromones eliciting specific reproductive behaviors. In murine species, V2Rs are organized into four families. Family-ABD V2Rs are expressed monogenically and coexpress with family-C V2Rs of either subfamily C1 (V2RC1) or subfamily C2 (V2RC2), according to a coordinate temporal diagram. Neurons expressing the phylogenetically ancient V2RC1 coexpress family-BD V2Rs or a specific group of subfamily-A V2Rs (V2RA8-10), whereas a second neuronal subset (V2RC2-positive) coexpresses a recently expanded group of five subfamily-A V2Rs (V2RA1-5) along with vomeronasal-specific Major Histocompatibility Complex molecules (H2-Mv). Through database mining and Sanger sequencing, we have analyzed the onset, diversification, and expansion of the V2R-families throughout the phylogeny of Rodentia. Our results suggest that the separation of V2RC1 and V2RC2 occurred in a Cricetidae ancestor in coincidence with the evolution of the H2-Mv genes; this phylogenetic event did not correspond with the origin of the coexpressing V2RA1-5 genes, which dates back to an ancestral myomorphan lineage. Interestingly, the evolution of receptors within the V2RA1-5 group may be implicated in the origin and diversification of some of the V2R putative cognate ligands, the exocrine secreting peptides. The establishment of V2RC2, which probably reflects the complex expansion and diversification of family-A V2Rs, generated receptors that have probably acquired a more subtle functional specificity.
Genome Biol Evol, 2014; 7
29662006: Arcidiacono D, Dedja A, Giacometti C, Fassan M, Nucci D, Francia S, Fabris F, Zaramella A, Gallagher EJ,

Cassaro M, Rugge M, LeRoith D, Alberti A, Realdon S
Hyperinsulinemia Promotes Esophageal Cancer Development in a Surgically-Induced Duodeno-Esophageal Reflux Murine Model.
Hyperinsulinemia could have a role in the growing incidence of esophageal adenocarcinoma (EAC) and its pre-cancerous lesion, Barrett's Esophagus, a possible consequence of Gastro-Esophageal Reflux Disease. Obesity is known to mediate esophageal carcinogenesis through different mechanisms including insulin-resistance leading to hyperinsulinemia, which may mediate cancer progression via the insulin/insulin-like growth factor axis. We used the hyperinsulinemic non-obese FVB/N (Friend leukemia virus B strain) MKR (muscle (M)-IGF1R-lysine (K)-arginine (R) mouse model to evaluate the exclusive role of hyperinsulinemia in the pathogenesis of EAC related to duodeno-esophageal reflux. FVB/N wild-type (WT) and MKR mice underwent jejunum-esophageal anastomosis side-to end with the exclusion of the stomach. Thirty weeks after surgery, the esophagus was processed for histological, immunological and insulin/Insulin-like growth factor 1 (IGF1) signal transduction analyses. Most of the WT mice (63.1%) developed dysplasia, whereas most of the MKR mice (74.3%) developed squamous cell and adenosquamous carcinomas, both expressing Human Epidermal growth factor receptor 2 (HER2). Hyperinsulinemia significantly increased esophageal cancer incidence in the presence of duodenal-reflux. Insulin receptor (IR) and IGF1 receptor (IGF1R) were overexpressed in the hyperinsulinemic condition. IGF1R, through ERK1/2 mitogenic pattern activation, seems to be involved in cancer onset. Hyperinsulinemia-induced IGF1R and HER2 up-regulation could also increase the possibility of forming of IGF1R/HER2 heterodimers to support cell growth/proliferation/progression in esophageal carcinogenesis.
Int J Mol Sci, 2018; 19
31875544: Zamparo I, Francia S, Franchi SA, Redolfi N, Costanzi E, Kerstens A, Fukutani Y, Battistutta R, Polverino de Laureto P, Munck S, De Strooper B, Matsunami H, Lodovichi C
Axonal Odorant Receptors Mediate Axon Targeting.
In mammals, odorant receptors not only detect odors but also define the target in the olfactory bulb, where sensory neurons project to give rise to the sensory map. The odorant receptor is expressed at the cilia, where it binds odorants, and at the axon terminal. The mechanism of activation and function of the odorant receptor at the axon terminal is, however, still unknown. Here, we identify phosphatidylethanolamine-binding protein 1 as a putative ligand that activates the odorant receptor at the axon terminal and affects the turning behavior of sensory axons. Genetic ablation of phosphatidylethanolamine-binding protein 1 in mice results in a strongly disturbed olfactory sensory map. Our data suggest that the odorant receptor at the axon terminal of olfactory neurons acts as an axon guidance cue that responds to molecules originating in the olfactory bulb. The dual function of the odorant receptor links specificity of odor perception and axon targeting.
Cell Rep, 2019; 29
34452614: Francia S, Lodovichi C
The role of the odorant receptors in the formation of the sensory map.
In the olfactory system, odorant receptors (ORs) expressed at the cell membrane of olfactory sensory neurons detect odorants and direct sensory axons toward precise target locations in the brain, reflected in the presence of olfactory sensory maps. This dual role of ORs is corroborated by their subcellular expression both in cilia, where they bind odorants, and at axon terminals, a location suitable for axon guidance cues. Here, we provide an overview and discuss previous work on the role of ORs in establishing the topographic organization of the olfactory system and recent findings on the mechanisms of activation and function of axonal ORs.
BMC Biol, 2021; 19
33376209: Maset A, Galla L, Francia S, Cozzolino O, Capasso P, Goisis RC, Losi G, Lombardo A, Ratto GM, Lodovichi C
Altered Cl homeostasis hinders forebrain GABAergic interneuron migration in a mouse model of intellectual disability.
Impairments of inhibitory circuits are at the basis of most, if not all, cognitive deficits. The impact of OPHN1, a gene associate with intellectual disability (ID), on inhibitory neurons remains elusive. We addressed this issue by analyzing the postnatal migration of inhibitory interneurons derived from the subventricular zone in a validated mouse model of ID (OPHN1 mice). We found that the speed and directionality of migrating neuroblasts were deeply perturbed in OPHN1 mice. The significant reduction in speed was due to altered chloride (Cl) homeostasis, while the overactivation of the OPHN1 downstream signaling pathway, RhoA kinase (ROCK), caused abnormalities in the directionality of the neuroblast progression in mutants. Blocking the cation-Cl cotransporter KCC2 almost completely rescued the migration speed while proper directionality was restored upon ROCK inhibition. Our data unveil a strong impact of OPHN1 on GABAergic inhibitory interneurons and identify putative targets for successful therapeutic approaches.
Proc Natl Acad Sci U S A, 2021; 118
35033138: Baumann J, Tsao CC, Patkar S, Huang SF, Francia S, Magnussen SN, Gassmann M, Vogel J, Köster-Hegmann C, Ogunshola OO

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Pericyte, but not astrocyte, hypoxia inducible factor-1 (HIF-1) drives hypoxia-induced vascular permeability in vivo.
Ways to prevent disease-induced vascular modifications that accelerate brain damage remain largely elusive. Improved understanding of perivascular cell signalling could provide unparalleled insight as these cells impact vascular stability and functionality of the neurovascular unit as a whole. Identifying key drivers of astrocyte and pericyte responses that modify cell-cell interactions and crosstalk during injury is key. At the cellular level, injury-induced outcomes are closely entwined with activation of the hypoxia-inducible factor-1 (HIF-1) pathway. Studies clearly suggest that endothelial HIF-1 signalling increases blood-brain barrier permeability but the influence of perivascular HIF-1 induction on outcome is unknown. Using novel mouse lines with astrocyte and pericyte targeted HIF-1 loss of function, we herein show that vascular stability in vivo is differentially impacted by perivascular hypoxia-induced HIF-1 stabilization.
Fluids Barriers CNS, 2022; 19

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BOARD NUMBER: S01-706

A CAV1.4 L-TYPE CALCIUM CHANNEL TRUNCATION MUTATION AFFECTS THE RETINAL ROD PATHWAY

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Matthias Ganglberger[1], Dagmar Knoflach[2], Hartwig Seitter[1], Lucia Zanetti[1], Emilio Casanova[2], Bettina Wagner[3], Thomas Rülicke[3], Alexandra Koschak[1]
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L-type calcium channels (LTCC) are the main regulators for cellular events, like excitation-contraction coupling, transmitter release and hormone secretion. In the retina, the Cav1.4 LTCCs are predominantly expressed at the synaptic terminals of photoreceptors in the outer plexiform layer (OPL) to support tonic glutamate release. In patients, mutations in the *CACNA1F* gene, which encodes Cav1.4 channels can cause congenital stationary night blindness type 2 (CSNB2). In this study we investigated a C-terminal truncation mutation in the Cav1.4 channel (Cav1.4-RX). Heterologously expressed Cav1.4-RX channels showed calcium-dependent inactivation, which is normally not present in this LTCC isoform. The aim of the current study is to examine morphological and functional changes in mice carrying the truncation mutation Cav1.4-R1827X (Cav1.4-RX) comparing to a gain-of-function mutation, Cav1.4-I756T (Cav1.4-IT) and wildtype mice via immunohistochemical analyses and functional (multielectrode array, MEA) analyses. Initial immunohistochemical studies showed significant changes in the retinal morphology comparable to the Cav1.4-IT retinas: in addition to changes in the presynaptic ribbon structure we also found neurite sprouting of second-order neurons. However, cones seemed to be largely unaffected; a finding that is in contrast to Cav1.4-IT. This difference is also seen in MEA recordings in which ganglion cell responses were mainly affected under scotopic conditions suggesting that the truncation primarily affects the rod pathway. This observation could be explained by a difference in the protein composition in rod and cone photoreceptor terminals. Further investigations will include a proteomic approach to fully understand the molecular mechanism of the distal C-terminal part of Cav1.4 channels.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S01-707

CHARACTERIZATION OF TWO PATHOLOGICAL CAV1.4 L-TYPE CALCIUM CHANNEL VARIANTS

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Thomas Heigl[1], Michael Netzer[1,2], Matthias Ganglberger[1], Xuechen Tang[3], Monica Fernandez-Quintero[3], Alexandra Koschak[1]
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Aims: Cav1.4 L-type calcium channels are predominantly expressed at retinal synapses, mediating neurotransmitter release. Mutations in its gene, *CACNA1F*, can cause congenital stationary night-blindness type 2 (CSNB2), an X-linked disease that can manifest in variable levels of night-blindness but also photophobia or low visual acuity. This project aims to elucidate the biophysical properties of two variants, Cav1.4-R964G and Cav1.4-R1288L (JF701915.1), both S4 charge-neutralizing mutants reported in CSNB2 patients. **Methods:** Cav1.4 variants were produced in Flp-In-293 cells, which stably expressed β3 and α2δ1 auxiliary subunits (electrophysiology), whereas tsA-201 cells were transiently co-transfected with their ancillary subunits (western blot). We combined homology modelling with molecular dynamics simulations to understand the functional effects at atomistic detail. **Results:** Cav1.4-R964G and Cav1.4-R1288L were less expressed in the membrane *in vitro*, indicated by western blot analyses. This finding is in accordance with whole-cell patch-clamp recordings, which revealed a reduction of current density compared to wild type channels, with Cav1.4-R964G having 23% and Cav1.4-R1288L 12.4% of the wild type current density. Consistent with a loss of gating charges, both substitutions lead to a significantly increased slope of voltage-dependent activation while Cav1.4-R1288L also shifts the voltage-dependence of activation towards more positive potentials. In agreement with these observations, we find that Cav1.4-R1288L destabilizes the activated state, while Cav1.4-R964G diminishes interactions in intermediate resting states with the inner negative cluster. **Conclusions:** The detected changes in expression level and biophysical properties for Cav1.4-R964G and Cav1.4-R1288L likely account for differences in signal transmission in retinal cells, thus leading to CSNB2.

Pubmed:

31525190: Misslinger M, Scheven MT, Hortschansky P, López-Berges MS, Heiss K, Beckmann N, Heigl T, Hermann M, Krüger T, Kniemeyer O, Brakhage AA, Haas H
The monothiol glutaredoxin GrxD is essential for sensing iron starvation in Aspergillus fumigatus.
Efficient adaptation to iron starvation is an essential virulence determinant of the most common human mold pathogen, Aspergillus fumigatus. Here, we demonstrate that the cytosolic monothiol glutaredoxin GrxD plays an essential role in iron sensing in this fungus. Our studies revealed that (i) GrxD is essential for growth; (ii) expression of the encoding gene, grxD, is repressed by the transcription factor SreA in iron replete conditions and upregulated during iron starvation; (iii) during iron starvation but not iron sufficiency, GrxD displays predominant nuclear localization; (iv) downregulation of grxD expression results in de-repression of genes involved in iron-dependent pathways and repression of genes involved in iron acquisition during iron starvation, but did not significantly affect these genes during iron sufficiency; (v) GrxD displays protein-protein interaction with components of the cytosolic iron-sulfur cluster biosynthetic machinery, indicating a role in this process, and with the transcription factors SreA and HapX, which mediate iron regulation of iron acquisition and iron-dependent pathways; (vi) UV-Vis spectra of recombinant HapX or the complex of HapX and GrxD indicate coordination of iron-sulfur clusters; (vii) the cysteine required for iron-sulfur cluster coordination in GrxD is in vitro dispensable for interaction with HapX; and (viii) there is a GrxD-independent mechanism for sensing iron sufficiency by HapX; (ix) inactivation of SreA suppresses the lethal effect caused by GrxD inactivation. Taken together, this study demonstrates that GrxD is crucial for iron homeostasis in A. fumigatus.
PLoS Genet, 2019; 15
34212239: Koschak A, Fernandez-Quintero ML, Heigl T, Ruzza M, Seitter H, Zanetti L
Cav1.4 dysfunction and congenital stationary night blindness type 2.
Cav1.4 L-type Ca channels are predominantly expressed in retinal neurons, particularly at the photoreceptor terminals where they mediate sustained Ca entry needed for continuous neurotransmitter release at their ribbon synapses. Cav1.4 channel gating properties are controlled by accessory subunits, associated regulatory proteins, and also alternative splicing. In

humans, mutations in the CACNA1F gene encoding for Cav1.4 channels are associated with X-linked retinal disorders such as congenital stationary night blindness type 2. Mutations in the Cav1.4 protein result in a spectrum of altered functional channel activity. Several mouse models broadened our understanding of the role of Cav1.4 channels not only as Ca source at retinal synapses but also as synaptic organizers. In this review, we highlight different structural and functional phenotypes of Cav1.4 mutations that might also occur in patients with congenital stationary night blindness type 2. A further important yet mostly neglected aspect that we discuss is the influence of alternative splicing on channel dysfunction. We conclude that currently available functional phenotyping strategies should be refined and summarize potential specific therapeutic options for patients carrying Cav1.4 mutations. Importantly, the development of new therapeutic approaches will permit a deeper understanding of not only the disease pathophysiology but also the physiological function of Cav1.4 channels in the retina. Pflugers Arch, 2021; 473

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-708

FUNCTIONAL RESPONSES OF THE RETINA AND STRUCTURE OF THE VISUAL PROJECTIONS IN ALBINO MICE

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Visual perception is based on a precise architecture of the retina and visual pathways. In albinism, the hypopigmentation of the retinal pigment epithelium (RPE) alters the structure of the visual pathways during development, resulting in poor visual acuity and abnormal binocular vision. Here, we characterize the deficits in the retina and visual projections of an albino mouse model ($Tyr^{c/c}$). First, we studied the ratio of RGC projections from both eyes into their brain targets as fewer RGCs project ipsilaterally in albino mammals. Using fluorescent tracers, iDISCO+ clearing and whole brain imaging with light-sheet microscopy, we showed that the volume of ipsilateral projections in the dorsal lateral geniculate nucleus of albino $Tyr^{c/c}$ mice are decreased compared to $Tyr^{c/+}$ mice, but similar in the suprachiasmatic nucleus (SCN). It suggests different targeting mechanisms for the RGCs innervating the SCN. Second, we compared the expression of various opsins labelling subtypes of photoreceptors (S- and M-opsin) or intrinsically photosensitive retinal ganglion cells (ipRGCs, melanopsin) in the retina, to determine whether their proportion and distribution are altered in albino mice. Finally, we recorded the electrophysiological responses of RGCs to light by multi-electrode array recording to determine whether a change in the RGC receptive field diameter could explain the low visual acuity in albino mice. Surprisingly, no difference was found, neither in the receptive field diameters nor in the functional population of RGCs, suggesting another origin for the low visual acuity. Multi-scale analysis of the albino phenotype is a prerequisite to study RPE regulation of retinal development.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-709

CONTEXT-DEPENDENT SELECTIVITY TO NATURAL IMAGES IN THE RETINA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Understanding how sensory neurons extract relevant information from natural scenes is a major challenge in neuroscience. Even in the retina, is not clear how Retinal ganglion cells (RGC) extract specific features from natural scenes and send this information to the brain. Many studies using simple, artificial stimuli have shown responses to local light increase (ON-responses), and/or decrease (OFF), but it is unclear if this selectivity is maintained when processing natural stimuli. Other works tried learning non-linear models to predict RGC responses to natural stimuli, but are hard to interpret. Here we address these issues using a novel perturbative approach taking the best of these two strategies, by probing selectivity with perturbations added to natural scenes. We stimulated mouse and axolotl retinas with natural images, adding small checkerboard-like perturbations on top. We found that ON-OFF selectivity strongly depended on the image. RGCs can thus switch from ON to OFF depending on the context. We designed a convolutional neural network model to explain this, and mapped it to specific retinal circuits. Pharmacological experiments and modeling showed that selectivity changes were due to the non-linear combination of retinal pathways. Finally, using dimensionality reduction and gradient field representations, we demonstrated that this context dependence is compatible with a robust computation of a more abstract feature: contrast. Our perturbative approach thus uncovers neuronal selectivity to more complex features than initially thought during natural scene stimulation and could be applied in other sensory systems to refine models or test hypotheses about features extracted from sensory inputs.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-710

RETINAL VASCULAR DYSREGULATION IN ALTITUDINAL VISUAL FIELD DEFECTS IN GLAUCOMA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Aims: Healthy vision depends on both, neuronal excitability and healthy neurovascular coupling (NVC). To relate the extend to vision loss to central neurovascular regulation, we imaged retinal blood vessels at high resolution to study the relationship between retinal vessel dynamics during neural activation and neurological (visual field) function in glaucoma. Methods: With the dynamic vessel analyzer (DVA) we quantified retinal vessel diameters and dilation responses following flickering light stimulation in 30 subjects with primary open angle glaucoma compared to 22 age-matched healthy subjects. Vessel dynamics was then related to severity of glaucomatous visual field loss. Results: The average vessels diameter in glaucoma was smaller than in controls (artery: 90.6 vs. 94.8 µm, p=0.003, vein: 104.9 vs. 107.3 µm, p=0.48), but when exposing the retina to flickering light, venous maximal dilation was greater in glacuoma patients (p<0.001), i.e. dilation reached normal values despite having smaller diameters. However, venous maximal dilation was smallest in regions of the retina correspond to regions of the visual field with moderate vision loss and largest in intact regions and those with mild or severe vision loss (p=0.029), with a similar tendency in arteries. Smaller vessels had the greatest vascular dysregulation. Conclusions: The observation that NVC-impairment was most pronounced in regions of moderate functional loss - not in regions of normal, mild or severe vision loss – was rather surprising. It suggests that vascular dysregulation is not distributed uniformly throughout the retina. The functional implications of why NVC is greatest in moderate functional loss needs further study.

Pubmed:
34924406: Sabel BA, Zhou W, Huber F, Schmidt F, Sabel K, Gonschorek A, Bilc M
Non-invasive brain microcurrent stimulation therapy of long-COVID-19 reduces vascular dysregulation and improves visual and cognitive impairment.
An effective treatment is needed for long-COVID patients which suffer from symptoms of vision and/or cognition impairment such as impaired attention, memory, language comprehension, or fatigue.
Restor Neurol Neurosci, 2021; 39
32953576: Zhou WS, Lin WX, Geng YY, Wang T
Combined phacoemulsification and goniosynechialysis with or without endoscopic cyclophotocoagulation in the treatment of PACG with cataract.
To investigate the efficacy and safety of combined phacoemulsification and goniosynechialysis with or without endoscopic cyclophotocoagulation (PGE group and PG group) for the treatment of patients with coexisting primary angle-closure glaucoma (PACG) and cataracts.
Int J Ophthalmol, 2020; 13

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-711

IDENTIFICATION OF TYROSINE HYDROXYLASE-IMMUNOREACTIVE AMACRINE CELLS IN THE GERBIL RETINA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Gerbil's diurnality, with a higher proportion of retinal cones compared to nocturnal mice and rats, gives specific advantages for studies on the central visual system. The purpose of this study was to characterize tyrosine hydroxylase (TH)-immunoreactive (IR) cells in the gerbil retina using immunocytochemistry and confocal microscopy. Based on dendritic morphologies and stratification patterns, at least four subtypes of TH-IR cells were observed. The first subtype had monostratified dense plexus in substrata (s) 1 of the inner plexiform layer. The second subtype had three stratifications of plexuses in s1, s3 and s5. The third subtype also had three stratifications like the second one, with additional processes ascending to the outer plexiform layer. The fourth subtype was displaced to the ganglion cell layer, which was less than 5 cells/retina. TH-IR cells were wide-field amacrine cells and typically had 2–5 primary processes, derived from the soma. The dendritic processes extensively overlapped with neighboring TH-IR cells forming thick dendritic plexuses. Double-immunofluorescence study with calcium-binding proteins showed that TH-IR cells colocalized only with some calbindin (CB)-IR cells, but not with calretinin (CR)- or parvalbumin (PV)-IR cells. Some CR-IR cells were encircled by the ring-like structures composed of processes of TH-IR cells. Vesicular nucleotide transporter expression was found in all TH-IR cells, indicating that ATP release from TH-IR cells. This study showed at least four subtypes of TH-IR cells in the gerbil retina and could provide valuable data for the diverse role of dopamine in the retina of diurnal animals.

Pubmed:
35052772: Kwon KM, Lee MJ, Chung HS, Pak JH, Jeon CJ
The Organization of Somatostatin-Immunoreactive Cells in the Visual Cortex of the Gerbil.
Somatostatin (SST) is widely expressed in the brain and plays various, vital roles involved in neuromodulation. The purpose of this study is to characterize the organization of SST neurons in the Mongolian gerbil visual cortex (VC) using immunocytochemistry, quantitative analysis, and confocal microscopy. As a diurnal animal, the Mongolian gerbil provides us with a different perspective to other commonly used nocturnal rodent models. In this study, SST neurons were located in all layers of the VC except in layer I; they were most common in layer V. Most SST neurons were multipolar round/oval or stellate cells. No pyramidal neurons were found. Moreover, 2-color immunofluorescence revealed that only 33.50%, 24.05%, 16.73%, 0%, and 64.57% of SST neurons contained gamma-aminobutyric acid, calbindin-D28K, calretinin, parvalbumin, and calcium/calmodulin-dependent protein kinase II, respectively. In contrast, neuropeptide Y and nitric oxide synthase were abundantly expressed, with 80.07% and 75.41% in SST neurons, respectively. Our immunocytochemical analyses of SST with D and D dopamine receptors and choline acetyltransferase, α and β nicotinic acetylcholine receptors suggest that dopaminergic and cholinergic fibers contact some SST neurons. The results showed some distinguishable features of SST neurons and provided some insight into their afferent circuitry in the gerbil VC. These findings may support future studies investigating the role of SST neurons in visual processing.
Biomedicines, 2022; 10

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-712

CELL-TYPE-SPECIFIC DENDRITIC CHANGES IN THE MOUSE RETINA DURING DEVELOPMENT AND IN RESPONSE TO INJURY

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Joana Santos[1,2,3], Luca Masin[2], Bram Nuttin[1,2,3], Chen Li[1,2,3], Lieve Moons[2], Karl Farrow[1,2,3]
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In the central nervous system of adult mammals, neurons naturally fail to regenerate their axons after injury. During development, by contrast, neurons grow axons effectively. It has been shown that neuronal types differ dramatically in their resilience to injury or other insults. For example, among all the retinal ganglion cells (RGCs) that survived best are the alpha-RGCs. However, even among these molecularly defined cell types, their resilience differs with stratification. This makes alpha-RGCs a great starting point to understand what makes transcriptionally similar cells respond differently to injury. Here, we aim to understand the relationship between dendritic morphology and the ability of axons to regrow after injury. In this project, first, we documented the emergence of the characteristic dendritic features of RGCs physiology during development; to find the cell type-specific rules of dendritic growth. Second, we studied the selective resilience of mouse retinal ganglion cells following optic nerve crush, which results in traumatic damage to axons and leads to the death of 80% of retinal ganglion cells within a period of 2 weeks. We characterized the changes in dendritic morphology that preceded degeneration. As numerous neurodegenerative diseases have been linked to early dendritic defects, we believe that comparing these relationships will provide an important basis for future work on promoting neuronal repair in the damaged mammalian central nervous system and possible treatments for visual neuropathies. Understanding the mechanisms involved in the developmental growth and response to injury may give us insights into how to restore functioning neuronal connectivity after injury.

Pubmed:
34073191: Claes M, Santos JRF, Masin L, Cools L, Davis BM, Arckens L, Farrow K, De Groef L, Moons L
A Fair Assessment of Evaluation Tools for the Murine Microbead Occlusion Model of Glaucoma.
Despite being one of the most studied eye diseases, clinical translation of glaucoma research is hampered, at least in part, by the lack of validated preclinical models and readouts. The most popular experimental glaucoma model is the murine microbead occlusion model, yet the observed mild phenotype, mixed success rate, and weak reproducibility urge for an expansion of available readout tools. For this purpose, we evaluated various measures that reflect early onset glaucomatous changes in the murine microbead occlusion model. Anterior chamber depth measurements and scotopic threshold response recordings were identified as an outstanding set of tools to assess the model's success rate and to chart glaucomatous damage (or neuroprotection in future studies), respectively. Both are easy-to-measure, in vivo tools with a fast acquisition time and high translatability to the clinic and can be used, whenever judged beneficial, in combination with the more conventional measures in present-day glaucoma research (i.e., intraocular pressure measurements and post-mortem histological analyses). Furthermore, we highlighted the use of dendritic arbor analysis as an alternative histological readout for retinal ganglion cell density counts.
Int J Mol Sci, 2021; 22
30767082: Santos JRF, Bauer C, Schuchhardt J, Wedekind D, Waniek K, Lachmann I, Wiltfang J, Vogelgsang J
Validation of a prototype tau Thr231 phosphorylation CSF ELISA as a potential biomarker for Alzheimer's disease.
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the presence of extracellular amyloid plaques (senile plaques) and intracellular neurofibrillary tangles formed by hyperphosphorylated tau protein. This process leads to neuronal degradation and neuronal death. Phosphorylation of tau protein at threonine 231 (p-tau231) has been shown to be characteristic in post-mortem brain tissue of patients with AD and it can be sensitively detected in cerebrospinal fluid (CSF). Therefore, it may serve as a biomarker to support the diagnosis of AD. In this study, we analysed how well p-tau231 could differentiate between patients suffering from dementia either due or not due to AD by a sandwich enzyme immunoassay. CSF p-tau231 was significantly higher in patients with dementia due to AD than in those with dementia due to other causes. In addition, we studied different factors affecting p-tau231 levels in CSF. We found that apolipoprotein E

genotype influences p-tau231 CSF levels. Gender and age did not affect p-tau231 levels in CSF. Our findings indicate that p-tau231 levels in CSF can be a valuable marker for the clinical diagnosis of AD.
J Neural Transm (Vienna), 2019; 126

FENS Forum 2022
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BOARD NUMBER: S01-713

THE INTERPHOTORECEPTOR MATRIX: INVESTIGATING THE ROLE OF IMPG2 IN ZEBRAFISH RETINAL DEVELOPMENT AND FUNCTION

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Retinitis pigmentosa (RP) is one of the most commonly inherited retinal dystrophies, characterized by progressive degeneration of photoreceptors. Recent studies have reported that nonsense mutations in the interphotoreceptor matrix proteoglycan 2 (IMPG2) gene are associated with autosomal recessive RP in humans. This gene encodes the proteoglycan IMPG2 expressed in the interphotoreceptor matrix that surrounds retinal photoreceptor outer segments and ellipsoids. We chose zebrafish to investigate Impg2 function and expression, as its retinal structure is similar to the human macula. Zebrafish has two Impg2 paralogues, Impg2a and Impg2b. Phylogenetic analysis confirmed that teleosts are the only vertebrate group with two paralogues, consistently with the whole genome duplication that occurred in the common teleosts ancestor. Homology modelling of IMPG2 conserved domains in human and in zebrafish, showed structure similarity of the domains in the two species. Moreover, expression analyses revealed that impg2a and impg2b starts to be expressed at 3 days post fertilization and their expression is eye-specific in the adult. Interestingly, we observed that Impg2 localization changes over time. Microinjection of antisense morpholino oligonucleotides, specific for impg2a and impg2b, provided preliminary evidence that Impg2 is involved in eye development. Finally, we are generating a zebrafish line carrying the human IMPG2 mutation, by using CRISPR/Cas9 technology. Preliminary experiments on Impg2a -/- mutant fish showed alteration of the photoreceptor layer with respect to the WT. We planned to better characterize the phenotype of single and double mutants and perform large-scale testing of therapeutic compounds on this new zebrafish model.

Pubmed:
29964155: Boschian C, Messina A, Bozza A, Castellini ME, Provenzano G, Bozzi Y, Casarosa S
Impaired Neuronal Differentiation of Neural Stem Cells Lacking the Engrailed-2 Gene.
The Engrailed-2 (En2) gene codes for a homeobox-containing transcription factor, involved in midbrain-hindbrain embryonic development. In postnatal brain, En2 is expressed in the ventral mesencephalon, cerebellum, hippocampus and neocortex. Two single-nucleotide polymorphisms (SNPs) that are associated to autism spectrum disorders (ASD) have been identified in the human EN2 gene. Accordingly, mice lacking the En2 homeodomain (En2, referred to as En2) show molecular, anatomical and behavioral "ASD-like" features. Among these, we previously showed a partial loss of GABAergic interneurons in the En2 postnatal hippocampus and neocortex, accompanied by a marked decrease of brain-derived neurotrophic factor (BDNF) signaling, a crucial determinant of GABAergic differentiation. In order to better investigate the role of En2 in GABAergic interneuron differentiation, we generated and subsequently differentiated neural stem cells (NSCs) from basal ganglia and neocortex of En2 and En2 mouse embryos. Wild-type NSCs from both basal ganglia and neocortex express En2, while mutant ones do not, as expected. As compared to En2 NSCs, En2 NSCs derived from basal ganglia show impaired GABAergic differentiation accompanied by a reduced expression of the BDNF receptor trkB. Conversely, En2 NSCs derived from the neocortex expressed high levels of trkB and readily differentiated into neurons, as En2 NSCs. Our results suggest that En2 contributes to GABAergic neuron differentiation from basal ganglia NSCs through a trkB-dependent BDNF signaling, thus providing a possible explanation for the reduced number of GABAergic interneurons detected in the En2 postnatal forebrain.
Neuroscience, 2018; 386

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-714

HOW THE USE OF DIM RED-LIGHT DURING PREPARATION AFFECTS ERG RESPONSES OF MICE WITH LONG-WAVELENGTH SHIFTED OPSIN

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Electroretinograms (ERGs) give insight in retinal integrity *in vivo*. For ERGs in mice, visible red light is often used for preparation. Here we show, how ERGs of (LIAIS) mice, expressing human L*-opsin instead of native M-opsin, are affected by the pre-recording use of visible red light, that may lead to L*-opsin activation. Recordings were performed on LIAIS and wildtype (WT) mice. Mice were dark adapted overnight and further handling was done either under dim red light or IR light performed in two separate sessions. Anesthetized animals were dark adapted for 10 min before scotopic flash ERGs with flash strengths increasing from 0.0002 to 6.3 cd.s/m² were recorded. For photopic flash ERGs, animals were adapted for 2 min to 25 cd/m² white background, before flashes between 0.63 to 16 cd.s/m² were presented upon the background. Responses to scotopic flash ERGs using red light were 10-16% smaller in LIAIS compared to WT mice. Use of IR light resulted in larger amplitudes of scotopic a- and b-wave for both genotypes, but the increase was 8-16% larger in LIAIS mice. OPs and implicit times did not depend on the light source used for preparation. For photopic flash ERGs, IR light usage pre-recording increased LIAIS amplitudes by 14%. The use of IR light for mouse handling resulted in larger ERG responses for both genotypes. Even though the use of IR light seems to ensure a more dark-adapted state for the animals, handling is quite challenging and substantially prolonged the total time of the experiment.

Pubmed:

34360929: Lux UT, Ehrenberg J, Joachimsthaler A, Atorf J, Pircher B, Reim K, Kremers J, Gießl A, Brandstätter JH
Cell Types and Synapses Expressing the SNARE Complex Regulating Proteins Complexin 1 and Complexin 2 in Mammalian Retina.
Complexins (Cplxs) 1 to 4 are components of the presynaptic compartment of chemical synapses where they regulate important steps in synaptic vesicle exocytosis. In the retina, all four Cplxs are present, and while we know a lot about Cplxs 3 and 4, little is known about Cplxs 1 and 2. Here, we performed in situ hybridization experiments and bioinformatics and exploited Cplx 1 and Cplx 2 single-knockout mice combined with immunocytochemistry and light microscopy to characterize in detail the cell type and synapse-specific distribution of Cplx 1 and Cplx 2. We found that Cplx 2 and not Cplx 1 is the main isoform expressed in normal and displaced amacrine cells and ganglion cells in mouse retinae and that amacrine cells seem to operate with a single Cplx isoform at their conventional chemical synapses. Surprising was the finding that retinal function, determined with electroretinographic recordings, was altered in Cplx 1 but not Cplx 2 single-knockout mice. In summary, the results provide an important basis for future studies on the function of Cplxs 1 and 2 in the processing of visual signals in the mammalian retina.
Int J Mol Sci, 2021; 22
33811381: Ryl M, Urbasik A, Gierke K, Babai N, Joachimsthaler A, Feigenspan A, Frischknecht R, Stallwitz N, Fejtová A, Kremers J, von Wittgenstein J, Brandstätter JH
Genetic disruption of bassoon in two mutant mouse lines causes divergent retinal phenotypes.
Bassoon (BSN) is a presynaptic cytomatrix protein ubiquitously present at chemical synapses of the central nervous system, where it regulates synaptic vesicle replenishment and organizes voltage-gated Ca channels. In sensory photoreceptor synapses, BSN additionally plays a decisive role in anchoring the synaptic ribbon, a presynaptic organelle and functional extension of the active zone, to the presynaptic membrane. In this study, we functionally and structurally analyzed two mutant mouse lines with a genetic disruption of Bsn-Bsn and Bsn -using electrophysiology and high-resolution microscopy. In both Bsn mutant mouse lines, full-length BSN was abolished, and photoreceptor synaptic function was similarly impaired, yet synapse structure was more severely affected in Bsn than in Bsn photoreceptors. The synaptic defects in Bsn retina coincide with remodeling of the outer retina-rod bipolar and horizontal cell sprouting, formation of ectopic ribbon synaptic sites-and death of cone photoreceptors, processes that did not occur in Bsn retina. An analysis of Bsn hybrid mice revealed that the divergent retinal phenotypes of Bsn and Bsn mice can be attributed to the expression of the Bsn allele, which triggers cone

photoreceptor death and neurite sprouting in the outer retina. These findings shed new light on the existing Bsn mutant mouse models and might help to understand mechanisms that drive photoreceptor death.
FASEB J, 2021; 35
33556541: Barboni MTS, Liber AMP, Joachimsthaler A, Saoudi A, Goyenvalle A, Rendon A, Roger JE, Ventura DF, Kremers J, Vaillend C
Altered visual processing in the mdx52 mouse model of Duchenne muscular dystrophy.
The mdx52 mouse model of Duchenne muscular dystrophy (DMD) is lacking exon 52 of the DMD gene that is located in a hotspot mutation region causing cognitive deficits and retinal anomalies in DMD patients. This deletion leads to the loss of the dystrophin proteins, Dp427, Dp260 and Dp140, while Dp71 is preserved. The flash electroretinogram (ERG) in mdx52 mice was previously characterized by delayed dark-adapted b-waves. A detailed description of functional ERG changes and visual performances in mdx52 mice is, however, lacking. Here an extensive full-field ERG repertoire was applied in mdx52 mice and WT littermates to analyze retinal physiology in scotopic, mesopic and photopic conditions in response to flash, sawtooth and/or sinusoidal stimuli. Behavioral contrast sensitivity was assessed using quantitative optomotor response (OMR) to sinusoidally modulated luminance gratings at 100% or 50% contrast. The mdx52 mice exhibited reduced amplitudes and delayed implicit times in dark-adapted ERG flash responses, particularly in their b-wave and oscillatory potentials, and diminished amplitudes of light-adapted flash ERGs. ERG responses to sawtooth stimuli were also diminished and delayed for both mesopic and photopic conditions in mdx52 mice and the first harmonic amplitudes to photopic sine-wave stimuli were smaller at all temporal frequencies. OMR indices were comparable between genotypes at 100% contrast but significantly reduced in mdx52 mice at 50% contrast. The complex ERG alterations and disturbed contrast vision in mdx52 mice include features observed in DMD patients and suggest altered photoreceptor-to-bipolar cell transmission possibly affecting contrast sensitivity. The mdx52 mouse is a relevant model to appraise the roles of retinal dystrophins and for preclinical studies related to DMD.
Neurobiol Dis, 2021; 152
33105896: Gierke K, von Wittgenstein J, Hemmerlein M, Atorf J, Joachimsthaler A, Kremers J, Cooper BH, Varoqueaux F, Regus-Leidig H, Brandstätter JH
Heterogeneous Presynaptic Distribution of Munc13 Isoforms at Retinal Synapses and Identification of an Unconventional Bipolar Cell Type with Dual Expression of Munc13 Isoforms: A Study Using Munc13-EXFP Knock-in Mice.
Munc13 isoforms are constituents of the presynaptic compartment of chemical synapses, where they govern important steps in preparing synaptic vesicles for exocytosis. The role of Munc13-1, -2 and -3 is well documented in brain neurons, but less is known about their function and distribution among the neurons of the retina and their conventional and ribbon-type chemical synapses. Here, we examined the retinae of Munc13-1-, -2-, and -3-EXFP knock-in (KI) mice with a combination of immunocytochemistry, physiology, and electron microscopy. We show that knock-in of Munc13-EXFP fusion proteins did not affect overall retinal anatomy or synapse structure, but slightly affected synaptic transmission. By labeling Munc13-EXFP KI retinae with specific antibodies against Munc13-1, -2 and -3, we found that unlike in the brain, most retinal synapses seem to operate with a single Munc13 isoform. A surprising exception to this rule was type 6 ON bipolar cells, which expressed two Munc13 isoforms in their synaptic terminals, ubMunc13-2 and Munc13-3. The results of this study provide an important basis for future studies on the contribution of Munc13 isoforms in visual signal processing in the mammalian retina.
Int J Mol Sci, 2020; 21
32049345: Barboni MTS, Vaillend C, Joachimsthaler A, Liber AMP, Khabou H, Roux MJ, Vacca O, Vignaud L, Dalkara D, Guillonneau X, Ventura DF, Rendon A, Kremers J
Rescue of Defective Electroretinographic Responses in Dp71-Null Mice With AAV-Mediated Reexpression of Dp71.
To study the potential effect of a gene therapy, designed to rescue the expression of dystrophin Dp71 in the retinas of Dp71-null mice, on retinal physiology.
Invest Ophthalmol Vis Sci, 2020; 61
31614616: Falk N, Joachimsthaler A, Kessler K, Lux UT, Noegel AA, Kremers J, Brandstätter JH, Gießl A, Falk N, Joachimsthaler A, Kessler K, Lux UT, Noegel AA, Kremers J, Brandstätter JH, Gießl A
Lack of a Retinal Phenotype in a Syne-2/Nesprin-2 Knockout Mouse Model.
Syne-2 (also known as Nesprin-2) is a member of a family of proteins that are found primarily in the outer nuclear membrane, as well as other subcellular compartments. Syne-2 contains a C-terminal KASH transmembrane domain and is part of a protein network that associates the nuclear envelope to the cytoskeleton via the binding to actin filaments. Syne-2 plays a role in nuclear migration, nuclear positioning during retinal development, and in ciliogenesis. In a previous study, we showed a connection between Syne-2 and the multifunctional scaffold protein Pericentrin (Pcnt). The elimination of the interaction of Syne-2 and Pcnt showed defects in nuclear migration and the formation of outer segments during retinal development, as well as disturbances in centrosomal migration at the beginning of ciliogenesis in general. In this study, the Syne-2 KO mouse model Nesprin-2△ABD (Syne-2, MGI) with special attention to Pcnt and ciliogenesis was analyzed. We show reduced

expression of Syne-2 in the retina of the Syne-2 KO mouse but found no significant structural-and only a minor functional-phenotype. For the first time, detailed expression analyses showed an expression of a Syne-2 protein larger than 400 kDa (~750 kDa) in the Syne2/Nesprin-2 KO mouse. In conclusion, the lack of an overt phenotype in Syne-2/Nesprin-2 KO mice suggests the usage of alternative translational start sites, producing Syne-2 splice variants with an intact Pcnt interaction site. Nevertheless, deletion of the actin-binding site in the Syne-2/Nesprin-2 KO mouse revealed a high variability in scotopic oscillatory potentials assuming a novel function of Syne-2 in synchronizing inner retinal processes.
Cells, 2019; 8
31100107: Joachimsthaler A, Kremers J
Mouse Cones Adapt Fast, Rods Slowly In Vivo.
To study rod- and cone-driven adaptation dynamics separately, we used the silent substitution technique to selectively stimulate rods or cones in the Opn1lwLIAIS (LIAIS) mouse, in which the native M-cone pigment is replaced by a human L-cone pigment (L*).
Invest Ophthalmol Vis Sci, 2019; 60
30696732: Müller TM, Gierke K, Joachimsthaler A, Sticht H, Izsvák Z, Hamra FK, Fejtová A, Ackermann F, Garner CC, Kremers J, Brandstätter JH, Regus-Leidig H
A Multiple Piccolino-RIBEYE Interaction Supports Plate-Shaped Synaptic Ribbons in Retinal Neurons.
Active zones at chemical synapses are highly specialized sites for the regulated release of neurotransmitters. Despite a high degree of active zone protein conservation in vertebrates, every type of chemical synapse expresses a given set of protein isoforms and splice variants adapted to the demands on neurotransmitter release. So far, we know little about how specific active zone proteins contribute to the structural and functional diversity of active zones. In this study, we explored the nanodomain organization of ribbon-type active zones by addressing the significance of Piccolino, the ribbon synapse-specific splice variant of Piccolo, for shaping the ribbon structure. We followed up on previous results, which indicated that rod photoreceptor synaptic ribbons lose their structural integrity in a knockdown of Piccolino. Here, we demonstrate an interaction between Piccolino and the major ribbon component RIBEYE that supports plate-shaped synaptic ribbons in retinal neurons. In a detailed ultrastructural analysis of three different types of retinal ribbon synapses in Piccolo/Piccolino-deficient male and female rats, we show that the absence of Piccolino destabilizes the superstructure of plate-shaped synaptic ribbons, although with variable manifestation in the cell types examined. Our analysis illustrates how the expression of a specific active zone protein splice variant (e.g., Piccolino) contributes to structural diversity of vertebrate active zones. Retinal ribbon synapses are a specialized type of chemical synapse adapted for the regulated fast and tonic release of neurotransmitter. The hallmark of retinal ribbon synapses is the plate-shaped synaptic ribbon, which extends from the release site into the terminals' cytoplasm and tethers hundreds of synaptic vesicles. Here, we show that Piccolino, the synaptic ribbon specific splice variant of Piccolo, interacts with RIBEYE, the main component of synaptic ribbons. This interaction occurs via several PxDLS-like motifs located at the C terminus of Piccolino, which can connect multiple RIBEYE molecules. Loss of Piccolino disrupts the characteristic plate-shaped structure of synaptic ribbons, indicating a role of Piccolino in synaptic ribbon assembly.
J Neurosci, 2019; 39
29049717: Tsai TI, Joachimsthaler A, Kremers J
Mesopic and Photopic Rod and Cone Photoreceptor-Driven Visual Processes in Mice With Long-Wavelength-Shifted Cone Pigments.
The clearer divergence in spectral sensitivity between native rod and human L-cone (L*-cone) opsins in the transgenic Opn1lwLIAIS mouse (LIAIS) allows normal visual processes mediated by these photoreceptor subtypes to be isolated effectively using the silent substitution technique. The objective of this study was to further characterize the influence of mean luminance and temporal frequency on the functional properties of signals originating in each photoreceptor separately and independently of adaptation state in LIAIS mice.
Invest Ophthalmol Vis Sci, 2017; 58
31740648: Joachimsthaler A, Tsai TI, Kremers J
Electrophysiological Studies on The Dynamics of Luminance Adaptation in the Mouse Retina.
To date, most studies involving in vivo electroretinography in mice are performed on steady state adapted animals. In this study, we focused on the dynamics of adaptation to high and low light levels in the mouse retina. Two flash electroretinogram (ERG) protocols and one flicker ERG protocol were employed. In the two flash ERG protocols, the animals were adapted to either 25 or 40 cd/m white light and ERGs were recorded for up to 15 min of adaptation. Afterwards, flash ERGs were recorded for up to 45 min of dark adaptation. Amplitudes of the flash ERG increased during light adaptation, while implicit times of the different wave components decreased. During subsequent dark adaptation, the amplitudes further increased. The increase in a-to-b-wave ratio indicated adaptational processes at the photoreceptor synapse. In the flicker ERG protocol, the responses to 12 Hz sinusoidal luminance modulation during the adaptation to 25 cd/m and a 1 cd/m mean luminances were recorded. The amplitudes of the first harmonic components in the flicker protocol decreased during light adaptation but

increased during dark adaptation. This is at odds with the changes in the flash ERG, indicating that adaptation may be different in different retinal pathways.
Vision (Basel), 2017; 1

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BOARD NUMBER: S01-715

GENERATION OF THREE DISTINCT INDUCIBLE NON-HUMAN PRIMATE MODELS OF OUTER RETINAL DEGENERATION

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Divya Ail[1], Diane Nava[1], In Pyo Hwang[1], Amin Benadjal[2], Elena Brazhnikova[3], Céline Nouvel-Jaillard[3], Corentin Joffrois[4], Lionel Rousseau[5], Julie Dégardin[3], Stephane Bertin[6], Olivier Goureau[7], Serge Picaud[4], Deniz Dalkara[1]
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Aim: The differences between the retinas of humans and most animal models poses a challenge for testing novel therapies. Non-human primate (NHP) retina is physiologically and anatomically closest to human retina. However, there is a lack of retinal degeneration NHP models for preclinical studies of vision restoration strategies. To address this unmet need we aimed to generate inducible NHP models of retinal degeneration. Methods: We generated cynomolgus macaque models – 1. by optogenetic ablation of rods; 2. by using a Crispr-Cas9 system disrupting the rhodopsin gene and 3. by inducing physical separation between the photoreceptors and Retinal Pigment Epithelium (RPE) using a polymer patch. In-vivo degeneration was evaluated by fundus imaging, optical coherence tomography (OCT), adaptive optics (AO) and electroretinography (ERG). The retinal tissues were fixed and immunolabelled after sacrifice. Results: Proof-of-concept experiments were first conducted in rodents to establish doses, light levels and adequate polymer materials. In the 'optogenetically-induced' and 'Crispr model' we observed punctuate areas of degeneration in the injected areas marked by disorganization of outer segments, loss of rod photoreceptors and thinning of the outer nuclear layer (ONL). In the 'physical model' the degeneration was faster, more severe involving both rods and cones but stayed restricted to the area of the patch. In all three models, there was a general trend in the reduction of ERG. Conclusion: We have generated three distinct non-human primate models with different features adequate for testing various gene and cell therapies.

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BOARD NUMBER: S01-716

MORPHOMETRIC ANALYSIS OF HORIZONTAL CELLS IN THE MOUSE RETINA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Alejandra Acevedo Harnecker[1], Matteo Spinelli[2], Ulrike Janssen-Bienhold[2], Christian Puller[2], Karin Dedek[1]
[1]Carl von Ossietzky University, Neurosensorics/animal Navigation, Institute For Biology And Environmental Sciences, Oldenburg, Germany, [2]Carl von Ossietzky University, Visual Neuroscience, Department Of Neuroscience, Oldenburg, Germany

Aim: Mice have dichromatic vision with enhanced sensitivity for green light in the dorsal retina, often facing the ground, and for UV light in the ventral retina, often facing the sky. This asymmetry is also reflected in the spectral composition of the mouse habitat (Qiu et al., Curr Biol, 2021, 31:3233-3247.e6.) and the distribution of S-opsin (short-wavelength-sensitive) and M-opsin (medium-wavelength-sensitive) along the dorso-ventral axis of the retina. Horizontal cells (HCs) in the mouse retina are postsynaptic to S- and M-opsin-expressing photoreceptors and send feedback to them. Here, we investigated whether individual HCs also show non-uniform properties (e.g., morphologies, synaptic contacts) across the retina. **Methods:** Intracellular dye injections of HCs in living retinal whole-mounts were followed by fixation of the tissue and immunohistochemical staining of synaptic proteins, keeping track of the retinal orientation. We reconstructed >20 HCs from different retinal regions and analyzed morphometric parameters, such as the length and branching order of dendrites. We also compared the number of contacts with photoreceptors and the presence of electrical synapses across the different retinal axes. **Results:** In general, HCs located close to the optic nerve had smaller dendritic fields than peripheral HCs. However, dorsal HCs were larger than ventral HCs of the same eccentricity. In addition, preliminary data shows that also the distribution of distinct HCs synaptic proteins differed between cells in dorsal and ventral retina. **Conclusions**: Further quantifications are needed to see whether differences in the mouse visual field are indeed reflected in the morphology and connectivity of mouse horizontal cells.

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BOARD NUMBER: S01-717

A MULTI-METHODOLOGICAL APPROACH TO STUDY HORIZONTAL CELLS IN THE VERTEBRATE RETINA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Bianca Pircher, Andreas Feigenspan
Friedrich-Alexander-University Erlangen-Nürnberg, Biology, Erlangen, Germany

Ever since the beginning of the study of the retina, horizontal cells (HCs) occupied a truly exceptional role. Research undertaken so far has HCs assigned to the tasks of modulating information flow from photoreceptors to bipolar cells as they provide negative feedback and feedforward signals to both cell types, respectively. However, there have been unclear and sometimes even contradictory results regarding feedback mechanisms and elementary functionality of HCs. Using electrophysiology, bioinformatics and immunocytochemistry we approached the signal generating properties of HCs. Therefore, we studied the expression and distribution of ionotropic glutamate receptors (iGluRs) in HCs and their respective subtypes in various vertebrate species.
First, we could show expression of AMPA, but not kainate receptors and found remarkable differences between mammalian and a non-mammalian species in their iGluR expression patterns. Additionally, we were able to reveal true indices for a "division of labor" among HC subtypes and even more noteworthy between individual HCs of the same subtype. Single cells showing different compositions of iGluR expression, which taken together shaping a characteristic composition of iGluRs in HC subtypes.
Second, we were investigating the role of calcium channels and calcium-based action potentials. Patch-clamp experiments revealed a detailed description of the biophysical properties of HCs, which in particular emphasizes the role of $Ca_V3.2$ for signal processing in the mouse retina.
Our results allow us to refine the picture of information processing and the functionality of HCs in the vertebrate retina.

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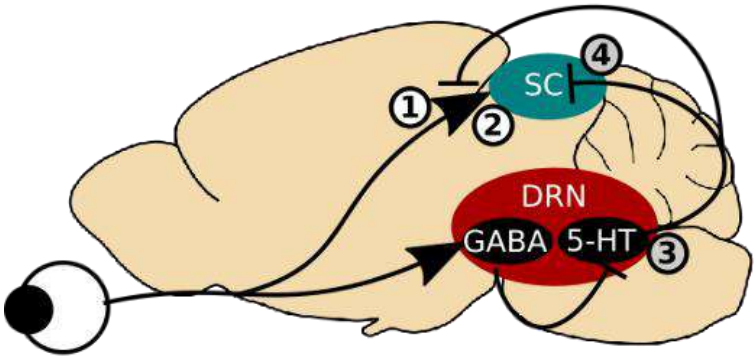
BOARD NUMBER: S01-718

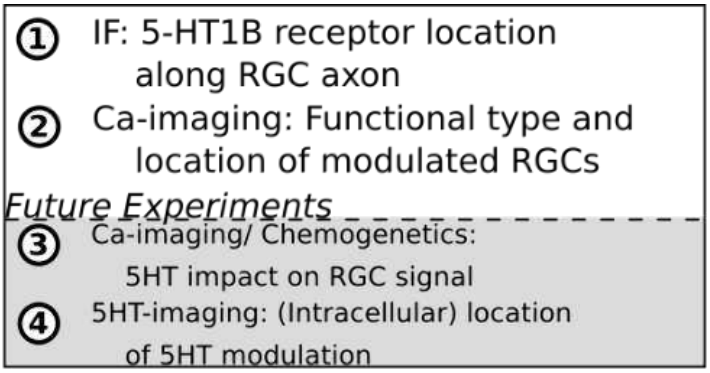
IMPACT AND SUBCELLULAR LOCATION OF SEROTONERGIC MODULATION ON RETINAL GANGLION CELL SIGNALLING

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Tristan Wiessalla
EMBL Rome, Neuroscience, Monterotondo, Italy

A recent study has described collateral projections from the retina to both the superior colliculus (SC) and the dorsal raphe nucleus (DRN). Earlier studies have shown that serotonergic DRN neurons in turn send projections to the superficial layers of SC. DRN responds strongly to expanding dark disks (looming stimulus, LS). LS causes freezing behavior in the absence of a shelter and flight response in its presence. However, the impact of the serotonergic modulation on the processing of visual stimuli remains unknown. We are studying the types of retinal ganglion cells (RGCs) impacted by DRN_{5HT} projections and the exact intracellular location of the modulation. We have performed functional and spatial characterization of RGC response modulation using *in-vivo* two-photon calcium-imaging. Further details on the location of the modulation were gained by immunofluorescence (IF) analysis of the $5\text{-}HT_{1B}$ receptor location along the RGC axon. However, 5-HT is known to be released not only as a response to LS, but rather playing a role as a neurotransmitter in a wide range of stimulus responses, behaviors and overall brain states. This highlights a potential impact of non-visual brain activity on RGC activity in the SC. Future experiments will investigate the modulation of retinal signal by stimulus independent serotonergic activity.





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BOARD NUMBER: S01-719

ESTABLISHMENT AND MEASUREMENT OF MYOPIA IN MICE WITH ON-BIPOLAR CELL DEFECTS

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Baptiste Wilmet[1], Robert Duvoisin[2], Jacques Callebert[3], Ruben Goulet[1], Christophe Tourain[4], Christelle Michiels[1], Julie Dégardin[1], Helen Frederiksen[1], Quenol Cesar[1], Manuel Simonutti[1], Olivier Marre[1], José-Alain Sahel[1,5,6], Isabelle Audo[1,5], Serge Picaud[1], Christina Zeitz[1]
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Aim: Myopia is the most common ocular dysfunction with an increasing prevalence world-wide. Complete congenital stationary night blindness (cCSNB) is a group of genetically and clinically heterogeneous retinal disorders caused by dysfunction of the retinal ON-bipolar cell (ON-BC) pathway. It is mainly characterized by impaired dim light vision but is also associated with high myopia. Here, we established a protocol to induce and measure myopia in cCSNB mouse models. **Method:** To assess refractive development and induced myopia, we evaluated the levels of retinal Dopamine (DOPA) and its metabolite 3,4-Dihydroxyphenylacetic acid (DOPAC), a commonly used marker of myopia, in three cCSNB mouse models lacking *Grm6, Gpr179* or *Lrit3*. We developed a lens-induced myopia (LIM) model and measured the refractive error using an infrared photorefractometer with and without myopia induction. **Results:** Our preliminary data revealed decreased levels of retinal DOPA and DOPAC in all tested cCSNB mouse models. WT Mice with LIM showed a myopic shift of ≈-10D after 21 days of induction. Therefore, LIM will be induced in cCSNB mouse models, myopic shift will be measured and compared to the respective wild-type mice to document the refractive errors induced by the ON-BC defect. **Conclusion:** These data suggest that cCSNB mouse models can mimic the human phenotype and ON-BC dysfunction can regulate the onset of myopia. These findings may help understand the development of high myopia in cCNSB patients, and myopia generally, as well as provide the basis for the development of pharmacological and optical therapies.

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BOARD NUMBER: S01-720

AN ADVANCED NANOZYME TO PREVENT ROS-INDUCED NEURODEGENERATION OF THE RETINA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Sara Cupini[1,2], Alessio Cavalli[1,2], Stefano Di Marco[2,3], Elisabetta Colombo[2,3], Luca Boselli[4], Valentina Mastronardi[4], Valentina Castagnola[2], Pier Paolo Pompa[5], Fabio Benfenati[2,3]
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Reactive oxygen species (ROS) are generated by light stimuli in the retina. Although the retina possesses strategies to cope with oxidative stress, genetic defects and environmental factors can interfere with these mechanisms, leading to Age-related Macular Degeneration (AMD). Moreover, high levels of ROS accelerate the progression of inherited photoreceptor degenerations like Retinitis Pigmentosa (RP). Platinum nanoparticles (PtNPs) can mimic the effects of antioxidant enzymes, such as catalase and superoxide dismutase, exhibiting high catalytic activity and acting as ROS scavengers in the biological environment. Here we show that PtNP intravitreal injection is an efficient therapeutic tool for lowering ROS in a light-damaged albino rat model (LD) mimicking AMD, and Royal College of Surgeons (RCS) rat, model of RP. LD was obtained by exposing the animals to intense light for 24 hours. Two days later, rats were intravitreally injected with PtNPs or albumin as a control. After fifteen days, electroretinogram recordings were performed to evaluate visual function. RCS rats were injected at early stage of degeneration. After one month, Pupillary Light Reflex recordings were performed, complemented by *ex-vivo* high-density multielectrode array electrophysiology of light-evoked ganglion cells. Both animal models were subjected the light/dark box test to evaluate visually driven behaviour. Finally, retinal morphology was evaluated using *in vivo* optical coherence tomography and immunofluorescence. PtNPs efficiently mitigate neurodegeneration induced by exposure to high-intensity light and gene mutations. The treatment preserves visual function, photoreceptor number and decreases the degeneration-induced inflammation. Behavioural tests confirm the amelioration of the degeneration in PtNPs-injected animals respect to controls.

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BOARD NUMBER: S01-721

THE TRANSITION OF PHOTORECEPTOR GUANYLATE CYCLASE TYPE 1 TO THE ACTIVE STATE

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Manisha Kumari Shahu[1], Fabian Schuhmann[2], Alexander Scholten[1], Ilia Solov'Yov[2], Karl-Wilhelm Koch[1]
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Introduction: Membrane-bound guanylate cyclases (GCs), which synthesize the second messenger guanosine-3',5'-cyclic monophosphate, differ in their activation modes to reach the active state. Hormone receptor type guanylate cyclases, for example undergo an 'α'-helix rotation upon encountering with peptide for activation, but the mechanism of calcium-regulated photoreceptor GCs is unclear. **Aim:** We aim to unravel the conformational switch of photoreceptor guanylate cyclase type 1, GC-E to the active state and compare it with a constitutionally active state, which is found in patients suffering from retinal cone-rod dystrophies. **Methods:** We simulated experimentally an 'α'-helix rotation by integration of alanine residues close to the transmembrane region by site directed mutagenesis and performed functional studies. We further investigated the enzymatic catalytic parameters of wildtype and the retinal disease-related mutant V902L of GC-E, and characterized the protein dynamics of a conformational switch by a computational approach based on molecular dynamics simulations. **Results:** Our data shows no involvement of an 'α'-helix rotation indicating a difference to hormone receptor GCs. The constitutively active state of the V902L mutant is therefore like the active state of wildtype GC-E that is reached by interacting with calcium-sensors. We detected a swinging movement of the dimerization domain in the V902L mutant as the critical conformational switch in the cyclase going from the low-to-high activity state. **Conclusion:** Our results show a principal difference in the activation modus between hormone receptor GCs and sensory GCs, which is relevant for understanding the molecular basis of retinal diseases.

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BOARD NUMBER: S01-722

IN VIVO BIOCOMPATIBILITY AND FUNCTIONALITY OF POROUS-GRAPHENE-BASED SUBRETINAL IMPLANTS FOR VISION RESTORATION.

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Julie Zhang[1], Vi Anh Nguyen[1], Julie Dégardin[1], Ruben Goulet[1], Quenol Cesar[1], Steven Walston[2], José Antonio Garrido[2], Fabrice Arcizet[1], Grégory Gauvain[1], Serge Picaud[1]
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Novel materials are sought to improve the unsatisfactory spatial resolution achieved by past or current retinal prostheses for vision restoration. The electrochemical properties of reduced graphene oxide (rGO) micro-electrodes (around 30 kΩ impedance at 1 kHz and up to 8 mC/cm² of CIC) are encouraging enough for efficient electric stimulation of the retinal neurons. We inserted porous graphene-based implants in the subretinal space of rodents (N=6) and investigated first their *in vivo* biocompatibility using post-hoc immunolabelling (DAPI, Iba1, GFAP and G0α). We performed chronic *in vivo* impedance spectroscopy and cyclic voltammetry tests for evaluation of rGO electrodes long-term electrochemical performance. Using functional ultrasound (fUS) imaging, we measured the neural activation in the visual cortex in response to monopolar stimulation of the rGO electrodes acutely and chronically implanted in the retina. We first noticed on the confocal images of the implanted retinas that there was no major inflammation above graphene electrodes compared to platinum electrodes (N=4) or sham polyimide device (N=5). Microglia cells numbering confirmed these results. Our preliminary results for the implant functionality indicate an increase of the impedance during the first week post-implantation with a stabilization occurring around one month, in concordance with literature. First results with fUS imaging show that the spatial resolution of neural activation in the cortex varies with increasing electrode size and frequency of stimulation. We also investigated the influence of pulse duration. Together, all these preliminary results provide great confidence in the graphene technology for the elaboration of the future generation of visual prostheses.

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UNDER THE SEE: DISCOVERING VISUAL CIRCUITS OF A PYGMY SQUID USING CONNECTOMICS

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Cephalopods present one of the most complex invertebrate nervous systems. Their large, centralised brains support idiosyncratic behaviours such as camouflage and mimicry, and present higher-order abilities such as learning from observing others and tool manipulation. While widely divergent from vertebrates, cephalopods independently evolved common characteristics. A salient example is their camera-like eye, similar in anatomy, optics and function to the vertebrate eye and an evidence of convergent evolution. We aim at mapping the cephalopod's visual circuits to identify any similarities with those of vertebrates and insects, and therefore, given their independent evolutionary history, to potentially find optimal neural circuits for visual processing.
To this end, we imaged a volume containing an eye and optic lobe of an *Idiosepius hallami* pygmy squid free-living hatchling using a customised FIB-SEM (Focused Ion Beam Scanning Electron Microscope), at a resolution sufficient to resolve all neuronal arbours and synapses. Preliminary data from the reconstruction of photoreceptors and their axonal projections to the optic neuropil show an intriguing organisation. Basal photoreceptor dendrites within the retina contact spatially adjacent photoreceptors. Photoreceptor axons present, as they emerge from the retina, a long mitochondria-rich proximal segment of large calibre that subsequently subdivides into numerous thinner (~200 nm diameter) parallel axons that run the remainder of the optic nerve prior to entering the optic lobe. The reconstruction of the postsynaptic partners of photoreceptor axons within the optic lobe will enable the comparison with circuits of the vertebrate retina and the insect optic lobes.

Pubmed:
34837731: Barsotti E, Correia A, Cardona A
Neural architectures in the light of comparative connectomics.
Since the Cambrian, animals diversified from a few body forms or bauplans, into many extinct and all extant species. A characteristic neural architecture serves each bauplan. How the connectome of each animal differs from that of closely related species or whether it converged into an optimal architecture shared with more distant ones is unknown. Recent technological innovations in molecular biology, microscopy, digital data storage and processing, and computational neuroscience have lowered the barriers for whole-brain connectomics. Comparative connectomics of suitable, relatively small, representative species across the phylogenetic tree can infer the archetypal neural architecture of each bauplan and identify any circuits that possibly converged onto a shared and potentially optimal, structure.
Curr Opin Neurobiol, 2021; 71
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Development of the electric organ in embryos and larvae of the knifefish, Brachyhypopomus gauderio.
South American Gymnotiform knifefish possess electric organs that generate electric fields for electro-location and electro-communication. Electric organs in fish can be derived from either myogenic cells (myogenic electric organ/mEO) or neurogenic cells (neurogenic electric organ/nEO). To date, the embryonic development of EOs has remained obscure. Here we characterize the development of the mEO in the Gymnotiform bluntnose knifefish, Brachyhypopomus gauderio. We find that EO primordial cells arise during embryonic stages in the ventral edge of the tail myotome, translocate into the ventral fin and develop into syncytial electrocytes at early larval stages. We also describe a pair of thick nerve cords that flank the dorsal aorta, the location and characteristic morphology of which are reminiscent of the nEO in Apteronotid species, suggesting a common evolutionary origin of these tissues. Taken together, our findings reveal the embryonic origins of the mEO and provide a basis for elucidating the mechanisms of evolutionary diversification of electric charge generation by myogenic and neurogenic EOs.
Dev Biol, 2020; 466
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C, Perry A, Wood ME, Hetheridge MJ, Currie RA, Tyler CR
Cardiovascular Effects and Molecular Mechanisms of Bisphenol A and Its Metabolite MBP in Zebrafish.
The plastic monomer bisphenol A (BPA) is one of the highest production volume chemicals in the world and is frequently detected in wildlife and humans, particularly children. BPA has been associated with numerous adverse health outcomes relating to its estrogenic and other hormonal properties, but direct causal links are unclear in humans and animal models. Here we simulated measured (1×) and predicted worst-case (10×) maximum fetal exposures for BPA, or equivalent concentrations of its metabolite MBP, using fluorescent reporter embryo-larval zebrafish, capable of quantifying Estrogen Response Element (ERE) activation throughout the body. Heart valves were primary sites for ERE activation by BPA and MBP, and transcriptomic analysis of microdissected heart tissues showed that both chemicals targeted several molecular pathways constituting biomarkers for calcific aortic valve disease (CAVD), including extra-cellular matrix (ECM) alteration. ECM collagen deficiency and impact on heart valve structural integrity were confirmed by histopathology for high-level MBP exposure, and structural defects (abnormal curvature) of the atrio-ventricular valves corresponded with impaired cardiovascular function (reduced ventricular beat rate and blood flow). Our results are the first to demonstrate plausible mechanistic links between ERE activation in the heart valves by BPA's reactive metabolite MBP and the development of valvular-cardiovascular disease states.
Environ Sci Technol, 2019; 53
29996700: Noventa S, Hacker C, Correia A, Drago C, Galloway T
Gold nanoparticles ingested by oyster larvae are internalized by cells through an alimentary endocytic pathway.
The biological fate of nanoparticles (NPs) taken up by organisms from their environment is a crucial issue for assessing ecological hazard. Despite its importance, it has scarcely been addressed due to the technical difficulties of doing so in whole organism studies. Here, by using transmission electron microscopy and energy dispersive X-ray spectroscopy (TEM-EDS), we describe the key aspects that characterize the interaction between an aquatic organism of global ecological and economic importance, the early larval stage of the Japanese oyster (), and model gold NPs dispersed in their environment. The small size of the model organism allowed for a high-throughput visualization of the subcellular distribution of NPs, providing a comprehensive and robust picture of the route of uptake, mechanism of cellular permeation, and the pathways of clearance counterbalancing bioaccumulation. We show that NPs are ingested by larvae and penetrate cells through alimentary pinocytic/phagocytic mechanisms. They undergo intracellular digestion and storage inside residual bodies, before excretion with feces or translocation to phagocytic coelomocytes of the visceral cavity for potential extrusion or further translocation. Our mechanistically-supported findings highlight the potential of oyster larvae and other organisms which feature intracellular digestion processes to be exposed to man-made NPs and thus any risks associated with their inherent toxicity.
Nanotoxicology, 2018; 12
29567712: Sakulkoo W, Osés-Ruiz M, Oliveira Garcia E, Soanes DM, Littlejohn GR, Hacker C, Correia A, Valent B, Talbot NJ
A single fungal MAP kinase controls plant cell-to-cell invasion by the rice blast fungus.
Blast disease destroys up to 30% of the rice crop annually and threatens global food security. The blast fungus invades plant tissue with hyphae that proliferate and grow from cell to cell, often through pit fields, where plasmodesmata cluster. We showed that chemical genetic inhibition of a single fungal mitogen-activated protein (MAP) kinase, Pmk1, prevents from infecting adjacent plant cells, leaving the fungus trapped within a single plant cell. Pmk1 regulates expression of secreted fungal effector proteins implicated in suppression of host immune defenses, preventing reactive oxygen species generation and excessive callose deposition at plasmodesmata. Furthermore, Pmk1 controls the hyphal constriction required for fungal growth from one rice cell to the neighboring cell, enabling host tissue colonization and blast disease.
Science, 2018; 359
28932931: Gorgulho R, Jacinto R, Lopes SS, Pereira SA, Tranfield EM, Martins GG, Gualda EJ, Derks RJE, Correia AC, Steenvoorden E, Pintado P, Mayboroda OA, Monteiro EC, Morello J
Usefulness of zebrafish larvae to evaluate drug-induced functional and morphological renal tubular alterations.
Prediction and management of drug-induced renal injury (DIRI) rely on the knowledge of the mechanisms of drug insult and on the availability of appropriate animal models to explore it. Zebrafish (Danio rerio) offers unique advantages for assessing DIRI because the larval pronephric kidney has a high homology with its human counterpart and it is fully mature at 3.5 days post-fertilization. Herein, we aimed to evaluate the usefulness of zebrafish larvae as a model of renal tubular toxicity through a comprehensive analysis of the renal alterations induced by the lethal concentrations for 10% of the larvae for gentamicin, paracetamol and tenofovir. We evaluated drug metabolic profile by mass spectrometry, renal function with the inulin clearance assay, the 3D morphology of the proximal convoluted tubule by two-photon microscopy and the ultrastructure of proximal convoluted tubule mitochondria by transmission electron microscopy. Paracetamol was metabolized by conjugation and oxidation with further detoxification with glutathione. Renal clearance was reduced with gentamicin and paracetamol. Proximal tubules were enlarged with paracetamol and tenofovir. All drugs induced mitochondrial alterations including

dysmorphic shapes ("donuts", "pancakes" and "rods"), mitochondrial swelling, cristae disruption and/or loss of matrix granules. These results are in agreement with the tubular effects of gentamicin, paracetamol and tenofovir in man and demonstrate that zebrafish larvae might be a good model to assess functional and structural damage associated with DIRI.
Arch Toxicol, 2018; 92

28671740: Steinberg G, Schuster M, Hacker C, Kilaru S, Correia A
ATP prevents Woronin bodies from sealing septal pores in unwounded cells of the fungus Zymoseptoria tritici.
Septa of filamentous ascomycetes are perforated by septal pores that allow communication between individual hyphal compartments. Upon injury, septal pores are plugged rapidly by Woronin bodies (WBs), thereby preventing extensive cytoplasmic bleeding. The mechanism by which WBs translocate into the pore is not known, but it has been suggested that wound-induced cytoplasmic bleeding "flushes" WBs into the septal opening. Alternatively, contraction of septum-associated tethering proteins may pull WBs into the septal pore. Here, we investigate WB dynamics in the wheat pathogen Zymoseptoria tritici. Ultrastructural studies showed that 3.4 ± 0.2 WBs reside on each side of a septum and that single WBs of 128.5 ± 3.6 nm in diameter seal the septal pore (41 ± 1.5 nm). Live cell imaging of green fluorescent ZtHex1, a major protein in WBs, and the integral plasma membrane protein ZtSso1 confirms WB translocation into the septal pore. This was associated with the occasional formation of a plasma membrane "balloon," extruding into the dead cell, suggesting that the plasma membrane rapidly seals the wounded septal pore wound. Minor amounts of fluorescent ZtHex1-enhanced green fluorescent protein (eGFP) appeared associated with the "ballooning" plasma membrane, indicating that cytoplasmic ZtHex1-eGFP is recruited to the extending plasma membrane. Surprisingly, in ~15% of all cases, WBs moved from the ruptured cell into the septal pore. This translocation against the cytoplasmic flow suggests that an active mechanism drives WB plugging. Indeed, treatment of unwounded and intact cells with the respiration inhibitor carbonyl cyanide m-chlorophenyl hydrazone induced WB translocation into the pores. Moreover, carbonyl cyanide m-chlorophenyl hydrazone treatment recruited cytoplasmic ZtHex1-eGFP to the lateral plasma membrane of the cells. Thus, keeping the WBs out of the septal pores, in Z. tritici, is an ATP-dependent process.
Cell Microbiol, 2017; 19

33145484: Hamied A, Alnedawy Q, Correia A, Hacker C, Ramsdale M, Hashimoto H, Kudoh T
Identification and Characterization of Highly Fluorescent Pigment Cells in Embryos of the Arabian Killifish ().
The Arabian killifish, , is a small tropical teleost fish living in wide range of habitats in sea water and fresh water in the Middle East. Here, we report extraordinary fluorescent pigment cells in the Arabian killifish embryo. These cells appear brown in transmitted light, yellowish white in reflected light, and as strong fluorescence in GFP and RFP filters. TEM and confocal microscopy analyses show the fluorescence emanates from leucosome-like pigment organelles. The cells express the gene encoding GTP cyclohydrolase (), a marker for leucophores and xanthophore. Gene knockdown and knockout of using morpholino or CRISPR-Cas9 induced loss of fluorescence in these embryos, indicating a crucial role of the enzyme and the associated pterine biosynthesis pathway in the generation of the fluorescence. We concluded that these cells are a highly fluorescent subtype of leucophores and have named them as fluoroleucophores.
iScience, 2020; 23

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BOARD NUMBER: S01-724

PROFILING GANGLION CELL TYPES BASED ON VISUAL RESPONSES AND ELECTRICAL INPUT FILTERS IN WILD TYPE AND RD10 DEGENERATED MOUSE RETINA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Retinal electronic devices make it possible to restore vision to patients with progressive retinal degenerative. However, current stimulation parameters cannot stimulate retinal pathways, leading to improper perception. Thus, We are exploring whether different Ganglion Cell types can be selectively activated using specific electrical input filters Microelectrode array (MEA) recording was used to record ganglion cells (GCs) from healthy and rd10 degenerated mouse. Firstly, visual stimuli were shone to retinas including moving bars, contrast and temporal frequency chirps, blue-green flashes for classifying different ganglion cell types. Then, we delivered Gaussian-distributed voltage pulses (mean -800 mV, sigma 280 mV) into the tissue and estimate the electrical input filter of each cell using electrical spike triggered averaging (eSTA). Finally, we applied noise-embedded, sine-wave, pulse train modulations to retina in order to selectively stimulate ON and OFF pathways. Based on GC light responses, 35 and 12 clusters were detected in wild-type and rd10 retinas. A hierarchical clustering of electrical input filters showed that ON GCs had upward eSTAs and OFF GCs had downward in both healthy and rd10 retinas. Noise-embedded, sine-wave, pulse train modulations were sufficient to elicit ON or OFF RGC-specific activation, depending on the direction of the modulation. GCs receiving ON pathway input responded more strongly to upward cathodic pulse train modulations, whereas GCs receiving OFF input responded more strongly to downward modulations. Deriving amplitude-modulated electrical pulse trains from the electrical input filter is a useful method to find out specific stimuli eliciting ON and OFF RGC pathway inputs.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S01-725

A CERTAIN TYPE OF PHOTORECEPTOR CONTRIBUTES TO THE IMMEDIATE EFFECT OF LIGHT ON ECLOSION BEHAVIOUR

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Daniel Bidell[1], Tilman Triphan[2], Natalie-Danielle Feige[1], Claudia Müller[1], Charlotte Helfrich-Förster[3], Mareike Selcho[1]
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For most organisms, adaption to altering external conditions is of critical importance. One of the most prominent external stimuli is light. While light has the ability to indirectly influence a wide variety of physiological processes and behaviours by entraining the circadian clock, it can also have a direct “masking” effect on circadian behaviour. In *Drosophila melanogaster*, light influences the emergence of the adult fly out of its puparium, called eclosion (Engelmann and Honegger, 1966). Eclosion is gated by the circadian clock to the morning to prevent desiccation and enhance survival rate. A light stimulus induces a rapid increase in eclosion rate, the so-called lights-on (LOn) effect that is eliminated in flies lacking eyes and ocelli (McNabb and Truman, 2008). Since eclosion is independent of the animal´s physiological state and motivation, we here use it as a readout to examine the immediate effects of light on eclosion behaviour. Light is perceived by the compound eyes, which consist of around 800 ommatidia, each equipped with six outer (R1-6) and two inner (R7, R8) photoreceptor cells. While the outer cells express Rhodopsin1, the inner ones express specific combinations of Rhodopsin3 and Rhodopsin4, Rhodopsin5 and Rhodopsin6 (Rister and Desplan, 2011). Besides the compound eyes, flies have three simple dorsal eyes called ocelli, which express Rhodopsin2 (Pollock and Benzer, 1988). Here we show that flies need the photoreceptors in the retina, but not the ocelli for the LOn response. Our results suggest that R8 photoreceptor cells appear to transmit the light signal that is responsible for the LOn response.

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BOARD NUMBER: S01-726

SOMATIC CONNEXIN PLAQUES ON AMACRINE CELLS OF THE MAMMALIAN RETINA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Katalin Fusz[1,2], Péter Kóbor[1,2], Tamás Kovács-Öller[2,3], Béla Völgyi[2,3], Ildikó Telkes[1,2], Péter Buzás[1,2]
[1]University of Pécs Medical School, Institute Of Phsyiology, Pécs, Hungary, [2]University of Pécs, Centre For Neuroscience, Pécs, Hungary, [3]University of Pécs, Szentágothai Research Centre, Pécs, Hungary

Aims: In the adult mammalian nervous system, gap junctions (GJs) are usually found on axon terminals and dendrites. Recently, we have also found plaques immunoreactive to the main neuronal GJ protein connexin-36 (Cx36) on certain amacrine cell bodies. Here, we aimed to characterize size and retinal distribution of these GJs as well as narrow down the cell types forming them. **Methods:** We used flat-mounted retinas of cats (n = 4), rats (n = 4) and mice (n = 5). Prox1 immunofluorescent labelling was used in combination with Cx36, calretinin (CaR) and parvalbumin (PV). The densities of labelled cells and Cx36 puncta were measured at several retinal locations. **Results:** Cx36 plaques were attached to about 8–10% of Prox1-positive amacrine cell somata. Co-expression patterns of CaR and PV showed that the majority of them are AII amacrine cells. The cross-sectional area of somatic plaques in the retina of cats (2.06±1.5µm^2), rats (1.40±0.6µm^2) and mice (0.81±0.39µm^2) was significantly larger than that of Cx36-plaques seen in the neuropil of the inner plexiform layer ($p < 0.001$). Somatic plaques were larger and their density was higher in the central areas of the retina in rats and mice. **Conclusions:** The presence of somatic plaques suggests strong coupling of pairs or small clusters of amacrine cell bodies via gap junctions. We hypothesize that these large gap junctions may enhance electrical synchronization or the exchange of signalling molecules between participating cells.

Pubmed:
34572046: Fusz K, Kovács-Öller T, Kóbor P, Szabó-Meleg E, Völgyi B, Buzás P, Telkes I
Regional Variation of Gap Junctional Connections in the Mammalian Inner Retina.
The retinas of many species show regional specialisations that are evident in the differences in the processing of visual input from different parts of the visual field. Regional specialisation is thought to reflect an adaptation to the natural visual environment, optical constraints, and lifestyle of the species. Yet, little is known about regional differences in synaptic circuitry. Here, we were interested in the topographical distribution of connexin-36 (Cx36), the major constituent of electrical synapses in the retina. We compared the retinas of mice, rats, and cats to include species with different patterns of regional specialisations in the analysis. First, we used the density of Prox1-immunoreactive amacrine cells as a marker of any regional specialisation, with higher cell density signifying more central regions. Double-labelling experiments showed that Prox1 is expressed in AII amacrine cells in all three species. Interestingly, large Cx36 plaques were attached to about 8-10% of Prox1-positive amacrine cell somata, suggesting the strong electrical coupling of pairs or small clusters of cell bodies. When analysing the regional changes in the volumetric density of Cx36-immunoreactive plaques, we found a tight correlation with the density of Prox1-expressing amacrine cells in the ON, but not in the OFF sublamina in all three species. The results suggest that the relative contribution of electrical synapses to the ON- and OFF-pathways of the retina changes with retinal location, which may contribute to functional ON/OFF asymmetries across the visual field.
Cells, 2021; 10

FENS Forum 2022
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BOARD NUMBER: S01-727

A COMPLEXIN-TRANSDUCIN-COMPLEX IN LIGHT ADAPTATION IN MAMMALIAN RETINA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Uwe Thorsten Lux[1], Anna Wartenberg[1], Jutta Meyer[2], Andreas Gießl[3], Olaf Jahn[2], Kerstin Reim[2], Johann Helmut Brandstätter[1]
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Complexins (Cplxs) are SNARE complex regulating proteins which control the speed and Ca^{2+} sensitivity of vesicle fusion at chemical synapses. In mammalian retina, all four known Cplx isoforms are present: Cplxs 1/2 at conventional synapses and Cplxs 3/4 primarily at the highly specialized ribbon synapses. The aim of our study was to decipher the synaptic function of Cplxs 3/4 to better understand tonic release at photoreceptor ribbon synapses. Previous results suggest that in addition to the role of Cplxs 3/4 in vesicle release, they may also be involved in light-regulated vesicle loading of the synaptic ribbon. Here we focused on the role of Cplx 4 in light adaptation at rod photoreceptor ribbon synapses. Electron microscopic analysis revealed that light-regulated vesicle loading of rod photoreceptor ribbons occurs on a timescale of minutes and that this process is compromised in Cplx 4 knockout mice. In affinity purification experiments with peptides representing the SNARE-binding domain of Cplx, we found the rod-specific alpha-subunit of the G-protein Transducin as a putative interactor of the Cplx 4-SNARE complex. Transducin, a key protein of the phototransduction process in the photoreceptor outer segments, is translocated to the rod photoreceptor synaptic terminals in light. Here its function is enigmatic. With *in situ* proximity ligation assays, we found a putative interaction between Cplx 4/Transducin, Cplx 4/Syt1, and Syt1/Transducin. In conclusion, our data suggest that the light-regulated function of Cplx 4 in vesicle loading of the synaptic ribbon is controlled by Transducin.

Pubmed:
34360929: Lux UT, Ehrenberg J, Joachimsthaler A, Atorf J, Pircher B, Reim K, Kremers J, Gießl A, Brandstätter JH
Cell Types and Synapses Expressing the SNARE Complex Regulating Proteins Complexin 1 and Complexin 2 in Mammalian Retina.
Complexins (Cplxs) 1 to 4 are components of the presynaptic compartment of chemical synapses where they regulate important steps in synaptic vesicle exocytosis. In the retina, all four Cplxs are present, and while we know a lot about Cplxs 3 and 4, little is known about Cplxs 1 and 2. Here, we performed in situ hybridization experiments and bioinformatics and exploited Cplx 1 and Cplx 2 single-knockout mice combined with immunocytochemistry and light microscopy to characterize in detail the cell type and synapse-specific distribution of Cplx 1 and Cplx 2. We found that Cplx 2 and not Cplx 1 is the main isoform expressed in normal and displaced amacrine cells and ganglion cells in mouse retinae and that amacrine cells seem to operate with a single Cplx isoform at their conventional chemical synapses. Surprising was the finding that retinal function, determined with electroretinographic recordings, was altered in Cplx 1 but not Cplx 2 single-knockout mice. In summary, the results provide an important basis for future studies on the function of Cplxs 1 and 2 in the processing of visual signals in the mammalian retina.
Int J Mol Sci, 2021; 22
33020147: Korn MA, Schmitt H, Angermüller S, Chambers D, Seeling M, Lux UT, Brey S, Royzman D, Brückner C, Popp V, Percivalle E, Bäuerle T, Zinser E, Winkler TH, Steinkasserer A, Nimmerjahn F, Nitschke L
Siglec-15 on Osteoclasts Is Crucial for Bone Erosion in Serum-Transfer Arthritis.
Siglec-15 is a conserved sialic acid-binding Ig-like lectin, which is expressed on osteoclasts. Deficiency of Siglec-15 leads to an impaired osteoclast development, resulting in a mild osteopetrotic phenotype. The role of Siglec-15 in arthritis is still largely unclear. To address this, we generated Siglec-15 knockout mice and analyzed them in a mouse arthritis model. We could show that Siglec-15 is directly involved in pathologic bone erosion in the K/BxN serum-transfer arthritis model. Histological analyses of joint destruction provided evidence for a significant reduction in bone erosion area and osteoclast numbers in Siglec-15 mice, whereas the inflammation area and cartilage destruction was comparable to wild-type mice. Thus, Siglec-15 on osteoclasts has a crucial function for bone erosion during arthritis. In addition, we generated a new monoclonal

anti-Siglec-15 Ab to clarify its expression pattern on immune cells. Whereas this Ab demonstrated an almost exclusive Siglec-15 expression on murine osteoclasts and hardly any other expression on various other immune cell types, human Siglec-15 was more broadly expressed on human myeloid cells, including human osteoclasts. Taken together, our findings show a role of Siglec-15 as a regulator of pathologic bone resorption in arthritis and highlight its potential as a target for future therapies, as Siglec-15 blocking Abs are available.
J Immunol, 2020; 205

31614616: Falk N, Joachimsthaler A, Kessler K, Lux UT, Noegel AA, Kremers J, Brandstätter JH, Gießl A, Falk N, Joachimsthaler A, Kessler K, Lux UT, Noegel AA, Kremers J, Brandstätter JH, Gießl A
Lack of a Retinal Phenotype in a Syne-2/Nesprin-2 Knockout Mouse Model.
Syne-2 (also known as Nesprin-2) is a member of a family of proteins that are found primarily in the outer nuclear membrane, as well as other subcellular compartments. Syne-2 contains a C-terminal KASH transmembrane domain and is part of a protein network that associates the nuclear envelope to the cytoskeleton via the binding to actin filaments. Syne-2 plays a role in nuclear migration, nuclear positioning during retinal development, and in ciliogenesis. In a previous study, we showed a connection between Syne-2 and the multifunctional scaffold protein Pericentrin (Pcnt). The elimination of the interaction of Syne-2 and Pcnt showed defects in nuclear migration and the formation of outer segments during retinal development, as well as disturbances in centrosomal migration at the beginning of ciliogenesis in general. In this study, the Syne-2 KO mouse model Nesprin-2△ABD (Syne-2, MGI) with special attention to Pcnt and ciliogenesis was analyzed. We show reduced expression of Syne-2 in the retina of the Syne-2 KO mouse but found no significant structural-and only a minor functional-phenotype. For the first time, detailed expression analyses showed an expression of a Syne-2 protein larger than 400 kDa (~750 kDa) in the Syne2/Nesprin-2 KO mouse. In conclusion, the lack of an overt phenotype in Syne-2/Nesprin-2 KO mice suggests the usage of alternative translational start sites, producing Syne-2 splice variants with an intact Pcnt interaction site. Nevertheless, deletion of the actin-binding site in the Syne-2/Nesprin-2 KO mouse revealed a high variability in scotopic oscillatory potentials assuming a novel function of Syne-2 in synchronizing inner retinal processes.
Cells, 2019; 8

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GLUCAGON AS A NOVEL NEUROMODULATOR OF RETINAL ROD BIPOLAR CELL INHIBITORY ACTIVITY, POSSIBLE IMPLICATIONS IN MYOPIA PATHOGENESIS

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Aims: Myopia incidence is steadily increasing worldwide, but the underlying pathophysiologic mechanisms are still only partially understood. Glucagon is a peptide thought to signal image defocus in the eye, which is considered the first step in myopia induction, and dopamine is known for its key role in myopia, being proposed as the main signaling molecule linked to its pathogenesis. The aim of this study was to corroborate glucagon signaling in the mammalian retina and find a possible link with dopaminergic signaling. **Methods:** Whole-cell patch-clamp was used to study the inhibitory activity of rod bipolar cells (RBCs), as this activity is known to be regulated by dopamine and RBCs have been implicated in glucagon-related activity. Inhibitory post-synaptic currents (IPSC) were measured in RBCs from wild-type and lens-induced myopia model mice. **Results:** Glucagon produced a dose-dependent and glucagon receptor-dependent increase in RBC glycinergic IPSC frequency. This effect was also dependent on dopaminergic activity as it was abolished by dopamine type 1 receptor (D1R) antagonism and in scotopic conditions. The effect was also abolished in the myopia murine model but could be recovered using D1R agonism. **Conclusions:** Glucagon is a novel retinal neuromodulator in mammals, regulating the glycinergic inhibitory activity acting on RBCs in a D1R-dependent manner. Its effects are abolished in a myopia model, suggesting it is one of the pathways affected in this condition. More research will be required to determine whether this pathway could be a target for myopia prevention or treatment.

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BOARD NUMBER: S01-729

SYNAPTIC DEGENERATION IN RETINAL ROD BIPOLAR CELLS IS A CAUSE OF AGE-RELATED LOSS IN VISUAL SENSITIVITY IN CHRONICALLY HYPOGLYCEMIC MICE

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Aims Mice rendered chronically hypoglycemic by a null mutation of the glucagon receptor gene (Gcgr) experience an age-related loss in retinal and visual sensitivity along with a loss of synaptic contacts in the outer retina. Acuity and contrast sensitivity begin to decline at 9 to 13 months. Retinal function decreases more than 100-fold in the same period. Because the ERG b-wave is the first signal to decrease in amplitude, we investigated the response of rod bipolar cells (RBCs) in $Gcgr^{-/-}$ mice. **Methods** We performed whole-cell patch-clamp recoding from RBCs. To mimic a dark-adapted condition we included L-AP4 (an agonist at the mGluR6 receptor) in the external solution. We applied CPPG using a puffer pipette positioned near the dendrites of RBCs to simulate light responses. CPPG antagonizes L-AP4 at the mGluR6 receptors in RB dendrites, resembling the decrease in glutamate release that follows photoreceptor light activation. **Results** We found that the responses of RBCs in $Gcgr^{-/-}$ mice are smaller than in control $Gcgr^{+/-}$ mice. In voltage-clamp mode, CPPG evoked maximal inward currents of 6.8 ± 4.7 pA and 18 ± 14 pA in $Gcgr^{-/-}$ and $Gcgr^{+/-}$ mice, respectively. In current-clamp mode the voltage responses peaked at 18 ± 14 mV and 31 ± 18 mV in $Gcgr^{-/-}$ and $Gcgr^{+/-}$ mice, respectively. Dendrites of RBCs appeared to be shorter in $Gcgr^{-/-}$ mice than in control animals. **Conclusions** These results suggest that chronic hypoglycemia causes postsynaptic alterations in the photoreceptor to RB synapse in agreement with observed losses in visual and retinal sensitivity.

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BOARD NUMBER: S01-730

THE SELECTIVITY OF RETINAL GANGLION CELLS DYNAMICALLY CHANGES WHEN STIMULATED WITH A MOVING OBJECT

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

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Some ganglion cells have been shown to compensate for delays in information transmission and anticipate motion. Yet, fundamental characteristics of retinal ganglion cells selectivity such as their receptive field polarity, i.e. sensitivity to local light increase (ON) and/or decrease (OFF), are usually assessed using static stimuli like flashed images. Here we developed a new approach to characterize the ganglion cell selectivity when the retina processes motion. We recorded the responses of mouse ganglion cells to stimuli composed of moving bars slightly perturbed by patterns of random noise. We repeated the same moving stimulus many times, but always changing the random noise patterns. We then estimated how these random noise patterns change the response of each ganglion cell to the moving bar, and measured a dynamical receptive field for each ganglion cell, for each position of the bar. Our preliminary results show that the polarity of ganglion cell receptive fields can be dynamically modulated as a function of the position of the moving bar. A ganglion cell that initially responds to a light increase can respond to a light decrease when stimulated by a moving object. The selectivity of RGCs has previously been shown to depend on image statistics such as background luminance and local content of static images. Here we show that it can also change in time. The perturbative approach proposed here is therefore able to provide insight on how ganglion cells integrate the visual stimuli information in time.

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BOARD NUMBER: S01-731

A NOVEL MEMBRANE-TARGETED PHOTOSWITCH RESTORES PHYSIOLOGICAL LIGHT-RESPONSES IN THE DEGENERATED RD10 MOUSE RETINA

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Gaia Ziraldo[1,2], Stefano Di Marco[1,2], Elisabetta Colombo[1], Fabio Benfenati[2,3]
[1]Istituto Italiano di Tecnologia, Center For Synaptic Neuroscience And Technology, Genova, Italy, [2]IRCCS, Ospedale Policlinico San Martino, Genova, Italy, [3]Istituto Italiano Di Tecnologia, Center For Synaptic Neuroscience And Technology, Genova, Italy

Age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are the principal pathologies leading to photoreceptor degeneration and blindness in humans. Unfortunately, no definitive cure exists, and the substitutive strategies currently available rely on prosthetic devices, whose application is strongly hampered by their poor sensitivity, low spatio-temporal resolution, wiring and size. In this context, we engineered a novel and injectable membrane-targeted photoswitchable molecule named Ziapin2, which can modulate membrane capacitance and trigger action potential firing. This work aims to investigate the capability of Ziapin2 to restore physiological responses to light and visual functions in blind retinas of Rd10 mice, a model of RP. We performed ex-vivo electrophysiological recordings on retinal explants freshly isolated from blind Rd10 mice: we assessed light-evoked response to light stimulation by combining patch-clamp experiments in current- and voltage-clamp configuration and high-density Multi-Electrode Array (HD-MEA) measurements. This work demonstrated that Ziapin2 could trigger light-evoked action potentials in RGCs of blind retinal explants at low luminance levels. Strikingly, the ability of Ziapin2 to increase membrane capacitance in the darkness and suddenly decrease it under light stimulation allows for restoration of both the ON- and OFF-response pathways, resembling the neuronal circuitry of a regular sighted retinal network. This work highlights the potential of Ziapin2 as a highly biocompatible, non-invasive injectable therapeutic tool to treat retinal dystrophies at single-cell resolution, paving the way to the development of novel therapeutic strategies that would finally overcome the significant obstacles currently existing to therapeutic intervention.

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BOARD NUMBER: S01-732

A NEW EXPERIMENTAL GLAUCOMA MODEL WITH CHRONIC OCULAR HYPERTENSION

POSTER SESSION 01 - SECTION: RETINAL CIRCUITRY AND FUNCTION

Qianbin Wang
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Progress in understanding the glaucomatous pathogenesis is greatly hampered by the lack of a reliable animal model. Experimental animal models that can mimic the retinal ganglion cells (RGC) and optic nerve pathology in human disease are of crucial importance in uncovering the mechanisms of the disease and developing therapies. Therefore, developing a reliable and controllable experimental glaucoma model to induce chronic and sustained intraocular pressure (IOP) elevation and glaucomatous neurodegeneration would be extremely useful. Here, we developed a novel technique to induce chronic ocular hypertension using size distributed polymeric viscobeads. These viscobeads can partially degrade into viscoelastic material to completely block the aqueous drainage and obtained a sustained IOP elevation, and their diameters that match the pores in the trabecular meshwork nets have facilitated the generation of sustained ocular hypertension. Reference: Tian, F., Cheng, Y., Zhou, S., Wang, Q.,... & He, Z. (2022). Core Transcription Programs Controlling Injury-Induced Neurodegeneration of Retinal Ganglion Cells. *Neuron* (accepted)

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Poster Session 02

- Poster Session 02 - Section: Spatial Memory, Navigation & Cognition
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- **BOARD NUMBER: S02-001**

ODOR CUES MAY BIAS HIPPOCAMPAL REACTIVATIONS DURING SPATIAL GOAL LEARNING

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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In the hippocampus, neuronal sequences encoding past experience reactivate during sharp wave ripple events. Sharp wave ripples during waking immobility (wSPW-Rs) have recently been associated with memory consolidation, but also with future trajectory planning and hippocampal map stabilization. These observations raise the exciting possibility that enhancing reactivations through wSPW-Rs could improve memory performance. Previous studies showed that presenting a sensory cue associated with a past experience can bias the content of neuronal reactivations occurring during sleep SPW-Rs. Similarly, we used olfactory cues in an attempt to bias wSPW-R content and probe their function in spatial learning. We recorded neurons from the dorsal hippocampus of mice trained to locate fixed hidden goal locations in two environments. During learning, an odor was added in the water rewards of one environment while a second odor was used on the other environment. Both environments were visited in a pseudo-random fashion. In order to manipulate wSPW-Rs, one of the two odors (designed as a 'reinforcing' cue) was dispensed during the inter-trial intervals. A retrieval test was then conducted in the absence of odors. Preliminary results suggest that, despite having little influence on the behavioral performance, the rate of reward-associated ripples was enhanced on the non-reinforced environment during learning. During the retrieval test, place cell representations did not significantly differ in quality between reinforced and non-reinforced environments. Altogether these data suggest that wSPW-Rs occurrence are subject to task-demand and sensory priming that could favor a homogeneous representation of different environments for optimal memory performance and retrieval.

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BOARD NUMBER: S02-002

AN EXAMINATION OF THE BEHAVIOURAL AND NEURAL CORRELATES OF HUMAN NAVIGATION AND SPATIAL MEMORY.

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Learning how to navigate our environment and recall important locations is an vital subconscious cognitive task we perform every day. Navigation, along with the required learning and memory, has been examined extensively in animals using the Morris water maze (Morris, 1981). In this task animals are required to find a platform, hidden somewhere in a large circular pool of water (below surface level). As animals cannot see the goal directly, they must use various cues in the environment to locate it and escape. With the advancements in virtual reality technologies, navigation can now be directly examined in humans using a virtual version of the task. For this project, we have used our open-source virtual water maze software NavWell (Commins et al., 2020) to record real-time navigation learning and recall in human participants. We have also simultaneously recorded the neural activity using Electroencephalography (EEG) with the BioSemi Active 32 system. Here, we examine 25 adult participants (mean age = 22.5 years). All participants showed good learning across 12 trials of the navigation task. Furthermore, we examine frequency-band power changes in both theta (4 - 8Hz) and alpha (8 - 12Hz) that correlate with learning. Results are discussed in terms of the interaction between the two bands and their possible role in place learning and recall. We aim to examine learning and neural differences across age.

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BOARD NUMBER: S02-003

EFFECTS OF TRANSCRANIAL MAGNETIC STIMULATION ON SPATIAL MEMORY AND RELATED BRAIN OXIDATIVE METABOLISM

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulation technique that allows modifying brain excitability outside the skull. The use of rTMS is promising to treat several disorders that affect our nervous system, however, there are still many unknowns about its mechanism of action, as well as in the modulation of memory processes. Our objective was to determine its effects on the nervous system of healthy rodents, taking into account brain oxidative metabolism, neuronal activation and the study of glial cells, the latter being indicators of possible inflammatory effects. We apply rTMS with different stimulation protocols, modifying the number of trials, pulses and days of administration and we study the oxidative metabolism through the histochemistry of cytochrome c oxidase (CCO), the neuronal activation through the immunohistochemistry of c -Fos, and the study of glial cells through the immunohistochemistry of GFAP and Iba1, selective markers of astrocytes and immunoreactive microglia, respectively. Regarding possible impact on spatial memory, we used the Morris water maze. The results showed that rTMS is able to modify CCO activity in cortical and hippocampal regions, produces an increase in neuronal activation, and does not lead to changes in glial cell density. We also observe that, although it does not lead to facilitation of spatial memory, it is capable of achieving a more efficient use of the brain networks that support it. In conclusion, these experiments add information about the mechanism of action of rTMS, and its potential use in the modulation of cognitive functions.

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BOARD NUMBER: S02-004

INFLUENCE OF SPATIAL LEARNING ON THE CONNECTOME OF ADULT-BORN NEURONS IN RATS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Experience such as learning enhances adult hippocampal neurogenesis in the dentate gyrus, a brain region essential for learning and memory, by promoting newborn neurons development into the dentate network. In return, adult-born neurons play an important role in the encoding of new spatial memories. Yet, it is still unknown if spatial learning influences the connectome of adult-born neurons. Here, using an approach allowing the visualization of the glutamatergic post-synaptic densities and a retroviral retrograde monosynaptic tracing method, we characterized the connectome of adult-born neurons following spatial learning at different time points of their maturation. First, we showed that the glutamatergic post-synaptic density on adult-born neurons immature at the time of learning is increased following spatial learning, suggesting an enhanced excitatory innervation. We evidenced that this enhancement corresponds to a transient increase in the innervation from other mature granule cells during the first days of life of newborn neurons. Once adult-born neurons reach full maturity, we showed that spatial learning similarly increased their total glutamatergic post-synaptic density and also their innervation from the hilus. The analyses of their intrinsic properties by patch clamp confirm that spatial learning increase the presence of glutamatergic receptors. All together our results indicated a crucial role of glutamatergic transmission in learning–induced modulation of adult-born neurons.

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BOARD NUMBER: S02-005

COMMUNICATION BETWEEN THE HIPPOCAMPUS, NUCLEUS ACCUMBENS AND VENTRAL TEGMENTAL AREA DURING LEARNING AND MEMROY

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The hippocampus is thought to form the brain substrate of a cognitive map. While the spatial component of this map involves hippocampal ‘place’ cells, non-spatial features may require coupling with other brain areas. This could involve ‘hippocampal sequences’, endogenous activations of successive place cells representing entire trajectories at a highly accelerated rate. These sequences are paced by theta or ripple oscillations, and could participate in learning and memory, as well as goal-directed decision making. However, the association between hippocampal sequences and reward- and goal-related signals remains poorly understood. The ventral tegmental area (VTA) and nucleus accumbens (Nacc) are involved in reward coding and goal-directed action selection, and have both been independently reported to activate during hippocampal ripples. These co-activations could be part of a broader mechanism, whereby the three structures coordinate at a precise timescale during hippocampal oscillations, with different characteristics depending on cognitive function. To explore these questions, we perform electrophysiological recordings from dozens of single units in the dorsal hippocampus, NAcc and VTA simultaneously, in rats trained to learn a complex spatial memory task : the ‘Hippodamos maze’. This novel task features a daily-changing set of reward and error zones, requiring the rats to learn elaborate spatial configurations, to flexibly adapt to changes in these configurations, and every day learn and remember trajectories never experienced before. We investigate how the hippocampus, NAcc and VTA coordinate during various cognitive functions (learning, recall, planning, long-term storage, etc.) and relate to the performance of the animals in the task.

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BOARD NUMBER: S02-006

THE RAT HEXMAZE: A STUDY INTO HOW PREVIOUS KNOWLEDGE AFFECTS LEARNING

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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How does sleep influence memory processing and which factors such as previous knowledge can influence this? To examine this, we developed a new behavioral task called the HexMaze, a large (9x5m) maze for rats. This task allows us to study how quickly rats build a knowledge network (i.e. cognitive map) of the environment, how they update this map with new information (i.e. goal location change) and paves the way for examination of neural correlates for these behavioral measures. The use of pharmacological manipulations additionally allows us to evaluate the role of different brain regions, such as the hippocampus, during different phases of the task. In the current study, we used cyanquixaline (CNQX) to temporarily inhibit the CA1 region of the hippocampus in rats during the different phases of the task (i.e. build-up and update phase), either while learning a new goal location or while retrieving a previously learned location. We show that rats are able to perform direct runs between start and goal location already in the first training sessions. Moreover, they show expedited long-term memory after updating approximately twelve weeks into training. Through inhibition of CA1 during retrieval in both the build-up and update phase, we additionally show that the lack of a fully working hippocampus does not seem to negatively affect the rats' performance in the HexMaze task. These results seem to hint to less of a role of the hippocampus already during the formation of a cognitive map.

Pubmed:
34913214: Aleman-Zapata A, van der Meij J, Genzel L
Disrupting ripples: Methods, results, and caveats in closed-loop approaches in rodents.
Hippocampal ripple oscillations have been associated with memory reactivations during wake and sleep. These reactivations should contribute to working memory and memory consolidation respectively. In the past decade studies have moved from being observational to actively disrupting ripple-related activity in closed-loop approaches to enable causal investigations into their function. All together these studies have been able to provide evidence that wake, task-related ripple activity is important for working memory and planning but less important for stabilisation of spatial representations. Rest and sleep-related ripple activity, in contrast, is important for long-term memory performance and thus memory consolidation. In this review, we summarise results from different closed-loop approaches in rodents. Further, we highlight differences in detection and stimulation methods as well as controls and discuss how these differences could influence outcomes.
J Sleep Res, 2021;
34135006: Alonso A, Bokeria L, van der Meij J, Samanta A, Eichler R, Lotfi A, Spooner P, Navarro Lobato I, Genzel L
The HexMaze: A Previous Knowledge Task on Map Learning for Mice.
New information is rarely learned in isolation; instead, most of what we experience can be incorporated into or uses previous knowledge networks in some form. Previous knowledge in form of a cognitive map can facilitate knowledge acquisition and will influence how we learn new spatial information. Here, we developed a new spatial navigation task where food locations are learned in a large, gangway maze to test how mice learn a large spatial map over a longer time period-the HexMaze. Analyzing performance across sessions as well as on specific trials, we can show simple memory effects as well as multiple effects of previous knowledge of the map accelerating both online learning and performance increases over offline periods when incorporating new information. We could identify the following three main phases: (1) learning the initial goal location; (2) faster learning after 2 weeks when learning a new goal location; and then (3) the ability to express one-session learning, leading to long-term memory effect after 12 weeks. Importantly, we are the first to show that buildup of a spatial map is dependent on how much time passes, not how often the animal is trained.
eNeuro, 2021 Jul-Aug; 8
32954007: Alonso A, van der Meij J, Tse D, Genzel L
Naïve to expert: Considering the role of previous knowledge in memory.
In humans, most of our new memories are in some way or another related to what we have already experienced. However, in

memory research, especially in non-human animal research, subjects are often mostly naïve to the world. But we know that previous knowledge will change how memories are processed and which brain areas are critical at which time point. Each process from encoding, consolidation, to memory retrieval will be affected. Here, we summarise previous knowledge effects on the neurobiology of memory in both humans and non-human animals, with a special focus on schemas - associative network structures. Furthermore, we propose a new theory on how there may be a continuous gradient from naïve to expert, which would modulate the importance and role of brain areas, such as the hippocampus and prefrontal cortex.
Brain Neurosci Adv, 2020 Jan-Dec; 4
31944434: van der Meij J, Rattenborg NC, Beckers GJL
Divergent neuronal activity patterns in the avian hippocampus and nidopallium.
Sleep-related brain activity occurring during non-rapid eye-movement (NREM) sleep is proposed to play a role in processing information acquired during wakefulness. During mammalian NREM sleep, the transfer of information from the hippocampus to the neocortex is thought to be mediated by neocortical slow-waves and their interaction with thalamocortical spindles and hippocampal sharp-wave ripples (SWRs). In birds, brain regions composed of pallial neurons homologous to neocortical (pallial) neurons also generate slow-waves during NREM sleep, but little is known about sleep-related activity in the hippocampus and its possible relationship to activity in other pallial regions. We recorded local field potentials (LFP) and analogue multiunit activity (AMUA) using a 64-channel silicon multi-electrode probe simultaneously inserted into the hippocampus and medial part of the nidopallium (i.e., caudal medial nidopallium; NCM) or separately into the caudolateral nidopallium (NCL) of adult female zebra finches (Taeniopygia guttata) anesthetized with isoflurane, an anesthetic known to induce NREM sleep-like slow-waves. We show that slow-waves in NCM and NCL propagate as waves of neuronal activity. In contrast, the hippocampus does not show slow-waves, nor sharp-wave ripples, but instead displays localized gamma activity. In conclusion, neuronal activity in the avian hippocampus differs from that described in mammals during NREM sleep, suggesting that hippocampal memories are processed differently during sleep in birds and mammals.
Eur J Neurosci, 2020; 52
31231182: Rattenborg NC, van der Meij J, Beckers GJL, Lesku JA
Local Aspects of Avian Non-REM and REM Sleep.
Birds exhibit two types of sleep that are in many respects similar to mammalian rapid eye movement (REM) and non-REM (NREM) sleep. As in mammals, several aspects of avian sleep can occur in a local manner within the brain. Electrophysiological evidence of NREM sleep occurring more deeply in one hemisphere, or only in one hemisphere - the latter being a phenomenon most pronounced in dolphins - was actually first described in birds. Such asymmetric or unihemispheric NREM sleep occurs with one eye open, enabling birds to visually monitor their environment for predators. Frigatebirds primarily engage in this form of sleep in flight, perhaps to avoid collisions with other birds. In addition to interhemispheric differences in NREM sleep intensity, the intensity of NREM sleep is homeostatically regulated in a local, use-depended manner within each hemisphere. Furthermore, the intensity and temporo-spatial distribution of NREM sleep-related slow waves varies across layers of the avian hyperpallium - a primary visual area - with the slow waves occurring first in, and propagating through and outward from, thalamic input layers. Slow waves also have the greatest amplitude in these layers. Although most research has focused on NREM sleep, there are also local aspects to avian REM sleep. REM sleep-related reductions in skeletal muscle tone appear largely restricted to muscles involved in maintaining head posture. Other local aspects of sleep manifest as a mixture of features of NREM and REM sleep occurring simultaneously in different parts of the neuroaxis. Like monotreme mammals, ostriches often exhibit brainstem-mediated features of REM sleep (muscle atonia and REMs) while the hyperpallium shows EEG slow waves typical of NREM sleep. Finally, although mice show slow waves in thalamic input layers of primary sensory cortices during REM sleep, this is not the case in the hyperpallium of pigeons, suggesting that this phenomenon is not a universal feature of REM sleep. Collectively, the local aspects of sleep described in birds and mammals reveal that wakefulness, NREM sleep, and REM sleep are not always discrete states.
Front Neurosci, 2019; 13
30983954: van der Meij J, Martinez-Gonzalez D, Beckers GJL, Rattenborg NC
Neurophysiology of Avian Sleep: Comparing Natural Sleep and Isoflurane Anesthesia.
Propagating slow-waves in electroencephalogram (EEG) or local field potential (LFP) recordings occur during non-rapid eye-movement (NREM) sleep in both mammals and birds. Moreover, in both, input from the thalamus is thought to contribute to the genesis of NREM sleep slow-waves. Interestingly, the general features of slow-waves are also found under isoflurane anesthesia. However, it is unclear to what extent these slow-waves reflect the same processes as those giving rise to NREM sleep slow-waves. Similar slow-wave spatio-temporal properties during NREM sleep and isoflurane anesthesia would suggest that both types of slow-waves are based on related processes. We used a 32-channel silicon probe connected to a transmitter to make intra-cortical recordings of the visual hyperpallium in naturally sleeping and isoflurane anesthetized pigeons () using a within-bird design. Under anesthesia, the amplitude of LFP slow-waves was higher when compared to NREM sleep. Spectral power density across all frequencies (1.5-100 Hz) was also elevated. In addition, slow-wave

coherence between electrode sites was higher under anesthesia, indicating higher synchrony when compared to NREM sleep. Nonetheless, the spatial distribution of slow-waves under anesthesia was more comparable to NREM sleep than to wake or REM sleep. Similar to NREM sleep, slow-wave propagation under anesthesia mainly occurred in the thalamic input layers of the hyperpallium, regions which also showed the greatest slow-wave power during both recording conditions. This suggests that the thalamus could be involved in the genesis of slow-waves under both conditions. Taken together, although slow-waves under isoflurane anesthesia are stronger, they share spatio-temporal activity characteristics with slow-waves during NREM sleep.
Front Neurosci, 2019; 13
30462347: van der Meij J, Martinez-Gonzalez D, Beckers GJL, Rattenborg NC
Intra-"cortical" activity during avian non-REM and REM sleep: variant and invariant traits between birds and mammals.
Several mammalian-based theories propose that the varying patterns of neuronal activity occurring in wakefulness and sleep reflect different modes of information processing. Neocortical slow-waves, hippocampal sharp-wave ripples, and thalamocortical spindles occurring during mammalian non-rapid eye-movement (NREM) sleep are proposed to play a role in systems-level memory consolidation. Birds show similar NREM and REM (rapid eye-movement) sleep stages to mammals; however, it is unclear whether all neurophysiological rhythms implicated in mammalian memory consolidation are also present. Moreover, it is unknown whether the propagation of slow-waves described in the mammalian neocortex occurs in the avian "cortex" during natural NREM sleep. We used a 32-channel silicon probe connected to a transmitter to make intracerebral recordings of the visual hyperpallium and thalamus in naturally sleeping pigeons (Columba livia). As in the mammalian neocortex, slow-waves during NREM sleep propagated through the hyperpallium. Propagation primarily occurred in the thalamic input layers of the hyperpallium, regions that also showed the greatest slow-wave activity (SWA). Spindles were not detected in both the visual hyperpallium, including regions receiving thalamic input, and thalamus, using a recording method that readily detects spindles in mammals. Interestingly, during REM sleep fast gamma bursts in the hyperpallium (when present) were restricted to the thalamic input layers. In addition, unlike mice, the decrease in SWA from NREM to REM sleep was the greatest in these layers. Taken together, these variant and invariant neurophysiological aspects of avian and mammalian sleep suggest that there may be associated mechanistic and functional similarities and differences between avian and mammalian sleep.
Sleep, 2019; 42
24580797: Beckers GJ, van der Meij J, Lesku JA, Rattenborg NC
Plumes of neuronal activity propagate in three dimensions through the nuclear avian brain.
In mammals, the slow-oscillations of neuronal membrane potentials (reflected in the electroencephalogram as high-amplitude, slow-waves), which occur during non-rapid eye movement sleep and anesthesia, propagate across the neocortex largely as two-dimensional traveling waves. However, it remains unknown if the traveling nature of slow-waves is unique to the laminar cytoarchitecture and associated computational properties of the neocortex.
BMC Biol, 2014; 12

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BOARD NUMBER: S02-007

SUB-CHRONIC PCP TREATMENT IN RATS DOES NOT IMPAIR HIPPOCAMPAL RAPID PLACE LEARNING ON THE WATERMAZE DELAYED-MATCHING-TO-PLACE TASK

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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NMDA receptor hypofunction caused by sub-chronic treatment with phencyclidine (scPCP) has been reported to cause impairments in GABAergic interneurons, including in the hippocampus (Neill et al.,2010,*Pharmacol.Ther.*). However, it is unclear how this impairment may contribute to cognitive deficits in the scPCP model. The watermaze delayed-matching-to-place (DMP) task requires hippocampal GABAergic inhibition, with hippocampal neural disinhibition markedly impairing task performance (McGarrity et al.,2017,*Cereb.Cortex*). Therefore, we investigated whether scPCP treatment would cause watermaze-DMP deficits, similar to those produced by hippocampal neural disinhibition. Young adult male and female Lister-hooded rats were tested on the watermaze DMP task at 1, 3 and 5 weeks following scPCP (males: 5 mg/kg; females: 2 mg/kg, bi-daily for 7 days). Rats also underwent testing of novel object recognition (NOR), locomotor activity (LMA), startle reactivity and prepulse inhibition (PPI). Sub-chronic PCP did not impair watermaze-DMP performance. In addition, sensorimotor processes measured using LMA, startle and PPI were unaffected by scPCP treatment. NOR testing at week 1 revealed a deficit in the scPCP group ($F_{(1, 28)}$=10.5, p<0.01), which was not significant at later time points. This is consistent with previous studies, although many previous studies reported a long-lasting NOR deficit (Rajagopal et al.,2014,*Curr.Pharm.Des.*). We hypothesise that handling and/or exercise during watermaze testing may have rescued later NOR deficits, as both handling and exercise were reported to improve scPCP-induced NOR impairments (Mitsadali et al.,2020,*J.Psychopharmacol.*; Watson et al.,2019,*J.Psychopharmacol.*). Overall, scPCP treatment did not disrupt hippocampal rapid place learning performance. This contrasts with the marked impairments observed following acute pharmacological hippocampal disinhibition.

Pubmed:
34980662: Williams SA, Gwilt M, Hock R, Taylor C, Loayza J, Stevenson CW, Cassaday HJ, Bast T
Hippocampal Disinhibition Reduces Contextual and Elemental Fear Conditioning While Sparing the Acquisition of Latent Inhibition.
Hippocampal neural disinhibition, i.e., reduced GABAergic inhibition, is a key feature of schizophrenia pathophysiology. The hippocampus is an important part of the neural circuitry that controls fear conditioning and can also modulate prefrontal and striatal mechanisms, including dopamine signaling, which play a role in salience modulation. Consequently, hippocampal neural disinhibition may contribute to impairments in fear conditioning and salience modulation reported in schizophrenia. Therefore, we examined the effect of ventral hippocampus (VH) disinhibition in male rats on fear conditioning and salience modulation, as reflected by latent inhibition (LI), in a conditioned emotional response (CER) procedure. A flashing light was used as the conditioned stimulus (CS), and conditioned suppression was used to index conditioned fear. In experiment 1, VH disinhibition via infusion of the GABA-A receptor antagonist picrotoxin before CS pre-exposure and conditioning markedly reduced fear conditioning to both the CS and context; LI was evident in saline-infused controls but could not be detected in picrotoxin-infused rats because of the low level of fear conditioning to the CS. In experiment 2, VH picrotoxin infusions only before CS pre-exposure did not affect the acquisition of fear conditioning or LI. Together, these findings indicate that VH neural disinhibition disrupts contextual and elemental fear conditioning, without affecting the acquisition of LI. The disruption of fear conditioning resembles aversive conditioning deficits reported in schizophrenia and may reflect a disruption of neural processing both within the hippocampus and in projection sites of the hippocampus.
eNeuro, 2022 Jan-Feb; 9

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BOARD NUMBER: S02-008

COMPARISON OF THE EFFECTS OF PACAP-38 AND ITS ANALOG ON SPATIAL MEMORY

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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BOARD NUMBER: S02, Faculty of Science of Bizerte, Carthage University, Life Sciences, Jarzouna, Tunisia, [2]Laboratory of Cognitive Neuroscience, UMR 7291, Aix Marseille University, Cnrs, Marseille, Cedex, France

The Pituitary Adenylate Cyclase-Activating Peptide 38 more commonly known as PACAP-38 (P38) is an endogenous human neuropeptide. In order to efficiently use its neuroprotective (Deguil *et al.,* 2010), vasodilatory (Tlili *et al.,* 2015) and antioxidant (Masmoudi-Kouki *et al.,* 2011) properties for potential therapeutical purposes, an analog (P38-alg), chemically more stable than P38 was synthesized by Bourgault *et al.,* in 2008. We studied the effects of a single intravenous injection of P38 (30 µg/kg), P38-alg (30 µg/kg) and NaCl 0.9% (SAL) on spatial memory in male Wistar rats and measured BDNF levels in the hippocampus. A fourth group received also an injection of NaCl (0.9%) but rats were neither trained nor tested (SALNT). Using a weak massed-learning procedure, we trained P38, P38-alg and SAL groups in a reference memory version of the navigation task in the Morris Water Maze. The 3 groups showed similar acquisition of the task but the P38 group displayed enhanced memory of the platform location in the probe trial with respect to both P38-alg and SAL groups. Training reduced BDNF expression in the hippocampus in SAL relative to SALNT rats. In contrast, P38 administration increased BDNF content that was restored to its basal level. BDNF in P38-alg group was similar to SAL group. Thus, the results suggest that P38 exerts a promnesic effect in rats that is mediated by hippocampal BDNF. In contrast, P38-alg did not mimick the effects of P38 on both spatial memory and BDNF.

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BOARD NUMBER: S02-009

EARLY EXPOSURE TO WESTERN-TYPE DIET AND STRESS BY MATERNAL SEPARATION PROGRAM BRAIN METABOLIC CAPACITY AND COGNITION IN ADULT RATS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

Saúl Sal Sarria, Isabel López Taboada, Nélida Conejo Jiménez, Héctor González Pardo
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Exposure to early-life stress and consumption of high-fat and high-sugar diets (HFS) [similar to Western-type diets] represent two of the most frequent and ubiquitous adverse environmental factors influencing neurodevelopment nowadays. Both factors have been widely associated with all kinds of problems, including learning or memory deficits and impaired cognitive flexibility. **Methods:** The combined effects after lifelong exposure to an HFS diet and early-life stress by maternal separation were evaluated in adult Wistar rats. Specifically, possible long-term effects on spatial memory and cognitive flexibility were evaluated in the Morris Water Maze. Regional brain metabolic capacity using quantitative cytochrome oxidase histochemistry was measured in selected key brain regions related to spatial memory and behavioral flexibility. **Results:** Significant effects of both factors were found for spatial learning and memory abilities, associated with changes in the metabolic capacity of the prefrontal cortex, the cingulate cortex and the dorsal hippocampus. **Conclusion:** Prolonged consumption of HFS diet impaired spatial memory, but particularly behavioral flexibility. Furthermore, these effects were accompanied by increased brain metabolic capacity in the previously mentioned regions, probably related with increased neuroinflammatory mechanisms. In summary, the study shows the relevance of the sensitive developmental periods of exposure to important environmental factors for brain development and adult cognition. Finally, our results suggests that the specific combination of particular environmental factors can program adult behavior and brain metabolic capacity.

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BOARD NUMBER: S02-010

THE ROLE OF MPFC SPATIAL CODING IN SUPPORTING A CONTEXTUAL ASSOCIATION TASK

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The medial prefrontal cortex (mPFC) has a broad role in decision making and executive functions, as well as consolidation of long-term memories. These distinct functions are reconciled by the mPFC's role in context-dependent decision making, which requires the evaluation and selection of representations that are relevant for a particular task or goal. Moreover, the mPFC has recently been shown to encode spatial information in well-trained animals during tasks that rely on spatial information for correct decisions. Importantly, this spatial encoding may not be present during purely exploratory behavior. Instead, it may arise as a result of coordination between the hippocampus and mPFC while animals engage in specific spatial tasks. Our work aims to identify at what point in the learning process these spatial representations appear in the mPFC. We use 32-tetrode microdrives to record from the hippocampus and mPFC while rats learn to associate a particular food cue with a specific reward location in an 8-arm maze. Two paired cue-location associations are learned in parallel. To find the reward, the rats must flexibly adapt their behavior based on which cue is presented, i.e. which "context" they find themselves in. During the acquisition of the behavioral data, we observed a sudden jump in performance after 6-7 days of training. This shift may coincide with the appearance of spatial representations in the mPFC. Determining when mPFC spatial representations first appear during context-dependent decision making will provide insight into how behavioral demands may drive the appearance of task-relevant information in the mPFC.

Pubmed:
29754750: Sjulson L, Peyrache A, Cumpelik A, Cassataro D, Buzsáki G
Cocaine Place Conditioning Strengthens Location-Specific Hippocampal Coupling to the Nucleus Accumbens.
Conditioned place preference (CPP) is a widely used model of addiction-related behavior whose underlying mechanisms are not understood. In this study, we used dual site silicon probe recordings in freely moving mice to examine interactions between the hippocampus and nucleus accumbens in cocaine CPP. We found that CPP was associated with recruitment of D2-positive nucleus accumbens medium spiny neurons to fire in the cocaine-paired location, and this recruitment was driven predominantly by selective strengthening of coupling with hippocampal place cells that encode the cocaine-paired location. These findings provide in vivo evidence suggesting that the synaptic potentiation in the accumbens caused by repeated cocaine administration preferentially affects inputs that were active at the time of drug exposure. This provides a potential physiological mechanism by which drug use becomes associated with specific environmental contexts.
Neuron, 2018; 98

FENS Forum 2022
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BOARD NUMBER: S02-011

CORTICO-STRIATAL NETWORK GUIDING STRATEGY DEPLOYMENT IN A TRIAL AND ERROR NAVIGATION TASK

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Unsupervised spatial navigation tasks involve deployment of search strategies, which are increasingly refined presumably as a function of action-outcome learning sessions. The brain mechanisms allowing this balance between flexibility and efficiency are poorly understood, but dorsal striatum is likely implicated, possibly under the direction of cortical areas related to the hippocampus. One of these areas, the retrosplenial cortex (RSC) has been shown to have a role in spatial learning and navigation in rodents and humans. In this study we show that RSC and dorsomedial striatum (DMS) have both an essential, although different role in implementing the deployment of search strategies in the Morris Water Maze. In particular, we demonstrate that RSC acts upstream of DMS to provide an instructive signal directing the relative use of different strategies in response to the goal and the knowledge of the task. On the other hand, activity in DMS is necessary for a fast learning progression and is more directly linked to the action. Moreover, the function of DMS is strongly dependent on validation, thus providing a fundamental feedback mechanism that helps promoting the gradual refinement of search strategies.

Pubmed:

34058387: Parrini M, Naskar S, Alberti M, Colombi I, Morelli G, Rocchi A, Nanni M, Piccardi F, Charles S, Ronzitti G, Mingozzi F, Contestabile A, Cancedda L
Restoring neuronal chloride homeostasis with anti-NKCC1 gene therapy rescues cognitive deficits in a mouse model of Down syndrome.
A common feature of diverse brain disorders is the alteration of GABA-mediated inhibition because of aberrant, intracellular chloride homeostasis induced by changes in the expression and/or function of chloride transporters. Notably, pharmacological inhibition of the chloride importer NKCC1 is able to rescue brain-related core deficits in animal models of these pathologies and in some human clinical studies. Here, we show that reducing NKCC1 expression by RNA interference in the Ts65Dn mouse model of Down syndrome (DS) restores intracellular chloride concentration, efficacy of gamma-aminobutyric acid (GABA)-mediated inhibition, and neuronal network dynamics in vitro and ex vivo. Importantly, adeno-associated virus (AAV)-mediated, neuron-specific NKCC1 knockdown in vivo rescues cognitive deficits in diverse behavioral tasks in Ts65Dn animals. Our results highlight a mechanistic link between NKCC1 expression and behavioral abnormalities in DS mice and establish a molecular target for new therapeutic approaches, including gene therapy, to treat brain disorders characterized by neuronal chloride imbalance.
Mol Ther, 2021; 29
31551698: Vagni P, Perlini LE, Chenais NAL, Marchetti T, Parrini M, Contestabile A, Cancedda L, Ghezzi D
Gene Editing Preserves Visual Functions in a Mouse Model of Retinal Degeneration.
Inherited retinal dystrophies (IRDs) are a large and heterogeneous group of degenerative diseases caused by mutations in various genes. Given the favorable anatomical and immunological characteristics of the eye, gene therapy holds great potential for their treatment. Our goal is to validate the preservation of visual functions by viral-free homology directed repair (HDR) in an autosomal recessive loss of function mutation. We used a tailored gene editing system based on clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) to prevent retinal photoreceptor death in the retinal degeneration 10 (Rd10) mouse model of retinitis pigmentosa. We tested the gene editing tool and then used subretinal electroporation to deliver it to one of the retinas of mouse pups at different stages of photoreceptor differentiation. Three months after gene editing, the treated eye exhibited a higher visual acuity compared to the untreated eye. Moreover, we observed preservation of light-evoked responses both in explanted retinas and in the visual cortex of treated animals. Our study validates a CRISPR/Cas9-based therapy as a valuable new approach for the treatment of retinitis pigmentosa caused by autosomal recessive loss-of-function point mutations.
Front Neurosci, 2019; 13
29203796: Parrini M, Ghezzi D, Deidda G, Medrihan L, Castroflorio E, Alberti M, Baldelli P, Cancedda L, Contestabile A
Aerobic exercise and a BDNF-mimetic therapy rescue learning and memory in a mouse model of Down syndrome.

Down syndrome (DS) is caused by the triplication of human chromosome 21 and represents the most frequent genetic cause of intellectual disability. The trisomic Ts65Dn mouse model of DS shows synaptic deficits and reproduces the essential cognitive disabilities of the human syndrome. Aerobic exercise improved various neurophysiological dysfunctions in Ts65Dn mice, including hippocampal synaptic deficits, by promoting synaptogenesis and neurotransmission at glutamatergic terminals. Most importantly, the same intervention also prompted the recovery of hippocampal adult neurogenesis and synaptic plasticity and restored cognitive performance in trisomic mice. Additionally, the expression of brain-derived neurotrophic factor (BDNF) was markedly decreased in the hippocampus of patients with DS. Since the positive effect of exercise was paralleled by increased BDNF expression in trisomic mice, we investigated the effectiveness of a BDNF-mimetic treatment with 7,8-dihydroxyflavone at alleviating intellectual disabilities in the DS model. Pharmacological stimulation of BDNF signaling rescued synaptic plasticity and memory deficits in Ts65Dn mice. Based on our findings, Ts65Dn mice benefit from interventions aimed at promoting brain plasticity, and we provide evidence that BDNF signaling represents a potentially new pharmacological target for treatments aimed at rescuing cognitive disabilities in patients with DS.
Sci Rep, 2017; 7

25774849: Deidda G, Parrini M, Naskar S, Bozarth IF, Contestabile A, Cancedda L

Reversing excitatory GABAAR signaling restores synaptic plasticity and memory in a mouse model of Down syndrome.
Down syndrome (DS) is the most frequent genetic cause of intellectual disability, and altered GABAergic transmission through Cl(-)-permeable GABAA receptors (GABAARs) contributes considerably to learning and memory deficits in DS mouse models. However, the efficacy of GABAergic transmission has never been directly assessed in DS. Here GABAAR signaling was found to be excitatory rather than inhibitory, and the reversal potential for GABAAR-driven Cl(-) currents (ECl) was shifted toward more positive potentials in the hippocampi of adult DS mice. Accordingly, hippocampal expression of the cation Cl(-) cotransporter NKCC1 was increased in both trisomic mice and individuals with DS. Notably, NKCC1 inhibition by the FDA-approved drug bumetanide restored ECl, synaptic plasticity and hippocampus-dependent memory in adult DS mice. Our findings demonstrate that GABA is excitatory in adult DS mice and identify a new therapeutic approach for the potential rescue of cognitive disabilities in individuals with DS.
Nat Med, 2015; 21

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BOARD NUMBER: S02-012

IMMEDIATE EARLY GENE EXPRESSION OF PREVIOUS KNOWLEDGE NETWORKS OF THE HEXMAZE: A LARGE NAVIGATIONAL TASK FOR RODENTS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Which brain regions play a key role in updating previous knowledge in a large navigational task? We studied the immediate early gene (IEG) expression of *c-Fos* and *Arc* in rodents trained in a large hexagonal maze (HexMaze, 2mX2m) for three months. We studied the IEG expression in medial prefrontal cortex, hippocampal structures, posterior parietal and retrosplenial cortex of mice and rats and compared the expression of IEGs during the first and fourth session of an update of a goal location after the animals were trained for three months on the maze and compared this to early training (2nd day on the maze) as well as a homecage control. We set up a double-labeling immunohistochemistry protocol and used widefield microscope and image data analysis with FIJI/ImageJ and Ilastik to count *Arc*+ and *c-Fos*+ cells. Our result indicate different brain regions show varying *Arc* and *cFOS* expression the different conditions. Overall, this effect of condition was especially pronounced in hippocampal regions as well as prelimbic and parietal cortex. In sum, we could show that previous knowledge will influence where in the brain memories are being consolidated.

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BOARD NUMBER: S02-013

CHOOSING MEMORY RETRIEVAL STRATEGIES: A CRITICAL ROLE FOR INHIBITION IN THE DENTATE GYRUS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

Alice Weiglein[1], Iris Müller[2], Gürsel Çalışkan[2], Oliver Stork[2], Anne Albrecht[1]
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Remembering the location of a worthwhile food source is not only crucial for survival but also a major faculty of neural networks. Humans and rodents alike exert two distinct strategies to learn and retrieve reward-featuring locations. The cue response strategy is based on striatal stimulus-response, while the spatial strategy depends on the hippocampus. Especially rodents appear to prefer a spatial strategy. When being stressed, however, a shift to the stimulus-response strategy is observed. Using transgenic mice susceptible to stress due to a lack of the GABA-synthesizing enzyme GAD65, we investigated whether a reduction of spatial learning preference could be validated. The respective mice were assessed for their memory performance in a dual solution task for which they could learn the location of a food reward in an open field setup utilizing either one of the above-described strategies via proximal or distal cues, respectively. Interestingly, GAD65 knock out mice lacked a spatial preference during retrieval, but showed undisrupted spatial retrieval when no proximal cue was provided. Regarding the neural activation marker cFos, a shift in the co-activation of the hippocampal dentate gyrus (DG) with frontal cortical brain areas was demonstrated. Further, a shRNA-mediated local knock down of GAD65 within the DG replicated the behavioral effect, thus pinpointing a central role of the dorsal DG for retrieval strategies, particularly modulating strategy choices.

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BOARD NUMBER: S02-014

DEGREE OF CONTEXTUAL MEMORY IS ENCODED BY PLACE CELLS REMAPPING

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Place cells (PC) are hippocampal neurons that are tuned to spatial location and are able to change their tuning when sensory inputs change (remapping). As a population, PCs are thought to form a cognitive map of events, providing the spatial dimension of episodic memory. Moreover, it has been suggested that the hippocampal ability of storing and distinguishing between different situations and contexts can be related with PC's remapping. Several studies have shown how PC can either remap or not as a consequence of changes in the environment. However, it is still unclear the role that PC have in episodic memory. The aim of this project is to understand how the PC activity of hippocampal's CA3 region correlates with the evocation of contextual memories. To tackle this question we performed electrophysiological recordings in CA3 while the animal was performing a task that allows us to discriminate if it recognizes a context as new, or as one it already knows. We found a significant correlation between CA3 PC activity and the memory that the animal is recalling. In particular the amount of remapping and the spatial correlation of PC activity between contexts is related with the animal's behavioral output. These results suggest that PC activity not only is important for spatial navigation but also for evocation of contextual memories.

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BOARD NUMBER: S02-015

NEURAL MECHANISMS OF SPATIAL MEMORY DEVELOPMENT

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Although as adults we can recall decades-old episodic/spatial memories with high precision this ability is not mature at birth; a phenomenon known as infantile amnesia. IA is thought to reflect the protracted development of the neuronal networks supporting memory recall and/or consolidation. Hippocampal replay – time-compressed reactivation of hippocampal cell sequences reflecting wakeful experiences – is a mechanism thought to support both these memory functions. Thus, we asked whether the delayed development of memory reflects the delayed maturation of replay. To this end, we recorded from large (~20-50) ensembles of hippocampal principal cells from developing (2-4weeks) rat pups while the pups engaged in a hippocampal-dependent working memory (WM) task and during rest. We observed the pups' performance on the task improved with age, reaching above-chance levels by P22. However, although the average performance improved gradually, individual pups displayed abrupt, over-night improvements in performance at different ages; showing the timing of WM emergence does not purely depend on learning time. Thus, we sought to investigate if the development of hippocampal reactivations mirrors these individual WM development profiles. *Preliminary* results show that although *candidate* hippocampal replay events become gradually more adult-like with age they also display a sharp transition in maturity at the time WM abruptly emerges. Further analyses are underway to elucidate the relationship between WM and replay development further. These results suggest the emergence of (working) memory may rely on the late development of replay and these findings indicate hippocampal replay as a prerequisite for different memory types and for planning.

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BOARD NUMBER: S02-016

INTERPLAY BETWEEN SLEEP AND NOVELTY IN SEMANTIC-LIKE MEMORY PROCESSING

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Memories are thought to be initially encoded in the hippocampus and then integrated into the neocortex for long term storage. Sleep has been shown to play a crucial role in this consolidation process. However, the consolidation of one of a kind, novel memories (i.e. flashbulb memories) might be differently regulated. Novel experiences are known to strengthen memories encoded surrounding the event. We hypothesize that memories tagged with a novel experience get retained in the hippocampus with all the episodic details for the long-term, in contrast sleep following learning should lead to more cortical memory consolidation. Rats and mice were trained in the Object Space Task to evaluate their performance on cumulative memory expression (i.e. semantic-like memory). In addition, they were trained in an adapted version of the task wherein an interference trial was included 24 hours after training followed by novelty and/or sleep deprivation. Memory retrieval was then tested at 24/72 hours. Finally, they were sacrificed at different timepoints and brain samples were collected for IEG expression analyses Both rats and mice showed cumulative memory expression and remembered the original location configuration if they were allowed to sleep after training. However, when interference was followed by a novelty exposure, the animals remembered the interference trial and not the previous cumulative memory.. Our results validate the notion that novelty exposure strengthens event memories while sleep is important for cumulative memory processing.

Pubmed:
34037203: Samanta A, van Rongen LS, Rossato JI, Jacobse J, Schoenfeld R, Genzel L
Sleep Leads to Brain-Wide Neural Changes Independent of Allocentric and Egocentric Spatial Training in Humans and Rats.
Sleep is important for memory consolidation and systems consolidation in particular, which is thought to occur during sleep. While there has been a significant amount of research regarding the effect of sleep on behavior and certain mechanisms during sleep, evidence that sleep leads to consolidation across the system has been lacking until now. We investigated the role of sleep in the consolidation of spatial memory in both rats and humans using a watermaze task involving allocentric- and egocentric-based training. Analysis of immediate early gene expression in rodents, combined with functional magnetic resonance imaging in humans, elucidated similar behavioral and neural effects in both species. Sleep had a beneficial effect on behavior in rats and a marginally significant effect in humans. Interestingly, sleep led to changes across multiple brain regions at the time of retrieval in both species and in both training conditions. In rats, sleep led to increased gene expression in the hippocampus, striatum, and prefrontal cortex. In the humans, sleep led to an activity increase in brain regions belonging to the executive control network and a decrease in activity in regions belonging to the default mode network. Thus, we provide cross-species evidence for system-level memory consolidation occurring during sleep.
Cereb Cortex, 2021; 31
34135006: Alonso A, Bokeria L, van der Meij J, Samanta A, Eichler R, Lotfi A, Spooner P, Navarro Lobato I, Genzel L
The HexMaze: A Previous Knowledge Task on Map Learning for Mice.
New information is rarely learned in isolation; instead, most of what we experience can be incorporated into or uses previous knowledge networks in some form. Previous knowledge in form of a cognitive map can facilitate knowledge acquisition and will influence how we learn new spatial information. Here, we developed a new spatial navigation task where food locations are learned in a large, gangway maze to test how mice learn a large spatial map over a longer time period-the HexMaze. Analyzing performance across sessions as well as on specific trials, we can show simple memory effects as well as multiple effects of previous knowledge of the map accelerating both online learning and performance increases over offline periods when incorporating new information. We could identify the following three main phases: (1) learning the initial goal location; (2) faster learning after 2 weeks when learning a new goal location; and then (3) the ability to express one-session learning, leading to long-term memory effect after 12 weeks. Importantly, we are the first to show that buildup of a spatial map is dependent on how much time passes, not how often the animal is trained.
eNeuro, 2021 Jul-Aug; 8

32531423: Schut EHS, Alonso A, Smits S, Khamassi M, Samanta A, Negwer M, Kasri NN, Navarro Lobato I, Genzel L
The Object Space Task reveals increased expression of cumulative memory in a mouse model of Kleefstra syndrome.
Kleefstra syndrome is a disorder caused by a mutation in the EHMT1 gene characterized in humans by general developmental delay, mild to severe intellectual disability and autism. Here, we characterized cumulative memory in the Ehmt1 mouse model using the Object Space Task. We combined conventional behavioral analysis with automated analysis by deep-learning networks, a session-based computational learning model, and a trial-based classifier. Ehmt1 mice showed more anxiety-like features and generally explored objects less, but the difference decreased over time. Interestingly, when analyzing memory-specific exploration, Ehmt1 show increased expression of cumulative memory, but a deficit in a more simple, control memory condition. Using our automatic classifier to differentiate between genotypes, we found that cumulative memory features are better suited for classification than general exploration differences. Thus, detailed behavioral classification with the Object Space Task produced a more detailed behavioral phenotype of the Ehmt1 mouse model.
Neurobiol Learn Mem, 2020; 173
26388493: Chakravarty S, Maitra S, Reddy RG, Das T, Jhelum P, Kootar S, Rajan WD, Samanta A, Samineni R, Pabbaraja S, Kernie SG, Mehta G, Kumar A
A novel natural product inspired scaffold with robust neurotrophic, neurogenic and neuroprotective action.
In search for drugs to treat neuropsychiatric disorders wherein neurotrophic and neurogenic properties are affected, two neurotrophically active small molecules specially crafted following natural product leads based on 2-oxa-spiro[5.5]-undecane scaffold, have been thoroughly evaluated for their neurotrophic, neurogenic and neuroprotective potential in ex vivo primary culture and in vivo zebrafish and mouse models. The outcome of in vivo investigations suggest that one of these molecules is more neurotrophic than neurogenic while the other one is more neurogenic than neurotrophic and the former exhibits remarkable neuroprotection in a mouse acute ischemic stroke model. The molecular mechanisms of action of these compounds appear to be through the TrkB-MEK-ERK-CREB-BDNF pathway as pre-treatment with neurotrophin receptor TrkB inhibitor ANA-12 and MEK inhibitor PD98059 attenuates the neurotrophic action of compounds.
Sci Rep, 2015; 5

FENS Forum 2022
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BOARD NUMBER: S02-017

THE HEXMAZE FOR MICE: HIPPOCAMPAL AND CORTICAL CONTRIBUTIONS TO SPATIAL NAVIGATION IN THE BACKGROUND OF PREVIOUS KNOWLEDGE

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Previous knowledge in the form of a cognitive map can facilitate knowledge acquisition and will influence how we learn new spatial information. Previous rat studies have shown that when memories fit to previous knowledge networks, consolidation can be very rapid, and memories can become hippocampal independent in a window of 48 hrs. However, which brain regions facilitate this effect in spatial memory is not clear yet. In the present study we perform pharmacological interventions of different brain regions at different stages of learning in a previous knowledge-based task. The mouse HexMaze is a large (2m x 2m) spatial environment, where animals learn to efficiently reach a reward location from different points of the maze. An initial 3 month build up period is followed by weekly changes to the maze, such as food location and introduction of barriers, and pharmacological inhibition takes place either during the initial build up period or during the stage of weekly updates. During weekly updates, inhibition took place either on the day of encoding, or 48 hrs later during retrieval. We replicated previous schema-effects and could show that the hippocampus was necessary for encoding of new information once the animals have extensive knowledge of the environment, but not necessary for retrieval. With this task we aim to disentangle current theories of memory consolidation taking into consideration the presence of previous knowledge.

Pubmed:

34330819: Vallianatou CA, Alonso A, Aleman AZ, Genzel L, Stella F
Learning-Induced Shifts in Mice Navigational Strategies Are Unveiled by a Minimal Behavioral Model of Spatial Exploration.
Shifts in spatial patterns produced during the execution of a navigational task can be used to track the effects of the accumulation of knowledge and the acquisition of structured information about the environment. Here, we provide a quantitative analysis of mice behavior while performing a novel goal localization task in a large, modular arena, the HexMaze. To demonstrate the effects of different forms of previous knowledge we first obtain a precise statistical characterization of animals' paths with sub-trial resolution and over different phases of learning. The emergence of a flexible representation of the task is accompanied by a progressive improvement of performance, mediated by multiple, multiplexed time scales. We then use a generative mathematical model of the animal behavior to isolate the specific contributions to the final navigational strategy. We find that animal behavior can be accurately reproduced by the combined effect of a goal-oriented component, becoming stronger with the progression of learning, and of a random walk component, producing choices unrelated to the task and only partially weakened in time.
eNeuro, 2021 Sep-Oct; 8
34135006: Alonso A, Bokeria L, van der Meij J, Samanta A, Eichler R, Lotfi A, Spooner P, Navarro Lobato I, Genzel L
The HexMaze: A Previous Knowledge Task on Map Learning for Mice.
New information is rarely learned in isolation; instead, most of what we experience can be incorporated into or uses previous knowledge networks in some form. Previous knowledge in form of a cognitive map can facilitate knowledge acquisition and will influence how we learn new spatial information. Here, we developed a new spatial navigation task where food locations are learned in a large, gangway maze to test how mice learn a large spatial map over a longer time period-the HexMaze. Analyzing performance across sessions as well as on specific trials, we can show simple memory effects as well as multiple effects of previous knowledge of the map accelerating both online learning and performance increases over offline periods when incorporating new information. We could identify the following three main phases: (1) learning the initial goal location; (2) faster learning after 2 weeks when learning a new goal location; and then (3) the ability to express one-session learning, leading to long-term memory effect after 12 weeks. Importantly, we are the first to show that buildup of a spatial map is dependent on how much time passes, not how often the animal is trained.
eNeuro, 2021 Jul-Aug; 8
32954007: Alonso A, van der Meij J, Tse D, Genzel L
Naïve to expert: Considering the role of previous knowledge in memory.

In humans, most of our new memories are in some way or another related to what we have already experienced. However, in memory research, especially in non-human animal research, subjects are often mostly naïve to the world. But we know that previous knowledge will change how memories are processed and which brain areas are critical at which time point. Each process from encoding, consolidation, to memory retrieval will be affected. Here, we summarise previous knowledge effects on the neurobiology of memory in both humans and non-human animals, with a special focus on schemas - associative network structures. Furthermore, we propose a new theory on how there may be a continuous gradient from naïve to expert, which would modulate the importance and role of brain areas, such as the hippocampus and prefrontal cortex.
Brain Neurosci Adv, 2020 Jan-Dec; 4
32531423: Schut EHS, Alonso A, Smits S, Khamassi M, Samanta A, Negwer M, Kasri NN, Navarro Lobato I, Genzel L
The Object Space Task reveals increased expression of cumulative memory in a mouse model of Kleefstra syndrome.
Kleefstra syndrome is a disorder caused by a mutation in the EHMT1 gene characterized in humans by general developmental delay, mild to severe intellectual disability and autism. Here, we characterized cumulative memory in the Ehmt1 mouse model using the Object Space Task. We combined conventional behavioral analysis with automated analysis by deep-learning networks, a session-based computational learning model, and a trial-based classifier. Ehmt1 mice showed more anxiety-like features and generally explored objects less, but the difference decreased over time. Interestingly, when analyzing memory-specific exploration, Ehmt1 show increased expression of cumulative memory, but a deficit in a more simple, control memory condition. Using our automatic classifier to differentiate between genotypes, we found that cumulative memory features are better suited for classification than general exploration differences. Thus, detailed behavioral classification with the Object Space Task produced a more detailed behavioral phenotype of the Ehmt1 mouse model.
Neurobiol Learn Mem, 2020; 173
30796293: Espinosa N, Alonso A, Lara-Vasquez A, Fuentealba P
Basal forebrain somatostatin cells differentially regulate local gamma oscillations and functionally segregate motor and cognitive circuits.
The basal forebrain delivers extensive axonal projections to the cortical mantle regulating brain states and cognitive processing. Recent evidence has established the basal forebrain as a subcortical node of the default mode network that directionally influences cortical dynamics trough gamma oscillations, yet their synaptic origin has not been established. Here, we used optogenetic stimulation and in vivo recordings of transgenic mice to show that somatostatin neurons exert an anatomically specialized role in the coordination of subcortical gamma oscillations of the rostral basal forebrain. Indeed, the spike timing of somatostatin cells was tightly correlated with gamma oscillations in the ventral pallidum, but not in the medial septum. Consequently, optogenetic inactivation of somatostatin neurons selectively disrupted the amplitude and coupling of gamma oscillations only in the ventral pallidum. Moreover, photosupression of somatostatin cells produced specific behavioral interferences, with the ventral pallidum regulating locomotor speed and the medial septum modulating spatial working memory. Altogether, these data suggest that basal forebrain somatostatin cells can selectively synchronize local neuronal networks in the gamma band directly impinging on cortical dynamics and behavioral performance. This further supports the role of the basal forebrain as a subcortical switch commanding transitions between internally and externally oriented brain states.
Sci Rep, 2019; 9
29161383: Espinosa N, Alonso A, Morales C, Espinosa P, Chávez AE, Fuentealba P
Basal Forebrain Gating by Somatostatin Neurons Drives Prefrontal Cortical Activity.
The basal forebrain provides modulatory input to the cortex regulating brain states and cognitive processing. Somatostatin-expressing neurons constitute a heterogeneous GABAergic population known to functionally inhibit basal forebrain cortically projecting cells thus favoring sleep and cortical synchronization. However, it remains unclear if somatostatin cells can regulate population activity patterns in the basal forebrain and modulate cortical dynamics. Here, we demonstrate that somatostatin neurons regulate the corticopetal synaptic output of the basal forebrain impinging on cortical activity and behavior. Optogenetic inactivation of somatostatin neurons in vivo rapidly modified neural activity in the basal forebrain, with the consequent enhancement and desynchronization of activity in the prefrontal cortex, reflected in both neuronal spiking and network oscillations. Cortical activation was partially dependent on cholinergic transmission, suppressing slow waves and potentiating gamma oscillations. In addition, recruitment dynamics was cell type-specific, with interneurons showing similar temporal profiles, but stronger responses than pyramidal cells. Finally, optogenetic stimulation of quiescent animals during resting periods prompted locomotor activity, suggesting generalized cortical activation and increased arousal. Altogether, we provide physiological and behavioral evidence indicating that somatostatin neurons are pivotal in gating the synaptic output of the basal forebrain, thus indirectly controlling cortical operations via both cholinergic and non-cholinergic mechanisms.
Cereb Cortex, 2019; 29

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BOARD NUMBER: S02-018

OPTOGENETIC ENTRAINMENT OF OSCILLATORY ACTIVITY IN THE PREFRONTAL CORTEX IMPAIR ACQUISITION OF SPATIAL MEMORY

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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During acquisition of spatial memory, subjects optimize the path to the goal across training, process supported by the prefrontal cortex (PFC). Theta oscillations (6-12 Hz) emerge in the PFC during spatial navigation, likely supporting large-scale neural communication during learning. Importantly, theta activity displays dynamical fluctuations along the behavioral task, which may represent different states of synchronization and desynchronization of neural populations as required for cortical computations. However, it is not known if this oscillatory dynamics in the PFC is necessary for spatial learning. To test this possibility, we perturbed prefrontal oscillatory dynamics during learning. Accordingly, we induced optogenetic continual entrainment of prefrontal activity at theta frequency band during training in the Barnes maze task in freely behaving mice expressing ChR2 in layer V cortical pyramidal neurons. Optogenetic stimulation at theta frequency reliably entrained neural firing and oscillatory activity in the PFC in opsin-expressing mice, but not in controls. Several measurements of oscillatory dynamics, as spectral power variation, incidence of phase resetting, theta-gamma cross-frequency coupling and multiscale entropy were significatively perturbed by optogenetic entrainment in opsin-expressing animals. Importantly, optogenetic entrainment during training sessions impaired learning performance parameters in opsin-expressing mice, as escape and nose-poke goal latency, as well as path optimization and navigation strategy progression across training. Similar behavioral impairments were observed in probe trials after training, when no optogenetic entrainment was delivered, suggesting an effect on memory formation. Altogether, our data revealed that entrainment of prefrontal activity during spatial training perturbed oscillatory dynamics and impaired memory formation.

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BOARD NUMBER: S02-019

HIPPOCAMPAL ASTROCYTES ENCODE REWARD LOCATION

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Astrocytic calcium dynamics are involved in sensory information encoding, and modulating them was shown to impact behavior. Recent studies have implicated hippocampal astrocytes in memory processes, but their real-time calcium activity in awake mice has not been investigated as of yet. We chronically imaged dozens of CA1 astrocytes using 2-photon microscopy when head-fixed mice performed a spatial task. Specifically, we trained mice to run on a linear treadmill and proceed in a virtual environment to obtain water rewards. We find that astrocytic activity persistently ramps towards the reward location in a familiar environment. When the reward location was changed in the same familiar environment or when mice were introduced to a novel context, the ramping was not apparent. Accordingly, using linear decoders we reconstructed mice location trajectories in a familiar environment from astrocyte activity alone, but could not do the same in the novel environment. Following additional training, as the mice were familiarized with the new reward location or novel context, the ramping was reestablished, suggesting that the astrocytic activity is experience dependent. This is the first indication that astrocytes are involved in spatial learning and can encode position related information in familiar contexts, thus implicating them as part of the neural computation involved in spatial processing.

Pubmed:
29804835: Adamsky A, Kol A, Kreisel T, Doron A, Ozeri-Engelhard N, Melcer T, Refaeli R, Horn H, Regev L, Groysman M, London M, Goshen I
Astrocytic Activation Generates De Novo Neuronal Potentiation and Memory Enhancement.
Astrocytes respond to neuronal activity and were shown to be necessary for plasticity and memory. To test whether astrocytic activity is also sufficient to generate synaptic potentiation and enhance memory, we expressed the Gq-coupled receptor hM3Dq in CA1 astrocytes, allowing their activation by a designer drug. We discovered that astrocytic activation is not only necessary for synaptic plasticity, but also sufficient to induce NMDA-dependent de novo long-term potentiation in the hippocampus that persisted after astrocytic activation ceased. In vivo, astrocytic activation enhanced memory allocation; i.e., it increased neuronal activity in a task-specific way only when coupled with learning, but not in home-caged mice. Furthermore, astrocytic activation using either a chemogenetic or an optogenetic tool during acquisition resulted in memory recall enhancement on the following day. Conversely, directly increasing neuronal activity resulted in dramatic memory impairment. Our findings that astrocytes induce plasticity and enhance memory may have important clinical implications for cognitive augmentation treatments.
Cell, 2018; 174
31951529: Doron A, Goshen I
Glia: The Glue Holding Memories Together.
Adult oligodendrogenesis is regulated by neuronal activity and learning. Can it affect memory processes? In this issue of Neuron, Steadman et al. (2020) found that newly generated oligodendrocytes are crucial for memory acquisition and consolidation and required for the neuronal coupling between brain regions known to be involved in memory.
Neuron, 2020; 105
28939475: Doron A, Goshen I
Investigating the transition from recent to remote memory using advanced tools.
Remote memories, weeks to decades long, are usually the ones most important to the organism, as the longevity of a memory is tightly connected to its significance. Retrograde amnesia studies in human patients as well as lesions and immediate early gene expression investigation in animal models, suggested that the hippocampus has a time dependent role in memory consolidation. Namely, that as a memory matures it becomes independent of the hippocampus and instead depends on extra-hippocampal areas. However, accumulating evidence implies that this temporal segregation is not as rigid

as originally proposed. In this review we will focus on the integration of new methods, such as chemogenetics, optogenetics and calcium imaging, which enable genetic specificity as well as high temporal and spatial resolution. Using these methods, recent studies have started to resolve the inconsistencies of past findings by observing and manipulating neural ensembles in different brain regions. We then discuss how these techniques can be applied to investigate the cellular underpinnings of memory across multiple time points, and employed to study the contribution of various cell types to remote memory.
Brain Res Bull, 2018; 141

34117643: Refaeli R, Doron A, Benmelech-Chovav A, Groysman M, Kreisel T, Loewenstein Y, Goshen I
Features of hippocampal astrocytic domains and their spatial relation to excitatory and inhibitory neurons.
The mounting evidence for the involvement of astrocytes in neuronal circuits function and behavior stands in stark contrast to the lack of detailed anatomical description of these cells and the neurons in their domains. To fill this void, we imaged >30,000 astrocytes in hippocampi made transparent by CLARITY, and determined the elaborate structure, distribution, and neuronal content of astrocytic domains. First, we characterized the spatial distribution of >19,000 astrocytes across CA1 lamina, and analyzed the morphology of thousands of reconstructed domains. We then determined the excitatory somatic content of CA1 astrocytes, and measured the distance between inhibitory neuronal somata to the nearest astrocyte soma. We find that on average, there are almost 14 pyramidal neurons per domain in the CA1, increasing toward the pyramidal layer midline, compared to only five excitatory neurons per domain in the amygdala. Finally, we discovered that somatostatin neurons are found in close proximity to astrocytes, compared to parvalbumin and VIP inhibitory neurons. This work provides a comprehensive large-scale quantitative foundation for studying neuron-astrocyte interactions.
Glia, 2021; 69

26505966: Doron A, Manassi M, Herzog MH, Ahissar M
Intact crowding and temporal masking in dyslexia.
Phonological deficits in dyslexia are well documented. However, there is an ongoing discussion about whether visual deficits limit the reading skills of people with dyslexia. Here, we investigated visual crowding and backward masking. We presented a Vernier (i.e., two vertical bars slightly offset to the left or right) and asked observers to indicate the offset direction. Vernier stimuli are visually similar to letters and are strongly affected by crowding, even in the fovea. To increase task difficulty, Verniers are often followed by a mask (i.e., backward masking). We measured Vernier offset discrimination thresholds for the basic Vernier task, under crowding, and under backward masking, in students with dyslexia (n = 19) and age and intelligence matched students (n = 27). We found no group differences in any of these conditions. Controls with fast visual processing (good backward masking performance), were faster readers. By contrast, no such correlation was found among the students with dyslexia, suggesting that backward masking does not limit their reading efficiency. These findings indicate that neither elevated crowding nor elevated backward masking pose a bottleneck to reading skills of people with dyslexia.
J Vis, 2015; 15

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BOARD NUMBER: S02-020

DISTINCT ROLES OF THE DORSAL AND VENTRAL HIPPOCAMPUS IN SPATIAL WORKING MEMORY AND IN SIGNALING SPATIAL INFORMATION TO THE MEDIAL PREFRONTAL CORTEX

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The cognitive process of spatial working memory (SWM) engages both the medial prefrontal cortex (mPFC) and the hippocampus. The interaction of the dorsal and ventral hippocampus (dHPC, vHPC) with the mPFC can be observed on the level of single cells and as local field potentials. But it is not clear in which phases of SWM - encoding, maintenance and retrieval - the two hippocampal poles are involved and which task-relevant information they transmit to the mPFC. To address these questions, we infused mice with the viral construct AAV5-CamKII-ArchT-GFP in dHPC or vHPC and trained them to perform a delayed non-match-to-sample T-maze task. Pyramidal neurons in either dHPC or vHPC were optogenetically inhibited during the different phases of SWM while simultaneously recording neuronal activity in the mPFC. Our results indicate a complementary function of the two hippocampal poles. dHPC inhibition during encoding and retrieval, but not during maintenance, strongly impaired behavioral performance. The vHPC on the other hand was only critically involved in encoding, but not in maintenance or retrieval. In the mPFC, a subpopulation of neurons was modulated both by dHPC or vHPC inhibition. Goal-selective firing of mPFC neurons was however only disrupted by vHPC inhibition during the encoding phase, and unaffected by dHPC inhibition. In contrast, mPFC neuronal encoding of the linearized position between start and goal of the maze was modified both by dHPC and vHPC inhibition. These findings indicate distinct roles of the dorsal and ventral hippocampal poles in signaling task-related spatial information to the mPFC.

Pubmed:
31665647: Babl SS, Rummell BP, Sigurdsson T
The Spatial Extent of Optogenetic Silencing in Transgenic Mice Expressing Channelrhodopsin in Inhibitory Interneurons.
Optogenetic stimulation of inhibitory interneurons has become a commonly used strategy for silencing neuronal activity. This is typically achieved using transgenic mice expressing excitatory opsins in inhibitory interneurons throughout the brain, raising the question of how spatially extensive the resulting inhibition is. Here, we characterize neuronal silencing in VGAT-ChR2 mice, which express channelrhodopsin-2 in inhibitory interneurons, as a function of light intensity and distance from the light source in several cortical and subcortical regions. We show that light stimulation, even at relatively low intensities, causes inhibition not only in brain regions targeted for silencing but also in their subjacent areas. In contrast, virus-mediated expression of an inhibitory opsin enables robust silencing that is restricted to the region of opsin expression. Our results reveal important constraints on using inhibitory interneuron activation to silence neuronal activity and emphasize the necessity of carefully controlling light stimulation parameters when using this silencing strategy.
Cell Rep, 2019; 29
33138915: van Wijngaarden JB, Babl SS, Ito HT
Entorhinal-retrosplenial circuits for allocentric-egocentric transformation of boundary coding.
Spatial navigation requires landmark coding from two perspectives, relying on viewpoint-invariant and self-referenced representations. The brain encodes information within each reference frame but their interactions and functional dependency remains unclear. Here we investigate the relationship between neurons in the rat's retrosplenial cortex (RSC) and entorhinal cortex (MEC) that increase firing near boundaries of space. Border cells in RSC specifically encode walls, but not objects, and are sensitive to the animal's direction to nearby borders. These egocentric representations are generated independent of visual or whisker sensation but are affected by inputs from MEC that contains allocentric spatial cells. Pharmaco- and optogenetic inhibition of MEC led to a disruption of border coding in RSC, but not vice versa, indicating allocentric-to-egocentric transformation. Finally, RSC border cells fire prospective to the animal's next motion, unlike those in MEC, revealing the MEC-RSC pathway as an extended border coding circuit that implements coordinate transformation to guide navigation behavior.
Elife, 2020; 9


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BOARD NUMBER: S02-021

EVENT STRUCTURE SCULPTS LATERAL ENTORHINAL DYNAMICS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The lateral entorhinal cortex (LEC) is a multisensory hub that processes information about objects and events which is formed into episodic memories in the hippocampus. How the moment-to-moment changes in an animal's experience are represented by LEC neural activity has remained elusive, in part because its anatomical position makes it difficult to access with high-density recording methods. In addition, many LEC neurons do not exhibit clear functional correlates in standard foraging experiments, as they do in the medial entorhinal cortex (MEC) and hippocampus. To overcome these challenges, we implanted newly developed multi-shank Neuropixels probes in rats to simultaneously record hundreds to thousands of units in LEC during various free behaviors and reward-based tasks. We simultaneously recorded from other areas including MEC and hippocampus for direct comparison. First, we found robust temporal coding in the dynamics of LEC activity that arose instantaneously during novel experience to support one-shot memory formation. Second, multiple behavioral timescales were encoded without interference to serve memories with different temporal resolutions. Third, temporal coding evolved relative to event structure, with salient events causing large changes in the population activity. Finally, such temporal coding was not observed in MEC and hippocampus. In ongoing work, we systematically manipulate event structure to test how these changes are reflected in LEC. Together, our results suggest that LEC may uniquely support episodic memory through one-shot encoding of experience at multiple behaviorally relevant timescales. In addition, these findings provide insight into how a continuous stream of multisensory information is processed into discrete events.

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BOARD NUMBER: S02-022

NEURONAL DISCRIMINATION OF VISUAL ENVIRONMENTS DIFFERENTIALLY DEPENDS ON BEHAVIOURAL CONTEXT IN THE HIPPOCAMPUS AND THE NEOCORTEX

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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To guide memory-based behavioural decisions, the hippocampus needs to discriminate between distinct sensory environments. Before reaching the hippocampus, sensory information is initially processed in sensory cortices. We hypothesised that primary sensory cortices provide a faithful representation of the sensory environment to distributed brain regions, whereas the hippocampus produces a cognitive map that is weighted according to the behavioural relevance of the sensory inputs. To test this hypothesis, we aimed to determine how complex sensory stimuli differentially depend on the behavioural context in the primary visual cortex (V1), CA1 and the dentate gyrus (DG). We performed two-photon calcium imaging of head-fixed mice navigating in a virtual-reality linear tract. Mice were exposed to alternating environments by changing visual textures along the virtual corridor. During active navigation, movements in the virtual environment were controlled by the animal motion on a running wheel. By contrast, in a passive open-loop condition, the visual scene was completely uncoupled from animal locomotion. We found that a binary probabilistic decoder could predict the environment being explored based on the neuronal activity in all regions during active navigation. During passive exposure, decoding accuracy remained high in the visual cortex while it decreased to chance level in the hippocampus (accuracy [active-passive] in V1: 92-77%; DG: 65-51%, CA1: 61-52%). Task engagement is therefore necessary for neuronal discrimination in the hippocampus but not in the visual cortex, suggesting that primary sensory cortices serve as robust general-purpose discriminators of sensory inputs, while the hippocampus selectively discriminates behaviourally relevant inputs.

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BOARD NUMBER: S02-023

NEURAL SIGNATURES OF CONTEXTUAL LEARNING STRATEGIES

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Like humans and primates, rats can employ various learning strategies to meet the demands of a task, depending on its complexity. It is still unclear, however, how memory representations evolve in different individuals throughout learning. Here we used in vivo electrophysiological recordings in rats, in the CA1 area of the hippocampus, to monitor the formation of representations related to context and reward-position associations. We recorded local field potentials, cell activity, and the animal's position during a spatial memory task, in which performance was assessed by animals 'digging' at correct reward locations. Contextual cues determined one reward location, whilst a second reward was context-independent. Context-reward associations were learned by all animals in 3-4 days, yet individual learning curves differed considerably. Whilst some animals improved performance in all contextual categories simultaneously over days, others initially learned a single association and only later learned the remaining ones. We then investigated whether the hippocampal representation of context differed between animals. At the neural population level, by day four, all animals showed a small but significant decorrelation between contexts around context-dependent reward positions. This was not observed in other maze positions, including the context-independent reward location. However, in earlier learning, the representation similarity was less homogenous between individuals. Our data demonstrate that individual animals can exhibit different learning strategies, yet ultimately, the hippocampal cognitive map can reliably code for both contextual-dependent representations and context-independent ones.

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BOARD NUMBER: S02-024

INCREASED CORTICAL PLASTICITY ENHANCES ONE-TRIAL MEMORY BUT LEADS TO INCREASED INTERFERENCE OF SEMANTIC-LIKE MEMORY AND CHANGES IN NONREM SLEEP OSCILLATIONS IN RATS.

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

Adrian Aleman-Zapata, Irene Navarro-Lobato, Shekhar Narayanan, Milan Bogers, Kopal Agarwal, Pelin Özsezer, Anumita Samanta, Alejandra Alonso, Lisa Genzel
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AIM: To investigate the effects of increased cortical plasticity on one-trial and semantic memories respectively, as well as to describe its effect on the cortical and hippocampal mechanisms involved in memory consolidation during NonREM sleep. **METHODS:** Cortical plasticity was increased by overexpressing the $RGS14_{414}$ gene in the prelimbic cortex of rats. The rodents were trained to perform the Object Space task to evaluate their performance for one-trial and semantic memories. Tetrodes were implanted in the medial prefrontal cortex and the hippocampus to record local field potentials and spiking activity. Sleep stages, sleep oscillations and the spiking activity of cortical pyramidal neurons during sleep were quantified and analyzed. **RESULTS:** $RGS14_{414}$ rats showed a significantly higher discrimination index compared to the control rats during the one-trial memory condition. In contrast, $RGS14_{414}$ rats had increased interference effect in the semantic-like memory condition. $RGS14_{414}$ rats displayed a decrease of NonREM sleep, accompanied by significant changes in sleep oscillations, as well as a shift in the phase-coupling of spindles and ripples with slow oscillations. $RGS14_{414}$ cortical pyramidal neurons exhibited significantly lower firing rates and less phase-locking to slow oscillations. **CONCLUSION:** These results confirm and have implications in multiple theories and hypotheses on sleep, memory and plasticity. The complementary learning-systems theory is confirmed with the interference of semantic-like memory in $RGS14_{414}$. Changes in delta waves correspond to the synaptic homeostasis hypothesis. Finally, our results replicate the finding that high-plasticity neurons show slower firing rates (Grosmark & Buzsáki, Science, 2016).

Pubmed:
34913214: Aleman-Zapata A, van der Meij J, Genzel L
Disrupting ripples: Methods, results, and caveats in closed-loop approaches in rodents.
Hippocampal ripple oscillations have been associated with memory reactivations during wake and sleep. These reactivations should contribute to working memory and memory consolidation respectively. In the past decade studies have moved from being observational to actively disrupting ripple-related activity in closed-loop approaches to enable causal investigations into their function. All together these studies have been able to provide evidence that wake, task-related ripple activity is important for working memory and planning but less important for stabilisation of spatial representations. Rest and sleep-related ripple activity, in contrast, is important for long-term memory performance and thus memory consolidation. In this review, we summarise results from different closed-loop approaches in rodents. Further, we highlight differences in detection and stimulation methods as well as controls and discuss how these differences could influence outcomes.
J Sleep Res, 2021;
26313560: Sensinger J, Aleman-Zapata A, Englehart K
Do Cost Functions for Tracking Error Generalize across Tasks with Different Noise Levels?
Control of human-machine interfaces are well modeled by computational control models, which take into account the behavioral decisions people make in estimating task dynamics and state for a given control law. This control law is optimized according to a cost function, which for the sake of mathematical tractability is typically represented as a series of quadratic terms. Recent studies have found that people actually use cost functions for reaching tasks that are slightly different than a quadratic function, but it is unclear which of several cost functions best explain human behavior and if these cost functions generalize across tasks of similar nature but different scale. In this study, we used an inverse-decision-theory technique to reconstruct the cost function from empirical data collected on 24 able-bodied subjects controlling a myoelectric interface. Compared with previous studies, this experimental paradigm involved a different control source (myoelectric control, which has inherently large multiplicative noise), a different control interface (control signal was mapped to cursor velocity), and a different task (the tracking position dynamically moved on the screen throughout each trial). Several cost functions, including a linear-quadratic; an inverted Gaussian, and a power function, accurately described the behavior of subjects throughout this

experiment better than a quadratic cost function or other explored candidate cost functions ($p<0.05$). Importantly, despite the differences in the experimental paradigm and a substantially larger scale of error, we found only one candidate cost function whose parameter was consistent with the previous studies: a power function (cost $\propto$ error$^{\alpha}$) with a parameter value of α = 1.69 (1.53-1.78 interquartile range). This result suggests that a power-function is a representative function of user's error cost over a range of noise amplitudes for pointing and tracking tasks.
PLoS One, 2015; 10

34330819: Vallianatou CA, Alonso A, Aleman AZ, Genzel L, Stella F
Learning-Induced Shifts in Mice Navigational Strategies Are Unveiled by a Minimal Behavioral Model of Spatial Exploration. Shifts in spatial patterns produced during the execution of a navigational task can be used to track the effects of the accumulation of knowledge and the acquisition of structured information about the environment. Here, we provide a quantitative analysis of mice behavior while performing a novel goal localization task in a large, modular arena, the HexMaze. To demonstrate the effects of different forms of previous knowledge we first obtain a precise statistical characterization of animals' paths with sub-trial resolution and over different phases of learning. The emergence of a flexible representation of the task is accompanied by a progressive improvement of performance, mediated by multiple, multiplexed time scales. We then use a generative mathematical model of the animal behavior to isolate the specific contributions to the final navigational strategy. We find that animal behavior can be accurately reproduced by the combined effect of a goal-oriented component, becoming stronger with the progression of learning, and of a random walk component, producing choices unrelated to the task and only partially weakened in time.
eNeuro, 2021 Sep-Oct; 8

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BOARD NUMBER: S02-025

INHIBITING THE DIRECT INPUTS FROM THE DORSAL SUBICULUM TO THE RETROSPLENIAL CORTEX IMPAIRS SPATIAL MEMORY IN THE RAT

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Background and Aims: The dorsal subiculum is the primary source of hippocampal projections to the granular retrosplenial cortex. Although, both regions have been repeatedly implicated in spatial memory and navigation, it remains unclear how the regions interact or if functional control is from the hippocampus to the retrosplenial cortex. The aim of the present experiment was to address this issue and to investigate the functional consequences of selectively inhibiting these projections in the rat. **Methods:** To address the issue, adeno-associated virus encoding either inhibitory designer-receptor exclusively activated by designer drugs (iDREADDs) or GFP control, was injected in the dorsal subiculum in two separate groups of adult male rats. These preparations were combined with intracerebral infusions of clozapine or saline within the retrosplenial cortex. Animals were tested on the reinforced T-maze alternation task, using five experimental manipulations that differently taxed the use of intra-maze, extra-maze, and egocentric cues. **Results and Conclusions:** iDREADDs mediated disruption of these efferent projections seemed sufficient to impair spatial working memory, particularly when intra-maze and extra-maze cues were incongruent or when animals were required to switch between different strategies to solve the mazes. Cumulatively, these findings suggest that the direct projections from the dorsal subiculum to the granular retrosplenial cortex, may be key for successful integration of visual spatial cues.

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BOARD NUMBER: S02-026

AWAKE HIPPOCAMPAL REPLAY IS NOT REQUIRED FOR SHORT-TERM MEMORY

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Short-term memory (STM) on a time scale of seconds to minutes is required to successfully perform day-to-day tasks, for example when engaging in a meaningful conversation. Previous studies in both rodents and primates have correlated hippocampal cellular activity and behavioural expression of STM. This research has led to models describing the putative neural mechanism in the hippocampus that mediate STM. In these models, a key role has been given to hippocampal replay – reactivation of neurons representing a trajectory through space– but definitive causal evidence that can challenge or confirm the model is missing. In this study, we aimed to address the uncertainty around the role of awake replay in STM by collecting direct causal evidence from behaving rats. Signatures of replay events were detected in the hippocampus and disrupted using electrical stimulation of the ventral hippocampal commissure in rats that were trained on three different spatial memory tasks in a multi-arm radial maze. All tasks required memory of the recent past, but varied in the time scale over which information needed to be retained: (1) a multiple trial match-to sample task, (2) a single trial non-match to sample task and (3) a spatial sequence memory paradigm. Rats readily learned the task rules, but disruption of awake replay did not affect task performance or other behavioural measures in any of the task. Altogether, our results show for the first time with definitive causal evidence that awake replay is not involved in STM of events or of their temporal order.

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BOARD NUMBER: S02-027

A SYNAPTIC SIGNAL FOR NOVELTY PROCESSING IN THE HIPPOCAMPUS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Episodic memory formation and recall are complementary processes that put conflicting requirements on neuronal computations in the hippocampus. How this challenge is resolved in hippocampal circuits is unclear. To address this question, we obtained in vivo whole-cell patch-clamp recordings from dentate gyrus granule cells in head-fixed mice trained to explore and distinguish between familiar and novel virtual environments. We find that granule cells consistently show a small transient depolarization of their membrane potential upon transition to a novel environment. A computational model suggests that the observed transient synaptic response to novel environments may lead to a bias in the granule cell population activity, which can, in turn, drive the downstream attractor networks to a new state, thereby favoring the switch from generalization to discrimination when faced with novelty. Such a novelty-driven switch may enable flexible encoding of new memories while preserving stable retrieval of familiar ones.

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BOARD NUMBER: S02-028

SPATIAL AND REWARD CODING OF DORSAL HIPPOCAMPUS PROJECTION NEURONS TO THE NUCLEUS ACCUMBENS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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[1]German Center for Neurodegenerative Diseases, Neuronal Networks Group, Bonn, Germany, [2]Leibniz Institute for Neurobiology, Cellular Neuroscience, Magdeburg, Germany

The hippocampal formation is a brain structure crucial for spatial navigation. Many neurons in the dorsal hippocampus (dHPC) show position-selective tuning characteristics. These place cells are thought to be part of a ‘cognitive map’ that animals can use to navigate their environment. How such a hippocampal map is used by downstream regions to drive goal-directed navigation behaviour, however, remains unclear. The hippocampus sends extensive projections to the nucleus accumbens (NAc), a basal ganglia structure involved in reward-related behaviours. Indeed, dHPC shows functional coupling to NAc in reward contexts, and dHPC>NAc projection neurons are required for conditioned place preference. Yet, it is unknown which information is conveyed by individual dHPC>NAc projection neurons. To visualise hippocampal neuronal activity in behaving animals, we used mice expressing the calcium indicator GCaMP6s pan-neuronally, and implanted a hippocampal window for optical access. To visualise and compare the activity of dHPC>NAc projection neurons with the general dHPC population, we used a retro-Cre/mCherry intersectional approach. Head-fixed food-restricted mice were trained to run on a textured belt to receive milk in a fixed reward zone. Dual-colour two-photon calcium imaging of hippocampal neurons in trained animals shows that, while dHPC>NAc projection neurons showed similar reward-related tuning characteristics, their spatial tuning was enhanced compared to the rest of the dHPC population. dHPC>NAc projection neurons contained a higher proportion of place cells that showed greater reliability and a higher density of spatial information content. These results suggest that dHPC conveys a robust and dense spatial code to the NAc.

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THE MORE THE MERRIER: IS MORE THAN ONE TRIAL NECESSARY FOR ACCURATE NAVIGATIONAL STRATEGY ASSESSMENT IN A DUAL-SOLUTION PLUS MAZE?

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Rodents navigate using different strategies. The dual-solution plus maze is designed to assess whether an animal is using place (following a flexible representation or a cognitive map of the space) or response (following a sequence of actions to go to a specific place) strategy. This is done by training the animal in a plus-shaped maze with one of the arms blocked (turning it into a T-maze) and probing which navigational strategy the animal is using when releasing it from the opposite previously blocked arm to the start. Animals have been classified as place or response learners in this task based on a single probe trial. This work aims to investigate whether classifying the navigation strategy based on one probe trial is representative of the strategy the animal uses for most trials. For this we trained BL6 mice (n=7) in a plus maze with one of the arms blocked for 6 days (10 correct trials per day) and probed them on the opposite arm for ten trials. We compared the number of total place and response trials with the first trial. All animals showed more response than place trials. The two animals that showed place strategy on the first trial had the lowest response trials number. Since most protocols for this test base the classification of place/response strategy from one single probe trial, we wonder from our results whether the animals demonstrating “place strategy” isn’t simply a byproduct of their error rate on the task rather than their actual navigation strategy.

Pubmed:
29277591: Andreoli L, Simplício H, Morya E
Egg Model Training Protocol for Stereotaxic Neurosurgery and Microelectrode Implantation.
Neuroscience research uses neurosurgery in animal models for several experimental techniques. To our knowledge, there is no published method for small animal neurosurgery training. Based on the similar thickness of chicken eggshells and mouse, rat, and some small primate skulls, here we propose an egg model training protocol for stereotaxic surgery.
World Neurosurg, 2018; 111

FENS Forum 2022
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BOARD NUMBER: S02-030

CLAUSTRUM LESIONS LEAD TO CHANGES IN BEHAVIOURAL STRATEGY DURING REVERSAL LEARNING IN A SPATIAL MEMORY TASK

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The Claustrum (CLA) is a small subcortical region located between the insula and the putamen. Even though it has been shown that the CLA is directly connected to key structures that regulate memory processing such as prefrontal (PFC), anterior cingulate (ACC), retrosplenial (RSC) and entorinal (MEC) cortex, its potential role in memory-related mechanisms has yet to be explored. Thus, we aim to investigate whether the CLA is involved in memory processes by focusing on spatial memory. To test this, we specifically lesioned CLA cells projecting to PFC, ACC, RSC and MEC via stereotaxic injections an of apoptosis-inducing virus. Two weeks later, lesioned and control mice were placed on a modified Barnes maze. Our 10-day protocol included 4 days of training, 3 days reversal training and ending with a probe trial 24h following the end of each training sessions. Control and lesioned mice exhibited similar behavior and were capable of spatial learning and spatial memory during the training phase and the probe test, respectively. However, during the reversal training phase, lesioned mice favoured spatial strategy, whereas control mice used preferentially serial strategy. Nevertheless, during the reversal probe trial, there was no significant difference in spatial memory between both groups. To conclude, lesioning the CLA seems to affect behavioural strategy choice while not having a direct impact on spatial memory.

Pubmed:
30273829: Cattaud V, Bezzina C, Rey CC, Lejards C, Dahan L, Verret L
Early disruption of parvalbumin expression and perineuronal nets in the hippocampus of the Tg2576 mouse model of Alzheimer's disease can be rescued by enriched environment.
Recent findings show that parvalbumin (PV) interneuron function is impaired in Alzheimer's disease (AD), and that this impairment in PV function can be linked to network dysfunction and memory deficits. PV cells are often associated with a specific extracellular matrix, the perineuronal net (PNN). PNNs are believed to protect PV cell integrity, and whether the amyloidopathy affects PNNs remains unclear. Here, we evaluated the number of PV cells with and without PNNs in the hippocampus of the Tg2576 mouse model of AD at different stages of the disease. We show a deficit of PV+ and/or PV+/PNN+ cells in the areas CA1, CA2, and CA3 in Tg2576 as young as 3 months of age. Importantly, transient exposure to an enriched environment, which has proven long-lasting beneficial effects on memory in AD subjects, rescues the PV/PNN cell number deficits. We conclude that cognitive improvements induced by enriched environment in AD mouse models could be supported by a remodeling of hippocampal PV cell network and their PNNs.
Neurobiol Aging, 2018; 72

FENS Forum 2022
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BOARD NUMBER: S02-031

TEMPORAL INSTABILITY OF CFOS EXPRESSION IN THE DENTATE GYRUS AS A MECHANISM FOR PATTERN SEPARATION

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The use of TetTag mice has advanced the understanding of memory acquisition and recall over the last 10 years. These mice allow for identification and control of specific ensembles that express plasticity-related transcription factors involved in memory formation (i.e. cFos) in several brain regions including the hippocampus. The dentate gyrus (DG) of the hippocampus is crucial for spatial memory tasks like the Morris water maze (WM). It is not clear, however, how a subset of $cFos^+$ neurons (engram cells) integrate the spatial information over the course of WM training. We found that despite the increase in spatial memory precision, cFos expression patterns were temporally unstable in granule cells (GCs). Optogenetic inhibition $cFos^+$ GCsof the first training day, impaired memory recall on subsequent days even though these cells did not express cFos again. Using CaMPARI, we identified that $cFos^+$ GCs were active when mice re-visit the WM tank but not a novel environment. These data challenge the notion that cFos re-expression in the same neuronal ensemble is a readout of memory recall. Additionally, we found that the observed cFos temporal instability is mediated via a different transcription factor (FosB/ΔFosB) in an inhibitory feedback loop fashion. Altogether, our results suggest that the temporal shift of cFos expression in GCs is a mechanism of temporal discrimination of past experiences

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BOARD NUMBER: S02-032

INVOLVEMENT OF THE CEREBELLAR MOLECULAR LAYER INTERNEURONS IN SEQUENCE-BASED NAVIGATION: DEVELOPMENT OF A COMPLEX NAVIGATION BEHAVIORAL TASK

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Learning and execution of sequence-based navigation involves activation of the posterior cerebellum, in particular lobule VI in mice. In a simple Y-maze paradigm, where animals need to remember the location of a reward, the chemogenetic inactivation of Molecular Layer Interneurons (MLIs) in lobule VI delayed the acquisition of the task. However, the role of MLIs in a more complex sequence-based navigation has not been addressed yet. The purpose of this work is to understand how cerebellar MLIs in Lobule VI contribute to the learning of complex sequence-based navigation. To answer this question, we developed a behavioral task in which mice learn a sequence of three successive turns to obtain a stimulation of the Medial Forebrain Bundle as a reward. Mice are first evaluated in a place preference task to determine the optimal intensity of the reward, then trained every day for seven days, 4 sessions a day. Our preliminary results show that control mice properly acquire the task, achieving more than 75% success within 7 days. To evaluate the role of MLIs in cerebellar lobule VI, we performed specific chemogenetic inhibition of MLIs in this region during learning. Sensorimotor abilities (e.g. locomotor skills, movement coordination and balance) and level of anxiety of mice, assessed through a battery of tasks, suggest no obvious abnormalities. Performances in the sequence-based navigation task are currently under evaluation. We also plan to investigate the consequence of MLI inhibition on the cerebellum, performing cerebellar recordings during this sequence-based navigation.

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BOARD NUMBER: S02-033

LEARNING FAST AND SLOW: THE EFFECT OF INCREASED CORTICAL PLASTICITY ON THE PREFRONTAL-HIPPOCAMPAL COMMUNICATION DURING WAKE AND REM SLEEP STATES

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Aims: In the contemporary view of memories' organization, the hippocampus is proposed to be involved initially in the rapid encoding of different events and their temporary storage (fast learning), while the neocortical system is critical for extracting information from several events and long-term storage of information (slow learning). Such differences between the two systems would protect our long-term memories from interference effects. Although the previous proposal is the basis of our current understanding of memory formation, it was not tested experimentally. **Methods**: We increased the cortical plasticity by overexpressing the RGS_{414} protein in the prelimbic prefrontal cortex (PFC). We then obtained simultaneous local field potential recording from the PFC and the HPC while rodents performed the object-space task (OST). The coherence between both regions and the theta-gamma dynamics were examined during wake and REM sleep states. **Results**: The sleep structure was altered in the RGS_{414} group showing shorter NREM and more prolonged REM bouts. Interestingly, during wake the theta coherence between the PFC and the HPC was higher in the RGS_{414} relative to the control group. Furthermore, the theta-gamma dynamics during wake and sleep states showed distinctive activity patterns in both groups. **Conclusion**: Our current results support the complementary learning system theory proposed by McClelland et al. (1995), emphasizing the role of synaptic plasticity in learning and memory consolidation and the possible top-down control of the neocortical system. Furthermore, they elucidate the underlying theta dynamics during wake and sleep states.

Pubmed:
28634444: Draht F, Zhang S, Rayan A, Schönfeld F, Wiskott L, Manahan-Vaughan D
Experience-Dependency of Reliance on Local Visual and Idiothetic Cues for Spatial Representations Created in the Absence of Distal Information.
Spatial encoding in the hippocampus is based on a range of different input sources. To generate spatial representations, reliable sensory cues from the external environment are integrated with idiothetic cues, derived from self-movement, that enable path integration and directional perception. In this study, we examined to what extent idiothetic cues significantly contribute to spatial representations and navigation: we recorded place cells while rodents navigated towards two visually identical chambers in 180° orientation via two different paths in darkness and in the absence of reliable auditory or olfactory cues. Our goal was to generate a conflict between local visual and direction-specific information, and then to assess which strategy was prioritized in different learning phases. We observed that, in the absence of distal cues, place fields are initially controlled by local visual cues that override idiothetic cues, but that with multiple exposures to the paradigm, spaced at intervals of days, idiothetic cues become increasingly implemented in generating an accurate spatial representation. Taken together, these data support that, in the absence of distal cues, local visual cues are prioritized in the generation of context-specific spatial representations through place cells, whereby idiothetic cues are deemed unreliable. With cumulative exposures to the environments, the animal learns to attend to subtle idiothetic cues to resolve the conflict between visual and direction-specific information.
Front Behav Neurosci, 2017; 11
28012274: Perea G, Gómez R, Mederos S, Covelo A, Ballesteros JJ, Schlosser L, Hernández-Vivanco A, Martín-Fernández M, Quintana R, Rayan A, Díez A, Fuenzalida M, Agarwal A, Bergles DE, Bettler B, Manahan-Vaughan D, Martín ED, Kirchhoff F, Araque A
Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks.
Interneurons are critical for proper neural network function and can activate Ca signaling in astrocytes. However, the impact of the interneuron-astrocyte signaling into neuronal network operation remains unknown. Using the simplest hippocampal Astrocyte-Neuron network, i.e., GABAergic interneuron, pyramidal neuron, single CA3-CA1 glutamatergic synapse, and

astrocytes, we found that interneuron-astrocyte signaling dynamically affected excitatory neurotransmission in an activity- and time-dependent manner, and determined the sign (inhibition potentiation) of the GABA-mediated effects. While synaptic inhibition was mediated by GABA receptors, potentiation involved astrocyte GABA receptors, astrocytic glutamate release, and presynaptic metabotropic glutamate receptors. Using conditional astrocyte-specific GABA receptor () knockout mice, we confirmed the glial source of the interneuron-induced potentiation, and demonstrated the involvement of astrocytes in hippocampal theta and gamma oscillations in vivo. Therefore, astrocytes decode interneuron activity and transform inhibitory into excitatory signals, contributing to the emergence of novel network properties resulting from the interneuron-astrocyte interplay.
Elife, 2016; 5

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BOARD NUMBER: S02-034

THE GENERALIZED SPATIAL REPRESENTATION IN THE PREFRONTAL CORTEX IS INHERITED FROM THE HIPPOCAMPUS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Hippocampal and neocortical neural activity is modulated by the position of the individual in space. While hippocampal neurons provide the basis for a spatial map, prefrontal cortical neurons generalize over environmental features. Whether these generalized representations result from a bidirectional interaction with, or are mainly derived from hippocampal spatial representations is not known. By examining simultaneously recorded hippocampal and medial prefrontal neurons, we observed that prefrontal spatial representations show a delayed coherence with hippocampal ones. We also identified subpopulations of cells in the hippocampus and medial prefrontal cortex that formed functional cross-area couplings; these resembled the optimal connections predicted by a probabilistic model of spatial information transfer and generalization. Moreover, cross-area couplings were strongest and had the shortest delay preceding spatial decision-making. Our results suggest that generalized spatial coding in the medial prefrontal cortex is inherited from spatial representations in the hippocampus and that the routing of information can change dynamically with behavioral demands.

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BOARD NUMBER: S02-035

DRIFTING MEMORIES: SPONTANEOUS LONG-TERM EVOLUTION OF MEMORY REPRESENTATIONS IN THE HIPPOCAMPUS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Sleep's fundamental role for the processing of memory and its consolidation has now received large experimental support. Nevertheless, sleep actually consists of multiple stages that can be broadly classified in the two main categories of REM and non-REM (nREM) sleep, which display very different characteristics. Importantly, their relative contribution to memory function is largely unknown and questions about their interaction during offline processing of newly acquired information have remained mostly untapped. In this study, we addressed these issues by combining a goal-directed learning task with long-term wireless electrophysiological recordings in the hippocampus of rats. After the acquisition of a novel episodic-like memory, place cells were continuously tracked for an extended period of time (>20hrs) while animals rested. We then combined multiple decoding approaches to obtain a time-resolved characterization of the evolution during sleep of a newly established memory representation. This innovative approach allowed us to identify a continuous drift in the reactivated activity patterns: the firing rate of multiple coexisting place cell populations was progressively and differentially modulated in time, resulting in a multiplexed cell code combining change with representational stability. Intriguingly, the direction of this drift was not constant: a closer inspection revealed opposing effects of REM and nREM phases. While nREM sleep 'pushed' the representation towards novel configurations, REM sleep coincided with enhanced reactivation of the recent memory and therefore partially resetted the ongoing drift. The emerging picture is thus one of a complex interplay between sleep dynamics, the age of memories and differential coding properties across cells.

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BOARD NUMBER: S02-036

COGNITIVE IMPAIRMENT IN DP(10)2YEY MOUSE MODEL OF DOWN SYNDROME IS ASSOCIATED WITH ALTERED NEURAL DYNAMICS AND CHANGES IN MEDIAL PREFRONTAL CORTEX AND HIPPOCAMPAL CELLULAR BIOLOGY.

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

Phillip Muza[1], Daniel Bush[2], Steven West[3], Marta Perez Gonzalez[1], Karen Cleverley[1], Suzanna Noy[1], Loukia Katsouri[4], Victor Tybulewicz[5], Mark Good[6], Matthew Walker[7], Elizabeth Fisher[1], Pishan Chang[8]
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Down syndrome (DS), caused by trisomy of human chromosome 21, is the most common genetic cause of intellectual disability with an incidence of 1 in 1000 worldwide. Using a mouse model of DS - Dp(10)2Yey - with only 37 out of a possible 234 protein coding genes implicated in DS expressed in three copies, we investigated how these genes modulate cognitive function and cellular biology in brain regions typically associated with spatial cognition. Depth recording electrodes were implanted in the medial prefrontal cortex (mPFC) and hippocampus of Dp(10)2Yey mice performing open field and T-maze alternation paradigms. Histological techniques, including optical clearing and immunohistochemistry, were conducted to examine changes in cell density of three common interneuron markers - calretinin, parvalbumin, and neuropeptide-Y (NPY) - within mPFC and hippocampus, as well as spine density on hippocampal pyramidal cells, and whole-brain volumetric changes. Dp(10)2Yey mice showed reduced mean running speed and an increase in time spent at the edge of the arena in the open field, indicative of anxiety-like behaviour; and impaired spatial memory in the T-maze alternation task. Both paradigms were associated with altered neural dynamics in the mPFC and hippocampus and, in addition, we observed increased NPY+ interneuron densities within the same cortical regions. DS genes expressed in three copies in Dp(10)2Yey mice are associated with impairments in spatial cognition, altered neural dynamics and the increased expression of interneurons in mPFC and hippocampus. The Dp(10)2Yey mouse is therefore a tractable model to investigate the genetic mechanisms of cognitive impairment in DS.

Pubmed:
30711612: Muza P, Bachmeier C, Mouzon B, Algamal M, Rafi NG, Lungmus C, Abdullah L, Evans JE, Ferguson S, Mullan M, Crawford F, Ojo JO
APOE Genotype Specific Effects on the Early Neurodegenerative Sequelae Following Chronic Repeated Mild Traumatic Brain Injury.
Repeated mild traumatic brain injury (r-mTBI) can potentially manifest into chronic traumatic encephalopathy (CTE). The apolipoprotein E (APOE4) genotype, a well-recognized potent genetic risk factor in age-related neurodegenerative diseases such as Alzheimer's disease, has been linked to worse outcome after TBI in individuals who carry this allele. The underlying molecular modifications triggered by APOE genotype following r-mTBI remain elusive. We addressed the influence of APOE genotype on TBI dependent tau pathology in middle-aged mice. Using a previously established experimental mTBI protocol in a new repetitive injury paradigm, we report the pathological changes that occurred following one-month of repetitive injuries in APOE3/4 gene targeted mice. Firstly, pathological assessment demonstrated evidence of microgliosis and astrogliosis in the corpus callosum of injured animals, but there was no APOE dependent genotype effect on injury. However, in the parietal cortex Iba1-immunoreactivity was significantly increased in injured versus sham APOE3 mice, but not in APOE4 mice. No effects were observed in soluble amyloid levels with injury or interaction with genotype. APOE4 mice showed significant increases in the tau conformational marker MC1, neurofilament H, brain phospholipids, and endothelial specific oxidized low density lipoprotein receptor in cortical homogenates obtained from injured mice compared to sham counterparts. This pilot work suggests APOE3 and APOE4 specific effects following injury in a mouse model of r-mTBI. These changes may underlie the molecular changes that trigger the vulnerability and increased risk of developing neurodegenerative diseases in aged individuals exposed to repetitive mTBI.

Neuroscience, 2019; 404
30079015: Algamal M, Ojo JO, Lungmus CP, Muza P, Cammarata C, Owens MJ, Mouzon BC, Diamond DM, Mullan M, Crawford F
Chronic Hippocampal Abnormalities and Blunted HPA Axis in an Animal Model of Repeated Unpredictable Stress.
Incidence of post-traumatic stress disorder (PTSD) ranges from 3 to 30% in individuals exposed to traumatic events, with the highest prevalence in groups exposed to combat, torture, or rape. To date, only a few FDA approved drugs are available to treat PTSD, which only offer symptomatic relief and variable efficacy. There is, therefore, an urgent need to explore new concepts regarding the biological responses causing PTSD. Animal models are an appropriate platform for conducting such studies. Herein, we examined the chronic behavioral and neurobiological effects of repeated unpredictable stress (RUS) in a mouse model. 12 weeks-old C57BL/6J male mice were exposed to a 21-day RUS paradigm consisting of exposures to a predator odor (TMT) whilst under restraint, unstable social housing, inescapable footshocks and social isolation. Validity of the model was assessed by comprehensive examination of behavioral outcomes at an acute timepoint, 3 and 6 months post-RUS; and molecular profiling was also conducted on brain and plasma samples at the acute and 6 months timepoints. Stressed mice demonstrated recall of traumatic memories, passive stress coping behavior, acute anxiety, and weight gain deficits when compared to control mice. Immunoblotting of amygdala lysates showed a dysregulation in the p75NTR/ProBDNF, and glutamatergic signaling in stressed mice at the acute timepoint. At 6 months after RUS, stressed mice had lower plasma corticosterone, reduced hippocampal CA1 volume and reduced brain-derived neurotrophic factor levels. In addition, glucocorticoid regulatory protein FKBP5 was downregulated in the hypothalamus of stressed mice at the same timepoint, together implicating an impaired hypothalamus-pituitary-adrenal-axis. Our model demonstrates chronic behavioral and neurobiological outcomes consistent with those reported in human PTSD cases and thus presents a platform through which to understand the neurobiology of stress and explore new therapeutic interventions.
Front Behav Neurosci, 2018; 12

FENS Forum 2022
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BOARD NUMBER: S02-037

EXERCISE INCREASES INFORMATION CONTENT AND PARADOXICALLY AFFECTS LONG-TERM STABILITY OF HIPPOCAMPAL PLACE CODES

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Physical exercise augments brain functioning, improving memory and cognition. While some of the physiological effects of physical activity on the brain are known (e.g., increased hippocampal neurogenesis), little is known about its effects on the neural code. Using Ca^{2+} imaging in freely behaving mice, we studied how voluntary exercise affects the quality and long-term stability of hippocampal place codes. In addition to increasing neurogenesis, we found that running accelerated the emergence of a more informative spatial code in novel environments and increased code stability over timescales of days to weeks. Paradoxically, although running mice demonstrated an overall more stable place code than their sedentary peers, their place code exhibited a higher degree of representational drift when controlling for code quality level. A model-based simulation showed that the combination of both improved code quality and faster representational drift in runners, but neither of these effects alone could account for our results. Thus, exercise may enhance hippocampal function via a more informative and dynamic place code.

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BOARD NUMBER: S02-038

EFFECTS OF REPRODUCTIVE STATUS ON COGNITIVE FUNCTION AND BEHAVIORAL FLEXIBILITY OF FEMALE MICE IN THE INTELLICAGE HOME CAGE ENVIRONMENT

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The impact of pregnancy and motherhood on the physiology and behavior of the mother is dramatic. Dynamic fluctuations in steroid and peptide hormones during pregnancy and lactation and also sensory inputs from the pups induce the adaptations by acting on neural circuits in the brain. Although research has investigated the hormonal, neural and behavioral alterations related to maternal behaviors, it is not established how pregnancy and motherhood affect hippocampus-dependent spatial learning and memory and prefrontal-cortex dependent cognitive flexibility. Our purpose was to study the exploratory behavior, water consumption, spatial learning and cognitive flexibility of female mice throughout the reproductive cycle using automatic monitoring IntelliCage system. The present study is the first to determine that motherhood improves spatial learning performance and cognitive flexibility using IntelliCage. Previously, standard behavioral tests were used to examine these phenomenons in connection with different reproductive stages, but the findings were inconclusive. Our results demonstrate that compared to control/pregnant females, cognitive flexibility is enhanced in mothers tested by reversed place learning and fixed schedule drinking task in IntelliCage. Alterations of the executive functions, such as attention and cognitive flexibility, are likely critical for maternal responsiveness and parenting quality not just in rodents but in humans as well. Funding: New National Excellence Programme ÚNKP-21-5-ELTE-1078 for MCS, NKFIH OTKA K134221, NKFIH-4300-1/2017-NKP_17, and Eötvös Loránd University Thematic Excellence Programme 2020 (TKP2020-IKA-05). Conflict of interest: The authors declare that they have no conflict of interest.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-039

INTERFERENCE-BASED FORGETTING IN A GOAL-DIRECTED SPATIAL NAVIGATION TASK FOR RODENTS.

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The number of memories that can be stored in the brain is limited. Active forgetting is one possible mechanism by which the brain can decide which information to store in long-term memory. Interference-based forgetting occurs when information acquired before or after a learning event attenuates memory strength. In our daily lives, we are constantly exposed to new associations, each with its own value and volatility, limiting the interpretation of results obtained under lab conditions using only a few associations. For this reason, we developed a novel task that allows us to observe memory interference under more ethological conditions. We used our high-throughput behavioral task where animals learn the location of the reward among 8 positions that changes randomly over days. We test the memory recall of the reward position 2hrs after the training session and we observe how memories from previous sessions (i.e. days) interfere with the latest acquired memory. To dissect the interference between previously learned memories, we infused highly-specific human N-Methyl-D-aspartic receptor antibodies into animals' brain ventricles to mimic an anterograde amnesic state. We confirmed that the memory strength of new associations is weaker due to interference with previous memories. We found that antibody-mediated amnesia significantly reduces interference ($p<0.01$) by enhancing recent memories at the expense of a substantial reduction (~40%) in the strength of old ones. Our results support the theory of retroactive interference as a mechanism for eliminating memories of associations with high volatility.

Pubmed:
29659262: Chaari M, Gaztelumendi N, Cabrera-González J, Peixoto-Moledo P, Viñas C, Xochitiotzi-Flores E, Farfán N, Ben Salah A, Nogués C, Núñez R
Fluorescent BODIPY-Anionic Boron Cluster Conjugates as Potential Agents for Cell Tracking.
A series of novel fluorescent BODIPY-anionic boron cluster conjugates bearing [BH] (5, 6), [3,3'-Co(1,2-CBH)] (7, 8), and [3,3'-Fe(1,2-CBH)] (9) anions have been readily synthesized from meso-(4-hydroxyphenyl)-4,4-difluoro-4-bora-3 a,4 a-diaza-s-indacene (BODIPY 4), and their structure and photoluminescence properties have been assessed. Linking anionic boron clusters to the BODIPY (4) does not alter significantly the luminescent properties of the final fluorophores, showing all of them similar emission fluorescent quantum yields (3-6%). Moreover, the cytotoxicity and cellular uptake of compounds 5-9 have been analyzed in vitro at different concentrations of B (5, 50, and 100 μg B/mL) using HeLa cells. At the lowest concentration, none of the compounds shows cytotoxicity and they are successfully internalized by the cells, especially compounds 7 and 8, which exhibit a strong cytoplasmic stain indicating an excellent internalization efficiency. To the best of our knowledge, these are the first BODIPY-anionic boron cluster conjugates developed as fluorescent dyes aiming at prospective biomedical applications. Furthermore, the cellular permeability of the starting BODIPY (4) was improved after the functionalization with boron clusters. The exceptional cellular uptake and intracellular boron release, together with the fluorescent and biocompatibility properties, make compounds 7 and 8 good candidates for in vitro cell tracking.
Bioconjug Chem, 2018; 29

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-040

LEC MODULATION OF CA1 ENSEMBLE DYNAMICS DURING MEMORY-GUIDED BEHAVIORS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The ability to form and store memories is crucial for daily human functioning. It allows us to form appropriate relationships with others and our ever-changing environments. Without new memories we become unable to adapt and progress through space and time. In Alzheimer's Disease (AD), patients are robbed of this ability and experience severe cognitive deficits in spatial navigation and memory formation. Hippocampal output region, area CA1, receives inputs directly from the entorhinal cortex (EC). EC serves as an integrative hub for different sensory modalities, allowing a multisensory environment to be encoded within CA1. The lateral subdivision of EC (LEC) shows early dysfunction in both human AD patients and mouse models. Therefore, in early-stage AD, CA1 begins to receive corrupted inputs. But, how this translates into CA1's representation of environment or an animal's cognitive performance is unknown. To identify LEC-CA1 as a circuit underlying AD impairments, we determine if loss of LEC activity produces AD-like deficits. We performed *in vivo* imaging of CA1 neurons with chemogenetic silencing of LEC-CA1 long-range projections and found a decrease in ensemble stability while performance on a spatial task was spared. Ongoing imaging experiments are being conducted with multisensory spatial tasks in wild type and AD model mice to further dissect the contribution of LEC to CA1 ensemble activity. These experiments will contribute to our understanding of (1) basic memory processing and hippocampal circuits, as well as (2) early degradation of the cortico-hippocampal circuit in AD.

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BOARD NUMBER: S02-041

IN SEARCH FOR THE AVIAN TRIGEMINAL MAGNETIC SENSOR: ORGANIZATION OF THE OPHTHALMIC SENSORY COMPLEX IN THE NIGHT-MIGRATORY EURASIAN BLACKCAP (SYLVIA ATRICAPILLA)

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

Katrin Haase[1], Isabelle Musielak[1], Leonie Warmuth-Moles[1], Bo Leberecht[1], Anna Zolotareva[2], Henrik Mouritsen[1,3], Dominik Heyers[1,3]
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Night-migratory songbirds can use the Earth's magnetic field to navigate between their breeding and wintering grounds. Neurobiological and behavioral evidence suggests the existence of a magnetic sense associated with the ophthalmic branch of the trigeminal nerve (V1), located in the bird's upper beak. Curiously, neither the unequivocal existence, structural nature, nor the exact location of any sensory structure has been revealed to date. To narrow in on the putative location of any magnetic sensor, we used neuronal tract tracing to map both the innervation fields in the upper beak and the detailed trigeminal brainstem terminations of the medial and lateral V1 subbranches in the night-migratory Eurasian Blackcap (*Sylvia atricapilla*). The medial V1 subbranch takes its course along the ventral part of the upper beak to innervate subepidermal layers and the mucosa of the nasal cavity, while the lateral V1 subbranch runs along dorsolateral levels until the nostrils to innervate mainly the skin of the upper beak. In the trigeminal brainstem, medial V1 terminals innervate both the dorsal part and the ventral, magnetically activated part of the principle sensory trigeminal brainstem nuclei (PrV). In contrast, the lateral V1 subbranch innervates only a small part of the ventral PrV. The spinal sensory trigeminal brainstem nuclei receive topographically ordered projections from both medial and lateral subbranches. The present findings provide valuable information for further analysis of the trigeminal magnetic sense of birds and indicate that both the medial as well as the lateral V1 subbranch could be involved in mediating magnetic information.

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BOARD NUMBER: S02-042

CIRCUIT LOGIC OF DESCENDING INTERNEURONS CONTROLLING STEERING IN DROSOPHILA

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

Tomke Stürner[1], Katharina Eichler[1], Paul Brooks[1], Han Cheong[2], Gwyneth Card[2], Elizabeth Marin[1], Marta Costa[1], Gregory Jefferis[3]
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Behavioural decisions result from a complex interplay between evolutionary predisposition and individual experience. In most animals a small set of descending interneurons (DNs) connect higher brain centres to the nerve cord to control a variety of complex behaviours. With the emergence of whole brain electron microscopy (EM) datasets for both the whole brain and Ventral Nerve Cord (VNC) of the fly, a systematic description of the DN population is now possible. In this project we identify and describe the entire set of 660 pairs of DNs that transfer pre-processed sensory information to motor centres in the VNC. This approach includes a comprehensive reconstruction of all DNs connecting brain and VNC and connectomics circuit reconstructions of VNC neurons involved in sensory-motor processing. Here, I present our collaborative work that has identified organisational principles across the DN population and their connections in the male VNC. Many DNs that relay information from the Lateral Accessory Lobes (LAL), a multimodal pre-motor integration centre in the brain, converge onto specific leg circuits involved in steering towards or away from a stimulus. Therefore a special focus of this project is the circuit logic of VNC local interneurons involved in steering during walking. The specific example of turning in response to learned or innately aversive stimuli gives us a general understanding of how the VNC executes a decision made in a higher brain centre.

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BOARD NUMBER: S02-043

MULTIMODAL REPRESENTATIONS OF CONTEXT IN GRADIENT NAVIGATION IN LARVAL ZEBRAFISH

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Animals use a plethora of sensory systems and behavioral strategies to navigate where conditions for survival are maximized. It has been extensively shown that a temporal sampling strategy (i.e. detecting changes in stimulus intensity over time) will lead to an increase in turning rate when a worsening of conditions is detected. Conversely, animals will continue performing forward motion if the conditions are improving. Since the majority of existing studies on gradient navigation focus on single sensory modalities it is difficult to disentangle whether the aforementioned action selection processes are driven by the sensory modality itself or by a shared representation. In the present study, we took advantage of the optical accessibility and well-defined behavior of larval zebrafish to first confirm fish perform chemotaxis and thermotaxis in a freely swimming setup. Then, we identified the experienced rate of change of the gradient as the sole information sufficient for navigation. Finally, to find brain areas detecting an improvement or worsening of conditions we developed an open-loop virtual gradient navigation assay in head-restrained larvae used in combination with lightsheet microscopy. Notably, we found the habenular-interpeduncular pathway to be tuned to the rate of change of the stimulus while the serotoninergic superior raphe showed context-dependent representations of movements. The importance of the habenular-interpeduncular pathway and the involvement of the superior raphe in gradient navigation was further verified through chemical treatments. Taken together our results uncovered a pathway processing relevant sensory and motor variables for successful gradient navigation irrespective of sensory modality identity.

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BOARD NUMBER: S02-044

A BRAINSTEM INTEGRATOR FOR SELF-LOCATION MEMORY AND POSITIONAL HOMEOSTASIS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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To track and control self-location, animals integrate their movements through space. While self-location is represented in the hippocampal formation, it is unknown how such representations arise from integrated self-motion, whether they exist in more ancient brain regions, and by what pathways they control locomotion. Fish can be carried by water currents to potentially dangerous areas; here we report that larval zebrafish track their displacements to later return to previous locations. Whole-brain functional imaging revealed the circuit enabling this 'positional homeostasis'. A newly identified brainstem positional integrator stores a memory of past displacements and induces an error signal in the inferior olive, which controls future corrective swimming. Optogenetically manipulating functionally-identified integrator cells evokes displacement-memory behavior; ablating them, or downstream olivary cells, abolishes positional homeostasis. These results reveal a multiregional hindbrain circuit in vertebrates for integration of self-motion, memory of self-location, and control of locomotor behavior.

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BOARD NUMBER: S02-045

SPINDLE–SLOW OSCILLATION COUPLING CORRELATES WITH MEMORY PERFORMANCE AND CONNECTIVITY CHANGES IN A HIPPOCAMPAL NETWORK AFTER SLEEP

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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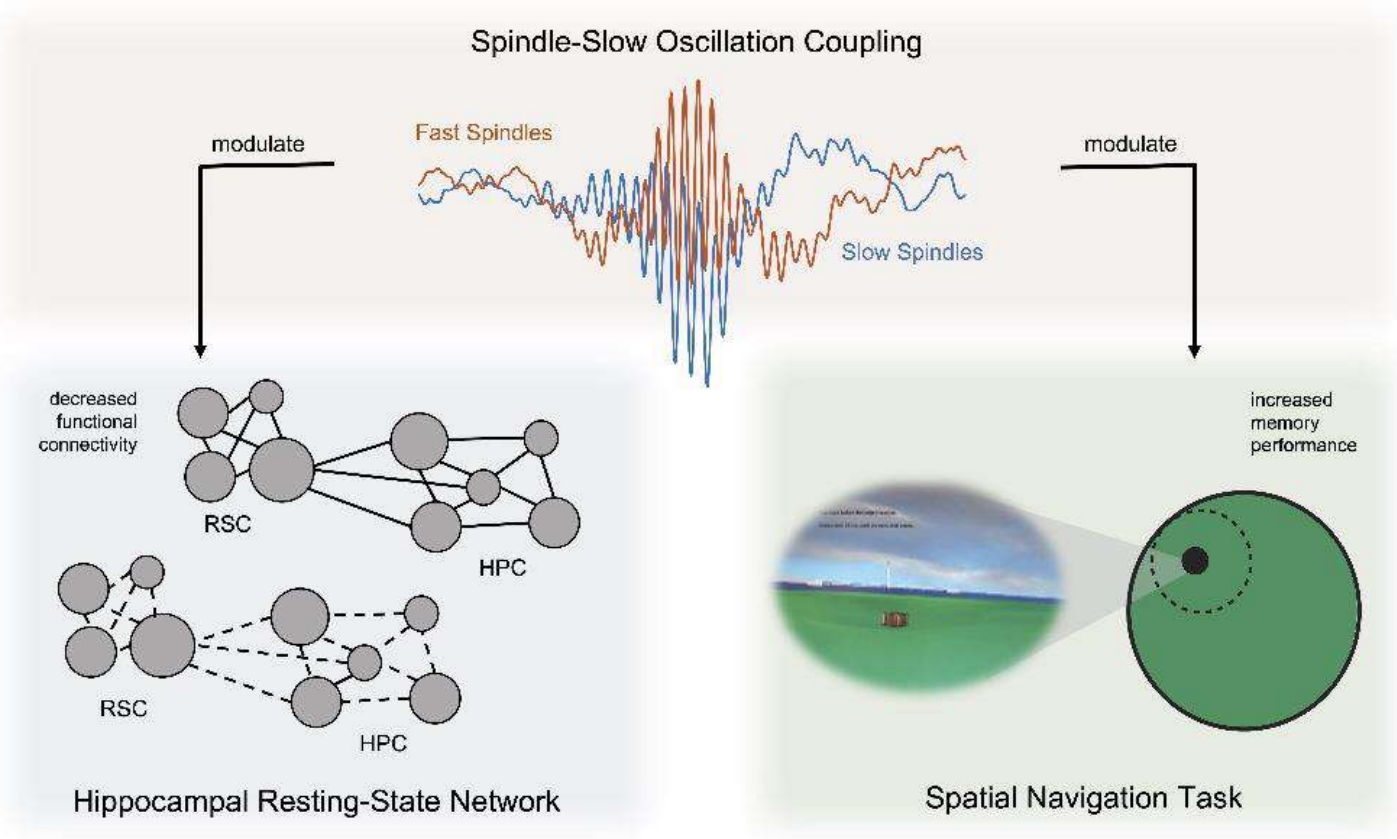
After experiences are encoded, post-encoding reactivations during sleep have been proposed to mediate long-term memory consolidation. Spindle–slow oscillation coupling during NREM sleep is a candidate mechanism through which a hippocampal-cortical dialogue may strengthen a newly formed memory engram. Here, we investigated the role of fast spindle- and slow spindle–slow oscillation coupling in the consolidation of spatial memory in humans with a virtual watermaze task involving allocentric and egocentric learning strategies. Furthermore, we analyzed how resting-state functional connectivity evolved across learning, consolidation, and retrieval of this task using a data-driven approach. Our results show task-related connectivity changes in the executive control network, the default mode network, and the hippocampal network at post-task rest. The hippocampal network could further be divided into two subnetworks of which only one showed modulation by sleep. Decreased functional connectivity in this subnetwork was associated with higher spindle–slow oscillation coupling power, which was also related to better memory performance at test. Overall, this study contributes to a more holistic understanding of the functional resting-state networks and the mechanisms during sleep associated to spatial memory

consolidation.



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BOARD NUMBER: S02-046

MEMORY IS REGULATED BY ASTROCYTIC RECEPTORS IN A SEX-SPECIFIC MANNER

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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Aims: Astrocytes regulate cognition and behavior, but the precise mechanisms are unclear. Previous studies have shown that astrocytic G protein-coupled receptors (GPCRs) are key regulators of astrocytic-neuronal interactions and cognitive function. However, these studies did not account for sex as a biological variable. Here, we tested whether astrocytic glutamate receptors and other GPCRs have sex-specific effects on spatial learning and memory. **Methods:** We interrogated the effects of astrocytic GPCRs in the hippocampus using complementary transgenic and chemogenetic approaches. CRISPR-Cas9 was used to selectively knock down astrocytic metabotropic glutamate receptor 3 (mGluR3), a $G_{i/o}$-coupled receptor that is highly enriched in astrocytes and implicated in various cognitive disorders. To complement this approach, we employed a Cre-dependent viral vector to selectively enhance astrocytic mGluR3 expression. Additionally, we used chemogenetics to activate either $G_{i/o}$- or G_s-coupled receptor signaling in astrocytes during spatial learning. **Results:** We found that knocking down astrocytic mGluR3 impaired memory in females, but improved memory in males. Conversely, increasing astrocytic mGluR3 expression improved memory in females, but not males. Analogous to the sexually dimorphic effects of manipulating astrocytic mGluR3, activating astrocytic $G_{i/o}$-coupled signaling improved memory in females, but impaired memory in males. Moreover, activating astrocytic G_s-coupled signaling impaired memory in females but not males. **Conclusions:** Thus, astrocytic glutamate receptor mGluR3 and related GPCR signaling regulate spatial memory in a sex-specific manner. Given that alterations in mGluR3 and astrocytic receptor signaling are linked to various neurological and neuropsychiatric disorders, our findings suggest that astrocytes may promote sex differences in disease-associated cognitive impairments.

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BOARD NUMBER: S02-047

SPATIAL REASONING VIA RECURRENT NEURAL DYNAMICS IN MOUSE RETROSPLENIAL CORTEX

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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From visual perception to language, sensory stimuli change their meaning depending on prior experience. Recurrent neural dynamics can interpret stimuli based on externally cued context, but it is unknown whether similar dynamics can compute and employ internal hypotheses to resolve ambiguities. Here, we show that mouse retrosplenial cortex (RSC) can form hypotheses over time and perform spatial reasoning through recurrent dynamics. In our task, mice navigated using ambiguous landmarks that are identified through their mutual spatial relationship, requiring sequential refinement of hypotheses. Neurons in RSC and in artificial neural networks encoded mixtures of hypotheses, location, and sensory information, and were constrained by robust low dimensional dynamics. RSC encoded hypotheses as locations in activity space with divergent trajectories for identical sensory inputs, enabling their correct interpretation. Our results indicate that interactions between internal hypotheses and external sensory data in recurrent circuits can provide a substrate for complex sequential cognitive reasoning.

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HIPPOCAMPAL REPRESENTATIONS OF HOMING BASED ON PATH INTEGRATION

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

Maryam Najafian Jazi, Adrian Tymorek, Ting-Yun Yen, Moritz Stingl, Benay Baskurt, Celia García Vilela, Kevin Allen
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Homing based on path integration (H/PI) is a form of navigation in which an animal processes self-motion cues to keep track of its location in order to return to a starting point. The neuronal representations supporting H/PI have remained largely unexplored because of a lack of H/PI tasks suitable for recordings of spatially selective neurons. Here we overcame this problem by developing an automated H/PI task for rodents. The task required a mouse to find a variably placed lever on a circular arena, before heading back to their home base. H/PI was assessed in complete darkness, where performance depended on path integration. Recordings from CA1 pyramidal neurons in mice performing the H/PI task showed that several firing fields were anchored to the lever position. The spatial selectivity of these fields was reduced during trials with lower homing accuracy. In a subset of lever-anchored neurons, the field position around the lever predicted the homing direction of the mouse. These results demonstrate how object-anchored firing fields convey behaviourally relevant information for homing and navigation beyond the object vicinity.

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THALAMIC HEAD-DIRECTION CELLS ARE ORGANIZED IRRESPECTIVE OF THEIR INPUTS

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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The thalamus relays sensory signals to the cortex and understanding its role in sensory processing is a central question. Thalamic head-directions (HD) neurons of the anterodorsal nucleus (ADN) increase their firing rates as a function of the direction of the head of the animal in the horizontal plane. ADN-HD cells maintain their mutual coordination during sleep, when sensory inputs are virtually absent, supporting the view that the HD signal arises from attractor dynamics. It has been proposed that the upstream structure, the lateral mammillary nucleus (LMN), is a central component of the HD signal generator circuit. Yet, whether the ADN passively relays LMN inputs or actively processes its inputs remains unclear. We investigated the organization of LMN activity across brain states in mice, and its relationship to ADN activity. While, during non-REM sleep, ADN-HD neurons maintained the same level of mutual coherence as during wake, LMN-HD cells showed reduced coordination. The decreased level of correlation in the LMN resulted, at least in part, by neurons co-firing irrespectively of their mutual preferred direction. Spike train dynamics were also strikingly different between structures. When the animal's HD was around a cell's preferred direction, the rate of LMN neurons varied linearly. ADN-HD neurons showed sharp transitions from low to high activity states. A model of nonlinear integration of LMN inputs accounts for the coordination of ADN-HD cell activity irrespective of the organization of the inputs. This suggests that intrinsic properties of thalamic cells are essential in shaping representation in thalamocortical networks.

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CAN EPILEPTIC RODENTS CREATE A REPRESENTATION OF SPACE THROUGH OBSERVATION ONLY?

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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In humans and many animals, new behaviors may be learned through observation of conspecific's experience. Patients with epilepsy and experimental models show memory deficits, but whether observational learning is affected is not known. We studied whether epileptic rats show cognitive deficit in learning by observation in a spatial task. We developed a new behavior task in an environment containing both directly experienced and purely observed areas. In this task a demonstrator animal performs a task under observation of a naïve animal which never physically experience the space where the task is occurring. After sufficient observational sessions, the observer animal is free to explore the observed space and perform the task. In control rats, we found that this observation led to highly significant improvements in both accuracy and latency towards the goal as compared to naïve animals. By contrast, epileptic animals failed to learn by observation. However, they were able to successfully complete the task through their own experience. This new behavior task allows us to confirm that rodents can create representations of a space they observed but never physically explored and that epileptic animals show a clear cognitive deficit in learning by observation.

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THE EFFECT OF PROBLEM CONTEXTUALIZATION IN PHYSICS : A NEUROPHYSIOLOGICAL APPROACH TOWARDS A BETTER UNDERSTANDING OF PROBLEM SOLVING

POSTER SESSION 02 - SECTION: SPATIAL MEMORY, NAVIGATION & COGNITION

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In their critical review of available studies related to contextualization of physics, Taasoobshirazi, & Carr (2008) showed a certain lack of quality in available empirical studies and called for more rigorous research on the topic. Considering the work of Haussler et al. (1998), who observed that certain types of contexts (technical or humanistic) do promote girls' or boys' engagement, the overall goal of the present project is to evaluate the extent to which contextualization of mechanical physics instruction can influence student's engagement and performance. The study was conducted on 65 university students (32 females/33 males) who were given a task consisting of 18 mechanical physics problems (6 technical; 6 humanistic contextualized; 6 decontextualized) on a computer. Drawing on neurophysiological methods, cognitive (electroencephalography, pupillometry), affective (electrodermal activity and facial recognition of emotions) and behavioral (traces, responses to questionnaires) engagement data was collected during the task. The data are now being analyzed and will be available for the presentation. These results may possibly provide support (or otherwise) for the relevance of contextualizing physics instruction.

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INVESTIGATING THE FUNCTIONAL SPECIALIZATION OF HUMAN DECLARATIVE MEMORY SUBSYSTEMS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

Svenja Klinkowski[1], Anna Seewald[1], Björn Fath[1], Panagiotis Iliopoulos[1], Silke Schmidt[1], Franziska Voss[1], Michael Erb[2], Klaus Scheffler[2], Steffen Gais[1], Svenja Brodt[1]
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Aims. While traditional models of systems memory consolidation postulate the reliance of freshly encoded memories on the hippocampus, recent evidence in humans and animals has shown that there are conditions under which the neocortex can rapidly acquire genuine memory engrams. The current study investigates the idea of concurrent memory encoding in the entire network, and specialized subsystems coding for different aspects of the memory. **Methods**. 80 participants encoded the same abstract visual stimuli during fMRI scanning and were instructed to either remember the detailed item-context combinations (DET) or to identify conceptual categories (CEP). 24h later, performance was tested in a categorization and an item-context recognition task. **Results**. CEP performed better in categorizing novel stimuli (t_{78}=-6.91*; p*<0.001), whereas DET had better memory for item-context combinations (t_{78}=6.31*; p*<0.001). In both groups, repetitions activated the precuneus, for exact items (p_{FWE}<0.05) as well as conceptual repetitions (p_{FWE}<0.05). Contrasting the two groups, exact item repetition elicited higher bilateral activation of the superior frontal gyrus (t=7.03; p_{FWE}<0.05), caudate and thalamus in DET (t=5.88; p_{FWE}<0.05), and higher activation in visual cortex extending towards precuneus and fusiform gyrus (t=7.51; p_{FWE}<0.05) in CEP. Comparing CEP with DET, activation in medial occipital cortex (t=6.83, p_{FWE}<0.05) increased over category repetitions only in CEP. **Discussion**. Our data suggest the precuneus as a hub for detailed as well as conceptual memory representations. Additionally, differences in memory performance and neural differences between the two groups indicate a functional specialization of neocortical and subcortical areas during prioritized encoding of similar vs. differential features.

Pubmed:
31820807: Bach P, Frischknecht U, Klinkowski S, Bungert M, Karl D, Vollmert C, Vollstädt-Klein S, Lis S, Kiefer F, Hermann D
Higher Social Rejection Sensitivity in Opioid-Dependent Patients Is Related to Smaller Insula Gray Matter Volume: A Voxel-Based Morphometric Study.
Opioid-dependent patients are highly sensitized to negative social feedback, and increased social rejection sensitivity was linked to adverse treatment outcome, but its neurobiological underpinnings have not been understood yet. The present study investigated gray matter (GM) volume differences between 19 opioid maintenance treatment (OMT) patients and 20 healthy controls using magnetic resonance imaging and voxel-based morphometry. Associations of GM volumes with subjective feelings of exclusion and inclusion during a social ostracism (Cyberball) paradigm, with rejection sensitivity, social interaction anxiety and social phobia were explored. OMT patients displayed smaller GM volume in the bilateral insula and inferior frontal gyri. Psychometric and task data showed that patients reported significantly higher rejection sensitivity, social anxiety and social phobia scores and felt more excluded and less included during the social ostracism paradigm. Smaller GM volume in the insula was associated with higher subjective exclusion, lower subjective inclusion and higher rejection sensitivity, social anxiety and social phobia scores. Findings indicate that structural deficits in emotion- and anxiety-processing brain regions in OMT patients are associated with increased social rejection sensitivity. As social rejection is a potential trigger for relapse, patients might benefit from therapeutic strategies that promote social integration.
Soc Cogn Affect Neurosci, 2019; 14

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BOARD NUMBER: S02-053

THE ROLES OF THE HUMAN ORBITOFRONTAL CORTEX, VMPFC, AND ANTERIOR CINGULATE CORTEX CONNECTOME IN EMOTION AND MEMORY

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

Edmund Rolls
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The human orbitofrontal cortex, ventromedial prefrontal cortex (vmPFC) and anterior cingulate cortex are implicated in reward and emotion, but also in memory. Analysis of the effective connectivity of these regions in humans using Human Connectome Project data and the HCPex brain atlas (Huang et al 2021) shows how the human orbitofrontal cortex connecting with the vmPFC and anterior cingulate provide a route to the hippocampus for reward and emotional value to be incorporated in episodic memory, enabling memory of where a reward was seen (Rolls et al 2022a,b). It is proposed that this value component results in primarily episodic memories with some value component to be repeatedly recalled from the hippocampus so that they are more likely to become incorporated into neocortical semantic and autobiographical memories. The same orbitofrontal and anterior cingulate regions also connect in humans to the septal and basal forebrain cholinergic nuclei, thereby helping to consolidate memory, and helping to account for why damage to the vMPFC impairs memory (Rolls et al 2022a,b). The human hippocampus and vmPFC thus contribute in complementary ways to forming episodic and semantic memories. Rolls,E.T., Deco,G., Huang,C-C. and Feng,J. (2022a) The human orbitofrontal cortex, vmPFC, and anterior cingulate cortex effective connectome: emotion, memory, and action. Cerebral Cortex doi:10.1093/cercor/bhac070. Rolls,E.T., Deco,G., Huang,C-C. and Feng,J. (2022b) The effective connectivity of the human hippocampal memory system. Cerebral Cortex doi:10.1093/cercor/bhab442. Huang,C-C., Rolls,E.T., Feng,J. and Lin,C-P. (2021) An extended Human Connectome Project multimodal parcellation atlas of the human cortex and subcortical areas. Brain Structure and Function doi:10.1007/

BOARD NUMBER: S00429-021-02421-6.

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BOARD NUMBER: S02-054

P300 LATENCY DEPEND ON WORKING MEMORY CAPACITY IN THE ELDERLY

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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The present study explored modulations of the event-related brain potentials (ERPs) in young and elder adults during a modified version of the ASMRT (Almeria Spatial Memory Recognition Test) modulated by working memory capacity. Forty adults (20 young) performed this task. As we expected, young adults showed better performance in the task. ERP results revealed significant differences in the P300 latency between groups. Latency was greater in the elderly. In addition, the P300 latency decreased with working memory capacity, only in this group. This results support previous research that suggest the relationship between age and latency in this ERP component.

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IS THERE EVIDENCE FOR REWARD-CONDITIONING INDUCED RETROSPECTIVE AND PROSPECTIVE MEMORY ENHANCEMENT EFFECTS?

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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The neurobiological synaptic tagging mechanism allows the brain to temporarily store episodic events which seem insignificant at encoding but are subsequently enhanced in memory by acquiring salience. Human behavioural studies have found evidence for retrospective memory enhancement of initially neutral stimuli from a semantic category when images from that category were later paired with a salient event, e.g. monetary reward [Patil et al., 2017]. Although this phenomenon has important implications for adaptive memory and reward-memory literature, the generalisability of these findings remains unexplored. Our initial aims to find the same effects in different learning and reward paradigms were inconclusive, leading us to conduct four experiments to (1) replicate category-specific retrospective memory enhancement through reward conditioning using the same approach as [Patil et al., 2017], and (2) investigate the possibility of prospective memory effects. None of our experiments provided reliable evidence for effect of reward-conditioning on recognition memory. Our findings were analysed with frequentist statistics, linear mixed-effects models and further supported by Bayesian hypothesis testing, indicating substantial evidence for the null hypothesis of no effect of reward category. Critically, we also did not find any evidence for category-specific retrospective or prospective enhancement effects. Overall, our results cast doubt on the robustness of the reward-conditioning paradigm used to probe retrospective enhancement of memory, and thus on the underlying reward-induced adaptive memory phenomenon itself. Patil, A., Murty, V. P., Dunsmoor, J. E., Phelps, E. A., and Davachi, L. (2017). Reward retroactively enhances memory consolidation for related items. Learning and Memory, 24(1):65–69

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BOARD NUMBER: S02-056

FILTERING EFFICIENCY AS AN UNDERLYING MECHANISM OF MODEL-BASED WORKING MEMORY TRAINING IN HEALTHY OLD ADULTS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Background: Working memory (WM) is crucial for cognitive functioning and amongst the first abilities to decline with age. It has been shown that older adults have difficulties to suppress distracting information during WM encoding, termed as filtering efficiency (FE). Own results revealed improved WM capacity in healthy older adults following combined training of WM and FE. Research is needed to establish how WM and FE interact in order to reach maximal improvement in WM functioning. **Aims:** To study FE as an underlying mechanism of WM capacity improvement in healthy older adults when trained in combination with WM by altering FE or WM load to a training version with high WM and low FE (MB+), high FE and high WM (MB++), and high FE but low WM (MB+-) load. **Methods**: This online, randomized, double-blind, parallel group study aims at including 171 healthy adults aged ≥ 50 years, 57 in each training condition (MB+, MB+-, MB++). Participants perform twelve 40-min training sessions during three weeks using the in-house developed application *SmartBrain* for smartphones and three web-based cognitive assessments, each assessing WM capacity by three complex span tasks and FE by a change detection task. The first two assessments serve as double-baseline to investigate practice effects. **Results:** The results will yield novel insights on underlying mechanisms of WM training in healthy elderly adults. These insights will allow to help elderly people overcome difficulties in daily life tasks due to their age-related cognitive decline and may be translated in rehabilitative strategies for clinical conditions.

Pubmed:

34845312: Zuber P, Gaetano L, Griffa A, Huerbin M, Pedullà L, Bonzano L, Altermatt A, Tsagkas C, Parmar K, Hagmann P, Wuerfel J, Kappos L, Sprenger T, Sporns O, Magon S
Additive and interaction effects of working memory and motor sequence training on brain functional connectivity.
Although shared behavioral and neural mechanisms between working memory (WM) and motor sequence learning (MSL) have been suggested, the additive and interactive effects of training have not been studied. This study aimed at investigating changes in brain functional connectivity (FC) induced by sequential (WM + MSL and MSL + WM) and combined (WM × MSL) training programs. 54 healthy subjects (27 women; mean age: 30.2 ± 8.6 years) allocated to three training groups underwent twenty-four 40-min training sessions over 6 weeks and four cognitive assessments including functional MRI. A double-baseline approach was applied to account for practice effects. Test performances were compared using linear mixed-effects models and t-tests. Resting state fMRI data were analysed using FSL. Processing speed, verbal WM and manual dexterity increased following training in all groups. MSL + WM training led to additive effects in processing speed and verbal WM. Increased FC was found after training in a network including the right angular gyrus, left superior temporal sulcus, right superior parietal gyrus, bilateral middle temporal gyri and left precentral gyrus. No difference in FC was found between double baselines. Results indicate distinct patterns of resting state FC modulation related to sequential and combined WM and MSL training suggesting a relevance of the order of training performance. These observations could provide new insight for the planning of effective training/rehabilitation.
Sci Rep, 2021; 11
34211390: Zuber P, Geiter E, de Quervain DJ, Magon S
Investigation of a Model-Based Working Memory Training With and Without Distractor Inhibition and Its Comparative Efficacy: A Randomized Controlled Trial on Healthy Old Adults.
: Various working memory (WM) trainings have been tested, but differences in experimental designs, the lack of theoretical background, and the need of identifying task-related processes such as filtering efficiency limit conclusions about their comparative efficacy. : In this study, we compared the efficacy of a model-based WM training with (MB) and without (MB) distractor inhibition on improving WM capacity to a dual and active control condition. : This randomized clinical trial included 123 healthy elderly adults (78 women, 45 men; aged 64.1 ± 8.3 years). All groups underwent 12 40-min training sessions

over 3 weeks and four cognitive testing sessions. The first two sessions served as double baseline to account for practice effects. Primary outcome was WM capacity post-training measured by complex span tasks. Near and far transfer was assessed by simple span, n-back, visuospatial and verbal learning, processing speed, and reasoning tasks. : Due to preliminary termination (COVID-19), 93 subjects completed the post-training and 60 subjects the follow-up session. On a whole group level, practice effects occurred from prebaseline to baseline in WM capacity (= 4.85, = 4.01, < 0.001, = 0.37). Linear mixed-effects models revealed a difference in WM capacity post-training between MB and MB (= -9.62, = -2.52, = 0.014, = 0.27) and a trend difference between MB and dual (= -7.59, = -1.87, = 0.065, = 0.20) and control training (= -7.08, = -1.86, = 0.067, = 0.20). Univariate analyses showed an increase between pre- and post-training for WM capacity within MB (= -3.34, < 0.05) only. There was no difference between groups pre- and post-training regarding near and far transfer. Univariate analyses showed improved visuospatial learning within MB (= -3.8, < 0.05), improved processing speed (= 2.19, < 0.05) and -back performance (= 2.12, < 0.05) in MB, and improved -back performance (= 3.83, < 0.001) in the dual -back training. : A model-based WM training including filtering efficacy may be a promising approach to increase WM capacity and needs further investigation in randomized controlled studies.
Front Aging Neurosci, 2021; 13

32124042: Zuber P, Tsagkas C, Papadopoulou A, Gaetano L, Huerbin M, Geiter E, Altermatt A, Parmar K, Ettlin T, Schuster-Amft C, Suica Z, Alrasheed H, Wuerfel J, Kesselring J, Kappos L, Sprenger T, Magon S
Efficacy of inpatient personalized multidisciplinary rehabilitation in multiple sclerosis: behavioural and functional imaging results.
Although multidisciplinary rehabilitation programs are commonly used in clinical practice for patients with multiple sclerosis (MS), they are currently underexamined.
J Neurol, 2020; 267

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BOARD NUMBER: S02-057

BORDERS BETWEEN DOMAIN-GENERAL AND TASK-SPECIFIC BRAIN REGIONS ARE CRITICAL FOR EXECUTIVE FUNCTIONS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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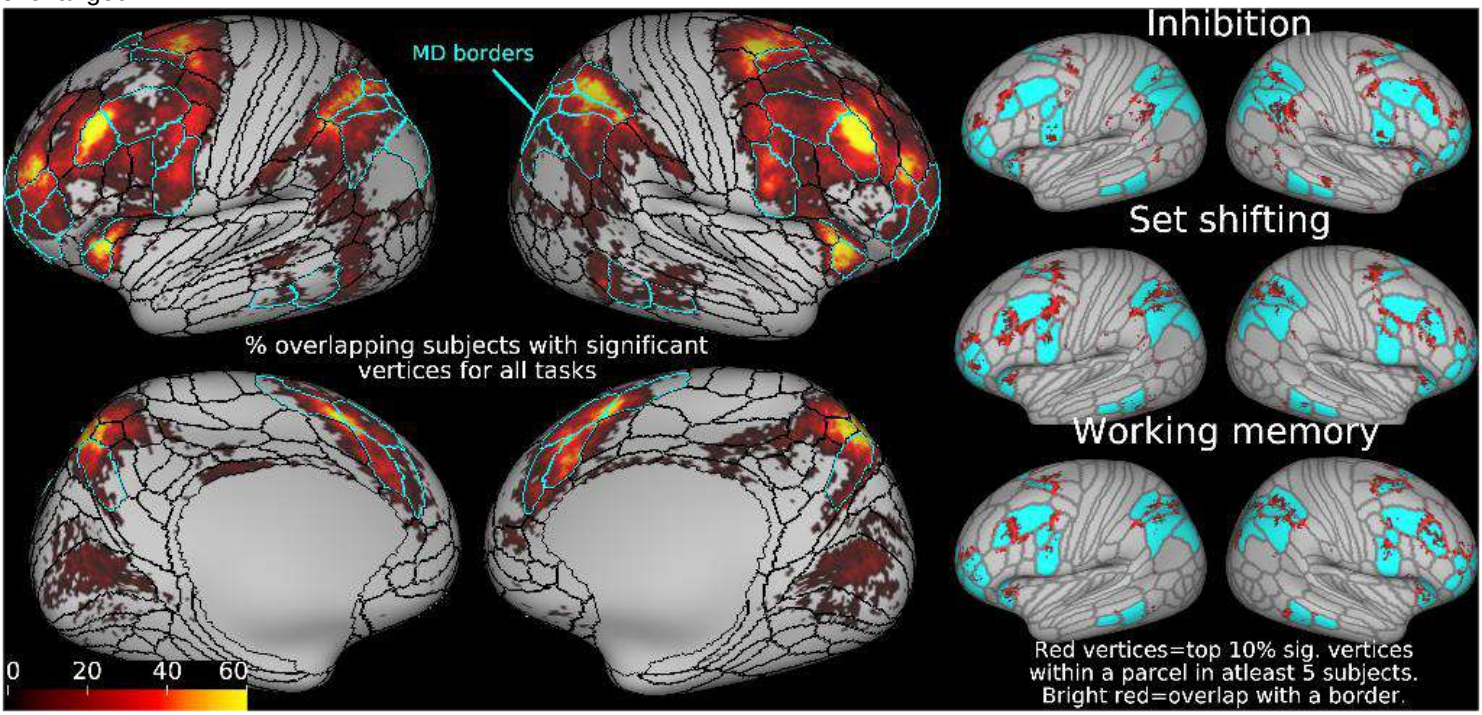
Executive functions are associated with fMRI activations that overlap with fronto-parieto-temporal domain-general or multiple-demand (MD) cortex. Mechanistic insight into how MD cortex supports executive functions remains lacking as the limited spatial resolution of traditional imaging methods obscured overlapping and segregated activations for different executive components. Here we used multimodal MRI techniques of the Human Connectome Project (HCP) to scan subjects performing three kinds of executive tasks: working memory, set shifting and inhibition, each contrasted with a matched baseline task. In line with a “common” executive function component, the three executive>baseline contrasts showed robust overlaps in MD cortex at the single subject level. Each executive demand, however, showed unique activations that overlapped with adjacent regions belonging to different networks. Strikingly, activation peaks clustered at the borders between MD and adjacent regions. Inhibition showed relatively right lateralized activations lying between MD and cingulo-opercular networks, working memory showed overlaps between MD and default-mode networks, while for set shifting, activations were relatively left lateralized lying between MD and dorsal attention networks. Using an independent HCP resting-state dataset, we found that seed regions within MD cortex show differential connectivity patterns that closely predict each task’s precise topography. These results bring a new level of precision to understanding how executive functions are assembled in the brain. Each task demand displays a unique intersection between the domain-general MD network and adjacent, more specialised networks. In this intersection, the strongest activity arises at network borders, where information may be most intensively
exchanged.

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BOARD NUMBER: S02-058

INVESTIGATING NEURAL SIGNATURES OF WORKING MEMORY IN FRONTOPARIETAL ELECTROCORTICOGRAPHY

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Aims
Working memory is an essential cognitive system involved in retaining and manipulating information. Mental workload describes the relationship between working memory capacity and task demand. Despite its importance, the relationship between working memory load and neural activity remains unclear. **Methods**
We aimed to decode task difficulty, a proxy for working memory load, from frontoparietal electrocorticography data collected from three subjects during an n-back memory task using regularized logistic regression. We fitted generalised models (using data from all subjects) and individualised models. We selected electrodes using either the average difference in amplitude between conditions or in common cortical regions across participants. Decoder performance was assessed by measuring the accuracy for classifying task type, i.e., 1-back, 2-back, or 3-back. **Results**
Working memory load can be decoded from neural activity in frontoparietal cortex with a mean accuracy of 57.23% (61.7%, 71.7%, and 38.3% individually). We show that individualised models produce greater decoding accuracy than generalised models (58.33% vs. 42%). Further, accuracy is not strongly influenced by the electrodes used for training, with 58.33% accuracy for electrodes chosen based on amplitude-differences and 57.23% accuracy for electrodes in common regions. **Conclusions**
Our results suggest that the neural signature of working memory load varies between participants, but some cross-participant features appear to be conserved. These results also suggest that working memory is not strongly localised to a specific cortical region, but is widely distributed in the frontoparietal cortex.

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EVIDENCE OF WORKING MEMORY IMPAIRMENT AT 8-11 MONTHS POSTPARTUM IN WOMEN WITH HISTORY OF SARS-COV-2 INFECTION DURING PREGNANCY

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

Margaret Kyle[1], Audrey Li[2], Cynthia Rodriguez[3,4], Violet Hott[1], Catherine Bianco[5], Sabrina Hyman[1], Mia Kyler[1], Morgan Firestein[3], Lauren Shuffrey[3], Maha Hussain[1], Sharon Ettinger[6], Grace Smotrich[1], Helen Tzul Lopez[1], Mary Bence[1], Catherine Monk[6], Melodie Winawer[2,7], Marla Hamberger[2], Dani Dumitriu[1,3]
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Aims: Several reports have demonstrated cognitive impairment in patients who have recovered from COVID-19. We conducted an objective screening of maternal cognitive functioning at 8-11 months postpartum in women with and without history of SARS-CoV-2 infection during pregnancy. **Methods:** We assessed cognition in 131 women (60 SARS-CoV-2 infected, 71 uninfected) who gave birth at Columbia University Irving Medical Center and enrolled in the COVID-19 Mother Baby Outcomes (COMBO) Study. Over Zoom, we administered the American National Adult Reading Test (AmNART) as an estimate of Full-Scale IQ (FSIQ), the Blind Montreal Cognitive Assessment (MoCA) screen for cognitive impairment, and the Trail Making Test A and B to assess processing speed and executive function. **Results:** Analysis of covariance (ANCOVA) models adjusting for maternal age, years of education, language of administration (English or Spanish), AmNART FSIQ, ethnicity, and race demonstrated that women infected with SARS-CoV-2 during pregnancy scored significantly lower on the global Blind MoCA cognitive screen (23.34±4.68 infected vs. 25.33±3.13 uninfected; $F(1,115)=10.56$; $p=0.002$), due primarily to reduced working memory performance (serial 7s: 2.50±0.87 infected vs. 2.82±0.52 uninfected [$F(1,115)=7.98$; $p=0.006$]; sentence repetition: 1.08±0.79 infected vs. 1.52±0.61 uninfected [$F(1,115)=21.27$; $p<0.001$]). No significant differences were observed in Trail Making. **Conclusions:** Women in our cohort who were infected with SARS-CoV-2 during pregnancy scored significantly lower than uninfected women on the global Blind MoCA screen administered more than 8 months after infection. More specifically, previously infected women exhibited impairment in tasks assessing working memory.

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NON-LINEAR FUNCTIONAL CO-ACTIVATIONS IN SHORT-TERM MEMORY TASK

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Human memory is prone to errors, that is why the neural correlates of true and false recognition have been investigated for years. Meanwhile, the influence of time-of-day on cognitive functions has been confirmed by numerous previous research. The aim of this study was to determine the effects of diurnal factors on true and false recognition in short-term memory with the use of a non-linear correlation analysis, originally designed for resting-state fMRI data (known as "resting BOLD event triggered averages"). Fifty-four young and healthy participants (32 females, mean age: 24.17 ± 3.56 y.o.) performed a task in an MR scanner twice, once in the morning and once in the evening. The task was based on the Deese-Roediger-McDermott paradigm adjusted to investigate the short-term memory and required global visual information processing. The regional BOLD activity was non-linearly correlated with the stimuli, separately for the encoding and retrieval phases of the task. The results of a model predicting true and false recognitions demonstrated that the correlations between the stimuli and BOLD interact with the time-of-day and the type of the probe (a previously seen versus a misleadingly similar one). Moreover, the interactions depended on the brain regions, with particular differences in correlations for the two types of probe within hippocampal areas. The study sheds new light on the influence of diurnal factors on memory functioning, on the neural correlates and predictive measures of memory distortions as well as it confirms the effectiveness of the non-linear correlation analysis method for the task fMRI paradigm.

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INFORMATION FLOWS FROM HIPPOCAMPUS TO AUDITORY CORTEX DURING REPLAY OF VERBAL WORKING MEMORY ITEMS.

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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The maintenance of items in working memory relies on a widespread network of cortical areas and hippocampus where synchronization between electrophysiological recordings reflects functional coupling. We investigated the direction of information flow between auditory cortex and hippocampus while participants heard and then mentally replayed strings of letters in working memory by activating their phonological loop. We recorded LFP from the hippocampus, reconstructed beamforming sources of scalp EEG, and - additionally in 3 participants – recorded from subdural cortical electrodes. When analyzing Granger causality, the information flow was from auditory cortex to hippocampus with a peak in the 4-8 Hz range while participants heard the letters. This flow was subsequently reversed during maintenance while participants maintained the letters in memory. The functional interaction between hippocampus and the cortex and the reversal of information flow provide a physiological basis for the encoding of memory items and their active replay during maintenance.

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UPPER ALPHA OSCILLATORY ACTIVITY REFLECTS SUB-PROCESSES OF CREATING, BUT NOT SOLVING GEOMETRIC MATRICES

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Although the relationship between fluid intelligence and creativity has been studied for many years, the underlying neural mechanisms of this relationship are still under debate. Typically, fluid intelligence is measured using convergent thinking tasks, whereas creativity is measured using divergent thinking tasks. Both fluid intelligence and creativity have been associated with working memory abilities (especially working memory capacity). In our study, we investigated how divergent and convergent thinking (as proxy for fluid intelligence and creativity) are related above and beyond activity related to working memory. We collected EEG data while 17 participants performed the Creative Reasoning Task (CRT) and the Raven's Advanced Matrices (APM), in which participants are asked to either create or solve geometric matrices, respectively. Furthermore, we measured working memory within the same knowledge domain and by using highly comparable stimulus material. We analyzed upper alpha (10-12 Hz) oscillatory activity in both CRT and APM while subtracting working memory related activity. To account for the temporal variability associated with the different cognitive stages in convergent and divergent thinking, we investigated early, intermediate and late stages of the self-paced trials in CRT and APM. In addition, we separated the CRT into phases according to predominantly divergent and convergent thinking processes. Beyond activity related to working memory, we observed differences in upper alpha synchronization in divergent thinking phases which were not found in convergent thinking phases. This implies that increased activity in the upper alpha band reflects sub-processes of creating - but not solving - geometrical matrices.

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SHORT-TERM MEMORY CAPACITY IS DETERMINED BY LONG-TERM MEMORY REPRESENTATIONS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Several studies have described the attentional bottleneck and tried to explore the nature of the limitation in short-term memory (e.g., Luck & Vogel, 1997). Most notably whether short-term memory limitations are feature- or object-based, and while alternate models add to the debate, most seem to omit the possible role of familiarity and expertise. In a range of behavioral studies, we investigate how attentional selection and encoding into short-term memory are modulated by prior expertise and the degree of familiarity. Sørensen & Kyllingsbæk (2012) demonstrate that memory capacity is modulated by expertise in a change detection design of various age groups ranging from children to adults, this is later replicated by Dall, Watanabe, & Sørensen (2016) in three groups of adults with varying degrees of knowledge to Japanese, and finally processing of Chinese characters are explored in a whole-report design revealing that familiarity solely drives processing over character complexity (Dall, Wang, Cai, Chan, & Sørensen, 2021). Across several studies we demonstrate the dynamics of how familiarity and expertise gradually tune mental representations involved in perceptual processing and attentional selection (see also Brogaard & Sørensen, *in press*), suggesting that long-term memory representations play a pivotal role in both attentional selection and the encoding into short-term memory.

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MISMATCH NEGATIVITY, A NEURAL MARKER OF PLASTICITY IN UNILATERAL HEARING LOSS PATIENTS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

Mariam Alzaher[1], Strelnikov Kuzma[2], Pascal Barone[3], Mathieu Marx[2]
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Objectives: Unilateral Hearing Loss (UHL) possess detrimental consequences on horizontal sound localization. However, some patients might present near-normal localization accuracies that could be related to spatial induced plasticity due to monaural spatial adaptation. In order to assess the neural mechanisms underlying auditory spatial adaptation in UHL, we used the Mismatch Negativity (MMN) as a neural marker of plasticity, combined with a behavioural evaluation of sound localization and speech recognition. **Methods:** Twenty-one UHL were assigned into three groups based on their behavioural performances using K-mean cluster analysis: better performers group (BPG n=6), moderate (MPG n=9) and poor (PPG n=6), and 20 normal hearing subjects (NHS) took part in the study. Participants underwent behavioural evaluations of sound localization and speech segregation, followed by a passive oddball paradigm to record the MMN as a response to 3 spatial deviations placed at 10°, 20° and 100° of deviation from the standard (50°). NHS underwent binaural (NHS_bin) and monaural (NH_mon) stimulation in both behavioural and neural investigations. **Results:** BPG showed normal localization performances, reflected by significant MMN at small spatial disparities of 10° similarly to NH_bin. In addition, despite their hearing loss, MPG had better spatial sensitivity than NH_mon with significant MMN starting from 20° of deviation. PPG and NH_mon had the highest localization errors, with an MMN response only at 100° of deviation. **Conclusion:** Our results suggest that some UHL patients are able to adapt to monaural hearing, and that MMN can be used as a neural marker to reflect this adaptation.

Pubmed:

34657730: Alzaher M, Vannson N, Deguine O, Marx M, Barone P, Strelnikov K
Brain plasticity and hearing disorders.
Permanently changed sensory stimulation can modify functional connectivity patterns in the healthy brain and in pathology. In the pathology case, these adaptive modifications of the brain are referred to as compensation, and the subsequent configurations of functional connectivity are called compensatory plasticity. The variability and extent of auditory deficits due to the impairments in the hearing system determine the related brain reorganization and rehabilitation. In this review, we consider cross-modal and intra-modal brain plasticity related to bilateral and unilateral hearing loss and their restoration using cochlear implantation. Cross-modal brain plasticity may have both beneficial and detrimental effects on hearing disorders. It has a beneficial effect when it serves to improve a patient's adaptation to the visuo-auditory environment. However, the occupation of the auditory cortex by visual functions may be a negative factor for the restoration of hearing with cochlear implants. In what concerns intra-modal plasticity, the loss of interhemispheric asymmetry in asymmetric hearing loss is deleterious for the auditory spatial localization. Research on brain plasticity in hearing disorders can advance our understanding of brain plasticity and improve the rehabilitation of the patients using prognostic, evidence-based approaches from cognitive neuroscience combined with post-rehabilitation objective biomarkers of this plasticity utilizing neuroimaging.
Rev Neurol (Paris), 2021; 177
33390347: Alzaher M, Serrano P, Tardieu J, Barone P, Marx M, Nieto P
Contribution of a method of assessing minimum audible angle in headphones.
The main objective of this study was to test the feasibility of measuring minimum audible angle in headphones with different reference positions in the horizontal plane, and comparing different types of pre-recorded head-related transfer functions. The secondary objective was to assess spatial discrimination performance in simulated unilateral hearing loss by measuring the minimum audible angle under monaural conditions using headphones.
Eur Ann Otorhinolaryngol Head Neck Dis, 2021; 138

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CHANGES IN HIPPOCAMPAL ACTIVITY AND MEMORY FUNCTION DURING NEUROFEEDBACK TRAINING WITH INTRACRANIAL ELECTRODES

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Aims: Resective surgery for mesial temporal lobe epilepsy carries the potential risk of memory decline. Although neurofeedback (NF) is thought to be a promising solution for preserving memory function, there have been no studies on its application to preserve memory function. Thus, we aimed to develop an NF system for memory function and investigate the changes in neural activity and memory task scores in the hippocampal area through NF training. ***Methods:*** We constructed a memory NF system that uses intracranial electrodes to acquire and visualize brain activity near the hippocampus while memorizing words. Twenty trials of tug-of-war per session were used for NF, and the game was designed so that the tug was pulled to the right (red team side) when brain activity increased and to the left (white team side) when brain activity decreased. Two patients participated in this NF training, and they were told which team to root for before the start of each session and were instructed to try to win (red team/white team root condition). ***Results:*** The difference in NF signal (hippocampal low-theta power ratio for the first trial) between the two conditions became larger as the number of sessions increased. On the other hand, there was no correlation between the hippocampal activity change and the memory task performance change. ***Conclusions:*** Our results indicated that NF could change hippocampal activity related to memorization. We plan to improve the NF system further and verify its effects on memory functions for future study.

Pubmed:
33192387: Koizumi K, Ueda K, Li Z, Nakao M
Effects of Transcranial Direct Current Stimulation on Brain Networks Related to Creative Thinking.
Human creative thinking is unique and capable of generating novel and valuable ideas. Recent research has clarified the contribution of different brain networks (default mode network, DN; executive control network; salience network) to creative thinking. However, the effects of brain stimulation on brain networks during creative thinking and on creative performance have not been clarified. The present study was designed to examine the changes in functional connectivity (FC) and effective connectivity (EC) of the large-scale brain network, and the ensuing changes in creative performance, induced by transcranial direct current stimulation (tDCS). Fourteen healthy male students underwent two tDCS sessions, one with actual stimulation and one with sham stimulation, on two separate days. Participants underwent tDCS (anode over the left dorsolateral prefrontal cortex, DLPFC; cathode over the right inferior parietal lobule, IPL) for 20 min. Before and after the tDCS session, electroencephalography signals were acquired from 32 electrodes over the whole head during the creative thinking task. On FC analysis, the delta band FC between the posterior cingulate cortex and IPL significantly increased only after real stimulation. We also found that the change of flexibility score was significantly correlated with the change in: (i) delta band FC between mPFC and left lateral temporal cortex (LTC) and (ii) alpha band FC between IPL and right LTC. On EC analysis, decreased flow within the DN (from left LTC to right IPL) was observed. Our results reveal that tDCS could affect brain networks, particularly the DN, during creative thinking and modulate key FC in the generation of flexible creative ideas.
Front Hum Neurosci, 2020; 14
30440574: Koizumi K, Ueda K, Nakao M
Development of a Cognitive Brain-Machine Interface Based on a Visual Imagery Method.
In the field of brain-machine interface (BMI) research, the development of cognitive BMI is a hot topic because it may lead to more intuitive and goal-directed findings than existing BMI technology. In this study, we devised a "visual-imagery method," which enables visual imaging of the operation of a target. We also investigated an "inner-speech method," which comprised internal pronunciation of words without emitting sounds, and an "inner-speech + visual-imagery method," which combined the two methods. When only the high γ band (60-120 Hz) power in the prefrontal cortex was used, the average accuracy of the 15 participants, with 20-fold crossvalidation, was 81.3% in inner speech, 84.6% in visual imagery, and 83.2% in inner speech + visual imagery. This study also found that the frontal pole was the most useful region in the prefrontal

cortex.
Annu Int Conf IEEE Eng Med Biol Soc, 2018; 2018

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BOARD NUMBER: S02-066

GRID-LIKE CODES IN THE HUMAN ENTORHINAL CORTEX MAP VISUAL SPACE DURING MEMORY FORMATION

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Whether we will remember or forget is partly determined by the processes engaged upon the initial encounter of new information. For instance, when presented with visual input, we tend to actively sample information with saccadic eye movements to visually explore and extract relevant image features which can support recognition memory thereafter. Grid cells in the entorhinal cortex of animals and humans were recently shown to map visual space, similar to their role in navigating physical space. Here, we asked whether grid-like coding of visual space supported memory formation. Fifty human participants studied face and scene images while undergoing functional magnetic resonance imaging (fMRI) and continuous eye tracking. To determine memory performance, participants completed a recognition memory test immediately after studying as well as one week later. Results revealed significantly increased activation in the medial temporal lobe (including the hippocampus, parahippocampal and entorhinal cortices), the ventromedial prefrontal cortex and occipital regions when studying later remembered compared to later forgotten images. Focusing on eye movement-related activity, we found significantly increased grid-like codes in the entorhinal cortex when participants performed saccades to study later remembered images, but not when they studied later forgotten images. We provide first evidence that entorhinal grid-like coding of visual space supports memory formation.

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HIPPOCAMPAL SUBFIELDS AND THEIR NEOCORTICAL INTERACTIONS DURING AUTOBIOGRAPHICAL MEMORY USING SUBMILLIMETER WHOLE-BRAIN FMRI AT 7 TESLA

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Re-living autobiographical memories (AM) is one of our most valued cognitive abilities and it is impaired in a broad range of psychiatric and neurological disorders with devastating effects for patients and caregivers. The hippocampus (HC) plays a key role in vivid AM retrieval. However, the HC comprises several subfields and it remains unclear how HC subfields differentially contribute to AM. Furthermore, while the HC is part of a distributed brain network supporting AM, due to technical limitations inherent to 3T fMRI, how HC subfields interact with this network is unknown. A novel 7T fMRI sequence covering the whole-brain at submillimeter resolution was employed using customized 3D Echo Planar Imaging (EPI). This sequence enabled us to examine both detailed HC subfield activity and whole-brain functional connectivity during AM retrieval. While scanning, 24 healthy participants were instructed to relive detail rich AM relevant to specific word cues or solve simple math problems as a control condition. HC subfields were manually segmented, and fMRI signals extracted for each subfield and condition in all participants. Our results suggest that, while all HC subfields were engaged during AM retrieval, the pre/parasubiculum contributed more so than other subfields. Subsequent, functional connectivity analyses also revealed that the pre/parasubiculum was strongly connected to neocortical regions typically associated with AM. Together, these technically innovative results indicate functional differentiation amongst HC subfields and their neocortical connectivity pattern, highlighting a crucial role of the pre/parasubiculum in AM retrieval.

Pubmed:
33180737: Banluesombatkul N, Ouppaphan P, Leelaarporn P, Lakhan P, Chaitusaney B, Jaimchariyatam N, Chuangsuwanich E, Chen W, Phan H, Dilokthanakul N, Wilaiprasitporn T
MetaSleepLearner: A Pilot Study on Fast Adaptation of Bio-Signals-Based Sleep Stage Classifier to New Individual Subject Using Meta-Learning.
Identifying bio-signals based-sleep stages requires time-consuming and tedious labor of skilled clinicians. Deep learning approaches have been introduced in order to challenge the automatic sleep stage classification conundrum. However, the difficulties can be posed in replacing the clinicians with the automatic system due to the differences in many aspects found in individual bio-signals, causing the inconsistency in the performance of the model on every incoming individual. Thus, we aim to explore the feasibility of using a novel approach, capable of assisting the clinicians and lessening the workload. We propose the transfer learning framework, entitled MetaSleepLearner, based on Model Agnostic Meta-Learning (MAML), in order to transfer the acquired sleep staging knowledge from a large dataset to new individual subjects (source code is available at https://github.com/IoBT-VISTEC/MetaSleepLearner). The framework was demonstrated to require the labelling of only a few sleep epochs by the clinicians and allow the remainder to be handled by the system. Layer-wise Relevance Propagation (LRP) was also applied to understand the learning course of our approach. In all acquired datasets, in comparison to the conventional approach, MetaSleepLearner achieved a range of 5.4% to 17.7% improvement with statistical difference in the mean of both approaches. The illustration of the model interpretation after the adaptation to each subject also confirmed that the performance was directed towards reasonable learning. MetaSleepLearner outperformed the conventional approaches as a result from the fine-tuning using the recordings of both healthy subjects and patients. This is the first work that investigated a non-conventional pre-training method, MAML, resulting in a possibility for human-machine collaboration in sleep stage classification and easing the burden of the clinicians in labelling the sleep stages through only several epochs rather than an entire recording.
IEEE J Biomed Health Inform, 2021; 25

32960771: Piriyajitakonkij M, Warin P, Lakhan P, Leelaarporn P, Kumchaiseemak N, Suwajanakorn S, Pianpanit T, Niparnan N, Mukhopadhyay SC, Wilaiprasitporn T
SleepPoseNet: Multi-View Learning for Sleep Postural Transition Recognition Using UWB.
Recognizing movements during sleep is crucial for the monitoring of patients with sleep disorders, and the utilization of ultra-wideband (UWB) radar for the classification of human sleep postures has not been explored widely. This study investigates the performance of an off-the-shelf single antenna UWB in a novel application of sleep postural transition (SPT) recognition. The proposed Multi-View Learning, entitled SleepPoseNet or SPN, with time series data augmentation aims to classify four standard SPTs. SPN exhibits an ability to capture both time and frequency features, including the movement and direction of sleeping positions. The data recorded from 38 volunteers displayed that SPN with a mean accuracy of 73.7 ±0.8 % significantly outperformed the mean accuracy of 59.9 ±0.7 % obtained from deep convolution neural network (DCNN) in recent state-of-the-art work on human activity recognition using UWB. Apart from UWB system, SPN with the data augmentation can ultimately be adopted to learn and classify time series data in various applications.
IEEE J Biomed Health Inform, 2021; 25
32816110: Helmstaedter C, Hansen N, Leelaarporn P, Schwing K, Oender D, Widman G, Racz A, Surges R, Becker A, Witt JA
Specific B- and T-cell populations are associated with cognition in patients with epilepsy and antibody positive and negative suspected limbic encephalitis.
Neuropsychological impairments are major symptoms of autoimmune limbic encephalitis (LE) epilepsy patients. In LE epilepsy patients with an autoimmune response against intracellular antigens as well as in antibody-negative patients, the antibody findings and magnetic resonance imaging pathology correspond poorly to the clinical features. Here, we evaluated whether T- and B-cells are linked to cognitive impairment in these groups.
J Neurol, 2021; 268
32199348: Hansen N, Önder D, Schwing K, Widman G, Leelaarporn P, Prusseit I, Surges R, Becker AJ, Witt JA, Helmstaedter C, Elger CE
CD19+ B-cells in autoantibody-negative limbic encephalitis.
Flow cytometry helps to elucidate the cellular immune repertoire's mechanisms in patients with temporal lobe epilepsy (TLE) due to limbic encephalitis (LE) subcategories and carries potential significance for subtype-specific treatment.
Epilepsy Behav, 2020; 106
31846897: Hansen N, Schwing K, Önder D, Widman G, Leelaarporn P, Prusseit I, Surges R, Melzer N, Gross C, Becker AJ, Witt JA, Elger CE, Helmstaedter C
Low CSF CD4/CD8+ T-cell proportions are associated with blood-CSF barrier dysfunction in limbic encephalitis.
Investigating immune cells in autoimmune limbic encephalitis (LE) will contribute to our understanding of its pathophysiology and may help to develop appropriate therapies. The aim of the present study was to analyze immune cells to reveal underlying immune signatures in patients with temporal lobe epilepsy (TLE) with LE.
Epilepsy Behav, 2020; 102

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BOARD NUMBER: S02-068

MEMORY SUPPRESSION RELIES ON TARGETED REPRESENTATIONAL CONTROL OF INDIVIDUAL MEMORIES

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Some memories haunt us. However, prior research has shown that the human brain can learn to control intrusive memories even when faced with reminders. By deliberately suppressing their retrieval, unwanted memories become less frequent, less vivid and can even be forgotten. This suggests that memory control depends on the targeted weakening of individual representations of unwanted memories. Here we tracked this gradual decrease in mnemonic strength of intrusive memories in the ventral visual stream and the hippocampus using fMRI. To isolate instances of involuntary recall, we used the Think/No-Think task in which people are asked to suppress retrieval of a memory, given a reminder to it. By introducing subjective reports after each reminder, we identified trials in which a memory came to mind, despite attempts to suppress it, indicating involuntary retrieval. We then correlated activation patterns of each suppression trial with patterns from a separate visual task that belonged to the same item. This allowed us to quantify the degree to which an individual memory was reinstated on each suppression trial. We hypothesized that reported intrusions will be accompanied by more reinstatement than trials without memory intrusions. However, irrespective of intrusion reports, reinstatement of memory specific activation should decline over suppression attempts. This reduction in reinstatement should be related to the decrease in the frequency of involuntary recalls and memory deficits on a later test. Hence, each time we push an intrusive memory out of awareness, its mnemonic strength weakens until its memory trace no longer supports effective remembering.

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BOARD NUMBER: S02-069

HIPPOCAMPAL NEURONS SPARSELY CODE INDIVIDUAL EPISODIC MEMORIES IN HUMANS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Introduction
The hippocampus is an essential hub for episodic memory processing. However, how human hippocampal single neurons code multi-feature associations remains unknown. Some argue that each hippocampal neuron codes for an invariant element within an episode (Quiroga, 2012). Instead, others have proposed that hippocampal neurons bind together all features present in a discrete episodic memory (Teyler and DiScenna, 1986). Methods
In search of an answer to this issue, we have recorded the activity of 585 single neurons in the hippocampus of human epileptic patients (N = 16). During a self-paced memory association task patients formed unique episodic memories comprising an animal cue and two associate images (places or faces). Results
We contrasted the empirical firing reinstatement (firing rate at encoding multiplied with retrieval) of every single unit during every episode with a shuffled distribution. Next, we excluded potential concept neurons that showed increased firing to the animal cue at encoding. In a second level shuffling procedure we show that a significant number (N = 144/585; $p < 0.001$) of single units showed a memory specific reinstatement of neural firing during individual episodes. We termed these single units Episode Specific Neurons (ESNs).

Conclusions
In conclusion, we provide evidence that individual neurons code discrete episodes via an increased firing rate during encoding and retrieval. We demonstrate that these Episode Specific Neurons do not reflect the coding of a particular feature in the episode (i.e., concept or time). Instead, they code for the conjunction of the different elements that make up the episode.

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BOARD NUMBER: S02-070

POST-STIMULUS INACTIVATION OF THE MAMMILLARY BODIES IMPAIRS PERFORMANCE ON SPATIAL RECOGNITION TASKS.

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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The mammillary bodies, and their connections to the anterior thalamic nuclei, are important for memory. Damage to the mammillary bodies can result in diencephalic amnesia in humans and spatial memory impairments in rodents. Previous studies have typically used permanent lesions to determine the contribution of the mammillary bodies making it difficult to differentiate specific roles during stimulus processing, post-stimulus encoding and retrieval. To address this, we used a temporary inactivation approach by infusing the Group II metabotropic glutamate receptor specific agonist, APDC, into the mammillary bodies of rats. Saline infusions were used as a control. Rats were tested on two spatial memory tasks: a place discrimination task in the Y maze and an object-in-place task. Both tasks involved a sample phase, followed by a 6h delay, then a test session. The extent to which animals spent exploring the novel place (Y-maze) or novel place-object combination during the test phase was taken as a measure of “memory” of the sample phase. When rats were infused with APDC immediately after the sample phase, their performance was impaired on both spatial tasks, compared to performance following saline infusions. In contrast, infusions of APDC 15 minutes before the test phase resulted in similar performance to that seen following saline infusions. This highlights a specific involvement of the mammillary bodies in post-stimulus processing.

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BOARD NUMBER: S02-071

INTERMITTENT WORKING MEMORY TRAINING PROTECTS FROM AGE-RELATED DECLINE OF SPATIAL MEMORY IN RATS.

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Engagement in cognitive activity in adulthood is one of the factors that enable successful cognitive aging, both in humans and rodents. However, some studies emphasize that the beneficial effect on cognition of such an activity may reflect carry over from one test situation to another, including memory for procedural aspects of the behavioral tasks, and thus question whether this effect can be limited to the trained cognitive domain or whether it can be transfer to an untrained one's. In the current study, we assessed whether adulthood intermittent working memory training has beneficial effect on long-term memory of aged rats using two very different test situations. To this aim, rats trained in a delayed non-matching to position task in operant box at 3 and 15 months of age were tested in a place learning task in water maze when they were 24 months. The two tasks differ with regard to the cognitive domain but also in their spatial ability requirement and the nature of the reinforcer used. During the memory tests, accuracy of the platform search indicated age-related impairment only in the aged-untrained group. In contrast to aged untrained rats, aged trained rats performed as young ones: they showed a specific search in the target quadrant. Thus, intermittent training during adult life in a task involving working memory protects against the effects of aging on spatial memory abilities. Working memory training has beneficial effect on an untrained cognitive domain in older animals.

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BOARD NUMBER: S02-072

RESPONSE BIASES IN A VISUOSPATIAL DELAYED RESPONSE TASK

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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In decision-making tasks, animal responses must sometimes be maintained in short-term memory before their execution, a period during which they are presumably prepared but they can also undergo alterations. NMDA receptors have been linked to such mnemonic maintenance. Aiming to better understand neural mechanisms of action selection, maintenance in short-term memory and execution, we developed a visuospatial delayed response task in mice. Subjects were trained to look at a visual stimulus briefly displayed on a touchscreen, maintain its position during a short mnemonic delay, and execute a response by touching at the remembered position. We found that animals' errors increased as a function of delay showing that, at least partially, mice made memory maintenance errors. Forgetting errors were idiosyncratically biased, suggesting that memory stability is not homogenous across possible responses but mice exhibit side preferences for one of the memorized choices which increases with delay. Responses in memory trials were also biased by subjects' tendency to repeat the previous choice, however, its magnitude did not vary with delay length. This means that, in some trials, animals use their previous choices instead of a stimulus-guided strategy to resolve the task. Finally, we pharmacologically blocked NMDA receptors systemically and found that animals increased their repeating bias, although their memory stability and overall accuracy were not affected. Our results suggest that mice accuracy is limited by biases in the stability of the different responses and that NMDA receptors might impact how often responses are memory-guided rather than memory stability.

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BOARD NUMBER: S02-073

PHYSICAL ACTIVITY - IS IT INVARIABLY BENEFICIAL?

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Introduction: A growing body of research indicates that regular exercise has a significant impact on cognitive function. The structural changes associated with exercise are reflected in improvements in synaptic plasticity. In addition, it appears that physical activity exerts neuroregenerative and neuroprotective influences. Exercise has been found to benefit working memory in late life, but its effects in young adults are less clear. **The Aim:** The aim is to investigate the effect of subchronic aerobic exercise on spatial memory, neurogenesis, and oxidative stress in young adult female rats. **Methods:** Adult female Wistar rats were divided into two groups: sedentary control (C) and exercise (E). E group ran 30 min/day on a treadmill for 15 consecutive days. The parameters of spatial memory: total number of errors, working, and reference memory errors were tested in the radial arm maze. The number of Ki67+ and NeuN+ cells in the dentate gyrus was determined immunohistochemically. Levels of proBDNF, mBDNF, and markers of oxidative stress have been determined in the hippocampus and the cortex. **Results:** Subchronic exercise increased the total number of errors and working memory errors. Exercise did not change the number of Ki67+ and NeuN+ cells. The expression of BDNF was significantly increased in the cortex of the E group. Exercise significantly decreased the activity of superoxide dismutase and glutathione peroxidase in the hippocampus and the cortex, respectively. **Conclusion:** Subchronic exercise impairs working memory in healthy female rats, which correlates with increased oxidative stress.

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INACTIVATION OF THE VENTRAL MIDLINE THALAMUS DOES NOT ALTER ENCODING, HOLDING OR RESTITUTION OF A SPATIAL WORKING MEMORY IN RATS.

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Tasks assessing spatial working memory (SWM) in animals encompass 3 phases: 1) a first trial allowing animals to encode information, 2) a holding time with various delays (from seconds to hours), and 3) an evaluation trial to test the restitution capabilities. In a delayed SWM water-escape task, we previously showed that activity of the reuniens nucleus (Re) positively correlated with performance on the evaluation trial (Morvan et al. 2021). Here, we used the same protocol in the Double-H maze: rats had to localize a platform submerged in water during the first trial (encoding) and after a 6-h delay, an evaluation trial was performed. Ventral midline thalamic nuclei (Re, Rhomboid, Rh) were inactivated using muscimol (0,26 and 0,70 nmol, 0,3 µL) or DREADD-inhibition (2 X 0.4 µL of 7.3 X 10^9 genomic copies /µL, AAV8-Camk2α-hM4Di-mCherry, Vigene, Biosciences) either during or right after the first trial, or before the second trial. With both amounts of muscimol, inhibition of the ReRh nuclei, be it during or after encoding, or during evaluation, did not alter performance. CNO administered before encoding in rats with DREADD-expressing neurons in ReRh was also ineffective. These findings are the first to challenge previous data showing an implication of ReRh nuclei in SWM. The main difference between our task and other standard SWM tasks, (usually in a T-maze), is the lower risk for proactive interference, especially due to the longer delay we used between encoding and restitution. The discrepancy calls for further investigation.

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BOARD NUMBER: S02-075

NMDAR BLOCKING BY MK801 ALTERS HIPPOCAMPAL AND PREFRONTAL CORTEX OSCILLATIONS AND IMPAIRS SPATIAL WORKING MEMORY IN MICE

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Abnormal NMDAr function has been linked to rhythmopathies, psychosis, and cognitive dysfunction in schizophrenia (SCZ). Here, we investigate the role of NMDAr hypofunction in pathological oscillations and behavior. We implanted mice with tetrodes in the dorsal hippocampus and medial prefrontal cortex (mPFC), administered the NMDAr antagonist MK801, and recorded oscillations during spontaneous exploration in an open field and in the y-maze spatial working memory test. Our results show that NMDAr blockade increased locomotor activity, impaired spatial working memory, and disrupted the correlation between oscillations and speed of movement, crucial for internal representations of distance. In the hippocampus, MK801 increased gamma oscillations and theta/gamma coupling, while in the mPFC, it increased the power of theta, gamma, and generated high-frequency oscillations (HFO 155-185 Hz). Spatial working memory tests in the y-maze revealed that theta/gamma coupling was consistently higher in correct trials after MK801 administration but lower in the baseline condition. Theta/gamma co-modulation mediated by NMDAr function might be essential to explaining several of SCZ's cognitive symptoms.

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BOARD NUMBER: S02-076

IN VIVO TWO-PHOTON IMAGING OF HIPPOCAMPAL NEURONS DURING THE FORCED ALTERNATION WORKING MEMORY TASK IN 5XFAD ALZHEIMER MOUSE MODEL

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Alzheimer's disease (AD) is associated with progressive memory decline and cognitive impairments. Spatial memory decline in transgenic mouse models of AD is believed to reflect neuropathological processing in the hippocampus. Here we present that two-photon imaging of hippocampal CA1 neurons in the 5xFAD model of AD during the performance of a spatial working memory task in an air-lifted T maze. 5xFAD and WT control mice were divided into two age groups: young (2-4 months) and old (7-9 months). AAV-jGCaMP7s were injected into the pyramidal cell body layer of the hippocampal CA1 subregion, then implanted a glass craniotomy imaging window by aspirating the cortex above the injection site and a metal head-plate. Water-restricted animals were trained using 1% sucrose water rewards under head-fixation in the air-lifted T maze cage for two sessions daily. We recorded hippocampal neural activity using two-photon calcium imaging in both 5xFAD and WT mice during both learning and testing. Our results demonstrate deficits in 5xFAD mice in the forced alternation working memory task in both age groups. Hippocampal CA1 neurons show neuronal hyperactivity in 5xFAD mice. Also, place cells are hyperactive only in animals with working memory deficits. Place cell quality is correlated to performance level in the air-lifted T maze alternation task. In this work, we have validated a new working memory task compatible with in vivo head-fixed two-photon calcium imaging, enabling quantitative assessment of deficits in and recovery of neural circuit function underpinning cognitive behaviour in mouse models of AD.

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BOARD NUMBER: S02-077

PHARMACOLOGICAL AND GENETIC PROBING OF THE ROLE OF NOCICEPTIN/ORPHANINFQ RECEPTORS IN CHRONIC STRESS-INDUCED MEMORY DEFICITS.

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Chronic stress causes memory deficits, together with impairments in structural plasticity in the hippocampus. The mechanisms underlying these deleterious effects remain to be fully understood. Beside classical mediators such as corticosterone (CORT), stress induces the release of an opioid-related neuropeptide named Nociceptin/OrphaninFQ (N/OFQ) which acts on a G protein-coupled receptors called NOP. Since this peptide has deleterious effects on memory when injected into the brain, we hypothesized that it could be a mediator of the negative effects of stress on memory and plasticity. In order to establish a causal link between stress-induced impairments in memory and structural plasticity and the dysregulation of the N/OFQ system, we performed systemic and local blockade of the nociceptin system *via* pharmacological and genetic tools in mice exposed to chronic CORT administration. We show that acute NOP receptor blockade restores long-term memory performance in the object location and recognition tasks. Our results also suggest a protective effect of the NOP antagonist against the hippocampus structural defects induced by stress. More specifically co-treatment with the antagonist prevents the deficits in spinogenesis observed in adult-born neurons following chronic CORT exposure. The region-specific knockout of NOP receptors in the hippocampus is in progress. Preliminary data suggest that this local knockout mimics the effect of systemic antagonist treatment. Our data demonstrate that blocking the N/OFQ system can be beneficial for hippocampus plasticity and memory in a pharmacological model of chronic stress. We therefore suggest that NOP antagonists could be useful for the treatment of memory deficits in stress-related disorders.

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INTERNALLY ORGANIZED TASK MAPS IN THE MOUSE MEDIAL FRONTAL CORTEX

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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New tasks are often similar in structure to old ones. Animals that take advantage of such conserved or “abstract” task structures can master new tasks with minimal training. To understand the neural basis of this abstraction, we developed a novel behavioural paradigm for mice and recorded from their medial frontal neurons as they learned. Freely moving mice learned multiple tasks where they had to visit 4 rewarded locations in sequence (ABCD) on a 3x3 spatial maze. Tasks within this "ABCD paradigm" shared the same circular transition structure (… ABCDABCD …) but differed in the locations and geometric arrangement of rewards. As well as improving across tasks, mice inferred that A followed D (i.e. completed the loop) on the very first trial of a new task. This “zero-shot inference” is only possible if animals had learned the abstract structure of the task. Medial frontal cortex (mFC) neurons were tuned to task space in a hierarchical manner. Moreover, this tuning showed multiple signatures of internal organization, including offline replay. These findings point to separable neuronal substrates for internally organised representations of task structure that may guide abstraction in the mammalian brain

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THE ROLE OF TRANSIENT RECEPTOR POTENTIAL CHANNELS IN MEMORY CONSOLIDATION IN THE PASSIVE AVOIDANCE TASK LEARNING MODEL IN ONE-DAY OLD CHICKS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Introduction An increase in intracellular Ca^{2+} concentration is an important step in memory consolidation processes that stabilize a memory trace after its initial acquisition. Stimulation of mGluR1 and mGluR5 leads to an increase in intracellular Ca^{2+} by stimulation of IP3 receptors and Ca^{2+} release from intracellular stores and by activation transient receptor potential (TRP) channels. Ca^{2+} fluxes tie mGluR1/5 and TRP channels with the effector kinase cascade (CaMKII) indicating that their activation affects memory formation. Thus, disclosure of the potential of each of these Ca^{2+} signals in memory formation may establish the premise for innovative strategy and treatment of memory impairment. **Methods** We used the passive avoidance task in one-day chicks. Animals trained to avoid pecking metal bead covered with bitter-tasting substance (methylanthranilate) were tested 2 h after training. The non-specific TRP channels antagonist or agonist, and specific mGluR1 mGluR5 antagonists or agonists were injected immediately after training into intermediate medial mesopallium, the chick brain region responsible for early stages of memory formation. **Results** Inhibition of mGluR1/5 or TRP channels resulted in significant memory impairment. Inhibition of TRP channels in the presence of mGluR1/5 agonist also disrupted memory formation. However, inhibition of mGluR1/5 in the presence of TRP channels agonist did not result in amnesia. TRP channels agonist prevented amnesia even though Ca^{2+} release from an intracellular store was inhibited due to IP3 agonist administration. **Conclusion** Our results show that the memory formation process can bypass the mGluR1/5 channels pathway, using the Ca^{2+} signal generated by TRP channels.

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BOARD NUMBER: S02-080

DISTRACTION AND PROACTIVE INTERFERENCE FOR OBJECT MEMORY IN THE SUB-CHRONIC PHENCYCLIDINE (SCPCP) MODEL OF SCHIZOPHRENIA; A COMPARISON OF RAT AND MOUSE BEHAVIOUR

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Introduction Cognitive deficits are a key, treatment-resistant symptom of schizophrenia. The rodent sub-chronic phencyclidine (scPCP) model of schizophrenia exhibits robust amnesia in the novel object recognition (NOR) task, which can be improved in both scPCP rats and mice by minimising distraction during the task inter-trial interval (Grayson *et al.,* 2014; Gigg *et al*., 2020). Minimising distraction allows the study of disease-relevant proactive interference via multiple consecutive NOR trials in scPCP rats using the continuous NOR (cNOR) task (Landreth et al. 2021). However, whether scPCP mice are also sensitive to proactive interference in this task is unknown. **Aims** To run the cNOR task with scPCP mice to determine if there are species-specific differences in task performance. **Methods** Female c57 mice (c. 12 weeks old) were dosed sub-chronically with phencyclidine (2mg/kg daily for 10 days) before undergoing standard NOR testing. Under mild food restriction, mice were then trained to perform 11 consecutive NOR trials using a continuous testing design (cNOR). Exploration of the novel versus familiar object was measured for each trial. **Results and Conclusions** Preliminary results indicate that scPCP mice are unable to identify object novelty in the standard NOR task, as expected, but their performance is rescued in trial one of the cNOR. Interestingly, initial analyses suggest that the effect of proactive interference on object memory is weaker in mice compared to rats for both scPCP and control groups. A full analysis of these data will be presented.

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BOARD NUMBER: S02-081

BINDING CELL ASSEMBLIES INTO MEMORY ENGRAMS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Memory storage is distributed in large brain territories. However, distributed information storage imposes the need for coordination to link the activated experience-relevant neurons into comprehensive memory engrams. Here we are concern with the mechanisms of activity coordination during contextual memory encoding and hypothesize that the dentate gyrus (DG) plays a critical role. Synaptic potentiation in the DG has been shown to enhance the functional coupling of a network of mesolimbic neocortical and subcortical structures important for memory formation. Furthermore, optogenetic activation of granule cells in the DG, tagged via c-Fos-dependent expression of channelrhodopsin, has been shown to recall complete contextual fear memories. We combined pharmacogenetic tools to bidirectionally modulate granule cells activity through the regulation of parvalbumin (PV) cell activity, while mice encoded spatial information in the Novel Object Location task. Disinhibition of DG enhanced the encoding of novel object-location associations whereas increased inhibition blocked it. Correlations between the number of c-Fos+ neurons in a network composed by different hippocampal structures, the prefrontal cortex and the nucleus accumbens, demonstrates that DG disinhibition enhances the functional coupling between these regions, while increased inhibition decorrelates cell assemblies in the network. We will further present our latest graph theory analysis on an extended brain network composed of more than 200 brain areas. Our results attribute a pivotal role to the DG in coordinating brain-wide functional connectivity and help explain why reactivation of engrams in the DG can efficiently induce recall of artificial memories.

Pubmed:
30874323: Garcia-Hernandez R
Towards developing meaningful MRI biomarkers of neuroinflammation.
J Neurosci Res, 2019; 97
32637601: De Santis S, Cosa-Linan A, Garcia-Hernandez R, Dmytrenko L, Vargova L, Vorisek I, Stopponi S, Bach P, Kirsch P, Kiefer F, Ciccocioppo R, Sykova E, Moratal D, Sommer WH, Canals S
Chronic alcohol consumption alters extracellular space geometry and transmitter diffusion in the brain.
Already moderate alcohol consumption has detrimental long-term effects on brain function. However, how alcohol produces its potent addictive effects despite being a weak reinforcer is a poorly understood conundrum that likely hampers the development of successful interventions to limit heavy drinking. In this translational study, we demonstrate widespread increased mean diffusivity in the brain gray matter of chronically drinking humans and rats. These alterations appear soon after drinking initiation in rats, persist into early abstinence in both species, and are associated with a robust decrease in extracellular space tortuosity explained by a microglial reaction. Mathematical modeling of the diffusivity changes unveils an increased spatial reach of extrasynaptically released transmitters like dopamine that may contribute to alcohol's progressively enhanced addictive potency.
Sci Adv, 2020; 6

FENS Forum 2022
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BOARD NUMBER: S02-082

TOWARDS NEURAL RECORDINGS AND NEUROSTIMULATION OF DISTRIBUTED BRAIN NETWORKS DURING COGNITIVE TASKS IN NON-HUMAN PRIMATES

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Cognitive deficits caused by ageing, neurodegenerative diseases or brain injury are associated with a disruption of neural oscillations in large-scale brain networks, but the exact signatures of physiological and impaired cognitive processes are unclear. Here, we propose a methodological framework for studying these oscillations in distributed brain networks and for applying neurostimulation protocols during cognitive tasks in non-human primates. We first trained 2 macaque monkeys to perform a memory task called “paired associates learning” on an instrumented touchscreen (MonkeyCANTAB, >100 sessions each). Animals were trained to select a stimulus presented at one among 1-4 possible locations. Task difficulty was further increased by presenting 2 consecutive stimuli, followed by two choice phases containing 2-3 distractors each. Preliminary results indicate that both monkeys perform well above chance level, and increasing task difficulty results in lower performance. This task, used to probe episodic memory, is known to rely on the hippocampal formation and the prefrontal cortex. To study neuronal oscillations across this network, we next designed a novel brain implant to record field potentials and apply electrical stimulation in three disctinct areas: the hippocampus and entorhinal cortex using two depth macroelectrodes, and the prefrontal cortex using a custom electrocorticographic (ECoG) grid. We performed a preliminary validation of the surgical procedures and neurophysiological recordings during an acute surgery in one anesthetized macaque monkey. These experiments lay the ground for future work that will involve neuronal recording and electrical stimulation in the large-scale memory network during an episodic memory task in non-human primates.

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BOARD NUMBER: S02-083

REPLAY OF MOTOR SEQUENCES IN DORSOLATERAL STRIATUM DURING OFFLINE CONSOLIDATION ARE REVEALED USING A UNSUPERVISED POINT PROCESS MODEL

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Memory is known to be supported by periods of offline consolidation, most significantly during sleep. For episodic memories, reactivation of previously active ensembles (replay) is thought to be the substrate of this consolidation. Like episodic, procedural memory is also improved by sleep but the offline dynamics that support consolidation are not known. Here we use unsupervised analysis methods of recordings from a multi-step sequence task to show that replay of procedural information occurs in the dorsolateral striatum during offline consolidation. Mapping dopamine-dependent transcriptional activation markers revealed that this region of dorsolateral striatum was engaged during the task. Lesion to this area impaired both sequence learning and execution, while pharmacologically blocking plasticity post task revealed that offline consolidation in this region was required for procedural memory. Neuropixel probe recordings revealed that, during the task, activity in the dorsolateral striatum tiles sequence execution with individual neuronal activity locked to distinct postural movements. Recordings were extended to include post-task sleep bouts and linear decoding of online activity, in parallel with novel application of an unsupervised point process model (PP-Seq), revealed multiple instances of replay. These instances were compressed in time and included both partial and full recapitulation of the sequential extent of task related activity. Consequently, our results provide evidence that procedural replay occurs in the striatum during offline consolidation of a motor sequence. These replay instances may represent a mechanism for procedural memory consolidation akin to but independent from those found for consolidation of episodic memory.

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BOARD NUMBER: S02-084

NAVIGATION TO A VIRTUAL PLATFORM TO EVALUATE ENCODING AND RETRIEVAL OF "EVERYDAY MEMORIES"

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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The study of behaviour allows us to contextualize and provide biological meaning to experimentally acquired physiological and molecular data. In the study of memory formation, often, loss- and gain-of-function experiments to investigate the mechanisms underlying specific memory processes, focus on misleading learning curves during manipulations. They commonly reflect their effect on encoding, storage, consolidation, and/or retrieval of memories, without discriminating relative contributions, nor identifying possible confounding factors due to performance alterations (i.e., attention and motor effects). Here we present a modification of the delayed-matching to place protocol of the water maze [1] to investigate “everyday memory” formation. The test is based on spatial navigation in a large arena and the finding of a virtual (invisible) “platform”. Online video tracking of the animals is used to operate the maze, regulting light intensity and opening the access door to a home location when the animal crosses the target virtual platform location. The task requires remembering the location of the previous day platform and encoding the current location, to be tested on the next day. The implementation in a “dry maze”, facilitates concomitant electrophysiological recordings and brain network manipulations based on deep brain electric or optogenetic stimulation (DBS). We show that, in a 3-day protocol with experimental pharmacological or DBS interventions in the second day, this procedure allows dissociation between memory encoding, retrieval and consolidation, measured in the behavioural performance. [1] Rossato et al. *Curr. Biol.* 2018; 28(21):3508-3515.e5.

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BOARD NUMBER: S02-085

MULTISENSORY LEARNING EXPANDS A MEMORY ENGRAM

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Life is a multisensory experience for most animals. Learning to associate and bind different sensory features into a scene is a fundamental process of the brain, which improves subsequent memory performance. However, neural mechanisms that bind sensory features during learning remain to be explained. We developed new multisensory aversive and appetitive memory paradigms in *Drosophila* which combine visual and olfactory cues. Flies trained with combinations of both modalities show improved memory performance, even when the sensory cues are presented separately during testing. We used temporal control of neuronal function to define the components of the mushroom body memory network that are required for multisensory learning and performance enhancement. Calcium imaging in head-fixed flies revealed that dopaminergic reinforcement during multisensory learning binds activity in streams of modality-specific Kenyon Cells. After learning, a unimodal sensory input generates a multimodal neuronal response. This cross-modal binding and expansion of the memory engram underlies improved memory performance.

Pubmed:
34146482: Jacob PF, Vargas-Gutierrez P, Okray Z, Vietti-Michelina S, Felsenberg J, Waddell S
Prior experience conditionally inhibits the expression of new learning in Drosophila.
Prior experience of a stimulus can inhibit subsequent acquisition or expression of a learned association of that stimulus. However, the neuronal manifestations of this learning effect, named latent inhibition (LI), are poorly understood. Here, we show that prior odor exposure can produce context-dependent LI of later appetitive olfactory memory performance in Drosophila. Odor pre-exposure forms a short-lived aversive memory whose lone expression lacks context-dependence. Acquisition of odor pre-exposure memory requires aversively reinforcing dopaminergic neurons that innervate two mushroom body compartments-one group of which exhibits increasing activity with successive odor experience. Odor-specific responses of the corresponding mushroom body output neurons are suppressed, and their output is necessary for expression of both pre-exposure memory and LI of appetitive memory. Therefore, odor pre-exposure attaches negative valence to the odor itself, and LI of appetitive memory results from a temporary and context-dependent retrieval deficit imposed by competition with the parallel short-lived aversive memory.
Curr Biol, 2021; 31
33376141: Silva V, Palacios-Muñoz A, Okray Z, Adair KL, Waddell S, Douglas AE, Ewer J
The impact of the gut microbiome on memory and sleep in .
The gut microbiome has been proposed to influence diverse behavioral traits of animals, although the experimental evidence is limited and often contradictory. Here, we made use of the tractability of for both behavioral analyses and microbiome studies to test how elimination of microorganisms affects a number of behavioral traits. Relative to conventional flies (i.e. with unaltered microbiome), microbiologically sterile (axenic) flies displayed a moderate reduction in memory performance in olfactory appetitive conditioning and courtship assays. The microbiological status of the flies had a small or no effect on anxiety-like behavior (centrophobism) or circadian rhythmicity of locomotor activity, but axenic flies tended to sleep for longer and displayed reduced sleep rebound after sleep deprivation. These last two effects were robust for most tests conducted on both wild-type Canton S and strains, as well for tests using an isogenized panel of flies with mutations in the gene, which causes altered circadian rhythmicity. Interestingly, the effect of absence of microbiota on a few behavioral features, most notably instantaneous locomotor activity speed, varied among wild-type strains. Taken together, our findings demonstrate that the microbiome can have subtle but significant effects on specific aspects of behavior, some of which are dependent on genetic background.
J Exp Biol, 2021; 224
28366741: Franco LM, Okray Z, Linneweber GA, Hassan BA, Yaksi E
Reduced Lateral Inhibition Impairs Olfactory Computations and Behaviors in a Drosophila Model of Fragile X Syndrome.
Fragile X syndrome (FXS) patients present neuronal alterations that lead to severe intellectual disability, but the underlying

neuronal circuit mechanisms are poorly understood. An emerging hypothesis postulates that reduced GABAergic inhibition of excitatory neurons is a key component in the pathophysiology of FXS. Here, we directly test this idea in a FXS Drosophila model. We show that FXS flies exhibit strongly impaired olfactory behaviors. In line with this, olfactory representations are less odor specific due to broader response tuning of excitatory projection neurons. We find that impaired inhibitory interactions underlie reduced specificity in olfactory computations. Finally, we show that defective lateral inhibition across projection neurons is caused by weaker inhibition from GABAergic interneurons. We provide direct evidence that deficient inhibition impairs sensory computations and behavior in an in vivo model of FXS. Together with evidence of impaired inhibition in autism and Rett syndrome, these findings suggest a potentially general mechanism for intellectual disability.
Curr Biol, 2017; 27
27385016: Yuan L, Hu S, Okray Z, Ren X, De Geest N, Claeys A, Yan J, Bellefroid E, Hassan BA, Quan XJ
The Drosophila neurogenin Tap functionally interacts with the Wnt-PCP pathway to regulate neuronal extension and guidance.
The neurogenin (Ngn) transcription factors control early neurogenesis and neurite outgrowth in mammalian cortex. In contrast to their proneural activity, their function in neurite growth is poorly understood. Drosophila has a single predicted Ngn homolog, Tap, of unknown function. Here we show that Tap is not a proneural protein in Drosophila but is required for proper axonal growth and guidance of neurons of the mushroom body, a neuropile required for associative learning and memory. Genetic and expression analyses suggest that Tap inhibits excessive axonal growth by fine regulation of the levels of the Wnt signaling adaptor protein Dishevelled.
Development, 2016; 143
25693964: Okray Z, de Esch CE, Van Esch H, Devriendt K, Claeys A, Yan J, Verbeeck J, Froyen G, Willemsen R, de Vrij FM, Hassan BA
A novel fragile X syndrome mutation reveals a conserved role for the carboxy-terminus in FMRP localization and function.
Loss of function of the FMR1 gene leads to fragile X syndrome (FXS), the most common form of intellectual disability. The loss of FMR1 function is usually caused by epigenetic silencing of the FMR1 promoter leading to expansion and subsequent methylation of a CGG repeat in the 5' untranslated region. Very few coding sequence variations have been experimentally characterized and shown to be causal to the disease. Here, we describe a novel FMR1 mutation and reveal an unexpected nuclear export function for the C-terminus of FMRP. We screened a cohort of patients with typical FXS symptoms who tested negative for CGG repeat expansion in the FMR1 locus. In one patient, we identified a guanine insertion in FMR1 exon 15. This mutation alters the open reading frame creating a short novel C-terminal sequence, followed by a stop codon. We find that this novel peptide encodes a functional nuclear localization signal (NLS) targeting the patient FMRP to the nucleolus in human cells. We also reveal an evolutionarily conserved nuclear export function associated with the endogenous C-terminus of FMRP. In vivo analyses in Drosophila demonstrate that a patient-mimetic mutation alters the localization and function of Dfmrp in neurons, leading to neomorphic neuronal phenotypes.
EMBO Mol Med, 2015; 7
23690751: Soldano A, Okray Z, Janovska P, Tmejová K, Reynaud E, Claeys A, Yan J, Atak ZK, De Strooper B, Dura JM, Bryja V, Hassan BA
The Drosophila homologue of the amyloid precursor protein is a conserved modulator of Wnt PCP signaling.
Wnt Planar Cell Polarity (PCP) signaling is a universal regulator of polarity in epithelial cells, but it regulates axon outgrowth in neurons, suggesting the existence of axonal modulators of Wnt-PCP activity. The Amyloid precursor proteins (APPs) are intensely investigated because of their link to Alzheimer's disease (AD). APP's in vivo function in the brain and the mechanisms underlying it remain unclear and controversial. Drosophila possesses a single APP homologue called APP Like, or APPL. APPL is expressed in all neurons throughout development, but has no established function in neuronal development. We therefore investigated the role of Drosophila APPL during brain development. We find that APPL is involved in the development of the Mushroom Body αβ neurons and, in particular, is required cell-autonomously for the β-axons and non-cell autonomously for the α-axons growth. Moreover, we find that APPL is a modulator of the Wnt-PCP pathway required for axonal outgrowth, but not cell polarity. Molecularly, both human APP and fly APPL form complexes with PCP receptors, thus suggesting that APPs are part of the membrane protein complex upstream of PCP signaling. Moreover, we show that APPL regulates PCP pathway activation by modulating the phosphorylation of the Wnt adaptor protein Dishevelled (Dsh) by Abelson kinase (Abl). Taken together our data suggest that APPL is the first example of a modulator of the Wnt-PCP pathway specifically required for axon outgrowth.
PLoS Biol, 2013; 11
23067575: Okray Z, Hassan BA
Genetic approaches in Drosophila for the study neurodevelopmental disorders.
The fruit fly Drosophila melanogaster is one of the premier genetic model organisms used in biomedical research today owing to the extraordinary power of its genetic tool-kit. Made famous by numerous seminal discoveries of basic developmental

mechanisms and behavioral genetics, the power of fruit fly genetics is becoming increasingly applied to questions directly relevant to human health. In this review we discuss how Drosophila research is applied to address major questions in neurodevelopmental disorders. This article is part of the Special Issue entitled 'Neurodevelopmental Disorders'. Neuropharmacology, 2013; 68

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BOARD NUMBER: S02-086

A LOCUS COERULEUS- DORSAL CA1 DOPAMINERGIC CIRCUIT MODULATES MEMORY LINKING

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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[1]UCLA, Neurobiology, Los Angeles, United States of America, [2]FORTH, Imbb, Heraklion, Crete, Greece

Our brain not only encodes memories of individual events, it can also link memories encoded close in time, such that the recall of one memory triggers the recall of the other. For example, our lab showed that when two contextual memories are encoded close in time (e.g., 5 hours apart) the recall of one triggers the recall of the other. Neuromodulatory projections are thought to have a role in sending information about novelty to dCA1, including dopaminergic/noradrenergic projections from Locus Coeruleus (LC) and serotonergic projections from the Raphe Nucleus (RN). Although both the LC and RN are activated by contextual exploration, only inhibition of LC to CA1-projecting neurons disrupted contextual memory linking. Importantly, this same manipulation did not disrupt contextual learning and memory. Furthermore, pharmacological manipulations demonstrated that this effect was mediated by dopamine and not by noradrenaline. Studies with head mounted fluorescent microscopes (UCLA miniscopes) in dCA1 showed that the manipulations that disrupted contextual memory linking also impaired the overlap between dCA1 contextual memory ensembles. We found that the inhibition of LC to CA1-projecting neurons also affected the synchrony of firing between subpopulations of dCA1 neurons activated by both contextual memories, as well as their activity patterns within cell assemblies. This discovery of a neuromodulatory system that specifically affects memory linking without affecting memory formation reveals a fundamental separation between the brain mechanisms that modulate these two distinct processes, and highlights a new mechanism through which memories can be encoded together.

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BOARD NUMBER: S02-087

AUDITORY PRIMING IN FREELY-MOVING MICE

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Priming is a form of implicit short-term memory, defined as a change in the ability to process an object as a result of previously encountering that object. A key part of our daily life, priming has been studied extensively. However, no behavioral model of priming in lab animals has been established, precluding the study of the neuronal network basis of priming. Here, we developed a novel paradigm for studying priming in freely-moving mice, consisting of three stages. In the first stage, the mouse learns a discrimination task, associating a long 5 kHz auditory “target” stimulus with water reward in one port, and a 10 kHz stimulus with reward in another port. In the third stage, a “prime” stimulus, namely a short tone, precedes the target stimulus. Two types of prime stimuli are used: congruent, at the same frequency as the target; and non-congruent. In an intermediate stage, the mouse learns to ignore prime stimuli that are not followed by target stimuli. Four mice were trained on the paradigm. The mice learned the discrimination task within two weeks, achieving 90-95% success. Within two additional weeks, animals learned to ignore isolated prime stimuli, with false alarm under 15%. Finally, we found a consistent prime effect: success rates in congruent trials were 5%-10% higher than in non-congruent trials. Detecting priming behavior in mice implies that priming is not limited to humans or primates. The novel mouse task can be combined with electrophysiological recordings for deciphering the network basis of priming. Funding: ERC #679253

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BOARD NUMBER: S02-088

UNRAVELLING THE ROLE OF HISTAMINE NEURONS IN MEMORY PROCESSES THROUGH CHEMOGENETICS SILENCING : POTENTIAL SEX DIFFERENCES

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

Lola Hardt[1], Eva-Gunnel Ducourneau[1,2], Ioannis Bakoyiannis[1], Gustavo Provensi[3], Essi Biyong[1], Maria Beatrice Passani[3], Pierre Trifilieff[1], Guillaume Ferreira[1]
[1]INRAE, Umr 1286 - Nutrineuro, Bordeaux, France, [2]INRAE, Nutrineuro Laboratory, Bordeaux CEDEX, France, [3]University of Florence, Dipartimento Di Scienze Della Salute, Florence, Italy

Histamine is a known modulator of memory processes, however its implication in consolidation and/or retrieval phases remains poorly understood. This is particularly worrisome because anti-histaminergic drugs are among the most commonly used drugs worldwide and display unwanted mnemonic side effects. **Aims:** Previous studies were based on histamine receptors pharmacology or histamine depletion which have inherent limitations, restricting the ability to clearly separate the implication of histamine transmission between different memory phases e.g., consolidation or retrieval. **Methods:** Our study used a chemogenetic approach in order to specifically silence histaminergic neurons during either memory formation/consolidation or retrieval in three different long-term memory tasks, i.e. Object Recognition Memory (ORM), Inhibitory Avoidance (IA) and Social Recognition Memory (SRM), in both male and female mice. **Results:** Chemogenetic histamine modulation specifically during training (*not* retreival) impairs ORM at 48h but not 24h, independently of sex. Similar manipulations during IA training *or* retrieval have an opposite effect in both sexes at 7 days but not 48h, impairing IA memory in males but enhancing it in females. Finally, histaminergic neurons silencing had no impact on SRM assessed at 24h in males (we observed sex differences with SRM protocol not being efficient in females). **Conclusions:** Our results confirm the implication of histamine in memory consolidation and further indicate an additional role in later consolidation phases necessary for long-term memory persistence. Furthermore, our results stress the complexity of histamine implication in memory processes through the discovery of opposite sex effects of histamine neuron inhibition in the aversive IA memory task.

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THE DEVELOPMENT OF THE RELAXIN3 INNERVATION OF THE MEDIAL SEPTUM

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

Francisco Olucha-Bordonau, Jorge Lucerón-Morales, Mónica Navarro Sánchez, Isis Gil-Miravet, Aroa Mañas-Ojeda, Clara García Mompó, Francisco Ros-Bernal
Universitat Jaume I, Unitat Predepartamental De Medicina, Castelló de la Plana, Spain

The hippocampal theta rhythm is a key element associated with the spatial navigation and memory. It depends on driving connections arising from the medial septum. Both the medial septum and the hippocampus are targeted by relaxin3 projections coming from the medial septum and these projections also contribute to the hippocampal theta rhythm. Thus, its development is important in understanding the emergence of these functions. In this work we have analyzed the postnatal development of the relaxin3 innervation of the medial septum. We have studied the relaxin3 fibers at postnatal (PN) days 3, 10, 15, 17, 20, 30 and 3 months. Along PN3 to PN15 there is a plateau with a scattered number of fibers dispersed along the medial septum. At these times, the different neuronal types progressively gain complexity in their dendritic trees. A few of these fibers contain some synaptic markers like synaptophysin and homer. At P17 there is a huge emergence of the RLN3 innervation of the entire complex. Also at this time, the neuronal types look to be fully configured. After P17, the innervation of the area declines smoothly. Thus, the RLN3 innervation of the medial septum is produced at two stages, the first comes before the birth and the second abruptly emerges at P17. It is worth to highlight that the appearance of these fibers comes just before weaning when the animal starts a free autonomous life. Supported by the PN Drogas, Spanish Ministry of Health (2020I012); Ministerio de Ciencia, Innovación (RTI2018095698-B-I00); Generalitat Valenciana (AICO21I376)

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BOARD NUMBER: S02-090

TEMPORAL COORDINATION BETWEEN SLEEP SLOW OSCILLATIONS, SPINDLES, AND RIPPLES IS ASSOCIATED WITH SPATIAL MEMORY FORMATION IN DEVELOPING RATS.

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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University of Tübingen, Institute Of Medical Psychology And Behavioral Neurobiology, Tübingen, Germany

Memory formation is a major function of sleep. Infant rats can form spatial memory representations from early life; however, the role of sleep oscillation coordination during brain maturation and memory formation is unknown. In this study, we used rats as a developmental model to investigate the association between the maturation of spatial memory capabilities and the temporal coordination between slow oscillation (SO), spindles, and hippocampal ripples. We compared rats which upon prior experience exposures, can express adult-like spatial memory. Starting on PD25, the Spatial experience group performed three exposures to the object-place recognition (OPR) task on subsequent days. The Control group performed three exposures to a pseudo-OPR task where the objects remained stationary. At the test phase on PD31, both groups were subjected to a classical OPR task, including a 3-hours consolidation period during which frontal and parietal EEGs and hippocampal LFP recordings were acquired. Behavioral results showed that only rats with prior spatial experiences formed a persistent spatial memory. Further, sleep analyses revealed an increase in the percentage of ripples that co-occurred with spindles nested in a SO in the Spatial experience group. Interestingly, this increase in the triple-coupling was restricted to the parietal SO-spindle events. Rats of the Spatial experience group also showed a significant ripple-spindle phase-locking and an increase in both the ripple activity and the ripple band's power after the parietal spindle onset. Overall, we demonstrated that the temporal association between the three major cardinal sleep oscillations is associated with the maturation of spatial memory in developing rats.

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BOARD NUMBER: S02-091

THE ROLE OF NORADRENALINE IN SLEEP-DEPENDENT MEMORY CONSOLIDATION

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

Yaroslav Sych[1,2], Tiago Campelo[2,3], Carolina Gutierrez Herrera[2], Thomas Rusterholz[2], Fritjof Helmchen[1,4], Antoine Adamantidis[2,3]
[1]University of Zurich, Brain Research Institute, Zürich, Switzerland, [2]Zentrum für Experimentelle Neurologie, Department Of Neurology, Bern, Switzerland, [3]University of Bern, Center For Experimental Neurology, Dept Of Neurology, Bern, Switzerland, [4]University of Zürich, Neuroscience Center Zürich, Zürich, Switzerland

Molecular and cellular mechanisms contribute to memory consolidation. Noradrenaline release controlled by the midbrain neurons of the locus coeruleus (LC) can modulate learning and memory. Here we investigate the role of noradrenaline in sleep-dependent memory consolidation. We used the genetically encoded fluorescent noradrenaline sensor $GRAB_{NE}$ and multi-fiber photometry to examine how salient experiences during wakefulness, such as learning a reward-based auditory discrimination task, relate to noradrenergic modulation of frontal cortical circuits. We identified region-specific noradrenaline changes on the short time scale of behavioral trials during task engagement, corresponding to memory encoding, as well as on the longer time scale of task learning, corresponding to memory consolidation. During task execution, sustained elevated levels of noradrenaline were present in lateral orbitofrontal cortex (lOFC) and anterolateral motor cortex (ALM). During the first episodes of NREM sleep, after reaching the expert criterion of task proficiency, noradrenaline levels in ALM increased as compared to the NREM sleep in the pre-learning phase. Conversely, noradrenaline levels decreased during NREM sleep for lOFC and prefrontal cortex as compared to the pre-learning state. Furthermore, we identified a fraction of sleep spindles that was coupled to the noradrenaline dynamics during NREM sleep. This coupling of sleep spindles and noradrenaline was also strengthened by task learning in the medial orbitofrontal cortex and ALM. Bilateral optogenetic silencing of LC neurons with ArchT slowed task learning and decreased the coupling between sleep spindles and noradrenaline. Our results underscore the region-specific role of noradrenaline at multiple time-scales for memory encoding and consolidation.

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BOARD NUMBER: S02-092

FUNCTIONAL IMAGING MEMORIES OF A MOTHER DURING FILIAL IMPRINTING IN AWAKE DOMESTIC CHICK NEWBORNS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Filial imprinting has been used as a powerful ethological paradigm to investigate the neurobiology of early learning that affects lifelong behaviors. When a visually naïve chick is exposed to one of a wide range of conspicuous objects, it may learn its characteristics and subsequently recognizes and selectively approaches this stimulus (usually the mother hen). While the initial phases of memory acquisition have been unravelled, the long-term storage and retrieval components of imprinting memories are still unknown. Here, we established an fMRI protocol to study awake brain activity in newborn chicks. We identified a neural network involved in the acquisition and long-term storage of imprinting memory, providing the first example of imaging in vivo memory formation, storage, and retrieval during imprinting. Our findings unveiled a prosencephalic neural network that, among others, comprises the Social Behavior Network, the Mesolimbic Reward System, and the medial meso-/nidopallium for long-term storage and retrieval of filial imprinting memory. As expected, the networks of memory formation and retrieval partially overlapped. Network activity was more pronounced during memory retrieval and further involved the avian analogue to the mammalian prefrontal cortex. Thus, consolidation of imprinting memory results in strengthening and expanding the neural system encoding the original engram, but in a more distributed manner. From this perspective, the storage of the imprinting memory is likely to comprise this wide and distributed network within which the “prefrontal” NCL could be a central hub. These results provide the first example of non-invasive imaging of the brain in a newborn vertebrate.

Pubmed:
34992520: Russo G, Helluy X, Behroozi M, Manahan-Vaughan D
Gradual Restraint Habituation for Awake Functional Magnetic Resonance Imaging Combined With a Sparse Imaging Paradigm Reduces Motion Artifacts and Stress Levels in Rodents.
Functional magnetic resonance imaging, as a non-invasive technique, offers unique opportunities to assess brain function and connectivity under a broad range of applications, ranging from passive sensory stimulation to high-level cognitive abilities, in awake animals. This approach is confounded, however, by the fact that physical restraint and loud unpredictable acoustic noise must inevitably accompany fMRI recordings. These factors induce marked stress in rodents, and stress-related elevations of corticosterone levels are known to alter information processing and cognition in the rodent. Here, we propose a habituation strategy that spans specific stages of adaptation to restraint, MRI noise, and confinement stress in awake rats and circumvents the need for surgical head restraint. This habituation protocol results in stress levels during awake fMRI that do not differ from pre-handling levels and enables stable image acquisition with very low motion artifacts. For this, rats were gradually trained over a period of three weeks and eighteen training sessions. Stress levels were assessed by analysis of fecal corticosterone metabolite levels and breathing rates. We observed significant drops in stress levels to below pre-handling levels at the end of the habituation procedure. During fMRI in awake rats, after the conclusion of habituation and using a non-invasive head-fixation device, breathing was stable and head motion artifacts were minimal. A task-based fMRI experiment, using acoustic stimulation, conducted 2 days after the end of habituation, resulted in precise whole brain mapping of BOLD signals in the brain, with clear delineation of the expected auditory-related structures. The active discrimination by the animals of the acoustic stimuli from the backdrop of scanner noise was corroborated by significant increases in BOLD signals in the thalamus and reticular formation. Taken together, these data show that effective habituation to awake fMRI can be achieved by gradual and incremental acclimatization to the experimental conditions. Subsequent BOLD recordings, even during superimposed acoustic stimulation, reflect low stress-levels, low motion and a corresponding high-quality image acquisition. Furthermore, BOLD signals obtained during fMRI indicate that effective habituation facilitates selective attention to sensory stimuli that can in turn support the discrimination of cognitive processes in the absence of

stress confounds.
Front Neurosci, 2021; 15
34618529: Wittek N, Matsui H, Behroozi M, Otto T, Wittek K, Sarı N, Stoecker S, Letzner S, Choudhary V, Peterburs J, Güntürkün O
Unihemispheric evidence accumulation in pigeons.
Perceptual decision making involves choices between alternatives based on sensory information. Studies in primates and rodents revealed a stochastic perceptual evidence accumulation process that, after reaching threshold, results in action execution. Birds represent a cognitively highly successful vertebrate class that has been evolving independent from mammals for more than 300 million years. The present study investigated whether perceptual decision making in pigeons shows behavioral and computational dynamics comparable to those in mammals and rodents. Using a novel "pigeon helmet" with liquid shutter displays that controls visual input to individual eyes/hemispheres with precise timing, we indeed revealed highly similar dynamics of perceptual decision making. Thus, both mammals and birds seem to share this core cognitive process that possibly represents a fundamental constituent of decision making throughout vertebrates. Interestingly, in our experiments we additionally discovered that both avian hemispheres start independent sensory accumulation processes without any major interhemispheric exchange. Because birds lack a corpus callosum and have only a small anterior commissure, they seem to be forced to decide on motor responses based on unihemispheric decisions under conditions of time pressure. (PsycInfo Database Record (c) 2021 APA, all rights reserved).
J Exp Psychol Anim Learn Cogn, 2021; 47
34544756: Tabrik S, Behroozi M, Schlaffke L, Heba S, Lenz M, Lissek S, Güntürkün O, Dinse HR, Tegenthoff M
Visual and Tactile Sensory Systems Share Common Features in Object Recognition.
Although we use our visual and tactile sensory systems interchangeably for object recognition on a daily basis, little is known about the mechanism underlying this ability. This study examined how 3D shape features of objects form two congruent and interchangeable visual and tactile perceptual spaces in healthy male and female participants. Since active exploration plays an important role in shape processing, a virtual reality environment was used to visually explore 3D objects called digital embryos without using the tactile sense. In addition, during the tactile procedure, blindfolded participants actively palpated a 3D-printed version of the same objects with both hands. We first demonstrated that the visual and tactile perceptual spaces were highly similar. We then extracted a series of 3D shape features to investigate how visual and tactile exploration can lead to the correct identification of the relationships between objects. The results indicate that both modalities share the same shape features to form highly similar veridical spaces. This finding suggests that visual and tactile systems might apply similar cognitive processes to sensory inputs that enable humans to rely merely on one modality in the absence of another to recognize surrounding objects.
eNeuro, 2021 Sep-Oct; 8
32948772: Behroozi M, Helluy X, Ströckens F, Gao M, Pusch R, Tabrik S, Tegenthoff M, Otto T, Axmacher N, Kumsta R, Moser D, Genc E, Güntürkün O
Event-related functional MRI of awake behaving pigeons at 7T.
Animal-fMRI is a powerful method to understand neural mechanisms of cognition, but it remains a major challenge to scan actively participating small animals under low-stress conditions. Here, we present an event-related functional MRI platform in awake pigeons using single-shot RARE fMRI to investigate the neural fundaments for visually-guided decision making. We established a head-fixated Go/NoGo paradigm, which the animals quickly learned under low-stress conditions. The animals were motivated by water reward and behavior was assessed by logging mandibulations during the fMRI experiment with close to zero motion artifacts over hundreds of repeats. To achieve optimal results, we characterized the species-specific hemodynamic response function. As a proof-of-principle, we run a color discrimination task and discovered differential neural networks for Go-, NoGo-, and response execution-phases. Our findings open the door to visualize the neural fundaments of perceptual and cognitive functions in birds-a vertebrate class of which some clades are cognitively on par with primates.
Nat Commun, 2020; 11
32009190: Billings BK, Behroozi M, Helluy X, Bhagwandin A, Manger PR, Güntürkün O, Ströckens F
A three-dimensional digital atlas of the Nile crocodile (Crocodylus niloticus) forebrain.
The phylogenetic position of crocodilians in relation to birds and mammals makes them an interesting animal model for investigating the evolution of the nervous system in amniote vertebrates. A few neuroanatomical atlases are available for reptiles, but with a growing interest in these animals within the comparative neurosciences, a need for these anatomical reference templates is becoming apparent. With the advent of MRI being used more frequently in comparative neuroscience, the aim of this study was to create a three-dimensional MRI-based atlas of the Nile crocodile (Crocodylus niloticus) brain to provide a common reference template for the interpretation of the crocodilian, and more broadly reptilian, brain. Ex vivo MRI acquisitions in combination with histological data were used to delineate crocodilian brain areas at telencephalic, diencephalic, mesencephalic, and rhombencephalic levels. A total of 50 anatomical structures were successfully identified

and outlined to create a 3-D model of the Nile crocodile brain. The majority of structures were more readily discerned within the forebrain of the crocodile with the methods used to produce this atlas. The anatomy outlined herein corresponds with both classical and recent crocodilian anatomical analyses, barring a few areas of contention predominantly related to a lack of functional data and conflicting nomenclature.
Brain Struct Funct, 2020; 225

29695446: Behroozi M, Billings BK, Helluy X, Manger PR, Güntürkün O, Ströckens F
Functional MRI in the Nile crocodile: a new avenue for evolutionary neurobiology.
Crocodilians are important for understanding the evolutionary history of amniote neural systems as they are the nearest extant relatives of modern birds and share a stem amniote ancestor with mammals. Although the crocodilian brain has been investigated anatomically, functional studies are rare. Here, we employed functional magnetic resonance imaging (fMRI), never tested in poikilotherms, to investigate crocodilian telencephalic sensory processing. Juvenile were placed in a 7 T MRI scanner to record blood oxygenation level-dependent (BOLD) signal changes during the presentation of visual and auditory stimuli. Visual stimulation increased BOLD signals in rostral to mid-caudal portions of the dorso-lateral anterior dorsal ventricular ridge (ADVR). Simple auditory stimuli led to signal increase in the rostromedial and caudocentral ADVR. These activation patterns are in line with previously described projection fields of diencephalic sensory fibres. Furthermore, complex auditory stimuli activated additional regions of the caudomedial ADVR. The recruitment of these additional, presumably higher-order, sensory areas reflects observations made in birds and mammals. Our results indicate that structural and functional aspects of sensory processing have been likely conserved during the evolution of sauropsids. In addition, our study shows that fMRI can be used to investigate neural processing in poikilotherms, providing a new avenue for neurobiological research in these critical species.
Proc Biol Sci, 2018; 285

29564025: Parhizi B, Daliri MR, Behroozi M
Decoding the different states of visual attention using functional and effective connectivity features in fMRI data.
The present paper concentrates on the impact of visual attention task on structure of the brain functional and effective connectivity networks using coherence and Granger causality methods. Since most studies used correlation method and resting-state functional connectivity, the task-based approach was selected for this experiment to boost our knowledge of spatial and feature-based attention. In the present study, the whole brain was divided into 82 sub-regions based on Brodmann areas. The coherence and Granger causality were applied to construct functional and effective connectivity matrices. These matrices were converted into graphs using a threshold, and the graph theory measures were calculated from it including degree and characteristic path length. Visual attention was found to reveal more information during the spatial-based task. The degree was higher while performing a spatial-based task, whereas characteristic path length was lower in the spatial-based task in both functional and effective connectivity. Primary and secondary visual cortex (17 and 18 Brodmann areas) were highly connected to parietal and prefrontal cortex while doing visual attention task. Whole brain connectivity was also calculated in both functional and effective connectivity. Our results reveal that Brodmann areas of 17, 18, 19, 46, 3 and 4 had a significant role proving that somatosensory, parietal and prefrontal regions along with visual cortex were highly connected to other parts of the cortex during the visual attention task. Characteristic path length results indicated an increase in functional connectivity and more functional integration in spatial-based attention compared with feature-based attention. The results of this work can provide useful information about the mechanism of visual attention at the network level.
Cogn Neurodyn, 2018; 12

28866684: Behroozi M, Ströckens F, Helluy X, Stacho M, Güntürkün O
Functional Connectivity Pattern of the Internal Hippocampal Network in Awake Pigeons: A Resting-State fMRI Study.
In the last two decades, the avian hippocampus has been repeatedly studied with respect to its architecture, neurochemistry, and connectivity pattern. We review these insights and conclude that we unfortunately still lack proper knowledge on the interaction between the different hippocampal subregions. To fill this gap, we need information on the functional connectivity pattern of the hippocampal network. These data could complement our structural connectivity knowledge. To this end, we conducted a resting-state fMRI experiment in awake pigeons in a 7-T MR scanner. A voxel-wise regression analysis of blood oxygenation level-dependent (BOLD) fluctuations was performed in 6 distinct areas, dorsomedial (DM), dorsolateral (DL), triangular shaped (Tr), dorsolateral corticoid (CDL), temporo-parieto-occipital (TPO), and lateral septum regions (SL), to establish a functional connectivity map of the avian hippocampal network. Our study reveals that the system of connectivities between CDL, DL, DM, and Tr is the functional backbone of the pigeon hippocampal system. Within this network, DM is the central hub and is strongly associated with DL and CDL BOLD signal fluctuations. DM is also the only hippocampal region to which large Tr areas are functionally connected. In contrast to published tracing data, TPO and SL are only weakly integrated in this network. In summary, our findings uncovered a structurally otherwise invisible architecture of the avian hippocampal formation by revealing the dynamic blueprints of this network.
Brain Behav Evol, 2017; 90

28474481: Behroozi M, Chwiesko C, Ströckens F, Sauvage M, Helluy X, Peterburs J, Güntürkün O
In vivo measurement of T and T relaxation times in awake pigeon and rat brains at 7T.
Establishment of regional longitudinal (T) and transverse (T) relaxation times in awake pigeons and rats at 7T field strength. Regional differences in relaxation times between species and between two different pigeon breeds (homing pigeons and Figurita pigeons) were investigated.
Magn Reson Med, 2018; 79
26400624: Behroozi M, Daliri MR, Shekarchi B
EEG phase patterns reflect the representation of semantic categories of objects.
Oscillations of electroencephalographic signals represent the cognitive processes arose from the behavioral task and sensory representations across the mental state activity. Previous studies have shown the relation between event-related EEG and sensory-cognitive representation and revealed that categorization of presented object can be successfully recognized using recorded EEG signals when subjects view objects. Here, EEG signals in conjunction with a multivariate pattern recognition technique were used for investigating the possibility to identify conceptual representation based on the presentation of 12 semantic categories of objects (5 exemplars per category). Using multivariate stimulus decoding methods, surprisingly, we demonstrate that how objects are discriminated from phase pattern of EEG signals across the time in low-frequency band (1-4 Hz), but not from power of oscillatory brain signals in the same frequency band. In contrast, discrimination accuracy from the power of EEG signals has significantly higher than the performance from phase of EEG signal in the high-frequency band (20-30 Hz). Moreover, our results indicate that how the accuracy of prediction changes between various areas of brain continuously across the time. In particular, we find that, during the object categorization task, the inter-trial phase coherence in low-frequency band is significantly higher than other frequency in various regions of interests. This measure is associated with decoding pattern across the time. These results suggest that the mechanism underlying conceptual representation can be mediated by the phase of oscillatory neural activity.
Med Biol Eng Comput, 2016; 54

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BOARD NUMBER: S02-093

FROM RECENCY EFFECTS TO CONTRACTION BIAS - A NEURAL NETWORK MODEL FOR HISTORY EFFECTS IN PARAMETRIC WORKING MEMORY TASKS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Contraction bias is a phenomenon where the judgment of the magnitude of items held in working memory (WM) is biased towards the average of past observations. This phenomenon has been first described more than a century ago and has been replicated in various decision making tasks in humans and rodents. Contraction bias is assumed to be an optimal strategy by the brain, given the noisy nature of WM. In proposed Bayesian frameworks, the progressive shift of the memory towards the mean of a prior distribution built from past sensory experience helps with more accurate estimates of it. We propose an alternative mechanism, via short-term sensory history biases. Our model is motivated by recent results from a delayed-discrimination task in rats, where the posterior parietal cortex (PPC) has been shown to be critical to these memory effects. In our model, a circuit with a WM function receives inputs from another network (PPC). Both modules are continuous 1D attractor networks that differ in the timescales over which they integrate external inputs; the neurons in the PPC are additionally equipped with neuronal adaptation. As a result, contraction bias emerges as a result of a volatile WM content which makes it susceptible to shifting towards the previous sensory experience. This can result in errors that are sampled from the full distribution of the stimuli, and not simply its mean. Our results are consistent with the role of the PPC in encoding such sensory history biases, and provide predictions of performance across different stimulus distributions.

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UNSUPERVISED, FREQUENT AND REMOTE: A NOVEL PLATFORM FOR PERSONALISED DIGITAL PHENOTYPING OF SPATIAL WORKING MEMORY AND IMAGE RECOGNITION IN HUMANS.

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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AIMS: Spatial working memory and image recognition tests are commonly used to facilitate the diagnosis of hippocampal-related neurological disorders such as Alzheimer's disease. Hence, there is an urgent need to implement them at scale and frequency. **METHODS:** We developed a novel digital platform, hAge ('healthy Age'), which integrates spatial alternation (SA), image recognition (IR) and visuospatial task (VS) for the frequent remote and unsupervised assessment of spatial and non-spatial working memory. **RESULTS:** We tested 191 healthy adults who continuously engaged with the hAge ~10 times/day for >eight weeks. Consistent with findings from analogous standard laboratory-based tests, we showed that Participants found VS task to be more challenging than IR and that performance on SA task negatively correlated with inter-trial periods. Importantly, we demonstrated that frequent engagement with the double SA task in older age groups (>50 years old) led to a strong practice effect. All of these measures were previously used to assess the cognitive decline and risks of developing AD in MCI patients. Unexpectedly, older Participants demonstrated higher absolute performance compared to the younger group. **CONCLUSIONS:** We demonstrated feasibility of our approach by showing good adherence levels. We also showed that the performance on hAge tasks is comparable to the performance observed in the analogous standard tests. Older participants performing better than younger ones, while unexpected, can be explained by unrelated confounds which could be addressed with some modifications to the current hAge.

Pubmed:
35030329: Perentos N, Krstulovic M, Morton AJ
Deep brain electrophysiology in freely moving sheep.
Although rodents are arguably the easiest animals to use for studying brain function, relying on them as model species for translational research comes with its own set of limitations. Here, we propose sheep as a practical large animal species to use for in vivo brain function studies performed in naturalistic settings. We conducted proof-of-principle deep brain electrophysiological recording experiments using unrestrained sheep during behavioral testing. Recordings were made from cortex and hippocampus, both while sheep performed goal-directed behaviors (two-choice discrimination tasks) and across states of vigilance, including sleep. Hippocampal and cortical oscillatory rhythms were consistent with those seen in rodents and non-human primates, and included cortical alpha oscillations and hippocampal sharp wave ripple oscillations (~150 Hz) during immobility and hippocampal theta oscillations (5-6 Hz) during locomotion. Recordings were conducted over a period of many months during which time the animals participated willingly in the experiments. Over 3,000 putative neurons were identified, including examples whose activity was modulated by task, speed of locomotion, spatial position, reward and vigilance states, and one whose firing rate was potentially modulated by the sight of the investigator. Together, these experiments demonstrate that sheep are excellent experimental animals to use for longitudinal studies requiring a large-brained mammal and/or large-scale recordings across distributed neuronal networks. Sheep could be used safely for studying not only neural encoding of decision-making and spatial-mapping in naturalistic environments outside the confines of the traditional laboratory but also the neural basis of both intra- and inter-species social interactions.
Curr Biol, 2022; 32

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BOARD NUMBER: S02-095

MOUSE MATERNAL LOW PROTEIN DIET AFFECTS WORKING MEMORY AND HIPPOCAMPAL GLIAL CELLS

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Brain development in late gestation and after birth are affected by poor maternal nutrition, influencing structural, biochemical and pathway dynamics for motor and cognitive function. However, the importance of nutrition during early embryogenesis for brain development is less known. We have previously shown mouse maternal low protein diet (LPD) confined to the preimplantation period (Emb-LPD) with normal nutrition thereafter is sufficient to alter brain neuron proportion and adult short-term memory in adult offspring (doi/10.1073/pnas.1721876115). Aim: Here, we explore mechanisms of offspring memory change related to maternal LPD. Methods: Female mice were fed from mating to term normal protein diet (NPD), LPD or Emb-LPD (LPD up to E3.5, NPD thereafter). T-Maze tested working memory at 14 weeks. Hippocampus were analysed at 14 weeks, using immunohistochemistry and RNAseq. Results: LPD and Emb-LPD offspring exhibited memory deficit in the T-Maze, except for LPD females. While radial glia were not affected in the dentate gyrus, astrocyte density increased in LPD females compared to NDP and Emb-LPD females. Microglia density decreased in Emb-LPD and LPD, males and females, likely excluding an inflammatory role of these cells. RNAseq analysis revealed minimal pathway modulation in LPD females, whereas several metabolism pathways were altered in Emb-LPD females, compared to NPD females. Conclusions: Transient Emb-LPD around conception was sufficient to induce adult memory defects and altered glial metabolism and density. LPD females' memory rescue may be linked to dentate gyrus astrocyte density increase, which may lead to normalisation of metabolism pathways. Funding: Wessex Medical Trust,Rosetrees Trust,Gerald Kerkut Trust, BBSRC

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TARGETED MEMORY REACTIVATION DURING POST-LEARNING SLEEP AFFECTS MEMORY CONSOLIDATION WITHIN CHANGES OF DENDRITIC SPINE PLASTICITY

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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Post-learning sleep is thought to be the critical period for the memory consolidation, during which the newly encoding memory is also susceptible to the external stimulus. Targeted memory reactivation (TMR), a procedure presenting the conditioned cues during sleep, has shown be able to trigger the replay of internal memory in recent studies. However, studies applying TMR in different sleep substages presented divergent outcomes, and the mechanism underlying TMR in memory consolidation is still unclear. Here we aim to investigate the effects of TMR at different sleep stages after fear conditioning in the frontal association cortex. We found that mice received TMR during the non-slow wave phases of non-rapid eye movement sleep (nS-NREM) significantly reduced the freezing rate in the recall test and inhibited the increased dendritic spine elimination in the frontal association cortex induced by fear conditioning when compared with controls. In contrast, mice subjected to TMR during slow wave sleep (SWS) showed enhanced fear memory with a stronger resistance to the later extinction trainings. In addition, the increased spine formation was also inhibited in SWS-TMR group when compared to normal extinction group. Furthermore, optogenetic inhibition in the frontal association cortex simultaneously with TMR cueing abolished the effects of TMR on animal behaviour. Our results indicated that synaptic plasticity and neuronal activity in the frontal association cortex at different sleep stages played an indispensable role in TMR.

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PATTERN SEPARATION DEFICITS IN THE 3XTG MOUSE MODEL OF ALZHEIMER'S DISEASE.

POSTER SESSION 02 - SECTION: WORKING MEMORY AND MEMORY CONSOLIDATION

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3xTgAD mice exhibit pronounced deficits in episodic-like memory encoding, akin to Alzheimer's disease patients. Pattern separation (PS) is required to encode similar episodic memories as distinct representations in the brain. This study aimed to determine whether impaired PS underlies episodic-like memory deficits in 3xTgAD. To achieve this, control and 3xTgAD mice performed a four-trial continuous novel object recognition task that individually familiarised them to a sample object before exposing them to the sample object and a novel object of either high or low similarity to that sample object. There was a significant effect of group but not trial on the novelty discrimination ratio (D2) for high similarity objects, with control mice D2s being significantly greater than those of 3xTgAD mice at trial four. There was no significant effect of group or trial on D2s for low similarity objects. Furthermore, proactive interference did not significantly affect D2s across trials for both groups and object types. Overall, these findings indicate that 3xTgAD mice had a moderate PS deficit; such that they preferentially explored low similarity object novel objects (which requires less PS) but only control mice had the PS capacity to detect novelty in high similarity objects.

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CONTEXTUAL MODULATION OF REWARD SIGNALS ACROSS OBJECT AND SPATIAL REVERSAL LEARNING TASKS IN THE RHESUS MACAQUE BRAIN

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aims. Decision-making situations could differ along many dimensions such the nature of the action to take, or the identity of the option to choose from. To make such decisions in a changing environment, the brain needs to assign a value to each option, compare them to make a choice and update them based on the obtained outcome. We investigated how learning about spatial position or object identity are represented in specific brain areas and rely on similar computational principles. **Methods.** We used fMRI data from rhesus macaque performing deterministic reversal discrimination tasks in which they had to choose between stimuli with either different identities or target locations. **Results**. In previous studies, we linked an area located on the lateral part of the orbital surface (area 47/12o) to win-stay-lose-shift behaviours in object learning. In a spatial context, this region is important during the learning stage but did not show the same functional role in trained animals. We further investigated the fundamental difference between the two task contexts and found that a different pattern of activation in the ventromedial prefrontal cortex in processing reward information seems to originate an adaptive coding across contexts. While this region was functionally connected with the putamen in the two tasks, its activity was only correlated with activity in area 47/12o in the object context. **Conclusion.** Our findings reveal different recruitments of areas of the prefrontal cortex in object and spatial learning contexts and suggest that reward encoding is a main driver of these different network activities.

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ACTIVITY IN DISTINCT NEURAL CIRCUITS PREDICTS RISK-SEEKING VERSUS RISK-AVERSE CHOICES

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Previous findings on risky decision-making have raised the question of whether a single system or distinct circuits contribute to risky choice. In this study, we investigated brain activation preceding risky choice using Functional Magnetic Resonance Imaging (FMRI) in three independent samples with two different gambling tasks and analysis methods. In original (n=20) and validation (n=48) datasets, participants were scanned as they chose between a safe and a risky gamble, all of which had equal expected value and variance but which differed in skewness. Consistent with the Affect-Integration-Motivation framework (Samanez-Larkin & Knutson, 2015), pre-choice activity in the Nucleus Accumbens (NAcc) and Medial Prefrontal Cortex (MPFC) predicted risk seeking, whereas activity in the Anterior Insula (AIns) predicted risk averse choices. This pattern of results replicated in an independent sample using univariate and multivariate methods, and generalized to data acquired with a different gambling task (i.e., the Mixed Gambles Task; Tom et al. (2007)). Results also provided convergent evidence that distinct neural predictors of trial-by-trial risky choice could be dissociated from activity in sensory and motor cortices associated with gamble perception and choice. Findings therefore indicated that risky choice may be controlled in opposite directions by two distinct circuits, rather than a single circuit.

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ECONOMIC VALUE IN THE BRAIN: AN ACTIVATION LIKELIHOOD ESTIMATION META-ANALYSIS OF WILLINGNESS-TO-PAY

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Forming and comparing subjective values of choice options is a critical stage of decision making. Previous literature has highlighted a complex network of brain regions involved in this process by utilising a wide variety of tasks and stimuli, varying in economic, hedonic and sensory qualities. To identify the core brain valuation system that processes economic value, we conducted a coordinate-based activation likelihood estimation meta-analysis of fMRI studies that employed the Becker-DeGroot-Marshack (BDM) auction, an incentivised demand-revealing mechanism which quantifies economic value of stimuli as willingness-to-pay (WTP). The systematic review revealed twenty-two studies (703 participants; 164 foci). Using two additional contrast analyses, we also investigated whether this encoding of subjective value would be invariant to the synchrony of auction task and fMRI recordings, and types of stimuli (food vs. non-food). WTP positively correlated with fMRI-BOLD activations in the bilateral ventral striatum and left ventromedial prefrontal cortex, with a sub-cluster reaching into the anterior cingulate cortex. Contrast analysis revealed no difference in brain activation patterns between outside versus inside scanner BDMs, or between food vs non-food stimuli. Our findings offer succinct empirical support for the ventral striatum and vmPFC as the core structures responsible for the formation of economic value, irrespective of the type, quality or quantity of item. The results also suggest that economic valuation is an automatic process, as the activation patterns were not affected by when and where the BDM was completed and are therefore unaffected by task relevancy.

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BOARD NUMBER: S02-101

NEUROIMAGING EVIDENCE OF THE FUNCTIONAL INTERPLAY WITHIN THE FRONTO-AMYGDALA NETWORK DURING BEHAVIOURAL ADAPTATION IN HUMAN

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aims: A hallmark of our survival in the real world is our ability to show behavioural adaptation (BA). BA is a challenging process that requires to know whether to link our failures to our own actions (i.e. our actions' feedback -FB-) or to unexpected changes in our environment (i.e. when facing Action-Independent Events -AIDEs-, e.g. rules changed). The aim of this study is to identify how the functional interplay in the network formed by the midcingulate cortex (MCC) and the amygdala (AMG) is modulated by BA. **Methods:** We scanned 20 human subjects with fMRI while they were performing a new behavioral task in which they had to learn by trial and error how to adapt when facing both FB and AiDEs, AiDEs influencing or not the meaning of the FB. **Results:** Results show that the functional interplay between the AMG and MCC changes during the learning process: 1) the AMG is activated and the MCC deactivated at the first occurrence of AiDEs (i.e. when subjects do not know yet the meaning of the AiDEs), 2) then the MCC becomes activated and the AMG deactivated when subjects understood that an AiDE signal a need to adapt. **Conclusions:** This study suggests the AMG detects salient information from the environment and constantly informs the MCC through a bottom-up pathway. When the MCC identifies a particular event that requires BA, it exerts a top-down control onto AMG to optimize behavioral adaptation.

Pubmed:
34974618: Giacometti C, Dureux A, Autran-Clavagnier D, Wilson CRE, Sallet J, Dirheimer M, Procyk E, Hadj-Bouziane F, Amiez C
Frontal Cortical Functional Connectivity Is Impacted by Anaesthesia in Macaques.
A critical aspect of neuroscience is to establish whether and how brain networks evolved across primates. To date, most comparative studies have used resting-state functional magnetic resonance imaging (rs-fMRI) in anaesthetized nonhuman primates and in awake humans. However, anaesthesia strongly affects rs-fMRI signals. The present study investigated the impact of the awareness state (anaesthesia vs. awake) within the same group of macaque monkeys on the rs-fMRI functional connectivity organization of a well-characterized network in the human brain, the cingulo-frontal lateral network. Results in awake macaques show that rostral seeds in the cingulate sulcus exhibited stronger correlation strength with rostral compared to caudal lateral frontal cortical areas, while more caudal seeds displayed stronger correlation strength with caudal compared to anterior lateral frontal cortical areas. Critically, this inverse rostro-caudal functional gradient was abolished under anaesthesia. This study demonstrated a similar functional connectivity (FC) organization of the cingulo-frontal cortical network in awake macaque to that previously uncovered in the human brain pointing toward a preserved FC organization from macaque to human. However, it can only be observed in awake state suggesting that this network is sensitive to anaesthesia and warranting significant caution when comparing FC patterns across species under different states.
Cereb Cortex, 2021;

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BOARD NUMBER: S02-102

THE ROAD NOT TAKEN: INVESTIGATING THE BEHAVIOURAL AND NEURAL CORRELATES OF CHANGING ONE'S MIND

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Cognitive flexibility is the ability to adapt to our constantly changing environment. Reduced flexibility can lead to worse life outcomes and heightened cognitive decline with age. However, a full picture of the behavioural and neural underpinnings of cognitive flexibility has not yet been established, as it is a multifaceted construct. Paradigms commonly used to study flexibility include task-switching, attentional set-shifting and reversal learning. In daily life, flexibility often involves a shift in behaviour being elicited volitionally. Individuals often choose to change behaviours based on environmental signals, but these are frequently accompanied by noise. We report results from a novel 'change your mind' task, which assesses proactive switching under uncertainty, without the need for ongoing rule-based learning. Participants completed a two-alternative choice task and following spurious feedback, were presented with the same stimulus again. They could repeat their previous response or change it, acting of their own volition. To our knowledge, no existing task provides participants with the opportunity to repeat their choice and assess whether they subsequently choose the 'road not taken'. We report findings from forty healthy participants who completed the task whilst undergoing a functional MRI scan. Behavioural findings indicate that participants predominantly repeat their choice but do change their response when the first response was incorrect, or feedback was negative. Brain circuitry including the anterior insula, anterior cingulate cortex and dorsolateral prefrontal cortex were found to be more active on change than repeat trials. The importance of the circuitry including anterior insula and occipital cortex was highlighted.

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BOARD NUMBER: S02-103

THE NEURAL NETWORK FOR SOCIAL DECISION-MAKING DEPENDENT ON A MULTI-CONTEXT ENVIRONMENT

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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We optimize our behaviors in social situations where we constantly interact with other individuals in various contexts. Social decisions can be affected by multi-social contexts such as others' intentions, our position, and interest. In this study, we aim to reveal the neural mechanism of social decision-making considering the above-mentioned contexts. We developed a modified Old-Maid card game experiment that enables us to parametrically control the computer opponents' honesty. The participants' roles were randomly assigned on each trial, and interests between subjects and the computer opponent occasionally changed. In the experiment, fifty-one subjects performed the task in an fMRI scanner and we acquired BOLD signals in the task. To explain the subjects' behaviors, we introduce a novel computational model, which integrates several internal parameters related to the opponents' intentions and other social contexts. Then, we performed the GLM analysis to identify the brain activity correlated to these parameters. We found that 1) Activities in the temporoparietal junction (TPJ) and the dorsomedial prefrontal cortex (dmPFC), which are implicated by the theory of mind function, were correlated with the subjects' estimation of the opponent's honesty; 2) Multiple prefrontal regions, especially the ventromedial prefrontal cortex (vmPFC) and TPJ were related to the changing of interests; and 3) The right anterior insula and the left temporal pole had a close correlation with the subject's honesty parameter. Based on these observations, we suggest the existence of a network including the above-mentioned brain regions would play an important role in social decision-making.

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BOARD NUMBER: S02-104

INTRACEREBRAL CORRELATES OF VISUAL FIXATIONS ENCODE VALUES AND PREDICT MULTIDIMENSIONAL CHOICES

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Whether it be in front of a restaurant menu or a library shelf, humans make decisions on a constant basis, shifting their gaze between stimuli of interest, evaluating, and integrating values before reaching a decision. Despite visual attention being a well-established component of the value-based decision-making process, little is known about the role fixations may play on underlying neural mechanisms. In the present study, we recorded neural activity from 38 patients with refractory epilepsy using stereoencephalography (sEEG) while they performed an economics choice task in which they were asked to integrate prospective gains and losses before accepting or declining a sensori-motor challenge. Behavioral results showed that the duration and pattern of visual fixations to gains and losses biased choices made. Neural results revealed that the ventromedial prefrontal cortex (vmPFC) and anterior insula (aINS) respectively encoded fixation-dependent gain and loss magnitudes, and that this gaze-related activity mediated the effect of prospective gains and losses on choices. These results offer novel evidence for a guiding role of visual fixations on the neural mechanisms underlying the biasing effects of gaze on choices.

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COMPARISON BETWEEN CLOSED- AND OPEN-SKILLS SPORT ENVIRONMENTS IN MODULATING PROACTIVE AND REACTIVE MOTOR INHIBITION VIA A MOUSE TRACKING SYSTEM

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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This study aimed to investigate the effect of different sport categories (open- and closed skills) on proactive and reactive inhibitory processes as two distinct components of motor inhibition. A mouse tracking procedure was employed to compare behavioural performance among three groups of participants (tennis players, swimmers and non-athletes) in non-sport-specific cued Go/No-Go (GNG) and Stop Signal Task (SST), which mainly engage proactive and reactive inhibitory control, respectively. Reaction times (RTs), inhibitory failures, and Stop Signal Reaction Times (SSRTs) were measured. To investigate dynamic aspects of inhibitory control, one-shot and non-one-shot movement profiles were analysed and compared among groups. Results showed no group differences in RTs in Go/No-Go and Stop conditions. SSRTs were significant shorter for the athletes than non-athletes in SST, but no differences emerged for inhibitory failures in cued GNG. During inhibitory failures athletes showed higher proportion of non-one-shot movements than non-athletes in both tasks. Higher proportion of non-one-shot profiles was observed in cued GNG compared to SST. Finally, no differences between two sport categories were found in both tasks. Our findings suggest that both proactive and reactive inhibitory controls do benefit from sport practice, but open- and closed-skills sports do not differ in influencing inhibitory processes. Movement profile analysis could be a promising, complementary behavioral analysis to integrate for more fine-grained evaluation and differentiation of inhibitory motor control in athletes, specifically when using GNG tasks.

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BOARD NUMBER: S02-106

AN FMRI-BASED BRAIN MARKER OF INDIVIDUAL DIFFERENCES IN DELAY DISCOUNTING PREDICTS OVERWEIGHT AND METABOLIC MARKERS

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Individual differences in delay discounting—how much we discount future compared to immediate rewards—are associated with general life outcomes, psychopathology, and obesity. Here, we use machine-learning on fMRI activity during an intertemporal choice task to develop a brain marker of these individual differences. Training the marker in one dataset (N = 110) resulted in a significant cross-validated prediction-outcome correlation (r = 0.49), and accuracy of the marker was replicated in an independent data set (N = 145, r = 0.45). Significant predictive weights were found in cingulate, insula, and frontoparietal areas, suggesting an interplay between regions associated with valuation and cognitive control. Responses of the marker also predicted discounting several weeks later, differed between overweight and lean individuals, and predicted blood markers related to glucose metabolism. This pattern (termed k-marker) is a step towards a generalizable brain model of delay discounting that can be tested as a potentially transdiagnostic phenotype in future studies.

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BOARD NUMBER: S02-107

NOVEL CONTINUOUS SENSORY PROCESSING AND DECISION-MAKING PARADIGM EXHIBITS HIGH TEST-RETEST RELIABILITY ACROSS NEURAL AND BEHAVIOURAL MEASURES

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aims: When conducting psychiatric research, we must balance the desire for high-quality data with the inherent attentional and time constraints of clinical populations. We have developed a novel paradigm that pairs a continuous random dot motion task with a roving auditory mismatch negativity (MMN) paradigm. By concurrently exposing participants to a continuous visual and auditory stimulus stream alongside electroencephalogram (EEG) recording, we can study both auditory processing and visual evidence accumulation. However, for this paradigm to be applicable in clinical and pharmacological settings, we must ensure that we can reliably measure the neural signatures of both task components within a single session and across multiple days. *Methods:* The present study examines the test-retest reliability of the MMN response, behavioural integration kernels, and neural responses in the visual task. Test-retest reliability was assessed both across continuous five-minute blocks within a single recording session and across separate recording days. *Results:* We found that our task elicited the expected neural responses for the visual and auditory paradigms. While participants' MMN waveforms were highly reliable even across separate recording days, significant between-subject variability emerged. Through our paradigm's blocked design, we were further able to classify the relationship between EEG recording length and signal reliability. Reliable behavioural integration kernels were also observed. *Conclusions:* These findings suggest that our combined task can successfully elicit the key neural and behavioural signatures of both the auditory and visual paradigms. This high reliability, paired with the between-subject heterogeneity, supports the suitability of our paradigm for clinical and pharmacological settings.

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BOARD NUMBER: S02-108

PUPILLOMETRY IN INSTRUMENTAL ACTION- AND VALENCE-BASED DECISION-MAKING

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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In decisions involving motor actions, we decide either to invigorate or to inhibit. This is the action (effect) axis of control over the decision. On the other hand, we make decisions based on whether the situation is rewarding or punishing, and this comprises the valence (affect) axis of decision. Although it seems like actions are instructed by valence, we can also decide on “no-go” to receive a reward, or on “go” to avoid a punishment. Therefore, conversely to what is suggested by the classical instrumental vs. Pavlovian learning framework, there should not be a deductive dependence between action and valence. Optimal decision-making also involves the ability to regulate between known sources of reward (exploitation) and new rewards (exploration). Brain’s locus coeruleus-noradrenergic (LC-NE) system, whose activity is tracked by pupil diameter, is thought to be critically involved in this regulation. The present study aims to investigate the role of the LC-NE system in valence-based decision-making by studying pupil diameter as an index of norepinephrine activity. To this end, a go/no-go task (as in Guitart-Massip et al., 2012) is used in which reaction times and accuracy rates for going and stopping are compared as a function of rewarding and punishing conditions. We hypothesize that “go to reward” and “no-go to avoid punishment” conditions result in less reaction times, higher accuracy rates and less task-evoked pupil dilation, compared to ”no-go to reward” and “go to avoid punishment” conditions. Our results may lead to a better understanding of neuromodulation processes involved in decision-making.

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BOARD NUMBER: S02-109

COORDINATED MULTI-REGION ACTIVITY DURING CHOICE BEHAVIOR REVEALED BY HUMAN INTRACRANIAL RECORDINGS

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Decision-making requires coordinated activity across multiple brain regions involved in evaluation, comparison, and choice. Understanding neural activations underlying choices therefore requires examination of distributed neural activity. Here, we probed activity across multiple brain regions involved in valuation and choice using intracranial electroencephalography (iEEG) in human neurosurgical patients (n=20) while they played a gambling game designed to probe value-based decisions. On each trial, participants chose between a certain reward and a risky gamble of varying win probability. We recorded iEEG activity from orbitofrontal cortex (OFC), lateral prefrontal cortex (LPFC), cingulate cortex (CC), amygdala, hippocampus, insula, parietal cortex, and pre- and post-central gyri. We analyzed iEEG local field potential data across six canonical frequency bands and nine regions during deliberation (1s prior to choice). By comparing pre-choice activity to a pre-stimulus baseline, we found significant widespread task-related power modulations across multiple power bands and regions. Specifically, lower frequency (<30Hz) activity increased in all regions except pre- and post-central gyri and parietal regions, whereas higher frequencies were more likely to decrease, particularly in LPFC, OFC and CC. Next, we examined power modulations related to patient choice (gamble or safe bet) and found that low (30 to 70 Hz) and high gamma (70 to 200 Hz) were associated with decisions taken, particularly in OFC, LPFC, and precentral gyrus. These results describe critical neural components underlying human decision-making and demonstrate that widespread and multi-frequency neural activity underlies human choices under uncertainty.

Pubmed:
28855286: Overton JA, Cooke DF, Goldring AB, Lucero SA, Weatherford C, Recanzone GH
Improved methods for acrylic-free implants in nonhuman primates for neuroscience research.
Traditionally, head fixation devices and recording cylinders have been implanted in nonhuman primates (NHP) using dental acrylic despite several shortcomings associated with acrylic. The use of more biocompatible materials such as titanium and PEEK is becoming more prevalent in NHP research. We describe a cost-effective set of procedures that maximizes the integration of headposts and recording cylinders with the animal's tissues while reducing surgery time. Nine rhesus monkeys were implanted with titanium headposts, and one of these was also implanted with a recording chamber. In each case, a three-dimensional printed replica of the skull was created based on computerized tomography scans. The titanium feet of the headposts were shaped, and the skull thickness was measured preoperatively, reducing surgery time by up to 70%. The recording cylinder was manufactured to conform tightly to the skull, which was fastened to the skull with four screws and remained watertight for 8.5 mo. We quantified the amount of regression of the skin edge at the headpost. We found a large degree of variability in the timing and extent of skin regression that could not be explained by any single recorded factor. However, there was not a single case of bone exposure; although skin retracted from the titanium, skin also remained adhered to the skull adjacent to those regions. The headposts remained fully functional and free of complications for the experimental life of each animal, several of which are still participating in experiments more than 4 yr after implant. Cranial implants are often necessary for performing neurophysiology research with nonhuman primates. We present methods for using three-dimensional printed monkey skulls to form and fabricate acrylic-free implants preoperatively to decrease surgery times and the risk of complications and increase the functional life of the implant. We focused on reducing costs, creating a feasible timeline, and ensuring compatibility with existing laboratory systems. We discuss the importance of using more biocompatible materials and enhancing osseointegration.
J Neurophysiol, 2017; 118
26936987: Overton JA, Recanzone GH
Effects of aging on the response of single neurons to amplitude-modulated noise in primary auditory cortex of rhesus

macaque.
Temporal envelope processing is critical for speech comprehension, which is known to be affected by normal aging. Whereas the macaque is an excellent animal model for human cerebral cortical function, few studies have investigated neural processing in the auditory cortex of aged, nonhuman primates. Therefore, we investigated age-related changes in the spiking activity of neurons in primary auditory cortex (A1) of two aged macaque monkeys using amplitude-modulated (AM) noise and compared these responses with data from a similar study in young monkeys (Yin P, Johnson JS, O'Connor KN, Sutter ML. J Neurophysiol 105: 582-600, 2011). For each neuron, we calculated firing rate (rate code) and phase-locking using phase-projected vector strength (temporal code). We made several key findings where neurons in old monkeys differed from those in young monkeys. Old monkeys had higher spontaneous and driven firing rates, fewer neurons that synchronized with the AM stimulus, and fewer neurons that had differential responses to AM stimuli with both a rate and temporal code. Finally, whereas rate and temporal tuning functions were positively correlated in young monkeys, this relationship was lost in older monkeys at both the population and single neuron levels. These results are consistent with considerable evidence from rodents and primates of an age-related decrease in inhibition throughout the auditory pathway. Furthermore, this dual coding in A1 is thought to underlie the capacity to encode multiple features of an acoustic stimulus. The apparent loss of ability to encode AM with both rate and temporal codes may have consequences for stream segregation and effective speech comprehension in complex listening environments.
J Neurophysiol, 2016; 115

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BOARD NUMBER: S02-110

THE NEURAL CORRELATES OF EFFORT-REWARD DECISION-MAKING IN OLDER ADULTS

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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BACKGROUND: Effort mobilization is critical for goal directed behaviour across the human lifespan. Effort discounting is the process of reducing the subjective value of a reward as increasing effort that is required to obtain that reward. However, the neurobehavioral substrates of effort discounting in older adults are poorly understood and identifying related brain regions can help us better understand factors affecting normal cognitive aging. **METHODS**: We examined the effect of effort discounting on functional brain connectivity in 19 cognitively normal older adults (10 males; mean age = 66±6 years). Participants completed a computerized cognitive task, the Effort Expenditure for Rewards Task, capturing decision making to expend effort for monetary rewards through binary choices between high-reward-high-effort or low-reward-low-effort options. We modelled subjective value to assess the *k* parameter, as a proxy of effort discounting, and then examined the association to brain functional connectivity. Regions associated to the salience network—right/left anterior insula (AI) and anterior cingulate cortex (ACC)—were selected in seed-to-voxel analyses with age, sex, and Bayesian information criterion as covariates. **RESULTS**: Increased willingness to exert effort for reward was associated with enhanced functional connectivity between ACC and right lateral occipital cortex, and between the left AI, cerebellum and precuneus cortex (voxel $p<0.001$, cluster $p<0.05$ FDR corrected). The effect size of effort discounting was greater for ACC ($r^2=0.737$ for ACC; $r^2=0.718$ for left AI). **CONCLUSIONS**: Effort discounting is associated with functional connectivity changes involving the anterior cingulate cortex and left anterior insula in cognitively healthy older adults.

Pubmed:
32692429: Brown KE, Neva JL, Mang CS, Chau B, Chiu LK, Francisco BA, Staines WR, Boyd LA
The influence of an acute bout of moderate-intensity cycling exercise on sensorimotor integration.
Acute cycling exercise can modulate motor cortical circuitry in the non-exercised upper-limb. Within the primary motor cortex, measures of intracortical inhibition are reduced and intracortical facilitation is enhanced following acute exercise. Further, acute cycling exercise decreases interhemispheric inhibition between the motor cortices and lowers cerebellar-to-motor cortex inhibition. Yet, investigations into the effects of acute exercise on sensorimotor integration, referring to the transfer of incoming afferent information from the primary somatosensory cortex to motor cortex, are lacking. The current work addresses this gap in knowledge with two experimental sessions. In the first session, we tested the exercise-induced changes in somatosensory and motor excitability by assessing somatosensory (SEP) and motor evoked potentials (MEPs). In the second session, we explored the effects of acute cycling exercise on short- (SAI) and long-latency afferent inhibition (LAI), and afferent facilitation. In both experimental sessions, neurophysiological measures were obtained from the non-exercised upper-limb muscle, tested at two time points pre-exercise separated by a 25-min period of rest. Next, a 25-min bout of moderate-intensity lower-limb cycling was performed with measures assessed at two time points post-exercise. Acute lower-limb cycling increased LAI, without modulation of SAI or afferent facilitation. Further, there were no exercise-induced changes to SEP or MEP amplitudes. Together, these results suggest that acute exercise has unique effects on sensorimotor integration, which are not accompanied by concurrent changes in somatosensory or motor cortical excitability.
Eur J Neurosci, 2020; 52

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BOARD NUMBER: S02-111

MCC-DLPFC NETWORK MODULATION BY PATHWAY-SPECIFIC DREADDS IN MACAQUE MONKEYS: BEHAVIORAL, RESTING-STATE FMRI AND HISTOLOGICAL VALIDATIONS.

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Brain perturbation methods are essential to investigate causal inferences of behavioral and neural processes. We present the first stages in a project to use pathway-specific DREADDs (*Designer Receptor Exclusively Activated by Designer Drugs)* in macaque monkeys to target and reversibly modulate these neural networks. DREADDs are well established in rodents, but to date there has been little use of them in a pathway-specific manner in primate species, an essential step toward translation to clinical applications.
We assessed pathway-specific DREADD activation in macaques with a behavioral task and resting-state fMRI analysis, and validated transfection with histology. We targeted an anatomically defined pathway between midcingulate cortex (MCC) and dorsolateral prefrontal cortex (dlPFC) by injecting floxed hM3D(Gq) DREADD genetic material carried by AAV5 to MCC, and Cre-recombinase carried by retrograde CAV-2 vector to the dlPFC. Only neurons transfected by both AAV and CAV-2 would be able to express the DREADDs.
We present behavioural results from a search task, indicating an alteration of adaptive behaviour under DREADD activation with Deschloroclozapine (DCZ). We also present data showing whether resting-state fMRI networks are modified in the presence of DCZ in lightly anesthetized monkeys with and without DREADDs. Finally, we present histological validation of pathway-specific DREADDs in the MCC-DLPFC network. Our data provide a first step towards reversible pathway-specific interventions in primates.

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BOARD NUMBER: S02-112

THALAMOCORTICAL BASES OF THE EXPLOITATION/EXPLORATION TRADE-OFF

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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In a constantly changing environment, deciding when to explore (to gain knowledge) instead of exploiting it (using available knowledge to secure a gain) is an essential aspect of adaptive decision-making. Recent studies have shown a crucial role of the orbitofrontal cortex (OFC) when arbitrating between exploitation and exploration is required. The OFC shares extensive reciprocal connections with various subcortical regions including the mediodorsal thalamus (MD), a thalamic nucleus previously demonstrated to be largely involved in higher-order cognitive functions. To probe the contribution of these cortical and thalamic regions in the rat, we developed a probabilistic three-armed bandit task, in which rats must identify the most valuable option among three possible choices. Three levers are differentially rewarded, defining a good (80% chance to get a reward) and two bad options (20%). Within a session, the contingencies are randomly shuffled once to prompt the animals to switch from exploitation to exploration. Post-training excitotoxic lesions of the OFC and the MD appear to selectively impair the transition between exploitation and exploration. Indeed, exploration strategies appear to be less efficient in these animals and OFC rats also exhibited perseverative responses indicating an impairment in flexible exploration. Further experiments are warranted to document the role of the reciprocal pathways connecting the OFC and the MD. Ongoing work relying on a fiber photometry approach suggests that calcic transients in the cortex and the thalamus are differentially associated with specific behavioral events as rats perform the three-armed bandit task.

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BOARD NUMBER: S02-113

VENTRAL HIPPOCAMPUS IS REQUIRED FOR UPDATING ACTION-OUTCOME ASSOCIATIONS THROUGH CONTEXT-OUTCOME LEARNING

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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In a changing environment, the ability to update our representation of previously learnt information is essential. Such goal-directed behaviour requires individuals to constantly monitor their knowledge about the causal relationships between available actions and their specific outcomes, as well as the value of those outcomes. Interestingly, the influence of the environmental context on goal-directed action and specifically on the ability to adapt to any changes in action-outcome relationships remains poorly understood. Thus, here, we studied whether goal-directed behaviour relies on activity in the ventral hippocampus (vHPC), a structure involved in the contextual regulation of other associative learning processes. We first assessed the effect of pre-training vHPC lesions on outcome devaluation, a task designed to assess whether an action is sensitive to changes in outcome value. We found that inhibition of vHPC left devaluation intact. We then assessed the effect of vHPC inhibition on contingency degradation, which requires the animal to learn that the environmental context (and not the action) is now the best predictor of outcome delivery. We showed that rats with vHPC inhibition were unable to readily adapt their behaviour when the causal relationship between the action and the outcome was disrupted, and the context became the best predictor of reward. This suggests that the vHPC may be required to form context-outcome relationships to guide adaptive behaviour and appropriate action selection. Overall, our results show that goal-directed behaviour relies on activity in the vHPC only when learning about the environment is required.

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BOARD NUMBER: S02-114

NORADRENERGIC SIGNALING IN THE RODENT ORBITOFRONTAL CORTEX IS REQUIRED TO UPDATE GOAL-DIRECTED ACTIONS

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aims: In a constantly changing world, an organism must be able to assign specific outcomes to distinct actions in order to meet basic needs and desires. Such adaptive behaviour relies on circuits involving cortical and subcortical structures. For instance, recent data from our laboratory indicate that the ventral (VO) and lateral (LO) parts of the orbitofrontal cortex are required to integrate changes in the relationships between actions and their specific outcomes. Moreover, among all neuromodulatory agents involved in prefrontal functioning, it has been proposed that adaptive behavior might heavily depend upon the noradrenergic drive. Therefore, we assessed the intriguing possibility that noradrenergic innervation of the VO and the LO might play a role in updating action-outcome relationships. **Methods**: To do so, we tested Long-Evans rats in an identity-based reversal task, employed toxins selective for noradrenergic and dopaminergic neurons, and a retrograde virus carrying a noradrenergic-specific promoter and inhibitory DREADDs. **Results**: We found that either depletion or chemogenetic silencing of noradrenergic inputs within the VO and the LO impaired the update of action-outcome associations. Silencing of noradrenergic inputs in the medial prefrontal cortex or depletion of dopaminergic inputs in the VO and the LO did not reproduce this deficit. **Conclusions**: Altogether, our results indicate that noradrenergic projections to the ventral and lateral parts of the orbitofrontal cortex are cardinal for updating action-outcome associations, blending in and enriching our knowledge of neuroanatomical and neuromodulatory substrates of adaptive behavior.

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BOARD NUMBER: S02-115

INSULAR CORTEX IS REQUIRED TO GUIDE ACTION SELECTION IN OUTCOME-SPECIFIC DEVALUATION AND PAVLOVIAN-TO-INSTRUMENTAL TRANSFER

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aims: Appropriate decision making relies on our ability to recall the causal relationship between our actions and their consequences, as well as the value of expected outcomes. Cues associated with rewarding or aversive outcomes have been shown to influence such decisions, altering motivation and choice between competing actions. The phenomenon by which these mental representations or Pavlovian expectancies are able to bias choice selection is called Pavlovian-to-Instrumental transfer (PIT). Decades of research have identified two forms of PIT (General and Specific), distinct in terms of both behaviour and neural substrates. However, little is known about the involvement of cortical areas in such behaviors. Here, we investigated the potential role of the rodent gustatory insular cortex in specific PIT, as we have previously shown that this cortical region is required to guide action selection based on current outcome value. **Methods:** We used chemogenetics to temporally inactivate the insular cortex in adult Long-Evans male and female rats during a satiety-induced outcome-specific devaluation choice test and a specific PIT test. **Results:** We found that silencing the insular cortex during the test phase of both tasks impaired the rats' ability to bias their choice towards the appropriate action. **Conclusions:** These results indicate that the gustatory insular cortex is required to bias (1) choice selection towards the most desirable outcome and (2) operant behavior using predictive cues, by recalling associated mental representations.

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BOARD NUMBER: S02-116

LOCUS COERULEUS PROJECTIONS TO THE ORBITOFRONTAL CORTEX ARE NECESSARY TO UPDATE PAVLOVIAN CONTINGENCIES.

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aims : The orbitofrontal cortex (OFC) is known to play a critical role in acquiring and updating outcomes to specific events in a changing environment. Specifically, it has been involved in updating pavlovian but not instrumental associations in contingency degradation experiments. Moreover, noradrenaline has been shown to play critical roles in adaptative behavior depending on prefrontal regions. As a result, we assessed the role of noradrenergic innervation onto the ventrolateral OFC (vlOFC) in updating stimulus and actions/outcomes associations. **Methods :** In order to adress these questions, we tested the ability of Long Evans rats to update stimulus-outcome and action-outcome contingency degradations using chemogenetical inhibition of noradrenergic fibers projecting to the vlOFC. To do so, retrograde viruses carrying inhibitory DREADDs were injected into the vlOFC targetting noradrenergic fibers using a specific promoter. **Results :** We found that selectively inhibiting noradrenergic fibers projecting to the vlOFC during contingency degradation specifically impaired the rats ability to update Pavlovian but not instrumental associations. **Conclusions :** Altogether, these results indicate that noradrenergic projections to the vlOFC are crucial to update stimulus-outcome but not actions-outcomes associations in a changing environment, thus further improving our understanding of the role of noradrenaline in flexible behavior.

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BOARD NUMBER: S02-117

MOUSE PREFRONTAL AREA MOS ENCODES TASK FEATURES AND BODY MOVEMENTS

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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[Aims] Area MOs in the mouse prefrontal cortex is implicated in sensory-based decision-making, but it is not known if its neurons also carry signals related to value-based decisions, or how these neurons are affected by 'task-irrelevant' signals such as spontaneous body movements. **[Methods]** We used two-photon calcium imaging to measure the activity of L2/3 cortical excitatory neurons in area MOs while mice performed a visually guided decision task, in which the location of a visual stimulus was reported with a wheel movement. In addition to the changing stimulus location, the value of choices also differed, with reward volume for left versus right correct choices alternating over blocks (Lak et al. *Neuron*, 2020). We imaged the same field of view over multiple sessions, and simultaneously recorded eye, face and forepaw movements with an infrared video camera. **[Results]** MOs neurons heterogeneously encoded multiple task variables, such as contraversive or ipsiversive choice direction, contralateral visual stimulus, and the presence or absence of reward. Moreover, they encoded spontaneous movements of the eye, forepaws and/or snout that occurred on single trials. In some sessions, we observed neurons whose pretrial activity correlated with the reward block, but the strength and direction of this encoding changed over repeated sessions in the same population. **[Conclusions]** MOs neurons encode features of a decision-making task, such as stimulus, choice direction and reward outcome, but their value encoding varies across sessions. Additionally, MOs neurons encode 'task-irrelevant' spontaneous body movements.

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BOARD NUMBER: S02-118

TASK-EXPERIENCE-DEPENDENT STABILISATION OF OUTCOME-SELECTIVE NEURONS IN SENSORY CORTEX DURING REVERSAL LEARNING

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Flexible behavioural adaptation relies on learning and adjusting complex 'rules' that link sensory experience to possible actions. While mechanisms of learning and generalising rules have been identified in the prefrontal cortex, their interactions with the primary sensory cortex remain unknown. We trained head-fixed mice on a 'Go/No-go' tactile serial reversal-learning task where the animal experienced two successive rule switches (R1 and R2, respectively). Functional responses were measured using two-photon Ca^{2+} imaging from excitatory layer 2/3 neurons in primary somatosensory cortex (S1) and lateral orbitofrontal cortex (lOFC). We used a receiver operating characteristic analysis to assess the selectivity of single-neuron activity for specific trial types and employed tensor decomposition to analyse inter-trial population dynamics across all task stages. Upon R1, S1 neurons lost outcome-selectivity acquired during initial learning, whereas lOFC neurons showed increased activity, signalling a feedback prediction error. Lateral OFC top-down feedback instructed S1 response-remapping, enabling neurons to slowly gain outcome-selectivity. Silencing lOFC-to-S1 projections following R1, but not R2, impaired behavioural performance (*d'*), indicating the involvement of lOFC when task demands are relatively high. Despite a comparable drop in *d'* across R1 and R2, lOFC and S1 showed distinct engagements. While lOFC showed no significant activity upon R2, a substantial fraction of S1 neurons retained outcome-selective and history-dependent responses during early R2. Our findings propose feedback-independent assignment of outcome values in S1 that switch between conserved states depending on task demand. This strengthens the emerging idea that local mechanisms in the primary sensory areas can crucially contribute to higher cognitive functions.

Pubmed:
32884146: Banerjee A, Parente G, Teutsch J, Lewis C, Voigt FF, Helmchen F
Value-guided remapping of sensory cortex by lateral orbitofrontal cortex.
Adaptive behaviour crucially depends on flexible decision-making, which in mammals relies on the frontal cortex, specifically the orbitofrontal cortex (OFC). How OFC encodes decision variables and instructs sensory areas to guide adaptive behaviour are key open questions. Here we developed a reversal learning task for head-fixed mice, monitored the activity of neurons of the lateral OFC using two-photon calcium imaging and investigated how OFC dynamically interacts with primary somatosensory cortex (S1). Mice learned to discriminate 'go' from 'no-go' tactile stimuli and adapt their behaviour upon reversal of stimulus-reward contingency ('rule switch'). Imaging individual neurons longitudinally across all behavioural phases revealed a distinct engagement of S1 and lateral OFC, with S1 neural activity reflecting initial task learning, whereas lateral OFC neurons responded saliently and transiently to the rule switch. We identified direct long-range projections from lateral OFC to S1 that can feed this activity back to S1 as value prediction error. This top-down signal updated sensory representations in S1 by functionally remapping responses in a subpopulation of neurons that was sensitive to reward history. Functional remapping crucially depended on top-down feedback as chemogenetic silencing of lateral OFC neurons disrupted reversal learning, as well as plasticity in S1. The dynamic interaction of lateral OFC with sensory cortex thus implements computations critical for value prediction that are history dependent and error based, providing plasticity essential for flexible decision-making.
Nature, 2020; 585
31507388: Teutsch J, Kätzel D
Operant Assessment of DMTP Spatial Working Memory in Mice.
Working memory (WM) is required to bridge the time between the moment of sensory perception and the usage of the acquired information for subsequent actions. Its frequent and pharmacoresistent impairment in mental health disorders urges the development of rodent paradigms through back-translation of human WM tests, ideally avoiding the confounds of alternation-based assays. Here we show, that mice can acquire a delayed-matching-to-position (DMTP) operant spatial WM (SWM) paradigm that is akin to the (CAM) task previously developed for rats, and that relies on a 5-choice wall [5-CSWM, 5-

choice based operant testing of SWM (5-CSWM)]. Requiring ca. 3 months of daily training with a non-illuminated operant box in the default state, mice could attain a performance level of ≥70% choice accuracy with short (2 s) delays in the DMTP 5-CSWM task. Performance decreased with extended delays, as expected for WM processes. Modafinil (15 and 30 mg/kg) and guanfacine (0.3 and 1 mg/kg) showed no consistent efficacy in enhancing task performance. We also found, that mice did not improve beyond chance level, when trained in the DNMTP-version of the 5-CSWM. Our results outline the methodical possibility and constraints of assessing spatial WM in mice with an operant paradigm that provides high control over potentially confounding variables, such as cue-directed attention, motivation or mediating strategies like body-positioning.
Front Behav Neurosci, 2019; 13
33888809: Kilonzo K, van der Veen B, Teutsch J, Schulz S, Kapanaiah SKT, Liss B, Kätzel D
Delayed-matching-to-position working memory in mice relies on NMDA-receptors in prefrontal pyramidal cells.
A hypofunction of N-methyl-D-aspartate glutamate receptors (NMDARs) has been implicated in the pathogenesis of schizophrenia by clinical and rodent studies. However, to what extent NMDAR-hypofunction in distinct cell-types across the brain causes different symptoms of this disease is largely unknown. One pharmaco-resistant core symptom of schizophrenia is impaired working memory (WM). NMDARs have been suggested to mediate sustained firing in excitatory neurons of the prefrontal cortex (PFC) that might underlie WM storage. However, if NMDAR-hypofunction in prefrontal excitatory neurons may indeed entail WM impairments is unknown. We here investigated this question in mice, in which NMDARs were genetically-ablated in PFC excitatory cells. This cell type-selective NMDAR-hypofunction caused a specific deficit in a delayed-matching-to-position (DMTP) 5-choice-based operant WM task. In contrast, T-maze rewarded alternation and several psychological functions including attention, spatial short-term habituation, novelty-processing, motivation, sociability, impulsivity, and hedonic valuation remained unimpaired at the level of GluN1-hypofunction caused by our manipulation. Our data suggest that a hypofunction of NMDARs in prefrontal excitatory neurons may indeed cause WM impairments, but are possibly not accounting for most other deficits in schizophrenia.
Sci Rep, 2021; 11
34791112: Gigliucci V, Teutsch J, Woodbury-Smith M, Luoni M, Busnelli M, Chini B, Banerjee A
Region-Specific KCC2 Rescue by rhIGF-1 and Oxytocin in a Mouse Model of Rett Syndrome.
Rett syndrome (RTT) is characterized by dysfunction in neuronal excitation/inhibition (E/I) balance, potentially impacting seizure susceptibility via deficits in K+/Cl- cotransporter 2 (KCC2) function. Mice lacking the Methyl-CpG binding protein 2 (MeCP2) recapitulate many symptoms of RTT, and recombinant human insulin-like growth factor-1 (rhIGF-1) restores KCC2 expression and E/I balance in MeCP2 KO mice. However, clinical trial outcomes of rhIGF-1 in RTT have been variable, and increasing its therapeutic efficacy is highly desirable. To this end, the neuropeptide oxytocin (OXT) is promising, as it also critically modulates KCC2 function during early postnatal development. We measured basal KCC2 expression levels in MeCP2 KO mice and identified 3 key frontal brain regions showing KCC2 alterations in young adult mice, but not in postnatal P10 animals. We hypothesized that deficits in an IGF-1/OXT signaling crosstalk modulating KCC2 may occur in RTT during postnatal development. Consistently, we detected alterations of IGF-1 receptor and OXT receptor levels in those brain areas. rhIGF-1 and OXT treatments in KO mice rescued KCC2 expression in a region-specific and complementary manner. These results suggest that region-selective combinatorial pharmacotherapeutic strategies could be most effective at normalizing E/I balance in key brain regions subtending the RTT pathophysiology.
Cereb Cortex, 2021;
30301879: Grimm CM, Aksamaz S, Schulz S, Teutsch J, Sicinski P, Liss B, Kätzel D
Schizophrenia-related cognitive dysfunction in the Cyclin-D2 knockout mouse model of ventral hippocampal hyperactivity.
Elevated activity at the output stage of the anterior hippocampus has been described as a physiological endophenotype of schizophrenia, and its development maps onto the transition from the prodromal to the psychotic state. Interventions that halt the spreading glutamatergic over-activity in this region and thereby the development of overt schizophrenia could be promising therapies. However, animal models with high construct validity to support such pre-clinical development are scarce. The Cyclin-D2 knockout (CD2-KO) mouse model shows a hippocampal parvalbumin-interneuron dysfunction, and its pattern of hippocampal over-activity shares similarities with that seen in prodromal patients. Conducting a comprehensive phenotyping of CD2-KO mice, we found that they displayed novelty-induced hyperlocomotion (a rodent correlate of positive symptoms of schizophrenia), that was largely resistant against D1- and D2-dopamine-receptor antagonism, but responsive to the mGluR2/3-agonist LY379268. In the negative symptom domain, CD2-KO mice showed transiently reduced sucrose-preference (anhedonia), but enhanced interaction with novel mice and objects, as well as normal nest building and incentive motivation. Also, unconditioned anxiety, perseveration, and motor-impulsivity were unaltered. However, in the cognitive domain, CD2-knockouts showed reduced executive function in assays of rule-shift and rule-reversal learning, and also an impairment in working memory, that was resistant against LY379268-treatment. In contrast, sustained attention and forms of spatial and object-related memory that are mediated by short-term habituation of stimulus-specific attention were intact. Our results suggest that CD2-KO mice are a valuable model in translational research targeted at the pharmacoresistant cognitive

symptom domain in causal relation to hippocampal over-activity in the prodrome-to-psychosis transition. Transl Psychiatry, 2018; 8

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BOARD NUMBER: S02-119

STATE REPRESENTATION OF NON-SENSORY NEURONS IN VISUAL CORTICAL AREAS TO VISUALLY GUIDED DECISIONS IN THE RAT

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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[1]Doshisha University, Graduate School Of Brain Science, Kyoto, Japan, [2]College of Life Sciences, Ritsumeikan University, Laboratory Of Brain Network Information, Kusatsu, Shiga, Japan, [3]Graduate School of Brain Science, Doshisha University, Laboratory Of Neural Information, Kyotanabe, Kyoto, Japan

It is widely assumed that trial-by-trial variability in visual detection performance is explained by the fidelity of visual responses in visual cortical areas influenced by fluctuations of internal states, such as vigilance and behavioral history. However, which neuronal ensembles represent such different internal states remain unclear. In this study, we utilized a visual detection task, which distinguishes rats' internal states in response to identical stimuli, while recording neurons simultaneously from the primary visual cortex (V1) and the posterior parietal cortex (PPC). We found that rats often fail to respond to visual stimuli despite the robust presence of V1 responses. Our unsupervised analysis revealed distinct population dynamics segregating hit responses from misses, orthogonally embedded to visual response dynamics in both V1 and PPC. Heterogeneous non-sensory neurons in V1 and PPC significantly contributed to population-level encoding accompanied with the modulation of noise correlation only in V1. These results highlight the non-trivial contributions of non-sensory neurons in V1 and PPC for population-level computations that reflect the animals' internal states to drive behavioral responses to visual stimuli.

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BOARD NUMBER: S02-120

ALPHA 2-ADRENERGIC MECHANISM IN STRESS-INDUCED DECISION-MAKING

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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The α2A-adrenergic receptor is closely associated with stress. Systemic administration of yohimbine, an α2A receptor antagonist increases stress and impulsivity in rats and humans. The stress response is associated with significant increases in noradrenaline in the hippocampus, and dopamine in the prefrontal cortex, which might stimulate the cortex to drive risky behaviors contributing to pathological tendencies towards gambling, substance abuse, and other high-risk behaviors. Here, we explored this possibility by combining a pharmacological stressor, FG7142 with adrenergic agonists and antagonists. We first trained rats on an operant touchscreen decision-making task in which rats chose between two visual stimuli. Responses to the 'safe' stimulus always resulted in the delivery of a certain reward (50µl sucrose). Responses to the 'risky' stimulus resulted in the delivery of an uncertain reward (10µl 75% of the time, and 170µl 25% of the time). When given a systemic 4 mg dose of FG7142, the rats shifted their baseline strategies by increasing their choice of the safe stimulus and hence the certain reward. In contrast, the co-administration of FG7142 and yohimbine made the rats riskier since these animals not only preferred the option that delivered the uncertain reward but also increased the speed of responding. The combination of FG7142 and guanfacine, an α2A receptor agonist had the opposite effect of making animals significantly slower in their choices especially following a loss. These data indicate that stress together with acute activation of the noradrenergic system may worsen or trigger risky behaviors observed in both clinical and non-clinical populations.

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BOARD NUMBER: S02-121

THE NEED FOR EXPLORATION AND ITS USEFULNESS AFFECT LEARNING AND DECISION-MAKING SIGNALS IN MACAQUES PREFRONTAL CORTEX

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

Jan Grohn[1], Caroline Jahn[1,2], Matthew Rushworth[1], Sebastien Bouret[2], Mark Walton[1], Nils Kolling[3], Jerome Sallet[1,4]
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Adapting one's choices and learning from their outcomes is critical for survival in complex and changing environments. Exploring available options is a strategy to solve this problem that requires identifying when to explore to avoid forgo valuable rewards unnecessarily. Here we showed that monkeys (rhesus macaques) assess the need for exploration and its usefulness for future choices when making strategic exploratory decisions. Using fMRI, we showed that only when there was a need to explore through information sampling did the mid-cingulate cortex (MCC) and the dorsolateral prefrontal cortex (dlPFC) show increased activity with decreasing value of the chosen option. This suggests a role in counteracting expected value signals, when exploration away from value needs to be considered. At the heart of this strategic assessment, exploration and learning are intertwined. To optimize exploration based on the need for exploratory choices, one must be able to process the counterfactual information that is obtained without active sampling. We suggest that this occurs in medial orbito-frontal cortex (OFC), where we showed that monkeys represent chosen and unchosen reward prediction errors. Overall, our study shows how MCC-dlPFC and OFC circuits together could support exploitation of available information to the fullest and drive behavior towards finding more information when the need arises.

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JUXTACELLULAR RECORDING AND LABELLING OF NEURONS IN THE ORBITOFRONTAL CORTEX OF FREELY-MOVING RATS DURING A CONFIDENCE REPORTING TASK

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Knowing the difference between a choice that was taken with high confidence and one with low, can make the difference between life and death. Monitoring confidence allows a decision maker to adapt to variable uncertainty for any given decision. The firing rates of individual neurons within the orbitofrontal cortex (OFC) encode decision confidence, the probability of a decision being correct, given choice and evidence. Pharmacological inhibition of the OFC prevents rats from adjusting their time investment based on decision confidence. However, the cortical location and connectivity of functionally distinct neurons within the OFC microcircuit remains unknown. We trained rats to perform a previously described confidence reporting task in rats. Using the freely moving juxtaxellular labelling technique, we first recorded OFC neurons in rats performing this confidence task, then labelled them with neurobiotin. Neurons were selected for labelling on the basis of precisely timed fluctuations in firing rates relative to task events. We combined this technique with retrograde tracer injections, into candidate projection target areas, allowing us to identify the axon target of neurons with known activity. The post-hoc anatomical evaluation allowed us to determine soma position, dendritic arborisation and axonal projections of the recorded neurons. Our results indicate a correspondence between the behaviourally-related firing patterns of a neuron, and distinct dendritic and axonal connectivity. We expect that our approach will reveal how specific elements of OFC circuits compute confidence and contribute to neural machinery of a mysterious cognitive capacity.

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BOARD NUMBER: S02-123

REPRESENTATION OF ACTION VALUE AND UNCERTAINTY ACROSS DORSAL STRIATUM

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aims The dorsal striatum plays a key role in reward-guided decision making. Its primary cell type, medium spiny neurons (MSNs), are largely segregated into a *direct* and an *indirect* pathway. However, it is still highly contentious *how* these coordinate their activity to allow beneficial decisions to be made. One recent theoretical idea is that the relative patterns of activity across these pathways encode the expected value *and* uncertainty of the options (Mikhael & Bogacz, 2016). We therefore aimed at testing this in a context where an agent chooses between options with different reward probabilities. **Methods** We trained D1- and A2a-*Cre* mice, to allow selective targeting of the direct or indirect pathways MSNs, respectively, in a 3-option probabilistic decision-making task. Mice were trained to initiate a trial and then choose between 3 spatially-distinct reward ports, each of which was associated with a specific reward probability (100%, 50% or 20%). We injected GCaMP6f into dorsomedial and dorsolateral striatum and recorded bulk signals from either the direct or indirect MSNs while mice performed the task. **Results** Our preliminary results confirm a strong movement- and outcome-driven activation of both pathways in both regions. Critically, we can observe that encoding of the value and uncertainty of the options might be reflected in the signals from both MSNs type when the animal selects an option, and as it waits for the outcome. **Conclusions** Our study is revealing how dorsal striatum MSNs in different regions reflect decision variables such as reward value and uncertainty associated with options.

Pubmed:

34060871: Cerpa JC, Coutureau E, Parkes SL
Dopamine and noradrenaline modulation of goal-directed behavior in orbital and medial prefrontal cortex: Toward a division of labor?
The prefrontal cortex is considered to be at the core of goal-directed behaviors. Notably, the medial prefrontal cortex (mPFC) is known to play an important role in learning action-outcome (A-O) associations, as well as in detecting changes in this contingency. Previous studies have also highlighted a specific engagement of the dopaminergic pathway innervating the mPFC in adapting to changes in action causality. While previous research on goal-directed actions has primarily focused on the mPFC region, recent findings have revealed a distinct and specific role of the ventral and lateral orbitofrontal cortex (vlOFC). Indeed, vlOFC is not necessary to learn about A-O associations but appears specifically involved when outcome identity is unexpectedly changed. Unlike the mPFC, the vlOFC does not receive a strong dopaminergic innervation. However, it receives a dense noradrenergic innervation which might indicate a crucial role for this neuromodulator. In addition, several lines of evidence highlight a role for noradrenaline in adapting to changes in the environment. We, therefore, propose that the vlOFC's function in action control might be under the strong influence of the noradrenergic system. In the present article, we review anatomical and functional evidence consistent with this proposal and suggest a direction for future studies that aim to shed light on the orbitofrontal mechanisms for flexible action control. Specifically, we suggest that dopaminergic modulation in the mPFC and noradrenergic modulation in the vlOFC may underlie distinct processes related to updating one's actions. (PsycInfo Database Record (c) 2021 APA, all rights reserved).
Behav Neurosci, 2021; 135
28541407: Parkes SL, Ravassard PM, Cerpa JC, Wolff M, Ferreira G, Coutureau E
Insular and Ventrolateral Orbitofrontal Cortices Differentially Contribute to Goal-Directed Behavior in Rodents.
The medial prefrontal cortex (mPFC) has long been considered a critical site in action control. However, recent evidence indicates that the contribution of cortical areas to goal-directed behavior likely extends beyond mPFC. Here, we examine the function of both insular (IC) and ventrolateral orbitofrontal (vlOFC) cortices in action-dependent learning. We used chemogenetics to study the consequences of IC or vlOFC inhibition on acquisition and performance of instrumental actions using the outcome devaluation task. Rats first learned to associate actions with desirable outcomes. Then, one of these outcomes was devalued and we assessed the rats' choice between the 2 actions. Typically, rats will bias their selection

towards the action that delivers the still valued outcome. We show that chemogenetic-induced inhibition of IC during choice abolishes goal-directed control whereas inhibition during instrumental acquisition is without effect. IC is therefore necessary for action selection based on current outcome value. By contrast, vlOFC inhibition during acquisition or the choice test impaired goal-directed behavior but only following a shift in the instrumental contingencies. Our results provide clear evidence that vlOFC plays a critical role in action-dependent learning, which challenges the popular idea that this region of OFC is exclusively involved in stimulus-dependent behaviors.
Cereb Cortex, 2018; 28
30630012: Cerpa JC, Marchand AR, Coutureau E
Distinct regional patterns in noradrenergic innervation of the rat prefrontal cortex.
The anatomy and functions of the rodent prefrontal cortex (PFC) have been extensively studied. It is now clear that the PFC is at the core of various executive functions and that these functions depend on monoaminergic neuromodulation. The PFC receives extensive projections from monoaminergic nuclei and, in particular, from the locus cœruleus (LC) which is the major source of noradrenaline (NA) in the cortex. Projections of this nucleus have long been considered to act diffusely and uniformly throughout the entire brain. However, recent studies have revealed a separate innervation of prefrontal sub-regions by non-collateralizing LC neurons, suggesting a specific modulation of their functions. Following this idea, we aimed at describing more precisely the pattern of noradrenergic innervation into different orbital (OFC) and medial (mPFC) sub-regions of the PFC. We focused on the lateral (LO), ventral (VO) and medial (MO) portions of the OFC, and on areas 32d (A32d), 32v (A32v) and 25 (A25) in the mPFC. Using Dopamine-β-Hydroxylase as a specific noradrenergic marker, we performed an automatic quantification of noradrenergic fibers and varicosities in each of these sub-regions. The results indicate that noradrenergic innervation is heterogeneous in some prefrontal sub-regions along the rostro-caudal axis. Functional dissociations have been recently reported in prefrontal sub-regions along the rostro-caudal direction. Our findings add neuroanatomical support to this emergent idea.
J Chem Neuroanat, 2019; 96
32719584: Cerpa JC, Marchand AR, Salafranque Y, Pape JR, Kremer EJ, Coutureau E
Targeting Catecholaminergic Systems in Transgenic Rats With a CAV-2 Vector Harboring a Cre-Dependent DREADD Cassette.
Techniques that allow the manipulation of specific neural circuits have greatly increased in the past few years. DREADDs (Designer receptors exclusively activated by designer drugs) provide an elegant way to manipulate individual brain structures and/or neural circuits, including neuromodulatory pathways. Considerable efforts have been made to increase cell-type specificity of DREADD expression while decreasing possible limitations due to multiple viral vectors injections. In line with this, a retrograde canine adenovirus type 2 (CAV-2) vector carrying a Cre-dependent DREADD cassette has been recently developed. In combination with Cre-driver transgenic animals, the vector allows one to target neuromodulatory pathways with cell-type specificity. In the present study, we specifically targeted catecholaminergic pathways by injecting the vector in knock-in rat line containing Cre recombinase cassette under the control of the tyrosine hydroxylase promoter. We assessed the efficacy of infection of the nigrostriatal pathway and the catecholaminergic pathways ascending to the orbitofrontal cortex (OFC) and found cell-type-specific DREADD expression.
Front Mol Neurosci, 2020; 13
30904918: de Medeiros GF, Lafenêtre P, Janthakhin Y, Cerpa JC, Zhang CL, Mehta MM, Mortessagne P, Helbling JC, Ferreira G, Moisan MP
Corticosteroid-Binding Globulin Deficiency Specifically Impairs Contextual and Recognition Memory Consolidation in Male Mice.
Glucocorticoids are essential in modulating memory processes of emotionally arousing experiences and we have shown that corticosteroid-binding globulin (CBG) influences glucocorticoid delivery to the brain. Here, we investigated the role of CBG in contextual and recognition long-term memory according to stress intensity.
Neuroendocrinology, 2019; 109

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BOARD NUMBER: S02-124

BILATERAL MAPPING OF ACTIONS IN THE SUPERIOR COLLICULUS

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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[Aims] Mouse superior colliculus (SC) plays a key role in selecting actions guided by sensory cues (e.g. Huda et al. 2020, Duan et al. 2021, Essig et al. 2021). SC neurons exhibit lateralized activity, responding after contralateral visual cues and before contraversive movements (Steinmetz et al. 2019). A common view therefore is that the two sides of the SC compete, each side promoting contralateral choices. [Methods] We inactivated the left SC in head-fixed mice trained to turn a steering wheel to indicate whether a stimulus appears on the left or right (Burgess et al. 2017, IBL et al. 2021), by expressing the inhibitory soma-targeting opsin stGtACR2 (Mahn et al. 2018) in deep and intermediate SC layers. [Results] Unilateral optogenetic manipulation had opposite effects depending on the region of SC that was inactivated. Inactivation of lateral SC suppressed contralateral choices (n=2 mice), whereas inactivation of medial SC promoted contralateral choices (n=3 mice). Inactivation of medial SC also markedly reduced the reaction times, possibly suggesting a different behavioral strategy. [Conclusions] These initial results reveal that each side of the SC contains regions that promote vs. suppress contralateral actions . This observation suggests that we need to revise the classical view of competition between two sides of the SC: for instance, competition may also take place within each side of the SC.

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BOARD NUMBER: S02-125

LIPOPOLYSACCHARIDE-INDUCED NEUROINFLAMMATION IN THE POSTERIOR DORSOMEDIAL STRIATUM FACILITATES GOAL-DIRECTED ACTION.

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Neuroinflammation has been identified in striatum of individuals with compulsive disorders. These individuals also display deficits in goal-directed action. Nevertheless, a direct, causal link between striatal neuroinflammation and impaired goal-directed action has yet to be established. In Experiment 1, rats received micro-injections in posterior dorsomedial striatum (pDMS) of either saline or lipopolysaccharide (LPS) (5mg/ml) to induce neuroinflammation. We then examined goal-directed decision-making performance using Pavlovian-instrumental transfer and outcome devaluation. In the Pavlovian phase, rats were trained to associate two unique auditory cues with pellets and sucrose (counterbalanced). In the instrumental phase, the same rats were trained to press left and right levers for pellet and sucrose outcomes respectively (counterbalanced). When animals were overfed and control effects were small, transfer and devaluation were comparatively enhanced in group LPS. During transfer testing, group LPS selectively responded on the lever associated with the same outcome (Same>Different) whereas group Sham did not. During devaluation testing, although both groups responded more on the lever that had earned the valued relative to the devalued outcome, this difference was larger in group LPS. Experiment 2 tested whether pDMS neuroinflammation increases motivation generally, or goal-directed action specifically. We trained sham and LPS pDMS animals to press a single lever for sucrose. When tested for progressive ratio performance, animals in group LPS reached consistently higher breakpoints than group Sham. Upon devaluation testing, group Sham demonstrated evidence of habits (Valued=Devalued) and group LPS demonstrated intact goal-directed actions (Valued>Devalued). These results suggest that LPS-induced neuroinflammation in pDMS increases both motivation and goal-directed action.

Pubmed:

34798235: Abiero AR, Ali Z, Vissel B, Bradfield LA
Outcome-selective reinstatement is predominantly context-independent, and associated with c-Fos activation in the posterior dorsomedial striatum.
Research from human and animal studies has found that after responding has been successfully reduced following treatment it can return upon exposure to certain contexts. An individual in recovery from alcohol use disorder, for example, might relapse to drinking upon visiting their favourite bar. However, most of these data have been derived from experiments involving a single (active) response, and the context-dependence of returned responding in situations involving choice between multiple actions and outcomes is less well-understood. We thus investigated how outcome-selective reinstatement - a procedure involving choice between two actions and outcomes - was affected by altering the physical context in rats. In Experiment 1, rats were trained over 6 days to press a left lever for one food outcome (pellets or sucrose) and a right lever for the other outcome. Then, rats received an extinction session in either the same context (A) as lever press training, or in a different context (B). Rats were tested immediately (5 min) after extinction in Context A or B such that there were four groups in total: AAA, ABB, ABA, and AAB. Reinstatement testing consisted of one food outcome being delivered 'freely' (i.e. unearned by lever pressing and unsignalled by cues) to the food magazine every 4 min in the following order: Sucrose, Pellet, Pellet, Sucrose. Selective reinstatement was considered intact if pellet delivery increased pressing selectively on the pellet lever, and sucrose delivery selectively increased pressing on the sucrose lever. This result (Reinstated > Nonreinstated) was observed for rats in group AAA and ABB, but not rats in groups ABA and AAB. Experiment 2 was conducted identically, except that rats received two extinction sessions over two days and tested one day later. This time, all groups demonstrated intact outcome-selective reinstatement regardless of context. Analysis of c-Fos expression in several brain regions revealed that only c-Fos expression in the posterior dorsomedial striatum (pDMS) was related to intact reinstatement performance. Overall, these results suggest that outcome-selective reinstatement is predominantly context-independent, and that intact reinstatement is related to neuronal activity in the pDMS.
Neurobiol Learn Mem, 2022; 187
33460722: Abiero A, Perez Custodio RJ, Botanas CJ, Ortiz DM, Sayson LV, Kim M, Lee HJ, Yoon S, Lee YS, Cheong JH,

Kim HJ
1-Phenylcyclohexan-1-amine hydrochloride (PCA HCl) alters mesolimbic dopamine system accompanied by neuroplastic changes: A neuropsychopharmacological evaluation in rodents.
The recreational use of N-methyl-D-aspartate (NMDA) antagonist phencyclidine (PCP) and ketamine have grown rapidly due to their psychotomimetic properties. These compounds induce both non-fatal and fatal adverse effects and despite the enhanced regulation, they are continuously synthesized and are being sold in the illegal drug market, including 1-phenylcyclohexan-1-amine hydrochloride (PCA). Therefore, we evaluated its abuse potential through the conditioned-place preference (CPP), self-administration, and locomotor sensitization paradigms. Pretreatment with SCH 2 3390 and haloperidol was also performed during a CPP test. We used ELISA to measure dopamine (DA) levels and western blotting to determine effects on the DA-related proteins as well as on phosphorylated CREB, deltaFosB, and brain-derived neurotrophic factor (BDNF) in the ventral tegmental area (VTA) and nucleus accumbens (NAc). Finally, we examined the effects on brain wave activity using electroencephalography (EEG). PCA induced CPP in mice and was self-administered by rats, suggesting that PCA has rewarding and reinforcing properties. PCA increased locomotor of mice on the first treatment and challenge days. SCH 23390 and haloperidol blocked the CPP. PCA altered the DA, tyrosine hydroxylase, dopamine D1 and D2 receptors as well as p-CREB and deltaFosB. Also, PCA altered the delta and gamma waves in the brain, which were then normalized by SCH 2 3390 and haloperidol. The present findings indicate that PCA may induce abuse potential through the dopaminergic system and probably accompanied with alterations in brain wave activity which is similar to that of other psychotomimetic NMDA antagonists. We advocate thorough monitoring of PCP analogs as they pose potential harm to public health.
Neurochem Int, 2021; 144
32648801: Custodio RJP, Sayson LV, Botanas CJ, Abiero A, Kim M, Lee HJ, Ryu HW, Lee YS, Kim HJ, Cheong JH
Two newly-emerging substituted phenethylamines MAL and BOD induce differential psychopharmacological effects in rodents.
Recently, the recreational use of substituted phenethylamines has grown rapidly. Among these are 2-(3,5-dimethoxy-4-((2-methylallyl)oxy)phenyl)ethanamine (MAL) and 2-(2,5-dimethoxy-4-methylphenyl)-2-methoxyethan-1-amine (BOD). However, studies characterizing their abuse potential are still lacking.
J Psychopharmacol, 2020; 34
33135332: Ortiz DM, Custodio RJP, Abiero A, Botanas CJ, Sayson LV, Kim M, Lee HJ, Kim HJ, Jeong Y, Yoon S, Lee YS, Cheong JH
The dopaminergic alterations induced by 4-F-PCP and 4-Keto-PCP may enhance their drug-induced rewarding and reinforcing effects: Implications for abuse.
Novel psychoactive substances remain the popular recreational drugs of use over the years. They continue to bypass government restrictions due to their synthesis and modifications. Recent additions to the lists are the 4-F-PCP and 4-Keto-PCP, analogs of the drug phencyclidine (PCP) known to induce adverse effects and abuse potential. However, studies on the abuse potential of 4-F-PCP and 4-Keto-PCP remain scarce. The rewarding and reinforcing effects of the drugs were assessed using conditioned place preference (CPP), self-administration, and locomotor sensitization tests. Dopamine (DA) receptor antagonists (SCH23390 and haloperidol) were administered during CPP to evaluate the involvement of the mesolimbic dopaminergic system. DA-related protein expression in the nucleus accumbens (NAcc) and ventral tegmental area (VTA) was measured. Additionally, phosphorylated cyclic-adenosine monophosphate-activated protein (AMP) response element-binding (p-CREB) protein, deltaFosB (ΔFosB), and brain-derived neurotrophic factor (BDNF) protein levels in the NAcc were measured to assess the addiction neural plasticity effect of the drugs. Both 4-F-PCP and 4-Keto-PCP-induced CPP and self-administration; however, only 4-F-PCP elicited locomotor sensitization. Treatment with DA receptor antagonists (SH23390 and haloperidol) inhibited the 4-F- and 4-Keto-induced CPP. Both substances altered the levels of DA receptor D1 (DRD1), thyroxine hydroxylase (TH), DA receptor D2 (DRD2), p-CREB, ΔFosB, and BDNF. The results suggest that 4-F-PCP and 4-Keto-PCP may induce abuse potential in rodents via alterations in dopaminergic system accompanied by addiction neural plasticity.
Addict Biol, 2021; 26
31739380: Kim M, Acharya S, Botanas CJ, Custodio RJ, Lee HJ, Sayson LV, Abiero A, Lee YS, Cheong JH, Kim KM, Kim HJ
Catalpol and Mannitol, Two Components of , Exhibit Anticonvulsant Effects Probably via GABA Receptor Regulation.
Epilepsy is a brain disorder that affects millions of people worldwide and is usually managed using currently available antiepileptic drugs, which result in adverse effects and are ineffective in approximately 20-25% of patients. Thus, there is growing interest in the development of new antiepileptic drugs with fewer side effects. In a previous study, we showed that a (RG) water extract has protective effects against electroshock- and pentylenetetrazol (PTZ)-induced seizures, with fewer side effects. In this study, the objective was to identify the RG components that are responsible for its anticonvulsant effects. Initially, a number of RG components (aucubin, acteoside, catalpol, and mannitol) were screened, and the anticonvulsant effects of different doses of catalpol, mannitol, and their combination on electroshock- and chemically (PTZ or strychnine)-

induced seizures in mice, were further assessed. Gamma-aminobutyric acid (GABA) receptor binding assay and electroencephalography (EEG) analysis were conducted to identify the potential underlying drug mechanism. Additionally, treated mice were tested using open-field and rotarod tests. Catalpol, mannitol, and their combination increased threshold against electroshock-induced seizures, and decreased the percentage of seizure responses induced by PTZ, a GABA antagonist. GABA receptor binding assay results revealed that catalpol and mannitol are associated with GABA receptor activity, and EEG analysis provided evidence that catalpol and mannitol have anticonvulsant effects against PTZ-induced seizures. In summary, our results indicate that catalpol and mannitol have anticonvulsant properties, and may mediate the protective effects of RG against seizures.
Biomol Ther (Seoul), 2020; 28
31230432: Abiero A, Ryu IS, Botanas CJ, Custodio RJP, Sayson LV, Kim M, Lee HJ, Kim HJ, Seo JW, Cho MC, Lee KW, Yoo SY, Jang CG, Lee YS, Cheong JH
Four Novel Synthetic Tryptamine Analogs Induce Head-Twitch Responses and Increase 5-HTR2a in the Prefrontal Cortex in Mice.
Tryptamines are monoamine alkaloids with hallucinogenic properties and are widely abused worldwide. To hasten the regulations of novel substances and predict their abuse potential, we designed and synthesized four novel synthetic tryptamine analogs: Pyrrolidino tryptamine hydrochloride (PYT HCl), Piperidino tryptamine hydrochloride (PIT HCl), N,N-dibutyl tryptamine hydrochloride (DBT HCl), and 2-Methyl tryptamine hydrochloride (2-MT HCl). Then, we evaluated their rewarding and reinforcing effects using the conditioned place preference (CPP) and self-administration (SA) paradigms. We conducted an open field test (OFT) to determine the effects of the novel compounds on locomotor activity. A head-twitch response (HTR) was also performed to characterize their hallucinogenic properties. Lastly, we examined the effects of the compounds on 5-HTR1a and 5-HTR2a in the prefrontal cortex using a quantitative real-time polymerase chain reaction (qRT-PCR) assay. None of the compounds induced CPP in mice or initiated SA in rats. PYT HCl and PIT HCl reduced the locomotor activity and elevated the 5-HTR1a mRNA levels in mice. Acute and repeated treatment with the novel tryptamines elicited HTR in mice. Furthermore, a drug challenge involving a 7-day abstinence from drug use produced higher HTR than acute and repeated treatments. Both the acute treatment and drug challenge increased the 5-HTR2a mRNA levels. Ketanserin blocked the induced HTR. Taken together, the findings suggest that PYT HCl, PIT HCl, DBT HCl, and 2-MT HCl produce hallucinogenic effects via 5-HTR2a stimulation, but may have low abuse potential.
Biomol Ther (Seoul), 2019;
30091820: Kim M, Custodio RJ, Botanas CJ, de la Peña JB, Sayson LV, Abiero A, Ryoo ZY, Cheong JH, Kim HJ
The circadian gene, Per2, influences methamphetamine sensitization and reward through the dopaminergic system in the striatum of mice.
Drug addiction is a chronic and relapsing brain disorder, influenced by complex interactions between endogenous and exogenous factors. Per2, a circadian gene, plays a role in drug addiction. Previous studies using Per2-knockout mice have shown a role for Per2 in cocaine, morphine and alcohol addiction. In the present study, we investigated the role of Per2 in methamphetamine (METH) addiction using Per2-overexpression and knockout mice. We observed locomotor sensitization responses to METH administration, and rewarding effects using a conditioned place preference test. In addition, we measured expression levels of dopamine and dopamine-related genes (monoamine oxidase A, DA receptor 1, DA receptor 2, DA active transporter, tyrosine hydroxylase and cAMP response element-binding protein 1) in the striatum of the mice after repeated METH treatments, using qRT-PCR. Per2-overexpressed mice showed decreased locomotor sensitization and rewarding effects of METH compared to the wildtype mice, whereas the opposite was observed in Per2 knockout mice. Both types of transgenic mice showed altered expression levels of dopamine-related genes after repeated METH administration. Specifically, we observed lower dopamine levels in Per2-overexpressed mice and higher levels in Per2-knockout mice. Taken together, Per2 expression levels may influence the addictive effects of METH through the dopaminergic system in the striatum of mice.
Addict Biol, 2019; 24
31749223: Custodio RJP, Sayson LV, Botanas CJ, Abiero A, You KY, Kim M, Lee HJ, Yoo SY, Lee KW, Lee YS, Seo JW, Ryu IS, Kim HJ, Cheong JH
25B-NBOMe, a novel N-2-methoxybenzyl-phenethylamine (NBOMe) derivative, may induce rewarding and reinforcing effects via a dopaminergic mechanism: Evidence of abuse potential.
An increasing number of N-2-methoxybenzyl-phenethylamine (NBOMe) derivatives are being misused worldwide, including the potent hallucinogen 2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25B-NBOMe). However, the number of studies characterizing the abuse potential and psychopharmacological properties of 25B-NBOMe is limited; thus, we examined its rewarding and reinforcing effects using conditioned place preference (CPP) and self-administration (SA) tests. Pretreatment with SCH23390 (SCH), Haloperidol (HAL), and ketanserin (KS), antagonists of dopamine D1 (DRD), dopamine D2 (DRD), and serotonin 2A (5-HT receptor) receptors, respectively, was utilized during a CPP test to investigate

the involvement of the dopaminergic and serotonergic systems in 25B-NBOMe-mediated effects. We also examined the effects of 25B-NBOMe on the expression of dopamine-related proteins in the nucleus accumbens (NAcc) and ventral tegmental area (VTA). Then, we measured the dopamine level, phosphorylated CREB (p-CREB), deltaFosB (ΔFosB), and brain-derived neurotrophic factor (BDNF) in the NAcc. In addition, we explored the involvement of 5-HT receptors in the 25B-NBOMe-induced head twitch response (HTR). We also examined the effects of 25B-NBOMe on brain wave activity using electroencephalography. 25B-NBOMe elicited CPP and SA. SCH and HAL blocked 25B-NBOMe-induced CPP, whereas KS did not. Moreover, 25B-NBOMe altered the DRD , DRD , and dopamine transporter expression and increased dopamine levels. It also induced changes in p-CREB, ΔFosB, and BDNF expression. 25B-NBOMe induced HTR and increased 5-HT receptor mRNA levels, effects inhibited by KS. Furthermore, 25B-NBOMe altered delta and gamma wave activity, which was normalized by SCH and HAL. These findings show that 25B-NBOMe may induce rewarding and reinforcing effects via a dopaminergic mechanism, suggesting its abuse potential.
Addict Biol, 2020; 25
31828394: Abiero A, Botanas CJ, Custodio RJ, Sayson LV, Kim M, Lee HJ, Kim HJ, Lee KW, Jeong Y, Seo JW, Ryu IS, Lee YS, Cheong JH
4-MeO-PCP and 3-MeO-PCMo, new dissociative drugs, produce rewarding and reinforcing effects through activation of mesolimbic dopamine pathway and alteration of accumbal CREB, deltaFosB, and BDNF levels.
A high number of synthetic dissociative drugs continue to be available through online stores, leading to their misuse. Recent inclusions in this category are 4-MeO-PCP and 3-MeO-PCMo, analogs of phencyclidine. Although the dissociative effects of these drugs and their recreational use have been reported, no studies have investigated their abuse potential.
Psychopharmacology (Berl), 2020; 237
30053461: Abiero A, Botanas CJ, Sayson LV, Custodio RJ, de la Peña JB, Kim M, Lee HJ, Seo JW, Ryu IS, Chang CM, Yang JS, Lee YS, Jang CG, Kim HJ, Cheong JH
5-Methoxy-α-methyltryptamine (5-MeO-AMT), a tryptamine derivative, induces head-twitch responses in mice through the activation of serotonin receptor 2a in the prefrontal cortex.
5-Methoxy-α-methyltryptamine (5-MeO-AMT) is a tryptamine derivative that is used recreationally because of its reported hallucinogenic and mood elevating effects. Studies suggest that the psychopharmacological effects of tryptamines involve serotonin receptor 2a (5-HTR2a) activation in the brain. The head-twitch response (HTR) is widely used as a behavioral correlate for assessing 5-HTR2a agonist activity of a drug. Thus, we investigated whether 5-MeO-AMT induces HTR in mice and explored its mechanism of action. 5-MeO-AMT (0.3, 1, 3, 10 mg/kg) was administered once a day for 7 days, and the HTR was measured after 1 day (acute) and 7 days (repeated) of administration. Another cohort of mice was treated with 5-HTR2a antagonist ketanserin (KS) before 5-MeO-AMT administration. We measured 5-HTR2a and 5-HTR2c mRNA levels in the prefrontal cortex of the mice treated acutely or repeatedly with 5-MeO-AMT. We performed western blotting to determine the effects of the drug on the expression of G protein (G), protein kinase C gamma (PKC-γ), and extracellular signal-regulated kinases 1/2 (ERK1/2), in addition to PKC-γ and ERK1/2 phosphorylation. Additionally, we evaluated potential rewarding and reinforcing effects of 5-MeO-AMT using locomotor sensitization, conditioned place preference (CPP), and self-administration (SA) paradigms. Acute 5-MeO-AMT administration elicited the HTR, while repeated administration resulted in tolerance. KS blocked the 5-MeO-AMT-induced HTR. 5-MeO-AMT increased 5-HTR2a mRNA levels and induced PKC-γ phosphorylation in the prefrontal cortex. 5-MeO-AMT did not induce locomotor sensitization, CPP, or SA. This study shows that 5-MeO-AMT induces HTR through 5-HTR2a activation in the prefrontal cortex, and may have low potential for abuse.
Behav Brain Res, 2019; 359

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BOARD NUMBER: S02-126

THE BASOLATERAL AMYGDALA TO NUCLEUS ACCUMBENS SHELL PATHWAY ENCODES, BUT DOESN'T RETRIEVE, OUTCOME-SPECIFIC PREDICTIONS TO GUIDE CHOICE BETWEEN ACTIONS

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

Elise Pepin, Beatrice Leung, Billy Chieng, Bernard Balleine, Vincent Laurent
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Aims: Outcome-specific predictions drive the accumulation of delta-opioid receptors (DOPR) on the membrane of cholinergic interneurons (CINs) in the nucleus accumbens shell (NAc-S) to promote choice between actions. Here, we aimed to provide decisive evidence that outcome-specific predictions control DOPR accumulation and to establish whether this control is mediated by projections from the basolateral amygdala (BLA) to the NAc-S. **Methods and Results:** We confirmed that outcome-specific predictions drive DOPR accumulation on NAc-S CINs and found that this accumulation can be removed by systemic administration of the DOPR agonist SNC80. Remarkably, DOPR accumulation was reinstated by a single session retraining the outcome-specific predictions, underscoring the causal relationship between these predictions and DOPR expression. To establish whether this relationship relies on the BLA→NAc-S pathway, we optically silenced this pathway during outcome-specific predictive learning and assessed the impact on subsequent choice between actions. BLA→NAc-S silencing abolished the traditional influence of outcome-specific predictions on choice between actions. In another experiment, we found that the same silencing had no effect when it was conducted at the time of choice. **Conclusions:** Taken together, these findings indicate that the BLA encodes outcome-specific predictions and drive DOPR accumulation on NAc-S CINs to guide choice between actions. However, they also show that the BLA does not control how this accumulation is then used to guide choice, suggesting that other brain regions may oversee this control

Pubmed:
32240599: Morse AK, Leung BK, Heath E, Bertran-Gonzalez J, Pepin E, Chieng BC, Balleine BW, Laurent V
Basolateral Amygdala Drives a GPCR-Mediated Striatal Memory Necessary for Predictive Learning to Influence Choice.
Predictive learning exerts a powerful influence over choice between instrumental actions. Nevertheless, how this learning is encoded in a sufficiently stable manner to influence choices that can occur much later in time is unclear. Here, we report that the basolateral amygdala (BLA) encodes predictive learning and establishes the memory necessary for future choices by driving the accumulation of delta-opioid receptors (DOPRs) on the somatic membrane of cholinergic interneurons in the nucleus accumbens shell (NAc-S). We found that the BLA controls DOPR accumulation via its influence on substance P release in the NAc-S, and that although DOPR accumulation is not necessary for predictive learning per se, it is necessary for the influence of this learning on later choice between actions. This study uncovers, therefore, a novel GPCR-based form of memory that is established by predictive learning and is necessary for such learning to guide the selection and execution of specific actions.
Neuron, 2020; 106
32153401: Pépin É, Jalinier T, Lemieux GL, Massicotte G, Cyr M
Sphingosine-1-Phosphate Receptors Modulators Decrease Signs of Neuroinflammation and Prevent Parkinson's Disease Symptoms in the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Mouse Model.
Sphingosine-1-phosphate (S1P) is a potent bioactive lipid mediator that acts as a natural ligand upon binding to five different receptors that are located in astrocytes, oligodendrocytes, microglial and neuronal cells. Recently, global activation of these receptors by FTY720 (fingolimod) has been suggested to provide neuroprotection in animal model of Parkinson's disease (PD). Among S1P receptors, the subtype 1 (S1P1R) has been linked to features of neuroprotection and, using the selective agonist SEW2871, the present investigation assessed potential benefits (and mechanisms) of this receptor subtype in an established animal model of PD. We demonstrated that oral treatments with SEW2871 are able to provide protection to the same levels as FTY720 against loss of dopaminergic neurons and motor deficits in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (30 mg/kg, i.p., 5 days) mouse model of PD. At the molecular level, we observed that the beneficial effects of both S1PR agonists were not associated with alterations in ERK and Akt levels, two markers of molecular adaptations in the striatum neurons. However, these compounds have the capacity to prevent signs of neuroinflammation such as the activation of astrocytes and glial cells, as well as MPTP-induced reduction of BDNF levels in key regions of the

brain implicated in motor functions. These findings suggest that selective S1P1R modulation has the ability to provide neuroprotection in response to MPTP neurotoxicity. Targeting S1P1R in PD therapy may represent a prominent candidate for treatment of this neurodegenerative conditions.
Front Pharmacol, 2020; 11

30688489: Ouimet B, Pépin É, Bergeron Y, Chagniel L, Beaulieu JM, Massicotte G, Cyr M
Motor learning deficits and striatal GSK-3 hyperactivity in Akt3 knockout mice.
Akt protein family (Akt1, Akt2 and Akt3) of serine/threonine kinases, also known as protein kinase B, are enzymes implicated in many physiological and pathological processes in the central nervous system. A striking feature of these enzymes is their ability to interact with several molecular targets such as the glycogen synthase kinase 3 (GSK-3). Among Akt isoforms, the Akt3 is significantly more expressed in the brain and the present investigation was designed to determine whether the Akt3/GSK-3 pathway plays a role in the learning of a complex motor skill. Using the accelerating rotarod task, known to reproduce different motor learning phases, we demonstrated in mouse models that genetic deletion of GSK-3α or GSK-3β had no effect on rotarod performances. However, Akt3 deletion robustly compromised rotarod learning when compared with wild-type animals. Biochemical analysis in the striatum revealed modifications in the levels of both phosphorylated GSK-3 and tau in Akt3-deficient mice, which are reminiscent of enhanced GSK-3 activity. In this line, we observed that both biochemical and motor learning impairments were prevented in Akt3-deficent mice by chronic treatments with lithium, a well-known GSK-3 inhibitor. Altogether, our findings raised the interesting possibility that interconnection between Akt3 and GSK-3 kinases is required in the learning of new complex motor tasks. (PsycINFO Database Record (c) 2019 APA, all rights reserved).
Behav Neurosci, 2019; 133

26260438: Attiori Essis S, Laurier-Laurin ME, Pépin É, Cyr M, Massicotte G
GluN2B-containing NMDA receptors are upregulated in plasma membranes by the sphingosine-1-phosphate analog FTY720P.
Sphingosine-1-phosphate (S1P) is a ceramide derivative serving not only as a regulator of immune properties but also as a modulator of brain functions. To better understand the mechanism underlying the effects of S1P on brain functions, we investigated the potential impact of S1P receptor (S1PR) activation on NMDA receptor subunits. We used acute rat hippocampal slices as a model system, and determined the effects of the active phosphorylated S1P analog, fingolimod (FTY720P) on various NMDA receptors. Treatment with FTY720P significantly increased phosphorylation of GluN2B-containing NMDA receptors at Tyr1472. This effect appears rather specific, as treatment with FTY720P did not modify GluN2B-Tyr1336, GluN2B-Ser1480, GluN2A-Tyr1325 or GluN1-Ser897 phosphorylation. Pre-treatment of hippocampal slices with the compounds W146 and PP1 indicated that FTY720P-induced GluN2B phosphorylation at Tyr1472 epitopes was dependent on activation of S1PR subunit 1 (S1PR1) and Src/Fyn kinase, respectively. Cell surface biotinylation experiments indicated that FTY720P-induced GluN2B phosphorylation at Tyr1472 was also associated with increased levels of GluN1 and GluN2B subunits on membrane surface, whereas no change was observed for GluN2A subunits. We finally demonstrate that FTY720P is inclined to favor Tau and Fyn accumulation on plasma membranes. These results suggest that activation of S1PR1 by FTY720P enhances GluN2B receptor phosphorylation in rat hippocampal slices, resulting in increased levels of GluN1 and GluN2B receptor subunits in neuronal membranes through a mechanism probably involving Fyn and Tau.
Brain Res, 2015; 1624

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BOARD NUMBER: S02-127

CONTRIBUTIONS OF ORBITOFRONTAL CORTEX NEURONS TO CHOICE ABANDONMENT IN A DECISION CONFIDENCE TASK

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Effective decision-making requires not just resolving difficult choices but also knowing when to abandon a failed strategy. The orbitofrontal cortex (OFC) plays a key role in decision-making and has been shown to represent metacognitive computations such as post-decision confidence, i.e. the probability of being correct given the subjective evidence. How neural representations of decision confidence contribute to the determination to abandon or persevere with a choice is as yet unclear. Here we examined the activity of neurons in the OFC of rats, while they were performing an auditory choice task with a post-decision time investment option. After making their perceptual decision, animals had to wait for reward delivery delayed by a pseudo-random period. The time animals were willing to invest into waiting for an uncertain reward before abandoning their choice served as a post-decision measure of confidence We utilised high-density silicon probes to record cells throughout the deep layers of the OFC, using a function driven strategy to search for neurons that were specifically active during time investment periods. We coupled these recordings with an optogenetic tagging approach that allowed us to identify neurons that had specific distal projection targets. We recorded populations of OFC neurons with distinctive activity profiles that reflect different aspects of the waiting period commitment. The firing patterns of identified cells with projections to distal sub-cortical regions will be analysed to reveal how OFC neurons contribute to the decision to abandon a choice commitment.

Pubmed:

22928838: Kulikova SP, Tolmacheva EA, Anderson P, Gaudias J, Adams BE, Zheng T, Pinault D
Opposite effects of ketamine and deep brain stimulation on rat thalamocortical information processing.
Sensory and cognitive deficits are common in schizophrenia. They are associated with abnormal brain rhythms, including disturbances in γ frequency (30-80 Hz) oscillations (GFO) in cortex-related networks. However, the underlying anatomofunctional mechanisms remain elusive. Clinical and experimental evidence suggests that these deficits result from a hyporegulation of glutamate N-methyl-D-aspartate receptors. Here we modeled these deficits in rats with ketamine, a non-competitive N-methyl-D-aspartate receptor antagonist and a translational psychotomimetic substance at subanesthetic doses. We tested the hypothesis that ketamine-induced sensory deficits involve an impairment of the ability of the thalamocortical (TC) system to discriminate the relevant information from the baseline activity. Furthermore, we wanted to assess whether ketamine disrupts synaptic plasticity in TC systems. We conducted multisite network recordings in the rat somatosensory TC system, natural stimulation of the vibrissae and high-frequency electrical stimulation (HFS) of the thalamus. A single systemic injection of ketamine increased the amount of baseline GFO, reduced the amplitude of the sensory-evoked TC response and decreased the power of the sensory-evoked GFO. Furthermore, cortical application of ketamine elicited local and distant increases in baseline GFO. The ketamine effects were transient. Unexpectedly, HFS of the TC pathway had opposite actions. In conclusion, ketamine and thalamic HFS have opposite effects on the ability of the somatosensory TC system to discriminate the sensory-evoked response from the baseline GFO during information processing. Investigating the link between the state and function of the TC system may conceptually be a key strategy to design innovative therapies against neuropsychiatric disorders.
Eur J Neurosci, 2012; 36
21733235: Jones NC, Reddy M, Anderson P, Salzberg MR, O'Brien TJ, Pinault D
Acute administration of typical and atypical antipsychotics reduces EEG γ power, but only the preclinical compound LY379268 reduces the ketamine-induced rise in γ power.

A single non-anaesthetic dose of ketamine, a non-competitive NMDA receptor (NMDAR) antagonist with hallucinogenic properties, induces cognitive impairment and psychosis, and aggravates schizophrenia symptoms in patients. In conscious rats an equivalent dose of ketamine induces key features of animal models of acute psychosis, including hyperlocomotor activity, deficits in prepulse inhibition and gating of auditory evoked potentials, and concomitantly increases the power of ongoing spontaneously occurring gamma (30-80 Hz) oscillations in the neocortex. This study investigated whether NMDAR antagonist-induced aberrant gamma oscillations could be modulated by acute treatment with typical and atypical antipsychotic drugs. Extradural electrodes were surgically implanted into the skull of adult male Wistar rats. After recovery, rats were subcutaneously administered either clozapine (1-5 mg/kg, n=7), haloperidol (0.05-0.25 mg/kg; n=8), LY379268 (a preclinical agonist at mGluR2/3 receptors: 0.3-3 mg/kg; n=5) or the appropriate vehicles, and 30 min later received ketamine (5 mg/kg s.c.). Quantitative measures of EEG gamma power and locomotor activity were assessed throughout the experiment. All three drugs significantly reduced the power of baseline EEG gamma oscillations by 30-50%, an effect most prominent after LY379268, and all inhibited ketamine-induced hyperlocomotor activity. However, only pretreatment with LY379268 attenuated trough-to-peak ketamine-induced gamma hyperactivity. These results demonstrate that typical and atypical antipsychotic drugs acutely reduce cortical gamma oscillations, an effect that may be related to their clinical efficacy.
Int J Neuropsychopharmacol, 2012; 15
24832766: Jones NC, Anderson P, Rind G, Sullivan C, van den Buuse M, O'Brien TJ
Effects of aberrant gamma frequency oscillations on prepulse inhibition.
Emerging literature implicates abnormalities in gamma frequency oscillations in the pathophysiology of schizophrenia, with hypofunction of N-methyl-D-aspartate (NMDA) receptors implicated as a key factor. Prepulse inhibition (PPI) is a behavioural measure of sensorimotor gating, which is disrupted in schizophrenia. We studied relationships between ongoing and sensory-evoked gamma oscillations and PPI using pharmacological interventions designed to increase gamma oscillations (ketamine, MK-801); reduce gamma oscillations (LY379268); or disrupt PPI (amphetamine). We predicted that elevating ongoing gamma power would lead to increased 'neural noise' in cortical circuits, dampened sensory-evoked gamma responses and disrupted behaviour. Wistar rats were implanted with EEG recording electrodes. They received ketamine (5 mg/kg), MK-801 (0.16 mg/kg), amphetamine (0.5 mg/kg), LY379268 (3 mg/kg) or vehicle and underwent PPI sessions with concurrent EEG recording. Ketamine and MK-801 increased the power of ongoing gamma oscillations and caused time-matched disruptions of PPI, while amphetamine marginally affected ongoing gamma power. In contrast, LY379268 reduced ongoing gamma power, but had no effect on PPI. The sensory gamma response evoked by the prepulse was reduced following treatment with all psychotomimetics, associating with disruptions in PPI. This was most noticeable following treatment with NMDA receptor antagonists. We found that ketamine and MK-801 increase ongoing gamma power and reduce evoked gamma power, both of which are related to disruptions in sensorimotor gating. This appears to be due to antagonism of NMDA receptors, since amphetamine and LY379268 differentially impacted these outcomes and possess different neuropharmacological substrates. Aberrant gamma frequency oscillations caused by NMDA receptor hypofunction may mediate the sensory processing deficits observed in schizophrenia.
Int J Neuropsychopharmacol, 2014; 17
24964190: Anderson PM, Pinault D, O'Brien TJ, Jones NC
Chronic administration of antipsychotics attenuates ongoing and ketamine-induced increases in cortical γ oscillations.
Noncompetitive N-methyl-d-aspartate receptor (NMDAr) antagonists can elicit many of the symptoms observed in schizophrenia in healthy humans, and induce a behavioural phenotype in animals relevant to psychosis. These compounds also elevate the power and synchrony of gamma (γ) frequency (30-80 Hz) neural oscillations. Acute doses of antipsychotic medications have been shown to reduce ongoing γ power and to inhibit NMDAr antagonist-mediated psychosis-like behaviour in rodents. This study aimed to investigate how a chronic antipsychotic dosing regimen affects ongoing cortical γ oscillations, and the electrophysiological and behavioural responses induced by the NMDAr antagonist ketamine. Male Wistar rats were chronically treated with haloperidol (0.25 mg/kg/d), clozapine (5 mg/kg/d), LY379268 (0.3 mg/kg/d) or vehicle for 28 d, delivered by subcutaneous (s.c.) osmotic pumps. Weekly electrocorticogram (ECoG) recordings were acquired. On day 26, ketamine (5 mg/kg, s.c.) was administered, and ECoG and locomotor activity were simultaneously measured. These results were compared with data generated previously following acute treatment with these antipsychotics. Sustained and significant decreases in ongoing γ power were observed during chronic administration of haloperidol (64%) or clozapine (43%), but not of LY379268 (2% increase), compared with vehicle. Acute ketamine injection concurrently increased γ power and locomotor activity in vehicle-treated rats, and these effects were attenuated in rats chronically treated with all three antipsychotics. The ability of haloperidol or clozapine to inhibit ketamine-induced elevation in γ power was not observed following acute administration of these drugs. These results indicate that modulation of γ power may be a useful biomarker of chronic antipsychotic efficacy.
Int J Neuropsychopharmacol, 2014; 17
25592157: Hill-Yardin EL, Argyropoulos A, Hosie S, Rind G, Anderson P, Hannan AJ, O'Brien TJ

Reduced susceptibility to induced seizures in the Neuroligin-3(R451C) mouse model of autism.
Epilepsy is a common comorbidity in patients with autism spectrum disorder (ASD) and several gene mutations are associated with both of these disorders. In order to determine whether a point mutation in the gene for the synaptic protein, Neuroligin-3 (Nlgn3, R451C), identified in patients with ASD alters seizure susceptibility, we administered the proconvulsant pentylenetetrazole (PTZ) to adult male Neuroligin-3(R451C) (NL3(R451C)) and wild type (WT) mice. It has previously been reported that NL3(R451C) mice show altered inhibitory GABAergic activity in brain regions relevant to epilepsy, including the hippocampus and somatosensory cortex. PTZ administration induces absence-seizures at low dose, and generalised convulsive seizures at higher dose. Susceptibility to absence seizures was examined by analysing the frequency and duration of spike-and-wave discharge (SWD) events and accompanying motor seizure activity induced by subcutaneous administration of low dosage (20 or 30mg/kg) PTZ. Susceptibility to generalised convulsive seizures was tested by measuring the response to high dosage (60mg/kg) PTZ using a modified Racine scale. There was no change in the number of SWD events exhibited by NL3(R451C) compared to WT mice following administration of both 20mg/kg PTZ (1.17±0.31 compared to 16.0±11.16 events/30min, NL3(R451C) versus WT, respectively) and 30mg/kg PTZ (7.5±6.54 compared with 27.8±19.9 events/30min, NL3(R451C) versus WT, respectively). NL3(R451C) mice were seizure resistant to generalised convulsive seizures induced by high dose PTZ compared to WT littermates (median latency to first >3s duration clonic seizure; 14.5min versus 7.25min, 95% CI: 1.625-2.375, p=0.0009, NL3(R451C) versus WT, respectively). These results indicate that the R451C mutation in the Nlgn3 gene, associated with ASD in humans, confers resistance to induced seizures, suggesting dysfunction of PTZ-sensitive GABAergic signalling in this mouse model of ASD.
Neurosci Lett, 2015; 589
25992564: Long LE, Anderson P, Frank E, Shaw A, Liu S, Huang XF, Pinault D, Karl T, O'Brien TJ, Shannon Weickert C, Jones NC
Neuregulin 1 expression and electrophysiological abnormalities in the Neuregulin 1 transmembrane domain heterozygous mutant mouse.
The Neuregulin 1 transmembrane domain heterozygous mutant (Nrg1 TM HET) mouse is used to investigate the role of Nrg1 in brain function and schizophrenia-like behavioural phenotypes. However, the molecular alterations in brain Nrg1 expression that underpin the behavioural observations have been assumed, but not directly determined. Here we comprehensively characterise mRNA Nrg1 transcripts throughout development of the Nrg1 TM HET mouse. In addition, we investigate the regulation of high-frequency (gamma) electrophysiological oscillations in this mutant mouse to associate molecular changes in Nrg1 with a schizophrenia-relevant neurophysiological profile.
PLoS One, 2015; 10
27261525: Anderson PM, Jones NC, O'Brien TJ, Pinault D
The N-Methyl d-Aspartate Glutamate Receptor Antagonist Ketamine Disrupts the Functional State of the Corticothalamic Pathway.
The non-competitive N-methyl d-aspartate glutamate receptor (NMDAR) antagonist ketamine elicits a brain state resembling high-risk states for developing psychosis and early stages of schizophrenia characterized by sensory and cognitive deficits and aberrant ongoing gamma (30-80 Hz) oscillations in cortical and subcortical structures, including the thalamus. The underlying mechanisms are unknown. The goal of the present study was to determine whether a ketamine-induced psychotic-relevant state disturbs the functional state of the corticothalamic (CT) pathway. Multisite field recordings were performed in the somatosensory CT system of the sedated rat. Baseline activity was challenged by activation of vibrissa-related prethalamic inputs. The sensory-evoked thalamic response was characterized by a short-latency (~4 ms) prethalamic-mediated negative sharp potential and a longer latency (~10 ms) CT-mediated negative potential. Following a single subcutaneous injection of ketamine (2.5 mg/kg), spontaneously occurring and sensory-evoked thalamic gamma oscillations increased and decreased in power, respectively. The power of the sensory-related gamma oscillations was positively correlated with both the amplitude and the area under the curve of the corresponding CT potential but not with the prethalamic potential. The present results show that the layer VI CT pathway significantly contributes in thalamic gamma oscillations, and they support the hypothesis that reduced NMDAR activation disturbs the functional state of CT and corticocortical networks.
Cereb Cortex, 2017; 27
30793082: Bielczyk NZ, Uithol S, van Mourik T, Anderson P, Glennon JC, Buitelaar JK
Disentangling causal webs in the brain using functional magnetic resonance imaging: A review of current approaches.
In the past two decades, functional Magnetic Resonance Imaging (fMRI) has been used to relate neuronal network activity to cognitive processing and behavior. Recently this approach has been augmented by algorithms that allow us to infer causal links between component populations of neuronal networks. Multiple inference procedures have been proposed to approach this research question but so far, each method has limitations when it comes to establishing whole-brain connectivity patterns. In this paper, we discuss eight ways to infer causality in fMRI research: Bayesian Nets, Dynamical Causal

Modelling, Granger Causality, Likelihood Ratios, Linear Non-Gaussian Acyclic Models, Patel's Tau, Structural Equation Modelling, and Transfer Entropy. We finish with formulating some recommendations for the future directions in this area. Netw Neurosci, 2019; 3

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IMPLICATION OF MEDIAL PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS DOPAMINE TRANSMISSION IN GOAL-DIRECTED BEHAVIORS: A ROLE FOR DOPAMINE AND NMDA RECEPTORS HETEROMERS ?

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Mesocorticolimbic dopamine transmission is believed to be a key modulator of goal-directed actions and reward processing through its action on dopaminoceptive neurons expressing either D1 (D1R) or D2 receptors (D2R) in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc). Through chemogenetic approaches coupled with operant conditioning tasks, we demonstrated an implication of dopaminergic projections from the Ventral Tegmental Area together with a complex interplay between D1R- and D2R-expressing neurons of the mPFC and the NAc in the flexible expression of food-oriented action as well as in the motivation to obtain a palatable reward. In both structures, the activity of dopaminoceptive neurons is strongly regulated by the convergence of glutamatergic and dopaminergic inputs. Heteromers formed by dopaminergic and glutamatergic N-methyl-d-aspartate receptors (NMDAR) recently emerged as molecular coincidence detectors of these transmissions, notably in the context of psychostimulant-induced adaptations. We therefore asked whether these receptor complexes could play a role in physiological processing. Expression of heteromers was mapped in substructures of the striatum and mPFC with Proximity Ligation Assay. Blockade of D1R-NMDAR or D2R-NMDAR heteromerization in either the mPFC or the NAc through the local viral-mediated expression of interfering peptides induced alterations of discrete components of goal directed behaviors. These findings contribute to decipher the role of dopaminoceptive neurons in executive functions and support a key role of heteromers formed by dopamine and NMDA receptors in such processes.

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VISUOMOTOR LEARNING ROUTES VISUAL SIGNALS TO MEDIAL PREFRONTAL CORTEX

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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[Aims] The medial prefrontal cortex is necessary for executing learned associations between visual stimuli and movement. This function is possibly supported by the presence of stimulus-driven activity specifically after learning, though it is unknown how this stimulus-driven activity develops across learning and what aspects of behavior it is linked to. [Methods] We performed longitudinal widefield calcium imaging of excitatory neurons across days while mice learned a visuomotor detection task. The task consisted of turning a wheel with the forelimbs in response to a single visual stimulus to receive a sucrose reward, which was successfully learned over the course of about one week. We monitored cortical responses to the task stimulus in both behavioral and passive contexts. [Results] We found that stimulus responses in the medial prefrontal cortex developed over the same time course as task learning. This stimulus activity was present during both the task and quiescent passive viewing, indicating that it was not tied to subsequent movement. Furthermore, both behavioral performance and medial prefrontal stimulus responses increased overnight rather than within training sessions, suggesting that training induces delayed plasticity. Neuropixels recordings revealed that the medial prefrontal stimulus response originates from both the secondary motor and anterior cingulate cortex, and the stimulus response was eliminated by visual cortical inactivation. [Conclusions] These results show that visual information is routed to the medial prefrontal cortex during visuomotor learning.

Pubmed:

33473213: Peters AJ, Fabre JMJ, Steinmetz NA, Harris KD, Carandini M
Striatal activity topographically reflects cortical activity.
The cortex projects to the dorsal striatum topographically to regulate behaviour, but spiking activity in the two structures has previously been reported to have markedly different relations to sensorimotor events. Here we show that the relationship between activity in the cortex and striatum is spatiotemporally precise, topographic, causal and invariant to behaviour. We simultaneously recorded activity across large regions of the cortex and across the width of the dorsal striatum in mice that performed a visually guided task. Striatal activity followed a mediolateral gradient in which behavioural correlates progressed from visual cue to response movement to reward licking. The summed activity in each part of the striatum closely and specifically mirrored activity in topographically associated cortical regions, regardless of task engagement. This relationship held for medium spiny neurons and fast-spiking interneurons, whereas the activity of tonically active neurons differed from cortical activity with stereotypical responses to sensory or reward events. Inactivation of the visual cortex abolished striatal responses to visual stimuli, supporting a causal role of cortical inputs in driving the striatum. Striatal visual responses were larger in trained mice than untrained mice, with no corresponding change in overall activity in the visual cortex. Striatal activity therefore reflects a consistent, causal and scalable topographical mapping of cortical activity.
Nature, 2021; 591
33096037: Jacobs EAK, Steinmetz NA, Peters AJ, Carandini M, Harris KD
Cortical State Fluctuations during Sensory Decision Making.
In many behavioral tasks, cortex enters a desynchronized state where low-frequency fluctuations in population activity are suppressed. The precise behavioral correlates of desynchronization and its global organization are unclear. One hypothesis holds that desynchronization enhances stimulus coding in the relevant sensory cortex. Another hypothesis holds that desynchronization reflects global arousal, such as task engagement. Here, we trained mice on tasks where task engagement could be distinguished from sensory accuracy. Using widefield calcium imaging, we found that performance-related desynchronization was global and correlated better with engagement than with accuracy. Consistent with this link between desynchronization and engagement, rewards had a long-lasting desynchronizing effect. To determine whether engagement-related state changes depended on the relevant sensory modality, we trained mice on visual and auditory tasks and found that in both cases desynchronization was global, including regions such as somatomotor cortex. We conclude that variations

in low-frequency fluctuations are predominately global and related to task engagement.
Curr Biol, 2020; 30
28932809: Steinmetz NA, Buetfering C, Lecoq J, Lee CR, Peters AJ, Jacobs EAK, Coen P, Ollerenshaw DR, Valley MT, de Vries SEJ, Garrett M, Zhuang J, Groblewski PA, Manavi S, Miles J, White C, Lee E, Griffin F, Larkin JD, Roll K, Cross S, Nguyen TV, Larsen R, Pendergraft J, Daigle T, Tasic B, Thompson CL, Waters J, Olsen S, Margolis DJ, Zeng H, Hausser M, Carandini M, Harris KD
Aberrant Cortical Activity in Multiple GCaMP6-Expressing Transgenic Mouse Lines.
Transgenic mouse lines are invaluable tools for neuroscience but, as with any technique, care must be taken to ensure that the tool itself does not unduly affect the system under study. Here we report aberrant electrical activity, similar to interictal spikes, and accompanying fluorescence events in some genotypes of transgenic mice expressing GCaMP6 genetically encoded calcium sensors. These epileptiform events have been observed particularly, but not exclusively, in mice with Emx1-Cre and Ai93 transgenes, of either sex, across multiple laboratories. The events occur at >0.1 Hz, are very large in amplitude (>1.0 mV local field potentials, >10% df/f widefield imaging signals), and typically cover large regions of cortex. Many properties of neuronal responses and behavior seem normal despite these events, although rare subjects exhibit overt generalized seizures. The underlying mechanisms of this phenomenon remain unclear, but we speculate about possible causes on the basis of diverse observations. We encourage researchers to be aware of these activity patterns while interpreting neuronal recordings from affected mouse lines and when considering which lines to study.
eNeuro, 2017 Sep-Oct; 4
28375768: Peters AJ, Liu H, Komiyama T
Learning in the Rodent Motor Cortex.
The motor cortex is far from a stable conduit for motor commands and instead undergoes significant changes during learning. An understanding of motor cortex plasticity has been advanced greatly using rodents as experimental animals. Two major focuses of this research have been on the connectivity and activity of the motor cortex. The motor cortex exhibits structural changes in response to learning, and substantial evidence has implicated the local formation and maintenance of new synapses as crucial substrates of motor learning. This synaptic reorganization translates into changes in spiking activity, which appear to result in a modification and refinement of the relationship between motor cortical activity and movement. This review presents the progress that has been made using rodents to establish the motor cortex as an adaptive structure that supports motor learning.
Annu Rev Neurosci, 2017; 40
28671694: Peters AJ, Lee J, Hedrick NG, O'Neil K, Komiyama T
Reorganization of corticospinal output during motor learning.
Motor learning is accompanied by widespread changes within the motor cortex, but it is unknown whether these changes are ultimately funneled through a stable corticospinal output channel or whether the corticospinal output itself is plastic. We investigated the consistency of the relationship between corticospinal neuron activity and movement through in vivo two-photon calcium imaging in mice learning a lever-press task. Corticospinal neurons exhibited heterogeneous correlations with movement, with the majority of movement-modulated neurons decreasing activity during movement. Individual cells changed their activity across days, which led to changed associations between corticospinal activity and movement. Unlike previous observations in layer 2/3, activity accompanying learned movements did not become more consistent with learning; instead, the activity of dissimilar movements became more decorrelated. These results indicate that the relationship between corticospinal activity and movement is dynamic and that the types of activity and plasticity are different from and possibly complementary to those in layer 2/3.
Nat Neurosci, 2017; 20
24239124: Kato HK, Gillet SN, Peters AJ, Isaacson JS, Komiyama T
Parvalbumin-expressing interneurons linearly control olfactory bulb output.
In the olfactory bulb, odor representations by principal mitral cells are modulated by local inhibitory circuits. While dendrodendritic synapses between mitral and granule cells are typically thought to be a major source of this modulation, the contributions of other inhibitory neurons remain unclear. Here we demonstrate the functional properties of olfactory bulb parvalbumin-expressing interneurons (PV cells) and identify their important role in odor coding. Using paired recordings, we find that PV cells form reciprocal connections with the majority of nearby mitral cells, in contrast to the sparse connectivity between mitral and granule cells. In vivo calcium imaging in awake mice reveals that PV cells are broadly tuned to odors. Furthermore, selective PV cell inactivation enhances mitral cell responses in a linear fashion while maintaining mitral cell odor preferences. Thus, dense connections between mitral and PV cells underlie an inhibitory circuit poised to modulate the gain of olfactory bulb output.
Neuron, 2013; 80
24805237: Peters AJ, Chen SX, Komiyama T

Emergence of reproducible spatiotemporal activity during motor learning.
The motor cortex is capable of reliably driving complex movements yet exhibits considerable plasticity during motor learning. These observations suggest that the fundamental relationship between motor cortex activity and movement may not be fixed but is instead shaped by learning; however, to what extent and how motor learning shapes this relationship are not fully understood. Here we addressed this issue by using in vivo two-photon calcium imaging to monitor the activity of the same population of hundreds of layer 2/3 neurons while mice learned a forelimb lever-press task over two weeks. Excitatory and inhibitory neurons were identified by transgenic labelling. Inhibitory neuron activity was relatively stable and balanced local excitatory neuron activity on a movement-by-movement basis, whereas excitatory neuron activity showed higher dynamism during the initial phase of learning. The dynamics of excitatory neurons during the initial phase involved the expansion of the movement-related population which explored various activity patterns even during similar movements. This was followed by a refinement into a smaller population exhibiting reproducible spatiotemporal sequences of activity. This pattern of activity associated with the learned movement was unique to expert animals and not observed during similar movements made during the naive phase, and the relationship between neuronal activity and individual movements became more consistent with learning. These changes in population activity coincided with a transient increase in dendritic spine turnover in these neurons. Our results indicate that a novel and reproducible activity-movement relationship develops as a result of motor learning, and we speculate that synaptic plasticity within the motor cortex underlies the emergence of reproducible spatiotemporal activity patterns for learned movements. These results underscore the profound influence of learning on the way that the cortex produces movements.
Nature, 2014; 510
26098758: Chen SX, Kim AN, Peters AJ, Komiyama T
Subtype-specific plasticity of inhibitory circuits in motor cortex during motor learning.
Motor skill learning induces long-lasting reorganization of dendritic spines, principal sites of excitatory synapses, in the motor cortex. However, mechanisms that regulate these excitatory synaptic changes remain poorly understood. Here, using in vivo two-photon imaging in awake mice, we found that learning-induced spine reorganization of layer (L) 2/3 excitatory neurons occurs in the distal branches of their apical dendrites in L1 but not in the perisomatic dendrites. This compartment-specific spine reorganization coincided with subtype-specific plasticity of local inhibitory circuits. Somatostatin-expressing inhibitory neurons (SOM-INs), which mainly inhibit distal dendrites of excitatory neurons, showed a decrease in axonal boutons immediately after the training began, whereas parvalbumin-expressing inhibitory neurons (PV-INs), which mainly inhibit perisomatic regions of excitatory neurons, exhibited a gradual increase in axonal boutons during training. Optogenetic enhancement and suppression of SOM-IN activity during training destabilized and hyperstabilized spines, respectively, and both manipulations impaired the learning of stereotyped movements. Our results identify SOM inhibition of distal dendrites as a key regulator of learning-related changes in excitatory synapses and the acquisition of motor skills.
Nat Neurosci, 2015; 18

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PROBING PREFRONTAL CORTICAL SIGNALS DURING RISKY ECONOMIC DECISIONS IN MICE

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Economic decision making under risk – the process of selecting between options with different values and uncertain outcomes – concerns many aspects of our lives. Past studies have identified the prefrontal cortex (PFC) as one of the crucial brain regions for such decisions. These studies, however, could either record signals across the brain with coarse resolution (e.g. using fMRI) or acquire high-resolution readouts (e.g. using electrophysiology) but often only in select brain regions. Therefore, it is not known how the activity of neurons across different regions of PFC underlie risky economic decisions. To address this, we developed a novel two-alternative economic decision task in head-fixed mice, and employed high-density large-scale electrophysiological recordings to measure the activity of thousands of neurons across PFC during the task. In each trial, mice chose between visual stimuli associated with different size and probabilities of reward, akin to previous tasks in primates. We show that mice successfully perform the task, selecting stimuli with higher expected value. Moreover, we demonstrate that each mouse exhibits a consistent risk attitude; some showing strong risk-seeking while others exhibit risk-neutral behavior. Preliminary analyses of neural responses show rich neural representations across PFC during the task. In our ongoing analysis, we use regression models to examine the relationship between neuronal responses and key variables inferred from the behavioral data. Our work provides a platform for investigating neural bases of economic decision-making at a large scale and high spatial and temporal resolution.

Pubmed:
31649556: Rathje CC, Randle SJ, Al Rawi S, Skinner BM, Nelson DE, Majumdar A, Johnson EEP, Bacon J, Vlazaki M, Affara NA, Ellis PJ, Laman H
A Conserved Requirement for During Male Germ Cell Cytoplasmic Remodeling.
Fbxo7 is the substrate-recognition subunit of an SCF-type ubiquitin E3 ligase complex. It has physiologically important functions in regulating mitophagy, proteasome activity and the cell cycle in multiple cell types, like neurons, lymphocytes and erythrocytes. Here, we show that in addition to the previously known Parkinsonian and hematopoietic phenotypes, male mice with reduced Fbxo7 expression are sterile. In these males, despite successful meiosis, nuclear elongation and eviction of histones from chromatin, the developing spermatids are phagocytosed by Sertoli cells during late spermiogenesis, as the spermatids undergo cytoplasmic remodeling. Surprisingly, despite the loss of all germ cells, there was no evidence of the symplast formation and cell sloughing that is typically associated with spermatid death in other mouse sterility models, suggesting that novel cell death and/or cell disposal mechanisms may be engaged in Fbxo7 mutant males. Mutation of the Fbxo7 ortholog, () also leads to sterility with germ cell death during cytoplasmic remodeling, indicating that the requirement for Fbxo7 at this stage is conserved. The phenotype was attributed to decreased levels of the proteasome regulator, DmPI31 and reduced proteasome activity. Consistent with the fly model, we observe a reduction in PI31 levels in mutant mice; however, there is no alteration in proteasome activity in whole mouse testes. Our results are consistent with findings that Fbxo7 regulates PI31 protein levels, and indicates that a defect at the late stages of spermiogenesis, possibly due to faulty spatial dynamics of proteasomes during cytoplasmic remodeling, may underlie the fertility phenotype in mice.
Front Physiol, 2019; 10
31071644: Hayes SH, Manohar S, Majumdar A, Allman BL, Salvi R
Noise-induced hearing loss alters hippocampal glucocorticoid receptor expression in rats.
Although the effects of intense noise exposure on the peripheral and central auditory pathway have been well characterized, its effects on non-classical auditory structures in the brain, such as the hippocampus, are less well understood. Previously, we demonstrated that noise-induced hearing loss causes a significant long-term reduction in hippocampal neurogenesis and cell proliferation. Given the known suppressive effects of stress hormones on neurogenesis, the goal of the present study was to determine if activation of the stress response is an underlying mechanism for the long-term reduction in hippocampal neurogenesis observed following noise trauma. To accomplish this, we monitored basal and reactive blood plasma levels of

the stress hormone corticosterone in rats for ten weeks following acoustic trauma, and quantified changes in hippocampal glucocorticoid and mineralocorticoid receptors. Our results indicate that long-term auditory deprivation does not cause a persistent increase in basal or reactive stress hormone levels in the weeks following noise exposure. Instead, we observed a greater decline in reactive corticosterone release in noise-exposed rats between the first and tenth week of sampling compared to control rats. We also observed a significant increase in hippocampal glucocorticoid receptor expression which may cause greater hippocampal sensitivity to circulating glucocorticoid levels and result in glucocorticoid-induced suppression of neurogenesis, as well as increased feedback inhibition on the HPA axis. No change in mineralocorticoid receptor expression was observed between control and noise exposed rats. These results highlight the adverse effect of intense noise exposure and auditory deprivation on the hippocampus.
Hear Res, 2019; 379

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BOARD NUMBER: S02-131

DOPAMINERGIC COMPUTATIONS UNDERLYING LEARNING OF A PERCEPTUAL DECISION TASK FROM NAIVE TO EXPERT

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Dopamine signals across the striatum reflect a range of computations necessary for perceptual decision making. How these computations emerge when first learning to make perceptual decisions remains unclear. When training animals, experimenters typically start with an easy task and increase its difficulty as animals learn, making it hard to study longitudinal learning and its associated dopaminergic signatures. Here, we trained mice on a two-alternative visual decision making task that was difficult from the start and did not change across several weeks of experimentation. Throughout learning, we imaged dopamine release in three regions of dorsal striatum: anterior dorsomedial (aDMS), posterior dorsomedial (pDMS), and dorsolateral (DLS) striatum. In all these regions stimulus-locked dopamine release increased gradually over learning. The dopamine responses in both aDMS and pDMS developed over learning to scale by contralateral sensory evidence. However, aDMS and pDMS differed in that only pDMS dopamine release encoded contralateral sensory evidence in naive animals. DLS dopamine developed over learning to predominantly reflect expectation of reward irrespective of stimulus laterality. To formalize these findings, we are developing a computational framework that provides trial-by-trial trajectories of latent variables underlying the learning process (see Liebana Garcia et al. poster). By showing distinct signatures of learning in dopamine release across dorsal striatum, our findings reveal previously unknown diversity in dopaminergic computations during longitudinal learning under perceptual uncertainty.

Pubmed:
32979522: Laffere A, Dick F, Holt LL, Tierney A
Attentional modulation of neural entrainment to sound streams in children with and without ADHD.
To extract meaningful information from complex auditory scenes like a noisy playground, rock concert, or classroom, children can direct attention to different sound streams. One means of accomplishing this might be to align neural activity with the temporal structure of a target stream, such as a specific talker or melody. However, this may be more difficult for children with ADHD, who can struggle with accurately perceiving and producing temporal intervals. In this EEG study, we found that school-aged children's attention to one of two temporally-interleaved isochronous tone 'melodies' was linked to an increase in phase-locking at the melody's rate, and a shift in neural phase that aligned the neural responses with the attended tone stream. Children's attention task performance and neural phase alignment with the attended melody were linked to performance on temporal production tasks, suggesting that children with more robust control over motor timing were better able to direct attention to the time points associated with the target melody. Finally, we found that although children with ADHD performed less accurately on the tonal attention task than typically developing children, they showed the same degree of attentional modulation of phase locking and neural phase shifts, suggesting that children with ADHD may have difficulty with attentional engagement rather than attentional selection.
Neuroimage, 2021; 224
32463356: Galloni AR, Laffere A, Rancz E
Apical length governs computational diversity of layer 5 pyramidal neurons.
Anatomical similarity across the neocortex has led to the common assumption that the circuitry is modular and performs stereotyped computations. Layer 5 pyramidal neurons (L5PNs) in particular are thought to be central to cortical computation because of their extensive arborisation and nonlinear dendritic operations. Here, we demonstrate that computations associated with dendritic Ca plateaus in mouse L5PNs vary substantially between the primary and secondary visual cortices. L5PNs in the secondary visual cortex show reduced dendritic excitability and smaller propensity for burst firing. This reduced excitability is correlated with shorter apical dendrites. Using numerical modelling, we uncover a universal principle underlying

the influence of apical length on dendritic backpropagation and excitability, based on a Na channel-dependent broadening of backpropagating action potentials. In summary, we provide new insights into the modulation of dendritic excitability by apical dendrite length and show that the operational repertoire of L5PNs is not universal throughout the brain.
Elife, 2020; 9
32165265: Laffere A, Dick F, Tierney A
Effects of auditory selective attention on neural phase: individual differences and short-term training.
How does the brain follow a sound that is mixed with others in a noisy environment? One possible strategy is to allocate attention to task-relevant time intervals. Prior work has linked auditory selective attention to alignment of neural modulations with stimulus temporal structure. However, since this prior research used relatively easy tasks and focused on analysis of main effects of attention across participants, relatively little is known about the neural foundations of individual differences in auditory selective attention. Here we investigated individual differences in auditory selective attention by asking participants to perform a 1-back task on a target auditory stream while ignoring a distractor auditory stream presented 180° out of phase. Neural entrainment to the attended auditory stream was strongly linked to individual differences in task performance. Some variability in performance was accounted for by degree of musical training, suggesting a link between long-term auditory experience and auditory selective attention. To investigate whether short-term improvements in auditory selective attention are possible, we gave participants 2 h of auditory selective attention training and found improvements in both task performance and enhancements of the effects of attention on neural phase angle. Our results suggest that although there exist large individual differences in auditory selective attention and attentional modulation of neural phase angle, this skill improves after a small amount of targeted training.
Neuroimage, 2020; 213
30131109: Holt LL, Tierney AT, Guerra G, Laffere A, Dick F
Dimension-selective attention as a possible driver of dynamic, context-dependent re-weighting in speech processing.
The contribution of acoustic dimensions to an auditory percept is dynamically adjusted and reweighted based on prior experience about how informative these dimensions are across the long-term and short-term environment. This is especially evident in speech perception, where listeners differentially weight information across multiple acoustic dimensions, and use this information selectively to update expectations about future sounds. The dynamic and selective adjustment of how acoustic input dimensions contribute to perception has made it tempting to conceive of this as a form of non-spatial auditory selective attention. Here, we review several human speech perception phenomena that might be consistent with auditory selective attention although, as of yet, the literature does not definitively support a mechanistic tie. We relate these human perceptual phenomena to illustrative nonhuman animal neurobiological findings that offer informative guideposts in how to test mechanistic connections. We next present a novel empirical approach that can serve as a methodological bridge from human research to animal neurobiological studies. Finally, we describe four preliminary results that demonstrate its utility in advancing understanding of human non-spatial dimension-based auditory selective attention.
Hear Res, 2018; 366

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NEURAL BASES OF DECISION MAKING: REINFORCEMENT, VARIABILITY AND EXPLORATION IN CHOICE BEHAVIOR

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aims: Behaving in an unusual, variable, or unpredictable manner is a fundamental ability, observed even when animals repeatedly face the same situation, for instance in decision-making settings (exploration). Yet, the neural bases underlying such behavior are still poorly understood. The dopaminergic (DA) system encodes rewards value, reinforces the best options and makes us choose them more frequently (exploitation). This work aims to assess DA dynamics in exploratory goal-directed decisions. **Methods:** We designed a conditioning task where mice learn to perform sequences of binary choices to obtain rewards. Animals are rewarded for non-repetitive choice sequences (when they choose the target leading to higher sequence complexity). Neural correlates of choice strategy are assessed using tetrodes recordings in the Ventral Tegmental Area (VTA) and fiber photometry (DA sensor) in the Nucleus Accumbens (NAc) during the task. **Results:** We show that mice progressively increase their choice variability and use a random-like choice strategy (Belkaïd et al, 2020), which seems to rely on DA dynamics. Indeed, when rewards are certain (all choices are rewarded), VTA DA neurons activity encode next choice's value, but this encoding is impaired in our complexity rule. Moreover, electrophysiological recordings suggest an adaptive modulation of DA neurons activity during the task, while DA release in the NAc displays continuous computations of Reward Prediction Errors (RPE) to adapt choice strategy online. **Conclusions:** We show evidence of mice ability to generate and reinforce a random-like choice strategy. Preliminary results suggest the involvement of meso-limbic DA signaling in this exploratory strategy.

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CONTEXTUAL MODULATION OF MESOSCALE FUNCTIONAL CONNECTIVITY

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Cognitive flexibility involves rapidly modifying input-output mappings of brain-wide networks to achieve context-appropriate behaviour. While context-dependent changes in neural responses have been studied locally in various brain regions, it is not clear to what extent different contexts lead to changes in functional connectivity between brain regions. We sought to examine how functional connectivity between dorsal cortical regions was modulated while mice performed a context dependent decision making task. We imaged widefield calcium activity from the entire dorsal cortex of mice as they switched between two distinct contexts in an attention-switching task. Different task contexts were distinctly represented across multiple cortical areas with both increases and decreases in average activity levels, which could not be accounted for by overt movements. We obtained measures of cortex-wide functional connectivity from the correlations in neural activity across brain regions. Different task contexts were associated with widespread, systematic changes in functional connectivity patterns. While some regions showed uniform, cortex-wide changes in functional connectivity with changing context, the majority of regions showed a heterogenous distribution of increased and decreased functional connectivity distributed across different brain regions. During visual attention, visual and retrosplenial cortex showed the greatest increase in functional coupling to most other regions of the cortex. Finally, to validate correlation-based functional connectivity measures, we developed a causal approach using widefield calcium imaging during patterned optogenetic stimulation. Our results demonstrate that changes in mesoscale functional connectivity provide a substrate for flexibly rerouting information across brain-wide networks with changing contextual demands.

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REINFORCEMENT LEARNING OF ABSTRACT RULES INVOLVES THE PREFRONTAL CORTEX AND THE STRIATUM

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Reinforcement learning offers a conceptual framework of how agents can learn different tasks by promoting the actions that maximize expected reward. Learning, however, is often based on more abstract rules based on action or stimulus categories. Rules are thought to be represented in prefrontal circuits but the neural bases of rule learning are not well understood. Here, we developed a two-alternative auditory discrimination task in which rats had to categorize stimuli with various levels of evidence. The stimulus sequence included trial blocks generated with two different statistical rules: a tendency to repeat the previous stimulus category or a preference to alternate it. We fitted three reinforcement learning (RL) models based on (1) action values (Left versus Right), (2) sequence values (Left-Left vs Left-Right and Right-Right vs Right-Left) and (3) rule values (Repetition versus Alternation). Only the rule RL described behavior accurately. To assess the role of different brain areas in rule encoding, we photo-inhibited neural activity during the inter-trial-interval and found that inactivation of mPFC, the dorso medial striatum (DMS) and the auditory striatum tail decreased the impact of the rule value on the immediately subsequent choice while sparing the stimulus impact. Moreover, inactivation impaired the rule updating in illuminated trials affecting choices in several upcoming trials. Finally, DMS neural recordings showed that a significant fraction of neurons encoded the rule value provided by the RL model. Our results suggest that, similarly to model-free RL, rule reinforcement may involve reward-based strengthening of connections between the prefrontal cortex and the striatum.

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ROLES OF PRELIMBIC AND INFRALIMBIC PREFRONTAL CORTICES IN AN APPETITIVE INHIBITORY DISCRIMINATION LEARNING TASK

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aims Inhibitory modulation of associative learning is crucial to healthy cognition. Impairment of inhibition can cause unchecked trains of associations to disturb thought processes, producing incoherence and impulsivity, as seen in various psychological disorders including schizophrenia and addiction. Neuromodulation in prelimbic (PL) versus infralimbic (IL) subregions of the medial prefrontal cortex has different effects in various cognitive tasks. We aim to investigate whether these subregions play dissociable roles in inhibitory learning. **Methods** We examined how bilateral PL or IL inactivation, using muscimol (GABA-A receptor agonist) micro-infusions compared to saline-controls, affected inhibitory learning in Wistar rats, using a within-subjects appetitive Pavlovian task (A+/AX-; Waite et al.,2021*,PhysiolBehav*). The task involved learning excitatory associations (rewarded presentations of stimulus A; micro-infusion-free), preceding inhibitory training (following micro-infusions) which introduced a non-rewarded compound of A with novel stimulus X (AX-). Learning required establishing X as a non-rewarded inhibitor of excitatory responding to A. Performance, measured by nose-pokes during stimulus presentation versus baseline responding, was analysed via mixed-factorial 2-Way-ANOVA (within-subjects factors: stimuli and task (1 session/day); between-subjects factor: micro-infusion group). **Results** Following excitatory training, the inhibitory discrimination was learned overall, with decreased responding to AX- vs A+. However, preliminary results (pre-histology) indicate that the IL group showed weaker discrimination than the PL and control groups. **Conclusions** PL and IL may contribute differently to inhibitory learning, as IL inactivation caused more impairment. Differences may reflect functionally dissociable components in cortico-striatal neurocircuitry, and the influence of dopaminergic modulation. Ongoing work is investigating effects of prefrontal D1-receptors on inhibitory learning.

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THE ROLE OF STRIATAL PARVALBUMIN INTERNEURONS IN DECISION-MAKING

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Parvalbumin interneurons (PVI) represent only 1 to 2% of the neuronal population of the striatum, a cerebral structure involved in the execution of cognitive and motor processes. Despite their low numbers, PVI receive many afferences ranging from associative to motor areas of the cortex allowing for a fast and powerful inhibition of the striatal networks. Recent studies suggested the implication of striatal PVI in cognitive processes and decision-making in particular (Gage et al., 2010; Martiros et al., 2018). In this project, we are interested in the role of striatal PVI in information processing during decision-making. Over this process, one need to accumulate evidence before taking action. We hypothesize that striatal PVI could have a crucial role in inhibiting striatal processing, thus delaying choice execution while accumulating more information to optimize choice, especially during difficult decision. To address this question, we developed a novel decision-making task implemented in innovative custom-built automatized operant chambers, which allow for collecting thousands of trials over several weeks. We could contrast the animals performance depending on the levels of choice-difficulty while recording striatal PVI activity using fiber-photometry. Our behavioural results showed the more difficult the choice, the longer the decision duration, validating the task. Preliminary neurophysiological recordings showed an increase of PVI activity when the mouse was exposed to the stimulus; suggesting the implication of PVI in the decision-making process.

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UNCERTAINTY-RELATED INTER-SUBJECT VARIABILITY IN HUMAN CHOICES DURING SEQUENTIAL DECISION-MAKING

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Aim. Humans often need to make choices in changing and uncertain environments. Previous studies found considerable between-subject variability in information-seeking and uncertainty-attitude, but its origin is unclear since typical experimental designs confound learning and decision-making. Here, we present a novel bandit task that dissociates reward and different forms of uncertainty during learning and decision making. **Methods.** Our task addresses two key challenges. First, reward and uncertainty are often inversely related because more rewarding options are sampled more frequently. We introduce forced choices to better decorrelate them. Second, we distinguish different forms of uncertainty, expected and unexpected, and dissociate them through intermittent changes in reward levels and noise. To explore the neural substrate of choice variability, we test subjects across two sessions (behavioral and fMRI) with two repeated sets of reward sequences. We use a Bayesian model for the learning process. **Results.** Our results show that expected reward is a major and consistent driver of choices across subjects. In contrast, the effect of uncertainty is smaller and variable across subjects in both direction (uncertainty-seeking or avoidance) and magnitude, but stable across sessions and reward sequences. In preliminary fMRI results, we localize effects of reward and uncertainty to previously reported neural substrates in subcortical regions and frontoparietal cortical networks. **Conclusion.** Our results contribute to a computational understanding of the roles of uncertainty in learning and choice. Our next steps are to map the behavioral variability in uncertainty-related choices to neuromodulatory and cortical brain correlates and to stable individual differences in psychological profiles.

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SLEEP DEPRIVATION INCREASES PERFORMANCE IN LARVAL ZEBRAFISH DECISION MAKING

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Humans and most animals need sleep to function well. Lack of sleep has a multitude of effects on the body including a decrease of cognitive and physical performance. Zebrafish are a popular model to study sleep. Here we set out to investigate the effects of sleep deprivation on simple behavior and cognition in larval zebrafish. We show that in zebrafish larvae sleep deprivation downregulates bouting the day after sleep deprivation but does not compromise visual-motor integration. In contrast to expectation, sleep-deprived larvae that are faced with a random-dot motion discrimination task perform better than a well-rested control. Previous work has demonstrated that zebrafish larvae accumulate motion evidence over time and their resulting decision can be explained with a bounded leaky integrator model. We hypothesize that the reduced bouting gives them more time to integrate the random-dot-motion and hence leads to an increased performance. The change in bouting frequency, however, leads to a decrease in exploratory behavior. Finally, we artificially reduce bouting in zebrafish larvae using the drug melatonin and show that the decrease bouting in fact leads to an increased performance.

Pubmed:
30417089: Michels B, Zwaka H, Bartels R, Lushchak O, Franke K, Endres T, Fendt M, Song I, Bakr M, Budragchaa T, Westermann B, Mishra D, Eschbach C, Schreyer S, Lingnau A, Vahl C, Hilker M, Menzel R, Kähne T, Leßmann V, Dityatev A, Wessjohann L, Gerber B
Memory enhancement by ferulic acid ester across species.
Cognitive impairments can be devastating for quality of life, and thus, preventing or counteracting them is of great value. To this end, the present study exploits the potential of the plant and identifies the constituent ferulic acid eicosyl ester [icosyl-(2)-3-(4-hydroxy-3-methoxyphenyl)-prop-2-enoate (FAE-20)] as a memory enhancer. We show that food supplementation with dried root material from dose-dependently improves odor-taste reward associative memory scores in larval and prevents the age-related decline of this appetitive memory in adult flies. Task-relevant sensorimotor faculties remain unaltered. From a parallel approach, a list of candidate compounds has been derived, including -derived FAE-20. Here, we show that both -derived FAE-20 and synthetic FAE-20 are effective as memory enhancers in larval . Synthetic FAE-20 also partially compensates for age-related memory decline in adult flies, as well as genetically induced early-onset loss of memory function in young flies. Furthermore, it increases excitability in mouse hippocampal CA1 neurons, leads to more stable context-shock aversive associative memory in young adult (3-month-old) mice, and increases memory scores in old (>2-year-old) mice. Given these effects, and given the utility of -the plant from which we discovered FAE-20-as a memory enhancer, these results may hold potential for clinical applications.
Sci Adv, 2018; 4
30740045: Zwaka H, Bartels R, Lehfeldt S, Jusyte M, Hantke S, Menzel S, Gora J, Alberdi R, Menzel R
Learning and Its Neural Correlates in a Virtual Environment for Honeybees.
The search for neural correlates of operant and observational learning requires a combination of two (experimental) conditions that are very difficult to combine: stable recording from high order neurons and free movement of the animal in a rather natural environment. We developed a virtual environment (VE) that simulates a simplified 3D world for honeybees walking stationary on an air-supported spherical treadmill. We show that honeybees perceive the stimuli in the VE as meaningful by transferring learned information from free flight to the virtual world. In search for neural correlates of learning in the VE, mushroom body extrinsic neurons were recorded over days during learning. We found changes in the neural activity specific to the rewarded and unrewarded visual stimuli. Our results suggest an involvement of the mushroom body extrinsic neurons in operant learning in the honeybee ().
Front Behav Neurosci, 2018; 12
27746723: Zwaka H, Münch D, Manz G, Menzel R, Rybak J

The Circuitry of Olfactory Projection Neurons in the Brain of the Honeybee, .
In the honeybee brain, two prominent tracts - the medial and the lateral antennal lobe tract - project from the primary olfactory center, the antennal lobes (ALs), to the central brain, the mushroom bodies (MBs), and the protocerebral lobe (PL). Intracellularly stained uniglomerular projection neurons were reconstructed, registered to the 3D honeybee standard brain atlas, and then used to derive the spatial properties and quantitative morphology of the neurons of both tracts. We evaluated putative synaptic contacts of projection neurons (PNs) using confocal microscopy. Analysis of the patterns of axon terminals revealed a domain-like innervation within the MB lip neuropil. PNs of the lateral tract arborized more sparsely within the lips and exhibited fewer synaptic boutons, while medial tract neurons occupied broader regions in the MB calyces and the PL. Our data show that uPNs from the medial and lateral tract innervate both the core and the cortex of the ipsilateral MB lip but differ in their innervation patterns in these regions. In the mushroombody neuropil collar we found evidence for ALT boutons suggesting the collar as a multi modal input site including olfactory input similar to lip and basal ring. In addition, our data support the conclusion drawn in previous studies that reciprocal synapses exist between PNs, octopaminergic-, and GABAergic cells in the MB calyces. For the first time, we found evidence for connections between both tracts within the AL.
Front Neuroanat, 2016; 10
30127725: Zwaka H, Bartels R, Grünewald B, Menzel R
Neural Organization of A3 Mushroom Body Extrinsic Neurons in the Honeybee Brain.
In the insect brain, the mushroom body is a higher order brain area that is key to memory formation and sensory processing. Mushroom body (MB) extrinsic neurons leaving the output region of the MB, the lobes and the peduncle, are thought to be especially important in these processes. In the honeybee brain, a distinct class of MB extrinsic neurons, A3 neurons, are implicated in playing a role in learning. Their MB arborisations are either restricted to the lobes and the peduncle, here called A3 lobe connecting neurons, or they provide feedback information from the lobes to the input region of the MB, the calyces, here called A3 feedback neurons. In this study, we analyzed the morphology of individual A3 lobe connecting and feedback neurons using confocal imaging. A3 feedback neurons were previously assumed to innervate each lip compartment homogenously. We demonstrate here that A3 feedback neurons do not innervate whole subcompartments, but rather innervate zones of varying sizes in the MB lip, collar, and basal ring. We describe for the first time the anatomical details of A3 lobe connecting neurons and show that their connection pattern in the lobes resemble those of A3 feedback cells. Previous studies showed that A3 feedback neurons mostly connect zones of the vertical lobe that receive input from Kenyon cells of distinct calycal subcompartments with the corresponding subcompartments of the calyces. We can show that this also applies to the neck of the peduncle and the medial lobe, where both types of A3 neurons arborize only in corresponding zones in the calycal subcompartments. Some A3 lobe connecting neurons however connect multiple vertical lobe areas. Contrarily, in the medial lobe, the A3 neurons only innervate one division. We found evidence for both input and output areas in the vertical lobe. Thus, A3 neurons are more diverse than previously thought. The understanding of their detailed anatomy might enable us to derive circuit models for learning and memory and test physiological data.
Front Neuroanat, 2018; 12
26592345: Zwaka H, Bartels R, Gora J, Franck V, Culo A, Götsch M, Menzel R
Context odor presentation during sleep enhances memory in honeybees.
Sleep plays an important role in stabilizing new memory traces after learning [1-3]. Here we investigate whether sleep's role in memory processing is similar in evolutionarily distant species and demonstrate that a context trigger during deep-sleep phases improves memory in invertebrates, as it does in humans. We show that in honeybees (Apis mellifera), exposure to an odor during deep sleep that has been present during learning improves memory performance the following day. Presentation of the context odor during wake phases or novel odors during sleep does not enhance memory. In humans, memory consolidation can be triggered by presentation of a context odor during slow-wave sleep that had been present during learning [3-5]. Our results reveal that deep-sleep phases in honeybees have the potential to prompt memory consolidation, just as they do in humans. This study provides strong evidence for a conserved role of sleep-and how it affects memory processes-from insects to mammals.
Curr Biol, 2015; 25
29938214: Haenicke J, Yamagata N, Zwaka H, Nawrot M, Menzel R
Neural Correlates of Odor Learning in the Presynaptic Microglomerular Circuitry in the Honeybee Mushroom Body Calyx.
The mushroom body (MB) in insects is known as a major center for associative learning and memory, although exact locations for the correlating memory traces remain to be elucidated. Here, we asked whether presynaptic boutons of olfactory projection neurons (PNs) in the main input site of the MB undergo neuronal plasticity during classical odor-reward conditioning and correlate with the conditioned behavior. We simultaneously measured Ca responses in the boutons and conditioned behavioral responses to learned odors in honeybees. We found that the absolute amount of the neural change for the rewarded but not for the unrewarded odor was correlated with the behavioral learning rate across individuals. The temporal profile of the induced changes matched with odor response dynamics of the MB-associated inhibitory neurons,

suggestive of activity modulation of boutons by this neural class. We hypothesize the circuit-specific neural plasticity relates to the learned value of the stimulus and underlies the conditioned behavior of the bees.
eNeuro, 2018 May-Jun; 5
25667342: Schallschmidt K, Becker R, Zwaka H, Menzel R, Johnen D, Fischer-Tenhagen C, Rolff J, Nehls I
In vitro cultured lung cancer cells are not suitable for animal-based breath biomarker detection.
In vitro cultured lung cancer cell lines were investigated regarding the possible identification of volatile organic compounds as potential biomarkers. Gas samples from the headspace of pure culture medium and from the cultures of human lung adenocarcinoma cell lines A549 and Lu7466 were exposed to polypropylene fleece in order to absorb odour components. Sniffer dogs were trained with loaded fleeces of both cell lines, and honey bees were trained with fleeces exposed to A549. Afterwards, their ability to distinguish between cell-free culture medium odour and lung cancer cell odour was tested. Neither bees nor dogs were able to discriminate between odours from the cancer cell cultures and the pure culture medium. Solid phase micro extraction followed by gas chromatography with mass selective detection produced profiles of volatiles from the headspace offered to the animals. The profiles from the cell lines were largely similar; distinct differences were based on the decrease of volatile culture medium components due to the cells' metabolic activity. In summary, cultured lung cancer cell lines do not produce any biomarkers recognizable by animals or gas chromatographic analysis.
J Breath Res, 2015; 9

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BOARD NUMBER: S02-139

INVOLVEMENT OF SEROTONINERGIC PROJECTIONS TO THE DORSAL STRIATUM IN BEHAVIOURAL SWITCHING UNDER UNCERTAINTY

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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In adaptive decision-making processes, behavioural switching is made based on past outcomes (positive or negative). While the neuromodulator serotonin (5-HT) has been shown to affect specific domains relevant for behavioral switching, such as patience for future rewards, impulse control and active persistence, little is known about the underlying circuit substrates. Here we investigated the involvement of dorsal raphe serotoninergic projections to the posterior dorsomedial and dorsolateral striatum ($DRN_{5\text{-}HT} \rightarrow$ pDMS and $DRN_{5\text{-}HT} \rightarrow$ DLS) in a probabilistic discrimination task, which critically requires the use of previous experience to maximize reinforcement. We trained mice to either clockwise or anticlockwise wheel turning for reward and shaped their preference by contrasting the probability of reinforcement upon the different options. We established probabilistic discrimination to be sensitive to uncertainty (evoked by tempering reward signalling) and to DRN optogenetic inhibition. By using fiber photometry during task, we found that population activity of non-dissected $DRN_{5\text{-}HT}$ neurons was differently related to time-locked reward consumption from DRN_{5HT} neurons projecting to the pDMS or DLS. Furthermore, the activity of $DRN_{5\text{-}HT} \rightarrow$ DLS neurons differed from that of their terminals, with the latter only increasing at reward collection, which is at the time of behavioural evaluation. Accordingly, optogenetic inhibition of DRN_{5HT}-→ DLS projections at wheel turning, or in-vivo 5-HT4R antagonism in the DLS before task, affected behavioural switching. Altogether, these results suggests that serotonergic transmission in the DLS is locally regulated to shape behavioural switching; the contribution of different striatal neuron subpopulations is currently under investigation.

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BOARD NUMBER: S02-140

SYSTEMATIC COMPARISON OF RISKY CHOICE PREFERENCE REVERSALS IN HUMAN AND MONKEY

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

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Monkeys have been used to study the neuronal mechanism of risky decision-making for more than a decade. Although some previous studies tried to compare the decision-making of humans and monkeys, these studies did not investigate the consistency of risk preference across these two primate species. We here designed a task allowing direct comparison of the two species' behaviour in a systematic way. We used a well-defined economic test for choice consistency, based on the independence axiom of expected utility theory. We measured the similarity in human's and monkey's stochastic choice behaviour in terms of violations of the independence axiom. More specifically, we tested whether adding a common gamble to two gamble options will change the preference of the subject (across 34 sessions and 26 sessions for two monkeys respectively). With this task, we found that monkeys exhibited preference reversal patterns similar to humans, except that our monkeys are more risk-taking than humans. Moreover, our machine learning models built with monkey data predicted human behaviour and vice versa, supporting the general preference reversal consistency across primates. These data suggest that (1) monkeys have similar risky choice behaviour to humans; (2) monkeys would be a good model organism to study decision-making under risk and the neuronal mechanism behind it, given that precise single-cell electrophysiology can be done in monkeys.

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BOARD NUMBER: S02-141

INVESTIGATING THE ROLE OF INHIBITORY CONTROL IN SCIENCE LEARNING USING EEG

POSTER SESSION 02 - SECTION: THE NEURAL CIRCUITS OF DECISION-MAKING

Lorie-Marlène Brault Foisy[1], Yannick Skelling[2], Patrice Potvin[2], Jérémie Blanchette Sarrasin[2], Hugo Lapierre[2], Steve Masson[2], Patrick Charland[2]
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An extensive research literature reveals that students hold misconceptions about various scientific phenomena. These misconceptions can interfere with the learning of scientific concepts and therefore make formal instruction more difficult. An example of such common misconception could be to believe that bigger objects sink more, that one wire is sufficient to light a bulb, and many more. Furthermore, it has been shown that those misconceptions do not only interfere during learning; they also persist after being given formal instruction. Recent neuroimaging and reaction time studies show that inhibitory control (IC) mechanisms are involved in understanding basic science concepts many scientific domains (e.g. Brault Foisy et al., 2015). The present research investigates the well-documented misconception in biology that “moving things are alive” that is known to interfere with the conceptual learning of what is considered alive or not. Electroencephalography (EEG) measures were selected for this study, as they allow for the detection of brain signal variations over short time frames in ecologically valid tasks. Twenty-eight undergraduate students solved a classification task in which they had to select between two images which one represented a living thing, while their EEG signals were recorded. Results show that ERP signals differ at N200 and LPP locations between counter-intuitive stimuli (for which IC is presumably required) and intuitive ones (counter-intuitive>intuitive). These locations are commonly involved in distinct cognitive processes associated to IC (Zhu et al., 2019). These results will be discussed as well as methodological considerations regarding the use of EEG to address science learning.

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BOARD NUMBER: S02-142

PHARMACOLOGICAL STIMULATION OF THE SEROTONIN RECEPTOR 7 RESCUES FEAR GENERALIZATION IN A PTSD MOUSE MODEL CARRYING A TRUNCATED FORM OF MECP2

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

Livia Di Crescenzo[1], Livia Cosentino[1], Chiara Urbinati[1], Enza Lacivita[2], Marcello Leopoldo[2], Bianca De Filippis[1]
[1]Istituto Superiore di Sanità, Center For Behavioural Sciences And Mental Health, Rome, Italy, [2]University of Bari, Department Of Pharmacy, Drug Sciences, Bari, Italy

Post-traumatic stress disorder (PTSD) is a chronic mental disorder occurring after trauma exposure, characterized by invalidating symptoms, that are often resistant to current interventions. The serotonin receptor 7 (5-HT7R) is involved in the endocrine and behavioural responses undertaken to cope with stress, and controls brain structural changes related to memory processes. Although these functions are relevant in PTSD neurobiology, the role of 5-HT7R in PTSD has not been investigated yet. **Aims**: we sought to evaluate the effects of 5-HT7R pharmacological stimulation on PTSD-like behavioural alterations in a mouse model of trauma vulnerability, carrying a truncated form of the X-linked epigenetic regulator methyl-CpG binding protein 2 (MeCP2). **Methods**: mutant males (MeCP2-308) and wild-type (wt) controls were systemically treated with the 5-HT7R agonist LP-211 for 7 days (0.25 mg/kg/day), starting 24 hours after the trauma (2x 3s, 0.8 mA unescapable footshocks). Mice were tested for cued and contextual fear memory recall (Day 1 and 7 of treatment) and for avoidance of trauma-related stimuli (Day 6). **Results**: Trauma-exposed MeCP2-308 mice performed freezing when exposed to a novel, non-threatening context, suggesting that they generalized fear to neutral stimuli. Consistently, traumatized mutants exhibited fear regardless of the presence of trauma-related stimuli in the avoidance task. LP-211 treatment rescued both aberrant behaviours. **Conclusions:** Present data provide novel evidence of a potential involvement of 5-HT7R in PTSD vulnerability, suggesting a role for 5-HT7R in counteracting fear generalization, a maladaptive memory-related process typically observed in patients with PTSD. Further studies are needed to clarify the mechanisms involved.

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TRANSCRIPTOMICS ANALYSIS FOR THE PRISMO STUDY: EXPRESSION PROFILES IN SUSCEPTIBLE VERSUS RESILIENT INDIVIDUALS IN A PROSPECTIVE PTSD COHORT.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aim: Post-traumatic Stress Disorder (PTSD) symptoms consist of, among others, re-experiencing traumatic experiences, avoidance of stimuli that remind of this traumatic experience, aggression, and sleep problems, leading to a strong adverse effect on patients' daily functioning. Epigenetic mechanisms, such as DNA methylation, have been proposed to underlie the relationship between exposure to traumatic stress and the susceptibility to develop PTSD. **Method:** Analysis of blood transcriptomics and DNA methylation profiles in relation to the development of PTSD symptoms in a prospective military cohort study (PRISMO). A subset (total n=96) of three similarly sized subgroups (susceptible, resilient, and control) of study participants was pre-selected based on the level of traumatic stress exposure and the presence of PTSD symptoms. **Result:** Transcriptomics data have been pre-processed and statistically analysed to compare susceptible to resilient profiles 6 months after return from deployment. Our preliminary findings suggest that there are 397 genes that are significantly differentially expressed in those who are susceptible to developing PTSD. Including control group (non-trauma exposed) expression levels, the changed genes have been further categorised into response classes, *e.g.*, being in- or decreased, or rather lacking in- or decrease in susceptible versus resilient individuals after trauma exposure. **Conclusion:** Genes in several response classes have been identified. Follow-up analyses, including pathway and Gene Ontology mapping, will be conducted to further biologically characterise these observed classes. Furthermore, differential expression results will be aligned with the epigenomics data we have collected previously, and the profiles correlated with the findings of our earlier published study.

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SEX-SPECIFIC CONDITIONED CONTEXT AVERSION IN OUTBRED MICE AS A MODEL OF ANTICIPATORY NAUSEA IN HUMANS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

Çınar Furkan Ilhan[1], Ayşe Seza Şahin[1], Gonzalo Urcelay[2], Sezen Kışlal[1]
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Aim: Anticipatory nausea and vomiting (ANV), which results from classical conditioning, develops mostly in female patients undergoing chemotherapy. Preclinical studies have shown that the administration of an illness-inducing agent in the presence of novel contextual cues can cause conditioned context aversion (CCA) in rodents as an animal model of ANV. This study investigated sex-specific development of CCA in mice after a single context-illness pairing. **Methods:** Twelve-week-old CD1 outbred mice were assigned to either the experimental (n = 15 for male, n = 14 for female) or control groups (n = 15 for male, n = 13 for female). A distinctive context consisting of tactile, auditory, and visual cues was paired with an intraperitoneal injection of illness-inducing lithium chloride (LiCl; 6 mEq/kg) in the experimental groups and sodium chloride (NaCl; 0.9%) in the control groups. To investigate the long-term retention of CCA, we conducted retention tests (20 minutes) measuring water intake in the trained context at three-day intervals. An independent sample t-test was used to measure the sex-specific significant differences between the experimental and control groups. **Results:** Both sexes acquired CCA learning after a single conditioning trial ($p < .001$). Retention of the CCA (decreased water intake) lasted 12 and 24 days for males and females, respectively (Figure 1,2). **Conclusion:** Female mice showed longer and more robust retention than male mice, similar to clinical findings showing sex differences among cancer patients. The results indicate the importance of using the CD1 outbred strain of mice as an animal model of
ANV.

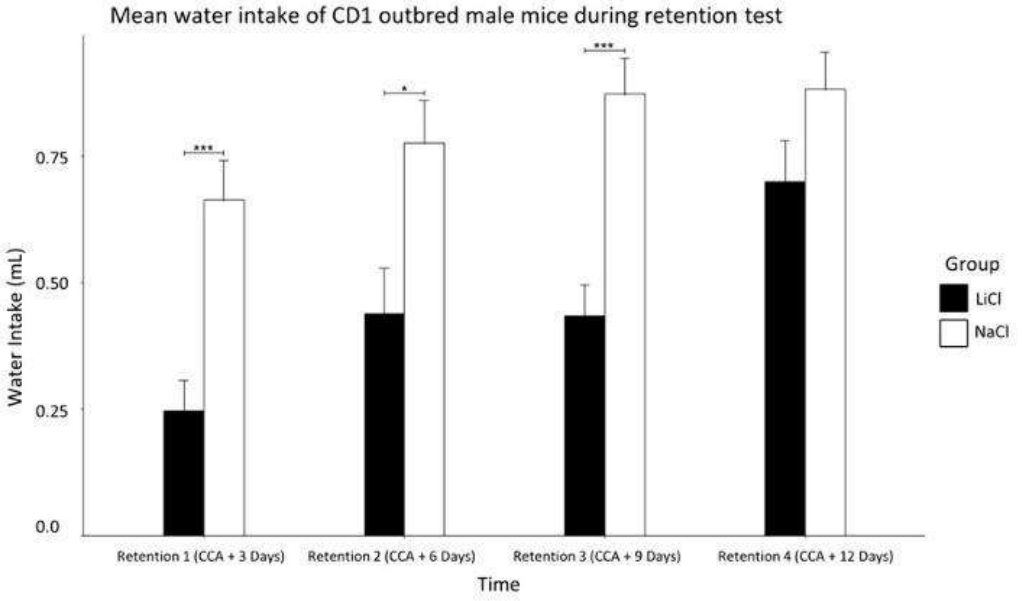

Figure 1

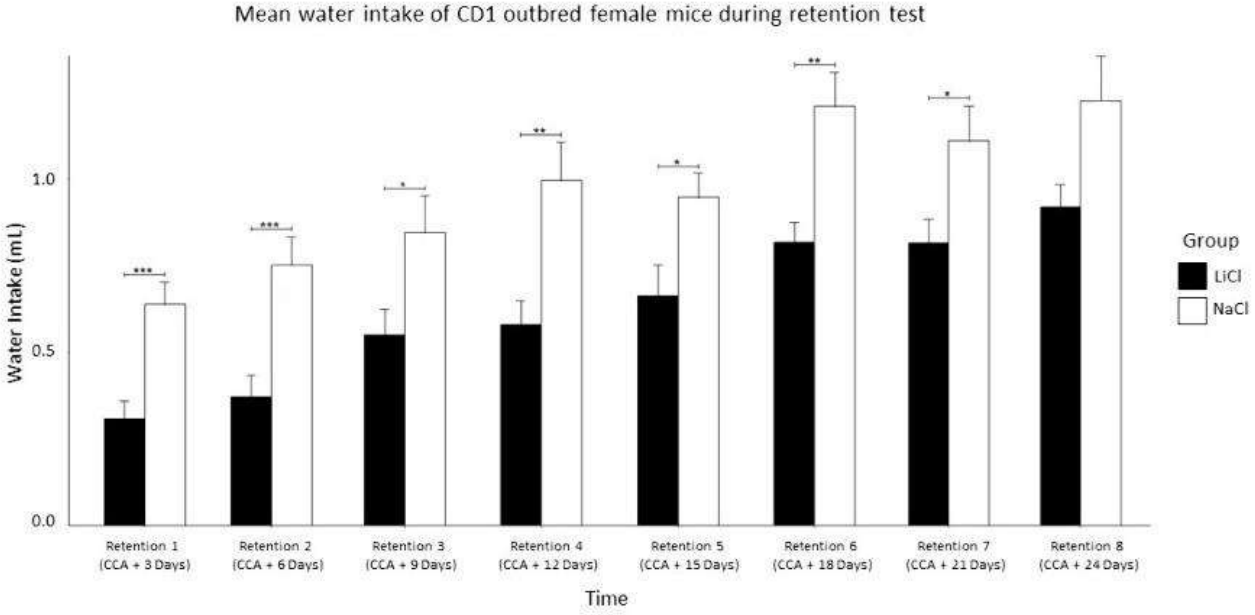

Figure 2


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FKBP51 IN GLUTAMATERGIC FOREBRAIN NEURONS MEDIATES BENEFICIAL EFFECTS OF MODERATE EARLY LIFE ADVERSITY ON HIPPOCAMPAL STRUCTURE AND FUNCTION.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Exposure to stressors in early in life can be a risk factor for later psychopathologies, but may also enhance stress resilience, dependent on the genetic background and later life conditions of an individual. The chaperone FKBP51 has been implicated in mediating such effects, but the underlying mechanism are still largely unclear. Furthermore, recent research has highlighted the differential responses to stress between sexes. This study investigates whether early-life stress (ELS) exposure can enhance cognitive performance in adulthood and if FKBP51 plays a role in this process. Mice with a conditional deletion of FKBP51 in forebrain glutamatergic neurons (FKBP51^{Nex}) or wild-type (WT) litter mates were either subjected to ELS in the form of limited nesting and bedding material or left undisturbed. Cognitive testing in adulthood at 5 - 7 months of age revealed that predominantly in female offspring ELS resulted in an improved cognitive performance specifically under high-stress conditions, and this effect was absent in FKBP51^{Nex} mice. Subsequent structural MRI revealed genotype or ELS-induced volumetric changes. Additionally, RNA bulk sequencing revealed interesting differences in expression profiles within the hippocampi of female mice, whereas field potential recordings in CA1 slices of a separate cohort of female FKBP51^{Nex} and WT mice exposed contrasting LTP induction profiles in response to a corticosterone application. Taken together, our results show that moderate exposure to early life adversity can enhance resilience in a sex-dependent manner by improving cognitive performance under high stress conditions as a result of molecular, functional and structural changes.

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BOARD NUMBER: S02-146

CANNABIDIOL PREVENTS COGNITIVE DEFICITS INDUCED BY ACUTE STRESS IN SERINE RACEMASE MUTANT MICE.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Introduction: Genetic variations in serine racemase (SRR), enzyme that forms D-serine endogenously, are associated to schizophrenia, and mice with mutations that inactivate SRR resemble cognitive aspects of schizophrenia. Acute stress decreases D-serine levels in pre-frontal cortex causing deficit in recognition memory. The endocannabinoid system modulates the stress response and evidence suggest that cannabidiol (CBD) may remediate cognitive deficits in animal models. However, we don't know whether CBD is able to prevents the memory deficit induced by acute stress in a schizophrenia mice model. Aim: To investigate the effects of CBD on memory deficits induced by acute stress on serine racemase mutant mice (SRR-/-). Methods: C57Bl/6 (WT) and SRR-/- male mice between 2 – 6 months old were exposed to acute restrained stress. CBD 5mg/kg was orally administrated 3h before acute stress. Animals were exposed to acute stress immediately after training trial of Novel Object Recognition task (NOR). The retention test occurred 24h after training. Results: Stressed animals showed higher levels of serum corticosterone than non-stressed animals. Acute stress reduced the performance at NOR in both WT and SRR-/- mice. CBD reduced performance at NOR test and failed to protect against the impairments in recognition memory induced by acute stress in WT mice. Interestingly, in SRR-/- mice CBD prevented the deficits in NOR induced by stress. Conclusion: CBD harmed recognition memory in WT mice, but prevented the induction of deficit by acute stress in serine racemase mutant mice.

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BOARD NUMBER: S02-147

ACUTE INSULIN ADMINISTRATION ENHANCES CONTEXTUAL FEAR MEMORY INDEPENDENTLY OF ADRENALINE, THROUGH INCREASED HIPPOCAMPUS BDNF EXPRESSION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Increased adrenaline and consequently hyperglycemia are effective in contextual fear memory enhancement[1]. Subsequently, hyperglycemia leads to insulin release which facilitates spatial memory-related and passive-avoidance tasks[2,3]. This physiological loop makes it difficult to distinguish the actions of both hormones in contextual fear memory. We aim to determine how insulin affects contextual fear memory in an adrenaline deficient mouse (phenylethanolamine-*N*-methyltransferase knock-out, Pnmt-KO) model. Wild type (WT) and Pnmt-KO (129x1/SvJ) mice were exposed to a fear conditioning protocol. On day 1, mice were exposed to 3-foot shocks (2 s, 0.6 mA) followed by contextual reminder exposure on day 2. Insulin (2 U/kg; i.p.) or vehicle (0.9% NaCl) were administered before training and context. Freezing percentage, tissues/plasma catecholamines, and *Bdnf* mRNA expression were evaluated. $P<0.05$ represents a significant difference. Insulin-treated WT mice have increased freezing behaviour and catecholamines in plasma when compared with vehicle-treated WT mice. Insulin-treated Pnmt-KO mice presented an increased freezing behaviour when compared with vehicle-treated Pnmt-KO mice, with no plasma catecholamines changes. Furthermore, they presented increased hippocampus *Bdnf* gene expression. In conclusion, insulin administration appears to strengthen contextual fear memory in a mice model with/without endogenous adrenaline. This memory enhancement could be related to the increased expression of *Bdnf* in the hippocampus of insulin-treated mice which can possibly lead to central molecular changes that promote contextual fear memory formation and strengthening. FCT (Grant SFRH/BD/138984/2018 to Ana Oliveira) Alves, E et al., Psychopharmacology 2016; 2. Park, C R et al., Physiol Behav 2000; 3. Moosavi M et al., Horm Behav 2006.

Pubmed:
30319349: Oliveira A, Martinho R, Serrão P, Moreira-Rodrigues M
Epinephrine Released During Traumatic Events May Strengthen Contextual Fear Memory Through Increased Hippocampus mRNA Expression of Transcription Factors.
Epinephrine (EPI) strengthens contextual fear memories by acting on peripheral β-adrenoceptors. Phenylethanolamine--methyltransferase-knockout (Pnmt-KO) mice are EPI-deficient mice and have reduced contextual fear learning. Our aim was to evaluate the molecular mechanisms by which peripheral EPI strengthens contextual fear memory and if a β-adrenoceptor antagonist can erase contextual fear memories. Pnmt-KO and wild-type (WT) mice were submitted to fear conditioning (FC) procedure after treatment with EPI, norepinephrine (NE), EPI plus ICI 118,551 (selective β-adrenoceptor antagonist), ICI 118,551 or vehicle (NaCl 0.9%). Catecholamines were separated and quantified by high performance liquid chromatography-electrochemical detection (HPLC-ED). Blood glucose was measured by coulometry. Real-time polymerase chain reaction (qPCR) was used to evaluate mRNA expression of nuclear receptor 4a1 (), and in hippocampus samples. In WT mice, plasma EPI concentration was significantly higher after fear acquisition (FA) compared with mice without the test. NE did not increase in plasma after FA and did not strengthen contextual fear memory, contrary to EPI. Freezing induced by EPI was blocked by ICI 118,551 in Pnmt-KO mice. In WT mice, ICI 118,551 blocked blood glucose release into the bloodstream after FA and decreased contextual fear memory. , and mRNA expression decreased in Pnmt-KO mice compared with WT mice after FC procedure. In Pnmt-KO mice, EPI induced an increase in mRNA expression of compared to vehicle. In conclusion, EPI increases in plasma after an aversive experience, possibly improving long-term and old memories, by acting on peripheral β-adrenoceptors. Glucose could be the mediator of peripheral EPI in the central nervous system, inducing the expression of transcription factor genes involved in consolidation of contextual fear memories.
Front Mol Neurosci, 2018; 11
33192300: Martinho R, Oliveira A, Correia G, Marques M, Seixas R, Serrão P, Moreira-Rodrigues M

Epinephrine May Contribute to the Persistence of Traumatic Memories in a Post-traumatic Stress Disorder Animal Model.
The importance of catecholamines in post-traumatic stress disorder (PTSD) still needs to be explored. We aimed to evaluate epinephrine's (EPI) causal role and molecular mechanism for the persistence of PTSD traumatic memories. Wild-type (WT) and EPI-deficient mice (phenylethanolamine--methyltransferase-knockout mice, Pnmt-KO) were induced with PTSD and behavioral tests were performed. Some Pnmt-KO mice were administered with EPI or vehicle. Catecholamines were quantified by HPLC-ED. , , and mRNA expression were evaluated by real-time PCR in hippocampus samples. It was observed an increase in EPI and freezing behavior, and a decrease in open arm entries in the elevated plus-maze test and time spent in the light in the light-dark test in WT mice in the PTSD-induction group compared to control. After induction of PTSD, Pnmt-KO mice showed a decrease in freezing, as well as an increase in open arm entries and transitions between compartments compared to WT. After PTSD induction, Pnmt-KO mice administered with EPI showed an increase in freezing compared with the vehicle. On day 0 of PTSD induction, it was observed an increase in mRNA expression of and genes in the hippocampus of WT mice compared to control, contrary to Pnmt-KO mice. In conclusion, our data suggest that EPI may be involved in the persistence of traumatic memories in PTSD, possibly through enhancement of the expression of and genes in the hippocampus. Peripheral administration of EPI restored contextual traumatic memories in Pnmt-KO mice, which suggests a causal role for EPI. The persistence of contextual traumatic memories may contribute to anxiety-like behavior and resistance of traumatic memory extinction in this PTSD mice model.
Front Mol Neurosci, 2020; 13
35173611: Martinho R, Seixas R, Azevedo M, Oliveira A, Serrão P, Moreira-Rodrigues M
Sotalol Treatment may Interfere With Retrieval, Expression, and/or Reconsolidation Processes Thus Disrupting Traumatic Memories in a Post-Traumatic Stress Disorder Mice Model.
The processes by which fear memory is encoded, consolidated, and re-consolidated are extremely complex and appear to require the release of stress hormones, especially adrenaline (AD). AD improves contextual fear memory, acting specifically on peripheral β2-adrenoceptors. Propranolol (peripheral and central β-adrenoceptor antagonist) treatment was shown to prevent post-traumatic stress disorder (PTSD) development and reduce its symptoms. However, propranolol has several side effects. Thus, we aimed to evaluate if sotalol (a peripheral β-adrenoceptor antagonist) treatment interferes with retrieval, expression, and/or reconsolidation of traumatic memories in a validated mice model that mimics the signs/symptoms of PTSD, thus intending to decrease them. Female mice were induced with PTSD following an established protocol. Sotalol (2.0 mg/kg) or vehicle were administered on days 2, 7, and 14. The percentage of freezing was calculated, and behavioral tests were carried out. Catecholamines in plasma were quantified by HPLC with electrochemical detection. Quantitative real-time polymerase chain reaction (qPCR) was used to evaluate mRNA expression of NR4A family genes in hippocampus. Following the submission of the animals to the same aversive context on days 2, 7, and 14, sotalol-treated mice exhibited significant less freezing behavior. In the elevated plus-maze test, the time spent and number of entries in the open arms, and total arm entries were increased in sotalol-treated mice. Also, the light-dark transition test revealed higher time spent, number of transitions to the light, and total number of transitions in sotalol-treated mice. Moreover, plasma AD was significantly decreased in sotalol-treated mice. On day 14, sotalol-treated mice exhibited a decrease in mRNA expression of in the hippocampus. In conclusion, in PTSD mice model, sotalol appears to decrease traumatic memories and anxiety-like behavior, probably due to a decrease in peripheral adrenergic activity, which influences traumatic memories. The effects of sotalol upon re-exposure to the traumatic context may be consistent with interference in the retrieval, expression, and/or reconsolidation processes of contextual traumatic memory, resulting in a long-term reduction of PTSD symptoms and signs. The decreased mRNA expression in the hippocampal formation may be crucial for these mice to develop diminished traumatic contextual memories after sotalol therapy in PTSD.
Front Pharmacol, 2021; 12
34630037: Martinho R, Correia G, Seixas R, Oliveira A, Silva S, Serrão P, Fernandes-Lopes C, Costa C, Moreira-Rodrigues M
Treatment With Nepicastat Decreases Contextual Traumatic Memories Persistence in Post-traumatic Stress Disorder.
Post-traumatic stress disorder (PTSD) is a common anxiety mental disorder and can be manifested after exposure to a real or perceived life-threatening event. Increased noradrenaline and adrenaline in plasma and urine have been documented in PTSD. Dopamine-β-hydroxylase (DBH) catalyzes the conversion of dopamine to noradrenaline and consequently, DBH inhibition reduces catecholamines. Our aim was to evaluate if nepicastat treatment decreases PTSD signs in an animal model. Wild-type (129x1/SvJ) female mice were submitted to PTSD induction protocol. DBH-inhibitor nepicastat (30 mg/kg) or vehicle (0.2% HPMC) were administered once daily since day 0 until day 7 or 12. The percentage of freezing was calculated on days 0, 1, 2, and 7, and behavioral tests were performed. Quantification of nepicastat in plasma and DBH activity in the adrenal gland was evaluated. Catecholamines were quantified by HPLC with electrochemical detection. mRNA expression of and in hippocampus was evaluated by qPCR.Mice in the PTSD-group and treated with nepicastat showed a decrease in freezing, and an increase in the time spent and entries in open arms in elevated plus maze test. In mice treated with

nepicastat, adrenal gland DBH activity was decreased, and catecholamines were also decreased in plasma and tissues. On day 7, in mice treated with nepicastat, there was an increase of and mRNA expression in the hippocampus.In conclusion, DBH inhibitor nepicastat has an effect consistent with a decrease in the persistence of traumatic memories and anxiety-like behavior in this PTSD mice model. The disruption of traumatic memories through interference with the formation, consolidation, retrieval, and/or expression processes may be important to decrease PTSD symptoms and signs. The increase in and mRNA expression in the hippocampus may be important to develop a weaker traumatic contextual memory after nepicastat treatment.
Front Mol Neurosci, 2021; 14

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BOARD NUMBER: S02-148

ROLE OF NLRP3 INFLAMMASOME AND METAFLAMMASOME IN THE COGNITIVE DYSFUNCTION AND ANXIETY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Neuroinflammation has been classified as a trigger of behavioral alterations and cognitive impairments. Given the role of metainflammation during aging, the way to regulate it may be an effective strategy to influence cognitive functions. We have studied the mechanisms of insulin signaling and metaflammation in the brain in Wild-type mice and NLRP3 KO mice. Aim: to assess NLRP3 inflammasome contribution to anxiety, acquisition of fear responses, hippocampal neurogenesis and brain insulin resistance. Methods: Behavioral, immunohistochemical and electrophysiological approaches were used. Results: NLRP3 KO mice demonstrate anxious behavior and deterioration in learning and memory; impairment of adult neurogenesis; reduction and altered morphology of astrocytes. We have found the hyperexcitability in basolateral amygdala; impaired activation in axons of pyramidal cells of CA1 zone in NLRP3 KO mice; and impaired synaptic transduction in pyramidal cells mediated by an embarrassment of neurotransmitter release from presynaptic site in CA3 zone. It was revealed that NLRP3 inflammasomes are required for insulin-dependent glucose transport in the brain and memory consolidation. Conclusions: The study has demonstrated the novel findings that basal level of NLRP3 inflammasome in the brain of young mice is required for conditioning-induced plasticity in the ventral hippocampus and the basolateral amygdala. The deletion of NLRP3 impair synaptic transduction and caused anxiety-like behavior and labored fear learning, suggesting that low grade inflammation, mediated by NLRP3 expression, play a key role in memory consolidation. This work was supported by a grant from the President of the Russian Federation for State support of young Russian scientists - MD-2368.2022.3.

Pubmed:
33358726: Komleva YK, Lopatina OL, Gorina IV, Shuvaev AN, Chernykh A, Potapenko IV, Salmina AB
NLRP3 deficiency-induced hippocampal dysfunction and anxiety-like behavior in mice.
Neuroinflammation has been classified as a trigger of behavioral alterations and cognitive impairments in many neurological conditions, including Alzheimer's disease, major depression, anxiety and others. Regardless of the cause of neuroinflammation, key molecules, which sense neuropathological conditions, are intracellular multiprotein signaling inflammasomes. Increasing evidence shows that the inflammatory response, mediated by activated nucleotide-binding oligomerization domain-, leucine-rich repeat- and pyrin domain-containing 3 (NLRP3) inflammasomes, is associated with the onset and progression of a wide range of diseases of the CNS. However, whether the NLRP3 inflammasome in the CNS is involved in the learning, development of anxiety and adult neurogenesis remains elusive. Therefore, the present study was designed to assess NLRP3 inflammasome contribution in anxiety and reveal its potential involvement in the experimental acquisition of fear responses and hippocampal neurogenesis. Behavioral, immunohistochemical and electrophysiological alterations were measured to evaluate role of neuroinflammation in the limbic system of mice. In this study, we describe interrelated neurophysiological mechanisms, which culminate in absence of NLRP3 inflammasome in young 4 months mice. These include the following: anxious behavior and deterioration in learning and memory of fear conditioning; impairment of adult neurogenesis; reduction and altered morphology of astrocytes in the brain; hyperexcitability in basolateral amygdala (BLA); impaired activation in axons of pyramidal cells of CA1 hippocampal zone in NLRP3 KO mice particularly via the Schaffer collateral pathway; and impaired synaptic transduction in pyramidal cells mediated by an embarrassment of neurotransmitter release from presynaptic site in CA3 hippocampal zone. The present study has demonstrated the novel findings that basal level of NLRP3 inflammasome in the brain of young mice is required for conditioning-induced plasticity in the ventral hippocampus and the basolateral amygdala. The deletion of NLRP3 impair synaptic transduction and caused anxiety-like behavior and labored fear learning, suggesting that low grade inflammation, mediated by NLRP3 expression, play a key role in memory consolidation.

Brain Res, 2021; 1752
34769018: Komleva YK, Potapenko IV, Lopatina OL, Gorina YV, Chernykh A, Khilazheva ED, Salmina AB, Shuvaev AN
NLRP3 Inflammasome Blocking as a Potential Treatment of Central Insulin Resistance in Early-Stage Alzheimer's Disease.
Alzheimer's disease (AD) is a devastating neurodegenerative disorder. In recent years, attention of researchers has increasingly been focused on studying the role of brain insulin resistance (BIR) in the AD pathogenesis. Neuroinflammation makes a significant contribution to the BIR due to the activation of NLRP3 inflammasome. This study was devoted to the understanding of the potential therapeutic roles of the NLRP3 inflammasome in neurodegeneration occurring concomitant with BIR and its contribution to the progression of emotional disorders.
Int J Mol Sci, 2021; 22
33519369: Komleva Y, Chernykh A, Lopatina O, Gorina Y, Lokteva I, Salmina A, Gollasch M
Inflamm-Aging and Brain Insulin Resistance: New Insights and Role of Life-style Strategies on Cognitive and Social Determinants in Aging and Neurodegeneration.
Over the past decades, the human life span has dramatically increased, and therefore, a steady increase in diseases associated with age (such as Alzheimer's disease and Parkinson's disease) is expected. In these neurodegenerative diseases, there is a cognitive decline and memory loss, which accompany increased systemic inflammation, the inflamm-aging, and the insulin resistance. Despite numerous studies of age-related pathologies, data on the contribution of brain insulin resistance and innate immunity components to aging are insufficient. Recently, much research has been focused on the consequences of nutrients and adiposity- and nutrient-related signals in brain aging and cognitive decline. Moreover, given the role of metainflammation in neurodegeneration, lifestyle interventions such as calorie restriction may be an effective way to break the vicious cycle of metainflammation and have a role in social behavior. The various effects of calorie restriction on metainflammation, insulin resistance, and neurodegeneration have been described. Less attention has been paid to the social determinants of aging and the possible mechanism by which calorie restriction might influence social behavior. The purpose of this review is to discuss current knowledge in the interdisciplinary field of geroscience-immunosenescence, inflamm-aging, and metainflammation-which makes a significant contribution to aging. A substantial part of the review is devoted to frontiers in the brain insulin resistance in relation to neuroinflammation. In addition, we summarize new data on potential mechanisms of calorie restriction that influence as a lifestyle intervention on the social brain. This knowledge can be used to initiate successful aging and slow the onset of neurodegenerative diseases.
Front Neurosci, 2020; 14
33392919: Komleva YK, Lopatina OL, Gorina YV, Chernykh AI, Trufanova LV, Vais EF, Kharitonova EV, Zhukov EL, Vahtina LY, Medvedeva NN, Salmina AB
Expression of NLRP3 Inflammasomes in Neurogenic Niche Contributes to the Effect of Spatial Learning in Physiological Conditions but Not in Alzheimer's Type Neurodegeneration.
A common feature of neurodegenerative disorders, in particular Alzheimer's disease (AD), is a chronic neuroinflammation associated with aberrant neuroplasticity. Development of neuroinflammation affects efficacy of stem and progenitor cells proliferation, differentiation, migration, and integration of newborn cells into neural circuitry. However, precise mechanisms of neurogenesis alterations in neuroinflammation are not clear yet. It is well established that expression of NLRP3 inflammasomes in glial cells marks neuroinflammatory events, but less is known about contribution of NLRP3 to deregulation of neurogenesis within neurogenic niches and whether neural stem cells (NSCs), neural progenitor cells (NPCs) or immature neuroblasts may express inflammasomes in (patho)physiological conditions. Thus, we studied alterations of neurogenesis in rats with the AD model (intra-hippocampal injection of Aβ1-42). We found that in Aβ-affected brain, number of CD133+ cells was elevated after spatial training in the Morris water maze. The number of PSA-NCAM+ neuroblasts diminished by Aβ injection was completely restored by subsequent spatial learning. Spatial training leads to elevated expression of NLRP3 inflammasomes in the SGZ (subgranular zones): CD133+ and PSA-NCAM+ cells started to express NLRP3 in sham-operated, but not AD rats. Taken together, our data suggest that expression of NLRP3 inflammasomes in CD133+ and PSA-NCAM+ cells may contribute to stimulation of adult neurogenesis in physiological conditions, whereas Alzheimer's type neurodegeneration abolishes stimuli-induced overexpression of NLRP3 within the SGZ neurogenic niche.
Cell Mol Neurobiol, 2022; 42
33304350: Lopatina OL, Komleva YK, Malinovskaya NA, Panina YA, Morgun AV, Salmina AB
CD157 and Brain Immune System in (Patho)physiological Conditions: Focus on Brain Plasticity.
Ectoenzyme and receptor BST-1/CD157 has been considered as a key molecule involved in the regulation of functional activity of cells in various tissues and organs. It is commonly accepted that CD157 catalyzes NAD+ hydrolysis and acts as a component of integrin adhesion receptor complex. Such properties are important for the regulatory role of CD157 in neuronal and glial cells: in addition to recently discovered role in the regulation of emotions, motor functions, and social behavior, CD157 might serve as an important component of innate immune reactions in the central nervous system. Activation of innate immune system in the brain occurs in response to infectious agents as well as in brain injury and neurodegeneration. As an

example, in microglial cells, association of CD157 with CD11b/CD18 complex drives reactive gliosis and neuroinflammation evident in brain ischemia, chronic neurodegeneration, and aging. There are various non-substrate ligands of CD157 belonging to the family of extracellular matrix proteins (fibronectin, collagen I, finbrinogen, and laminin) whose activity is required for controlling cell adhesion and migration. Therefore, CD157 could control structural and functional integrity of the blood-brain barrier and barriergenesis. On the other hand, contribution of CD157 to the regulation of brain development is rather possible since in the embryonic brain, CD157 expression is very high, whereas in the adult brain, CD157 is expressed on neural stem cells and, presumably, is involved in the neurogenesis. Besides, CD157 could mediate astrocytes' action on neural stem and progenitor cells within neurogenic niches. In this review we will summarize how CD157 may affect brain plasticity acting as a molecule at the crossroad of neurogenesis, cerebral angiogenesis, and immune regulation.
Front Immunol, 2020; 11
31152644: Lopatina OL, Malinovskaya NA, Komleva YK, Gorina YV, Shuvaev AN, Olovyannikova RY, Belozor OS, Belova OA, Higashida H, Salmina AB
Excitation/inhibition imbalance and impaired neurogenesis in neurodevelopmental and neurodegenerative disorders.
The excitation/inhibition (E/I) balance controls the synaptic inputs to prevent the inappropriate responses of neurons to input strength, and is required to restore the initial pattern of network activity. Various neurotransmitters affect synaptic plasticity within neural networks via the modulation of neuronal E/I balance in the developing and adult brain. Less is known about the role of E/I balance in the control of the development of the neural stem and progenitor cells in the course of neurogenesis and gliogenesis. Recent findings suggest that neural stem and progenitor cells appear to be the target for the action of GABA within the neurogenic or oligovascular niches. The same might be true for the role of neuropeptides (i.e. oxytocin) in neurogenic niches. This review covers current understanding of the role of E/I balance in the regulation of neuroplasticity associated with social behavior in normal brain, and in neurodevelopmental and neurodegenerative diseases. Further studies are required to decipher the GABA-mediated regulation of postnatal neurogenesis and synaptic integration of newly-born neurons as a potential target for the treatment of brain diseases.
Rev Neurosci, 2019; 30
30820471: Yamamoto Y, Liang M, Munesue S, Deguchi K, Harashima A, Furuhara K, Yuhi T, Zhong J, Akther S, Goto H, Eguchi Y, Kitao Y, Hori O, Shiraishi Y, Ozaki N, Shimizu Y, Kamide T, Yoshikawa A, Hayashi Y, Nakada M, Lopatina O, Gerasimenko M, Komleva Y, Malinovskaya N, Salmina AB, Asano M, Nishimori K, Shoelson SE, Yamamoto H, Higashida H
Vascular RAGE transports oxytocin into the brain to elicit its maternal bonding behaviour in mice.
Oxytocin sets the stage for childbirth by initiating uterine contractions, lactation and maternal bonding behaviours. Mice lacking secreted oxcytocin (,) or its receptor ( ) fail to nurture. Normal maternal behaviour is restored by peripheral oxcytocin replacement in and , but not mice, implying that circulating oxcytocin crosses the blood-brain barrier. Exogenous oxcytocin also has behavioural effects in humans. However, circulating polypeptides are typically excluded from the brain. We show that oxcytocin is transported into the brain by receptor for advanced glycation end-products (RAGE) on brain capillary endothelial cells. The increases in oxcytocin in the brain which follow exogenous administration are lost in male mice lacking RAGE, and behaviours characteristic to abnormalities in oxcytocin signalling are recapitulated in mice, including deficits in maternal bonding and hyperactivity. Our findings show that RAGE-mediated transport is critical to the behavioural actions of oxcytocin associated with parenting and social bonding.
Commun Biol, 2019; 2
30287150: Lopatina OL, Komleva YK, Gorina YV, Olovyannikova RY, Trufanova LV, Hashimoto T, Takahashi T, Kikuchi M, Minabe Y, Higashida H, Salmina AB
Oxytocin and excitation/inhibition balance in social recognition.
Social recognition is the sensitive domains of complex behavior critical for identification, interpretation and storage of socially meaningful information. Social recognition develops throughout childhood and adolescent, and is affected in a wide variety of psychiatric disorders. Recently, new data appeared on the molecular mechanisms of these processes, particularly, the excitatory-inhibitory (E/I) ratio which is modified during development, and then E/I balance is established in the adult brain. While E/I imbalance has been proposed as a mechanism for schizophrenia, it also seems to be the common mechanism in autism spectrum disorder (ASD). In addition, there is a strong suggestion that the oxytocinergic system is related to GABA-mediated E/I control in the context of brain socialization. In this review, we attempt to summarize the underpinning molecular mechanisms of E/I balance and its imbalance, and related biomarkers in the brain in healthiness and pathology. In addition, because there are increasing interest on oxytocin in the social neuroscience field, we will pay intensive attention to the role of oxytocin in maintaining E/I balance from the viewpoint of its effects on improving social impairment in psychiatric diseases, especially in ASD.
Neuropeptides, 2018; 72
30210321: Lopatina OL, Komleva YK, Gorina YV, Higashida H, Salmina AB
Neurobiological Aspects of Face Recognition: The Role of Oxytocin.

Face recognition is an important index in the formation of social cognition and neurodevelopment in humans. Changes in face perception and memory are connected with altered sociability, which is a symptom of numerous brain conditions including autism spectrum disorder (ASD). Various brain regions and neuropeptides are implicated in face processing. The neuropeptide oxytocin (OT) plays an important role in various social behaviors, including face and emotion recognition. Nasal OT administration is a promising new therapy that can address social cognition deficits in individuals with ASD. New instrumental neurotechnologies enable the assessment of brain region activation during specific social tasks and therapies, and can characterize the involvement of genes and peptides in impaired neurodevelopment. The present review sought to discuss some of the mechanisms of the face distinguishing process, the ability of OT to modulate social cognition, as well as new perspectives and technologies for research and rehabilitation of face recognition.
Front Behav Neurosci, 2018; 12

30135279: Komleva YK, Lopatina OL, Gorina YV, Chernykh AI, Shuvaev AN, Salmina AB
[Early changes in hyppocampal neurogenesis induced by soluble Ab1-42 oligomers].
Alzheimer's disease is characterized by the loss of neurons, the accumulation of intracellular neurofibrillary tangles and extracellular amyloid plaques in the brain. However, there are contradicting data on differences in neurogenesis at the onset of the disease or before the formation of amyloid plaques. As awareness of the importance of the pre-symptom phase in neurodegenerative diseases grows in the context of early diagnosis and pathogenesis, we analyzed the critical periods of adult hippocampal neurogenesis at an early stage under the action of soluble Ab1-42 beta-amyloid. The proliferation, migration and neuronal cells survival were evaluated in mice with an injection of soluble amyloid beta-oligomers. It was found that the injection of Ab1-42 oligomers causes a decrease in cell proliferation in the mouse hippocampus. Despite the preservation of the neuroblast pool in animals after beta-amyloid injection, the process of radial migration is disrupted, and an increase in apoptosis in the neurogenic niche was revealed. Thus, our results demonstrate damage of neurogenesis critical stages: the progenitor cells, neuroblast migration, the integration of immature neurons, and the survival of neurons under application of soluble beta-amyloid oligomers. The obtained data indicate decline in proliferation rate in the subgranular zone, that is accompanied by ectopic differentiation and disturbed migration, producing, apparently, abnormal neurons that have lower survival rates. That could lead to a decrease in mature neurons numbers and the number of cells in the granular layer of the dentate gyrus.
Biomed Khim, 2018; 64

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BOARD NUMBER: S02-149

CONTRIBUTION OF ADULT HIPPOCAMPAL NEUROGENESIS TO PRENATAL STRESS-INDUCED VULNERABILITY TO PTSD-LIKE MEMORY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

Mohamed-Lyès Kaci[1], Chloé Bouarab[1], Fanny Farrugia[2], Djoher Nora Abrous[1], Aline Desmedt[1], Muriel Koehl[1]
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Life events in childhood, experienced as early as during *in utero* life, play a pivotal role in shaping adult behavior, and studies have shown that exposure to prenatal stress (PS) could constitute a developmental risk factor for psychiatric disorders, with behavioral features reminiscent to those of post-traumatic stress disorder (PTSD). One of the cardinal feature of PTSD is a paradoxical pattern of memory with both emotional hypermnesia and contextual amnesia. Furthermore, it has recently been proposed that alteration in contextual pattern separation, which depends on adult hippocampal neurogenesis, could contribute to this abnormal memory profile. Altogether, this led us to examine the impact of PS in mice in a fear paradigm allowing to evidence PTSD-associated memory disturbances, and to analyze the contribution of adult neurogenesis in the PS-induced phenotype. We used optogenetics to target and activate 6-weeks old newborn neurons when mice were exposed to the fear paradigm. We then measured the effects of this stimulation on freezing behavior when mice were re-exposed to the fear conditioning context or to a neutral context. We report that PS mice exhibit a PTSD-like aberrant memory profile, while control mice develop a normal fear memory. Activating adult-born neurons prevents this PTSD-like memory in PS mice while it has no effect in control mice. In conclusion these results indicate that PS alters trauma contextualization, hence increasing the risk of developing PTSD-like memory disturbances, and that acting on adult-born neurons activity could help restoring a normal memory profile.

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REPEATED STRESS IN THE EARLY ADOLESCENT PERIOD: ARE CB1 RECEPTORS MEDIATING STRESS-REACTIVITY IN A SEX-SPECIFIC MANNER?

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

Emmanuelle Person, Alexandre Savard, Helene Plamondon
University of Ottawa, School Of Psychology, Ottawa, Canada

The importance of adolescent living experiences in shaping ensuing social and cognitive responses has been reported, although mechanisms remained to be determined. This study examined the effects of a heterotypic stress in prepubescent rats on social behaviours, memory and corticosterone levels, and possible mediation of stress outcomes by cannabinoid CB1 receptor antagonism. Sixty-four (N = 32 per sex) male and female rats were randomly assigned to a stress or a no-stress condition. Rats in the stress condition underwent a 10-day heterotypic stress paradigm alternating between a restrain stressor and a modified forced swim between postnatal day (PND) 30 and PND39. On PND42 and PND44, half of the rats received a dose of cannabinoid 1 receptor (CB1) antagonist AM251 (1mg/kg) before behavioural testing on the social interaction and social preference test (SIT), and the Y-Maze passive avoidance test (YMPAT), respectively. Blood droplets were collected via tail puncture at 5 different timepoints (PND30, PND35, PND39, PND42, PND46) to assess sex-related time-related changes in circulatory levels of corticosterone prior, during and post stress exposure experiment. Corticosterone (CORT) ELISA immunoassays indicated that while male rats habituated to stress exposure, females showed heightened secretion compared to males from baseline and CORT secretion remained elevated throughout assessments days. CB1 antagonism using AM251 failed to influence CORT levels. Analysis of the impact of pre-testing blockade of CB1 receptors in the SIT and the YMPAT is pending. Our findings are consistent with increased CORT secretion reported in females, and further suggest sex-specific differences in habituation to stress exposure.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-151

CHEMICAL SIGNALS FROM LIPOPOLYSACCHARIDE-TREATED MALE MICE DO NOT ELICIT AVOIDANCE IN FEMALES

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

Camila Savarelli-Balsamo[1], Adrián Martínez-Requena[2], Anna Teruel-Sanchis[2], Manuel-Esteban Vila-Martin[1,2], Enrique Lanuza[2]
[1]Universitat de València, Human Anatomy And Embriology, Faculty Of Medicine And Odontology, Valencia, Spain, [2]Facultat de Biològiques, Universitat de València, Biologia Cel·lular, Tissular I Antropologia Física, Burjassot, Spain

Animals have developed complex physical and behavioural systems in response to chemosensory stimuli. The ability to detect, sort and respond to those signals might be critical for their survival, as it reveals key information about the environment. In rodents, chemical cues are detected by the olfactory and vomeronasal systems, responsible respectively, for the detection of volatile and non-volatile molecules. The vomeronasal organ of mice can detect both attractive sexual pheromones and cues from sick conspecifics, thus avoiding social interaction with them, reducing the risk of infection. In this work, we studied whether signals from sick males can counteract the attraction elicited by male pheromones present in urine. We also tested whether attractive or aversive responses are mediated by volatile molecules or require direct contact with male-derived stimuli. Using a model of infection based on the immune response to lipopolysaccharides (LPS) we performed two-choice preference tests in female subjects confronting soiled bedding from female, healthy male and LPS-treated males. Our results reveal that female mice show preference for either LPS-treated or healthy male-soiled bedding when presented against female-soiled bedding, both in volatile and contact-allowing tests. However, females showed no preference when presented with LPS-infected vs. healthy male bedding. We concluded that the LPS treatment is not robust enough to elicit aversive behaviour in females. Therefore, we suggest employing pathogenic infection models when seeking aversive behaviour. Funded by the Spanish Ministry of Science and Innovation-FEDER (PID2019-108562GB-I00). The author is an “Atracció al talent” program predoctoral fellow at the University of Valencia.

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BOARD NUMBER: S02-152

VARIABILITY IN ESCAPE BEHAVIOUR IS CONSERVED ACROSS SPECIES AND EXPLAINED BY AN ACTIVE INFERENCE PROCESS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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[1]Italian Institute of Technology, Neuroscience And Behaviour, Rome, Italy, [2]University of St Andrews, School Of Biology, St Andrews, United Kingdom, [3]UCL, Neuroscience Physiology And Pharmacology, London, United Kingdom, [4]Sainsbury wellcome Centre, Swc, LONDON, United Kingdom, [5]Sainsbury Wellcome Centre, Sainsbury Wellcome Centre, London, United Kingdom, [6]University of Trento, Cimec - Center For Mind/brain Sciences, Rovereto, Italy, [7]Italian Institute of Technology, Neuroscience And Behaviour Laboratory, Rome, Italy

Animals must effectively escape from sudden threats to ensure survival. Despite this strong selective pressure, in many species an individual's escape trajectories are quite variable, sometimes even driving prey in the direction of a predator. This seemingly random trial-by-trial variability has been interpreted as a strategy to prevent predators from predicting escape behaviour, thereby improving population fitness. However, an overarching formal explanation of this variability is missing. Here we investigated escape behaviour evoked by looming visual stimuli in four phylogenetically distant organisms (fish, chicks, mice and humans). We discovered that a large part of the trial-to-trial variability in escapes is not random, but predictable from sensory and behavioural state variables. Specifically, we found that in all species 1) early escapes are more frequently directed towards the threat; 2) escapes are more frequently directed in the direction of gaze. In addition, fish and chicks' propensity to escape is higher when gaze direction and threat position overlap. Using an active inference framework, we demonstrate that these observations can be explained *respectively* by independent, competing drives aiming to 1) reduce sensory uncertainty, and 2) pursue existing movement goals, all the while additionally avoiding threats. The inclusion of new-borns (fish larvae and chicks) and a precocial species (chicks) suggests that these principles are mostly innate. In sum, we show that a large part of the seemingly random variability in escape behaviour is in fact predictable and propose that it is a consequence of the interplay between perceptual and motor goals.

Pubmed:
30337061: Bufacchi RJ, Iannetti GD
An Action Field Theory of Peripersonal Space.
Predominant conceptual frameworks often describe peripersonal space (PPS) as a single, distance-based, in-or-out zone within which stimuli elicit enhanced neural and behavioural responses. Here we argue that this intuitive framework is contradicted by neurophysiological and behavioural data. First, PPS-related measures are not binary, but graded with proximity. Second, they are strongly influenced by factors other than proximity, such as walking, tool use, stimulus valence, and social cues. Third, many different PPS-related responses exist, and each can be used to describe a different space. Here, we reconceptualise PPS as a set of graded fields describing behavioural relevance of actions aiming to create or avoid contact between objects and the body. This reconceptualisation incorporates PPS into mainstream theories of action selection and behaviour.
Trends Cogn Sci, 2018; 22
34588085: Bufacchi RJ, Iannetti GD
Movement vigor: Frameworks, exceptions, and nomenclature.
Shadmehr and Ahmed cogently argue that vigor of appetitive movements is positively correlated with their value, and that value can therefore be inferred by measuring vigor. Here, we highlight three points to consider when interpreting this account: (1) The correlation between vigor and value is not obligatory, (2) the vigor effect also arises in frameworks other than optimal foraging, and (3) the term vigor can be misinterpreted, thereby affecting rigor.
Behav Brain Sci, 2021; 44
32282798: Guo Y, Bufacchi RJ, Novembre G, Kilintari M, Moayedi M, Hu L, Iannetti GD
Ultralow-frequency neural entrainment to pain.
Nervous systems exploit regularities in the sensory environment to predict sensory input, adjust behavior, and thereby

maximize fitness. Entrainment of neural oscillations allows retaining temporal regularities of sensory information, a prerequisite for prediction. Entrainment has been extensively described at the frequencies of periodic inputs most commonly present in visual and auditory landscapes (e.g., >0.5 Hz). An open question is whether neural entrainment also occurs for regularities at much longer timescales. Here, we exploited the fact that the temporal dynamics of thermal stimuli in natural environment can unfold very slowly. We show that ultralow-frequency neural oscillations preserved a long-lasting trace of sensory information through neural entrainment to periodic thermo-nociceptive input as low as 0.1 Hz. Importantly, revealing the functional significance of this phenomenon, both power and phase of the entrainment predicted individual pain sensitivity. In contrast, periodic auditory input at the same ultralow frequency did not entrain ultralow-frequency oscillations. These results demonstrate that a functionally significant neural entrainment can occur at temporal scales far longer than those commonly explored. The non-supramodal nature of our results suggests that ultralow-frequency entrainment might be tuned to the temporal scale of the statistical regularities characteristic of different sensory modalities.
PLoS Biol, 2020; 18
30824228: Bufacchi RJ, Iannetti GD
The Value of Actions, in Time and Space.
Trends Cogn Sci, 2019; 23
32871034: John JB, Anderson M, Dutton T, Stott M, Crundwell M, Llewelyn R, Gemmell A, Bufacchi R, Spiers A, Campain N
Percutaneous microwave ablation of renal masses in a UK cohort.
To report a tertiary referral centre's experience of microwave ablation (MWA) for suspected renal cell carcinoma (RCC), describing complications and oncological outcomes.
BJU Int, 2021; 127
27825445: Bufacchi RJ, Iannetti GD
Gravitational cues modulate the shape of defensive peripersonal space.
The potential damage caused by an environmental threat increases with proximity to the body, so animals perform more effective and stronger defensive responses when threatening stimuli occur nearby the body, in a region termed the defensive peripersonal space (DPPS) [1,2]. We recently characterized the fine-grained geometry of the face's DPPS by recording the enhancement of the blink reflex elicited by electrical stimulation of the median nerve (hand-blink reflex, HBR), when the hand is closer to the face [3]. The resulting DPPS has the shape of a bubble, elongated asymmetrically along the rostro-caudal axis, extending further above eye-level [4]. We hypothesized that this vertical asymmetry is determined by gravitational cues: the probability that a threat will hit the body is higher when it comes from above. By systematically altering body posture, we show that the extent of DPPS asymmetry is defined in an earth-centred coordinate frame. This observation suggests the brain takes gravitational cues to automatically update threat value in an adaptive mechanism that accounts for the simple fact that objects fall down.
Curr Biol, 2016; 26
31085301: Novembre G, Pawar VM, Kilintari M, Bufacchi RJ, Guo Y, Rothwell JC, Iannetti GD
The effect of salient stimuli on neural oscillations, isometric force, and their coupling.
Survival in a suddenly-changing environment requires animals not only to detect salient stimuli, but also to promptly respond to them by initiating or revising ongoing motor processes. We recently discovered that the large vertex brain potentials elicited by sudden supramodal stimuli are strongly coupled with a multiphasic modulation of isometric force, a phenomenon that we named cortico-muscular resonance (CMR). Here, we extend our investigation of the CMR to the time-frequency domain. We show that (i) both somatosensory and auditory stimuli evoke a number of phase-locked and non-phase-locked modulations of EEG spectral power. Remarkably, (ii) some of these phase-locked and non-phase-locked modulations are also present in the Force spectral power. Finally, (iii) EEG and Force time-frequency responses are correlated in two distinct regions of the power spectrum. An early, low-frequency region (~4 Hz) reflects the previously-described coupling between the phase-locked EEG vertex potential and force modulations. A late, higher-frequency region (beta-band, ~20 Hz) reflects a second coupling between the non-phase-locked increase of power observed in both EEG and Force. In both time-frequency regions, coupling was maximal over the sensorimotor cortex contralateral to the hand exerting the force, suggesting an effect of the stimuli on the tonic corticospinal drive. Thus, stimulus-induced CMR occurs across at least two different types of cortical activities, whose functional significance in relation to the motor system should be investigated further. We propose that these different types of corticomuscular coupling are important to alter motor behaviour in response to salient environmental events.
Neuroimage, 2019; 198
29378865: Novembre G, Pawar VM, Bufacchi RJ, Kilintari M, Srinivasan M, Rothwell JC, Haggard P, Iannetti GD
Saliency Detection as a Reactive Process: Unexpected Sensory Events Evoke Corticomuscular Coupling.
Survival in a fast-changing environment requires animals not only to detect unexpected sensory events, but also to react. In humans, these salient sensory events generate large electrocortical responses, which have been traditionally interpreted within the sensory domain. Here we describe a basic physiological mechanism coupling saliency-related cortical responses

with motor output. In four experiments conducted on 70 healthy participants, we show that salient substartle sensory stimuli modulate isometric force exertion by human participants, and that this modulation is tightly coupled with electrocortical activity elicited by the same stimuli. We obtained four main results. First, the force modulation follows a complex triphasic pattern consisting of alternating decreases and increases of force, time-locked to stimulus onset. Second, this modulation occurs regardless of the sensory modality of the eliciting stimulus. Third, the magnitude of the force modulation is predicted by the amplitude of the electrocortical activity elicited by the same stimuli. Fourth, both neural and motor effects are not reflexive but depend on contextual factors. Together, these results indicate that sudden environmental stimuli have an immediate effect on motor processing, through a tight corticomuscular coupling. These observations suggest that saliency detection is not merely perceptive but reactive, preparing the animal for subsequent appropriate actions. Salient events occurring in the environment, regardless of their modalities, elicit large electrical brain responses, dominated by a widespread "vertex" negative-positive potential. This response is the largest synchronization of neural activity that can be recorded from a healthy human being. Current interpretations assume that this vertex potential reflects sensory processes. Contrary to this general assumption, we show that the vertex potential is strongly coupled with a modulation of muscular activity that follows the same pattern. Both the vertex potential and its motor effects are not reflexive but strongly depend on contextual factors. These results reconceptualize the significance of these evoked electrocortical responses, suggesting that saliency detection is not merely perceptive but reactive, preparing the animal for subsequent appropriate actions.
J Neurosci, 2018; 38

34668519: Somervail R, Bufacchi RJ, Salvatori C, Neary-Zajiczek L, Guo Y, Novembre G, Iannetti GD
Brain Responses to Surprising Stimulus Offsets: Phenomenology and Functional Significance.
Abrupt increases of sensory input (onsets) likely reflect the occurrence of novel events or objects in the environment, potentially requiring immediate behavioral responses. Accordingly, onsets elicit a transient and widespread modulation of ongoing electrocortical activity: the Vertex Potential (VP), which is likely related to the optimisation of rapid behavioral responses. In contrast, the functional significance of the brain response elicited by abrupt decreases of sensory input (offsets) is more elusive, and a detailed comparison of onset and offset VPs is lacking. In four experiments conducted on 44 humans, we observed that onset and offset VPs share several phenomenological and functional properties: they (1) have highly similar scalp topographies across time, (2) are both largely comprised of supramodal neural activity, (3) are both highly sensitive to surprise and (4) co-occur with similar modulations of ongoing motor output. These results demonstrate that the onset and offset VPs largely reflect the activity of a common supramodal brain network, likely consequent to the activation of the extralemniscal sensory system which runs in parallel with core sensory pathways. The transient activation of this system has clear implications in optimizing the behavioral responses to surprising environmental changes.
Cereb Cortex, 2022; 32

33174497: Bufacchi RJ, Magri C, Novembre G, Iannetti GD
Local spatial analysis: an easy-to-use adaptive spatial EEG filter.
Spatial EEG filters are widely used to isolate event-related potential (ERP) components. The most commonly used spatial filters (e.g., the average reference and the surface Laplacian) are "stationary." Stationary filters are conceptually simple, easy to use, and fast to compute, but all assume that the EEG signal does not change across sensors and time. Given that ERPs are intrinsically nonstationary, applying stationary filters can lead to misinterpretations of the measured neural activity. In contrast, "adaptive" spatial filters (e.g., independent component analysis, ICA; and principal component analysis, PCA) infer their weights directly from the spatial properties of the data. They are, thus, not affected by the shortcomings of stationary filters. The issue with adaptive filters is that understanding how they work and how to interpret their output require advanced statistical and physiological knowledge. Here, we describe a novel, easy-to-use, and conceptually simple adaptive filter (local spatial analysis, LSA) for highlighting local components masked by large widespread activity. This approach exploits the statistical information stored in the trial-by-trial variability of stimulus-evoked neural activity to estimate the spatial filter parameters adaptively at each time point. Using both simulated data and real ERPs elicited by stimuli of four different sensory modalities (audition, vision, touch, and pain), we show that this method outperforms widely used stationary filters and allows to identify novel ERP components masked by large widespread activity. Implementation of the LSA filter in MATLAB is freely available to download. EEG spatial filtering is important for exploring brain function. Two classes of filters are commonly used: stationary and adaptive. Stationary filters are simple to use but wrongly assume that stimulus-evoked EEG responses (ERPs) are stationary. Adaptive filters do not make this assumption but require solid statistical and physiological knowledge. Bridging this gap, we present local spatial analysis (LSA), an adaptive, yet computationally simple, spatial filter based on linear regression that separates local and widespread brain activity (https://www.iannettilab.net/lsa.html or https://github.com/rorybufacchi/LSA-filter).
J Neurophysiol, 2021; 125

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BOARD NUMBER: S02-153

INTEGRATED CARDIO-BEHAVIOURAL DEFENSIVE STATES

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Fear and anxiety are brain states that evolved to mediate defensive responses to threat. While it is clear that the defence reaction includes multiple interacting behavioural, autonomic and endocrine adjustments, their integrative nature is poorly understood. In particular, under seemingly identical threat conditions, both deceleration (bradycardia) as well as acceleration (tachycardia) of heart rate (HR) have been reported, hampering consensus on the relevance and meaning of cardiac changes for the integrated defence reaction. By identifying stereotypical behavioural and HR dynamic associations at different timescales and under various conditions, we here define cardio-behavioural, rapid “microstates” and “macrostates”, characterized by slower dynamics. Interestingly, both micro-and macrostates reflect context-dependent threat levels, but we also found that macrostate dynamics can critically affect the expression of the most elemental cardio-behavioural elements (i.e. microstates), encompassing the most common readout for fear, i.e. the freezing response. In turn, optogenetics experiments designed accordingly revealed how integrated cardio-behavioural micro- and macrostates are mediated by specific circuit elements in the midbrain periaqueductal grey. Our work puts forth a framework for systematic integration of cardiac and behavioural readouts that presents the basis for a better understanding of complex neural defensive states and their associated systemic functions.

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BOARD NUMBER: S02-154

NEUROBIOLOGICAL CONSEQUENCES OF THE ADOLESCENT EXPOSURE TO THE SYNTHETIC CANNABINOID JWH-018 IN MALE AND FEMALE MICE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Spice/K2 herbal mixtures containing synthetic cannabinoids such as JWH-018, have been marketed as marijuana surrogates. Early abuse of cannabinoids has been related to the development of psychiatric disorders in adulthood as adolescence is a critical period for the maturation of the central nervous system. This study aims to analyze the short and long-term effects of the exposure to JWH-018 during adolescence, on aversive emotional memory, anxiety, and psychotic-like behaviours. Mice were treated from postnatal day 35 to 49 with increasing doses of JWH-018. Short and long-term effects were analyzed 5 and 20 days, respectively, after the end of the treatment. Adolescent treated males, but not females, showed significant anxiety-like behaviour 5 days after treatment. This effect was not maintained at long-term in males. However, JWH-018 administration induced a clear anxiogenic tendency in females. Fear extinction was not modified neither in males nor in females both in short and long-term. Males, but not females, chronically exposed to JWH-018, showed a reduction in pre-pulse inhibition of startle reflex in short and long-term suggesting the development of a psychotic-like behaviour. Alterations in the expression of parvalbumin-containing neurons and perineuronal nets have been associated to changes in the plasticity and maturation of the prefrontal cortex. Therefore, immunohistochemical studies are also being carried out in JWH-018 exposed mice to analyze the expression of these neuronal features in this brain area. To conclude, the exposure to JWH-018 during adolescence leads to the appearance of different behavioural alterations which could be dependent on gender.

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BOARD NUMBER: S02-155

EFFECTS OF A NOVEL POSITIVE NMDA RECEPTOR MODULATOR IN A MOUSE MODEL OF IMPAIRED FEAR EXTINCTION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aims: Approved treatments for PTSD, such as antidepressants or cognitive behavioral therapy (CBT), leave many patients with an unsatisfying clinical outcome to control their symptoms. The search for more effective drugs and augmentation strategies for CBT to improve the quality of life of these patients poses a great challenge for preclinical and clinical research. This subgroup of treatment-resistant individuals is closely modeled by the 129S1/SvImJ (S1) inbred mouse strain, which exhibits impaired fear extinction and anxiety-like behaviors. The novel, positive N-methyl-D-aspartate (NMDA) receptor modulator NYX-783 has already shown promising results in exploratory clinical studies in PTSD patients, but has not been tested before in a mouse model of deficient fear extinction. **Methods & Results**: In our experiments, acute NYX-783 administration improved fear extinction in extinction-competent C57BL/6J mice, but had no such effect in S1 animals. Here, NYX-783 strengthened fear memories, most likely because no within-session fear extinction was achieved in this test setting. Interestingly, however, in naïve (not fear-conditioned) S1 mice, NYX-783 had an anxiolytic-like effect in the elevated plus maze. **Conclusions**: In summary, our preliminary data suggest that NYX-783 may acutely reduce anxiety and serve as a powerful cognitive enhancer for emotional memories acquired during drug action, regardless of their valence. Further experiments are needed to determine mechanism and site(s) of action in the brain, as well as to gain more detailed information on how PTSD patients may benefit best from an individualized therapy regimen that utilizes NYX-783 to provide anxiolysis and selectively enhance fear-inhibitory memories. Supported by Aptinyx.

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BOARD NUMBER: S02-156

NEUROCIRCUITRY OF SOCIAL FEAR EXTINCTION. INVOLVEMENT OF THE SEPTAL OXYTOCIN SYSTEM?

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Social anxiety disorder (SAD), is the second most common anxiety disorder and characterized by fear and avoidance of social situations. Understanding the neuronal mechanisms underlying SAD is of utmost importance to develop new therapeutic strategies. Using the social fear conditioning (SFC) paradigm, we identified oxytocin (OXT) signaling within the lateral septum (LS) as a critical regulator of social fear extinction. The LS is reciprocally connected with various brain regions which control different drive states and motivated responses. Therefore, the LS is thought to serve as an essential converging point, that relays incoming cognitive information to downstream affective regions that then adjust the behavioral output in response to varied environmental stimuli. We were able to show, that OXT receptors (OXTR) are expressed in high levels specifically in the caudal part of the LS (LSc). Infusion of OXT in the LSc facilitates social fear extinction, but the exact signaling mechanisms of OXTR-expressing neurons in the LSc in the context of social fear extinction are still unknown. Using a viral anterograde tracer in combination with an OXTR-Cre mouse line, we were able to identify the medial habenula (MHb) as a downstream target of OXTR-expressing neurons. We now aim to identify the functional role of OXTR-expressing neurons in the LSc within the framework of the SFC paradigm using an in vivo calcium imaging approach. This study may provide a deeper understanding on how septal oxytocin signaling modulates emotional states, and might shed light on the mechanisms that underlie the extinction of social fear.

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BOARD NUMBER: S02-157

SEX-SPECIFIC DISRUPTION OF SOCIAL, BUT NOT EMOTIONAL BEHAVIOR AFTER SOCIAL TRAUMA IN ADOLESCENT MICE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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The developmental period of adolescence is highly vulnerable towards stress, especially social trauma, leading to a higher susceptibility for mental disorders such as social anxiety disorder (SAD). We aimed to investigate the acute and long-lasting effects of adolescent social trauma on socio-emotional behavior and the underlying neuronal mechanisms. Hence, adolescent male and female mice were exposed to the social fear conditioning (SFC) paradigm, a mouse model of social trauma generating social avoidance as symptom of SAD. Social fear extinction was performed either on the subsequent day or in adulthood. To assess neuronal activation and oxytocin receptor (OXTR) binding, SFC mice were subjected to singular social stimuli in either adolescence or adulthood. We confirmed that SFC generates robust social fear in adolescent male and female mice, without affecting general anxiety- and depressive-like behavior. However, SFC exposure in adolescence lead to social fear persisting into adulthood in male mice only. Analyzing the neuronal activation of mice following a social encounter the day after SFC revealed a sex-dependent activity of the lateral septum (LS). Here, SFC mice showed reduced activation within either the ventral LS in females or the dorsal LS in males. Moreover, we found lower OXTR binding within the ventromedial hypothalamus of adult SFC male mice. In conclusion, we demonstrate higher vulnerability of adolescent SFC male compared to female mice with respect to disruption of social behavior during adulthood. Further, adolescent SFC leads to changes in adolescent neuronal activation and the adult oxytocin system, possible contributing factors for SAD development.

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NICOTINE EFFECTS ON ANXIETY BEHAVIOR AND BRAIN BIOCHEMICAL MARKERS IN ADULT MALE MICE AND IN RAT HIPPOCAMPAL SLICES: PATCH CLAMP RECORDINGS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aims Nicotine has been described as the main component of cigarette, which is responsible for a wide variety of neurochemical and behavioral effects. The aims of the present study were to a) investigate the effects of nicotine intake on behavioral parameters (anxiety-like behavior, motility), as well as activity of acetylcholinesterase's (AChE) isoforms (G1, G4) on specific brain regions of adult male mice and b) evaluate, by patch-clamp recordings, the effect of acute slice perfusion of various concentrations (1-10 mM) of nicotine on inhititory and excitatory inputs on hippocampal CA1 neurons. **Methods** Mice were divided into 3 groups: control, cigarettes' smoke and Tobacco Heating System 2.2 (THS2.2') aerosol exposed groups. The exposure was carried out in a unique smoking device with whole body exposure to smoke. The behavioral analysis was assessed by using the open field test, by which anxiety-like behavior and motility will be addressed. The activity of both G1 and G4 AChE's isoforms was determined by using Ellman's colorimetric method. Whole cell patch-clamp recording of spontaneous GABAergic and Glutamatergic currents was performed in CA1 hippocampal neurons. **Results** Our results show that both the smoke and aerosol exposures affected the anxiety-like behavior and inhibits AChE's isoform activity. Moreover, nicotine increases in a dose dependent way both the amplitude and the frequency of glutammatergic or GABAergic currents, respectively, recorded in CA1 neurons **Conclusion** Nicotine exposure inhibits affects anxiety-like behavior, AChE activity in brain areas and may affect hippocampal function and may decrease its function at least when is somministrated acutely.

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BOARD NUMBER: S02-159

HARNESSING THE HETEROGENEITY OF FREEZING RESPONSES IN MICE TO UNDERSTAND BIOLOGICAL BASES OF SPECTRUM OF ANXIETY DISORDERS.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Preclinical studies rely on functional patterns of defensive behaviors in rodents as basis for conceptualizing human fear/anxiety. However, very often animal models do not account for the role of inter-individual differences of stimulus perception and processing in shaping the behavioral response. By applying cued fear conditioning paradigm with prolonged retrieval session we confirmed that mice respond to fearful stimuli in an individual manner. Using unsupervised clustering approach, we identified 2 distinct endophenotypes (phasic and sustained responders) in the population of wild-type mice (C57BL6/J). The phenotypes are stable at least through two retrievals in both male and female mice. Interestingly, distribution of phasic/sustained responders is 60/30% for males but 30/60% for females consistent with higher occurrence of anxiety disorders in women compared to men. Also freezing behaviour during retrieval correlated with approach-avoidance behavior in anxiety tests. Transcriptomic analysis of key brain regions in defence circuit of phasic (n=6) and sustained (n=6) female responders revealed substantial differences in transcriptomes of ACC, BLA, CeA, and BNST. At 24h post-conditioning time-point (Retrieval I) the largest transcriptomic changes were detected in CeA (2922 DEGs), followed by ACC (378 DEGs). Functional annotation analysis revealed that DEGs from both regions are enriched for genes involved in inflammatory response. Additionally, extracellular matrix organization pathway was significantly deregulated in CeA. Interestingly, when sequencing was repeated using the same brain regions 28d post-conditioning (Retrieval II) the number of DEGs decreased in all regions except BLA, where it almost doubled (140 vs 253 DEGs).

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BOARD NUMBER: S02-160

CHEMOGENETIC CONTROL OF TMNHA NEURONS ACTIVITY AFFECTS MALADAPTIVE COGNITIVE RESPONSES TO CHRONIC STRESS IN MICE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Background: Exposure to repeated social stress may cause maladaptive emotional reactions. Histaminergic neurotransmission has a central role in orchestrating specific behavioural responses depending on the homeostatic state of a subject, but it remains to be established if it participates in stress susceptibility and resilience. Aim: The study aims at understanding the full implications of activating or silencing hypothalamic histaminergic neurons in the development of cognitive and behavioural impairments induced by repeated exposures to stressful events. Methods: we used the DREADDs-driven technology injecting HDC-Cre male mice bilaterally into the TMN with excitatory or inhibitory DREADDs to transfect histaminergic neurons. Mice underwent the chronic social defeat (CSD) stress protocol for variable periods of time during which they received clozapine N-oxide i.p. daily. Mice were then tested for social interaction and short-term memory by using the novel object recognition test. Results: Chemogenetic activation of histaminergic neurons during a 10-day CSD protocol improved the social interaction index and memory in the novel object recognition test. On the other hand, chemogenetic inhibition of histaminergic neurons during a 3-day CSD worsened the social interaction index and short-term memory. Conclusions: Our data strongly suggest that the chemogenetic activation of TMN^{HA} neurons confers a pro-resilient phenotype to repeated exposure to stressful events, whereas their inactivation renders the subject more susceptible. Our results pave the way for future studies to deconstruct specific histaminergic neural pathways involved in the observed effects and may open new strategies for the treatment of specific maladaptive behaviours manifested in psychiatric disorders.

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BOARD NUMBER: S02-161

EFFECTS OF D-AMPHETAMINE ON NOSE-POKE RESPONDING IN AN APPETITIVE PAVLOVIAN INHIBITORY LEARNING TASK USING WISTAR RATS.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aim: Amphetamine is an indirect dopamine agonist with generally performance enhancing effects, tested here in an appetitive within-subjects inhibitory learning (A+/AX-) procedure (Waite *et al*. 10.1016/j.physbeh.2021.113557). **Methods:** Excitatory training was followed by inhibitory discrimination, summation and retardation test stages. Experiments 2-3 included some variations in the behavioural procedure, e.g. increased inter-trial-interval. D-amphetamine (sub-cutaneous) was administered at inhibitory discrimination and summation in Experiments 1-2 and before summation and retardation tests in Experiment 3. Data were analysed in mixed ANOVAs with between-subjects factors of dose (Experiment 1: 1.5mg/kg, 1mg/kg and saline and Experiments 2-3: 1.0mg/kg, 0.5mg/kg and saline). **Results:** Experiment 1 showed no clear inhibitory discrimination (A+/AX-) across groups, an overall effect of dose because of reduced nose-poking at 1.5mg/kg, but no interaction between dose and discrimination. The expression of inhibitory learning at the retardation test was impaired in animals which acquired the inhibitory discrimination under *d*-amphetamine (1.5mg/kg). Experiment 2 showed overall clear inhibitory discrimination, and nose-poking was generally increased at 0.5mg/kg, but there was no expression of inhibitory learning at the drug-free retardation test. In Experiment 3, the inhibitory discrimination was learned, but there was no expression of inhibitory learning (now under drug) at retardation. In all experiments, the summation test was overall passed by the weak control (C>CX). **Conclusions:** D-amphetamine had dose-dependent effects, though these differed in profile by experiment: 1.5mg/kg reduced and 0.5mg/kg increased nose-poking. Despite these shifts in baseline responding, there were no consistent effects of d-amphetamine on the acquisition of inhibitory discrimination, summation or retardation test performance.

Pubmed:
34400194: Waite L, Bonardi C, Stevenson CW, Cassaday HJ
Strain comparisons in inhibitory discrimination learning and novel object recognition procedures.
Strain differences in visual abilities and exploratory tendencies can confound rats' performance in cognitive tests of learning and memory. In the present study we compared the performance of albino Wistar and pigmented Lister Hooded rats in appetitive conditioning and recognition memory procedures, specifically within-subjects inhibitory learning (A+ /AX-) and novel object recognition (NOR) variants. The inhibition task included an excitatory training stage and summation and retardation tests. Difference scores were used to help control for individual variation in baseline nosepoke responding. NOR was tested after a 10 min delay, following 24hr delay and using a recency variant. Discrimination ratios were used to control for individual variation in exploratory activity. In the inhibitory learning procedure, Lister Hooded showed more magazine activity prior to stimulus presentations than Wistar rats but this was a transient effect restricted to day 1 of excitatory training. There was no strain difference in associative learning at the excitatory training stage. The Wistars went on to show some performance advantage at the inhibitory discrimination stage and marginally stronger retardation test performance. In the NOR tasks, there was no significant effect of strain on cognitive performance, but the Wistars showed some advantage in the 10 min delay variant, whereas in the 24hr delay and relative recency NOR variants, the Lister Hooded rats showed some advantage. Overall the results of the present study confirm the suitability of Wistar rats for use in associative learning and basic NOR procedures.
Physiol Behav, 2021; 240
26827956: Orme RP, Middleditch C, Waite L, Fricker RA
The Role of Vitamin D_3 in the Development and Neuroprotection of Midbrain Dopamine Neurons.
Vitamin D has long been synonymous with bone health. More recently, new health benefits are continually being associated with vitamin D, including a burgeoning field on neuroprotective properties. This has generated a huge explosion of interest in recent years in the potential for vitamin D to be used not only as a therapeutic in neurodegenerative disease, including

Parkinson's disease, but also as biomarkers and for risk association. With an emphasis on Parkinson's disease, this chapter will discuss recent evidence supporting the assertion that vitamin D can be a useful therapeutic agent used as an intervention therapy to be combined with existing treatments; and the case for further development of novel treatments utilizing the potential of vitamin D. In addition, we present novel, previously unpublished evidence showing that in a unilateral model of Parkinson's disease, vitamin D can not only reduce the extent of denervation, but that this is also reflected in functional benefit to the animals. The potential of vitamin D is slowly being realized; in the future, it will be widely associated with far more than just bone health and may even contribute to an elusive treatment of neurodegenerative illness.
Vitam Horm, 2016; 100

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-162

SYNAPTIC INTEGRATION IN THE MOUSE MIDBRAIN DURING INSTINCTIVE DEFENSIVE BEHAVIOUR

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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When faced with threat, animals instinctively react with defensive behaviours, such as escape to a safe place or freezing in a motionless crouching posture. It has recently been shown that activation of neurons in the dorsal periaqueductal gray (dPAG) is the main step for commanding escape initiation. The biophysical and synaptic integration properties in these neurons are therefore critical for controlling escape behaviour but they have not been measured in behaving animals. Here we have investigated how synaptic inputs are integrated during escape initiation and how this process is modulated in conditions that suppress or enhance the likelihood of escape. We have developed a paradigm where awake head-restrained mice navigate between a shelter and a threat zone while exposed to sensory stimuli mimicking approaching predators. Using whole-cell recordings from dPAG neurons we characterized synaptic responses during threat presentation and escape initiation. We found that escape-eliciting stimuli cause depolarizations that are temporally summated across stimuli repetitions and are correlated with escape vigour. Presentation of stimuli that increase escape probability caused sustained depolarization that lasted for several seconds and reduced the amount of synaptic input needed to reach action potential threshold. Conversely, freezing-eliciting stimuli caused hyperpolarization, indicating that escape initiation is actively suppressed by inhibitory input. Our findings suggest that modulating the baseline rate of excitatory and inhibitory synaptic input onto dPAG neurons bidirectionally control the escape initiation threshold. These modulations provide a means for rapid adjustments of escape threshold and may thus be a crucial element supporting survival in dynamic environments.

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CACNAC 1C GENETIC MODEL OF PSYCHOSIS IEG´S EXPRESSION INCREASES IN THE PREFRONTAL CORTEX AND AMYGDALA AFTER PAVLOVIAN APPETITIVE EXTINCTION AND RENEWAL.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Most studies on the neural bases of extinction learning dealt with the phenomenon of extinction learning from the perspective of aversive conditioning. However, the neural bases underlying the altered behavioural responses in natural or everyday forms of extinction learning is still controversial. Extinction of appetitive associations is indeed a key to understanding clinical disorders such as abnormal persistent appetite, contextual drug associations and some psychotic symptoms in which failure of extinction may play a major role. Psychosis disorders such as schizophrenia or bipolar disorders affect 3% of the population. These patients present symptoms as delusions and hallucinations that are related to extinction learning (EL) failures. While it is known that psychosis is accompanied by changes in neuronal excitability and synaptic plasticity, the neural circuits modifications associated with the cognitive impairments remain largely unknown In this study, we investigated the extinction and reactivation of a pavlovian conditioned response in the hemizigotic deletion rat model (cacna1c $^{+}/_{-}$). This gene plays an important role in regulating gene expression of L-Type Voltage-Gated Calcium Channels involved in synaptic plasticity and learning, being strongly associated by GWAS with Schizophrenia and Bipolar Disorder. Here, we used fluorescence in situ hybridization of somatic immediate early gene (IEG) expression to scrutinize genotype related brain regional changes at the prefrontal cortex and amygdaloid nuclei in the psychosis model as compared to healthy subjects.
This work was supported by a German Research Foundation (DFG) grant to MMC and PG (SFB 1280/Treasureproject. Project number: 316803389)

Pubmed:
34838984: Méndez-Couz M, González-Pardo H, Arias JL, Conejo NM
Hippocampal neuropeptide Y receptor blockade improves spatial memory retrieval and modulates limbic brain metabolism.
The neuropeptide Y (NPY) is broadly distributed in the central nervous system (CNS), and it has been related to neuroprotective functions. NPY seems to be an important component to counteract brain damage and cognitive impairment mediated by drugs of abuse and neurodegenerative diseases, and both NPY and its Y receptor (YR) are highly expressed in the hippocampus, critical for learning and memory. We have recently demonstrated its influence on cognitive functions; however, the specific mechanism and involved brain regions where NPY modulates spatial memory by acting on YR remain unclear.
Neurobiol Learn Mem, 2022; 187
33994970: Méndez-Couz M, Krenzek B, Manahan-Vaughan D
Genetic Depletion of BDNF Impairs Extinction Learning of a Spatial Appetitive Task in the Presence or Absence of the Acquisition Context.
Brain derived neurotropic factor (BDNF) supports neuronal survival, growth, and differentiation and is involved in forms of hippocampus-dependent and independent learning, as well as hippocampus-dependent learning. Extinction learning comprises active inhibition of no-longer relevant learned information, in conjunction with a decreased response of a previously learned behavior. It is highly dependent on context, and evidence exists that it requires hippocampal activation. The participation of BDNF in memory processing is experience-dependent. For example, BDNF has been associated with synaptic plasticity needed for spatial learning, and it is involved in acquisition and extinction learning of fear conditioning. However, little is known about its role in spatial appetitive extinction learning. In this study, we evaluated to what extent BDNF contributes to spatial appetitive extinction learning in the presence (ABA) or absence (AAA) of exposure to the acquisition context. Daily training, of BDNF-mice or their wildtype (WT) littermates, to reach acquisition criterion in a T-maze, resulted in a similar performance outcome. However, extinction learning was delayed in the AAA, and impaired in the ABA-paradigm compared to performance in WT littermates. Trial-by-trial learning analysis indicated differences in the integration of the

context into extinction learning by BDNF-mice compared to WT littermates. Taken together, these results support an important role for BDNF in processes that relate to information updating and retrieval that in turn are crucial for effective extinction learning.
Front Behav Neurosci, 2021; 15
33631483: Méndez-Couz M, Manahan-Vaughan D, Silva AP, González-Pardo H, Arias JL, Conejo NM
Corrigendum to "Metaplastic contribution of neuropeptide Y receptors to spatial memory" [Behav. Brain Res. 396 (2020) 112864].
Behav Brain Res, 2021; 404
32827566: Méndez-Couz M, Manahan-Vaughan D, Silva AP, González-Pardo H, Arias JL, Conejo NM
Metaplastic contribution of neuropeptide Y receptors to spatial memory acquisition.
Neuropeptide Y (NPY) is highly abundant in the brain and is released as a co-transmitter with plasticity-related neurotransmitters such as glutamate, GABA and noradrenaline. Functionally, its release is associated with appetite, anxiety, and stress regulation. NPY acting on Y2 receptors (YR), facilitates fear extinction, suggesting a role in associative memory. Here, we explored to what extent NPY action at YR contributes to hippocampus-dependent spatial memory and found that dorsal intrahippocampal receptor antagonism improved spatial reference memory acquired in a water maze in rats, without affecting anxiety levels, or spontaneous motor activity. Water maze training resulted in an increase of YR, but not YR expression in the hippocampus. By contrast, in the prefrontal cortex there was a decrease in YR, and an increase of YR expression. Our results indicate that neuropeptide YR are significantly involved in hippocampus-dependent spatial memory and that receptor expression is dynamically regulated by this learning experience. Effects are consistent with a metaplastic contribution of NPY receptors to cumulative spatial learning.
Behav Brain Res, 2021; 396
31798429: Méndez-Couz M, Becker JM, Manahan-Vaughan D
Functional Compartmentalization of the Contribution of Hippocampal Subfields to Context-Dependent Extinction Learning.
During extinction learning (EL), an individual learns that a previously learned behavior no longer fulfills its original purpose, or is no longer relevant. Recent studies have contradicted earlier theories that EL comprises forgetting, or the inhibition of the previously learned behavior, and indicate that EL comprises new associative learning. This suggests that the hippocampus is involved in this process. Empirical evidence is lacking however. Here, we used fluorescence hybridization of somatic immediate early gene (IEG) expression to scrutinize if the hippocampus processes EL. Rodents engaged in context-dependent EL and were also tested for renewal of (the original behavioral response to) a spatial appetitive task in a T-maze. Whereas distal and proximal CA1 subfields processed both EL and renewal, effects in the proximal CA1 were more robust consistent with a role of this subfield in processing context. The lower blade of the dentate gyrus (DG) and the proximal CA3 subfields were particularly involved in renewal. Responses in the distal and proximal CA3 subfields suggest that this hippocampal subregion may also contribute to the evaluation of the reward outcome. Taken together, our findings provide novel and direct evidence for the involvement of distinct hippocampal subfields in context-dependent EL and renewal.
Front Behav Neurosci, 2019; 13
27102086: Méndez-Couz M, González-Pardo H, Vallejo G, Arias JL, Conejo NM
Spatial memory extinction differentially affects dorsal and ventral hippocampal metabolic activity and associated functional brain networks.
Previous studies showed the involvement of brain regions associated with both spatial learning and associative learning in spatial memory extinction, although the specific role of the dorsal and ventral hippocampus and the extended hippocampal system including the mammillary body in the process is still controversial. The present study aimed to identify the involvement of the dorsal and ventral hippocampus, together with cortical regions, the amygdaloid nuclei, and the mammillary bodies in the extinction of a spatial memory task. To address these issues, quantitative cytochrome c oxidase histochemistry was applied as a metabolic brain mapping method. Rats were trained in a reference memory task using the Morris water maze, followed by an extinction procedure of the previously acquired memory task. Results show that rats learned successfully the spatial memory task as shown by the progressive decrease in measured latencies to reach the escape platform and the results obtained in the probe test. Spatial memory was subsequently extinguished as shown by the descending preference for the previously reinforced location. A control naïve group was added to ensure that brain metabolic changes were specifically related with performance in the spatial memory extinction task. Extinction of the original spatial learning task significantly modified the metabolic activity in the dorsal and ventral hippocampus, the amygdala and the mammillary bodies. Moreover, the ventral hippocampus, the lateral mammillary body and the retrosplenial cortex were differentially recruited in the spatial memory extinction task, as shown by group differences in brain metabolic networks. These findings provide new insights on the brain regions and functional brain networks underlying spatial memory, and specifically spatial memory extinction. © 2016 Wiley Periodicals, Inc.
Hippocampus, 2016; 26

25813749: Méndez-Couz M, Conejo NM, Vallejo G, Arias JL
Brain functional network changes following Prelimbic area inactivation in a spatial memory extinction task.
Several studies suggest a prefrontal cortex involvement during the acquisition and consolidation of spatial memory, suggesting an active modulating role at late stages of acquisition processes. Recently, we have reported that the prelimbic and infralimbic areas of the prefrontal cortex, among other structures, are also specifically involved in the late phases of spatial memory extinction. This study aimed to evaluate whether the inactivation of the prelimbic area of the prefrontal cortex impaired spatial memory extinction. For this purpose, male Wistar rats were implanted bilaterally with cannulae into the prelimbic region of the prefrontal cortex. Animals were trained during 5 consecutive days in a hidden platform task and tested for reference spatial memory immediately after the last training session. One day after completing the training task, bilateral infusion of the GABAA receptor agonist Muscimol was performed before the extinction protocol was carried out. Additionally, cytochrome c oxidase histochemistry was applied to map the metabolic brain activity related to the spatial memory extinction under prelimbic cortex inactivation. Results show that animals acquired the reference memory task in the water maze, and the extinction task was successfully completed without significant impairment. However, analysis of the functional brain networks involved by cytochrome oxidase activity interregional correlations showed changes in brain networks between the group treated with Muscimol as compared to the saline-treated group, supporting the involvement of the mammillary bodies at a the late stage in the memory extinction process.
Behav Brain Res, 2015; 287
25680583: Méndez-Couz M, Conejo NM, González-Pardo H, Arias JL
Functional interactions between dentate gyrus, striatum and anterior thalamic nuclei on spatial memory retrieval.
The standard model of memory system consolidation supports the temporal reorganization of brain circuits underlying long-term memory storage, including interactions between the dorsal hippocampus and extra-hippocampal structures. In addition, several brain regions have been suggested to be involved in the retrieval of spatial memory. In particular, several authors reported a possible role of the ventral portion of the hippocampus together with the thalamus or the striatum in the persistence of this type of memory. Accordingly, the present study aimed to evaluate the contribution of different cortical and subcortical brain regions, and neural networks involved in spatial memory retrieval. For this purpose, we used cytochrome c oxidase quantitative histochemistry as a reliable method to measure brain oxidative metabolism. Animals were trained in a hidden platform task and tested for memory retention immediately after the last training session; one week after completing the task, they were also tested in a memory retrieval probe. Results showed that retrieval of the previously learned task was associated with increased levels of oxidative metabolism in the prefrontal cortex, the dorsal and ventral striatum, the anterodorsal thalamic nucleus and the dentate gyrus of the dorsal and ventral hippocampus. The analysis of functional interactions between brain regions suggest that the dorsal and ventral dentate gyrus could be involved in spatial memory retrieval. In addition, the results highlight the key role of the extended hippocampal system, thalamus and striatum in this process. Our study agrees with previous ones reporting interactions between the dorsal hippocampus and the prefrontal cortex during spatial memory retrieval. Furthermore, novel activation patterns of brain networks involving the aforementioned regions were found. These functional brain networks could underlie spatial memory retrieval evaluated in the Morris water maze task.
Brain Res, 2015; 1605
24315832: Méndez-Couz M, Conejo NM, Vallejo G, Arias JL
Spatial memory extinction: a c-Fos protein mapping study.
While the neuronal basis of spatial memory consolidation has been thoroughly studied, the substrates mediating the process of extinction remain largely unknown. This study aimed to evaluate the functional contribution of selected brain regions during the extinction of a previously acquired spatial memory task in the Morris water maze. For that purpose, we used adult male Wistar rats trained in a spatial reference memory task. Learning-related changes in c-Fos inmunoreactive cells after training were evaluated in cortical and subcortical regions. Results show that removal of the hidden platform in the water maze induced extinction of the previously reinforced escape behavior after 16 trials, without spontaneous recovery 24h later. Extinction was related with significantly higher numbers of c-Fos positive nuclei in amygdala nuclei and prefrontal cortex. On the other hand, the lateral mammillary bodies showed higher number of c-Fos positive cells than the control group. Therefore, in contrast with the results obtained in studies of classical conditioning, we show the involvement of diencephalic structures mediating this kind of learning. In summary, our findings suggest that medial prefrontal cortex, the amygdala complex and diencephalic structures like the lateral mammillary nuclei are relevant for the extinction of spatial memory.
Behav Brain Res, 2014; 260
23724089: Conejo NM, Cimadevilla JM, González-Pardo H, Méndez-Couz M, Arias JL
Hippocampal inactivation with TTX impairs long-term spatial memory retrieval and modifies brain metabolic activity.
Functional inactivation techniques enable studying the hippocampal involvement in each phase of spatial memory formation in the rat. In this study, we applied tetrodotoxin unilaterally or bilaterally into the dorsal hippocampus to evaluate the role of

this brain structure in retrieval of memories acquired 28 days before in the Morris water maze. We combined hippocampal inactivation with the assessment of brain metabolism using cytochrome oxidase histochemistry. Several brain regions were considered, including the hippocampus and other related structures. Results showed that both unilateral and bilateral hippocampal inactivation impaired spatial memory retrieval. Hence, whereas subjects with bilateral hippocampal inactivation showed a circular swim pattern at the side walls of the pool, unilateral inactivation favoured swimming in the quadrants adjacent to the target one. Analysis of cytochrome oxidase activity disclosed regional differences according to the degree of hippocampal functional blockade. In comparison to control group, animals with bilateral inactivation showed increased CO activity in CA1 and CA3 areas of the hippocampus during retrieval, while the activity of the dentate gyrus substantially decreased. However, unilateral inactivated animals showed decreased CO activity in Ammon's horn and the dentate gyrus. This study demonstrated that retrieval recruits differentially the hippocampal subregions and the balance between them is altered with hippocampal functional lesions.
PLoS One, 2013; 8

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BOARD NUMBER: S02-164

INHIBITION OF THE AGE-RELATED METABOLIC RISK FACTOR QUINONE REDUCTASE 2 (QR2) REDUCES BRAIN PATHOLOGIES AND IMPROVES MEMORY OF AGED ALZHEIMER'S DISEASE MODEL MICE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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The main risk factor for developing neurodegenerative diseases, including Alzheimer's disease (AD), is biological ageing, namely, the prolonged and accumulating metabolic stress within cells, tissues and organs. Finding targetable processes contributing to age-related metabolic stress, that will serve at the same time as a cognitive enhancers, is therefore of major importance. We have recently found that the age-related metabolic risk factor quinone reductase 2 (QR2) functions in the brain. Normally, QR2 acts as a metabolic buffer, downstream of acetylcholine (ACh) or dopamine (DA), depending on brain region and sensory input. Following a salient experience ACh/DA release drive removal of QR2 via micro-RNA-182 (miR-182), which reduces physiological levels of reactive oxygen species (ROS) that QR2 generates within cells. This affects neuronal intrinsic properties, primarily reducing interneuron excitability, thus enabling a transient shift in brain excitation/inhibition balance. The outcome is enhanced memory for salient/important events, which makes them stand out compared to more trivial information, for example - "first time" experiences. However, with age DA/ACh and miR-182 are diminished, while QR2 is overexpressed. This natural process places QR2 as a potentially novel contributor to AD aetiology. We therefore developed novel, selective, non-toxic and orally bioavailable QR2 inhibitors (QR2i), and evaluated their effect on wild-type- as well as aged, AD model mice. We found that mice were able to perform significantly better in a number of memory tasks, while brain pathologies were significantly reduced. QR2i, therefore, represent a novel way to reduce age-related metabolic stress, and a potential AD drug target.

Pubmed:

34518366: Gould NL, Kolatt Chandran S, Kayyal H, Edry E, Rosenblum K
Somatostatin Interneurons of the Insula Mediate QR2-Dependent Novel Taste Memory Enhancement.
Forming long-term memories is crucial for adaptive behavior and survival in changing environments. The molecular consolidation processes which underlie the formation of these long-term memories are dependent on protein synthesis in excitatory and SST-expressing neurons. A centrally important, parallel process to this involves the removal of the memory constraint quinone reductase 2 (QR2), which has been recently shown to enhance memory consolidation for novel experiences in the cortex and hippocampus, via redox modulation. However, it is unknown within which cell type in the cortex removal of QR2 occurs, nor how this affects neuronal function. Here, we use novel taste learning in the mouse anterior insular cortex (aIC) to show that similarly to mRNA translation, QR2 removal occurs in excitatory and SST-expressing neurons. Interestingly, both novel taste and QR2 inhibition reduce excitability specifically within SST, but not excitatory neurons. Furthermore, reducing QR2 expression in SST, but not in PV or excitatory neurons, is sufficient to enhance taste memory. Thus, QR2 mediated intrinsic property changes of SST interneurons in the aIC is a central removable factor to allow novel taste memory formation. This previously unknown involvement of QR2 and SST interneurons in resetting aIC activity hours following learning, describes a molecular mechanism to define cell circuits for novel information. Therefore, the QR2 pathway in SST interneurons provides a fresh new avenue by which to tackle age-related cognitive deficits, while shedding new light onto the functional machinations of long-term memory formation for novel information.
eNeuro, 2021 Sep-Oct; 8
33046554: Gould NL, Sharma V, Hleihil M, Kolatt Chandran S, David O, Edry E, Rosenblum K
Dopamine-Dependent QR2 Pathway Activation in CA1 Interneurons Enhances Novel Memory Formation.
The formation of memory for a novel experience is a critical cognitive capacity. The ability to form novel memories is sensitive to age-related pathologies and disease, to which prolonged metabolic stress is a major contributing factor. Presently, we describe a dopamine-dependent redox modulation pathway within the hippocampus of male mice that promotes memory consolidation. Namely, following novel information acquisition, quinone reductase 2 (QR2) is suppressed by miRNA-182 (miR-182) in the CA1 region of the hippocampus via dopamine D1 receptor (D1R) activation, a process largely facilitated by locus coeruleus activity. This pathway activation reduces ROS generated by QR2 enzymatic activity, a process that alters the

intrinsic properties of CA1 interneurons 3 h following learning, in a form of oxidative eustress. Interestingly, novel experience decreases QR2 expression predominately in inhibitory interneurons. Additionally, we find that in aged animals this newly described QR2 pathway is chronically under activated, resulting in miR-182 underexpression and QR2 overexpression. This leads to accumulative oxidative stress, which can be seen in CA1 via increased levels of oxidized, inactivated potassium channel Kv2.1, which undergoes disulfide bridge oligomerization. This newly described interneuron-specific molecular pathway lies alongside the known mRNA translation-dependent processes necessary for long-term memory formation, entrained by dopamine in CA1. It is a process crucial for the distinguishing features of novel memory, and points to a promising new target for memory enhancement in aging and age-dependent diseases. One way in which evolution dictates which sensory information will stabilize as an internal representation, relies on information novelty. Dopamine is a central neuromodulator involved in this process in the mammalian hippocampus. Here, we describe for the first time a dopamine D1 receptor-dependent quinone reductase 2 pathway in interneurons. This is a targeted redox event necessary to delineate a novel experience to a robust long-term internal representation. Activation of this pathway alone can explain the effect novelty has on "flashbulb" memories, and it can become dysfunctional with age and diseases, such as Alzheimer's disease.
J Neurosci, 2020; 40

32217627: Gould NL, Elkobi A, Edry E, Daume J, Rosenblum K

Muscarinic-Dependent miR-182 and QR2 Expression Regulation in the Anterior Insula Enables Novel Taste Learning.

In a similar manner to other learning paradigms, intact muscarinic acetylcholine receptor (mAChR) neurotransmission or protein synthesis regulation in the anterior insular cortex (aIC) is necessary for appetitive taste learning. Here we describe a parallel local molecular pathway, where GABA receptor control of mAChR activation causes upregulation of miRNA-182 and quinone reductase 2 (QR2) mRNA destabilization in the rodent aIC. Damage to long-term memory by prevention of this process, with the use of mAChR antagonist scopolamine before novel taste learning, can be rescued by local QR2 inhibition, demonstrating that QR2 acts downstream of local muscarinic activation. Furthermore, we prove for the first time the presence of endogenous QR2 cofactors in the brain, establishing QR2 as a functional reductase there. In turn, we show that QR2 activity causes the generation of reactive oxygen species, leading to modulation in Kv2.1 redox state. QR2 expression reduction therefore is a previously unaccounted mode of mAChR-mediated inflammation reduction, and thus adds QR2 to the cadre of redox modulators in the brain. The concomitant reduction in QR2 activity during memory consolidation suggests a complementary mechanism to the well established molecular processes of this phase, by which the cortex gleans important information from general sensory stimuli. This places QR2 as a promising new target to tackle neurodegenerative inflammation and the associated impediment of novel memory formation in diseases such as Alzheimer's disease.
eNeuro, 2020 May/Jun; 7

32499677: David O, Barrera I, Gould N, Gal-Ben-Ari S, Rosenblum K

D1 Dopamine Receptor Activation Induces Neuronal eEF2 Pathway-Dependent Protein Synthesis.

Dopamine, alongside other neuromodulators, defines brain and neuronal states, through regulation of global and local mRNA translation. Yet, the signaling pathways underlying the effects of dopamine on mRNA translation and psychiatric disorders are not clear. In order to examine the molecular pathways downstream of dopamine receptors, we used genetic, pharmacologic, biochemical, and imaging methods, and found that activation of dopamine receptor D1 but not D2 leads to rapid dephosphorylation of eEF2 at Thr but not eIF2α in cortical primary neuronal culture in a time-dependent manner. NMDA receptor, mTOR, and ERK pathways are upstream of the D1 receptor-dependent eEF2 dephosphorylation and essential for it. Furthermore, D1 receptor activation resulted in a major reduction in dendritic eEF2 phosphorylation levels. D1-dependent eEF2 dephosphorylation results in an increase of BDNF and synapsin2b expression which was followed by a small yet significant increase in general protein synthesis. These results reveal the role of dopamine D1 receptor in the regulation of eEF2 pathway translation in neurons and present eEF2 as a promising therapeutic target for addiction and depression as well as other psychiatric disorders.
Front Mol Neurosci, 2020; 13

33930301: Yiannakas A, Kolatt Chandran S, Kayyal H, Gould N, Khamaisy M, Rosenblum K

Parvalbumin interneuron inhibition onto anterior insula neurons projecting to the basolateral amygdala drives aversive taste memory retrieval.

Memory retrieval refers to the fundamental ability of organisms to make use of acquired, sometimes inconsistent, information about the world. Although memory acquisition has been studied extensively, the neurobiological mechanisms underlying memory retrieval remain largely unknown. Conditioned taste aversion (CTA) is a robust associative paradigm, through which animals can be trained to express aversion toward innately appetitive tastants. The anterior insula (aIC) is indispensable in the ability of mammals to retrieve associative information regarding tastants that have been previously linked with gastric malaise. Here, we show that CTA memory retrieval promotes cell-type-specific activation in the aIC. Using chemogenetic tools in the aIC, we found that CTA memory acquisition requires activation of excitatory neurons and inhibition of inhibitory neurons, whereas retrieval necessitates activation of both excitatory and inhibitory aIC circuits. CTA memory retrieval at the

aIC activates parvalbumin (PV) interneurons and increases synaptic inhibition onto activated pyramidal neurons projecting to the basolateral amygdala (aIC-BLA). Unlike innately appetitive taste memory retrieval, CTA retrieval increases synaptic inhibition onto aIC-BLA-projecting neurons that is dependent on activity in aIC PV interneurons. PV aIC interneurons coordinate CTA memory retrieval and are necessary for its dominance when conflicting internal representations are encountered over time. The reinstatement of CTA memories following extinction is also dependent on activation of aIC PV interneurons, which increase the frequency of inhibition onto aIC-BLA-projecting neurons. This newly described interaction of PV and a subset of excitatory neurons can explain the coherency of aversive memory retrieval, an evolutionary pre-requisite for animal survival.
Curr Biol, 2021; 31
34219650: Kayyal H, Chandran SK, Yiannakas A, Gould N, Khamaisy M, Rosenblum K
Insula to mPFC reciprocal connectivity differentially underlies novel taste neophobic response and learning in mice.
To survive in an ever-changing environment, animals must detect and learn salient information. The anterior insular cortex (aIC) and medial prefrontal cortex (mPFC) are heavily implicated in salience and novelty processing, and specifically, the processing of taste sensory information. Here, we examined the role of aIC-mPFC reciprocal connectivity in novel taste neophobia and memory formation, in mice. Using pERK and neuronal intrinsic properties as markers for neuronal activation, and retrograde AAV (rAAV) constructs for connectivity, we demonstrate a correlation between aIC-mPFC activity and novel taste experience. Furthermore, by expressing inhibitory chemogenetic receptors in these projections, we show that aIC-to-mPFC activity is necessary for both taste neophobia and its attenuation. However, activity within mPFC-to-aIC projections is essential only for the neophobic reaction but not for the learning process. These results provide an insight into the cortical circuitry needed to detect, react to- and learn salient stimuli, a process critically involved in psychiatric disorders.
Elife, 2021; 10

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BOARD NUMBER: S02-165

MITOCHONDRIA, ADULT HIPPOCAMPAL NEUROGENESIS, AND ANXIETY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Hippocampal adult neurogenesis involves the generation of new granule neurons in the dentate gyrus and this process has been linked to hippocampal-related behaviors, such as spatial navigation, learning, anxiety, stress regulation, and social cognition. Adult hippocampal neurogenesis is increased by antidepressants, and is required for some of their behavioral effects. However, it remains unclear whether expanding the population of adult-born neurons is sufficient to affect anxiety and depression-related behaviors. Emerging evidence indicates that mitochondria can regulate stem cell fate decisions and are crucial for adult neurogenesis. Nevertheless, the role of both mitochondrial molecular components as well as their functions in adult neurogenesis remains barely understood. In this study, we investigated the molecular underpinnings that link basal anxiety with differences in mitochondrial function in stem cells and newly generated neurons in the dentate gyrus. To this end, we exploited an animal model of natural diversity in anxiety trait and applied different behavioral manipulations (i.e., spontaneous wheel running; chronic stress) to modulate both anxiety and neurogenesis. Our results identify molecules implicated in mitochondrial dynamics as differently expressed in the dentate gyrus of high and low anxious rats. These differences were observed along with variation in mitochondrial respiration. Our findings provide critical information to understand how anxiety affects adult neurogenesis and which factors are important for the regulation of neural stem cell proliferation and the generation of neurons.

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BOARD NUMBER: S02-166

THE ROLE OF PREFRONTAL SOMATOSTATIN INTERNEURONS AND NEUROTROPHIN SIGNALING IN STRESS COPING

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aims. Adequate coping during stressful challenges are essential to adapt, hence, it is regulated by highly conserved brain mechanisms. Alterations of this circuit contribute to the development of affective disorders characterized by shifts towards passive coping (avoidance, inactivity). Accumulating evidence show significant alterations in prefrontal networks with marked changes in somatostatin (SST) interneurons involving altered neurotrophic signaling (Brain-derived neurotrophic factor-BDNF) under these conditions, however causal pathogenetic mechanisms are to be clarified. Methods. We investigated how SST neuron-specific knockout of the tyrosine receptor kinase B (TrkB) receptors alters coping behavior using sst-ires-cre x $TrkB^{flox/flox}$ transgenic male mice (SST-TrkB-CKO). We assessed coping in the Forced swim, Tail suspension, and Back tests. We assessed whole-brain activity using immunohistochemical labeling of c-Fos and other cell-type markers with subsequent quantification of signal counts by an automated method. We also explored major gene expression changes related to TrkB dysfunction using qPCR in order to uncover subcellular mechanisms. Results. We found that TrkB dysfunction of SST neurons resulted in significantly increased active coping indicated by reduced immobility in all tests. We identified complex network alterations involving neuronal populations e.g. in the amygdala, hippocampus and sensory systems. We also describe expression changes of major gene candidates altered by TrkB dysfunction. Conclusions. Altered development of prefrontal SST neurons and their altered neurotrophin signaling pathways significantly modulates coping responses. Accordingly, these subcellular and neuronal network alterations can contribute to the development of affective disorders characterized by dominant passive coping.

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BOARD NUMBER: S02-167

FUNCTIONAL DISSOCIATION OF VENTRAL HIPPOCAMPAL INHIBITORY CIRCUITS DURING ANXIETY AND FEAR BEHAVIORS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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The ability to predict potentially harmful environments and escape from dangerous circumstances is vital for wildlife. Mounting evidence suggests that while the dorsal subdivision of the hippocampus is associated with spatial and episodic memory formation, the ventral hippocampus is mostly involved in emotional behaviors. However, the neural circuits and mechanisms within the ventral hippocampus underlying innate or learned emotional behaviors are poorly understood. In the present work, we investigated whether anxiety and conditioned fear are represented by distinct ventral CA1 (vCA1) neural circuits. By utilizing cell-type-specific expression of calcium indicator GCaMPs and in vivo calcium imaging in freely behaving mice via miniature fluorescence microscope, we monitored the neuronal activity of vCA1 pyramidal cells and three sub-classes of GABAergic interneurons (PV+, VIP+, and SST+) during innate anxiety and conditioned fear behaviors. Our data indicated that vCA1 pyramidal cells and interneurons have distinct activity patterns during anxiety and fear behaviors. Different subpopulations of vCA1 pyramidal cells showed preferential responses to either anxiogenic experiences or fear-conditioned cues. The majority of PV+ interneurons were recruited during anxiety behavior but barely during a cued fear test. By contrast, about half of VIP+ interneurons were involved in conditioned fear learning but not in anxiety behaviors, while SST+ interneurons displayed inhibition during fear learning, suggesting that an inhibitory microcircuit may gate pyramidal cell activity during fear conditioning. Altogether, our data suggested a division of labor among various vCA1 GABAergic interneurons during different forms of emotional behaviors.

Pubmed:

29890412: Maslyukov A, Li K, Su X, Kovalchuk Y, Garaschuk O
Spontaneous calcium transients in the immature adult-born neurons of the olfactory bulb.
Spontaneous neuronal activity and concomitant intracellular Ca signaling are abundant during early perinatal development and are well known for their key role in neuronal proliferation, migration, differentiation and wiring. However, much less is known about the in vivo patterns of spontaneous Ca signaling in immature adult-born cells. Here, by using two-photon Ca imaging, we analyzed spontaneous in vivo Ca signaling in adult-born juxtaglomerular cells of the mouse olfactory bulb over the time period of 5 weeks, from the day of their arrival in the glomerular layer till their stable integration into the preexisting neural network. We show that spontaneous Ca transients are ubiquitously present in adult-born cells right after their arrival, require activation of voltage-gated Na channels and are little sensitive to isoflurane anesthesia. Interestingly, several parameters of this spontaneous activity, such as the area under the curve, the time spent in the active state as well as the fraction of continuously active cells show a bell-shaped dependence on cell's age, all peaking in 3-4 weeks old cells. This data firmly document the in vivo presence of spontaneous Ca signaling during the layer-specific maturation of adult-born neurons in the olfactory bulb and motivate further analyses of the functional role(s) of this activity.
Cell Calcium, 2018; 74
28076784: Askew K, Li K, Olmos-Alonso A, Garcia-Moreno F, Liang Y, Richardson P, Tipton T, Chapman MA, Riecken K, Beccari S, Sierra A, Molnár Z, Cragg MS, Garaschuk O, Perry VH, Gomez-Nicola D
Coupled Proliferation and Apoptosis Maintain the Rapid Turnover of Microglia in the Adult Brain.
Microglia play key roles in brain development, homeostasis, and function, and it is widely assumed that the adult population is long lived and maintained by self-renewal. However, the precise temporal and spatial dynamics of the microglial population are unknown. We show in mice and humans that the turnover of microglia is remarkably fast, allowing the whole population to be renewed several times during a lifetime. The number of microglial cells remains steady from late postnatal stages until aging and is maintained by the spatial and temporal coupling of proliferation and apoptosis, as shown by pulse-chase studies, chronic in vivo imaging of microglia, and the use of mouse models of dysregulated apoptosis. Our results reveal that the microglial population is constantly and rapidly remodeled, expanding our understanding of its role in the maintenance of brain homeostasis.

Cell Rep, 2017; 18
27174051: Liang Y, Li K, Riecken K, Maslyukov A, Gomez-Nicola D, Kovalchuk Y, Fehse B, Garaschuk O
Long-term in vivo single-cell tracking reveals the switch of migration patterns in adult-born juxtaglomerular cells of the mouse olfactory bulb.
The behavior of adult-born cells can be easily monitored in cell culture or in lower model organisms, but longitudinal observation of individual mammalian adult-born cells in their native microenvironment still proves to be a challenge. Here we have established an approach named optical cell positioning system for long-term in vivo single-cell tracking, which integrates red-green-blue cell labeling with repeated angiography. By combining this approach with in vivo two-photon imaging technique, we characterized the in vivo migration patterns of adult-born neurons in the olfactory bulb. In contrast to the traditional view of mere radial migration of adult-born cells within the bulb, we found that juxtaglomerular cells switch from radial migration to long distance lateral migration upon arrival in their destination layer. This unique long-distance lateral migration has characteristic temporal (stop-and-go) and spatial (migratory, unidirectional or multidirectional) patterns, with a clear cell age-dependent decrease in the migration speed. The active migration of adult-born cells coincides with the time period of initial fate determination and is likely to impact on the integration sites of adult-born cells, their odor responsiveness, as well as their survival rate.
Cell Res, 2016; 26
20127820: Feng Z, Li K, Liu M, Wen C
NRAGE is a negative regulator of nerve growth factor-stimulated neurite outgrowth in PC12 cells mediated through TrkA-ERK signaling.
NRAGE, also denominated as MAGE-D1 or Dlxin-1, is firstly identified as a molecule interacting with NGF low affinity receptor p75NTR. It facilitates cell cycle arrest and NGF-dependent neuronal apoptosis. Here we report that NRAGE is downregulated while p75NTR is upregulated during the process of NGF-induced neuronal differentiation of PC12 cells. Knockdown of NRAGE by RNA interference accelerates NGF-mediated neurite outgrowth. In addition, in the NRAGE-suppressed cells, NGF-induced ERK activation is increased and this activation is MEK-dependent. Conversely, NRAGE overexpression significantly represses NGF-induced ERK activation. Further studies revealed that NRAGE downregulates TrkA expression through a post-transcriptional manner and thereby blocks NGF-induced TrkA phosphrylation at tyrosine-490. Altogether, these data indicate for the first time that NRAGE is an endogenous inhibitor for NGF-induced neuronal differentiation of PC12 cells by regulating TrkA-ERK signaling.
J Neurosci Res, 2010; 88

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BOARD NUMBER: S02-168

AN INTEROCEPTIVE ROLE FOR GLYCINERGIC PERIAQUEDUCTAL GREY CIRCUITS DURING DEFENSIVE STATES

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Fear and anxiety are defensive states that evolved as adaptive responses to a threat. Inappropriate selection and inability to rapidly switch between defensive states are hallmarks of anxiety-related disorders. Defensive states encompass a multitude of coordinated and integrated responses, such as behavioral and autonomic adaptations, which in turn result in afferent information flow reporting the body's physiological state to the brain. These interoceptive processes are believed to be crucial for regulation of central emotional states. Neuronal circuits of the midbrain periaqueductal grey (PAG) critically contribute to defensive states, however, it remains poorly understood if and how they encode and integrate interoceptive signals as part of a defense reaction. Using in vivo miniscope calcium imaging, we observed activation of glycinergic neurons within the ventrolateral PAG (vlPAG) during the switch between a freezing/bradycardia state to a no-freezing/tachycardia (HR) state, in which HR precedes neuronal activity, suggesting that these neurons receive cardiac interoceptive information. Furthermore, optogenetic manipulation confirmed an involvement of vlPAG glycinergic neurons in regulating macrostate dynamics, in optoactivation caused an increase of HR variability, whereas optoinhibion had the opposite effect. Moreover, trans-synaptic retrograde tracing demonstrated monosynaptic connectivity with cardiac regulatory areas such as nucleus of solitary tract. Anterograde tracing demonstrated that PAG glycinergic neurons project almost exclusively to forebrain regions, suggesting that these neurons might be crucial to report the cardiac state to higher order brain regions. Overall, our data suggest that vlPAG glycinergic neurons contribute to defensive states via regulating cardiac interoception, a process that plays a major role in fear, anxiety and related disorders.

Pubmed:
31274188: Reis SL, Silva HB, Almeida M, Cunha RA, Simões AP, Canas PM
Adenosine A and A receptors differently control synaptic plasticity in the mouse dorsal and ventral hippocampus.
The hippocampus is a brain region involved in processing both memory and emotions, through a preferential involvement of the dorsal hippocampus (DH) and ventral hippocampus (VH), respectively. Adenosine A and A receptors (A R and A R) control both mood and memory, but it is not known if there is a different adenosine modulation of synaptic plasticity along the hippocampal axis. Using adult, C57BL/6 male mice, we show that both A R and A R were more abundant in DH compared with VH. However, recordings of field excitatory postsynaptic potentials at Schaffer collaterals-CA1 pyramidal synapses revealed that A R were equi-effective to inhibit basal excitatory synaptic transmission in DH and VH, but endogenous A R activation was more effective to depress the probability of release in VH. In contrast, the selective A R antagonist (SCH58261, 50 nM) controlled both long-term potentiation (induced by a high frequency stimulation protocol) and long-term depression (induced by a low frequency stimulation protocol) selectively in DH rather than VH, whereas the selective A R antagonist (DPCPX, 100 nM) revealed a similar tonic inhibition of long-term depression in DH and VH. These findings show a different control of synaptic plasticity by the adenosine modulation system in the dorsal and ventral poles of the hippocampus, which may underlie a different efficiency of the adenosine system to control mood and memory.
J Neurochem, 2019; 151

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BOARD NUMBER: S02-169

GLUCOSE-SENSING NEURONS IN THE INSULAR CORTEX MODULATE THE FEAR BALANCE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Glucose is the primary energy source for the brain. Its levels are constantly monitored by specialized glucose-sensing neuronal populations that have been described so far mainly in subcortical areas contributing to the regulation of energy homeostasis. The insular cortex (IC) is modulated by blood glucose levels and has been described as an interface between interoception and emotions. The aim of our work was to examine whether glucose-sensing cells are present in IC and, if so, to understand their role in emotional behavior. Monitoring c-Fos expression, we identified a cell population activated by hypoglycemia in the mouse IC. We used a genetic mouse model to target and record those cells in vitro. With this approach we confirmed that they are glucose sensing neurons. Morphological reconstructions showed that they are thick-tufted layer 5 pyramidal cells in the posterior insula. We then devised an intersectional strategy to address those cells in vivo with chemogenetic actuators. With this tool we determined that IC glucose sensing neurons affect fear-like behavior through projection to the central nucleus of the amygdala (CeA). Finally, we identified PKC delta+ neurons of the CeA as downstream connectivity of IC glucose-sensing neurons. Altogether, our data provides the first evidence of the existence of glucose-sensing neurons in the insular cortex and of a neuronal circuit linking directly interoception and emotional behavior.

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BOARD NUMBER: S02-170

ENRICHED ENVIRONMENT ATTENUATES ENHANCED TRAIT-ANXIETY AND ASSOCIATED NEURO-INFLAMMATORY DYSBALANCE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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[1]University of Innsbruck, Pharmacology & Toxicology, Innsbruck, Austria, [2]Charitè University Hospital, Berlin, Department Of Ophthalmology, Berlin, Germany, [3]Max-Delbrück-Center for Molecular Medicine in the Helmholtz Society, Berlin, Department Of Psychoneuroimmunology, Berlin, Germany

Neuroinflammation is discussed to play a role in specific subgroups of different psychiatric disorders. We have previously shown that a mouse model of trait-anxiety (HAB) displays enhanced microglial-density and phagocytic-activity in key regions of anxiety-circuits in comparison to normal anxiety controls (NAB). Using minocycline we provided proof of principle evidence that reducing microglia activation within the dentate gyrus (DG) attenuated enhanced-anxiety in HABs. Besides pharmacological interventions; “positive-stimuli”, which have the advantage of exerting no or negligible side-effects, have been shown to attenuate inflammation in humans. Therefore, in the current study, we investigated whether environmental-enrichment (EE) as a “positive intervention” would be sufficient to modulate inflammation in high anxiety HABs. We now show that EE can also attenuate enhanced-anxiety when presented during adulthood, complimenting our previous observations of such EE effects in early development. Using immunohistochemistry, we found that EE-induced anxiolysis was associated with attenuation of enhanced microglial-density in the DG and medial-prefrontal cortex. Furthermore, EE also reduced phagocytic-activity of microglia within the DG. Hence, successful attenuation of trait-anxiety by EE was associated with normalization of part of the identified neuro-inflammatory imbalances. Together with our previous pharmacological findings, these results indicate that beneficial environmental cues can partly mimic anti-inflammatory effects of minocycline in individuals predisposed to trait-anxiety. Recently, we also found sex differences in microglial-expression, microglial-morphology as well as synaptic-pruning pathways in the DG of HABs compared to NABs and we are currently investigating whether and how these contribute to EE-induced anxiolytic-like effect. Funding:FWF I3875

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BOARD NUMBER: S02-171

STRESS-INDUCED MODULATION OF MEMORY CONSOLIDATION IN THE HIPPOCAMPUS-AMYGDALA NETWORK DURING SLEEP

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aversive memories tend to be more vividly remembered than neutral ones, suggesting a differential consolidation of memories depending on their emotional valence. Stress hormones such as corticosterone (CORT), secreted during threatening events, have been proposed to mediate this selective consolidation. Associating a threat to a particular context requires the hippocampus (HPC), which integrates the spatial context, and the amygdala (BLA) which processes fear. During sleep, the coordinated HPC-BLA representation of an aversive experience is reactivated during hippocampal events called "sharp-wave ripples" (SWRs), potentially sustaining the consolidation of context-threat associations. However, how stress modifies sleep-dependent consolidation mechanisms remains unknown. To tackle this question, we are investigating how stress induced by CORT injections affects the consolidation of contextual fear learning in the HPC-BLA network. Pilot experiments assessing the effects of CORT, without prior learning, on HPC-BLA sleep oscillations show that injecting CORT increases the SWRs rate during subsequent sleep. This could be one of the mechanisms by which stress hormones modulate memory consolidation during sleep.

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BOARD NUMBER: S02-172

ADOLESCENT NICOTINE EXPOSURE DISRUPTS ITS ANXIOGENIC PROPERTIES IN ADULTHOOD

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Dopamine (DA) circuity is increasingly considered as a “plasticity system” where its structure and function is shaped by experience during development, creating adaptive behavioral profiles that can endure throughout the lifetime. This plasticity may also demarcate a period of increased vulnerability to environmental insult: adolescent initiation of drug use, in particular, is associated with an increased risk of psychopathologies linked with DA dysfunction. This may be especially true for nicotine, as up to 90% of adult smokers began as adolescents. Nicotine acts on distinct DA pathways to produce both reinforcing and anxiogenic effects: the activation of nucleus accumbens (NAc)-projecting DA neurons produces reinforcement, whereas the simultaneous inhibition of amygdala (AMG)-projecting DA neurons produces anxiety-like behavior. How experience with nicotine in adolescence modulates the anxiogenic effects of later re-exposure is largely unexplored. Remarkably, we found that the anxiogenic response to nicotine injection is abolished in adult mice pre-exposed to nicotine in adolescence, but not those pre-exposed in adulthood. Using iDISCO and ClearMap, we found that adolescent nicotine exposure augments brain-wide activation in response to an acute nicotine injection in adulthood, with notably increased cFos+ neurons in the NAc and AMG. Using single-unit recordings we found that nicotine-induced activation was stronger in adult mice exposed to nicotine in adolescence, nicotine-induced inhibition, however, was unchanged. Together, our results highlight how diverse DA pathways can be impacted by experience in adolescence, and further suggest that developmentally induced “imbalance” of these pathways may alter vulnerability profiles for later DA-dependent psychopathologies.

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ANXIETY-LIKE BEHAVIOR IN ADOLESCENT MICE PRENATALLY EXPOSED TO DIFFERENT DOSES OF LEVETIRACETAM

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

Jelena Podgorac[1], Branka Petković[1], Gordana Stojadinović[1], Ljiljana Martać[1], Slobodan Sekulić[2], Vesna Pešić[3]
[1]Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, University of Belgrade, Department Of Neurophysiology, Belgrade, Serbia, [2]Clinical Center of Vojvodina, Department Of Neurology, Novi Sad, Serbia, [3]Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, University of Belgrade, Department Of Neurobiology, Belgrade, Serbia

Aims: Levetiracetam (LEV), which belongs to a new generation of antiepileptic drugs, has been recognized as a relatively safe antiepileptic therapy, according to studies on neurodevelopmental outcomes in children exposed to LEV *in utero*, although some animal studies reported skeletal abnormalities. This study deals with the influence of prenatal exposure to LEV in the doses that cover therapeutic range in humans on gross skeletal structure and anxiety-like behavior in adolescent mice. Methods: Adult 8-weeks old female NMRI mice were randomly divided into four groups and treated subcutaneously during breeding and gestation with saline (control) or LEV in the doses of 158 mg/kg/day (LEV-158), 211 mg/kg/day (LEV-211), or 316 mg/kg/day (LEV-316). After delivery, each female with the progeny was housed separately. Weaning and separation by sex were done on the 20th postnatal day (P20). The elevated plus-maze test was used to assess the anxiety-like behavior in both male and female offspring on P37. Results: Females and males in the LEV-158 and LEV-316 group displayed less anxiety-like behavior compared to the control, while in the LEV-211 group such behavior was not observed. Sex-related differences in anxiety-like behavior were not detected within any LEV group, as well as visible skeletal malformations. Conclusion: The findings in the mouse model suggest that prenatal exposure to LEV could be associated with less anxiety-like behavior in adolescence, paying attention to the U-shaped dose-response and highlighting the behavioral outcomes of small LEV doses. Support: Ministry of Education, Science, and Technological Development of the Republic of Serbia (Contract 451-03-9/2021-14/200007).

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BOARD NUMBER: S02-174

THE NEUROMETABOLIC UNDERPINNINGS OF SOCIAL RANK – IS THERE A ROLE FOR ACCUMBAL SIRTUIN 1?

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

Silvie Ruigrok[1], Dogukan Ulgen[1], Laia Morato[1], Marie-Isabelle Guillot De Suduiraut[1], Jocelyn Grosse[1], Cristina Cudalbu[2], Carmen Sandi[1]
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Aims: Mental disorders are a major problem in modern society, and social status affects ones' susceptibility to develop such disorders. However, the neural mechanisms whereby social status impacts on mental health is still unclear. The nucleus accumbens (NAc) is a key brain region involved in motivated behaviors, including the establishment of social hierarchies. Our recent work has implicated NAc mitochondrial function as critical underlying mechanism of social status. Sirtuin 1 (SIRT1) is a central regulator of cellular metabolism, and has been related to anxiety-like behaviors. In this project, we asked whether dominant and subordinate individuals differ in their NAc SIRT1 content and whether SIRT1 is causally involved in social status and the NAc metabolic profile. Methods: We first measured SIRT1 protein levels in the NAc of dominant and submissive mice. To study a causal role for SIRT1 in social rank and anxiety, male *Sirt1*^{loxP} mice received a viral injection (AAV2-SYN-eGFP-T2A-iCre-WPRE) in the NAc, resulting in neuron-specific knockdown (KD) of SIRT1. ^{1}H-magnetic resonance spectroscopy was used to study brain metabolite levels *in vivo*. Results: We show that NAc SIRT1 protein levels are lower in subordinate mice. SIRT1 KD affected social but not anxiety-like behavior. Whether SIRT1 KD modulates dominance status and NAc metabolites is currently under investigation. Conclusion: Here, we show that social rank is related to levels of SIRT1 in the NAc, and investigate a causal role for SIRT1 in social rank establishment. This will deepen our understanding of the underpinnings of social rank and the molecular determinants of neurometabolism.

Pubmed:
34980257: González-Domínguez R, Castellano-Escuder P, Lefèvre-Arbogast S, Low DY, Du Preez A, Ruigrok SR, Lee H, Helmer C, Pallàs M, Urpi-Sarda M, Sánchez-Pla A, Korosi A, Lucassen PJ, Aigner L, Manach C, Thuret S, Samieri C, Andres-Lacueva C
Apolipoprotein E and sex modulate fatty acid metabolism in a prospective observational study of cognitive decline.
Fatty acids play prominent roles in brain function as they participate in structural, metabolic and signaling processes. The homeostasis of fatty acids and related pathways is known to be impaired in cognitive decline and dementia, but the relationship between these metabolic disturbances and common risk factors, namely the ε4 allele of the apolipoprotein E (ApoE-ε4) gene and sex, remains elusive.
Alzheimers Res Ther, 2022; 14
34661340: González-Domínguez R, Castellano-Escuder P, Carmona F, Lefèvre-Arbogast S, Low DY, Du Preez A, Ruigrok SR, Manach C, Urpi-Sarda M, Korosi A, Lucassen PJ, Aigner L, Pallàs M, Thuret S, Samieri C, Sánchez-Pla A, Andres-Lacueva C
Food and Microbiota Metabolites Associate with Cognitive Decline in Older Subjects: A 12-Year Prospective Study.
Diet is considered an important modulator of cognitive decline and dementia, but the available evidence is, however, still fragmented and often inconsistent.
Mol Nutr Food Res, 2021; 65
34402599: Du Preez A, Lefèvre-Arbogast S, Houghton V, de Lucia C, Low DY, Helmer C, Féart C, Delcourt C, Proust-Lima C, Pallàs M, Ruigrok SR, Altendorfer B, González-Domínguez R, Sánchez-Pla A, Urpi-Sardà M, Andres-Lacueva C, Aigner L, Lucassen PJ, Korosi A, Manach C, Samieri C, Thuret S
The serum metabolome mediates the concert of diet, exercise, and neurogenesis, determining the risk for cognitive decline and dementia.
Diet and exercise influence the risk of cognitive decline (CD) and dementia through the food metabolome and exercise-triggered endogenous factors, which use the blood as a vehicle to communicate with the brain. These factors might act in concert with hippocampal neurogenesis (HN) to shape CD and dementia.
Alzheimers Dement, 2022; 18

34366778: Ruigrok SR, Stöberl N, Yam KY, de Lucia C, Lucassen PJ, Thuret S, Korosi A
Modulation of the Hypothalamic Nutrient Sensing Pathways by Sex and Early-Life Stress.
There are sex differences in metabolic disease risk, and early-life stress (ES) increases the risk to develop such diseases, potentially in a sex-specific manner. It remains to be understood, however, how sex and ES affect such metabolic vulnerability. The hypothalamus regulates food intake and energy expenditure by sensing the organism's energy state via metabolic hormones (leptin, insulin, ghrelin) and nutrients (glucose, fatty acids). Here, we investigated if and how sex and ES alter hypothalamic nutrient sensing short and long-term. ES was induced in mice by limiting the bedding and nesting material from postnatal day (P)2-P9, and the expression of genes critical for hypothalamic nutrient sensing were studied in male and female offspring, both at P9 and in adulthood (P180). At P9, we observed a sex difference in both and expression, while the latter was also increased in ES-exposed animals relative to controls. In adulthood, we found sex differences in , , and expression, whereas ES did not affect the expression of genes involved in hypothalamic nutrient sensing. Thus, we observe a pervasive sex difference in nutrient sensing pathways and a targeted modulation of this pathway by ES early in life. Future research is needed to address if the modulation of these pathways by sex and ES is involved in the differential vulnerability to metabolic diseases.
Front Neurosci, 2021; 15
34277896: Ruigrok SR, Kotah JM, Kuindersma JE, Speijer E, van Irsen AAS, la Fleur SE, Korosi A
Adult food choices depend on sex and exposure to early-life stress: Underlying brain circuitry, adipose tissue adaptations and metabolic responses.
Exposure to early-life stress (ES) increases the risk to develop obesity later in life, and these effects may be sex-specific, but it is currently unknown what underlies the ES-induced metabolic vulnerability. We have previously shown that ES leads to a leaner phenotype under standard chow diet conditions, but to increased fat accumulation when exposed to an unhealthy obesogenic diet. However these diets were fed without a choice. An important, yet under investigated, element contributing to the development of obesity in humans is the choice of the food. There is initial evidence that ES leads to altered food choices but a thorough testing on how ES affects the choice of both the fat and sugar component, and if this is similar in males and females, is currently missing. We hypothesized that ES increases the choice for unhealthy foods, while it at the same time also affects the response to such a diet. In a mouse model for ES, in which mice are exposed to limited nesting and bedding material from postnatal day (P)2-P9, we investigated if ES exposure affected i) food choice with a free choice high-fat high-sugar diet (fcHFHS), ii) the response to such a diet, iii) the brain circuits that regulate food intake and food reward and iv) if such ES effects are sex-specific. We show that there are sex differences in food choice under basal circumstances, and that ES increases fat intake in females when exposed to a mild acute stressor. Moreover, ES impacts the physiologic response to the fcHFHS and the brain circuits regulating food intake in sex-specific manner. Our data highlight sex-specific effects of ES on metabolic functioning and food choice.
Neurobiol Stress, 2021; 15
34274734: Ruigrok SR, Yim K, Emmerzaal TL, Geenen B, Stöberl N, den Blaauwen JL, Abbink MR, Kiliaan AJ, van Schothorst EM, Kozicz T, Korosi A
Effects of early-life stress on peripheral and central mitochondria in male mice across ages.
Exposure to early-life stress (ES) increases the vulnerability to develop metabolic diseases as well as cognitive dysfunction, but the specific biological underpinning of the ES-induced programming is unknown. Metabolic and cognitive disorders are often comorbid, suggesting possible converging underlying pathways. Mitochondrial dysfunction is implicated in both metabolic diseases and cognitive dysfunction and chronic stress impairs mitochondrial functioning. However, if and how mitochondria are impacted by ES and whether they are implicated in the ES-induced programming remains to be determined. ES was applied by providing mice with limited nesting and bedding material from postnatal day (P)2-P9, and metabolic parameters, cognitive functions and multiple aspects of mitochondria biology (i.e. mitochondrial electron transport chain (ETC) complex activity, mitochondrial DNA copy number, expression of genes relevant for mitochondrial function, and the antioxidant capacity) were studied in muscle, hypothalamus and hippocampus at P9 and late adulthood (10-12 months of age). We show that ES altered bodyweight (gain), adiposity and glucose levels at P9, but not in late adulthood. At this age, however, ES exposure led to cognitive impairments. ES affected peripheral and central mitochondria in an age-dependent manner. At P9, both muscle and hypothalamic ETC activity were affected by ES, while in hippocampus, ES altered the expression of genes involved in fission and antioxidant defence. In adulthood, alterations in ETC complex activity were observed in the hypothalamus specifically, whereas in muscle and hippocampus ES affected the expression of genes involved in mitophagy and fission, respectively. Our study demonstrates that ES affects peripheral and central mitochondria biology throughout life, thereby uncovering a converging mechanism that might contribute to the ES-induced vulnerability for both metabolic diseases and cognitive dysfunction, which could serve as a novel target for intervention.
Psychoneuroendocrinology, 2021; 132
33508744: Lefèvre-Arbogast S, Hejblum BP, Helmer C, Klose C, Manach C, Low DY, Urpi-Sarda M, Andres-Lacueva C,

González-Domínguez R, Aigner L, Altendorfer B, Lucassen PJ, Ruigrok SR, De Lucia C, Du Preez A, Proust-Lima C, Thuret S, Korosi A, Samieri C
Early signature in the blood lipidome associated with subsequent cognitive decline in the elderly: A case-control analysis nested within the Three-City cohort study.
Brain lipid metabolism appears critical for cognitive aging, but whether alterations in the lipidome relate to cognitive decline remains unclear at the system level.
EBioMedicine, 2021; 64
33498469: Ruigrok SR, Abbink MR, Geertsema J, Kuindersma JE, Stöberl N, van der Beek EM, Lucassen PJ, Schipper L, Korosi A
Effects of Early-Life Stress, Postnatal Diet Modulation and Long-Term Western-Style Diet on Peripheral and Central Inflammatory Markers.
Early-life stress (ES) exposure increases the risk of developing obesity. Breastfeeding can markedly decrease this risk, and it is thought that the physical properties of the lipid droplets in human milk contribute to this benefit. A concept infant milk formula (IMF) has been developed that mimics these physical properties of human milk (Nuturis, N-IMF). Previously, we have shown that N-IMF reduces, while ES increases, western-style diet (WSD)-induced fat accumulation in mice. Peripheral and central inflammation are considered to be important for obesity development. We therefore set out to test the effects of ES, Nuturis and WSD on adipose tissue inflammatory gene expression and microglia in the arcuate nucleus of the hypothalamus. ES was induced in mice by limiting the nesting and bedding material from postnatal day (P) 2 to P9. Mice were fed a standard IMF (S-IMF) or N-IMF from P16 to P42, followed by a standard diet (STD) or WSD until P230. ES modulated adipose tissue inflammatory gene expression early in life, while N-IMF had lasting effects into adulthood. Centrally, ES led to a higher microglia density and more amoeboid microglia at P9. In adulthood, WSD increased the number of amoeboid microglia, and while ES exposure increased microglia coverage, Nuturis reduced the numbers of amoeboid microglia upon the WSD challenge. These results highlight the impact of the early environment on central and peripheral inflammatory profiles, which may be key in the vulnerability to develop metabolic derangements later in life.
Nutrients, 2021; 13
29973407: Rajani RM, Quick S, Ruigrok SR, Graham D, Harris SE, Verhaaren BFJ, Fornage M, Seshadri S, Atanur SS, Dominiczak AF, Smith C, Wardlaw JM, Williams A
Reversal of endothelial dysfunction reduces white matter vulnerability in cerebral small vessel disease in rats.
Dementia is a major social and economic problem for our aging population. One of the most common of dementia in the elderly is cerebral small vessel disease (SVD). Magnetic resonance scans of SVD patients typically show white matter abnormalities, but we do not understand the mechanistic pathological link between blood vessels and white matter myelin damage. Hypertension is suggested as the cause of sporadic SVD, but a recent alternative hypothesis invokes dysfunction of the blood-brain barrier as the primary cause. In a rat model of SVD, we show that endothelial cell (EC) dysfunction is the first change in development of the disease. Dysfunctional ECs secrete heat shock protein 90α, which blocks oligodendroglial differentiation, contributing to impaired myelination. Treatment with EC-stabilizing drugs reversed these EC and oligodendroglial pathologies in the rat model. EC and oligodendroglial dysfunction were also observed in humans with early, asymptomatic SVD pathology. We identified a loss-of-function mutation in ATPase11B, which caused the EC dysfunction in the rat SVD model, and a single-nucleotide polymorphism in ATPase11B that was associated with white matter abnormalities in humans with SVD. We show that EC dysfunction is a cause of SVD white matter vulnerability and provide a therapeutic strategy to treat and reverse SVD in the rat model, which may also be of relevance to human SVD.
Sci Transl Med, 2018; 10
28027926: Hoeijmakers L, Ruigrok SR, Amelianchik A, Ivan D, van Dam AM, Lucassen PJ, Korosi A
Early-life stress lastingly alters the neuroinflammatory response to amyloid pathology in an Alzheimer's disease mouse model.
Exposure to stress during the sensitive period of early-life increases the risk to develop cognitive impairments and psychopathology later in life. In addition, early-life stress (ES) exposure, next to genetic causes, has been proposed to modulate the development and progression of Alzheimer's disease (AD), however evidence for this hypothesis is currently lacking. We here tested whether ES modulates progression of AD-related neuropathology and assessed the possible contribution of neuroinflammatory factors in this. We subjected wild-type (WT) and transgenic APP/PS1 mice, as a model for amyloid neuropathology, to chronic ES from postnatal day (P)2 to P9. We next studied how ES exposure affected; 1) amyloid β (Aβ) pathology at an early (4month old) and at a more advanced pathological (10month old) stage, 2) neuroinflammatory mediators immediately after ES exposure as well as in adult WT mice, and 3) the neuroinflammatory response in relation to Aβ neuropathology. ES exposure resulted in a reduction of cell-associated amyloid in 4month old APP/PS1 mice, but in an exacerbation of Aβ plaque load at 10months of age, demonstrating that ES affects Aβ load in the hippocampus in an age-dependent manner. Interestingly, ES modulated various neuroinflammatory mediators in the hippocampus of WT mice as well

as in response to Aβ neuropathology. In WT mice, immediately following ES exposure (P9), Iba1-immunopositive microglia exhibited reduced complexity and hippocampal interleukin (IL)-1β expression was increased. In contrast, microglial Iba1 and CD68 were increased and hippocampal IL-6 expression was decreased at 4months, while these changes resolved by 10months of age. Finally, Aβ neuropathology triggered a neuroinflammatory response in APP/PS1 mice that was altered after ES exposure. APP/PS1 mice exhibited increased CD68 expression at 4months, which was further enhanced by ES, whereas the microglial response to Aβ neuropathology, as measured by Iba1 and CD11b, was less prominent after ES at 10months of age. Finally, the hippocampus appears to be more vulnerable for these ES-induced effects, since ES did not affect Aβ neuropathology and neuroinflammation in the entorhinal cortex of adult ES exposed mice. Overall, our results demonstrate that ES exposure has both immediate and lasting effects on the neuroinflammatory response. In the context of AD, such alterations in neuroinflammation might contribute to aggravated neuropathology in ES exposed mice, hence altering disease progression. This indicates that, at least in a genetic context, ES could aggravate AD pathology.
Brain Behav Immun, 2017; 63

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-175

BLOOD BRAIN BARRIER DIFFERENCES IN THE NUCLEUS ACCUMBENS RELATE TO NATURAL VARIATION IN TRAIT ANXIETY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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The nucleus accumbens (NAc), a brain region involved in reward and motivation, is implicated in the regulation of anxiety-related behaviors. Astrocytes are powerful modulators of neuronal activity, and their localization around blood vessels is important for brain metabolism. In this study, exploiting natural phenotypic variation in anxiety-like behaviors in outbred Wistar male rats, we aimed at investigating anxiety-related differences in astrocytic and endothelial features in the NAc. We classified animals as high (HA) or low anxious (LA) according to the time spent in the open arms of the elevated plus maze. We assessed astrocyte's morphology using GFAP immunostaining and did not find morphological differences. However, gene expression analyses indicated a significant decrease in the expression levels of Aquaporine 4 (Aqp4) and Claudine 5 (Cldn5) in HA animals. Aqp4 is specifically expressed at the level of astrocytic endfeet around blood vessels. Therefore, we asked whether HA and LA animals show differences in blood brain barrier (BBB) permeability. Using Evan's Blue injections, we observed higher blood vessels' permeability in the NAc of HA animals. Using electron microscopy, we observed a lower number of endfeet processes around blood vessels in the NAc of HA animals. Furthermore, we observed a lower number of contacts between the mitochondria and the endoplasmic reticulum in those endfeets. Therefore, we show differences in the coverage of blood vessels by astrocytic processes, and in the associated BBB permeability, as a function of individuals' anxiety. Our findings suggest that alterations in accumbal astrocytes may contribute to the vulnerability of high anxious individuals.

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BOARD NUMBER: S02-176

KISSPEPTIN-13 MAY INDUCE ANXIETY-LIKE BEHAVIOUR VIA MODULATION OF CENTRAL VASOPRESSIN IN RATS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aims: Kisspeptin-13 (Kp-13), an endogenous derivative of Kisspeptin, in our previous experiments induced anxiety-like behaviour in rats. In the present experiments, we investigated the possible mechanism of Kp-13's anxiogenic action in adult male Wistar rats. **Methods:** After Kp-13 (2 µg/2 µl) injection intracerebroventricularly (icv) hypothalamic, amygdalar and hippocampal samples were obtained, to measure the relative gene expression of the corticotropin-releasing hormone (CRH) and vasopressin (VP) system via qPCR and the protein level of CRH and VP via ELISA. The behavior of animals, pretreated with CRH or VP receptor antagonists prior to Kp-13, were recorded via elevated plus maze (EPM) and open field (OF) tests. In addition to assess Kp-13 long-term effects, OF test was performed 8 and 24 h after Kp-13 injection. **Results:** Our results showed that Kp-13 upregulated the relative gene expression of VP in amygdala, CRH receptor 1 and VP1b receptors in the hippocampus. VP concentration in the amygdala was elevated. In addition, KISS1R and VP receptor antagonist pretreatment was able to abolish KP-13's anxiogenic effect in behavioral tests, whereas CRH blockage had little effect. Kp-13's anxiogenic effect remained after 24 h. **Conclusion:** KP-13's long-term anxiogenic action might be mediated by central release of the stress responsive VP, rather than CRH. Indeed, increased amygdalar VP expression may alter amygdala processing to induce anxiety and trigger downstream activation of the hypothalamic-pituitary-adrenal axis in rats.

Pubmed:
33503835: Ibos KE, Bodnár É, Bagosi Z, Bozsó Z, Tóth G, Szabó G, Csabafi K
Kisspeptin-8 Induces Anxiety-Like Behavior and Hypolocomotion by Activating the HPA Axis and Increasing GABA Release in the Nucleus Accumbens in Rats.
Kisspeptins (Kp) are RF-amide neuropeptide regulators of the reproductive axis that also influence anxiety, locomotion, and metabolism. We aimed to investigate the effects of intracerebroventricular Kp-8 (an N-terminally truncated octapeptide) treatment in Wistar rats. Elevated plus maze (EPM), computerized open field (OF), and marble burying (MB) tests were performed for the assessment of behavior. Serum LH and corticosterone levels were determined to assess kisspeptin1 receptor (Kiss1r) activation and hypothalamic-pituitary-adrenal axis (HPA) stimulation, respectively. GABA release from the nucleus accumbens (NAc) and dopamine release from the ventral tegmental area (VTA) and NAc were measured via ex vivo superfusion. Kp-8 decreased open arm time and entries in EPM, and also raised corticosterone concentration, pointing to an anxiogenic effect. Moreover, the decrease in arm entries in EPM, the delayed increase in immobility accompanied by reduced ambulatory activity in OF, and the reduction in interactions with marbles show that Kp-8 suppressed exploratory and spontaneous locomotion. The increase in GABA release from the NAc might be in the background of hypolocomotion by inhibiting the VTA-NAc dopaminergic circuitry. As Kp-8 raised LH concentration, it could activate Kiss1r and stimulate the reproductive axis. As Kiss1r is associated with hyperlocomotion, it is more likely that neuropeptide FF receptor activation is involved in the suppression of locomotor activity.
Biomedicines, 2021; 9

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BOARD NUMBER: S02-177

THE “CUFF” MODEL OF SCIATIC NERVE COMPRESSION INDUCES CHRONIC HYPERSENSITIVITY BUT NOT ANXIODEPRESSIVE-LIKE SYMPTOMS IN SPRAGUE-DAWLEY RATS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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AIMS: About 10-20% of the population is currently suffering from chronic pain. This condition is thus a major cause of disability and disease burden of this century. Although pain syndromes result from different underlying mechanisms, they share common comorbidities such as neurocognitive changes, sleep disturbances and in 50% of the patients suffering from chronic pain, mood alterations leading to anxiety and/or depression. Our aim was to study the mechanism underlying the development of the anxiodepressive-like consequences of chronic pain in a rat model of sciatic nerve compression. METHODS: The “Cuff” model consists in the implantation of a polyethylene tube around the main branch of the right sciatic nerve to induce chronic pain. Calibrated forceps were used to assess the mechanical sensitivity of both hind paws and behavioral tests evaluating anxious- and depressive-like symptoms were done at different time points after the cuff surgery. RESULTS: The sciatic nerve compression induced a chronic mechanical hypersensitivity in male Sprague-Dawley rats, similar to previous results obtained in mice. While this hypersensitivity was accompanied by time-dependent anxiodepressive-like symptoms in the cuffed mice, the rats showed surprisingly neither anxiety nor depression-related behaviors. CONCLUSION: These results suggest that anxiodepressive-like behaviors are not necessarily a consequence of chronic pain, and they provide an interesting model to further investigate the basis of resilience vs susceptibility to this pain comorbidity. AKNOWLEDGEMENTS: This work has been supported by Centre National de la Recherche Scientifique (UPR3212), University of Strasbourg (UPR3212), Agence Nationale de la Recherche (Euridol ANR-17-EURE-0022) and Région Grand Est (ClueDol).

FENS Forum 2022
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BOARD NUMBER: S02-178

SEEKING COMFORT IN STRESSFUL SITUATIONS: ERP AND ATTACHMENT DIMENSIONS AS PREDICTORS OF CARE OR COMFORT FOOD CHOICE.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aims: Threatening stimuli induce specific EEG response patterns and, in agreement to the attachment theory, motivate subjects to seek comfort. Both care-seeking and comfort food-seeking behaviour are known strategies of negative emotion regulation, and in this study we investigated in threatening conditions the association of the Late Positive Potential (LPP) response and attachment dimensions in the choice of care/food and its timing (RT). Methods: Fifty-two participants responded to the ECR questionnaire and were subjected to visual stimuli from IAPS, BAPS-Adult and Food-Pics_Extended, during EEG recording. Results: Threatening stimuli increase the choice of care, decrease RT, and increase LPP magnitude in right central-parietal areas (Cpz, Pz, P4), while comfort food choice was reduced, with increased RT. Further, regression analysis show that high increments in LPP magnitude in P4 channel are associated with increased choice of care, while an increase in RT is associated with high LPP increments in Cpz, Pz and P4. When considering the dimensions of attachment, in the threatening conditions, while anxiety is not associated with RT and care choice, avoidance is associated to an increase in care choice and to an increased RT. Conclusions: The specific increment in care choice RT associated with high LPP magnitude in right central-parietal areas, may be explained in terms of an interference of these brain areas in the choice of care, but not of comfort food. Further, we found that the defences of avoidant individuals can be by-passed, and the longer RT may reflect a more articulated elaboration of the choice process.

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BOARD NUMBER: S02-179

FORAGING UNDER THREAT AND ITS RELATIONSHIP TO REAL-LIFE ANXIETY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Background: When foraging for food under threat of predation, animals or humans must be vigilant and switch between behavioural strategies. Understanding how we decide between attending to rewards versus risks may have clinical relevance to understanding disordered responses to threatening stimuli in psychopathology. Methods: To investigate foraging under threat, we designed a task and a cognitive model to explore what information humans consider when choosing freely and continuously among foraging, vigilance, and hiding. We used 7T fMRI to investigate brain activity associated with this task in healthy adults. We additionally collected 400 participants over the internet on the task together with a standard psychiatric questionnaire about real-life anxiety (as well as apathy and compulsivity). Results: We found that real-life anxiety influenced behaviour in our task, leading to threat avoidance. We hypothesised that the ACC will be linked with encoding of action timing and task switching, and that the amygdala and periaqueductal gray will show activity relevant to transitioning from foraging to vigilance. Discussion: We developed a computer-based objective measure mirroring subjective reports related to anxiety. This task could be interesting for clinical collaborations on anxiety disorders or putative novel treatments.

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BOARD NUMBER: S02-180

REGION-SPECIFIC CREB FUNCTION REGULATES DISTINCT FORMS OF REGRET ASSOCIATED WITH RESILIENCE VERSUS SUSCEPTIBILITY TO CHRONIC STRESS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

Romain Durand-De Cuttoli, Freddyson Martinez-Rivera, Long Li, Angelica Minier-Toribio, Flurin Cathomas, Leanne Holt, Farzana Yasmin, Salma Elhassa, Sanjana Ahmed, Jasmine Shaikh, Scott Russo, Eric Nestler, Brian Sweis
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Regret describes recognizing that alternative actions could have led to better outcomes. This can transform into behavioral consequences, altering subsequent valuations, but remains unclear if regret derives from a generalized computation for mistake appraisal or instead is made up of dissociable action-specific processes. Using a novel neuroeconomic decision-making paradigm, we found mice were differentially sensitive to fundamentally distinct types of missed opportunities following exposure to chronic social defeat stress or manipulations of CREB, a key transcription factor implicated in chronic stress action. Bias to make compensatory decisions after rejecting high-value offers (regret type I) was unique to stress-susceptible mice. Bias following the converse operation, accepting low-value offers (regret type II), was enhanced in stress-resilient and absent in stress-susceptible mice. CREB function in either the medial prefrontal cortex or nucleus accumbens was required to suppress regret type I but differentially affected regret type II. We provide insight into how adaptive versus maladaptive stress-response traits may be related to fundamentally distinct forms of counterfactual thinking and could steer psychotherapy for mood disorders such as depression toward unveiling circuit-specific computations through a careful description of decision narrative.

Pubmed:
34836948: Dongelmans M, Durand-de Cuttoli R, Nguyen C, Come M, Duranté EK, Lemoine D, Brito R, Ahmed Yahia T, Mondoloni S, Didienne S, Bousseyrol E, Hannesse B, Reynolds LM, Torquet N, Dalkara D, Marti F, Mourot A, Naudé J, Faure P
Chronic nicotine increases midbrain dopamine neuron activity and biases individual strategies towards reduced exploration in mice.
Long-term exposure to nicotine alters brain circuits and induces profound changes in decision-making strategies, affecting behaviors both related and unrelated to drug seeking and consumption. Using an intracranial self-stimulation reward-based foraging task, we investigated in mice the impact of chronic nicotine on midbrain dopamine neuron activity and its consequence on the trade-off between exploitation and exploration. Model-based and archetypal analysis revealed substantial inter-individual variability in decision-making strategies, with mice passively exposed to nicotine shifting toward a more exploitative profile compared to non-exposed animals. We then mimicked the effect of chronic nicotine on the tonic activity of dopamine neurons using optogenetics, and found that photo-stimulated mice adopted a behavioral phenotype similar to that of mice exposed to chronic nicotine. Our results reveal a key role of tonic midbrain dopamine in the exploration/exploitation trade-off and highlight a potential mechanism by which nicotine affects the exploration/exploitation balance and decision-making.
Nat Commun, 2021; 12
34242565: Nguyen C, Mondoloni S, Le Borgne T, Centeno I, Come M, Jehl J, Solié C, Reynolds LM, Durand-de Cuttoli R, Tolu S, Valverde S, Didienne S, Hannesse B, Fiancette JF, Pons S, Maskos U, Deroche-Gamonet V, Dalkara D, Hardelin JP, Mourot A, Marti F, Faure P
Nicotine inhibits the VTA-to-amygdala dopamine pathway to promote anxiety.
Nicotine stimulates dopamine (DA) neurons of the ventral tegmental area (VTA) to establish and maintain reinforcement. Nicotine also induces anxiety through an as yet unknown circuitry. We found that nicotine injection drives opposite functional responses of two distinct populations of VTA DA neurons with anatomically segregated projections: it activates neurons that project to the nucleus accumbens (NAc), whereas it inhibits neurons that project to the amygdala nuclei (Amg). We further show that nicotine mediates anxiety-like behavior by acting on β2-subunit-containing nicotinic acetylcholine receptors of the VTA. Finally, using optogenetics, we bidirectionally manipulate the VTA-NAc and VTA-Amg pathways to dissociate their

contributions to anxiety-like behavior. We show that inhibition of VTA-Amg DA neurons mediates anxiety-like behavior, while their activation prevents the anxiogenic effects of nicotine. These distinct subpopulations of VTA DA neurons with opposite responses to nicotine may differentially drive the anxiogenic and the reinforcing effects of nicotine.
Neuron, 2021; 109
32284606: Flanigan ME, Aleyasin H, Li L, Burnett CJ, Chan KL, LeClair KB, Lucas EK, Matikainen-Ankney B, Durand-de Cuttoli R, Takahashi A, Menard C, Pfau ML, Golden SA, Bouchard S, Calipari ES, Nestler EJ, DiLeone RJ, Yamanaka A, Huntley GW, Clem RL, Russo SJ
Orexin signaling in GABAergic lateral habenula neurons modulates aggressive behavior in male mice.
Heightened aggression is characteristic of multiple neuropsychiatric disorders and can have various negative effects on patients, their families and the public. Recent studies in humans and animals have implicated brain reward circuits in aggression and suggest that, in subsets of aggressive individuals, domination of subordinate social targets is reinforcing. In this study, we showed that, in male mice, orexin neurons in the lateral hypothalamus activated a small population of glutamic acid decarboxylase 2 (GAD2)-expressing neurons in the lateral habenula (LHb) via orexin receptor 2 (OxR2) and that activation of these GAD2 neurons promoted male-male aggression and conditioned place preference for aggression-paired contexts. Moreover, LHb GAD2 neurons were inhibitory within the LHb and dampened the activity of the LHb as a whole. These results suggest that the orexin system is important for the regulation of inter-male aggressive behavior and provide the first functional evidence of a local inhibitory circuit within the LHb.
Nat Neurosci, 2020; 23
32152102: Durand-de Cuttoli R, Chauhan PS, Pétriz Reyes A, Faure P, Mourot A, Ellis-Davies GCR
Optofluidic control of rodent learning using cloaked caged glutamate.
Glutamate is the major excitatory neurotransmitter in the brain, and photochemical release of glutamate (or uncaging) is a chemical technique widely used by biologists to interrogate its physiology. A basic prerequisite of these optical probes is bio-inertness before photolysis. However, all caged glutamates are known to have strong antagonism toward receptors of γ-aminobutyric acid, the major inhibitory transmitter. We have developed a caged glutamate probe that is inert toward these receptors at concentrations that are effective for photolysis with violet light. Pharmacological tests in vitro revealed that attachment of a fifth-generation (G5) dendrimer (i.e., cloaking) to the widely used 4-methoxy-7-nitro-indolinyl(MNI)-Glu probe prevented such off-target effects while not changing the photochemical properties of MNI-Glu significantly. G5-MNI-Glu was used with optofluidic delivery to stimulate dopamine neurons of the ventral tegmental area of freely moving mice in a conditioned place-preference protocol so as to mediate Pavlovian conditioning.
Proc Natl Acad Sci U S A, 2020; 117
31965053: Belkaid M, Bousseyrol E, Durand-de Cuttoli R, Dongelmans M, Duranté EK, Ahmed Yahia T, Didienne S, Hanesse B, Come M, Mourot A, Naudé J, Sigaud O, Faure P
Mice adaptively generate choice variability in a deterministic task.
Can decisions be made solely by chance? Can variability be intrinsic to the decision-maker or is it inherited from environmental conditions? To investigate these questions, we designed a deterministic setting in which mice are rewarded for non-repetitive choice sequences, and modeled the experiment using reinforcement learning. We found that mice progressively increased their choice variability. Although an optimal strategy based on sequences learning was theoretically possible and would be more rewarding, animals used a pseudo-random selection which ensures high success rate. This was not the case if the animal is exposed to a uniform probabilistic reward delivery. We also show that mice were blind to changes in the temporal structure of reward delivery once they learned to choose at random. Overall, our results demonstrate that a decision-making process can self-generate variability and randomness, even when the rules governing reward delivery are neither stochastic nor volatile.
Commun Biol, 2020; 3
31400823: Mondoloni S, Durand-de Cuttoli R, Mourot A
Cell-Specific Neuropharmacology.
Neuronal communication involves a multitude of neurotransmitters and an outstanding diversity of receptors and ion channels. Linking the activity of cell surface receptors and ion channels in defined neural circuits to brain states and behaviors has been a key challenge in neuroscience, since cell targeting is not possible with traditional neuropharmacology. We review here recent technologies that enable the effect of drugs to be restricted to specific cell types, thereby allowing acute manipulation of the brain's own proteins with circuit specificity. We highlight the importance of developing cell-specific neuropharmacology strategies for decoding the nervous system with molecular and circuit precision, and for developing future therapeutics with reduced side effects.
Trends Pharmacol Sci, 2019; 40
30293722: Forget B, Scholze P, Langa F, Morel C, Pons S, Mondoloni S, Besson M, Durand-de Cuttoli R, Hay A, Tricoire L, Lambolez B, Mourot A, Faure P, Maskos U

A Human Polymorphism in CHRNA5 Is Linked to Relapse to Nicotine Seeking in Transgenic Rats.
Tobacco addiction is a chronic and relapsing disorder with an important genetic component that represents a major public health issue. Meta-analysis of large-scale human genome-wide association studies (GWASs) identified a frequent non-synonymous SNP in the gene coding for the α5 subunit of nicotinic acetylcholine receptors (α5SNP), which significantly increases the risk for tobacco dependence and delays smoking cessation. To dissect the neuronal mechanisms underlying the vulnerability to nicotine addiction in carriers of the α5SNP, we created rats expressing this polymorphism using zinc finger nuclease technology and evaluated their behavior under the intravenous nicotine-self-administration paradigm. The electrophysiological responses of their neurons to nicotine were also evaluated. α5SNP rats self-administered more nicotine at high doses and exhibited higher nicotine-induced reinstatement of nicotine seeking than wild-type rats. Higher reinstatement was associated with altered neuronal activity in several discrete areas that are interconnected, including in the interpeduncular nucleus (IPN), a GABAergic structure that strongly expresses α5-containing nicotinic receptors. The altered reactivity of IPN neurons of α5SNP rats to nicotine was confirmed electrophysiologically. In conclusion, the α5SNP polymorphism is a major risk factor for nicotine intake at high doses and for relapse to nicotine seeking in rats, a dual effect that reflects the human condition. Our results also suggest an important role for the IPN in the higher relapse to nicotine seeking observed in α5SNP rats.
Curr Biol, 2018; 28
30176987: Durand-de Cuttoli R, Mondoloni S, Marti F, Lemoine D, Nguyen C, Naudé J, d'Izarny-Gargas T, Pons S, Maskos U, Trauner D, Kramer RH, Faure P, Mourot A
Manipulating midbrain dopamine neurons and reward-related behaviors with light-controllable nicotinic acetylcholine receptors.
Dopamine (DA) neurons of the ventral tegmental area (VTA) integrate cholinergic inputs to regulate key functions such as motivation and goal-directed behaviors. Yet the temporal dynamic range and mechanism of action of acetylcholine (ACh) on the modulation of VTA circuits and reward-related behaviors are not known. Here, we used a chemical-genetic approach for rapid and precise optical manipulation of nicotinic neurotransmission in VTA neurons in living mice. We provide direct evidence that the ACh tone fine-tunes the firing properties of VTA DA neurons through β2-containing (β2*) nicotinic ACh receptors (nAChRs). Furthermore, locally photo-antagonizing these receptors in the VTA was sufficient to reversibly switch nicotine reinforcement on and off. By enabling control of nicotinic transmission in targeted brain circuits, this technology will help unravel the various physiological functions of nAChRs and may assist in the design of novel therapies relevant to neuropsychiatric disorders.
Elife, 2018; 7

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BOARD NUMBER: S02-181

CORTISOL IN CORTISOL LEVELS ACROSS THE LIFESPAN IN COMMON MARMOSETS (CALLITHRIX JACCHUS)

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

Kimberley Phillips, Matthew Lopez, Amaya Seidl
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Human aging is associated with senescence of the hypothalamic-pituitary-adrenal (HPA) axis, leading to progressive dysregulation characterized by increased cortisol exposure. This key hormone is implicated in the pathogenesis of many age-related diseases. Common marmosets display a wide spectrum of naturally-occurring age-related pathologies that compare similarly to humans, and are increasingly used as translational models of aging and age-related disease. Whether the marmoset HPA axis also shows senescence with increasing age is unknown. We analyzed hair cortisol concentration (HCC) across the lifespan of 50 captive common marmosets via a cross-sectional design. Samples were processed and analyzed for cortisol using enzyme immunoassay. HCC ranged from 1,416 to 15,343 pg/mg. We found significant main effects of age group ($F(4, 39) = 3.46$, $p = 0.016$) and sex ($F(1, 39) = 4.66$, $p = 0.037$), and no interaction effects. Infants had significantly higher levels of HCC compared to all other age groups. Females had higher HCC than males. These results suggest marmosets do not show dysregulation of the HPA axis with increasing age.

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BOARD NUMBER: S02-182

ASSESSING THE IMPACT OF HUMAN BODY DISSECTION ON FIRST YEAR UNDERGRADUATE MEDICAL STUDENTS AT BPKIHS.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aims: The present study aimed to assess the stress experienced just after one week of first dissection (IES1) and compare it with impact after 12 weeks (IES2) of first experience by using Impact Event Scale (IES) on first year medical students of B.P. Koirala Institute of Health Sciences, Nepal. **Methods:** Questionnaires for Impact Event Scale (IES) were administered to 100 students attending the cadaveric dissection. The questionnaire provides an overall scale, sub-scales of intrusion and avoidance. The sub-scale scores of 0-8 as a minor reaction;9-19 as a moderate reaction, and a score of 20 or above as a clinically important reaction. The cut-off over all score of 30 on IES indicates a traumatic stress reaction. The results considered significant at the usual 5 per cent level or below. **Results:** The percentage of students having clinically important reaction (a score of 20 or above) after one week of first cadaveric dissection in both sub-scales of Intrusion and Avoidance were 2% and 6% respectively but were found to be reduced to 1% each after 12 weeks. The overall scores indicating traumatic stress reaction was found to be reduced to 2% from 8%. The bivariate correlation analysis between IES 1 and IES 2 was found to have positive correlation with statistical significant level (r_s=0.252;p<0.001). **Conclusion:** The results of present study indicate that the stress related to the human body dissection in medical students reduced significantly after the period of 12 weeks of first cadaveric dissection. **Key words:** dissection; impact Event Scale; intrusion

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BOARD NUMBER: S02-183

EFFECT OF RADIATION AND EMOTIONAL STRESS ON BEHAVIOR OF RATS IN THE OPEN FIELD TEST

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Aims: to determine the characteristics of behavioral responses in male and female rats under emotional (immobilization stress) and physical (external irradiation and electromagnetic radiation (EMR)) stress. **Methods:** experiments were carried out on male and female rats (6 months' age). The rats were exposed to the outside of the radiation in a doses of 0.5 and 1.0 Gy (^{137}Cs, 46.2 sGy/min) and prolonged EMR (0.9 GHz for 10 days/8 hours a day). Model of emotional stress were caused by placing the animals in the narrow plastic tubes ("soft" immobilization) for 2 hours per day for 5 days. The animals were examined in the Open Field test (OF) on the next day after last immobilization session. **Results:** emotional stress was resulted in complex degenerative changes of internal organs. It was shown that immobilization leads to inhibition of the horizontal and vertical motor activity in experimental animals in OF test. It was shown that chronic immobilization stress leads to inhibition of the horizontal and vertical motor activity in experimental animals (male and female) in OF test. Thus, the horizontal locomotors activity was reduced by 43.8%; the level of rearing was reduced by 37.6 % (male rats). **Conclusions:** exposure of EMR modifies the level of integrative reaction of CNS rats which were exposed to ionizing radiation in the OF test. It was found that more pronounced negative changes of orientation and exploratory activity and of the levels of emotionality observed in the experimental groups after exposure to ionizing radiation and immobilization.

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BOARD NUMBER: S02-184

CHRONIC EXPOSURE TO HIGH FAT DIET AFFECTS THE DOPAMINE MODULATION IN NUCLEUS ACCUMBENS OF ADOLESCENT MALE RATS: IMPLICATIONS IN HEDONIC FOOD INTAKE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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Obesity is a worldwide health problem, which are caused by an excess of hypercaloric and obesogenic food intake and high palatability, which is increased from puberty. However, the neuronal circuits that regulate hedonic mechanisms of food intake are still poor understanding. **Aim**: To determine the molecular and functional changes of dopaminergic and glutamatergic modulation of nucleus accumbens (NAcc) in male rats exposed to chronic consumption of high fat diet (HFD). **Methods**: Sprague-Dawley male rats were exposed from postnatal day (PND) 21 to 62 to chow or HFD food. Dopamine (DA), DOPAC content and mRNA-DAT levels was measured in NAcc using HPLC-ED and RT-qPCR. DA release and glutamatergic postsynaptic currents properties were assess using voltammetry and patch clamp recordings from the spiny medium neurons (MSN) of NAcc slices obtained after PND 62. In addition, hedonic behavior was measured. **Results**: Our results show that chronic exposure to HFD reduces DOPAC content and DAT levels without affects DA tissue levels. In addition, amphetamine-induced DA release showed a decay time (t) higher than control. In HFD rats, we found a higher number of D_2-type MSN, while amphetamine induced a higher increase of glutamatergic postsynaptic currents in D_2-MSN compared than D_1-MSN. HFD rats showed an increased sucrose intake compared to controls. **Conclusions**: Chronic exposure to HFD reduces DA reuptake due to a decrease in DAT and higher D_2 levels, which could be associated to findings of the glutamatergic modulation from cortical projections. This research was funded by ANID-Chile through FONDECYT Grant Nº 120–0474 and DIUV-CI Grant N°01/2006.

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BOARD NUMBER: S02-185

A ROLE FOR NEURONS OF THE MEDIAL DIVISION OF THE CENTRAL AMYGDALA IN APPETITIVE BEHAVIOURS.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND APPETITIVE LEARNING

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The central amygdala (CeA) with its lateral (CeL) and medial divisions (CeM) orchestrates a wide range of behaviours, including defensive and appetitive responses. The CeA is composed of GABAergic neuron subpopulations that are marked by expression of neuropeptides, receptors and kinases, and elicit distinct, sometimes opposite behavioural phenotypes. The relative contributions of subpopulations in CeL versus CeM is, however, incompletely understood. Here, we took advantage of an intersectional genetic approach, to investigate how neurons marked by expression of serotonin receptor 2a (Htr2a) or somatostatin (Sst) located in either the CeL or CeM subdivisions regulate feeding, drinking, and promote positive reinforcement. To do so, we generated a novel transgenic mouse line that expresses a Tamoxifen-inducible version of the Flp recombinase (FlpOER) specifically and widely in the CeL, but not in the CeM (Wfs1-FlpOER). Double transgenic mice carrying Wfs1-FlpOER and Htr2a-Cre or Sst-Cre in combination with intersectional viral vectors allowed precise manipulation of CeL cells (positive for both Cre and Flp) versus CeM cells (positive for Cre only). We made the surprising observation that optogenetic activation of the CeM subpopulations mediated appetitive responses. Stimulation of CeM^{Htr2a} and CeM^{Sst} neurons promoted drinking and positive reinforcement behaviour. Stimulation of CeM^{Htr2a}, but not CeM^{Sst}, neurons increased food intake. Photoactivation of the corresponding CeL subpopulations failed to elicit appetitive responses. Ongoing analysis of intra-CeA and long-range projections will provide further insights into the specific roles of CeL and CeM subpopulations in appetitive responses.

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EARLY LIFE STRESS INDUCES EPIGENETICALLY REGULATED CHANGES OF PREFRONTAL ENDOCANNABINOID RECEPTOR 1 EXPRESSION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Early life stress (ELS) represents a major programming factor in the aetiology of behavioral and mental disorders, as well as in the development of stress resilience. However, the underlying brain mechanisms are still poorly understood. Recent studies revealed evidence for an involvement of the endocannabinoid system as a mediator of vulnerability and resilience. The aim of this study was to test the hypothesis that the endocannabinoid receptor 1 (eCB1R) might play a critical role in this context. Female rats were exposed to ELS using the "neglectful mother" paradigm in which dams had limited access to nesting material from postnatal day 7 to P14. Anxiety-like behavior and gene expression of eCB1R in the prefrontal cortex (PFC) of adult rats were examined. Our results revealed that ELS significantly increased eCB1R expression compared to unstressed controls. We also found that these gene expression changes were mediated by CpG-site specific changes of DNA methylation within or near eCB1R promoter. Behavioral analysis in the same animal cohorts revealed that ELS exposed females exhibit reduced anxiety-like behavior indicated by higher activity and more time spent in the center of an Open Field arena when compared to controls. Taken together we show here that one week of stress exposure at early childhood induces long-term changes of eCB1R gene expression and DNA methylation. We suggest that these ELS-induced changes are associated with the observed behavioral alterations indicating the development of stress resilience.

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ENDOCANNABINOID SIGNALLING MODULATES STRESS-INDUCED STEREOTYPIC BEHAVIORS AND HPA AXIS ACTIVATION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Introduction: Endocannabinoid (eCB) signalling is known to regulate many aspects of the stress response including the hypothalamic-pituitary-adrenal (HPA) axis. A nexus of the HPA axis is a cluster of corticotropin releasing hormone (CRH) producing neurons in the paraventricular nucleus of the hypothalamus (PVN). ECB signalling mediates the glucocorticoid dependent negative feedback mechanism on CRH neurons following stress, suggesting that pharmacological upregulation of this system may dampen stress-induced consequences associated with CRH neuron activity. As such, using an array of cellular, endocrine and behavioral readouts associated with activation of CRH neurons in the PVN, we examined how upregulating eCB signaling ameliorates stress-induced generation of a stress response. **Methods:** Our endpoint measurements of a stress response included the examination of self-directed homecage behaviors (i.e., grooming), activation of CRH neurons in the PVN and corticosterone release. Phyisological readouts were measured using respective immunohistochemical and ELISA approaches. Cannula were implanted for central delivery of eCB enzyme inhibitors prior to foot shock stress. **Results:** Foot shock stress elevated grooming, PVN CRH neuron activation and circulating corticosterone. While FAAH inhibition had no effect, systemic MAGL inhibition ameliorated stress-induced grooming with no influence on HPA measures. Interestingly, preliminary results suggest that intra-PVN MAGL inhibition may ameliorate grooming as well as HPA measures following foot shock stress. **Conclusions:** These data suggest that pharmacological upregulation of 2-AG, specifically in the PVN, can alter the behavioral and HPA response to stress. These results lay the foundation for the development of cannabinoid based therapies targeting symptoms of stress-related disorders.

Pubmed:
34116110: Petrie GN, Nastase AS, Aukema RJ, Hill MN
Endocannabinoids, cannabinoids and the regulation of anxiety.
Cannabis has been used for hundreds of years, with its ability to dampen feelings of anxiety often reported as a primary reason for use. Only recently has the specific role cannabinoids play in anxiety been thoroughly investigated. Here we discuss the body of evidence describing how endocannabinoids and exogenous cannabinoids are capable of regulating the generation and termination of anxiety states. Disruption of the endogenous cannabinoid (eCB) system following genetic manipulation, pharmacological intervention or stress exposure reliably leads to the generation of an anxiety state. On the other hand, upregulation of eCB signaling is capable of alleviating anxiety-like behaviors in multiple paradigms. When considering exogenous cannabinoid administration, cannabinoid receptor 1 (CB1) agonists have a biphasic, dose-dependent effect on anxiety such that low doses are anxiolytic while high doses are anxiogenic, a phenomenon that is evident in both rodent models and humans. Translational studies investigating a loss of function mutation in the gene for fatty acid amide hydrolase, the enzyme responsible for metabolizing AEA, have also shown that AEA signaling regulates anxiety in humans. Taken together, evidence reviewed here has outlined a convincing argument for cannabinoids being powerful regulators of both the manifestation and amelioration of anxiety symptoms, and highlights the therapeutic potential of targeting the eCB system for the development of novel classes of anxiolytics. This article is part of the special issue on 'Cannabinoids'.
Neuropharmacology, 2021; 195
30993360: Petrie GN, Wills KL, Piscitelli F, Smoum R, Limebeer CL, Rock EM, Humphrey AE, Sheppard-Perkins M, Lichtman AH, Mechoulam R, Di Marzo V, Parker LA
Oleoyl glycine: interference with the aversive effects of acute naloxone-precipitated MWD, but not morphine reward, in male Sprague-Dawley rats.
Oleoyl glycine (OlGly), a recently discovered fatty acid amide that is structurally similar to N- acylethanolamines, which include the endocannabinoid, anandamide (AEA), as well as endogenous peroxisome proliferator-activated receptor alpha (PPARα) agonists oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), has been shown to interfere with nicotine reward and dependence in mice.

Psychopharmacology (Berl), 2019; 236
34907248: Baglot SL, Hume C, Petrie GN, Aukema RJ, Lightfoot SHM, Grace LM, Zhou R, Parker L, Rho JM, Borgland SL, McLaughlin RJ, Brechenmacher L, Hill MN
Pharmacokinetics and central accumulation of delta-9-tetrahydrocannabinol (THC) and its bioactive metabolites are influenced by route of administration and sex in rats.
Up to a third of North Americans report using cannabis in the prior month, most commonly through inhalation. Animal models that reflect human consumption are critical to study the impact of cannabis on brain and behaviour. Most animal studies to date utilize injection of delta-9-tetrahydrocannabinol (THC; primary psychoactive component of cannabis). THC injections produce markedly different physiological and behavioural effects than inhalation, likely due to distinctive pharmacokinetics. The current study directly examined if administration route (injection versus inhalation) alters metabolism and central accumulation of THC and metabolites over time. Adult male and female Sprague-Dawley rats received either an intraperitoneal injection or a 15-min session of inhaled exposure to THC. Blood and brains were collected at 15, 30, 60, 90 and 240-min post-exposure for analysis of THC and metabolites. Despite achieving comparable peak blood THC concentrations in both groups, our results indicate higher initial brain THC concentration following inhalation, whereas injection resulted in dramatically higher 11-OH-THC concentration, a potent THC metabolite, in blood and brain that increased over time. Our results provide evidence of different pharmacokinetic profiles following inhalation versus injection. Accordingly, administration route should be considered during data interpretation, and translational animal work should strongly consider using inhalation models.
Sci Rep, 2021; 11
32393901: Mock ED, Mustafa M, Gunduz-Cinar O, Cinar R, Petrie GN, Kantae V, Di X, Ogasawara D, Varga ZV, Paloczi J, Miliano C, Donvito G, van Esbroeck ACM, van der Gracht AMF, Kotsogianni I, Park JK, Martella A, van der Wel T, Soethoudt M, Jiang M, Wendel TJ, Janssen APA, Bakker AT, Donovan CM, Castillo LI, Florea BI, Wat J, van den Hurk H, Wittwer M, Grether U, Holmes A, van Boeckel CAA, Hankemeier T, Cravatt BF, Buczynski MW, Hill MN, Pacher P, Lichtman AH, van der Stelt M
Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice.
N-acylethanolamines (NAEs), which include the endocannabinoid anandamide, represent an important family of signaling lipids in the brain. The lack of chemical probes that modulate NAE biosynthesis in living systems hamper the understanding of the biological role of these lipids. Using a high-throughput screen, chemical proteomics and targeted lipidomics, we report here the discovery and characterization of LEI-401 as a CNS-active N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) inhibitor. LEI-401 reduced NAE levels in neuroblastoma cells and in the brain of freely moving mice, but not in NAPE-PLD KO cells and mice, respectively. LEI-401 activated the hypothalamus-pituitary-adrenal axis and impaired fear extinction, thereby emulating the effect of a cannabinoid CB receptor antagonist, which could be reversed by a fatty acid amide hydrolase inhibitor. Our findings highlight the distinctive role of NAPE-PLD in NAE biosynthesis in the brain and suggest the presence of an endogenous NAE tone controlling emotional behavior.
Nat Chem Biol, 2020; 16
33381644: DeVuono MV, Hrelja KM, Petrie GN, Limebeer CL, Rock EM, Hill MN, Parker LA
Nausea-Induced Conditioned Gaping Reactions in Rats Produced by High-Dose Synthetic Cannabinoid, JWH-018.
Cannabinoid hyperemesis syndrome is becoming a more prominently reported side effect of cannabis containing high-dose Δ-tetrahydrocannabinol (THC) and designer cannabinoid drugs such as "Spice." One active ingredient that has been found in "Spice" is 1-pentyl-3-(1-naphthoyl)indole (JWH-018), a synthetic full agonist of the cannabinoid 1 (CB) receptor. In this study, we evaluated the potential of different doses of JWH-018 to produce conditioned gaping in rats, an index of nausea. Rats received 3 daily conditioning trials in which saccharin was paired with JWH-018 (0.0, 0.1, 1, and 3 mg/kg, intraperitoneal [i.p.]). Then the potential of pretreatment with the CB antagonist, rimonabant (SR), to prevent JWH-018-induced conditioned gaping was determined. To begin to understand the potential mechanism underlying JWH-018-induced nausea, serum collected from trunk blood was subjected to a corticosterone (CORT) analysis in rats receiving three daily injections with vehicle (VEH) or JWH-018 (3 mg/kg). At doses of 1 and 3 mg/kg (i.p.), JWH-018 produced nausea-like conditioned gaping reactions. The conditioned gaping produced by 3 mg/kg JWH-018 was reversed by pretreatment with rimonabant, which did not modify gaping on its own. Treatment with JWH-018 elevated serum CORT levels compared to vehicle-treated rats. As we have previously reported with high-dose THC, JWH-018 produced conditioned gaping in rats, reflective of a nausea effect mediated by its action on CB receptors and accompanied by elevated CORT, reflective of hypothalamic-pituitary-adrenal (HPA) activation.
Cannabis Cannabinoid Res, 2020; 5
32399633: DeVuono MV, La Caprara O, Sullivan MT, Bath A, Petrie GN, Limebeer CL, Rock EM, Hill MN, Parker LA
Role of the stress response and the endocannabinoid system in Δ-tetrahydrocannabinol (THC)-induced nausea.
Dysregulation of the endocannabinoid (eCB) system by high doses of Δ-tetrahydrocannabinol (THC) is hypothesized to

generate a dysfunctional hypothalamic-pituitary-adrenal (HPA) axis contributing to cannabinoid hyperemesis syndrome (CHS).
Psychopharmacology (Berl), 2020; 237
33998876: DeVuono MV, La Caprara O, Petrie GN, Limebeer CL, Rock EM, Hill MN, Parker LA
Cannabidiol Interferes with Establishment of Δ-Tetrahydrocannabinol-Induced Nausea Through a 5-HT Mechanism.
Cannabinoid hyperemesis syndrome (CHS) is characterized by intense nausea and vomiting brought on by the use of high-dose Δ-tetrahydrocannabinol (THC), the main psychotropic compound in cannabis. Cannabidiol (CBD), a nonpsychotropic compound found in cannabis, has been shown to interfere with some acute aversive effects of THC. In this study, we evaluated if CBD would interfere with THC-induced nausea through a 5-HT receptor mechanism as it has been shown to interfere with nausea produced by lithium chloride (LiCl). Since CHS has been attributed to a dysregulated stress response, we also evaluated if CBD would interfere with THC-induced increase in corticosterone (CORT). The potential of CBD (5 mg/kg, ip) to suppress THC-induced conditioned gaping (a measure of nausea) was evaluated in rats, as well as the potential of the 5-HT receptor antagonist, WAY-100635 (WAY; 0.1 mg/kg, ip), to reverse the suppression of THC-induced conditioned gaping by CBD. Last, the effect of CBD (5 mg/kg, ip) on THC-induced increase in serum CORT concentration was evaluated. Pretreatment with CBD (5 mg/kg, ip) interfered with the establishment of THC-induced conditioned gaping (=0.007, relative to vehicle [VEH] pretreatment), and this was reversed by pretreatment with 0.1 mg/kg WAY. This dose of WAY had no effect on gaping on its own. THC (10 mg/kg, ip) significantly increased serum CORT compared with VEH-treated rats (=0.04). CBD (5 mg/kg, ip) pretreatment reversed the THC-induced increase in CORT. CBD attenuated THC-induced nausea as well as THC-induced elevation in CORT. The attenuation of THC-induced conditioned gaping by CBD was mediated by its action on 5-HT receptors, similar to that of LiCl-induced nausea.
Cannabis Cannabinoid Res, 2022; 7
31953372: Freels TG, Baxter-Potter LN, Lugo JM, Glodosky NC, Wright HR, Baglot SL, Petrie GN, Yu Z, Clowers BH, Cuttler C, Fuchs RA, Hill MN, McLaughlin RJ
Vaporized Cannabis Extracts Have Reinforcing Properties and Support Conditioned Drug-Seeking Behavior in Rats.
Recent trends in cannabis legalization have increased the necessity to better understand the effects of cannabis use. Animal models involving traditional cannabinoid self-administration approaches have been notoriously difficult to establish and differences in the drug used and its route of administration have limited the translational value of preclinical studies. To address this challenge in the field, we have developed a novel method of cannabis self-administration using response-contingent delivery of vaporized Δ-tetrahydrocannabinol-rich (CAN) or cannabidiol-rich (CAN) whole-plant cannabis extracts. Male Sprague-Dawley rats were trained to nose-poke for discrete puffs of CAN, CAN, or vehicle (VEH) in daily 1 h sessions. Cannabis vapor reinforcement resulted in strong discrimination between active and inactive operanda. CAN maintained higher response rates under fixed ratio schedules and higher break points under progressive ratio schedules compared with CAN or VEH, and the number of vapor deliveries positively correlated with plasma THC concentrations. Moreover, metabolic phenotyping studies revealed alterations in locomotor activity, energy expenditure, and daily food intake that are consistent with effects in human cannabis users. Furthermore, both cannabis regimens produced ecologically relevant brain concentrations of THC and CBD and CAN administration decreased hippocampal CB1 receptor binding. Removal of CAN reinforcement (but not CAN) resulted in a robust extinction burst and an increase in cue-induced cannabis-seeking behavior relative to VEH. These data indicate that volitional exposure to THC-rich cannabis vapor has bona fide reinforcing properties and collectively support the utility of the vapor self-administration model for the preclinical assessment of volitional cannabis intake and cannabis-seeking behaviors. The evolving legal landscape concerning recreational cannabis use has increased urgency to better understand its effects on the brain and behavior. Animal models are advantageous in this respect; however, current approaches typically used forced injections of synthetic cannabinoids or isolated cannabis constituents that may not capture the complex effects of volitional cannabis consumption. We have developed a novel model of cannabis self-administration using response-contingent delivery of vaporized cannabis extracts containing high concentrations of Δ tetrahydrocannabinol (THC) or cannabidiol. Our data indicate that THC-rich cannabis vapor has reinforcing properties that support stable rates of responding and conditioned drug-seeking behavior. This approach will be valuable for interrogating effects of cannabis and delineating neural mechanisms that give rise to aberrant cannabis-seeking behavior.
J Neurosci, 2020; 40
33845076: Roebuck AJ, Greba Q, Smolyakova AM, Alaverdashvili M, Marks WN, Garai S, Baglot SL, Petrie G, Cain SM, Snutch TP, Thakur GA, Hill MN, Howland JG, Laprairie RB
Positive allosteric modulation of type 1 cannabinoid receptors reduces spike-and-wave discharges in Genetic Absence Epilepsy Rats from Strasbourg.
Childhood Absence Epilepsy (CAE) accounts for approximately 10% of all pediatric epilepsies. Current treatments for CAE are ineffective in approximately 1/3 of patients and can be associated with severe side effects such as hepatotoxicity. Certain cannabinoids, such as cannabidiol (CBD), have shown promise in the treatment of pediatric epilepsies. However, CBD

remains limited or prohibited in many jurisdictions, and has not been shown to have efficacy in CAE. Modulation of the type 1 cannabinoid receptor (CB1R) may provide more desirable pharmacological treatments. Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model many aspects of CAE, including cortical spike and wave discharges (SWDs). We have recently demonstrated that Δ-tetrahydrocannabinol (THC) increases SWDs in GAERS whereas CBD decreases these events. Here, we characterized aspects of the endocannabinoid system in brain areas relevant to seizures in GAERS and tested whether positive allosteric modulators (PAMs) of CB1R reduced SWDs. Both female and male GAERS had reduced (>50%) expression of CB1R and elevated levels of the endocannabinoid 2-AG in cortex compared to non-epileptic controls (NEC). We then administered the CB1R PAMs GAT211 and GAT229 to GAERS implanted with cortical electrodes. Systemic administration of GAT211 to male GAERS reduced SWDs by 40%. Systemic GAT229 administration reduced SWDs in female and male GAERS. Intracerebral infusion of GAT229 into the cortex of male GAERS reduced SWDs by >60% in a CB1R-dependent manner that was blocked by SR141716A. Together, these experiments identify altered endocannabinoid tone in GAERS and suggest that CB1R PAMs should be explored for treatment of absence seizures.
Neuropharmacology, 2021; 190

29567093: Donvito G, Piscitelli F, Muldoon P, Jackson A, Vitale RM, D'Aniello E, Giordano C, Ignatowska-Jankowska BM, Mustafa MA, Guida F, Petrie GN, Parker L, Smoum R, Sim-Selley L, Maione S, Lichtman AH, Damaj MI, Di Marzo V, Mechoulam R

N-Oleoyl-glycine reduces nicotine reward and withdrawal in mice.

Cigarette smokers with brain damage involving the insular cortex display cessation of tobacco smoking, suggesting that this region may contribute to nicotine addiction. In the present study, we speculated that molecules in the insular cortex that are sensitive to experimental traumatic brain injury (TBI) in mice might provide leads to ameliorate nicotine addiction. Using targeted lipidomics, we found that TBI elicited substantial increases of a largely uncharacterized lipid, N-acyl-glycine, N-oleoyl-glycine (OlGly), in the insular cortex of mice. We then evaluated whether intraperitoneal administration of OlGly would alter withdrawal responses in nicotine-dependent mice as well as the rewarding effects of nicotine, as assessed in the conditioned place preference paradigm (CPP). Systemic administration of OlGly reduced mecamylamine-precipitated withdrawal responses in nicotine-dependent mice and prevented nicotine CPP. However, OlGly did not affect morphine CPP, demonstrating a degree of selectivity. Our respective in vitro and in vivo observations that OlGly activated peroxisome proliferator-activated receptor alpha (PPAR-α) and the PPAR-α antagonist GW6471 prevented the OlGly-induced reduction of nicotine CPP in mice suggests that this lipid acts as a functional PPAR-α agonist to attenuate nicotine reward. These findings raise the possibility that the long chain fatty acid amide OlGly may possess efficacy in treating nicotine addiction.
Neuropharmacology, 2019; 148

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ENHANCING ANANDAMIDE PREVENTS A STRESS PHENOTYPE VIA β-CATENIN IN THE PFC IN A RAT MODEL OF PTSD

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Introduction: Emerging evidence suggests that enhancing anandamide signaling ameliorates anxiety and depression, although the underlying mechanisms require further investigation. We aimed to determine whether increasing anandamide signaling, using the fatty acid amide hydrolase (FAAH) inhibitor URB597, prevents a stress phenotype via a mechanism involving the Wnt/β-catenin pathway in the medial PFC (mPFC). **Methods:** Rats were exposed to shock and reminders model of PTSD and injected with URB597 (0.4 mg/kg, i.p.). The expression of β-catenin protein levels and the stress markers corticotrophin releasing factor (CRF) and CRF type 1 receptor (CRFr1) were examined 3 weeks after the shock. In a following experiment, rats were taken to stereotaxic surgery in which β-catenin was down-regulated in the mPFC by viral-mediated gene transfer. **Results:** Exposure to shock and reminders induced a depression- and anxiety-like phenotype, upregulated CRF and CRFr1 and downregulated the expression of β-catenin protein levels in the mPFC. URB597 restored the behavioral phenotype and the alterations in CRF, CRFr1 and β-catenin. Importantly, viral downregulation of PFC β-catenin in rats exposed to shock and reminders blocked the therapeutic-like effects of URB597 on the behavioral phenotype and the alterations In CRF and CRFr1 levels. Hence, the therapeutic-like effects of URB597 are mediated via β-catenin in the PFC. **Conclusions:** The findings further support a potential therapeutic role for increasing anandamide signaling in stress-induced depression and anxiety and suggest that the effects of URB597 on stress phenotype are associated with β-catenin function in the PFC.

Pubmed:
32311373: Sbarski B, Akirav I
Cannabinoids as therapeutics for PTSD.
Post-traumatic stress disorder (PTSD) is a complex disorder that involves dysregulation of multiple neurobiological systems. The traumatic stressor plays a causal role in producing psychological dysfunction and the pattern of findings suggests that the hypothalamic-pituitary-adrenal (HPA) axis, which is instrumental for stress adaptation, is critically dysfunctional in PTSD. Given the lack of understanding of the basic mechanisms and underlying pathways that cause the disorder and its heterogeneity, PTSD poses challenges for treatment. Targeting the endocannabinoid (ECB) system to treat mental disorders, and PTSD in particular, has been the focus of research and interest in recent years. The ECB system modulates multiple functions, and drugs enhancing ECB signaling have shown promise as potential therapeutic agents in stress effects and other psychiatric and medical conditions. In this review, we focus on the interaction between the ECB-HPA systems in animal models for PTSD and in patients with PTSD. We summarize evidence supporting the use of cannabinoids in preventing and treating PTSD in preclinical and clinical studies. As the HPA system plays a key role in the mediation of the stress response and the pathophysiology of PTSD, we describe preclinical studies suggesting that enhancing ECB signaling is consistent with decreasing PTSD symptoms and dysfunction of the HPA axis. Overall, we suggest that a pharmacological treatment targeted at one system (e.g., HPA) may not be very effective because of the heterogeneity of the disorder. There are abnormalities across different neurotransmitter systems in the pathophysiology of PTSD and none of these systems function uniformly among all patients with PTSD. Hence, conceptually, enhancing ECB signaling may be a more effective avenue for pharmacological treatment.
Pharmacol Ther, 2020; 211
30026011: Sbarski B, Akirav I
Chronic exposure to cannabinoids before an emotional trauma may have negative effects on emotional function.
Chronic direct activation of cannabinoid CB1 receptors (CB1r) may lead to downregulation of CB1r which may in turn result in a depression-like phenotype in certain individuals. We examined the effects of chronic cannabinoid receptor activation before

exposure to an emotional traumatic event on CB1r expression in the basolateral amygdala (BLA) and CA1 and on protracted anxiety- and depression-like behaviors. We used exposure to severe shock and situational reminders (SRs) in an inhibitory apparatus as a model for emotional trauma. Chronic treatment with the CB1/2 receptor agonist WIN55,212-2 (1.2 mg/kg, i.p.) before shock exposure had differential effects on depression- and anxiety-like behavioral measures depending on withdrawal periods. In the 24 hrs withdrawal condition, WIN55,212-2 enhanced fear retrieval and impaired extinction, increased anhedonia and despair, but had a therapeutic effect in the startle test. In the 10 days withdrawal condition, WIN55,212-2 enhanced fear retrieval and impaired extinction without preventing the shock/SR-induced negative effects on anhedonia or startle response, but had a therapeutic effect in the despair test. Chronic treatment with WIN55,212-2 was found to down regulate CB1r protein levels in the BLA in the 10 days withdrawal condition, and to upregulate CB1r protein levels in the 24 hrs condition. In the CA1, rats chronically injected with vehicle or WIN55,212-2 demonstrated downregulation of CB1r protein levels. Chronic exposure to cannabinoids prior to an emotional trauma may have deleterious effects on emotional function suggesting that direct CB1/2 receptor activation may not be an optimal way to manipulate the endocannabinoid system in stressful individuals.
Eur Neuropsychopharmacol, 2018; 28

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DOSE-DEPENDENT EFFECT OF REPEATED EXPOSURE TO EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELD (ELF-EMF) ON STRESS-RELATED BEHAVIOUR IN RATS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Angelika Klimek, Maciej Klimiuk, Hanna Kletkiewicz, Justyna Maliszewska, Agnieszka Siejka, Milena Jankowska, Joanna Wyszkowska, Maria Stankiewicz, Justyna Rogalska
Nicolaus Copernicus University, Department Of Animal Physiology And Neurobiology, Toruń, Poland

The ELF-EMF is regarded as a mild stress factor, which has been linked to the development of depression and anxiety in humans and anxiety-like behaviours in animals. Recently we have found the bidirectional effect of ELF-EMF on hypothalamic-pituitary-adrenal axis activity. We hypothesized that there is a safe limit of ELF-EMF intensity to which the organism can adapt and beyond which the behavioural changes can occur. Therefore, the study was aimed at determining the influence of repeated exposure to ELF-EMF 50 Hz of the low and high intensity on stress-related behaviour in rats. Adult male Wistar rats were divided into groups: 1) animals exposed to ELF-EMF of 1 mT, or 2) 7 mT, 3) control animals subjected to the same experimental procedure except for ELF-EMF exposure. Rats were exposed one (E1), two (E2) or three times (E3) for ELF-EMF (each: 7-days, 1 hour per day). After the end of each exposure period, the open-field test was performed. In rats exposed to 1 mT ELF-EMF, the activity was not changed significantly. While in animals exposed to 7 mT ELF-EMF, the activity was increased after E1 and E2, but it reached the control level after E3. We suggest that the stress system response evoked by the low-stress factor - 1 mT EMF - was not strong enough to change the behaviour. However, exposure to 7 mT ELF-EMF can cause the disturbance of stress response determining the increase of sensitivity to subsequent stress and probably the risk of stress-induced disorders.

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EFFECT OF DAUN02 INACTIVATION OF THE VENTRAL HIPPOCAMPUS ON BEHAVIORAL RESPONSES EVOKED BY CHRONIC RESTRAINT IN C-FOS LACZ TRANSGENIC RATS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Aims: Present work used Daun02 inactivation method in c-Fos lacZ transgenic rat in order to evaluate the effect of ventral hippocampus (VH) inactivation on behavioral responses habituation related to repeated chronic stress (CRS). Daun02 is substrate of β-galactosidase that catalyses Daun02 into daunomycin, causing apoptosis and calcium channels blockade. Methods: Guide cannulas were implanted into the VH of c-Fos LacZ transgenic rats. We submitted rats to CRS (2 h/day for 21 days) and studied the effect of vehicle or Daun02 microinjection into the VH on CRS-induced anxiety- (elevated plus maze and open field) and depressive-like (forced swimming test and splash test) responses observed, respectively, 24 and 48h after the last stress session. Results: Preliminary results showed that vehicle group presented a tendency in increase the number of open arms entries when compared to naive group (no stress). Daun02 group did not change the number of entries and the time spent in the open arms when compared to the vehicle. Vehicle group caused no change in time spent in the center of the arena when compared to naive, however Daun02 group showed a tendency of decrease it. Vehicle group showed a tendency in decrease grooming and immobility times when compared to naive. Daun02 group presented a tendency of increase the grooming time when compared to vehicle. Conclusion: Results show that, probably, VH participate in the neural pathway which is involved with behavioral responses observed during CRS. Financial Support: The State of São Paulo Research Foundation (FAPESP # 2018/04899-1; 2021/00148-4; 2021/04572-5; 2021/06709-8).

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INVESTIGATING THE EFFECTS OF ACUTE THC VAPOUR EXPOSURE ON STRESS REACTIVITY AND FEAR CONDITIONING

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Management of stress and anxiety in the general population, and management of PTSD symptoms in patients, are often listed as primary motivations behind cannabis use, yet the understanding of how acute exposure to cannabis modulates the neurobehavioral and endocrine response to negatively valanced stimuli is not well characterized. The aims of this study are twofold, to investigate the effects of acute cannabis exposure on stress reactivity and extinction training in a fear conditioning paradigm. Using a highly translational model of THC vapour inhalation, male and female rats were exposed to THC or vehicle for 15 minutes. In study 1, animals were subjected to one of the two vapour exposure conditions followed by exposure to an acute stressor or no stress condition. Blood samples were taken at several time points and plasma corticosterone (CORT) levels were quantified using an ELISA. In study 2, animals underwent fear conditioning, followed by vapour exposure and extinction training and finally extinction retrieval. Both passive (freezing) and active (darting) conditioned responses were quantified. This research has shown that acute THC exposure immediately elevates CORT levels in males but not female rats. It also shows that THC exposure prior to extinction training does not alter fear extinction in males but did impair fear extinction in females. This research suggests that acute exposure to THC vapour has sex-dependent effects on both neuroendocrine and behavioural responses to aversive stimuli.

Pubmed:

34907248: Baglot SL, Hume C, Petrie GN, Aukema RJ, Lightfoot SHM, Grace LM, Zhou R, Parker L, Rho JM, Borgland SL, McLaughlin RJ, Brechenmacher L, Hill MN
Pharmacokinetics and central accumulation of delta-9-tetrahydrocannabinol (THC) and its bioactive metabolites are influenced by route of administration and sex in rats.
Up to a third of North Americans report using cannabis in the prior month, most commonly through inhalation. Animal models that reflect human consumption are critical to study the impact of cannabis on brain and behaviour. Most animal studies to date utilize injection of delta-9-tetrahydrocannabinol (THC; primary psychoactive component of cannabis). THC injections produce markedly different physiological and behavioural effects than inhalation, likely due to distinctive pharmacokinetics. The current study directly examined if administration route (injection versus inhalation) alters metabolism and central accumulation of THC and metabolites over time. Adult male and female Sprague-Dawley rats received either an intraperitoneal injection or a 15-min session of inhaled exposure to THC. Blood and brains were collected at 15, 30, 60, 90 and 240-min post-exposure for analysis of THC and metabolites. Despite achieving comparable peak blood THC concentrations in both groups, our results indicate higher initial brain THC concentration following inhalation, whereas injection resulted in dramatically higher 11-OH-THC concentration, a potent THC metabolite, in blood and brain that increased over time. Our results provide evidence of different pharmacokinetic profiles following inhalation versus injection. Accordingly, administration route should be considered during data interpretation, and translational animal work should strongly consider using inhalation models.
Sci Rep, 2021; 11
30061817: Noye Tuplin EW, Lightfoot SHM, Holahan MR
Comparison of the Time-Dependent Changes in Immediate Early Gene Labeling and Spine Density Following Abstinence From Contingent or Non-contingent Chocolate Pellet Delivery.
: Incubation of craving is a phenomenon whereby responding for cues associated with a reward increases over extended periods of abstinence. Both contingent and non-contingent behavioral designs have been used to study the incubation of

craving phenomenon with differing results. The present study directly compares behavioral and neural changes following contingent or non-contingent administration of chocolate flavored pellets. : The current study examined whether an incubation of craving response would be observed at the behavioral and neural levels following delays of abstinence from chocolate pellets in a contingent or non-contingent reinforcement design. : Rats were trained for 10 days to bar press for chocolate pellets (contingent) or received chocolate pellets in a non-contingent design (classical conditioning). Groups were then subjected to abstinence from the reward for 24 h, 7, 14 or 28 days at which point they were tested for responding for reward associated cues. Following the test, brains from all rats were processed and assessed for c-Fos and FosB labeling as well as dendritic spine density in the nucleus accumbens (NAc). : Behavioral measures during the test (lever presses, food hopper entries and locomotor activity) revealed similar behavioral outcomes across all delays indicating the lack of an incubation of craving response on both the contingent and non-contingent designs. Overall, labeling of c-Fos in the NAc was lower for the non-contingent group compared to the operant-trained and food restricted control. Compared to the operant-trained and non-trained control groups, a significantly reduced FosB labeling was noted in the NAc of the classically conditioned groups across all abstinence periods. Spine density in the NAc was elevated in both the classically and operant conditioned compared to the food-restricted, non-trained controls. : Chocolate pellet reward did not result in incubation of craving but did produce behavioral learning that was associated with increased spine density. This suggests that chocolate pellet administration results in long-term structural and functional changes that are present for at least 28 days following abstinence. Contingent and non-contingent administration resulted in differential immediate early gene labeling in the NAc, but the functional significance of this has yet to be elucidated.
Front Behav Neurosci, 2018; 12

FENS Forum 2022
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BOARD NUMBER: S02-192

VOLATILE ORGANIC COMPOUNDS AS A PREDICTOR OF STRESS IN RATS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Stress is one of the most dangerous latent conditions in our lives and the leading cause of several types of disease. Today, stress is underdiagnosed. This could be attributed to the fact that part of the current diagnostic methods is based mainly on self-reporting and clinical checklists, which are subjective and inaccurate. The other part of the methods includes physiological measurement, such as heart rate variability, which are more accurate but are still affected by other factors and may not be suitable for daily monitoring. Here, we report a novel way to measure stress in a fast, non-invasive, and accurate manner. The approach relies on detecting volatile organic compounds (VOCs) that are emitted as a response to a changes in a bodily condition or a disease and in exchange with the skin, which has been previously shown as a reliable method to detect several diseases. VOCs linked with traumatic stress in rats (Sprague-Dawley male rats, *n*=32) were measured before, during, and after induction of a traumatic event. The results show a significant difference between the VOCs pattern of stressed rats and control in a predictive model with ROC AUC of 0.81 (90% sensitivity and 70% specificity) as well as a long-term effect after one week with *p*-value=0.0091 by Monte Carlo correlation analysis (and *p*-value=0.0044 during stress). These results signify the potential of using this method for a non-invasive, automatic, and real-time prediction of stress for diagnostics and monitoring mental health.

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ULTRASTRUCTURAL MITOCHONDRIAL ALTERATIONS IN RODENT MODEL OF DEPRESSION AND POTENTIAL CLINICAL RELEVANCE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Aims: Chronic stress models represent valid animal models for major depressive disorder (MDD) and can mimic core symptoms of the disease, e.g. anhedonia. It is well documented that chronic stress alters the functional and morphological integrity of neurons in limbic areas such as the hippocampus and prefrontal cortex (PFC). Typical stress-induced structural changes are the dendritic debranching, loss of dendritic spines and synapses. Recent theories suggest that stress may also alter mitochondrial structure and function. The purpose of the present study was to investigate putative changes of mitochondrial morphology and number in neurons of chronically stressed rats and depressed patients. Methods: We carried out a transmission electron microscopic analysis to quantify the number and morphological features of mitochondria in the medial PFC of control (n=4) and stressed (n=4) rats. A random systematic sampling was performed to take ultrastructural images at 40 000x magnification. Images were analysed with an unbiased stereology protocol. Results: Circa 8 000 EM images and nearly 100 000 mitochondria were counted and examined. We found a significant reduction in the number of mitochondria in the infralimbic cortex of rats, yet there were no changes in mitochondrial morphology. Mitochondrial parameters were also analysed in samples collected from the hippocampi of control (n=4) and MDD patients (n=4). Conclusion: In a rodent chronic stress model, we found significant reduction of mitochondrial numbers, but mitochondrial morphology was not altered. Further data from human samples will be presented on the poster.

Pubmed:
29440995: Csabai D, Wiborg O, Czéh B
Reduced Synapse and Axon Numbers in the Prefrontal Cortex of Rats Subjected to a Chronic Stress Model for Depression. Stressful experiences can induce structural changes in neurons of the limbic system. These cellular changes contribute to the development of stress-induced psychopathologies like depressive disorders. In the prefrontal cortex of chronically stressed animals, reduced dendritic length and spine loss have been reported. This loss of dendritic material should consequently result in synapse loss as well, because of the reduced dendritic surface. But so far, no one studied synapse numbers in the prefrontal cortex of chronically stressed animals. Here, we examined synaptic contacts in rats subjected to an animal model for depression, where animals are exposed to a chronic stress protocol. Our hypothesis was that long term stress should reduce the number of axo-spinous synapses in the medial prefrontal cortex. Adult male rats were exposed to daily stress for 9 weeks and afterward we did a post mortem quantitative electron microscopic analysis to quantify the number and morphology of synapses in the infralimbic cortex. We analyzed asymmetric (Type I) and symmetric (Type II) synapses in all cortical layers in control and stressed rats. We also quantified axon numbers and measured the volume of the infralimbic cortex. In our systematic unbiased analysis, we examined 21,000 axon terminals in total. We found the following numbers in the infralimbic cortex of control rats: 1.15 × 10 asymmetric synapses, 1.06 × 10 symmetric synapses and 1.00 × 10 myelinated axons. The density of asymmetric synapses was 5.5/µm and the density of symmetric synapses was 0.5/µm. Average synapse membrane length was 207 nm and the average axon terminal membrane length was 489 nm. Stress reduced the number of synapses and myelinated axons in the deeper cortical layers, while synapse membrane lengths were increased. These stress-induced ultrastructural changes indicate that neurons of the infralimbic cortex have reduced cortical network connectivity. Such reduced network connectivity is likely to form the anatomical basis for the impaired functioning of this brain area. Indeed, impaired functioning of the prefrontal cortex, such as cognitive deficits are common in stressed individuals as

well as in depressed patients.
Front Cell Neurosci, 2018; 12

FENS Forum 2022
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BOARD NUMBER: S02-194

CRH-ERGIC NEURONS OF MEDIAN RAPHE REGION REGULATE STRESS AND ANXIETY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Dysfunctions of median raphe region (MRR) were associated with stress-related psychiatric disorders, assumably due to its serotoninergic content. Recently we have found that corticotropin-releasing hormone (CRH), the main hypothalamic regulator of stress axis, is also abundant in this area. Therefore, we aimed to reveal the contribution of these peptidergic cells in stress adaptation. Pharmacogenetic technique was used in male CRH-Cre mice. Control, or stimulatory DREADD sequence was injected into the MRR using adeno-associated viral vector. Clozapine-N-oxide was used as a ligand. Parallel with changes in stress-hormone concentration, anxiety- and depression-like behavior was also measured. Stress-induced activation of MRR-CRH-ergic cells were studied in CRH-Cre tdTomato crossbred animals. Accuracy of injections and c-Fos activation were investigated by immunohistochemistry. Stimulation of CRH neurons in MRR increased corticosterone levels and relative spleen weight parallel with anxiety-like behavior in elevated plus maze, light-dark box, and fox odor test compared to controls. Difference was not found in tests measuring depression-like behavior. Acute stress activated the MRR-CRH neurons measured by c-Fos. To summarize, stimulation of MRR-CRH neurons may induce stress-hormone elevation as well as anxiety-like, but not depression-like behavior. Thus, our results support the existence of a new, acute stress regulatory brainstem CRH population, which may play a key role in regulating stress adaptation both at hormonal and behavioral level.

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BOARD NUMBER: S02-195

MIR-34 FAMILY IS INVOLVED IN CHRONIC SOCIAL DEFEAT-INDUCED VULNERABILITY TO MOOD DISORDERS AND CARDIAC MITOCHONDRIAL DYSFUNCTIONS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Chronic stress exposure increases vulnerability to mood disorders. Interestingly, a bidirectional relationship between depressive disorders and cardiovascular disease has been observed in clinical and pre-clinical studies, and chronic stress can favour the onset of both pathologies. This could be due to shared genetic predispositions as well as dysregulated stress-coping response. MicroRNAs (miRs) are involved in brain responses to stressful stimuli. Our previous studies have linked the miR-34 family with neurobiological mechanisms that underlie the regulation of stress response, and the subsequent adopted coping mechanism. Interestingly, we recently observed that rats exposed to Chronic Social Defeat (CSD) showed increased miR-34a levels in heart, coupled with increased risk of cardiovascular dysfunctions, and RNA sequencing suggested compromised mitochondria's function. Here, we hypothesize that miR-34 family is involved in the onset of stress-induced mood disorders as well as cardiovascular disease, affecting coping response and heart's mitochondrial respiration. Wild Type (WT) and knockout (TKO) mice for miR-34 family were tested to assess anxious- and depressive-like behavior. Then they underwent to CSD and retested to evaluate the effect of CSD exposure. A separate cohort of subject underwent to CSD or left unstressed, then heart's Oxygen Consumption Rate (OCR) was measured. We found that TKO coping behavior is less affected by CSD exposure compared to WT. Moreover, we found that TKO mice exhibit lower heart's OCR after CSD compared to other groups. Our results suggest that miR-34 family is involved in CSD-induced vulnerability to cardiovascular and mood disorders, affecting coping behavior and cellular respiratory functions.

Pubmed:
34227918: Andolina D, Savi M, Ielpo D, Barbetti M, Bocchi L, Stilli D, Ventura R, Lo Iacono L, Sgoifo A, Carnevali L
Elevated miR-34a expression and altered transcriptional profile are associated with adverse electromechanical remodeling in the heart of male rats exposed to social stress.
This study investigated epigenetic risk factors that may contribute to stress-related cardiac disease in a rodent model. Experiment 1 was designed to evaluate the expression of microRNA-34a (miR-34a), a known modulator of both stress responses and cardiac pathophysiology, in the heart of male adult rats exposed to a single or repeated episodes of social defeat stress. Moreover, RNA sequencing was conducted to identify transcriptomic profile changes in the heart of repeatedly stressed rats. Experiment 2 was designed to assess cardiac electromechanical changes induced by repeated social defeat stress that may predispose rats to cardiac dysfunction. Results indicated a larger cardiac miR-34a expression after repeated social defeat stress compared to a control condition. This molecular modification was associated with increased vulnerability to pharmacologically induced arrhythmias and signs of systolic left ventricular dysfunction. Gene expression analysis identified clusters of differentially expressed genes in the heart of repeatedly stressed rats that are mainly associated with morphological and functional properties of the mitochondria and may be directly regulated by miR-34a. These results suggest the presence of an association between miR-34a overexpression and signs of adverse electromechanical remodeling in the heart of rats exposed to repeated social defeat stress, and point to compromised mitochondria efficiency as a potential mediator of this link. This rat model may provide a useful tool for investigating the causal relationship between miR-34a expression, mitochondrial (dys)function, and cardiac alterations under stressful conditions, which could have important implications in the context of stress-related cardiac disease.
Stress, 2021; 24
33937445: D'Addario SL, Di Segni M, Ledonne A, Piscitelli R, Babicola L, Martini A, Spoleti E, Mancini C, Ielpo D, D'Amato

FR, Andolina D, Ragozzino D, Mercuri NB, Cifani C, Renzi M, Guatteo E, Ventura R
Resilience to anhedonia-passive coping induced by early life experience is linked to a long-lasting reduction of I current in VTA dopaminergic neurons.
Exposure to aversive events during sensitive developmental periods can affect the preferential coping strategy adopted by individuals later in life, leading to either stress-related psychiatric disorders, including depression, or to well-adaptation to future adversity and sources of stress, a behavior phenotype termed "resilience". We have previously shown that interfering with the development of mother-pups bond with the Repeated Cross Fostering (RCF) stress protocol can induce resilience to depression-like phenotype in adult C57BL/6J female mice. Here, we used patch-clamp recording in midbrain slice combined with both and pharmacology to test our hypothesis of a link between electrophysiological modifications of dopaminergic neurons in the intermediate Ventral Tegmental Area (VTA) of RCF animals and behavioral resilience. We found reduced hyperpolarization-activated (I) cation current amplitude and evoked firing in VTA dopaminergic neurons from both young and adult RCF female mice. , VTA-specific pharmacological manipulation of the I current reverted the pro-resilient phenotype in adult early-stressed mice or mimicked behavioral resilience in adult control animals. This is the first evidence showing how pro-resilience behavior induced by early events is linked to a long-lasting reduction of I current and excitability in VTA dopaminergic neurons.
Neurobiol Stress, 2021; 14
33333214: Babicola L, Ventura R, D'Addario SL, Ielpo D, Andolina D, Di Segni M
Long term effects of early life stress on HPA circuit in rodent models.
Adaptation to environmental challenges represents a critical process for survival, requiring the complex integration of information derived from both external cues and internal signals regarding current conditions and previous experiences. The Hypothalamic-pituitary-adrenal axis plays a central role in this process inducing the activation of a neuroendocrine signaling cascade that affects the delicate balance of activity and cross-talk between areas that are involved in sensorial, emotional, and cognitive processing such as the hippocampus, amygdala, Prefrontal Cortex, Ventral Tegmental Area, and dorsal raphe. Early life stress, especially early critical experiences with caregivers, influences the functional and structural organization of these areas, affects these processes in a long-lasting manner and may result in long-term maladaptive and psychopathological outcomes, depending on the complex interaction between genetic and environmental factors. This review summarizes the results of studies that have modeled this early postnatal stress in rodents during the first 2 postnatal weeks, focusing on the long-term effects on molecular and structural alteration in brain areas involved in Hypothalamic-pituitary-adrenal axis function. Moreover, a brief investigation of epigenetic mechanisms and specific genetic targets mediating the long-term effects of these early environmental manipulations and at the basis of differential neurobiological and behavioral effects during adulthood is provided.
Mol Cell Endocrinol, 2021; 521
33845072: Lo Iacono L, Ielpo D, Parisi C, Napoli G, Accoto A, Di Segni M, Babicola L, D'Addario SL, Guzzo SM, Pascucci T, Ventura R, Andolina D
MicroRNA-34a regulates 5-HT2C expression in dorsal raphe and contributes to the anti-depressant-like effect of fluoxetine.
Selective serotonin reuptake inhibitors (SSRIs) are designed to improve mood by raising extracellular serotonin levels through the blockade of the serotonin transporter. However, they exhibit a slow onset of action, suggesting the involvement of adaptive regulatory mechanisms. We hypothesized that the microRNA-34 family facilitates the therapeutic activity of SSRIs. We show that genetic deletion of these microRNAs in mice impairs the response to chronic, but not acute, fluoxetine treatment, with a specific effect on behavioral constructs that are related to depression, rather than anxiety. Moreover, using a pharmacological strategy, we found that an increased expression of the serotonin 2C (5-HT2C) receptor in the dorsal raphe region of the brain contributes to this phenotype. The onset of the therapeutic efficacy of SSRIs is paralleled by the desensitization of the 5-HT2C receptor in the dorsal raphe, and 5-HT2C is a putative target of microRNA-34. In this study, acute and chronic fluoxetine treatment differentially alters the expression of 5-HT2C and microRNA-34a in the dorsal raphe. Moreover, by in vitro luciferase assay, we demonstrated the repressive regulatory activity of microRNA-34a against 5-HT2C mRNA. Specific blockade of this interaction through local infusion of a target site blocker was sufficient to prevent the behavioral effects of chronic fluoxetine. Our results demonstrate a new miR-34a-mediated regulatory mechanism of 5-HT2C expression in the dorsal raphe and implicate it in eliciting the behavioral responses to chronic fluoxetine treatment.
Neuropharmacology, 2021; 190
32113966: Di Segni M, D'Addario SL, Babicola L, Ielpo D, Lo Iacono L, Andolina D, Accoto A, Luchetti A, Mancini C, Parisi C, D'Onofrio M, Arisi I, Brandi R, Pascucci T, Cifani C, D'Amato FR, Ventura R
Xlr4 as a new candidate gene underlying vulnerability to cocaine effects.
Although several studies have been performed in rodents, non-human primates and humans, the biological basis of vulnerability to develop cocaine addiction remains largely unknown. Exposure to critical early events (as Repeated Cross Fostering (RCF)) has been reported to increase sensitivity to cocaine effects in adult C57BL/6J female mice. Using a

microarray approach, here we report data showing a strong engagement of X-linked lymphocyte-regulated 4a and 4b (Xlr4) genes in cocaine effects. The expression of Xlr4, a gene involved in chromatin remodeling and dendritic spine morphology, was reduced into the Nucleus Accumbens (NAc) of adult RCF C57BL/6J female. We used virally mediated accumbal Xlr4 down-modulation (AAVXlr4-KD) to investigate the role of this gene in vulnerability to cocaine effects. AAVXlr4-KD animals show a potentiated behavioral and neurochemical response to cocaine, reinstatement following cocaine withdrawal and cocaine-induced spine density alterations in the Medium-Sized Spiny Neurons of NAc. We propose Xlr4 as a new candidate gene mediating the cocaine effects.
Neuropharmacology, 2020; 168
32169579: Babicola L, Pietrosanto M, Ielpo D, D'Addario SL, Cabib S, Ventura R, Ferlazzo F, Helmer-Citterich M, Andolina D, Lo Iacono L
RISC RNA sequencing in the Dorsal Raphè reveals microRNAs regulatory activities associated with behavioral and functional adaptations to chronic stress.
The Dorsal Raphe (DR) is the primary source of serotonergic input in the brain and a center for the homeostatic maintenance of the serotonergic tone. Under repeated stimulation, it can undergo adaptive modifications that alter serotonergic neurotransmission, which can lead to behavioral dysfunction. Post-transcriptional regulation by microRNAs is implicated in these adaptations. However, a global microRNA/target network effect on the DR neuroplasticity has yet to be elucidated. Here we investigate the microRNAs/mRNAs regulatory activity in the mouse DR after a chronic stress experience. First, we assessed the behavioral consequences of repeated restraint stress exposure and the functional adaptations of the DR by measuring the change in acute stress-induced serotonin release. Then, through next generation RNA-Seq of Argonaute2-bound RNA (RISC-Seq) we identified microRNAs and their targets that are associated to the RISC complex of the DR in unstressed and stressed mice. We mapped the potential microRNA/mRNA network within the stress-altered transcripts, uncovering new interactions that contribute to the chronic stress-induced DR modifications.
Brain Res, 2020; 1736
31228589: Di Segni M, Andolina D, D'Addario SL, Babicola L, Ielpo D, Luchetti A, Pascucci T, Lo Iacono L, D'Amato FR, Ventura R
Sex-dependent effects of early unstable post-natal environment on response to positive and negative stimuli in adult mice.
Alterations in early environmental conditions that interfere with the creation of a stable mother-pup bond have been suggested to be a risk factor for the development of stress-related psychopathologies later in life. The long-lasting effects of early experiences are mediated by changes in various cerebral circuits, such as the corticolimbic system, which processes aversive and rewarding stimuli. However, it is evident that the early environment is not sufficient per se to induce psychiatric disorders; interindividual (eg, sex-based) differences in the response to environmental challenges exist. To examine the sex-related effects that are induced by an early experience on later events in adulthood, we determine the enduring effects of repeated cross-fostering (RCF) in female and male C57BL/6J mice. To this end, we assessed the behavioral phenotype of RCF and control (male and female) mice in the saccharine preference test and cocaine-induced conditioned place preference to evaluate the response to natural and pharmacological stimuli and in the elevated plus maze test and forced swimming test to measure their anxiety- and depression-like behavior. We also evaluated FST-induced c-Fos immunoreactivity in various brain regions that are engaged in the response to acute stress exposure (FST). Notably, RCF has opposing effects on the adult response to these tests between sexes, directing male mice toward an "anhedonia-like" phenotype and increasing the sensitivity for rewarding stimuli in female mice.
Neuroscience, 2019; 413
31482401: Lo Iacono L, Ielpo D, Accoto A, Di Segni M, Babicola L, D'Addario SL, Ferlazzo F, Pascucci T, Ventura R, Andolina D
MicroRNA-34a Regulates the Depression-like Behavior in Mice by Modulating the Expression of Target Genes in the Dorsal Raphè.
Chronic stress exposure is known to increase vulnerability to the expression of psychiatric disorders, such as depression. Clinical and preclinical evidences support the involvement of the microRNA-34 family in stress-related psychiatric conditions and in the regulation of stress responses. However, the mechanism and the multiple targets by which the microRNA-34 family can affect the stress response and stress-related behavioral alteration are not fully known. Here, with the aid of constitutive and conditional genetic strategy, we examined the role of microRNA-34 family in the expression of depression-like phenotype in mice induced by chronic stress exposure, and we identified their "in vivo" targets during the stressful challenge. We found that microRNA-34a, under chronic stress, is significantly up-regulated in the mouse raphe nuclei, where its recruitment is necessary to induce depression-like behavioral alterations and impact the function of the serotonergic system. Moreover, by next-generation RNA-seq of Ago-2-bound mRNAs, we identified genes that are targeted by microRNA-34a in response to chronic stress and that are likely to mediate its effects.
Mol Neurobiol, 2020; 57

29417477: Andolina D, Di Segni M, Accoto A, Lo Iacono L, Borreca A, Ielpo D, Berretta N, Perlas E, Puglisi-Allegra S, Ventura R

MicroRNA-34 Contributes to the Stress-related Behavior and Affects 5-HT Prefrontal/GABA Amygdalar System through Regulation of Corticotropin-releasing Factor Receptor 1.

Recent studies show that microRNA-34 (miR-34) family is critical in the regulation of stress response also suggesting that it may contribute to the individual responsiveness to stress. We have recently demonstrated that mice carrying a genetic deletion of all miR-34 isoforms (triple knockout, TKO) lack the stress-induced serotonin (5-HT) and GABA release in the medial prefrontal cortex (mpFC) and basolateral amygdala (BLA), respectively. Here, we evaluated if the absence of miR-34 was also able to modify the stress-coping strategy in the forced swimming test. We found that the blunted neurochemical response to stress was associated with lower levels of immobility (index of active coping behavior) in TKO compared to WT mice. Interestingly, among the brain regions mostly involved in the stress-related behaviors, the miR-34 displayed the strongest expression in the dorsal raphe nuclei (DRN) of wild-type (WT) mice. In the DRN, the corticotropin-releasing factor receptors (CRFR) 1 and 2, contribute to determine the stress-coping style and the CRFR1 is a target of miR-34. Thus, we hypothesized that the miR-34-dependent modulation of CRFR1 expression may be involved in the DRN regulation of stress-coping strategies. In line with this hypothesis, we found increased CRFR1 levels in the DNR of TKO compared to WT mice. Moreover, infusion of CRFR1 antagonist in the DRN of TKO mice reverted their behavioral and neurochemical phenotype. We propose that miR-34 modulate the mpFC 5-HT/BLA GABA response to stress acting on CRFR1 in the DRN and that this mechanism could contribute to determine individual stress-coping strategy.

Mol Neurobiol, 2018; 55

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-196

DOPAMINE SYSTEM, NMDA RECEPTOR AND EGF FAMILY EXPRESSIONS IN BRAIN STRUCTURES OF BL6 AND 129SV STRAINS DISPLAYING DIFFERENT BEHAVIORAL ADAPTATION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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C57BL/6NTac (Bl6) and 129S6/SvEvTac (129Sv) mice display different coping strategies in stressful conditions. Our aim was to evaluate biomarkers related to different adaptation strategies in the brain of male 129Sv and Bl6 mice. We focused on signaling pathways related to the dopamine (DA) system, N-methyl-D-aspartate (NMDA) receptor and epidermal growth factor (EGF) family, shown as the key players in behavioral adaptation. Mice from Bl6 and 129Sv lines were divided into either home cage controls (HCC group) or exposed to repeated motility testing and treated with saline for 11 days (RMT group). Distinct stress responses were reflected in severe body weight loss in 129Sv and the increased exploratory behavior in Bl6 mice. Besides that, amphetamine caused significantly stronger motor stimulation in Bl6. Together with the results from gene expression (particularly *Maob*), this study supports higher baseline activity of DA system in Bl6. Interestingly, the adaptation is reflected with opposite changes of DA markers in dorsal and ventral striatum. In forebrain, stress increased the gene expressions of *Egf-Erbb1* and *Nrg1/Nrg2-Erbb4* pathways more clearly in 129Sv, whereas the corresponding proteins were significantly elevated in Bl6. We suggest that not only inhibited activity of the DA system, but also reduced activity of EGF family and NMDA receptor signaling underlies higher susceptibility to stress in 129Sv. Altogether, this study underlines the better suitability of 129Sv for modelling neuropsychiatric disorders than Bl6.

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THE EFFECT OF SOCIAL ISOLATION ON CANCER METASTASES AND THE NUCLEUS ACCUMBENS IN RATS.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Background: Social isolation (SI) was found to negatively impact wellbeing, is a risk factor for malignancy, morbidity, and mortality, and is associated with immune suppression, accelerated cancer progression, and neuroendocrine changes. Here we study the impact of SI in rats on metastatic progression and gene expression in the Nucleus Accumbens (NAc). **Methods:** Female and male Fischer-344 rats, 4-6 months old, were randomly assigned to control or SI conditions 1 or 2 weeks prior to IV injection of 1x105 MADB106 mammary adenocarcinoma cells. On day 21 post-injection, lungs, brain and blood were obtained for analysis: Lung metastases were enumerated; Corticosterone (CORT) plasma levels were quantified using ELISA; mRNA was isolated from the NAc, and the expression of OXTR, CRF1, CRF2, 5-HTR, and D1r genes were assessed with RT-PCR. **Results:** One and two weeks of isolation was associated with increased metastases numbers compared to controls ($p=0.055$; $p<0.04$ respectively). In females only, 6 weeks of SI significantly elevated baseline CORT levels ($p=0.004$). SI-female rats showed increased levels of OXTr and 5HTr mRNAs in the NAc (2-fold, $p=0.02$; 3-fold, $p=0.01$; respectively), while SI-males showed decreased levels of 5HTr (0.5-Fold, $p=0.03$) and increased levels of D1r mRNAs (2-Fold, $p=0.04$). CRF1r and CRF2r mRNA levels were not affected. **Conclusion:** SI was associated with metastasis growth and sex-dependent alterations in neuroendocrine signaling. These changes may be part of a mediating mechanism altering immunity; our ongoing studies look deeper to understand the influence of the social environment on immune function through neuro-immune pathways.

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BOARD NUMBER: S02-198

H3K4ME3 EPIGENETIC SIGNATURES IN SOCIAL DEFEAT STRESS OF VARYING DURATION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Stress-induced psychiatric disorders are a worldwide problem of contemporary society due to the complexity of therapy and the variety of forms. In this study, we analyzed the effects of social defeat stress (SDS) of varying duration (10, 15 and 30 days) on the genome-wide H3K4me3 landscape in the prefrontal cortex (PFC) of C57BL/6 mice using MNase-ChIP-seq method. For downstream analyses we selected peaks located in promoter regions (±2Kb from TSS) and integrated these results with previously obtained RNA-seq data after 10, 15 and 30 days of SDS. We analyzed data using relaxed thresholds of p-value <0.05 resulting in 1637 deferentially enriched peaks (DEP) for SDS10 and 2033 DEP for SDS30 compared to control. With increase in duration of SDS we observed more genes with DEP that are involved in synaptic plasticity regulation. We identified a cluster of 71 genes with at least one DEP present in all three groups (SDS10, SDS15, SDS30). Only DEP in 4 genes (*Numbl, Gal3st3, Cntn2* and *Gria2)* had unidirectional changes, while others formed unique patterns. Changes in the epigenetic landscape after SDS did not correlate with the level of gene expression. We found few DE genes (7-12%) with DEP in its promoter region. Among them only *Pde10a* gene had DEP in its promoter and altered expression in all groups. Our results demonstrate that the effects of SDS probably depend on combination of changes in multiple H3K4me3 promoter peaks instead of individual peak density. This work was supported by the Russian Science Foundation (21-15-00142).

Pubmed:
34877386: Ryabushkina YA, Shevelev OB, Kisaretova PE, Sozonov NG, Ayriyants KA, Bondar NP, Reshetnikov VV
High-resolution MRI data of the brain of C57BL/6J and BTBR mice in three anatomical views.
The research on strain-, sex-, and stress-specific differences in structural and functional connectivity of the brain is important for elucidating various behavioral features and etiologies of psychiatric disorders. Socially impaired BTBR mice are considered a model of autism spectrum disorders. Here we present high-resolution magnetic resonance imaging data from the brain of 89 adolescent mice (C57BL/6J and BTBR) in axial, sagittal, and coronal views. The study [1] includes both females and males differed in early-life experience (normally reared or subjected to prolonged maternal separation: 3 h daily from postnatal day 2 to 15). The MRI data were obtained on a horizontal tomograph Biospec 117/16 instrument with a magnetic field strength of 11.7 T. Thus, multislice Turbo RARE T-weighted images of the brain were captured in eight groups of mice. Altogether, these data allow to evaluate strain-, sex-, and stress-specific alterations in the volumes of various brain structures and to better understand the relation between brain structural differences and behavioral abnormalities.
Data Brief, 2021; 39
34259424: Dolgova EV, Andrushkevich OM, Kisaretova PE, Proskurina AS, Ritter GS, Dubatolova TD, Romanenko MV, Taranov OS, Efremov YR, Zavyalov EL, Romaschenko AV, Mishinov SV, Kirikovich SS, Levites EV, Potter EA, Ostanin AA, Chernykh ER, Roshchin SY, Bervitskiy AV, Moysak GI, Rzaev JA, Bogachev SS
Efficacy of the new therapeutic approach in curing malignant neoplasms on the model of human glioblastoma.
Glioma is a highly invasive tumor, frequently disposed in essential areas of the brain, which makes its surgical excision extremely difficult; meanwhile adjuvant therapy remains quite ineffective.
Cancer Biol Med, 2021;
33659850: Ritter GS, Nikolin VP, Popova NA, Proskurina AS, Kisaretova PE, Taranov OS, Dubatolova TD, E V Dolgova EV, Potter EA, Kirikovich SS, Efremov YR, Bayborodin SI, Romanenko MV, Meschaninova MI, Venyaminova AG, Kolchanov NA, Bogachev SS
[Characteristic of the active substance of the Saccharomyces cerevisiae preparation having radioprotective properties].
The paper describes some biological features of the radioprotective effect of double-stranded RNA preparation. It was found that yeast RNA preparation has a prolonged radioprotective effect after irradiation by a lethal dose of 9.4 Gy. 100 % of

animals survive on the 70th day of observation when irradiated 1 hour or 4 days after 7 mg RNA preparation injection, 60 % animals survive when irradiated on day 8 or 12. Time parameters of repair of double-stranded breaks induced by gamma rays were estimated. It was found that the injection of the RNA preparation at the time of maximum number of double-stranded breaks, 1 hour after irradiation, reduces the efficacy of radioprotective action compared with the injection 1 hour before irradiation and 4 hours after irradiation. A comparison of the radioprotective effect of the standard radioprotector B-190 and the RNA preparation was made in one experiment. It has been established that the total RNA preparation is more efficacious than B-190. Survival on the 40th day after irradiation was 78 % for the group of mice treated with the RNA preparation and 67 % for those treated with B-190. In the course of analytical studies of the total yeast RNA preparation, it was found that the preparation is a mixture of single-stranded and double-stranded RNA. It was shown that only double-stranded RNA has radioprotective properties. Injection of 160 μg double-stranded RNA protects 100 % of the experimental animals from an absolutely lethal dose of gamma radiation, 9.4 Gy. It was established that the radioprotective effect of double-stranded RNA does not depend on sequence, but depends on its double-stranded form and the presence of "open" ends of the molecule. It is supposed that the radioprotective effect of double-stranded RNA is associated with the participation of RNA molecules in the correct repair of radiation-damaged chromatin in blood stem cells. The hematopoietic pluripotent cells that have survived migrate to the periphery, reach the spleen and actively proliferate. The newly formed cell population restores the hematopoietic and immune systems, which determines the survival of lethally irradiated animals.
Vavilovskii Zhurnal Genet Selektsii, 2020; 24
33328525: Reshetnikov VV, Kisaretova PE, Ershov NI, Shulyupova AS, Oshchepkov DY, Klimova NV, Ivanchihina AV, Merkulova TI, Bondar NP
Genes associated with cognitive performance in the Morris water maze: an RNA-seq study.
Learning and memory are among higher-order cognitive functions that are based on numerous molecular processes including changes in the expression of genes. To identify genes associated with learning and memory formation, here, we used the RNA-seq (high-throughput mRNA sequencing) technology to compare hippocampal transcriptomes between mice with high and low Morris water maze (MWM) cognitive performance. We identified 88 differentially expressed genes (DEGs) and 24 differentially alternatively spliced transcripts between the high- and low-MWM-performance mice. Although the sets of DEGs and differentially alternatively spliced transcripts did not overlap, both were found to be enriched with genes related to the same type of biological processes: trans-synaptic signaling, cognition, and glutamatergic transmission. These findings were supported by the results of weighted-gene co-expression network analysis (WGCNA) revealing the enrichment of MWM-cognitive-performance-correlating gene modules with very similar Gene Ontology terms. High-MWM-performance mice manifested mostly higher expression of the genes associated with glutamatergic transmission and long-term potentiation implementation, which are processes necessary for memory acquisition and consolidation. In this set, there were genes participating in the regulation of trans-synaptic signaling, primarily AMPA receptor signaling (Nrn1, Nptx1, Homer3, Prkce, Napa, Camk2b, Syt7, and Nrgn) and calcium turnover (Hpca, Caln1, Orai2, Cpne4, and Cpne9). In high-MWM-performance mice, we also demonstrated significant upregulation of the "flip" splice variant of Gria1 and Gria2 transcripts encoding subunits of AMPA receptor. Altogether, our data helped to identify specific genes in the hippocampus that are associated with learning and long-term memory. We hypothesized that the differences in MWM cognitive performance between the mouse groups are linked with increased long-term potentiation, which is mainly mediated by increased glutamatergic transmission, primarily AMPA receptor signaling.
Sci Rep, 2020; 10
33102649: Reshetnikov VV, Kisaretova PE, Ershov NI, Merkulova TI, Bondar NP
Data of correlation analysis between the density of H3K4me3 in promoters of genes and gene expression: Data from RNA-seq and ChIP-seq analyses of the murine prefrontal cortex.
H3K4me3 is typically found in the promoter region of genes and is a mark associated with an open chromatin state and active gene transcription. Nonetheless, the role of H3K4me3 in the regulation of transcription is still debated. To improve the understanding of the connection between H3K4me3 density in promoters and gene expression, we assessed the correlation between these two parameters. We utilized genome-wide high-throughput RNA sequencing (RNA-seq) data and H3K4me3-based chromatin immunoprecipitation with high-throughput sequencing (ChIP-seq), carried out on the same samples of the prefrontal cortex from 10 male C57Bl6 mice with different stress experience [Social defeat stress in adult mice causes alterations in gene expression, alternative splicing, and the epigenetic landscape of H3K4me3 in the prefrontal cortex: an impact of early-life stress, 1]. In addition, we assessed the correlation between H3K4me3 density and gene expression in datasets of cell-specific genes. Altogether, the results are useful for the elucidation of H3K4me3 involvement in the regulation of transcription in the murine prefrontal cortex.
Data Brief, 2020; 33
32810572: Reshetnikov VV, Kisaretova PE, Ershov NI, Merkulova TI, Bondar NP
Social defeat stress in adult mice causes alterations in gene expression, alternative splicing, and the epigenetic landscape of

H3K4me3 in the prefrontal cortex: An impact of early-life stress.
Chronic stress is the leading risk factor of a broad range of severe psychopathologies. Nonetheless, the molecular mechanisms triggering these pathological processes are not well understood. In our study, we investigated the effects of 15-day social defeat stress (SDS) on the genome-wide landscape of trimethylation at the 4th lysine residue of histone H3 (H3K4me3) and on the transcriptome in the prefrontal cortex of mice that were reared normally (group SDS) or subjected to maternal separation early in life (group MS+SDS). The mice with the history of stress early in life showed increased susceptibility to SDS in adulthood and demonstrated long-lasting genome-wide alterations in gene expression and splicing as well as in the H3K4me3 epigenetic landscape in the prefrontal cortex. Thus, the high-throughput techniques applied here allowed us to simultaneously detect, for the first time, genome-wide epigenetic and transcriptional changes in the murine prefrontal cortex that are associated with both chronic SDS and increased susceptibility to this stressor.
Prog Neuropsychopharmacol Biol Psychiatry, 2021; 106
32658564: Ritter GS, Nikolin VP, Popova NA, Proskurina AS, Kisaretova PE, Taranov OS, Dubatolova TD, Dolgova EV, Potter EA, Kirikovich SS, Efremov YR, Bayborodin SI, Romanenko MV, Meschaninova MI, Venyaminova AG, Kolchanov NA, Shurdov MA, Bogachev SS
Characterization of biological peculiarities of the radioprotective activity of double-stranded RNA isolated from .
Protection from ionizing radiation is the most important component in the curing malignant neoplasms, servicing atomic reactors, and resolving the situations associated with uncontrolled radioactive pollutions. In this regard, discovering new effective radioprotectors as well as novel principles of protecting living organisms from high-dose radiation is the most important factor, determining the new approaches in medical and technical usage of radiation.
Int J Radiat Biol, 2020; 96
32014922: Kisaretova PE, Kirikovich SS, Ritter GS, Efremov YR, Taranov OS, Dubatolova TD, Proskurina AS, Potter EA, Dolgova EV, Sidorov SV, Ostanin AA, Chernykh ER, Bogachev SS
Approbation of the Cancer Treatment Approach Based on the Eradication of TAMRA+ Cancer Stem Cells in a Model of Murine Cyclophosphamide Resistant Lymphosarcoma.
We previously have described the "3+1" tumors cure approach consisting of individual time schedule of cyclophosphamide and dsDNA preparation administrations. The aim of the study was to adapt the "3+1" approach based on eradication of cancer stem cells to the model of murine ascitic cyclophosphamide-resistant lymphosarcoma (RLS).
Anticancer Res, 2020; 40
31080361: Dolgova EV, Petrova DD, Proskurina AS, Ritter GS, Kisaretova PE, Potter EA, Efremov YR, Bayborodin SI, Karamysheva TV, Romanenko MV, Netesov SV, Taranov OS, Ostanin AA, Chernykh ER, Bogachev SS
Identification of the xenograft and its ascendant sphere-forming cell line as belonging to EBV-induced lymphoma, and characterization of the status of sphere-forming cells.
We have characterized the human cell line arised from the Epstein-Barr virus (EBV) positive multiple myeloma aspirate subjected to the long-term cultivation. This cell line has acquired the ability to form free-floating spheres and to produce a xenograft upon transplantation into NOD/SCID mice.
Cancer Cell Int, 2019; 19

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THE INTERACTION OF GR AND TET3 REPRESENTS A POTENTIAL MECHANISTIC LINK TO THE EPIGENETIC EMBEDDING OF GR ACTIVATION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Upon **stress exposure** the hypothalamic-pituitary-adrenal axis gets activated which ultimately leads to the release of glucocorticoid hormones. Glucocorticoids bind the **glucocorticoid receptor (GR)** leading to its nuclear translocation, where it binds to DNA regions, known as GR response elements (GREs) and promotes both transcriptional and epigenetic alterations, including changes in **DNA methylation**. However, the exact molecular mechanism remains unclear. The known interaction of **TET3, a DNA demethylase**, with nuclear receptors, such as the thyroid hormone receptor, led us to hypothesize that a similar interaction could exist between GR and TET3. In HeLa cells overexpressing TET3 we have shown using co-immunoprecipitation that **TET3 and GR interact** after activation of GR with dexamethasone (dex), a synthetic glucocorticoid agonist. Additionally, using chromatin immunoprecipitation (ChIP)-qPCR at known intronic GREs of three dex-induced genes (TSC22D3, ZBTB16 and FKBP51), we show significant enrichment of TET3 after dex treatment (Student's t test, veh vs dex for the respective regions p = 0.056, p=0.002, p=0.043, N=2) compared to a control region (DSCAM, p = 0.163). These findings suggest that glucocorticoid stimulation leads to the recruitment of TET3 to GREs, where it potentially contributes through **epigenetic modulation** to the regulation of gene expression. Targeted bisulfite- and assay for transposase-accessible chromatin sequencing will help unravel the changes in the level of DNA methylation and chromatin accessibility at these specific loci. Ultimately, these results highlight the importance to understand the molecular mechanisms driving the **pathological embedding of environmental stimuli**, especially relevant in the aetiology of **stress-related psychiatric disorders**.

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BOARD NUMBER: S02-200

ALTERED UNFOLDED PROTEIN RESPONSE IN A DOUBLE-HIT MODEL OF NEURODEVELOPMENT IN CORTEX AND HIPPOCAMPUS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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[1]Universidad Complutense de Madrid, Farmacología Y Toxicología, Madrid, Spain, [2](CIBERSAM), Centro De Investigación Biomédica En Red De Salud Mental, Madrid, Spain, [3](IUIN), Instituto Universitario De Investigación En Neuroquímica, Madrid, Spain, [4]i+12, Instituto De Investigación Hospital 12 De Octubre, Madrid, Spain, [5]Complutense University of Madrid, Pharmacology And Toxicology, School Of Medicine, Madrid, Spain

Neuropsychiatric diseases have a multifactorial origin where the exposure to stressors in vulnerable phases of neurodevelopment (pre/post-natal and adolescence) is critical. Stressful stimuli produce a disruption of homeostasis that generates a series of complex physiological changes, including immune/inflammatory activation that requires a large protein synthesis machinery that depends on the endoplasmic reticulum. A noxious stimulus, like oxidative stress, can lead to the accumulation of misfolded proteins in the ER lumen; when these accumulate above a critical threshold, cells activate the unfolded protein response (UPR). UPR alterations could be implicated in the pathophysiology of some neurodevelopmental disorders. This study aimed to characterise some key elements of the UPR pathway in the frontal cortex (FC) and hippocampus (Hp) in a Two-Hit model of neurodevelopment. Methods: Wistar rats (eight weeks old) were used (n=9 per group). First prenatal hit was Poly(I:C) exposure on GD15, an immune-activating viral mimetic, and the second hit was isolation (Iso) during adolescence. After sacrifice, FC and Hp were obtained. RT-PCR analysed BDNF and UPR components gene expression. Statistical analysis employed One Way-ANOVA with a Tukey´s post-hoc test. Results: Two-hit animals exhibited an activation of the UPR pathway. In FC, BiP, XBP1s, ATF6, CHOP and BDNF showed elevated gene expression levels in two-hit animals vs control group. In Hp, it only showed upregulated XBP1s and BDNF. Conclusions: This model showed alterations in key elements of the UPR pathway. Furthermore, some of the changes appear to depend on the brain area. These results could appoint new therapeutic strategies and need further investigation.

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BOARD NUMBER: S02-201

EFFECTS OF EARLY LIFE STRESS ON HIPPOCAMPAL NPY-Y2 GENE EXPRESSION CHANGES IN MALE MICE VIA DNA METHYLATION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Early life stress (ELS) can induce epigenetically mediated long-lasting changes of gene expression, which may underlie altered responses towards stress at adulthood. The Neuropeptide Y system is involved the regulation of stress response throughout development via its receptors, specifically the activation of NPY-Y2 receptors has been shown to reduce NPY levels and enhance stress vulnerability. Here, we hypothesized that ELS (maternal separation) as well as adult stress (AS, as swim stress) changes the expression of hippocampal NPY-Y2 receptor gene. We investigated (i) if ELS and AS affect NPY-Y2 gene expression in the hippocampus of male mice via DNA methylation (ii) if ELS-induced epigenetic changes exert a programming effect on NPY-Y2 gene expression in response to AS. Animals were assigned to the following experimental groups: 1) controls, 2) ELS exposure, 3) AS exposure, 4) ELS+AS exposure. We found that ELS and AS have a main effect on NPY-Y2 gene expression and mean DNA-methylation. Especially, increased NPY-Y2 gene expression was found in the AS animals compared to other groups. Also, decreased mean DNA-methylation was found in the AS animals compared to control and ELS animals and increased mean DNA-methylation in ELS+AS animals compared to AS animals. In addition, we determined a strong negative correlation between NPY-Y2 gene expression and mean DNA-methylation. In conclusion, ELS seems to have a protective programming effect on NPY-Y2 gene expression in response to AS via DNA methylation. Therefore, it might be considered that ELS induces protective effects in males against consecutive stressors at later life periods.

Pubmed:
29952147: Yılmaz ES, Sapmaz T, Kazgan H, Yildiz ŞM, Kocamaz D, Akpolat N, Sapmaz E
Examination of the antioxidant effects of pre-HSG melatonin use on ovarian surface epithelium in rats: An experimental study. There is no study of whether the dysplastic changes in the ovarian surface epithelium of X-ray-exposed rats during hysterosalpingography (HSG) decrease or not with the use of Lipiodol and melatonin given both intraperitoneally (i.p.) and into the suspensorium ovarii.
Adv Clin Exp Med, 2018; 27

FENS Forum 2022
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BOARD NUMBER: S02-202

ENDOPLASMIC RETICULUM STRESS DYSREGULATION CAN CAUSE NEURODEVELOPMENTAL ALTERATIONS IN THE FRONTAL CORTEX OF A "DOUBLE-HIT" NEURODEVELOPMENTAL DISORDER LIKE-MODEL

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Aims: Some mental disease constitutes a class of neurodevelopmental disorders, characterize for malfunctions affecting the central nervous system development. Endoplasmic reticulum (ER) disruptions through accumulation of misfolded proteins induces ER stress activating unfolded protein response (UPR). UPR impairments have high impact in cognitive malfunctions and neurodevelopment. We aim to characterize Nrf2 antioxidant pathway and BDNF/UPR key components in frontal cortex in a "Double-Hit" model of neurodevelopment. **Methods:** Young Wistar rats were used (N=11 rats/group). First prenatal hit was maternal deprivation (MD) as first activator of the immune system. Second hit was isolation (Iso). Both MD and Iso groups had their pertinent control groups. Frontal cortex (FC) was obtained after sacrifice. BDNF and Nrf2 pathway parameters protein expression was analyzed by Western Blot (WB). UPR components gene expression was analysed by RT-PCR. Data were analysed employing a One Way-ANOVA with a Tukey´s *post-hoc* test. **Results:** CHOP, XBP1s and Total XBP1 (UPR factors activated in ER stress condition) showed lower levels of gene expression in double-hit group versus control (CT). BDNF gene expression and protein levels as well as Nrf2 and PPARg protein levels were also lower compared to CT. Protein expression of Nrf2 pathway component HO1 was higher, while SOD1 had lower levels of expression compared to CT. **Conclusions:** This MD+Iso model indicates the importance of UPR regulation through Nrf2 and BDNF to compensate elevated levels of ER stress and neurodevelopmental impairments in FC. Thus, this model is a promising candidate to reproduce ER stress dysregulation and potential neurodevelopmental disfunctions.

Pubmed:
34576238: Vera-Montecinos A, Rodríguez-Mias R, MacDowell KS, García-Bueno B, Bris ÁG, Caso JR, Villén J, Ramos B Analysis of Molecular Networks in the Cerebellum in Chronic Schizophrenia: Modulation by Early Postnatal Life Stressors in Murine Models.
Despite the growing importance of the cerebellum as a region highly vulnerable to accumulating molecular errors in schizophrenia, limited information is available regarding altered molecular networks with potential therapeutic targets. To identify altered networks, we conducted one-shot liquid chromatography-tandem mass spectrometry in postmortem cerebellar cortex in schizophrenia and healthy individuals followed by bioinformatic analysis (PXD024937 identifier in ProteomeXchange repository). A total of 108 up-regulated proteins were enriched in stress-related proteins, half of which were also enriched in axonal cytoskeletal organization and vesicle-mediated transport. A total of 142 down-regulated proteins showed an enrichment in proteins involved in mitochondrial disease, most of which were also enriched in energy-related biological functions. Network analysis identified a mixed module of mainly axonal-related pathways for up-regulated proteins with a high number of interactions for stress-related proteins. Energy metabolism and neutrophil degranulation modules were found for down-regulated proteins. Further, two double-hit postnatal stress murine models based on maternal deprivation combined with social isolation or chronic restraint stress were used to investigate the most robust candidates of generated networks. CLASP1 from the axonal module in the model of maternal deprivation was combined with social isolation, while YWHAZ was not altered in either model. METTL7A from the degranulation pathway was reduced in both models and was identified as altered also in previous gene expression studies, while NDUFB9 from the energy network was reduced only in the model of maternal deprivation combined with social isolation. This work provides altered stress- and mitochondrial disease-related proteins involved in energy, immune and axonal networks in the cerebellum in schizophrenia as possible novel targets for therapeutic interventions and suggests that METTL7A is a possible relevant altered stress-related protein in this context.
Int J Mol Sci, 2021; 22

33290967: MacDowell KS, Martín-Hernández D, Ulecia-Morón C, Bris ÁG, Madrigal JLM, García-Bueno B, Caso JR
Paliperidone attenuates chronic stress-induced changes in the expression of inflammasomes-related protein in the frontal cortex of male rats.
Several stress-related neuropsychiatric diseases are related to inflammatory phenomena. Thus, a better understanding of stress-induced immune responses could lead to enhanced treatment alternatives. Little is known about the possible involvement of inflammasomes in the stress-induced proinflammatory response. Antipsychotics have anti-inflammatory effects, but the possible antipsychotic treatment actions on inflammasomes remain unexplored. Our aim was to study whether inflammasomes are involved in the neuroinflammation induced by a paradigmatic model of chronic stress and whether the monoamine receptor antagonist paliperidone can modulate the possible stress-induced inflammasomes activation in the frontal cortex (FC). Thus, the effects of paliperidone (1 mg/Kg, oral gavage) administered during a chronic restraint stress protocol (6 h/day for 21 days) on the possible stress-related inflammasomes protein induction were evaluated through Western blot in the FC of male Wistar rats. Stress increased protein expression levels of the inflammasome complexes NALP1, NLRP3 and AIM2 and augmented caspase-1 and mature interleukin (IL)-1β protein levels. Paliperidone pre-treatment normalized the protein expression of the inflammasome pathway. In conclusion, our data indicate an induction of inflammasome complexes by chronic restraint stress in the FC of rats. The antipsychotic paliperidone has an inhibitory action on some of the stress-induced inflammasomes stimulation trying to normalize the neuroinflammatory scenario caused by stress. Considering the emerging role of inflammation in neuropsychiatric diseases, the development of new drugs targeting inflammasome pathways is a promising approach for future therapeutic interventions.
Int Immunopharmacol, 2021; 90
26686392: Martín-Hernández D, Caso JR, Bris ÁG, Maus SR, Madrigal JL, García-Bueno B, MacDowell KS, Alou L, Gómez-Lus ML, Leza JC
Bacterial translocation affects intracellular neuroinflammatory pathways in a depression-like model in rats.
Recent studies have suggested that depression is accompanied by an increased intestinal permeability which would be related to the inflammatory pathophysiology of the disease. This study aimed to evaluate whether experimental depression presents with bacterial translocation that in turn can lead to the TLR-4 in the brain affecting the mitogen-activated protein kinases (MAPK) and antioxidant pathways. Male Wistar rats were exposed to chronic mild stress (CMS) and the intestinal integrity, presence of bacteria in tissues and plasma lipopolysaccharide levels were analyzed. We also studied the expression in the prefrontal cortex of activated forms of MAPK and some of their activation controllers and the effects of CMS on the antioxidant Nrf2 pathway. Our results indicate that after exposure to a CMS protocol there is increased intestinal permeability and bacterial translocation. CMS also increases the expression of the activated form of the MAPK p38 while decreasing the expression of the antioxidant transcription factor Nrf2. The actions of antibiotic administration to prevent bacterial translocation on elements of the MAPK and Nrf2 pathways indicate that the translocated bacteria are playing a role in these effects. In effect, our results propose a role of the translocated bacteria in the pathophysiology of depression through the p38 MAPK pathway which could aggravate the neuroinflammation and the oxidative/nitrosative damage present in this pathology. Moreover, our results reveal that the antioxidant factor Nrf2 and its activators may be involved in the consequences of the CMS on the brain.
Neuropharmacology, 2016; 103
26686388: Martín-Hernández D, Bris ÁG, MacDowell KS, García-Bueno B, Madrigal JL, Leza JC, Caso JR
Modulation of the antioxidant nuclear factor (erythroid 2-derived)-like 2 pathway by antidepressants in rats.
Patients with major depression who are otherwise medically healthy have activated inflammatory pathways in their organism. It has been described that depression is not only escorted by inflammation but also by induction of multiple oxidative/nitrosative stress pathways. Nevertheless, there are finely regulated mechanisms involved in preserving cells from damage, such as the antioxidant nuclear transcription factor Nrf2. We aim to explore in a depression-like model the Nrf2 pathway in the prefrontal cortex (PFC) and the hippocampus of rats and to analyze whether antidepressants affect the antioxidant activity of the Nrf2 pathway. Male Wistar rats were exposed to chronic mild stress (CMS) and some of them were treated with desipramine, escitalopram or duloxetine. We studied the expression of upstream and downstream elements of the Nrf2 pathway and the oxidative damage induced by the CMS. After CMS, there is an inhibition of upstream and downstream elements of the Nrf2 pathway in the PFC (e.g. PI3K/Akt, GPx…). Moreover, antidepressant treatments, particularly desipramine and duloxetine, are able to recover some of these elements and to reduce the oxidative damage induced by the CMS. However, in the hippocampus, Nrf2 pathways are not that affected and antidepressants do not have many actions. In conclusion, Nrf2 pathway is differentially regulated by antidepressants in the PFC and hippocampus. The Nrf2 pathway is involved in the oxidative/nitrosative damage detected in the PFC and antidepressants have a therapeutic action through this pathway. However, it seems that Nrf2 is not involved in the effects caused by CMS in the hippocampus.
Neuropharmacology, 2016; 103
33432590: Martínez M, Martín-Hernández D, Virto L, MacDowell KS, Montero E, González-Bris Á, Marín MJ, Ambrosio N,

Herrera D, Leza JC, Sanz M, García-Bueno B, Figuero E
Periodontal diseases and depression: A pre-clinical in vivo study.
To analyse, through a pre-clinical in vivo model, the possible mechanisms linking depression and periodontitis at behavioural, microbiological and molecular levels.
J Clin Periodontol, 2021; 48

28376896: González N, Moreno-Villegas Z, González-Bris A, Egido J, Lorenzo Ó
Regulation of visceral and epicardial adipose tissue for preventing cardiovascular injuries associated to obesity and diabetes.
Nowadays, obesity is seriously increasing in most of the populations all over the world, and is associated with the development and progression of high-mortality diseases such as type-2 diabetes mellitus (T2DM) and its subsequent cardiovascular pathologies. Recent data suggest that both body fat distribution and adipocyte phenotype, can be more determinant for fatal outcomes in obese patients than increased general adiposity. In particular, visceral adiposity is significantly linked to long term alterations on different cardiac structures, and in developed forms of myocardial diseases such as hypertensive and ischaemic heart diseases, and diabetic cardiomyopathy. Interestingly, this depot may be also related to epicardial fat accumulation through secretion of lipids, adipokines, and pro-inflammatory and oxidative factors from adipocytes. Thus, visceral adiposity and its white single-lipid-like adipocytes, are risk factors for different forms of heart disease and heart failure, mainly in higher degree obese subjects. However, under specific stimuli, some of these adipocytes can transdifferentiate to brown multi-mitochondrial-like adipocytes with anti-inflammatory and anti-apoptotic proprieties. Accordingly, in order to improve potential cardiovascular abnormalities in obese and T2DM patients, several therapeutic strategies have been addressed to modulate the visceral and epicardial fat volume and phenotypes. In addition to lifestyle modifications, specific genetic manipulations in adipose tissue and administration of PPARγ agonists or statins, have improved fat volume and phenotype, and cardiovascular failures. Furthermore, incretin stimulation reduced visceral and epicardial fat thickness whereas increased formation of brown adipocytes, alleviating insulin resistance and associated cardiovascular pathologies.
Cardiovasc Diabetol, 2017; 16

29325553: Ramírez E, Picatoste B, González-Bris A, Oteo M, Cruz F, Caro-Vadillo A, Egido J, Tuñón J, Morcillo MA, Lorenzo Ó
Sitagliptin improved glucose assimilation in detriment of fatty-acid utilization in experimental type-II diabetes: role of GLP-1 isoforms in Glut4 receptor trafficking.
The distribution of glucose and fatty-acid transporters in the heart is crucial for energy consecution and myocardial function. In this sense, the glucagon-like peptide-1 (GLP-1) enhancer, sitagliptin, improves glucose homeostasis but it could also trigger direct cardioprotective actions, including regulation of energy substrate utilization.
Cardiovasc Diabetol, 2018; 17

FENS Forum 2022
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BOARD NUMBER: S02-203

HYPOTHALAMIC EXPRESSION OF THREAT AND PRECAUTION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Tamas Fuzesi[1,2], Neilen Rasiah[1], David Rosenegger[1], Nuria Daviu[1], Leonardo Molina[2], Taylor Chomiak[2], Toni-Lee Sterley[1], Wilten Nicola[1], Jaideep Bains[1]
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A decrease in presumptive safety elicits behaviors to probe the environment for additional information and engages precautionary physiological responses to prepare the organism in case of threat. The physiological response includes an anticipatory increase in circulating glucocorticoids in the body. Glucocorticoids levels are controlled by corticotropin-releasing hormone neurons in the paraventricular nucleus of the hypothalamus (CRH^{PVN}). Here we show that CRH^{PVN} cells in freely behaving mice show a scalable precautionary response to a decrease in safety. This precautionary response consists of a stable increase in the activity of CRH^{PVN} neurons in low safety environments. This response shows no habituation during repeated trials with individual cells exhibiting faithful activity profiles over multiple days. The stable increase in activity on repeated days is matched by increases in circulating stress hormone, but there is a marked shift in behavior. The precautionary response is scaled by prior experience. Specifically, CRH^{PVN} neurons show an enhanced precautionary state after exposure to a threat (footshock) in a low safety environment. This increase in the precautionary response requires the recruitment of cells that respond faithfully to the footshocks but have low basal precautionary activity, consistent with a perceptron-like learning rule. These findings delineate a scalable precautionary representation in the brain. An increase in the precautionary assembly of CRH^{PVN} neurons following an acute threat may contribute to sensitization to environmental cues in neuropsychiatric stress disorders.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-204

POST-STRESS ACTIVITY OF CALRETININ POSITIVE CELLS IN THE PARAVENTRICULAR THALAMIC NUCLEUS UNDERLIES LONG TERM, STRESS INDUCED DISTURBANCE OF BEHAVIOR

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Anna Jász[1], László Biró[1], Zsolt Buday[1], Bálint Király[2], Balazs Hangya[3], László Acsády[4]
[1]Institute of Experimental Medicine, Laboratory Of Thalamus Research, Budapest, Hungary, [2]Institute of Experimental Medicine, Lendület Laboratory Of Systems Neuroscience, Budapest, Hungary, [3]Institute of Experimental Medicine, Lendület Laboratory, Budapest, Hungary, [4]Institute of Experimental Medicine, Budapest, Hungary, Laboratory Of Thalamus Research, Budapest, Hungary

Behavioral disorders caused by stress affect millions of people around the world, but its neurobiological bases are still unclear. The calretinin-positive neurons of the paraventricular thalamus (PVT/CR+) are in a unique position to participate in stress induced sleep disturbances since their activity is significantly affected by stress and by sleep-wake transitions. In the accompanying study, using optogenetic inhibition, we found that the post-stress activity of PVT/CR+ cells is critical to establish the acute stress induced behavioral changes. Thus, in this study we aimed to determine the activity of PVT/CR+ cells before and after the exposure to a natural stressor (2MT, 10 min). We also tested whether stress induced long term changes in firing activity can be reversed by post-stress photoinhibition. Recordings involved 3 hours sessions for five days before and after stress using movable optrodes. During pre-stress days PVT/CR+ cells displayed strongly state dependent activity. Unit activity within the nest during wakefulness was lower than outside the nest and further decreased during sleep. Following the exposure to 2MT firing rate was elevated for four days with strongest increase in the nest. Both high frequency spike clusters and synchrony among PVT/CR+ cells significantly increased in a state dependent manner. Photoinhibition of PVT/CR+ neurons after the 2MT presentation prevented altered firing rate, increase in high frequency clusters and cross correlations on the poststress days. These data together strongly suggest that altered post-stress activity of PVT/CR+ cells is crucial to establish the neuronal network responsible for the emergence of stress induced behavioral phenotype.

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BOARD NUMBER: S02-205

CHRONIC OVERCROWDING STRESS AFFECTS DIFFERENTLY THE NEUROPLASTICITY-RELATED SIGNALING PATHWAYS IN THE RATS' HIPPOCAMPUS AND AMYGDALA

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Maj Institute of Pharmacology, Polish Academy of Sciences, Department Of Brain Biochemistry, Krakow, Poland

Undisturbed functioning of synaptic plasticity seems to be critical for computational processes occurring in the brain. Among factors influencing synaptic plasticity is chronic stress. Brain regions especially vulnerable to chronic stress-induced neuroplasticity**,** include the hippocampus, and the amygdala. Interestingly, opposite effects of stressful conditions have been shown in these structures. Despite increasing knowledge, there is still not enough information on which molecules may be involved in stress-related neuroplasticity in these regions. Thus, our goal was to characterize an influence of chronic stress on the mRNA expression of genes encoding for proteins related to the synaptic organization, and on the level of neuroplasticity-related proteins in the amygdala and the hippocampus. Rats underwent overcrowding stress for 3, 7, and 14 days. The TaqMan arrays were used for evaluation of 24 genes in a single sample, together with the RT-qPCR to assess mRNA expression of genes. The level of selected proteins was assessed using western blot. Our results demonstrate region-dependent alterations in the mRNA expression of genes encoding for several intracellular proteins, and similarly, region-dependent changes in the protein abundance. Interestingly, chronic (14 days) overcrowding decreased mRNA expression of focal adhesion kinase (FAK) and protein tyrosine phosphatase non-receptor type 5 in the hippocampus but not in the amygdala. Furthermore, alterations in the level of the FAK after exposure to the chronic stress were consistent with changes in the mRNA expression, suggesting its involvement in stress-related processes. These results imply selected proteins and theirs downstream signaling pathways as potential targets for new pharmacotherapy strategies.

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BOARD NUMBER: S02-206

STRESS-INDUCED SOCIAL AVOIDANCE BEHAVIOR AND INCREASE IN INFLAMMATORY CYTOKINES ARE NORMALIZED BY PERIPHERAL INHIBITION OF FAAH IN RATS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Margherita Barbetti[1], Luca Carnevali[1], Federica Vacondio[2], Francesca Ferlenghi[2], Marco Mor[2], Silvia Rivara[2], Andrea Sgoifo[1]
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Peripheral inflammation occurring after a traumatic/stressful event has been implicated in the development of post-traumatic stress disorder (PTSD). Therefore, targeting inflammatory mediators in the aftermath of stress exposure may be a valid approach for the prevention of PTSD-related symptoms. **AIMS:** (i) to demonstrate the association between increased pro-inflammatory cytokines and disrupted social behavior in rats exposed to acute social defeat stress; (ii) to verify whether peripheral pharmacological inhibition of the enzyme fatty acid amide hydrolase (FAAH), which terminates the signaling of the anti-inflammatory palmitoylethanolamide (PEA), exerts favorable effects on social behavior. **METHODS**: Male wistar rats were exposed to an episode of social defeat stress/control condition and 30 min later they were injected with either the peripheral FAAH inhibitor URB937 (3mg/kg) or vehicle. 24 hours later, social behavior (social approach/avoidance test) and plasma levels of corticosterone were analyzed. Pro-inflammatory cytokines (IL-1beta, TNFalpha and IL-6) and PEA levels were measured in plasma and brain 6 and 24 hours after stress exposure. **RESULTS:** Stressed rats showed clear social avoidance behavior and increased corticosterone levels 24 hours after social defeat, as well as a significant increase in plasmatic pro-inflammatory cytokines after 6 hours. URB937 increased plasma PEA levels, normalized social behavior, and reduced corticosterone and peripheral cytokines levels. **CONCLUSIONS:** Peripheral inflammation occurring after social defeat may be implicated in the development of social avoidance behavior. Peripheral inhibition of FAAH activity may exert positive effects on social behavior via anti-inflammatory mechanisms and may represent a promising strategy for the prevention of PTSD-like symptomatology.

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BOARD NUMBER: S02-207

THE ROLE OF CALRETININ-POSITIVE MIDLINE THALAMIC NEURONS IN STRESS INDUCED BEHAVIOURAL ALTERATIONS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Zsolt Buday[1], László Biró[1], Anna Jász[1], Gergely Komlósi[1], Róbert Bódizs[2], Orsolya Szalárdy[2], Krisztina Kovács[3], Dániel Kuti[3], László Acsády[1]
[1]Institute of Experimental Medicine, Laboratory Of Thalamus Research, Budapest, Hungary, [2]Semmelweis University, Institute Of Behavioural Sciences, Budapest, Hungary, [3]Institute of Experimental Medicine, Laboratory Of Molecular Neuroendocrinology, Budapest, Hungary

Severe acute stress could induce the emergence of psychiatric disorders, although the underlying neuronal mechanisms are presently unresolved. Calretinin expressing neurons in the paraventricular nucleus of the thalamus (PVT/CR+) form a critical hub between brainstem and forebrain and play essential roles in arousal, anxiety and stress regulating circuit operations. Thus, in this study we tested whether the activity of PVT/CR+ neurons, following an exposure to a natural stressor (fox odour, 2MT), contributes to acute stress-induced behavioural changes. We inhibited PVT/CR+ neurons after the stress event using optogenetic inhibition (SwiChR) and measured nesting behavior, locomotion, sleep, and stress hormone levels. We also quantified c-Fos expression in the PVT/CR+ cells and their projection areas. EYFP injected animals served as control. During the stress exposure, both groups showed similar levels of defensive behaviours. Following the stress exposure, the control EYFP group displayed increased EMG activity, disturbed nesting behavior, and elevated corticosterone level. c-Fos expression was increased both in the PVT/CR+ cells and their projection areas. The behavioral changes persisted for five days following the stress exposure. Photoinhibition of PVT/CR+ cells after the stress exposure prevented all these changes, with the exception of acute hormonal stress response, which remained unaffected. This suggests that the post-stress activity of PVT/CR+ cells shapes stress induced behaviour, independently from the hormonal stress response. Collectively, our findings indicate that post-stress activity of PVT/CR+ neurons plays a fundamental role in the emergence of stress-induced behavioral changes, and post-stress inhibition of PVT/CR+ cells is sufficient to prevent these changes.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S02-208

THE CONSEQUENCES OF CONCURRENT STRESS AND HYPERGLYCEMIA ON REDOX HOMEOSTASIS IN THE ADULT ZEBRAFISH BRAIN.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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[1]Jawaharlal Nehru University, School Of Life Sciences, Delhi, India, [2]Jawaharlal Nehru University, School Of Life Sciences,, New Delhi, India

Aim Diabetes mellitus, a metabolic disorder involving abnormally elevated blood glucose levels (hyperglycemia), may negatively affect the central nervous system. Compromised brain health increases susceptibility towards psychiatric illnesses including depression and anxiety. Patients afflicted with diabetes are vulnerable to developing “diabetic distress”, a term used to describe psychological stress that ensues from a diabetes diagnosis. While similar in nature to the clinical manifestations of depression, diabetic distress is not synonymous to depression, although it may lead to its development. The zebrafish is rapidly gaining impetus as a vertebrate model to study metabolic as well as neurological disorders. Hyperglycemic zebrafish display abnormalities in blood-brain barrier integrity, neuronal signaling pathways, and neural cell proliferation. Redox imbalance appears to be a significant player in this association. However, thorough research examining the neurobiological implications of coexisting hyperglycemia and chronic stress is lacking. For this, we established a model of simultaneous stress and hyperglycemia in adult zebrafish to investigate consequences on brain redox homeostasis. Methods Fishes were exposed to dextrose solution (111mM) alongside chronic unpredictable mild stress (CUMS) for 14 days. Fasting blood glucose levels were measured with a glucometer, after which whole brain samples were utilized for gene expression and biochemical studies. Results Blood glucose levels were dramatically elevated in stressed-hyperglycemic fishes compared to control. Furthermore, there were alterations in the levels and activities of several antioxidant molecules, accompanied by increased brain lipid peroxidation. Conclusions Further investigation could uncover underlying disease mechanisms of comorbid hyperglycemia and chronic stress, opening doors for therapeutic drug development.

Pubmed:
33529409: Subba R, Sandhir R, Singh SP, Mallick BN, Mondal AC
Pathophysiology linking depression and type 2 diabetes: Psychotherapy, physical exercise, and fecal microbiome transplantation as damage control.
Diabetes increases the likelihood of developing depression and vice versa. Research on this bidirectional association has somewhat managed to delineate the interplay among implicated physiological processes. Still, further exploration is required in this context. This review addresses the comorbidity by investigating suspected common pathophysiological mechanisms. One such factor is psychological stress which disturbs the hypothalamic-pituitary-adrenal axis causing hormonal imbalance. This includes elevated cortisol levels, a common biomarker of both depression and diabetes. Disrupted insulin signaling drives the hampered neurotransmission of serotonin, dopamine, and norepinephrine. Also, adipokine hormones such as adiponectin, leptin, and resistin and the orexigenic hormone, ghrelin, are involved in both depression and T2DM. This disarray further interferes with physiological processes encompassing sleep, the gut-brain axis, metabolism, and mood stability. Behavioral coping mechanisms, such as unhealthy eating, mediate disturbed glucose homeostasis, and neuroinflammation. This is intricately linked to oxidative stress, redox imbalance, and mitochondrial dysfunction. However, interventions such as psychotherapy, physical exercise, fecal microbiota transplantation, and insulin-sensitizing agents can help to manage the distressing condition. The possibility of glucagon-like peptide 1 possessing a therapeutic role has also been discussed. Nonetheless, there stands an urgent need for unraveling new correlating targets and biological markers for efficient treatment.
Eur J Neurosci, 2021; 53

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BOARD NUMBER: S02-209

DIFFBRAINNET: A COMBINED RESOURCE OF DIFFERENTIAL NETWORKS AND DIFFERENTIAL EXPRESSION TO ANALYZE TRANSCRIPTOMIC RESPONSES TO GLUCOCORTICOIDS IN 8 MOUSE BRAIN REGIONS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Genome-wide gene expression analyses are invaluable tools for increasing our knowledge of biological and disease processes, allowing a hypothesis-free comparison of gene expression profiles across experimental groups, tissues and cell types. Traditionally, transcriptomic data analysis has focused on gene-level effects found by differential expression (DE). In recent years, co-expression network analysis has emerged as an important additional level of investigation, providing information on the molecular connectivity level. We demonstrate how combined DE and network analysis can be used to explore complex datasets. As an example, we analyzed the transcriptional responses following administration of the glucocorticoid/stress hormone receptor agonist dexamethasone in C57Bl/6 mice, in 8 brain regions important for stress processing: the prefrontal cortex, the amygdala, the paraventricular nucleus of the hypothalamus, 4 subregions of the hippocampus as well as the cerebellar cortex. By applying a combination of differential network and DE analyses, we find that these explain distinct but complementary aspects of the responses to stimulus and that network analysis may unravel different biological mechanisms that are not obvious at the level of DE. In addition, network analysis identifies new differentially connected partners of important genes and can be used to generate hypotheses of specific molecular pathways affected. We provide an analysis framework and a publicly available resource for the study of the transcriptional landscape of 8 mouse brain regions named DiffBrainNet (http://134.76.24.68/app/diffbrainnet), that can be used to pinpoint molecular pathways important for basic functioning and response to glucocorticoids in a brain-region specific manner.

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BOARD NUMBER: S02-210

SPHK2 DELETION IS INVOLVED IN STRUCTURAL ABNORMALITIES AND TH17 RESPONSE BUT DOES NOT AGGRAVATE COLON INFLAMMATION INDUCED BY SUB-CHRONIC STRESS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

David Martín-Hernández[1,2], Irene López Gutiérrez[2], Marta González-Prieto[2], Karina Macdowell[2], Javier Robledo-Montaña[2], Hiram Tendilla-Beltrán[3], Natalia Calleja-Rodríguez[2], Álvaro G. Bris[2], Cristina Ulecia-Moron[2], Beatriz Moreno[2], Javier Caso[2], Borja García-Bueno[2], Sandra Rodrígues-Mascarenhas[4], Ignacio Marín-Jiménez[5], Juan Carlos Leza[2], Luis Menchén[5]
[1]Centro de Investigación Biomédica en Red de Salud Mental, Pharmacology And Toxicology (ucm), Madrid, Spain, [2]Universidad Complutense de Madrid, Pharmacology And Toxicology, Madrid, Spain, [3]Benemérita Universidad Autónoma de Puebla (BUAP), Laboratorio De Neuropsiquiatría, Instituto De Fisiología, Puebla, Mexico, [4]Universidade Federal da Paraíba (UFPB), Laboratório De Imunobiotecnologia, Centro De Biotecnologia, João Pessoa - PB, Brazil, [5]Hospital General Universitario Gregorio Marañón, Servicio De Aparato Digestivo, Madrid, Spain

Background: inflammation in inflammatory bowel diseases (IBD) is mainly driven by T-cell response to microbial and environmental antigens. Psychological stress is a potential trigger of clinical flares of IBD and drugs based on the sphingosine-1-phosphate (S1P) pathways, which control T-cell recruitment, are currently under clinical trials for IBD. **Aims:** to analyze the sub-chronic stress impact and absence of sphingosine kinase 2 (Sphk2), an enzyme of S1P metabolism, in the colon of mice. **Methods:** wild-type (WT) and Sphk2-/- mice were exposed to a sub-chronic stress mixed model: Control WT (n=5), Control Sphk2-/- (n=5), Stress WT (n=8), and Stress Sphk2-/- (n=8). Tissue and biochemical assays were performed to evaluate stress and immune responses, S1P pathways, and barrier integrity. **Results:** sub-chronic stress increased S1P in the colon, possibly decreasing its degradation enzymes and Sphk2. S1P accumulation could lead to inflammation and immune dysregulation reflected by upregulation of toll-like receptor 4 (TLR4) pathway, inhibition of anti-inflammatory mechanisms, cytokine-expression profile towards a T-helper lymphocyte 17 (Th17) polarization, plasmacytosis, decrease in some IgA+ immune cells, and increase in IgM+ B cells. Stress also enhanced intestinal permeability. Sphk2 knockout mice presented a cytokine-expression profile towards a boosted Th17 response, lower expression of claudin 3,4,5,7, and 8, and structural abnormalities in the colon. **Conclusions:** sub-chronic stress causes colon S1P increase, immune dysregulation, and intestinal permeability. Sphk2 deletion is involved in structural abnormalities and Th17 response but did not aggravate the inflammation originated by stress. Intestinal pathophysiology should consider stress and S1P as modulators of the immune response.

Pubmed:
33513342: Florensa-Zanuy E, Garro-Martínez E, Adell A, Castro E, Díaz Á, Pazos Á, Mac-Dowell KS, Martín-Hernández D, Pilar-Cuéllar F
Cannabidiol antidepressant-like effect in the lipopolysaccharide model in mice: Modulation of inflammatory pathways.
Major Depression is a severe psychiatric condition with a still poorly understood etiology. In the last years, evidence supporting the neuroinflammatory hypothesis of depression has increased. In the current clinical scenario, in which the available treatments for depression is far from optimal, there is an urgent need to develop fast-acting drugs with fewer side effects. In this regard, recent pieces of evidence suggest that cannabidiol (CBD), the major non-psychotropic component of Cannabis sativa with anti-inflammatory properties, appears as a drug with antidepressant properties. In this work, CBD 30 mg/kg was administered systemically to mice 30 min before lipopolysaccharide (LPS; 0.83 mg/kg) administration as a neuroinflammatory model, and behavioral tests for depressive-, anhedonic- and anxious-like behavior were performed. NF-κB, IκBα and PPARγ levels were analyzed by western blot in nuclear and cytosolic fractions of cortical samples. IL-6 and TNFα levels were determined in plasma and prefrontal cortex using ELISA and qPCR techniques, respectively. The precursor tryptophan (TRP), and its metabolites kynurenine (KYN) and serotonin (5-HT) were measured in hippocampus and cortex by HPLC. The ratios KYN/TRP and KYN/5-HT were used to estimate indoleamine 2,3-dioxygenase (IDO) activity and the balance of both metabolic pathways, respectively. CBD reduced the immobility time in the tail suspension test and increased sucrose preference in the LPS model, without affecting locomotion and central activity in the open-field test. CBD diminished cortical NF-κB activation, IL-6 levels in plasma and brain, and the increased KYN/TRP and KYN/5-HT ratios in hippocampus

and cortex in the LPS model. Our results demonstrate that CBD produced antidepressant-like effects in the LPS neuroinflammatory model, associated to a reduction in the kynurenine pathway activation, IL-6 levels and NF-κB activation. As CBD stands out as a promising antidepressant drug, more research is needed to completely understand its mechanisms of action in depression linked to inflammation.
Biochem Pharmacol, 2021; 185
33432590: Martínez M, Martín-Hernández D, Virto L, MacDowell KS, Montero E, González-Bris Á, Marín MJ, Ambrosio N, Herrera D, Leza JC, Sanz M, García-Bueno B, Figuero E
Periodontal diseases and depression: A pre-clinical in vivo study.
To analyse, through a pre-clinical in vivo model, the possible mechanisms linking depression and periodontitis at behavioural, microbiological and molecular levels.
J Clin Periodontol, 2021; 48
31519825: Tendilla-Beltrán H, Meneses-Prado S, Vázquez-Roque RA, Tapia-Rodríguez M, Vázquez-Hernández AJ, Coatl-Cuaya H, Martín-Hernández D, MacDowell KS, Garcés-Ramírez L, Leza JC, Flores G
Risperidone Ameliorates Prefrontal Cortex Neural Atrophy and Oxidative/Nitrosative Stress in Brain and Peripheral Blood of Rats with Neonatal Ventral Hippocampus Lesion.
Reduction of the dendritic arbor length and the lack of dendritic spines in the pyramidal cells of the prefrontal cortex (PFC) are prevalent pathological features in schizophrenia (SZ). Neonatal ventral hippocampus lesion (NVHL) in male rats reproduces these neuronal characteristics and here we describe how this is a consequence of BDNF/TrkB pathway disruption. Moreover, COX-2 proinflammatory state, as well as Nrf-2 antioxidant impairment, triggers oxidative/nitrosative stress, which also contributes to dendritic spine impairments in the PFC. Interestingly, oxidative/nitrosative stress was also detected in the periphery of NVHL animals. Furthermore, risperidone treatment had a neurotrophic effect on the PFC and antioxidant effects on the brain and periphery of NVHL animals; these cellular effects were related to behavioral improvement. Our data highlight the link between brain development and immune response, as well as several other factors to understand mechanisms related to the pathophysiology of SZ. Prefrontal cortex dysfunction in schizophrenia can be a consequence of morphological abnormalities and oxidative/nitrosative stress, among others. Here, we detailed how impaired plasticity-related pathways and oxidative/nitrosative stress are part of the dendritic spine pathology and their modulation by atypical antipsychotic risperidone treatment in rats with neonatal ventral hippocampus lesion. Moreover, we found that animals with neonatal ventral hippocampus lesion had oxidative/nitrosative stress in the brain as well as in the peripheral blood, an important issue for the translational approaches of this model. Then, risperidone restored plasticity and reduced oxidative/nitrosative stress of prefrontal cortex pyramidal cells, and ultimately improved the behavior of lesioned animals. Moreover, risperidone had differential effects than the brain on peripheral blood oxidative/nitrosative stress.
J Neurosci, 2019; 39
31509716: Giménez-Gómez P, Pérez-Hernández M, O'Shea E, Caso JR, Martín-Hernandez D, Cervera LA, Centelles MLG, Gutiérrez-Lopez MD, Colado MI
Changes in brain kynurenine levels gut microbiota and gut-barrier disruption induced by chronic ethanol exposure in mice.
Inflammatory processes have been shown to modify tryptophan (Trp) metabolism. Gut microbiota appears to play a significant role in the induction of peripheral and central inflammation. Ethanol (EtOH) exposure alters gut permeability, but its effects on Trp metabolism and the involvement of gut microbiota have not been studied. We analyzed several parameters of gut-barrier and of peripheral and central Trp metabolism following 2 different EtOH consumption patterns in mice, the binge model, drinking in the dark (DID), and the chronic intermittent (CI) consumption paradigm. Antibiotic treatment was used to evaluate gut microbiota involvement in the CI model. Mice exposed to CI EtOH intake, but not DID, show bacterial translocation and increased plasma LPS immediately after EtOH removal. Gut-barrier permeability to FITC-dextran is increased by CI, and, furthermore, intestinal epithelial tight-junction (TJ) disruption is observed (decreased expression of zonula occludens 1 and occludin) associated with increased matrix metalloproteinase (MMP)-9 activity and iNOS expression. CI EtOH, but not DID, increases kynurenine (Kyn) levels in plasma and limbic forebrain. Intestinal bacterial decontamination prevents the LPS increase but not the permeability to FITC-dextran, TJ disruption, or the increase in MMP-9 activity and iNOS expression. Although plasma Kyn levels are not affected by antibiotic treatment, the elevation of Kyn in brain is prevented, pointing to an involvement of microbiota in CI EtOH-induced changes in brain Trp metabolism. Additionally, CI EtOH produces depressive-like symptoms of anhedonia, which are prevented by the antibiotic treatment thus pointing to an association between anhedonia and the increase in brain Kyn and to the involvement of gut microbiota.-Giménez-Gómez, P., Pérez-Hernández, M., O'Shea, E., Caso, J. R., Martín-Hernández, D., Cervera, L. A., Centelles. M. L. G.-L., Gutiérrez-Lopez, M. D., Colado, M. I. Changes in brain kynurenine levels gut microbiota and gut-barrier disruption induced by chronic ethanol exposure in mice.
FASEB J, 2019; 33
31054078: Martín-Hernández D, Pereira MP, Tendilla-Beltrán H, Madrigal JLM, García-Bueno B, Leza JC, Caso JR

Modulation of Monoaminergic Systems by Antidepressants in the Frontal Cortex of Rats After Chronic Mild Stress Exposure.
The standard pharmacological treatment of the major depressive disorder (MDD) is still grounded in a monoaminergic approach. Consequently, antidepressant treatments pursue to heighten serotonergic and noradrenergic neurotransmissions. Thus, the aim of this study was to assess the impact of exposure to a well-characterized animal model, the chronic mild stress (CMS) on serotonin (5-HT) and noradrenaline (NE) levels, and reuptake transporters and receptors in the frontal cortex (FC) of CMS-exposed rats. Moreover, considering the diverse pharmacological profiles of existing antidepressants and the large number of patients not responding to treatments, we have investigated whether generally utilized antidepressants can modulate their expression. Male Wistar rats were exposed to CMS and some of them treated with desipramine, escitalopram, or duloxetine. Possible changes in the described monoaminergic neurotransmission elements were evaluated. CMS induced differences in the expression of reuptake transporters and receptors involved in the monoaminergic neurotransmission pointing towards the weakening of their signaling. CMS antidepressant-treated rats showed an improvement of the monoaminergic tone, being desipramine and duloxetine more influential than escitalopram over noradrenergic elements and having the three antidepressant-tested effects on serotonergic transmission. In summary, there are molecular alterations on the monoaminergic neurotransmission in FC induced by CMS exposure. Besides, antidepressant treatments modulate the elements of these neurotransmission systems differentially.
Mol Neurobiol, 2019; 56
30872094: Oriolo G, Blanco-Hinojo L, Navines R, Mariño Z, Martín-Hernández D, Cavero M, Gimenez D, Caso J, Capuron L, Forns X, Pujol J, Sola R, Martin-Santos R
Association of chronic inflammation and perceived stress with abnormal functional connectivity in brain areas involved with interoception in hepatitis C patients.
Sickness behavioral changes elicited by inflammation may become prolonged and dysfunctional in patients with chronic disease, such as chronic hepatitis C (CHC). Neuroimaging studies show that the basal ganglia and insula are sensitive to systemic inflammation.
Brain Behav Immun, 2019; 80
30180869: Martín-Hernández D, Caso JR, Javier Meana J, Callado LF, Madrigal JLM, García-Bueno B, Leza JC
Intracellular inflammatory and antioxidant pathways in postmortem frontal cortex of subjects with major depression: effect of antidepressants.
Studies show that Toll-like receptors (TLRs), members of the innate immune system, might participate in the pathogenesis of the major depressive disorder (MDD). However, evidence of this participation in the brain of patients with MDD has been elusive.
J Neuroinflammation, 2018; 15
29725904: Martín-Hernández D, Tendilla-Beltrán H, Madrigal JLM, García-Bueno B, Leza JC, Caso JR
Chronic Mild Stress Alters Kynurenine Pathways Changing the Glutamate Neurotransmission in Frontal Cortex of Rats.
Immune stimulation might be involved in the pathophysiology of major depressive disorder (MDD). This stimulation induces indoleamine 2,3-dioxygenase (IDO), an enzyme that reduces the tryptophan bioavailability to synthesize serotonin. IDO products, kynurenine metabolites, exert neurotoxic/neuroprotective actions through glutamate receptors. Thus, we study elements of these pathways linked to kynurenine metabolite activity examining whether antidepressants (ADs) can modulate them. Male Wistar rats were exposed to chronic mild stress (CMS), and some of them were treated with ADs. The expression of elements of the IDO pathway, including kynurenine metabolites, and their possible modulation by ADs was studied in the frontal cortex (FC). CMS increased IDO expression in FC compared to control group, and ADs restored the IDO expression levels to control values. CMS-induced IDO expression led to increased levels of the excitotoxic quinolinic acid (QUINA) compared to control, and ADs prevented the rise in such levels. Neither CMS nor ADs changed significantly the antiexcitotoxic kynurenic acid (KYNA) levels. The QUINA/KYNA ratio, calculated as excitotoxicity risk indicator, increased after CMS and ADs prevented this increase. CMS lowered excitatory amino acid transporter (EAAT)-1 and EAAT-4 expression, and some ADs restored their expression levels. Furthermore, CMS decreased N-methyl-D-aspartate receptor (NMDAR)-2A and 2B protein expression, and ADs mitigated this decrease. Our research examines the link between CMS-induced pro-inflammatory cytokines and the kynurenine pathway; it shows that CMS alters the kynurenine pathway in rat FC. Importantly, it also reveals the ability of classic ADs to prevent potentially harmful situations related to the brain scenario caused by CMS.
Mol Neurobiol, 2019; 56
26686392: Martín-Hernández D, Caso JR, Bris ÁG, Maus SR, Madrigal JL, García-Bueno B, MacDowell KS, Alou L, Gómez-Lus ML, Leza JC
Bacterial translocation affects intracellular neuroinflammatory pathways in a depression-like model in rats.
Recent studies have suggested that depression is accompanied by an increased intestinal permeability which would be related to the inflammatory pathophysiology of the disease. This study aimed to evaluate whether experimental depression

presents with bacterial translocation that in turn can lead to the TLR-4 in the brain affecting the mitogen-activated protein kinases (MAPK) and antioxidant pathways. Male Wistar rats were exposed to chronic mild stress (CMS) and the intestinal integrity, presence of bacteria in tissues and plasma lipopolysaccharide levels were analyzed. We also studied the expression in the prefrontal cortex of activated forms of MAPK and some of their activation controllers and the effects of CMS on the antioxidant Nrf2 pathway. Our results indicate that after exposure to a CMS protocol there is increased intestinal permeability and bacterial translocation. CMS also increases the expression of the activated form of the MAPK p38 while decreasing the expression of the antioxidant transcription factor Nrf2. The actions of antibiotic administration to prevent bacterial translocation on elements of the MAPK and Nrf2 pathways indicate that the translocated bacteria are playing a role in these effects. In effect, our results propose a role of the translocated bacteria in the pathophysiology of depression through the p38 MAPK pathway which could aggravate the neuroinflammation and the oxidative/nitrosative damage present in this pathology. Moreover, our results reveal that the antioxidant factor Nrf2 and its activators may be involved in the consequences of the CMS on the brain.
Neuropharmacology, 2016; 103
26686388: Martín-Hernández D, Bris ÁG, MacDowell KS, García-Bueno B, Madrigal JL, Leza JC, Caso JR
Modulation of the antioxidant nuclear factor (erythroid 2-derived)-like 2 pathway by antidepressants in rats.
Patients with major depression who are otherwise medically healthy have activated inflammatory pathways in their organism. It has been described that depression is not only escorted by inflammation but also by induction of multiple oxidative/nitrosative stress pathways. Nevertheless, there are finely regulated mechanisms involved in preserving cells from damage, such as the antioxidant nuclear transcription factor Nrf2. We aim to explore in a depression-like model the Nrf2 pathway in the prefrontal cortex (PFC) and the hippocampus of rats and to analyze whether antidepressants affect the antioxidant activity of the Nrf2 pathway. Male Wistar rats were exposed to chronic mild stress (CMS) and some of them were treated with desipramine, escitalopram or duloxetine. We studied the expression of upstream and downstream elements of the Nrf2 pathway and the oxidative damage induced by the CMS. After CMS, there is an inhibition of upstream and downstream elements of the Nrf2 pathway in the PFC (e.g. PI3K/Akt, GPx…). Moreover, antidepressant treatments, particularly desipramine and duloxetine, are able to recover some of these elements and to reduce the oxidative damage induced by the CMS. However, in the hippocampus, Nrf2 pathways are not that affected and antidepressants do not have many actions. In conclusion, Nrf2 pathway is differentially regulated by antidepressants in the PFC and hippocampus. The Nrf2 pathway is involved in the oxidative/nitrosative damage detected in the PFC and antidepressants have a therapeutic action through this pathway. However, it seems that Nrf2 is not involved in the effects caused by CMS in the hippocampus.
Neuropharmacology, 2016; 103

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BOARD NUMBER: S02-211

INCREASED OXIDATIVE STRESS AS A COMMON MECHANISM FOR DIFFERENT PRENATAL STRESSORS: LONG-TERM EFFECTS ON ADOLESCENT MALE AND FEMALE MICE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Chiara Musillo[1,2], Alessandra Berry[1], Kerstin Creutzberg[3], Veronica Begni[3], Marco Riva[3], Francesca Cirulli[1]
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Stressful experiences *in utero* can affect fetal development, increasing the risk for psychiatric disorders. We hypothesized that stressors as diverse as maternal obesity and maternal psychophysical stress might disrupt fetal programming resulting in long-lasting effects on offspring brain development by acting through shared oxidative stress (OS)-mediated mechanisms. We compared a mouse model (C57Bl/6N) of maternal high-fat diet (HFD) consumption (13 weeks, until delivery) to prenatal restraint stress (PNS) repeatedly administered during the last week of pregnancy. To counteract the negative effects of both stressors, the antioxidant N-acetyl-cysteine (NAC, 1 g/kg) was administered to female breeders for 5 weeks until delivery. Emotionality was assessed in adolescent male and female offspring through the elevated-plus-maze (EPM). Moreover, hippocampal gene expression levels of Brain-Derived-Neurotrophic-Factor (*Bdnf*), Nuclear factor erythroid 2–related factor 2 (*Nrf-2*) and Kelch-like ECH-associated protein 1 (*Keap-1*) were measured, by qPCR, as markers of brain plasticity and antioxidant capacity. Prenatal exposure to both HFD and PNS enhanced behavioral disinhibition, increasing time spent in the open arms of the EPM and decreasing the frequency of stretch-attend-posture, especially in female offspring. Moreover, both prenatal stressors led to decreased *Bdnf* (in females) and *Nrf-2* levels, and disrupted *Keap-1* levels. Prenatal NAC was able to counteract these effects on the brain. Overall, different prenatal stressors result in long-term negative effects on the adolescent offspring, increasing risk assessment behaviors and affecting brain antioxidant defenses: rescue by NAC suggests that OS may be a common mechanism, playing a pivotal role in fetal programming of mental disorders. *ERANET-NEURON-JTC-2018-Mental Disorders-"EMBED".*

Pubmed:
32032668: Cirulli F, Musillo C, Berry A
Maternal Obesity as a Risk Factor for Brain Development and Mental Health in the Offspring.
Maternal obesity plays a key role in the health trajectory of the offspring. Although research on this topic has largely focused on the potential of this condition to increase the risk for child obesity, it is becoming more and more evident that it can also significantly impact cognitive function and mental health. The mechanisms underlying these effects are starting to be elucidated and point to the placenta as a critical organ that may mediate changes in the response to stress, immune function and oxidative stress. Long-term effects of maternal obesity may rely upon epigenetic changes in selected genes that are involved in metabolic and trophic regulations of the brain. More recent evidence also indicates the gut microbiota as a potential mediator of these effects. Overall, understanding cause-effect relationships can allow the development of preventive measures that could rely upon dietary changes in the mother and the offspring. Addressing diets appears more feasible than developing new pharmacological targets and has the potential to affect the multiple interconnected physiological pathways engaged by these complex regulations, allowing prevention of both metabolic and mental disorders.
Neuroscience, 2020; 447
33711185: Berry A, Mazzelli M, Musillo C, Riva MA, Cattaneo A, Cirulli F
High-fat diet during adulthood interacts with prenatal stress, affecting both brain inflammatory and neuroendocrine markers in male rats.
Prenatal stress (PNS) affects foetal programming and, through an interaction with subsequent challenges, can increase vulnerability to mood and metabolic disorders. We have previously shown that, following PNS, adult male rats are characterized by increased vulnerability to a metabolic stressor experienced at adulthood (8-week-high-fat diet-HFD). In this study, we specifically assessed whether PNS might interact with an adult metabolic challenge to induce an inflammatory phenotype. Changes in the expression levels of inflammatory (Il-1β, Tnf-α, Il-6) and of stress response mediators (Nr3c1, Fkbp5) as well as of mood and metabolic regulators (Bdnf, Ghs-R) were investigated in the hippocampus, prefrontal cortex

and hypothalamus, brain regions involved in the pathogenesis of depression and prone to inflammation in response to stress. Overall, PNS reduced the expression of Bdnf and Tnf-α, while HFD administered at adulthood counteracted this effect suggesting that PNS impinges upon the same pathways regulating responses to a metabolic challenge at adulthood. Furthermore, HFD and PNS affected the expression of both Nr3c1 and Fkbp5, two neuroendocrine mediators involved in the response to stress, metabolic challenges and in the modulation of the emotional profile (as shown by the correlation between Fkbp5 and the time spent in the open arms of the elevated plus-maze). Overall, these results indicate that the same metabolic and neuroendocrine effectors engaged by PNS are affected by metabolic challenges at adulthood, providing some mechanistic insight into the well-known comorbidity between mood and metabolic disorders.
Eur J Neurosci, 2022; 55

33309907: Musillo C, Borgi M, Saul N, Möller S, Luyten W, Berry A, Cirulli F
Natural products improve healthspan in aged mice and rats: A systematic review and meta-analysis.
Over the last decades a decrease in mortality has paved the way for late onset pathologies such as cardiovascular, metabolic or neurodegenerative diseases. This evidence has led many researchers to shift their focus from researching ways to extend lifespan to finding ways to increase the number of years spent in good health; "healthspan" is indeed the emerging concept of such quest for ageing without chronic or disabling diseases and dysfunctions. Regular consumption of natural products might improve healthspan, although the mechanisms of action are still poorly understood. Since preclinical studies aimed to assess the efficacy and safety of these compounds are growing, we performed a systematic review and meta-analysis on the effects of natural products on healthspan in mouse and rat models of physiological ageing. Results indicate that natural compounds show robust effects improving stress resistance and cognitive abilities. These promising data call for further studies investigating the underlying mechanisms in more depth.
Neurosci Biobehav Rev, 2021; 121

31676426: Berry A, Marconi M, Musillo C, Chiarotti F, Bellisario V, Matarrese P, Gambardella L, Vona R, Lombardi M, Foglieni C, Cirulli F
Trehalose administration in C57BL/6N old mice affects healthspan improving motor learning and brain anti-oxidant defences in a sex-dependent fashion: a pilot study.
Aim of this study was to characterize the effects of oral trehalose administration (2%w/v) on healthspan in old mice. Trehalose was administered in drinking water for 1 month to male and female C57BL/6N mice aged 25-months. After behavioral phenotyping (grip strength, beam walking and rotarod tests), autophagy (LC3-II/actin) and oxidative stress were tested in the cerebral cortex and gastrocnemius muscle. The latter parameter was indirectly assessed by evaluating carbonyl groups added to proteins as a result of oxidative reactions, in addition to central levels of NRF2 protein, a transcription factor that regulates the expression of antioxidant enzymes. In comparison with sex-matched controls, trehalose-treated males performed better in motor planning and coordination tasks. This behavioral phenotype was associated with an activation of the ubiquitin-proteasome system, autophagy and antioxidant defences in cerebral cortex. Independently from trehalose administration, females were characterized by better motor performance and showed higher levels of ubiquitinated proteins and NRF2 in cerebral cortex, suggesting an up-regulation of basal antioxidant defences. In conclusion, trehalose was effective in counteracting some aspects of age-related decay, with specific effects in male and female subjects.
Exp Gerontol, 2020; 129

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SEX-DEPENDENT NEURODEVELOPMENTAL VULNERABILITY IN PRENATALLY STRESSED MOUSE OFFSPRING IS MEDIATED BY OXIDATIVE STRESS AND PLACENTAL IMMUNE ACTIVATION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Alessandra Berry[1], Chiara Musillo[1,2], Roberta Tassinari[3], Sabrina Tait[3], Francesca Maranghi[3], Francesca Cirulli[1]
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Prenatal stress (PNS) plays a key role in foetal development; inflammation and oxidative stress (OS) may mediate its effects with long-term consequences on the offspring mental health. We set up a PNS mouse model and characterized the effects of such condition on the placenta, maternal behaviour and pups' neurodevelopment, focussing on OS and inflammation pathways. Pregnant C57BL6/N mice underwent restraint-stress during the last week of pregnancy; controls were left undisturbed. Five weeks before mating half subjects from each group were administered with the antioxidant N-Acetyl-cysteine (NAC-1g/kg) until delivery. Both PNS and NAC treatment contained body weight gain during pregnancy; NAC reduced the number of pups/litter. The placenta's labyrinth layer in the PNS-NAC group showed increased macrophages number suggesting that NAC might prime the immune system to respond more efficiently to sub-optimal intrauterine conditions. PNS overall disrupted the maternal circadian alternation between nursing and foraging that was partially restored upon NAC administration. When challenged in the homing test, both PNS and NAC-treated 10-day-old female pups showed greater reactivity being characterised by a reduced latency to reach the nest; male pups performance was not affected by PNS. Our results suggest that PNS affects OS and immune activation in both pre- and post-natal maternal environment leading to a sex-dependent vulnerability in the offspring. Data on NAC administration deserve a thorough investigation to better characterize its efficacy and specific mechanisms of action. Proteomic analyses are ongoing aimed at characterizing placental immunomodulation; targeted gene expression will be carried-out on pups' brain inflammatory mediators. "EMBED"-ERANET-NEURON-JTC-2018.

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BOARD NUMBER: S02-213

META-ANALYSIS OF PREFRONTAL CORTEX RNA-SEQ DATA AFTER SOCIAL DEFEAT STRESS OF VARYING DURATION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Ksenia Ayriyants[1], Natalia Bondar[1], Vasiliy Reshetnikov[1], Polina Kisaretova[1,2]
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Depression covering over 5% of the world's adult population is enhanced by chronic stress. Chronic social defeat stress (SDS) is an ethologically valid animal model of depression. We conducted a meta-analysis of the prefrontal cortex transcriptomes in the in 5 datasets after 10-day SDS and 2 datasets after 30-day SDS. Moreover, we identified genes bonded with susceptibility and resistance to stress after 10-day SDS. Analysis showed 20 most reproducible genes changed under 10-day SDS. These genes are connected with neuroinflammation (*Vwf, Il1r1, Il6ra*) and expression change of glucocorticoid signaling-related genes (*Fkbp5, Hsd11b*). Stress sensitive mice after 10 days SDS showed a steady decrease in expression of 45 oligodendrocyte-specific genes. Most of these genes are linked with myelination (*Mbp, Mal, Mobp, Plp1, Mag, Ugt8a, Olig2*). Finally, a 30-day SDS leads to a strong increase in the expression of genes associated with transcription, translation, and ATP synthesis. Besides, animals after 30 days of SDS showed increased gene expression linked with microglia activation (*Ifngr1, Tyrobp, Aif1*) and opposite trend in expression of genes contributing to neuroplasticity (*Grin1, Grin2c, Htt*). Surprisingly, DE genes after 10 and 30 days SDS don't overlap. Taking everything into consideration, RNA-seq meta-analysis shed light onto time-dependence of molecular changes in social defeat stress paradigm. This work was supported by the Russian Science Foundation (21-15-00142).

Pubmed:
34877386: Ryabushkina YA, Shevelev OB, Kisaretova PE, Sozonov NG, Ayriyants KA, Bondar NP, Reshetnikov VV
High-resolution MRI data of the brain of C57BL/6J and BTBR mice in three anatomical views.
The research on strain-, sex-, and stress-specific differences in structural and functional connectivity of the brain is important for elucidating various behavioral features and etiologies of psychiatric disorders. Socially impaired BTBR mice are considered a model of autism spectrum disorders. Here we present high-resolution magnetic resonance imaging data from the brain of 89 adolescent mice (C57BL/6J and BTBR) in axial, sagittal, and coronal views. The study [1] includes both females and males differed in early-life experience (normally reared or subjected to prolonged maternal separation: 3 h daily from postnatal day 2 to 15). The MRI data were obtained on a horizontal tomograph Biospec 117/16 instrument with a magnetic field strength of 11.7 T. Thus, multislice Turbo RARE T-weighted images of the brain were captured in eight groups of mice. Altogether, these data allow to evaluate strain-, sex-, and stress-specific alterations in the volumes of various brain structures and to better understand the relation between brain structural differences and behavioral abnormalities.
Data Brief, 2021; 39
34303728: Reshetnikov VV, Ayriyants KA, Ryabushkina YA, Sozonov NG, Bondar NP
Sex-specific behavioral and structural alterations caused by early-life stress in C57BL/6 and BTBR mice.
Lately, the development of various mental illnesses, such as depression, personality disorders, and autism spectrum disorders, is often associated with traumatic events in childhood. Nonetheless, the mechanism giving rise to this predisposition is still unknown. Because the development of a disease often depends on a combination of a genetic background and environment, we decided to evaluate the effect of early-life stress on BTBR mice, which have behavioral, neuroanatomical, and physiological features of autism spectrum disorders. As early-life stress, we used prolonged separation of pups from their mothers in the first 2 weeks of life (3 h once a day). We assessed effects of the early-life stress on juvenile (postnatal day 23) and adolescent (postnatal days 37-38) male and female mice of strains C57BL/6 (B6) and BTBR. We found that in both strains, the early-life stress did not lead to changes in the level of social behavior, which is an important characteristic of autism-related behavior. Nonetheless, the early-life stress resulted in increased locomotor activity in juvenile BTBR mice. In adolescent mice, the stress early in life caused a low level of anxiety in B6 males and BTBR females and increased exploratory activity in adolescent BTBR males and females. In addition, adolescent B6 male and female mice with a history of the early-life stress tended to have a thinner motor cortex as assessed by magnetic resonance imaging. As

compared to B6 mice, BTBR mice showed reduced levels of social behavior and exploratory activity but their level of locomotor activity was higher. BTBR mice had smaller whole-brain, cortical, and dorsal hippocampal volumes; decreased motor cortex thickness; and increased ventral-hippocampus volume as compared to B6 mice, and these parameters correlated with the level of exploratory behavior of BTBR mice. Overall, the effects of early postnatal stress are sex- and strain-dependent.
Behav Brain Res, 2021; 414
28320276: Dygalo NN, Bannova AV, Sukhareva EV, Shishkina GT, Ayriyants KA, Kalinina TS
Effects of Short-Term Exposure to Lithium on Antiapoptotic Bcl-xL Protein Expression in Cortex and Hippocampus of Rats after Acute Stress.
The antiapoptotic protein Bcl-xL is involved in development of neurobiological resilience to stress; hence, the possibility of use of psychotropic drugs to increase its expression in brain in response to stress is of considerable interest. Lithium is a neurotropic drug widely used in psychiatry. In work, we studied effects of lithium administration (for 2 or 7 days) on the expression of Bcl-xL mRNA and protein in the hippocampi and cortices of rats subjected to stress that induced depression-like behavior in the animals. In contrast to the brain-derived neurotrophic factor (BDNF), whose expression decreased in the hippocampus in response to acute stress, stress increased the level of Bcl-xL mRNA in the hippocampus, but decreased it in the frontal cortex. Treatment of stressed animals with lithium for 2 or 7 days increased Bcl-xL protein levels 1.5-fold in the hippocampus, but it decreased them in the cortex. Therefore, Bcl-xL expression in the brain can be modulated by both stress and psychotropic drugs, and the effects of these factors are brain region-specific: both stress exposure and lithium administration activated Bcl-xL expression in the hippocampus and suppressed it in the frontal cortex. The activation of Bcl-xL expression in the hippocampus by lithium, demonstrated for the first time in this study, suggests an important role of this protein in the therapeutic effects of lithium in the treatment of stress-induced psychoemotional disorders.
Biochemistry (Mosc), 2017; 82

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PREDICTING CHRONIC STRESS AMONG HEALTHY FEMALES USING DAILY-LIFE PHYSIOLOGICAL AND LIFESTYLE FEATURES FROM WEARABLE SENSORS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Noa Magal[1], Sharona Rab[1], Pavel Goldstein[2], Lisa Simon[1], Talita Jiryis[1], Roee Admon[1,3]
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Chronic stress is a highly prevalent condition that may stem from different sources and can substantially impact physiology and behavior, leading to impaired mental and physical health. Multiple physiological and behavioral lifestyle features can now be recorded unobtrusively in daily-life using wearable sensors. The aim of the current study was to identify a distinct set of physiological and behavioral lifestyle features that are associated with elevated levels of chronic stress across different stress sources. In here, 140 healthy female participants completed the TICS chronic stress inventory before wearing the Fitbit Charge3 sensor for seven consecutive days while maintaining their daily routine. Physiological and lifestyle features that were extracted from sensor data, alongside demographic features, were used to predict high vs. low chronic stress with support vector machine classifiers, applying out-of-sample model testing. The results showed that the model achieved 79% classification accuracy for chronic stress from a social tension source. A mixture of physiological (resting heart-rate, heart-rate circadian characteristics), lifestyle (steps count, sleep onset and sleep regularity) and non-sensor demographic features (smoking status) contributed to this classification. For conclusion, As wearable technologies continue to rapidly evolve, integration of daily-life indicators could improve our understanding of chronic stress and its impact of physiology and behavior.

Pubmed:
34286052: Magal N, Hendler T, Admon R
Is neuroticism really bad for you? Dynamics in personality and limbic reactivity prior to, during and following real-life combat stress.
The personality trait of neuroticism is considered a risk factor for stress vulnerability, putatively via its association with elevated limbic reactivity. Nevertheless, majority of evidence to date that relates neuroticism, neural reactivity and stress vulnerability stems from cross-sectional studies conducted in a "stress-free" environment. Here, using a unique prospective longitudinal design, we assessed personality, stress-related symptoms and neural reactivity at three time points over the course of four and a half years; accounting for prior to, during, and long-time following a stressful military service that included active combat. Results revealed that despite exposure to multiple potentiality traumatic events, majority of soldiers exhibited none-to-mild levels of posttraumatic and depressive symptoms during and following their military service. In contrast, a quadratic pattern of change in personality emerged overtime, with neuroticism being the only personality trait to increase during stressful military service and subsequently decrease following discharge. Elevated neuroticism during military service was associated with reduced amygdala and hippocampus activation in response to stress-related content, and this association was also reversed following discharge. A similar pattern was found between neuroticism and hippocampus-anterior cingulate cortex (ACC) functional connectivity in response to stress-related content. Taken together these findings suggest that stressful military service at young adulthood may yield a temporary increase in neuroticism mediated by a temporary decrease in limbic reactivity, with both effects being reversed long-time following discharge. Considering that participants exhibited low levels of stress-related symptoms throughout the study period, these dynamic patterns may depict behavioral and neural mechanisms that facilitate stress resilience.
Neurobiol Stress, 2021; 15

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BOARD NUMBER: S02-215

THE ROLE OF LOCUS COERULEUS-NORADRENERGIC SYSTEM IN RESILIENCE FOLLOWING CHILD ABUSE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Déa Slavova[1], Stéphanie De Gois[1], Bruno Giros[1,2], Elsa Isingrini[1]
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The capacity to adapt positively following stressful events, called resilience, involves several neurobiological systems including the noradrenergic (NE) system. Being one of the main effectors of the stress response, the NE system also plays an important role for brain development, especially during critical periods. In the last years, growing evidences in mice and humans showed that its activity is implicated in resilience following stressful events during adulthood. However, there is less evidence for its importance in resilience following early life stress. Here, we used a translational approach to investigate the role of the locus coeruleus-noradrenergic (LC-NE) system in resilience following early life stress. In human LC, we demonstrated by immunohistochemistry that the number of LC-NE neurons was different between post mortem tissues from depressed patients with a history of child abuse (DS-CA) or without (DS), and matched samples from psychiatrically healthy individuals with a history of child abuse (RES-CA) or without (CTL). In parallel, we developed a mice model of maternal separation in which, using a battery of anxio-depressive tests, we identified two groups of mice: one with depressive-like behavior and another with resilient-like phenotype with behavior similar to the control group. Immunofluorescence analysis of the early activated gene c-fos showed a differential activity state of NE neurons in depressive-like mice compared with resilient mice. By demonstrating both a structural and functional role of the LC-NE system, these data highlight the implication of the LC-NE system in resilience following child abuse.

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BOARD NUMBER: S02-216

REARING ENVIRONMENT MODULATES THE PHENOTYPE OF GENETICALLY HOMOGENEOUS MICE: A MULTI-CENTER STUDY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Aim: To investigate possible causes of poor replicability in behavioral neuroscience research, we determined the extent to which common differences in housing and husbandry conditions between rearing facilities (RFs) contribute to the replicability problem by modulating the phenotype of genetically homogenous (inbreed) mice. **Methods:** We reared inbred C57BL/6JRj mice from a single breeding stock in five different RFs throughout early life and adolescence, before transporting them to a single test laboratory. We examined the extent and persistence of variation in the composition of the gut microbiota associated with the different RFs and measured differences in behavioral and physiological phenotypic traits. We also assessed chromatin accessibility in the ventral hippocampus to explore the biological basis of behavioral differences. **Results:** We found persistent effects of RFs on the composition and heterogeneity of the gut microbiota. These effects were paralleled by persistent differences in body weight and behavior. We show that the facility-specific environment influenced developmental programs by affecting neuronal chromatin accessibility profiles, likely modulating the mice' behavioral phenotypes. We detected changes in chromatin organization of genes involved in the regulation of behavior, neurogenesis and presynaptic plasticity events, targeting mainly GABAergic and glutamatergic transmission. **Conclusion:** Our findings demonstrate that common environmental differences between RFs may produce facility-specific phenotypes, from the molecular to the behavioral level, which might explain inconsistency of results between different studies. The results highlight that the animals' environmental background should be accounted for by study design to produce robust and replicable research findings.

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BOARD NUMBER: S02-217

SOCIAL DEFEAT DURING ADOLESCENCE INCREASES THE SUSCEPTIBILITY TO AN IMMUNE CHALLENGE LATER IN LIFE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Aims Early-life adverse experiences (first hit) may increase vulnerability to immune challenges experienced later in life (second hit) and subsequently induce the development of stress-related psychopathologies. Microglia have been proposed to play a role in this phenomenon. In the present study, we investigated whether exposure to social adversity during adolescence induces brain and behavioural alterations and whether such adversity increases the susceptibility to an immune challenge experienced in adulthood. **Methods** Males Wistar rats were exposed to repeated social defeat (RSD, first hit) during adolescence (postnatal day [PND] 35-39) and then to an immune challenge consisting of a single injection of lipopolysaccharide (LPS, second hit) at adulthood (PND90). Positron emission tomography scans using [^{11}C]-PBR28 at the tracer and ELISA were performed on PND46, 89, and 93 to measure neuroinflammation and plasma corticosterone respectively. Sucrose preference and social interaction tests were performed on PND40-67-91 to measure anhedonia and social behaviour. **Results** RSD alone induced an increase in neuroinflammation and plasma corticosterone that remained until adulthood. Rats exposed to the combination of RSD and LPS exhibited susceptibility towards the development of anhedonia and social preference dysfunction characterized by a reduction in sucrose preference and a lack of social preference towards a rat over an object, respectively. Rats subjected to both hits also displayed a synergistic increase in brain inflammation characterized by a higher [^{11}C]-PBR28 tracer brain uptake. **Conclusions** Our findings indicate that exposure to social stress during adolescence primes microglia and increases the susceptibility to an immune challenge later in life.

Pubmed:
34715148: Guerrin CGJ, Doorduin J, Sommer IE, de Vries EFJ
The dual hit hypothesis of schizophrenia: Evidence from animal models.
Schizophrenia is a heterogeneous psychiatric disorder, which can severely impact social and professional functioning. Epidemiological and clinical studies show that schizophrenia has a multifactorial aetiology comprising genetic and environmental risk factors. Although several risk factors have been identified, it is still not clear how they result in schizophrenia. This knowledge gap, however, can be investigated in animal studies. In this review, we summarise animal studies regarding molecular and cellular mechanisms through which genetic and environmental factors may affect brain development, ultimately causing schizophrenia. Preclinical studies suggest that early environmental risk factors can affect the immune, GABAergic, glutamatergic, or dopaminergic system and thus increase the susceptibility to another risk factor later in life. A second insult, like social isolation, stress, or drug abuse, can further disrupt these systems and the interactions between them, leading to behavioural abnormalities. Surprisingly, first insults like maternal infection and early maternal separation can also have protective effects. Single gene mutations associated with schizophrenia did not have a major impact on the susceptibility to subsequent environmental hits.
Neurosci Biobehav Rev, 2021; 131

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BOARD NUMBER: S02-218

MULTI-TRAJECTORY ANALYSIS UNCOVERS ADAPTIVE AND MALADAPTIVE PSYCHO-PHYSIOLOGICAL ACUTE STRESS RESPONSE PATTERNS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Everyday life involves exposure to challenging acute stressful situations. Encounter with such acute stressor(s) elicits multiple physiological and psychological response trajectories that travel at different times-scales and directions. Hence, associating a specific psycho-physiological response pattern as adaptive or maladaptive represents a substantial challenge. The current study aimed, for the first time, to assess multiple physiological stress response trajectories that are simultaneously recorded and investigate their putative relation to psychological responses. Using a within-subject design, 96 healthy female participants underwent an acute laboratory stress induction procedure. Psychological, physiological, and endocrinal responses were assessed before, during and after stress via positive affect and negative affect (PANAS), heart rate (HR) and heart rate variability (HRV) and saliva cortisol, respectively. Fitting an unsupervised multi-trajectory cluster analysis on these data uncovered that acute stress responsivity across multiple trajectories was best described by three latent classes. These three classes were identified based on their stress-induced psychological response patterns as an adaptive stress response (n=55), heightened affective response during stress (n=24) and lack of recovery after stress relief (n=17). All groups exhibited a stress-induced increase in HR and cortisol. Interestingly, only the adaptive group showed the expected stress-induced HRV response, while the two smaller and presumably maladaptive response groups exhibited blunted HRV. Our findings support the important notion that adaptive response to stress depends on psycho-physiological coherence, while its violation may be considered as a core mechanism that promotes stress vulnerability and related psychopathologies.

Pubmed:
34573169: Simon L, Jiryis T, Admon R
Now or Later? Stress-Induced Increase and Decrease in Choice Impulsivity Are Both Associated with Elevated Affective and Endocrine Responses.
Exposure to acute stress elicit physiological and psychological responses that can impact decision-making, often expressed as an increased tendency to act in an impulsive manner following stress. Delay discounting (DD) task has emerged as a reliable measure of impulsive behavior in the form of choice impulsivity (CI). Interestingly, studies that examined the effect of acute stress on DD performance reported mixed results. To address this, we conducted a within-subject examination of the impact of acute stress on CI, focusing on individual differences in response patterns. One hundred and fifty healthy female participants completed the DD task twice, before and after undergoing an acute laboratory stress induction procedure. Saliva samples and self-report mood and affect measures were collected at four time points throughout the session. Fifty-nine matched healthy control participants completed only the DD task twice, with no stress in between. Results indicate that the acute stress procedure elicited the expected effects of increased cortisol release and increased negative mood and affect, at the group level. With respect to DD, stress indeed increased CI at the group level, yet participants differed in the magnitude and direction of this effect. Interestingly, regression analysis revealed quadratic relations between stress-induced changes in CI and cortisol release. Indeed, dividing the sample into three sub-groups based on the impact of stress on CI revealed that, compared to participants that exhibited no substantial change in their CI following stress, participants that exhibited either stress-induced increase or decrease in their CI also exhibited more stress-induced cortisol release, as well as more negative affect. Taken together, these findings suggest that elevated physiological and psychological responses to stress are associated with either increased or decreased choice impulsivity, thus depicting quadratic relations between stress and impulsivity.
Brain Sci, 2021; 11

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BOARD NUMBER: S02-219

MULTIMODAL ASSOCIATIONS OF FKBP5 METHYLATION WITH EMOTION-REGULATORY PREFRONTAL-LIMBIC BRAIN CIRCUITS AND REAL-LIFE STRESS RESPONSIVITY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Aims *FKBP5*, a molecular stress regulator, contributes to individual differences in major categories of mental health, such as emotion regulation and stress responsivity. Here we examine the neural and real-world correlates of *FKBP5* methylation in a large sample of 395 non-affected individuals. **Methods** *FKBP5* methylation (cg20813374 and cg00130530) was assessed using Infinium EPIC arrays and residualized for age, sex, cell composition and population structure. In SPM12 we prerpocessed structural and functional MRI data following standard procedures. We built general linear models defining *FKBP5* methylation as regressor of interest to predict (i) vmPFC grey matter volume using voxel based morphometry, (ii) vmPFC habituation during emotional face matching and (iii) emotion-regulatory vmPFC-amygdala coupling estimated using psychophysiological interaction. We calculated a Spearman correlation between the *T*-map resulting from the voxel based morphometric analysis and *FKBP5* expression from the allen human brain atlas. Using ambulatory assessment we estimated modulatory effects of *FKBP5* methylation and *FKBP5*-related neural connectivity on daily-life stress responsivity. **Results** *FKBP5* DNAm is associated with (i) anxiety-related morphometric variance in the vmPFC (*FKBP5*: T=3.99, P_{FWE}=.039), (ii) vmPFC habituation to repeated emotional stimuli (T=3.13, P_{FWE}=.024) and (iii) emotion-regulatory vmPFC-amygdala coupling (T=3.45, P_{FWE}=0.020). The brain-wide pattern of morphometric correlation with *FKBP5* methylation is co-located with the brain-wide distribution of *FKBP5* expression (R=-.195, P_{PERM}=.034). *FKBP5* methylation (T=5.43, P<.001) and *FKBP5*-related emotion-regulatory vmPFC-amygdala coupling (T=-2.91, P=.004) modulate daily-life stress responsivity. **Conclusions** Our findings suggest that *FKBP5* DNAm and *FKBP5*-related neural mechanisms are critically important in affective stress responsivity and in maintaining mental health.

Pubmed:

32049268: Chen J, Zang Z, Braun U, Schwarz K, Harneit A, Kremer T, Ma R, Schweiger J, Moessnang C, Geiger L, Cao H, Degenhardt F, Nöthen MM, Tost H, Meyer-Lindenberg A, Schwarz E
Association of a Reproducible Epigenetic Risk Profile for Schizophrenia With Brain Methylation and Function.
Schizophrenia is a severe mental disorder in which epigenetic mechanisms may contribute to illness risk. Epigenetic profiles can be derived from blood cells, but to our knowledge, it is unknown whether these predict established brain alterations associated with schizophrenia.
JAMA Psychiatry, 2020; 77

FENS Forum 2022
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BOARD NUMBER: S02-220

EARLY-LIFE ADVERSITY INDUCES SEX-SPECIFIC DYSFUNCTION OF THE MOFC AND LEADS TO IMPULSIVE, HYPERACTIVE, AND RISK-TAKING BEHAVIORS IN JUVENILE MALE MICE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Exposure to early-life adversity, such as that caused by neglect and abuse is known to induce maladaptive behaviors and to increase the vulnerability to substance abuse, neuropsychiatric disorders, and other negative outcomes later in life. These behavioral alterations are also accompanied with long lasting changes in the volume, activity and connectivity of several brain regions related to emotional processing, impulse control and decision making, such as the pre-frontal cortex (PFC), nucleus accumbens and the amygdala. However, the mechanisms and cellular correlates that lead to these alterations are yet poorly understood. In our laboratory, we employed a model of maternal separation and maternal stress (MSUS) to study the impact of early-life stress on juvenile behavior, as well as on the inhibitory system of the PFC, which is known to experience intense remodeling during the first weeks of life. Using behavioral, stereological, and electrophysiological analysis, we show that MSUS male mice, but not females, present increased hyperactive, impulsive, and risk-taking behaviors, together with a layer-specific increase in the number of parvalbumin positive interneurons. In addition, we observed an increase in inhibitory activity within the medial orbito-frontal cortex (mOFC), a region reported to play a role in the modulation of reward sensitivity and impulsivity. Chemogenetic tools were also employed to further probe the relationship between the observed mOFC dysfunction and the behavioral phenotypes. Taken together our results show that ELS alters the inhibitory balance of the mOFC, potentially contributing to a risk-taking and impulsive phenotype that is more striking in males.

Pubmed:
34022277: Costa J, Martins S, Ferreira PA, Cardoso AMS, Guedes JR, Peça J, Cardoso AL
The old guard: Age-related changes in microglia and their consequences.
Among all major organs, the brain is one of the most susceptible to the inexorable effects of aging. Throughout the last decades, several studies in human cohorts and animal models have revealed a plethora of age-related changes in the brain, including reduced neurogenesis, oxidative damage, mitochondrial dysfunction and cell senescence. As the main immune effectors and first responders of the nervous tissue, microglia are at the center of these events. These cells experience irrevocable changes as a result from cumulative exposure to environmental triggers, such as stress, infection and metabolic dysregulation. The age-related immunosenescent phenotype acquired by microglia is characterized by profound modifications in their transcriptomic profile, secretome, morphology and phagocytic activity, which compromise both their housekeeping and defensive functions. As a result, aged microglia are no longer capable of establishing effective immune responses and sustaining normal synaptic activity, directly contributing to age-associated cognitive decline and neurodegeneration. This review discusses how lifestyle and environmental factors drive microglia dysfunction at the molecular and functional level, also highlighting possible interventions to reverse aging-associated damage to the nervous and immune systems.
Mech Ageing Dev, 2021; 197
32492699: Franco LO, Carvalho MJ, Costa J, Ferreira PA, Guedes JR, Sousa R, Edfawy M, Seabra CM, Cardoso AL, Peça J
Social subordination induced by early life adversity rewires inhibitory control of the prefrontal cortex via enhanced Npy1r signaling.
Social hierarchies are present in most mammalian species. In nature, hierarchies offer a tradeoff between reduction of in-group fighting between males, at the expense of an asymmetric sharing of resources. Early life experiences and stress are known to influence the rank an individual attains in adulthood, but the associated cellular and synaptic alterations are poorly understood. Using a maternal separation protocol, we show that care-deprived mice display a long-lasting submissive phenotype, increased social recognition, and enhanced explorative behavior. These alterations are consistent with an adaptation that favors exploration rather than confrontation within a group setting. At the neuronal level, these animals display dendritic atrophy and enhanced inhibitory synaptic inputs in medial prefrontal cortex (mPFC) neurons. To determine what

could underlie this synaptic modification, we first assessed global gene expression changes via RNAseq, and next focused on a smaller subset of putatively altered synaptic receptors that could explain the changes in synaptic inhibition. Using different cohorts of maternally deprived mice, we validated a significant increase in the expression of Npy1r, a receptor known to play a role in maternal care, anxiety, foraging, and regulation of group behavior. Using electrophysiological recordings in adult mice while blocking NPY1R signaling, we determined that this receptor plays a key role in enhancing GABAergic currents in mice that experience maternal deprivation. Taken together, our work highlights the potential of regulating NPY1R in social anxiety disorders and the alterations induced in brain circuitry as a consequence of early life stress and adversity.
Neuropsychopharmacology, 2020; 45

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BOARD NUMBER: S02-221

THE ROLE OF FGF14 IN SUSCEPTIBILITY TO DEPRESSION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Depression is a major health problem in modern society and currently available therapies require prolonged treatments and have limited efficacy. The lack of knowledge about underlying mechanisms limits the development of specific treatments. The main theory is based on the loss of balance between excitation and inhibition in neuronal circuits implicated in depression. FGF14 is an accessory protein of voltage-dependent sodium channels, able to regulate the intrinsic excitability of the cells. The complex Ffg14-voltage dependent sodium channels is a key determinant of the excitation/inhibition balance in brain circuits. Our hypothesis is that Fgf14 can be a target gene for the pharmacological treatment of depression. **Aim:** This study aimed to better understand the role of Fgf14 in brain circuits involved in depression. **Methods:** We analyzed female *Fgf14*$^{-/-}$ mice and wild-type littermates in estrous phase. To induce acute stress in mice test suspension test was used. To mark activation of neuronal circuits cFos labeling was evaluated in different brain regions. **Results:** *Fgf14*$^{-/-}$ showed a resilience to depression induced by tail suspension test. Moreover, we found a statistically significant increase in cFos positive cell density *Fgf14*$^{-/-}$ mice in ventral tegmental area and nucleus accumbens. **Conclusions:** The *Fgf14*$^{-/-}$ mouse could be a valid model to understand the mechanisms altered in depression. These results could be helpful for new therapeutic treatment that could properly act to counterbalance the alterations present in this psychiatric disorder.

Pubmed:
34139039: Hoxha E, Balbo I, Parolisi R, Audano M, Montarolo F, Ravera F, Guglielmotto M, Muratori L, Raimondo S, DiGregorio E, Buffo A, Brusco A, Borroni B, Mitro N, Caruso D, Tempia F
Elovl5 is required for proper action potential conduction along peripheral myelinated fibers.
Elovl5 elongates fatty acids with 18 carbon atoms and in cooperation with other enzymes guarantees the normal levels of very long-chain fatty acids, which are necessary for a proper membrane structure. Action potential conduction along myelinated axons depends on structural integrity of myelin, which is maintained by a correct amount of fatty acids and a proper interaction between fatty acids and myelin proteins. We hypothesized that in Elovl5 mice, the lack of elongation of Elovl5 substrates might cause alterations of myelin structure. The analysis of myelin ultrastructure showed an enlarged periodicity with reduced G-ratio across all axonal diameters. We hypothesized that the structural alteration of myelin might affect the conduction of action potentials. The sciatic nerve conduction velocity was significantly reduced without change in the amplitude of the nerve compound potential, suggesting a myelin defect without a concomitant axonal degeneration. Since Elovl5 is important in attaining normal amounts of polyunsaturated fatty acids, which are the principal component of myelin, we performed a lipidomic analysis of peripheral nerves of Elovl5-deficient mice. The results revealed an unbalance, with reduction of fatty acids longer than 18 carbon atoms relative to shorter ones. In addition, the ratio of saturated to unsaturated fatty acids was strongly increased. These findings point out the essential role of Elovl5 in the peripheral nervous system in supporting the normal structure of myelin, which is the key element for a proper conduction of electrical signals along myelinated nerves.
Glia, 2021; 69
33994961: Balbo I, Montarolo F, Boda E, Tempia F, Hoxha E
Elovl5 Expression in the Central Nervous System of the Adult Mouse.
(Elongase of Very-Long Fatty Acid 5) gene encodes for an enzyme that elongates long chain fatty acids, with a marked preference for polyunsaturated molecules. In particular, it plays an essential role in the elongation of omega-3 and omega-6 fatty acids, precursors for long-chain polyunsaturated fatty acids (PUFAs). Mutations of cause the spino-cerebellar ataxia type 38 (SCA38), a rare autosomal neurological disease characterized by gait abnormality, dysarthria, dysphagia, hyposmia and peripheral neuropathy, conditions well represented by a mouse model with a targeted deletion of this gene (mice). However, the expression pattern of this enzyme in neuronal and glial cells of the central nervous system (CNS) is still uninvestigated. This work is aimed at filling this gap of knowledge by taking advantage of an Elovl5-reporter mouse line and immunofluorescence analyses on adult mouse CNS sections and glial cell primary cultures. Notably, Elovl5 appears

expressed in a region- and cell type-specific manner. Abundant Elovl5-positive cells were found in the cerebellum, brainstem, and primary and accessory olfactory regions, where mitral cells show the most prominent expression. Hippocampal pyramidal cells of CA2/CA3 where also moderately labeled, while in the rest of the telencephalon Elovl5 expression was high in regions related to motor control. Analysis of primary glial cell cultures revealed Elovl5 expression in oligodendroglial cells at various maturation steps and in microglia, while astrocytes showed a heterogeneous expression of Elovl5. The elucidation of Elovl5 CNS distribution provides relevant information to understand the physiological functions of this enzyme and its PUFA products, whose unbalance is known to be involved in many pathological conditions.
Front Neuroanat, 2021; 15
30542279: Hoxha E, Lippiello P, Zurlo F, Balbo I, Santamaria R, Tempia F, Miniaci MC
The Emerging Role of Altered Cerebellar Synaptic Processing in Alzheimer's Disease.
The role of the cerebellum in Alzheimer's disease (AD) has been neglected for a long time. Recent studies carried out using transgenic mouse models have demonstrated that amyloid-β (Aβ) is deposited in the cerebellum and affects synaptic transmission and plasticity, sometimes before plaque formation. A wide variability of motor phenotype has been observed in the different murine models of AD, without a consistent correlation with the extent of cerebellar histopathological changes or with cognitive deficits. The loss of noradrenergic drive may contribute to the impairment of cerebellar synaptic function and motor learning observed in these mice. Furthermore, cerebellar neurons, particularly granule cells, have been used as model of Aβ-induced neuronal damage. An unexpected conclusion is that the cerebellum, for a long time thought to be somehow protected from AD pathology, is actually considered as a region vulnerable to Aβ toxic damage, even at the early stage of the disease, with consequences on motor performance.
Front Aging Neurosci, 2018; 10
30189289: Hoxha E, Marcinnò A, Montarolo F, Masante L, Balbo I, Ravera F, Laezza F, Tempia F
Emerging roles of Fgf14 in behavioral control.
Sexual disturbances, and aggressivity are a major social problem. However, the molecular mechanisms involved in the control of these behaviors are largely unknown. FGF14, which is an intracellular protein controlling neuronal excitability and synaptic transmission, has been implied in neurologic and psychiatric disorders. Mice with Fgf14 deletion show blunted responses to drugs of abuse. By behavioral tests we show that male Fgf14 knockout mice have a marked reduction of several behaviors including aggressivity and sexual behavior. Other behaviors driven by spontaneous initiative like burying novel objects and spontaneous digging and climbing are also reduced in Fgf14 knockout mice. These deficits cannot be attributed to a generalized decrease of activity levels, because in the open field test Fgf14 knockout mice have the same spontaneous locomotion as wild types and increased rearing. Our results show that Fgf14 is important to preserve a set of behaviors and suggest that fine tuning of neuronal function by Fgf14 is an important mechanism of control for such behaviors.
Behav Brain Res, 2019; 356
29760657: Hoxha E, Balbo I, Miniaci MC, Tempia F
Purkinje Cell Signaling Deficits in Animal Models of Ataxia.
Purkinje cell (PC) dysfunction or degeneration is the most frequent finding in animal models with ataxic symptoms. Mutations affecting intrinsic membrane properties can lead to ataxia by altering the firing rate of PCs or their firing pattern. However, the relationship between specific firing alterations and motor symptoms is not yet clear, and in some cases PC dysfunction precedes the onset of ataxic signs. Moreover, a great variety of ionic and synaptic mechanisms can affect PC signaling, resulting in different features of motor dysfunction. Mutations affecting Na channels (Na1.1, Na1.6, Naβ4, Fgf14 or Rer1) reduce the firing rate of PCs, mainly via an impairment of the Na resurgent current. Mutations that reduce Kv3 currents limit the firing rate frequency range. Mutations of Kv1 channels act mainly on inhibitory interneurons, generating excessive GABAergic signaling onto PCs, resulting in episodic ataxia. Kv4.3 mutations are responsible for a complex syndrome with several neurologic dysfunctions including ataxia. Mutations of either Cav or BK channels have similar consequences, consisting in a disruption of the firing pattern of PCs, with loss of precision, leading to ataxia. Another category of pathogenic mechanisms of ataxia regards alterations of synaptic signals arriving at the PC. At the parallel fiber (PF)-PC synapse, mutations of glutamate delta-2 (GluD2) or its ligand Crbl1 are responsible for the loss of synaptic contacts, abolishment of long-term depression (LTD) and motor deficits. At the same synapse, a correct function of metabotropic glutamate receptor 1 (mGlu1) receptors is necessary to avoid ataxia. Failure of climbing fiber (CF) maturation and establishment of PC mono-innervation occurs in a great number of mutant mice, including mGlu1 and its transduction pathway, GluD2, semaphorins and their receptors. All these models have in common the alteration of PC output signals, due to a variety of mechanisms affecting incoming synaptic signals or the way they are processed by the repertoire of ionic channels responsible for intrinsic membrane properties. Although the PC is a final common pathway of ataxia, the link between specific firing alterations and neurologic symptoms has not yet been systematically studied and the alterations of the cerebellar contribution to motor signals are still unknown.
Front Synaptic Neurosci, 2018; 10

29163054: Hoxha E, Gabriele RMC, Balbo I, Ravera F, Masante L, Zambelli V, Albergo C, Mitro N, Caruso D, Di Gregorio E, Brusco A, Borroni B, Tempia F
Motor Deficits and Cerebellar Atrophy in Knock Out Mice.
Spino-Cerebellar-Ataxia type 38 (SCA38) is caused by missense mutations in the very long chain fatty acid elongase 5 gene, . The main clinical findings in this disease are ataxia, hyposmia and cerebellar atrophy. Mice in which has been knocked out represent a model of the loss of function hypothesis of SCA38. In agreement with this hypothesis, knock out mice reproduced the main symptoms of patients, motor deficits at the beam balance test and hyposmia. The cerebellar cortex of knock out mice showed a reduction of thickness of the molecular layer, already detectable at 6 months of age, confirmed at 12 and 18 months. The total perimeter length of the Purkinje cell (PC) layer was also reduced in knock out mice. Since Elovl5 transcripts are expressed by PCs, whose dendrites are a major component of the molecular layer, we hypothesized that an alteration of their dendrites might be responsible for the reduced thickness of this layer. Reconstruction of the dendritic tree of biocytin-filled PCs, followed by Sholl analysis, showed that the distribution of distal dendrites was significantly reduced in Elovl5 knock out mice. Dendritic spine density was conserved. These results suggest that knock out mice recapitulate SCA38 symptoms and that their cerebellar atrophy is due, at least in part, to a reduced extension of PC dendritic arborization.
Front Cell Neurosci, 2017; 11

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9-13 July 2022 | Paris, France

BOARD NUMBER: S02-222

INVESTIGATING THE EFFECTS OF A STABLE STIMULUS IN AN UNSTABLE ENVIRONMENT INDUCED BY ATTACHMENT BOND INTERFERENCE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Attachment Bond is an emotional, reciprocal bond and experiences affecting its formation may lead to psychopathologies, such as depression, later in life. The neuropeptide Oxytocin (Oxt) is involved in forming attachment bond and is very sensitive to early life experiences. Interestingly, Repeated Cross Fostering (RCF) manipulation increases sensitivity to anhedonic-like behavior and passive coping strategies in DBA female mice by interfering with attachment bond. Moreover, RCF mice show reduced brain Oxt levels. Here, we hypothesized that introducing a Stable Attachment Figure (SAF) during the RCF procedure (inducing instability) may prevent the long-lasting effects of attachment bond alteration, rescuing the brain Oxt levels. To test our hypothesis, control and RCF DBA mice experienced the SAF from PND1 to PND4. Maternal behavior, USV distress calls and Homing test were evaluated in childhood. In adulthood, animals were tested in Social Interaction Test (SIT), Saccharin Preference Test (SPT), Forced Swimming Test (FST) and Tail Suspension Test (TST) to evaluate the rescue effects of SAF on RCF-induced phenotype. SAF did not alter maternal behavior in both Control and RCF groups. No significant effect of SAF was evident in adult Control mice. However, interestingly, SAF prevents the effects of RCF in adulthood probably acting *via* Oxt system.
Overall, our data indicate that the instability induced by RCF is mediated by altered Oxt system. The experience of a stable stimulus (SAF) in the unstable environment (RCF) prevents adulthood negative consequences restoring normal Oxt levels.

Pubmed:
34660854: Lo Iacono L, Mancini C, Babicola L, Pietrosanto M, Di Segni M, D'Addario SL, Municchi D, Ielpo D, Pascucci T, Cabib S, Ferlazzo F, D'Amato FR, Andolina D, Helmer-Citterich M, Cifani C, Ventura R
Early life adversity affecting the attachment bond alters ventral tegmental area transcriptomic patterning and behavior almost exclusively in female mice.
Early life experiences that affect the attachment bond formation can alter developmental trajectories and result in pathological outcomes in a sex-related manner. However, the molecular basis of sex differences is quite unknown. The dopaminergic system originating from the ventral tegmental area has been proposed to be a key mediator of this process. Here we exploited a murine model of early adversity (Repeated Cross Fostering, RCF) to test how interfering with the attachment bond formation affects the VTA-related functions in a sex-specific manner. Through a comprehensive behavioral screening, within the NiH RDoC framework, and by next-generation RNA-Seq experiments, we analyzed the long-lasting effect of RCF on behavioral and transcriptional profiles related to the VTA, across two different inbred strains of mouse in both sexes. We found that RCF impacted to an extremely greater extent VTA-related behaviors in females than in males and this result mirrored the transcriptional alterations in the VTA that were almost exclusively observed in females. The sexual dimorphism was conserved across two different inbred strains in spite of their divergent long lasting consequences of RCF exposure. Our data suggest that to be female primes a sub-set of genes to respond to early environmental perturbations. This is, to the best of our knowledge, the first evidence of an almost exclusive effect of early life experiences on females, thus mirroring the extremely stronger impact of precocious aversive events reported in clinical studies in women.
Neurobiol Stress, 2021; 15
33937445: D'Addario SL, Di Segni M, Ledonne A, Piscitelli R, Babicola L, Martini A, Spoleti E, Mancini C, Ielpo D, D'Amato FR, Andolina D, Ragozzino D, Mercuri NB, Cifani C, Renzi M, Guatteo E, Ventura R
Resilience to anhedonia-passive coping induced by early life experience is linked to a long-lasting reduction of I current in VTA dopaminergic neurons.
Exposure to aversive events during sensitive developmental periods can affect the preferential coping strategy adopted by individuals later in life, leading to either stress-related psychiatric disorders, including depression, or to well-adaptation to

future adversity and sources of stress, a behavior phenotype termed "resilience". We have previously shown that interfering with the development of mother-pups bond with the Repeated Cross Fostering (RCF) stress protocol can induce resilience to depression-like phenotype in adult C57BL/6J female mice. Here, we used patch-clamp recording in midbrain slice combined with both and pharmacology to test our hypothesis of a link between electrophysiological modifications of dopaminergic neurons in the intermediate Ventral Tegmental Area (VTA) of RCF animals and behavioral resilience. We found reduced hyperpolarization-activated (I) cation current amplitude and evoked firing in VTA dopaminergic neurons from both young and adult RCF female mice. , VTA-specific pharmacological manipulation of the I current reverted the pro-resilient phenotype in adult early-stressed mice or mimicked behavioral resilience in adult control animals. This is the first evidence showing how pro-resilience behavior induced by early events is linked to a long-lasting reduction of I current and excitability in VTA dopaminergic neurons.
Neurobiol Stress, 2021; 14
32113966: Di Segni M, D'Addario SL, Babicola L, Ielpo D, Lo Iacono L, Andolina D, Accoto A, Luchetti A, Mancini C, Parisi C, D'Onofrio M, Arisi I, Brandi R, Pascucci T, Cifani C, D'Amato FR, Ventura R
Xlr4 as a new candidate gene underlying vulnerability to cocaine effects.
Although several studies have been performed in rodents, non-human primates and humans, the biological basis of vulnerability to develop cocaine addiction remains largely unknown. Exposure to critical early events (as Repeated Cross Fostering (RCF)) has been reported to increase sensitivity to cocaine effects in adult C57BL/6J female mice. Using a microarray approach, here we report data showing a strong engagement of X-linked lymphocyte-regulated 4a and 4b (Xlr4) genes in cocaine effects. The expression of Xlr4, a gene involved in chromatin remodeling and dendritic spine morphology, was reduced into the Nucleus Accumbens (NAc) of adult RCF C57BL/6J female. We used virally mediated accumbal Xlr4 down-modulation (AAVXlr4-KD) to investigate the role of this gene in vulnerability to cocaine effects. AAVXlr4-KD animals show a potentiated behavioral and neurochemical response to cocaine, reinstatement following cocaine withdrawal and cocaine-induced spine density alterations in the Medium-Sized Spiny Neurons of NAc. We propose Xlr4 as a new candidate gene mediating the cocaine effects.
Neuropharmacology, 2020; 168

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BOARD NUMBER: S02-223

FEAR EXTINCTION IMPAIRMENTS AND SLEEP ABNORMALITIES IN RATS SELECTED FOR BLUNTED GLUCOCORTICOID RESPONSIVENESS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Silvia Monari[1], Marie-Isabelle Guillot De Suduiraut[1], Sophie Walker[1], Jocelyn Grosse[1], Olivia Zanoletti[1], Diana Cash[2], Simone Astori[1], Carmen Sandi[1]
[1]École polytechnique fédérale de Lausanne, Brain Mind Institute, Lausanne, Switzerland, [2]King's College London, Neuroimaging Department, London, United Kingdom

Humans show inter-individual differences in vulnerability to develop post-traumatic stress disorder (PTSD) following exposure to trauma. Although low cortisol levels have been linked to PTSD pathophysiology, a causative role for blunted glucocorticoid responsiveness in PTSD is still a matter of debate. We have addressed this question by examining fear extinction in lines of Wistar rats genetically selected for their differential glucocorticoid responsiveness to stress. We observed that rats with blunted corticosterone responsiveness exhibited strong deficits in extinction consolidation compared to rats with 'normative' glucocorticoid responses, and showed higher fear response both few days and several weeks after trauma exposure. Notably, similar to PTSD patients, low-responder rats presented smaller hippocampi, regardless of trauma exposure. Given the hypothesized role of norepinephrine (NE) in PTSD and in sleep-dependent memory consolidation, we monitored hippocampal NE levels with fiber photometry combined with polysomnographic recordings. Our data indicate that low-responder rats had an excess of NE during REM sleep. In addition, during post-extinction sleep, they failed to increase the %time spent in REM sleep, which has been shown to support extinction consolidation. Importantly, the memory deficits in low-responder rats could be normalized by corticosterone injections provided after the extinction session. Conversely, blocking the corticosterone synthesis in rats with normative glucocorticoid levels induced an extinction impairment. Our findings strongly support a causative role for blunted glucocorticoid responsiveness in the biobehavioral traits that provide higher vulnerability to PTSD.

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STRESS-ACTIVATED VENTRAL HIPPOCAMPAL NUCLEI SHOW DIFFERENTIAL METHYLATION LANDSCAPE OF TRANSCRIPTION AND SYNAPTIC GTPASE SIGNALLING REGULATION IN THE RESILIENT MICE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Monika Chanu Chongtham[1], Dewi Hartwich[2], Hristo Todorov[2], Nicolas Ruffini[3], Tamer Butto[2], Eva Weiss[2], Matthias Linke[2], Susanne Gerber[2], Jennifer Winter[2,3]
[1]Johannes Gutenberg University, Leibniz Institute For Resilience Research, Mainz, Germany, [2]University Medical Centre of the Johannes Gutenberg University, Institute Of Human Genetics, Mainz, Germany, [3]Johannes Gutenberg University, Leibniz Institute For Resilience, Mainz, Germany

Introduction: Chronic stress has resulted in a general decline of mental health, worldwide. However, despite being exposed to similar types of stress, differences in resilience to these stressors can be observed between individuals. Such differences result from multiple factors, including genetic/existing epigenetic build-up, prior successful adaptation to similar stress, or presence of social buffering. Increasing number of studies investigate the biological embedding of such experiences in the cell-specific epigenome. Our study examines specific ventral hippocampal nuclei (activated during stress recall) to dissect the DNA methylation landscape in the alternative behavioral states of resilience and susceptibility. **Methods:** We adapted the chronic social defeat stress paradigm on *Arc*$^{creERT2/+}$.R26$^{CAG\text{-}LSL\text{-}Sun1\text{-}sfGFP\text{-}Myc/+}$. This mouse line allows for the trapping of specific nuclei activated (expressing immediate early gene, *Arc*) during social interaction test (stress-recall). Activated nuclei showed *sfGFP* expression on the nuclear membrane and were sorted using “Fluorescent activated nuclei sorting”. DNA methylation states were accessed using Reduced Represented Bisulfite Sequencing (RRBS). **Results:** We identified differential modifications at the basal cytosine level and regional methylation of multiple genes involved in transcription and GTPase signalling between resilience and susceptibility states. Most of these genes are involved in synaptic remodelling. Apart from the genic regions, we also identified differential methylation of important regulatory regions lying in the intergenic regions. **Conclusion:** Differential methylation of genes regulating synaptic function in resilient mice indicates a different synaptic threshold towards environmental insults. Additional longitudinal studies along with exploration of the intergenic regions in a 3D context, could reveal further insights in stress-resilience mechanisms.

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LATE-LIFE INFLUENCE OF CHILDHOOD MALTREATMENT ON BRAIN STRUCTURE IS MEDIATED BY PARALLEL AND SEQUENTIAL PATHWAYS OF STRESS, IMMUNE, METABOLIC PHYSIOLOGY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Sofia Orellana Aguirre[1], Richard Bethlehem[1], Ivan Simpson-Kent[2], Petra Vértes[1], Edward Bullmore[1], Anne-Laura Van Harmelen[3]
[1]University of Cambridge, Department Of Psychiatry, Cambridge, United Kingdom, [2]University of Pennsylvania, Department Of Psychology, Philadelphia, United States of America, [3]Leiden University, Institute Of Education And Child Studies, Leiden, Netherlands

Childhood maltreatment (CM) is associated with abnormal physiology and lifelong psychopathology risks. However, the role of altered physiological systems in enabling temporally distal effects of CM on neurobiology is unclear. We test immune function, metabolic function, and adult stress, three factors reported to influence brain structure, as pathways of distal CM influence on grey matter subcortical volume and cortical thickness (CT). We use an MR imaging subsample of the UK BIOBANK (N=21,738, ages 39-73), where CM was retrospectively assessed, immune and metabolic function measured via circulating C-reactive protein (CRP) levels and body-mass index (BMI) respectively, and stress indexed through self-reported adult trauma (AT). Implementing path modelling at each of 187 bilateral regions, we find and examine the best fitting of two models of distal CM influence on brain structure: (i) a “parallel” model where BMI, AT and CPR act as direct and simultaneous mediators of CM’s distal relationship to brain structure and (ii) an “sequential” model, where CM only influences brain structure through its BMI and AT mediated effects on CRP. We find that CM is “sequentially” related to lower temporo-parietal thickness through BMI’s effects on CRP. In contrast, at the ROIs of best “parallel” fit, CM was related to greater subcortical volume and frontotemporal thickness *directly t*hrough BMI, and lower subcortical volumes and temporo-occipital thickness *directly* through AT. Findings highlight for the first time how the biological embedding of CM on physiology influences neurobiology in late life.

Pubmed:
33472787: Smith AJ, Moreno-López L, Davidson E, Dauvermann M, Orellana S, Soneson E, Ioannidis K, Kaser M, van Harmelen AL
REACT study protocol: resilience after the COVID-19 threat (REACT) in adolescents.
COVID-19-related social isolation and stress may have significant mental health effects, including post-traumatic stress, anxiety and depression. These factors are thought to disproportionately affect populations at risk of psychopathology, such as adolescents with a history of childhood adversity (CA). Therefore, examining which factors may buffer the impact of COVID-19-related stress and isolation in vulnerable adolescents is critical. The Resilience After the COVID-19 Threat (REACT) study assesses whether emotion regulation capacity, inflammation and neuroimmune responses to stress induced in the laboratory prior to the pandemic predict responses to COVID-19-related social isolation and stress in adolescents with CA. We aim to elucidate the mechanisms that enable vulnerable adolescents to maintain or regain good mental health when confronted with COVID-19.
BMJ Open, 2021; 11
33436466: Moreno-López L, Sallie SN, Ioannidis K, Kaser M, Schueler K, Askelund AD, Turner L, van Harmelen AL,
RAISE study protocol: a cross-sectional, multilevel, neurobiological study of resilience after individual stress exposure.
This paper describes the protocol for an ongoing project funded by the Royal Society, the Resilience After Individual Stress Exposure (RAISE) study; which aims to examine the factors and mechanisms that facilitate resilient functioning after childhood adversity (CA).
BMJ Open, 2021; 11

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BOARD NUMBER: S02-226

A NOVEL GENE CONTROLS A NEW STRUCTURE: PIGGYBAC TRANSPOSABLE ELEMENT-DERIVED 1, UNIQUE TO MAMMALS, CONTROLS MAMMAL-SPECIFIC NEURONAL PARASPECKLES

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Tamas Rasko[1], Amit Pande[1], Kathrin Radscheit[1], Annika Zink[2], Manvendra Singh[3], Christian Sommer[1], Matthias Selbach[4], Mario Bunse[5], Alessandro Prigione[6], Laurence Hurst[7], Zsuzsanna Izsvak[1]
[1]MDC Berlin, Mobile Dna, Berlin, Germany, [2]Heinrich Heine University, General Pediatrics, Neonatologz And Pediatric Cardiology, Duesseldorf, Germany, [3]Cornell University, Molecular Biology And Genetics, Ithaca, Germany, [4]MDC Berlin, Cardiovascular Research, Berlin, Germany, [5]MDC Berlin, Mario.bunse@mdc-berlin.de, Berlin, Germany, [6]University Children's Hospital, Heinrich Heine University Duesseldorf, Department Of General Pediatrics, Neonatology And Pediatric Cardiology, Duesseldorf, Germany, [7]Millner Center for Evolution, Biology And Biochemistry, Bath, United Kingdom

While new genes can arrive from modes other than duplication, few examples are well characterised. Given a putative link to psychological disorders (e.g. schizophrenia), suggestive of brain functionality, here we characterize *piggyBac* transposable element derived 1 (*PGBD1*). *PGBD1* is non-monotreme mammal-specific and under purifying selection, consistent with functionality. The gene body of human *PGBD1* retains much of the original DNA transposon but has additionally captured SCAN and KRAB domains. Despite gene body retention, PGBD1 has lost transposition abilities, thus transposase functionality is absent. PGBD1 no longer recognises *piggyBac* transposon-like inverted repeats, nonetheless PGBD1 has DNA binding activity. Genome scale analysis identifies enrichment of binding sites in and around genes involved in neuronal development, with association with both histone activating and repressing marks. We focus on one of the repressed genes, the long non-coding RNA *NEAT1*, also dysregulated in schizophrenia, the core structural RNA of paraspeckles. DNA binding assays confirm specific binding of PGBD1 both in the NEAT1 promoter and in the gene body. Depletion of PGBD1 in neuronal progenitor cells (NPCs) results in increased NEAT1/paraspeckles and differentiation, suggesting that PGBD1 has evolved core regulatory functionality for the maintenance of NPCs. In neurons, by contrast, PGBD1 and NEAT1 DNA binding are partially intertwined, and share multiple target genes. We conclude that as paraspeckles are a mammal-specific structure, PGBD1 is a rare example of the evolution of a novel gene coupled to the evolution of a contemporaneous new structure.

Pubmed:
28192174: van den Buuse M, Biel D, Radscheit K
Does genetic BDNF deficiency in rats interact with neurotransmitter control of prepulse inhibition? Implications for schizophrenia.
Several studies have suggested a role of BDNF in the development of schizophrenia. For example, post-mortem studies have shown significantly reduced levels of BDNF protein expression in the brain of schizophrenia patients. We investigated the relationship between reduced levels of BDNF in the brain and the regulation of prepulse inhibition (PPI), a behavioral endophenotype of schizophrenia. We used BDNF heterozygous mutant rats which display a 50% decrease of mature BDNF protein levels. Previously, we observed normal baseline PPI and responses to the dopamine D1/D2 receptor agonist, apomorphine, in these rats. Here, we focused on the effects of the NMDA receptor antagonist, MK-801, its interaction with mGluR2/3 and mGluR5 receptors, and the PPI response to serotonergic drugs. MK-801 administration caused a dose-dependent reduction of PPI and increase of startle amplitudes. Baseline PPI and the effect of 0.02-0.1mg/kg of MK-801 were not significantly altered in male or female BDNF heterozygous rats, although the MK-801-induced increase in startle levels was reduced. Co-treatment with the mGluR2/3 agonist, LY379,268, or the mGluR5 antagonist, MPEP, did not alter the effect of MK-801 on PPI in controls or BDNF mutant rats. Treatment with the serotonin-1A receptor agonist, 8-OH-DPAT, the serotonin-2A receptor agonist, DOI, or the serotonin releaser, fenfluramine, induced differential effects on PPI and startle but these effects were not different between the genotypes. These results show that a significant decrease of BDNF protein expression does not lead to reduced PPI at baseline or changes in the regulation of PPI via NMDA receptors or serotonergic mechanisms. These findings in a genetic rat model of BDNF deficiency do not support a role for similar reductions of BDNF levels in schizophrenia in the disruption of PPI, widely reported as an endophenotype of the illness. The potential implications

of these results for our understanding of changes in PPI and BDNF expression in schizophrenia are discussed.
Prog Neuropsychopharmacol Biol Psychiatry, 2017; 75
27575199: Täuber D, Radscheit K, von Borczyskowski C, Schulz M, Osipov VA
Fluorescence correlation spectroscopy in thin films at reflecting substrates as a means to study nanoscale structure and dynamics at soft-matter interfaces.
Structure and dynamics at soft-matter interfaces play an important role in nature and technical applications. Optical single-molecule investigations are noninvasive and capable to reveal heterogeneities at the nanoscale. In this work we develop an autocorrelation function (ACF) approach to retrieve tracer diffusion parameters obtained from fluorescence correlation spectroscopy (FCS) experiments in thin liquid films at reflecting substrates. This approach then is used to investigate structure and dynamics in 100-nm-thick 8CB liquid crystal films on silicon wafers with five different oxide thicknesses. We find a different extension of the structural reorientation of 8CB at the solid-liquid interface for thin and for thick oxide. For the thin oxides, the perylenediimide tracer diffusion dynamics in general agrees with the hydrodynamic modeling using no-slip boundary conditions with only a small deviation close to the substrate, while a considerably stronger decrease of the interfacial tracer diffusion is found for the thick oxides.
Phys Rev E, 2016; 94

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MEDIAL PREFRONTAL CORTEX ENCODES BEHAVIORAL STRATEGY DURING STRESS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Ole Christian Sylte[1,2], Mateo N'Diaye[1,2], Jonas-Frederic Sauer[1]
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In stressful situations, individuals use different strategies to cope with stressors, dysfunction of which may underlie individuals' vulnerability to stress-related disorders. The medial prefrontal cortex (mPFC) is involved in behavioral responses to stress, but the dynamics of encoding stress-related behavioral strategies by mPFC neurons remains poorly understood. Here, we used 1-photon calcium imaging to record large populations of layer 5 mPFC pyramidal neurons in mice during repeated exposure to stress in a tail suspension test (TST). In this paradigm, mice rapidly switch between active and passive coping styles, thus allowing us to directly assess stress coping-related neuronal responses using various correlational and decoding-approaches. In line with previous research, we observed that some cells were selectively activated by either active or passive stress coping. From neuronal activity alone, we were able to predict the stress coping strategy of the animal with 99% accuracy. Recording from the same set of neurons during baseline movement in the home-cage revealed that neuronal responses during stress coping were unrelated to general movement. Furthermore, we found that neurons retained consistent responses during active and passive coping styles over multiple days of TST exposure, with only minor drift in the response profile. These results implicate a strong and temporally consistent involvement of mPFC populations in stress responses, which might be of importance to understand mechanisms underlying stress-related disorders.

Pubmed:
34557946: Sylte OC, Johansen JS, Heinla I, Houwing DJ, Olivier JDA, Heijkoop R, Snoeren EMS
Effects of perinatal fluoxetine exposure on novelty-induced social and non-social investigation behaviors in a seminatural environment.
Selective serotonin reuptake inhibitors (SSRIs) are increasingly prescribed as medication for various affective disorders during pregnancy. SSRIs cross the placenta and affect serotonergic neurotransmission in the fetus, but the neurobehavioral consequences for the offspring remain largely unclear. Recent rodent research has linked perinatal SSRI exposure to alterations in both social and non-social aspects of behavior. However, this research has mainly focused on behavior within simplified environments. The current study investigates the effects of perinatal SSRI exposure on social and non-social investigation behaviors of adult rat offspring upon introduction to a novel seminatural environment with unknown conspecifics. During the perinatal period (gestational day 1 until postnatal day 21), rat dams received daily treatment with either an SSRI (fluoxetine, 10 mg/kg) or vehicle. Adult male and female offspring were observed within the first hour after introduction to a seminatural environment. The results showed that perinatal fluoxetine exposure altered aspects of non-social investigation behaviors, while not altering social investigation behaviors. More specifically, both fluoxetine-exposed males and females spent more total time on locomotor activity than controls. Furthermore, fluoxetine-exposed females spent less time exploring objects and specific elements in the environment. The data suggest that perinatal exposure to SSRIs leads to a quicker, less detailed investigation strategy in novel environments and that the alteration is mostly pronounced in females.
Psychopharmacology (Berl), 2021; 238

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BOARD NUMBER: S02-228

CELL-TYPE-SPECIFIC DOPAMINE SIGNALING IN VENTRAL HIPPOCAMPUS TRACKS ANXIETY

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Despite accumulating evidence for a role of both the ventral hippocampus (vHipp) and the mesocorticolimbic dopamine system in encoding anxiety-relevant information, surprisingly little is known about how dopamine signaling selectively affects vHipp representations of emotionally-salient stimuli to guide innate approach/avoidance behaviors. To address these shortcomings, we here study dopaminoceptive neurons in mouse vHipp – which can be segregated based on their expression of either the dopamine D1 or D2 receptor – to delineate a model of dopamine action in vHipp. At the histological level, D1- and D2-expressing cells exhibit a precise topographical organization across vHipp subfields, which we further dissected using RNA-sequencing of single, sorted nuclei from D1- and D2-cells. Functionally, we showed that anxiogenic environments trigger distinct patterns of calcium activity in D1 and D2 cells, together with higher dopamine release in vHipp. Bidirectional chemogenetic and optogenetic manipulation of D1- or of D2-neurons' activity causally demonstrated their distinct roles in mediating anxiety and approach/avoidance behaviors. Intriguingly, cocaine exposure modified vHipp dopamine dynamics, with D1- and D2-expressing neurons differently modulating drug-related behaviors, suggesting drug-induced plastic changes in this circuit. Together, we propose that dopamine dynamics in vHipp operate as a feedback loop that bidirectionally tracks anxiety levels to gate exploratory behaviors through differential recruitment of vHipp D1- and D2-neurons, which in turn mediate opposite approach/avoidance and anxiety-like responses. This work paves the way for further studies of dopamine signal processing in limbic regions, and underscores the complexity of the circuit mechanisms that govern affective states.

Pubmed:
33723435: Kronman H, Torres-Berrío A, Sidoli S, Issler O, Godino A, Ramakrishnan A, Mews P, Lardner CK, Parise EM, Walker DM, van der Zee YY, Browne CJ, Boyce BF, Neve R, Garcia BA, Shen L, Peña CJ, Nestler EJ
Long-term behavioral and cell-type-specific molecular effects of early life stress are mediated by H3K79me2 dynamics in medium spiny neurons.
Animals susceptible to chronic social defeat stress (CSDS) exhibit depression-related behaviors, with aberrant transcription across several limbic brain regions, most notably in the nucleus accumbens (NAc). Early life stress (ELS) promotes susceptibility to CSDS in adulthood, but associated enduring changes in transcriptional control mechanisms in the NAc have not yet been investigated. In this study, we examined long-lasting changes to histone modifications in the NAc of male and female mice exposed to ELS. Dimethylation of lysine 79 of histone H3 (H3K79me2) and the enzymes (DOT1L and KDM2B) that control this modification are enriched in D2-type medium spiny neurons and are shown to be crucial for the expression of ELS-induced stress susceptibility. We mapped the site-specific regulation of this histone mark genome wide to reveal the transcriptional networks it modulates. Finally, systemic delivery of a small molecule inhibitor of DOT1L reversed ELS-induced behavioral deficits, indicating the clinical relevance of this epigenetic mechanism.
Nat Neurosci, 2021; 24
33706932: Salery M, Godino A, Nestler EJ
Drug-activated cells: From immediate early genes to neuronal ensembles in addiction.
Beyond their rapid rewarding effects, drugs of abuse can durably alter an individual's response to their environment as illustrated by the compulsive drug seeking and risk of relapse triggered by drug-associated stimuli. The persistence of these associations even long after cessation of drug use demonstrates the enduring mark left by drugs on brain reward circuits. However, within these circuits, neuronal populations are differently affected by drug exposure and growing evidence indicates that relatively small subsets of neurons might be involved in the encoding and expression of drug-mediated associations. The identification of sparse neuronal populations recruited in response to drug exposure has benefited greatly from the study of immediate early genes (IEGs) whose induction is critical in initiating plasticity programs in recently activated neurons. In

particular, the development of technologies to manipulate IEG-expressing cells has been fundamental to implicate broadly distributed neuronal ensembles coincidently activated by either drugs or drug-associated stimuli and to then causally establish their involvement in drug responses. In this review, we summarize the literature regarding IEG regulation in different learning paradigms and addiction models to highlight their role as a marker of activity and plasticity. As the exploration of neuronal ensembles in addiction improves our understanding of drug-associated memory encoding, it also raises several questions regarding the cellular and molecular characteristics of these discrete neuronal populations as they become incorporated in drug-associated neuronal ensembles. We review recent efforts towards this goal and discuss how they will offer a more comprehensive understanding of addiction pathophysiology.
Adv Pharmacol, 2021; 90
32641757: Dash S, Balasubramaniam M, Martínez-Rivera FJ, Godino A, Peck EG, Patnaik S, Suar M, Calipari ES, Nestler EJ, Villalta F, Dash C, Pandhare J
Cocaine-regulated microRNA miR-124 controls poly (ADP-ribose) polymerase-1 expression in neuronal cells.
MiR-124 is a highly expressed miRNA in the brain and regulates genes involved in neuronal function. We report that miR-124 post-transcriptionally regulates PARP-1. We have identified a highly conserved binding site of miR-124 in the 3'-untranslated region (3'UTR) of Parp-1 mRNA. We demonstrate that miR-124 directly binds to the Parp-1 3'UTR and mutations in the seed sequences abrogate binding between the two RNA molecules. Luciferase reporter assay revealed that miR-124 post-transcriptionally regulates Parp-1 3'UTR activity in a dopaminergic neuronal cell model. Interestingly, the binding region of miR-124 in Parp-1 3'UTR overlapped with the target sequence of miR-125b, another post-transcriptional regulator of Parp-1. Our results from titration and pull-down studies revealed that miR-124 binds to Parp-1 3'UTR with greater affinity and confers a dominant post-transcriptional inhibition compared to miR-125b. Interestingly, acute or chronic cocaine exposure downregulated miR-124 levels concomitant with upregulation of PARP-1 protein in dopaminergic-like neuronal cells in culture. Levels of miR-124 were also downregulated upon acute or chronic cocaine exposure in the mouse nucleus accumbens (NAc)-a key reward region of brain. Time-course studies revealed that cocaine treatment persistently downregulated miR-124 in NAc. Consistent with this finding, miR-124 expression was also significantly reduced in the NAc of animals conditioned for cocaine place preference. Collectively, these studies identify Parp-1 as a direct target of miR-124 in neuronal cells, establish miR-124 as a cocaine-regulated miRNA in the mouse NAc, and highlight a novel pathway underlying the molecular effects of cocaine.
Sci Rep, 2020; 10
31477236: Browne CJ, Godino A, Salery M, Nestler EJ
Epigenetic Mechanisms of Opioid Addiction.
Opioid use kills tens of thousands of Americans each year, devastates families and entire communities, and cripples the health care system. Exposure to opioids causes long-term changes to brain regions involved in reward processing and motivation, leading vulnerable individuals to engage in pathological drug seeking and drug taking that can remain a lifelong struggle. The persistence of these neuroadaptations is mediated in part by epigenetic remodeling of gene expression programs in discrete brain regions. Although the majority of work examining how epigenetic modifications contribute to addiction has focused on psychostimulants such as cocaine, research into opioid-induced changes to the epigenetic landscape is emerging. This review summarizes our knowledge of opioid-induced epigenetic modifications and their consequential changes to gene expression. Current evidence points toward opioids promoting higher levels of permissive histone acetylation and lower levels of repressive histone methylation as well as alterations to DNA methylation patterns and noncoding RNA expression throughout the brain's reward circuitry. Additionally, studies manipulating epigenetic enzymes in specific brain regions are beginning to build causal links between these epigenetic modifications and changes in addiction-related behavior. Moving forward, studies must leverage advanced chromatin analysis and next-generation sequencing approaches combined with bioinformatics pipelines to identify novel gene networks regulated by particular epigenetic modifications. Improved translational relevance also requires increased focus on volitional drug-intake models and standardization of opioid exposure paradigms. Such work will significantly advance our understanding of how opioids cause persistent changes to brain function and will provide a platform on which to develop interventions for treating opioid addiction.
Biol Psychiatry, 2020; 87
31092585: Calipari ES, Godino A, Salery M, Damez-Werno DM, Cahill ME, Werner CT, Gancarz AM, Peck EG, Jlayer Z, Rabkin J, Landry JA, Smith ACW, Defilippi P, Kenny PJ, Hurd YL, Neve RL, Dietz DM, Nestler EJ
Synaptic Microtubule-Associated Protein EB3 and SRC Phosphorylation Mediate Structural and Behavioral Adaptations During Withdrawal From Cocaine Self-Administration.
Addictive behaviors, including relapse, are thought to depend in part on long-lasting drug-induced adaptations in dendritic spine signaling and morphology in the nucleus accumbens (NAc). While the influence of activity-dependent actin remodeling in these phenomena has been studied extensively, the role of microtubules and associated proteins remains poorly understood. We report that pharmacological inhibition of microtubule polymerization in the NAc inhibited locomotor

sensitization to cocaine and contextual reward learning. We then investigated the roles of microtubule end-binding protein 3 (EB3) and SRC kinase in the neuronal and behavioral responses to volitionally administered cocaine. In synaptoneurosomal fractions from the NAc of self-administering male rats, the phosphorylation of SRC at an activating site was induced after 1 d of withdrawal, while EB3 levels were increased only after 30 d of withdrawal. Blocking SRC phosphorylation during early withdrawal by virally overexpressing SRCIN1, a negative regulator of SRC activity known to interact with EB3, abolished the incubation of cocaine craving in both male and female rats. Conversely, mimicking the EB3 increase observed after prolonged withdrawal increased the motivation to consume cocaine in male rats. In mice, the overexpression of either EB3 or SRCIN1 increased dendritic spine density and altered the spine morphology of NAc medium spiny neurons. Finally, a cocaine challenge after prolonged withdrawal recapitulated most of the synaptic protein expression profiles observed at early withdrawal. These findings suggest that microtubule-associated signaling proteins such as EB3 cooperate with actin remodeling pathways, notably SRC kinase activity, to establish and maintain long-lasting cellular and behavioral alterations following cocaine self-administration. Drug-induced morphological restructuring of dendritic spines of nucleus accumbens neurons is thought to be one of the cellular substrates of long-lasting drug-associated memories. The molecular basis of these persistent changes has remained incompletely understood. Here we implicate for the first time microtubule function in this process, together with key players such as microtubule-bound protein EB3 and synaptic SRC phosphorylation. We propose that microtubule and actin remodeling cooperate during withdrawal to maintain the plastic structural changes initially established by cocaine self-administration. This work opens new translational avenues for further characterization of microtubule-associated regulatory molecules as putative drug targets to tackle relapse to drug taking.
J Neurosci, 2019; 39

29861096: Walker DM, Cates HM, Loh YE, Purushothaman I, Ramakrishnan A, Cahill KM, Lardner CK, Godino A, Kronman HG, Rabkin J, Lorsch ZS, Mews P, Doyle MA, Feng J, Labonté B, Koo JW, Bagot RC, Logan RW, Seney ML, Calipari ES, Shen L, Nestler EJ

Cocaine Self-administration Alters Transcriptome-wide Responses in the Brain's Reward Circuitry.

Global changes in gene expression underlying circuit and behavioral dysregulation associated with cocaine addiction remain incompletely understood. Here, we show how a history of cocaine self-administration (SA) reprograms transcriptome-wide responses throughout the brain's reward circuitry at baseline and in response to context and/or cocaine re-exposure after prolonged withdrawal (WD).
Biol Psychiatry, 2018; 84

29339724: Calipari ES, Godino A, Peck EG, Salery M, Mervosh NL, Landry JA, Russo SJ, Hurd YL, Nestler EJ, Kiraly DD

Granulocyte-colony stimulating factor controls neural and behavioral plasticity in response to cocaine.

Cocaine addiction is characterized by dysfunction in reward-related brain circuits, leading to maladaptive motivation to seek and take the drug. There are currently no clinically available pharmacotherapies to treat cocaine addiction. Through a broad screen of innate immune mediators, we identify granulocyte-colony stimulating factor (G-CSF) as a potent mediator of cocaine-induced adaptations. Here we report that G-CSF potentiates cocaine-induced increases in neural activity in the nucleus accumbens (NAc) and prefrontal cortex. In addition, G-CSF injections potentiate cocaine place preference and enhance motivation to self-administer cocaine, while not affecting responses to natural rewards. Infusion of G-CSF neutralizing antibody into NAc blocks the ability of G-CSF to modulate cocaine's behavioral effects, providing a direct link between central G-CSF action in NAc and cocaine reward. These results demonstrate that manipulating G-CSF is sufficient to alter the motivation for cocaine, but not natural rewards, providing a pharmacotherapeutic avenue to manipulate addictive behaviors without abuse potential.
Nat Commun, 2018; 9

27046646: Cadet JL, Brannock C, Krasnova IN, Jayanthi S, Ladenheim B, McCoy MT, Walther D, Godino A, Pirooznia M, Lee RS

Genome-wide DNA hydroxymethylation identifies potassium channels in the nucleus accumbens as discriminators of methamphetamine addiction and abstinence.

Epigenetic consequences of exposure to psychostimulants are substantial but the relationship of these changes to compulsive drug taking and abstinence is not clear. Here, we used a paradigm that helped to segregate rats that reduce or stop their methamphetamine (METH) intake (nonaddicted) from those that continue to take the drug compulsively (addicted) in the presence of footshocks. We used that model to investigate potential alterations in global DNA hydroxymethylation in the nucleus accumbens (NAc) because neuroplastic changes in the NAc may participate in the development and maintenance of drug-taking behaviors. We found that METH-addicted rats did indeed show differential DNA hydroxymethylation in comparison with both control and nonaddicted rats. Nonaddicted rats also showed differences from control rats. Differential DNA hydroxymethylation observed in addicted rats occurred mostly at intergenic sites located on long and short interspersed elements. Interestingly, differentially hydroxymethylated regions in genes encoding voltage (Kv1.1, Kv1.2, Kvb1 and Kv2.2)- and calcium (Kcnma1, Kcnn1 and Kcnn2)-gated potassium channels observed in the NAc of

nonaddicted rats were accompanied by increased mRNA levels of these potassium channels when compared with mRNA expression in METH-addicted rats. These observations indicate that changes in differentially hydroxymethylated regions and increased expression of specific potassium channels in the NAc may promote abstinence from drug-taking behaviors. Thus, activation of specific subclasses of voltage- and/or calcium-gated potassium channels may provide an important approach to the beneficial treatment for METH addiction.
Mol Psychiatry, 2017; 22
26023847: Godino A, Jayanthi S, Cadet JL
Epigenetic landscape of amphetamine and methamphetamine addiction in rodents.
Amphetamine and methamphetamine addiction is described by specific behavioral alterations, suggesting long-lasting changes in gene and protein expression within specific brain subregions involved in the reward circuitry. Given the persistence of the addiction phenotype at both behavioral and transcriptional levels, several studies have been conducted to elucidate the epigenetic landscape associated with persistent effects of drug use on the mammalian brain. This review discusses recent advances in our comprehension of epigenetic mechanisms underlying amphetamine- or methamphetamine-induced behavioral, transcriptional, and synaptic plasticity. Accumulating evidence demonstrated that drug exposure induces major epigenetic modifications-histone acetylation and methylation, DNA methylation-in a very complex manner. In rare instances, however, the regulation of a specific target gene can be correlated to both epigenetic alterations and behavioral abnormalities. Work is now needed to clarify and validate an epigenetic model of addiction to amphetamines. Investigations that include genome-wide approaches will accelerate the speed of discovery in the field of addiction.
Epigenetics, 2015; 10
34785784: Forget B, Garcia EM, Godino A, Rodriguez LD, Kappes V, Poirier P, Andrianarivelo A, Marchan ES, Allichon MC, Marias M, Vanhoutte P, Girault JA, Maldonado R, Caboche J
Cell-type- and region-specific modulation of cocaine seeking by micro-RNA-1 in striatal projection neurons.
The persistent and experience-dependent nature of drug addiction may result in part from epigenetic alterations, including non-coding micro-RNAs (miRNAs), which are both critical for neuronal function and modulated by cocaine in the striatum. Two major striatal cell populations, the striato-nigral and striato-pallidal projection neurons, express, respectively, the D1 (D1-SPNs) and D2 (D2-SPNs) dopamine receptor, and display distinct but complementary functions in drug-evoked responses. However, a cell-type-specific role for miRNAs action has yet to be clarified. Here, we evaluated the expression of a subset of miRNAs proposed to modulate cocaine effects in the nucleus accumbens (NAc) and dorsal striatum (DS) upon sustained cocaine exposure in mice and showed that these selected miRNAs were preferentially upregulated in the NAc. We focused on miR-1 considering the important role of some of its predicted mRNA targets, Fosb and Npas4, in the effects of cocaine. We validated these targets in vitro and in vivo. We explored the potential of miR-1 to regulate cocaine-induced behavior by overexpressing it in specific striatal cell populations. In DS D1-SPNs miR-1 overexpression downregulated Fosb and Npas4 and reduced cocaine-induced CPP reinstatement, but increased cue-induced cocaine seeking. In DS D2-SPNs miR-1 overexpression reduced the motivation to self-administer cocaine. Our results indicate a role of miR1 and its target genes, Fosb and Npas4, in these behaviors and highlight a precise cell-type- and region-specific modulatory role of miR-1, illustrating the importance of cell-specific investigations.
Mol Psychiatry, 2022; 27

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THE ROLE OF REV-ERBα CIRCADIAN CLOCK GENE IN STRESS RESILIENCE AND DEVELOPMENT OF DEPRESSION-LIKE BEHAVIOUR.

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Major depressive disorder is one of the most frequently diagnosed disabling mental illnesses. Resilience to stress is critical for the development of depression. While there is emerging evidence in depression for disruption of circadian rhythms, the role of circadian clock in stress resilience is not well understood. Here, we have investigated the effects of repeated swim stress on the development of depression-like behavior in a mouse model with genetically modified circadian clockwork – mice expressing dominant negative clock gene Rev-ERBα (dnRev-ERBα). The development of depression-like behavior was analyzed by utilizing the chronic despair mouse model (CDM) for depression. In this paradigm the mice undergo 10 minutes of forced swimming per day for 5 consecutive days (induction phase). The effects on mood-related behavior are examined 1 week later (test phase) via forced swim (FST) and the tail suspension test (TST). dnRev-ERBα mice showed strong and significant resistance towards development of chronic despair behavior. WT mice had gradual increase of the immobility time during the induction phase of CDM, while the dnRev-ERBα mice showed constant low immobility time. FST and TST performed during the test phase confirmed the stress resilient phenotype of the dnRev-ERBα. The analyses of locomotor and exploratory behavior in open field test (OFT) indicate no significant changes. Moreover, dnRev-ERBα mice had an increased anxyolitic behavior in OFT – shorter time spent in the center of the arena. Our data suggest the potential role of Rev-ERBα in response to stress and development of depression.

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THE ANXIOLYTIC AND ANTIDEPRESSANT EFFECTS OF UROCORTIN 2 AND UROCORTIN 3 FRAGMENTS IN MICE

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Urocortin 2 (Ucn2) and urocortin 3 (Ucn3) are selective agonists of the corticotropin-releasing factor receptor type 2 (CRFR2). Previous studies indicated that Ucn2 and Ucn3 have anxiolytic and antidepressant properties in mice. In the present study we aimed to identify the biologically active center of these peptides, through which they might exert their beneficial effects. Therefore, male C57BL/6 mice were treated intracerebroventricularly (icv) with smaller fragments of Ucn2 and Ucn3, and then they were investigated in an elevated plus-maze test and a forced swim test for anxiety-like and depression-like behavior, respectively. The following fragments were tested: Ucn2(1-21), Ucn2(22-38), Ucn3(18-38), Ucn3(19-27), Ucn3(28-38), Ucn3(34-38), Ucn3(36-38), Ucn3(34-36), and Ucn3(34-38). The only effective fragment of Ucn2 inducing both anxiolytic and antidepressant behavior was Ucn2(1-21). Many of the fragments of Ucn3 were effective, but the most significant anxiolytic and antidepressant actions were produced by Ucn3(36-38). The present study demonstrates that the whole sequences of the selective CRFR2 agonists are not necessary to exert their anxiolytic and antidepressant properties, and that the biologically active center is the N-terminal for Ucn2 and the C-terminal for Ucn3. Future studies are yet to demonstrate the efficacy of these peptide fragments in treating anxiety and depression in humans.

Pubmed:
29247629: Bagosi Z, Csabafi K, Balangó B, Pintér D, Szolomájer-Csikós O, Bozsó Z, Tóth G, Telegdy G, Szabó G
Anxiolytic- and antidepressant-like actions of Urocortin 2 and its fragments in mice.
The aim of the present study was to investigate the potential anxiolytic- and antidepressant-like actions of Urocortin 2 (Ucn2) and its two fragments, Ucn2 (1-21) and Ucn2 (22-38), in mice, in an attempt to identify the biologically active sequence of this 38 amino acid neuropeptide. In this purpose, male C57BL/6 mice were treated intracerebroventricularly (icv) with 0.125, 0.25, 0.5 and 1 μg/2 μl of Ucn2, Ucn2 (1-21) or Ucn2 (22-38). After 30 min, the mice were evaluated in an elevated plus-maze test and a forced swim test for anxiety- and depression-like behavior, respectively. Each test lasted 5 min. Ucn2 at dose of 0.25 μg/2 μl and Ucn2 (1-21) at dose of 0.125 μg/2 μl, but not Ucn2 (22-38), increased significantly the number of entries into and the time spent in the open-arms, without influencing the total number of entries. In parallel, the same doses of Ucn2 and Ucn2 (1-21), but not Ucn2 (22-38), increased significantly the climbing and the swimming activity, while decreasing significantly the time of immobility. In addition, Ucn2 at doses of 0.125 μg/2 μl and 0.5 μg/2 μl decreased significantly the time of immobility, but they did not change the other parameters. The present study demonstrates that Ucn2 exerts anxiolytic- and antidepressant-like effects in C57BL/6 mice, which are mediated by the N-terminal, but not the C-terminal fragment of the peptide. The establishment of the smallest active sequence by further fragmentation of Ucn2 (1-21) may allow the synthesis of new anxiolytic and antidepressant drugs.
Brain Res, 2018; 1680

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OBESE MOTHERS HAVE A HIGHER RISK OF DEVELOPING DEPRESSIVE-LIKE BEHAVIOUR DUE TO HORMONAL ALTERATIONS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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[1]School of Medicine, University of Belgrade, Institute Of Medical Physiology, Belgrade, Serbia, [2]School of Medicine, University of Belgrade, Institute Of Medical And Clinical Biochemistry, Belgrade, Serbia, [3]Serbian Academy of Sciences and Arts, Medical Sciences, Belgrade, Serbia

Introduction: Maternal obesity is inseparably linked to adverse health outcomes for the mother and her children. It is associated with an increased risk of neurobehavioral problems in the offspring such are neurodevelopmental disorder, anxiety and depression-spectrum disorders. **Our objective** was to determine the influence of maternal HFD on post-weaning depression-like behavior and hormonal state. **Methods:** Adult naive female Wistar rats were assigned to either standard diet (CD) or HFD. A mixture of vegetable oils was used as a source of fat (HFD 40% and CD 4% energy derived from fat). The animals were fed for four weeks before breeding, during mating, pregnancy, and lactation, and until behavioural tests were completed. Sucrose preference testing (SPT) and forced swimming (FST) have been used to assess anhedonia and depression-like behavior. **Results:** HFD-fed dams showed significant increases in body weight (g) and BMI ($p<0.05$). HFD group showed significantly increased climbing time but did not affect immobility time and sucrose preference. However, the correlation analysis revealed a strong negative correlation between total fluid consumption and climbing time in HFD ($r=-0.81$) and total fluid consumption and E2 levels ($r=-0.52$). In fact, HFD lowered estradiol serum levels ($p<0.05$) and slightly elevated corticosterone levels ($p>0.05$). Further analysis revealed a moderate negative correlation between E2 and corticosterone levels ($r=-0.51$) in HFD dams. **Conclusion:** According to the data presented, HFD dams have a higher risk of developing depressive-like behavior, likely due to lower estradiol levels.

Pubmed:
27494641: Rasic-Markovic A, Hrncic D, Krstic D, Colovic M, Djuric E, Rankov-Petrovic B, Susic V, Stanojlovic O, Djuric D
The effect of subchronic supplementation with folic acid and l-arginine on homocysteine-induced seizures.
The aim of the present study was to examine the effect of subchronic co-administration of folic acid (F) and l-arginine (A) on behavioural and electroencephalographic (EEG) characteristics of dl homocysteine thiolactone (H) induced seizures in adult rats. The activity of membrane ATPases in different brain regions were also investigated. Rats were treated with F, A, or vehicle for 15 days (regimen: F 5 mg/kg + A 500 mg/kg (F5A500); F 10 mg/kg + A 300 mg/kg (F10A300)). Seizures were elicited by convulsive dose of H (H, F5A500H, F10A300H) Subchronic supplementation with F and A did not affect seizure incidence, number of seizure episodes, and severity in F5A500H and F10A300H groups vs. H group. However, a tendency to increase latency and decrease the number of seizure episodes was noticed in the F10A300H group. EEG mean spectral power densities during ictal periods were significantly lower in F10A300H vs. H group. The activity of Na/K-ATPase and Mg-ATPase was significantly increased in almost all examined structures in rats treated with F and A. We can conclude that subchronic supplementation with folic acid and l-arginine has an antiepileptic effect in dl homocysteine thiolactone induced epilepsy.
Can J Physiol Pharmacol, 2016; 94

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GLUTAMATERGIC AND CYTOSKELETAL PROTEIN PHOSPHORYLATION ASSOCIATED WITH THE ANTIDEPRESSANT-LIKE PROPERTIES OF THE IRON CHELATOR DEFERIPRONE IN A MOUSE MODEL OF DEPRESSION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

Volkan Uzungil[1], Isaline Mees[1], Shanshan Li[2], Anthony Hannan[2], Thibault Renoir[2]
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Aims: Depression severity has recently been linked to increased levels of iron in various regions of the brain. The serotonin transporter knock-out (5-HTT KO) mouse is utilised as a model of depression, as individuals who are carriers of the short ('s') allele of the promoter region of the gene having increased vulnerability to stress and reduced response to first-line antidepressant therapies. Deferiprone is an iron chelator drug which we have found to have acute antidepressant-like properties in a battery of depression-related behaviours. Methods: A phosphoproteomics analysis of the prefrontal cortex was conducted following acute deferiprone treatment in wild-type (WT) controls and 5-HTT KO mice to determine the rapid post-translational modifications following treatment. Due to batch effects, multiple comparisons were not conducted for the differential expression analysis. Under- or over-phosphorylated phosphopeptides were matched to their respective molecular mechanisms using gene ontology and pathway analysis. Results: The analysis revealed that in both WT and 5-HTT KO mice there were differential expression in the phosphorylation of proteins involved in cytoskeletal organisation following deferiprone treatment. In comparison, there were selective differentially expressed phosphorylation in 5-HTT KO mice of proteins involved in glutamatergic signalling. Additionally, tyrosine hydroxylase was also shown to be down-phosphorylated by deferiprone treatment in 5-HTT KO mice, which has been linked to both iron regulation and depression. Conclusions: These data indicate that the antidepressant-like properties of deferiprone may be mediated by molecular mechanisms underpinned by glutamatergic signalling and cytoskeletal organisation.

Pubmed:
33195226: Gubert C, Kong G, Uzungil V, Zeleznikow-Johnston AM, Burrows EL, Renoir T, Hannan AJ
Microbiome Profiling Reveals Gut Dysbiosis in the Metabotropic Glutamate Receptor 5 Knockout Mouse Model of Schizophrenia.
Schizophrenia (SZ) is a psychiatric disorder that constitutes one of the top 10 global causes of disability. More recently, a potential pathogenic role for the gut microbial community (microbiota) has been highlighted, with numerous studies describing dysregulated microbial profiles in SZ patients when compared to healthy controls. However, no animal model of SZ has previously recapitulated the gut dysbiosis observed clinically. Since the metabotropic glutamate receptor 5 (mGlu5) knockout mice provide a preclinical model of SZ with strong face and predictive validity, in the present study we performed gut microbiome profiling of mGlu5 knockout (KO) and wild-type (WT) mice by 16S rRNA sequencing of bacterial genomic DNA from fecal samples, analyzing bacterial diversity and taxonomic composition, as well as gastrointestinal parameters as indicators of gut function. We found a significant genotype difference in microbial beta diversity. Analysis of composition of microbiomes (ANCOM) models were performed to evaluate microbiota compositions, which identified a decreased relative abundance of the family and genus in this mouse model of SZ. We also identified a signature of bacteria discriminating between the genotypes (KO and WT), consisting of the Erysipelotrichales, Bacteroidales, and Clostridiales orders and macroscopic gut differences. We thus uncovered global differential community composition in the gut microbiota profile between mGlu5 KO and WT mice, outlining the first evidence for gut dysbiosis in a genetic animal model of SZ. Our findings suggest that this widely used preclinical model of SZ also has substantial utility for investigations of gut dysbiosis and associated signaling via the microbiota-gut-brain axis, as potential modulators of SZ pathogenesis. Our discovery opens up new avenues to explore gut dysbiosis and its proposed links to brain dysfunction in SZ, as well as novel therapeutic approaches to this devastating disorder.
Front Cell Dev Biol, 2020; 8

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THE COGNITIVE MECHANISMS OF CREDIT ASSIGNMENT, AND ITS RELATIONSHIP TO LOW MOOD

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Cognitive theories of depression have proposed that depression is caused by negative attributional styles. Yet, we still lack an understanding how attributional styles arise and are maintained in the healthy and depressed population. Here, we investigated the underlying cognitive mechanisms of credit assignment, i.e. learning about the causes of events. For this, we developed a novel behavioural paradigm. In this new task, participants play several 'mini games' together with another player. The participants' task was to infer across trials how well they and the other player perform, and how much influence each of them exert over the environment. Using this paradigm, we investigated how people learn about the causes of positive and negative events in a social context. We employed this paradigm in a large online study which allowed us to develop computational models quantifying how people attribute outcomes. We found that people vary in how they attribute positive and negative feedback to themselves, others and their influence over the environment. Furthermore, they actively explored their environment in order to reduce uncertainty. In the future, these results will be important for improving existing as well as proposing novel treatments for depression.

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ASSOCIATION OF CANONICAL NF-κB SIGNALING PATHWAY WITH APOPTOTIC CELL DEATH AND CELL PROLIFERATION IN GLUCOCORTICOID-INDUCED NEUROTOXICITY AND AFTER VITAMIN D3 SUPPLEMENTATION

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Aim: To investigate the canonical pathway of nuclear factor κB (NF-κB) activation in association with the intensity of apoptotic and proliferative processes in different structural areas of the brain in glucocorticoid(GC)-induced neurotoxicity and under vitamin D_3 (VD_3) action. **Methods:** Female Wistar rats received prednisolone (5 mg/kg b.w.) with/without VD_3 (1000 IU/kg b.w., 30 days). NF-κB/p65 and its phosphorylated forms, as well as molecular markers of apoptosis (AIF, Bax, Bcl-2, caspase-8, and p17 subunit of caspase-3) were determined by qRT-PCR and/or Western blotting. The number of apoptotic cells was studied by the TUNEL. Proliferative activity of cells in histological sections of the cerebral cortex and hippocampus was evaluated using double immunofluorescent labeling with 5-bromo-2'-deoxyuridine or Ki67 and marker proteins of neurons, micro- and macroglia (NG2, Iba-1, GFAP respectively). **Results:** We showed an increase in the expression of the NF-κB p65 subunit and an intensification of its specific phosphorylation at Ser311, Ser536, and Thr435. There was a significant decrease in the number of NG2-positive cells in the hippocampus and increasing number of astroglial cells in different brain sections. GC activated apoptotic cell death predominantly by intrinsic (mitochondral) mechanism. Elevated apoptosis was accompanied by inhibition of cell proliferation in different areas of the brain, mainly due to microglia. VD_3 supplementation partially restored most of the GC-induced abnormalities in the brain, but exacerbated the inhibition of cell proliferation. **Conclusions:** VD_3 attenuated GC-induced changes in rat brain suggesting modulation of NF-κB-associated processes in the mechanism of antiapoptic, antiproliferative and neuroprotective actions of VD_3.

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OXIDATIVE BALANCE ALTERATIONS IN THE RAT VENTRAL HIPPOCAMPUS ARE ASSOCIATED TO THE VULNERABILITY AND RESILIENCE TO STRESS-INDUCED ANHEDONIA

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Aims Major Depressive Disorder (MDD) is a severe psychiatric disorder with an aetiology and pathophysiology partially unknown. Although its insurgence is the result of the interaction between vulnerability genes and stressful events, only the so-called "vulnerable" subjects develop the psychopathology while the resilient population overcomes the stressful experience. Accordingly, the identification of the molecular alteration underlying MDD vulnerability and resilience is essential to develop novel and more efficient therapeutic intervention strategies. **Methods** Adult male rats were exposed to 2 weeks of chronic mild stress (CMS) paradigm and the sucrose consumption test was used to divide the animals in stress-vulnerable (anhedonic) and resilient (non anhedonic). 24 hours after the last stress, gene and protein expression of specific mediators of the oxidative balance and related cell survival pathways have been assessed within the ventral hippocampus. **Results** We observed that CMS has a significant impact on oxidative balance in vulnerable animals, which showed a lower activation of both Nrf2 antioxidant pathway and mTOR/p62 pathway in comparison with resilient animals. **Conclusions**
Our data suggest that Nrf2-related antioxidant pathway might represents a novel pharmacological target for stress-related disorders. Hence, favouring its activation could redirect the molecular stress response toward a resilient coping behaviour.

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EFFECTS OF ACUPUNCTURE AT VARIOUS DEPTHS OF NEUROGENIC INFLAMMATORY SPOTS ON IMMOBILIZATION STRESS-INDUCED HYPERTENSION IN RATS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Introduction: We proved that neurogenic inflammatory spots exhibit the characteristics as acupuncture points. In addition, stimulation of neurogenic spots alleviates pathological conditions in rat hypertension model via endogenous opioid systems. It is not clear which depth of neurogenic spots in skin generates therapeutic effects when stimulated. **Methods:** The effects of acupuncture at various needle depths on blood pressure was examined in a rat immobilization-induced hypertension (IMH) model. In vivo extracellular recordings and immunohistochemistry of c-Fos were performed in ventrolateral periaqueductal gray (vlPAG) or rostral ventrolateral medulla (rVLM). **Results**: IMH rats revealed neurogenic spots bilaterally or unilaterally on the wrist, and 67% of those spots matched with acupoints, such as PC6, PC7,HT7. When electrical acupuncture at PC6 in the depths of 1,2,3mm was applied, acupuncture of 3mm-depth most effectively reduced the blood pressure, compared to those of 1 or 2mm depths or control groups. Following electrical stimulation of PC6 areas, c-Fos expression was dominant in 3mm-group. In vivo extracellular recording of RVLM showed that the electrical discharge rates of rVLM following acupuncture stimulation increased up to 150% of baseline 5min after acupuncture at 3mm depth, while that of 1mm group reached about 120% of baseline after stimulation. **Conclusion**: Our data suggest that neurogenic spots generated therapeutic effects at deep skin rather than shallow skin, when stimulation. **Key words**: IMH, rVLM, vlPAG, neurogenic inflammatory spot, acupuncture **Acknowledgement:** This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (Nos. 2018R1A5A2025272, 2019R1A2C1002555 and KMDF_PR_20200901_01179991006790).

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BOARD NUMBER: S02-237

ALTERATIONS OF KYNURENINE PATHWAY IN PERIPHERAL AND CENTRAL NERVOUS SYSTEM UNDER HYPOBARIC HYPOXIC STRESS

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Aim: The present study is an attempt to understand the alterations of kynurenine pathway (KP) under hypobaric hypoxia (HH) and its interrelation with depression-like behaviour. **Methods:** Male Sprague-Dawley rats weighing 230–250 g were exposed to HH (at 25,000 ft.) for 1, 3, and 7 days. Forced Swim Test was used to record the immobility as a parameter of passive behaviour, reflecting depression in rats. The level of KP metabolites in serum, cerebrospinal fluid (CSF), and hippocampus was estimated using high-performance liquid chromatography. **Results:** Tryptophan levels in serum, CSF, and the hippocampus increased after a single day of HH exposure, but decreased over a longer period of HH exposure. Up to 3-day HH, kynurenines were found to be reduced in serum (peripheral system) but increased in CSF and the hippocampus (central system), indicating an influx of kynurenines. However, serotonin levels were significantly reduced both peripherally and centrally. On behavioural assessment, a significant increase in immobility, indicating the depression state was found to be highest on 3day HH exposure. A time-dependent positive correlation between immobility time, kynurenine, and quinolinic acid was observed. On 7-day HH, 3-hydroxyanthranillic acid and quinolinic acid levels were lower in comparison to the control, signifying impairment in the formation of NAD+ which consequently dysfunctions oxidative phosphorylation. **Conclusion:** Under HH stress, alteration in KP's metabolic status was found which has a positive correlation with depression-like behaviour. This study signifies that KP can be used as a therapeutic target to treat HH related psychological and physiological abnormalities.

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BOARD NUMBER: S02-237a

5-HT RAPHE - VENTRAL HIPPOCAMPUS PATHWAY: WHAT ROLE IN AVERSIVE BEHAVIORS?

POSTER SESSION 02 - SECTION: STRESS, ANXIETY AND DEPRESSION

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Serotonin (5-HT) is clearly involved in modulating emotions but the precise mechanisms through which serotonergic neurons are recruited and how they react to aversive stimuli remains to be characterized. In particular, the ventral hippocampus (vHP) is densely innervated by serotonergic fibers and has also been involved in emotional behaviour. Thus, the serotonergic raphe - vHP pathway is ideally positioned to modulate emotional behaviours. This study aims at investigating how the activity of 5-HT neurons changes to adapt to aversive situations. Our objectives are 1- to analyze the correlation between serotonin neural dynamics and anxiety-like behaviors and 2- to investigate the impact of optogenetic activation of vHP-projecting serotonergic neurons on these behaviors. We used viral strategies in SERT-cre mice to enable conditional expression of the calcium sensor GCaMP6s in vHP-projecting 5-HT neurons to perform fiber photometry recordings during exploration of aversive environments. Furthermore, the opsin ChETA was conditionally expressed in SERT-cre mice to photoactivate the 5-HT raphe – vHP pathway during anxiety tests. We found that the activity of serotonergic neurons is modulated during exploration of an aversive environment and that activation of vHP-projecting 5-HT neurons increases anxiety-like behaviors in female but not male mice. The results generated by this project will provide a deeper insight into the neuronal circuits underlying emotional behaviors to adapt to aversive situations.

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BOARD NUMBER: S02-238

GHRELIN INDUCES HEDONIC FEEDING THROUGH THE ACTIVATION OF CENTRAL AMYGDALA HTR2A NEURONS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Ghrelin, known as the hunger hormone, promotes homeostatic feeding influencing the activity of the hypothalamus, and hedonic feeding through extra-hypothalamic areas. The latter behavior may involve the amygdala, a highly conserved brain region important for the expression of defensive and appetitive behaviors. Previous work from our lab showed that the activation of Htr2a+ neurons in central amygdala (CeA) increases food intake through a positive valence signal (Douglass et al, 2017). We hypothesized that part of the rewarding mechanism induced by ghrelin to increase appetite occurs through CeA^{Htr2a} neurons. Our results show that ghrelin increases c-Fos staining and excitatory neurotransmission in CeA^{Htr2a}, but not $CeA^{PKC\delta}$ neurons, a major anorexigenic subpopulation. Chemogenetic inhibition of CeA^{Htr2a} neurons completely blocked the orexigenic effects of systemic application of ghrelin. Direct infusion of ghrelin into the CeA increased food consumption of satiated mice, an effect that was dependent on ghrelin (GHSR) and opioid receptors. In vivo calcium imaging in freely moving animals showed that systemic ghrelin injections increased the activities of CeA^{Htr2a} neurons in a pattern similar to the presence of food. Our findings also indicate that the neurons activated by ghrelin in CeA project to the parabrachial nucleus (PBN), inhibiting PBN neurons and promoting feeding. This conclusion is consistent with our previous observations that at least part of the orexigenic activities of CeA^{Htr2a} neurons are mediated by projections to the PBN. These findings suggest that ghrelin enters the brain and directly activates a cell type-specific appetitive CeA-to-PBN circuit to induce hedonic feeding.

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BOARD NUMBER: S02-239

THE DEVELOPMENT OF MC3R NEURONS, AGRP AND POMC NEURONAL PROJECTIONS AND THE MAINTENANCE OF INTRA-HYPOTHALAMIC NEURONAL CIRCUITS.

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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The melanocortin system has been extensively studied for its role in regulating feeding behavior and the metabolism. However, the role of this system in early development and the establishment of these neuronal circuits are less well known. We sought to study the establishment of key connections from the arcuate nucleus of the hypothalamus (ARC) neurons, namely agouti-related peptide (AgRP) and Pro-opiomelanocortin (POMC), in early development and their persistence into adulthood. In addition to this, we analyzed how the loss of the melanocortin 3 receptor (MC3R), expressed by AgRP neurons, affect these AgRP and POMC neuronal projections specifically within the hypothalamus. qPCR data collected throughout the development suggest a dynamic regulation for the MC3R expression in the hypothalamus. Therefore, using the MC3R-GFP mouse model, we were able to assess the dynamic development of MC3R neurons in the hypothalamus. These results suggest that MC3R is absolutely critical for the development of POMC and AgRP neuronal connections within the hypothalamus. Elaborating on previous literature assessing the structural development of ARC neuronal projections, we identified, throughout development, the positive labelling of the individual of AgRP and POMC neuropeptides themselves to intra but also interesting extra-hypothalamic structures. The dynamic regulation of MC3R expression and establishment of neuronal connectivity in early development underscores the probable role of the melanocortin system not only in adult feeding behavior, but also in early formation of AgRP and POMC neurocircuits. **Keywords**: Hypothalamus, Melanocortin, brain development.

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BOARD NUMBER: S02-240

H3K27 DEMETHYLASE KDM6A/UTX CONTROLS NGN3, POMC AND NPY EXPRESSION IN A SEX-SPECIFIC WAY IN THE DEVELOPING NEUROENDOCRINE HYPOTHALAMUS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Kdm6a is an X-linked H3K27me3/2 demethylase and component of COMPASS/COMPASS-like H3K4 methyltransferase complexes promoting transcription genome-wide critical for tissue/cell-specific differentiation. Previous results showed higher Kdm6a levels in XX than in XY hypothalamic neurons and a female-specific requirement for Kdm6a in mediating increased axogenesis before brain masculinization. Here we explored Kdm6a role in the specification of hypothalamic neuronal subtypes using sex-segregated E14 mouse hypothalamic neuronal cultures transfected with siRNAs to knockdown Kdm6a expression (Kdm6a-KD). First, we evaluated by immunocytochemistry Kdm6a-KD effect on Ngn3, a transcription factor regulating neuronal sub-specification in hypothalamus. Kdm6a-KD downregulated Ngn3 in females but not in males, abolishing basal sex differences previously reported. Then, we analyzed Kdm6a-KD effect on *Ascl1*, *Pomc*, *Npy*, *Sf1*, *Gad1* and *Th* expression by RT-qPCR. While Kdm6a-KD downregulated *Ascl1* in both sexes equally, sex-specific results were obtained for *Pomc*, *Npy* and *Th*. *Pomc* and *Th* expressed higher in female than in male neurons and Kdm6a-KD reduced their levels only in females, while *Npy* expressed higher in male than in female neurons and Kdm6a-KD upregulated its expression only in females. Identical results were found by immunocytochemistry for Pomc and Npy. No effects of sex/treatment on *Sf1* or *Gad1* expression were found. Finally, using ChIP-qPCR we found higher H3K27me3 levels at *Ngn3*, *Pomc* and *Npy* promoters in male than in female neurons, in line with Kdm6a higher expression and demethylase activity in females. These results indicate that Kdm6a plays a key sex-specific role in controlling the differentiation of neuronal populations regulating food intake and energy homeostasis.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-241

NEURAL NETWORKS INVOLVED IN OLFACTION - FOOD INTAKE INTERACTIONS : ROLE OF THE HYPOTHALAMUS IN ODOR PROCESSING

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Body weight of most animals is remarkably stable over time, suggesting that a complex physiological system balances food intake and energy expenditure over the long term. This homeostatic control system is the arcuate nucleus (ARC) of the hypothalamus. By integrating internal (hormones) as well as external (sensory cues) feeding signals, the ARC controls eating behaviour. It contains 2 populations of neurons, Agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) neurons, whose activity is respectively modulated by hunger and satiety hormones such as ghrelin and leptin. Recent studies showed that the activity of AgRP neurons was also modulated by food odors, since the presentation of palatable but hidden food leads to a clear decrease in the activity of these neurons (Betley et al., 2015, Chen et al., 2015). However, the functional connection between AgRP neurons and the Olfactory Bulb (OB) - first cortical relay of the olfactory system- remains unexplored. The aim of this study is to determine whether hypothalamic AgRP neurons modulate odor processing in the OB. In order to investigate this question, AgRP DTR mice were used. In this transgenic mouse model, perinatal ablation of AgRP neurons is achieved by toxin-mediated ablation of AgRP neurons in the first week after birth (Luquet et al., 2005). Control mice and mice lacking AgRP neurons were then compared and subjected to multiple behavioural Tests. Our data show that mice with AgRP neuron’s ablation tend to have different Olfactory behaviour, suggesting that beside regulating food intake, hypothalamic AgRP neurons impact OB-mediated food-odor processing.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-242

CENTRAL OXYTOCIN AMPLIFIES CARDIORESPIRATORY COUPLING

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Heart rate increases during inspiration and decreases during expiration, a phenomenon called respiratory sinus arrhythmia (RSA). We recently showed that brainstem preBötzinger complex (preBötC) neurons, which generate the inspiratory rhythm, also generate RSA by providing an inhibitory modulation of adjacent cardiac parasympathetic neurons of the nucleus ambiguus (nA). A high RSA amplitude is physiologically and psychologically beneficial. The neuromodulator oxytocin (OT) is known to beneficially influence individuals' health and OTergic axons project to brainstem cardiorespiratory nuclei. We hypothesized that OT amplifies RSA by modulating the synaptic connectivity between preBötC and nA neurons. Optogenetic stimulation of OTergic fibers in the preBötC/nA area amplified RSA, in OTcre::Ai27 anesthetized mice. This effect was abolished after OT receptor antagonist injection. To investigate the intrinsic brainstem mechanism involved, we used the *in situ* working heart-brainstem preparation and we recorded inspiratory, cardiac parasympathetic and sympathetic nerve activities, as well as heart rate and blood pressure. After OT agonist microinjection into the preBötC/nA area, the respiratory modulation of cardiac parasympathetic activity and RSA amplitude were amplified, without modifications of respiratory and other cardiovascular parameters. Anatomically, OTergic fibers make synaptic contacts with a subset of preBötC inhibitory neurons that express the oxytocin receptor. These experiments identify a new pathway for RSA amplification: OT modulates the inhibitory connection between preBötC/nA neurons, leading to an amplification of the respiratory modulation of cardiac parasympathetic activity, which amplifies RSA. We will next perform behavioral experiments to identify the physiological conditions in which this mechanism is activated.

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BOARD NUMBER: S02-243

CENTRAL REGULATION OF AUTONOMIC AND CARDIAC FUNCTION

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Much of neuroscience is focused on the role of the brain in generating adaptive behaviors based on external sensory stimuli. However, another crucial role of the brain is to govern the operation of the organs of the body and to optimize their function for current or predicted environmental and internal conditions. While the direct neural regulators of organ function in the autonomic nervous system (vagal and sympathetic) are known, little is known about how the central nervous systems orchestrate their activity. Here we try to understand how the brain of the larval zebrafish integrates sensory information about threatening environmental changes to generate appropriate changes in heart rate. Using pharmacological perturbations, we find that the brain must be modulating cholinergic vagal activity. We then perform brain-wide imaging during threat presentation, where we observe that heart rate correlates with switches in ventral habenular functional states, as well as the activity of a glutamatergic nucleus within the thalamus. Optogenetic stimulation of this nucleus leads to abrupt reductions of cardiac activity, suggesting it is involved in upregulating vagal activity. Together our results push toward the brainwide description of a circuit responsible for transforming sensory input into changes of visceral dynamics.

Pubmed:
34145235: Fei A, Wu W, Tan L, Tang C, Xu Z, Huo X, Bao H, Kong Y, Johnson M, Hartmann G, Talay M, Yang C, Riegler C, Herrera KJ, Engert F, Xie XS, Barnea G, Liberles SD, Yang H, Li Q
Coordination of two enhancers drives expression of olfactory trace amine-associated receptors.
Olfactory sensory neurons (OSNs) are functionally defined by their expression of a unique odorant receptor (OR). Mechanisms underlying singular OR expression are well studied, and involve a massive cross-chromosomal enhancer interaction network. Trace amine-associated receptors (TAARs) form a distinct family of olfactory receptors, and here we find that mechanisms regulating Taar gene choice display many unique features. The epigenetic signature of Taar genes in TAAR OSNs is different from that in OR OSNs. We further identify that two TAAR enhancers conserved across placental mammals are absolutely required for expression of the entire Taar gene repertoire. Deletion of either enhancer dramatically decreases the expression probabilities of different Taar genes, while deletion of both enhancers completely eliminates the TAAR OSN populations. In addition, both of the enhancers are sufficient to drive transgene expression in the partially overlapped TAAR OSNs. We also show that the TAAR enhancers operate in cis to regulate Taar gene expression. Our findings reveal a coordinated control of Taar gene choice in OSNs by two remote enhancers, and provide an excellent model to study molecular mechanisms underlying formation of an olfactory subsystem.
Nat Commun, 2021; 12
34115116: Zhu ML, Herrera KJ, Vogt K, Bahl A
Navigational strategies underlying temporal phototaxis in Drosophila larvae.
Navigating across light gradients is essential for survival for many animals. However, we still have a poor understanding of the algorithms that underlie such behaviors. Here, we developed a novel closed-loop phototaxis assay for Drosophila larvae in which light intensity is always spatially uniform but updates depending on the location of the animal in the arena. Even though larvae can only rely on temporal cues during runs, we find that they are capable of finding preferred areas of low light intensity. Further detailed analysis of their behavior reveals that larvae turn more frequently and that heading angle changes increase when they experience brightness increments over extended periods of time. We suggest that temporal integration of brightness change during runs is an important - and so far largely unexplored - element of phototaxis.
J Exp Biol, 2021; 224
33338431: Herrera KJ, Panier T, Guggiana-Nilo D, Engert F
Larval Zebrafish Use Olfactory Detection of Sodium and Chloride to Avoid Salt Water.
Salinity levels constrain the habitable environment of all aquatic organisms. Zebrafish are freshwater fish that cannot tolerate high-salt environments and would therefore benefit from neural mechanisms that enable the navigation of salt gradients to

avoid high salinity. Yet zebrafish lack epithelial sodium channels, the primary conduit land animals use to taste sodium. This suggests fish may possess novel, undescribed mechanisms for salt detection. In the present study, we show that zebrafish indeed respond to small temporal increases in salt by reorienting more frequently. Further, we use calcium imaging techniques to identify the olfactory system as the primary sense used for salt detection, and we find that a specific subset of olfactory receptor neurons encodes absolute salinity concentrations by detecting monovalent anions and cations. In summary, our study establishes that zebrafish larvae have the ability to navigate and thus detect salinity gradients and that this is achieved through previously undescribed sensory mechanisms for salt detection.
Curr Biol, 2021; 31

31866367: Johnson RE, Linderman S, Panier T, Wee CL, Song E, Herrera KJ, Miller A, Engert F
Probabilistic Models of Larval Zebrafish Behavior Reveal Structure on Many Scales.
Nervous systems have evolved to combine environmental information with internal state to select and generate adaptive behavioral sequences. To better understand these computations and their implementation in neural circuits, natural behavior must be carefully measured and quantified. Here, we collect high spatial resolution video of single zebrafish larvae swimming in a naturalistic environment and develop models of their action selection across exploration and hunting. Zebrafish larvae swim in punctuated bouts separated by longer periods of rest called interbout intervals. We take advantage of this structure by categorizing bouts into discrete types and representing their behavior as labeled sequences of bout types emitted over time. We then construct probabilistic models-specifically, marked renewal processes-to evaluate how bout types and interbout intervals are selected by the fish as a function of its internal hunger state, behavioral history, and the locations and properties of nearby prey. Finally, we evaluate the models by their predictive likelihood and their ability to generate realistic trajectories of virtual fish swimming through simulated environments. Our simulations capture multiple timescales of structure in larval zebrafish behavior and expose many ways in which hunger state influences their action selection to promote food seeking during hunger and safety during satiety.
Curr Biol, 2020; 30

30929901: Thyme SB, Pieper LM, Li EH, Pandey S, Wang Y, Morris NS, Sha C, Choi JW, Herrera KJ, Soucy ER, Zimmerman S, Randlett O, Greenwood J, McCarroll SA, Schier AF
Phenotypic Landscape of Schizophrenia-Associated Genes Defines Candidates and Their Shared Functions.
Genomic studies have identified hundreds of candidate genes near loci associated with risk for schizophrenia. To define candidates and their functions, we mutated zebrafish orthologs of 132 human schizophrenia-associated genes. We created a phenotype atlas consisting of whole-brain activity maps, brain structural differences, and profiles of behavioral abnormalities. Phenotypes were diverse but specific, including altered forebrain development and decreased prepulse inhibition. Exploration of these datasets identified promising candidates in more than 10 gene-rich regions, including the magnesium transporter cnnm2 and the translational repressor gigyf2, and revealed shared anatomical sites of activity differences, including the pallium, hypothalamus, and tectum. Single-cell RNA sequencing uncovered an essential role for the understudied transcription factor znf536 in the development of forebrain neurons implicated in social behavior and stress. This phenotypic landscape of schizophrenia-associated genes prioritizes more than 30 candidates for further study and provides hypotheses to bridge the divide between genetic association and biological mechanism.
Cell, 2019; 177

30402545: Jordi J, Guggiana-Nilo D, Bolton AD, Prabha S, Ballotti K, Herrera K, Rennekamp AJ, Peterson RT, Lutz TA, Engert F
High-throughput screening for selective appetite modulators: A multibehavioral and translational drug discovery strategy.
How appetite is modulated by physiological, contextual, or pharmacological influence is still unclear. Specifically, the discovery of appetite modulators is compromised by the abundance of side effects that usually limit in vivo drug action. We set out to identify neuroactive drugs that trigger only their intended single behavioral change, which would provide great therapeutic advantages. To identify these ideal bioactive small molecules, we quantified the impact of more than 10,000 compounds on an extended series of different larval zebrafish behaviors using an in vivo imaging strategy. Known appetite-modulating drugs altered feeding and a pleiotropy of behaviors. Using this multibehavioral strategy as an active filter for behavioral side effects, we identified previously unidentified compounds that selectively increased or reduced food intake by more than 50%. The general applicability of this strategy is shown by validation in mice. Mechanistically, most candidate compounds were independent of the main neurotransmitter systems. In addition, we identified compounds with multibehavioral impact, and correlational comparison of these profiles with those of known drugs allowed for the prediction of their mechanism of action. Our results illustrate an unbiased and translational drug discovery strategy for ideal psychoactive compounds and identified selective appetite modulators in two vertebrate species.
Sci Adv, 2018; 4

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BOARD NUMBER: S02-244

ARGININE VASOPRESSIN INCREASES THE EXCITATORY SYNAPTIC DRIVE AND FIRING ACTIVITY OF DEVELOPING SEROTONERGIC NEURONS IN NEONATAL DORSAL RAPHE NUCLEUS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Birth stress, a risk factor for psychiatric disorders, triggers a surge of the stress hormone arginine vasopressin (AVP). The effect of AVP on developing neuronal networks such as the dorsal raphe nucleus (DRN) serotonin system are largely unknown. **Aims:** To investigate the effect of AVP on the synaptic drive and firing activity of neonatal serotonergic neurons *in vitro* and *in vivo* and to reveal the origin of the vasopressinergic innervation of neonatal DRN. **Methods:** Electrophysiological recordings were performed *in vitro* in acute brain slices containing DRN from P10-12 rat pups with the whole-cell patch clamp method and *in vivo* in urethane anaesthetised rat pups by juxtacellular recording and labelling. The origin of the vasopressinergic innervation in neonatal DRN was determined by retrograde tracer injections (Fluorogold) combined with immunohistochemistry against AVP. **Results:** We found that 10 nM AVP significantly increased the frequency and amplitude of sEPSCs in serotonergic neurons while the effect on sIPSCs was less pronounced. AVP also strongly augmented their action potential firing. Neonatal serotonergic neurons display a wide range of firing patterns *in vivo* but are characterised by a broad action potential waveform. They also seem to respond to AVP *in vivo*. The vasopressinergic innervation of neonatal DRN emerges nearly exclusively from sparse cell groups in medial amygdala and BNST. **Conclusions:** AVP is a powerful modulator of neonatal serotonergic activity in DRN. Abnormal AVP release by birth stress may alter the functional development of the DRN serotonin system and contribute to increased susceptibility to psychiatric disorders later on.

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BOARD NUMBER: S02-245

BASOLATERAL AMYGDALA CIRCUITRY DURING STRESS EXPOSURE

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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AIMS. Anxiety disorders are increasingly prevalent, highly disabling, and often resistant to treatment. To develop more targeted, effective treatments, we must better understand how the brain identifies environmental stimuli as stressful. Importantly, which specific brain circuits and cell types respond to threat? Here, we investigate temporal, spatial, and circuit-specific activation patterns within the basolateral amygdala, a key brain region involved in emotional processing. **METHODS.** In adult rats, fiber photometry recorded temporal patterns of activation within the BLA to novel stimuli: swim, restraint, footshock, bobcat odor, citral odor, and crackers. In a different subset of rats, brains were collected to plot the spatial pattern of activation via labelling of FOS+ neurons following exposure to each stimulus. Using restraint as a model for stress, we then used retrograde tracing (CTb) and immunohistochemistry (cFos) to map topographical distribution and circuit-specific activation of BLA projection populations targeting six downstream brain regions. Finally, we employed chemogenetics or optogenetics, respectively, to inhibit or stimulate BLA projection neurons, and blood was subsequently collected to measure plasma levels of corticosterone. **RESULTS.** BLA projection neurons exhibited unique spatial and temporal patterns of activation to different novel stimuli. Exposure to restraint stress increased FOS expression in discrete BLA projection populations within the anterior basal amygdala. Modulation of this cluster of BLA projection neurons bidirectionally influenced plasma corticosterone levels. **CONCLUSIONS.** Aversive stimuli elicit unique spatiotemporal patterns of activation in the BLA, and activation of the BLA is both necessary and sufficient to generate an endocrine response to aversive stimuli.

Pubmed:
34882838: Baglot SL, VanRyzin JW, Marquardt AE, Aukema RJ, Petrie GN, Hume C, Reinl EL, Bieber JB, McLaughlin RJ, McCarthy MM, Hill MN
Maternal-fetal transmission of delta-9-tetrahydrocannabinol (THC) and its metabolites following inhalation and injection exposure during pregnancy in rats.
Cannabis use during pregnancy has increased over the past few decades, with recent data indicating that, in youth and young adults especially, up to 22% of people report using cannabis during pregnancy. Animal models provide the ability to study prenatal cannabis exposure (PCE) with control over timing and dosage; however, these studies utilize both injection and inhalation approaches. While it is known that Δ9-tetrahydrocannabinol (THC; primary psychoactive component of cannabis) can cross the placenta, examination of the transmission and concentration of THC and its metabolites from maternal blood into the placenta and fetal brain remains relatively unknown, and the influence of route of administration has never been examined. Pregnant female rats were exposed to either vaporized THC-dominant cannabis extract for pulmonary consumption or subcutaneous injection of THC repeatedly during the gestational period. Maternal blood, placenta, and fetal brains were collected following the final administration of THC for analysis of THC and its metabolites, as well as endocannabinoid concentrations, through mass spectrometry. Both routes of administration resulted in the transmission of THC and its metabolites in placenta and fetal brain. Repeated exposure to inhaled THC vapor resulted in fetal brain THC concentrations that were about 30% of those seen in maternal blood, whereas repeated injections resulted in roughly equivalent concentrations of THC in maternal blood and fetal brain. Neither inhalation nor injection of THC during pregnancy altered fetal brain endocannabinoid concentrations. Our data provide the first characterization of maternal-fetal transmission of THC and its metabolites following both vaporized delivery and injection routes of administration. These data are important to establish the maternal-fetal transmission in preclinical injection and inhalation models of PCE and may provide insight into predicting fetal exposure in human studies.
J Neurosci Res, 2022; 100
34916909: Vecchiarelli HA, Aukema RJ, Hume C, Chiang V, Morena M, Keenan CM, Nastase AS, Lee FS, Pittman QJ,

Sharkey KA, Hill MN
Genetic Variants of Fatty Acid Amide Hydrolase Modulate Acute Inflammatory Responses to Colitis in Adult Male Mice.
Cannabinoids, including derived phytocannabinoids and endogenous cannabinoids (endocannabinoids), are typically considered anti-inflammatory. One such endocannabinoid is -arachidonoylethanolamine (anandamide, AEA), which is metabolized by fatty acid amide hydrolase (FAAH). In humans, there is a loss of function single nucleotide polymorphism (SNP) in the FAAH gene (C385A, rs324420), that leads to increases in the levels of AEA. Using a mouse model with this SNP, we investigated how this SNP affects inflammation in a model of inflammatory bowel disease. We administered 2,4,6-trinitrobenzene sulfonic acid (TNBS) intracolonically, to adult male FAAH SNP mice and examined colonic macroscopic tissue damage and myeloperoxidase activity, as well as levels of plasma and amygdalar cytokines and chemokines 3 days after administration, at the peak of colitis. We found that mice possessing the loss of function alleles (AC and AA), displayed no differences in colonic damage or myeloperoxidase activity compared to mice with wild type alleles (CC). In contrast, in plasma, colitis-induced increases in interleukin (IL)-2, leukemia inhibitory factor (LIF), monocyte chemoattractant protein (MCP)-1, and tumor necrosis factor (TNF) were reduced in animals with an A allele. A similar pattern was observed in the amygdala for granulocyte colony stimulating factor (G-CSF) and MCP-1. In the amygdala, the mutant A allele led to lower levels of IL-1α, IL-9, macrophage inflammatory protein (MIP)-1β, and MIP-2 independent of colitis-providing additional understanding of how FAAH may serve as a regulator of inflammatory responses in the brain. Together, these data provide insights into how FAAH regulates inflammatory processes in disease.
Front Cell Neurosci, 2021; 15
34907248: Baglot SL, Hume C, Petrie GN, Aukema RJ, Lightfoot SHM, Grace LM, Zhou R, Parker L, Rho JM, Borgland SL, McLaughlin RJ, Brechenmacher L, Hill MN
Pharmacokinetics and central accumulation of delta-9-tetrahydrocannabinol (THC) and its bioactive metabolites are influenced by route of administration and sex in rats.
Up to a third of North Americans report using cannabis in the prior month, most commonly through inhalation. Animal models that reflect human consumption are critical to study the impact of cannabis on brain and behaviour. Most animal studies to date utilize injection of delta-9-tetrahydrocannabinol (THC; primary psychoactive component of cannabis). THC injections produce markedly different physiological and behavioural effects than inhalation, likely due to distinctive pharmacokinetics. The current study directly examined if administration route (injection versus inhalation) alters metabolism and central accumulation of THC and metabolites over time. Adult male and female Sprague-Dawley rats received either an intraperitoneal injection or a 15-min session of inhaled exposure to THC. Blood and brains were collected at 15, 30, 60, 90 and 240-min post-exposure for analysis of THC and metabolites. Despite achieving comparable peak blood THC concentrations in both groups, our results indicate higher initial brain THC concentration following inhalation, whereas injection resulted in dramatically higher 11-OH-THC concentration, a potent THC metabolite, in blood and brain that increased over time. Our results provide evidence of different pharmacokinetic profiles following inhalation versus injection. Accordingly, administration route should be considered during data interpretation, and translational animal work should strongly consider using inhalation models.
Sci Rep, 2021; 11
34116110: Petrie GN, Nastase AS, Aukema RJ, Hill MN
Endocannabinoids, cannabinoids and the regulation of anxiety.
Cannabis has been used for hundreds of years, with its ability to dampen feelings of anxiety often reported as a primary reason for use. Only recently has the specific role cannabinoids play in anxiety been thoroughly investigated. Here we discuss the body of evidence describing how endocannabinoids and exogenous cannabinoids are capable of regulating the generation and termination of anxiety states. Disruption of the endogenous cannabinoid (eCB) system following genetic manipulation, pharmacological intervention or stress exposure reliably leads to the generation of an anxiety state. On the other hand, upregulation of eCB signaling is capable of alleviating anxiety-like behaviors in multiple paradigms. When considering exogenous cannabinoid administration, cannabinoid receptor 1 (CB1) agonists have a biphasic, dose-dependent effect on anxiety such that low doses are anxiolytic while high doses are anxiogenic, a phenomenon that is evident in both rodent models and humans. Translational studies investigating a loss of function mutation in the gene for fatty acid amide hydrolase, the enzyme responsible for metabolizing AEA, have also shown that AEA signaling regulates anxiety in humans. Taken together, evidence reviewed here has outlined a convincing argument for cannabinoids being powerful regulators of both the manifestation and amelioration of anxiety symptoms, and highlights the therapeutic potential of targeting the eCB system for the development of novel classes of anxiolytics. This article is part of the special issue on 'Cannabinoids'.
Neuropharmacology, 2021; 195
30573646: Morena M, Aukema RJ, Leitl KD, Rashid AJ, Vecchiarelli HA, Josselyn SA, Hill MN
Upregulation of Anandamide Hydrolysis in the Basolateral Complex of Amygdala Reduces Fear Memory Expression and Indices of Stress and Anxiety.

Increased anandamide (AEA) signaling through inhibition of its catabolic enzyme fatty acid amide hydrolase (FAAH) in the basolateral complex of amygdala (BLA) is thought to buffer against the effects of stress and reduces behavioral signs of anxiety and fear. However, examining the role of AEA signaling in stress, anxiety, and fear through pharmacological depletion has been challenging due to the redundant complexity of its biosynthesis and the lack of a pharmacological synthesis inhibitor. We developed a herpes simplex viral vector to rapidly yet transiently overexpress FAAH specifically within the BLA to assess the impact of suppressing AEA signaling on stress, fear, and anxiety in male rats. Surprisingly, FAAH overexpression in BLA dampened stress-induced corticosterone release, reduced anxiety-like behaviors, and decreased conditioned fear expression. Interestingly, depleting AEA signaling in the BLA did not prevent fear conditioning itself or fear reinstatement. These effects were specific to the overexpression of FAAH because they were reversed by intra-BLA administration of an FAAH inhibitor. Moreover, the fear-suppressive effects of FAAH overexpression were also mitigated by intra-BLA administration of a low dose of a GABA receptor antagonist, but not an NMDA/AMPA/kainate receptor antagonist, suggesting that they were mediated by an increase in GABAergic neurotransmission. Our data suggest that a permissive AEA tone within the BLA might gate GABA release and that loss of this tone through elevated AEA hydrolysis increases inhibition in the BLA, which in turn reduces stress, anxiety, and fear. These data provide new insights on the mechanisms by which amygdalar endocannabinoid signaling regulates emotional behavior. Amygdala endocannabinoid signaling is involved in the regulation of stress, anxiety, and fear. Our data indicate that viral-mediated augmentation of anandamide hydrolysis within the basolateral amygdala reduces behavioral indices of stress, anxiety, and conditioned fear expression. These same effects have been previously documented with inhibition of anandamide hydrolysis in the same brain region. Our results indicate that the ability of anandamide signaling to regulate emotional behavior is nonlinear and may involve actions at distinct neuronal populations, which could be influenced by the basal level of anandamide. Modulation of anandamide signaling is a current clinical therapeutic target for stress-related psychiatric illnesses, so these data underscore the importance of fully understanding the mechanisms by which anandamide signaling regulates amygdala-dependent changes in emotionality.
J Neurosci, 2019; 39
30120421: Mayo LM, Asratian A, Lindé J, Holm L, Nätt D, Augier G, Stensson N, Vecchiarelli HA, Balsevich G, Aukema RJ, Ghafouri B, Spagnolo PA, Lee FS, Hill MN, Heilig M
Protective effects of elevated anandamide on stress and fear-related behaviors: translational evidence from humans and mice.
Post-traumatic stress disorder (PTSD) is a common, debilitating condition with limited treatment options. Extinction of fear memories through prolonged exposure therapy, the primary evidence-based behavioral treatment for PTSD, has only partial efficacy. In mice, pharmacological inhibition of fatty acid amide hydrolase (FAAH) produces elevated levels of anandamide (AEA) and promotes fear extinction, suggesting that FAAH inhibitors may aid fear extinction-based treatments. A human FAAH 385C->A substitution encodes an FAAH enzyme with reduced catabolic efficacy. Individuals homozygous for the FAAH 385A allele may therefore offer a genetic model to evaluate the impact of elevations in AEA signaling in humans, helping to inform whether FAAH inhibitors have the potential to facilitate fear extinction therapy for PTSD. To overcome the challenge posed by low frequency of the AA genotype (appr. 5%), we prospectively genotyped 423 individuals to examine the balanced groups of CC, AC, and AA individuals (n = 25/group). Consistent with its loss-of-function nature, the A allele was dose dependently associated with elevated basal AEA levels, facilitated fear extinction, and enhanced the extinction recall. Moreover, the A-allele homozygotes were protected against stress-induced decreases in AEA and negative emotional consequences of stress. In a humanized mouse model, AA homozygous mice were similarly protected against stress-induced decreases in AEA, both in the periphery, and also in the amygdala and prefrontal cortex, brain structures critically involved in fear extinction and regulation of stress responses. Collectively, these data suggest that AEA signaling can temper aspects of the stress response and that FAAH inhibition may aid the treatment for stress-related psychiatric disorders, such as PTSD.
Mol Psychiatry, 2020; 25
30257799: Guo Z, Tse YC, Zhang Y, Sun Q, Vecchiarelli HA, Aukema R, Hill MN, Wong TP, Boksa P
Prenatal immune activation potentiates endocannabinoid-related plasticity of inhibitory synapses in the hippocampus of adolescent rat offspring.
There is strong evidence that immune activation from prenatal infection increases the risk for offspring to develop schizophrenia. The endocannabinoid (eCB) system has been implicated in the pathophysiology of schizophrenia while models of cortical dysfunction postulate an imbalance between neuronal excitation and inhibition in the disorder. The current study examined the impact of prenatal immune activation on eCB-mediated inhibitory mechanisms. We compared two forms of eCB-related plasticity of evoked inhibitory postsynaptic currents, namely depolarization-induced suppression of inhibition (DSI) and metabotropic glutamate receptor-induced long term depression (mGluR-iLTD), in both the dorsal and ventral hippocampus between adolescent offspring from rat dams that received either saline or bacterial lipopolysaccharide (LPS) during pregnancy. Compared to prenatal saline offspring, prenatal LPS offspring displayed prolonged DSI and stronger

mGluR-iLTD in the dorsal and ventral hippocampus, respectively. The sensitivity of mGluR-iLTD to the CB1 receptor antagonist AM251 was also lower in the dorsal hippocampus of prenatal LPS compared to prenatal saline offspring. Testing whether changes in eCB receptor signaling or levels could contribute to these changes in inhibitory transmission, we found region specific increases in 2-arachidonoylglycerol-stimulated signaling and in basal and mGluR-induced levels of anandamide in prenatal LPS offspring when compared to prenatal saline offspring. Our findings indicate that prenatal immune activation can lead to long-term changes in eCB-related plasticity of hippocampal inhibitory synaptic transmission in adolescent rat offspring. Perturbation of the eCB system resulting from prenatal immune activation could represent a mechanism linking early life immune events to the development of psychopathology in adolescence.
Eur Neuropsychopharmacol, 2018; 28

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BOARD NUMBER: S02-246

PHASIC AND TONIC LOCUS COERULEUS STIMULATIONS LEAD TO OPPOSITE VALENCE LEARNING VIA DISTINCT ADRENOCEPTORS IN THE BASOLATERAL AMYGDALA

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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AIMS: The locus coeruleus (LC) signals opposing valences via two different firing modes: tonic and phasic. LC activation engages the basolateral amygdala (BLA) for the formation of opposite valences. Light-induced LC phasic and tonic firing patterns preferentially recruited BLA neurons projecting to the nucleus accumbens (NAc) reward circuitry and to the central nucleus of the amygdala (CeA) aversive circuitry, respectively. Here, we ask if different adrenoceptors in the BLA are involved in opposite valences generated by either LC light patterns, or natural conditioning. **METHODS**: α1-adrenoceptor antagonist prozasin or β1/2-adrenoceptor antagonist propranolol was infused bilaterally in the BLA 30 min before behavioral training. A conditioned odor preference test was carried out in TH-CRE rats expressing ChR2 in the LC. LC light stimulation (10 Hz phasic or 25 Hz tonic) was paired with a conditioned odor. Odor reward learning using food pellet pairing and odor aversive learning using foot shock pairing were carried out in separate cohorts. Retrograde CTB tracers were infused in the CeA and NAc to map fear- and reward circuitry. Adrenoceptor expressions in the BLA were measured by immunohistochemistry. **RESULTS**: Reward conditioning, by either LC phasic stimulation or food reward training, activated more NAc-projecting cells in the BLA and was impaired by either α1- or β1/2-adrenoceptor blockade. Aversive learning, by either LC high tonic stimulation, or shock training, was dependent on β1/2-, but not α1-adrenoceptor activation. **CONCLUSIONS**: Positive and negative valence learning may induce different patterns of NE release and engage diverge populations of the neurons in the BLA.

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BOARD NUMBER: S02-247

THE ROLE OF MICRORNA-34A ON DORSAL RAPHE NUCLEI NEUROTRANSMISSION IN RESPONSE TO SPECIFIC VALENCE STIMULI

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

Serafina Manila Guzzo[1], Donald Ielpo[2], Giovanni Porcheddu[3], Luisa Lo Iacono[2], Cristina Marchetti[3], Rossella Ventura[2,4], Carlo Cifani[1], Diego Andolina[2,4]
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To survive, an individual must be able to discriminate aversive and appetitive stimuli within the environment and elicit adaptation behavioral responses.
Serotoninergic (5-HT) neurons in the Dorsal Raphe Nucleus (DRN) play critical roles in behaviors motivated by environmental stimuli valence. Within the DRN, 5-HT neurons are modulated by GABAergic activity. However, it is still unclear how GABAergic neurons are modulated to process environmental information.
MicroRNAs are important molecules for the regulation of transcriptional changes induced by environmental challenges. Our previous studies have shown that MicroRNA-34 (MiR-34) is highly expressed in the DRN and that its blockade alters 5-HT release while improving the stress-induced phenotype.
Here, we tested the hypothesis that MiR-34 regulates the 5-HT response via GABAergic modulation after negative stimuli. First, by using RNA scope in situ hybridization, we demonstrate that MiR-34 is predominantly expressed on DRN GABAergic neurons. We then use microdialysis and electrophysiology on a mouse model that has MiR-34 deletion on GABAergic neurons (GAD2 MiR-34loxP/loxP) and we showed that, only after negative stimuli such as restraint (and not positive such as white chocolate), MiR-34 regulates GABAergic release on 5-HT neurons. In particular, GAD2 MiR-34loxP/loxP mice have altered behavioral responses to negative stimuli (forced swimming test) compared to control groups (miR-34loxP/loxP and Gad2-CRE mice). Additionally, these mice models did not show any behavioral changes after positive stimuli (sucrose preference test) compared to control groups.
Overall, our results demonstrated a new regulatory mechanism in which MiR-34 modulates GABAergic neurotransmission on DRN 5-HT neurons in response to negative stimuli valence.

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BOARD NUMBER: S02-248

MALE MICE ENGAGING DIFFERENTLY IN EMOTIONAL EATING PRESENT DISTINCT PLASMATIC AND NEUROLOGICAL PROFILES

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Objective: When exposed to stressful events, individuals tend to turn to “comfort food” which provides a temporary relief from stress. The emotional eating drive is highly variable among subjects. We explored the plasmatic and neurobiological differences between “high and low emotional eaters” (HEE and LEE) in male mice. Methods: Male mice were daily exposed for 5 weeks to an unpredictable chronic mild stress paradigm. More, every 3 or 4 days, they were submitted to a 1-hr restraint stress, immediately followed by a 3-hr period during which a choice between chow and chocolate sweet cereals was proposed. Plasmatic and neurobiological characteristics were analyzed and compared in mice displaying high vs low intakes. Results: LEE displayed higher plasma corticosterone and lower levels of the neuropeptide Y (NPY) than HEE, but acylated and total ghrelin were similar in both groups. In the brain, the abundance of NPY neurons in the arcuate nucleus of the hypothalamus was similar in both groups, but was higher in the ventral hippocampus and the basal lateral amygdala of LEE. Surprisingly in the lateral hypothalamus LEE had also more orexin positive neurons but no more melanin concentrating hormone neurons. Both NPY and orexin are orexigenic peptides, and mood regulators. Discussion: The difference in the responses to post-stress food was reflected in plasma and brain structures implicated in emotion and eating regulation. These results concur with the psychological side of food consumption. A subsequent question is whether the abundance of orexin and NPY neurons was stress-acquired or a trait.

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COMPLEMENTARY LATERAL HYPOTHALAMIC POPULATIONS RESIST HUNGER PRESSURE TO BALANCE NUTRITIONAL AND SOCIAL NEEDS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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[1]Max Planck Institute, Metabolism Research, Cologne, Germany, [2]University of Cologne, Institute For Vegetative Physiology, Cologne, Germany

Diets often fail due to the inability to resist even moderate hunger pressure. However, neuronal mechanisms of sensing and resisting metabolic pressure such as hunger remain poorly understood. The lateral hypothalamus (LH) regulates feeding and drinking, in part through leptin receptor-expressing ($LepR^{LH}$) and neurotensin-expressing (Nts^{LH}) neurons. In this study, we show how these neural populations enable resistance to metabolic deprivation to enable behavioural flexibility. Using single cell Ca^{2+} imaging in freely moving animals, we found that $LepR^{LH}$ neurons encode food stimuli, whereby the magnitude of food-elicited responses reflected food intake under moderate, but not under strong hunger pressure. Failure to resist moderate hunger pressure was encoded by escalating inhibition of a leptin-sensitive $LepR^{LH}$ subpopulation at a fast time scale. Similarly, $LepR^{LH}$ neurons of thirsty animals encode water stimuli. Optogenetic activation of $LepR^{LH}$ neurons suppressed food or water intake in moderately hungry or thirsty animals, and promoted the exploration of social stimuli. Conversely, hunger pressure intensified water encoding of Nts^{LH} neurons, whereby the magnitude of water-elicited responses reflected food intake. Detailed behavioural analysis reveals that Nts^{LH} neurons track food intake to scale water intake accordingly, ensuring the balance between feeding and drinking, even discounting social interaction in favour of water. In summary, hunger pressure gates $LepR^{LH}$ and Nts^{LH} populations in a complementary manner to enable the flexible fulfilment of competing essential needs. We gratefully acknowledge support by the ERC Consolidator Grant (772994, FeedHypNet, to T.K.) and DFG (Project-ID 431549029 – SFB 1451, to T.K., EXC2030 CECAD, to T.K., 233886668-GRK1960, to R.F.).

Pubmed:

26582977: Petzold A, Valencia M, Pál B, Mena-Segovia J
Decoding brain state transitions in the pedunculopontine nucleus: cooperative phasic and tonic mechanisms.
Cholinergic neurons of the pedunculopontine nucleus (PPN) are most active during the waking state. Their activation is deemed to cause a switch in the global brain activity from sleep to wakefulness, while their sustained discharge may contribute to upholding the waking state and enhancing arousal. Similarly, non-cholinergic PPN neurons are responsive to brain state transitions and their activation may influence some of the same targets of cholinergic neurons, suggesting that they operate in coordination. Yet, it is not clear how the discharge of distinct classes of PPN neurons organize during brain states. Here, we monitored the in vivo network activity of PPN neurons in the anesthetized rat across two distinct levels of cortical dynamics and their transitions. We identified a highly structured configuration in PPN network activity during slow-wave activity that was replaced by decorrelated activity during the activated state (AS). During the transition, neurons were predominantly excited (phasically or tonically), but some were inhibited. Identified cholinergic neurons displayed phasic and short latency responses to sensory stimulation, whereas the majority of non-cholinergic showed tonic responses and remained at high discharge rates beyond the state transition. In vitro recordings demonstrate that cholinergic neurons exhibit fast adaptation that prevents them from discharging at high rates over prolonged time periods. Our data shows that PPN neurons have distinct but complementary roles during brain state transitions, where cholinergic neurons provide a fast and transient response to sensory events that drive state transitions, whereas non-cholinergic neurons maintain an elevated firing rate during global activation.
Front Neural Circuits, 2015; 9
25724412: Petzold A, Psotta L, Brigadski T, Endres T, Lessmann V
Chronic BDNF deficiency leads to an age-dependent impairment in spatial learning.
Brain-derived neurotrophic factor (BDNF) is a crucial mediator of neural plasticity and, consequently, of memory formation. In hippocampus-dependent learning tasks BDNF also seems to play an essential role. However, there are conflicting results concerning the spatial learning ability of aging BDNF(+/-) mice in the Morris water maze paradigm. To evaluate the effect of chronic BDNF deficiency in the hippocampus on spatial learning throughout life, we conducted a comprehensive study to test

differently aged BDNF(+/-) mice and their wild type littermates in the Morris water maze and to subsequently quantify their hippocampal BDNF protein levels as well as expression levels of TrkB receptors. We observed an age-dependent learning deficit in BDNF(+/-) animals, starting at seven months of age, despite stable hippocampal BDNF protein expression and continual decline of TrkB receptor expression throughout aging. Furthermore, we detected a positive correlation between hippocampal BDNF protein levels and learning performance during the probe trial in animals that showed a good learning performance during the long-term memory test.
Neurobiol Learn Mem, 2015; 120

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BOARD NUMBER: S02-250

TUBERAL NUCLEUS SOMATOSTATIN NEURONS IN THE REGULATION OF HIGH-FAT EATING RELATED BEHAVIORS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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[1]Agency for Science, Technology and Research, Institute Of Molecular Cell And Biology, Singapore, Singapore, [2]National University of Singapore, Physiology, Yong Loo Lin School Of Medicine, Singapore, Singapore, [3]Nanyang Technological University, Lee Kong Chian School Of Medicine, Singapore, Singapore

Even though high-fat food is preferentially chosen over regular food, the neuronal regulation of high-fat intake is not well identified. Diverse hypothalamic neuronal populations, including arcuate agouti-related protein expressing neurons (^{ARC}AgRP), tuberal nucleus somatostatin neurons (^{TN}SST), GABAergic neurons in the lateral hypothalamus (^{LH}VGAT) have been identified over the decade in the feeding regulation of mice; however, how they differentially participate in high-fat intake regulation is still unclear. By staining neuronal activity marker, cFos, we observed a specific higher cFos positive neurons after consuming high-fat in ^{TN}SST neurons but not in ^{ARC}AgRP or ^{LH}VGAT. Subsequently, to investigate the effect of post-ingestive mechanisms on ^{TN}SST activity, we infused different caloric solutions to the mice by gavaging. We found no significant difference among different caloric solutions; thus, the ^{TN}SST response to different caloric solutions mainly not through the post-ingestive mechanisms. Furthermore, in vivo imaging of ^{TN}SST neurons revealed that majority of them demonstrated preferential response to high-fat even before consuming it. However, the preferential response of ^{TN}SST neurons did not dictate choice of high-fat in hungry mice, since chemogenetic activation of the either there three population led to preferential consumption in sated mice and inhibition of ^{TN}SST or ^{ARC}AgRP or ^{LH}VGAT neurons resulted in less consumption of high-fat. Interestingly, only inhibiting ^{TN}SST neurons, but not other two neuronal populations, abolished the conditioned place preference induced by high-fat, suggesting that reinforcing effect of high-fat specifically requires ^{TN}SST neurons. Therefore, we revealed an unique high-fat-specific response and role of ^{TN}SST neurons.

Pubmed:
34985643: Xiao W, Jiao ZL, Senol E, Yao J, Zhao M, Zhao ZD, Chen X, Cao P, Fu Y, Gao Z, Shen WL, Xu XH
Neural circuit control of innate behaviors.
All animals possess a plethora of innate behaviors that do not require extensive learning and are fundamental for their survival and propagation. With the advent of newly-developed techniques such as viral tracing and optogenetic and chemogenetic tools, recent studies are gradually unraveling neural circuits underlying different innate behaviors. Here, we summarize current development in our understanding of the neural circuits controlling predation, feeding, male-typical mating, and urination, highlighting the role of genetically defined neurons and their connections in sensory triggering, sensory to motor/motivation transformation, motor/motivation encoding during these different behaviors. Along the way, we discuss possible mechanisms underlying binge-eating disorder and the pro-social effects of the neuropeptide oxytocin, elucidating the clinical relevance of studying neural circuits underlying essential innate functions. Finally, we discuss some exciting brain structures recurrently appearing in the regulation of different behaviors, which suggests both divergence and convergence in the neural encoding of specific innate behaviors. Going forward, we emphasize the importance of multi-angle and cross-species dissections in delineating neural circuits that control innate behaviors.
Sci China Life Sci, 2022; 65
34168339: Mohammad H, Senol E, Graf M, Lee CY, Li Q, Liu Q, Yeo XY, Wang M, Laskaratos A, Xu F, Luo SX, Jung S, Augustine GJ, Fu Y
A neural circuit for excessive feeding driven by environmental context in mice.
Despite notable genetic influences, obesity mainly results from the overconsumption of food, which arises from the interplay of physiological, cognitive and environmental factors. In patients with obesity, eating is determined more by external cues than by internal physiological needs. However, how environmental context drives non-homeostatic feeding is elusive. Here, we identify a population of somatostatin (SST) neurons in the mouse hypothalamic tuberal nucleus that are preferentially activated by palatable food. Activation of SST neurons enabled a context to drive non-homeostatic feeding in sated mice and required inputs from the subiculum. Pairing a context with palatable food greatly potentiated synaptic transmission between

the subiculum and SST neurons and drove non-homeostatic feeding that could be selectively suppressed by inhibiting SST neurons or the subiculum but not other major orexigenic neurons. These results reveal how palatable food, through a specific hypothalamic circuit, empowers environmental context to drive non-homeostatic feeding.
Nat Neurosci, 2021; 24
33962958: Phua SC, Tan YL, Kok AMY, Senol E, Chiam CJH, Lee CY, Peng Y, Lim ATJ, Mohammad H, Lim JX, Fu Y
A distinct parabrachial-to-lateral hypothalamus circuit for motivational suppression of feeding by nociception.
The motivation to eat is not only shaped by nutrition but also competed by external stimuli including pain. How the mouse hypothalamus, the feeding regulation center, integrates nociceptive inputs to modulate feeding is unclear. Within the key nociception relay center parabrachial nucleus (PBN), we demonstrated that neurons projecting to the lateral hypothalamus (PBN) are nociceptive yet distinct from danger-encoding central amygdala-projecting (PBN) neurons. Activation of PBN strongly suppressed feeding by limiting eating frequency and also reduced motivation to work for food reward. Refined approach-avoidance paradigm revealed that suppression of PBN, but not PBN, sustained motivation to obtain food. The effect of PBN neurons on feeding was reversed by suppressing downstream LH neurons. Thus, distinct from a circuit for fear and escape responses, PBN neurons channel nociceptive signals to LH neurons to suppress motivational drive for feeding. Our study provides a new perspective in understanding feeding regulation by external competing stimuli.
Sci Adv, 2021; 7

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ANTAGONISTIC CONTROL OF SOCIAL INTERACTION BY LEPTIN RECEPTOR-EXPRESSING AND NEUROTENSIN-EXPRESSING NEURONS IN THE LATERAL HYPOTHALAMUS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

Rebecca Figge[1,2], Anne Petzold[1,2], Hanna Van Den Munkhof[1,2], Tatiana Korotkova[1,2]
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The lateral hypothalamus (LH) plays a major role in regulating consummatory behaviors to maintain homeostasis. However, hunger and thirst are continuously weighed against competing needs, according to state and opportunity. The neuronal mechanisms of representation and prioritization of multiple innate rewards remain poorly understood. We investigated how distinct neuronal populations of leptin receptor- (LepR) and neurotensin-expressing cells (Nts) in the LH guide increasingly hungry animals through behavioral choices. For that purpose, we used opto- and chemogenetic stimulation as well as single cell, deep-brain calcium imaging in freely moving mice in various nutritional states. Stimulation of LepR neurons promoted interaction with females but not males despite moderate hunger pressure. Furthermore, LepR neurons exhibited stronger responses to females than to males during spontaneous social interaction. Hunger pressure led to increase of food-selective and decrease of social-selective LepR neurons, suggesting need-dependent competitive coding of these orthogonal stimuli by LepR neurons. In contrast, activation of Nts neurons reduced social interaction, and activity of Nts neurons did not differentiate the sex of conspecifics. Here, we demonstrate that LepR and Nts populations exert opposite effects on social interaction with LepR neurons promoting social exploration and Nts neurons restraining social drive. This complementary control of innate drives enables flexible fulfillment of orthogonal needs according to current opportunities and gated by physiological demands. **We gratefully acknowledge support by the ERC Consolidator Grant (772994, FeedHypNet, to T.K.) and the DFG (233886668-GRK1960, to R.F., Project-ID 431549029 – SFB 1451, to T.K., EXC2030 CECAD, to T.K.).**

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BOARD NUMBER: S02-252

DOPAMINERGIC NEURONS RESPONSES TO INTRAGASTRIC DELIVERY OF DIFFERENT REINFORCERS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Background: Infusions of sucrose directly into the stomach modulates food seeking behavior and activates ventral tegmental area (VTA) dopaminergic neurons. However, how information regarding different macronutrients is transmitted and detected in brain reward areas remains unknown. Here we tested if VTA dopaminergic activity is differently modulated by distinct reinforcing macronutrients. Methods: Dopamine transporter (DAT)-Cre mice were injected with GcaMP6f, a genetically encoded fluorescent calcium indicator, and implanted with a gradient index lens, in the VTA. Fluorescent imaging of VTA neurons was recorded using a microendoscope during a 25-minute session. During each session a different reinforcer - sucrose, sucralose, or corn oil - was delivered into the stomach through a previously implanted intragastric catheter. Reinforcer order was randomized. Corn oil and sucrose solutions were isocaloric and sucralose, a non-caloric sweetener, was used as control. Results: The number of neurons detected on average for the sessions of each reinforcer did not differ significantly. However, the mean activity of VTA dopaminergic neurons increased significantly after intragastric infusions of sucrose when compared with isocaloric corn oil or sucralose sessions. Accordingly, the percentage of positively modulated neurons after reinforcer delivery was significantly higher in sessions when sucrose was infused when compared with the other two reinforcers, with no lateralization of the response. Furthermore, the neurons that are positively modulated by sucrose are not positively modulated by corn oil. Conclusion: This preliminary data indicates that VTA dopaminergic neurons are modulated by sucrose but not by corn oil or sucralose, indicating a nutrient-specific response.

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BOARD NUMBER: S02-253

CENTRAL AMYGDALA AND FEEDING BEHAVIOR: SINGLE CELL TRANSCRIPTOME ANALYSIS AND REGULATION BY FASTING

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

Songwei He, Christian Peters, Rüdiger Klein
Max-Planck Institute of Neurobiology, Department Of Molecules – Signaling - Development, Martinsried, Germany

The relationship between different cell types and brain functions is of central interest in understanding mouse behavior. The development of single cell sequencing technologies has greatly accelerated our ability to classify and study cell types based on their transcriptomes. It has been used to characterize transcriptional changes in specific cell types in response to external cues or changes in internal states. Here, we explored cell types and their transcriptional variations in the central amygdala (CeA), a brain region that mediates defensive and appetitive responses. We performed single nuclei RNA sequencing in adult mice and compared the results with known genetic and functionally defined populations, which have been studied in different behavioral contexts. We identified at least seven transcriptionally and spatially discrete cell types (three in the lateral division, CeL; three in the medial division, CeM; and one in the capsular division, CeC). Five of these cell types have been previously characterized for their role in feeding behavior, while the other two seem novel and uncharacterized. To begin investigating whether these cell types show individual responses during appetitive behavior, we compared their transcriptomes in food-deprived and satiated mice. We found alternations of the transcriptomes at different dimensions across cell types. Particularly, we see changes in synaptic-related genes in the Htr2a/Sst neurons, a population that is known to promote hedonic feeding (Douglass et al., Nat. Neuro., 2017).

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IDENTIFYING NEURONAL CIRCUITS THAT COORDINATE SUGAR AND WATER INGESTION

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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While the mechanisms animals use to eat is beautifully diverse, all organisms need to acquire nutrients from their environment to survive. Food ingestion is tightly regulated by internal signals of nutrient abundance in order to maintain homeostasis. Recent studies have identified four neurons called Interoceptive Subesophageal zone Neurons (ISNs) in *Drosophila melanogaster* that respond to endogenous hunger and thirst signals to oppositely regulate sugar and water ingestion. Upon activation, ISNs increase sugar ingestion but decrease water ingestion. While the key molecular pathways that lead to ISN activation have been identified, it is still unknown how ISN activity translates to a behavioral response. The goal of this study is to examine how competing needs are coordinated by the nervous system by identifying and characterizing the neural circuit downstream of the ISNs. We used the full adult fly brain electron microscopy volume to trace the ISNs and their downstream targets. Interestingly, we have identified two distinct pathways downstream of the ISNs: a neuroendocrine circuit that connects the ISNs to *Drosophila* insulin-like peptide producing cells (IPCs), and a local circuit that connects the ISNs to motor neurons controlling feeding. Future experiments involve gaining genetic access to these neurons to conduct behavioral and functional connectivity experiments to characterize their involvement in sucrose and water ingestion. This study will provide insight into how the nervous system organizes competing internal needs to drive opposing behavioral responses.

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BOARD NUMBER: S02-255

A NEURAL MECHANISM INVOLVED IN THE MOTIVATIONAL SUPPRESSION OF FEEDING BY NOCICEPTION.

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Our understanding of neural circuits of feeding regulation have focused on a top down approach of the influence of the hypothalamus in regulating feeding. However, the mechanism in how suppression of activity of these neurons can lead to an 'off switch' in feeding behaviour is unclear. The presence of competing states, such as pain and hunger, will result in mice displaying protective behaviour towards pain which results in suppressed motivation in fulfilling the needs of hunger. Utilizing an acute shock-food reward behavioural paradigm, we showed that attenuation of lateral hypothalamus projecting parabrachial nucleus (^{LH}PBN) neurons resulted in hungry mice obtaining food even in the presence of shock; thus demonstrating that ^{LH}PBN is involved in modulating the prioritization of pain in a pain-reward conflict. In addition, the activation of ^{LH}PBN neurons in fasted mice trained in a progressive operant task reduced its motivation to work for rewards. This behavioural change was not due to an effect from changes in valuation towards reward as mice were able to obtain maximum number of rewards when they were put through a simpler fixed ratio task. We also confirmed that both activation and attenuation of ^{LH}PBN neurons had no effect on the physical sensation of pain in mice, as mice showed no altered paw sensitivity in a von frey test. Thus, the findings of this study have revealed a neural mechanism involved in the motivational suppression of feeding by nociception.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-256

RXFP3 EXPRESSION IN DOPAMINERGIC NEURONS OF THE HYPOTHALAMUS AND THE VENTRAL TEGMENTAL AREA OF MICE

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Introduction: RXFP3 (relaxin-family peptide 3 receptor) is the cognate G-protein-coupled receptor for the neuropeptide, relaxin-3. RXFP3 is expressed widely throughout the brain, including the hypothalamus, where it has been shown to modulate feeding behaviour and neuroendocrine activity in rodents. **Aim:** In studies to better characterise potential mechanisms that underlie these effects, this study determined whether RXFP3 is expressed by dopaminergic neurons within the arcuate nucleus (ARC) and dorsomedial hypothalamus (DMH), in addition to the ventral tegmental area (VTA). **Methods:** Neurons that express RXFP3 were visualised in coronal brain sections from RXFP3-Cre/tdTomato mice, which express the tdTomato fluorophore within RXFP3-positive cells, and dopaminergic neurons in these areas were visualised by simultaneous immunohistochemical detection of tyrosine hydroxylase-immunoreactivity (TH-IR). **Results:** Approximately 20% of ARC neurons containing TH-IR co-expressed tdTomato fluorescence, suggesting RXFP3 can influence the dopamine pathway from the ARC to the pituitary gland that controls prolactin release. The ability of prolactin to reduce leptin sensitivity and increase food consumption therefore represents a potential mechanism by which RXFP3 activation mediates its demonstrated effects on feeding. Notably, intra-hypothalamic injection of relaxin-3 increased plasma prolactin levels (McGowan et al. 2014). A similar proportion of DMH neurons containing TH-IR expressed RXFP3-related tdTomato fluorescence, consistent with a possible RXFP3-mediated regulation of stress and neuroendocrine circuits. In contrast, RXFP3 was barely detected within the VTA. **Conclusion:** These findings identify potential hypothalamic mechanisms by which RXFP3 influences neuroendocrine control of metabolism, and further highlight the therapeutic potential of targeting this receptor in feeding-related disorders

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BOARD NUMBER: S02-257

THE NEURONAL LOGIC OF HOW INTERNAL STATES CONTROL FOOD CHOICE

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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When deciding what to eat, animals evaluate sensory information about food quality alongside multiple ongoing internal states. How internal states interact to alter sensorimotor processing and shape decisions such as food choice remains poorly understood. Here, we use pan-neuronal volumetric activity imaging in the *Drosophila* brain to investigate the neuronal basis of internal state dependent nutrient appetites. We created a functional atlas of the ventral fly brain and find that metabolic state shapes sensorimotor processing across large sections of the neuropil. Reproductive state, in contrast, acts locally to define how sensory information is translated into feeding motor output. Thereby, these two states synergistically modulate protein-specific food intake and thus food choice. Finally, using a novel computational strategy, we identify driver lines innervating state-modulated regions and show that the newly identified “borboleta” region is sufficient to direct food choice towards protein-rich food. We therefore identify a generalizable principle by which distinct internal states are integrated to shape decision-making and propose a strategy to uncover and functionally validate how internal states shape behavior.

Pubmed:
32692973: Baden T, Maina MB, Maia Chagas A, Mohammed YG, Auer TO, Silbering A, von Tobel L, Pertin M, Hartig R, Aleksic J, Akinrinade I, Awadelkareem MA, Koumoundourou A, Jones A, Arieti F, Beale A, Münch D, Salek SC, Yusuf S, Prieto-Godino LL
TReND in Africa: Toward a Truly Global (Neuro)science Community.
TReND is a volunteer-scientist run charity dedicated to promoting research and education on the African continent. Focusing on neuroscience, we discuss approaches to address some of the factors that currently stifle Africa's scientific development and our experience in implementing them.
Neuron, 2020; 107
31816522: Münch D, Ezra-Nevo G, Francisco AP, Tastekin I, Ribeiro C
Nutrient homeostasis - translating internal states to behavior.
Behavioral neuroscience aims to describe a causal relationship between neuronal processes and behavior. Animals' ever-changing physiological needs alter their internal states. Internal states then alter neuronal processes to adapt the behavior of the animal enabling it to meet its needs. Here, we describe nutrient-specific appetites as an attractive framework to study how internal states shape complex neuronal processes and resulting behavioral outcomes. Understanding how neurons detect nutrient states and how these are integrated at the level of neuronal circuits will provide a multilevel description of the mechanisms underlying complex feeding and foraging decisions.
Curr Opin Neurobiol, 2020; 60
30315166: Sánchez-Alcañiz JA, Silbering AF, Croset V, Zappia G, Sivasubramaniam AK, Abuin L, Sahai SY, Münch D, Steck K, Auer TO, Cruchet S, Neagu-Maier GL, Sprecher SG, Ribeiro C, Yapici N, Benton R
An expression atlas of variant ionotropic glutamate receptors identifies a molecular basis of carbonation sensing.
Through analysis of the Drosophila ionotropic receptors (IRs), a family of variant ionotropic glutamate receptors, we reveal that most IRs are expressed in peripheral neuron populations in diverse gustatory organs in larvae and adults. We characterise IR56d, which defines two anatomically-distinct neuron classes in the proboscis: one responds to carbonated solutions and fatty acids while the other represents a subset of sugar- and fatty acid-sensing cells. Mutational analysis indicates that IR56d, together with the broadly-expressed co-receptors IR25a and IR76b, is essential for physiological responses to carbonation and fatty acids, but not sugars. We further demonstrate that carbonation and fatty acids both promote IR56d-dependent attraction of flies, but through different behavioural outputs. Our work provides a toolkit for investigating taste functions of IRs, defines a subset of these receptors required for carbonation sensing, and illustrates how the gustatory system uses combinatorial expression of sensory molecules in distinct neurons to coordinate behaviour.
Nat Commun, 2018; 9
28852844: Münch D, Galizia CG

Take time: odor coding capacity across sensory neurons increases over time in Drosophila.
Due to the highly efficient olfactory code, olfactory sensory systems are able to reliably encode enormous numbers of olfactory stimuli. The olfactory code consists of combinatorial activation patterns across sensory neurons, thus its capacity exceeds the number of involved classes of sensory neurons by a manifold. Activation patterns are not static but vary over time, caused by the temporally complex response dynamics of the individual sensory neuron responses. We systematically analyzed the temporal dynamics of olfactory sensory neuron responses to a diverse set of odorants. We find that response dynamics depend on the combination of sensory neuron and odorant and that information about odorant identity can be extracted from the time course of the response. We also show that new response dynamics can arise when mixing two odorants. Our data show that temporal dynamics of odorant responses are able to significantly enhance the coding capacity of olfactory sensory systems.
J Comp Physiol A Neuroethol Sens Neural Behav Physiol, 2017; 203
27746723: Zwaka H, Münch D, Manz G, Menzel R, Rybak J
The Circuitry of Olfactory Projection Neurons in the Brain of the Honeybee, .
In the honeybee brain, two prominent tracts - the medial and the lateral antennal lobe tract - project from the primary olfactory center, the antennal lobes (ALs), to the central brain, the mushroom bodies (MBs), and the protocerebral lobe (PL). Intracellularly stained uniglomerular projection neurons were reconstructed, registered to the 3D honeybee standard brain atlas, and then used to derive the spatial properties and quantitative morphology of the neurons of both tracts. We evaluated putative synaptic contacts of projection neurons (PNs) using confocal microscopy. Analysis of the patterns of axon terminals revealed a domain-like innervation within the MB lip neuropil. PNs of the lateral tract arborized more sparsely within the lips and exhibited fewer synaptic boutons, while medial tract neurons occupied broader regions in the MB calyces and the PL. Our data show that uPNs from the medial and lateral tract innervate both the core and the cortex of the ipsilateral MB lip but differ in their innervation patterns in these regions. In the mushroombody neuropil collar we found evidence for ALT boutons suggesting the collar as a multi modal input site including olfactory input similar to lip and basal ring. In addition, our data support the conclusion drawn in previous studies that reciprocal synapses exist between PNs, octopaminergic-, and GABAergic cells in the MB calyces. For the first time, we found evidence for connections between both tracts within the AL.
Front Neuroanat, 2016; 10
27337300: Silbering AF, Bell R, Münch D, Cruchet S, Gomez-Diaz C, Laudes T, Galizia CG, Benton R
Ir40a neurons are not DEET detectors.
Nature, 2016; 534
26912260: Münch D, Galizia CG
DoOR 2.0--Comprehensive Mapping of Drosophila melanogaster Odorant Responses.
Odors elicit complex patterns of activated olfactory sensory neurons. Knowing the complete olfactome, i.e. the responses in all sensory neurons for all relevant odorants, is desirable to understand olfactory coding. The DoOR project combines all available Drosophila odorant response data into a single consensus response matrix. Since its first release many studies were published: receptors were deorphanized and several response profiles were expanded. In this study, we add unpublished data to the odor-response profiles for four odorant receptors (Or10a, Or42b, Or47b, Or56a). We deorphanize Or69a, showing a broad response spectrum with the best ligands including 3-hydroxyhexanoate, alpha-terpineol, 3-octanol and linalool. We include all of these datasets into DoOR, provide a comprehensive update of both code and data, and new tools for data analyses and visualizations. The DoOR project has a web interface for quick queries (http://neuro.uni.kn/DoOR), and a downloadable, open source toolbox written in R, including all processed and original datasets. DoOR now gives reliable odorant-responses for nearly all Drosophila olfactory responding units, listing 693 odorants, for a total of 7381 data points.
Sci Rep, 2016; 6
24389870: Strauch M, Lüdke A, Münch D, Laudes T, Galizia CG, Martinelli E, Lavra L, Paolesse R, Ulivieri A, Catini A, Capuano R, Di Natale C
More than apples and oranges--detecting cancer with a fruit fly's antenna.
Cancer cells and non-cancer cells differ in their metabolism and they emit distinct volatile compound profiles, allowing to recognise cancer cells by their scent. Insect odorant receptors are excellent chemosensors with high sensitivity and a broad receptive range unmatched by current gas sensors. We thus investigated the potential of utilising the fruit fly's olfactory system to detect cancer cells. Using in vivo calcium imaging, we recorded an array of olfactory receptor neurons on the fruit fly's antenna. We performed multidimensional analysis of antenna responses, finding that cell volatiles from different cell types lead to characteristic response vectors. The distances between these response vectors are conserved across flies and can be used to discriminate healthy mammary epithelial cells from different types of breast cancer cells. This may expand the repertoire of clinical diagnostics, and it is the first step towards electronic noses equipped with biological sensors, integrating artificial and biological olfaction.

Sci Rep, 2014; 4
24564474: Strauch M, Müthing C, Broeg MP, Szyszka P, Münch D, Laudes T, Deussen O, Galizia CG, Merhof D
The looks of an odour--visualising neural odour response patterns in real time.
Calcium imaging in insects reveals the neural response to odours, both at the receptor level on the antenna and in the antennal lobe, the first stage of olfactory information processing in the brain. Changes of intracellular calcium concentration in response to odour presentations can be observed by employing calcium-sensitive, fluorescent dyes. The response pattern across all recorded units is characteristic for the odour.
BMC Bioinformatics, 2013; 14 Suppl 19
23315042: Münch D, Schmeichel B, Silbering AF, Galizia CG
Weaker ligands can dominate an odor blend due to syntopic interactions.
Most odors in natural environments are mixtures of several compounds. Perceptually, these can blend into a new "perfume," or some components may dominate as elements of the mixture. In order to understand such mixture interactions, it is necessary to study the events at the olfactory periphery, down to the level of single-odorant receptor cells. Does a strong ligand present at a low concentration outweigh the effect of weak ligands present at high concentrations? We used the fruit fly receptor dOr22a and a banana-like odor mixture as a model system. We show that an intermediate ligand at an intermediate concentration alone elicits the neuron's blend response, despite the presence of both weaker ligands at higher concentration, and of better ligands at lower concentration in the mixture. Because all of these components, when given alone, elicited significant responses, this reveals specific mixture processing already at the periphery. By measuring complete dose-response curves we show that these mixture effects can be fully explained by a model of syntopic interaction at a single-receptor binding site. Our data have important implications for how odor mixtures are processed in general, and what preprocessing occurs before the information reaches the brain.
Chem Senses, 2013; 38

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BOARD NUMBER: S02-258

ASSESSMENT OF THE INSULAR CORTEX SUBREGIONS ACTIVITY INDUCED BY TASTE EXPOSURE.

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Previous research on the role of particular insular cortex regions in processing taste and taste novelty recognition has pointed to the insular disgranular gustatory cortex. However, studies dissociating disgranular (gustatory), granular (visceral) and agranular insular cortex regions are lacking. The present study aimed to assess activity changes associated with taste novelty and familiarization in the insular cortex subregions of adult Wistar rats. As an index of neural activation, the number of positive c-Fos cells was counted in different groups during the water baseline (BL), first (Novel), second (Familiar 2) and sixth (Familiar 6) exposures to a sodium saccharin solution (0,5%). All groups showed reduced saccharin consumption on Day 1 indicating taste neophobia which was attenuated on the following exposures. The results showed lower number of Fos positive nuclei in the agranular than granular and disgranular insular cortex regions with no differences between the groups so that this region was used as the control area. Higher number of Fos positive cells was found in the disgranular insular cortex when compared with the control region in the Novel group, this supporting the role in processing taste novelty found in previous studies. The granular insular cortex exhibited higher number of Fos positive cells than the control agranular area in all the groups, this indicating a nonspecific involvement of the insular granular cortex receiving visceral afferents in fluids intake which can be related with basal hydromineral regulation. Supported by PSI2017-86381-P (MINECO) and PID2020-114269GB-I00 (MICIU, Spain). Key Words: Neophobia, c-fos, taste learning, insular cortex, rats.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-259

MARMITE DEFINES A NEW CONSERVED NEUROPEPTIDE FAMILY MEDIATING PROTEIN-SPECIFIC SATIETY

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

Ana Patricia Francisco[1], Ibrahim Tastekin[1], Gili Ezra-Nevo[1], Bart Deplancke[2], Alisson Gontijo[3], Carlos Ribeiro[1]
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Neuropeptides play a key role in regulating animal's physiology and behavior. One of their important functions is to mediate information about the internal state of the animal, which is essential for controlling several behaviors, such as feeding. While we know that animals modify their food choices to respond to the lack of specific nutrients, the mechanisms mediating nutrient-specific appetites remain unclear. We have identified *marmite*, an uncharacterized gene encoding a secreted peptide with unknown function in *Drosophila*. Bioinformatic analyses suggest that it is a member of a broad family of poorly characterized conserved neuropeptides including vertebrate orthologues, which have previously been proposed to control feeding. We found that *marmite* expression is upregulated in amino acid-rich diets compared to amino-acid-poor diets. To characterize whether *marmite* is involved in feeding decisions, we used the flyPAD technology, a capacitance-based setup that enables a quantitative feeding analysis, together with CRISPR-Cas9-based genome engineering techniques. Neuronal knockdown of *marmite* or neuronal silencing of *marmite*-expressing neurons increases yeast feeding in fully-fed flies. Moreover, activation of these neurons is sufficient to reduce yeast feeding in a *marmite*-dependent manner, suggesting that *marmite* acts as an amino acid specific satiety signal. We also investigated the functional conservation of *marmite* and its vertebrate orthologues and we found that neuronal expression of either *marmite* or the human orthologue is sufficient to decrease feeding in flies. Thus, we have identified a novel class of conserved neuropeptides that play a key role in nutritional homeostasis and feeding decisions across phyla.

Pubmed:
32843654: Henriques SF, Dhakan DB, Serra L, Francisco AP, Carvalho-Santos Z, Baltazar C, Elias AP, Anjos M, Zhang T, Maddocks ODK, Ribeiro C
Metabolic cross-feeding in imbalanced diets allows gut microbes to improve reproduction and alter host behaviour.
The impact of commensal bacteria on the host arises from complex microbial-diet-host interactions. Mapping metabolic interactions in gut microbial communities is therefore key to understand how the microbiome influences the host. Here we use an interdisciplinary approach including isotope-resolved metabolomics to show that in Drosophila melanogaster, Acetobacter pomorum (Ap) and Lactobacillus plantarum (Lp) a syntrophic relationship is established to overcome detrimental host diets and identify Ap as the bacterium altering the host's feeding decisions. Specifically, we show that Ap uses the lactate produced by Lp to supply amino acids that are essential to Lp, allowing it to grow in imbalanced diets. Lactate is also necessary and sufficient for Ap to alter the fly's protein appetite. Our data show that gut bacterial communities use metabolic interactions to become resilient to detrimental host diets. These interactions also ensure the constant flow of metabolites used by the microbiome to alter reproduction and host behaviour.
Nat Commun, 2020; 11
31816522: Münch D, Ezra-Nevo G, Francisco AP, Tastekin I, Ribeiro C
Nutrient homeostasis - translating internal states to behavior.
Behavioral neuroscience aims to describe a causal relationship between neuronal processes and behavior. Animals' ever-changing physiological needs alter their internal states. Internal states then alter neuronal processes to adapt the behavior of the animal enabling it to meet its needs. Here, we describe nutrient-specific appetites as an attractive framework to study how internal states shape complex neuronal processes and resulting behavioral outcomes. Understanding how neurons detect nutrient states and how these are integrated at the level of neuronal circuits will provide a multilevel description of the mechanisms underlying complex feeding and foraging decisions.
Curr Opin Neurobiol, 2020; 60
28441450: Leitão-Gonçalves R, Carvalho-Santos Z, Francisco AP, Fioreze GT, Anjos M, Baltazar C, Elias AP, Itskov PM, Piper MDW, Ribeiro C

Commensal bacteria and essential amino acids control food choice behavior and reproduction.
Choosing the right nutrients to consume is essential to health and wellbeing across species. However, the factors that influence these decisions are poorly understood. This is particularly true for dietary proteins, which are important determinants of lifespan and reproduction. We show that in Drosophila melanogaster, essential amino acids (eAAs) and the concerted action of the commensal bacteria Acetobacter pomorum and Lactobacilli are critical modulators of food choice. Using a chemically defined diet, we show that the absence of any single eAA from the diet is sufficient to elicit specific appetites for amino acid (AA)-rich food. Furthermore, commensal bacteria buffer the animal from the lack of dietary eAAs: both increased yeast appetite and decreased reproduction induced by eAA deprivation are rescued by the presence of commensals. Surprisingly, these effects do not seem to be due to changes in AA titers, suggesting that gut bacteria act through a different mechanism to change behavior and reproduction. Thus, eAAs and commensal bacteria are potent modulators of feeding decisions and reproductive output. This demonstrates how the interaction of specific nutrients with the microbiome can shape behavioral decisions and life history traits.
PLoS Biol, 2017; 15
24041769: Jones-Dias D, Manageiro V, Francisco AP, Martins AP, Domingues G, Louro D, Ferreira E, Caniça M
Assessing the molecular basis of transferable quinolone resistance in Escherichia coli and Salmonella spp. from food-producing animals and food products.
Enterobacteriaceae resistant to quinolones frequently arise in animals, being easily disseminated through the food-chain. The aim of this study was to investigate the presence of plasmid-mediated quinolone resistance (PMQR) determinants in Salmonella spp. (n=183) and Escherichia coli (n=180) isolates, collected from food-producing animals and food products among swine, poultry, rabbits and cattle. All isolates were subjected to antimicrobial susceptibility testing and molecular screening of PMQR determinants. β-Lactamase-encoding genes, and the quinolone resistance determining region (QRDR) of gyrA, gyrB, parC and parE genes were also investigated in PMQR-positive isolates. Plasmid characterization was performed by conjugation, followed by replicon-typing. Genetic relatedness of PMQR-positive E. coli was examined by Multilocus Sequence Typing, while Salmonella was previously serotyped. The association of mobile genetic elements and PMQR was investigated through PCR mapping assays. Overall, 4.1% (15/363) isolates harbored qnrB2 (n=3), qnrB19 (n=3), and qnrS1 (n=9) genes. All but one isolate presented one to four mutations in QRDR of gyrA or parC genes, which is consistent with the range of MIC values detected (0.19-64 mg/L) for ciprofloxacin; 60% (9/15) of qnr-harboring isolates were non-susceptible to β-lactam antibiotics which was justified by the presence of β-lactamases from TEM (TEM-1, n=8; TEM-135, n=1) and SHV (SHV-108, n=1) families. Analysis of mobile genetic elements revealed that qnr genes were detected nearby relevant genetic elements like intI1, ISEcl2, IS26 and ISCR1 and enclosed in diverse Inc. type plasmids. This study illustrated the existence of Qnr-producing E. coli and Salmonella from food-producing animals, associated to specific mobile elements that might mediate their transference between species and among distinct settings.
Vet Microbiol, 2013; 167

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BOARD NUMBER: S02-260

VENTRAL TEGMENTAL AREA GLUTAMATERGIC NEURONS PLAY A ROLE IN FEAR-INDUCED HYPOPHAGIA THROUGH LATERAL HYPOTHALAMIC GLUTAMATERGIC INPUTS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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We have shown that Ventral Tegmental Area (VTA) dopamine neurons are intermixed with glutamate neurons that receive glutamatergic inputs from lateral hypothalamus (LH), and this LH-VTA pathway plays a role in innate escape behavior. Here, we tested the role of this pathway in another innate behavior, feeding. By viral vectors, we selectively expressed channelrhodopsin (ChR2-mice), halorhodopsin (Halo-mice) or eYFP (control-mice) in LH-glutamatergic neurons. After food restriction, we found that VTA photostimulation of LH-glutamate fibers in ChR2-mice, but not in Halo- or control-mice, suppressed eating, indicating that VTA release of glutamate from LH inputs plays a role in feeding behavior. By in vivo recordings, we found that mice approach to an anesthetized rat induced activation of LH-glutamate neurons innervating VTA, indicating that VTA release of glutamate from LH inputs plays a role in innate fear behavior. Next, we tested food restricted Halo- and control-mice in absence of phostimulation and found that all mice decreased their food intake in the presence of the anesthetized rat. In contrast, VTA photoinhibition increased food intake in the presence of the anesthetized rat just in the Halo-mice, suggesting that inhibition of VTA glutamate release from LH neurons blocks fear-induced hypophagia. By genetic ablation of VTA-glutamatergic neurons, we found that in the presences or absence of the anesthetized rat, ablated mice ate the same amount of food, suggesting that fear-induced hypophagia is mediated by VTA glutamate neurons. We conclude that LH-glutamate neurons convey information to VTA-glutamate neurons for feeding modulation in response to innate threats.

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BOARD NUMBER: S02-261

CB1 RECEPTORS IN POMC NEURONS COORDINATE RESPONSES TO FEAR AND FOOD

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Pro-opiomelanocortin (POMC) expressing neurons in the arcuate nucleus of the hypothalamus are typically activated by energy availability and promote satiety. Recent findings from our lab have demonstrated that POMC neurons activity is affected in the first stages of dietary conditions leading to obesity, probably through a signaling pathway involving the cannabinoid CB1 receptors. Brain CB1 receptors are key physiological determinants of synaptic and behavioral functions. However, the role of CB1 receptors specifically in POMC neurons remains poorly understood. Here we have generated a novel mouse line that lacks CB1 only in POMC-expressing cells (POMC-CB1-KO). As compared to their control littermates, POMC-CB1-KO mice do not show any relevant change in the control of energy homeostasis. However, our behavioral analyses have shown that POMC-CB1-KO mice have a clear exacerbation of the fear-conditioned response. Although CB1 has been already described to control fear memories in other brain areas, the role of CB1 receptor in POMC neurons in the regulation of fear memory have never been assessed before. Considering that the canonical function of POMC neurons is the control of food intake, we hypothesized that CB1 receptors-dependent signaling in these neurons is at the intersection of fear and feeding responses. Accordingly, POMC-CB1-KO mice displayed blunted fear-induced suppression of feeding. While further studies are currently ongoing to decipher the potential underlying circuits involved in these responses, these results demonstrate that CB1 receptor in POMC neurons might play a key role in the balance between fear and feeding behavior, two motivational states essential for survival.

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MELANOCORTIN SIGNALING IN THE PVH CAUSES SATIETY BY INPUT-SPECIFIC REGULATION OF SYNAPTIC PLASTICITY

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Melanocortin-4 receptor (MC4R) signaling in the paraventricular hypothalamus (PVH) is fundamental for appetite regulation. Arcuate nucleus POMC (ARC^{POMC}) neurons release the anorexigenic MC4R agonist α-MSH in the PVH, but it remains unclear which downstream events in PVH^{MC4R} satiety neurons are critical for α-MSH's effects to reduce food intake. Here, by combining cell-type-specific chemogenetics and optogenetics, we electrophysiologically identify that α-MSH released by ARC^{POMC} neurons strengthens glutamatergic synaptic activity onto PVH^{MC4R} neurons. This occurs postsynaptically, and remarkably is selective for input coming from parallel-projecting ARC^{Glut} satiety neurons; glutamatergic input to these same PVH^{MC4R} neurons from median preoptic nucleus neurons is unaffected by α-MSH/MC4R signaling. Importantly, genetic deficiency of α-MSH, or fasting which naturally reduces activity of ARC^{POMC} neurons, greatly diminishes the ability of ARC^{Glut} satiety neurons to evoke action-potential firing in downstream PVH^{MC4R} neurons, and α-MSH deficiency completely blocks the ability of ARC^{Glut} satiety neurons to decrease feeding. Thus, ARC^{POMC} neurons and α-MSH/MC4R signaling in PVH^{MC4R} neurons induce satiety by adjusting the gain on transmission across the ARC^{Glut}àPVH^{MC4R} synapse.

Pubmed:
32302532: Jais A, Paeger L, Sotelo-Hitschfeld T, Bremser S, Prinzensteiner M, Klemm P, Mykytiuk V, Widdershooven PJM, Vesting AJ, Grzelka K, Minère M, Cremer AL, Xu J, Korotkova T, Lowell BB, Zeilhofer HU, Backes H, Fenselau H, Wunderlich FT, Kloppenburg P, Brüning JC
PNOC Neurons Promote Hyperphagia and Obesity upon High-Fat-Diet Feeding.
Calorie-rich diets induce hyperphagia and promote obesity, although the underlying mechanisms remain poorly defined. We find that short-term high-fat-diet (HFD) feeding of mice activates prepronociceptin (PNOC)-expressing neurons in the arcuate nucleus of the hypothalamus (ARC). PNOC neurons represent a previously unrecognized GABAergic population of ARC neurons distinct from well-defined feeding regulatory AgRP or POMC neurons. PNOC neurons arborize densely in the ARC and provide inhibitory synaptic input to nearby anorexigenic POMC neurons. Optogenetic activation of PNOC neurons in the ARC and their projections to the bed nucleus of the stria terminalis promotes feeding. Selective ablation of these cells promotes the activation of POMC neurons upon HFD exposure, reduces feeding, and protects from obesity, but it does not affect food intake or body weight under normal chow consumption. We characterize PNOC neurons as a novel ARC neuron population activated upon palatable food consumption to promote hyperphagia.
Neuron, 2020; 106
32976803: Reinoß P, Ciglieri E, Minére M, Bremser S, Klein A, Löhr H, Fuller PM, Büschges A, Kloppenburg P, Fenselau H, Hammerschmidt M
Hypothalamic Pomc Neurons Innervate the Spinal Cord and Modulate the Excitability of Premotor Circuits.
Locomotion requires energy, yet animals need to increase locomotion in order to find and consume food in energy-deprived states. While such energy homeostatic coordination suggests brain origin, whether the central melanocortin 4 receptor (Mc4r) system directly modulates locomotion through motor circuits is unknown. Here, we report that hypothalamic Pomc neurons in zebrafish and mice have long-range projections into spinal cord regions harboring Mc4r-expressing V2a interneurons, crucial components of the premotor networks. Furthermore, in zebrafish, Mc4r activation decreases the excitability of spinal V2a neurons as well as swimming and foraging, while systemic or V2a neuron-specific blockage of Mc4r promotes locomotion. In contrast, in mice, electrophysiological recordings revealed that two-thirds of V2a neurons in lamina X are excited by the Mc4r agonist α-MSH, and acute inhibition of Mc4r signaling reduces locomotor activity. In addition, we found other Mc4r neurons in spinal lamina X that are inhibited by α-MSH, which is in line with previous studies in rodents where Mc4r agonists reduced locomotor activity. Collectively, our studies identify spinal V2a interneurons as evolutionary conserved second-order neurons of the central Mc4r system, providing a direct anatomical and functional link between energy homeostasis and locomotor

control systems. The net effects of this modulatory system on locomotor activity can vary between different vertebrate species and, possibly, even within one species. We discuss the biological sense of this phenomenon in light of the ambiguity of locomotion on energy balance and the different living conditions of the different species.
Curr Biol, 2020; 30

32513829: Hannan S, Affandi AHB, Minere M, Jones C, Goh P, Warnes G, Popp B, Trollmann R, Nizetic D, Smart TG
Differential Coassembly of α1-GABARs Associated with Epileptic Encephalopathy.
GABA receptors (GABARs) are profoundly important for controlling neuronal excitability. Spontaneous and familial mutations to these receptors feature prominently in excitability disorders and neurodevelopmental deficits following disruption to GABA-mediated inhibition. Recent genotyping of an individual with severe epilepsy and Williams-Beuren syndrome identified a frameshifting variant in a major GABAR gene, This truncated the α1 subunit between the third and fourth transmembrane domains and introduced 24 new residues forming the mature protein, α1* Cell surface expression of mutant murine GABARs is severely impaired compared with WT, due to retention in the endoplasmic reticulum. Mutant receptors were differentially coexpressed with β3, but not with β2, subunits in mammalian cells. Reduced surface expression was reflected by smaller IPSCs, which may underlie the induction of seizures. The mutant does not have a dominant-negative effect on native neuronal GABAR expression since GABA current density was unaffected in hippocampal neurons, although mutant receptors exhibited limited GABA sensitivity. To date, the underlying mechanism is unique for epileptogenic variants and involves differential β subunit expression of GABAR populations, which profoundly affected receptor function and synaptic inhibition. GABARs are critical for controlling neural network excitability. They are ubiquitously distributed throughout the brain, and their dysfunction underlies many neurologic disorders, especially epilepsy. Here we report the characterization of an α1-GABAR variant that results in severe epilepsy. The underlying mechanism is structurally unusual, with the loss of part of the α1 subunit transmembrane domain and part-replacement with nonsense residues. This led to compromised and differential α1 subunit cell surface expression with β subunits resulting in severely reduced synaptic inhibition. Our study reveals that disease-inducing variants can affect GABAR structure, and consequently subunit assembly and cell surface expression, critically impacting on the efficacy of synaptic inhibition, a property that will orchestrate the extent and duration of neuronal excitability.
J Neurosci, 2020; 40

30794836: Hannan S, Minere M, Harris J, Izquierdo P, Thomas P, Tench B, Smart TG
GABAR isoform and subunit structural motifs determine synaptic and extrasynaptic receptor localisation.
GABA receptors (GABARs) are the principal inhibitory neurotransmitter receptors in the central nervous system. They control neuronal excitability by synaptic and tonic forms of inhibition mostly mediated by different receptor subtypes located in specific cell membrane subdomains. A consensus suggests that α1-3βγ comprise synaptic GABARs, whilst extrasynaptic α4βδ, α5βγ and αβ isoforms largely underlie tonic inhibition. Although some structural features that enable the spatial segregation of receptors are known, the mobility of key synaptic and extrasynaptic GABARs are less understood, and yet this is a key determinant of the efficacy of GABA inhibition. To address this aspect, we have incorporated functionally silent α-bungarotoxin binding sites (BBS) into prominent hippocampal GABAR subunits which mediate synaptic and tonic inhibition. Using single particle tracking with quantum dots we demonstrate that GABARs that are traditionally considered to mediate synaptic or tonic inhibition are all able to access inhibitory synapses. These isoforms have variable diffusion rates and are differentially retained upon entering the synaptic membrane subdomain. Interestingly, α2 and α4 subunits reside longer at synapses compared to α5 and δ subunits. Furthermore, a high proportion of extrasynaptic δ-containing receptors exhibited slower diffusion compared to δ subunits at synapses. A chimera formed from δ-subunits, with the intracellular domain of γ2L, reversed this behaviour. In addition, we observed that receptor activation affected the diffusion of extrasynaptic, but not of synaptic GABARs. Overall, we conclude that the differential mobility profiles of key synaptic and extrasynaptic GABARs are determined by receptor subunit composition and intracellular structural motifs. This article is part of the special issue entitled 'Mobility and trafficking of neuronal membrane proteins'.
Neuropharmacology, 2020; 169

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MULTIMODAL MAPPING OF LATERAL HYPOTHALAMIC CIRCUITS THAT MEDIATE THE REGULATORY METABOLIC FUNCTIONS OF AGRP NEURONS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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The ability to identify and characterise the cellular components of neural circuits is crucial for furthering our understanding of how the brain orchestrates complex homeostatic processes such as energy balance and peripheral glucose metabolism. Starvation-sensitive, agouti-related peptide (AgRP)-expressing neurons of the arcuate nucleus are known to control feeding behaviour and insulin resistance through inhibition of downstream neurons. However, the genetic identity of such synaptically-connected neurons remains elusive in most second-order sites, including the lateral hypothalamic area (LHA). Here, using a dual Cre- and Dre-recombinase-dependent strategy, we performed targeted ChR2-assisted circuit mapping (CRACM) studies to elucidate AgRP neuron connections to genetically-defined LHA neurons. We demonstrate that discrete neuronal populations receive monosynaptic, GABAergic input from AgRP neurons, and reveal their relative topographical distribution throughout the rostro-caudal extent of the LHA. Furthermore, immunohistochemical and fluorescent *in situ* hybridisation analyses show the extent of overlap between these identified downstream neuronal populations as well as their innervation by AgRP neurons. Subsequent systematic investigation of these circuits with complementary opto- and chemo-genetic approaches demonstrated the relevant contributions of these pathways to the feeding behaviour and insulin resistance that are evoked by AgRP terminal activation in the LHA. These findings identify not only previously unknown effector populations in the LHA that are involved in homeostatic control of metabolism, but also demonstrate the ability of dual recombinatorial strategies to precisely map neural circuits in the brain.

Pubmed:
32892984: Milton LK, Mirabella PN, Greaves E, Spanswick DC, van den Buuse M, Oldfield BJ, Foldi CJ
Suppression of Corticostriatal Circuit Activity Improves Cognitive Flexibility and Prevents Body Weight Loss in Activity-Based Anorexia in Rats.
The ability to adapt behavior to changing environmental circumstances, or cognitive flexibility, is impaired in multiple psychiatric conditions, including anorexia nervosa (AN). Exaggerated prefrontal cortical activity likely underpins the inflexible thinking and rigid behaviors exhibited by patients with AN. A better understanding of the neural basis of cognitive flexibility is necessary to enable treatment approaches that may target impaired executive control.
Biol Psychiatry, 2021; 90

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SINGLE-CELL MOLECULAR AND FUNCTIONAL MAPPING OF POMC NEURONS IN OBESITY: A MULTI-MODAL APPROACH

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

Stéphane Léon[1], Vincent Simon[1], Thomas Lee[1], Samantha Clark[1], Nathalie Dupuy[1], Yves Le Feuvre[1], Xavier Fioramonti[2], Daniela Cota[3], Carmelo Quarta[3]
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Aims: The brain plays a crucial role in maintaining the body's energy needs, a process involving the activity of a group of hypothalamic neurons that express the neuropeptidergic marker pro-opiomelanocortin (POMC). POMC neuronal dysfunction can cause obesity and its associated metabolic sequelae. However, this population of neurons is highly diverse at a molecular and functional level, and whether or not such heterogeneity is implicated in disease establishment or progression has yet to be elucidated. Methods: Here, using a fate-tracing approach in combination with histological and electrophysiological tools, we have characterized POMC neuronal cells at a single-cell resolution in control lean and diet-induced obese (DIO) mice. We 'traced' with a reporter protein POMC neurons in adult mice, thus studying these neuronal cells independently from the expression of their main marker POMC. We used immunohistochemistry, fluorescent in-situ hybridization, and RNAscope to cluster genetically 'traced' POMC neuronal cells based on their expression of POMC. Results: These different approaches allowed the identification of a previously uncharacterized sub-population that expresses negligible POMC mRNA which we named ''Ghost''-POMC neurons. Intriguingly, DIO mice present an increased number of Ghost-POMC neurons relative to control animals. Furthermore, using whole-cell patch-clamp of traced POMC neurons with subsequent single-cell molecular profiling by qPCR, we observed that DIO leads to selective electrical alterations in these clusters with an atypical molecular profile. Conclusions: Thus, Ghost-POMC neurons might constitute a novel subpopulation of POMC neurons that undergo dysfunction in response to prolonged dietary cues, perhaps contributing to obesity establishment or progression.

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OXYTOCIN INFLUENCES THE DEVELOPMENT OF THE MELANOCORTIN SYSTEM AND ADIPOSITY DURING DISTINCT PERIODS OF POSTNATAL LIFE

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Aims. The melanocortin system plays a critical role in the central regulation of metabolism. It consists of hypothalamic neurons producing pro-opiomelanocortin (POMC) and neurons producing agouti-related peptide (AgRP). It is now accepted that abnormal development of the melanocortin system contributes to obesity in later life. Recent data indicated that oxytocin (OT) treatment has beneficial effects on appetite, particularly when the neurohormone is administered neonatally. Here, we investigated whether OT influences the development of the melanocortin system with enduring consequences on adiposity, whether there is a critical period during which OT exerts maximal neurodevelopmental effects. Methods. A chemogenetic approach was used to silence OT neurons in *Ot*-Cre::hM4Di-DREADD mice during distinct periods of life (P0-P7, *early postnatal*; P25-P32, *juvenile period*; and P63-P70, *adulthood*). Neuroanatomical analysis was used to examine the effect of OT neurons' inhibition on the development of melanocortin circuits. We also evaluated the long-term impact of OT neurons silencing on body weight, fat mass, and adipocyte size. Results. Neonatal inhibition of OT neurons disrupted the development of POMC and AgRP axonal projections. It also increases body weight without affecting adiposity. In contrast, OT neurons inhibition during juvenile or adult life does not alter melanocortin circuits but it increases body weight. Juvenile OT neurons' inhibition also increased fat mass associated with bigger adipocytes. Conclusions. OT influences the development of melanocortin circuits during a critical period restricted to neonatal life. However, OT can still exert metabolic effects in later life through mechanisms that remain to be investigated.

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INTERSECTIONAL GENETIC TARGETING OF DISTINCT SENSORY NEURON POPULATIONS FOR FUNCTIONAL ANALYSIS OF GUT-BRAIN COMMUNICATION

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

Diba Borgmann[1], Leonie Cabot[2], Juliet Erlenbeck-Dinkelmann[2], Elisa Ciglieri[2], Nasim Biglari[3], Claus Brandt[3], Anna-Lena Cremer[3], Heiko Backes[3], Marc Tittgemeyer[4], Thomas Wunderlich[3], Jens Brüning[3], Henning Fenselau[2]
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Communication between the gut and the brain is critical for homeostatic regulation of metabolism. Sensory neurons relay nutrient-related signals to the brain, but investigating the functional neurocircuits of distinct populations has been limited by the difficulty of targeting the ganglia they reside in. Here, we describe an intersectional genetic approach for mapping and manipulating molecularly distinct sensory neurons. We reconstruct the gut innervation patterns of numerous discrete vagal and spinal populations and define their downstream targets in the brainstem and spinal cord. Chemogenetic manipulations, coupled with behavioral analysis and optogenetic mapping, demonstrate that stomach-innervating vagal afferents relay anorexigenic signals via the nucleus of the solitary tract to parabrachial nucleus neurons that control meal termination. Further, we show that stomach-innervating vagal afferent activation improves glucose tolerance, and that their inhibition elevates blood glucose levels independent of food intake. Small intestine-innervating vagal afferents, in contrast, increase hepatic glucose production upon stimulation, and activate parabrachial neurons that control normoglycemia, but are dispensable for feeding regulation. These findings highlight functionally distinct sensory neuron circuits in the control of feeding and glucose metabolism.

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EPIGENETIC REGULATION OF OREXIN NEURONS BY MIRNAS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Narcolepsy type 1 is a sleep disorder characterized by excessive daytime sleepiness and cataplexy. Postmortem brain analysis showed a large reduction of orexin-producing neurons in the lateral hypothalamus of narcolepsy patients. Transcriptional control of orexin is not well-known. In this study the role of MicroRNAs (miRNAs) as epigenetic regulators of orexin gene expression were tested. MiRNAs are small, non-coding, highly conserved RNAs which can block mRNA translation into protein. Dicer is an essential protein in the production of mature and functional miRNAs that can regulate or silence expression of target genes. Thus, we inactivated miRNAs in orexin neurons by deleting the floxed-Dicer alleles in orexin-Cre-ki mice (Orexin-Dicer-ko). We found that orexin expression is completely lost in Orexin-Dicer-ko mice, both at mRNA and protein levels, resulting in the typical narcolepsy symptoms such as cataplexy, sleepiness, difficulties to maintain long wakefulness and shorter Rapid-Eyes-Movements (REM) sleep latency. Interestingly, conditional deletion of miRNAs maturation in adult mice by using a tet-off system (Orexin-Dicer-cko) did not lead to the loss of orexin neurons 4 weeks after shutting down of miRNA maturation but a decrease of orexin neurons is observed 8 weeks after Dicer deletion. These results suggest that orexin neurons could survive without mature miRNAs during 4 weeks but not 8 weeks. Our findings suggest a major role for miRNAs in the development and maintenance of orexin neurons and add Orexin-Dicer-ko and Orexin-Dicer-cko as news mouse models of narcolepsy.

Pubmed:
33247179: Seifinejad A, Li S, Possovre ML, Vassalli A, Tafti M
Hypocretinergic interactions with the serotonergic system regulate REM sleep and cataplexy.
Loss of muscle tone triggered by emotions is called cataplexy and is the pathognomonic symptom of narcolepsy, which is caused by hypocretin deficiency. Cataplexy is classically considered to be an abnormal manifestation of REM sleep and is treated by selective serotonin (5HT) reuptake inhibitors. Here we show that deleting the 5HT transporter in hypocretin knockout mice suppressed cataplexy while dramatically increasing REM sleep. Additionally, double knockout mice showed a significant deficit in the buildup of sleep need. Deleting one allele of the 5HT transporter in hypocretin knockout mice strongly increased EEG theta power during REM sleep and theta and gamma powers during wakefulness. Deleting hypocretin receptors in the dorsal raphe neurons of adult mice did not induce cataplexy but consolidated REM sleep. Our results indicate that cataplexy and REM sleep are regulated by different mechanisms and both states and sleep need are regulated by the hypocretinergic input into 5HT neurons.
Nat Commun, 2020; 11
34835975: Zinni M, Pansiot J, Colella M, Faivre V, Delahaye-Duriez A, Guillonneau F, Bruce J, Salnot V, Mairesse J, Knoop M, Possovre ML, Vaiman D, Baud O
Impact of Fetal Growth Restriction on the Neonatal Microglial Proteome in the Rat.
Microglial activation is a key modulator of brain vulnerability in response to intra-uterine growth restriction (IUGR). However, the consequences of IUGR on microglial development and the microglial proteome are still unknown. We used a model of IUGR induced by a gestational low-protein diet (LPD) in rats. Microglia, isolated from control and growth-restricted animals at P1 and P4, showed significant changes in the proteome between the two groups. The expression of protein sets associated with fetal growth, inflammation, and the immune response were significantly enriched in LPD microglia at P1 and P4. Interestingly, upregulation of protein sets associated with the oxidative stress response and reactive oxygen species production was observed at P4 but not P1. During development, inflammation-associated proteins were upregulated between P1 and P4 in both control and LPD microglia. By contrast, proteins associated with DNA repair and senescence pathways were upregulated in only LPD microglia. Similarly, protein sets involved in protein retrograde transport were significantly downregulated in only LPD microglia. Overall, these data demonstrate significant and multiple effects of LPD-induced IUGR

on the developmental program of microglial cells, leading to an abnormal proteome within the first postnatal days. Nutrients, 2021; 13

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VISUALIZING INPUT-OUTPUT ARCHITECTURE OF OREXIN NEURONS WITH RETROGRADE TRACING VECTORS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Orexin-producing neurons (orexin neurons), located in the lateral hypothalamic area (LHA), play a highly important role in maintaining wakefulness. Orexin neurons send widespread projections to nuclei containing monoaminergic neurons such as ventral tegmental area (VTA), locus coeruleus (LC), tuberomammillary nucleus (TMN), and raphe nuclei (Nambu, et al., 1999), all of which contain monoaminergic neurons. We previously identified input neurons that make direct synaptic contacts to orexin neurons with modified rabies vector-based retrograde tracing (Saito, et al., 2018). This study showed orexin neurons receive input from various regions of the brain. It has been still unknown whether orexin neuron populations that send projections to particular regions receive biased input circuits. In this study, to identify the neuronal inputs to the orexin neurons with projections to particular regions, we used projection sites-specific rabies monosynaptic retrograde tracing (Piñol, et al., 2018). We used newly generated *orexin-iCre KI* mice to analyze the input-output relationship of orexin neuronal circuits using the modified multi-color simple-cTRIO method. This new method allowed us to detect more than two different input-output pathways in the same brain. This study revealed that orexin neurons projecting to each output region also send projections to all brain regions we examined previously. Orexin neurons integrate information from the broad areas of the brain and broadcast it to all monoaminergic nuclei and the other regions. However, we found some biased input and output architectures.

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GATING OF HUNGER AND ANXIETY SIGNALING THROUGH NPY-DEPENDENT SYNAPTIC PLASTICITY IN THE BNST

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Effective coordination of hunger drive requires suppression of competing motivational systems, such as anxiety-related behavior and fear action. Hypothalamic agouti-related peptide (**AgRP**)-expressing neurons are a central element in the control of hunger. They are activated by energy deficit, such as fasting, and their acute stimulation triggers voracious food consumption. One projection site through which AgRP neurons influence feeding behavior is the bed nucleus of the stria terminalis (**BNST**). While the BNST is also a key node for the control of anxiety, it is unknown whether and how AgRP neurons' projections to the BNST affect hunger state-dependent modulation of anxiety signaling. Here, using optogenetics combined with electrophysiological recordings, we show that synaptic connectivity between AgRP hunger neurons and neurons in the BNST is strengthened in response to fasting as well as upon an exclusive stimulation of AgRP neurons. Moreover, analysis of GABAergic synaptic transmission onto the same BNST neurons from the central amygdala (CeA), which is essential for driving anxiety, is profoundly reduced in the fasted state. Importantly, these effects are mediated by NPY released from AgRP neurons, since there are no comparable effects in NPY-KO mice, whereas selective re-expression of NPY exclusively in AgRP neurons of NPY-KO mice is sufficient to reproduce the fasting-induced adaptations of both AgRP and CeA synapses. Our data provide evidence that AgRP neuron-mediated release of NPY triggers plastic changes in the synaptic formation of AgRP neurons in the BNST while it also causes heterosynaptic adaptation of another neuronal circuit which controls anxiety.

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LRP2 FUNCTION IN THE CONTROL OF LEPTIN TRANSPORT BY HYPOTHALAMIC TANYCYTES

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Introduction. Obesity is the primary risk factor for the development of type 2 diabetes and cardiovascular diseases. Obese patients show high circulating leptin levels that fail to reduce appetite or increase energy expenditure. Although the leptin receptor (LepR) is thought to be a key candidate for leptin transport, emerging data suggest that low-density lipoprotein receptor-related protein-2 (LRP2) function as potential leptin transporter that can deliver circulating leptin into the brain, through tanycytes, likely via its association with the LepR. Methods. To investigate the physiological role of LRP2 in leptin transport by tanycytes, we will investigate the effect of selectively knocking out LRP2 in tanycytes on energy expenditure (O_2 consumption and locomotor activity will be measured using the metabolic cages) and body composition (fat and lean body mass will be assessed with MRI) using the Rax-CreER^{T2}; LRP2$^{lox/lox}$ mouse model. We will also determine whether deleting LRP2 from tanycytes blocks leptin uptake in the ME and its transport into the mediobasal hypothalamus (MBH) in vivo by assessing leptin-induced Stat3 phosphorylation in the hypothalamus. Results/expected results. If tanycytic LRP2 plays a role in leptin transport mice lacking LRP2 in tanycytes will display hyperphagia and obesity. Conclusions. Our results will establish the role of tanycytic LRP2 in the transport of blood-borne leptin into the hypothalamus in association or not with the LepR.

Pubmed:

33539777: Mimouni NEH, Paiva I, Barbotin AL, Timzoura FE, Plassard D, Le Gras S, Ternier G, Pigny P, Catteau-Jonard S, Simon V, Prevot V, Boutillier AL, Giacobini P
Polycystic ovary syndrome is transmitted via a transgenerational epigenetic process.
Polycystic ovary syndrome (PCOS) is the most common reproductive and metabolic disorder affecting women of reproductive age. PCOS has a strong heritable component, but its pathogenesis has been unclear. Here, we performed RNA sequencing and genome-wide DNA methylation profiling of ovarian tissue from control and third-generation PCOS-like mice. We found that DNA hypomethylation regulates key genes associated with PCOS and that several of the differentially methylated genes are also altered in blood samples from women with PCOS compared with healthy controls. Based on this insight, we treated the PCOS mouse model with the methyl group donor S-adenosylmethionine and found that it corrected their transcriptomic, neuroendocrine, and metabolic defects. These findings show that the transmission of PCOS traits to future generations occurs via an altered landscape of DNA methylation and propose methylome markers as a possible diagnostic landmark for the condition, while also identifying potential candidates for epigenetic-based therapy.
Cell Metab, 2021; 33

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FOOD CONSUMPTION FOLLOWING BLOOD-BRAIN BARRIER-PENETRATING RXFP3-ANTAGONIST INJECTION IN MICE.

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

Izel Eraslan[1], Justin Read[2], Eslam Bastawy[1], Lara Voglsanger[1], Rasika Samarasinghe[1], Adam Walker[1], Olivia Dean[3], Andrew Gundlach[3], Ross Bathgate[3], Johan Rosengren[4], Craig Smith[1]
[1]Deakin Univeristy, Faculty Of Health, School Of Medicine, Institute For Innovation In Mental And Physical Health And Clinical Translation,, Waurn Ponds, Australia, [2]Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia, [3]Florey Institute of Neuroscience and Mental Health, The University Of Melbourne, Parkville, Australia, [4]Faculty of Medicine, The University of Queensland,, School Of Biomedical Sciences, Brisbane, Australia

The Relaxin-Family Peptide 3 receptor (RXFP3) is a G-protein-coupled receptor that modulates several physiological processes, such as appetite. RXFP3 has promise as a target for treating obesity and other neurological disorders, but realising its clinical potential requires developing an RXFP3-specific drug that penetrates the blood-brain barrier. **AIM:** These studies involved the *in vivo* testing of a recently developed RXFP3 antagonist (inhibitor) drug, that has been designed to cross the blood-brain barrier. **METHODS:** On the test day, adult male C57Bl-6 mice were intraperitoneally injected with an RXFP3 antagonist drug (dose: 0.25 µmol/kg and 0.50 µmol/kg) and given highly palatable food from 30–60 min post-injection. To determine the pharmacological selectivity of this effect, RXFP3 knock-out mice were tested in parallel. P-values were calculated using two-way ANOVA (repeated measures/multiple comparisons) **RESULTS:** Mice that received a dose of 0.25 µmol/kg displayed no significant differences in food consumption compared to saline-injected controls. By increasing the dose to 0.50 µmol/kg, RXFP3 antagonist treatment significantly reduced food consumption to approximately 30% of saline values (P=0.0055). RXFP3 antagonist treatment did not alter food consumption in RXFP3 knock-out mice, suggesting that the anorexigenic effects of this drug during the 30–60 min period are primarily driven by 'on target' activity at the RXFP3 receptor. However, video analysis of home-cage behaviours revealed 'off-target' sedative effects in the 20 mins after drug injection for both wild-type and RXFP3 knock-out mice. **CONCLUSION:** Characterisation of the pharmacological profile of this and related compounds assist the further refinement and development of RXFP3 antagonists.

Pubmed:
31398430: Smith CM, Cross J, Eraslan IM, Attawar A, Ch'ng S, Dhar P, Samarasinghe R, Gray L, Lawrence AJ
Phenotyping neurons activated in the mouse brain during restoration of salt debt.
Salt overconsumption contributes to hypertension, which is a major risk factor for stroke, heart and kidney disease. Characterising neuronal pathways that may control salt consumption is therefore important for developing novel approaches for reducing salt overconsumption. Here, we identify neurons within the mouse central amygdala (CeA), lateral parabrachial nucleus (LPBN), intermediate nucleus of the solitary tract (iNTS), and caudal NTS (cNTS) that are activated and display Fos immunoreactivity in mice that have consumed salt in order to restore a salt debt, relative to salt replete and salt depleted controls. Double-label immunohistochemical studies revealed that salt restoring mice had significantly greater densities of activated enkephalin neurons within the CeA and iNTS, while statistically significant changes within the LPBN and cNTS were not observed. Furthermore, within the CeA, restoration of salt debt conferred a significant increase in the density of activated calretinin neurons, while there was no change relative to control groups in the density of activated neurons that co-expressed protein kinase C delta (PKC-δ). Taken together, these studies highlight the importance of opioid systems within the CeA and iNTS in neuronal processes associated with salt restoration, and may aid the development of future pharmacological and other strategies for reducing salt overconsumption.
J Chem Neuroanat, 2019; 101
33584175: Voglsanger LM, Read J, Ch'ng SS, Zhang C, Eraslan IM, Gray L, Rivera LR, Hamilton LD, Williams R, Gundlach AL, Smith CM
Differential Level of RXFP3 Expression in Dopaminergic Neurons Within the Arcuate Nucleus, Dorsomedial Hypothalamus and Ventral Tegmental Area of RXFP3-Cre/tdTomato Mice.
RXFP3 (relaxin-family peptide 3 receptor) is the cognate G-protein-coupled receptor for the neuropeptide, relaxin-3. RXFP3 is expressed widely throughout the brain, including the hypothalamus, where it has been shown to modulate feeding behavior

and neuroendocrine activity in rodents. In order to better characterize its potential mechanisms of action, this study determined whether RXFP3 is expressed by dopaminergic neurons within the arcuate nucleus (ARC) and dorsomedial hypothalamus (DMH), in addition to the ventral tegmental area (VTA). Neurons that express RXFP3 were visualized in coronal brain sections from RXFP3-Cre/tdTomato mice, which express the tdTomato fluorophore within RXFP3-positive cells, and dopaminergic neurons in these areas were visualized by simultaneous immunohistochemical detection of tyrosine hydroxylase-immunoreactivity (TH-IR). Approximately 20% of ARC neurons containing TH-IR coexpressed tdTomato fluorescence, suggesting that RXFP3 can influence the dopamine pathway from the ARC to the pituitary gland that controls prolactin release. The ability of prolactin to reduce leptin sensitivity and increase food consumption therefore represents a potential mechanism by which RXFP3 activation influences feeding. A similar proportion of DMH neurons containing TH-IR expressed RXFP3-related tdTomato fluorescence, consistent with a possible RXFP3-mediated regulation of stress and neuroendocrine circuits. In contrast, RXFP3 was barely detected within the VTA. TdTomato signal was absent from the ARC and DMH in sections from Rosa26-tdTomato mice, suggesting that the cells identified in RXFP3-Cre/tdTomato mice expressed authentic RXFP3-related tdTomato fluorescence. Together, these findings identify potential hypothalamic mechanisms through which RXFP3 influences neuroendocrine control of metabolism, and further highlight the therapeutic potential of targeting RXFP3 in feeding-related disorders.
Front Neurosci, 2020; 14

FENS Forum 2022
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BOARD NUMBER: S02-272

DIFFERENTIAL LEARNING-RELATED ALTERATIONS IN MEDIAL PREFRONTAL AND LATERAL HYPOTHALAMIC RESPONSE PROFILES DURING RESTRICTIVE AND BINGE-LIKE EATING.

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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For effective foraging, animals will often restrict their food intake, passing up a nutritional food source if their surroundings indicate that a preferred or more nutritionally dense option will be available in the near future, thus allowing them to maximize their energy input. This behavioural construct is known as anticipatory negative contrast (ANC) and has been experimentally investigated for determining the psychological processes regarding restrictive eating and those involving over-consumption (i.e. binge-like eating). Using a rat model of ANC where a carbohydrate-rich solution (maltodextrin 2%, saccharine 0.2%) is followed by a preferred condensed milk solution (50/50% condensed milk/carbohydrate-rich solution), the current study aims to relate activity in the medial prefrontal cortex (mPFC) and lateral hypothalamus (LH) – brain regions critical for impulse control and food-seeking behaviour, respectively - to licking behaviour during different phases of ANC. Using a fibre photometry approach where the Ca^{2+} indicator GCamp6s was expressed and imaged for measuring population activity, PFC and LH activity was monitored in separate animals and related to food intake during both phases throughout ANC training. Our preliminary results suggest that both the mPFC and LH neural populations respond to licking for both solutions, each showing differential learning-related alterations in their response profiles as ANC develops. These differential changes in PFC and LH activity may underlie different neural mechanisms for establishing ANC.

Pubmed:
33444639: Hayes J, Laursen B, Eneberg E, Kehler J, Rasmussen LK, Langgard M, Bastlund JF, Gerdjikov TV
Phosphodiesterase type 1 inhibition alters medial prefrontal cortical activity during goal-driven behaviour and partially reverses neurophysiological deficits in the rat phencyclidine model of schizophrenia.
Positive modulation of cAMP signalling by phosphodiesterase (PDE) inhibitors has recently been explored as a potential target for the reversal of cognitive and behavioural deficits implicating the corticoaccumbal circuit. Previous studies show that PDE type 1 isoform B (PDE1B) inhibition may improve memory function in rodent models; however, the contribution of PDE1B inhibition to impulsivity, attentional and motivational functions as well as its neurophysiological effects have not been investigated. To address this, we recorded single unit activity in medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) in Lister Hooded rats treated with the PDE1B inhibitor Lu AF64386 and tested in the 5-choice serial reaction time task (5-CSRTT). We also asked whether PDE1B inhibition modulates neurophysiological deficits produced by subchronic phencyclidine (PCP) treatment, a rat pharmacological model of schizophrenia. Lu AF64386 significantly affected behavioural parameters consistent with a reduction in goal-directed behaviour, however without affecting accuracy. Additionally, it reduced mPFC neuronal activity. Pre-treatment with PCP did not affect behavioural parameters, however it significantly disrupted overall neuronal firing while increasing phasic responses to reward-predicting cues and disrupting mPFC-NAc cross-talk. The latter two effects were reversed by Lu AF64386. These findings suggest PDE1B inhibition may be beneficial in disorders implicating a dysfunction of the mPFC-NAc network.
Neuropharmacology, 2021; 186
34562439: Hayes J, Garau C, Chiacchierini G, Urcelay GP, McCutcheon JE, Apergis-Schoute J
Predictive and motivational factors influencing anticipatory contrast: A comparison of contextual and gustatory predictors in food restricted and free-fed rats.
In anticipation of palatable food, rats can learn to restrict consumption of a less rewarding food type resulting in an increased consumption of the preferred food when it is made available. This construct is known as anticipatory negative contrast (ANC) and can help elucidate the processes that underlie binge-like behavior as well as self-control in rodent motivation models. In the current investigation we aimed to shed light on the ability of distinct predictors of a preferred food choice to generate contrast effects and the motivational processes that underlie this behavior. Using a novel set of rewarding solutions, we directly compared contextual and gustatory ANC predictors in both food restricted and free-fed Sprague-Dawley rats. Our

results indicate that, despite being food restricted, rats are selective in their eating behavior and show strong contextually-driven ANC similar to free-fed animals. These differences mirrored changes in palatability for the less preferred solution across the different sessions as measured by lick microstructure analysis. In contrast to previous research, predictive cues in both food restricted and free-fed rats were sufficient for ANC to develop although flavor-driven ANC did not relate to a corresponding change in lick patterning. These differences in the lick microstructure between context- and flavor-driven ANC indicate that the motivational processes underlying ANC generated by the two predictor types are distinct. Moreover, an increase in premature port entries to the unavailable sipper - a second measure of ANC - in all groups reveals a direct influence of response competition on ANC development.
Physiol Behav, 2021; 242

FENS Forum 2022
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BOARD NUMBER: S02-273

CHEMOGENETIC CONTROL OF TMN-HA NEURONS ACTIVITY MODULATES THE EXPRESSION OF MEMORY AND FEEDING BEHAVIOUR.

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Background: The histaminergic system has a cardinal role in regulating animals' performance in various learning paradigms and feeding behaviour. However, our current knowledge of the role of neuronal histamine (HA) in memory and feeding behaviour is based on its depletion, or acute local injections of histaminergic ligands, which present inherent technical limitations. Aim: Here, we investigated the impact of activating or silencing endogenous histaminergic neurotransmission using chemogenetic tools. Methods: To interrogate the function of brain HA we used the DREADDs-driven technology injecting HDC-Cre mice bilaterally into the TMN with excitatory or inhibitory DREADDs to transfect histaminergic cells. The mice were then tested for social, fear memories and feeding behaviour. Results: We observed an opposite effect when TMN^{HA} cells are modulated. When stimulated the HA cells lead to an improvement of social and fear memories, while if inhibited an impairment is apparent. Consistently, TMN^{HA} stimulation induces a reduction of food intake. TMN^{HA} inhibition, instead, is responsible for increased food intake. Conclusions: We revealed that selective chemogenetic activation or inhibition of TMN^{HA} cells results in facilitation or impairment of memory, respectively. Similarly, HA cells activation reduces, whereas its inhibition increases food intake. These results confirm and expand previous reports regarding the role of neuronal HA in the regulation of memory and feeding behaviour and pave the way for future studies to deconstruct specific histaminergic neural pathways involved in different types and memory phases as well as the regulatory mechanisms underlying the control of feeding.

Pubmed:
34844019: Costa A, Ai M, Nunn N, Culotta I, Hunter J, Boudjadja MB, Valencia-Torres L, Aviello G, Hodson DJ, Snider BM, Coskun T, Emmerson PJ, Luckman SM, D'Agostino G
Anorectic and aversive effects of GLP-1 receptor agonism are mediated by brainstem cholecystokinin neurons, and modulated by GIP receptor activation.
Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are effective medications to reduce appetite and body weight. These actions are centrally mediated; however, the neuronal substrates involved are poorly understood.
Mol Metab, 2022; 55

FENS Forum 2022
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BOARD NUMBER: S02-274

HIGH FAT DIET FEEDING DISRUPTS NUCLEUS ACCUMBENS CORE REGULATED MOTIVATIONAL CONTROL OVER FOOD-SEEKING BEHAVIOUR

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

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Food intake is governed by homeostatic and hedonic mechanisms and is disrupted in obesity. The nucleus accumbens core (NAcC) is a hedonic regulator, mediating the metabolic control of food intake. This function can be studied using the general Pavlovian Instrumental Transfer (gPIT) paradigm, during which a stimulus predicting a food outcome is found to energize performance of an instrumental action earning another food outcome. This energizing effect is distorted in obesity-prone rats, in rats tested sated and in rats with abnormal functioning of the NAcC or its cholinergic interneurons (CINs). However, whether obesity-related brain insulin resistance can modulate gPIT and if this modulation involves a loss of insulin function on NAcC CINs is unknown. Here we investigated the effect of high fat diet (HFD) feeding and excision of the insulin receptor on NAcC CINs (Knockout) on gPIT. We used a dietary induced obesity protocol and Cre-Lox transgenics to assess gPIT when mice were hungry or sated. We found that gPIT was not disrupted in HFD and Knockout mice when tested hungry. When tested sated though, gPIT was abolished in Knockout and control mice but was left intact in HFD mice. This persistent food-seeking was associated with changes in NAcC CINs functioning, but not with changes to NAcC CINs insulin signalling. These results indicate that a HFD can disrupt the ability for motivational states to control gPIT, but that overeating despite a lack of hunger may not be driven by insulin resistance or insulin dependent mechanisms.

Pubmed:
30619085: Gladding JM, Abbott KN, Antoniadis CP, Stuart A, Begg DP
The Effect of Intrahippocampal Insulin Infusion on Spatial Cognitive Function and Markers of Neuroinflammation in Diet-induced Obesity.
Obesity and high fat diet consumption contribute to the development of metabolic disorders, insulin resistance, neuroinflammation, and cognitive impairments. CNS administration of insulin into the brain can attenuate these cognitive impairments. The present study investigated whether hippocampal-dependent spatial memory impairments in a dietary induced mouse model of obesity could be improved by the direct administration of insulin into the hippocampus and whether this was associated with markers of hippocampal inflammation. C57Bl/6J mice consumed a low fat or high fat diet for 16 weeks and continuous intrahippocampal saline or insulin infusion for the final 4 weeks, during a period of behavioral testing, before gene expression analysis was performed. The high fat diet group demonstrated poorer spatial memory performance in the Morris water maze and Y-maze, supporting the hypothesis that high fat diet leads to hippocampal dependent cognitive impairment. Insulin infusion into the hippocampus reversed the deficit of high fat diet consumption on both of the tasks. Increased expression of inflammatory markers was detected in the hippocampus in the high fat diet group and expression of these markers was ameliorated in insulin infused mice. This demonstrates that CNS insulin can improve hippocampal-dependent memory and that hippocampal inflammation may be a factor in the development of cognitive deficits associated with diet-induced obesity. Furthermore, these data suggest that insulin may act to attenuate high fat diet induced cognitive deficits by reducing neuroinflammation.
Front Endocrinol (Lausanne), 2018; 9
35164442: Goodman EK, Mitchell CS, Teo JD, Gladding JM, Abbott KN, Rafiei N, Zhang L, Herzog H, Begg DP
The effect of insulin receptor deletion in neuropeptide Y neurons on hippocampal dependent cognitive function in aging mice.
Insulin is known to act in the central nervous system to regulate several physiological and behavioural outcomes, including energy balance, glucose homeostasis and cognitive functioning. However, the neuronal populations through which insulin enhances cognitive performance remain unidentified. Insulin receptors are found in neuropeptide-Y (NPY) expressing neurons, which are abundant in the hypothalamus and hippocampus; regions involved in feeding behaviour and spatial memory, respectively. Here we show that mice with a tissue specific knockout of insulin receptors in NPY expressing neurons (IR□o□x/l□o□x; NPYC□r□e□/+) display an impaired performance in the probe trial of the Morris Water Maze compared with control mice at both the 6 and the 12, but not at the 24 months time point, consistent with a crucial role of insulin and NPY in

cognitive functioning. By 24 months of age all groups demonstrated similar reductions in spatial memory performance. Together, these data suggest that the mechanisms through which insulin influences cognitive functioning are, at least in part, via insulin receptor signaling in NPY expressing neurons. These results also highlight that cognitive impairments observed in aging may be due to impaired insulin signaling.

J Integr Neurosci, 2022; 21

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BOARD NUMBER: S02-275

EFFECT OF SATURATED VS UNSATURATED DIETARY FAT ON LEPTIN RECEPTOR SIGNALLING IN THE PREFRONTAL CORTEX AND HIPPOCAMPUS

POSTER SESSION 02 - SECTION: HYPOTHALAMIC CIRCUITS, HUNGER AND SATIETY

Jesús Fernández Felipe[1], Ana Belén Sanz Martos[2], Victoria Cano[1], Nuria Del Olmo[2], Mariano Ruiz Gayo[1], Beatriz Merino[1]
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Introduction: High-fat diets (HFDs) have a negative impact on cognition and neurotransmission when consumed during the juvenile period. The mechanism remains unknown, but a role of leptin receptors (LepR) has been proposed. Indeed, HFDs increase leptin production, able to promote leptin resistance in tissues like the hippocampus (HIP). However, the contribution of diet components to this endocrine alteration remains to be characterized. **AIM:** To evaluate in prefrontal cortex (PFC) and HIP the influence of **u**nsaturated **o**i**l**-enriched (UOLF) *vs* **s**aturated **o**i**l**-enriched **f**oods (SOLF) on i) LepR signalling pathways and ii) suppressor of cytokine signalling 3 (SOCS3) expression. **Methods**: Five-week-old male mice fed standard chow or UOLF/SOLF. After eight weeks of dietary treatment, mice received a single dose of leptin (1 mg/kg), and PFC/HIP samples were handled for PCR/western blot analysis. **Results**: In SOLF-fed mice, pSTAT3 was not enhanced by leptin neither in PFC nor in HIP. In contrast, in UOLF-mice, pSTAT3 was increased ($p<0.05$) by leptin in both areas. In regard to pAKT and pAMPK, leptin increased pAKT ($p<0.05$) and pAMPK levels ($p<0.05$) in SOLF mice but failed to promote pAKT and pAMPK in UOLF animals. Secondly, SOCS3 density was specifically augmented in the PFC of UOLF-mice. Finally, none of the two diets modified *Lepr* gene expression in PFC/HIP, compared to chow-fed mice. **Conclusions**: Our study shows that saturated and unsaturated fat intake negatively affects LepR signalling in the PFC/HIP. Differences between diets might account for the specific effects these diets have on cognition and HIP synaptic transmission and plasticity.

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BOARD NUMBER: S02-276

INTERROGATING MODULATORY EFFECTS OF CA2 ON THE PERSISTENCE AND ASSOCIATIVITY OF CA1 PLASTICITY IN MICE HIPPOCAMPUS

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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National University of Singapore, Yong Loo Lin School Of Medicine Department Of Physiology, Singapore, Singapore

The hippocampus plays an integral role in episodic memory, particularly by neurons in CA1 subfield. Beyond the canonical circuitry is the CA2 region, implicated in social memory. There also exists monosynaptic connections from CA2 innervating CA1, which functional relevance is relatively unknown. We aimed to examine how the CA2-CA1 connections can modulate maintenance of long-term potentiation (LTP) in the Schaffer collateral (SC)-CA1 synapses. Field electrophysiology of hippocampal slices from young male C57BL/6 mice was performed to measure synaptic modification via long-term potentiation (LTP), a cellular correlate of learning and memory. Subthreshold stimulation of SC-CA1 synapses exhibit early form of LTP (early-LTP) but not persistent late form of LTP (late-LTP). However, when “primed” by activation of CA2, SC-CA1 synapses exhibit protein synthesis-dependent late-LTP upon subthreshold stimulation within a temporal window. Following this, we investigated the associativity of the CA2-primed late-LTP via the synaptic tagging and capture hypothesis (STC). When a second set of SC-CA1 synapses is weakly stimulated following CA2 priming, both the first and second population of SC-CA1 synapses can only exhibit early-LTP, thus not participating in STC. The CA2-primed late-LTP can only participate in STC when prior CA2 activation is increased in magnitude. This set of results demonstrate that CA2 connections onto CA1 can influence the synaptic plasticity of CA1, suggesting possible implications of how social behavioural states can modulate persistence of memory. Our next steps will involve analysing the molecular pathways and players involved in plasticity and signalling, and replicating the electrophysiological result using behavioural models.

Pubmed:

34561235: Benoy A, Bin Ibrahim MZ, Behnisch T, Sajikumar S
Metaplastic Reinforcement of Long-Term Potentiation in Hippocampal Area CA2 by Cholinergic Receptor Activation. Hippocampal CA2, an inconspicuously positioned area between the well-studied CA1 and CA3 subfields, has captured research interest in recent years because of its role in social memory formation. However, the role of cholinergic inputs to the CA2 area for the regulation of synaptic plasticity remains to be fully understood. We show that cholinergic receptor activation with the nonselective cholinergic agonist, carbachol (CCh), triggers a protein synthesis-dependent and NMDAR-independent long-term synaptic depression (CCh-LTD) at entorhinal cortical (EC)-CA2 and Schaffer collateral (SC)-CA2 synapses in the hippocampus of adult male Wistar rats. The activation of muscarinic acetylcholine receptors (mAChRs) is critical for the induction of CCh-LTD with the results suggesting an involvement of M3 and M1 mAChRs in the early facilitation of CCh-LTD, while nicotinic AChR activation plays a role in the late maintenance of CCh-LTD at CA2 synapses. Remarkably, we find that CCh priming lowers the threshold for the subsequent induction of persistent long-term potentiation (LTP) of synaptic transmission at EC-CA2 and the plasticity-resistant SC-CA2 pathways. The effects of such a cholinergic-dependent synaptic depression on subsequent LTP at EC-CA2 and SC-CA2 synapses have not been previously explored. Collectively, the results demonstrate that CA2 synaptic learning rules are regulated in a metaplastic manner, whereby modifications triggered by prior cholinergic stimulation can dictate the outcome of future plasticity events. Moreover, the reinforcement of LTP at EC inputs to CA2 following the priming stimulus coexists with concurrent sustained CCh-LTD at the SC-CA2 pathway and is dynamically scaled by modulation of SC-CA2 synaptic transmission. The release of the neuromodulator acetylcholine is critically involved in processes of hippocampus-dependent memory formation. Cholinergic afferents originating in the medial septum and diagonal bands of Broca terminating in the hippocampal area CA2 might play an important role in the modulation of area-specific synaptic plasticity. Our findings demonstrate that cholinergic receptor activation induces an LTD of synaptic transmission at entorhinal cortical- and Schaffer collateral-CA2 synapses. This cholinergic activation-mediated LTD displays a bidirectional metaplastic switch to LTP on a future timescale. This suggests that such bidirectional synaptic modifications triggered by the dynamic modulation of tonic cholinergic receptor activation may support the formation of CA2-dependent memories given the increased hippocampal cholinergic tone during active wakefulness observed in exploratory behavior.
J Neurosci, 2021; 41

FENS Forum 2022
9-13 July 2022 | Paris, France

34109726: Bin Ibrahim MZ, Benoy A, Sajikumar S

Long-term plasticity in the hippocampus: maintaining within and 'tagging' between synapses.

Synapses between neurons are malleable biochemical structures, strengthening and diminishing over time dependent on the type of information they receive. This phenomenon known as synaptic plasticity underlies learning and memory, and its different forms, long-term potentiation (LTP) and long-term depression (LTD), perform varied cognitive roles in reinforcement, relearning and associating memories. Moreover, both LTP and LTD can exist in an early transient form (early-LTP/LTD) or a late persistent form (late-LTP/LTD), which are triggered by different induction protocols, and also differ in their dependence on protein synthesis and the involvement of key molecular players. Beyond homosynaptic modifications, synapses can also interact with one another. This is encapsulated in the synaptic tagging and capture hypothesis (STC), where synapses expressing early-LTP/LTD present a 'tag' that can capture the protein synthesis products generated during a temporally proximal late-LTP/LTD induction. This 'tagging' phenomenon forms the framework of synaptic interactions in various conditions and accounts for the cellular basis of the time-dependent associativity of short-lasting and long-lasting memories. All these synaptic modifications take place under controlled neuronal conditions, regulated by subcellular elements such as epigenetic regulation, proteasomal degradation and neuromodulatory signals. Here, we review current understanding of the different forms of synaptic plasticity and its regulatory mechanisms in the hippocampus, a brain region critical for memory formation. We also discuss expression of plasticity in hippocampal CA2 area, a long-overlooked narrow hippocampal subfield and the behavioural correlate of STC. Lastly, we put forth perspectives for an integrated view of memory representation in synapses.

FEBS J, 2022; 289

FENS Forum 2022
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BOARD NUMBER: S02-277

LEARNING-INDUCED ENHANCED PREDISPOSITION FOR LLD AND LLP OF INHIBITORY SYNAPTIC TRANSMISSION

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Aim: To study the mechanisms and functional significance of learning-induced long-lasting modulation of inhibitory synaptic transmission. Olfactory discrimination (OD) rule learning results with simultaneous enhancement in excitatory and inhibitory connections onto piriform cortex layer-II principal neurons. Ground cause of this increased inhibitory synaptic modifications and its intimate assistance in memory formation is yet to be described. **Methods:** We used transgenic mice (VGAT-ChR2-EYFP), optogenetic stimulation protocol and whole-cell patch clamp recording in piriform cortex brain slices, five days after learning. **Results:** Employment of quantal analysis to explore the dominancy of GABA mediated presynaptic inputs, revealed no significant changes in quantal release probability and number of released vesicles between neurons from trained and control animals, though a significant increase in the quantal size of the inhibitory post synaptic currents (IPSCs) after learning, indicating learning-induced enhancement of inhibition is maintained via a post-synaptic mechanism. Next, we examined how such learning-induced modifications affect subsequent plasticity of synaptic inhibition on principal neurons by inducing long-lasting changes with repetitive firing of postsynaptic neuron. To that end, we elicited brief repetitive depolarizing current pulses at resting (-70mV) and hyperpolarizing (-90mV) membrane potential. As expected, we found that predisposition for long-lasting depression (LLD, at resting state) is increased after learning. Such enhancement is L-type Ca^{+2} channels dependent. Surprisingly, the predisposition for long-lasting potentiation (LLP, at hyperpolarising state) was also enhanced after learning. **Conclusion:** In sharp contrast to excitatory synaptic transmission, learning enhances greatly the ability to modify the strength of synaptic inhibition onto principal neurons in both directions.

Pubmed:
31969605: Kfir A, Awasthi R, Ghosh S, Kundu S, Paul B, Lamprecht R, Barkai E
A Cellular Mechanism of Learning-Induced Enhancement of Synaptic Inhibition: PKC-Dependent Upregulation of KCC2 Activation.
Long-term memory of complex olfactory learning is expressed by wide spread enhancement in excitatory and inhibitory synaptic transmission onto piriform cortex pyramidal neurons. A particularly interesting modification in synaptic inhibition is the hyperpolarization of the reversal potential of the fast post synaptic inhibitory potential (fIPSP). Here we study the mechanism underlying the maintenance of such a shift in the fIPSP. Blocking of the neuronal specific K-Cl co-transporter (KCC2) in neurons of trained rats significantly depolarized the averaged fIPSP reversal potential of the spontaneous miniature inhibitory post synaptic currents (mIPSCs), to the averaged pre-training level. A similar effect was obtained by blocking PKC, which was previously shown to upregulate KCC2. Accordingly, the level of PKC-dependent phosphorylation of KCC2, at the serine 940 site, was significantly increased after learning. In contrast, blocking two other key second messenger systems CaMKII and PKA, which have no phosphorylation sites on KCC2, had no effect on the fIPSP reversal potential. Importantly, the PKC inhibitor also reduced the averaged amplitude of the spontaneous miniature excitatory synaptic currents (mEPSCs) in neurons of trained rats only, to the pre-training level. We conclude that learning-induced hyper-polarization of the fIPSP reversal potential is mediated by PKC-dependent increase of KCC2 phosphorylation.
Sci Rep, 2020; 10
34988597: Shridhar S, Singh VP, Bhatt R, Kundu S, Balaji J
A new paradigm for investigating temporal order memory shows higher order associations are present in recent but not in remote retrieval.
Memory of a sequence of distinct events requires encoding the temporal order as well as the intervals that separates these events. In this study, using order-place association task where the animal learns to associate the location of the food pellet to the order of entry into the event arena, we probe the nature of temporal order memory in mice. In our task, individual trials become distinct events, as the animal is trained to form a unique association between entry order and a correct location. The inter-trial intervals (> 30 min) are chosen deliberately to minimize the inputs from working memory. We develop this paradigm initially using four order-place associates and later extend it to five paired associates. Our results show that animals not only

acquire these explicit (entry order to place) associations but also higher order associations that can only be inferred implicitly (temporal relation between the events) from the temporal order of these events. As an indicator of such higher order learning during the probe trial, the mice exhibit predominantly prospective errors that decline proportionally with temporal distance. On the other hand, prior to acquiring the sequence, the retrospective errors are dominant. In addition, we also tested the nature of such acquisitions when temporal order CS is presented along with flavored pellet as a compound stimulus comprising of order and flavor both simultaneously being paired with location. Results from these experiments indicate that the animal learns both order-place and flavor-place associations. Comparing with pure order-place training, we find that the additional flavor stimulus in a compound training paradigm did not interfere with the ability of the animals to acquire the order-place associations. When tested remotely, pure order-place associations could be retrieved only after a reminder training. Further higher order associations representing the temporal relationship between the events is markedly absent in the remote time.
Exp Brain Res, 2022; 240

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-278

INHIBITION OF DOPAMINE D3 RECEPTORS IMPROVES HIPPOCAMPAL SYNAPTIC PLASTICITY AND MEMORY

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Dopamine D3 receptors (D3-Rs) are involved in several functions such as reward, social behavior, and movement control. Indeed, D3Rs have been proposed as a therapeutical target for addiction, schizophrenia, and Parkinson's disease. More recently, some studies have evidenced the involvement of D3-Rs in cognition, demonstrating that activation of D3-Rs impairs attention and memory, whereas their inhibition induced a pro-cognitive effect. However, the specific contribution of D3-Rs in hippocampal synaptic plasticity and memory has not been thoroughly investigated. Here, we examined the role of D3-Rs in hippocampal long-term potentiation (LTP) and memory in mice. For this purpose, we first performed extracellular electrophysiological recordings at CA3-CA1 synapses of hippocampal slices treated with a D3-Rs antagonist. Then, we investigated the effect of D3-Rs inhibition on recognition memory studied by Object Recognition Test on wild-type mice treated with a D3-Rs antagonist and on D3 knock out (D3-KO) mice. We found that perfusion of hippocampal slices with a D3-Rs antagonist was able to convert early-LTP into late-LTP. These effects were depended upon protein kinase A (PKA). Behavioral experiments confirmed the cognitive enhancing effect of D3-Rs inhibition, as wild type mice treated with a D3-Rs antagonist and D3-KO animals showed a conversion from short-term into long-term memory. We also demonstrated that D3-Rs inhibition rescued the impairment of synaptic plasticity and memory typically found in aged mice. In conclusion, our findings suggest that D3-Rs plays a role in synaptic plasticity and memory and that their inhibition can be useful to rescue cognitive dysfunction occurring during aging.

Pubmed:

35002742: Caruso G, Grasso M, Fidilio A, Torrisi SA, Musso N, Geraci F, Tropea MR, Privitera A, Tascedda F, Puzzo D, Salomone S, Drago F, Leggio GM, Caraci F
Antioxidant Activity of Fluoxetine and Vortioxetine in a Non-Transgenic Animal Model of Alzheimer's Disease.
Depression is a risk factor for the development of Alzheimer's disease (AD). A neurobiological and clinical continuum exists between AD and depression, with neuroinflammation and oxidative stress being involved in both diseases. Second-generation antidepressants, in particular selective serotonin reuptake inhibitors (SSRIs), are currently investigated as neuroprotective drugs in AD. By employing a non-transgenic AD model, obtained by intracerebroventricular (i.c.v.) injection of amyloid-β (Aβ) oligomers in 2-month-old C57BL/6 mice, we recently demonstrated that the SSRI fluoxetine (FLX) and the multimodal antidepressant vortioxetine (VTX) reversed the depressive-like phenotype and memory deficits induced by Aβ oligomers rescuing the levels of transforming growth factor-β1 (TGF-β1). Aim of our study was to test FLX and VTX for their ability to prevent oxidative stress in the hippocampus of Aβ-injected mice, a brain area strongly affected in both depression and AD. The long-term intraperitoneal (i.p.) administration of FLX (10 mg/kg) or VTX (5 and 10 mg/kg) for 24 days, starting 7 days before Aβ injection, was able to prevent the over-expression of inducible nitric oxide synthase (iNOS) and NADPH oxidase 2 (Nox2) induced by Aβ oligomers. Antidepressant pre-treatment was also able to rescue the mRNA expression of glutathione peroxidase 1 (Gpx1) antioxidant enzyme. FLX and VTX also prevented Aβ-induced neurodegeneration in mixed neuronal cultures treated with Aβ oligomers. Our data represent the first evidence that the long-term treatment with the antidepressants FLX or VTX can prevent the oxidative stress phenomena related to the cognitive deficits and depressive-like phenotype observed in a non-transgenic animal model of AD.
Front Pharmacol, 2021; 12
34924388: Tropea MR, Sanfilippo G, Giannino F, Davì V, Gulisano W, Puzzo D
Innate Preferences Affect Results of Object Recognition Task in Wild Type and Alzheimer's Disease Mouse Models.
Object recognition task (ORT) is a widely used behavioral paradigm to assess memory in rodent models, due to its easy technical execution, the lack of aversive stressful stimuli, and the possibility to repeat the test on the same animals. However, mouse exploration might be strongly influenced by a variety of variables.
J Alzheimers Dis, 2022; 85

34453977: Tropea MR, Li Puma DD, Melone M, Gulisano W, Arancio O, Grassi C, Conti F, Puzzo D
Genetic deletion of α7 nicotinic acetylcholine receptors induces an age-dependent Alzheimer's disease-like pathology.
The accumulation of amyloid-beta peptide (Aβ) and the failure of cholinergic transmission are key players in Alzheimer's disease (AD). However, in the healthy brain, Aβ contributes to synaptic plasticity and memory acting through α7 subtype nicotinic acetylcholine receptors (α7nAChRs). Here, we hypothesized that the α7nAChR deletion blocks Aβ physiological function and promotes a compensatory increase in Aβ levels that, in turn, triggers an AD-like pathology. To validate this hypothesis, we studied the age-dependent phenotype of α7 knock out mice. We found that α7nAChR deletion caused an impairment of hippocampal synaptic plasticity and memory at 12 months of age, paralleled by an increase of Amyloid Precursor Protein expression and Aβ levels. This was accompanied by other classical AD features such as a hyperphosphorylation of tau at residues Ser 199, Ser 396, Thr 205, a decrease of GSK-3β at Ser 9, the presence of paired helical filaments and neurofibrillary tangles, neuronal loss and an increase of GFAP-positive astrocytes. Our findings suggest that α7nAChR malfunction might precede Aβ and tau pathology, offering a different perspective to interpret the failure of anti-Aβ therapies against AD and to find novel therapeutical approaches aimed at restoring α7nAChRs-mediated Aβ function at the synapse.
Prog Neurobiol, 2021; 206
31293421: Torrisi SA, Geraci F, Tropea MR, Grasso M, Caruso G, Fidilio A, Musso N, Sanfilippo G, Tascedda F, Palmeri A, Salomone S, Drago F, Puzzo D, Leggio GM, Caraci F
Fluoxetine and Vortioxetine Reverse Depressive-Like Phenotype and Memory Deficits Induced by Aβ Oligomers in Mice: A Key Role of Transforming Growth Factor-β1.
Depression is a risk factor for the development of Alzheimer's disease (AD), and the presence of depressive symptoms significantly increases the conversion of mild cognitive impairment (MCI) into AD. A long-term treatment with antidepressants reduces the risk to develop AD, and different second-generation antidepressants such as selective serotonin reuptake inhibitors (SSRIs) are currently being studied for their neuroprotective properties in AD. In the present work, the SSRI fluoxetine and the new multimodal antidepressant vortioxetine were tested for their ability to prevent memory deficits and depressive-like phenotype induced by intracerebroventricular injection of amyloid-β (1-42) (Aβ) oligomers in 2-month-old C57BL/6 mice. Starting from 7 days before Aβ injection, fluoxetine (10 mg/kg) and vortioxetine (5 and 10 mg/kg) were intraperitoneally injected daily for 24 days. Chronic treatment with fluoxetine and vortioxetine (both at the dose of 10 mg/kg) was able to rescue the loss of memory assessed 14 days after Aβ injection by the passive avoidance task and the object recognition test. Both antidepressants reversed the increase in immobility time detected 19 days after Aβ injection by forced swim test. Vortioxetine exerted significant antidepressant effects also at the dose of 5 mg/kg. A significant deficit of transforming growth factor-β1 (TGF-β1), paralleling memory deficits and depressive-like phenotype, was found in the hippocampus of Aβ-injected mice in combination with a significant reduction of the synaptic proteins synaptophysin and PSD-95. Fluoxetine and vortioxetine completely rescued hippocampal TGF-β1 levels in Aβ-injected mice as well as synaptophysin and PSD-95 levels. This is the first evidence that a chronic treatment with fluoxetine or vortioxetine can prevent both cognitive deficits and depressive-like phenotype in a non-transgenic animal model of AD with a key contribution of TGF-β1.
Front Pharmacol, 2019; 10
31127002: Gulisano W, Melone M, Ripoli C, Tropea MR, Li Puma DD, Giunta S, Cocco S, Marcotulli D, Origlia N, Palmeri A, Arancio O, Conti F, Grassi C, Puzzo D
Neuromodulatory Action of Picomolar Extracellular Aβ42 Oligomers on Presynaptic and Postsynaptic Mechanisms Underlying Synaptic Function and Memory.
Failure of anti-amyloid-β peptide (Aβ) therapies against Alzheimer's disease (AD), a neurodegenerative disorder characterized by high amounts of the peptide in the brain, raised the question of the physiological role of Aβ released at low concentrations in the healthy brain. To address this question, we studied the presynaptic and postsynaptic mechanisms underlying the neuromodulatory action of picomolar amounts of oligomeric Aβ (oAβ) on synaptic glutamatergic function in male and female mice. We found that 200 pm oAβ induces an increase of frequency of miniature EPSCs and a decrease of paired pulse facilitation, associated with an increase in docked vesicle number, indicating that it augments neurotransmitter release at presynaptic level. oAβ also produced postsynaptic changes as shown by an increased length of postsynaptic density, accompanied by an increased expression of plasticity-related proteins such as cAMP-responsive element binding protein phosphorylated at Ser133, calcium-calmodulin-dependent kinase II phosphorylated at Thr286, and brain-derived neurotrophic factor, suggesting a role for Aβ in synaptic tagging. These changes resulted in the conversion of early into late long-term potentiation through the nitric oxide/cGMP/protein kinase G intracellular cascade consistent with a cGMP-dependent switch from short- to long-term memory observed after intrahippocampal administration of picomolar amounts of oAβ These effects were present upon extracellular but not intracellular application of the peptide and involved α7 nicotinic acetylcholine receptors. These observations clarified the physiological role of oAβ in synaptic function and memory formation providing solid fundamentals for investigating the pathological effects of high Aβ levels in the AD brains. High levels of

oligomeric amyloid-β (oAβ) induce synaptic dysfunction leading to memory impairment in Alzheimer's disease (AD). However, at picomolar concentrations, the peptide is needed to ensure long-term potentiation (LTP) and memory. Here, we show that extracellular 200 pm oAβ concentrations increase neurotransmitter release, number of docked vesicles, postsynaptic density length, and expression of plasticity-related proteins leading to the conversion of early LTP into late LTP and of short-term memory into long-term memory. These effects require α7 nicotinic acetylcholine receptors and are mediated through the nitric oxide/cGMP/protein kinase G pathway. The knowledge of Aβ function in the healthy brain might be useful to understand the causes leading to its increase and detrimental effect in AD.
J Neurosci, 2019; 39
30333723: Costa L, Sardone LM, Bonaccorso CM, D'Antoni S, Spatuzza M, Gulisano W, Tropea MR, Puzzo D, Leopoldo M, Lacivita E, Catania MV, Ciranna L
Activation of Serotonin 5-HT Receptors Modulates Hippocampal Synaptic Plasticity by Stimulation of Adenylate Cyclases and Rescues Learning and Behavior in a Mouse Model of Fragile X Syndrome.
We have previously demonstrated that activation of serotonin 5-HT receptors (5-HTR) reverses metabotropic glutamate receptor-mediated long term depression (mGluR-LTD) in the hippocampus of wild-type (WT) and Knockout (KO) mice, a model of Fragile X Syndrome (FXS) in which mGluR-LTD is abnormally enhanced. Here, we have investigated intracellular mechanisms underlying the effect of 5-HTR activation using patch clamp on hippocampal slices. Furthermore, we have tested whether administration of LP-211, a selective 5-HTR agonist, can rescue learning and behavior in KO mice. In the presence of an adenylate cyclase blocker, mGluR-LTD was slightly enhanced in WT and therefore the difference between mGluR-LTD in WT and KO slices was no longer present. Conversely, activation of adenylate cyclase by either forskolin or Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) completely reversed mGluR-LTD in WT and KO. 5-HTR activation reversed mGluR-LTD in WT and corrected exaggerated mGluR-LTD in KO; this effect was abolished by blockade of either adenylate cyclase or protein kinase A (PKA). Exposure of hippocampal slices to LP-211 caused an increased phosphorylation of extracellular signal regulated kinase (ERK), an intracellular effector involved in mGluR-LTD, in WT mice. Conversely, this effect was barely detectable in KO mice, suggesting that 5-HTR-mediated reversal of mGluR-LTD does not require ERK stimulation. Finally, an acute administration of LP-211 improved novel object recognition (NOR) performance in WT and KO mice and reduced stereotyped behavior in KO mice. Our results indicate that mGluR-LTD in WT and KO slices is bidirectionally modulated in conditions of either reduced or enhanced cAMP formation. Activation of 5-HT receptors reverses mGluR-LTD by activation of the cAMP/PKA intracellular pathway. Importantly, a systemic administration of a 5-HTR agonist to KO mice corrected learning deficits and repetitive behavior. We suggest that selective 5-HTR agonists might become novel pharmacological tools for FXS therapy.
Front Mol Neurosci, 2018; 11
30092511: Gulisano W, Melone M, Li Puma DD, Tropea MR, Palmeri A, Arancio O, Grassi C, Conti F, Puzzo D
The effect of amyloid-β peptide on synaptic plasticity and memory is influenced by different isoforms, concentrations, and aggregation status.
The increase of oligomeric amyloid-beta (oAβ) has been related to synaptic dysfunction, thought to be the earliest event in Alzheimer's disease pathophysiology. Conversely, the suppression of endogenous Aβ impaired synaptic plasticity and memory, suggesting that the peptide is needed in the healthy brain. However, different species, aggregation forms and concentrations of Aβ might differently influence synaptic function/dysfunction. Here, we have tested the contribution of monomeric and oligomeric Aβ42 and Aβ40 at 200 nM and 200 pM concentrations on hippocampal long-term potentiation and spatial memory. We found that, when at 200 nM, oAβ40, oAβ42, and monomeric Aβ42 impaired long-term potentiation and memory, whereas only oAβ42 200 pM enhanced synaptic plasticity and memory and rescued the detrimental effect due to depletion of endogenous Aβ. Interestingly, quantification of monomer-like and oligomer-like species carried out by transmission electron microscopy revealed an increase of the monomer/oligomer ratio in the oAβ42 200 pM preparation, suggesting that the content of monomers and oligomers depends on the final concentration of the solution.
Neurobiol Aging, 2018; 71
29885420: Gulisano W, Tropea MR, Arancio O, Palmeri A, Puzzo D
Sub-efficacious doses of phosphodiesterase 4 and 5 inhibitors improve memory in a mouse model of Alzheimer's disease.
Cyclic nucleotides cAMP and cGMP cooperate to ensure memory acquisition and consolidation. Increasing their levels by phosphodiesterase inhibitors (PDE-Is) enhanced cognitive functions and rescued memory loss in different models of aging and Alzheimer's disease (AD). However, side effects due to the high doses used limited their application in humans. Based on previous studies suggesting that combinations of sub-efficacious doses of cAMP- and cGMP-specific PDE-Is improved synaptic plasticity and memory in physiological conditions, here we aimed to study whether this treatment was effective to counteract the AD phenotype in APPswe mice. We found that a 3-week chronic treatment with a combination of sub-efficacious doses of the cAMP-specific PDE4-I roflumilast (0.01 mg/kg) and the cGMP-specific PDE5-I vardenafil (0.1 mg/kg) improved recognition, spatial and contextual fear memory. Importantly, the cognitive enhancement persisted for 2 months

beyond administration. This long-lasting action, and the possibility to minimize side effects due to the low doses used, might open feasible therapeutic strategies against AD.
Neuropharmacology, 2018; 138
28696204: Puzzo D, Piacentini R, Fá M, Gulisano W, Li Puma DD, Staniszewski A, Zhang H, Tropea MR, Cocco S, Palmeri A, Fraser P, D'Adamio L, Grassi C, Arancio O
LTP and memory impairment caused by extracellular Aβ and Tau oligomers is APP-dependent.
The concurrent application of subtoxic doses of soluble oligomeric forms of human amyloid-beta (oAβ) and Tau (oTau) proteins impairs memory and its electrophysiological surrogate long-term potentiation (LTP), effects that may be mediated by intra-neuronal oligomers uptake. Intrigued by these findings, we investigated whether oAβ and oTau share a common mechanism when they impair memory and LTP in mice. We found that as already shown for oAβ, also oTau can bind to amyloid precursor protein (APP). Moreover, efficient intra-neuronal uptake of oAβ and oTau requires expression of APP. Finally, the toxic effect of both extracellular oAβ and oTau on memory and LTP is dependent upon APP since APP-KO mice were resistant to oAβ- and oTau-induced defects in spatial/associative memory and LTP. Thus, APP might serve as a common therapeutic target against Alzheimer's Disease (AD) and a host of other neurodegenerative diseases characterized by abnormal levels of Aβ and/or Tau.
Elife, 2017; 6
28626017: Palmeri A, Ricciarelli R, Gulisano W, Rivera D, Rebosio C, Calcagno E, Tropea MR, Conti S, Das U, Roy S, Pronzato MA, Arancio O, Fedele E, Puzzo D
Amyloid-β Peptide Is Needed for cGMP-Induced Long-Term Potentiation and Memory.
High levels of amyloid-β peptide (Aβ) have been related to Alzheimer's disease pathogenesis. However, in the healthy brain, low physiologically relevant concentrations of Aβ are necessary for long-term potentiation (LTP) and memory. Because cGMP plays a key role in these processes, here we investigated whether the cyclic nucleotide cGMP influences Aβ levels and function during LTP and memory. We demonstrate that the increase of cGMP levels by the phosphodiesterase-5 inhibitors sildenafil and vardenafil induces a parallel release of Aβ due to a change in the approximation of amyloid precursor protein (APP) and the β-site APP cleaving enzyme 1. Moreover, electrophysiological and behavioral studies performed on animals of both sexes showed that blocking Aβ function, by using anti-murine Aβ antibodies or APP knock-out mice, prevents the cGMP-dependent enhancement of LTP and memory. Our data suggest that cGMP positively regulates Aβ levels in the healthy brain which, in turn, boosts synaptic plasticity and memory. Amyloid-β (Aβ) is a key pathogenetic factor in Alzheimer's disease. However, low concentrations of endogenous Aβ, mimicking levels of the peptide in the healthy brain, enhance hippocampal long-term potentiation (LTP) and memory. Because the second messenger cGMP exerts a central role in LTP mechanisms, here we studied whether cGMP affects Aβ levels and function during LTP. We show that cGMP enhances Aβ production by increasing the APP/BACE-1 convergence in endolysosomal compartments. Moreover, the cGMP-induced enhancement of LTP and memory was disrupted by blockade of Aβ, suggesting that the physiological effect of the cyclic nucleotide on LTP and memory is dependent upon Aβ.
J Neurosci, 2017; 37

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BOARD NUMBER: S02-279

ASTROCYTIC EPHB3 RECEPTORS CONTROL NMDA RECEPTOR FUNCTIONS AND MEMORY

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Long-term synaptic plasticity is considered as the cellular substrate of learning and memory. This includes long-term synaptic potentiation (LTP), corresponding to a persistent increase of synaptic transmission. The most common form of LTP depends on glutamatergic NMDA receptors whose activation requires the binding of glutamate and of a co-agonist that was identified to be D-serine at hippocampal CA3-CA1 synapses. Our previous work in the hypothalamus has indicated that the close apposition of the astrocytic processes with synaptic neuronal elements was essential for the D-serine availability in the synaptic cleft. Interestingly, activation of cell adhesion molecules depends on the close apposition of two membrane elements, making them very likely to participate in this glia-neuron interaction process. Data obtained in astrocytic cultures have shown that the D-serine release was induced by the activation of astrocytic EphB3 receptors. However, we do not know whether this requires the interaction of the astrocytic EphB3 receptor with its neuronal synaptic partner and whether it could impact NMDAR functions. Here, we first established that the activation of EphB3 receptors in acute hippocampal slices from the brain of adult mice led to an increase of D-serine availability at CA3-CA1 synapses inducing an increase of NMDAR activity. Then, selective inhibition of astrocytic EphB3 using a viral approach impaired the induction of LTP as well as novel object recognition memory. Importantly, exogenous application of D-serine was able to rescue LTP and memory deficits. Altogether, our data indicate that astrocytic EphB3 receptors play a key role in NMDAR functions.

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COLLABORATION AND COMPETITION LEAD TO LONG-TERM SPATIAL HETEROSYNAPTIC PLASTICITY

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Activity-dependent synaptic plasticity is a crucial mechanism in the formation of memory in neuronal networks. Experimental results indicate that postsynaptic dendritic spine plasticity occurs at the stimulated (homosynaptic plasticity) and non-stimulated spines (heterosynaptic plasticity) over minutes or even hours. The disruption of this plasticity is linked to neurodegenerative disorders and memory deficits. However, the exact mechanisms that drive the growth and shrinkage of synaptic spines, particularly those not directly stimulated, are not fully understood. We will present a mathematical model that can provide insight into processes that define these dynamics. Inspired by the calcium levels hypothesis, where different amounts of calcium lead to either growth or shrinkage, we introduce a dual-role protein that governs the proposed dynamics in conjunction with calcium. Comparing our model predictions with the experimental results, we observed that (i) both collaboration as well as competition, are key drivers of heterosynaptic plasticity and (ii) the temporal and spatial arrangements of simultaneously stimulated spines have a crucial impact on the resulting spine dynamics. We discuss our results in relation to previous models and show how a number of disparate experimental results that reported heterosynaptic LTP or LTD can be understood using our model.

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BOARD NUMBER: S02-281

THE ROLE OF IMPAIRED SYNAPTIC PLASTICITY IN ABERRANT SALIENCE AND PSYCHOSIS

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Impaired synaptic plasticity is prevalent in conditions ranging from Alzheimer's Disease to depression and schizophrenia and may explain transdiagnostic symptom overlap. Here we present a preclinical analysis of rodent models relevant to psychosis in behavioural habituation assays, to see how disrupted synaptic plasticity could generate aberrant salience. Increased locomotor responding in these models may reflect increased attending to environmental stimuli and impairments in habituation of translational relevance to psychosis. We investigated two commonly used models of disrupted synaptic plasticity; NMDAR receptor blockade (MK-801), and genetic ablation of the *Gria1* gene encoding the GluA1 AMPA receptor subunit (GluA1$^{-/-}$), and one widely used model of hyperdopaminergia (amphetamine). While all models generated robust hyperactivity to novelty, only amphetamine led to hyperlocomotion in a familiar environment. MK-801 and GluA1$^{-/-}$ impaired short-term habituation over several assays but left long-term habituation intact. Intriguingly, responding over prolonged novelty exposure led to an *increase* in responding, indicating sensitisation, of potential relevance to clinical psychosis. Furthermore, this sensitisation was shown to be stimulus-specific, further suggesting this may be a driver of aberrant salience. Amphetamine, however, failed to disrupt expression of habituation, though locomotion increased independent of stimulus novelty. This work suggests a causal role of synaptic plasticity in responding to novel and familiar stimuli and demonstrates mechanisms through which impaired plasticity can lead to impaired habituation and aberrant salience. Impaired plasticity aberrantly increased subjects responding to stimuli and represents a potential mechanism of how psychosis may develop not just in schizophrenia, but also other disorders of impaired hippocampal plasticity.

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BOARD NUMBER: S02-282

MULTIPLE MECHANISTICALLY DISTINCT TIMESCALES OF NEOCORTICAL PLASTICITY OCCUR DURING HABITUATION

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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The recognition of familiar but innocuous stimuli limits their cognitive burden and minimizes energy use, while enabling detection of novel, potentially significant stimuli. A unique, long-lasting form of neocortical plasticity, known as stimulus-selective response potentiation (SRP), occurs as mice develop long-lasting familiarity. This form of response potentiation is manifest in layer 4 of primary visual cortex (V1) over days of repeated presentation of phase-reversing sinusoidal grating stimuli, while behaviourally manifest habituation occurs across a similar timescale. Habituation and accompanying SRP are highly stimulus-selective and reliant on NMDA receptors and parvalbumin-expressing (PV+) interneurons in V1. However, modification of cortical response and visual-evoked behaviour have yet to be investigated over shorter timescales within the same paradigm. Here, we characterise several timescales of plasticity, revealing mechanistically distinct processes and elucidating interactions between them. Notably, while the behavioural response habituates both over the course of minutes and days, layer 4 visual evoked potentials (VEPs) depress over minutes but increase over days. Adaptation of the VEP also occurs over the shorter timescale of seconds. This very short-term form of plasticity is modulated by familiarity, such that adaptation is pronounced for novel but not familiar stimuli. Genetic knock-down of NMDA receptors impairs all timescales of plasticity while only long-term familiarity requires unhindered PV+ neuronal function. Notably, however, the modulation of short-term adaptation by long-term familiarity is reliant on PV+ interneurons. Our findings therefore indicate that distinct forms of plasticity and habituation occur across different timescales and provide insight into their underlying mechanisms and interactions.

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BOARD NUMBER: S02-283

REACTIVATION OF HIPPOCAMPAL NEURONS ENABLES ASSOCIATIVE PLASTICITY OF TEMPORALLY DISCONTIGUOUS INPUTS

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Reactivation of neuronal activity is thought to support the consolidation of hippocampus-dependent memory traces. However, to date, direct evidence for an effect of neuronal reactivation on synaptic plasticity is missing. Here we found that Hebbian pairing followed by postsynaptic reactivation in the presence of dopamine activates a two-step coincidence detector mechanism for rapid protein synthesis-dependent LTP. Using computational modeling, we show that this synaptic learning rule adds specificity to traditional reinforcement learning models by controlling memory allocation in the network. We predicted that this mechanism would make reactivated neurons activate more strongly and carry more spatial information than non-reactivated cells. Using calcium imaging in freely moving mice performing a reward-based navigation task, we show that neurons that were reactivated after finding a reward have increased calcium responses and place map peaks compared to non-reactivated cells. Taken together, our findings suggest that reactivation-induced synaptic plasticity enables the selective encoding of salient events in long-term memory.

Pubmed:
34314698: Hay YA, Deperrois N, Fuchsberger T, Quarrell TM, Koerling AL, Paulsen O
Thalamus mediates neocortical Down state transition via GABA-receptor-targeting interneurons.
Slow-wave sleep is characterized by near-synchronous alternation of active Up states and quiescent Down states in the neocortex. Although the cortex itself can maintain these oscillations, the full expression of Up-Down states requires intact thalamocortical circuits. Sensory thalamic input can drive the cortex into an Up state. Here we show that midline thalamic neurons terminate Up states synchronously across cortical areas. Combining local field potential, single-unit, and patch-clamp recordings in conjunction with optogenetic stimulation and silencing in mice in vivo, we report that thalamic input mediates Down transition via activation of layer 1 neurogliaform inhibitory neurons acting on GABA receptors. These results strengthen the evidence that thalamocortical interactions are essential for the full expression of slow-wave sleep, show that Down transition is an active process mediated by cortical GABA receptors, and demonstrate that thalamus synchronizes Down transitions across cortical areas during natural slow-wave sleep.
Neuron, 2021; 109
32686528: Lloret A, Monllor P, Fuchsberger T, Giraldo E, Perluigi M, Vina J
Increased basal antioxidant levels in RCAN1 - deficient mice lowers oxidative injury after acute paraquat insult.
RCAN1 is an inhibitor of the phosphatase calcineurin, which is involved in the regulation of oxidative stress and apoptosis, among other important cell processes. Here we have used RCAN1 deficient mice (RCAN1) to elucidate its role after an acute oxidative insult such as paraquat injection. We have observed that RCAN1 mice show less oxidative damage than wildtype (WT) mice after treatment. Under basal conditions, RCAN1 animals express more calcineurin, heme oxygenase-1, Nrf2, and catalase compared to WT mice (controls). This may explain the less severe effect of paraquat treatment on RCAN1 mice compared to WT. We showed that oxidative stress is involved in the early stages of apoptosis, thus we determined the apoptotic effector BAD and found that decreases in RCAN1 mice after treatment with paraquat compared with WT in similar experimental conditions. Our results suggest that RCAN1 may be involved in the balance between oxidant and antioxidant species production .
Free Radic Res, 2020; 54
31658055: Fuchsberger T, Yuste R, Martinez-Bellver S, Blanco-Gandia MC, Torres-Cuevas I, Blasco-Serra A, Arango R, Miñarro J, Rodríguez-Arias M, Teruel-Marti V, Lloret A, Viña J
Oral Monosodium Glutamate Administration Causes Early Onset of Alzheimer's Disease-Like Pathophysiology in APP/PS1

Mice.
Glutamate excitotoxicity has long been related to Alzheimer's disease (AD) pathophysiology, and it has been shown to affect the major AD-related hallmarks, amyloid-β peptide (Aβ) accumulation and tau phosphorylation (p-tau). We investigated whether oral administration of monosodium glutamate (MSG) has effects in a murine model of AD, the double transgenic mice APP/PS1. We found that AD pathogenic factors appear earlier in APP/PS1 when supplemented with MSG, while wildtype mice were essentially not affected. Aβ and p-tau levels were increased in the hippocampus in young APP/PS1 animals upon MSG administration. This was correlated with increased Cdk5-p25 levels. Furthermore, in these mice, we observed a decrease in the AMPA receptor subunit GluA1 and they had impaired long-term potentiation. The Hebb-Williams Maze revealed that they had memory deficits. We show here for the first time that oral MSG supplementation can accelerate AD-like pathophysiology in a mouse model of AD.
J Alzheimers Dis, 2019; 72
30472273: Koerling AL, Fuchsberger T, Paulsen O, Hay YA
Partial restoration of physiological UP-state activity by GABA pathway modulation in an acute brain slice model of epilepsy.
In addition to reducing seizures, anti-epileptic treatments should preserve physiological network activity. Here, we used a thalamocortical slice preparation displaying physiological slow oscillations to investigate the effects of anticonvulsant drugs on physiological activity and epileptiform activity in two pharmacological epilepsy models. Thus, we compared the effects of GABA pharmacology on spontaneous physiological and pathological events in slices of the mouse barrel cortex. We show that both reducing inhibition using GABAR blockers and enhancing excitation by lowering Mg concentration allow for the transition from physiological slow oscillations to epileptiform activity. Our results indicate that GABAR antagonists have pro-convulsive properties by increasing event duration in the low inhibition model and event frequency in the high excitation model. Moreover, we show that GABAR agonists and GABA uptake blockers, known for their anticonvulsant properties, act primarily on epileptiform burst frequency and allow for a partial restoration of physiological events. As a proof of principle, these results indicate that a slice model with spontaneous network events may be a useful pipeline to investigate the effects of anti-epileptic drugs on both epileptiform and physiological network activity.
Neuropharmacology, 2019; 148
28505105: Fuchsberger T, Lloret A, Viña J
New Functions of APC/C Ubiquitin Ligase in the Nervous System and Its Role in Alzheimer's Disease.
The E3 ubiquitin ligase Anaphase Promoting Complex/Cyclosome (APC/C) regulates important processes in cells, such as the cell cycle, by targeting a set of substrates for degradation. In the last decade, APC/C has been related to several major functions in the nervous system, including axon guidance, synaptic plasticity, neurogenesis, and neuronal survival. Interestingly, some of the identified APC/C substrates have been related to neurodegenerative diseases. There is an accumulation of some degradation targets of APC/C in Alzheimer's disease (AD) brains, which suggests a dysregulation of the protein complex in the disorder. Moreover, recently evidence has been provided for an inactivation of APC/C in AD. It has been shown that oligomers of the AD-related peptide, Aβ, induce degradation of the APC/C activator subunit cdh1, in vitro in neurons in culture and in vivo in the mouse hippocampus. Furthermore, in the AD mouse model APP/PS1, lower cdh1 levels were observed in pyramidal neurons in CA1 when compared to age-matched wildtype mice. In this review, we provide a complete list of APC/C substrates that are involved in the nervous system and we discuss their functions. We also summarize recent studies that show neurobiological effects in cdh1 knockout mouse models. Finally, we discuss the role of APC/C in the pathophysiology of AD.
Int J Mol Sci, 2017; 18
27514492: Fuchsberger T, Martínez-Bellver S, Giraldo E, Teruel-Martí V, Lloret A, Viña J
Aβ Induces Excitotoxicity Mediated by APC/C-Cdh1 Depletion That Can Be Prevented by Glutaminase Inhibition Promoting Neuronal Survival.
The E3 ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C) is activated by the fizzy-related protein homolog/CDC20-like protein 1 (cdh1) in post-mitotic neurons. Growing evidence suggests that dysregulation of APC/C-Cdh1 is involved in neurodegenerative diseases. Here we show in neurons that oligomers of amyloid beta (Aβ), a peptide related to Alzheimer's disease, cause proteasome-dependent degradation of cdh1. This leads to a subsequent increase in glutaminase (a degradation target of APC/C-Cdh1), which causes an elevation of glutamate levels and further intraneuronal Ca(2+) dysregulation, resulting in neuronal apoptosis. Glutaminase inhibition prevents glutamate excitotoxicity and apoptosis in Aβ treated neurons. Furthermore, glutamate also decreases cdh1 and leads to accumulation of glutaminase, suggesting that there may be a positive feedback loop of cdh1 inactivation. We confirmed the main findings in vivo using microinjection of either Aβ or glutamate in the CA1 region of the rat hippocampus. We show here for the first time in vivo that both Aβ and glutamate cause nuclear exclusion of cdh1 and an increase in glutaminase. These results show that maintaining normal APC/C-Cdh1 activity may be a useful target in Alzheimer's disease treatment.
Sci Rep, 2016; 6

26391042: Lloret A, Fuchsberger T, Giraldo E, Vina J
Reductive Stress: A New Concept in Alzheimer's Disease.
Reactive oxygen species play a physiological role in cell signaling and also a pathological role in diseases, when antioxidant defenses are overwhelmed causing oxidative stress. However, in this review we will focus on reductive stress that may be defined as a pathophysiological situation in which the cell becomes more reduced than in the normal, resting state. This may occur in hypoxia and also in several diseases in which a small but persistent generation of oxidants results in a hormetic overexpression of antioxidant enzymes that leads to a reduction in cell compartments. This is the case of Alzheimer's disease. Individuals at high risk of Alzheimer's (because they carry the ApoE4 allele) suffer reductive stress long before the onset of the disease and even before the occurrence of mild cognitive impairment. Reductive stress can also be found in animal models of Alzheimer's disease (APP/PS1 transgenic mice), when their redox state is determined at a young age, i.e. before the onset of the disease. Later in their lives they develop oxidative stress. The importance of understanding the occurrence of reductive stress before any signs or symptoms of Alzheimer's has theoretical and also practical importance as it may be a very early marker of the disease.
Curr Alzheimer Res, 2016; 13
25746773: Lloret A, Fuchsberger T, Giraldo E, Viña J
Molecular mechanisms linking amyloid β toxicity and Tau hyperphosphorylation in Alzheimer′s disease.
Neurofibrillary tangles (aggregates of cytoskeletal Tau protein) and senile plaques (aggregates mainly formed by amyloid β peptide) are two landmark lesions in Alzheimer′s disease. Some researchers have proposed tangles, whereas others have proposed plaques, as primary lesions. For a long time, these were thought of as independent mechanisms. However, experimental evidence suggests that both lesions are intimately related. We review here some molecular pathways linking amyloid β and Tau toxicities involving, among others, glycogen synthase kinase 3β, p38, Pin1, cyclin-dependent kinase 5, and regulator of calcineurin 1. Understanding amyloid β and Tau toxicities as part of a common pathophysiological mechanism may help to find molecular targets to prevent or even treat the disease.
Free Radic Biol Med, 2015; 83
25061569: Giraldo E, Lloret A, Fuchsberger T, Viña J
Aβ and tau toxicities in Alzheimer's are linked via oxidative stress-induced p38 activation: protective role of vitamin E.
Oxidative stress is a hallmark of Alzheimer's disease (AD). We propose that rather than causing damage because of the action of free radicals, oxidative stress deranges signaling pathways leading to tau hyperphosphorylation, a hallmark of the disease. Indeed, incubation of neurons in culture with 5 μM beta-amyloid peptide (Aβ) causes an activation of p38 MAPK (p38) that leads to tau hyperphosphorylation. Inhibition of p38 prevents Aβ-induced tau phosphorylation. Aβ-induced effects are prevented when neurons are co-incubated with trolox (the water-soluble analog of vitamin E). We have confirmed these results in vivo, in APP/PS1 double transgenic mice of AD. We have found that APP/PS1 transgenic mice exhibit a high level of P-p38 in the hippocampus but not in cortex and this is prevented by feeding animals with a diet supplemented with vitamin E. Our results underpin the role of oxidative stress in the altered cell signaling in AD pathology and suggest that antioxidant prevention may be useful in AD therapeutics.
Redox Biol, 2014; 2

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MECHANISMS OF PRESYNAPTIC CAMP DEPENDENT POTENTIATION

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Hippocampal mossy fiber (MF) synapses play a key role in the processing of spatial information in the CA3 region of the hippocampus. These synapses exhibit a presynaptic cyclic adenosine monophosphate (cAMP)-dependent long-term potentiation (LTP) that switches the MF mode of action from a conditional to full detonator synapse, thus facilitating plasticity in the CA3 network. The downstream targets of cAMP, protein kinase A and Epac2, have been proposed to contribute to the mechanisms of elevated glutamate release at MF synapses. However, it is still unclear how each of these signaling pathways contributes to the induction and maintenance of MF LTP. In particular, Epac2 was only recently discovered to play a role. This guanine nucleotide exchanging factor is highly expressed in the dentate gyrus and has been recognized to regulate neurotransmitters release in other neurons and insulin secretion in the pancreas. To directly evaluate the role of presynaptic Epac2 in cAMP potentiation at MF synapses, we employed a novel mouse with loxP sites inserted in the Epac2 locus. We are using both a dentate specific Cre mouse cross for chronic ablation of Epac2 throughout development and a dentate specific viral strategy for acute ablation in adult mice. Electrophysiological recordings from CA3 neurons are utilized to evaluate basal synaptic properties and the integrity of short- and long-term potentiation of MF synapses following Epac2 ablation. Taken together, these experiments will provide a further understanding of the basic mechanisms underlying MF LTP, a process that is disrupted in normal aging and neuropsychiatric disorders.

Pubmed:
33754629: Chiesa M, Rabiei H, Riffault B, Ferrari DC, Ben-Ari Y
Brain Volumes in Mice are Smaller at Birth After Term or Preterm Cesarean Section Delivery.
The rate of cesarean section (CS) delivery has steadily increased over the past decades despite epidemiological studies reporting higher risks of neonatal morbidity and neurodevelopmental disorders. Yet, little is known about the immediate impact of CS birth on the brain, hence the need of experimental studies to evaluate brain parameters following this mode of delivery. Using the solvent clearing method iDISCO and 3D imaging technique, we report that on the day of birth, whole-brain, hippocampus, and striatum volumes are reduced in CS-delivered as compared to vaginally-born mice, with a stronger effect observed in preterm CS pups. These results stress the impact of CS delivery, at term or preterm, during parturition and at birth. In contrast, cellular activity and apoptosis are reduced in mice born by CS preterm but not term, suggesting that these early-life processes are only impacted by the combination of preterm birth and CS delivery.
Cereb Cortex, 2021; 31
29771287: Chiesa M, Guimond D, Tyzio R, Pons-Bennaceur A, Lozovaya N, Burnashev N, Ferrari DC, Ben-Ari Y
Term or Preterm Cesarean Section Delivery Does Not Lead to Long-term Detrimental Consequences in Mice.
Epidemiological studies have provided contradictory data on the deleterious sequels of cesarean section (C-section) delivery and their links with developmental brain disorders such as Autism Spectrum Disorders. To gain better insight on these issues, we have now compared physiological, morphological, and behavioral parameters in vaginal, term, and preterm C-section delivered mice. We report that C-section delivery does not lead to long-term behavioral alterations though preterm C-section delivery modifies communicative behaviors in pups. Moreover, C-section delivery neither alters the gamma-aminobutyric acid (GABA) developmental excitatory to inhibitory shift nor the frequency or amplitude of glutamatergic and GABAergic postsynaptic currents in hippocampal pyramidal neurons. However, these neurons present an underdeveloped dendritic arbor at birth in pups born by C-section delivery, but this difference disappears 1 day later suggesting an accelerated growth after birth. Therefore, C-section delivery, with prematurity as an aggravating factor, induces transient developmental delays but neither impacts the GABA developmental sequence nor leads to long-term consequences in mice. The deleterious sequels of C-section delivery described in epidemiological studies might be due to a perinatal insult that could be aggravated by C-section delivery.
Cereb Cortex, 2019; 29

31354805: Chiesa M, Nardou R, Lozovaya N, Eftekhari S, Tyzio R, Guimond D, Ferrari DC, Ben-Ari Y
Enhanced Glutamatergic Currents at Birth in Shank3 KO Mice.
Autism spectrum disorders (ASD) are neurodevelopmental disorders induced by genetic and environmental factors. In our recent studies, we showed that the GABA developmental shifts during delivery and the second postnatal week are abolished in two rodent models of ASD. Maternal treatment around birth with bumetanide restored the GABA developmental sequence and attenuated the autism pathogenesis in offspring. Clinical trials conducted in parallel confirmed the usefulness of bumetanide treatment to attenuate the symptoms in children with ASD. Collectively, these observations suggest that an alteration of the GABA developmental sequence is a hallmark of ASD. Here, we investigated whether similar alterations occur in the Shank3 mouse model of ASD. We report that in CA3 pyramidal neurons, the driving force and inhibitory action of GABA are not different in naïve and Shank3-mutant age-matched animals at birth and during the second postnatal week. In contrast, the frequency of spontaneous excitatory postsynaptic currents is already enhanced at birth and persists through postnatal day 15. Therefore, in CA3 pyramidal neurons of Shank3-mutant mice, glutamatergic but not GABAergic activity is affected at early developmental stages, hence reflecting the heterogeneity of mechanisms underlying the pathogenesis of ASD.
Neural Plast, 2019; 2019
32151280: Chiesa M, Ferrari DC, Ben-Ari Y
Alteration in the time and/or mode of delivery differentially modulates early development in mice.
Delivery is a complex biological process involving hormonal and mechanical stimuli that together condition the survival and development of the fetus out of the womb. Accordingly, changes in the time or way of being born are associated with an alteration of fundamental biological functions and hypothesized to promote the emergence of neurodevelopmental disorders. Hence, the steadily rise in preterm birth and cesarean section (CS) delivery rates over the past years has become a worldwide health concern. In our previous work, we reported that even though no long-term autistic-like deficits were observed, mice born preterm by CS presented early transient neuronal and communicative defects. However, understanding if these alterations were due to an early birth combined with CS delivery, or if prematurity solely could lead to a similar outcome remained to be evaluated. Using mice born either at term or preterm by vaginal or CS delivery, we assessed early life ultrasonic vocalizations and the onset of eye opening. We report that alterations in communicative behaviors are finely attuned and specifically affected either by preterm birth or by the association between CS delivery and preterm birth in mice, while delayed onset of eye opening is due to prematurity. Moreover, our work further underlies a gender-dependent vulnerability to changes in the time and/or way of being born with distinct outcomes observed in males and females. Thus, our results shed light on the intricacy of birth alterations and might further explain the disparities reported in epidemiological studies.
Mol Brain, 2020; 13
31239460: Lozovaya N, Nardou R, Tyzio R, Chiesa M, Pons-Bennaceur A, Eftekhari S, Bui TT, Billon-Grand M, Rasero J, Bonifazi P, Guimond D, Gaiarsa JL, Ferrari DC, Ben-Ari Y
Early alterations in a mouse model of Rett syndrome: the GABA developmental shift is abolished at birth.
Genetic mutations of the Methyl-CpG-binding protein-2 (MECP2) gene underlie Rett syndrome (RTT). Developmental processes are often considered to be irrelevant in RTT pathogenesis but neuronal activity at birth has not been recorded. We report that the GABA developmental shift at birth is abolished in CA3 pyramidal neurons of Mecp2 mice and the glutamatergic/GABAergic postsynaptic currents (PSCs) ratio is increased. Two weeks later, GABA exerts strong excitatory actions, the glutamatergic/GABAergic PSCs ratio is enhanced, hyper-synchronized activity is present and metabotropic long-term depression (LTD) is impacted. One day before delivery, maternal administration of the NKCC1 chloride importer antagonist bumetanide restored these parameters but not respiratory or weight deficits, nor the onset of mortality. Results suggest that birth is a critical period in RTT with important alterations that can be attenuated by bumetanide raising the possibility of early treatment of the disorder.
Sci Rep, 2019; 9

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DIFFERENT LTP MECHANISMS AMONG DENTATE GRANULE CELLS WITH DIFFERENT TYPES OF SPIKING PATTERN

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

Yoonsub Kim, Suk-Ho Lee, Won-Kyung Ho
Seoul National University College of Medicine, Physiology, Seoul, Korea, Republic of

Mature granule cells (GCs) in the dentate gyrus are characterized by low excitability due to low input resistance and hyperpolarized resting membrane potential. Nevertheless, burst firing is often observed in GCs as in other pyramidal neurons where it plays important roles for neuronal signaling and synaptic plasticity. However, physiological significance of bursting in GCs is not well understood. In the present study, we classified mature GCs into regular-spiking (RS-GCs) and burst-spiking (BS-GCs) based on their initial firing frequency, and investigated how they responded differently to synaptic inputs. Excitatory postsynaptic potentials evoked by stimulating lateral perforant pathway were potentiated after a high-frequency stimulation (HFS, 10 stimuli at 100 Hz) with a subthreshold intensity. This potentiation, referred to as LTP-sub, was found to be NMDAR-dependent. There was no significance difference in the magnitude of LTP-sub between groups (128.90 ± 5.00%, BS-GCs; 139.69 ± 5.30%, RS-GCs). Interestingly, we could induce further potentiation in BS-GCs (138.73 ± 6.70%), but not in RS-GCs (98.67 ± 6.42%), by applying the second HFS with a higher intensity that evoked at least 3 action potentials (APs). This AP-dependent LTP (referred to as LTP-AP) was dependent on L-type calcium channel (LTCC). We found that initial AP frequency induced by the second HFS was higher in BS-GCs (133.66 ± 12.17 Hz) than in RS-GCs (81.17 ± 10.68 Hz). Taken together, it is suggested that intrinsic firing properties affect synaptically driven firing, and that bursting behavior is critical for inducing LTP-AP in GCs.

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CHANGES OF SPIKE TIMING IN TOPOLOGICALLY CONSTRAINED NETWORKS UNDER REPEATED ELECTRICAL STIMULATION

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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The exact mechanisms of how learning and memory is achieved is arguably one of the fundamental questions tackled by the field of neuroscience. However, after many years of research, the answer remains elusive. To find the exact mechanism, we believe it is important to observe neuronal activity with single cell resolution. It is difficult to achieve this in mammal model organisms. Instead, we propose using small primary neuronal networks cultured in-vitro on top of multi-electrode arrays (MEA) in order to record the electrical activity of neurons. By physically confining the space in which neurites can grow using polydimethylsiloxane (PDMS) microstructures, the topology of such networks can be constrained [1]. It is possible to highly parallelize experiments by compartmentalizing the MEA into multiple independent networks. By stimulating networks of primary rat hippocampal cells repeatedly every 250 ms, we have recently shown that the spiking response of the network stays remarkably constant for multiple hours [2]. However some networks showed highly reproducible changes in spike timing. Changes in spike timing can be interpreted as a form of plasticity. We found that roughly 20% of the spikes expressed depressing effects, while 5% experienced potentiating responses. We further observed that the number of depressing spike responses increased over time, while there was no significant change in potentiating effects. These results show that topologically constrained networks are a useful tool for plasticity research. [1]: Forró, et al. *Biosensors and Bioelectronics* 122 (2018): 75-87. [2]: Ihle, et al. *Biosensors and Bioelectronics* 201 (2022): 113896.

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GROUP I METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED MODULATION OF EXCITATORY SYNAPTIC TRANSMISSION SHOWS INTERNEURON SPECIFICITY IN THE HUMAN NEOCORTEX

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Activation of group I metabotropic glutamate receptors (mGluR) in the brain mediates changes in neuronal excitability, synaptic transmission, and network activity of cortical circuits. Influence of mGluRs on neural activity in the neocortex has been linked to learning-related plasticity, brain state-modulation as well as various human neurological disorders but studies investigating their effect in human brain are scarce. We performed intracellular recordings from synaptically-connected glutamatergic pyramidal cell-to-GABAergic interneuron pairs in layer 2/3 of human neocortical slices to study the effect of group I mGluR activation on these neurons and their synaptic communication. We found that activation of group I mGluRs by agonist (S)-3,5-dihydroxyphenylglycine (DHPG) modulated interneurons in subtype-dependent manner. We observed depression of excitatory synaptic transmission strength in non fast-spiking adaptive firing interneurons whereas most fast-spiking basket cells and axo-axonic cells exhibited potentiation of their synaptic excitatory input by the agonist. Parallel experiments in Wistar rat showed DHPG-mediated strengthening of glutamatergic input to fast-spiking basket cells. Our results demonstrate cell type-specific modulation of human neocortical neurons and their synaptic excitation by group I metabotropic receptor activation.

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STUDY OF SYNAPTIC PLASTICITY MECHANISMS UNDERLYING MEMORY CONSOLIDATION IN THE HIPPOCAMPO-NEOCORTICAL NETWORK

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Learning and consolidation of a behavioural response is a multistep process, taking place over multiple days, which implicates an active communication between the hippocampus and multiple associative cortical areas. The consolidation phase happens during slow wave sleep when hippocampal Sharp Wave-Ripples (SW-Rs) events occur. SW-Rs are replays of wakeful-acquired memory and are known to be involved in memory consolidation. SW-Rs are thought to strengthen synaptic connections within memory-related assemblies via synaptic plasticity mechanisms, however direct proof of this concept has been hard to obtain. The aim of my research is to unravel the synaptic mechanism underlying hippocampal to associative cortical areas communication in the context of memory consolidation and address whether and when synaptic plasticity takes place at hippocampal to cortical synapses. To this aim, I record local field potentials and unitary activity from extracellular electrodes placed in the dorsal hippocampus, medial prefrontal (mPFC) and posterior parietal (PPC) cortices in freely behaving animals performing a spatial memory task (Delayed Spatial Alternation task) and during a 3 hours resting period following the task. My results show that neuronal firing of excitatory pyramidal cells and inhibitory interneurons in the mPFC, during the occurrence of hippocampal SW-Rs, is enhanced after learning suggesting a change in synaptic strength between these two brain areas. My goal is now to manipulate synaptic plasticity at these afferences to unravel the synaptic mechanisms underlying memory consolidation in cortical associative areas.

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INTERPLAY OF THALAMO- AND CORTICOSTRIATAL PLASTICITY IN BEHAVIORAL FLEXIBILITY

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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The striatum is a major site of memory formation as the acquisition of a behavioral repertoire has been associated with cortico-striatal (CS) plasticity. Striatum integrates inputs from the cortex and thalamus that display concomitant or sequential activity. If a vast literature has focused on CS plasticity, little remains known about thalamo-striatal (TS) plasticity rules, their interplay with CS plasticity, and involvement in behavioral flexibility. We recorded in vivo plasticity in mice engaged in procedural learning on a horizontal ladder and found CS-LTP of opto-evoked LFP upon learning of a rung pattern#1 (without modification of TS-evoked-LFP), whereas this plasticity was canceled upon novelty (change for a rung pattern#2) to the profit of a TS-LTP. In vivo recording, of TS/CS activity during this task revealed activity patterns reminiscent of time-coding plasticity. Therefore, we characterized the input-timing dependent plasticity at CS and TS synapses in brain slices, in supra- and subthreshold regimes. We found that most temporal combinations induced LTP except when thalamus (supra) activity precedes cortical one, where LTD was observed, highlighting the crucial impact of timing in cortical and thalamic activities for the memory engram at striatal synapses. Ex vivo occlusion experiment showed that CS-LTP and TS-LTP were engaged during procedural learning and novelty, respectively. Interestingly, the modification of the rung patterning induced an increase in TS synaptic weight, while CS transmission decreases. The ability of TS plasticity to override CS plasticity could allow for flexible behavior, helping the exit of an existing automatism in favor of a new behavioral strategy.

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ENDOCANNABINOID-MEDIATED PLASTICITY AT CORTICO-STRIATAL SYNAPSES DURING FAST LEARNING

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Fast learning designates the mechanisms underlying the acquisition of a long-term memory after a unique and brief experience. Considering that a brief exposure to a stimulus involves only a small number of spiking events and based on our earlier *in vitro* work uncovering a new form of synaptic potentiation induced by a very low number of temporally coupled cortical and striatal spikes and dependent on the endocannabinoid system (Cui et al., 2015), we hypothesize that the endocannabinoid system could underlie the acquisition of fast learning in the dorsolateral striatum. We developed a fast learning task in which mice learn to avoid contacting an adhesive tape after a single brief exposure. This experience induces a long-lasting memory, along with a cortico-striatal potentiation observed *in vivo*, by following the striatal response to optogenetically-evoked cortical stimulation. *Ex vivo* occlusion plasticity experiments confirmed the involvement of the endocannabinoid pathway. Furthermore, mice in which endocannabinoid-mediated potentiation (eCB-LTP) is altered, by either genetically knocking-out dopaminergic type-2 receptors (Xu et al., 2018) or inhibiting striatal CB1 receptors via striatal micro-infusions of AM251, exhibited degraded fast learning performance. Recordings of cortical and striatal units performed using Neuropixel probes in acute behaving animals in the Mobile Home Cage (Neurotar) allow us to extract neuronal activity patterns that may be responsible for eCB-LTP induction. The strength of cortico-striatal synapses is modified during the encoding of one-shot memories. Importantly, the endocannabinoid system, which can be activated with low spiking activity levels, appears to be a key molecular substrate for fast learning.

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VALENCE-DEPENDENT SYNAPTIC PLASTICITY IN SOCIAL CONTEXT INSTRUCTS APPROACH/AVOIDANCE BEHAVIOR

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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To decide whether to approach or avoid a conspecific, individuals need first to recognize the possibly positive (appetitive) or negative (aversive) valence of the stimulus and learn this association. The Nucleus Accumbens (NAc) is a key brain region of the mesocorticolimbic circuits for evaluating valence. However, how valence is codified at the synaptic level in a social context is still an open question. Within the NAc, D1 receptor-containing Medium Spiny Neurons (MSNs) have been related to rewarding and motivational aspects of social behavior. Using a free social interaction paradigm and calcium imaging techniques, we demonstrated that D1-MSNs respond to positive and negative social valence stimuli. Using anatomical tracing and *in vitro*electrophysiological recordings, we found D1-MSN strongly connected with glutamatergic neurons from the Anterior Insular Cortex (AIC) that also express D1Rs revealing a novel D1R-D1R top-down circuit between AIC and NAc. A day after the first social interaction, we evaluated glutamatergic synaptic parameters and found a dichotomous, long-term valence-dependent synaptic plasticity that occurs specifically in these neurons. Interestingly, these forms of plasticity are triggered by different firing frequencies from AIC inputs. Specifically, we showed that low-frequency stimulation drives positive valence-like plasticity, whereas high-frequency stimulation induces negative valence-like plasticity. By recruiting the same D1R positive neurons within the AIC-NAc circuit, we demonstrated long-term synaptic plasticity signature of social valence tuned by firing frequency.

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INTACT INDUCTION AND PRESYNAPTIC OCCLUSION OF SHORT AND LONG-TERM POTENTIATION IN SYNAPTOPHYSIN FAMILY KNOCKOUTS

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Synaptophysin/gyrin family proteins are ubiquitous components of synaptic vesicle membranes. Baseline synaptic strength is elevated in quadruple knockouts owing to elevated probability of release of neurotransmitter from readily releasable vesicles within presynaptic terminals. And, both short- and long-term synaptic potentiation are severely degraded. Deficits in short-term potentiation could result directly from the elevated baseline release via an occlusion mechanism. However, key types of long-term potentiation that require NMDA receptors for induction (NMDAR-LTP) are thought to be expressed by unrelated mechanisms, such as more AMPA receptors. Nevertheless, we now find that NMDAR-LTP in knockouts can be rescued by reducing extracellular Ca^{2+}, which lowers release probability back to wildtype levels. Likewise, increasing Ca^{2+} occludes LTP expression at wildtype synapses without blocking induction. The results indicate that both short- and long-term synaptic plasticity deficits in synaptophysin knockouts are caused by the elevated baseline release, and that the longest-lasting components of NMDAR-LTP are expressed by presynaptic mechanisms subject to the same kinds of endogenous limitations that shape short-term plasticity.

Pubmed:

31118412: Fernández-Chacón M, Casquero-García V, Luo W, Francesca Lunella F, Ferreira Rocha S, Del Olmo-Cabrera S, Benedito R
iSuRe-Cre is a genetic tool to reliably induce and report Cre-dependent genetic modifications.
Most biomedical research aimed at understanding gene function uses the Cre-Lox system, which consists of the Cre recombinase-dependent deletion of genes containing LoxP sites. This system enables conditional genetic modifications because the expression and activity of the recombinase Cre/CreERT2 can be regulated in space by tissue-specific promoters and in time by the ligand tamoxifen. Since the precise Cre-Lox recombination event is invisible, methods were developed to report Cre activity and are widely used. However, numerous studies have shown that expression of a given Cre activity reporter cannot be assumed to indicate deletion of other LoxP-flanked genes of interest. Here, we report the generation of an inducible dual reporter-Cre mouse allele, iSuRe-Cre. By significantly increasing Cre activity in reporter-expressing cells, iSuRe-Cre provides certainty that these cells have completely recombined floxed alleles. This genetic tool increases the ease, efficiency, and reliability of conditional mutagenesis and gene function analysis.
Nat Commun, 2019; 10
31090538: Raja MK, Preobraschenski J, Del Olmo-Cabrera S, Martinez-Turrillas R, Jahn R, Perez-Otano I, Wesseling JF
Elevated synaptic vesicle release probability in synaptophysin/gyrin family quadruple knockouts.
Synaptophysins 1 and 2 and synaptogyrins 1 and 3 constitute a major family of synaptic vesicle membrane proteins. Unlike other widely expressed synaptic vesicle proteins such as vSNAREs and synaptotagmins, the primary function has not been resolved. Here, we report robust elevation in the probability of release of readily releasable vesicles with both high and low release probabilities at a variety of synapse types from knockout mice missing all four family members. Neither the number of readily releasable vesicles, nor the timing of recruitment to the readily releasable pool was affected. The results suggest that family members serve as negative regulators of neurotransmission, acting directly at the level of exocytosis to dampen connection strength selectively when presynaptic action potentials fire at low frequency. The widespread expression suggests that chemical synapses may play a frequency filtering role in biological computation that is more elemental than presently envisioned.
Elife, 2019; 8
28802047: Pontes-Quero S, Heredia L, Casquero-García V, Fernández-Chacón M, Luo W, Hermoso A, Bansal M, Garcia-Gonzalez I, Sanchez-Muñoz MS, Perea JR, Galiana-Simal A, Rodriguez-Arabaolaza I, Del Olmo-Cabrera S, Rocha SF, Criado-Rodriguez LM, Giovinazzo G, Benedito R

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Dual ifgMosaic: A Versatile Method for Multispectral and Combinatorial Mosaic Gene-Function Analysis.

Improved methods for manipulating and analyzing gene function have provided a better understanding of how genes work during organ development and disease. Inducible functional genetic mosaics can be extraordinarily useful in the study of biological systems; however, this experimental approach is still rarely used in vertebrates. This is mainly due to technical difficulties in the assembly of large DNA constructs carrying multiple genes and regulatory elements and their targeting to the genome. In addition, mosaic phenotypic analysis, unlike classical single gene-function analysis, requires clear labeling and detection of multiple cell clones in the same tissue. Here, we describe several methods for the rapid generation of transgenic or gene-targeted mice and embryonic stem (ES) cell lines containing all the necessary elements for inducible, fluorescent, and functional genetic mosaic (ifgMosaic) analysis. This technology enables the interrogation of multiple and combinatorial gene function with high temporal and cellular resolution.

Cell, 2017; 170

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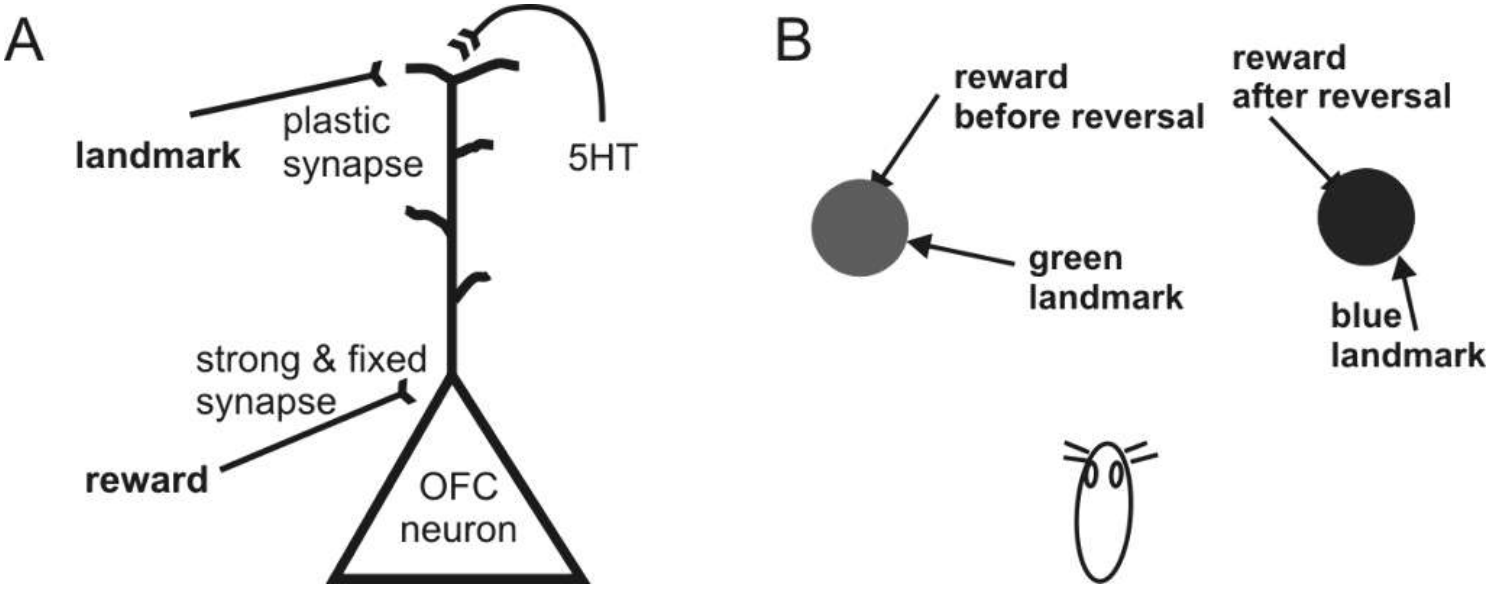
BOARD NUMBER: S02-293

BEYOND THE REWARD PREDICTION ERROR: ACHIEVING REVERSAL LEARNING WITH HEBBIAN CORTICAL PLASTICITY AND SEROTONERGIC MODULATION

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

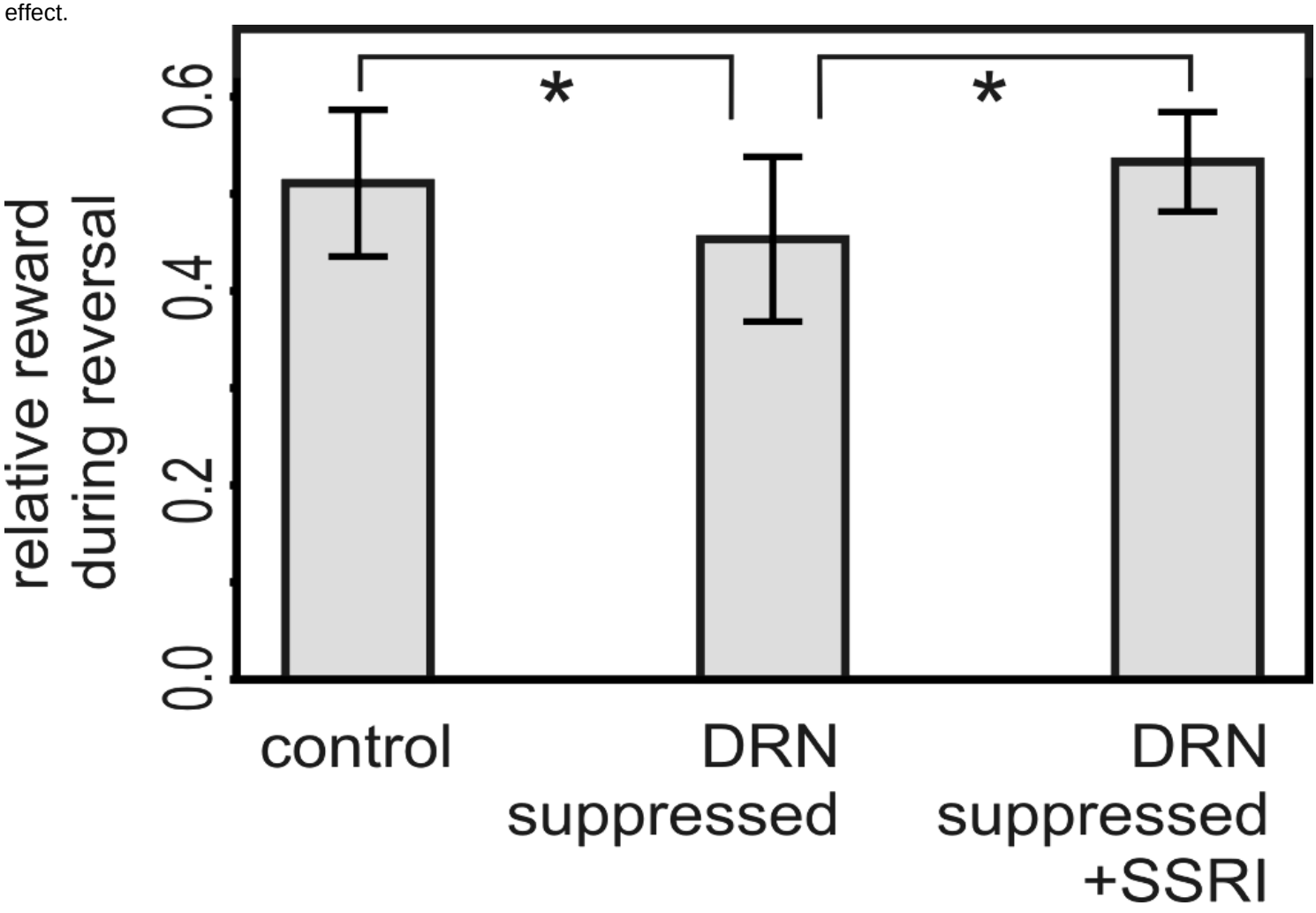
Bernd Porr, Gillian Burnet
University of Glasgow, Biomedical Engineering, Glasgow, United Kingdom

We present a model of reversal learning which uses cortical neurons for its computations and won't require a reward prediction
error.



It utilises the structure and functional differentiation of cortical pyramidal neurons, in particular here of orbitofrontal cortex (OFC) neurons (see Panel A). The primary reward drives non-plastic synapses close to the soma of pyramidal neurons and causes strong postsynaptic responses which in turn cause a large calcium influx. New associations such as place or landmark information (Panel B) are established with Hebbian plasticity via plastic synapses in the dendritic tree (Panel A). LTP is triggered when strong somatic reward inputs drive a cortical neuron into spiking and cause a strong influx of calcium. LTD is triggered when the primary reward is not driving the OFC pyramidal neurons causing less and slower changing postsynaptic activity. Serotonin here acts as an accelerator for both LTP and LTD being a rectified reward prediction error. Experiments of a simulated animal (Panel B) performing a reversal task were run for a control scenario, for one with less serotonin and one with a simulated application of serotonin reuptake inhibitors. We show that Hebbian plasticity is able to model reversal learning and that serotonin controls the rate of plasticity which in turn regulates the animal's flexibility. Reduced serotonin increases the number of non-rewarding actions during reversal. SSRIs can help to overcome this deficiency, but we'd predict that any drug boosting plasticity, such as psychedelics, will have this

effect.


relative reward during reversal
0.0
0.2
0.4
0.6
*
*
control
DRN suppressed
DRN suppressed +SSRI

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BOARD NUMBER: S02-294

CLOSED-LOOP OPTOGENETIC STIMULATION CHANGES SPIKE TRANSMISSION GAIN IN FREELY MOVING MICE

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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The precise timing and temporal ordering of neural spikes leads to the strengthening or weakening of synapses, and are thought to play a crucial role in circuit refinement, learning and memory. Spike timing-dependent plasticity (STDP) has been studied extensively in vitro and quantified mainly using the amplitude or the slope of the postsynaptic potentials. However, the extent to which spike transmission itself is affected by spike timing in intact animals is unknown. Here, we studied how spike transmission between two neurons is modified by inducing spikes in postsynaptic neurons, contingent on presynaptic spike timing. For that, we performed closed-loop optogenetic stimulation experiments, in which a pyramidal cell (PYR) spike triggered light activation of parvalbumin-immunoreactive interneurons (INT). Experiments were carried out in neocortex and hippocampal region CA1 of six freely-moving mice. The closed-loop manipulation resulted in new multi-neuronal spatiotemporal spike patterns, in which the presynaptic PYR fired several milliseconds before increased firing rate of the postsynaptic INT. We found that following repetitive timed spiking of the pre- and postsynaptic cells, spike transmission gain increased in most PYR-INT pairs in which the INT was light activated. In contrast, PYR-INT pairs in which the INT was not activated by light did not show a consistent increase of spike transmission gain. Thus, our results show that the temporal ordering of multi-neuronal spike trains induces persistent changes in spike transmission gain. **Funding: ERC #679253, ISF #638/16, and CIHR-IDRC-ISF #2558/18**

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BOARD NUMBER: S02-295

ALL OPTICAL INTERROGATION OF CA1 SYNAPSES AND NEURONS IN VIVO

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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The hippocampus encodes information about an animal's position in space, integrating internal representations about the environment with sensory inputs, including tactile, auditory and visual stimuli. Most prominently, spatial information is encoded in CA1 place cells. However, while spatial memories can be retained over long time periods, place cell ensembles reorganize over days. While the cellular dynamics of hippocampal coding are well-described, less is known about functional and structural dynamics of individual Schaffer collateral synapses. There is no consensus with regard to the stability of synapses over time. While some studies suggest high turnover of spines, others found high stability. Moreover, a link between synaptic function and structural plasticity is missing. While shown *in vitro* that spine survival is influenced by functional plasticity, it is not known to what extent this link between synapse stability and activity *in vivo* is preserved. Thus, to better understand how dynamic remapping of cellular ensembles is related to synapse dynamics, we need to establish a link between functional and structural synaptic plasticity *in vivo*. In this study, we chronically imaged dendritic spines on CA1 cells in the awake mouse, monitoring their synaptic responses by optogenetically stimulating presynaptic CA3 cells. Using this approach, we were able to induce local, synaptic calcium responses at individual spines and to assess the stability of these functionally identified synapses over a time period of two weeks.

Pubmed:
33793545: Yang W, Chini M, Pöpplau JA, Formozov A, Dieter A, Piechocinski P, Rais C, Morellini F, Sporns O, Hanganu-Opatz IL, Wiegert JS
Anesthetics fragment hippocampal network activity, alter spine dynamics, and affect memory consolidation.
General anesthesia is characterized by reversible loss of consciousness accompanied by transient amnesia. Yet, long-term memory impairment is an undesirable side effect. How different types of general anesthetics (GAs) affect the hippocampus, a brain region central to memory formation and consolidation, is poorly understood. Using extracellular recordings, chronic 2-photon imaging, and behavioral analysis, we monitor the effects of isoflurane (Iso), medetomidine/midazolam/fentanyl (MMF), and ketamine/xylazine (Keta/Xyl) on network activity and structural spine dynamics in the hippocampal CA1 area of adult mice. GAs robustly reduced spiking activity, decorrelated cellular ensembles, albeit with distinct activity signatures, and altered spine dynamics. CA1 network activity under all 3 anesthetics was different to natural sleep. Iso anesthesia most closely resembled unperturbed activity during wakefulness and sleep, and network alterations recovered more readily than with Keta/Xyl and MMF. Correspondingly, memory consolidation was impaired after exposure to Keta/Xyl and MMF, but not Iso. Thus, different anesthetics distinctly alter hippocampal network dynamics, synaptic connectivity, and memory consolidation, with implications for GA strategy appraisal in animal research and clinical settings.
PLoS Biol, 2021; 19

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DENDRITIC SPINE NECK RESTRICTION REGULATES HETEROSYNAPTIC PLASTICITY

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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[1]University of Bristol, Department Of Computer Sciences, Bristol, United Kingdom, [2]Magee Campus-Ulster University, School Of Computing, Engineering And Intelligent Systems, Londonderry, United Kingdom

Synaptic plasticity is a widely recognised physiological correlate of learning and memory. An important but poorly understood phenomenon is heterosynaptic plasticity. Long-term potentiation (LTP) and depression (LTD) at an stimulated synapse (homosynaptic plasticity) can cause either LTP or LTD at neighbouring synapses, depending on experimental conditions. The factors determining these forms of heterosynaptic plasticity are currently unknown. Here, we propose a new theory that explains how differential restrictions imposed by dendritic spine neck plasticity on membrane and cytosolic diffusions of plasticity-associated molecular signals may control the rules of heterosynaptic plasticity. To demonstrate this theory in practice, we developed a compartment-based reaction-diffusion model of Ca^{2+}/Calmodulin-dependent Kinase II (CaMKII)-Calcineurin synaptic signalling and AMPA receptor (AMPAR) trafficking in a 100 micronmeter long dendritic branch bearing hundreds of spines. We fit the AMPAR trafficking parameters to previously published fluorescence imaging data from glutamate uncaging experiments on single spine in CA1 pyramidal neurons (Patterson et al, PNAS 2010). We found that the model could replicate all four forms of heterosynaptic plasticity, depending on where a spine neck sits in the two-dimensional parameter space of membrane vs. cytosolic diffusion restrictions. For high neck surface restriction, homosynaptic LTP and LTD consistently lead to heterosynaptic LTD. For lesser neck surface restriction, homosynaptic LTP and LTD cause heterosynaptic LTP or LTD depending on relative intensities of synaptic CaMKII and Calcineurin activation, and neck restriction to Calcineurin diffusion. This model makes new predictions for experiments and offers a theoretical bridge between molecular processes and learning rules in the brain.

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BOARD NUMBER: S02-297

CAMP MODULATES NEURONAL EXCITABILITY, NOT LONG-TERM PLASTICITY

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Center for Molecular Neurobiology Hamburg (ZMNH), Institute For Synaptic Physiology, Hamburg, Germany

Cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger that, when raised by forskolin, usually together with picrotoxin and rolipram to block GABAA and phosphodiesterases induces long-term potentiation (LTP) of synaptic transmission and expression of immediate early genes such as cFos by activating the postsynaptic PKA-CREB pathway. Most studies, however, have relied on pharmacological tools whose actions are not confined to specific cells or synaptic compartments. Here, we take advantage of recent developments in photoactivatable adenylyl cyclases to raise cAMP in single postsynaptic hippocampal CA1 neurons, in many presynaptic CA3 neurons, many postsynaptic CA1 neurons or in all excitatory neurons to investigate compartment-specific effects of cAMP at Schaffer collateral synapses. Surprisingly, optogenetically raising cAMP in single postsynaptic CA1 neurons induced neither LTP nor cFos expression. Whereas, similarly to the effects of forskolin, optogenetically increasing cAMP in excitatory neurons throughout the whole hippocampal slice, induced LTP and strong cFos expression. Interestingly, raising cAMP in only presynaptic or only postsynaptic neurons was insufficient to induce LTP and moderately increased the intensity of cFos labeling. In contrast to current models of intracellular signaling cascades, there is no direct pathway leading from cAMP to cFos induction in individual neurons. Rather cAMP increases excitability and transmitter release and these are required for cFos and LTP. The function of neuronal cAMP as a plasticity trigger thus requires an intact, synaptically connected network.

Pubmed:

34663304: Yang S, Constantin OM, Sachidanandan D, Hofmann H, Kunz TC, Kozjak-Pavlovic V, Oertner TG, Nagel G, Kittel RJ, Gee CE, Gao S
PACmn for improved optogenetic control of intracellular cAMP.
Cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger that transduces extracellular signals in virtually all eukaryotic cells. The soluble Beggiatoa photoactivatable adenylyl cyclase (bPAC) rapidly raises cAMP in blue light and has been used to study cAMP signaling pathways cell-autonomously. But low activity in the dark might raise resting cAMP in cells expressing bPAC, and most eukaryotic cyclases are membrane-targeted rather than soluble. Our aim was to engineer a plasma membrane-anchored PAC with no dark activity (i.e., no cAMP accumulation in the dark) that rapidly increases cAMP when illuminated.
BMC Biol, 2021; 19
30333716: Beck S, Yu-Strzelczyk J, Pauls D, Constantin OM, Gee CE, Ehmann N, Kittel RJ, Nagel G, Gao S
Synthetic Light-Activated Ion Channels for Optogenetic Activation and Inhibition.
Optogenetic manipulation of cells or living organisms became widely used in neuroscience following the introduction of the light-gated ion channel channelrhodopsin-2 (ChR2). ChR2 is a non-selective cation channel, ideally suited to depolarize and evoke action potentials in neurons. However, its calcium (Ca) permeability and single channel conductance are low and for some applications longer-lasting increases in intracellular Ca might be desirable. Moreover, there is need for an efficient light-gated potassium (K) channel that can rapidly inhibit spiking in targeted neurons. Considering the importance of Ca and K in cell physiology, light-activated Ca-permeant and K-specific channels would be welcome additions to the optogenetic toolbox. Here we describe the engineering of novel light-gated Ca-permeant and K-specific channels by fusing a bacterial photoactivated adenylyl cyclase to cyclic nucleotide-gated channels with high permeability for Ca or for K, respectively. Optimized fusion constructs showed strong light-gated conductance in oocytes and in rat hippocampal neurons. These constructs could also be used to control the motility of larvae, when expressed in motoneurons. Illumination led to body contraction when motoneurons expressed the light-sensitive Ca-permeant channel, and to body extension when expressing the light-sensitive K channel, both effectively and reversibly paralyzing the larvae. Further optimization of these constructs will be required for application in adult flies since both constructs led to eclosion failure when expressed in motoneurons.
Front Neurosci, 2018; 12

29799525: Scheib U, Broser M, Constantin OM, Yang S, Gao S, Mukherjee S, Stehfest K, Nagel G, Gee CE, Hegemann P
Rhodopsin-cyclases for photocontrol of cGMP/cAMP and 2.3 Å structure of the adenylyl cyclase domain.
The cyclic nucleotides cAMP and cGMP are important second messengers that orchestrate fundamental cellular responses. Here, we present the characterization of the rhodopsin-guanylyl cyclase from Catenaria anguillulae (CaRhGC), which produces cGMP in response to green light with a light to dark activity ratio >1000. After light excitation the putative signaling state forms with τ = 31 ms and decays with τ = 570 ms. Mutations (up to 6) within the nucleotide binding site generate rhodopsin-adenylyl cyclases (CaRhACs) of which the double mutated YFP-CaRhAC (E497K/C566D) is the most suitable for rapid cAMP production in neurons. Furthermore, the crystal structure of the ligand-bound AC domain (2.25 Å) reveals detailed information about the nucleotide binding mode within this recently discovered class of enzyme rhodopsin. Both YFP-CaRhGC and YFP-CaRhAC are favorable optogenetic tools for non-invasive, cell-selective, and spatio-temporally precise modulation of cAMP/cGMP with light.
Nat Commun, 2018; 9

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AVERSION LEARNING MEDIATED BY DOPAMINERGIC NEUROTRANSMISSION IN THE ANTERIOR CINGULATE CORTEX

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

Lotte Jonker, Walter Senn, Thomas Nevian
University of Bern, Department Of Physiology, Bern, Switzerland

Synaptic plasticity is instrumental for cognitive functions. Moreover, cortical plasticity rules can be influenced by neuromodulatory inputs. The anterior cingulate cortex (ACC) is a brain region involved in error detection, pain processing and aversive learning. The ACC is highly regulated by dopaminergic inputs, mainly from the ventral tegmental area (VTA). However, the relationship between synaptic plasticity and the role of dopamine (DA) in the ACC in the context of aversive learning has not yet been characterized. We found that chemogenetic inhibition of ACC neuronal activity suppressed aversive learning. Likewise, silencing ACC inputs from the VTA impaired aversive learning. Moreover, at a cellular level, our results demonstrate that at L5 pyramidal neurons in the ACC, DA facilitates plasticity at electrically-evoked proximal, but not distal, synapses in an NMDAR-mediated, dopamine-1-receptor dependent manner. These results suggest that DA contributes to strengthen synaptic connections of ACC L5 pyramidal neurons, a mechanism that in vivo is partly mediated by VTA inputs underlying aversive learning.

Pubmed:

35078926: Hogrefe N, Blom SM, Valentinova K, Ntamati NR, Jonker LJE, Nevian NE, Nevian T
Long-Lasting, Pathway-Specific Impairment of a Novel Form of Spike-Timing-Dependent Long-Term Depression by Neuropathic Pain in the Anterior Cingulate Cortex.
Malfunctioning synaptic plasticity is one of the major mechanisms contributing to the development of chronic pain. We studied spike-timing dependent depression (tLTD) in the anterior cingulate cortex (ACC) of male mice, a brain region involved in processing emotional aspects of pain. tLTD onto layer 5 pyramidal neurons depended on postsynaptic calcium-influx through GluN2B-containing NMDARs and retrograde signaling via nitric oxide to reduce presynaptic release probability. After chronic constriction injury of the sciatic nerve, a model for neuropathic pain, tLTD was rapidly impaired; and this phenotype persisted even beyond the time of recovery from mechanical sensitization. Exclusion of GluN2B-containing NMDARs from the postsynaptic site specifically at projections from the anterior thalamus to the ACC caused the tLTD phenotype, whereas signaling downstream of nitric oxide synthesis remained intact. Thus, transient neuropathic pain can leave a permanent trace manifested in the disturbance of synaptic plasticity in a specific afferent pathway to the cortex. Synaptic plasticity is one of the main mechanisms that contributes to the development of chronic pain. Most studies have focused on potentiation of excitatory synaptic transmission, but very little is known about the reduction in synaptic strength. We have focused on the ACC, a brain region associated with the processing of emotional and affective components of pain. We studied spike-timing dependent LTD, which is a biologically plausible form of synaptic plasticity, that depends on the relative timing of presynaptic and postsynaptic activity. We found a long-lasting and pathway-specific suppression of the induction mechanism for spike-timing dependent LTD from the anterior thalamus to the ACC, suggesting that this pathology might be involved in altered emotional processing in pain.
J Neurosci, 2022; 42
33046549: Marti Mengual U, Wybo WAM, Spierenburg LJE, Santello M, Senn W, Nevian T
Efficient Low-Pass Dendro-Somatic Coupling in the Apical Dendrite of Layer 5 Pyramidal Neurons in the Anterior Cingulate Cortex.
Signal propagation in the dendrites of many neurons, including cortical pyramidal neurons in sensory cortex, is characterized by strong attenuation toward the soma. In contrast, using dual whole-cell recordings from the apical dendrite and soma of layer 5 (L5) pyramidal neurons in the anterior cingulate cortex (ACC) of adult male mice we found good coupling, particularly of slow subthreshold potentials like NMDA spikes or trains of EPSPs from dendrite to soma. Only the fastest EPSPs in the ACC were reduced to a similar degree as in primary somatosensory cortex, revealing differential low-pass filtering capabilities. Furthermore, L5 pyramidal neurons in the ACC did not exhibit dendritic Ca spikes as prominently found in the apical dendrite of S1 (somatosensory cortex) pyramidal neurons. Fitting the experimental data to a NEURON model revealed

that the specific distribution of , , , and was sufficient to explain the electrotonic dendritic structure causing a leaky distal dendritic compartment with correspondingly low input resistance and a compact perisomatic region, resulting in a decoupling of distal tuft branches from each other while at the same time efficiently connecting them to the soma. Our results give a biophysically plausible explanation of how a class of prefrontal cortical pyramidal neurons achieve efficient integration of subthreshold distal synaptic inputs compared with the same cell type in sensory cortices. Understanding cortical computation requires the understanding of its fundamental computational subunits. Layer 5 pyramidal neurons are the main output neurons of the cortex, integrating synaptic inputs across different cortical layers. Their elaborate dendritic tree receives, propagates, and transforms synaptic inputs into action potential output. We found good coupling of slow subthreshold potentials like NMDA spikes or trains of EPSPs from the distal apical dendrite to the soma in pyramidal neurons in the ACC, which was significantly better compared with S1. This suggests that frontal pyramidal neurons use a different integration scheme compared with the same cell type in somatosensory cortex, which has important implications for our understanding of information processing across different parts of the neocortex.
J Neurosci, 2020; 40

27641766: Smit-Rigter L, Rajendran R, Silva CA, Spierenburg L, Groeneweg F, Ruimschotel EM, van Versendaal D, van der Togt C, Eysel UT, Heimel JA, Lohmann C, Levelt CN
Mitochondrial Dynamics in Visual Cortex Are Limited In Vivo and Not Affected by Axonal Structural Plasticity.
Mitochondria buffer intracellular Ca and provide energy [1]. Because synaptic structures with high Ca buffering [2-4] or energy demand [5] are often localized far away from the soma, mitochondria are actively transported to these sites [6-11]. Also, the removal and degradation of mitochondria are tightly regulated [9, 12, 13], because dysfunctional mitochondria are a source of reactive oxygen species, which can damage the cell [14]. Deficits in mitochondrial trafficking have been proposed to contribute to the pathogenesis of Parkinson's disease, schizophrenia, amyotrophic lateral sclerosis, optic atrophy, and Alzheimer's disease [13, 15-19]. In neuronal cultures, about a third of mitochondria are motile, whereas the majority remains stationary for several days [8, 20]. Activity-dependent mechanisms cause mitochondria to stop at synaptic sites [7, 8, 20, 21], which affects synapse function and maintenance. Reducing mitochondrial content in dendrites decreases spine density [22, 23], whereas increasing mitochondrial content or activity increases it [7]. These bidirectional interactions between synaptic activity and mitochondrial trafficking suggest that mitochondria may regulate synaptic plasticity. Here we investigated the dynamics of mitochondria in relation to axonal boutons of neocortical pyramidal neurons for the first time in vivo. We find that under these circumstances practically all mitochondria are stationary, both during development and in adulthood. In adult visual cortex, mitochondria are preferentially localized at putative boutons, where they remain for several days. Retinal-lesion-induced cortical plasticity increases turnover of putative boutons but leaves mitochondrial turnover unaffected. We conclude that in visual cortex in vivo, mitochondria are less dynamic than in vitro, and that structural plasticity does not affect mitochondrial dynamics.
Curr Biol, 2016; 26

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TWO-PHOTON CALCIUM IMAGING EXPERIMENTS UNCOVER CEREBELLAR CORTEX MICROCIRCUIT PROPERTIES

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Investigation of microcircuits functioning is growing in interest in neuroscience research, as they have a key role in processing incoming inputs. The study of the dynamic interactions between the different elements generating the microcircuit activity requires the acquisition of data from several single neurons, which must be uniquely identified and whose activity must be monitored over time. We investigated the cerebellar cortex microcircuit by performing two-photon calcium imaging experiments in acute cerebellar slices with a scanless spatial-light modulator two-photon microscope (SLM-2PM) that allows to monitor the activity of hundreds of neurons simultaneously over time, while maintaining single-cell resolution. We acquired stimulus-induced calcium signals (10 pulses@50Hz delivered to the afferent mossy fibers) from neurons located in different regions of the circuit (granular layer, Purkinje cells layer and molecular interneurons layer), showing different trends and temporal activations. We monitored these activities before and after the induction of long-term synaptic plasticity at the mossy fibers-granule cells synapses. The expression of long-term plasticity at the input stage of the circuit reverberated throughout the entire network, resulting in the expression of both short-term and long-term plasticity in Purkinje cells and molecular layer interneurons. These data are crucial in order to understand the role of different neuronal types in shaping the cerebellar cortex activity and to uncover how the latter is modulated by different forms of plasticity, providing experimental validation of both model predictions of circuit properties and hypothesis about cerebellar cortex functioning.

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INVOLVEMENT OF DOPAMINE D1/D5 AND BETA- ADRENERGIC RECEPTORS IN HIPPOCAMPAL LTD ENABLED BY LOCUS COERULEUS STIMULATION

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Aims: Synaptic plasticity is a cellular mechanism that crucially supports hippocampus-dependent memory formation. This phenomenon, in turn, is potently influenced by catecholaminergic neuromodulation. The locus coeruleus (LC) is a major source of catecholamines for the brain. Changes in LC tonus that is triggered by novel experience leads to noradrenaline (NA) release in the hippocampus, particularly in the dentate gyrus (DG). Patterned stimulation of the LC in frequency ranges related to phasic activation of the LC triggers long-term depression (LTD) in the hippocampus *in vivo.*In recent years it has emerged that the LC co-releases dopamine (DA) under particular experimental circumstances. In this study, we explored whether both neurotransmitters contribute equally to hippocampal LTD triggered by LC stimulation (LC-LTD). **Methods:** Adult male rats underwent chronic implantation of a stimulating electrode into both the LC and the medial perforant path, and a recording electrode into the DG. A cannula was implanted into the lateral cerebral ventricle to permit pharmacological treatment. We examined the effect of pharmacological antagonism of dopamine D1/D5 receptors (D1/5R) and beta-adrenergic receptors (b-AR) on synaptic plasticity evoked in the DG by LC stimulation using different stimulation patterns ranging from 2 -100Hz. **Results:** Whereas D1/D5R are critically involved in LC-LTD that is triggered by low frequency stimulation of the LC, b-AR are predominantly involved in LC-LTD induced by high-frequency stimulation. **Conclusions:** Our results indicate that D1/D5R and b-AR contribute in a differentiated manner to frequency-dependent hippocampal LC-LTD.
This work was funded by the Deutsche Forschungsgemeinschaft (SFB 874/B3, project number: 122679504).

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BOARD NUMBER: S02-301

SYNAPTIC AND INTRINSIC POTENTIATION IN O-LM INTERNEURONS IS INDUCED BY THETA PATTERNS OF STIMULATION

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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[1]INSERM UMR1072, AMU, Faculté De Médecine, Marseille, France, [2]Inserm UMR1072, AMU, Faculté De Médecine, Marseille, France

Oriens lacunosum-moleculare (O-LM) interneurons display a non-conventional form of long-term synaptic potentiation (LTP) conferred by calcium-permeable AMPA receptors (CP-AMPAR). So far, this form of LTP has been induced in O-LM cells by physiologically unrealistic protocols. We report here the induction of both synaptic and intrinsic potentiation in O-LM interneurons following stimulation of afferent glutamatergic inputs in the theta (θ) frequency range. LTP is induced by synaptic activation of CP-AMPAR whereas long-term potentiation of intrinsic excitability (LTP-IE) results from the mGluR1-dependent down-regulation of Kv7 voltage-dependent potassium channel and hyperpolarization activated and cyclic nucleotide-gated (HCN) channel through the depletion of phosphatidylinositol-4,5-bi-phosphate (PIP2). LTP and LTP-IE are reversible, demonstrating that both synaptic and intrinsic changes are bidirectional in O-LM cells. We conclude that physiological stimuli such as θ patterns induce synaptic and intrinsic potentiation in O-LM interneurons. Funded by FRM

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BOARD NUMBER: S02-302

CAPTURING DYNAMICS OF INHIBITORY SYNAPTIC CONNECTIVITY UNDERLYING LEARNING USING IN VIVO TWO-PHOTON OPTICAL IMAGING OF HIPPOCAMPAL CA1

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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The formation of new episodic memories requires the hippocampus. The CA1 hippocampal area exhibits high level synaptic structural plasticity, which is thought to support temporary information storage. By tracking excitatory synapses, it has indeed been shown that hippocampal CA1 has more excitatory structural turnover than neocortical areas, and that such turnover negatively correlates with the ability of mice to recall a hippocampal-dependent memory. However, little is known about the dynamics of inhibitory synapses. Inhibitory neurons have a critical role in episodic memory and the position of inhibitory synapses on CA1 pyramidal neurons strongly influences the computations these neurons carry out. Thus, understanding the dynamics of inhibitory synapses is fundamental to decipher the circuit-level mechanisms underlying encoding of new information. Inhibitory synapses, however, lack a morphological feature, which makes it difficult to visualize them *in vivo.* To solve this issue, we tested and optimized different labelling methods with the aim to track inhibitory synapses on excitatory CA1 pyramidal neurons using *in vivo* time-lapse, deep-brain, 2-photon microscopy in mice. The most promising method was a double viral injection in which one virus expressed cytosolic tdTomato in frame with Cre recombinase under the control of an excitatory promotor and thereby driving the expression of a second virus carrying a Cre-dependent GFP, fused to the inhibitory post-synaptic protein: Gephyrin. Utilizing this labelling method, I tracked inhibitory synapses before and after fear conditioning training to investigate the relationship between CA1 inhibitory synaptic dynamics and hippocampal-dependent learning.

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MAINTAINING NEUROPLASTICITY IN HEALTHY AGEING: SEX-DEPENDENT ROLE OF NEUROPEPTIDE Y

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Aging-related cognitive decline is associated with a reduced number of neuropeptide Y (NPY)-expressing interneurons and a loss of cholinergic function in the hippocampus. Furthermore, NPY-expressing interneurons in the dorsal DG (dDG) are modulated by the cholinergic system and are critically involved in memory formation and storage. In the current study, we therefore investigated with extracellular field potential recordings the effect of NPYergic neurotransmission on synaptic plasticity in a sex- and age-dependent manner, under intact inhibitory neurotransmission, in mice. Our data show that theta burst stimulation induced long-term potentiation (LTP) in aged mice is dependent on cholinergic and NPYergic neurotransmission in a sex-specific manner. This is indicated by a loss of LTP in old male mice, which could be restored by increasing the cholinergic activity through physostigmine. Strikingly, this cholinergic effect was strictly dependent on NPY and could be blocked by an Y1 receptor antagonist BIBP3226. In contrast, in old female mice, LTP was not abolished during ageing but dependent on NPYergic neurotransmission. Interestingly, LTP was independent of Y1-R blockade under moderate cholinergic activation. Together, these observations suggest that NPYergic neurotransmission becomes critical for maintaining dDG LTP in old female mice and for the recovery of synaptic plasticity under moderate cholinergic activation in old male mice. Observed alterations in baseline excitability might contribute to these phenomena. The mechanisms of NPY-mediated facilitation of plasticity in the aged dDG are currently under investigation.

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STRAIN-SPECIFIC DIFFERENCES IN DOPAMINE-RELATED HIPPOCAMPAL SYNAPTIC PLASTICITY IN MICE

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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The C57Bl/6 mouse strain is very popular in brain research. Recent findings reported that synaptic plasticity in the form of long-term potentiation (LTP) is markedly reduced in hippocampal slice preparations from the C57Bl/6, compared to the CBA/CaOlaHsd mice. Furthermore, LTP has been shown to depend on D1-like receptors in rodents, and that the D1-like receptor variants, D1 and D5, have shown different functional relevance for hippocampal synaptic plasticity. In this study, we investigated whether strain-dependent differences in persistent hippocampal synaptic plasticity are also evident in freely behaving mice. We also explored how pharmacological agonism or antagonism of D1-like receptors differently modulates LTP in these strains. Finally, we assessed differences in D1-like expression between strains using immunohistochemistry. Male CBA/CaOlaHsd and C57Bl/6 mice (7-8 weeks old) underwent implantation of a stimulating electrode in the Schaffer collaterals and a recording electrode in Stratum radiatum to obtain synaptic responses in the CA1 region of the hippocampus. A cannula was implanted i.c.v. for pharmacological treatment. Freely behaving C57Bl/6 mice expressed significantly poorer LTP and short-term potentiation (STP) compared to CBA/CaOlaHsd mice. Synaptic potentiation also differed markedly between the mouse strains in the presence of a D1-like receptor agonist or antagonist. The plasticity outcome was considered in light of D1-like expression in the hippocampus. Our findings show that C57Bl/6 mice exhibit poorer CA1 synaptic potentiation in comparison with CBA/CaOlaHsd mice. Effects may be mediated partially by the dopaminergic system. This work was supported by a German Research Foundation (DFG) grant to DMV (SFB 874/B1, project no.:122679504).

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CHOLECYSTOKININERGIC SIGNALING EXERTS MAJOR CONTROL ON CORTICO-STRIATAL SYNAPTIC PLASTICITY AND MOTOR BEHAVIOR.

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Cholecystokinin (CCK) is well-known as the intestine satiety hormone, but this molecule is also detected at high concentrations in the brain, and plays a major role, yet not totally unraveled, on synaptic plasticity and memory. In order to better understand processes by which CCK impact plasticity, we chose to focus our attention on the striatum. This CCK2R rich region, presents a high level of plasticity and is involved in procedural learning and motor behavior. To elucidate the role of CCK in the striatal synapse, we use an *in-vivo* and *ex-vivo* approaches. In our *ex-vivo* approach, we evaluated the impact of CCK2R antagonist on cortico-striatal plasticity using patch-clamp of MSNs of horizontal brain slices of juvenile rats (20-35 days). To induce plasticity, we used a Spike Timing Dependent Plasticity (STDP) protocol. To confirm these electrophysiology data, we evaluated the effect of an injection of CCK2R antagonist or CCK on the motor behavior of juvenile rats *in-vivo*. Different motor tests were performed: rotarod, stepping test, open-field. C-fos immunohistochemistry performed on the brains collected at the end of the protocol would allow us to confirm the direct implication of striatum CCK2R in this function. Interestingly, we demonstrated that the CCK2R antagonist was able to completely reverse the cortico-striatal synaptic plasticity (i.e. LTP protocol leads to LTD). Moreover, *in-vivo* experiments showed a sex-dependent impairment of motor behavior following an injection of CCK2R antagonist. Overall, our results demonstrated that CCK and its receptor CCK2R are essential for encoding information processing in the cortico-striatal network.

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DIVERGENCE OF FREQUENCY-DEPENDENT INDUCTION OF LTP AND LTD BY THE LATERAL AND MEDIAL PERFORANT PATH INPUTS TO THE DENTATE GYRUS

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Aim: The entorhinal cortex segregates information sent to the hippocampus via the lateral (LPP) and medial perforant paths (MPP). Whether frequency-dependent LPP and MPP information-relay results in expression of different forms of homosynaptic plasticity within the respective synapses is unclear. We compared frequency-dependent long-term potentiation (LTP) and long-term depression (LTD) at MPP and LPP synapses in the dentate gyrus (DG) of freely behaving rats. We also compared neurotransmitter receptor expression at the level of synaptic inputs of the MPP and LPP in the molecular layer of the DG. **Methods:** Male Wistar rats underwent implantation of a recording electrode in the dentate gyrus and a stimulation electrode in the MPP or LPP. Frequency-dependent synaptic plasticity was tested using afferent frequencies in the range of 1- 200Hz. Immunohistochemistry was performed to examine the expression of GluN1, GluN2A and GluN2B subunits of the NMDA receptor in the molecular layer. **Results:** MPP–DG synapses exhibit a predisposition toward LTP expression, whereas LPP-DG synapses prefer to express synaptic depression at the afferent frequencies tested. GluN1, GluN2A, or GluN2B subunits were equally expressed in the molecular layer of the DG, suggesting that input properties, and not receptor distribution, determine the response of LPP or MPP synapses in the DG. **Conclusion:** LPP and MPP enable distinct forms of synaptic plasticity in response to the same frequencies and thus, may support the functional differentiation of information input to, and experience-dependent information storage in, the hippocampus. This work was funded by a Deutsche Forschungsgemeinschaft grant (SFB 1280/A04, project number: 316803389) to DMV.

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SYNAPTIC MECHANISMS UNDERLYING INNATE AND LEARNED SOCIAL FEAR

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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When animals encounter danger in a social context, they usually respond to the situation by freezing or flying. The fear that accompanies these behaviors can be innate or learned and in both cases is critical for survival. Indeed, animals innately fear predators while learning to fear aggressive conspecifics. Whether innate and learned social fears share common neuronal circuits and mechanisms is still largely unknown. Here, we first developed a behavioral paradigm that allows us to induce and test learned and innate social fear. cFos immunohistochemistry and fiber photometry recordings revealed that the olfactory tubercle (OT), situated in the ventral striatum, it is active during both the expression of learned and innate fear. Using tracing approaches, we anatomically characterized the different glutamatergic inputs to this region. We found that the two main inputs to the OT are the olfactory bulb (OB) and the basolateral amygdala (BLA). We characterized shared and unique forms of synaptic plasticity induced by innate and learned social fear and their role in the expression of the fear response. Our study will provide the foundation for understanding the synaptic mechanisms underlying learned and innate social fear responses.

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DOPAMINE D2-LIKE RECEPTORS BIDIRECTIONALLY REGULATE CA1 SYNAPTIC PLASTICITY AND MODULATE CUMULATIVE SPATIAL MEMORY IN RATS

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Dopamine plays an important role in modulating hippocampal synaptic plasticity and memory. Dopamine D1/ D5 receptors (D1/5R) and D2-like receptors (D2R) are positively and negatively coupled to adenylyl cyclase, respectively. Substantial evidence supports a role for D1/5R in supporting the persistency of hippocampal long-term potentiation (LTP) and depression (LTD). Less is known about the role of D2R in these phenomena. In this study, we explored the influence of D2R on synaptic plasticity at Schaffer collateral (SC)-CA1 synapses *in vivo*, and on spatial memory in rats. Intracerebral pharmacological antagonism of D2R prevented the expression of both short-term (<1 h) and long-term potentiation (>4 h), as well as the expression of short-term depression (STD, <1 h) at CA1-SC synapses of freely behaving adult rats. The same dose of D2R antagonist also altered the retention of a cumulative (semantic-like) spatial memory task and significantly impaired retention of recent spatiotemporal aspects of an episodic-like memory task. Taken together, our findings indicate that the D2R bidirectionally modulate synaptic plasticity in the hippocampal CA1 region, and play an important role in enabling cumulative and episodic-like forms of spatial learning. This work was funded by the Deutsche Forschunggemeinschaft (SFB 874/B10, project number: 122679504).

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CELLULAR MECHANISM OF SILENT SYNAPSES FORMATION IN CENTRAL AMYGDALA IN COCAINE-INDUCED LOCOMOTOR SENSITIZATION

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Drugs of abuse, such as cocaine, are known to induce extremely durable forms of memory. One of the features of cocaine-induced plasticity is the induction of silent synapses - immature excitatory connections, that represent the brain's increased capacity to learn. They possess NMDA receptors but lack AMPA receptors on their surface. We showed that exposure to cocaine induces silent synaptic connections in the neurons of the amygdalar complex - a key brain structure in aversive and appetitive learning (memorizing negative and positive events respectively). Such silent synaptic contacts are transient and their disappearance could indicate their integration into the circuitry by accumulation of AMPA receptors in LTP-like manner. Alternatively, silent synapses could be formed via endocytosis of AMPA receptors, hence representing an ongoing LTD process in response to cocaine exposure. To elucidate the cellular mechanism of silent synapses formation in central amygdala in cocaine-induced locomotor sensitization we used MMP-9 (matrix metalloproteinase 9) KO mice, as the lack of MMP-9 function blocks LTP. MMP-9 KO mice and their wild type littermates received daily i.p. injections of cocaine for 1 or 7 days. Locomotor activity was measured and electrophysiological recordings of silent synapses levels were conducted. The results showed lower cocaine sensitization in KO mice, as well as decreased percentage of silent synapses in central amygdala. This suggests the requirement of amygdalar LTP in the formation of silent synapses during cocaine-induced locomotor sensitization.

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ACTIVITY-DEPENDENT MODULATION OF ACTIN DYNAMICS BY CDC42 MODULATES SYNAPTIC COOPERATION AND COMPETITION

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Persistent forms of LTP require the synthesis of plasticity-related proteins (PRPs) to be maintained. Thus, the maintenance of synaptic plasticity relies on the interplay between input-specific synaptic tags and the capture of PRPs at activated synapses. Given this interplay, different groups of activated synapses can interact by cooperation, if PRPs are available, or competition, if PRPs are limited. Synaptic cooperation and competition are two forms of heterosynaptic plasticity that allow neurons to integrate events occurring within large time windows of minutes to hours. Previous results from our laboratory showed a critical role of actin dynamics in synaptic tagging and maintenance of LTP (Fonseca, 2012) and LTD (Szabó, 2016). Our data support the hypothesis that a local activity-dependent remodelling of actin, through CaMKII activation, renders the synapse locally and transiently permissive to plasticity modifications. We found that continuous synaptic activation is necessary for the capture of PRPs by activated synapses, suggesting a critical role of continuous neuronal activity for the consolidation of plasticity. Interestingly, the activation of Cdc42, a GTPase also involved in the modulation of the actin cytoskeleton is necessary for the maintenance of LTP through tagging and capture of PRPs. Cdc42 activity is necessary for LTP maintenance in the first hour, after induction, and for the capture of PRPs by cooperation. Interestingly, synaptic competition is also blocked by Cdc42 inhibition. Given that Cdc42 activation is spatially restricted, our results show that local remodelling of actin, by CaMKII and Cdc42 activation are key modulators of heterosynaptic cooperation and competition.

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MIDBRAIN DOPAMINE NEURONS TRIGGER HIPPOCAMPAL LONG TERM POTENTIATION AND CONTEXTUAL LEARNING

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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The classical unsupervised model of hebbian Long Term Potentiation (LTP) fails to explain why some events are learnt while others are not. An alternative model postulates that a neuromodulator may act as a teaching signal triggering LTP. We used optogenetic tools to specifically manipulate the dopamine pathway originating from the midbrain and innervating the dorsal hippocampus. We show that dopamine midbrain projections can trigger LTP at Schaeffer Collaterals *in vivo,* in anaesthetized mice. In order to test for the role of this pathway in contextual learning, we used a variation of the contextual fear conditioning in which the context and its association to the electric shock take place on two consecutive days. We show that inhibiting the midbrain to hippocampus dopamine pathway impairs contextual learning while its stimulation promotes it. We thus discovered a new form of LTP triggered by dopamine which is compatible with computational models of hippocampo-dependent learning and we show that midbrain dopamine projections to the dorsal hippocampus are involved in contextual learning. We propose that, when something has to be learnt, midbrain dopamine provides a teaching signal to the hippocampus which induces hippocampal LTP responsible for learning and memory.

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ASTROCYTE-MEDIATED SWITCH IN SPIKE TIMING-DEPENDENT PLASTICITY DURING HIPPOCAMPAL DEVELOPMENT

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Presynaptic spike timing-dependent long-term depression (t-LTD) at hippocampal CA3-CA1 synapses is evident until the 3rd postnatal week in mice, disappearing during the 4th week. At more mature stages, we found that the protocol that induced t-LTD induced t-LTP. We characterized this form of t-LTP and the mechanisms involved in its induction, as well as that driving this switch from t-LTD to t-LTP. We found that this t-LTP is expressed presynaptically at CA3-CA1 synapses, as witnessed by coefficient of variation, number of failures, pairedpulse ratio and miniature responses analysis. Additionally, this form of presynaptic t-LTP does not require NMDARs but the activation of mGluRs and the entry of Ca2+ into the postsynaptic neuron through L-type voltage-dependent Ca2+ channels and the release of Ca2+ from intracellular stores. Nitric oxide is also required as a messenger from the postsynaptic neuron. Crucially, the release of adenosine and glutamate by astrocytes is required for t-LTP induction and for the switch from t-LTD to t-LTP. Thus, we have discovered a developmental switch of synaptic transmission from t-LTD to t-LTP at hippocampal CA3-CA1 synapses in which astrocytes play a central role and revealed a form of presynaptic LTP and the rules for its induction.

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EXTENDED TIMESCALE PLASTICITY IN THE PREFRONTAL CORTEX PROVIDES EVIDENCE OF ELIGIBILITY TRACES PERMISSIVE TO SUPERVISED LEARNING

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Learning is required to optimize behavioural choices. Synaptic plasticity mechanisms, such as Long-term potentiation (LTP), are widely believed to be cellular correlates of learning and memory. LTP can be induced by Spike-Timing-Dependent Plasticity protocols that typically require dozens of near-simultaneous (*i.e.,* tens of milliseconds) firing of pre- and postsynaptic neurons, a timescale fundamentally incompatible with that expected for supervised learning (200ms-2s), and a repetition requirement that is far from that of quasi one-shot learning (1-5x). Eligibility traces, an unknown biochemical process priming synapses to remain eligible for potentiation for an extended period of time (hundreds of milliseconds), have long been hypothesized to provide an appealing solution to this temporal credit assignment problem. However, their existence is unclear. Here, using cellular electrophysiology and 2-photon microscopy, we examined the ability of temporally separated pre- and postsynaptic events to induce potentiation in pyramidal neurons of mice prefrontal cortex. We observed that a few pairings of pre- and postsynaptic events at behaviourally relevant timescale (0.5s–1.5s) reliably and robustly potentiated synaptic strength. This form of plasticity followed unsuspected temporal rules that were dependent on bursting and that were modulated by norepinephrine, a neuromodulator believed to provide saliency signal. We are currently developing a model that captures the core aspects of these plasticity rules. The features of these plasticity rules support the existence of synaptic eligibility traces, and provide a potential avenue of solution to the temporal credit assignment problem.

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POSTSYNAPTIC GABAB RECEPTORS INHIBIT SYNAPTIC PLASTICITY AT MOSSY FIBRE INPUT ONTO THE BASKET CELLS OF THE DENTATE GYRUS

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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$GABA_B$ receptors are important modulators of synaptic plasticity in principal cells, promoting plasticity via disinhibition. In contrast, in inhibitory interneurons, $GABA_B$ receptors were recently found to inhibit plasticity (Booker et al., 2019). Thus, the modulatory role of $GABA_B$ receptor may differ in a cell type-specific manner. Here, we investigated the effects of tonic $GABA_B$ receptor activation on the induction of synaptic plasticity in basket cells of the dentate gyrus (DG). We performed whole-cell patch-clamp recordings from DG basket cells and induced long-term potentiation (LTP) at their mossy fibre input by non-associative theta burst stimulation (TBS) in acute slices of the rat hippocampus. Under control conditions, TBS resulted in a robust LTP of the EPSP amplitude to 226.2 ± 33.57% of preceding baseline (at 30 min). Pre-application of Baclofen (5 µM), a $GABA_B$ receptor agonist, to the bath massively reduced the LTP to 126.8±13.20% (p=0.0328, compared to control conditions). When baclofen was co-applied with SCH-23390, a Kir3.1 channel blocker, the inhibitory effect on plasticity was reversed (192.7±20.07%, p=0.5733, compared to control conditions), pointing to the involvement of these channels. Finally, intracellular perfusion of Gallein, an inhibitor of G-protein signaling, prior to induction partially reversed the inhibitory effect of $GABA_B$ receptor activation (163.6±26.76%, p=0.3046, compared to control conditions). These results show that at the excitatory input synapses of basket cells in the DG, GABA can modulate synaptic plasticity post-synaptically through $GABA_B$ receptor-Kir3.1 channel cascade, and inhibit LTP induction. At a network level, $GABA_B$ receptors may act to readjust excitation-inhibition balance.

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MOTOR LEARNING SELECTIVELY STRENGTHENS CORTICAL AND STRIATAL SYNAPSES OF MOTOR ENGRAM NEURONS

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Learning and consolidation of new motor skills require adaptations of neuronal activity and connectivity in the motor cortex and striatum, two key motor regions of the brain. Yet, how neurons undergo synaptic changes and become recruited during motor learning to form a memory engram remains an open question. Here, we train mice on a single-pellet reaching motor learning task and use a genetic approach to identify and manipulate behavior-relevant neurons selectively in the primary motor cortex (M1). We find that the degree of reactivation of M1 engram neurons correlates strongly with motor performance. We further demonstrate that learning-induced dendritic spine reorganization specifically occurs in these M1 engram neurons. In addition, we find that motor learning leads to an increase in the number and strength of outputs from M1 engram neurons onto striatal spiny projection neurons (SPNs) and that these synapses form local clusters along SPN dendrites. These results identify a highly specific synaptic plasticity during the formation of long-lasting motor memory traces in the corticostriatal circuit.

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ROLE OF PNN-MEDIATED SEMAPHORIN3A IN LEARNING AND MEMORY

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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Learning relies mainly on neuroplasticity, i.e. the ability of neuronal cells to perform structural and functional adaptations throughout life. Plasticity comes in two forms: synaptic plasticity (changes in synaptic strength), and structural plasticity (rewiring of synaptic networks). Plasticity events involve cellular components, including neurons and glial cells, and extracellular components such as the extracellular matrix. This resulted in a formulation of a concept called the tetrapartite synapse: pre and post synaptic elements, glial cells and the surrounding extracellular matrix. Around some neurons, proteins of the extracellular matrix are densely organised in a reticular pattern to form the perineuronal net (PNN). The PNN plays an important role in controlling neuroplasticity but the mechanisms by which the PNN acts remain unclear. The chemorepulsive axon guidance protein semaphorin3A (Sema3A) is highly expressed in PNNs. Our hypothesis is that Sema3A is a crucial functional component of PNNs, regulating learning and memory. To investigate this idea, we used 3-months-old mutant mice in which the ability of Sema3A to bind to its receptor complex is reduced and evaluated the cognitive changes with a discrimination and reversal learning test. The results indicate that impaired Sema3A signalling leads to an improvement of reversal learning. Potential sources of Sema3A in PNNs are certain classes of neurons and the choroid plexus. Therefore, we created tools to knock-out Sema3A in specific cell types using a CRE/LoxP approach and adeno-associated viral vectors. These tools will be employed to study the consequences of sema3A deletion for various types of neuroplasticity and learning.

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EXPERIENCE-DEPENDENT PLASTICITY OF BURST ACTIVITY IN LATERAL HABENULA NEURONS

POSTER SESSION 02 - SECTION: SYNAPTIC PLASTICITY, LEARNING AND MEMORY

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The lateral habenula (LHb) is a subcortical structure controlling monoamimergic systems contributing to valence processing. In contrast, its defective activity leads to the emergence of depressive like states typical of depression and addiction. This suggests that LHb neuronal dynamics adapt accordingly to the animal's affective state, although the underlying mechanism remains elusive. Burst activity in LHb neurons, defined as a transitory high-frequency spikes that mingles with regular firing, is a candidate substrate disrupted in depression. Yet its ionic substrate, plasticity and relevance in behavior remains understudied. We used ex-vivo and in-vivo electrophysiology together with an observational threat paradigm in mice in which animals observe a cage-mate undergoing a traumatic experience (i.e. foot shocks). First, we observed that bath application of T-type and Ca2+-dependent potassium (Sk) channels blockers as well as serotonin decreased the number of bursts, unraveling the conductances and neuromodulatory systems relevant for this activity modality. Then, we demonstrated that observational threat downregulates LHb burst activity. Finally, serotonin-induced bursts reduction was occluded after the observational threat paradigm. In conclusion, our evidence support that *i.* LHb bursts rely on specific ion-channels conductances, *ii.* bursts adapt after observing a conspecific under threat, and *iii.* this burst plasticity requires serotonin release. This supports that LHb neuronal activity is vulnerable to the affective state of individuals, which may in turn have repercussions on motivated behaviors.

Pubmed:
35013094: Mondoloni S, Mameli M, Congiu M
Reward and aversion encoding in the lateral habenula for innate and learned behaviours.
Throughout life, individuals experience a vast array of positive and aversive events that trigger adaptive behavioural responses. These events are often unpredicted and engage actions that are likely anchored on innate behavioural programs expressed by each individual member of virtually all animal species. In a second step, environmental cues, that are initially neutral, acquire value through the association with external sensory stimuli, and become instrumental to predict upcoming positive or negative events. This process ultimately prompts learned goal-directed actions allowing the pursuit of rewarding experience or the avoidance of a danger. Both innate and learned behavioural programs are evolutionarily conserved and fundamental for survival. Among the brain structures participating in the encoding of positive/negative stimuli and contributing to innate and learned behaviours is the epithalamic lateral habenula (LHb). The LHb provides top-down control of monoaminergic systems, responds to unexpected appetitive/aversive stimuli as well as external cues that predict the upcoming rewards or punishments. Accordingly, the LHb controls a number of behaviours that are innate (originating from unpredicted stimuli), and learned (stemming from predictive cues). In this review, we will discuss the progresses that rodent's experimental work made in identifying how LHb activity governs these vital processes, and we will provide a view on how these findings integrate within a complex circuit connectivity.
Transl Psychiatry, 2022; 12
34836948: Dongelmans M, Durand-de Cuttoli R, Nguyen C, Come M, Duranté EK, Lemoine D, Brito R, Ahmed Yahia T, Mondoloni S, Didienne S, Bousseyrol E, Hannesse B, Reynolds LM, Torquet N, Dalkara D, Marti F, Mourot A, Naudé J, Faure P
Chronic nicotine increases midbrain dopamine neuron activity and biases individual strategies towards reduced exploration in mice.
Long-term exposure to nicotine alters brain circuits and induces profound changes in decision-making strategies, affecting behaviors both related and unrelated to drug seeking and consumption. Using an intracranial self-stimulation reward-based foraging task, we investigated in mice the impact of chronic nicotine on midbrain dopamine neuron activity and its consequence on the trade-off between exploitation and exploration. Model-based and archetypal analysis revealed substantial inter-individual variability in decision-making strategies, with mice passively exposed to nicotine shifting toward a more exploitative profile compared to non-exposed animals. We then mimicked the effect of chronic nicotine on the tonic

activity of dopamine neurons using optogenetics, and found that photo-stimulated mice adopted a behavioral phenotype similar to that of mice exposed to chronic nicotine. Our results reveal a key role of tonic midbrain dopamine in the exploration/exploitation trade-off and highlight a potential mechanism by which nicotine affects the exploration/exploitation balance and decision-making.
Nat Commun, 2021; 12

34273390: Nuno-Perez A, Mondoloni S, Tchenio A, Lecca S, Mameli M
Biophysical and synaptic properties of NMDA receptors in the lateral habenula.
Excitatory synaptic transmission in the lateral habenula (LHb), an evolutionarily ancient subcortical structure, encodes aversive stimuli and affective states. Habenular glutamatergic synapses contribute to these processes partly through the activation of AMPA receptors. Yet, N-methyl-d-aspartate receptors (NMDARs) are also expressed in the LHb and support the emergence of depressive symptoms. Indeed, local NMDAR blockade in the LHb rescues anhedonia and behavioral despair in rodent models of depression. However, the subunit composition and biophysical properties of habenular NMDARs remain unknown, thereby hindering their study in the context of mental health. Here, we performed electrophysiological recordings and optogenetic-assisted circuit mapping in mice, to study pharmacologically-isolated NMDAR currents in LHb neurons that receive innervation from different brain regions (entopeduncular nucleus, lateral hypothalamic area, bed nucleus of the stria terminalis, or ventral tegmental area). This systematic approach revealed that habenular NMDAR currents are sensitive to TCN and ifenprodil - drugs that specifically inhibit GluN2A- and GluN2B-containing NMDARs, respectively. Whilst these pharmacological effects were consistently observed across inputs, we detected region-specific differences in the current-voltage relationship and decay time of NMDAR currents. Finally, inspired by the firing of LHb neurons in vivo, we designed a burst protocol capable of eliciting calcium-dependent long-term potentiation of habenular NMDAR transmission ex vivo. Altogether, we define basic biophysical and synaptic properties of NMDARs in LHb neurons, opening new avenues for studying their plasticity processes in physiological as well as pathological contexts.
Neuropharmacology, 2021; 196

34242565: Nguyen C, Mondoloni S, Le Borgne T, Centeno I, Come M, Jehl J, Solié C, Reynolds LM, Durand-de Cuttoli R, Tolu S, Valverde S, Didienne S, Hannesse B, Fiancette JF, Pons S, Maskos U, Deroche-Gamonet V, Dalkara D, Hardelin JP, Mourot A, Marti F, Faure P
Nicotine inhibits the VTA-to-amygdala dopamine pathway to promote anxiety.
Nicotine stimulates dopamine (DA) neurons of the ventral tegmental area (VTA) to establish and maintain reinforcement. Nicotine also induces anxiety through an as yet unknown circuitry. We found that nicotine injection drives opposite functional responses of two distinct populations of VTA DA neurons with anatomically segregated projections: it activates neurons that project to the nucleus accumbens (NAc), whereas it inhibits neurons that project to the amygdala nuclei (Amg). We further show that nicotine mediates anxiety-like behavior by acting on β2-subunit-containing nicotinic acetylcholine receptors of the VTA. Finally, using optogenetics, we bidirectionally manipulate the VTA-NAc and VTA-Amg pathways to dissociate their contributions to anxiety-like behavior. We show that inhibition of VTA-Amg DA neurons mediates anxiety-like behavior, while their activation prevents the anxiogenic effects of nicotine. These distinct subpopulations of VTA DA neurons with opposite responses to nicotine may differentially drive the anxiogenic and the reinforcing effects of nicotine.
Neuron, 2021; 109

33112237: Lemoine D, Mondoloni S, Tange J, Lambolez B, Faure P, Taly A, Tricoire L, Mourot A
Probing the ionotropic activity of glutamate GluD2 receptor in HEK cells with genetically-engineered photopharmacology.
Glutamate delta (GluD) receptors belong to the ionotropic glutamate receptor family, yet they don't bind glutamate and are considered orphan. Progress in defining the ion channel function of GluDs in neurons has been hindered by a lack of pharmacological tools. Here, we used a chemo-genetic approach to engineer specific and photo-reversible pharmacology in GluD2 receptor. We incorporated a cysteine mutation in the cavity located above the putative ion channel pore, for site-specific conjugation with a photoswitchable pore blocker. In the constitutively open GluD2 Lurcher mutant, current could be rapidly and reversibly decreased with light. We then transposed the cysteine mutation to the native receptor, to demonstrate with high pharmacological specificity that metabotropic glutamate receptor signaling triggers opening of GluD2. Our results assess the functional relevance of GluD2 ion channel and introduce an optogenetic tool that will provide a novel and powerful means for probing GluD2 ionotropic contribution to neuronal physiology.
Elife, 2020; 9

31400823: Mondoloni S, Durand-de Cuttoli R, Mourot A
Cell-Specific Neuropharmacology.
Neuronal communication involves a multitude of neurotransmitters and an outstanding diversity of receptors and ion channels. Linking the activity of cell surface receptors and ion channels in defined neural circuits to brain states and behaviors has been a key challenge in neuroscience, since cell targeting is not possible with traditional neuropharmacology. We review here recent technologies that enable the effect of drugs to be restricted to specific cell types, thereby allowing

acute manipulation of the brain's own proteins with circuit specificity. We highlight the importance of developing cell-specific neuropharmacology strategies for decoding the nervous system with molecular and circuit precision, and for developing future therapeutics with reduced side effects.
Trends Pharmacol Sci, 2019; 40
30293722: Forget B, Scholze P, Langa F, Morel C, Pons S, Mondoloni S, Besson M, Durand-de Cuttoli R, Hay A, Tricoire L, Lambolez B, Mourot A, Faure P, Maskos U
A Human Polymorphism in CHRNA5 Is Linked to Relapse to Nicotine Seeking in Transgenic Rats.
Tobacco addiction is a chronic and relapsing disorder with an important genetic component that represents a major public health issue. Meta-analysis of large-scale human genome-wide association studies (GWASs) identified a frequent non-synonymous SNP in the gene coding for the α5 subunit of nicotinic acetylcholine receptors (α5SNP), which significantly increases the risk for tobacco dependence and delays smoking cessation. To dissect the neuronal mechanisms underlying the vulnerability to nicotine addiction in carriers of the α5SNP, we created rats expressing this polymorphism using zinc finger nuclease technology and evaluated their behavior under the intravenous nicotine-self-administration paradigm. The electrophysiological responses of their neurons to nicotine were also evaluated. α5SNP rats self-administered more nicotine at high doses and exhibited higher nicotine-induced reinstatement of nicotine seeking than wild-type rats. Higher reinstatement was associated with altered neuronal activity in several discrete areas that are interconnected, including in the interpeduncular nucleus (IPN), a GABAergic structure that strongly expresses α5-containing nicotinic receptors. The altered reactivity of IPN neurons of α5SNP rats to nicotine was confirmed electrophysiologically. In conclusion, the α5SNP polymorphism is a major risk factor for nicotine intake at high doses and for relapse to nicotine seeking in rats, a dual effect that reflects the human condition. Our results also suggest an important role for the IPN in the higher relapse to nicotine seeking observed in α5SNP rats.
Curr Biol, 2018; 28
30176987: Durand-de Cuttoli R, Mondoloni S, Marti F, Lemoine D, Nguyen C, Naudé J, d'Izarny-Gargas T, Pons S, Maskos U, Trauner D, Kramer RH, Faure P, Mourot A
Manipulating midbrain dopamine neurons and reward-related behaviors with light-controllable nicotinic acetylcholine receptors.
Dopamine (DA) neurons of the ventral tegmental area (VTA) integrate cholinergic inputs to regulate key functions such as motivation and goal-directed behaviors. Yet the temporal dynamic range and mechanism of action of acetylcholine (ACh) on the modulation of VTA circuits and reward-related behaviors are not known. Here, we used a chemical-genetic approach for rapid and precise optical manipulation of nicotinic neurotransmission in VTA neurons in living mice. We provide direct evidence that the ACh tone fine-tunes the firing properties of VTA DA neurons through β2-containing (β2*) nicotinic ACh receptors (nAChRs). Furthermore, locally photo-antagonizing these receptors in the VTA was sufficient to reversibly switch nicotine reinforcement on and off. By enabling control of nicotinic transmission in targeted brain circuits, this technology will help unravel the various physiological functions of nAChRs and may assist in the design of novel therapies relevant to neuropsychiatric disorders.
Elife, 2018; 7
29236669: Durand-de Cuttoli R, Mondoloni S, Mourot A
[Optically dissecting brain nicotinic receptor function with photo-controllable designer receptors].
Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels widely expressed in the central nervous system and the periphery. They play an important modulatory role in learning, memory and attention, and have been implicated in various diseases such as Alzheimer's disease, Parkinson's disease, epilepsy, schizophrenia and addiction. These receptors are activated by the endogenous neurotransmitter acetylcholine, or by nicotine, the alkaloid found in tobacco leaves. Both molecules open the ion channel and cause the movement of cations across the membrane, which directly affects neuronal excitability and synaptic plasticity. nAChRs are very heterogeneous in their subunit composition (α2-10 et β2-4), in their brain distribution (cortex, midbrain, striatum…) and in their sub-cellular localization (pre- vs post-synaptic, axonal, dendritic…). This heterogeneity highly contributes to the very diverse roles these receptors have in health and disease. The ability to activate or block a specific nAChR subtype, at a defined time and space within the brain, would greatly help obtaining a clearer picture of these various functions. To this aim, we are developing novel optogenetic pharmacology strategies for optically controlling endogenous nAChR isoforms within the mouse brain. The idea is to tether a chemical photoswitch on the surface of a cysteine-modified nAChR, and use light for rapidly and reversibly turning that receptor mutant on and off. Here we will discuss the history of optogenetic pharmacology, and the recent advances for the optical control of brain nicotinic receptors in vivo.
Biol Aujourdhui, 2017; 211

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BOARD NUMBER: S02-318

MIR-124-DEPENDENT TAGGING OF GLUTAMATERGIC SYNAPSES BY SYNAPTOPODIN CONTROLS NON-UNIFORM AND INPUT-SPECIFIC HOMEOSTATIC SYNAPTIC PLASTICITY

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Homeostatic synaptic plasticity (HSP) is a process by which neurons adjust synaptic strengths to compensate for various perturbations and which allows to stabilize neuronal activity. Yet, whether the highly diverse synapses harboring a neuron respond uniformly to a same perturbation is unclear and the underlying molecular determinants remain to be identified. Here, using patch-clamp recordings, immunolabeling and imaging approaches, we report that the ability of individual synapses to undergo HSP in response to activity-deprivation paradigms depends on the local expression of the spine apparatus related protein synaptopodin (SP) acting as a synaptic tag to promote AMPA receptors synaptic accumulation and spine growth. Gain and loss-of-function experiments indicate that this process relies on the local de-repression of SP translation by miR124 which supports both non-uniform and synapse-autonomous HSP induced by global or input specific activity deprivation, respectively. Our findings uncover an unexpected synaptic-tagging mechanism for HSP, whose molecular actors are intriguingly shared with Hebbian plasticity and linked to multiple neurological diseases.

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BOARD NUMBER: S02-319

LONGITUDINAL TRACKING OF SYNAPTIC STRUCTURAL HOMEOSTATIC MECHANISMS IN THE HIPPOCAMPAL CA1 REGION OF LIVE MICE USING TWO-PHOTON IMAGING

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

Bhargavi Keerthana Boovaraga Murthy[1], Hannah Klimmt[1], Inna Slutsky[2], Alessio Attardo[1]
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Experience induces specific neural activity patterns and plasticity of synaptic connections, thus enabling information storage. In addition, homeostatic mechanisms at the synaptic level ensure stability by diverse functional and structural modifications such as, change in dendritic spine density and size – as demonstrated in cortical brain regions *in vivo*. Owing to the hippocampus being buried deep in the brain, little is known on how homeostatic compensation affects long-term structural stability of synapses in the hippocampus of live mammals. The aim of this project is to investigate *in vivo*, if changes in the density and stability of excitatory and inhibitory synapses in the hippocampus, constitute adaptive mechanisms in response to perturbed neuronal activity. By employing deep-brain two-photon time lapse imaging in live mice, we are chronically tracking and measuring the synaptic dynamics of excitatory and inhibitory synapses on pyramidal neurons in the CA1 region, in response to chemogenetically manipulated neuronal activity. We image dendritic spines as a proxy for excitatory synapses and for inhibitory synapses, we image a fluorescent protein fused to Gephyrin, a postsynaptic protein in inhibitory synapses. Through this project, we will recognize the timescale required for synaptic structural homeostatic mechanisms to emerge in the hippocampus. The study results will contribute to bridging the gap between the properties of individual neurons and the emerging properties of neuronal populations, and lead to a deeper understanding of how neural circuits in the hippocampus maintain functional stability upon perturbation of activity.

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BOARD NUMBER: S02-320

DYNAMIC CHANGES IN MUNC13-1 DISTRIBUTION AT HIPPOCAMPAL MOSSY FIBER BOUTONS DURING SYNAPTIC PLASTICITY

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Despite a large body of work on the morphological and physiological properties of synapses, the links between structure and function remain enigmatic. Although recent studies elucidated a potential correlation between physiological and morphological properties of synapses during synaptic plasticity (Vandael *et al.*, 2020, Neuron 107: 509–521), the corresponding molecular changes remain unknown. To pinpoint changes in the molecular architecture at hippocampal mossy fiber bouton (MFB) synapses during short- and long-term potentiation (STP and LTP), we combined chemical potentiation, by the adenylyl cyclase activator forskolin, and freeze-fracture replica immunolabeling of calcium channels and synaptic proteins in mouse hippocampus. This method allowed us to localize membrane-bound proteins with nanometer precision within the active zone (AZ), in particular, $Ca_V2.1$ calcium channels and Munc13-1 protein, a putative marker of primed vesicles. First, the mean number of clusters of Munc13-1 in the MFB active zone significantly increased from 2.4 in control to 3.3 clusters during STP (~5 min; 90 and 51 AZs respectively), but it decreased below the control value to 1.9 clusters during LTP (~30 min; 44 AZs). Secondly, although the mean pairwise distance between the $Ca_V2.1$s and Munc13-1s did not significantly change in the STP phase (113.5 nm; control: 105.8 nm), it shortened during LTP (80.8 nm). These results suggest the existence of two distinct mechanisms that govern STP and LTP at MFB synapses: an increase in the readily releasable pool in the case of STP and a potential increase in release probability during LTP.

Pubmed:
31928842: Borges-Merjane C, Kim O, Jonas P
Functional Electron Microscopy, "Flash and Freeze," of Identified Cortical Synapses in Acute Brain Slices.
How structural and functional properties of synapses relate to each other is a fundamental question in neuroscience. Electrophysiology has elucidated mechanisms of synaptic transmission, and electron microscopy (EM) has provided insight into morphological properties of synapses. Here we describe an enhanced method for functional EM ("flash and freeze"), combining optogenetic stimulation with high-pressure freezing. We demonstrate that the improved method can be applied to intact networks in acute brain slices and organotypic slice cultures from mice. As a proof of concept, we probed vesicle pool changes during synaptic transmission at the hippocampal mossy fiber-CA3 pyramidal neuron synapse. Our findings show overlap of the docked vesicle pool and the functionally defined readily releasable pool and provide evidence of fast endocytosis at this synapse. Functional EM with acute slices and slice cultures has the potential to reveal the structural and functional mechanisms of transmission in intact, genetically perturbed, and disease-affected synapses.
Neuron, 2020; 105

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CKAMP44 MODULATES PROCESSING OF VISUAL INFORMATION BY DLGN RELAY NEURONS

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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The dorsolateral geniculate nucleus (dLGN) is the first relay station for visual information. Relay cells in the dLGN receive excitatory inputs from retinal ganglion cells via retinogeniculate synapses. We showed previously that the AMPA receptors (AMPARs) auxiliary subunit CKAMP44 increases the number of AMPARs and reduces short-term depression in retinogeniculate synapses. The influence of CKAMP44 on short-term plasticity results from the slow rate of recovery from desensitization of AMPARs that contain CKAMP44. *In vivo* recordings showed that the alteration in short-term plasticity affects relay cell spike probability in response to visual input. To investigate in more detail how deletion of CKAMP44 alters computational properties of relay cells, I analyzed relay cell activity in response to diverse visual stimuli by performing *in-vivo* tetrode recordings in awake head-fixed mice. The results indicate that the influence of CKAMP44 on relay cells is particularly high when retinal ganglion cells respond to external input with high firing frequency. Furthermore, to understand the relevance of main mechanisms involved in short-term plasticity of retinogeniculate synapses and information processing in relay cells, I generated a computational model of relay cells with retinogeniculate synapses based on realistic anatomical and physiological parameters. Results from simulations revealed that input integration at retinogeniculate synapses depends strongly on the slow recovery from desensitization of AMPARs, but also on glutamate spillover. Taken together, these findings show that CKAMP44 via its influence on AMPAR gating has an important influence on processing of visual information.

Pubmed:
34884469: Back MK, Kurzawa J, Ruggieri S, von Engelhardt J
C57BL/6 Background Attenuates mHTT Toxicity in the Striatum of YAC128 Mice.
Mouse models are frequently used to study Huntington's disease (HD). The onset and severity of neuronal and behavioral pathologies vary greatly between HD mouse models, which results from different huntingtin expression levels and different CAG repeat length. HD pathology appears to depend also on the strain background of mouse models. Thus, behavioral deficits of HD mice are more severe in the FVB than in the C57BL/6 background. Alterations in medium spiny neuron (MSN) morphology and function have been well documented in young YAC128 mice in the FVB background. Here, we tested the relevance of strain background for mutant huntingtin (mHTT) toxicity on the cellular level by investigating HD pathologies in YAC128 mice in the C57BL/6 background (YAC128/BL6). Morphology, spine density, synapse function and membrane properties were not or only subtly altered in MSNs of 12-month-old YAC128/BL6 mice. Despite the mild cellular phenotype, YAC128/BL6 mice showed deficits in motor performance. More pronounced alterations in MSN function were found in the HdhQ150 mouse model in the C57BL/6 background (HdhQ150/BL6). Consistent with the differences in HD pathology, the number of inclusion bodies was considerably lower in YAC128/BL6 mice than HdhQ150/BL6 mice. This study highlights the relevance of strain background for mHTT toxicity in HD mouse models.
Int J Mol Sci, 2021; 22
34208315: Back MK, Ruggieri S, Jacobi E, von Engelhardt J
Amyloid Beta-Mediated Changes in Synaptic Function and Spine Number of Neocortical Neurons Depend on NMDA Receptors.
Onset and progression of Alzheimer's disease (AD) pathophysiology differs between brain regions. The neocortex, for example, is a brain region that is affected very early during AD. NMDA receptors (NMDARs) are involved in mediating amyloid beta (Aβ) toxicity. NMDAR expression, on the other hand, can be affected by Aβ. We tested whether the high vulnerability of neocortical neurons for Aβ-toxicity may result from specific NMDAR expression profiles or from a particular regulation of NMDAR expression by Aβ. Electrophysiological analyses suggested that pyramidal cells of 6-months-old wildtype mice express mostly GluN1/GluN2A NMDARs. While synaptic NMDAR-mediated currents are unaltered in 5xFAD

mice, extrasynaptic NMDARs seem to contain GluN1/GluN2A and GluN1/GluN2A/GluN2B. We used conditional GluN1 and GluN2B knockout mice to investigate whether NMDARs contribute to Aβ-toxicity. Spine number was decreased in pyramidal cells of 5xFAD mice and increased in neurons with 3-week virus-mediated Aβ-overexpression. NMDARs were required for both Aβ-mediated changes in spine number and functional synapses. Thus, our study gives novel insights into the Aβ-mediated regulation of NMDAR expression and the role of NMDARs in Aβ pathophysiology in the somatosensory cortex.
Int J Mol Sci, 2021; 22

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ROLE OF PRESYNAPTIC PLASTICITY AT MOSSY FIBER-CA3 SYNAPSES: CONSEQUENCES OF SYT7 ABROGATION IN DG CELLS ON CA3 CIRCUITS AND MEMORY ENCODING

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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The hippocampus is known to play a major role in the storage and recall of information depending on different forms of activity dependent synaptic plasticity. Mossy fibers synapses between the axons of dentate gyrus (DG) cells and CA3 pyramidal cells (Mf-CA3 synapses) display a high dynamic range of presynaptic plasticity which endow these synaptic connections with detonator properties. The pattern of action potential firing, in the form of high frequency bursts in the DG strongly controls the amplitude of synaptic responses and information transfer to CA3. Presynaptic short-term plasticity is thought to play a major role in the process of spike transfer within local circuits. Here we aim at investigating the role of presynaptic facilitation at Mf- CA3 synapses in the operation of CA3 circuits *in vivo* and in memory encoding. Syt7 is a calcium sensor that has been showed to be necessary for a presynaptic facilitation. We have selectively abrogated Syt7 expression in DG granule cells using conditional Syt7 KO mice. We confirm that presynaptic facilitation is suppressed at Mf-CA3 synapses in the absence of presynaptic Syt7. We have next used simultaneous silicon probe recordings in DG and CA3 to understand how the network adapts to changes in presynaptic plasticity, in reference to brain states. Finally, we describe the behavioral consequences of DG-selective Syt7 deletion in DG-dependent tasks, with a focus on memory encoding. This approach will bring new understanding of the role in presynaptic facilitation and specifically on the detonator properties of DG-CA3 synapses.

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BOARD NUMBER: S02-323

ORGANIZATION AND DYNAMICS OF THE ENDOGENOUS CAV2.1 NANOCLUSTERS SHAPE THE SHORT-TERM PLASTICITY IN HIPPOCAMPAL SYNAPSES

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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The kinetic properties of presynaptic calcium channels, including the activation state and conductivity but also their spatial arrangement, are critical parameters shaping the release probability of synapses. $Ca_V2.1$ (P/Q-type channels) represent the main population of voltage-gated calcium channels in many synapses of the mammalian brain. Within the presynaptic membrane, the $Ca_V2.1$ were found to be clustered in the active zone (AZ). However, it remains unclear whether such clusters are synapse type-specific and whether they depend on the history of presynaptic activation. To address these questions, we used a Cacna1a-Citrine knock-in mouse model together with an anti-GFP nanobody in combination with super-resolution microscopy (STED and dSTORM) to directly target and characterize the nanocluster organization of the endogenous population of Cav2.1 in AZ. We found that the cluster size of calcium channels is smaller in inhibitory synapses as compared to excitatory synapses. Furthermore, we generated an anti-GFP intrabody expression vector to monitor the local dynamics of Cav2.1. Our mobility data suggests an ongoing rearrangement that depends on the network activity levels. Interestingly, the manipulation with presynaptic G protein-coupled receptors (GPCRs) also influenced the Cav2.1 dynamics. Artificial clustering of Cav2.1 by photoactivatable cross-linkers induced a shrinkage of nanoclusters, which affected the impact of GPCR modulation on the synaptic function that was evaluated by the glutamate and calcium imaging. Taken together, this data suggests that the short-term plasticity is influenced by the re-arrangement of Cav2.1 nanoclusters inside the AZ, which can serve as an initial step leading to sustained changes in synaptic transmission.

Pubmed:

34706225: Yoo S, Santos C, Reynders A, Marics I, Malapert P, Gaillard S, Charron A, Ugolini S, Rossignol R, El Khallouqi A, Springael JY, Parmentier M, Saurin AJ, Goaillard JM, Castets F, Clerc N, Moqrich A
TAFA4 relieves injury-induced mechanical hypersensitivity through LDL receptors and modulation of spinal A-type K current.
Pain, whether acute or persistent, is a serious medical problem worldwide. However, its management remains unsatisfactory, and new analgesic molecules are required. We show here that TAFA4 reverses inflammatory, postoperative, and spared nerve injury (SNI)-induced mechanical hypersensitivity in male and female mice. TAFA4 requires functional low-density lipoprotein receptor-related proteins (LRPs) because their inhibition by RAP (receptor-associated protein) dose-dependently abolishes its antihypersensitive actions. SNI selectively decreases A-type K current (I) in spinal lamina II outer excitatory interneurons (L-IIo ExINs) and induces a concomitant increase in I and decrease in hyperpolarization-activated current (I) in lamina II inner inhibitory interneurons (L-IIi InhINs). Remarkably, SNI-induced ion current alterations in both IN subtypes were rescued by TAFA4 in an LRP-dependent manner. We provide insights into the mechanism by which TAFA4 reverses injury-induced mechanical hypersensitivity by restoring normal spinal neuron activity and highlight the considerable potential of TAFA4 as a treatment for injury-induced mechanical pain.
Cell Rep, 2021; 37
34133920: Klatt O, Repetto D, Brockhaus J, Reissner C, El Khallouqi A, Rohlmann A, Heine M, Missler M
Endogenous β-neurexins on axons and within synapses show regulated dynamic behavior.
Neurexins are key organizer molecules that regulate synaptic function and are implicated in autism and schizophrenia. β-neurexins interact with numerous cell adhesion and receptor molecules, but their neuronal localization remains elusive. Using single-molecule tracking and high-resolution microscopy to detect neurexin1β and neurexin3β in primary hippocampal neurons from knockin mice, we demonstrate that endogenous β-neurexins are present in fewer than half of excitatory and inhibitory synapses. Moreover, we observe a large extrasynaptic pool of β-neurexins on axons and show that axonal β-neurexins diffuse with higher surface mobility than those transiently confined within synapses. Stimulation of neuronal activity further increases the mobility of synaptic and axonal β-neurexins, whereas inhibition causes the opposite. Blocking

ectodomain cleavage by metalloproteases also reduces β-neurexin mobility and enhances glutamate release. These findings suggest that the surface mobility of endogenous β-neurexins inside and outside of synapses is dynamically regulated and linked to neuronal activity.
Cell Rep, 2021; 35
34107849: Heck J, Palmeira Do Amaral AC, Weißbach S, El Khallouqi A, Bikbaev A, Heine M
More than a pore: How voltage-gated calcium channels act on different levels of neuronal communication regulation.
Voltage-gated calcium channels (VGCCs) represent key regulators of the calcium influx through the plasma membrane of excitable cells, like neurons. Activated by the depolarization of the membrane, the opening of VGCCs induces very transient and local changes in the intracellular calcium concentration, known as calcium nanodomains, that in turn trigger calcium-dependent signaling cascades and the release of chemical neurotransmitters. Based on their central importance as concierges of excitation-secretion coupling and therefore neuronal communication, VGCCs have been studied in multiple aspects of neuronal function and malfunction. However, studies on molecular interaction partners and recent progress in omics technologies have extended the actual concept of these molecules. With this review, we want to illustrate some new perspectives of VGCCs reaching beyond their function as calcium-permeable pores in the plasma membrane. Therefore, we will discuss the relevance of VGCCs as voltage sensors in functional complexes with ryanodine receptors, channel-independent actions of auxiliary VGCC subunits, and provide an insight into how VGCCs even directly participate in gene regulation. Furthermore, we will illustrate how structural changes in the intracellular C-terminus of VGCCs generated by alternative splicing events might not only affect the biophysical channel characteristics but rather determine their molecular environment and downstream signaling pathways.
Channels (Austin), 2021; 15

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BOARD NUMBER: S02-324

PRIORITIZED DOCKING OF SYNAPTIC VESICLES PROVIDED BY A RAPID RECYCLING PATHWAY

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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To manage computational demands of synaptic transmission, synaptic vesicles (SVs) are organised into multiple functional pools within a synapse. The readily releasable pool (RRP) is responsible for the immediate release of neurotransmitter in response to action potential stimulation. The RRP is replenished either by recycling fused SVs or by recruiting new SVs from an upstream pool. Collectively, all SVs that take part in synaptic transmission constitute the recycling pool. The remaining SVs, which form the reserve pool, only undergo exocytosis during intense and prolonged activity. **Aims:** We investigated the recycling of SVs during repeated train stimulation at cerebellar synapses formed by granule cells (GCs) onto molecular layer interneurons (MLIs). **Methods:** Electrophysiological recordings were obtained from individual MLIs while a single presynaptic GC was being stimulated. **Results:** We found evidence for a rapid route of SV recycling during repeated train stimulation with short inter-train intervals. This route, which requires activation of the myosin light chain kinase, uses recently endocytosed vesicles to delay the reduction in synaptic output. It has a limited capacity and contributes minimally during steady-state release with short intervals. Nonetheless, SVs that are recycled through this pathway are preferentially docked compared to those coming from the reserve pool. **Conclusions:** Prioritized docking reveals a functional link between specific recycling pathways and SV sorting within the RRP. During and after extended synaptic stimulation, prioritized docking enhances the synaptic response to the first stimulation in a train, thus protecting responses to scarce stimuli from depression.

FENS Forum 2022
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BOARD NUMBER: S02-325

IMPAIRED EXCITATORY AND INHIBITORY SYNAPTIC PLASTICITY IN THE NL3-R451C MOUSE MODEL OF AUTISM SPECTRUM DISORDER

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Autistic spectrum disorder (ASD) has been associated to genetic alterations of proteins involved in synaptic function such as the point mutation R451C in neuroligin 3 (NLGN3), a postsynaptic adhesion molecule which binds its presynaptic partner neurexin at both excitatory and inhibitory synapses. In the present study, by exploiting the transgenic NL3R451C knock-in (KI) mice as an ASD animal model, we aimed at investigating the role of this mutation in the coordination of both excitatory and inhibitory synaptic plasticity. We found that, with respect to WT condition, the $NL3^{R451C}$ protein was less expressed at the neuronal surface and showed increased lateral diffusion at GABAergic synapses. In WT animals, we observed that, in response to a chemical protocol for the induction of synaptic plasticity (NMDA treatment), excitatory synaptic currents were depressed (LTD) whereas the inhibitory ones were potentiated (iLTP). Interestingly, such opposed synaptic plasticity was abolished in KI neurons. These effects were paralleled by changes in synaptic receptors and scaffold proteins at both excitatory and inhibitory synapses in WT and KI neurons. In addition, the quantification of NLGN3 clusters fluorescence intensity revealed that, after the NMDA treatment, surface NLGN3 decreased in WT neurons while it was unaffected in KI neurons. Collectively, our results reveal that the perturbed synaptic molecular composition of both glutamatergic and GABAergic synapses induced by the NL3 R451C mutation disrupts the coordination of excitatory and inhibitory synaptic plasticity thus potentially contributing to the pathophysiology of ASD.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-326

DESCRIBING THE LONG-RANGE TRAFFICKING DYNAMICS OF THE AMPA RECEPTORS IN HIPPOCAMPAL NEURONS USING A QUANTITATIVE MODEL FRAMEWORK

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Aims: AMPA receptors (AMPARs) are crucial ionotropic glutamate receptors directly involved in synaptic plasticity. The number of AMPARs at a synapse serves as a proxy for its synaptic strength. Experimental studies show that neurons can modulate the number of AMPARs locally in a synapse during synaptic plasticity by adjusting the surface mobility of the receptors or by altering the endocytosis/exocytosis frequency or both [1]. The active molecular motor-based transport is essential for the long-distance delivery of the receptors [2]. Recent advances in molecular and imaging techniques have improved our understanding of these trafficking mechanisms in dendrites and spines, and provided a careful characterization of the kinetic parameters involved in AMPAR trafficking. **Methods:** To keep up with this growing experimental data and to link changes in molecular mechanisms to changes in synaptic or dendritic receptor distributions, we developed a quantitative reaction diffusion model for local and long-range AMPAR trafficking. In parallel, we advanced the corresponding automated analysis of neural imaging data to obtain endogenous long-range mRNA and protein distributions in situ. **Results and Conclusions:** This allowed us to build a data-driven model framework describing the endogenous distribution of AMPA receptor subunits globally and locally. Next, we plan to study the temporal and spatial extent of heterosynaptic plasticity using our model. **References:** [1] Anggono & Huganir *Curr. Opin. Neurobiol.* **22**, 461–469 (2012).; [2] Hangen et al .*Cell Rep.* **24**, 1001-1012.e3.

Pubmed:
34226615: Wagle S, Ghosh A, Karthic P, Ghosh A, Pervaiz T, Kapoor R, Patil K, Gupta N
Development and testing of a game-based digital intervention for working memory training in autism spectrum disorder.
Autism spectrum disorder (ASD) is prevalent globally, yet it lacks cost-effective treatment approaches. Deficits in executive functions occur frequently in autism spectrum disorder and present a target for intervention. Here we report the design and development of five smartphone-based games for training working memory in children with ASD. These open-source games, available free of cost to the community, were designed to match the behavioral preferences and sensorimotor abilities of children with ASD. We then conducted a preliminary trial to test the effectiveness of a month-long intervention using these games. Although we did not see a significant change in the working memory of all children with a month-long training, children who performed better on the games also showed more improvement in their working memory, suggesting that a longer intervention with the games might be useful in improving working memory. Using a Hindi translation of the autism treatment evaluation checklist, we also tested the collateral gains of the training in reducing autistic symptoms. We found no significant change in the autistic symptoms after the intervention. Further, there was no correlation between the change in the working memory and the change in the autistic symptoms.
Sci Rep, 2021; 11

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BOARD NUMBER: S02-327

REVERSE ENGINEERING OF THE SYNAPTIC TAGGING AND CAPTURE MECHANISMS

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Memory and learning require fine-tuning of the connections between neurons. To strengthen a connection, a neuron needs to deliver hundreds of proteins from a range of different signaling pathways, but only to synapses that have been specifically stimulated. These proteins, known as plasticity-related proteins (PRP) can be locally recruited and produced in response to synaptic stimulation. Despite decades of research, molecules and signaling pathways that participate in this mechanism, known as "synaptic tagging" have not been categorically identified, possibly because there are many different tags. Instead of asking “What is the synaptic tag?”, we have focused on “What does it take to be a synaptic tag?” aiming to identify the biophysical characteristics PRPs should have. To address these questions, we have engineered artificial proteins, Synthetic PRPs (SynPRPs), to test mechanisms of activity-dependent targeting and determine how they collaborate to strengthen connections. We have demonstrated that these synPRPs are phosphorylated by CaMKII and consequently bind postsynaptic density protein 95 (PSD95) in a phospho-dependent manner. Additionally, we have tested if they are captured in activated synapses by expressing synPRPs in mouse hippocampal neuronal cultures. Subsequently, we have analyzed synaptic distribution and colocalization with PSD95 after an in vitro chemical long term potentiation (cLTP). We have observed that synaptic clusters of SynPRPs+PSD95 increase 2-fold their synaptic localization after cLTP in cultured hippocampal neurons. Our results could indicate that SynPRPs fulfill the criteria to be captured by a synaptic tag in activated synapses. Moreover, biophysically understanding synaptic tag and capture mechanisms could lead to prevent alterations of synaptic tagging in cognitive dysfunctions

Pubmed:
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L-Serine dietary supplementation is associated with clinical improvement of loss-of-function -related pediatric encephalopathy.
Autosomal dominant mutations in are associated with severe encephalopathy, but little is known about the pathophysiological outcomes and any potential therapeutic interventions. Genetic studies have described the association between de novo mutations of genes encoding the subunits of the -methyl-d-aspartate receptor (NMDAR) and severe neurological conditions. Here, we evaluated a missense mutation in , causing a proline-to-threonine switch (P553T) in the GluN2B subunit of NMDAR, which was found in a 5-year-old patient with Rett-like syndrome with severe encephalopathy. Structural molecular modeling predicted a reduced pore size of the mutant GluN2B-containing NMDARs. Electrophysiological recordings in a HEK-293T cell line expressing the mutated subunit confirmed this prediction and showed an associated reduced glutamate affinity. Moreover, GluN2B(P553T)-expressing primary murine hippocampal neurons showed decreased spine density, concomitant with reduced NMDA-evoked currents and impaired NMDAR-dependent insertion of the AMPA receptor subunit GluA1 at stimulated synapses. Furthermore, the naturally occurring coagonist d-serine restored function to GluN2B(P553T)-containing NMDARs. l-Serine dietary supplementation of the patient was hence initiated, resulting in the increased abundance of d-serine in the plasma and brain. The patient has shown notable improvements in motor and cognitive performance and communication after 11 and 17 months of l-serine dietary supplementation. Our data suggest that l-serine supplementation might ameliorate -related severe encephalopathy and other neurological conditions caused by glutamatergic signaling deficiency.
Sci Signal, 2019; 12
30140203: Gómez de Salazar M, Grau C, Ciruela F, Altafaj X
Phosphoproteomic Alterations of Ionotropic Glutamate Receptors in the Hippocampus of the Ts65Dn Mouse Model of Down Syndrome.
Down syndrome (DS), the main genetic cause of intellectual disability, is associated with an imbalance of excitatory/inhibitory

neurotransmitter systems. The phenotypic assessment and pharmacotherapy interventions in DS murine models strongly pointed out glutamatergic neurotransmission alterations (specially affecting ionotropic glutamate receptors [iGluRs]) that might contribute to DS pathophysiology, which is in agreement with DS condition. iGluRs play a critical role in fast-mediated excitatory transmission, a process underlying synaptic plasticity. Neuronal plasticity is biochemically modulated by post-translational modifications, allowing rapid and reversible adaptation of synaptic strength. Among these modifications, phosphorylation/dephosphorylation processes strongly dictate iGluR protein-protein interactions, cell surface trafficking, and subsynaptic mobility. Hence, we hypothesized that dysregulation of phosphorylation/dephosphorylation balance might affect neuronal function, which in turn could contribute to the glutamatergic neurotransmitter alterations observed in DS. To address this point, we biochemically purified subsynaptic hippocampal fractions from adult Ts65Dn mice, a trisomic mouse model recapitulating DS phenotypic alterations. Proteomic analysis showed significant alterations of the molecular composition of subsynaptic compartments of hippocampal trisomic neurons. Further, we characterized iGluR phosphopattern in the hippocampal glutamatergic synapse of trisomic mice. Phosphoenrichment-coupled mass spectrometry analysis revealed specific subsynaptic- and trisomy-associated iGluR phosphorylation signature, concomitant with differential subsynaptic kinase and phosphatase composition of Ts65Dn hippocampal subsynaptic compartments. Furthermore, biochemical data were used to build up a genotype-kinome-iGluR phosphopattern matrix in the different subsynaptic compartments. Overall, our results provide a precise profile of iGluR phosphopattern alterations in the glutamatergic synapse of the Ts65Dn mouse model and support their contribution to DS-associated synaptopathy. The alteration of iGluR phosphoresidues in Ts65Dn hippocampi, together with the kinase/phosphatase signature, identifies potential novel therapeutic targets for the treatment of glutamatergic dysfunctions in DS.
Front Mol Neurosci, 2018; 11

FENS Forum 2022
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BOARD NUMBER: S02-328

ACTIVITY-DEPENDENT REGULATION OF SYNAPTIC INTEGRATION IN PARVALBUMIN-POSITIVE INTERNEURONS

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Neuronal networks require precise coordination of excitatory and inhibitory synaptic connectivity to function properly. Regulation of parvalbumin-positive (PV+) interneuron activity is especially important for network function, and changes in PV+ interneuron activity correlate with an animal's performance in learning tasks. Previously, changes in PV+ interneuron connectivity have been shown to regulate excitatory pyramidal cell activity. However, the mechanism via which PV+ interneurons control their own activity remains unknown. Here we used a chemogenetic approach to change the activity of PV+ interneurons and performed whole-cell electrophysiological recordings to assess compensatory changes in the excitatory and inhibitory inputs they received. We find that increasing or decreasing the activity of PV+ interneuron activity specifically leads to a cell-autonomous compensatory change in inhibitory, but not excitatory, synaptic connectivity. Further optogenetic experiments revealed that these synaptic changes originate exclusively from other PV+ interneurons. We next performed ribosome profiling experiments to identify the molecular mechanisms mediating the activity-dependent scaling of PV+ synapses onto PV+ interneurons. To this end, we combined chemogenetics with viral Translating Ribosome Affinity Purification (vTRAP) to isolate mRNAs that are specifically translated in activated PV+ interneurons. Subsequent RNA-sequencing revealed differentially regulated genes that are specifically induced following an increase in the activity of PV+ interneurons, some of which are specifically required for the compensatory synaptic changes induced by activity in these cells. Our experiments identify specific molecular programmes through which PV+ interneurons control their activity by regulating the amount of inhibition they receive from other PV+ interneurons.

Pubmed:
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Cadherin-13 is a critical regulator of GABAergic modulation in human stem-cell-derived neuronal networks.
Activity in the healthy brain relies on a concerted interplay of excitation (E) and inhibition (I) via balanced synaptic communication between glutamatergic and GABAergic neurons. A growing number of studies imply that disruption of this E/I balance is a commonality in many brain disorders; however, obtaining mechanistic insight into these disruptions, with translational value for the patient, has typically been hampered by methodological limitations. Cadherin-13 (CDH13) has been associated with autism and attention-deficit/hyperactivity disorder. CDH13 localizes at inhibitory presynapses, specifically of parvalbumin (PV) and somatostatin (SST) expressing GABAergic neurons. However, the mechanism by which CDH13 regulates the function of inhibitory synapses in human neurons remains unknown. Starting from human-induced pluripotent stem cells, we established a robust method to generate a homogenous population of SST and MEF2C (PV-precursor marker protein) expressing GABAergic neurons (iGABA) in vitro, and co-cultured these with glutamatergic neurons at defined E/I ratios on micro-electrode arrays. We identified functional network parameters that are most reliably affected by GABAergic modulation as such, and through alterations of E/I balance by reduced expression of CDH13 in iGABAs. We found that CDH13 deficiency in iGABAs decreased E/I balance by means of increased inhibition. Moreover, CDH13 interacts with Integrin-β1 and Integrin-β3, which play opposite roles in the regulation of inhibitory synaptic strength via this interaction. Taken together, this model allows for standardized investigation of the E/I balance in a human neuronal background and can be deployed to dissect the cell-type-specific contribution of disease genes to the E/I balance.
Mol Psychiatry, 2022; 27
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Distinct Pathogenic Genes Causing Intellectual Disability and Autism Exhibit a Common Neuronal Network Hyperactivity Phenotype.

Pathogenic mutations in either one of the epigenetic modifiers EHMT1, MBD5, MLL3, or SMARCB1 have been identified to be causative for Kleefstra syndrome spectrum (KSS), a neurodevelopmental disorder with clinical features of both intellectual disability (ID) and autism spectrum disorder (ASD). To understand how these variants lead to the phenotypic convergence in KSS, we employ a loss-of-function approach to assess neuronal network development at the molecular, single-cell, and network activity level. KSS-gene-deficient neuronal networks all develop into hyperactive networks with altered network organization and excitatory-inhibitory balance. Interestingly, even though transcriptional data reveal distinct regulatory mechanisms, KSS target genes share similar functions in regulating neuronal excitability and synaptic function, several of which are associated with ID and ASD. Our results show that KSS genes mainly converge at the level of neuronal network communication, providing insights into the pathophysiology of KSS and phenotypically congruent disorders.
Cell Rep, 2020; 30
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Optimization of interneuron function by direct coupling of cell migration and axonal targeting.
Neural circuit assembly relies on the precise synchronization of developmental processes, such as cell migration and axon targeting, but the cell-autonomous mechanisms coordinating these events remain largely unknown. Here we found that different classes of interneurons use distinct routes of migration to reach the embryonic cerebral cortex. Somatostatin-expressing interneurons that migrate through the marginal zone develop into Martinotti cells, one of the most distinctive classes of cortical interneurons. For these cells, migration through the marginal zone is linked to the development of their characteristic layer 1 axonal arborization. Altering the normal migratory route of Martinotti cells by conditional deletion of Mafb-a gene that is preferentially expressed by these cells-cell-autonomously disrupts axonal development and impairs the function of these cells in vivo. Our results suggest that migration and axon targeting programs are coupled to optimize the assembly of inhibitory circuits in the cerebral cortex.
Nat Neurosci, 2018; 21
29375819: Selten M, van Bokhoven H, Nadif Kasri N
Inhibitory control of the excitatory/inhibitory balance in psychiatric disorders.
Neuronal networks consist of different types of neurons that all play their own role in order to maintain proper network function. The two main types of neurons segregate in excitatory and inhibitory neurons, which together regulate the flow of information through the network. It has been proposed that changes in the relative strength in these two opposing forces underlie the symptoms observed in psychiatric disorders, including autism and schizophrenia. Here, we review the role of alterations to the function of the inhibitory system as a cause of psychiatric disorders. First, we explore both patient and post-mortem evidence of inhibitory deficiency. We then discuss the function of different interneuron subtypes in the network and focus on the central role of a specific class of inhibitory neurons, parvalbumin-positive interneurons. Finally, we discuss genes known to be affected in different disorders and the effects that mutations in these genes have on the inhibitory system in cortex and hippocampus. We conclude that alterations to the inhibitory system are consistently identified in animal models of psychiatric disorders and, more specifically, that mutations affecting the function of parvalbumin-positive interneurons seem to play a central role in the symptoms observed in these disorders.
F1000Res, 2018; 7
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Ptchd1 deficiency induces excitatory synaptic and cognitive dysfunctions in mouse.
Synapse development and neuronal activity represent fundamental processes for the establishment of cognitive function. Structural organization as well as signalling pathways from receptor stimulation to gene expression regulation are mediated by synaptic activity and misregulated in neurodevelopmental disorders such as autism spectrum disorder (ASD) and intellectual disability (ID). Deleterious mutations in the PTCHD1 (Patched domain containing 1) gene have been described in male patients with X-linked ID and/or ASD. The structure of PTCHD1 protein is similar to the Patched (PTCH1) receptor; however, the cellular mechanisms and pathways associated with PTCHD1 in the developing brain are poorly determined. Here we show that PTCHD1 displays a C-terminal PDZ-binding motif that binds to the postsynaptic proteins PSD95 and SAP102. We also report that PTCHD1 is unable to rescue the canonical sonic hedgehog (SHH) pathway in cells depleted of PTCH1, suggesting that both proteins are involved in distinct cellular signalling pathways. We find that Ptchd1 deficiency in male mice (Ptchd1) induces global changes in synaptic gene expression, affects the expression of the immediate-early expression genes Egr1 and Npas4 and finally impairs excitatory synaptic structure and neuronal excitatory activity in the hippocampus, leading to cognitive dysfunction, motor disabilities and hyperactivity. Thus our results support that PTCHD1 deficiency induces a neurodevelopmental disorder causing excitatory synaptic dysfunction.
Mol Psychiatry, 2018; 23
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Celikel T, Schubert D
Reduced Inhibition within Layer IV of Sert Knockout Rat Barrel Cortex is Associated with Faster Sensory Integration.
Neural activity is essential for the maturation of sensory systems. In the rodent primary somatosensory cortex (S1), high extracellular serotonin (5-HT) levels during development impair neural transmission between the thalamus and cortical input layer IV (LIV). Rodent models of impaired 5-HT transporter (SERT) function show disruption in their topological organization of S1 and in the expression of activity-regulated genes essential for inhibitory cortical network formation. It remains unclear how such alterations affect the sensory information processing within cortical LIV. Using serotonin transporter knockout (Sert-/-) rats, we demonstrate that high extracellular serotonin levels are associated with impaired feedforward inhibition (FFI), fewer perisomatic inhibitory synapses, a depolarized GABA reversal potential and reduced expression of KCC2 transporters in juvenile animals. At the neural population level, reduced FFI increases the excitatory drive originating from LIV, facilitating evoked representations in the supragranular layers II/III. The behavioral consequence of these changes in network excitability is faster integration of the sensory information during whisker-based tactile navigation, as Sert-/- rats require fewer whisker contacts with tactile targets and perform object localization with faster reaction times. These results highlight the association of serotonergic homeostasis with formation and excitability of sensory cortical networks, and consequently with sensory perception.
Cereb Cortex, 2017; 27
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Increased GABA receptor signaling in a rat model for schizophrenia.
Schizophrenia is a complex disorder that affects cognitive function and has been linked, both in patients and animal models, to dysfunction of the GABAergic system. However, the pathophysiological consequences of this dysfunction are not well understood. Here, we examined the GABAergic system in an animal model displaying schizophrenia-relevant features, the apomorphine-susceptible (APO-SUS) rat and its phenotypic counterpart, the apomorphine-unsusceptible (APO-UNSUS) rat at postnatal day 20-22. We found changes in the expression of the GABA-synthesizing enzyme GAD67 specifically in the prelimbic- but not the infralimbic region of the medial prefrontal cortex (mPFC), indicative of reduced inhibitory function in this region in APO-SUS rats. While we did not observe changes in basal synaptic transmission onto LII/III pyramidal cells in the mPFC of APO-SUS compared to APO-UNSUS rats, we report reduced paired-pulse ratios at longer inter-stimulus intervals. The GABA receptor antagonist CGP 55845 abolished this reduction, indicating that the decreased paired-pulse ratio was caused by increased GABA signaling. Consistently, we find an increased expression of the GABA receptor subunit in APO-SUS rats. Our data provide physiological evidence for increased presynaptic GABA signaling in the mPFC of APO-SUS rats, further supporting an important role for the GABAergic system in the pathophysiology of schizophrenia.
Sci Rep, 2016; 6
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Histone Methylation by the Kleefstra Syndrome Protein EHMT1 Mediates Homeostatic Synaptic Scaling.
Homeostatic plasticity, a form of synaptic plasticity, maintains the fine balance between overall excitation and inhibition in developing and mature neuronal networks. Although the synaptic mechanisms of homeostatic plasticity are well characterized, the associated transcriptional program remains poorly understood. We show that the Kleefstra-syndrome-associated protein EHMT1 plays a critical and cell-autonomous role in synaptic scaling by responding to attenuated neuronal firing or sensory drive. Chronic activity deprivation increased the amount of neuronal dimethylated H3 at lysine 9 (H3K9me2), the catalytic product of EHMT1 and an epigenetic marker for gene repression. Genetic knockdown and pharmacological blockade of EHMT1 or EHMT2 prevented the increase of H3K9me2 and synaptic scaling up. Furthermore, BDNF repression was preceded by EHMT1/2-mediated H3K9me2 deposition at the Bdnf promoter during synaptic scaling up, both in vitro and in vivo. Our findings suggest that H3K9me2-mediated changes in chromatin structure govern a repressive program that controls synaptic scaling.
Neuron, 2016; 91
26854232: Ba W, Selten MM, van der Raadt J, van Veen H, Li LL, Benevento M, Oudakker AR, Lasabuda RSE, Letteboer SJ, Roepman R, van Wezel RJA, Courtney MJ, van Bokhoven H, Nadif Kasri N
ARHGAP12 Functions as a Developmental Brake on Excitatory Synapse Function.
The molecular mechanisms that promote excitatory synapse development have been extensively studied. However, the molecular events preventing precocious excitatory synapse development so that synapses form at the correct time and place are less well understood. Here, we report the functional characterization of ARHGAP12, a previously uncharacterized Rho GTPase-activating protein (RhoGAP) in the brain. ARHGAP12 is specifically expressed in the CA1 region of the hippocampus, where it localizes to the postsynaptic compartment of excitatory synapses. ARHGAP12 negatively controls spine size via its RhoGAP activity and promotes, by interacting with CIP4, postsynaptic AMPA receptor endocytosis.

Arhgap12 knockdown results in precocious maturation of excitatory synapses, as indicated by a reduction in the proportion of silent synapses. Collectively, our data show that ARHGAP12 is a synaptic RhoGAP that regulates excitatory synaptic structure and function during development.
Cell Rep, 2016; 14

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Cadherin-13, a risk gene for ADHD and comorbid disorders, impacts GABAergic function in hippocampus and cognition.
Cadherin-13 (CDH13), a unique glycosylphosphatidylinositol-anchored member of the cadherin family of cell adhesion molecules, has been identified as a risk gene for attention-deficit/hyperactivity disorder (ADHD) and various comorbid neurodevelopmental and psychiatric conditions, including depression, substance abuse, autism spectrum disorder and violent behavior, while the mechanism whereby CDH13 dysfunction influences pathogenesis of neuropsychiatric disorders remains elusive. Here we explored the potential role of CDH13 in the inhibitory modulation of brain activity by investigating synaptic function of GABAergic interneurons. Cellular and subcellular distribution of CDH13 was analyzed in the murine hippocampus and a mouse model with a targeted inactivation of Cdh13 was generated to evaluate how CDH13 modulates synaptic activity of hippocampal interneurons and behavioral domains related to psychopathologic (endo)phenotypes. We show that CDH13 expression in the cornu ammonis (CA) region of the hippocampus is confined to distinct classes of interneurons. Specifically, CDH13 is expressed by numerous parvalbumin and somatostatin-expressing interneurons located in the stratum oriens, where it localizes to both the soma and the presynaptic compartment. Cdh13(-/-) mice show an increase in basal inhibitory, but not excitatory, synaptic transmission in CA1 pyramidal neurons. Associated with these alterations in hippocampal function, Cdh13(-/-) mice display deficits in learning and memory. Taken together, our results indicate that CDH13 is a negative regulator of inhibitory synapses in the hippocampus, and provide insights into how CDH13 dysfunction may contribute to the excitatory/inhibitory imbalance observed in neurodevelopmental disorders, such as ADHD and autism.
Transl Psychiatry, 2015; 5

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BOARD NUMBER: S02-329

THE KINESIN KIF21B REGULATES ACTIN DYNAMICS AT SPINE SYNAPSES AND CONTRIBUTES TO HOMEOSTATIC SYNAPTIC DOWNSCALING

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Homeostatic regulation of synaptic strength preserves the balance of excitation and inhibition (E-I), which is critical for brain function and behavior. Accordingly, E-I imbalance contributes to neuronal pathology in neurodegenerative and neurodevelopmental disorders. Current evidence suggests that neurons counterbalance chronic activity changes by modulating glutamate receptor trafficking and adapting scaffold protein dynamics at postsynaptic sites. However, underlying molecular mechanisms and the potential contribution of actin remodeling at spines are barely understood. Here we report that the processive motor protein kinesin KIF21B plays a role in homeostatic synaptic downscaling. We demonstrate that KIF21B is localized to synaptic spines in a myosin-dependent manner and associates with postsynaptic densities. Furthermore, Kif21B regulates actin dynamics and interacts with scaffold proteins, essential for homeostatic plasticity. Our study provides novel insights into the role of kinesin motors in the homeostatic regulation of synaptic function.

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HOMEOSTATIC CONTROL OF NEUROMUSCULAR SYNAPTIC TRANSMISSION

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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The neuromuscular synapse plays a relay function that requires a homeostatic control. This control adapts the neurotransmitter release to the post-synaptic excitability, resulting in a unity synaptic gain (one post-synaptic action potential for one pre-synaptic action potential). For this control process, the post-synaptic cell evaluates the synaptic efficacy and exercises a retrograde feedback on the pre-synaptic terminal. Our work focuses on the postsynaptic sensors that evaluate the synaptic efficacy. We have previously proposed (Ouanounou et al. eLife 2016;5:e12190) that postsynaptic calcium signaling reports distinctly the occurrence of the pre- and postsynaptic events, and that this dual detection is used to adjust the neurotransmitter release to a level matching the postsynaptic excitability. Here we confirm the calcium nature of the sensor of the synaptic efficacy, and we refine the understanding of its mechanism. We show that in addition to the detection of activities, the postsynaptic sensor precisely reports the synaptic strength. Contrary to the common belief, we discovered that this operation is not done by the quantification of the postsynaptic potential amplitude, masked by the action potential, but of the repolarization kinetics. We show that the sub-synaptic calcium build up, due to the nicotinic receptor permeability to calcium, strongly depends on repolarization kinetics and perfectly encodes the ratio between the synaptic conductance and the postsynaptic input conductance. This signal is used by the postsynaptic cell to balance homeostatic synaptic plasticity and to hold synaptic strength at a set point.

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ENTORHINAL CORTEX LESION INDUCES HOMEOSTATIC SYNAPTIC PLASTICITY OF CA3 PYRAMIDAL NEURONS

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Aims: A common aspect of many neurological diseases is the denervation of brain regions as a result of either demyelination or cell death. Nonetheless, the underlying mechanisms involved in lesion-induced reorganization of neural networks warrant further investigation. In this study, we addressed the effects of a partial denervation of CA3 pyramidal neurons. **Methods:** Lesion of the entorhinal cortex in organotypic entorhino-hippocampal tissue cultures — prepared from wild type mice of both sexes — was used to denervate distal apical dendrites of hippocampal granule cells and CA3 pyramidal neurons. Changes in excitatory neurotransmission were assessed with single and paired whole-cell patch-clamp recordings and morphological alterations were analyzed with electron microscopy. Moreover, a region specific transcriptome analysis was performed. **Results:** Partial denervation resulted in homeostatic synaptic adaptions of dentate granule cells and CA3 pyramidal cells. These changes in excitatory neurotransmission occurred predominantly in the strongest synapses as shown by a hierarchical analysis of spontaneous excitatory postsynaptic currents. The homeostatic adjustment was accompanied by characteristic region-specific transcriptomic changes. Consistent with these findings, paired recordings of dentate granule cells and CA3 pyramidal neurons and ultrastructural analysis of mossy fiber synapses revealed denervation-induced homeostatic structural and functional changes at the single synapse level. **Conclusion:** Lesion-induced homeostatic synaptic plasticity of dentate granule cells and CA3 pyramidal neurons leads to hetero- and transsynaptic adaptations of hippocampal networks. Supported by Else-Kröner Fresenius Stiftung (EKFS).

Pubmed:

33781382: Lenz M, Kruse P, Eichler A, Straehle J, Beck J, Deller T, Vlachos A
All-trans retinoic acid induces synaptic plasticity in human cortical neurons.
A defining feature of the brain is the ability of its synaptic contacts to adapt structurally and functionally in an experience-dependent manner. In the human cortex, however, direct experimental evidence for coordinated structural and functional synaptic adaptation is currently lacking. Here, we probed synaptic plasticity in human cortical slices using the vitamin A derivative all-trans retinoic acid (atRA), a putative treatment for neuropsychiatric disorders such as Alzheimer's disease. Our experiments demonstrated that the excitatory synapses of superficial (layer 2/3) pyramidal neurons underwent coordinated structural and functional changes in the presence of atRA. These synaptic adaptations were accompanied by ultrastructural remodeling of the calcium-storing spine apparatus organelle and required mRNA translation. It was not observed in synaptopodin-deficient mice, which lack spine apparatus organelles. We conclude that atRA is a potent mediator of synaptic plasticity in the adult human cortex.
Elife, 2021; 10
34723795: Lenz M, Eichler A, Kruse P, Muellerleile J, Deller T, Jedlicka P, Vlachos A
All-trans retinoic acid induces synaptopodin-dependent metaplasticity in mouse dentate granule cells.
Previously we showed that the vitamin A metabolite all-trans retinoic acid (atRA) induces synaptic plasticity in acute brain slices prepared from the mouse and human neocortex (Lenz et al., 2021). Depending on the brain region studied, distinct effects of atRA on excitatory and inhibitory neurotransmission have been reported. Here, we used intraperitoneal injections of atRA (10 mg/kg) in adult C57BL/6J mice to study the effects of atRA on excitatory and inhibitory neurotransmission in the mouse fascia dentata-a brain region implicated in memory acquisition. No major changes in synaptic transmission were observed in the ventral hippocampus while a significant increase in both spontaneous excitatory postsynaptic current frequencies and synapse numbers were evident in the dorsal hippocampus 6 hr after atRA administration. The intrinsic properties of hippocampal dentate granule cells were not significantly different and hippocampal transcriptome analysis revealed no essential neuronal changes upon atRA treatment. In light of these findings, we tested for the metaplastic effects of atRA, that is, for its ability to modulate synaptic plasticity expression in the absence of major changes in baseline synaptic

strength. Indeed, in vivo long-term potentiation (LTP) experiments demonstrated that systemic atRA treatment improves the ability of dentate granule cells to express LTP. The plasticity-promoting effects of atRA were not observed in synaptopodin-deficient mice, therefore, extending our previous results regarding the relevance of synaptopodin in atRA-mediated synaptic strengthening in the mouse prefrontal cortex. Taken together, our data show that atRA mediates synaptopodin-dependent metaplasticity in mouse dentate granule cells.
Elife, 2021; 10
33391287: Lenz M, Eichler A, Kruse P, Strehl A, Rodriguez-Rozada S, Goren I, Yogev N, Frank S, Waisman A, Deller T, Jung S, Maggio N, Vlachos A
Interleukin 10 Restores Lipopolysaccharide-Induced Alterations in Synaptic Plasticity Probed by Repetitive Magnetic Stimulation.
Systemic inflammation is associated with alterations in complex brain functions such as learning and memory. However, diagnostic approaches to functionally assess and quantify inflammation-associated alterations in synaptic plasticity are not well-established. In previous work, we demonstrated that bacterial lipopolysaccharide (LPS)-induced systemic inflammation alters the ability of hippocampal neurons to express synaptic plasticity, i.e., the long-term potentiation (LTP) of excitatory neurotransmission. Here, we tested whether synaptic plasticity induced by repetitive magnetic stimulation (rMS), a non-invasive brain stimulation technique used in clinical practice, is affected by LPS-induced inflammation. Specifically, we explored brain tissue cultures to learn more about the direct effects of LPS on neural tissue, and we tested for the plasticity-restoring effects of the anti-inflammatory cytokine interleukin 10 (IL10). As shown previously, 10 Hz repetitive magnetic stimulation (rMS) of organotypic entorhino-hippocampal tissue cultures induced a robust increase in excitatory neurotransmission onto CA1 pyramidal neurons. Furthermore, LPS-treated tissue cultures did not express rMS-induced synaptic plasticity. Live-cell microscopy in tissue cultures prepared from a novel transgenic reporter mouse line [] confirms that LPS administration triggers microglial tumor necrosis factor alpha (TNFα) expression, which is ameliorated in the presence of IL10. Consistent with this observation, IL10 hampers the LPS-induced increase in TNFα, IL6, IL1β, and IFNγ and restores the ability of neurons to express rMS-induced synaptic plasticity in the presence of LPS. These findings establish organotypic tissue cultures as a suitable model for studying inflammation-induced alterations in synaptic plasticity, thus providing a biological basis for the diagnostic use of transcranial magnetic stimulation in the context of brain inflammation.
Front Immunol, 2020; 11

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BOARD NUMBER: S02-332

HOMEOSTATIC SYNAPTIC PLASTICITY RECRUITS EPITRANSCRIPTOMIC MODIFICATIONS IN MURINE AND HUMAN CORTICAL TISSUE

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Aims: Homeostatic synaptic plasticity aims at compensating for perturbations in network activity, thereby keeping synaptic transmission in a dynamic range. Among the mechanisms that mediate and control homeostatic plasticity, mRNA translation in dendrites plays a crucial role for the local control of synaptic properties. Nevertheless, the precise regulatory mechanisms and the relevance of homeostatic plasticity in human cortical networks remain unknown. **Methods:** In this study, we investigated the impact of neural network silencing through pharmacological inhibition of voltage-gated sodium channels or glutamatergic neurotransmission (i.e., common targets of anticonvulsant substances) on functional and structural properties of murine and human cortical neurons. **Results:** Using mouse organotypic tissue cultures and adult human cortical slices we showed that network silencing promotes a compensatory functional reorganization of excitatory synapses consistent with a homeostatic synaptic adjustment. This form of homeostatic plasticity was accompanied by epitranscriptomic changes and required *de novo* protein synthesis. **Conclusion:** Our findings provide first experimental evidence for homeostatic synaptic plasticity in the adult human neocortex. They suggest an important role for epitranscriptomic modifications and protein synthesis in the regulation of synaptic homeostasis in murine and human cortical networks.

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INACTIVITY INDUCED HOMEOSTATIC SYNAPTIC PLASTICITY REQUIRES ECM REMODELING

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Neuronal networks are balanced by mechanisms of homeostatic plasticity, which adjust synaptic strength via molecular and morphological changes in the pre- and post-synapse. The perineuronal extracellular matrix (ECM) of the adult brain, which is mainly composed of chondroitin sulfate proteoglycans such as brevican stabilizes synapses and thereby reduces neuronal plasticity. Previous data indicate that brevican is cleaved during homoeostatic plasticity and thereby may facilitate synapse remodeling. The proteases ADAMTS4, -5 and the closely related ADAMTS8, -9 and -15 may share brevican as substrate and are presumably responsible for ECM regulation. In order to elucidate the role of ADAMTS family members in homeostatic plasticity we quantified ADAMTS expression by qPCR in dissociated neuronal cultures after prolonged network silencing. Further, we investigated substrate specificity of ADAMTS family members *in vitro*. We used siRNAs to knock-down selected ADAMTS in neuronal cultures and quantified abundance of specific synaptic proteins regulated during network silencing. We found ADAMTS4 and -5 mRNA regulated during homeostatic plasticity. In contrast to ADAMTS4 and -5, neither ADAMTS8 nor ADAMTS15 cleaved brevican *in vitro*. In line with this finding downregulation of ADAMTS4 and -5, but not ADAMTS8 diminished cleavage of brevican in neuronal cultures. Furthermore, homeostatic regulation of synaptic proteins was abolished when interfering with ADAMTS function. In conclusion we found that ECM remodeling via ADAMTS4 and -5 derived cleavage of brevican is necessary for homeostatic regulation of synaptic plasticity and thus adjustment of neuronal networks.

Pubmed:
32043120: Parcerisas A, Pujadas L, Ortega-Gascó A, Perelló-Amorós B, Viais R, Hino K, Figueiro-Silva J, La Torre A, Trullás R, Simó S, Lüders J, Soriano E
NCAM2 Regulates Dendritic and Axonal Differentiation through the Cytoskeletal Proteins MAP2 and 14-3-3.
Neural cell adhesion molecule 2 (NCAM2) is involved in the development and plasticity of the olfactory system. Genetic data have implicated the NCAM2 gene in neurodevelopmental disorders including Down syndrome and autism, although its role in cortical development is unknown. Here, we show that while overexpression of NCAM2 in hippocampal neurons leads to minor alterations, its downregulation severely compromises dendritic architecture, leading to an aberrant phenotype including shorter dendritic trees, retraction of dendrites, and emergence of numerous somatic neurites. Further, our data reveal alterations in the axonal tree and deficits in neuronal polarization. In vivo studies confirm the phenotype and reveal an unexpected role for NCAM2 in cortical migration. Proteomic and cell biology experiments show that NCAM2 molecules exert their functions through a protein complex with the cytoskeletal-associated proteins MAP2 and 14-3-3γ and ζ. We provide evidence that NCAM2 depletion results in destabilization of the microtubular network and reduced MAP2 signal. Our results demonstrate a role for NCAM2 in dendritic formation and maintenance, and in neural polarization and migration, through interaction of NCAM2 with microtubule-associated proteins.
Cereb Cortex, 2020; 30

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RAPID HOMEOSTATIC MODULATION OF TRANSSYNAPTIC NANOCOLUMN RINGS

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Robust neural information transfer relies on a delicate molecular architecture of chemical synapses and even subtle changes in the molecular organization of synapses may profoundly affect synaptic transmission and animal behavior. Neurotransmitter release is controlled by a specific arrangement of proteins within presynaptic active zones. How the specific presynaptic molecular architecture relates to postsynaptic organization, and how synaptic nano-architecture is transsynaptically regulated to achieve stable synaptic transmission remains enigmatic. Using STED microscopy, we here discovered that presynaptic nano-rings formed by the active-zone cytomatrix protein scaffold Bruchpilot (Brp) precisely align with glutamate receptors (GluRs) nano-rings composed of ~ 6 GluR clusters at the *Drosophila* NMJ. Individual rings harbor ~ 5 transsynaptically-aligned Brp-GluR 'nanocolumns'. Transsynaptic nanocolumn rings are partially masked by unaligned GluR clusters. Genetic manipulations of GluR subunits and the auxiliary GluR subunit Neto revealed a GluR subtype-specific nano-organization: While GluRs containing the GluRIIA subunit predominantly localize to nanorings, GluRIIB 'nanoclusters' can be found both, inside and outside of nanorings. Interestingly, acute GluR impairment rapidly triggers the formation of new transsynaptic nanocolumns on the minute time scale during homeostatic plasticity. We reveal distinct phases of structural transsynaptic homeostatic plasticity, with postsynaptic reorganization preceding presynaptic modulation. Finally, the auxiliary GluR subunit Neto promotes structural and functional homeostatic plasticity. Thus, transsynaptic nanocolumns arrange in stereotypic rings that are rapidly modulated during homeostatic plasticity to stabilize synaptic efficacy. We are in in the process of linking synaptic nano-architecture to synaptic physiology. Preliminary data suggest a correlation between nano-column number and miniature frequency

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DENDRITIC SIGNALING PATHWAYS UNDERLYING ENDOCANNABINOID-MEDIATED INHIBITORY BOUTON GROWTH

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Coordination between excitation and inhibition is essential for proper functioning of neuronal circuits. Inhibitory synapses can locally control the activity of excitatory inputs by their strategic placement on the same dendrite. We recently discovered that excitatory and inhibitory plasticity is regulated at the synaptic level. By repeated stimulation of excitatory synapses, the formation of a nearby inhibitory bouton onto the same dendrite can be triggered. Our findings show that this process is mediated by the dendritic production of the 2-AG which activates axonal CB1 receptors. In addition, we found that long-term structural plasticity of excitatory synapses was affected by manipulations of the endocannabinoid system.
We further explored the local dendritic signaling mechanism that underlies coordinated inhibitory plasticity. We found that NMDA receptor activation is required, but not sufficient, to induce bouton growth. We hypothesized that additional activation of mGluR5 is necessary. We used two-photon microscopy to image crossings between CA1 pyramidal cell dendrites and GFP-labeled GABAergic axons in organotypic hippocampal slices of GAD65-GFP mice. We stimulated multiple dendritic spines close to the crossing axon using two-photon glutamate uncaging (30x @0.5Hz) paired with somatic postsynaptic depolarization (0 mV, 100 msec). Pharmacological blockade of mGluR5 throughout the experiment did not prevent bouton growth, indicating that inhibitory bouton growth is independent of mGluR5 activation. To visualize the local dendritic activity upon two-photon uncaging we are now performing functional imaging experiments with an calcium indicator. Our results help to further unravel the activity-dependent signaling pathways that regulate coordination between excitatory and inhibitory synapses within dendrites.

Pubmed:
32853970: Herstel LJ, Wierenga CJ
Network control through coordinated inhibition.
Coordinated excitatory and inhibitory activity is required for proper brain functioning. Recent computational and experimental studies have demonstrated that activity patterns in recurrent cortical networks are dominated by inhibition. Whereas previous studies have suggested that inhibitory plasticity is important for homeostatic control, this new framework puts inhibition in the driver's seat. Complex neuronal networks in the brain comprise many configurations in parallel, controlled by external and internal 'switches'. Context-dependent modulation and plasticity of inhibitory connections play a key role in memory and learning. It is therefore important to realize that synaptic plasticity is often multisynaptic and that a proper balance between excitation and inhibition is not fixed, but depends on context and activity level.
Curr Opin Neurobiol, 2021; 67
32940601: Lindhout FW, Kooistra R, Portegies S, Herstel LJ, Stucchi R, Snoek BL, Altelaar AM, MacGillavry HD, Wierenga CJ, Hoogenraad CC
Quantitative mapping of transcriptome and proteome dynamics during polarization of human iPSC-derived neurons.
The differentiation of neuronal stem cells into polarized neurons is a well-coordinated process which has mostly been studied in classical non-human model systems, but to what extent these findings are recapitulated in human neurons remains unclear. To study neuronal polarization in human neurons, we cultured hiPSC-derived neurons, characterized early developmental stages, measured electrophysiological responses, and systematically profiled transcriptomic and proteomic dynamics during these steps. The neuron transcriptome and proteome shows extensive remodeling, with differential expression profiles of ~1100 transcripts and ~2200 proteins during neuronal differentiation and polarization. We also identified a distinct axon developmental stage marked by the relocation of axon initial segment proteins and increased microtubule remodeling from the distal (stage 3a) to the proximal (stage 3b) axon. This developmental transition coincides with action potential maturation. Our comprehensive characterization and quantitative map of transcriptome and proteome dynamics provides a solid framework for studying polarization in human neurons.
Elife, 2020; 9
33835529: Lindhout FW, Portegies S, Kooistra R, Herstel LJ, Stucchi R, Hummel JJA, Scheefhals N, Katrukha EA, Altelaar

M, MacGillavry HD, Wierenga CJ, Hoogenraad CC
Centrosome-mediated microtubule remodeling during axon formation in human iPSC-derived neurons.
Axon formation critically relies on local microtubule remodeling and marks the first step in establishing neuronal polarity. However, the function of the microtubule-organizing centrosomes during the onset of axon formation is still under debate. Here, we demonstrate that centrosomes play an essential role in controlling axon formation in human-induced pluripotent stem cell (iPSC)-derived neurons. Depleting centrioles, the core components of centrosomes, in unpolarized human neuronal stem cells results in various axon developmental defects at later stages, including immature action potential firing, mislocalization of axonal microtubule-associated Trim46 proteins, suppressed expression of growth cone proteins, and affected growth cone morphologies. Live-cell imaging of microtubules reveals that centriole loss impairs axonal microtubule reorganization toward the unique parallel plus-end out microtubule bundles during early development. We propose that centrosomes mediate microtubule remodeling during early axon development in human iPSC-derived neurons, thereby laying the foundation for further axon development and function.
EMBO J, 2021; 40
33074225: Ruiter M, Herstel LJ, Wierenga CJ
Reduction of Dendritic Inhibition in CA1 Pyramidal Neurons in Amyloidosis Models of Early Alzheimer's Disease.
In an early stage of Alzheimer's disease (AD), before the formation of amyloid plaques, neuronal network hyperactivity has been reported in both patients and animal models. This suggests an underlying disturbance of the balance between excitation and inhibition. Several studies have highlighted the role of somatic inhibition in early AD, while less is known about dendritic inhibition.
J Alzheimers Dis, 2020; 78

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DOES ENVIRONMENTAL ENRICHMENT OR MONOCULAR DEPRIVATION MODIFY THE DEVELOPMENT OF SYNAPTOME ARCHITECTURE?

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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The synaptome describes the diversity of brain synapses and the synaptome architecture describes the spatial distribution of these synapses in neurons, brain regions and the whole brain. Systematic mapping of the molecular and morphological properties of individual excitatory synapses on a brain-wide scale in the mouse reveals high synapse diversity (Zhu et al, *Neuron* 2018 doi.org/10.1016/j.neuron.2018.07.007). Excitatory synapse diversity expands during postnatal development and each brain region develops a unique signature of synapse composition (Cizeron et al, *Science* 2020 doi: 10.1126/science.aba3163). How much the synaptome architecture of excitatory synapses is controlled by activity and experience is poorly understood, especially for large parts of the brain that remain unexplored. We asked if the development of the synaptome architecture was modified in two well-established paradigms used to study how experience modifies synaptic properties, environmental enrichment (EE) and monocular deprivation (MD). PSD95$^{eGFP/eGFP}$/SAP102$^{MKO2/MKO2}$ mice expressing fluorescently labelled post-synaptic proteins underwent MD from P25-31 or EE rearing until P30 and P90 and synaptome mapping was performed on a brain-wide scale. Preliminary results indicate no significant differences in excitatory synapse number in any brain regions. Analysis of synapse types, subtypes and synapse diversity will be presented. These findings will help to clarify diverse, region-specific changes in excitatory synapse populations in response to experience-dependent activity.

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GIRK CHANNELS ARE INVOLVED IN THE MODULATION OF DORSAL HIPPOCAMPUS METAPLASTICITY MECHANISMS THAT SUPPORT COGNITIVE HEALTH

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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GirK (G-protein-gated inwardly rectifying potassium) channels conductance is constitutively active in dorsal CA1 neurons, contributing to their resting membrane potential and ability to self-regulate their excitability in response to neuronal activity, a key aspect of homeostatic plasticity. This open a research avenue to explore GirK-mediated homeostatic regulation as a therapeutic tool in pathologies were hyperexcitabilitiy and altered synaptic plasticity are a hallmark. Here, we aimed to explore the role GirK-mediated basal activity in the regulation of metaplasticity mechanisms supporting hippocampal plasticity processes and dorsal hippocampus-dependent cognitive capabilities. Towards this aim, first, using mice dorsal hippocampal slice preparations, we examined pharmacological GirK channel activity modulation effect on the metaplasticity of long-term plasticity processes induction at CA3-CA1 synapse. Additionally, using an *in vivo* approach, we performed acute intracerebroventricular injections of GirK selective modulators to study their contribution to CA3-CA1 synaptic plasticity and subsequent learning and memory functions. Our data shows that GirK activity is required for the establishment of long-term synaptic plasticity processes in dorsal hippocampus, as its modulation can modify the metaplasticity of LTP/LTD induction threshold. Also, the disruption of such mechanism leads to a decline in hippocampal-dependent tasks performance. Together, these results provide evidence that GirK basal activity governs hippocampal synaptic plasticity direction, which has a significant impact on hippocampal-dependent cognitive functions. **Acknowledgements**: This work was supported by grants no. BFU2017-82494-P and PID2020-115823-GB100 funded by MCIN/AEI/10.13039/501100011033 and by “ERDF A way of making Europe”, both to LJ-D y JDN-L. Fundación Tatiana Perez de Guzmán el Bueno and Plan Propio UCLM.

Pubmed:
34261700: Djebari S, Iborra-Lázaro G, Temprano-Carazo S, Sánchez-Rodríguez I, Nava-Mesa MO, Múnera A, Gruart A, Delgado-García JM, Jiménez-Díaz L, Navarro-López JD
G-Protein-Gated Inwardly Rectifying Potassium (Kir3/GIRK) Channels Govern Synaptic Plasticity That Supports Hippocampal-Dependent Cognitive Functions in Male Mice.
The G-protein-gated inwardly rectifying potassium (Kir3/GIRK) channel is the effector of many G-protein-coupled receptors (GPCRs). Its dysfunction has been linked to the pathophysiology of Down syndrome, Alzheimer's and Parkinson's diseases, psychiatric disorders, epilepsy, drug addiction, or alcoholism. In the hippocampus, GIRK channels decrease excitability of the cells and contribute to resting membrane potential and inhibitory neurotransmission. Here, to elucidate the role of GIRK channels activity in the maintenance of hippocampal-dependent cognitive functions, their involvement in controlling neuronal excitability at different levels of complexity was examined in C57BL/6 male mice. For that purpose, GIRK activity in the dorsal hippocampus CA3-CA1 synapse was pharmacologically modulated by two drugs: ML297, a GIRK channel opener, and Tertiapin-Q (TQ), a GIRK channel blocker. , using dorsal hippocampal slices, we studied the effect of pharmacological GIRK modulation on synaptic plasticity processes induced in CA1 by Schaffer collateral stimulation. , we performed acute intracerebroventricular (i.c.v.) injections of the two GIRK modulators to study their contribution to electrophysiological properties and synaptic plasticity of dorsal hippocampal CA3-CA1 synapse, and to learning and memory capabilities during hippocampal-dependent tasks. We found that pharmacological disruption of GIRK channel activity by i.c.v. injections, causing either function gain or function loss, induced learning and memory deficits by a mechanism involving neural excitability impairments and alterations in the induction and maintenance of long-term synaptic plasticity processes. These results support the contention that an accurate control of GIRK activity must take place in the hippocampus to sustain cognitive functions. Cognitive processes of learning and memory that rely on hippocampal synaptic plasticity processes are critically ruled by a finely tuned neural excitability. G-protein-gated inwardly rectifying K (GIRK) channels play a key role in maintaining resting membrane potential, cell excitability and inhibitory neurotransmission. Here, we demonstrate that modulation of GIRK channels activity, causing either function gain or function loss, transforms high-frequency stimulation (HFS)-induced long-

term potentiation (LTP) into long-term depression (LTD), inducing deficits in hippocampal-dependent learning and memory. Together, our data show a crucial GIRK-activity-mediated mechanism that governs synaptic plasticity direction and modulates subsequent hippocampal-dependent cognitive functions.
J Neurosci, 2021; 41
31375981: Hernández RG, Djebari S, Vélez-Ortiz JM, de la Cruz RR, Pastor AM, Benítez-Temiño B
Short-term plasticity after partial deafferentation in the oculomotor system.
Medial rectus motoneurons are innervated by two main pontine inputs. The specific function of each of these two inputs remains to be fully understood. Indeed, selective partial deafferentation of medial rectus motoneurons, performed by the lesion of either the vestibular or the abducens input, initially induces similar changes in motoneuronal discharge. However, at longer time periods, the responses to both lesions are dissimilar. Alterations on eye movements and motoneuronal discharge induced by vestibular input transection recover completely 2 months post-lesion, whereas changes induced by abducens internuclear lesion are more drastic and permanent. Functional recovery could be due to some kind of plastic process, such as reactive synaptogenesis, developed by the remaining intact input, which would occupy the vacant synaptic spaces left after lesion. Herein, by means of confocal microscopy, immunocytochemistry and retrograde labeling, we attempt to elucidate the possible plastic processes that take place after partial deafferentation of medial rectus motoneuron. 48 h post-injury, both vestibular and abducens internuclear lesions produced a reduced synaptic coverage on these motoneurons. However, 96 h after vestibular lesion, there was a partial recovery in the number of synaptic contacts. This suggests that there was reactive synaptogenesis. This recovery was preceded by an increase in somatic neurotrophin content, suggesting a role of these molecules in presynaptic axonal sprouting. The rise in synaptic coverage might be due to terminal sprouting performed by the remaining main input, i.e., abducens internuclear neurons. The present results may improve the understanding of this apparently redundant input system.
Brain Struct Funct, 2019; 224
31875959: Sánchez-Rodríguez I, Djebari S, Temprano-Carazo S, Vega-Avelaira D, Jiménez-Herrera R, Iborra-Lázaro G, Yajeya J, Jiménez-Díaz L, Navarro-López JD
Hippocampal long-term synaptic depression and memory deficits induced in early amyloidopathy are prevented by enhancing G-protein-gated inwardly rectifying potassium channel activity.
Hippocampal synaptic plasticity disruption by amyloid-β (Aβ) peptides + thought to be responsible for learning and memory impairments in Alzheimer's disease (AD) early stage. Failures in neuronal excitability maintenance seems to be an underlying mechanism. G-protein-gated inwardly rectifying potassium (GirK) channels control neural excitability by hyperpolarization in response to many G-protein-coupled receptors activation. Here, in early in vitro and in vivo amyloidosis mouse models, we study whether GirK channels take part of the hippocampal synaptic plasticity impairments generated by Aβ . In vitro electrophysiological recordings from slices showed that Aβ alters synaptic plasticity by switching high-frequency stimulation (HFS) induced long-term potentiation (LTP) to long-term depression (LTD), which led to in vivo hippocampal-dependent memory deficits. Remarkably, selective pharmacological activation of GirK channels with ML297 rescued both HFS-induced LTP and habituation memory from Aβ action. Moreover, when GirK channels were specifically blocked by Tertiapin-Q, their activation with ML297 failed to rescue LTP from the HFS-dependent LTD induced by Aβ . On the other hand, the molecular analysis of the recorded slices by western blot showed that the expression of GIRK1/2 subunits, which form the prototypical GirK channel in the hippocampus, was not significantly regulated by Aβ . However, immunohistochemical examination of our in vivo amyloidosis model showed Aβ to down-regulate hippocampal GIRK1 subunit expression. Together, our results describe an Aβ-mediated deleterious synaptic mechanism that modifies the induction threshold for hippocampal LTP/LTD and underlies memory alterations observed in amyloidosis models. In this scenario, GirK activation assures memory formation by preventing the transformation of HFS-induced LTP into LTD.
J Neurochem, 2020; 153
32698467: Mayordomo-Cava J, Iborra-Lázaro G, Djebari S, Temprano-Carazo S, Sánchez-Rodríguez I, Jeremic D, Gruart A, Delgado-García JM, Jiménez-Díaz L, Navarro-López JD
Impairments of Synaptic Plasticity Induction Threshold and Network Oscillatory Activity in the Hippocampus Underlie Memory Deficits in a Non-Transgenic Mouse Model of Amyloidosis.
In early Alzheimer disease (AD) models synaptic failures and upstreaming aberrant patterns of network synchronous activity result in hippocampal-dependent memory deficits. In such initial stage, soluble forms of Amyloid- (A) peptides have been shown to play a causal role. Among different A species, A has been identified as the biologically active fragment, as induces major neuropathological signs related to early AD stages. Consequently, it has been extensively used to acutely explore the pathophysiological events related with neuronal dysfunction induced by soluble A forms. However, the synaptic mechanisms underlying its toxic effects on hippocampal-dependent memory remain unresolved. Here, in an in vivo model of amyloidosis generated by intracerebroventricular injections of A we studied the synaptic dysfunction mechanisms underlying hippocampal cognitive deficits. At the synaptic level, long-term potentiation (LTP) of synaptic excitation and inhibition was induced in CA1

region by high frequency simulation (HFS) applied to collaterals. A was found to alter metaplastic mechanisms of plasticity, facilitating long-term depression (LTD) of both types of LTP. In addition, aberrant synchronization of hippocampal network activity was found while at the behavioral level, deficits in hippocampal-dependent habituation and recognition memories emerged. Together, our results provide a substrate for synaptic disruption mechanism underlying hippocampal cognitive deficits present in A amyloidosis model.
Biology (Basel), 2020; 9
29116174: Sánchez-Rodríguez I, Temprano-Carazo S, Nájera A, Djebari S, Yajeya J, Gruart A, Delgado-García JM, Jiménez-Díaz L, Navarro-López JD
Activation of G-protein-gated inwardly rectifying potassium (Kir3/GirK) channels rescues hippocampal functions in a mouse model of early amyloid-β pathology.
The hippocampus plays a critical role in learning and memory. Its correct performance relies on excitatory/inhibitory synaptic transmission balance. In early stages of Alzheimer's disease (AD), neuronal hyperexcitability leads to network dysfunction observed in cortical regions such as the hippocampus. G-protein-gated potassium (GirK) channels induce neurons to hyperpolarize, contribute to the resting membrane potential and could compensate any excesses of excitation. Here, we have studied the relationship between GirK channels and hippocampal function in a mouse model of early AD pathology. Intracerebroventricular injections of amyloid-β (Aβ) peptide-which have a causal role in AD pathogenesis-were performed to evaluate CA3-CA1 hippocampal synapse functionality in behaving mice. Aβ increased the excitability of the CA3-CA1 synapse, impaired long-term potentiation (LTP) and hippocampal oscillatory activity, and induced deficits in novel object recognition (NOR) tests. Injection of ML297 alone, a selective GirK activator, was also translated in LTP and NOR deficits. However, increasing GirK activity rescued all hippocampal deficits induced by Aβ due to the restoration of excitability values in the CA3-CA1 synapse. Our results show a synaptic mechanism, through GirK channel modulation, for the prevention of the hyperexcitability that causally contributes to synaptic, network, and cognitive deficits found in early AD pathogenesis.
Sci Rep, 2017; 7

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MECHANICAL ACTIONS OF DENDRITIC-SPINE ENLARGEMENT ON THE PRESYNAPTIC TERMINAL AND THE DISCOVERY OF THE PRESSURE SENSATION AND TRANSDUCTION (PREST) MECHANISM.

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Dendritic spines in the CNS enlarge during learning. Within the compact brain tissue, this spine enlargement may have mechanical effects on the presynaptic terminals. In this work, we studied this phenomenon and we have identified a mechanosensory and transduction mechanism where presynaptic activity is enhanced by sensing the mechanical pressure emerging from spine enlargement. We used the Schaffer collateral (SC) in hippocampal slice cultures and we have found that fine and transient pushing of the boutons by a glass pipette markedly promoted the evoked neurotransmitter release and vesicle binding in the presynaptic terminals. To our surprise, both effects persisted over 20 min. Here, transmitter release was measured by presynaptic expression of iGluSnFR, and vesicle binding was estimated by the assembly of SNARE proteins, whose FRET between Syntaxin1A and VAMP2 was measured with fluorescence lifetime imaging (FLIM). Both effects were reproduced by a low hypertonic sucrose solution. Finally, we identified synapse candidates by labeling boutons (trans-SNARE or iGluSnFR probe) and dendritic spines (Alexa-dye by whole-cell patch-clamping). Here, the spine enlargement induced by two-photon glutamate uncaging enhanced evoked release and FRET only when the spines pushed the boutons by their elongation. In summary, we have found a PREST (Pressure Sensation and Transduction) mechanism in the boutons which enables boutons to sense mechanical forces for enhancing evoked release by increasing the ternary-SNARE formation. And we suggest that the spine enlargement, in addition to its roles in the well-recognized postsynaptic mechanisms, can potentiate the synaptic transmission by direct mechanical coupling of the presynaptic terminal.

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BOARD NUMBER: S02-339

CONTROL OF POST-SYNAPTIC DENSITY PROTEIN RECRUITMENT BY OPTOGENETIC MANIPULATION OF THE SPINE CYTOSKELETON

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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In glutamatergic synapses of the mammalian brain, the sizes of synaptic structures are strongly correlated with each other and with synaptic strength. In particular the size of the post-synaptic density (PSD) correlates with that of the supporting spine. How this correlation is established and maintained is not clear. Spine size depends on the mechanical support and outward physical pressure exerted by the spine's actin cytoskeleton. Since some of the PSD proteins are also stabilized by binding to actin filaments, one possibility is that assembly of spine actin may directly control the extent of PSD growth.
To test this hypothesis, using cultured hippocampal neurons, we have investigated changes in the PSD component Homer 1c following pharmacological stabilization of actin filaments or direct optogenetic stimulation of actin filament nucleation in single spines.
We find that the effects of enhanced actin assembly are consistent with a homeostatic role of spine actin for regulating the size of the PSD at the single synapse level. The findings are discussed in the framework of structural plasticity.

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ROLE OF INTRACELLULAR CA2+ STORES IN SYNAPTIC TAG AND CAPTURE IN MOUSE HIPPOCAMPAL SLICES

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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LTP induced by a weak stimulus can be enhanced if it is preceded by LTP induced by a strong stimulus at neighbouring synapses due to a process known as synaptic tagging and capture (STC). Despite the importance of calcium in synaptic plasticity, there remains a large gap as to the exact mechanism by which this occurs and the source of calcium that govern different forms of synaptic plasticity. To study this, we performed a two-pathway experiment. In the first pathway (

BOARD NUMBER: S0), we induced LTP with a strong spaced TBS protocol (sTBS) and followed that with a weak TBS (wTBS) applied to an independent pathway (S1) 30 mins later. We recorded fEPSPs from stratum radiatum in response to stimulation of the Schaffer Collateral-Commissural pathway of 10-12 week old C57/BL6J M mice. We found that when Cyclopiazonic acid (CPA, 30 uM), Ryanodine (Ry, 10uM) and IEM (30 uM) a Calcium Permeable -AMPAR inhibitor were present during the strong TBS, LTP in both pathways were inhibited. However, when group 1 mGluRs inhibtors (1 uM YM 298198 and MTEP) were used, STC was unaffected. To see if the deficit was due to a disruption of setting of the synaptic tag, we washed on CPA 30 mins later during only the weak TBS and showed that LTP was inhibited in the second pathway but not the first. Together these results highlight the importance of intracellular calcium stores in the generation (during the sTBS in

BOARD NUMBER: S0) and setting of the synaptic tag (during wTBS in S1).

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EFFECT OF CANNABIDIOL ON SYNAPTIC PLASTICITY IN PRIMARY EMBRYONIC RAT CORTICAL NEURONS

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Aims: Neuropsychiatric disorders are on a steep rise in the last decades. With their complex etiology and poorly defined molecular basis, it is very difficult to aim for efficient treatment. Their pathology is usually connected to a dysregulation of neurotransmitters, disruption of neuroplasticity and neurogenesis. Cannabidiol (CBD) is a non-psychoactive compound harvested from *Cannabis sativa* with a considerable therapeutic potential for treating anxiety and depression. Proposed molecular mechanisms involved in its antidepressant and anxiolytic features are connected to cannabinoid (CB) and serotonin 5-HT_{1A} receptors. However, we still lack sufficient amount of clear scientific data proving these effects. Methods: First, we examined viability of embryonic rat cortical neuronal cultures (E18, DIV16) after CBD treatment in various concentrations (0.1 – 100 μM). Next, we examined the effect of CBD (0.1 – 10 μM) on synaptic plasticity with immunocytochemistry, using synapsin I and PSD-95 as presynaptic and postsynaptic markers, respectively, in dendrites of pyramidal neurons. Data were analysed using ImageJ software. Results: 24h treatment with 100 μM CBD significantly decreased viability of the cells, while lower doses did not affect it. Interestingly, CBD dose-dependently decreased numbers of both pre– and postsynaptic puncta and also number of synapses. Conclusions: CBD diminished number of synaptic proteins puncta. Our data show new insights into the molecular effects of CBD and indicate that further research on the actions of CBD is required to trace the basis of therapeutic potential of CBD.

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PRION PROTEIN TURNOVER AT SYNAPSES AND ENDOLYSOSOMAL COMPARTMENTS DURING SYNAPTIC PLASTICITY

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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The cellular prion protein (PrP^C) is highly expressed in the brain and in neurons. It is known for its role in transmissible spongiform encephalopathies and is suggested to be involved in the pathophysiology of other neurodegenerative diseases including Alzheimer's disease. Although PrP^C structure, misfolding, and mechanisms of pathogenesis have been extensively studied, its physiological functions in neurons largely remain elusive.
Recently, we have shown that the coordination of dynein- and kinesin-mediated bidirectional PrP^C vesicle trafficking impacts on prion disease progression. The regulation of motor protein complexes was found to operate as a critical switch for PrP^C lysosomal degradation versus its recycling and release in exosomes. In the current project, we aimed to investigate the role of PrP^C at hippocampal synapses and for activity-regulated processes. Our analysis revealed PrP^C localization at both excitatory and inhibitory synapses. Interestingly, we identified two cell adhesion molecules as novel interaction partners of PrP^C. Synaptically localized PrP^C only showed moderate response to different chemical stimulation protocols. In contrast, induction of chemical LTP or LTD triggered prominent changes in the vesicular PrP^C pool and in its active transport and targeting to endolysosomal compartments. Furthermore, neuronal live imaging revealed PrP^C and cell adhesion molecules to undergo cotransport within the same vesicles. Functionally, PrP^C facilitated the targeting of cell adhesion molecules to endolysosomes. Future experiments are planned to ask whether and how PrP^C neuronal turnover links to synaptic function.

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LONG-TERM MEMORY AND PLASTICITY CONTROLLED BY THE UNCONVENTIONAL TRANSLATION INITIATION FACTOR EIF2A

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Protein synthesis is a highly regulated process that is pivotal for long-term memory formation. Mutations in translation factors, or genes modulating translation initiation, have been associated with a wide range of neurological disorders, including cognitive disorders. Compared to the relatively well characterized AUG-mediated classical model of translation, little is known about the role of alternative translation that utilizes non-AUG start codons in mnemonic processing. Here we generated and characterized a new mouse model lacking the unconventional translation initiation factor eIF2A, which has been shown to regulate non-AUG translation initiation. Mice lacking eIF2A exhibit deficits in several long-term memory tasks. Interestingly, eIF2A deficient mice show specific deficits in protein synthesis-dependent mGluR long-term potentiation (LTP) and depression (LTD). Finally, using an in vivo ribosome profiling, we investigated the translation landscape controlled by eIF2A in the mammalian brain. Our findings suggest a crucial role for non-canonical translational control by eIF2A in long-term memory formation.

Pubmed:
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An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike.
The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus enters host cells via an interaction between its Spike protein and the host cell receptor angiotensin-converting enzyme 2 (ACE2). By screening a yeast surface-displayed library of synthetic nanobody sequences, we developed nanobodies that disrupt the interaction between Spike and ACE2. Cryo-electron microscopy (cryo-EM) revealed that one nanobody, Nb6, binds Spike in a fully inactive conformation with its receptor binding domains locked into their inaccessible down state, incapable of binding ACE2. Affinity maturation and structure-guided design of multivalency yielded a trivalent nanobody, mNb6-tri, with femtomolar affinity for Spike and picomolar neutralization of SARS-CoV-2 infection. mNb6-tri retains function after aerosolization, lyophilization, and heat treatment, which enables aerosol-mediated delivery of this potent neutralizer directly to the airway epithelia.
Science, 2020; 370
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Inhibition of Upf2-Dependent Nonsense-Mediated Decay Leads to Behavioral and Neurophysiological Abnormalities by Activating the Immune Response.
In humans, disruption of nonsense-mediated decay (NMD) has been associated with neurodevelopmental disorders (NDDs) such as autism spectrum disorder and intellectual disability. However, the mechanism by which deficient NMD leads to neurodevelopmental dysfunction remains unknown, preventing development of targeted therapies. Here we identified novel protein-coding UPF2 (UP-Frameshift 2) variants in humans with NDD, including speech and language deficits. In parallel, we

found that mice lacking Upf2 in the forebrain (Upf2 fb-KO mice) show impaired NMD, memory deficits, abnormal long-term potentiation (LTP), and social and communication deficits. Surprisingly, Upf2 fb-KO mice exhibit elevated expression of immune genes and brain inflammation. More importantly, treatment with two FDA-approved anti-inflammatory drugs reduced brain inflammation, restored LTP and long-term memory, and reversed social and communication deficits. Collectively, our findings indicate that impaired UPF2-dependent NMD leads to neurodevelopmental dysfunction and suggest that anti-inflammatory agents may prove effective for treatment of disorders with impaired NMD.
Neuron, 2019; 104
30610193: Wu Y, Du S, Johnson JL, Tung HY, Landers CT, Liu Y, Seman BG, Wheeler RT, Costa-Mattioli M, Kheradmand F, Zheng H, Corry DB
Microglia and amyloid precursor protein coordinate control of transient Candida cerebritis with memory deficits.
Bloodborne infections with Candida albicans are an increasingly recognized complication of modern medicine. Here, we present a mouse model of low-grade candidemia to determine the effect of disseminated infection on cerebral function and relevant immune determinants. We show that intravenous injection of 25,000 C. albicans cells causes a highly localized cerebritis marked by the accumulation of activated microglial and astroglial cells around yeast aggregates, forming fungal-induced glial granulomas. Amyloid precursor protein accumulates within the periphery of these granulomas, while cleaved amyloid beta (Aβ) peptides accumulate around the yeast cells. CNS-localized C. albicans further activate the transcription factor NF-κB and induce production of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor (TNF), and Aβ peptides enhance both phagocytic and antifungal activity from BV-2 cells. Mice infected with C. albicans display mild memory impairment that resolves with fungal clearance. Our results warrant additional studies to understand the effect of chronic cerebritis on cognitive and immune function.
Nat Commun, 2019; 10
30338063: Jiang X, Zhang T, Wang H, Wang T, Qin M, Bao P, Wang R, Liu Y, Chang HC, Yan J, Xu J
Neurodegeneration-associated FUS is a novel regulator of circadian gene expression.
Circadian rhythms are oscillating physiological and behavioral changes governed by an internal molecular clock, and dysfunctions in circadian rhythms have been associated with ageing and various neurodegenerative diseases. However, the evidence directly connecting the neurodegeneration-associated proteins to circadian control at the molecular level remains sparse.
Transl Neurodegener, 2018; 7

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METHAMPHETAMINE-INDUCED REMODELLING OF HIPPOCAMPAL NEURONS IS ORCHESTRATED VIA CDC42 PATHWAY

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Aims: Psychostimulant substances, such as methamphetamine (Meth), affect the function and morphology of neuronal cells. Here, we aimed at uncovering the signalling pathways regulating this complex process, focusing on cdc42, a RhoGTPase important for the regulation of the actin cytoskeleton and synaptic function. **Methods:** Primary hippocampal neurons were exposed to Meth to assess alterations on morphology, function and to monitor cdc42 activation through FRET assays. Synaptoneurosomes from these cultures were used to evaluate cdc42 downstream proteins. Tying to prevent Meth effects on neuronal morphology, neurons were transfected with a miRNA to knockdown Intersectin1, a specific activator of cdc42, or treated with Zcl278, an inhibitor of cdc42/Intersectin1 interaction. Finally, WT mice received a binge pattern of Meth (4x5mg/kg, 2h intervals) and cdc42 activation and neuronal remodelling on the hippocampus were assessed. **Results:** Meth increased neurite outgrowth, dendritic spine density and decreased spontaneous neuronal activity. Cdc42 activity was increased at dendritic spines 5 min after drug exposure. Concurrently, the downstream pathway (N-Wasp, Arp3) was activated 15 min following Meth. Inhibition of Intersectin1, as well as Intersectin1 knockdown prevented Meth-induced effects on neuronal remodelling. In WT mice exposed to Meth, cdc42 activity was increased in hippocampal synaptoneurosomes 15 min after drug administration. Meth also increased neurite length and dendritic spine density *in vivo*. **Conclusions:** Collectively, our data demonstrate that Meth affects neuronal function and morphology in the hippocampus, which is driven, at least in part, by cdc42. Future work will determine the role of cdc42 on the development of addictive behaviour and relapse.

Pubmed:
35159165: Bravo J, Ribeiro I, Terceiro AF, Andrade EB, Portugal CC, Lopes IM, Azevedo MM, Sousa M, Lopes CDF, Lobo AC, Canedo T, Relvas JB, Summavielle T
Neuron-Microglia Contact-Dependent Mechanisms Attenuate Methamphetamine-Induced Microglia Reactivity and Enhance Neuronal Plasticity.
Exposure to methamphetamine (Meth) has been classically associated with damage to neuronal terminals. However, it is now becoming clear that addiction may also result from the interplay between glial cells and neurons. Recently, we demonstrated that binge Meth administration promotes microgliosis and microglia pro-inflammation via astrocytic glutamate release in a TNF/IPR2-Ca-dependent manner. Here, we investigated the contribution of neuronal cells to this process. As the crosstalk between microglia and neurons may occur by contact-dependent and/or contact-independent mechanisms, we developed co-cultures of primary neurons and microglia in microfluidic devices to investigate how their interaction affects Meth-induced microglia activation. Our results show that neurons exposed to Meth do not activate microglia in a cell-autonomous way but require astrocyte mediation. Importantly, we found that neurons can partially prevent Meth-induced microglia activation via astrocytes, which seems to be achieved by increasing arginase 1 expression and strengthening the CD200/CD200r pathway. We also observed an increase in synaptic individual area, as determined by co-localization of pre- and post-synaptic markers.

The present study provides evidence that contact-dependent mechanisms between neurons and microglia can attenuate pro-inflammatory events such as Meth-induced microglia activation.
Cells, 2022; 11

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Astrocyte-derived TNF and glutamate critically modulate microglia activation by methamphetamine.
Methamphetamine (Meth) is a powerful illicit psychostimulant, widely used for recreational purposes. Besides disrupting the monoaminergic system and promoting oxidative brain damage, Meth also causes neuroinflammation, contributing to synaptic dysfunction and behavioral deficits. Aberrant activation of microglia, the largest myeloid cell population in the brain, is a common feature in neurological disorders triggered by neuroinflammation. In this study, we investigated the mechanisms underlying the aberrant activation of microglia elicited by Meth in the adult mouse brain. We found that binge Meth exposure caused microgliosis and disrupted risk assessment behavior (a feature that usually occurs in individuals who abuse Meth), both of which required astrocyte-to-microglia crosstalk. Mechanistically, Meth triggered a detrimental increase of glutamate exocytosis from astrocytes (in a process dependent on TNF production and calcium mobilization), promoting microglial expansion and reactivity. Ablating TNF production, or suppressing astrocytic calcium mobilization, prevented Meth-elicited microglia reactivity and re-established risk assessment behavior as tested by elevated plus maze (EPM). Overall, our data indicate that glial crosstalk is critical to relay alterations caused by acute Meth exposure.
Neuropsychopharmacology, 2021; 46

33225275: Gomes JR, Lobo A, Nogueira R, Terceiro AF, Costelha S, Lopes IM, Magalhães A, Summavielle T, Saraiva MJ
Neuronal megalin mediates synaptic plasticity-a novel mechanism underlying intellectual disabilities in megalin gene pathologies.
Donnai-Barrow syndrome, a genetic disorder associated to LRP2 (low-density lipoprotein receptor 2/megalin) mutations, is characterized by unexplained neurological symptoms and intellectual deficits. Megalin is a multifunctional endocytic clearance cell-surface receptor, mostly described in epithelial cells. This receptor is also expressed in the CNS, mainly in neurons, being involved in neurite outgrowth and neuroprotective mechanisms. Yet, the mechanisms involved in the regulation of megalin in the CNS are poorly understood. Using transthyretin knockout mice, a megalin ligand, we found that transthyretin positively regulates neuronal megalin levels in different CNS areas, particularly in the hippocampus. Transthyretin is even able to rescue megalin downregulation in transthyretin knockout hippocampal neuronal cultures, in a positive feedback mechanism via megalin. Importantly, transthyretin activates a regulated intracellular proteolysis mechanism of neuronal megalin, producing an intracellular domain, which is translocated to the nucleus, unveiling megalin C-terminal as a potential transcription factor, able to regulate gene expression. We unveil that neuronal megalin reduction affects physiological neuronal activity, leading to decreased neurite number, length and branching, and increasing neuronal susceptibility to a toxic insult. Finally, we unravel a new unexpected role of megalin in synaptic plasticity, by promoting the formation and maturation of dendritic spines, and contributing for the establishment of active synapses, both in and hippocampal neurons. Moreover, these structural and synaptic roles of megalin impact on learning and memory mechanisms, since megalin heterozygous mice show hippocampal-related memory and learning deficits in several behaviour tests. Altogether, we unveil a complete novel role of megalin in the physiological neuronal activity, mainly in synaptic plasticity with impact in learning and memory. Importantly, we contribute to disclose the molecular mechanisms underlying the cognitive and intellectual disabilities related to megalin gene pathologies.
Brain Commun, 2020; 2

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RNF10: A SYNAPTONUCLEAR MESSENGER LINKING NMDA RECEPTOR SYNAPTIC ACTIVITY AT CA1 SYNAPSES TO COGNITIVE FLEXIBILITY

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Background: Synaptonuclear protein messengers are fundamental players for the regulation of neuronal activation and morphology both in health and diseases. The Ring Finger Protein 10 (RNF10) is a synapse-to-nucleus signaling protein that specifically links activation of synaptic NMDA receptors to modulation of gene expression. *Aims:* To gain new insight into the *in vivo* functional and structural role of this synaptonuclear protein messenger in hippocampal neurons in orchestrating NMDA receptor-dependent signalling and learning behavior. *Methods:* We used an AAV-shRNA mediated strategy to decrease RNF10 expression selectively in CA1 region. 6 weeks after AAV-injection we performed *in vivo* behavioral evaluations of hippocampal dependent tasks and *ex-vivo* morphological and molecular (biochemistry, confocal imaging, RNAseq) analyses. RNF10 ko mice were also used for proof of concept experiments. *Results: In vivo* silencing of RNF10 in CA1 hippocampal neurons induced morphological and molecular alterations at dendritic spines strictly associated to its role as nuclear messenger. These events led to profound behavioral impairments in learning and cognitive flexibility tests. *Conclusions:* RNF10 activity as synaptonuclear messenger orchestrates the molecular composition, morphology and signalling at CA1 dendritic spines, playing also a key role in NMDAR-dependent learning and cognitive flexibility.

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BOARD NUMBER: S02-346

MMP-9 EXERTS CONTROL OVER INDIVIDUAL DENDRITIC SPINE PLASTICITY

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Understanding molecular mechanisms of morphological and functional synaptic plasticity is a major research challenge. Matrix metalloproteinase-9 (MMP-9) has repeatedly been implicated in plasticity of excitatory synapses, however the exact mechanistic insight into its function is missing. To address this issue, we have followed glutamate uncaging protocol (uLTP) to produce long-term potentiation of individual synapses in organotypic hippocampal cultures. The cultures were incubated in the presence of MNI-glutamate with stimulation protocol of 30 laser pulses (0.5 Hz) delivered specifically at CA1 individual spines (harboring the excitatory synapses). Fluorescent lifetime imaging microscopy (FLIM) together with MMP-9 FRET sensor was used to measure MMP-9 activity. uLTP protocol resulted in a marked spine growth (up to 3-fold peak, followed by a 50% sustained increase lasting for at least 20 min.). The increase in spine volume was taken as a measure of a structural LTP of the synapses. Treatment with MMP-9 inhibitors (Inhibitor I and GM 6001) significantly impaired the spine growth both during its peak and sustained phase. Similarly, uLTP protocol induced significantly smaller spine growth in cultures derived from the MMP-9 KO mice in comparison to their WT littermates. This structural plasticity impairment was reversed by MMP-9 overexpression in KO cultures. Furthermore, we have shown a rapid local activity of MMP-9 during uLTP induction on dendritic spines. Our results suggest the MMP-9 involvement in dendritic spines plasticity from the onset of the LTP induction.

Pubmed:
33739386: Salamian A, Legutko D, Nowicka K, Badyra B, Kaźmierska-Grębowska P, Caban B, Kowalczyk T, Kaczmarek L, Beroun A
Inhibition of Matrix Metalloproteinase 9 Activity Promotes Synaptogenesis in the Hippocampus.
Information coding in the hippocampus relies on the interplay between various neuronal ensembles. We discovered that the application of a cholinergic agonist, carbachol (Cch), which triggers oscillatory activity in the gamma range, induces the activity of matrix metalloproteinase 9 (MMP-9)-an enzyme necessary for the maintenance of synaptic plasticity. Using electrophysiological recordings in hippocampal organotypic slices, we show that Cch potentiates the frequency of miniature inhibitory and excitatory postsynaptic currents (mIPSCs and mEPSCs, respectively) in CA1 neurons and this effect is MMP-9 dependent. Interestingly, though MMP-9 inhibition prevents the potentiation of inhibitory events, it further boosts the frequency of excitatory mEPSCs. Such enhancement of the frequency of excitatory events is a result of increased synaptogenesis onto CA1 neurons. Thus, the function of MMP-9 in cholinergically induced plasticity in the hippocampus is to maintain the fine-tuned balance between the excitatory and the inhibitory synaptic transmission.
Cereb Cortex, 2021; 31
27312902: Stefaniuk M, Gualda EJ, Pawlowska M, Legutko D, Matryba P, Koza P, Konopka W, Owczarek D, Wawrzyniak M, Loza-Alvarez P, Kaczmarek L
Light-sheet microscopy imaging of a whole cleared rat brain with Thy1-GFP transgene.
Whole-brain imaging with light-sheet fluorescence microscopy and optically cleared tissue is a new, rapidly developing research field. Whereas successful attempts to clear and image mouse brain have been reported, a similar result for rats has proven difficult to achieve. Herein, we report on creating novel transgenic rat harboring fluorescent reporter GFP under control of neuronal gene promoter. We then present data on clearing the rat brain, showing that FluoClearBABB was found superior over passive CLARITY and CUBIC methods. Finally, we demonstrate efficient imaging of the rat brain using light-sheet fluorescence microscopy.
Sci Rep, 2016; 6
28409571: Pawłowska M, Legutko D, Stefaniuk M

[Getting an insight into the brain - new optical clearing techniques and imaging using light-sheet microscope].
One of the biggest challenges in neuroscience is to understand how brain operates. For this, it would be the best to image the whole brain with at least cellular resolution, preserving the three-dimensional structure in order to capture the connections between different areas. Most currently available high-resolution imaging techniques are based on preparing thin brain sections that are next photographed one by one and subsequently bigger structures are reconstructed. These techniques are laborious and create artifacts. Recent optical clearing methods allow to obtain literally transparent brains that can be imaged using light-sheet microscope. The present review summarizes the most popular optical clearing techniques, describing their different mechanisms and comparing advantages and disadvantages of different approaches, and presents the principle of light-sheet microscopy and its use in imaging. Finally, it gives examples of application of optical tissue clearing and light-sheet imaging in neuroscience and beyond it.
Postepy Biochem, 2017; 63
32552916: Florkowska A, Meszka I, Zawada M, Legutko D, Proszynski TJ, Janczyk-Ilach K, Streminska W, Ciemerych MA, Grabowska I
Pax7 as molecular switch regulating early and advanced stages of myogenic mouse ESC differentiation in teratomas.
Pluripotent stem cells present the ability to self-renew and undergo differentiation into any cell type building an organism. Importantly, a lot of evidence on embryonic stem cell (ESC) differentiation comes from in vitro studies. However, ESCs cultured in vitro do not necessarily behave as cells differentiating in vivo. For this reason, we used teratomas to study early and advanced stages of in vivo ESC myogenic differentiation and the role of Pax7 in this process. Pax7 transcription factor plays a crucial role in the formation and differentiation of skeletal muscle precursor cells during embryonic development. It controls the expression of other myogenic regulators and also acts as an anti-apoptotic factor. It is also involved in the formation and maintenance of satellite cell population.
Stem Cell Res Ther, 2020; 11

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BOARD NUMBER: S02-347

DESIGN OF AN ULTRAPOTENT GENETICALLY ENCODED BLOCKER OF THE POTASSIUM CHANNEL KV4.2 FOR GATING NEURAL PLASTICITY

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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The voltage gated potassium channel Kv4.2 is critical in neuronal plasticity, learning and memory. However, the exact mechanism(s) by which Kv4.2 modulates neuronal plasticity are poorly understood and, at times, diverging. Current means to study the roles of Kv4.2, whether genetic or pharmacologic, are limited in specificity, spatial and temporal resolutions. To address the roles of Kv4.2 in neurons, we engineered a novel targeted tethered toxin, **Me**mbrane **t**ethered Hetero**Poda**toxin-2 (**MetaPoda**) and systematically characterized it. We found that MetaPoda is potent at inhibiting Kv4.2 currents and instigated a drastic reduction in the expression of Kv4.2. In neurons, MetaPoda shortened the action potential, but other intrinsic properties were unaffected. Using single cell RNA Sequencing of neurons expressing MetaPoda showed that inhibition of Kv4.2 causes very subtle changes in transcriptome, consistent with studies using Kv4.2-knock-out animals. However, we did observe an increase in cFOS and Arc/Arg3.1 which have been shown to increase during LTP and are necessary for it. cFos immunostaining showed an increase of 2.5-fold in MetaPoda infected neurons. This led us to suggest an additional role for Kv4.2 beyond its function as a potassium channel which negatively influences plasticity. Kv4.2 could act as a plasticity regulator with the following proposed mechanism: downregulation of Kv4.2 in neurons drives the neuron towards a post-potentiation state, as if potentiation has ensued. This post-potentiation state thereby hinders the ability of the neuron to further undergo potentiation which affects learning and memory. This counterintuitive role could help reveal the mechanisms through which Kv4.2 affects plasticity.

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BOARD NUMBER: S02-348

VASOPRESSIN ACTS AS A SYNAPSE ORGANIZER IN LIMBIC REGIONS BY BOOSTING PSD95 AND GLUA1 EXPRESSION

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Previous results from our group have shown that AVP magnocellular neurons project to the hippocampus, extended amygdala, habenula, and locus coeruleus, with dense-core vesicles close to the active zone of excitatory synapses. The current study was conducted to determine whether AVP regulates excitatory synapse formation in those areas. Western blot analysis showed that 48-hour water deprivation increases the expression of PSD95 and GluA1 in the amygdala, lateral habenula, and ventral hippocampus, but not in the visual cortex. In *ex vivo* experiments with hippocampal brain slices incubated with AVP for 1-2 hours, we observed an upregulation of both PSD95 and GluA1. The co-incubation with the antagonists for the V1a (SR49059) or V1b (SSR149415) receptors blocked this AVP-induced increase in PSD95 and GLUA1. Using expansion microscopy, we showed that the density of PSD95 and GluA1 puncta in the dendrites of neurons innervated by vasopressinergic fibers is increased after 48-hours of water deprivation. These results suggest that vasopressin can act as a synapse organizer by modulating the synaptic strength in specific synapses of hypothalamic-limbic circuits.

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BOARD NUMBER: S02-349

FULLY-PRIMED SLOWLY-RECOVERING VESICLES MEDIATE LTP AT NEOCORTICAL NEURONS

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

Iron Weichard[1], Holger Taschenberger[2], Grit Bornschein[1], Andreas Ritzau-Jost[1], Hartmut Schmidt[1], Robert Kittel[3], Jens Eilers[1], Erwin Neher[4], Stefan Hallermann[1], Jana Nerlich[1]
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Pre- and postsynaptic forms of long-term potentiation (LTP) are candidate synaptic mechanisms underlying learning and memory. A classical form of presynaptic LTP, referred to as synaptic redistribution, has been described for layer 5 pyramidal neurons. However, how this apparent increase in the release probability relates to recent advances in the understanding of priming of synaptic vesicles remains unclear. We therefore performed whole-cell recordings from layer 5 pyramidal neurons in acute cortical slices of rats in combination with extracellular stimulation of local excitatory inputs and analyzed the presynaptic function before and after the induction of LTP. LTP increased the EPSC amplitude by a median factor of 1.5 in half of the synapses. In these responder synapses, LTP increased synaptic depression during high-frequency transmission and slowed the recovery from depression by adding a second slow component to the time course of recovery. Analysis with a recently established two-step vesicle priming model indicates an increase in the number of fully-primed vesicles that recover slowly following stimulation. To further test this hypothesis, we pharmacologically stimulated the cyclic adenosine monophosphate (cAMP) and diacylglycerol (DAG) pathways, which are both known to promote synaptic vesicle priming. Both pharmacological manipulations indeed mimicked all features of electrically-induced LTP. Comparing presynaptic plasticity at various synapses revealed a general correlation that stronger synapses recover slower from synaptic depression, indicating that fully-primed vesicles rely on a slowly maturating release machinery. Our data show that LTP at layer 5 pyramidal neurons increases the synaptic strength by enlarging a subpool of fully-primed slowly-recovering vesicles.

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BOARD NUMBER: S02-350

GEPHYRIN E DOMAIN DIMERIZATION-DEPENDENT GLYCINE RECEPTOR BINDING

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Gephyrin is the main scaffold protein at inhibitory synapses and interacts with γ-aminobutyric acid type A receptors ($GABA_AR$) or glycine receptors (GlyR), thus restricting their lateral movement and ensuring proper inhibitory signal transmission. Gephyrin is a three-domain protein which assembles as a trimer and adopts various conformations. High-affinity interaction towards GlyRs and $GABA_ARs$ is primarily mediated via the E domain (GephE), which forms dimers in solution and builds up a hydrophobic pocket for receptor binding. However, full-length gephyrin assembles as a homotrimer in which GephE was found in a monomeric state. In this work we utilized electron microscopy with single particle analysis (SPA) of gephyrin alone or in complex with 49 residue peptide derived from the GlyR β subunit (GlyR-bloop) harboring the gephyrin binding motif. In addition, we used a pentameric GlyR intracellular domain (ICD) mimicking protein to obtain structural insights of the GlyR-gephyrin complex. Analyzing those complexes revealed a ligand-induced GephE dimerization which formed within one gephyrin trimer, thus creating the previously observed binding pocket for high affinity receptor interaction. Consequently, gephyrin variants with altered GephE dimerization showed an impairment in GlyR binding *in vitro* and in neurons. Furthermore, the obtained structural data excluded the formation of GephE dimers between different gephyrin trimers, which argues against the formation of a previously proposed synapse spanning hexagonal lattice. Nonetheless, complex formation between gephyrin pentameric GlyR-ICD mimicking protein resulted in the formation of large assemblies suggesting a bridging function of gephyrin in connecting various receptors at the postsynaptic membrane.

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BOARD NUMBER: S02-351

BNDF MRNA TRACKING IN LIVE NEURONS

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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The neurotrophin brain-derived neurotrophic factor (BDNF) plays a key role in neuronal survival and neurite out-growth, synaptogenesis and synaptic plasticity. BDNF mRNA can be transported in neuronal dendrites in an activity-dependent manner in particular, following seizures but also in response to antidepressants or physical activity. At present, a clear demonstration that BDNF mRNA is locally transported and translated at activated synapses in response to the induction of long-term potentiation (LTP) is still lacking. Here, we study the dynamics of BDNF mRNA trafficking during neuronal plasticity induced by chemical-LTP. The project explores the two hypotheses of selective vs. non-selective dendritic transport of BDNF transcripts after synaptic potentiation and the related methodological constrains using the MS2 system for mRNA visualization in living neurons.

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BOARD NUMBER: S02-352

ALL-OPTICAL INVESTIGATION OF LONG-TERM PLASTICITY IN THE HIPPOCAMPUS

POSTER SESSION 02 - SECTION: MECHANISMS OF SYNAPTIC PLASTICITY

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Synaptic plasticity, the long-lasting change in synaptic efficacy and structure, is a major mechanism of information storage in the brain. We took advantage of optogenetic tools to investigate the role of postsynaptic CaMKIIα in synaptic plasticity up to 3 days following induction. We induced spike-timing-dependent plasticity (STDP) at Schaffer collateral synapses in rat hippocampal slice cultures by pairing independent optogenetic stimulation of pre and postsynaptic neurons expressing spectrally separated channelrhodopsins. We found that optically induced STDP induces timing-dependent long-term potentiation (tLTP) of synaptic strength up to 30 minutes after stimulation. Even 3 days later, we could detect strengthened synaptic input onto tLTP neurons. When we optically inhibited the activity of CaMKIIα, one of the very important memory molecules that transform transient synaptic activity events into long-lasting synaptic plasticity, we observed a complete blockade of the acute tLTP. Unexpectedly, 3 days later, stimulated neurons received significantly stronger input than their neighbors, a potentiation that appears to be independent of CaMKIIα activity. We then tested the direct effects of CaMKIIα activation with a photoactivatable CaMKII. We found that optical activation of CaMKIIα is sufficient to acutely induce functional LTP. In line with the effects of acute CaMKII inhibition, this CaMKIIα activation-induced LTP returned to baseline after a couple of days. In conclusion, the potentiation of synaptic inputs seems to have two phases: CaMKIIα is necessary and sufficient to induce acute LTP. Whereas a second CaMKIIα independent mechanism is responsible for the selective strengthening of inputs days later.

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BOARD NUMBER: S02-353

REGULATION OF THE GABAERGIC SYNAPSE BY THE WNK PATHWAY.

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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We have shown that the chloride-sensing WNK kinase and its effector, SPAK, rapidly regulates neuronal Cl- homeostasis in mature hippocampal neurons by controlling the membrane dynamics and clustering of the chloride transporters KCC2 and NKCC1 near GABAergic synapses, thereby impacting the efficacy of GABAergic transmission. It is still unknown whether this pathway regulates the ionotropic $GABA_A$ receptor ($GABA_AR$) and/or its scaffolding protein gephyrin. Upon pharmacological blockade of the WNK/SPAK pathway, Single-Particle Tracking (SPT) experiments on live neurons show no effect of inhibitors on the lateral diffusion of the $GABA_AR$ α1 subunit ($GABA_AR\alpha1$) at synapses but a reduced diffusion and an increased confinement of the $GABA_AR\alpha1$ at extrasynaptic site. This effect was prevented by applying a peptide blocking clathrin-dependent endocytosis indicating that the restricted motion of $GABA_AR\alpha1$ upon WNK/SPAK inhibition was due to receptor undergoing endocytosis. Super-resolution microscopy upon WNK/SPAK inhibition reported an increase in the number of $GABA_AR\alpha1$ clusters with reduced size and density of detections, indicating cluster fragmentation. These results suggest that the WNK/SPAK pathway stabilizes $GABA_AR\alpha1$ at the plasma membrane. To confirm this hypothesis, we activated the pathway by lowering intracellular chloride levels and checked its impact on $GABA_AR\alpha1$ diffusion. Preliminary results show that activation of the pathway releases diffusion constraints on extrasynaptic GABAARα1 and confines GABAARα1 to synapses. This suggests increased receptor recruitment to synapses, which will need to be confirmed in super-resolution. Altogether, our data point toward a regulation of the GABAergic synapse by the WNK pathway.

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BOARD NUMBER: S02-354

PRESYNAPTIC LOCALIZATION OF ATG-9 IS REGULATED BY SCAMP5 ASSOCIATED WITH AP-4 COMPLEX

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

Unghwi Lee, Seung Hyun Ryu, Jungmin Lee, Sunghoe Chang
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Autophagy is one of major cellular catabolic systems for regulating degradation of a wide range of damaged proteins and dysfunctional organelles. In neurons, autophagy is important for controlling turnover of synaptic proteins and removal of protein aggregates to maintain the neuronal activity and synaptic transmission. In recent studies, ATG-9 which is a transmembrane component of core autophagosome formation machineries has been suggested as a mediator of synaptic autophagy at presynaptic terminal. ATG-9 is known to be exported from *trans*-Golgi network (TGN) and transported to its destination via AP-4 complex, however, how ATG-9 trafficking to presynaptic bouton is regulated remains unknown despite its importance for adjusting the presynaptic autophagy and synaptic activity. Here, we found that secretory carrier membrane protein 5 (SCAMP5) enriched at TGN interacted with AP-4 complex to promote the recruitment of ATG-9 to presynaptic site for mediating autophagy process. Biochemical analysis and optical imaging revealed that the N-terminal region of SCAMP5 is associated with mu subunit of AP-4 complex and shRNA-mediated knockdown (KD) of SCAMP5 significantly impaired the localization of ATG-9 and autophagosome formation which led to the accumulation of excessive proteins at presynaptic terminal. Together, our results exhibit that the interaction between SCAMP5 and AP-4 complex is crucial for the export and transport of ATG-9 from TGN to presynaptic compartment. Since ATG-9 is one of core autophagic machinery members, understanding the localization mechanism of ATG-9 to synaptic boutons may propose a better insight about working system of synaptic autophagy and underpinning mechanisms of autophagy-related neuronal disease.

Pubmed:
33372133: Lee U, Choi C, Ryu SH, Park D, Lee SE, Kim K, Kim Y, Chang S
SCAMP5 plays a critical role in axonal trafficking and synaptic localization of NHE6 to adjust quantal size at glutamatergic synapses.
Glutamate uptake into synaptic vesicles (SVs) depends on cation/H exchange activity, which converts the chemical gradient (ΔpH) into membrane potential (Δψ) across the SV membrane at the presynaptic terminals. Thus, the proper recruitment of cation/H exchanger to SVs is important in determining glutamate quantal size, yet little is known about its localization mechanism. Here, we found that secretory carrier membrane protein 5 (SCAMP5) interacted with the cation/H exchanger NHE6, and this interaction regulated NHE6 recruitment to glutamatergic presynaptic terminals. Protein-protein interaction analysis with truncated constructs revealed that the 2/3 loop domain of SCAMP5 is directly associated with the C-terminal region of NHE6. The use of optical imaging and electrophysiological recording showed that small hairpin RNA-mediated knockdown (KD) of SCAMP5 or perturbation of SCAMP5/NHE6 interaction markedly inhibited axonal trafficking and the presynaptic localization of NHE6, leading to hyperacidification of SVs and a reduction in the quantal size of glutamate release. Knockout of NHE6 occluded the effect of SCAMP5 KD without causing additional defects. Together, our results reveal that as a key regulator of axonal trafficking and synaptic localization of NHE6, SCAMP5 could adjust presynaptic strength by regulating quantal size at glutamatergic synapses. Since both proteins are autism candidate genes, the reduced quantal size by interrupting their interaction may underscore synaptic dysfunction observed in autism.
Proc Natl Acad Sci U S A, 2021; 118
33663553: Lee U, Ryu SH, Chang S
SCAMP5 mediates activity-dependent enhancement of NHE6 recruitment to synaptic vesicles during synaptic plasticity.
Na(K)/H exchanger 6 (NHE6) on synaptic vesicle (SV) is critical for the presynaptic regulation of quantal size at the glutamatergic synapses by converting the chemical gradient (ΔpH) into membrane potential (Δψ) across the SV membrane. We recently found that NHE6 directly interacts with secretory carrier membrane protein 5 (SCAMP5), and SCAMP5-dependent recruitment of NHE6 to SVs controls the strength of synaptic transmission by modulation of quantal size of glutamate release at rest. It is, however, unknown whether NHE6 recruitment by SCAMP5 plays a role during synaptic plasticity. Here, we found that the number of NHE6-positive presynaptic boutons was significantly increased by the chemical long-term potentiation (cLTP). Since cLTP involves new synapse formation, our results indicated that NHE6 was recruited not

only to the existing presynaptic boutons but also to the newly formed presynaptic boutons. Knock down of SCAMP5 completely abrogated the enhancement of NHE6 recruitment by cLTP. Interestingly, despite an increase in the number of NHE6-positive boutons by cLTP, the quantal size of glutamate release at the presynaptic terminals remained unaltered. Together with our recent results, our findings indicate that SCAMP5-dependent recruitment of NHE6 plays a critical role in manifesting presynaptic efficacy not only at rest but also during synaptic plasticity. Since both are autism candidate genes, reduced presynaptic efficacy by interfering with their interaction may underlie the molecular mechanism of synaptic dysfunction observed in autism.
Mol Brain, 2021; 14
27226294: Lee SE, Kim Y, Han JK, Park H, Lee U, Na M, Jeong S, Chung C, Cestra G, Chang S
nArgBP2 regulates excitatory synapse formation by controlling dendritic spine morphology.
Neural Abelson-related gene-binding protein 2 (nArgBP2) was originally identified as a protein that directly interacts with synapse-associated protein 90/postsynaptic density protein 95-associated protein 3 (SAPAP3), a postsynaptic scaffolding protein critical for the assembly of glutamatergic synapses. Although genetic deletion of nArgBP2 in mice leads to manic/bipolar-like behaviors resembling many aspects of symptoms in patients with bipolar disorder, the actual function of nArgBP2 at the synapse is completely unknown. Here, we found that the knockdown (KD) of nArgBP2 by specific small hairpin RNAs (shRNAs) resulted in a dramatic change in dendritic spine morphology. Reintroducing shRNA-resistant nArgBP2 reversed these defects. In particular, nArgBP2 KD impaired spine-synapse formation such that excitatory synapses terminated mostly at dendritic shafts instead of spine heads in spiny neurons, although inhibitory synapse formation was not affected. nArgBP2 KD further caused a marked increase of actin cytoskeleton dynamics in spines, which was associated with increased Wiskott-Aldrich syndrome protein-family verprolin homologous protein 1 (WAVE1)/p21-activated kinase (PAK) phosphorylation and reduced activity of cofilin. These effects of nArgBP2 KD in spines were rescued by inhibiting PAK or activating cofilin combined with sequestration of WAVE. Together, our results suggest that nArgBP2 functions to regulate spine morphogenesis and subsequent spine-synapse formation at glutamatergic synapses. They also raise the possibility that the aberrant regulation of synaptic actin filaments caused by reduced nArgBP2 expression may contribute to the manifestation of the synaptic dysfunction observed in manic/bipolar disorder.
Proc Natl Acad Sci U S A, 2016; 113
29562188: Park D, Lee U, Cho E, Zhao H, Kim JA, Lee BJ, Regan P, Ho WK, Cho K, Chang S
Impairment of Release Site Clearance within the Active Zone by Reduced SCAMP5 Expression Causes Short-Term Depression of Synaptic Release.
Despite being a highly enriched synaptic vesicle (SV) protein and a candidate gene for autism, the physiological function of SCAMP5 remains mostly enigmatic. Here, using optical imaging and electrophysiological experiments, we demonstrate that SCAMP5 plays a critical role in release site clearance at the active zone. Truncation analysis revealed that the 2/3 loop domain of SCAMP5 directly interacts with adaptor protein 2, and this interaction is critical for its role in release site clearance. Knockdown (KD) of SCAMP5 exhibited pronounced synaptic depression accompanied by a slower recovery of the SV pool. Moreover, it induced a strong frequency-dependent short-term depression of synaptic release, even under the condition of sufficient release-ready SVs. Super-resolution microscopy further proved the defects in SV protein clearance induced by KD. Thus, reduced expression of SCAMP5 may impair the efficiency of SV clearance at the active zone, and this might relate to the synaptic dysfunction observed in autism.
Cell Rep, 2018; 22
28698220: Park D, Na M, Kim JA, Lee U, Cho E, Jang M, Chang S
Activation of CaMKIV by soluble amyloid-β impedes trafficking of axonal vesicles and impairs activity-dependent synaptogenesis.
The prefibrillar form of soluble amyloid-β (sAβ) impairs synaptic function and is associated with the early phase of Alzheimer's disease (AD). We investigated how sAβ led to presynaptic defects using a quantum dot-based, single particle-tracking method to monitor synaptic vesicle (SV) trafficking along axons. We found that sAβ prevented new synapse formation induced by chemical long-term potentiation (cLTP). In cultured rat hippocampal neurons, nanomolar amounts of sAβ impaired Ca clearance from presynaptic terminals and increased the basal Ca concentration. This caused an increase in the phosphorylation of Ca/calmodulin-dependent protein kinase IV (CaMKIV) and its substrate synapsin, which markedly inhibited SV trafficking along axons between synapses. Neurons derived from a transgenic AD mouse model had similar defects, which were prevented by an inhibitor of CaMK kinase (CaMKK; which activates CaMKIV), by antibodies against Aβ, or by expression a phosphodeficient synapsin mutant. The CaMKK inhibitor also abolished the defects in activity-dependent synaptogenesis caused by sAβ Our results suggest that by disrupting SV reallocation between synapses, sAβ prevents neurons from forming new synapses or adjusting strength and activity among neighboring synapses. Targeting this mechanism might prevent synaptic dysfunction in AD patients.
Sci Signal, 2017; 10

32989096: Kim Y, Lee U, Choi C, Chang S
Release Mode Dynamically Regulates the RRP Refilling Mechanism at Individual Hippocampal Synapses.
Synaptic strength and reliability are determined by the number of vesicles released per action potential and the availability of release-competent vesicles in the readily releasable pool (RRP). Compared with release of a single vesicle (univesicular release), multivesicular release (MVR) would speed up RRP depletion, yet whether the RRP is refilled differently during the two different release modes has not been investigated. Here, we address this question by quantitative optical imaging with an axon-targeting glutamate sensor, iGluSnFRpre. We found that hippocampal synapses preferentially release multiple vesicles per action potential at high extracellular calcium or by paired-pulse stimulation. When MVR prevails, the RRP is recovered very rapidly with a time constant of 430 ms. This rapid recovery is mediated by dynamin-dependent endocytosis followed by direct reuse of retrieved vesicles. Furthermore, our simulation proved that the portion of retrieved vesicles that directly refill the RRP increases dramatically (>70%) in MVR compared with that in univesicular release (<10%). These results suggest that the contribution of rapid and direct recruitment of retrieved vesicle to the RRP changes dynamically with release mode at the level of individual synapses, which suggests a form of presynaptic homeostatic plasticity for reliable synaptic transmission during various synaptic activity. The number of vesicles released in response to an action potential and the number of release competent vesicles in the readily releasable pool (RRP) are the fundamental determinants of synaptic efficacy. Despite its functional advantages, releasing multiple vesicles, especially at small synapses, can deplete the RRP after a couple of action potentials. To prevent failure of synaptic transmission, the RRP should be refilled rapidly, yet whether the RRP replenishment process is regulated by the release mode has not been investigated. Here, using quantitative optical glutamate imaging and simulation, we demonstrate that the contribution of the fast refilling mechanism changes with release mode at the level of individual synapses, suggesting a rapid form of presynaptic homeostatic plasticity during various synaptic activity.
J Neurosci, 2020; 40

31053155: Lee SE, Jeong S, Lee U, Chang S
SGIP1α functions as a selective endocytic adaptor for the internalization of synaptotagmin 1 at synapses.
Proper sorting of exocytosed synaptic vesicle (SV) proteins into individual SVs during endocytosis is of the utmost importance for the fidelity of subsequent neurotransmission. Recent studies suggest that each SV protein is sorted into individual SVs by its own dedicated adaptors as well as by association between SV proteins. The SH3-containing GRB2-like protein 3-interacting protein 1 (SGIP1), an ortholog of Fer/Cip4 homology domain-only (FCHo) proteins, contains a μ-homology domain (μHD) and binds AP-2 and Eps15, thus functioning as an endocytic regulator of clathrin-mediated endocytosis (CME). Its longest isoform SGIP1α is predominantly expressed in the brain but the functional significance of SGIP1 in SV recycling remains unknown. Here, we found that SGIP1α, a brain-specific long isoform of SGIP1 binds synaptotagmin1 (Syt1) via its μHD and promotes the internalization of Syt1 on the neuronal surface. The small hairpin RNA (shRNA)-mediated knockdown (KD) of SGIP1α caused selective impairment of Syt1 internalization at hippocampal synapses and it was fully rescued by coexpression of the shRNA-resistant form of SGIP1α in KD neurons. We further found that the μHD of SGIP1α is structurally similar to those of AP-2 and stonin2, and mutations at Trp771 and Lys781, which correspond to Syt1-recognition motifs of AP-2 and stonin2, to Ala bound less efficiently to Syt1 and failed to rescue the endocytic defect of Syt1 caused by KD. Our results indicate that SGIP1α is an endocytic adaptor dedicated to the retrieval of surface-stranded Syt1. Since endocytic sorting of Syt1 is also mediated by the overlapping activities of synaptic vesicle glycoprotein 2A/B (SV2A/B) and stonin2, our results suggest that complementary fail-safe mechanism by these proteins ensures high fidelity of Syt1 retrieval.
Mol Brain, 2019; 12

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THE TIMING OF THE GABA SHIFT AFFECTS THE POSTNATAL DEVELOPMENT OF INHIBITORY SYNAPSES

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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Chloride-mediated GABA signaling shifts from depolarizing to hyperpolarizing during the second postnatal week in rodents, due to increased activity of the chloride exporter KCC2 relative to the chloride importer NKCC1. The GABA shift is delayed in patients and mouse models for neurodevelopmental disorders. It is unclear whether a delayed GABA shift causes alterations in synaptic transmission . Here we assessed the consequences of mistimed GABA shift on synapse development in hippocampal slice cultures, in which we can follow the GABA shift between *day in vitro* (DIV) 2 and 22. We set out to delay the GABA shift by blocking KCC2 with VU0463271 or furosemide during DIV2-8 and assessed the consequences for inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs) using patch clamp recordings at DIV9 and DIV21. As expected, blocking KCC2 with VU0463271 resulted in a depolarizing GABA reversal potential at DIV8, which normalized at DIV20. IPSCs and EPSCs were not affected at DIV9. However, at DIV21, when chloride levels were restored, the frequency of spontaneous IPSCs was increased. Unexpectedly, GABA signaling was strongly hyperpolarizing at DIV 8 after furosemide treatment. This was associated with a decrease in the frequency of spontaneous IPSCs at DIV9. We observed that IPSCs were suppressed in an activity-dependent manner, which was independent of BDNF and endocannabinoids. When chloride levels were restored at DIV 21, IPSC frequency was increased compared to control slices. Our results indicate that the timing of the GABA shift affects postnatal development of inhibitory synapses .

Pubmed:
33549742: Peerboom C, Wierenga CJ
The postnatal GABA shift: A developmental perspective.
GABA is the major inhibitory neurotransmitter that counterbalances excitation in the mature brain. The inhibitory action of GABA relies on the inflow of chloride ions (Cl), which hyperpolarizes the neuron. In early development, GABA signaling induces outward Cl currents and is depolarizing. The postnatal shift from depolarizing to hyperpolarizing GABA is a pivotal event in brain development and its timing affects brain function throughout life. Altered timing of the postnatal GABA shift is associated with several neurodevelopmental disorders. Here, we argue that the postnatal shift from depolarizing to hyperpolarizing GABA represents the final shift in a sequence of GABA shifts, regulating proliferation, migration, differentiation, and finally plasticity of developing neurons. Each developmental GABA shift ensures that the instructive role of GABA matches the circumstances of the developing network. Sensory input may be a crucial factor in determining proper timing of the postnatal GABA shift. A developmental perspective is necessary to interpret the full consequences of a mismatch between connectivity, activity and GABA signaling during brain development.
Neurosci Biobehav Rev, 2021; 124

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BOARD NUMBER: S02-356

TSPAN15 DEPLETION AFFECTS EXTRACELLULAR VESICLE UPTAKE BY RECIPIENT NEURONS

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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Extracellular vesicles (EVs) provide a unique mode of intercellular communication. EVs serve as vehicles for transferring proteins, lipids, and RNAs between cells. They represent a heterogeneous population of membranous vesicles released by several cell types, such as glia or neurons. EVs are known to alter recipient cell physiology depending on their membrane and luminal cargoes and play critical roles in pathological processes. The functions of EVs have been extensively studied in the context of neurodegenerative diseases and their bioengineering provides great therapeutic potential. EVs are taken up by recipient cells through different modes. However, detailed molecular mechanisms and critical molecular players are presently barely understood. Using biochemical and cell biology techniques, we show that Tspan15 is an EV membrane protein. Fluorescent EV labeling demonstrates that TSPAN15 depletion does not affect EV release, but significantly impairs EV uptake by recipient neurons.

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GABA EVOKES DEPOLARIZATIONS AND CALCIUM TRANSIENTS IN ADULT CEREBROSPINAL FLUID-CONTACTING NEURONS OF MOUSE SPINAL CORD

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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Cerebrospinal fluid-contacting neurons (CSF-cNs) form an evolutionary conserved bipolar cells population localized in mammalian spinal cord. They lie at the interface between CSF and the parenchyma while their activity is modulated by extracellular pH and osmolarity variations, suggesting that CSF-cNs are polymodal sensory neurons able to carry information from the CSF to spinal neuronal network. Alongside their sensory features, CSF-cNs were shown to express molecular markers of neuronal immaturity during adulthood, though the functional relevance of their incomplete maturation is still unknown. Here, we used a combination of electrophysiology and calcium imaging to investigate the properties of $GABA_A$ receptors-dependent signaling in experimental conditions that maintain unchanged the concentration of intracellular chloride in CSF-cNs. We found that CSF-cNs of adult mice spinal cord respond by either depolarization or hyperpolarization of their membrane potential upon GABA application. Moreover, depolarizing-GABA responses trigger intracellular calcium elevations in CSF-cNs through the activation of voltage-gated calcium channels. Our findings represent the first evidence that a subpopulation of adult spinal CSF-cNs around the central canal maintain immature properties in their GABA signaling, with GABA-dependent depolarization and calcium modulation. Hence, GABAergic transmission in CSF-cNs may be part of mechanisms regulating their functional maturation and integration in spinal circuits.

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SYNAPTOTAGMIN-11 CONTROLS GABAB RECEPTOR INTERNALIZATION

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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$GABA_B$ receptors (GBRs) regulate neurotransmitter release and postsynaptic inhibition. Proteomic approaches have revealed that the Amyloid Precursor Protein APP is required for axonal transport of GBRs (Dinamarca et al., Nature Communications 10, 2019). Synaptotagmin-11 (Syt11), an atypical member of the synaptotagmin family, was found to form a multi-protein complex with GBRs and APP (Schwenk et al., Nature Neuroscience 19, 2016). Using biochemical approaches, we characterized the interaction between Syt11, APP, and GBRs. We found that Syt11 interacts indirectly with GBRs through APP and the auxiliary receptor subunit KCTD16 (K^+ channel tetramerization-domain containing protein 16). We show that the GBR/Syt11 complex is transported in NPY^+ cargo vesicles in axons and dendrites and co-localizes with pre- and postsynaptic structures at the plasma membrane. Consistent with its reported role as a clamp for endocytosis (Wang et al., Embo reports 17, 2016), Syt11 inhibited GBR internalization from the cell surface. Accordingly, $Syt11^{-/-}$ neurons exhibit a reduced number of GBRs at the plasma membrane and a deficit in GBR-mediated inhibition of neurotransmitter release.

Pubmed:
33279506: Werthmann RC, Tzouros M, Lamerz J, Augustin A, Fritzius T, Trovò L, Stawarski M, Raveh A, Diener C, Fischer C, Gassmann M, Lindemann L, Bettler B
Symmetric signal transduction and negative allosteric modulation of heterodimeric mGlu1/5 receptors.
For a long time metabotropic glutamate receptors (mGluRs) were thought to regulate neuronal functions as obligatory homodimers. Recent reports, however, indicate the existence of heterodimers between group-II and -III mGluRs in the brain, which differ from the homodimers in their signal transduction and sensitivity to negative allosteric modulators (NAMs). Whether the group-I mGluRs, mGlu1 and mGlu5, form functional heterodimers in the brain is still a matter of debate. We now show that mGlu1 and mGlu5 co-purify from brain membranes and hippocampal tissue and co-localize in cultured hippocampal neurons. Complementation assays with mutants deficient in agonist-binding or G protein-coupling reveal that mGlu1/5 heterodimers are functional in heterologous cells and transfected cultured hippocampal neurons. In contrast to heterodimers between group-II and -III mGluRs, mGlu1/5 receptors exhibit a symmetric signal transduction, with both protomers activating G proteins to a similar extent. NAMs of either protomer in mGlu1/5 receptors partially inhibit signaling, showing that both protomers need to be able to reach an active conformation for full receptor activity. Complete heterodimer inhibition is observed when both protomers are locked in their inactive state by a NAM. In summary, our data show that mGlu1/5 heterodimers exhibit a symmetric signal transduction and thus intermediate signaling efficacy and kinetic properties. Our data support the existence of mGlu1/5 heterodimers in neurons and highlight differences in the signaling transduction of heterodimeric mGluRs that influence allosteric modulation.
Neuropharmacology, 2021; 190
32032735: Trovò L, Fuchs C, De Rosa R, Barbiero I, Tramarin M, Ciani E, Rusconi L, Kilstrup-Nielsen C
The green tea polyphenol epigallocatechin-3-gallate (EGCG) restores CDKL5-dependent synaptic defects in vitro and in vivo.
CDKL5 deficiency disorder (CDD) is a rare X-linked neurodevelopmental disorder that is characterised by early-onset seizures, intellectual disability, gross motor impairment, and autistic-like features. CDD is caused by mutations in the cyclin-dependent kinase-like 5 (CDKL5) gene that encodes a serine/threonine kinase with a predominant expression in the brain. Loss of CDKL5 causes neurodevelopmental alterations in vitro and in vivo, including defective dendritic arborisation and spine maturation, which most likely underlie the cognitive defects and autistic features present in humans and mice. Here, we show that treatment with epigallatocathechin-3-gallate (EGCG), the major polyphenol of green tea, can restore defects in dendritic and synaptic development of primary Cdkl5 knockout (KO) neurons. Furthermore, defective synaptic maturation in the hippocampi and cortices of adult Cdkl5-KO mice can be rescued through the intraperitoneal administration of EGCG, which is however not sufficient to normalise behavioural CDKL5-dependent deficits. EGCG is a pleiotropic compound with numerous cellular targets, including the dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) that is selectively inhibited by EGCG. DYRK1A controls dendritic development and spine formation and its deregulation has been

implicated in neurodevelopmental and degenerative diseases. Treatment with another DYRK1A inhibitor, harmine, was capable of correcting neuronal CDKL5-dependent defects; moreover, DYRK1A levels were upregulated in primary Cdkl5-KO neurons in concomitance with increased phosphorylation of Tau, a well-accepted DYRK1A substrate. Altogether, our results indicate that DYRK1A deregulation may contribute, at least in part, to the neurodevelopmental alterations caused by CDKL5 deficiency.
Neurobiol Dis, 2020; 138

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MOLECULAR MECHANISMS OF UNCONVENTIONAL NMDA RECEPTORS CONTAINING GLUN3A SUBUNITS

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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NMDA receptors (N-Methyl-D-Aspartate) are ligand gated ionotropic channels belonging to the ionotropic glutamate receptor (iGluR) family that drives excitatory transmission in the central nervous system (CNS). NMDARs assemble as tetramers, composed of two GluN1s with two GluN2 (GluN2A-D), or two GluN3 subunits (GluN3A-B). Among NMDARs, di-heteromeric GluN1/GluN3A NMDARs exhibit peculiar properties: i) they are activated by glycine alone and not glutamate; ii) they display atypical gating kinetics with glycine acting as an activator at GluN3A subunits and an antagonist as GluN1 subunits. In recent years, we and others have revealed that GluN1/GluN3A receptors are functionally expressed in the CNS where they form excitatory glycine receptors. Still, little is known about the molecular mechanisms of GluN1/GluN3A receptor function involved in neuronal excitability. In other iGluRs, it is well established that the Ligand Binding Domain (LBD) interfaces control important features of receptor gating. We thus aimed at characterizing the role of LBD interfaces in GluN3As. Structural modelling allowed us to highlight key residues located in these regions. Using two electrodes voltage clamp recordings in Xenopus oocytes, we studied the impact of mutating these residues on the main biophysical properties of GluN1/GluN3A receptors, among which current phenotypes, channel open probability, desensitization kinetics, and sensitivity to pharmacological agents. Some of these mutants display gain of function phenotypes unveiling distinct properties of the GluN3A receptor LBD interface. Altogether, our research individuates structural features underpinning the atypical behavior of glycine-gated GluN1/GluN3A receptors, representing a step forward in our understanding of their differences with canonical GluN1/GluN2 NMDARs.

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COMPLEX REGULATION OF GEPHYRIN SPLICING IS A DETERMINANT OF INHIBITORY POSTSYNAPTIC DIVERSITY

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

Fabrice Ango
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Gephyrin (*GPHN*) regulates the clustering of postsynaptic components at inhibitory synapses and is involved in pathophysiology of neuropsychiatric disorders. Here, we uncover an extensive diversity of *GPHN* transcripts that are tightly controlled by splicing during mouse and human brain development. Proteomic analysis reveals at least a hundred isoforms of GPHN incorporated at inhibitory Glycine and GABA-A receptors containing synapses. They exhibit different localization and postsynaptic clustering properties, and altering the expression level of one isoform is sufficient to affect the number, size, and density of inhibitory synapses in cerebellar Purkinje cells. Furthermore, we discovered that splicing defects reported in neuropsychiatric disorders are carried by multiple alternative *GPHN* transcripts, demonstrating the need for a thorough analysis of the *GPHN* transcriptome in patients. Overall, we show that alternative splicing of *GPHN* is an important genetic variation to consider in neurological diseases and a determinant of the diversity of postsynaptic inhibitory synapses.

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EFFECTS OF PKA-MEDIATED PHOSPHORYLATION IN THE GLYCINE RECEPTOR ALPHA 3K VARIANT.

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

Katherine Fariña, Nicole Espinoza, David Flaig, Santiago Quintana, Paul Soto, Patricio Castro, Jorge Fuentealba, Carola Munoz Montesino, Gonzalo Yévenes, Gustavo Moraga-Cid
University of Concepción, Department Of Physiology, Concepción, Chile

Glycine receptors (GlyRs) are anion-permeable pentameric ligand-gated ion channels (pLGICs). Glycinergic neurotransmission is critical controlling key functions, such as motor coordination, respiratory control, muscle tone and pain processing. In this context, previous studies have shown that the functional inhibition of GlyRs containing the α3 subunit is a pivotal mechanism of pain hypersensitivity. In glycinergic neurons, two GlyRsα3 variants are generated by post-transcriptional processing; alpha 3K and alpha 3L, with the later containing a 15 amino acids insert located in the large intracellular domain (ICD). Interestingly, pain hypersensitivity has been associated to the inhibition of glycine activated currents by phosphorylation by PKA in the S346 residue located in the ICD of the α3L. This reduced activity of α3L is due to a diminished single channel conductance. Despite to the importance of the expression of both variants in glycinergic neurons, the effects of PKA phosphorylation in the α3K variant is still unknown. Here, using optogenetics and electrophysiological approach combined with site-directed mutagenesis we evaluated the effects of PKA activation in the unitary conductance of the wild type and phospomimetic mutants (S346E) α3K variant. Single channel currents obtained in cell attached configurations showed similar amplitude in both variants. PKA activation by a photoactivatable adenylyl cyclases (bPACs) or a phosphomimetic mutation (S346E) significantly reduced the unitary conductance in the α3K, similar to observed in the α3L variant. These results reveal that the activation of PKA and the subsequent phosphorylation of the S346 residue, have a similar functional impact on both GlyRsa3 variants.

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ACTIVATION MECHANISMS OF THE ORPHAN RECEPTOR GPR158

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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G Protein Coupled Receptors (GPCR) are important receptor family for therapeutics development since a vast number of drugs in clinical use target GPCRs. Orphan GPCRs represent promising candidates for development of novel therapeutics. GPR158 is an orphan GPCR highly expressed in brain. It is enriched in the prefrontal cortex, and its genetic suppression in mice results in prominent antidepressant phenotype and stress resiliency making GPR158 an attractive target for development of novel anti-depressants. One of the biggest mysteries related to GPR158 biology is its ligand and mechanism of activation. In this work, we are addressing this challenge in several ways. First, we studied an ability of GPR158 to constitutively activate various heterotrimeric G proteins and detected none. We then analyzed whether it could modulate an activity of its intracellular partner – a GTPase activating complex consisting of RGS7 and Gb5 subunits. Indeed, we found that manipulations with an extracellular domain of GPR158 result in RGS7/Gb5-dependent regulation of G protein deactivation. This indicates that GPR158 may signal non-canonically to regulate activity of RGS proteins rather than activate G proteins. Finally, we determined a high-resolution structure of GPR158 in complex with RGS7/Gb5. In addition to showing how GPR158 engages RGS7/Gb5 complex, it revealed the presence of an extracellular Cache domain, a putative ligand-binding module. Work is underway to determine the nature of the ligand that binds to the Cache domain and activates GPR158 to result in modulation of RGS7/Gb5 activity. Collectively, these results offer new insights into exciting pharmacological target for intervention in depression.

Pubmed:
34793198: Patil DN, Singh S, Laboute T, Strutzenberg TS, Qiu X, Wu D, Novick SJ, Robinson CV, Griffin PR, Hunt JF, Izard T, Singh AK, Martemyanov KA
Cryo-EM structure of human GPR158 receptor coupled to the RGS7-Gβ5 signaling complex.
GPR158 is an orphan G protein–coupled receptor (GPCR) highly expressed in the brain, where it controls synapse formation and function. GPR158 has also been implicated in depression, carcinogenesis, and cognition. However, the structural organization and signaling mechanisms of GPR158 are largely unknown. We used single-particle cryo–electron microscopy (cryo-EM) to determine the structures of human GPR158 alone and bound to an RGS signaling complex. The structures reveal a homodimeric organization stabilized by a pair of phospholipids and the presence of an extracellular Cache domain, an unusual ligand-binding domain in GPCRs. We further demonstrate the structural basis of GPR158 coupling to RGS7-Gβ5. Together, these results provide insights into the unusual biology of orphan receptors and the formation of GPCR-RGS complexes.
Science, 2022; 375

FENS Forum 2022
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BOARD NUMBER: S02-363

THE ORPHAN GPCR RECEPTOR, GPR88, INTERACTS WITH NUCLEAR PROTEIN PARTNERS IN THE CEREBRAL CORTEX.

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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GPR88 is an orphan G-protein-coupled receptor (GPCR) highly expressed in striatal medium spiny neurons (MSN), also found in cortical neurons at low level. In MSN, GPR88 has a canonical GPCR plasma membrane/cytoplasmic expression, whereas in cortical neurons, we previously reported an atypical intranuclear localization. Molecular size analysis suggests that GPR88, expressed in plasma membrane of MSN or in nuclear compartment of cortical neurons, corresponds to the full-length protein. By transfection of cortical neurons, we showed that GPR88 fluorescent chimeras exhibit a nuclear localization. This localization is contingent on the third intracytoplasmic loop and C-terminus domains, even though these domains do not contain any known nuclear localization signals (NLS). Using yeast two-hybrid screening with these domains, we identified the nuclear proteins ATRX, TOP2B, and BAZ2B, all involved in chromatin remodeling, as potential protein partners of GPR88. We also validated the interaction of GPR88 with these nuclear proteins by proximity ligation assay on cortical neurons in culture and coimmunoprecipitation experiments on cortical extracts from GPR88 wild-type (WT) and knockout (KO) mice. The identification of GPR88 subcellular partners may provide novel functional insights for nonclassical modes of GPCR action that could be relevant in the maturating process of neocortical neurons.

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AGE DIFFERENTIALLY MODULATES L-TYPE CALCIUM CHANNEL AND N-METHYL-D-ASPARTATE RECEPTOR EXPRESSIONS IN THE HIPPOCAMPUS AND PIRIFORM CORTEX

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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AIMS: Calcium signalling is essential for learning and memory, and its dysregulation is a key contributor in age-related cognitive decline. Ca^{2+} influx in neurons is mediated mainly by L-type calcium channels (LTCCs) and N-methyl-D-aspartate receptors (NMDARs). Here we measured LTCC and NMDAR expressions in Sprague-Dawley rats of various ages. We focused on the piriform cortex (PC) and the hippocampus, two areas integral to olfactory and spatial learning. **METHODS**: We measured synaptic and extrasynaptic levels of LTCCs (Cav1.2) and NMDAR subunits (GluN1, GluN2A, and GluN2B) in neonatal, adult and aged rats using western blot. We further investigated the somatic and dendritic expression of the Cav1.2 subunit using immunohistochemistry. Synaptic and extrasynaptic (PSD95 colocalizing and non-colocalizing) Cav1.2 subunit expressions in the dendritic layer were compared between adult and aged brains using confocal microscopy. **RESULTS**: In adults and aged hippocampus, there was a higher expression of synaptic, but not extrasynaptic NMDARs compared to neonates. However, GluN2A/2B ratios and synaptic:extrasynaptic ratios of NMDAR subunits were similar across age groups. In contrast, in the PC, GluN2A/2B and synaptic:extrasynaptic ratios were higher in adult PC compared to neonates. In hippocampal CA1 and PC, the soma:dendritic ratio of Cav1.2 expression increased with aging and extrasynaptic Cav1.2 (PSD95 non-colocalizing) expression was also found to have higher expression in the aged rats PC compared to adults. **CONCLUSIONS**: Hippocampus and PC differ in age-related channel expression. Higher somatic Cav1.2 expression in CA1 and higher extrasynaptic Cav1.2 in the PC may correlate with aging-associated disruption of calcium homeostasis and cognitive decline.

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REDEFINING THE GABAERGIC TRANSPORTER SYSTEMS IN C. ELEGANS

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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Functional neuronal circuits rely on a combination of both excitation and inhibition. In mature neurons, the main inhibitory neurotransmitter is GABA. Traditionally, neurons have been classified as GABAergic if they co-expressed three protein determinants: 1) GAD/UNC-25, which synthesizes GABA from glutamate, 2) VGAT/UNC-47, a vesicular transporter that packages GABA into synaptic vesicles, and 3) GAT/SNF-11, a plasma membrane transporter that recaptures extracellular GABA. In *C. elegans*, only 26 out of 302 neurons were considered GABAergic, until an improved immunostaining enabled the identification of 15 additional GABA-positive neurons. Although they stain for GABA and contact neurons expressing post synaptic $GABA_A$ receptors, they do not all coexpress GAD/UNC-25, VGAT/UNC-47 and GAT/SFN-11. In particular, three pairs of neurons express none of those. They are thus unable to synthesize, uptake or package GABA the way we know it. **We hypothesize that those neurons use unidentified transporters for GABA uptake and vesicular packaging. The present work aims to identify them.** Therefore, we are probing 51 putative amino acid transporters, analyzing both their expression pattern and how they affect GABA localisation. Currently, 18 available mutant strains are being characterized for GABA staining. For the other genes without any null allele available, we already found four out of nine to be neuronally expressed. One has been further characterized and is likely to be expressed in two of our neurons of interest. Using Crispr/Cas9 technology, we will generate a knockout allele of that candidate to test it for GABA transport.

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ROLE OF THE ORGANIC ANION TRANSPORTER 1 IN MEMORY AND SYNAPTIC PLASTICITY

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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The molecular mechanisms of synaptic plasticity have been extensively studied. Nevertheless, many events occurring at the synapse during physiological and pathological neuroadaptations are still unclear. New modulators of synaptic physiology are recurringly discovered. The organic anion transporter 1 (OAT1), a multi-specific kidney transporter involved in drug excretion and regulation of systemic metabolite levels, could belong to this category of newly identified plasticity modulators. Until recently, very little was known about OAT1 expression and function in the central nervous system. A few reports indicated its expression in the brain and choroid plexus. Our group recently showed that OAT1 expression in the mouse spinal cord is key to the establishment and maintenance of hypersensitivity in chronic inflammatory pain. Thus, a function for OAT1 as a mediator of maladaptive plasticity was identified for the first time. Whether OAT1 is involved in additional plasticity mechanisms has not been studied. Here, we systematically characterized OAT1 expression in the adult mouse brain. Using OAT1-knockout mice, we studied the involvement of OAT1 in hippocampus-dependent memory tasks. We found that OAT1 deficiency results in changes in several molecular players of synaptic plasticity and their downstream signaling events in both primary hippocampal cultures and hippocampal tissue. Furthermore, metabolomics analysis of the OAT1-KO hippocampus and cerebrospinal fluid revealed pathway alterations similar to those previously reported in the plasma, hinting at candidate OAT1 substrates that might explain the involvement of OAT1 in synaptic signaling. Taken together, our results suggest OAT1 as a novel regulator of plasticity in the central nervous system.

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NON-CANONICAL ROLE OF NKCC1 IN NEURONS BY REGULATING KCC2 FUNCTION

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

Erwan Pol[1,2], Marion Russeau[2], Christophe Piesse[3], Nicolas Pietrancosta[1,3], Sabine Levi[2]
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Chloride neuronal homeostasis, which is determined by the KCC2 and NKCC1 transporters, responsible for Cl- efflux and influx respectively, regulates the efficiency and polarity of GABAergic transmission in the brain. The dysfunction of these transporters leads to a pathological state by a build-up of chloride in neurons. Drugs aimed at reducing neuronal chloride levels therefore represent promising therapeutic strategies. We have shown in mature neurons that the regulation of the membrane trafficking/activity of KCC2 and NKCC1 involves the With-No-lysine-(K)-serine-threonine kinase WNK1 and its effector, Ste20-Proline-Asparagine-Rich-Kinase (SPAK). Once activated, SPAK is recruited below the plasma membrane allowing a local control of its activity. NKCC1 but not KCC2 directly binds SPAK. We propose that NKCC1 regulates KCC2 by recruiting SPAK near inhibitory synapses. We developed several peptides that prevent SPAK binding to NKCC1 and showed that they regulate KCC2 as a proof of concept. We want now to validate the SPAK scaffold hypothesis and identif new small compounds, through interdisciplinary approaches (chemistry/molecular modeling/cell biology/imaging/animal behavior), able to interact with the NKCC1/SPAK complex and inhibit SPAK activity and thus prevent KCC2 internalization and chloride build-up in neurons. This project will shed light on the virtually unknown role of a signaling pathway in the nervous system and may lead to the discovery of a non-canonical role for NKCC1 in neurons. It may also lead to the discovery of new therapeutic strategies in diseases with inhibition defects.

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KCC2-MEDIATED REGULATION OF CHRONIC BENZODIAZEPINE ACTION

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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Benzodiazepines remain to be routinely used as rapidly acting, short-term therapy for status epilepticus, anxiety, panic attacks and insomnia. However, limitations related to acute and chronic tolerance, leading to escalating doses and heightened risk of dependence, hampers their clinical usage. Adaptations in $GABA_A$Rs' subunit composition, intracellular mechanisms or changes in other neurotransmitter systems have been extensively studied as main components underlying such drug tolerance. In contrast, what happens to Cl^- homeostasis – the principal mechanisms of ensuring the inward, hyperpolarizing Cl^- gradient - has remained poorly understood. In this study, we aimed at exploring possible alterations in KCC2-mediated chloride homeostasis underlying tolerance development to diazepam. Mice were treated chronically with diazepam (elevating doses from 5 to 25 mg/kg, s.c.) or vehicle twice a day for 28 days. Tolerance development to motor-impairing effects was confirmed using the Rotarod performance and beam walking tests. Surprisingly, light-dark box measurements conducted at the end of the treatment period showed a strong anxiety-like phenotype in the diazepam-treated group, independent of the timing of the last drug administration (30 min or 11 h). Brain regions known to mediate the effects of diazepam, including the cerebellum and thalamus, were subjected to immunoblotting using antibodies against pan-KCC2 and its phosphorylated forms (S940 and T1007). Immunoblotting analysis did not show any statistically significant differences in brain regional KCC2 protein expression and phosphorylation between the diazepam- and vehicle-treated mice. These preliminary results should still be confirmed by additionally examining possible alterations in KCC2 transporter functionality.

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BOARD NUMBER: S02-369

A NEW MOLECULAR TOOLBOX FOR EXPLORING GEPHYRIN AND INHIBITORY SYNAPSE BIOLOGY

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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Progress in neurobiology is often limited by the availability, tractability, and specificity of molecular tools. This especially concerns the use of antibodies to label synaptic marker proteins which define the number, size, and dynamics of synapses as a proxy for function. Gephyrin is the major post-synaptic scaffold at GABAergic and glycinergic synapses in the nervous system where it interacts with other synaptic organizing proteins and receptors to facilitate inhibitory neurotransmission. Gephyrin itself is modified by post-translational modifications including phosphorylation to control its synaptic function. Despite the importance of using gephyrin as a canonical inhibitory post-synaptic marker, there are currently no widely available tools that can probe gephyrin specifically across applications and independently of its modified state. We therefore employed a synthetic library screening approach to identify Designed Ankyrin Repeat Proteins (DARPins) which specifically interact with gephyrin. From this screen we discovered several DARPin clones which bind to gephyrin, and then characterised their morphological use to examine compartment-specific phosphorylation of gephyrin in neurons. We additionally used these anti-gephyrin DARPins as biochemical tools to determine the most comprehensive gephyrin protein interactome to date, revealing putative novel functions of gephyrin beyond synapses. These anti-gephyrin DARPins offer a new resource to the study of both gephyrin biology and inhibitory synapses in general.

Pubmed:
33020478: van Oostrum M, Campbell B, Seng C, Müller M, Tom Dieck S, Hammer J, Pedrioli PGA, Földy C, Tyagarajan SK, Wollscheid B
Surfaceome dynamics reveal proteostasis-independent reorganization of neuronal surface proteins during development and synaptic plasticity.
Neurons are highly compartmentalized cells with tightly controlled subcellular protein organization. While brain transcriptome, connectome and global proteome maps are being generated, system-wide analysis of temporal protein dynamics at the subcellular level are currently lacking. Here, we perform a temporally-resolved surfaceome analysis of primary neuron cultures and reveal dynamic surface protein clusters that reflect the functional requirements during distinct stages of neuronal development. Direct comparison of surface and total protein pools during development and homeostatic synaptic scaling demonstrates system-wide proteostasis-independent remodeling of the neuronal surface, illustrating widespread regulation on the level of surface trafficking. Finally, quantitative analysis of the neuronal surface during chemical long-term potentiation (cLTP) reveals fast externalization of diverse classes of surface proteins beyond the AMPA receptor, providing avenues to investigate the requirement of exocytosis for LTP. Our resource (neurosurfaceome.ethz.ch) highlights the importance of subcellular resolution for systems-level understanding of cellular processes.
Nat Commun, 2020; 11
31596629: Country MW, Campbell BFN, Jonz MG
Spontaneous action potentials in retinal horizontal cells of goldfish () are dependent upon L-type Ca channels and ryanodine receptors.
Horizontal cells (HCs) are interneurons of the outer retina that undergo graded changes in membrane potential during the light response and provide feedback to photoreceptors. We characterized spontaneous Ca-based action potentials (APs) in isolated goldfish () HCs with electrophysiological and intracellular imaging techniques. Transient changes in intracellular Ca concentration ([Ca]) were observed with fura-2 and were abolished by removal of extracellular Ca or by inhibition of Ca channels by 50 µM Cd or 100 µM nifedipine. Inhibition of Ca release from stores with 20 µM ryanodine or 50 µM dantrolene abolished Ca transients and increased baseline [Ca]. This increased baseline was prevented by blocking L-type Ca channels with nifedipine, suggesting that Ca-induced Ca release from stores may be needed to inactivate membrane Ca channels. Caffeine (3 mM) increased the frequency of Ca transients, and the store-operated channel antagonist 2-aminoethyldiphenylborinate (100 µM) counteracted this effect. APs were detected with voltage-sensitive dye imaging

(FluoVolt) and current-clamp electrophysiology. In current-clamp recordings, regenerative APs were abolished by removal of extracellular Ca or in the presence of 5 mM Co or 100 µM nifedipine, and APs were amplified with 15 mM Ba. Collectively, our data suggest that during APs Ca enters through L-type Ca channels and that Ca stores (gated by ryanodine receptors) contribute to the rise in [Ca]. This work may lead to further understanding of the possible role APs have in vision, such as transitioning from light to darkness or modulating feedback from HCs to photoreceptors. Horizontal cells (HCs) are interneurons of the outer retina that provide inhibitory feedback onto photoreceptors. HCs respond to light via graded changes in membrane potential. We characterized spontaneous action potentials in HCs from goldfish and linked action potential generation to a rise in intracellular Ca via plasma membrane channels and ryanodine receptors. Action potentials may play a role in vision, such as transitioning from light to darkness, or in modulating feedback from HCs to photoreceptors.
J Neurophysiol, 2019; 122

31456660: Campbell BFN, Tyagarajan SK

Cellular Mechanisms Contributing to the Functional Heterogeneity of GABAergic Synapses.

GABAergic inhibitory neurotransmission contributes to diverse aspects of brain development and adult plasticity, including the expression of complex cognitive processes. This is afforded for in part by the dynamic adaptations occurring at inhibitory synapses, which show great heterogeneity both in terms of upstream signaling and downstream effector mechanisms. Single-particle tracking and live imaging have revealed that complex receptor-scaffold interactions critically determine adaptations at GABAergic synapses. Super-resolution imaging studies have shown that protein interactions at synaptic sites contribute to nano-scale scaffold re-arrangements through post-translational modifications (PTMs), facilitating receptor and scaffold recruitment to synaptic sites. Additionally, plasticity mechanisms may be affected by the protein composition at individual synapses and the type of pre-synaptic input. This mini-review article examines recent discoveries of plasticity mechanisms that are operational within GABAergic synapses and discusses their contribution towards functional heterogeneity in inhibitory neurotransmission.
Front Mol Neurosci, 2019; 12

30142695: Früh S, Tyagarajan SK, Campbell B, Bosshard G, Fritschy JM

The catalytic function of the gephyrin-binding protein IQSEC3 regulates neurotransmitter-specific matching of pre- and post-synaptic structures in primary hippocampal cultures.

In dissociated neuronal cultures the absence of spatial and temporal cues causes the emergence of mismatched synapses, where post-synaptic proteins of GABAergic synapses are in part apposed to glutamatergic pre-synaptic terminals and vice versa. This mismatch offers an opportunity to study the mechanisms that regulate correct apposition of pre- and post-synaptic elements. We report here that the IQ motif and Sec7 domain-containing protein 3 (IQSEC3; BRAG3; synArfGEF) specifically regulates the mislocalization of GABAergic post-synaptic density (PSD) proteins. Over-expression of IQSEC3 constructs harboring mutations that ablate Sec7 domain or IQ motif function revealed that IQSEC3 catalytic activity is involved in the control of apposition between the GABAergic PSD and glutamatergic terminals. Neurons co-expressing eGFP-gephyrin with IQSEC3 Sec7 mutant displayed a drastically increased fraction of mismatched eGFP-gephyrin clusters compared to other IQSEC3 constructs. Along with eGFP-gephyrin, endogenous GABA receptor cluster mismatching was increased by IQSEC3 Sec7 mutant over-expression. Conversely, GFP-PSD-95 clusters were unaffected by over-expression of any IQSEC3 construct. The GABAergic PSD mismatch phenotype was recapitulated by Arf6 dominant-negative mutant over-expression, suggesting that Arf6 activation by IQSEC3 is an essential step in this pathway. In addition, we provide biochemical evidence to confirm gephyrin/IQSEC3 interaction near the IQSEC3 IQ motif, which in turn binds calmodulin at low Ca concentrations. Taken together, our findings identify a post-synaptic protein which specifically regulates correct apposition of the GABAergic PSD to pre-synaptic terminals.
J Neurochem, 2018; 147

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BENZODIAZEPINE DIAZEPAM INDUCES DENDRITIC SPINE LOSS VIA 18 KDA TRANSLOCATOR PROTEIN

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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[1]Ludwig Maximilian University of Munich, Center For Neuropathology, Munich, Germany, [2]German Center for Neurodegenerative Diseases (DZNE), Translational Brain Research, Munich, Germany, [3]Munich Medical Research School, Ludwig Maximilian University Of Munich, Munich, Germany, [4]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

Benzodiazepines are widely prescribed drugs to treat insomnia and anxiety. However, they have been long suspected of causing cognitive impairment and increasing the risk for dementia. Here we report that diazepam, a commonly used benzodiazepine, impairs the structural plasticity of dendritic spines and leads to cognitive impairment in mice. After daily administration of diazepam for one week (5mg/kg) or eight weeks (1mg/kg), we observed significant, yet reversible, reductions of cortical dendritic spines in mice, accompanied with behavioural alterations. Given that diazepam exerts sedative and anxiolytic effects primarily via $GABA_A$ receptors ($GABA_AR$), we blocked the binding of diazepam to $GABA_AR$ by employing *Gabra RRRR* transgenic mice, which lack the histidine residue necessary for diazepam binding. However, blocking of diazepam's binding to $GABA_AR$ failed to prevent diazepam-induced dendritic spine reduction or behavioural changes. We then extend our exploration to another binding site of diazepam in the central nervous system, the 18kDa translocator protein (TSPO), which is highly expressed in microglia – the brain resident macrophage. Interestingly, in the TSPO knockout mice, diazepam administration did not induce changes in dendritic spines and behaviours. Using *in vivo* imaging and super-resolution microscopy, we discovered that via TSPO, diazepam administration alters microglia functionally and morphologically. In diazepam-fed mice, we observed that microglia contacted dendritic spines more frequently, we also observed microglia became hypertrophic and engulfed more synaptic materials. Collectively, we discovered a benzodiazepine diazepam-induced microglial TSPO-mediated dendritic spine regulation, independent of the $GABA_AR$-mediated signalling pathway.

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COOPERATIVE EFFECT OF GABA AND CA2+ FOR THE DIFFUSION AND TRAPPING OF GABAARS AT SYNAPSES: REGULATION OF RECEPTOR NUMBER

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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$GABA_AR$s diffuse on the surface of neurons and are trapped by scaffold proteins at synapses. Receptor number at synapses determines the efficiency of neurotransmission. Thus, mechanisms regulating $GABA_AR$ diffusion and synaptic trapping are crucial for the tuning of GABAergic transmission. Bindings of agonists to $GABA_AR$ and its post-translational modification modify its trapping at synapses. Hence, precise regulation of pre-and post-synapse is involved in the regulation of $GABA_AR$ clustering. However, the cooperation of pre-and post-synaptic activity for the regulation of the diffusion-trapping of $GABA_AR$ is poorly understood due to technical difficulty for the simultaneous single-molecule imaging combined with uncaging and optogenetic stimulation. Actually, the UV photoconversion of dye/fluorescent protein could release the caged compound, we used a spontaneously blinking fluorophore (HMSiR) instead for the single-molecule-localization-microscopy (SMLM) to track $GABA_AR$s. Overexpression of tagged receptors is another issue because it biases the ratio of the number of synaptic-scaffolds vs receptors. This is why we raised a knock-in mouse whose $GABA_AR$g2-subunit contains a spot-tag recognized by a dye-coupled nanobody. We found in mature synapses from cultured (> 21 div) dissociated neurons that repetitive GABA uncaging combined with Ca^{2+} elevation induced a rapid and persistent reduction of $GABA_AR$g2-subunit-containing receptors from the stimulated synapse. Either sole Ca^{2+}-elevation or GABA-uncaging alone couldn't induce this phenomenon. Thus, both the binding of GABA which induce dissociation of $GABA_AR$s from scaffolds combined with neuronal activity, and Ca^{2+} elevation prevents re-accumulation of receptors to synaptic sites could be indispensable for the long-lasting loss of $GABA_AR$s from synapses and a subsequent dis-inhibition.

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SINGLE MOLECULE CHARACTERISATION OF α3-CONTAINING GABAARS REVEALS A UNIQUE ROLE AT INHIBITORY SYNAPSES

POSTER SESSION 02 - SECTION: GABAERGIC TRANSMISSION AND SYNAPTIC FUNCTION

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Aims: GABA-A receptors (GABAARs) are the main inhibitory receptors in the brain. They are pentameric oligomers comprised of combinations of 19 subunits. Of these, little is known about the α3 subunit despite significant expression in the brain. Mutations in α3 have been linked to epilepsy, anxiety and intellectual disabilities. We characterised synaptic properties of α3-containing GABAARs (α3-GABAARs) in comparison to α1-GABAARs (the most well-studied GABAARs), to understand the role of α3 in synaptic transmission. **Methods:** We recorded synaptic currents mediated by specific α3-GABAAR isoforms in heterosynapses of transfected HEK293 cells and neuronal presynaptic terminals, and used super-resolution microscopy to track receptor molecules in neurons. We characterised the shape and size of synaptic clusters, rate of receptor trapping at synapses, diffusion probability and diffusion state transitions at synapses. Distinct mobility patterns were identified using moment scaling spectrum analysis. **Results:** We found that α3-GABAARs cluster at synapses, and diffuse 10-fold faster than α1-GABAARs. Synaptic currents mediated by α3-GABAARs have longer decay times and altered frequency compared to α1. Per length of neuronal processes, α3-GABAARs are more abundant at synapses, but dwell for significantly shorter times. **Conclusions:** Synaptic α3-GABAARs mediate phasic neurotransmission. The slow decay times, fast diffusion and shorter dwell times indicate that unlike the stably-anchored α1-GABAARs, α3-GABAARs might be more mobile at synapses in response to neuronal activity, maintaining the excitatory/inhibitory balance at a synapse level. These results show the first functional characterisation of α3-GABAARs, their integration into synapses and provide unique insights into their specialised role in inhibitory synaptic plasticity.

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BOARD NUMBER: S02-373

THE GLUTAMATERGIC SYNAPSE: A CHAT ROOM FOR AMYLOID-BETA PEPTIDE AND THE NUCLEUS

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Introduction: Synaptonuclear messengers translate specific synaptic signaling into gene expression, modulating synaptic dynamics. Alterations of such proteins lead to dendritic spine failure, indicating a contribution to synaptopathies. The synaptonuclear messenger RING Finger Protein 10 (RNF10) operates as a mobile hub that docks NMDAR-derived signalosomes to nuclear target sites, regulating genes involved in spine morphology and Alzheimer's disease (AD) pathogenesis, suggesting an implication in AD-synaptic dysfunction. *Methods:* RNF10 expression and subcellular localization, were investigated by biochemical and imaging approaches in AD patients autoptic specimens and primary hippocampal neurons exposed to Amyloid β (Aβ) oligomers. RNA-Seq analysis elucidated the impact of Aβ-triggered RNF10 trafficking on gene expression. Mitochondrial morphology and activity were examined by imaging techniques and oxygen consumption measurements. *Results:* RNF10 expression and localization are altered in AD patients' hippocampi at the earlier stages of the disease. Furthermore, Aβ oligomers trigger a calcium-dependent NMDAR-induced RNF10 nuclear translocation, in hippocampal neurons. RNA-Seq analysis in hippocampal neurons silenced for RNF10 and exposed to Aβ oligomers showed that Aβ-mediated RNF10 signaling pathway triggers mitochondrial dysfunctions in neurons. Importantly, the down-regulation of RNF10 prevents the Aβ-triggered mitochondrial defects. *Conclusions:* Several mitochondrial functions have been found severely compromised in AD models linked to Aβ, nevertheless the molecular mechanisms underlying such changes are still unclear. Here we show that RNF10 can play a key role in translating Aβ synaptotoxicity into mitochondrial dysfunction. Overall, our findings suggest that the RNF10-mediated signaling contributes to AD and provides new potential pharmacological tools and novel biomarkers of synaptic dysfunction in AD.

Pubmed:
34572474: D'Andrea L, Stringhi R, Di Luca M, Marcello E
Looking at Alzheimer's Disease Pathogenesis from the Nuclear Side.
Alzheimer's disease (AD) is a neurodegenerative disorder representing the most common form of dementia. It is biologically characterized by the deposition of extracellular amyloid-β (Aβ) senile plaques and intracellular neurofibrillary tangles, constituted by hyperphosphorylated tau protein. The key protein in AD pathogenesis is the amyloid precursor protein (APP), which is cleaved by secretases to produce several metabolites, including Aβ and APP intracellular domain (AICD). The greatest genetic risk factor associated with AD is represented by the () allele. Importantly, all of the above-mentioned molecules that are strictly related to AD pathogenesis have also been described as playing roles in the cell nucleus. Accordingly, evidence suggests that nuclear functions are compromised in AD. Furthermore, modulation of transcription maintains cellular homeostasis, and alterations in transcriptomic profiles have been found in neurodegenerative diseases. This report reviews recent advancements in the AD players-mediated gene expression. Aβ, tau, AICD, and localize in the nucleus and regulate the transcription of several genes, part of which is involved in AD pathogenesis, thus suggesting that targeting nuclear functions might provide new therapeutic tools for the disease.
Biomolecules, 2021; 11
34017076: Carotti S, Zingariello M, Francesconi M, D'Andrea L, Latasa MU, Colyn L, Fernandez-Barrena MG, Flammia RS, Falchi M, Righi D, Pedini G, Pantano F, Bagni C, Perrone G, Rana RA, Avila MA, Morini S, Zalfa F
Fragile X mental retardation protein in intrahepatic cholangiocarcinoma: regulating the cancer cell behavior plasticity at the leading edge.
Intrahepatic cholangiocarcinoma (iCCA) is a rare malignancy of the intrahepatic biliary tract with a very poor prognosis. Although some clinicopathological parameters can be prognostic factors for iCCA, the molecular prognostic markers and potential mechanisms of iCCA have not been well investigated. Here, we report that the Fragile X mental retardation protein (FMRP), a RNA binding protein functionally absent in patients with the Fragile X syndrome (FXS) and also involved in several types of cancers, is overexpressed in human iCCA and its expression is significantly increased in iCCA metastatic tissues.

The silencing of FMRP in metastatic iCCA cell lines affects cell migration and invasion, suggesting a role of FMRP in iCCA progression. Moreover, we show evidence that FMRP is localized at the invasive front of human iCCA neoplastic nests and in pseudopodia and invadopodia protrusions of migrating and invading iCCA cancer cells. Here FMRP binds several mRNAs encoding key proteins involved in the formation and/or function of these protrusions. In particular, we find that FMRP binds to and regulates the expression of Cortactin, a critical regulator of invadopodia formation. Altogether, our findings suggest that FMRP could promote cell invasiveness modulating membrane plasticity and invadopodia formation at the leading edges of invading iCCA cells.
Oncogene, 2021; 40
31919630: Nobile V, Palumbo F, Lanni S, Ghisio V, Vitali A, Castagnola M, Marzano V, Maulucci G, De Angelis C, De Spirito M, Pacini L, D'Andrea L, Ragno R, Stazi G, Valente S, Mai A, Chiurazzi P, Genuardi M, Neri G, Tabolacci E
Altered mitochondrial function in cells carrying a premutation or unmethylated full mutation of the FMR1 gene.
Fragile X-related disorders are due to a dynamic mutation of the CGG repeat at the 5' UTR of the FMR1 gene, coding for the RNA-binding protein FMRP. As the CGG sequence expands from premutation (PM, 56-200 CGGs) to full mutation (> 200 CGGs), FMRP synthesis decreases until it is practically abolished in fragile X syndrome (FXS) patients, mainly due to FMR1 methylation. Cells from rare individuals with no intellectual disability and carriers of an unmethylated full mutation (UFM) produce slightly elevated levels of FMR1-mRNA and relatively low levels of FMRP, like in PM carriers. With the aim of clarifying how UFM cells differ from CTRL and FXS cells, a comparative proteomic approach was undertaken, from which emerged an overexpression of SOD2 in UFM cells, also confirmed in PM but not in FXS. The SOD2-mRNA bound to FMRP in UFM more than in the other cell types. The high SOD2 levels in UFM and PM cells correlated with lower levels of superoxide and reactive oxygen species (ROS), and with morphological anomalies and depolarization of the mitochondrial membrane detected through confocal microscopy. The same effect was observed in CTRL and FXS after treatment with MC2791, causing SOD2 overexpression. These mitochondrial phenotypes reverted after knock-down with siRNA against SOD2-mRNA and FMR1-mRNA in UFM and PM. Overall, these data suggest that in PM and UFM carriers, which have high levels of FMR1 transcription and may develop FXTAS, SOD2 overexpression helps to maintain low levels of both superoxide and ROS with signs of mitochondrial degradation.
Hum Genet, 2020; 139
29590342: Jacquemont S, Pacini L, Jønch AE, Cencelli G, Rozenberg I, He Y, D'Andrea L, Pedini G, Eldeeb M, Willemsen R, Gasparini F, Tassone F, Hagerman R, Gomez-Mancilla B, Bagni C
Protein synthesis levels are increased in a subset of individuals with fragile X syndrome.
Fragile X syndrome (FXS) is a monogenic form of intellectual disability and autism spectrum disorder caused by the absence of the fragile X mental retardation protein (FMRP). In biological models for the disease, this leads to upregulated mRNA translation and as a consequence, deficits in synaptic architecture and plasticity. Preclinical studies revealed that pharmacological interventions restore those deficits, which are thought to mediate the FXS cognitive and behavioral symptoms. Here, we characterized the de novo rate of protein synthesis in patients with FXS and their relationship with clinical severity. We measured the rate of protein synthesis in fibroblasts derived from 32 individuals with FXS and from 17 controls as well as in fibroblasts and primary neurons of 27 Fmr1 KO mice and 20 controls. Here, we show that levels of protein synthesis are increased in fibroblasts of individuals with FXS and Fmr1 KO mice. However, this cellular phenotype displays a broad distribution and a proportion of fragile X individuals and Fmr1 KO mice do not show increased levels of protein synthesis, having measures in the normal range. Because the same Fmr1 KO animal measures in fibroblasts predict those in neurons we suggest the validity of this peripheral biomarker. Our study offers a potential explanation for the comprehensive drug development program undertaken thus far yielding negative results and suggests that a significant proportion, but not all individuals with FXS, may benefit from the reduction of excessive levels of protein synthesis.
Hum Mol Genet, 2018; 27
29114037: Santini E, Huynh TN, Longo F, Koo SY, Mojica E, D'Andrea L, Bagni C, Klann E
Reducing eIF4E-eIF4G interactions restores the balance between protein synthesis and actin dynamics in fragile X syndrome model mice.
Fragile X syndrome (FXS) is the most common form of inherited intellectual disability and autism spectrum disorder. FXS is caused by silencing of the gene, which encodes fragile X mental retardation protein (FMRP), an mRNA-binding protein that represses the translation of its target mRNAs. One mechanism by which FMRP represses translation is through its association with cytoplasmic FMRP-interacting protein 1 (CYFIP1), which subsequently sequesters and inhibits eukaryotic initiation factor 4E (eIF4E). CYFIP1 shuttles between the FMRP-eIF4E complex and the Rac1-Wave regulatory complex, thereby connecting translational regulation to actin dynamics and dendritic spine morphology, which are dysregulated in FXS model mice that lack FMRP. Treating FXS mice with 4EGI-1, which blocks interactions between eIF4E and eIF4G, a critical interaction partner for translational initiation, reversed defects in hippocampus-dependent memory and spine morphology. We also found that 4EGI-1 normalized the phenotypes of enhanced metabotropic glutamate receptor (mGluR)-mediated long-

term depression (LTD), enhanced Rac1-p21-activated kinase (PAK)-cofilin signaling, altered actin dynamics, and dysregulated CYFIP1/eIF4E and CYFIP1/Rac1 interactions in FXS mice. Our findings are consistent with the idea that an imbalance in protein synthesis and actin dynamics contributes to pathophysiology in FXS mice, and suggest that targeting eIF4E may be a strategy for treating FXS.
Sci Signal, 2017; 10
28012946: Sabanov V, Braat S, D'Andrea L, Willemsen R, Zeidler S, Rooms L, Bagni C, Kooy RF, Balschun D
Impaired GABAergic inhibition in the hippocampus of Fmr1 knockout mice.
Many clinical and molecular features of the fragile X syndrome, a common form of intellectual disability and autism, can be modeled by deletion of the Fmr1 protein (Fmrp) in mice. Previous studies showed a decreased expression of several components of the GABAergic system in Fmr1 knockout mice. Here, we used this mouse model to investigate the functional consequences of Fmrp deletion on hippocampal GABAergic inhibition in the CA1-region of the hippocampus. Whole-cell patch-clamp recordings demonstrated a significantly reduced amplitude of evoked inhibitory postsynaptic currents (eIPSCs) and a decrease in the amplitude and frequency of spontaneous IPSCs. In addition, miniature IPSCs were reduced in amplitude and frequency and decayed significantly slower than mIPSCs in controls. Quantitative real-time PCR revealed a significantly lower expression of α2, β1 and δ GABA receptor subunits in the hippocampus of the juvenile mice (P22) compared to wild-type littermates. Correspondingly, we found also at the protein level reduced amounts of α2, β1 and δ subunits in Fmr1 knockout mice. Overall, these results demonstrate that the reduction in several components of the GABAergic system is already present at young age and that this reduction results in measurable abnormalities on GABA receptor-mediated phasic inhibition. These abnormalities might contribute to the behavioral and cognitive deficits of this fragile X mouse model.
Neuropharmacology, 2017; 116
26182420: Pasciuto E, Ahmed T, Wahle T, Gardoni F, D'Andrea L, Pacini L, Jacquemont S, Tassone F, Balschun D, Dotti CG, Callaerts-Vegh Z, D'Hooge R, Müller UC, Di Luca M, De Strooper B, Bagni C
Dysregulated ADAM10-Mediated Processing of APP during a Critical Time Window Leads to Synaptic Deficits in Fragile X Syndrome.
The Fragile X mental retardation protein (FMRP) regulates neuronal RNA metabolism, and its absence or mutations leads to the Fragile X syndrome (FXS). The β-amyloid precursor protein (APP) is involved in Alzheimer's disease, plays a role in synapse formation, and is upregulated in intellectual disabilities. Here, we show that during mouse synaptogenesis and in human FXS fibroblasts, a dual dysregulation of APP and the α-secretase ADAM10 leads to the production of an excess of soluble APPα (sAPPα). In FXS, sAPPα signals through the metabotropic receptor that, activating the MAP kinase pathway, leads to synaptic and behavioral deficits. Modulation of ADAM10 activity in FXS reduces sAPPα levels, restoring translational control, synaptic morphology, and behavioral plasticity. Thus, proper control of ADAM10-mediated APP processing during a specific developmental postnatal stage is crucial for healthy spine formation and function(s). Downregulation of ADAM10 activity at synapses may be an effective strategy for ameliorating FXS phenotypes.
Neuron, 2015; 87
25453757: Panja D, Kenney JW, D'Andrea L, Zalfa F, Vedeler A, Wibrand K, Fukunaga R, Bagni C, Proud CG, Bramham CR
Two-stage translational control of dentate gyrus LTP consolidation is mediated by sustained BDNF-TrkB signaling to MNK.
BDNF signaling contributes to protein-synthesis-dependent synaptic plasticity, but the dynamics of TrkB signaling and mechanisms of translation have not been defined. Here, we show that long-term potentiation (LTP) consolidation in the dentate gyrus of live rodents requires sustained (hours) BDNF-TrkB signaling. Surprisingly, this sustained activation maintains an otherwise labile signaling pathway from TrkB to MAP-kinase-interacting kinase (MNK). MNK activity promotes eIF4F translation initiation complex formation and protein synthesis in mechanistically distinct early and late stages. In early-stage translation, MNK triggers release of the CYFIP1/FMRP repressor complex from the 5'-mRNA cap. In late-stage translation, MNK regulates the canonical translational repressor 4E-BP2 in a synapse-compartment-specific manner. This late stage is coupled to MNK-dependent enhanced dendritic mRNA translation. We conclude that LTP consolidation in the dentate gyrus is mediated by sustained BDNF signaling to MNK and MNK-dependent regulation of translation in two functionally and mechanistically distinct stages.
Cell Rep, 2014; 9
26220900: Pasciuto E, Borrie SC, Kanellopoulos AK, Santos AR, Cappuyns E, D'Andrea L, Pacini L, Bagni C
Autism Spectrum Disorders: Translating human deficits into mouse behavior.
Autism Spectrum Disorders are a heterogeneous group of neurodevelopmental disorders, with rising incidence but little effective therapeutic intervention available. Currently two main clinical features are described to diagnose ASDs: impaired social interaction and communication, and repetitive behaviors. Much work has focused on understanding underlying causes of ASD by generating animal models of the disease, in the hope of discovering signaling pathways and cellular targets for drug intervention. Here we review how ASD behavioral phenotypes can be modeled in the mouse, the most common animal

model currently in use in this field, and discuss examples of genetic mouse models of ASD with behavioral features that recapitulate various symptoms of ASD.
Neurobiol Learn Mem, 2015; 124

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BOARD NUMBER: S02-374

LINE-1 ORF1P IS TARGETTING NUCLEAR ENVELOPE COMPONENTS IN HUMAN NEURONAL MODEL OF AGING

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Aims. Transposable elements (TEs) are emerging as novel unsuspected culprits in neurodegenerative diseases. The effects of a derepression of young, coding TEs from the LINE-1 family are not well known but include genomic instability and inflammation. We are studying the mechanisms through which LINE-1 RNA and proteins are linked to age-related neurodegenerative diseases like Parkinson's and Alzheimer's disease. **Methods**. We are using a human dopaminergic neuron cell line (LUHMES) as a model in which we induce oxidative stress or an aging-like condition by arsenite treatment (NaAsO2) or multiple passaging, respectively. Levels of LINE-1 RNA and proteins (ORF1p/ORF2p) were analyzed by immunofluorescence (IF), Western blot and RT-qPCR. Nuclear envelope (NE) integrity (LaminB1/RAN/KPNB/Nup153), heterochromatin (H3K9me3, H4K20me3, HP1) and DNA damage (yH2aX) were analyzed by IF. Direct interaction of ORF1p with NE proteins was analyzed by proximal ligation assay (PLA) and IF colocalization. **Results and Conclusion**. LINE-1 expression in neurons increased upon oxidative stress or multiple passaging. Upon stress, ORF1p translocated to the nucleus and co-localized with NE proteins (LaminB1/RAN). This was accompanied by NE distortion as revealed by LaminB1 staining. NE proteins (e.g. LaminB1, RAN, PIN1, KPNB, Nup153) were also identified as ORF1p partners (mass spectroscopy/co-immunoprecipitation). Direct interaction of ORF1p with LaminB1 and Nup153 was confirmed by PLA. ORF1p could thus potentially sequester NE proteins, compromising NE integrity and function, altering nucleocytoplasmic transport and induce genomic instability (DNA damage and cytosolic leakage) and inflammation events. NE distortion also resulted in heterochromatin disorganisation, with potential consequences on gene expression.

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BOARD NUMBER: S02-375

A NOVEL DUAL INDUCIBLE SYSTEM FOR AGEING HUMAN INDUCED NEURONS REVEALS CHANGES IN EPIGENETIC LANDSCAPE OF THE AGED CELLS

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

Koby Baranes[1,2], Daniel Ives[3], Brendan Swain[3], Mohamed Ashick[4], Noah Poulin[1,2], Joana Tavares[1,2], Grant Belgard[4], Steve Horvath[5,6], Mark Kotter[1,2]
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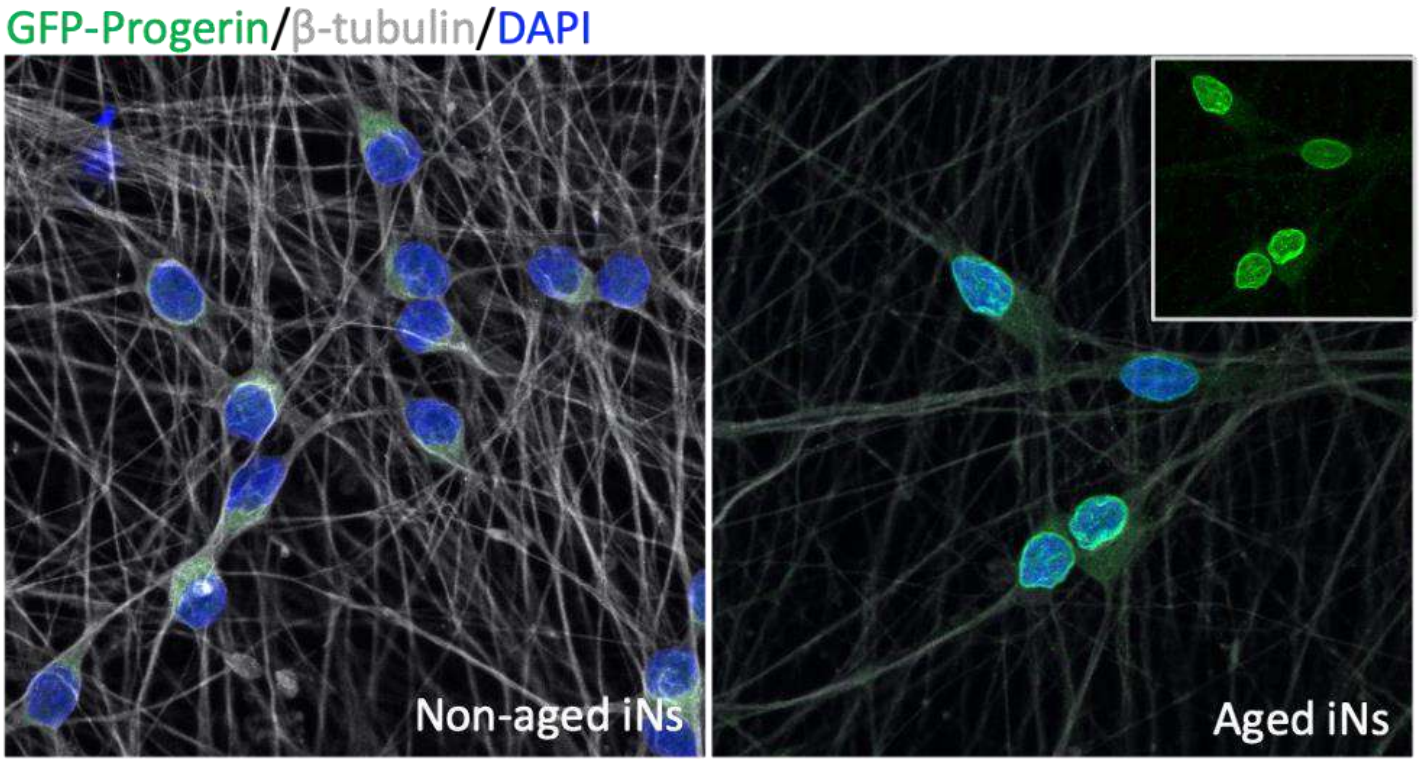
Modeling late-onset disorders using induced pluripotent stem cells (iPSCs) technology holds a challenge due to iPSCs cellular rejuvenation during reprogramming. Hallmarks of aging are often difficult to distinguish from disease-related degenerative processes but are usually associated with a gradual deterioration in structure and function. Extreme perturbations of cellular ageing occur in the context of genetic progeria syndromes, especially in Hutchison-Gilford Progeria Syndrome (HGPS). These provide unique opportunities to investigate the ageing program. Our aim is to generate a cellular model system of human ageing based on progerin overexpression. To that end, we have harnessed our inducible reprogramming system, OptiOX, and engineered human iPSCs to overexpress progerin in parallel with neurogenin 2 (NGN2). This enables direct conversion of iPSCs into cortical glutamatergic neurons by overexpressing NGN2, while ageing the cells by overexpressing progerin. Aged induced neurons (iNs) show phenotypic changes in neuronal process outgrowth, connectivity, and in morphology. Selected hallmarks of physiological ageing were observed, among these downregulation of peripheral heterochromatin markers and increased double strand DNA breaks. In addition, the epigenetic clock of the aged iNs was studied. These findings confirm our base hypothesis that our system enables independent control of progerin expression and cellular reprogramming. The resulting neurons remain viable and demonstrate select hallmarks of ageing, which provides a cellular model for both physiological and chronological

ageing.



Pubmed:

30704243: Baranes K, Hibsh D, Cohen S, Yamin T, Efroni S, Sharoni A, Shefi O
Comparing Transcriptome Profiles of Neurons Interfacing Adjacent Cells and Nanopatterned Substrates Reveals Fundamental Neuronal Interactions.
Developing neuronal axons are directed by chemical and physical signals toward a myriad of target cells. According to current dogma, the resulting network architecture is critically shaped by electrical interconnections, the synapses; however, key mechanisms translating neuronal interactions into neuronal growth behavior during network formation are still unresolved. To elucidate these mechanisms, we examined neurons interfacing nanopatterned substrates and compared them to natural interneuron interactions. We grew similar neuronal populations under three connectivity conditions, (1) the neurons are isolated, (2) the neurons are interconnected, and (3) the neurons are connected only to artificial substrates, then quantitatively compared both the cell morphologies and the transcriptome-expression profiles. Our analysis shows that whereas axon-guidance signaling pathways in isolated neurons are predominant, in isolated neurons interfacing nanotopography, these pathways are downregulated, similar to the interconnected neurons. Moreover, in nanotopography, interfacing neuron genes related to synaptogenesis and synaptic regulation are highly expressed, that is, again resembling the behavior of interconnected neurons. These molecular findings demonstrate that interactions with nanotopographies, although not leading to electrical coupling, play a comparable functional role in two major routes, neuronal guidance and network formation, with high relevance to the design of regenerative interfaces.
Nano Lett, 2019; 19
28640544: Marcus M, Baranes K, Park M, Choi IS, Kang K, Shefi O
Interactions of Neurons with Physical Environments.
Nerve growth strongly relies on multiple chemical and physical signals throughout development and regeneration. Currently, a cure for injured neuronal tissue is an unmet need. Recent advances in fabrication technologies and materials led to the development of synthetic interfaces for neurons. Such engineered platforms that come in 2D and 3D forms can mimic the native extracellular environment and create a deeper understanding of neuronal growth mechanisms, and ultimately advance the development of potential therapies for neuronal regeneration. This progress report aims to present a comprehensive discussion of this field, focusing on physical feature design and fabrication with additional information about considerations of chemical modifications. We review studies of platforms generated with a range of topographies, from micro-scale features

down to topographical elements at the nanoscale that demonstrate effective interactions with neuronal cells. Fabrication methods are discussed as well as their biological outcomes. This report highlights the interplay between neuronal systems and the important roles played by topography on neuronal differentiation, outgrowth, and development. The influence of substrate structures on different neuronal cells and parameters including cell fate, outgrowth, intracellular remodeling, gene expression and activity is discussed. Matching these effects to specific needs may lead to the emergence of clinical solutions for patients suffering from neuronal injuries or brain-machine interface (BMI) applications.
Adv Healthc Mater, 2017; 6
27179923: Marcus M, Karni M, Baranes K, Levy I, Alon N, Margel S, Shefi O
Iron oxide nanoparticles for neuronal cell applications: uptake study and magnetic manipulations.
The ability to direct and manipulate neuronal cells has important potential in therapeutics and neural network studies. An emerging approach for remotely guiding cells is by incorporating magnetic nanoparticles (MNPs) into cells and transferring the cells into magnetic sensitive units. Recent developments offer exciting possibilities of magnetic manipulations of MNPs-loaded cells by external magnetic fields. In the present study, we evaluated and characterized uptake properties for optimal loading of cells by MNPs. We examined the interactions between MNPs of different cores and coatings, with primary neurons and neuron-like cells.
J Nanobiotechnology, 2016; 14
26674672: Baranes K, Shevach M, Shefi O, Dvir T
Gold Nanoparticle-Decorated Scaffolds Promote Neuronal Differentiation and Maturation.
Engineered 3D neuronal networks are considered a promising approach for repairing the damaged spinal cord. However, the lack of a technological platform encouraging axonal elongation over branching may jeopardize the success of such treatment. To address this issue we have decorated gold nanoparticles on the surface of electrospun nanofiber scaffolds, characterized the composite material, and investigated their effect on the differentiation, maturation, and morphogenesis of primary neurons and on an immature neuronal cell line. We have shown that the nanocomposite scaffolds have encouraged a longer outgrowth of the neurites, as judged by the total length of the branching trees and the length and total distance of neurites. Moreover, neurons grown on the nanocomposite scaffolds had less neurites originating out of the soma and lower number of branches. Taken together, these results indicate that neurons cultivated on the gold nanoparticle scaffolds prefer axonal elongation over forming complex branching trees. We envision that such cellular constructs may be useful in the future as implantable cellular devices for repairing damaged neuronal tissues, such as the spinal cord.
Nano Lett, 2016; 16
26507853: Betzer O, Meir R, Dreifuss T, Shamalov K, Motiei M, Shwartz A, Baranes K, Cohen CJ, Shraga-Heled N, Ofir R, Yadid G, Popovtzer R
In-vitro Optimization of Nanoparticle-Cell Labeling Protocols for In-vivo Cell Tracking Applications.
Recent advances in theranostic nanomedicine can promote stem cell and immune cell-based therapy. Gold nanoparticles (GNPs) have been shown to be promising agents for in-vivo cell-tracking in cell-based therapy applications. Yet a crucial challenge is to develop a reliable protocol for cell upload with, on the one hand, sufficient nanoparticles to achieve maximum visibility of cells, while on the other hand, assuring minimal effect of particles on cell function and viability. Previous studies have demonstrated that the physicochemical parameters of GNPs have a critical impact on their efficient uptake by cells. In the current study we have examined possible variations in GNP uptake, resulting from different incubation period and concentrations in different cell-lines. We have found that GNPs effectively labeled three different cell-lines - stem, immune and cancer cells, with minimal impairment to cell viability and functionality. We further found that uptake efficiency of GNPs into cells stabilized after a short period of time, while GNP concentration had a significant impact on cellular uptake, revealing cell-dependent differences. Our results suggest that while heeding the slight variations within cell lines, modifying the loading time and concentration of GNPs, can promote cell visibility in various nanoparticle-dependent in-vivo cell tracking and imaging applications.
Sci Rep, 2015; 5
25880565: Shilo M, Sharon A, Baranes K, Motiei M, Lellouche JP, Popovtzer R
The effect of nanoparticle size on the probability to cross the blood-brain barrier: an in-vitro endothelial cell model.
During the last decade nanoparticles have gained attention as promising drug delivery agents that can transport through the blood brain barrier. Recently, several studies have demonstrated that specifically targeted nanoparticles which carry a large payload of therapeutic agents can effectively enhance therapeutic agent delivery to the brain. However, it is difficult to draw definite design principles across these studies, owing to the differences in material, size, shape and targeting agents of the nanoparticles. Therefore, the main objective of this study is to develop general design principles that link the size of the nanoparticle with the probability to cross the blood brain barrier. Specifically, we investigate the effect of the nanoparticle size on the probability of barbiturate coated GNPs to cross the blood brain barrier by using bEnd.3 brain endothelial cells as an in vitro blood brain barrier model.

J Nanobiotechnology, 2015; 13
24559496: Baranes K, Moshe H, Alon N, Schwartz S, Shefi O
Neuronal growth on L- and D-cysteine self-assembled monolayers reveals neuronal chiral sensitivity.
Studying the interaction between neuronal cells and chiral molecules is fundamental for the design of novel biomaterials and drugs. Chirality influences all biological processes that involve intermolecular interaction. One common method used to study cellular interactions with different enantiomeric targets is the use of chiral surfaces. Based on previous studies that demonstrated the importance of cysteine in the nervous system, we studied the effect of L- and D-cysteine on single neuronal growth. L-Cysteine, which normally functions as a neuromodulator or a neuroprotective antioxidant, causes damage at elevated levels, which may occur post trauma. In this study, we grew adult neurons in culture enriched with L- and D-cysteine as free compounds or as self-assembled monolayers of chiral surfaces and examined the effect on the neuronal morphology and adhesion. Notably, we have found that exposure to the L-cysteine enantiomer inhibited, and even prevented, neuronal attachment more severely than exposure to the D-cysteine enantiomer. Atop the L-cysteine surfaces, neuronal growth was reduced and degenerated. Since the cysteine molecules were attached to the surface via the thiol groups, the neuronal membrane was exposed to the molecular chiral site. Thus, our results have demonstrated high neuronal chiral sensitivity, revealing chiral surfaces as indirect regulators of neuronal cells and providing a reference for studying chiral drugs.
ACS Chem Neurosci, 2014; 5
22572872: Baranes K, Kollmar D, Chejanovsky N, Sharoni A, Shefi O
Interactions of neurons with topographic nano cues affect branching morphology mimicking neuron-neuron interactions.
We study the effect of topographic nano-cues on neuronal growth-morphology using invertebrate neurons in culture. We use photolithography to fabricate substrates with repeatable line-pattern ridges of nano-scale heights of 10-150 nm. We plate leech neurons atop the patterned-substrates and compare their growth pattern to neurons plated atop non-patterned substrates. The model system allows us the analysis of single neurite-single ridge interactions. The use of high resolution electron microscopy reveals small filopodia processes that attach to the line-pattern ridges. These fine processes, that cannot be detected in light microscopy, add anchoring sites onto the side of the ridges, thus additional physical support. These interactions of the neuronal process dominantly affect the neuronal growth direction. We analyze the response of the entire neuronal branching tree to the patterned substrates and find significant effect on the growth patterns compared to non-patterned substrates. Moreover, interactions with the nano-cues trigger a growth strategy similarly to interactions with other neuronal cells, as reflected in their morphometric parameters. The number of branches and the number of neurites originating from the soma decrease following the interaction demonstrating a tendency to a more simplified neuronal branching tree. The effect of the nano-cues on the neuronal function deserves further investigation and will strengthen our understanding of the interplay between function and form.
J Mol Histol, 2012; 43
22252990: Baranes K, Chejanovsky N, Alon N, Sharoni A, Shefi O
Topographic cues of nano-scale height direct neuronal growth pattern.
We study the role of nano-scale cues in controlling neuronal growth. We use photolithography to fabricate substrates with repeatable line-pattern ridges of nano-scale heights. We find that neuronal processes, which are of micron size, have strong interactions with ridges even as low as 10 nm. The interaction between the neuronal process and the ridge leads to a deflection of growth direction and a preferred alignment with the ridges. The interaction strength clearly depends on the ridges' height. For 25 nm ridges approximately half of the neuronal processes are modified, while at 100 nm the majority of neurites change their original growth direction post interaction. In addition, the effect on growth correlates with the incoming angle between the neuronal process and the ridge. We underline the adhesion as a key mechanism in directing neuronal growth. Our study highlights the sensitivity of growing neurites to nano-scale cues thus opens a new avenue of research for pre-designed neuronal growth and circuitry.
Biotechnol Bioeng, 2012; 109
19345213: Baranes K, Raz-Prag D, Nitzan A, Galron R, Ashery-Padan R, Rotenstreich Y, Assaf Y, Shiloh Y, Wang ZQ, Barzilai A, Solomon AS
Conditional inactivation of the NBS1 gene in the mouse central nervous system leads to neurodegeneration and disorganization of the visual system.
Nijmegen breakage syndrome (NBS) is a genomic instability disease caused by hypomorphic mutations in the NBS1 gene encoding the Nbs1 (nibrin) protein. Nbs1 is a component of the Mre11/Rad50/Nbs1 (MRN) complex that acts as a sensor of double strand breaks (DSBs) in the DNA and is critical for proper activation of the broad cellular response to DSBs. Conditional disruption of the murine ortholog of the human NBS1, Nbs1, in the CNS of mice was previously reported to cause microcephaly, severe cerebellar atrophy and ataxia. Here we report that conditional targeted disruption of the murine NBS1 gene in the CNS results in mal-development, degeneration, disorganization and dysfunction of the murine visual system, especially in the optic nerve. Nbs1 deletion resulted in reduced diameters of Nbs1-CNS-Delta eye and optic nerve. MRI

analysis revealed defective white matter development and organization. Nbs1 inactivation altered the morphology and organization of the glial cells. Interestingly, at the age of two-month-old the levels of the axonal guidance molecule semaphorin-3A and its receptor neuropilin-1 were up-regulated in the retina of the mutant mice, a typical injury response. Electroretinogram analysis revealed marked reduction in a- and b-waves, indicative of decreased retinal function. Our study points to a novel role for Nbs1 in the development, organization and function of the visual system.
Exp Neurol, 2009; 218

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BOARD NUMBER: S02-376

EXPLORING THE MISSING HERITABILITY IN SPG7 HETEROZYGOUS CARRIERS WITH WHOLE GENOME SEQUENCING

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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SPG7 biallelic mutations are the most frequent cause of autosomal recessive spastic paraplegia. The associated clinical picture is either a pure spastic paraplegia, or a complex phenotype encompassing mitochondrial features, optic atrophy, and cerebellar signs. In the recent years, the phenotype has been widened to cerebellar ataxia more generally, with or without pyramidal signs; and to clinical presentations associating extrapyramidal features, mimicking Parkinson's disease or Multisystemic Atrophy of the cerebellar type in some cases. In 731 patients with cerebellar ataxia, we sequenced known ataxia genes, either with amplicon-based panel sequencing (n=412) or whole exome sequencing (n=319). We found biallelic mutations in 23 patients (3.1%), often associating spastic or mitochondrial presentations. We also identified 19 heterozygous carriers of loss of function or previously described missense variants in *SPG7*, without a second mutation. Dominant transmission has been discussed in the literature. While it is suggested in some patients, a recent report described a deep intronic change responsible for an alteration of *SPG7* expression, in trans with a missense mutation, advising genetic reexamination of heterozygous carriers. We included 19 index cases with a phenotype characteristic of *SPG7*-related cerebellar ataxia, associating either spasticity or parkinsonism, and an established causative but monoallelic *SPG7* variant. We performed short-read Whole Genome Sequencing in those patients and their relatives, when available, and report our results on missing heritability identification.

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BOARD NUMBER: S02-377

STUDYING THE OPTIC NERVE STRUCTURE IN CONGENITAL NON-SYNDROMIC RETINAL DETACHMENT (NCRNA) FROM THE PERSPECTIVE OF HISTOPATHOLOGY AND RADIOLOGY

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

Fatemeh Sadat Rashidi[1], Ehsan Ahmadipour[1], Fahimeh Asadi Amoli[2], Amir Hosein Falahian[3], Hamideh Gholamhoseini[4], Mohammad Ismail Zibaii[5], Nader Maghsoudi[1], Alireza Zali[6], Mostafa Soltan Sanjari[7], Reza Ahadi[8], Noor Mohamad Ghiasvand[1]
[1]Shahid Beheshti University of medical science, Neuroscience Research Center, Tehran, Iran, [2]Tehran University of Medical science, Farabi Comprehensive Center Of Excellence In Ophthalmology, Tehran, Iran, [3]Kansas City Veterans Medical Center, Department(s) Of Radiology,, Kansas, United States of America, [4]Modarres Hospital, Shahid Beheshti University of Medical Sciences, Radiology Department, Tehran, Iran, [5]Laser and Plasma Research Institute-Shahid Beheshti University, Laser And Plasma, Sari, Iran, [6]Shohada Hospital, Shahid Beheshti University of Medical Sciences, Functional Neurosurgery Research Center, Tehran, Iran, [7]Eye Research Center, Rasoul-Akram Hospital, Iran University of Medical Sciences (IUMS), Department Of Ophthalmology, Tehran, Iran, [8]Rasoul-Akram Hospital, Iran University of Medical Sciences (IUMS), Department Of Anatomy, Tehran, Iran

Transient expression of the *ATOH7* gene in the embryonic neural retina is required for the genesis of retinal ganglion cells (RGC), hence for optic nerve (ON) development. Different *ATOH7* disease alleles could result in different degrees of optic nerve abnormalities; from optic nerve aplasia (ONA) to a wide spectrum of optic nerve hypoplasia (ONH). We have previously shown that the deletion of a cis controlling element upstream of *ATOH7* results in nonsyndromic congenital retinal non-attachment (NCRNA) and congenital blindness with no perception of light. In this study, we investigate the histology of optic pathways in a 16-week NCRNA (*ATOH7*-/-) fetus and an age-matched *ATOH7*+/+ fetus as control. We reported this phenotype as a case of ONA. However, because, in the magnetic resonance images of an NCRNA patient, thin linear structures were present in his orbital cones, and the MR images did not allow us to resolve the components of these structures, we could not rule out the presence of thin atrophic/hypoplastic optic nerves. In this report, we describe the histopathological differences between optic pathways in these fetuses.

Pubmed:
33775777: Javadpour P, Askari S, Rashidi FS, Dargahi L, Ahmadiani A, Ghasemi R
Imipramine alleviates memory impairment and hippocampal apoptosis in STZ-induced sporadic Alzheimer's rat model: Possible contribution of MAPKs and insulin signaling.
Alzheimer's disease (AD) is the most common age-related neurodegenerative disease, associated with several pathophysiological complaints. Impaired insulin signaling in the brain, is one of the important characteristic features of AD which is accompanied by cognitive deficits. According to the multifactorial and complicated pathology of AD, no modifying therapy has been approved yet. Imipramine is a kind of tricyclic antidepressant with reported anti-inflammatory and anti-oxidant effects in the brain. There are controversial studies about the effect of this drug on spatial memory. This study investigates the effect of imipramine on streptozotocin (STZ) induced memory impairment in rats. Pursuing this objective, rats were treated with imipramine 10 or 20 mg/kg i.p. once a day for 14 days. 24 h after the last injection, memory function was evaluated by the Morris water maze (MWM) test in 4 consecutive days. Then, hippocampi were removed and the activity of caspase-3, mitogen activated protein kinases (MAPKs) family and inhibitory phosphorylation of insulin receptor substrate-1 (IRS-1) were analyzed using Western blotting. Results showed that imipramine prevents memory impairment in STZ induced rats and this improvement was accompanied with an increase in ERK activity, reduction of caspase-3 and JNK activity, as well as partial restoration of P38 and IRS-1 activity. In conclusion, our study demonstrated that at least some members of the MAPK family are involved in the neuroprotective effect of imipramine.
Behav Brain Res, 2021; 408
28691545: Rashidi FS, Ahmadipour E, Shiravand S, Ahmadiani A, Asadi S, Shams J
Association of the functional serotonin transporter haplotype with familial form of obsessive compulsive disorder in Iranian patients.
Several polymorphisms have been reported in the 5-HTTLPR of the serotonin transporter gene (SLC6A4). Family-based

evidences for the association of 5-HTTLPR polymorphisms with OCD were previously reported but results were controversial. The present study investigated the possible correlation of SLC6A4 polymorphisms (5-HTTLPR, rs25532, rs25531) in Iranian OCD patients considering gender, age of onset, family history of psychiatric disorders, obsessive and compulsive subtypes and severities.
Int J Psychiatry Clin Pract, 2018; 22
28164936: Salehi MS, Namavar MR, Tamadon A, Bahmani R, Jafarzadeh Shirazi MR, Khazali H, Dargahi L, Pandamooz S, Mohammad-Rezazadeh F, Rashidi FS
The Effects of Acoustic White Noise on the Rat Central Auditory System During the Fetal and Critical Neonatal Periods: A Stereological Study.
To evaluate the effects of long-term, moderate level noise exposure during crucial periods of rat infants on stereological parameters of medial geniculate body (MGB) and auditory cortex.
Noise Health, 2017 Jan-Feb; 19
28261314: Karbaschi R, Zardooz H, Khodagholi F, Dargahi L, Salimi M, Rashidi F
Maternal high-fat diet intensifies the metabolic response to stress in male rat offspring.
The mother's consumption of high-fat food can affect glucose metabolism and the hypothalamic-pituitary-adrenal axis responsiveness in the offspring and potentially affect the metabolic responses to stress as well. This study examines the effect of maternal high-fat diet on the expression of pancreatic glucose transporter 2 and the secretion of insulin in response to stress in offspring.
Nutr Metab (Lond), 2017; 14

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THE ROLE OF THE CHIP/STUB1 PATHWAY ON MECHANISMS OF NEURODEGENERATION.

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

Dominika Pankanin[1], Erisa Nita[2], Jakub Faktor[1], Ailish Tynan[2], Artur Piróg[1], Georges Bedran[1], Irena Dapic[1], Sachin Kote[1], Kathryn Ball[2], Ted Hupp[1]
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Neurodegenerative diseases affect millions of people worldwide. Until now no effective treatment have been developed and currently available options are targeted to help reduce symptoms and relieve patient's pain. C terminus of HSC70-Interacting Protein (CHIP) is acknowledged as a protector of neurons and cells of central nervous system (CNS) from oxidative stress which plays fundamental role in a common pathophysiology of neurodegenerative diseases. Previous studies have shown that CHIP regulates cellular membrane integrity under conditions of acute stress, at the same time acting as a proteostasis sensor that models the proteome. Protein misfolding and aggregation are hallmarks of age-related proteinopathies. To determine the role of CHIP in the process of neurodegeneration, we proposed 'network level' view on signalling in SH-SY5Y cell models. Our team investigated protein homeostasis inside the WT and CHIP KO SH-SY5Y cell models. The key readout of changes in proteostasis obtained with use of liquid chromatography mass spectrometry (LC-MS) showed overexpression of Pro-neuropeptide Y (NPY) and Neurofilament medium polypeptide (NEFM) in the SH-SY5Y CHIP KO cells when compared to SH-SY5Y WT. Results were confirmed by SDS-PAGE. Both proteins play an important role in neurodevelopmental processes. This study could be fundamental for development of effective anti-neurodegeneration treatment therapies.

Pubmed:
30656911: Węgrzyn G, Pierzynowska K, Podlacha M, Brokowska J, Gaffke L, Mantej J, Cyske Z, Rintz E, Osiadły M, Bartkowski M, Puchalski M, Grabski M, Pierzynowski M, Pankanin D, Piotrowska E, Tukaj S
[Molecular mechanisms of genistein action in the light of therapies for genetic and immunological diseases].
Genetic and immunological diseases, despite many attempts to develop effective treatments, still remain a great challenge for medicine. Current therapies of these diseases consist of pharmacological alleviation of symptoms, rehabilitation and psychological help which, although very important, are not sufficient. Therefore, searching for new therapeutics which could remove the major causes of these diseases is of particular importance for the society. Natural compounds reveal many biological activities which makes them candidates for drugs in such diseases. One of them is genistein, a compound from the group of flavonoids. As it affects multiple processes, genistein has become in the center of interest of many scientists working on diseases of various etiology, course and inheritance. It was used in experimental therapies of some genetic diseases (Huntington's disease, amyotrophic lateral sclerosis Parkinson disease, cystic fibrosis), as well as autoimmunological diseases and allergies. Clinical trials with the use of genistein in treatment of patients suffering from Alzheimer's diseases and mucopolysaccharidosis type III are ongoing. The employment of differential properties of genistein in attempts to treat each of these diseases is of special interest. In this review, detailed molecular mechanisms of genistein action are summarized in the light of therapies of the above mentioned genetic and immunological diseases, including description of therapeutic potentials of each activity of this isoflavone, efficiency of its action, and its potential use as a drug in the future.
Postepy Biochem, 2018; 64

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PROTEOMIC CHARACTERIZATION OF THE HUMAN ENTORRINAL CORTEX IN ALZHEIMER'S DISEASE.

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

Veronica Astillero-Lopez[1], Sandra Villar-Conde[1], Melania Gonzalez-Rodriguez[1], Alicia Flores-Cuadrado[1], Isabel Ubeda-Banon[1], Daniel Saiz-Sanchez[1], Alino Martinez-Marcos[2]
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Alzheimer´s disease (AD) is clinically characterized by cognitive deficits. Neuropathologically, AD brains accumulate deposits of amyloid-β and tau proteins. Furthermore, these misfolded proteins can propagate cell-to-cell in a prion-like manner and induce native proteins to become pathological. Particularly, the entorhinal cortex (EC) is the earliest affected area by tau accumulation. Previous proteomic study in our lab revealed very interesting up- (S100A6, PPP1R1B, BAG3 and PRDX6) and downregulated (GSK3B, SYN1, DLG4 and RAB3A) proteins related with neurodegeneration and astrogliosis in the human EC in AD. However, several relevant proteins were neglected. Therefore, the aim of this work was to characterize additional different proteins between AD and non-AD cases in the human EC.
Post-mortem tissue, including 12 AD and 10 non-AD age-matched cases, was provided by IDIBAPS, BTCIEN, BIOBANC-MUR, BPA and NAVARRABIOMED Spanish National Biobanks. Experimental procedures were approved by the Ethical Committee of Clinical Research at Ciudad Real University Hospital (PID2019-108659RB-I00). Metaboanalyst, Reactome and STRING databases were used for bioinformatic analysis. Immunofluorescence and western blot were performed to evaluate selected proteins.
Data analysis indicate several pathological pathways involving synaptic processes, neuroinflammatory and chaperone response, as well as identify proteins that could constitute potential biomarkers in AD such as HSP90AA1, PTK2B and ANXA2. These results provide new insights on proteomic changes in the human entorhinal cortex in AD.
Sponsored by the UCLM/ERDF (2020-GRIN-31233), the Spanish Ministries of Economy and Competitiveness/ERDF (SAF2016-75768-R) and Science and Innovation (PID2019-108659RB-I00) and the Autonomous Government of Castilla-La Mancha/ERDF (SBPLY/17/180501/000430). SVC and MGR held a predoctoral fellowship UCLM/ESF.

Pubmed:
35142030: Astillero-Lopez V, Gonzalez-Rodriguez M, Villar-Conde S, Flores-Cuadrado A, Martinez-Marcos A, Ubeda-Banon I, Saiz-Sanchez D
Neurodegeneration and astrogliosis in the entorhinal cortex in Alzheimer's disease: Stereological layer-specific assessment and proteomic analysis.
The entorhinal cortex is among the earliest areas involved in Alzheimer's disease. Volume reduction and neural loss in this area have been widely reported. Human entorhinal cortex atrophy is, in part, due to neural loss, but microglial and/or astroglial involvement in the different layers remains unclear. Additionally, -omic approaches in the human entorhinal cortex are scarce.
Alzheimers Dement, 2022;

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-380

ACCELERATED COGNITIVE DECLINE IN OBESE MOUSE MODEL OF ALZHEIMER'S DISEASE IS LINKED TO SIALIC ACID-DRIVEN IMMUNE DEREGULATION

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Systemic immunity supports healthy brain homeostasis. Accordingly, conditions causing systemic immune deregulation may accelerate onset of neurodegeneration in predisposed individuals. Here we show that, in the 5xFAD mouse model of Alzheimer's disease (AD), high-fat diet-induced obesity accelerated cognitive decline, which was associated with immune deviations comprising increased splenic frequencies of exhausted $CD4^+$ T effector memory cells and $CD4^+FOXP3^+$ regulatory T cells (Tregs). Non-targeted plasma metabolomics identified *N*-acetylneuraminic acid (NANA), the predominant sialic acid, as the major obesity-induced metabolite in 5xFAD mice, the levels of which directly correlated with Tregs abundance and inversely correlated with cognitive performance. Visceral adipose tissue macrophages were identified by sNuc-Seq as one potential source of NANA. Exposure to NANA led to immune deregulation in middle-aged wild-type mice, and *ex vivo* in human T cells. Our study identified diet-induced immune deregulation, potentially via sialic acid, as a previously unrecognized link between obesity and AD.

Pubmed:
35108978:
35108879:
34516550: Suzzi S, Ahrendt R, Hans S, Semenova SA, Chekuru A, Wirsching P, Kroehne V, Bilican S, Sayed S, Winkler S, Spieß S, Machate A, Kaslin J, Panula P, Brand M
Deletion of lrrk2 causes early developmental abnormalities and age-dependent increase of monoamine catabolism in the zebrafish brain.
LRRK2 gain-of-function is considered a major cause of Parkinson's disease (PD) in humans. However, pathogenicity of LRRK2 loss-of-function in animal models is controversial. Here we show that deletion of the entire zebrafish lrrk2 locus elicits a pleomorphic transient brain phenotype in maternal-zygotic mutant embryos (mzLrrk2). In contrast to lrrk2, the paralog gene lrrk1 is virtually not expressed in the brain of both wild-type and mzLrrk2 fish at different developmental stages. Notably, we found reduced catecholaminergic neurons, the main target of PD, in specific cell populations in the brains of mzLrrk2 larvae, but not adult fish. Strikingly, age-dependent accumulation of monoamine oxidase (MAO)-dependent catabolic signatures within mzLrrk2 brains revealed a previously undescribed interaction between LRRK2 and MAO biological activities. Our results highlight mzLrrk2 zebrafish as a tractable tool to study LRRK2 loss-of-function in vivo, and suggest a link between LRRK2 and MAO, potentially of relevance in the prodromic stages of PD.
PLoS Genet, 2021; 17
24941947: Gomez-Nicola D, Suzzi S, Vargas-Caballero M, Fransen NL, Al-Malki H, Cebrian-Silla A, Garcia-Verdugo JM, Riecken K, Fehse B, Perry VH
Temporal dynamics of hippocampal neurogenesis in chronic neurodegeneration.
The study of neurogenesis during chronic neurodegeneration is crucial in order to understand the intrinsic repair mechanisms of the brain, and key to designing therapeutic strategies. In this study, using an experimental model of progressive chronic neurodegeneration, murine prion disease, we define the temporal dynamics of the generation, maturation and integration of new neurons in the hippocampal dentate gyrus, using dual pulse-chase, multicolour γ-retroviral tracing, transmission electron microscopy and patch-clamp. We found increased neurogenesis during the progression of prion disease, which partially counteracts the effects of chronic neurodegeneration, as evidenced by blocking neurogenesis with cytosine arabinoside, and helps to preserve the hippocampal function. Evidence obtained from human post-mortem samples, of both variant

Creutzfeldt-Jakob disease and Alzheimer's disease patients, also suggests increased neurogenic activity. These results open a new avenue into the exploration of the effects and regulation of neurogenesis during chronic neurodegeneration, and offer a new model to reproduce the changes observed in human neurodegenerative diseases.
Brain, 2014; 137
23392676: Gómez-Nicola D, Fransen NL, Suzzi S, Perry VH
Regulation of microglial proliferation during chronic neurodegeneration.
An important component of chronic neurodegenerative diseases is the generation of an innate inflammatory response within the CNS. Microglial and astroglial cells play a key role in the development and maintenance of this inflammatory response, showing enhanced proliferation and activation. We studied the time course and regulation of microglial proliferation, using a mouse model of prion disease. Our results show that the proliferation of resident microglial cells accounts for the expansion of the population during the development of the disease. We identify the pathway regulated by the activation of CSF1R and the transcription factors PU.1 and C/EBPα as the molecular regulators of the proliferative response, correlating with the chronic human neurodegenerative conditions variant Creutzfeldt-Jakob disease and Alzheimer's disease. We show that targeting the activity of CSF1R inhibits microglial proliferation and slows neuronal damage and disease progression. Our results demonstrate that microglial proliferation is a major component in the evolution of chronic neurodegeneration, with direct implications for understanding the contribution of the CNS innate immune response to disease progression.
J Neurosci, 2013; 33

FENS Forum 2022
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BOARD NUMBER: S02-381

THE APOE ε4 GENETIC POLYMORPHISM ALTERS CHOLESTEROL METABOLISM AND CHOLINERGIC SIGNALLING PATHWAY PROMOTING NEUROTOXIC EFFECTS

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Background. Physiologically, ApoE is produced by astrocytes and microglia in the central nervous system and is involved in the maintenance of normal brain functions, promoting neuronal integrity/repair and cholesterol redistribution. In neuropathological conditions, ApoE is also produced by neurons, in response to neuronal damage/stress; however, when ε4 isoform is expressed, the ApoE neuroprotective/repairing role is lost. Indeed, ApoEε4 promotes neurotoxic effects, increasing the APP transcription and Aβ production/accumulation, and leads to Alzheimer's Disease (AD), a neurodegenerative disorder characterized by cholinergic synapses alterations. Despite the cholinergic neurotransmission changes and ApoE-dependent pathogenic mechanisms in AD development have been extensively investigated, their cause-effect connection remains elusive. **Aim.** The current study aimed to explore the ApoEε4-induced neurotoxic effects in relation to cholesterol metabolism and cholinergic neurotransmission alterations. **Results.** A cholinergic neuron cell model overexpressing ApoEε4 was performed, thus reproducing a neuronal damage model. It showed increased intracellular cholesterol accumulation given by dysregulation of cholesterol metabolism genes (increased PLIN2; decreased HMGCR, PLIN2, NCEH, and CYP46A1). Furthermore, the overexpression of ApoEε4 altered ACh homeostasis (increased ChAT protein expression; increased VChAT and reduced AChE genes expression), leading to extracellular accumulation of ACh and intracellular accumulation of IP_3 and Ca^{2+}. This mediates apoptotic effects by reducing the expression/activation of PKCε, which in turn leads to higher APP/Aβ accumulation and GSK-3β expression and increased cleaved Cas-3 protein production. **Conclusions.** Overall, the obtained data suggest a possible involvement of ApoEε4-dependent cholesterol metabolism dysregulation in the alteration of cholinergic synapses and neurotoxicity. Further experiments are ongoing to investigate this crucial aspect.

Pubmed:
30826968: Baldacci F, Daniele S, Piccarducci R, Giampietri L, Pietrobono D, Giorgi FS, Nicoletti V, Frosini D, Libertini P, Lo Gerfo A, Petrozzi L, Donadio E, Betti L, Trincavelli ML, Siciliano G, Ceravolo R, Tognoni G, Bonuccelli U, Martini C
Potential Diagnostic Value of Red Blood Cells α-Synuclein Heteroaggregates in Alzheimer's Disease.
A plethora of complex misfolded protein combinations have been found in Alzheimer disease (AD) brains besides the classical pathological hallmarks. Recently, α-synuclein (α-syn) and its heterocomplexes with amyloid-β (Aβ) and tau have been suggested to be involved in the pathophysiological processes of neurodegenerative diseases. These pathological features are not limited to the brain, but can be also found in peripheral fluids. In this respect, red blood cells (RBCs) have been suggested as a good model to investigate the biochemical alterations of neurodegeneration. Our aim is to find whether RBC concentrations of α-syn and its heterocomplexes (i.e., α-syn/Aβ and α-syn/tau) were different in AD patients compared with healthy controls (HC). The levels of homo- and heteroaggregates of α-syn, Aβ and tau, were analyzed in a cohort of AD patients at early stage either with dementia or prodromal symptoms (N = 39) and age-matched healthy controls (N = 39). All AD patients received a biomarker-based diagnosis (low cerebrospinal fluid levels of Aβ peptide combined with high cerebrospinal fluid concentrations of total tau and/or phospho-tau proteins; alternatively, a positivity to cerebral amyloid-PET scan). Our results showed lower concentrations of α-syn and its heterocomplexes (i.e., α-syn/Aβ and α-syn/tau) in RBCs of AD patients with respect to HC. RBC α-syn/Aβ as well as RBC α-syn/tau heterodimers discriminated AD participants from HC with fair accuracy, whereas RBC α-syn concentrations differentiated poorly the two groups. Although additional investigations are required, these data suggest α-syn heteroaggregates in RBCs as potential tool in the diagnostic work-up of early AD diagnosis.
Mol Neurobiol, 2019; 56
31281579: Piccarducci R, Pietrobono D, Pellegrini C, Daniele S, Fornai M, Antonioli L, Trincavelli ML, Blandizzi C, Martini C
High Levels of -Amyloid, Tau, and Phospho-Tau in Red Blood Cells as Biomarkers of Neuropathology in Senescence-Accelerated Mouse.
Alzheimer's Disease (AD) is the most common Neurodegenerative Disease (ND), primarily characterised by

neuroinflammation, neuronal plaques of -amyloid (A), and neurofibrillary tangles of hyperphosphorylated tau. -Synuclein (-syn) and its heteroaggregates with A and tau have been recently included among the neuropathological elements of NDs. These pathological traits are not restricted to the brain, but they reach peripheral fluids as well. In this sense, Red Blood Cells (RBCs) are emerging as a good model to investigate the biochemical alterations of aging and NDs. Herein, the levels of homo- and heteroaggregates of ND-related proteins were analysed at different stages of disease progression. In particular, a validated animal model of AD, the SAMP8 (Senescence-Accelerated Mouse-Prone) and its control strain SAMR1 (Senescence-Accelerated Mouse-Resistant) were used in parallel experiments. The levels of the aforementioned proteins and of the inflammatory marker interleukin-1 (IL-1) were examined in both brain and RBCs of SAMP8 and SAMR1 at 6 and 8 months. Brain A, tau, and phospho-tau (p-tau) were higher in SAMP8 mice than in control mice and increased with AD progression. Similar accumulation kinetics were found in RBCs, even if slower. By contrast, -syn and its heterocomplexes (-syn-A and -syn-tau) displayed different accumulation kinetics between brain tissue and RBCs. Both brain and peripheral IL-1 levels were higher in SAMP8 mice, but increased sooner in RBCs, suggesting that inflammation might initiate at a peripheral level before affecting the brain. In conclusion, these results confirm RBCs as a valuable model for monitoring neurodegeneration, suggesting peripheral A, tau, and p-tau as potential early biomarkers of AD.
Oxid Med Cell Longev, 2019; 2019

31717561: Piccarducci R, Daniele S, Fusi J, Chico L, Baldacci F, Siciliano G, Bonuccelli U, Franzoni F, Martini C
Impact of ApoE Polymorphism and Physical Activity on Plasma Antioxidant Capability and Erythrocyte Membranes.
The allele epsilon 4 (ε4) of apolipoprotein E (ApoE) is the strongest genetic risk factor for Alzheimer's disease (AD). ApoE protein plays a pivotal role in the synthesis and metabolism of amyloid beta (Aβ), the major component of the extracellular plaques that constitute AD pathological hallmarks. Regular exercise is an important preventive/therapeutic tool in aging and AD. Nevertheless, the impact of physical exercise on the well-being of erythrocytes, a good model of oxidative stress and neurodegenerative processes, remains to be investigated, particularly depending on ApoE polymorphism. Herein, we evaluate the oxidative status, Aβ levels, and the membrane's composition of erythrocytes in a cohort of human subjects. In our hands, the plasma antioxidant capability (AOC), erythrocytes membrane fluidity, and the amount of phosphatidylcholine (PC) were demonstrated to be significantly decreased in the ApoE ε4 genotype and non-active subjects. In contrast, erythrocyte Aβ content and lipid peroxidation increased in ε4 carriers. Regular physical exercise was associated with an increased plasma AOC and membrane fluidity, as well as to a reduced amount of erythrocytes Aβ. Altogether, these data highlight the influence of the ApoE genotype on erythrocytes' well-being and confirm the positive impact of regular physical exercise.
Antioxidants (Basel), 2019; 8

32429301: Pellegrini C, Daniele S, Antonioli L, Benvenuti L, D'Antongiovanni V, Piccarducci R, Pietrobono D, Citi V, Piragine E, Flori L, Ippolito C, Segnani C, Palazon-Riquelme P, Lopez-Castejon G, Martelli A, Colucci R, Bernardini N, Trincavelli ML, Calderone V, Martini C, Blandizzi C, Fornai M
Prodromal Intestinal Events in Alzheimer's Disease (AD): Colonic Dysmotility and Inflammation Are Associated with Enteric AD-Related Protein Deposition.
Increasing evidence suggests that intestinal dysfunctions may represent early events in Alzheimer's disease and contribute to brain pathology. This study examined the relationship between onset of cognitive impairment and colonic dysfunctions in a spontaneous AD model before the full development of brain pathology. SAMP8 mice underwent Morris water maze and assessment of faecal output at four, six and eight months of age. In vitro colonic motility was examined. Faecal and colonic Aβ, tau proteins, α-synuclein and IL-1β were assessed by ELISA. Colonic citrate synthase activity was assessed by spectrophotometry. Colonic NLRP3, caspase-1 and ASC expression were evaluated by Western blotting. Colonic eosinophil density and claudin-1 expression were evaluated by immunohistochemistry. The effect of Aβ on NLRP3 signalling and mitochondrial function was tested in cultured cells. Cognitive impairment and decreased faecal output occurred in SAMP8 mice from six months. When compared with SAMR1, SAMP8 animals displayed: (1) impaired in vitro colonic contractions; (2) increased enteric AD-related proteins, IL-1β, active-caspase-1 expression and eosinophil density; and (3) decreased citrate synthase activity and claudin-1 expression. In THP-1 cells, Aβ promoted IL-1β release, which was abrogated upon incubation with caspase-1 inhibitor or in ASC cells. Aβ decreased mitochondrial function in THP-1 cells. In SAMP8, enteric AD-related proteins deposition, inflammation and impaired colonic excitatory neurotransmission, occurring before the full brain pathology development, could contribute to bowel dysmotility and represent prodromal events in AD.
Int J Mol Sci, 2020; 21

32571879: Raimondi S, Mangione PP, Verona G, Canetti D, Nocerino P, Marchese L, Piccarducci R, Mondani V, Faravelli G, Taylor GW, Gillmore JD, Corazza A, Pepys MB, Giorgetti S, Bellotti V
Comparative study of the stabilities of synthetic and natural transthyretin amyloid fibrils.
Systemic amyloidosis caused by extracellular deposition of insoluble fibrils derived from the pathological aggregation of circulating proteins, such as transthyretin, is a severe and usually fatal condition. Elucidation of the molecular pathogenic

mechanism of the disease and discovery of effective therapies still represents a challenging medical issue. The preparation of amyloid fibrils that exhibit structural and biochemical properties closely similar to those of natural fibrils is central to improving our understanding of the biophysical basis of amyloid formation and may offer an important tool for drug discovery. Here, we compared the morphology and thermodynamic stability of natural transthyretin fibrils with those of fibrils generated either using the common acidification procedure or primed by limited selective cleavage by plasmin. The free energies for fibril formation were -12.36, -8.10, and -10.61 kcal mol, respectively. The fibrils generated via plasmin cleavage were more stable than those prepared at low pH and were thermodynamically and morphologically similar to natural fibrils extracted from human amyloidotic tissue. Determination of thermodynamic stability is an important tool that is complementary to other methods of structural comparison between fibrils and fibrils generated Our finding that fibrils created via an amyloidogenic pathway are structurally similar to human amyloid fibrils does not necessarily establish that the fibrillogenic pathway is the same for both, but it narrows the current knowledge gap between models and pathophysiology.
J Biol Chem, 2020; 295
33488947: Piccarducci R, Daniele S, Polini B, Carpi S, Chico L, Fusi J, Baldacci F, Siciliano G, Bonuccelli U, Nieri P, Martini C, Franzoni F
Apolipoprotein E Polymorphism and Oxidative Stress in Human Peripheral Blood Cells: Can Physical Activity Reactivate the Proteasome System through Epigenetic Mechanisms?
Alzheimer's disease (AD) is characterized by proteasome activity impairment, oxidative stress, and epigenetic changes, resulting in -amyloid (A) production/degradation imbalance. Apolipoprotein E (ApoE) is implicated in A clearance, and particularly, the ApoE 4 isoform predisposes to AD development. Regular physical activity is known to reduce AD progression. However, the impact of ApoE polymorphism and physical exercise on A production and proteasome system activity has never been investigated in human peripheral blood cells, particularly in erythrocytes, an emerging peripheral model used to study biochemical alteration. Therefore, the influence of ApoE polymorphism on the antioxidant defences, amyloid accumulation, and proteasome activity was here evaluated in human peripheral blood cells depending on physical activity, to assess putative peripheral biomarkers for AD and candidate targets that could be modulated by lifestyle. Healthy subjects were enrolled and classified based on the ApoE polymorphism (by the restriction fragment length polymorphism technique) and physical activity level (Borg scale) and grouped into ApoE 4/non-4 carriers and active/non-active subjects. The plasma antioxidant capability (AOC), the erythrocyte A production/accumulation, and the nuclear factor erythroid 2-related factor 2 (Nrf2) mediated proteasome functionality were evaluated in all groups by the chromatographic and immunoenzymatic assay, respectively. Moreover, epigenetic mechanisms were investigated considering the expression of histone deacetylase 6, employing a competitive ELISA, and the modulation of two key miRNAs (miR-153-3p and miR-195-5p), through the miRNeasy Serum/Plasma Mini Kit. ApoE 4 subjects showed a reduction in plasma AOC and an increase in the Nrf2 blocker, miR-153-3p, contributing to an enhancement of the erythrocyte concentration of A. Physical exercise increased plasma AOC and reduced the amount of A and its precursor, involving a reduced miR-153-3p expression and a miR-195-5p enhancement. Our data highlight the impact of the ApoE genotype on the amyloidogenic pathway and the proteasome system, suggesting the positive impact of physical exercise, also through epigenetic mechanisms.
Oxid Med Cell Longev, 2021; 2021
33579836: Daniele S, Baldacci F, Piccarducci R, Palermo G, Giampietri L, Manca ML, Pietrobono D, Frosini D, Nicoletti V, Tognoni G, Giorgi FS, Lo Gerfo A, Petrozzi L, Cavallini C, Franzoni F, Ceravolo R, Siciliano G, Trincavelli ML, Martini C, Bonuccelli U
α-Synuclein Heteromers in Red Blood Cells of Alzheimer's Disease and Lewy Body Dementia Patients.
Red blood cells (RBCs) contain the majority of α-synuclein (α-syn) in blood, representing an interesting model for studying the peripheral pathological alterations proved in neurodegeneration.
J Alzheimers Dis, 2021; 80
34658885: D'Antongiovanni V, Pellegrini C, Antonioli L, Benvenuti L, Di Salvo C, Flori L, Piccarducci R, Daniele S, Martelli A, Calderone V, Martini C, Fornai M
Palmitoylethanolamide Counteracts Enteric Inflammation and Bowel Motor Dysfunctions in a Mouse Model of Alzheimer's Disease.
Palmitoylethanolamide (PEA), an endogenous lipid mediator, is emerging as a promising pharmacological agent in multiple neurodegenerative disorders for its anti-inflammatory and neuroprotective properties. However, its effects on enteric inflammation and colonic dysmotility associated with Alzheimer's disease (AD) are lacking. This study was designed to investigate the beneficial effect of PEA administration in counteracting the enteric inflammation and relieving the bowel motor dysfunctions in an AD mouse model, SAMP8 mice. In addition, the ability of PEA in modulating the activation of enteric glial cells (EGCs), pivotally involved in the pathophysiology of bowel dysfunctions associated with inflammatory conditions, has also been examined. SAMP8 mice at 4 months of age were treated orally with PEA (5 mg/kg/day) for 2 months. SAMR1 animals were employed as controls. At the end of treatment, parameters dealing with colonic motility, inflammation, barrier

integrity and AD protein accumulation were evaluated. The effect of PEA on EGCs was tested in cultured cells treated with lipopolysaccharide (LPS) plus β-amyloid 1-42 (Aβ). SAMP8 treated with PEA displayed: 1) an improvement of colonic motor activity, citrate synthase activity and intestinal epithelial barrier integrity and 2) a decrease in colonic Aβ and α-synuclein (α-syn) accumulation, S100-β expression as well as enteric IL-1β and circulating LPS levels, as compared with untreated SAMP8 mice. In EGCs, treatment with PEA counteracted the increment of S100-β, TLR-4, NF-κB p65 and IL-1β release induced by LPS and Aβ. These results suggest that PEA, under a condition of cognitive decline, prevents the enteric glial hyperactivation, reduces AD protein accumulation and counteracts the onset and progression of colonic inflammatory condition, as well as relieves intestinal motor dysfunctions and improves the intestinal epithelial barrier integrity. Therefore, PEA represents a viable approach for the management of the enteric inflammation and motor contractile abnormalities associated with AD.
Front Pharmacol, 2021; 12

34687672: Giacomelli C, Piccarducci R, Marchetti L, Romei C, Martini C
Pulmonary fibrosis from molecular mechanisms to therapeutic interventions: lessons from post-COVID-19 patients.
Pulmonary fibrosis (PF) is characterised by several grades of chronic inflammation and collagen deposition in the interalveolar space and is a hallmark of interstitial lung diseases (ILDs). Recently, infectious agents have emerged as driving causes for PF development; however, the role of viral/bacterial infections in the initiation and propagation of PF is still debated. In this context, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the current coronavirus disease 2019 (COVID-19) pandemic, has been associated with acute respiratory distress syndrome (ARDS) and PF development. Although the infection by SARS-CoV-2 can be eradicated in most cases, the development of fibrotic lesions cannot be precluded; furthermore, whether these lesions are stable or progressive fibrotic events is still unknown. Herein, an overview of the main molecular mechanisms driving the fibrotic process together with the currently approved and newly proposed therapeutic solutions was given. Then, the most recent data that emerged from post-COVID-19 patients was discussed, in order to compare PF and COVID-19-dependent PF, highlighting shared and specific mechanisms. A better understanding of PF aetiology is certainly needed, also to develop effective therapeutic strategies and COVID-19 pathology is offering one more chance to do it. Overall, the work reported here could help to define new approaches for therapeutic intervention in the diversity of the ILD spectrum.
Biochem Pharmacol, 2021; 193

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BOARD NUMBER: S02-382

TARGETING OF H3K4 DEMETHYLASES AS A THERAPEUTIC STRATEGY TO TREAT ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Aim: Mutations in genes that control epigenetic gene-expression, especially the machinery that controls Histone 3 lysine 4 (H3K4me) methylation, are over-represented in intellectual disability disorders. Moreover, there is evidence that H3K4me3 levels decrease in neurodegenerative diseases such as Alzheimer's disease. Methods: In this study, we have analyzed H3K4me3 levels in Alzheimer's disease and tested the therapeutic potential of H3K4me demethylases (KDMs) in *vitro* and in *vivo.* Results: Our data suggest that decreasing the levels of H3K4 KMDs can improve neuronal synapse plasticity and reduce inflammatory responses. Furthermore, inhibition of KDMs in mouse models for age-associated memory decline or amyloid deposition ameliorated memory impairment. Conclusion: Our data suggest that the H3K4me demethylases are therapeutic targets to treat cognitive diseases.

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BOARD NUMBER: S02-383

THE CYCLASE-ASSOCIATED PROTEIN 2 CONTROLS COFILIN-ACTIN RODS FORMATION IN ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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[1]Università degli studi di Milano, Department Of Pharmocological And Biomolecular Sciences, Milano, Italy, [2]Università degli Studi di Milano, Unitech Nolimits, Mialno, Italy

Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by Amyloid β(Aβ)-driven synaptic dysfunction in the early phases of pathogenesis. In addition to spine loss, cytoskeletal abnormalities, such as cofilin-actin rods, have been reported in AD patients and animal models. We have recently demonstrated that the actin-binding protein Cyclase-associated protein 2 (CAP2) is a master regulator of cofilin localization and activity, through the Cys32-dependent CAP2 dimerization. These mechanisms are altered in AD, suggesting an involvement of CAP2/cofilin pathway in AD pathogenesis. *Aims:* This work aims to explore the involvement of CAP2 in Aβ-induced actin rods formation during the early phases of AD pathogenesis. *Methods:* Experiments were mainly carried out using imaging and biochemical approaches, in order to evaluate the effect of Aβ oligomers treatment on CAP2/cofilin pathway. In particular, three-dimensional imaging analysis was performed to identify CAP2 as a core component of Aβ-induced actin rods. *Results:* Firstly, we demonstrated that Aβ oligomers impair CAP2/cofilin pathway both after a short exposure and when cofilin-actin rods are generated, although the resulting effects are different. Then, we found that CAP2 accumulates in actin rods, when specifically induced by Aβ exposure, but not when neurons are exposed to a different stressor. Finally, we show that CAP2 overexpression can prevent rods formation and spine loss, through a mechanism that requires CAP2 capability to form Cys32-dependent dimers. *Conclusions:* Overall, our data support the involvement of cofilin/CAP2 cooperation in different biological aspects of AD pathogenesis in neuronal cells, thus providing novel potential therapeutic target for AD.

Pubmed:
34572474: D'Andrea L, Stringhi R, Di Luca M, Marcello E
Looking at Alzheimer's Disease Pathogenesis from the Nuclear Side.
Alzheimer's disease (AD) is a neurodegenerative disorder representing the most common form of dementia. It is biologically characterized by the deposition of extracellular amyloid-β (Aβ) senile plaques and intracellular neurofibrillary tangles, constituted by hyperphosphorylated tau protein. The key protein in AD pathogenesis is the amyloid precursor protein (APP), which is cleaved by secretases to produce several metabolites, including Aβ and APP intracellular domain (AICD). The greatest genetic risk factor associated with AD is represented by the () allele. Importantly, all of the above-mentioned molecules that are strictly related to AD pathogenesis have also been described as playing roles in the cell nucleus. Accordingly, evidence suggests that nuclear functions are compromised in AD. Furthermore, modulation of transcription maintains cellular homeostasis, and alterations in transcriptomic profiles have been found in neurodegenerative diseases. This report reviews recent advancements in the AD players-mediated gene expression. Aβ, tau, AICD, and localize in the nucleus and regulate the transcription of several genes, part of which is involved in AD pathogenesis, thus suggesting that targeting nuclear functions might provide new therapeutic tools for the disease.
Biomolecules, 2021; 11
32019166: Pelucchi S, Stringhi R, Marcello E
Dendritic Spines in Alzheimer's Disease: How the Actin Cytoskeleton Contributes to Synaptic Failure.
Alzheimer's disease (AD) is a neurodegenerative disorder characterized by Aβ-driven synaptic dysfunction in the early phases of pathogenesis. In the synaptic context, the actin cytoskeleton is a crucial element to maintain the dendritic spine architecture and to orchestrate the spine's morphology remodeling driven by synaptic activity. Indeed, spine shape and synaptic strength are strictly correlated and precisely governed during plasticity phenomena in order to convert short-term alterations of synaptic strength into long-lasting changes that are embedded in stable structural modification. These functional and structural modifications are considered the biological basis of learning and memory processes. In this review we discussed the existing evidence regarding the role of the spine actin cytoskeleton in AD synaptic failure. We revised the physiological function of the actin cytoskeleton in the spine shaping and the contribution of actin dynamics in the endocytosis

mechanism. The internalization process is implicated in different aspects of AD since it controls both glutamate receptor membrane levels and amyloid generation. The detailed understanding of the mechanisms controlling the actin cytoskeleton in a unique biological context as the dendritic spine could pave the way to the development of innovative synapse-tailored therapeutic interventions and to the identification of novel biomarkers to monitor synaptic loss in AD.
Int J Mol Sci, 2020; 21

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BOARD NUMBER: S02-384

HIGH-CONTENT SCREENING OF ALZHEIMER'S DISEASE GENETIC RISK FACTORS BASED ON SYNAPTIC DENSITY ANALYSIS

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Objectives: Synaptic loss is one of the earliest pathological hallmarks and the strongest marker of cognitive decline in Alzheimer's disease (AD). A strong genetic predisposition is linked to AD, and genome-wide association studies have pointed out hundreds of genes associated with the risk of developing the disease. With this background, our objective is to develop a cell-based screen to assess the impact of each genetic risk factor on synaptic density. **Methods**: We screened a lentiviral shRNA library targeting 200 AD genetic risk factors using primary rat hippocampal neurons in 384-wells plates. Immunofluorescence was performed to reveal pre- and post-synaptic compartments and the neuronal network. Synaptic density was then assessed through high content analysis by assigning each post-synaptic structure to the nearest pre-synaptic structure using *Columbus* and *Matlab* software. **Results**: We identified 9 shRNAs targeting AD risk genes which strongly impacted synaptic density in our model. These results could help us to point out a role for some unexpected AD genetic risk factors in synaptic function. **Conclusion**: We have developed a high-content screening approach which allows us to define the genetic risk factors that are susceptible to be involved in synaptic dysregulation observed in the early stages of AD.

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BOARD NUMBER: S02-385

THE IMPORTANCE OF S-DEPALMITOYLATION IN NEURODEGENERATION IS EMPHASIZED BY THE IDENTIFICATION OF CLN5 AS A NEW TYPE OF CYSTEINE-BASED S-DEPALMITOYLASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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The Neuronal Ceroid Lipofuscinoses (NCL) are the most common neurodegenerative disorders of childhood and presently 13 different genetic NCL variants are known. The CLN5 variant is caused by mutations in the *CLN5* gene and is associated with variable types of dementias ranging from late infantile epileptic encephalopathy to familial Alzheimer's disease. Cln5 shows no significant homology to other proteins by primary amino acid sequence, and the molecular functions of Cln5 have not been previously demonstrated. We crystallized the glycosylated human Cln5 protein, determined its protein structure and revealed unexpected thioesterase activity of Cln5. We demonstrated complete loss of enzymatic activity for mutations affecting the catalytic H166 and C280 as well as for the two patient mutations Y258D and D279N and discovered that Cln5-deficient neuronal progenitor cells showed reduced thioesterase activity. These findings represent an important step toward understanding the neurodegenerative disease mechanisms associated with *CLN5* mutations and opens new avenues for developing causative therapies against this fatal disorder.

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BOARD NUMBER: S02-386

DYSREGULATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR RECEPTOR IN ALZHEIMER'S DISEASE IS MIRRORED IN EXTRACELLULAR VESICLES

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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[1]Instituto de Medicina Molecular João Lobo Antunes, Faculdade De Medicina Da Universidade De Lisboa, Lisboa, Portugal, [2]Instituto de Farmacologia e Neurociências, Faculdade De Medicina Da Universidade De Lisboa, Lisboa, Portugal, [3]Research Institute for Medicines (iMed.ULisboa), Faculdade De Farmácia Da Universidade De Lisboa, Lisboa, Portugal, [4]Neuroscience Center, University Of Helsinki, Helsinki, Finland

Introduction: In Alzheimer's disease (AD), the neuroprotective BDNF/TrkB-FL system is impaired due to an amyloid-beta-mediated TrkB-FL receptor cleavage and subsequent formation of an intracellular fragment (TrkB-ICD). TrkB-ICD retains its kinase activity, promotes cognitive impairments, and modifies gene expression, contributing to intracellular toxicity. Interestingly, AD pathological features may be disseminated by small (sEVs) or large (lEVs) extracellular vesicles, but also by soluble factors on the secretome, causing cell-to-cell toxicity. Importantly, previous studies have already detected TrkB-ICD in cerebrospinal fluid of humans, raising the possibility of its secretion. **Aims:** Accordingly, this work aims to assess TrkB-ICD presence in EVs and secretome. **Methods**: EVs were isolated from media of control (non-transduced, CTR), GFP- and TrkB-ICD-V5-transduced (ICD-V5) differentiated SH-SY5Y cells. After purification, lEVs and sEVs were characterized regarding their morphology, size, and protein cargo using transmission electron microscopy (TEM), dynamic light scattering (DLS) and western-blot (WB) methods, respectively. **Results**: TEM and DLS analysis showed that isolated EVs exhibited a cup-shaped morphology and expected size profile for both lEVs and sEVs. WB experiments confirmed the presence of typical EV protein markers. Interestingly, we were able not only to confirm the existence of ICD-V5 in both EV types, but also the presence of the endogenous TrkB-ICD fragment. Additionally, this fragment was also detected in the EV-depleted cell secretome. **Conclusions**: Altogether, both EV populations were correctly isolated and the detection of TrkB-ICD in both lEVs and sEVs, and in the secretome soluble fraction, strongly suggest the paracrine dissemination of this fragment from cell-to-cell, propagating its putative toxicity.

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ADNP-SIRT1 NEW COMPLEX REGULATES HISTONE METHYLATION: DRAMATICALLY DYSREGULATED IN ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Activity-dependent neuroprotective protein (ADNP) is essential for brain formation and function. As such, de novo mutations in ADNP lead to the autistic ADNP syndrome and somatic ADNP mutations may drive Alzheimer's disease (AD) tauopathy. Sirtuin 1 (SIRT1) is positively associated with aging, the major risk for AD. Here, we revealed DNA/chromatin interaction site for ADNP and SIRT1, with yin yang 1, histone deacetylase 2, and ADNP, sharing a DNA binding motif and regulating SIRT1, ADNP, and EB1 (MAPRE1). This interaction was linked to sex- and age-dependent altered histone modification, associated with ADNP/SIRT1/WD repeat-containing protein 5, which mediates the assembly of histone modification complexes. Single-cell RNA and protein expression analyses as well as gene expression correlations placed SIRT1–ADNP and either MAPRE1 (EB1), MAPRE3 (EB3), or both in the same mouse and human cell; however, while MAPRE1 seemed to be similarly regulated to ADNP and SIRT1, MAPRE3 seemed to deviate. Finally, we demonstrated an extremely tight correlation for the gene transcripts described above, including related gene products. This correlation was specifically abolished in affected postmortem AD and Parkinson's disease brain select areas compared to matched controls, while being maintained in blood samples. Thus, we identified an ADNP–SIRT1 complex that may serve as a new target for the understanding of brain degeneration. Citation: Hadar, A*., Kapitansky, O*., Ganaiem, M., Sragovich, S., Lobyntseva, A., Giladi, E., ... & Gozes, I. Introducing ADNP and SIRT1 as new partners regulating microtubules and histone methylation. *Equal contribution. Molecular psychiatry, 2021 Nov;26(11):6550-6561.

Pubmed:
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Introducing ADNP and SIRT1 as new partners regulating microtubules and histone methylation.
Activity-dependent neuroprotective protein (ADNP) is essential for brain formation and function. As such, de novo mutations in ADNP lead to the autistic ADNP syndrome and somatic ADNP mutations may drive Alzheimer's disease (AD) tauopathy. Sirtuin 1 (SIRT1) is positively associated with aging, the major risk for AD. Here, we revealed two key interaction sites for ADNP and SIRT1. One, at the microtubule end-binding protein (EB1 and EB3) Tau level, with EB1/EB3 serving as amplifiers for microtubule dynamics, synapse formation, axonal transport, and protection against tauopathy. Two, on the DNA/chromatin site, with yin yang 1, histone deacetylase 2, and ADNP, sharing a DNA binding motif and regulating SIRT1, ADNP, and EB1 (MAPRE1). This interaction was linked to sex- and age-dependent altered histone modification, associated with ADNP/SIRT1/WD repeat-containing protein 5, which mediates the assembly of histone modification complexes. Single-cell RNA and protein expression analyses as well as gene expression correlations placed SIRT1-ADNP and either MAPRE1 (EB1), MAPRE3 (EB3), or both in the same mouse and human cell; however, while MAPRE1 seemed to be similarly regulated to ADNP and SIRT1, MAPRE3 seemed to deviate. Finally, we demonstrated an extremely tight correlation for the gene transcripts described above, including related gene products. This correlation was specifically abolished in affected postmortem AD and Parkinson's disease brain select areas compared to matched controls, while being maintained in blood samples. Thus, we identified an ADNP-SIRT1 complex that may serve as a new target for the understanding of brain degeneration.
Mol Psychiatry, 2021; 26

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Single Cell ADNP Predictive of Human Muscle Disorders: Mouse Knockdown Results in Muscle Wasting.
Activity-dependent neuroprotective protein (ADNP) mutations are linked with cognitive dysfunctions characterizing the autistic-like ADNP syndrome patients, who also suffer from delayed motor maturation. We thus hypothesized that ADNP is deregulated in versatile myopathies and that local ADNP muscle deficiency results in myopathy, treatable by the ADNP fragment NAP. Here, single-cell transcriptomics identified as a major constituent of the developing human muscle. transcript concentrations further predicted multiple human muscle diseases, with concentrations negatively correlated with the ADNP target interacting protein, microtubule end protein 1 (EB1). Reverting back to modeling at the single-cell level of the male mouse transcriptome, mRNA concentrations age-dependently correlated with motor disease as well as with sexual maturation gene transcripts, while expressing limb muscle cells significantly decreased with aging. Mouse heterozygous deficiency exhibited muscle microtubule reduction and myosin light chain () deregulation coupled with motor dysfunction. CRISPR knockdown of adult gastrocnemius muscle Adnp in a Cas9 mouse resulted in treadmill (male) and gait (female) dysfunctions that were specifically ameliorated by treatment with the ADNP snippet, microtubule interacting, -regulating, NAP (CP201). Taken together, our studies provide new hope for personalized diagnosis/therapeutics in versatile myopathies.
Cells, 2020; 9
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Age and Sex-Dependent ADNP Regulation of Muscle Gene Expression Is Correlated with Motor Behavior: Possible Feedback Mechanism with PACAP.
The activity-dependent neuroprotective protein (ADNP), a double-edged sword, sex-dependently regulates multiple genes and was previously associated with the control of early muscle development and aging. Here we aimed to decipher the involvement of ADNP in versatile muscle gene expression patterns in correlation with motor function throughout life. Using quantitative RT-PCR we showed that heterozygous deficiency in mice resulted in aberrant gastrocnemius (GC) muscle, tongue and bladder gene expression, which was corrected by the Adnp snippet, drug candidate, NAP (CP201). A significant sexual dichotomy was discovered, coupled to muscle and age-specific gene regulation. As such, Adnp was shown to regulate myosin light chain () in the gastrocnemius (GC) muscle, the language acquisition gene forkhead box protein P2 () in the tongue and the pituitary-adenylate cyclase activating polypeptide (PACAP) receptor PAC1 mRNA () in the bladder, with PACAP linked to bladder function. A tight age regulation was observed, coupled to an extensive correlation to muscle function (gait analysis), placing ADNP as a muscle-regulating gene/protein.
Int J Mol Sci, 2020; 21
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Discovery of autism/intellectual disability somatic mutations in Alzheimer's brains: mutated ADNP cytoskeletal impairments and repair as a case study.
With Alzheimer's disease (AD) exhibiting reduced ability of neural stem cell renewal, we hypothesized that de novo mutations controlling embryonic development, in the form of brain somatic mutations instigate the disease. A leading gene presenting heterozygous dominant de novo autism-intellectual disabilities (ID) causing mutations is activity-dependent neuroprotective protein (ADNP), with intact ADNP protecting against AD-tauopathy. We discovered a genomic autism ADNP mutation (c.2188C>T) in postmortem AD olfactory bulbs and hippocampi. RNA-Seq of olfactory bulbs also identified a novel ADNP hotspot mutation, c.2187_2188insA. Altogether, 665 mutations in 596 genes with 441 mutations in AD patients (389 genes, 38% AD-exclusive mutations) and 104 genes presenting disease-causing mutations (OMIM) were discovered. OMIM AD mutated genes converged on cytoskeletal mechanisms, autism and ID causing mutations (about 40% each). The number and average frequencies of AD-related mutations per subject were higher in AD subjects compared to controls. RNA-seq datamining (hippocampus, dorsolateral prefrontal cortex, fusiform gyrus and superior frontal gyrus-583 subjects) yielded similar results. Overlapping all tested brain areas identified unique and shared mutations, with ADNP singled out as a gene associated with autism/ID/AD and presenting several unique aging/AD mutations. The large fusiform gyrus library (117 subjects) with high sequencing coverage correlated the c.2187_2188insA ADNP mutation frequency to Braak stage (tauopathy) and showed more ADNP mutations in AD specimens. In cell cultures, the ADNP-derived snippet NAP inhibited mutated-ADNP-microtubule (MT) toxicity and enhanced Tau-MT association. We propose a paradigm-shifting concept in the perception of AD whereby accumulating mosaic somatic mutations promote brain pathology.
Mol Psychiatry, 2021; 26
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Peripheral transcriptomic biomarkers for early detection of sporadic Alzheimer disease?
Alzheimer disease (AD) is the major epidemic of the 21 century, its prevalence rising along with improved human longevity. Early AD diagnosis is key to successful treatment, as currently available therapeutics only allow small benefits for diagnosed

AD patients. By contrast, future therapeutics, including those already in preclinical or clinical trials, are expected to afford neuroprotection prior to widespread brain damage and dementia. Brain imaging technologies are developing as promising tools for early AD diagnostics, yet their high cost limits their utility for screening at-risk populations. Blood or plasma transcriptomics, proteomics, and/or metabolomics may pave the way for cost-effective AD risk screening in middle-aged individuals years ahead of cognitive decline. This notion is exemplified by data mining of blood transcriptomics from a published dataset. Consortia blood sample collection and analysis from large cohorts with mild cognitive impairment followed longitudinally for their cognitive state would allow the development of a reliable and inexpensive early AD screening tool.
Dialogues Clin Neurosci, 2018; 20
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SIRT1, miR-132 and miR-212 link human longevity to Alzheimer's Disease.
Alzheimer's Disease (AD) is the most common cause of dementia in the elderly. Centenarians - reaching the age of >100 years while maintaining good cognitive skills - seemingly have unique biological features allowing healthy aging and protection from dementia. Here, we studied the expression of SIRT1 along with miR-132 and miR-212, two microRNAs known to regulate SIRT1, in lymphoblastoid cell lines (LCLs) from 45 healthy donors aged 21 to 105 years and 24 AD patients, and in postmortem olfactory bulb and hippocampus tissues from 14 AD patients and 20 age-matched non-demented individuals. We observed 4.0-fold ($P = 0.001$) lower expression of SIRT1, and correspondingly higher expression of miR-132 (1.7-fold; $P = 0.014$) and miR-212 (2.1-fold; $P = 0.036$), in LCLs from AD patients compared with age-matched healthy controls. Additionally, SIRT1 expression was 2.2-fold ($P = 0.001$) higher in centenarian LCLs compared with LCLs from individuals aged 56-82 years; while centenarian LCLs miR-132 and miR-212 indicated 7.6-fold and 4.1-fold lower expression, respectively. Correlations of SIRT1, miR-132 and miR-212 expression with cognitive scores were observed for AD patient-derived LCLs and postmortem AD olfactory bulb and hippocampus tissues, suggesting that higher SIRT1 expression, possibly mediated by lower miR-132 and miR-212, may protect aged individuals from dementia and is reflected in their peripheral tissues.
Sci Rep, 2018; 8
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RNA sequencing of bipolar disorder lymphoblastoid cell lines implicates the neurotrophic factor HRP-3 in lithium's clinical efficacy.
Lithium remains the oldest and most effective treatment for mood stabilisation in bipolar disorder (BD), even though at least half of patients are only partially responsive or do not respond. This study aimed to identify biomarkers associated with lithium response in BD, based on comparing RNA sequencing information derived from lymphoblastoid cell lines (LCLs) of lithium-responsive (LR) versus lithium non-responsive (LNR) BD patients, to assess gene expression variations that might bear on treatment outcome. RNA sequencing was carried out on 24 LCLs from female BD patients (12 LR and 12 LNR) followed by qPCR validation in two additional independent cohorts (41 and 17 BD patients, respectively). Fifty-six genes showed nominal differential expression comparing LR and LNR ($FC \geq |1.3|$, ≤ 0.01). The differential expression of and was validated by qPCR in the independent cohorts. We observed higher expression levels of and in BD patients who favourably respond to lithium. Both of these genes are involved in neurogenesis, and has been suggested to be a neurotrophic factor. Additional studies in larger BD cohorts are needed to confirm the potential of and expression levels in blood cells as tentative favourable lithium response biomarkers.
World J Biol Psychiatry, 2019; 20
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RGS2 expression predicts amyloid-β sensitivity, MCI and Alzheimer's disease: genome-wide transcriptomic profiling and bioinformatics data mining.
Alzheimer's disease (AD) is the most frequent cause of dementia. Misfolded protein pathological hallmarks of AD are brain deposits of amyloid-β (Aβ) plaques and phosphorylated tau neurofibrillary tangles. However, doubts about the role of Aβ in AD pathology have been raised as Aβ is a common component of extracellular brain deposits found, also by in vivo imaging, in non-demented aged individuals. It has been suggested that some individuals are more prone to Aβ neurotoxicity and hence more likely to develop AD when aging brains start accumulating Aβ plaques. Here, we applied genome-wide transcriptomic profiling of lymphoblastoid cells lines (LCLs) from healthy individuals and AD patients for identifying genes that predict sensitivity to Aβ. Real-time PCR validation identified 3.78-fold lower expression of RGS2 (regulator of G-protein signaling 2; $P=0.0085$) in LCLs from healthy individuals exhibiting high vs low Aβ sensitivity. Furthermore, RGS2 showed 3.3-fold lower expression ($P=0.0008$) in AD LCLs compared with controls. Notably, RGS2 expression in AD LCLs correlated with the patients' cognitive function. Lower RGS2 expression levels were also discovered in published expression data sets from

postmortem AD brain tissues as well as in mild cognitive impairment and AD blood samples compared with controls. In conclusion, Aβ sensitivity phenotyping followed by transcriptomic profiling and published patient data mining identified reduced peripheral and brain expression levels of RGS2, a key regulator of G-protein-coupled receptor signaling and neuronal plasticity. RGS2 is suggested as a novel AD biomarker (alongside other genes) toward early AD detection and future disease modifying therapeutics.
Transl Psychiatry, 2016; 6

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Insulin-like Growth Factor 1 Differentially Affects Lithium Sensitivity of Lymphoblastoid Cell Lines from Lithium Responder and Non-responder Bipolar Disorder Patients.
Bipolar disorder (BD) is a chronic psychiatric illness with an unknown etiology. Lithium is considered the cornerstone in the management of BD, though about 50-60 % of patients do not respond sufficiently to chronic treatment. Insulin-like growth factor 1 (IGF1) has been identified as a candidate gene for BD susceptibility, and its low expression has been suggested as a putative biomarker for lithium unresponsiveness. In this study, we examined the in vitro effects of insulin-like growth factor 1 (IGF-1) on lithium sensitivity in lymphoblastoid cell lines (LCLs) from lithium responder (R) and non-responder (NR) bipolar patients. Moreover, we evaluated levels of microRNA let-7c, a small RNA predicted to target IGF1. We found that exogenous IGF-1 added to serum-free media increased lithium sensitivity selectively in LCLs from NR BD patients. However, no significant differences were observed when comparing let-7c expression in LCLs from R vs. NR BD patients. Our data support a key role for IGF-1 in lithium resistance/response in the treatment of bipolar disorder.
J Mol Neurosci, 2015; 56

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BOARD NUMBER: S02-388

REGULATION OF THE DNA DAMAGE RESPONSE BY E2F4 PHOSPHORYLATION IN ITS T249/T251 CONSERVED MOTIF AND ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Alzheimer's disease (AD) has a multifactorial etiology that includes DNA damage in neurons. The transcription factor E2F4, which can potentially regulate DNA repair, has two conserved threonines (T249/T251 in the mouse) that can be phosphorylated by $p38^{MAPK}$ (p38). The expression *in vivo* of the T249A/T251A E2F4 mutant form (E2F4DN) has been shown to be a multifactorial therapeutic agent against AD. In this work, we have analyzed the effects of E2F4 phosphorylation in T249/T251 on the DNA repair response (DDR). To this aim, we used N2a mouse neuroblastoma cells treated with 10 µM camptothecin (CPT), a treatment known to induce *Cited2* expression and subsequent cell death. In this paradigm, the repression of *Cited2* by E2F4 is abolished upon CPT treatment, thus allowing its E2F1-dependent expression followed by cell death. While E2F4 can be detected in both the nucleus and cytoplasm of N2a cells, phosphoT249-E2F4-specific immunoreactivity is specifically observed in the nucleus 4 h after treatment with CPT. Therefore, the known activation of p38 in response to CPT could lead to T249 phosphorylation of E2F4, thus suppressing its inhibition on E2F1 activity and allowing *Cited2* expression. This hypothesis, is being tested in CPT-treated N2a cells co-transduced with adenoviral vectors expressing E2F1 together with either wild-type E2F4 or E2F4DN, in either the presence or absence of p38 inhibitors. In summary, our work provides support for a novel mechanism used by E2F4 to regulate the response to DNA damage in pathological situations, which could participate in the therapeutic capacity of E2F4DN against AD.

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BOARD NUMBER: S02-389

CHARACTERIZATION OF HIPSC-DERIVED ENDOTHELIAL CELLS ROLE IN THE FORMATION OF CEREBRAL AMYLOID ANGIOPATHY RELATED TO ALZHEIMER'S DISEASE.

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Sporadic Alzheimer's disease (sAD), familial AD with duplication of the Amyloid Precursor Protein gene (Dup-APP), and Down Syndrome (DS) with three copies of *APP*, share common hallmarks including amyloid and tau pathologies. However, the amount of Aβ peptides deposits in the walls of cerebral blood vessels leading to cerebral amyloid angiopathy (CAA) vary. Our aim is to characterize and understand the role of endothelial cells (ECs) forming the blood brain barrier (BBB) in CAA severity. For this purpose, we used human iPSC-derived ECs (hiPSC-EC) from patients with Dup-APP and from mosaic DS. We found that hiPSC-EC with Dup-APP and DS secreted higher levels of Aβ compared to their isogenic controls using MesoScaleDiscovery Aβ triplex assay. In addition, staining of adherent and tight junction proteins using immunofluorescence, showed changes in expression and distribution of these proteins at the cell surface of ECs. To better understand these changes, we analyzed hiPSC-ECs transcripts using RNAseq and revealed multiple dysregulated genes linked to cell adhesion and tight junction proteins between Dup-APP, DS and their isogenic controls. A monolayer of iPSC-EC was cultivated on Transwell microporous membranes to study the alteration of permeability in Dup-APP and DS. Our results suggest that APP duplication may intrinsically alter ECs properties, possibly leading to differential CAA and BBB alterations in AD patients carrying Dup-APP and in individuals with DS.

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POSSIBLE NEUROENDOCRINE MODULATIONS IN HIPPOCAMPI OF 3XTG-AD MICE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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AIM: Alzheimer's disease (AD) is the most common neurodegenerative disease all over the world. However, cellular mechanism of AD is not fully understood yet. The aim of this study was to detect possible pathophysiological factors in AD on examining differentially expressed genes in 3xTg-AD mice model by using *in silico* tools. **METHODS:** GSE144459 dataset downloaded from Gene Expression Omnibus (GEO) database was re-examined for this research. In the dataset, 3xTg-AD male mice (n=8 at 12 weeks-old-age (woa); n=8 at 52 woa) and B6129SF2 male mice (n=8 at 12 woa.; n=8 at 52 woa) were examined and gene expression profiles of their hippocampi were re-analyzed in R program, based on Benjamini-Hochberg correction, adjusted *p*-values <0.05 were accepted as significant. Gene set enrichment analyses were performed in Gene Ontology and ENRICHR tools. **RESULTS:** Gene expression levels indicated that prolactin receptor (PRLR), periostin (POSTN), gonadotropin-releasing hormone (GNRH1), galectin (LGALS-2,3,9), diazepam binding inhibitor (DBIL5), resistin (RETN), period circadian clock (PER1-2), insulin receptor (INSR) were upregulated ($p<0.05$); and arginine vasopressin receptor (AVPR1A), gastrin (GAST), glucagon (GCG), leptin (LEP), neuropeptid Y receptor (NPY-1R,4R), galanin (GAL), parathyroid hormone (PTH2), orexin receptor (HCRTR1), nerve growth factor (NGF), urotensin-2 receptor (UTS2R) were downregulated ($p<0.05$) in AD group, compared with control group. **CONCLUSION:** Results indicate imbalances in the expression levels (up- and down-regulation) of genes known to be involved in many neuroendocrine signaling in hippocampal cells during the developmental process of AD. **Keywords:** Alzheimer's disease, neuroendocrine signaling, bioinformatics. ***Acknowledgements:*** Thanks to my dear supervisor, Prof.Dr. Ahmet AYAR, for his scientific and spiritual support.

Pubmed:
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Investigation of Effects of Gadolinium-Based Contrast Agents on Uterine Contractility Using Isolated Rat Myometrium.
Despite concerns about safety, gadolinium-based contrast agents (GBCAs) are still used for abdominal and pelvic imaging during pregnancy. Researchers have mainly focused on teratogenicity, while very little is known about their possible direct effects on uterine contractility, yet free gadolinium potentially impacts contractility through interaction with calcium channels.
J Magn Reson Imaging, 2022; 55
35168243: Kurt A, Salihoglu AK, Ayar A
An In Vitro Study on the Contractility of Epileptic Myometrium and Effects of Antiepileptic Agents on Oxytocin-Induced Contractions of Myometrium Isolated from Absence Epileptic WAG/Rij Rats.
To determine whether spontaneous and stimulated contractile activity of myometrium in epileptic rats is different from healthy ones and whether anti-epileptic drugs (AEDs) have any direct influence on myometrial contractility.
Neuroendocrinology, 2022;

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THE CDK5-CDH1-ROCK2 SIGNALLING AXIS MEDIATES AMYLOID-ß NEUROTOXICITY

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Aims Alzheimer's disease (AD) is the main cause of dementia and one of the most lethal and burdening diseases. The main mechanisms responsible for the cognitive decline developed by the patients are synaptic and neuronal loss, triggered by Amyloid-beta (Aβ). Cdh1, the main APC/C cofactor in neurons, is essential for neuronal survival both by preserving neuronal homeostasis as well as by avoiding apoptosis. Neuronal survival is highly dependent on the maintenance of dendritic stability. One key protein involved in dendritic integrity regulation is ROCK2. Previous studies of our group demonstrate that APC/C-Cdh1 complex maintains dendritic integrity in cortex and hippocampus by regulating ROCK2 levels, preserving cognitive function. We have also demonstrated that Cdk5 hyperactivation directly phosphorylates and inactivates Cdh1. Our working hypothesis is that CDK5-APC/C-Cdh1-ROCK2 pathway regulates neuronal apoptosis against Aβ. Methods To assess this issue, we used an $A\beta_{25-35}$toxicity model in primary neurons, and in intracerebroventricularly-injected mice. Results We demonstrate that Aβ induces Cdk5 activation, which in turn, promotes Cdh1 phosphorylation and its disassembly from APC. This Cdh1 inactivation triggers Rock2 stabilization and activation, mediating amyloid-β neurotoxicity. Moreover, using a ROCK2 selective inhibitor, we were able to reduce the Aβ-induced memory impairment in vivo. Conclusions These results demonstrate that Cdk5-APC/C-Cdh1-ROCK2 axis actively modulates the neuronal response to Aβ damage, making them potential molecular targets for AD therapy. Funded by Instituto de Salud Carlos III (PI21/00727; RD21/0006/0005); FEDER; Junta de Castilla y León (CSI151P20; co-financed with FEDER funds) and Bodegas R. López de Heredia Viña Tondonia.

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PSEN1 EXPRESSION IS REPRESSED IN ADULT TGCRND8 MICE VIA PERINATAL S-ADENOSYLMETHIONINE SUPPLEMENTATION AFFECTING ON CPG AND NON-CPG METHYLATION.

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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AIMS: The metabolic Homocysteine (Hcy) pathway, also called the “one-carbon metabolism”, leads to the production of S-adenosylmethionine (SAM), the first endogenous methyl donor in methylation reactions, and is modulated by B vitamins. The pathophysiology of neurodegenerative diseases is considerably affected by DNA methylation, the main epigenetic gene expression regulator. Specifically, previous evidence indicates that promoter hypomethylation of PSEN1, a gene involved in the amyloidogenic pathway in AD, increases the Alzheimer’s Disease-like phenotype in transgenic TgCRND8 mice. On the contrary, supplementation with S-adenosylmethionine, reverts the pathological phenotype. A large body of evidence indicates that epigenetic signatures driving the drift between the normal-diseased aging can be acquired during the first stage of life, even in utero, and becoming manifest later on. METHODS: In this study we compared the effect of post-weaning and perinatal SAM treatment in TgCRND8 mice, assessing PSEN1 methylation and expression. DNA from mice brain samples is modified by the sodium bisulfite reaction and amplified by PCR. PCR products are ligated in plasmids that are sequenced by Sanger method. The PSEN1 gene expression is assessed by Real-time PCR. Histological techniques have allowed the study of amyloid plaque spreading. RESULTS: The results show that short term perinatal supplementation is more effective than post-weaning chronic supplementation in repressing PSEN1 expression in adult mice, with comparable effect in reducing amyloid deposition. CONCLUSIONS: These results point out the importance of methyl-donors availability during the early life in order to promote healthy aging.

Pubmed:

32267165: Tarashi S, Badi SA, Moshiri A, Ebrahimzadeh N, Fateh A, Vaziri F, Aazami H, Siadat SD, Fuso A
The inter-talk between and the epigenetic mechanisms.
Epigenetics regulate gene function without any alteration in the DNA sequence. The epigenetics represent one of the most important regulators in different cellular processes and have initially been developed in microorganisms as a protective strategy. The evaluation of the epigenetic mechanisms is also important in achieving an efficient control strategy in tuberculosis (TB). TB is one of the most significant epidemiological concerns in human history. Despite several and studies that have evaluated different epigenetic modifications in TB, many aspects of the association between epigenetics and TB are not fully understood. The current paper is aimed at reviewing our knowledge on histone modifications and DNA methylation modifications, as well as miRNAs regulation in TB.
Epigenomics, 2020; 12
32061806: Fuso A, Raia T, Orticello M, Lucarelli M
The complex interplay between DNA methylation and miRNAs in gene expression regulation.
The short, non-coding RNAs, also called microRNAs (miRNAs) can bind complementary sequences on cellular mRNAs. The consequence of this binding is generally the degradation of mRNA and the inhibition of its translation. For this reason, miRNAs are included among the epigenetic factors acting as a modulator of gene expression. How miRNAs expression is, in turn, regulated is still the object of active investigation, but DNA methylation, another epigenetic modification, seems to play a central role in this sense. The "one-carbon" metabolism is responsible for the metabolic regulation of trans-methylation reactions and, therefore, DNA methylation. For this reason, to investigate the possible correlations between alterations of the one-carbon metabolism and differential DNA methylation sounds interesting. Moreover, recent evidence indicates that, vice-versa, miRNAs are associated with DNA methylation modulation, in a mutual cross-talk. The present review will discuss the interplay between miRNAs and DNA methylation and its fall-out on gene expression regulation.
Biochimie, 2020; 173
32019393: Monti N, Cavallaro RA, Stoccoro A, Nicolia V, Scarpa S, Kovacs GG, Fiorenza MT, Lucarelli M, Aronica E, Ferrer I, Coppedè F, Troen AM, Fuso A
CpG and non-CpG Presenilin1 methylation pattern in course of neurodevelopment and neurodegeneration is associated with

gene expression in human and murine brain.
The Presenilin1 () gene encodes the catalytic peptide of the γ-secretase complex, a key enzyme that cleaves the amyloid-β protein precursor (AβPP), to generate the amyloid-β (Aβ) peptides, involved in Alzheimer's Disease (AD). Other substrates of the γ-secretase, such as E-cadherin and Notch1, are involved in neurodevelopment and haematopoiesis. Gene-specific DNA methylation influences expression in AD animal models. Here we evaluated canonical and non-canonical cytosine methylation patterns of the 5'-flanking during brain development and AD progression, in DNA extracted from the frontal cortex of AD transgenic mice (TgCRND8) and post-mortem human brain. Mapping CpG and non-CpG methylation revealed different methylation profiles in mice and humans. expression only correlated with DNA methylation in adult female mice. However, in post-mortem human brain, lower methylation, both at CpG and non-CpG sites, correlated closely with higher expression during brain development and in disease progression. methylation in blood DNA was significantly lower in AD patients than in controls. The present study is the first to demonstrate a temporal correlation between dynamic changes in CpG and non-CpG methylation patterns and mRNA expression during neurodevelopment and AD neurodegeneration. These observations were made possible by the use of an improved bisulphite methylation assay employing primers that are not biased towards non-CpG methylation. Our findings deepen the understanding of γ-secretase regulation and support the hypothesis that epigenetic changes can promote the pathophysiology of AD. Moreover, they suggest that DNA methylation in peripheral blood may provide a biomarker for AD.
Epigenetics, 2020; 15
31842376: Lucarelli M, Ferraguti G, Fuso A
Active Demethylation of Non-CpG Moieties in Animals: A Neglected Research Area.
The functional role of cytosine methylation in the CpG moieties of DNA, is well established in several biological functions. The interplay between CpG methylation and hypomethylation is a well-known mechanism of modulation of gene expression. However, the role of non-CpG methylation and active dynamics of demethylation is not clearly recognized. Although some evidence exists of a role of active non-CpG demethylation in the fast dynamics of transcriptional activation in animals, few studies deal with this topic. At present, active demethylation of non-CpG moieties is a neglected research area, in spite of the promise of significant novelties.
Int J Mol Sci, 2019; 20
31202182: Delfino D, Rossetti DV, Martelli C, Inserra I, Vincenzoni F, Castagnola M, Urbani A, Scarpa S, Fuso A, Cavallaro RA, Desiderio C
Exploring the brain tissue proteome of TgCRND8 Alzheimer's Disease model mice under B vitamin deficient diet induced hyperhomocysteinemia by LC-MS top-down platform.
The multifactorial nature of Late Onset Alzheimer's Disease (LOAD), the AD form of major relevance on epidemiological and social aspects, has driven the original investigation by LC-MS and top-down proteomics approach of the protein repertoire of the brain tissue of TgCRND8 model mice fed with a diet deficient in B vitamins. The analysis of the acid-soluble fraction of brain tissue homogenates identified a list of proteins and peptides, proteoforms and PTMs. In order to disclose possible modulations, their relative quantification in wild type and AD model mice under both B vitamin deficient and control diets was performed. The levels of metallothionein III, guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2 and brain acid soluble protein 1 showed statistically significant alterations depending on genotype, diet or both effects, respectively. Particularly, metallothionein III exhibited increased levels in TgCRND8 mice under B vitamin deficient diet with respect to wild type mice under both diets. Brain acid soluble protein 1 showed the opposite, revealing decreased levels in all diet groups of AD model mice with respect to wild type mice in control diet. Lower levels of brain acid soluble protein 1 were also observed in wild type mice under deficiency of B vitamins. These results, besides contributing to increase the knowledge of AD at molecular level, give new suggestions for deeply investigating metallothionein III and brain acid soluble protein 1 in AD.
J Chromatogr B Analyt Technol Biomed Life Sci, 2019; 1124
30887425: Fuso A, Lucarelli M
CpG and Non-CpG Methylation in the Diet-Epigenetics-Neurodegeneration Connection.
Unraveling the diet-epigenetics-neurodegeneration connection may disclose associated mechanisms and novel approaches to the neurodegenerative diseases. This review summarizes the basic concepts and the innovative results in this field focusing on the relevance of non-CpG methylation.
Curr Nutr Rep, 2019; 8
28973985: Cavallaro RA, Nicolia V, Fiorenza MT, Scarpa S, Fuso A
S-Adenosylmethionine and Superoxide Dismutase 1 Synergistically Counteract Alzheimer's Disease Features Progression in TgCRND8 Mice.
Recent evidence emphasizes the role of dysregulated one-carbon metabolism in Alzheimer's Disease (AD). Exploiting a nutritional B-vitamin deficiency paradigm, we have previously shown that PSEN1 and BACE1 activity is modulated by one-carbon metabolism, leading to increased amyloid production. We have also demonstrated that S-adenosylmethionine (SAM)

supplementation contrasted the AD-like features, induced by B-vitamin deficiency. In the present study, we expanded these observations by investigating the effects of SAM and SOD (Superoxide dismutase) association. TgCRND8 AD mice were fed either with a control or B-vitamin deficient diet, with or without oral supplementation of SAM + SOD. We measured oxidative stress by lipid peroxidation assay, PSEN1 and BACE1 expression by Real-Time Polymerase Chain Reaction (PCR), amyloid deposition by ELISA assays and immunohistochemistry. We found that SAM + SOD supplementation prevents the exacerbation of AD-like features induced by B vitamin deficiency, showing synergistic effects compared to either SAM or SOD alone. SAM + SOD supplementation also contrasts the amyloid deposition typically observed in TgCRND8 mice. Although the mechanisms underlying the beneficial effect of exogenous SOD remain to be elucidated, our findings identify that the combination of SAM + SOD could be carefully considered as co-adjuvant of current AD therapies.
Antioxidants (Basel), 2017; 6

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CYP46A1 IN THE CHOROID PLEXUS: AN UNEXPECTED SAFEGUARD OF BRAIN FUNCTION LOST IN ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Type-I interferon (IFN-I) signaling at the choroid plexus (CP) negatively affects brain function in aging and Alzheimer's disease (AD). Emerging evidence shows that oxidized cholesterol metabolites control immune responses. Here, we hypothesized that 24-hydroxycholesterol (24-OH), produced by the brain-specific enzyme cholesterol 24-hydroxylase (CYP46A1), affects the inflammatory state of the CP, and that the CP itself expresses this enzyme. Transcriptomic analysis of primary mouse CP cultures exposed to 24-OH revealed down-regulation of IFN-I signaling. Using single-cell published transcriptomic data, we could demonstrate that epithelial cells within the CP express CYP46A1. We further verified that CYP46A1 is expressed in both mice and human postmortem CP samples at the protein level, and that its levels are reduced with AD in both an AD mouse model (5xFAD) and human patients. We further found that *Cyp46a1* gene expression at the CP is reduced by tumor necrosis factor alpha (TNFα), an inflammatory signature of the brain in AD, known to also induce IFN-I signaling at the CP. In addition, systemic PD-L1 blockade, which transiently elevates IFN-II signaling at the CP , induced *Cyp46a1* gene expression at the CP. Finally, overexpression of *Cyp46a1* at the CP in 5xFAD mice, reduced the expression of IFN-I associated genes and attenuated cognitive loss and neuroinflammation. Our results suggest that CP CYP46A1 provides a safeguard against chronic inflammation that is lost with disease, but amenable to rescue.

Pubmed:

26284939: Baruch K, Rosenzweig N, Kertser A, Deczkowska A, Sharif AM, Spinrad A, Tsitsou-Kampeli A, Sarel A, Cahalon L, Schwartz M
Breaking immune tolerance by targeting Foxp3(+) regulatory T cells mitigates Alzheimer's disease pathology.
Alzheimer's disease (AD) is a neurodegenerative disorder in which chronic neuroinflammation contributes to disease escalation. Nevertheless, while immunosuppressive drugs have repeatedly failed in treating this disease, recruitment of myeloid cells to the CNS was shown to play a reparative role in animal models. Here we show, using the 5XFAD AD mouse model, that transient depletion of Foxp3(+) regulatory T cells (Tregs), or pharmacological inhibition of their activity, is followed by amyloid-β plaque clearance, mitigation of the neuroinflammatory response and reversal of cognitive decline. We further show that transient Treg depletion affects the brain's choroid plexus, a selective gateway for immune cell trafficking to the CNS, and is associated with subsequent recruitment of immunoregulatory cells, including monocyte-derived macrophages and Tregs, to cerebral sites of plaque pathology. Our findings suggest targeting Treg-mediated systemic immunosuppression for treating AD.
Nat Commun, 2015; 6
30692527: Rosenzweig N, Dvir-Szternfeld R, Tsitsou-Kampeli A, Keren-Shaul H, Ben-Yehuda H, Weill-Raynal P, Cahalon L, Kertser A, Baruch K, Amit I, Weiner A, Schwartz M
PD-1/PD-L1 checkpoint blockade harnesses monocyte-derived macrophages to combat cognitive impairment in a tauopathy mouse model.
Alzheimer's disease (AD) is a heterogeneous disorder with multiple etiologies. Harnessing the immune system by blocking the programmed cell death receptor (PD)-1 pathway in an amyloid beta mouse model was shown to evoke a sequence of immune responses that lead to disease modification. Here, blocking PD-L1, a PD-1 ligand, was found to have similar efficacy to that of PD-1 blocking in disease modification, in both animal models of AD and of tauopathy. Targeting PD-L1 in a tau-driven disease model resulted in increased immunomodulatory monocyte-derived macrophages within the brain parenchyma. Single cell RNA-seq revealed that the homing macrophages expressed unique scavenger molecules including

macrophage scavenger receptor 1 (MSR1), which was shown here to be required for the effect of PD-L1 blockade in disease modification. Overall, our results demonstrate that immune checkpoint blockade targeting the PD-1/PD-L1 pathway leads to modification of common factors that go awry in AD and dementia, and thus can potentially provide an immunotherapy to help combat these diseases.
Nat Commun, 2019; 10
26779813: Baruch K, Deczkowska A, Rosenzweig N, Tsitsou-Kampeli A, Sharif AM, Matcovitch-Natan O, Kertser A, David E, Amit I, Schwartz M
PD-1 immune checkpoint blockade reduces pathology and improves memory in mouse models of Alzheimer's disease. Systemic immune suppression may curtail the ability to mount the protective, cell-mediated immune responses that are needed for brain repair. By using mouse models of Alzheimer's disease (AD), we show that immune checkpoint blockade directed against the programmed death-1 (PD-1) pathway evokes an interferon (IFN)-γ-dependent systemic immune response, which is followed by the recruitment of monocyte-derived macrophages to the brain. When induced in mice with established pathology, this immunological response leads to clearance of cerebral amyloid-β (Aβ) plaques and improved cognitive performance. Repeated treatment sessions were required to maintain a long-lasting beneficial effect on disease pathology. These findings suggest that immune checkpoints may be targeted therapeutically in AD.
Nat Med, 2016; 22
28959042: Deczkowska A, Matcovitch-Natan O, Tsitsou-Kampeli A, Ben-Hamo S, Dvir-Szternfeld R, Spinrad A, Singer O, David E, Winter DR, Smith LK, Kertser A, Baruch K, Rosenzweig N, Terem A, Prinz M, Villeda S, Citri A, Amit I, Schwartz M
Mef2C restrains microglial inflammatory response and is lost in brain ageing in an IFN-I-dependent manner.
During ageing, microglia acquire a phenotype that may negatively affect brain function. Here we show that ageing microglial phenotype is largely imposed by interferon type I (IFN-I) chronically present in aged brain milieu. Overexpression of IFN-β in the CNS of adult wild-type mice, but not of mice lacking IFN-I receptor on their microglia, induces an ageing-like transcriptional microglial signature, and impairs cognitive performance. Furthermore, we demonstrate that age-related IFN-I milieu downregulates microglial myocyte-specific enhancer factor 2C (Mef2C). Immune challenge in mice lacking Mef2C in microglia results in an exaggerated microglial response and has an adverse effect on mice behaviour. Overall, our data indicate that the chronic presence of IFN-I in the brain microenvironment, which negatively affects cognitive function, is mediated via modulation of microglial activity. These findings may shed new light on other neurological conditions characterized by elevated IFN-I signalling in the brain.Microglia cells in the brain regulate immune responses, but in ageing can negatively affect brain function. Here the authors show that the chronic presence of type I interferon in aged mouse brain impedes cognitive ability by altering microglia transcriptome and limiting Mef2C, a microglia 'off' signal.
Nat Commun, 2017; 8

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ROLE OF THE TYROSINE KINASE PYK2 IN SYNAPTIC FUNCTION AND IN THE PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Aims: A genome wild association study has identified PTK2B, a gene which encodes for Pyk2, a neuronal Ca^{2+}-activated non-receptor tyrosine kinase implicated in synaptic function, as a major risk factor for Alzheimer's disease (AD). Several studies have validated the implication of Pyk2 in the pathophysiology of AD but contradictory findings have been reported. The objective of this project is to further understand the role of Pyk2 in physiological and pathophysiological synaptic functions in the context of AD. Methods: Murine primary neuronal culture (DIV 14) were transfected with Life-actin GFP and Pyk2 plasmids with altered kinase activity to assess the influence of PyK2 kinase activity on dendritic spines density and morphology. Furthermore, we measured Pyk2 expression and phosphorylation profile in the hippocampus and cortex of APPxPS1-21 transgenic mice, a model of AD. Results: Pyk2 overexpression leads to a decrease in synaptic density when it is overexpressed in neurons. This effect does not appear to be related to its kinase activity since this decrease is also observed with the inactive mutant Y402F of Pyk2. In APPxPS1 mice, we observed a strong decrease of Pyk2 phosphorylation in the cortex at 6 and 9 months. Conclusions: Together these results highlight the physiological role of PyK2 in the structural features of excitatory synapses. Because PyK2 phosphorylation displays a long lasting reduction in APPxPS1-21 mouse model of AD, we will study further the consequences of an altered PyK2 activity on excitatory neurotransmission in the context of AD.

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FORMULATING NOVEL RESEARCH QUESTIONS TO INVESTIGATE A MECHANISTIC RELATIONSHIP BETWEEN THE BRAIN GLYMPHATIC SYSTEM AND ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Since it was first described in 1906, Alzheimer's disease (AD) has remained one of the most insidious brain diseases to claim the lives of the aging population. Despite the progress in drug development, an efficacious approach to preventing and treating AD is still needed. Although amyloid-beta and tau protein aggregation have been highly investigated for their roles in the pathogenesis of AD, little is still known regarding how dysfunction in the brain's microenvironment may result in conditions conducive to neuronal and glial cell dysfunction. An essential mechanism for homeostatic regulation of the brain microenvironment is the glymphatic system, comprised of multicellular and interstitial components that efficiently clear potential neurotoxins from the brain. The glymphatic system, most active during sleep, facilitates the elimination of waste macromolecules via fluid flux through paraventricular channels formed by glial cells, particularly astrocytes. Although the anatomical and physiological properties of the glymphatic system have been well-characterized, its potential role in AD is newly emerging. We performed a systematic literature review using the PubMed, Google Scholar, and NIH REPORTER databases using the keywords: sleep, glymphatic system, and AD. Forty-seven separate articles collectively suggested strong correlations between glymphatic function, sleep, and AD as of February-2022, with a small subset of studies dedicated to elucidating potential mechanistic relationships. Therefore, the existing rigor of the prior research indicates a premise to propose and conduct future studies elucidating the mechanisms of AD as it involves alterations in the brain microenvironment secondary to glymphatic system effects.

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INTEGRATED NETWORK-BASED AND DIFFERENTIAL GENE EXPRESSION ANALYSIS IDENTIFIES POTENTIAL THERAPEUTIC TARGETS BASED ON ENDOTHELIAL AND MYELOID CELL TRANSCRIPTOME CHANGES IN PATIENTS WITH ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Aims: Etiopathology of Alzheimer's dementia (AD) comprises complex interactions, including the interplay of disrupted neurovasculature and inflammatory processes. This *in silico* study investigates the transcriptomic changes in endothelial (EC) and myeloid cells (MC) obtained from superior frontal lobe. **Methods:** RNA-seq dataset GSE125050 was acquired from NCBI GEO database. Differentially expressed genes (DEGs) were identified with *DESeq2*, while weighted gene co-expression network analysis (WGCNA) pipeline was applied to identify highly correlated gene modules and key genes. Functional enrichment analysis was performed on DEGs and modules of interest. Transcriptomics-based drug repurposing approach using LINCS connectivity map was applied to identify drug candidates. **Results:** Distinct cell-type-specific transcriptomic changes were observed using different approaches. Six modules with 1695 key genes were significantly correlated with AD MC, while two modules with 378 key genes with AD EC, respectively. FZD3 was recognized as hub gene in both cell types, supporting evidence that Wnt signaling pathway is disrupted in AD. 53 and 1384 DEGs were identified in AD MC and EC, respectively, of which UVRAG was upregulated, and ANKRD26P3 and GLT1D1 were downregulated in both cell types. AD EC revealed altered cell adhesion and organization, whereas pathways related to synaptic modulation and immune response activation were more prominent in MC. 17 drug candidates, including temsirolimus, exhibited potential to reverse the AD transcriptomic signature. **Conclusions:** This integrated study identified several cell-specific genes that may serve as biomarkers or therapeutic targets and supports further investigations of rapamycin and its derivatives which exhibit protective effects in animal AD models.

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THE NEURONAL SEIZURE PROTEIN 6 (SEZ6) IS A SUBSTRATE OF THE ALZHEIMER PROTEASE BACE1 AND A LIGAND FOR THE LDL-RELATED PROTEIN 1 (LRP1)

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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The protease BACE1 has fundamental functions in the nervous system, including in myelination, but also is a major drug target for Alzheimer's disease, because it generates the pathogenic amyloid beta peptide. However, BACE1-targeted inhibitors induced side effects in clinical trials, including cognitive worsening. While the underlying mechanism is not yet known, the side effects are assumed to be mechanism-based and to results from inhibition of one or several of the numerous BACE1 substrates in the nervous system. One key substrate for BACE1, that we identified, is the transmembrane protein seizure protein 6 (SEZ6), which controls dendritic branching, kainate receptor signaling, dendritic spine density and LTP. The BACE1-cleaved soluble SEZ6 ectodomain (sSEZ6) may act as a ligand and contribute to these neuronal processes, but a receptor for sSEZ6 has not yet been identified. Using mass spectrometry and biochemical strategies, we identified and validated the LDL receptor-related protein 1 (LRP1) as a receptor for sSEZ6. We provide evidence that sSEZ6 binding to LRP1 contributes to neurite arborization and spine morphology. Taken together, our study identifies a receptor for sSEZ6, demonstrates a molecular function for the sSEZ6-LRP1 interaction in the nervous system and may help to better understand the mechanistic basis of the side effects observed in BACE inhibitor trials.

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EXPLORING THE IMPACT OF APOE POLYMORPHISM ON THE MOLECULAR, MORPHOLOGICAL AND FUNCTIONAL PROFILE OF IPSC-DERIVED ASTROCYTES FROM ALZHEIMER'S PATIENTS

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Alzheimer's disease (AD) is pathologically characterised by the presence of amyloid-β plaques, neurofibrillary tangles containing hyperphosphorylated Tau protein, neuroinflammation and neuronal death leading to progressive cognitive impairment. The ε4 allele of the gene encoding apolipoprotein E (APOE), which is mainly expressed in glial cells, is the strongest genetic risk factor for sporadic AD. Increasing evidence has shown that APOE4 may disrupt normal astrocyte activity, potentially contributing to AD pathology, but the impact of different APOE alleles on astrocyte differentiation, maturation and function is not yet fully understood. To go in depth on these questions, we obtained induced pluripotent stem cells (iPSCs) from fibroblasts of AD patients carrying ε3 and ε4 alleles (in homozygosis) and from healthy patients. We also used gene-edited iPSC lines homozygous for the main APOE variants and an APOE knock-out line. iPSC-derived human astrocytes were generated by establishing a differentiation protocol through the consecutive addition of small molecules and growth factors, and the expression of typical markers (GFAP, GLT1, AQP4 and S100β) and APOE was confirmed. In addition, astrocytes exhibited functional features like glutamate uptake capacity and calcium waves production. They also responded to an inflammatory stimulus (IL-1β and TNF-α) or to the presence of amyloid-β 1-42 peptide by changing their morphology and increasing the expression levels of pro-inflammatory factors and cytokines. Our results shed light on the potential dual role of APOE polymorphism and the individual´s genetic background in favouring or perhaps preventing AD pathology.

Pubmed:
34298892: Barrio E, Vecino R, Sánchez-Morán I, Rodríguez C, Suárez-Pindado A, Bolaños JP, Almeida A, Delgado-Esteban M
Preconditioning-Activated AKT Controls Neuronal Tolerance to Ischemia through the MDM2-p53 Pathway.
One of the most important mechanisms of preconditioning-mediated neuroprotection is the attenuation of cell apoptosis, inducing brain tolerance after a subsequent injurious ischemia. In this context, the antiapoptotic PI3K/AKT signaling pathway plays a key role by regulating cell differentiation and survival. Active AKT is known to increase the expression of murine double minute-2 (MDM2), an E3-ubiquitin ligase that destabilizes p53 to promote the survival of cancer cells. In neurons, we recently showed that the MDM2-p53 interaction is potentiated by pharmacological preconditioning, based on subtoxic stimulation of NMDA glutamate receptor, which prevents ischemia-induced neuronal apoptosis. However, whether this mechanism contributes to the neuronal tolerance during ischemic preconditioning (IPC) is unknown. Here, we show that IPC induced PI3K-mediated phosphorylation of AKT at Ser, which in turn phosphorylated MDM2 at Ser. This phosphorylation triggered the nuclear stabilization of MDM2, leading to p53 destabilization, thus preventing neuronal apoptosis upon an ischemic insult. Inhibition of the PI3K/AKT pathway with wortmannin or by AKT silencing induced the accumulation of cytosolic MDM2, abrogating IPC-induced neuroprotection. Thus, IPC enhances the activation of PI3K/AKT signaling pathway and promotes neuronal tolerance by controlling the MDM2-p53 interaction. Our findings provide a new mechanistic pathway involved in IPC-induced neuroprotection via modulation of AKT signaling, suggesting that AKT is a potential therapeutic target against ischemic injury.
Int J Mol Sci, 2021; 22
29687302: Ramos-Araque ME, Rodriguez C, Vecino R, Cortijo Garcia E, de Lera Alfonso M, Sanchez Barba M, Colàs-Campàs L, Purroy F, Arenillas JF, Almeida A, Delgado-Esteban M
The Neuronal Ischemic Tolerance Is Conditioned by the Tp53 Arg72Pro Polymorphism.
Cerebral preconditioning (PC) confers endogenous brain protection after stroke. Ischemic stroke patients with a prior transient ischemic attack (TIA) may potentially be in a preconditioned state. Although PC has been associated with the

activation of pro-survival signals, the mechanism by which preconditioning confers neuroprotection is not yet fully clarified. Recently, we have described that PC-mediated neuroprotection against ischemic insult is promoted by p53 destabilization, which is mediated by its main regulator MDM2. Moreover, we have previously described that the human Tp53 Arg72Pro single nucleotide polymorphism (SNP) controls susceptibility to ischemia-induced neuronal apoptosis and governs the functional outcome of patients after stroke. Here, we studied the contribution of the human Tp53 Arg72Pro SNP on PC-induced neuroprotection after ischemia. Our results showed that cortical neurons expressing the Pro72-p53 variant exhibited higher PC-mediated neuroprotection as compared with Arg72-p53 neurons. PC prevented ischemia-induced nuclear and cytosolic p53 stabilization in Pro72-p53 neurons. However, PC failed to prevent mitochondrial p53 stabilization, which occurs in Arg72-p53 neurons after ischemia. Furthermore, PC promoted neuroprotection against ischemia by controlling the p53/active caspase-3 pathway in Pro72-p53, but not in Arg72-p53 neurons. Finally, we found that good prognosis associated to TIA within 1 month prior to ischemic stroke was restricted to patients harboring the Pro72 allele. Our findings demonstrate that the Tp53 Arg72Pro SNP controls PC-promoted neuroprotection against a subsequent ischemic insult by modulating mitochondrial p53 stabilization and then modulates TIA-induced ischemic tolerance.
Transl Stroke Res, 2019; 10

29371613: Vecino R, Burguete MC, Jover-Mengual T, Agulla J, Bobo-Jiménez V, Salom JB, Almeida A, Delgado-Esteban M
The MDM2-p53 pathway is involved in preconditioning-induced neuronal tolerance to ischemia.
Brain preconditioning (PC) refers to a state of transient tolerance against a lethal insult that can be evoked by a prior mild event. It is thought that PC may induce different pathways responsible for neuroprotection, which may involve the attenuation of cell damage pathways, including the apoptotic cell death. In this context, p53 is a stress sensor that accumulates during brain ischemia leading to neuronal death. The murine double minute 2 gene (MDM2), a p53-specific E3 ubiquitin ligase, is the main cellular antagonist of p53, mediating its degradation by the proteasome. Here, we study the role of MDM2-p53 pathway on PC-induced neuroprotection both in cultured neurons (in vitro) and rat brain (in vivo). Our results show that PC increased neuronal MDM2 protein levels, which prevented ischemia-induced p53 stabilization and neuronal death. Indeed, PC attenuated ischemia-induced activation of the p53/PUMA/caspase-3 signaling pathway. Pharmacological inhibition of MDM2-p53 interaction in neurons abrogated PC-induced neuroprotection against ischemia. Finally, the relevance of the MDM2-p53 pathway was confirmed in rat brain using a PC model in vivo. These findings demonstrate the key role of the MDM2-p53 pathway in PC-induced neuroprotection against a subsequent ischemic insult and poses MDM2 as an essential target in ischemic tolerance.
Sci Rep, 2018; 8

26109654: Veas-Pérez de Tudela M, Delgado-Esteban M, Maestre C, Bobo-Jiménez V, Jiménez-Blasco D, Vecino R, Bolaños JP, Almeida A
Regulation of Bcl-xL-ATP Synthase Interaction by Mitochondrial Cyclin B1-Cyclin-Dependent Kinase-1 Determines Neuronal Survival.
The survival of postmitotic neurons needs continuous degradation of cyclin B1, a mitotic protein accumulated aberrantly in the damaged brain areas of Alzheimer's disease and stroked patients. Degradation of cyclin B1 takes place in the proteasome after ubiquitylation by the anaphase-promoting complex/cyclosome (APC/C)-cadherin 1 (Cdh1), an E3 ubiquitin ligase that is highly active in neurons. However, during excitotoxic damage-a hallmark of neurological disorders-APC/C-Cdh1 is inactivated, causing cyclin B1 stabilization and neuronal death through an unknown mechanism. Here, we show that an excitotoxic stimulus in rat cortical neurons in primary culture promotes cyclin B1 accumulation in the mitochondria, in which it binds to, and activates, cyclin-dependent kinase-1 (Cdk1). The cyclin B1-Cdk1 complex in the mitochondria phosphorylates the anti-apoptotic protein B-cell lymphoma extra-large (Bcl-xL), leading to its dissociation from the β subunit of F1Fo-ATP synthase. The subsequent inhibition of ATP synthase activity causes complex I oxidative damage, mitochondrial inner membrane depolarization, and apoptotic neuronal death. These results unveil a previously unrecognized role for mitochondrial cyclin B1 in the oxidative damage associated with neurological disorders.
J Neurosci, 2015; 35

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-399

OLFACTORY MUCOSAL CELLS OF ALZHEIMER'S DISEASE PATIENTS DISPLAY DISEASE SPECIFIC TRANSCRIPTIONAL AND FUNCTIONAL ALTERATIONS, AND DYSHOMEOSTASIS OF BIOMETALS

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Olfactory dysfunction manifests early in several neurodegenerative disorders including Alzheimer's disease (AD), however, disease-related alterations to olfactory mucosal (OM) cells remain poorly described. Furthermore, biometals functionally influence to olfaction and are altered in AD-affected brains. We harvested OM biopsies from cognitively healthy individuals and patients with late-onset AD to first profile disease-linked transcriptomic alterations at single-cell level. The single-cell RNA sequencing (scRNA-seq) unveiled 240 differentially expressed disease-associated genes in AD OM cells compared to the controls, and five distinct cell populations. Furthermore, the alterations in gene expression of mitochondrially located genes in AD OM cells were verified by functional assays. AD OM cells displayed increased secretion of beta-amyloid. Since alterations in the balance of metal ions are linked to the deposition of beta-amyloid, total RNA sequencing was applied to larger cohort of samples to discover transcriptional changes related to metals in AD and control OM cells. Furthermore, analysis of the elemental content by inductively coupled plasma mass spectrometry (ICP-MS) was performed. Consequently, the levels of zinc, calcium and sodium were observed to be significantly increased in the AD OM cells concomitantly with alterations to 17 genes associated with a metal-related function. A significant elevation in alpha-2-macroglobulin, a known metal-binding biomarker correlated with brain disease burden, was observed on the gene and protein level in the OM cells of AD patients. Our results reveal disease-related changes of OM cells in AD and suggest that impairments in biometal homeostasis in the OM can recapitulate the alterations observed in the disease affected brain.

Pubmed:
35203328: Lampinen R, Fazaludeen MF, Avesani S, Örd T, Penttilä E, Lehtola JM, Saari T, Hannonen S, Saveleva L, Kaartinen E, Fernández Acosta F, Cruz-Haces M, Löppönen H, Mackay-Sim A, Kaikkonen MU, Koivisto AM, Malm T, White AR, Giugno R, Chew S, Kanninen KM
Single-Cell RNA-Seq Analysis of Olfactory Mucosal Cells of Alzheimer's Disease Patients.
Olfaction is orchestrated by olfactory mucosal cells located in the upper nasal cavity. Olfactory dysfunction manifests early in several neurodegenerative disorders including Alzheimer's disease, however, disease-related alterations to the olfactory mucosal cells remain poorly described. The aim of this study was to evaluate the olfactory mucosa differences between cognitively healthy individuals and Alzheimer's disease patients. We report increased amyloid-beta secretion in Alzheimer's disease olfactory mucosal cells and detail cell-type-specific gene expression patterns, unveiling 240 differentially expressed disease-associated genes compared to the cognitively healthy controls, and five distinct cell populations. Overall, alterations of RNA and protein metabolism, inflammatory processes, and signal transduction were observed in multiple cell populations, suggesting their role in Alzheimer's disease-related olfactory mucosa pathophysiology. Furthermore, the single-cell RNA-sequencing proposed alterations in gene expression of mitochondrially located genes in AD OM cells, which were verified by functional assays, demonstrating altered mitochondrial respiration and a reduction of ATP production. Our results reveal disease-related changes of olfactory mucosal cells in Alzheimer's disease and demonstrate the utility of single-cell RNA

sequencing data for investigating molecular and cellular mechanisms associated with the disease.
Cells, 2022; 11
32487172: Chew S, Lampinen R, Saveleva L, Korhonen P, Mikhailov N, Grubman A, Polo JM, Wilson T, Komppula M, Rönkkö T, Gu C, Mackay-Sim A, Malm T, White AR, Jalava P, Kanninen KM
Urban air particulate matter induces mitochondrial dysfunction in human olfactory mucosal cells.
The adverse effects of air pollutants including particulate matter (PM) on the central nervous system is increasingly reported by epidemiological, animal and post-mortem studies in the last decade. Oxidative stress and inflammation are key consequences of exposure to PM although little is known of the exact mechanism. The association of PM exposure with deteriorating brain health is speculated to be driven by PM entry via the olfactory system. How air pollutants affect this key entry site remains elusive. In this study, we investigated effects of urban size-segregated PM on a novel cellular model: primary human olfactory mucosal (hOM) cells.
Part Fibre Toxicol, 2020; 17
32201281: Kanninen KM, Lampinen R, Rantanen LM, Odendaal L, Jalava P, Chew S, White AR
Olfactory cell cultures to investigate health effects of air pollution exposure: Implications for neurodegeneration.
Air pollution is a major, global public health concern. A growing body of evidence shows that exposure to air pollutants may impair the brain. Living in highly polluted areas has been linked to several neurodegenerative diseases, where exposure to complex mixtures of air pollutants in urban environments may have harmful effects on brain function. These harmful effects are thought to originate from elevated inflammation and oxidative stress. The olfactory epithelium is a key entry site of air pollutants into the brain as the particles are deposited in the upper airways and the nasal region. A potential source of patient-derived cells for study of air pollutant effects is the olfactory mucosa, which constitutes a central part of the olfactory epithelium. This review first summarizes the current literature on the available in vitro models of the olfactory epithelium. It then describes how alterations of the olfactory mucosa are linked to neurodegeneration and discusses potential therapeutic applications of these cells for neurodegenerative diseases. Finally, it reviews the research performed on the effects of air pollutant exposure in cells of the olfactory epithelium. Patient-derived olfactory epithelial models hold great promise for not only elucidating the molecular and cellular pathophysiology of neurodegenerative disorders, but for providing key understanding about air pollutant particle entry and effects at this key brain entry site.
Neurochem Int, 2020; 136
30453390: Konttinen H, Gureviciene I, Oksanen M, Grubman A, Loppi S, Huuskonen MT, Korhonen P, Lampinen R, Keuters M, Belaya I, Tanila H, Kanninen KM, Goldsteins G, Landreth G, Koistinaho J, Malm T
PPARβ/δ-agonist GW0742 ameliorates dysfunction in fatty acid oxidation in PSEN1ΔE9 astrocytes.
Astrocytes are the gatekeepers of neuronal energy supply. In neurodegenerative diseases, bioenergetics demand increases and becomes reliant upon fatty acid oxidation as a source of energy. Defective fatty acid oxidation and mitochondrial dysfunctions correlate with hippocampal neurodegeneration and memory deficits in Alzheimer's disease (AD), but it is unclear whether energy metabolism can be targeted to prevent or treat the disease. Here we show for the first time an impairment in fatty acid oxidation in human astrocytes derived from induced pluripotent stem cells of AD patients. The impairment was corrected by treatment with a synthetic peroxisome proliferator activated receptor delta (PPARβ/δ) agonist GW0742 which acts to regulate an array of genes governing cellular metabolism. GW0742 enhanced the expression of CPT1a, the gene encoding for a rate-limiting enzyme of fatty acid oxidation. Similarly, treatment of a mouse model of AD, the APP/PS1-mice, with GW0742 increased the expression of Cpt1a and concomitantly reversed memory deficits in a fear conditioning test. Although the GW0742-treated mice did not show altered astrocytic glial fibrillary acidic protein-immunoreactivity or reduction in amyloid beta (Aβ) load, GW0742 treatment increased hippocampal neurogenesis and enhanced neuronal differentiation of neuronal progenitor cells. Furthermore, GW0742 prevented Aβ-induced impairment of long-term potentiation in hippocampal slices. Collectively, these data suggest that PPARβ/δ-agonism alleviates AD related deficits through increasing fatty acid oxidation in astrocytes and improves cognition in a transgenic mouse model of AD.
Glia, 2019; 67
30042655: Abdalkader M, Lampinen R, Kanninen KM, Malm TM, Liddell JR
Targeting Nrf2 to Suppress Ferroptosis and Mitochondrial Dysfunction in Neurodegeneration.
Ferroptosis is a newly described form of regulated cell death, distinct from apoptosis, necroptosis and other forms of cell death. Ferroptosis is induced by disruption of glutathione synthesis or inhibition of glutathione peroxidase 4, exacerbated by iron, and prevented by radical scavengers such as ferrostatin-1, liproxstatin-1, and endogenous vitamin E. Ferroptosis terminates with mitochondrial dysfunction and toxic lipid peroxidation. Although conclusive identification of ferroptosis is challenging, several salient and very well established features of neurodegenerative diseases are consistent with ferroptosis, including lipid peroxidation, mitochondrial disruption and iron dysregulation. Accordingly, interest in the role of ferroptosis in neurodegeneration is escalating and specific evidence is rapidly emerging. One aspect that has thus far received little attention is the antioxidant transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2). This transcription factor

regulates hundreds of genes, of which many are either directly or indirectly involved in modulating ferroptosis, including metabolism of glutathione, iron and lipids, and mitochondrial function. This potentially positions Nrf2 as a key deterministic component modulating the onset and outcomes of ferroptotic stress. The minimal direct evidence currently available is consistent with this and indicates that Nrf2 may be critical for protection against ferroptosis. In contrast, abundant evidence demonstrates that enhancing Nrf2 signaling is potently neuroprotective in models of neurodegeneration, although the exact mechanism by which this is achieved is unclear. Further studies are required to determine to extent to which the neuroprotective effects of Nrf2 activation involve the prevention of ferroptosis.
Front Neurosci, 2018; 12

28611094: Lempiäinen JK, Niskanen EA, Vuoti KM, Lampinen RE, Göös H, Varjosalo M, Palvimo JJ
Agonist-specific Protein Interactomes of Glucocorticoid and Androgen Receptor as Revealed by Proximity Mapping.
Glucocorticoid receptor (GR) and androgen receptor (AR) are steroid-inducible transcription factors (TFs). The GR and the AR are central regulators of various metabolic, homeostatic and differentiation processes and hence important therapeutic targets, especially in inflammation and prostate cancer, respectively. Hormone binding to these steroid receptors (SRs) leads to DNA binding and activation or repression of their target genes with the aid of interacting proteins, coregulators. However, protein interactomes of these important drug targets have remained poorly defined. We used proximity-dependent biotin identification to map the protein interaction landscapes of GR and AR in the presence and absence of their cognate agonist (dexamethasone, 5α-dihydrotestosterone) and antagonist (RU486, enzalutamide) in intact human cells. We reproducibly identified more than 30 proteins that interacted with the GR in an agonist-specific manner and whose interactions were significantly influenced by the DNA-binding function of the receptor. Interestingly, the agonist-dependent interactome of the GR overlapped considerably with that of the AR. In addition to known coactivators, corepressors and components of BAF (SWI/SNF) chromatin-remodeling complex, we identified a number of proteins, including lysine methyltransferases and demethylases that have not been previously linked to glucocorticoid or androgen signaling. A substantial number of these novel agonist-dependent GR/AR-interacting proteins, BCOR, IRF2BP2, RCOR1, and TLE3, have previously been implicated in transcription repression. This together with our data on the effect of BCOR, IRF2BP2, and RCOR1 on GR target gene expression suggests multifaceted functions and roles for SR coregulators. These first high confidence SR interactomes will aid in therapeutic targeting of the GR and the AR.
Mol Cell Proteomics, 2017; 16

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BOARD NUMBER: S02-400

COLLAGEN XVIII KNOCKOUT MICE AS A MODEL FOR EARLY CEREBRAL SMALL VESSEL DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Aims: The pathological alterations of small arteries and capillaries defined as cerebral small vessel disease (CSVD) are characterized by age-dependent blood-brain barrier (BBB) breakdown and vessel wall remodeling related to small vessel occlusions and perivascular bleeds. As collagen XVIII (Col18a1) is an abundant heparan sulfate proteoglycan in vascular basement membranes, here we focused on studying the role of Col18a1 in the vascular integrity and neurovascular unit maintenance in the context of CSVD. Methods: In this study, using qPCR we compared the expression levels of genes encoding tight junction and basement membrane proteins, inflammatory markers, neural ECM proteins and proteinases in 12-month-old Col18a1-/- andCol18a1+/+ mice. Furthermore, we examined the BBB breakdown and pre/peri-synaptic proteins alterations using immunohistochemistry. Results: Our immunohistochemical analysis revealed a progression of small vessel leakage in Col18a1-/- mice between 5 and 12 months. A large fraction (60%) of small vessel area was mouse IgG-immunopositive in the hippocampus and retrosplenial cortex of 12-month-old Col18a1-/- mice. However, none of Col18a1-/- mice displayed hallmarks typical of more advanced stages of CSVD, such as perivascular or large bleeds or infarcts. Col18a1 deficiency-induced BBB leakage was accompanied by activation of microglia and astrocytes, leading to perivascular ECM remodeling, upregulation of TIMP3 and accumulation of perisynaptic ECM proteoglycan brevican and complement protein C1q, which may underlie impaired synaptic plasticity and loss of synapses. Conclusion: Our findings highlight that Col18a1-/- mice represent a valuable model of early CSVD and call for more mechanistic analysis of underlying mechanisms and cognitive dysfunction in this model.

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BOARD NUMBER: S02-401

DECIPHERING PROK2 IMPLICATION IN THE DEVELOPMENT OF DEMENTIA RELATED TO ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

Hadi Younes, Zineb Mahrougui, Marie Amourette, Nadia Alfaidy-Benharougua, Christel Marquette
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A broad consensus is emerging that neuroinflammation and cerebrovascular dysfunction are central processes in the blood-brain barrier (BBB) disruption and dementia-related Alzheimer's disease (AD) pathogenesis. AD is the most common cause of dementia, with progressive and irreversible mental decline related to loss of cognitive abilities and memory functions, as well as cognitive impairment, and increasing neuroinflammation. Important inflammatory factors include prokineticin 2 (PROK2), a chemokine-like molecule that appears to mediate Aβ cytotoxicity in an acute model of AD. PROK2 acts through specific G protein-coupled receptors, the PROK receptor 2 (PROKR2) to control multiple biological functions, including circadian rhythm, angiogenesis, olfactory bulb neurogenesis, neuronal survival, reproduction, and inflammation. PROK2 increases in inflamed tissues and exacerbates local inflammation. Therefore, the objective of this study is to decipher PROK2's actions in the early vascular inflammatory steps leading to Aβ deposition and ultimately AD. Methods: First, PROK2/PROK2 antagonists' effects on vascular permeability and tight junction protein expression were investigated using in vitro BBB co-culture systems including endothelial/astrocytic cells and neurons. In parallel, using AD transgenic mice (APP/PSENdE9), which develop the pathology throughout their lives, we determined PROKR2 expression at different stages of the disease using WB, immunohistochemistry and Elisa assay to measure circulating PROK2 levels. We demonstrate that PROK2/PROKR2 are regulated during the development of the disease and notably as a neuroinflammatory agent at the vascular level. This is important to understand other neuroinflammatory cases related to cognitive dysfunction and the development of dementia, such as in preeclampsia, following hypertensive complications and vascular dysfunction.

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BOARD NUMBER: S02-402

TRANSCRIPTIONAL MODULATION OF ER-MITOCHONDRIA CROSSTALK IN ALZHEIMER'S DISEASE – ROLE OF HISTONE DEACETYLASES

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Center for Neuroscience and Cell Biology, Neuroscience And Disease, Coimbra, Portugal

Background: Several molecular mechanisms have been described in Alzheimer's disease (AD), including repressed gene transcription and mitochondrial and endoplasmic reticulum (ER) dysfunction. Chromatin remodeling by class I histone deacetylases (HDACs) inhibitors was recently reported to rescue AD-like phenotype, highlighting transcription regulation as a potential therapeutic approach. **Aims**: In this study, we aimed to evaluate the potential efficacy of transcriptional modifications exerted by class I HDACs inhibition or knockdown in ameliorating ER-mitochondria crosstalk in AD hippocampal neural cells. **Methods**: We investigated changes in intracellular Ca^{2+} homeostasis following HDAC inhibitor tacedinaline (Tac) treatment in HT22 cells exposed to Abeta1-42 oligomers (AbetaO). We also evaluated the expression of proteins involved in Ca^{2+} signaling at mitochondrial-associated ER membranes (MAMs) and ER-mitochondria contacts (MERCs) by electron microscopy. To study the selective effect of class I HDACs on ER-mitochondria crosstalk, we silenced HDAC2 or HDAC3. **Results**: We show that Tac prevents enhanced ER-Ca^{2+} retention and mitochondrial Ca^{2+} accumulation induced by AbetaO. Concomitantly, exposure to AbetaO leads to increased mRNA levels of IP3R1, VDAC1, Sig1R and Grp75, associated with increased MERCs length and a narrower gap between the two organelles, which are prevented after Tac treatment. Moreover, HDAC2 siRNA ameliorates mitochondrial-Ca^{2+} retention and ER-mitochondria Ca^{2+} transfer, whereas HDAC3 silencing reverts ER-Ca^{2+} accumulation in AbetaO-treated cells, suggesting differential epigenetic regulation of ER-mitochondrial communication and ER function. Interestingly, knockdown of HDAC2 or HDAC3 reverts mitochondrial depolarization induced by AbetaO. **Conclusions**: These findings support ER/mitochondrial-related transcriptional regulation as a promising target for innovative therapeutics in AD.

Pubmed:

33679301: Toledo JP, Fernández-Pérez EJ, Ferreira IL, Marinho D, Riffo-Lepe NO, Pineda-Cuevas BN, Pinochet-Pino LF, Burgos CF, Rego AC, Aguayo LG
Boldine Attenuates Synaptic Failure and Mitochondrial Deregulation in Cellular Models of Alzheimer's Disease.
Alzheimer's disease (AD) is the most common cause of senile dementia worldwide, characterized by both cognitive and behavioral deficits. Amyloid beta peptide (Aβ) oligomers (AβO) have been found to be responsible for several pathological mechanisms during the development of AD, including altered cellular homeostasis and synaptic function, inevitably leading to cell death. Such AβO deleterious effects provide a way for identifying new molecules with potential anti-AD properties. Available treatments minimally improve AD symptoms and do not extensively target intracellular pathways affected by AβO. Naturally-derived compounds have been proposed as potential modifiers of Aβ-induced neurodysfunction and cytotoxicity based on their availability and chemical diversity. Thus, the aim of this study was to evaluate boldine, an alkaloid derived from the bark and leaves of the Chilean tree , and its capacity to block some dysfunctional processes caused by AβO. We examined the protective effect of boldine (1-10 μM) in primary hippocampal neurons and HT22 hippocampal-derived cell line treated with AβO (24-48 h). We found that boldine interacts with Aβ affecting its aggregation and protecting hippocampal neurons from synaptic failure induced by AβO. Boldine also normalized changes in intracellular Ca levels associated to mitochondria or endoplasmic reticulum in HT22 cells treated with AβO. In addition, boldine completely rescued the decrease in mitochondrial membrane potential (ΔΨm) and the increase in mitochondrial reactive oxygen species, and attenuated AβO-induced decrease in mitochondrial respiration in HT22 hippocampal cells. We conclude that boldine provides neuroprotection in AD models by both direct interactions with Aβ and by preventing oxidative stress and mitochondrial dysfunction. Additional studies are required to evaluate the effect of boldine on cognitive and behavioral deficits induced by Aβ .
Front Neurosci, 2021; 15

FENS Forum 2022
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BOARD NUMBER: S02-403

THE Q-JUNCTION AND THE INFLAMMATORY RESPONSE DETERMINE THE PATHOLOGICAL FEATURES AND THE THERAPEUTIC SUCCESS IN A MODEL OF FATAL MITOCHONDRIAL ENCEPHALOPATHY.

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Defects in Coenzyme Q (CoQ) metabolism have been associated to primary mitochondrial disorders, neurodegenerative diseases and metabolic conditions. The consequences of CoQ deficiency have not been fully addressed, and the therapeutic approaches remain challenging. Here, we carried out multi-omics, molecular and morphologic analyses and we demonstrate that CoQ deficiency induces reactive gliosis, which mediates a neuroinflammatory response, both leading to a fatal encephalopathic phenotype in the *Coq9*^{R239X} mouse model. Also, CoQ deficiency profoundly alters the Q-junction, which lead to a major remodelling of the mitochondrial proteome and metabolism in the kidneys, and to a lesser extent in the brain. Importantly, the treatment with either vanillic acid (VA) or β-resorcylic acid (β-RA), two analogs of the natural precursor for CoQ biosynthesis, partially restores CoQ metabolism, particularly in the kidneys and liver, and induce a profound normalization of the mitochondrial proteome and metabolism, ultimately leading to the reduction of gliosis, neuroinflammation and spongiosis and, consequently, rescuing the phenotype. Together, these results add key mechanistic insights about defects in CoQ metabolism, and identify potential disease biomarkers. Furthermore, our findings are not only important for the use of analogs of the CoQ biosynthetic precursor in the treatment of mitochondrial encephalopathies associated with CoQ deficiency but also for the therapeutic use of those compounds in more common neurodegenerative and metabolic diseases that occur with secondary CoQ deficiency.

Pubmed:
32975579: González-García P, Hidalgo-Gutiérrez A, Mascaraque C, Barriocanal-Casado E, Bakkali M, Ziosi M, Abdihankyzy UB, Sánchez-Hernández S, Escames G, Prokisch H, Martín F, Quinzii CM, López LC
Coenzyme Q10 modulates sulfide metabolism and links the mitochondrial respiratory chain to pathways associated to one carbon metabolism.
Abnormalities of one carbon, glutathione and sulfide metabolisms have recently emerged as novel pathomechanisms in diseases with mitochondrial dysfunction. However, the mechanisms underlying these abnormalities are not clear. Also, we recently showed that sulfide oxidation is impaired in Coenzyme Q10 (CoQ10) deficiency. This finding leads us to hypothesize that the therapeutic effects of CoQ10, frequently administered to patients with primary or secondary mitochondrial dysfunction, might be due to its function as cofactor for sulfide:quinone oxidoreductase (SQOR), the first enzyme in the sulfide oxidation pathway. Here, using biased and unbiased approaches, we show that supraphysiological levels of CoQ10 induces an increase in the expression of SQOR in skin fibroblasts from control subjects and patients with mutations in Complex I subunits genes or CoQ biosynthetic genes. This increase of SQOR induces the downregulation of the cystathionine β-synthase and cystathionine γ-lyase, two enzymes of the transsulfuration pathway, the subsequent downregulation of serine biosynthesis and the adaptation of other sulfide linked pathways, such as folate cycle, nucleotides metabolism and glutathione system. These metabolic changes are independent of the presence of sulfur aminoacids, are confirmed in mouse models, and are recapitulated by overexpression of SQOR, further proving that the metabolic effects of CoQ10 supplementation are mediated by the overexpression of SQOR. Our results contribute to a better understanding of how sulfide metabolism is integrated in one carbon metabolism and may explain some of the benefits of CoQ10 supplementation observed in mitochondrial diseases.
Hum Mol Genet, 2020; 29
34829558: González-García P, Barriocanal-Casado E, Díaz-Casado ME, López-Herrador S, Hidalgo-Gutiérrez A, López LC
Animal Models of Coenzyme Q Deficiency: Mechanistic and Translational Learnings.
Coenzyme Q (CoQ) is a vital lipophilic molecule that is endogenously synthesized in the mitochondria of each cell. The CoQ biosynthetic pathway is complex and not completely characterized, and it involves at least thirteen catalytic and regulatory proteins. Once it is synthesized, CoQ exerts a wide variety of mitochondrial and extramitochondrial functions thank to its

redox capacity and its lipophilicity. Thus, low levels of CoQ cause diseases with heterogeneous clinical symptoms, which are not always understood. The decreased levels of CoQ may be primary caused by defects in the CoQ biosynthetic pathway or secondarily associated with other diseases. In both cases, the pathomechanisms are related to the CoQ functions, although further experimental evidence is required to establish this association. The conventional treatment for CoQ deficiencies is the high doses of oral CoQ supplementation, but this therapy is not effective for some specific clinical presentations, especially in those involving the nervous system. To better understand the CoQ biosynthetic pathway, the biological functions linked to CoQ and the pathomechanisms of CoQ deficiencies, and to improve the therapeutic outcomes of this syndrome, a variety of animal models have been generated and characterized in the last decade. In this review, we show all the animal models available, remarking on the most important outcomes that each model has provided. Finally, we also comment some gaps and future research directions related to CoQ metabolism and how the current and novel animal models may help in the development of future research studies.
Antioxidants (Basel), 2021; 10
34680574: Hidalgo-Gutiérrez A, Barriocanal-Casado E, Díaz-Casado ME, González-García P, Zenezini Chiozzi R, Acuña-Castroviejo D, López LC
β-RA Targets Mitochondrial Metabolism and Adipogenesis, Leading to Therapeutic Benefits against CoQ Deficiency and Age-Related Overweight.
Primary mitochondrial diseases are caused by mutations in mitochondrial or nuclear genes, leading to the abnormal function of specific mitochondrial pathways. Mitochondrial dysfunction is also a secondary event in more common pathophysiological conditions, such as obesity and metabolic syndrome. In both cases, the improvement and management of mitochondrial homeostasis remain challenging. Here, we show that beta-resorcylic acid (β-RA), which is a natural phenolic compound, competed in vivo with 4-hydroxybenzoic acid, which is the natural precursor of coenzyme Q biosynthesis. This led to a decrease in demethoxyubiquinone, which is an intermediate metabolite of CoQ biosynthesis that is abnormally accumulated in mice. As a consequence, β-RA rescued the phenotype of mice, which is a model of primary mitochondrial encephalopathy. Moreover, we observed that long-term treatment with β-RA also reduced the size and content of the white adipose tissue (WAT) that is normally accumulated during aging in wild-type mice, leading to the prevention of hepatic steatosis and an increase in survival at the elderly stage of life. The reduction in WAT content was due to a decrease in adipogenesis, an adaptation of the mitochondrial proteome in the kidneys, and stimulation of glycolysis and acetyl-CoA metabolism. Therefore, our results demonstrate that β-RA acted through different cellular mechanisms, with effects on mitochondrial metabolism; as such, it may be used for the treatment of primary coenzyme Q deficiency, overweight, and hepatic steatosis.
Biomedicines, 2021; 9
33810539: Hidalgo-Gutiérrez A, González-García P, Díaz-Casado ME, Barriocanal-Casado E, López-Herrador S, Quinzii CM, López LC
Metabolic Targets of Coenzyme Q10 in Mitochondria.
Coenzyme Q10 (CoQ) is classically viewed as an important endogenous antioxidant and key component of the mitochondrial respiratory chain. For this second function, CoQ molecules seem to be dynamically segmented in a pool attached and engulfed by the super-complexes I + III, and a free pool available for complex II or any other mitochondrial enzyme that uses CoQ as a cofactor. This CoQ-free pool is, therefore, used by enzymes that link the mitochondrial respiratory chain to other pathways, such as the pyrimidine de novo biosynthesis, fatty acid β-oxidation and amino acid catabolism, glycine metabolism, proline, glyoxylate and arginine metabolism, and sulfide oxidation metabolism. Some of these mitochondrial pathways are also connected to metabolic pathways in other compartments of the cell and, consequently, CoQ could indirectly modulate metabolic pathways located outside the mitochondria. Thus, we review the most relevant findings in all these metabolic functions of CoQ and their relations with the pathomechanisms of some metabolic diseases, highlighting some future perspectives and potential therapeutic implications.
Antioxidants (Basel), 2021; 10
31540029: Díaz-Casado ME, Quiles JL, Barriocanal-Casado E, González-García P, Battino M, López LC, Varela-López A
The Paradox of Coenzyme Q in Aging.
Coenzyme Q (CoQ) is an essential endogenously synthesized molecule that links different metabolic pathways to mitochondrial energy production thanks to its location in the mitochondrial inner membrane and its redox capacity, which also provide it with the capability to work as an antioxidant. Although defects in CoQ biosynthesis in human and mouse models cause CoQ deficiency syndrome, some animals models with particular defects in the CoQ biosynthetic pathway have shown an increase in life span, a fact that has been attributed to the concept of mitohormesis. Paradoxically, CoQ levels decline in some tissues in human and rodents during aging and coenzyme Q (CoQ) supplementation has shown benefits as an anti-aging agent, especially under certain conditions associated with increased oxidative stress. Also, CoQ has shown therapeutic benefits in aging-related disorders, particularly in cardiovascular and metabolic diseases. Thus, we discuss the paradox of health benefits due to a defect in the CoQ biosynthetic pathway or exogenous supplementation of CoQ.

Nutrients, 2019; 11
30898651: Barriocanal-Casado E, Hidalgo-Gutiérrez A, Raimundo N, González-García P, Acuña-Castroviejo D, Escames G, López LC
Rapamycin administration is not a valid therapeutic strategy for every case of mitochondrial disease.
The vast majority of mitochondrial disorders have limited the clinical management to palliative care. Rapamycin has emerged as a potential therapeutic drug for mitochondrial diseases since it has shown therapeutic benefits in a few mouse models of mitochondrial disorders. However, the underlying therapeutic mechanism is unclear, the minimal effective dose needs to be defined and whether this therapy can be generally used is unknown.
EBioMedicine, 2019; 42

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PATIENT-DERIVED IPSCS AND CORTICAL DIFFERENTIATION: A NOVEL MODEL FOR CEREBRAL METHYLMALONIC ACIDURIA

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Methylmalonic aciduria (MMA) is an autosomal recessive disorder commonly caused by deficiency of the enzyme methylmalonyl-CoA mutase (MMUT) or production of its vitamin B12 derived cofactor (adenosyl-cobalamin). Although MMA affects multiple organs through impaired anaplerosis and energetic depletion; the pathomechanisms causing cerebral symptoms, such as lesions to the globus pallidus and developmental delay remain unclear. Patient-derived iPSCs were generated from fibroblasts with non-incorporating Sendai-delivered Yamanaka factors. A dual-SMAD inhibition approach was used to generate neural stem cells (NSCs) and patterning towards dorsal telencephalon was achieved with N2-B27 medium. We demonstrate that control and patient iPSCs progressively adopt PAX6, EOMES, TBR1 and NeuN. However, patient-derived neurons adopt excitatory markers (EOMES) at an accelerated rate. Throughout, we characterised the cellular location of MMUT. We identify heterogeneous populations of patient mitochondria, those with localized and with mis-localized MMUT. Mis-localisation of MMUT is associated to decreased mitochondrial membrane potential (assessed through TMRM) and increased mitochondrial superoxide production (assessed through mitoSOX). In neurons we demonstrate increased autophagy in patient cells using p62 and LC3-I/II. In NSCs, we identified the opposite effect - decreased autophagy. Whilst Caspase-3 was elevated in all time-points of patient cells, the relative increase in NSCs was more pronounced. In this project we have generated and characterised the first physiologically relevant, human neuronal model for MMA. Additionally, we can demonstrate a mitochondrial phenotype that may be associated with MMUT mis-localisation, as well as impaired autophagy and apoptosis.

Pubmed:
29887794: Denley MCS, Gatford NJF, Sellers KJ, Srivastava DP
Estradiol and the Development of the Cerebral Cortex: An Unexpected Role?
The cerebral cortex undergoes rapid folding in an "inside-outside" manner during embryonic development resulting in the establishment of six discrete cortical layers. This unique cytoarchitecture occurs via the coordinated processes of neurogenesis and cell migration. In addition, these processes are fine-tuned by a number of extracellular cues, which exert their effects by regulating intracellular signaling pathways. Interestingly, multiple brain regions have been shown to develop in a sexually dimorphic manner. In many cases, estrogens have been demonstrated to play an integral role in mediating these sexual dimorphisms in both males and females. Indeed, 17β-estradiol, the main biologically active estrogen, plays a critical organizational role during early brain development and has been shown to be pivotal in the sexually dimorphic development and regulation of the neural circuitry underlying sex-typical and socio-aggressive behaviors in males and females. However, whether and how estrogens, and 17β-estradiol in particular, regulate the development of the cerebral cortex is less well understood. In this review, we outline the evidence that estrogens are not only present but are engaged and regulate molecular machinery required for the fine-tuning of processes central to the cortex. We discuss how estrogens are thought to regulate the function of key molecular players and signaling pathways involved in corticogenesis, and where possible, highlight if these processes are sexually dimorphic. Collectively, we hope this review highlights the need to consider how estrogens may influence the development of brain regions directly involved in the sex-typical and socio-aggressive behaviors as well as development of sexually dimorphic regions such as the cerebral cortex.
Front Neurosci, 2018; 12
32314480: Sellers KJ, Denley MCS, Saito A, Foster EM, Salgarella I, Delogu A, Kamiya A, Srivastava DP
Brain-synthesized oestrogens regulate cortical migration in a sexually divergent manner.
Oestrogens play an important role in brain development where they have been implicated in controlling various cellular processes. Several lines of evidence have been presented showing that oestrogens can be synthesized locally within the brain. Studies have demonstrated that aromatase, the enzyme responsible for the conversion of androgens to oestrogens, is expressed during early development in both male and female cortices. Furthermore, 17β-oestradiol has been measured in

foetal brain tissue from multiple species. 17β-oestradiol regulates neural progenitor proliferation as well as the development of early neuronal morphology. However, what role locally derived oestrogens play in regulating cortical migration and, moreover, whether these effects are the same in males and females are unknown. Here, we investigated the impact of knockdown expression of Cyp19a1, which encodes aromatase, between embryonic day (E) 14.5 and postnatal day 0 (P0) had on neural migration within the cortex. Aromatase was expressed in the developing cortex of both sexes, but at significantly higher levels in male than female mice. Under basal conditions, no obvious differences in cortical migration between male and female mice were observed. However, knockdown of Cyp19a1 resulted in an increase in cells within the cortical plate, and a concurrent decrease in the subventricular zone/ventricular zone in P0 male mice. Interestingly, the opposite effect was observed in females, who displayed a significant reduction in cells migrating to the cortical plate. Together, these findings indicate that brain-derived oestrogens regulate radial migration through distinct mechanisms in males and females.
Eur J Neurosci, 2020; 52

34524466: Luciani A, Denley MCS, Govers LP, Sorrentino V, Froese DS
Mitochondrial disease, mitophagy, and cellular distress in methylmalonic acidemia.
Mitochondria-the intracellular powerhouse in which nutrients are converted into energy in the form of ATP or heat-are highly dynamic, double-membraned organelles that harness a plethora of cellular functions that sustain energy metabolism and homeostasis. Exciting new discoveries now indicate that the maintenance of this ever changing and functionally pleiotropic organelle is particularly relevant in terminally differentiated cells that are highly dependent on aerobic metabolism. Given the central role in maintaining metabolic and physiological homeostasis, dysregulation of the mitochondrial network might therefore confer a potentially devastating vulnerability to high-energy requiring cell types, contributing to a broad variety of hereditary and acquired diseases. In this Review, we highlight the biological functions of mitochondria-localized enzymes from the perspective of understanding-and potentially reversing-the pathophysiology of inherited disorders affecting the homeostasis of the mitochondrial network and cellular metabolism. Using methylmalonic acidemia as a paradigm of complex mitochondrial dysfunction, we discuss how mitochondrial directed-signaling circuitries govern the homeostasis and physiology of specialized cell types and how these may be disturbed in disease. This Review also provides a critical analysis of affected tissues, potential molecular mechanisms, and novel cellular and animal models of methylmalonic acidemia which are being used to develop new therapeutic options for this disease. These insights might ultimately lead to new therapeutics, not only for methylmalonic acidemia, but also for other currently intractable mitochondrial diseases, potentially transforming our ability to regulate homeostasis and health.
Cell Mol Life Sci, 2021; 78

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MITOCHONDRIAL DYSFUNCTION AND DEPRESSION : THE CHICKEN OR THE EGG ?

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Major depressive disorder (MDD) is a leading cause of disability. Although the precise pathomechanisms underlying MDD development are still not completely understood, mitochondria and energy metabolism have been increasingly considered to be involved in the pathomechanisms of depression. Cells from a cohort of MDD patients and non-depressed controls were previously analyzed and provide a data pool, which we now use as a reference to conduct case studies. Our purpose is to confront unique patients to our reference cohort, and unravel their specificities. The case study consists of a patient diagnosed with a mitochondriopathy, but whose precise pathology is still unclear, and of an anti-depressant (AD) resistant patient. We investigated cellular and mitochondrial functions in the peripheral, neural and neuronal lineage. In those different cell types, bioenergetic functions and Ca^{2+} homeostasis were assessed. In addition, mitochondrial content and cell size were evaluated. iNeurons were analyzed by whole-cell voltage- and current-clamp recording. In the AD-resistant patient, we found hyperactive oxidative phosphorylation and increased oxidative stress. iNeurons showed a more negative resting membrane potential (RMP). In the mitochondriopathy patient, we observed generally impaired bioenergetics functions and higher RMP in iNeurons. Interestingly, in both patients and across all cell types, as well as in the full cohort, patients cells were significantly smaller. Our findings reveal an interesting physiology in both case study patients, which is also consistent across reprogramming and further neural and neuronal differentiation. The case studies offer a singular perspective on MDD and mitochondrial dysfunctions, and how they relate to each other.

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BRAIN PENETRATION OF CAMELID SINGLE-DOMAIN ANTIBODIES (VHH) : A PRIVILEGE OR A MORE COMMON TRAIT ?

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Monoclonal antibodies have been increasingly used over the last decades in several pathologies such as inflammation and cancers. However, their use for the treatment of neurological and neurodegenerative diseases is not common yet. Their delivery into the brain parenchyma is challenging because of the blood-brain barrier (BBB). It makes the development of immunotherapy for brain diseases difficult. In the past few years, camelid single-domain antibodies (also known as Nanobody® or VHH) have gained interest in the biomedical field. Their small size (15kDa), conformational specificity, easy engineering, low immunogenicity and low cost production make them a good alternative to conventional antibodies. Besides, some VHHs have been reported to naturally cross the BBB (Wouters J. et al., 2020 ; Ruiz-Lopez E. and Schumacher A. J., 2021, ; Pothin E. et al., 2020). The present work describes the discovery of the first brain-penetrating VHHs specific to one metabotropic receptor subtype. An approach based on immunohistochemistry showed labelling in brain parenchyma after their peripheral administration in wild-type mice. Our results suggest that such VHH may be of interest for therapeutic intervention into the brain.

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INCREASED EEG ALPHA POWER IS ASSOCIATED WITH HIGHER VITAMIN D3 AND LOWER IL-8 LEVELS IN BORNA-POSITIVE DEPRESSED PATIENTS

POSTER SESSION 02 - SECTION: NEW PERSPECTIVES IN NEURODEGENERATION

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Borna Disease Virus (BDV) is a neurotropic, negative single-stranded RNA virus that induces inflammation in olfactory and limbic structures within a broad range of warm-blooded animals. Yet, there is no conclusive evidence that BDV is infecting man nor that it might constitute a co-factor in the etiology of human psychiatric disorders, although increases in BDV-specific RNA and BDV antibodies were observed in the blood of patients suffering from Major Depressive Disorder (MDD). Some studies indicate that human reactive antibodies exhibit low avidity for BDV antigens, offering the possibility that they might have been produced by exposure to a related cellular immunogen, which is up-regulated in MDD. The aim of the present study was to examine possible associations between MDD patient´s BDV-status (positive vs. negative), vitamin D3 level, plasma level of proinflammatory Interleukin 8 (IL-8), and resting-state EEG activity. We demonstrated a higher fronto-central EEG alpha power and lower IL-8 level in BDV-positive compared to BDV-negative patients. Both groups do not differ in their severity of depression. In BDV-positive patients a negative correlation between concentrations of proinflammatory IL-8 and anti-inflammatory vitamin D3 was observed. Thus, lower IL-8 levels are accompanied by high concentrations of vitamin D3. To summarize, our results suggest that MDD patients show BDV-dependent alterations in their immune and neural system. We suggest that in BDV-positive MDD patients, high levels of vitamin D3 serve to protect from a long-lasting overreaction of the immune system by inhibition of the NF-κB signaling pathway, which is responsible for an up-regulation of IL-8.

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NR2F1 HAPLOINSUFFICIENCY ALTERS THE MORPHOLOGY OF ADULT-BORN NEURONS IN THE HIPPOCAMPUS OF A MOUSE MODEL OF THE NEURODEVELOPMENTAL DISORDER BBSOAS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Intellectual disability (ID) is a neurodevelopmental pathological condition characterised by limitations in intellectual functioning and adaptive behaviour. Although the causes are highly heterogeneous, genetic factors take a large part in the etiology of ID. The Bosch-Boonstra-Schaaf optic atrophy-intellectual syndrome (BBSOAS; OMIN#615722), is a rare disorder caused by mutations in the NR2F1 gene, characterized by ID associated to global developmental delay, optic nerve atrophy, hypotonia, seizure and autistic traits. The transcriptional regulator Nr2f1 (i.e., COUP-TFI) is a key player in multiple cellular processes during brain development. Interestingly, alterations in postnatal hippocampal neurogenesis have been reported in animal models of ID and recent findings suggest that a deficit in hippocampal plasticity may contribute to BBSOAS. Here, to investigate the possible effects of Nr2f1 haploinsufficiency on the hippocampal circuit we took advantage of a recently validated BBSOAS mouse model (i.e., constitutive Nr2f1-heterozygous mice) and focussed on the adult hippocampal dentate gyrus (DG). Although, no differences were found in the density of newly generated doublecortin (DCX)-positive immature neurons, data from 3D morphometric reconstruction strongly suggests that Nr2f1 haploinsufficiency influences dendritic architecture and proper development of adult-born DG neurons, leading to the appearance of atypical and peculiar neuronal morphologies that are usually associated with pathological conditions and aberrant hippocampal circuitry rearrangements. Interestingly, preliminary data on the expression of the neuronal activity-dependent gene Npas4 started to depict altered recruitment of neuronal ensemble in the DG of Nr2f1-heterozygous mice. Ongoing and future investigations are aimed at elucidating the mechanisms underlying of the observed defects.

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STXBP1 ENCEPHALOPATHY IS CAUSED BY THE FAILURE OF EXCITATORY SYNAPSES TO RECRUIT INHIBITION IN FEEDFORWARD INHIBITORY MICROCIRCUITS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Neurodevelopmental disorders affect 2-5% of children at birth. Most have a genetic origin and are caused by *de novo* mutations affecting a restricted number of genes. We studied the functional consequences of mutations affecting the presynaptic protein Munc18-1 (encoded by *STXBP1*) because of its high prevalence in intellectual disabilities (ID), epilepsy, autism, and movement disorders. Munc18-1 chaperones the formation of the SNARE-complex, which is necessary to fuse synaptic vesicles. Most currently studied human disease mutations result in an unstable protein and in haploinsufficiency. Therefore, the disease can be modeled in mice by disrupting one of the two alleles (*STXBP1* heterozygotes). *STXBP1*$^{+/-}$ mice show cortical hyperexcitability and intermittent epilepsy, but the underlying synaptic deficits are not understood, since Munc18-1 is essential for both excitatory and inhibitory synapses and haploinsufficiency is, in principle, expected to affect both equally. We investigated the underlying synaptic deficits by simultaneously recording the activity of parvalbumin (PV) expressing interneurons and connected pyramidal cells. Our results demonstrate that inhibition is deficient in feedforward inhibition microcircuits in the neocortex of *STXBP1*$^{+/-}$ animals. Surprisingly, we found that inhibitory synapses are almost unaffected by the mutation. Instead, the deficit in inhibition is caused by the inability of excitatory synapses to recruit PV interneurons, which results in a lack of feedforward inhibition leading to the hyperexcitability of principal cells. The data show that deficits in excitatory synapses are the main explanation for cortical hyperexcitability in mouse models for STXBP1 syndrome, which may shift the focus of therapy design for human patients.

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ANALYSIS OF THE SYNAPTIC CONTRIBUTION OF THE DPYSL5 GENE INVOLVED IN NEURODEVELOPMENTAL DISORDERS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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The *DPYSL5* (Dihydropyrimidinase like 5) gene encodes a cytosolic protein strongly expressed in the developing brain and which plays a role in neuronal migration, axonal guidance and neuritic growth. We recently described missense mutations (p.E41K, recurrent ; p.G47R) in nine individuals presenting intellectual disability and brain malformations (agenesis of corpus callosum, cerebellar hypoplasia), causing abnormal dendritic development and impaired interaction with the cytoskeleton. Interestingly, various studies showed the presence of DPYSL5 in synaptic fractions, suggesting a role in the mature neuron. Here, our main objective was to clarify the physiological and pathological role of DPYSL5 at the synapse. We studied primary cultures of mouse hippocampal neurons transfected with normal (WT) and mutated variants of DPYSL5-GFP to evaluate their impact on synapse development and maturation. In parallel, we analyzed brains lysates from adult mouse models (*Dpysl5*$^{-/-}$, *Dpysl5*$^{+/-}$) to assess the consequences of *Dpysl5* inactivation on the expression of synaptic proteins. We found that the overexpression of both WT and mutated forms of DPYSL5 increased the synaptic density in vitro. However, while WT DPYSL5-GFP caused an increased number of mature spines when compared to GFP neurons, this effect was absent for both variants (i.e. similar to GFP neurons). Our study also revealed a significant alteration of the expression of excitatory synaptic protein (PSD95, vGLUT1) in *Dpysl5*$^{-/-}$ mice compared to WT mice. Taken together, these results suggest an involvement of DPYSL5 in synapse formation and maturation of excitatory neuronal networks, and they further highlight the contribution of glutamatergic synapse dysfunction in neurodevelopmental disorders.

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CONTRIBUTION OF THE ADIPOCYTE HORMONE LEPTIN IN THE PATHOGENESIS OF RETT SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder characterized by a loss of acquired speech, hand stereotypies, gait abnormalities, breathing difficulties and seizures appearing after an apparently normal postnatal development. This syndrome is caused by mutations in the X-linked chromosome gene *Methyl-CpG-binding protein 2* (*MECP2*) encoding a transcriptional regulator. Currently there are no cures for this disease. In spite of different causative mutated gene and biological pathways affected, epidemiological and animal studies revealed abnormally elevated circulating levels of the adipocyte hormone leptin in patients with RTT and *Mecp2*-deficient mice. Besides its canonical role in the control of satiety and energy expenditure, leptin acts on many brain areas modulating cognitive functions, anxiety, breathing and more. Consequently, altered leptin levels may contribute to the RTT-associated neuronal network dysfunctions. In the present study, we show that serum leptin levels are elevated in early (postnatal day (P) 30) and late symptomatic (P60) male *Mecp2-null* mice. We also show that daily subcutaneous injections of a validated leptin antagonist (Peg-SMLA, a competitive inhibitor of leptin BBB transport), during 10 days from P40, prevent the body weight loss, restore the hippocampal excitatory/inhibitory (E/I) balance, slows down the progression of breathing dysfunctions in the symptomatic male *Mecp2-null* mice. Conversely, leptin-treatment of wild type mice mimics some of the phenotypic manifestations observed in *Mecp2-null* mice, i.e. increased hippocampal E/I balance and increased number of apneas. These data provide further insights on the possible contribution of leptin in RTT pathogenesis and offer therapeutic perspective to alleviate RTT symptoms.

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MULTI-SCALE ANALYSIS OF A NOVEL KNOCK-IN MOUSE MODEL REVEALS NEW PATHOGENIC MECHANISMS UNDERLYING SEVERE PAK3-LINKED NEURODEVELOPMENTAL DISORDERS.

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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The aetiology of neurodevelopmental diseases is complex, involving environmental and genetic factors: numerous genes were identified in several pathologies such as intellectual disability (ID) and autism spectrum disorder (ASD). *PAK3*, one of the first genes identified in X-linked ID, is involved in brain development and plasticity. Mutations in the *PAK3* gene are responsible for a large clinical spectrum, including ID, ASD, microcephaly or macrocephaly, epilepsy, and other neuropsychiatric symptoms. The pathogenic mechanisms underlying these symptoms are not well-known. Consequently, uncovering the precise genotype/phenotype correlation associated with *PAK3* mutations is challenging. We are deciphering the biochemical properties of PAK3 variants using *in-vitro* analyses of a broad mutation collection responsible for ID of varying severity. Our results helped us hypothesise that the biochemical anomalies of PAK3 variants affecting protein expression and stability, kinase activity, and protein-protein interactions are at the core of *PAK3* mutation pathogenicity. To go further, we generated a knock-in mouse expressing the *PAK3*-G424R mutation responsible for a severe clinical case. The *PAK3*-G424R mouse presents hyperactivity, stereotypies, microcephaly and cognitive defects, reminiscent of the patient's clinical data, confirming the relevance of this new model. Our results helped us put forward a new molecular pathogenic mechanism that could be at the root of the behavioural symptoms, electrophysiological dysfunctions and developmental defects observed in severe disease cases. Advances in our understanding of the pathophysiology of *PAK3*-linked disorders will pave the way for substantial advances in personalised treatment. Supported by the National Research Agency (ANR-CE17-0053), Phenomin-Fondation Maladies rares, CNRS and Université Paris-Saclay.

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CHARACTERIZATION OF DE NOVO GABAB2 VARIANTS LINKED TO RETT SYNDROME AND ENCEPHALOPATHIC EPILEPSY

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Dysfunction of $GABA_B$ receptor (GBR) signaling has long been associated with neurological and psychiatric conditions, including epilepsy, spasticity, chronic pain, depression and schizophrenia. Recently, several *de novo* monoallelic variants have been identified in the $GABA_{B2}$ transmembrane domain 3 (TM3) of Rett Syndrome (RTT/A567T) and TM6 of Epileptic Encephalopathy (EE1/S695I, EE2/I705N) patients. In biochemical assays with heterologous cells, GABA exhibited decreased efficacy at the variant receptors (Yoo et al., 2017), presumably relating to increased constitutive receptor activity (Vuillaume et al., 2018). Using electrophysiology with transfected HEK293 cells, we now confirm increased constitutive activity of the variant receptors, which in turn causes an increase in tonic activity of Kir3-type effector potassium channels. Mutant receptors expressed in HEK293 cells also showed decreased responses to saturating concentrations of GABA. Patients carrying these mutations suffer from epileptic seizures and intellectual disability. To study the synaptic effects of a $GABA_{B2}$ variant in the brain, we developed a mouse line carrying the amino acid substitution corresponding to the human EE2 variant in the mouse gene (EE2/I704N). We have started to analyze these mice for electrophysiological phenotypes. Preliminary data indicate that the induction of LTP in hippocampal slices of EE2/I704N mice is compromised. We will provide an update on the characterization of homozygous and heterozygous EE2/I704N mice. References: Vuillaume et al. A Novel Mutation in the Transmembrane 6 Domain of GABBR2 Leads to a Rett-like Phenotype. 2018 *Annals of Neurology*, *83*(2): 437–439 Yoo et al. GABBR2 mutations determine phenotype in rett syndrome and epileptic encephalopathy. 2017 *Annals of Neurology*, *82*(3): 466–478

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-414

IDENTIFYING NEW MOLECULES TARGETING BKCA CHANNELS FOR THE TREATMENT OF AUDITORY IMPAIRMENTS IN TWO MOUSE MODELS OF NEURODEVELOPMENTAL DISORDERS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Auditory impairments, such as hearing loss and hyperacusis (i.e. increased perception of sounds), affect a large part of the human population with a dramatic impact on life quality. These auditory dysfunctions are commonly described in patients with neurodevelopmental disorders (NDDs) and often exacerbate the communication and social deficits typical of these conditions. In subjects with two major NDDs characterized by auditory abnormalities – namely, Fragile X syndrome (FXS) and Williams-Beuren syndrome (WBS) - a reduction in the expression and functionality of a specific population of ion channels, the Big conductance calcium-activated potassium (BK_{Ca}) channels, has been reported. This reduction could be linked to the hearing disorders manifested by these patients. Indeed, BK_{Ca} channels are highly expressed in the auditory pathway and play a key role in regulating the membrane potential of cochlear hair cells as well as neurons. BK_{Ca} channels are therefore potential major therapeutic candidates for auditory impairments in the context of NDDs. Here we tested the effects of a BK_{Ca} channel opener molecule (Chlorzoxazone) on the abnormal auditory phenotypes of two mouse models of FXS and WBS, i.e., respectively the Fmr1-KO and CD mouse lines. To this end, we combined acute injections of Chlorzoxazone with behavioural, electrophysiological and molecular analyses, in order to elucidate the role of these ion channels in the etiopathology of acoustic dysfunction. Our results clearly demonstrate that acting on BK_{Ca} channels is a valuable therapeutic strategy to treat acoustic dysfunction in these NDDs.

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GENE THERAPY TARGETING THE BLOOD-BRAIN BARRIER IMPROVES NEUROLOGICAL SYMPTOMS IN A MODEL OF GENETIC MCT8 DEFICIENCY

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Adriana Arrulo Pereira[1], Sivaraj Sundaram[1], Hannes Köpke[1], Helge Müller-Fielitz[1], Meri De Angelis[2], Timo Müller[3], Heike Heuer[4], Jakob Körbelin[2], Markus Krohn[1], Jens Mittag[5], Ruben Nogueiras[6], Vincent Prevot[7], Markus Schwaninger[1]
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The monocarboxylate transporter 8 (MCT8) transports thyroid hormones across brain barriers. Inactivating mutations in the gene encoding for MCT8 (*SLC16A2)* results in MCT8 deficiency also known as Allan-Herndon-Dudley syndrome (AHDS). AHDS is an important cause of X-linked intellectual and motor disability. While thyroid hormone analogues improve peripheral changes of MCT8 deficiency, no treatment of the neurological symptoms is available so far. Therefore, we developed a gene replacement therapy in *Mct8*- and *Oatp1c1*-deficient mice, a well-established animal model for AHDS. In our study we show that targeting brain endothelial cells by intravenous injection of AAV-BR1-*Mct8* in neonatal mice increased the T3 levels in the brain and ameliorated morphological and functional parameters associated with the disease. This gene replacement therapy also resulted in long-lasting improvement in motor coordination. Together, our results support the concept that MCT8 mediates the transport of thyroid hormones into the brain and indicates that a readily accessible vascular target can help overcome the consequences of the severe disability associated with MCT8 deficiency.

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EVALUATION OF A PRE/PERINATAL TREATMENT IN A DOWN SYNDROME MOUSE MODEL.

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Manon Moreau[1], Rodolphe Dard[2], Janany Kandiah[1], Nadim Kassis[3], Julien Dairou[4], Boris Matrot[5], Anne-Claude Camproux[6], François Vialard[2], Nathalie Janel[1]
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Down syndrome (DS) is a genetic disease characterized by a supernumerary chromosome 21. Intellectual deficiency (ID) is one of the most prominent features of DS. Central nervous system defects lead to learning disabilities, motor and language delays and memory impairments. Previous studies showed a decrease in neurogenesis and proliferation, an increase of neuron apoptosis and astrogliogenesis, deregulations related to chronic neuroinflammation. So far, there is no prenatal treatment that is targeting the ID in DS. A natural treatment, based on the administration of a small molecule, has showed some neuroprotective effects such as decreased apoptosis with anti-inflammatory properties. We therefore evaluated the administration of this treatment during the gestation in a mouse model, the Dp1(16)Yey mice, that carry a duplication of a relevant orthologous region of the human chromosome 21 (HSA21). The effect of the treatment on mice brain is studied at a juvenile stage and adult stage by molecular and histological analysis and behavioral tests. Our results at postnatal day 11 (P11) show beneficial effects on microglia and astrocytes markers as well on a marker of HSA21, the Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A (DYRK1A) which is overexpressed and contributes to the specific cognitive defects in DS. Taken together, our results show that this treatment could represent a clinical candidate for cognitive deficits in DS patients and an alternative for parents to decide the fate of the pregnancy of a fetus with a DS.

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BOARD NUMBER: S02-417

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS WITH CACNA1A VARIANTS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Marina Hommersom[1], Bart Van De Warrenburg[2], Nael Nadif Kasri[1], Hans Van Bokhoven[1]
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Episodic ataxia type 2 (EA2) is an autosomal dominant disorder in which patients experience recurrent episodes, characterized by ataxia, slurred speech, interictal nystagmus and vertigo. Also, chronic progressive ataxia can manifest in patients, both with and without previous episodic ataxia, carrying these variants. EA2 is caused by loss-of-function variants in *CACNA1A*, encoding the pore-forming α1 subunit ($Ca_V2.1$) of the P/Q-type voltage-gated calcium channel. $Ca_V2.1$ is widely expressed in the central nervous system, and one of its most important functions is the coupling of Ca^{2+} influx to neurotransmitter release in presynaptic terminals. As this channel has never been investigated in a human model, we aimed to study the electrophysiological phenotype of human induced pluripotent stem cell (hiPSC)-derived neurons with *CACNA1A* variants. We have derived hiPSCs from three ataxia patients with heterozygous loss-of-function variants in *CACNA1A*, and edited the *CACNA1A* gene by CRISPR/Cas9 in one control and one patient cell line to form isogenic pairs. These hiPSCs were differentiated to neurons and recorded on micro electrode arrays (MEAs). Upon development of an *in vitro* neuronal network, patient and control lines showed a regular pattern of network bursts and no distinct electrophysiological phenotype of the patient neurons was detected. However, when synaptic plasticity was induced, neurons with complete *CACNA1A* loss-of-function, showed a distinct MEA pattern. Our data show that basal neuronal network activity is not affected by loss-of-function of *CACNA1A*, but that a phenotype can be induced by triggering the network. This MEA phenotype offers opportunities for diagnostic pipelines and drug screens in *CACNA1A*.

Pubmed:

34806130: Hommersom MP, van Prooije TH, Pennings M, Schouten MI, van Bokhoven H, Kamsteeg EJ, van de Warrenburg BPC
The complexities of CACNA1A in clinical neurogenetics.
Variants in CACNA1A are classically related to episodic ataxia type 2, familial hemiplegic migraine type 1, and spinocerebellar ataxia type 6. Over the years, CACNA1A has been associated with a broader spectrum of phenotypes. Targeted analysis and unbiased sequencing of CACNA1A result not only in clear molecular diagnoses, but also in large numbers of variants of uncertain significance (VUS), or likely pathogenic variants with a phenotype that does not directly match the CACNA1A spectrum. Over the last years, targeted and clinical exome sequencing in our center has identified 41 CACNA1A variants. Ultimately, variants were considered pathogenic or likely pathogenic in 23 cases, with most phenotypes ranging from episodic or progressive ataxia to more complex ataxia syndromes, as well as intellectual disability and epilepsy. In two cases, the causality of the variant was discarded based on non-segregation or an alternative diagnosis. In the remaining 16 cases, the variant was classified as uncertain, due to lack of opportunities for segregation analysis or uncertain association with a non-classic phenotype. Phenotypic variability and the large number of VUS make CACNA1A a challenging gene for neurogenetic diagnostics. Accessible functional read-outs are clearly needed, especially in cases with a non-classic phenotype.
J Neurol, 2022; 269
34286667: Linda K, Lewerissa EI, Verboven AHA, Gabriele M, Frega M, Klein Gunnewiek TM, Devilee L, Ulferts E, Hommersom M, Oudakker A, Schoenmaker C, van Bokhoven H, Schubert D, Testa G, Koolen DA, de Vries BBA, Nadif Kasri N
Imbalanced autophagy causes synaptic deficits in a human model for neurodevelopmental disorders.
Macroautophagy (hereafter referred to as autophagy) is a finely tuned process of programmed degradation and recycling of proteins and cellular components, which is crucial in neuronal function and synaptic integrity. Mounting evidence implicates chromatin remodeling in fine-tuning autophagy pathways. However, this epigenetic regulation is poorly understood in neurons. Here, we investigate the role in autophagy of KANSL1, a member of the nonspecific lethal complex, which

acetylates histone H4 on lysine 16 (H4K16ac) to facilitate transcriptional activation. Loss-of-function of KANSL1 is strongly associated with the neurodevelopmental disorder Koolen-de Vries Syndrome (KdVS). Starting from KANSL1-deficient human induced-pluripotent stem cells, both from KdVS patients and genome-edited lines, we identified SOD1 (superoxide dismutase 1), an antioxidant enzyme, to be significantly decreased, leading to a subsequent increase in oxidative stress and autophagosome accumulation. In KANSL1-deficient neurons, autophagosome accumulation at excitatory synapses resulted in reduced synaptic density, reduced GRIA/AMPA receptor-mediated transmission and impaired neuronal network activity. Furthermore, we found that increased oxidative stress-mediated autophagosome accumulation leads to increased MTOR activation and decreased lysosome function, further preventing the clearing of autophagosomes. Finally, by pharmacologically reducing oxidative stress, we could rescue the aberrant autophagosome formation as well as synaptic and neuronal network activity in KANSL1-deficient neurons. Our findings thus point toward an important relation between oxidative stress-induced autophagy and synapse function, and demonstrate the importance of H4K16ac-mediated changes in chromatin structure to balance reactive oxygen species- and MTOR-dependent autophagy.: APO: apocynin; ATG: autophagy related; BAF: bafilomycin A; BSO: buthionine sulfoximine; CV: coefficient of variation; DIV: days in vitro; H4K16ac: histone 4 lysine 16 acetylation; iPSC: induced-pluripotent stem cell; KANSL1: KAT8 regulatory NSL complex subunit 1; KdVS: Koolen-de Vries Syndrome; LAMP1: lysosomal associated membrane protein 1; MAP1LC3/LC3: microtubule associated protein 1 light chain 3; MEA: micro-electrode array; MTOR: mechanistic target of rapamycin kinase; NSL complex: nonspecific lethal complex; 8-oxo-dG: 8-hydroxydesoxyguanosine; RAP: rapamycin; ROS: reactive oxygen species; sEPSCs: spontaneous excitatory postsynaptic currents; SOD1: superoxide dismutase 1; SQSTM1/p62: sequestosome 1; SYN: synapsin; WRT: wortmannin.
Autophagy, 2022; 18

34143952: Polla DL, Edmondson AC, Duvet S, March ME, Sousa AB, Lehman A, , Niyazov D, van Dijk F, Demirdas S, van Slegtenhorst MA, Kievit AJA, Schulz C, Armstrong L, Bi X, Rader DJ, Izumi K, Zackai EH, de Franco E, Jorge P, Huffels SC, Hommersom M, Ellard S, Lefeber DJ, Santani A, Hand NJ, van Bokhoven H, He M, de Brouwer APM
Bi-allelic variants in the ER quality-control mannosidase gene EDEM3 cause a congenital disorder of glycosylation.
EDEM3 encodes a protein that converts ManGlcNAc isomer B to ManGlcNAc. It is involved in the endoplasmic reticulum-associated degradation pathway, responsible for the recognition of misfolded proteins that will be targeted and translocated to the cytosol and degraded by the proteasome. In this study, through a combination of exome sequencing and gene matching, we have identified seven independent families with 11 individuals with bi-allelic protein-truncating variants and one individual with a compound heterozygous missense variant in EDEM3. The affected individuals present with an inherited congenital disorder of glycosylation (CDG) consisting of neurodevelopmental delay and variable facial dysmorphisms. Experiments in human fibroblast cell lines, human plasma, and mouse plasma and brain tissue demonstrated decreased trimming of ManGlcNAc isomer B to ManGlcNAc, consistent with loss of EDEM3 enzymatic activity. In human cells, ManGlcNAc to ManGlcNAc conversion is also diminished with an increase of GlcManGlcNAc. Furthermore, analysis of the unfolded protein response showed a reduced increase in EIF2AK3 (PERK) expression upon stimulation with tunicamycin as compared to controls, suggesting an impaired unfolded protein response. The aberrant plasma N-glycan profile provides a quick, clinically available test for validating variants of uncertain significance that may be identified by molecular genetic testing. We propose to call this deficiency EDEM3-CDG.
Am J Hum Genet, 2021; 108

34031815: Hommersom MP, Buijsen RAM, van Roon-Mom WMC, van de Warrenburg BPC, van Bokhoven H
Human Induced Pluripotent Stem Cell-Based Modelling of Spinocerebellar Ataxias.
Dominant spinocerebellar ataxias (SCAs) constitute a large group of phenotypically and genetically heterogeneous disorders that mainly present with dysfunction of the cerebellum as their main hallmark. Although animal and cell models have been highly instrumental for our current insight into the underlying disease mechanisms of these neurodegenerative disorders, they do not offer the full human genetic and physiological context. The advent of human induced pluripotent stem cells (hiPSCs) and protocols to differentiate these into essentially every cell type allows us to closely model SCAs in a human context. In this review, we systematically summarize recent findings from studies using hiPSC-based modelling of SCAs, and discuss what knowledge has been gained from these studies. We conclude that hiPSC-based models are a powerful tool for modelling SCAs as they contributed to new mechanistic insights and have the potential to serve the development of genetic therapies. However, the use of standardized methods and multiple clones of isogenic lines are essential to increase validity and reproducibility of the insights gained.
Stem Cell Rev Rep, 2022; 18

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BOARD NUMBER: S02-418

LOSS-OF-FUNCTION MUTATIONS IN ANK2 LINKED TO EARLY ONSET EPILEPSY: TO FIRE OR NOT TO FIRE

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Elly Lewerissa[1], Maria Teunissen[2], Eline Van Hugte[1], Shan Wang[1], Han Brunner[1], Nael Nadif Kasri[3]
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Mutations in *Ankyrin 2* (*ANK2*), previously known to cause cardiac arrythmia, may also cause epilepsy. In this study we delineate epilepsy phenotypes in patients with pathogenic variants in *ANK2*, and explore the effects on neuronal network dynamics and homeostatic plasticity in human neurons to explain the pathophysiological mechanism of epilepsy in these patients. We collected clinical data of twelve patients with heterozygous *de novo* loss-of-function (LoF) variants in *ANK2*. In addition, we generated a heterozygous LoF allele of *ANK2*using CRISPR/Cas9 in human induced pluripotent stem cells (hiPSCs). hiPSCs were differentiated towards excitatory neurons of which we characterized axon initial segment (AIS) structure and plasticity, somatodendritic morphology, and measured spontaneous electrophysiological responses using micro-electrode arrays (MEAs). We found epilepsy in seven of twelve (58%) of patients, all with early onset and variable severity of epilepsy. Autism spectrum disorder and intellectual disability were also frequently reported. Using MEAs, we found that hiPSCs-derived neurons with heterozygous LoF of *ANK2* show a hyperactive and desynchronized neuronal network. *ANK2* deficient neurons also showed altered AIS structure and plasticity of which is impaired upon activity-dependent modulation. The altered AIS structure may contribute to the observed increased neuronal network excitability. In conclusion, early onset epilepsy with variable severity is an important phenotype of *ANK2* LoF variants. Functional *in vitro* data of *ANK2*-deficient human neurons show a specific neuronal phenotype in which reduced ANKB disrupts AIS structure and thereby impairs activity-dependent plasticity of the AIS, resulting in increased neuronal excitability.

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BOARD NUMBER: S02-419

A REPROGRAMMING-BASED STRATEGY FOR DRUG REPOSITIONING IN MITOCHONDRIAL DNA-ASSOCIATED LEIGH SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Jesper Bergmans[1], Nael Nadif Kasri[2], Werner Koopman[1], Merel Adjobo-Hermans[3]
[1]Radboud University Medical Center, Pediatrics, Nijmegen, Netherlands, [2]Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Human Genetics, Nijmegen, Netherlands, [3]Radboud University Medical Center, Biochemistry, Nijmegen, Netherlands

Mitochondrial DNA (mtDNA)-associated Leigh syndrome (MILS) is an untreatable brain disease affecting 1/100,000 newborns. MILS is typically caused by mtDNA mutations in the *MT-ATP6* gene encoding the a-subunit of ATP-synthase, a vital part of the mitochondrial oxidative phosphorylation system. Drug discovery is particularly challenging for MILS. The limited access to patient neural tissue and the challenges in mtDNA engineering hinder the development of the cellular and animal models needed for intervention development. Here we will show our consortium's reprogramming-based strategy for large-scale phenotype-driven screening of repurposable drugs in cells derived from MILS patients, allowing the identification of novel therapeutics. This strategy includes screening a large library of repurposable compounds, followed by validation of the top hits. This validation will include both mitochondrial profiling of induced pluripotent stem cell (iPSC)-derived neural progenitor cells and electrophysiological characterization of iPSC-derived neuronal cultures on micro-electrode arrays, as well as multi-omics analysis and subsequent computation-based modeling. A proof-of-concept study already demonstrated that this approach is feasible and relevant [1]. By extending this approach, our consortium aims to identify existing drugs suited for repositioning as interventions in MILS, laying the foundation for a multi-national clinical trial and a concrete path towards a treatment for MILS. Moreover, we will establish a paradigmatic working pipeline for reprogramming-driven drug discovery in rare neurological disorders. [1] Lorenz, C., et al., *Human iPSC-Derived Neural Progenitors Are an Effective Drug Discovery Model for Neurological mtDNA Disorders.* Cell Stem Cell, 2017. **20**(5): p. 659-674.e9.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S02-420

SIALIC ACID BIOSYNTHESIS IS ESSENTIAL FOR NETWORK FORMATION OF IPSC-DERIVED EXCITATORY NEURONS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Rachel Mijdam[1], Raisa Veizaj[1], Hanneke Kwast[2], Marek Noga[2], Eline Van Hugte[3], Chantal Schoenmaker[3], Emma Dyke[3], Thomas Boltje[4], Dirk Schubert[5], Nael Nadif Kasri[3], Dirk Lefeber[1]
[1]Radboudumc, Neurology, Nijmegen, Netherlands, [2]Radboudumc, Translational Metabolic Laboratory, Nijmegen, Netherlands, [3]Radboudumc, Donders Institute for Brain, Cognition, and Behaviour, Human Genetics, Nijmegen, Netherlands, [4]Radboud University, Institute for Molecules and Materials, Department Of Organic Chemistry, Nijmegen, Netherlands, [5]Donders Institute for Brain, Cognition and Behavior, Department Of Cognitive Neuroscience, Nijmegen, Netherlands

Multiple congenital disorders of glycosylation (CDG) are linked to sialic acid, a sugar commonly found in all human tissues, with the highest expression in the brain. Of these, only NANS-CDG is characterized by severe neurological symptoms. How changes in sialic acid biosynthesis lead to neurological symptoms is poorly understood. Here, we studied how the sialic acid biosynthesis pathway (SBP) influences neuronal network formation. To assess the requirement of sialic acid and the effect of disease causing variants in this pathway, excitatory neurons (iNeurons) were derived from control induced pluripotent stem cells (iPSC) treated with the sialyltransferase inhibitor (SiaFEt) and from three NANS-CDG patient-derived iPSC lines. Changes in sugar metabolite levels were followed by mass spectrometry-based metabolomics and spontaneous electrophysiological activity was monitored with micro electrode arrays. We found that SiaFEt inhibits the entire SBP and disturbs network formation in iNeurons, leading to a lower firing rate and shorter but more frequent bursts. NANS-CDG iNeurons presented with a delayed network formation. Metabolomics revealed high ManNAc-6P levels, but no reduction in sialic acid. Surprisingly, also the hexosamine biosynthesis pathway was affected, since GlcNAc-1P, GalNAc-1P and UDP-HexNAc levels were decreased. In conclusion, we demonstrate that disturbances in the SBP lead to abnormal neuronal network formation, but in NANS-CDG this cannot directly be related to a lack of sialic acid.

Pubmed:
31022214: Jussen L, Lagro-Janssen T, Leenders J, Logie C, Mijdam R
Underreported and unknown student harassment at the Faculty of Science.
Reports of sexual harassment at medical faculties throughout the world, including the Radboud University, raised the question how prevalent this is at the Faculty of Science. We performed a survey among students to assess their experiences with harassment. This questionnaire consisted of questions from the EGERA survey, a questionnaire held among staff of multiple European Universities. We found that 9% of the respondents had observed or experienced harassment at the Faculty. Hardly any of these cases were reported to one of the institutional services. Moreover, most students did not now any of the provided services. We therefore suggest raising awareness on harassment and to make students more familiar with the trust person.
PLoS One, 2019; 14

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-421

IDENTIFYING DEREGULATED AUTOPHAGY AS UNDERLYING MECHANISM OF NEURODEVELOPMETNAL DISORDERS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Katrin Linda[1], Elly Lewerissa[1], Ummi Ciptasari[1], Shan Wang[2], Hans Van Bokhoven[1], Dirk Schubert[2], Nael Nadif Kasri[1]
[1]Donders Institute for Brain, Cognition and Behaviour, Radboudumc, Human Genetics, Nijmegen, Netherlands, [2]Donders Institute for Brain, Cognition and Behavior, Cognitive Neuroscience, Nijmegen, Netherlands

Autophagy is an evolutionary highly conserved, catabolic process, that is important for the clearance of cytosolic contents to maintain cellular homeostasis and survival. Recent findings point towards a critical role for autophagy in brain function, not only by preserving neuronal health, but especially by controlling different aspects of neuronal development and functioning. More precisely, knockdown of different autophagy genes showed to affect neural stem cell proliferation and differentiation. Furthermore, reduced autophagic activity revealed to result in structural neuronal changes, like decreased synaptic pruning. In line with this, mutations in autophagy regulating genes could be linked to various key characteristics and symptoms of neurodevelopmental disorders, including autism, micro-/ macrocephaly, and epilepsy. Given the importance of tightly regulated autophagic activity for neuronal development and functioning, we questioned, whether deregulated autophagy is a more widely shared underlying mechanism among neurodevelopental disorders. To examine this, we focused on a group of neurological disorders that are caused by mutations in epigenetic modifier genes, so-called chromatinopathies. We made use of CRISPR/Cas9 genome editing and created different chromatinopathy-associated mutations in iPSC control cells. Subsequently, we performed a battery of standardized autophagy assays to compare autophagic protein expression and activity to isogenic control cells. Results indicate deregulated autophagy in most of our mutated iPSC lines, which provides an auspicious new approach to a better understanding of disease pathology and, more importantly, might help to develop more targeted therapies for a larger group of neuropsychiatric disorders.

Pubmed:
34329594: Mossink B, Verboven AHA, van Hugte EJH, Klein Gunnewiek TM, Parodi G, Linda K, Schoenmaker C, Kleefstra T, Kozicz T, van Bokhoven H, Schubert D, Nadif Kasri N, Frega M
Human neuronal networks on micro-electrode arrays are a highly robust tool to study disease-specific genotype-phenotype correlations in vitro.
Micro-electrode arrays (MEAs) are increasingly used to characterize neuronal network activity of human induced pluripotent stem cell (hiPSC)-derived neurons. Despite their gain in popularity, MEA recordings from hiPSC-derived neuronal networks are not always used to their full potential in respect to experimental design, execution, and data analysis. Therefore, we benchmarked the robustness of MEA-derived neuronal activity patterns from ten healthy individual control lines, and uncover comparable network phenotypes. To achieve standardization, we provide recommendations on experimental design and analysis. With such standardization, MEAs can be used as a reliable platform to distinguish (disease-specific) network phenotypes. In conclusion, we show that MEAs are a powerful and robust tool to uncover functional neuronal network phenotypes from hiPSC-derived neuronal networks, and provide an important resource to advance the hiPSC field toward the use of MEAs for disease phenotyping and drug discovery.
Stem Cell Reports, 2021; 16
34286667: Linda K, Lewerissa EI, Verboven AHA, Gabriele M, Frega M, Klein Gunnewiek TM, Devilee L, Ulferts E, Hommersom M, Oudakker A, Schoenmaker C, van Bokhoven H, Schubert D, Testa G, Koolen DA, de Vries BBA, Nadif Kasri N
Imbalanced autophagy causes synaptic deficits in a human model for neurodevelopmental disorders.
Macroautophagy (hereafter referred to as autophagy) is a finely tuned process of programmed degradation and recycling of proteins and cellular components, which is crucial in neuronal function and synaptic integrity. Mounting evidence implicates chromatin remodeling in fine-tuning autophagy pathways. However, this epigenetic regulation is poorly understood in neurons. Here, we investigate the role in autophagy of KANSL1, a member of the nonspecific lethal complex, which acetylates histone H4 on lysine 16 (H4K16ac) to facilitate transcriptional activation. Loss-of-function of KANSL1 is strongly associated with the neurodevelopmental disorder Koolen-de Vries Syndrome (KdVS). Starting from KANSL1-deficient human

induced-pluripotent stem cells, both from KdVS patients and genome-edited lines, we identified SOD1 (superoxide dismutase 1), an antioxidant enzyme, to be significantly decreased, leading to a subsequent increase in oxidative stress and autophagosome accumulation. In KANSL1-deficient neurons, autophagosome accumulation at excitatory synapses resulted in reduced synaptic density, reduced GRIA/AMPA receptor-mediated transmission and impaired neuronal network activity. Furthermore, we found that increased oxidative stress-mediated autophagosome accumulation leads to increased MTOR activation and decreased lysosome function, further preventing the clearing of autophagosomes. Finally, by pharmacologically reducing oxidative stress, we could rescue the aberrant autophagosome formation as well as synaptic and neuronal network activity in KANSL1-deficient neurons. Our findings thus point toward an important relation between oxidative stress-induced autophagy and synapse function, and demonstrate the importance of H4K16ac-mediated changes in chromatin structure to balance reactive oxygen species- and MTOR-dependent autophagy.: APO: apocynin; ATG: autophagy related; BAF: bafilomycin A; BSO: buthionine sulfoximine; CV: coefficient of variation; DIV: days in vitro; H4K16ac: histone 4 lysine 16 acetylation; iPSC: induced-pluripotent stem cell; KANSL1: KAT8 regulatory NSL complex subunit 1; KdVS: Koolen-de Vries Syndrome; LAMP1: lysosomal associated membrane protein 1; MAP1LC3/LC3: microtubule associated protein 1 light chain 3; MEA: micro-electrode array; MTOR: mechanistic target of rapamycin kinase; NSL complex: nonspecific lethal complex; 8-oxo-dG: 8-hydroxydesoxyguanosine; RAP: rapamycin; ROS: reactive oxygen species; sEPSCs: spontaneous excitatory postsynaptic currents; SOD1: superoxide dismutase 1; SQSTM1/p62: sequestosome 1; SYN: synapsin; WRT: wortmannin.
Autophagy, 2022; 18

33972691: Mossink B, van Rhijn JR, Wang S, Linda K, Vitale MR, Zöller JEM, van Hugte EJH, Bak J, Verboven AHA, Selten M, Negwer M, Latour BL, van der Werf I, Keller JM, Klein Gunnewiek TM, Schoenmaker C, Oudakker A, Anania A, Jansen S, Lesch KP, Frega M, van Bokhoven H, Schubert D, Nadif Kasri N

Cadherin-13 is a critical regulator of GABAergic modulation in human stem-cell-derived neuronal networks.

Activity in the healthy brain relies on a concerted interplay of excitation (E) and inhibition (I) via balanced synaptic communication between glutamatergic and GABAergic neurons. A growing number of studies imply that disruption of this E/I balance is a commonality in many brain disorders; however, obtaining mechanistic insight into these disruptions, with translational value for the patient, has typically been hampered by methodological limitations. Cadherin-13 (CDH13) has been associated with autism and attention-deficit/hyperactivity disorder. CDH13 localizes at inhibitory presynapses, specifically of parvalbumin (PV) and somatostatin (SST) expressing GABAergic neurons. However, the mechanism by which CDH13 regulates the function of inhibitory synapses in human neurons remains unknown. Starting from human-induced pluripotent stem cells, we established a robust method to generate a homogenous population of SST and MEF2C (PV-precursor marker protein) expressing GABAergic neurons (iGABA) in vitro, and co-cultured these with glutamatergic neurons at defined E/I ratios on micro-electrode arrays. We identified functional network parameters that are most reliably affected by GABAergic modulation as such, and through alterations of E/I balance by reduced expression of CDH13 in iGABAs. We found that CDH13 deficiency in iGABAs decreased E/I balance by means of increased inhibition. Moreover, CDH13 interacts with Integrin-β1 and Integrin-β3, which play opposite roles in the regulation of inhibitory synaptic strength via this interaction. Taken together, this model allows for standardized investigation of the E/I balance in a human neuronal background and can be deployed to dissect the cell-type-specific contribution of disease genes to the E/I balance.
Mol Psychiatry, 2022; 27

33824500: Polla DL, Farazi Fard MA, Tabatabaei Z, Habibzadeh P, Levchenko OA, Nikuei P, Makrythanasis P, Hussain M, von Hardenberg S, Zeinali S, Fallah MS, Schuurs-Hoeijmakers JHM, Shahzad M, Fatima F, Fatima N, Kaat LD, Bruggenwirth HT, Fleming LR, Condie J, Ploski R, Pollak A, Pilch J, Demina NA, Chukhrova AL, Sergeeva VS, Venselaar H, Masri AT, Hamamy H, Santoni FA, Linda K, Ahmed ZM, Nadif Kasri N, de Brouwer APM, Bergmann AK, Hethey S, Yavarian M, Ansar M, Riazuddin S, Riazuddin S, Silawi M, Ruggeri G, Pirozzi F, Eftekhar E, Taghipour Sheshdeh A, Bahramjahan S, Mirzaa GM, Lavrov AV, Antonarakis SE, Faghihi MA, van Bokhoven H

Biallelic variants in TMEM222 cause a new autosomal recessive neurodevelopmental disorder.

To elucidate the novel molecular cause in families with a new autosomal recessive neurodevelopmental disorder.
Genet Med, 2021; 23

31914384: Frega M, Selten M, Mossink B, Keller JM, Linda K, Moerschen R, Qu J, Koerner P, Jansen S, Oudakker A, Kleefstra T, van Bokhoven H, Zhou H, Schubert D, Nadif Kasri N

Distinct Pathogenic Genes Causing Intellectual Disability and Autism Exhibit a Common Neuronal Network Hyperactivity Phenotype.

Pathogenic mutations in either one of the epigenetic modifiers EHMT1, MBD5, MLL3, or SMARCB1 have been identified to be causative for Kleefstra syndrome spectrum (KSS), a neurodevelopmental disorder with clinical features of both intellectual disability (ID) and autism spectrum disorder (ASD). To understand how these variants lead to the phenotypic convergence in KSS, we employ a loss-of-function approach to assess neuronal network development at the molecular, single-cell, and

network activity level. KSS-gene-deficient neuronal networks all develop into hyperactive networks with altered network organization and excitatory-inhibitory balance. Interestingly, even though transcriptional data reveal distinct regulatory mechanisms, KSS target genes share similar functions in regulating neuronal excitability and synaptic function, several of which are associated with ID and ASD. Our results show that KSS genes mainly converge at the level of neuronal network communication, providing insights into the pathophysiology of KSS and phenotypically congruent disorders.
Cell Rep, 2020; 30
31666522: Frega M, Linda K, Keller JM, Gümüş-Akay G, Mossink B, van Rhijn JR, Negwer M, Klein Gunnewiek T, Foreman K, Kompier N, Schoenmaker C, van den Akker W, van der Werf I, Oudakker A, Zhou H, Kleefstra T, Schubert D, van Bokhoven H, Nadif Kasri N
Neuronal network dysfunction in a model for Kleefstra syndrome mediated by enhanced NMDAR signaling.
Kleefstra syndrome (KS) is a neurodevelopmental disorder caused by mutations in the histone methyltransferase EHMT1. To study the impact of decreased EHMT1 function in human cells, we generated excitatory cortical neurons from induced pluripotent stem (iPS) cells derived from KS patients. Neuronal networks of patient-derived cells exhibit network bursting with a reduced rate, longer duration, and increased temporal irregularity compared to control networks. We show that these changes are mediated by upregulation of NMDA receptor (NMDAR) subunit 1 correlating with reduced deposition of the repressive H3K9me2 mark, the catalytic product of EHMT1, at the GRIN1 promoter. In mice EHMT1 deficiency leads to similar neuronal network impairments with increased NMDAR function. Finally, we rescue the KS patient-derived neuronal network phenotypes by pharmacological inhibition of NMDARs. Summarized, we demonstrate a direct link between EHMT1 deficiency and NMDAR hyperfunction in human neurons, providing a potential basis for more targeted therapeutic approaches for KS.
Nat Commun, 2019; 10
29128445: Linda K, Fiuza C, Nadif Kasri N
The promise of induced pluripotent stem cells for neurodevelopmental disorders.
A major challenge in clinical genetics and medicine is represented by genetically and phenotypically highly diverse neurodevelopmental disorders, like for example intellectual disability and autism. Intellectual disability is characterized by substantial limitations in cognitive function and adaptive behaviour. At the cellular level, this is reflected by deficits in synaptic structure and plasticity and therefore has been coined as a synaptic disorder or "synaptopathy". In this review, we summarize the findings from recent studies in which iPSCs have been used to model specific neurodevelopmental syndromes, including Fragile X syndrome, Rett syndrome, Williams-Beuren syndrome and Phelan-McDermid syndrome. We discuss what we have learned from these studies and what key issues need to be addressed to move the field forward.
Prog Neuropsychopharmacol Biol Psychiatry, 2018; 84
28117798: Frega M, van Gestel SH, Linda K, van der Raadt J, Keller J, Van Rhijn JR, Schubert D, Albers CA, Nadif Kasri N
Rapid Neuronal Differentiation of Induced Pluripotent Stem Cells for Measuring Network Activity on Micro-electrode Arrays.
Neurons derived from human induced Pluripotent Stem Cells (hiPSCs) provide a promising new tool for studying neurological disorders. In the past decade, many protocols for differentiating hiPSCs into neurons have been developed. However, these protocols are often slow with high variability, low reproducibility, and low efficiency. In addition, the neurons obtained with these protocols are often immature and lack adequate functional activity both at the single-cell and network levels unless the neurons are cultured for several months. Partially due to these limitations, the functional properties of hiPSC-derived neuronal networks are still not well characterized. Here, we adapt a recently published protocol that describes production of human neurons from hiPSCs by forced expression of the transcription factor neurogenin-2. This protocol is rapid (yielding mature neurons within 3 weeks) and efficient, with nearly 100% conversion efficiency of transduced cells (>95% of DAPI-positive cells are MAP2 positive). Furthermore, the protocol yields a homogeneous population of excitatory neurons that would allow the investigation of cell-type specific contributions to neurological disorders. We modified the original protocol by generating stably transduced hiPSC cells, giving us explicit control over the total number of neurons. These cells are then used to generate hiPSC-derived neuronal networks on micro-electrode arrays. In this way, the spontaneous electrophysiological activity of hiPSC-derived neuronal networks can be measured and characterized, while retaining interexperimental consistency in terms of cell density. The presented protocol is broadly applicable, especially for mechanistic and pharmacological studies on human neuronal networks.
J Vis Exp, 2017;

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BOARD NUMBER: S02-422

ABERRANT HIPPOCAMPAL TRANSMISSION AND BEHAVIOR IN MICE WITH A STARGAZIN MUTATION LINKED TO INTELLECTUAL DISABILITY

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Mutations linked to neurodevelopmental disorders, such as intellectual disability (ID), are frequently found in genes that encode for proteins of the excitatory synapse. Transmembrane AMPA receptor regulatory proteins (TARPs) are AMPA receptor auxiliary proteins that regulate crucial aspects of receptor function. Here, we investigate a mutant form of the TARP family member stargazin, described in an ID patient. Our data revealed that the ID-associated stargazin variant, V143L, presents increased cell surface mobility and disrupts synaptic AMPA receptor trafficking in cultured neurons. Knock-in mice harboring the V143L stargazin mutation manifest cognitive and social deficits and hippocampal synaptic transmission defects, resembling phenotypes displayed by ID patients. In the hippocampus of stargazin V143L mice, CA1 neurons show impaired spine maturation, abnormal synaptic transmission and long-term potentiation specifically in basal dendrites, and synaptic ultrastructural alterations. These observations indicate that stargazin has a specific role in maintaining spine structure and synaptic function and plasticity in CA1 basal dendrites, which agrees with the higher expression levels of stargazin in the hippocampal *stratum oriens*, where basal dendrites are located, compared with the *stratum radiatum*, where apical dendrites are located. These data suggests that apart from the well-described brain region- and cell type-specific roles of TARPs, there may be subfield-specific roles that are determined by the subcellular distribution pattern of different TARPs. Altogether, our results point to a causal role for mutated stargazin in the pathogenesis of ID and unveil a new role for stargazin in the development and function of hippocampal synapses.

Pubmed:
32138985: Santos-Ferreira N, Mesquita JR, Rivadulla E, Inácio ÂS, Martins da Costa P, Romalde JL, Nascimento MSJ
Hepatitis E virus genotype 3 in echinoderms: First report of sea urchin (Paracentrotus lividus) contamination.
Hepatitis E virus (HEV) deriving from manure application runoffs and faecal waste spill over of swine and human origin bypass wastewater treatment plants and contaminate coastal waters. Shellfish bioaccumulate enteric viruses such as HEV from fecally contaminated coastal waters and under current European Regulations, shellfish sanitary status surveillance is mandatory but only by means of bacterial faecal indicators. The sea urchins are under the same regulations and their vulnerability to fecal contamination has been pointed out. Since they are consumed raw and with no steps to control/reduce hazards, sea urchin contamination with enteric viruses can represent a food safety risk. Hence, the aim of the present study was to screen sea urchin gonads destined for human consumption for the presence of HEV. HEV was detected and quantified in gonads of sea urchins collected in north Portugal by a reverse transcription-quantitative PCR (RT-qPCR) assay targeting the ORF3 region, followed by genotyping by a nested RT-PCR targeting the ORF2 region. Sequencing and phylogenetic analysis clustered the HEV sequence within genotype 3, subgenotype e. This the first study reporting HEV contamination of sea urchins. We hypothesize that like shellfish, sea urchins can also be a food vehicle for HEV transmission to humans.
Food Microbiol, 2020; 89
30473355: Freitas-Silva J, Inácio ÂS, Mourão J, Antunes P, Mendes Â, de Carvalho AP, Vasconcelos V, Peixe L, da Costa PM
Occurrence of mcr-1 in Escherichia coli from rabbits of intensive farming.
The emergence of mobile colistin resistance genes (mcr) is yet another challenge in the fight against antimicrobial resistance, with reports proving the dissemination of these genes in different countries and different environments being of great concern. In the present study, we describe the recovery of three E. coli strains with mcr-1 gene in IncHI2 plasmids from intestinal content of necropsied meat rabbits reared in two intensive production systems in Portugal. Our findings are

worrisome, given the high level of dependence on the usage of antibiotics in rabbit rearing and call for the development and implementation of an active surveillance system in this species.
Vet Microbiol, 2018; 227
30322037: Resende DISP, Pereira-Terra P, Inácio ÂS, Costa PMD, Pinto E, Sousa E, Pinto MMM
Lichen Xanthones as Models for New Antifungal Agents.
Due to the emergence of multidrug-resistant pathogenic microorganisms, the search for new antimicrobial compounds plays an important role in current medicinal chemistry research. Inspired by lichen antimicrobial xanthones, a series of novel chlorinated xanthones was prepared using five chlorination methods (Methods A⁻E) to obtain different patterns of substitution in the xanthone scaffold. All the synthesized compounds were evaluated for their antimicrobial activity. Among them, 3-chloro-4,6-dimethoxy-1-methyl-9-xanthen-9-one showed promising antibacterial activity against (ATCC 29212 and 29213) and ATCC 29213. 2,7-Dichloro-3,4,6-trimethoxy-1-methyl-9-xanthen-9-one revealed a potent fungistatic and fungicidal activity against dermatophytes clinical strains (, , and (MIC = 4⁻8 µg/mL)). Moreover, when evaluated for its synergistic effect for , compound exhibited synergy with fluconazole (ΣFIC = 0.289). These results disclosed new hit xanthones for both antibacterial and antifungal activity.
Molecules, 2018; 23
29642369: Buttachon S, Ramos AA, Inácio Â, Dethoup T, Gales L, Lee M, Costa PM, Silva AMS, Sekeroglu N, Rocha E, Pinto MMM, Pereira JA, Kijjoa A
Bis-Indolyl Benzenoids, Hydroxypyrrolidine Derivatives and Other Constituents from Cultures of the Marine Sponge-Associated Fungus Aspergillus candidus KUFA0062.
A previously unreported -indolyl benzenoid, candidusin D () and a new hydroxypyrrolidine alkaloid, preussin C () were isolated together with fourteen previously described compounds: palmitic acid, clionasterol, ergosterol 5,8-endoperoxides, chrysophanic acid (), emodin (), six -indolyl benzenoids including asterriquinol D dimethyl ether (), petromurin C (), kumbicin B (), kumbicin A (), 2″-oxoasterriquinol D methyl ether (), kumbicin D (), the hydroxypyrrolidine alkaloid preussin (), (3, 6)-3,6-dibenzylpiperazine-2,5-dione () and 4-(acetylamino) benzoic acid (), from the cultures of the marine sponge-associated fungus KUFA 0062. Compounds , , , , , and were tested for their antibacterial activity against Gram-positive and Gram-negative reference and multidrug-resistant strains isolated from the environment. Only exhibited an inhibitory effect against ATCC 29213 and ATCC29212 as well as both methicillin-resistant (MRSA) and vancomycin-resistant enterococci (VRE) strains. Both and also reduced significant biofilm formation in ATCC 25922. Moreover, and revealed a synergistic effect with oxacillin against MRSA 66/1 while exhibited a strong synergistic effect with the antibiotic colistin against 1410/1. Compound , , , , , and were also tested, together with the crude extract, for cytotoxic effect against eight cancer cell lines: HepG2, HT29, HCT116, A549, A 375, MCF-7, U-251, and T98G. Except for , , , and , all the compounds showed cytotoxicity against all the cancer cell lines tested.
Mar Drugs, 2018; 16
26976875: Inácio ÂS, Nunes A, Milho C, Mota LJ, Borrego MJ, Gomes JP, Vaz WL, Vieira OV
In Vitro Activity of Quaternary Ammonium Surfactants against Streptococcal, Chlamydial, and Gonococcal Infective Agents.
Quaternary ammonium compounds (QAC) are widely used, cheap, and chemically stable disinfectants and topical antiseptics with wide-spectrum antimicrobial activities. Within this group of compounds, we recently showed that there are significant differences between the pharmacodynamics of n-alkyl quaternary ammonium surfactants (QAS) with a short (C12) alkyl chain when in vitro toxicities toward bacterial and mammalian epithelial cells are compared. These differences result in an attractive therapeutic window that justifies studying short-chain QAS as prophylactics for sexually transmitted infections (STI) and perinatal vertically transmitted urogenital infections (UGI). We have evaluated the antimicrobial activities of short-chain (C12) n-alkyl QAS against several STI and UGI pathogens as well as against commensal Lactobacillus species. Inhibition of infection of HeLa cells by Neisseria gonorrhoeae and Chlamydia trachomatis was studied at concentrations that were not toxic to the HeLa cells. We show that the pathogenic bacteria are much more susceptible to QAS toxic effects than the commensal vaginal flora and that QAS significantly attenuate the infectivity of N. gonorrhoeae and C. trachomatis without affecting the viability of epithelial cells of the vaginal mucosa. N-Dodecylpyridinium bromide (C12PB) was found to be the most effective QAS. Our results strongly suggest that short-chain (C12) n-alkyl pyridinium bromides and structurally similar compounds are promising microbicide candidates for topical application in the prophylaxis of STI and perinatal vertical transmission of UGI.
Antimicrob Agents Chemother, 2016; 60
26679255: Inácio ÂS, Domingues NS, Nunes A, Martins PT, Moreno MJ, Estronca LM, Fernandes R, Moreno AJ, Borrego MJ, Gomes JP, Vaz WL, Vieira OV
Quaternary ammonium surfactant structure determines selective toxicity towards bacteria: mechanisms of action and clinical implications in antibacterial prophylaxis.
Broad-spectrum antimicrobial activity of quaternary ammonium surfactants (QAS) makes them attractive and cheap topical

prophylactic options for sexually transmitted infections and perinatal vertically transmitted urogenital infections. Although attributed to their high affinity for biological membranes, the mechanisms behind QAS microbicidal activity are not fully understood. We evaluated how QAS structure affects antimicrobial activity and whether this can be exploited for use in prophylaxis of bacterial infections.
J Antimicrob Chemother, 2016; 71
23529737: Inácio ÂS, Costa GN, Domingues NS, Santos MS, Moreno AJ, Vaz WL, Vieira OV
Mitochondrial dysfunction is the focus of quaternary ammonium surfactant toxicity to mammalian epithelial cells.
Surfactants have long been known to have microbicidal action and have been extensively used as antiseptics and disinfectants for a variety of general hygiene and clinical purposes. Among surfactants, quaternary ammonium compounds (QAC) are known to be the most useful antiseptics and disinfectants. However, our previous toxicological studies showed that QAC are also the most toxic surfactants for mammalian cells. An understanding of the mechanisms that underlie QAC toxicity is a crucial first step in their rational use and in the design and development of more effective and safer molecules. We show that QAC-induced toxicity is mediated primarily through mitochondrial dysfunction in mammalian columnar epithelial cell cultures in vitro. Toxic effects begin at sublethal concentrations and are characterized by mitochondrial fragmentation accompanied by decreased cellular energy charge. At very low concentrations, several QAC act on mitochondrial bioenergetics through a common mechanism of action, primarily by inhibiting mitochondrial respiration initiated at complex I and, to a lesser extent, by slowing down coupled ADP phosphorylation. The result is a reduction of cellular energy charge which, when reduced below 50% of its original value, induces apoptosis. The lethal effects are shown to be primarily a result of this process. At higher doses (closer to the critical micellar concentration), QAC induce the complete breakdown of cellular energy charge and necrotic cell death.
Antimicrob Agents Chemother, 2013; 57
20506358: Carreira BP, Morte MI, Inácio A, Costa G, Rosmaninho-Salgado J, Agasse F, Carmo A, Couceiro P, Brundin P, Ambrósio AF, Carvalho CM, Araújo IM
Nitric oxide stimulates the proliferation of neural stem cells bypassing the epidermal growth factor receptor.
Nitric oxide (NO) was described to inhibit the proliferation of neural stem cells. Some evidence suggests that NO, under certain conditions, can also promote cell proliferation, although the mechanisms responsible for a potential proliferative effect of NO in neural stem cells have remained unaddressed. In this work, we investigated and characterized the proliferative effect of NO in cell cultures obtained from the mouse subventricular zone. We found that the NO donor NOC-18 (10 microM) increased cell proliferation, whereas higher concentrations (100 microM) inhibited cell proliferation. Increased cell proliferation was detected rapidly following exposure to NO and was prevented by blocking the mitogen-activated kinase (MAPK) pathway, independently of the epidermal growth factor (EGF) receptor. Downstream of the EGF receptor, NO activated p21Ras and the MAPK pathway, resulting in a decrease in the nuclear presence of the cyclin-dependent kinase inhibitor 1, p27(KIP1), allowing for cell cycle progression. Furthermore, in a mouse model that shows increased proliferation of neural stem cells in the hippocampus following seizure injury, we observed that the absence of inducible nitric oxide synthase (iNOS(-/-) mice) prevented the increase in cell proliferation observed following seizures in wild-type mice, showing that NO from iNOS origin is important for increased cell proliferation following a brain insult. Overall, we show that NO is able to stimulate the proliferation of neural stem cells bypassing the EGF receptor and promoting cell division. Moreover, under pathophysiological conditions in vivo, NO from iNOS origin also promotes proliferation in the hippocampus.
Stem Cells, 2010; 28
22378242: Carreira BP, Morte MI, Lourenço AS, Santos AI, Inácio A, Ambrósio AF, Carvalho CM, Araújo IM
Differential contribution of the guanylyl cyclase-cyclic GMP-protein kinase G pathway to the proliferation of neural stem cells stimulated by nitric oxide.
Nitric oxide (NO) is an important inflammatory mediator involved in the initial boost in the proliferation of neural stem cells following brain injury. However, the mechanisms underlying the proliferative effect of NO are still unclear. The aim of this work was to investigate whether cyclic GMP (cGMP) and the cGMP-dependent kinase (PKG) are involved in the proliferative effect triggered by NO in neural stem cells. For this purpose, cultures of neural stem cells isolated from the mouse subventricular zone (SVZ) were used. We observed that long-term exposure to the NO donor (24 h), NOC-18, increased the proliferation of SVZ cells in a cGMP-dependent manner, since the guanylate cyclase inhibitor, ODQ, prevented cell proliferation. Similarly to NOC-18, the cGMP analogue, 8-Br-cGMP, also increased cell proliferation. Interestingly, shorter exposures to NO (6 h) increased cell proliferation in a cGMP-independent manner via the ERK/MAP kinase pathway. The selective inhibitor of PKG, KT5823, prevented the proliferative effect induced by NO at 24 h but not at 6 h. In conclusion, the proliferative effect of NO is initially mediated by the ERK/MAPK pathway, and at later stages by the GC/cGMP/PKG pathway. Thus, our work shows that NO induces neural stem cell proliferation by targeting these two pathways in a biphasic manner.
Neurosignals, 2013; 21
17585341: Araújo IM, Carreira BP, Pereira T, Santos PF, Soulet D, Inácio A, Bahr BA, Carvalho AP, Ambrósio AF, Carvalho

CM

Changes in calcium dynamics following the reversal of the sodium-calcium exchanger have a key role in AMPA receptor-mediated neurodegeneration via calpain activation in hippocampal neurons.

Proteolytic cleavage of the Na(+)/Ca(2+) exchanger (NCX) by calpains impairs calcium homeostasis, leading to a delayed calcium overload and excitotoxic cell death. However, it is not known whether reversal of the exchanger contributes to activate calpains and trigger neuronal death. We investigated the role of the reversal of the NCX in Ca(2+) dynamics, calpain activation and cell viability, in alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor-stimulated hippocampal neurons. Selective overactivation of AMPA receptors caused the reversal of the NCX, which accounted for approximately 30% of the rise in intracellular free calcium concentration ([Ca(2+)](i)). The NCX reverse-mode inhibitor, 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea (KB-R7943), partially inhibited the initial increase in [Ca(2+)](i), and prevented a delayed increase in [Ca(2+)](i). In parallel, overactivation of AMPA receptors strongly activated calpains and led to the proteolysis of NCX3. KB-R7943 prevented calpain activation, cleavage of NCX3 and was neuroprotective. Silencing of NCX3 reduced Ca(2+) uptake, calpain activation and was neuroprotective. Our data show for the first time that NCX reversal is an early event following AMPA receptor stimulation and is linked to the activation of calpains. Since calpain activation subsequently inactivates NCX, causing a secondary Ca(2+) entry, NCX may be viewed as a new suicide substrate operating in a Ca(2+)-dependent loop that triggers cell death and as a target for neuroprotection.

Cell Death Differ, 2007; 14

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THE X-LINKED PTCHD1 GENE ASSOCIATED WITH ID AND/OR ASD INTERACTS AS A PROTEIN RECEPTOR WITH RAC1 A KEY REGULATOR OF CYTOSKELETON REMODELING AND SYNAPSE PLASTICITY.

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Universite de Tours, Umr1253, Ibrain, Inserm, Tours, France

Intellectual disability (ID) is a prevalent neurodevelopmental condition with enormous burden for patients and families and without effective treatment. Deleterious mutations in the *PTCHD1* (Patched domain containing 1) gene have been described in male patients with X-linked ID and/or ASD (Autism Spectrum Disorders) and accounting for up to 1% of diagnoses in males. I have used neurobiology and molecular biology techniques, applied to mouse models, to discover that genetically deleting *Ptchd1* affects the synaptic transcriptome and impairs synaptic structure and activity, leading to cognitive dysfunction, motor disabilities and hyperactivity (Ung et al., *MolPsych*, 2018). I have also identified a PDZ-binding motif at the C-terminal end of synaptic receptor PTCHD1 that binds to the major postsynaptic scaffolding proteins PSD-95 and SAP102. Recently, we characterized 13 novel missense variants in the *PTCHD1* gene (Halewa et al, 2021) causing alteration of its membrane subcellular localization and leading to ID and/or ASD in patients. Nevertheless several novel missense mutations have not been studied yet. We therefore continue our effort by focusing on additional variants (L38Q, N152E, G303R, F420L, P605L) by studying protein expression and subcellular localization in HEK293T cell line and in mouse hippocampal primary neuronal cultures. In parallel using specific protein interaction assays, I determined that the C-terminal region of PTCHD1 interacts with Rac1, a RhoGTPase associated with ID, essential for cytoskeleton remodeling and synapse plasticity. Highlighting PTCHD1 novel interacting proteins and *PTCHD1* mutations functional impact will allow a better understanding of those neurodevelopmental disorders and give potential answers to patients and families.

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LOSS-OF-FUNCTION VARIANTS IN THE SCHIZOPHRENIA RISK GENE SETD1A ALTER NEURONAL NETWORK ACTIVITY IN HUMAN NEURONS THROUGH CAMP/PKA PATHWAY

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Heterozygous loss-of-function (LoF) mutations in *SETD1A*, which encodes a subunit of histone H3 lysine 4 methyltransferase, were shown to cause a novel neurodevelopmental syndrome and increase the risk for schizophrenia. We generated *in vitro* excitatory/inhibitory neuronal networks from human induced pluripotent stem cells with a *SETD1A* heterozygous LoF mutation (*SETD1A*$^{+/-}$) using CRISPR/Cas9. Our data show that *SETD1A* haploinsufficiency resulted in morphologically increased dendritic complexity and functionally increased bursting activity. This network phenotype was primarily driven by *SETD1A* haploinsufficiency in glutamatergic neurons. In accordance with the functional changes, transcriptomic profiling revealed perturbations in gene sets associated with glutamatergic synaptic function. At the molecular level, we identified specific changes in the cAMP/PKA pathway pointing toward a hyperactive cAMP pathway in *SETD1A*$^{+/-}$ neurons. Finally, by pharmacologically targeting the cAMP pathway we were able to rescue major aspects of the network deficits in *SETD1A*$^{+/-}$ cultures. Our results demonstrate a link between SETD1A and the cAMP-dependent pathway in human neurons.

Pubmed:
33972691: Mossink B, van Rhijn JR, Wang S, Linda K, Vitale MR, Zöller JEM, van Hugte EJH, Bak J, Verboven AHA, Selten M, Negwer M, Latour BL, van der Werf I, Keller JM, Klein Gunnewiek TM, Schoenmaker C, Oudakker A, Anania A, Jansen S, Lesch KP, Frega M, van Bokhoven H, Schubert D, Nadif Kasri N
Cadherin-13 is a critical regulator of GABAergic modulation in human stem-cell-derived neuronal networks.
Activity in the healthy brain relies on a concerted interplay of excitation (E) and inhibition (I) via balanced synaptic communication between glutamatergic and GABAergic neurons. A growing number of studies imply that disruption of this E/I balance is a commonality in many brain disorders; however, obtaining mechanistic insight into these disruptions, with translational value for the patient, has typically been hampered by methodological limitations. Cadherin-13 (CDH13) has been associated with autism and attention-deficit/hyperactivity disorder. CDH13 localizes at inhibitory presynapses, specifically of parvalbumin (PV) and somatostatin (SST) expressing GABAergic neurons. However, the mechanism by which CDH13 regulates the function of inhibitory synapses in human neurons remains unknown. Starting from human-induced pluripotent stem cells, we established a robust method to generate a homogenous population of SST and MEF2C (PV-precursor marker protein) expressing GABAergic neurons (iGABA) in vitro, and co-cultured these with glutamatergic neurons at defined E/I ratios on micro-electrode arrays. We identified functional network parameters that are most reliably affected by GABAergic modulation as such, and through alterations of E/I balance by reduced expression of CDH13 in iGABAs. We found that CDH13 deficiency in iGABAs decreased E/I balance by means of increased inhibition. Moreover, CDH13 interacts with Integrin-β1 and Integrin-β3, which play opposite roles in the regulation of inhibitory synaptic strength via this interaction. Taken together, this model allows for standardized investigation of the E/I balance in a human neuronal background and can be deployed to dissect the cell-type-specific contribution of disease genes to the E/I balance.
Mol Psychiatry, 2022; 27
34803610: Wang S, Bleeck A, Nadif Kasri N, Kleefstra T, van Rhijn JR, Schubert D
SETD1A Mediated H3K4 Methylation and Its Role in Neurodevelopmental and Neuropsychiatric Disorders.
Posttranslational modification of histones and related gene regulation are shown to be affected in an increasing number of neurological disorders. SETD1A is a chromatin remodeler that influences gene expression through the modulation of mono- di- and trimethylation marks on Histone-H3-Lysine-4 (H3K4me1/2/3). H3K4 methylation is predominantly described to result in transcriptional activation, with its mono- di- and trimethylated forms differentially enriched at promoters or enhancers. Recently, dominant mostly variants in have clinically been linked to developmental delay, intellectual disability (DD/ID), and

schizophrenia (SCZ). Affected individuals often display both developmental and neuropsychiatric abnormalities. The primary diagnoses are mainly dependent on the age at which the individual is assessed. Investigations in mouse models of SETD1A dysfunction have been able to recapitulate key behavioral features associated with ID and SCZ. Furthermore, functional investigations suggest disrupted synaptic and neuronal network function in these mouse models. In this review, we provide an overview of pre-clinical studies on the role of SETD1A in neuronal development. A better understanding of the pathobiology underlying these disorders may provide novel opportunities for therapeutic intervention. As such, we will discuss possible strategies to move forward in elucidating the genotype-phenotype correlation in associated disorders.
Front Mol Neurosci, 2021; 14
30316911: Wang S, Huang L, Zhang Y, Peng Y, Wang X, Peng Y
Protective Effects of L-3-n-Butylphthalide Against HO-Induced Injury in Neural Stem Cells by Activation of PI3K/Akt and Mash1 Pathway.
It has been reported that oxidative stress could result in damage to the developing brain. L-3-n-butylphthalide (L-NBP) could inhibit neuronal cell apoptosis and has neurogenesis effect in different animal and cellular models. However, whether L-NBP could protect the process of neurogenesis in neural stem cells (NSCs) against oxidative stress injury is still unclear. Here, in the present study, we evaluated the neuroprotective effect of L-NBP in NSCs against HO-induced injury and the possible mechanisms. The results showed that L-NBP elevated the proliferation of NSCs by upregulating cyclin D1, and PI3K/Akt might be a possible target in this process. Subsequently, L-NBP was found to promote the migration of NSCs and N-cadherin might be involved in. NSC differentiation was measured using immunofluorescence staining and the results demonstrated that L-NBP could promote the NSCs to differentiate more into neurons. The elevation of achaete-scute homolog1 (Mash1) expression might be a key factor as attenuation of endogenous Mash1 expression by short-interfering RNA could block L-NBP-promoted neuronal differentiation. In summary, L-NBP exerts protective effects in NSCs against HO-induced injury by promoting the proliferation, migration and neural differentiation of NSCs, indicating that L-NBP might be a potential therapeutic agent for the neurogenesis-based treatment for some brain diseases, such as Alzheimer's disease (AD).
Neuroscience, 2018; 393
29895257: Wang S, Ma F, Huang L, Zhang Y, Peng Y, Xing C, Feng Y, Wang X, Peng Y
Dl-3-n-Butylphthalide (NBP): A Promising Therapeutic Agent for Ischemic Stroke.
Stroke is a leading cause of morbidity and mortality in both developed and developing countries all over the world. The only drug for ischemic stroke approved by FDA is recombinant tissue plasminogen activator (rtPA). However, only 2-5% stroke patients receive rtPAs treatment due to its strict therapeutic time window. As ischemic stroke is a complex disease involving multiple mechanisms, medications with multi-targets may be more powerful compared with single-target drugs. Dl-3-n-Butylphthalide (NBP) is a synthetic compound based on l-3-n- Butylphthalide that is isolated from seeds of Apium graveolens. The racemic 3-n-butylphthalide (dl- NBP) was approved by Food and Drug Administration of China for the treatment of ischemic stroke in 2002. A number of clinical studies indicated that NBP not only improved the symptoms of ischemic stroke, but also contributed to the long-term recovery. The potential mechanisms of NBP for ischemic stroke treatment may target different pathophysiological processes, including anti-oxidant, antiinflammation, anti-apoptosis, anti-thrombosis, and protection of mitochondria et al. Conclusion: In this review, we have summarized the research progress of NBP for the treatment of ischemic stroke during the past two decades.
CNS Neurol Disord Drug Targets, 2018; 17
30103000: Huang L, Wang S, Ma F, Zhang Y, Peng Y, Xing C, Feng Y, Wang X, Peng Y
From stroke to neurodegenerative diseases: The multi-target neuroprotective effects of 3-n-butylphthalide and its derivatives.
Discovering effective agents to slow or stop neurodegeneration is a challenging task. Over decades, only a few drugs were approved by Food and Drug Administration (FDA) and most ended in failure. The lessons learned have switched the strategy of drug discovery from designing highly selective ligands to a network pharmacology approach. This enables many natural products like butylphthalide (NBP) once again to be regarded as a valuable source of leads for drug discovery. In this review, we first start with the neuroprotective effects of NBPs on acute ischemic stroke, and later spread to their applications in major neurodegenerative diseases. The underlying mechanisms are also discussed in order to provide a direction for further study. Hopefully, this review could bring some new insights for drug development in this struggling field.
Pharmacol Res, 2018; 135
29035776: Liu XY, Wang S, Li CJ, Ma J, Chen FY, Peng Y, Wang XL, Zhang DM
Dammarane-type saponins from the leaves of Panax notoginseng and their neuroprotective effects on damaged SH-SY5Y cells.
Seven dammarane-type saponins previously undescribed (notoginsenosides Fh1-Fh7) were isolated from the aqueous extract leaves of Panax notoginseng (Burk.) F.H.Chen (Araliaceae), together with eleven known saponins. Their structures of were elucidated by spectroscopic analysis (NMR, UV, IR, ect.), HR-ESI-MS techniques and chemical methods. Screening results indicated that compounds 4, 5, 12, 13 showed moderate neuroprotective effects on HO-induced cellular damage in

human neuroblastoma SH-SY5Y cells (10 μM).
Phytochemistry, 2018; 145
30450970: Peng YC, Wang S, Zhang Y, Huang LJ, Wang XL, Peng Y
Hsp90β inhibitors prevent GLT-1 degradation but have no beneficial efficacy on absence epilepsy.
The loss of glutamate transporter-1 (GLT-1) is associated with temporal lobe epilepsy (TLE). A recent study reported that Hsp90β interacted with GLT-1 and recruited it to 20S proteasome for degradation. Therefore, inhibiting Hsp90β may be a new strategy for treating epilepsy. So far, no studies have shown whether the inhibition of Hsp90β had therapeutic effects on absence epilepsy. Using a model of absence epilepsy, we demonstrated that 17-allylamino-17-demethoxygeldanamycin (17AAG) and Ganetespib (STA9090) had no therapeutic effect. Although this is a negative result, it also has a meaningful exploration value for whether Hsp90 inhibitors have therapeutic effects on other epilepsy types.
J Asian Nat Prod Res, 2019; 21

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BOARD NUMBER: S02-425

SCN1A-DEFICIENT EXCITATORY NEURONAL NETWORKS DISPLAY MUTATION SPECIFIC NETWORK PHENOTYPES

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Eline Van Hugte[1,2], Elly Lewerissa[1], Ka Man Wu[1], Monica Frega[3], Jurgen Schelhaas[2], Judith Verhoeven[2], Marian Majoie[2], Hans Van Bokhoven[1], Nael Nadif Kasri[1]
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Dravet syndrome (DS) is a severe epileptic encephalopathy, characterized by (febrile) seizures and neurological problems. 80% of DS patients have a mutation in *SCN1A*, encoding $Na_v1.1$. While there are over 1500 different *SCN1A* mutations known, the clinical predictive value remains challenging, even if one mutation is inherited within one family. Both this clinical and genetic heterogeneity complicate tailored prescription of anti-epileptic drugs (AEDs). Moreover, it is currently debated if excitatory neurons contribute to the DS phenotype, it could either depend on the patient clinical phenotype, or to the type of mutation studied. Studies sometimes only investigate a few *SCN1A* mutations, often in isolation of the patient background, or do not include multiple clinical phenotypes. Therefore, we investigated a panel of hiPSC-derived lines from patients with different phenotypes, harboring different mutations in *SCN1A*, including members of a family where the same mutation leads to different clinical phenotypes. Using Micro-electrode arrays, we provide further evidence that excitatory neurons are implicated in DS syndrome. The excitatory networks are affected by different *SCN1A* mutations to different extend, and show altered neuronal network organization upon febrile temperatures. However, the network phenotypes could not be distinguished based on patient clinical severity. Interestingly, the network phenotype could be partially rescued with valproic acid, the main AED prescribed for DS, while carbamazepine, a contraindicated AED, leads to aggrevation of the phenotype. In conclusion, our results indicate a mutation-specific excitatory neuronal network phenotype, which responds to the main DS-linked triggers, suggesting a useful platform for precision therapies for DS.

Pubmed:

30911152: Kroon T, van Hugte E, van Linge L, Mansvelder HD, Meredith RM
Early postnatal development of pyramidal neurons across layers of the mouse medial prefrontal cortex.
Mammalian neocortex is a highly layered structure. Each layer is populated by distinct subtypes of principal cells that are born at different times during development. While the differences between principal cells across layers have been extensively studied, it is not known how the developmental profiles of neurons in different layers compare. Here, we provide a detailed morphological and functional characterisation of pyramidal neurons in mouse mPFC during the first postnatal month, corresponding to known critical periods for synapse and neuron formation in mouse sensory neocortex. Our data demonstrate similar maturation profiles of dendritic morphology and intrinsic properties of pyramidal neurons in both deep and superficial layers. In contrast, the balance of synaptic excitation and inhibition differs in a layer-specific pattern from one to four postnatal weeks of age. Our characterisation of the early development and maturation of pyramidal neurons in mouse mPFC not only demonstrates a comparable time course of postnatal maturation to that in other neocortical circuits, but also implies that consideration of layer- and time-specific changes in pyramidal neurons may be relevant for studies in mouse models of neuropsychiatric and neurodevelopmental disorders.
Sci Rep, 2019; 9
31705501: van Hugte E, Nadif Kasri N
Modeling Psychiatric Diseases with Induced Pluripotent Stem Cells.
Neuropsychiatric disorders are a heterogeneous group of disorders that are challenging to model and treat, due to their underlying complex genetic architecture and clinical variability. Presently, increasingly more studies are making use of induced pluripotent stem cell (iPSC)-derived neurons, reprogrammed from patient somatic cells, to model neuropsychiatric disorders. iPSC-derived neurons offer the possibility to recapitulate relevant disease biology in the context of the individual patient genetic background. In addition to disease modeling, iPSC-derived neurons offer unprecedented opportunities in drug screening. In this chapter, the current status of iPSC disease modeling for neuropsychiatric disorders is presented. Both 2D and 3D disease modeling approaches are discussed as well as the generation of different neuronal cell types that are

relevant for studying neuropsychiatric disorders. Moreover, the advantages and limitations are highlighted in addition to the future perspectives of using iPSC-derived neurons in the uncovering of robust cellular phenotypes that consecutively have the potential to lead to clinical developments.
Adv Exp Med Biol, 2019; 1192
33972691: Mossink B, van Rhijn JR, Wang S, Linda K, Vitale MR, Zöller JEM, van Hugte EJH, Bak J, Verboven AHA, Selten M, Negwer M, Latour BL, van der Werf I, Keller JM, Klein Gunnewiek TM, Schoenmaker C, Oudakker A, Anania A, Jansen S, Lesch KP, Frega M, van Bokhoven H, Schubert D, Nadif Kasri N
Cadherin-13 is a critical regulator of GABAergic modulation in human stem-cell-derived neuronal networks.
Activity in the healthy brain relies on a concerted interplay of excitation (E) and inhibition (I) via balanced synaptic communication between glutamatergic and GABAergic neurons. A growing number of studies imply that disruption of this E/I balance is a commonality in many brain disorders; however, obtaining mechanistic insight into these disruptions, with translational value for the patient, has typically been hampered by methodological limitations. Cadherin-13 (CDH13) has been associated with autism and attention-deficit/hyperactivity disorder. CDH13 localizes at inhibitory presynapses, specifically of parvalbumin (PV) and somatostatin (SST) expressing GABAergic neurons. However, the mechanism by which CDH13 regulates the function of inhibitory synapses in human neurons remains unknown. Starting from human-induced pluripotent stem cells, we established a robust method to generate a homogenous population of SST and MEF2C (PV-precursor marker protein) expressing GABAergic neurons (iGABA) in vitro, and co-cultured these with glutamatergic neurons at defined E/I ratios on micro-electrode arrays. We identified functional network parameters that are most reliably affected by GABAergic modulation as such, and through alterations of E/I balance by reduced expression of CDH13 in iGABAs. We found that CDH13 deficiency in iGABAs decreased E/I balance by means of increased inhibition. Moreover, CDH13 interacts with Integrin-β1 and Integrin-β3, which play opposite roles in the regulation of inhibitory synaptic strength via this interaction. Taken together, this model allows for standardized investigation of the E/I balance in a human neuronal background and can be deployed to dissect the cell-type-specific contribution of disease genes to the E/I balance.
Mol Psychiatry, 2022; 27
34329594: Mossink B, Verboven AHA, van Hugte EJH, Klein Gunnewiek TM, Parodi G, Linda K, Schoenmaker C, Kleefstra T, Kozicz T, van Bokhoven H, Schubert D, Nadif Kasri N, Frega M
Human neuronal networks on micro-electrode arrays are a highly robust tool to study disease-specific genotype-phenotype correlations in vitro.
Micro-electrode arrays (MEAs) are increasingly used to characterize neuronal network activity of human induced pluripotent stem cell (hiPSC)-derived neurons. Despite their gain in popularity, MEA recordings from hiPSC-derived neuronal networks are not always used to their full potential in respect to experimental design, execution, and data analysis. Therefore, we benchmarked the robustness of MEA-derived neuronal activity patterns from ten healthy individual control lines, and uncover comparable network phenotypes. To achieve standardization, we provide recommendations on experimental design and analysis. With such standardization, MEAs can be used as a reliable platform to distinguish (disease-specific) network phenotypes. In conclusion, we show that MEAs are a powerful and robust tool to uncover functional neuronal network phenotypes from hiPSC-derived neuronal networks, and provide an important resource to advance the hiPSC field toward the use of MEAs for disease phenotyping and drug discovery.
Stem Cell Reports, 2021; 16
30581017: Nadadhur AG, Alsaqati M, Gasparotto L, Cornelissen-Steijger P, van Hugte E, Dooves S, Harwood AJ, Heine VM
Neuron-Glia Interactions Increase Neuronal Phenotypes in Tuberous Sclerosis Complex Patient iPSC-Derived Models.
Tuberous sclerosis complex (TSC) is a rare neurodevelopmental disorder resulting from autosomal dominant mutations in the TSC1 or TSC2 genes, leading to a hyperactivated mammalian target of rapamycin (mTOR) pathway, and gray and white matter defects in the brain. To study the involvement of neuron-glia interactions in TSC phenotypes, we generated TSC patient induced pluripotent stem cell (iPSC)-derived cortical neuronal and oligodendrocyte (OL) cultures. TSC neuron mono-cultures showed increased network activity, as measured by calcium transients and action potential firing, and increased dendritic branching. However, in co-cultures with OLs, neuronal defects became more apparent, showing cellular hypertrophy and increased axonal density. In addition, TSC neuron-OL co-cultures showed increased OL cell proliferation and decreased OL maturation. Pharmacological intervention with the mTOR regulator rapamycin suppressed these defects. Our patient iPSC-based model, therefore, shows a complex cellular TSC phenotype arising from the interaction of neuronal and glial cells and provides a platform for TSC disease modeling and drug development.
Stem Cell Reports, 2019; 12
32320658: Klein Gunnewiek TM, Van Hugte EJH, Frega M, Guardia GS, Foreman K, Panneman D, Mossink B, Linda K, Keller JM, Schubert D, Cassiman D, Rodenburg R, Vidal Folch N, Oglesbee D, Perales-Clemente E, Nelson TJ, Morava E, Nadif Kasri N, Kozicz T
m.3243A > G-Induced Mitochondrial Dysfunction Impairs Human Neuronal Development and Reduces Neuronal Network

Activity and Synchronicity.
Epilepsy, intellectual and cortical sensory deficits, and psychiatric manifestations are the most frequent manifestations of mitochondrial diseases. How mitochondrial dysfunction affects neural structure and function remains elusive, mostly because of a lack of proper in vitro neuronal model systems with mitochondrial dysfunction. Leveraging induced pluripotent stem cell technology, we differentiated excitatory cortical neurons (iNeurons) with normal (low heteroplasmy) and impaired (high heteroplasmy) mitochondrial function on an isogenic nuclear DNA background from patients with the common pathogenic m.3243A > G variant of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). iNeurons with high heteroplasmy exhibited mitochondrial dysfunction, delayed neural maturation, reduced dendritic complexity, and fewer excitatory synapses. Micro-electrode array recordings of neuronal networks displayed reduced network activity and decreased synchronous network bursting. Impaired neuronal energy metabolism and compromised structural and functional integrity of neurons and neural networks could be the primary drivers of increased susceptibility to neuropsychiatric manifestations of mitochondrial disease.
Cell Rep, 2020; 31
25693726: van Schendel RV, Dondorp WJ, Timmermans DR, van Hugte EJ, de Boer A, Pajkrt E, Lachmeijer AM, Henneman L
NIPT-based screening for Down syndrome and beyond: what do pregnant women think?
The aim of the study is to study pregnant women's views on noninvasive prenatal testing (NIPT) for Down syndrome and the potential to test for a broader range of conditions.
Prenat Diagn, 2015; 35

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BOARD NUMBER: S02-426

MOLECULAR AND CELLULAR STUDY OF THE IMPACT OF NR2F1 MISSENSE VARIANTS BY USING STRUCTURAL BIOLOGY AND THE GENETIC CODE EXPANSION (GCE) APPROACH

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Wanchana Phromkrasae[1], Valerio Marino[2], Michele Bertacchi[1], Krittalak Chakrabandhu[1], Daniele Dell'Orco[2], Michèle Studer[1]
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Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS) is a rare neurodevelopmental disorder caused by mutations in the *NR2F1* gene. NR2F1 acts as a transcriptional regulator and plays an important role in brain development. Several pathogenic mutations were reported in *NR2F1* leading to variable clinical severities among patients. However, the way single mutations affect protein structural stability and neuronal function is still poorly understood. By modeling *NR2F1* missense variants *in silico*, we found that several mutations destabilize the structure of the isolated functional domains and have a negative effect on either the affinity or the stability of the complexes. By using a synthetic biology technique, called genetic code expansion (GCE), that incorporates the photo-crosslinker azido-L-phenylalanine (AzF) at pre-determined sites within the NR2F1 protein, and transient transfections *in vitro*, we unravelled a whole series of putative co-factor protein complexes of different sizes. GCE-captured protein complexes were abolished in the presence of the mutation and restored by amber codon suppression of the full-length NR2F1 protein. Transient transfection of different NR2F1 mutant proteins in HEK293T cells show severe reduction in cell proliferation and increased cell apoptosis together with abnormal accumulation in the cytoplasm of some variants, such as the truncated Q244* and E400* forms, in line with affected protein structural stability. By evaluating the impact of single mutations on NR2F1 nuclear localization, on protein complex formation and on NR2F1-mediated control of cell proliferation and survival, we propose here a genotype to phenotype correlation of BBSOAS from a mechanistic point of view.

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BOARD NUMBER: S02-427

CONVERGENCE OF BEHAVIOURAL AND MOLECULAR PHENOTYPES IN MOUSE AND RAT MODELS FOR DOWN SYNDROME AND CONSEQUENCE FOR FURTHER THERAPIES IN HUMAN.

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Yann Herault, Marion Pellen, Arnaud Duchon, Claire Chevalier, Véronique Brault
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Down syndrome (DS) is the most common genetic form of neurodevelopmental disorder with intellectual disabilities and caused by the presence of an additional copy of human chromosome 21 (Hsa21). The phenotype-genotype relationship, the identification of main driver genes and various proof-of-concepts for therapeutic improvement have benefited from the development of many mouse models and their analysis. To provide novel insights into genotype–phenotype correlations for cognition and other comorbidities found in DS, we have used several new mouse models, refine old one such as the Ts(1716)65Dn/J (Ts65Dn), and develop rat models for DS. We have performed a standardized behavioural and brain pipeline with hippocampal gene expression. We have confirmed the important role of DYRK1A as a main driver of cognitive dysfunction in DS but we have also found new loci which are involved in cognition and behaviour, highlighting the complexity of the altered neurological mechanisms in DS. Our ongoing study has unravelled several interacting loci homologous to Hsa21 gene, contributing to altering brain cognition, function and structure. Our next in-depth analysis of misregulated expressed genes showed that synaptic dysfunction was the consequence of a few altered biological cascades, with more impact in the hippocampus compared to the Entorhinal cortex in some models. Rat models highlighted the importance of investigating a complete DS models with all the regions triplicated. Finally, we provide a novel vision of the existing altered gene-gene crosstalk and molecular mechanisms in DS models that should become central to better understanding of DS and improving the development of therapies in human.

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BOARD NUMBER: S02-428

MUTATIONS IN THE MCPH GENE, RRP7A, CONVERGE ON TGFB/BMP SIGNALING AT THE PRIMARY CILIUM

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Jindriska Leischner Fialova[1], Josefine Stensbøl[1], Emma Dyke[2], Daniel Baird[3], Lars Larsen[3], Nael Nadif Kasri[2], Lotte Pedersen[1], Søren Christensen[1]
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Emerging evidence suggests that neurodevelopmental disorders are closely linked to dysfunction of primary cilia that project as solitary antenna-like organelles on the surface of most vertebrate cell types, including radial glial progenitor cells (RGCs). However, little is still known on the mechanisms by which primary cilia control brain development, and how defects in their function cause congenital brain disorders. We previously identified a homozygous missense mutation (p.W155C) in *Ribosomal RNA Processing 7 Homolog A*, *RRP7A*, segregating with MCPH in a consanguineous family with 10 affected individuals [Farooq et al. (2020) Nat. Comm. 11, 5816]. RRP7A is highly expressed in RGCs in the developing human neocortex and functions in the borderline between nucleoli and primary cilia to control neurogenesis and cell cycle entry, which in part is associated with defects in the timely resorption of primary cilia. The aim of our current work is to decipher the molecular mechanisms by which defects in RRP7A impede ciliary resorption and affect neurodevelopmental signaling in turn leading to MCPH. To this end, we show that RRP7A mutations in patient fibroblasts and (stem) cell models lead to defects in coordination of TGFB/BMP signaling at the primary cilium, and that this signaling phenotype links to defects in *in vitro* neurogenesis. These results provide novel insight into the function of primary cilia in brain development and MCPH, and experiments are underway to delineate in more detail how RRP7A at the cilium orchestrate neural and neuronal development in cerebral organoids and zebrafish models.

Pubmed:
33213355: Fialova JL, Raudenska M, Jakubek M, Kejik Z, Martasek P, Babula P, Matkowski A, Filipensky P, Masarik M
Novel Mitochondria-targeted Drugs for Cancer Therapy.
The search for mitochondria-targeted drugs has dramatically risen over the last decade. Mitochondria are essential organelles serving not only as a powerhouse of the cell but also as a key player in cell proliferation and cell death. Their central role in the energetic metabolism, calcium homeostasis and apoptosis makes them an intriguing field of interest for cancer pharmacology. In cancer cells, many mitochondrial signaling and metabolic pathways are altered. These changes contribute to cancer development and progression. Due to changes in mitochondrial metabolism and changes in membrane potential, cancer cells are more susceptible to mitochondria-targeted therapy. The loss of functional mitochondria leads to the arrest of cancer progression and/or a cancer cell death. Identification of mitochondrial changes specific for tumor growth and progression, rational development of new mitochondria-targeted drugs and research on delivery agents led to the advance of this promising area. This review will highlight the current findings in mitochondrial biology, which are important for cancer initiation, progression and resistance, and discuss approaches of cancer pharmacology with a special focus on the anti-cancer drugs referred to as 'mitocans'.
Mini Rev Med Chem, 2021; 21
34854883: Kuzmić M, Castro Linares G, Leischner Fialová J, Iv F, Salaün D, Llewellyn A, Gomes M, Belhabib M, Liu Y, Asano K, Rodrigues M, Isnardon D, Tachibana T, Koenderink GH, Badache A, Mavrakis M, Verdier-Pinard P
Septin-microtubule association via a motif unique to isoform 1 of septin 9 tunes stress fibers.
Septins, a family of GTP-binding proteins that assemble into higher order structures, interface with the membrane, actin filaments and microtubules, and are thus important regulators of cytoarchitecture. Septin 9 (SEPT9), which is frequently overexpressed in tumors and mutated in hereditary neuralgic amyotrophy (HNA), mediates the binding of septins to microtubules, but the molecular determinants of this interaction remained uncertain. We demonstrate that a short microtubule-associated protein (MAP)-like motif unique to SEPT9 isoform 1 (SEPT9_i1) drives septin octamer-microtubule interaction in cells and in vitro reconstitutions. Septin-microtubule association requires polymerizable septin octamers harboring SEPT9_i1. Although outside of the MAP-like motif, HNA mutations abrogate this association, identifying a putative regulatory domain. Removal of this domain from SEPT9_i1 sequesters septins on microtubules, promotes microtubule

stability and alters actomyosin fiber distribution and tension. Thus, we identify key molecular determinants and potential regulatory roles of septin-microtubule interaction, paving the way to deciphering the mechanisms underlying septin-associated pathologies. This article has an associated First Person interview with the first author of the paper.
J Cell Sci, 2022; 135

31355636: Sojka M, Fojtu M, Fialova J, Masarik M, Necas M, Marek R
Locked and Loaded: Ruthenium(II)-Capped Cucurbit[]uril-Based Rotaxanes with Antimetastatic Properties.
We report here the first coupling of Ru(II) units with cucurbit[6/7]uril-based pseudorotaxane ligands meant for biological application. The resulting ruthenium-capped rotaxanes were fully characterized, and a structure of one supramolecular system was determined by X-ray diffraction. Because the biological properties of Ru-based metallodrugs are tightly linked to the ligand-exchange processes, the effect of salt concentration on the hydrolysis of chlorides from the Ru(II) center was monitored by using H NMR spectroscopy. The biological activity of Ru(II)-based rotaxanes was evaluated for three selected mammalian breast cell lines, HBL-100, MCF-7, and MDA-MB-231. The antimetastatic activity of the assembled cationic Ru(II)-rotaxane systems, evaluated in migration assays against MCF-7 and MDA-MB-231 cell lines, is notably enhanced compared to that of RAPTA-C, a reference that was used. The indicated synergistic effect of combining Ru(II) with a pseudorotaxane unit opens a new direction in searching for anticancer supramolecular metallodrugs.
Inorg Chem, 2019; 58

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BOARD NUMBER: S02-429

IDENTIFICATION OF DEFECTS OF HUMAN CORTICAL NEURON DEVELOPMENT USING SINGLE CELL TRANSCRIPTOMICS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Down syndrome is a common neurodevelopmental condition and the most common genetic cause of intellectual disability. Although the underlying cause, a trisomy of chromosome 21, is known for more than 60 years, it is not well understood how the increased gene dosage of the more than 400 genes on chromosome 21 leads to the observed neurodevelopmental defects, and causal therapies are lacking. Mouse models suggest molecular mechanisms impairing neural progenitor proliferation and neuronal differentiation in the cerebral cortex, but it is unclear to what extent these alterations are recapitulated in the human brain, and whether additional defects occur during neuronal development in the much more complex human cortex. To address these questions, we use single cell transcriptomics to identify molecular and cellular alterations in human cortical neuron development in Down syndrome in different experimental settings, including primary fetal brain tissue and a novel in vivo graft model. Here, we present preliminary data from our graft model, suggesting that progenitor proliferation and excitatory neuron generation are also impaired in human neuron development. This is supported by a re-analysis of a published scRNA-Seq dataset of cortical organoids, which also suggests potential regulators that may mediate these phenotypes. Overall, our work will help to clarify the mechanisms underlying neurodevelopmental alterations in Down syndrome and may indicate new strategies to ameliorate cognitive impairments in this common neurodevelopmental condition.

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BOARD NUMBER: S02-430

MODELLING GENETIC DISORDERS OF LYSINE METABOLISM IN A DISH

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

Imke Schuurmans[1,2,3], Katrin Linda[4], Tessa Peters[5,6], Udo Engelke[7], Karlien Coene[8], Clara Van Karnebeek[9], Nael Nadif Kasri[10,11], Alex Garanto[1,3,12]
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Pyridoxine-dependent epilepsy (PDE-ALDH7A1) and glutaric aciduria type 1 (GA1) are two rare neurometabolic disorders of lysine metabolism, caused by pathogenic variants in *ALDH7A1* and *GCDH,* respectively. Deficiency of the encoded enzymes results in accumulation of neurotoxic metabolites causing debilitating neurological sequelae in patients. Currently, no suitable human PDE-ALHD7A1 and GA1 models are available. Therefore, we here aim to develop human cellular PDE-ALDH7A1 and GA1 models to investigate the disease mechanism and identify therapeutic targets. In this study, patient-derived fibroblasts were reprogrammed into induced pluripotent stem cells (iPSCs). In addition, isogenic knock-out lines derived from control iPSCs have been generated using the CRISPR/Cas9 system. Because *ALDH7A1* and *GCDH* expression is enriched in astrocytes, we differentiated iPSC lines towards neurons and astrocytes. We show that iPSC-derived astrocytes generated using a two-step differentiation protocol, are both morphologically and functionally mature within 5 weeks of differentiation. In addition, when co-cultured together with cortical excitatory neurons, the iPSC-derived astrocytes support neuronal maturation and synapse formation confirmed by immunostainings with MAP2 and Synapsin1/2 antibodies respectively. Co-cultures of patient-derived neurons and astrocytes on micro-electrode arrays are then used for electrophysiological and molecular measurements to identify the disease-specific neural network activity. Moreover, cell extracts and medium of both the fibroblasts and neuronal and astrocyte co-cultures are collected for metabolomic analysis. By in-depth molecular, electrophysiological and metabolomic characterization of fibroblasts and neuronal and astrocyte co-cultures, we aim to understand the neurotoxicity mechanism underlying these two neurometabolic disorders.

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BOARD NUMBER: S02-431

CEREBELLUM-POSTNATAL DEVELOPMENT AND BEHAVIOR ONTOGENESIS IN A MOUSE MODEL OF THE INTELLECTUAL DISABILITY-ASSOCIATED COFFIN-LOWRY SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Coffin-Lowry syndrome (CLS) is a rare neurodevelopmental disorder characterized by severe intellectual disability associated with physical and neurological abnormalities. CLS is due to mutations in the *rps6ka3* gene that encodes the RSK2 kinase, which acts at the end of the MAPK signaling pathway, known for its key role in cognitive processes. Once activated by ERK, RSK2 has numerous cytosolic and nuclear targets, such as CREB, Histone 3, PDL1, suggesting a key role in different cellular processes (proliferation, differentiation, and neuronal maturation). We therefore characterized postnatal brain development of the *Rsk2*-KO mouse model of CLS. Our *in vivo* MRI analyses revealed reduced volumes of the whole brain, hippocampus and cerebellum in *Rsk2*-KO mice from postnatal day (PND) 14. Interestingly, total brain volume spontaneously recovers WT level in adulthood, highlighting a delay in postnatal development. Behavioral tests revealed that *Rsk2*-KO mice display slight motor or spontaneous behavior deficits during the postnatal period, suggesting altered cerebellum integrity. We then studied cellular processes in the cerebellum and found that at PND7 the absence of RSK2 does not affect the proliferation and migration of granular cells from the external to the internal granular layer, but impacts the development of Purkinje-cell dendritic tree. Biochemical analyses further revealed a dysregulation of the MAPK signaling pathway that appears at PND14, which could be responsible for the developmental alterations observed in the cerebellum. Supported by Fondation Jerome Lejeune, Fondation Xtraordinaire, PhD (LG) supported by FRM, Graduate School Life Science and Health and Paris-Saclay University.

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BOARD NUMBER: S02-432

TREATMENT WITH THE CB1R ANTAGONIST RIMONABANT RESCUES BRAIN MITOCHONDRIAL DYSFUNCTION VIA INHIBITION OF INTRA-MITOCHONDRIAL PROTEIN KINASE A SIGNALLING IN A MOUSE MODEL OF RETT SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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AIMS. Cannabinoid receptors 1 (CB1R) have attracted much attention as potential therapeutic targets for intellectual disability disorders; in fact, treatment with the CB1R antagonist rimonabant normalizes neurobehavioural alterations in Fragile X and Down syndromes murine models. Importantly, increased CB1R activation at brain mitochondria membranes (mtCB1R) can affect mitochondrial activity and memory processes via inhibition of intra-mitochondrial protein kinase A (PKA), suggesting CB1R inhibitors as promising drug candidates for intellectual disability disorders characterized by brain mitochondrial alterations. Defective brain mitochondria and the resulting increased levels of free radicals are emerging crucial factors in the pathogenesis of Rett syndrome (RTT), a rare disorder and a major cause of intellectual disability in females. In the present study, we explored whether a systemic treatment with rimonabant can rescue brain mitochondrial dysfunction in a RTT mouse model. **METHODS**. Fully symptomatic MeCP2-308 heterozygous female mice (HET) and wild-type littermates received a systemic treatment with rimonabant or vehicle (4-day-long intraperitoneal injections at the dose of 0.3 mg/kg/day) at the end of which brains were cryopreserved for bioenergetic analyses. **RESULTS**. Treatment with rimonabant normalized the reduced mitochondrial ATP production and ATP levels in the brain of HET mice. Rimonabant also rescued the overexpression of the cerebral mtCB1R and the intra-mitochondrial hypoactivation of PKA in the hippocampus of HET mice. **CONCLUSIONS**. Present results outline a potential mechanism underlying mitochondrial alterations in RTT mouse brain which could represent a novel target for the treatment of this severe disease. Funded by the Italian Ministry of Health (#GR-2018-12366210).

Pubmed:
34201747: Urbinati C, Cosentino L, Germinario EAP, Valenti D, Vigli D, Ricceri L, Laviola G, Fiorentini C, Vacca RA, Fabbri A, De Filippis B
Treatment with the Bacterial Toxin CNF1 Selectively Rescues Cognitive and Brain Mitochondrial Deficits in a Female Mouse Model of Rett Syndrome Carrying a MeCP2-Null Mutation.
Rett syndrome (RTT) is a rare neurological disorder caused by mutations in the X-linked gene and a major cause of intellectual disability in females. No cure exists for RTT. We previously reported that the behavioural phenotype and brain mitochondria dysfunction are widely rescued by a single intracerebroventricular injection of the bacterial toxin CNF1 in a RTT mouse model carrying a truncating mutation of the gene (MeCP2-308 mice). Given the heterogeneity of mutations in RTT patients, we tested the CNF1 therapeutic efficacy in a mouse model carrying a null mutation (MeCP2-Bird mice). CNF1 selectively rescued cognitive defects, without improving other RTT-related behavioural alterations, and restored brain mitochondrial respiratory chain complex activity in MeCP2-Bird mice. To shed light on the molecular mechanisms underlying the differential CNF1 effects on the behavioural phenotype, we compared treatment effects on relevant signalling cascades in the brain of the two RTT models. CNF1 provided a significant boost of the mTOR activation in MeCP2-308 hippocampus, which was not observed in the MeCP2-Bird model, possibly explaining the differential effects of CNF1. These results demonstrate that CNF1 efficacy depends on the mutation beared by MeCP2-mutated mice, stressing the need of testing potential therapeutic approaches across RTT models.
Int J Mol Sci, 2021; 22
32492904: Zuliani I, Urbinati C, Valenti D, Quattrini MC, Medici V, Cosentino L, Pietraforte D, Di Domenico F, Perluigi M, Vacca RA, De Filippis B
The Anti-Diabetic Drug Metformin Rescues Aberrant Mitochondrial Activity and Restrains Oxidative Stress in a Female

Mouse Model of Rett Syndrome.
Metformin is the first-line therapy for diabetes, even in children, and a promising attractive candidate for drug repurposing. Mitochondria are emerging as crucial targets of metformin action both in the periphery and in the brain. The present study evaluated whether treatment with metformin may rescue brain mitochondrial alterations and contrast the increased oxidative stress in a validated mouse model of Rett syndrome (RTT), a rare neurologic disorder of monogenic origin characterized by severe behavioral and physiological symptoms. No cure for RTT is available. In fully symptomatic RTT mice (12 months old MeCP2-308 heterozygous female mice), systemic treatment with metformin (100 mg/kg ip for 10 days) normalized the reduced mitochondrial ATP production and ATP levels in the whole-brain, reduced brain oxidative damage, and rescued the increased production of reactive oxidizing species in blood. A 10-day long treatment with metformin also boosted pathways related to mitochondrial biogenesis and antioxidant defense in the brain of metformin-treated RTT mice. This treatment regimen did not improve general health status and motor dysfunction in RTT mice at an advanced stage of the disease. Present results provide evidence that systemic treatment with metformin may represent a novel, repurposable therapeutic strategy for RTT.
J Clin Med, 2020; 9

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BOARD NUMBER: S02-433

MIGRATION DEFECTS IN FRAGILE X SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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The Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability and is caused by the absence of the mRNA-binding protein FMRP (Fragile X Mental Retardation Protein). FXS patients and animal models present numerous neurodevelopmental and plasticity defects. Neuronal migration is crucial to the establishment of functional neural circuits but remains mostly unexplored in FXS. We analyzed the migration defects induced by FMRP absence in the context of the postnatal Rostral Migratory Stream (RMS). In the RMS, neurons perform cyclic saltatory migration from the ventricular/subventricular zone to the olfactory bulb along with stereotypical movement of their centrosome. Through *in vivo* intraventricular electroporation of plasmids expressing fluorescent proteins followed by confocal live imaging, we show that migrating neurons in *Fmr1-null* mice display slowed-down migration along with altered trajectory and defective centrosomal dynamic. This phenotype is recapitulated by RNA interference and hence cell autonomous. Mutated neurons also display altered morphology, suggesting cytoskeletal defects. The microtubule (MT) network is essential during neuronal migration. By immunostaining, we analyzed the subcellular compartmentalization of dynamic tyrosinated MTs and observed strong defects in MT tyrosination in mutant neurons. MAP1B (Microtubule Associated Protein 1B) is a known FMRP mRNA target involved in the control of MT tyrosination. We will thus test whether increased MAP1B in mutant neurons is causal in defective MT dynamics leading to abnormal migration. Our results hence unveil a new neurodevelopmental role of FMRP, as a key element of neuronal migration, likely to be essential for the understanding of FXS pathophysiology.

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BOARD NUMBER: S02-434

ALTERATIONS OF CORTICAL CONNECTIVITY IN A MOUSE MODEL OF PREMATURE BRAIN INJURY

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Cortical circuits are built at perinatal times and gradually refined in an activity- dependent manner during the postnatal period of critical plasticity. Although lesions of the CNS occurring during this period recover better than those occurring later in life, they are often associated with long-term cognitive deficits, which suggests that neuronal circuits rewiring, in particular within the cortex, may either be incomplete or inappropriate. Here we used chronic hypoxia, a mouse model of very premature birth, to study the long-term impact of premature brain injuries on glutamatergic neuron's maturation and cortical circuit's formation. Our results reveal gradual and profound alterations of glutamatergic neurons dendritic arborizations following chronic hypoxia, that differentially affect their apical and basal dendritic compartments. Using retrograde tracing, we show that these dendritic alterations are paralleled by a global cortical hyperconnectivity as well as a redistribution of long-distance cortical connections. Finally, testing of sociability reveals an impairment for social novelty in young adult hypoxic mice, which amplifies in adulthood. Altogether, our results highlight how premature brain injuries, such as those resulting from very premature births, impact cortical neuron maturation and connectivity, as well as associated behaviors.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S02-435

CORTICOGENESIS IS IMPAIRED IN A MOUSE MODEL OF DDX3X SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Background: DDX3X syndrome is caused by mutations in the *DDX3X* gene and accounts for 1-3% of unexplained intellectual disability (ID) in females, presenting also behavioral problems and motor impairments. Even though the genetic cause of the syndrome is known, the cellular and molecular mechanisms driving it remain elusive. DDX3X is an X-linked gene that regulates mRNA translation and has emerging functions in corticogenesis and synaptogenesis. We previously generated the first mouse model (*Ddx3x*$^{+/-}$) with construct and face validity for *DDX3X* loss-of-function mutations, which showed developmental and behavioral alterations accompanied by defective cortical lamination. **Aims:** The aim of this study is to elucidate the cellular and molecular functions of DDX3X syndrome during corticogenesis. **Methods:** We used our Ddx3x haploinsufficient mouse model (*Ddx3x*$^{+/-}$) to study the impact of *Ddx3x* haploinsufficiency on cortical development, by examining cortical progenitors and post-mitotic neurons using cell-specific markers at different time points of embryogenesis. We also performed *in vivo* birth dating of specific populations of glutamatergic neurons. **Results:** *Ddx3x*$^{+/-}$ mice present alterations in cortical neurogenesis and cell fate specification compared to their *Ddx3x*$^{+/+}$ littermates. *Ddx3x*$^{+/-}$ mice have changes in the abundance and/or laminar position of specific populations of cortical projection neurons. These changes are area-specific. *Ddx3x*$^{+/-}$ mice also show alterations of cortical progenitors. **Conclusion:** Our data shed new light on the cellular mechanisms driving the neurodevelopmental phenotypes of DDX3X syndrome.

Pubmed:
33218123: Garcia-Forn M, Boitnott A, Akpinar Z, De Rubeis S
Linking Autism Risk Genes to Disruption of Cortical Development.
Autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder characterized by impairments in social communication and social interaction, and the presence of repetitive behaviors and/or restricted interests. In the past few years, large-scale whole-exome sequencing and genome-wide association studies have made enormous progress in our understanding of the genetic risk architecture of ASD. While showing a complex and heterogeneous landscape, these studies have led to the identification of genetic loci associated with ASD risk. The intersection of genetic and transcriptomic analyses have also begun to shed light on functional convergences between risk genes, with the mid-fetal development of the cerebral cortex emerging as a critical nexus for ASD. In this review, we provide a concise summary of the latest genetic discoveries on ASD. We then discuss the studies in postmortem tissues, stem cell models, and rodent models that implicate recently identified ASD risk genes in cortical development.
Cells, 2020; 9
34344536: Boitnott A, Garcia-Forn M, Ung DC, Niblo K, Mendonca D, Park Y, Flores M, Maxwell S, Ellegood J, Qiu LR, Grice DE, Lerch JP, Rasin MR, Buxbaum JD, Drapeau E, De Rubeis S
Developmental and Behavioral Phenotypes in a Mouse Model of DDX3X Syndrome.
Mutations in the X-linked gene DDX3X account for approximately 2% of intellectual disability in females, often comorbid with behavioral problems, motor deficits, and brain malformations. DDX3X encodes an RNA helicase with emerging functions in corticogenesis and synaptogenesis.
Biol Psychiatry, 2021; 90
33345999: Meneses-Salas E, Garcia-Forn M, Castany-Pladevall C, Lu A, Fajardo A, Jose J, Wahba M, Bosch M, Pol A, Tebar F, Klein AD, Zanlungo S, Pérez-Navarro E, Grewal T, Enrich C, Rentero C
Lack of Annexin A6 Exacerbates Liver Dysfunction and Reduces Lifespan of Niemann-Pick Type C Protein-Deficient Mice.
Niemann-Pick type C (NPC) disease is a lysosomal storage disorder characterized by cholesterol accumulation caused by loss-of-function mutations in the Npc1 gene. NPC disease primarily affects the brain, causing neuronal damage and affecting

motor coordination. In addition, considerable liver malfunction in NPC disease is common. Recently, we found that the depletion of annexin A6 (ANXA6), which is most abundant in the liver and involved in cholesterol transport, ameliorated cholesterol accumulation in Npc1 mutant cells. To evaluate the potential contribution of ANXA6 in the progression of NPC disease, double-knockout mice (Npc1/Anxa6) were generated and examined for lifespan, neurologic and hepatic functions, as well as liver histology and ultrastructure. Interestingly, lack of ANXA6 in NPC1-deficient animals did not prevent the cerebellar degeneration phenotype, but further deteriorated their compromised hepatic functions and reduced their lifespan. Moreover, livers of Npc1/Anxa6 mice contained a significantly elevated number of foam cells congesting the sinusoidal space, a feature commonly associated with inflammation. We hypothesize that ANXA6 deficiency in Npc1 mice not only does not reverse neurologic and motor dysfunction, but further worsens overall liver function, exacerbating hepatic failure in NPC disease.
Am J Pathol, 2021; 191
33369245: Alcalá-Vida R, Garcia-Forn M, Castany-Pladevall C, Creus-Muncunill J, Ito Y, Blanco E, Golbano A, Crespí-Vázquez K, Parry A, Slater G, Samarajiwa S, Peiró S, Di Croce L, Narita M, Pérez-Navarro E
Neuron type-specific increase in lamin B1 contributes to nuclear dysfunction in Huntington's disease.
Lamins are crucial proteins for nuclear functionality. Here, we provide new evidence showing that increased lamin B1 levels contribute to the pathophysiology of Huntington's disease (HD), a CAG repeat-associated neurodegenerative disorder. Through fluorescence-activated nuclear suspension imaging, we show that nucleus from striatal medium-sized spiny and CA1 hippocampal neurons display increased lamin B1 levels, in correlation with altered nuclear morphology and nucleocytoplasmic transport disruption. Moreover, ChIP-sequencing analysis shows an alteration of lamin-associated chromatin domains in hippocampal nuclei, accompanied by changes in chromatin accessibility and transcriptional dysregulation. Supporting lamin B1 alterations as a causal role in mutant huntingtin-mediated neurodegeneration, pharmacological normalization of lamin B1 levels in the hippocampus of the R6/1 mouse model of HD by betulinic acid administration restored nuclear homeostasis and prevented motor and cognitive dysfunction. Collectively, our work points increased lamin B1 levels as a new pathogenic mechanism in HD and provides a novel target for its intervention.
EMBO Mol Med, 2021; 13
35053183: Pérez-Sisqués L, Solana-Balaguer J, Campoy-Campos G, Martín-Flores N, Sancho-Balsells A, Vives-Isern M, Soler-Palazón F, Garcia-Forn M, Masana M, Alberch J, Pérez-Navarro E, Giralt A, Malagelada C
RTP801/REDD1 Is Involved in Neuroinflammation and Modulates Cognitive Dysfunction in Huntington's Disease.
RTP801/REDD1 is a stress-regulated protein whose levels are increased in several neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's diseases (HD). RTP801 downregulation ameliorates behavioral abnormalities in several mouse models of these disorders. In HD, RTP801 mediates mutant huntingtin (mhtt) toxicity in in vitro models and its levels are increased in human iPSCs, human postmortem putamen samples, and in striatal synaptosomes from mouse models of the disease. Here, we investigated the role of RTP801 in the hippocampal pathophysiology of HD. We found that RTP801 levels are increased in the hippocampus of HD patients in correlation with gliosis markers. Although RTP801 expression is not altered in the hippocampus of the R6/1 mouse model of HD, neuronal RTP801 silencing in the dorsal hippocampus with shRNA containing AAV particles ameliorates cognitive alterations. This recovery is associated with a partial rescue of synaptic markers and with a reduction in inflammatory events, especially microgliosis. Altogether, our results indicate that RTP801 could be a marker of hippocampal neuroinflammation in HD patients and a promising therapeutic target of the disease.
Biomolecules, 2021; 12
31365052: Creus-Muncunill J, Badillos-Rodríguez R, Garcia-Forn M, Masana M, Garcia-Díaz Barriga G, Guisado-Corcoll A, Alberch J, Malagelada C, Delgado-García JM, Gruart A, Pérez-Navarro E
Increased translation as a novel pathogenic mechanism in Huntington's disease.
Huntington's disease is a neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the huntingtin gene. Striatal projection neurons are mainly affected, leading to motor symptoms, but molecular mechanisms involved in their vulnerability are not fully characterized. Here, we show that eIF4E binding protein (4E-BP), a protein that inhibits translation, is inactivated in Huntington's disease striatum by increased phosphorylation. Accordingly, we detected aberrant de novo protein synthesis. Proteomic characterization indicates that translation specifically affects sets of proteins as we observed upregulation of ribosomal and oxidative phosphorylation proteins and downregulation of proteins related to neuronal structure and function. Interestingly, treatment with the translation inhibitor 4EGI-1 prevented R6/1 mice motor deficits, although corticostriatal long-term depression was not markedly changed in behaving animals. At the molecular level, injection of 4EGI-1 normalized protein synthesis and ribosomal content in R6/1 mouse striatum. In conclusion, our results indicate that dysregulation of protein synthesis is involved in mutant huntingtin-induced striatal neuron dysfunction.
Brain, 2019; 142
31023421: Molina-Porcel L, Pérez-Navarro E, García-Forn M, Westaway D, Colom-Cadena M, Gelpi E

Teaching case 3-2019: Are nuclear clefts or invaginations the niche of intranuclear inclusions in FTLD-TDP?
Clin Neuropathol, 2019 May/Jun; 38
30176350: García-Forn M, Martínez-Torres S, García-Díaz Barriga G, Alberch J, Milà M, Azkona G, Pérez-Navarro E
Pharmacogenetic modulation of STEP improves motor and cognitive function in a mouse model of Huntington's disease.
Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an expansion of a CAG repeat in the huntingtin (htt) gene, which results in an aberrant form of the protein (mhtt). This leads to motor and cognitive deficits associated with corticostriatal and hippocampal alterations. The levels of STriatal-Enriched protein tyrosine Phosphatase (STEP), a neural-specific tyrosine phosphatase that opposes the development of synaptic strengthening, are decreased in the striatum of HD patients and also in R6/1 mice, thereby contributing to the resistance to excitotoxicity described in this HD mouse model. Here, we aimed to analyze whether STEP inactivation plays a role in the pathophysiology of HD by investigating its effect on motor and cognitive impairment in the R6/1 mouse model of HD. We found that genetic deletion of STEP delayed the onset of motor dysfunction and prevented the appearance of cognitive deficits in R6/1 mice. This phenotype was accompanied by an increase in pERK1/2 levels, a delay in the decrease of striatal DARPP-32 levels and a reduction in the size of mhtt aggregates, both in the striatum and CA1 hippocampal region. We also found that acute pharmacological inhibition of STEP with TC-2153 improved cognitive function in R6/1 mice. In conclusion, our results show that deletion of STEP has a beneficial effect on motor coordination and cognition in a mouse model of HD suggesting that STEP inhibition could be a good therapeutic strategy in HD patients.
Neurobiol Dis, 2018; 120

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ALTERED BEHAVIOUR, METABOLISM AND NEURAL ACTIVITY IN A MOUSE MODEL OF INTELLECTUAL DISABILITY WITH A MUTATION IN PDZD8 GENE

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Intellectual disability (ID) is the most common childhood neurodevelopmental disorder that affects learning ability, impairs cognitive functioning, and is defined by an intelligence quotient below 70. Although ID is caused by both environmental and genetic causes, severe forms are often associated with defects in single genes. We have identified four children from two unrelated families diagnosed with intellectual disability, developmental delay, macrocephaly and other autistic features caused by a specific mutation in PDZ domain containing protein 8 (PDZD8) gene. PDZD8 is an ER-mitochondria tethering protein which regulates calcium dynamics in mammalian neurons and has not previously been associated with any human disease. As PDZD8 mutation is a novel mutation linked to ID, our aim was to further characterise PDZD8 disruption in a mouse model in terms of behaviour analysis, molecular characterization and *in vivo* two-photon calcium imaging of neuronal dynamics. Mice homozygous for a premature termination codon in PDZD8 displayed abnormal brain structure, cognitive impairment, and altered neuronal activity *in vivo.* These data demonstrate the involvement of a homozygous loss of function mutation in PDZD8 in a neurodevelopmental cognitive disorder.

Pubmed:
27535874: O'Neill P, Lindsay SL, Pantiru A, Guimond SE, Fagoe N, Verhaagen J, Turnbull JE, Riddell JS, Barnett SC Sulfatase-mediated manipulation of the astrocyte-Schwann cell interface.
Schwann cell (SC) transplantation following spinal cord injury (SCI) may have therapeutic potential. Functional recovery is limited however, due to poor SC interactions with host astrocytes and the induction of astrogliosis. Olfactory ensheathing cells (OECs) are closely related to SCs, but intermix more readily with astrocytes in culture and induce less astrogliosis. We previously demonstrated that OECs express higher levels of sulfatases, enzymes that remove 6-O-sulfate groups from heparan sulphate proteoglycans, than SCs and that RNAi knockdown of sulfatase prevented OEC-astrocyte mixing in vitro. As human OECs are difficult to culture in large numbers we have genetically engineered SCs using lentiviral vectors to express sulfatase 1 and 2 (SC-S1S2) and assessed their ability to interact with astrocytes. We demonstrate that SC-S1S2s have increased integrin-dependent motility in the presence of astrocytes via modulation of NRG and FGF receptor-linked PI3K/AKT intracellular signaling and do not form boundaries with astrocytes in culture. SC-astrocyte mixing is dependent on local NRG concentration and we propose that sulfatase enzymes influence the bioavailability of NRG ligand and thus influence SC behavior. We further demonstrate that injection of sulfatase expressing SCs into spinal cord white matter results in less glial reactivity than control SC injections comparable to that of OEC injections. Our data indicate that sulfatase-mediated modification of the extracellular matrix can influence glial interactions with astrocytes, and that SCs engineered to express sulfatase may be more OEC-like in character. This approach may be beneficial for cell transplant-mediated spinal cord repair. GLIA 2016 GLIA 2017;65:19-33.
Glia, 2017; 65

FENS Forum 2022
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BOARD NUMBER: S02-437

COMPREHENSIVE DELINEATION AND PRECISION MEDICINE OF GRIN-RELATED NEURODEVELOPMENTAL DISORDERS, A PRIMARY DISTURBANCE OF THE NMDA RECEPTOR

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Glutamate, the main excitatory amino acid neurotransmitter plays a crucial role in neuronal physiology. Glutamatergic neurotansmission disturbance can result from primary *de novo* mutations of *GRIN* genes, encoding for the *N*-methyl-D-Aspartate receptor (NMDAR) subunits. These rare autosomic dominant conditions cause severe neurodevelopmental encephalopathies namely GRIN-related disorders (GRD, also called Grinpathies). GRD display a clinical spectrum including intellectual disability, hypotonia, ASD traits, motor impairment, epilepsy, and gastro-intestinal distress in a gene- and residue-dependent manner. Accordingly, as for other channelopathies, the functional annotation of *GRIN de novo* variants is critical i) to understand GRD pathophysiology, ii) to evaluate potential therapeutic strategies and iii) to define personalised therapeutic approaches. Accordingly, we have created a multi-angled GRIN cluster initiative, merging computational, experimental, translational, and clinical neuroscience approaches. Bioinformatic analysis allowed to build-up a comprehensive and specific *GRIN* variants database compiling genetic, structural, functional and clinical annotations. This database allowed to define a superimposition structural algorithm drastically increasing *GRIN* variants annotations with a high predictive likelihood ultimately accelerating *GRIN* variants functional annotations. Further, an experimental pipeline has been developed for the annotation of GRIN-orphan variants and their functional stratification. Finally, we evaluated and experimentally demonstrated the potential therapeutic benefit of nutraceutical interventions for the rescue of LoF *GRIN* variants, both in preclinical models and in proof-of-concept GRD cases. These findings opened the avenue for the first reported GRD clinical trial (ongoing) as well as for future treatments of genetic conditions perturbing the glutamatergic synapse.

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DE NOVO MISSENSE MUTATION IN CERS6 IDENTIFIED IN A PATIENT WITH CORTICAL HETEROTOPIA

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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A *de novo* missense mutation in CERS6 has been identified by human exome sequencing in a patient with subcortical heterotopia. This is a rare disorder associated with epilepsy and intellectual disability, which may be caused by disturbances in cortical progenitors and /or migrating neurons. Ceramide synthases are important for the biogenesis of ceramides in the endoplasmic reticulum, which will then be transported to the Golgi apparatus for the formation of complex sphingolipids to be transported to the membranes. The major aim of this project is to understand the role of ceramide synthases in normal cortical development, for which we use knockdown and overexpression approaches. Importantly, we will question the impact of the CERS6 patient-specific mutation in the processes mentioned above leading to the formation of heterotopia. Our preliminary analyses suggest delayed migration of neurons upon CerS6 knockdown in mouse cortex. Future experiments will continue loss of function experiments and phenotypic analyses. Furthermore, our future experiments will be aimed at understanding the subcellular mechanisms impacted by normal and mutant CerS6. These studies highlight the understudied role played by lipid biogenesis factors in cortical development.

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BOARD NUMBER: S02-439

ANTI-NKCC1 GENE THERAPY RESCUES COGNITIVE DEFICITS IN A MOUSE MODEL OF DOWN SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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A common feature of diverse brain disorders is the alteration of GABA-mediated inhibition because of aberrant, intracellular chloride homeostasis induced by changes in the expression and/or function of chloride transporters. Notably, pharmacological inhibition of the chloride importer NKCC1 is able to rescue brain-related core deficits in animal models of these pathologies and in some human clinical studies. In this study, we aimed to test the potential therapeutic applications of NKCC1 gene knock down in the Ts65Dn mouse model of Down syndrome (DS) by intraparenchymal injection of two different doses of adeno-associated virus (AAV) expressing artificial microRNA (amiR) targeted to NKCC1. We performed bilateral hippocampal stereotaxic injections into 2-3 month-old Ts65Dn mice with either control or NKCC1 amiR. AAV-mediated expression of NKCC1 amiR effectively diminished NKCC1 expression in vivo in a dose-dependent manner, without inducing appreciable inflammation. Ts65Dn mice showed significant deficits in learning and memory tests including a marked impairment in long-term discriminative and spatial memory, short-term working memory, and associative memory. NKCC1 amiR effectively rescued the cognitive deficits in the behavioral tasks in a dose-dependent manner. We are currently differentiating an isogenic pair of IPSCs (WT and DS) lines into neural progenitor stem cells (NPCs) into neuronal and glial cells to assess whether our NKCC1 amiR approach may be successful when applied also to human cells. Our results provide compelling evidence that NKCC1 is implicated in cognitive dysfunctions in DS adults, thereby firmly supporting the potential breakthrough of NKCC1 knockdown gene therapy in DS.

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BOARD NUMBER: S02-440

NOVEL INSIGHT INTO THE NEURODEVELOPMENTAL DISORDER BBSOAS: NR2F1 CONTROLS MITOCHONDRIAL ARCHITECTURE IN ADULT-BORN MOUSE HIPPOCAMPAL NEURONS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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The Bosch-Boonstra-Schaaf optic atrophy-intellectual syndrome (BBSOAS), is a rare neurodevelopmental disorder due to mutations in the NR2F1 gene, which code for the strong transcriptional regulator Nr2f1, also known as COUP-TFI. The BBSOAS is characterized by several symptoms, including optic nerve atrophy, seizures, intellectual disability and autistic traits, that are often linked to mitochondrial dysfunction. Moreover, alterations in mitochondrial energy supply have been recently reported in BBSOAS patients. In this study, we combined genome-wide and in silico analyses to mouse genetics, neuroanatomical and imaging approaches to test the hypothesis that Nr2f1controls mitochondrial function. First, we found that key mitochondrial proteins are potential genomic targets under direct Nr2f1 transcriptional control in neurons. Next, we focused on the adult mouse hippocampal dentate gyrus (DG), a site of persistent neurogenesis, to genetically manipulate Nr2f1 expression in newborn neurons. These experiments showed that both Nr2f1 loss- and gain-of-function result in altered mitochondrial architecture in newborn neurons, suggesting a role for Nr2f1 on mitochondrial dynamics. In line, we identified the mitochondrial membrane protein Mfn2, a key factor for mitochondrial fusion, as a direct target gene under the Nr2f1 transcriptional activity. We found that Mfn2 is downregulated in the DG of mice heterozygous for Nr2f1, a validated BBSOAS mouse model, and we confirmed its downregulation in adult-born DG neurons conditionally depleted for Nr2f1. Our study provides the first evidence of a key role for Nr2f1 in controlling mitochondria architecture in DG neurons and open new perspectives on the mechanisms underlying the human disease BBSOAS.

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BOARD NUMBER: S02-441

NEUROANATOMICAL STUDIES IDENTIFY VPS13B AS AN IMPORTANT REGULATOR OF BRAIN ARCHITECTURE AND HIPPOCAMPAL FORMATION

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Vacuolar Protein Sorting 13 homolog B (VPS13B) is a highly conserved protein through evolution, but its function is not well understood yet. Human mutations in *VPS13B* cause Cohen Syndrome, a rare recessive developmental disease characterized by intellectual disability, acquired microcephaly and hypotonia. Mouse *Vps13b* is highly expressed across several brain structures including the hippocampus and the cortex. Using a knockout mouse model approach, we set out to identify the role of VPS13B in the brain. We first showed that homozygous mutant mice of *Vps13b* are sub-viable, half dying progressively during the first week of life. The survivor homozygous mutant mice exhibit growth delay and microcephaly (-27% for weight and -20% for microcephaly in adult male mice). Using a systematic neuroanatomical study at multiple ages (embryonic E18.5 and postnatal 5, 7, 18 and 33 weeks), we then found severe neuroanatomical changes appearing within the first few weeks after birth in homozygous mutant mice when compared to matched controls. The hippocampus is the most affected brain region with a reduction of 34% of the size of the dentate gyrus. Finally, we performed a battery of behavioral tests and showed hyperactivity, hypotonia and altered memory but enhanced sociability and resilience to anxiety and depression in *Vps13b* homozygous mutant mice. It is noteworthy that heterozygous mutant mice did not show any apparent phenotype. Together, these findings indicate a highly specific role of VPS13B in the regulation of brain structure and an association with previously unreported features of Cohen Syndrome.

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DECIPHERING THE GENETICS OF BRAIN MALFORMATION DISORDERS REVEALS THE IMPORTANCE OF WD-REPEAT 47 (WDR47) AND WD-REPEAT 91 (WDR91) GENES IN MICE

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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WD-repeat (WDR) proteins are one of largest eukaryotic family, however little is known about their role in neurodevelopment. In a previous report, we manually curated 246 WDR proteins and found that WDR genes are 3-times more likely to be linked to brain malformation disorders. Here we set out to explain how mutations in two WDR genes, WDR47 and WDR91, lead to brain structural anomalies. Using the CRISPR/cas9 system, we generated two mouse models with mutations at position p.Val660Ala for WDR47 and p.Tyr80^{STOP} for WDR91, to model variants found in human patients and assess their pathogenesis. Because both mouse models were not viable at the homozygous state, we conducted the experiments in heterozygous mutants. More specifically, we carried out systematic and ultra-standardized histological studies of brain anatomy at various timepoints, assessing 39 different brain parameters. We compared neuroanatomical defects between WDR47 and WDR91 and found the most severe brain malformations in WDR91 p.Tyr80^{STOP}. Indeed, this mutation resulted in reduced size of four main brain regions known to be implicated in cognition, motor activity and coordination, namely the hippocampus, neocortex, thalamus and cerebellum. The cerebellar area was the most affected region with a size reduction of 28%. The area of the neuroepithelial ventricular zone in the neocortex, where the cells decide to proliferate or differentiate, was the only parameter among the 39 evaluated whose size was significantly increased by 39%. These findings indicate that WDR91 has a more important role in the regulation of brain architecture from earlier developmental stages than WDR47.

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TOWARDS A CURE FOR CREATINE TRANSPORTER DEFICIENCY: A GENE THERAPY APPROACH

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Aims: Creatine Transporter Deficiency (CTD) is an X-linked neurodevelopmental disorder caused by mutations in the Creatine Transporter (CrT) gene presenting with cerebral creatine (Cr) depletion, intellectual disability, epilepsy and autistic-like behaviour. CTD has a major impact on patient quality of life and no cure is available. Here, we evaluated gene therapy as a possible solution to reverse CTD pathology.
Methods: We used Adeno-Associated Viral vectors to deliver a functional copy of CrT gene (AAV/CrT) to newborn CrT KO mice through intracerebroventricular injection. After six weeks we assessed the distribution of the vector and measured Cr levels in the brain, and we longitudinally evaluated the effectiveness of this strategy in reversing the CTD phenotype by combining behavioral and imaging analyses.
Results: Preliminary data showed that AAV/CrT administration resulted in the expression of transgenic CrT and increased Cr levels in the cerebral cortex and hippocampus. However, toxicity and inflammation were observed with high titres of the vector. Behavioural investigation on mice treated with non-toxic doses demonstrated that the treatment improved cognitive performance. We are currently optimising the vector design to obtain a widespread, physiological expression of CrT reducing the toxicity caused by creatine overload.
Conclusions: Our results indicate that AAV-mediated delivery of exogenous CrT increases cerebral Cr levels and may improve cognitive performances in a CTD mouse model, providing an encouraging first step towards the proof-of-concept for the feasibility of CrT replacement therapy.

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ALTERED GABA-MEDIATED INHIBITION DURING DEVELOPMENT IN NEURONAL NETWORKS FROM THE TS65DN MOUSE MODEL OF DOWN SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Down syndrome (DS) is a genetic disorder that causes intellectual disability in children and adults. People with DS also present higher susceptibility to seizures and often hyperactivity. Altered GABAergic signaling through chloride-permeable $GABA_A$ receptors plays a major role in several brain disorders including DS and epilepsy. In particular, higher intracellular chloride concentration due to impairment of the expression ratio of the Cl transporters NKCC1/KCC2 is present in adult animals of the Ts65Dn mouse model of DS and in epilepsy. Accordingly, treatment with the FDA-approved, NKCC1 inhibitor bumetanide during adulthood rescues inhibitory GABAergic transmission and cognitive deficits in DS mice, although the beneficial effect of the treatment is rapidly lost upon drug withdrawal. Moreover, bumetanide treatment in adult Ts65Dn mice fails to rescue their higher susceptibility to seizure and hyperactivity. Given the crucial role of $GABA_A$ergic transmission for healthy brain development, an early pharmacological NKCC1 inhibition acting when neuronal networks are still plastic may in fact ameliorate symptoms that treatments in adult ages were not able to reverse. To this aim, we first characterized the NKCC1/KCC2 ratio and the GABAergic signaling in the hippocampus of developing Ts65Dn and WT mice. Our results showed that alterations of GABAergic signaling and NKCC1/KCC2 expression ratio are present already in early development. This opens the possibility of an early treatment with bumetanide that may have long-lasting positive effects later in life.

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INVESTIGATION OF MICROGLIAL AND ASTROCYTIC REACTIVITY IN MOUSE MODELS OF DOWN SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Introduction Down syndrome (DS) is a developmental disorder caused by trisomy of human chromosome 21 (Hsa21) that constitutes the most common genetic form of intellectual disability. Although it arises from an increased dosage of Hsa21 genes (Antonarakis, 2017), the specific mechanisms underlying cognitive dysfunction remain unclear. Multiple studies have shown defective cell proliferation and neurogenesis (Stagni et al, 2018; Reiche et al, 2019) in DS. Indeed, increased numbers of glial cells, especially astrocytes, have been reported in the DS brain (Zdaniuk et al, 2011: Chen et al., 2014). These reports support the possibility that a gliogenic shift occurs in DS and contributes to intellectual disability (Lee et al., 2016). However, there is still controversy about this hypothesis. **Aims** Here, we seek to study glial alterations in DS and their contribution to cognitive impairment– ultimately aiming to identify causative genes through studying mouse models of DS. **Methods** Previously using Dp(10)2Yey and Dp1Tyb mouse models of DS, which present duplications of Hsa21-orthologous genes on mouse chromosomes 10 and 16 respectively, we observed cognitive impairments in the T-maze Alternation task. We have now performed a protein-based investigation of different neuroinflammatory related markers in the hippocampus of 3 month-old mice by immunoblotting. Currently, we are undertaking immunofluorescence assays in cleared brains to confirm alterations in glial cell numbers. **Results** Our data so far suggest changes in the maturation state of astrocytes in Dp(10)2Yey mice and a gliocentric shift in Dp1Tyb mice. **Conclusion** Alterations in glial-related markers are potentially associated with cognitive impairment in DS.

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CILIOPATHY IN MENINGEAL FIBROBLAST ACCOMPANYING THE DELAYED MATURATION OF PIAL BASEMENT MEMBRANE DURING CORTICAL DEVELOPMENT OF FMR1-/Y MICE

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Three layer of tissue membrane known as meninges contributes to protect the brain as well as form the normal brain, at early embryonic stages. The meningeal cells produce basement membrane (BM) proteins and extracellular matrix components to form the glial limitans, and they control the migration of neurons during early brain development. Cerebrospinal fluid (CSF) is mainly secreted via cilia into ventricles, and 30% of CSF is produced in the leptomeninges. While the meninges are important roles in the mechanisms of cerebral pathology, their function has not been explored in developmental disorders such as Fragile X Syndrome (FXS). We found the abnormal cilia formation in the meninges of *Fmr1*$^{-/y}$ (*Fmr1* KO) mouse including the reduction in the number of cilia in both the meninges of cortex and the primary meningeal fibroblasts (MFs). In addition, the abnormalities of cell integrity and differentiation of Meningeal Fibroblasts (MFs) are observed in the neocortex of *Fmr1* KO mice. These results suggest that the phenotypic ciliary deficit is related to the meningeal cell integrity causing abnormal meningeal differentiation during the cortical development of *Fmr 1* KO mice.

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A NEW MOLECULAR PATHWAY FOR RIBOSOMAL S6 ACTIVATION IN THE BRAIN

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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During the last decade, the mTOR pathway has been unraveled as a critical biological pathway, which its dysfunction has been observed in an important number of neurodevelopmental disorders. mTOR signalling regulates, differentially, cell shape, migration and differentiation during brain development, and synaptic plasticity during adulthood, but how the principal targets of mTORC1 are specifically regulated during different stages of development in the nervous brain is unknown currently. Studying the principal readout of mTOR pathway, ribosomal S6 protein, we revealed how one of the component of the KICSTOR complex, Kaptin, has an essential role in the brain, regulating S6 phosphorylation through a S6 kinase-independent form. Through *in vitro* and *in vivo* analysis of full *Kptn* knockout, we uncovered how a new regulator of the mTOR pathway has a complementary function in the phosphorylation of S6 during the postnatal development. Molecular (using lysosomal and immunoprecipitation analysis), physiological (immunohistochemical and electrophysiological analysis) and behavioral data from *Kptn* full KOs and Emx1-Cre conditional *Kptn* KOs pointed to the subcortical nuclei, as a brain part where S6 has to be critically controlled. Surprisingly, our data shown a new molecular partner of Kaptin, which it has a fundamental function *in vivo* and *in vitro*. With this work, and through the specific function of Kaptin, we show a new mechanism of regulation of ribosomal protein S6 in the brain. This new mechanism should bring new pharmacological strategies to treat specific neurodevelopmental mTORopathies.

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ABERRANT CELLULAR SIGNALLING AND A SHIFT IN EXCITATION/INHIBITION BALANCE CONTRIBUTE TO LTP IMPAIRMENTS IN A MOUSE MODEL OF NOONAN SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Noonan syndrome (NS) belongs to a disease family called RASopathies, linked to mutations in genes encoding components and regulators of the RAS/MAPK-signalling pathway. NS is accompanied by craniofacial features, congenital heart diseases and neurocognitive impairments. Germ-line mutations in various genes can lead to NS. KRASV14I is a NS-linked mutation that results in an increased KRAS activity and presents with mild to moderate cognitive and behavioural defects in patients. Previous experiments indicated behavioural and memory alterations and a shift in excitation/inhibition (E/I) balance in mice expressing KRASV14I mutation in the brain. In this project we wanted to address the molecular and cellular mechanisms behind the impaired long-term potentiation (LTP). This information is instrumental for the development of treatment for neurocognitive impairments seen in NS patients. We recorded extracellular field potentials from CA1 stratum radiatum evoked by the stimulation of Schaffer collaterals in acute cortico-hippocampal brain slices from 7-8 week-old WT and KRASV14I mice treated with specific drugs targeting RAS downstream signalling and E/I balance. Our results revealed specific contributions of downstream RAS targets to aberrant transmission and neuroplasticity.

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MODELLING KOOLEN-DE VRIES SYNDROME IN CEREBRAL ORGANOIDS

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Koolen-de Vries syndrome (KdVS) is a rare genetic neurodevelopmental disorder with multisystemic phenotypes. Patients suffer from developmental delay, intellectual disability, facial dysmorphisms, epilepsy, and congenital malformations in multiple organ systems, including the brain. KdVS is caused by haploinsufficiency of *KANSL1*, which encodes for a protein involved in chromatin remodeling and microtubule stabilisation**.** KANSL1 is widely expressed early during development, but its role during early brain development is not well-established. Here we generated neuronal progenitor cells and human cerebral organoids to define the effect of KANSL1 mutations on human cortical development. Using transcriptomic analysis of KANSL1-deficient human induced-pluripotent stem cells (iPSCs), both from KdVS patients and genome-edited lines, we identified aberrant cell-cycle regulation. Analysis of cell-cycle pointed towards reduced mitotic activity in KdVS patient-derived iPSCs as well as neuronal progenitor cells (NPC), which was further accompanied with decreased proliferative capacity of NPCs. In human cerebral organoids we found that *KANSL1* haploinsufficiency caused a major disruption of neurodevelopmental trajectories with a decrease in cortical layer thickness, a reduced percentage of deep and upper layer neurons. This aberration in cortical layer formation was further accompanied by a reduction of outer radial glial cells. Taken together our results point towards a critcial role of KANSL1 in early aspects of brain development.

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TUBB3 MISSENSE MUTATION IS ASSOCIATED WITH CONGENITAL FIBROSIS OF EXTRA OCULAR MUSCLES TYPE 3 IN AN IRANIAN FAMILY.

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Congenital fibrosis of the extraocular muscles (CFEOM) as a vertical disorder of ocular movement is grouped among the congenital cranial dysinnervation disorders. There are different types of CFEOM phenotypes: CFEOM3A is accompanied with ptosis, mild hypotropic gaze position, bilateral external ophthalmoplegia, inability to elevate one or both eyes above the midline with restriction in lateral rectus and inferior rectus extraocular muscles. We describe a large Iranian family with twenty patients suffering from autosomal dominant CFEOM3A with high penetrance and variable expression. In this family surgery did not correct patients' strabismus or ptosis. Genome-wide sequence analysis of six patients and six healthy controls revealed C784>T (R262C) mutation in the *TUBB3* gene.

Pubmed:
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Association of the functional serotonin transporter haplotype with familial form of obsessive compulsive disorder in Iranian patients.
Several polymorphisms have been reported in the 5-HTTLPR of the serotonin transporter gene (SLC6A4). Family-based evidences for the association of 5-HTTLPR polymorphisms with OCD were previously reported but results were controversial. The present study investigated the possible correlation of SLC6A4 polymorphisms (5-HTTLPR, rs25532, rs25531) in Iranian OCD patients considering gender, age of onset, family history of psychiatric disorders, obsessive and compulsive subtypes and severities.
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Helicobacter pylori in patients suffering from pulmonary disease.
Recently, research of indirect evidence suggested a possible association between Helicobacter pylori and pulmonary disease. This study aimed to determine if H. pylori could be detected in endobronchial specimens collected from patients undergoing bronchoscopy.
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BOARD NUMBER: S02-451

HYPERSYNCHRONISED GAMMA OSCILLATIONS IN THE MEDIAL PREFRONTAL CORTEX AND HIPPOCAMPUS IN A RAT MODEL OF FRAGILE X SYNDROME

POSTER SESSION 02 - SECTION: GENE THERAPY AND GENETIC DISORDERS

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Fragile X Syndrome (FXS) is one of the most common causes of intellectual disability and autism spectrum disorder, and is often associated with severe learning deficits. A rat model of the disorder, the *Fmr1*$^{-/y}$ rat, is impaired in associative memory tasks known to be dependent on connections between the medial prefrontal cortex (mPFC) and hippocampus. Both human FXS patients and rodent models show deficits in cognitive flexibility which could also be associated with these connections. The current study aimed to assess functional connectivity between the mPFC and hippocampus in *Fmr1*$^{-/y}$rats using *in vivo* electrophysiology. Local field potentials were recorded from tetrodes implanted into the mPFC prelimbic area and dorsal hippocampus of adult male *Fmr1*$^{-/y}$rats and their wild-type (WT) littermates as they freely explored a familiar and a novel environment, and as they performed a spatial memory task on a double Y maze. In the mPFC, average power of gamma oscillations (30-80 Hz) during exploration was significantly higher in *Fmr1*$^{-/y}$ rats compared to WTs. Furthermore, average mPFC-hippocampus gamma frequency coherence was significantly higher in *Fmr1*$^{-/y}$ rats, measured using phase coherence and weighted phase lag index. This hypersynchronous network activity may underlie cognitive inflexibility seen in *Fmr1*$^{-/y}$ rodents and FXS patients. The results also provide further evidence for gamma oscillation abnormalities as a useful cross-species biomarker of FXS.

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BOARD NUMBER: S02-452

INVESTIGATING THE REGION-SPECIFIC FUNCTION OF ZFHX3 IN THE MOUSE BRAIN, AND CHARACTERISING ITS MOLECULAR ACTIVITY

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Aims: Zinc finger homeobox 3 (*Zfhx3*) is a brain-region enriched transcription factor that binds to AT motifs in promoter and enhancer regions. Multiple coding sequence mutations have been linked to a range of diseases, including schizophrenia. Data from the Allen Brain Atlas show *Zfhx3* expression within dopaminergic regions, such as the ventral tegmental area (VTA). Given the dopaminergic hypothesis of schizophrenia, and the genetic associations, we wish to investigate the contribution of *Zfhx3* to schizophrenia-related behavioural changes in mice. **Methods:** A DAT-*Cre* (dopamine active transporter) driven *Zfhx3* knockout mouse line was established – a conditional knockout mouse model which deletes *Zfhx3* in dopaminergic cells. These mice were subjected to a behavioural phenotype pipeline focused on schizophrenia-related endophenotypes, and sub-dissected dopaminergic brain regions are undergoing molecular analysis via immunofluorescent imaging and qPCR to investigate changes in gene expression. **Results:** DAT-*Cre*; *Zfhx3*-flox homozygous knockout female mice show attenuated anxiety behaviours in open field and light-dark box; and both male and female homozygotes show social dominance, sleep fragmentation differences, reduced interest in a novel object, and pre-pulse inhibition dysfunction. Analysis of the sub-dissected striatum – a dopaminergic pathway terminus - of homozygotes shows expression of dopamine receptors is significantly reduced. **Conclusions:** Homozygous knockout of *Zfhx3* in dopaminergic neurons shows significant behavioural changes in mice, when assessed with a phenotyping pipeline focused on schizophrenia endophenotypes. Further molecular analysis will elucidate the role of *Zfhx3* in schizophrenia-related behaviours in mice, and its wider function within the dopaminergic system.

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BOARD NUMBER: S02-453

DECREASED NDEL1 OLIGOPEPTIDASE ACTIVITY IS ASSOCIATED WITH ABERRANT NEURODEVELOPMENT AND IMPAIRED ANIMAL BEHAVIOR IN A TRANSGENIC RAT MODEL FOR SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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The purpose of this study was to investigate the relation of nuclear-distribution element-like 1 (Ndel1) activity with neurodevelopment and dopamine associated schizophrenia-related phenotypes, using a rat model overexpressing the human non-mutant *Disrupted-in-schizophrenia 1* (*DISC1*) and presenting dysfunctional dopamine signaling. In the present work, we evaluated the neuronal cell distribution in striatum and cortex, by histology and X-ray microtomography, as well as we quantified the basal and amphetamine-stimulated locomotion and Ndel1 activity of transgenic rats compared with wild-type littermate controls. 3D assessment of neuronal cell body number and spatial organization of mercury-impregnated neurons showed altered neuronal positioning, characteristic of impaired cell migration, in striatum/nucleus accumbens, and prefrontal cortex of transgenic compared with wild-type rats. Locomotion and Ndel1 activity were significantly increased by low doses of amphetamine in transgenic but not in wild-type animals. Our present findings suggest that decreased Ndel1 activity may reflect both a trait (neurodevelopmental phenotype) and state (amphetamine-induced dopamine release), leading to hypothesize that abnormal brain structure due to impaired neuronal migration may be responsible for amphetamine sensitivity. In a previous report we have demonstrated significant lower Ndel1 activity in treatment resistant schizophrenia (TRS) which is line with the hypothesis that TRS patients could present neurodevelopmental deficits, as also observed here in the transgenic animal model. The employment of this transgenic rat, combined with Ndel1 activity measures, may be a potential model for evaluating novel treatments for TRS or any other brain disorder, including schizophrenia, in which decreased Ndel1 activity may be a biomarker of impaired neuronal functioning.

Pubmed:

31916893: Nani JV, Fonseca MC, Engi SA, Perillo MG, Dias CS, Gazarini ML, Korth C, Cruz FC, Hayashi MA
Decreased nuclear distribution nudE-like 1 enzyme activity in an animal model with dysfunctional disrupted-in-schizophrenia 1 signaling featuring aberrant neurodevelopment and amphetamine-supersensitivity.
Interaction of nuclear-distribution element-like 1 with disrupted-in-schizophrenia 1 protein is crucial for neurite outgrowth/neuronal migration, and this interaction competitively inhibits nuclear-distribution element-like 1 peptidase activity. Nuclear-distribution element-like 1 activity is reduced in antipsychotic-naïve first-episode psychosis and in medicated chronic schizophrenia, with even lower activity in treatment-resistant schizophrenia.
J Psychopharmacol, 2020; 34
32696960: Nani JV, Dal Mas C, Yonamine CM, Ota VK, Noto C, Belangero SI, Mari JJ, Bressan R, Cordeiro Q, Gadelha A, Hayashi MAF
A study in first-episode psychosis patients: does angiotensin I-converting enzyme (ACE) activity associated with genotype predict symptoms severity reductions after treatment with the atypical antipsychotic risperidone?
Our previous studies showed increased angiotensin I-converting enzyme (ACE) activity in chronic schizophrenia (SCZ) patients compared to healthy control (HC) volunteers, and the relevance of combining ACE genotype and activity for predicting SCZ was suggested.
Int J Neuropsychopharmacol, 2020;
33116174: Nani JV, Lee RS, Yonamine CM, Sant'Anna OA, Juliano MA, Gadelha A, Mari JJ, Hayashi MAF
Evaluation of NDEL1 oligopeptidase activity in blood and brain in an animal model of schizophrenia: effects of psychostimulants and antipsychotics.
Nuclear distribution element-like 1 (NDEL1) enzyme activity is important for neuritogenesis, neuronal migration, and neurodevelopment. We reported previously lower NDEL1 enzyme activity in blood of treated first episode psychosis and chronic schizophrenia (SCZ) compared to healthy control subjects, with even lower activity in treatment resistant chronic SCZ patients, implicating NDEL1 activity in SCZ. Herein, higher NDEL1 activity was observed in the blood and several brain regions of a validated animal model for SCZ at baseline. In addition, long-term treatment with typical or atypical

antipsychotics, under conditions in which SCZ-like phenotypes were reported to be reversed in this animal model for SCZ, showed a significant NDEL1 activity reduction in blood and brain regions which is in line with clinical data. Importantly, these results support measuring NDEL1 enzyme activity in the peripheral blood to predict changes in NDEL1 activity in the CNS. Also, acute administration of psychostimulants, at levels reported to induce SCZ-like phenotype in normal rat strains, increased NDEL1 enzyme activity in blood. Therefore, alterations in NDEL1 activity after treatment with antipsychotics or psychostimulants may suggest a possible modulation of NDEL1 activity secondary to neurotransmission homeostasis and provide new insights into the role of NDEL1 in SCZ pathophysiology.
Sci Rep, 2020; 10
30806143: Nani JV, Yonamine CM, Castro Musial D, Dal Mas C, Mari JJ, Hayashi MAF
ACE activity in blood and brain axis in an animal model for schizophrenia: Effects of dopaminergic manipulation with antipsychotics and psychostimulants.
Angiotensin I-converting enzyme (ACE) was initially correlated with schizophrenia (SCZ) in studies showing a correlation of ACE increased enzyme activity with memory impairments. Possible role for ACE in SCZ was also suggested by ACE activity interaction with dopaminergic mechanisms to modulate abnormalities of sensorimotor gating. In addition, we have demonstrated higher ACE activity in blood of SCZ subjects, its implication in cognitive performance in SCZ and its power as a predictor for SCZ diagnosis. ACE activity was determined in the serum and in selected brain regions of an animal model presenting SCZ-like behaviour, before and after the treatment with typical and atypical antipsychotics, and also in the serum of animals receiving the psychostimulants amphetamine/lisdexamphetamine. Dopaminergic manipulations with antipsychotics and psychostimulants influenced the ACE activity, but with no correlation with the animal blood pressure. The validity of measuring ACE activity in animal blood to predict activity in the CNS, as well as the lack of correlation between the activity and blood pressure, before and after the treatment with antipsychotics, were confirmed here. Correlations of the present findings with data from clinical studies also strengthen the value of this animal model for studying several aspects of SCZ.
World J Biol Psychiatry, 2020; 21
33872680: Campeiro JD, Nani JV, Monte GG, Almeida PGC, Mori MA, Hayashi MAF
Regulation of monoamine levels by typical and atypical antipsychotics in Caenorhabditis elegans mutant for nuclear distribution element genes.
Mammalian nuclear distribution genes encode proteins with essential roles in neuronal migration and brain formation during embryogenesis. The implication of human nuclear distribution genes, namely nudC and NDE1 (Nuclear Distribution Element 1)/NDEL1 (Nuclear Distribution Element-Like 1), in psychiatric disorders including schizophrenia and bipolar disorder, has been recently described. The partial loss of NDEL1 expression results in neuronal migration defects, while ndel1 null knockout (KO) leads to early embryonic lethality in mice. On the other hand, loss-of-function of the orthologs of nuclear distribution element genes (nud) in Caenorhabditis elegans renders viable worms and influences behavioral endophenotypes associated with dopaminergic and serotoninergic pathways. In the present work, we evaluated the role of nud genes in monoamine levels at baseline and after the treatment with typical or atypical antipsychotics. Dopamine, serotonin and octopamine levels were significantly lower in homozygous loss-of-function mutant worms KO for nud genes compared with wild-type (WT) C. elegans at baseline. While treatment with antipsychotics determined significant differences in monoamine levels in WT, the nud KO mutant worms appear to respond differently to the treatment. According to the best of our knowledge, we are the first to report the influence of nud genes in the monoamine levels changes in response to antipsychotic drugs, ultimately placing the nuclear distribution genes family at the cornerstone of pathways involved in the modulation of monoamines in response to different classes of antipsychotic drugs.
Neurochem Int, 2021; 147
34262063: Altaf-Ul-Amin M, Hirose K, Nani JV, Porta LC, Tasic L, Hossain SF, Huang M, Ono N, Hayashi MAF, Kanaya S
A system biology approach based on metabolic biomarkers and protein-protein interactions for identifying pathways underlying schizophrenia and bipolar disorder.
Mental disorders (MDs), including schizophrenia (SCZ) and bipolar disorder (BD), have attracted special attention from scientists due to their high prevalence and significantly debilitating clinical features. The diagnosis of MDs is still essentially based on clinical interviews, and intensive efforts to introduce biochemical based diagnostic methods have faced several difficulties for implementation in clinics, due to the complexity and still limited knowledge in MDs. In this context, aiming for improving the knowledge in etiology and pathophysiology, many authors have reported several alterations in metabolites in MDs and other brain diseases. After potentially fishing all metabolite biomarkers reported up to now for SCZ and BD, we investigated here the proteins related to these metabolites in order to construct a protein-protein interaction (PPI) network associated with these diseases. We determined the statistically significant clusters in this PPI network and, based on these clusters, we identified 28 significant pathways for SCZ and BDs that essentially compose three groups representing three major systems, namely stress response, energy and neuron systems. By characterizing new pathways with potential to innovate the diagnosis and treatment of psychiatric diseases, the present data may also contribute to the proposal of new

intervention for the treatment of still unmet aspects in MDs.
Sci Rep, 2021; 11
33652776: Correia BSB, Nani JV, Waladares Ricardo R, Stanisic D, Costa TBBC, Hayashi MAF, Tasic L
Effects of Psychostimulants and Antipsychotics on Serum Lipids in an Animal Model for Schizophrenia.
Schizophrenia (SCZ) treatment is essentially limited to the use of typical or atypical antipsychotic drugs, which suppress the main symptoms of this mental disorder. Metabolic syndrome is often reported in patients with SCZ under long-term drug treatment, but little is known about the alteration of lipid metabolism induced by antipsychotic use. In this study, we evaluated the blood serum lipids of a validated animal model for SCZ (Spontaneously Hypertensive Rat, SHR), and a normal control rat strain (Normotensive Wistar Rat, NWR), after long-term treatment (30 days) with typical haloperidol (HAL) or atypical clozapine (CLZ) antipsychotics. Moreover, psychostimulants, amphetamine (AMPH) or lisdexamfetamine (LSDX), were administered to NWR animals aiming to mimic the human first episode of psychosis, and the effects on serum lipids were also evaluated. Discrepancies in lipids between SHR and NWR animals, which included increased total lipids and decreased phospholipids in SHR compared with NWR, were similar to the differences previously reported for SCZ patients relative to healthy controls. Administration of psychostimulants in NWR decreased omega-3, which was also decreased in the first episode of psychosis of SCZ. Moreover, choline glycerophospholipids allowed us to distinguish the effects of CLZ in SHR. Thus, changes in the lipid metabolism in SHR seem to be reversed by the long-term treatment with the atypical antipsychotic CLZ, which was under the same condition described to reverse the SCZ-like endophenotypes of this validated animal model for SCZ. These data open new insights for understanding the potential influence of the treatment with typical or atypical antipsychotics on circulating lipids. This may represent an outcome effect from metabolic pathways that regulate lipids synthesis and breakdown, which may be reflecting a cell lipids dysfunction in SCZ.
Biomedicines, 2021; 9
32914395: Maes M, Nani JV, Noto C, Rizzo L, Hayashi MAF, Brietzke E
Impairments in Peripheral Blood T Effector and T Regulatory Lymphocytes in Bipolar Disorder Are Associated with Staging of Illness and Anti-cytomegalovirus IgG Levels.
There is now evidence that, based on cytokine profiles, bipolar disorder (BD) is accompanied by simultaneous activation of the immune-inflammatory response system (IRS) and the compensatory immune-regulatory system (CIRS), and that both components may be associated with the staging of illness. Nevertheless, no BD studies have evaluated the IRS/CIRS ratio using CD (cluster of differentiation) molecules expressed by peripheral blood activated T effector (Teff) and T regulatory (Treg) subpopulations. This study examined Teff/Treg subsets both before and after ex vivo anti-CD3/CD28 stimulation using flow cytometric immunophenotyping in 25 symptomatic remitted BD patients and 21 healthy controls and assessed human cytomegalovirus (HCMV)-specific IgG antibodies. BD is associated with a significantly lowered frequency of unstimulated CD3 + CD8 + CD71+ and CD4 + CD25 + FOXP3 and increased CD4 + CD25 + FOXP3 + CD152+ frequencies and with lowered stimulated frequencies of CD3 + CD8 + CD71+, CD4 + CD25 + FOXP3 + CD152+, and CD4 + CD25 + FOXP3 + GARP cells and, consequently, by an increased stimulated Teff/Treg ratio. Moreover, the number of manic, but not hypomanic or depressive episodes, is significantly and negatively associated with the stimulated proportions of CD3 + CD4 + CD154+, and CD69+ and CD71+ expression on CD4+ and CD8+ cells, while duration of illness (≥ 10 years) is accompanied by a depleted frequency of stimulated CD152+ Treg, and CD154+ and CD71+ CD4+ T cells. BD and anti-human cytomegalovirus (HCMV) IgG levels significantly interact to decrease the expression of CD4 + CD25 + FOXP+GARP T phenotypes. In conclusion, in BD patients, immune injuries, staging, and HCMV seropositivity interact and cause CIRS dysfunctions and exaggerated IRS responses, which play a key role in parainflammation and neuroaffective toxicity. HCMV seropositivity contributes to an immune-risk phenotype in BD.
Mol Neurobiol, 2021; 58
30857875: Dal Mas C, Nani JV, Noto C, Yonamine CM, da Cunha GR, Mansur RB, Ota VK, Belangero SI, Cordeiro Q, Kapczinski F, Brietzke E, Bressan RA, Gadelha A, Hayashi MAF
Ndel1 oligopeptidase activity as a potential biomarker of early stages of schizophrenia.
Our previous studies showed reduced Ndel1 enzyme activity in patients with chronic schizophrenia (SCZ), and only a subtle NDEL1 mRNA increases in antipsychotic-naïve first-episode psychosis (FEP) individuals compared to matched healthy controls (HC). Aiming to refine the evaluation of Ndel1 enzyme activity in early stages of psychosis, we compared 3 groups composed by (1) subjects at ultra-high-risk (UHR) for psychosis, (2) a cohort comprising antipsychotic-naïve FEP individuals (assessed in three moments, at baseline (FEP-0), and after 2 months (FEP-2 M) and one year (FEP-1Y) of treatment with risperidone), and (3) a HC group. There was no significant difference in Ndel1 enzyme activity between UHR and HC, but this activity was significantly lower in FEP compared to HC. Conversely, Ndel1 activity in HC groups was higher than in FEP even before (FEP-0) or after the treatment with risperidone (FEP-2 M and FEP-1Y), and with progressive decrease of Ndel1 activity and significant improvement of symptoms observed after this treatment. In addition, a positive correlation was observed for Ndel1 activity with clinical symptoms as assessed by PANSS, while a negative correlation was seen for GAF scores. Our

results suggest that reductions in Ndel1 activity in FEP may be possibly related to responses to the illness, rather than to the pharmacological effects of antipsychotics, which might be acting essentially in the symptoms suppression. This hypothesis might be further evaluated in prospective long-term follow-up studies with a larger sample cohort.
Schizophr Res, 2019; 208

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BOARD NUMBER: S02-454

THOUGHT-PATTERNS AMONG SCHIZOPHRENIA PATIENTS WITH NEGATIVE SYMPTOMS; FIRST FINDINGS.

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Background: In recent years thought-patterns and their underlying neuronal mechanisms have been investigated mainly in healthy populations, e.g., in the context of problem-solving thoughts (Mckeen et al., 2020). In our preliminary study, we characterized thought-patterns in patients with schizophrenia (SZ) with predominantly negative symptoms and compared them to healthy controls and related thought patterns to measures of neural connectivity during resting-state fMRI. **Method:** We measured functional connectivity for 9.8 minutes using resting-state fMRI in 72 SZ patients and 61 controls matched for age and sex. Immediately afterwards, participants answered the Multi-Dimensional Experience Sampling (MDES; Gorgolewski et al., 2014) to describe their thoughts during the scan. We extracted thought-components using Principal Component Analysis (PCA) based on this questionnaire data. The association between these thought-patterns and brain-functional connectivity measures will be assessed using whole-brain functional gradient analyses. **Results:** PCA of the MDES data revealed a seven-component solution explaining 63.86% of the total variance (KMO=0.71). The first component comprised highly intrusive negative thoughts (image 1) and the SZ group displayed significantly higher values compared to controls [$t(129.35) = 3.19$, $p = .002$]. Another component was characterized by regular-vivid-positive-thoughts and showed significantly higher values among the HC. No significant group differences were found for the other components. Analyses of association between thought components and rsMRI measures are work-in-progress. **Conclusion:** Our findings showed that the MDES is an efficient tool for assessing thought-patterns with the ability to significantly distinguish between HC and SZ groups. We will assess the connection between thought-patterns and brain functional

connectivity.

Image 1: 1st component represented as a word cloud. Font size describes the influence, ink color indicates the polarity (red = positive, blue = negative)

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BOARD NUMBER: S02-455

D-NEURON, LIGAND NEURON OF TRACE AMINE-ASSOCIATED RECEPTOR 1 (TAAR1): KEY OF NOVEL NON-D2 RECEPTOR-BINDING ANTIPSYCHOTICS

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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The latest psychopharmacological study showed effectiveness of a novel non-D2-receptor-binding drug, SEP-363856, for the treatment of schizophrenia. The compound is trace amine-associated receptor 1 (TAAR1) full agonist and also 5-hydroxytryptamin 1A (5-HT 1A) receptor partial agonist. I found the TAAR1 ligand neuron, D-neuron, in the striatum and nucleus accumbens (Acc), a neuroleptic acting site, of human brains, though failed to find in the homologous area of monkey brains. To study human D-neuron functions, total of 154 post-mortem brains, and a modified immunohistochemical method using high qualified antibodies against monoamine-related substances, was applied. As the number of immunoreactive neurons reduced accoding to the length of post-mortem interval to fixation (PMI), quantification of immunoreactive neurons has been done by using post-mortem brains with PMI shorter than 8 hours. The number of D-neurons in the caudate nucleus, putamen, and Acc was reduced in post-mortem brains with schizophrenia. The reduction was significant ($p<0.05$) in Acc. I proposed “D-cell hypothesis of schizophrenia”, that NSC dysfunction-based D-neuron reduction is cellular and molecular basis of mesolimbic dopamine hyperactivity, progressive pathophysiology of mental disorders, and prospectiveness of TAAR1 agonist as a novel antipsychotic agent. Reference: Koblan KS, et al. A Non-D2-Receptor-Binding Drug for the Treatment of Schizophrenia. *N Engl J Med 2020;* **382:** 1497-1506. Ikemoto K, Study on trace amine-associated receptor 1 (TAAR1) ligand neuron, D-neuron. In: Recent Developments in Medicine and Medical Research. Chapter 20, Vol.4, pp.166-174, 2021 (BP International, UK)

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BOARD NUMBER: S02-456

SOCIAL ANHEDONIA AS A DISRUPTED-IN-SCHIZOPHRENIA 1-DEPENDENT PHENOTYPE

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Mohammad Seidisarouei[1], Sandra Schäble[2], Marijn Wingerden[3], Svenja V Trossbach[4], Carsten Korth[4], Tobias Kalenscher[2]
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Deficits in social interaction or social cognition are key phenotypes in a variety of chronic mental diseases, yet, their modeling and molecular dissection are only in their infancy. The Disrupted-in-Schizophrenia 1 (DISC1) signaling pathway is considered to play a role in different psychiatric disorders such as schizophrenia, depression, and biopolar disorders. DISC1 is involved in regulating the dopaminergic neurotransmission in, among others, the mesolimbic reward system. A transgenic rat line tgDISC1 has been introduced as a model system to study behavioral phenotypes associated with abnormal DISC1 signaling pathways. Here, we evaluated the impact of impaired DISC1 signaling on social (social interaction) and non-social (sucrose) reward preferences in the tgDISC1 animal model. In a plus-maze setting, rats chose between the opportunity for social interaction with an unfamiliar juvenile conspecific (social reward) or drinking sweet solutions with variable sucrose concentrations (non-social reward). tgDISC1 rats differed from wild-type rats in their social, but not in their non-social reward preferences. Specifically, DISC1 rats showed a lower interest in interaction with the juvenile conspecific, but did not differ from wild-type rats in their preference for higher sucrose concentrations. These results suggest that disruptions of the DISC1 signaling pathway that is associated with altered dopamine transmission in the brain result in selective deficits in social motivation reminiscent of phenotypes seen in neuropsychiatric illness.

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TRACKING DEVELOPMENTAL CONNECTOPATHY IN 22Q11.2 DELETION SYNDROME WITH CROSS-SPECIES FMRI

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Background: 22q11.2 deletion syndrome (22q11DS) is a genetic syndrome with high penetrance for developmental neuropsychiatric disorders such as autism spectrum disorders and schizophrenia. Human imaging studies have shown alterations in functional connectivity across brain regions in individuals with 22q11DS. However, the neural underpinnings and developmental course of brain connectopathy in 22q11DS remain undetermined. **Aim:** This study was aimed at tracking the developmental trajectory of connectopathy in 22q11DS in both a mouse model and human patients. **Methods:** We longitudinally mapped resting-state fMRI (rsfMRI) connectivity and socio-cognitive behavior in juvenile and adult LgDel mice, a well characterized mouse model of 22q11DS. Guided by mouse findings, we mapped functional connectivity in prepubertal and adult 22q11DS patients. To determine the neural underpinnings of the observed rsfMRI dysconnectivity, we next investigated whether pharmacological inhibition of the GSK3β pathway produce a developmental rescue of connectivity alterations, as previous research showed that this procedure normalizes fronto-hippocampal synchrony in an analogous mouse model. **Results:** We found that 22q11DS is associated with prominent prepubertal fMRI hyperconnectivity that reverts to focal hypoconnectivity in adult mice. We also found a similar developmental trajectory across development in 22q11DS patients. Importantly, using pharmacological studies in the mouse, we linked juvenile hyperconnectivity (but not adult hypoconnectivity) to aberrant GSK3β signaling. Interestingly, the same treatment completely rescued cognitive functions in adult mice but no other socio-behavioral alteration assayed at both developmental timepoints. **Conclusions:** Our data shed light on the course and etiopathological determinants of brain dysconnectivity in 22q11DS.

Pubmed:
34998465: Gutierrez-Barragan D, Singh NA, Alvino FG, Coletta L, Rocchi F, De Guzman E, Galbusera A, Uboldi M, Panzeri S, Gozzi A
Unique spatiotemporal fMRI dynamics in the awake mouse brain.
Human imaging studies have shown that spontaneous brain activity exhibits stereotypic spatiotemporal reorganization in awake, conscious conditions with respect to minimally conscious states. However, whether and how this phenomenon can be generalized to lower mammalian species remains unclear. Leveraging a robust protocol for resting-state fMRI (rsfMRI) mapping in non-anesthetized, head-fixed mice, we investigated functional network topography and dynamic structure of spontaneous brain activity in wakeful animals. We found that rsfMRI networks in the awake state, while anatomically comparable to those observed under anesthesia, are topologically configured to maximize interregional communication, departing from the underlying community structure of the mouse axonal connectome. We further report that rsfMRI activity in wakeful animals exhibits unique spatiotemporal dynamics characterized by a state-dependent, dominant occurrence of coactivation patterns encompassing a prominent participation of arousal-related forebrain nuclei and functional anti-coordination between visual-auditory and polymodal cortical areas. We finally show that rsfMRI dynamics in awake mice exhibits a stereotypical temporal structure, in which state-dominant coactivation patterns are configured as network attractors. These findings suggest that spontaneous brain activity in awake mice is critically shaped by state-specific involvement of basal forebrain arousal systems and document that its dynamic structure recapitulates distinctive, evolutionarily relevant principles that are predictive of conscious states in higher mammalian species.
Curr Biol, 2022; 32
32541823: Marrocco E, Indrieri A, Esposito F, Tarallo V, Carboncino A, Alvino FG, De Falco S, Franco B, De Risi M, De Leonibus E
α-synuclein overexpression in the retina leads to vision impairment and degeneration of dopaminergic amacrine cells.
The presence of α-synuclein aggregates in the retina of Parkinson's disease patients has been associated with vision

impairment. In this study we sought to determine the effects of α-synuclein overexpression on the survival and function of dopaminergic amacrine cells (DACs) in the retina. Adult mice were intravitreally injected with an adeno-associated viral (AAV) vector to overexpress human wild-type α-synuclein in the inner retina. Before and after systemic injections of levodopa (L-DOPA), retinal responses and visual acuity-driven behavior were measured by electroretinography (ERG) and a water maze task, respectively. Amacrine cells and ganglion cells were counted at different time points after the injection. α-synuclein overexpression led to an early loss of DACs associated with a decrease of light-adapted ERG responses and visual acuity that could be rescued by systemic injections of L-DOPA. The data show that α-synuclein overexpression affects dopamine neurons in the retina. The approach provides a novel accessible method to model the underlying mechanisms implicated in the pathogenesis of synucleinopathies and for testing novel treatments.
Sci Rep, 2020; 10
34108486: De Risi M, Tufano M, Alvino FG, Ferraro MG, Torromino G, Gigante Y, Monfregola J, Marrocco E, Pulcrano S, Tunisi L, Lubrano C, Papy-Garcia D, Tuchman Y, Salleo A, Santoro F, Bellenchi GC, Cristino L, Ballabio A, Fraldi A, De Leonibus E
Altered heparan sulfate metabolism during development triggers dopamine-dependent autistic-behaviours in models of lysosomal storage disorders.
Lysosomal storage disorders characterized by altered metabolism of heparan sulfate, including Mucopolysaccharidosis (MPS) III and MPS-II, exhibit lysosomal dysfunctions leading to neurodegeneration and dementia in children. In lysosomal storage disorders, dementia is preceded by severe and therapy-resistant autistic-like symptoms of unknown cause. Using mouse and cellular models of MPS-IIIA, we discovered that autistic-like behaviours are due to increased proliferation of mesencephalic dopamine neurons originating during embryogenesis, which is not due to lysosomal dysfunction, but to altered HS function. Hyperdopaminergia and autistic-like behaviours are corrected by the dopamine D1-like receptor antagonist SCH-23390, providing a potential alternative strategy to the D2-like antagonist haloperidol that has only minimal therapeutic effects in MPS-IIIA. These findings identify embryonic dopaminergic neurodevelopmental defects due to altered function of HS leading to autistic-like behaviours in MPS-II and MPS-IIIA and support evidence showing that altered HS-related gene function is causative of autism.
Nat Commun, 2021; 12
30074247: Iannotti FA, Pagano E, Moriello AS, Alvino FG, Sorrentino NC, D'Orsi L, Gazzerro E, Capasso R, De Leonibus E, De Petrocellis L, Di Marzo V
Effects of non-euphoric plant cannabinoids on muscle quality and performance of dystrophic mdx mice.
Duchenne muscular dystrophy (DMD), caused by dystrophin deficiency, results in chronic inflammation and irreversible skeletal muscle degeneration. Moreover, the associated impairment of autophagy greatly contributes to the aggravation of muscle damage. We explored the possibility of using non-euphoric compounds present in Cannabis sativa, cannabidiol (CBD), cannabidivarin (CBDV) and tetrahydrocannabidivarin (THCV), to reduce inflammation, restore functional autophagy and positively enhance muscle function in vivo.
Br J Pharmacol, 2019; 176
29118420: Gatto F, Rossi B, Tarallo A, Polishchuk E, Polishchuk R, Carrella A, Nusco E, Alvino FG, Iacobellis F, De Leonibus E, Auricchio A, Diez-Roux G, Ballabio A, Parenti G
AAV-mediated transcription factor EB (TFEB) gene delivery ameliorates muscle pathology and function in the murine model of Pompe Disease.
Pompe disease (PD) is a metabolic myopathy due to acid alpha-glucosidase deficiency and characterized by extensive glycogen storage and impaired autophagy. We previously showed that modulation of autophagy and lysosomal exocytosis by overexpression of the transcription factor EB (TFEB) gene was effective in improving muscle pathology in PD mice injected intramuscularly with an AAV-TFEB vector. Here we have evaluated the effects of TFEB systemic delivery on muscle pathology and on functional performance, a primary measure of efficacy in a disorder like PD. We treated 1-month-old PD mice with an AAV2.9-MCK-TFEB vector. An animal cohort was analyzed at 3 months for muscle and heart pathology. A second cohort was followed at different timepoints for functional analysis. In muscles from TFEB-treated mice we observed reduced PAS staining and improved ultrastructure, with reduced number and increased translucency of lysosomes, while total glycogen content remained unchanged. We also observed statistically significant improvements in rotarod performance in treated animals compared to AAV2.9-MCK-eGFP-treated mice at 5 and 8 months. Cardiac echography showed significant reduction in left-ventricular diameters. These results show that TFEB overexpression and modulation of autophagy result in improvements of muscle pathology and of functional performance in the PD murine model, with delayed disease progression.
Sci Rep, 2017; 7

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BOARD NUMBER: S02-458

MICRORNA THERAPEUTICS FOR STRATIFIED TREATMENT OF SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Aim: Schizophrenia (SZ) is a devastating psychiatric illness affecting 1% of the world population. In addition to genetic predisposition, environmental factors contribute to the risk for developing SZ. Such genome environment interactions frequently activate epigenetic and epitranscriptomic mechansims. There are emerging evidence that genetic and environmental risk factors merge at the level of microRNA expression, which are discussed as biomarker and therapeutic target in various disorders including neuropsychiatric diseases. Methods: In this study we analyzed the blood microRNAome of 331 healthy individuals and 244 SZ patients via small RNA sequencing. Results: By combining these data with a corresponding analysis of post-mortem human brain tissue, we identify one candidate microRNA that is down-regulated in patients. Moreover, its expression is significantly correlated to disease phenotypes. Manipulation of this microRNAs in mouse prefrontal cortex causes schizophrenia-like phenotypes. Functional analysis revealed the cellular processes affected by this microRNA and allowed us to develop an arsenal of RNA-based therapeutic approaches that are able to ameliorate molecular disease phenotypes in mouse and human-based cellular systems as well as the behavioral phenotypes. In conclusion, we identify a novel microRNA as target for stratified RNA-therapeutics in schizophrenia.

Pubmed:

34806773: Michurina A, Sakib MS, Kerimoglu C, Krüger DM, Kaurani L, Islam MR, Joshi PD, Schröder S, Centeno TP, Zhou J, Pradhan R, Cha J, Xu X, Eichele G, Zeisberg EM, Kranz A, Stewart AF, Fischer A
Postnatal expression of the lysine methyltransferase SETD1B is essential for learning and the regulation of neuron-enriched genes.
In mammals, histone 3 lysine 4 methylation (H3K4me) is mediated by six different lysine methyltransferases. Among these enzymes, SETD1B (SET domain containing 1b) has been linked to syndromic intellectual disability in human subjects, but its role in the mammalian postnatal brain has not been studied yet. Here, we employ mice deficient for Setd1b in excitatory neurons of the postnatal forebrain, and combine neuron-specific ChIP-seq and RNA-seq approaches to elucidate its role in neuronal gene expression. We observe that Setd1b controls the expression of a set of genes with a broad H3K4me3 peak at their promoters, enriched for neuron-specific genes linked to learning and memory function. Comparative analyses in mice with conditional deletion of Kmt2a and Kmt2b histone methyltransferases show that SETD1B plays a more pronounced and potent role in regulating such genes. Moreover, postnatal loss of Setd1b leads to severe learning impairment, suggesting that SETD1B-dependent regulation of H3K4me levels in postnatal neurons is critical for cognitive function.
EMBO J, 2022; 41
34764281: Kettwig M, Ternka K, Wendland K, Krüger DM, Zampar S, Schob C, Franz J, Aich A, Winkler A, Sakib MS, Kaurani L, Epple R, Werner HB, Hakroush S, Kitz J, Prinz M, Bartok E, Hartmann G, Schröder S, Rehling P, Henneke M, Boretius S, Alia A, Wirths O, Fischer A, Stadelmann C, Nessler S, Gärtner J
Interferon-driven brain phenotype in a mouse model of RNaseT2 deficient leukoencephalopathy.
Infantile-onset RNaseT2 deficient leukoencephalopathy is characterised by cystic brain lesions, multifocal white matter alterations, cerebral atrophy, and severe psychomotor impairment. The phenotype is similar to congenital cytomegalovirus brain infection and overlaps with type I interferonopathies, suggesting a role for innate immunity in its pathophysiology. To date, pathophysiological studies have been hindered by the lack of mouse models recapitulating the neuroinflammatory

encephalopathy found in patients. In this study, we generated Rnaset2 mice using CRISPR/Cas9-mediated genome editing. Rnaset2 mice demonstrate upregulation of interferon-stimulated genes and concurrent IFNAR1-dependent neuroinflammation, with infiltration of CD8 effector memory T cells and inflammatory monocytes into the grey and white matter. Single nuclei RNA sequencing reveals homeostatic dysfunctions in glial cells and neurons and provide important insights into the mechanisms of hippocampal-accentuated brain atrophy and cognitive impairment. The Rnaset2 mice may allow the study of CNS damage associated with RNaseT2 deficiency and may be used for the investigation of potential therapies.
Nat Commun, 2021; 12
34633146: Islam MR, Kaurani L, Berulava T, Heilbronner U, Budde M, Centeno TP, Elerdashvili V, Zafieriou MP, Benito E, Sertel SM, Goldberg M, Senner F, Kalman JL, Burkhardt S, Oepen AS, Sakib MS, Kerimoglu C, Wirths O, Bickeböller H, Bartels C, Brosseron F, Buerger K, Cosma NC, Fliessbach K, Heneka MT, Janowitz D, Kilimann I, Kleinedam L, Laske C, Metzger CD, Munk MH, Perneczky R, Peters O, Priller J, Rauchmann BS, Roy N, Schneider A, Spottke A, Spruth EJ, Teipel S, Tscheuschler M, Wagner M, Wiltfang J, Düzel E, Jessen F, , Rizzoli SO, Zimmermann WH, Schulze TG, Falkai P, Sananbenesi F, Fischer A
A microRNA signature that correlates with cognition and is a target against cognitive decline.
While some individuals age without pathological memory impairments, others develop age-associated cognitive diseases. Since changes in cognitive function develop slowly over time in these patients, they are often diagnosed at an advanced stage of molecular pathology, a time point when causative treatments fail. Thus, there is great need for the identification of inexpensive and minimal invasive approaches that could be used for screening with the aim to identify individuals at risk for cognitive decline that can then undergo further diagnostics and eventually stratified therapies. In this study, we use an integrative approach combining the analysis of human data and mechanistic studies in model systems to identify a circulating 3-microRNA signature that reflects key processes linked to neural homeostasis and inform about cognitive status. We furthermore provide evidence that expression changes in this signature represent multiple mechanisms deregulated in the aging and diseased brain and are a suitable target for RNA therapeutics.
EMBO Mol Med, 2021; 13
34524839: Kerimoglu C, Pham L, Tonchev AB, Sakib MS, Xie Y, Sokpor G, Ulmke PA, Kaurani L, Abbas E, Nguyen H, Rosenbusch J, Michurina A, Capece V, Angelova M, Maricic N, Brand-Saberi B, Esgleas M, Albert M, Minkov R, Kovachev E, Teichmann U, Seong RH, Huttner WB, Nguyen HP, Stoykova A, Staiger JF, Fischer A, Tuoc T
H3 acetylation selectively promotes basal progenitor proliferation and neocortex expansion.
Increase in the size of human neocortex—acquired in evolution—accounts for the unique cognitive capacity of humans. This expansion reflects the evolutionarily enhanced proliferative ability of basal progenitors (BPs), including the basal radial glia and basal intermediate progenitors (bIPs) in mammalian cortex, which may have been acquired through epigenetic alterations in BPs. However, how the epigenome in BPs differs across species is not known. Here, we report that histone H3 acetylation is a key epigenetic regulation in bIP amplification and cortical expansion. Through epigenetic profiling of sorted bIPs, we show that histone H3 lysine 9 acetylation (H3K9ac) is low in murine bIPs and high in human bIPs. Elevated H3K9ac preferentially increases bIP proliferation, increasing the size and folding of the normally smooth mouse neocortex. H3K9ac drives bIP amplification by increasing expression of the evolutionarily regulated gene, , in developing cortex. Our findings demonstrate a previously unknown mechanism that controls cortical architecture.
Sci Adv, 2021; 7
31992760: Mehani B, Narta K, Paul D, Raj A, Kumar D, Sharma A, Kaurani L, Nayak S, Dash D, Suri A, Sarkar C, Mukhopadhyay A
Fusion transcripts in normal human cortex increase with age and show distinct genomic features for single cells and tissues.
Fusion transcripts can contribute to diversity of molecular networks in the human cortex. In this study, we explored the occurrence of fusion transcripts in normal human cortex along with single neurons and astrocytes. We identified 1305 non-redundant fusion events from 388 transcriptomes representing 59 human cortices and 329 single cells. Our results indicate while the majority of fusion transcripts in human cortex are intra-chromosomal (85%), events found in single neurons and astrocytes were primarily inter-chromosomal (80%). The number of fusions in single neurons was significantly higher than that in single astrocytes ($p < 0.05$), indicating fusion as a possible contributor towards transcriptome diversity in neuronal cells. The identified fusions were largely private and 4 specific recurring events were found both in cortex and in single neurons but not in astrocytes. We found a significant increase in the number of fusion transcripts in human brain with increasing age both in single cells and whole cortex ($p < 0.0005$ and < 0.005, respectively). This is likely one of the many possible contributors for the inherent plasticity of the adult brain. The fusion transcripts in fetal brain were enriched for genes for long-term depression; while those in adult brain involved genes enriched for long-term potentiation pathways. Our findings demonstrate fusion transcripts are naturally occurring phenomenon spanning across the health-disease continuum, and likely contribute to the diverse molecular network of human brain.

Sci Rep, 2020; 10
31591382: Jain G, Stuendl A, Rao P, Berulava T, Pena Centeno T, Kaurani L, Burkhardt S, Delalle I, Kornhuber J, Hüll M, Maier W, Peters O, Esselmann H, Schulte C, Deuschle C, Synofzik M, Wiltfang J, Mollenhauer B, Maetzler W, Schneider A, Fischer A
A combined miRNA-piRNA signature to detect Alzheimer's disease.
Alzheimer's disease (AD) is the most common neurodegenerative disorder causing huge emotional and economic burden to our societies. An effective therapy has not been implicated yet, which is in part also due to the fact that pathological changes occur years before clinical symptoms manifest. Thus, there is a great need for the development of a translatable biomarker. Recent evidence highlights microRNAs as candidate biomarkers. In this study, we use next-generation sequencing to study the small noncoding RNAome (sncRNAome) in exosomes derived from human cerebrospinal fluid (CSF). We show that the sncRNAome from CSF-derived exosomes is dominated not only by microRNAs (miRNAs) but also by PIWI-interacting RNAs (piRNAs). We define a combined signature consisting of three miRNAs and three piRNAs that are suitable to detect AD with an AUC of 0.83 in a replication cohort and furthermore predict the conversion of mild-cognitive impaired (MCI) patients to AD dementia with an AUC of 0.86 for the piRNA signature. When combining the smallRNA signature with pTau and Aβ 42/40 ratio the AUC reaches 0.98. Our study reports a novel exosomal small noncoding RNA signature to detect AD pathology and provides the first evidence that in addition to miRNAs, piRNAs should also be considered as a candidate biomarker for AD.
Transl Psychiatry, 2019; 9
29643512: Wendeln AC, Degenhardt K, Kaurani L, Gertig M, Ulas T, Jain G, Wagner J, Häsler LM, Wild K, Skodras A, Blank T, Staszewski O, Datta M, Centeno TP, Capece V, Islam MR, Kerimoglu C, Staufenbiel M, Schultze JL, Beyer M, Prinz M, Jucker M, Fischer A, Neher JJ
Innate immune memory in the brain shapes neurological disease hallmarks.
Innate immune memory is a vital mechanism of myeloid cell plasticity that occurs in response to environmental stimuli and alters subsequent immune responses. Two types of immunological imprinting can be distinguished-training and tolerance. These are epigenetically mediated and enhance or suppress subsequent inflammation, respectively. Whether immune memory occurs in tissue-resident macrophages in vivo and how it may affect pathology remains largely unknown. Here we demonstrate that peripherally applied inflammatory stimuli induce acute immune training and tolerance in the brain and lead to differential epigenetic reprogramming of brain-resident macrophages (microglia) that persists for at least six months. Strikingly, in a mouse model of Alzheimer's pathology, immune training exacerbates cerebral β-amyloidosis and immune tolerance alleviates it; similarly, peripheral immune stimulation modifies pathological features after stroke. Our results identify immune memory in the brain as an important modifier of neuropathology.
Nature, 2018; 556
29208638: Martinez Hernandez A, Urbanke H, Gillman AL, Lee J, Ryazanov S, Agbemenyah HY, Benito E, Jain G, Kaurani L, Grigorian G, Leonov A, Rezaei-Ghaleh N, Wilken P, Arce FT, Wagner J, Fuhrmann M, Caruana M, Camilleri A, Vassallo N, Zweckstetter M, Benz R, Giese A, Schneider A, Korte M, Lal R, Griesinger C, Eichele G, Fischer A
The diphenylpyrazole compound anle138b blocks Aβ channels and rescues disease phenotypes in a mouse model for amyloid pathology.
Alzheimer's disease is a devastating neurodegenerative disease eventually leading to dementia. An effective treatment does not yet exist. Here we show that oral application of the compound anle138b restores hippocampal synaptic and transcriptional plasticity as well as spatial memory in a mouse model for Alzheimer's disease, when given orally before or after the onset of pathology. At the mechanistic level, we provide evidence that anle138b blocks the activity of conducting Aβ pores without changing the membrane embedded Aβ-oligomer structure. In conclusion, our data suggest that anle138b is a novel and promising compound to treat AD-related pathology that should be investigated further.
EMBO Mol Med, 2018; 10
28723559: Kerimoglu C, Sakib MS, Jain G, Benito E, Burkhardt S, Capece V, Kaurani L, Halder R, Agís-Balboa RC, Stilling R, Urbanke H, Kranz A, Stewart AF, Fischer A
KMT2A and KMT2B Mediate Memory Function by Affecting Distinct Genomic Regions.
Kmt2a and Kmt2b are H3K4 methyltransferases of the Set1/Trithorax class. We have recently shown the importance of Kmt2b for learning and memory. Here, we report that Kmt2a is also important in memory formation. We compare the decrease in H3K4 methylation and de-regulation of gene expression in hippocampal neurons of mice with knockdown of either Kmt2a or Kmt2b. Kmt2a and Kmt2b control largely distinct genomic regions and different molecular pathways linked to neuronal plasticity. Finally, we show that the decrease in H3K4 methylation resulting from Kmt2a knockdown partially recapitulates the pattern previously reported in CK-p25 mice, a model for neurodegeneration and memory impairment. Our findings point to the distinct functions of even closely related histone-modifying enzymes and provide essential insight for the development of more efficient and specific epigenetic therapies against brain diseases.
Cell Rep, 2017; 20

28533418: Bahari-Javan S, Varbanov H, Halder R, Benito E, Kaurani L, Burkhardt S, Anderson-Schmidt H, Anghelescu I, Budde M, Stilling RM, Costa J, Medina J, Dietrich DE, Figge C, Folkerts H, Gade K, Heilbronner U, Koller M, Konrad C, Nussbeck SY, Scherk H, Spitzer C, Stierl S, Stöckel J, Thiel A, von Hagen M, Zimmermann J, Zitzelsberger A, Schulz S, Schmitt A, Delalle I, Falkai P, Schulze TG, Dityatev A, Sananbenesi F, Fischer A
HDAC1 links early life stress to schizophrenia-like phenotypes.
Schizophrenia is a devastating disease that arises on the background of genetic predisposition and environmental risk factors, such as early life stress (ELS). In this study, we show that ELS-induced schizophrenia-like phenotypes in mice correlate with a widespread increase of histone-deacetylase 1 () expression that is linked to altered DNA methylation. overexpression in neurons of the medial prefrontal cortex, but not in the dorsal or ventral hippocampus, mimics schizophrenia-like phenotypes induced by ELS. Systemic administration of an HDAC inhibitor rescues the detrimental effects of ELS when applied after the manifestation of disease phenotypes. In addition to the hippocampus and prefrontal cortex, mice subjected to ELS exhibit increased expression in blood. Moreover, levels are increased in blood samples from patients with schizophrenia who had encountered ELS, compared with patients without ELS experience. Our data suggest that HDAC1 inhibition should be considered as a therapeutic approach to treat schizophrenia.
Proc Natl Acad Sci U S A, 2017; 114

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DYSREGULATION OF PARVALBUMIN- AND CALRETININ-EXPRESSING NEURONS IN THE LATERAL SEPTUM OF THE DF(16)A+/- MOUSE MODEL OF SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Paula Sierra Diaz[1], Antonia Ruiz[1], Olivia Lofaro[2], Felix Leroy[1]
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Schizophrenia is a major disabling psychiatric disease, with major consequences on patients' social life. The lateral septum (LS), a brain region connecting the cortex to the hypothalamus and critical for the regulation of affect and social interactions, is implicated in schizophrenia. We supposed that specific populations of LS neurons regulating social interactions are dysregulated during schizophrenia and leveraged the *Df(16)A*$^{+/-}$ mouse model of schizophrenia to test our hypothesis. This model contains the human 22q11 microdeletion, which in humans, confers a 30% probability to develop schizophrenia. These mice exhibit impairments in social interaction and, specifically, in social recognition reminiscent of the human patients' symptoms. We investigated the populations of LS neurons expressing the parvalbumin (PV^{+}) or calretinin (CALB2^{+}) proteins in mutant mice and their wild-type (WT) littermates. WT mice displayed a striking difference between males and females with twice more PV^{+} and CALB2^{+} neurons in males. In mutant male mice, the densities of PV^{+} and CALB2^{+} expressing neurons are specifically decreased in the ventral-lateral region of posterior LS, a region known to project to downstream hypothalamic areas involved in social interactions. Unlike male mice, mutant female mice showed little differences compared to WT. This strong gender bias in mice parallel human studies showing that the course of schizophrenia is more severe in male than female. As LS is necessary for social recognition, we are currently using chemogenetic, optogenetic and calcium imaging to probe the function of these LS neurons to evaluate whether and how their dysregulation leads to the social deficits.

Pubmed:
30518859: Leroy F, Park J, Asok A, Brann DH, Meira T, Boyle LM, Buss EW, Kandel ER, Siegelbaum SA
A circuit from hippocampal CA2 to lateral septum disinhibits social aggression.
Although the hippocampus is known to be important for declarative memory, it is less clear how hippocampal output regulates motivated behaviours, such as social aggression. Here we report that pyramidal neurons in the CA2 region of the hippocampus, which are important for social memory, promote social aggression in mice. This action depends on output from CA2 to the lateral septum, which is selectively enhanced immediately before an attack. Activation of the lateral septum by CA2 recruits a circuit that disinhibits a subnucleus of the ventromedial hypothalamus that is known to trigger attack. The social hormone arginine vasopressin enhances social aggression by acting on arginine vasopressin 1b receptors on CA2 presynaptic terminals in the lateral septum to facilitate excitatory synaptic transmission. In this manner, release of arginine vasopressin in the lateral septum, driven by an animal's internal state, may serve as a modulatory control that determines whether CA2 activity leads to declarative memory of a social encounter and/or promotes motivated social aggression.
Nature, 2018; 564

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TRANSCRIPTOMIC ANALYSIS OF CYTOPLASMIC POLYADENYLATION ELEMENT BINDING PROTEINS (CPEBS) IN SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Ivana Ollà[1,2], Alberto Parras[1,2], María Santos-Galindo[1,2], Ainara Elorza[1,2], Sara Pico[1,2], Ivó Hernandez[1,2], Jose Lucas[1,2]
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Aim: Schizophrenia (SCZ) is a severe psychiatric disorder. Both environmental and complex genetic factors contribute to the pathology. This is common to other diseases affecting the nervous system, such as autism spectrum disorders (ASD). Our group has previously demonstrated a role of the cytoplasmatic polyadenylation element (CPE) binding protein 4 (CPEB4) in the aetiology of ASD. CPEBs regulate the translation of specific mRNAs in response to extracellular stimuli. Regarding ASD, we have previously shown that patients show a CPEB4 splicing alteration, generating an imbalance in its isoforms that correlates with concerted mis-expression of multiple ASD risk genes whose transcripts harbour CPE sequences. The parallelism between SCZ and ASD as neurodevelopmental disorders with complex genetics led us to characterize CPEBs in SCZ. Method: First, we searched for bioinformatics evidence of a potential enrichment of CPE-containing and CPEB-binding transcripts within Schizophrenia-associated genes. Then, we performed a screening of the status of CPEBs in SCZ exploiting RNA-seq data from the PsychENCODE repository, to explore possible changes regarding their RNA level and/or splicing. Results: The bioinformatics analysis revealed that SCZ-associated genes are enriched in CPE sequences and CPEB targets. Moreover, CPEB4 splicing shows an altered distribution in SCZ when compared with controls, with an imbalance in its isoforms, as previously reported in the case of ASD. Conclusions: Our results suggest that CPEBs could potentially play a role in the aetiology of Schizophrenia, therefore underlying a novel common feature between SCZ and ASD.

Pubmed:
33122998: Ollà I, Santos-Galindo M, Elorza A, Lucas JJ
P2X7 Receptor Upregulation in Huntington's Disease Brains.
Huntington's disease (HD) is a fatal degenerative disorder affecting the nervous system. It is characterized by motor, cognitive, and psychiatric dysfunctions, with a late onset and an autosomal dominant pattern of inheritance. HD-causing mutation consists in an expansion of repeated CAG triplets in the huntingtin gene (), encoding for an expanded polyglutamine (polyQ) stretch in the huntingtin protein (htt). The mutation causes neuronal dysfunction and loss through multiple mechanisms, affecting both the nucleus and cytoplasm. P2X7 receptor (P2X7R) emerged as a major player in neuroinflammation, since ATP - its endogenous ligand - is massively released under this condition. Indeed, P2X7R stimulation in the central nervous system (CNS) is known to enhance the release of pro-inflammatory cytokines from microglia and of neurotransmitters from neuronal presynaptic terminals, as well as to promote apoptosis. Previous experiments performed with neurons expressing the mutant huntingtin and exploiting HD mouse models demonstrated a role of P2X7R in HD. On the basis of those results, here, we explore for the first time the status of P2X7R in HD patients' brain. We report that in HD postmortem striatum, as earlier observed in HD mice, the protein levels of the full-length form of P2X7R, also named P2X7R-A, are upregulated. In addition, the exclusively human naturally occurring variant lacking the C-terminus region, P2X7R-B, is upregulated as well. As we show here, this augmented protein levels can be explained by elevated mRNA levels. Furthermore, in HD patients' striatum, P2X7R shows not only an augmented total transcript level but also an alteration of its splicing. Remarkably, P2X7R introns 10 and 11 are more retained in HD patients when compared with controls. Taken together, our data confirm that P2X7R is altered in brains of HD subjects and strengthen the notion that P2X7R may represent a potential therapeutic target for HD.
Front Mol Neurosci, 2020; 13
33070315: Conte G, Parras A, Alves M, Ollà I, De Diego-Garcia L, Beamer E, Alalqam R, Ocampo A, Mendez R, Henshall DC, Lucas JJ, Engel T
High concordance between hippocampal transcriptome of the mouse intra-amygdala kainic acid model and human temporal lobe epilepsy.
Pharmacoresistance and the lack of disease-modifying actions of current antiseizure drugs persist as major challenges in the

treatment of epilepsy. Experimental models of chemoconvulsant-induced status epilepticus remain the models of choice to discover potential antiepileptogenic drugs, but doubts remain as to the extent to which they model human pathophysiology. The aim of the present study was to compare the molecular landscape of the intra-amygdala kainic acid model of status epilepticus in mice with findings in resected brain tissue from patients with drug-resistant temporal lobe epilepsy (TLE).
Epilepsia, 2020; 61
32594159: Parras A, de Diego-Garcia L, Alves M, Beamer E, Conte G, Jimenez-Mateos EM, Morgan J, Ollà I, Hernandez-Santana Y, Delanty N, Farrell MA, O'Brien DF, Ocampo A, Henshall DC, Méndez R, Lucas JJ, Engel T
Polyadenylation of mRNA as a novel regulatory mechanism of gene expression in temporal lobe epilepsy.
Temporal lobe epilepsy is the most common and refractory form of epilepsy in adults. Gene expression within affected structures such as the hippocampus displays extensive dysregulation and is implicated as a central pathomechanism. Post-transcriptional mechanisms are increasingly recognized as determinants of the gene expression landscape, but key mechanisms remain unexplored. Here we show, for first time, that cytoplasmic mRNA polyadenylation, one of the post-transcriptional mechanisms regulating gene expression, undergoes widespread reorganization in temporal lobe epilepsy. In the hippocampus of mice subjected to status epilepticus and epilepsy, we report >25% of the transcriptome displays changes in their poly(A) tail length, with deadenylation disproportionately affecting genes previously associated with epilepsy. Suggesting cytoplasmic polyadenylation element binding proteins (CPEBs) being one of the main contributors to mRNA polyadenylation changes, transcripts targeted by CPEBs were particularly enriched among the gene pool undergoing poly(A) tail alterations during epilepsy. Transcripts bound by CPEB4 were over-represented among transcripts with poly(A) tail alterations and epilepsy-related genes and CPEB4 expression was found to be increased in mouse models of seizures and resected hippocampi from patients with drug-refractory temporal lobe epilepsy. Finally, supporting an adaptive function for CPEB4, deletion of Cpeb4 exacerbated seizure severity and neurodegeneration during status epilepticus and the development of epilepsy in mice. Together, these findings reveal an additional layer of gene expression regulation during epilepsy and point to novel targets for seizure control and disease-modification in epilepsy.
Brain, 2020; 143
31496937: Siano G, Caiazza MC, Ollà I, Varisco M, Madaro G, Quercioli V, Calvello M, Cattaneo A, Di Primio C
Identification of an ERK Inhibitor as a Therapeutic Drug Against Tau Aggregation in a New Cell-Based Assay.
Formation of Tau aggregates is a common pathological feature of tauopathies and their accumulation directly correlates with cytotoxicity and neuronal degeneration. Great efforts have been made to understand Tau aggregation and to find therapeutics halting or reversing the process, however, progress has been slowed due to the lack of a suitable method for monitoring Tau aggregation. We developed a cell-based assay allowing to detect and quantify Tau aggregation in living cells. The system is based on the FRET biosensor CST able to monitor the molecular dynamic of Tau aggregation in different cellular conditions. We probed candidate compounds that could block Tau hyperphosphorylation. In particular, to foster the drug discovery process, we tested kinase inhibitors approved for the treatment of other diseases. We identified the ERK inhibitor PD-901 as a promising therapeutic molecule since it reduces and prevents Tau aggregation. This evidence establishes the CST cell-based aggregation assay as a reliable tool for drug discovery and suggests that PD-901 might be a promising compound to be tested for further preclinical studies on AD.
Front Cell Neurosci, 2019; 13
31189515: Hernández F, Cuadros R, Ollá I, García C, Ferrer I, Perry G, Avila J
Differences in structure and function between human and murine tau.
The main difference between the primary structures of human and mouse tau can be found at the N-terminal end of the protein. Residues 17 to 28 in human tau are not present in the mouse form of the molecule. Here we tested the capacity of these human tau residues to bind to specific proteins. Several proteins were observed to bind to these residues. Among those that showed the greatest binding were three related to energetic processes: enolase, glyceraldehyde 3 phosphate dehydrogenase and creatine kinase B. The latter did not bind to tau from brain extracts taken from patients with Alzheimer's disease (AD). This lack of binding could be due to the modification of CKB by oxidation in AD.
Biochim Biophys Acta Mol Basis Dis, 2019; 1865
30668577: Sayas CL, Medina M, Cuadros R, Ollá I, García E, Pérez M, Ferrer I, Hernández F, Avila J
Role of tau N-terminal motif in the secretion of human tau by End Binding proteins.
For unknown reasons, humans appear to be particular susceptible to developing tau pathology leading to neurodegeneration. Transgenic mice are still undoubtedly the most popular and extensively used animal models for studying Alzheimer's disease and other tauopathies. While these murine models generally overexpress human tau in the mouse brain or specific brain regions, there are differences between endogenous mouse tau and human tau protein. Among them, a main difference between human and mouse tau is the presence of a short motif spanning residues 18 to 28 in the human tau protein that is missing in murine tau, and which could be at least partially responsible for that different susceptibility across species. Here we report novel data using affinity chromatography analysis indicating that the sequence containing human tau residues 18 to

28 acts a binding motif for End Binding proteins and that this interaction could facilitate tau secretion to the extracellular space.
PLoS One, 2019; 14

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BOARD NUMBER: S02-461

MATERNAL IMMUNE ACTIVATION INDUCES OFFSPRING GLIAL CELL DYSFUNCTION AND ABERRANT PERINEURONAL NET FORMATION, WITH IMPLICATIONS FOR COGNITIVE DEFICITS IN SCHIZOPHRENIA.

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Rebecca Woods[1], Jennifer Fletcher[2], Hannah Mellor[2], Harry Potter[3], Jocelyn Glazier[1], Jo Neill[2], Mike Harte[2], Reinmar Hager[1]
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Introduction: Maternal immune activation (mIA) is a risk factor for schizophrenia. It is hypothesised that mIA-induced inflammatory cytokines influence fetal neurodevelopment and predispose offspring to neuropathology. Adult offspring of pregnant rat dams treated with the viral mimetic poly(I:C) exhibit cognitive deficits comparable to those in schizophrenia, thought to be underpinned by dysfunctional fast-spiking parvalbumin (PV)-interneurons. **Aims:** Determine the relationship between inflammation-responsive glial cells and PV-interneuron development, using cell densitometry analysis and gene expression of structural proteins produced primarily by glia, notably myelin and perineuronal net (PNN) components. **Methods:** Pregnant Wistar rats received 10mg/kg bodyweight (i.p.) poly(I:C) or saline on gestational day 15, with plasma cytokines measured at 3h to confirm mIA. Adolescent and adult offspring were assessed using novel object recognition and/or attentional set-shifting tasks. Prefrontal cell densitometry (immunohistochemistry) and mRNA expression (qPCR) analysis were performed using a satellite group of age-matched animals. Results were analysed using general linear mixed modelling. **Results:** Poly(I:C) significantly increased maternal plasma IL-6 and TNF-α concentration and increased lD/ED shift in adult offspring, with no observed deficits in adolescence. PV-interneuron density was unchanged, but accompanied by decreased astrocyte and ramified microglia, alongside increased oligodendrocyte, activated microglia and PNN densities. These cellular changes were associated with altered expression of myelin (*Mag*, *Mbp*), PNN/Perinodal (*Acan, Bcan, Ncan*, *Vcan*, *Nfasc)* and adhesion/extracellular matrix (*Gpc4, Nrxn2, Ank3*) genes. Interestingly, molecular changes presented in adolescence, preceding adult behavioural deficits. **Conclusion:** mIA induced developmental alterations in glial function and PNN formation. Aberrant PNN development could promote PV-interneuron dysfunction and cognitive deficits.

Pubmed:
34280428: Woods RM, Lorusso JM, Potter HG, Neill JC, Glazier JD, Hager R
Maternal immune activation in rodent models: A systematic review of neurodevelopmental changes in gene expression and epigenetic modulation in the offspring brain.
Maternal immune activation (mIA) during pregnancy is hypothesised to disrupt offspring neurodevelopment and predispose offspring to neurodevelopmental disorders such as schizophrenia. Rodent models of mIA have explored possible mechanisms underlying this paradigm and provide a vital tool for preclinical research. However, a comprehensive analysis of the molecular changes that occur in mIA-models is lacking, hindering identification of robust clinical targets. This systematic review assesses mIA-driven transcriptomic and epigenomic alterations in specific offspring brain regions. Across 118 studies, we focus on 88 candidate genes and show replicated changes in expression in critical functional areas, including elevated inflammatory markers, and reduced myelin and GABAergic signalling proteins. Further, disturbed epigenetic markers at nine of these genes support mIA-driven epigenetic modulation of transcription. Overall, our results demonstrate that current outcome measures have direct relevance for the hypothesised pathology of schizophrenia and emphasise the importance of mIA-models in contributing to the understanding of biological pathways impacted by mIA and the discovery of new drug targets.
Neurosci Biobehav Rev, 2021; 129

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BOARD NUMBER: S02-462

THE SHALLOW COGNITIVE MAP HYPOTHESIS: A HIPPOCAMPAL FRAMEWORK FOR THOUGHT DISORDER IN SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Memories are not formed in isolation. They are associated and organized into relational knowledge structures that allow coherent thought. Failure to express such coherent thought is a key hallmark of Schizophrenia. Here we explore the hypothesis that thought disorder arises from disorganized Hippocampal cognitive maps. In doing so, we combine insights from two key lines of investigation, one concerning the neural signatures of cognitive mapping, and another that seeks to understand lower-level cellular mechanisms of cognition within a dynamical systems framework. Specifically, we propose that multiple distinct pathological pathways converge on the shallowing of Hippocampal attractors, giving rise to disorganized Hippocampal cognitive maps and driving conceptual disorganization. We discuss the available evidence at the computational, behavioural, network and cellular levels. We also outline testable predictions from this framework including how it could unify major chemical and psychological theories of schizophrenia and how it can provide a rationale for understanding the aetiology and treatment of the disease.

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BOARD NUMBER: S02-463

INCREASED DOPAMINE SIGNALING IN CAUDATE NUCLEUS IS ASSOCIATED WITH STRIATAL GENE CO-EXPRESSION IN INDIVIDUALS AT GENETIC RISK FOR SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Leonardo Sportelli[1,2], Daniel Eisenberg[3], Enrico D'Ambrosio[4,5], Roberta Passiatore[2], Alessandro Bertolino[2,4], Qiang Chen[1], Jasmine Czarapatac[3], Michael Gregory[3], Kira Griffiths[5], Thomas Hyde[1,6], Joel Kleinman[1,6], Antonio F. Pardiñas[7], Joo Heon Shin[1], Mattia Veronese[5], Caroline F. Zink[1,8], Oliver Howes[5], Karen Berman[3], Daniel R. Weinberger[1,8], Giulio Pergola[1,2]
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Schizophrenia pathogenesis involves synaptic function and neurotransmitter systems critical for brain circuit functionality. Diverse biological pathways with polygenic architecture are potentially affected. We aimed to identify a set of genes implicated both in genetic risk and in differential expression in a brain circuit of three brain regions. We studied how gene-set-specific schizophrenia risk translated into brain circuit function. RNA-sequencing data from dorsolateral prefrontal cortex, caudate nucleus, and hippocampus from 238 individuals (neurotypical controls [NC]:154, patients with schizophrenia[SCZ]:84) comprised 22,356 genes clustered into 69 co-expression components. We associated components with schizophrenia diagnosis and genetic risk via linear models and performed gene enrichment analyses. Parsed genetic risk was indexed by scores based on risk polymorphisms within PGC3 schizophrenia loci (GRS). We associated the GRS with fDOPA uptake in the striatum in NC (N=91) and SCZ (N=47; fixed-effect model metanalysis of linear coefficients). We replicated PET results in an independent NC cohort (N=150). Finally, we associated the GRS with reward anticipation-related fMRI activation in 86 NC. One component with striatal dopaminergic specificity overrepresented PGC3 schizophrenia loci (p[corrected]<.05), was associated with diagnosis (t[210]=3.3;p=.001), and GRS (t[93]=2.7;p=.005). The positive association of the GRS with increased striatal fDOPA uptake in NC and SCZ (coefficient[95% CI]: 0.23[0.06, 0.39]) was replicated (t[149]=3.95;16 voxels;p_{FWE}<.05). The GRS was positively correlated to striatal activation during reward anticipation (p_{TFCE}<.05;Z=3.09;68 voxels). Both in post-mortem and in PET/fMRI data, increased GRS in a striatal co-expression gene set linked with dopaminergic transmission is associated with increased estimated striatal dopamine function and related functional phenotypes.

Pubmed:
31496088: Pergola G, Papalino M, Gelao B, Sportelli L, Vollerbergh W, Grattagliano I, Bertolino A
Evocative gene-environment correlation between genetic risk for schizophrenia and bullying victimization.
World Psychiatry, 2019; 18
33866994: Rampino A, Torretta S, Gelao B, Veneziani F, Iacoviello M, Marakhovskaya A, Masellis R, Andriola I, Sportelli L, Pergola G, Minelli A, Magri C, Gennarelli M, Vita A, Beaulieu JM, Bertolino A, Blasi G
Evidence of an interaction between and polymorphisms on levels of Negative Symptoms of Schizophrenia and their response to antipsychotics.
Genome-Wide Association Studies (GWASs) have identified several genes associated with Schizophrenia (SCZ) and exponentially increased knowledge on the genetic basis of the disease. In addition, products of GWAS genes interact with neuronal factors coded by genes lacking association, such that this interaction may confer risk for specific phenotypes of this brain disorder. In this regard, fragile X mental retardation syndrome-related 1 (FXR1) gene has been GWAS associated with SCZ. FXR1 protein is regulated by glycogen synthase kinase-3β (GSK3β), which has been implicated in pathophysiology of SCZ and response to antipsychotics (APs). rs496250 and rs12630592, two eQTLs (Expression Quantitative Trait Loci) of FXR1 and GSK3β, respectively, interact on emotion stability and amygdala/prefrontal cortex activity during emotion processing. These two phenotypes are associated with Negative Symptoms (NSs) of SCZ suggesting that the interaction

between these SNPs may also affect NS severity and responsiveness to medication.
Eur Psychiatry, 2021; 64

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BOARD NUMBER: S02-464

EXPLORING THE ASSOCIATION BETWEEN BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) LEVELS AND LONGITUDINAL PSYCHOPATHOLOGICAL AND COGNITIVE CHANGES IN SARDINIAN PSYCHOTIC PATIENTS

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Background and aims: Schizophrenia spectrum disorders are among the most debilitating mental disorders and evidence on its pathophysiological underpinnings is scant. The brain-derived neurotrophic factor (BDNF) appears to be involved in the pathophysiology of these complex psychiatric disorders. The present study investigates the longitudinal variation of serum BDNF levels in a 24-month observational cohort study of Sardinian psychotic patients (LABSP). This study assessed the variation in BDNF serum levels and its relationship with psychopathological and cognitive changes. Further, we also examined if genetic variations within the BDNF gene could moderate these relationships. **Methods:** Every six months 105 LABSP patients were assessed for their BDNF levels, as well as for a series of psychopathological, cognitive, and drug-related measures. Four tag single nucleotide polymorphisms (SNPs) within the BDNF gene were selected and analyzed using Polymerase Chain Reaction (PCR). Longitudinal data were analyzed using mixed-effects linear regression models (MLRM). **Results:** Analysis showed a declining trajectory of BDNF levels in psychotic patients with increased severity of depressive and negative symptoms. BDNF levels were also decreased in patients scoring lower in cognitive measures such as symbol coding and semantic fluency. Val66Met polymorphism within BDNF gene significantly moderated the relationship between the severity of negative symptoms and BDNF levels. **Conclusions:** Our findings are consistent with previous literature suggesting that peripheral BDNF levels are associated with some cognitive domains and mood disruption in major psychosis. These findings in real-life patients suggest a plausible role of peripheral BDNF levels as a marker of illness burden in major psychoses.

Pubmed:
34315098: Eskin M, Baydar N, Harlak H, Hamdan M, Mechri A, Isayeva U, Abdel-Khalek AM, Rezaeian M, Asad N, El-Nayal M, Buhairan FA, Noor IM, Khader Y, Khan A, Sayyari AA, Khader A, Behzadi B, Öztürk CŞ, Agha H, Hendarmin LA, Khan MM
Cultural and interpersonal risk factors for suicide ideation and suicide attempts among Muslim college students from 11 nations.
Research on suicidality in low to middle-income countries is scarce. We addressed this issue by investigating suicidality in a cross-national college student samples from 11 predominantly low to middle-income majority Muslim countries.
J Affect Disord, 2021; 294
33007656: Eskin M, Baydar N, El-Nayal M, Asad N, Noor IM, Rezaeian M, Abdel-Khalek AM, Al Buhairan F, Harlak H, Hamdan M, Mechri A, Isayeva U, Khader Y, Khan A, Al Sayyari A, Khader A, Behzadi B, Öztürk CŞ, Agha H, Hendarmin LA, Khan MM
Associations of religiosity, attitudes towards suicide and religious coping with suicidal ideation and suicide attempts in 11 muslim countries.
The study investigated the associations of religiosity, religious coping and suicide acceptance to suicide ideation and attempts in 7427 young adults affiliating with Islam from 11 Muslim countries.
Soc Sci Med, 2020; 265
30498939: Eskin M, AlBuhairan F, Rezaeian M, Abdel-Khalek AM, Harlak H, El-Nayal M, Asad N, Khan A, Mechri A, Noor IM, Hamdan M, Isayeva U, Khader Y, Al Sayyari A, Khader A, Behzadi B, Öztürk CŞ, Hendarmin LA, Khan MM, Khatib S
Suicidal Thoughts, Attempts and Motives Among University Students in 12 Muslim-Majority Countries.

There is a scarcity of research on suicidal phenomena in the Muslim world. Therefore, this study aimed at investigating the self-reported prevalence of suicidal thoughts, attempts and motives in 12 Muslim countries. A total of 8417 (54.4% women) university students were surveyed by means of a self-report questionnaire. Overall, 22% of the participants reported suicidal ideation and 8.6% reported attempting suicide. The odds of suicidal thoughts were elevated in Azerbaijan, Indonesia and Saudi Arabia, while reduced ORs were recorded in Egypt, Jordan, Lebanon and Malaysia. While odds of suicide attempts were high in Azerbaijan, Palestine and Saudi Arabia reduced odds ratios (OR) were detected in Indonesia, Iran, Jordan, Lebanon, Malaysia and Tunisia. Taking drugs and using a sharp instrument were the two most frequently used methods to attempt suicide. Only 32.7% of attempts required medical attention. Escape motives were endorsed more than social motives by participants who attempted suicide. Suicidal behaviors were more frequent in women than in men. Compered to men, fewer attempts by women required medical attention. Moreover, our results show that making suicide illegal does not reduce the frequency of suicidal behavior. Results from this comparative study show that suicidal thoughts and attempts are frequent events in young adults in countries where religious scripture explicitly prohibit suicide and the frequencies of nonfatal suicidal behavior show large variation in nations adhering to the same religion.
Psychiatr Q, 2019; 90

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BOARD NUMBER: S02-465

DOPAMINE D4 RECEPTOR MODULATION OF ELECTRICALLY STIMULATED DOPAMINE RELEASE IN RAT BRAIN SLICES: IMPLICATION FOR THE TREATMENT OF SCHIZOPHRENIA.

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Schizophrenia is a debilitating mental illness that affects around 0.5% of the population. Although several antipsychotic drugs are used, around 70% of patients do not respond well, and many experience severe side effects from the drugs, emphasizing the need to understand better the changes occurring in schizophrenia, and to develop better medications to treat it. Behavioural studies have suggested that dopamine D4-receptor agonists may exhibit an antipsychotic-like profile (Sood et al, 2011; J. Psychopharmacology 25, 792-800). This experiment aims to study the action of the D4-receptor agonist A412997 on dopamine release in nucleus accumbens in the context of changes in schizophrenia and potential novel treatment. Brain slices (400 µM) containing nucleus accumbens were taken from male and female juvenile Wistar rats and fast-scan cyclic voltammetry (FSCV) was used to measure electrically-stimulated dopamine release from nucleus accumbens in real-time. Repeated stimulations were given at 3 min intervals: after four baseline (no drug) stimulations, drugs were applied for the next four stimulations (12 min) to assess effects on stimulated release. . Results revealed that the D4-receptor agonist, A412997 causes a concentration-dependent attenuation of electrically stimulated dopamine release, which was abolished by the D4 specific antagonist, L-741,742. Clozapine, which has D4 antagonist properties, amongst many other pharmacological actions, reduced but did not completely abolish the attenuation caused by A412997. This demonstrates a local modulation of dopamine release by D4- receptors, providing a better understanding of the actions of this drug in behavioural models of schizophrenia perhaps suggesting a potential novel treatment for schizophrenia.

Pubmed:

33848365: Ferdinand JM, Peters KZ, Yavas E, Young AMJ
Modulation of stimulated dopamine release in rat nucleus accumbens shell by GABA in vitro: Effect of sub-chronic phencyclidine pretreatment.
Dopamine signaling in nucleus accumbens (NAc) is modulated by γ-aminobutyric acid (GABA), acting through GABA-A and GABA-B receptors: dysregulation of GABAergic control of dopamine function may be important in behavioral deficits in schizophrenia. We investigated the effect of GABA-A (muscimol) and GABA-B (baclofen) receptor agonists on electrically stimulated dopamine release. Furthermore, we explored whether drug-induced changes were disrupted by pretreatment with phencyclidine, which provides a well-validated model of schizophrenia. Using brain slices from female rats, fast-scan cyclic voltammetry was used to measure electrically stimulated dopamine release in NAc shell. Both muscimol and baclofen caused concentration-dependent attenuation of evoked dopamine release: neither effect was changed by dihydro-β-erythroidine, a nicotinic acetylcholine receptor antagonist, or the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), precluding indirect mechanisms using these transmitter systems in the GABAergic actions. In slices taken from rats pretreated with phencyclidine, the attenuation of evoked dopamine release by baclofen was abolished, but the attenuation by muscimol was unaffected. Since phencyclidine pretreatment was followed by drug-free washout period of at least a week, the drug was not present during recording. Therefore, disruption of GABA-B modulation of dopamine is due to long-term functional changes resulting from the treatment, rather than transient changes due to the drug's presence at test. This enduring dysregulation of GABA-B modulation of accumbal dopamine release provides a plausible mechanism through which GABA dysfunction influences accumbal dopamine leading to behavioral changes seen in schizophrenia and may provide a route for novel therapeutic strategies to treat the condition.
J Neurosci Res, 2021; 99
33426718: Peters KZ, Young AMJ, McCutcheon JE
Distracting stimuli evoke ventral tegmental area responses in rats during ongoing saccharin consumption.
Disruptions in attention, salience and increased distractibility are implicated in multiple psychiatric conditions. The ventral

tegmental area (VTA) is a potential site for converging information about external stimuli and internal states to be integrated and guide adaptive behaviours. Given the dual role of dopamine signals in both driving ongoing behaviours (e.g., feeding) and monitoring salient environmental stimuli, understanding the interaction between these functions is crucial. Here, we investigate VTA neuronal activity during distraction from ongoing feeding. We developed a task to assess distraction exploiting self-paced licking in rats. Rats trained to lick for saccharin were given a distraction test, in which three consecutive licks within 1 s triggered a random distractor (e.g. light and tone stimulus). On each trial they were quantified as distracted or not based on the length of their pauses in licking behaviour. We expressed GCaMP6s in VTA neurons and used fibre photometry to record calcium fluctuations during this task as a proxy for neuronal activity. Distractor stimuli caused rats to interrupt their consumption of saccharin, a behavioural effect which quickly habituated with repeat testing. VTA neural activity showed consistent increases to distractor presentations and, furthermore, these responses were greater on distracted trials compared to non-distracted trials. Interestingly, neural responses show a slower habituation than behaviour with consistent VTA responses seen to distractors even after they are no longer distracting. These data highlight the complex role of the VTA in maintaining ongoing appetitive and consummatory behaviours while also monitoring the environment for salient stimuli.
Eur J Neurosci, 2021; 53

32853750: Yavas E, Young AMJ

Repeated phencyclidine disrupts nicotinic acetylcholine regulation of dopamine release in nucleus accumbens: Implications for models of schizophrenia.

Dopaminergic dysregulation in nucleus accumbens has been implicated in the origin of schizophrenia. Accumbal cholinergic interneurons exert powerful modulatory control of local dopamine function, through nicotinic receptors located on dopamine terminals. Fast-scan cyclic voltammetry in rat brain slices in vitro was used to measure dopamine release evoked by high-frequency electrical stimulation, mimicking phasic dopamine activity. We investigated whether cholinergic regulation of stimulated dopamine release was disrupted by pretreatment with phencyclidine, a non-competitive NMDA receptor antagonist, which provides a well validated animal model of schizophrenia. Dihydro-β-erythroidine, an antagonist at β2-subuit containing nicotinic receptors, caused a concentration-dependent enhancement of stimulated dopamine release, indicating cholinergic inhibitory control over dopamine release. The agonist, nicotine, also caused concentration-dependent increases in release, consistent with rapid desensitisation of the receptors previously described. In slices taken from animals pretreated with phencyclidine, the augmentation of electrically-stimulated dopamine release elicited by both drugs was attenuated, particularly when each drug was applied at high concentration. In addition, the concentration-dependence of each drug effect was lost. Taken together these findings indicate that pretreatment with phencyclidine causes changes in acetylcholine systems modulating dopamine release in accumbens. Since phencyclidine treatment was terminated at least a week before the slices were taken, the effects are due to long-term changes in function resulting from the treatment, rather than from transient changes due to the presence of the drug at test. Such enduring dysregulation of cholinergic control of phasic dopamine release could account for deficits in behaviours mediated by accumbal dopamine seen in schizophrenia, and may provide a route for novel therapeutic strategies to treat the disease.
Neurochem Int, 2020; 140

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BOARD NUMBER: S02-466

UNVEILING SUBTYPE-SPECIFIC UPPER CORTICAL LAYER VULNERABILITY IN SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Aims: Schizophrenia is a serious mental disorder caused by a chemical imbalance between neuronal circuits. However, due to the high complexity of these neuronal networks, the aetiology of schizophrenia remains poorly explored. While many other studies have identified the global impact of schizophrenia on the cerebral cortex, the aim of our study is to uncover specific neuronal subtypes with schizophrenia-associated transcriptional changes. Methods: We carried out single-nucleus RNA sequencing (snRNA-seq) on post-mortem brain tissue of more than 220 000 neurons from the dorsolateral prefrontal cortex of patients with schizophrenia and matched controls. Furthermore, we performed immunohistochemistry to bridge cortical anatomy with snRNA-seq findings. Finally, to validate the detected subtype-specific impairment, we performed in situ hybridization and spatial transcriptomics. Results: Using snRNA-seq, we show a decrease in abundance of all GABAergic neurons, and an increase in principal neurons with the highest perturbations in upper layers. Histological analysis confirmed density reduction in supragranular layers of GABAergic neurons particularly in layer 2. Spatial transcriptomics confirmed previously detected hotspot changes in layer 2-3 subtypes which exhibit functional downregulation of genes related to energy metabolism and upregulation of genes related to neurotransmission. Conclusions: Our results show an impairment of specific neuronal subtypes occupying upper cortical layers. Moreover, we also show that schizophrenia-associated changes affect multiple neuronal subtypes which indicates a general network impairment.

Pubmed:
34769425: Bukovac A, Dragičević K, Kafka A, Orešković D, Cesarec-Augustinović S, Pećina-Šlaus N
Decoding the Role of DVL1 in Intracranial Meningioma.
In the search for molecular candidates for targeted meningioma therapies, increasing attention has been paid to the role of signaling pathways in the development and progression of intracranial meningiomas. Although it is well known that the Wnt signaling pathway is involved in meningioma progression, the role of its central mediator, DVL1, is still unclear. In order to investigate the influence of gene alterations on the progression of human intracranial meningioma, we focused on its central PDZ domain, which is responsible for DVL interaction with the Fzd receptor and the phosphorylation of DVL mediated through the casein kinases CK1 and CK2. A genetic analysis of genomic instability revealed the existence of microsatellite instability in 9.09% and the loss of heterozygosity in 6.06% of the samples. The sequencing of the PDZ gene region showed repetitive deletions of two bases located in intron 7 and exon 8, and a duplication in intron 8 in most samples, with different outcomes on the biological function of the DVL1 protein. Immunohistochemistry revealed that the nuclear expression of DVL1 was significantly correlated with a higher expression of active β-catenin (= 0.029) and a higher meningioma grade (= 0.030), which leads to the conclusion that it could be used as biomarker for meningioma progression and the activation of the Wnt signaling pathway.
Int J Mol Sci, 2021; 22
33915799: Bukovac A, Kafka A, Raguž M, Brlek P, Dragičević K, Müller D, Pećina-Šlaus N
Are We Benign? What Can Wnt Signaling Pathway and Epithelial to Mesenchymal Transition Tell Us about Intracranial Meningioma Progression.
Epithelial to mesenchymal transition (EMT), which is characterized by the reduced expression of E-cadherin and increased expression of N-cadherin, plays an important role in the tumor invasion and metastasis. Classical Wnt signaling pathway has a tight link with EMT and it has been shown that nuclear translocation of β-catenin can induce EMT. This research has showed that genes that are involved in cadherin switch, and , play a role in meningioma progression. Increased N-cadherin expression in relation to E-cadherin was recorded. In meningioma, transcription factors SNAIL, SLUG, and TWIST1 demonstrated strong expression in relation to E- and N-cadherin. The expression of SNAIL and SLUG was significantly associated with higher grades (= 0.001), indicating their role in meningioma progression. Higher grades also recorded an

increased expression of total β-catenin followed by an increased expression of its active form (= 0.000). This research brings the results of genetic and protein analyzes of important molecules that are involved in Wnt and EMT signaling pathways and reveals their role in intracranial meningioma. The results of this study offer guidelines and new markers of progression for future research and reveal new molecular targets of therapeutic interventions.
Cancers (Basel), 2021; 13

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BOARD NUMBER: S02-467

EARLY-LIFE CHANGES IN PREFRONTAL CORTICAL SPONTANEOUS ACTIVITY, GABAERGIC TRANSMISSION AND RECENCY MEMORY IN THE MAM MOUSE MODEL OF SCHIZOPHRENIA.

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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[1]University of Crete, Department Of Biology, Heraklion Crete, Greece, [2]Foundation for Research and Technology Heraklion, Greece, Institute Of Molecular Biology And Biotechnology, Heraklion Crete, Greece

Schizophrenia is a severe and multifactorial neuropsychiatric disorder, for which current medication mainly focuses on treating the positive symptoms of the disease, leaving cognitive deficits unaltered. An important research direction for schizophrenia is that of early diagnosis. Aim: In our study we aim to identify early-life neurophysiological changes in the methylazoxymethanol acetate (MAM) mouse model of schizophrenia compared to control mice (saline-treated). Methods: Neonatal (postnatal days (P)8-11), juvenile (P15-21) and adolescent (P40-45), female and male MAM or control prefrontal cortical (PFC) brain slices were acquired for extracellular local field recordings to measure Up states and neuronal oscillations. Adolescent MAM and control mice performed the temporal order object recognition (TOR) task, and were additionally investigated for $GABA_A$ receptor ($GABA_AR$) reversal potential in PFC through voltage-clamp recordings. Results: Delta, theta, alpha and beta frequency neuronal oscillations are significantly reduced in neonatal, but not in juvenile or adolescent MAM PFC, while juvenile MAM mice exhibit a significantly increased frequency of up states, compared to controls. In control adolescent mice, ketamine application in PFC brain slices increases the beta and gamma frequencies, but not in MAM adolescent mice. Furthermore, adolescent MAM mice display a significantly reduced discrimination index in TOR task, and a more positive $GABA_AR$ reversal potential relative to control mice. Conclusion: Early-life alterations of neuronal oscillations and up states generation could affect PFC development and network activity (depolarizing $GABA_AR$ reversal potential), leading to cognitive deficits (TOR deficits) observed in adolescent MAM mice.

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BOARD NUMBER: S02-468

DYSREGULATED MRNA TRANSLATION AND SCHIZOPHRENIA-RELEVANT BEHAVIOURS IN MICE

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Schizophrenia (SCZ) is one of the leading causes of disability worldwide. SCZ arises by alterations in neurodevelopment, involving multiple etiological factors that influence the function of several neurotransmitter systems. For example, dysfunction of the dopaminergic (DAergic) system has been consistently implicated in the pathology of SCZ. Although the role of DA has been well established in SCZ, the developmental factors that lead to dysfunctional DAergic neurotransmission are still a matter of active research. mRNA translation is a key regulator of neural development and is regulated through the mammalian target of rapamycin complex 1 (mTORC1) pathway, among others. Upon its activation, mTORC1 phosphorylates the eukaryotic initiation factor 4E (eIF4E) binding proteins (4E-BPs), allowing eIF4E to recruit the remainder of the translation initiation factors. Considering the importance of the mTORC1-4E-BP pathway in development, we examined the role of 4E-BP2 in the mesolimbic dopaminergic system using a 4E-BP2 mutant mouse model. In male and female mice, we found that absence or haploinsufficiency of 4E-BP2 impaired pre-pulse inhibition of acoustic startle and exaggerated amphetamine-induced locomotion, compared to wildtype littermates (but no difference in locomotion induced by the NMDA receptor antagonist MK-801). These results demonstrate that 4E-BP2 plays an essential role in the development of the dopamine system and potentially in the pathophysiology of SCZ.

Pubmed:
33061910: Aguilar-Valles A, Rodrigue B, Matta-Camacho E
Maternal Immune Activation and the Development of Dopaminergic Neurotransmission of the Offspring: Relevance for Schizophrenia and Other Psychoses.
Prenatal infections have been linked to the development of schizophrenia (SCZ) and other neurodevelopmental disorders in the offspring, and work in animal models indicates that this is to occur through the maternal inflammatory response triggered by infection. Several studies in animal models demonstrated that acute inflammatory episodes are sufficient to trigger brain alterations in the adult offspring, especially in the mesolimbic dopamine (DA) system, involved in the pathophysiology of SCZ and other disorders involving psychosis. In the current review, we synthesize the literature on the clinical studies implicating prenatal infectious events in the development of SCZ. Then, we summarize evidence from animal models of maternal immune activation (MIA) and the behavioral and molecular alterations relevant for the function of the DAergic system. Furthermore, we discuss the evidence supporting the involvement of maternal cytokines, such as interleukin 6 (IL-6) and leptin (a hormone with effects on inflammation) in mediating the effects of MIA on the fetal brain, leading to the long-lasting effects on the offspring. In particular, IL-6 has been involved in mediating the effects of MIA animal models in the offspring through actions on the placenta, induction of IL-17a, or triggering the decrease in non-heme iron (hypoferremia). Maternal infection is very likely interacting with additional genetic and environmental risk factors in the development of SCZ; systematically investigating how these interactions produce specific phenotypes is the next step in understanding the etiology of complex psychiatric disorders.
Front Psychiatry, 2020; 11

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-469

SELECTIVE GSK3α AND β INHIBITION DURING EARLY POSTNATAL DEVELOPMENT MODIFIES WORKING MEMORY-RELATED BEHAVIOR IN A SEX-BIASED MANNER IN A MOUSE MODEL OF 22Q11.2 DELETION SYNDROME

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Johannes Passecker[1,2,3], Chloe Aloimonos[3], Chia-Yuan Chang[2], Aleksandra Dagunts[3], Maxym Myroshnychenko[3], David Kupferschmidt[3], Joseph Gogos[2], Joshua Gordon[3,4]
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Working memory, a cognitive process involving short-term encoding and maintenance of information relies on prefrontal cortex (PFC) functionality, is reliably modeled in preclinical animal models and is profoundly impaired in schizophrenia. The 22q11.2 Deletion Syndrome (DS) is a clinically relevant genetic cause of schizophrenia and non-selective Glycogen Synthase Kinase (GSK)3 (α and β isoform) inhibitors have been found to rescue cognitive deficits in preclinical models, but their non-selective profiles hamper their preclinical utility. Recently developed selective inhibitors allow targeted inhibition of each isoform. Here, we tested their potential to rescue PFC-dependent spatial working memory deficits in the *Df(16)A*$^{+/-}$ mouse model of the 22q11.2DS. The selective GSK3β inhibitor BRD3731 administered during postnatal development rescued acquisition deficits in spatial working memory in male but not female *Df(16)A*$^{+/-}$ mice. Developmental medial PFC and ventral hippocampus bulk-seq transcriptomics data support the findings. GSK3β inhibition also rescued deficits in theta-frequency coherence between ventral hippocampus and medial PFC, a neurophysiological correlate of spatial working memory performance, in *Df(16)A*$^{+/-}$ mice. Ongoing analysis of medial PFC single-unit recordings aims to further verify behavioral and neurophysiological findings. Conversely, selective postnatal GSK3α inhibition by BRD0705 failed to rescue task acquisition deficits but did reverse deficits in task performance under conditions of increased working memory demand in both male and female *Df(16)A*$^{+/-}$ mice. Overall, the experiments indicate differential roles of GSK3α and β isoforms in the development of the prefrontal-hippocampal circuitry supporting spatial working memory, its disease-relevant dysfunction in mice and highlights the import of sex-specific analysis in pre-clinical research.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-470

A PROSPECTIVE STUDY OF ANTIPSYCHOTIC THERAPY INFLUENCE ON CYTOKINE PROFILE IN PATIENTS WITH SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Katerina Basharova[1], Tatiana Usenko[1,2], Anastasia Bezrukova[1], Elena Shagimardanova[3], Natalia Blatt[3], Albert Rizvanov[3], Anna Zabotina[1,2], Regina Nasyrova[4], Nikolay Neznanov[4], Sofya Pchelina[1,2], Anastasia Taraskina[1,2,4]
[1]Petersburg Nuclear Physics Institute named by B.P.Konstantinov of NRC «Kurchatov Institute», Molecular And Radiation Biophysics Division, Gatchina, Russian Federation, [2]Pavlov First Saint Petersburg State Medical University, Research Center, Saint-Petersburg, Russian Federation, [3]Kazan Federal University, Institute of Fundamental Medicine and Biology, Research Center "regulatory Genomics", Kazan', Russian Federation, [4]National Medical Research Center for Psychiatry and Neurology named after N.N. V.M. Bekhtereva, Center For Personalized Psychiatry And Neurology, Saint-Petersburg, Russian Federation

Background. Schizophrenia (SCZ) is a chronic brain disorder that is characterized by alterations in perception, thoughts, mood, behavior. Molecular mechanisms of SCZ are unknown today. However, a cytokine imbalance has been described in SCZ pathogenesis. **The aim** of this study was to investigate the role of over-time cytokine profile changes in predicting the pharmacotherapyeffectiveness of SCZ. **Methods**. Blood serum samples were collected from 61 SCZ patients during antipsychotic therapy (haloperidol/olanzapine) twice: before (T1) and after 28 days (T2) of treatment. Patients were divided according to the therapy effectiveness, depending on the reduction in Positive and Negative Syndrome Scale (PANSS) (>20% - effective, <20% - ineffective). Profile of 48 cytokines was assessed by Immunology Multiplex Assay (HCYTOMAG-48,Merk-Millipore,USA). **Results**. Decreased plasma proinflammatory cytokine concentrations of Il-5, Il-6, Il-7, Il-15, TNF-alpha-2, CXCL1/GRO were revealed in SCZ patients with effective response to therapy in T2 compared to T1 ($p<0.05$). Also, increased plasma proinflammatory cytokine concentrations of IL-4, CCL-22/MDC, PDGF-AB-BB were observed in SCZ patients with poor response to therapy T2 compared to T1 ($p<0.05$). Receiver-operating characteristic (ROC) analysis has been conducted for all studied cytokines in SCZ patients with effective response to therapy compared to ineffective. Cut-off value was estimated as 7.99 ng/ml for TGF-alpha (AUC=0.67;p=0.0315) and 17816 ng/ml for sCD40-L (AUC=0.65;p=0.039634). **Conclusions**. Our data showed a more pronounced decrease of proinflammatory cytokine secretion during antipsychotic therapy in SCZ patients with an effective response to therapy. Concentration of TGF-alpha and sCD40-L may be proposed as as potential biomarkers for predicting the antipsychotic therapy effectiveness.

Pubmed:
35066761: Usenko TS, Senkevich KA, Bezrukova AI, Baydakova GV, Basharova KS, Zhuravlev AS, Gracheva EV, Kudrevatykh AV, Miliukhina IV, Krasakov IV, Khublarova LA, Fursova IV, Zakharov DV, Timofeeva AA, Irishina YA, Palchikova EI, Zalutskaya NM, Emelyanov AK, Zakharova EY, Pchelina SN
Impaired Sphingolipid Hydrolase Activities in Dementia with Lewy Bodies and Multiple System Atrophy.
The synucleinopathies are a group of neurodegenerative diseases characterized by the oligomerization of alpha-synuclein protein in neurons or glial cells. Recent studies provide data that ceramide metabolism impairment may play a role in the pathogenesis of synucleinopathies due to its influence on alpha-synuclein accumulation. The aim of the current study was to assess changes in activities of enzymes involved in ceramide metabolism in patients with different synucleinopathies (Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA)). The study enrolled 163 PD, 44 DLB, and 30 MSA patients as well as 159 controls. Glucocerebrosidase, alpha-galactosidase, acid sphingomyelinase enzyme activities, and concentrations of the corresponding substrates (hexosylsphingosine, globotriaosylsphingosine, lysosphingomyelin) were measured by liquid chromatography tandem-mass spectrometry in blood. Expression levels of GBA, GLA, and SMPD1 genes encoding glucoceresobridase, alpha-galactosidase, and acid sphingomyelinase enzymes, correspondently, were analyzed by real-time PCR with TaqMan assay in CD45 + blood cells. Increased hexosylsphingosine concentration was observed in DLB and MSA patients in comparison to PD and controls ($p < 0.001$) and it was associated with earlier age at onset (AAO) of DLB ($p = 0.0008$). SMPD1 expression was decreased in MSA compared to controls ($p = 0.015$). Acid sphingomyelinase activity was decreased in DLB, MSA patients compared to PD patients ($p < 0.0001$, $p < 0.0001$, respectively), and in MSA compared to controls ($p < 0.0001$). Lower acid sphingomyelinase activity was

associated with earlier AAO of PD (p = 0.012). Our data support the role of lysosomal dysfunction in the pathogenesis of synucleinopathies, namely, the pronounced alterations of lysosomal activities involved in ceramide metabolism in patients with MSA and DLB.
Mol Neurobiol, 2022; 59
34680941: Usenko T, Bezrukova A, Basharova K, Panteleeva A, Nikolaev M, Kopytova A, Miliukhina I, Emelyanov A, Zakharova E, Pchelina S
Comparative Transcriptome Analysis in Monocyte-Derived Macrophages of Asymptomatic Mutation Carriers and Patients with GBA-Associated Parkinson's Disease.
Mutations of the gene, encoding for lysosomal enzyme glucocerebrosidase (GCase), are the greatest genetic risk factor for Parkinson's disease (PD) with frequency between 5% and 20% across the world. N370S and L444P are the two most common mutations in the gene. PD carriers of severe mutation L444P in the gene is characterized by the earlier age at onset compared to N370S. Not every carrier of mutations develop PD during one's lifetime. In the current study we aimed to find common gene expression signatures in PD associated with mutation in the gene (GBA-PD) using RNA-seq. We compared transcriptome of monocyte-derived macrophages of 5 patients with GBA-PD (4 L444P/N, 1 N370S/N) and 4 asymptomatic mutation carriers (GBA-carriers) (3 L444P/N, 1 N370S/N) and 4 controls. We also conducted comparative transcriptome analysis for L444P/N only GBA-PD patients and GBA-carriers. Revealed deregulated genes in GBA-PD independently of mutations (L444P or N370S) were involved in immune response, neuronal function. We found upregulated pathway associated with zinc metabolism in L444P/N GBA-PD patients. The potential important role of in the pathogenesis of GBA-PD was suggested.
Genes (Basel), 2021; 12

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9-13 July 2022 | Paris, France

BOARD NUMBER: S02-471

COMMON PATTERNS OF SCHIZOPHRENIA AND LYSOSOMAL STORAGE DISORDERS

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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[1]Petersburg Nuclear Physics Institute named by B.P.Konstantinov of NRC «Kurchatov Institute», Molecular And Radiation Biophysics Division, Gatchina, Russian Federation, [2]Pavlov First Saint Petersburg State Medical University, Research Center, Saint-Petersburg, Russian Federation, [3]V.M. Bekhterevs National Medical Research Center Psychiatry and Neurology, Department Of Geriatric Psychiatry, Saint-Petersburg, Russian Federation, [4]N.P. Bechtereva Institute of the Human Brain, Center For Neurodegenerative Diseases And Botulinum Therapy, Saint-Petersburg, Russian Federation, [5]Research Center for Medical Genetics, Scientific Departments, Moscow, Russian Federation

Background. Schizophrenia (SCZ) is a mental disorder with a prevalence of 0.7-1% of the general population and is characterized by impairment of dopamine transmission in neurons. Last data demonstrated that lysosomal storage disorders (LSDs) accompanied by a decrease of lysosomal activity may manifest with a wide spectrum of clinical presentations including psychosis, schizophrenia, mood disorders, early-onset dementia. **The aim** was to estimate whether alteration of lysosomal activities contributes to SCZ pathogenesis. **Methods.** 34 SCZ patients and 181 patients with Parkinson's disease (PD) as patients with disease also associated with dopamine metabolism dysfunction and 176 controls were enrolled for the current study. Enzyme activities (glucoceresobridase (GCase), alpha-galactosidase (GLA), acid sphingomyelinase (ASMase)), and concentration of their corresponded substrates (hexosylsphingosine (HexSph), globotriaosylsphingosine (LysoGb3), lysosphingomyelin (LysoSM)) in blood were measured by liquid chromatography tandem-mass spectrometry. **Results.** Decreased ASMase activity was found in SCZ patients compared to PD patients ($p<0.0001$) and controls ($p<0.0001$) with increase of LysoSM concentration in SCZ patients compared to PD patients ($p<0.0001$) and controls ($p=0.00081$). Elevated GLA activity in SCZ patients compared to PD patients ($p=0.0039$) and controls ($p=0.0004$) with increase of LysoGb3 concentration in SCZ patients compared to PD patients ($p<0.0001$) and controls ($p<0.0001$) was found. HexSph concentration was increased in SCZ patients compared to PD patients and controls ($p<0.0001$) with no differences in GCase activity between all studied groups ($p>0.05$). **Conclusion.** Our data suggested possible link between SCZ and LSDs, characterized by alteration of lysosomal enzyme activities with more pronounce decreased of ASMase and increase of LysoSM.

Pubmed:
35066761: Usenko TS, Senkevich KA, Bezrukova AI, Baydakova GV, Basharova KS, Zhuravlev AS, Gracheva EV, Kudrevatykh AV, Miliukhina IV, Krasakov IV, Khublarova LA, Fursova IV, Zakharov DV, Timofeeva AA, Irishina YA, Palchikova EI, Zalutskaya NM, Emelyanov AK, Zakharova EY, Pchelina SN
Impaired Sphingolipid Hydrolase Activities in Dementia with Lewy Bodies and Multiple System Atrophy.
The synucleinopathies are a group of neurodegenerative diseases characterized by the oligomerization of alpha-synuclein protein in neurons or glial cells. Recent studies provide data that ceramide metabolism impairment may play a role in the pathogenesis of synucleinopathies due to its influence on alpha-synuclein accumulation. The aim of the current study was to assess changes in activities of enzymes involved in ceramide metabolism in patients with different synucleinopathies (Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA)). The study enrolled 163 PD, 44 DLB, and 30 MSA patients as well as 159 controls. Glucocerebrosidase, alpha-galactosidase, acid sphingomyelinase enzyme activities, and concentrations of the corresponding substrates (hexosylsphingosine, globotriaosylsphingosine, lysosphingomyelin) were measured by liquid chromatography tandem-mass spectrometry in blood. Expression levels of GBA, GLA, and SMPD1 genes encoding glucoceresobridase, alpha-galactosidase, and acid sphingomyelinase enzymes, correspondently, were analyzed by real-time PCR with TaqMan assay in CD45 + blood cells. Increased hexosylsphingosine concentration was observed in DLB and MSA patients in comparison to PD and controls ($p < 0.001$) and it was associated with earlier age at onset (AAO) of DLB ($p = 0.0008$). SMPD1 expression was decreased in MSA compared to controls ($p = 0.015$). Acid sphingomyelinase activity was decreased in DLB, MSA patients compared to PD patients ($p < 0.0001$, $p < 0.0001$, respectively), and in MSA compared to controls ($p < 0.0001$). Lower acid sphingomyelinase activity was

associated with earlier AAO of PD (p = 0.012). Our data support the role of lysosomal dysfunction in the pathogenesis of synucleinopathies, namely, the pronounced alterations of lysosomal activities involved in ceramide metabolism in patients with MSA and DLB.
Mol Neurobiol, 2022; 59
34680941: Usenko T, Bezrukova A, Basharova K, Panteleeva A, Nikolaev M, Kopytova A, Miliukhina I, Emelyanov A, Zakharova E, Pchelina S
Comparative Transcriptome Analysis in Monocyte-Derived Macrophages of Asymptomatic Mutation Carriers and Patients with GBA-Associated Parkinson's Disease.
Mutations of the gene, encoding for lysosomal enzyme glucocerebrosidase (GCase), are the greatest genetic risk factor for Parkinson's disease (PD) with frequency between 5% and 20% across the world. N370S and L444P are the two most common mutations in the gene. PD carriers of severe mutation L444P in the gene is characterized by the earlier age at onset compared to N370S. Not every carrier of mutations develop PD during one's lifetime. In the current study we aimed to find common gene expression signatures in PD associated with mutation in the gene (GBA-PD) using RNA-seq. We compared transcriptome of monocyte-derived macrophages of 5 patients with GBA-PD (4 L444P/N, 1 N370S/N) and 4 asymptomatic mutation carriers (GBA-carriers) (3 L444P/N, 1 N370S/N) and 4 controls. We also conducted comparative transcriptome analysis for L444P/N only GBA-PD patients and GBA-carriers. Revealed deregulated genes in GBA-PD independently of mutations (L444P or N370S) were involved in immune response, neuronal function. We found upregulated pathway associated with zinc metabolism in L444P/N GBA-PD patients. The potential important role of in the pathogenesis of GBA-PD was suggested.
Genes (Basel), 2021; 12
33227372: Usenko TS, Bezrukova AI, Bogdanova DA, Kopytova AE, Senkevich KA, Gracheva EV, Timofeeva AA, Miliukhina IV, Zakharova EY, Emelyanov AK, Pchelina SN
Genetics variants and expression of the SCARB2 gene in the pathogenesis of Parkinson's disease in Russia.
Lysosomal integral membrane protein-2 (LIMP-2), encoded by the SCARB2 gene, is the specific lysosomal receptor for glucocerebrosidase enzyme. Association between rs6812193 and rs68250047 of SCARB2 with PD has been shown in genetic studies, including large genome-wide association studies. The aim of the current study was to determine whether rs6812193 and rs8475 are associated with PD in Russia. rs6812193 and rs8475 were genotyped in a total of 604 PD patients (65 PD patients with positive (fPD) and 539 PD patients with negative family history (sPD)) and 413 controls and also in 17 patients with PD associated with GBA mutations (PD-GBA) and 18 asymptomatic GBA mutation carriers (GBA-Carriers). SCARB2 expression was measured by real-time PCR in CD45+ blood cells in part of individuals in the studied groups. No linkage disequilibrium was shown between rs6812193 and rs8475 in Russian population. Increased PD risk for TT variant of rs8475 (OR = 2.02; p < 0.001) was found in sPD patients but not in fPD. rs6812193 and rs8475 were not associated with age at onset (AAO) of PD. SCARB2 expression level was decreased in GBA-PD patients and GBA-Carriers compared to PD patients (p = 0.02, p = 0.003, respectively) and GBA-Carriers compared to controls (p = 0.013) with no significant difference in PD patients and controls. SCARB2 expression was not modified with rs6812193 and rs8475. In conclusion, rs8475 was associated with PD status. rs6812193 and rs8475 are not genetic modifier of AAO of PD and do not influence on SCARB2 mRNA level in CD45+ blood cells in studied groups. SCARB2 expression could be modified with GBA mutations and is independent of PD status.
Neurosci Lett, 2021; 741
32336641: Usenko TS, Nikolaev MA, Miliukhina IV, Bezrukova AI, Senkevich KA, Gomzyakova NA, Beltceva YA, Zalutskaya NM, Gracheva EV, Timofeeva AA, Petrova OA, Semenov AV, Lubimova NE, Totolyan AA, Pchelina SN
Plasma cytokine profile in synucleinophaties with dementia.
Immune response may play a pivotal role in the pathogenesis of the common synucleinopathy as Parkinson's disease (PD) and could be mediated with the accumulation of neurotoxic alpha-synuclein. There is limited evidence for immune response in another synucleinopathy as dementia with Lewy bodies (DLB). Recent data suggest that immune response may contribute to cognitive impairment. We aimed to estimate plasma cytokine profile in patients with synucleinopathies with dementia (PD dementia (PDD), DLB). Plasma cytokine levels (interferon-gamma (IFN-gamma), interleukin (IL)-4 (IL-4), IL-6, IL-10, tumor necrosis factor alpha (TNF-alpha), monocyte chemoattractant protein-1 (MCP-1)). were estimated in 16 patients with DLB, 19 patients with PDD, 28 patients with PD without dementia (PD) and 19 individuals without neurological disorders (controls) using Luminex array system. Cognitive status was assessed with the Mini-Mental State Examination (MMSE). TNF-alpha and IL-6 plasma levels were elevated in patients with synucleinopathies with dementia (DLB, PDD) compared to controls and IL-10 plasma level was increased in PDD compared to controls (p < 0.05). IFN-gamma levels were decreased in PD and PDD patients compared to controls (p < 0.001, p = 0.026, respectively) and in PD patients than in DLB patients (p = 0.032). Patients with PD, PDD, and DLB were characterized by increased plasma levels of MCP-1 compared to controls (p < 0.001). At the same time, no differences in TNF-alpha, IL-10, IL-6 plasma levels in PD patients compared to controls were found. Our

study demonstrated more pronounced immune response in synucleinopathies associated with dementia compared to PD without demetia.
J Clin Neurosci, 2020; 78

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-472

OXIDATIVE STRESS MARKERS AS POTENTIAL PREDICTORS OF THE TRANSITION TO PSYCHOSIS IN INDIVIDUALS AT ULTRA-HIGH RISK.

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Victoria Rambaud[1], Ariel Frajerman[1], Margot Fournier[2], Daniella Dwir[2], Ines Khadimallah[2], Oussama Kebir[1,3], Aude Marzo[1], Marie-Odile Krebs[1,3], Kim Q Do[2], Boris Chaumette[1,3,4]
[1]Université de Paris, INSERM U1266, Institute Of Psychiatry And Neuroscience Paris, Paris, France, [2]Center for Psychiatric Neuroscience, Lausanne University Hospital (CHUV), Department Of Psychiatry, Lausanne, Switzerland, [3]Hôpital Sainte Anne, Ghu-paris Psychiatrie Et Neurosciences, PARIS, France, [4]McGill University, Department Of Psychiatry, Montréal, France

Background: Ultra-high-risk state (UHR) concept was initially applied to promote the early detection of young help-seeking patients with higher risk of psychotic transition. However, most UHR individuals do not evolve to psychosis, stressing the need for biomarkers allowing the prediction of the transition. Therefore, phase-specific approaches and mechanism-based-biomarkers are necessary to develop early prevention strategies. The interaction between redox dysregulation and neuroinflammation was shown to play a major role in the pathophysiology of psychotic disorders. **Aim:** The aim of this project is to investigate the role of blood markers for redox dysregulation in the risk of transition to psychosis in UHR. **Methods:** Blood samples were collected from 48 UHR at their first visit and 6 to 12 months later for those who did not transition to psychosis (UHR-NC), or at the time of the transition (UHR-C). Markers for redox dysregulation, including the glutathione antioxidant system, superoxide dismutase, thioredoxin, TBARS, macrophage migration inhibitory factor, peroxiredoxin 4, MMP9 and sRAGE, were assessed in erythrocytes, serum and plasma. **Results:** Statistical analyses revealed a combination of peripheral redox markers as indicators of the transition to psychosis. These markers were able to discriminate between UHR-C and UHR-NC subjects at baseline. Importantly, some of the redox markers were associated with the severity of symptoms at the final timepoint. **Conclusion:** These findings support the use of mechanism-based peripheral biomarkers of oxidative stress as predictors of the transition to psychosis. Overall, these findings hold promises for early detection and stratification of UHR, targeting redox pathways.

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BOARD NUMBER: S02-473

COMPLEMENT RESPONSES AND SYNAPTIC CHANGES AFTER TRANSIENT MICROGLIA DEFICIENCY IN THE ADOLESCENT PREFRONTAL CORTEX

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Sina-Maria Schalbetter[1], Anina Von Arx[1], Natalia Cruz-Ochoa[2], Han-Yu Lin[1], René Amport[1], Csaba Földy[2], Melanie Greter[3], Tina Notter[1], Urs Meyer[1]
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Microglia are the resident immune cells of the brain parenchyma and account for 5-12% and 0.5-15% of the total cell population in the adult mouse and human brain, respectively. Besides their classical immunological functions, microglia also contribute to the remodeling of synaptic connections through phagocytic mechanisms. Commonly referred to as “synaptic pruning”, this process involves microglia-mediated engulfment of pre- and postsynaptic material that is tagged with components of the complement system. In the present study we used a mouse model of selective and transient depletion of microglia in the adolescent prefrontal cortex (PFC) to examine its effects on synaptic densities and functions, as well as on the complement system in the adult PFC. Using a selective and transient microglia loss-of-function approach, our study identified dynamic changes in complement component expression (i.e. an upregulation of C3 and C4) and microglial uptake of synaptic particles in the adolescent PFC. Our data further show that prefrontal microglia deficiency in adolescence has lasting effects on synaptic structures and functions in adulthood. Given the implication of C3 and C4 in neurodevelopmental disorders, such as schizophrenia, our model system offers unique opportunities for preclinical research and to examine the neurobiological consequences of non-genetic upregulation of C3 and C4b arising from transient microglia deficiency during specific stages of brain maturation.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S02-474

A COMPARISON OF PSYCHOTROPIC DRUG PREFERENCES AND SIDE EFFECTS IN OLD AND YOUNG PATıENTS WITH SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Zehra Uçar-Hasanlı, Betül Yıldırım, Zeynep Tatlı, Erguvan Tuğba Özel-Kızıl
Ankara University Faculty of Medicine, Psychiatry, Ankara, Turkey

AIM: In parallel with the aging population, the treatment of elderly schizophrenic patients has become important. Antipsychotic therapy, which is the mainstay in the treatment of schizophrenia, is different due to both the course of the disease and pharmacokinetic/pharmacodynamic changes in elderly. The current study investigates the preferred psychotropic drug treatments and their tolerability in elderly patients (EP) with a diagnosis of schizophrenia or schizoaffective disorder compared to younger patients (YP). **METHOD:** The study included 154 EP and 195 YP with schizophrenia/schizoaffective disorder admitted to a university hospital in the last decade. The medical records of the patients were reviewed. The types and doses of antipsychotic drugs used by each patient, their use of other psychotropic drugs, ongoing complaints, drug-related side effects and compliance with treatment were also examined. **RESULTS:** Second generation antipsychotic use was higher in YP($p=0.046$). Antipsychotic equivalent doses were also higher in the YP group($p<0.001$). The use of clozapine and mood stabilizers were higher in YP($p<0.001$). There was a higher rate of dose reduction of antipsychotic drugs in EP($p<0.001$). When the groups were compared in terms of the reasons of antipsychotic dose reduction, it was more frequent in EP due to side effects, while the dose reduction due to remission was more common in YP($p=0.040$). **CONCLUSIONS:** Lower doses of antipsychotics in EP suggests milder symptoms or lower tolerability. The higher clozapine and mood stabilizer use in YP can be explained by avoiding side effects like extrapyramidal, cardiovascular and metabolic side effects that are more frequent in EP.

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BOARD NUMBER: S02-475

A STUDY OF THE TRANSCRIPTOMIC SIGNATURES IN MALE AND FEMALE RATS EXPOSED TO MATERNAL IMMUNE ACTIVATION AND THC DURING ADOLESCENCE

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Mario Moreno-Fernández, Roberto Capellán, Marcos Ucha, Alberto Marcos, Emilio Ambrosio, Alejandro Higuera-Matas
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AIMS: To use a double-hit animal model of neurodevelopmental disorders relevant to schizophrenia and study the transcriptomic signatures of maternal immune activation (MIA) and THC administration during adolescence in peripheral blood mononuclear cells (PBMCs) with the goal of finding vulnerability markers for the disease. METHODS: Pregnant Sprague-Dawley rats were treated with lipopolysaccharides or saline at gestational days 15 and 16. During adolescence, male and female offspring were injected with incremental doses of THC or vehicle. When the animals were adults PBMCs were obtained from the blood, and RNA sequencing was performed to search for transcriptomic signatures of either each hit alone or in combination. RESULTS: In the females, MIA differentially affected 15 genes while in the males it affected 389. THC exposure affected 148 genes in the females and 604 in the males. Interestingly, THC affected the expression of 254 genes in females exposed to MIA and 109 in MIA males. A gene ontology analysis suggested that, in the males, there was an increased representation of genes related to the inflammatory response due to the MIA+THC combination. In the females, the normal response to THC of gene categories related to interferon I or interleukine was absent among MIA rats and there was an increased presence of the innate immune response ontology due to the synergy of both hits. CONCLUSIONS: The combination of a vulnerability factor (MIA) and a triggering event (exposure to THC) results in specific effects in the transcriptome that could guide the search for early biomarkers.

Pubmed:

31787482: Capellán R, Moreno-Fernández M, Orihuel J, Roura-Martínez D, Ucha M, Ambrosio E, Higuera-Matas A
Ex vivo H-MRS brain metabolic profiling in a two-hit model of neurodevelopmental disorders: Prenatal immune activation and peripubertal stress.
Prenatal infections are environmental risk factors for neurodevelopmental disorders. In addition, traumatic experiences during adolescence in individuals exposed to infections during gestation could increase the risk of schizophrenia. It is of the most crucial importance to discover potential markers of the disease in its early stages or before its onset, so that therapeutic strategies may be implemented. In the present study, we combined a proposed two-hit model of schizophrenia-related symptoms with proton magnetic resonance spectroscopy (H-MRS) to discover potential biomarkers. To this end, we i.p. injected 100 µg/kg/ml of lipopolysaccharide (LPS) or saline on gestational days 15 and 16 to pregnant rats. Their male offspring were then subjected to five episodes of stress or handling on alternate days during postnatal days (PND) 28-38. Once the animals reached adulthood (PND70), we evaluated prepulse inhibition (PPI). At PND90, we performed an ex vivo H-MRS study in the cortex and striatum. While we did not detect alterations in PPI at the age tested, we found neurochemical disturbances induced by LPS, stress or (more interestingly) their interaction. LPS decreased glucose levels in the cortex and striatum and altered glutamate, glutamine and N-acetylaspartate levels. Glutamate and glutamine levels in the left (but not right) striatum were differentially affected by prenatal LPS exposure in a manner that depended on stress experiences. These results suggest that alterations in the glutamate cycle in the striatum could be used as early markers of developmental disorders.
Schizophr Res, 2019;
32203565: Marcos A, Moreno M, Orihuel J, Ucha M, Mª de Paz A, Higuera-Matas A, Capellán R, Crego AL, Martínez-Larrañaga MR, Ambrosio E, Anadón A
The effects of combined intravenous cocaine and ethanol self-administration on the behavioral and amino acid profile of young adult rats.
Under paradigms of combined intravenous cocaine and ethanol self-administration, the effects on behavior have been poorly explored. Numerous studies have found sex differences in amino acids profile and behavioral responses to each drug, yet few have focused on the interactions between cocaine and ethanol. The main objective of this work was to explore the acquisition and maintenance of intravenous self-administration behavior with a combination of cocaine and ethanol in male

and female young adult rats. Likewise, the amino acids profile in blood plasma was quantified 48 hours after the last self-administration session. Male and female 52 days old Wistar rats were randomly assigned to one of 3 groups: i) saline control, ii) cocaine (1 mg/kg bodyweight/injection) and iii) cocaine and ethanol (1 mg + 133 mg/kg bodyweight/ injection). After 24 self-administration sessions carried out on a fixed-ratio-1 schedule, with a limit of 15 doses per session, 14 plasma amino acids were quantified by mean Capillary Electrophoresis technique. The curve of cocaine and ethanol combined self-administration was similar to that associated with cocaine administration alone, with females acquiring self-administration criterion before males. The self-administration of cocaine and ethanol altered the plasma concentration and relative ratios of the amino acid L-Tyrosine. In our intravenous self-administration model, females appeared more vulnerable to acquire abusive consumption of the cocaine and ethanol combination, which altered plasma L-Tyrosine levels.
PLoS One, 2020; 15

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CHRONIC IN VIVO ELECTROPHYSIOLOGICAL RECORDINGS OF DOPAMINE NEURONS IN A MOUSE MODEL OF THE 22Q11.2 MICRODELETION SYNDROME

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Dopamine (DA) dysregulation, particularly elevated striatal DA neurotransmission, as revealed by more recent imaging studies, has been at the focus of schizophrenia (SCZ) psychopathology, but its mechanistic contributions to disease manifestations are still unresolved. To examine DA dysregulation relevant to human disease, we made use of the *Df(16)A*$^{+/-}$ mouse model of the 22q11.2 Deletion Syndrome, which represents the highest genetic risk factor for SCZ. Previous work on the *Df(16)A*$^{+/-}$ mice revealed several schizophrenia-like deficits, however, the function of the DA system has not yet been studied. For this, we chronically recorded DA neuronal firing activity *and* extracellular striatal DA dynamics in awake freely moving *Df(16)A*$^{+/-}$ mice and wild-type littermates. We established chronic *in vivo* single-unit extracellular recordings of pharmacologically identified DA neurons in the medial substantia nigra (mSN) during open field exploration. For these DA mSN neurons, projecting to either dorsal or ventral striatum, we detected persistent electrophysiological hyperactivity in *Df(16)A*$^{+/-}$ mice compared to controls. This was characterized by increased mean firing frequencies (1.5–fold; $p = 0.0002$, n = 7, N =53 for WT and N=97 for *Df(16)A*$^{+/}$) and elevated bursting activity (1.7-fold; $p < 0.0001$). Using dLight1 fluorometry, we detected a (1.3-fold; $p < 0,01$, n = 7) higher rate of DA transients in *Df(16)A*$^{+/-}$ mice in DMS, as would have been predicted by an *in vivo* DA hyperexcitability phenotype, if tightly coupled to striatal DA dynamics. In summary, we made first steps towards an electrical and neurochemical characterization of DA dysfunction in a 22q11 deletion mouse model.

Pubmed:
34455393: Freudenberg F, Candemir E, Chen X, Li LL, Esen-Sehir D, Schenk N, Kinoshita M, Grünewald L, Frerichs V, Fattakhov N, Manchen J, Bikas S, Kumar A, OLeary A, Slattery DA, von Engelhardt J, Courtney MJ, Reif A
Hippocampal overexpression of NOS1AP promotes endophenotypes related to mental disorders.
Nitric oxide synthase 1 adaptor protein (NOS1AP; previously named CAPON) is linked to the glutamatergic postsynaptic density through interaction with neuronal nitric oxide synthase (nNOS). NOS1AP and its interaction with nNOS have been associated with several mental disorders. Despite the high levels of NOS1AP expression in the hippocampus and the relevance of this brain region in glutamatergic signalling as well as mental disorders, a potential role of hippocampal NOS1AP in the pathophysiology of these disorders has not been investigated yet.
EBioMedicine, 2021; 71

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FOXP2 IN THE MAMMALIAN THALAMUS: HUMANS, INCLUDING PATIENTS WITH SCHIZOPHRENIA, AND ANIMAL MODELS.

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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FOXP2 is a transcriptional factor involved in the development of speech and language. It has been considered a candidate gene in schizophrenia. Indeed, deficits in FOXP2 function can lead to failure of structures relevant to normal language processing; also, FOXP2 has been associated with psychotic symptoms, such as auditory hallucinations. One of the relevant regions of the brain in schizophrenia is the thalamus, a region of gray matter composed of multiple nuclei where sensory, motor, emotional, and cognitive neural pathways relay and connect with the cerebral cortex. We have previously shown the association of FOXP2 gene variants with reductions of gray matter density and gene expression in various brain regions of patients with schizophrenia. In the present study, we investigated the expression of FOXP2 and related markers in the thalamus of postmortem brains from patients with schizophrenia and in a rat “dual hit” animal model of schizophrenia. In addition, we studied the distributions of cells containing FOXP2 protein in the macaque and rat thalamic nuclei. FOXP2 gene expression is reduced in the thalamus of postmortem brains of patients with schizophrenia. Furthermore, FOXP2 protein is differentially expressed in the macaque and rat thalamic nuclei and in the rat model of schizophrenia. These observations point to a possible role of this transcriptional factor in the regulation of thalamic nuclei and in the pathogenesis and/or pathophysiology of the disease. This work is supported by a grant from Universidad Autónoma de Madrid - Comunidad Autónoma de Madrid (SI3-PJI-2021-00417).

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BOARD NUMBER: S02-478

GUT MICROBIOTA – HIPPOCAMPUS SYNERGISMS IN NON-CLINICAL SUBJECTS WITH HIGH POSITIVE SCHIZOTYPY

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Background and rationale: Schizotypy can be understood as a developmental mediator between early endophenotypes and later risk for developing overt psychosis. Hippocampal dysregulation might be a key factor for the developmental trajectory of psychotic disorders and is related to poor clinical outcomes. While preliminary studies indicate alterations in gut microbiota in patients with schizophrenia, it remains unknown whether they are evident in healthy people with high level of schizotypy. **Objectives:** By exploring aberrant gut microbiota-hippocampus interactions in non-clinical individuals with high positive schizotypy we aim to investigate differences in 1) hippocampus function and neurochemistry, 2) gut microbiota composition between populations with low and high positive schizotypy and 3) whether hippocampal measures can predict different microbial profiles. **Methods:** 1) Hippocampal perfusion and activation will be measured with arterial spin labelling, fMRI, and magnetic resonance spectroscopy. 2) Quantitative Microbiome Profiling will be based on 16S ribosomal RNA sequencing. Differences in species abundance will be calculated using Bray-Curtis distances and group differences will be assessed with permutational analysis of variance. 3) Clusters of enterotypes will be determined based on Dirichlet Multinomial Modelling. **Expected results:** Most prominent hippocampus differences between high and low schizotypy will be hypothesized in GABA and glutamate concentrations, with lower GABA and higher glutamate concentrations in people with high positive schizotypy. We hypothesize enterotypes to be predicted by hippocampal measures, with GABA and glutamate providing the most explanatory power. This project may deliver novel prognostic and predictive biomarkers supporting the improvement of psychosis prediction and the development of novel interventions.

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ANALYSIS OF SCHIZOPHRENIA-ASSOCIATED LIPIDOME CHANGES ON THE WHOLE-BRAIN SCALE

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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There is growing evidence for changes in lipid metabolism associated with psychiatric diseases, including schizophrenia, and the brain itself has the second-highest concentration of lipids in the human body. The goal of our study was to investigate changes in lipidome composition of various brain regions associated with schizophrenia on the whole-brain scale. We assessed the lipidome composition of 75 anatomically distinct regions of four healthy and four schizophrenic individuals. Lipid fraction was extracted and measured using liquid chromatography coupled with mass spectrometry. After recalibration peaks were filtered based on their retention time, mean intensity, number of missed values, and their intensity in blank samples. Selected peaks were manually annotated, yielding 379 lipid species, covering 21 lipid classes. Comparison of lipid abundance in the brain of healthy and schizophrenic individuals showed different degrees of changes for various lipid categories with the greatest changes in the myelin-associated and brain function-associated lipids. Regions with the most pronounced changes in lipid abundance were identified: thalamus, associative and secondary cortex (increased levels); white matter, and basal ganglia (decreased levels). The results of our work indicate statistically significant differences in the behavior of different types of lipids in various regions of the brain, which might be associated with cytoarchitectural and myeloarchitectural differences. Moreover, the association to the functional architecture of the brain makes lipids a potential target for the diagnosis of mental disorders. The reported study was funded by RFBR, project number №20-34-90146.

Pubmed:
34822371: Smirnov D, Mazin P, Osetrova M, Stekolshchikova E, Khrameeva E
The Hitchhiker's Guide to Untargeted Lipidomics Analysis: Practical Guidelines.
Lipidomics is a newly emerged discipline involving the identification and quantification of thousands of lipids. As a part of the omics field, lipidomics has shown rapid growth both in the number of studies and in the size of lipidome datasets, thus, requiring specific and efficient data analysis approaches. This paper aims to provide guidelines for analyzing and interpreting lipidome data obtained using untargeted methods that rely on liquid chromatography coupled with mass spectrometry (LC-MS) to detect and measure the intensities of lipid compounds. We present a state-of-the-art untargeted LC-MS workflow for lipidomics, from study design to annotation of lipid features, focusing on practical, rather than theoretical, approaches for data analysis, and we outline possible applications of untargeted lipidomics for biological studies. We provide a detailed R notebook designed specifically for untargeted lipidome LC-MS data analysis, which is based on software.
Metabolites, 2021; 11
29900295: Arapidi G, Osetrova M, Ivanova O, Butenko I, Saveleva T, Pavlovich P, Anikanov N, Ivanov V, Govorun V
Peptidomics dataset: Blood plasma and serum samples of healthy donors fractionated on a set of chromatography sorbents.
Blood as connective tissue potentially contains evidence of all processes occurring within the organism, at least in trace amounts (Petricoin et al., 2006) [1]. Because of their small size, peptides penetrate cell membranes and epithelial barriers more freely than proteins. Among the peptides found in blood, there are both fragments of proteins secreted by various tissues and performing their function in plasma and receptor ligands: hormones, cytokines and mediators of cellular response (Anderson et al., 2002) [2]. In addition, in minor amounts, there are peptide disease markers (for example, oncomarkers) and even foreign peptides related to pathogenic organisms and infection agents. To propose an approach for detailed peptidome characterization, we carried out an LC-MS/MS analysis of blood serum and plasma samples taken from 20 healthy donors on a TripleTOF 5600+ mass-spectrometer. We prepared samples based on our previously developed method of peptide desorption from the surface of abundant blood plasma proteins followed by standard chromatographic steps (Ziganshin et al., 2011) [3]. The mass-spectrometry peptidomics data presented in this article have been deposited to the ProteomeXchange Consortium (Deutsch et al., 2017) [4] via the PRIDE partner repository with the dataset identifier PXD008141 and 10.6019/PXD008141.

Data Brief, 2018; 18
28729867: Lomakin Y, Arapidi GP, Chernov A, Ziganshin R, Tcyganov E, Lyadova I, Butenko IO, Osetrova M, Ponomarenko N, Telegin G, Govorun VM, Gabibov A, Belogurov A
Exposure to the Epstein-Barr Viral Antigen Latent Membrane Protein 1 Induces Myelin-Reactive Antibodies .
Multiple sclerosis (MS) is an autoimmune chronic inflammatory disease of the central nervous system (CNS). Cross-reactivity of neuronal proteins with exogenous antigens is considered one of the possible mechanisms of MS triggering. Previously, we showed that monoclonal myelin basic protein (MBP)-specific antibodies from MS patients cross-react with Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1). In this study, we report that exposure of mice to LMP1 results in induction of myelin-reactive autoantibodies . We posit that chronic exposure or multiple acute exposures to viral antigen may redirect B cells from production of antiviral antibodies to antibodies, specific to myelin antigen. However, even in inbred animals, which are almost identical in terms of their genomes, such an effect is only observed in 20-50% of animals, indicating that this change occurs by chance, rather than systematically. Cross-immunoprecipitation analysis showed that only part of anti-MBP antibodies from LMP1-immunized mice might simultaneously bind LMP1. In contrast, the majority of anti-LMP1 antibodies from MBP-immunized mice bind MBP. sequencing of anti-LMP1 and anti-MBP antibodies by mass spectrometry demonstrated enhanced clonal diversity in LMP1-immunized mice in comparison with MBP-immunized mice. We suggest that induction of MBP-reactive antibodies in LMP1-immunized mice may be caused by either Follicular dendritic cells (FDCs) or by T cells that are primed by myelin antigens directly in CNS. Our findings help to elucidate the still enigmatic link between EBV infection and MS development, suggesting that myelin-reactive antibodies raised as a response toward EBV protein LMP1 are not truly cross-reactive but are primarily caused by epitope spreading.
Front Immunol, 2017; 8
29116070: Kudryavtseva AA, Osetrova MS, Livinyuk VY, Manukhov IV, Zavilgelsky GB
[The importance of C-terminal aspartic acid residue (D141) to the antirestriction activity of the ArdB (R64) protein].
Antirestriction proteins of the ArdB/KlcA family are specific inhibitors of restriction (endonuclease) activity of type-I restriction/modification enzymes. The effect of conserved amino acid residues on the antirestriction activity of the ArdB protein encoded by the transmissible R64 (IncI1) plasmid has been investigated. An analysis of the amino acid sequences of ArdB homologues demonstrated the presence of four groups of conserved residues ((1) R16, E32, and W51; (2) Y46 and G48; (3) S81, D83 and E132, and (4) N77, L(I)140, and D141) on the surface of the protein globule. Amino acid residues of the fourth group showed a unique localization pattern with the terminal residue protruding beyond the globule surface. The replacement of two conserved amino acids (D141 and N77) located in the close vicinity of each other on the globule surface showed that the C-terminal D141 is essential for the antirestriction activity of ArdB. The deletion of this residue, as well as replacement by a hydrophobic threonine residue (D141T), completely abolished the antirestriction activity of ArdB. The synonymous replacement of D141 by a glutamic acid residue (D141E) caused an approximately 30-fold decrease of the antirestriction activity of ArdB, and the point mutation N77A caused an approximately 20-fold decrease in activity. The residues D141 and N77 located on the surface of the protein globule are presumably essential for the formation of a contact between ArdB and a currently unknown factor that modulates the activity of type-I restriction/modification enzymes.
Mol Biol (Mosk), 2017 Sep-Oct; 51

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BOARD NUMBER: S02-480

PHENCYCLIDINE AND NICOTINE INTERACTIVE EFFECTS IN AN ADOLESCENT MOUSE MODEL OF SCHIZOPHRENIA-LIKE PSYCHOSIS AND NICOTINE MISUSE CO-MORBIDITY

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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The association between schizophrenia and nicotine addiction is already evident during adolescence. Here, to investigate the mechanisms that underlie the early establishment of this comorbidity, we used phencyclidine-evoked locomotor sensitization, a proxy model of psychotic behavior, and nicotine minipump infusions. Considering the involvement of dopamine D_2 receptors in both schizophrenia and addiction mechanisms, we further tested the role of these receptors by treating mice with raclopride. Adolescent mice exposed to nicotine (24mg/Kg/day) or not, received single daily raclopride (0.5mg/kg, s.c.) or saline followed by phencyclidine injections (10mg/Kg, s.c.). Drugs administration was followed by open field testing for 6 days (acquisition period, ACQ1-6). Phencyclidine and nicotine challenges (sensitization tests, ST) were carried out after a 5-day withdrawal. Ambulation both escalated in response to repeated phencyclidine exposure during ACQ and was increased after phencyclidine challenge, evidencing development and expression of locomotor sensitization. Raclopride prevented phencyclidine-evoked development of sensitization. However, raclopride pretreatment during ACQ only partially inhibited its expression in phencyclidine-challenged mice. Nicotine failed to interfere with phencyclidine stimulatory effects during ACQ but potentiated raclopride inhibitory outcomes during the first ACQ days. During ST, nicotine history prevented the expression of phencyclidine-evoked sensitization. Nicotine challenge had no relevant impact on locomotion, which is consistent with a lack of nicotine/phencyclidine cross-sensitization. In conclusion, nicotine does not worsen, and may even ameliorate phencyclidine-sensitized psychotic-like behavior in adolescent mice. The potentiation of raclopride-mediated inhibition further suggests that nicotine might improve the therapeutic efficacy of medication on psychotic symptoms through mechanisms that converge on D_2 receptors.

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TOXIC EFFECTS AND OVERDOSE OF CARBAMAZEPINE AFTER PSYCHIATRIC CONDITIONS: POSTMORTEM ANALYSIS IN HUMAN BONE

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Javier Navarro-Zaragoza[1], Lucia Fernandez-Lopez[1], Pilar Almela[1], Manuela Pellegrini[2], Rosanna Mancini[2], Maria Falcon[3]
[1]University of Murcia, Department Of Pharmacology, Murcia, Spain, [2]National Centre on Drug Addiction and Doping, Istituto Superiore Di Sanità, Roma, Italy, [3]University of Murcia, Health And Social Sciences, Murcia, Spain

Carbamazepine is the main option to be used as a preventive medication to treat bipolar disorder when there is no response to lithium. One of the reasons why carbamazepine is not the first choice to treat bipolar disorder is the high rate of toxicity that this drug produces. According to the US Poison Control Center, nearly 2000 cases each year, only in the US. So, it is necessary a validated tool to determine carbamazepine levels in post-mortem studies to discard a suicide or to know if the death has been a consequence of an ADR Sometimes. blood and urine are not available so other specimens are submitted, i.e., stomach contents, bile, vitreous humour, liver and other tissues, muscle, or bone marrow. In these cases, it can be hard to test and interpret data as these matrices are not frequently analysed, and therefore there is a lack of literature and published results to compare with. The aim of this work was to analyse different post-mortem samples obtained from human ribs randomly arrived at our laboratory to find a correlation to concentration levels in blood using gas chromatography–mass spectrometry. The method was applied to bone samples from a forensic case where carbamazepine was found in blood and the level detected in bone was 46 ng/mg.

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TARGETED MODULATION OF MD-PFC THALAMOCORTICAL LOOPS – A NOVEL THERAPEUTIC STRATEGY TOWARDS THE TREATMENT OF SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Schizophrenia ranks within the top 15 leading causes of disability worldwide. Despite severe side effects and limited effectivity, antipsychotics from the 1950's continue to be the primary treatment option for the disorder. To develop much needed targeted therapies at par with modern medicine we need to isolate dysfunctions of discrete brain circuits that underlie different symptoms of schizophrenia. While prefrontal cortex dysfunction is well established in Schizophrenia, recent studies have shown that its interactions with the MD thalamus, is also perturbed. I have discovered genetically identifiable MD neurons that required for prefrontal control over flexible behavior. Specifically, one MD projection that targets disinhibitory (VIP+) PFC interneurons is required to amplify task relevant neural activity and maintain task performance; while another MD projection that targets inhibitory parvalbumin (PV+) neurons within the PFC suppresses task irrelevant activity for task switching. Thus, rational targeting of MD-PFC sub circuits with neurostimulation may result in targeted therapeutics for schizophrenia and is the central aim of this project. Here using virus-based circuit tracing, vivo electrophysiology and optogenetic manipulations I uncover specific anatomical and functional perturbations in MD-PFC sub circuits in a mouse model of Schizophrenia. Furthermore, this mouse model exhibits a specific deficit in prefrontal driven task switching which can be ameliorated with precise optogenetic manipulation of a MD-PFC sub-circuit. In conclusions, given the role of the MD as a bottleneck for information flow into the PFC, my work provides exciting evidence for the MD as a particularly attractive target for intervention in schizophrenia.

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EFFECT OF MANGIFERIN DURING ADOLESCENCE AND ADULTHOOD IN A RAT MODEL OF SCHIZOPHRENIA

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

Sonia Torres-Sanchez[1,2,3], Jose Antonio Garcia-Partida[2], Karina Macdowell[4,5,6,7], Maria Teresa Fernández-Ponce[8], Lourdes Casas[8], Maria Luisa Soto-Montenegro[9], Casimiro Mantell[8], Diego Romero-Miguel[9], Nicolás Lamanna-Rama[9], Juan Carlos Leza[5,6,7,10], Manuel Desco[11], Esther Berrocoso[1]
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There is evidence that in schizophrenia, imbalances in inflammatory and oxidative processes occur during pregnancy and in the early postnatal period, generating interest in the potential therapeutic efficacy of anti-inflammatory and antioxidant compounds. Mangiferin (MG) is a polyphenolic compound abundant in mango tree with robust antioxidant and anti-inflammatory properties, could be a candidate for treating schizophrenia. Hence, this study set-out to evaluate the effect of MG in a model of schizophrenia based on maternal immune activation by Poly I:C administration to pregnant rats. Young adult or adolescent male offspring received a MG extract daily, and the effects of MG in adolescence were compared to those of risperidone, assessing behavior, brain magnetic resonance images (MRI), and oxidative/inflammatory and antioxidant mediators in the adult offspring. MG treatment in adulthood reversed the deficit in prepulse inhibition (PPI) but it failed to attenuate the sensitivity to amphetamine and the deficit in novel object recognition (NOR) induced. By contrast, adolescent MG treatment prevented the sensorimotor gating deficit in the PPI test, producing an effect similar to that of risperidone. This MG treatment also produced a reduction in grooming behaviour and an anxiolytic-like effect, but it had no effect in the NOR test. MRI studies revealed that adolescent MG administration partially dampened the cortical shrinkage, and cerebellum and ventricle enlargement. In addition, MG administration in adolescence reduced iNOS mediated inflammatory activation and it promoted compensatory antioxidant activity in the prefrontal cortex and hippocampus, as witnessed through Keap1 reduction and NRF2, NQO1 and HO1 enhancement.

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MOSAIC CAS9 FUSIONS TO INVESTIGATE REGULATORY PHENOTYPES OF SCHIZOPHRENIA RISK GENES IN THE RODENT BRAIN

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Large-scale patient sequencing suggests that neuropsychiatric genetics often involve polygenic variants in regulatory regions of risk genes. These affect the timing and dosage of expression rather than the protein structure of gene products. In order to study the consequences of polygenic schizophrenia risk gene dysregulation in the rodent brain, we developed an in utero electroporation approach using Cas9 fusions to investigate novel phenotypes produced by changing expression dosage and timing of multiple risk genes. We targeted cell adhesion molecules of the immunoglobulin superfamily implicated in the risk for schizophrenia. We demonstrate that dosing multiple genes affects local development of the cerebral cortex. The broader aim is to establish a generalizable approach to investigate dysregulation of dosage and timing across risk genes in the developing brain to reveal impacts on cortical wiring across neurodevelopmental and neuropsychiatric conditions.

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A NOVEL PGX TEST SUPPORTING DRUG SELECTION FOR DISEASES OF THE CENTRAL NERVOUS SYSTEM.

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Background Pharmacogenomics studies the association between gene variations and drug responses. Here we present a novel pharmacogenetic panel (iDNA PGx CNS), providing clinically useful information to Psychiatrists and Neurologists for the treatment of diseases of the Central Nervous System (CNS). Materials For the construction of the pharmacogenetic panel, 24 SNPs on 13 genes were analyzed to optimize the selection of the treatment and support medical decisions for diseases of the CNS, by providing individualized pharmacogenetic information about the response, the efficacy of the treatment, and the ADEs of 31 drugs. A bioinformatics platform that contains an in-house database and a user interface, was built and used for the analysis of 1387 patient-derived samples from a southeastern European population. The samples were genotyped, using the TaqMan Drug Metabolism and Custom Genotyping Assays at the QuantStudio™ 12 K Flex Real-Time PCR System (Applied Biosystems by Life Technologies). Results Results reveal that the frequencies of each SNP in the genotyped samples are consistent with European population frequencies. The metabolic efficiency (poor, intermediate, extensive, ultra-rapid) of members of the CYP family and the frequency of clinical useful pharmacogenetic, associations in the population (drug relevance), are also presented. These results are indicating the strong potential of the PGX CNS panel as a companion diagnostic assay. Conclusion Overall, the results illustrate that the PGx-CNS panel is a valuable tool providing statistically accurate clinically relevant information, supporting therapeutic medical decisions, and highlighting its broad clinical implementation.

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STAGE DEPENDENT OSCILLATORY PATTERN ANALYSIS IN WISKET SCHIZOPHRENIA RAT MODEL

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Schizophrenia is defined as a severe neuropsychiatric condition with positive, negative symptoms and cognitive impairments. Patients with schizophrenia steadily report sleep disturbances which are contributed to cognitive or affective symptoms. The current study aimed to evaluate the oscillatory pattern of Wisket animals during awake, REM, and NREM sleep stages. Male, adult control, and Wisket rats were involved in the study. After chronic implantation of cortical electrodes, continuous electroencephalographic (EEG) registration was performed in freely moving condition. Automated stage discrimination was carried out using software developed in-house. The Wisket rats showed a clear light-dark cycle similar to controls, and their sleep-wake rhythm displayed only a tendency to spend more time in NREM and less in REM stage. In contrast, the analysis of the oscillatory pattern resulted in significant group differences in the relative power of different frequency bands by stages compared to controls,
accordingly:

Bands (Hz)	Awake		NREM		REM	
	↓	↑	↓	↑	↓	↑
Delta: 0.5–4			3 – 4	1.2 – 2	1.9 – 2.5	
Theta: 4–8	6.4 – 7.6		4.12– 5.6		6.5 – 7.5	
Alpha: 8–12		8.4 – 9.7				8.25 – 10
Beta: 12–30		12.1 – 19.3		12.9 – 17.5		12.1 – 15.7
Gamma: 30–48	44.2– 48					

In conclusion, the investigation of the oscillatory pattern by frequency bands revealed complex alterations in Wisket animals, which might be involved in the cognitive dysfunctions obtained in this schizophrenia animal model.

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BOARD NUMBER: S02-487

CANNABINOIDS MODULATE SOCIABILITY DEFICITS IN PSYCHOSIS THROUGH A CDK5-DEPENDENT MECHANISM

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Aims: Negative and cognitive symptoms, including social deficits, are associated with greater functional disability in first-episode psychosis (FEP) patients. We investigated the implication of cyclin-dependent kinase 5 (CDK5) and postsynaptic density protein 95 (PSD95) in these traits in a population of FEP with and without a history of cannabis use (FEP/c and FEP/nc, respectively). Methods: In a cross-sectional study, patients underwent clinical and neuropsychological evaluations before the extraction of olfactory neuroepithelium (ON) cells. Samples were then processed to quantify the expression of PSD95 and CDK5 via western blot. In a parallel dual-hit mouse model of psychosis (phencyclidine (PCP) and WIN 55,212-2 (WIN) administration during adolescence), we evaluated enduring social and cognitive alterations, and changes in PSD95 and CDK5 in the hippocampus and the prefrontal cortex. Results: Higher levels of CDK5 and lower levels of PSD95 in ON cells from FEP/nc correlated with fewer premorbid social skills. Contrastingly, FEP/c patients exhibited better social functioning than FEP/nc and the expression of CDK5 and PSD95 was normalized to control levels. We corroborated these findings in a mouse model of psychosis, where mice exposed to PCP alone showed more sociability deficits, higher CDK5 and lower PSD95 expression than mice exposed concomitantly to PCP and WIN. The blockade of CDK5 activity restored sociability impairments in PCP-treated mice. Conclusions: We provide translational evidence showing that CDK5 overexpression is linked to social deficits in psychosis, and that a history of cannabis use in FEP is associated with better social functioning and normal levels of CDK5 and PSD95.

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BOARD NUMBER: S02-488

THE HIPPOCAMPAL CA2 SUBREGION IN THE NMDA RECEPTOR HYPOFUNCTION PATHOLOGY OF PSYCHIATRIC DISORDERS

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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The *Cornu Ammonis* (CA) 2 subregion of the hippocampus has been linked to social memory, a higher brain function strongly impaired in patients with psychiatric disorders. Moreover, alterations in the balance of excitation and inhibition (E/I) of networks - key for the proper function of neuronal networks - have been described in CA2 of patients. These findings lead to the suggestion of a causal link between E/I balance in CA2, alterations upon disease and symptomatology. Nevertheless, there is a lack in the deeper understanding of molecular alterations in disease. We hypothesize that the E/I balance in CA2 is disrupted in mice displaying a subset of psychiatric symptoms. A common and well-known model showing cognitive deficits is the N-methyl-D-aspartate glutamate receptor (NMDAR) hypofunction model, induced by the treatment with a NMDAR antagonist. In order to probe our hypothesis, we performed immunohistochemistry on subchronic phencyclidine (scPCP) treated adult mice followed by high-throughput confocal imaging combined with automated multiparameter analysis. Exploring the expression levels of key proteins in CA2 and the intrahippocampal E/I balance in scPCP model broadened the understanding of molecular abnormalities following chronic NMDAR hypofunction. Furthermore, this study points out the importance of better understanding molecular alterations of models for drug discovery and translational research.

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DEEP BRAIN STIMULATION OF THE VENTRAL TEGMENTAL AREA TO CONTROL POSITIVE SYMPTOMS OF SCHIZOPHRENIA: A CASE REPORT.

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Background: Current treatments for schizophrenia modulate global neurotransmission through medication but are neither specific nor anatomically directed. Tailored stimulation of target nuclei could increase treatment efficacy while reducing side effects. **Aims:** To assess safety and clinical impact of deep brain stimulation (DBS) of the ventral tegmental area (VTA) on a patient with Treatment-Resistant Schizophrenia (TRS). **Methods:** In a registered clinical trial, one 41-year-old male with a 22-year history of schizophrenia underwent a bilateral DBS surgery targeting the VTA. The first stimulation trial period included eleven blind-random stimulation patterns to assess safety and potential adverse events. The best four parameters were selected for a second blind trial in outpatient settings during one month each, with a wash-out period of a week between every stimulation phase. The final stimulation pattern set at chronic bases was controlled by PET and DAT-Scans, and monthly clinical & neuropsychological double-blinded assessments. **Results:** After six months of ultimate stimulation pattern, the patient was clinically stable, maintained a reduction of PANSS-positive score in 58%, PANSS-negative score in 30%, and PANSS-GP score in 52%. During this period of DBS, the patient did not need further sessions of Electroconvulsive Therapy. The neuropsychological assessment showed a decreased performance in visual processing and impairments in memory retrieval. Some improvement in social cognition was also detected. In the following months, the patient had mood fluctuations, adding clozapine. **Conclusion:** DBS stimulation in VTA may work as an alternative for patients with TRS. Further studies are needed to see whether this brings even more benefits.

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BOARD NUMBER: S02-490

POPULATION-LEVEL LINKS BETWEEN SCHIZOPHRENIA AND THE GUT MICROBIOME

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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The human gut microbiome has received increased interest for its mediating role in neuropsychiatric disorders as an effect of the gut-brain-axis. Multiple studies proposed a link with schizophrenia. Therapeutic measures via the easily accessible, scalable, and cost-effective modulation of the gut microbiome could aid in the less-than-optimal treatment options currently available for schizophrenia. However, great inter-individual variability, small sample sizes and a confounding effect of antipsychotic medication on microbiota limitate studies on the relationship between the two and reproducibility across them. Links between the gut microbiome and schizophrenia were therefore assessed at a population level using epidemiological methods. Standard microbiome analyses were conducted on samples from two multidimensional large-scale datasets grouped according to their location and corresponding prevalence of schizophrenia. Additionally, a machine learning model was employed to determine whether samples could be accurately attributed to high or low prevalence groups based on their characteristics. Diversity differed significantly between samples from high and low prevalence locations. Several taxa were significantly enriched as well as differentially abundant between groups. The machine learning model determined the prevalence level with high accuracy. Multiple taxa implicated in the analyses were linked with bile acid metabolism, which might provide an interesting avenue for future research. However, effect sizes were relatively small and direct correlation was hard to establish, emphasising the need for greater data availability and consistent data collection methods. Still, these results present an additional strengthening of the proposed link between gut microbiome and schizophrenia, pointing to a potential avenue for therapeutic measures.

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THE EFFECT OF LIRAGLUTIDE ON STRATEGY-SHIFTING IN A SUBCHRONIC KETAMINE MODEL OF COGNITIVE DYSFUNCTION

POSTER SESSION 02 - SECTION: SCHIZOPHRENIA

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Cognitive impairment is an enduring feature of schizophrenia that remains untreated by current medications. To address this, preclinical tools used to measure cognitive impairment must be improved to screen novel drug candidates. In the current study, a novel dynamic strategy-shifting task (SST) was designed with automated shifts between a cue-light task, fixed location task, continuous detection task, and an auditory continuous detection task. The SST was used to examine the effects of the glucagon-like peptide-1 receptor agonist liraglutide on strategy-shifting. Liraglutide exerts neuroprotective actions within the brain and is a promising therapeutic candidate for improving cognitive flexibility impaired in individuals with schizophrenia. Sixteen Sprague-Dawley rats were trained to lever-press on the SST. After completing training, they were administered 30 mg/kg ketamine to disrupt strategy-shifting, or saline via intraperitoneal injection over 10 days. After a drug washout of 14 days and open-field testing, rats were administered either 0.4 mg/kg liraglutide or saline, 1 hour before testing on the SST (N=4 per group). This study shows that acute liraglutide administration does not improve strategy-shifting performance at any stage of the novel SST. Furthermore, subchronic ketamine did not consistently impair strategy-shifting performance as shown in similar tasks. Interestingly, rats given liraglutide and ketamine showed a perseverative strategy and were unable to shift away from the response required in the cue-light task when tested on the fixed location task. Importantly, animals showed rapid training times and limited omissions on the SST suggesting this task may be a promising preclinical tool to screen therapeutic candidates.

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BOARD NUMBER: S02-492

BRAIN AGE PREDICTION OF HEALTHY ADULTS BASED ON STRUCTURAL AND FUNCTIONAL CONNECTIVITY USING CONVOLUTIONAL NEURAL NETWORKS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Aims Brain age prediction can help characterize conditions that may affect the normal aging trajectory. In this work, we used functional and structural connectivity measures for brain age prediction using convolutional neural networks. **Methods** We used resting-state functional MR and diffusion-weighted imaging data from 619 healthy participants (18-89 yrs) included in the CamCAN cohort to construct functional and structural matrices based on functional correlation and structural strength (number of streamlines resulted from fiber tracking) between 1133 parcels. We further estimated three nodal graph metrics including degree (K), clustering coefficient (C), and local efficiency (E) from each connectivity matrix. A convolutional neural network (CNN) was then trained and evaluated using the 5-fold cross-validation strategy for the brain age prediction using connection strengths (S) and each of the nodal graph features. We further used principal component analysis (PCA) to reduce the dimensionality of features. **Results** Among the structural connectivity features, our method achieved the lowest mean absolute error (MAE) of 7.4 yrs using structural connection strengths (S) with 50 principal components (PCs) with a strong correlation ($\rho = 0.9$) in comparison with other features. The age prediction with the combined structural features (K, C, and E) also yielded an MAE of 7.6 yrs with 40 PCs. The CNN based on functional strengths (S) achieved age prediction errors higher than 9 yrs. **Conclusions** Our findings suggest higher predictive values for brain age prediction using structural connectivity features in comparison with the topological properties of resting-state functional networks.

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INTRODUCING STRUCTURAL DISCONNECTION MASKS IN WHOLE-BRAIN MODELS: A MECHANISTIC EXPLANATION OF STROKE PATIENTS' EFFECTIVE CONNECTIVITY

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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The understanding of the stroke lesions' consequences is limited, relying mostly on behavioral reports and descriptive information from neuroimaging techniques. Here we present a generative model that introduces a mechanistic explanation for these phenomena. The whole-brain model combines the structural and functional information, next to structural disconnection maps, by optimizing it with an Effective Connectivity. As a result, the prediction of behavioral responses got enhanced compared to previous models while the classification of the stroke behavior severity showed a higher accuracy compared to the most common techniques. These results, together with the proposed topological metrics, provided by the asymmetricity of the model, allow a more personalized treatment in order to achieve a better recovery of the patients. Furthermore, it opens the possibility to apply external manipulations such as neurostimulation in order to treat such a word-wide disease as stroke lesions.

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SYNCHRONIZATION IN THE CONNECTOME: METASTABLE OSCILLATORY MODES EMERGE FROM INTERACTIONS IN THE BRAIN SPACETIME NETWORK

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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The brain exhibits a rich repertoire of oscillatory patterns organized in space, time and frequency, detected from human brains with electro- and magnetoencephalography (EEG/MEG). However, the principles driving coherent oscillations and their link with neural activity remain unclear. Here, we propose that the transient emergence of brain rhythms is a signature of weakly stable synchronization between spatially distributed brain areas, occurring at network-specific collective frequencies due to non-negligible conduction times. We test this mechanism using a reduced network model representing interactions between neural mass potentials (resonating at 40Hz) in the Connectome. Despite the deliberate reduction, we find a critical regime where metastable oscillatory modes emerge spontaneously in the delta (0.5-4Hz), theta (4-8Hz), alpha (8-13Hz) and beta (13-30Hz) frequency bands from weak synchronization of different subsystems, approximating the MEG power spectra from heathy individuals. Grounded on the physics of delay-coupled oscillators, this work demonstrates the role of the Connectome spacetime structure in modulating brain activity in the frequency domain.

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THE CAUSAL STRUCTURE OF BAND-LIMITED CORTICAL DYNAMICS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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The instantaneous correlation of cortical activity reflects functional interactions between brain regions, but the directed, causal structure of these interactions remains unclear. To address this, we investigated the dynamics of human cortical activity using source-reconstructed magnetoencephalography (MEG) and by considering the time-antisymmetric cross-correlation of neural activity across different frequency bands and the full cortical space. We employed a tensor decomposition to identify components of this cross-correlation that are interpretable as causal patterns of lagged correlation across cortical regions and frequency bands. We identified several fundamental, causal patterns that were consistent across subjects, that were selectively modulated by cognitive tasks, and that were expressed across distinct frequency bands and wide-spread cortical networks. Our results provide new insights into the causal structure of large-scale human brain dynamics and open a new window to study these dynamics in the healthy and diseased brain.

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MACHINE LEARNING-BASED SUPPORT NETWORK EXTRACTION FOR NEURAL STATE CHARACTERIZATION

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Discriminative support network extraction is a relatively new technique which involves two principal steps. First, machine learning classifiers are employed to distinguish the classes, or neural states, of interest. Second, the most important features are extracted and visualized. This support network of important features serves as a signature of the classification and can be used, considering the feature set upon which the classifiers were trained, to characterize the neural states being analyzed.Though discriminative support networks have been used for neural state characterization in the past, a thorough analysis of the methods used for their extraction as well as their statistical relevance, remains to be performed. Absent such analyses, support networks remain difficult to interpret and require additional analyses to be performed post-extraction to establish their scientific relevance. Thus, we developed a discriminative support network extraction pipeline involving three classifiers and three feature importance algorithms in combination with statistical analyses to elucidate the statistical relevance of the extracted networks. Furthermore, we evaluated the techniques based on their computational complexity, classification accuracy, and replicability over repeated extractions. Multinomial logistic regression (MLR), k-nearest neighbors (KNN), 1D convolutional neural networks (CNN) were used to classify five neuro-motor states associated with a reach-to-grasp task. Subsequently, recursive feature elimination (RFE), beta regression coefficients, and perturbation-based feature importance algorithms were used to derive the support networks. CNN-based methods resulted in the highest classification accuracy though the derived support network exhibits lower replicability and statistical significance compared to the other techniques. MLR+RFE, however, resulted in the highest replicability and statistical relevance.

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RELIABLE DETECTION OF CLAUSTRUM CONNECTIONS INTO THE FOREBRAIN BY TRACTOGRAPHY OF TWO LARGE HUMAN SAMPLES

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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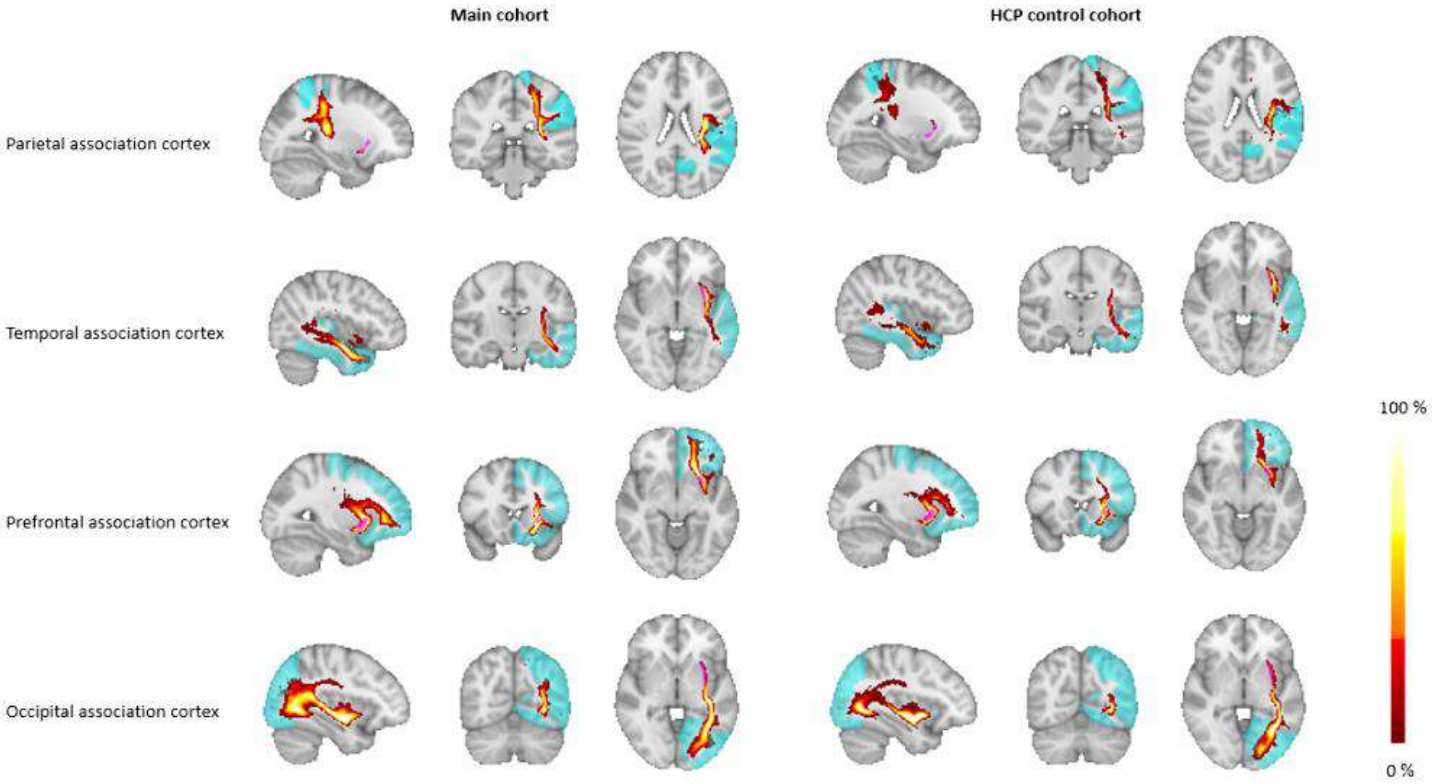
Aim: The claustrum, a thin layer of neurons located between the external and extreme capsule and below the insular cortex, is widely connected in the mammalian forebrain. However, despite numerous ex- and in-vivo studies in non-human mammals, investigations into the claustrum's connections in humans are few with small sample sizes. To address this problem, we performed diffusion imaging-based tractography of claustrum connections in two large human adult samples. **Methods:** We used diffusion imaging in a cohort of 81 healthy young adults and 60 subjects from the human connectome project (HCP) to assess in-vivo claustrum connectivity. The bilateral claustra were individually delineated using deep learning-based automated segmentation. Tracts between the claustrum and 13 cortical and 10 subcortical regions were reconstructed in each subject using probabilistic tractography. Probabilistic group average maps and connection density were generated to assess the claustrum's connectivity profile. **Results:** Robust streamline probability maps were found to most cortical areas as well as to a selection of subcortical areas, notably the pallidum and thalamus. We replicated these findings in the HCP cohort (see Figure 1 for some examples). Further evidence of connectivity to the contralateral hemisphere was also found. **Conclusion:** This study is the first to investigate the white matter connections of the claustrum to cortical and subcortical regions in large human samples. Our results demonstrate the reliable reconstruction of claustrum connectivity in humans and provide a base for further examinations of claustrum connectivity in health and in the context of different pathologies. Figure

1


Main cohort
HCP control cohort
Parietal association cortex
Temporal association cortex
Prefrontal association cortex
Occipital association cortex
100 %
0 %

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INFERRING BRAIN-WIDE CIRCUIT MODULES LINKING STRUCTURAL AND FUNCTIONAL CONNECTIVITY IN ZEBRAFISH LARVAE

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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How neuronal circuits support the processing of the sensory information and the coordination of an appropriate motor outcome is still an open challenge. In zebrafish larvae, optical methods allow for the reconstruction of the activity from several thousand neurons across the entire brain at once, while tracking the behavioral outcome, in response to a sensory stimulation. This kind of datasets provides an ideal workbench for the application of theoretical approaches aiming at generating plausible circuit models where to test hypotheses on the circuit mechanisms, to assess the network properties and its functional organization. This approach is also taking advantage from everyday-more-detailed reconstructions of the neuronal wiring diagrams and connectomes whose layouts offer a sort of structural blueprint underlying the circuit activity. However, it's not yet clear if and to what extent the patterns extracted from the connectome reconstruction can explain the observed functional information, and viceversa. Here, we address these aspects. We started with the generation of a structural connectome from the publicly available dataset of single cell reconstructions to characterize the network properties of the fish brain. An algorithm for graph analysis was implemented to reveal distinct circuit modules based on the relative connection strength between cells. We are currently comparing the structure of the identified modules and the activity recorded from neurons, both registered into the same anatomical space. This method could represent a valid approach for the analysis of the brain functioning and its underlying organization.

Pubmed:

32368416: Bruzzone M, Gatto E, Lucon Xiccato T, Dalla Valle L, Fontana CM, Meneghetti G, Bisazza A
Measuring recognition memory in zebrafish larvae: issues and limitations.
Recognition memory is the capacity to recognize previously encountered objects, events or places. This ability is crucial for many fitness-related activities, and it appears very early in the development of several species. In the laboratory, recognition memory is most often investigated using the novel object recognition test (NORt), which exploits the tendency of most vertebrates to explore novel objects over familiar ones. Despite that the use of larval zebrafish is rapidly increasing in research on brain, cognition and neuropathologies, it is unknown whether larvae possess recognition memory and whether the NORt can be used to assess it. Here, we tested a NOR procedure in zebrafish larvae of 7-, 14- and 21-days post-fertilization (dpf) to investigate when recognition memory first appears during ontogeny. Overall, we found that larvae explored a novel stimulus longer than a familiar one. This response was fully significant only for 14-dpf larvae. A control experiment evidenced that larvae become neophobic at 21-dpf, which may explain the poor performance at this age. The preference for the novel stimulus was also affected by the type of stimulus, being significant with tri-dimensional objects varying in shape and bi-dimensional geometrical figures but not with objects differing in colour. Further analyses suggest that lack of effect for objects with different colours was due to spontaneous preference for one colour. This study highlights the presence of recognition memory in zebrafish larvae but also revealed non-cognitive factors that may hinder the application of NORt paradigms in the early developmental stages of zebrafish.
PeerJ, 2020; 8
34040051: Bruzzone M, Chiarello E, Albanesi M, Miletto Petrazzini ME, Megighian A, Lodovichi C, Dal Maschio M
Whole brain functional recordings at cellular resolution in zebrafish larvae with 3D scanning multiphoton microscopy.
Optical recordings of neuronal activity at cellular resolution represent an invaluable tool to investigate brain mechanisms. Zebrafish larvae is one of the few model organisms where, using fluorescence-based reporters of the cell activity, it is possible to optically reconstruct the neuronal dynamics across the whole brain. Typically, leveraging the reduced light scattering, methods like lightsheet, structured illumination, and light-field microscopy use spatially extended excitation profiles to detect in parallel activity signals from multiple cells. Here, we present an alternative design for whole brain imaging based on sequential 3D point-scanning excitation. Our approach relies on a multiphoton microscope integrating an electrically

tunable lens. We first apply our approach, adopting the GCaMP6s activity reporter, to detect functional responses from retinal ganglion cells (RGC) arborization fields at different depths within the zebrafish larva midbrain. Then, in larvae expressing a nuclear localized GCaMP6s, we recorded whole brain activity with cellular resolution. Adopting a semi-automatic cell segmentation, this allowed reconstructing the activity from up to 52,000 individual neurons across the brain. In conclusion, this design can easily retrofit existing imaging systems and represents a compact, versatile and reliable tool to investigate neuronal activity across the larva brain at high resolution.
Sci Rep, 2021; 11
34755638: Gatto E, Bruzzone M, Lucon-Xiccato T
Innate visual discrimination abilities of zebrafish larvae.
The ability to discriminate between objects visually plays a key role in animals' interactions with their environment because it enables them to recognise companions, prey, and predators. In the zebrafish, Danio rerio, hatching occurs early on during development (48-72 h post fertilisation), and the larvae must forage and evade predators despite their immature sensory and cognitive systems. Using a preference paradigm, we investigated whether larval zebrafish are nonetheless capable of discriminating between visual stimuli. We found that larvae discriminated not only between figures with different colours or different shapes, but also between two identical figures with different orientations and between sets of figures with different numerosities. By manipulating larvae's exposure to objects before the test, we demonstrated that their discrimination abilities are innate and do not depend upon experience. This study highlighted that zebrafish possess relatively sophisticated visual discrimination abilities even at the larval stage. These abilities likely improve larval survival via the recognition of biologically relevant stimuli.
Behav Processes, 2021; 193

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BOARD NUMBER: S02-499

PARCELLATION AND CONNECTIVITY OF THE HUMAN SUPERIOR COLLICULUS.

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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The Superior Colliculus (SC) serves basic functions of everyday human life thanks to the unique architecture of its connections with almost the entire brain, which is well documented in other mammals. Here, we performed several analyses using data from the Human Connectome Project to parcellate and profile the connectivity of SC in the human brain. To this end, we first manually recorded the SC ROIs in MNI space and calculated probabilistic tractography in 200 subjects. Then, we clustered the SC ROI of each subject into 3 components and created a probabilistic map for each cluster among the participants. Finally, machine learning and searchlight approaches were applied to determine the specificity of the probabilistic map and the accuracy of connectivity among 3 clusters. The first cluster (Superficial Division - SUP) is located in the anterior and superior part of SC. Its connectivity is closely related to the visual cortices, such as the primary visual cortex (V1), LGN, Amygdala, and the MT + complex. The second cluster (Intermediate-Deep Division - INTDP) is located more ventrally in the posterior-dorsal part of the SC and its connectivity profile suggests preferential connectivity with auditory, somatosensory, and parietal cortices. The third cluster (Deep Division - DEEP) is located in the most ventral part of the SC and its main connectivity is with the pons, cerebellum, corticospinal tracts and contralateral SC. In addition, each cluster shows a specific functional activity that links SUP and INTDP to each other and in opposition to the DEEP compartment.

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BOARD NUMBER: S02-500

THE POTENTIAL OF VOLTAGE IMAGING FOR ACCURATE INFERENCE OF NEURON CONNECTIONS IN VIVO

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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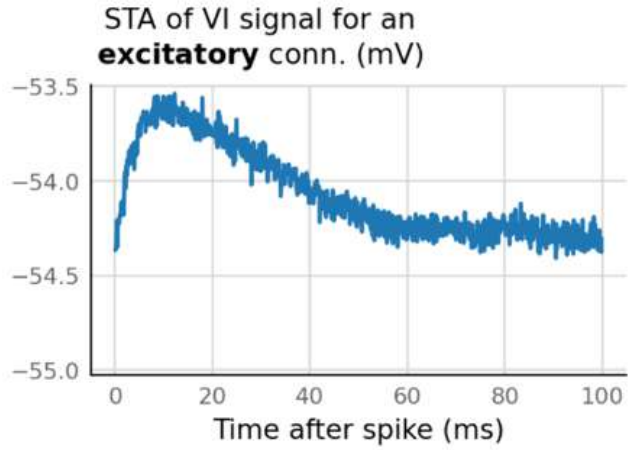
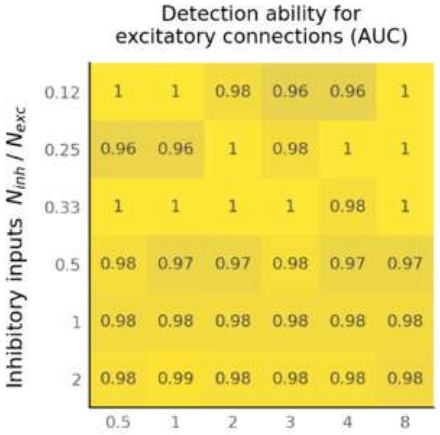
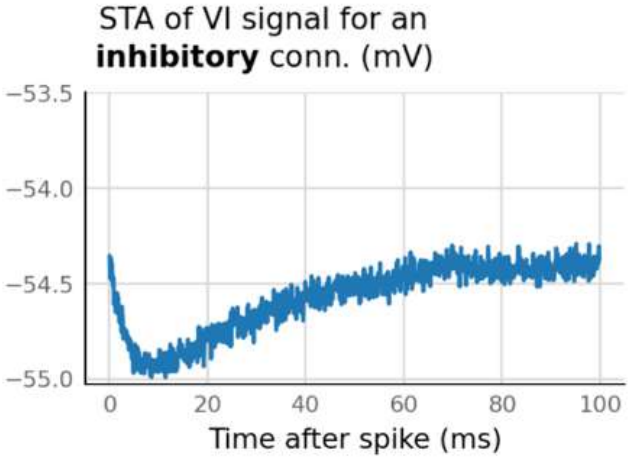
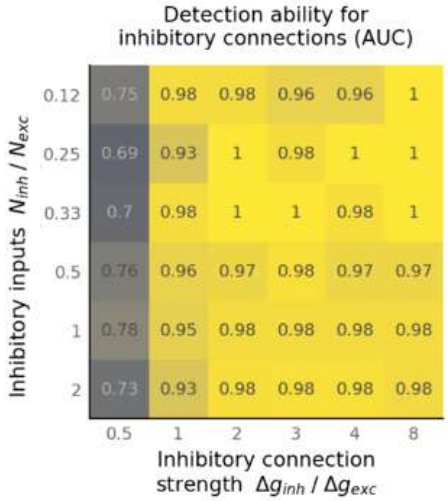
In the ideal systems neuroscience experiment, we would be able see the connections between neurons in living animals. We could then track how this wiring changes after an experimental intervention — or even over the animal's lifetime. Wire tracing from thin tissue slices is the gold standard for connectivity mapping, but cannot be done in vivo. Hence, we turn to *network inference* (**NI**) methods, based on in-vivo recordings of neural activity. These methods are typically spike-based, working from calcium imaging or extracellular electrode recordings. This makes network inference challenging, as spiking is sparse, and spike-based NI algorithms typically perform poorly. We propose to use *voltage imaging* (**VI**) recordings for network inference. Because such recordings can be sub-threshold, we can exploit a direct causal link between connectivity and activity: a successful spike will elicit a precisely-timed post-synaptic potential (PSP) in the downstream neuron's membrane voltage. We simulate VI data using a spiking model neuron, stimulated by Poisson inputs. We find that we can detect connections using spike-triggered averaging (STA) of PSPs alone. This simple method detects both excitatory and inhibitory connections with unusually high sensitivity and specificity, under realistic VI conditions (10-minute recording, 20 dB spike-SNR) — *iff* the input has a high spike rate. Based on these encouraging results, we plan to extend our experiment to full spiking neural networks. Our results suggest necessary voltage imaging conditions for network inference, and highlight the potential of this technique for accurate and large-scale *in-vivo* connectivity

mapping.


STA of VI signal for an
excitatory conn. (mV)
−53.5
−54.0
−54.5
−55.0
0
20
40
60
80
100
Time after spike (ms)


Detection ability for
excitatory connections (AUC)
Inhibitory inputs N_{inh} / N_{exc}
0.12 1 1 0.98 0.96 0.96 1
0.25 0.96 0.96 1 0.98 1 1
0.33 1 1 1 1 0.98 1
0.5 0.98 0.97 0.97 0.98 0.97 0.97
1 0.98 0.98 0.98 0.98 0.98 0.98
2 0.98 0.99 0.98 0.98 0.98 0.98
0.5 1 2 3 4 8


STA of VI signal for an
inhibitory conn. (mV)
−53.5
−54.0
−54.5
−55.0
0
20
40
60
80
100
Time after spike (ms)


Detection ability for
inhibitory connections (AUC)
Inhibitory inputs N_{inh} / N_{exc}
0.12 0.75 0.98 0.98 0.96 0.96 1
0.25 0.69 0.93 1 0.98 1 1
0.33 0.7 0.98 1 1 0.98 1
0.5 0.76 0.96 0.97 0.98 0.97 0.97
1 0.78 0.95 0.98 0.98 0.98 0.98
2 0.73 0.93 0.98 0.98 0.98 0.98
0.5 1 2 3 4 8
Inhibitory connection
strength Δg_{inh} / Δg_{exc}

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BAYESIAN INFERENCE OF SPIKE-TIMING-DEPENDENT PLASTICITY LEARNING RULES FROM DATA

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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The brain can be thought of as a network of neurons that interact through a complex system of connections. These connections are dynamic and develop over time according to unknown or partially unknown rules. Recent advances in recording technology have made large neural recordings common, but to date, there is no established method for inferring learning rules from them. We look at this from a statistical point of view and assume the system of connections can be modeled with a parameterized spike-timing-dependent plasticity learning rule. The goal is to infer the parameters from neural recordings, and we choose a Bayesian approach, with which we are able to get the uncertainty of the learning rule parameters as well as incorporate prior beliefs on their values. We use a Metropolis-Hastings sampling algorithm with particle filtering to obtain a posterior distribution of the parameters of the learning rule, which we can use to say something about the neural connections. We have tested the algorithm with both simulated and real data, and the results are promising.

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TRAVELLING WAVES IN THE FRONTAL LOBE OF PRIMATES DURING A COUNTERMANDING REACHING TASK.

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

Vasiliki Bougou[1], Giampiero Bardella[2], Franco Giarrocco[2], Emiliano Brunamonti[2], Michaël Vanhoyland[1], Alexander Bertrand[3], Pierpaolo Pani[2], Peter Janssen[1], Tom Theys[1], Stefano Ferraina[2]
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Increasingly evidence is emerging to support the organization of neural activity in travelling waves (TWs), which have been reported in the hippocampus, visual, auditory, motor, and somatosensory systems. Previous work suggested that β oscillations (18 – 30 Hz) over motor areas propagate as planar waves during movement planning and execution, but no studies reported on motor inhibition. We recorded neural activity from Utah arrays in the dorsal premotor cortex (PMd) of three rhesus monkeys and from the middle frontal gyrus of one epilepsy patient. All subjects performed a countermanding reaching task, where randomly presented Stop signals instructed to suppress the planned movement after the Go signal. We used a novel approach by fitting the linear equation of a planar wave to the instantaneous phases of the LFPs. Wave parameters (i.e., direction, speed) were then computed from the estimated coefficients of the fitted models. We detected TWs in a narrow frequency range of the β band (~ 20 Hz) during movement planning and, more interestingly, during movement inhibition. Wave parameters were comparable across subjects, species, and behavioural conditions, but during inhibition, the TWs exhibited greater intensity. With graph theory methods, the functional interactions between the phase of the TWs and the average local spiking activity (SA) were quantified. We found a relationship between the topological arrangement of local SA changes across behavioural outcomes and the wave parameters. Thus, motor decisions depend on the variations of the TWs parameters and intensity and on a topological reorganization of the premotor network.

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DYNAMIC MULTIPLEX NETWORK ANALYSIS OF HUMAN INTRACRANIAL RECORDINGS DURING POSTICTAL APHASIA

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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We analyse long intracranial EEG recordings of a few patients suffering from pharmaco-resistant epilepsy and postictal aphasia, whose language capabilities are tested after a seizure until their full recovery. We study the functional connectivity between different cortical sites in the time-frequency domain, to capture how the flexible dynamics of inter-regional interactions is affected by the seizure onset and recovery. Specifically, for each recording we compute the wavelet coherence, in 5 frequency bands peaked around 1 Hz, 5 Hz, 10 Hz, 20 Hz, 40 Hz, of each pair of electrodes at each time stamp, thus representing each recording as a time-varying, multilayer network (dynamic multiplex). Within each layer of the dynamic multiplex, we characterizee the flow between consecutive network frames, identifying and comparing different types of network dynamics depending on the task stage. We studied in particular: the rate of overall reconfiguration of links from one frame to the next (dynamic Functional Connectivity speed); and the flexibility/crystallisation of network modules through time, by means of a dynamic Allegiance matrix (dAm) analysis. We find different dynamics types corresponding to baseline, seizure and long post-seizure recovery phases. Importantly, we persistently recover a pathological state whose occurrence is correlated with the language-test fallacies reported by the physicians. This pathological state corresponds to momentary drops of the speed of network reconfiguration during the recovery phase. In conclusion, in our work we devise a data driven pipeline that can help both shed light on general mechanisms underlying postictal aphasia and address patient specific issues.

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OBJECTIVE AND EASILY PERFORMED ASSESSMENT OF FINE MOTOR SKILLS TO SUPPORT THE DIFFERENTIAL DIAGNOSIS OF PARKINSON'S DISEASE (PD) AND OTHER MOVEMENT DISORDERS WITH TREMOR

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Aim: The differential diagnosis between PD and other tremor disorders still poses a challenge, especially at the early stages of the disease. An objective assessment of the patients' tremor and motor skills is a valuable tool in clinical practice, assisting in the diagnosis of PD and other movement disorders with tremor. We aim to assess the ability of a handheld device and its accompanying data analytics to distinguish between tremor disorders. Methods: We assessed fine motor skills in 108 patients with movement disorders (68 PD, 40 non-PD patients). Data were collected while the patients were completing drawing and writing tasks on a tablet with a proprietary multi-sensor digital pen (NeuroMotor Pen) intended to measure and analyse neuromotor function. We extracted and analysed 123 features quantifying multiple symptoms of the disease. Results: The analysis revealed a subset of five features which serve as biomarkers for differential diagnosis between PD and non-PD patients. A classifier was built based on those five features, and it was tested on a separate set of 12 PD and 15 patients with other tremor disorders, achieving 85.2% accuracy (93.3% specificity, 75.0% sensitivity). Conclusions: The device shows potential in assisting in the differential diagnosis between PD and other tremor disorders. The accuracy level of the classifier is comparable to current diagnostic imaging practices (DaTSCAN). Clinical use of the device will enable cost and workflow benefits as well as patient benefits. It will also allow identifying early stage patients for clinical trials on medications.

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TOWARD A NEW DIMENSIONAL APPROACH TO ADDICTION: LINKING ADDICTION MARKERS TO THE CONNECTIVITY PROFILES OF STRIATUM SUBDIVISIONS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Aims: Examining a dimensional approach to addiction is essential for refining possible treatments and prevention strategies, particularly because there is significant heterogeneity of the disease across addiction to different substances. One promising avenue to inform this approach is to advance our understanding of large-scale variation in neurobiological circuits mediating addiction to different substances. In this new study, we aim at capitalizing on the natural variation in 1)latent behaviours related to different substances and 2)connectivity profiles of several subdivisions of the striatum in a large population extracted from the Human Connectome Project (HCP). Methods: In this study, we used factor analyses on the HCP questionnaires' answers from 564 individuals to extract latent behaviours relevant for addiction and impulsivity. We then explored variations in functional brain connectivity related to these behavioural factors. We first segmented the striatum according to anatomically and functionally defined landmarks. We also used 23 cortical and 13 subcortical regions that are anatomically or functionally known to be linked to the striatum to infer the Resting-State-Functional Connectivity (RSFC) of its subdivisions. Results: We found three addiction-related factors underlying general behavioural variation in (i) use of tobacco, (ii) alcohol and (iii) illicit drugs as well as one transdiagnostic factor defining (v) impulsivity. We postulate that distinct RSFC of the striatum are related to distinct substances abuse disorders. Conclusion: We hope that by building on the effectiveness of large-scale imaging and online data collected on a general population, we will provide a reliable link between anatomical relationships and markers of addiction.

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GENE CONNECTIVITY ANALYSIS OF CO-EXPRESSION NETWORKS PROVIDES INSIGHTS INTO THE OMNIGENIC MODEL AND IDENTIFIES NOVEL GENETIC HUBS OF SCHIZOPHRENIA RISK

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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The omnigenic model posits that polygenic risk arises from cumulative effects of many peripheral genes on “core genes” that mechanistically drive disease traits. However, understanding omnigenic architecture and identifying core genes in gene co-expression networks remains challenging. In co-expression networks derived from postmortem brain RNAseq parsed for age and region (LIBD, GTEx), we found that within gene sets enriched for schizophrenia (SCZ) risk – and not within others – the most central genes accumulated SCZ-GWAS signal. This suggests a definition of core genes as those most connected with SCZ-GWAS genes. This phenomenon was more prevalent in SCZ than Alzheimer’s disease or multiple sclerosis, and strongest in the developmental stage prefrontal cortex. We re-performed this analysis in simulations from variable numbers of random seeds (representing core genes) displaying either cis-heritability (only the seed genes had high importance), trans-heritability (the seed genes only conferred importance to the genes most connected to them), or cis-trans mixture. The simulations that most closely matched real SCZ networks had a number of seeds in the thousands, and a cis-trans importance mixture of 70%-30%. Finally, we prioritized candidate SCZ core genes as those most highly connected to SCZ-GWAS genes. In consensus across LIBD and GTEX networks, this procedure revealed cerebrum wide genes (across 12 networks), and tissues specific genes in the striatum (across 5 networks) and the hippocampus (across 3 networks). Our results revealed properties of the genetic architecture of SCZ, and leveraged omnigenic model predictions to identify targets to further assess disease risk pathways.

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RADIOMICS FEATURES CAN IDENTIFY LATERALITY IN MRI-NEGATIVE TEMPORAL LOBE EPILEPSY PATIENTS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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In this study, we hypothesized that radiomic features from MRI can predict the lateralization of the epileptogenic focus of the patients with MRI-negative Temporal lobe epilepsy(TLE). And we also assumed that the radiomic features of hippocampus and extrahippocampal regions, especially, would provide useful information in patients with hippocampal sclerosis (MRI-positive) and those with MRI-negative TLE. Participants for this study consisted of training set and validation set. The training set used to develop a radiomic model includes patients with MRI-positive TLE, and age- and sex-matched healthy controls (n=50). To validate the radiomic model, internal verification set consisted of patients with MRI-negative TLE. Radiology features for identifying lateralization were extracted from the hippocampus and extrahippocampal temporal region of the participant's affected and unaffected areas, respectively. Based on these features, an evaluation was conducted to predict the laterization of epileptogenic focus using supervised learning. Then, feature selection and modeling were performed. Radiomic features that can predict to identify laterization of the epileptogenic focus were extracted 48 from Hippocampus and 99 from Extrahippocampal regions. The models for predicting laterality in MRI-negative TLE showed an AUC of 0.67 and 0.90, respectively. In the results, radiomic features representing the difference between the affected and the unaffected side had a larger number of features in the extrahippocampal model than in the hippocampal model and better performance, which indicates that extrahippocampal model can more effectively predict epileptogenic focus on the affected side of patients with MRI-negative TLE. In conclusion, radiomic analysis could potentially help to identify MRI-negative TLE.

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PREDICTING INTELLIGENCE FROM FMRI DATA OF THE HUMAN BRAIN

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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In recent years, the prediction of individual behaviour from the fMRI-based functional connectome has become a major focus of research. The motivation behind this research is to find generalizable neuromarkers of cognitive functions. However, insufficient prediction accuracies and long scan time requirements are still unsolved issues. Here we propose a new machine learning algorithm for predicting intelligence scores of healthy human subjects from resting state (rsfMRI) or task-based fMRI (tfMRI). In a cohort of 390 unrelated test subjects of the Human Connectome Project, we found correlations between the observed and the predicted general intelligence of more than 50~percent in tfMRI, and of around 59~percent when results from two tasks are combined. Surprisingly, we found that the tfMRI data were significantly more predictive of intelligence than rsfMRI even though they were acquired at much shorter scan times (approximately 10~minutes versus 1~hour). Existing methods that we investigated in a benchmark comparison underperformed on tfMRI data and produced prediction accuracies well below our results. Our proposed algorithm differs from existing methods in that it achieves dimensionality reduction via ensemble learning and partial least squares regression rather than via brain parcellations or ICA decompositions. In addition, it introduces Ricci-Forman curvature as a novel type of edge weight. Reference: G. Lohmann, E. Lacosse, T. Ethofer, V.J. Kumar, K. Scheffler, J. Jost, Predicting intelligence from fMRI data of the human brain in a few minutes of scan time, biorxiv (2021), doi: https://doi.org/10.1101/2021.03.18.435935

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MULTI-CHANNEL AUTOMATED SPIKE SORTING ACHIEVES NEAR-HUMAN PERFORMANCE

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

Tali Remenick[1,2], Lidor Spivak[1,3], Amir Levi[1,3], Shirly Someck[1,3], Hadas Sloin[1,3], Roni Gattegno[1,3], Eran Stark[1,4]
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Identifying spike timing of individual neurons recorded simultaneously is essential for investigating neuronal circuits that underlie cognitive functions. Recent developments allow simultaneous recording of multi-channel extracellular spikes from hundreds of neurons. However, even state-of-the-art spike sorters require a substantial degree of manual curation, making the process time consuming and lacking in uniformity. Here, we developed a fully automated alternative to manual curation. As input to the automated curator (AC), we over-cluster spikes using standard software. From this point onwards we refer to manual curation as a sequence of two binary classification problems. To solve the first problem, we developed a “noise classifier” that uses adaptive boosting to remove non-neuronal clusters. To solve the second problem, we developed a “merge classifier” that uses a deep convolutional neural network to determine whether any given pair of non-noise clusters should be merged, resulting in a set of well-isolated units. To train the classifiers, we used multi-channel spike data recorded by high-density silicon probes from freely moving mice, curated manually by expert neurophysiologists. To evaluate AC performance, test data were manually curated by five human experts and by the novel AC. We quantified pairwise similarity between sorting solutions using the adjusted Rand index. We found that the AC reached a high similarity to human results, within inter-expert variance. Thus, the novel automated curator can replace human curation of spikes without compromising performance. Funding: ERC #679253, ISF #638/16, CIHR-IDRC-ISF #2558/18

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RECONSTRUCTING VOICE FROM FMRI USING DEEP NEURAL NETWORKS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

Charly Lamothe[1,2], Etienne Thoret[1,3,4], Stéphane Ayache[1], Régis Trapeau[2], Bruno L Giordano[2], Sylvain Takerkart[2], Thierry Artières[1,5], Pascal Belin[2,4,6]
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Recently, neural decoding has been used in conjunction with deep neural networks, leading to important advances in our understanding of higher-level visual cerebral representations, allowing the reconstruction of visual stimuli from fMRI patterns (van Gerven MAJ et al., 2019; Dado et al., 2020). We are interested in establishing a linear relationship between a deep-derived 'Voice latent space' and neural activity measured by fMRI. The goodness of fit in different cerebral regions will be probed via brain-based voice reconstruction. The main idea is to learn a deep auto-encoder to reconstruct spectrograms of human voice, then to learn a linear mapping from the encoding space of the autoencoder to the fMRI space, a strategy assumed to be more relevant for learning such a mapping between fMRI maps and sounds. Then, an fMRI acquisition of test stimuli is mapped to the autoencoder space and subsequently decoded as a spectrogram, enabling stimulus reconstruction. We developed and proposed a simple framework to perform brain voice decoding from fMRI patterns using DNNs. We applied our voice decoding method using different functionally-defined ROIs. We found that the decoded spectrograms only contained human voice when the ROI consisted of the fMRI-defined Temporal Voicer Areas. Ongoing analyses aim to further refine reconstruction quality and systematically test it as a function of anatomical cerebral location. The reconstruction obtained by the linear regression will allow us to better understand the functional representation of voice stimuli in the brain and potential analogies to the DNN-generated latent representations.

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A STOCHASTIC SIMULATION EXPERIMENT FOR A BETTER UNDERSTANDING OF UNCERTAINTY IN SELF-ORGANIZING MAPS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Self Organizing Maps (SOM) were developed by Kohonen, as a simplified abstract version of the Wilshaw-von der Malsburg modelling of the retina-cortex connection development. Statistically speaking, SOM is a clustering technique assigning observations from an original p-dimensional space, to a typically two-dimensional map, in such way that the distances between the corresponding points in the map reflect those in the original space. The original Kohonen algorithm is deterministic, but work also exists under a stochastic approach. In this paper, a computational experiment is performed to learn about the propagation of uncertainties along the SOM algorithm. We assume there exists, in the original dimensión of the data (p), a data generating process or probability distribution, which is transformed, through the (SOM) algorithm, into a stochastic process. This process is indexed by the iterations of the algorithm, so we formulate the estimation of a stochastic model in terms of the (estimation of) its (the process) limit distribution. Thus, two sources of uncertainty follow: the aforementioned SOM final weight variability and the one associated to the estimation of the parameters of the weight stochastic model. In our computational experiment, a large (Monte Carlo simulated) sample is generated from a mixture of tri-variate Gaussian distributions. We then divide the sample into subsamples of and apply the Kohonen algorithm to each subsample on a 2-D map, thus obtaining a sample of the final SOM weights, and subsequently estimating their statistical properties. Additionally, uncertainty in the distribution parameters is estimated by means of bootstrap resampling.

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STATISTICAL LIMITATIONS IN THE RECONSTRUCTION OF LOW-DIMENSIONAL NEURAL TRAJECTORIES FROM HIGH-DIMENSIONAL ACTIVITY RECORDINGS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Multi-electrode or calcium-imaging techniques now make possible to record the activity of hundreds or thousands of neurons in a brain area. A common assumption is that this high-dimensional activity is embedded on a low-dimensional manifold, and describes a trajectory informative about the ongoing e.g. sensory or motor computations. Principal component analysis, among other manifold-reconstruction methods, is routinely used to infer the relevant neural subspace and visualize the corresponding trajectories. An important issue is the reliability of this trajectory reconstruction process. The limited recording time, the size of the recorded population, the presence of dynamical neural noise, as well as intrinsic limitations of the recording methods, such as errors or biases in spike identification from voltage or fluorescence signals may considerably affect the reconstructed trajectories, and our understanding of the underlying computational processes. In our work, we present a systematic characterization of these effects on low-dimensional trajectory inference. We derive analytical expressions for the reconstruction error using the tools of the statistical physics of disordered systems, and derive phase diagrams locating regions in which the error is under control, or in which reconstruction is not possible. Our results are confirmed by extensive numerical simulations. We then show, on various existing recordings in the visual cortex, the hippocampus, and in the prefrontal cortex, how our results can be used to characterize the expected error on trajectory obtained from real datasets.

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SCALABLE NEURAL NETWORK-BASED PREDICTION OF NEURODEGENERATIVE DISEASES USING CLINICAL AND GENOMIC DATA FROM THE UK BIOBANK

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Predictive modeling with clinical patient data is anticipated to drive personalized medicine and improve healthcare quality. Constructing predictive statistical models typically requires extraction of curated predictor variables from normalized clinical data, a labor-intensive process that discards most of the information in each patient's record. Representation of health data using Fast Healthcare Interoperability Resources (FHIR) - an international standard for exchanging digital health data increasingly used in health information technology - should represent clinical data in a consistent, hierarchical, and extensible container format, regardless of the health system, which simplifies data interchange between sites. We propose a representation of participants from the UK Biobank entire raw clinical records based on the FHIR format, including molecular and genomic data, to demonstrate that deep learning methods using this representation are capable of accurately predicting multiple neurodegenerative diseases (e.g. Alzheimer's, Parkinson's) using integrative data from multiple data sources. We believe that this approach would allow to create accurate and scalable predictions for a variety of clinical scenarios, where molecular data can be supplemented to clinical patient data to improve prediction quality of neurodegenerative conditions and other ageing-related conditions.

Pubmed:

34635696: Zoabi Y, Kehat O, Lahav D, Weiss-Meilik A, Adler A, Shomron N
Predicting bloodstream infection outcome using machine learning.
Bloodstream infections (BSI) are a main cause of infectious disease morbidity and mortality worldwide. Early prediction of BSI patients at high risk of poor outcomes is important for earlier decision making and effective patient stratification. We developed electronic medical record-based machine learning models that predict patient outcomes of BSI. The area under the receiver-operating characteristics curve was 0.82 for a full featured inclusive model, and 0.81 for a compact model using only 25 features. Our models were trained using electronic medical records that include demographics, blood tests, and the medical and diagnosis history of 7889 hospitalized patients diagnosed with BSI. Among the implications of this work is implementation of the models as a basis for selective rapid microbiological identification, toward earlier administration of appropriate antibiotic therapy. Additionally, our models may help reduce the development of BSI and its associated adverse health outcomes and complications.
Sci Rep, 2021; 11
33398013: Zoabi Y, Deri-Rozov S, Shomron N
Machine learning-based prediction of COVID-19 diagnosis based on symptoms.
Effective screening of SARS-CoV-2 enables quick and efficient diagnosis of COVID-19 and can mitigate the burden on healthcare systems. Prediction models that combine several features to estimate the risk of infection have been developed. These aim to assist medical staff worldwide in triaging patients, especially in the context of limited healthcare resources. We established a machine-learning approach that trained on records from 51,831 tested individuals (of whom 4769 were confirmed to have COVID-19). The test set contained data from the subsequent week (47,401 tested individuals of whom 3624 were confirmed to have COVID-19). Our model predicted COVID-19 test results with high accuracy using only eight binary features: sex, age ≥60 years, known contact with an infected individual, and the appearance of five initial clinical symptoms. Overall, based on the nationwide data publicly reported by the Israeli Ministry of Health, we developed a model that detects COVID-19 cases by simple features accessed by asking basic questions. Our framework can be used, among other considerations, to prioritize testing for COVID-19 when testing resources are limited.
NPJ Digit Med, 2021; 4
34568830: Catav A, Fu B, Zoabi Y, Weiss-Meilik A, Shomron N, Ernst J, Sankararaman S, Gilad-Bachrach R
Marginal Contribution Feature Importance - an Axiomatic Approach for Explaining Data.
In recent years, methods were proposed for assigning feature importance scores to measure the contribution of individual

features. While in some cases the goal is to understand a specific model, in many cases the goal is to understand the contribution of certain properties (features) to a real-world phenomenon. Thus, a distinction has been made between feature importance scores that explain a and scores that explain the . When explaining the data, machine learning models are used as proxies in settings where conducting many real-world experiments is expensive or prohibited. While existing feature importance scores show great success in explaining models, we demonstrate their limitations when explaining the data, especially in the presence of correlations between features. Therefore, we develop a set of axioms to capture properties expected from a feature importance score when explaining data and prove that there exists only one score that satisfies all of them, the (MCI). We analyze the theoretical properties of this score function and demonstrate its merits empirically.
Proc Mach Learn Res, 2021; 139
33606253: Zoabi Y, Shomron N
Processing and Analysis of RNA-seq Data from Public Resources.
Advances in next generation sequencing (NGS) technologies resulted in a broad array of large-scale gene expression studies and an unprecedented volume of whole messenger RNA (mRNA) sequencing data, or the transcriptome (also known as RNA sequencing, or RNA-seq). These include the Genotype Tissue Expression project (GTEx) and The Cancer Genome Atlas (TCGA), among others. Here we cover some of the commonly used datasets, provide an overview on how to begin the analysis pipeline, and how to explore and interpret the data provided by these publicly available resources.
Methods Mol Biol, 2021; 2243
35015838: Sharon M, Vinogradov E, Argov CM, Lazarescu O, Zoabi Y, Hekselman I, Yeger-Lotem E
The differential activity of biological processes in tissues and cell subsets can illuminate disease-related processes and cell type identities.
The distinct functionalities of human tissues and cell types underlie complex phenotype-genotype relationships, yet often remain elusive. Harnessing the multitude of bulk and single-cell human transcriptomes while focusing on processes can help reveal these distinct functionalities.
Bioinformatics, 2022;
31960892: Basha O, Argov CM, Artzy R, Zoabi Y, Hekselman I, Alfandari L, Chalifa-Caspi V, Yeger-Lotem E
Differential network analysis of multiple human tissue interactomes highlights tissue-selective processes and genetic disorder genes.
Differential network analysis, designed to highlight network changes between conditions, is an important paradigm in network biology. However, differential network analysis methods have been typically designed to compare between two conditions and were rarely applied to multiple protein interaction networks (interactomes). Importantly, large-scale benchmarks for their evaluation have been lacking.
Bioinformatics, 2020; 36

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BOARD NUMBER: S02-514

ASSESSING THE INFLUENCE OF LOCAL NEURAL ACTIVITY ON GLOBAL CONNECTIVITY FLUCTUATIONS: APPLICATION TO HUMAN INTRACRANIAL EEG DURING A COGNITIVE TASK

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Aims. Cognitive-relevant information is processed by different brain areas that cooperate to eventually produce a response. The relationship between local activity and global brain states during such processes, however, remains for the most part unexplored. **Methods**. To address this question, we designed a simple face-recognition task performed in drug-resistant epileptic patients with intracranial EEG. Based on our observations, we developed a novel analytical framework (named “local-global” framework) to statistically correlate the brain activity in every recorded gray-matter region with the widespread connectivity functions as proxy to assess the level of influence of local neural activations into the brain’s global state during cognition. **Results**. The application of our analysis to the data from two subjects was able to detect the local gamma activity in task-relevant brain areas including the primary visual and motor cortices. Despite substantial differences in the recorded regions of each subject, the connectivity functions (functional connectivity derived from Pearson correlation and phase-locking value) consistently showed a significant global decrease around alpha and beta bands occurring a few hundred milliseconds after the stimulus onset. In this context, the local-global framework revealed that the reported desynchronization was better explained by the local activity of brain areas involved in face information processing, providing evidence that the global measures might be a novel signature of functional brain activity reorganization taking place when a stimulus is processed in a task context. **Conclusion**. Overall, the findings of this study suggest that task-driven connectivity fluctuations might be interpreted of relevant local neural activity.

Pubmed:
28433856: Vila-Vidal M, Principe A, Ley M, Deco G, Tauste Campo A, Rocamora R
Detection of recurrent activation patterns across focal seizures: Application to seizure onset zone identification.
We introduce a method that quantifies the consistent involvement of intracranially monitored regions in recurrent focal seizures.
Clin Neurophysiol, 2017; 128
29620007: Quevedo-Diaz M, Campo AT, Vila-Vidal M, Principe A, Ley M, Rocamora R
Ictal spitting in non-dominant temporal lobe epilepsy: an anatomo-electrophysiological correlation.
We report a patient presenting drug-resistant, non-dominant temporal lobe epilepsy with ictal spitting and prosopometamorphopsia, both extremely rare semiologies. Second-phase pre-surgical monitoring was performed using SEEG due to lesion-negative imaging and the rare semiology. The seizure onset zone was delimited to the right anterior hippocampus and the temporobasal cortex, with the propagation zone within the entorhinal cortex. Interestingly, direct electrical stimulation to the entorhinal cortex, which was reproduced in a number of trials, evoked spitting without leading to seizures or post-discharges. After the resection of the epileptogenic zone, the patient remained seizure-free without AEDs for a follow-up period of five years (Engel Class 1a). The neuropathology revealed a focal cortical dysplasia type FCD-Ia. Spectral analysis of intracranial ictal EEG (iEEG) data suggested a possible role of the basal temporal and entorhinal cortex as a necessary node in ictal spitting. [Published with video sequences on www.epilepticdisorders.com].
Epileptic Disord, 2018; 20
31706923: Vila-Vidal M, Capouskova K, Atasoy S, Kringelbach ML, Deco G
Uncovering the spatiotemporal scales of common neuro-mental constructs: Comment on "Is temporo-spatial dynamics the 'common currency' of brain and mind? In Quest of 'Spatiotemporal Neuroscience'" by Georg Northoff et al.
Phys Life Rev, 2020; 33

32052354: Clemente A, Vila-Vidal M, Pearce MT, Aguiló G, Corradi G, Nadal M
A Set of 200 Musical Stimuli Varying in Balance, Contour, Symmetry, and Complexity: Behavioral and Computational Assessments.
We present a novel set of 200 Western tonal musical stimuli (MUST) to be used in research on perception and appreciation of music. It consists of four subsets of 50 stimuli varying in balance, contour, symmetry, or complexity. All are 4 s long and designed to be musically appealing and experimentally controlled. We assessed them behaviorally and computationally. The behavioral assessment (Study 1) aimed to determine whether musically untrained participants could identify variations in each attribute. Forty-three participants rated the stimuli in each subset on the corresponding attribute. We found that inter-rater reliability was high and that the ratings mirrored the design features well. Participants' ratings also served to create an abridged set of 24 stimuli per subset. The computational assessment (Study 2) required the development of a specific battery of computational measures describing the structural properties of each stimulus. We distilled nonredundant composite measures for each attribute and examined whether they predicted participants' ratings. Our results show that the composite measures indeed predicted participants' ratings. Moreover, the composite complexity measure predicted complexity ratings as well as existing models of musical complexity. We conclude that the four subsets are suitable for use in studies that require presenting participants with short musical motifs varying in balance, contour, symmetry, or complexity, and that the stimuli and the computational measures are valuable resources for research in music psychology, empirical aesthetics, music information retrieval, and musicology. The MUST set and MATLAB toolbox codifying the computational measures are freely available at osf.io/bfxz7.
Behav Res Methods, 2020; 52
31785422: Vila-Vidal M, Pérez Enríquez C, Principe A, Rocamora R, Deco G, Tauste Campo A
Low entropy map of brain oscillatory activity identifies spatially localized events: A new method for automated epilepsy focus prediction.
The spatial mapping of localized events in brain activity critically depends on the correct identification of the pattern signatures associated with those events. For instance, in the context of epilepsy research, a number of different electrophysiological patterns have been associated with epileptogenic activity. Motivated by the need to define automated seizure focus detectors, we propose a novel data-driven algorithm for the spatial identification of localized events that is based on the following rationale: the distribution of emerging oscillations during confined events across all recording sites is highly non-uniform and can be mapped using a spatial entropy function. By applying this principle to EEG recording obtained from 67 distinct seizure epochs, our method successfully identified the seizure focus on a group of ten drug-resistant temporal lobe epilepsy patients (average sensitivity: 0.94, average specificity: 0.90) together with its characteristic electrophysiological pattern signature. Cross-validation of the method outputs with postresective information revealed the consistency of our findings in long follow-up seizure-free patients. Overall, our methodology provides a reliable computational procedure that might be used as in both experimental and clinical domains to identify the neural populations undergoing an emerging functional or pathological transition.
Neuroimage, 2020; 208

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BOARD NUMBER: S02-515

FROM NEURAL CORRELATIONS TO POPULATION CODES AND BACK- UNDERSTANDING A DUALITY IN THE TOPOLOGICAL ANALYSIS OF NEURAL DATA

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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There are two distinct but complementary ways to approach the analysis of recorded neural spikes. The first one is to analyse the correlation structure between neurons, while the second is concerned with understanding the population vector or *state space* representation. As an example, consider electrophysiological recordings from head direction neurons with single-peaked receptive fields. The circular shape of possible head directions can in principle be recovered both in the correlation structure of the neurons and in the space of observed population vectors. While in this simple example, the two approaches provide equivalent descriptions, they differ in general, for example if one of the head direction neurons has multiple fields. A natural question is then how to relate and reconcile the two views. We try to give an answer by using topological data analysis - the science of shapes in datasets. This is motivated by many recent studies that showcase the relevance of shapes for understanding the brain. Using the principle of Dowker duality we provide a theoretical framework for their comparison and discuss implications for the analysis and interpretation of neural data.

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THE ROLE OF EXCITATORY-INHIBITORY HOMEOSTASIS IN THE RECOVERY OF FUNCTIONAL CONNECTIVITY AFTER FOCAL LESION – A COMPUTATIONAL ACCOUNT

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Stroke-related disruptions in functional connectivity (FC) often spread beyond lesioned areas. Thus, it is unclear how the recovery of FC can be orchestrated on a global scale. Given that post-stroke recovery is accompanied by long-term increases in excitability, we propose excitatory-inhibitory homeostasis as a mechanism of recovery and aim to model its role in restoring FC properties, relating it to observed changes in excitability. In this study, we modelled stroke in connectome-based networks of Wilson-Cowan masses with homeostatic scaling of local inhibition. Activity was recorded pre-lesion (T0), post-lesion (T1) and post-lesion after stabilization of inhibitory weights (T2). Effects were quantified through changes in FC and local inhibitory weights were used as a proxy for excitability. Results showed decreased interhemispheric connectivity at T1, strongly correlated with lesion severity, and further recovered towards baseline levels at T2, independently of lesion strength. The similarity of FC with baseline was improved from T1 to T2 and, similarly to interhemispheric connectivity, it correlated with lesion severity at T1, but not at T2, revealing independence between recovery and lesion severity. We further observed long-term increases in local excitability, correlated with structural connectivity to lesioned areas, with severe lesions requiring larger and more widespread changes. Thus, our model not only replicated acute effects of stroke in FC but also showed an ability to recover it towards pre-stroke levels through the regulation of local excitability. Therefore, we present excitatory-inhibitory homeostasis as a key driver of stroke recovery, tying long-term changes in local excitability to the restoration of FC.

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BOARD NUMBER: S02-517

LESION-NETWORK MAPPING FOR POST-STROKE PREDICTION OF MOTOR AND ACTION SPEED DEFICITS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Aims Stroke lesions can cause structural disconnection leading to network disruption and cognitive deficits. Our study aims at modeling the association between lesion-derived structural network disconnections and motor-action speed deficits. **Methods** We applied the lesion-network mapping to MR images from 93 stroke patients (right-handed, 40-80 yrs) with lesions in the left corticospinal tract to predict motor and action speed deficits assessed by the standardized GRECogVASC battery of clinical tests at six-month post-injury. For each lesion, a probability disconnection map was first generated after removing white matter (WM) pathways passing through the lesion from the tractography (diffusion-weighted imaging) profile of 403 healthy participants (40-80 yrs) in the Human Connectome Project. *A voxel-wise multiple regression analysis (p<0.01 FDR corrected) was then* carried out with lesion binary masks-disconnection maps (dependent variables) and motor-action speed scores (independent variables). **Results** The statistical maps of lesions showed a voxel cluster in the left corticospinal tract (-26,-7,16), significantly associated with motor deficits. No association was found between the lesion location and action speed deficits using the voxelwise lesion-symptom mapping (VLSM). The lesion-network mapping, however, showed that WM disconnections in the left corticospinal and internal capsule were significantly correlated with motor deficits. The action speed deficit was significantly correlated with disconnected WM pathways along the right corticospinal tract and internal capsule between supplementary motor areas, inferior frontal gyrus, thalamus, caudate, and putamen. **Conclusions** Compared to VLSM, our findings revealed that the lesion-network mapping could better identify regions impacted by network disruption causing motor-action speed deficits.

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THE FUNCTIONAL NETWORK STRUCTURE OF LEXICAL DECISION-MAKING

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Abstract The Functional Network Structure of Lexical Decision-Making Decision-making is arguably an important human cognitive function and an area of research prioritisation. Mapping anatomical cortical regions, including structural and functional networks in decision-making is yet to be achieved in detail. Exposing these neuronal circuits will improve understanding of the underlying structure and functional properties involved in lexical decision-making processes. **Aims:** Further analysing an existing lexical decision-making dataset (Dufau et al., 2015) to establish specific network structural properties in healthy adults, to increase understanding of the complexities of human decision-making. **Methods:** Brain graph theory was applied to explore structural properties. Binary and weighted matrices were constructed, whereby nodes = electrodes and edges exist where ERP amplitude is r >0.7 at 280ms post-stimulus, and nodes = electrode and weighted edges = correlation co-efficient of ERP amplitude at 280ms post-stimulus, respectively. **Results:** Findings revealed a gradient in average degree from anterior to posterior frontal regions in both the binary (10→14.5→18) and weighted (15.34→18.52→20.45) networks. Results also showed greater average degree in frontal regions in both binary and weighted networks (weighted=18.36 [average = 16.95], binary = 14.7 [average = 11.44]) and a shorter than average path length in frontal regions in the binary network (1.09 [average 1.72]). **Conclusions:** Findings indicate greater connectivity and efficiency of prefrontal compared to other cortical networks in lexical decision-making. Additionally, these findings align with anatomical evidence of connectivity gradient from anterior-posterior in the prefrontal cortex, forming an important basis on which to build future developments in the understanding of decision-making networks.

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USING WHOLE-BRAIN MODELS TO IDENTIFY CRITICAL NETWORKS IN EARLY PSYCHOSIS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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[1]Universitat Pompeu Fabra, Centre For Brain And Cognition, Barcelona, Spain, [2]Universitat Pompeu Fabra, Center For Brain And Cognition, Barcelona, Spain, [3]Lausanne University Hospital and University of Lausanne, Connectomics Lab, Department Of Radiology, Lausanne, Switzerland, [4]Lausanne University Hospital, Centre For Psychiatric Neuroscience, Department Of Psychiatry, Lausanne, Switzerland, [5]Center for Psychiatric Neuroscience, Lausanne University Hospital (CHUV), Department Of Psychiatry, Lausanne, Switzerland, [6]Universitat Pompeu Fabra, Department Of Technology And Information, Barcelona, Spain

Aims: Psychotic disorders are characterized by heterogeneity in etiopathology, clinical presentation and individual trajectory, complicating diagnosis and treatment. Despite the increasing amount of studies investigating significant alterations, the mechanisms underlying the emergence and progression of these disorders remains unclear. It is therefore fundamental to improve our ability to differentiate between clinical subgroups and to move towards a more personalised approach. Here, we aim to highlight critical neural correlates in early stages of psychosis, and to identify relevant biomarkers correlating with clinical staging. **Methods:** In this study, we included resting-state fMRI and DTI data from a cohort of 129 healthy controls and 94 patients with early psychosis stratified into four distinct groups based on the severity of their condition and their ability to recover after the first episode. We used a whole-brain model that combines functional dynamics and anatomical structure to fit the empirical data and to extract global and local properties of the brain network. **Results:** This model allowed us to highlight significant differences between subgroups and to identify critical networks involved in damaging mechanisms relevant for emergence of the disease as well as in potential compensatory mechanisms involved in the recovery process. In particular, the disruption of local dynamical properties in areas previously identified as hubs of connectivity emerged as a critical biomarker of progression of disease. **Conclusions:** These preliminary results allow us to progress in understanding of the mechanisms underlying the emergence and the disruption progression of psychosis, and could open the way for possible future therapeutic applications.

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INFORMATION PROCESSING IN SUBCLINICAL PSYCHOSIS: INCREASED PRECISION OF SENSORY EVIDENCE IN PERCEPTUAL INFERENCE ASSOCIATED WITH HALLUCINATION- AND DELUSION-LIKE EXPERIENCES.

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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AIMS We aim to identify neurocomputational patterns possibly involved in hallucinations- and delusion-like experiences. In predictive coding, the precision (inverse of variance) of neural signals quantifies the noise in the information source. Psychosis is hypothesised to emerge from overestimating the precision of prior beliefs or sensory evidence. Evidence from the Beads Task (BT) (cognitive inference) shows delusion-like experiences predict impulsive sampling and increased sensory precision. It is unclear whether these patterns feature also in perceptual inference. We investigated this question evaluating how psychotic-like experiences affect performance in the Random Dot Motion task (RDM). METHODS We fitted RDM data (N=191) to hierarchical drift-diffusion models that allowed drift-rate (proxy of precision of sensory evidence) and decision-threshold (proxy of impulsive sampling) parameters to vary: 1) between groups with higher vs. lower scores of subclinical psychotic symptoms; 2) as response variables of a regression model having subclinical psychotic symptoms as predictors. We also attempted to replicate impulsivity associated with delusion-like symptomatology in BT. RESULTS In RDM, both delusion- and hallucination-like experiences were associated with higher precision of sensory evidence (higher drift-rates). Hallucination-like experiences were also associated with lower decision-thresholds. In BT, we did not find impulsive sampling associated with psychotic-like experiences. CONCLUSIONS Our findings suggest increased precision of sensory evidence in perceptual inference may represent a candidate neurocomputational mechanism contributing to subclinical psychotic traits. Significantly, they indicate a link between perceptual mechanisms and abnormal beliefs, hinting that alterations of perceptual processing could precede and contribute to changes in the belief system.

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COMPUTATIONAL PSYCHIATRY: DATA DRIVEN IN SILICO MODELS FOR SCHIZOPHRENIA AND BI-POLAR DISORDER.

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Mental disorders are among the leading causes of morbidity globally, and some of the costliest disorders to affect humanity. Identifying the underlying pathophysiology is an imperative step to unlocking better treatment and prevention strategies, and can lead to major health benefits. Mental disorders are complex, combining biological and psychosocial mechanisms, and hence a multidisciplinary approach is necessary to generate new knowledge about the pathology of severe mental disorders. Based on integrated statistical genetics, brain imaging, and electrophysiological measurements on patient-derived brain organoids, we present a framework for building computational models for mental phenotypes that are relevant in Schizophrenia and Bipolar Disorder.

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MODELING THE CONTEXT-DEPENDENT EFFECTS OF CHOLINERGIC NEUROMODULATION ON FUNCTIONAL NETWORK TOPOLOGY

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Integration and segregation are recognized as two fundamental principles of brain organization. The brain manage the transitions between different functional states, more segregated or integrated, through neuromodulatory systems. Recently computational and experimental works suggest a pro-integration (segregation) role of noradrenergic (cholinergic) neuromodulation. Here, we studied the effects of the cholinergic system on brain functional connectivity using both empirical fMRI data and computational modeling. The model, informed with neuroimaging data, was used to find causal mechanisms about the effects of cholinergic system on brain dynamics. We first analyzed the effects of nicotine on functional connectivity and network topology in resting-state conditions and during an attentional task. Then, we built a whole-brain neural mass model embedded into a human connectome for simulating the effects of nicotine. The drug was modeled decreasing both the global coupling and local feedback inhibition parameters of the model. We found that nicotine incremented functional segregation in both empirical and simulated data, and the effects are context-dependent: observed during task, but not in resting-state. In-task performance correlates with functional segregation, finding a link between functional network topology and behavior. Also, we observed that the particular regional density of the nicotinic acetylcholine α4β2 receptor is meaningful to explain the effects of nicotine at the whole-brain level. Our results suggest that cholinergic neuromodulation promotes functional segregation in a context-dependent fashion, propose biophysical mechanisms to simulate cholinergic neuromodulation, and explain how functional segregation could be desirable in visual-attentional tasks.

Pubmed:
34335217: Coronel-Oliveros C, Castro S, Cofré R, Orio P
Structural Features of the Human Connectome That Facilitate the Switching of Brain Dynamics via Noradrenergic Neuromodulation.
The structural connectivity of human brain allows the coexistence of segregated and integrated states of activity. Neuromodulatory systems facilitate the transition between these functional states and recent computational studies have shown how an interplay between the noradrenergic and cholinergic systems define these transitions. However, there is still much to be known about the interaction between the structural connectivity and the effect of neuromodulation, and to what extent the connectome facilitates dynamic transitions. In this work, we use a whole brain model, based on the Jasen and Rit equations plus a human structural connectivity matrix, to find out which structural features of the human connectome network define the optimal neuromodulatory effects. We simulated the effect of the noradrenergic system as changes in filter gain, and studied its effects related to the global-, local-, and meso-scale features of the connectome. At the global-scale, we found that the ability of the network of transiting through a variety of dynamical states is disrupted by randomization of the connection weights. By simulating neuromodulation of partial subsets of nodes, we found that transitions between integrated and segregated states are more easily achieved when targeting nodes with greater connection strengths-local feature-or belonging to the rich club-meso-scale feature. Overall, our findings clarify how the network spatial features, at different levels, interact with neuromodulation to facilitate the switching between segregated and integrated brain states and to sustain a richer brain dynamics.
Front Comput Neurosci, 2021; 15
33600402: Coronel-Oliveros C, Cofré R, Orio P

Cholinergic neuromodulation of inhibitory interneurons facilitates functional integration in whole-brain models.
Segregation and integration are two fundamental principles of brain structural and functional organization. Neuroimaging studies have shown that the brain transits between different functionally segregated and integrated states, and neuromodulatory systems have been proposed as key to facilitate these transitions. Although whole-brain computational models have reproduced this neuromodulatory effect, the role of local inhibitory circuits and their cholinergic modulation has not been studied. In this article, we consider a Jansen & Rit whole-brain model in a network interconnected using a human connectome, and study the influence of the cholinergic and noradrenergic neuromodulatory systems on the segregation/integration balance. In our model, we introduce a local inhibitory feedback as a plausible biophysical mechanism that enables the integration of whole-brain activity, and that interacts with the other neuromodulatory influences to facilitate the transition between different functional segregation/integration regimes in the brain.
PLoS Comput Biol, 2021; 17
29171010: Coronel-Oliveros CM, Pacheco-Calderón R
Prenatal exposure to ketamine in rats: Implications on animal models of schizophrenia.
Schizophrenia is a complex neuropsychiatric disorder characterized by hallucinations, delusions, anhedonia, flat affect and cognitive impairments. The aim of this study was to propose a prenatal treatment with ketamine, a psychedelic drug that acts as a non-competitive inhibitor of glutamate NMDA receptors, as a neurodevelopmental animal model of schizophrenia. The drug was applied (i.m. 60 mg.kg h) in pregnant Sprague-Dawley rats on gestational Day 14. Offspring behavior was studied on pubertal (4 weeks old) and adult (10 weeks old) stages. Also, hippocampal CA1-CA3 morphology was assessed in adult animals through a Nissl stain. Results showed a disinhibition and hyperactive behavior in pubertal animals exposed to ketamine, followed in adulthood with cognitive impairments, social withdrawal, anxiety, depression, and aggressive-like behaviors. In the hippocampus, a reduction of the CA3 layer thickness was observed, without changes in cell density. These results strongly suggest a robust link between prenatal pharmacologic manipulation of NMDA receptors and schizophrenia.
Dev Psychobiol, 2018; 60

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BOARD NUMBER: S02-523

EEG SPATIAL PATTERNS RELATED TO RHYTHM PROCESSING ACCURATELY CLASSIFY DYSLEXIA AND SHOW TRANSFER LEARNING CAPABILITIES ACROSS RECEPTIVE SPEECH TASKS.

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Slow cortical oscillations play a crucial role in processing the speech envelope, which is perceived atypically by children with developmental dyslexia. Here, we use EEG and natural speech listening paradigms to identify neural speech processing patterns that characterise and classify dyslexic children. Using a powerful linear method to derive supervised EEG spatial filters (CSP), we were able to accurately classify dyslexic and typically-developing children during a story listening task. Delta-band oscillations were particularly discriminative. Furthermore, accurate classifications for this story listening dataset could be derived from oscillatory spatial patterns from another EEG dataset, in which typically-developing and dyslexic children attended to rhythmic speech - the repetition of the syllable (“ba”) presented isochronously at 2Hz. The data demonstrate the feasibility of cross-dataset transfer learning of CSP features and the role of spatially-filtered delta-band oscillations in classifying dyslexia across very different speech tasks. This cross-dataset transfer may be due to the role of delta-band modulations in speech rhythm processing – a mechanism that is known to be atypical in dyslexia.

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BOARD NUMBER: S02-524

A STEREOTACTIC ATLAS BASED 3D MODEL OF MR GUIDED FOCUSED ULTRASOUND THALAMOTOMY TARGETS.

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

Nada Yousif[1], Ayesha Jameel[2], Joely Smith[3], Sena Akgun[4], Brynmor Jones[3], Dipankar Nandi[2], Peter Bain[2], Wladyslaw Gedroyc[3]
[1]University of Hertfordshire, School Of Physics, Engoineering, Hatfield, United Kingdom, [2]Imperial College London, Department Of Brain Sciences, London, United Kingdom, [3]Imperial College London Healthcare NHS Trust, Radiology, London, United Kingdom, [4]La Sapienza University, Medicine, Rome, Italy

Aims: Essential tremor (ET) is considered the most common movement disorder (National Tremor Foundation, 2022), affecting up to one percent of adults over 40 years of age. Magnetic Resonance guided focused ultrasound (MRgFUS) thalamotomy is a proven treatment for ET (Miller et al, 2022), however its success is highly dependent on the accuracy of lesioning the target thalamic nucleus – the ventral intermediate nucleus (VIM). This study aimed to improve our understanding of target location via a data driven computational approach to visualise the treatment. **Methods:** The Schaltenbrand and Wahren (1977) stereotactic atlas was used to construct a model via the open-source software 3D Slicer (Fedorov, 2012). First, 20 of these atlas images were stacked in Matlab (R2021a, Mathworks Inc.), to obtain a resolution of 0.1mm per pixel. This data was imported into 3D Slicer to allow segmentation of the thalamus (including key nuclei such as VIM), and adjacent structures such as the internal capsule. The resulting structures were smoothed to produce the final three-dimensional model. **Results:** The resulting model was used to plot patient treatments and assess accuracy of targeting. Furthermore, the target locations from several international MRgFUS centres were mapped to compare the evolution of targeting in MRgFUS across centres and over time. **Conclusions:** This model provides a visualisation of targeting within the thalamus. The model will be developed into a thermal finite element model to fully understand clinical efficacy with regards to lesion location and sonication, to improve the clinical effect for people with essential tremor.

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BOARD NUMBER: S02-525

A GENERATIVE MODEL OF BRAIN STATES DYNAMIC IN NON-HUMAN PRIMATES BASED ON VARIATIONAL AUTO-ENCODER

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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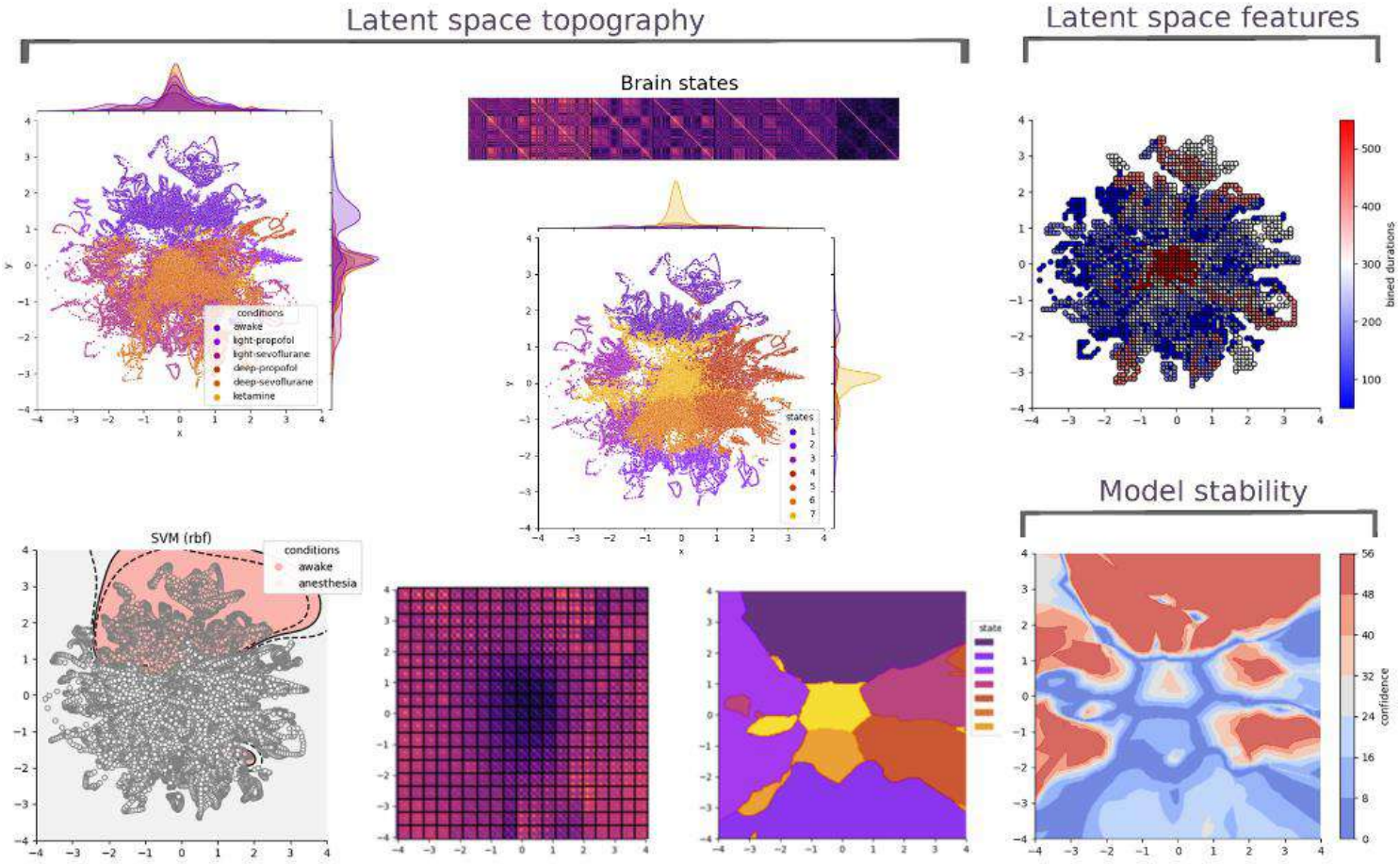

Aims The states of consciousness are characterized by different configurations of resting-state fMRI (rs-fMRI) patterns. By applying k-means clustering to dynamic functional connectivity matrices (dFCs), we could identify repertoires of brain states that are specific to wakefulness and anesthesia-induced loss of consciousness. Recent studies show that a low-dimensional manifold keep information on the main properties of the different dynamic configurations. We hypothesize that by using deep learning models, we might characterize the structure of the brain states repertoire. **Methods** We previously acquired rs-fMRI in non-human primates during wakefulness or under anesthesia. We trained a dense-Variational Auto-Encoder to encode the dFCs to probability distributions in a 2D-space. Then we used the generative decoder to produce new realistic samples and extrapolate configurations. **Results** The latent space obtained contains ordered topography of brain states. The brain state

that is more correlated to the underlying brain structure occupies the central place surrounded by the other brain states. The generative approach of the model makes possible to reconstruct new brain states in a coherent and stable way despite the limited dataset size. **Conclusions** This is consistent with our previous work in which we demonstrated that the repertoire of brain states associated with wakefulness is rich, flexible and uncorrelated with brain anatomy; while the opposite occurs for the one associated with consciousness loss. By capturing essential information, our model helps visualizing and interpreting the dynamic brain states repertoire. In the future, we will model the transition probabilities, and trajectories across states of consciousness.

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BOARD NUMBER: S02-526

SEIZURE PREDICTION IN MEA SIGNALS WITH DEEP LEARNING

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Epilepsy affects a large part of the world's population causing not only a lot of problems from a pathilogy point of view but also from the economic and social point of view of the patients. One of the treatment paradigms when the pharmacological approach is not effective is deep brain stimulation.
Currently, brain stimulation devices use predefined stimulation protocols that do not take into account the underlying situation of the brain at any given moment. Therefore, in this work we propose the development of an artificial intelligence algorithm to predict epileptic seizures from Microelectrode Array signals and thus be able to control the stimulation in a responsive way. Using a neural network based on Long Short-term memory neurons, an architecture has been established that is able to predict ictal events 2 minutes in advance, which is enough time to initiate seizure-avoiding stimulation. As an experimental basis, hippocampal slices recorded with a multi-electrode array have been used.
This algorithm opens the door to implementations in clinical devices and to a change in the current design of deep brain stimulation systems.

Pubmed:
32528245: Díaz-Rodríguez SM, López-López D, Herrero-Turrión MJ, Gómez-Nieto R, Canal-Alonso A, Lopéz DE
Inferior Colliculus Transcriptome After Status Epilepticus in the Genetically Audiogenic Seizure-Prone Hamster GASH/Sal.
The Genetic Audiogenic Seizure Hamster from Salamanca (GASH/Sal), an animal model of reflex epilepsy, exhibits generalized tonic-clonic seizures in response to loud sound with the epileptogenic focus localized in the inferior colliculus (IC). Ictal events in seizure-prone strains cause gene deregulation in the epileptogenic focus, which can provide insights into the epileptogenic mechanisms. Thus, the present study aimed to determine the expression profile of key genes in the IC of the GASH/Sal after the status epilepticus. For such purpose, we used RNA-Seq to perform a comparative study between the IC transcriptome of GASH/Sal and that of control hamsters both subjected to loud sound stimulation. After filtering for normalization and gene selection, a total of 36 genes were declared differentially expressed from the RNA-seq analysis in the IC. A set of differentially expressed genes were validated by RT-qPCR showing significant differentially expression between GASH/Sal hamsters and Syrian control hamsters. The confirmed differentially expressed genes were classified on ontological categories associated with epileptogenic events similar to those produced by generalized tonic seizures in humans. Subsequently, based on the result of metabolomics, we found the interleukin-4 and 13-signaling, and nucleoside transport as presumably altered routes in the GASH/Sal model. This research suggests that seizures in GASH/Sal hamsters are generated by multiple molecular substrates, which activate biological processes, molecular processes, cellular components and metabolic pathways associated with epileptogenic events similar to those produced by tonic seizures in humans. Therefore, our study supports the use of the GASH/Sal as a valuable animal model for epilepsy research, toward establishing correlations with human epilepsy and searching new biomarkers of epileptogenesis.
Front Neurosci, 2020; 14
31731446: Casado-Vara R, Canal-Alonso A, Martin-Del Rey A, De la Prieta F, Prieto J
Smart Buildings IoT Networks Accuracy Evolution Prediction to Improve Their Reliability Using a Lotka-Volterra Ecosystem Model.
Internet of Things (IoT) is the paradigm that has largely contributed to the development of smart buildings in our society. This technology makes it possible to monitor all aspects of the smart building and to improve its operation. One of the main challenges encountered by IoT networks is that the the data they collect may be unreliable since IoT devices can lose accuracy for several reasons (sensor wear, sensor aging, poorly constructed buildings, etc.). The aim of our work is to study the evolution of IoT networks over time in smart buildings. The hypothesis we have tested is that, by amplifying the Lotka-Volterra equations as a community of living organisms (an ecosystem model), the reliability of the system and its components can be predicted. This model comprises a set of differential equations that describe the relationship between an IoT network

and multiple IoT devices. Based on the Lotka-Volterra model, in this article, we propose a model in which the predators are the non-precision IoT devices and the prey are the precision IoT devices. Furthermore, a third species is introduced, the maintenance staff, which will impact the interaction between both species, helping the prey to survive within the ecosystem. This is the first Lotka-Volterra model that is applied in the field of IoT. Our work establishes a proof of concept in the field and opens a wide spectrum of applications for biology models to be applied in IoT.
Sensors (Basel), 2019; 19

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BOARD NUMBER: S02-527

SELF-SUPERVISED LEARNING AS A GATEWAY TO REVEAL UNDERLYING DYNAMICS IN ANIMAL BEHAVIOUR

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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The brain is a dynamical system and its dynamics are reflected in the actions it performs. In order to understand brain functions, observable motion is a fundamental resource. Technological approaches towards understanding and dissecting the spatiotemporal order of animal motion are still in its infancy compared to approaches aiming at dissecting brain functions. Recent advances in representation and self-supervised learning can be leveraged to learn robust representations of animal behavior in an unprecedented manner. In this work, we explore combining autoregressive models with the notion of variational auto-encoding to learn robust spatiotemporal embeddings of animal behavior. By using a mouse model of beta amyloidosis as a use case, we show that our framework (VAME) not only identifies discrete behavioral motifs, but also captures their hierarchical representation. Thus, we present a novel and robust approach to learn the representation of animal motion, which is applicable to a wide range of experimental setups, models and conditions without requiring supervised or a-priori human interference.

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BOARD NUMBER: S02-528

ULTRA-RAPID VISUAL SEARCH IN NATURAL IMAGES USING ACTIVE DEEP LEARNING

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Visual search, or the simultaneous localization and detection of a visual target of interest, is a vital task. Applied to the case of natural scenes, searching for example an animal (either a prey, a predator or a partner) constitutes a challenging problem due to large variability over numerous visual dimensions. Yet, biological visual systems are able to perform such detection efficiently in briefly flashed scenes in a very short amount of time. Deep convolutional neuronal networks (DCNNs) were shown to be well fitted to the image classification task. Previous models also managed to solve the visual search task by roughly dividing the image into sub-areas. This is at the cost, however, of computer-intensive parallel processing on relatively low-resolution image. Taking inspiration from natural vision systems, we develop here a model builds over the anatomical visual processing pathways observed in mammals, namely the "What" and the "Where" pathways. It operates in two steps, first by selecting regions of interest, before knowing their actual visual content, through an ultra-fast/low resolution analysis of the full visual field, and the second providing a detailed categorization over the detailed "foveal" selected region attained with a saccade. Modeling this dual-pathways architecture brings an efficient model of visual search as active vision and may allows to fill the gap with the shortcomings of DCNNs with respect to physiological performances. In the future, we expect to apply this model to better understand visual pathologies involving one of the two pathways while contributing to the computer vision field.

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BOARD NUMBER: S02-529

EXPLORING NEUROPHYSIOLOGICAL AND PSYCHOLOGICAL PAIN BIOMARKERS WITH MACHINE LEARNING

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

Greta Preatoni[1], Noemi Gozzi[1], Federico Ciotti[1], Natalija Katic[1], Michele Hubli[2], Petra Schweinhardt[2], Stanisa Raspopovic[1]
[1]ETH Zürich, Department Of Health Sciences And Technology, Zürich, Switzerland, [2]Spinal Cord Injury Center, Research Balgrist University Hospital, Zurich, Switzerland

Aims Pain is a subjective experience typically measured through patient's self-report. Researchers have tried to achieve an objective measurement of pain and its relative biomarkers by developing machine learning (ML) algorithms. However, most of these studies do not consider that pain has also emotional components. Consequently, today there is no reliable model of pain prediction considering all its multifaceted aspects. We aim at investigating how emotional, cognitive, and physiological components are impacting pain perception. **Methods** To achieve this goal, ML techniques have been applied to data collected from 40 healthy and chronic pain patients, who underwent experimentally induced pain while their physiological and complete psychological profiles have been collected. Through explainable AI models we investigated which are the most important features to distinguish pain and to what type of measurement (physical or psychological) they belong. **Results** Our models were able to classify pain vs no pain with more than 90% of accuracy. We found that the most important features to detect pain in the physiological recordings belong to the EEG power spectral density and the derivative features of the SC. Most importantly, the pain level was identifiable through physiological recordings adjusted by a subjective bias, meaning that the reported pain level was dependent on a subjective perception of pain. **Conclusions** For the first time, ML is applied to give neuroscientific insights on biomarkers of the subjective pain perception. Our model has the potential of opening the way to personalized therapies based on a comprehensive evaluation of each individual's pain experience.

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BOARD NUMBER: S02-530

MODEL BASED META REINFORCEMENT LEARNING FOR ALCHEMY

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Meta-learning emphasises the 'learning to learn' paradigm where the similar characteristics between the tasks generate a common basis bearing relevance that extends beyond the trained tasks and acts as an inductive bias that helps the learning agent to quickly generalize to unseen tasks from the same distribution. For example during navigation, understanding the underlying structure of the environment can aid inferences when going back to an explored state from an unexplored point of view. Recent work by Whittington et al 2020 has shown that the underlying graph defining the structure once learnt is reusable across different environments consisting of different sensory observations, thus forming an invariant feature basis. This can be extenteded to the relational inference scenarios encountered by reinforcement learning (RL) agents. A recently introduced benchmark 'Alchemy' aims at challenging meta-RL by providing both a convenient interface and an optimal solution for assessing agent efficiency. The meta-RL agent navigating the alchemy environment is challenged with understanding the latent causal structure to excel at this task with maximum reward. We aim to train model-based meta-RL agents to solve alchemy, by introducing useful inductive bias for capturing the underlying structure of explored environments. We hypothesize that training such an agent, in addition to improving the meta-RL knowledge base, may help shed light on the interactions between Prefrontal cortex and Entorhinal cortex. Here, we present preliminary results from initial studies that show promising performance on the task compared to the two deep RL agents (VMPO, IMPALA) previously deployed to solve Alchemy.

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BOARD NUMBER: S02-531

EXPLORATION OF THE RESPECTIVE ROLES OF CORTEX AND BASAL GANGLIA IN CATATONIA WITH A COMPUTATIONAL MODEL.

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Catatonia is a psychomotor syndrome associating both motor and affective symptoms. One of the most characteristic symptoms of catatonia –posturing– corresponds to patients assuming unusual and uncomfortable body postures. No animal model of catatonia is available, impairing deeper experimental exploration of its physiological mechanisms. As such, the physiopathology of the syndrome is not fully understood. Its first-line treatment is high doses of benzodiazepines and related drugs, which suggests the implication of an excitation-inhibition imbalance. The dominant conception of catatonia focuses on frontal cortex disorders that would be relayed by the basal ganglia, resulting in the observed motor disorders. However, a large naturalistic cohort study shows significant organic morbidity with implication of basal ganglia dysfunction. As most of the basal ganglia neural populations are inhibitory, we hypothesize that the basal ganglia may indeed have a more direct role in catatonia. To investigate this hypothesis, we present here a biologically constrained computational model of the cortico-baso-thalamo-cortical loops, with which we explore the effects of attenuating the weight of inhibitions. For this first attempt at modeling catatonia with computational tools, we focus on the study of neural dynamics, and explore how irregular activity collapses to oscillatory regimes when inhibition is attenuated in the cortex, the basal ganglia, or both, and we use disconnection approaches to identify which circuit causes the oscillations.

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BOARD NUMBER: S02-532

THE FRACTAL CORTEX: A MULTI-SCALE SURFACE-PRESERVING ANALYSIS SUGGESTS ALL CORTICES ARE APPROXIMATIONS OF A SINGLE UNIVERSAL SHAPE

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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The mammalian cerebral cortex is probably one of the most complex structures ever studied by science. At first glance, any attempt to express its diversity, or significant features thereof, from universal principles, would seem doomed to failure. Indeed, there are many ways of comparing the morphological features of cerebral cortices. In what follows, working from first principles, we will express cortical morphology using a more natural set of variables across length scales, rather than across spatial position. We will introduce an algorithm for 'melting' cortical shape that erases structural features smaller than a set scale while maintaning surface self-avoidance and topological structure, and show that this process occurs in a universal self-similar manner. Using this new framework, we will finally show that the morphology of all cerebral cortices analyzed so far, across both mammalian species and individuals, are approximations of a single, universal fractal shape; and that the main distinction between different cortical shapes is the range of scales at which this approximation remains valid. This universal description of the cortical shape is at the same time mechanistically insightful and in full agreement with empirical data across species and individuals. Prospectively, we hope this new framework for expressing and analyzing cortical morphology, besides revealing a hitherto hidden regularity of nature, can become a powerful tool to characterize and compare cortices of different species and individuals, across development and aging, and across health and disease.

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BOARD NUMBER: S02-533

DEEP LEARNING BASED PERSONALIZED OUTCOME PREDICTION AFTER ACUTE ISCHEMIC STROKE

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Purpose The long-term risk of major vascular events after acute ischemic stroke (AIS) cannot be reliably assessed by traditional survival analysis at an individual level. We aimed to develop a personalized outcome prediction model using deep learning after AIS. **Methods** A total of 6,247 patients with AIS were enrolled in this study. The primary outcome was the occurrence of major adverse cerebro/cardiovascular events (MACE) over 12 months. We compared deep learning models (DeepSurv and DeepHit) with traditional survival models (Cox proportional hazards [CoxPH] and random survival forest [RSF]) using the time-dependent concordance (C^{td}) index and the mean absolute day error (MADE). **Results** The performances of the deep learning models (DeepSurv and DeepHit) were improved after adding the brain image features to clinical data (C^{td} index = 0.8303 and 0.8137, respectively). The performances were similar with the survival models of CoxPH (C^{td} index = 0.8059) and RSF (C^{td} index = 0.8352) for predicting MACE. The deep learning model of DeepHit had the lowest day error for predicting MACE. The MADE of the DeepSurv, DeepHit, CoxPH, and RSF models to predict MACE were 235.99 (95% CI, 200.25-273.73), 113.57 (95% CI, 88.87-141.45), 215.24 (95% CI, 178.34-253.02), and 303.24 (95% CI, 275.48-328.35), respectively, in the analyses with clinical and image factors. **Conclusion** This study showed that deep learning models developed using clinical data and brain images outperformed traditional survival models predicting MACE after AIS. Deep learning can improve the prediction of major vascular events and provide personalized outcome prediction in patients with AIS.

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BOARD NUMBER: S02-535

COMMUNICATION AND CAUSATION IN THE HUMAN BRAIN

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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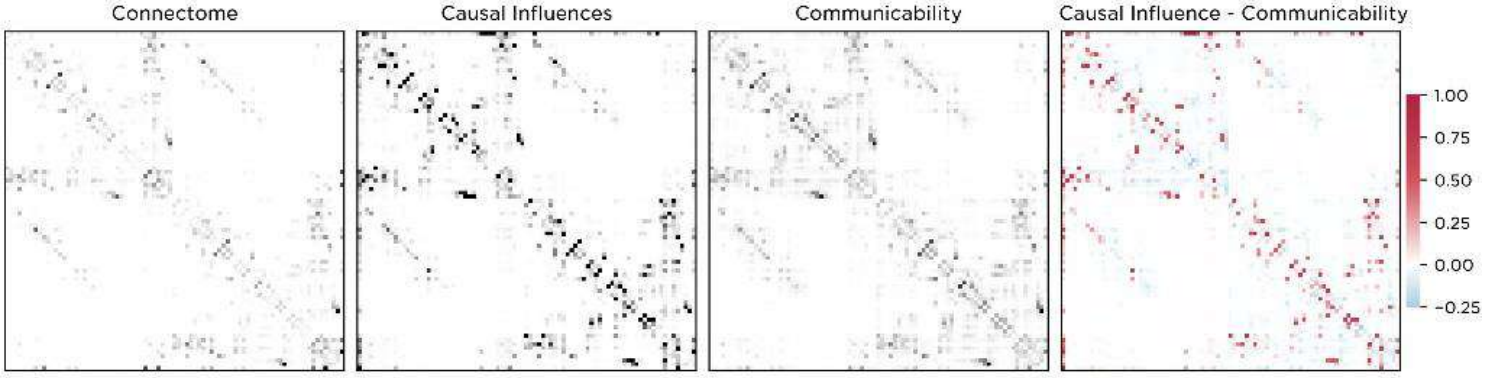
The human brain is a complex network where nodes communicate with one another via direct and indirect pathways. Graph-theoretical metrics are employed to understand how information is transmitted in such networks. Among these metrics, communicability describes the communication efficiency of two nodes given all direct and indirect paths existing between them. However, it is unclear if the brain harnesses its infrastructure optimally, such that the most efficient routes are the most influential ones. To address this point, we first represented each node of the human connectome as a dynamical system to model information propagation. We then used a game-theoretical framework called Multi-perturbation Shapley value Analysis (MSA) that systematically perturbs every possible combination of nodes to quantify their causal influences on each other. We found that communicability and MSA contributions provide qualitatively similar measures, indicating that nodes largely influence each other via direct connections and to a lesser extent also via indirect paths. However, contrasting results from the two approaches revealed that nodes deviate from the efficient communication channels by overusing the less efficient routes and underutilizing the more efficient ones (warm and cold entries in the figure below, respectively). Our results show that knowledge of the structural connectome of the brain by itself is insufficient for understanding its causal functional interactions.



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ESTIMATING THE EXPOSOME SCORE FOR STRESS-RELATED MENTAL DISORDERS USING PREDICTIVE MODELLING

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Aims: Environmental risk factors for mental disorders are often studied in isolation and with no consideration of high correlations between exposures. The exposome approach addresses this problem by incorporating a multitude of environmental exposures over the entire life span and using advanced statistical techniques to appropriately model environmental risk. We aimed to demonstrate the validity of this approach for a transdiagnostic phenotype by constructing an exposome score for stress-related mental disorders using predictive modelling. **Methods:** The sample consisted of 269 cases with lifetime diagnoses of depression, anxiety disorders, or PTSD and 104 healthy controls. Measures of childhood abuse and neglect, lifetime trauma and loss events, and current and parental sociodemographic information were used as predictors. A simple sum score of dichotomized predictors was compared to a Gaussian Naïve Bayes model, simple logistic regression, and LASSO and Ridge penalized logistic regression models. Models were trained and tested via repeated 10x10 cross-validation. The area under the ROC curve was chosen as the primary outcome measure. **Results:** The LASSO-regularized model outperformed all other predictive models including the simple sum score (AUC_{LASSO}=.68, $AUC_{other\ models}$=.65-.67). The exposome score resulting from the coefficients of the LASSO model successfully discriminated cases from controls (OR=2.69, Nagelkerke's R^2=.28, p<.001) and significantly predicted several continuous measures of anxiety and depression (R^2=.09-.30, p<.05). **Conclusions:** These results indicate that the exposome approach is a valid model for studying environmental risk, yields better results than the use of simple unweighted sum scores, and can be applied to transdiagnostic phenotypes.

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BOARD NUMBER: S02-537

CLASSIFICATION OF COGNITIVE PERFORMANCE FROM HOME-BASED EEG REPEATED MEASUREMENTS IN OLDER ADULTS

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Aims: The earliest cognitive effects of neurodegeneration are challenging to detect on an individual basis. Common laboratory-based face-to-face assessments are usually snapshots of performance, hindering the detection of early cognitive changes. This can be addressed with remote digital assessment of cognition which enable repeated measurements in more naturalistic settings. This is the case of home-based electro-encephalography (EEG) combined with gamified laboratory tasks. Here we identified neurotypical participants who have low performance in the MoCA assessment (Montreal Cognitive Assessment) using home-based EEG recordings. **Methods:** Neurotypical older adults (N=91) were asked to perform a session 5 times a week during a 6-week or 12-week period while neurophysiology was recorded with a dry-EEG headset designed for unsupervised use in the home. Participants performed a gamified 2-stimulus visual oddball paradigm and a resting state (RS) paradigm. EEG features during the task and RS paradigm were extracted. Reaction time to the infrequent stimuli was extracted as a benchmark metric. Participants were labelled as high/low performers based on the median split of the MoCA score (27). Machine learning methods were applied to subsets of the data using repeated 10-fold nested cross validation to classify high/low performers. **Results:** Data with all features and only EEG features classified participants with an accuracy of 65-75% using random forests. This was above the accuracy obtained with behavioural data only (59%). **Discussion:** Features extracted from home-based EEG recordings during the visual oddball task and resting state can be used to identify participants with high (or low) cognitive performance.

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PREDICTION OF CHRONIC PAIN ONSET FROM UK BIOBANK DATA

POSTER SESSION 02 - SECTION: MACHINE LEARNING FOR NEUROSCIENCE & PSYCHIATRY

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Aims: The purpose of this work is to apply machine learning methods to the UK Biobank dataset to identify potential mechanisms that predict the development of chronic pain. **Methods:** Participant data (n=8776) was included if s/he: i) reported no pain-related medical condition; ii) did not experience pain at the time of the FMRI vist (visit 2); and iii) completed the online follow-up pain questionnaire after visit 2 was completed. Those participants that answered “yes” to the question “troubled by pain or discomfort present for more than 3 months” were defined those that developed chronic pain (CP+). Data categories included: 1) clinical questionnaires; 2) brain imaging-derived phenotypes; and 3) preprocessed multimodal brain MRI data. Data compression was completed using the FSL tool BigFLICA. Random forest classification was then applied, and the most relevant classifier features were identified (Python; sklearn v0.24.2). Hyperparameters were tuned using 4-fold cross-validation (Optuna v2.9.1). **Results:** A total of 3163 (36%) of the participants developed CP+ (mean age: 63.3 s.d.=7.14; M/F ratio: 0.465) versus n=5613 (64%) that did not (mean age: 63.2 s.d.=7.31; M/F ratio:0.503). The best performing classifier that correctly identified CP+ participants achieved a balanced accuracy of 57.4% and an AUC of 60.1%. This classifier contains 298 features across all data categories. **Conclusions:** Preliminary analyses show that a combination of questionnaire (e.g., health satisfaction, insomnia, neuroticism, frequency of unenthusiasm, miserableness) and brain activation maps (task-evoked FMRI) are most important for predicting which UKB participants are likely to develop chronic pain at follow-up.

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GEOMETRY OF POPULATION ACTIVITY IN SPIKING NETWORKS WITH LOW-RANK STRUCTURE

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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An influential paradigm posits that computations in the brain can be understood at the collective level by studying the geometry of dynamics in the state space of joint activity of all neurons [Vyas et al 2020]. Within that framework, low-rank recurrent neural networks (RNNs) have emerged as a rich class of computational models for linking connectivity, the geometry of dynamics and computations [Mastrogiuseppe and Ostojic 2018]. RNNs are however highly abstract models, in which activity is represented in terms of continuous firing rates and basic biological constraints are lacking. Here we examine how results obtained using low-rank RNNs extend to more biologically plausible models where neurons interact through discrete action potentials. We consider randomly connected networks of excitatory and inhibitory integrate-and-fire neurons [Brunel 2000], to which we add a low-rank connectivity structure. We then compare the resulting geometry of spiking activity to the predictions of rate-based low-rank RNNs. We find that the predictions for the geometry of dynamics hold in spiking networks irrespective of the underlying regime of activity. The dynamics induced by the low-rank structure can moreover be highly nonlinear and develop multiple attractors or oscillations, and we show how they can be employed to perform nonlinear computations such as perceptual decision-making. Altogether our results demonstrate that the mechanistic insights obtained in abstract RNN models remain valid when taking into account additional biological constraints.

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SHAPING ACTIVITY MANIFOLDS IN LOW-RANK RECURRENT NEURAL NETWORKS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Mounting evidence across cortical circuits suggests that continuous variables are encoded in the brain in terms of continuous manifolds in neural activity space. For example head direction or position are represented by ring-shaped or torus-shaped manifolds [Chaudhuri et al. 2019, Gardner et al. 2022], and it has been hypothesized that such structures are signatures of continuous attractors generated by recurrent connectivity. A variety of computational models have been developed to explain how connectivity determines the properties of the resulting manifold, but a unifying picture has yet to emerge. For instance, ring attractors can be generated from different connectivity structures, giving rise to different properties at the individual neuron as well as the population level. In this work, we aim to identify general principles for how connectivity structures shape particular continuous attractors such as rings or tori. We approach this problem through the scope of low-rank recurrent neural networks, which are a recently introduced class of models providing a mathematical link between recurrent connectivity and neural latent dynamics [Mastrogiuseppe & Ostojic 2018]. We show how hidden symmetries in the low-rank structure of the connectivity matrix directly determine the geometrical properties of the continuous attractor. Using these principles, we derive a general blueprint to build continuous attractors of various dimensionalities, and with various levels of neural heterogeneity, unifying different previously proposed computational models.

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BOARD NUMBER: S02-541

DYNAMICAL ENTROPY IN POPULATION ACTIVITY OF ZEBRAFISH LARVAE

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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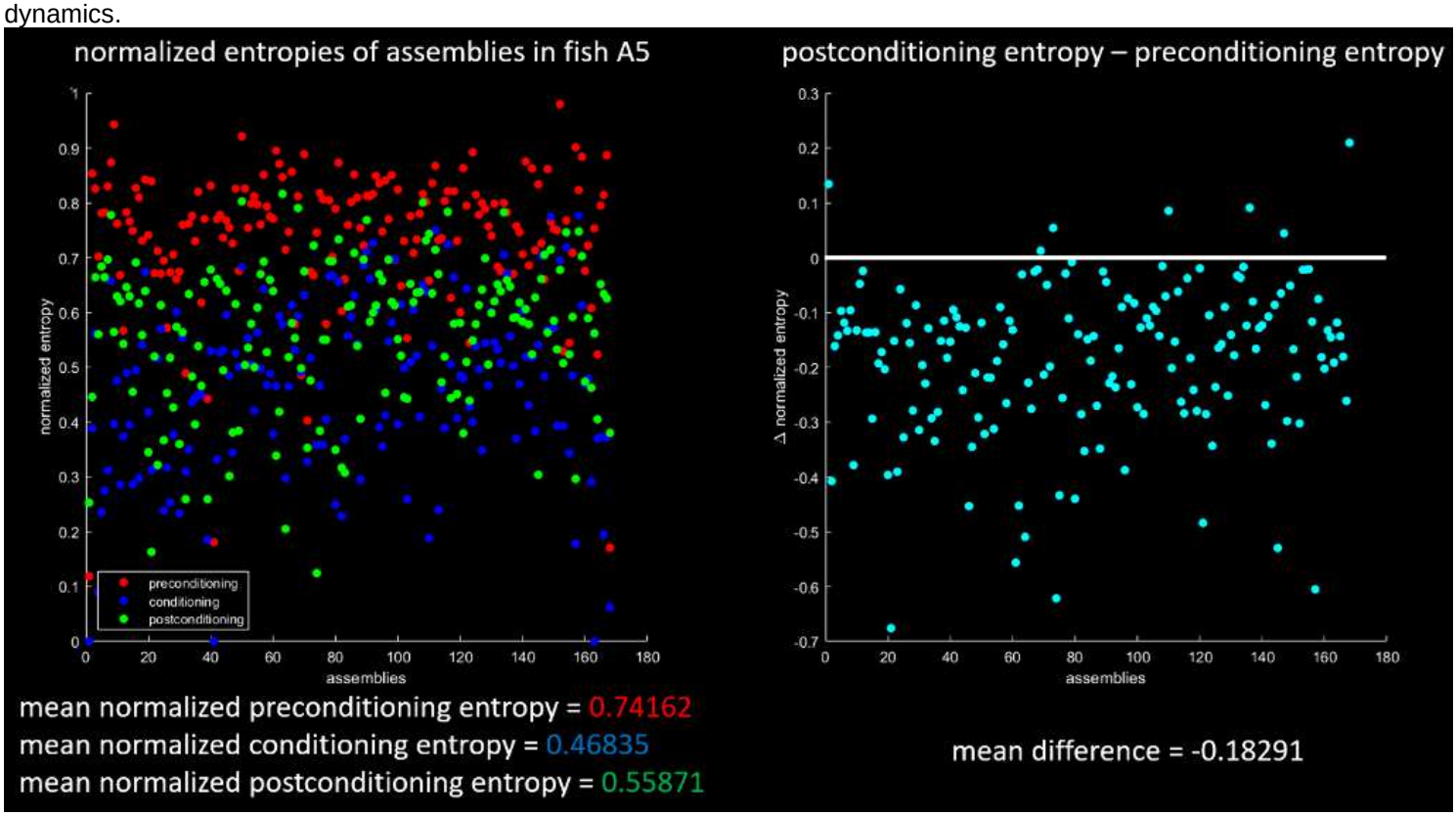
The optic tectum in zebrafish larvae displays rich assembly dynamics that can be observed via calcium imaging. The population activity is shaped by an abundance of inhibition, and displays attractor-like phenomena. To investigate the effects of stimulation on assembly dynamics, we defined a mass distribution on neurons that assigns to each neuron its fraction of the total population activity. The Shannon entropy of this distribution measures whether activity is concentrated on a few neurons or spread out evenly across the entire population. Using light sheet microscopy, spontaneous activity of ~2000 neurons was recorded for a period of two hours. In the experimental fish, there was a five-minute period of light dot stimulation presented one hour into the recording. We found that in the stimulated fish, the entropy of the mass distribution dropped significantly between the pre- and post-conditioning periods. This was true both for individual assemblies (see Figure) and the entire population. However, this entropy drop was not seen in control fish. Next, we investigated whether a simple inhibition-dominated network model could explain the entropy behavior. We constructed a random threshold-linear network of 1000 excitatory neurons in an inhibition dominated regime. In the conditioning phase, 30% of neurons were stimulated, which sharply decreased the population entropy. Surprisingly, these effects on the dynamics remained even after stimulation was removed: population entropy in the postconditioning period was significantly lower than in preconditioning. These findings suggest that the observed changes in entropy following stimulation may be a generic feature of inhibition-dominated network
dynamics.

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BOARD NUMBER: S02-542

IDENTIFYING THE ROLE OF TEMPORAL DYNAMICS IN THE AUDITORY SYSTEM.

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Neural processing across sensory areas is often conceptualized as a series of static, feed-forward steps analogous to successive layers in a deep network. In contrast, the role of temporal dynamics generated by recurrent connectivity is only starting to be explored. In this study, we sought to uncover the role of temporal dynamics in sensory representations of static and dynamical sound features across the mouse auditory pathway. We examined large-scale calcium-imaging and electrophysiological recordings performed across the Inferior Colliculus, the Auditory Thalamus and the Auditory Cortex in response to a set of 140 auditory stimuli. The stimuli consisted of pure tones of various frequencies and amplitudes, as well as temporal modulations and superpositions thereof. We hypothesized that at a population level, frequency and amplitude are represented as continuous variables that give rise to continuous surfaces –manifolds– in the neural activity space. We first exploited dimensionality reduction methods on responses to static, pure sounds to identify the static frequency-amplitude manifolds in the different areas. We then compared the response to temporally modulated stimuli with the predictions of the static manifolds, and reasoned that the difference between the two corresponds to temporal processing. Altogether, our approach allows us to extract the representation of dynamical sound properties as it unfolds along the auditory pathway.

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BOARD NUMBER: S02-543

EXTRACTING COMPUTATIONAL MECHANISMS FROM NEURAL ACTIVITY USING LOW-RANK RECURRENT NEURAL NETWORKS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

Adrian Valente[1], Jonathan Pillow[2], Srdjan Ostojic[1]
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An influential proposal within systems neuroscience posits that neural computations can be understood in terms of low-dimensional dynamics [Vyas et al 2020]. A key issue within that framework is that any given computational task may be compatible with a variety of dynamical mechanisms. To identify the mechanisms at play in the brain, it is therefore crucial to constrain models using recorded neural activity. Here we introduce a new approach for extracting low-dimensional dynamics from data, based on inferring low-rank recurrent neural networks (lrRNNs), a highly expressive and interpretable class of models [Mastrogiuseppe 2018, Dubreuil-Valente 2022]. We first apply our technique to synthetic data generated from full-rank RNNs trained on a variety of neuroscience tasks. We show that lrRNNs fitted to neural activity allow us to identify the collective computational mechanisms, and generate novel predictions for inactivations in the original RNNs. We then apply our methodology to data recorded from the primate prefrontal cortex in a context-dependent decision making task. Our approach allows us to assign computational roles to the different latent variables and provides us with a mechanistic model of the recorded dynamics, on which *in silico* experiments like inactivations can be performed.

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UNIFIED SPIKING MODEL OF STRUCTURED ACTIVITY AND TRAVELLING SPARSE WAVES IN THE RESTING STATE OF THE PRIMARY VISUAL CORTEX.

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Charles University, Prague, Department Of Software And Computer Science Education, Prague, Czech Republic

In the primary visual cortex (V1) of higher mammals, spontaneous activity is modular and reflects the underlying structure - co-active regions are highly correlated to orientation maps[1]. This activity travels in sparse waves, in line with the propagation speed of unmyelinated lateral connections [2]. These two intertwined phenomena have so far only been studied separately, both experimentally [1,4] and computationally [5,2]. Furthermore, there is a lack of theoretical and mechanistic understanding of how these two phenomena interact with evoked activity. In this study we offer a unifying computational theory of structured spontaneous activity and traveling waves, by presenting a single large-scale spiking model of cat V1 that exhibits both phenomena. Our model exhibits spontaneous activity, travelling in sparse waves matching the conduction speed of V1 layer 2/3 horizontal connections, and is simultaneously correlated with the underlying orientation map. From the outset [3], our model demonstrates a wide variety of previously reported visually evoked properties, which shows the compatibility of the identified mechanisms of spontaneous travelling waves and structured activity with the mechanisms required by the evoked regime. 1. Smith, G.B. et al., M. Nat Neurosci 21, 1600–1608 (2018). 2. Davis, Z.W. et al. Nat Commun 12, (2021). 3. Antolík, J. et al., (2018) doi:10.1101/416156. 4. Muller, L. et al., Nat Commun 5, (2014). 5. Cai, D. et al., Proceedings of the National Academy of Sciences 102, 5868–5873 (2005)

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FLEXIBLE SENSORY REPRESENTATION LEARNING IN NON-STATIONARY ENVIRONMENTS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

Matthias Tsai[1], Jasper Teutsch[2], Fritjof Helmchen[3], Abhishek Banerjee[2], Walter Senn[1]
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Neural representations of sensory stimuli are modulated by internal states and beliefs about the world. Such top-down modulations—informed by context and task contingencies—are crucial to the perception and behaviour of animals but remain poorly understood. Inspired by the theoretical framework of temporal difference (TD) learning, we propose a task-dependent attentional feedback model of layer 2/3 pyramidal neurons in the primary somatosensory cortex (S1). It has long been proposed that top-down excitation of apical dendrites mediates attention. Our computational model learns to attend to stimuli that predict future rewards or punishments by raising their apical excitation proportionally to the magnitude of the TD error they produce. The TD error is fed back to apical dendrites through top-down signals involving a disinhibitory circuit of VIP+ and somatostatin (SST) expressing interneurons. If the learned attention allocation is no longer appropriate for the ongoing task, apical inhibition can revoke it. The lateral orbitofrontal cortex (lOFC) is responsive to changes in task contingency and has been found to target SST neurons, which are known to inhibit apical dendrites. By incorporating recorded lOFC activity into our model, we show that attention can be flexibly reallocated to in case a task change. Finally, the evolution of sensory representations predicted by the model is confirmed by S1 population imaging in mice. In summary, this work contributes to solving the credit assignment problem and understanding sensory representation learning in non-stationary environments by explaining how top-down apical attention prolongs and strengthens the neural representation of task-relevant information.

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DOPAMINE-DEPENDENT PLASTICITY AND ATTRACTOR DYNAMICS COOPERATE IN BASAL GANGLIA-CORTICAL LOOP FOR MOTOR LEARNING

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Aims: The basal ganglia (BG) are subcortical nuclei known for their implication in motor control, sensorimotor integration and procedural learning. The BG shape activity patterns in their thalamocortical target structures to optimize actions. In particular, plasticity in BG output implements corrections in behavior to maximize reward and correct errors during learning. While BG models typically rely on action repertoires represented in discrete populations, shaping the right motor pattern during reward-driven learning is more difficult than simply choosing from separately encoded actions. **Methods**: Building on anatomical, physiological and behavioral evidence, we propose a model for the generation, learning and adaptation of reaching movement in the BG-cortical loop. In the motor cortex, a distributed representation of actions (population vector) drives continuous motor patterns. **Results**: We show that the BG shape sensorimotor transformation, driving cortical motor output patterns as a function of sensory cortical activity, corticostriatal synaptic weights and overall gains of the feedback loops. The attractor dynamics of the network confers the system with resistance to noisy input, persistent activity and complex spatiotemporal activity profiles in response to transient inputs. In a reinforcement learning paradigm reflecting classical conditioning tasks implemented labs, dopamine-dependent corticostriatal plasticity enables robust learning in the sensorimotor transformation. Following striatal dopamine depletion, the network loses its ability to shape cortical activity through attractor dynamics. Strong depletion may ultimately lead to the emergence of pathological beta oscillations. **Conclusion**: Our study suggests the BG-cortical network shapes motor output based on its rich closed-loop dynamics and corticostriatal dopamine-dependent plasticity.

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EARLY SELECTION OF RELEVANT AUDITORY FEATURES THROUGH CONTEXT-DEPENDENT POPULATION GATING

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Understanding how stable neural circuits implement flexible, context-dependent behavior is crucial to understanding cognition. How different brain areas interact through fixed communication channels to select relevant stimuli and ignore irrelevant ones remains to be fully elucidated. Here, we focus on the population dynamics previously recorded from the primary auditory cortex (A1) and prefrontal cortex (PFC), while rats exhibited flexible, context-dependent behavior. Specifically, rats performed stimuli discrimination (go/no-go) according to either the location or the pitch of the auditory stimuli, depending on the context that was not explicitly cued. Using linear decoders, we found that A1 represented relevant and irrelevant stimuli during both contexts. On the other hand, PFC exclusively represented the stimuli relevant for the ongoing context. This raised the question of how these areas interacted to effectively select the relevant stimulus in PFC, specifically assuming a fixed readout from A1. To address this question, we trained recurrent neural networks (RNN) with back-propagation on a similar task. We found that both the relevant and irrelevant stimuli were represented, similar to A1. We reverse-engineered the mechanism employed by these networks, predicting that different populations that selectively integrate the relevant stimuli can be identified by different context-specific activity during the pre-stimulus period. Motivated by these predictions, we found two distinct populations in A1, each discriminating more strongly the relevant stimuli in its preferred context. Finally, we built a multi-area RNN in which decision and context were communicated feedforward and feedback, respectively, through fixed channels, inspired by the communication subspace hypothesis.

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EMERGENCE OF TIME PERSISTENCE IN AN INTERPRETABLE DATA-DRIVEN NEURAL NETWORK MODEL

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

Sebastien Wolf[1], Guillaume Le Goc[2], Simona Cocco[3], Georges Debrégeas[2], Remi Monasson[3]
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Establishing accurate as well as interpretable models of neural networks activity is an open challenge in systems neuroscience. Here we infer an energy-based generative network model of the anterior rhombencephalic turning region (ARTR) of zebrafish larvae using calcium-imaging recordings of the spontaneous activity of hundreds of neurons. While our data-driven model is trained to solely reproduce the short-term statistics of the neural activity, its dynamics exhibits persistence on much longer time scales. The model's persistence time decreases with water temperature in agreement with neuronal and behavioral observations. Mathematical analysis of the model unveils a low-dimensional landscape-based representation of the population activity where the long-term dynamics reflects slow Arrhenius-like activated processes between metastable activity states. We show how this effective landscape is modified in the presence of light stimuli, which allows us to reinterpret previous experiments characterizing the visually-driven operation of the ARTR.

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MEMORY REPLAY IN A DISINHIBITION-BASED MODEL OF HIPPOCAMPAL SHARP-WAVES

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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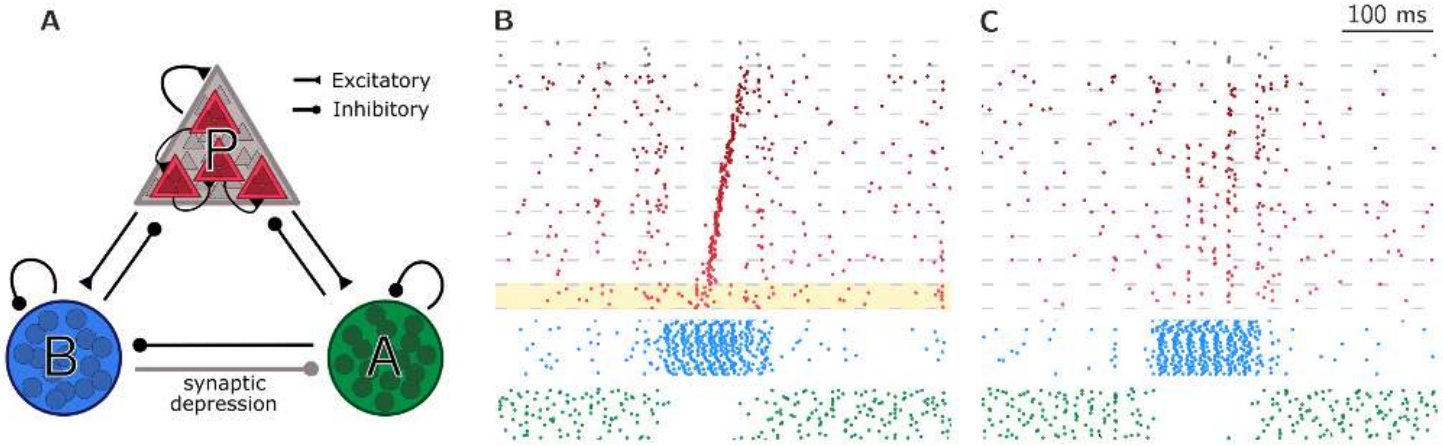
The hippocampus encodes new episodic memories during active wakefulness (Buzsáki, *Neuroscience*, 1989), which are gradually consolidated into the neocortex through sharp wave-ripple (SWR) associated replays. Chenkov et al. (*PLOS Comput. Biol.*, 2017) showed that balanced recurrent spiking networks are capable of replaying memory sequences, but sharp-wave dynamics were not considered. Evangelista et al. (*J Neurosci*, 2020) introduced a disinhibition-based homogeneous network with two different classes of interneurons, explaining sharp-wave dynamics, but replay was not investigated. Our work combines these results and shows how disinhibition-based networks can produce spontaneous sharp-waves while replaying encoded sequences. We model neuronal activity in a slice of CA3 rodent hippocampus with a three-population spiking network governed by disinhibition. To investigate replay, we embed sequentially connected assemblies in the pyramidal cell population (Fig-A). We find that a stored sequence can be replayed during spontaneous SWRs if we bias the network, e.g., by a small enduring depolarization of the initial assembly (Fig-B); replay can also be elicited by transient stimulation of a specific assembly (as in Chenkov et al., 2017). Without bias, there is no replay, and the network exhibits global bursts of activity (Fig-C). These bursts emerge from the SWR-state's tendency to generate oscillations in the low-gamma frequency
range.



Overall, we specify conditions under which replay succeeds or fails in such models, improving the understanding of the mechanisms governing sharp-waves and memory replay.

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SIMULATIONS APPROACHING DATA: CORTICAL SLOW WAVES IN INFERRED MODELS OF THE WHOLE MOUSE HEMISPHERE

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

Cristiano Capone[1], Chiara De Luca[2,3], Giulia De Bonis[3], Robin Gutzen[4], Irene Bernava[3], Cosimo Lupo[3], Elena Pastorelli[3], Francesco Simula[3], Leonardo Tonielli[3], Francesco Resta[5], Anna Allegra Mascaro[5], Francesco Pavone[5], Michael Denker[4], Pier Stanisalo Paolucci[1]
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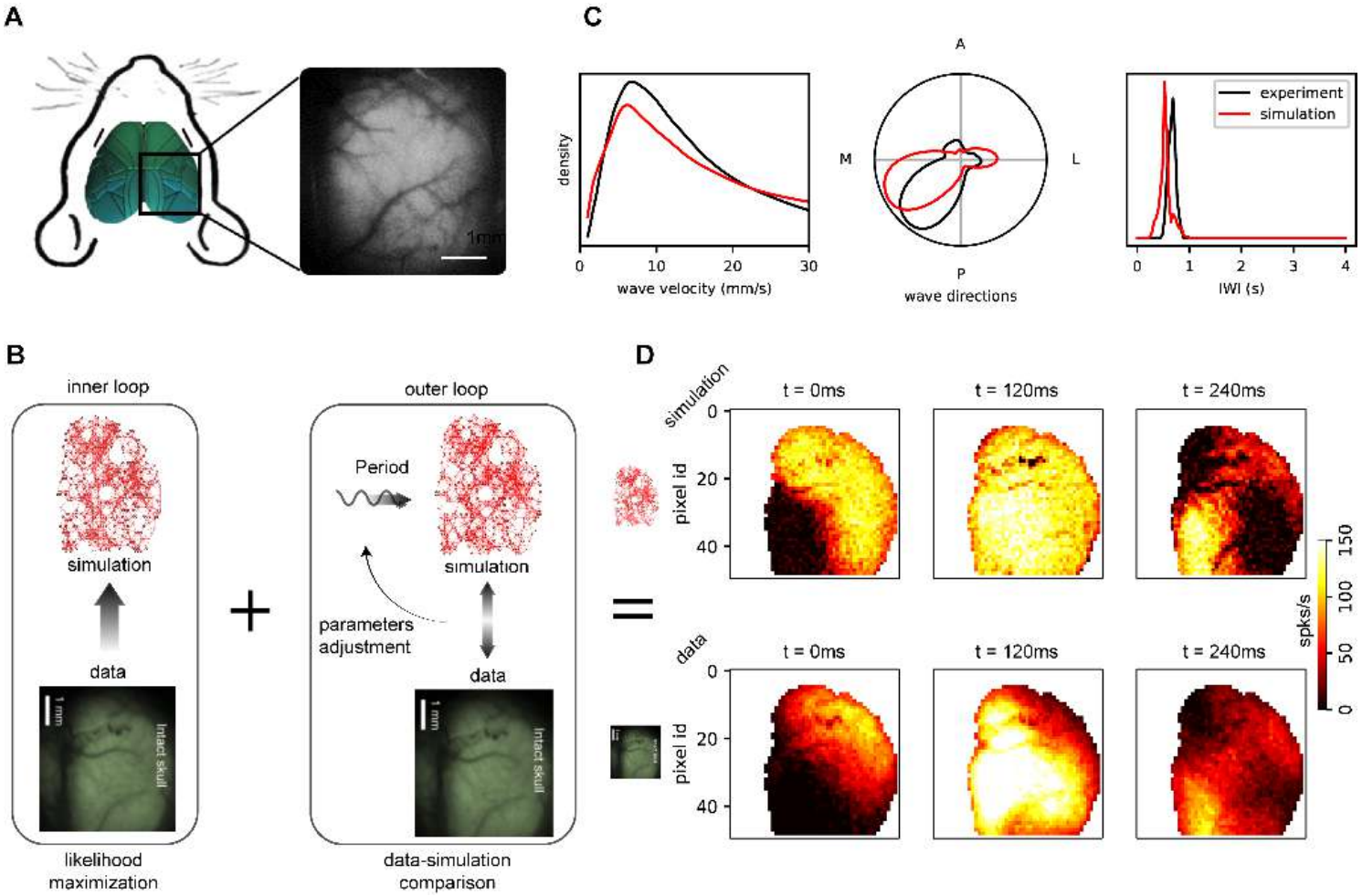

Recent developments of new and powerful brain activity recording techniques, combined with the increasing anatomical knowledge provided by atlases and the growing understanding of neuromodulation principles, are allowing the study of the brain at a whole new level, paving the way to the creation of data-driven extremely detailed effective network models. In this

work, aiming at reproducing the complex spatio-temporal dynamics of slow waves observed in experimental recordings of the cortical hemisphere of a mouse under ketamine anesthesia (A), a two-step inference procedure is proposed (B). First, *inner loop*, parameters of a mean-field model are optimized by likelihood maximization, exploiting anatomical knowledge to define connectivity priors; then, *outer loop*, the space of “external" parameters is explored in search of an optimal match between the simulation outcome and the data, to enrich the spontaneous activity generated by the model with the desired variety of dynamical features observed in the data. The validation of the model relies on the development of a versatile ensemble of analysis tools, applicable to both experimental and simulated data and capable of identifying and quantifying the spatio-temporal propagation of waves across the cortex; the tools enable the comparison of wave dynamics, evaluating the differences in the distribution of local observables (speed, direction, frequency, C), and, in the channel space, through the multivariate gaussian approach. Thanks to the interplay between data analysis and inference, the so-tuned model is capable of reproducing most of the non-stationary and non-linear dynamics displayed by biological networks (D).

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MOTION INTENTION PREDICTION

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Motion intention prediction is the key to robot-assisted rehabilitation systems. These can rely on various biological signals. One commonly used signal is the muscle activity measured by an electromyogram that occurs between 50-100 milliseconds before the actual movement, allowing a real-world application to assist in time. We show that upper limb motion can be estimated from the corresponding muscle activity. To this end, eight-arm muscles are mapped to the joint angle, velocity, and acceleration of the shoulder, elbow, and wrist. For this purpose, we specifically develop an artificial neural network that estimates complex motions involving multiple upper limb joints. The network model is evaluated concerning its ability to generalize across subjects as well as for new motions. This is achieved through training on multiple subjects and additional transfer learning methods so that the prediction for new subjects is significantly improved. In particular, this is beneficial for a robust real-world application. Furthermore, we investigate the importance of the different parameters such as angle, velocity, and acceleration for simple and complex motions. Predictions for simple motions along with the main components of complex motions achieve excellent accuracy while joints that do not play a dominant role during the motion have comparatively lower accuracy.

Pubmed:
32694988: Brickwedde M, Schmidt MD, Krüger MC, Dinse HR
20 Hz Steady-State Response in Somatosensory Cortex During Induction of Tactile Perceptual Learning Through LTP-Like Sensory Stimulation.
The induction of synaptic plasticity requires the presence of temporally patterned neural activity. Numerous cellular studies in animals and brain slices have demonstrated that long-term potentiation (LTP) enhances synaptic transmission, which can be evoked by high-frequency intermittent stimulation. In humans, plasticity processes underlying perceptual learning can be reliably induced by repetitive, LTP-like sensory stimulation. These protocols lead to improvement of perceptual abilities parallel to widespread remodeling of cortical processing. However, whether maintained rhythmic cortical activation induced by the LTP-like stimulation is also present during human perceptual learning experiments, remains elusive. To address this question, we here applied a 20 Hz intermittent stimulation protocol for 40 min to the index-, middle- and ring-fingers of the right hand, while continuously recording EEG over the hand representation in primary somatosensory cortex in young adult participants. We find that each train of stimulation initiates a transient series of sensory-evoked potentials which accumulate after about 500 ms into a 20 Hz steady-state response persisting over the entire period of the 2-s-train. During the inter-train interval, no consistent evoked activity can be detected. This response behavior is maintained over the whole 40 min of stimulation without any indication of habituation. However, the early stimulation evoked potentials (SEPs) and the event-related desynchronization (ERD) during the steady-state response change over the 40 min of stimulation. In a second experiment, we demonstrate in a separate cohort of participants that the here-applied pneumatic type of stimulation results in improvement of tactile acuity as typically observed for electrically applied 20 Hz intermittent stimulation. Our data demonstrate that repetitive stimulation using a 20 Hz protocol drives rhythmic activation in the hand representation of somatosensory cortex, which is sustained during the entire stimulation period. At the same time, cortical excitability increases as indicated by altered ERD and SEP amplitudes. Our results, together with previous data underlining the dependence of repetitive sensory stimulation effects on NMDA-receptor activation, support the view that repetitive sensory stimulation elicits LTP-like processes in the cortex, thereby facilitating perceptual learning processes.
Front Hum Neurosci, 2020; 14
30803484: Taylor GJ, Tichit P, Schmidt MD, Bodey AJ, Rau C, Baird E
Bumblebee visual allometry results in locally improved resolution and globally improved sensitivity.
The quality of visual information that is available to an animal is limited by the size of its eyes. Differences in eye size can be observed even between closely related individuals, yet we understand little about how this affects vision. Insects are good

models for exploring the effects of size on visual systems because many insect species exhibit size polymorphism. Previous work has been limited by difficulties in determining the 3D structure of eyes. We have developed a novel method based on x-ray microtomography to measure the 3D structure of insect eyes and to calculate predictions of their visual capabilities. We used our method to investigate visual allometry in the bumblebee and found that size affects specific aspects of vision, including binocular overlap, optical sensitivity, and dorsofrontal visual resolution. This reveals that differential scaling between eye areas provides flexibility that improves the visual capabilities of larger bumblebees.
Elife, 2019; 8

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AN INHIBITORY NETWORK MODEL EXPLAINS THE TRANSIENT DYNAMICS OF HIPPOCAMPAL RIPPLE OSCILLATIONS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Hippocampal ripple oscillations have been implicated in important cognitive functions such as memory consolidation (Buzsáki, 1989). The mechanisms underlying ripple generation however are still debated. Modeling studies have shown that interneuron networks can produce oscillations in the ripple-band (140-220 Hz) (Brunel & Hakim, 1999; Donoso, 2018) and even exhibit the experimentally observed intra-ripple frequency accommodation (IFA) — an asymmetric decrease in the instantaneous frequency within a ripple event (Donoso, 2018; Ponomarenko, 2004).
Here we show that IFA is an inherent feature of the inhibition-based ripple model and explain its mechanism. We consider a network of leaky integrate-and-fire units with delayed inhibitory coupling receiving white noise and a common excitatory drive. In the mean-field limit the population rate and the density of membrane potentials are described by the Fokker-Planck Equation. Focusing only on the mean-driven dynamics for strong drive we can approximate analytically the frequency and the amplitude of the network oscillation as a function of the external drive. Numerical simulations verify that the approximation works in a large parameter regime. We show that for fast changing, sharp wave-like drive the network frequency response is asymmetric due to a speed-dependent hysteresis effect in the oscillation amplitude of the mean membrane potential. We predict that IFA vanishes in the limit of slowly changing drive, which can be tested optogenetically.
Our results demonstrate how understanding transient features of ripple oscillations can guide model selection. Buzsáki, Neuroscience, 1989
Brunel, Hakim., Neural Comput., 1999
Donoso et al., J Neurosci, 2018
Ponomarenko et al., EJN, 2004

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BOARD NUMBER: S02-553

FLYING CLOSE TO THE PRECIPICE - THE BREAKING POINT OF THE CONTINUOUS QUASI-ATTRACTOR

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Small mammals, like larger ones, navigate in complex territories. It is believed that they create *cognitive maps* of the environments they explore. Spatially selective cells in the hippocampal formation are neuronal candidates involved in this capability. In the development of mathematically defined network models, it has been hypothesized that the dynamics underlying the retrieval of place cell maps could be driven by *continuous attractors*. Such models typically assume homogeneity across units. However, the wilder the environment the more place cells seem to be activated unevenly. Recently published recordings of place cells in bats flying in a 200*mt* long track show, for example, multiple fields of variable width and peak rate. Can a 1D continuous attractor be established, to encode also such complex nonuniform activity? In this poster we argue that it can, if relaxing the requirement of a continuous manifold of fixed points, to a continuous dynamical flow. We see, indeed, that the stable states which persist with noisy maps, still lie on the manifold, inducing an effective flow along it. We call this mathematical object a *continuous quasi-attractor*. We find that there is a critical level of noise at which the quasi-attractive manifold abruptly breaks, through a phase transition. Applying our analysis to the experimental recordings measured in bats, we see that they lie close to the edges of the transition. This result leads us to hypothesize that place maps may be effectively memorized in a 1D continuous quasi-attractive manifold.

Pubmed:
33480759: Schönsberg F, Roudi Y, Treves A
Efficiency of Local Learning Rules in Threshold-Linear Associative Networks.
We derive the Gardner storage capacity for associative networks of threshold linear units, and show that with Hebbian learning they can operate closer to such Gardner bound than binary networks, and even surpass it. This is largely achieved through a sparsification of the retrieved patterns, which we analyze for theoretical and empirical distributions of activity. As reaching the optimal capacity via nonlocal learning rules like back propagation requires slow and neurally implausible training procedures, our results indicate that one-shot self-organized Hebbian learning can be just as efficient.
Phys Rev Lett, 2021; 126

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BOARD NUMBER: S02-554

CONTEXTUAL CONTROL OVER EPISODIC MEMORY THROUGH HIERARCHICAL PREDICTIVE PROCESSING

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Aims: The ability to rapidly form pattern-separated memories in the hippocampus comes with a risk of storing mutually inconsistent memory traces. Cognitive control over memory encoding and retrieval leverages context and goal information to resolve conflicts between similar episodes and to improve behavioral flexibility. Experimental evidence pointing to the prominent role of prefrontal-hippocampal interactions for achieving this have accumulated. However, the implementation details of this function are not yet known. Methods: We consider two existing biologically-inspired neural networks that have been proposed to account for hippocampal and prefrontal functions separately. The hippocampal module is a self-supervised autoencoder that learns to predict incoming sensory information. The prefrontal component is a hierarchical network in which individual layers learn to predict lower layers' prediction errors given the current context, and bias their predictions accordingly. We explore how the prefrontal cortex can be used to bias hippocampal activity by comparing different combinations of these two modules. Results: We confront the resulting models to experimental data of rodents learning two conflicting lists of stimulus pairs with selective inactivations of the prefrontal cortex and the medial temporal lobe. The ability of the resulting models to reproduce these findings is evaluated and contributes to the understanding of the functional involvement of different prefrontal-hippocampal anatomical pathways. Conclusion: Our results participate in guiding the development of prefrontal-hippocampal models. Such models will play a pivotal role in understanding high-level episodic memory functions beyond stimulus-driven recall, including goal-directed memory retrieval and imagination.

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DISCOVERING LOW-DIMENSIONAL INTERPRETABLE DYNAMICS FROM HIGH-DIMENSIONAL NEURONAL ACTIVITY

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Recent improvements in brain recording techniques have provided tools to record activity from hundreds to thousands of neurons simultaneously. Modeling and interpreting the resulting spike train data is a challenging task that is central to the quest of understanding brain functions. For this task, data-driven models such as point-process generalized linear models (PPGLMs) have been developed to fit and predict high-dimensional neuronal activity. Continuous approximations and basis projections of PPGLMs have previously been introduced to uncover the latent underlying dynamics. Yet, the question of interpreting those equations with regards to functional behavior remains difficult. On the other hand, frameworks such as structured flows on manifolds (SFM) propose a compelling description of behavior using interpretable dynamical systems, but remain detached from actual recordings. To overcome this gap between models, we analytically performed separation between two time scales of the continuous approximation of the PPGLM equations. In the first fast time-scale, high-dimensional dynamics collapse into a low-dimensional manifold, effectively constraining the set of possible behaviors. In the second slow time-scale, trajectories are traced out on the manifold to represent a particular behavior. We demonstrated the functional relevance of this decomposition in simulations of neuronal networks, and derived the resulting constraints on the network connectivity matrices. These simulations provide a testbed for the proposed framework, which will be next applied to human microelectrode array recordings during speech processing task, and opens new avenues for the analyses of neural data.

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DYNAMICAL EVOLUTION OF ELECTRICALLY COUPLED NEURONS THAT PARTICIPATE IN THE COORDINATION OF SEQUENTIAL NEURAL ACTIVITY

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Many central pattern generator circuits (CPGs) contain electrically coupled cells whose activity show different synchronization degrees. CPGs generate robust sequential activations that result in highly coordinated motor activity. We assessed the contribution of electrically coupled cells in shaping cycle-by-cycle intervals that define robust sequences. By using a mixed experimental and modeling approach, we characterized the level of synchronization, the dynamical evolution of membrane potential in electrically coupled cells, and the relationship between the level of synchronization and the duration and variability of time intervals that build robust sequences.

Experiments in the pyloric CPG of *Carcinus maenas* were performed with intracellular recordings of the two electrically coupled PD neurons and the LP cell. The coupled PDs showed a variable level of synchronization progressing along the bursts. The spike timing was measured unveiling an adaptive delay and transient desynchronizations. Time intervals between the LP bursts were also quantified. A conductance-based model of the CPG was employed to study the effect of altering the electrical conductance between the PD neurons to match the synchronization level observed in the experiments. We also evaluated the variability of the intervals which build the sequence cycle-by-cycle in the model.

We related the observed interval variability to the evolving synchronization between the PD neurons. Our joint experimental/modeling results indicate that the coordination of time intervals that define robust CPG sequences is shaped both by the inhibitory chemical interaction between cells and the dynamical coupling of the electrical synapses.

SM., Pablo and B., Blanca contributed equally to this work.

Pubmed:
34309826: Sánchez P, Sánchez ML, Mangas A, de Souza E, Aguilar LA, Coveñas R
Neuroanatomical distribution of the enkephalinergic and tachykininergic systems in the alpaca brainstem: an immunohistochemical study.
A recent study has shown a close neuroanatomical relationship between the enkephalinergic (methionine-enkephalin) and tachykininergic (substance P) systems in the alpaca diencephalon. In this study, our aim is to show this relationship in the alpaca brainstem.
Folia Histochem Cytobiol, 2021; 59
32412087: Sánchez P, Sánchez ML, Mangas A, Aguilar LÁ, Coveñas R
A close neuroanatomical relationship between the enkephalinergic (methionine-enkephalin) and tachykininergic (substance P) systems in the alpaca diencephalon.
In the alpaca diencephalon, the distribution of immunoreactive cell bodies and fibers containing methionine-enkephalin (MET) or substance P (SP) has been studied.
Folia Histochem Cytobiol, 2020; 58

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BOARD NUMBER: S02-557

MODELING PARALLEL SPECIALIZED PATHWAYS OF MAMMALIAN VISUAL SYSTEM

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Aims: Visual system of mammals is comprised of two parallel pathways: the “what” and the “where” pathways which are specialized for object categorization and movement, respectively. Therefore, an artificial neural network (ANN) model of mammalian visual systems should also have the same specialized parallel pathways. In this work, we studied the sufficient and necessary factors for learning the “what” and “where” representations in an ANN. **Methods:** We trained an ANN with two segregated parallel pathways with a combination of path-specific and shared loss functions. We used two methods to evaluate the type of learned representations in our model: 1) linear evaluation on downstream tasks, such as motion discrimination and object categorization, and 2) evaluatng the similarity of the ANN with the “what” and “where” pathways of mouse and monkey visual systems using Representational Similarity Analysis (RSA). **Results:** We show that training an ANN that has two parallel pathways with a self-supervised prediction loss function is sufficient for learning both “what”- and “where”-like representations. However, we show that additional, path-specific loss functions are necessary to further encourage and stabilize the learning of each type of representation within the two pathways. For example, adding the self-motion estimation loss to one of the two pathways leads to learning a better “where”-like pathway in our ANN. **Conclusions:** Our results show that an ANN with segregated parallel pathways trained with a combination of a global self-supervised prediction and path-specific loss functions can explain the development of parallel specialized pathways in mammalian visual systems.

Pubmed:

35213337: Awada A, Bakhtiari S, Legault C, Odier C, Pack CC
Training with optic flow stimuli promotes recovery in cortical blindness.
Cortical blindness is a form of severe vision loss that is caused by damage to the primary visual cortex (V1) or its afferents. This condition has devastating effects on quality of life and independence. While there are few treatments currently available, accumulating evidence shows that certain visual functions can be restored with appropriate perceptual training: Stimulus sensitivity can be increased within portions of the blind visual field. However, this increased sensitivity often remains highly specific to the trained stimulus, limiting the overall improvement in visual function.
Restor Neurol Neurosci, 2022; 40
33683287: Awada A, Bakhtiari S, Pack CC
Visual perceptual learning generalizes to untrained effectors.
Visual perceptual learning (VPL) is an improvement in visual function following training. Although the practical utility of VPL was once thought to be limited by its specificity to the precise stimuli used during training, more recent work has shown that such specificity can be overcome with appropriate training protocols. In contrast, relatively little is known about the extent to which VPL exhibits motor specificity. Previous studies have yielded mixed results. In this work, we have examined the effector specificity of VPL by training observers on a motion discrimination task that maintains the same visual stimulus (drifting grating) and task structure, but that requires different effectors to indicate the response (saccade vs. button press). We find that, in these conditions, VPL transfers fully between a manual and an oculomotor response. These results are consistent with the idea that VPL entails the learning of a decision rule that can generalize across effectors.
J Vis, 2021; 21
32214128: Bakhtiari S, Altinkaya A, Pack CC, Sadikot AF
The Role of the Subthalamic Nucleus in Inhibitory Control of Oculomotor Behavior in Parkinson's Disease.
Inhibiting inappropriate actions in a context is an important part of the human cognitive repertoire, and deficiencies in this ability are common in neurological and psychiatric disorders. An anti-saccade is a simple oculomotor task that tests this ability by requiring inhibition of saccades to peripheral targets (pro-saccade) and producing voluntary eye movements toward the mirror position (anti-saccades). Previous studies provide evidence for a possible contribution from the basal ganglia in anti-

saccade behavior, but the precise role of different components is still unclear. Parkinson's disease patients with implanted deep brain stimulators (DBS) in subthalamic nucleus (STN) provide a unique opportunity to investigate the role of the STN in anti-saccade behavior. Previous attempts to show the effect of STN DBS on anti-saccades have produced conflicting observations. For example, the effect of STN DBS on anti-saccade error rate is not yet clear. Part of this inconsistency may be related to differences in dopaminergic states in different studies. Here, we tested Parkinson's disease patients on anti- and pro-saccade tasks ON and OFF STN DBS, in ON and OFF dopaminergic medication states. First, STN DBS increases anti-saccade error rate while patients are OFF dopamine replacement therapy. Second, dopamine replacement therapy and STN DBS interact: L-dopa reduces the effect of STN DBS on anti-saccade error rate. Third, STN DBS induces different effects on pro- and anti-saccades in different patients. These observations provide evidence for an important role for the STN in the circuitry underlying context-dependent modulation of visuomotor action selection.
Sci Rep, 2020; 10
30626723: Bakhtiari S
Can Deep Learning Model Perceptual Learning?
J Neurosci, 2019; 39

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NONLINEAR OPTIMAL CONTROL OF NEURAL POPULATIONS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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We apply methods from nonlinear optimal control theory to a biophysical neural mass model to study optimal control strategies and their effects on neural populations. External control is realized by time-dependent inputs to two mutually coupled populations of excitatory and inhibitory neurons. Optimality is defined by cost functions that trade the deviation of the controlled variable from its target value against the ”strength” of the control, which is quantified by the 1- and 2-norms of the control signal. We focus on a bistable region in state space where one low- (”down state”) and one high-activity (”up state”) stable fixed point coexist. We search for the most cost-efficient control function to switch between both states. We compare two types of control signals, which mimic either the ion flow across a neuron’s cell membrane ("current control"), or synaptic inputs from other connected neurons ("rate control"). We analyze optimal external stimulation for a deterministic system, and for a more realistic, stochastic system. Cost-efficient control strategies consist of a pulse of finite duration, which – for deterministic systems – pushes the state variables only minimally into the basin of attraction of the target state. For stochastic processes, control amplitudes are increased to reliably push the system into another state. Penalizing control strength via the averaged 1-norm (2-norm) yields finite inputs along one (vs. several) input channels. Restrictions in transition time result in faster transitions produced by larger inputs. Our study highlights the applicability of nonlinear optimal control to understand neuronal processing under constraints better.

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NETWORK MECHANIMS UNDERLYING LONG-DISTANCE DEPENDENCIES

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Sequential behaviors such as language or bird songs are structured in time. This structure relies on the notion of long-distance-dependencies: transitions between words depend on the identity of words produced in the past. Here we propose network mechanisms supporting such dependencies. To do so we trained artificial neural networks to produce a minimal set of sequences exhibiting long-distance-dependencies. By reverse-engineering the trained networks we found this to rely on two superposing neural sequences, one responsible for the production of the motor sequence and another one encoding a contextual memory. We show how these two sequences are supported by neural activity and network connectivity and how they interact with each other to decide on transitions between words. We discuss similarities between the neural activity of our artificial neural networks and neural correlates of long-distance-dependencies that have recently been exposed in songbirds.

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A HYBRID COMPUTATIONAL MODEL OF THE HIPPOCAMPAL FORMATION TO REPLICATE THETA-NESTED GAMMA OSCILLATIONS AND THETA PHASE RESET DURING NEUROSTIMULATION

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

Nikolaos Vardalakis[1,2], Amélie Aussel[1,2], Nicolas Rougier[1,2], Fabien Wagner[1]
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Theta and gamma oscillations in the hippocampal formation play a crucial role in memory processes and are disrupted in several neurological disorders. Recently, electrical neurostimulation of the human entorhinal area has been shown to enhance memory encoding, but the underlying mechanisms need to be investigated. Here, we aimed to validate the hypothesis that neurostimulation entrains theta-gamma oscillations and induces phase reset of theta oscillations, through a novel hybrid computational model that replicates both theta-gamma hippocampal oscillations and theta phase reset. Specifically, we used an existing biophysically realistic model of the hippocampal formation able to generate theta-nested gamma oscillations, and interfaced it with abstract Kuramoto oscillators that dynamically generate theta oscillations and theta phase reset. Biophysically realistic neurons were modeled using the Hodgkin-Huxley formalism and connected based on anatomical data from the literature. The set of Kuramoto oscillators had reciprocal connections with the CA1 field of the hippocampus and was meant as an abstract representation of the generation of hippocampal theta, which is thought to originate mainly from the medial septum. Our model replicates both theta-nested gamma oscillations and theta phase reset, and can be used to probe the effects of different neurostimulation protocols on theta and gamma oscillations and their couplings. In particular, we show that neurostimulation pulses delivered in-phase with the underlying theta rhythm can rescue impaired oscillations caused by changes in the model parameters that reflect pathophysiological circuit alterations. Overall, our novel computational framework opens new avenues for studying the effects of neurostimulation on hippocampal circuits

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FOUR GRID CELL MODULES CAN CODE FOR A PRECISE LOCATION AT A LOW ERROR

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

Isabelle Hoxha[1], Alvaro Feito Boirac[2], Gabriel Orellana[3], Lianne Klaver[4], Jonathan Coutinho[5], Fabio Solari[6]
[1]Université Paris-Saclay, Ciams, Faculté Des Sciences Du Sport, Orsay Cedex, France, [2]Bermuda Automation, Bermuda Automation, Warwick, Bermuda, [3]Protera, Protera Biosciences, Santiago, Chile, [4]Virginia Tech, School Of Neuroscience, Blacksburg, United States of America, [5]Queen's University, Centre For Neuroscience Studies, Kingston, Canada, [6]University of Genova, Department Of Informatics, Bioengineering, Robotics And Systems Engineering, Genova, Italy

Aims: Grid cells in the rat entorhinal cortex fire according to the animal's location in space. Depending on their anatomical location, grid cells belong to modules with different field sizes, which combine to encode location precisely. While previous studies have addressed the importance of modular architecture for location accuracy, we aim at computing the optimal number of modules for accurate location. **Methods:** We built two artificial neural networks, whose input layers contain N modules * 16 cells each. Our initial model rests on the observation that one cell encodes the rat's position ambiguously with possible overlap of firing patterns between cells. The first model multiplies those spatial firing patterns to obtain the position. To explore more optimal encodings of the animal's location, we added two fully connected hidden layers between noisy activation patterns and the output layer, trained to predict the rat's 2D Cartesian coordinates. **Results:** Our initial model can map 75% of the output space, with 25% of four-cell combinations coding position ambiguously. Using a model with one spatial module results in inaccurate location, either due to high error rates if the separation is large, or to high ambiguity with small field sizes. Increasing the number of layers reduces ambiguity and error. **Conclusions:** Increasing the number of modules from 1 to 4 increases the performance of our model on predicting location at a reduced error. Further research could investigate the relation between the number of modules and the animal's range of motion, and the energetic cost of modular architecture.

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INHIBITION STABILIZATION AND PARADOXICAL EFFECTS IN NETWORKS WITH SHORT-TERM PLASTICITY

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

Yue Kris Wu[1,2], Julijana Gjorgijeva[1,2]
[1]Max Planck Institute for Brain Research, Computation In Neural Circuits Group, Frankfurt am Main, Germany, [2]Technical University of Munich, School Of Life Sciences, Freising, Germany

Inhibition stabilization is a ubiquitous property of cortical networks, whereby inhibition ensures that network activity remains at a realistic level despite the presence of strong recurrent excitation. Networks operating in the inhibition stabilized regime are capable of performing various computations, including input amplification, response normalization, and network multistability. In excitatory and inhibitory networks with static connectivity, an identifying characteristic of inhibition stabilization is that increasing (decreasing) excitatory input to the inhibitory population leads to a decrease (increase) in its firing, known as the paradoxical effect. However, synaptic strengths are subject to short-term plasticity (STP) on a time scale of milliseconds to seconds. How inhibition stabilization relates to paradoxical effect in networks with short-term plasticity is unclear. Here, we analytically identified the conditions of being inhibition stabilized and the conditions of having paradoxical effects in networks with STP. We found that while the paradoxical effect implies inhibition stabilization, inhibition stabilization does not imply the paradoxical effect. Interestingly, in the presence of STP, networks can undergo non-monotonic transitions between inhibition stabilized regime and non-inhibition-stabilized regime when the excitatory drive to the inhibitory population increases. Furthermore, networks with STP can exhibit a novel response profile, which cannot be observed in conventional static networks, in response to excitatory input to the inhibitory population. In summary, our work establishes the link between inhibition stabilization and paradoxical effects in the presence of STP. This makes important predictions for future experimental studies that aim to identify cortical operating regimes in the brain.

Pubmed:
34895468: Wu YK, Zenke F
Nonlinear transient amplification in recurrent neural networks with short-term plasticity.
To rapidly process information, neural circuits have to amplify specific activity patterns transiently. How the brain performs this nonlinear operation remains elusive. Hebbian assemblies are one possibility whereby strong recurrent excitatory connections boost neuronal activity. However, such Hebbian amplification is often associated with dynamical slowing of network dynamics, non-transient attractor states, and pathological run-away activity. Feedback inhibition can alleviate these effects but typically linearizes responses and reduces amplification gain. Here, we study nonlinear transient amplification (NTA), a plausible alternative mechanism that reconciles strong recurrent excitation with rapid amplification while avoiding the above issues. NTA has two distinct temporal phases. Initially, positive feedback excitation selectively amplifies inputs that exceed a critical threshold. Subsequently, short-term plasticity quenches the run-away dynamics into an inhibition-stabilized network state. By characterizing NTA in supralinear network models, we establish that the resulting onset transients are stimulus selective and well-suited for speedy information processing. Further, we find that excitatory-inhibitory co-tuning widens the parameter regime in which NTA is possible in the absence of persistent activity. In summary, NTA provides a parsimonious explanation for how excitatory-inhibitory co-tuning and short-term plasticity collaborate in recurrent networks to achieve transient amplification.
Elife, 2021; 10
32917810: Wu YK, Hengen KB, Turrigiano GG, Gjorgjieva J
Homeostatic mechanisms regulate distinct aspects of cortical circuit dynamics.
Homeostasis is indispensable to counteract the destabilizing effects of Hebbian plasticity. Although it is commonly assumed that homeostasis modulates synaptic strength, membrane excitability, and firing rates, its role at the neural circuit and network level is unknown. Here, we identify changes in higher-order network properties of freely behaving rodents during prolonged visual deprivation. Strikingly, our data reveal that functional pairwise correlations and their structure are subject to homeostatic regulation. Using a computational model, we demonstrate that the interplay of different plasticity and homeostatic mechanisms can capture the initial drop and delayed recovery of firing rates and correlations observed experimentally. Moreover, our model indicates that synaptic scaling is crucial for the recovery of correlations and network

structure, while intrinsic plasticity is essential for the rebound of firing rates, suggesting that synaptic scaling and intrinsic plasticity can serve distinct functions in homeostatically regulating network dynamics.
Proc Natl Acad Sci U S A, 2020; 117
31366632: Torrado Pacheco A, Tilden EI, Grutzner SM, Lane BJ, Wu Y, Hengen KB, Gjorgjieva J, Turrigiano GG
Rapid and active stabilization of visual cortical firing rates across light-dark transitions.
The dynamics of neuronal firing during natural vision are poorly understood. Surprisingly, mean firing rates of neurons in primary visual cortex (V1) of freely behaving rodents are similar during prolonged periods of light and darkness, but it is unknown whether this reflects a slow adaptation to changes in natural visual input or insensitivity to rapid changes in visual drive. Here, we use chronic electrophysiology in freely behaving rats to follow individual V1 neurons across many dark-light (D-L) and light-dark (L-D) transitions. We show that, even on rapid timescales (1 s to 10 min), neuronal activity was only weakly modulated by transitions that coincided with the expected 12-/12-h L-D cycle. In contrast, a larger subset of V1 neurons consistently responded to unexpected L-D and D-L transitions, and disruption of the regular L-D cycle with 60 h of complete darkness induced a robust increase in V1 firing on reintroduction of visual input. Thus, V1 neurons fire at similar rates in the presence or absence of natural stimuli, and significant changes in activity arise only transiently in response to unexpected changes in the visual environment. Furthermore, although mean rates were similar in light and darkness, pairwise correlations were significantly stronger during natural vision, suggesting that information about natural scenes in V1 may be more strongly reflected in correlations than individual firing rates. Together, our findings show that V1 firing rates are rapidly and actively stabilized during expected changes in visual input and are remarkably stable at both short and long timescales.
Proc Natl Acad Sci U S A, 2019; 116

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RELATING LOCAL CONNECTIVITY AND GLOBAL DYNAMICS IN EXCITATORY-INHIBITORY NETWORKS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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One of the key questions in neuroscience is how the cortical connectivity structure determines the collective dynamics of neural activity. Two complementary approaches have been developed to address this question: (i) representing connectivity in terms of local statistics of excitatory-inhibitory motifs; (ii) representing connectivity through a global low-rank structure that determines the low-dimensional dynamics. It is however currently unclear how local connectivity statistics are related to the global structure and shape the low-dimensional activity. To bridge this gap, we map local EI statistics onto global statistics of low-rank connectivity and examine the dynamics. We consider a randomly connected, block-like network composed of excitatory and inhibitory subpopulations. Connections in each block are specified by cell-type-dependent statistics and consist of independent and reciprocal parts. We first determine the dominant eigenvalues and eigenvectors of the connectivity matrix, and show that the statistics of their entries universally obey a mixture of Gaussian distribution. We then approximate random EI networks by Gaussian-mixture low-rank networks and show that mean connectivity determines the dominant low-rank structure, which the reciprocal motifs further modify by modulating the dominant eigenvalue. Comparing the dynamics in the original EI network and their low-rank approximations, we find that the mean and variance of population activity closely match. In both cases, reciprocal motifs enhance feedback and induce dynamic state transition. Altogether, our analytical mapping of the local EI statistics to low-rank description provides an intuitive picture of how local connectivity statistics determine global low-dimensional dynamics and resulting computations.

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THE IMPACT OF SPARSITY ON THE DYNAMICS OF LOW-RANK RECURRENT NEURAL NETWORKS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Neural population dynamics are often highly coordinated, allowing task-related computations to be understood as neural trajectories through low-dimensional subspaces. How the connectivity and input structure give rise to such activity can be investigated with the aid of low-rank recurrent neural networks, a recently-developed class of computational models which offer a rich theoretical framework linking the underlying connectivity structure to emergent low-dimensional dynamics. However, this framework has so far relied on the assumption of all-to-all connectivity, while cortical networks are known to be highly sparse. Here we investigate the influence of sparsity on the dynamics of recurrent networks in which the underlying connectivity structure is low rank. We first analyse how the eigenvalue spectrum of low-rank connectivity matrices is modified when the network connections are sparsified, and then examine the implications for the dynamics. We find that in the presence of sparsity, the eigenspectra consist of a central bulk distribution and isolated outliers, analogously to matrices composed of a low-rank and a random, full-rank component. This analogy allows us to characterise distinct dynamical regimes of the sparsified low-rank network as a function of key network parameters. Altogether, we find that the low-dimensional dynamics induced by low-rank structure in the connectivity are preserved even at high levels of sparsity, and therefore give rise to highly robust computations.

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DYNAMICS CLOSE TO CRITICALITY SUPPORT LONG SYNAPTIC LIFETIMES IN CORTICAL CIRCUITS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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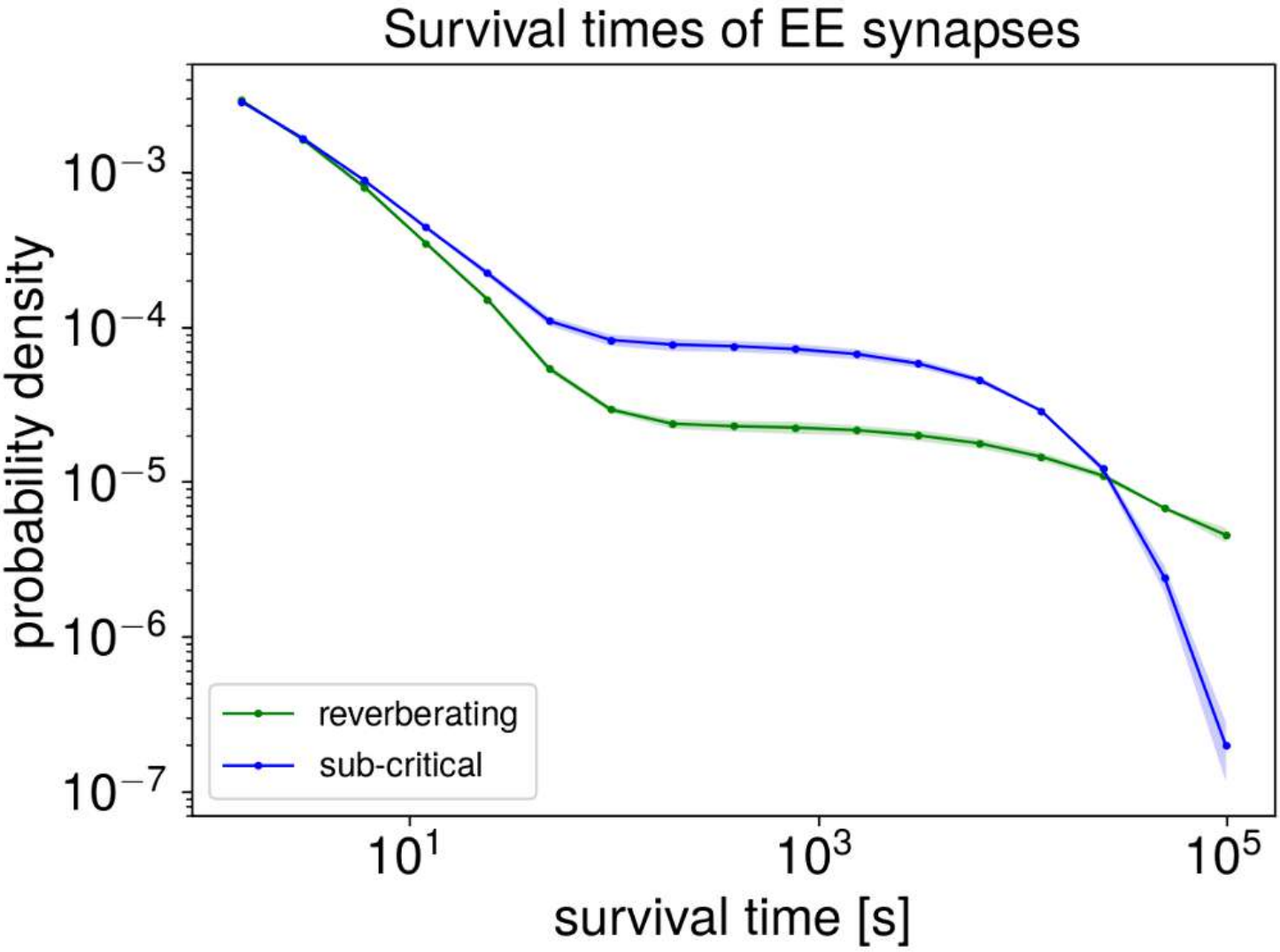
The connectome of the brain is highly dynamic, exhibiting high turnover of synaptic connections even under basal conditions. Nevertheless, our brains are able to maintain life-long memories. How are such memories formed and safeguarded in such a dynamic environment? We hypothesize that plastic recurrent neural networks can generate self-sustaining connectivity patterns if they operate in the reverberating regime close to criticality. To test this hypothesis, we simulate spiking neural networks with spike timing-dependent, homeostatic, and structural plasticity. Structural plasticity creates new excitatory-to-excitatory (EE) synapses and randomly prunes those below a certain threshold. We find that the reverberating regime promotes long survival times of EE synapses, while the sub-critical regime shows more pruning/creation events and a lack of very long synaptic life times. Furthermore, we find that random Kesten processes, a popular model of synaptic fluctuations, reproduce the short survival times of the sub-critical regime but fail to account for the long synaptic life times of the reverberating regime. Together, these findings indicate that long synaptic and memory life times may be explained by self-sustaining synaptic weight patterns shaping network activity so as to reinforce their own existence.



The reverberating regime (green, branching factor m≥0.9) promotes long survival times of excitatory-to-excitatory synapses. In contrast, the sub-critical regime (blue, branching factor m~0.3) is characterized by frequent creation/pruning events, leading to more synapses with short and medium survival times. The shaded area indicates the standard deviation from the mean calculated using three random initializations of the simulation for each regime.

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META-LEARNT PLASTICITY RULES IN SPIKING NETWORKS AND THEIR IMPLICATIONS FOR NEUROSCIENCE RESEARCH

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Synaptic plasticity is essential for learning and memory throughout the animal kingdom. However, the link between changes at individual synapses and emerging network-level computations remains obtuse. Theoretical studies model synaptic plasticity with plasticity rules, collapsing various molecular contributions into the evolution of a scalar synaptic weight across time. Deriving a complete set of functional plasticity rules analytically in a spiking neural network (SNNs) remains largely out of reach. Here, we approach the problem numerically (Confavreux *et al.* NeurIPS 2020), and meta-learn plasticity rules in large SNNs in silico to solve memory tasks. In our attempt to meta-learn and interpret plasticity rules, we use an evolutionary strategy (CMA-ES) to recover rules that robustly solve a familiarity detection task in a biologically plausible way. We discuss the challenges in designing meta-objectives that combine performance while ensuring biologically relevant solutions. We compare two search spaces for plasticity rules (both comprising around 100 meta-parameters). One is a polynomial with interpretable terms, considering quantities classically involved in plasticity: spike timing, spike history, membrane potential *etc*. The other is a multilayer perceptron, combining the same variables as the polynomial search space in a non interpretable, more flexible way. These search spaces give rise to different solutions. To understand and compare these high-dimensional meta-learnt rules, we use the covariance matrix learned along the optimisation with CMA-ES. Preliminary analysis revealed that the learned rules used inhibitory plasticity both for stability and computation, and operated mainly via codependent terms, corroborating recent theoretical work.

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STRUCTURAL PLASTICITY WITH A GAUSSIAN ACTIVITY RULE PREDICTS DEPRIVATION-INDUCED NETWORK REMODELING

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Aims: Homeostatic plasticity is fundamental for neuronal networks to maintain network activity within a dynamic range. In this context, structural synaptic plasticity—including changes in spine sizes, densities, and synapse numbers—is not consistently regulated in a homeostatic manner as seen for the synaptic weights. Increased network activity induces homeostatic down-scaling of excitatory neurotransmission and spine density reduction. Conversely, a decrease in network activity increases excitatory synaptic weights, while either reducing, increasing, or not changing spine densities. To reconcile the rich scenarios of activity-dependent structural plasticity, we proposed a homeostatic structural plasticity model with a Gaussian law with two firing activity setpoints. **Methods**: We studied the divergent behaviors of the Gaussian rule in a large Brunel neural network with spiking neurons in response to excitation and input deprivation to the excitatory subpopulation. **Results**: Simulations showed that this model maintains homeostatic properties upon stimulation whereas formation or loss of dendritic spines is observed depending on the extent of deprivation. When dendritic spines are lost due to silencing or denervation, homeostatic synaptic up-scaling or weak external stimulation rescues firing activity and pushes the structural dynamics into regeneration. **Conclusions**: Our study suggests that (i) a Gaussian structural plasticity rule predicts divergent activity-dependent structural remodeling following activity perturbations, (ii) homeostatic synaptic scaling and homeostatic structural plasticity are not redundant but complementary under certain conditions. i.e., after deprivation. (iii) Applying weak stimulation achieves similar effects and prevents partially denervated neurons from disconnecting from the network—with important implications for clinically applied (non-)invasive brain stimulation techniques.

Pubmed:
30778732: Humo M, Lu H, Yalcin I
The molecular neurobiology of chronic pain-induced depression.
The increasing number of individuals with comorbidities poses an urgent need to improve the management of patients with multiple co-existing diseases. Among these comorbidities, chronic pain and mood disorders, two long-lasting disabling conditions that significantly reduce the quality of life, could be cited first. The recent development of animal models accelerated the studies focusing on the underlying mechanisms of the chronic pain and depression/anxiety comorbidity. This review provides an overview of clinical and pre-clinical studies performed over the past two decades addressing the molecular aspects of the comorbid relationship of chronic pain and depression. We thus focused on the studies that investigated the molecular characteristics of the comorbid relationship between chronic pain and mood disorders, especially major depressive disorders, from the genetic and epigenetic point of view to key neuromodulators which have been shown to play an important role in this comorbidity.
Cell Tissue Res, 2019; 377
31637332: Lu H, Gallinaro JV, Rotter S
Network remodeling induced by transcranial brain stimulation: A computational model of tDCS-triggered cell assembly formation.
Transcranial direct current stimulation (tDCS) is a variant of noninvasive neuromodulation, which promises treatment for brain diseases like major depressive disorder. In experiments, long-lasting aftereffects were observed, suggesting that persistent plastic changes are induced. The mechanism underlying the emergence of lasting aftereffects, however, remains elusive. Here we propose a model, which assumes that tDCS triggers a homeostatic response of the network involving growth and decay of synapses. The cortical tissue exposed to tDCS is conceived as a recurrent network of excitatory and inhibitory

neurons, with synapses subject to homeostatically regulated structural plasticity. We systematically tested various aspects of stimulation, including electrode size and montage, as well as stimulation intensity and duration. Our results suggest that transcranial stimulation perturbs the homeostatic equilibrium and leads to a pronounced growth response of the network. The stimulated population eventually eliminates excitatory synapses with the unstimulated population, and new synapses among stimulated neurons are grown to form a cell assembly. Strong focal stimulation tends to enhance the connectivity within new cell assemblies, and repetitive stimulation with well-chosen duty cycles can increase the impact of stimulation even further. One long-term goal of our work is to help in optimizing the use of tDCS in clinical applications.
Netw Neurosci, 2019; 3

27695433: Wang K, Lu H, Cheung EF, Neumann DL, Shum DH, Chan RC
"Female Preponderance" of Depression in Non-clinical Populations: A Meta-Analytic Study.
Clinical observations and research suggest a female preponderance in major depressive disorder. However, it is unclear whether a similar gender difference is found for the reporting of depressive symptoms in non-clinical populations. The present meta-analysis was conducted to address this issue. We searched for published papers targeting non-clinical populations in which the 21-item Beck Depression Inventory (BDI) was used. Eighty-four papers (91 studies) published between 1977 and 2014 were included in the final meta-analysis, which comprised 23,579 males and 29,470 females. Females in the general population reported higher level of depressive symptoms than males (= -0.187, corresponding to 1.159 points in the 21-item BDI). This pattern was not found to influence by years of publication, socioeconomic status, or version of the BDI used. Using age group as a moderator, studies with adolescents and young adults were found to show a smaller effect size than studies with older participants. Our results appear to confirm the "female preponderance" in the level of self-report depressive symptoms in the general population, and support the social gender role theory in explaining gender difference over biological susceptibility theory and evolutionary theory.
Front Psychol, 2016; 7

34607362: Lu H, Gallinaro JV, Normann C, Rotter S, Yalcin I
Time Course of Homeostatic Structural Plasticity in Response to Optogenetic Stimulation in Mouse Anterior Cingulate Cortex.
Plasticity is the mechanistic basis of development, aging, learning, and memory, both in healthy and pathological brains. Structural plasticity is rarely accounted for in computational network models due to a lack of insight into the underlying neuronal mechanisms and processes. Little is known about how the rewiring of networks is dynamically regulated. To inform such models, we characterized the time course of neural activity, the expression of synaptic proteins, and neural morphology employing an in vivo optogenetic mouse model. We stimulated pyramidal neurons in the anterior cingulate cortex of mice and harvested their brains at 1.5 h, 24 h, and $48\,\mathrm{h}$ after stimulation. Stimulus-induced cortical hyperactivity persisted up to 1.5 h and decayed to baseline after $24\,\mathrm{h}$ indicated by c-Fos expression. The synaptic proteins VGLUT1 and PSD-95, in contrast, were upregulated at $24\,\mathrm{h}$ and downregulated at $48\,\mathrm{h}$, respectively. Spine density and spine head volume were also increased at $24\,\mathrm{h}$ and decreased at $48\,\mathrm{h}$. This specific sequence of events reflects a continuous joint evolution of activity and connectivity that is characteristic of the model of homeostatic structural plasticity. Our computer simulations thus corroborate the observed empirical evidence from our animal experiments.
Cereb Cortex, 2022; 32

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A MODEL OF THE TEMPORAL DYNAMICS OF THE SONGBIRD'S PREMOTOR NUCLEUS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Aim: Temporal processing in the range of tens to hundreds of milliseconds is critical for sensory tasks such as interval and duration discrimination, whereas temporal control is crucial for the generation of a wide range of actions. In the setting of the acquisition and performance of a complex sensorimotor task, we aim to understand the underlying mechanisms encoding timing. Methods: Zebra finches' premotor nucleus HVC (proper name) is responsible for the precise control of song timing. To model the sequential pattern of neuronal activity in the HVC, we use attractor dynamics, the Ring Model. Thereafter, we use a reward covariance learning rule (Williams, 1992) to investigate the behaviour of the model in a widely used lab conditioning paradigm. Results: We show that the ring model is able to robustly reproduce the activity of HVC neurons observed in recordings. Moreover, the duration of a syllable can be modified in either direction (lengthened or shortened) and, consistent with behavioral data, the change in duration is specific to the targeted syllable. Conclusion: Although, current computational models of HVC rely on synfire chains, we propose that it may be modelled using the ring attractor (Zhang, 1996; Hansel and Sompolinsky, 1998), which is more robust and can sustain a localized activity profile for a wide range of weights. Using reinforcement learning, we acquire a duration change consistent with experimental results. Lastly, we derive a prediction from the model, showing that a local inhibition in HVC only perturbs and delays song initiation, while singing is sustained.

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BOARD NUMBER: S02-569

CONTROL OF WORKING MEMORY IN A RECURRENT NEURAL NETWORK

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Aim. We aimed to model and understand how retrospective cues (retrocues) benefit performance and drive neural geometry during multi-item delayed recall tasks. A recent study in macaques studied the neural patterns that stimuli evoke before (pre-cue) and after (post-cue) retrocue onset. They found that in the pre-cue period, individual stimuli were maintained in short-term memory in orthogonal neural subspaces, whereas in the post-cue period, the prioritized items were rotated into a parallel subspace. To understand how such representational shifts can be learnt through error minimisation, we trained recurrent neural networks (RNNs) to perform an equivalent task. *Methods*. RNNs were trained with supervision to perform a cued-recall task. Networks were presented with two conjunctive colour-location stimuli, followed by a *pre-cue* memory delay*,* a retro-cue*,* and a *post-cue* memory delay. We used dimensionality reduction methods to analyse the neural geometry of the cued item representations formed in the delay periods. *Results and conclusions*. We found that the neural geometry previously observed in monkeys emerged naturally in RNNs optimized to perform the task. This is in accordance with a growing literature showing that neural networks project information into orthogonal subspaces to minimize interference, and into parallel subspaces to permit readout generalisation. Additionally, only RNNs trained with longer *post-cue* delay intervals learnt to transform the cued item information from orthogonal to parallel subspaces. This suggests that the parallel geometry might aid robust memory maintenance. Overall, our findings are consistent with the view that retro-cues benefit performance by transforming memories into a prospective, action-oriented format.

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SEGREGATED NETWORKS ARE MORE PRONE TO SYNERGY AND DYNAMICAL FUNCTIONAL CONNECTIVITY

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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How the brain generates complex behavior –metastable dynamics, synergistic interactions– has many standing issues to be answered. As the brain ages, decreased structural segregation correlates with less dynamic and more redundant functional connectivity (FC). Structural and dynamical features have been identified as sources of rich dynamics: modular/hierarchical structure, time delays, and others. However, a systematic assessment of the relationship between topology and landmarks of complex behavior has not been undertaken. We built a series of structural matrices of different nature: small-world, scale-free, modular, and hierarchical, spanning a range of topological measures. Then, dynamics were simulated using either a neural mass model or an oscillatory neuron model sweeping the global connectivity parameter G. Dynamics were assessed for dynamic FC (dFC) and for the emergence of high-order statistics (redundancy, synergy) in triplets and quintuplets of nodes. For dFC, the variance of the dFC matrix was taken as metastability index. High-order statistics was measured using the information theory-based O-information, that discriminates between synergy- and redundancy-predominating interactions. Metastability and synergy were more frequently observed in segregated networks and in those classified as regular by the small-world index ω. Networks of random nature (including scale-free networks) display a steeper transition to synchrony and more redundant interactions. Synergistic interactions are more frequent in triplets with few connections, while redundancy governs when measured in more connected tuples. We show for the first time that synergistic interactions emerge more frequently with metastable dynamics, contributing to understand how complex behavior relates to structural constraints.

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BOARD NUMBER: S02-571

CHARACTERIZATION OF NEURAL NETWORK DYNAMICS: INSIGHTS FROM DISSIPATION

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Aim: Models of neural networks are often built by using single-neuron models describing neural excitability interacting together with appropriate connectivity. Different dynamical behaviors observed in experiments may be reproduced by varying parameters. Such large networks may have highly chaotic dynamics that are very difficult to understand, even with a good knowledge of the individual modeled neurons that constitute it. We study these dynamics and focus on the fluctuations around the time average activity and how they can influence the response of the network to perturbations.
Method: We ran numerous simulations with different neural networks models. Inspired by statistical physics, we consider dissipation, an observable that gives the contraction of phase space around a given point of the dynamics.

Results: A stable network has on average a negative dissipation, meaning that fluctuations converge to a global attractor of the trajectories, but punctual positive dissipation can arise too and show how the system can fluctuate. We observed that dissipations are correlated to the response of a system to perturbations. We identified that the connectivity of each individual single neuron may strongly change their impact on the global dynamics as their dissipation differs. We specifically found that positive dissipation has an important impact on the dynamic and can help characterize different models, even when they have similar firing rates.

Conclusion: Our results show that dissipation can be used to characterize more accurately neural networks dynamics, and may help to understand the intrinsic activity leading to the global behavior observed.

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FACILITATING SYNAPSES AND BROAD RECURRENT CONNECTIONS CAN INCREASE THE SELECTIVITY OF CORTICAL POPULATIONS TO SENSORY STIMULI

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Even the simpler sensory stimuli trigger the activation of large groups of neurons in the cortex. This finding has motivated the hypothesis that the neural representation of sensory stimuli is encoded at the population level. The particular activity pattern of a cortical neuronal population in response to a sensory stimulus is determined by the characteristics of the circuit: its feed-forward and recurrent connections, the type of synaptic dynamics, or the degree of excitation-inhibition balance. However, the causal relationship between each of these circuit features and its impact on the population tuning response remains difficult to study in experimental settings. Here, we propose a theoretical framework to dissect the role of recurrent connectivity and synaptic plasticity in the establishment of the population tuning properties. This framework relies on the assumption of balanced excitation and inhibition, a phenomenon that has been observed in cortical circuits. Using mean-field models and spiking network simulations we show that the type of synapses controls the width of the population tuning — facilitating synapses increase the network selectivity by decreasing tuning width while depressing synapses have the opposite effect. We also demonstrate that the width of the recurrent connections plays a crucial role in the regulation of the population tuning width. Counter-intuitively, the broader the recurrent connections extend in space, the lower the width of the population tuning. These results lead to specific experimentally-testable predictions and map the physiology of recurrency and plasticity to the population response to sensory stimuli.

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MANIFOLD PROPAGATION THROUGH RECURRENT NEURAL NETWORKS

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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For a long time neuroscientists have attempted to understand neural computation through simplified neural network models. Recurrent neural networks (RNNs) with rate dynamics are one of the simplest examples of such models. Due to their conceptual and mathematical simplicity, a large amount of effort has been spent on understanding their formal properties. Another area that has gained significant traction in the last few years is that of neural manifolds, which allow one to think of the activity of neural populations as tracing out a surface in high dimensional space. The geometry and topology of such a surface are informative of the nature of computation in the neural population, but it has been difficult to relate the structure of a neural network to that of the manifolds it produces. In this work we address this problem by studying projection mappings from a manifold with a particular topology to RNNs with differing weight matrix structures. We then study how the topological and geometric properties of neural manifolds change through time depending on the properties of a network. We study two main properties that determine the network dynamics, the first being the eigenvalue spectrum of the weight matrix and the second - the orientation of the projection mapping from the stimulus manifold to the network with respect to the weight basis. By simulating neural network dynamics using random RNNs we seek conditions that allow for the topology of a neural manifold to be retrieved from a network.

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MODELING EFFECTS OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION PROTOCOLS IN RECURRENT NEURAL NETWORKS WITH HOMEOSTATIC STRUCTURAL PLASTICITY: EXPLORING THE RTMS PARAMETER SPACE

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Aims: rTMS is a non-invasive brain stimulation technique that induces neuronal plasticity in the human cortex. Despite its clinical use, mechanisms through which rTMS affects neural networks remain elusive. To complement our experimental efforts and explore the vast parameter space of rTMS, we employed a computational approach to explore the impact of stimulation parameters on network dynamics and connectivity. **Methods:** rTMS was modeled as a train of brief and strong depolarizations of the somatic membrane potential. We explored the effects of rTMS on network dynamics and remodeling in a balanced recurrent network with and without homeostatic structural plasticity (HSP). When subject to HSP, excitatory neurons dynamically form/retract synapses with each other to homeostatically maintain their firing-rate. **Results:** Simulations of static networks showed that rTMS influenced network dynamics (i.e.,neural firing-rate) in a frequency- and intensity-dependent manner. The firing-rate versus intensity relationship was found to be non-linear. We suspect feedback-inhibition as a cause of this non-linearity. Plastic network simulations supported a similar relationship between stimulation intensity and network remodeling. In the plastic network, we further observed an increase and saturation of connectivity after “long-enough” stimulation at a given frequency. **Conclusion:** Our simulations confirmed that the exact protocol design has a profound impact on network activity and resulting remodeling. We identified an overstimulation risk in terms of both intensity and duration. Intensities above an ‘optimal’ value cause detrimental effects, and durations exceeding a certain length do not contribute to further remodeling. rTMS initiated feedback-inhibition influences the net stimulation outcome, highlighting the role of inhibitory neurons in rTMS induced plasticity.

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ENTROPY, FREE ENERGY, SYMMETRY AND DYNAMICS IN THE BRAIN

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Neuroscience is home to concepts and theories with roots in a variety of domains including information theory, dynamical systems theory, and cognitive psychology. Not all of those can be coherently linked, some concepts are incommensurable, and domain-specific language poses an obstacle to integration. Still, conceptual integration is a form of understanding that provides intuition and consolidation, without which progress remains unguided. This work is concerned with the integration of deterministic and stochastic processes within an information theoretic framework, linking information entropy and free energy to mechanisms of emergent dynamics and self-organization in brain networks. We identify basic properties of neuronal populations leading to an equivariant matrix in a network, in which complex behaviors can naturally be represented through structured flows on manifolds establishing the internal model relevant to theories of brain function. We propose a neural mechanism for the generation of internal models from symmetry breaking in the connectivity of brain networks. The emergent perspective illustrates how free energy can be linked to internal models and how they arise from the neural substrate.

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SHAPING CIRCUIT CONNECTIVITY BY INHIBITION

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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It is widely accepted that activity-dependent plasticity plays a fundamental role in determining andreshaping connectivity in the brain. Numerous studies considered the effects of plasticity on smallgroups of neurons (circuit motifs), or larger populations, modeling how the interplay of plasticity rulesand correlated external inputs shapes circuit connectivity(Ocker et al.2015; Ocker and Doiron2019).For example, it has been shown that, even in the absence of structured external inputs, simple plasticityrules can give rise to organized structures, such as neural assemblies or feedforward chains(Montangieet al.2020; Tannenbaum and Burak2016). Most of these studies focus on the excitatory neural populations, and include an inhibitory componentfor the sole purpose of stabilizing the excitatory activity. However structured inhibitory interactionscan change the correlation statistics of the excitatory activity, which in turn determines the effect ofspike-timing dependent plasticity (STDP) on the circuit. In this work we use both analytic and numerical approaches to account for the role of inhibition inshaping connectivity through STDP rules. We consider small-scale circuit motifs that include inhibitorycomponents, and we show how the presence of inhibitory interactions influences the firing patternsof the excitatory population, and what are the consequences on circuit connectivity when plasticity ispresent.

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MECHANISMS OF PLASTICITY FOR PUP CALL SOUNDS IN THE MATERNAL AUDITORY CORTEX

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Distress calls of mice pups outside their nest elicit pup retrieval in maternal female mice but not in their virgin ('naive') conspecifics. However, when co-housed with maternal mice, naive mice become 'experienced' and learn to perform pup retrieval. This process correlates with neuronal changes in the primary auditory cortex: While excitatory (E) neuron responses are sharply tuned to a certain inter- pup-call interval and inhibitory (I) neuron responses are broadly tuned in naive mice, the two neuron types are co-tuned in experienced mice. This change in behavior and tuning is mediated by oxytocin (Schiavo et al., 2020). Here, we aim to dissect the underlying mechanisms behind the behaviorally-relevant changes in tuning from naive to experienced mice by combining computational modeling and in-vitro experiments. Using optogenetic targeting of somatostatin-positive (SST) or parvalbumin-positive (PV) inhibitory neurons, we quantified short-term plasticity at SST-to-E and PV-to-E connections. Furthermore, pairing experiments reveal sufficient long-term plasticity at SST-to-E but not PV-to-E connections. Using a model, we study the interaction of three neuron populations with synapses experiencing short- and long-term plasticity. We show that 1) short-term plasticity leads to the tuning of excitatory and inhibitory neurons to inter-stimulus intervals; 2) oxytocin-gated long-term plasticity of E-to-E and SST-to-E connections lead to changes in tuning from naive to experienced mice. Furthermore, 3) short-term plasticity can control the signal amplitude without changing the tuning properties. Our results reveal that short- and long-term plasticity cooperate to generate tuning of excitatory and inhibitory neurons in microcircuits with important implications for maternal behavior.

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BOARD NUMBER: S02-578

ADDING DENDRITES TO SPIKING NEURAL NETWORKS WITH DENDRIFY

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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Computational modelling has been indispensable for understanding how subcellular features of neurons can influence circuit processing. Yet, the influence of dendritic computations on network-level operations remains largely unexplored. This is partly because existing tools do not allow the development of realistic and efficient network models that account for dendrites. Current spiking neural networks, although efficient, are usually overly simplistic, overlooking essential dendritic properties. Conversely, circuit models with morphologically detailed neuron models are computationally costly, thus impractical for large-network simulations. To bridge the gap between these two, we introduce *Dendrify*, an open-source Python package compatible with *Brian2,* designed to facilitate the development of bioinspired spiking neural networks. *Dendrify*, through simple commands, automatically generates reduced compartmental neuron models with realistic dendritic and synaptic integrative properties that match experimental findings. Such models strike a good balance between flexibility, performance, and biological accuracy, allowing us to explore dendritic contributions to network-level functions while paving the way for the development of more powerful neuromorphic systems.

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BOARD NUMBER: S02-579

THE OPTIMAL STATE FOR COMPUTATION CHANGES DEPENDING ON THE AVAILABLE PROCESSING TIME

POSTER SESSION 02 - SECTION: NEURAL DYNAMICS AND NETWORK COMPUTATIONS

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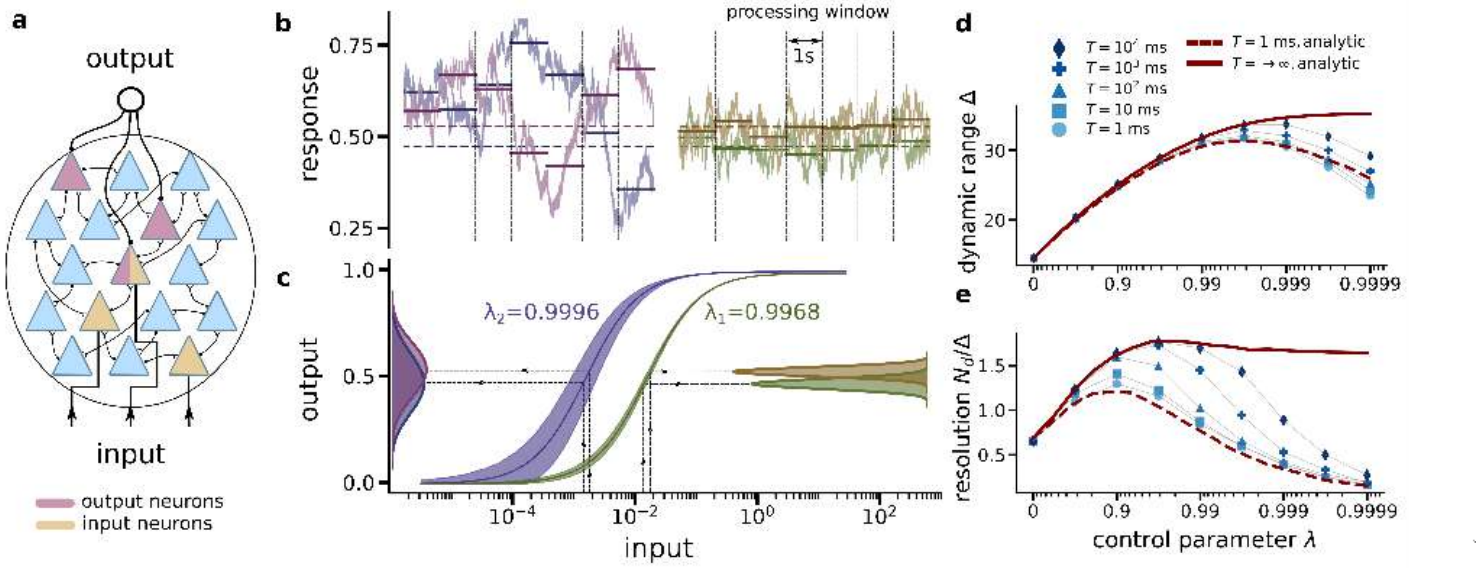
Common estimates of information processing capabilities such as the dynamic range are defined in terms of expected values that do not incorporate effects from finite processing time. However, in real-world tasks, animals and humans have only a limited amount of time to process input and make a decision. Here, using a stochastic neural network with integrate-and-fire units as an example, we propose new definitions for dynamic range and perceptual resolution that explicitly include uncertainty arising from the finite processing time. We evaluate these measures both analytically and using simulations and find that the network's ability to discriminate inputs within a finite time is maximized in a subcritical regime. Interestingly, with a longer processing time, the optimal regime moves closer to criticality (Fig.1d-e). This result highlights the importance of incorporating the constraints of finite processing time when studying the information processing capacity of any system in noisy real-world situations. (for a decent-quality figure visit https://www.dropbox.com/s/d7fazthbgoa1hr5/FENS_highQuality.png?dl=0)



FENS Forum 2022
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BOARD NUMBER: S02-580

V2A RETICULOSPINAL NEURONS IN THE MEDULLA ENCODE SPEED AND DURATION OF EXPLORATORY LOCOMOTION

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Aims: In the absence of direct sensory stimuli, animals locomote in a way adapted to their internal needs to explore their environment. In all vertebrates, these commands are driven by the mesencephalic locomotor region (MLR) that controls forward locomotion through the reticulospinal neurons (RSNs) that directly contact the spinal effector circuits. However, the nature of the RSNs the MLR recruits to initiate and modulate forward locomotion remains obscure due to the heterogeneity and difficulty to access these systems. Here, we leverage the optically and genetically accessible larval zebrafish to investigate the recruitment of a genetically-defined population of RSNs conserved across vertebrates, referred to as V2a RSNs, during MLR-induced forward swimming. Methods: We monitored the calcium activity of V2a neurons in the medulla while electrically stimulating the MLR to elicit forward locomotion in head-embedded larval zebrafish. Results: We identified that 20% of the medial V2a RSNs in the medulla were recruited during MLR-induced forward swims. Although a subset of these neurons maintained their activity throughout the duration of the swim, the kinematic of the calcium responses differed. Using multiple linear regression to model activity with motor regressors, we found that a cluster of V2a RSNs in the caudal medulla encoded locomotor frequency and number of oscillations, while another cluster in the rostral medulla encoded locomotor frequency and the increase in the movement amplitude. Conclusions: Our results reveal that a previously unappreciated population of V2a RSNs in the medulla act as maintain neurons encoding speed and duration during MLR induced exploratory locomotion.

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BOARD NUMBER: S02-581

TARGETED ACTIVATION OF MIDBRAIN NEURONS RESTORES LOCOMOTOR FUNCTION IN MOUSE MODELS OF PARKINSONISM

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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The pedunculopontine nucleus (PPN) is together with the cuneiform nucleus (CnF) a locomotor command area containing glutamatergic neurons that control locomotor initiation, speed and maintenance. These motor actions are deficient in Parkinson's disease (PD), where dopaminergic neurodegeneration alters basal ganglia activity. Of the two locomotor-initiating areas, the PPN is directly downstream of the basal ganglia and it is therefore a suitable target for ameliorating parkinsonian motor symptoms. In this study we have used in vivo cell-type specific PPN activation to recover motor function in mouse models of parkinsonism made by acute pharmacological blockage of dopamine transmission. With a combination of chemo- and opto-genetics, we show that excitation of caudal glutamatergic PPN neurons can induce locomotion and is sufficient to normalize the otherwise severe locomotor deficit observed in parkinsonian mice. Targeting the local GABAergic population only leads to partial recovery. The motor rescue driven by glutamatergic PPN activation is independent of activity in nearby locomotor promoting glutamatergic Cuneiform area. Moreover, the locomotor phenotype induced by caudal glutamatergic PPN in parkinsonian mice is proficient and shares all the characteristics of normal locomotion found in wild type mice. Our observations point to caudal glutamatergic PPN neurons as a potential target for neuromodulatory restoration of locomotor function in PD. This work was supported by the Lundbeck Foundation and the Novo Foundation.

Pubmed:

35135875: Smedler E, Louhivuori L, Romanov RA, Masini D, Dehnisch Ellström I, Wang C, Caramia M, West Z, Zhang S, Rebellato P, Malmersjö S, Brusini I, Kanatani S, Fisone G, Harkany T, Uhlén P
Disrupted gene expression perturbs spontaneous Ca activity causing abnormal brain development and increased anxiety. The L-type voltage-gated Ca channel gene is a risk gene for various psychiatric conditions, including schizophrenia and bipolar disorder. However, the cellular mechanism by which contributes to psychiatric disorders has not been elucidated. Here, we report that the embryonic deletion of in neurons destined for the cerebral cortex using an strategy disturbs spontaneous Ca activity and causes abnormal brain development and anxiety. By combining computational modeling with electrophysiological membrane potential manipulation, we found that neural network activity was driven by intrinsic spontaneous Ca activity in distinct progenitor cells expressing marginally increased levels of voltage-gated Ca channels. MRI examination of the knockout mouse brains revealed volumetric differences in the neocortex, hippocampus, and periaqueductal gray. These results suggest that acts as a molecular switch and that its disruption during embryogenesis can perturb Ca handling and neural development, which may increase susceptibility to psychiatric disease.
Proc Natl Acad Sci U S A, 2022; 119
35082287: Masini D, Kiehn O
Targeted activation of midbrain neurons restores locomotor function in mouse models of parkinsonism.
The pedunculopontine nucleus (PPN) is a locomotor command area containing glutamatergic neurons that control locomotor initiation and maintenance. These motor actions are deficient in Parkinson's disease (PD), where dopaminergic neurodegeneration alters basal ganglia activity. Being downstream of the basal ganglia, the PPN may be a suitable target for ameliorating parkinsonian motor symptoms. Here, we use in vivo cell-type specific PPN activation to restore motor function in two mouse models of parkinsonism made by acute pharmacological blockage of dopamine transmission. With a combination of chemo- and opto-genetics, we show that excitation of caudal glutamatergic PPN neurons can normalize the otherwise severe locomotor deficit in PD, whereas targeting the local GABAergic population only leads to recovery of slow locomotion. The motor rescue driven by glutamatergic PPN activation is independent of activity in nearby locomotor promoting glutamatergic Cuneiform neurons. Our observations point to caudal glutamatergic PPN neurons as a potential target for neuromodulatory restoration of locomotor function in PD.
Nat Commun, 2022; 13

34070345: Masini D, Plewnia C, Bertho M, Scalbert N, Caggiano V, Fisone G
A Guide to the Generation of a 6-Hydroxydopamine Mouse Model of Parkinson's Disease for the Study of Non-Motor Symptoms.
In Parkinson's disease (PD), a large number of symptoms affecting the peripheral and central nervous system precede, develop in parallel to, the cardinal motor symptoms of the disease. The study of these conditions, which are often refractory to and may even be exacerbated by standard dopamine replacement therapies, relies on the availability of appropriate animal models. Previous work in rodents showed that injection of the neurotoxin 6-hydroxydopamine (6-OHDA) in discrete brain regions reproduces several non-motor comorbidities commonly associated with PD, including cognitive deficits, depression, anxiety, as well as disruption of olfactory discrimination and circadian rhythm. However, the use of 6-OHDA is frequently associated with significant post-surgical mortality. Here, we describe the generation of a mouse model of PD based on bilateral injection of 6-OHDA in the dorsal striatum. We show that the survival rates of males and females subjected to this lesion differ significantly, with a much higher mortality among males, and provide a protocol of enhanced pre- and post-operative care, which nearly eliminates animal loss. We also briefly discuss the utility of this model for the study of non-motor comorbidities of PD.
Biomedicines, 2021; 9
29686643: Masini D, Bonito-Oliva A, Bertho M, Fisone G
Inhibition of mTORC1 Signaling Reverts Cognitive and Affective Deficits in a Mouse Model of Parkinson's Disease.
Non-motor symptoms, including cognitive deficits and affective disorders, are frequently diagnosed in Parkinson's disease (PD) patients and are only partially alleviated by dopamine replacement therapy. Here, we used a 6-hydroxydopamine (6-OHDA) mouse model of PD to examine the effects exerted on non-motor symptoms by inhibition of the mammalian target of rapamycin complex 1 (mTORC1), which is involved in the control of protein synthesis, cell growth, and metabolism. We show that rapamycin, which acts as an allosteric inhibitor of mTORC1, counteracts the impairment of novel object recognition. A similar effect is produced by PF-4708671, an inhibitor of the downstream target of mTORC1, ribosomal protein S6 kinase (S6K). Rapamycin is also able to reduce depression-like behavior in PD mice, as indicated by decreased immobility in the forced swim test. Moreover, rapamycin exerts anxiolytic effects, thereby reducing thigmotaxis in the open field and increasing exploration of the open arm in the elevated plus maze. In contrast to rapamycin, administration of PF-4708671 to PD mice does not counteract depression- and anxiety-like behaviors. Altogether, these results identify mTORC1 as a target for the development of drugs that, in combination with standard antiparkinsonian agents, may widen the efficacy of current therapies for the cognitive and affective symptoms of PD.
Front Neurol, 2018; 9
28398344: Rivetti di Val Cervo P, Romanov RA, Spigolon G, Masini D, Martín-Montañez E, Toledo EM, La Manno G, Feyder M, Pifl C, Ng YH, Sánchez SP, Linnarsson S, Wernig M, Harkany T, Fisone G, Arenas E
Induction of functional dopamine neurons from human astrocytes in vitro and mouse astrocytes in a Parkinson's disease model.
Cell replacement therapies for neurodegenerative disease have focused on transplantation of the cell types affected by the pathological process. Here we describe an alternative strategy for Parkinson's disease in which dopamine neurons are generated by direct conversion of astrocytes. Using three transcription factors, NEUROD1, ASCL1 and LMX1A, and the microRNA miR218, collectively designated NeAL218, we reprogram human astrocytes in vitro, and mouse astrocytes in vivo, into induced dopamine neurons (iDANs). Reprogramming efficiency in vitro is improved by small molecules that promote chromatin remodeling and activate the TGFβ, Shh and Wnt signaling pathways. The reprogramming efficiency of human astrocytes reaches up to 16%, resulting in iDANs with appropriate midbrain markers and excitability. In a mouse model of Parkinson's disease, NeAL218 alone reprograms adult striatal astrocytes into iDANs that are excitable and correct some aspects of motor behavior in vivo, including gait impairments. With further optimization, this approach may enable clinical therapies for Parkinson's disease by delivery of genes rather than cells.
Nat Biotechnol, 2017; 35
25221486: Bonito-Oliva A, Masini D, Fisone G
A mouse model of non-motor symptoms in Parkinson's disease: focus on pharmacological interventions targeting affective dysfunctions.
Non-motor symptoms, including psychiatric disorders, are increasingly recognized as a major challenge in the treatment of Parkinson's disease (PD). These ailments, which often appear in the early stage of the disease, affect a large number of patients and are only partly resolved by conventional antiparkinsonian medications, such as L-DOPA. Here, we investigated non-motor symptoms of PD in a mouse model based on bilateral injection of the toxin 6-hydroxydopamine (6-OHDA) in the dorsal striatum. This model presented only subtle gait modifications, which did not affect horizontal motor activity in the open-field test. Bilateral 6-OHDA lesion also impaired olfactory discrimination, in line with the anosmia typically observed in early stage parkinsonism. The effect of 6-OHDA was then examined for mood-related dysfunctions. Lesioned mice showed

increased immobility in the forced swim test and tail suspension test, two behavioral paradigms of depression. Moreover, the lesion exerted anxiogenic effects, as shown by reduced time spent in the open arms, in the elevated plus maze test, and by increased thigmotaxis in the open-field test. L-DOPA did not modify depressive- and anxiety-like behaviors, which were instead counteracted by the dopamine D2/D3 receptor agonist, pramipexole. Reboxetine, a noradrenaline reuptake inhibitor, was also able to revert the depressive and anxiogenic effects produced by the lesion with 6-OHDA. Interestingly, pre-treatment with desipramine prior to injection of 6-OHDA, which is commonly used to preserve noradrenaline neurons, did not modify the effect of the lesion on depressive- and anxiety-like behaviors. Thus, in the present model, mood-related conditions are independent of the reduction of noradrenaline caused by 6-OHDA. Based on these findings we propose that the anti-depressive and anxiolytic action of reboxetine is mediated by promoting dopamine transmission through blockade of dopamine uptake from residual noradrenergic terminals.
Front Behav Neurosci, 2014; 8

24291518: Mouton-Liger F, Sahún I, Collin T, Lopes Pereira P, Masini D, Thomas S, Paly E, Luilier S, Même S, Jouhault Q, Bennaï S, Beloeil JC, Bizot JC, Hérault Y, Dierssen M, Créau N
Developmental molecular and functional cerebellar alterations induced by PCP4/PEP19 overexpression: implications for Down syndrome.
PCP4/PEP19 is a modulator of Ca(2+)-CaM signaling. In the brain, it is expressed in a very specific pattern in postmitotic neurons. In particular, Pcp4 is highly expressed in the Purkinje cell, the sole output neuron of the cerebellum. PCP4, located on human chromosome 21, is present in three copies in individuals with Down syndrome (DS). In a previous study using a transgenic mouse model (TgPCP4) to evaluate the consequences of 3 copies of this gene, we found that PCP4 overexpression induces precocious neuronal differentiation during mouse embryogenesis. Here, we report combined analyses of the cerebellum at postnatal stages (P14 and adult) in which we identified age-related molecular, electrophysiological, and behavioral alterations in the TgPCP4 mouse. While Pcp4 overexpression at P14 induces an earlier neuronal maturation, at adult stage it induces increase in cerebellar CaMK2alpha and in cerebellar LTD, as well as learning impairments. We therefore propose that PCP4 contributes significantly to the development of Down syndrome phenotypes through molecular and functional changes.
Neurobiol Dis, 2014; 63

29342142: Caggiano V, Leiras R, Goñi-Erro H, Masini D, Bellardita C, Bouvier J, Caldeira V, Fisone G, Kiehn O
Midbrain circuits that set locomotor speed and gait selection.
Locomotion is a fundamental motor function common to the animal kingdom. It is implemented episodically and adapted to behavioural needs, including exploration, which requires slow locomotion, and escape behaviour, which necessitates faster speeds. The control of these functions originates in brainstem structures, although the neuronal substrate(s) that support them have not yet been elucidated. Here we show in mice that speed and gait selection are controlled by glutamatergic excitatory neurons (GlutNs) segregated in two distinct midbrain nuclei: the cuneiform nucleus (CnF) and the pedunculopontine nucleus (PPN). GlutNs in both of these regions contribute to the control of slower, alternating-gait locomotion, whereas only GlutNs in the CnF are able to elicit high-speed, synchronous-gait locomotion. Additionally, both the activation dynamics and the input and output connectivity matrices of GlutNs in the PPN and the CnF support explorative and escape locomotion, respectively. Our results identify two regions in the midbrain that act in conjunction to select context-dependent locomotor behaviours.
Nature, 2018; 553

28398338: Masini D, Lopes-Aguiar C, Bonito-Oliva A, Papadia D, Andersson R, Fisahn A, Fisone G
The histamine H3 receptor antagonist thioperamide rescues circadian rhythm and memory function in experimental parkinsonism.
Parkinson's disease (PD) is a common neurodegenerative disorder, characterized by motor impairment and a wide range of non-motor symptoms, including sleep disorders and cognitive and affective deficits. In this study, we used a mouse model of PD based on 6-hydroxydopamine (6-OHDA) to examine the effect of thioperamide, a histamine H3 receptor antagonist, on circadian activity, recognition memory and anxiety. A partial, bilateral 6-OHDA lesion of the striatum reduces motor activity during the active phase of the 24 h cycle. In addition, the lesion disrupts the endogenous circadian rhythm observed when mice are maintained in constant darkness. Administration of thioperamide to 6-OHDA-lesion mice rescues the normal rest/activity cycle. Moreover, thioperamide counteracts the deficit of novel object recognition produced by 6-OHDA. Our experiments show that this memory impairment is accompanied by disrupted gamma oscillations in the hippocampus, which are also rescued by thioperamide. In contrast, we do not observe any modification of the anxiogenic effect of 6-OHDA in response to administration of thioperamide. Our results indicate that thioperamide may act as a multifunctional drug, able to counteract disruptions of circadian rhythm and cognitive deficits associated with PD.
Transl Psychiatry, 2017; 7


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BOARD NUMBER: S02-582

ANATOMICAL AND FUNCTIONAL ORGANIZATION OF RED NUCLEUS CIRCUITS

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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[1]Friedrich Miescher Institute for Biomedical Research, Neurobiology, Basel, Switzerland, [2]University of Basel, Biozentrum, Basel, Switzerland

Many types of descending projection neurons distributed throughout the brain drive motor output and modulate distinct movement features. Isolating individual projection-specific circuit modules has recently proven successful to stratify spatially intermingled but functionally distinct subpopulations in the brainstem, opening new avenues to address long-standing questions in the field of motor control. Linking cortical and cerebellar output to spinal cord circuits, the red nucleus represents one of most ancient and conserved structures implicated in limb control and skill regulation in vertebrates. Rubrospinal neurons in the magnocellular division of the red nucleus have been shown to play a key role in processes ranging from controlling forelimb and digit mobilization to compensating for motor impairment following cortical lesions. Yet, the precise cellular and circuit mechanisms involved are not understood and their relevance in humans remains debated. In stark contrast, evidence for parvicellular rubral circuits organization and function are extremely sparse, leaving their contribution to movement control unknown. To address this century-old problem, we first use a combination of viral tracing and 3D cellular reconstruction approaches to map the topographic organization of red nucleus neurons and their full projection profiles. Importantly, this strategy identifies a previously uncharacterized rubral module projecting to limb control-specific brainstem structures. We then take advantage of novel anatomical entry points to study the function of rubral subpopulations through neuronal recordings, optogenetic manipulations, and behavioral analysis in freely moving mice. Altogether, this work reveals the first comprehensive functional map of rubral circuits and how it relates to the control of movement.

Pubmed:
34146485: Wu MY, Carbo-Tano M, Mirat O, Lejeune FX, Roussel J, Quan FB, Fidelin K, Wyart C
Spinal sensory neurons project onto the hindbrain to stabilize posture and enhance locomotor speed.
In the spinal cord, cerebrospinal fluid-contacting neurons (CSF-cNs) are GABAergic interoceptive sensory neurons that detect spinal curvature via a functional coupling with the Reissner fiber. This mechanosensory system has recently been found to be involved in spine morphogenesis and postural control but the underlying mechanisms are not fully understood. In zebrafish, CSF-cNs project an ascending and ipsilateral axon reaching two to six segments away. Rostralmost CSF-cNs send their axons ipsilaterally into the hindbrain, a brain region containing motor nuclei and reticulospinal neurons (RSNs), which send descending motor commands to spinal circuits. Until now, the synaptic connectivity of CSF-cNs has only been investigated in the spinal cord, where they synapse onto motor neurons and premotor excitatory interneurons. The identity of CSF-cN targets in the hindbrain and the behavioral relevance of these sensory projections from the spinal cord to the hindbrain are unknown. Here, we provide anatomical and molecular evidence that rostralmost CSF-cNs synapse onto the axons of large RSNs including Mauthner cells and V2a neurons. Functional anatomy and optogenetically assisted mapping reveal that rostral CSF-cNs also synapse onto the soma and dendrites of cranial motor neurons innervating hypobranchial muscles. During acousto-vestibular evoked escape responses, ablation of rostralmost CSF-cNs results in a weaker escape response with a decreased C-bend amplitude, lower speed, and deficient postural control. Our study demonstrates that spinal sensory feedback enhances speed and stabilizes posture, and reveals a novel spinal gating mechanism acting on the output of descending commands sent from the hindbrain to the spinal cord.
Curr Biol, 2021; 31
31291987: Bercier V, Hubbard JM, Fidelin K, Duroure K, Auer TO, Revenu C, Wyart C, Del Bene F
Dynactin1 depletion leads to neuromuscular synapse instability and functional abnormalities.
Dynactin subunit 1 is the largest subunit of the dynactin complex, an activator of the molecular motor protein complex dynein. Reduced levels of DCTN1 mRNA and protein have been found in sporadic amyotrophic lateral sclerosis (ALS) patients, and mutations have been associated with disease, but the role of this protein in disease pathogenesis is still unknown.
Mol Neurodegener, 2019; 14
28623664: Knafo S, Fidelin K, Prendergast A, Tseng PB, Parrin A, Dickey C, Böhm UL, Figueiredo SN, Thouvenin O, Pascal-

Moussellard H, Wyart C
Mechanosensory neurons control the timing of spinal microcircuit selection during locomotion.
Despite numerous physiological studies about reflexes in the spinal cord, the contribution of mechanosensory feedback to active locomotion and the nature of underlying spinal circuits remains elusive. Here we investigate how mechanosensory feedback shapes active locomotion in a genetic model organism exhibiting simple locomotion-the zebrafish larva. We show that mechanosensory feedback enhances the recruitment of motor pools during active locomotion. Furthermore, we demonstrate that inputs from mechanosensory neurons increase locomotor speed by prolonging fast swimming at the expense of slow swimming during stereotyped acoustic escape responses. This effect could be mediated by distinct mechanosensory neurons. In the spinal cord, we show that connections compatible with monosynaptic inputs from mechanosensory Rohon-Beard neurons onto ipsilateral V2a interneurons selectively recruited at high speed can contribute to the observed enhancement of speed. Altogether, our study reveals the basic principles and a circuit diagram enabling speed modulation by mechanosensory feedback in the vertebrate spinal cord.
Elife, 2017; 6
27524486: Sternberg JR, Severi KE, Fidelin K, Gomez J, Ihara H, Alcheikh Y, Hubbard JM, Kawakami K, Suster M, Wyart C
Optimization of a Neurotoxin to Investigate the Contribution of Excitatory Interneurons to Speed Modulation In Vivo.
Precise control of speed during locomotion is essential for adaptation of behavior in different environmental contexts [1-4]. A central question in locomotion lies in understanding which neural populations set locomotor frequency during slow and fast regimes. Tackling this question in vivo requires additional non-invasive tools to silence large populations of neurons during active locomotion. Here we generated a stable transgenic line encoding a zebrafish-optimized botulinum neurotoxin light chain fused to GFP (BoTxBLC-GFP) to silence synaptic output over large populations of motor neurons or interneurons while monitoring active locomotion. By combining calcium imaging, electrophysiology, optogenetics, and behavior, we show that expression of BoTxBLC-GFP abolished synaptic release while maintaining characterized activity patterns and without triggering off-target effects. As chx10(+) V2a interneurons (V2as) are well characterized as the main population driving the frequency-dependent recruitment of motor neurons during fictive locomotion [5-14], we validated our silencing method by testing the effect of silencing chx10(+) V2as during active and fictive locomotion. Silencing of V2as selectively abolished fast locomotor frequencies during escape responses. In addition, spontaneous slow locomotion occurred less often and at frequencies lower than in controls. Overall, this silencing approach confirms that V2a excitation is critical for the production of fast stimulus-evoked swimming and also reveals a role for V2a excitation in the production of slower spontaneous locomotor behavior. Altogether, these results establish BoTxBLC-GFP as an ideal tool for in vivo silencing for probing the development and function of neural circuits from the synaptic to the behavioral level.
Curr Biol, 2016; 26
27306044: Hernandez O, Papagiakoumou E, Tanese D, Fidelin K, Wyart C, Emiliani V
Three-dimensional spatiotemporal focusing of holographic patterns.
Two-photon excitation with temporally focused pulses can be combined with phase-modulation approaches, such as computer-generated holography and generalized phase contrast, to efficiently distribute light into two-dimensional, axially confined, user-defined shapes. Adding lens-phase modulations to 2D-phase holograms enables remote axial pattern displacement as well as simultaneous pattern generation in multiple distinct planes. However, the axial confinement linearly degrades with lateral shape area in previous reports where axially shifted holographic shapes were not temporally focused. Here we report an optical system using two spatial light modulators to independently control transverse- and axial-target light distribution. This approach enables simultaneous axial translation of single or multiple spatiotemporally focused patterns across the sample volume while achieving the axial confinement of temporal focusing. We use the system's capability to photoconvert tens of Kaede-expressing neurons with single-cell resolution in live zebrafish larvae.
Nat Commun, 2016; 7
26752076: Fidelin K, Djenoune L, Stokes C, Prendergast A, Gomez J, Baradel A, Del Bene F, Wyart C
State-Dependent Modulation of Locomotion by GABAergic Spinal Sensory Neurons.
The cerebrospinal fluid (CSF) constitutes an interface through which chemical cues can reach and modulate the activity of neurons located at the epithelial boundary within the entire nervous system. Here, we investigate the role and functional connectivity of a class of GABAergic sensory neurons contacting the CSF in the vertebrate spinal cord and referred to as CSF-cNs. The remote activation of CSF-cNs was shown to trigger delayed slow locomotion in the zebrafish larva, suggesting that these cells modulate components of locomotor central pattern generators (CPGs). Combining anatomy, electrophysiology, and optogenetics in vivo, we show that CSF-cNs form active GABAergic synapses onto V0-v glutamatergic interneurons, an essential component of locomotor CPGs. We confirmed that activating CSF-cNs at rest induced delayed slow locomotion in the fictive preparation. In contrast, the activation of CSF-cNs promptly inhibited ongoing slow locomotion. Moreover, selective activation of rostral CSF-cNs during ongoing activity disrupted rostrocaudal propagation of descending excitation along the spinal cord, indicating that CSF-cNs primarily act at the premotor level. Altogether, our results

demonstrate how a spinal GABAergic sensory neuron can tune the excitability of locomotor CPGs in a state-dependent manner by projecting onto essential components of the excitatory premotor pool.
Curr Biol, 2015; 25
24440416: Fidelin K, Wyart C
Inhibition and motor control in the developing zebrafish spinal cord.
Vertebrate locomotion relies on oscillatory activity along the spinal cord. Inhibition is involved in controlling the alternation of activity between each side and contributes in modulating propagation and termination of locomotor activity. Spinal inhibitory neurons are thought to regulate these mechanisms but the exact contribution of specific cell types remains difficult to tackle during active locomotion. In the past two decades, use of the transparent zebrafish larva has enabled morphological, functional, and genetic characterization of specific inhibitory spinal neurons. A wide range of new optical tools has been developed to monitor and to manipulate the activity of genetically targeted spinal populations. Combining these techniques with conventional electrophysiology will provide a better understanding of the contribution of inhibitory spinal interneurons in regulating essential features of locomotor patterns.
Curr Opin Neurobiol, 2014; 26
24360962: Morishita J, Kang MJ, Fidelin K, Ryoo HD
CDK7 regulates the mitochondrial localization of a tail-anchored proapoptotic protein, Hid.
The mitochondrial outer membrane is a major site of apoptosis regulation across phyla. Human and C. elegans Bcl-2 family proteins and Drosophila Hid require the C-terminal tail-anchored (TA) sequence in order to insert into the mitochondrial membrane, but it remains unclear whether cytosolic proteins actively regulate the mitochondrial localization of these proteins. Here, we report that the cdk7 complex regulates the mitochondrial localization of Hid and its ability to induce apoptosis. We identified cdk7 through an in vivo RNAi screen of genes required for cell death. Although CDK7 is best known for its role in transcription and cell-cycle progression, a hypomorphic cdk7 mutant suppressed apoptosis without impairing these other known functions. In this cdk7 mutant background, Hid failed to localize to the mitochondria and failed to bind to recombinant inhibitors of apoptosis (IAPs). These findings indicate that apoptosis is promoted by a newly identified function of CDK7, which couples the mitochondrial localization and IAP binding of Hid.
Cell Rep, 2013; 5
23791726: Ninov N, Hesselson D, Gut P, Zhou A, Fidelin K, Stainier DY
Metabolic regulation of cellular plasticity in the pancreas.
Obese individuals exhibit an increase in pancreatic β cell mass; conversely, scarce nutrition during pregnancy has been linked to β cell insufficiency in the offspring [reviewed in 1, 2]. These phenomena are thought to be mediated mainly through effects on β cell proliferation, given that a nutrient-sensitive β cell progenitor population in the pancreas has not been identified. Here, we employed the fluorescent ubiquitination-based cell-cycle indicator system to investigate β cell replication in real time and found that high nutrient concentrations induce rapid β cell proliferation. Importantly, we found that high nutrient concentrations also stimulate β cell differentiation from progenitors in the intrapancreatic duct (IPD). Furthermore, using a new zebrafish line where β cells are constitutively ablated, we show that β cell loss and high nutrient intake synergistically activate these progenitors. At the cellular level, this activation process causes ductal cell reorganization as it stimulates their proliferation and differentiation. Notably, we link the nutrient-dependent activation of these progenitors to a downregulation of Notch signaling specifically within the IPD. Furthermore, we show that the nutrient sensor mechanistic target of rapamycin (mTOR) is required for endocrine differentiation from the IPD under physiological conditions as well as in the diabetic state. Thus, this study reveals critical insights into how cells modulate their plasticity in response to metabolic cues and identifies nutrient-sensitive progenitors in the mature pancreas.
Curr Biol, 2013; 23

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-583

BASAL GANGLIA-SPINAL CORD PATHWAY THAT COMMANDS LOCOMOTOR ASYMMETRIES

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Motor impairments in Parkinson's disease are caused by loss of dopamine input to basal ganglia circuits. Although the basal ganglia are important for locomotion in particular, mechanisms underlying basal ganglia control over spinal locomotor networks remain unclear. One hallmark feature of human Parkinsonism is an exacerbated turning gait and failure to negotiate turns. *Chx10* gigantocellular (Gi) neurons are required for turning gait asymmetries (Cregg et al., 2020; Usseglio et al., 2020), suggesting that turning deficits in Parkinson's disease may arise via this spinal projection pathway. Using deep brainstem calcium recording in mice, we found that D1 and D2 striatal projection neurons (SPNs) evoke discrete changes in *Chx10* Gi activity during locomotor turns. Leveraging *Chx10* Gi neurons as an entry point, we used a reverse dissection approach to uncover the dominant basal ganglia-spinal cord pathway for locomotor asymmetries in mammals: striatal projection neurons → substantia nigra pars reticulata (SNr) → pontine nucleus oralis (PnO) → *Chx10* Gi neurons → spinal locomotor networks. PnO was identified using an intersectional viral screening strategy, where a subset of PnO neurons defined by *Vglut2* expression and commissural projection proved to act as the critical link between basal ganglia output and *Chx10* Gi neurons. Stimulation of this small cluster of neurons evoked contralateral turning, whereas photoinhibition evoked ipsilateral turning. Our results reveal the circuit logic underlying a critical motor program, from action commitment in the basal ganglia to execution by spinal locomotor networks.

This work was supported by the Novo Nordisk Foundation and the Lundbeck Foundation.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-584

FUNCTIONAL COUPLING OF THE MESENCEPHALIC LOCOMOTOR REGION AND V2A RETICULOSPINAL NEURONS.

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Aims: Throughout vertebrate species, locomotion relies on high brain centres converging onto the mesencephalic locomotor region (MLR). How the MLR recruits downstream reticulospinal neurons (RSNs) to initiate locomotor movements remains poorly understood due to the difficulty of recording brainstem neurons during locomotion. Methods: To tackle this question, we leveraged the transparency and genetic accessibility of larval zebrafish. Results: We identified for the first time, functionally and anatomically, the MLR in larval zebrafish as a small region located medially and dorsal to the locus coeruleus, containing glutamatergic and cholinergic neurons. MLR stimulation reliably controlled the duration and locomotor frequency of forward swimming bouts. Using calcium imaging in combination with MLR stimulation, we investigated whether the MLR recruits V2a RSNs shown to be involved in starting, steering and stopping locomotion. We show that the MLR sequentially recruits a subset of V2a RSNs throughout the brainstem. While pontine and retropontine V2a RSNs are recruited at once at low MLR stimulation strength, increasing intensities of the MLR stimulation recruits gradually medullary V2a RSNs, suggesting that V2a RSNs in the medulla could set the speed of forward locomotion. Conclusion: Our study reveals that the mesencephalic locomotor region specifically recruits a subset of V2a RSNs to control forward locomotion. The description of the MLR in larval zebrafish represents a breakthrough for future investigation of the supraspinal motor centres as virtually each and all RSNs can be identified, monitored and manipulated in this transparent genetic model organism

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BOARD NUMBER: S02-585

LIGHT ON THE SPINAL PROJECTIONS AND ROLES OF MEDULLARY V2A RETICULOSPINAL NEURONS IN LOCOMOTION

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Sorbonne Université, Paris Brain Institute (icm), Paris, France

Across vertebrate species, locomotion relies on reticulospinal neurons (RSNs) in the brainstem who provide descending commands to spinal locomotor circuits. Among them, V2a RSNs are necessary to initiate locomotion. Due to the difficulty to record these cells during movement, we lack information on the projection of the V2a RSNs to spinal circuits and their role in eliciting locomotion at different speeds. Here we tackle this question by leveraging the transparency and genetic accessibility of larval zebrafish using advanced optical methods. We used a photoconvertible protein to identify RSNs among V2a brainstem neurons to backfill optically their axons in the spinal cord. To survey their projection range, photoconversion was performed at different rostrocaudal positions in the spinal cord. We found that early-born V2a RSNs recruited during powerful movements project to caudal spinal cord while late-born V2a RSNs recruited during weak exploratory locomotion project more rostrally. To test their roles, we used 2D optogenetics to selectively activate a column of V2a RSNs in the brainstem of head-restrained tail-free larval zebrafish. We observed that the patterned activation of late born V2a RSNs was effective at eliciting forward locomotion at a set tail-beat frequency. Altogether our work reveals in a simple vertebrate species the structure and function of medullary V2a RSNs in the control of locomotion.

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BOARD NUMBER: S02-586

RECOVERY OF TURNING GAITS IN PARKINSONIAN MICE BY TARGETED STIMULATION OF BRAINSTEM NEURONS

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Parkinson's disease (PD) is caused by loss of dopaminergic neurons of the nigrostriatal pathway, leading to disrupted function of basal ganglia circuits. PD is associated with a variety of locomotor impairments, including freezing and difficulties in making turns. With the recent identification of a basal ganglia-spinal cord pathway for turning gait asymmetries (Cregg et al., FENS Forum 2022)—a pathway that includes the substantia nigra pars reticulata, pontine nucleus oralis (PnO), and *Chx10* reticulospinal neurons—we hypothesized that turning deficits in PD may be alleviated by targeted activation of this pathway. To induce Parkinsonian turning gait deficiencies, we used the unilateral 6-hydroxydopamine (6-OHDA) mouse model of PD. Unilateral 6-OHDA lesioned mice exhibited loss of dopaminergic neurons of the ipsilateral substantia nigra pars compacta and their axonal terminals in the striatum. 6-OHDA lesioned mice developed pronounced turning behavior with a dominance to the ipsilateral (lesioned) side within days, which persisted into the chronic state (>2 weeks). Using excitatory DREADDS, we demonstrate that stimulation of *Chx10* reticulospinal neurons contralateral to the lesion restores contralateral turning gaits in both acutely and chronically lesioned states. In accordance, optogenetic stimulation of excitatory PnO neurons ipsilateral to the lesion—projecting to *Chx10* reticulospinal neurons on the contralateral side—also restores contralateral turning gaits with millisecond precision. Our data outline promising brainstem targets for improving locomotor function in PD. This work was supported by the Novo Nordisk Foundation and the Lundbeck Foundation.

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BOARD NUMBER: S02-587

PASSIVE LIMB TRAINING MODULATES THE RESPIRATORY RHYTHM

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Initial modifications of breathing at the onset of exercise are mainly provided by afferent feedback from exercising limbs and descending input from the suprapontine areas. To better characterize neural systems controlling respiration during physical activity, we designed a novel experimental in vitro platform. Thus, an isolated preparation of the central nervous system from neonatal rodents had the limbs attached to an ad-hoc robot (BIKE) driving passive pedaling at calibrated speeds. In these conditions, a stable spontaneous respiratory rhythm was extracellularly recorded for more than four hours from all cervical ventral roots. The duration of single respiratory bursts was already reduced at lower pedaling speeds (1 Hz), while only an intense exercise (3.5 Hz) modulated the pace of breathing. Brief sessions (5 min) of BIKE at 3.5 Hz augmented the respiratory rate of preparations that showed slow bursting in control (slower breathers), whereas slowed down the rhythm of faster breathers. Accordingly, when spontaneous breathing was accelerated by high potassium concentrations, BIKE reduced bursting frequency. Regardless of the baseline respiratory pace, BIKE at 3.5 Hz always decreased single burst duration with unaffected amplitude. The surgical ablation of suprapontine structures completely prevented modulation of breathing by intense training. Albeit the variability in baseline breathing rates, intense passive cyclic movement of limbs tuned the frequency of fictive respiration toward a common value, while each respiratory event became shorter. These modulatory effects of passive training rely on suprapontine areas. The observations presented contribute to defining how the respiratory system integrates sensory input from limbs.

Pubmed:
32760254: Taccola G, Salazar BH, Apicella R, Hogan MK, Horner PJ, Sayenko D
Selective Antagonism of A1 Adenosinergic Receptors Strengthens the Neuromodulation of the Sensorimotor Network During Epidural Spinal Stimulation.
Although epidural spinal stimulation (ESS) results in promising therapeutic effects in individuals with spinal cord injury (SCI), its potential to generate functional motor recovery varies between individuals and remains largely unclear. However, both preclinical and clinical studies indicate the capacity of electrical and pharmacological interventions to synergistically increase the engagement of spinal sensorimotor networks and regain motor function after SCI. This study explored whether selective pharmacological antagonism of the adenosine A1 receptor subtype synergizes with ESS, thereby increasing motor response. We hypothesized that selective pharmacological antagonism of A1 receptors during ESS would produce facilitatory effects in spinal sensorimotor networks detected as an increased amplitude of spinally-evoked motor potentials and sustained duration of ESS induced activity. Terminal experiments were performed in adult rats using trains of stereotyped pulses at 40 Hz delivered at L5 with the local administration to the cord of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). We demonstrated that ESS combined with the blockage of A1 receptors increased the magnitude of the endogenous modulation and postponed the decay of responses that occur during ESS alone. Although DPCPX significantly increased the yield of repetitive stimulation in intact spinal cords, the effects of A1 antagonism on motor evoked responses after an acute spinal transection was not detected. These studies support the future investigation of the optimal dosage, methods of delivery, and systemic effects of the synergistic application of A1 antagonists and spinal stimulation in the intact and injured spinal cord.
Front Syst Neurosci, 2020; 14

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BOARD NUMBER: S02-588

THE ROLE OF HINDBRAIN SEGMENTATION AND THE MAUTHNER ARRAY DURING VISUAL ESCAPE BEHAVIOUR IN LARVAL ZEBRAFISH

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Segmentation in the hindbrain plays an important role in the organization of motor circuits in vertebrates and gives rise to sets of homologous neurons that share similar morphology and functionality. A key question in the field is what purpose does segmental homology serve? One explanation that has been advanced is that using homologous neurons in different combinations allows for modulation of the kinematics of a particular movement. Another possibility is that the homologues allow for sequences of behavior to play out over time. Larval zebrafish serve as an ideal model in which to test these hypotheses, and in particular the Mauthner array (M-array) are known to contribute to the generation of the fast escape swim, known as the Short Latency C-start (SLC). The M-array consists of three homologous pairs of neurons: the Mauthner cells, and two pairs of homologues, MiD2cm and MiD3cm. Using genetic ablations of the Mauthner cells, we show they are not necessary for SLCs in response to a visual threat, but that there is a reduction in the length of sequences of SLCs, suggesting that the homologue array may play a role in sustaining chains of behavior. In ongoing work we are recording the M-array using fast volumetric light-sheet imaging, while presenting larvae with a visual predator-like stimulus that evokes sequences of SLCs in order to evaluate how the M-array is activated. With this work we hope to contribute to the debate over why segmentation is fundamental to motor systems.

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BOARD NUMBER: S02-589

CHARACTERIZATION OF PHOX2B-EXPRESSING PREMOTORS IN THE HINDBRAIN RETICULAR FORMATION

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Aims The reticular formation of the pons and medulla is known to contain neurons involved in vital functions (respiratory and cardiovascular) and in orofacial movements, but remains poorly characterized in terms of genetically defined neuron types. The transcription factor *Phox2b* specifies most autonomic neurons, and orofacial motor nuclei. Moreover, genetic anterograde tracing of all hindbrain glutamatergic Phox2b+ interneurons reveals terminals in jaw opening and closing motor nuclei, pointing to orofacial Phox2b+ premotor populations. Previous work from the lab identified jaw-opener Phox2b+ premotor neurons in the intermediate reticular formation (Dempsey et al., 2021). Here we explore the function of another Phox2b+ interneuronal population (Sup5^{Phox2b}) located in the supratrigeminal nucleus, a region known to project to the trigeminal (jaw-closing) motor nucleus. **Methods** In the Sup5^{Phox2b} of *Phox2b::Cre* mice, we stereotaxically injected a virally encoded Cre-dependent opsin or calcium indicator, combined with fiber optic cannula implantation, then video-recorded and motion-tracked head-fixed animals by DeepLabCut. **Results** We found that a 100ms pulse of blue light minimally adducts the jaw from its resting position, while a 1000ms light pulse interrupts a spontaneous licking sequence. Changes in bulk calcium fluorescence in spontaneously chewing animals suggest that Sup5^{Phox2b} is recruited during this behavior. **Conclusion** Expression of the transcription factor *Phox2b* in the supratrigeminal nucleus identifies a likely jaw-closing premotor center and provides access to its further functional characterization. Dempsey B., Sungeelee S., et al. (2021). A medullary lapping center in mice. Nat Commun *12*, 6307.

FENS Forum 2022
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BOARD NUMBER: S02-590

IMAGING NEURAL ACTIVITY DYNAMICS DURING GAIT SWITCHING IN LARVAL ZEBRAFISH.

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Animals need to perform a diverse range of behaviours to navigate their environment successfully. Larval zebrafish swim using discrete episodes of propulsion called bouts that can be classified into 13 categories of movement. We aim to elucidate the mechanism of transition between different movements, particularly in the context of forward swimming, on both a behavioural and neural level. To characterise gait-switching behaviour, larval zebrafish were presented with gratings moving from tail to head at different speeds eliciting forward swimming, known as the optomotor response. Collecting various kinematic parameters, through tracking fish position and tail angle, allowed swims to be classified into different categories of movement. In response to slow gratings, larval zebrafish predominantly exhibited slow swims, whereas fast gratings elicited a rapid transition from slow to sustained trains of fast swims. To identify the neural correlates of slow and fast swims and elucidate the population dynamics underlying gait transitions, we recorded activity from genetically labelled neural populations in the brainstem of head-fixed larval zebrafish while they performed the optomotor response in a closed-loop configuration, utilising a 'SCAPE' light-sheet microscope. Head-fixed fish showed differences in bout kinematics from freely swimming fish, with longer movements that included switches in frequency within a single bout. We therefore use regression analysis, based on kinematics of individual half-beats within each bout to identify neuronal populations associated with different modes of swimming. By showing how the brain selects dynamically between two distinct motor outputs we strive to understand fundamental principles in the supra-spinal control of locomotion.

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BOARD NUMBER: S02-591

DESCENDING EXCITATORY RETICULOSPINAL DRIVE TO SPINAL NEURONS IN SALAMANDERS.

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

Jordan Swiegers[1], Alberto Joven[2], Auke Ijspeert[3], Andras Simon[2], Dimitri Ryczko[1]
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Salamanders are the only tetrapods that recover voluntary locomotion after full spinal transection. However, the neurons that play a key role in the recovery of locomotor control are still largely unknown. As in other vertebrates, excitatory reticulospinal neurons likely play an important role in the initiation of locomotion, while excitatory spinal neurons likely generate the locomotor rhythm. However, there is little information about these neurons in salamanders compared to lamprey, zebrafish or mouse models. Here, we used neural tracing, in situ hybridization (RNAScope), and immunofluorescence to anatomically study glutamatergic markers (vesicular glutamate transporter Vglut2, and Chx10) in reticulospinal and spinal neurons of the Iberian newt (Pleurodeles waltl). We also used calcium imaging in an ex vivo brainstem-spinal cord preparation to examine the role of the descending glutamatergic reticulospinal drive in the activation pattern of spinal neurons. Our results suggest that the glutamatergic locomotor neurons described in other species are present in salamanders. These neurons are good candidates to play a role in the recovery of locomotor control after spinal cord regeneration in salamanders.

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SENSORY-EVOKED CALCIUM RESPONSES IN NEURONS OF THE MESENCEPHALIC LOCOMOTOR REGION IN FREELY BEHAVING MICE.

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

Cornelis Immanuel Van Der Zouwen[1], Joël Boutin[1], Léonie Dejas[1], Aurélie Flaive[1], Ted Hsu[2], Vaibhav Konanur[2], Mitchell Roitman[2], Dimitri Ryczko[1]
[1]Université de Sherbrooke, Pharmacology-physiology, Sherbrooke, Canada, [2]University of Illinois at Chicago, Department Of Psychology, Chicago, United States of America

Considerable knowledge has been gained on the role of Mesencephalic Locomotor Region (MLR) in locomotor control. However, little is known about the sensory events that increase MLR activity. Locomotion potentially needs to be initiated in various environmental contexts, which could be channeled to the MLR through sensory modalities. Here, we investigated whether MLR neurons show increases in activity in response to unconditioned sensory stimulations in freely moving mice. First, to validate our ability to target the glutamatergic (Vglut2$^+$) MLR neurons generating the locomotor drive, we injected a virus encoding for a channelrhodopsin in a Cre-dependent manner in the MLR of Vglut2-Cre mice. Applying blue light to the MLR initiated locomotion. Then, we recorded the activity of MLR neurons with fiber photometry by injecting a virus encoding for a calcium sensor (GCaMP7f) in a Cre-dependent manner in the MLR of Vglut2-Cre mice. When mice were made to walk on a motorized treadmill, calcium signals increased in Vglut2$^+$ MLR neurons, confirming the locomotor nature of these neurons. An air puff applied to the mouse body, a brief unexpected sound, or a looming stimulus all evoked calcium increases in Vglut2$^+$ MLR neurons, indicating that these neurons can encode sensory information. Interestingly, calcium responses were not always associated with an escape response. In contrast, our preliminary data suggest that no calcium responses were evoked by the same sensory stimulations in GABAergic (VGAT$^+$) MLR neurons. This indicates that sensory events encoding potentially dangerous environmental conditions are channeled to a specific cell type in the MLR.

Pubmed:

34670837: Fougère M, van der Zouwen CI, Boutin J, Neszvecsko K, Sarret P, Ryczko D
Optogenetic stimulation of glutamatergic neurons in the cuneiform nucleus controls locomotion in a mouse model of Parkinson's disease.
In Parkinson's disease (PD), the loss of midbrain dopaminergic cells results in severe locomotor deficits, such as gait freezing and akinesia. Growing evidence indicates that these deficits can be attributed to the decreased activity in the mesencephalic locomotor region (MLR), a brainstem region controlling locomotion. Clinicians are exploring the deep brain stimulation of the MLR as a treatment option to improve locomotor function. The results are variable, from modest to promising. However, within the MLR, clinicians have targeted the pedunculopontine nucleus exclusively, while leaving the cuneiform nucleus unexplored. To our knowledge, the effects of cuneiform nucleus stimulation have never been determined in parkinsonian conditions in any animal model. Here, we addressed this issue in a mouse model of PD, based on the bilateral striatal injection of 6-hydroxydopamine, which damaged the nigrostriatal pathway and decreased locomotor activity. We show that selective optogenetic stimulation of glutamatergic neurons in the cuneiform nucleus in mice expressing channelrhodopsin in a Cre-dependent manner in Vglut2-positive neurons (Vglut2-ChR2-EYFP mice) increased the number of locomotor initiations, increased the time spent in locomotion, and controlled locomotor speed. Using deep learning-based movement analysis, we found that the limb kinematics of optogenetic-evoked locomotion in pathological conditions were largely similar to those recorded in intact animals. Our work identifies the glutamatergic neurons of the cuneiform nucleus as a potentially clinically relevant target to improve locomotor activity in parkinsonian conditions. Our study should open avenues to develop the targeted stimulation of these neurons using deep brain stimulation, pharmacotherapy, or optogenetics.
Proc Natl Acad Sci U S A, 2021; 118
33897379: van der Zouwen CI, Boutin J, Fougère M, Flaive A, Vivancos M, Santuz A, Akay T, Sarret P, Ryczko D
Freely Behaving Mice Can Brake and Turn During Optogenetic Stimulation of the Mesencephalic Locomotor Region.
A key function of the mesencephalic locomotor region (MLR) is to control the speed of forward symmetrical locomotor movements. However, the ability of freely moving mammals to integrate environmental cues to brake and turn during MLR stimulation is poorly documented. Here, we investigated whether freely behaving mice could brake or turn, based on

environmental cues during MLR stimulation. We photostimulated the cuneiform nucleus (part of the MLR) in mice expressing channelrhodopsin in Vglut2-positive neurons in a Cre-dependent manner (Vglut2-ChR2-EYFP) using optogenetics. We detected locomotor movements using deep learning. We used patch-clamp recordings to validate the functional expression of channelrhodopsin and neuroanatomy to visualize the stimulation sites. In the linear corridor, gait diagram and limb kinematics were similar during spontaneous and optogenetic-evoked locomotion. In the open-field arena, optogenetic stimulation of the MLR evoked locomotion, and increasing laser power increased locomotor speed. Mice could brake and make sharp turns (~90°) when approaching a corner during MLR stimulation in the open-field arena. The speed during the turn was scaled with the speed before the turn, and with the turn angle. Patch-clamp recordings in Vglut2-ChR2-EYFP mice show that blue light evoked short-latency spiking in MLR neurons. Our results strengthen the idea that different brainstem neurons convey braking/turning and MLR speed commands in mammals. Our study also shows that Vglut2-positive neurons of the cuneiform nucleus are a relevant target to increase locomotor activity without impeding the ability to brake and turn when approaching obstacles, thus ensuring smooth and adaptable navigation. Our observations may have clinical relevance since cuneiform nucleus stimulation is increasingly considered to improve locomotion function in pathological states such as Parkinson's disease, spinal cord injury, or stroke.
Front Neural Circuits, 2021; 15

33224027: Flaive A, Fougère M, van der Zouwen CI, Ryczko D
Serotonergic Modulation of Locomotor Activity From Basal Vertebrates to Mammals.
During the last 50 years, the serotonergic (5-HT) system was reported to exert a complex modulation of locomotor activity. Here, we focus on two key factors that likely contribute to such complexity. First, locomotion is modulated directly and indirectly by 5-HT neurons. The locomotor circuitry is directly innervated by 5-HT neurons in the caudal brainstem and spinal cord. Also, indirect control of locomotor activity results from ascending projections of 5-HT cells in the rostral brainstem that innervate multiple brain centers involved in motor action planning. Second, each approach used to manipulate the 5-HT system likely engages different 5-HT-dependent mechanisms. This includes the recruitment of different 5-HT receptors, which can have excitatory or inhibitory effects on cell activity. These receptors can be located far or close to the 5-HT release sites, making their activation dependent on the level of 5-HT released. Here we review the activity of different 5-HT nuclei during locomotor activity, and the locomotor effects of 5-HT precursors, exogenous 5-HT, selective 5-HT reuptake inhibitors (SSRI), electrical or chemical stimulation of 5-HT neurons, genetic deletions, optogenetic and chemogenetic manipulations. We highlight both the coherent and controversial aspects of 5-HT modulation of locomotor activity from basal vertebrates to mammals. This mini review may hopefully inspire future studies aiming at dissecting the complex effects of 5-HT on locomotor function.
Front Neural Circuits, 2020; 14

32869307: Fougère M, van der Zouwen CI, Boutin J, Ryczko D
Heterogeneous expression of dopaminergic markers and Vglut2 in mouse mesodiencephalic dopaminergic nuclei A8-A13.
Co-transmission of glutamate by brain dopaminergic (DA) neurons was recently proposed as a potential factor influencing cell survival in models of Parkinson's disease. Intriguingly, brain DA nuclei are differentially affected in Parkinson's disease. Whether this is associated with different patterns of co-expression of the glutamatergic phenotype along the rostrocaudal brain axis is unknown in mammals. We hypothesized that, as in zebrafish, the glutamatergic phenotype is present preferentially in the caudal mesodiencephalic DA nuclei. Here, we used in mice a cell fate mapping strategy based on reporter protein expression (ZsGreen) consecutive to previous expression of the vesicular glutamate transporter 2 (Vglut2) gene, coupled with immunofluorescence experiments against tyrosine hydroxylase (TH) or dopamine transporter (DAT). We found three expression patterns in DA cells, organized along the rostrocaudal brain axis. The first pattern (TH-positive, DAT-positive, ZsGreen-positive) was found in A8-A10. The second pattern (TH-positive, DAT-negative, ZsGreen-positive) was found in A11. The third pattern (TH-positive, DAT-negative, ZsGreen-negative) was found in A12-A13. These patterns should help to refine the establishment of the homology of DA nuclei between vertebrate species. Our results also uncover that Vglut2 is expressed at some point during cell lifetime in DA nuclei known to degenerate in Parkinson's disease and largely absent from those that are preserved, suggesting that co-expression of the glutamatergic phenotype in DA cells influences their survival in Parkinson's disease.
J Comp Neurol, 2021; 529

32155428: van der Zouwen CI, Ryczko D
Motor Control: Swim Harder, Faster, Stronger.
A new study provides evidence in zebrafish that dopamine increases the activity of motor neurons in the spinal cord, and this translates into faster swimming bouts in response to visual stimulation.
Curr Biol, 2020; 30

FENS Forum 2022
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BOARD NUMBER: S02-593

DEEP LEARNING-BASED MOVEMENT ANALYSES DURING WALKING AND SWIMMING IN SALAMANDERS

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Salamanders swim underwater and walk on ground. They are an ideal animal model to study how the locomotor circuitry can generate two different behaviors. They are also able to recover voluntary locomotion after full spinal transection. However, a precise description of the gradual recovery of swimming and walking movements after full spinal transection is lacking. In order to do such analysis, here we introduce the use of a deep-learning based software to analyze the locomotor movements of the salamander *Pleurodeles waltl*. We recorded swimming and walking movements from below at 300 frames per second in a motorater. We used DeepLabCut software to track twenty-five user-defined anatomical points distributed on the body axis, limbs and head without the need to place any physical markers. Tracking of the axial body parts allowed us to measure the phase coupling and the amplitude of the angular excursion between the anatomical points along the body axis as a function of time. Tracking of the limbs allowed us to measure the speed of limb movements, from which we can evaluate the footfall pattern, and therefore swing and stance duration, cycle duration and stride length. Our study shows that the use of DeepLabCut provides a powerful approach to obtain a detailed analysis of salamander locomotion. This will allow us to study the recovery of swimming and walking movements during regeneration of the locomotor circuitry.

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BOARD NUMBER: S02-594

SACCADIC PREMOTOR BURST NEURONS AND HISTOCHEMICAL CORRELATES OF THEIR FIRING PATTERNS IN RHESUS MONKEY

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Bursting behavior of brainstem premotor burst neurons (BNs) is essential for initiation of saccades and calibrating their metrics. Several ion channel families such as voltage-gated potassium (Kv) channels, low-voltage-activated calcium (Cav3) channels and hyperpolarization-activated cyclic nucleotide–gated (HCN) channels are major regulators of the bursting in neurons. Therefore, it was speculated that ion channels with rapid kinematics are essential for characteristic firing patterns of the BNs and rapid saccade velocities. However, the expression patterns of ion channels are yet to be confirmed in order to support the neuromimetic model predictions for saccade generation in brainstem, and also to support contemporary views that channelopathy can cause saccade disorders in humans. For the proof of concept, we examined excitatory BNs in rostral interstitial nucleus of medial longitudinal fasciculus (RIMLF, vertical saccades) and inhibitory BNs in nucleus paragigantocellularis dorsalis (PGD, horizontal saccades) histochemically in macaque monkeys. We found strong expression of Kv channels, HCN1&2 and Cav3.2&3.3 in both BN populations. These channels are known for their fast-kinematic properties and are essential for post-inhibitory rebound. Moreover, PGD was found to host multiple neuron groups regarding calretinin immunoreactivity. Our results provide histochemical evidence in support for proposed post-inhibitory rebound bursting model of BNs. Furthermore, our findings confirm that deduction can be made about electrophysiological firing properties by histochemical examination of functional groups of the brainstem saccadic circuitry. This development is an important building block supporting the concept of channelopathies in saccadic disorders. Future histological studies in humans will confirm this theory for saccadic disorders.

Pubmed:
33083961: Zeeh C, Mayadali ÜS, Horn AKE
Histochemical Characterization of the Vestibular Y-Group in Monkey.
The Y-group plays an important role in the generation of upward smooth pursuit eye movements and contributes to the adaptive properties of the vertical vestibulo-ocular reflex. Malfunction of this circuitry may cause eye movement disorders, such as downbeat nystagmus. To characterize the neuron populations in the Y-group, we performed immunostainings for cellular proteins related to firing characteristics and transmitters (calretinin, GABA-related proteins and ion channels) in brainstem sections of macaque monkeys that had received tracer injections into the oculomotor nucleus. Two histochemically different populations of premotor neurons were identified: The calretinin-positive population represents the excitatory projection to contralateral upgaze motoneurons, whereas the GABAergic population represents the inhibitory projection to ipsilateral downgaze motoneurons. Both populations receive a strong supply by GABAergic nerve endings most likely originating from floccular Purkinje cells. All premotor neurons express nonphosphorylated neurofilaments and are ensheathed by strong perineuronal nets. In addition, they contain the voltage-gated potassium channels Kv1.1 and Kv3.1b which suggests biophysical similarities to high-activity premotor neurons of vestibular and oculomotor systems. The premotor neurons of Y-group form a homogenous population with histochemical characteristics compatible with fast-firing projection neurons that can also undergo plasticity and contribute to motor learning as found for the adaptation of the vestibulo-ocular reflex in response to visual-vestibular mismatch stimulation. The histochemical characterization of premotor neurons in the Y-group allows the identification of the homologue cell groups in human, including their transmitter inputs and will serve as basis for correlated anatomical-neuropathological studies of clinical cases with downbeat nystagmus.
Cerebellum, 2021; 20
34181058: Mayadali ÜS, Fleuriet J, Mustari M, Straka H, Horn AKE
Transmitter and ion channel profiles of neurons in the primate abducens and trochlear nuclei.
Extraocular motoneurons initiate dynamically different eye movements, including saccades, smooth pursuit and vestibulo-

ocular reflexes. These motoneurons subdivide into two main types based on the structure of the neuro-muscular interface: motoneurons of singly-innervated (SIF), and motoneurons of multiply-innervated muscle fibers (MIF). SIF motoneurons are thought to provoke strong and brief/fast muscle contractions, whereas MIF motoneurons initiate prolonged, slow contractions. While relevant for adequate functionality, transmitter and ion channel profiles associated with the morpho-physiological differences between these motoneuron types, have not been elucidated so far. This prompted us to investigate the expression of voltage-gated potassium, sodium and calcium ion channels (Kv1.1, Kv3.1b, Nav1.6, Cav3.1-3.3, KCC2), the transmitter profiles of their presynaptic terminals (vGlut1 and 2, GlyT2 and GAD) and transmitter receptors (GluR2/3, NMDAR1, GlyR1α) using immunohistochemical analyses of abducens and trochlear motoneurons and of abducens internuclear neurons (INTs) in macaque monkeys. The main findings were: (1) MIF and SIF motoneurons express unique voltage-gated ion channel profiles, respectively, likely accounting for differences in intrinsic membrane properties. (2) Presynaptic glutamatergic synapses utilize vGlut2, but not vGlut1. (3) Trochlear motoneurons receive GABAergic inputs, abducens neurons receive both GABAergic and glycinergic inputs. (4) Synaptic densities differ between MIF and SIF motoneurons, with MIF motoneurons receiving fewer terminals. (5) Glutamatergic receptor subtypes differ between MIF and SIF motoneurons. While NMDAR1 is intensely expressed in INTs, MIF motoneurons lack this receptor subtype entirely. The obtained cell-type-specific transmitter and conductance profiles illuminate the structural substrates responsible for differential contributions of neurons in the abducens and trochlear nuclei to eye movements.
Brain Struct Funct, 2021; 226

31325972: Mayadali ÜS, Lienbacher K, Mustari M, Strupp M, Horn AKE
Potassium channels in omnipause neurons.
Potassium (K) channels are major contributors to fast and precise action potential generation. The aim of this study was to establish the immunoreactivity profile of several potassium channels in omnipause neurons (OPNs), which play a central role in premotor saccadic circuitry. To accomplish this, we histochemically examined monkey and human brainstem sections using antibodies against the voltage gated K-channels K1.1, K3.1b and K-Cl cotransporter (KCC2). We found that OPNs of both species were positive for all three K-antibodies and that the staining patterns were similar for both species. In individual OPNs, K3.1b was detected on the somatic membrane and proximal dendrites, while K1.1 was mainly confined to soma. Further, KCC2 immunoreactivity was strong in distal dendrites, but was weak in the somatic membrane. Our findings allow the speculation that the alterations in K-channel expression in OPNs could be the underlying mechanism for several saccadic disorders through neuronal and circuit-level malfunction.
Prog Brain Res, 2019; 249

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BOARD NUMBER: S02-595

PEDUNCULOPONTINE NEURONS FOR GLOBAL MOTOR ARREST

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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The episodic nature of movement requires neural mechanisms for movement initiation and arrest. Motor arrest may happen in diverse contexts such as the termination of a goal-directed movement, or in behavioral responses that lead to global motor arrest, for example fear/defensive freezing and behavioral interruption upon detection of salient environmental cues. Within global motor arrest, previous studies have mainly focused on the circuits mediating defensive freezing. However, the neuronal circuits that bridge with the executive motor circuits to implement a global motor arrest in non-defensive contexts are poorly understood. Using a combined anatomical, physiological, and behavioral approach, we report the discovery that glutamatergic Chx10^{+} neurons in the pedunculopontine nucleus (PPN) evoke global motor arrest in mice. First, we describe a subpopulation of glutamatergic PPN neurons that expresses the transcription factor Chx10 (Chx10-PPN) and is preferentially located in the rostral-half of the nucleus. Second, we show that optogenetic activation of Chx10-PPN neurons arrests all ongoing movements including locomotion, grooming, and rearing. Third, we find that Chx10-PPN evoked motor arrest is accompanied by apnea and bradycardia. Moreover, we demonstrate that the arrest evoked from Chx10-PPN neurons differs from the defensive freezing evoked from glutamatergic neurons in the ventrolateral periaqueductal gray (vlPAG), both in its motor and autonomic components. Thus, our study defines a cluster of excitatory neurons that evokes a global motor arrest that is different from defensive freezing, and identifies a locomotor-opposing role for Chx10^{+} glutamatergic neurons in the rostral PPN.

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BOARD NUMBER: S02-596

REPRESENTATION OF BODY ORIENTATION IN VESTIBULAR-DEFECTIVE PATIENTS BEFORE AND AFTER UNILATERAL VESTIBULAR LOSS

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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Aims: The unilateral vestibular syndrome results in postural, oculomotor, perceptive, and cognitive symptoms. The present study was designed to investigate the role of vestibular signals in the representation of body orientation, which remains poorly considered in vestibular patients. **Methods:** We investigated the subjective straight ahead (SSA) direction using a horizontal rod allowing to dissociate the translation and rotation components of the body midline representation in 21 patients with unilateral vestibular loss (right = 8; left = 13) and in 12 healthy controls. Patients were tested the day before surgery and during the recovery period, 7 days and 2 months after the surgery. **Results and Conclusions:** In the chronic phases, i.e., before and two months after unilateral vestibular neurotomy, the patients showed a rightward translation bias of their SSA, without rotation bias, whatever the side of the vestibular loss. This biais could be considered as a sign of spatial neglect. In the early phase after surgery, SSA moved toward the operated side both in translation and in rotation, as typically found for biases occurring after unilateral vestibular loss. Therefore, the recovery time-course of the SSA differed depending on the side of the vestibular loss, with more extensive changes over time before and after left vestibular loss. This study strongly supports the notion that the vestibular system plays a major role in body representation processes and more broadly in spatial cognition. From a clinical point of view, SSA appeared to be a reliable indicator for the presence of a vestibular disorder.

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BOARD NUMBER: S02-597

FUNCTIONAL REGIONALIZATION OF THE VESTIBULO-CEREBELLUM IN RATS

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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The vestibulo-cerebellum (VC) encompasses cerebellar regions targeted by fibers originating from vestibular end organs and/or brainstem vestibular nuclei. At the level of the vermis, the VC corresponds to the most posterior lobules designated as nodulus and uvula (NU) which are themselves subdivided in a series of parasagittal modules. Despite the involvement of the VC in multiple pathways, from the control of gaze and posture to spatial orientation, these modules have not yet been linked with specific functions, with the exception of the processing of specific patterns of optic flow in pigeons. To unravel the functional regionalization of the NU, we chronically recorded NU units at various antero-posterior and medio-lateral locations in rats while tracking the 3D rotations and accelerations of their head using inertial measurements units, both during active (self-generated) or passively-applied movements. The sensitivity of the recorded units to head kinematics was assessed using a model-free approach linking their instantaneous firing rate with head angular velocity and/or tilt. In our preliminary analysis, rotation-sensitive vs tilt-sensitive units were preferentially found in medial vs lateral regions. NU units within a medial band appeared to anticipate head angular velocity, consistent with the encoding of either a sensory prediction or of angular acceleration. Additionally, many medially-located units exhibited larger modulations during passive vs active movement, a pervasive feature of a number of other vestibular structures. Taken together, these results are consistent with the existence of specialized medio-lateral bands in the NU, whose associated functions within specific vestibular pathways remain to be precisely established.

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BOARD NUMBER: S02-598

MAGNETIC ACTUATION OF EAR STONES ALLOWS BEHAVIORAL AND NEURONAL EXPLORATION OF VESTIBULO-MOTOR PROCESSING IN LARVAL ZEBRAFISH

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

Natalia Beiza[1], Thomas Pujol[1,2], Hippolyte Moulle[1], Thomas Panier[1], Geoffrey Migault[1], Nicolas Desprat[3], Pierre Tapis[1], Georges Debrégeas[1], Volker Bormuth[1]
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Whether animals walk, fly, or swim, active sensorimotor control is required to maintain balance while moving. The vestibular system of the inner ear plays a central role in this process as it continuously informs the brain of the gravitational field and the head acceleration. Monitoring the neural circuits that process vestibular information remains challenging as vestibular stimulations require a rotation or a translation of the animal's head. Here, we report on a new method to deliver in vivo fictive vestibular stimulation in zebrafish larvae through magnetic actuation of the otoliths. After injecting a ferrofluid into the inner ear, we were able to exert controlled forces on the ear stone of immobilized larvae, eliciting consistent eye and tail compensatory movements. We showed that these behavioral responses corresponded to normal body rotations of up to 20$degree$ in amplitude. Using simultaneous light-sheet based calcium imaging, we were able to record the brain-wide neuronal response to this fictive vestibular stimuli, showing evoked activity in the vestibular nucleus and downstream nuclei. We disentangled the contribution of both ears using unilateral ferrofluid injection. The recorded neuronal activity shows contralateral inhibition between the ears. As they are compatible with fluorescence microscopy, optogenetics, and electrophysiological techniques, magnetic stimulations open new perspectives for dissecting the neural circuits processing vestibular information.

FENS Forum 2022
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BOARD NUMBER: S02-599

FUNCTIONAL AND MOLECULAR HETEROGENEITY IN THE DEVELOPING VESTIBULO-OCULAR REFLEX CIRCUIT

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

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All vertebrates need to stabilize gaze during movement. As animals develop, gaze stabilization improves. However, it is unclear how the developing brain comes to stabilize gaze. It is well established that mature neurons in the reflex arc (sensory; central; motor neurons) responsible for gaze stabilization have heterogeneous response dynamics. I hypothesize that such heterogeneities will correlate with—and potentially control—different features of behavioral maturation, reflecting differential roles in development. We generated lines of transgenic zebrafish to express the calcium indicator GCaMP6s in two of these nuclei: the central vestibular nucleus and extraocular motor neurons of the trochlear nuclei. I measured neural activity with a multiphoton microscope while delivering whole-body tilts in pitch and roll axes in the same cells at 3, 5, and 7 days post fertilization, tiling the time before and during maturation of gaze stabilization. I observed systematic changes in neural activity, including a strengthening period of tuning. To probe response dynamics of neurons, I recorded evoked activity as a function of increasing stimulus amplitude and direction. These events were correlated, consistent with prior studies of mature neuron function. However, the slope of this relationship varied considerably, implying differential contributions to gaze stabilizing behavior. I have adopted a multipronged approach including correlative imaging and perturbative assays to directly address how the heterogeneity of response profiles differentially influences the maturation of behavior. Additionally, we are working to characterize the molecular correlates of these changes with transcriptomic approaches. This work will speak to the mechanisms responsible for development of gaze stabilization.

Pubmed:
35077698: Leary P, Schoppik D
Efference copies: Side-eyeing across species.
Efference copies of movement-inducing neural signals have been proposed to serve a role in gaze stabilization. Prior work has demonstrated a spino-extraocular motor circuit in the tadpole that relays copies of spinal commands to extraocular motor neurons. A recent study demonstrates the presence of this circuitry in mice, suggesting a unique method of gaze stabilization in the locomoting mouse.
Curr Biol, 2022; 32
31775052: Botterill JJ, Lu YL, LaFrancois JJ, Bernstein HL, Alcantara-Gonzalez D, Jain S, Leary P, Scharfman HE
An Excitatory and Epileptogenic Effect of Dentate Gyrus Mossy Cells in a Mouse Model of Epilepsy.
The sparse activity of hippocampal dentate gyrus (DG) granule cells (GCs) is thought to be critical for cognition and behavior, whereas excessive DG activity may contribute to disorders such as temporal lobe epilepsy (TLE). Glutamatergic mossy cells (MCs) of the DG are potentially critical to normal and pathological functions of the DG because they can regulate GC activity through innervation of GCs or indirectly through GABAergic neurons. Here, we test the hypothesis that MC excitation of GCs is normally weak, but under pathological conditions, MC excitation of GCs is dramatically strengthened. We show that selectively inhibiting MCs during severe seizures reduced manifestations of those seizures, hippocampal injury, and chronic epilepsy. In contrast, selectively activating MCs was pro-convulsant. Mechanistic in vitro studies using optogenetics further demonstrated the unanticipated ability of MC axons to excite GCs under pathological conditions. These results demonstrate an excitatory and epileptogenic effect of MCs in the DG.
Cell Rep, 2019; 29
31073064: Luna VM, Anacker C, Burghardt NS, Khandaker H, Andreu V, Millette A, Leary P, Ravenelle R, Jimenez JC, Mastrodonato A, Denny CA, Fenton AA, Scharfman HE, Hen R
Adult-born hippocampal neurons bidirectionally modulate entorhinal inputs into the dentate gyrus.
Young adult-born granule cells (abGCs) in the dentate gyrus (DG) have a profound impact on cognition and mood. However, it remains unclear how abGCs distinctively contribute to local DG information processing. We found that the actions of abGCs in the DG depend on the origin of incoming afferents. In response to lateral entorhinal cortex (LEC) inputs, abGCs exert

monosynaptic inhibition of mature granule cells (mGCs) through group II metabotropic glutamate receptors. By contrast, in response to medial entorhinal cortex (MEC) inputs, abGCs directly excite mGCs through -methyl-d-aspartate receptors. Thus, a critical function of abGCs may be to regulate the relative synaptic strengths of LEC-driven contextual information versus MEC-driven spatial information to shape distinct neural representations in the DG.
Science, 2019; 364
30837829: Ojo JO, Algamal M, Leary P, Abdullah L, Mouzon B, Evans JE, Mullan M, Crawford F
Converging and Differential Brain Phospholipid Dysregulation in the Pathogenesis of Repetitive Mild Traumatic Brain Injury and Alzheimer's Disease.
Repetitive mild traumatic brain injury (rmTBI) is a major epigenetic risk factor for Alzheimer's disease (AD). The precise nature of how rmTBI leads to or precipitates AD pathology is currently unknown. Numerous neurological conditions have shown an important role for dysfunctional phospholipid metabolism as a driving factor for the pathogenesis of neurodegenerative diseases. However, the precise role in rmTBI and AD remains elusive. We hypothesized that a detailed phospholipid characterization would reveal profiles of response to injury in TBI that overlap with age-dependent changes in AD and thus provide insights into the TBI-AD relationship. We employed a lipidomic approach examining brain phospholipid profiles from mouse models of rmTBI and AD. Cortex and hippocampal tissue were collected at 24 h, 3, 6, 9, and 12 months post-rmTBI, and at ages representing 'pre', 'peri' and 'post' onset of amyloid pathology (i.e., 3, 9, 15 months-old). Total levels of phosphatidylcholine (PC), phosphatidylethanolamine (PE), LysoPE, and phosphatidylinositol (PI), including their monounsaturated, polyunsaturated and saturated fatty acid (FA) containing species were significantly increased at acute and/or chronic time points post-injury in both brain regions. However, levels of most phospholipid species in PS1/APP mice were nominal in the hippocampus, while in the cortex, levels were significantly decreased at ages post-onset of amyloid pathology. Sphingomyelin and LysoPC levels showed coincidental trends in our rmTBI and AD models within the hippocampus, an increase at acute and/or chronic time points examined. The ratio of arachidonic acid (omega-6 FA) to docosahexaenoic acid (omega-3 FA)-containing PE species was increased at early time points in the hippocampus of injured versus sham mice, and in PS1/APP mice there was a coincidental increase compared to wild type littermates at all time points. This study demonstrates some overlapping and diverse phospholipid profiles in rmTBI and AD models. Future studies are required to corroborate our findings in human post-mortem tissue. Investigation of secondary mechanisms triggered by aberrant downstream alterations in bioactive metabolites of these phospholipids, and their modulation at the appropriate time-windows of opportunity could help facilitate development of novel therapeutic strategies to ameliorate the neurodegenerative consequences of rmTBI or the potential triggering of AD pathogenesis by rmTBI.
Front Neurosci, 2019; 13
30564087: Ojo JO, Algamal M, Leary P, Abdullah L, Mouzon B, Evans JE, Mullan M, Crawford F
Disruption in Brain Phospholipid Content in a Humanized Tau Transgenic Model Following Repetitive Mild Traumatic Brain Injury.
Repetitive mild traumatic brain injury (mTBI) is a risk factor for the development of neurodegenerative diseases such as chronic traumatic encephalopathy typified by immunoreactive tau aggregates in the depths of the sulci. However, the underlying neurobiological mechanisms involved have not been largely explored. Phospholipids are important molecules which form membrane lipid bilayers; they are ubiquitous to every cell in the brain, and carry out a host of different functions. Imbalance in phospholipid metabolism, signaling and transport has been documented in some neurological conditions. However, not much is currently known about their roles in repetitive mTBI and how this may confer risk for the development of age-related neurodegenerative diseases. To address this question, we designed a longitudinal study (24 h, 3, 6, 9, and 12 months post-injury) to comprehensively investigate mTBI dependent brain phospholipid profiles compared to sham counterparts. We use our established mouse model of repetitive mTBI that has been extensively characterized up to 1-year post-injury in humanized tau (hTau) mice, which expresses all six human tau isoforms, on a null murine background. Our data indicates a significant increase in sphingomyelin, phosphatidylethanolamine (PE), phosphatidylcholine (PC), and derivative lysoPE and lysoPC at acute and/or sub-acute time points post-injury within the cortex and hippocampus. There was also a parallel increase at early time points in monounsaturated, polyunsaturated and saturated fatty acids. Omega-6 (arachidonic acid) to omega-3 (docosahexaenoic acid) fatty acid ratio for PE and PC species was increased also at 24 h and 3 months post-injury in both hippocampus and cortex. The long-term consequences of these early changes in phospholipids on neuronal and non-neuronal cell function is unclear, and warrants further study. Understanding phospholipid metabolism, signaling and transport following TBI could be valuable; they may offer novel targets for therapeutic intervention not only in TBI but other neurodegenerative diseases.
Front Neurosci, 2018; 12
30395237: Ojo JO, Leary P, Lungmus C, Algamal M, Mouzon B, Bachmeier C, Mullan M, Stewart W, Crawford F
Subchronic Pathobiological Response Following Chronic Repetitive Mild Traumatic Brain Injury in an Aged Preclinical Model of Amyloid Pathogenesis.

Repetitive mild traumatic brain injury (r-mTBI) is a risk factor for Alzheimer disease (AD). The precise nature of how r-mTBI leads to, or precipitates, AD pathogenesis remains unclear. In this study, we explore subchronic effects of chronic r-mTBI (12-impacts) administered over 1-month in aged-PS1/APP mice and littermate controls. We investigate specific mechanisms that may elucidate the molecular link between AD and r-mTBI, focusing primarily on amyloid and tau pathology, amyloid processing, glial activation states, and associated clearance mechanisms. Herein, we demonstrate r-mTBI in aged PS1/APP mice does not augment, glial activation, amyloid burden, or tau pathology (with exception of pS202-positive Tau) 1 month after exposure to the last-injury. However, we observed a decrease in brain soluble Aβ42 levels without any appreciable change in peripheral soluble Aβ42 levels. This was accompanied by an increase in brain insoluble to soluble Aβ42 ratio in injured PS1/APP mice compared with sham injury. A parallel reduction in phagocytic receptor, triggering receptor expressed on myeloid cells 2, was also observed. This study demonstrates very subtle subchronic effects of r-mTBI on a preexisting amyloid pathology background, which may be on a continuum toward a slow and worsening neurodegenerative outcome compared with sham injury, and therefore, have many implications, especially in the elderly population exposed to TBI.
J Neuropathol Exp Neurol, 2018; 77
25002839: Ojo JO, Greenberg MB, Leary P, Mouzon B, Bachmeier C, Mullan M, Diamond DM, Crawford F
Neurobehavioral, neuropathological and biochemical profiles in a novel mouse model of co-morbid post-traumatic stress disorder and mild traumatic brain injury.
Co-morbid mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) has become the signature disorder for returning combat veterans. The clinical heterogeneity and overlapping symptomatology of mTBI and PTSD underscore the need to develop a preclinical model that will enable the characterization of unique and overlapping features and allow discrimination between both disorders. This study details the development and implementation of a novel experimental paradigm for PTSD and combined PTSD-mTBI. The PTSD paradigm involved exposure to a danger-related predator odor under repeated restraint over a 21 day period and a physical trauma (inescapable footshock). We administered this paradigm alone, or in combination with a previously established mTBI model. We report outcomes of behavioral, pathological and biochemical profiles at an acute timepoint. PTSD animals demonstrated recall of traumatic memories, anxiety and an impaired social behavior. In both mTBI and combination groups there was a pattern of disinhibitory like behavior. mTBI abrogated both contextual fear and impairments in social behavior seen in PTSD animals. No major impairment in spatial memory was observed in any group. Examination of neuroendocrine and neuroimmune responses in plasma revealed a trend toward increase in corticosterone in PTSD and combination groups, and an apparent increase in Th1 and Th17 proinflammatory cytokine(s) in the PTSD only and mTBI only groups respectively. In the brain there were no gross neuropathological changes in any groups. We observed that mTBI on a background of repeated trauma exposure resulted in an augmentation of axonal injury and inflammatory markers, neurofilament L and ICAM-1 respectively. Our observations thus far suggest that this novel stress-trauma-related paradigm may be a useful model for investigating further the overlapping and distinct spatio-temporal and behavioral/biochemical relationship between mTBI and PTSD experienced by combat veterans.
Front Behav Neurosci, 2014; 8

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9-13 July 2022 | Paris, France

BOARD NUMBER: S02-600

THE FUNDAMENTALS OF GAZE STABILIZATION – WHAT THE EYES CAN TELL US ABOUT SENSORY INTEGRATION

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

Tobias Wibble[1,2], Sten Grillner[2], Tony Pansell[1], Juan Pérez-Fernández[3]
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Aims Gaze stabilization is primarily achieved through the optokinetic (OKR) and vestibulo-ocular (VOR) reflexes, which work in constant synergy to compensate for movements across the retina. This project aims to illustrate how these primordial circuits operate using the lamprey animal model, and expand on their relevance in humans using eye-tracking. **Methods** Trials involved participants sitting in a mechanized sled facing a projected visual scene. The scene and the chair would then be rotated with different levels of visual and vestibular intensities while subjects' eyes were recorded. Trials were also performed on an eye-brain preparation of the lamprey exposed to similar stimulations as in the human experiments, which allowed us to map what neural structures were involved in producing gaze-stabilizing eye movements. **Results** The human studies showed that rotational stimulations evoke both torsional and vergence eye movements, and that the torsional response was increased to greater visual clutter (n=13), while the vergence increased to higher accelerations (n=12). We were able to quantify the percentual influence of visual and vestibular input during any given stimulation (n=25). Experiments on the lamprey showed that the mechanisms underlying gaze-stabilization are conserved and performed by circuits in the brainstem, not requiring cortical or cerebellar contributions. **Conclusions** Gaze-stabilization offered clear insights into how the brain integrates sensory information, which is reflected in its fundamental brainstem network. As several aspects of our results are relevant for postural control, ocular torsion and vergence may hold important clinical utility when treating patients with diffuse motion hypersensitivity, particularly visually induced dizziness.

Pubmed:
34668924: Frattini D, Wibble T
Alertness and Visual Attention Impact Different Aspects of the Optokinetic Reflex.
Assessing visual attention and alertness is of great importance in visual and cognitive neuroscience, providing objective measures valuable for both researchers and clinicians. This study investigates how the optokinetic response differs between levels of visual attention in healthy adults while controlling for alertness.
Invest Ophthalmol Vis Sci, 2021; 62
32968160: Wibble T, Pansell T
Optokinetic stimulation induces vertical vergence, possibly through a non-visual pathway.
Vertical vergence is generally associated with one of three mechanisms: vestibular activation during a head tilt, induced by vertical visual disparity, or as a by-product of ocular torsion. However, vertical vergence can also be induced by seemingly unrelated visual conditions, such as optokinetic rotations. This study aims to investigate the effect of vision on this latter form of vertical vergence. Eight subjects (4m/4f) viewed a visual scene in head erect position in two different viewing conditions (monocular and binocular). The scene, containing white lines angled at 45° against a black background, was projected at an eye-screen distance of 2 m, and rotated 28° at an acceleration of 56°/s. Eye movements were recorded using a Chronos Eye-Tracker, and eye occlusions were carried out by placing an infrared-translucent cover in front of the left eye during monocular viewing. Results revealed vergence amplitudes during binocular viewing to be significantly lower than those seen for monocular conditions ($p = 0.003$), while torsion remained unaffected. This indicates that vertical vergence to optokinetic stimulation, though visually induced, is visually suppressed during binocular viewing. Considering that vertical vergence is generally viewed as a vestibular signal, the findings may reflect a visually induced activation of a vestibular pathway.
Sci Rep, 2020; 10
32392313: Wibble T, Engström J, Pansell T
Visual and Vestibular Integration Express Summative Eye Movement Responses and Reveal Higher Visual Acceleration Sensitivity than Previously Described.
Acceleration plays a great impact on the vestibular system, but is attributed little influence over vision. This study aims to

explore how visual and vestibular acceleration affect roll-plane oculomotor responses, including their addiative effect.
Invest Ophthalmol Vis Sci, 2020; 61
32077140: Wibble T, Engström J, Verrecchia L, Pansell T
The effects of meclizine on motion sickness revisited.
Antihistamines make up the first line of treatments against motion-sickness. Still, their efficacy and specific mechanism have come into question. The aim of this study was to investigate the effect of meclizine on motion-sensitivity.
Br J Clin Pharmacol, 2020; 86
31900468: Wibble T, Södergård U, Träisk F, Pansell T
Intensified visual clutter induces increased sympathetic signalling, poorer postural control, and faster torsional eye movements during visual rotation.
Many dizzy patients express a hypersensitivity to visual motion and clutter. This study aims to investigate how exposure to rotating visual clutter affects ocular torsion, vertical skewing, body-sway, the autonomic pupillary response, and the subjective feeling of discomfort to the stimulation. Sixteen healthy subjects were exposed to 20 seconds rotational visual stimulation (72 deg/s; 50 deg visual field). Visual stimuli were comprised of black lines on a white background, presented at low and high intensity levels of visual clutter, holding 19 lines and 38 lines respectively. Ocular torsion and vertical skewing were recorded using the Chronos Eye Tracker, which also measured pupil size as a reflection of the autonomic response. Postural control was evaluated by measuring body-sway area on the Wii Balance Board. Values were compared to data retrieved 20 seconds before and after the optokinetic stimulation, as subjects viewed the stationary visual scene. The high intensity stimulus resulted in significantly higher torsional velocities. Subjects who were exposed to low intensity first exhibited higher velocities for both intensities. Both pupil size and body sway increased for the higher intensity to both the moving and stationary visual scene, and were positively correlated to torsional velocity. In conclusion, exposure to visual clutter was reflected in the eye movement response, changes in postural control, and the autonomic response. This response may hold clinical utility when assessing patients suffering from visual motion hypersensitivity, while also providing some context as to why some healthy people feel discomfort in visually cluttered surroundings.
PLoS One, 2020; 15
31296565: Suzuki DG, Pérez-Fernández J, Wibble T, Kardamakis AA, Grillner S
The role of the optic tectum for visually evoked orienting and evasive movements.
As animals forage for food and water or evade predators, they must rapidly decide what visual features in the environment deserve attention. In vertebrates, this visuomotor computation is implemented within the neural circuits of the optic tectum (superior colliculus in mammals). However, the mechanisms by which tectum decides whether to approach or evade remain unclear, and also which neural mechanisms underlie this behavioral choice. To address this problem, we used an eye-brain-spinal cord preparation to evaluate how the lamprey responds to visual inputs with distinct stimulus-dependent motor patterns. Using ventral root activity as a behavioral readout, we classified 2 main types of fictive motor responses: () a unilateral burst response corresponding to orientation of the head toward slowly expanding or moving stimuli, particularly within the anterior visual field, and () a unilateral or bilateral burst response triggering fictive avoidance in response to rapidly expanding looming stimuli or moving bars. A selective pharmacological blockade revealed that the brainstem-projecting neurons in the deep layer of the tectum in interaction with local inhibitory interneurons are responsible for selecting between these 2 visually triggered motor actions conveyed through downstream reticulospinal circuits. We suggest that these visual decision-making circuits had evolved in the common ancestor of vertebrates and have been conserved throughout vertebrate phylogeny.
Proc Natl Acad Sci U S A, 2019; 116
30897617: Wibble T, Pansell T
Vestibular Eye Movements Are Heavily Impacted by Visual Motion and Are Sensitive to Changes in Visual Intensities.
Eye movement evaluation constitutes the basis of diagnosis in dizzy patients. Through evaluating ocular torsion and vertical skewing during balance provoking stimulation, the aim of this study was to investigate the impact of vision on a typical vestibular eye movement response.
Invest Ophthalmol Vis Sci, 2019; 60

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BOARD NUMBER: S02-602

DEVELOPMENTAL SWITCH IN VESTIBULO-SPINAL NEURONAL PHENOTYPES DURING THE XENOPUS METAMORPHOSIS.

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

Francois Lambert, Gabriel Barrios, Anne Olechowski, Laura Cardoit, Muriel Thoby-Brisson
University of Bordeaux, Incia Cnrs Umr 5287, Bordeaux, France

Vestibular neurons involved in vestibular pathways exhibit distinct intrinsic membrane properties tuned to ensure various sensory-motor tasks. The amphibian metamorphosis represents a relevant neuronal plasticity model to investigate this neural computation. In adult frog, vestibular neurons present two phenotypes according to their discharge dynamic: 1) Phasic neurons exhibiting a high-frequency burst of 1-3 spikes, without subsequent continuous discharge and 2) tonic neurons firing continuously. These neuronal sub-populations act as band-pass and low-pass neuronal filters, respectively. However such a characterization remains unrelated to a specific vestibular function and so far nothing is known about the maturation of these neuronal dynamics. This study aims to investigate the maturation of membrane properties specifically expressed in vestibulospinal neurons involved in postural control, during the complete re-organization of the body induced by the metamorphosis. Discharge dynamics of vestibulospinal neurons were characterized in larval and juvenile *Xenopus laevis* frog. Vestibulo-spinal neurons were identified by retrograde labeling from the spinal cord. Patch-clamp recordings of VST neurons were performed on brainstem slices in voltage and current clamp configurations. Both developmental stages exhibited classical phasic and tonic VST neurons with some variations in their membrane properties. Surprisingly the proportion of tonic and phasic neurons was reversed from larva (70% of tonic) to juvenile (70% of phasic). These results revealed a developmental switch in the expression of neuronal phenotypes dedicated to a specific vestibular function and lead to promising hypothesis: 1/ which maturation mechanism is responsible for such a switch; 2/ does this switch exist in other functional 2°OVN groups?

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BOARD NUMBER: S02-603

CHARACTERIZATION OF ANATOMICAL AND FUNCTIONAL VESTIBULOSPINAL PATHWAYS IN LARVAL XENOPUS LAEVIS

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

Gabriel Barrios, Laura Cardoit, Didier Le Ray, Francois Lambert
University of Bordeaux, Incia Cnrs Umr 5287, Bordeaux, France

In all vertebrates vestibulo-spinal reflexes participate to postural adjustments needed during passive and active motion. So far this modality of postural control is barely understood in aquatic animals, facing different constrains than terrestrial models. The amphibian metamorphosis, provoking the complete remodeling of the locomotor system, is a developmental context particularly interesting to address this question. This study aims to characterize vestibulo-spinal pathways in larval xenopus laevis. Neuronal tracing of tail axial motoneurons and *synapsin* immunostaining were combined with confocal microscopy to explore anatomical vestibular motor pathways in the larval cord. Vestibular-evoked activity was measured from extracellular nerve recordings of spinal roots activity in response to vestibular nuclei electrical stimulation or galvanic stimulation of horizontal semicircular canal afferents, mimicking head horizontal rotations. Multi-level retrograde labelling from spinal segments (5^{th}, 12^{th} and 16^{th}) revealed that vestibulospinal neurons projected specifically either in conserved or in degenerated spinal regions after metamorphosis, respectively. Both anterograde labelling and electrical stimulation from bilateral vestibulospinal nuclei also showed functional vestibular terminals on rostral, mid-caudal and caudal axial. Galvanic stimulation elicited postural reflexive or various locomotor responses in rostro-caudal spinal motor nerves. Vestibular-induced responses remained in caudal spinal segments despite the blocking of rostral segments, demonstrating independent vestibulospinal pathways from rostral to caudal spinal regions. Altogether, our results showed vestibulospinal functional pathways inducing either reflexive or locomotor postural motor outcomes, suggesting 1/ the implication of other networks (reticular formation, CPGs) and 2/ the existence of a vestibular control specific to larval stage giving a flexible behavioural repertoire.

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ALTERED OPTOKINETIC REFLEXES IN PATIENTS WITH POST-CONCUSSION SYNDROME AND VISUAL VERTIGO

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

Davide Frattini[1], Mariagrazia Benassi[2], Roberto Bolzani[2], Tony Pansell[1], Tobias Wibble[1]
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Aims Visual vertigo is characterized by a heightened sensitivity to visual motion and is often associated with vestibular symptoms. While it is a common sequela after a concussion, many patients receive little rehabilitation, likely due to a limited number of diagnostic tools. This study investigates how optokinetic gaze-stabilization may be used to identify visual vertigo, focusing on ocular torsion (OT) and vertical vergence (VV). **Methods** Nine concussed patients with visual vertigo (7m, 2f; age 36±11.22) and nine healthy controls (7m, 2f; age 39.66±10.06) were recruited. Subjects were exposed to rotational optokinetic stimulations while wearing a head-mounted eye-tracker measuring slow-phase velocities. Stimulations differed in terms of movement coherence between central and peripheral visual fields, rotating either clockwise, counter-clockwise, or randomly. **Results** A generalized estimation equations analysis revealed a significant increase in VV slow phase velocity in the patient group (p = .002), VV peak velocity (p = .002), and OT peak velocity (p = .019). The VV-OT ratio was significantly increased in the patient group during coherent full-field stimulation (p = .029). **Conclusions** Both optokinetic gaze-stabilizing eye movements presented increased velocities in patients, indicating heightened neural sensitivity to visual motion. The VV-OT ratio was increased in patients during full-field rotations, showing that vergence was more readily affected in these patients. Vertical vergence is generally considered a vestibular response, and so a heightened VV velocity may suggest a greater visual influence over the vestibular nuclei. Altogether, vertical vergence may prove a valuable clinical tool when assessing patients with visual vertigo.

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CHRONIC STRESS TRIGGERS DISMANTLEMENT OF THE CALYCEAL JUNCTION BETWEEN TYPE I VESTIBULAR HAIR CELLS AND CALYX AFFERENT TERMINALS

POSTER SESSION 02 - SECTION: MOVEMENT: BRAINSTEM AND VESTIBULAR CONTROL

Jordi Llorens[1], Mireia Borrajo[1], Angela Callejo[2], Marta Perez-Grau[2], Emilio Amilibia[2], Francesc Roca-Ribas[2], Elisabeth Castellanos[3], Alberto Maroto[1]
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Reversible synaptic uncoupling between vestibular hair cells (HC) and afferent terminals, including early dismantlement of the calyceal junction in the contact between type I HC (HCI) and calyx afferent terminals, has been described in the IDPN model of sub-chronic ototoxicity in rats. In this study, we evaluated whether this pathological process has wider significance. First, we studied if calyceal junction dismantlement occurs in rats treated with a clinically relevant ototoxin, streptomycin. Weaning rats received streptomycin once or twice a day for 3 to 8 weeks. Vestibular function was assessed using the tail-lift reflex. Sensory epithelia were studied by SEM, TEM, and confocal microscopy. Streptomycin caused reversible loss of vestibular function. In the sensory epithelia, a partial loss of HC occurred. Moreover, decreased expression of CASPR1, denoting calyceal junction dismantlement, was observed in the calyces encasing the surviving HCI. We also recorded reduced numbers of pre-synaptic (ribeye) and post-synaptic (PSD95) puncta per cell, and KCNQ4 mislocalization. After washout, the CASPR1 label revealed junction rebuilding. Second, we studied by confocal microscopy human sensory epithelia from vestibular schwannomma patients; these tumors cause inflammatory stress on the sensory epithelia. Some samples had intact calyceal junctions, but others presented patched label indicating junction dismantlement. In conclusion, dismantlement of the calyceal junction is a common response triggered by chronic stress in the vestibular sensory epithelium. This effect precedes HC loss, is reversible, and may explain, at least in part, reversible function loss. Support: grants RTI2018-096452-B-100 (MINECO/FEDER, EU), 2017SGR621 (Generalitat Catalunya), 202007-30-31 (Marató TV3).

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BOARD NUMBER: S02-606

DIFFERENTIAL ENCODING OF ODOR INFORMATION BY DISTINCT SUBPOPULATIONS OF OLFACTORY CORTEX PROJECTION NEURONS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Sensory cortexes contain a multitude of neuron types projecting to different target areas. Determining their distinct functional properties is essential to better understand how stimulus information is transmitted across brain areas. Piriform cortex (PCx), the main recipient of sensory input from the olfactory bulb, contains neurons that project to numerous areas, including the Olfactory Bulb (PCx_{OB}) and the Medial Prefrontal Cortex (PCx_{mPFC}). Both PCx_{OB} and PCx_{mPFC} cells are deep-layer pyramidal cells, however, whether they selectively encode distinct stimulus information is unknown. Here, we record neuronal activity in the PCx of awake behaving mice using GRIN lens technology and two-photon calcium imaging microscopy. We label PCx_{OB} and PCx_{mPFC}subpopulations using retrogradely transported Adeno-associated virus, and we determine their response properties under passive odor presentation and associative tasks. We show that PCx_{OB} and PCx_{mPFC} subpopulations can accurately be identified based on their odor response profiles. Moreover, odor identity is more efficiently encoded in PCx_{mPFC} neurons compared to PCx_{OB} neurons. Odor coding efficiency dynamically change in both ensembles with behavioral state. Our results suggest that odor information coding differs between PCx neuron types, providing new insights into how olfactory information is routed across brain areas.

Pubmed:
34353899: DuBreuil DM, Lopez Soto EJ, Daste S, Meir R, Li D, Wainger B, Fleischmann A, Lipscombe D
Heat But Not Mechanical Hypersensitivity Depends on Voltage-Gated Ca2.2 Calcium Channel Activity in Peripheral Axon Terminals Innervating Skin.
Voltage-gated Ca2.2 calcium channels are expressed in nociceptors at presynaptic terminals, soma, and axons. Ca2.2 channel inhibitors applied to the spinal cord relieve pain in humans and rodents, especially during pathologic pain, but a biological function of nociceptor Ca2.2 channels in processing of nociception, outside presynaptic terminals in the spinal cord, is underappreciated. Here, we demonstrate that functional Ca2.2 channels in peripheral axons innervating skin are required for capsaicin-induced heat hypersensitivity in male and female mice. We show that Ca2.2 channels in TRPV1-nociceptor endings are activated by capsaicin-induced depolarization and contribute to increased intracellular calcium. Capsaicin induces hypersensitivity of both thermal nociceptors and mechanoreceptors, but only heat hypersensitivity depends on peripheral Ca2.2 channel activity, and especially a cell-type-specific Ca2.2 splice isoform. Ca2.2 channels at peripheral nerve endings might be important therapeutic targets to mitigate certain forms of chronic pain. It is generally assumed that nociceptor termini in the spinal cord dorsal horn are the functionally significant sites of Ca2.2 channel in control of transmitter release and the transmission of sensory information from the periphery to central sites. We show that peripheral Ca2.2 channels are essential for the classic heat hypersensitivity response to develop in skin following capsaicin exposure. This function of Ca2.2 is highly selective for heat, but not mechanical hypersensitivity induced by capsaicin exposure, and is not a property of closely related Ca2.1 channels. Our findings suggest that interrupting Ca2.2-dependent calcium entry in skin might reduce heat hypersensitivity that develops after noxious heat exposure and may limit the degree of heat hypersensitivity associated with certain other forms of pain.
J Neurosci, 2021; 41

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BOARD NUMBER: S02-607

NEURAL CIRCUIT BASIS OF AVERSIVE ODOUR PROCESSING IN DROSOPHILA FROM SENSORY INPUT TO DESCENDING OUTPUT

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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How are sensorimotor transformations in the nervous system instantiated in the circuitry of the brain? *Drosophila melanogaster* is a powerful model to answer this question, thanks to its extensive genetic toolkit and the release of multiple synaptic-resolution electron-microscopy volumes (Zheng et al., 2018, Scheffer et al., 2020). Previous work has shown that the Or56a receptor neurons are narrowly tuned to the microbial odorant geosmin. These microbes interrupt egg-development, and female flies avoid it when laying eggs (Stensmyr et al., 2012). This circuit has been characterised using a combination of light and electron microscopy, electrophysiology, functional imaging, and egg-laying assays to establish the necessary behavioural components (Huoviala et al., 2018). Within this circuit multiple levels of convergence have been identified, including an axo-axonic input onto neurons conveying another danger signal, the pheromone of parasitoid wasps. We also observe extensive divergence: second order geosmin neurons connect with a diverse array of ~80 third order cell types. At the next synaptic layer we have shown that a descending neuron (DN) from the brain to the ventral nerve cord (VNC) is required for females to avoid geosmin during egg-laying. Light-activation of this DN triggers backwards movement, and it shares downstream targets in the VNC with Moonwalker neurons (Williamson et al. 2018). These findings show that olfactory pathways from brain to nerve cord may involve as few as 4 synaptic levels, as previously suggested for pheromone responses (Ruta et al., 2010), and fewer than thought to exist in visual escape responses (von Reyn et al., 2017).

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PREVALENCE OF OLFACTORY DYSFUNCTION AMONG POST-PARTUM WOMEN WITH AND WITHOUT PRENATAL SARS-COV-2 INFECTIONS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

Maha Hussain[1], Fatimah Dawood[2], Melissa Stockwell[1], Gabriella Newes-Adeyi[3], Michael Varner[4], Alan Tita[5,6], Diego Alvarez[1], Ashley Battarbee[5,6], Ann Bruno[4], Siri Doddi[1], Cristina Fernández[1], Kaylee Fisher[1], Violet Hott[1], Yunzhe Hu[1], Mia Kyler[1], Helen Tzul Lopez[1], Margaret Kyle[1], Julie Mennella[7], Mirella Mourad[8], Emily Powers[4], Lawrence Reichle[3], Grace Smotrich[1], Melanie Tejeda Romero[1], Kelly Vorwaller[4], Kristina Wielgosz[2], Jonathan Overdevest[9], Dani Dumitriu[1,10]
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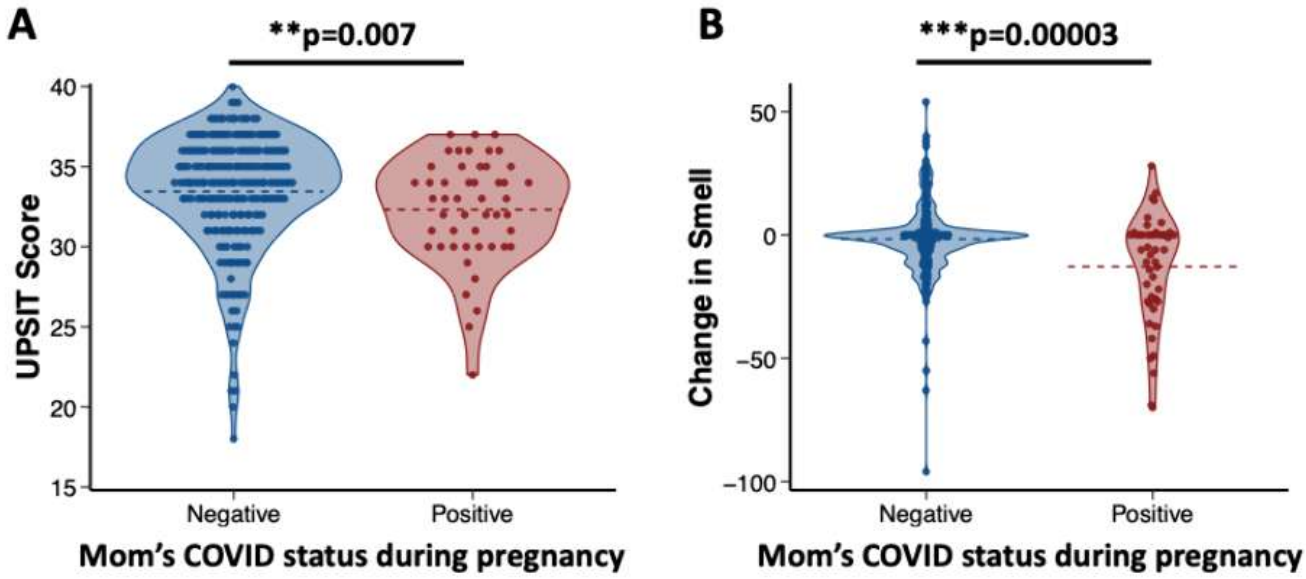
Aims: Post-partum olfaction is critical to mother-baby bonding. We compare post-partum olfaction among women with and without prenatal SARS-CoV-2 infection. Methods: We enrolled pregnant women (n=453) in New York, Utah, and Alabama. Participants self-reported history of COVID-19, participated in surveillance for SARS-CoV-2 infection with symptom monitoring, weekly nasal swabs for SARS-CoV-2 PCR-testing, and up to three blood draws for SARS-CoV-2 antibody-testing. A subset completed objective (University of Pennsylvania Smell Identification Test (UPSIT)) and subjective (self-reported smell) measures of olfaction within 6-months post-partum. We compared unadjusted olfactory function among women with (cases) and without (controls) prenatal SARS-CoV-2 infection using chi-square and T-tests. Current analyses excluded women with pre-pregnancy and/or postpartum infections. Mean±SDs are reported. Results: 195 controls and 48 cases completed olfaction testing. Among cases, none were hospitalized and infections were asymptomatic for 8 and symptomatic for 32 (severity missing for 8 cases with self-reported infections prior to surveillance). Olfaction testing was completed 227±12 days post-infection (range: 68-417). Compared to controls, a smaller proportion of cases had olfactory function within the UPSIT normosmic range (25% vs. 48%, chi-square=8.4, p=0.004). Cases also had greater post-partum microsmia severity (1=anosmia: 5=normosmia, 3.92±0.12 vs. 4.31±0.06, p=0.003), lower total UPSIT scores (32.3±0.5 vs. 33.8±0.2 of 40, p=.007, Figure 1a), and larger loss of self-reported sense of smell post-partum compared to pre-pregnancy (vs. -13±3 vs. -2±1 of 100, p=0.00003, Figure 1b). Conclusion: Maternal prenatal SARS-CoV-2 infection is associated with objective and subjective post-partum olfactory dysfunction in unadjusted models, which could have implications for mother-baby

bonding.

Figure 1



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γ-SYNCHRONIZATION ENHANCES TRANSMISSION OF SENSORY INFORMATION IN THE BRAIN

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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γ-oscillatory activity is ubiquitous across brain areas, but the ways it contributes to sensory processing and cognitive function remain unclear. Numerous studies and theoretical models have suggested that γ-synchrony should enhance the transmission of sensory information in the brain . However, direct causal evidence is still lacking. Furthermore, γ-oscillations are typically generated by the increased rhythmic activity of inhibitory neurons, which reduces the number of output spikes transmitted to the next brain region, thus raising the question of whether synchrony can compensate for the reduction in firing rate . Here we tested this hypothesis in the mouse olfactory system, where local GABAergic granule cells in the olfactory bulb shape mitral/tufted cell (MTC) excitatory output from the olfactory bulb to downstream piriform cortex. By optogenetically modulating granule cell activity, we successfully dissociated MTC γ-synchronization from MTC firing rates. By recording odor responses in the downstream piriform cortex neurons, we found that increasing MTC γ-synchronization enhanced cortical neurons' odor-evoked firing rates, reduced their response variability, and improved their odor ensemble representation. These improvements occurred despite a reduction in MTC firing rates. Furthermore, reducing MTC γ-synchronization without changing its firing rates via suppression of granule cells activity, degraded piriform cortex odor-evoked responses. Finally, directly or indirectly suppressing MTC firing rates without increasing synchrony degraded piriform cortex odor responses. These findings provide causal evidence that increased γ-synchronization enhances the transmission of sensory information between two brain regions.

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GPCR VOLTAGE DEPENDENCE CONTROLS NEURONAL PLASTICITY AND BEHAVIOR

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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G-protein coupled receptors (GPCRs) play a paramount role in diverse brain functions. Almost 20 years ago, GPCR activity was shown to be regulated by membrane potential *in vitro*, but whether the voltage dependence of GPCRs contributes to neuronal coding and behavioral output under physiological conditions *in vivo* has never been demonstrated. We show, that muscarinic GPCR mediated neuronal potentiation *in vivo* is voltage dependent. This potentiation voltage dependency is abolished in mutant animals expressing a voltage independent receptor. Depolarization alone, without a muscarinic agonist, resulted in a nicotinic ionotropic receptor potentiation that was mediated by muscarinic receptor voltage dependency. Most important, muscarinic receptor voltage independence caused a strong behavioral effect of increased odor habituation. Together, this study provides the very first demonstration of a physiological role for the voltage dependency of GPCRs by demonstrating crucial involvement of GPCR voltage dependence in neuronal plasticity and behavior. Thus, this study suggests that GPCR voltage dependency plays a role in many diverse neuronal functions including learning and memory and may serve as a target for novel drug development.

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IMPACT OF ODOR-INDUCED REDUCTION IN ANXIETY ON BRAIN'S ACTIVITY AND CONNECTIVITY.

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Anxiety disorders are a group of mental illnesses that is characterized by feelings of fear or worry. Odor exposure can be used to reduce anxiety; however, the neural basis of this emotional improvement is unclear. In this context, we investigated brain activity and connectivity in mice enriched with pleasant odorants that showed a reduction of anxiety compared to mice enriched with neutral odorants showing no change in anxiety level. Analysis of c-Fos expression across twenty brain regions involved in olfactory and anxiety circuits allowed the identification of a pattern of correlated activity specific of the odor-induced reduction in anxiety. Within the network of correlated activity, we identified key brain structures that could sustain this emotional improvement by presenting important increased connectivity, the paraventricular thalamic nucleus, the hypothalamic paraventricular nucleus and the postero-lateral-cortical amygdaloid area. These finding confirm the efficiency of odorant stimulation on well-being and further propose neural mechanism and regions that may play privileged roles in this process.

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INVESTIGATING SPARSE CODING ADAPTATION IN KENYON CELLS OF DROSOPHILA MELANOGASTER

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Stimulus-specificity of associative memories requires sparse coding in the neurons storing the memory. Effective sparse coding requires that the memory-storing neurons have approximately equal probabilities of firing across all stimuli. Otherwise, some cells will be disproportionately active or silent, and thereby be less informative about stimulus identity. We study the problem of how distributed sparse coding is maintained in Kenyon cells (KCs). KCs are the third order olfactory neurons in Drosophila melanogaster that store olfactory associative memories. We are investigating the hypothesis that KCs homeostatically adjust their intrinsic properties to ensure even activity across the population of KCs by using ex-vivo whole-cell patch-clamping, two-photon imaging and genetic manipulation of ion channel expression. Acute overexpression of the bacterial voltage-gated sodium channel, NaChBac, in KCs increases their excitability (as measured by amplitude of odour responses); while chronic expression of NaChBac throughout development paradoxically decreased KC excitability. This suggests KC can compensate for excitability and even overcompensate by homeostatic mechanisms. Calcium imaging results indicate that overexpressing a voltage-gated sodium channel in KCs paradoxically decreases their excitability, as measured by amplitude of odour responses. Additionally, initial results suggest that the inclusion of NaChBac causes the axon initial segment of the KCs to decrease the number of para channels. Lastly, knocking down or disrupting potassium channels appears to either weaken odour responses or have no effect in specific mushroom body lobes. We are currently investigating the acute expression of the potassium channels.

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MICE CAN EXTRACT INFORMATION FROM TEMPORALLY COMPLEX ODOUR PLUMES TO LEARN ABOUT DISTANCE TO AN ODOUR SOURCE

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Natural odour plumes are shaped by airflow turbulence, resulting in high frequency odour intensity fluctuations that may contain information about odour source location. We aimed to investigate whether mice can extract and use this information in a distance discrimination task. We built a wind tunnel and odour delivery devices to reliably generate and record odour plumes. In addition, we created an “olfactory virtual reality” by replicating the recorded odour plumes with a high-temporal-bandwidth multichannel odour delivery device. In a high-throughput behavioural conditioning system, we trained mice to perform distance discrimination tasks, either in “olfactory virtual reality”, or directly using odour plumes generated in the wind tunnel. Using odour sources placed at different distances in a wind tunnel, we found that mice learnt to respond differently to odour sources based on their distance. In a separate cohort of mice, we performed calcium imaging of neurons in the mouse Olfactory Bulb while presenting distant odour plumes in olfactory virtual reality, and found a subset of Mitral and Tufted Cells that responded differently to different distances. As total odour concentration was kept constant, we suggest these neurons respond to features of the temporal structures of odour plumes, which are informative of source distance. We conclude that mice are able to perform distance discrimination using olfactory information available in temporally complex odour plumes, and that the differential processing of this information can already be observed at the level of the Olfactory Bulb.

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BOARD NUMBER: S02-614

STIMULUS DURATION ENCODING BY MOTH OLFACTORY RECEPTOR NEURONS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Insects heavily rely on olfactory cues in their search for a mate, food, and a suitable place for egg-laying. Flying insects encounter turbulent environments, where chemotaxis along a concentration gradient makes little sense. Detection of the onset and offset of discrete odor pulses is predicted to become crucial for navigation in this environment. It is well understood how the olfactory system encodes the onset, but surprisingly, not the offset, i.e., the duration of the odor pulse. Typically, the reported duration of moth olfactory receptor neuron's (ORN) spike firing response greatly exceeds the pulse duration. We built a precise olfactometer to eliminate possible artefacts caused by low pheromone volatility and performed extracellularly single sensillum recordings from the ORN. We found that the sharp pheromone pulses generated by the new olfactometer evoked firing responses in the ORN that faithfully tracked the pulse duration, provided the pulse lasted at least 200ms. A transient inhibition marked the termination of the pulse and disappeared with shorter pulses. We could precisely predict the firing response from the receptor potential with a linear filter harboring several time constants. Particularly, a slow (±500ms) adaptation of the spike generating mechanism is necessary to reproduce the qualitatively different response to different pulse durations. The observed limits in temporal resolution are in agreement with behavioral studies. Our findings show that moth ORNs are better at encoding stimulus duration than previously thought and help understand how their olfactory system encodes the fine structure of the pheromone plume in the turbulent environment.

Pubmed:
33736083: Barta T, Kostal L
Regular spiking in high-conductance states: The essential role of inhibition.
Strong inhibitory input to neurons, which occurs in balanced states of neural networks, increases synaptic current fluctuations. This has led to the assumption that inhibition contributes to the high spike-firing irregularity observed in vivo. We used single compartment neuronal models with time-correlated (due to synaptic filtering) and state-dependent (due to reversal potentials) input to demonstrate that inhibitory input acts to decrease membrane potential fluctuations, a result that cannot be achieved with simplified neural input models. To clarify the effects on spike-firing regularity, we used models with different spike-firing adaptation mechanisms, and we observed that the addition of inhibition increased firing regularity in models with dynamic firing thresholds and decreased firing regularity if spike-firing adaptation was implemented through ionic currents or not at all. This fluctuation-stabilization mechanism provides an alternative perspective on the importance of strong inhibitory inputs observed in balanced states of neural networks, and it highlights the key roles of biologically plausible inputs and specific adaptation mechanisms in neuronal modeling.
Phys Rev E, 2021; 103
31790384: Barta T, Kostal L
The effect of inhibition on rate code efficiency indicators.
In this paper we investigate the rate coding capabilities of neurons whose input signal are alterations of the base state of balanced inhibitory and excitatory synaptic currents. We consider different regimes of excitation-inhibition relationship and an established conductance-based leaky integrator model with adaptive threshold and parameter sets recreating biologically relevant spiking regimes. We find that given mean post-synaptic firing rate, counter-intuitively, increased ratio of inhibition to excitation generally leads to higher signal to noise ratio (SNR). On the other hand, the inhibitory input significantly reduces the dynamic coding range of the neuron. We quantify the joint effect of SNR and dynamic coding range by computing the metabolic efficiency-the maximal amount of information per one ATP molecule expended (in bits/ATP). Moreover, by calculating the metabolic efficiency we are able to predict the shapes of the post-synaptic firing rate histograms that may be tested on experimental data. Likewise, optimal stimulus input distributions are predicted, however, we show that the optimum can essentially be reached with a broad range of input distributions. Finally, we examine which parameters of the used

neuronal model are the most important for the metabolically efficient information transfer.
PLoS Comput Biol, 2019; 15

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BOARD NUMBER: S02-615

COMPARATIVE STUDY OF OLFACTORY SYSTEM ONTOGENY IN MUS MUSCULUS AND OCTODON DEGUS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Olfaction is critical for survival of the individual. Olfactory reception is mediated by olfactory sensory neurons (OSNs), which are located in convoluted turbinates within the nasal cavity. The axons of OSNs coalesce into globose structures called glomeruli within the olfactory bulb. In mouse, olfactory development begins with the invagination of the olfactory pit at E10.5 and proceeds by establishing the olfactory epithelium. Here, we take a step further in understanding the complexity of the olfactory system by studying its development in a lesser-known species, the *Octodon degus*, a rodent endemic to central Chile with an exceptionally long gestation period. We performed a comparative study to identify differences in the ontogeny of the olfactory system between mouse and degu. First, we compared developmental stage-specific features in mouse and degu. After determining developmentally equivalent stages, mouse and degu embryos were collected and processed for immunofluorescence. With OSN specific markers, we found that the olfactory system is more established at birth in degu than in mouse. Glomeruli in the olfactory bulb are further developed in degu and are akin to glomeruli at later stages in mouse. In addition, the structural organization of turbinates appears more convoluted in degu than in mouse. Several structural differences were also notable at earlier developmental stages, such as glomerular formation and axon/mitral cell connectivity.

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BOARD NUMBER: S02-616

BINGE EATING SUPPRESSES FLAVOR REPRESENTATIONS IN THE MOUSE OLFACTORY CORTEX

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Appropriate feeding behavior is the foundation of maintaining homeostasis. Elevated feeding speed (binge eating) is a common trait of eating disorders and is associated with obesity. It is also known that flavor perception has an active role in regulating feeding. However, the effects of feeding speed on flavor sensory feedback remain unknown. By using miniscope in mice, we show that binge eating suppresses neuronal activity in the anterior olfactory (piriform) cortex (aPC). The strength of binge-induced inhibition in the aPC is correlated with animals' appetite. This suppression is partially due to local GABAergic interneurons (PV & SOM) in the aPC. Odor inputs from olfactory bulb mitral cells remain stable upon binge eating, suggesting the suppression is not due to degraded odor inputs. We further excluded the inhibitory effect from serotonergic modulation in the aPC by using *in vivo* serotonin imaging. Taken together, our results provide clear circuit mechanisms of binge-induced flavor modulation, which may explain binge-induced overeating due to suppression of sensory feedback of food items.

Pubmed:
33788834: Jambor H, Antonietti A, Alicea B, Audisio TL, Auer S, Bhardwaj V, Burgess SJ, Ferling I, Gazda MA, Hoeppner LH, Ilangovan V, Lo H, Olson M, Mohamed SY, Sarabipour S, Varma A, Walavalkar K, Wissink EM, Weissgerber TL
Creating clear and informative image-based figures for scientific publications.
Scientists routinely use images to display data. Readers often examine figures first; therefore, it is important that figures are accessible to a broad audience. Many resources discuss fraudulent image manipulation and technical specifications for image acquisition; however, data on the legibility and interpretability of images are scarce. We systematically examined these factors in non-blot images published in the top 15 journals in 3 fields; plant sciences, cell biology, and physiology (n = 580 papers). Common problems included missing scale bars, misplaced or poorly marked insets, images or labels that were not accessible to colorblind readers, and insufficient explanations of colors, labels, annotations, or the species and tissue or object depicted in the image. Papers that met all good practice criteria examined for all image-based figures were uncommon (physiology 16%, cell biology 12%, plant sciences 2%). We present detailed descriptions and visual examples to help scientists avoid common pitfalls when publishing images. Our recommendations address image magnification, scale information, insets, annotation, and color and may encourage discussion about quality standards for bioimage publishing.
PLoS Biol, 2021; 19
32457067: Moreno-Velasquez L, Lo H, Lenzi S, Kaehne M, Breustedt J, Schmitz D, Rüdiger S, Johenning FW
Circuit-Specific Dendritic Development in the Piriform Cortex.
Dendritic geometry is largely determined during postnatal development and has a substantial impact on neural function. In sensory processing, postnatal development of the dendritic tree is affected by two dominant circuit motifs, ascending sensory feedforward inputs and descending and local recurrent connections. In the three-layered anterior piriform cortex (aPCx), neurons in the sublayers 2a and 2b display vertical segregation of these two circuit motifs. Here, we combined electrophysiology, detailed morphometry, and Ca imaging in acute mouse brain slices and modeling to study circuit-specific aspects of dendritic development. We observed that determination of branching complexity, dendritic length increases, and pruning occurred in distinct developmental phases. Layer 2a and layer 2b neurons displayed developmental phase-specific differences between their apical and basal dendritic trees related to differences in circuit incorporation. We further identified functional candidate mechanisms for circuit-specific differences in postnatal dendritic growth in sublayers 2a and 2b at the mesoscale and microscale levels. Already in the first postnatal week, functional connectivity of layer 2a and layer 2b neurons during early spontaneous network activity scales with differences in basal dendritic growth. During the early critical period of sensory plasticity in the piriform cortex, our data are consistent with a model that proposes a role for dendritic NMDA-spikes

in selecting branches for survival during developmental pruning in apical dendrites. The different stages of the morphologic and functional developmental pattern differences between layer 2a and layer 2b neurons demonstrate the complex interplay between dendritic development and circuit specificity.
eNeuro, 2020 May/Jun; 7

FENS Forum 2022
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BOARD NUMBER: S02-617

EXPLORING THE ROLE OF THE LATERAL ENTORHINAL CORTEX IN THE MODULATION OF THE PIRIFORM CORTEX NEURONAL ACTIVITY

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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The piriform cortex (PC), the main region of the olfactory cortex, receives afferent sensory inputs from the olfactory bulb through the lateral olfactory tract (LOT), and extensive inputs from higher-order areas such as the lateral entorhinal cortex (LEC). To understand the contribution of LEC to the processing of odors, we study its functional and anatomical connectivity to excitatory and inhibitory neurons in the PC. We infected LEC with adeno-associated virus expressing channelrhodopsin under CamKIIa promoter to activate excitatory LEC afferents arriving to PC. We recorded then, in acute brain slices, postsynaptic currents and spiking in pyramidal L2/3 neurons and interneurons, in response to photostimulation. We found that excitatory long-range projections arriving from LEC evoke different excitation to inhibition balance in each type of neuron. L2 neurons and PV interneurons receive more excitation than inhibition along a 10Hz stimulation train. In addition, we observed that LEC projections preferentially contact L2 pyramidal neurons. We then explored how the PC microcircuit is reorganized in the presence of the cholinergic agonist, carbachol (CBC). We found that a diminished response to LEC activation is due to the reduction in recurrent activity, but the response to the monosynaptic input remains intact after bath infusion of CBC. Last, to assess the role of LEC in the processing of odors in vivo, we conducted experiments to inactivate this region during an odor-visual context associative task and evaluate the effect of LEC silencing after learning this olfactory behavior.

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BOARD NUMBER: S02-618

EARLY-LIFE OLFACTORY EXPERIENCE SHAPES THE CONNECTIVITY OF THE ODORANT-RESPONDING BRAIN NETWORK IN MICE

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Early-life experience shapes behavior and the anatomical and functional organization of the brain with life-long consequences. In this context, olfactory processing and related brain connectivity or plasticity remain poorly documented. Here, we measured brain connectivity based on between-region correlations of c-Fos positive cell density, in different experimental groups including male and female mice, submitted or not to olfactory enrichment from birth to weaning. The pattern of brain connectivity in the adult brain in response to an odorant stimulation was greatly modified by early-life olfactory enrichment in the direction of enhanced connection density in both males and females. Increased connectivity held for both sides of the brain to the exception of enriched females showing a left hemisphere dominance. Increased connectivity expanded beyond olfactory regions to other functional brain systems and this effect was broader in females than in males. Altogether, these findings revealed a strong and long-lasting effect of early-life olfactory enrichment on brain connectivity including lateralization and a sex-related susceptibility to these effects.

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BOARD NUMBER: S02-619

EXPRESSION OF C-FOS IN THE VCA1, DCA2, CHEMOSENSORY AMYGDALA AND REWARD SYSTEM OF FEMALE MICE INDUCED BY MALE PHEROMONAL SIGNALS.

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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[1]Facultat de Biològiques, Universitat de València, Biologia Cel·lular, Tissular I Antropologia Física, Burjassot, Spain, [2]Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Department Of Neurophysiology And Chronobiology, Kraków, Poland, [3]Universitat de València, Human Anatomy And Embriology, Faculty Of Medicine And Odontology, Valencia, Spain

Individual recognition in mice is based on the major urinary proteins present in urine, detected by the vomeronasal organ. These signals are processed in the chemosensory amygdala, where the posteromedial cortical nucleus (PMCo) constitutes the primary vomeronasal cortex and influences the hippocampal formation via direct and indirect pathways. Social memory is stored in the ventral hippocampus (vCA1), where PMCo directly projects. In addition, social information is integrated into spatial memory through an amygdalo-entorhino-hippocampal (dCA1) indirect circuit, which is highly activated in female mice exploring male urine. Although hippocampal CA2 region (dCA2) is essential for social memory, its role in vomeronasal processing is still unknown. To assess the effect of urine stimuli on c-Fos activity in the chemosensory amygdala, the vCA1 and the dCA2, we tested the preference of CD1 female mice to investigate male urine or a control odorant (citralva). Behavioral analysis showed that male urine induced a significantly longer exploration, accompanied by a significant increase in c-Fos expression in vCA1 and dCA2. In the chemosensory amygdala, higher c-Fos expression was found in the cortex-amygdala transition area and the dorsal and ventral subdivisions of the posterior medial amygdala. Since male urine induces a preferential exploration, we hypothesized a higher activation of the mesolimbic reward system and the basolateral complex of the amygdala (the interface between the chemosensory amygdala and the reward system). However, our results showed non-significant differences in these structures, where a low level of c-Fos expression was found in both experimental groups.
Funded by: MINECO-FEDER: PID2019-108562GB-I00

Pubmed:
34489431: Villafranca-Faus M, Vila-Martín ME, Esteve D, Merino E, Teruel-Sanchis A, Cervera-Ferri A, Martínez-Ricós J, Lloret A, Lanuza E, Teruel-Martí V
Integrating pheromonal and spatial information in the amygdalo-hippocampal network.
Vomeronasal information is critical in mice for territorial behavior. Consequently, learning the territorial spatial structure should incorporate the vomeronasal signals indicating individual identity into the hippocampal cognitive map. In this work we show in mice that navigating a virtual environment induces synchronic activity, with causality in both directionalities, between the vomeronasal amygdala and the dorsal CA1 of the hippocampus in the theta frequency range. The detection of urine stimuli induces synaptic plasticity in the vomeronasal pathway and the dorsal hippocampus, even in animals with experimentally induced anosmia. In the dorsal hippocampus, this plasticity is associated with the overexpression of pAKT and pGSK3β. An amygdalo-entorhino-hippocampal circuit likely underlies this effect of pheromonal information on hippocampal learning. This circuit likely constitutes the neural substrate of territorial behavior in mice, and it allows the integration of social and spatial information.
Nat Commun, 2021; 12
33544856: Ulloa-Navas MJ, Rubio L, Teruel-Sanchis A, Peña-Peña J, García-Verdugo JM, Herranz-Pérez V, Ferrer-Lozano J
Heterogeneous Pattern of Differentiation With BCAS1/NABC1 Expression in a Case of Oligodendroglioma.
J Neuropathol Exp Neurol, 2021; 80

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BOARD NUMBER: S02-620

SAMPLE PREPARATION AND WARPING ACCURACY FOR CORRELATIVE MULTIMODAL IMAGING IN THE MOUSE OLFACTORY BULB USING 2-PHOTON, SYNCHROTRON X-RAY AND VOLUME ELECTRON MICROSCOPY

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

Yuxin Zhang[1,2], Tobias Ackels[1,2], Alexandra Pacureanu[1,2,3], Marie-Christine Zdora[4,5,6,7], Anne Bonnin[7], Andreas Schaefer[1,2], Carles Bosch[1]
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Integrating physiology with structural insights of the same neuronal circuit provides a unique approach to understanding how the mammalian brain computes information. However, combining the techniques that provide both streams of data represents an experimental challenge. When studying glomerular column circuits in the mouse olfactory bulb, this approach involves e.g. recording the neuronal activity with *in vivo* 2-photon (2P) calcium imaging, retrieving the circuit structure with synchrotron X-ray computed tomography with propagation-based phase contrast (SXRT) and/or serial block-face electron microscopy (SBEM) and correlating these datasets. Sample preparation and dataset correlation are two key bottlenecks in this correlative workflow. Here, we first quantify the occurrence of different artefacts when staining tissue slices with heavy metals to generate X-ray or electron contrast. We report improvements in the staining procedure, ultimately achieving perfect staining in ~67% of the 0.6 mm thick olfactory bulb slices that were previously imaged *in vivo* with 2P. Secondly, we characterise the accuracy of the spatial correlation between functional and structural datasets. We demonstrate that direct, single-cell precise correlation between *in vivo* 2P and SXRT tissue volumes is possible and as reliable as correlating between 2P and SBEM. Altogether, these results pave the way for experiments that require retrieving physiology, circuit structure and synaptic signatures in targeted regions. These correlative function-structure studies will bring a more complete understanding of mammalian olfactory processing across length scales and time.

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BOARD NUMBER: S02-621

POTENTIAL NEUROANATOMICAL PATHWAYS FOR THE INTEGRATION OF PHEROMONAL AND SPATIAL INFORMATION.

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

Manuel E. Vila-Martín[1,2], Maria Villafranca-Faus[2], Anna Teruel-Sanchis[1], Camila Savarelli-Balsamo[2], Esteban Merino[2], Joana Martínez-Ricòs[2], Vicent Teruel-Martí[2], Enrique Lanuza[1]
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Vomeronasal information plays an essential role in rodents' individual recognition, which is crucial for orienting and navigating through their environment. This particular type of sensory information must necessarily reach the hippocampal formation to provide the identity of the conspecific owner of each territory, constituting the "who" component of episodic memory. Through tract-tracing methods we have investigated neural pathways between the chemosensory amygdala and the hippocampus. Since no direct connection was observed after retrograde tracer (FluoroGold) injection in the dorsal CA1, we looked for indirect projections through the entorhinal cortex. FluoroGold injections in the dorsolateral entorhinal cortex resulted in labelled neurons in all the cortical areas of the chemosensory amygdala (including the posteromedial nucleus of the amygdala). Conversely, the subcortical structures of the chemosensory amygdala (medial amygdala and posteromedial bed nucleus of the stria terminalis) have no direct or indirect connections with the hippocampus. This amygdalo-entorhino-hippocampal circuit may constitute the neural substrate for the integration of pheromonal information into spatial memory. In order to assess the activation of these pathways in social recognition tasks including spatial information, we have designed behavioural experiments to evaluate c-Fos expression in paradigms in which female mice explore social stimulus from different males or objects that change their spatial location over the days. Funded by the Spanish Ministry of Science and Innovation-FEDER (PID2019-108562GB-I00). M.E. Vila-Martin is a predoctoral fellow of the "Atracció de Talent" program of the University of Valencia, and this work is part of his PhD.

Pubmed:
34489431: Villafranca-Faus M, Vila-Martín ME, Esteve D, Merino E, Teruel-Sanchis A, Cervera-Ferri A, Martínez-Ricós J, Lloret A, Lanuza E, Teruel-Martí V
Integrating pheromonal and spatial information in the amygdalo-hippocampal network.
Vomeronasal information is critical in mice for territorial behavior. Consequently, learning the territorial spatial structure should incorporate the vomeronasal signals indicating individual identity into the hippocampal cognitive map. In this work we show in mice that navigating a virtual environment induces synchronic activity, with causality in both directionalities, between the vomeronasal amygdala and the dorsal CA1 of the hippocampus in the theta frequency range. The detection of urine stimuli induces synaptic plasticity in the vomeronasal pathway and the dorsal hippocampus, even in animals with experimentally induced anosmia. In the dorsal hippocampus, this plasticity is associated with the overexpression of pAKT and pGSK3β. An amygdalo-entorhino-hippocampal circuit likely underlies this effect of pheromonal information on hippocampal learning. This circuit likely constitutes the neural substrate of territorial behavior in mice, and it allows the integration of social and spatial information.
Nat Commun, 2021; 12

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-622

TARGET-SPECIFIC CONTROL OF OLFACTORY BULB PERIGLOMERULAR CELLS BY GABAERGIC AND CHOLINERGIC BASAL FOREBRAIN INPUTS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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CNRS, Institut Des Neurosciences Cellulaires Et Intégratives, Strasbourg, France

The olfactory bulb (OB), the first relay for odor processing in the brain, receives dense GABAergic and cholinergic long-range projections from basal forebrain (BF) nuclei that provide information about the internal state and behavioral context of the animal. However, the targets, impact and dynamic of these afferents are still unclear. I studied how BF synaptic inputs modulate activity in diverse subtypes of periglomerular (PG) interneurons using optogenetic stimulation and loose cell-attached or whole-cell patch-clamp recording in OB slices from adult mice. GABAergic BF inputs potently blocked PG cells firing except in a minority of calretinin-expressing cells in which GABA release elicited spiking. Parallel cholinergic projections excited a previously overlooked PG cell subtype via synaptic activation of M1 muscarinic receptors. Low frequency stimulation of the cholinergic axons drove persistent firing in these PG cells thereby increasing tonic inhibition in principal neurons. Taken together, these findings suggest that modality-specific BF inputs can orchestrate synaptic inhibition in OB glomeruli using multiple, potentially independent, inhibitory or excitatory target-specific pathways.

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BOARD NUMBER: S02-623

POPULATION CODING OF ODOR CONCENTRATION IN THE GLOMERULAR LAYER OF THE MOUSE OLFACTORY BULB

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

Mathieu Loizeau[1], Alexandra Angelova[1], Harold Cremer[1], Hervé Rouault[2], Jean Claude Platel[2]
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The first step of olfactory processing takes place in the glomeruli of the olfactory bulb (OB) where a variety of interneurons integrate the sensory information, before it is transmitted to OB output neurons. Yet, due to the high density and diversity of interneurons, their role in odor processing and in odor concentration coding remains unknown. We chose to focus on a specific population of excitatory interneurons of the glomerular layer, the external tufted cells (ETCs). We use the ND6-Cre transgenic mouse line crossed with a fluorescent reporter line and a calcium genetic sensor line to study this population. We combine high resolution two-photon structural imaging to reconstruct the dendritic morphology of ETCs and assign them to a specific glomerulus, with *in vivo* calcium imaging. By using this approach, we show that ETCs can be activated by olfactory stimulation and surprisingly, they can also be inhibited or generate complex responses. Interestingly, responding ETCs connected to the same glomerulus have significantly correlated responses. Then, we stimulate with different odor concentrations and we observe an increase of the percentage of responding ETCs within the same glomerulus meaning that ETCs are progressively recruited. This process creates a specific ETCs recruitment map in each glomerulus to discriminate odor concentration. We demonstrate that these cellular recruitment maps for a specific odor are stable over a month. Our work describes for the first time the *in vivo* properties of ETCs and a new network mechanism to decode odor concentration in the glomerular layer.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-624

THE PECULIAR CILIA OF OLFACTORY SENSORY NEURONS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

Mert Ege[1], Dheeraj Rayamajhi[2], Kirill Ukhanov[3], Christa Ringers[4], Yiliu Zhang[2], Inyoung Jeong[1], Percival Paul D'Gama[4], Hae-Chul Park[5], Jeffrey Martens[3], Sudipto Roy[2], Nathalie Jurisch-Yaksi[1]
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Cilia are hair-like structures enriched in receptors and signaling molecules, which play key roles in signal transduction. Notably, olfactory sensory neurons (OSN) cilia harbor all the machinery necessary for odor detection, and thus are indispensable for olfaction. To date, the regulatory pathways involved in controlling cilia formation in OSN remain elusive. Particularly, OSN cilia have properties of motile cilia, akin to those on respiratory and ependymal cells, yet in most vertebrates, OSN cilia lack motility. In our study, we reveal that mammalian and zebrafish OSN express the master regulator of motile ciliogenesis, *foxj1*. Interestingly, the expression of *foxj1* target genes involved in cilia motility are actively repressed in OSN as compared to motile ciliated respiratory cells, explaining their immotility. Next, we investigated the function of *foxj1* in cilia formation in zebrafish OSNs. We observed that OSN cilia are lacking in *foxj1* mutants. In line with ciliogenesis defects, we identified that *foxj1* mutant display aberrant responses to olfactory cues. Altogether, we identified a novel and critical role of *foxj1* in OSN cilia formation but not motility. We argue that understanding the regulatory pathways underlying diversity in cilia formation and function is necessary to better understand sensory deficits in patients with ciliary disorders.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-625

PERIPHERAL OLFACTORY CODING IN DROSOPHILA MELANOGASTER

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Insects, like many animals, rely strongly on their sense of smell for locating potential food sources, mates and predators. Flying insects such as *Drosophila melanogaster* possess an exceptional ability to interpret complex odour plumes. In addition to identifying odorants and their intensities, plume navigation also requires differentiating relevant odorant mixtures originating from one source from irrelevant odorants emitted from separate sources. This capacity is typically attributed to lateral inhibition in the antennae lobe. However, we propose that non-synaptic “ephaptic” interactions (NSIs) between olfactory receptor neurons (ORNs) co-located in sensilla on the antenna could also play an important role by pre-processing olfactory information. In this work, we are investigating the feasibility of this hypothesis using single sensillum recordings and a two-channel high-precision olfactory stimulator for delivering precisely timed pulses of odours selective for each of two co-localised ORNs in *Drosophila* sensilla. We demonstrate that NSIs can occur on short timescales as seen in natural odour plumes. When a brief pulse of one odorant is delivered at increasing delays from the start of a second odorant, this inhibition increases as the delay increases, and eventually saturates. These results provide not only insights into the processing speed of the insect olfactory system, but also elucidate the significance of potential interactions in other examples of compartmentalised sensory neurons, including mammalian taste buds and a wide range of invertebrate sensilla. This work was funded by a Leverhulme Trust Research Project Grant.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-626

ANATOMICAL AND FUNCTIONAL CONNECTIVITY AT THE DENDRODENDRITIC RECIPROCAL MITRAL CELL-GRANULE CELL SYNAPSE: IMPACT ON RECURRENT AND LATERAL INHIBITION

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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In the olfactory bulb, reciprocal dendrodendritic interactions between its principal neurons, the mitral and tufted cells (MTCs), and inhibitory interneurons in the external plexiform layer mediate both recurrent and lateral inhibition, with the most numerous of these interneurons being granule cells (GCs). Here, we use recently established anatomical data (most importantly the density of reciprocal synapses on MC lateral dendrites, ~ 1 per µm) and functional data (most importantly the properties of recurrent unitary GABAergic inputs from GC spines onto MCs, ~ 200 pS) to simulate the strength of recurrent MC inhibition specifically from the reciprocal spines of rat olfactory bulb GCs in a compartmental NEURON model. We predict a total recurrent inhibition in the wake of a MC action potential $\Delta V_m \approx 2$ mV. Based on these recent data sets we also update and refine a preexisting model of lateral connectivity between any two MCs (Egger & Urban 2006), including functional ensembles of MCs belonging to a glomerular column. We compute the average dendrodendritic connectivity via interconnecting GCs as a function of the distance between MCs and/or entire glomerular MC ensembles. Our results predict the extents of three regimes of anatomical connectivity between ensembles: high connectivity within a glomerulus and the first three rings of adjacent glomeruli, considerable connectivity to up to seven glomeruli away and negligible connectivity beyond. We plan to combine this anatomical connectivity with the above simulations of inhibitory output from GC spines to estimate the functional impact of lateral active columns.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-627

MOLECULAR SIGNATURES OF OLFACTORY CIRCUITS REVEALED BY SINGLE CELL MULTIOMICS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Mammals co-evolved ancient conserved cortical circuits such as the *paleocortex* together with novel ones, the *neocortex*. A *paleo* principal cell stands apart from a *neocortex* principal cell for its developmental origin, morphology, and co-expression of genetic determinants for neuronal identity. However, the mechanisms driving cell type diversification across distinct cortical traits remain unknown. Here, we characterize the gene regulatory network (GRN) that defines principal cell type identity in the mouse olfactory cortex, and we compare GRN activity across *paleo*-, *peri-paleo*- and *neo*cortical traits. Using single-nucleus dual omics (ATAC and RNA) sequencing, we reveal *cis* enhancer-gene pair interactions and identify transcription factors cross-repression as a key mechanism for cell type diversification. Finally, we propose semilunar cells of the olfactory cortex as the ancestral principal cell in the mammalian cortex. Our data provide the first comprehensive molecular description of cell types in the mouse olfactory cortex and identify epigenetic mechanisms underlying cell type diversification during evolution.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-628

ODOR HEDONIC RATINGS ARE RELATED TO INDIVIDUAL ODOR DETECTION THRESHOLDS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Aims Odor hedonic perception is considered as one of the most prominent dimensions in olfaction and is known to depend on several parameters (odorant properties, contexts, subject characteristics…). Among them, the relation between the stimulus concentration and the hedonic estimation has been widely studied. However, few studies have considered odor hedonic ratings in relation to individual detection thresholds. Thus, the aim of this study was to determine olfactory detection thresholds and to describe the evolution of hedonic ratings from the individual threshold to higher concentrations. Methods Odor detection thresholds were assessed for four odorants (two pleasant and two unpleasant) using a classical psychophysical method. Then, different odorant concentrations, above individual odor detection thresholds, were presented to the participants in a randomized order. Participants had to rate odor pleasantness of these stimuli on a visual analog scale. Results Individual detections thresholds presented a large dispersion in relation to the dilutions as function of the odorant stimulus and subjects. When individual thresholds were considered, hedonic scores evolved in the same way for all subjects. For a specific odorant, results showed a similar evolution of hedonic ratings in relation to stimulus concentration whatever the olfactory thresholds. Conclusion These findings suggest a relation between odor hedonic ratings and individual detection thresholds. Given the strong interindividual differences in olfactory sensitivity, these data contribute to explain the large variability of the hedonic tone at specific concentration in the general population, and could serve future research in this field.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-629

IMPACT OF OXYTOCIN IN THE PIRIFORM CORTEX AND EFFECT ON SOCIAL BEHAVIORS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Social interactions are crucial for the survival of most species. In rodents, these interactions are strongly influenced by olfactory cues emitted by individuals, collected during social investigation and processed by the olfactory system. Previous works indicated that the neuropeptide oxytocin (OXT) promotes social interactions and social memory in rodents and that it is released during interactions with conspecifics. Interestingly, OXT receptor expression is enriched in the piriform cortex (PIR), suggesting a role for OXT in the processing of social olfactory cues in this major part of the olfactory cortex. Yet, such role and the neurophysiological impact of OXT in PIR remains obscure. Using a local pharmacological approach and a three-chamber behavioral test, we showed that blocking OXT receptors in the PIR does not impair sociability nor social memory. However, using the Live Mouse Tracker which allows for more naturalistic social behaviors and automatic profiling of interactions motives, we showed that OXT receptor blockade in PIR induces an increase in nose-to-nose contacts between mice. To decipher how OXT modulates PIR circuits, we implanted mice with multisite electrodes in the PIR as well as nasal cannulas to monitor respiration and sniffing. Our preliminary data indicated that intraperitoneal injections of a selective OXT receptor agonist increased the firing rate of putative excitatory cells, and decreased their entrainment by the respiration signal. Overall, our work suggests that endogenous OXT modulates specific aspects of social interactions involving olfactory investigation and that neuronal activity in the PIR is sensitive to OXT manipulations.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-630

PHYSIOLOGICAL CHARACTERIZATION OF PIRIFORM CORTEX MODULATION BY RESPIRATORY SIGNAL ACROSS BRAIN STATES

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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A number of studies using time-controlled odorant presentation have demonstrated that the piriform cortex plays a key role in olfaction. However, the baseline physiological description of piriform cortex activity during odor-free baseline states remains largely unexplored. To answer this, we examined the activity of large ensembles of extracellularly recorded piriform cortex cells from freely moving mice implanted with intra-nasal pressure sensors to monitor instantaneous respiration patterns. Mice were engaged in various tasks mimicking naturalistic conditions and rest/sleep states. Close examination of respiratory traces revealed that they encompass intermingled oscillatory (inhalation/exhalation) and linear (pauses) components which piriform cortex populations are differently responsive to. Moreover, we found that when nasal pressure fluctuations are treated as an oscillatory signal, a large fraction of piriform cortex neurons are entrained by the oscillation. Interestingly, their phase locking is either altered or preserved depending on the respiration frequency and internal brain state. Next, we explored whether gamma rhythm, the most prominent oscillation typical of the piriform cortex, could be similarly modulated by the respiration phase and frequency during different brain states. Consistent with previous results, we observed two independent non-harmonical gamma bands resonating around 40 Hz and 60 Hz. During wake state, their prominence was differentially modulated by the phase and frequency of the respiratory oscillation pattern. Together, these results highlight several physiological features of the piriform cortex during different brain states and behavioral tasks, enriching our global understanding of its complexity and computation supporting olfactory representation.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-631

ADAPTIVE TRAITS IN THE DROSOPHILA MUSHROOM BODY

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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How the brain adapts as species evolve remains largely unknown. Thus far, adaptive traits have been primarily identified in sensory systems, suggesting that sensory neurons might be more malleable to evolutionary pressures than the neurons embedded in higher brain centers. In *Drosophila*, comparative studies of closely related species that live in drastically different ecology revealed that olfactory sensory neurons adapt by developing new detection capabilities. For instance, the obligate noni specialist *Drosophila sechellia* is equipped with olfactory sensory neurons finely tuned to noni volatiles whereas its close generalist relatives — *Drosophila melanogaster* and *Drosophila simulans* — is equipped with olfactory sensory neurons tuned to more general fruit volatiles . Here, we use different neuronal tracing techniques to determine how the olfactory circuit connects to higher brain centers in these three species. Statistical analyses of these connections enable us to test which, if any, global architectural features of higher brain centers vary across these species. We found that — although most features do not vary — the projection neurons downstream of the olfactory sensory neurons detecting noni and fruit volatiles have adapted in *D. sechellia*through different cellular mechanisms: these projection neurons either change in number, vary in their connectivity rates or target different neuropiles. Altogether, this study shows that higher brain centers are just as malleable to evolutionary pressures as sensory systems, suggesting that the brain might adapt to novel sensory environments through concomitant cellular changes distributed along neuronal circuits.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-632

THE ENDOCANNABINOID SYSTEM REGULATES OLFACTORY PERCEPTION IN THE ANTERIOR PIRIFORM CORTEX

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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The endocannabinoid system (ECS) is a potent modulator of cognitive processes, but it is still unclear how it controls sensory perception. In the olfactory system, a major structure involved in sensory processing is the anterior piriform cortex (aPC). Although recent evidence suggests that the ECS regulates aPC functions through its main cannabinoid type 1 receptor (CB1R), little is known about how ECS impacts aPC circuits *in vivo* and whether it influences olfactory perception. Using a combination of pharmacology and aPC multi-electrode recordings in freely moving mice, we showed that an antagonist/inverse agonist of the CB1R increases local field potential power selectively in the gamma frequency band as well as the phase-locking of aPC units to gamma oscillations. Moreover, we found that CB1R antagonist reduces unit synchrony, mainly during active sniffing. The latter observation was in line with a reduced occurrence of spontaneous and odor-evoked calcium transients recorded with fiber photometry following local CB1R blockade in aPC, consistent with a lower neuronal synchronization. This increased gamma power combined with reduced unit synchrony suggested that the ECS could tune olfactory perception in the aPC. Indeed, as compared to vehicle injected animals, mice injected with CB1R antagonist in aPC had a lower threshold for odor detection. We propose that the endocannabinoids in aPC tend to dampen olfactory perception by limiting gamma oscillations and odor-induced neuronal desynchronization.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-633

DRAMATIC CHANGES IN THE RABBIT VOMERONASAL ORGAN TRANSCRIPTOME BEFORE BIRTH AND ITS CRITICAL ROLE IN FIRST MILK INTAKE

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Mother-offspring chemical communication is essential to ensure pup survival. The maternal mammary pheromone (2MB2), which has only been identified in rabbits, triggers nipple-search and suckling behavior. While some studies suggest that this pheromone is solely detected by the main olfactory epithelium, the importance of the vomeronasal system in innate pheromone-mediated behaviours makes the study of the pup vomeronasal organ (VNO) at perinatal stages very promising to look for cues involved in mother-pup interaction. A total of 48 VNO samples from individuals at embryonic day 28 (E28), postnatal day 0 (P0) before and 2.5 h after fist milk intake, and postnatal day 4 (P4) were dissected out and processed for RNA extraction, library construction and sequencing using Illumina Nova-Seq 75 bp PE run. Gene expression was analyzed using Kallisto and DESeq2. The VNO gene expression showed significant differences between the four groups. Importantly, P0 pre and P0 post suckling showed 399 differentially expressed genes, supporting that cues delivered in milk might be detected through the VNO. Vomeronasal receptors were barely expressed at E28, their expression increased after birth and reached a maximum at P4, suggesting that exposure to the outside world activates the VNO not only innately but also after prior experience. These results suggest that the VNO has an essential role in dam-kits communication after birth. This vital interaction guarantees kits survival since birth. Further studies are required to assess the nature of the pheromone-receptor interaction and how to apply this information to improve mother-offspring bond.

Pubmed:

35158632: Villamayor PR, Gullón J, Yáñez U, Sánchez M, Sánchez-Quinteiro P, Martínez P, Quintela L
Assessment of Biostimulation Methods Based on Chemical Communication in Female Doe Reproduction.
Biostimulation is an animal management practice that helps improve reproductive parameters by modulating animal sensory systems. Chemical signals, mostly known as pheromones, have a great potential in this regard. This study was conducted to determine the influence of short-term female rabbit exposure to different conditions, mainly pheromone-mediated, on reproductive parameters of inseminated does. Groups of 60 females/each were exposed to (1) female urine, (2) male urine, (3) seminal plasma and (4) female-female (F-F) separated, just before artificial insemination, and compared to a 'golden method' female-female interaction. The following reproductive parameters were analyzed for each group: receptivity (vulvar color), fertility (kindling rate), prolificacy and number of born alive and dead kits/litter. Our results showed that the biostimulation methods employed in this experiment did not significantly improve any of the analyzed parameters. However, female doe exposure to urine, especially to male urine, showed no significant higher fertility values (95.4%) when compared to the rest of the experimental conditions (on average 92.4%). Female-female interaction before artificial insemination, which is a common practice in rabbit farms, showed similar results as not establishing social interaction (F-F separated), which suggests that F-F interaction could be replaced by F-F separated, therefore avoiding unnecessary animal management and time cost. On the other hand, fertility ranges were lower for animals with a pale vulvar color whereas no differences were noticed among the other three colors which measure receptivity (pink, red, purple), thus suggesting that these three colors could be grouped together. Future studies should aim at determining potential chemical cues/pheromones released through bodily secretions that influence reproduction in rabbits, therefore contributing to animal welfare and to a natural image of animal production.
Animals (Basel), 2022; 12
34896556: Ortiz-Leal I, Torres MV, Villamayor PR, Fidalgo LE, López-Beceiro A, Sanchez-Quinteiro P
Can domestication shape Canidae brain morphology? The accessory olfactory bulb of the red fox as a case in point.
The accessory olfactory bulb (AOB) is the first integrative center of the vomeronasal system (VNS), and the general macroscopic, microscopic, and neurochemical organizational patterns of the AOB differ fundamentally among species.

Therefore, the low degree of differentiation observed for the dog AOB is surprising. As the artificial selection pressure exerted on domestic dogs has been suggested to play a key role in the involution of the dog VNS, a wild canid, such as the fox, represents a useful model for studying the hypothetical effects of domestication on the AOB morphology.
Ann Anat, 2022; 240

34800143: Torres MV, Ortiz-Leal I, Villamayor PR, Ferreiro A, Rois JL, Sanchez-Quinteiro P
Does a third intermediate model for the vomeronasal processing of information exist? Insights from the macropodid neuroanatomy.
The study of the α-subunit of Gi2 and Go proteins in the accessory olfactory bulb (AOB) was crucial for the identification of the two main families of vomeronasal receptors, V1R and V2R. Both families are expressed in the rodent and lagomorph AOBs, according to a segregated model characterized by topographical anteroposterior zonation. Many mammal species have suffered from the deterioration of the Gαo pathway and are categorized as belonging to the uniform model. This scenario has been complicated by characterization of the AOB in the tammar wallaby, Notamacropus eugenii, which appears to follow a third model of vomeronasal organization featuring exclusive Gαo protein expression, referred to as the intermediate model, which has not yet been replicated in any other species. Our morphofunctional study of the vomeronasal system (VNS) in Bennett's wallaby, Notamacropus rufogriseus, provides further information regarding this third model of vomeronasal transduction. A comprehensive histological, lectin, and immunohistochemical study of the Bennett's wallaby VNS was performed. Anti-Gαo and anti-Gαi2 antibodies were particularly useful because they labeled the transduction cascade of V2R and V1R receptors, respectively. Both G proteins showed canonical immunohistochemical labeling in the vomeronasal organ and the AOB, consistent with the anterior-posterior zonation of the segregated model. The lectin Ulex europaeus agglutinin selectively labeled the anterior AOB, providing additional evidence for the segregation of vomeronasal information in the wallaby. Overall, the VNS of the Bennett's wallaby shows a degree of differentiation and histochemical and neurochemical diversity comparable to species with greater VNS development. The existence of the third intermediate type in vomeronasal information processing reported in Notamacropus eugenii is not supported by our lectin-histochemical and immunohistochemical findings in Notamacropus rufogriseus.
Brain Struct Funct, 2022; 227

34015461: Villamayor PR, Robledo D, Fernández C, Gullón J, Quintela L, Sánchez-Quinteiro P, Martínez P
Analysis of the vomeronasal organ transcriptome reveals variable gene expression depending on age and function in rabbits.
The vomeronasal organ (VNO) is a chemosensory organ specialized in pheromone detection that shows a broad morphofunctional and genomic diversity among mammals. However, its expression patterns have only been well-characterized in mice. Here, we provide the first comprehensive RNA sequencing study of the rabbit VNO across gender and sexual maturation stages. We characterized the VNO transcriptome, updating the number and expression of the two main vomeronasal receptor families, including 128 V1Rs and 67 V2Rs. Further, we defined the expression of formyl-peptide receptor and transient receptor potential channel families, both known to have specific roles in the VNO. Several sex hormone-related pathways were consistently enriched in the VNO, highlighting the relevance of this organ in reproduction. Moreover, whereas juvenile and adult VNOs showed significant transcriptome differences, male and female did not. Overall, these results contribute to understand the genomic basis of behavioural responses mediated by the VNO in a non-rodent model.
Genomics, 2021; 113

33893372: Villamayor PR, Arana ÁJ, Coppel C, Ortiz-Leal I, Torres MV, Sanchez-Quinteiro P, Sánchez L
A comprehensive structural, lectin and immunohistochemical characterization of the zebrafish olfactory system.
Fish chemosensory olfactory receptors allow them to detect a wide range of water-soluble chemicals, that mediate fundamental behaviours. Zebrafish possess a well-developed sense of smell which governs reproduction, appetite, and fear responses. The spatial organization of functional properties within the olfactory epithelium and bulb are comparable to those of mammals, making this species suitable for studies of olfactory differentiation and regeneration and neuronal representation of olfactory information. The advent of genomic techniques has been decisive for the discovery of specific olfactory cell types and the identification of cell populations expressing vomeronasal receptors. These advances have marched ahead of morphological and neurochemical studies. This study aims to fill the existing gap in specific histological, lectin-histochemical and immunohistochemical studies on the olfactory rosette and the olfactory bulb of the zebrafish. Tissue dissection and microdissection techniques were employed, followed by histological staining techniques, lectin-histochemical labelling (UEA, LEA, BSI-B) and immunohistochemistry using antibodies against G proteins subunits αo and αi2, growth-associated protein-43, calbindin, calretinin, glial-fibrillary-acidic-protein and luteinizing-hormone-releasing-hormone. The results obtained enrich the available information on the neurochemical patterns of the zebrafish olfactory system, pointing to a greater complexity than the one currently considered, especially when taking into account the peculiarities of the nonsensory epithelium.
Sci Rep, 2021; 11

32764621: Torres MV, Ortiz-Leal I, Villamayor PR, Ferreiro A, Rois JL, Sanchez-Quinteiro P

The vomeronasal system of the newborn capybara: a morphological and immunohistochemical study.
The vomeronasal system (VNS) is responsible for the perception mainly of pheromones and kairomones. Primarily studied in laboratory rodents, it plays a crucial role in their socio-sexual behaviour. As a wild rodent, the capybara offers a more objective and representative perspective to understand the significance of the system in the Rodentia, avoiding the risk of extrapolating from laboratory rodent strains, exposed to high levels of artificial selection pressure. We have studied the main morphological and immunohistochemical features of the capybara vomeronasal organ (VNO) and accessory olfactory bulb (AOB). The study was done in newborn individuals to investigate the maturity of the system at this early stage. We used techniques such as histological stains, lectins-labelling and immunohistochemical characterization of a range of proteins, including G proteins (Gαi2, Gαo) and olfactory marking protein. As a result, we conclude that the VNS of the capybara at birth is capable of establishing the same function as that of the adult, and that it presents unique features as the high degree of differentiation of the AOB and the active cellular migration in the vomeronasal epithelium. All together makes the capybara a promising model for the study of chemical communication in the first days of life.
Sci Rep, 2020; 10
32584430: Ortiz-Leal I, Torres MV, Villamayor PR, López-Beceiro A, Sanchez-Quinteiro P
The vomeronasal organ of wild canids: the fox (Vulpes vulpes) as a model.
The vomeronasal system (VNS) has been extensively studied within specific animal families, such as Rodentia. However, the study of the VNS in other families, such as Canidae, has long been neglected. Among canids, the vomeronasal organ (VNO) has only been studied in detail in the dog, and no studies have examined the morphofunctional or immunohistochemical characteristics of the VNS in wild canids, which is surprising, given the well-known importance of chemical senses for the dog and fox and the likelihood that the VNS plays roles in the socio-reproductive physiology and behaviours of these species. In addition, characterising the fox VNS could contribute to a better understanding of the domestication process that occurred in the dog, as the fox would represent the first wild canid to be studied in depth. Therefore, the aim of this study was to analyze the morphological and immunohistochemical characteristics of the fox VNO. Tissue dissection and microdissection techniques were employed, followed by general and specific histological staining techniques, including with immunohistochemical and lectin-histochemical labelling strategies, using antibodies against olfactory marker protein (OMP), growth-associated protein 43 (GAP-43), calbindin (CB), calretinin (CR), α-tubulin, Gαo, and Gαi2 proteins, to highlight the specific features of the VNO in the fox. This study found significant differences in the VNS between the fox and the dog, particularly concerning the expression of Gαi2 and Gαo proteins, which were associated with the expression of the type 1 vomeronasal receptors (V1R) and type 2 vomeronasal receptors (V2R), respectively, in the vomeronasal epithelium. Both are immunopositive in foxes, as opposed to the dog, which only expresses Gαi2. This finding suggests that the fox possesses a well-developed VNO and supports the hypothesis that a profound transformation in the VNS is associated with domestication in the canid family. Furthermore, the unique features identified in the fox VNO confirm the necessity of studying the VNS system in different species to better comprehend specific phylogenetic aspects of the VNS.
J Anat, 2020; 237
31802255: Villamayor PR, Cifuentes JM, Quintela L, Barcia R, Sanchez-Quinteiro P
Structural, morphometric and immunohistochemical study of the rabbit accessory olfactory bulb.
The accessory olfactory bulb (AOB) is the first neural integrative centre of the vomeronasal system (VNS), which is associated primarily with the detection of semiochemicals. Although the rabbit is used as a model for the study of chemocommunication, these studies are hampered by the lack of knowledge regarding the topography, lamination, and neurochemical properties of the rabbit AOB. To fill this gap, we have employed histological stainings: lectin labelling with Ulex europaeus (UEA-I), Bandeiraea simplicifolia (BSI-B4), and Lycopersicon esculentum (LEA) agglutinins, and a range of immunohistochemical markers. Anti-G proteins Gαi2/Gαo, not previously studied in the rabbit AOB, are expressed following an antero-posterior zonal pattern. This places Lagomorpha among the small groups of mammals that conserve a double-path vomeronasal reception. Antibodies against olfactory marker protein (OMP), growth-associated protein-43 (GAP-43), glutaminase (GLS), microtubule-associated protein-2 (MAP-2), glial fibrillary-acidic protein (GFAP), calbindin (CB), and calretinin (CR) characterise the strata and the principal components of the BOA, demonstrating several singular features of the rabbit AOB. This diversity is accentuated by the presence of a unique organisation: four neuronal clusters in the accessory bulbar white matter, two of them not previously characterised in any species (the γ and δ groups). Our morphometric study of the AOB has found significant differences between sexes in the numerical density of principal cells, with larger values in females, a pattern completely opposite to that found in rats. In summary, the rabbit possesses a highly developed AOB, with many specific features that highlight the significant role played by chemocommunication among this species.
Brain Struct Funct, 2020; 225
30255591: Villamayor PR, Cifuentes JM, Fdz-de-Troconiz P, Sanchez-Quinteiro P
Morphological and immunohistochemical study of the rabbit vomeronasal organ.

The characterization of the rabbit mammary pheromone, which is sensed by the main olfactory system, has made this species a unique model for the study of pheromonal communication in mammals. This discovery has brought attention to the global understanding of chemosensory communication in this species. Chemocommunication is mediated by two distinct organs located in the nasal cavity, the main olfactory epithelium and the vomeronasal organ (VNO). However, there is a lack of knowledge about the vomeronasal system in rabbits. To understand the role of this system, an exhaustive anatomical and histological study of the rabbit VNO was performed. The rabbit VNO was studied macroscopically by light microscopy, and by histochemical and immunohistochemical techniques. We employed specific histological staining techniques (periodic acid-Schiff, Alcian blue, Gallego's trichrome), confocal autofluorescence, histochemical labelling with the lectin Ulex europaeus agglutinin (UEA-I), and immunohistochemical studies of the expression of the Gαi2 and Gαo proteins and olfactory marker protein. The opening of the vomeronasal duct into the nasal cavity and its indirect communication with the oral cavity through a functional nasopalatine duct was demonstrated by classical dissection and microdissection. In a series of transverse histological sections, special attention was paid to the general distribution of the various soft-tissue components of this organ (duct, glands, connective tissue, blood vessels and nerves) and to the nature of the capsule of the organ. Among the main morphological features that distinguish the rabbit VNO, the presence of a double envelope, which is bony externally and cartilaginous internally, and highly developed venous sinuses stand out. This observation indicates the crucial role played in this species by the pumping mechanism that introduces chemical signals into the vomeronasal duct. The functional properties of the organ are also confirmed by the presence of a well-developed neuroepithelium and profuse glandular tissue that is positive for neutral mucopolysaccharides. The role of glycoconjugates was assessed by the identification of the α1-2 fucose glycan system in the neuroepithelium of the VNO employing UEA-I lectin. The pattern of labelling, which was concentrated around the commissures of the sensory epithelium and more diffuse in the central segments, is different from that found in most mammals studied. According to the expression of G-proteins, two pathways have been described in the VNOs of mammals: neuroreceptor cells expressing the Gαi2 protein (associated with vomeronasal receptor type 1); and cells expressing Gαo (associated with vomeronasal receptor type 2). The latter pathway is absent in most mammals studied. The expression of both G-protein families in the rabbit VNO places Lagomorpha together with rodents and insectivores in a small group of mammals belonging to the two-path model. These findings support the notion that the rabbit possesses a highly developed VNO, with many specific morphological features, which highlights the significance of chemocommunication in this species.
J Anat, 2018; 233

33483429: Galliano E, Hahn C, Browne LP, R Villamayor P, Tufo C, Crespo A, Grubb MS
Brief Sensory Deprivation Triggers Cell Type-Specific Structural and Functional Plasticity in Olfactory Bulb Neurons.
Can alterations in experience trigger different plastic modifications in neuronal structure and function, and if so, how do they integrate at the cellular level? To address this question, we interrogated circuitry in the mouse olfactory bulb responsible for the earliest steps in odor processing. We induced experience-dependent plasticity in mice of either sex by blocking one nostril for one day, a minimally invasive manipulation that leaves the sensory organ undamaged and is akin to the natural transient blockage suffered during common mild rhinal infections. We found that such brief sensory deprivation produced structural and functional plasticity in one highly specialized bulbar cell type: axon-bearing dopaminergic neurons in the glomerular layer. After 24 h naris occlusion, the axon initial segment (AIS) in bulbar dopaminergic neurons became significantly shorter, a structural modification that was also associated with a decrease in intrinsic excitability. These effects were specific to the AIS-positive dopaminergic subpopulation because no experience-dependent alterations in intrinsic excitability were observed in AIS-negative dopaminergic cells. Moreover, 24 h naris occlusion produced no structural changes at the AIS of bulbar excitatory neurons, mitral/tufted and external tufted cells, nor did it alter their intrinsic excitability. By targeting excitability in one specialized dopaminergic subpopulation, experience-dependent plasticity in early olfactory networks might act to fine-tune sensory processing in the face of continually fluctuating inputs. Sensory networks need to be plastic so they can adapt to changes in incoming stimuli. To see how cells in mouse olfactory circuits can change in response to sensory challenges, we blocked a nostril for just one day, a naturally relevant manipulation akin to the deprivation that occurs with a mild cold. We found that this brief deprivation induces forms of axonal and intrinsic functional plasticity in one specific olfactory bulb cell subtype: axon-bearing dopaminergic interneurons. In contrast, intrinsic properties of axon-lacking bulbar dopaminergic neurons and neighboring excitatory neurons remained unchanged. Within the same sensory circuits, specific cell types can therefore make distinct plastic changes in response to an ever-changing external landscape.
J Neurosci, 2021; 41

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BOARD NUMBER: S02-634

MEASURING PERK (PHOSPHORYLATED EXTRACELLULAR-SIGNAL REGULATED KINASE) ACTIVITY IN ACUTE IN-VITRO SLICES OF THE RAT OLFACTORY BULB

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

Lisa Kindler, Hajime Suyama, Veronica Egger, Michael Lukas
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Vasopressin (VP), when released in the olfactory bulb, enables the discrimination of conspecifics in rats. Intriguingly, tufted cells in the olfactory bulb that express VP (VPCs) show IPSPs following olfactory nerve (ON) stimulation in-vitro, whereas other tufted cell types are excited. However, following social interaction in-vivo, we measured increased pERK (*phosphorylated extracellular-signal regulated kinase*) immunohistochemistry in VPCs, indicating that excitation of VPCs is possible. Interestingly, cholinergic modulation also enables a switch from inhibition to excitation following in-vitro ON stimulation. The aim of this study was to establish pERK activity as a reliable population marker to study excitation patterns in acute in-vitro OB slices following pharmacological and/or electrical stimulation, using VPCs and their known acetylcholine-modulated switch from inhibition to excitation as an indicator. Thus, we examined the effect of general depolarization, ON stimulation and cholinergic modulation on pERK activity in OB VPCs. Incubation of acute OB slices in KCl-ACSF (*artificial cerebrospinal fluid, 90mM*) only led to a pERK activation in the mitral cell and the granule cell layer but not in VPCs. Further, we could show that electrical ON stimulation results in pERK activation within columnar units, including mitral and granule cells. Finally, ON stimulation coupled with acetylcholine application resulted in a significant increase in pERK+/VPCs, thereby confirming that the combination of ACh presence and ON stimulation is necessary to obtain excitation of VPCs, as previously shown in our electrophysiological experiments on the single-cell level.

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BOARD NUMBER: S02-635

VASOPRESSIN INHIBITS PROJECTION NEURONS IN THE OLFACTORY BULB VIA INCREASED EXCITATION OF INHIBITORY INTERNEURONS

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Vasopressin (VP) is essential for social memory already at the level of the olfactory bulb (OB). Further, we previously showed that OB VP cells are activated by social interaction. However, it remains unclear how VP modulates odor processing to enable enhanced discrimination of very similar odors, e.g., rat body odors. So far, it was shown that VP reduces firing rates in mitral cells (MCs) during odor presentation *in-vivo* and decreases the amplitudes of olfactory nerve-evoked excitatory postsynaptic potentials (ON-evoked EPSPs) in external tufted cells *in-vitro*.
We performed whole-cell patch-clamp recordings and Ca^{2+} imaging in acute rat OB slices. We recorded ON-evoked EPSPs as well as spontaneous inhibitory postsynaptic currents (IPSCs) from both projection neurons, middle tufted cells (mTCs) and MCs. VP bath-application reduced the amplitudes of ON-evoked EPSPs and the frequencies of spontaneous IPSCs in mTCs but did not change those in MCs. To search for the origin of increased inhibition in mTCs, we analyzed ON evoked-EPSPs in inhibitory interneurons, i.e., periglomerular cells (PGCs) and granule cells (GCs). However, VP did not increase EPSPs in either type of interneuron. We next performed two-photon Ca^{2+} imaging in PGCs and GCs to record postsynaptic activity during stronger ON stimulation, evoking action potentials in the interneurons. We observed that ON-evoked Ca^{2+} influx at the proximal apical dendrite of PGCs and the soma of GCs was increased during VP application. Thus, our findings imply that VP could mediate inhibition of projection neurons via enhanced inhibitory interneuron activity upon strong olfactory inputs.

Pubmed:
31217196: Lukas M, Suyama H, Egger V
Vasopressin Cells in the Rodent Olfactory Bulb Resemble Non-Bursting Superficial Tufted Cells and Are Primarily Inhibited upon Olfactory Nerve Stimulation.
The intrinsic vasopressin system of the olfactory bulb is involved in social odor processing and consists of glutamatergic vasopressin cells (VPCs) located at the medial border of the glomerular layer. To characterize VPCs in detail, we combined various electrophysiological, neuroanatomical, and two-photon Ca imaging techniques in acute bulb slices from juvenile transgenic rats with eGFP-labeled VPCs. VPCs showed regular non-bursting firing patterns, and displayed slower membrane time constants and higher input resistances versus other glutamatergic tufted cell types. VPC axons spread deeply into the external plexiform and superficial granule cell layer (GCL). Axonal projections fell into two subclasses, with either denser local columnar collaterals or longer-ranging single projections running laterally within the internal plexiform layer and deeper within the granule cell layer. VPCs always featured lateral dendrites and a tortuous apical dendrite that innervated a single glomerulus with a homogenously branching tuft. These tufts lacked Ca transients in response to single somatically-evoked action potentials and showed a moderate Ca increase upon prolonged action potential trains.Notably, electrical olfactory nerve stimulation did not result in synaptic excitation of VPCs, but triggered substantial GABA receptor-mediated IPSPs that masked excitatory barrages with yet longer latency. Exogenous vasopressin application reduced those IPSPs, as well as olfactory nerve-evoked EPSPs recorded from external tufted cells. In summary, VPCs can be classified as non-bursting, vertical superficial tufted cells. Moreover, our findings imply that sensory input alone cannot trigger excitation of VPCs, arguing for specific additional pathways for excitation or disinhibition in social contexts.
eNeuro, 2019 Jul/Aug; 6
34021245: Suyama H, Egger V, Lukas M
Top-down acetylcholine signaling via olfactory bulb vasopressin cells contributes to social discrimination in rats.
Social discrimination in rats requires activation of the intrinsic bulbar vasopressin system, but it is unclear how this system comes into operation, as olfactory nerve stimulation primarily inhibits bulbar vasopressin cells (VPCs). Here we show that stimulation with a conspecific can activate bulbar VPCs, indicating that VPC activation depends on more than olfactory cues during social interaction. A series of in vitro electrophysiology, pharmacology and immunohistochemistry experiments implies that acetylcholine, probably originating from centrifugal projections, can enable olfactory nerve-evoked action potentials in

VPCs. Finally, cholinergic activation of the vasopressin system contributes to vasopressin-dependent social discrimination, since recognition of a known rat was blocked by bulbar infusion of the muscarinic acetylcholine receptor antagonist atropine and rescued by additional bulbar application of vasopressin. Thus, our results implicate that top-down cholinergic modulation of bulbar VPC activity is involved in social discrimination in rats.
Commun Biol, 2021; 4

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BOARD NUMBER: S02-636

DEVELOPMENT OF SPARSE, COMBINATORIAL CONNECTIVITY IN THE DROSOPHILA MUSHROOM BODY CALYX

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Detection of environmental stimuli is essential for survival. The total number of sensory stimuli encountered by an organism is unpredictable and nearly limitless, making genetic coding of detectors for all possible stimuli impossible. Instead, evolution has selected for amplifying the number of discriminable stimuli from a limited number of genetically encoded sensors to the number of combinations among them by expanding sensory neuron inputs via sparse, combinatorial wiring to higher order neurons involved in perceptual processing. It is not understood in any species what developmental mechanisms give rise to the input sparseness necessary for sensory amplification. To address this, we use the fly olfactory system where 50 odor channels are dispersed, via Projection Neurons (PNs), among ~2,500 higher order neurons called Kenyon Cells (KCs) in the mushroom body calyx. Previous work in the lab demonstrated that in the absence of KCs during development, adult PN axons no longer provide input to the calyx. We subsequently found this is because developing PNs never initiate collateral formation when KCs are not present. These results have led to the hypothesis that KCs dictate their input density via retrograde feedback to PNs. To test this hypothesis, we generated a bulk RNA sequencing dataset for developing KCs and used this as the basis for a candidate screen to search for KC-derived retrograde signals required for the production of odor channel inputs. Furthermore, we utilized fixed brain samples along with *ex vivo* live imaging to ask whether cell biological events of calyx development support the hypothesis that KCs direct PN input production.

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BOARD NUMBER: S02-637

NMDA RECEPTORS SHAPE SENSORY PROCESSING IN THE PIRIFORM CORTEX

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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The piriform cortex (PCx) is the first cortical destination of olfactory information and is essential for discriminating and learning odours. As in other cortical regions, synapses in the PCx can express activity-dependent plasticity that requires NMDA receptors. However, the extent to which NMDA receptors contribute to other operations of the PCx remains unknown. Here, we used *in vivo* patch clamping to examine the role of NMDA receptors in the encoding of odour stimuli in the PCx. Blind whole-cell recordings were made in the PCx of anaesthetised mice (P30-P50) while applying odorants to the nares using an olfactometer. Neurons were identified using soma depth, intrinsic electrical properties and recovered morphology. In a subset of experiments, a non-competitive NMDA receptor blocker, MK-801 (1 mM), was applied to the PCx. We found that MK-801 had no effect on intrinsic electrical properties of excitatory neurons. In contrast, MK-801 significantly suppressed spontaneous EPSPs and action potentials in these neurons. Furthermore, in the presence of MK-801, odour responses were more stereotyped and coupled to respiration, while the fraction of odours to which neurons responded was not significantly different from control. Our results show that NDMA receptors play a key role in a variety of functions of the PCx beyond synaptic plasticity. NMDA receptors are fundamentally involved in olfaction by altering network activity under quiescent conditions and by shaping the responses of neurons to odour stimulation. Further work on NMDA receptors will provide insight into the intricate link between odour processing and sensory learning in the PCx.

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BOARD NUMBER: S02-638

BREATHING- AND OLFACTORY-DRIVEN ACTIVITY IN THE OLFACTORY CORTEX

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Modulation of breathing rate is crucial to flexibly explore the olfactory environment. Inhalation gates the perception of scents, and the act of sniffing is necessary for the olfactory percept.
However, different airflows due to distinct types of inhalations, such as during regular breathing and sniffing, alter the concentration of an odorant inside the nose regardless of its concentration at the source. Yet, animals can discriminate the concentration of an odor regardless of the speed of their inhalations. This ability is fundamental in instances such as foraging as the navigating system must be able to assess the concentration gradient towards an odor source regardless of how fast the animal is breathing. The olfactory cortex is arguably important for olfactory perception, therefore we asked how distinct types of inhalation affect the activity of the cortex.
We recorded the activity of hundreds of neurons along with the respiratory activity using high-density electrodes implanted in the primary olfactory cortex of awake head-fixed mice. Our results push further our understanding of how the brain can reliably discriminate olfactory features regardless of breathing fluctuations.

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BOARD NUMBER: S02-639

THE ROLE OF THE OLFACTORY GNRH SYSTEM IN THE CONTROL OF CHEMOSENSORY PROCESSING AND NEUROENDOCRINE CHANGES

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

Laurine Decoster[1], Sara Trova[1], Stefano Zucca[2], Gaëtan Ternier[1], Tori Lhomme[1], Paolo Peretto[2], Serena Bovetti[2], Mauro Batista Da Silva[1], Paolo Giacobini[1]
[1]Univ Lille, INSERM, CHU Lille, U - LilNCog - Lille Neuroscience and Cognition, Laboratory Of The Development And Plasticity Of Neuroendocrine Brain, Lille Cedex, France, [2]University of Turin, Neuroscience Institute Cavalieri Ottolenghi, Torino, Italy

The role of the olfactory GnRH system in the control of chemosensory processing and neuroendocrine changes in the mouse The role of gonadotropin releasing hormone (GnRH) neurons as the final neural output controlling reproduction fitness and fertility is well established among the different vertebrates. An extra-hypothalamic GnRH neuron population is present in areas dedicated to olfactory processing and more precisely within the olfactory bulbs. We named this population OB-GnRH neurons. We hypothesized that this newly identified GnRH neuronal population may convey olfactory and/or pheromonal information to participate to the neuroendocrine responses controlling reproduction. Accordingly, we evaluated whether OB-GnRH neurons can be activated by opposite-sex smell exposure. The use of *in vivo* calcium imaging coupled with two-photons microscopy confirmed that olfactory and pheromonal stimulations can activate the OB-GnRH neurons. We next showed using a combination of viral tracing experiments and electrophysiological recordings that the OB-GnRH neurons are connected with the neuroendocrine GnRH population located in the rostral preoptic area. The function of OB-GnRH neurons on sex-related sensory processing was investigated using bidirectional chemogenetic neuromodulation combined with behavioral testing. Remarkably, our data demonstrate that OB-GnRH neurons are essential to drive the opposite-sex preference as well as a robust Luteinizing Hormone (LH) and testosterone secretion in males. Together, our study highlights a novel role for olfactory GnRH neurons as a central regulatory hub linking pheromonal stimulations with sexual behavior and reproductive functions.

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BOARD NUMBER: S02-640

PREFRONTAL TO OLFACTORY CORTEX VENTRAL TENIA TECTA INPUTS SHARE ODOR-EVOKED BEHAVIORAL-STATE SIGNALS TO AFFECT CONTEXT-DEPENDENT LEARNING

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

Yuta Tanisumi[1], Kazuki Shiotani[2], Yuma Osako[1], Tomoya Ohnuki[1], Shogo Takamiya[1], Junya Hirokawa[1], Yoshio Sakurai[1], Hiroyuki Manabe[1]
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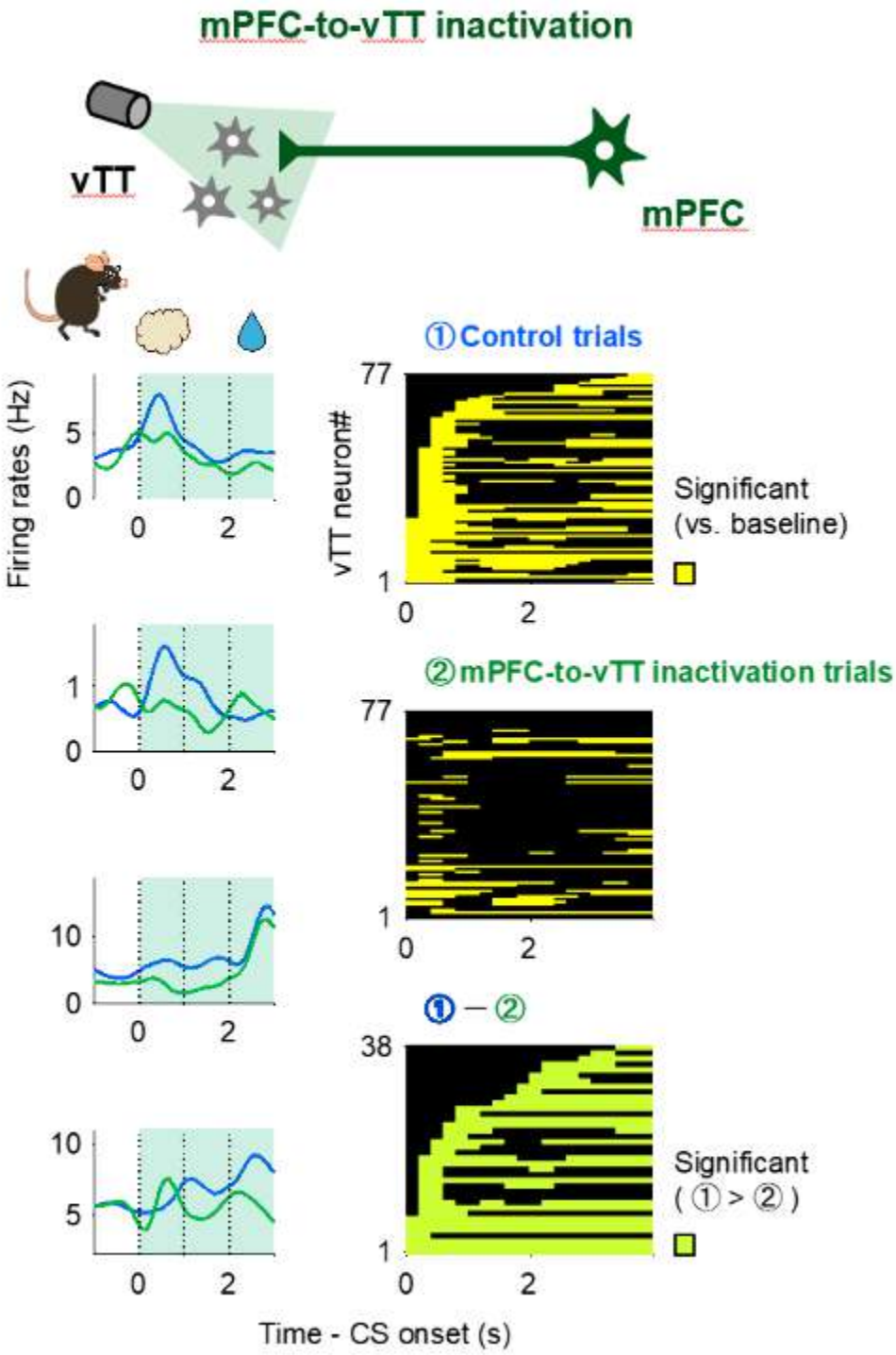

How sensory cortex shares top-down signals from higher-order regions is a significant question. We previously provided the first recordings of neuronal activity in the ventral tenia tecta (vTT) in the olfactory cortex, which receives top-down projections from the medial prefrontal cortex (mPFC) and projects to the broad olfactory areas. Here, we developed and combined electrophysiological recordings in the vTT with optogenetic mPFC-silencing while mice associated different four different odor cues with appetitive and aversive outcomes. First, we found individual vTT neurons encoded not only encoded odor-presentation associated with

positive and negative value, but also the odor-evoked outcome waiting and positive/negative outcome phases. Second, we investigated whether the neural activity undergoes temporal scaling from the short to long intervals using the different-interval timing task. The neural tunings to the extension of short-delay to long-delay were expanded, suggesting the scalable vTT state encoding. Third, to reveal the source of the top-down signals, we expressed inhibitory opsin Arch3.0 in the mPFC and recorded from vTT neurons with and without the optogenetic silencing of mPFC axons while mice conducted the task. Without mPFC-to-vTT inputs, the vTT-state representations were degraded and less integrated. Finally, we performed optogenetic silencing during the odor-outcome association task and the reversal learning to ask whether the mPFC-to-vTT inputs contribute to the learning of appetitive associations. Mice that experienced mPFC-to-vTT axons inhibition exhibited learning deficits. Taken together, our data suggest that the vTT acts as a hub that sends various context-dependent signals for learning from the mPFC to broad olfactory areas.

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BOARD NUMBER: S02-641

BENEFICIAL EFFECTS OF PROLONGED 2-PHENYLETHYL ALCOHOL INHALATION ON ALTERED FEEDING BEHAVIOR AND NEURAL ACTIVITY IN CHRONICALLY DISTRESSED FEMALE MICE

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Chronic distress is core to the pathophysiology of neuropsychiatric disorders, such as anxiety and depression. Due to the diverse side effects of available classical medications, there has been a growing interest in developing safer therapeutic strategies based on substances derived from aromatic plants. Among them, rose essential oil is commonly used in aromatherapy which is considered as an adjuvant treatment for the improvement of patients' quality of life. The purpose of this study was to investigate the potential beneficial effects of 2-phenylethyl alcohol (PEA), one of the pharmacologically active constituents of rose oil, on behavioral and neurobiological changes that occur in a neuroendocrine mouse model of anxiety-depression. Female C57BL/6JRJ mice were orally treated with corticosterone (CORT) for 8 weeks. After a prolonged PEA inhalation (30 min per day, for 15 consecutive days), animals were submitted to the Novelty-Suppressed Feeding task (NSF). Immunohistochemistry was performed to provide a global map of functional neural network activity based on cerebral cFos expression analysis. Our results show that chronic CORT administration induces an anxio-depressive-like phenotype, evidenced by an increased latency to feed in the NSF. The altered feeding behavior negatively correlates with the olfactory bulb cFos-positive cells density. Moreover, cross-correlation-based network analysis reveals CORT-induced altered brain connectivity. These behavioral and neurobiological changes are reversed by prolonged PEA inhalation in chronically distressed female mice. The presented data suggest that the olfactory system contributes to the beneficial behavioral effects of prolonged PEA inhalation. Additional investigations are needed to further characterize the exact underlying neurobiological mechanisms.

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STIMULUS NOVELTY DIFFERENTIALLY MODULATES ODOR RESPONSES IN THE ANTERIOR OLFACTORY NUCLEUS AND PIRIFORM CORTEX

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

Martijn Broux[1,2], Cagatay Aydin[1,2], José Esquivelzeta Rabell[1,2], Eleonore Schiltz[1,2], Sebastian Haesler[1,2,3,4]
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When animals detect novel stimuli in their environment, they react with distinct orienting and exploratory behaviors. Detection of stimulus novelty is fast – behavioral novelty responses are observed already less than 100ms after stimulus presentation. In order to accomplish such rapid detection, the brain needs to perform a remarkably efficient computation involving a memory-based classification of incoming sensory stimuli. To begin to unravel the mechanism by which the brain discriminates novel from familiar olfactory stimuli, we performed multielectrode recordings in the anterior olfactory nucleus (AON) and piriform cortex (PCx) of awake, behaving mice, while presenting novel and familiar odorants. As described previously, novel stimuli evoked rapid increases in respiration frequency, also referred to as exploratory sniffing, which diminished with subsequent stimulus presentations. Neurons in the AON and PCx showed robust responses to novel and familiar odorants. However, odor responses in the AON responded much stronger to the initial presentations of novel stimuli than neurons in the PCx. To quantify this effect, we calculated the mean Euclidean distance from each trial population response to the last odor presentations as described previously (Bolding et al. 2020). The distance-to-stable was much larger in the neuronal population of the AON than the PCx. Consistent with this, a linear support vector machine showed superior performance in classifying novel from familiar trials when trained with AON compared to PCx neurons. Collectively, these results suggest the sensory representation in the AON is modulated by stimulus experience, while it appears relatively invariant to experience in the PCx.

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BOARD NUMBER: S02-643

LONG TERM STABILITY AND VOLATILITY OF ODOR-EVOKED RESPONSES IN THE MOUSE OLFACTORY BULB

POSTER SESSION 02 - SECTION: OLFACTORY SYSTEM

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Recent developments in methods to measure neuronal activity led to a general shift from recordings in anesthetized to awake animals. In sensory systems, and particularly olfaction, the differences between sensory coding in anesthetized versus awake states received only limited attention. Here, we used in vivo two-photon calcium imaging of odor responses in mitral cells from the olfactory bulb of mice in wakefulness and under anesthesia. Using a diverse panel of stimuli, including simple and complex odorants, we show an extensive change in mitral cells responses between states, supporting previous findings. Unlike previous finding we find heterogeneous, bidirectional changes by state rather than uniform modulation. Further, odor coding under anesthesia carries more sensory-relevant information, supporting the role of strong modulatory contextual information in awake mice. Using time-lapse imaging experiments, weeks apart, we found that despite the large differences between states, coding in each one of the states alone is highly stable over time. These findings show that different brain states dictate distinct, yet stable, coding schemes in the olfactory bulb.

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BOARD NUMBER: S02-644

DOUBLE-CODING: A MECHANISTIC EVALUATION OF NEURONAL MULTI-CISTRONIC GENES PERFORMED ON THE EXAMPLE OF THE P/Q-TYPE CALCIUM CHANNEL CAV2.1

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Besides their classical role as calcium-conducting elements controlling neuronal communication, voltage-gated calcium channels (VGCCs) have been shown to act on different regulatory levels of neuronal physiology by initiating signaling pathways that coordinate downstream gene expression. Observations have been made underpinning the idea that fragments of VGCCs might also directly act as gene regulators. In particular, the neuronal P/Q-type channel ($Ca_v2.1$) has been reported to express a C-terminal fragment (α1ACT) which translocates to nuclei of neurons *in vitro* and *in vivo*, where it binds to promotor regions of the DNA and orchestrates genes associated with neurogenesis, synaptic transmission, and cell adhesion. Although previous reports describe a cap-independent translation mechanism, an internal ribosomal entry site (IRES), to initiate the expression of these VGCC fragments, other studies rather suggest a transcriptional regulation of two independent mRNA transcripts via an exonic (cryptic) promoter in the *CACNA1A* gene, coding for $Ca_v2.1$. Thus, we aim to elucidate the nature of the underlying expression mechanism and how the expression of α1ACT is regulated during neuronal differentiation and upon pathological stimuli (e.g., elevated intracellular calcium levels or oxidative stress). After confirming the expression of α1ACT in various cellular model systems, we are now applying enhanced fluorescence in situ hybridization together with proximity labelling to dissect transcriptional and translational regulation mechanisms. Resolving the mechanistic underpinnings of multi-cistronic genes, by the example of α1ACT, will provide new insights into how functional neuronal complexity is achieved with a limited number of genes.

Pubmed:
34107849: Heck J, Palmeira Do Amaral AC, Weißbach S, El Khallouqi A, Bikbaev A, Heine M
More than a pore: How voltage-gated calcium channels act on different levels of neuronal communication regulation.
Voltage-gated calcium channels (VGCCs) represent key regulators of the calcium influx through the plasma membrane of excitable cells, like neurons. Activated by the depolarization of the membrane, the opening of VGCCs induces very transient and local changes in the intracellular calcium concentration, known as calcium nanodomains, that in turn trigger calcium-dependent signaling cascades and the release of chemical neurotransmitters. Based on their central importance as concierges of excitation-secretion coupling and therefore neuronal communication, VGCCs have been studied in multiple aspects of neuronal function and malfunction. However, studies on molecular interaction partners and recent progress in omics technologies have extended the actual concept of these molecules. With this review, we want to illustrate some new perspectives of VGCCs reaching beyond their function as calcium-permeable pores in the plasma membrane. Therefore, we will discuss the relevance of VGCCs as voltage sensors in functional complexes with ryanodine receptors, channel-independent actions of auxiliary VGCC subunits, and provide an insight into how VGCCs even directly participate in gene regulation. Furthermore, we will illustrate how structural changes in the intracellular C-terminus of VGCCs generated by alternative splicing events might not only affect the biophysical channel characteristics but rather determine their molecular environment and downstream signaling pathways.
Channels (Austin), 2021; 15
32414783: Bikbaev A, Ciuraszkiewicz-Wojciech A, Heck J, Klatt O, Freund R, Mitlöhner J, Enrile Lacalle S, Sun M, Repetto D, Frischknecht R, Ablinger C, Rohlmann A, Missler M, Obermair GJ, Di Biase V, Heine M
Auxiliary α2δ1 and α2δ3 Subunits of Calcium Channels Drive Excitatory and Inhibitory Neuronal Network Development.
VGCCs are multisubunit complexes that play a crucial role in neuronal signaling. Auxiliary α2δ subunits of VGCCs modulate trafficking and biophysical properties of the pore-forming α1 subunit and trigger excitatory synaptogenesis. Alterations in the expression level of α2δ subunits were implicated in several syndromes and diseases, including chronic neuropathic pain, autism, and epilepsy. However, the contribution of distinct α2δ subunits to excitatory/inhibitory imbalance and aberrant network connectivity characteristic for these pathologic conditions remains unclear. Here, we show that α2δ1 overexpression enhances spontaneous neuronal network activity in developing and mature cultures of hippocampal neurons. In contrast,

overexpression, but not downregulation, of α2δ3 enhances neuronal firing in immature cultures, whereas later in development it suppresses neuronal activity. We found that α2δ1 overexpression increases excitatory synaptic density and selectively enhances presynaptic glutamate release, which is impaired on α2δ1 knockdown. Overexpression of α2δ3 increases the excitatory synaptic density as well but also facilitates spontaneous GABA release and triggers an increase in the density of inhibitory synapses, which is accompanied by enhanced axonaloutgrowth in immature interneurons. Together, our findings demonstrate that α2δ1 and α2δ3 subunits play distinct but complementary roles in driving formation of structural and functional network connectivity during early development. An alteration in α2δ surface expression during critical developmental windows can therefore play a causal role and have a profound impact on the excitatory-to-inhibitory balance and network connectivity. The computational capacity of neuronal networks is determined by their connectivity. Chemical synapses are the main interface for transfer of information between individual neurons. The initial formation of network connectivity requires spontaneous electrical activity and the calcium channel-mediated signaling. We found that, in early development, auxiliary α2δ3 subunits of calcium channels foster presynaptic release of GABA, trigger formation of inhibitory synapses, and promote axonal outgrowth in inhibitory interneurons. In contrast, later in development, α2δ1 subunits promote the glutamatergic neurotransmission and synaptogenesis, as well as strongly enhance neuronal network activity. We propose that formation of connectivity in neuronal networks is associated with a concerted interplay of α2δ1 and α2δ3 subunits of calcium channels.
J Neurosci, 2020; 40
30851307: Heine M, Heck J, Ciuraszkiewicz A, Bikbaev A
Dynamic compartmentalization of calcium channel signalling in neurons.
Calcium fluxes through the neuronal membrane are strictly limited in time due to biophysical properties of voltage-gated and ligand-activated ion channels and receptors. Being embedded into the crowded dynamic environment of biological membranes, Ca-permeable receptors and channels undergo perpetual spatial rearrangement, which enables their temporary association and formation of transient signalling complexes. Thus, efficient calcium-mediated signal transduction requires mechanisms to support very precise spatiotemporal alignment of the calcium source and Ca-binding lipids and proteins in a highly dynamic environment. The mobility of calcium channels and calcium-sensing proteins themselves can be considered as a physiologically meaningful variable that affects calcium-mediated signalling in neurons. In this review, we will focus on voltage-gated calcium channels (VGCCs) and activity-induced relocation of stromal interaction molecules (STIMs) in the endoplasmic reticulum (ER) to show that particularly in time ranges between milliseconds to minutes, dynamic rearrangement of calcium conducting channels and sensor molecules is of physiological relevance. This article is part of the special issue entitled 'Mobility and trafficking of neuronal membrane proteins'.
Neuropharmacology, 2020; 169
31104951: Heck J, Parutto P, Ciuraszkiewicz A, Bikbaev A, Freund R, Mitlöhner J, Andres-Alonso M, Fejtova A, Holcman D, Heine M
Transient Confinement of Ca2.1 Ca-Channel Splice Variants Shapes Synaptic Short-Term Plasticity.
The precision and reliability of synaptic information transfer depend on the molecular organization of voltage-gated calcium channels (VGCCs) within the presynaptic membrane. Alternative splicing of exon 47 affects the C-terminal structure of VGCCs and their affinity to intracellular partners and synaptic vesicles (SVs). We show that hippocampal synapses expressing VGCCs either with exon 47 (Ca2.1) or without (Ca2.1) differ in release probability and short-term plasticity. Tracking single channels revealed transient visits (~100 ms) of presynaptic VGCCs in nanodomains (~80 nm) that were controlled by neuronal network activity. Surprisingly, despite harboring prominent binding sites to scaffold proteins, Ca2.1 persistently displayed higher mobility within nanodomains. Synaptic accumulation of Ca2.1 was accomplished by optogenetic clustering, but only Ca2.1 increased transmitter release and enhanced synaptic short-term depression. We propose that exon 47-related alternative splicing of Ca2.1 channels controls synapse-specific release properties at the level of channel mobility-dependent coupling between VGCCs and SVs.
Neuron, 2019; 103
31827157: Parutto P, Heck J, Heine M, Holcman D
Biophysics of high density nanometer regions extracted from super-resolution single particle trajectories: application to voltage-gated calcium channels and phospholipids.
The cellular membrane is very heterogenous and enriched with high-density regions forming microdomains, as revealed by single particle tracking experiments. However the organization of these regions remain unexplained. We determine here the biophysical properties of these regions, when described as a basin of attraction. We develop two methods to recover the dynamics and local potential wells (field of force and boundary). The first method is based on the local density of points distribution of trajectories, which differs inside and outside the wells. The second method focuses on recovering the drift field that is convergent inside wells and uses the transient field to determine the boundary. Finally, we apply these two methods to the distribution of trajectories recorded from voltage gated calcium channels and phospholipid anchored GFP in the cell

membrane of hippocampal neurons and obtain the size and energy of high-density regions with a nanometer precision.
Sci Rep, 2019; 9
27872869: Voigt A, Freund R, Heck J, Missler M, Obermair GJ, Thomas U, Heine M
Dynamic association of calcium channel subunits at the cellular membrane.
High voltage gated calcium channels (VGCCs) are composed of at least three subunits, one pore forming [Formula: see text]-subunit, an intracellular [Formula: see text]-variant, and a mostly extracellular [Formula: see text]-variant. Interactions between these subunits determine the kinetic properties of VGCCs. It is unclear whether these interactions are stable over time or rather transient. Here, we used single-molecule tracking to investigate the surface diffusion of [Formula: see text]- and [Formula: see text]-subunits at the cell surface. We found that [Formula: see text]-subunits show higher surface mobility than [Formula: see text]-subunits, and that they are only transiently confined together, suggesting a weak association between [Formula: see text]- and [Formula: see text]-subunits. Moreover, we observed that different [Formula: see text]-subunits engage in different degrees of association with the [Formula: see text]-subunit, revealing the tighter interaction of [Formula: see text] with [Formula: see text]. These data indicate a distinct regulation of the [Formula: see text] interaction in VGCC subtypes. We modeled their membrane dynamics in a Monte Carlo simulation using experimentally determined diffusion constants. Our modeling predicts that the ratio of associated [Formula: see text]- and [Formula: see text]-subunits mainly depends on their expression density and confinement in the membrane. Based on the different motilities of particular [Formula: see text]-subunit combinations, we propose that their dynamic assembly and disassembly represent an important mechanism to regulate the signaling properties of VGCC.
Neurophotonics, 2016; 3
26891382: Heine M, Ciuraszkiewicz A, Voigt A, Heck J, Bikbaev A
Surface dynamics of voltage-gated ion channels.
Neurons encode information in fast changes of the membrane potential, and thus electrical membrane properties are critically important for the integration and processing of synaptic inputs by a neuron. These electrical properties are largely determined by ion channels embedded in the membrane. The distribution of most ion channels in the membrane is not spatially uniform: they undergo activity-driven changes in the range of minutes to days. Even in the range of milliseconds, the composition and topology of ion channels are not static but engage in highly dynamic processes including stochastic or activity-dependent transient association of the pore-forming and auxiliary subunits, lateral diffusion, as well as clustering of different channels. In this review we briefly discuss the potential impact of mobile sodium, calcium and potassium ion channels and the functional significance of this for individual neurons and neuronal networks.
Channels (Austin), 2016; 10

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BOARD NUMBER: S02-645

THE LONG NON-CODING RNA MIMI SCAFFOLDS NEURONAL GRANULES TO MAINTAIN NERVOUS SYSTEM MATURITY

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Dominika Grzejda, Valérie Hilgers
Max Planck Institute of Immunobiology and Epigenetics, Valérie Hilgers Lab, Freiburg, Germany

RNA-binding proteins and messenger RNAs assemble into ribonucleoprotein granules that regulate mRNA trafficking, local translation, and turnover. RNP granule-mediated regulation of gene expression is abundantly utilized by neurons— cells that particularly rely on precise spatiotemporal compartmentalization of cellular events. Dysregulation of RNA-protein condensation can disturb neuronal survival and function: granule hypo- and hyper-assembly are associated with human neurological diseases, including Alzheimer's disease, ALS/FTD (amyotrophic lateral sclerosis/ frontotemporal dementia), SMA (spinal muscular atrophy) or Huntington's disease. Neuronal granules are thought to condense around particular proteins. Contrasting with this prevailing model, here, we show in Drosophila that a previously uncharacterized long non-coding RNA, *mimi*, is required to scaffold large neuronal granules in the adult nervous system. Neuronal ELAV-like proteins directly bind *mimi* and mediate granule assembly, while Staufen maintains *mimi* expression and condensate integrity. *mimi* is undetectable outside of condensates, suggesting that its main cellular function is to support RNP granules. *mimi* mutant flies constitute a unique animal model for granule hypo-assembly that provides a handle to interrogate functions of a condensate independently from those of its constituents. *mimi* granules contain mRNAs and proteins involved in synaptic processes; granule loss in *mimi* mutant flies impairs nervous system maturity, neuropeptide-mediated signaling, causes memory defects and phenotypes of neurodegeneration. Our work reports the first architectural RNA for a neuronal granule and showcases how the molecular functions of highly conserved RNA-binding proteins converge in phase-separated condensates to regulate neuronal function and behavior.

Pubmed:

33007255: Carrasco J, Rauer M, Hummel B, Grzejda D, Alfonso-Gonzalez C, Lee Y, Wang Q, Puchalska M, Mittler G, Hilgers V
ELAV and FNE Determine Neuronal Transcript Signatures through EXon-Activated Rescue.
The production of alternative RNA variants contributes to the tissue-specific regulation of gene expression. In the animal nervous system, a systematic shift toward distal sites of transcription termination produces transcript signatures that are crucial for neuron development and function. Here, we report that, in Drosophila, the highly conserved protein ELAV globally regulates all sites of neuronal 3' end processing and directly binds to proximal polyadenylation sites of target mRNAs in vivo. We uncover an endogenous strategy of functional gene rescue that safeguards neuronal RNA signatures in an ELAV loss-of-function context. When not directly repressed by ELAV, the transcript encoding the ELAV paralog FNE acquires a mini-exon, generating a new protein able to translocate to the nucleus and rescue ELAV-mediated alternative polyadenylation and alternative splicing. We propose that exon-activated functional rescue is a more widespread mechanism that ensures robustness of processes regulated by a hierarchy, rather than redundancy, of effectors.
Mol Cell, 2020; 80
30390702: Marchut-Mikolajczyk O, Drożdżyński P, Pietrzyk D, Antczak T
Biosurfactant production and hydrocarbon degradation activity of endophytic bacteria isolated from Chelidonium majus L.
The process of plant growth in the contaminated environment is often inhibited and entails the neutralization of harmful compounds. To reduce the negative impact of harmful compounds microorganisms produce unique compounds called biosurfactants. This paper describes the potential of culturable endophytic microorganisms from synanthropic plant-Chelidonium majus L. for the production of biosurfactants, as indirect plant promoting factors as well as their degradation activity. Emulsifying activity and degradation potential of tested strains were assessed by cultivation of isolates in the presence of diesel oil and waste engine oil.
Microb Cell Fact, 2018; 17

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BOARD NUMBER: S02-646

ROLE OF DEVELOPMENTAL REGULATORS OF AXONAL LOCAL TRANSLATION IN ADULT AXONS

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Marina Vidaki, Nikoletta Triantopoulou, Sofia Pasadaki
University of Crete, School Of Medicine, Heraklion, Greece

Local mRNA translation (LT) is vital for axon development. Disruption of LT has been linked to the emergence of neurodevelopmental disorders, while several studies suggest that LT is related to the intrinsic regenerative capacity of mature neurons. Axonal regeneration displays similarities with axon growth during development, exhibiting a number of cellular processes that require LT. Although mature PNS axons maintain the ability for LT, maturing CNS axons exhibit a gradually decreasing LT potential, highly correlated with their limited regenerative capacity. Despite the evident role of LT in axon stability and regeneration, the regulatory mechanisms behind the process and their role in post-injury regeneration remain elusive. We have previously identified a ribonucleoprotein complex (Mena-RNP) that regulates axonal LT in the developing brain. It consists of the cytoskeleton-associated protein Mena that interacts with known regulators of translation (i.e. HnrnpK, PCBP1), thus controlling the translation of the RNP-bound mRNAs. Here, we investigate the conservation of Mena-RNP in the adult nervous system, in an attempt to elucidate the role of Mena and Mena-dependent translation in adult axon regeneration. Employing both *in vivo* and *ex vivo* injury assays we observe that the Mena-RNP components differ between the developing and adult nervous system and in CNS vs PNS axons. In an additional, unbiased line of work we are currently exploring other molecular components that function locally in axons and could potentially explain the differential capacity of adult CNS and PNS axons for regeneration.

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TALKING TO THE NUCLEUS: TUNING OF CORTICAL INHIBITION BY BMP-SMAD1 SIGNALING

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Zeynep Okur, Nadia Schlauri, Vassilis Bitsikas, Peter Scheiffele
Biozentrum, University Of Basel, Basel, Switzerland

Neocortical networks are responsive to environmental changes during development and at adult stages. Parvalbumin expressing fast spiking interneurons (PV-INs) are the most abundant population of inhibitory neurons in the neocortex. PV-INs and their plasticity are critically involved in the generation of network oscillations, learning, and maintaining the excitation - inhibition in balance. During late postnatal development, PV-INs mature significantly as evidenced by functional properties, increases in parvalbumin expression, and the elaboration of perineuronal nets. Moreover, mature PV-INs undergo reversible state changes during learning. However, the molecular mechanisms underlying such transitions are largely unknown. Growth factors are candidate regulators as they can be mobilized upon neuronal activation and through effects on transcription factors direct neuronal transcriptome changes. We demonstrate that Bone Morphogenic Proteins (BMPs), a class of morphogens acting in early development, remain expressed in adult neocortex and are significantly elevated in response to neuronal stimulation. Neuron specific BMP signaling loss of function from cultured neocortical cells results in elevated network activity and pervasive disruption of the neuronal transcriptome. Postnatal conditional knock-out of Smad1, selectively in PV-INs ($Smad1^{cKO}$), results in reduced parvalbumin levels, perineuronal nets and parvalbumin driven inhibitory synapses in adult mice. Electrophysiological recordings show delayed action potential firing of PV-INs in adult $Smad1^{cKO}$ mice. Consistent with a hypofunction of PV-INs, $Smad1^{cKO}$ mice exhibit spontaneous seizures. In ongoing work, we are investigating the activity-dependent mobilization of BMPs in the adult cortex. We propose that BMP-SMAD1 signaling balances PV-IN connectivity by driving functional PV-IN state shifts in mature neocortical networks.

Pubmed:
32572071: Vickers E, Osypenko D, Clark C, Okur Z, Scheiffele P, Schneggenburger R
LTP of inhibition at PV interneuron output synapses requires developmental BMP signaling.
Parvalbumin (PV)-expressing interneurons (PV-INs) mediate well-timed inhibition of cortical principal neurons, and plasticity of these interneurons is involved in map remodeling of primary sensory cortices during critical periods of development. To assess whether bone morphogenetic protein (BMP) signaling contributes to the developmental acquisition of the synapse- and plasticity properties of PV-INs, we investigated conditional/conventional double KO mice of BMP-receptor 1a (BMPR1a; targeted to PV-INs) and 1b (BMPR1a/1b (c)DKO mice). We report that spike-timing dependent LTP at the synapse between PV-INs and principal neurons of layer 4 in the auditory cortex was absent, concomitant with a decreased paired-pulse ratio (PPR). On the other hand, baseline synaptic transmission at this connection, and action potential (AP) firing rates of PV-INs were unchanged. To explore possible gene expression targets of BMP signaling, we measured the mRNA levels of the BDNF receptor TrkB and of P/Q-type Ca channel α-subunits, but did not detect expression changes of the corresponding genes in PV-INs of BMPR1a/1b (c)DKO mice. Our study suggests that BMP-signaling in PV-INs during and shortly after the critical period is necessary for the expression of LTP at PV-IN output synapses, involving gene expression programs that need to be addressed in future work.
Sci Rep, 2020; 10
30998895: Okur Z, Scheiffele P
The Yin and Yang of Arnt2 in Activity-Dependent Transcription.
Spatiotemporal regulation of neuronal gene expression is essential for proper functioning of neuronal circuits. In this issue of Neuron, Sharma et al. (2019) discover a dual role for Arnt2-NcoR2 protein complexes in the activity-dependent regulation of neuronal transcriptomes.
Neuron, 2019; 102
29770392: Okur Z, Senturk OI, Yilmaz C, Gulseren G, Mammadov B, Guler MO, Tekinay AB
Promotion of neurite outgrowth by rationally designed NGF-β binding peptide nanofibers.
Promotion of neurite outgrowth is an important limiting step for regeneration in nerve injury and depends strongly on the local expression of nerve growth factor (NGF). The rational design of bioactive materials is a promising approach for the

development of novel therapeutic methods for nerve regeneration, and biomaterials capable of presenting NGF to nerve cells are especially suitable for this purpose. In this study, we show bioactive peptide amphiphile (PA) nanofibers capable of promoting neurite outgrowth by displaying high density binding epitopes for NGF. A high-affinity NGF-binding sequence was identified by phage display and combined with a beta-sheet forming motif to produce a self-assembling PA molecule. The bioactive nanofiber had higher affinity for NGF compared to control nanofibers and in vitro studies revealed that the NGF binding peptide amphiphile nanofibers (NGFB-PA nanofiber) significantly promote the neurite outgrowth of PC-12 cells. In addition, the nanofibers induced differentiation of PC-12 cells into neuron-like cells by enhancing NGF/high-activity NGF receptor (TrkA) interactions and activating MAPK pathway elements. The NGFB-PA nanofiber was further shown as a promising material to support axonal outgrowth from primary sensory neurons. These materials will pave the way for the development of new therapeutic agents for peripheral nervous system injuries.
Biomater Sci, 2018; 6

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A NEW TRIPLE FLUORESCENCE REPORTER SYSTEM FOR DISCRIMINATION OF APOBEC1 AND APOBEC3 C-TO-U RNA EDITING ACTIVITIES AND EDITING-DEPENDENT PROTEIN EXPRESSION

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Jochen Meier[1], Magnus Harnau[1], Barbara Schweissthal[1], Kea Brunken[1], Anton Marques[1], Julia Brach[1], Leonie Emde[1], Julia Leonhard[1], Florian Hetsch[2], Steffen Fricke[1]
[1]Technische Universität Braunschweig, Cell Physiology, Braunschweig, Germany, [2]Johannes Gutenberg-University of Mainz, Institute Of Pathophysiology, Mainz, Germany

The human body is composed of many different cell types which communicate with each other. In particular, the brain consists of billions of neurons and non-neuronal cells which are interconnected and require tight and precise regulation of cellular processes. RNA editing is a cellular process that diversifies gene function by enzymatic deamination of cytidine or adenine. This can result in changes of protein structure and function. Altered RNA editing is becoming increasingly associated with all kind of disease, but most approaches use advanced sequencing technologies to analyze bulk material. However, it is also becoming progressively evident that changes in RNA editing have to be analyzed and considered in a cell type specific way. We present here a triple fluorescence reporter system that discriminates between Apobec1- and Apobec3-dependent C-to-U RNA editing at the single cell level. In particular, the Apobec3 reporter enables C-to-U RNA editing inducible protein expression through generation of a RNA splice donor site. We used the new system here to analyze Apobec1- and Apobec3-dependent RNA editing in primary neuron culture. The results reveal a large heterogeneity of C-to-U RNA editing in neurons and glia cells. The new system can be the foundation of therapeutic application systems that counteract changes in Apobec3-dependent RNA editing in disease while simultaneously monitoring Apobec1-dependent RNA editing at the single cell level.

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BOARD NUMBER: S02-649

TRANSCRIPTIONAL BIOMARKERS OF ORBITOFRONTAL CORTEX EFFERENT PROJECTIONS

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Marta Solano Mateos[1], Suelynn Ren[2], Ben-Orli Nathanson[1], Torben Ott[3], Romana Hauer[1], Adam Kepecs[2], Thomas Klausberger[1]
[1]Medical University of Vienna, Centre For Brain Research, Vienna, Austria, [2]Washington University School of Medicine, Department Of Neuroscience, St Louis, United States of America, [3]Humboldt University of Berlin, Bernstein Centre For Computational Neuroscience, Berlin, Germany

The orbitofrontal cortex (OFC) plays a fundamental role in post-decision confidence and value-based decision-making. The integrative function of this ventral subregion of the prefrontal cortex makes it heavily circuit dependent; OFC projection efferents include superior colliculus and ventral striatum. The network underlying economic value has been dissected by neuroimaging, anatomic tracing and single-unit recordings, but the transcriptomic profile remains unclear. Within neurons, translation occurs in different subcellular locations allowing dynamic adaptation to the anatomic location and cellular environment. Research shows transcriptional differences between neuronal types are more prominent in somata than dendritic arbours, therefore gene expression patterns and subcellular localization of mRNA transcripts can help narrow projecting neuronal types in the OFC. Here, we aimed to identify expression markers of OFC efferent projection neurons from a set of pre-selected putative biomarkers, by combining retrograde viral vector tracing with RNA Scope. RNA Scope is an in-situ hybridization method; It consists of sequence-specific probes and a two-step amplification allowing specific detection of single mRNA molecules. Co-localization of the mRNA with retrograde tracer labelled neurons enabled accurate spatial context detection, cellular localization and quantification of candidate gene expression in OFC efferent projecting neurons. We identified specific transcriptomic markers of OFC efferent projections, which can prove useful as predictive markers for projection target identification of neurons. Overall, our approach allowed transcriptomic profiling of OFC circuitry enabling the genetic and anatomic bottom-up understanding of the connectivity underlying value-based decision making and post-decision confidence. Research supported by the Austrian Science Fund (I 5458).

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CHARACTERIZATION OF THE EXPRESSION OF TRANSCRIPTION FACTOR 4 MRNA AND PROTEIN ISOFORMS IN THE DEVELOPING AND ADULT RODENT AND HUMAN BRAIN AND PERIPHERAL TISSUES

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Anastassia Shubina, Alex Sirp, Jürgen Tuvikene, Laura Tamberg, Carl Sander Kiir, Tõnis Timmusk
Tallinn University of Technology, Department Of Chemistry And Biotechnology, Tallinn, Estonia

Transcription factor 4 (TCF4) belongs to a family of basic helix-loop-helix transcription factors, also known as E-proteins, and is vital for the development of a normally functioning nervous system. TCF4 has been associated with several mental disorders such as schizophrenia, intellectual disability, and a very rare but severe disease known as Pitt-Hopkins syndrome (PTHS). We have previously demonstrated that human TCF4 gene is transcribed using numerous alternative 5'-exons, potentially yielding in many TCF4 protein isoforms with different N-termini that vary in their subcellular distribution and transcription activation capability. We have found that PTHS-associated mutations impair the functions of TCF4 by diverse mechanisms ranging from hypomorphic to dominant-negative effects. Our results have also demonstrated that TCF4 transcriptional activity is induced by neuronal activity via direct phosphorylation of TCF4 by protein kinase A. Additionally, our recently published data showed that different TCF4 isoforms have differential synergism with class II basic helix-loop-helix transcription factors. TCF4 expression studies have been mainly focused on TCF4 mRNA levels whereas expression of protein isoforms has been less characterized. Here we present our recent results of the expression pattern of different TCF4 mRNA and protein isoforms in the brain and peripheral organs during rodent and human development.

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ROLE OF NETRIN-1 IN EXPERIMENTAL MODEL OF MULTIPLE SCLEROSIS

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Fritz Kagerer[1], Almir Aljovic[1], Clara De La Rosa Del Val[1], Arek Kendirli[1], Florence Bareyre[2], Martin Kerschensteiner[3]
[1]Ludwig-Maximilians University Munich, Neuroimmunology, Planegg-Martinsried, Germany, [2]University Hospital LMU Munich, Institute For Clinical Neuroimmunology, Munich, Germany, [3]Biomedical Center of LMU, Institute Of Clinical Neuroimmunology, Martinsried, Germany

Multiple sclerosis (MS) is a disease affecting 2,8 million people worldwide, however, there are still many open questions about this disease and targeted treatments are lacking. MS is mostly caused by an autoimmune reaction against myelination of neurons. Therefore, first order treatments are mostly restricted to immunomodulators that downregulate the immune system. This immunomodulation is rather unspecific and we are interested in finding regulators that could modulate inflammatory environment specifically in CNS. Recent studies suggest that the axon guidance protein Netrin-1 was shown to also play a significant role in inflammation. We hypothesized that Netrin-1 could be a potent candidate for immune modulation specific to CNS environment. In line with our hypothesis, we found that Netrin-1 expression not only significantly increases in tissue of experimental autoimmune encephalomyelitis (EAE) mice, but also that the protein expression correlates almost perfectly with the stage of the disease. Additionally, we found a significant increase of Netrin-1-positive cells at EAE-induced lesion sites in the spinal cord. These cells were mostly CD11b positive, suggesting that Netrin-1 gets upregulated in macrophages and microglia in EAE lesion site. We therefore want to further study this up-regulation of Netrin-1 in tissue of EAE mice and the role of Netrin-1 in EAE inflammation.

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BOARD NUMBER: S02-652

NOVEL STIMULUS-DEPENDENT REGULATORS OF BDNF GENE EXPRESSION

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Eli-Eelika Esvald, Andra Moistus, Jürgen Tuvikene, Karin Lehe, Annela Avarlaid, Tõnis Timmusk
Tallinn University of Technology, Department Of Chemistry And Biotechnology, Tallinn, Estonia

Brain-derived neurotrophic factor (BDNF) is a widely expressed neurotrophin that supports the survival, differentiation and signaling of various neuronal populations. To utilize the great potential of neurotrophic factors in treating nervous system disorders, it is crucial to understand their regulation. Here, we aimed to elucidate the BDNF gene regulation after different stimuli to better understand how cells express BDNF gene to regulate the development and maintenance of the nervous system. To this end, we have used an unbiased approach using *in vitro* DNA pulldown coupled with mass-spectrometry to discover the transcription factors binding to BDNF promoters I and IV in cultured cortical neurons after different stimuli. Our results confirm many previously described regulators of BDNF and suggest novel regulators of BDNF gene. We next studied a selection of the novel candidate regulators using coexpression with promoter constructs in cultured neurons, then deciphered the role of selected factors in the regulation of BDNF gene in the endogenous context, and finally confirmed their binding to BDNF promoters in the endogenous context using chromatin immunoprecipitation. Our data reveals complicated fine-tuning of BDNF expression at the promoter regions and provides insight to how neurons respond to different stimuli and control BDNF transcription.

Pubmed:
33560226: Tuvikene J, Esvald EE, Rähni A, Uustalu K, Zhuravskaya A, Avarlaid A, Makeyev EV, Timmusk T
Intronic enhancer region governs transcript-specific expression in rodent neurons.
Brain-derived neurotrophic factor (BDNF) controls the survival, growth, and function of neurons both during the development and in the adult nervous system. is transcribed from several distinct promoters generating transcripts with alternative 5' exons. transcripts initiated at the first cluster of exons have been associated with the regulation of body weight and various aspects of social behavior, but the mechanisms driving the expression of these transcripts have remained poorly understood. Here, we identify an evolutionarily conserved intronic enhancer region inside the gene that regulates both basal and stimulus-dependent expression of the transcripts starting from the first cluster of 5' exons in mouse and rat neurons. We further uncover a functional E-box element in the enhancer region, linking the expression of and various pro-neural basic helix-loop-helix transcription factors. Collectively, our results shed new light on the cell-type- and stimulus-specific regulation of the important neurotrophic factor BDNF.
Elife, 2021; 10
31915257: Esvald EE, Tuvikene J, Sirp A, Patil S, Bramham CR, Timmusk T
CREB Family Transcription Factors Are Major Mediators of BDNF Transcriptional Autoregulation in Cortical Neurons.
BDNF signaling via its transmembrane receptor TrkB has an important role in neuronal survival, differentiation, and synaptic plasticity. Remarkably, BDNF is capable of modulating its own expression levels in neurons, forming a transcriptional positive feedback loop. In the current study, we have investigated this phenomenon in primary cultures of rat cortical neurons using overexpression of dominant-negative forms of several transcription factors, including CREB, ATF2, C/EBP, USF, and NFAT. We show that CREB family transcription factors, together with the coactivator CBP/p300, but not the CRTC family, are the main regulators of rat gene expression after TrkB signaling. CREB family transcription factors are required for the early induction of all the major transcripts, whereas CREB itself directly binds only to promoter IV, is phosphorylated in response to BDNF-TrkB signaling, and activates transcription from promoter IV by recruiting CBP. Our complementary reporter assays with promoter constructs indicate that the regulation of by CREB family after BDNF-TrkB signaling is generally conserved between rat and human. However, we demonstrate that a nonconserved functional cAMP-responsive element in promoter IXa in humans renders the human promoter responsive to BDNF-TrkB-CREB signaling, whereas the rat ortholog is unresponsive. Finally, we show that extensive BDNF transcriptional autoregulation, encompassing all major transcripts, occurs also in the adult rat hippocampus during BDNF-induced LTP. Collectively, these results improve the understanding of the intricate mechanism of BDNF transcriptional autoregulation. Deeper understanding of stimulus-specific regulation of gene expression is essential to precisely adjust BDNF levels that are dysregulated in various neurological disorders. Here, we have

elucidated the molecular mechanisms behind TrkB signaling-dependent mRNA induction and show that CREB family transcription factors are the main regulators of gene expression after TrkB signaling. Our results suggest that BDNF-TrkB signaling may induce gene expression in a distinct manner compared with neuronal activity. Moreover, our data suggest the existence of a stimulus-specific distal enhancer modulating gene expression.
J Neurosci, 2020; 40

26818516: Tuvikene J, Pruunsild P, Orav E, Esvald EE, Timmusk T
AP-1 Transcription Factors Mediate BDNF-Positive Feedback Loop in Cortical Neurons.
Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, regulates both survival and differentiation of several neuronal populations in the nervous system during development, as well as synaptic plasticity in the adult brain. BDNF exerts its biological functions through its receptor TrkB. Although the regulation of BDNF transcription by neuronal activity has been widely studied, little is known about TrkB signaling-dependent expression of BDNF. Using rat primary cortical neuron cultures, we show that the BDNF gene is a subject to an extensive autoregulatory loop, where TrkB signaling upregulates the expression of all major BDNF transcripts, mainly through activating MAPK pathways. Investigating the mechanisms behind this autoregulation, we found that AP-1 transcription factors, comprising Jun and Fos family members, participate in the induction of BDNF exon I, III, and VI transcripts. AP-1 transcription factors directly upregulate the expression of exon I transcripts by binding two novel AP-1 cis-elements in promoter I. Moreover, our results show that the effect of AP-1 proteins on the activity of rat BDNF promoters III and VI is indirect, because AP-1 proteins were not detected to bind the respective promoter regions by chromatin immunoprecipitation (ChIP). Collectively, we describe an extensive positive feedback system in BDNF regulation, adding a new layer to the elaborate control of BDNF gene expression.
J Neurosci, 2016; 36

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STUDY OF THE DYNAMIC EFFECTS OF GLUCOCORTICOID PULSATILITY ON BRAIN-DERIVED NEUROTROPHIC FACTOR EXPRESSION IN ASTROCYTES

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Alexandros Tsimpolis[1,2], Aris Logothetis[1,2], Konstantinos Kalafatakis[1,2], Ioannis Charalampopoulos[1,2]
[1]University of Crete, Medical Department, Heraklion, Greece, [2]Foundation of Research & Technology, Institute Of Molecular Biology & Biotechnology, Heraklion, Greece

Both glucocorticoids (GCs) and neurotrophins, like brain-derived neurotrophic factor (BDNF), are strongly implicated in the pathophysiology of stress-related diseases through their defined effects on adult hippocampal neurogenesis and synaptic plasticity. Functional interactions between BDNF and GCs have been recently demonstrated indicating a potential synergy or antagonism on neuroplasticity under physiological or neuropathological conditions. In order to further investigate this neuro-immunological crosstalk, we examined the baseline effects of a physiologically-relevant GC concentration on BDNF expression in primary cultures of hippocampal neurons, astrocytes and co-cultures of the two cell populations, in consecutive timepoints during a 24-hour exposure. Our results, demonstrate a different reaction of each cell type to administrated GCs and characterize the existence, in astrocytes, of two temporally distinct functions with opposing outcomes on BDNF expression: an early, very fast effect that occurs well within the first hour of GC administration and leads to BDNF overexpression and a late inhibitory effect, that can last for up to 24 hours. Using selective pharmacological inhibitors, we were able to match the involvement of Glucocorticoid receptor to the late effect and interestingly, the necessity of both Mineralocorticoid receptor and TrkB (BDNF receptor) for the regulation of the early effect. Finally, by simulating *in vitro* the physiological pulsatile secretion of GCs, we demonstrated astrocyte's ability to restore BDNF expression, back to its baseline untreated level, thus highlighting the well-established homeostatic capabilities of GCs and the important role of astrocyte susceptibility to GC pulsatility.

Pubmed:
32601750: Kalafatakis I, Kalafatakis K, Tsimpolis A, Giannakeas N, Tsipouras M, Tzallas A, Karagogeos D
Using the Allen gene expression atlas of the adult mouse brain to gain further insight into the physiological significance of TAG-1/Contactin-2.
The anatomic gene expression atlas (AGEA) of the adult mouse brain of the Allen Institute for Brain Science is a transcriptome-based atlas of the adult C57Bl/6 J mouse brain, based on the extensive in situ hybridization dataset of the Institute. This spatial mapping of the gene expression levels of mice under baseline conditions could assist in the formation of new, reasonable transcriptome-derived hypotheses on brain structure and underlying biochemistry, which could also have functional implications. The aim of this work is to use the data of the AGEA (in combination with Tabula Muris, a compendium of single cell transcriptome data collected from mice, enabling direct and controlled comparison of gene expression among cell types) to provide further insights into the physiology of TAG-1/Contactin-2 and its interactions, by presenting the expression of the corresponding gene across the adult mouse brain under baseline conditions and to investigate any spatial genomic correlations between TAG-1/Contactin-2 and its interacting proteins and markers of mature and immature oligodendrocytes, based on the pre-existing experimental or bibliographical evidence. The across-brain correlation analysis on the gene expression intensities showed a positive spatial correlation of TAG-1/Contactin-2 with the gene expression of Plp1, Myrf, Mbp, Mog, Cldn11, Bace1, Kcna1, Kcna2, App and Nfasc and a negative spatial correlation with the gene expression of Cspg4, Pdgfra, L1cam, Ncam1, Ncam2 and Ptprz1. Spatially correlated genes are mainly expressed by mature oligodendrocytes (like Cntn2), while spatially anticorrelated genes are mainly expressed by oligodendrocyte precursor cells. According to the data presented in this work, we propose that even though Contactin-2 expression during development correlates with high plasticity events, such as neuritogenesis, in adulthood it correlates with pathways characterized by low plasticity.
Brain Struct Funct, 2020; 225

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BOARD NUMBER: S02-654

GENE-REGULATORY DYNAMICS OF MICROGLIA STATES DURING NEUROINFLAMMATION

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Gene expression and genetic variation studies suggest an important contribution of microglia cells to the onset and progression of most prevalent neurodegenerative conditions, such as Alzheimer's disease (AD), where a putative diversity of microglia states with divergent homeostatic or pathophysiological roles has been proposed. However, very little is known on the mechanisms regulating the ability of these highly plastic cells of the brain to adopt specialized roles when exposed to different conditions. Here, we combine high-throughput genomics and in situ RNA expression analysis at the single-cell level with functional assays to reveal the molecular underpinnings of the transitions and maintenance of the distinct phenotypic and functional states of brain's innate immune cells through the initiation, activation and resolution of the neuroinflammatory response. These data deepen our understanding of microglia heterogeneity which is critical to devise new therapeutics for most prevalent neurodegenerative diseases.

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BOARD NUMBER: S02-655

A CELL-BASED MODEL TO VALIDATE THE PATHOGENIC NATURE OF EPILEPSY-ASSOCIATED VARIANTS OF THE RORB GENE.

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Epilepsy is a complex disease with a high contribution of genetic factors, including several hundreds of Mendelian disorders. In those case, pathogenic variants disrupt the expression or function of proteins essential for neuronal development, synaptic transmission, or ion currents. Yet the identification of variants of unknown clinical relevance in patients raises the need to validate that the mutations affect some of the functions of the gene. We focused on *RORB*, which encodes the retinoid-related orphan receptor beta (RORß), and was recently associated to moderate intellectual disability and epilepsy. We characterized that knockdown of RORß expression altered axon morphogenesis in mouse cortical neurons. Conversely, overexpression of RORß led to a tractable axonal phenotype (ie. increased axonal complexity). Taking advantage of this phenotype, we generated plasmids expressing mutant RORß proteins mimicking variants identified in epileptic patients, and validated that these mutations impair the functions of the RORß protein. Overall, our study demonstrates the pathogenic nature of the variants tested in our cell model.

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A FAR UPSTREAM ENHANCER IS A CRUCIAL REGULATOR OF BDNF GENE EXPRESSION IN RODENT NEURONS AND ASTROCYTES

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Annela Avarlaid[1], Eli-Eelika Esvald[1], Jürgen Tuvikene[1], Indrek Koppel[1], Anna Zhuravskaya[2], Eugene Makeyev[2], Tõnis Timmusk[1]
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Brain derived neurotrophic factor (BDNF) has a fundamental role in the developing and adult nervous system, where BDNF assists the development of neuronal circuits, promotes neuronal survival, differentiation and synaptic plasticity. Therefore, dysregulation of BDNF expression has been implicated in many neurological disorders, for example Rett syndrome, schizophrenia and Alzheimer's disease. The stimulus- and tissue-specific expression of BDNF is ensured with a complex transcriptional regulation. BDNF gene contains several promoters that are activated in response to various stimuli, including neuronal activity and TrkB-signalling in neurons, and catecholamine-dependent signalling in astrocytes. Although the promoter regions of the BDNF gene have been extensively studied, very little is known about the enhancer regions regulating the transcription of BDNF. In this study, we investigated five potential enhancer regions and report that an enhancer far upstream of BDNF gene regulates the BDNF gene expression in rodent neurons and astrocytes. Specifically, the far upstream enhancer participates in basal and stimulus-dependent expression of BDNF in rodent neurons and is responsible for catecholamine-induced CREB and AP1 family-dependent expression of BDNF in cortical astrocytes. Altogether, these findings reveal a new enhancer region crucial for BDNF gene expression in the nervous system.

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BOARD NUMBER: S02-657

THE BASIC HELIX-LOOP-HELIX TRANSCRIPTION FACTOR TCF4 REGULATES ACTIVITY-DEPENDENT TRANSCRIPTIONAL PROGRAMS IN NEURONS

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Jürgen Tuvikene[1], Eli-Eelika Esvald[1], Alex Sirp[1], Anastassia Shubina[1], Hanna Vihma[1], Mari Urb[1], Kaur Jaanson[1], Kaja Nurm[1], Andra Moistus[1], Indrek Koppel[1], Kristi Luberg[1], Derek Blake[2], Mari Sepp[1], Tõnis Timmusk[1]
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Transcription factor 4 (TCF4), belonging to the basic helix-loop-helix transcription factor superfamily, plays an important role in the development of the nervous system. Dysregulation of TCF4 is associated with various neurological diseases, including autism spectrum disorder Pitt-Hopkins syndrome, intellectual disability, and schizophrenia. We have previously reported that TCF4 is a neuronal activity-dependent transcriptional activator in neurons. Although numerous studies have described the TCF4 target genes and processes in various cellular and animal model systems, little is known about the gene expression programs regulated by TCF4 upon neuronal activity. Here, we combined TCF4 knock-down in rat cultured cortical neurons and depolarization with KCl to mimic neuronal activity, and measured the gene expression changes with RNA-seq. We also carried out TCF4 ChIP-seq analysis to determine the binding of endogenous TCF4 in neurons and identified direct transcriptional targets of TCF4 upon neuronal activity. We further used *in vitro* DNA pulldown coupled with mass-spectrometry to elucidate the transcription factors binding to different E-box *cis*-elements, and identified TCF4 interaction partners in neurons using TCF4 co-IP and mass-spectrometry. Collectively, our results reveal novel neuronal activity-dependent transcriptional programs regulated by TCF4.

Pubmed:

35091341: Pupina N, Avarlaid A, Sadam H, Pihlak A, Jaago M, Tuvikene J, Rähni A, Planken A, Planken M, Kalso E, Tienari PJ, Nieminen JK, Seppänen MRJ, Vaheri A, Lindholm D, Sinisalo J, Pussinen P, Timmusk T, Palm K
Immune response to a conserved enteroviral epitope of the major capsid VP1 protein is associated with lower risk of cardiovascular disease.
Major cardiac events including myocardial infarction (MI) are associated with viral infections. However, how specific infections contribute to the cardiovascular insults has remained largely unclear.
EBioMedicine, 2022; 76
34748727: Sirp A, Roots K, Nurm K, Tuvikene J, Sepp M, Timmusk T
Functional consequences of TCF4 missense substitutions associated with Pitt-Hopkins syndrome, mild intellectual disability, and schizophrenia.
Transcription factor 4 (TCF4) is a basic helix-loop-helix transcription factor essential for neurocognitive development. The aberrations in TCF4 are associated with neurodevelopmental disorders including schizophrenia, intellectual disability, and Pitt-Hopkins syndrome, an autism-spectrum disorder characterized by developmental delay. Several disease-associated missense mutations in TCF4 have been shown to interfere with TCF4 function, but for many mutations, the impact remains undefined. Here, we tested the effects of 12 functionally uncharacterized disease-associated missense mutations and variations in TCF4 using transient expression in mammalian cells, confocal imaging, in vitro DNA-binding assays, and reporter assays. We show that Pitt-Hopkins syndrome-associated missense mutations within the basic helix-loop-helix domain of TCF4 and a Rett-like syndrome-associated mutation in a transcription activation domain result in altered DNA-binding and transcriptional activity of the protein. Some of the missense variations found in schizophrenia patients slightly increase TCF4 transcriptional activity, whereas no effects were detected for missense mutations linked to mild intellectual disability. We in addition find that the outcomes of several disease-related mutations are affected by cell type, TCF4 isoform, and dimerization partner, suggesting that the effects of TCF4 mutations are context-dependent. Together with previous work, this study provides a basis for the interpretation of the functional consequences of TCF4 missense variants.
J Biol Chem, 2021; 297
34518368: Nurm K, Sepp M, Castany-Pladevall C, Creus-Muncunill J, Tuvikene J, Sirp A, Vihma H, Blake DJ, Perez-Navarro E, Timmusk T

Isoform-Specific Reduction of the Basic Helix-Loop-Helix Transcription Factor TCF4 Levels in Huntington's Disease.
Huntington's disease (HD) is an inherited neurodegenerative disorder with onset of characteristic motor symptoms at midlife, preceded by subtle cognitive and behavioral disturbances. Transcriptional dysregulation emerges early in the disease course and is considered central to HD pathogenesis. Using wild-type (wt) and HD knock-in mouse striatal cell lines we observed a HD genotype-dependent reduction in the protein levels of transcription factor 4 (TCF4), a member of the basic helix-loop-helix (bHLH) family with critical roles in brain development and function. We characterized mouse gene structure and expression of alternative mRNAs and protein isoforms in cell-based models of HD, and in four different brain regions of male transgenic HD mice (R6/1) from young to mature adulthood. The largest decrease in the levels of TCF4 at mRNA and specific protein isoforms were detected in the R6/1 mouse hippocampus. Translating this finding to human disease, we found reduced expression of long TCF4 isoforms in the postmortem hippocampal CA1 area and in the cerebral cortex of HD patients. Additionally, TCF4 protein isoforms showed differential synergism with the proneural transcription factor ASCL1 in activating reporter gene transcription in hippocampal and cortical cultured neurons. Induction of neuronal activity increased these synergistic effects in hippocampal but not in cortical neurons, suggesting brain region-dependent differences in TCF4 functions. Collectively, this study demonstrates isoform-specific changes in TCF4 expression in HD that could contribute to the progressive impairment of transcriptional regulation and neuronal function in this disease.
eNeuro, 2021 Sep-Oct; 8
33560226: Tuvikene J, Esvald EE, Rähni A, Uustalu K, Zhuravskaya A, Avarlaid A, Makeyev EV, Timmusk T
Intronic enhancer region governs transcript-specific expression in rodent neurons.
Brain-derived neurotrophic factor (BDNF) controls the survival, growth, and function of neurons both during the development and in the adult nervous system. is transcribed from several distinct promoters generating transcripts with alternative 5' exons. transcripts initiated at the first cluster of exons have been associated with the regulation of body weight and various aspects of social behavior, but the mechanisms driving the expression of these transcripts have remained poorly understood. Here, we identify an evolutionarily conserved intronic enhancer region inside the gene that regulates both basal and stimulus-dependent expression of the transcripts starting from the first cluster of 5' exons in mouse and rat neurons. We further uncover a functional E-box element in the enhancer region, linking the expression of and various pro-neural basic helix-loop-helix transcription factors. Collectively, our results shed new light on the cell-type- and stimulus-specific regulation of the important neurotrophic factor BDNF.
Elife, 2021; 10
33116252: Sirp A, Leite K, Tuvikene J, Nurm K, Sepp M, Timmusk T
The Fuchs corneal dystrophy-associated CTG repeat expansion in the TCF4 gene affects transcription from its alternative promoters.
The CTG trinucleotide repeat (TNR) expansion in Transcription factor 4 (TCF4) intron 3 is the main cause of Fuchs' endothelial corneal dystrophy (FECD) and may confer an increased risk of developing bipolar disorder (BD). Usage of alternative 5' exons for transcribing the human TCF4 gene results in numerous TCF4 transcripts which encode for at least 18 N-terminally different protein isoforms that vary in their function and transactivation capability. Here we studied the TCF4 region containing the CTG TNR and characterized the transcription initiation sites of the nearby downstream 5' exons 4a, 4b and 4c. We demonstrate that these exons are linked to alternative promoters and show that the CTG TNR expansion decreases the activity of the nearby downstream TCF4 promoters in primary cultured neurons. We confirm this finding using two RNA sequencing (RNA-seq) datasets of corneal endothelium from FECD patients with expanded CTG TNR in the TCF4 gene. Furthermore, we report an increase in the expression of various other TCF4 transcripts in FECD, possibly indicating a compensatory mechanism. We conclude that the CTG TNR affects TCF4 expression in a transcript-specific manner both in neurons and in the cornea.
Sci Rep, 2020; 10
32641419: Tamberg L, Jaago M, Säälik K, Sirp A, Tuvikene J, Shubina A, Kiir CS, Nurm K, Sepp M, Timmusk T, Palgi M
Daughterless, the orthologue of TCF4, is required for associative learning and maintenance of the synaptic proteome.
Mammalian transcription factor 4 (TCF4) has been linked to schizophrenia and intellectual disabilities, such as Pitt-Hopkins syndrome (PTHS). Here, we show that similarly to mammalian TCF4, fruit fly orthologue Daughterless (Da) is expressed widely in the brain. Furthermore, silencing of , using several central nervous system-specific Gal4 driver lines, impairs appetitive associative learning of the larvae and leads to decreased levels of the synaptic proteins Synapsin (Syn) and Discs large 1 (Dlg1), suggesting the involvement of Da in memory formation. Here, we demonstrate that and are direct target genes of Da in adult heads, as Da binds to the regulatory regions of these genes and the modulation of Da levels alter the levels of and mRNA. Silencing of also affects negative geotaxis of the adult flies, suggesting the impairment of locomotor function. Overall, our findings suggest that Da regulates larval memory and adult negative geotaxis, possibly via its synaptic target genes and These behavioural phenotypes can be further used as a PTHS model to screen for therapeutics.This article has an associated First Person interview with the first author of the paper.

Dis Model Mech, 2020; 13
31915257: Esvald EE, Tuvikene J, Sirp A, Patil S, Bramham CR, Timmusk T
CREB Family Transcription Factors Are Major Mediators of BDNF Transcriptional Autoregulation in Cortical Neurons.
BDNF signaling via its transmembrane receptor TrkB has an important role in neuronal survival, differentiation, and synaptic plasticity. Remarkably, BDNF is capable of modulating its own expression levels in neurons, forming a transcriptional positive feedback loop. In the current study, we have investigated this phenomenon in primary cultures of rat cortical neurons using overexpression of dominant-negative forms of several transcription factors, including CREB, ATF2, C/EBP, USF, and NFAT. We show that CREB family transcription factors, together with the coactivator CBP/p300, but not the CRTC family, are the main regulators of rat gene expression after TrkB signaling. CREB family transcription factors are required for the early induction of all the major transcripts, whereas CREB itself directly binds only to promoter IV, is phosphorylated in response to BDNF-TrkB signaling, and activates transcription from promoter IV by recruiting CBP. Our complementary reporter assays with promoter constructs indicate that the regulation of by CREB family after BDNF-TrkB signaling is generally conserved between rat and human. However, we demonstrate that a nonconserved functional cAMP-responsive element in promoter IXa in humans renders the human promoter responsive to BDNF-TrkB-CREB signaling, whereas the rat ortholog is unresponsive. Finally, we show that extensive BDNF transcriptional autoregulation, encompassing all major transcripts, occurs also in the adult rat hippocampus during BDNF-induced LTP. Collectively, these results improve the understanding of the intricate mechanism of BDNF transcriptional autoregulation. Deeper understanding of stimulus-specific regulation of gene expression is essential to precisely adjust BDNF levels that are dysregulated in various neurological disorders. Here, we have elucidated the molecular mechanisms behind TrkB signaling-dependent mRNA induction and show that CREB family transcription factors are the main regulators of gene expression after TrkB signaling. Our results suggest that BDNF-TrkB signaling may induce gene expression in a distinct manner compared with neuronal activity. Moreover, our data suggest the existence of a stimulus-specific distal enhancer modulating gene expression.
J Neurosci, 2020; 40
28983964: Koppel I, Jaanson K, Klasche A, Tuvikene J, Tiirik T, Pärn A, Timmusk T
Dopamine cross-reacts with adrenoreceptors in cortical astrocytes to induce BDNF expression, CREB signaling and morphological transformation.
Expression of brain-derived neurotrophic factor (BDNF) is induced in cultured rat cortical astrocytes by catecholamines norepinephrine and dopamine as well as selective α1 and β adrenergic agonists. However, it has remained unknown which receptors mediate dopamine-induced BDNF upregulation in astrocytes. Here, we demonstrate that β adrenoreceptors are the main mediators of this effect in cultured cortical astrocytes, while α1 adrenoreceptors and D1 dopamine receptors contribute to a lesser extent. We show that in cortical astrocytes BDNF exon IV and exon VI containing mRNAs are induced by dopamine and norepinephrine via CREB-dependent signaling and that this regulation is mediated by a mechanism that is distinct from activity-dependent CREB-mediated activation of BDNF transcription in neurons. We also show that regulation of BDNF promoters IV and VI by catecholamines requires a distal regulatory element in the BDNF locus. Finally, we demonstrate that dopamine-induced astrocyte stellation and induction of CREB signaling are mediated by cross-reaction of dopamine with β adrenoreceptors.
Glia, 2018; 66
26818516: Tuvikene J, Pruunsild P, Orav E, Esvald EE, Timmusk T
AP-1 Transcription Factors Mediate BDNF-Positive Feedback Loop in Cortical Neurons.
Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, regulates both survival and differentiation of several neuronal populations in the nervous system during development, as well as synaptic plasticity in the adult brain. BDNF exerts its biological functions through its receptor TrkB. Although the regulation of BDNF transcription by neuronal activity has been widely studied, little is known about TrkB signaling-dependent expression of BDNF. Using rat primary cortical neuron cultures, we show that the BDNF gene is a subject to an extensive autoregulatory loop, where TrkB signaling upregulates the expression of all major BDNF transcripts, mainly through activating MAPK pathways. Investigating the mechanisms behind this autoregulation, we found that AP-1 transcription factors, comprising Jun and Fos family members, participate in the induction of BDNF exon I, III, and VI transcripts. AP-1 transcription factors directly upregulate the expression of exon I transcripts by binding two novel AP-1 cis-elements in promoter I. Moreover, our results show that the effect of AP-1 proteins on the activity of rat BDNF promoters III and VI is indirect, because AP-1 proteins were not detected to bind the respective promoter regions by chromatin immunoprecipitation (ChIP). Collectively, we describe an extensive positive feedback system in BDNF regulation, adding a new layer to the elaborate control of BDNF gene expression.
J Neurosci, 2016; 36
25868795: Koppel I, Tuvikene J, Lekk I, Timmusk T
Efficient use of a translation start codon in BDNF exon I.
The brain-derived neurotrophic factor (BDNF) gene contains a number of 5' exons alternatively spliced with a common 3'

exon. BDNF protein is synthesized from alternative transcripts as a prepro-precursor encoded by the common 3' exon IX, which has a translation start site 21 bp downstream of the splicing site. BDNF mRNAs containing exon I are an exception to this arrangement as the last three nucleotides of this exon constitute an in-frame AUG. Here, we show that this AUG is efficiently used for translation initiation in PC12 cells and cultured cortical neurons. Use of exon I-specific AUG produces higher levels of BDNF protein than use of the common translation start site, resulting from a higher translation rate. No differences in protein degradation, constitutive or regulated secretion were detected between BDNF isoforms with alternative 5' termini. As the BDNF promoter preceding exon I is known to be highly regulated by neuronal activity, our results suggest that the function of this translation start site may be efficient stimulus-dependent synthesis of BDNF protein. The brain-derived neurotrophic factor (BDNF) gene contains multiple untranslated 5' exons alternatively spliced to one common protein-coding 3' exon. However, exon I contains an in-frame ATG in a favorable translation context. Here, we show that use of this ATG is associated with more efficient protein synthesis than the commonly used ATG in exon IX.
J Neurochem, 2015; 134

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BOARD NUMBER: S02-658

ROLE OF DEVELOPMENTAL REGULATORS OF AXONAL LOCAL TRANSLATION IN ADULT AXONS

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Local mRNA translation (LT) is vital for axon development. Disruption of LT has been linked to the emergence of neurodevelopmental disorders, while several studies suggest that LT is related to the intrinsic regenerative capacity of mature neurons. Axonal regeneration displays similarities with axon growth during development, exhibiting a number of cellular processes that require LT. Although mature PNS axons maintain the ability for LT, maturing CNS axons exhibit a gradually decreasing LT potential, highly correlated with their limited regenerative capacity. Despite the evident role of LT in axon stability and regeneration, the regulatory mechanisms behind the process and their role in post-injury regeneration remain elusive. We have previously identified a ribonucleoprotein complex (Mena-RNP) that regulates axonal LT in the developing brain. It consists of the cytoskeleton-associated protein Mena that interacts with known regulators of translation (i.e. HnrnpK, PCBP1), thus controlling the translation of the RNP-bound mRNAs. Here, we investigate the conservation of Mena-RNP in the adult nervous system, in an attempt to elucidate the role of Mena and Mena-dependent translation in adult axon regeneration. Employing both *in vivo* and *ex vivo* injury assays we observe that the Mena-RNP components differ between the developing and adult nervous system and in CNS vs PNS axons. In an additional, unbiased line of work we are currently exploring other molecular components that function locally in axons and could potentially explain the differential capacity of adult CNS and PNS axons for regeneration.

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ROLE OF DEVELOPMENTAL REGULATORS OF AXONAL LOCAL TRANSLATION IN ADULT AXONS

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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CNS CAPILLARY PERICYTES EXPRESS MRNA OF CARDINAL CONTRACTILE PROTEINS, α-SMA AND MYH11: AN IN-SITU HYBRIDIZATION STUDY

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Aims: Pericytes are contractile cells located on the walls of microvessels. Although physiological and pathological impacts of pericyte contractility on microcirculation have recently been disclosed, the molecular machinery that imparts contractility to pericytes needs further elucidation because of the inconsistencies between protein and single cell transcriptomic data regarding the presence of the contractile protein alpha-smooth muscle actin (α-SMA) in pericytes. **Methods:** In this study, expression of the Acta2 and Myh11 genes encoding α-SMA and myosin heavy chain-11, was investigated in pericytes along the microvascular tree in intact retinal tissue by RNAscope in situ hybridization. **Results:** We found that both genes were intensely expressed in peri-nuclear cytoplasm and processes of pericytes on upstream capillaries (<4th order). Although both transcripts considerably decreased in downstream branches (≥4th order), they were unambiguously detectable and overlapping in about 2/3 of pericytes and mostly localized in the peri-nuclear cytoplasm, in parallel with our immunohistochemical findings. **Discussion:** These data support the view that contraction and relaxation of the upstream pericytes is mediated by actomyosin bridging as in vascular smooth muscle cells (vSMCs) for distributing blood flow between sister branches, whereas pericytes located on the downstream branches express low amounts of contractile proteins sufficient for regulating flow by changing capillary resistance with subtle contractions and relaxations. Thus, our study provides an *in situ* answer to the decades-long debate about α-SMA expression in pericytes, providing supporting evidence for the view that microvascular pericytes express contractile protein sets and employ the same contractile mechanisms as in vSMCs.

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CONTRIBUTION OF CELL TYPE-SPECIFIC ALTERNATIVE SPLICING PROGRAMS IN SPECIFICATION OF NEURONAL PROPERTIES

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Brain function relies on complex assemblies of multiple neuron types, each characterized by distinctive anatomical, physiological and molecular features. To a large extent, these features are instructed by genetic programs, including alternative splicing. Recent evidence suggests that the selective expression of splicing regulators in certain neuron classes may contribute to their differentiation. However, whether and how these cell type-specific splicing factors specify molecular programs is largely unknown. In a survey of ribosome-associated mRNAs from genetically-defined neuron populations of the mouse brain we identified the RNA-binding protein Rbm20 to be selectively expressed in glutamatergic mitral and tufted cells of the olfactory bulb. Thus far, Rbm20 expression had been thought to be heart and skeletal muscle specific, where RBM20 drives the alternative splicing of Titin and calcium signaling components (CACNA1C, CAMK2D).
We hypothesized that RBM20 contributes to the regulation of intrinsic properties and plasticity of neurons, driving alternative splicing programs encoding calcium signaling proteins. To uncover Rbm20 dependent splicing regulation we performed affinity isolation of ribosome-associated mRNAs from conditional Rbm20 knock-out neurons of the olfactory bulb. Moreover, using Crosslinking-Immuno-Precipitation and sequencing experiments (CLIP-seq) we identified endogenous mRNAs bound by neuronal RBM20. Our analysis identifies brain-specific targets and highlights the unexpected function of RBM20 in neuronal cell type-specific transcript isoform regulation in vivo.

Pubmed:
29581545: Weinhard L, di Bartolomei G, Bolasco G, Machado P, Schieber NL, Neniskyte U, Exiga M, Vadisiute A, Raggioli A, Schertel A, Schwab Y, Gross CT
Microglia remodel synapses by presynaptic trogocytosis and spine head filopodia induction.
Microglia are highly motile glial cells that are proposed to mediate synaptic pruning during neuronal circuit formation. Disruption of signaling between microglia and neurons leads to an excess of immature synaptic connections, thought to be the result of impaired phagocytosis of synapses by microglia. However, until now the direct phagocytosis of synapses by microglia has not been reported and fundamental questions remain about the precise synaptic structures and phagocytic mechanisms involved. Here we used light sheet fluorescence microscopy to follow microglia-synapse interactions in developing organotypic hippocampal cultures, complemented by a 3D ultrastructural characterization using correlative light and electron microscopy (CLEM). Our findings define a set of dynamic microglia-synapse interactions, including the selective partial phagocytosis, or trogocytosis (trogo-: nibble), of presynaptic structures and the induction of postsynaptic spine head filopodia by microglia. These findings allow us to propose a mechanism for the facilitatory role of microglia in synaptic circuit remodeling and maturation.
Nat Commun, 2018; 9
29239126: Weinhard L, Neniskyte U, Vadisiute A, di Bartolomei G, Aygün N, Riviere L, Zonfrillo F, Dymecki S, Gross C
Sexual dimorphism of microglia and synapses during mouse postnatal development.
Microglia participate in synapse remodeling in the cortex and hippocampus during mouse postnatal development. Although sex differences in microglia activity during embryonic development have been reported in these regions, it remains unexplored whether microglia show sexually dimorphic features during the early postnatal period, a critical window for synapse formation and maturation. Here, we investigated morphological and functional features of microglia across early postnatal development as well as morphological features of both pre- and postsynaptic neuronal compartments in the mouse hippocampus. We found a sex-dependent shift in microglia volume and phagocytic capacity across the first four postnatal weeks. Measurements of synaptic features revealed sex differences in the density of synaptic spines and boutons during the second postnatal week. These data are consistent with a precocious development of both microglia and synapses in the female brain. We further hypothesize that this bias may contribute to sex-specific brain wiring. © 2017 The Authors.

Developmental Neurobiology Published by Wiley Periodicals, Inc. Develop Neurobiol 78: 618-626, 2018.
Dev Neurobiol, 2018; 78

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BOARD NUMBER: S02-662

JAK2-STAT3-DEPENDENT MOLECULAR SIGNATURE IN REACTIVE ASTROCYTES OF THE MOUSE STRIATUM

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Astrocytes undergo morphological and functional changes in disease that remain to be fully characterized. JAK2-STAT3 signalling pathway is a central regulator of astrocyte reactive response in neurodegenerative disorders. These include Huntington's disease (HD), which mainly affects the striatum. Therefore, the objective is to establish the molecular changes induced by the JAK2-STAT3 pathway in mouse striatal astrocytes. To activate the STAT3-JAK2 pathway, astrocyte-targeting adeno-associated vectors encoding a constitutively active form of JAK2 (AAV-JAK2ca) were generated. The striatum of WT mice was injected with AAV-JAK2ca and AAV-GFP to label infected GFP+ astrocytes. Control mice were injected with AAV-GFP only. Histological analysis demonstrated increased soma surface and branching index in striatal astrocytes together with GFAP and Vimentin overexpression, following the activation of the JAK2-STAT3 cascade. Microarray analysis of sorted infected astrocytes revealed extensive transcriptional expression changes with 689 genes differentially expressed by JAK2ca, including genes linked to inflammation, cytokine signalling, proteostasis and energy metabolism. Interestingly, many of these functions were also confirmed by RNA sequencing analysis of astrocytes isolated from the striatum of a HD murine model when activating JAK2-STAT3 pathway (Abjean, in press, Brain). Last, by label-free mass spectrometry, 87 proteins were found differentially expressed between JAK2ca- and GFP-astrocytes. They were involved in cell adhesion, cytoskeleton and oxidative phosphorylation, revealing only limited overlap between mRNA and protein changes. Our multi-omics analysis of the JAK2-STAT3 signature identifies coordinated morphological and molecular changes in striatal reactive astrocytes, which helps defining a specific reactive state that could be observed in different brain diseases.

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BOARD NUMBER: S02-663

EXPLORATION OF A NOVEL MECHANISM FOR RAPID GENE REGULATION IN NEURONAL PLASTICITY AND LEARNING

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Neuronal development and plasticity rely on the dynamic modification of molecular repertoires through a variety of mechanisms. Activity-dependent transcription has emerged as a major source of gene products that regulate neuronal excitability, connectivity and synaptic properties. However, the elongation rate of RNA polymerases imposes a significant temporal constraint for transcript synthesis, in particular for long genes where new synthesis requires tens of hours. In our work, we uncovered a novel, transcription-independent mechanism that releases transcripts within minutes of neuronal stimulation. It relies on nuclear storage of highly stable transcripts retaining select introns. Within few minutes after neuronal stimulation, these transcripts undergo splicing completion, they are then exported to the cytosol and loaded onto the ribosome - thereby representing a pool of mRNAs readily available for synthesis of plasticity regulators. Remarkably, we found that distinct groups of intron-retaining transcripts are regulated in response to different forms of stimulation and this selectivity arises from the activation of specific intracellular signaling pathways. Finally, we demonstrate that sensory stimulation in the mouse visual system triggers regulated intron excision in vivo.

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THE HETEROGENEITY OF ASTROCYTES IN STROKE: SPATIALLY RESOLVED GENE EXPRESSION REVEALS THE DYNAMICS OF ASTROCYTES OVER TIME AND THEIR INTERACTIONS WITH NEIGHBORING CELLS

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Erica Scott[1], Nickie Safarian[2], Lozano Casasbuenas[3], Teodora Tockovska[4], Shreejoy Tripathy[2], Aaron Wheeler[5], Scott Yuzwa[4], Maryam Faiz[3]
[1]University of Toronto, Surgery, Biomedical Engineering, Toronto, Canada, [2]University of Toronto, Krembil Centre For Neuroinformatics, Toronto, Canada, [3]University of Toronto, Surgery, Toronto, Canada, [4]University of Toronto, Laboratory Medicine & Pathobiology, Toronto, Canada, [5]University of Toronto, Terrence Donnelly Centre For Cellular & Biomolecular Research, Toronto, Canada

Stroke recovery is a dynamic process that evolves over time. Here, we investigated the processes that mediate recovery from cortical stroke at different time points post-injury in a mouse model of endothelin-1 stroke model. We utilized three sequencing strategies: Visium Spatial Transcriptomics, 10X Chromium and a new platform DISCO (Digital microfluidic Single Cell -Omic) to gain temporally and spatially defined RNA sequencing datasets. Visium was performed on acute (d2), subacute (d10), and chronic (d21) post-stroke brains. With this data we saw an astrocyte-dominant signature at day 10 post-stroke. To investigate potential roles of astrocytes during the subacute phase we performed single cell RNA-sequencing using 10X Chromium on GLAST+ cells isolated from the d14 stroke-injured cortex. Finally, with DISCO, we were able to selectively isolate individual astrocytes within and outside the penumbra to identify, validate and discover cell markers and roles of astrocytes governing repair at the injury site. Our datasets compare and highlight the strength of each sequencing platform and the compatibility of each approach. Altogether, a unique combination of sequencing approaches led to a novel, highly resolved, chronicle of the molecular signatures underlying cortical ischemic stroke.

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FEATURES OF LNCRNA EXPRESSIONS AND ITS REGULATION IN ACROSS BRAIN REGIONS OF PRIMATES

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Mohit Navandar[1], Susanne Gerber[2]
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Human and non-human primates like chimpanzee have strikingly similar genomes, but differs phenotypically, behaviourally and cognitive wise. It is fascinating to understand how brain was evolved. With the advancement of next generation sequencing techniques, function of protein coding genes underlying evolution is widely studied. Looking at the complexity of the eukaryotic genome, little is known about the non-coding parts such as long non-coding RNAs (lncRNAs). In this study, we analysed transcriptional expression pattern of lncRNAs across different brain regions from human and non-human primates (chimpanzee, gorilla and gibbon). Our analysis identified shared/orthologous and species specific lncRNAs based on orthology studies. Orthologous lncRNAs differentially expressed in human and non-human primate showed alike expression pattern (clusters) across brain regions with potential regulation involved in cognition, neurogenesis and central nervous system development. Our downstream analysis also predicted the potential transcription factors driven regulation of lncRNAs. We further characterized the number of co-expression pairs (lncRNAs and protein coding genes), which are different in human and non-human primates. Network analysis of each cluster pair shades light on the potential gene regulation by novel predicted orthologous lncRNAs. Overall, our study provides the insight of gene regulatory landscape, evolutionary conservation, and a resource for candidate lncRNAs in human and non-human primate's brain.

Pubmed:
32034128: Domingo-Rodriguez L, Ruiz de Azua I, Dominguez E, Senabre E, Serra I, Kummer S, Navandar M, Baddenhausen S, Hofmann C, Andero R, Gerber S, Navarrete M, Dierssen M, Lutz B, Martín-García E, Maldonado R
A specific prelimbic-nucleus accumbens pathway controls resilience versus vulnerability to food addiction.
Food addiction is linked to obesity and eating disorders and is characterized by a loss of behavioral control and compulsive food intake. Here, using a food addiction mouse model, we report that the lack of cannabinoid type-1 receptor in dorsal telencephalic glutamatergic neurons prevents the development of food addiction-like behavior, which is associated with enhanced synaptic excitatory transmission in the medial prefrontal cortex (mPFC) and in the nucleus accumbens (NAc). In contrast, chemogenetic inhibition of neuronal activity in the mPFC-NAc pathway induces compulsive food seeking. Transcriptomic analysis and genetic manipulation identified that increased dopamine D2 receptor expression in the mPFC-NAc pathway promotes the addiction-like phenotype. Our study unravels a new neurobiological mechanism underlying resilience and vulnerability to the development of food addiction, which could pave the way towards novel and efficient interventions for this disorder.
Nat Commun, 2020; 11
33907201: Navandar M, Martín-García E, Maldonado R, Lutz B, Gerber S, Ruiz de Azua I
Transcriptional signatures in prefrontal cortex confer vulnerability versus resilience to food and cocaine addiction-like behavior.
Addiction is a chronic relapsing brain disease characterized by compulsive reward-seeking despite harmful consequences. The mechanisms underlying addiction are orchestrated by transcriptional reprogramming in the reward system of vulnerable subjects. This study aims at revealing gene expression alterations across different types of addiction. We analyzed publicly available transcriptome datasets of the prefrontal cortex (PFC) from a palatable food and a cocaine addiction study. We found 56 common genes upregulated in the PFC of addicted mice in these two studies, whereas most of the differentially expressed genes were exclusively linked to either palatable food or cocaine addiction. Gene ontology analysis of shared genes revealed that these genes contribute to learning and memory, dopaminergic synaptic transmission, and histone phosphorylation. Network analysis of shared genes revealed a protein-protein interaction node among the G protein-coupled receptors (Drd2, Drd1, Adora2a, Gpr6, Gpr88) and downstream targets of the cAMP signaling pathway (Ppp1rb1, Rgs9, Pde10a) as a core network in addiction. Upon extending the analysis to a cell-type specific level, some of these common molecular players were selectively expressed in excitatory neurons, oligodendrocytes, and endothelial cells. Overall,

computational analysis of publicly available whole transcriptome datasets provides new insights into the molecular basis of addiction-like behaviors in PFC.
Sci Rep, 2021; 11
32064328: Gerber S, Pospisil L, Navandar M, Horenko I
Low-cost scalable discretization, prediction, and feature selection for complex systems.
Finding reliable discrete approximations of complex systems is a key prerequisite when applying many of the most popular modeling tools. Common discretization approaches (e.g., the very popular -means clustering) are crucially limited in terms of quality, parallelizability, and cost. We introduce a low-cost improved quality scalable probabilistic approximation (SPA) algorithm, allowing for simultaneous data-driven optimal discretization, feature selection, and prediction. We prove its optimality, parallel efficiency, and a linear scalability of iteration cost. Cross-validated applications of SPA to a range of large realistic data classification and prediction problems reveal marked cost and performance improvements. For example, SPA allows the data-driven next-day predictions of resimulated surface temperatures for Europe with the mean prediction error of 0.75°C on a common PC (being around 40% better in terms of errors and five to six orders of magnitude cheaper than with common computational instruments used by the weather services).
Sci Adv, 2020; 6
28418928: Navandar M, Garding A, Sahu SK, Pataskar A, Schick S, Tiwari VK
ERK signalling modulates epigenome to drive epithelial to mesenchymal transition.
The series of events that allow the conversion from adherent epithelial cells into migratory cells is collectively known as epithelial-mesenchymal transition (EMT). EMT is employed during embryonic development such as for gastrulation and neural crest migration and is misused in diseases, such as cancer metastasis. ERK signalling is known to be essential for EMT, however its influence on the epigenetic and transcriptional programme underlying EMT is poorly understood. Here, using a comprehensive genome-wide analysis of H3K27ac mark and gene expression in mammary epithelial cells undergoing EMT, we found that ERK signalling is essential for the epigenetic reprogramming underlying hallmark gene expression and phenotypic changes of EMT. We show that the chemical inhibition of Erk signalling during EMT prevents the loss and gain of the H3K27ac mark at regulatory regions of epithelial and mesenchymal genes, respectively, and results in a transcriptome and epigenome closer to those of epithelial cells. Further computational analyses identified a distinct set of transcription factor motifs enriched at distal regulatory regions that are epigenetically remodelled by ERK signalling. Altogether, our findings reveal an ERK-dependent epigenetic remodelling of regulatory elements that results in a gene expression programme essential for driving EMT.
Oncotarget, 2017; 8

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BOARD NUMBER: S02-666

GLIAL-SECRETED FACTORS REGULATE NEURONAL GENE EXPRESSION AND FUNCTION

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Glial cells secrete a wide variety of proteins and metabolites to the extracellular space (Dowell et al., 2009). We and others have shown the importance of these factors for neuron survival, morphological development, and excitatory synapse formation (Banker et al., 1980; Turko et al., 2018). However, our knowledge of precisely how these factors mediate their effects is incomplete. Our **aims** therefore are to further investigate the effects of glial-secreted factors on neuron physiology and to identify the roles of specific proteins on neuron function. We have employed a combination of **methods**, including cell sorting, RNA sequencing and mass spectrometry to explore the regulatory effects of glial-secreted factors on neuronal gene expression, and to identify secreted proteins interacting with distinct neuron types (Eichelbaum et al., 2012).
Our **results** demonstrate that glial-secreted factors exert a profound effect on neuronal gene expression, with hundreds of genes being significantly up or down regulated by the presence of glial-secreted factors. Interestingly, some gene expression changes are neuron-type specific, with glial cells regulating, for example, genes associated with synapse assembly in glutamatergic but not GABAergic neurons. Finally, we have identified several glial-secreted protein candidates which may physically interact with neurons to promote cell growth and survival. In **conclusion**, our results suggest that secreted factors are a central means by which glial cells can dynamically communicate with neurons to regulate their gene expression and function.

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CAN THE BRAIN BE TAKEN AS A WHOLE WHEN CHOOSING HOUSEKEEPING GENES FOR STUDDING SEX DIFFERENCES?

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Florencia Dadam[1,2], Laura Vivas[1,3], Ximena Caeiro[1]
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Expression of sexual dimorphic genes at different brain areas are been extensively studied. But can the brain be taken as a whole when choosing housekeeping genes (HKG) for studying sex differences? Are there sex differences in HKG expression? Are these due to sex chromosome complement or hormonal factors? To analyze these questions, we used mice of the "four core genotypes" mouse model, in which the effect of gonadal sex (testes/ovaries) and SCC(XX/XY) are uncoupled. Brains were collected from gonadectomized mice and relative gene expression of basal *Gapdh, Cyc and 18s* genes at fore/hindbrain nuclei were assessed using q-PCR. Sex-dependent expressions of commonly used housekeeping genes at the SFO and AP brain levels were observed. The statistical analysis at the SFO of *Gapdh* and *Cyc* genes showed a female phenotype ($F(1,20)=6,05$; $p=0,02$/$F(1,12)=6,12$; $p=0,03$). Furthermore, a SCC effect on *Cyc* gene expression in the SFO as well as for *18s* at the AP were observed ($F(1,12)=5,75$; $p=0,03$/$F(1,12)=5,52$; $p=0,04$). At the NTS, OVLT and PVN no differences in gene expression were shown. Moreover, the analysis of the M-value (HKGs stability test- geNorm/Normfinder) for each HKG demonstrated not only the importance of taking into account sex as a factor (since the stability values were always much higher when males and females worked together) but also studying each area independently (obtaining in this way lower M-values than working all the brain tissue together). Our findings underscore the importance of empirical determination of reference genes to effectively and accurately normalize q-PCR data before quantifying target genes.

Pubmed:

28254489: Dadam FM, Cisternas CD, Macchione AF, Godino A, Antunes-Rodrigues J, Cambiasso MJ, Vivas LM, Caeiro XE
Sex chromosome complement involvement in angiotensin receptor sexual dimorphism.
This study aimed to define whether sex chromosome complement (SCC) may differentially modulate sex differences in relative gene expression of basal Agtr1a, Agtr2, and Mas1 receptors at fore/hindbrain nuclei and at medulla/cortical kidney. Samples were collected from gonadectomized male (XX and XY) and female (XX and XY) mice of the "four core genotypes" model. At brain level, a SCC effect at the area postrema was demonstrated. An increase in mRNA level of Agtr1a and Agtr1a/Agtr2 ratio in XY-SCC mice was associated with a decrease in Mas1 compared to XX-SCC mice. In the renal cortex, a SCC effect for Agtr2 and Mas1 was observed. Regardless of sex (male or female), XX-SCC mice expressed higher levels of mRNA Agtr2 and Mas1 than XY-SCC mice {$F(1,12) = 6,126, p < 0.05$; $F(1,21) = 5,143, p < 0.05$}. Furthermore, XX-female mice showed a significant increase in Mas1 expression compared to XY-female mice. These results reveal a SCC modulatory effect at central and kidney level on angiotensin receptor expression, with an enhancement of the vasodilatory arm in XX-mice and an increase in the vasoconstriction arm in XY-mice, which may underlie sex differences in the regulation of arterial pressure.
Mol Cell Endocrinol, 2017; 447
27856342: Marchese NA, Paz MC, Caeiro X, Dadam FM, Baiardi G, Perez MF, Bregonzio C
Angiotensin II AT receptors mediate neuronal sensitization and sustained blood pressure response induced by a single injection of amphetamine.
A single exposure to amphetamine induces neurochemical sensitization in striatal areas. The neuropeptide angiotensin II, through AT receptors (AT-R) activation, is involved in these responses. However, amphetamine-induced alterations can be extended to extra-striatal areas involved in blood pressure control and their physiological outcomes. Our aim for the present study was to analyze the possible role for AT-R in these events using a two-injection protocol and to further characterize the proposed AT-R antagonism protocol. Central effect of orally administered AT-R blocker (Candesartan, 3mg/kg p.o.×5days) in male Wistar rats was analyzed by spontaneous activity of neurons within locus coeruleus. In another group of animals

pretreated with the AT-R blocker or vehicle, sensitization was achieved by a single administration of amphetamine (5mg/kg i.p. - day 6) followed by a 3-week period off drug. On day 27, after receiving an amphetamine challenge (0.5mg/kg i.p.), we evaluated: (1) the sensitized c-Fos expression in locus coeruleus (LC), nucleus of the solitary tract (NTS), caudal ventrolateral medulla (A1) and central amygdala (CeAmy); and (2) the blood pressure response. AT-R blockade decreased LC neurons' spontaneous firing rate. Moreover, sensitized c-Fos immunoreactivity in TH+neurons was found in LC and NTS; and both responses were blunted by the AT-R blocker pretreatment. Meanwhile, no differences were found neither in CeAmy nor A1. Sensitized blood pressure response was observed as sustained changes in mean arterial pressure and was effectively prevented by AT-R blockade. Our results extend AT-R role in amphetamine-induced sensitization over noradrenergic nuclei and their cardiovascular output.
Neuroscience, 2017; 340
24259464: Dadam FM, Caeiro XE, Cisternas CD, Macchione AF, Cambiasso MJ, Vivas L
Effect of sex chromosome complement on sodium appetite and Fos-immunoreactivity induced by sodium depletion.
Previous studies indicate a sex chromosome complement (SCC) effect on the angiotensin II-sexually dimorphic hypertensive and bradycardic baroreflex responses. We sought to evaluate whether SCC may differentially modulate sexually dimorphic-induced sodium appetite and specific brain activity due to physiological stimulation of the rennin angiotensin system. For this purpose, we used the "four core genotype" mouse model, in which the effect of gonadal sex and SCC is dissociated, allowing comparisons of sexually dimorphic traits between XX and XY females as well as in XX and XY males. Gonadectomized mice were sodium depleted by furosemide (50 mg/kg) and low-sodium diet treatment; control groups were administered with vehicle and maintained on normal sodium diet. Twenty-one hours later, the mice were divided into two groups: one group was submitted to the water-2% NaCl choice intake test, while the other group was perfused and their brains subjected to the Fos-immunoreactivity (FOS-ir) procedure. Sodium depletion, regardless of SCC (XX or XY), induced a significantly lower sodium and water intake in females than in males, confirming the existence in mice of sexual dimorphism in sodium appetite and the organizational involvement of gonadal steroids. Moreover, our results demonstrate a SCC effect on induced brain FOS-ir, showing increased brain activity in XX-SCC mice at the paraventricular nucleus, nucleus of the solitary tract, and lateral parabrachial nucleus, as well as an XX-SCC augmented effect on sodium depletion-induced brain activity at two circumventricular organs, the subfornical organ and area postrema, nuclei closely involved in fluid and blood pressure homeostasis.
Am J Physiol Regul Integr Comp Physiol, 2014; 306
29486102: Dadam F, Zádor F, Caeiro X, Szűcs E, Erdei AI, Samavati R, Gáspár R, Borsodi A, Vivas L
The effect of increased NaCl intake on rat brain endogenous μ-opioid receptor signalling.
Numerous studies demonstrate the significant role of central β-endorphin and its receptor, the μ-opioid receptor (MOR), in sodium intake regulation. The present study aimed to investigate the possible relationship between chronic high-NaCl intake and brain endogenous MOR functioning. We examined whether short-term (4 days) obligatory salt intake (2% NaCl solution) in rats induces changes in MOR mRNA expression, G-protein activity and MOR binding capacity in brain regions involved in salt intake regulation. Plasma osmolality and electrolyte concentrations after sodium overload and the initial and final body weight of the animals were also examined. After 4 days of obligatory hypertonic sodium chloride intake, there was clearly no difference in MOR mRNA expression and G-protein activity in the median preoptic nucleus (MnPO). In the brainstem, MOR binding capacity also remained unaltered, although the maximal efficacy of MOR G-protein significantly increased. Finally, no significant alterations were observed in plasma osmolality and electrolyte concentrations. Interestingly, animals that received sodium gained significantly less weight than control animals. In conclusion, we found no significant alterations in the MnPO and brainstem in the number of available cell surface MORs or de novo syntheses of MOR after hypertonic sodium intake. The increased MOR G-protein activity following acute sodium overconsumption may participate in the maintenance of normal blood pressure levels and/or in enhancing sodium taste aversion and sodium overload-induced anorexia.
J Neuroendocrinol, 2018; 30
26260434: Vivas L, Dadam FM, Caeiro XE
Sex differences in body fluid homeostasis: Sex chromosome complement influences on bradycardic baroreflex response and sodium depletion induced neural activity.
Clinical and basic findings indicate that angiotensin II (ANG II) differentially modulates hydroelectrolyte and cardiovascular responses in male and female. But are only the activational and organizational hormonal effects to blame for such differences? Males and females not only differ in their sex (males are born with testes and females with ovaries) but also carry different sex chromosome complements and are thus influenced throughout life by different genomes. In this review, we discuss our recent studies in order to evaluate whether sex chromosome complement is in part responsible for gender differences previously observed in ANG II bradycardic-baroreflex response and sodium depletion-induced sodium appetite and neural activity. To test the hypothesis that XX or XY contributes to the dimorphic ANG II bradycardic-baroreflex response, we used the four core genotype mouse model, in which the effects of gonadal sex (testes or ovaries) and sex chromosome

complement (XX or XY) are dissociated. The results indicate that ANG II bradycardic-baroreflex sexual dimorphic response may be ascribed to differences in sex chromosomes, indicating an XX-sex chromosome complement facilitatory bradycardic-baroreflex control of heart rate. Furthermore, we evaluated whether genetic differences within the sex chromosome complement may differentially modulate the known sexually dimorphic sodium appetite as well as basal or induced brain activity due to physiological stimulation of the renin-angiotensin system by furosemide and low-sodium treatment. Our studies demonstrate an organizational hormonal effect on sexually dimorphic induced sodium intake in mice, while at the brain level (subfornical organ and area postrema) we showed a sex chromosome complement effect in sodium-depleted mice, suggesting a sex chromosome gene participation in the modulation of neural pathways underlying regulatory response to renin-angiotensin stimulation.
Physiol Behav, 2015; 152
25872186: Macchione AF, Beas C, Dadam FM, Caeiro XE, Godino A, Ponce LF, Amigone JL, Vivas L
Early free access to hypertonic NaCl solution induces a long-term effect on drinking, brain cell activity and gene expression of adult rat offspring.
Exposure to an altered osmotic environment during a pre/postnatal period can differentially program the fluid intake and excretion pattern profile in a way that persists until adulthood. However, knowledge about the programming effects on the underlying brain neurochemical circuits of thirst and hydroelectrolyte balance, and its relation with behavioral outputs, is limited. We evaluated whether early voluntary intake of hypertonic NaCl solution may program adult offspring fluid balance, plasma vasopressin, neural activity, and brain vasopressin and angiotensinergic receptor type 1a (AT1a)-receptor gene expression. The manipulation (M) period covered dams from 1 week before conception until offspring turned 1-month-old. The experimental groups were (i) Free access to hypertonic NaCl solution (0.45 M NaCl), food (0.18% NaCl) and water [M-Na]; and (ii) Free access to food and water only [M-Ctrol]. Male offspring (2-month-old) were subjected to iv infusion (0.15 ml/min) of hypertonic (1.5M NaCl), isotonic (0.15M NaCl) or sham infusion during 20 min. Cumulative water intake (140 min) and drinking latency to the first lick were recorded from the start of the infusion. Our results indicate that, after systemic sodium overload, the M-Na group had increased water intake, and diminished neuronal activity (Fos-immunoreactivity) in the subfornical organ (SFO) and nucleus of the solitary tract. They also showed reduced relative vasopressin (AVP)-mRNA and AT1a-mRNA expression at the supraoptic nucleus and SFO, respectively. The data indicate that the availability of a rich source of sodium during the pre/postnatal period induces a long-term effect on drinking, neural activity, and brain gene expression implicated in the control of hydroelectrolyte balance.
Neuroscience, 2015; 298
26231585: Cisternas CD, Tome K, Caeiro XE, Dadam FM, Garcia-Segura LM, Cambiasso MJ
Sex chromosome complement determines sex differences in aromatase expression and regulation in the stria terminalis and anterior amygdala of the developing mouse brain.
Aromatase, which converts testosterone in estradiol, is involved in the generation of brain sex dimorphisms. Here we used the "four core genotypes" mouse model, in which the effect of gonadal sex and sex chromosome complement is dissociated, to determine if sex chromosomes influence the expression of brain aromatase. The brain of 16 days old XY mouse embryos showed higher aromatase expression in the stria terminalis and the anterior amygdaloid area than the brain of XX embryos, independent of gonadal sex. Furthermore, estradiol or dihydrotestosterone increased aromatase expression in cultures of anterior amygdala neurons derived from XX embryos, but not in those derived from XY embryos. This effect was also independent of gonadal sex. The expression of other steroidogenic molecules, estrogen receptor-α and androgen receptor was not influenced by sex chromosomes. In conclusion, sex chromosomes determine sex dimorphisms in aromatase expression and regulation in the developing mouse brain.
Mol Cell Endocrinol, 2015; 414

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THE FUNCTIONAL RELATIONSHIP BETWEEN C-JUN AND NNOS IN VSC 4.1 CELLS

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Introduction Ventral Spinal Cord 4.1 (VSC 4.1) provides a good model to study motor neuron response to injury, especially the involvement of c-Jun and nNOS signaling pathways. This study was designed to explore the functional relationship between these signaling pathways. **Methods** Cell viability was tested using the CCK-8 kit. Hydrogen peroxide was used as a cell irritatant. c-Jun/JNK inhibitor SP600125 and nNOS-specific inhibitor 7-nitroindazole were used as neuroprotective agents when VSC 4.1 cells were exposed to hydrogen peroxide insult. The cellular responses to injury were evaluated by total nitric oxide assay, immunofluorescence and western blotting as well as coimmunoprecipitation. **Results** Increasing concentrations of hydrogen peroxide (0 to 1000μM) given over 24 hours resulted in a dose depended death of the motor neurons whereby concentrations above 600μM killed all the cells while above 80% survived dosages of 200μM or less. The cells pre-treated with neuroprotective treatments were spared death when incubated on hydrogen dosages of 50-200μM. c-Jun and nNOS as well as proapoptotic markers Bim and Caspase 3 were overexpressed in the VSC 4.1 cells after hydrogen peroxide insult but were all significantly reduced by neuroprotective treatments relative to vehicle control made from less than 1% DMSO in normal medium. Nitric oxide production was increased by injury and attenuated by neuroprotective treatments. We also observed coimmunoprecipitation of c-Jun and nNOS from injured VSC 4.1 cell lysates suggesting that an interaction between c-Jun and nNOS exists. **Conclusion** Taken together, these results indicate a functional relationship between c-Jun and nNOS warranting further research.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-669

CYCLASE-ASSOCIATED PROTEIN 1 (CAP1) REPRESSES MRTF-SRF-DEPENDENT GENE EXPRESSION IN THE MOUSE BRAIN

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

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Serum response factor (SRF) is a ubiquitously expressed transcription factor essential for brain development and function. SRF activity is controlled by two classes of coactivators, myocardin-related transcription factors (MRTF) and ternary complex factors (TCF), which compete for SRF binding and introduce specificity into gene expression programs. To date, only few brain studies investigated regulatory mechanisms upstream of these coactivators, which mainly focused on TCF. Since an inhibitory function of monomeric actin (G-actin) towards MRTF-SRF signaling is well established, we hypothesized a role for the key actin-depolymerizing protein cofilin in this pathway. Surprisingly, cofilin was largely dispensable for neuronal MRTF-SRF activity. Instead, reporter assays combined with pharmacological and genetic approaches in isolated neurons from mutant mice identified cyclase-associated protein 1 (CAP1) as an important regulator of the MRTF-SRF pathway. Mechanistically, CAP1 promotes cytosolic MRTF retention and represses neuronal SRF activity by regulating cellular G-actin levels. RNAseq and mass spectrometry analysis of mice brain cortices deficient in CAP1 showed dysregulation of MRTF/SRF-dependent gene regulation, thus implying *in vivo* relevance of CAP1 in this process. Together, we identified CAP1 as a crucial repressor of the MRTF-SRF pathway in the brain.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-670

SEX-BIASED RNA POPULATIONS ALONG THE HIPPOCAMPAL LONGITUDINAL AXIS AND THEIR PUTATIVE TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATORY NETWORKS

POSTER SESSION 02 - SECTION: GENE EXPRESSION & REGULATION IN NEURAL FUNCTION

Luciano Román Albasini, Wladimir Corrales, Juan Silva, Felipe Olave, Felipe Aguayo, Pablo González, Matías Alarcón Mardones, Julia Catalán Casanellas, Jenny Fiedler
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Numerous studies have unveiled sex differences across several aspects of health and disease. While multiple brain areas are known to display sex-biased transcriptomic signatures, the sex-biased transcriptomic profiles of the dorsal and ventral hippocampus –which have molecular and circuitry differences– has not been studied. By employing RNA sequencing, we report the sex biases in large- and mature micro-RNA populations of the dorsal and ventral hippocampus in adult rats. Furthermore, we aimed at discovering transcriptional and post-transcriptional regulatory interactions that may govern these sex differences in both hippocampal poles. In the dorsal hippocampus, 769 genes and 22 miRNAs had a female-biased expression, whereas 723 genes and 31 miRNAs displayed a male-biased expression. In the ventral hippocampus, 293 genes and 11 miRNAs showed a female-biased expression, while 264 genes and 22 miRNAs were male-biased. Overrepresentation analyses revealed that the female-biased genes in the dorsal hippocampus were functionally enriched in ubiquitin mediated proteolysis, while the male-biased genes were enriched in neuronal morphology modulation and actin dynamics. In the ventral hippocampus, the female-biased genes were overrepresented for inflammatory signaling pathways and the male-biased genes were enriched in glycine transport and actin dynamics. Next, we constructed regulatory networks based on predicted and validated transcriptional regulators and miRNA targets that were present in each sex-biased dataset, revealing discreet modules within the networks that were largely sex- and pole-specific Our results demonstrate a sex-biased transcriptomic profile in both hippocampal regions that may help understanding the sex differences in resilience/susceptibility to internal or external insults. Grant FONDECYT 119-0899

FENS Forum 2022
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BOARD NUMBER: S02-671

INVESTIGATING THE DISEASE-CAUSING MECHANISMS OF NRROS-ASSOCIATED MICROGLIOPATHY

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Previously, our group and others have identified a fatal, incurable neurodevelopmental disorder caused by loss-of-function mutations in the microglial-associated protein negative regulator of reactive oxygen species (NRROS), leading to an aberrant microglial phenotype. However, the mechanisms mediating the impact of these mutations haven't been ascertained. Moreover, the biological function of NRROS isn't fully understood, with others showing that it regulates activation of latent microglial homeostatic mediator transforming growth factor beta 1 (TGF-β1). However, aspects of this regulation, such as whether it enables microglial-intrinsic and/or extrinsic activation, remain unclear. Here we show that a NRROS-deficient mouse model displays white matter pathology and microglial phenotypic changes, including increased CD45 and CD163 and lowered CD11b and P2RY12 expression. We then examined *in vitro* NRROS-deficient mouse microglia responses to exogenous TGF-β1. Additionally, we investigated the functional effects of NRROS deletion on microglia by assessing phagocytic capacity and responses to inflammatory stimuli. We also performed scRNAseq on CNS macrophages from P7 and P42 NRROS-deficient mice to examine whether microglial populations are affected uniformly. Our results demonstrate extensive abnormalities in NRROS knockout microglia, including a loss of homeostatic signature possibly due to defective TGF-β1 signalling, and which are associated with white matter dysfunction that will be further investigated through spatial transcriptomics. We will also explore *in vitro*, whether NRROS/TGF-β1 signalling is microglial-intrinsic/extrinsic, and if microglial elimination/repopulation can rescue disease progression. Our work may advance understanding of fundamental microglial biology aspects, which may pertain to other sporadic conditions across the human lifespan associated with microglial dysfunction.

Pubmed:
34107350: Sewell MDE, Jiménez-Sánchez L, Shen X, Edmondson-Stait AJ, Green C, Adams MJ, Rifai OM, McIntosh AM, Lyall DM, Whalley HC, Lawrie SM
Associations between major psychiatric disorder polygenic risk scores and blood-based markers in UK biobank.
Major depressive disorder (MDD), schizophrenia (SCZ), and bipolar disorder (BD) have both shared and discrete genetic risk factors, and are associated with peripheral abnormalities. The relationships between such genetic architectures and blood-based markers are, however, unclear. We investigated relationships between polygenic risk scores (PRS) for these disorders and peripheral markers in the UK Biobank cohort. We calculated polygenic risk scores for n = 367,329 (MDD PRS), n = 366,465 (SCZ PRS), and n = 366,383 (BD PRS) UK Biobank cohort subjects. We then examined associations between disorder PRS and 58 inflammatory/immune, hematological, bone, cardiovascular, hormone, liver, renal and diabetes-associated blood markers using two generalized linear regression models: 'minimally adjusted' controlling for variables such as age and sex, and 'fully adjusted' including additional lifestyle covariates: BMI, alcohol and smoking status, and medication intake. There were 38/58 MDD PRS, 32/58 SCZ PRS, and 20/58 BD PRS-blood marker associations detected for our minimally adjusted model. Of these, 13/38 (MDD PRS), 14/32 (SCZ PRS), and 10/20 (BD PRS) associations remained significant after controlling for lifestyle factors. Many were disorder-specific, with 8/13 unique MDD PRS associations identified. Several disorder-specific associations for MDD and SCZ were immune-related, with mostly positive and negative associations identified for MDD and SCZ PRS respectively. This study suggests that MDD, SCZ and BD have both shared and distinct peripheral markers associated with disorder-specific genetic risk. The results also implicate inflammatory dysfunction in MDD and SCZ, albeit with differences in patterns between the two conditions, and enrich our understanding of potential underlying pathophysiological mechanisms in major psychiatric disorders.
Brain Behav Immun, 2021; 97
30725465: Blacker TS, Sewell MDE, Szabadkai G, Duchen MR
Metabolic Profiling of Live Cancer Tissues Using NAD(P)H Fluorescence Lifetime Imaging.
Altered metabolism is a hallmark of cancer, both resulting from and driving oncogenesis. The NAD and NADP redox couples play a key role in a large number of the metabolic pathways involved. In their reduced forms, NADH and NADPH, these

molecules are intrinsically fluorescent. As the average time for fluorescence to be emitted following excitation by a laser pulse, the fluorescence lifetime, is exquisitely sensitive to changes in the local environment of the fluorophore, imaging the fluorescence lifetime of NADH and NADPH offers the potential for label-free monitoring of metabolic changes inside living tumors. Here, we describe the biological, photophysical, and methodological considerations required to establish fluorescence lifetime imaging (FLIM) of NAD(P)H as a routine method for profiling the metabolism of living cancer cells and tissues.
Methods Mol Biol, 2019; 1928

FENS Forum 2022
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BOARD NUMBER: S02-672

FUNCTIONAL CHARACTERIZATION OF A NOVEL LNCRNA IN THE AGING BRAIN

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Long non-coding RNAs (lncRNAs) are non-protein coding transcripts that have emerged as major regulators of cellular and molecular function in the central nervous system (CNS). In this study, we aimed to identify and characterize lncRNA that are de-regulated in the aging brain**.** We employed FACS to sort neuronal and non-neuronal nuclei from the hippocampus of 3- and 16-month-old mice and performed total RNA sequencing. Computational analysis of the sequencing results revealed several candidate lncRNAs. Further expression analysis identified a novel candidate lncRNA enriched in microglia, the resident immune cells of the CNS that play a central role in age-related CNS disorders. Knockdown of this candidate lncRNA in primary mouse microglia and in human iPSC-derived microglia led to an upregulation of inflammatory pathways and increased phagocytic activity. In conclusion, we identify a novel lncRNA that is deregulated in the aging brain and orchestrates microglia function.

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BOARD NUMBER: S02-673

THE S1PR2-CCL2-BDNF-TRKB PATHWAY MEDIATES NEUROINFLAMMATION, ALTERATIONS IN GABAERGIC NEUROTRANSMISSION AND MOTOR INCOORDINATION IN HYPERAMMONEMIC RATS

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Aims: Chronic hyperammonemia and inflammation synergistically induce neurological impairment including motor incoordination in hepatic encephalopathy. Hyperammonemic rats show neuroinflammation in cerebellum which enhances GABAergic neurotransmission leading to motor incoordination. We aimed to identify underlying mechanisms. The aims were: 1) assess if S1PR2 is involved in microglia and astrocytes activation in cerebellum of hyperammonemic rats; 2) identify pathways by which enhanced S1PR2 activation induces neuroinflammation and alters neurotransmission; 3) assess if blocking S1PR2 reduces neuroinflammation and restores motor coordination in hyperammonemic rats. Methods: We performed ex vivo studies in cerebellar slices from control or hyperammonemic rats to identify pathways by which neuroinflammation enhances GABAergic neurotransmission in hyperammonemia. Neuroinflammation and neurotransmission were assessed by immunochemistry/immunofluorescence and western blot. The S1PR2 was blocked by intracerebral treatment with JTE-013 using osmotic mini-pumps. Motor coordination was assessed in the beam walking Catwalk and motorater. Results: Chronic hyperammonemia enhances S1PR2 activation in cerebellum by increasing its membrane expression. This increases CCL2, especially in Purkinje neurons. CCL2 activates CCR2 in microglia, leading to microglia activation, increased P2X4 membrane expression and BDNF in microglia. BDNF enhanced TrkB activation in neurons, increasing KCC2 membrane expression. This enhances GABAergic neurotransmission, leading to motor incoordination in hyperammonemic rats. Blocking the S1PR2 in hyperammonemic rats by intracerebral administration of JTE-013 normalizes the S1PR2-CCL2-CCR2-BDNF-TrkB-KCC2 pathway, reduces glial activation and restores motor coordination in hyperammonemic rats. Conclusions: Enhanced S1PR2-CCL2-BDNF-TrkB pathway activation mediates neuroinflammation and incoordination in hyperammonemia. The data raise a promising therapy for patients with hepatic encephalopathy using compounds targeting this pathway.

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BOARD NUMBER: S02-674

CHARACTERISATION OF A NOVEL NOX2 INHIBITOR, GSK2795039, USING IN VITRO MODELS OF MICROGLIAL ACTIVATION

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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NADPH oxidase 2 (NOX2) is an enzyme complex responsible for reactive oxygen species (ROS) production in microglia. NOX2 also acts as a priming signal for NLRP3 inflammasome activation, which is implicated in microglial-mediated neuroinflammation during chronic neurodegeneration. GSK2795039 is a novel small molecule drug that inhibits NOX2 in an NADPH competitive manner. The goal of this in *vitro* study was to characterise GSK2795039 in models of microglial activation. Immortalised Microglial (IMG) cell line or primary microglia from p1 Wistar rat pups were pre-treated with GSK2795039 (NOX2 inhibitor; 1-40μM) or diphenyleneiodonium (DPI; broad antioxidant; 0.005-0.1μM) and stimulated with either lipopolysaccharide (LPS; 100ng/ml) or LPS (100ng/ml)/ATP(1mM) to induce NOX2/ROS and NLRP3 inflammasome activation. ROS production and cell viability were measured using CM-H2DCFDA and MTT assays, respectively. The conditioned media was analysed for IL-1β, TNF-α by ELISA, and levels of nitric oxide (NO) using a Griess assay. Protein expression of iNOS, Arginase 1 and NLRP3 were determined by Western immunoblot. In IMG and primary microglia, GSK2795039 attenuated LPS-induced ROS production, iNOS expression, NO and upregulated anti-inflammatory marker, Arginase-1. In the LPS/ATP model, GSK2795039 attenuated ROS, IL-1β release and NLRP3 expression. Thus, GSK2795039 attenuated pro-inflammatory activation of LPS-stimulated microglia and concurrently upregulated an anti-inflammatory response. GSK2795039 also inhibited NLRP3 inflammasome activation which may be due to reduced NOX2/ROS signalling. Preliminary results indicate that GSK2795039 may induce a phenotypic switch in microglia by reducing pro-inflammatory and upregulating anti-inflammatory responses. Thus, GSK2795039 may be a promising therapeutic drug for mitigating the damaging effects of microglial-mediated neuroinflammation

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BOARD NUMBER: S02-675

MICROGLIAL BRAIN COLONIZATION: LOCAL CROSSTALK WITH THE NEURAL ENVIRONMENT

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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[1]Institut de Biologie de l'ENS (IBENS), Ecole Normale Supérieure, CNRS, Inserm, Université Psl, Paris, France, [2]Centre Interdisciplinaire de Recherche en Biologie (CIRB), Collège de France, CNRS, Inserm, Université Psl, Paris, France, [3]Collège de France, Université Psl, Paris, France, [4]Institut de Biologie de l'ENS (IBENS), Ecole Normale Supérieure, CNRS, INSERM, Université Psl, Paris, France

Microglia, the brain resident macrophages, are instrumental for cerebral development, functioning and homeostasis. Generated in the embryonic yolk-sac, these immigrants colonize the central nervous system during early embryogenesis, proliferate, distribute themselves and self-renew throughout life. Their colonization follows a remarkable stereotypical spatiotemporal pattern which has been associated with specific microglial developmental functions. While little is known about the mechanisms that regulate their pattern of colonization, microglial survival, proliferation and maturation have been shown to rely on CSF-1 receptor signaling. Herein, we performed a detailed analysis of murine microglial colonization and highlighted two waves of global microglial proliferation, matching changes in numbers as well as some regional specificities. Moreover, two-photon live-imaging on acute brain slices allowed us to appreciate the dynamic of microglial colonization, showing little contribution of migration over local proliferation. Noteworthy, using conditional inactivation of *Csf-1* in specific neural populations, we elucidated the sources of CSF-1 during embryonic microglial colonization and highlighted its essential local, transient and dose-dependent roles. Consequently, we developed a specific embryonic microglial depletion model in which they are transiently locally deprived. Altogether, our study sheds light on how microglia proliferate and distribute to colonize the brain as well as on their focal dependency on the CSF-1 receptor pathway. Importantly, it provides a groundbreaking tool to study the local roles of microglia during development. Sonia Garel and Morgane S. Thion contributed equally to this work.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-676

THE DARK MICROGLIAL SUBSET DISPLAYS ULTRASTRUCTURAL AND METABOLIC ALTERATIONS IN AN AGED MOUSE MODEL OF BETA-AMYLOID PATHOLOGY

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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AIMS Recent technical advances helped to reveal the considerable heterogeneity of microglia, the resident immune cells of the central nervous system. One of these microglial subsets, the dark microglia (DM), was characterized by ultrastructural signs of cellular stress such as dilated endoplasmic reticulum (ER), altered mitochondria and loss of heterochromatin pattern. These dark cells were previously identified notably near amyloid beta plaques in age-matched APP-PS1 mice, a mouse model of amyloid beta deposition. However, their ultrastructural features and interactions with hallmarks of amyloid deposition (plaques, dystrophic neurites) were not yet investigated in aging. **METHODS** Using high-magnification chip mapping by scanning electron microscopy, we first analyzed the density of both typical and DM in the ventral hippocampus CA1 (*strata lacunosum-moleculare* and *radiatum*) of 20-month-old male WT *versus* APP-PS1 mice. **RESULTS** In the *stratum lacunosum-moleculare,* dark microglia represented nearly 43% of all microglial cells found near AD hallmarks. We found DM interacting more with dystrophic neurites, while contacting less healthy synaptic elements, myelinated axons and brain vasculature compared to typical microglia. **CONCLUSIONS** The present study further highlights the close interactions between DM and amyloid beta deposition hallmarks, suggesting a specialized involvement, whether beneficial or detrimental, compared to typical microglia in this context. Further investigations into the prevalence and parenchymal interactions of DM with Alzheimer's disease hallmarks will be performed in *post-mortem* brain samples from Alzheimer's patients and aged-matched controls. Studying DM close interactions with disease hallmarks could help us find new therapeutic targets modulating these cells in a context-dependent manner.

Pubmed:
34803607: Carrier M, Šimončičová E, St-Pierre MK, McKee C, Tremblay MÈ
Psychological Stress as a Risk Factor for Accelerated Cellular Aging and Cognitive Decline: The Involvement of Microglia-Neuron Crosstalk.
The relationship between the central nervous system (CNS) and microglia is lifelong. Microglia originate in the embryonic yolk sac during development and populate the CNS before the blood-brain barrier forms. In the CNS, they constitute a self-renewing population. Although they represent up to 10% of all brain cells, we are only beginning to understand how much brain homeostasis relies on their physiological functions. Often compared to a double-edged sword, microglia hold the potential to exert neuroprotective roles that can also exacerbate neurodegeneration once compromised. Microglia can promote synaptic growth in addition to eliminating synapses that are less active. Synaptic loss, which is considered one of the best pathological correlates of cognitive decline, is a distinctive feature of major depressive disorder (MDD) and cognitive aging. Long-term psychological stress accelerates cellular aging and predisposes to various diseases, including MDD, and cognitive decline. Among the underlying mechanisms, stress-induced neuroinflammation alters microglial interactions with the surrounding parenchymal cells and exacerbates oxidative burden and cellular damage, hence inducing changes in microglia and neurons typical of cognitive aging. Focusing on microglial interactions with neurons and their synapses, this review discusses the disrupted communication between these cells, notably involving fractalkine signaling and the triggering receptor expressed on myeloid cells (TREM). Overall, chronic stress emerges as a key player in cellular aging by altering the microglial sensome, notably via fractalkine signaling deficiency. To study cellular aging, novel positron emission tomography radiotracers for TREM and the purinergic family of receptors show interest for human study.

Front Mol Neurosci, 2021; 14
33587954: Picard K, St-Pierre MK, Vecchiarelli HA, Bordeleau M, Tremblay MÈ
Neuroendocrine, neuroinflammatory and pathological outcomes of chronic stress: A story of microglial remodeling.
Microglia, the resident macrophage cells of the central nervous system (CNS), are involved in a myriad of processes required to maintain CNS homeostasis. These cells are dynamic and can adapt their phenotype and functions to the physiological needs of the organism. Microglia rapidly respond to changes occurring in their microenvironment, such as the ones taking place during stress. While stress can be beneficial for the organism to adapt to a situation, it can become highly detrimental when it turns chronic. Microglial response to prolonged stress may lead to an alteration of their beneficial physiological functions, becoming either maladaptive or pro-inflammatory. In this review, we aim to summarize the effects of chronic stress exerted on microglia through the neuroendocrine system and inflammation at adulthood. We also discuss how these effects of chronic stress could contribute to microglial involvement in neuropsychiatric and sleep disorders, as well as neurodegenerative diseases.
Neurochem Int, 2021; 145
32755645: Lecours C, St-Pierre MK, Picard K, Bordeleau M, Bourque M, Awogbindin IO, Benadjal A, Ibanez FG, Gagnon D, Cantin L, Parent M, Di Paolo T, Tremblay ME
Levodopa partially rescues microglial numerical, morphological, and phagolysosomal alterations in a monkey model of Parkinson's disease.
Parkinson's disease (PD) is the most common neurodegenerative motor disorder. The mechanisms underlying the onset and progression of Levodopa (L-Dopa)-induced dyskinesia (LID) during PD treatment remain elusive. Emerging evidence implicates functional modification of microglia in the development of LID. Thus, understanding the link between microglia and the development of LID may provide the knowledge required to preserve or promote beneficial microglial functions, even during a prolonged L-Dopa treatment. To provide novel insights into microglial functional alterations in PD pathophysiology, we characterized their density, morphology, ultrastructure, and degradation activity in the sensorimotor functional territory of the putamen, using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) cynomolgus monkeys. A subset of MPTP monkeys was treated orally with L-Dopa and developed LID similar to PD patients. Using a combination of light, confocal and transmission electron microscopy, our quantitative analyses revealed alterations of microglial density, morphology and phagolysosomal activity following MPTP intoxication that were partially normalized with L-Dopa treatment. In particular, microglial density, cell body and arborization areas were increased in the MPTP monkeys, whereas L-Dopa-treated MPTP animals presented a microglial phenotype similar to the control animals. At the ultrastructural level, microglia did not differ between groups in their markers of cellular stress or aging. Nevertheless, microglia from the MPTP monkeys displayed reduced numbers of endosomes, compared with control animals, that remained lower after L-Dopa treatment. Microglia from MPTP monkeys treated with L-Dopa also had increased numbers of primary lysosomes compared with non-treated MPTP animals, while secondary and tertiary lysosomes remained unchanged. Moreover, a decrease microglial immunoreactivity for CD68, considered a marker of phagocytosis and lysosomal activity, was measured in the MPTP monkeys treated with L-Dopa, compared with non-treated MPTP animals. Taken together, these findings revealed significant changes in microglia during PD pathophysiology that were partially rescued by L-Dopa treatment. Albeit, this L-Dopa treatment conferred phagolysosomal insufficiency on microglia in the dyskinetic Parkinsonian monkeys.
Brain Behav Immun, 2020; 90
32544086: Gratuze M, Leyns CE, Sauerbeck AD, St-Pierre MK, Xiong M, Kim N, Serrano JR, Tremblay MÈ, Kummer TT, Colonna M, Ulrich JD, Holtzman DM
Impact of TREM2R47H variant on tau pathology-induced gliosis and neurodegeneration.
Alzheimer's disease (AD) is characterized by plaques containing amyloid-β (Aβ) and neurofibrillary tangles composed of aggregated, hyperphosphorylated tau. Beyond tau and Aβ, evidence suggests that microglia play an important role in AD pathogenesis. Rare variants in the microglia-expressed triggering receptor expressed on myeloid cells 2 (TREM2) gene increase AD risk 2- to 4-fold. It is likely that these TREM2 variants increase AD risk by decreasing the response of microglia to Aβ and its local toxicity. However, neocortical Aβ pathology occurs many years before neocortical tau pathology in AD. Thus, it will be important to understand the role of TREM2 in the context of tauopathy. We investigated the impact of the AD-associated TREM2 variant (R47H) on tau-mediated neuropathology in the PS19 mouse model of tauopathy. We assessed PS19 mice expressing human TREM2CV (common variant) or human TREM2R47H. PS19-TREM2R47H mice had significantly attenuated brain atrophy and synapse loss versus PS19-TREM2CV mice. Gene expression analyses and CD68 immunostaining revealed attenuated microglial reactivity in PS19-TREM2R47H versus PS19-TREM2CV mice. There was also a decrease in phagocytosis of postsynaptic elements by microglia expressing TREM2R47H in the PS19 mice and in human AD brains. These findings suggest that impaired TREM2 signaling reduces microglia-mediated neurodegeneration in the setting of tauopathy.
J Clin Invest, 2020; 130

32443939: St-Pierre MK, Šimončičová E, Bögi E, Tremblay MÈ
Shedding Light on the Dark Side of the Microglia.
Microglia, the resident immune cells of the central nervous system, are not a homogeneous population; their morphology, molecular profile, and even their ultrastructure greatly vary from one cell to another. Recent advances in the field of neuroimmunology have helped to demystify the enigma that currently surrounds microglial heterogeneity. Indeed, numerous microglial subtypes have been discovered such as the disease-associated microglia, neurodegenerative phenotype, and Cd11c-positive developmental population. Another subtype is the dark microglia (DM), a population defined by its ultrastructural changes associated with cellular stress. Since their first characterization using transmission electron microscopy, they have been identified in numerous disease conditions, from mouse models of Alzheimer's disease, schizophrenia, fractalkine signaling deficiency to chronic stress, just to name a few. A recent study also identified the presence of cells with a similar ultrastructure to the DM in brain samples from schizophrenic patients, underlining the importance of understanding the function of these cells. In this minireview, we aim to summarize the current knowledge on the DM, from their initial ultrastructural characterization to their documentation in various pathological contexts across multiple species. We will also highlight the current limitations surrounding the study of these cells and the future that awaits the DM.
ASN Neuro, 2020 Jan-Dec; 12
32241286: Savage JC, St-Pierre MK, Carrier M, El Hajj H, Novak SW, Sanchez MG, Cicchetti F, Tremblay MÈ
Microglial physiological properties and interactions with synapses are altered at presymptomatic stages in a mouse model of Huntington's disease pathology.
Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder that affects cognitive and motor abilities by primarily targeting the striatum and cerebral cortex. HD is caused by a mutation elongating the CAG repeats within the Huntingtin gene, resulting in HTT protein misfolding. Although the genetic cause of HD has been established, the specific susceptibility of neurons within various brain structures has remained elusive. Microglia, which are the brain's resident macrophages, have emerged as important players in neurodegeneration. Nevertheless, few studies have examined their implication in HD.
J Neuroinflammation, 2020; 17
31920505: Savage JC, St-Pierre MK, Hui CW, Tremblay ME
Microglial Ultrastructure in the Hippocampus of a Lipopolysaccharide-Induced Sickness Mouse Model.
Sickness behavior is a set of behavioral changes induced by infections and mediated by pro-inflammatory cytokines. It is characterized by fatigue, decreased appetite and weight loss, changes in sleep patterns, cognitive functions, and lost interest in social activity. It can expedite recovery by conserving energy to mount an immune response involving innate immunity. To provide insights into microglial implication in sickness behavior with special focus on cognitive and social impairment, we investigated changes in their ultrastructure and interactions with synapses using a toxemia mouse model. Adult mice were injected with 1 mg/kg lipopolysaccharide (LPS) or saline, and assayed for signs of sickness behavior. LPS treated mice displayed reduced activity in open-field tests 24 h post-injection, while social avoidance and weight gain/loss were not significantly different between treatment groups. Microglia were investigated using electron microscopy to describe changes in their structure and function at nanoscale resolution. Microglial cell bodies and processes were investigated in the hippocampus CA1, a region responsible for learning and memory that is often impacted after peripheral LPS administration. Microglia in LPS treated animals displayed larger cell bodies as well as less complex processes at the time point examined. Strikingly, microglial processes in LPS injected animals were also more likely to contact excitatory synapses and contained more phagocytic material compared with saline injected controls. We have identified at the ultrastructural level significant changes in microglia-synapse interactions shortly after LPS administration, which draws attention to studying the roles of microglia in synaptic rewiring after inflammatory stimuli.
Front Neurosci, 2019; 13
31392680: St-Pierre MK, Bordeleau M, Tremblay MÈ
Visualizing Dark Microglia.
Dark microglia, a recently described phenotype, are found in high numbers in nonhomeostatic conditions (e.g., Alzheimer's disease pathology, aging, chronic stress). As a specific protein marker has not yet been defined, they cannot be studied using conventional cellular biology techniques. They are recognized by their unique ultrastructural features visible under electron microscopy. This nanoscale resolution imaging technique allows the identification of cells based on their ultrastructure or immunoreactivity to certain proteins. In this protocol, we describe the steps necessary for the preparation of high-quality brain tissues for transmission electron microscopy, the imaging, the identification of dark microglia, and the ultrastructural analysis of various parameters that can be studied in these cells.
Methods Mol Biol, 2019; 2034
30702751: Nichols MR, St-Pierre MK, Wendeln AC, Makoni NJ, Gouwens LK, Garrad EC, Sohrabi M, Neher JJ, Tremblay ME, Combs CK

Inflammatory mechanisms in neurodegeneration.
This review discusses the profound connection between microglia, neuroinflammation, and Alzheimer's disease (AD). Theories have been postulated, tested, and modified over several decades. The findings have further bolstered the belief that microglia-mediated inflammation is both a product and contributor to AD pathology and progression. Distinct microglia phenotypes and their function, microglial recognition and response to protein aggregates in AD, and the overall role of microglia in AD are areas that have received considerable research attention and yielded significant results. The following article provides a historical perspective of microglia, a detailed discussion of multiple microglia phenotypes including dark microglia, and a review of a number of areas where microglia intersect with AD and other pathological neurological processes. The overall breadth of important discoveries achieved in these areas significantly strengthens the hypothesis that neuroinflammation plays a key role in AD. Future determination of the exact mechanisms by which microglia respond to, and attempt to mitigate, protein aggregation in AD may lead to new therapeutic strategies.
J Neurochem, 2019; 149
29908963: Hui CW, St-Pierre MK, Detuncq J, Aumailley L, Dubois MJ, Couture V, Skuk D, Marette A, Tremblay JP, Lebel M, Tremblay MÈ
Nonfunctional mutant Wrn protein leads to neurological deficits, neuronal stress, microglial alteration, and immune imbalance in a mouse model of Werner syndrome.
Werner syndrome (WS) is a premature aging disorder caused by mutations in a RecQ-family DNA helicase, WRN. Mice lacking part of the helicase domain of the WRN orthologue exhibit many phenotypic features of WS, including metabolic abnormalities and a shorter lifespan. Yet, little is known about the impact of WRN mutations on the central nervous system in both humans and mouse models of WS. In the current study, we have performed a longitudinal behavioral assessment on mice bearing a Wrn helicase deletion. Behavioral tests demonstrated a loss of motor activity and coordination, reduction in perception, increase in repetitive behavior, and deficits in both spatial and social novelty memories in Wrn mutant mice compared to age-matched wild type mice. These neurological deficits were associated with biochemical and histological changes in the brain of aged Wrn mutant mice. Microglia, resident immune cells that regulate neuronal plasticity and function in the brain, were hyper-ramified in multiple regions involved with the behavioral deficits of Wrn mutant mice. Furthermore, western analyses indicated that Wrn mutant mice exhibited an increase of oxidative stress markers in the prefrontal cortex. Supporting these findings, electron microscopy studies revealed increased cellular aging and oxidative stress features, among microglia and neurons respectively, in the prefrontal cortex of aged Wrn mutant mice. In addition, multiplex immunoassay of serum identified significant changes in the expression levels of several pro- and anti-inflammatory cytokines. Taken together, these findings indicate that microglial dysfunction and neuronal oxidative stress, associated with peripheral immune system alterations, might be important driving forces leading to abnormal neurological symptoms in WS thus suggesting potential therapeutic targets for interventions.
Brain Behav Immun, 2018; 73

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BOARD NUMBER: S02-677

PROTECTIVE ROLE OF MICROGLIA AND T LYMPHOCYTES IN A MOUSE MODEL OF TEMPORAL LOBE EPILEPSY.

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

Maria Moreno Montano[1], Paola Nobili[1], Laurine Martins[1], Anais Virenque[1], Julio Mateos-Languerak[2], Valentin Garcia[1], Geoffrey Canet[3], Valérie Dardalhon[4], Laurent Givalois[3], Hélène Hirbec[1], Nicola Marchi[1], Etienne Audinat[1]
[1]IGF, UM, INSERM, CNRS,, Neurosciences, MONTPELLIER, France, [2]IGH, CNRS,, Mri, MONTPELLIER, France, [3]MMDN, Neurosciences, Montpellier, France, [4]IGMM, CNRS, Immunology, Montpellier, France

Temporal lobe epilepsy (TLE) is characterized by focal recurrent seizures generated in the temporal structures. TLE is often accompanied by neuronal death, gliosis, and synaptic reorganization. Sixty percent of patients do not respond to treatments, highlighting the urgency of better understanding the mechanisms involved in the pathogenesis to identify new therapeutic targets. **Aims:** Microglia develop a dual profile, pro and anti-inflammatory, and the balance between these two states influences disease progression. Our working hypothesis is that interactions between microglia and T cells drive a protective anti-inflammatory effect in the epileptic hippocampus. **Methods:** We used a unilateral intracortical kainate model of TLE in mice lacking T cells (CD3-/- mouse) or depleted in regulatory T cells (Treg). We performed *in vivo* electrophysiological recordings and immunostainings to test the effect of T cell absence on epileptic activity and hippocampal sclerosis. **Results:** We observed an increase of seizure frequency, an increase of neuronal death and a loss microglial anti-inflammatory response in the hippocampus of CD3-/- mouse. Treg have been shown to promote anti-inflammatory reactions in different models of neurological disorders. Depleting Treg by administrating anti-CD25 antibodies during early epileptogenesis led to a loss of the microglial anti-inflammatory response and to an increase of neuronal death and of seizure frequency in the hippocampus. **Conclusions:** These observations suggest that interactions between Treg and microglia have a protective role in TLE. These findings may allow the identification of new targets to enhance the endogenous anti-inflammatory response and ultimately improve the treatment of TLE.

Pubmed:
31051234: Girard B, Tuduri P, Moreno MP, Sakkaki S, Barboux C, Bouschet T, Varrault A, Vitre J, McCort-Tranchepain I, Dairou J, Acher F, Fagni L, Marchi N, Perroy J, Bertaso F
The mGlu7 receptor provides protective effects against epileptogenesis and epileptic seizures.
Finding new targets to control or reduce seizure activity is essential to improve the management of epileptic patients. We hypothesized that activation of the pre-synaptic and inhibitory metabotropic glutamate receptor type 7 (mGlu7) reduces spontaneous seizures. We tested LSP2-9166, a recently developed mGlu7/4 agonist with unprecedented potency on mGlu7 receptors, in two paradigms of epileptogenesis. In a model of chemically induced epileptogenesis (pentylenetetrazole systemic injection), LSP2-9166 induces an anti-epileptogenic effect rarely observed in preclinical studies. In particular, we found a bidirectional modulation of seizure progression by mGlu4 and mGlu7 receptors, the latter preventing kindling. In the intra-hippocampal injection of kainic acid mouse model that mimics the human mesial temporal lobe epilepsy, we found that LSP2-9166 reduces seizure frequency and hippocampal sclerosis. LSP2-9166 also acts as an anti-seizure drug on established seizures in both models tested. Specific modulation of the mGlu7 receptor could represent a novel approach to reduce pathological network remodeling.
Neurobiol Dis, 2019; 129

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MICROGLIAL CASPASE-3 IS ESSENTIAL FOR MODULATING HIPPOCAMPAL NEUROGENESIS

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Adult hippocampal neurogenesis (AHN) is a process involved in numerous neurodegenerative diseases. Many researchers have described microglia as a key component in modulating this process, regulating the formation and migration of new neurons along the rostral migratory stream. Caspase-3 is a cysteine-aspartate-protease classically considered as one of the main effector caspases in the cell death program process. In addition to this classical function, we have identified the role of this protein as a modulator of microglial function; however, its action on neurogenic processes is unknown. The aim of the present study is to identify the role of caspase-3 in neurogenesis-related microglial functions. To address this study, caspase-3 conditional mutant mice in the microglia were used. Using this tool, we wanted to elucidate the role of this protein in microglial function in the hippocampus, the main region where adult neurogenesis takes place. After the deletion of caspase-3 in microglia, mutant mice showed a reduction of microglia in the hippocampus, showing a less active morphology. Using high-resolution image analysis, we also observed a reduction in the phagocytic capacity of microglia lacking caspase-3. Furthermore, we found a reduction in doublecortin-positive neurons in caspase-3 knockout mice, which corresponds to a reduction in neurogenic neurons. Behavioral analysis using object recognition and Y-maze tests showed altered memory and learning in the absence of caspase-3. Taken together, these results showed the essential role of caspase-3 in microglial function and highlight the relevant role of this specific microglial phenotype in the maintenance of AHN in the hippocampus.

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MAGNETOFECTION IN HMC3 HUMAN MICROGLIA IS NOT AFFECTED BY DEFINED-MEDIUM CONDITIONS

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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[1]Faculty of Biology, University of Bucharest, Department Of Anatomy, Animal Physiology And Biophysics, Bucharest, Romania, [2]Institute of Biochemistry, Romanian Academy, Department Of Molecular Cell Biology, Bucharest, Romania

Aims: Serum-free microglia culture models supplemented with primary components of astrocyte-derived factors (defined-medium) are required for the cells *in vitro* to resemble the mature, resting microglial phenotype. Here we hypothesize that using magnetofection to deliver siRNA in HMC3 human microglia is equally effective in both serum-supplemented and defined-medium cultures. **Methods:** The polarization towards the resting phenotype of HMC3 microglial cells maintained in defined-medium was established by quantifying the degree of cell elongation across the cell nucleus. Additionally, extracellular acidification rate and oxygen consumption rate were measured using Seahorse XFe24 analyser. Magnetofection is a novel transfection method that allows the delivery of nucleic acids upon exploitation of magnetic force. The efficiency of magnetofection was assessed by siRNA molecule labelled with Alexa Fluor 594 and analysed quantitatively as mean integrated density per cell area. The efficacy of magnetofection was tested on Iba1 protein expression, by Western blot and LC-MS analysis, using a customized anti-Iba1 siRNA molecule. **Results:** The degree of cell elongation as well as the metabolic measurements confirmed that polarization occurs until 7 days of culturing the microglial cells in defined-medium. The siRNA delivery efficiency showed comparable results in terms of siRNA concentration and ratio between transfection reagent:siRNA. The siRNA delivery efficacy indicated a decrease in Iba1 expression, even though the results were not striking: surprisingly, we confronted a low level of Iba1 protein intrinsically expressed by HMC3 microglia. **Conclusions:** High-yield delivery of siRNA in HMC3 human microglia can be achieved by means of magnetofection, in both serum-supplemented and defined-medium cultures.

Pubmed:
31950314: Gheorghe RO, Deftu A, Filippi A, Grosu A, Bica-Popi M, Chiritoiu M, Chiritoiu G, Munteanu C, Silvestro L, Ristoiu V
Silencing the Cytoskeleton Protein Iba1 (Ionized Calcium Binding Adapter Protein 1) Interferes with BV2 Microglia Functioning.
Iba1 (ionized calcium binding adapter protein 1) is a cytoskeleton protein specific only for microglia and macrophages, where it acts as an actin-cross linking protein. Although frequently regarded as a marker of activation, its involvement in cell migration, membrane ruffling, phagocytosis or in microglia remodeling during immunological surveillance of the brain suggest that Iba1 is not a simple cytoskeleton protein, but a signaling molecule involved in specific signaling pathways. In this study we investigated if Iba1 could also represent a drug target, and tested the hypothesis that its specific silencing with customized Iba1-siRNA can modulate microglia functioning. The results showed that Iba1-silenced BV2 microglia migrate less due to reduced proliferation and cell adhesion, while their phagocytic activity and P2x7 functioning was significantly increased. Our data are the proof of concept that Iba1 protein is a new microglia target, which opens a new therapeutic avenue for modulating microglia behavior.
Cell Mol Neurobiol, 2020; 40

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THE POSSIBLE THERAPEUTIC ROLE OF ITACONATE AND MESACONATE ON THE DELETERIOUS EFFECTS OF INFLUENZA A VIRUS INFECTION IN THE BRAIN

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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[1]TU Braunschweig, Department Of Bioinformatics And Biochemistry, Braunschweig, Germany, [2]TU Braunschweig, Department Of Cellular Neurobiology, Zoological Institute, Braunschweig, Germany, [3]Helmholtz Centre for Infection Research, Neuroinflammation And Neurodegeneration Group, Braunschweig, Germany

Numerous studies indicate that strong inflammatory responses in the periphery lead to infiltration of peripheral leukocytes into the central nervous system, resulting in activation of brain resident immune cells, neuroinflammation and even neurodegeneration. The inflammatory response is primarily a protective mechanism. However, excessive and chronic inflammation can lead to deleterious effects on neuronal cells. Indeed, neuroinflammation underlies the pathogenesis and accelerates the progression of many neurological disorders. Therefore, inflammatory signaling pathways have been implicated as potential therapeutic targets for many neurological diseases. A growing number of reports demonstrates that products of the cell metabolism play an important role in mediating the immune response. The TCA cycle-derived metabolite itaconate is strongly upregulated in activated macrophages and has been shown to act as an immunomodulator with anti-inflammatory functions. Mesaconate, an isomer of itaconate, also decreases the inflammatory response in macrophages. To investigate the immunomodulatory and therapeutic potential of itaconate and mesaconate in neuroinflammatory processes, the metabolites were administered in two mouse models: LPS-induced peripheral immune activation and influenza A virus infection. To analyze the extent of inflammation, pro-inflammatory cytokines were determined in the brain by ELISA. Interestingly, mice that received mesaconate in combination with influenza infection showed an improvement in the clinical score and lower cytokine levels compared to the corresponding control animals. Ongoing experiments address whether administration of itaconate or mesaconate may modulate microglial activation and neuronal structure and function. These results could lead to new therapeutic strategies to prevent immediate and delayed neurological manifestations associated with bacterial and viral infections.

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EXPLORING THE POTENTIAL ROLES OF THE PIWI PATHWAY IN MICROGLIA AND NEUROINFLAMMATION

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Piwi proteins and the small noncoding RNAs interacting with them i.e., piRNAs (henceforth referred to as the Piwi pathway), are best known in gonads where they act as an RNA-mediated immune mechanism that safeguards genomic integrity and male fertility. Although accumulating evidence indicates that the Piwi pathway is also involved in neuroinflammation and neurodegeneration (Penning et al., 2022), mechanisms remain elusive. Our data indicates that the induction of an inflammatory state in murine microglia, the immune-resident cells of the brain, alters the expression of the Piwi pathway, suggesting that it might be involved and contribute to the mechanisms of microglia activation. As microglia are key players in neuroinflammation, which is considered a risk factor for a wide range of age-related diseases of the central nervous system, such as dementia, depression and neurodegeneration, this finding has potential implications for successful brain aging.

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BOARD NUMBER: S02-682

EFFECTS OF INFLUENZA A VIRUS INFECTION ON HIPPOCAMPAL NEURON STRUCTURE AND _FUNCTION IN AGED WILD-TYPE MICE

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

Shirin Hosseini[1,2], Hendrik Demuth[1,2], Kristin Michaelsen-Preusse[2], Martin Korte[1,2]
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Influenza A virus (IAV) infections are primarily known to cause a self-limiting respiratory syndrome. It has rarely been systematically studied in what ways and with what long-term consequences the infection and/or the immune response to it might affect the nervous system. However, an expanded spectrum of cerebral manifestations has been reported following IAV infection. Evidence suggests that susceptibility to pulmonary infections caused by respiratory viruses increases with age. Indeed, a progressive decline in the integrity of the immune system, termed immunosenescence, is one of the prominent physiological changes during mammalian aging. Accordingly, we aimed to investigate whether age is a risk factor for the development of a more pronounced chronic immune response in the CNS of elderly individuals. Therefore, 15-month-old C57BL/6J mice were intranasally infected with non-neurotropic (H1N1 and H3N2) and neurotropic H7N7 IAV subtypes to determine potential long-term effects on hippocampal structure and function. Synapse loss detected at 30 dpi was associated with deficits in spatial learning and impaired synaptic plasticity. In addition, evidence of synaptic stripping by increased activated phagocytic microglia engulfing the postsynaptic compartment was detected in the hippocampus. While neuroinflammation induced by H7N7 IAV showed the strongest effect, systemic infection with H1N1 and H3N2 subtypes resulted in long-term impairment of hippocampal structure and function. Remarkably, the deficit in spatial learning at 120 dpi was still detectable in aged H7N7 IAV-infected mice, in contrast to fully recovered young mice. These results suggest that IAV infection has longer-lasting and more severe effects on hippocampal function in older animals.

Pubmed:

33994946: Hosseini S, Michaelsen-Preusse K, Schughart K, Korte M
Long-Term Consequence of Non-neurotropic H3N2 Influenza A Virus Infection for the Progression of Alzheimer's Disease Symptoms.
Influenza viruses until today are a leading cause of worldwide severe pandemics and represent a major threat to human and animal health. Although the primary target of influenza viruses is the lung, infection may manifest with acute and even chronic neurological complications (e.g., status epilepticus, encephalopathies, and encephalitis) potentially increasing the long-term risk for neurodegenerative diseases. We previously described that a peripheral influenza A virus (IAV) infection caused by non-neurotropic H3N2 (maHK68) variant leads to long-term neuroinflammation and synapse loss together with impaired memory formation in young adult mice. Processes of neuroinflammation have been associated with neurodegenerative diseases such as Alzheimer's disease (AD) and prolonged or excessive innate immune responses are considered a risk factor for AD. Here, the role of purely peripheral IAV infection for the development and progression of AD in a transgenic mouse model (APP/PS1) was investigated. At 2 months of age, mice were infected with H3N2 IAV and the detailed analysis of microglia morphology revealed neuroinflammation in the hippocampus already of 6 months old non-infected APP/PS1 mice together with impaired spatial learning, however, microglia activation, amyloid-β plaques load and cognitive impairments were even more pronounced in APP/PS1 mice upon H3N2 infection. Moreover, CA1 hippocampal dendritic spine density was reduced even at 120 dpi compared to wild-type and also to non-infected APP/PS1 mice, whereas neuronal cells number was not altered. These findings demonstrate that non-neurotropic H3N2 IAV infection as a peripheral immune stimulation may exacerbate AD symptoms possibly by triggering microglial hyperactivation.
Front Cell Neurosci, 2021; 15
33257576: Lonnemann N, Hosseini S, Marchetti C, Skouras DB, Stefanoni D, D'Alessandro A, Dinarello CA, Korte M
The NLRP3 inflammasome inhibitor OLT1177 rescues cognitive impairment in a mouse model of Alzheimer's disease.
Numerous studies demonstrate that neuroinflammation is a key player in the progression of Alzheimer's disease (AD). Interleukin (IL)-1β is a main inducer of inflammation and therefore a prime target for therapeutic options. The inactive IL-1β precursor requires processing by the the nucleotide-binding oligomerization domain-like receptor family, pyrin domain

containing 3 (NLRP3) inflammasome into a mature and active form. Studies have shown that IL-1β is up-regulated in brains of patients with AD, and that genetic inactivation of the NLRP3 inflammasome improves behavioral tests and synaptic plasticity phenotypes in a murine model of the disease. In the present study, we analyzed the effect of pharmacological inhibition of the NLRP3 inflammasome using dapansutrile (OLT1177), an oral NLRP3-specific inhibitor that is safe in humans. Six-month-old WT and APP/PS1 mice were fed with standard mouse chow or OLT1177-enriched chow for 3 mo. The Morris water maze test revealed an impaired learning and memory ability of 9-mo-old APP/PS1 mice (= 0.001), which was completely rescued by OLT1177 fed to mice (= 0.008 to untreated APP/PS1). Furthermore, our findings revealed that 3 mo of OLT1177 diet can rescue synaptic plasticity in this mouse model of AD (= 0.007 to untreated APP/PS1). In addition, microglia were less activated (= 0.07) and the number of plaques was reduced in the cortex (= 0.03) following NLRP3 inhibition with OLT1177 administration. We also observed an OLT1177 dose-dependent normalization of plasma metabolic markers of AD to those of WT mice. This study suggests the therapeutic potential of treating neuroinflammation with an oral inhibitor of the NLRP3 inflammasome.
Proc Natl Acad Sci U S A, 2020; 117
32951602: Cornelius ADA, Hosseini S, Schreier S, Fritzsch D, Weichert L, Michaelsen-Preusse K, Fendt M, Kröger A
Langat virus infection affects hippocampal neuron morphology and function in mice without disease signs.
Tick-borne encephalitis virus (TBEV) is an important human pathogen that can cause the serious illness tick-borne encephalitis (TBE). Patients with clinical symptoms can suffer from severe meningoencephalitis with sequelae that include cognitive disorders and paralysis. While less than 30% of patients with clinical symptoms develop meningoencephalitis, the number of seropositive individuals in some regions indicates a much higher prevalence of TBEV infections, either with no or subclinical symptoms. The functional relevance of these subclinical TBEV infections and their influence on brain functions, such as learning and memory, has not been investigated so far.
J Neuroinflammation, 2020; 17
32433975: Hosseini S, Michaelsen-Preusse K, Grigoryan G, Chhatbar C, Kalinke U, Korte M
Type I Interferon Receptor Signaling in Astrocytes Regulates Hippocampal Synaptic Plasticity and Cognitive Function of the Healthy CNS.
Type I interferon receptor (IFNAR) signaling is a hallmark of viral control and host protection. Here, we show that, in the hippocampus of healthy IFNAR-deficient mice, synapse number and synaptic plasticity, as well as spatial learning, are impaired. This is also the case for IFN-β-deficient animals. Moreover, antibody-mediated IFNAR blocking acutely interferes with neuronal plasticity, whereas a low-dose application of IFN-β has a positive effect on dendritic spine structure. Interfering with IFNAR signaling in different cell types shows a role for cognitive function and synaptic plasticity specifically mediated by astrocytes. Intriguingly, levels of the astrocytic glutamate-aspartate transporter (GLAST) are reduced significantly upon IFN-β treatment and increase following inhibition of IFNAR signaling. These results indicate that, besides the prominent role for host defense, IFNAR is important for synaptic plasticity as well as cognitive function. Astrocytes are at the center stage of this so-far-unknown signaling cascade.
Cell Rep, 2020; 31
29487124: Hosseini S, Wilk E, Michaelsen-Preusse K, Gerhauser I, Baumgärtner W, Geffers R, Schughart K, Korte M
Long-Term Neuroinflammation Induced by Influenza A Virus Infection and the Impact on Hippocampal Neuron Morphology and Function.
Acute influenza infection has been reported to be associated with neurological symptoms. However, the long-term consequences of an infection with neurotropic and non-neurotropic influenza A virus (IAV) variants for the CNS remain elusive. We can show that spine loss in the hippocampus after infection with neurotropic H7N7 (rSC35M) and non-neurotropic H3N2 (maHK68) in female C57BL/6 mice persists well beyond the acute phase of the disease. Although spine number was significantly reduced at 30 d postinfection (dpi) with H7N7 or H3N2, full recovery could only be observed much later at 120 dpi. Infection with H1N1 virus, which was shown previously to affect spine number and hippocampus-dependent learning acutely, had no significant long-term effects. Spine loss was associated with an increase in the number of activated microglia, reduced long-term potentiation in the hippocampus, and impairment in spatial memory formation, indicating that IAV-associated inflammation induced functional and structural alterations in hippocampal networks. Transcriptome analyses revealed regulation of many inflammatory and neuron- and glia-specific genes in H3N2- and H7N7-infected mice at day 18 and in H7N7-infected mice at day 30 pi that related to the structural and functional alterations. Our data provide evidence that neuroinflammation induced by neurotropic H7N7 and infection of the lung with a non-neurotropic H3N2 IAV result in long-term impairments in the CNS. IAV infection in humans may therefore not only lead to short-term responses in infected organs, but may also trigger neuroinflammation and associated chronic alterations in the CNS. In the acute phase of influenza infection, neuroinflammation can lead to alterations in hippocampal neuronal morphology and cognitive deficits. The results of this study now also provide evidence that neuroinflammation induced by influenza A virus (IAV) infection can induce longer-lasting, virus-specific alterations in neuronal connectivity that are still detectable 1 month after infection and are

associated with impairments in spatial memory formation. IAV infection in humans may therefore not only lead to short-term responses in infected organs, but may also trigger neuroinflammation and associated chronic alterations in the CNS.
J Neurosci, 2018; 38
24800043: Alizadeh Z, Fereidoni M, Behnam-Rassouli M, Hosseini S
Role of C-fibers in pain and morphine induced analgesia/hyperalgesia in rats.
Usual dosage of morphine (10 mg/kg) induces analgesia and ultra-low dose (ULD) of morphine (1 µg/kg); hyperalgesia, and C-fibers are also bearing µ-opioid receptors; here the importance of C-fibers on pain and morphine induced analgesia/hyperalgesia is questioned and investigated using pain evaluation methods and infant capsaicin treating for C-fibers lesioning.
Iran J Neurol, 2014; 13
24250922: Fereidoni M, Sabouni F, Moghimi A, Hosseini S
The effects of trypsin on rat brain astrocyte activation.
Astrocytes are cells within the central nervous system which are activated in a wide spectrum of infections, and autoimmune and neurodegenerative diseases. In pathologic states, they produce inflammatory cytokines, chemokines, and nitric oxide (NO), and sometimes they induce apoptosis. Their protease-activated receptors (PARs) can be activated by proteases, e.g. thrombin and trypsin, which are important in brain inflammation. The current study aimed to investigate the effects of different concentrations of trypsin (1 to 100U/ml) on cultured astrocytes.
Iran J Neurol, 2013; 12

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NANOPARTICLE-MEDIATED DELIVERY OF A NEW TSPO LIGAND SUPPRESSES INFLAMMATION IN LPS-STIMULATED MICROGLIA AND IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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The translocator protein 18kDa (TSPO) is a conserved outer mitochondrial membrane protein, implicated in inflammation, cell survival and proliferation. In the central nervous system, the expression of TSPO is markedly upregulated in activated microglia during various disease states such as Alzheimer's Disease. Synthetic as well as endogenous ligands with agonistic or antagonistic properties modulate the function of TSPO. Thus, TSPO ligands can act as putative therapeutic agents during neuroinflammatory processes. In the present study, we examined the effectiveness of a new TSPO ligand, named TEMNAP, in mitigating inflammatory processes associated to dementia. Lipopolysaccharide (LPS)-activated microglial cells were used as an *in vitro* model to study the anti-inflammatory effects of TEMNAP. In particular, we explored the efficacy of nanoparticle-mediated delivery of TEMNAP by investigating the molecular and the morphological properties of LPS-stimulated BV2 microglia. We demonstrated that the exposure of BV2 microglia to TEMNAP significantly reduced the LPS-induced microglia proliferation and strongly prevented the expression of the pro-inflammatory marker iNOS, as well as, the production of nitric oxide. In addition, we found that nanoparticle-mediated delivery of TEMNAP was more effective in preventing the LPS-induced proliferation of hyperactivated microglia and in reducing the upregulation of the M1 pro-inflammatory markers iNOS and CD86. Accordingly to our *in vitro* observations, *in vivo* experiments carried out in transgenic mice Tg2576, a well-known model of Alzheimer, confirmed the neuroprotective effects of TEMNAP systemically administered. Collectively, our results revealed a strong anti-inflammatory efficacy of the new compound TEMNAP.

Pubmed:

32240869: Cuomo O, Casamassa A, Brancaccio P, Laudati G, Valsecchi V, Anzilotti S, Vinciguerra A, Pignataro G, Annunziato L
Sumoylation of sodium/calcium exchanger in brain ischemia and ischemic preconditioning.
The small ubiquitin-like modifier (SUMO) conjugation (or SUMOylation) is a post-translational protein modification mechanism activated by different stress conditions that has been recently investigated in experimental models of cerebral ischemia. The expression of SUMOylation enzymes and substrates is not restricted to the nucleus, since they are present also in the cytoplasm and on plasma membrane and are involved in several physiological and pathological conditions. In the last decades, convincing evidence have supported the idea that the increased levels of SUMOylated proteins may induce tolerance to ischemic stress. In particular, it has been established that protein SUMOylation may confer neuroprotection during ischemic preconditioning. Considering the increasing evidence that SUMO can modify stability and expression of ion channels and transporters and the relevance of controlling ionic homeostasis in ischemic conditions, the present review will resume the main aspects of SUMO pathways related to the key molecules involved in maintenance of ionic homeostasis during cerebral ischemia and ischemic preconditioning, with a particular focus on the on Na/Ca exchangers.
Cell Calcium, 2020; 87
27458821: Boscia F, Begum G, Pignataro G, Sirabella R, Cuomo O, Casamassa A, Sun D, Annunziato L
Glial Na(+) -dependent ion transporters in pathophysiological conditions.
Sodium dynamics are essential for regulating functional processes in glial cells. Indeed, glial Na(+) signaling influences and regulates important glial activities, and plays a role in neuron-glia interaction under physiological conditions or in response to injury of the central nervous system (CNS). Emerging studies indicate that Na(+) pumps and Na(+) -dependent ion transporters in astrocytes, microglia, and oligodendrocytes regulate Na(+) homeostasis and play a fundamental role in modulating glial activities in neurological diseases. In this review, we first briefly introduced the emerging roles of each glial cell type in the pathophysiology of cerebral ischemia, Alzheimer's disease, epilepsy, Parkinson's disease, Amyotrophic Lateral Sclerosis, and myelin diseases. Then, we discussed the current knowledge on the main roles played by the different

glial Na(+) -dependent ion transporters, including Na(+) /K(+) ATPase, Na(+) /Ca(2+) exchangers, Na(+) /H(+) exchangers, Na(+) -K(+) -Cl(-) cotransporters, and Na(+) - HCO3- cotransporter in the pathophysiology of the diverse CNS diseases. We highlighted their contributions in cell survival, synaptic pathology, gliotransmission, pH homeostasis, and their role in glial activation, migration, gliosis, inflammation, and tissue repair processes. Therefore, this review summarizes the foundation work for targeting Na(+) -dependent ion transporters in glia as a novel strategy to control important glial activities associated with Na(+) dynamics in different neurological disorders. GLIA 2016;64:1677-1697.
Glia, 2016; 64

25633096: Boscia F, Casamassa A, Secondo A, Esposito A, Pannaccione A, Sirabella R, Pignataro G, Cuomo O, Vinciguerra A, de Rosa V, Annunziato L
NCX1 Exchanger Cooperates with Calretinin to Confer Preconditioning-Induced Tolerance Against Cerebral Ischemia in the Striatum.
Recently, the Na(+)/Ca(+2) exchanger NCX1 and the calcium binding protein calretinin have emerged as new molecular effectors of delayed preconditioning in the brain. In the present study, we investigated whether NCX1 and calretinin cooperate within the preconditioned striatum to confer neurons greater resistance to degeneration. Confocal microscopy analysis revealed that NCX1 expression was upregulated in calretinin-positive interneurons in the rat striatum after tolerance induction. Consistently, coimmunoprecipitation assays performed on human SHSY-5Y cells, a neuronal cell line which constitutively expresses calretinin, revealed a binding between NCX1 and calretinin. Finally, silencing of calretinin expression, both in vitro and in vivo, significantly prevented preconditioning-induced neuroprotection. Interestingly, our biochemical and functional studies showed that the selective silencing of calretinin in brain cells significantly prevented not only the preconditioning-induced upregulation of NCX1 expression and activity but also the activation of the prosurvival protein kinase Akt, which is involved in calretinin and NCX1 protective actions. Collectively, our results indicate that the Na(+)/Ca(+2) exchanger NCX1 and the calcium binding protein calretinin cooperate within the striatum to confer tolerance against cerebral ischemia.
Mol Neurobiol, 2016; 53

23069678: Formisano L, Guida N, Valsecchi V, Pignataro G, Vinciguerra A, Pannaccione A, Secondo A, Boscia F, Molinaro P, Sisalli MJ, Sirabella R, Casamassa A, Canzoniero LM, Di Renzo G, Annunziato L
NCX1 is a new rest target gene: role in cerebral ischemia.
The Na(+)-Ca(2+) exchanger 1 (NCX1), a bidirectional transporter that mediates the electrogenic exchange of one calcium ion for three sodium ions across the plasma membrane, is known to be involved in brain ischemia. Since the RE1-silencing transcription factor (REST) is a key modulator of neuronal gene expression in several neurological conditions, we studied the possible involvement of REST in regulating NCX1 gene expression and activity in stroke. We found that: (1) REST binds in a sequence specific manner and represses through H4 deacetylation, ncx1 gene in neuronal cells by recruting CoREST, but not mSin3A. (2) In neurons and in SH-SY5Y cells REST silencing by siRNA and site-direct mutagenesis of REST consensus sequence on NCX1 brain promoter determined an increase in NCX1 promoter activity. (3) By contrast, REST overexpression caused a reduction in NCX1 protein expression and activity. (4) Interestingly, in rats subjected to transient middle cerebral artery occlusion (tMCAO) and in organotypic hippocampal slices or SH-SY5Y cells exposed to oxygen and glucose deprivation (OGD) plus reoxygenation (RX), the increase in REST was associated with a decrease in NCX1. However, this reduction was reverted by REST silencing. (5) REST knocking down, along with the deriving NCX1 overexpression in the deep V and VIb cortical layers caused a marked reduction in infarct volume after tMCAO. Double silencing of REST and NCX1 completely abolished neuroprotection induced by siREST administration. Collectively, these results demonstrate that REST, by regulating NCX1 expression, may represent a potential druggable target for the treatment of brain ischemia.
Neurobiol Dis, 2013; 50

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BOARD NUMBER: S02-684

DETAILED ANALYSIS OF CORTICAL MICROGLIA MORPHOLOGY THROUGHOUT LIFE

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Aims. Microglia are long-lived cells that constantly monitor their microenvironment. In order to accomplish this task, microglia constantly change their morphologies under normal physiological conditions in both the short and long term. We investigated the morphological variation that microglia undergo across lifespan. **Methods.** Using semimanual and semiautomatic methods to quantify cortical microglia morphology. Data was obtained by confocal imaging the cortex of heterozygous TgH(CX3CR1-EGFP) mice. We were able to generate a detailed quantification of physiological morphologies of microglia at different time points of age, starting immediately postnatal (5, 10 and 20 days) and in young adult (20 weeks), early aged (58 and 84 weeks) and old aged (110 weeks) animals. **Results.** Basic microglia characteristics such as cellular density or the mean space occupied by each cell change over time. This is accompanied by a plethora of changes in the microglia ramifications, with its total length, number of branches also changes as time passes. Detailed cellular arbor analysis revealed differences in microglia morphology that are age-related, with mean branch length and the number of terminal processes changing the most over time. **Conclusions.** Our study provides insight into changes in microglia morphology across the lifespan and details the morphological appearance of microglia under normal conditions, with changes that are the result of the unique identity and nature of microglia.

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BOARD NUMBER: S02-685

MICROGLIA ARE REQUIRED FOR BRAIN INTEGRITY DURING EMBRYONIC MORPHOGENESIS

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Microglia, the brain resident macrophages, can adopt various cellular and transcriptional states, a remarkable feature initially associated to ageing and disease, but also found during normal postnatal development. In the embryonic brain, microglia transiently accumulate at stereotyped "hotspots" such as the corticostriatoamygdalar boundary (CSA), but their roles have remained elusive. Here, using transient depletion and genetic models, we showed that embryonic absence of microglia leads to a cavitary lesion at the CSA, a site where accumulating microglia share a common signature with the postnatal state previously identified as axon tract associated microglia (ATM). This embryonic ATM-like profile was observed at sites of morphogenetic stress that display fragilities during normal development - such as the CSA but also the subcallosal midline - and was strikingly induced by experimentally-driven mechanical damage. Remarkably, these cavitary lesions were transient, as they resorbed in concert with the progressive postnatal repopulation of microglia following embryonic depletion. At the molecular level, we found that core ATM factor Spp1/Osteopontin is partially required for brain integrity at the CSA and subcallosal midline. Taken together, our study reveals a novel role for embryonic microglia in the maintenance of brain integrity during morphogenesis and highlights a link between a specific microglial state and tissue cohesion, with major implications for our understanding of microglial functions in health and disease. * Alice Canzi and Cécile Bridlance contributed equally to this work ° Morgane S. Thion, Ludmilla Lokmane and Sonia Garel contributed equally to this work

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MICROGLIAL P2X4 IN NEUROPATHIC PAIN AND ASSOCIATED COMORBIDITIES: A GENDER INDEPENDENT STORY?

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Université de Montpellier, CNRS, INSERM, Institut De Génomique Fonctionnelle, Montpellier, France

Aims P2X4 purinergic receptors are involved in spinal hyperexcitability during neuropathic pain. In males, it has been described that their activation by ATP in spinal reactive microglia leads to an autocrine release of BDNF (Brain Derived Neurotrophic Factor), responsible for the development of a hyperexcitability and a hyperalgesia. However, recent studies suggest that a P2X4-independent pathway is developed in females. Yet, detailed mechanisms of this potential dimorphism are not understood. Moreover, neuropathic pain is associated with chronic comorbidities such as anxiety and depression-like states that have been suggested to be due to microglia and P2X4 receptors. The aims of this study are 1/to determine whether microglial P2X4 is involved in neuropathic pain in female 2/in which extent P2X4 is involved in neuropathic pain associated comorbidities. **Methods** We perform neuropathy using WT, $P2X4^{-/-}$ and specific microglial P2X4 invalidated mice ($Cx3Cr1Cre^{ERT2}$ x $P2X4^{lox/lox}$ and $Sall1Cre^{ERT2}$ x $P2X4^{lox/lox}$) or inhibit specific P2X4 dependent pathway with pharmacological tools. **Results** Our results indicate a similar *de novo* expression of P2X4 in spinal microglia in male and female mice that is correlated with the neuropathy-induced mechanical hyperalgesia. The genetic deletion or the pharmacological inhibition of P2X4 also reverse hyperalgesia in both sexes. Whereas WT mice of both genders develop chronic anxio-depressive behaviors, microglial P2X4 invalidated mice do not present such comorbidities. **Conclusions** P2X4 in spinal microglia is required for the neuropathy-induced mechanical hyperalgesia in both sexes. Our results also indicate that P2X4 are involved in the development of associated psychiatric comorbidities.

Pubmed:
29458097: Crouzier L, Gilabert D, Rossel M, Trousse F, Maurice T
Topographical memory analyzed in mice using the Hamlet test, a novel complex maze.
The Hamlet test is an innovative device providing a complex environment for testing topographic memory in mice. Animals were trained in groups for weeks in a small village with a central agora, streets expanding from it towards five functionalized houses, where they can drink, eat, hide, run, interact with a stranger mouse. Memory was tested by depriving mice from water or food and analyzing their ability to locate the Drink/Eat house. Exploration and memory were analyzed in different strains, gender, and after different training periods and delays. After 2 weeks training, differences in exploration patterns were observed between strains, but not gender. Neuroanatomical structures activated by training, identified using FosB/ΔFosB immunolabelling, showed an involvement of the hippocampus-subiculum-parahippocampal gyrus axis and dopaminergic structures. Training increased hippocampal neurogenesis (cell proliferation and neuronal maturation) and modified the amnesic efficacy of muscarinic or nicotinic cholinergic antagonists. Moreover, topographical disorientation in Alzheimer's disease was addressed using intracerebroventricular injection of amyloid β peptide in trained mice. When retested after 7 days, Aβ-treated mice showed memory impairment. The Hamlet test specifically allows analysis of topographical memory in mice, based on complex environment. It offers an innovative tool for various ethological or pharmacological research needs. For instance, it allowed to examine topographical disorientation, a warning sign in Alzheimer's disease.
Neurobiol Learn Mem, 2018; 149

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BOARD NUMBER: S02-687

NEONATAL MICROGLIA PHENOTYPING BY FLOW CYTOMETRY: IMPACT OF INFLAMMATION

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Background: Perinatal inflammation is a leading cause of neurodevelopmental injuries including periventricular white matter injury (PWMI) in the premature newborn. Brain inflammation alters development through processes including activation of microglia toward a pro-inflammatory and neurotoxic phenotype causing white matter defect. Investigating microglial phenotype in the context of inflammatory insult could be an effective approach to find therapeutic targets. **Methods:** PWMI was induced by intra-peritoneal interleukine-1-beta (IL-1b) injections from P1 to P3. At P3, whole brain was dissociated (Miltenyi Biotec) and immunostained with fluorophore-conjugated antibodies (Sony Biotechnology, Biolegend, BD Biosciences) and a viability probe (BD). FACS analysis was done within 24h. After doublets and dead cells exclusion, the impact of IL-1b was evaluated in microglia, defined as $CD11b^{+}/CD45^{int}$ cells, on P2RY12, CX3CR1, and CD18 expression. In addition, intracellular presence of VGLUT1 was analyzed to evaluate the level of synaptic phagocytosis. Infiltrating myeloid cells, defined as $CD11b^{+}/CD45^{high}$ cells, were further characterized with CCR2, Ly-6G and Ly-6C expression, to evaluate polymorphonuclear neutrophils (PMNs) and monocytes (Mo) proportions. **Results:** In microglia, IL-1b increased CD11b, P2RY12 and CD18 and decreased CX3CR1. Intracellular VGLUT1 was higher in IL-1b than in control animals, suggesting that inflammation enhanced synaptic phagocytosis, that might be deleterious. IL-1b also leads to brain infiltration of myeloid cells, which express PMNs and Mo markers. **Conclusion:** Multi-parameters flow cytometry is a convenient tool to analyze microglia phenotype and function, and brain recruitment of immune cells in early cerebral inflammation. Further analysis could precise the phenotype variations along time, and the impact of myeloid infiltrate on PWMI.

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3D MICROSCOPY REVEALS NEW DETAILS IN AMYLOID PLAQUE-MICROGLIA CROSSTALK IN ALZHEIMER'S DISEASE

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Maria H. Gotkiewicz, Heikki Tanila.
A. I. Virtanen Institute, University of Eastern Finland, 70210 Kuopio, FINLAND **Background:** In the past years, research on Alzheimer's Disease (AD) has emphasized the role of microglia and neuroinflammation in the disease progression. **Aims:** We aimed to define morphological details of microglia clustered around different types of amyloid plaques (AP) and the types of contact they make with them. **Methods:** We cross-bred APP/PS1 mice with CX3CR1-GFP mice exhibiting green fluorescent microglia, and studied them at 6 and 13 months. We stained 100 µm sections at the dorsal hippocampus with anti-beta-amyloid D54D2 antibody. Sections were mounted with DAPI in the mounting medium. Z-stack imaging and 3D rendering was performed with ZEISS LSM800 Airyscan x63 objective. **Results:** We collected 3D images of 54 clusters in 13-month-old mice and 29 clusters in 6-month-old mice. We found that DAPI selectively stained the plaque core. We created our own scale for the DAPI + core intensity. The DAPI+ score correlated positively (Spearman rho) with the plaque diameter (13 months: r=0.63, p=0.0001; 6 months: r=0.43 p=0.02. We also observed that number of microglia in a cluster correlated positively with the plaque diameter. **Conclusion:** 3D imaging and rendering of AP microglia cluster shed new light on microglia reaction to APs. Images in 3D show details that are not visible in 2D light microscopy and might be crucial for understanding the function of microglia in AD. Supported by the Doctoral Progamme of Molecular Medicine at UEF

Pubmed:

32560730: Kolosowska N, Gotkiewicz M, Dhungana H, Giudice L, Giugno R, Box D, Huuskonen MT, Korhonen P, Scoyni F, Kanninen KM, Ylä-Herttuala S, Turunen TA, Turunen MP, Koistinaho J, Malm T
Intracerebral overexpression of miR-669c is protective in mouse ischemic stroke model by targeting MyD88 and inducing alternative microglial/macrophage activation.
Ischemic stroke is a devastating disease without a cure. The available treatments for ischemic stroke, thrombolysis by tissue plasminogen activator, and thrombectomy are suitable only to a fraction of patients and thus novel therapeutic approaches are urgently needed. The neuroinflammatory responses elicited secondary to the ischemic attack further aggravate the stroke-induced neuronal damage. It has been demonstrated that these responses are regulated at the level of non-coding RNAs, especially miRNAs.
J Neuroinflammation, 2020; 17

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EXPLORING THE CONTRIBUTION OF MICROGLIAL NGF-TRKA SIGNALING IN HEALTH AND DISEASE

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Aim. Alterations in the Nerve Growth Factor (NGF) and its high-affinity Tropomyosin Receptor Kinase A (TrkA) signaling is thought to be a driving cause of the striking loss of cholinergic neurons in Alzheimer's disease (AD). However, being strongly multifactorial, AD cannot be simply recapitulated by the NGF-TrkA signaling disruption in neurons of the Central Nervous System (CNS), leading to hypothesize other NGF-responsive cell populations in the CNS. We identified neuroinflammation in an AD mouse model induced by NGF neutralization, pointing our attention to the immune cells resident in the CNS, microglia. Moreover, recently we also discovered the potent immunomodulatory properties of NGF via TrkA on microglia. Hence, our work aims to grasp the role of NGF-TrkA signaling in microglia and how this may contribute to neurodegeneration. **Results and Methods**. To achieve this, we have generated a transgenic mouse carrying a specific and tamoxifen-inducible TrkAKO in microglia. First, we report that microglia express TrkA *in vivo*, both via immunofluorescence staining on mouse brain slices and via mRNA/protein detection in microglia cells isolated from adult mouse brains, and that this TrkA expression is efficiently dampened by tamoxifen administration. Moreover, we report that knocking out TrkA in microglia leads to remarkable differences in microglial density and morphology in several brain and spinal cord areas. Transgenic mice have also revealed deficits in motor learning and a significantly reduced response to pain. **Conclusion**. This indicates the importance of TrkA signaling in microglia and calls for a closer look at their role in AD neurodegeneration.

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MICROGLIA POLARIZATION AND ITS MODULATION BY ENVIRONMENTAL FACTORS

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Microglia are the immune cells of the central nervous system (CNS). These cells are typically in a resting state, characterized by elongated, ramified processes and a small cellular body. They constantly probe their environment with cytoplasmic extensions and are “activated” in response to a variety of stimuli. Upon recruitment, these cells can acquire diverse phenotypes that display morphological and phenotypic changes. During the M1 state, considered neurotoxic, microglia retract their processes to adopt an amoeboid morphology promoting their multiplication and migration. In this state, they secrete cytotoxic and pro-inflammatory molecules. Conversely, in the M2 state, considered protective and anti-inflammatory, microglial cells have immunomodulatory functions. It was suggested that microglia play multiple and complex roles in the pathophysiology of numerous CNS diseases. While the M2 is beneficial, the M1 state precedes and underlies disease processes. In this context, the possibility of manipulating the activation of microglia and more importantly to trigger their passage from an M1 to an M2 state is a major therapeutic issue. We characterized the impact of the substrate and different soluble molecules on the phenotype of a murine microglial cell line. Our results indicate that some molecules (polymers, amino acids, etc.) induce morphological changes that may be associated with M1 or M2 states. Calcium imaging and molecular biology experiments are underway and should allow us to identify, in living cells, the resting state as well as the M1 and M2 states. Our study should ultimately make it possible to selectively control the functional states of microglia.

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MICROGLIAL FUNCTIONS: FROM THE CONTROL OF IMMUNE RESPONSE TO THE REGULATION OF ENERGY HOMEOSTASIS

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Microglia are brain resident immune cells. They are the first line of defense in CNS disturbances. In order to better understand the role of microglial function, we genetically ablate ASC, an essential component of intracellular inflammasome, in microglia in mouse. Mice expressing tamoxifen-inducible Cre-recombinase in CX3CR1^{+} cells were crossed with mice harboring conditional alleles for *Asc*. Treating progeny with tamoxifen generated *Asc*^{MgKO} mice in which ASC was deleted specifically in CX3CR1^{+} cells. Contrary to peripheral CX3CR1^{+} cells which are rapidly replaced by precursors, microglia present a slow turnover in the adult brain. Thus, 5-weeks after tamoxifen injections, the genetic recombination persisted in microglia while it was transient in periphery. We generated a transgenic mouse line with specific *Asc* gene deletion in microglia. After intraperitoneal injection of lipopolysaccharide, body weight and rectal temperature were higher in *Asc*^{MgKO} mice than controls. However, cell blood count revealed similar peripheral immune response in both groups. This indicates that *Asc*^{MgKO} mice were resistant to LPS challenge, and suggests that microglia are key factors that aggravate sepsis response. Furthermore, *Asc*^{MgKO} mice exhibited aberrant control of food intake during normal physiology. Food intake of *Asc*^{MgKO} mice was normal under standard diet but increased under high-fat-diet. This hyperphagic behaviour under HFD reveals that microglial ASC is involved in the acute homeostatic response to dietary fat, limiting excessive calorie intake during short-term HFD. These findings confirm that microglial inflammasome is implicated in the control of immune response, and reveal its contribution in the energy homeostasis regulation during normal physiology.

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OPTOGENETIC INVESTIGATION OF MICROGLIA DYNAMICS DURING ZEBRAFISH BRAIN DEVELOPMENT

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

Odessa Tanvé[1,2], Éric Bergeron[1], Gabriel Byatt[1,3], Mado Lemieux[1], Paul De Koninck[1,3]
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Microglia are brain cells involved in different functions, such as immune response or brain development. Through their phagocytic activity, microglia can eliminate cells or prune synapses, thereby controlling the formation of neuronal networks. A potentially important influence in this process is the intestinal microbiota, which has been shown to modulate the microglia. To learn more on the impact of the gut microbiota on the phagocytic function of microglia during CNS development, we are establishing an optogenetic larval zebrafish model. We are using a cross between two transgenic lines to visualize glutamatergic neurons in red (DsRed) and microglia in green (GFP). Larvae from 3-16dpf (day post-fertilization), either rendered germ-free or colonized with specific bacteria, are imaged live using confocal or 2-photon microscopy. We are developing deep-learning based tools to categorize microglia morphology and quantify phagocytosis. Our preliminary results suggest that microglia change their morphology during the development of zebrafish brain circuits. In the optic tectum, they are minimally branched and very mobile in 3-5dpf larvae. At 5-6dpf, their protrusions are longer and more branched. At 7dpf, microglia are mostly immobile, while their branched protrusions are moving to scan the nearby environment. Phagocytic function is detected by the inclusion of DsRed from the neurons inside the microglia, as phagosome-like structures. We find that the extent of phagocytosis differentially changes over the 3-7dpf period. We are now comparing how this activity is affected by changes in gut microbiota. We aim to exploit this model to characterize the role of microglia in synaptic pruning.

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DIFFERENTIAL EFFECTS OF URBAN PARTICULATE MATTER ON BV2 MICROGLIAL-LIKE AND C17.2 NEURAL STEM/PRECURSOR CELLS

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

Rebecca Morris[1,2], Claire Thornton[3], Serena Counsell[2], Imelda Mcgonnell[3], Gwladys Chabrier[3]
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Intro Air pollution affects the majority of the world's population and has been linked to over 7 million premature deaths per year. There is increasing evidence that exposure to air pollution *in utero* and in early childhood is associated with altered brain development. While oxidative stress and neuroinflammation are documented consequences of PM exposure, cell-specific mechanisms that may be triggered in response to air pollution exposure are less well defined. **Aims** We aimed to investigate the effect of urban (U)PM exposure on microglial-like BV2 and neural stem/precursor-like C17.2, two cell lines differing in source and maturity, to identify common and cell-specific mechanisms. **Methods** BV2 and C17.2 cells were treated with UPM (0-100µg/ml) for 24-72h. Cell health measurements (LDH, proliferation), neuroinflammation (cytokine expression) and oxidative stress (ROS production, NRF2 expression) were quantified. The effect of UPM exposure on mitochondrial function was also determined (TMRM, mitoTEMPO). **Results** We found that BV2 cells were more susceptible to UPM-mediated oxidative stress and cell death than immature C17.2 cells, evidence by a ~70% decrease in cell number in the 100µg/ml UPM-treated BV2 cells compared to control, whereas there was a ~20% decrease in the C17.2 cells. UPM exposure resulted in decreased mitochondrial TMRM intensity and increased mitochondrial ROS in BV2 cells, which could be prevented by mitoTEMPO antioxidant treatment. **Conclusions** UPM has cell-specific mechanistic outcomes dependent on cell type and maturity. Our data suggest that mitochondrial dysfunction may drive UPM-mediated cytotoxicity in BV2 cells, linked to inadequate antioxidant defences.

Pubmed:
33751627: Morris RH, Counsell SJ, McGonnell IM, Thornton C
Early life exposure to air pollution impacts neuronal and glial cell function leading to impaired neurodevelopment.
The World Health Organisation recently listed air pollution as the most significant threat to human health. Air pollution comprises particulate matter (PM), metals, black carbon and gases such as ozone (O), nitrogen dioxide (NO) and carbon monoxide (CO). In addition to respiratory and cardiovascular disease, PM exposure is linked with increased risk of neurodegeneration as well as neurodevelopmental impairments. Critically, studies suggest that PM crosses the placenta, making direct in utero exposure a reality. Rodent models reveal that neuroinflammation, neurotransmitter imbalance and oxidative stress are triggered following gestational/early life exposure to PM, and may be exacerbated by concomitant mitochondrial dysfunction. Gestational PM exposure (potentiated by mitochondrial impairment in the metabolically active neonatal brain) not only impacts neurodevelopment but may sensitise the brain to subsequent cognitive impairment. Having reviewed this field, we conclude that strategies are urgently required to reduce exposure to PM during this sensitive developmental period.
Bioessays, 2021; 43

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IGF-1 GENE THERAPY ON DOPAMINERGIC NEURONS AND GLIAL CELLS INTERACTION IN EARLY COGNITIVE DEFICITS IN A NEURODEGENERATIVE ANIMAL MODEL

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Parkinson´s disease is a neurodegenerative disorder with a progressive dopaminergic (DA) neuronal loss and a variety of non-motor symptoms, such as cognitive dysfunctions. IGF-1 neuroprotective effects could be, in part, due to changes in the activity of neurons and glia. Aims: 1) to determine the early cognitive decline and the correlation of hippocampal changes in 6OHDA model, 2) to carry out therapeutic approaches with IGF-1 and 3) to analyze modifications on glial cells through different brain areas involved in the proposed circuit. Male Wistar rats were divided into 6 groups according to CPu bilaterally injection with 6OHDA or vehicle (SHAM) and the adenoviral therapy in hippocampus with RAd-DSRed or RAd-IGF1. At 3 weeks, rats were tested for behavioral tasks of cognitive performance and locomotor activity induced by amphetamine. Then rats were perfused, the brains fixed and IHC performed for TH and Iba-1 and GFAP for glial cells. Results: At 20 days post-lesion, memory deficits were observed in 6OHDA rats compared to SHAM rats. This cognitive decline was partially modified with IGF1 gene therapy. We observed changes in GFAP+ astrocytes mainly in the group with IGF-1 and changes of TH expression. IGF-1 gene therapy restored memory impairments and modified cellular activity. Knowledge of this potential therapeutic strategy with IGF-1 gene therapy motivates us to further studies under this experimental model

Pubmed:

34339780: Herrera ML, Bandín S, Champarini LG, Hereñú CB, Bellini MJ
Intramuscular insulin-like growth factor-1 gene therapy modulates reactive microglia after traumatic brain injury.
Reactive gliosis is a key feature and an important pathophysiological mechanism underlying chronic neurodegeneration following traumatic brain injury (TBI). In this study, we have explored the effects of intramuscular IGF-1 gene therapy on reactive gliosis and functional outcome after an injury of the cerebral cortex. Young adult male rats were intramuscularly injected with a recombinant adenoviral construct harboring the cDNA of human IGF-1 (RAd-IGF1), with a control vector expressing green fluorescent protein (RAd-GFP) or PBS as control. Three weeks after the intramuscular injections of adenoviral vectors, animals were subjected to a unilateral penetrating brain injury. The data revealed that RAd-IGF1 gene therapy significantly increased serum IGF1 levels and improved working memory performance after one week of TBI as compared to PBS or RAd-GFP lesioned animals. At the same time, when we analyzed the effects of therapy on glial scar formation, the treatment with RAd-IGF1 did not modify the number of glial fibrillary acidic protein (GFAP) positive cells, but we observed a decrease in vimentin immunoreactive astrocytes at 7 days post-lesion in the injured hemisphere compared to RAd-GFP group. Moreover, IGF-1 gene therapy reduced the number of Iba1+ cells with reactive phenotype and the number of MHCII + cells in the injured hemisphere. These results suggest that intramuscular IGF-1 gene therapy may represent a new approach to prevent traumatic brain injury outcomes in rats.
Brain Res Bull, 2021; 175
32048766: Herrera ML, Basmadjian OM, Falomir-Lockhart E, Dolcetti FJ, Hereñú CB, Bellini MJ
Sex frailty differences in ageing mice: Neuropathologies and therapeutic projections.
It is well-established that females live longer than males. Paradoxically, women tend to have poorer health, a condition often named sex frailty. The aim of this study was to evaluate possible frailty predictors in older mice in a sex-specific manner, in order to employ these predictors to follow-up therapy efficiency. To further evaluate therapy effects, we also investigated the use of neurotrophic insulin-like growth factor 1 (IGF-1) gene therapy and its correlation with the expression of this frailty and emotional behaviour. In order to evaluate frailty, we employed two different approaches. We performed a frailty assessment through a 31-Item Clinical Frailty Index and through a Performance-Based 8-Item Frailty Index. Our results show that both indexes are in concordance to evaluate sex differences, but they do not correlate when evaluating IGF-1 therapy effects. Moreover, in order to reduce test-to-test variability for measures of dependent variables, we compared open field results

across studies assessing sex and treatment by means of the z-score normalization. The data show that regular open field parameters submitted to z-score normalization analysis could be a useful tool to identify sex differences in ageing mice after growth factor therapies. Taking this into account, sex is a factor that influences the incidence and/or nature of all major complex diseases; the main outcome of our investigation is the development of an efficient tool that compares the use of different frailty index calculations. This represents an important strategy in order to identify sex differences and therapy efficiency in ageing models.
Eur J Neurosci, 2020; 52
31229647: Herrera ML, Basmadjian OM, Falomir Lockhart E, Dolcetti FJ, Hereñú CB, Bellini MJ
Novel adenoviral IGF-1 administration modulates the association between depressive symptoms and aging: Does gender matter?
Depression is an illness of multifactorial origin and it seems to involve the dysregulation of many physiological processes. It also has been associated with age and a decreased in the expression of some neurotrophins. However, there are not unique animal models to assay depressive-like behavior, with male and females responding differently. In this study, we report the effects of gender on aged associated depressive signs as frailty, muscular strength and motor activity, as well as the role of intramuscular IGF-1 gene therapy in these processes. We found that male mice had higher general discomfort than females. Moreover, we observed that IGF-1 treatment did not modify this index in females. Regarding male mice, adenoviral IGF-1 injection reduced frailty scores compared to its adenoviral control. According to data, IGF-1 gene therapy had a positive effect on depressive associated hypo-locomotion activity as indicate by delta of total distance and the increment observed in time of mobility in male mice. This neurotrophic factor also increased the latency of time to fall in grip strength in male mice compared to female mice. Moreover, we observed that, while the therapy had no effect on the digging behavior, IGF-1 treatment diminished the latency to dig and increase the number of buried marbles in male mice, having no effect on female. The present study demonstrates that, in order to establish an animal model of depression both, gender and age are relevant variables/factors to consider. We also conclude that a frailty phenotype underlies depressive-like symptoms in an experimental mouse model. Furthermore, we demonstrated that intramuscular injection represents a less invasive, feasible and controllable route of IGF-1 gene delivery for the treatment of the depressive phenotype in old mice.
Behav Brain Res, 2019; 372
29936317: Deza-Ponzio R, Herrera ML, Bellini MJ, Virgolini MB, Hereñú CB
Aldehyde dehydrogenase 2 in the spotlight: The link between mitochondria and neurodegeneration.
Growing body of evidence suggests that mitochondrial dysfunctions and resultant oxidative stress are likely responsible for many neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). Aldehyde dehydrogenase (ALDH) superfamily plays a crucial role in several biological processes including development and detoxification pathways in the organism. In particular, ALDH2 is crucial in the oxidative metabolism of toxic aldehydes in the brain, such as catecholaminergic metabolites (DOPAL and DOPEGAL) and the principal product of lipid peroxidation process 4-HNE. This review aims to deepen the current knowledge regarding to ALDH2 function and its relation with brain-damaging processes that increase the risk to develop neurodegenerative disorders. We focused on relevant literature of what is currently known at molecular and cellular levels in experimental models of these pathologies. The understanding of ALDH2 contributions could be a potential target in new therapeutic approaches for PD and AD due to its crucial role in mitochondrial normal function maintenance that protects against neurotoxicity.
Neurotoxicology, 2018; 68
34234671: Montivero AJ, Ghersi MS, Silvero C MJ, Artur de la Villarmois E, Catalan-Figueroa J, Herrera M, Becerra MC, Hereñú CB, Pérez MF
Early IGF-1 Gene Therapy Prevented Oxidative Stress and Cognitive Deficits Induced by Traumatic Brain Injury.
Traumatic Brain Injury (TBI) remains a leading cause of morbidity and mortality in adults under 40 years old. Once primary injury occurs after TBI, neuroinflammation and oxidative stress (OS) are triggered, contributing to the development of many TBI-induced neurological deficits, and reducing the probability of critical trauma patients´ survival. Regardless the research investment on the development of anti-inflammatory and neuroprotective treatments, most pre-clinical studies have failed to report significant effects, probably because of the limited blood brain barrier permeability of no-steroidal or steroidal anti-inflammatory drugs. Lately, neurotrophic factors, such as the insulin-like growth factor 1 (IGF-1), are considered attractive therapeutic alternatives for diverse neurological pathologies, as they are neuromodulators linked to neuroprotection and anti-inflammatory effects. Considering this background, the aim of the present investigation is to test early IGF-1 gene therapy in both OS markers and cognitive deficits induced by TBI. Male Wistar rats were injected Cisterna Magna with recombinant adenoviral vectors containing the IGF-1 gene cDNA 15 min post-TBI. Animals were sacrificed after 60 min, 24 h or 7 days to study the advanced oxidation protein products (AOPP) and malondialdehyde (MDA) levels, to recognize the protein oxidation damage and lipid peroxidation respectively, in the TBI neighboring brain areas. Cognitive deficits were assessed by evaluating working memory 7 days after TBI. The results reported significant increases of AOPP and MDA levels at 60 min,

24 h, and 7 days after TBI in the prefrontal cortex, motor cortex and . In addition, at day 7, TBI also reduced working memory performance. Interestingly, AOPP, and MDA levels in the studied brain areas were significantly reduced after IGF-1 gene therapy that in turn prevented cognitive deficits, restoring TBI-animals working memory performance to similar values regarding control. In conclusion, early IGF-1 gene therapy could be considered a novel therapeutic approach to targeting neuroinflammation as well as to preventing some behavioral deficits related to TBI.
Front Pharmacol, 2021; 12
32466659: Herrera ML, Deza-Ponzio R, Ghersi MS, de la Villarmois EA, Virgolini MB, Pérez MF, Molina VA, Bellini MJ, Hereñú CB
Early Cognitive Impairment Behind Nigrostriatal Circuit Neurotoxicity: Are Astrocytes Involved?
Cognitive dysfunction is one of the most severe nonmotor symptoms of nigrostriatal impairment. This occurs as a result of profound functional and morphological changes of different neuronal circuits, including modifications in the plasticity and architecture of hippocampal synapses. Such alterations can be implicated in the genesis and progression of dementia associated with neurodegenerative diseases including Parkinson-like symptoms. There are few studies regarding cognitive changes in nigrostriatal animal models. The aim of this study was to characterize the onset of memory deficit after induction of neurotoxicity with 6-hydroxydopamine (6-OHDA) and its correlation with hippocampal dysfunction. For this, we bilaterally microinjected 6-OHDA in dorsolateral Caudate-Putamen unit (CPu) and then, animals were tested weekly for working memory, spatial short-term memory, and motor performance. We evaluated tyrosine hydroxylase (TH) as a dopamine marker, aldehyde dehydrogenase 2 (ALDH2), a mitochondria detoxification enzyme and astrocyte glial fibrillar acid protein (GFAP) an immunoreactivity marker involved in different areas: CPu, substantia nigra, prefrontal cortex, and hippocampus. We observed a specific prefrontal cortex and nigrostriatal pathway TH reduction while ALDH2 showed a decrease-positive area in all the studied regions. Moreover, GFAP showed a specific CPu decrease and hippocampus increase of positively stained area on the third week after toxicity. We also evaluated the threshold to induce long-term potentiation in hippocampal excitability. Our findings showed that reduced hippocampal synaptic transmission was accompanied by deficits in memory processes, without affecting motor performance on the third-week post 6-OHDA administration. Our results suggest that 3 weeks after neurotoxic administration, astrocytes and ALDH2 mitochondrial enzyme modifications participate in altering the properties that negatively affect hippocampal function and consequently cognitive behavior.
ASN Neuro, 2020 Jan-Dec; 12

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BOARD NUMBER: S02-695

SORLA SHAPES FUNCTIONAL PROPERTIES OF ACTIVATED MICROGLIA

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Aims: VPS10P domain receptors are primarily recognized for their roles in intracellular sorting in neurons. They are crucial for maintaining neuronal integrity and viability, while their dysfunctions are linked to neurodegenerative disorders. Emerging evidence suggests that VPS10P receptors can also play important roles in non-neuronal cells, including microglia. Those cells acquire diverse functional properties depending on the brain's pathophysiological context. We hypothesized that SorLA, a member of the VPS10P family, shapes functional properties of microglia thereby influencing the pathogenesis of brain disorders. As microglia supports the progression of glioma, we explored the function of SorLA in experimental models of glioma. **Methods**: Available RNA-seq datasets and our *in vitro* experiments were examined to compare *SorLA* expression levels in microglia under various conditions. We evaluated the impact of pro- and anti-inflammatory factors on SorLA levels and compared the composition of secretomes of wild type (WT) and *SorLA*($^{-/-}$) murine microglia under control and pro-inflammatory conditions, by using unbiased mass spectrometry and targeted ELISA assays. **Results:** We confirmed a dependence of the *SorLA* expression in microglia on their pro- or anti-inflammatory activation states. The proteomics study revealed potential partners of SorLA, which likely participate in microglial responses to different pathological conditions. **Conclusions**: SorLA controls the pro- versus anti-inflammatory properties of microglia by regulating secretion of bioactive factors. Studies were supported by the National Science Center, Poland (2020/37/B/NZ3/00761, AM) and Foundation for Polish Science (POIR.04.04.00 00 5CEF/18 00, AM).

Pubmed:
34200797: Zaręba-Kozioł M, Bartkowiak-Kaczmarek A, Roszkowska M, Bijata K, Figiel I, Halder AK, Kamińska P, Müller FE, Basu S, Zhang W, Ponimaskin E, Włodarczyk J
S-Palmitoylation of Synaptic Proteins as a Novel Mechanism Underlying Sex-Dependent Differences in Neuronal Plasticity. Although sex differences in the brain are prevalent, the knowledge about mechanisms underlying sex-related effects on normal and pathological brain functioning is rather poor. It is known that female and male brains differ in size and connectivity. Moreover, those differences are related to neuronal morphology, synaptic plasticity, and molecular signaling pathways. Among different processes assuring proper synapse functions are posttranslational modifications, and among them, S-palmitoylation (S-PALM) emerges as a crucial mechanism regulating synaptic integrity. Protein S-PALM is governed by a family of palmitoyl acyltransferases, also known as DHHC proteins. Here we focused on the sex-related functional importance of DHHC7 acyltransferase because of its S-PALM action over different synaptic proteins as well as sex steroid receptors. Using the mass spectrometry-based PANIMoni method, we identified sex-dependent differences in the S-PALM of synaptic proteins potentially involved in the regulation of membrane excitability and synaptic transmission as well as in the signaling of proteins involved in the structural plasticity of dendritic spines. To determine a mechanistic source for obtained sex-dependent changes in protein S-PALM, we analyzed synaptoneurosomes isolated from DHHC7-/- (DHHC7KO) female and male mice. Our data showed sex-dependent action of DHHC7 acyltransferase. Furthermore, we revealed that different S-PALM proteins control the same biological processes in male and female synapses.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S02-696

DIESEL EXHAUST PARTICLES (DEP) IN THE ONSET AND PROGRESSION OF NEUROINFLAMMATION

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Ambient air pollution (AP) is becoming a major health problem that affects millions of people worldwide. AP is contamination of the outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere. Pollutants of major public health concern include particulate matter (PM) of different size, mostly ascribed to diesel exhaust particles (DEP), organic compounds and metals. It is well known that AP causes respiratory and cardiovascular diseases, but recent extensive evidence indicates that it may also negatively affect the brain and contribute to central nervous system (CNS) diseases. AP is believed to exert its neurotoxic function through oxidative stress, glial activation and cerebrovascular damage. The aim of our study is to explore the effects of AP on CNS elements by focusing on microglia, brain cells of nonneural origin, as predominant regulators of neuroinflammation. To explore the effects of AP on microglia we treated, at different time-points, an immortalized line of murine microglia (i.e. BV2) and primary microglia with different concentration of a standard reference material of DEP. After the treatment, we evaluated viability, proliferation, cell morphology and intracellular calcium waves by means of calcium imaging technique. Our preliminary data indicate that DEP affect intracellular Ca^{2+} dynamics after a trigger of ATP and interfere with cell morphology and proliferation.

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HEMATOPOIETIC STEM CELL TRANSPLANTATION CHEMOTHERAPY CAUSES MICROGLIA SENESCENCE AND PERIPHERAL MACROPHAGE ENGRAFTMENT IN THE BRAIN

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

Kurt Sailor[1], George Agoranos[1], Sergio López-Manzaneda[2], Satoru Tada[2], Beatrix Gillet-Legrand[2], Corentin Guerinot[3], Jean-Baptiste Masson[3], Christian Vestergaard[3], Melissa Bonner[4], Khatuna Gagnidze[4], Gabor Veres[4], Pierre-Marie Lledo[1], Nathalie Cartier[2]
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Hematopoietic stem cell transplantation (HSCT) is a treatment for various malignant and non-malignant blood diseases with over 50,000 procedures annually worldwide. Yet, despite its prevalence, it is still unclear how HSCT affects the brain. Here we performed HSCT in mice using the myeloablative chemotherapy compound busulfan. Histological and *in vivo* imaging showing gradual donor macrophage engraftment of the brain between 6 and 24 weeks post-HSCT becoming resident while having similar surveillant processes comparedto the microglia they replaced. Microglia density halved by 2 weeks and the cells doubled their process area along with the induction of broad senescence markers. All adult neurogenesis was permanently abolished within the first few days. At 6 weeks microglia Ki67 expression peaked which was co-labeled with cell cycle induction markers, a DNA damage marker while lack of DNA incorporation indicated microglia underwent cell cycle arrest at S-G1 phase, unable to pass to G2. In vivo monitoring of post-HSCT microglia recovery after chemical depletion showed a complete lack of regenerative capacity whereas peripheral macrophages engrafted ~95 percent of the brain. Our results suggest busulfan DNA crosslinking causes microglia cell cycle arrest, with the induction of senescence along an anti-apoptotic p21 pathway. After HSCT, the gradual loss of microglia, due to their inability to proliferate, causes a minimal microglia density threshold, allowing permissive engraftment of macrophages in the brain. This strong effect of busulfan induced microglia senescence provides a therapeutic strategy for future cell transplantation-based brain gene therapies.

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LIVE-CELL OBSERVATION OF CYTOSOLIC DISC1 SUGGESTS ITS INTERACTIONS WITH CYTOSKELETON AND EXISTENCE OF DISC1 OLIGOMERS

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Disrupted In Schizophrenia (DISC1) gene, first identified in a Scottish pedigree is now regarded an important molecular lead to decoding etiology and molecular pathology of major mental illnesses. DISC1, being a scaffold protein, plays many distinct roles in the development and functioning of the brain. Microglia initiates the primary immune response to an injury or an invasion by pathogens in the CNS via phagocytosis, a process that involves continuous re-organization of cytoskeleton. An impairment in this process leads to onset of mental disorders. To probe a connection between impaired microglial phagocytosis and DISC1, we used advanced fluctuation microscopy techniques to quantify the diffusion and interaction of DISC1 in real-time in BV2 microglial cells. We expressed DISC1 in BV2 microglial cells to understand the cellular localization of DISC1 and we observed that that DISC1 diffuses rather slowly in cytoplasm. We also noted that diffusion of DISC1 is further reduced significantly near Actin enriched and Tubulin enriched regions of the cells, and is significantly faster in non-enriched regions of Actin and Tubulin, indicating an affinity for binding with these proteins. Our findings also suggested that DISC1 exists as an oligomer when interacting with Actin and Tubulin. However, further investigation is required to characterize the oligomeric state of DISC1 for interaction with the other proteins and to establish a link between DISC1 and microglial phagocytosis.

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BOARD NUMBER: S02-699

GAMMA-FREQUENCY OSCILLATIONS INDUCE CHANGES TO THE MORPHOLOGY AND ACTIVATION OF MICROGLIA

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Microglia are the immune cells of the central nervous system, which are responsible for mediating neuroinflammation in both health and disease. Their activity has been implicated in several neurological diseases such as dementia, and it is currently debated as to whether the inflammatory response observed in neurodegenerative disease is causative or compensatory. A recent series of studies has demonstrated that inducing neuronal network oscillations at the gamma frequency of 40 Hz is capable of reducing the neuropathology and cognitive deficits associated with dementia, which appears to occur as a result of microglia activation. To investigate the molecular mechanisms underlying this novel neuron-microglia interaction, we have developed an *ex vivo* assay, using hippocampal brain slices prepared from two mouse models (C57BL/6J and CX3CR1-GFP$^{+/-}$, which express GFP exclusively in microglia). This assay involves pharmacologically inducing gamma oscillations in the CA3 region of the hippocampus, fixing the brain tissue and then assessing microglia morphology following staining and mounting using two-photon microscopy. We have demonstrated that microglia morphology is altered in response to gamma oscillations, as microglia that have been exposed to these network oscillations have a significantly more amoeboid morphology than those in control conditions, where no oscillations were induced. These data suggest that sustained gamma oscillations result in the activation of microglia. Therefore, we have developed a new reduced model of gamma oscillation-microglia signalling that can be used to gain mechanistic understanding of this novel neuroimmune interaction.

Pubmed:

31310964: Wiese H, Ingram BT, Elley ML, Tüttenberg SC, Burton AM, Young AW
Later but not early stages of familiar face recognition depend strongly on attentional resources: Evidence from event-related brain potentials.
In everyday life we usually recognise personally familiar faces efficiently and without apparent effort. This study examined to which extent the neural processes involved in recognising personally familiar faces depend on attentional resources by analysing event-related brain potentials. In two experiments, participants were presented with multiple ambient images of highly personally familiar and unfamiliar faces and pictures of butterflies, with a letter string superimposed on each image. Their task was either to indicate when a butterfly occurred (effectively ignoring the letter strings) or to indicate whether each letter string contained the letter X or N. Attentional resource load was manipulated in the letter task by presenting the target among different distractor letters (high load; Experiment 1) or by using only a single repeated letter in each string (low load; Experiment 2). ERPs revealed more negative amplitudes for familiar relative to unfamiliar faces under both high and low load conditions, both in the N250, reflecting the activation of perceptual face representations, and in the subsequent Sustained Familiarity Effect (SFE). Nonetheless, while the magnitude of the N250 effect was not substantially affected by attentional load, the SFE was still present but reduced in the high relative to the low load experiment. These findings suggest that perceptual face representations are activated independent of the demands of a competing task. However, the subsequent SFE, presumably reflecting more sustained activation needed to access identity-specific knowledge that can guide potential interactions, strongly relies on the availability of attentional resources.
Cortex, 2019; 120
33444809: Clennell B, Steward TGJ, Elley M, Shin E, Weston M, Drinkwater BW, Whitcomb DJ
Transient ultrasound stimulation has lasting effects on neuronal excitability.
Transcranial ultrasound stimulation can acutely modulate brain activity, but the lasting effects on neurons are unknown.
Brain Stimul, 2021 Mar-Apr; 14

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BOARD NUMBER: S02-700

NIMODIPINE REDUCES LPS-INDUCED MICROGLIAL ACTIVATION IN PRIMARY MIXED AND ISOLATED MICROGLIA CELL CULTURE

POSTER SESSION 02 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Background: Microglia is the principal immune cell in the central nervous system. Microglial activation is an early response to brain ischemia which can induce an inflammatory response. Voltage-gated calcium channels (VGCCs) are implicated in microglia activation, which significantly contributes to the progression of neurodegenerative diseases. **Materials and methods**: Primary mixed cell cultures and isolated microglia cultures were prepared from the cortex of neonatal Sprague Dawley rats. On day 9, the plated cells were treated with LPS (20 ng/ml) or nimodipine (an L-type VGCC blocker) alone (5-10-20 uM), or in combination with LPS for 24 h. Microglial activation was evaluated by Iba1 immunolabeling (degree of arborization expressed by a transformation index - Ti) and Western blot analysis, and the visualization of phagocytotic activity with fluorescent microbeads. **Results:** Microglia displayed decreased area, perimeter and Ti in response to the LPS challenge, indicative of amoeboid transformation and activation. When the LPS-challenged cell cultures were treated with nimodipine, significantly more ramified cells were seen already at the lowest concentration. Increased Iba1 signal intensity in Western blot analysis confirmed microglial activation due to LPS treatment, which was decreased particularly by 10 and 20 uM nimodipine. Control microglia engulfed a few microbeads. In contrast, LPS challenge increased microglial phagocytic activity, which was significantly attenuated by nimodipine. **Conclusions**: Nimodipine is a potent vasodilator applied to alleviate vasospasm in acute cerebrovascular conditions. Our data suggest that nimodipine may be applicable in neuroinflammatory conditions including dementia, where neurodegeneration is believed to be linked to neurotoxic microglial activation.

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FUNCTIONAL INTEGRATION OF GRAFTED HIPSC DERIVED DOPAMINE NEURONS IN A MOUSE MODEL OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Mainly characterized by a progressive degeneration of mesencephalic dopaminergic (DA) neurons of the substancia nigra pars compacta (SNc), Parkinson's disease (PD) induces major imbalances of basal ganglia loop activity resulting mainly in motor symptoms. Therapeutic proposals are multiple but among the most promising ones, the cell therapy is emerging as a major research axis. In this context, we propose to study the functional integration of an intranigral graft of DA progenitor neurons derived from human induced-pluripotent stem cells (DA-hiPSCs) in 6-OHDA RAG2-KO mouse model of PD. Nine months after 6-OHDA lesion and transplantation, we investigated the electrophysiological functionality of the identified grafted-DA neurons by performing unit extracellular recordings in anesthetized mice to compare their electrophysiological characteristics to SNc dopaminergic neurons collected in control intact mice. We have found essentially similar electrophysiological criteria (frequency -1 to 10 Hz-; typical waveform of biphasic action potential - large duration above 2 ms-; inflection on the positive phase) of described firing patterns (burst and irregular ones) in grafted-DA-hiPSCs and native-DA neurons. The dorsal striatum is the main target structure of DA nigral pathway. In lack of DA release, dorsolateral striatal medium spiny neuron (MSNs) activities are imbalanced. Additional experiments are underway to assesse recovery of MSNs in grafted mice. For this purpose, we will combine electrophysiological recordings of MSN activities and optogenetics to manipulate grafted-DA neuron (expressing photosensible ArchT channels) spontaneous activity. The results of these experiments will provide valuable insights regarding the neuroanatomical and functional integration of grafted neurons.

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HOST TO GRAFT PROPAGATION OF ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE: INTRA-NIGRAL VERSUS INTRA-STRIATAL TRANSPLANTATION

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the degeneration of nigro-striatal dopaminergic (DA) neurons and by an abnormal accumulation of aggregated α-synuclein (α-syn), the main protein component of Lewy bodies. Intrastriatal transplantation of DA neurons from fetal ventral mesencephalic (fVM) tissue in PD patients have provided proof-of-principle for the cell replacement strategy in this disorder. However, 10–22 years after transplantation, some of the grafted neurons contained Lewy bodies similar to those observed in the host brain. We hypothesize that the striatum represents an unfavorable environment for the development of transplanted neurons, making DA neurons more vulnerable to the disease process. Our project aims to investigate the host-to-graft propagation of α-syn by comparing two transplantation sites, the substantia nigra (SN) and the striatum. For this purpose, we set up two mouse models of PD by injecting AAV2-human α-synuclein A53T viral vector into the SN or the striatum of mice. In these models, DA progenitors originating from mouse fVM or human induced-pluripotent stem cells (iPSC) were transplanted into the SN or the striatum. The host-to-graft propagation of human and phosphorylated α-syn were quantified from 1 month up to 12 months after transplantation. The polarization of the inflammatory response was assessed in astrocytes and microglia to determine whether the inflammatory state is correlated with the level of α-syn propagation. Our preliminary results show 5 months after transplantation that the amount of human α-syn is higher in striatal grafts of striatal viral injected animals compared to other conditions, confirming our hypothesis.

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LONG-TERM EVALUATION OF INTRANIGRAL TRANSPLANTATION OF HUMAN IPSC-DERIVED DOPAMINE NEURONS IN A PARKINSON'S DISEASE MICE MODEL

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Parkinson's disease (PD) is a neurodegenerative disorder associated with loss of dopaminergic (DA) neurons in the Substantia Nigra *pars compacta* (SNpc). **Cell replacement therapy** holds great promise for effective treatment of PD. Major advances have been made to generate **midbrain DA neurons** from human pluripotent stem cells. Before their use in clinical trials, extensive preclinical safety evaluations are required. One of the main issue to be addressed is the long-term therapeutic effectiveness of these cells. In most of transplantation studies using human cells, the maturation of DA neurons was analyzed over a relatively short period not exceeding 6 months. In present study, we generated midbrain DA progenitors from **human induced pluripotent stem cells (hiPSCs)** and grafted these cells into the SNpc in an animal model of PD. For the first time, we investigated the i**mpact of long-term transplantation up to 12 months** post-transplantation (mpt) and we report long-term survival and functionality of the grafted neurons. Indeed, intranigral grafted neurons express midbrain specific DA markers and allow the **reconstruction of degenerated nigrostriatal pathway**. Surprisingly, at 12 mpt, grafts still contained **immature neurons**, as evidenced by the presence of DCX+ cells. In addition, we observed a decrease in the proportion of SNpc DA neuron subtype compared to 6 mpt. Our results suggest that **longer-term evaluation** of the maturation of neurons derived from human stem cells is mandatory for the safe application of **cell therapy for PD**.

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THE ROLE OF SEROTONERGIC RECEPTOR 5-HTR4 IN MOTOR SYMPTOMS AND L-DOPA-INDUCED DYSKINESIA IN PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Parkinson's disease (PD) is caused by a selective loss of midbrain dopaminergic neurons that results in dopamine denervation of the stratum, an imbalance of striatal output pathways and hypersensitivity of direct pathway medium spiny neurons (dMSNs). L-DOPA pharmacotherapy alleviates PD motor symptoms, but causes abnormal involuntary movements termed L-DOPA induced dyskinesia (LID). The serotonergic system has been implicated in LID, since striatal serotonergic terminals take up L-DOPA, convert it to dopamine and uncontrollably release it. We hypothesize that the postsynaptic 5-HT4 receptor represents an interesting player in dyskinesia modulation, due to its high striatal expression and ability of regulating not only the activity of medium spiny neurons but also dopaminergic release. Using the unilateral 6-OHDA PD mouse model, we assessed the effect of 5-HTR4 partial agonist RS 67333 on dyskinesia and locomotion of chronically L-DOPA treated mice. We showed that RS 67333 on its own has an anti-akinetic, i.e. rotational, effect on lesioned mice. Furthermore, the severity of LID is significantly reduced by administration of RS 67333. Immunohistological analysis did not show any significant changes in known dyskinesia markers in striatal neurons. Based on transcriptomic studies, 5-HTR4 appears to be preferentially expressed in indirect pathway neurons, prompting us to perform cell-specific analysis of dyskinesia markers in Drd1a-Cre-RFP and Drd2-Cre-GFP reporter mice. This should enable us to interrogate the mechanisms by which agonism of 5-HTR4 results in a significant reduction of LID in our mouse model.

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BOARD NUMBER: S02-705

CELL-TYPE SPECIFIC ALTERATION OF EXCITABILITY IN THE SUBSTANTIA NIGRA PARS RETICULATA OF PARKINSONIAN MICE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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The *substantia nigra pars reticulata* (SNr) is the main output nucleus of the basal ganglia (BG), a subcortical network involved in motor, cognitive and associative functions. Recently, parvalbumin-expressing SNr subpopulation has been associated with motor functions; however the impact of dopamine depletion on the excitability of motor-related SNr-PV neurons is unknown. By combining immunohistochemical and electrophysiological *in vitro* approaches in PVCre::Ai9T mice, we demonstrated that SNr-PV neurons exhibit different anatomical, molecular and electrophysiological profiles than non PV-expressing SNr neurons. Moreover, we revealed that only SNr-PV neurons lose their excitability in the unilateral 6-OHDA mouse model of PD, due to a reduction in NALCN-currents in these neurons. Finally, we have used chemogenetic approaches to improve motor symptoms in parkinsonian mice and to demonstrate a causative relationship between SNr-PV excitability and motor function.

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BOARD NUMBER: S02-706

SPOT-ON! SPATIAL TRANSCRIPTOMIC ANALYSIS OF L-DOPA-INDUCED GENE EXPRESSION IN A MOUSE MODEL OF PARKINSONISM

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

Anna Radlicka[1], Magdalena Ziemiańska[1], Lukasz Szumiec[1], Marcin Piechota[1], Mateusz Zięba[1], Monika Bagińska[2], Grzegorz Kreiner[2], Joanna Pera[3], Jan Rodriguez Parkitna[1]
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Aims: Here we employ spatial transcriptomics to detect L-DOPA-induced gene expression changes in the forebrain sections of a mouse model of Parkinson's disease. **Methods:** Adult TIF-IA^{DATCreERT2} male mice with extensive loss of midbrain dopaminergic neurons underwent 14-day treatment with L-DOPA (18 mg/kg) or saline and behavioural testing. Brains were extracted 1 hour after the last dose of L-DOPA and processed to obtain spatial transcriptomic cDNA libraries. mRNA-Seq reads were analysed with the Space Ranger software package and a custom analysis pipeline adapting the Model-based Analysis of ChIP-Seq for validating transcript detection. **Results:** A total of 12 sections from 12 individual mice were used to construct spatially-barcoded cDNA libraries: 5 from each mutant treatment group and additional 2 from control animals that received saline. We observed induction of activity-dependent genes expression (e.g., *Fos*, *Junb*, *Egr2*) in the dorsal parts of the cortex and striatum but not the nucleus accumbens or olfactory tubercle of mutant mice treated with L-DOPA. Furthermore, we note that clustering of the of the gene expression results closely recapitulate the anatomical subdivisions in the forebrain. **Conclusions:** Our results show that L-DOPA evokes transcriptional changes in multiple cortical regions and the dorsal striatum in mice with loss of dopaminergic neurons. These observations indicate that treatment of L-DOPA affects, to a varying extent, diverse forebrain structures that regulate distinct motor and cognitive processes. This study was supported by the National Science Centre, Poland with a grant PRELUDIUM no. UMO-2020/37/N/NZ4/03672.

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BOARD NUMBER: S02-707

PHOSPHODIESTERASE 2A : FUNCTIONAL ROLE IN THE STRIATUM AND POTENTIALLY NEW THERAPEUTIC TARGET IN PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Aims: In Parkinson's disease (PD), the degeneration of dopaminergic neurons results in a deficit of dopamine. This situation is commonly treated pharmacologically by the administration of L-DOPA, a precursor of dopamine. However, after about 10 years of treatment, 80% of patients develop L-DOPA-induced dyskinesia (LID). In the dopamine depleted striatum, D1 type medium-sized spiny neurons (D1 MSN) become hyper-responsive to the stimulation of type 1 dopamine receptors. This hypersensitivity leads to an over-activation of the cAMP/PKA signaling pathway, resulting in the progressive development of LID. Our aim is to evaluate the potential of phosphodiesterase 2A (PDE2A), which degrades cAMP, to reduce D1 MSN hypersensitivity associated with LID. Because of its low affinity for cAMP, the stimulation of PDE2A activity through the nitric oxide (NO)/cGMP pathway could reduce excessive cAMP levels while preserving proper responses. **Methods**: Biosensor imaging reports the dynamics of cAMP/PKA signaling in MSNs in striatal brain slices from young mice, or adult mice in PD and dyskinetic situation. **Results**: In PD and dyskinetic mouse model, D1 MSN display a larger cAMP response to transient dopamine compared to normal mice. The larger cAMP response is similar to the response measured in immature brain. Interestingly, PDE2A activation by the NO/cGMP pathway efficiently reduces the amplitude of the dopamine response in PD and dyskinetic mouse model. **Conclusion**: the stimulation of PDE2A activity moderates excessive cAMP levels in the response to dopamine in dyskinetic mice. These results highlight the therapeutic potential of PDE2A stimulation in the treatment of LID.

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HIPPOCAMPAL SLEEP SPINDLE DYNAMICS DURING REM SLEEP AND THEIR DISTINCT UNDERLYING PARVALBUMIN AND SYNAPTIC PROTEINS EXPRESSION IN THE RETICULO-THALAMIC NUCLEUS OF THE PARKINSONIAN RATS

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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We investigated the alterations of reticulo-thalamic (RT) GABAergic parvalbumin (PV+) interneurons and synaptic re-organization underlying the altered hippocampal high voltage sleep spindle (HVS) dynamics during REM sleep in the rat models of Parkinson's disease (PD). Adult male Wistar rats were implanted for 6h sleep recording during light phase in four experimental groups: control (implanted controls), PD cholinopathy (bilateral lesion of *the nucleus pedunculopontinus tegmentalis*−PPT), hemiparkinsonism (unilateral lesion of *the nucleus substantiae nigrae pars compacta*−SNpc) and hemiparkinsonism with PD cholinopathy (unilateral SNpc/bilateral PPT lesion). Following 14 days of the surgical procedure we differentiated the Wake/NREM/REM 10s epochs, and the HVSs detection and extraction was done automatically (4.1–10 Hz band pass filter, 1s minimum duration) and visually validated. Hippocampal HVS dynamics were analyzed during 1h of NREM/REM sleep. Alterations of the PV+ interneurons and synaptic re-organization within the RT were determined by the parvalbumin, MAP2 and PSD-95 immunostaining. REM sleep is a predisposing state for the HVSs induction in all experimental models of PD neuropathology. Whereas the PD cholinopathy induced the prolongation and higher density of hippocampal HVSs, the hemiparkinsonism with PD cholinopathy increased the hippocampal HVSs intrinsic frequency during REM sleep. In contrast to the unaltered PV+ interneurons/partially enhanced MAP2/suppressed PSD-95 expression during PD cholinopathy, we evidenced the PV+ interneurons reduction/enhanced MAP2/no change of PSD-95 expression in the RT during hemiparkinsonism with PD cholinopathy. Distinct PV+ interneurons alteration and inhibition/excitation balance in the RT could be the underlying mechanisms of HVS generation/alteration during REM sleep in the parkinsonian rats.

Pubmed:

34445628: Radovanovic L, Petrovic J, Saponjic J
Hippocampal and Reticulo-Thalamic Parvalbumin Interneurons and Synaptic Re-Organization during Sleep Disorders in the Rat Models of Parkinson's Disease Neuropathology.
We investigated the alterations of hippocampal and reticulo-thalamic (RT) GABAergic parvalbumin (PV) interneurons and their synaptic re-organizations underlying the prodromal local sleep disorders in the distinct rat models of Parkinson's disease (PD). We demonstrated for the first time that REM sleep is a predisposing state for the high-voltage sleep spindles (HVS) induction in all experimental models of PD, particularly during hippocampal REM sleep in the hemiparkinsonian models. There were the opposite underlying alterations of the hippocampal and RT GABAergic PV+ interneurons along with the distinct MAP2 and PSD-95 expressions. Whereas the PD cholinopathy enhanced the number of PV+ interneurons and suppressed the MAP2/PSD-95 expression, the hemiparkinsonism with PD cholinopathy reduced the number of PV+ interneurons and enhanced the MAP2/PSD-95 expression in the hippocampus. Whereas the PD cholinopathy did not alter PV+ interneurons but partially enhanced MAP2 and suppressed PSD-95 expression remotely in the RT, the hemiparkinsonism with PD cholinopathy reduced the PV+ interneurons, enhanced MAP2, and did not change PSD-95 expression remotely in the RT. Our study demonstrates for the first time an important regulatory role of the hippocampal and RT GABAergic PV+ interneurons and the synaptic protein dynamic alterations in the distinct rat models of PD neuropathology.
Int J Mol Sci, 2021; 22
33038348: Petrovic J, Radovanovic L, Saponjic J
Prodromal local sleep disorders in a rat model of Parkinson's disease cholinopathy, hemiparkinsonism and hemiparkinsonism with cholinopathy.
We investigated the prodromal alterations of local sleep, particularly the motor cortical and hippocampal sleep, along with spontaneous locomotor activity in the rat models of Parkinson's disease (PD). We performed our experiments in adult, male

Wistar rats, chronically implanted for sleep recording and divided into four experimental groups: the control (implanted controls), the bilateral pedunculopontine tegmental nucleus (PPT) lesions (PD cholinopathy), the unilateral substantia nigra pars compacta (SNpc) lesions (hemiparkinsonism) and the unilateral SNpc/bilateral PPT lesions (hemiparkinsonism with PD cholinopathy). We followed their sleep, basal locomotor activity and spatial habituation for 14 days following the surgical procedures. Severe prodromal local sleep disturbances in the hemiparkinsonian rats were expressed as sleep fragmentation and distinct local NREM/REM EEG microstructure alterations in both the motor cortex and the hippocampus. Alongside the state-unrelated role of the dopaminergic control of theta oscillations and NREM/REM related sigma and beta oscillations, we demonstrated that the REM neurochemical regulatory substrate is particularly important in the dopaminergic control of beta oscillations. In addition, hippocampal prodromal sleep disorders in the hemiparkinsonian rats were expressed as NREM/REM fragmentation and the opposite impact of dopaminergic versus cholinergic control of the NREM delta and beta oscillation amplitudes in the hippocampus, likewise in the motor cortex versus the hippocampus. All these distinct prodromal local sleep disorders and the dopaminergic vs. cholinergic impact on NREM/REM EEG microstructure alterations are of fundamental importance for the further development and follow-up of PD-modifying therapies, and for the identification of patients who are at risk of developing PD.
Behav Brain Res, 2021; 397

32472657: Petrovic J, Radovanovic L, Saponjic J

Diversity of simultaneous sleep in the motor cortex and hippocampus in rats.

We investigated the homogeneity/heterogeneity of spontaneous sleep, simultaneously recorded in the motor cortex and the hippocampus of control rats, and particularly analysed simultaneous and non-simultaneous motor cortical and hippocampal non-rapid eye movement (NREM)/rapid eye movement (REM) sleep. We demonstrate that the sleep architectures of the motor cortex and hippocampus are different in control rats. There was an increase of NREM duration and a decrease of REM duration in the hippocampus versus the motor cortex. In terms of duration, NREM state is the most heterogeneous in the hippocampus, whereas the REM state is the most heterogeneous in the motor cortex. Whereas the hippocampal NREM duration was increased due to the prolongation of NREM episodes, the hippocampal REM duration decreased due to the decreased number of REM episodes. The heterogeneity of sleep in the motor cortex and hippocampus in control rats was particularly expressed through the inverse alteration of sigma amplitude during NREM sleep and beta/gamma amplitudes during REM sleep in the hippocampus, along with the delta, sigma, beta and gamma amplitudes only during non-simultaneous NREM/REM sleep in the hippocampus. We demonstrated the brain structure-related and NREM/REM state-related heterogeneity of the motor cortical and hippocampal local sleep in control rats. The distinctly altered local NREM/REM states, alongside their episode dynamics and electroencephalographic (EEG) microstructures, suggest the importance of both the local neuronal network substrate and the NREM/REM neurochemical substrate in the control mechanisms of sleep.
J Sleep Res, 2021; 30

30641119: Selakovic V, Arsenijevic L, Jovanovic M, Sivcev S, Jovanovic N, Leontijevic M, Stojanovic M, Radenkovic M, Andjus P, Radenovic L

Functional and pharmacological analysis of agmatine administration in different cerebral ischemia animal models.

Agmatine (AgM, 100 mg/kg i.p.) effect was tested in parallel at two animal models of cerebral ischemia - rat MCAO model (60'/24 h, 60'/48 h, 90'/24 h, 90'/48 h) and gerbil global ischemia (10') model, administrated 5 min after reperfusion. Aim was to evaluate AgM effect on functional outcome 24 and 48 h after MCAO on neurological and sensor-motor function, and coordination in rats. AgM administration significantly reduced infarct volume, improved neurological score and improved post-ischemic oxidative status. Results of behavioral tests (cylinder test, beam walking test, and adhesive removal test) have shown very effective functional recovery after AgM administration. Efficiency of AgM administration in gerbils was observed in forebrain cortex, striatum, hippocampus, and cerebellum at the level of each examined oxidative stress parameter (nitric oxide level, superoxide production, superoxide dismutase activity, and index of lipid peroxidation) measured in four different time points starting at 3 h up to 48 h after reperfusion. The highest levels were obtained 6 h after the insult. The most sensitive oxidative stress parameter to AgM was nitric oxide. Additionally, we performed pharmacological analysis of AgM on rat isolated common carotid arteries. The findings imply that mixed population of potassium channels located on the smooth muscle cells was involved in common carotid artery response to AgM, with predominance of inward rectifying K channels. In our comparative experimental approach, judged by behavioral, biochemical, as well as pharmacological data, the AgM administration showed an effective reduction of ischemic neurological damage and oxidative stress, hence indicating a direction towards improving post-stroke recovery.
Brain Res Bull, 2019; 146

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BOARD NUMBER: S02-709

CONVENTIONAL OPEN-LOOP DBS AND ON-OFF CLOSED-LOOP DBS RESULT IN SIMILAR BEHAVIOURAL IMPROVEMENT IN PARKINSONIAN RATS

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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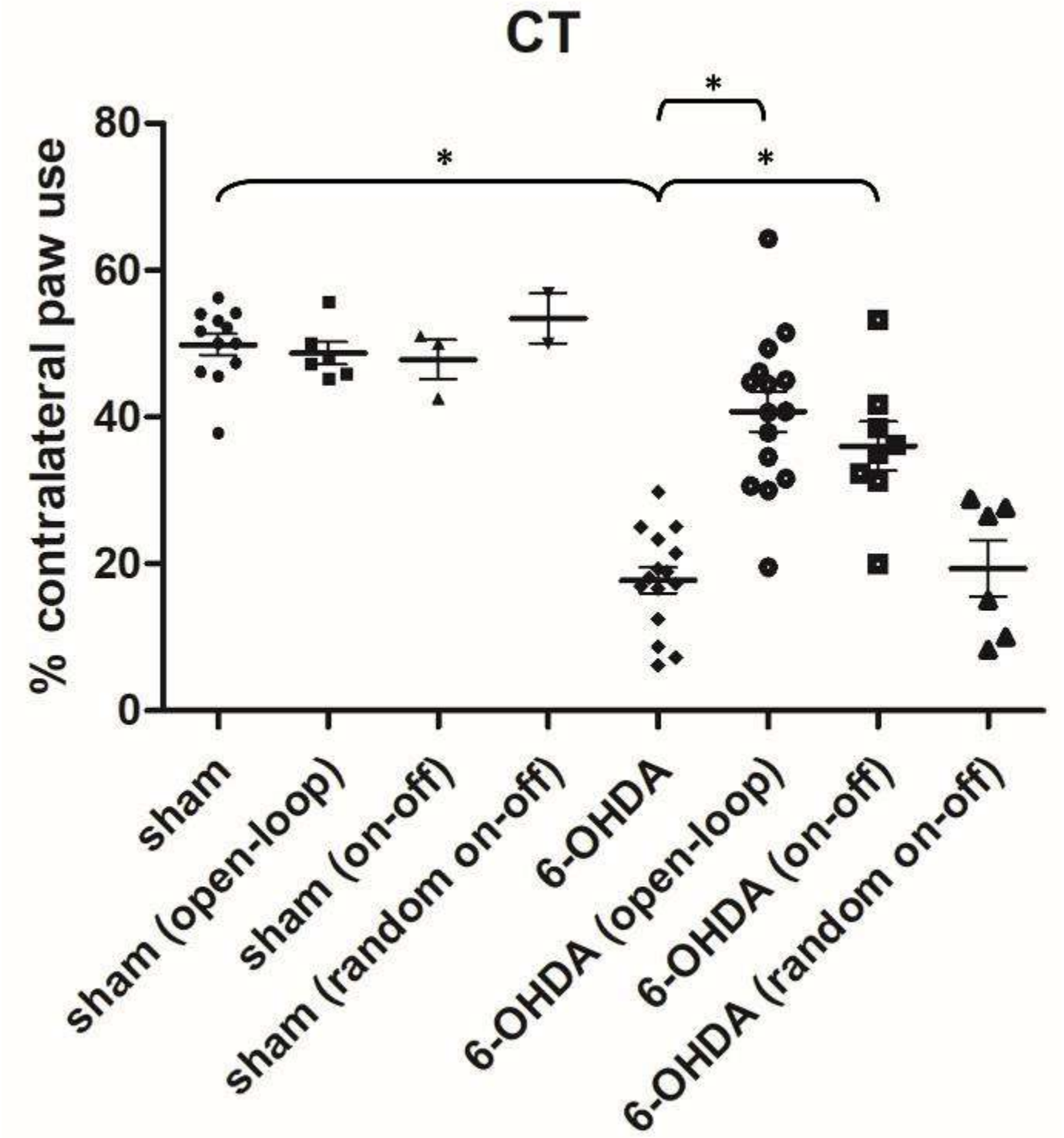
Aims: Closed-loop deep brain stimulation (DBS) has the potential automatically adjust the stimulation delivered to improve efficacy and reduce side effects in the treatment of medically refractory Parkinson's disease (PD). PD animal models provide an effective platform for testing different closed-loop algorithms before trialling in patients. Here we compared on-off closed-loop DBS to open-loop DBS in parkinsonian rats. Methods: A multi-electrode array (Microprobes) for stimulation and recording was implanted into the left subthalamic nucleus and 15 ug 6-OHDA (n=4), or vehicle only (n=3), were injected into the left medial forebrain bundle in 7 rats. Starting 3 weeks post-surgery, open-loop DBS, on-off closed-loop DBS based on recorded STN beta-power and random on-off stimulation were applied using W2100 system (Multichannel systems). Behaviour was assessed during the cylinder and stepping tests. Successful model creation was confirmed via apomorphine-induced rotation test and TH-immunocytochemistry, and electrode location was histologically confirmed. Data were analysed using linear mixed models (R Studio). Results: Contralateral paw use in parkinsonian rats was reduced to 20% in the cylinder test and 25% in the stepping test. Open-loop DBS and on-off closed-loop DBS, but not random on-off stimulation, improved motor function significantly (42%, 38%, 22% in cylinder test (Figure), 38%,38%, 30% in stepping test respectively).



Conclusions: Wireless closed-loop DBS is feasible in the rat and allows natural behaviour. On-off closed-loop stimulation was as effective as open-loop DBS in reducing motor symptoms of PD. In the future, the developed platform can be used to study more complex closed-loop algorithms, for example proportional control.

Pubmed:

28481856: Evers J, Jones JFX, O'Connell PR
Systematic Review of Animal Models Used in Research of Origins and Treatments of Fecal Incontinence.
Fecal incontinence is a common disorder, but its pathophysiology is not completely understood.
Dis Colon Rectum, 2017; 60
25167953: Carrington EV, Evers J, Grossi U, Dinning PG, Scott SM, O'Connell PR, Jones JF, Knowles CH
A systematic review of sacral nerve stimulation mechanisms in the treatment of fecal incontinence and constipation.
Sacral nerve stimulation (SNS) is now well established as a treatment for fecal incontinence (FI) resistant to conservative measures and may also have utility in the management of chronic constipation; however, mechanism of action is not fully understood. End organ effects of SNS have been studied in both clinical and experimental settings, but interpretation is difficult due to the multitude of techniques used and heterogeneity of reported findings. The aim of this study was to systematically review available evidence on the mechanisms of SNS in the treatment of FI and constipation.
Neurogastroenterol Motil, 2014; 26
33074305: Evers J, Lowery M
The Active Electrode in the Living Brain: The Response of the Brain Parenchyma to Chronically Implanted Deep Brain Stimulation Electrodes.
Deep brain stimulation is an established symptomatic surgical therapy for Parkinson disease, essential tremor, and a number of other movement and neuropsychiatric disorders. The well-established foreign body response around implanted electrodes is marked by gliosis, neuroinflammation, and neurodegeneration. However, how this response changes with the application of chronic stimulation is less well-understood.
Oper Neurosurg (Hagerstown), 2021; 20
31946513: Sridhar K, Evers J, Botelho DP, Lowery MM
Estimation of dispersive properties of encapsulation tissue surrounding deep brain stimulation electrodes in the rat.
The aim of this study was to estimate the electrical properties of the encapsulation tissue surrounding chronically implanted electrodes for deep brain stimulation in the rat. The impedance spectrum of a concentric bipolar microelectrode implanted in the rat brain was measured immediately following surgery and after 8 weeks of implantation. The experimental impedance data were used in combination with a finite element model of the rat brain using a parametric sweep method to estimate the electrical properties of the tissue surrounding the electrode in acute and chronic conditions. In the acute case, the conductivity and relative permittivity of the peri-electrode space were frequency independent with an estimated conductivity of 0.38 S/m and relative permittivity of 123. The electrical properties of the encapsulation tissue in the chronic condition were fitted to a dispersive Cole-Cole model. The estimated conductivity and relative permittivity in the chronic condition at 1 kHz were 0.028 S/m and 2×10, respectively. The estimated tissue properties can be used in combination with computational modeling as a basis for optimization of chronically implanted electrodes to increase the efficacy of long-term neural recording and stimulation.
Annu Int Conf IEEE Eng Med Biol Soc, 2019; 2019
22872659: Evers J, Buffini M, Craven S, O'Connell PR, Jones JF
Is there a nitrergic modulation of the rat external anal sphincter?
Nitric oxide is known to relax the internal anal sphincter, but its effect on the external anal sphincter (EAS) is unknown. The aim of this study was to investigate whether there is a nitrergic nerve plexus that modulates the EAS, similar to that found in oesophageal striated muscle. An in vitro ring preparation of rat anal canal was used to evaluate the effects of the nitric oxide synthase inhibitor N(ω)-nitro-L-arginine (L-NNA) and the NO donor sodium nitroprusside (SNP) on the EAS in conditions of neuromuscular blockade and the effect of SNP on nerve-evoked contractions. Immunohistological experiments were conducted to determine whether the neuronal isoform of nitric oxide synthase (nNOS) is present in the EAS. During direct muscle stimulation neither L-NNA (P = 0.32) nor SNP (P = 0.19) significantly changed the EF(50), which is the frequency at which 50% of maximal contraction is reached, compared with a time-dependent control. Nerve-evoked contractions were also not altered by addition of SNP to the tissue bath. Immunohistohistological experiments clearly showed co-localization of nNOS-positive nerve fibres at motor endplates of the oesophagus but not in the EAS. The internal anal sphincter was richly innervated by nitrergic fibres, but these did not extend into the EAS. In conclusion, there are no nitrergic motor fibres innervating the EAS, neurotransmission at the motor endplates is not affected by NO, and NO does not affect muscle force directly in conditions of neuromuscular blockade. There is, therefore, no evidence that EAS contraction is directly modulated by NO or by pudendal nitrergic fibres or diffusion from neighbouring nitrergic plexuses of the anal canal.
Exp Physiol, 2013; 98
26612206: Devane LA, Evers J, Scott MS, Knowles CH, O'Connell P, Jones JF
Acute lumbosacral nerve stimulation does not affect anorectal motor function in a rodent model.
Sacral nerve stimulation has become a first line treatment for fecal incontinence, however, its effect on the motor function of

the anorectum is uncertain. The aim of this study was to apply acute lumbosacral nerve stimulation in an animal model and to determine its effect on the external and internal anal sphincter forces, the rectoanal inhibitory and excitatory reflexes, and the slow wave frequency of the internal anal sphincter.
Neurogastroenterol Motil, 2016; 28
26821877: Evers J, Devane L, Carrington EV, Scott SM, Knowles CH, O'Connell PR, Jones JF
Reversal of sensory deficit through sacral neuromodulation in an animal model of fecal incontinence.
Sacral neuromodulation (SNM) is a treatment option for intractable fecal incontinence. The mechanism of action is unclear, however, increasing evidence for afferent somatosensory effects exists. This study's aim was to elucidate effects of acute SNM on the cerebral cortex in a rodent model of pudendal nerve injury.
Neurogastroenterol Motil, 2016; 28
26363190: Carrington EV, Evers J, Scott SM, Knowles CH, O'Connell PR, Jones JF
Mechanically evoked cortical potentials: A physiological approach to assessment of anorectal sensory pathways.
Normal defaecation involves activation of anorectal mechanoreceptors responsive to pressure and stretch. The aim of this study was to develop selective anal and rectal mucosal light-touch stimulation suitable for measurement of cortical evoked potentials (EPs) in order to explore the sensory arm of these pathways.
J Neurosci Methods, 2015; 256
34990427: Evers J, O'Connell PR, Jones JFX
The Relationship Between Cortical Activation in Response to Anorectal Stimuli and Continence Behavior in Freely Behaving Rats Before and After Application of Sacral Nerve Stimulation.
Changes in anorectal sensation have been reported in patients with fecal incontinence, and there is limited evidence that sacral nerve stimulation can restore normal sensation.
Dis Colon Rectum, 2022; 65
25069873: Evers J, Devane L, Carrington EV, Scott SM, Knowles CH, O'Connell PR, Jones JF
Effects of stimulation frequency and intensity in sacral neuromodulation on anorectal inputs to the somatosensory cortex in an experimental model.
Although sacral neuromodulation (SNM) is an established treatment for faecal incontinence, stimulation parameters have been derived empirically and only one frequency (14 Hz) is employed clinically. The aim of this study was to test a range of stimulation frequencies to establish an optimal frequency of SNM for maximum augmentation of anal canal cortical evoked potentials (EPs) in an animal model.
Br J Surg, 2014; 101

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BOARD NUMBER: S02-710

STIMULATION OF GABA-Aα2/3, BUT NOT α1, RECEPTORS INHIBITS ESSENTIAL AND PARKINSONIAN-LIKE TREMORS IN RATS

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

Barbara Kosmowska[1], Martyna Paleczna[1], Dominika Biała[1], Justyna Kadłuczka[1], Jadwiga Wardas[1], Jeffrey Witkin[2,3], James Cook[2,3], Dishary Sharmin[2], Monika Marcinkowska[4], Katarzyna Kuter-Nowak[1]
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Tremors are common symptoms of many neurological diseases, like Parkinson's disease or essential tremor (ET). Unfortunately, current tremor pharmacotherapy is often ineffective, and the molecular basis is not yet fully understood. It is known, however, that the basal ganglia with the key dopamine and the GABA-regulated cerebello-thalamo-cortical loop are involved in parkinsonian tremor. Disturbances in GABAergic transmission are observed also in ET. Interestingly, $GABA_A$ receptors play an important role in the pathophysiology of both tremors. The study aimed to investigate the antitremor potential of $GABA_A$ positive allosteric modulators, zolpidem (selective for α1 subunits) and methyl 8-ethynyl-6-(pyridin-2-yl)-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate, MP-III-024 (α2/3) in two different rat tremor models. Harmaline (15 mg/kg) was used to model ET, while parkinsonian-like tremor, observed as tremulous jaw movements (TJMs), was induced by pimozide (1 mg/kg ip, 7 days). Zolpidem (0.34, 0.67, 1.01 mg/kg ip) was administered simultaneously with the model compounds, while MP-III-024 (3.2, 10 mg/kg ip) 30 min before them. Harmaline-induced tremor was measured using Force Plate Actimeters, and TJMs after pimozide were counted manually by an observer blind to treatments. Zolpidem did not influence harmaline-induced tremor or pimozide-induced TJMs. MP-III-024 significantly reduced harmaline tremor in both doses used, but the effect was stronger for the lower dose. Furthermore, MP-III-024 also inhibited pimozide-induced TJMs, at both doses. The obtained results suggest that stimulation of the α2/3, but not α1, subunit of the $GABA_A$ receptor can inhibit both ET and parkinsonian-like tremors. These findings encourage further research in this direction with the goal of developing new potential therapies.

Pubmed:

34944457: Kosmowska B, Wardas J
The Pathophysiology and Treatment of Essential Tremor: The Role of Adenosine and Dopamine Receptors in Animal Models.
Essential tremor (ET) is one of the most common neurological disorders that often affects people in the prime of their lives, leading to a significant reduction in their quality of life, gradually making them unable to independently perform the simplest activities. Here we show that current ET pharmacotherapy often does not sufficiently alleviate disease symptoms and is completely ineffective in more than 30% of patients. At present, deep brain stimulation of the motor thalamus is the most effective ET treatment. However, like any brain surgery, it can cause many undesirable side effects; thus, it is only performed in patients with an advanced disease who are not responsive to drugs. Therefore, it seems extremely important to look for new strategies for treating ET. The purpose of this review is to summarize the current knowledge on the pathomechanism of ET based on studies in animal models of the disease, as well as to present and discuss the results of research available to date on various substances affecting dopamine (mainly D3) or adenosine A1 receptors, which, due to their ability to modulate harmaline-induced tremor, may provide the basis for the development of new potential therapies for ET in the future.
Biomolecules, 2021; 11
34536428: Kosmowska B, Ossowska K, Wardas J
Blockade of adenosine A receptors inhibits Tremulous Jaw Movements as well as expression of zif-268 and GAD65 mRNAs in brain motor structures.
Tremor is one of the motor symptoms of Parkinson's disease (PD), present also in neuroleptic-induced parkinsonism. Tremulous Jaw Movements (TJMs) are suggested to be a well-validated rodent model of PD resting tremor. TJMs can be induced by typical antipsychotics and are known to be reduced by different drugs, including adenosine A receptor

antagonists. The aim of the present study was to search for brain structures involved in the tremorolytic action of SCH58261, a selective A receptor antagonist, in TJMs induced by subchronic pimozide. Besides TJMs, we evaluated in the same animals the expression of zif-268 mRNA (neuronal responsiveness marker), and mRNA levels for glutamic acid decarboxylase 65-kDa isoform (GAD65) and vesicular glutamate transporters 1 and 2 (vGluT1/2) in selected brain structures, as markers of GABAergic and glutamatergic neurons, respectively. We found that SCH58261 reduced the pimozide-induced TJMs. Pimozide increased the zif-268 mRNA level in the striatum, nucleus accumbens (NAc) core, and substantia nigra pars reticulata (SNr). Additionally, it increased GAD65 mRNA in the striatum and SNr, and vGluT2 mRNA levels in the subthalamic nucleus (STN). A positive correlation between zif-268, GAD65 and vGluT2 mRNAs and TJMs was found. SCH58261 reversed the pimozide-increased zif-268 mRNA in the striatum and NAc core and GAD65 mRNA in the striatum and SNr. In contrast, SCH58261 did not influence vGluT2 mRNA in STN. The present study suggests an importance of the striato-subthalamo-nigro-thalamic circuit in neuroleptic-induced TJMs. The tremorolytic effect of A receptor blockade seems to involve this circuit bypassing, however, STN.
Behav Brain Res, 2022; 417
32219695: Ossowska K, Kosmowska B, Wardas J
Potential antipsychotic action of the selective agonist of adenosine A1 receptors, 5'-Cl-5'-deoxy-ENBA, in amphetamine and MK-801 rat models.
Disturbances of dopaminergic and glutamatergic transmissions have been suggested to be involved in the pathomechanisms underlying psychotic symptoms of schizophrenia. In line with this concept, hyperlocomotion induced by the dopaminomimetic amphetamine and the uncompetitive antagonist of NMDA receptors MK-801 (dizocilpine) in rodents is a generally established model for screening of new potential antipsychotic drugs. Since recent studies have indicated that receptors for adenosine may be targets for antipsychotic therapy, the aim of the present study was to investigate an influence of 5'-Cl-5'-deoxy-ENBA, a potent and selective adenosine A receptor agonist, on hyperlocomotion induced by amphetamine and MK-801.
Pharmacol Rep, 2020; 72
32172399: Kosmowska B, Ossowska K, Wardas J
Pramipexole Reduces zif-268 mRNA Expression in Brain Structures involved in the Generation of Harmaline-Induced Tremor.
Essential tremor is one of the most common neurological disorders, however, it is not sufficiently controlled with currently available pharmacotherapy. Our recent study has shown that pramipexole, a drug efficient in inhibiting parkinsonian tremor, reduced the harmaline-induced tremor in rats, generally accepted to be a model of essential tremor. The aim of the present study was to investigate brain targets for the tremorolytic effect of pramipexole by determination of the early activity-dependent gene zif-268 mRNA expression. Tremor in rats was induced by harmaline administered at a dose of 15 mg/kg ip. Pramipexole was administered at a low dose of 0.1 mg/kg sc. Tremor was measured by Force Plate Actimeters where four force transducers located below the corners of the plate tracked the animal's position on a Cartesian plane. The zif-268 mRNA expression was analyzed by in situ hybridization in brain slices. Harmaline induced tremor and increased zif-268 mRNA levels in the inferior olive, cerebellar cortex, ventroanterior/ventrolateral thalamic nuclei and motor cortex. Pramipexole reversed both the harmaline-induced tremor and the increase in zif-268 mRNA expression in the inferior olive, cerebellar cortex and motor cortex. Moreover, the tremor intensity correlated positively with zif-268 mRNA expression in the above structures. The present results seem to suggest that the tremorolytic effect of pramipexole is related to the modulation of the harmaline-increased neuronal activity in the tremor network which includes the inferior olive, cerebellar cortex and motor cortex. Potential mechanisms underlying the above pramipexole action are discussed.
Neurochem Res, 2020; 45
31935489: Kosmowska B, Ossowska K, Konieczny J, Lenda T, Berghauzen-Maciejewska K, Wardas J
Inhibition of Excessive Glutamatergic Transmission in the Ventral Thalamic Nuclei by a Selective Adenosine A1 Receptor Agonist, 5'-Chloro-5'-Deoxy-(±)-ENBA Underlies its Tremorolytic Effect in the Harmaline-Induced Model of Essential Tremor.
The primary cause of harmaline tremor, which is a model of essential tremor (ET) in animals, is excessive activation of olivocerebellar glutamatergic climbing fibers. Our recent study indicated that 5'-chloro-5'-deoxy-(±)-N6-(±)-(endo-norborn-2-yl)adenosine (5'Cl5'd-(±)-ENBA), a potent and selective adenosine A1 receptor (A1) agonist, inhibited harmaline tremor. The present study was aimed to evaluate the role of glutamatergic transmission system in 5'Cl5'd-(±)-ENBA tremorolytic action in the harmaline model in rats, by analyzing glutamate release in the motor nuclei of the thalamus and mRNA expression of glutamatergic neuron markers (vGlut1/2) in reference to the general neuronal activity marker (zif-268) in different brain structures. The extracellular glutamate level in the motor thalamus was evaluated by in vivo microdialysis and the vGlut1/vGlut2 and zif-268 mRNA expression was analyzed by in situ hybridization. The intensity of tremor was measured automatically using Force Plate Actimeters (FPAs). 5'Cl5'd-(±)-ENBA (0.5 mg/kg) given 30 min before harmaline (30 mg/kg) decreased the harmaline-induced excessive glutamate release in the motor thalamus and reversed harmaline-induced molecular effects, such as elevation of the vGlut1 mRNA expression in the inferior olive (IO) and decrease in the motor cortex, as well as an increase of the zif-268 mRNA expression in the IO, motor thalamus and motor cortex. Moreover, 5'Cl5'd-

(±)-ENBA reduced harmaline tremor by lowering its power in 9-15 Hz frequency band. Our findings show that A1 stimulation decreases glutamate release in the motor thalamic nuclei in the harmaline model of ET, suggesting that A1 receptors, especially in this structure, may be a potential therapeutic target in this disorder.
Neuroscience, 2020; 429
28371468: Kosmowska B, Ossowska K, Głowacka U, Wardas J
Tremorolytic effect of 5'-chloro-5'-deoxy-(±)-ENBA, a potent and selective adenosine A1 receptor agonist, evaluated in the harmaline-induced model in rats.
The aim of this study was to examine the role of adenosine A receptors in the harmaline-induced tremor in rats using 5'-chloro-5'-deoxy-(±)-ENBA (5'Cl5'd-(±)-ENBA), a brain-penetrant, potent, and selective adenosine A receptor agonist.
CNS Neurosci Ther, 2017; 23
26628402: Berghauzen-Maciejewska K, Wardas J, Kosmowska B, Domin H, Śmiałowska M, Głowacka U, Ossowska K
Adaptive down-regulation of the serotonin transporter in the 6-hydroxydopamine-induced rat model of preclinical stages of Parkinson's disease and after chronic pramipexole treatment.
Our recent study has indicated that a moderate lesion induced by bilateral 6-hydroxydopamine (6-OHDA) injections into the ventrolateral region of the caudate-putamen (CP) in rats, modeling preclinical stages of Parkinson's disease, induces a "depressive-like" behavior which is reversed by chronic treatment with pramipexole (PRA). The aim of the present study was to examine the influence of the above lesion and chronic PRA treatment on binding to the serotonin transporter (SERT) in different brain regions. As before, 6-OHDA (15 μg/2.5 μl) was administered bilaterally into the CP. PRA (1mg/kg) was injected subcutaneously twice a day for 2 weeks. Serotonergic and dopaminergic neurons of the dorsal raphe (DR) were immunostained for tryptophan hydroxylase and tyrosine hydroxylase, respectively, and were counted stereologically. Binding of [(3)H]GBR 12,935 to the dopamine transporter (DAT) and [(3)H]citalopram to SERT was analyzed autoradiographically. Intrastriatal 6-OHDA injections decreased the number of dopaminergic, but not serotonergic neurons in the DR. 6-OHDA reduced the DAT binding in the CP, and SERT binding in the nigrostriatal system (CP, substantia nigra (SN)), limbic system (ventral tegmental area (VTA), nucleus accumbens (NAC), amygdala, prefrontal cortex (PFCX), habenula, hippocampus) and DR. A significant positive correlation was found between DAT and SERT binding in the CP. Chronic PRA did not influence DAT binding but reduced SERT binding in the above structures, and deepened the lesion-induced losses in the core region of the NAC, SN, VTA and PFCX. The present study indicates that both the lesion of dopaminergic neurons and chronic PRA administration induce adaptive down-regulation of SERT binding. Moreover, although involvement of stimulation of dopaminergic transmission by chronic PRA in its "antidepressant" effect seems to be prevalent, additional contribution of SERT inhibition cannot be excluded.
Neuroscience, 2016; 314
26459182: Kosmowska B, Wardas J, Głowacka U, Ananthan S, Ossowska K
Pramipexole at a Low Dose Induces Beneficial Effect in the Harmaline-induced Model of Essential Tremor in Rats.
The aim of the study was to examine the effects of preferential agonists of dopamine D3 receptors: pramipexole and 7-OH-DPAT on the harmaline-induced tremor in rats (a model of essential tremor, ET). To study receptor mechanisms of these drugs, rats were pretreated with dopamine D3 receptor antagonists--SB-277011-A and SR-21502, an antagonist of presynaptic D2/D3 receptors--amisulpride, or a nonselective antagonist of D2-like receptors, haloperidol, at a postsynaptic dose.
CNS Neurosci Ther, 2016; 22
25933950: Ossowska K, Głowacka U, Kosmowska B, Wardas J
Apomorphine enhances harmaline-induced tremor in rats.
Harmaline-induced tremor is a well-known model of essential tremor in humans. The aim of the present study was to examine the influence of apomorphine, a non-selective dopamine receptor agonist, on the tremor induced by harmaline in rats. Propranolol (a first-line drug in essential tremor) was used as a reference compound.
Pharmacol Rep, 2015; 67
25739024: Berghauzen-Maciejewska K, Wardas J, Kosmowska B, Głowacka U, Kuter K, Ossowska K
Alterations of BDNF and trkB mRNA expression in the 6-hydroxydopamine-induced model of preclinical stages of Parkinson's disease: an influence of chronic pramipexole in rats.
Our recent study has indicated that a moderate lesion of the mesostriatal and mesolimbic pathways in rats, modelling preclinical stages of Parkinson's disease, induces a depressive-like behaviour which is reversed by chronic treatment with pramipexole. The purpose of the present study was to examine the role of brain derived neurotrophic factor (BDNF) signalling in the aforementioned model of depression. Therefore, we investigated the influence of 6-hydoxydopamine (6-OHDA) administration into the ventral region of the caudate-putamen on mRNA levels of BDNF and tropomyosin-related kinase B (trkB) receptor. The BDNF and trkB mRNA levels were determined in the nigrostriatal and limbic structures by in situ hybridization 2 weeks after the operation. Pramipexole (1 mg/kg sc twice a day) and imipramine (10 mg/kg ip once a day)

were injected for 2 weeks. The lesion lowered the BDNF and trkB mRNA levels in the hippocampus [CA1, CA3 and dentate gyrus (DG)] and amygdala (basolateral/lateral) as well as the BDNF mRNA content in the habenula (medial/lateral). The lesion did not influence BDNF and trkB expression in the caudate-putamen, substantia nigra, nucleus accumbens (shell and core) and ventral tegmental area (VTA). Chronic imipramine reversed the lesion-induced decreases in BDNF mRNA in the DG. Chronic pramipexole increased BDNF mRNA, but decreased trkB mRNA in the VTA in lesioned rats. Furthermore, it reduced BDNF and trkB mRNA expression in the shell and core of the nucleus accumbens, BDNF mRNA in the amygdala and trkB mRNA in the caudate-putamen in these animals. The present study indicates that both the 6-OHDA-induced dopaminergic lesion and chronic pramipexole influence BDNF signalling in limbic structures, which may be related to their pro-depressive and antidepressant activity in rats, respectively.
PLoS One, 2015; 10

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BOARD NUMBER: S02-711

CELL-TYPE SPECIFIC TRANSCRIPTIONAL CHANGES IN THE STRIATUM OF A 6-HYDROXYDOPAMINE MOUSE MODEL

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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The neurodegenerative movement disorder Parkinson's disease (PD) is the neuronal disorder with the fastest growing case numbers worldwide but disease mechanisms on cellular level are still not fully understood. One hallmark seen in human PD patients, neuronal loss in the substantia nigra (SN) causing a profound lack of dopaminergic signalling in the striatum, can be reproduced in mice using the neurotoxin 6-hydroxydopamine (6-OHDA). Applying single-cell RNA-sequencing to identify striatal cell populations, cell-type specific transcriptomic changes and the resulting alterations in gene regulatory patterns, can therefore allow valuable insides into cause and progression of PD. CD1 male mice, age 9-10 weeks, received intracranial unilateral injections with 6-OHDA or vehicle, either in the SN or the medial forebrain bundle (MFB). Thereby, the mice lose dopaminergic signalling in one half of the striatum, providing the possibility to compare changes within animals, as well as between groups. Striatal tissue was extracted 6-8 weeks after injection, cells were isolated, sequenced and six major cell classes were identified. A compositional analysis indicated alterations in the neuronal part of the data set after MFB lesion. Furthermore, differential expression analysis identified a set of statistically significant regulated genes in the striatum of mice receiving the 6-OHDA lesion. These preliminary findings indicate cell-type specific changes in the striatal gene regulation due to dopamine depletion and are a first step towards identifying new potential treatment targets.

Pubmed:
29377458: Harder L, Dudazy-Gralla S, Müller-Fielitz H, Hjerling Leffler J, Vennström B, Heuer H, Mittag J
Maternal thyroid hormone is required for parvalbumin neurone development in the anterior hypothalamic area.
Thyroid hormone (TH) is crucial for brain development and function. This becomes most evident in untreated congenital hypothyroidism, leading to irreversible mental retardation. Likewise, maternal hypothyroxinaemia, a lack of TH during pregnancy, is associated with neurological dysfunction in the offspring, such as autism and reduced intellectual capacity. In the brain, TH acts mainly through TH receptor α1 (TRα1). Consequently, mice heterozygous for a dominant-negative mutation in TRα1 display profound neuroanatomical abnormalities including deranged development of parvalbumin neurones. However, the exact timing and orchestration of TH signalling during parvalbumin neurone development remains elusive. In the present study, we dissect the development of parvalbumin neurones in the anterior hypothalamic area (AHA) in male mice using different mouse models with impaired pre- and postnatal TH signalling in combination with bromodeoxyuridine birth dating and immunohistochemistry. Our data reveal that hypothalamic parvalbumin neurones are born at embryonic day 12 and are first detected in the AHA at postnatal day 8, reaching their full population number at P13. Interestingly, they do not require TH postnatally because their development is not impaired in mice with impaired TH signalling after birth. By contrast, however, these neurones crucially depend on TH through TRα1 signalling in the second half of pregnancy, when the hormone is almost exclusively provided by the mother. For the first time, our findings directly link a maternal hormone to a neuroanatomical substrate in the foetal brain, and underline the importance of proper TH signalling during pregnancy for offspring mental health. Given the role of hypothalamic parvalbumin neurones in the central control of blood pressure, the present study advocates the inclusion of cardiovascular parameters in the current discussion on possible TH substitution in maternal hypothyroxinaemia.
J Neuroendocrinol, 2018; 30
27145010: Hoefig CS, Harder L, Oelkrug R, Meusel M, Vennström B, Brabant G, Mittag J
Thermoregulatory and Cardiovascular Consequences of a Transient Thyrotoxicosis and Recovery in Male Mice.
Thyroid hormones play a major role in body homeostasis, regulating energy expenditure and cardiovascular function. Given that obese people or athletes might consider rapid weight loss as beneficial, voluntary intoxication with T4 preparations is a growing cause for thyrotoxicosis. However, the long-lasting effects of transient thyrotoxicosis are poorly understood. Here we examined metabolic, thermoregulatory, and cardiovascular function upon induction and recovery from a 2-week thyrotoxicosis

in male C57BL/6J mice. Our results showed that T4 treatment caused tachycardia, decreased hepatic glycogen stores, and higher body temperature as expected; however, we did not observe an increase in brown fat thermogenesis or decreased tail heat loss, suggesting that these tissues do not contribute to the hyperthermia induced by thyroid hormone. Most interestingly, when the T4 treatment was ended, a pronounced bradycardia was observed in the animals, which was likely caused by a rapid decline of T3 even below baseline levels. On the molecular level, this was accompanied by an overexpression of cardiac phospholamban and Serca2a mRNA, supporting the hypothesis that the heart depends more on T3 than T4. Our findings therefore demonstrate that a transient thyrotoxicosis can have pathological effects that even persist beyond the recovery of serum T4 levels, and in particular the observed bradycardia could be of clinical relevance when treating hyperthyroid patients.
Endocrinology, 2016; 157
29594048: Harder L, Schanze N, Sarsenbayeva A, Kugel F, Köhrle J, Schomburg L, Mittag J, Hoefig CS
In vivo Effects of Repeated Thyronamine Administration in Male C57BL/6J Mice.
Thyronamines are decarboxylated and deiodinated metabolites of thyroid hormones (THs). Of all possible thyronamine variants, only 3-iodothyronamine (3-TAM) and iodine-free thyronamine (TAM) have been detected in vivo. While intensive research has been done on the (patho-)physiological action of 3-TAM, the role of TAM has been studied less intensively.
Eur Thyroid J, 2018; 7
29031714: Oelkrug R, Herrmann B, Geissler C, Harder L, Koch C, Lehnert H, Oster H, Kirchner H, Mittag J
Dwarfism and insulin resistance in male offspring caused by α1-adrenergic antagonism during pregnancy.
Maternal and environmental factors control the epigenetic fetal programming of the embryo, thereby defining the susceptibility for metabolic or endocrine disorders in the offspring. Pharmacological interventions required as a consequence of gestational problems, e.g. hypertension, can potentially interfere with correct fetal programming. As epigenetic alterations are usually only revealed later in life and not detected in studies focusing on early perinatal outcomes, little is known about the long-term epigenetic effects of gestational drug treatments. We sought to test the consequences of maternal α1-adrenergic antagonism during pregnancy, which can occur e.g. during hypertension treatment, for the endocrine and metabolic phenotype of the offspring.
Mol Metab, 2017; 6
31189119: Johann K, Cremer AL, Fischer AW, Heine M, Pensado ER, Resch J, Nock S, Virtue S, Harder L, Oelkrug R, Astiz M, Brabant G, Warner A, Vidal-Puig A, Oster H, Boelen A, López M, Heeren J, Dalley JW, Backes H, Mittag J
Thyroid-Hormone-Induced Browning of White Adipose Tissue Does Not Contribute to Thermogenesis and Glucose Consumption.
Regulation of body temperature critically depends on thyroid hormone (TH). Recent studies revealed that TH induces browning of white adipose tissue, possibly contributing to the observed hyperthermia in hyperthyroid patients and potentially providing metabolic benefits. Here, we show that browning by TH requires TH-receptor β and occurs independently of the sympathetic nervous system. The beige fat, however, lacks sufficient adrenergic stimulation and is not metabolically activated despite high levels of uncoupling protein 1 (UCP1). Studies at different environmental temperatures reveal that TH instead causes hyperthermia by actions in skeletal muscle combined with a central body temperature set-point elevation. Consequently, the metabolic and thermogenic effects of systemic hyperthyroidism were maintained in UCP1 knockout mice, demonstrating that neither beige nor brown fat contributes to the TH-induced hyperthermia and elevated glucose consumption, and underlining that the mere presence of UCP1 is insufficient to draw conclusions on the therapeutic potential of browning agents.
Cell Rep, 2019; 27
31398374: Jerome H, Taylor C, Sreenu VB, Klymenko T, Filipe ADS, Jackson C, Davis C, Ashraf S, Wilson-Davies E, Jesudason N, Devine K, Harder L, Aitken C, Gunson R, Thomson EC
Metagenomic next-generation sequencing aids the diagnosis of viral infections in febrile returning travellers.
Travel-associated infections are challenging to diagnose because of the broad spectrum of potential aetiologies. As a proof-of-principle study, we used MNGS to identify viral pathogens in clinical samples from returning travellers in a single center to explore its suitability as a diagnostic tool.
J Infect, 2019; 79
32183611: Nock S, Johann K, Harder L, Wirth EK, Renko K, Hoefig CS, Kracke V, Hackler J, Engelmann B, Rauner M, Köhrle J, Schomburg L, Homuth G, Völker U, Brabant G, Mittag J
CD5L Constitutes a Novel Biomarker for Integrated Hepatic Thyroid Hormone Action.
Pathological conditions of the thyroid hormone (TH) system are routinely diagnosed by using serum concentrations of thyrotropin (TSH), which is sufficient in most cases. However, in certain conditions, such as resistance to TH due to mutations in (RTHb) or TSH-releasing pituitary adenoma (TSHoma), TSH may be insufficient for a correct diagnosis, even in combination with serum TH concentrations. Likewise, under TH replacement therapy, these parameters can be misleading

and do not always allow optimal treatment. Hence, additional biomarkers to assess challenging clinical conditions would be highly beneficial. Data from untargeted multi-omics analyses of plasma samples from experimental thyrotoxicosis in human and mouse were exploited to identify proteins that might represent possible biomarkers of TH function. Subsequent mouse studies were used to identify the tissue of origin and the involvement of the two different TH receptors (TR). For in-depth characterization of the underlying cellular mechanisms, primary mouse cells were used. The analysis of the plasma proteome data sets revealed 16 plasma proteins that were concordantly differentially abundant under thyroxine treatment compared with euthyroid controls across the two species. These originated predominantly from liver, spleen, and bone. Independent studies in a clinical cohort and different mouse models identified CD5L as the most robust putative biomarker under different serum TH states and treatment periods. studies revealed that CD5L originates from proinflammatory M1 macrophages, which are similar to liver-residing Kupffer cells, and is regulated by an indirect mechanism requiring the secretion of a yet unknown factor from hepatocytes. In agreement with the role of TRα1 in immune cells and the TRβ-dependent hepatocyte-derived signaling, the regulation of expression depended on both TR isoforms. Our results identify several novel targets of TH action in serum, with CD5L as the most robust marker. Although further studies will be needed to validate the specificity of these targets, CD5L seems to be a promising candidate to assess TH action in hepatocyte-macrophage crosstalk.
Thyroid, 2020; 30
32188347: Herrmann B, Harder L, Oelkrug R, Chen J, Gachkar S, Nock S, Resch J, Korkowski M, Heuer H, Mittag J
Central Hypothyroidism Impairs Heart Rate Stability and Prevents Thyroid Hormone-Induced Cardiac Hypertrophy and Pyrexia.
Tachycardia, cardiac hypertrophy, and elevated body temperature are major signs of systemic hyperthyroidism, which are considered to reflect the excessive thyroid hormone (TH) action in the respective peripheral tissues. However, recent observations indicate that the central actions of TH also contribute substantially to cardiovascular regulation and thermogenesis. In this study, we dissect the individual contributions of peripheral TH action versus the central effects in body temperature regulation and cardiovascular functions by taking advantage of mice lacking the TH transporters monocarboxylate transporter 8 (MCT8) and organic anion transporting polypeptide 1C1 (OATP1C1) (double knock-out [dko]), which exhibit elevated serum triiodothyronine (T3) levels while their brain is in a profoundly hypothyroid state. We compared these animals with wild-type (WT) mice that were treated orally with T3 to achieve similarly elevated serum T3 levels, but are centrally hyperthyroid. For the studies, we used radiotelemetry, infrared thermography, gene expression profiling, Western blot analyses, and enzyme linked immunosorbent assays (ELISA) assays. Our analyses revealed mild hyperthermia and cardiac hypertrophy in T3-treated WT mice but not in dko animals, suggesting that central actions of TH are required for these hyperthyroid phenotypes. Although the average heart rate was unaffected in either model, the dko exhibited an altered heart rate frequency distribution with tachycardic bursts in active periods and bradycardic episodes during resting time, demonstrating that the stabilization of heart rate by the autonomic nervous system can be impaired in centrally hypothyroid animals. Our studies unravel distinct phenotypical traits of hyperthyroidism that depend on an intact central nervous system, and provide valuable insight into the cardiovascular pathology of the Allan-Herndon-Dudley syndrome, a condition caused by the lack of MCT8 in humans.
Thyroid, 2020; 30
32115725: Harder L, Oster H
The Tissue Clock Network: Driver and Gatekeeper of Circadian Physiology: Circadian rhythms are integrated outputs of central and peripheral tissue clocks interacting in a complex manner - from drivers to gatekeepers.
In mammals, a network of cellular circadian clocks organizes physiology and behavior along the 24-h day cycle. The traditional hierarchical model of circadian clock organization with a central pacemaker and peripheral slave oscillators has recently been challenged by studies combining tissue-specific mouse mutants with transcriptome analyses. First, a surprisingly small number of tissue rhythms are lost when only local clocks are ablated and, second, transcriptional circadian rhythms appear to be regulated by a complex mix of local and systemic factors. As reviewed here, these findings suggest a more integrated model of clock network interaction with the central pacemaker as the main source of behavioral and systemic-physiological rhythms and peripheral clocks controlling some local rhythms while at the same time acting as gatekeepers that temporally adjust cellular responses to external stimuli.
Bioessays, 2020; 42
25765843: Hoefig CS, Jacobi SF, Warner A, Harder L, Schanze N, Vennström B, Mittag J
3-Iodothyroacetic acid lacks thermoregulatory and cardiovascular effects in vivo.
3-Iodothyronamine (3-T1 AM) is an endogenous thyroid hormone derivative reported to induce strong hypothermia and bradycardia within minutes upon injection in rodents. Although 3-T1 AM is rapidly converted to several other metabolites in vivo, these strong pharmacological responses were solely attributed to 3-T1 AM, leaving potential contributions of downstream products untested. We therefore examined the cardiometabolic effects of 3-iodothyroacetic acid (TA1), the main

degradation product of 3-T1 AM.
Br J Pharmacol, 2015; 172

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DEEP PHENOTYPIC ANALYSIS OF ZEBRAFISH MODELS OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Parkinson's disease (PD) is a progressive disorder of the nervous system characterised by degeneration of dopaminergic neurons. While the pathogenesis remains unclear, there is evidence that changes in dopamine (DA) storage and release contribute to it. However, the precise effects on the dopaminergic circuitry are unknown. Here, pharmacological and genetic zebrafish models are used, involving incubation with the neurotoxicant MPTP or CRISPR/Cas9-induced knockout of PD-associated genes, respectively. The aim is to analyse the activity of the dopaminergic circuitry using genetically-encoded DA sensors. Using transgenesis, the $GRAB_{DA}$ and d.Light1.1 sensors are used to quantify the dynamics of DA signalling in wildtype conditions, in order to have an idea of the circuit activity. Subsequently, MPTP treatment is integrated to visualise the effects of dopaminergic neuron loss on the dopaminergic circuit. Additionally, a deep characterisation of locomotor patterns is conducted in the distinct PD models in order to identify any micromovement alterations compared to wildtype. Using high-throughput behavioural assays, swimming events of individual fish are recorded simultaneously at a high frequency over large timescales. Kinematic parameters are analysed in two manoeuvres: fast acoustovestibular escape responses, as well as slow forward swims and routine turns. Preliminary data currently point towards a long-latency escape response in PD mutants, which could be regarded as a delayed response. Moreover, mutants exhibit less swimming with higher tail beat amplitude compared to control. Ultimately, this work could help build a phenotypic profile of PD, which in the long term could enable screening of candidate chemicals for PD in zebrafish.

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SPATIO-TEMPORAL DEPENDENCY OF STRIATAL DOPAMINE IN THE CONTROL OF MOVEMENT KINEMATICS IN RATS

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Performing movements and quickly adapting them is essential in the ever-changing environment we are living in. It is well established that this capital function recruits dopaminergic transmission in basal ganglia circuit. Indeed, the role of dopamine (DA) in these processes is illustrated by the consequences of its loss in Parkinson's disease (PD) patients, suffering from severe motor symptoms such as bradykinesia and akinesia. Despite years of research, the spatio-temporal dynamics of DA during motor control remain largely unknown. In this study, we investigate how DA impact the execution of dexterous movements in rats trained to perform a 'reach-to-grasp' motor task. Our first set of experiments aimed to assess the effect of the chronic lack of DA in a model of parkinsonism induced by 6-OHDA injection in the striatum of trained animals. This depletion in the dorso-lateral region shortly induced bradykinetic movements while akinesia appears in the following days of the lesion. Although the motor deficits quickly reached their peaks, they often did not persist in time and we observed a motor symptoms reversion which expression and timing depends on the size and location of the striatal DA depletion. We highlighted that this functional motor recovery requires DA signaling and relies on neuronal networks located in the striatal depleted area. We are currently investigating using fiber photometry the local adaptations of specific striatal populations that could underlie the motor restoration. We propose that those compensatory mechanisms occur early on PD to compensate for the slow decrease of DA.

FENS Forum 2022
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BOARD NUMBER: S02-714

DOPAMINE DYSREGULATION SYNDROME IN A MOUSE MODEL OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Treatment of Parkinson's disease (PD) is based on the use of dopaminergic drugs, such as L-Dopa and dopamine receptor agonists. These substances compensate for the lack of dopamine and counteract motor symptoms, but long-term treatment is accompanied by motor and non-motor complications. Among these latter conditions a neurobehavioral disorder similar to drug abuse, known as dopamine dysregulation syndrome (DDS), is attracting increasing interest because of its profound negative impact on the patients' quality of life. We used a PD mouse model based on a bilateral injection of 6-hydroxydopamine into the dorsal striatum to reproduce some of the features of DDS. Using the conditioned place preference paradigm, we found that L-Dopa induces place preference in PD mice, but not in control mice, suggesting that this drug acquires addictive-like properties following dopamine depletion. This effect is accompanied by abnormal signaling in dopamine D1 receptor (D1R) striatal projection neurons, leading to hyperactivation of the cAMP signaling cascade and accumulation of the transcription factor ΔFosB. Pharmacological inactivation of D1Rs abolished these effects and prevented the development of place preference, thereby counteracting the psychostimulant effect of L-Dopa. Interestingly, blockade of dopamine D2 receptors was also able to reduce the accumulation of ΔFosB and abolish place preference. Our results suggest that, in PD, combined activation of D1 and D2 receptors by L-Dopa results in DDS, and that this effect may be linked to abnormal accumulation of ΔFosB in striatal neurons.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-715

CONTRIBUTION OF ARKYPALLIDAL GLOBUS PALLIDUS NEURONS TO LEVODOPA-INDUCED DYSKINESIAS

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Basal ganglia (BG) circuits play a key role in motor initiation and suppression and the loss of dopamine in these circuits triggers Parkinson's disease (PD) characterized by devastating motor impairments known as akinesia and bradykinesia. The supplement of DA, through the intake of the precursor levodopa, improves movements execution in the early stage of the disease but quickly triggers abnormal and involuntary movements known as levodopa-induced dyskinesias (LIDs). LIDs are very debilitating because refractory to any further drug treatments, hence understanding the neuronal mechanism of their generation is fundamental to develop new treatment strategies. Recent studies have shown that LIDs are caused by aberrant neuronal activity in the striatum, however how such striatal activity impacts on downstream BG circuits to generate LIDs is not known. The external globus pallidus (GPe) integrates striatal inputs and is an important hub nucleus that orchestrate normal and abnormal activity in BG circuit. In addition, arkypallidal GPe neurons, directly form a negative feedback loop to the striatum that powerfully control action inhibition in normal condition. Importantly, the activity and contribution of this action-suppressing loop during LIDs is totally unknown. In this work, we used optical methods (fiber photometry and miniscope) to monitor the calcium activity of arkypallidal neurons across different behavioral states: from healthy condition to the pathological state of PD and LIDs. Having characterized the abnormal change of activity of arkypallidal neurons during LIDs, we then used optogenetic manipulation to restore their activity and test their contribution to LIDs generation.

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BOARD NUMBER: S02-716

INVOLVEMENT OF 5-HT DESCENDING PATHWAY IN PAIN IN A MOUSE MODEL OF PARKINSONISM

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Parkinson disease (PD) is characterised by the dopamine (DA) depletion in basal ganglia (BG) especially in the substantia nigra and the striatum. PD is a multi-factory disorder leading to well-known motor symptoms but also to non-motor symptoms. The major one is chronic pain which is only partly explained by DA depletion in BG. The pain ascending signal coming from the periphery is strongly modulated by pain descending pathways and most particularly by serotonergic (5-HT) descending neurons coming from the nucleus raphe magnus (NRM) and acting at the level of the dorsal horn (DH) of the spinal cord. As the NRM seems to be affected by PD and as 5-HT is intricately involved in pain perception modulation, we hypothesized that this PD induced pain modulation may be associated to a modification of pain transmission by 5HT descending pathways. To assess this, we used a transgenic mice expressing cre-recombinase in 5-HT neurons in which we induced DA depletion with 6-OHDA intracerebral injection. After the validation of this model, we were able record the animal pain behaviour while optogenetically inhibiting specifically 5-HT descending pathways. We showed that unilateral DA depletion impairs bilaterally pain sensitivity. We showed then that 6-OHDA injection changes the role of 5-HT descending pathways on pain perception as 5-HT action shifted from an anti-nociceptive effect in Sham animal to proalgesic one in 6-OHDA. These results raise now the question of causes of this changes which will be investigated further to better understand and treat chronic pain syndromes in PD patients.

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BOARD NUMBER: S02-717

RELATIONSHIP BETWEEN CLOCK GENES AND PARKINSON'S PATHOPHYSIOLOGY IN ZEBRAFISH

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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It is known that Parkinson's disease (PD) patients report sleep disturbances and decreased levels of melatonin. The sleep/wake rhythm is controlled by the biological clock, a group of neurons expressing clock genes. These genes regulate not only melatonin production but also innate immunity and mitochondrial function, which are affected in PD. Thus, chronodisruption may be an early stage of this disease. **Objectives:** to evaluate the connection between clock genes and PD and to assess whether melatonin administration can improve PD restoring the normal functioning of the biological clock. **Methods:** Parkinsonism was induced with MPTP in 24-120 hours post fertilization (hpf) zebrafish embryos. Melatonin was administered at doses of 1 µM**. Results:** Day-night melatonin rhythm disappeared in MPTP-treated embryos, which showed an advance in the activity phase and a parallel changes in the acrophases of Bmal1 and Per2 were detected. We also found an alteration in the mitochondrial dynamics and mitochondrial function with MPTP. In all cases, melatonin restored the normal phenotype and clock rhythm expression, normalizing the mitochondrial dynamics. **Conclusions:** The parkinsonian zebrafish shows state there is an alteration of the biological clock, which explain phenotype changes that are restored by treatment with melatonin.

Pubmed:
29185873: Díaz-Casado ME, Rusanova I, Aranda P, Fernández-Ortiz M, Sayed RKA, Fernández-Gil BI, Hidalgo-Gutiérrez A, Escames G, López LC, Acuña-Castroviejo D
In Vivo Determination of Mitochondrial Respiration in 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Treated Zebrafish Reveals the Efficacy of Melatonin in Restoring Mitochondrial Normalcy.
Although mitochondria dysfunction is related to multiple diseases, no in vivo studies are available on mitochondrial respiration in animal parkinsonian models. Our aim is to analyze in vivo mitochondrial respiration, which reflects changes in mitochondrial bioenergetics more precisely than in vitro mitochondrial preparations. These experiments can be carried out in zebrafish embryos, which were treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) from 24 to 72 hours postfertilization (hpf). A reduction in electron transfer system capacity, ATP turnover, and increased proton leak were observed at 72 hpf in MPTP-treated embryos. These changes were followed by a significant oxidative stress due to inhibition in antioxidative defense and autophagy impairment. After removing MPTP from the treatment at 72 hpf, these bioenergetic deficiencies persisted up to 120 hpf. The administration of melatonin to zebrafish embryos at 72 hpf, when mitochondrial dysfunction is already present, restored the respiratory capacity and ATP production, reduced oxidative stress, and normalized autophagy after 48 h. Melatonin also counteracted mortality and embryonic malformations due to MPTP. Our results confirm for the first time the efficacy of melatonin in restoring parkinsonian phenotypes in animals.
Zebrafish, 2018; 15

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-718

BDNF/TRKB PATHWAY ACTIVATION IN D1 RECEPTOR-EXPRESSING STRIATAL PROJECTION NEURONS PLAYS A PROTECTIVE ROLE AGAINST L-DOPA-INDUCED DYSKINESIA

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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L-DOPA-induced dyskinesia (LID) is a frequent complication of the medication for Parkinson's disease (PD). LID is a motor disorder that affects the patient's quality of life. Therefore, understanding the mechanisms underlying its development is key to prevent them. To this aim, we investigated the role of TrkB receptor in L-DOPA-induced dyskinesia in hemiparkinsonian mice treated chronically with L-DOPA administration. Repeated L-DOPA injections specifically increased full-length TrkB receptor mRNA and protein levels in the dopamine-depleted dorsal striatum as compared to the contralateral non-lesioned striatum or to the striatum of sham-operated animals. In addition, chronic L-DOPA treatment activated TrkB receptor as indicated by its increased tyrosine phosphorylation. Using specific D1 or D2 receptors agonists, we found that TrkB increase was D1 receptor-dependent. To determine the consequences of these effects we invalidated the TrkB gene in striatal neurons expressing D1 receptor, by injecting Cre-expressing adeno-associated viruses into mice with TrkB floxed gene, or by crossing these mice with Drd1-Cre transgenic mice. After unilateral lesion of dopamine neurons in both these mice lines, we found an aggravation of LID compared to the control groups. No change was found when TrkB deletion was induced in the indirect pathway A2A receptor-expressing neurons. Our study suggests that BDNF/TrkB signaling plays a protective role against the development of LID and that agonists specifically activating TrkB could reduce the severity of LID.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-719

EVOLUTION OF THE SLEEP-WAKING CYCLE IN TRANSGENIC MICE WITH AN AGE-DEPENDENT ACCUMULATION OF NEUROMELANIN IN CATECHOLAMINERGIC NEURONS, A NEW HUMANIZED MODEL OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Parkinson's disease (PD) is characterized by a preferential degeneration of neurons accumulating with age neuromelanin in PD-vulnerable areas as substantia nigra (SN) and locus coeruleus (LC). Since this pigment is not naturally produced in most animals including rodents, we recently generated a neuromelanin-producing mouse model based on the tissue-specific constitutive expression of melanin-producing enzyme tyrosinase under the tyrosine hydroxylase promoter (Tg-TH-Tyr). As in humans, these animals age-dependently accumulate neuromelanin within catecholaminergic neurons. In parallel to its intracellular buildup, Tg-TH-Tyr mice exhibit an early degeneration of noradrenergic LC neurons that precedes nigral dopaminergic dysfunction, equivalent to prodromal PD, as well as PD-like motor and non-motor (i.e. olfactory, cognitive) deficits. Here we assessed whether Tg-TH-Tyr mice may also exhibit sleep alteration and REM sleep behaviour disorder (RBD), two documented prodromal manifestations of PD, likely linked to a degeneration of the LC/subcoeruleus area critical for REM sleep. For this goal, Tg-TH-Tyr and WT littermate mice (5 months old), prepared for polysomnography and video, were recorded for 58 weeks until natural death (5 Tg-6 WT). Preliminary results evidenced modifications of daily amounts and bout numbers of REM sleep, as their circadian distribution in Tg-TH-Tyr *vs.* WT mice, independently of the age factor. While clear occurrence of RBD was not detected by rapid video scrutinization, ongoing analyses of both EMG and EEG signals will allow to determine whether tonic/phasic motor events during REM sleep and the overall sleep quality are differentially affected by aging, along the presymptomatic PD period, in Tg-TH-Tyr *vs.* WT mice.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-720

CEREBELLAR STIMULATION PREVENTS LEVODOPA-INDUCED DYSKINESIA IN MICE AND NORMALIZES BRAIN ACTIVITY IN A WIDE MOTOR NETWORK

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Chronic Levodopa therapy, the gold-standard treatment for Parkinson’s Disease (PD), leads to the emergence of involuntary movements, called levodopa-induced dyskinesia (LID). Cerebellar stimulation has been shown to decrease LID severity in PD patients. Here, in order to determine how cerebellar stimulation induces LID alleviation, we performed daily short trains of optogenetic stimulations of Purkinje cells (PCs) in freely moving LID mice. We demonstrated that these stimulations are sufficient to suppress LID or even prevent their development. This symptomatic relief is accompanied by the normalization of aberrant neuronal discharge in the cerebellar nuclei, the motor cortex, and the parafascicular thalamus. Inhibition of the cerebello-parafascicular pathway counteracted the beneficial effects of cerebellar stimulation. Moreover, cerebellar stimulation reversed plasticity in D1 striatal neurons and normalized the overexpression of FosB, a transcription factor causally linked to LID. These findings demonstrate LID alleviation and prevention by daily PCs’ stimulations, which restore the function of a wide brain motor network, and may be valuable for LID treatment.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-721

ALTERED PARABRACHIAL NUCLEUS NOCICEPTIVE PROCESSING MAY UNDERLIE CENTRAL NEUROPATHIC PAIN IN PARKINSON'S DISEASE.

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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The presence of central neuropathic pain in Parkinson's disease suggests that the brain circuits that allow us to perceive and process pain could be dysfunctional in this disease. However, there is to date no clear pathophysiological mechanism to explain these specific symptoms. In the present work, we present evidence that the parabrachial nucleus, a low level primary nociceptive structure in the brainstem, and associated key structures in the basal ganglia, may be dysfunctional in Parkinson's disease. In rat models of Parkinson's disease with partial or total dopaminergic lesions of the substantia nigra pars compacta, we found that the subthalamic nucleus and the substantia nigra pars reticulata showed enhanced nociceptive responses with a Partial dopaminergic lesion while a Total dopaminergic lesion produced an increase in both the nociceptive responses and the baseline firing rate. At the level of the parabrachial nucleus, inhibited nociceptive responses and increased expression of $GABA_A$ receptors were found in the Total dopaminergic lesion group only. However, anatomical neuro-adaptations at the level of dendritic spine density and post-synaptic density were found in both dopaminergic lesion groups. We suggest that these neuro-adaptations are responsible for the substantial nociceptive rebound we found in the two groups after the pharmacological blockade of the substantia nigra pars reticulata. We propose that these neuro-adaptations follow increased inhibitory tone from the substantia nigra pars reticulata and may represent the mechanism generating central neuropathic pain in Parkinson's disease. **Keywords:** Basal Ganglia; Parabachial nucleus; Nociceptive processing; Parkinson's disease; Central neuropathic pain

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-722

SLEEP DISTURBANCES IN A MOUSE MODEL OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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A large spectrum of sleep disorders is frequently present in Parkinson's disease (PD). These co-morbidities significantly impair the patients' quality of life and are commonly refractory or exacerbated by standard anti-parkinsonian treatments. We studied sleep architecture in a mouse model of PD based on unilateral injection of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) in the medial forebrain bundle. Electrodes for electroencephalogram and electromyogram were implanted over the left parietal cortex and the neck muscles of sham-lesion and 6-OHDA-lesion mice. The recording was performed for 24 hours and awake state, REM, and non-REM sleep were sorted using a MATLAB script. The 6-OHDA lesion mice showed reduced awaken state and increased non-REM sleep during the dark (active) phase of the sleep-wake cycle. In the same animals, we also observed a higher number of NREM episodes during the light (inactive) phase, indicative of sleep fragmentation. Notably, this effect was reversed by preserving the noradrenergic system using desipramine prior to the 6-OHDA injection. Levodopa and pramipexole, administered to 6-OHDA lesion mice for seven consecutive days, significantly augmented NREM sleep during the active period. The data show that the PD mouse model used in this study reproduces sleep abnormalities reminiscent of the excessive daytime sleepiness and sleep fragmentation frequently observed in PD patients. Importantly, they also show that loss of nigrostriatal dopamine is sufficient to produce excessive daytime sleepiness, whereas sleep fragmentation depends on noradrenaline dysfunction. Finally, the sleep disturbances found in the present PD model are refractory to, and even exacerbated by common anti-parkinsonian medications.

Pubmed:
33281577: Medeiros DC, Cota VR, Oliveira ACP, Moreira FA, Moraes MFD
The Endocannabinoid System Activation as a Neural Network Desynchronizing Mediator for Seizure Suppression.
The understanding that hyper-excitability and hyper-synchronism in epilepsy are indissociably bound by a cause-consequence relation has only recently been challenged. Thus, therapeutic strategies for seizure suppression have often aimed at inhibiting excitatory circuits and/or activating inhibitory ones. However, new approaches that aim to desynchronize networks or compromise abnormal coupling between adjacent neural circuitry have been proven effective, even at the cost of enhancing local neuronal activation. Although most of these novel perspectives targeting circuitry desynchronization and network coupling have been implemented by non-pharmacological devices, we argue that there may be endogenous neurochemical systems that act primarily in the desynchronization component of network behavior rather than dampening excitability of individual neurons. This review explores the endocannabinoid system as one such possible pharmacological landmark for mimicking a form of "on-demand" desynchronization analogous to those proposed by deep brain stimulation in the treatment of epilepsy. This essay discusses the evidence supporting the role of the endocannabinoid system in modulating the synchronization and/or coupling of distinct local neural circuitry; which presents obvious implications on the physiological setting of proper sensory-motor integration. Accordingly, the process of ictogenesis involves pathological circuit coupling that could be avoided, or at least have its spread throughout the containment of other areas, if such endogenous mechanisms of control could be activated or potentiated by pharmacological intervention. In addition, we will discuss evidence that supports not only a weaker role played on neuronal excitability but the potential of the endocannabinoid system strengthening its modulatory effect, only when circuitry coupling surpasses a level of activation.
Front Behav Neurosci, 2020; 14
31827439: Medeiros DC, Lopes Aguiar C, Moraes MFD, Fisone G
Sleep Disorders in Rodent Models of Parkinson's Disease.
Sleep disorders are frequently diagnosed in Parkinson's disease and manifested in the prodromal and advanced stages of the disease. These conditions, which in some cases affect more than 50% of Parkinson's disease (PD) patients, include

hypersomnia, often manifested as excessive daytime sleepiness, insomnia, characterized by delayed initiation and fragmentation of sleep at night, and disruption of rapid eye movement (REM) sleep, resulting in loss of atonia and dream enactment. Standard dopamine replacement therapies for the treatment of motor symptoms are generally inadequate to combat sleep abnormalities, which seriously affect the quality of life of PD patients. Rodent models still represent a major tool for the study of many aspects of PD. They have been primarily designed to eliminate midbrain dopamine neurons and elicit motor impairment, which are the traditional pathological features of PD. However, rodent models are increasingly employed to investigate non-motor symptoms, which are often caused by degenerative processes affecting multiple monoaminergic and peptidergic structures. This review describes how neurotoxic and genetic manipulations of rats and mice have been utilized to reproduce some of the major sleep disturbances associated with PD and to what extent these abnormalities can be linked to nondopaminergic dysfunction, affecting for instance noradrenaline, serotonin, and orexin transmission. Strengths and limitations are discussed, as well as the consistency of results obtained so far, and the need for models that better reproduce the multisystemic neurodegenerative nature of PD, thereby allowing to replicate the complex etiology of sleep-related disorders.
Front Pharmacol, 2019; 10
31130472: Camargos VN, Foureaux G, Medeiros DC, da Silveira VT, Queiroz-Junior CM, Matosinhos ALB, Figueiredo AFA, Sousa CDF, Moreira TP, Queiroz VF, Dias ACF, Santana KTO, Passos I, Real ALCV, Silva LC, Mourão FAG, Wnuk NT, Oliveira MAP, Macari S, Silva T, Garlet GP, Jackman JA, Soriani FM, Moraes MFD, Mendes EMAM, Ribeiro FM, Costa GMJ, Teixeira AL, Cho NJ, Oliveira ACP, Teixeira MM, Costa VV, Souza DG
In-depth characterization of congenital Zika syndrome in immunocompetent mice: Antibody-dependent enhancement and an antiviral peptide therapy.
Zika virus (ZIKV) infection during pregnancy may cause major congenital defects, including microcephaly, ocular, articular and muscle abnormalities, which are collectively defined as Congenital Zika Syndrome. Here, we performed an in-depth characterization of the effects of congenital ZIKV infection (CZI) in immunocompetent mice.
EBioMedicine, 2019; 44
24472684: Medeiros Dde C, Moraes MF
Focus on desynchronization rather than excitability: a new strategy for intraencephalic electrical stimulation.
Epilepsy is a severely debilitating brain disease, often associated with premature death, which has an urgent need for alternative methods of treatment. In fact, roughly 25% of patients with epilepsy do not have seizures satisfactorily controlled by pharmacological treatment, and 30% of these patients with treatment-refractory seizures are not even eligible for ablative surgery. Epilepsy is most readily identifiable by its seizures and/or paroxysmal events, mostly viewed as spontaneously recurrent and unpredictable, which are caused by stereotyped changes in neurological function associated with hyperexcitability and hypersynchronicity of the underlying neural networks. Treatment has strongly been based on the fixed goal of depressing neuronal activity, working under the veiled assumption that hyperexcitability would lead to synchronous neuronal activity and, therefore, to seizure. Over the last 20-30 years, the emergence of electrical (ES) of deep brain structures, a practicable option for treating patients with otherwise untreatable seizures, has broadened our understanding of anticonvulsant mechanisms that conceptually differ from those of pharmacological treatment. Conversely, the research on ES therapy applied to epilepsy is contributing significantly to untwine the phenomena of excitation from that of synchronization as potential target mechanisms for abolishing seizures and predicting paroxysmal events. This paper is, thus, an addendum to other reviews on the subject of ES therapy in epilepsy which focuses on the desynchronization effect ES has on epileptogenic neural networks rather than its effect on overall brain excitability.
Epilepsy Behav, 2014; 38
24332185: Medeiros DC, Oliveira LB, Mourão FA, Bastos CP, Cairasco NG, Pereira GS, Mendes EM, Moraes MF
Temporal rearrangement of pre-ictal PTZ induced spike discharges by low frequency electrical stimulation to the amygdaloid complex.
Epilepsy is a common neurological disease affecting over 40 million people worldwide. The foremost important challenge of epileptologists has been to control and predict the recurrent and spontaneous seizures of epileptic patients. The application of low frequency electrical stimulation (LFS) in deep brain structures has shown promising results in seizure control. However, the use of LFS as a probing strategy for seizure prediction, thus contributing to a closed loop solution, is still poorly explored.
Brain Stimul, 2014 Mar-Apr; 7
22370119: Medeiros Dde C, Cota VR, Vilela MR, Mourão FA, Massensini AR, Moraes MF
Anatomically dependent anticonvulsant properties of temporally-coded electrical stimulation.
In the PTZ animal model of epilepsy, electrical stimulation applied to the amygdaloid complex may result in either pro-convulsive or anticonvulsant effect, depending on the temporal pattern used (i.e. periodic-PS and non-periodic-NPS electrical stimulation). Our hypothesis is that the anatomical target is a determinant factor for the differential effect of temporally-coded

patterns on seizure outcome. The threshold dose of PTZ to elicit forelimb clonus and generalized tonic-clonic seizure behavior was measured. The effect of amygdaloid complex PS on forelimb clonus threshold showed a pro-convulsive effect while NPS was anticonvulsant. NPS also significantly increased generalized tonic-clonic threshold; while PS, although at lower threshold levels, did not present statistical significance. Thalamus stimulation did not affect forelimb clonus threshold and showed similar anticonvulsant profiles for both PS and NPS on generalized tonic-clonic threshold. In summary, the anatomical target is a determinant factor on whether temporally-coded ES differentially modulates seizure outcome.
Epilepsy Behav, 2012; 23
31780904: Pinto HPP, de Oliveira Lucas EL, Carvalho VR, Mourão FAG, de Oliveira Guarnieri L, Mendes EMAM, de Castro Medeiros D, Moraes MFD
Seizure Susceptibility Corrupts Inferior Colliculus Acoustic Integration.
Evidence suggests that the pathophysiology associated with epileptic susceptibility may disturb the functional connectivity of neural circuits and compromise the brain functions, even when seizures are absent. Although memory impairment is a common comorbidity found in patients with epilepsy, it is still unclear whether more caudal structures may play a role in cognitive deficits, particularly in those cases where there is no evidence of hippocampal sclerosis. This work used a genetically selected rat strain for seizure susceptibility (Wistar audiogenic rat, WAR) and distinct behavioral (motor and memory-related tasks) and electrophysiological (inferior colliculus, IC) approaches to access acoustic primary integrative network properties. The IC neural assemblies' response was evaluated by auditory transient (focusing on bottom-up processing) and steady-state evoked response (ASSR, centering on feedforward and feedback forces over neural circuitry). The results show that WAR displayed no disturbance in motor performance or hippocampus-dependent memory tasks. Nonetheless, WAR animals exhibited significative impairment for auditory fear conditioning (AFC) along with no indicative of IC plastic changes between the pre-conditioning and test phases (ASSR coherence analysis). Furthermore, WAR's IC response to transient stimuli presented shorter latency and higher amplitude compared with Wistar; and the ASSR analysis showed similar results for WAR and Wistar animals under subthreshold dose of pentylenetetrazol (pro-convulsive drug) for seizure-induction. Our work demonstrated alterations at WAR IC neural network processing, which may explain the associated disturbance on AFC memory.
Front Syst Neurosci, 2019; 13
30352775: de Castro Medeiros D, Raspante LBP, Mourão FAG, Carvalho VR, Mendes EMAM, Moraes MFD
Deep brain stimulation probing performance is enhanced by pairing stimulus with epileptic seizure.
The unpredictability of spontaneous and recurrent seizures significantly impairs the quality of life of patients with epilepsy. Probing neural network excitability with deep brain electrical stimulation (DBS) has shown promising results predicting pathological shifts in brain states. This work presents a proof-of-principal that active electroencephalographic (EEG) probing, as a seizure predictive tool, is enhanced by pairing DBS and the electrographic seizure itself. The ictogenic model used consisted of inducing seizures by continuous intravenous infusion of pentylenetetrazol (PTZ - 2.5 mg/ml/min) while a probing DBS was delivered to the thalamus (TH) or amygdaloid complex to detect changes prior to seizure onset. Cortical electrophysiological recordings were performed before, during, and after PTZ infusion. Thalamic DBS probing, but not amygdaloid, was able to predict seizure onset without any observable proconvulsant effects. However, previously pairing amygdaloid DBS and epileptic polyspike discharges (day-1) elicited distinct preictal cortically recorded evoked response (CRER) (day-2) when compared with control groups that received the same amount of electrical pulses at different moments of the ictogenic progress at day-1. In conclusion, our results have demonstrated that the pairing strategy potentiated the detection of an altered brain state prior to the seizure onset. The EEG probing enhancement method opens many possibilities for both diagnosis and treatment of epilepsy.
Epilepsy Behav, 2018; 88
25609241: Mourão FAG, Lockmann ALV, Castro GP, de Castro Medeiros D, Reis MP, Pereira GS, Massensini AR, Moraes MFD
Triggering Different Brain States Using Asynchronous Serial Communication to the Rat Amygdala.
Inputting information to the brain through direct electrical microstimulation must consider how underlying neural networks encode information. One unexplored possibility is that a single electrode delivering temporally coded stimuli, mimicking an asynchronous serial communication port to the brain, can trigger the emergence of different brain states. This work used a discriminative fear-conditioning paradigm in rodents in which 2 temporally coded microstimulation patterns were targeted at the amygdaloid complex. Each stimulus was a binary-coded "word" made up of 10 ms bins, with 1's representing a single pulse stimulus: A-1001111001 and B-1110000111. During 3 consecutive retention tests (i.e., day-word: 1-B; 2-A, and 3-B), only binary-coded words previously paired with a foot-electroshock elicited proper aversive behavior. To determine the neural substrates recruited by the different stimulation patterns, c-Fos expression was evaluated 90 min after the last retention test. Animals conditioned to word-B, after stimulation with word-B, demonstrated increased hypothalamic c-Fos staining. Animals conditioned to word-A, however, showed increased prefrontal c-Fos labeling. In addition, prefrontal-cortex and hypothalamic

c-Fos staining for, respectively, word-B- and word-A-conditioned animals, was not different than that of an unpaired control group. Our results suggest that, depending on the valence acquired from previous learning, temporally coded microstimulation activates distinct neural networks and associated behavior.
Cereb Cortex, 2016; 26

34107264: Cid E, Marquez-Galera A, Valero M, Gal B, Medeiros DC, Navarron CM, Ballesteros-Esteban L, Reig-Viader R, Morales AV, Fernandez-Lamo I, Gomez-Dominguez D, Sato M, Hayashi Y, Bayés À, Barco A, Lopez-Atalaya JP, de la Prida LM

Sublayer- and cell-type-specific neurodegenerative transcriptional trajectories in hippocampal sclerosis.
Hippocampal sclerosis, the major neuropathological hallmark of temporal lobe epilepsy, is characterized by different patterns of neuronal loss. The mechanisms of cell-type-specific vulnerability and their progression and histopathological classification remain controversial. Using single-cell electrophysiology in vivo and immediate-early gene expression, we reveal that superficial CA1 pyramidal neurons are overactive in epileptic rodents. Bulk tissue and single-nucleus expression profiling disclose sublayer-specific transcriptomic signatures and robust microglial pro-inflammatory responses. Transcripts regulating neuronal processes such as voltage channels, synaptic signaling, and cell adhesion are deregulated differently by epilepsy across sublayers, whereas neurodegenerative signatures primarily involve superficial cells. Pseudotime analysis of gene expression in single nuclei and in situ validation reveal separated trajectories from health to epilepsy across cell types and identify a subset of superficial cells undergoing a later stage in neurodegeneration. Our findings indicate that sublayer- and cell-type-specific changes associated with selective CA1 neuronal damage contribute to progression of hippocampal sclerosis.
Cell Rep, 2021; 35

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BOARD NUMBER: S02-723

OPTOGENETIC MODULATIONS OF EXTERNAL GLOBUS PALLIDUS NEURONS DIFFERENTIALLY IMPACT MOTOR BEHAVIOUR IN NORMAL AND HEMIPARKINSONIAN MICE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Motor symptoms of Parkinson’s disease (PD) result from the loss of nigrostriatal dopamine (DA) neurons and subsequent dysfunction of cortico-basal-ganglia (CBG) network. Recent anatomical and electrophysiological studies suggest that altered neuronal activity of the external globus pallidus (GPe) may be a critical mechanism contributing to the CBG network dysfunction because of its widespread projections to all BG nuclei and to the thalamus and the cortex. Yet, behavioural evidence supporting this view are scarce and the reported results are controversial. Here, we used unilateral 6-hydroxydopamine (6-OHDA) nigrostriatal DA lesion model of PD and optogenetic approach in mice to explore GPe contribution to the motor deficits of PD. We first examined whether photoinhibition of GPe neurons in normal BL6 mice using the inhibitory opsin IC++ could reproduce the classical motor deficits (ipsilateral circling, forelimb akinesia and locomotor hypoactivity) of 6-OHDA lesion. Bilateral GPe photoinhibition had no impact on spontaneous locomotor activity while unilateral photoinhibition induced few ipsilateral rotations, indicating that GPe may not be recruited in all testing conditions. We next examined whether photostimulation of GPe neurons could alleviate lesion-induced motor deficits. GPe photostimulation using the excitatory opsin ChR2(E123T/T159C) improved motor deficits at stimulation parameters ineffective in non-lesioned mice. Interestingly, selective photostimulation of parvalbumin (PV)-expressing neurons, which preferentially innervate BG output structures, was sufficient for restoring all motor deficits of 6-OHDA lesion. Collectively, these findings support the central role of GPe in the pathophysiology of PD and suggest that compensatory alterations of GPe activity, namely dyfunction of PV-neurons, may underly motor impairments.

FENS Forum 2022
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BOARD NUMBER: S02-724

UNDERSTANDING THE BEHAVIOURAL AND MORPHOLOGICAL CHANGES IN AN ALPHA-SYNUCLEIN RAT MODEL OF PARKINSON´S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Parkinson’s disease (PD) is an age-related neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons in the substantia nigra (SN). Surviving neurons contain alpha-synuclein-positive intracellular inclusions mainly in nerve terminals. The aim of the present work is to investigate the effects of alpha-synuclein (alpha-syn) in the SN and striatum and its role on pathogenesis and symptomatology of PD. Methods: we used a rat model for Parkinson’s disease based on overexpression of alpha-syn with adeno-associated viral vectors. 1 µl of rAAV9-CMVie/SynP-wtsyn-WPRE (wt alpha-syn) was injected bilaterally in SN of 2 months Sprague Dawley male rats. After 2 months of alpha-syn overexpression open field and novel object recognition tests were realized. Animals were perfused and Tyrosine hydroxylase (TH) immunopositive striatal optical density, SNreticulata optical density and SNcompacta TH positive neuron density were measured. In addition, alpha-syn and alpha-syn -phosphorylated (p-alpha-syn) expression were observed by immunofluorescence. Results: behavioural tests had shown that alpha-syn animals have longer resting time and lower ability to recognize novel objects. Alpha-syn expression reached different brain regions in the rostro-caudal axis while p-alpha-syn expression remains near substantia nigra. TH immunostaining revealed a significant decrease of the TH-immunopositive striatal optical density, loss of TH-immunoreactive neurons in the SNcompacta and axodendritic network in the SNreticulata. Conclusions: These findings support the effectiveness of alpha-syn model to reproduce an animal model of PD. The morphological and behavioural description of this model could be a promising tool to investigate new strategies to implement against PD. Funding: PIBA19-0038; PUE21-03; COLAB20/07; GIU19/339; Transborder Joint Laboratory: CoMorPD.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-725

THERAPEUTIC REACTIVATION OF DORMANT NEUROMELANIN-LADEN NEURONS IN THE SUBSTANTIA NIGRA PARS COMPACTA BY OPTOGENETIC STIMULATION.

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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With age, humans accumulate the dark-brown pigment neuromelanin (NM) inside catecholaminergic neurons reaching the highest levels in dopaminergic neurons of the substantia nigra pars compacta (SNC) which become susceptible to degeneration that later leads to Parkinson's disease (PD). It has been shown that during PD early stages dopaminergic neurons undergo homeostatic changes that submerge them in a "dormancy state" characterized by neuronal activity decrease and a loss of tyrosine-hydroxylase enzyme (TH) expression. We hypothesize that pacemaking of dopaminergic neurons (either optogenetically or chemogenetically) would alleviate early signs in an NM accumulating PD animal model. In DAT-Cre and TH-Cre mice we overexpressed the melanin-producing enzyme tyrosinase (hTyr) specifically in midbrain dopaminergic neurons along with the red light-sensitive opsin Chrimson or the excitatory DREADD receptor HM3d. After seven weeks of viral expression and NM accumulation, the mice were optically (635nm laser) or chemically (J60) stimulated during 4 weeks accordingly. We found that neurons in the ventral tegmental area and SNC accumulate NM differentially when they are non-stimulated (control), optically stimulated, or chemogenetically stimulated. Significantly fewer NM-positive neurons (NM+) were found in light stimulated dopaminergic regions compared to the control non-stimulated contralateral ones. Although both control and stimulated dopaminergic areas showed similar numbers of TH-positive cells (TH+), the total number of them is overall diminished compared to intact animals. In contrast, the DREADD stimulation led to a dramatic decrease in the number of TH+ neurons in the stimulated regions while the NM+ cells countings remained similar compared to control non-stimulated contralateral regions.

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BOARD NUMBER: S02-726

THE THRESHOLD OF BENEFICIAL EFFECT OF REGULAR PHYSICAL ACTIVITY ON BEHAVIORAL DEFICITS IN ANIMAL MODEL OF PARKINSON'S DISEASE.

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

Martyna Paleczna, Justyna Kadłuczka, Dominika Biała, Barbara Kosmowska, Katarzyna Kuter-Nowak, Joanna Osika
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Background: Exercise is recommended for PD treatment, adjunct with pharmacotherapy. After partial degeneration of dopaminergic system surviving neurons can spontaneously compensate, to maintain almost normal system functioning but it strongly depends on the lesion size. Exercise could help to naturally move this threshold. The aim was to create reliable animal model to study potential neuroprotection, regeneration and compensation of the nigrostriatal dopaminergic system in PD. Comparison of medium vs large lesion resembling early vs late stage of PD will allow to determine when physiotherapeutic interventions are still possible and what kind of improvement can be expected. **Methods**: Regular training was performed for 4 weeks before and after unilateral 6-OHDA injection into the MFB (4 or 8 µg). Locomotor activity, asymmetric paw use and apomorphine induced rotation tests were assessed at different time points. **Results**: Locomotion of running lesioned animals was not different than their sham operated controls while sedentary rats showed decreased activity as compared to sedentary sham control. Asymmetric paw use resulted in lower ipsi/contra ratio in medium lesioned, exercised rats. Number of contralateral rotations in exercised medium lesioned animals was reduced. Medium lesion induced locomotor deficit was spontaneously compensated in contrast to large lesioned animals. **Conclusions:** Treadmill training may ameliorate some behavioral deficits but threshold of lesion size responsive to benefits of running is visible. The studies on exercise will bring us closer to understanding mechanisms prolonging independent functioning of patients in multiple neurodegenerative diseases. **Funding:** NCN 2019/35/B/NZ7/02862 and statutory funds of Maj Institute of Pharmacology, PAS, Poland.

Pubmed:
30295916: Kuter K, Olech Ł, Głowacka U, Paleczna M
Astrocyte support is important for the compensatory potential of the nigrostriatal system neurons during early neurodegeneration.
Glial pathology precedes symptoms of Parkinson's disease and multiple other neurodegenerative diseases. Prolonged impairment of astrocytic functions could increase the vulnerability of dopaminergic neurons in the substantia nigra (SN), accelerate their degeneration and affect ability to compensate for partial degeneration at the presymptomatic stages of the disease. The aim of this study was to investigate the astrocyte depletion in the SN, its impact on the dopaminergic system functioning and multiple markers of energy metabolism during the early stages of neurodegeneration and compensation. We induced death of 30% of astrocytes by chronic infusion of fluorocitrate (FC) into the SN, simultaneously activating microglia response but sparing the dopaminergic neurons. The FC effect was reversible after toxin withdrawal. Dopaminergic neurons were killed by 6-hydroxydopamine causing transient locomotor disability, reversed with time showing compensatory potential. Death of astrocytes diminished the capability of the dopaminergic system to compensate for the degeneration of neurons and caused a local energy deprivation by decreasing lactate and glycogen amount. Studied markers suggest a shift in the usage of energy substrates, via increased glycogenolysis and glycolysis markers, ketone bodies availability and fatty acid transport in remaining cells. Peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1alpha) and AMP-activated protein kinase (AMPK), the energy sensors, showed different regulation between the cell-types. Increased neuronal expression of carnitine palmitoyltransferase 1c could play a role in the adaptation to metabolic stress in response to glia dysfunction. Astrocyte energetic support is one of the essential factors for neuronal compensatory mechanisms of dopaminergic system and might have a leading role in the presymptomatic Parkinson's disease stages. OPEN SCIENCE BADGES: This article has received a badge for *Open Materials* because it provided all relevant information to reproduce the study in the manuscript. The complete Open Science Disclosure form for this article can be found at the end of the article. More information about the Open Practices badges can be found at https://cos.io/our-services/open-science-badges/.
J Neurochem, 2019; 148
34572572: Jurga AM, Paleczna M, Kadluczka J, Kuter KZ

Beyond the GFAP-Astrocyte Protein Markers in the Brain.
The idea of central nervous system as one-man band favoring neurons is long gone. Now we all are aware that neurons and neuroglia are team players and constant communication between those various cell types is essential to maintain functional efficiency and a quick response to danger. Here, we summarize and discuss known and new markers of astroglial multiple functions, their natural heterogeneity, cellular interactions, aging and disease-induced dysfunctions. This review is focused on newly reported facts regarding astrocytes, which are beyond the old stereotypes. We present an up-to-date list of marker proteins used to identify a broad spectrum of astroglial phenotypes related to the various physiological and pathological nervous system conditions. The aim of this review is to help choose markers that are well-tailored for specific needs of further experimental studies, precisely recognizing differential glial phenotypes, or for diagnostic purposes. We hope it will help to categorize the functional and structural diversity of the astroglial population and ease a clear readout of future experimental results.
Biomolecules, 2021; 11
32848611: Jurga AM, Paleczna M, Kuter KZ
Overview of General and Discriminating Markers of Differential Microglia Phenotypes.
Inflammatory processes and microglia activation accompany most of the pathophysiological diseases in the central nervous system. It is proven that glial pathology precedes and even drives the development of multiple neurodegenerative conditions. A growing number of studies point out the importance of microglia in brain development as well as in physiological functioning. These resident brain immune cells are divergent from the peripherally infiltrated macrophages, but their precise discrimination is surprisingly difficult. Microglial heterogeneity in the brain is especially visible in their morphology and cell density in particular brain structures but also in the expression of cellular markers. This often determines their role in physiology or pathology of brain functioning. The species differences between rodent and human markers add complexity to the whole picture. Furthermore, due to activation, microglia show a broad spectrum of phenotypes ranging from the pro-inflammatory, potentially cytotoxic M1 to the anti-inflammatory, scavenging, and regenerative M2. A precise distinction of specific phenotypes is nowadays essential to study microglial functions and tissue state in such a quickly changing environment. Due to the overwhelming amount of data on multiple sets of markers that is available for such studies, the choice of appropriate markers is a scientific challenge. This review gathers, classifies, and describes known and recently discovered protein markers expressed by microglial cells in their different phenotypes. The presented microglia markers include qualitative and semi-quantitative, general and specific, surface and intracellular proteins, as well as secreted molecules. The information provided here creates a comprehensive and practical guide through the current knowledge and will facilitate the choosing of proper, more specific markers for detailed studies on microglia and neuroinflammatory mechanisms in various physiological as well as pathological conditions. Both basic research and clinical medicine need clearly described and validated molecular markers of microglia phenotype, which are essential in diagnostics, treatment, and prevention of diseases engaging glia activation.
Front Cell Neurosci, 2020; 14
34299176: Kuter KZ, Olech Ł, Głowacka U, Paleczna M
Increased Beta-Hydroxybutyrate Level Is Not Sufficient for the Neuroprotective Effect of Long-Term Ketogenic Diet in an Animal Model of Early Parkinson's Disease. Exploration of Brain and Liver Energy Metabolism Markers.
The benefits of a ketogenic diet in childhood epilepsy steered up hope for neuroprotective effects of hyperketonemia in Parkinson's disease (PD). There are multiple theoretical reasons but very little actual experimental proof or clinical trials. We examined the long-term effects of the ketogenic diet in an animal model of early PD. A progressive, selective dopaminergic medium size lesion was induced by 6-OHDA injection into the medial forebrain bundle. Animals were kept on the stringent ketogenic diet (1% carbohydrates, 8% protein, 70% fat) for 3 weeks prior and 4 weeks after the brain operation. Locomotor activity, neuron count, dopaminergic terminal density, dopamine level, and turnover were analyzed at three time-points post-lesion, up to 4 weeks after the operation. Energy metabolism parameters (glycogen, mitochondrial complex I and IV, lactate, beta-hydroxybutyrate, glucose) were analyzed in the brain and liver or plasma. Protein expression of enzymes essential for gluconeogenesis (PEPCK, G6PC) and glucose utilization (GCK) was analyzed in the liver. Despite long-term hyperketonemia pre- and post-lesion, the ketogenic diet did not protect against 6-OHDA-induced dopaminergic neuron lesions. The ketogenic diet only tended to improve locomotor activity and normalize DA turnover in the striatum. Rats fed 7 weeks in total with a restrictive ketogenic diet maintained normoglycemia, and neither gluconeogenesis nor glycogenolysis in the liver was responsible for this effect. Therefore, potentially, the ketogenic diet could be therapeutically helpful to support the late compensatory mechanisms active via glial cells but does not necessarily act against the oxidative stress-induced parkinsonian neurodegeneration itself. A word of caution is required as the stringent ketogenic diet itself also carries the risk of unwanted side effects, so it is important to study the long-term effects of such treatments. More detailed metabolic long-term studies using unified diet parameters are required, and human vs. animal differences should be taken under

consideration.
Int J Mol Sci, 2021; 22

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-727

THE ANTI-DYSKINETIC EFFECTS OF CANNABIDIOL AND HUF-101, A FLUORINATED CANNABIDIOL DERIVATIVE, IN HEMIPARKINSONIAN RATS: A POSSIBLE ROLE OF ENDOCANNABINOIDS AND TRPV-1 RECEPTORS

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Pharmacological manipulation of endocannabinoid system represents a promising therapy to alleviate L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia (LID) in Parkinson's disease (PD). We investigated here whether CBD (10, 30 and 60 mg/kg) and HUF-101 (1, 3 and 30 mg/kg), a fluorinated CBD analogue, would induce antidyskinetic effects. Unilateral striatal 6-hydroxydopamine-lesioned rats received L-DOPA (10 mg/kg, s.c.) for one week, developing severe axial, limb, locomotor and orofacial abnormal involuntary movements (AIMs). Following that, the animals were treated with CBD or HUF-101 (i.p.) before L-DOPA for two weeks. Independent groups received a combination of CBD with antagonists of the Transient Receptor Potential Vanniloid-1 (CPZ), cannabinoid type 1 (AM251), cannabinoid type 2 (AM630) or Peroxisome Proliferator-Activated type gamma (GW9662) receptors. An inverted U-shaped curve was observed in the CBD effect, the 30 mg/kg dose was effective in decreasing AIMs. HUF-101 at 3 and 30 mg/kg doses present antidyskinetic effects. Both, CBD and HUF-101 do not interfere in motor benefits produced by L-DOPA. AM251 or GW9662 reversed the antidyskinetic effect of CBD and AM630 had no action in this effect. CPZ (5 mg/kg) potentiated the anti-dyskinetic effect of CBD (30mg/kg) and was able to reduce AIMs in association with a subeffective dose of CBD (60 mg/kg). In-tube solid-phase microextraction was used for determination of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in rat striatum. The decrease of LID by CBD was associated with an increase in the endocannabinoids AEA and 2-AG, which may be a possible mechanism of CBD prior to L-DOPA treatment to prevent the onset of LID.

Pubmed:
34246682: Vivanco-Estela AN, Dos-Santos-Pereira M, Guimaraes FS, Del-Bel E, Nascimento GCD
Cannabidiol has therapeutic potential for myofascial pain in female and male parkinsonian rats.
The musculoskeletal orofacial pain is a complex symptom of Parkinson's disease (PD) resulting in stomatognathic system dysfunctions aggravated by the disease rigidity and postural instability. We tested the effect of cannabidiol (CBD), a non-psychotomimetic constituent of Cannabis sativa, in PD-related myofascial pain. Wistar adult female and male rats orofacial allodynic and hyperalgesic responses were tested by Von Frey and formalin tests, before and 21 days past 6-OHDA lesion. Algesic response was tested after masseter muscle injection of CBD (10, 50, 100 µg in 10 µL) or vehicle. Males compared to females in all estrous cycles' phases presented reduced orofacial allodynia and hyperalgesia. According to the estrous cycle's phases, females presented distinct orofacial nociceptive responses, being the estrus phase well-chosen for nociceptive analysis after 6-OHDA lesion (phase with fewer hormone alterations and adequate length). Dopaminergic neuron lesion decreased mechanical and inflammatory nociceptive thresholds in females and males in a higher proportion in females. CBD local treatment reduced the increased orofacial allodynia and hyperalgesia, in males and females. The female rats were more sensitive to CBD effect considering allodynia, responding to the lowest dose. Although females and males respond to the effect of three doses of CBD in the formalin test, males showed a superior reduction in the hyperalgesic response. These results indicate that hemiparkinsonian female in the estrus phase and male answer differently to the different doses of CBD therapy and nociceptive tests. CBD therapy is effective for parkinsonism-induced orofacial nociception.
Neuropharmacology, 2021; 196
30109452: Bortolanza M, Nascimento GC, Socias SB, Ploper D, Chehín RN, Raisman-Vozari R, Del-Bel E
Tetracycline repurposing in neurodegeneration: focus on Parkinson's disease.
The prevalence of Parkinson's disease, which affects millions of people worldwide, is increasing due to the aging population. In addition to the classic motor symptoms caused by the death of dopaminergic neurons, Parkinson's disease encompasses a wide range of nonmotor symptoms. Although novel disease-modifying medications that slow or stop Parkinson's disease

progression are being developed, drug repurposing, which is the use of existing drugs that have passed numerous toxicity and clinical safety tests for new indications, can be used to identify treatment compounds. This strategy has revealed that tetracyclines are promising candidates for the treatment of Parkinson's disease. Tetracyclines, which are neuroprotective, inhibit proinflammatory molecule production, matrix metalloproteinase activity, mitochondrial dysfunction, protein misfolding/aggregation, and microglial activation. Two commonly used semisynthetic second-generation tetracycline derivatives, minocycline and doxycycline, exhibit effective neuroprotective activity in experimental models of neurodegenerative/ neuropsychiatric diseases and no substantial toxicity. Moreover, novel synthetic tetracyclines with different biological properties due to chemical tuning are now available. In this review, we discuss the multiple effects and clinical properties of tetracyclines and their potential use in Parkinson's disease treatment. In addition, we examine the hypothesis that the anti-inflammatory activities of tetracyclines regulate inflammasome signaling. Based on their excellent safety profiles in humans from their use for over 50 years as antibiotics, we propose the repurposing of tetracyclines, a multitarget antibiotic, to treat Parkinson's disease.
J Neural Transm (Vienna), 2018; 125
33751546: Bortolanza M, do Nascimento GC, Raisman-Vozari R, Del-Bel E
Doxycycline and its derivative, COL-3, decrease dyskinesia induced by l-DOPA in hemiparkinsonian rats.
l-DOPA-induced dyskinesia is a debilitating effect of treating Parkinson's disease with this drug. New therapeutic approaches that prevent or attenuate this side effect are needed.
Br J Pharmacol, 2021; 178
33611790: Nascimento GC, De Paula BB, Gerlach RF, Leite-Panissi CRA
Temporomandibular inflammation regulates the matrix metalloproteinases MMP-2 and MMP-9 in limbic structures.
Temporomandibular disorder (TMD) is characterized by acute or chronic orofacial pain, which can be associated with inflammatory processes in the temporomandibular joint (TMJ) and emotional disorders. Peripheral and central sensitization in painful orofacial processes is common, and it can be triggered by peripheral inflammatory challenge with consequent neuroinflammation phenomena. Such neuroinflammation comes from inflammatory products from supportive cells, blood-brain barrier, and extracellular matrix. Here, we evaluated the possible recruitment of limbic structures for modified matrix metalloproteinases (MMPs) expression and activity during temporomandibular inflammation-induced orofacial persistent pain. The inflammatory process in TMJs of rats was induced by Freund's Complete Adjuvant (CFA) administration. The activity and expression of MMPs-2 and 9 were assessed by in situ zymography and conventional zymography, respectively. A glial colocalization with the MMPs was performed using immunofluorescence. The results evidenced both short- and long-term alterations on MMP-2 and -9 expression in the limbic structures following CFA-induced temporomandibular inflammation. The gelatinolytic activity was increased in the central amygdala, hippocampus, hypothalamus, ventrolateral periaqueductal gray (vlPAG), superior colliculus, and inferior colliculus. Finally, an increase of colocalization of MMP-2/GFAP and MMP-9/GFAP in CFA-induced inflammation groups was observed when compared with saline groups in the central amygdala and vlPAG. It is possible to suggest that glial activation is partly responsible for the production of gelatinases in the persistent orofacial pain, and it is involved in the initiation and maintenance of this process, indicating that inhibition of MMPs might be pursued as a potential new therapeutic target for TMD.
J Cell Physiol, 2021; 236
34500037: Nascimento GC, de Paula BB, Ferrari DP, Iyomasa DM, Pereira YCL, Pedrazzi JF, Bortolanza M, Issy AC, Issa JPM, Leite-Panissi CRA, Iyomasa MM, Del-Bel E
Upregulation of FosB/ΔFosB in limbic circuits after tooth exodontia-induced occlusal instability in an experimental model of unpredictable chronic stress.
Psychological stress and occlusal alterations are contributing etiologic factors for temporomandibular and muscular disorders in the orofacial area. The neural modulation recruited for this relationship, however, is not elucidated. The aim of this study was to investigate potential central mechanisms involved in the exodontia-induced occlusal instability associated with unpredictable chronic stress (UCS). Male adult Wistar rats were submitted to occlusal instability (unilateral molar teeth extraction) and/or to a UCS protocol and treated with diazepam or vehicle. The anxiety-like behavior was evaluated by elevated plus maze (EPM) and open field (OF) tests. Limbic structures such as the central nucleus of the amygdala (CeA), paraventricular nucleus of the hypothalamus (PVN), dorsal periaqueductal gray matter (dPAG) and nucleus accumbens core (NAc) were analyzed for expression of FosB/ΔFosB (immediate early genes) by immunohistochemistry. Exodontia and/or UCS decreased the time spent in the open arms at the EPM and the distance travelled at the OF, and increased the immobility time at the OF, suggesting anxiety-like behavior. In addition, exodontia induction resulted in an upregulation of FosB/ΔFosB in the CeA, PVN and dPAG, while UCS and exodontia + UCS upregulate FosB/ΔFosB immunoreactivity in the CeA, PVN, dPAG and NAc. Treatment with diazepam decreased the expression of FosB/ΔFosB in all analyzed structures of animals subject to UCS and exodontia + UCS, while promoted a reduction in the FosB/ΔFosB expression in the CeA, PVN and dPAG in animals subject to exodontia. Our findings showed an anxiogenic effect of exodontia and UCS, which is

correlated with intranuclear neuron activation of limbic structures in a spatially dependent manner and that is prevented by the administration of diazepam.
Brain Res Bull, 2021; 176
31604145: Garattini EG, Santos BM, Ferrari DP, Capel CP, Francescato HDC, Coimbra TM, Leite-Panissi CRA, Branco LGS, Nascimento GC
Propargylglycine decreases neuro-immune interaction inducing pain response in temporomandibular joint inflammation model.
The mechanisms underlying temporomandibular disorders following orofacial pain remain unclear. Hydrogen sulfide (HS), a newly identified gasotransmitter, has been reported to modulate inflammation. Cystathionine γ-lyase (CSE) is responsible for the systemical production of HS, which exerts both pro- and antinociceptive effects through inflammation. In the current study, we investigated whether the endogenous HS production pathway contributes to arousal and maintenance of orofacial inflammatory pain, through the investigation of the effects of a CSE inhibitor, propargyglycine (PAG), in a rat CFA (Complete Freund Adjuvant)-induced temporomandibular inflammation model to mimic persistent pain in the orofacial region. For this, rats received either CFA or saline in the temporomandibular joints (TMJs), and after 3 or 14 days, they received a single injection of PAG or saline and were evaluated for nociception with the von Frey and formalin test. Also, pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) were analyzed in TMJs and trigeminal ganglion (TG). In this last one, glial cells reactivity was also verified. Endogenous HS production rate were measured in both, TMJ and TG. Our results indicated decreased allodynia and hyperalgesic responses in rats submitted to CFA after injection of PAG. Moreover, PAG inhibited leucocyte migration to temporomandibular synovial fluid after 3 and 14 days of inflammation. PAG was able to reduce levels of CBS, CSE, TNF-α, and IL-1β in the TMJ and TG, after 13 days of CFA injection. The observed increased activation of glial cells in the trigeminal ganglia on the 14th day of inflammation can be prevented by the highest dose of PAG. Finally, CBS and CSE expression, and endogenous HS production rate in the TMJ and TG was found higher in rats with persistent temporomandibular inflammation compared to rats injected with saline and PAG was able to prevent this elevation. Our results elucidated the molecular mechanisms by which HS exerts its pro-inflammatory and pro-nociceptive role in the orofacial region by alterations in both local tissue and TG.
Nitric Oxide, 2019; 93
32472004: Nascimento GC, Malzone BL, Iyomasa DM, Pereira YCL, Issa JPM, Leite-Panissi CRA, Watanabe IS, Iyomasa MM, Fuentes R, Del Bel E, Dias FJ
Beneficial effects of benzodiazepine on masticatory muscle dysfunction induced by chronic stress and occlusal instability in an experimental animal study.
Psychological stress and occlusal alteration are important etiologic factors for temporomandibular/masticatory muscular disorders. In particular, the exact physiologic mechanism underlying the relation by occlusal alteration and temporomandibular disorders remains unclear. Our purpose was to test the hypothesis that benzodiazepine therapy is able to prevent metabolic and vascular changes in the medial pterygoid muscle of rats under chronic stress after 14 days of unilateral exodontia. Adult Wistar rats were submitted to unpredictable chronic mild stress (10 days) and/or unilateral exodontia and their plasma and medial pterygoid muscles were removed for analysis. A pre-treatment with diazepam was used to verify its effect on stress. The parameters evaluated included anxiety behavior, plasma levels of corticosterone, metabolic activity by succinate dehydrogenase, capillary density by laminin staining and ultrastructural findings by transmission electron microscopy. Occlusal instability induced anxiety-like behavior on elevated plus-maze test and diazepam administration blocked the appearance of this behavior. Unilateral exodontia promoted in the contralateral muscle an increase of oxidative fibers and capillaries and modification of sarcoplasmic reticulum. Chronic stress caused increased glycolytic metabolism, reduced capillary density and morphological changes in mitochondria on both sides. Association of both factors induced a glycolytic pattern in muscle and hemodynamic changes. Pharmacological manipulation with diazepam inhibited the changes in the medial pterygoid muscle after stress. Our results reveal a preventive benzodiazepine treatment for stress and occlusal instability conditions affecting masticatory muscle disorders. In addition, provide insights into the mechanisms by which chronic stress and exodontia might be involved in the pathophysiology of masticatory muscular dysfunctions.
Sci Rep, 2020; 10
24291383: do Nascimento GC, Leite-Panissi CR
Time-dependent analysis of nociception and anxiety-like behavior in rats submitted to persistent inflammation of the temporomandibular joint.
Temporomandibular disorder (TMD) is prevalent in dental clinics and can involve problems with the masticatory muscles or the temporomandibular joints (TMJ). The pain of TMD is frequently associated with inflammation in the TMJs, but it's etiology is considered to be multifactorial and includes biologic, behavioral, environmental, social, emotional and cognitive factors. The purpose of this investigation was to evaluate the anxiety-like behavior in rats exposed to temporomandibular inflammation via injection of Freund's Adjuvant (CFA) with the elevated plus maze (EPM) and light/dark box (LDB) tests and

to evaluate nociceptive behavior with the von Frey test at different periods. Moreover, this study measured TMJ inflammation using plasma extravasation (Evans blue test) and the intraarticular infiltration of polymorphonuclear neutrophils (myeloperoxidase quantification). The results showed that rats that were submitted to TMJ inflammation exhibited a decreased number of entries into the open arms of the EPM and a decrease in the time spent in the light compartment and in the number of transitions in the LDB. Additionally, the number of entries in closed arms in the EPM, used as indicator of locomotor activity, did not alter between treatments. Furthermore, increases in mechanical sensitivity and increases in plasma extravasation in the joint tissue occurred throughout the inflammation process, along with an increase in myeloperoxidase in the synovial fluid of TMJ. Our results suggest that the temporomandibular inflammation induced by CFA produced anxiety-like behaviors in rats and induced nociceptive behavior across different periods of inflammation.
Physiol Behav, 2014; 125
31706993: Crivelaro do Nascimento G, Ferrari DP, Guimaraes FS, Del Bel EA, Bortolanza M, Ferreira-Junior NC
Cannabidiol increases the nociceptive threshold in a preclinical model of Parkinson's disease.
Medications that improve pain threshold can be useful in the pharmacotherapy of Parkinson's disease (PD). Pain is a prevalent PD's non-motor symptom with a higher prevalence of analgesic drugs prescription for patients. However, specific therapy for PD-related pain are not available. Since the endocannabinoid system is expressed extensively in different levels of pain pathway, drugs designed to target this system have promising therapeutic potential in the modulation of pain. Thus, we examined the effects of the 6-hydroxydopamine- induced PD on nociceptive responses of mice and the influence of cannabidiol (CBD) on 6-hydroxydopamine-induced nociception. Further, we investigated the pathway involved in the analgesic effect of the CBD through the co-administration with a fatty acid amide hydrolase (FAAH) inhibitor, increasing the endogenous anandamide levels, and possible targets from anandamide, i.e., the cannabinoid receptors subtype 1 and 2 (CB1 and CB2) and the transient receptor potential vanilloid type 1 (TRPV1). We report that 6-hydroxydopamine- induced parkinsonism decreases the thermal and mechanical nociceptive threshold, whereas CBD (acute and chronic treatment) reduces this hyperalgesia and allodynia evoked by 6-hydroxydopamine. Moreover, ineffective doses of either FAAH inhibitor or TRPV1 receptor antagonist potentialized the CBD-evoked antinociception while an inverse agonist of the CB1 and CB2 receptor prevented the antinociceptive effect of the CBD. Altogether, these results indicate that CBD can be a useful drug to prevent the parkinsonism-induced nociceptive threshold reduction. They also suggest that CB1 and TRPV1 receptors are important for CBD-induced analgesia and that CBD could produce these analgesic effects increasing endogenous anandamide levels.
Neuropharmacology, 2020; 163
29611150: Nascimento GC, Bariotto-Dos-Santos K, Leite-Panissi CRA, Del-Bel EA, Bortolanza M
Nociceptive Response to L-DOPA-Induced Dyskinesia in Hemiparkinsonian Rats.
Non-motor symptoms are increasingly identified to present clinical and diagnostic importance for Parkinson's disease (PD). The multifactorial origin of pain in PD makes this symptom of great complexity. The dopamine precursor, L-DOPA (L-3,4-dihydroxyphenylalanine), the classic therapy for PD, seems to be effective in pain threshold; however, there are no studies correlating L-DOPA-induced dyskinesia (LID) and nociception development in experimental Parkinsonism. Here, we first investigated nociceptive responses in a 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's disease to a hind paw-induced persistent inflammation. Further, the effect of L-DOPA on nociception behavior at different times of treatment was investigated. Pain threshold was determined using von Frey and Hot Plate/Tail Flick tests. Dyskinesia was measured by abnormal involuntary movements (AIMs) induced by L-DOPA administration. This data is consistent to show that 6-OHDA-lesioned rats had reduced nociceptive thresholds compared to non-lesioned rats. Additionally, when these rats were exposed to a persistent inflammatory challenge, we observed increased hypernociceptive responses, namely hyperalgesia. L-DOPA treatment alleviated pain responses on days 1 and 7 of treatment, but not on day 15. During that period, we observed an inverse relationship between LID and nociception threshold in these rats, with a high LID rate corresponding to a reduced nociception threshold. Interestingly, pain responses resulting from CFA-induced inflammation were significantly enhanced during established dyskinesia. These data suggest a pro-algesic effect of L-DOPA-induced dyskinesia, which is confirmed by the correlation founded here between AIMs and nociceptive indexes. In conclusion, our results are consistent with the notion that central dopaminergic mechanism is directly involved in nociceptive responses in Parkinsonism condition.
Neurotox Res, 2018; 34

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BOARD NUMBER: S02-728

CAUSAL ROLE OF DOPAMINE D2 RECEPTOR IN GENERATION OF A DISTINCT MOTOR MOTIF IN RODENT MODEL OF LEVODOPA INDUCED DYSKINESIA

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Levadopa Induced Dyskinesia (LID) is a series of abnormal involuntary movements that may arise after treating parkinsonian patients with levodopa. We used a deep-learning opensource package to track posture and movement of a rodent model of LID. Thereafter we used an unsupervised clustering algorithm to classify the subcomponents of movements. Our investigation into pharmacological components of this distinct episode demonstrated a causal role for D2 receprot in producing a distinct set of abnormal movements. We have followed up our behavioral study with in-vivo recordings of striatal activity to unravel the mechanism underlying D2-receptor modulation the mentioned behavioral episode.

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BOARD NUMBER: S02-729

PHARMACOLOGICAL MODULATION OF GLIAL PHENOTYPE IN ANIMAL MODEL OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

Justyna Kadłuczka, Martyna Paleczna, Dominika Biała, Agnieszka Jurga, Barbara Kosmowska, Katarzyna Kuter-Nowak
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Background: There is no available therapy counteracting Parkinson's disease (PD). Astrocyte dysfunction and microglia activation lead to neuroinflammation and consequently to degeneration of dopaminergic neurons. However, the exact mechanism is unknown. One of the proposed approaches to improve neuronal survival is to shift the glial phenotype towards a neuroprotective state. The aim of our project was to study the potential of zolpidem - a $GABA_A\alpha1$ receptor agonist and Compound 21 (C21) – an agonist of angiotensin type 2 receptor (AT2R) to modulate glial phenotype. Additionally we assessed how a selective depletion of microglia (PLX3397) influences motor behavior in rats. **Methods:** 6-ODHA was injected into the medial forebrain bundle to induce medium lesion of dopaminergic neurons. Fluorocitrate (FC) was infused for 7 days into the substantia nigra to promote astrocyte degeneration and microglia activation. Zolpidem and C21 were administered for 7 days after the surgery and PLX3397 7 days prior to and after the surgery. Locomotor activity was measured three and six days after the surgery using ACTIFRAME-SYSTEM and compensatory potential was calculated. **Results:** Lesion-induced behavioral deficit was spontaneously compensated 6 days after the surgery. Only in rats treated with FC, C21 improved motor behavior 3 days after the surgery. Neither zolpidem nor microglia depletion affected motor dysfunction. **Conclusions:** Improvement of motor behavior after AT2R activation suggests that C21 may possess astroprotective properties. To unravel, whether this effect is associated with glial phenotype shift requires further studies. **Funding:** Supported by the National Science Centre grant OPUS14 2017/27/B/NZ7/00289.

Pubmed:
34572572: Jurga AM, Paleczna M, Kadluczka J, Kuter KZ
Beyond the GFAP-Astrocyte Protein Markers in the Brain.
The idea of central nervous system as one-man band favoring neurons is long gone. Now we all are aware that neurons and neuroglia are team players and constant communication between those various cell types is essential to maintain functional efficiency and a quick response to danger. Here, we summarize and discuss known and new markers of astroglial multiple functions, their natural heterogeneity, cellular interactions, aging and disease-induced dysfunctions. This review is focused on newly reported facts regarding astrocytes, which are beyond the old stereotypes. We present an up-to-date list of marker proteins used to identify a broad spectrum of astroglial phenotypes related to the various physiological and pathological nervous system conditions. The aim of this review is to help choose markers that are well-tailored for specific needs of further experimental studies, precisely recognizing differential glial phenotypes, or for diagnostic purposes. We hope it will help to categorize the functional and structural diversity of the astroglial population and ease a clear readout of future experimental results.
Biomolecules, 2021; 11
31185306: Pydyn N, Kadluczka J, Kus E, Pospiech E, Losko M, Fu M, Jura J, Kotlinowski J
RNase MCPIP1 regulates hepatic peroxisome proliferator-activated receptor gamma via TXNIP/PGC-1alpha pathway.
Monocyte chemoattractant protein-1-induced protein-1 (MCPIP1) acts as an endonuclease that degrades selected mRNAs, viral RNAs and pre-miRNAs. MCPIP1 inhibits adipogenesis by degradation of C/EBPβ mRNA and adipogenesis-related miRNA, however its role in the regulation of hepatic lipid homeostasis is unknown. In this study, we investigated the role of MCPIP1 in the regulation of lipid metabolism in hepatocytes. C57BL/6 mice were fed a high-fat diet (HFD) for 2-20 weeks and next primary hepatocytes and adipose tissue were isolated. For in vitro experiments we used murine primary hepatocytes, control HepG2 cells and HepG2 with overexpressed or silenced MCPIP1. We found that Mcpip1 levels were lower in primary hepatocytes isolated from HFD-fed mice than in control cells starting at 4 weeks of a HFD. Level of Mcpip1 was also depleted in visceral fat isolated from obese and glucose-intolerant mice characterized by fatty liver disease. We showed that MCPIP1 overexpression in HepG2 cells treated with oleate induces the level and activity of peroxisome proliferator-activated receptor γ (PPARγ). This phenotype was reverted upon silencing of MCPIP1 in HepG2 cells and in primary hepatocytes lacking Mcpip1 protein. MCPIP1 activated the PPARγ transcription factor via the thioredoxin-interacting protein (TXNIP)/peroxisome

proliferator-activated receptor γ coactivator 1- α (PGC-1α) pathway. MCPIP1 contributes to lipid metabolism in hepatocytes by regulating the TXNIP/PGC-1α/PPARγ pathway.
Biochim Biophys Acta Mol Cell Biol Lipids, 2019; 1864

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BOARD NUMBER: S02-730

INTERMITTENT THETA BURST STIMULATION AMELIORATES MOTOR DYSFUNCTION IN THE 6-HYDROXYDOPAMINE MODEL OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Background: Parkinson's disease (PD) is a progressive movement disorder, and the second most common neurodegeneration without effective treatment, stemming the need for further research. Repetitive transcranial magnetic stimulation (rTMS) refers to a non-invasive stimulation protocol designed to modulate neuronal excitability, by applying magnetic pulses delivered in predefined patterns. This type of brain stimulation has been applied to several neurological and psychiatric diseases to alleviate motor and cognitive symptoms. Aim: We aimed to evaluate the effect of intermittent theta-burst stimulation (iTBS), a highly effective excitatory protocol of rTMS with shorter stimulation duration, on motor dysfunction in the 6-hydroxydopamine (6-OHDA) model of Parkinson's disease. Methods: Two-month-old male Wistar rats were divided into 4 experimental groups: controls, rats injected unilaterally with 6-OHDA (10 µg/kg) into the right SNpc, 6-OHDA rats subjected to iTBS protocol (2 times/day/three weeks), and iTBS sham group. To evaluate the therapeutic effect of iTBS in the 6-OHDA model, we performed the Rotarod test, Cylinder test, and Open field test. Results: Animals treated with iTBS showed significant improvement of motor deficit which reflected in increased time on Rotarod, compared to the 6-OHDA and sham group ($p < 0.01$). The iTBS treated rats also used the impaired forelimbs more than the 6-OHDA rats ($p < 0.01$) during lateral exploration. Finally, iTBS-treated rats exhibited reduced anxiety-like behavior when compared to 6-OHDA and sham groups ($p < 0. 01$). Conclusions: Administrations of iTBS showed a considerable beneficial effect on the motor behavior of hemiparkinsonian rats, which could candidate this protocol as a potential therapeutic strategy for PD.

Pubmed:

33508420: Mirkov I, Popov Aleksandrov A, Ninkov M, Tucovic D, Kulas J, Zeljkovic M, Popovic D, Kataranovski M
Immunotoxicology of cadmium: Cells of the immune system as targets and effectors of cadmium toxicity.
Cadmium (Cd) has been listed as one of the most toxic substances affecting numerous tissues/organs, including the immune system. Due to variations in studies examining Cd effects on the immune system (exposure regime, experimental systems, immune endpoint measured), data on Cd immunotoxicity in humans and experimental animals are inconsistent. However, it is clear that Cd can affect cells of the immune system and can modulate some immune responses. Due to the complex nature of the immune system and its activities which are determined by multiple interactions, the underlying mechanisms involved in the immunotoxicity of this metal are still vague. Here, the current knowledge regarding the interaction of Cd with cells of the immune system, which may affect immune responses as well as potential mechanisms of consequent biological effects of such activities, is reviewed. Tissue injury caused by Cd-induced effects on innate cell activities depicts components of the immune system as mediators/effectors of Cd tissue toxicity. Cd-induced immune alterations, which may compromise host defense against pathogenic microorganisms and homeostatic reparative activities, stress this metal as an important health hazard.
Food Chem Toxicol, 2021; 149
33766215: Kulas J, Tucovic D, Zeljkovic M, Popovic D, Popov Aleksandrov A, Kataranovski M, Mirkov I
Aryl Hydrocarbon Receptor is Involved in the Proinflammatory Cytokine Response to Cadmium.
To investigate involvement of the aryl hydrocarbon receptor (AhR) in the immunomodulatory effects of cadmium (Cd).
Biomed Environ Sci, 2021; 34
34688722: Popov Aleksandrov A, Mirkov I, Tucovic D, Kulas J, Zeljkovic M, Popovic D, Ninkov M, Jankovic S, Kataranovski M
Immunomodulation by heavy metals as a contributing factor to inflammatory diseases and autoimmune reactions: Cadmium as an example.
Cadmium (Cd) represents a unique hazard because of the long biological half-life in humans (20-30 years). This metal

accumulates in organs causing a continuum of responses, with organ disease/failure as extreme outcome. Some of the cellular and molecular alterations in target tissues can be related to immune-modulating potential of Cd. This metal may cause adverse responses in which components of the immune system function as both mediators and effectors of Cd tissue toxicity, which, in combination with Cd-induced alterations in homeostatic reparative activities may contribute to tissue dysfunction. In this work, current knowledge concerning inflammatory/autoimmune disease manifestations found to be related with cadmium exposure are summarized. Along with epidemiological evidence, animal and in vitro data are presented, with focus on cellular and molecular immune mechanisms potentially relevant for the disease susceptibility, disease promotion, or facilitating development of pre-existing pathologies.
Immunol Lett, 2021; 240
32599126: Dragic M, Zeljkovic M, Stevanovic I, Ilic T, Ilic N, Nedeljkovic N, Ninkovic M
Theta burst stimulation ameliorates symptoms of experimental autoimmune encephalomyelitis and attenuates reactive gliosis.
Multiple sclerosis (MS) is a chronic neurodegenerative disease caused by inflammatory processes in the central nervous system (CNS). Decades of research led to discovery of several disease-modifying therapeutics strategies with moderate success. Experimental autoimmune encephalomyelitis (EAE) is currently the most commonly used experimental model for MS and for studying various therapeutic approaches. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neurostimulation technique with multiple beneficial effects on healthy as well as CNS with pathology. However, the molecular and cellular mechanisms of rTMS on acute EAE are scarce. Our study demonstrated beneficial effects of theta-burst stimulation (TBS), an experimental paradigm of rTMS, on disease course of acute EAE. TBS treatment attenuated reactive gliosis, restored myelin sheet and down-regulated expression of vimentin in EAE rats. These effects were reflected through reduced clinical parameters, shorter duration of illness and days spent in paralysis. Based on our research, rTMS deserves further considerations for its neuroprotective effect on EAE, and is an excellent candidate for further research and points that it could be used for more than for simple symptomatic therapy.
Brain Res Bull, 2020; 162
33197509: Kulas J, Tucovic D, Zeljkovic M, Popovic D, Popov Aleksandrov A, Ukropina M, Cakic Milosevic M, Glamoclija J, Kataranovski M, Mirkov I
Proinflammatory effects of environmental cadmium boost resistance to opportunistic pathogen Aspergillus fumigatus: Implications for sustained low-level pulmonary inflammation?
Cadmium (Cd) is one of the most toxic environmental heavy metals to which the general population is exposed mainly via the oral route. Owing to its immunomodulatory potential, orally acquired Cd affects antimicrobial immune defense in several organs, including the lungs. While there are data concerning Cd and viral and bacterial pulmonary infections, effects on fungal infections are not studied yet. In the present study, the effect of the Cd (5 mg/L for 30 days, in drinking water, the average daily Cd intake 0.641 ± 0.089 mg/kg) on the immune response of rats to pulmonary A. fumigatus infection was examined. Data obtained showed that orally acquired cadmium does not affect the elimination of the fungus in immunocompetent rats owing to the preservation of some aspects of innate immune responses (lung leukocyte infiltration and NBT reduction) and an increase in other (increased numbers of mucus-producing goblet cells, MPO release). Cd does not affect an IFN-γ response in lung leukocytes during the infection (despite suppression of cytokine production in cells of lung-draining lymph nodes), while it stimulates IL-17 and suppresses IL-10 response to the fungus. As a result, the elimination of the fungus occurs in a milieu with the prevailing proinflammatory response in Cd-exposed animals that preserved fungal elimination from the lungs, though with more intense injury to the lung tissue. Therefore, the proinflammatory microenvironment in the lungs created by Cd that sustains inflammatory/immune response to the fungus to which humans are exposed for a lifetime, raises a concern of orally acquired Cd as a risk factor for the development of chronic low-grade pulmonary inflammation.
Toxicology, 2021; 447
34205965: Dragić M, Zeljković M, Stevanović I, Adžić M, Stekić A, Mihajlović K, Grković I, Ilić N, Ilić TV, Nedeljković N, Ninković M
Downregulation of CD73/AR-Mediated Adenosine Signaling as a Potential Mechanism of Neuroprotective Effects of Theta-Burst Transcranial Magnetic Stimulation in Acute Experimental Autoimmune Encephalomyelitis.
Multiple sclerosis (MS) is a chronic neurodegenerative disease caused by autoimmune-mediated inflammation in the central nervous system. Purinergic signaling is critically involved in MS-associated neuroinflammation and its most widely applied animal model-experimental autoimmune encephalomyelitis (EAE). A promising but poorly understood approach in the treatment of MS is repetitive transcranial magnetic stimulation. In the present study, we aimed to investigate the effect of continuous theta-burst stimulation (CTBS), applied over frontal cranial bone, on the adenosine-mediated signaling system in EAE, particularly on CD73/AR/AR in the context of neuroinflammatory activation of glial cells. EAE was induced in two-month-old female DA rats and in the disease peak treated with CTBS protocol for ten consecutive days. Lumbosacral spinal cord was analyzed immunohistochemically for adenosine-mediated signaling components and pro- and anti-inflammatory factors.

We found downregulated IL-1β and NF- κB- and upregulated IL-10 pointing towards a reduction in the neuroinflammatory process in EAE animals after CTBS treatment. Furthermore, CTBS attenuated EAE-induced glial eN/CD73 expression and activity, while inducing a shift in AR expression from glia to neurons, contrary to EAE, where tight coupling of eN/CD73 and AR on glial cells is observed. Finally, increased glial AR expression following CTBS supports anti-inflammatory adenosine actions and potentially contributes to the overall neuroprotective effect observed in EAE animals after CTBS treatment.
Brain Sci, 2021; 11

31924569: Tucovic D, Mirkov I, Kulas J, Zeljkovic M, Popovic D, Zolotarevski L, Djurdjic S, Mutic J, Kataranovski M, Popov Aleksandrov A

Dermatotoxicity of oral cadmium is strain-dependent and related to differences in skin stress response and inflammatory/immune activity.

Adverse effects of non-occupational exposure to cadmium (Cd) are increasingly acknowledged. Since our previous study has showed that orally acquired Cd affects skin, the contribution of genetic background to dermatotoxicity of oral cadmium was examined in two rat strains, Albino Oxford (AO) and Dark Agouti (DA), which differed in response to chemicals. While similar accumulation of Cd in the skin of both strains was noted, the skin response to the metal differed. DA rat individuals mounted antioxidant enzyme defense in the skin already at lower Cd dose, in contrast to AO rats which reacted to higher metal dose solely (and less pronounced), implying higher susceptibility of DA strain to Cd dermatotoxicity. Epidermal cells from both strains developed stress response, but higher intensity of antioxidant response in AO rats implied this strain`s better ability to defend against Cd insult. Cd induced epidermal cells' proinflammatory cytokine response only in DA rats. Increased IL-10 seems responsible for the lack of response in AO rats. Differences in the pattern of skin/epidermal cell responsiveness to cadmium give a new insight into repercussion of genetic variability to dermatotoxicity of orally acquired cadmium, bearing relevance for variations in the link between dietary cadmium and inflammation-based skin pathologies.
Environ Toxicol Pharmacol, 2020; 75

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BOARD NUMBER: S02-731

NEUROPROTECTIVE EFFECTS OF L-THEANINE AGAINST TRAMADOL INDUCED PARKINSON'S LIKE SYMPTOMS IN EXPERIMENTAL RATS

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Abstract Parkinson's disease (PD) is a chronic neurodegenerative disorder triggered by degeneration of dopaminergic neurons in substantia nigra pars compacta (SNpc). Experimental Animals: Wistar Rats (180-200 g). Drugs and Chemicals: Tramadol, L- theanine, Naloxone and Sinemet. Experimental procedure: The animals were divided into 7 groups. Behavioral observations were done on 1, 7, 14, 21 and 28 day after tramadol treatment. On 29 day, animals were sacrificed and striatum was isolated for biochemical, neuroinflammation, histopathological and neurotransmitters analysis. Administration of tramadol (50 mg/ kg, i.p.) for 28 days in rats produces impaired motor functions and locomotor activity as evidenced by rotarod, open field, narrow beam walk and grip strength performance. In addition, there was increased oxidative stress (MDA, nitrite) and neuroinflammatory markers (TNF-α, IL-1β and IL-17) and decreased levels of catecholamines, GABA and glutamate. The treatment drug L-theanine at dose (25, 50, 100 mg/kg) significantly and dose- dependently improved alterations in motor impairments and locomotor activity, attenuated oxidative stress, neuroinflammatory markers and restored catecholamines, GABA and glutamate level in striatum. Chronic tramadol administration produces impaired motor functions, increased oxidative stress, neuroinflammation and altered neurotransmitters level was significantly ameliorated by L-theanine, through antioxidant, anti-inflammatory and neuroprotective mechanisms.

Pubmed:
34792011: Dhami M, Raj K, Singh S
Neuroprotective Effect of Fucoxanthin against Intracerebroventricular Streptozotocin (ICV-STZ) Induced Cognitive Impairment in Experimental Rats.
Alzheimer's disease (AD) is a neurological disorder characterized by loss of memory and cognitive functions caused by oxidative stress, neuroinflammation, change in neurotransmitter levels, and excessive deposition of Aβ plaques. Fucoxanthin is a carotenoid with potential antioxidant, anti-inflammatory, and neuroprotective actions.
Curr Alzheimer Res, 2021; 18
34727278: Raj K, Gupta GD, Singh S
Spermine protects aluminium chloride and iron-induced neurotoxicity in rat model of Alzheimer's disease via attenuation of tau phosphorylation, Amyloid-β (1-42) and NF-κB pathway.
Alzheimer's disease (AD) is the most prevalent type of dementia, characterized by a gradual decline in cognitive and memory functions of the aged peoples. Long-term exposure to heavy metals (aluminium and iron) cause neurotoxicity by amyloid plaques accumulation, tau phosphorylation, increased oxidative stress, neuroinflammation, and cholinergic neurons degeneration, contributes to the development of AD-like symptoms. The present research work is designed to investigate the neuroprotective effect of spermine in aluminium chloride (AlCl), and iron (Fe) induced AD-like symptoms in rats. Rats were administered of AlCl (100 mg/kg p.o.) alone and in combination with iron (120 µg/g, p.o.) for 28 days. Spermine (5 and 10 mg/kg) through intraperitoneal (i.p.) route was given for 14 days. The recognition and spatial memory impairment were tasted using Morris water maze (MWM), actophotometer, and Novel Object Recognition test (NORT). All the rats were sacrificed on day 29, brains were isolated, and tissue homogenate was used for neuroinflammatory, biochemical, neurotransmitters, metals concentration, and nuclear factor-kappa B (NF-κB) analysis. In the present study, AlCl and iron administration elevated oxidative stress, cytokines release, dysbalanced neurotransmitters concentration, and biochemical changes. Rats treated with spermine dose-dependently improved the recognition and spatial memory, attenuated proinflammatory cytokine release, and restored neurotransmitters concentration and antioxidant enzymes. Spermine also mitigated the increased beta-amyloid (Aβ42), with downregulation of tau phosphorylation. Furthermore, spermine augmented the hippocampal levels of B cell leukaemia/lymphoma-2 (Bcl-2), diminished nuclear factor-kappa B (NF-κB) and caspase-3 (casp-3) expression. Moreover, spermine exhibited the neuroprotective effect through anti-inflammatory, antioxidant, neurotransmitters restoration, anti-apoptotic Aβ42 concentration.

Inflammopharmacology, 2021; 29
34674598: Sharma S, Raj K, Singh S
Protective effects of cerebrolysin against chemotherapy (carmustine) induced cognitive impairment in Albino mice.
Chemotherapy-induced cognitive impairment (CICI) comprises different neurological problems, including difficulty in learning new things, concentrating and making decisions that affect daily life activities. Clinical reports indicate that around 70% of cancer patients receiving chemotherapy suffer from cognitive impairment. The purpose of the present study is to examine the effects of widely used anticancer medication (Carmustine) on cognitive function using mice model and investigation of the neuroprotective effects of Cerebrolysin (CBN). Cerebrolysin (CBN) is a mixture of several neurotrophic factors and active peptides with anti-inflammatory, antioxidant, and neuroprotective actions. Our study aimed to establish a mice model of Carmustine (BCNU)-induced cognitive deficits and determine the protective effects of CBN. BCNU (10 mg/kg, i.v.) was administered to mice for 28 days, and behavioral parameters were measured on a weekly basis. CBN (44 and 88 mg/kg, i.p.) was administered daily from day 1 to 28 to BCNU treatment mice. All animals were sacrificed on day 29 and brain hippocampus tissues were used for biochemical, neuroinflammatory, neurotransmitters analysis. BCNU administration animals showed impaired cognition and memory, confirmed from behavioral analysis. Further, BCNU increased oxidative stress, inflammatory cytokines release and altered neurotransmitters concentration as compared to the control group (< 0.01). However, mice treated with CBN (44 and 88 mg/kg, i.p.) significantly and dose-dependently improved cognitive functions, reduced oxidative stress markers, inflammatory cytokines and restored neurotransmitters concentration as compared to BCNU administered mice (< 0.05). The finding of current study suggested that CBN could be the promising compound to reverse cognitive impairment associated with use of chemotherapy.
Drug Chem Toxicol, 2021;
34210222: Raj K, Gupta GD, Singh S
l-Theanine ameliorates motor deficit, mitochondrial dysfunction, and neurodegeneration against chronic tramadol induced rats model of Parkinson's disease.
Parkinson's disease (PD) is the second most prevalent progressive neurodegenerative disease, characterized by loss of dopaminergic neurons in substantia nigra, with deficiency of dopamine in the striatum. Tramadol is safe analgesic but long-term use confirmed to elevate oxidative stress, neuroinflammation, mitochondrial dysfunction, in brain leads to motor deficits. l-Theanine is an active constituent of green tea which prevents neuronal loss, mitochondrial failure and improves dopamine, gamma-aminobutyric acid (GABA), serotonin levels and in the central nervous system (CNS) via antioxidant, anti-inflammatory, and neuromodulatory properties. In the present study, tramadol was injected intraperitoneally to Wister rats for 28 days at a dose of 50 mg/kg. l-Theanine (25, 50, and 100 mg/kg) was administered orally 3 h before tramadol administration from day 14 to day 28. Behavioral analyses including rotarod, narrow beam walk, open field, and grip strength were used to evaluate motor coordination on a weekly basis. On the day 29, all Wistar rats were sacrificed and striatum homogenates were used for biochemical (lipid peroxidation, nitrite, glutathione, glutathione peroxidase activity, superoxide dismutase, catalase, mitochondrial complex I, IV, and cyclic adenosine monophosphate), neuroinflammatory markers (tumor necrosis factor-α, interleukin-1β, and interleukin-17), and neurotransmitters (dopamine, norepinephrine, serotonin, GABA, and glutamate) analysis. Chronic tramadol treatment caused motor deficits reduced antioxidant enzymes level, increased striatal proinflammatory cytokines release, dysbalanced neurotransmitters, and reduced mitochondrial complex activity I, IV, and cAMP activity. However, l-theanine administration attenuated behavioral, biochemical, neuroinflammatory, neurotransmitters, and mitochondrial activity indicated it as a promising neuroprotective potential against degenerative changes in experimental model of PD.
Drug Chem Toxicol, 2021;
34023945: Kaur S, Raj K, Gupta YK, Singh S
Allicin ameliorates aluminium- and copper-induced cognitive dysfunction in Wistar rats: relevance to neuro-inflammation, neurotransmitters and Aβ analysis.
Alzheimer's disease (AD) is a multifactorial neurological disorder associated with neuropathological and neurobehavioral changes, like cognition and memory loss. Pathological hallmarks of AD comprise oxidative stress, formation of insoluble β-amyloid (Aβ) plaques, intracellular neurofibrillary tangles constituted by hyperphosphorylated tau protein (P-tau), neurotransmitters dysbalanced (DA, NE, 5-HT, GABA and Glutamate) and metal deposition. Chronic exposure to metals like aluminium and copper causes accumulation of Aβ plaques, promotes oxidative stress, neuro-inflammation, and degeneration of cholinergic neurons results in AD-like symptoms. In the present study, rats were administered with aluminium chloride (200 mg/kg p.o) and copper sulfate (0.5 mg/kg p.o) alone and in combination for 28 days. Allicin (10 and 20 mg/kg i.p) was administered from day 7 to day 28. Spatial and recognition memory impairment analysis was performed using Morris water maze, Probe trial, and Novel Object Recognition test. Animals were sacrificed on day 29, brain tissue was isolated, and its homogenate was used for biochemical (lipid peroxidation, nitrite, and glutathione), neuro-inflammatory (IL-1β, IL-6 and TNF-α), neurotransmitters (DA, NE, 5-HT, GABA and Glutamate), Aβ level, Al concentration estimation, and Na/K-ATPase activity.

In the present study, aluminium chloride and copper sulfate administration increased oxidative stress, inflammatory cytokines release, imbalanced neurotransmitters' concentration, and promoted β-amyloid accumulation and Na/K-ATPase activity. Treatment with allicin dose-dependently attenuated these pathological events via restoration of antioxidants, neurotransmitters concentration, and inhibiting cytokine release and β-amyloid accumulation. Moreover, allicin exhibited the neuroprotective effect through antioxidant, anti-inflammatory, neurotransmitters restoration, attenuation of neuro-inflammation and β-amyloid-induced neurotoxicity.
J Biol Inorg Chem, 2021; 26
33961065: Raj K, Kaur K, Gupta GD, Singh S
Current understanding on molecular drug targets and emerging treatment strategy for novel coronavirus-19.
SARS-CoV-2 is an enveloped positive-sense RNA virus, contain crown-like spikes on its surface, exceptional of large RNA genome, and a special replication machinery. Common symptoms of SARS-CoV-2 include cough, common cold, fever, sore throat, and a variety of severe acute respiratory disease (SARD) such as pneumonia. SARS-CoV-2 infects epithelial cells, T-cells, macrophages, and dendritic cells and also influences the production and implantation of pro-inflammatory cytokines and chemokines. Repurposing of various drugs during this emergency condition can reduce the rate of mortality as well as time and cost. Two druggable protein and enzyme targets have been selected in this review article due to their crucial role in the viral life cycle. The eukaryotic translation initiation factor (eIF4A), cyclophilin, nucleocapsid protein, spike protein, Angiotensin-converting enzyme 2 (ACE2), 3-chymotrypsin-like cysteine protease (3CLpro), and RNA-dependent RNA polymerase (RdRp) play significant role in early and late phase of SARS-CoV-2 replication and translation. This review paper is based on the rationale of inhibiting of various SARS-CoV-2 proteins and enzymes as novel therapeutic approaches for the management and treatment of patients with SARS-CoV-2 infection. We also discussed the structural and functional relationship of different proteins and enzymes to develop therapeutic approaches for novel coronavirus SARS-CoV-2.
Naunyn Schmiedebergs Arch Pharmacol, 2021; 394
33812934: Raj K, Kaur P, Gupta GD, Singh S
Metals associated neurodegeneration in Parkinson's disease: Insight to physiological, pathological mechanisms and management.
Parkinson's disease (PD) is a deliberately progressive neurological disorder, arises due to degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The loss of dopaminergic nerves and dopamine deficiency leads to motor symptoms characterized by rigidity, tremor, and bradykinesia. Heavy metals and trace elements play various physiological and pathological roles in the nervous system. Excessive exposure to toxic metals like mercury (Hg), lead (Pb), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), aluminium (Al), arsenic (As), cadmium(cd), and selenium (Se) cross the blood-brain barrier to enter into the brain and leads to dopaminergic neuronal degeneration. Excessive concentrations of heavy metals in the brain promote oxidative stress, mitochondrial dysfunction, and the formation of α-synuclein leads to dopaminergic neuronal damage. There is increasing evidence that heavy metals normally present in the human body in minute concentration also cause accumulation to initiate the free radical formation and affecting the basal ganglia signaling. In this review, we explored how these metals affect brain physiology and their roles in the accumulation of toxic proteins (α-synuclein and Lewy bodies). We have also discussed the metals associated with neurotoxic effects and their prevention as management of PD. Our goal is to increase the awareness of metals as players in the onset and progression of PD.
Neurosci Lett, 2021; 753
32026359: Paudel R, Raj K, Gupta YK, Singh S
Oxiracetam and Zinc Ameliorates Autism-Like Symptoms in Propionic Acid Model of Rats.
Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by restrictive behaviour, deficit in social skills and interaction. The multifactorial etiology, complex pathophysiology and different combination of symptoms (unusual speech patterns, frequent repetition of phrases) make it difficult to treat. Thus, present study aimed to find the protective effects of oxiracetam alone and in combination with zinc on brain behavioral, biochemical, pro-inflammatory cytokines and neurotransmitters level. Rats were administered with propionic acid (250 mg/kg p.o.) for 3 days and immediately on next day treatment were given with oxiracetam (25, 50 mg/kg i.p), zinc (4 mg/kg) as well as oxiracetam (25 mg/kg i.p) in combination with zinc (4 mg/kg p.o). Behavioral parameters were performed from 22th to 28th day. On 29th day, all the animals were sacrificed by cervical dislocation and the brain was preserved for biochemical (LPO, GSH, nitrite, mitochondrial complex I, IV and cAMP), neuroinflammatory (TNF-α, IL-1β, IL-6) and neurotransmitters (5-HT, GABA, glutamate and acetylcholine) analysis. The propionic acid administration showed memory impairment, restrictive behavior, increased proinflammatory cytokines level, biochemical and neurotransmitters alteration. However, treatment with oxiracetam alone and in combination with zinc significantly attenuated behavioral, biochemical, inflammatory cytokines and restored neurotransmitters level. The finding of present study demonstrated that oxiracetam alone and in combination with zinc afforded superior anti-autistic effect through antioxidant, anti-inflammatory and anti-excitotoxic mechanisms and could serve as attractive strategy in managing autism.

Neurotox Res, 2020; 37
31721720: Raj K, Chawla P, Singh S
Neurobehavioral Consequences Associated with Long Term Tramadol Utilization and Pathological Mechanisms.
Tramadol is a synthetic analog of codeine used to treat pain of moderate to severe intensity and is reported to have neurotoxic potential. At therapeutic dose, tramadol does not cause major side effects in comparison to other opioid analgesics, and is useful for the management of neurological problems like anxiety and depression. Long term utilization of tramadol is associated with various neurological disorders like seizures, serotonin syndrome, Alzheimer's disease and Parkinson's disease. Tramadol produces seizures through inhibition of nitric oxide, serotonin reuptake and inhibitory effects on GABA receptors. Extensive tramadol intake alters redox balance through elevating lipid peroxidation and free radical leading to neurotoxicity and produces neurobehavioral deficits. During Alzheimer's disease progression, low level of intracellular signalling molecules like cGMP, cAMP, PKC and PKA affect both learning and memory. Pharmacologically tramadol produces actions similar to Selective Serotonin Reuptake Inhibitors (SSRIs), increasing the concentration of serotonin, which causes serotonin syndrome. In addition, tramadol also inhibits GABAA receptors in the CNS has been evidenced to interfere with dopamine synthesis and release, responsible for motor symptoms. The reduced level of dopamine may produce bradykinesia and tremors which are chief motor abnormalities in Parkinson's Disease (PD).
CNS Neurol Disord Drug Targets, 2019; 18
31229625: Kumar Sahel D, Kaira M, Raj K, Sharma S, Singh S
Mitochondrial dysfunctioning and neuroinflammation: Recent highlights on the possible mechanisms involved in Traumatic Brain Injury.
Traumatic brain injury (TBI) is the injury to the vasculature of brain while trauma caused by physical, chemical and biological stimuli. TBI is the leading cause of mortality and morbidity around the world. In this, primary insult leads to secondary injury through the involvement and initiation of various pathological processes. The most citable includes excitotoxicity, Blood Brain Barrier (BBB) dysfunction, inflammation, mitochondrial dysfunction, oxidative stress, calcium efflux, microglial mediated release of proinflammatory mediators (cytokine, chemokines, interleukin, tissue necrosis factor etc.). The morphological changes in TBI are proportional to mitochondrial dysfunctioning and microglial activation, which play an assorted role in neurodegeneration following traumatic brain injury. It is also assumed that the release of nitric oxide, activation of microglial cells plays a diversive role in maintaining the physiological and pathological balance. This review cites different pathophysiological mechanisms that are involved in progenesis of secondary injury after primary insult. These targets further are useful to explore the deep molecular mechanisms and to analyse the effectiveness of available drugs. Moreover, the present review reflects the underlying inflammatory cascade responsible for neuronal loss and neurological deficit in TBI.
Neurosci Lett, 2019; 710

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BOARD NUMBER: S02-732

EFFECT OF A COMBINATION TREATMENT OF BEE VENOM PHARMACOPUNCTURE IN GB34 AND L-DOPA IN A MOUSE MODEL OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Aim: L-DOPA-induced dyskinesias (LID) remain a major problem of long-term therapy of Parkinson's disease. After several years, however, the patients develop l-dopa–induced dyskinesia, or abnormal involuntary movements. Previous studies reported that bee venom (BV) had neuroprotective effect in mouse model of Parkinson's disease (PD). The present study aimed to investigate whether bee venom (BV) pharmacopuncture in GB34 would have synergistic effects with L-3,4-dihydroxyphenylalanine (L-DOPA)and antidyskinetic effects caused by L-dopa as well. **Methods:** MPTP (30 mg/kg) was injected intraperitoneally for 5 consecutive days. Then, 1 week after the last MPTP injection, L-dopa (15mg/kg) to test synergistic effects with BV pharmacopuncture in GB34. After the final treatments, behavioral changes were assessed in all mice using pole test. The antidyskinetic effects of BV in GB34 on pathological movements triggered by L-dopa were investigated by testing abnormal involuntary movements (AIMs) and measuring the expression of TH (tyrosine hydroxylase), Iba-1 in the substantia nigra and activation of FosB in striatum. **Results:** BV pharmacopuncture in GB34 synergistically improved the motor function when L-dopa was co-administered. In addition, it significantly reversed MPTP-induced improved expression of Iba-1, TH in the SN and FosB in the striatum. Furthermore, BV pharmacopuncture in GB34 significantly lowered AIMs and controlled abnormal levels. **Conclusion:** BV pharmacopuncture in GB34 lowered the effective dose of L-dopa and alleviated LID. These findings suggest that BV in GB34 may be used as an adjunct therapy to enhance the efficacy of l-dopa and alleviate its adverse effects in patients with PD.

FENS Forum 2022
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BOARD NUMBER: S02-733

ASSESSMENT OF REPETITIVE AND COMPULSIVE BEHAVIOURS INDUCED BY PRAMIPEXOLE IN RATS: EFFECT OF ALPHA-SYNUCLEIN-INDUCED NIGROSTRIATAL DEGENERATION

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

Mélina Decourt, Eric Balado, Haritz Jiménez-Urbieta, Maureen Francheteau, Pierre-Olivier Fernagut, Marianne Benoit-Marand
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Parkinson’s disease is characterized by the degeneration of *substantia nigra pars compacta* dopaminergic neurons, leading to motor and cognitive symptoms. Pramipexole (PPX, D2/D3 dopaminergic agonist) is effective to treat motor symptoms but induces non-motor side effects called impulse control disorders developing in 30% of treated patients. Several risk factors are known such individual vulnerability traits, medications and neurodegenerative process. Here, we explored the impact of nigrostriatal degeneration induced by bilateral injection of a viral vector expressing human mutated alpha-synuclein (AAV-2 hA53Talpha-syn) and chronic PPX treatment on repetitive and compulsive-like behaviours with spontaneous hoarding task and operant post-training signal attenuation task (PTSA). Our results highlight that PPX increases the time spent interacting with food (similar to punding behaviour) at the expense of hoarding in the same way in sham and lesioned rats. In PTSA task, the combination of nigrostriatal lesion and PPX decreased the number of completed trials and increased the number of uncompleted trials. The lesion led to an increased compulsive-like behaviour after attenuation, and PPX shifted the overall behavioural output towards an increased proportion of compulsive lever-presses. Given the magnitude of the behavioural effects and the lack of strong interaction between PPX and nigral degeneration, these results suggest that extra-nigral pathology may be critical to increase the vulnerability to develop compulsive-like behaviours following treatment.

FENS Forum 2022
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BOARD NUMBER: S02-734

SEROTONERGIC AND DOPAMINERGIC NEURONS IN DORSAL RAPHE NUCLEUS: FROM PHYSIOLOGY TO PARKINSON'S DISEASE PATHOLOGY.

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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The Dorsal Raphe Nucleus (DRN) contains different populations of serotonergic ($DRN_{5\text{-}HT}$) and dopaminergic (DRN_{DA}) neurons. Because of their anatomic and genetic features, attempts to target these populations are difficult and have often generated contrasting results. Parkinson's Disease (PD) is associated with non-motor comorbidities, which may involve the $DRN_{5\text{-}HT}$ and DRN_{DA}. To test this possibility, we first investigated the electrophysiological and morphological properties of $DRN_{5\text{-}HT}$ and DRN_{DA} in physiological conditions and in a mouse model of PD. Wild-type and DAT-tdTomato mice were bilaterally injected in the Dorsal Striatum with vehicle or 6-hydroxydopamine (6-OHDA). Three weeks later, mice were deeply anaesthetized and midbrain slices were prepared for whole-cell patch-clamp recordings of DRN neurons. Recorded neurons were stained and classified as $DRN_{5\text{-}HT}$ or DRN_{DA} using immunohistochemistry. We found that in control mice, $DRN_{5\text{-}HT}$ and DRN_{DA} neurons differ in many of their electrophysiological and morphological profiles. In 6-OHDA lesioned mice, $DRN_{5\text{-}HT}$ showed changes in the excitability and smaller and more circular cell bodies. DRN_{DA} neurons in lesioned mice had altered action potential properties as well as morphological changes such as swelling of cell bodies and increased dendritic branching. Our study identifies several electrophysiological properties that can be used to differentiate $DRN_{5\text{-}HT}$ and DRN_{DA} neurons. Importantly, both populations are affected by the 6-OHDA lesion. Taking into account the involvement of DRN in affective and sleep disorders, the present results provide useful information to determine the relative contribution of $DRN_{5\text{-}HT}$ and DRN_{DA} to non-motor comorbidities in PD.

Pubmed:
29541852: Pisanu A, Boi L, Mulas G, Spiga S, Fenu S, Carta AR
Neuroinflammation in L-DOPA-induced dyskinesia: beyond the immune function.
Neuroinflammation is a main component of Parkinson's disease (PD) neuropathology, where unremitting reactive microglia and microglia-secreted soluble molecules such as cytokines, contribute to the neurodegenerative process as part of an aberrant immune reaction. Besides, pro-inflammatory cytokines, predominantly TNF-α, play an important neuromodulatory role in the healthy and diseased brain, being involved in neurotransmitter metabolism, synaptic scaling and brain plasticity. Recent preclinical studies have evidenced an exacerbated neuroinflammatory reaction in the striatum of parkinsonian rats that developed dyskinetic responses following L-DOPA administration. These findings prompted investigation of non-neuronal mechanisms of L-DOPA-induced dyskinesia (LID) involving glial cells and glial-secreted soluble molecules. Hence, besides the classical mechanisms of LID that include abnormal corticostriatal neurotransmission and maladaptive changes in striatal medium spiny neurons (MSNs), here we review studies supporting a role of striatal neuroinflammation in the development of LID, with a focus on microglia and the pro-inflammatory cytokine TNF-α. Moreover, we discuss several mechanisms that have been involved in the development of LID, which are directly or indirectly under the control of TNF-α, and might be abnormally affected by its chronic overproduction and release by microglia in PD. It is proposed that TNF-α may contribute to the altered neuronal responses occurring in LID by targeting receptor trafficking and function in MSNs, but also dopamine synthesis in preserved dopaminergic terminals and serotonin metabolism in serotonergic neurons. Therapeutic approaches specifically targeting glial-secreted cytokines may represent a novel target for preventing or treating LID.
J Neural Transm (Vienna), 2018; 125
29570770: Lecca D, Janda E, Mulas G, Diana A, Martino C, Angius F, Spolitu S, Casu MA, Simbula G, Boi L, Batetta B, Spiga S, Carta AR
Boosting phagocytosis and anti-inflammatory phenotype in microglia mediates neuroprotection by PPARγ agonist MDG548 in Parkinson's disease models.
Microglial phenotype and phagocytic activity are deregulated in Parkinson's disease (PD). PPARγ agonists are

neuroprotective in experimental PD, but their role in regulating microglial phenotype and phagocytosis has been poorly investigated. We addressed it by using the PPARγ agonist MDG548.
Br J Pharmacol, 2018; 175
29755317: Janda E, Boi L, Carta AR
Microglial Phagocytosis and Its Regulation: A Therapeutic Target in Parkinson's Disease?
The role of phagocytosis in the neuroprotective function of microglia has been appreciated for a long time, but only more recently a dysregulation of this process has been recognized in Parkinson's disease (PD). Indeed, microglia play several critical roles in central nervous system (CNS), such as clearance of dying neurons and pathogens as well as immunomodulation, and to fulfill these complex tasks they engage distinct phenotypes. Regulation of phenotypic plasticity and phagocytosis in microglia can be impaired by defects in molecular machinery regulating critical homeostatic mechanisms, including autophagy. Here, we briefly summarize current knowledge on molecular mechanisms of microglia phagocytosis, and the neuro-pathological role of microglia in PD. Then we focus more in detail on the possible functional role of microglial phagocytosis in the pathogenesis and progression of PD. Evidence in support of either a beneficial or deleterious role of phagocytosis in dopaminergic degeneration is reported. Altered expression of target-recognizing receptors and lysosomal receptor CD68, as well as the emerging determinant role of α-synuclein (α-SYN) in phagocytic function is discussed. We finally discuss the rationale to consider phagocytic processes as a therapeutic target to prevent or slow down dopaminergic degeneration.
Front Mol Neurosci, 2018; 11
31335998: Boi L, Pisanu A, Greig NH, Scerba MT, Tweedie D, Mulas G, Fenu S, Carboni E, Spiga S, Carta AR
Immunomodulatory drugs alleviate l-dopa-induced dyskinesia in a rat model of Parkinson's disease.
Thalidomide and closely related analogues are used clinically for their immunomodulatory and antiangiogenic properties mediated by the inhibition of the proinflammatory cytokine tumor necrosis factor α. Neuroinflammation and angiogenesis contribute to classical neuronal mechanisms underpinning the pathophysiology of l-dopa-induced dyskinesia, a motor complication associated with l-dopa therapy in Parkinson's disease. The efficacy of thalidomide and the more potent derivative 3,6'-dithiothalidomide on dyskinesia was tested in the 6-hydroxydopamine Parkinson's disease model.
Mov Disord, 2019; 34
31817711: Cardia MC, Carta AR, Caboni P, Maccioni AM, Erbì S, Boi L, Meloni MC, Lai F, Sinico C
Trimethyl Chitosan Hydrogel Nanoparticles for Progesterone Delivery in Neurodegenerative Disorders.
Progesterone is a sex hormone which shows neuroprotective effects in different neurodegenerative disorders, including Parkinson's disease, stroke, and Alzheimer's disease. However, the pharmacokinetic limitations associated with the peripheral administration of this molecule highlight the need for more efficient delivery approaches to increase brain progesterone levels. Since the nose-to-brain administration of mucoadhesive hydrogel nanoparticles is a non-invasive and convenient strategy for the delivery of therapeutics to the central nervous system, in this work, progesterone-loaded hydrogel nanoparticle formulations have been prepared, characterized, and tested in vivo. Nanoparticles, loaded with different progesterone concentrations, have been obtained by polyelectrolyte complex formation between trimethyl chitosan and sodium alginate, followed by ionotropic gelation with sodium tripolyphosphate as a cross-linking agent. All formulations showed a mean diameter ranging from 200 nm to 236 nm, a polydispersity index smaller than 0.23, and a high progesterone encapsulation efficiency (83-95%). The zeta potential values were all positive and greater than 28 mV, thus ensuring nanoparticles stability against aggregation phenomena as well as interaction with negative sialic residues of the nasal mucosa. Finally, in vivo studies on Sprague-Dawley male rats demonstrated a 5-fold increase in brain progesterone concentrations compared to basal progesterone level after 30 min of hydrogel nanoparticle inhalation.
Pharmaceutics, 2019; 11
31857091: Talani G, Biggio F, Mostallino MC, Locci V, Porcedda C, Boi L, Saolini E, Piras R, Sanna E, Biggio G
Treatment with gut bifidobacteria improves hippocampal plasticity and cognitive behavior in adult healthy rats.
At the present time, gut microbiota inspires great interest in the field of neuroscience as a function of its role in normal physiology and involvement in brain function. This aspect suggests a specific gut-brain pathway, mainly modulated by gut microbiota activity. Among the multiple actions controlled by microbiota at the brain level, neuronal plasticity and cognitive function represent two of the most interesting aspects of this cross-talk communication. We address the possible action of two-months implementation of gut Bifidobacteria using a mixture of three different strains (B-MIX) on hippocampal plasticity and related cognitive behavior in adult healthy Sprague Dawley rats. B-MIX treatment increases the hippocampal BDNF with a parallel gain in dendritic spines' density of hippocampal CA1 pyramidal neurons. Electrophysiological experiments revealed a significant increment of HFS-induced LTP formation on the CA1 hippocampal region in B-MIX treated rats. All these effects are accompanied by a better cognitive performance observed in B-MIX treated animals with no impairments in locomotion activity. Therefore, in adult rats, the treatment with different strains of bifidobacteria is able to markedly enhance neuronal plasticity and the CNS function influencing cognitive behavior, an effect that may suggest a potential therapeutic treatment in

brain diseases associated with cognitive functions.
Neuropharmacology, 2020; 165
32116655: Casu MA, Mocci I, Isola R, Pisanu A, Boi L, Mulas G, Greig NH, Setzu MD, Carta AR
Neuroprotection by the Immunomodulatory Drug Pomalidomide in the LRRK2 Genetic Model of Parkinson's Disease.
The search for new disease-modifying drugs for Parkinson's disease (PD) is a slow and highly expensive process, and the repurposing of drugs already approved for different medical indications is becoming a compelling alternative option for researchers. Genetic variables represent a predisposing factor to the disease and mutations in leucine-rich repeat kinase 2 (LRRK2) locus have been correlated to late-onset autosomal-dominant PD. The common fruit fly melanogaster carrying the mutation LRRK2 loss-of-function in the WD40 domain (LRRK2), is a simple model of PD and is a valid tool to first evaluate novel therapeutic approaches to the disease. Recent studies have suggested a neuroprotective activity of immunomodulatory agents in PD models. Here the immunomodulatory drug Pomalidomide (POM), a Thalidomide derivative, was examined in the LRRK2 genetic model of PD. Mutant and wild type flies received increasing POM doses (1, 0.5, 0.25 mM) through their diet from day 1 post eclosion, until postnatal day (PN) 7 or 14, when POM's actions were evaluated by quantifying changes in climbing behavior as a measure of motor performance, the number of brain dopaminergic neurons and T-bars, mitochondria integrity. LRRK2 flies displayed a spontaneous age-related impairment of climbing activity, and POM significantly and dose-dependently improved climbing performance both at PN 7 and PN 14. LRRK2 fly motor disability was underpinned by a progressive loss of dopaminergic neurons in posterior clusters of the protocerebrum, which are involved in the control of locomotion, by a low number of T-bars density in the presynaptic bouton active zones. POM treatment fully rescued the cell loss in all posterior clusters at PN 7 and PN 14 and significantly increased the T-bars density. Moreover, several damaged mitochondria with dilated cristae were observed in LRRK2 flies treated with vehicle but not following POM. This study demonstrates the neuroprotective activity of the immunomodulatory agent POM in a genetic model of PD. POM is an FDA-approved clinically available and well-tolerated drug used for the treatment of multiple myeloma. If further validated in mammalian models of PD, POM could rapidly be clinically tested in humans.
Front Aging Neurosci, 2020; 12
33198335: Boi L, Pisanu A, Palmas MF, Fusco G, Carboni E, Casu MA, Satta V, Scherma M, Janda E, Mocci I, Mulas G, Ena A, Spiga S, Fadda P, De Simone A, Carta AR
Modeling Parkinson's Disease Neuropathology and Symptoms by Intranigral Inoculation of Preformed Human α-Synuclein Oligomers.
The accumulation of aggregated α-synuclein (αSyn) is a hallmark of Parkinson's disease (PD). Current evidence indicates that small soluble αSyn oligomers (αSynOs) are the most toxic species among the forms of αSyn aggregates, and that size and topological structural properties are crucial factors for αSynOs-mediated toxicity, involving the interaction with either neurons or glial cells. We previously characterized a human αSynO (H-αSynO) with specific structural properties promoting toxicity against neuronal membranes. Here, we tested the neurotoxic potential of these H-αSynOs in vivo, in relation to the neuropathological and symptomatic features of PD. The H-αSynOs were unilaterally infused into the rat substantia nigra pars compacta (SNpc). Phosphorylated αSyn (p129-αSyn), reactive microglia, and cytokine levels were measured at progressive time points. Additionally, a phagocytosis assay in vitro was performed after microglia pre-exposure to αsynOs. Dopaminergic loss, motor, and cognitive performances were assessed. H-αSynOs triggered p129-αSyn deposition in SNpc neurons and microglia and spread to the striatum. Early and persistent neuroinflammatory responses were induced in the SNpc. In vitro, H-αSynOs inhibited the phagocytic function of microglia. H-αsynOs-infused rats displayed early mitochondrial loss and abnormalities in SNpc neurons, followed by a gradual nigrostriatal dopaminergic loss, associated with motor and cognitive impairment. The intracerebral inoculation of structurally characterized H-αSynOs provides a model of progressive PD neuropathology in rats, which will be helpful for testing neuroprotective therapies.
Int J Mol Sci, 2020; 21
32173400: Carta AR, Boi L, Pisanu A, Palmas MF, Carboni E, De Simone A
Advances in modelling alpha-synuclein-induced Parkinson's diseases in rodents: Virus-based models versus inoculation of exogenous preformed toxic species.
Aggregates of alpha-synuclein (αSyn) have been described in Parkinson's disease (PD) patients, and recent evidence has suggested that the most toxic αSyn species in PD are small soluble aggregates including oligomers, prefibrils, protofibrils. The physiological function of αSyn is still highly debated, with a possible role in synaptic vesicle trafficking and release at the presynaptic compartment, and in the regulation of gene expression in the nucleus. Emerging evidence indicate that most of αSyn functions are related with the crucial ability to bind biological membranes, which is associated with structural conversion from a disordered monomer to an α-helical enriched structure. Conformational properties of αSyn can be modulated by a number of factors including post-translational modifications, gene duplication and triplication-driven overexpression, single point mutations, environmental changes, which affect membrane binding and the protein propensity to aggregate in toxic species. The recognized toxic role of αSyn in PD has laid the rational for purposing of αSyn-based, neuropathologically

relevant preclinical models of PD. Different approaches have led to the establishment of transgenic models, viral vector-based models, and more recently models based on the intracerebral inoculation of exogenous αSyn preformed fibrils/oligomers. Here, we overview and compare viral vector-based models of αSyn overexpression and models obtained by direct intracerebral infusion of in vitro preformed αSyn species. The advantages and pitfalls associated with these different approaches are discussed.
J Neurosci Methods, 2020; 338
32937957: Murgia F, Atzori L, Carboni E, Santoru ML, Hendren A, Pisanu A, Caboni P, Boi L, Fusco G, Carta AR
Metabolomics Fingerprint Induced by the Intranigral Inoculation of Exogenous Human Alpha-Synuclein Oligomers in a Rat Model of Parkinson's Disease.
Parkinson's disease (PD) is considered a synucleinopathy because of the intraneuronal accumulation of aggregated α-synuclein (αSyn). Recent evidence points to soluble αSyn-oligomers (αSynO) as the main cytotoxic species responsible for cell death. Given the pivotal role of αSyn in PD, αSyn-based models are crucial for the investigation of toxic mechanisms and the identification of new therapeutic targets in PD. By using a metabolomics approach, we evaluated the metabolic profile of brain and serum samples of rats infused unilaterally with preformed human αSynOs (HαSynOs), or vehicle, into the substantia nigra pars compacta (SNpc). Three months postinfusion, the striatum was dissected for striatal dopamine (DA) measurements via High Pressure Liquid Chromatography (HPLC) analysis and mesencephalon and serum samples were collected for the evaluation of metabolite content via gas chromatography mass spectrometry analysis. Multivariate, univariate and correlation statistics were applied. A 40% decrease of DA content was measured in the HαSynO-infused striatum as compared to the contralateral and the vehicle-infused striata. Decreased levels of dehydroascorbic acid, myo-inositol, and glycine, and increased levels of threonine, were found in the mesencephalon, while increased contents of fructose and mannose, and a decrease in glycine and urea, were found in the serum of HαSynO-infused rats. The significant correlation between DA and metabolite content indicated that metabolic variations reflected the nigrostriatal degeneration. Collectively, the metabolomic fingerprint of HαSynO-infused rats points to an increase of oxidative stress markers, in line with PD neuropathology, and provides hints for potential biomarkers of PD.
Int J Mol Sci, 2020; 21

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-735

RATHER A FANTASTIC DRUG THAN A FANTASTIC BEAST: DOXY (DOXYCYCLINE) WORKS AS AN ANTI-DYSKINETIC DRUG TO PARTIALLY LESIONED HEMIPARKINSONIAN MICE DUE TO ITS ANTI-INFLAMMATORY PROPERTIES.

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Pharmacologic manipulation of neuroinflammation is a promising strategy to alleviate L-DOPA-induced dyskinesia (LID). Recently, our group has demonstrated that anti-inflammatory drugs alleviate LID in hemiparkinsonian mice. Here we addressed the hypothesis that LID could be reversed by doxycycline (Doxy) in doses that present anti-inflammatory actions without affecting bacterial structure. To this aim, we used 6-OHDA-lesioned C57BL/6 hemiparkinsonian mice that present a partial lesion of the nigrostriatal pathway and treated them with L-DOPA (L-DOPA 25 mg/kg + benserazide 10 mg/kg i.p. for 21 days). These animals developed severe axial, limb and orofacial abnormal involuntary movements (AIMs). Following this period, the animals were exposed to doxy (20 or 40 mg/kg s.c.) for 5 more days. Sub-chronic treatment with Doxy at both doses significantly reduced the stablished L-DOPA-induced AIMs without affecting the locomotor activity improved by L-DOPA. As expected, Doxy at 40 mg/kg had a more robust effect over AIMs compared to Doxy 20 mg/kg. However, since doxy 20 mg/kg (the lower dose without antibiotic activity) also caused a significant decrease of AIMs, we chose this dose for molecular analysis. After confirmation of LID attenuation, we performed immunohistochemistry (for COX-2-immunoreactive cells) and ELISA (for cytokines TNF-α, IL-1β and IL-6 and for COX-2 metabolite PGE2) in the lesioned dorsal striatum of hemiparkinsonian mice. LID decrease was accompanied by the reduction of COX-2 expression, PGE2 and the cytokines TNF-α and IL-1β. Overall, we conclude that sub-antibiotic doses of doxy are responsible for anti-inflammatory actions that are mandatory for LID attenuation and might be an interesting alternative way to use doxy as treatment for LID in Parkinson's disease.

Pubmed:

34637050: Kubrusly RCC, da Rosa Valli T, Ferreira MNMR, de Moura P, Borges-Martins VPP, Martins RS, Ferreira DDP, Sathler MF, de Melo Reis RA, Ferreira GC, Manhães AC, Dos Santos Pereira M
Caffeine Improves GABA Transport in the Striatum of Spontaneously Hypertensive Rats (SHR).
The spontaneously hypertensive rat (SHR) is an excellent animal model that mimics the behavioral and neurochemical phenotype of attention-deficit/hyperactivity disorder (ADHD). Here, we characterized the striatal GABA transport of SHR and investigated whether caffeine, a non-selective antagonist of adenosine receptors, could influence GABAergic circuitry. For this purpose, ex vivo striatal slices of SHR and Wistar (control strain) on the 35th postnatal day were dissected and incubated with [3H]-GABA to quantify the basal levels of uptake and release. SHR exhibited a reduced [3H]-GABA uptake and release, suggesting a defective striatal GABAergic transport system. GAT-1 appears to be the primary transporter for [3H]-GABA uptake in SHR striatum, as GAT-1 selective blocker, NO-711, completely abolished it. We also verified that acute exposure of striatal slices to caffeine improved [3H]-GABA uptake and release in SHR, whereas Wistar rats were not affected. GABA-uptake increase and cAMP accumulation promoted by caffeine was reverted by A1R activation with N6-cyclohexyl adenosine (CHA). As expected, the pharmacological blockade of cAMP-PKA signaling by H-89 also prevented caffeine-mediated [3H]-GABA uptake increment. Interestingly, a single caffeine exposure did not affect GAT-1 or A1R protein density in SHR, which was not different from Wistar protein levels, suggesting that the GAT-1-dependent transport in SHR has a defective functional activity rather than lower protein expression. The current data support that caffeine regulates GAT-1 function and improves striatal GABA transport via A1R-cAMP-PKA signaling, specifically in SHR. These results reinforce that caffeine may have therapeutic use in disorders where the GABA transport system is impaired.
Neurotox Res, 2021; 39
34440932: Ferreira Junior NC, Dos Santos Pereira M, Francis N, Ramirez P, Martorell P, González-Lizarraga F, Figadère B, Chehin R, Del Bel E, Raisman-Vozari R, Michel PP
The Chemically-Modified Tetracycline COL-3 and Its Parent Compound Doxycycline Prevent Microglial Inflammatory Responses by Reducing Glucose-Mediated Oxidative Stress.

We used mouse microglial cells in culture activated by lipopolysaccharide (LPS) or α-synuclein amyloid aggregates (αSa) to study the anti-inflammatory effects of COL-3, a tetracycline derivative without antimicrobial activity. Under LPS or αSa stimulation, COL-3 (10, 20 µM) efficiently repressed the induction of the microglial activation marker protein - and the stimulated-release of the pro-inflammatory cytokine TNF-α. COL-3's inhibitory effects on TNF-α were reproduced by the tetracycline antibiotic doxycycline (DOX; 50 µM), the glucocorticoid dexamethasone, and apocynin (APO), an inhibitor of the superoxide-producing enzyme NADPH oxidase. This last observation suggested that COL-3 and DOX might also operate themselves by restraining oxidative stress-mediated signaling events. Quantitative measurement of intracellular reactive oxygen species (ROS) levels revealed that COL-3 and DOX were indeed as effective as APO in reducing oxidative stress and TNF-α release in activated microglia. ROS inhibition with COL-3 or DOX occurred together with a reduction of microglial glucose accumulation and NADPH synthesis. This suggested that COL-3 and DOX might reduce microglial oxidative burst activity by limiting the glucose-dependent synthesis of NADPH, the requisite substrate for NADPH oxidase. Coherent with this possibility, the glycolysis inhibitor 2-deoxy-D-glucose reproduced the immunosuppressive action of COL-3 and DOX in activated microglia. Overall, we propose that COL-3 and its parent compound DOX exert anti-inflammatory effects in microglial cells by inhibiting glucose-dependent ROS production. These effects might be strengthened by the intrinsic antioxidant properties of DOX and COL-3 in a self-reinforcing manner.
Cells, 2021; 10

34246682: Vivanco-Estela AN, Dos-Santos-Pereira M, Guimaraes FS, Del-Bel E, Nascimento GCD
Cannabidiol has therapeutic potential for myofascial pain in female and male parkinsonian rats.
The musculoskeletal orofacial pain is a complex symptom of Parkinson's disease (PD) resulting in stomatognathic system dysfunctions aggravated by the disease rigidity and postural instability. We tested the effect of cannabidiol (CBD), a non-psychotomimetic constituent of Cannabis sativa, in PD-related myofascial pain. Wistar adult female and male rats orofacial allodynic and hyperalgesic responses were tested by Von Frey and formalin tests, before and 21 days past 6-OHDA lesion. Algesic response was tested after masseter muscle injection of CBD (10, 50, 100 µg in 10 µL) or vehicle. Males compared to females in all estrous cycles' phases presented reduced orofacial allodynia and hyperalgesia. According to the estrous cycle's phases, females presented distinct orofacial nociceptive responses, being the estrus phase well-chosen for nociceptive analysis after 6-OHDA lesion (phase with fewer hormone alterations and adequate length). Dopaminergic neuron lesion decreased mechanical and inflammatory nociceptive thresholds in females and males in a higher proportion in females. CBD local treatment reduced the increased orofacial allodynia and hyperalgesia, in males and females. The female rats were more sensitive to CBD effect considering allodynia, responding to the lowest dose. Although females and males respond to the effect of three doses of CBD in the formalin test, males showed a superior reduction in the hyperalgesic response. These results indicate that hemiparkinsonian female in the estrus phase and male answer differently to the different doses of CBD therapy and nociceptive tests. CBD therapy is effective for parkinsonism-induced orofacial nociception.
Neuropharmacology, 2021; 196

33510643: Dos Santos Pereira M, Abreu GHD, Rocca J, Hamadat S, Raisman-Vozari R, Michel PP, Del Bel E
Contributive Role of TNF-α to L-DOPA-Induced Dyskinesia in a Unilateral 6-OHDA Lesion Model of Parkinson's Disease.
Our present objective was to better characterize the mechanisms that regulate striatal neuroinflammation in mice developing L-DOPA-induced dyskinesia (LID). For that, we used 6-hydroxydopamine (6-OHDA)-lesioned mice rendered dyskinetic by repeated intraperitoneal injections of 3,4-dihydroxyphenyl-L-alanine (L-DOPA) and quantified ensuing neuroinflammatory changes in the dopamine-denervated dorsal striatum. LID development was associated with a prominent astrocytic response, and a more moderate microglial cell reaction restricted to this striatal area. The glial response was associated with elevations in two pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β. Treatment with the phytocannabinoid cannabidiol and the transient receptor potential vanilloid-1 (TRPV-1) channel antagonist capsazepine diminished LID intensity and decreased TNF-α levels without impacting other inflammation markers. To possibly reproduce the neuroinflammatory component of LID, we exposed astrocyte and microglial cells in culture to candidate molecules that might operate as inflammatory cues during LID development, i.e., L-DOPA, dopamine, or glutamate. Neither L-DOPA nor dopamine produced an inflammatory response in glial cell cultures. However, glutamate enhanced TNF-α secretion and GFAP expression in astrocyte cultures and promoted Iba-1 expression in microglial cultures. Of interest, the antidyskinetic treatment with cannabidiol + capsazepine reduced TNF-α release in glutamate-activated astrocytes. TNF-α, on its own, promoted the synaptic release of glutamate in cortical neuronal cultures, whereas cannabidiol + capsazepine prevented this effect. Therefore, we may assume that the release of TNF-α by glutamate-activated astrocytes may contribute to LID by exacerbating corticostriatal glutamatergic inputs excitability and maintaining astrocytes in an activated state through a self-reinforcing mechanism.
Front Pharmacol, 2020; 11

31647138: Dos-Santos-Pereira M, Guimarães FS, Del-Bel E, Raisman-Vozari R, Michel PP
Cannabidiol prevents LPS-induced microglial inflammation by inhibiting ROS/NF-κB-dependent signaling and glucose

consumption.
We used mouse microglial cells in culture activated by lipopolysaccharide (LPS, 10 ng/ml) to study the anti-inflammatory potential of cannabidiol (CBD), the major nonpsychoactive component of cannabis. Under LPS stimulation, CBD (1-10 μM) potently inhibited the release of prototypical proinflammatory cytokines (TNF-α and IL-1β) and that of glutamate, a noncytokine mediator of inflammation. The effects of CBD were predominantly receptor-independent and only marginally blunted by blockade of CB2 receptors. We established that CBD inhibited a mechanism involving, sequentially, NADPH oxidase-mediated ROS production and NF-κB-dependent signaling events. In line with these observations, active concentrations of CBD demonstrated an intrinsic free-radical scavenging capacity in the cell-free DPPH assay. Of interest, CBD also prevented the rise in glucose uptake observed in microglial cells challenged with LPS, as did the inhibitor of NADPH oxidase apocynin and the inhibitor of IκB kinase-2, TPCA-1. This indicated that the capacity of CBD to prevent glucose uptake also contributed to its anti-inflammatory activity. Supporting this view, the glycolytic inhibitor 2-deoxy-d-glucose (2-DG) mimicked the antioxidant/immunosuppressive effects of CBD. Interestingly, CBD and 2-DG, as well as apocynin and TPCA-1 caused a reduction in glucose-derived NADPH, a cofactor required for NADPH oxidase activation and ROS generation. These different observations suggest that CBD exerts its anti-inflammatory effects towards microglia through an intrinsic antioxidant effect, which is amplified through inhibition of glucose-dependent NADPH synthesis. These results also further confirm that CBD may have therapeutic utility in conditions where neuroinflammatory processes are prominent.
Glia, 2020; 68
31637586: Junior NCF, Dos-Santos-Pereira M, Guimarães FS, Del Bel E
Cannabidiol and Cannabinoid Compounds as Potential Strategies for Treating Parkinson's Disease and L-DOPA-Induced Dyskinesia.
Parkinson's disease (PD) and L-DOPA-induced dyskinesia (LID) are motor disorders with significant impact on the patient's quality of life. Unfortunately, pharmacological treatments that improve these disorders without causing severe side effects are not yet available. Delay in initiating L-DOPA is no longer recommended as LID development is a function of disease duration rather than cumulative L-DOPA exposure. Manipulation of the endocannabinoid system could be a promising therapy to control PD and LID symptoms. In this way, phytocannabinoids and synthetic cannabinoids, such as cannabidiol (CBD), the principal non-psychotomimetic constituent of the Cannabis sativa plant, have received considerable attention in the last decade. In this review, we present clinical and preclinical evidence suggesting CBD and other cannabinoids have therapeutic effects in PD and LID. Here, we discuss CBD pharmacology, as well as its neuroprotective effects and those of other cannabinoids. Finally, we discuss the modulation of several pro- or anti-inflammatory factors as possible mechanisms responsible for the therapeutic/neuroprotective potential of Cannabis-derived/cannabinoid synthetic compounds in motor disorders.
Neurotox Res, 2020; 37
30394585: Dos-Santos-Pereira M, Acuña L, Hamadat S, Rocca J, González-Lizárraga F, Chehín R, Sepulveda-Diaz J, Del-Bel E, Raisman-Vozari R, Michel PP
Microglial glutamate release evoked by α-synuclein aggregates is prevented by dopamine.
When activated, microglial cells have the potential not only to secrete typical proinflammatory mediators but also to release the neurotransmitter glutamate in amounts that may promote excitotoxicity. Here, we wished to determine the potential of the Parkinson's disease (PD) protein α-Synuclein (αS) to stimulate glutamate release using cultures of purified microglial cells. We established that glutamate release was robustly increased when microglial cultures were treated with fibrillary aggregates of αS but not with the native monomeric protein. Promotion of microglial glutamate release by αS aggregates (αSa) required concomitant engagement of TLR2 and P2X7 receptors. Downstream to cell surface receptors, the release process was mediated by activation of a signaling cascade sequentially involving phosphoinositide 3-kinase (PI3K) and NADPH oxidase, a superoxide-producing enzyme. Inhibition of the Xc- antiporter, a plasma membrane exchange system that imports extracellular l-cystine and exports intracellular glutamate, prevented the release of glutamate induced by αSa, indicating that system Xc- was the final effector element in the release process downstream to NADPH oxidase activation. Of interest, the stimulation of glutamate release by αSa was abrogated by dopamine through an antioxidant effect requiring D dopamine receptor activation and PI3K inhibition. Altogether, present data suggest that the activation of microglial cells by αSa may possibly result in a toxic build-up of extracellular glutamate contributing to excitotoxic stress in PD. The deficit in dopamine that characterizes this disorder may further aggravate this process in a vicious circle mechanism.
Glia, 2018; 66
27373843: Dos-Santos-Pereira M, da-Silva CA, Guimarães FS, Del-Bel E
Co-administration of cannabidiol and capsazepine reduces L-DOPA-induced dyskinesia in mice: Possible mechanism of action.
Neurobiol Dis, 2016; 94

26509840: dos Santos Pereira M, Sathler MF, Valli Tda R, Marques RS, Ventura AL, Peccinalli NR, Fraga MC, Manhães AC, Kubrusly R
Long Withdrawal of Methylphenidate Induces a Differential Response of the Dopaminergic System and Increases Sensitivity to Cocaine in the Prefrontal Cortex of Spontaneously Hypertensive Rats.
Methylphenidate (MPD) is one of the most prescribed drugs for alleviating the symptoms of Attention Deficit/Hyperactivity Disorder (ADHD). However, changes in the molecular mechanisms related to MPD withdrawal and susceptibility to consumption of other psychostimulants in normal individuals or individuals with ADHD phenotype are not completely understood. The aims of the present study were: (i) to characterize the molecular differences in the prefrontal dopaminergic system of SHR and Wistar strains, (ii) to establish the neurochemical consequences of short- (24 hours) and long-term (10 days) MPD withdrawal after a subchronic treatment (30 days) with Ritalin® (Methylphenidate Hydrochloride; 2.5 mg/kg orally), (iii) to investigate the dopaminergic synaptic functionality after a cocaine challenge in adult MPD-withdrawn SHR and Wistar rats. Our results indicate that SHR rats present reduced [3H]-Dopamine uptake and cAMP accumulation in the prefrontal cortex (PFC) and are not responsive to dopaminergic stimuli in when compared to Wistar rats. After a 24-hour withdrawal of MPD, SHR did not present any alterations in [3H]-Dopamine Uptake, [3H]-SCH 23390 binding and cAMP production; nonetheless, after a 10-day MPD withdrawal, the results showed a significant increase of [3H]-Dopamine uptake, of the quantity of [3H]-SCH 23390 binding sites and of cAMP levels in these animals. Finally, SHR that underwent a 10-day MPD withdrawal and were challenged with cocaine (10 mg/kg i.p.) presented reduced [3H]-Dopamine uptake and increased cAMP production. Wistar rats were affected by the 10-day withdrawal of MPD in [3H]-dopamine uptake but not in cAMP accumulation; in addition, cocaine was unable to induce significant modifications in [3H]-dopamine uptake and in cAMP levels after the 10-day withdrawal of MPD. These results indicate a mechanism that could explain the high comorbidity between ADHD adolescent patients under methylphenidate treatment and substance abuse in adult life.
PLoS One, 2015; 10
26009769: Bortolanza M, Padovan-Neto FE, Cavalcanti-Kiwiatkoski R, Dos Santos-Pereira M, Mitkovski M, Raisman-Vozari R, Del-Bel E
Are cyclooxygenase-2 and nitric oxide involved in the dyskinesia of Parkinson's disease induced by L-DOPA?
Inflammatory mechanisms are proposed to play a role in L-DOPA-induced dyskinesia. Cyclooxygenase-2 (COX2) contributes to inflammation pathways in the periphery and is constitutively expressed in the central nervous system. Considering that inhibition of nitric oxide (NO) formation attenuates L-DOPA-induced dyskinesia, this study aimed at investigating if a NO synthase (NOS) inhibitor would change COX2 brain expression in animals with L-DOPA-induced dyskinesia. To this aim, male Wistar rats received unilateral 6-hydroxydopamine microinjection into the medial forebrain bundle were treated daily with L-DOPA (21 days) combined with 7-nitroindazole or vehicle. All hemi-Parkinsonian rats receiving l-DOPA showed dyskinesia. They also presented increased neuronal COX2 immunoreactivity in the dopamine-depleted dorsal striatum that was directly correlated with dyskinesia severity. Striatal COX2 co-localized with choline-acetyltransferase, calbindin and DARPP-32 (dopamine-cAMP-regulated phosphoprotein-32), neuronal markers of GABAergic neurons. NOS inhibition prevented L-DOPA-induced dyskinesia and COX2 increased expression in the dorsal striatum. These results suggest that increased COX2 expression after L-DOPA long-term treatment in Parkinsonian-like rats could contribute to the development of dyskinesia.
Philos Trans R Soc Lond B Biol Sci, 2015; 370

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-736

TIME-COURSE OF MOTOR BEHAVIOURAL, NEURODEGENERATIVE AND NEUROINFLAMMATORY CHANGES AFTER VIRAL VECTOR-MEDIATED OVEREXPRESSION OF ALPHA-SYNUCLEIN IN THE MOUSE SUBSTANTIA NIGRA

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Alpha-synuclein (α-syn), being the main protein in Lewy bodies, plays a key role in the pathological hallmarks of Parkinson's disease (PD). However, the mechanisms underlying the degeneration of dopaminergic (DA) neurons and the involvement of α-syn and neuroinflammation in this process remain unclear. We aimed to investigate the temporal relationship between α-syn overexpression, motor impairment, glial activation and nigrostriatal degeneration in a PD mouse model. Mice received uni or bilateral intranigral injection of adeno-associated viral vectors (AAV) encoding human α-syn (AAV-hα-syn) and were sacrificed 15, 60 or 120 days post-injection. At these time points, motor behavioural tests and immunohistochemistry for DA degeneration (TH) and neuroinflammation (microglia (Iba-1) and astrocytes (GFAP)) were carried out. Uni or bilateral intranigral injection of AAV-hα-syn led to significant nigral α-syn aggregation at day 15 post-surgery, reaching the peak at day 60. Significant reductions in striatal and nigral TH^+ fibres were observed 60 and 120 days post-surgery, whereas the number of nigral TH^+ cells was unchanged. Behavioural analysis revealed motor impairments highly correlated to striatal TH loss 60 days after the viral transduction. In both models, a significant nigral microglial activation was observed 60 and 120 days post-injection, but no striatal or nigral astroglial activation was found. Although these preliminary data indicate that the AAV-mediated overexpression of α-syn in the mouse *substantia nigra* is appropriate to model PD-like pathology, additional time points need to be explored to elucidate whether the neuroinflammation contributes to DA degeneration. Funding: PIBA2019-38; PUE21-03; COLAB20/07; GIU19/092. MZ is supported by UPV/EHU fellowship.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S02-737

INVOLVEMENT OF STRIATO-PALLIDAL DARPP32 IN SLEEP REGULATION

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Aim: DARPP32 is highly enriched in the striatal formation and has been intensively studied for its role in the regulation of the activity of the striato-nigral and striato-pallidal projection neurons, belonging to the direct and indirect pathway. The present study investigates the involvement of DARPP32 in different types of striato-pallidal driven behaviors, including locomotion and motor coordination, sleep, and cognition. Methods: *Animals:* we used a mouse strain where DARPP32 is specifically depleted in the A2A expressing striato-pallidal neurons. *Behavioral tests:* The motor phenotype was studied using the open-field and rotarod test. The novel object recognition test and the water Y maze were used to examine cognition and memory. Anxiety and obsessive behaviors were assessed by light-dark box, elevated plus maze and marble burying test. *Sleep analysis:* sleep architecture was analyzed by EEG and EMG recording using a MATLAB script. Results: Depletion of DARPP32 in A2A expressing neurons increases basal exploratory behavior in the open-field. This effect is accompanied by a significant increase in wakefulness and decrease in slow-wave sleep during the active period of the 24hr light-dark cycle. The other behavioral tests did not show impairment in cognition or learning. Conclusion: Our findings show that DARPP32 deficiency in striato-pallidal neurons increases motor behavior and reduces sleep during the active period of the sleep-wake cycle. Further studies will be necessary to clarify the possible involvement of dysregulated striato-pallidal DARPP32-dependent transmission in pathological states associated with sleep disruption, including neurodegenerative and psychiatric disorders.

Pubmed:
30689087: Mercatelli D, Pisanò CA, Novello S, Morari M
NOP Receptor Ligands and Parkinson's Disease.
Nociceptin/Orphanin FQ (N/OFQ) and its NOP receptor are highly expressed in motor areas of the rodent, nonhuman, and human primate brain, such as primary motor cortex, thalamus, globus pallidus, striatum, and substantia nigra. Endogenous N/OFQ negatively regulates motor behavior and dopamine transmission through NOP receptors expressed by dopaminergic neurons of the substantia nigra compacta. Consistent with the existence of an N/OFQ tone over dopaminergic transmission, blockade of NOP receptor antagonists increases striatal dopamine release. In this chapter, we will review the evidence linking the N/OFQ-NOP receptor system to Parkinson's disease (PD). We will first discuss data showing that the central N/OFQ-NOP receptor system undergoes plastic changes in different basal ganglia nuclei following dopamine depletion. Then we will show that NOP receptor antagonists relieve motor deficits in different rodent and nonhuman primate models of PD. Mechanistically, NOP receptor blockade in substantia nigra reticulata results in rebalancing of the inhibitory GABAergic and excitatory glutamatergic inputs impinging on nigro-thalamic GABAergic neurons, leading to thalamic disinhibition. We will also present data showing that, in addition to motor symptoms, N/OFQ also plays a role in the parkinsonian neurodegeneration. In fact, NOP receptor antagonists possess neuroprotective/neurorescue properties in in vitro and in vivo models of PD.
Handb Exp Pharmacol, 2019; 254
29232769: Arcuri L, Novello S, Frassineti M, Mercatelli D, Pisanò CA, Morella I, Fasano S, Journigan BV, Meyer ME, Polgar WE, Brambilla R, Zaveri NT, Morari M
Anti-Parkinsonian and anti-dyskinetic profiles of two novel potent and selective nociceptin/orphanin FQ receptor agonists.
We previously showed that nociceptin/orphanin FQ opioid peptide (NOP) receptor agonists attenuate the expression of levodopa-induced dyskinesia in animal models of Parkinson's disease. We now investigate the efficacy of two novel, potent and chemically distinct NOP receptor agonists, AT-390 and AT-403, to improve Parkinsonian disabilities and attenuate dyskinesia development and expression.
Br J Pharmacol, 2018; 175
32798726: Brugnoli A, Pisanò CA, Morari M
Striatal and nigral muscarinic type 1 and type 4 receptors modulate levodopa-induced dyskinesia and striato-nigral pathway

activation in 6-hydroxydopamine hemilesioned rats.
Acetylcholine muscarinic receptors (mAChRs) contribute to both the facilitation and inhibition of levodopa-induced dyskinesia operated by striatal cholinergic interneurons, although the receptor subtypes involved remain elusive. Cholinergic afferents from the midbrain also innervate the substantia nigra reticulata, although the role of nigral mAChRs in levodopa-induced dyskinesia is unknown. Here, we investigate whether striatal and nigral M1 and/or M4 mAChRs modulate dyskinesia and the underlying striato-nigral GABAergic pathway activation in 6-hydroxydopamine hemilesioned rats. Reverse microdialysis allowed to deliver the mAChR antagonists telenzepine (M1 subtype preferring), PD-102807 and tropicamide (M4 subtype preferring), as well as the selective M4 mAChR positive allosteric modulator VU0152100 in striatum or substantia nigra, while levodopa was administered systemically. Dyskinetic movements were monitored along with nigral GABA (and glutamate) and striatal glutamate dialysate levels, taken as neurochemical correlates of striato-nigral pathway and cortico-basal ganglia-thalamo-cortical loop activation. We observed that intrastriatal telenzepine, PD-102807 and tropicamide alleviated dyskinesia and inhibited nigral GABA and striatal glutamate release. This was partially replicated by intrastriatal VU0152100. The M2 subtype preferring antagonist AFDX-116, used to elevate striatal acetylcholine levels, blocked the behavioral and neurochemical effects of PD-102807. Intranigral VU0152100 prevented levodopa-induced dyskinesia and its neurochemical correlates whereas PD-102807 was ineffective. These results suggest that striatal, likely postsynaptic, M1 mAChRs facilitate dyskinesia and striato-nigral pathway activation in vivo. Conversely, striatal M4 mAChRs can both facilitate and inhibit dyskinesia, possibly depending on their localization. Potentiation of striatal and nigral M4 mAChR transmission leads to powerful multilevel inhibition of striato-nigral pathway and attenuation of dyskinesia.
Neurobiol Dis, 2020; 144
32086070: Pisanò CA, Brugnoli A, Novello S, Caccia C, Keywood C, Melloni E, Vailati S, Padoani G, Morari M
Safinamide inhibits in vivo glutamate release in a rat model of Parkinson's disease.
To investigate whether the reversible MAO-B inhibitor and sodium channel blocker safinamide impairs glutamate release under parkinsonian conditions in vivo, and this effect is dependent on MAO-B inhibition, safinamide (and rasagiline as a comparator) were administered to 6-hydroxydopamine hemilesioned rats, a model of Parkinson's disease, and haloperidol-treated rats, a model of neuroleptic-induced parkinsonism. A microdialysis probe was implanted in the dopamine-depleted dorsolateral striatum, globus pallidus, subthalamic nucleus or substantia nigra reticulata of 6-hydroxydopamine hemilesioned rats. Glutamate and GABA release was stimulated by reverse dialysis of veratridine, and safinamide or rasagiline were acutely administered before veratridine at doses inhibiting MAO-B >50%. A microdialysis probe was implanted in the substantia nigra reticulata of naïve rats to monitor glutamate and GABA release following acute haloperidol and safinamide administration. Safinamide inhibited the veratridine-evoked glutamate release in the globus pallidus and subthalamic nucleus but not in the striatum and substantia nigra. Moreover, it reduced pallidal and nigral GABA release. Conversely, rasagiline failed to modify the veratridine-induced glutamate and GABA release in the basal ganglia. Safinamide also inhibited the haloperidol-induced nigral glutamate release. MAO-B inhibitors safinamide and rasagiline differ in their abilities to inhibit depolarization-evoked glutamate release in the basal ganglia of parkinsonian rats. The ineffectiveness of rasagiline suggests that MAO-B inhibition does not contribute to the antiglutamatergic activity of safinamide. The glutamate-inhibiting action of safinamide within the subthalamo-external pallidal loop, which shows abnormal activity in Parkinson's disease, might contribute to its therapeutic actions of improving motor performance without provoking troublesome dyskinesia.
Neuropharmacology, 2020; 167
34767639: Pisanò CA, Mercatelli D, Mazzocchi M, Brugnoli A, Morella I, Fasano S, Zaveri NT, Brambilla R, O'Keeffe GW, Neubig RR, Morari M
Regulator of G-Protein Signalling 4 (RGS4) negatively modulates nociceptin/orphanin FQ opioid receptor signalling: Implication for l-Dopa-induced dyskinesia.
Regulator of G-protein signalling 4 (RGS4) is a signal transduction protein that accelerates intrinsic GTPase activity of Gα and Gα subunits, suppressing GPCR signalling. Here, we investigate whether RGS4 modulates nociceptin/orphanin FQ (N/OFQ) opioid (NOP) receptor signalling and if this modulation has relevance for l-Dopa-induced dyskinesia.
Br J Pharmacol, 2021;
32464686: Vegas-Suárez S, Pisanò CA, Requejo C, Bengoetxea H, Lafuente JV, Morari M, Miguelez C, Ugedo L
6-Hydroxydopamine lesion and levodopa treatment modify the effect of buspirone in the substantia nigra pars reticulata.
l-DOPA-induced dyskinesia (LID) is considered a major complication in the treatment of Parkinson's disease (PD). Buspirone (5-HT partial agonist) have shown promising results in the treatment of PD and LID, however no 5-HT-based treatment has been approved in PD. The present study was aimed to investigate how the substantia nigra pars reticulata (SNr) is affected by buspirone and whether it is a good target to study 5-HT antidyskinetic treatments.
Br J Pharmacol, 2020; 177
31951130: Kamakolanu UG, Meyer ME, Yasuda D, Polgar WE, Marti M, Mercatelli D, Pisanò CA, Brugnoli A, Morari M, Zaveri NT

Discovery and Structure-Activity Relationships of Nociceptin Receptor Partial Agonists That Afford Symptom Ablation in Parkinson's Disease Models.
A novel series of C(3)-substituted piperdinylindoles were developed as nociceptin opioid receptor (NOP) partial agonists to explore a pharmacological hypothesis that NOP partial agonists would afford a dual pharmacological action of attenuating Parkinson's disease (PD) motor symptoms and development of levodopa-induced dyskinesias. SAR around the C-3 substituents investigated effects on NOP binding, intrinsic activity, and selectivity and showed that while the C(3)-substituted indoles are selective, high affinity NOP ligands, the steric, polar, and cationic nature of the C-3 substituents affected intrinsic activity to afford partial agonists with a range of efficacies. Compounds , , and with agonist efficacies between 25% and 35% significantly attenuated motor deficits in the 6-OHDA-hemilesioned rat model of PD. Further, unlike NOP antagonists, which appear to worsen dyskinesia expression, these NOP partial agonists did not attenuate or worsen dyskinesia expression. The NOP partial agonists and their SAR reported here may be useful to develop nondopaminergic treatments for PD.
J Med Chem, 2020; 63
29167350: Morari M, Brugnoli A, Pisanò CA, Novello S, Caccia C, Melloni E, Padoani G, Vailati S, Sardina M
Safinamide Differentially Modulates In Vivo Glutamate and GABA Release in the Rat Hippocampus and Basal Ganglia.
Safinamide has been recently approved as an add-on to levodopa therapy for Parkinson disease. In addition to inhibiting monoamine oxidase type B, it blocks sodium channels and modulates glutamate (Glu) release in vitro. Since this property might contribute to the therapeutic action of the drug, we undertook the present study to investigate whether safinamide inhibits Glu release also in vivo and whether this effect is consistent across different brain areas and is selective for glutamatergic neurons. To this aim, in vivo microdialysis was used to monitor the spontaneous and veratridine-induced Glu and GABA release in the hippocampus and basal ganglia of naive, awake rats. Brain levels of safinamide were measured as well. To shed light on the mechanisms underlying the effect of safinamide, sodium currents were measured by patch-clamp recording in rat cortical neurons. Safinamide maximally inhibited the veratridine-induced Glu and GABA release in hippocampus at 15 mg/kg, which reached free brain concentrations of 1.89-1.37 M. This dose attenuated veratridine-stimulated Glu (but not GABA) release in subthalamic nucleus, globus pallidus, and substantia nigra reticulata, but not in striatum. Safinamide was ineffective on spontaneous neurotransmitter release. In vitro, safinamide inhibited sodium channels, showing a greater affinity at depolarized (IC = 8 M) than at resting (IC = 262 M) potentials. We conclude that safinamide inhibits in vivo Glu release from stimulated nerve terminals, likely via blockade of sodium channels at subpopulations of neurons with specific firing patterns. These data are consistent with the anticonvulsant and antiparkinsonian actions of safinamide and provide support for the nondopaminergic mechanism of its action.
J Pharmacol Exp Ther, 2018; 364
31149340: Mercatelli D, Bolognesi P, Frassineti M, Pisanò CA, Longo F, Shimshek DR, Morari M
Leucine-rich repeat kinase 2 (LRRK2) inhibitors differentially modulate glutamate release and Serine935 LRRK2 phosphorylation in striatal and cerebrocortical synaptosomes.
Mutations in leucine-rich repeat kinase 2 () gene have been pathogenically linked to Parkinson's disease, and pharmacological inhibition of LRRK2 is being pursued to tackle nigro-striatal dopaminergic neurodegeneration. However, LRRK2 kinase inhibitors may have manifold actions, affecting not only pathological mechanisms in dopaminergic neurons but also physiological functions in nondopaminergic neurons. Therefore, we investigated whether LRRK2 kinase inhibitors differentially modulate dopamine and glutamate release from the mouse striatum and cerebral cortex. Spontaneous and KCl-evoked [H]-dopamine and glutamate release from superfused synaptosomes obtained from wild-type and LRRK2 knock-out, kinase-dead or G2019S knock-in mice was measured. Two structurally unrelated inhibitors, LRRK2-IN-1 and GSK2578215A, were tested. LRRK2, phosphoSerine1292 and phosphoSerine935 LRRK2 levels were measured in all genotypes, and target engagement was evaluated by monitoring phosphoSerine935 LRRK2. LRRK2-IN-1 inhibited striatal glutamate but not dopamine release; GSK2578215A inhibited striatal dopamine and cortical glutamate but enhanced striatal glutamate release. LRRK2-IN-1 reduced striatal and cortical phosphoSerine935 levels whereas GSK2578215A inhibited only the former. Neither LRRK2 inhibitor affected neurotransmitter release in LRRK2 knock-out and kinase-dead mice; however, they facilitated dopamine without affecting striatal glutamate in G2019S knock-in mice. GSK2578215A inhibited cortical glutamate release in G2019S knock-in mice. We conclude that LRRK2-IN-1 and GSK2578215A modulate exocytosis by blocking LRRK2 kinase activity, although their effects vary depending on the nerve terminal examined. The G2019S mutation unravels a dopamine-promoting action of LRRK2 inhibitors while blunting their effects on glutamate release, which highlights their positive potential for the treatment of PD, especially of LRRK2 mutation carriers.
Pharmacol Res Perspect, 2019; 7

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BOARD NUMBER: S02-738

GLUCOCORTICOID RECEPTORS IN ASTROCYTES REGULATE ALPHA-SYNUCLEIN PATHOLOGICAL ACTIONS IMPACTING MOTOR AND NON-MOTOR SYMPTOMOLOGY OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Parkinson's disease (PD) is a complex neurodegenerative disease, characterized by both motor and non-motor symptoms. In MPTP model of PD, we previously showed that glucocorticoids (GCs), through their receptor GR in astrocytes, regulate degeneration of dopamine neurons in substantia nigra through connexin-43 hemichannel activity. In addition, we showed the number of astrocytes expressing GR was significantly lower in substantia nigra of PD samples compared to controls suggesting that GR activity is affected in PD (Cell Death and Differentiation, 2019). To dissect further the role of GR in astrocytes in PD pathology, we have undertaken experiments to study its role in alpha-synuclein pathology, using mice conditionally inactivated for GR gene specifically in parenchymal astrocytes. Stereotaxic injection of AAV human A53T alpha-synuclein viral vector in substantia nigra resulted, after 8 weeks, in greater dopamine neuronal loss with increased glial reactivity in astrocytic GR mutant mice compared to controls, suggesting astrocyte GR modulates alpha-synuclein pathology. As GC-GRs are known to affect profoundly mood and cognition, firstly we verified whether astrocytic GR mutant mice have behavioral, cognitive or motor anomalies. The results showed increased anxiety-like behavior and reduced motor performance in astrocytic GR mutant mice with no change observed in cognitive tests. Interestingly, expression of A53T alpha-synuclein in prefrontal cortex of astrocytic GR mutant and control mice resulted in impairment of working memory only in the mutant mice. We are investigating the cellular and biochemical mechanisms through which astrocyte GR regulates cognitive processes in face of alpha-synuclein pathology.

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BOARD NUMBER: S02-739

ENCAPSULATED-CELL BIO-DELIVERY OF PROSAPOSIN COUNTERACTS AAV-α-SYNUCLEIN-INDUCED PARKINSONISM

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Aims Prosaposin (PSAP) is a pleiotropic protein with neurotrophic feature and interacts with progranulin (PGRN) when trafficking in cells. PSAP is genetically linked to Parkinson's disease (PD). Encapsulated-cell biodelivery (ECB) is an effective way for therapeutic factor delivery. The objective of the study is to examine the therapeutic potential of PSAP, PGRN, and PSAP/PGRN complex delivered by ECB devices in α-synuclein-induced parkinsonism. ***Methods*** ARPE-19 cells overexpressing PSAP, PGRN, and PSAP/PGRN complex were encapsulated to generate corresponding factor-secreting ECB devices. AAV-α-synuclein was injected in the right substantia nigra of rats. Meanwhile, control ECB, ECB-PSAP, ECB-PGRN, and ECB-PSAP-PGRN devices were implanted into the right striatum of AAV-α-synuclein-injected rats. Open field test was conducted at 6-, 10-, and 14wks after surgery, followed by apomorphine rotation test at 14wks. Postmortem immunohistochemical stainings were performed to examine dopaminergic marker alterations. ***Results*** ECB devices were tested to be actively secreting corresponding factors before implantation. Overexpression of α-synuclein was detected in the substantia nigra and striatum and caused behavior deficiencies and dopaminergic degeneration in rats. ECB-PSAP and ECB-PSAP-PGRN protected rats from locomotion deterioration. ECB-PSAP prevented rats from rotating to the contralateral side induced by apomorphine. ECB-PSAP protected dopamine transporter (DAT) and vesicle monoamine transporter levels, and ECB-PSAP-PGRN protected DAT levels. However, ECB-PGRN did not show neuroprotective effect. ***Conclusions*** PSAP with or without PGRN delivered by ECB devices counteracted behavior impairments and dopaminergic loss induced by intranigral AAV-α-synuclein overexpression. Thus, PSAP replenishment might be beneficial in halting PD progression.

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BOARD NUMBER: S02-740

MITOCHONDRIAL ACTIVITY AS BIOMARKER IN A PRE-SYMPTOMATIC RODENT MODEL OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Parkinson´s Disease (PD) is a neurodegenerative disorder characterized by neuron loss in different brain areas, especially dopaminergic neurons in the Substancia Nigra (SN). The alterations cause motor symptoms such as tremor, rigidity and bradykinesia. It is not until these symptoms appear that the pathology can be diagnosed, when the neuron loss in SN is over 60%, due to a lack of early stage biomarkers that allow the diagnosis. The trigger of the degeneration remains unknown but some features have been associated with neuron death, such as the presence of Lewy bodies. These structures are formed by the accumulation of alpha-synuclein and its presence could lead to mitochondrial dysfunction, inducing oxidative stress and cell death. The aim of this study is to identify PD biomarkers in blood samples. In order to achieve this purpose, we have stablished a rodent model based on the overexpression of alpha-synuclein by injecting adeno-associated virus in the SN. Animals were tested four, eight and twelve weeks after the injection showing intact motor and non-motor performance, however, alterations in mitochondrial activity were observed in blood samples three months after the injection. With the current study we identified potential early stage PD biomarkers that could allow a sooner diagnosis of the disease. This project is financed by PIBA19-0038; PUE21-03; Bikaintek program: 007-B2/2020; COLAB20/07; GIU19/339. This research was conducted in the scope of the Transborder Joint Laboratory (LTC) “non-motor CoMorbidities in Parkinson’s Disease (CoMorPD)”. No conflict of interest.

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BOARD NUMBER: S02-741

THE MODULATORY ROLE OF CURCUMIN AND QUERCETIN ON DROSOPHILA GSK-3: A POTENTIAL THERAPEUTIC INTERVENTION IN PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Loss of GSK 3beta function leads to neuronal function in Shaggy strain of drosophila. Loss of neural function invariably leads to neurodegenerative diseases such as Parkinson's disease (PD). Natural compounds with antioxidant properties such as flavonoids is thought to be involved in protection against cellular oxidative stress. Oxidative stress is believed to be a major factor for the onset of PD. In this study, we have investigated oxidative status in transgenic *Drosophila* model of PD. Our results revealed elevated levels of reactive oxygen species (ROS) in shaggy PD model flies compared to control. We have demonstrated for the first time the ameliorating potential of synergistic natural antioxidants quercetin and curcumin PD model flies. Feeding of transgenic flies with co-administration of curcumin and quercetin for 5 days significantly improved their climbing ability and circadian rhythm of locomotor activity which was associated with reduction in levels of ROS and enhancement in the activities of catalase (CAT) and superoxide dismutase (SOD). The co-administration treatment protected against transgenic flies and delayed the onset of PD-like symptoms which appears to be mediated by suppression of oxidative stress.

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BOARD NUMBER: S02-743

GLYCATION OF ALPHA-SYNUCLEIN MODULATES AGGREGATION AND PARKINSON'S DISEASE-LIKE PHENOTYPES

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Posttranslational modifications (PTMs) are major determinants of protein folding, localization and function. Glycation is a non-enzymatic PTM that is increased in the brains of hyperglycemic patients, such as those affected by type 2 diabetes (T2D). Since it typically leads to irreversible changes in protein properties and behavior, it has attracted a great deal of attention in the last years. Alpha-synuclein (aSyn) can be glycated at lysine residues, thereby reducing fibril formation *in vitro* and modulating aggregation in cells. However, the molecular basis for these effects is unclear. To elucidate this, we are investigating the effect of different physiologically-relevant glycating agents on the aggregation of aSyn. The dicarbonyl methylglyoxal (MGO) and the sugar ribose modify aSyn to the greatest extent, and these glycation agents are the most efficient inhibitors of fibril formation. Glycation primarily inhibits elongation rather than nucleation of aSyn, and has only a modest effect on the level of oligomerization. Furthermore, glycated aSyn is not significantly incorporated into fibrils. In vivo, MGO-glycation alters dopaminergic pathways, consistent with alterations observed in PD. Our results are not only relevant for other aSyn PTMs, but also for understanding PTMs affecting other fibrillogenic proteins, and may thus open novel avenues for therapeutic intervention in protein aggregation disorders.

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BOARD NUMBER: S02-744

ROLE OF PARKINSONISM-ASSOCIATED PROTEIN FBXO7 IN SYNAPTIC INTEGRITY OF THE STRIATUM & OLFACTORY BULB

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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F-box protein only protein 7 (FBXO7) is a subunit of the SCF (SKP1/cullin-1/F-box protein) E3 ubiquitin ligase complex that plays an important role in the ubiquitin proteasome system. Mutations in *FBXO7* are associated with autosomal recessive juvenile parkinsonism, also known as Parkinsonian-pyramidal syndrome (PARK15) characterized by motor and non-motor dysfunctions. Here, we investigated the role of Fbxo7 in early pathological changes of the synapse in parkinsonism in the PARK15 mouse model by examining the basal ganglia and the olfactory bulb. First, we analyzed the striatal synaptic integrity and the proteome of the PARK15 mice by immunohistochemistry and mass spectrometry, respectively. In PARK15 mice, we found astrocytosis and microgliosis, increased complement system and dopamine transporters, as well as abnormal regulation of endocytic and exocytic proteins. Second, owing to the finding that PARK15 is associated with hyposmia, we performed immunohistochemical analysis of the olfactory bulb of PARK15 mice and found an increase in astrocytes, microglia, complement system and Vgat, but a decrease in Vglut1, tyrosine hydroxylase. In conclusion, our study demonstrated that loss of Fbxo7 resulted in activated dopaminergic pathway, complement system and immune system, and inhibited endocytosis and exocytosis in the striatum, and activated GABAergic pathway, complement system and immune system, and inhibited glutamatergic pathway and dopaminergic pathway in olfactory bulb.

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BOARD NUMBER: S02-745

EXOSOMAL MIR-128 IS ALTERED IN THE PARKINSON'S PATIENT PLASMA AND PROTECTS THE NEURONS AGAINST APOPTOSIS AND MAINTAINS SYNAPTIC INTEGRITY IN PARKINSON'S DISEASE MODELS

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Aim: Parkinson's disease (PD) is a progressive motor neuron disorder of the mid-brain. We aim at identifying specific brain-enriched exosomal miRNAs circulating in the PD patient blood (plasma) and characterizing their role in PD pathogenesis. **Methods**: 1.Exosome isolation; 2.qRT-PCR; 3.Immunoblotting; 4.Flow cytometry; 5.Confocal imaging **Results**: We collected blood samples from PD patients and age-matched controls and isolated exosomes from the respective plasma. Interestingly, we found a decrease in the expression of exosomal miR-128 in the PD patient plasma. Next, we treated SH-SY5Y and PC-12 cells with 6-OHDA (neurotoxin) which showed a decrease in the miR-128 expression upon treatment. Flow cytometry revealed that miR-128 over-expression could reduce the percentage of dying cells from 6-OHDA treatment. Since earlier reports from our lab showed that 6-OHDA can induce neuronal apoptosis via activation of the transcription factor FoxO3a, we decided to check the effect of miR-128 on apoptosis via FoxO3a activation, which revealed that 6-OHDA-mediated FoxO3a activation was significantly reversed upon over-expression of miR-128. Interestingly, miR-128 over-expression could also lead to down-regulation of early pro-apoptotic proteins like FasL and PUMA and attenuate the downstream activation of caspases-8 and -9 by 6-OHDA. Furthermore, miR-128 over-expression could not only improve neurite length formation, but also increase the expressions of synaptic proteins Synaptophysin and PSD-95 which was reduced during 6-OHDA treatment. **Conclusion**: We identified neuron-enriched miR-128 which was characteristically reduced in the PD patient plasma-derived exosomes and determined for the first time, its comprehensive role in neuronal apoptosis and maintenance of synaptic integrity in context to PD.

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BOARD NUMBER: S02-746

CHARACTERIZATION OF NEURONAL MODELS OF GLUCOCEREBROSIDASE DEFICIENCY: TOWARDS A BETTER UNDERSTANDING OF PARKINSON'S DISEASE

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Aims Parkinson's disease is characterized by a progressive degeneration of dopaminergic neurons, a pathological accumulation of alpha-synuclein and a chronic neuroinflammation. Loss-of-function mutations in the GBA gene encoding lysosomal glucocerebrosidase are common genetic factor for Parkinson's disease. However, how glucocerebrosidase deficiency contributes to Parkinson's disease pathophysiology is poorly understood. The research project aims to study the impact of the loss of glucocerebrosidase activity on the susceptibility of dopaminergic neurons exposed to cell death stimuli. **Methods**
Conduritol-beta-epoxide (CBE) was used to inhibit glucocerebrosidase activity in Human dopaminergic LUHMES cells. LUHMES cells were exposed to various cell-death inductors leading to apoptosis, autophagy, ferroptosis or mitochondrial respiratory chain alteration death. Cell viability, reactive oxygen species production and protein levels are assessed using resazurin, flow cytometry and western blot. **Results** Short-term inhibition of glucocerebrosidase activity didn't impact LUHMES cells susceptibility to inductors of neuronal death. Interestingly, prolonged inhibition of glucocerebrosidase activity decreased the sensitivity of dopaminergic LUHMES cells towards autophagic cell death and RSL3-induced ferroptosis. Ongoing experiments aim to determine the basis of the observed neuroprotective effect caused by long-term inhibition of glucocerebrosidase. **Conclusions**
Our data suggest that glucocerebrosidase deficiency in neurons decreases their susceptibility to cellular death suggesting that others mechanisms intrinsic to neurons of other cells such as microglia may increase the susceptibility of dopaminergic neurons to cell death. Ongoing experiments should determine whether the link between glucocerebrosidase and neurodegeneration could involve excessive activation of microglial cells.

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BOARD NUMBER: S02-747

NEUROPROTECTION OF MIDBRAIN CULTURED DOPAMINE NEURONS BY THE NON-PSYCHOACTIVE PHYTOCANNABINOID CANNABIDIOL

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

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Cannabidiol (CBD) is a non-psychoactive phytocannabinoid with interesting pharmacological properties that may have therapeutic utility for CNS-related disorders. In particular, CBD has been reported to have analgesic, anti-inflammatory, anti-convulsant, anti-dyskinetic and anxiolytic effects (dos Santos Pereira et al, Glia, 2020). Some studies also suggested that CBD might operate as a neuroprotectant. The present work aimed to determine whether CBD has the capacity to prevent dopamine (DA) cell death as it might occur in Parkinson disease (PD). For that, we established mouse primary midbrain cultures under experimental conditions in which DA neurons degenerate spontaneously and progressively by ferroptosis, a mode of cell death reported to contribute to PD neurodegeneration (Angelova et al, Cell Death Differ, 2020). We found that CBD efficiently protected DA neurons under such conditions. CBD effects were long lasting, not dependent on cell culture age and observable in a range of concentrations comprised between 0.2-1µM. Antagonists of CB1 (AM 251), CB2 (SCH 336) and PPARy (GW 9662) receptors as well as of TRPV1 channels (capsazepine) failed to curtail CBD neuroprotection, suggesting that CBD operated through receptor-independent mechanisms. Live-cell imaging of reactive oxygen species with DHR-123 revealed that CBD efficiently curtailed intracellular oxidative stress. The iron chelator desferrioxamine, the inhibitor of lipid peroxidation Trolox and the ferroptosis inhibitor liproxstatin-1, all reproduced CBD neuroprotective effects, confirming that CBD operated by curtailing iron-dependent ferroptosis. Overall, our data indicate that CBD has the potential to interfere efficiently with ferroptosis pathways that could possibly intervene in PD neurodegeneration.

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BOARD NUMBER: S02-748

PEGASUS: A NOVEL DOPAMINE AGONIST WITH NEUROPROTECTIVE EFFECT

POSTER SESSION 02 - SECTION: PARKINSON'S DISEASE AND MOVEMENT DISORDERS

Rosana Chehin[1,2], Agustin Pernicone[2], Verónica Manzano[1], Esteban Vera Pingitore[1], Martin Luong[3], Cesar Ávila[1], Adriana Kolender[3], Florencia González-Lizárraga[1], Rodrigo Tomas-Grau[1], Silvina Chaves[1], Oscar Varela[3], Diego Ploper[1]
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Aims: continuous and safe delivery of dopamine across the blood brain barrier (BBB) is a main obstacle for successful substitution therapy in Parkinson's disease. However, the presence of the neurotransmitter does not interfere the progression of neuronal death. Our aim is taking advantage of the well-known capacity of doxycycline (DOXY) to cross the BBB together with its neuroprotective activity to obtain a DOXY-DOPA hybrid molecule that we call Pegasus. **Methods**: *Pegasus* was synthetized by removal of the dimethylamino function at C4 and introducing an amino group at C-9, which was covalently linked via a linker to dopamine. Activation of dopaminergic receptors was performed by cAMPNOMAD Cells (Innoprot). Cytotoxicity was studied by MTT assay in SH-SY5Y/Bv2 cell lines. Neuroprotective properties were studied as follows: *i)* inhibition of α-Synuclein (AS) aggregation by ThT assay; *ii)* antioxidant capability by CellRox™ assay; *iii)* inhibition of proinflammatory cytokine IL-1β release by microglial cells; *iv*) antiapoptotic properties on HEK293T Cytochrome C-tGFP cell line, and *v*) influence on lysosomal biogenesis in SH-SY5Y cells with LysoTraker™ assay. **Results and conclusion:** *Pegasus* activated dopaminergic receptors 10-fold less efficiently than dopamine but resulted in improved theoretical ability to cross the BBB. *Pegasus* did not show toxicity in two different cell lines, and efficiently inhibited AS amyloid aggregation as well as LPS or AS fibrils-induced neuroinflammation. The new molecule also preserved antioxidant properties, characteristic of tetracyclines, and did not interfere with lysosomes biogenesis. We present *Pegasus* as a dopamine agonist with neuroprotective activity, and an ideal candidate for further preclinical studies.

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Poster Session 03

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BOARD NUMBER: S03-001

BLUE LIGHT MODULATES TASK-DEPENDENT SUBCORTICO-CORTICAL CONNECTIVITY DURING AN AUDITORY ATTENTIONAL TASK

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Aim: Exposure to blue light stimulates alertness and performance by affecting a widespread set of task-dependent brain regions. Light information is considered to reach first subcortical regions which in turn affects non-visual regional cortical activity. Here, we aimed to provide an empirical demonstration of this putative scenario in humans. **Methods:** Fourteen healthy young subjects (24.3y ± 3; 9 women) completed an attentional oddball task consisting in detecting rare (20%) deviant tones (100Hz) among more frequent (80%) standard (500Hz) ones. While performing the task, participants were exposed to 30s-blocks of blue-enriched (61 $\mu W/cm^2$) or orange monochromatic light (5.28 10^{12} photons/cm^2/s) interleaved by ~15s darkness periods. We used Dynamic Causal Modeling (DCM) to investigate the effective connectivity between areas significantly involved in the task. **Results:** Standard univariate analysis showed that the left intraparietal sulcus (IPS), involved in attention, and the inferior colliculus (IC), a brainstem nuclei part of the auditory pathway involved in multisensory integration, were significantly recruited by deviant tone detection ($p<.001$ uncorrected). Bayesian model selection and averaging of several DCMs in which light conditions modulated either the connection between IC to IPS, between IPS to IC, or both, revealed that the model with best posterior probability consisted in the model where blue, but not orange light, strengthened IC-to-IPS connection ($t=2.48$, $p=.02$). **Conclusion:** Our results provide empirical data suggesting that blue light affects cognitive activity by modulating task-dependent information flow from subcortical to cortical areas. Support: FNRS, ULiège, GIGA Doctoral School for Health Sciences, Fondation Léon Frédéric, LIGHTCAP EU-ETN-MSCA

Pubmed:
33479211: Veer IM, Riepenhausen A, Zerban M, Wackerhagen C, Puhlmann LMC, Engen H, Köber G, Bögemann SA, Weermeijer J, Uściłko A, Mor N, Marciniak MA, Askelund AD, Al-Kamel A, Ayash S, Barsuola G, Bartkute-Norkuniene V, Battaglia S, Bobko Y, Bölte S, Cardone P, Chvojková E, Damnjanović K, De Calheiros Velozo J, de Thurah L, Deza-Araujo YI, Dimitrov A, Farkas K, Feller C, Gazea M, Gilan D, Gnjidić V, Hajduk M, Hiekkaranta AP, Hofgaard LS, Ilen L, Kasanova Z, Khanpour M, Lau BHP, Lenferink DB, Lindhardt TB, Magas DÁ, Mituniewicz J, Moreno-López L, Muzychka S, Ntafouli M, O'Leary A, Paparella I, Põldver N, Rintala A, Robak N, Rosická AM, Røysamb E, Sadeghi S, Schneider M, Siugzdaite R, Stantić M, Teixeira A, Todorovic A, Wan WWN, van Dick R, Lieb K, Kleim B, Hermans EJ, Kobylińska D, Hendler T, Binder H, Myin-Germeys I, van Leeuwen JMC, Tüscher O, Yuen KSL, Walter H, Kalisch R
Psycho-social factors associated with mental resilience in the Corona lockdown.
The SARS-CoV-2 pandemic is not only a threat to physical health but is also having severe impacts on mental health. Although increases in stress-related symptomatology and other adverse psycho-social outcomes, as well as their most important risk factors have been described, hardly anything is known about potential protective factors. Resilience refers to the maintenance of mental health despite adversity. To gain mechanistic insights about the relationship between described psycho-social resilience factors and resilience specifically in the current crisis, we assessed resilience factors, exposure to Corona crisis-specific and general stressors, as well as internalizing symptoms in a cross-sectional online survey conducted in 24 languages during the most intense phase of the lockdown in Europe (22 March to 19 April) in a convenience sample of N = 15,970 adults. Resilience, as an outcome, was conceptualized as good mental health despite stressor exposure and measured as the inverse residual between actual and predicted symptom total score. Preregistered hypotheses (osf.io/r6btn) were tested with multiple regression models and mediation analyses. Results confirmed our primary hypothesis that positive appraisal style (PAS) is positively associated with resilience ($p < 0.0001$). The resilience factor PAS also partly mediated the positive association between perceived social support and resilience, and its association with resilience was in turn partly mediated by the ability to easily recover from stress (both $p < 0.0001$). In comparison with other resilience factors, good stress response recovery and positive appraisal specifically of the consequences of the Corona crisis were the strongest factors.

Preregistered exploratory subgroup analyses (osf.io/thka9) showed that all tested resilience factors generalize across major socio-demographic categories. This research identifies modifiable protective factors that can be targeted by public mental health efforts in this and in future pandemics.
Transl Psychiatry, 2021; 11

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BOARD NUMBER: S03-002

PRETERM NEONATES DISTINGUISH RHYTHM VIOLATION THROUGH A HIERARCHY OF CORTICAL PROCESSING

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Rhythm is a fundamental component of the auditory world, present even during the prenatal life. While there is evidence that some auditory capacities are already present before birth, whether and how the premature neural networks process auditory rhythm is yet not known. We investigated the neural response of premature neonates at 30-34 weeks gestational age to violations from rhythmic regularities in an auditory sequence using high-resolution electroencephalography and event-related potentials. Unpredicted rhythm violations elicited a fronto-central mismatch response, indicating that the premature neonates detected the rhythmic regularities. Next, we examined the cortical effective connectivity underlying the elicited mismatch response using dynamic causal modeling. We examined the connectivity between cortical sources using a set of 16 generative models that embedded alternate hypotheses about the role of the frontal cortex as well as backward fronto-temporal connection. Our results demonstrated that the processing of rhythm violations was not limited to the primary auditory areas, and as in the case of adults, encompassed a hierarchy of temporo-frontal cortical structures. The result also emphasized the importance of top-down (backward) projections from the frontal cortex in explaining the mismatch response. Our findings demonstrate a sophisticated cortical structure underlying predictive rhythm processing at the onset of the thalamocortical and cortico-cortical circuits, two months before term.

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BOARD NUMBER: S03-003

CHARACTERIZING THE FUNCTIONAL MATURATION OF THE BRAIN ACTIVITY USING RESTING-STATE EEG IN PREMATURE INFANTS

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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By interfering with the normal sequence of mechanisms serving the brain maturation, premature birth and perinatal stress can alter peri-natal experiences, with potential long-term consequences on the child development. The early characterization of brain functioning is thus of critical interest in premature infants who are at high risk of atypical outcomes. We aimed to characterize the resting-state activity in infants studied at the equivalent age of pregnancy term (n=20, gestational age at birth: 25-31weeks) and longitudinally 2-months later (n=12), using high-density electroencephalography (EEG; recording duration: 2.8-8.3mins). The temporal dynamics of brain activity were characterized by parsing it into “microstates”, defined as sequences of short-lasting scalp topographies (Michel & Koenig, NeuroImage, 2018). K-means clustering algorithm identified 7 dominant microstate templates that explained more than 50% of the signal variance across infants. While the duration of all microstates decreased between the two ages, their occurrence frequency mainly increased. Furthermore, the coverage of 4/7 microstates changed with development. These suggest both an acceleration of the temporal dynamics and an evolution in the spatiotemporal organization of brain activity with maturation. This preliminary work highlights the potential of microstates characterization to investigate resting-state functional networks in infants. This approach may provide clinically-useful quantitative measures of the developing brain activity, and help to evaluate atypical deviations related to early

insults.

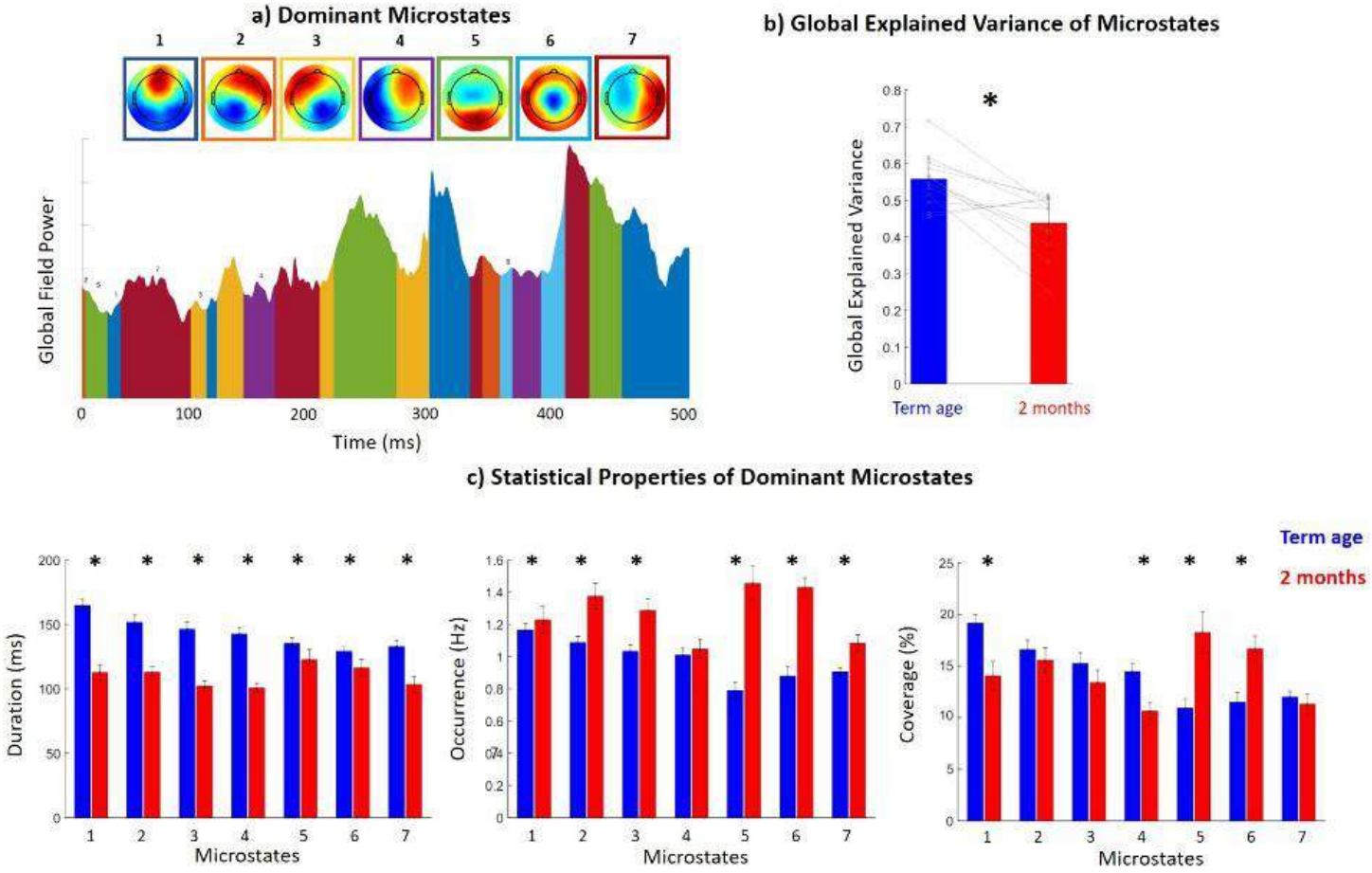

Figure. a) Continuous activity was parsed into 7 dominant microstates identified across all infants. These templates were back fitted to each infant recording and individual characteristics were measured. **b)** The microstates explained the global variance better at term-age than 2-month later (n=12). **c)** Metrics of Duration (i.e. average time a microstate lasts before changing to a new one), Occurrence (i.e. rate per second) and Coverage (i.e. proportion along recording time) were measured for each microstate and compared across ages (* paired t-test: $p<0.05$).

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BOARD NUMBER: S03-004

UNCOVERING THE FUNCTIONAL CONNECTIVITY OF THE HUMAN CORTICAL FACE NETWORK WITH CONCURRENT VISUAL FREQUENCY TAGGING AND INTRACEREBRAL ELECTRICAL STIMULATION

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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The neural basis of human face recognition has been extensively studied for decades, but the functional organization of the cortical face network remains largely unknown. Here we apply direct electrical stimulation (DES) through intracerebral electrodes (SEEG) combined with fast periodic visual stimulation (FPVS) to define the cortical face network functional connectivity. Electrodes implanted intracerebrally in drug-resistant epilepsy patients allow us to stimulate a local node of the network and record the functional activity of other implanted regions, with high spatial and temporal resolution.
We describe this original combination of techniques in one case: CJ, a right-handed 43-year-old woman with refractory focal epilepsy, excellent face recognition ability and key implantations in face-selective regions of the right lateral FG and the ventral ATL. The patient observed 60-second stimulation sequences of natural images of familiar faces alternating at a 6Hz rate, while focal stimulation (1,2mA at 55Hz) was applied to the face-selective right latFG for 10s. During stimulation, we found a reduction of significant 6Hz responses to famous faces not only locally (right latFG), but also in remote face-responsive electrodes of the right and left vATL. Interestingly, the stimulations that lead to significant electrophysiological effects, which suggest functional connectivity, were also associated with the clearest behavioural effects: during the stimulation, the patient could not recognize the famous faces displayed on the screen. This original combination of techniques appears to be effective and its application on a wide sample of individual brains could provide key information regarding the connectivity of well-defined functional brain networks.

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BOARD NUMBER: S03-005

EFFECT OF STATIC TRANSCRANIAL MAGNETIC STIMULATION OVER LEFT-DLPFC ON FATIGUE PRODUCED BY HUMAN GAIT

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Aims: We search for a role of left dorsolateral prefrontal cortex (DLPFC) on gait fatigue. For this purpose, we stimulated left DLPFC with a 0.5T magnet while participants walking. Static magnetic fields applied over the cortex are reported to reduce cortical excitability. **Methods:** 12 healthy participants (age-range [20-29 yrs]; 6 women) walked over a level-treadmill during 25 bouts of 5 min/each (15 secs rest in between), in two different sessions. Fatigue perception was recorded with a visual analogue scale (VAS, 0=not fatigue at all, 10=exhausted) every 5 min (we also recorded kinematics and muscle activity continuously). While walking, left DLPFC was stimulated with a real/sham magnet each session, sessions order counterbalanced across participants. Walking repetitions #2, 3, 24 and 25 also included the concurrent execution of a cognitive task (Stroop: color interfering or reading). Treadmill walking speed matched each participant's preferred walking speed, calculated over-ground in a previous session. **Results:** While walking at 4.6 Km/h (SE 0.1) along sessions, fatigue perception increased in a lineal manner ($p<0.001$), from ≈0 to 4.2 in the 10 points VAS. Fatigue perception and its increase along sessions did not differ for real/sham sessions ($p=0.437$ and $p=0.821$). The Stroop interference mildly modulated fatigue perception ($p=0.002$), not differently for early (#2 and 3) and late (#24 and 25) repetitions, and did not interact with the DLPFC stimulation mode either. **Conclusions:** Increasing cognitive demands and while walking has weak effects on fatigue perception.

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BOARD NUMBER: S03-006

RESPONSES TO LOCAL AND GLOBAL DEVIANCE IN SUBCORTICAL STRUCTURES

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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The ability to detect deviance in the environment is crucial in order to adapt our behavior. Deviance detection recruits the sympathetic nervous system, particularly the arousal system mainly driven by noradrenaline through the activity of the Locus Coeruleus (LC). However in humans, it is unclear whether the recruitment of this arousal system is specific or if it also relates to other systems such as dopamine, acetylcholine, and serotonin systems. Here, we performed a systematic mapping of response to deviants in subcortical structures using dedicated fMRI methods. Twenty participants performed the local-global task, an extension of a classical auditory oddball task that discriminates the bottom-up and top-down mechanisms of deviance detection. The auditory sequence exhibited two types of deviance as a lack of repetition, both locally (within a pattern of sound) and globally (across patterns). . During the task, pupil size was also recorded (indirect measure of the LC activity). We showed that the response to global deviants is very much distributed: it is found in the LC but also in many regions involving other neuromodulators, other subcortical nuclei, and cortical areas. Activity in the subject-specific LC region increased for both types of global deviants (locally standard or locally deviant patterns), like pupil size but unlike activity in an atlas-based LC region.Responses to global deviants were uncorrelated between the LC activity and pupil size. We discuss our results in terms of a hierarchy of processes involved in deviance detection. Moreover, our results validate a method to investigate neuromodulator-specific subcortical structures.

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BOARD NUMBER: S03-007

WHAT IS AROUSAL? AN AUTOMATED ANALYSIS OF A CORPUS OF 48.000 SCIENTIFIC ARTICLES

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Arousal refers to brain and body modulations within the awake state. Historically arousal has been associated with different neuromodulatory systems, and, in consequence, to different aspects of the general body and brain states. Different measurements have been used as a proxy: from heart rate and respiratory rhythm to skin conductance, or, more recently, pupil diameter. Despite the heterogeneity of measures, arousal is often conceptually seen as uni-dimensional, reflecting an "internal state". While some authors have attempted at bringing attention to the problems and limitations of the notion of arousal, the term has been and is still widely used: as of February 2022 sixty thousand scientific articles mention the term 'arousal' (Pubmed). Proper systematization of the concept would require pondering over tens of thousands of individual uses. Here, we use modern natural language processing tools which facilitate extraction of semantic meaning across very large datasets, without having to manually analyze the text. Using automatic term extraction and word embedding we recover semantic clusters within an arousal-related literature data set of ~48.000 abstracts. In contrast with the classical view of arousal, the semantic clusters seem to point to a heterogeneous, multi-dimensional concept of arousal. We further characterize the clusters by assessing the prevalence of neuromodulators and measurement-related terms in abstracts and titles of papers associated with each cluster. Finally, we attempt to relate the semantic associations with the brain using automatic fMRI meta-analytic tools.

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BOARD NUMBER: S03-008

WHAT IS THE CONTRIBUTION OF CEREBROVASCULAR REACTIVITY TO MEASURES OF HAEMODYNAMIC LAGS IN PATIENTS WITH LEFT HEMISPHERE STROKE?

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Resting-state fMRI is increasingly used to study post-stroke recovery. Recent evidence suggests that haemodynamic response (HRF) is impaired following stroke, showing temporal signal delays ('lag') which can correlate with behavioural outcomes, functional connectivity, and cerebral perfusion measures. **Aims:** To investigate the relationship between haemodynamic delays and cerebrovascular reactivity (CVR) in patients after a left hemisphere infarct. **Methods:** Voxelwise Lag maps were calculated relative to the mean grey-matter reference signal for 58 patients and 27 heathy controls, sub-acutely (~2wks) and chronically (~4m) post-stroke, using both a resting-state and a breath-holding fMRI task. The latter produced measures of CVR induced by hypercapnia. **Results:** Voxelwise cross-correlation between lag maps derived from the resting-state and breath-hold fMRI showed a moderately high spatial correlation (mean *r=0.46-0.55*) of lag between the two brain states, both at a whole brain level and within regions we have previously shown reduced CVR (lesion and perilesional tissue). Across both brain states there was a relative haemodynamic lead in primary sensorimotor cortices. Breath-hold derived lag showed no significant correlation at group level with CVR maps (mean *r=-0.03-0.06*). Lag maps were highly replicable irrespective of the choice of reference signal (whole-brain grey matter, right hemisphere, or left hemisphere). **Conclusions:** The lack of correlation between lag and CVR and the high spatial correlation of lag maps derived from the two brain states would suggest that alterations in CVR post stroke have negligible effect on the temporal lag in the HRF. We argue that the lag is predominantly driven by differential neural network dynamics.

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BOARD NUMBER: S03-009

SINGLE SESSION OF PREFRONTAL THETA BURST STIMULATION MODULATES METABOLIC ACTIVITY

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Background: Intermittent theta burst stimulation (iTBS) to the left dorsolateral prefrontal cortex (DLPFC) has emerged as a new treatment for depression. Effects of iTBS in humans however are not well understood. In this sham controlled double blind randomized cross-over study, we tested the effects of a single session of neuronavigated DLPFC iTBS on [18F]FDG uptake. **Methods**: Planned unblinding and interim analyses were carried out on 8 healthy individuals (6 females, age 27.5 ± 8.2). Subjects underwent standard 3 minute iTBS stimulation and a sham stimulation on separate days. [18F]FDG was injected immediately after both stimulations. [18F]FDG emission data was collected 40 minutes after injection for 30 minutesusing a Siemens PET/MRI scanner. Standardized uptake value ratios (SUVRs) were computed using preprocessed [18F]FDG scans. Difference between iTBS and sham in SUVRs were calculated. Effects of stimulation were analyzed using voxel-wise one sample t-tests. All tests were thresholded at voxel-wise p-values < 0.01, uncorrected. **Results**: Compared to sham stimulation, iTBS decreased [18F]FDG uptake in the subgenual anterior cingulate cortex, the anterior insula, and the left caudate. **Conclusions**: To our knowledge this is the first iTBS PET/MRI study. Our interim analysis suggests that a single session of iTBS is sufficient to modulate the metabolic activity of the core depression network. These results are in line with previous studies and if confirmed in the final sample, they will help design more personalized iTBS treatments in clinical populations.

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BOARD NUMBER: S03-010

PHENOMENOLOGY AND FUNCTIONAL SIGNIFICANCE OF THE VERTEX POTENTIAL

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Living in rapidly changing and potentially dangerous environments has shaped animal brains toward high sensitivity to sudden sensory events - often signaling threats or affordances requiring swift motor reactions. Unsurprisingly, such events elicit one of the largest electrocortical responses recordable from the scalp of several animals: the widespread Vertex Potential (VP). While often assumed to reflect sensory-specific processing, we have shown that the VP instead largely reflects *supramodal* neural activity, sensitive to the *behavioural-relevance* of the eliciting stimulus. Specifically the VP amplitude is determined by the magnitude of sensory changes, rather than their absolute intensity, and this sensitivity is conserved across sensory modalities and species. Additionally highly similar VPs are elicited regardless of the direction of this sensory change (i.e. increases or decreases of sensory intensity). All together, these results suggest that, rather than reflecting the canonical “lemniscal” sensory processing in primary sensory regions, the VP instead reflects a supramodal, widespread activation of cortex mediated by the non-specific “extralemniscal” projections from non-specific thalamic nuclei (and associated subcortical structures, such as the locus coeruleus and reticular activating system). We hypothesise that the negative-positive sequence of waves composing the VP reflects a process similar to the cortical up and down states observed during slow-wave sleep, presumably to set the cortex into a more reactive state, allowing the organism to better prepare swift motor reactions. We present novel electrophysiological data from monkeys and mice to test these ideas, using simultaneous surface and invasive recordings of cortex, and sudden sensory stimuli of several modalities.

Pubmed:
34668519: Somervail R, Bufacchi RJ, Salvatori C, Neary-Zajiczek L, Guo Y, Novembre G, Iannetti GD
Brain Responses to Surprising Stimulus Offsets: Phenomenology and Functional Significance.
Abrupt increases of sensory input (onsets) likely reflect the occurrence of novel events or objects in the environment, potentially requiring immediate behavioral responses. Accordingly, onsets elicit a transient and widespread modulation of ongoing electrocortical activity: the Vertex Potential (VP), which is likely related to the optimisation of rapid behavioral responses. In contrast, the functional significance of the brain response elicited by abrupt decreases of sensory input (offsets) is more elusive, and a detailed comparison of onset and offset VPs is lacking. In four experiments conducted on 44 humans, we observed that onset and offset VPs share several phenomenological and functional properties: they (1) have highly similar scalp topographies across time, (2) are both largely comprised of supramodal neural activity, (3) are both highly sensitive to surprise and (4) co-occur with similar modulations of ongoing motor output. These results demonstrate that the onset and offset VPs largely reflect the activity of a common supramodal brain network, likely consequent to the activation of the extralemniscal sensory system which runs in parallel with core sensory pathways. The transient activation of this system has clear implications in optimizing the behavioral responses to surprising environmental changes.
Cereb Cortex, 2022; 32
33026425: Somervail R, Zhang F, Novembre G, Bufacchi RJ, Guo Y, Crepaldi M, Hu L, Iannetti GD
Waves of Change: Brain Sensitivity to Differential, not Absolute, Stimulus Intensity is Conserved Across Humans and Rats.
Living in rapidly changing environments has shaped the mammalian brain toward high sensitivity to abrupt and intense sensory events-often signaling threats or affordances requiring swift reactions. Unsurprisingly, such events elicit a widespread electrocortical response (the vertex potential, VP), likely related to the preparation of appropriate behavioral reactions. Although the VP magnitude is largely determined by stimulus intensity, the relative contribution of the differential and absolute components of intensity remains unknown. Here, we dissociated the effects of these two components. We systematically varied the size of abrupt intensity increases embedded within continuous stimulation at different absolute

intensities, while recording brain activity in humans (with scalp electroencephalography) and rats (with epidural electrocorticography). We obtained three main results. 1) VP magnitude largely depends on differential, and not absolute, stimulus intensity. This result held true, 2) for both auditory and somatosensory stimuli, indicating that sensitivity to differential intensity is supramodal, and 3) in both humans and rats, suggesting that sensitivity to abrupt intensity differentials is phylogenetically well-conserved. Altogether, the current results show that these large electrocortical responses are most sensitive to the detection of sensory changes that more likely signal the sudden appearance of novel objects or events in the environment.
Cereb Cortex, 2021; 31

30842481: Somervail R, Bufacchi RJ, Guo Y, Kilintari M, Novembre G, Swapp D, Steed A, Iannetti GD
Movement of environmental threats modifies the relevance of the defensive eye-blink in a spatially-tuned manner.
Subcortical reflexive motor responses are under continuous cortical control to produce the most effective behaviour. For example, the excitability of brainstem circuitry subserving the defensive hand-blink reflex (HBR), a response elicited by intense somatosensory stimuli to the wrist, depends on a number of properties of the eliciting stimulus. These include face-hand proximity, which has allowed the description of an HBR response field around the face (commonly referred to as a defensive peripersonal space, DPPS), as well as stimulus movement and probability of stimulus occurrence. However, the effect of stimulus-independent movements of objects in the environment has not been explored. Here we used virtual reality to test whether and how the HBR-derived DPPS is affected by the presence and movement of threatening objects in the environment. In two experiments conducted on 40 healthy volunteers, we observed that threatening arrows flying towards the participant result in DPPS expansion, an effect directionally-tuned towards the source of the arrows. These results indicate that the excitability of brainstem circuitry subserving the HBR is continuously adjusted, taking into account the movement of environmental objects. Such adjustments fit in a framework where the relevance of defensive actions is continually evaluated, to maximise their survival value.
Sci Rep, 2019; 9

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-011

HEART RATE DECELERATION AS A BIOMARKER OF IMPLICIT PROCESSING: A DEMONSTRATION OF ITS SENSITIVITY IN A REAL-WORLD TEACHING TASK

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Heart rate deceleration (HRD) following the presentation of new stimulus is a component of the orienting response (Lacey & Lacey, 1974). HRD magnitude varies depending on the attentional requirement of the task, increasing when attention is drawn outward and diminishing when internally focused reflection is required, such as mentalizing and moral reasoning (Yang, 2013). Here we provide a proof-of-concept study of the utility of HRD as a biomarker for reflective thinking using a real-world teaching task. While grading work from a familiar student (compared to an unknown student), teachers spontaneously engage in more internally focused reflection to consider the rich history of social interactions, and therefore we predicted less HRD.19 secondary teachers (10 females, aged 28-57 years) designed and administered subject-specific assignments to their students. We collected students' answers and created matched controls, which we told teachers were from unknown students. Teachers viewed for the first time 10 students' answers and 10 controls during continuous pulse oximetry recording. Following a 2-second fixation, each answer was presented for 20 seconds, during which teachers evaluated answer quality and assigned a grade. For each trial, we calculated heart rate change relative to the pre-trial fixation, and averaged changes over a 6-second window after stimulus onset to capture HRD to the stimulus. As hypothesized, teachers showed less HRD when evaluating their own students' answers compared to equivalent answers from unknown students ($t(18)=2.08$; one-tailed $p=.03$)[CK1] . Our findings suggest that HRD is a useful biomarker to reveal differences in reflective processing during real-world social tasks.

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BOARD NUMBER: S03-012

A ROLE OF PARAHIPPOCAMPAL CORTEX IN FORWARD-LOOKING CHOICES DURING MULTI-STEP REINFORCEMENT LEARNING IN HUMANS

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Many real-life decisions involve a tension between short-term and long-term outcomes. In the reinforcement learning framework, an optimal solution to this trade off consists in computing forward-looking (or prospective) values using a model of the environment (model-based RL). Experimentally, this trade-off is often instantiated by multi-step decision-making problems, where decisions taken at an initial stage concomitantly generate an outcome and the transition to a new state. In the present study we wanted to compare the neural correlates of choices involving a forward-looking value computation. To do so we administered a new version of a two-step learning task to subjects (N=28) undergoing fMRI scanning. Analysis of the behavioral results during and after learning revealed that subjects learned both the reward values associated to each state and an explicit model of the task (i.e., state-transition probabilities). Turning to the neural data, we directly contrasted neural activity of choices in the first stage (involving a prospective component) to those in the second stage (without a prospective component) at the options onset. Results (significant after controlling for multiple comparisons) indicated that the parahippocampal cortex (BA37) was more activated in forward-looking choices. Another set of areas, including the bilateral orbitofrontal cortex (BA11) was more activated in short-term choices. Our results shed new light on the neural bases of model-based reinforcement learning by suggesting a specific role of the parahippocampal cortex in forward planning and OFC in choices involving immediate rewards.

FENS Forum 2022
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BOARD NUMBER: S03-013

CORTICAL GREY MATTER AND ITS RELATIONSHIP TO COGNITIVE PERFORMANCE AND METABOLIC PARAMETERS IN ADULTS WITH EARLY-TREATED PHENYLKETONURIA

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Background: Despite strict dietary adherence, the gold-standard treatment of phenylketonuria (PKU) seems unable to prevent damages to brain structure and function. How structural characteristics of the brain relate to cognitive outcomes in adult patients is still unknown.
Aims: We investigated cortical thickness (CT) and cortical surface area (CSA) in adults with PKU and their relationship to metabolic parameters and cognitive functions.
Methods: Twenty-three adult patients with early-treated PKU and fifty-one healthy controls were included. Surface-based morphometry was derived from T1-weighted MRI using FreeSurfer, and general intelligence, executive functions, and attention were assessed. In patients, concurrent plasma phenylalanine (Phe), tyrosine, and tryptophan levels were measured.
Results: Patients showed a thinner cortex than controls primarily in temporal, parietal, and occipital regions-of-interest (ROIs) (d=0.47 to 0.67), while group differences in CSA were more pronounced in frontal areas (d=0.47 to 0.73). Phe and tyrosine were negatively related to CT of occipital (r=-.46) and frontal ROIs (r=-.46), respectively. Conversely, tryptophan was positively related to a parietal ROI (r=.44). In patients, executive functions correlated with CT and CSA of frontal ROIs (r=-.59 to .56), and attention was associated with CT of temporal, parietal, and occipital ROIs (r=-.50 to .47). In controls, attention was correlated with CT of parietal and occipital ROIs (r=.29 to .30).
Conclusions: Our results reflect the heterogeneity of grey matter characteristics in adults with early-treated PKU and suggest that PKU has a widespread impact on cortical architecture. Such structural changes are partially related to metabolic control and cognitive performance in adults with PKU.

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BOARD NUMBER: S03-014

DOES THE VERTEX POTENTIAL REFLECT THE RAPID SUCCESSION OF CORTICAL DOWN AND UP STATES?

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Sudden sensory changes elicit a transient, large and widespread biphasic negative-positive deflection in the ongoing EEG: the Vertex Potential (VP). The VP has a number of transient behavioural effects, such as a multidirectional modulation of motor output. Here, we tested whether the VP affects already early sensory processing, which we probed by recording early somatosensory evoked potentials (SEPs). We elicited the VP using sudden auditory stimuli delivered at variable intervals (0.1-0.17 Hz), while participants were continuously receiving median nerve somatosensory stimuli at 9.5 Hz. We grouped single-trial SEPs as a function of the phase of the average VP waveform, and thereby provided a dynamic evaluation of the VP-dependent modulation of early somatosensory processing. We observed that the N20 component, which represents the arrival of the first thalamocortical volley, was unaffected by the VP phase. In contrast, the amplitude of the P45 component, which reflects later cortical processing, was reduced during the negative and enhanced during the positive VP wave. Crucially, these effects were enhanced when single-trial SEPs were grouped accounting for the trial-by-trial latency jitter of the VP (i.e. using the phase of single-trial VPs), compared to when they were aligned to the onset of the auditory stimulus. These results provide strong evidence that the VP exerts phase-dependent modulation of early lemniscal sensory processing. The biphasic nature of this modulation is reminiscent of the effects on early sensory processing of slow waves in non-REM sleep, suggesting that the negative-positive VP waves reflects a rapid succession of cortical down-state and up-state.

Pubmed:
32698726: Loconsole M, Perovic S, Regolin L
A leftward bias negatively correlated with performance is selectively displayed by domestic chicks during rule reversal (not acquisition).
In order to face a constantly changing environment, animals need to be able to update their knowledge of the world on the basis of new information. Often, this means to inhibit a previously acquired response and flexibly change their behaviour to produce a new response. Here, we measured such abilities in young domestic chicks, employing a Colour Reversal Learning Task. During the acquisition phase, 17 one-week-old male chicks had to learn to peck on one of two coloured boxes to obtain a food reward. After reaching criterion, chicks underwent a reversal phase in which the previously learned colour-reward contingency was swapped. As expected from the literature, chicks performed better in the acquisition phase with respect to the reversal phase. Results moreover highlighted the presence of a lateralized bias selectively during reversal: chicks performed better if the stimulus rewarded was located in the left hemispace (processed by the right hemisphere). Interestingly, the bias correlated with the individual difficulty, i.e., it was stronger in those chicks which needed more trials to complete the reversal session. The present study contributes evidence in support of behavioural flexibility in young chicks, along with a novel perspective on lateralized mechanisms that might underlie such ability.
Laterality, 2021 Jan-Mar; 26

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BOARD NUMBER: S03-015

LINK BETWEEN DORSOMEDIAL PREFRONTAL CORTEX AND ANTERIOR INSULA METABOLISM AND FMRI CORRELATES OF MOTIVATED BEHAVIOR

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Aims: Individuals continuously decide whether it is worth exerting effort to obtain rewards. Research on the physiological mechanisms that explain inter-individual differences in motivated behavior is essential to comprehend its neural underpinnings. A network encompassing the dorsomedial prefrontal cortex (dmPFC) and the anterior insula (aIN) plays a major role in several aspects of motivated behavior (i.e., exploration/exploitation tradeoff, saliency, uncertainty, effort, cognitive control). Here, we aim to understand how the metabolic state of the dmPFC and the aIN, in terms of concentrations of different metabolites (Glutamine, Glutamate and GABA), relates to differences in the brain activity associated to different aspects of motivated behavior and decision-making. Methods: The metabolic profile from healthy participants (25-40 years old) is acquired with proton magnetic resonance spectroscopy (^{1}H-MRS) in a 7T scanner. Immediately afterwards, participants perform two binary choice tasks while acquiring functional magnetic resonance imaging (fMRI). These tasks involve choices between options associated to different levels of monetary incentive, either to win or to lose, and of physical (squeezing a handgrip) or mental (2-back task) effort. Results: Our experiment allowed us to identify key metabolites (Glutamine/Glutamate, GABA) in the dmPFC and in the aIN relating to differences in the activity of the fMRI brain correlates of motivated behavior, in particular regarding the processing of different types of efforts, and, therefore, to differences in motivational profiles, assessed with computational modelling. Conclusion: Our results pave the way for a new approach of motivated behavior and brain imaging, by bridging baseline ^{1}H-MRS with fMRI BOLD activity.

Pubmed:
33067452: Pinho AL, Amadon A, Gauthier B, Clairis N, Knops A, Genon S, Dohmatob E, Torre JJ, Ginisty C, Becuwe-Desmidt S, Roger S, Lecomte Y, Berland V, Laurier L, Joly-Testault V, Médiouni-Cloarec G, Doublé C, Martins B, Salmon E, Piazza M, Melcher D, Pessiglione M, van Wassenhove V, Eger E, Varoquaux G, Dehaene S, Hertz-Pannier L, Thirion B
Individual Brain Charting dataset extension, second release of high-resolution fMRI data for cognitive mapping.
We present an extension of the Individual Brain Charting dataset -a high spatial-resolution, multi-task, functional Magnetic Resonance Imaging dataset, intended to support the investigation on the functional principles governing cognition in the human brain. The concomitant data acquisition from the same 12 participants, in the same environment, allows to obtain in the long run finer cognitive topographies, free from inter-subject and inter-site variability. This second release provides more data from psychological domains present in the first release, and also yields data featuring new ones. It includes tasks on e.g. mental time travel, reward, theory-of-mind, pain, numerosity, self-reference effect and speech recognition. In total, 13 tasks with 86 contrasts were added to the dataset and 63 new components were included in the cognitive description of the ensuing contrasts. As the dataset becomes larger, the collection of the corresponding topographies becomes more comprehensive, leading to better brain-atlasing frameworks. This dataset is an open-access facility; raw data and derivatives are publicly available in neuroimaging repositories.
Sci Data, 2020; 7
30844395: Pessiglione M, Clairis N
Looking into the Brain of Buridan's Ass.
To survive, animals must maximize the minimum-take care of the least satisfied among their basic needs. In this issue of Neuron, a study by Juechems et al. (2019) illustrates that this core principle might shape the way medial prefrontal regions of the human brain drive and valuate sequential choices between different types of reward.
Neuron, 2019; 101
27482097: Garrido Zinn C, Clairis N, Silva Cavalcante LE, Furini CR, de Carvalho Myskiw J, Izquierdo I
Major neurotransmitter systems in dorsal hippocampus and basolateral amygdala control social recognition memory.
Social recognition memory (SRM) is crucial for reproduction, forming social groups, and species survival. Despite its

importance, SRM is still relatively little studied. Here we examine the participation of the CA1 region of the dorsal hippocampus (CA1) and the basolateral amygdala (BLA) and that of dopaminergic, noradrenergic, and histaminergic systems in both structures in the consolidation of SRM. Male Wistar rats received intra-CA1 or intra-BLA infusions of different drugs immediately after the sample phase of a social discrimination task and 24-h later were subjected to a 5-min retention test. Animals treated with the protein synthesis inhibitor, anisomycin, into either the CA1 or BLA were unable to recognize the previously exposed juvenile (familiar) during the retention test. When infused into the CA1, the β-adrenoreceptor agonist, isoproterenol, the D1/D5 dopaminergic receptor antagonist, SCH23390, and the H2 histaminergic receptor antagonist, ranitidine, also hindered the recognition of the familiar juvenile 24-h later. The latter drug effects were more intense in the CA1 than in the BLA. When infused into the BLA, the β-adrenoreceptor antagonist, timolol, the D1/D5 dopamine receptor agonist, SKF38393, and the H2 histaminergic receptor agonist, ranitidine, also hindered recognition of the familiar juvenile 24-h later. In all cases, the impairment to recognize the familiar juvenile was abolished by the coinfusion of agonist plus antagonist. Clearly, both the CA1 and BLA, probably in that order, play major roles in the consolidation of SRM, but these roles are different in each structure vis-à-vis the involvement of the β-noradrenergic, D1/D5-dopaminergic, and H2-histaminergic receptors therein.
Proc Natl Acad Sci U S A, 2016; 113

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BOARD NUMBER: S03-016

HOW COGNITIVE CONTROL IS REPRESENTED IN THE BRAIN: AN EEG REPRESENTATIONAL SIMILARITY ANALYSIS STUDY

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Aims: Cognitive control is a fundamental human ability that allows pursuing specific relevant goals. Several theories postulate that cognitive control relies on neural representations. However, univariate approaches that have been classically used to investigate it are not suitable for studying such representations. Therefore, the aim of our EEG study was to explore how cognitive control representations are encoded at the neural level and to investigate the contribute of different theory-based representations. Methods: To this aim, we used Representational Similarity Analysis (RSA) to model theory-based representations and correlate them to the observed brain patterns.
We designed a spatial Stroop task, in which both list-wide and item-specific proportion of congruency were manipulated, respectively, to measure the effects of proactive and reactive control on Stroop interference resolution. We assessed the similarity between control-related representational models and temporal and spatial multivariate patterns of EEG activity, while controlling for low-level confounding effects. Results: RSA revealed specific spatiotemporal EEG correlates not only of low-level sensorial, motor, and cognitive representations, but also of both proactive and reactive control representations. Specifically, significant similarities were found between proactive control representational models and pre-stimulus multivariate patterns of EEG activity, as well as between reactive control representational models and post-stimulus multivariate patterns of EEG activity, in line with theoretical accounts of Stroop interference resolution. Conclusions: Our results suggest that RSA better informs cognitive control theory by revealing the dynamics of its neural representations. Indeed, temporal and spatial RSA patterns provided insights into cognitive control theory-based representations but also into low-level representations.

Pubmed:
34704157: Viviani G, De Luca F, Antonucci G, Yankouskaya A, Pecchinenda A
It is not always positive: emotional bias in young and older adults.
Healthy ageing has been associated with a bias toward positive information and greater psychological well-being. However, to what extent this positivity bias also applies to prioritizing positive information under emotional competition is unclear. Old and young adults performed a word-face interference task, in which they responded to the valence of positive and negative target-words while ignoring happy or angry distractor-faces that could be affectively congruent or incongruent. A control condition with scrambled neutral distractor-faces was also used. Findings showed small facilitation effects with faster responses when targets and distractors were affectively congruent and large interference effects with slower responses when targets and distractors were affectively incongruent compared to the control condition. Importantly, whereas for younger adults there was a similar pattern of interference from happy and angry distractor-faces, for older adults there was greater interference from angry distractor-faces. The present findings are discussed in the context of emotional bias literature. Psychol Res, 2021;
34117795: Viviani G, Vallesi A
EEG-neurofeedback and executive function enhancement in healthy adults: A systematic review.
Electroencephalographic (EEG)-neurofeedback training (NFT) is a promising technique that supports individuals in learning to modulate their brain activity to obtain cognitive and behavioral improvements. EEG-NFT is gaining increasing attention for its potential "peak performance" applications on healthy individuals. However, evidence for clear cognitive performance enhancements with healthy adults is still lacking. In particular, whether EEG-NFT represents an effective technique for enhancing healthy adults' executive functions is still controversial. Therefore, the main objective of this systematic review is to assess whether the existing EEG-NFT studies targeting executive functions have provided reliable evidence for NFT effectiveness. To this end, we conducted a qualitative analysis of the literature since the limited number of retrieved studies did not allow us meta-analytical comparisons. Moreover, a second aim was to identify optimal frequencies as NFT targets for

specifically improving executive functions. Overall, our systematic review provides promising evidence for NFT effectiveness in boosting healthy adults' executive functions. However, more rigorous NFT studies are required in order to overcome the methodological weaknesses that we encountered in our qualitative analysis.
Psychophysiology, 2021; 58

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BOARD NUMBER: S03-017

PSYCHOBIOLOGICAL APPROACH TO STRESS AND ANXIETY: A VIRTUAL REALITY STUDY UNCOVERING NEURAL AND PHYSIOLOGICAL READOUTS OF STRESS ANTICIPATION AND THEIR CAPACITY TO PREDICT PERFORMANCE

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

Catherine Bratschi, Jonathan Benhaiem, João Rodrigues, Carmen Sandi
EPFL, Life Sciences, Lausanne, Switzerland

Aim. Anticipating potential threats is essential for an organism to maximize its chances of survival. When exposed to a stressor, organisms undergo physiological, behavioral and neural responses. Repeated activations of these responses, either triggered by a threatening or a non-threatening event, can be detrimental to health. Sustaining high levels of anxiety and stress is maladaptive for the organism. Therefore, it is important to identify and characterize predictive biomarkers of individual differences in stress vulnerability. The present study aims to characterize anticipatory anxiety and stress responses based on locomotion, brain activity and physiological markers including respiration, cortisol and heart rate variability (HRV), to predict performance in completing a stressful task. **Methods**. Physiological recordings (respiration, electrocardiogram, photoplethysmogram, electrodermal activity), motion capture, salivary cortisol and EEG were collected before, during and after immersion in virtual reality scenarios, including a modified version of the Trier Social Stress Test (TSST) and two scenarios inspired by the animal literature to capture behavioral responses related to stress and anxiety. **Results**. We found that data on locomotor responses during novelty exploration reliably predicts interindividual differences in HRV triggered by exposure to a threatening VR environment. Several features from locomotor response commonly considered in rodent studies significantly contributed to our HRV predictive model and indicated a trade-off between area exploration and remaining in a safe environment. **Conclusion**. These findings pave the way for further research on biofeedback manipulation and development of phenotyping techniques to detect stress-vulnerable individuals.

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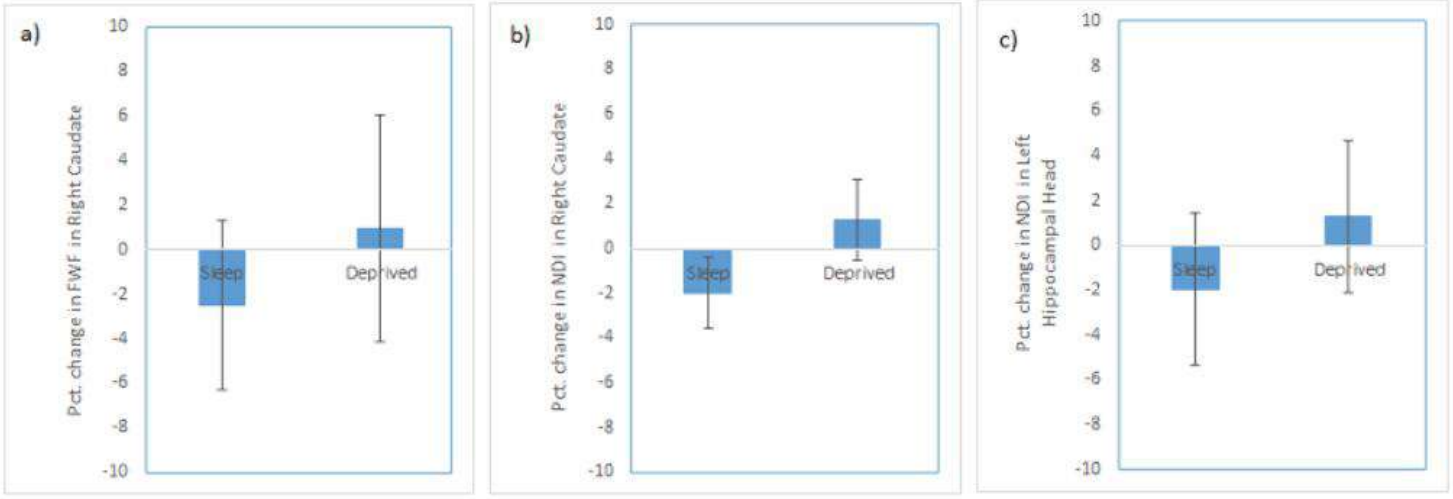
BOARD NUMBER: S03-018

SLEEP-DEPENDENT STRUCTURAL NEUROPLASTICITY AFTER A NAVIGATION TASK: A DIFFUSION WEIGHTED IMAGING (DWI) STUDY

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

Thomas Villemonteix[1,2], Michele Guerreri[3], Michele Deantoni[2,4], Evelyne Balteau[4], Christina Schmidt[4], Whitney Stee[2,4], Hui Zhang[3], Philippe Peigneux[2,4]
[1]Paris 8 University, Laboratoire Psychopathologie Et Processus De Changement, Saint-Denis, France, [2]Université Libre de Bruxelles, Ur2nf-neuropsychology And Functional Neuroimaging Research Unit Affiliated At Crcn – Centre For Research In Cognition And Neurosciences And Uni - Ulb Neuroscience Institute, Brussels, Belgium, [3]University College London, Department Of Computer Science & Centre For Medical Image Computing, London, United Kingdom, [4]University of Liège (ULiège), Giga - Cyclotron Research Centre - In Vivo Imaging, Liège, Belgium

Aims: Evidence for sleep-dependent changes in micro-structural neuroplasticity remains scarce, despite the fact that it is a mandatory corelate of the reorganization of learning-related functional networks. We investigated the effects of post-training sleep on structural neuroplasticity markers measuring standard diffusion tensor imaging mean diffusivity (MD) and the revised biophysical neurite orientation dispersion and density imaging (NODDI) free water fraction (FWF) and neurite density (NDI) parameters that enable disentangling whether MD changes result from modifications in neurites or in other cellular components (e.g., glial cells). **Methods:** 34 healthy adults (18-30y) were scanned using diffusion weighted imaging [DWI] on Day1 before and after 40-minutes route learning (navigation) in a virtual environment, then were sleep deprived (SD) or slept normally (RS) for the night. After recovery sleep for 2 nights, they were scanned again (Day4) before and after 40-minutes route learning (navigation) in an extended environment. Sleep-related microstructural changes were computed on DTI (MD) and NODDI (NDI and FWF) parameters in the cortical ribbon and subcortical regions of interest (ROIs). **Results and conclusions:** Results disclosed navigation learning-related decreased DWI parameters in the cortical ribbon (MD, FWF) and subcortical (MD, FWF, NDI) areas. Post-learning sleep-related changes were found at Day4 in the extended learning session (pre- to post-relearning percentage changes; see Figure) in the caudate nucleus (FWF and NDI: RS decrease vs. SD increase) and the hippocampus (NDI: RS decrease vs. SD increase), suggesting a sleep-related remodelling of neurites and glial cells subtending learning and memory processes in basal ganglia and hippocampal structures.



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BOARD NUMBER: S03-019

FUNCTIONAL MAPS OF THE MOUSE PREFRONTAL CORTEX DURING AUDITORY PROCESSING: HIGH-DENSITY RECORDINGS ACROSS PREFRONTAL LAYERS, SUBREGIONS, AND FUNCTIONS.

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

Pierre Le Merre[1], Sofie Ährlund-Richter[2], Daniela Calvigioni[1], Marina Slashcheva[1], Felix Jung[1], Nicolas Guyon[3], Cantin Ortiz[4], Sasa Lekic[5], Ram Yahya[1], Hyunsoo Park[1], Fredrik Wernstål[1], Konstantinos Meletis[1], Marie Carlen[1]
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The mouse prefrontal cortex (PFC) lacks a definition. Further, the function(s) of the PFC remains to be established, regardless of species. Traditionally, subregions of the PFC have been delineated based on cytoarchitectural features. Based on recent large-scale mapping of the connectivity of the mouse cortex, a prefrontal module has been identified. The cortical subregions within this module (anterior cingulate (ACA), prelimbic area (PL), infralimbic area (ILA), orbitofrontal cortex (OFC)) display particularly high interconnectivity to each other than to other cortical regions. Hodology thus gives support for the presence of a distinct prefrontal region (module) in the mouse brain. However, how hodology and cytoarchitectural features relate to function is highly unclear. We here use a series of auditory tasks and high-density (Neuropixels probes) recordings in a head-restrained mice to establish functional maps of the mouse PFC. Thousands of single units and the local field potential (LFP) activity were recorded across the layers and the proposed subregions of the PFC. The exact location of the individual electrodes was mapped after tissue clearing and reconstruction of the probe location. At first, we focused on spontaneous activity and used functional connectivity and intrinsic timescales to establish a hierarchical organization of PFC regions. We next used auditory stimuli to engage the subregions of the PFC while mice either passively listen to sounds or engage in distinct tasks. The presented data will focus on how functions segregate across the PFC during distinct auditory tasks, and how different prefrontal functions relate to traditional delineations of the PFC.

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BOARD NUMBER: S03-020

SPATIOTEMPORAL REORGANIZATION OF CORTICOSTRIATAL NETWORKS ENCODES MOTOR SKILL LEARNING

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Motor skill learning is fundamental for behavioral adaptation as it leads to automatization and refinement of common behaviors. Dorsal striatum has been described as central to motor learning with a differential global implication of the dorsomedial striatum (DMS) and the dorsolateral striatum (DLS) in the learning process. Aim: Our aim is to determine whether and how specific striatal neuronal ensembles encode the acquisition and consolidation of a motor skill. Methods and Results: Here, using ex vivo two-photon calcium imaging, we report the existence of striatal ensembles formed by highly active (HA) cells, which arise from distinct spatiotemporal reorganization in DMS and DLS networks over the course of motor learning. The overall activity of DMS decreases during the early phase, with few and sparsely distributed HA cells. In contrast, the DLS shows a progressive and long-lasting formation of HA cell clusters. In both territories, the HA cells have specific properties and are correlated with the learning performance of the mice. These two types of network reorganization arise from distinct levels of plasticity: early reinforcement of synaptic connections from cingulate cortex on DMS HA cells, followed by late and long-lasting anatomical rearrangements of somatosensory projections to the DLS. Using an AAV cFos-TRAP strategy combined with chemogenetics, we further demonstrate that silencing HA cells in DMS or DLS strongly impairs the individual performance. Conclusion: We therefore conclude that discrete domains of HA neuronal ensembles encode early acquisition in DMS and long-lasting retention of motor skill learning in DLS.

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BOARD NUMBER: S03-021

DEVELOPMENTAL CHANGES OF THE PULVINO-CORTICAL FUNCTIONAL CONNECTIVITY

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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The pulvinar is a complex posterior thalamic nucleus involved in numerous cognitive and socio-emotional functions. However, although we know that the pulvinar, and notably the dorsal part, acts as a key functional hub for these functions and that pulvino-cortical functional connectivity (PCFC) dysfunctions are reported in the context of neurodevelopmental disorder (ADHD and ASD), little is known about changes in functional pulvinar connectivity across development, how this relates to the emergence of specific higher-level cognitive and socio-emotional functions, or how factors in the early social environment may impact this development. Here, we aimed to characterize PCFC across childhood, adolescence and early adulthood, and consider the impact of early social deprivation (ESD) on this connectivity, as ESD is linked to increased levels of ADHD and “quasi-autistic” patterns of behaviour. To this aim, we utilized a macaque model of ESD to longitudinally examine changes in PCFC using resting-state fMRI. We found that connectivity became more adult-like from childhood into adulthood, with prefrontal cortex, anterior cingulate cortex, pre-supplementary motor area, and insula connectivity increasing with age, and lateral sulcus, superior temporal sulcus, intraparietal sulcus and posterior cingulate cortex connectivity decreasing. Changes were particularly marked in cortical areas associated with vocal production and social auditory perception suggesting that development of pulvino-cortical networks play a crucial role in the refinement of cognitive and socio-emotional skills during this period. Effects of early social adversity are currently being analysed.

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BOARD NUMBER: S03-022

EARLY SOCIAL ADVERSITY IN NON-HUMAN PRIMATES INTERFERES WITH THE DEVELOPMENTAL TRAJECTORY OF AMYGDALO-CORTICAL FUNCTIONAL CONNECTIVITY

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Neuroimaging of the non-human social primate brain has been instrumental to forge our current understanding of primate social cognition. However, little is known to date on how this cognitive function develops from childhood into adolescence, and how early social adversity (ESA) interferes with typical brain functional developmental trajectories. Here, we used a longitudinal neuroimaging protocol and collected anesthetized resting-state fMRI data in 21 juvenile monkeys (10 peer-reared, 11 mother-reared), at three time points (av. 2.7 years -childhood; av. 3.8 years -adolescence; av. 5.0 years -early adulthood). We focus on the amygdala, a subcortical structure which plays a key role in socioemotional and attentional processing. In the left and right amygdala, we quantified the functional connectivity between this structure and the rest of the brain. We applied a linear mixed-effects modelling approach to dissociate sources of variability associated with age from those associated with ESA. We show a progressive refinement of the amygdalo-cortical connectivity through adolescence, from a broad distributed pattern to a more adult-like restrained pattern. Independently from this age effect, we show that the peer-reared group, as compared to the mother-reared group, retains part of its functional connectivity with the medial posterior parietal cortex (precuneus), the mid-cingulate and specific premotor and prefrontal regions. We propose that altered amygdalo-cortical functional connectivity in the peer-reared group accounts for the specific differences in affect-biased attention and high emotional reactivity observed in this group.

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BOARD NUMBER: S03-023

A HEAD-FIXED ASSAY FOR LARVAL ZEBRAFISH TO STUDY BEHAVIORAL STATE CHANGES ACROSS MULTIPLE TIMESCALES

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Animals produce spontaneous patterns of behaviour that are modulated by internal states on timescales that range from seconds to hours. A challenge to studying the underlying neural mechanisms has been that these states are difficult to elicit in a controlled and reliable form in preparations amenable to large-scale physiology. We developed an assay to evoke a simple behavioural cycle repeatedly in head-fixed larval zebrafish. A photochemical, noxious stimulus results in a brief nocifensive response and a sustained period of immobility, which is followed by a stereotyped transition into rhythmic swimming behaviour. We characterised these behavioural states and assigned key temporal signatures to transitions in the bout kinematics. We see that the duration of the immobility period correlates with the time to establish rhythmic swimming. Swim bouts during the rhythmic swimming state occur more frequently and with less variation in timing and kinematics than observed during spontaneous swimming, before any presentation of aversive stimuli. While all fish showed a state of rhythmic swimming, some fish did not repeat a single stereotyped bout type but had multiple modes and would alternate between low and high amplitude forward swims. This assay was developed in an arena that was designed to be compatible with light-sheet imaging. Therefore, all behaviour was recorded, while also imaging neural activity in the brain using panneurally expressed GCaMP. Using this data, we hope not only to map neural states onto behavioural states, but also to shed light on the mechanisms by which these internal states switch over long timescales.

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BOARD NUMBER: S03-024

LEAKY INTEGRATION OF PAST NEURONAL AND BEHAVIORAL STATES BY HIPPOCAMPAL ASTROCYTES

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Recent years have seen a surge of research on astrocytes and their possible roles for computations and memory processes. However, a systematic study of natural activity patterns of astrocytes in the context of distinct behavioral, neuromodulatory, and neuronal network states is still lacking. Here, we perform simultaneous two-photon calcium imaging of astrocyte and neuronal populations in the hippocampus of head-fixed mice – together with recordings of locomotion, body movements, and pupil diameter – to investigate their behavioral, neuromodulatory and neuronal state dependence. We find that astrocytic activity in vivo, albeit exhibiting localized activation patterns, is dominated by a global mode that is largely maintained within and across hippocampal layers. This global activity pattern is highly correlated with the *current* pupil diameter. In addition, the global mode can also be well explained as a leaky integration of *past* neuronal activity and *past* body movement. The time constants of integration define a temporal sequence, with neuronal activity, motor movement and pupil changes preceding astrocytic activity in this order with consistent delays on the order of seconds. Furthermore, we find that integration of such past events in individual astrocytes occurs in a specific spatio-temporal pattern, starting in their distal processes followed by a slow propagation to the soma region. Together, our data provide evidence for a role of hippocampal astrocytes in the integration of past events, and put forward a quantitative description of the spatio-temporal integration in the context of behavioral, modulatory and neuronal signals.

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BOARD NUMBER: S03-025

INVESTIGATING HIPPOCAMPAL SYNAPTIC DEFICITS IN THE SUB-CHRONIC PHENCYCLIDINE RAT MODEL FOR SCHIZOPHRENIA

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Background: Schizophrenia is a complex neuropsychiatric disorder characterised by three main symptom domains, namely positive, negative, and cognitive deficits. Recent evidence suggests that the cognitive impairments associated with schizophrenia (CIAS) are the main determinant of functional outcomes. However, to date, there is no licenced treatment for CIAS. N-methyl-D-aspartate (NMDA) receptor antagonist rodent models are routinely used to model CIAS and provide a reliable model for investigating underlying mechanisms. **Methods:** Animals received either phencyclidine (scPCP) 2 mg/kg or vehicle bi-daily for 7 days followed by a 7 day washout period prior to behavioural testing (novel object recognition). Synaptic function in the CA1 region of the dorsal hippocampus was then assessed by acute electrophysiology under urethane anaesthesia *in vivo*. CA3-evoked field excitatory postsynaptic potential (fEPSP) in CA1 were analysed to investigate differences in synaptic connectivity and short/long-term synaptic plasticity. **Results:** The scPCP group showed expected novel object recognition deficits in behavioural testing. Our electrophysiology data shows that whilst short-term plasticity (paired-pulse facilitation) was similar between two groups, synaptic connectivity (input-output function) was significantly decreased in the scPCP group. Early data from ongoing experiments show that: (a) both groups support long-term synaptic potentiation in CA1 following high-frequency stimulation; and (b) depotentiation of fEPSP slope and amplitude after LTP induction appears similar between groups, with both reversing towards the baseline. Full results will be presented on the poster. **Conclusion:** In summary, our data indicate that the well-established cognitive deficit in the scPCP rat model is accompanied by a complex change in hippocampal synaptic networks.

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BOARD NUMBER: S03-026

SOCIAL PLAY DURING ADOLESCENCE IS CRITICAL FOR THE DEVELOPMENT OF PREFRONTAL INHIBITORY SYNAPSES IN RATS

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Experience-dependent organization of neuronal connectivity is critical during brain development. We recently demonstrated that social play, a form of social interaction that is abundant during development, is essential for fine-tuning of inhibitory synapses the prefrontal cortex (PFC) in rats. Here we asked how social play deprivation (SPD) during adolescence affects the developmental time course of two subregions of the PFC: the mPFC and OFC. Rats were social play deprived from postnatal day (PND) 21-42 by housing with a conspecific, while separated by a perforated screen. To address PFC circuit changes, spontaneous excitatory, inhibitory and miniature inhibitory postsynaptic currents (sEPSCs; sIPSCs; mIPSCs) were recorded in layer 5 pyramidal neurons in mPFC and OFC slices from young (PND 21), adolescent (PND 42) and adult (PND 85) rats. Our data reveal that the mPFC and OFC develop in a differential manner. GABAergic and glutamatergic synaptic inputs onto L5 pyramidal cells are already abundant in the OFC at P21 while these are much reduced in the mPFC. IPSC frequency in the mPFC gradually increased between P21 and P85, while IPSC frequency in the OFC remained stable between P21 and P42, after which a sharp increase was seen in adulthood. SPD did not affect glutamatergic development in either brain region, but SPD differentially affects the development of inhibitory synapses in these two brain regions. This suggests that the deprivation results in a direct reduction in mPFC inhibitory synapses, which leads to subsequent changes in the OFC.

Pubmed:
31570861: Kole K, Zhang Y, Jansen EJR, Brouns T, Bijlsma A, Calcini N, Yan X, Lantyer ADS, Celikel T
Assessing the utility of Magneto to control neuronal excitability in the somatosensory cortex.
Nat Neurosci, 2020; 23
30521020: da Silva Lantyer A, Calcini N, Bijlsma A, Kole K, Emmelkamp M, Peeters M, Scheenen WJJ, Zeldenrust F, Celikel T
A databank for intracellular electrophysiological mapping of the adult somatosensory cortex.
Neurons in the supragranular layers of the somatosensory cortex integrate sensory (bottom-up) and cognitive/perceptual (top-down) information as they orchestrate communication across cortical columns. It has been inferred, based on intracellular recordings from juvenile animals, that supragranular neurons are electrically mature by the fourth postnatal week. However, the dynamics of the neuronal integration in adulthood is largely unknown. Electrophysiological characterization of the active properties of these neurons throughout adulthood will help to address the biophysical and computational principles of the neuronal integration.
Gigascience, 2018; 7

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BOARD NUMBER: S03-027

EMERGENCE OF STRUCTURE IN HIPPOCAMPAL NETWORK DYNAMICS DURING LEARNING OF A DELAYED NON-MATCH TO SAMPLE TASK

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Recent technical advances enable the recording of large neuronal populations during behavior resulting in increasingly complex datasets. We have established the McGill-Mouse-Miniscope platform (www.m3platform.org) to combine miniscope calcium imaging with standardized touchscreen-based behavioral testing. Our mission is to curate an open-source and standardized framework for acquiring, analyzing, and accessing high-quality data of the neuronal dynamics that underly behavior and cognition throughout the brain in mice. Each experiment provides up to 1000 simultaneously recorded neurons from a single brain region over the course of ~3 months as animals learn and master the task. To highlight the feasibility of this approach, we have collected hippocampal CA1 and CA3 recordings during the delayed trial-unique nonmatching-to-location (TUNL) task. In this task, a square is presented in one of five positions on the touchscreen. A nose poke to this square starts a delay period. Following this delay, two squares are displayed, and the mouse must choose the nonmatching square to receive reward. As expected, CA1 and CA3 neurons are spatially modulated, however, they are also sensitive to other behavioral and task-related features that allow for decoding behavioral performance from neuron activity. Approximately 10% of hippocampal neurons participate in sequences that tile the delay period, which are unique to the sample square location, the fidelity of sequence predicts performance, and delay sequence cells are reliably activated during reward. We are now examining how this structure emerges in both regions during the initial learning of this task, and how network activity restructures when memory load is changed.

Pubmed:
34460812: van der Veldt S, Etter G, Mosser CA, Manseau F, Williams S
Conjunctive spatial and self-motion codes are topographically organized in the GABAergic cells of the lateral septum.
The hippocampal spatial code's relevance for downstream neuronal populations-particularly its major subcortical output the lateral septum (LS)-is still poorly understood. Here, using calcium imaging combined with unbiased analytical methods, we functionally characterized and compared the spatial tuning of LS GABAergic cells to those of dorsal CA3 and CA1 cells. We identified a significant number of LS cells that are modulated by place, speed, acceleration, and direction, as well as conjunctions of these properties, directly comparable to hippocampal CA1 and CA3 spatially modulated cells. Interestingly, Bayesian decoding of position based on LS spatial cells reflected the animal's location as accurately as decoding using the activity of hippocampal pyramidal cells. A portion of LS cells showed stable spatial codes over the course of multiple days, potentially reflecting long-term episodic memory. The distributions of cells exhibiting these properties formed gradients along the anterior-posterior and dorsal-ventral axes of the LS, directly reflecting the topographical organization of hippocampal inputs to the LS. Finally, we show using transsynaptic tracing that LS neurons receiving CA3 and CA1 excitatory input send projections to the hypothalamus and medial septum, regions that are not targeted directly by principal cells of the dorsal hippocampus. Together, our findings demonstrate that the LS accurately and robustly represents spatial, directional as well as self-motion information and is uniquely positioned to relay this information from the hippocampus to its downstream regions, thus occupying a key position within a distributed spatial memory network.
PLoS Biol, 2021; 19
32691490: Mosser CA, Haqqee Z, Nieto-Posadas A, Murai KK, Stifani S, Williams S, Brandon MP
The McGill-Mouse-Miniscope platform: A standardized approach for high-throughput imaging of neuronal dynamics during behavior.
Understanding the rules that govern neuronal dynamics throughout the brain to subserve behavior and cognition remains one of the biggest challenges in neuroscience research. Recent technical advances enable the recording of increasingly larger neuronal populations to produce increasingly more sophisticated datasets. Despite bold and important open-science and data-sharing policies, these datasets tend to include unique data acquisition methods, behaviors, and file structures.

Discrepancies between experimental protocols present key challenges in comparing data between laboratories and across different brain regions and species. Here, we discuss our recent efforts to create a standardized and high-throughput research platform to address these issues. The McGill-Mouse-Miniscope (M3) platform is an initiative to combine miniscope calcium imaging with standardized touchscreen-based animal behavioral testing. The goal is to curate an open-source and standardized framework for acquiring, analyzing, and accessing high-quality data of the neuronal dynamics that underly cognition throughout the brain in mice, marmosets, and models of disease. We end with a discussion of future developments and a call for users to adopt this standardized approach.
Genes Brain Behav, 2021; 20
32659044: Cansell C, Stobbe K, Sanchez C, Le Thuc O, Mosser CA, Ben-Fradj S, Leredde J, Lebeaupin C, Debayle D, Fleuriot L, Brau F, Devaux N, Benani A, Audinat E, Blondeau N, Nahon JL, Rovère C
Dietary fat exacerbates postprandial hypothalamic inflammation involving glial fibrillary acidic protein-positive cells and microglia in male mice.
In humans, obesity is associated with brain inflammation, glial reactivity, and immune cells infiltration. Studies in rodents have shown that glial reactivity occurs within 24 hr of high-fat diet (HFD) consumption, long before obesity development, and takes place mainly in the hypothalamus (HT), a crucial brain structure for controlling body weight. Here, we sought to characterize the postprandial HT inflammatory response to 1, 3, and 6 hr of exposure to either a standard diet (SD) or HFD. HFD exposure increased gene expression of astrocyte and microglial markers (glial fibrillary acidic protein [GFAP] and Iba1, respectively) compared to SD-treated mice and induced morphological modifications of microglial cells in HT. This remodeling was associated with higher expression of inflammatory genes and differential regulation of hypothalamic neuropeptides involved in energy balance regulation. DREADD and PLX5622 technologies, used to modulate GFAP-positive or microglial cells activity, respectively, showed that both glial cell types are involved in hypothalamic postprandial inflammation, with their own specific kinetics and reactiveness to ingested foods. Thus, recurrent exacerbated postprandial inflammation in the brain might promote obesity and needs to be characterized to address this worldwide crisis.
Glia, 2021; 69
31365857: Thion MS, Mosser CA, Férézou I, Grisel P, Baptista S, Low D, Ginhoux F, Garel S, Audinat E
Biphasic Impact of Prenatal Inflammation and Macrophage Depletion on the Wiring of Neocortical Inhibitory Circuits.
The etiology of neurodevelopmental disorders is linked to defects in parvalbumin (PV)-expressing cortical interneurons and to prenatal immune challenges. Mouse models of maternal immune activation (MIA) and microglia deficits increase the postnatal density of PV interneurons, raising the question of their functional integration. Here, we show that MIA and embryonic depletion of macrophages including microglia have a two-step impact on PV interneurons wiring onto their excitatory target neurons in the barrel cortex. In adults, both challenges reduced the inhibitory drive from PV interneurons, as reported in neurodevelopmental disorders. In juveniles, however, we found an increased density of PV neurons, an enhanced strength of unitary connections onto excitatory cells, and an aberrant horizontal inhibition with a reduced lateral propagation of sensory inputs in vivo. Our results provide a comprehensive framework for understanding the impact of prenatal immune challenges onto the developmental trajectory of inhibitory circuits that leads to pathological brain wiring.
Cell Rep, 2019; 28
28143732: Mosser CA, Baptista S, Arnoux I, Audinat E
Microglia in CNS development: Shaping the brain for the future.
Microglial cells are the resident macrophages of the central nervous system (CNS) and are mainly known for their roles in neuropathologies. However, major recent developments have revealed that these immune cells actively interact with neurons in physiological conditions and can modulate the fate and functions of synapses. Originating from myeloid precursors born in the yolk sac, microglial cells invade the CNS during early embryonic development. As a consequence they can potentially influence neuronal proliferation, migration and differentiation as well as the formation and maturation of neuronal networks, thereby contributing to the entire shaping of the CNS. We review here recent evidence indicating that microglial cells are indeed involved in crucial steps of the CNS development, including neuronal survival and apoptosis, axonal growth, migration of neurons, pruning of supernumerary synapses and functional maturation of developing synapses. We also discuss current hypotheses proposing that diverting microglial cells of their physiological functions, by promoting the expression of an immune phenotype during development, may be central to neurodevelopmental disorders such as autism, schizophrenia and epilepsy.
Prog Neurobiol, 2017 Feb - Mar; 149-150

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BOARD NUMBER: S03-028

DEEP BRAIN STIMULATION OF THE THALAMUS RESTORES SIGNATURES OF CONSCIOUSNESS IN A NONHUMAN PRIMATE MODEL

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Aims : Loss of consciousness is associated with the disruption of long-range thalamo-cortical and cortico-cortical brain communication. We tested the hypothesis that deep brain stimulation (DBS) of central thalamus might restore both arousal and awareness following consciousness loss. Methods : We modelled unconsciousness using finely tuned anesthesia in primates (Barttfeld et al., PNAS, 2015; Uhrig et al., Anesthesiology, 2018). Under propofol anesthesia, the implanted DBS leads stimulated either central thalamus or a control structure, the ventro-lateral thalamic nucleus. We monitored the effects of DBS on both behavior and brain activity using functional MRI (fMRI) and EEG. We investigated both the cortical dynamics of spontaneous resting-state activity and the brain evoked activity by the “local-global” auditory paradigm. Results : During anesthesia, central thalamic stimulation induced arousal in an on-off manner and increased fMRI activity in prefrontal, parietal and cingulate cortices. Moreover, DBS restored a broad dynamic repertoire of spontaneous resting-state activity, previously described as a signature of consciousness. None of these effects were obtained during the stimulation of the control site in the ventro-lateral thalamus. Finally, DBS restored a broad hierarchical response to auditory violations that was disrupted under anesthesia. Conclusions : DBS restored the two dimensions of consciousness, arousal and conscious access, following consciousness loss, paving the way to its therapeutical translation in patients with disorders of consciousness.

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BOARD NUMBER: S03-029

THE 6-ZONE TRACK: A NOVEL SPATIAL MEMORY TEST FOR AWAKE HEAD-FIXED CA2+ IMAGING

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Two-photon Ca^{2+} imaging is one of the state-of-the-art methods to record the activity of large neuronal populations in awake and behaving animals. Despite the emergence of miniature microscopes – which allow imaging of freely moving animals – many experimental systems still rely on head-fixation for better controlled conditions. Spatial memory tests in head-fixed configuration are still missing. Here, we introduce the novel 6-zone track, a spatial memory test that allows testing for different aspects and features of spatial memory processing in awake head-fixed mice. For this, we utilize a 360 cm long linear treadmill equipped with textures, separating the track into six reward zones with unique textual patterns and uniform transition zones. The local cues guide the mice along the track and allow them to identify zones associated with reward. The 6-zone track can be flexibly set-up in different ways – by introduction of fake reward zones or a delayed start – in order to test for working memory or different types of reference memory. Food-restricted and habituated mice were able to learn the tasks within a testing period of five days and showed significant improvements in several performance parameters. These parameters could be used to measure efficiency as well as specificity and thereby distinguish behavioral phenotypes. For these reasons we are convinced that the 6-zone track will be a an efficient tool to routinely perform spatial memory tests in head-fixed mice in combination with *in vivo* two-photon Ca^{2+} imaging.

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BOARD NUMBER: S03-030

NEUROVASCULAR DYSFUNCTION AND COGNITIVE IMPAIRMENT IN A MODEL OF HEART FAILURE WITH PRESERVED EJECTION FRACTION

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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[1]Maastricht University Medical Center, Department Of Neurology, Maastricht, Netherlands, [2]Maastricht University, Pharmacology & Toxicology, Maastricht, Netherlands, [3]Maastricht University, School For Mental Health And Neuroscience, Maastricht, Netherlands, [4]Maastricht University, School For Cardiovascular Diseases (carim), Maastricht, Netherlands, [5]University Maastricht, Pharmacology And Toxicology, Maastricht, Netherlands, [6]Maastricht University, Department Of Psychiatry And Neuropsychology, Maastricht, Netherlands, [7]School for Cardiovascular Diseases, Carim, Maastricht, Netherlands, [8]KU Leuven, Centre For Molecular And Vascular Biology, Department Of Cardiovascular Sciences, Leuven, Belgium

Background – The development of vascular cognitive impairment (VCI) and heart failure with preserved ejection fraction (HFpEF) is associated with the presence of comorbidities (obesity, diabetes, hypertension, aging). Microvascular dysfunction and subsequent rarefaction may be a key pathological step for the development of HFpEF and VCI. **Aim** - To study the impact of combined vascular risk factors on cerebral blood flow (CBF) and cognition in a rat model of comorbidities with HFpEF. **Methods** – Cognitive function of Lean (Ln, n=15) and Obese (Ob, n=14) ZSF1 rats was studied using a series of behavioural tasks. At 33-34 weeks old, CBF was measured over the barrel cortex via a thinned skull window using laser speckle contrast imaging (LSCI). Neurovascular coupling (NVC) was assessed by whisker stimulation (5Hz, 30sec). Animals were sacrificed at 34-35w for plasma and brain analyses. **Results** – Cardiac hypertrophy was observed in Ob vs Ln (heart-to-tibia: Ln=0.03; Ob=0.04g/mm; $p<0.0001$). At 31w, short-term memory was impaired in Ob vs Ln rats ($p<0.01$) in the novel object recognition task ($p_{group}<0.01$). Furthermore, spatial learning was impaired in Ob vs Ln in the Barnes maze at 22 and 32w ($p_{group}<0.05$; $p_{time\ x\ group}<0.0001$), as well as long-term memory in the probe trial ($p_{group}<0.05$). Although no difference was observed in baseline CBF, NVC was decreased in Ob vs Ln rats (Ln=23.7±1.6; Ob=18.8±1.4, $p<0.05$). **Conclusion** – Cognitive function and NVC were impaired in a HFpEF model. The development of cerebral microvascular dysfunction and rarefaction and their underlying mechanisms are being assessed in a longitudinal study.

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BOARD NUMBER: S03-031

NEURAL SUBSTRATES OF LATENT LEARNING IN THE RETROSPLENIAL CORTEX

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Latent learning occurs when associations are formed between stimuli in the absence of explicit reinforcement. It is thought to be critical for the creation of internal models of the world's structure used in decision making. But how stimulus representations are encoded in neural activity in the absence of direct reinforcement is still unclear. Silencing retrosplenial cortex (RSC) activity during the preexposure phase of a sensory preconditioning drastically impairs task performance, demonstrating the necessity of RSC for non-spatial latent learning. This makes RSC a suitable brain area to study neural activity in latent learning. Here, we set out to understand what task variables are encoded in RSC activity and how these change over the course of a latent learning task. We use head-mounted, miniature microscopes to chronically image calcium activity in GCaMP7s-expressing RSC neurons in freely moving, adult mice performing a non-spatial sensory preconditioning task. We relate neural activity from individual neurons in the superficial layers of RSC to task variables and show how stimulus representations evolve over the course of the task. Our results expand the current understanding of latent learning and its involvement in the creation of internal models required for model-based learning.

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BOARD NUMBER: S03-032

IMPACT OF COGNITIVE TRAINING IN RESTING STATE NETWORKS IN THE TGF344-AD RAT MODEL

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Introduction
TgF344-AD rat model (TG) of Alzheimer's disease shows time-dependent alterations revealing dysfunction in resting state networks (default mode, sensorimotor, somatosensorial [Tudela et al., 2019]). Our aim was to study the impact of cognitive training during 8 months on somatosensory networks and other resting state networks in wild type (WT) and TG rats. Methods: Training and delayed non-matched to sample (DMNS) test were performed in WT and TG rats starting at 3 months of age until 11 months, when MRI was acquired. rs-fMRI was acquired in a 7T scanner periodically to evaluate different resting state networks. The standard deviation of the time-series (Amplitude) of the component was computed for each subject and the differences between groups were evaluated using mixed ANOVA (genotype and treatment as between factors and time as within-group factor of variation). Differences were considered significant if $p< 0,05$. Results: Cognitive stimulation, by DNMS task performed between, 3 and 11 months had a positive effect in TgF344-AD rats compared to non-trained TG animals in resting state networks related with sensorial processing such as somatosensory S1, S2 and insular cortices (Figure1). While trained TgF344-AD rats showed a similar temporal evolution than their WT littermates, non-trained TgF344-AD animals revealed alterations in the amplitude of these networks. Conclusion: Our preliminary results point to a positive impact of early cognitive training in specific resting state networks in the TgF344-AD rat model of Alzheimer's

disease, which could prevent from the cognitive decline induced by the pathology at later time-

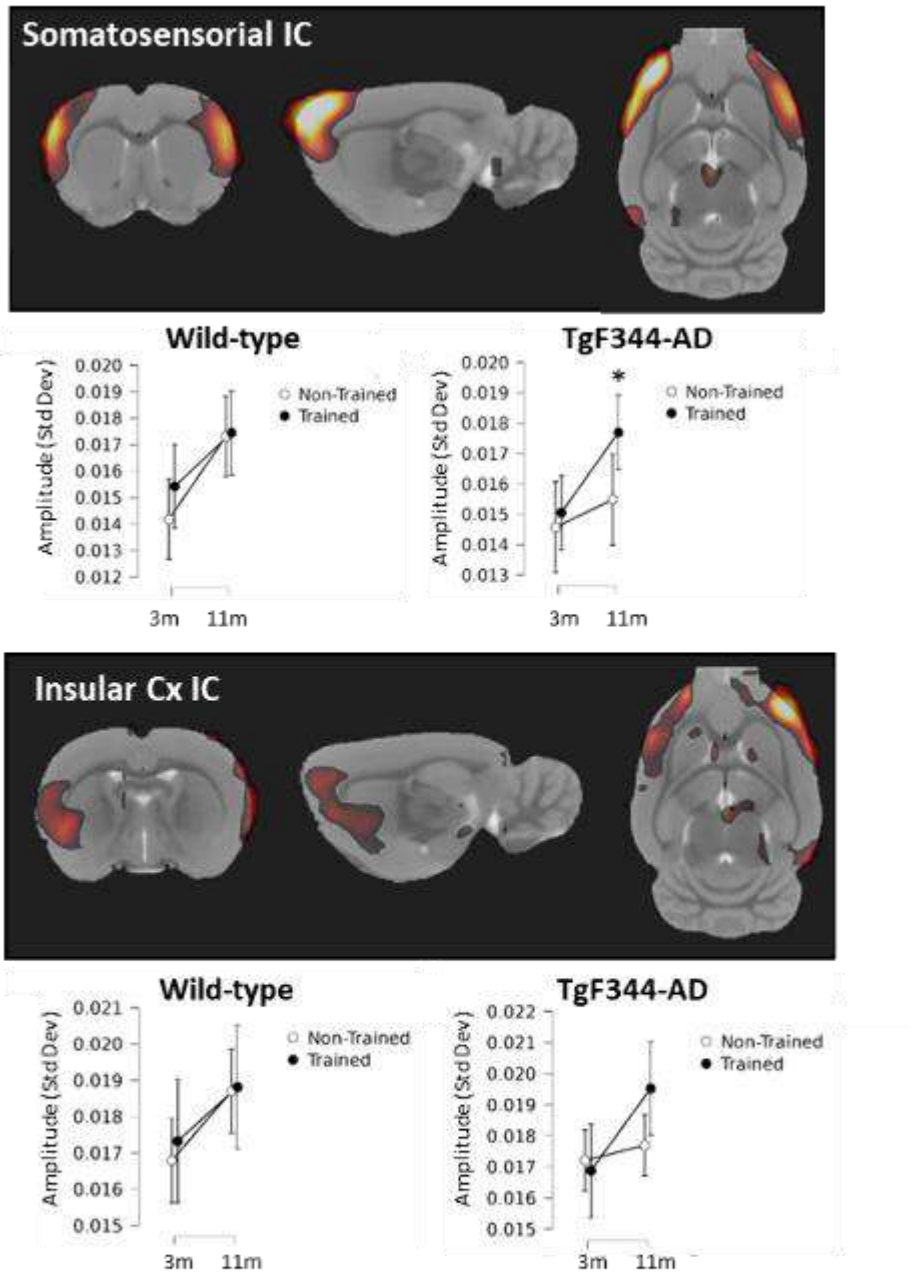

Figure 1. Somatosensorial and Insular cortices independent components (IC) and the corresponding longitudinal changes in the amplitude of the time series in trained and non-trained WT and TG animals. * Indicates significant difference $p<0,05$ vs non-trained group

points.

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BOARD NUMBER: S03-033

VENTRAL PALLIDUM ACTIVATION DIFFERENTIALLY MODULATES THE MEDIODORSAL THALAMUS AND THE LATERAL HABENULA OF RATS

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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University of Fribourg, Department Of Medicine, Fribourg, Switzerland

The Default Mode Network (DMN) is a collection of mainly cortical brain areas, the simultaneous activation of which is typically associated with mental inward focus and disengagement from environmental stimuli. Recent convergent literature suggests that the basal forebrain plays a major role in DMN regulation and more specifically, has implicated the ventral pallidum (VP) as a key brain region that triggers and maintains cortical DMN activation. Here, we investigate which efferent neural pathways mediate DMN regulation by VP. While the VP projects to a plethora of regions, particularly robust projections target the mediodorsal thalamus (MD) and the lateral habenula (LHb). Based on the apparent connectivity of MD and LHb with areas linked to the DMN, we hypothesize that VP impacts DMN activation through projections to these areas. To elucidate this, we optogenetically manipulated VP in different stimulation frequencies while recording single-unit and multi-unit activity in the MD and LHb. Towards this, we employed an AAV-hSyn1-hChR2 construct and activated the general population of neurons in the VP, which led to robust suppression in both MD and LHb. The inhibitory effect correlated positively with the stimulation frequency. Notably, while both areas were downregulated during VP activation, the prominent rebound excitation following the laser pulse and the low baseline activity of LHb resulted in an overall excitation of the latter relative to pre-stimulation baseline. Our preliminary findings indicate robust VP-induced regulation of MD and LHb and suggest therefore two candidate pathways by which VP might affect DMN activity.

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MORPHOLOGICAL ANALYSIS OF TWO SULCI IN THE VENTROLATERAL FRONTAL CORTEX OF THE CHIMPANZEE (PAN TROGLODYTES) BRAIN

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

Trisanna Sprung-Much[1], Dirk Jan Ardesch[2], Claude Lepage[1], Michael Petrides[1], William Hopkins[3]
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In the language dominant hemisphere of the human brain, the ventrolateral frontal cortex is necessary for speech production (Broca's area). Investigations of the homologous region in the brains of non-human primates are, therefore, relevant for understanding the evolution of language and related functional processes. In the present study, we examined the morphology of the fronto-orbital sulcus (*fo*) and the opercular sulcus (*op*) (Figure 1), which are characteristic features of the ventrolateral and neighbouring orbitofrontal cortex of the chimpanzee (*Pan troglodytes*) brain. Cortical surfaces were reconstructed from 77 in-vivo MRI scans (3T) from the National Chimpanzee Brain Resource, and the two sulci of interest were labelled manually to examine their morphological features and relations to each other. Morphological patterns were then categorized across hemispheres and individuals. The results demonstrate that the *fo* can be subdivided into a ventral component that remains on the orbital surface and a more dorsal component that extends onto the ventrolateral surface. Both the dorsal and ventral *fo* can exist as a single branch or can bifurcate. The *op* extends from the lateral fissure and its position varies considerably along the anterior-posterior axis. In the majority of cases, a gyral bridge separates the *fo* from the *op*, although this bridge can, occasionally, be submerged, resulting in the superficial joining of these two sulci. These results contribute to a better understanding of the structural organization of a region that, in the human brain, evolved to play a critical role in language

functions.

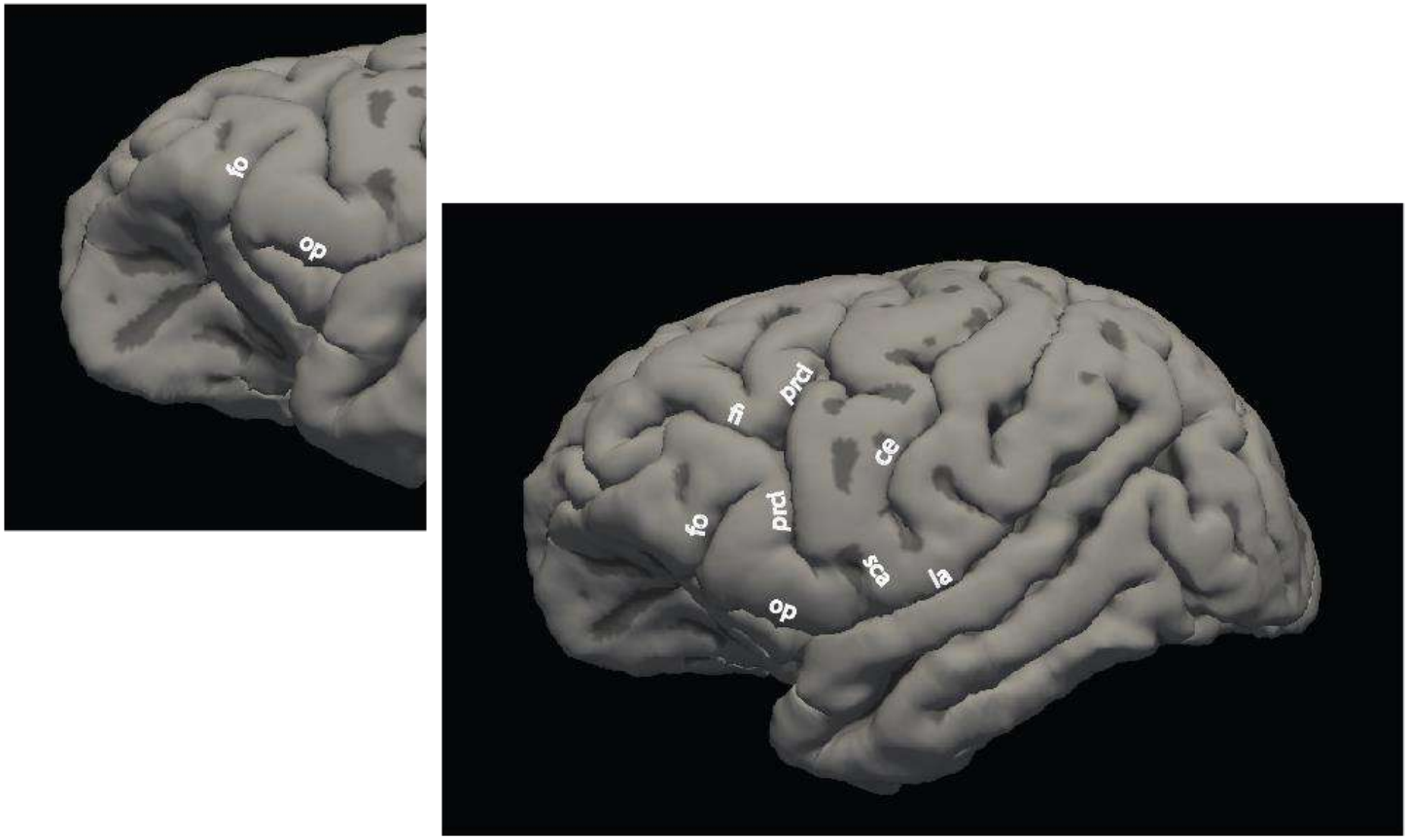

Figure 1: Surface reconstruction of the left hemisphere of a chimpanzee MRI brain scan. Top left image shows a more orbital view of the frontal lobe. ce, central sulcus; fi, inferior frontal sulcus; fo, fronto-orbital sulcus; la, lateral fissure; op, opercular sulcus; prci, inferior precentral sulcus; sca, anterior subcentral sulcus

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BOARD NUMBER: S03-035

INTERACTIONS BETWEEN THE EDINGER-WESTPHAL AND DORSAL RAPHE NUCLEI PROMOTE PARENTAL NESTING

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

Ana Marta Capaz
Paris Brain Institute, Laboratory Of Structural Plasticity, Paris, France

Preparatory nesting is an essential parental care behavioral adaption, as it anticipates the need to provide protection for the upcoming litter. Nevertheless, the neural mechanisms regulating it are largely unknown. We showed previously that the CART-expressing neurons in the Edinger-Westphal Nucleus (EWcp) are necessary for the expression of preparatory nest building behavior in pregnant female mice. However, the downstream regions that these neurons contact, as well as their inputs promoting such behavior are yet unknown. The Dorsal Raphe Nucleus (DR) is also known to be needed for the expression of nest building and maternal care behaviors. Therefore, we hypothesized that the 2 nuclei might interact to promote parental nesting. Here, we aimed to elucidate how the two nuclei interact, and whether the EWcp-DRN system might play a role in the plasticity of nesting behavior, its variability among individuals and its gender-specific expression.

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BOARD NUMBER: S03-036

REGULATION OF SOCIAL BEHAVIORS BY THE LATERAL SEPTUM

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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[1]Max Planck Institute, Metabolism Research, Köln, Germany, [2]University of Cologne, Institute Of Vegetative Physiology, Cologne, Germany, [3]University of Cologne, Institut For Vegetative Physiology, Faculty Of Medicine, Cologne, Germany

Social behaviors, conflictive or cooperative, are crucial for the survival and reproduction. Neural circuit mechanisms underlying the regulation of various social behaviors remain largely elusive. Further, little is known about how the brain computes choices when competing stimuli for mutually exclusive behaviors occur simultaneously. Aggression and feeding-related behaviors are regulated by the lateral septum (LS), which is connected with hypothalamic areas, the prefrontal cortex and hippocampus. We previously showed that somatostatin-expressing (Sst) neurons in the LS promote food-seeking (Carus-Cadavieco et al., Nature 2017). Here we investigated functions of two cell populations in the LS, Sst-, and neurotensin-expressing (NT) cells, in social interactions, from stressful to rewarding, as well as feeding-related behaviors. Combining opto-, chemogenetics and calcium imaging in behaving mice, we found differential neuronal activity in the LS selectively changing during different stages of social behaviors. Optogenetic activation of NT cells resulted in increased social interaction, accompanied by decreased dominant behavior to conspecifics. Simultaneously, activation of NT cells in the LS decreased food intake in the absence of conspecifics. Subsecond analysis of behavior using MoSeq, an unsupervised machine learning algorithm, upon chemogenetic activation of NT cells, revealed changes of multiple behavioral modules on a subsecond scale. Conversely, optogenetic activation of Sst cells in LS promoted food approach and decreased social interactions. Taken together, our results suggest that Sst- and NT-expressing populations in LS complementary regulate multiple aspects of innate behaviors. We gratefully acknowledge support by the ERC Consolidator Grant (772994, FeedHypNet, to T.K.) and DFG (SFB1089 and EXC2030-CECAD, 233886668-GRK1960).

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BOARD NUMBER: S03-037

CHEMOGENETIC MANIPULATIONS OF PARVALBUMIN INTERNEURONS AS AN ANIMAL MODEL OF SCHIZOPHRENIA: IMPLICATIONS ON BEHAVIOR AND ELECTROPHYSIOLOGY

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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In schizophrenia, the power of gamma oscillation is abnormally increased during resting states and fails to upregulate during cognitively demanding tasks. As parvalbumin interneruons (PVIs) participates in generation of gamma oscillations, their altered function could therefore participate in development of schizophrenia. Also, changes in PVIs were confirmed in post mortem studies in schizophrenia patients. In our study we aimed to explore the effect of brain-wide chemogenetic inhibition of PVIs on brain wave oscillations and on behavioral in PV-cre mice. The animals were transduced by AAV PHP.eB capsids with double floxed hM4D(Gi) DREADD receptors coupled with mCherry via the left jugular vein. In two different mice cohorts we either studied changes of gamma and other band oscillations in prefrontal cortex and hippocampus or observed changes in schizophrenia-like behavior. The electrophysiological measurements revealed higher network excitability in the dorsal hippocampus in the alpha band in contrast with activity in the prefrontal cortex where the alpha activity was reduced. In preliminary behavior data, we found group and sex differences in elevated plus maze test, open-field and prepulse inhibition task. In conclusion, the animal model of schizophhrenia based on chemogenetic inhibition of PVIs seems to be promising for further studies of various schizophrenia-like symptoms and disruptions in behavior and electrophysiology. This work was supported by Czech Science Foundation (GACR) grants 20-00939S and 21-16667K.

Pubmed:

34838932: Maleninska K, Janikova M, Radostova D, Vojtechova I, Petrasek T, Kirdajova D, Anderova M, Svoboda J, Stuchlik A
Selective deficits in attentional set-shifting in mice induced by maternal immune activation with poly(I:C).
Maternal immune activation has been identified as a significant risk factor for schizophrenia. Using rodent models, past work has demonstrated various behavioral and brain impairments in offspring after immune-activating events. We applied 5 mg/kg of poly(I:C) on gestation day 9 to pregnant mouse dams, whose offspring were then stressed during puberty. We show impairments in attentional set-shifting in a T-maze, and a decreased number of parvalbumin-positive interneurons in the hippocampus as a result of peripubertal stress specifically in females.
Behav Brain Res, 2022; 419
34265319: Malenínská K, Rudolfová V, Šulcová K, Koudelka V, Brunovský M, Horáček J, Nekovářová T
Is short-term memory capacity (7±2) really predicted by theta to gamma cycle length ratio?
Several studies suggest that EEG parameters, reflecting top-down processes in the brain, may predict cognitive performance, e.g. short-term memory (STM) capacity. According to Lisman and Idiart's model, STM capacity is predicted by theta and gamma EEG waves and their ratio. This model suggests that the more periods of gamma band waves fit into one period of theta band waves, the more information can be stored. We replicated the study by Kaminski et al. (2011), which recorded spontaneous EEG activity and measured verbal STM capacity with a modified digit span task from the Wechsler battery. Our study included more subjects and two EEG recording sessions. We discuss the possible limits of EEG correlates of STM capacity as EEG parameters were not stable across the two measurements and no correlation was found between the theta/gamma ratio and performance in the digit span task.
Behav Brain Res, 2021; 414
34788697: Maleninska K, Jandourkova P, Brozka H, Stuchlik A, Nekovarova T
Selective impairment of timing in a NMDA hypofunction animal model of psychosis.
Schizophrenia is severe neuropsychiatric disease, which is commonly accompanied not only by positive or negative symptoms, but also by cognitive impairment. To study neuronal mechanisms underlying cognitive distortions and mechanisms underlying schizophrenia, animal pharmacological models of cognitive symptoms are commonly used. Between various cognitive impairments in schizophrenia patients, disturbed time perception has often been reported. Here, we examined temporal and spatial cognition in a modified Carousel maze task in the animal model of schizophrenia induced by

non-competitive NMDA-receptor antagonists MK-801. Male Long-Evans rats (n = 18) first learned to avoid the aversive sector on a rotating arena in both dark and light intervals. We verified that during dark, rats used temporal cues, while during light they relied predominantly on spatial cues. We demonstrated that the timing strategy depends on the stable rotation speed of the arena and on the repositioning clues such as aversive stimuli. During testing (both in light and dark intervals), half of the rats received MK-801 and the control half received saline solution. We observed dose-dependent disruptions of both temporal and spatial cognition. Namely, both doses of MK-801 (0.1 and 0.12 mg/kg) significantly impaired timing strategy in the dark and increased locomotor activity. MK-801 dose 0.1 mg/kg, but not 0.12, also impaired spatial avoidance strategy in light. We found that the timing strategy is more sensitive to NMDA antagonist MK-801 than the spatial strategy. To conclude, a modified version of the Carousel maze is a useful and sensitive tool for detecting timing impairments in the MK-801 induced rodent model of schizophrenia.
Behav Brain Res, 2022; 419
33806936: Vojtechova I, Maleninska K, Kutna V, Klovrza O, Tuckova K, Petrasek T, Stuchlik A
Behavioral Alterations and Decreased Number of Parvalbumin-Positive Interneurons in Wistar Rats after Maternal Immune Activation by Lipopolysaccharide: Sex Matters.
Maternal immune activation (MIA) during pregnancy represents an important environmental factor in the etiology of schizophrenia and autism spectrum disorders (ASD). Our goal was to investigate the impacts of MIA on the brain and behavior of adolescent and adult offspring, as a rat model of these neurodevelopmental disorders. We injected bacterial lipopolysaccharide (LPS, 1 mg/kg) to pregnant Wistar dams from gestational day 7, every other day, up to delivery. Behavior of the offspring was examined in a comprehensive battery of tasks at postnatal days P45 and P90. Several brain parameters were analyzed at P28. The results showed that prenatal immune activation caused social and communication impairments in the adult offspring of both sexes; males were affected already in adolescence. MIA also caused prepulse inhibition deficit in females and increased the startle reaction in males. Anxiety and hypolocomotion were apparent in LPS-affected males and females. In the 28-day-old LPS offspring, we found enlargement of the brain and decreased numbers of parvalbumin-positive interneurons in the frontal cortex in both sexes. To conclude, our data indicate that sex of the offspring plays a crucial role in the development of the MIA-induced behavioral alterations, whereas changes in the brain apparent in young animals are sex-independent.
Int J Mol Sci, 2021; 22
27148049: Petrasek T, Skurlova M, Maleninska K, Vojtechova I, Kristofikova Z, Matuskova H, Sirova J, Vales K, Ripova D, Stuchlik A
A Rat Model of Alzheimer's Disease Based on Abeta42 and Pro-oxidative Substances Exhibits Cognitive Deficit and Alterations in Glutamatergic and Cholinergic Neurotransmitter Systems.
Alzheimer's disease (AD) is one of the most serious human, medical, and socioeconomic burdens. Here we tested the hypothesis that a rat model of AD (Samaritan; Taconic Pharmaceuticals, USA) based on the application of amyloid beta42 (Abeta42) and the pro-oxidative substances ferrous sulfate heptahydrate and L-buthionine-(S, R)-sulfoximine, will exhibit cognitive deficits and disruption of the glutamatergic and cholinergic systems in the brain. Behavioral methods included the Morris water maze (MWM; long-term memory version) and the active allothetic place avoidance (AAPA) task (acquisition and reversal), testing spatial memory and different aspects of hippocampal function. Neurochemical methods included testing of the NR1/NR2A/NR2B subunits of NMDA receptors in the frontal cortex and CHT1 transporters in the hippocampus, in both cases in the right and left hemisphere separately. Our results show that Samaritan rats(™) exhibit marked impairment in both the MWM and active place avoidance tasks, suggesting a deficit of spatial learning and memory. Moreover, Samaritan rats exhibited significant changes in NR2A expression and CHT1 activity compared to controls rats, mimicking the situation in patients with early stage AD. Taken together, our results corroborate the hypothesis that Samaritan rats are a promising model of AD in its early stages.
Front Aging Neurosci, 2016; 8
29729302: Vojtechova I, Petrasek T, Maleninska K, Brozka H, Tejkalova H, Horacek J, Stuchlik A, Vales K
Neonatal immune activation by lipopolysaccharide causes inadequate emotional responses to novel situations but no changes in anxiety or cognitive behavior in Wistar rats.
Infection during the prenatal or neonatal stages of life is considered one of the major risk factors for the development of mental diseases such as schizophrenia or autism. However, the impacts of such an immune challenge on adult behavior are still not clear. In our study, we used a model of early postnatal immune activation by the application of bacterial endotoxin lipopolysaccharide (LPS) to rat pups at a dose of 2 mg/kg from postnatal day (PD) 5 to PD 9. In adulthood, the rats were tested in a battery of tasks probing various aspects of behavior: spontaneous activity (open field test), social behavior (social interactions and female bedding exploration), anxiety (elevated plus maze), cognition (active place avoidance in Carousel) and emotional response (ultrasonic vocalization recording). Moreover, we tested sensitivity to acute challenge with MK-801, a psychotomimetic drug. Our results show that the application of LPS led to increased self-grooming in the female bedding

exploration test and inadequate emotional reactions in Carousel maze displayed by ultrasonic vocalizations. However, it did not have serious consequences on exploration, locomotion, social behavior or cognition. Furthermore, exposition to MK-801 did not trigger social or cognitive deficits in the LPS-treated rats. We conclude that the emotional domain is the most sensitive to the changes induced by neonatal immune activation in rats, including a disrupted response to novel and stressful situations in early adulthood (similar to that observed in human patients suffering from schizophrenia or autism), while other aspects of tested behavior remain unaffected.
Behav Brain Res, 2018; 349

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BOARD NUMBER: S03-038

OPTICAL INFERENCE OF FUNCTIONAL CONNECTIVITY IN THE AWAKE MOUSE CORTEX

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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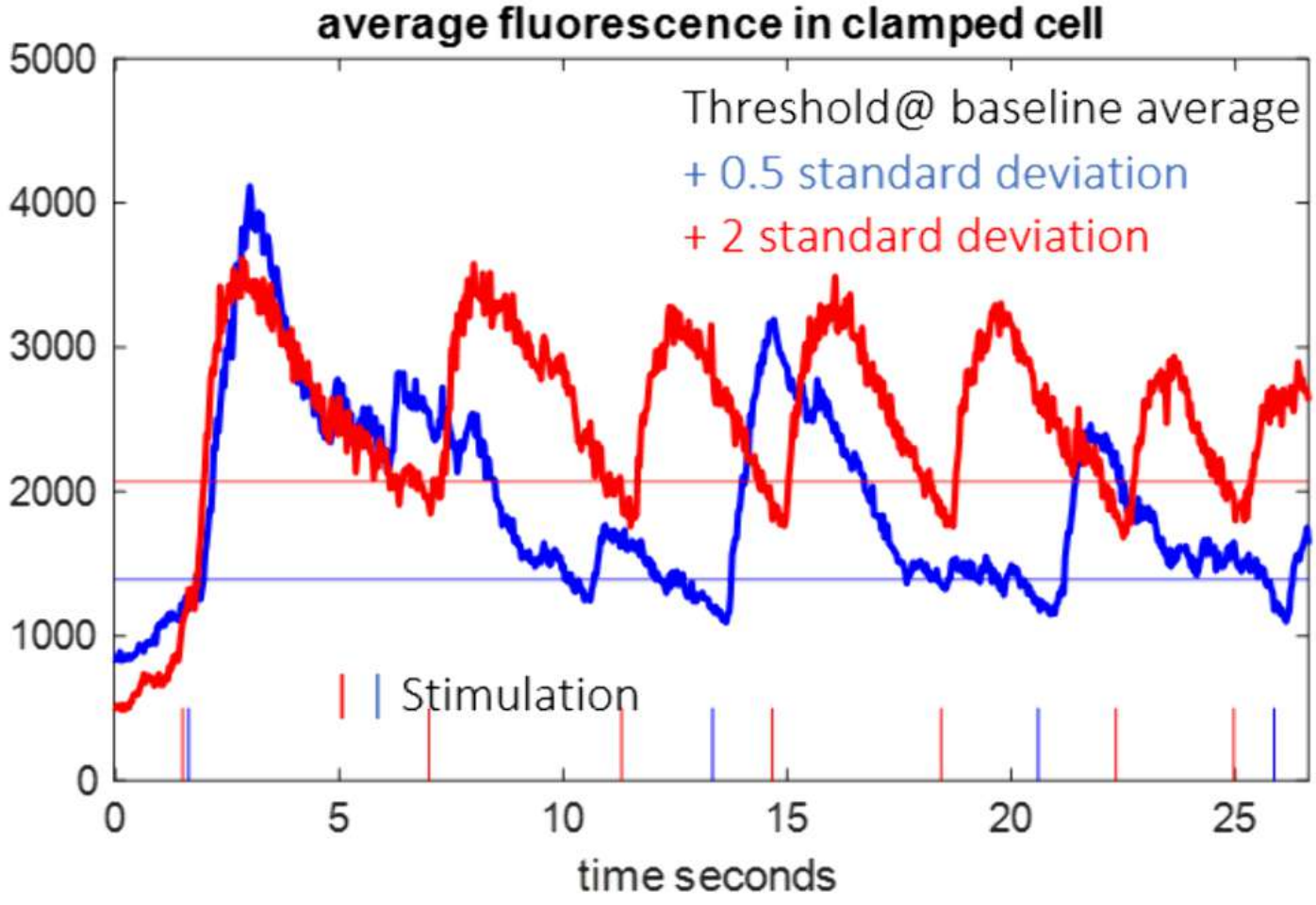
[1]University of Florida, Ece, Gainesville, United States of America, [2]University of Florida, Electrical Engineering, Gainesville, United States of America

Mapping functional connectivity among many simultaneously observed neurons is critical to understanding the neural basis of behavior. Ideally, each putative pre-synaptic cell is stimulated and the evoked membrane voltage in post-synaptic cells is measured using whole-cell patch clamp recording. While this approach is standard in vitro, its invasiveness and low-yield make it extremely challenging in vivo, particularly in awake behaving animals. We aim to develop an all-optical approach to infer connectivity between many simultaneously observed neurons in awake, behaving mice. We have previously developed an algorithm that utilizes parallel, multi neuron photostimulation, subthreshold voltage recording and compressive sensing to fully reconstruct synaptic connectivity with drastically fewer measurements. This approach, however, requires imaging/recording voltage which remains limited to few neurons. Here, we rely on measuring suprathreshold voltage dynamics (Ca^{2+} fluorescence) during optical stimulation of each presynaptic cell while stabilizing its suprathreshold output via an 'optical clamp'. Mice are injected with viral-encoded calcium indicator GCamp8 and opsins (soma-targeted Chrome or ChRMine) in the primary visual cortex and installed a cranial window and headplate. Awake mice are head-fixed and a cell expressing opsins is photo-stimulated, while the calcium signal in individual cells imaged with a two-photon system. The clamped cell is stimulated when its output falls below a threshold. We show that different clamp levels produce different spatiotemporal activation patterns in the neuronal ensemble within the field of view. Our method allows timely mapping of connectivity in a behaving animal, which would be a critical tool in decoding the neural basis of behavior.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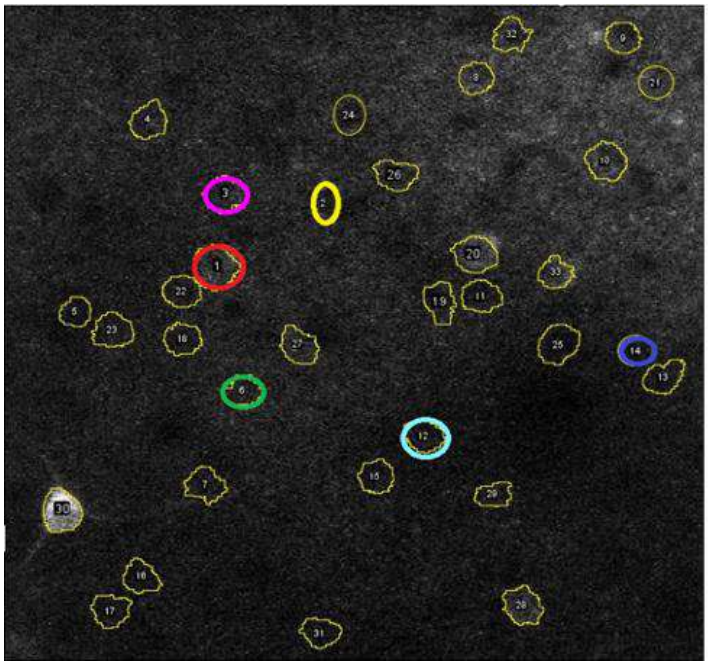

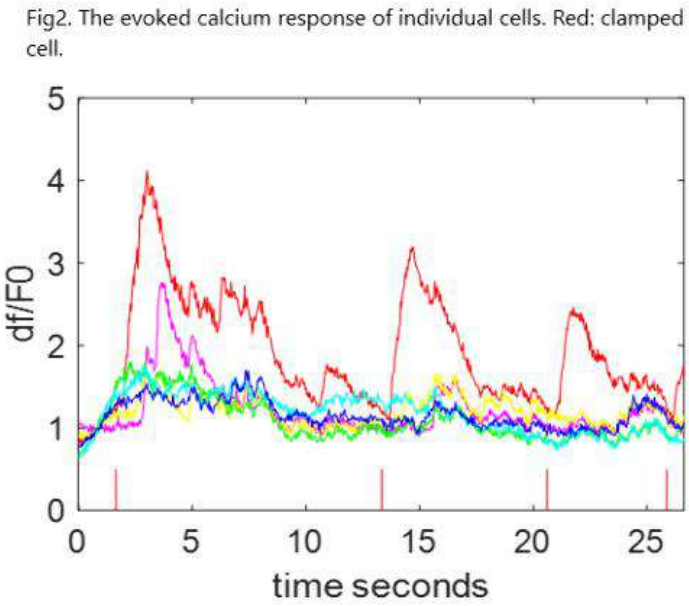

Pubmed:

33893166: Zheng HJV, Meagher JP, Xu D, Patel YA, O'Connor DH, Kwon HB
Environmental Enrichment Sharpens Sensory Acuity by Enhancing Information Coding in Barrel Cortex and Premotor Cortex.
Environmental enrichment (EE) is beneficial to sensory functions. Thus, elucidating the neural mechanism underlying improvement of sensory stimulus discrimination is important for developing therapeutic strategies. We aim to advance the understanding of such neural mechanism. We found that tactile enrichment improved tactile stimulus feature discrimination. The neural correlate of such improvement was revealed by analyzing single-cell information coding in both the primary somatosensory cortex and the premotor cortex of awake behaving animals. Our results show that EE enhances the decision-information coding capacity of cells that are tuned to adjacent whiskers, and of premotor cortical cells.
eNeuro, 2021 May-Jun; 8
31150385: Sederberg AJ, Pala A, Zheng HJV, He BJ, Stanley GB
State-aware detection of sensory stimuli in the cortex of the awake mouse.
Cortical responses to sensory inputs vary across repeated presentations of identical stimuli, but how this trial-to-trial variability impacts detection of sensory inputs is not fully understood. Using multi-channel local field potential (LFP) recordings in primary somatosensory cortex (S1) of the awake mouse, we optimized a data-driven cortical state classifier to predict single-trial sensory-evoked responses, based on features of the spontaneous, ongoing LFP recorded across cortical layers. Our findings show that, by utilizing an ongoing prediction of the sensory response generated by this state classifier, an ideal observer improves overall detection accuracy and generates robust detection of sensory inputs across various states of ongoing cortical activity in the awake brain, which could have implications for variability in the performance of detection tasks across brain states.
PLoS Comput Biol, 2019; 15
25787959: Zheng HJ, Wang Q, Stanley GB
Adaptive shaping of cortical response selectivity in the vibrissa pathway.
One embodiment of context-dependent sensory processing is bottom-up adaptation, where persistent stimuli decrease neuronal firing rate over hundreds of milliseconds. Adaptation is not, however, simply the fatigue of the sensory pathway, but shapes the information flow and selectivity to stimulus features. Adaptation enhances spatial discriminability (distinguishing stimulus location) while degrading detectability (reporting presence of the stimulus), for both the ideal observer of the cortex and awake, behaving animals. However, how the dynamics of the adaptation shape the cortical response and this detection and discrimination tradeoff is unknown, as is to what degree this phenomenon occurs on a continuum as opposed to a switching of processing modes. Using voltage-sensitive dye imaging in anesthetized rats to capture the temporal and spatial characteristics of the cortical response to tactile inputs, we showed that the suppression of the cortical response, in both magnitude and spatial spread, is continuously modulated by the increasing amount of energy in the adapting stimulus, which is nonuniquely determined by its frequency and velocity. Single-trial ideal observer analysis demonstrated a tradeoff between

detectability and spatial discriminability up to a moderate amount of adaptation, which corresponds to the frequency range in natural whisking. This was accompanied by a decrease in both detectability and discriminability with high-energy adaptation, which indicates a more complex coupling between detection and discrimination than a simple switching of modes. Taken together, the results suggest that adaptation operates on a continuum and modulates the tradeoff between detectability and discriminability that has implications for information processing in ethological contexts.
J Neurophysiol, 2015; 113

24607233: Ollerenshaw DR, Zheng HJV, Millard DC, Wang Q, Stanley GB
The adaptive trade-off between detection and discrimination in cortical representations and behavior.
It has long been posited that detectability of sensory inputs can be sacrificed in favor of improved discriminability and that sensory adaptation may mediate this trade-off. The extent to which this trade-off exists behaviorally and the complete picture of the underlying neural representations that likely subserve the phenomenon remain unclear. In the rodent vibrissa system, an ideal observer analysis of cortical activity measured using voltage-sensitive dye imaging in anesthetized animals was combined with behavioral detection and discrimination tasks, thalamic recordings from awake animals, and computational modeling to show that spatial discrimination performance was improved following adaptation, but at the expense of the ability to detect weak stimuli. Together, these results provide direct behavioral evidence for the trade-off between detectability and discriminability, that this trade-off can be modulated through bottom-up sensory adaptation, and that these effects correspond to important changes in thalamocortical coding properties.
Neuron, 2014; 81

22327024: Wang Q, Millard DC, Zheng HJ, Stanley GB
Voltage-sensitive dye imaging reveals improved topographic activation of cortex in response to manipulation of thalamic microstimulation parameters.
Voltage-sensitive dye imaging was used to quantify in vivo, network level spatiotemporal cortical activation in response to electrical microstimulation of the thalamus in the rat vibrissa pathway. Thalamic microstimulation evoked a distinctly different cortical response than natural sensory stimulation, with response to microstimulation spreading over a larger area of cortex and being topographically misaligned with the cortical column to which the stimulated thalamic region projects. Electrical stimulation with cathode-leading asymmetric waveforms reduced this topographic misalignment while simultaneously increasing the spatial specificity of the cortical activation. Systematically increasing the asymmetry of the microstimulation pulses revealed a continuum between symmetric and asymmetric stimulation that gradually reduced the topographic bias. These data strongly support the hypothesis that manipulation of the electrical stimulation waveform can be used to selectively activate specific neural elements. Specifically, our results are consistent with the prediction that cathode-leading asymmetric waveforms preferentially stimulate cell bodies over axons, while symmetric waveforms preferentially activate axons over cell bodies. The findings here provide some initial steps toward the design and optimization of microstimulation of neural circuitry, and open the door to more sophisticated engineering tools, such as nonlinear system identification techniques, to develop technologies for more effective control of activity in the nervous system.
J Neural Eng, 2012; 9

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BOARD NUMBER: S03-039

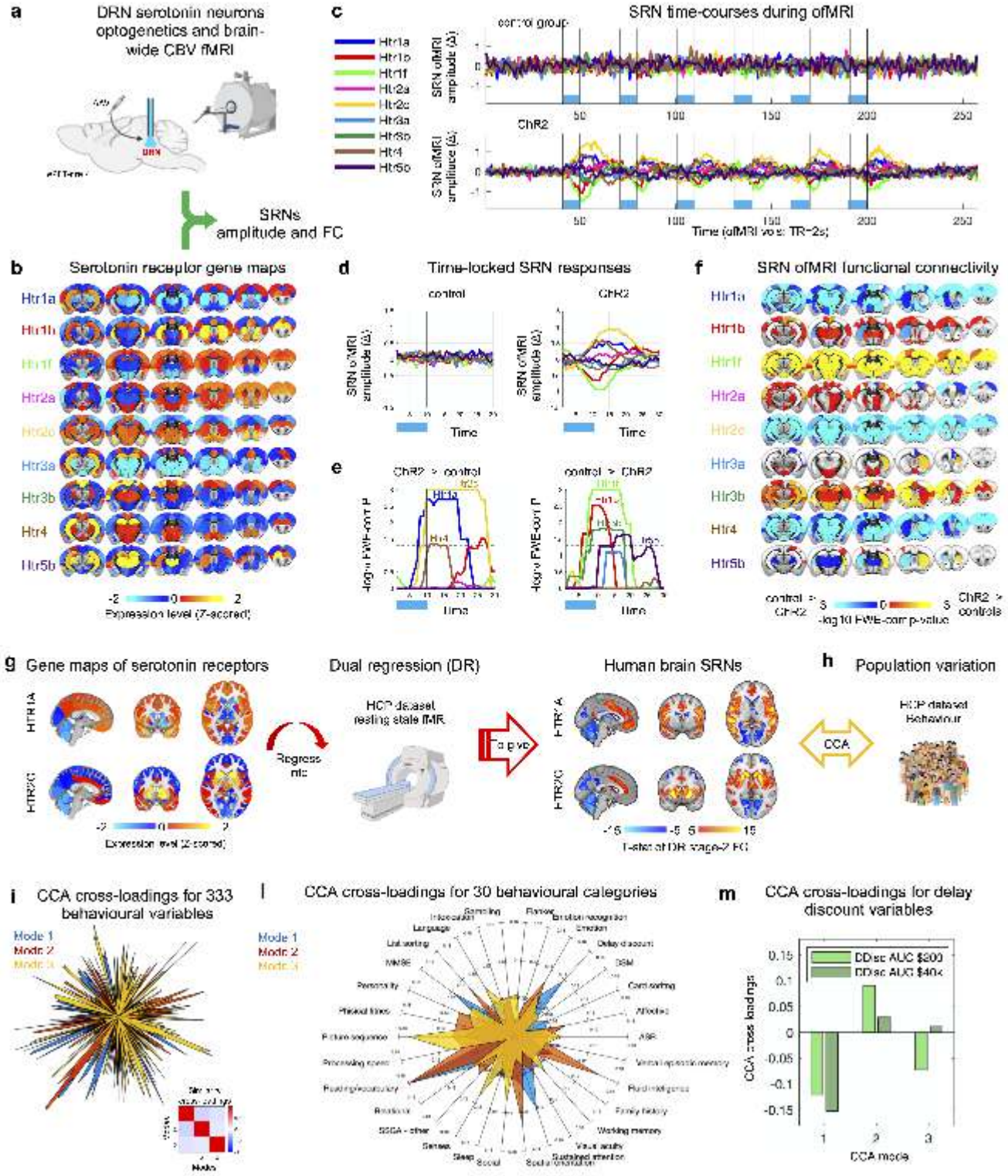
SEROTONIN REGULATION OF BEHAVIOUR VIA LARGE-SCALE NEUROMODULATION OF SEROTONIN RECEPTOR NETWORKS

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

Piergiorgio Salvan[1], Madalena Fonseca[1], Anderson Winkler[2], Antoine Beauchamp[3], Jason Lerch[1], Heidi Johansen-Berg[1]
[1]University of Oxford, Clinical Neurosciences, Oxford, United Kingdom, [2]National Institute of Mental Health, National Institutes Of Health, Bethesda, United States of America, [3]Hospital for Sick Children, Mouse Imaging Centre, Toronto, Canada

Although we understand how serotonin receptors function at the single-cell level, what role different serotonin receptors play in regulating brain-wide activity and, in turn, human behaviour, remains unknown. Here, we developed transcriptomic-neuroimaging mapping to characterise brain-wide functional signatures associated with specific serotonin receptors: serotonin receptor networks (SRNs). Probing SRNs with optogenetics-fMRI and pharmacology in mice, we show that activation of dorsal raphe serotonin neurons differentially modulates the amplitude and functional connectivity of different SRNs, showing that receptors' spatial distributions can confer specificity not only at the local, but also at the brain-wide, network-level (fig.a-f). In humans, using resting state fMRI, different sets of SRNs are linked to different behavioural phenotypes (fig.g-m). These results provide compelling evidence that heterogeneous brain-wide distributions of different serotonin receptor types may underpin behaviourally-distinct modes of serotonin regulation. This suggests that dorsal raphe

serotonin neurons may regulate multiple aspects of human behaviour via modulation of large-scale receptor



networks.

Pubmed:
35118774: Wheatley C, Wassenaar TM, Beale N, Salvan P, Dawes H, Davies E, Johansen-Berg H
The importance of prototype similarity for physical activity: Cross-sectional and longitudinal associations in a large sample of

young adolescents.
Physical activity declines during adolescence. The Theory of Planned Behaviour (TPB) is a useful framework for investigating activity but leaves variance unexplained. We explored the utility of a dual-process approach using the TPB and the Prototype Willingness Model (PWM) to investigate correlates of physical activity, and 1-year change in physical activity, among a large sample of adolescents.
Br J Health Psychol, 2022;
34742100: Lazari A, Salvan P, Cottaar M, Papp D, Jens van der Werf O, Johnstone A, Sanders ZB, Sampaio-Baptista C, Eichert N, Miyamoto K, Winkler A, Callaghan MF, Nichols TE, Stagg CJ, Rushworth MFS, Verhagen L, Johansen-Berg H
Reassessing associations between white matter and behaviour with multimodal microstructural imaging.
Several studies have established specific relationships between White Matter (WM) and behaviour. However, these studies have typically focussed on fractional anisotropy (FA), a neuroimaging metric that is sensitive to multiple tissue properties, making it difficult to identify what biological aspects of WM may drive such relationships. Here, we carry out a pre-registered assessment of WM-behaviour relationships in 50 healthy individuals across multiple behavioural and anatomical domains, and complementing FA with myelin-sensitive quantitative MR modalities (MT, R1, R2∗). Surprisingly, we only find support for predicted relationships between FA and behaviour in one of three pre-registered tests. For one behavioural domain, where we failed to detect an FA-behaviour correlation, we instead find evidence for a correlation between behaviour and R1. This hints that multimodal approaches are able to identify a wider range of WM-behaviour relationships than focusing on FA alone. To test whether a common biological substrate such as myelin underlies WM-behaviour relationships, we then ran joint multimodal analyses, combining across all MRI parameters considered. No significant multimodal signatures were found and power analyses suggested that sample sizes of 40-200 may be required to detect such joint multimodal effects, depending on the task being considered. These results demonstrate that FA-behaviour relationships from the literature can be replicated, but may not be easily generalisable across domains. Instead, multimodal microstructural imaging may be best placed to detect a wider range of WM-behaviour relationships, as different MRI modalities provide distinct biological sensitivities. Our findings highlight a broad heterogeneity in WM's relationship with behaviour, suggesting that variable biological effects may be shaping their interaction.
Cortex, 2021; 145
34731612: Salvan P, Lazari A, Vidaurre D, Mandino F, Johansen-Berg H, Grandjean J
Frequency modulation of entorhinal cortex neuronal activity drives distinct frequency-dependent states of brain-wide dynamics.
Human neuroimaging studies have shown that, during cognitive processing, the brain undergoes dynamic transitions between multiple, frequency-tuned states of activity. Although different states may emerge from distinct sources of neural activity, it remains unclear whether single-area neuronal spiking can also drive multiple dynamic states. In mice, we ask whether frequency modulation of the entorhinal cortex activity causes dynamic states to emerge and whether these states respond to distinct stimulation frequencies. Using hidden Markov modeling, we perform unsupervised detection of transient states in mouse brain-wide fMRI fluctuations induced via optogenetic frequency modulation of excitatory neurons. We unveil the existence of multiple, frequency-dependent dynamic states, invisible through standard static fMRI analyses. These states are linked to different anatomical circuits and disrupted in a frequency-dependent fashion in a transgenic model of cognitive disease directly related to entorhinal cortex dysfunction. These findings provide cross-scale insight into basic neuronal mechanisms that may underpin flexibility in brain-wide dynamics.
Cell Rep, 2021; 37
34650202: Mandino F, Vrooman RM, Foo HE, Yeow LY, Bolton TAW, Salvan P, Teoh CL, Lee CY, Beauchamp A, Luo S, Bi R, Zhang J, Lim GHT, Low N, Sallet J, Gigg J, Lerch JP, Mars RB, Olivo M, Fu Y, Grandjean J
A triple-network organization for the mouse brain.
The triple-network model of psychopathology is a framework to explain the functional and structural neuroimaging phenotypes of psychiatric and neurological disorders. It describes the interactions within and between three distributed networks: the salience, default-mode, and central executive networks. These have been associated with brain disorder traits in patients. Homologous networks have been proposed in animal models, but their integration into a triple-network organization has not yet been determined. Using resting-state datasets, we demonstrate conserved spatio-temporal properties between triple-network elements in human, macaque, and mouse. The model predictions were also shown to apply in a mouse model for depression. To validate spatial homologies, we developed a data-driven approach to convert mouse brain maps into human standard coordinates. Finally, using high-resolution viral tracers in the mouse, we refined an anatomical model for these networks and validated this using optogenetics in mice and tractography in humans. Unexpectedly, we find serotonin involvement within the salience rather than the default-mode network. Our results support the existence of a triple-network system in the mouse that shares properties with that of humans along several dimensions, including a disease condition. Finally, we demonstrate a method to humanize mouse brain networks that opens doors to fully

data-driven trans-species comparisons.
Mol Psychiatry, 2022; 27
34053250: Collett J, Fleming MK, Meester D, Al-Yahya E, Wade DT, Dennis A, Salvan P, Meaney A, Cockburn J, Dawes J, Johansen-Berg H, Dawes H
Dual-task walking and automaticity after Stroke: Insights from a secondary analysis and imaging sub-study of a randomised controlled trial.
To test the extent to which initial walking speed influences dual-task performance after walking intervention, hypothesising that slow walking speed affects automatic gait control, limiting executive resource availability.
Clin Rehabil, 2021; 35
33436528: Salvan P, Wassenaar T, Wheatley C, Beale N, Cottaar M, Papp D, Bastiani M, Fitzgibbon S, Duff E, Andersson J, Winkler AM, Douaud G, Nichols TE, Smith S, Dawes H, Johansen-Berg H
Multimodal Imaging Brain Markers in Early Adolescence Are Linked with a Physically Active Lifestyle.
The World Health Organization promotes physical exercise and a healthy lifestyle as means to improve youth development. However, relationships between physical lifestyle and human brain development are not fully understood. Here, we asked whether a human brain-physical latent mode of covariation underpins the relationship between physical activity, fitness, and physical health measures with multimodal neuroimaging markers. In 50 12-year old school pupils (26 females), we acquired multimodal whole-brain MRI, characterizing brain structure, microstructure, function, myelin content, and blood perfusion. We also acquired physical variables measuring objective fitness levels, 7 d physical activity, body mass index, heart rate, and blood pressure. Using canonical correlation analysis, we unravel a latent mode of brain-physical covariation, independent of demographics, school, or socioeconomic status. We show that MRI metrics with greater involvement in this mode also showed spatially extended patterns across the brain. Specifically, global patterns of greater gray matter perfusion, volume, cortical surface area, greater white matter extra-neurite density, and resting state networks activity covaried positively with measures reflecting a physically active phenotype (high fit, low sedentary individuals). Showing that a physically active lifestyle is linked with systems-level brain MRI metrics, these results suggest widespread associations relating to several biological processes. These results support the notion of close brain-body relationships and underline the importance of investigating modifiable lifestyle factors not only for physical health but also for brain health early in adolescence. An active lifestyle is key for healthy development. In this work, we answer the following question: How do brain neuroimaging markers relate with young adolescents' level of physical activity, fitness, and physical health? Combining advanced whole-brain multimodal MRI metrics with computational approaches, we show a robust relationship between physically active lifestyles and spatially extended, multimodal brain imaging-derived phenotypes. Suggesting a wider effect on brain neuroimaging metrics than previously thought, this work underlies the importance of studying physical lifestyle, as well as other brain-body relationships in an effort to foster brain health at this crucial stage in development.
J Neurosci, 2021; 41
33088584: Wheatley C, Wassenaar T, Salvan P, Beale N, Nichols T, Dawes H, Johansen-Berg H
Associations between fitness, physical activity and mental health in a community sample of young British adolescents: baseline data from the Fit to Study trial.
To examine relationships between fitness, physical activity and psychosocial problems among English secondary school pupils and to explore how components of physically active lifestyles are associated with mental health and well-being.
BMJ Open Sport Exerc Med, 2020; 6
28470961: Salvan P, Tournier JD, Batalle D, Falconer S, Chew A, Kennea N, Aljabar P, Dehaene-Lambertz G, Arichi T, Edwards AD, Counsell SJ
Language ability in preterm children is associated with arcuate fasciculi microstructure at term.
In the mature human brain, the arcuate fasciculus mediates verbal working memory, word learning, and sublexical speech repetition. However, its contribution to early language acquisition remains unclear. In this work, we aimed to evaluate the role of the direct segments of the arcuate fasciculi in the early acquisition of linguistic function. We imaged a cohort of 43 preterm born infants (median age at birth of 30 gestational weeks; median age at scan of 42 postmenstrual weeks) using high b value high-angular resolution diffusion-weighted neuroimaging and assessed their linguistic performance at 2 years of age. Using constrained spherical deconvolution tractography, we virtually dissected the arcuate fasciculi and measured fractional anisotropy (FA) as a metric of white matter development. We found that term equivalent FA of the left and right arcuate fasciculi was significantly associated with individual differences in linguistic and cognitive abilities in early childhood, independent of the degree of prematurity. These findings suggest that differences in arcuate fasciculi microstructure at the time of normal birth have a significant impact on language development and modulate the first stages of language learning. Hum Brain Mapp 38:3836-3847, 2017. © 2017 Wiley Periodicals, Inc.
Hum Brain Mapp, 2017; 38
24368264: Salvan P, Froudist Walsh S, Allin MP, Walshe M, Murray RM, Bhattacharyya S, McGuire PK, Williams SC, Nosarti

C

Road work on memory lane--functional and structural alterations to the learning and memory circuit in adults born very preterm.

Very preterm (VPT) birth is considered a risk factor not only for neurological impairment, but also for reduced function in several cognitive domains in childhood and later in life. Individuals who were born VPT are more likely to demonstrate learning and memory difficulties compared to term-born controls. These problems contribute to more VPT-born children repeating grades and underachieving in school. This, in turn, affects their prospects in adult life. Here we aimed to 1) study how the VPT-born adult brain functionally recruited specific areas during learning, i.e. encoding and recall across four repeated blocks of verbal stimuli, and to investigate how these patterns of activation differed from term-born subjects; and 2) probe the microstructural differences of white-matter tracts connecting these areas to other parts of the learning and memory network. To investigate these functional-structural relationships we analyzed functional and diffusion-weighted MRI. Functional-MRI and a verbal paired associate learning (VPAL) task were used to extract Blood Oxygenation Level Dependent (BOLD) activity in 21 VPT-born adults (<33 weeks of gestation) (mean age: 19.68 years ± 0.85; IQ: 99.86 ± 11.20) and 10 term-born controls (mean age: 19.87 years ± 2.04; IQ: 108.9 ± 13.18). Areas in which differences in functional activation were observed between groups were used as seed regions for tractography. Fractional anisotropy (FA) of the tract-skeleton was then compared between groups on a voxel-wise basis. Results of functional MRI analysis showed a significantly different pattern of activation between groups during encoding in right anterior cingulate-caudate body, and during retrieval in left thalamus, hippocampus and parts of left posterior parahippocampal gyrus. The number of correctly recalled word pairs did not statistically differ between individuals who were born VPT and controls. The VPT-born group was found to have reduced FA in tracts passing through the thalamic/hippocampal region that was differently activated during the recall condition, with the hippocampal fornix, inferior longitudinal fasciculus and inferior fronto-occipital fasciculus particularly affected. Young adults who were born very preterm display a strikingly different pattern of activation during the process of learning in key structures of the learning and memory network, including anterior cingulate and caudate body during encoding and thalamus/parahippocampal gyrus during cued recall. Altered activation in thalamus/parahippocampal gyrus may be explained by reduced connections between these areas and the hippocampus, which may be a direct consequence of neonatal hypoxic/ischemic injury. These results could reflect the effect of adaptive plastic processes associated with high-order cognitive functions, at least when the cognitive load remains relatively low, as ex-preterm young adults displayed unimpaired performance in completing the verbal paired associate learning task.

Neuroimage, 2014; 102 Pt 1

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BOARD NUMBER: S03-040

HOW SPATIAL EMBEDDING SHAPES FEEDBACK CORTICAL PROJECTIONS TO VISUAL CORTEX IN MACAQUE

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Projections from area V1 target higher-order cortical areas, and these ascending, feedforward pathways are reciprocated by feedback projections. Within the cortical connectome there are two functional streams: an occipital-parietal dorsal stream ensuring visuo-spatial motor information processing and an occipital-temporal stream ensuring visual object recognition. Elsewhere we have proposed that the exponential decline of connection weight with distance constitutes an important rule-based principle (EDR **E**xponential **D**istance **R**ule) of brain connectivity explaining numerous observed features of the cortex (Ercsey-Ravasz et al., Neuron 2013). Representation of the retina in V1 ensures that the far periphery lower field is located in dorsal cortex and the far periphery upper field in ventral cortex. To test the role of the EDR in shaping the functional streams we made tracer injections at different retinotopic locations and quantified the weight distribution in extrastriate cortical areas. The results show: (i) 5-8 areas project uniquely to peripheral retinotopic locations and could subserve specific functions ranging from multimodal integration to regulation of body posture; (ii) Injections in the far periphery lower-field label preferentially areas in the dorsal stream while injections in the fovea and upper-field representation label neurons preferentially in the ventral stream. Analysis of the distances between injected regions and preferentially labeling confirmed that the results obeyed the EDR. They suggest that the cortical connectome of the macaque is anchored in the visual cortex and propose that future large-scale models of the cortex embrace the ecological theory of vision (Previc, Behav Brain Sci 1990).

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BOARD NUMBER: S03-041

IN-VIVO INVESTIGATION OF CEREBELLO-PREFRONTAL CORTEX CONNECTIONS IN ANESTHETIZED MICE

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Alterations in cerebello-prefrontal cortex (mPFC) connections characterize several cognitive dysfunctions (such as schizophrenia), suggesting that the cerebellum has a crucial impact on mPFC functioning. Cerebellar activity might regulate mPFC neuronal activity through a dopaminergic pathway relayed by the ventral tegmental area (VTA), and a glutamatergic pathway relayed by the mediodorsal and ventrolateral nuclei of the thalamus. At present, the mechanisms of cerebellar regulation of mPFC are unknown. To get insight into the process of cerebellum-mPFC communication, we used single-unit recordings *in vivo* in the prelimbic area (PrL) of mPFC in anesthetized mice. Electrical stimulation of the contralateral cerebellar dentate nucleus elicited a pause in PrL neurons firing, sometimes followed by an excitation rebound. To investigate the nature of PrL responses, we co-applied D1-like and D2-like dopamine receptor antagonists (SCH23390 and sulpiride, respectively), a GABAA receptor antagonist (gabazine), and NMDA and AMPA glutamate receptors antagonists (NBQX, D-APV and 7Cl-kynurenate). The blockade of dopaminergic transmission modulated PrL neurons spontaneous firing rate without abolishing pause responses, which were suppressed by gabazine perfusion. Perfusion of glutamate receptor antagonists almost completely abolished the basal discharge of PrL neurons along with any response to dentate nucleus stimulation. Our data show that cerebellar activation transiently silences PrL activity engaging local inhibitory circuits (probably activated by thalamic projections), while dopaminergic receptors regulate PrL basal discharge. Thus, the combined effect of the two pathways tuned the PrL signal-to-noise ratio through pauses in neuronal discharge. Overall, these findings provide evidence for a complex cerebellar functional control over the PrL.

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BOARD NUMBER: S03-042

GENE-ENVIRONMENT CAUSAL PATHWAY TO IMPOVERISHED COGNITIVE DEVELOPMENT CONTRIBUTES TO PSYCHOTIC-LIKE EXPERIENCE IN CHILDREN

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Identifying the social and biological mechanisms of cognitive and psychological development of children is essential for optimizing preventive and educational efforts. However, the causal pathways by which genetic and environmental factors affect cognitive and psychiatric outcomes remain unknown, especially in early childhood. We examined the causal relationships among genes, the environment, intelligence, and psychotic-like experiences in 7,632 multiethnic (5,905 with European ancestry) children aged 9-10 years old from the Adolescent Brain Cognitive Development (ABCD) Study. Using up-to-date computational causal analysis and robust path modeling, we found a significant causal influence of residential, family, and school environments and genome-wide polygenic scores of cognitive capacities on preadolescents' psychotic-like experiences mediated by intelligence. Mitigation of good parenting behavior and positive school environments on psychotic-like experiences dominated the pernicious effects of genetic and residential adversities. Our findings support that intelligence may be a biological resilience factor for psychosis. To the best of our knowledge, this is the first study to identify casual trajectories of neurocognitive development in early childhood and the first to provide empirical evidence that positive parenting behavior and school environment can impose a considerable degree of causal impact on children's cognitive and psychiatric outcomes. We suggest the implementation of socioeconomic policies to improve family and school environments and promote local economic development to enhance children's cognitive ability and mental health.

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BOARD NUMBER: S03-043

MACHINE LEARNING TO PERSONALIZE COGNITIVE TRAINING

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Introduction Executive functions are a class of processes critical for purposeful goal-directed behavior. Cognitive training is the adequate stimulation of executive functions and has been extensively studied and applied. However, there is still a lack of solid consensus about its potential to elicit consistent improvements in untrained domains. Individual differences are considered one of the most important factors of inconsistent reports on cognitive training benefits, as differences in cognitive functioning are both genetic and context-dependent, and might be affected by age and socioeconomic status. **Aims and Methods** We aimed to predict if a child would benefit or not from a certain training, based on some of his/her baseline individual qualities. We explored supervised machine learning techniques to predict improvements, using baseline performance in previous cognitive tasks as features to every model. We used a dataset from an investigation in which 73 6-year-olds tested and challenged their executive functions using an online software with a fixed protocol. **Results and conclusions** We trained multiple algorithms, and the best one in the task was a support vector classifier that successfully predicted (average accuracy = .67, AUC=.707) improvements after the cognitive stimulation, using baseline individual differences as features. We also performed permutation feature importance analysis that suggested that features contribute equally to the model's performance. Our study is meant as a proof of concept showing that Machine Learning tools can be implemented to personalize cognitive training interventions. In the long term, these algorithms might allow us to personalize training protocols to maximize the stimulation.

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BOARD NUMBER: S03-044

EVIDENCE FOR FUNCTIONAL CONNECTIVITY CHANGES IN THE AMYGDALA ACROSS THE MENSTRUAL CYCLE - A RESTING-STATE FMRI STUDY.

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Over the last decade, neuroimaging research has pointed out changes in brain structure and function over the menstrual cycle, particularly in emotion-processing centers such as the amygdala. However, results are sparse, and the amygdala modulation during the menstrual cycle is still poorly understood. In the present study, we used resting-state fMRI (rs-fMRI) to identify menstrual cycle-related connectivity changes in the amygdala-based network. We scanned 126 naturally cycling women either in their early follicular (N=42; days 1-7), late follicular (N=51; days 8-14), or mid-luteal phase (N=33; days 17-26) and performed a separate seed-based connectivity analysis for each amygdala. We found that functional connectivity between the right amygdala and the left lateral prefrontal cortex (LFPC) was more negative during the late follicular and mid-luteal phases compared to the early follicular phase. These results are aligned with research that points to a negative correlation between the amygdala and the LPFC during cognitive reappraisal tasks. Thus, our findings suggest that phase-dependent differences in functional connectivity between these two regions could be related to shifts in cognitive reappraisal mechanisms throughout the menstrual cycle. The present research contributes to understanding the impact of the menstrual cycle on affective and cognitive processes in menstruating people.

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BOARD NUMBER: S03-045

STRUCTURES IN SPACE AND TIME – HIERARCHICAL NETWORK DYNAMICS IN THE AMYGDALA

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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In addition to its role in the learning and expression of conditioned behavior, the amygdala has long been implicated in the regulation of persistent states, such as anxiety and drive. Yet, it is not evident what features of the neuronal activity capture the functional role of the network across such different timescales, specifically when behavior and neuronal spaces are complex and high-dimensional. We applied a dynamical approach for the analysis of calcium imaging data from the basolateral amygdala, collected while mice performed complex, self-paced behavior, including goal directed actions. The seemingly complex network dynamics could be effectively described by a hierarchical, modular structure, with robust correspondence to behavior on multiple timescales. We describe the remapping of the network dynamics in response to perturbations along different dimensions and the interplay between slow, state-like representation and the fast processing of specific events and actions. The emergent spatio-temporal symmetries between nested dynamics, provide specific hypotheses regarding the principles and mechanisms that support the updating and maintenance of abstract task representations. We suggest hierarchical dynamical models offer a unified framework to capture amygdala involvement in such different functions as associative learning, action selection and emotional processing.

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BOARD NUMBER: S03-046

DYNAMIC FUNCTIONAL CONNECTIVITY ASSOCIATED WITH PROSPECTIVE MEMORY SUCCESS IN CHILDREN

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Prospective memory (PM) requires multiple process including memory and higher cognitive function. To remember the intention successfully, back-and-forth between background task and intention or dynamics of process can be critical. Executive function is associated with PM success in children but neural mechanism of PM in children has not been investigated. The purpose of this study in revealing dynamic functional connectivity underlying PM success in children. Healthy 108 children aged 7 to 15 were participated. This study was approved by the local ethics committee, and written informed consent was obtained from the parents of each participant based on the Declaration of Helsinki. PM task was a single trial with 30 min delay that children were given a post-it, then they were asked to return it to the experimenter when they come back to the initial room. Background task was watching animation in the room next-door to answer questions. The resting-state fMRI scan was done before or after PM task. About 70% of children successfully remembered the intention. No significant success/failure group differences in age, male/female ratio, and background task performance. Dynamics in connectivity between the right dorsolateral prefrontal cortex (DLPFC) and intraparietal sulcus, and between the right DLPFC and insula showed significant differences between children with PM success and failure. Attention and monitoring for background task in right fronto-parietal network and internal-state monitoring for prospective intention in insula network and those dynamics might be associated with PM success in children.

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BOARD NUMBER: S03-047

PHOTOBIOMODULATION DOES NOT LEAD TO VISIBLE EFFECTS ON MALE NOR FEMALE RAT BRAIN DEVELOPMENT

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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The role of light in our biological processes and systems is extensively known and the use of light devices has been introduced in the health field as an opportunity to administer light. Photobiomodulation (PBM) is a non-invasive technique that uses red to infrared light to stimulate, heal, regenerate and protect tissue. Numerous studies have documented several brain positive effects in adult subjects (improve metabolic function, anti-apoptotic proteins upregulation, stimulation of neurogenesis, synaptogenesis, migration, secretion of neurotrophins…), due to the light absorption by the cytochrome c-oxidase (CCO), terminal enzyme in the mitochondrial electron transport chain. This study aims to examine the effects of PBM in the developing brain (prefrontal cortex, striatum, thalamus, amygdala, hippocampus) of healthy 23 days-old male (n=31) and female (n=30) Wistar rats. Three groups of each sex were used: photobiomodulation group received five days of light therapy, a device group submitted to the same conditions but without light radiation and a control basal group. Brain metabolic activity and immediate early genes activation were analysed by CCO histochemistry and c-Fos immunostaining, respectively. Results displayed no metabolic differences between the three groups in both sexes. Same results were found in the analysis of c-Fos positive cells, reporting no differences between groups. This research, in contrast to the PBM consequences reported in adult healthy subjects, showed the lack of PBM effects in young healthy rat brain. Further studies should be necessary to deeply examine the effects of PBM in the developing brain. Funding: MINECO PID2020-117259RB-I00, FICYT AYUD/2021/51378 and MCIU-19-PRE2018-086220 to A.G.M

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BOARD NUMBER: S03-048

BRAIN-HEART INTERACTIONS DURING MOTOR RESPONSES IN REACTIVE AND PROACTIVE CONTEXTS

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Aims The brain and the heart continuously interact to optimize adaptive responses to the environment, including motor preparation, stop/go signal processing and motor execution. However, such interactions have not been systematically studied so far. Here we investigated Heartbeat Evoked Potential (HEP) modulations during different phases of motor responses and their relationships with co-occurring motor event-related potentials: the Bereitschaftspotential (BP) occurring during motor preparation, and the prefrontal Positive1 (pP1) associated to sensorimotor processing of go signals. We hypothesized that brain-heart interactions might adapt to the context, that could be either reactive (inhibitory) or proactive. ***Methods*** We used electroencephalography to measure HEP, BP and pP1 components. Participants performed two Go/No-Go tasks in which the different percentages of go and no-go trials created either a proactive or a reactive context. ***Results*** Results showed that HEP increases the closer we get to the motor responses. Moreover, HEP differently modulates according to the context. Specifically, the modulation is stronger in the reactive (inhibitory) than in the proactive context. Lastly, only in the proactive context, HEPs falling during motor preparation and sensorimotor processing of go signals correlate with the co-occurring BP and pP1 components, respectively. ***Conclusion*** Our findings are the first to empirically demonstrate context-dependent modulations of HEPs directly linked to different phases of motor responses.

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BOARD NUMBER: S03-049

MIRROR INVARIANCE FOR OBJECTS AND BRAILLE LETTERS IN CONGENITALLY BLIND PEOPLE; A BEHAVIORAL AND FMRI STUDY.

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Mirror images of objects are recognized as the same object, but letters are not (“d” is not “b”). Unlearning this mirror invariance for letters is achieved when learning to read. Its neuronal correlate can be observed in sighted individuals for objects, words, and letters (Pegado et al., 2011). Previous studies have shown that the fusiform cortex is important formirror discrimination. De Heering and colleagues (2018) showed that the congenitally blind humans can break mirror invariance for Braille letters like the sighted with the visual alphabet. Here, we investigated which neural mechanisms underlie this process. Nineteen congenitally blind adults participated in behavioural and fMRI experiments. First, they performed two behavioural same-different tactile comparisons tasks. Stimuli were pairs of Braille letters, geometric shapes, and everyday objects (e.g., toothbrush) wich were presented in same (“p” and “p”), mirror orientation (“p” and “q”) and different (“p” and “z”) pairs. In the fMRI part, Braille letters and everyday objects in the above-mentioned formats were presented in a priming paradigm. Participants had shorter response-times and higher accuracy for Braille letters and objects than for shapes. fMRI results showed mirror priming for objects in the fusiform cortex. Moreover, left lingual gyrus seems to distinguish between left-right orientation of Braille letters. Our results demonstrate that the fusiform cortex of blind individuals exhibits mirror invariance for tactile objects similar to the one observed for visual objects in the sighted. Moreover, language area (lingual) is involved in Braille letters’ mirror discrimination.

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BOARD NUMBER: S03-050

IMPLICIT READING IN TACTILE DOMAIN - A LONGITUDINAL FMRI STUDY OF SIGHTED PARTICIPANTS LEARNING BRAILLE ALPHABET

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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In skilled readers, reading occurs implicitly, even without explicit instruction to read. After intensive training, sighted participants can also read in the tactile domain, though it is not clear whether such reading could be implicit. In the current study, 18 Braille-naïve sighted participants took part in an extensive 7 month-long tactile Braille reading course. 19 age and gender-matched participants were recruited as a passive control group. We employed longitudinal functional magnetic resonance imaging with 5 time-points (TP) and a non-linguistic target detection task for both groups. We tested for a group-by-time interaction to answer the questions about if and when participants start to process Braille implicitly. After the course, participants in the experimental group could read by touch on average up to 17 letters and nearly 7 words per minute. At the neuronal level, we found significant interaction in the lingual gyrus, the superior parietal lobule and the lateral occipital cortex. Additional analyses showed that the neuronal responses to implicit reading were stronger in the experimental group at the last TP when participants were most proficient in Braille reading. Since the above-mentioned brain regions are crucial to letter recognition and spatial processing, our results suggest that learning to read the Braille alphabet in sighted participants could lead to implicit processing in the tactile domain. Nevertheless, this phenomenon is strongly modulated by reading proficiency.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-051

INCREASING CORTICO-SUBCORTICAL CONNECTIVITY PREDICTS A BURSTING EVENT DURING SEVOFLURANE-INDUCED BURST SUPPRESSION STATE IN HUMANS

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Burst suppression is a global electroencephalogram (EEG) pattern characterized by an alternating high-voltage activity (bursts) and isoelectric quiescence (flat line or suppression). This pattern has been identified as a global state of profound brain inactivation, physiologically seen during comatose states but also artificially induced by deep anesthesia. Evidence from animal and human data suggest the participation of the cortex, thalamus, and the ascending arousal system (ARAS) in the dynamics of a burst, however the driving mechanism of this process is still unclear. Using simultaneous EEG-fMRI previously published data of 19 male subjects during anesthesia-induced burst suppression state, we have studied functional connectivity of fMRI BOLD fluctuations from cortical, thalamus and ARAS regions ~10seconds before a bursting event. Events where labelled using EEG. To analyze time-resolved inter-regional connectivity prior to burst, we have applied a jackknife correlation approach, consisting of a repetitive re-estimation of the pair-wise correlation value between two regions after the removal of a single observation. The characteristic strong signal amplitude variations were accounted for by using global signal regression. Finally, using a principal component analysis (PCA) of the correlation results averaged per parcel (cortex, thalamus, ARAS), we calculated the amount of variance explained per parcel per time-point before the burst, enabling a reconstruction of the connectivity trajectory before an event. Our results indicate that just before an event, the cortical-ARAS and cortical-thalamic connectivity increase while cortico-cortical connectivity decreases ($p=0.03$). This supports the idea that subcortical areas are causal in the generation of individual bursts in sevoflurane anesthesia.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-052

CHALLENGING THE ROLE OF THE THALAMUS IN COGNITION: THE NEUROPSYCHOLOGICAL IMPACT OF CHRONIC THALAMIC STROKE

POSTER SESSION 03 - SECTION: FUNCTIONAL CONNECTIVITY AND COGNITION

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Aims: The thalamus is a deep structure of the diencephalon. Until the 20th century, case studies revealed major cognitive deficits following thalamic lesions, ranging from visual blindness to amnesia. However, the recent literature, which relies on neuroimaging and group studies, seems to highlight the opposite pattern, with rather moderate cognitive deficits. This implies that we don't fully understand the neuropsychological consequences of thalamic lesions. Methods: We collected data from 40 patients with ischemic thalamic lesions (27 left, 6 right, 7 bilateral) and 45 matched healthy subjects. Patients underwent a detailed neuropsychological assessment and a high-resolution MRI scan at the chronic phase (>3 months after the stroke). Lesions were localized using Morel's atlas and were correlated to cognitive functions using AnaCOM2. Results: Group analyses revealed impaired verbal memory, and moderate deficits in executive functions, attention and naming. A cluster analysis revealed that the majority of patients (60%) did not show major impairment. However, a subgroup of patients (37%) showed more severe verbal memory impairment (median Z-score = -6), related to lesions of the left or bilateral thalamus. Lesion of the MTT was not a predictor of the subgroup of the patient. Only one young patient with large isolated left thalamic lesions showed a major impairment in several cognitive domains. The majority of lesions were located in the dorsomedian and ventrolateral regions. Conclusion: The majority of thalamic strokes only moderately alter the patients' cognitive status. It highlights a misunderstanding of the role of the thalamus in cognition that needs to be resolved.

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BOARD NUMBER: S03-053

QUANTIFYING SOCIAL BEHAVIORS IN JUVENILE SHANK3 MICE USING ANIMAL POSE ESTIMATION TOOLS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Social interaction is a core aspect of mammalian behavior, and alterations in social behaviors are found across many neurodevelopmental and psychiatric conditions. Mice display a range of socioemotional behaviors and are commonly used as models to investigate the neuronal circuits and molecular mechanisms underlying differences in social behaviors. Analyzing social interactions in mice is often done by manual quantification of videos. Although multiple methods for automated tracking have been developed in recent years, reliable tracking and automated behavioral classification of multiple freely-moving unmarked animals have remained challenging. The use of an unbiased classification system in a more naturalistic environment could help obtain a translatable way to study social behaviors, in particular in mouse models for neuropsychiatric conditions. Here, we use open-source toolkits including DeepLabCut and SimBA to track and quantify reciprocal self-selected social interactions in freely-moving sex-and age-matched animals in a home cage. To study differences in social interaction we use juvenile female and male mice that lack the autism-associated gene Shank3. We show that DeepLabCut can track the movement of two size-matched juvenile mice freely interacting in a home-cage, and behavioral classification using supervised and unsupervised methods can detect differences in social interaction between Shank3 knockout and control mice. Quantifying behavior in an unbiased way remains a challenge in animal research. We confirm that freely-available open-source toolkits can be used to track and classify social interactions in freely-moving mice in a home cage, thereby providing a simple, low-cost solution to analyze social behaviors in age and sex-matched mice.

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BOARD NUMBER: S03-054

SPONTANEOUS ULTRASONIC VOCALISATIONS IN C57BL/6J MICE REVEAL SEX- AND CONTEXT-SPECIFICITY

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Social interactions and communication are key behaviors in the life of mice. Mice interact socially to regulate reproduction, inter-individual coordination and protection from predators. Despite their broad use as models for neuropsychiatric disorders, the knowledge on their social communication system is still fragmented. Our goal is to decipher the functions of ultrasonic vocalizations (USV) in mice during spontaneous social interactions. We automaticaly synchronize the behavioural annotation of live mouse tracker https://livemousetracker.org and automatic USV detections https://usv.pasteur.cloud over three days in freely behaving same-sex pairs of familiar C57BL/6J mice. We compute correlation between USVs and behaviors. We also provide specific software to explore and label USVs and behaviors that are not yet labeled automatically. We highlight significant variations between sexes in the spontaneous usage and structure of ultrasonic vocalizations, with females emitting more USVs in longer sequences and with longer duration and more frequency modulations compared to males. Also, females emit most of their calls in social contacts while males emit USVs when isolated. In females, the acoustic features of USVs vary according to the behavioral context. We also extend the study to manual deep exploration of the data to point out specific acoustic traits correlated with very specific behavioral situations. These results suggest USVs do not have the same functions in both sexes. Further investigations on other mouse strains should help understand the functions of these communicative signals, and better integrate them in the behavioral phenotyping of mouse models of communicative disorders.

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BOARD NUMBER: S03-055

LACK OF FMR1-GENE IMPACTS EARLY DEVELOPMENT OF VOCAL COMMUNICATION PARTICULARLY IN FEMALE MOUSE PUPS.

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Fragile X syndrome (FXS) is the most prevalent form of inheritable cognitive disability, and also the most frequent monogenic cause of autism. Individuals with FXS are suffering from a range of symptoms including impaired cognition, communication, and social behavior. Similar deficits have been reported in the FXS mouse model (*Fmr1*-KO mouse). Alterations in vocal communication have been shown even in early postnatal development, however, mostly focusing on a narrow age range. In this study, mouse pup isolation calls from both male and female *Fmr1*-KO and sighted FVB (FVB.129P2-Pde6b^{+}Tyr$^{c\text{-}ch}$/AntJ; wt) control mice are examined in a developmental range from before hearing onset until shortly after (P2 – P12). We noticed alterations in the temporal domain of mouse pup isolation calls mainly in female *Fmr1*-KO pups. These alterations encompassed changes in the number of calls emitted and an on-average constant call duration. Most strikingly however, throughout their developmental trajectory female *Fmr1*-KO mice displayed an altered temporal organization of the calls they emitted, which was expressed by longer breaks between single calls. The in-depth analysis of the impact of the lack of the *Fmr1*-gene on the spectral call parameters is currently still ongoing. Our results will provide further insights into the alterations during early development of vocal communication in the *Fmr1*-KO mouse that might be beneficial for our understanding of the impaired communication in FXS.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-056

FMR1-KO MOUSE MODEL, A SUITABLE TOOL TO STUDY AUTISM SPECTRUM DISORDER (ASD)

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Objectives Autism Spectrum Disorder (ASD) is a group of neurodevelopmental conditions which affect approximately 1.5% of the population worldwide. Individuals with ASD exhibit communication and socialization impairments, hyperactivity, repetitive behaviors, and restricted interests. Several genetic mutations have been tightly linked to ASD, including mutations in the *FMR1* (fragile X mental retardation 1) gene that leads to the Fragile X syndrome, a monogenic cause of ASD. Rodent models of ASD recapitulate behavior abnormalities observed in humans and serve as valuable tools to validate pharmacological compounds alleviating ASD behaviors. Thi study aimed to behaviorally characterize the *Fmr1*-Knockout (KO) mouse model compared with C57BL/6JRj animals. Additionally, the effects of the widely used GABAergic drug R-Baclofen on behavior were investigated. **Methods** Male B6.129P2-*Fmr1*^{tm1Cgr}/J (*Fmr1*-KO) mice were allocated to two different groups and intraperitoneally treated once with R-Baclofen or vehicle. Additionally, C57BL/6JRj mice received vehicle. Open field, grooming, three-chamber-social-interaction test, and ultrasonic vocalization (USV) emission recording test were conducted at the age of 7-10 weeks. **Results** *Fmr1*-KO vehicle-treated mice showed significantly higher activity, hyperactivity, grooming duration/episodes, as well as impaired social interaction and interest compared to C57BL/6JRj mice. USV recordings revealed a significant reduction in the number of calls in *Fmr1*-KO vehicle-treated mice compared to C57BL/6JRj animals. Acute R-Baclofen treatment alleviated repetitive grooming behavior in *Fmr1*-KO mice. **Conclusions** From our data, we concluded that the *Fmr1*-KO mouse model can serve as a valuable tool to investigate some core and secondary symptoms of ASD. Intriguingly, R-Baclofen treatment alleviated repetitive behavior of grooming in *Fmr1*-KO mice.

Pubmed:
31623187: Nogueira F, Sharghi S, Kuchler K, Lion T
Pathogenetic Impact of Bacterial-Fungal Interactions.
Polymicrobial infections are of paramount importance because of the potential severity of clinical manifestations, often associated with increased resistance to antimicrobial treatment. The intricate interplay with the host and the immune system, and the impact on microbiome imbalance, are of importance in this context. The equilibrium of microbiota in the human host is critical for preventing potential dysbiosis and the ensuing development of disease. Bacteria and fungi can communicate via signaling molecules, and produce metabolites and toxins capable of modulating the immune response or altering the efficacy of treatment. Most of the bacterial-fungal interactions described to date focus on the human fungal pathogen and different bacteria. In this review, we discuss more than twenty different bacterial-fungal interactions involving several clinically important human pathogens. The interactions, which can be synergistic or antagonistic, both and , are addressed with a focus on the quorum-sensing molecules produced, the response of the immune system, and the impact on clinical outcome.
Microorganisms, 2019; 7

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-057

THE IMPACT OF C-TACTILE LOW THRESHOLD MECHANORECEPTORS ON AFFECTIVE TOUCH AND SOCIAL INTERACTIONS IN MICE.

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Affective touch is necessary for proper neurodevelopment and sociability. However, it is still unclear how the neurons innervating the skin detect affective and social behaviours. The C-low threshold mechanoreceptors (C-LTMRs), a specific population of somatosensory neurons in mice, appears particularly well suited, physiologically and anatomically, to perceive affective and social touch. However, their contribution to sociability has not been resolved yet. Our observations revealed that C-LTMRs functional deficiency from birth induced social isolation and reduced tactile interactions in adulthood. Conversely, transient increase in C-LTMRs excitability in adults, using chemogenetics, was rewarding, promoted touch-seeking behaviours and had pro-social influences on group dynamics. This work provides the first empirical evidence that specific peripheral inputs alone can drive complex social behaviours. It demonstrates the existence of a specialised neuronal circuit, originating in the skin, wired to promote interactions with other individuals.

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BOARD NUMBER: S03-058

SOCIAL BEHAVIOR DEFICITS FOLLOWING SEROTONIN 2A RECEPTOR CONSTITUTIVE DELETION

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Social behavior (SB) is defined as interactions among individuals that offer mutual benefits and comprise different actions. Deficits in SB are a hallmark of different psychiatric disorders, including autism spectrum disorders. Changes in 5-HT levels, as well as some activity of key molecules within the system have been associated with deficits in SB. Serotonin 2A receptors (5-HT2aR) are one of the main excitatory serotonergic receptors. Social deficits in humans were associated with 5-HT2aR hypofunction. Interestingly, 5-HT2aR agonists increase social interaction (SI) in humans and animal models suggesting that 5-HT2aR might modulate SB. We used genetically modified male and female mice (htr2a-/-) and their littermates' controls (htr2a+/+) to study the specific role of the receptor in SB. P90 or older animals were exposed to different behavioral paradigms. In the three-chambers SI test *htr2a-/-* male and female mice show decreased discrimination indexes compared with same sex *htr2+/+* mice. Moreover, this deficit was rescued by the genetic restoration of the 5-HT2aR expression in the cortex suggesting a specific role of the receptor in this area. However, pharmacological manipulations in *htr2+/+* adult mice before the SI test showed no effect suggesting that 5-HT2aR is not acutely recruited for SB. Taken together, these results suggest that 5-HT2aR has a role in SB and its involvement appears to be due to a developmental or chronic action.

Pubmed:
33026440: Morales C, Morici JF, Espinosa N, Sacson A, Lara-Vasquez A, García-Pérez MA, Bekinschtein P, Weisstaub NV, Fuentealba P
Dentate Gyrus Somatostatin Cells are Required for Contextual Discrimination During Episodic Memory Encoding.
Memory systems ought to store and discriminate representations of similar experiences in order to efficiently guide future decisions. This problem is solved by pattern separation, implemented in the dentate gyrus (DG) by granule cells to support episodic memory formation. Pattern separation is enabled by tonic inhibitory bombardment generated by multiple GABAergic cell populations that strictly maintain low activity levels in granule cells. Somatostatin-expressing cells are one of those interneuron populations, selectively targeting the distal dendrites of granule cells, where cortical multimodal information reaches the DG. Nonetheless, somatostatin cells have very low connection probability and synaptic efficacy with both granule cells and other interneuron types. Hence, the role of somatostatin cells in DG circuitry, particularly in the context of pattern separation, remains uncertain. Here, by using optogenetic stimulation and behavioral tasks in mice, we demonstrate that somatostatin cells are required for the acquisition of both contextual and spatial overlapping memories.
Cereb Cortex, 2021; 31

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-059

STUDY OF MITOCHONDRIAL FUNCTION AND SOCIAL BEHAVIORS UNDER PSYCHOGENIC STRESS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Aims: Stress conditions threaten the organism's homeostasis, triggering adaptative responses. For instance, during stress, the brain adapts to cope with the increased energy demand by augmenting metabolism, glucose oxidation and oxygen consumption. We aim to unveil the molecular mechanisms triggered during stress and mediated by corticosterone, that if maladaptive, can lead to neuropsychiatric disorders. Considering that mitochondria and bioenergetics are emerging as important key players in psychopathologies, our goal is to study mitochondrial structure and function in Nucleus Accumbens (NAc). This brain region is implicated in stress-related pathologies and can be altered after stress exposure affecting social behavior. Here we study the effect of stress on mitochondrial-endoplasmic reticulum interactions, key for mitochondrial activity and ATP production in NAc. **Methods:** To this end, we evaluated the effect of the corticosterone and stress on mouse behavior, inter-organelle contacts using protein ligation assay, mitochondrial respiration assessed by high-resolution respirometry and ATP production. **Results:** Interestingly, our data show that in a situational dominance tube test confrontation against non-stressed animals, acutely stressed mice had a social advantage. The acutely stressed mice won almost all trials immediately after acute stress. Corticosterone levels correlated with success rate and ATP levels in NAc. In vitro studies showed that corticosterone can induce mito-ER contacts, modifying mitochondrial respiration and ATP levels, and our results indicate that this effect could be cell-specific. **Conclusions:** Corticosterone -through modulatory actions on mitochondrial inter-organelle interactions- seem to modulate mitochondrial activity to fulfil the energy demand that occurs during behavioral adaptation to stress.

Pubmed:
33795747: Ramos-Fernández E, Arrázola MS, Oliva CA, Arredondo SB, Varela-Nallar L, Inestrosa NC
Wnt5a promotes hippocampal postsynaptic development and GluN2B-induced expression via the eIF2α HRI kinase.
Wnt signaling plays a key role in neurodevelopment and neuronal maturation. Specifically, Wnt5a stimulates postsynaptic assemblies, increases glutamatergic neurotransmission and, through calcium signaling, generates nitric oxide (NO). Trying to unveil the molecular pathway triggering these postsynaptic effects, we found that Wnt5a treatment induces a time-dependent increases in the length of the postsynaptic density (PSD), elicits novel synaptic contacts and facilitates F-actin flow both in in vitro and ex vivo models. These effects were partially abolished by the inhibition of the Heme-regulated eukaryotic initiation factor 2α (HRI) kinase, a kinase which phosphorylates the initiation translational factor eIF2α. When phosphorylated, eIF2α normally avoids the translation of proteins not needed during stress conditions, in order to avoid unnecessary energetic expenses. However, phosphorylated eIF2α promotes the translation of some proteins with more than one open reading frame in its 5' untranslated region. One of these proteins targeted by Wnt-HRI-eIF2α mediated translation is the GluN2B subunit of the NMDA receptor. The identified increase in GluN2B expression correlated with increased NMDA receptor function. Considering that NMDA receptors are crucial for excitatory synaptic transmission, the molecular pathway described here contributes to the understanding of the fast and plastic translational mechanisms activated during learning and memory processes.
Sci Rep, 2021; 11
32609904: Guillot de Suduiraut I, Grosse J, Ramos-Fernández E, Sandi C, Hollis F
Astrocytic release of ATP through type 2 inositol 1,4,5-trisphosphate receptor calcium signaling and social dominance behavior in mice.
Brain mitochondrial function is critical for numerous neuronal processes. We recently identified a link between brain energy and social dominance, where higher levels of mitochondrial function resulted in increased social competitive ability. The underlying mechanism of this link, however, remains unclear. Here, we investigated the contribution of astrocytic release of adenosine triphosphate (ATP) through the type 2 inositol 1,4,5-triphosphate receptor to social dominance behavior. Mice lacking the type 2 inositol 1,4,5-triphosphate receptor were characterized for their social dominance behavior, as well as their performance on a nonsocial task, the Morris Water Maze. In parallel, we also examined mitochondrial function in the medial

prefrontal cortex, nucleus accumbens, and hippocampus to investigate how deficiencies in astrocytic ATP could modulate overall mitochondrial function. While knockout mice showed similar competitive ability compared with their wild-type littermates, dominant knockout mice exhibited a significant delay in exerting their dominance during the initial encounter. Otherwise, there were no differences in anxiety and exploratory traits, spatial learning and memory, or brain mitochondrial function in either light or dark circadian phases. Our findings point to a marginal role of astrocytic ATP through IP R2 in social competition, suggesting that, under basal conditions, the neuronal compartment is predominant for social dominance exertion.
Eur J Neurosci, 2021; 53
32438253: Zalachoras I, Hollis F, Ramos-Fernández E, Trovo L, Sonnay S, Geiser E, Preitner N, Steiner P, Sandi C, Morató L
Therapeutic potential of glutathione-enhancers in stress-related psychopathologies.
The mammalian brain has high energy demands, which may become higher in response to environmental challenges such as psychogenic stress exposure. Therefore, efficient neutralization of reactive oxygen species that are produced as a by-product of ATP synthesis is crucial for preventing oxidative damage and ensuring normal energy supply and brain function. Glutathione (GSH) is arguably the most important endogenous antioxidant in the brain. In recent years, aberrant GSH levels have been implicated in different psychiatric disorders, including stress-related psychopathologies. In this review, we examine the available data supporting a role for GSH levels and antioxidant function in the brain in relation to anxiety and stress-related psychopathologies. Additionally, we identify several promising compounds that could raise GSH levels in the brain by either increasing the availability of its precursors or the expression of GSH-regulating enzymes through activation of Nuclear factor erythroid-2-related factor 2 (Nrf2). Given the high tolerability and safety profile of these compounds, they may represent attractive new opportunities to complement existing therapeutic manipulations against stress-related psychopathologies.
Neurosci Biobehav Rev, 2020; 114
31110288: Carreras-Sureda A, Jaña F, Urra H, Durand S, Mortenson DE, Sagredo A, Bustos G, Hazari Y, Ramos-Fernández E, Sassano ML, Pihán P, van Vliet AR, González-Quiroz M, Torres AK, Tapia-Rojas C, Kerkhofs M, Vicente R, Kaufman RJ, Inestrosa NC, Gonzalez-Billault C, Wiseman RL, Agostinis P, Bultynck G, Court FA, Kroemer G, Cárdenas JC, Hetz C
Non-canonical function of IRE1α determines mitochondria-associated endoplasmic reticulum composition to control calcium transfer and bioenergetics.
Mitochondria-associated membranes (MAMs) are central microdomains that fine-tune bioenergetics by the local transfer of calcium from the endoplasmic reticulum to the mitochondrial matrix. Here, we report an unexpected function of the endoplasmic reticulum stress transducer IRE1α as a structural determinant of MAMs that controls mitochondrial calcium uptake. IRE1α deficiency resulted in marked alterations in mitochondrial physiology and energy metabolism under resting conditions. IRE1α determined the distribution of inositol-1,4,5-trisphosphate receptors at MAMs by operating as a scaffold. Using mutagenesis analysis, we separated the housekeeping activity of IRE1α at MAMs from its canonical role in the unfolded protein response. These observations were validated in vivo in the liver of IRE1α conditional knockout mice, revealing broad implications for cellular metabolism. Our results support an alternative function of IRE1α in orchestrating the communication between the endoplasmic reticulum and mitochondria to sustain bioenergetics.
Nat Cell Biol, 2019; 21
29967987: Ramos-Fernández E, Tapia-Rojas C, Ramírez VT, Inestrosa NC
Wnt-7a Stimulates Dendritic Spine Morphogenesis and PSD-95 Expression Through Canonical Signaling.
Wnt signaling regulates brain development and synapse maturation; however, the precise molecular mechanism remains elusive. Here, we report that Wnt-7a stimulates dendritic spine morphogenesis in the hippocampus via glycogen synthase kinase-3 β (GSK-3β) inhibition, triggering β-catenin/T cell factor/lymphoid enhancer factor (TCF/LEF)-dependent gene transcription and promoting postsynaptic density-95 (PSD-95) protein expression. In addition, wild-type mice treated with an inhibitor of β-catenin/TCF/LEF-mediated transcription showed a reduction in spatial memory acquisition accompanied by a reduction in PSD-95 and decreases in spine density measured by Golgi staining, suggesting that PSD-95 is a novel Wnt target gene. Together, our data strongly demonstrate that Wnt-dependent target gene transcription is essential to hippocampal synaptic plasticity.
Mol Neurobiol, 2019; 56
28060833: Arrázola MS, Ramos-Fernández E, Cisternas P, Ordenes D, Inestrosa NC
Wnt Signaling Prevents the Aβ Oligomer-Induced Mitochondrial Permeability Transition Pore Opening Preserving Mitochondrial Structure in Hippocampal Neurons.
Alzheimer's disease (AD) is a neurodegenerative disorder mainly known for synaptic impairment and neuronal cell loss, affecting memory processes. Beside these damages, mitochondria have been implicated in the pathogenesis of AD through the induction of the mitochondrial permeability transition pore (mPTP). The mPTP is a non-selective pore that is formed under apoptotic conditions, disturbing mitochondrial structure and thus, neuronal viability. In AD, Aβ oligomers (Aβos) favor the

opening of the pore, activating mitochondria-dependent neuronal cell death cascades. The Wnt signaling activated through the ligand Wnt3a has been described as a neuroprotective signaling pathway against amyloid-β (Aβ) peptide toxicity in AD. However, the mechanisms by which Wnt signaling prevents Aβos-induced neuronal cell death are unclear. We proposed here to study whether Wnt signaling protects neurons earlier than the late damages in the progression of the disease, through the preservation of the mitochondrial structure by the mPTP inhibition. To study specific events related to mitochondrial permeabilization we performed live-cell imaging from primary rat hippocampal neurons, and electron microscopy to analyze the mitochondrial morphology and structure. We report here that Wnt3a prevents an Aβos-induced cascade of mitochondrial events that leads to neuronal cell death. This cascade involves (a) mPTP opening, (b) mitochondrial swelling, (c) mitochondrial membrane potential loss and (d) cytochrome c release, thus leading to neuronal cell death. Furthermore, our results suggest that the activation of the Wnt signaling prevents mPTP opening by two possible mechanisms, which involve the inhibition of mitochondrial GSK-3β and/or the modulation of mitochondrial hexokinase II levels and activity. This study suggests a possible new approach for the treatment of AD from a mitochondrial perspective, and will also open new lines of study in the field of Wnt signaling in neuroprotection.
PLoS One, 2017; 12
27557499: Ramos-Fernández E, Tajes M, Ill-Raga G, Vargas L, Busquets-García A, Bosch-Morató M, Guivernau B, Valls-Comamala V, Gomis M, Grau C, Fandos C, Rosen MD, Rabinowitz MH, Inestrosa N, Maldonado R, Altafaj X, Ozaita A, Alvarez A, Vicente R, Valverde MA, Muñoz FJ
Glutamatergic stimulation induces GluN2B translation by the nitric oxide-Heme-Regulated eIF2α kinase in cortical neurons.
The activation of N-Methyl D-Aspartate Receptor (NMDAR) by glutamate is crucial in the nervous system function, particularly in memory and learning. NMDAR is composed by two GluN1 and two GluN2 subunits. GluN2B has been reported to participate in the prevalent NMDAR subtype at synapses, the GluN1/2A/2B. Here we studied the regulation of GluN2B expression in cortical neurons finding that glutamate up-regulates GluN2B translation through the action of nitric oxide (NO), which induces the phosphorylation of the eukaryotic translation initiation factor 2 α (eIF2α). It is a process mediated by the NO-heme-regulated eIF2α kinase (HRI), as the effect was avoided when a specific HRI inhibitor or a HRI small interfering RNA (siHRI) were used. We found that the expressed GluN2B co-localizes with PSD-95 at the postsynaptic ending, which strengthen the physiological relevance of the proposed mechanism. Moreover the receptors bearing GluN2B subunits upon NO stimulation are functional as high Ca2+ entry was measured and increases the co-localization between GluN2B and GluN1 subunits. In addition, the injection of the specific HRI inhibitor in mice produces a decrease in memory retrieval as tested by the Novel Object Recognition performance. Summarizing our data suggests that glutamatergic stimulation induces HRI activation by NO to trigger GluN2B expression and this process would be relevant to maintain postsynaptic activity in cortical neurons.
Oncotarget, 2016; 7
27402827: Ramírez VT, Ramos-Fernández E, Henríquez JP, Lorenzo A, Inestrosa NC
Wnt-5a/Frizzled9 Receptor Signaling through the Gαo-Gβγ Complex Regulates Dendritic Spine Formation.
Wnt ligands play crucial roles in the development and regulation of synapse structure and function. Specifically, Wnt-5a acts as a secreted growth factor that regulates dendritic spine formation in rodent hippocampal neurons, resulting in postsynaptic development that promotes the clustering of the PSD-95 (postsynaptic density protein 95). Here, we focused on the early events occurring after the interaction between Wnt-5a and its Frizzled receptor at the neuronal cell surface. Additionally, we studied the role of heterotrimeric G proteins in Wnt-5a-dependent synaptic development. We report that FZD9 (Frizzled9), a Wnt receptor related to Williams syndrome, is localized in the postsynaptic region, where it interacts with Wnt-5a. Functionally, FZD9 is required for the Wnt-5a-mediated increase in dendritic spine density. FZD9 forms a precoupled complex with Gαo under basal conditions that dissociates after Wnt-5a stimulation. Accordingly, we found that G protein inhibition abrogates the Wnt-5a-dependent pathway in hippocampal neurons. In particular, the activation of Gαo appears to be a key factor controlling the Wnt-5a-induced dendritic spine density. In addition, we found that Gβγ is required for the Wnt-5a-mediated increase in cytosolic calcium levels and spinogenesis. Our findings reveal that FZD9 and heterotrimeric G proteins regulate Wnt-5a signaling and dendritic spines in cultured hippocampal neurons.
J Biol Chem, 2016; 291
24503620: Ramos-Fernández E, Tajes M, Palomer E, Ill-Raga G, Bosch-Morató M, Guivernau B, Román-Dégano I, Eraso-Pichot A, Alcolea D, Fortea J, Nuñez L, Paez A, Alameda F, Fernández-Busquets X, Lleó A, Elosúa R, Boada M, Valverde MA, Muñoz FJ
Posttranslational nitro-glycative modifications of albumin in Alzheimer's disease: implications in cytotoxicity and amyloid-β peptide aggregation.
Glycation and nitrotyrosination are pathological posttranslational modifications that make proteins prone to losing their physiological properties. Since both modifications are increased in Alzheimer's disease (AD) due to amyloid-β peptide (Aβ) accumulation, we have studied their effect on albumin, the most abundant protein in cerebrospinal fluid and blood. Brain and

plasmatic levels of glycated and nitrated albumin were significantly higher in AD patients than in controls. In vitro turbidometry and electron microscopy analyses demonstrated that glycation and nitrotyrosination promote changes in albumin structure and biochemical properties. Glycated albumin was more resistant to proteolysis and less uptake by hepatoma cells occurred. Glycated albumin also reduced the osmolarity expected for a solution containing native albumin. Both glycation and nitrotyrosination turned albumin cytotoxic in a cell type-dependent manner for cerebral and vascular cells. Finally, of particular relevance to AD, these modified albumins were significantly less effective in avoiding Aβ aggregation than native albumin. In summary, nitrotyrosination and especially glycation alter albumin structural and biochemical properties, and these modifications might contribute for the progression of AD.
J Alzheimers Dis, 2014; 40

22488900: Guix FX, Wahle T, Vennekens K, Snellinx A, Chávez-Gutiérrez L, Ill-Raga G, Ramos-Fernandez E, Guardia-Laguarta C, Lleó A, Arimon M, Berezovska O, Muñoz FJ, Dotti CG, De Strooper B
Modification of γ-secretase by nitrosative stress links neuronal ageing to sporadic Alzheimer's disease.
Inherited familial Alzheimer's disease (AD) is characterized by small increases in the ratio of Aβ42 versus Aβ40 peptide which is thought to drive the amyloid plaque formation in the brain of these patients. Little is known however whether ageing, the major risk factor for sporadic AD, affects amyloid beta-peptide (Aβ) generation as well. Here we demonstrate that the secretion of Aβ is enhanced in an in vitro model of neuronal ageing, correlating with an increase in γ-secretase complex formation. Moreover we found that peroxynitrite (ONOO(-)), produced by the reaction of superoxide anion with nitric oxide, promoted the nitrotyrosination of presenilin 1 (PS1), the catalytic subunit of γ-secretase. This was associated with an increased association of the two PS1 fragments, PS1-CTF and PS1-NTF, which constitute the active catalytic centre. Furthermore, we found that peroxynitrite shifted the production of Aβ towards Aβ(42) and increased the Aβ(42) /Aβ(40) ratio. Our work identifies nitrosative stress as a potential mechanistic link between ageing and AD.
EMBO Mol Med, 2012; 4

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BOARD NUMBER: S03-060

UNDERSTANDING THE COMMUNICATIVE VALUE OF ULTRASONIC CALLS IN MALE AND FEMALE LABORATORY MICE: LESSONS FROM MOUSE MODELS OF NEURODEVELOPMENTAL DISORDERS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Ultrasonic vocalizations (USVs) are a major tool for assessing social communication in laboratory mice during their entire lifespan. At adulthood, male mice preferentially emit USVs towards a female conspecific, while females mostly produce ultrasonic calls when facing an adult intruder of the same sex. Recent studies have largely developed several sophisticated tools to analyze pup and adult mouse USVs, especially in males, because of the relevance of communication abnormalities in the behavioral phenotyping of mouse models of autism spectrum disorder (ASD). Nonetheless, little is still known about the communicative value of the different types of ultrasonic calls emitted by male and female mice. Little attention has been indeed devoted to the impact of these calls on the behavior of the receiver, a crucial issue to understand the significance of certain call characteristics in the communication context. Here we have performed playback studies in adult male and female mice, in order to assess the behavioral impact of sex differences both on the emitting and the receiving subject. Moreover, we have combined these findings with data obtained from an extensive qualitative analysis of the ultrasonic calls emitted by adult male and female transgenic mice modelling major neurodevelopmental disorders (NDDs). Our results show that male- and female-specific calls have different attractive/aversive properties for male and female adult mice and their perception is clearly altered in mouse models of NDDs.

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BOARD NUMBER: S03-061

A SPECIALIZED GENETIC ARCHITECTURE FOR SOCIAL LEARNING IN DROSOPHILA MELANOGASTER

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Social and asocial learning are required to cope with the complexity of the environment. They are behaviourally distinct. However, whether they use a single (general-purpose) or distinct (special-purpose) cognitive mechanisms remains elusive. A general-purpose mechanism should comprise a single set of genes, while a special-purpose mechanism should comprise two sets of genes, possibly regulating different biochemical pathways and/or neural circuits. This study aims to disentangle the genetic architecture of social and asocial learning in *Drosophila melanogaster*. We firstly tested social and asocial learning abilities in 40 lines of the DGRP, a panel constituted of hundreds of isogenic sequenced lines that together represent the genetic variation of a natural *Drosophila* population. We used aversive conditioning paradigms for oviposition sites. We obtained significant learning performance variation across the tested lines. Secondly, we performed a Genome-Wide Association Study – a statistical correlation between the genetic variants in the DGRP and each learning phenotype. We obtained two completely different sets of candidate genes for social and asocial learning, ranging from genes with unknown biological activity to genes already known to be related to learning. Thirdly, to functionally validate the role of each candidate gene on each learning phenotype, we used GAL4/RNAi-UAS lines, with knockdown induced by the pan-neuronal *nsyb-GAL4* driver. We found genes with no effect, genes with similar or opposite (trade-off) effects, and genes with social learning specialization. This study is the first experimental evidence supporting the special-purpose cognitive mechanism hypothesis. Future work will address this specialization at the neural circuitry level.

Pubmed:
31454441: Simões-Henriques C, Mateus-Pinheiro M, Gaspar R, Pinheiro H, Mendes Duarte J, Baptista FI, Canas PM, Fontes-Ribeiro CA, Cunha RA, Ambrósio AF, Gomes CA
Microglia cytoarchitecture in the brain of adenosine A receptor knockout mice: Brain region and sex specificities.
Microglia cells exert a critical role in brain development, mainly supported by their immune functions, which predicts an impact on the genesis of psychiatric disorders. In fact, microglia stress during gestation is, for instance, associated with chronic anxiety and cognitive deficits accompanied by long-lasting, region- and sex-specific changes in microglia morphology. We recently reported that the pattern of microglia morphologic plasticity, which is sex-determined, impacts on anxious-like behaviour and cognition. We also reported that the pharmacologic blockade of adenosine A receptors (A R) is able to reshape microglia morphology, in a sex-specific manner and with behavioural sequelae. In order to better understand the role of A R in the sex differentiation of microglia, we now compared their morphology in wild-type and A R knockout male and female C57BL/6 mice in two cardinal brain regions implicated in anxiety-like behaviour and cognition, the prefrontal cortex (PFC) and the dorsal hippocampus (dHIP). We report interregional differences between PFC and dHIP in a sex-specific manner: while males presented more complex microglia in the dHIP, microglia from females had a more complex morphology in the PFC. Surprisingly, the genetic deletion of A R did not alter these sex differences, but promoted the exclusive remodelling (increase in complexity) in PFC microglia from females. These findings further support the existence of a heterogeneous microglial network, distinct between sexes and brain regions, and help characterizing the role of A R in the sex- and brain region-specific morphologic differentiation of microglia.
Eur J Neurosci, 2020; 51

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BOARD NUMBER: S03-062

EMERGENCE OF INDIVIDUAL PERSONALITY TRAITS IN MICE LIVING IN SAME SEX COLONIES FROM WEANING: SOCIAL BEHAVIORS, ACOUSTIC COMMUNICATION AND MOTIVATION PROFILES

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Origin of individual variability has not yet been fully defined in pathological or in healthy states despite its impact on cognition. We expect variability to be promoted by early life social interaction and, therefore, that an environment offering more stimulations will favor the emergence of individual profiles. **Aim:** Our aim was to study individual behavioral variability regarding social behaviors. In a group of healthy adolescent mice living in a small stable colony, we assessed the range of behavioral individual variation over time, and differences between individuals. Some behaviors -within the social repertoire- might change throughout development, whereas some others might be stable, thus reflecting individual profile. **Methods:** We focused on housing conditions that would allow animals to develop a large behavioral repertoire and maximize behavioral variability detection. We expect to evaluate the influence of an enriched environment compared to standard housing conditions on mice behavior from adolescence to adulthood. **Results:** For that, we evaluated how an enriched environment influences individual differences in reward preference, social interactions, ultrasonic vocalizations or social motivation. We further investigated gender effects. **Conclusions:** We expect this work to increase our knowledge about the development of social profiles in mice. We shall also get information about the influence of a naturalistic environment and of gender on social and communication behaviors, and eventually on the development of social profiles.

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BOARD NUMBER: S03-063

PREGNANCY-INDUCED INDUCTION OF PARENTAL BEHAVIOUR

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Understanding how physiological states shape information processing in neural circuits is a fundamental question in neuroscience. Parenting is an instinctive behaviour that is crucial for survival and wellbeing of the offspring in many species. While considerable progress has been made in dissecting parental behaviour in rodents and other species, very little is known about the modulation of this behaviour and its underlying circuits by the females' reproductive state. Specifically, while the onset of parental behaviour is assumed to be linked to parturition and motherhood, whether, and when, pregnancy affects parental behaviour remains unexplored. Here we characterise pup-directed interactions in female mice throughout pregnancy. We find that parental interactions significantly increase during pregnancy, with the most pronounced changes occurring in the last trimester. By also tracking parental interactions in (1) ovariectomised females and (2) pregnant females not repeatedly exposed to pups, we identify aspects of parenting that are affected by pregnancy hormones, rather than by sensitisation due to frequent pup exposure. Moreover, we find that in addition to individual parenting parameters, entire pup-directed behavioural sequences are affected by pregnancy. Intriguingly, hormonally mediated increases in parental behaviour persist until at least one month after parturition, suggesting that they result from (semi)permanent remodelling of the brain by pregnancy hormones. We are currently investigating the neuronal and circuit-level changes underlying these behavioural adaptations. Uncovering the behavioural and neural effects of pregnancy hormone action will provide insights into how physiological states modulate brain function to instruct adaptive behavioural changes.

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BOARD NUMBER: S03-064

CHRONIC MATERNAL SSRI EXPOSURE DURING THE PRENATAL AND/OR POSTNATAL PERIOD INDUCED VOCAL BEHAVIOR DEFICITS AND AFFECTED SEROTONERGIC NEUROGENESIS IN THE MOUSE OFFSPRING

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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The mechanism of autism spectrum disorders (ASD) remains unclear, even though more than a thousand of genes have been identified as genetic factors. Regarding environmental factors, maternal infection and drug exposure are paid attention as increased risks. Clinical reports suggest that maternal intake of Fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), during pregnancy may cause an increased risk of ASD in their offspring. To investigate how maternal SSRI exposure impacts on behavior of the offspring, we used wild type (C57BL/6J) mice as a model. We recorded maternal separation-induced ultrasonic vocalizations (USVs), an important indicator of vocal communication, previously reported as an ASD-related behavior. The tested mouse pups were divided into four groups according to the period of maternal FLX administration; pups raised with mothers that were orally administrated with FLX during both pregnancy and lactation periods (FLX), during the pregnancy (F-Sa), during the lactation (Sa-F), and with those administrated with vehicle water (with saccharin) during both pregnancy and lactation periods (Sa). We observed impaired production of USVs in FLX and Sa-F groups, suggesting that FLX exposure in lactation periods could be risky for deficits of vocal communication. Additionally, we performed immunohistochemistry using the same cohorts, in which the number of serotonergic neurons was reduced in all three groups treated with FLX maternally. This result indicated that persistent FLX exposure during pregnancy and/or lactation may inhibit serotonergic neurogenesis. Overall, our results provide potential insights for ASD mouse model research and clues associated with maternal SSRI intake during pregnancy and/or lactation.

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BOARD NUMBER: S03-065

CORTICOTROPIN-RELEASING HORMONE FROM THE PREFRONTAL CORTEX REGULATES SOCIAL PREFERENCE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Social preference is a key feature of optimizing social interactions. In mice, social preference is based on anxiety, sex, strain and kinship but also on social memory. For example, adult rodents prefer to interact with novel versus familiar individuals (social novelty preference) whereas young pups prefer to interact with familiar littermates. Despite social novelty preference being used routinely to assess social memory, it is still unclear which neuronal circuits guide social preference and whether such circuits promote social interactions with the preferred individuals or prevent interactions with the non-preferred ones. The infra-limbic area of the pre-frontal cortex (ILA) - involved in social decision-making - and the lateral septum (LS) - involved in the inhibition of motivated behaviors, including social interactions - are necessary for social novelty preference but the neuronal circuits and molecular mechanisms allowing them to regulate social interactions are still unknown. Here, we show how the release of corticotropin-releasing hormone (CRH, a 41 amino acid neuropeptide) from ILA into the rostro-dorsal region of LS (rdLS) inhibits social interaction with familiar mice. This circuit therefore participates in the process of familiarization (decrease in interaction as a novel mouse becomes familiar) and contributes to social novelty preference in adult mice. We also demonstrate how the maturation of CRH expression during the first two post-natal weeks enables a shift of social preference from familiar to novel mice.

Pubmed:

31591398: De León Reyes NS, Mederos S, Varela I, Weiss LA, Perea G, Galazo MJ, Nieto M
Transient callosal projections of L4 neurons are eliminated for the acquisition of local connectivity.
Interhemispheric axons of the corpus callosum (CC) facilitate the higher order functions of the cerebral cortex. According to current views, callosal and non-callosal fates are determined early after a neuron's birth, and certain populations, such as cortical layer (L) 4 excitatory neurons of the primary somatosensory (S1) barrel, project only ipsilaterally. Using a novel axonal-retrotracing strategy and GFP-targeted visualization of Rorb neurons, we instead demonstrate that L4 neurons develop transient interhemispheric axons. Locally restricted L4 connectivity emerges when exuberant contralateral axons are refined in an area- and layer-specific manner during postnatal development. Surgical and genetic interventions of sensory circuits demonstrate that refinement rates depend on distinct inputs from sensory-specific thalamic nuclei. Reductions in input-dependent refinement result in mature functional interhemispheric hyperconnectivity, demonstrating the plasticity and bona fide callosal potential of L4 neurons. Thus, L4 neurons discard alternative interhemispheric circuits as instructed by thalamic input. This may ensure optimal wiring.
Nat Commun, 2019; 10
32988974: De León Reyes NS, Bragg-Gonzalo L, Nieto M
Development and plasticity of the corpus callosum.
The corpus callosum (CC) connects the cerebral hemispheres and is the major mammalian commissural tract. It facilitates bilateral sensory integration and higher cognitive functions, and is often affected in neurodevelopmental diseases. Here, we review the mechanisms that contribute to the development of CC circuits in animal models and humans. These species comparisons reveal several commonalities. First, there is an early period of massive axonal projection. Second, there is a postnatal temporal window, varying between species, in which early callosal projections are selectively refined. Third, sensory-derived activity influences axonal refinement. We also discuss how defects in CC formation can lead to mild or severe CC congenital malformations.
Development, 2020; 147
29273751: Pose-Utrilla J, García-Guerra L, Del Puerto A, Martín A, Jurado-Arjona J, De León-Reyes NS, Gamir-Morralla A, Sebastián-Serrano Á, García-Gallo M, Kremer L, Fielitz J, Ireson C, Pérez-Álvarez MJ, Ferrer I, Hernández F, Ávila J, Lasa M, Campanero MR, Iglesias T
Excitotoxic inactivation of constitutive oxidative stress detoxification pathway in neurons can be rescued by PKD1.
Excitotoxicity, a critical process in neurodegeneration, induces oxidative stress and neuronal death through mechanisms

largely unknown. Since oxidative stress activates protein kinase D1 (PKD1) in tumor cells, we investigated the effect of excitotoxicity on neuronal PKD1 activity. Unexpectedly, we find that excitotoxicity provokes an early inactivation of PKD1 through a dephosphorylation-dependent mechanism mediated by protein phosphatase-1 (PP1) and dual specificity phosphatase-1 (DUSP1). This step turns off the IKK/NF-κB/SOD2 antioxidant pathway. Neuronal PKD1 inactivation by pharmacological inhibition or lentiviral silencing in vitro, or by genetic inactivation in neurons in vivo, strongly enhances excitotoxic neuronal death. In contrast, expression of an active dephosphorylation-resistant PKD1 mutant potentiates the IKK/NF-κB/SOD2 oxidative stress detoxification pathway and confers neuroprotection from in vitro and in vivo excitotoxicity. Our results indicate that PKD1 inactivation underlies excitotoxicity-induced neuronal death and suggest that PKD1 inactivation may be critical for the accumulation of oxidation-induced neuronal damage during aging and in neurodegenerative disorders.
Nat Commun, 2017; 8
34030948: Bragg-Gonzalo L, De León Reyes NS, Nieto M
Genetic and activity dependent-mechanisms wiring the cortex: Two sides of the same coin.
The cerebral cortex is responsible for the higher-order functions of the brain such as planning, cognition, or social behaviour. It provides us with the capacity to interact with and transform our world. The substrates of cortical functions are complex neural circuits that arise during development from the dynamic remodelling and progressive specialization of immature undefined networks. Here, we review the genetic and activity-dependent mechanisms of cortical wiring focussing on the importance of their interaction. Cortical circuits emerge from an initial set of neuronal types that engage in sequential forms of embryonic and postnatal activity. Such activities further complement the cells' genetic programs, increasing neuronal diversity and modifying the electrical properties while promoting selective connectivity. After a temporal window of enhanced plasticity, the main features of mature circuits are established. Failures in these processes can lead to neurodevelopmental disorders whose treatment remains elusive. However, a deeper dissection of cortical wiring will pave the way for innovative therapies.
Semin Cell Dev Biol, 2021; 118

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BOARD NUMBER: S03-066

AUTOMATICALLY ANNOTATED MOTION TRACKING IDENTIFIES A DISTINCT SOCIAL BEHAVIORAL PROFILE FOLLOWING CHRONIC SOCIAL DEFEAT STRESS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Severe stress exposure is a global problem with long-lasting negative behavioral and physiological consequences, which can increase the risk for stress-related disorders such as major depressive disorder (MDD). Studying the role of social behavior in relation to the development of MDD is crucial, as poor social networks have been linked to lowered mental and physical health. An important animal model for the research in MDD is chronic social defeat stress (CSDS), which induces a cascade of stress-related physiological and behavioral responses. Here, we introduce the open-source tool “DeepOF”, to specifically investigate the social behavioral profile in mice by providing supervised and unsupervised behavioral profiling of DeepLabCut annotated pose-estimation data. The supervised pipeline relies on pretrained classifiers to automatically detect already defined traits for both single and dyadic animal behavior. Subsequently, the unsupervised pipeline aims to explore the behavioral repertoire of the animals without label priming. A distinct social behavioral profile is observed following CSDS using the DeepOF social behavioral classifiers and unsupervised profiling. In both cases, the observed social behavioral pattern was exclusively observed in the first 2.5 minutes of the interaction with a novel conspecific, which coincides with a state of social arousal, that disappears after 2.5 minutes due to habituation. Moreover, the classical social avoidance task does identify the social behavioral profile induced by CSDS, but both DeepOF behavioral pipelines provide a stronger profile. This study shows that the DeepOF module can contribute to further unravel the role of social behavior in stress-related disorders, such as MDD.

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BOARD NUMBER: S03-067

VGLUT3 AS A MARKER OF VULNERABILITY TO STRESS AND ASSOCIATED PSYCHIATRIC DISORDERS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Aims: Stress is a major risk factor that can lead to psychiatric and sleep disorders. Emotional responses to stress are characterized by an inter-individual variability which is influenced by environmental and genetic factors. The glutamatergic transmission, mediated by vesicular glutamate transporters, type 1-3 (VGLUT1-3), is involved in mood disorders. In humans, the p.T8I-VGLUT3 variant is linked to addictive behaviors. The aim of this study was to understand the role of this mutation in stress-related behaviors and its consequences on sleep patterns. **Methods:** VGLUT3$^{T8I/T8I}$ and VGLUT3$^{+/+}$ adult mice (male and female) were subjected to the chronic social defeat stress paradigm (CSDS). Social, anxiety- and depressive-like behaviors were assessed before and after stress exposure. Polysomnographic sleep recordings were done before, during and after CSDS. **Results:** Social behavior was differently impacted in control and mutant male mice. VGLUT3$^{T8I/T8I}$ male mice showed a higher susceptibility to stress characterized by a stronger social avoidance after CSDS. These mice also showed greater levels of anxiety-like behaviors, suggesting that the p.T8I variant plays a role in susceptibility to stress. In female mice, social and anxiety-like behaviors were similarly impacted after CSDS between control and mutant mice, suggesting sex differences in the role of the mutation in stress-related behaviors. CSDS also impaired sleep in both groups, with a long term increased wake time suggesting an insomniac-like phenotype. A stress-anticipatory arousal was found in control mice but not in mutant mice after CSDS. **Conclusion:** Our results revealed the role of the p.T8I mutation in susceptibility to stress.

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BOARD NUMBER: S03-068

DISSECTING THE NEURAL BASES UNDERLYING OBSERVATIONAL LEARNING OF PROSOCIAL AND SELFISH BEHAVIORS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Background: Humans constantly observe and learn from others (ie, observational learning). Through observation, humans model another's individual behavior, skills, emotions, and may replicate complex social outcomes such as prosocial or selfish acts. Rodent studies have helped defining the neural circuitry underlying spatial and fear learning by observation of their conspecifics. However, whether rodents can learn refined social behaviors through observation is unknown. The hippocampus is crucial for learning and has a key role (ie, dorsal CA1) in the representation of self-others location and action in the space. Given this evidence, neurodegenerative diseases affecting hippocampal activity might compromise observational learning ability. **Aims:** The major goal was dissecting the neural bases underlying observational learning. We studied observational learning in mice specifically focusing on the role of dorsal CA1. We also analyzed observational learning in a model of Alzheimer's dementia (ie, AD mice) characterized by hippocampal dysfunction. **Methods:** We used an operant social decision-making task where mice observe their conspecifics and learn to make prosocial or selfish choices. We investigated the role of dorsal CA1 by inhibitory chemogenetics. Then, we tested AD mice in our task. **Results:** Our results show that mice, after observation, display faster learning compared to mice not observing the task. Chemogenetic silencing of dorsal CA1 and AD mice showed impairments in observational learning. **Conclusions:** Our study starts addressing the role of dorsal CA1 in observational learning of complex social behaviors. Besides, our results with AD mice highlight how hippocampal dysfunction might compromise observational learning potentially disrupting social interactions.

Pubmed:
34246733: Scheggia D, Stanic J, Italia M, La Greca F, Zianni E, Benussi A, Borroni B, Di Luca M, Gardoni F
GluA3 autoantibodies induce alterations in dendritic spine and behavior in mice.
Autoantibodies targeting the GluA3 subunit of AMPA receptors (AMPARs) have been found in patients with Rasmussen's encephalitis and different types of epilepsy and were associated with the presence of learning and attention deficits. Our group recently identified the presence of anti-GluA3 immunoglobulin G (IgG) in about 25% of patients with frontotemporal dementia (FTD), thus suggesting a novel pathogenetic role also in chronic neurodegenerative diseases. However, the in vivo behavioral, molecular and morphological effects induced these antibodies are still unexplored. We injected anti-GluA3 IgG purified from the serum of FTD patients, or control IgG, in mice by intracerebroventricular infusion. Biochemical analyses showed a reduction of synaptic levels of GluA3-containing AMPARs in the prefrontal cortex (PFC), and not in the hippocampus. Accordingly, animals injected with anti-GluA3 IgG showed significant changes in recognition memory and impairments in social behavior and in social cognitive functions. As visualized by confocal imaging, functional outcomes were paralleled by profound alterations of dendritic spine morphology in the PFC. All observed behavioral, molecular and morphological alterations were transient and not detected 10-14 days from anti-GluA3 IgG injection. Overall, our in vivo preclinical data provide novel insights into autoimmune encephalitis associated with anti-GluA3 IgG and indicate an additional pathological mechanism affecting the excitatory synapses in FTD patients carrying anti-GluA3 IgG that could contribute to clinical symptoms.
Brain Behav Immun, 2021; 97

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BOARD NUMBER: S03-069

DOES PATERNAL METHAMPHETAMINE EXPOSURE CAUSE SUCH A SERIOUS IMPACT TO RAT OFFSPRING DURING DEVELOPMENT AND IN ADULTHOOD AS MATERNAL DRUG EXPOSURE?

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Drug addiction and its influence on behavior and development of offspring has become a serious problem in our society. Methamphetamine (MA) is one of the most abused psychostimulants in the Czech Republic and worldwide. Previous studies have demonstrated the adverse effects of maternal drug abuse. However, the father's contribution is unclear. The present study aimed to examine the effect of MA exposure on male sexual behavior and the paternal MA exposure on development and locomotor activity in rat offspring. MA was administrated for 30 days in dose of 5mg/kg s.c. to adult male rats. First, the effect of MA on sexual behavior and locomotor activity of adult male rats was examined. Second, the impact of paternal MA exposure on rat pups was investigated using behavioral tests during development and locomotor activity tests in adulthood. Prior to testing, adult offspring were exposed to an acute challenge dose of MA (1 mg/kg) to examine the possible sensitizing effect of the paternal treatment. Our results demonstrated that MA exposure does not affect sexual behavior. The data from Laboras test showed that MA administration has significant effect on locomotor activity only in case of acute MA application. As a matter of paternal administration effect on offspring, there were no significant differences in development or locomotor activity in adulthood. In conclusion, in contrast to the maternal MA administration, our results demonstrated that paternal MA administration does not affect behavior during development and in adult offspring as it was shown after maternal administration. Financial support: Research program Cooperatio Neurosciences and project PharmaBrain CZ.02.1.01/0.0/0.0/16_0250007444.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-070

EFFECTS OF SEX AND FAMILIARITY ON HELPING BEHAVIOR WITH AND WITHOUT SOCIAL REWARD IN RATS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Keren Ruzal[1], Estherina Trachtenberg[1], Einat Bigelman[2], Inbal Ben-Ami Bartal[1,2]
[1]Tel Aviv Univerity, Sagol School Of Neuroscience, Tel Aviv, Israel, [2]Tel Aviv University, School Of Psychological Sciences, Tel Aviv, Israel

Introduction: Prosocial behavior, like helping others in distress, relies on empathic responding. To investigate the neurobiological mechanisms underlying this behavior rats were tested in the Helping Behavior Test (HBT), where they help a trapped conspecific by opening the door to a restrainer. To dissociate the contribution of social reward, rats were physically separated and could not interact after helping. We found that helping selectively occurs for ingroup members even in the lack of social reward across strain and sex. **Methods**: Experiments were conducted in adult rats (8-13 pairs per group), with different sex, strain (Sprague Dawley/Long Evans/Fischer344, or SD/LE/F344), and familiarity (cagemate/stranger). Social reward was manipulated by using a single arena where rats could interact (non-separated) or a split arena where rats enter when released (separated). **Results**: In the non-separated HBT, SD males released cagemates more than LE strangers, reproducing previous results. Female F344 also released cagemates, as opposed to male F344. In the separated HBT, LE males typically released an LE. Movement analysis show differing social motivations across conditions, and point to anxiety as detrimental for helping. **Conclusions**: Social reward is not required for helping behavior in rats, who continue to release a trapped conspecific without contact. Social context is the main predictor of helping, whereas variations in anxiety, cognitive and motor abilities explain strain differences in door-opening. Follow-up experiments study the effects of co-housing across strains on the neural mechanisms involved in helping in the absence of social reward.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-071

SOCIAL ISOLATION DURING ADOLESCENCE TRIGGERS EMOTIONAL DYSFUNCTION IN BOTH FEMALE AND MALE MICE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Istituto superiore di sanità, Centro Di Neuroscienze Comportamentali, Roma, Italy

Aims: Depressive disorder in adolescents constitutes a huge socio-economic issue, represents the second leading cause of death among teenagers, and is more than twice as likely among girls than boys. Since social deprivation during adolescence triggers adolescent depression, we aimed to investigate in a preclinical model how adolescent social isolation leads to emotional dysfunction in both sexes. **Methods:** C57BL/6 male and female mice were exposed from weaning to adulthood either to standard housing conditions or to a social isolation protocol: in the latter, control mice were housed four per cage, while in the former, mice were singly housed. The behavioral phenotype was assessed during this period through saccharin preference, open field, and elevated plus-maze tests. **Results:** Following adolescent isolation, both male and female mice displayed increased anhedonic and anxiety-like profiles. Isolated mice showed a significantly decreased saccharin preference, compared to control mice. In the elevated plus-maze, isolated mice spent less time in the open arms performing longer rearing behavior, an index of increased fear. Moreover, isolated mice spent less time in the center of the open field arena displaying a longer stretch attend posture duration, a risk-assessment defensive mouse behavior. **Conclusion:** Our findings show that social isolation during adolescence leads to the onset of emotional dysfunction concerning depressive- and anxiety-like domains in both males and females mice. Translationally, the similar vulnerability in the two sexes suggests that the gender differences in adolescent depression might be ascribed not only to social deprivation but also to the interaction of several factors.

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BOARD NUMBER: S03-072

CO-MODULATION OF MULTIBRAIN BROADBAND DYNAMICS OF THE DORSOMEDIAL PREFRONTAL CORTEX BY SOCIAL CONTEXT

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Jeongyoon Lee[1], Gwang Ung Lee[2], Damhyeon Kwak[2], Chan Yeong Kim[2], Jee Hyun Choi[3], Sung Q. Lee[4], Han Kyoung Choe[2]
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Although mice are social animals, studies that explore the simultaneously recorded neural activities of multiple mice, especially in a naturalistic social setting, are still lacking. This study used a minimized head-mounted wireless edge-computing solution to record electrophysiological signals from multiple awake, freely-moving mice simultaneously. Four mice were allowed to interact freely with each other or were individually separated, while their behaviors and electrophysiological signals were recorded for three hours. Power spectral density (PSD) estimates of local field potential (LFP) recordings in the dorsomedial prefrontal cortex (dmPFC) showed differential patterns of powers at low- and high- frequencies when the mice were in a group and when they were separated. High- and Low-power ratio (HLR) was low when the mice were inactive, and it was high when they were active—whether or not they were moving—, and the mice in the group showed higher HLR in any behavioral states. However, the group/single HLR disparity disappeared when the mice were huddling (gathering together), suggesting the importance of active gathering or affiliative touch. The mice in the 'group' condition showed correlated patterns of HLR in the time domain, while the mice in the 'single' condition showed no correlation of HLR, suggesting the existence of the interbrain synchrony. Granger causality analysis indicated there is unilateral temporal precedence of the HLR among the mice, implying a dominant directed influence exists in the group of mice. Overall, this study shows that the neural activities of the multiple mice can be co-modulated by social context and locomotive status.

Pubmed:
31270159: Lee J, Lin J, Rabang C, Wu GK
Differential Inhibitory Configurations Segregate Frequency Selectivity in the Mouse Inferior Colliculus.
Receptive fields and tuning curves of sensory neurons represent the neural substrates that allow animals to efficiently detect and distinguish external stimuli. They are progressively refined to create diverse sensitivity and selectivity for neurons along ascending central pathways. However, the neural circuitry mechanisms have not been directly determined for such fundamental qualities in relation to sensory neurons' functional organizations, because of the technical difficulty of correlating neurons' input and output. Here, we obtained spike outputs and synaptic inputs from the same neurons within characteristically defined neural ensembles, to determine the synaptic mechanisms driving their diverse frequency selectivity in the mouse inferior colliculus. We find that the synaptic strength and timing of excitatory and inhibitory inputs are configured differently and independently within individual neurons' receptive fields, which segregate sensitive and selective neurons and endow neural populations with broad receptive fields and sharp frequency tuning. By computationally modeling spike outputs from integrating synaptic inputs and comparing them with real spike responses of the same neurons, we show that space-clamping errors did not qualitatively affect the estimation of spike responses derived from synaptic currents in voltage-clamp recordings. These data suggest that heterogeneous inhibitory circuits coexist locally for a parallel but differentiated representation of incoming signals. Sensitivity and selectivity are functional qualities of sensory systems to facilitate animals' survival. There is little direct evidence for the synaptic basis of neurons' functional variance within neural ensembles. Here we adopted a novel framework to fill such a long-standing gap by uniting population activities with single cells' spike outputs and their synaptic inputs. Furthermore, the effects of space-clamping errors on subcortical synaptic currents were evaluated , by comparing recorded spike responses and simulated spike outputs from computationally integrating synaptic inputs. Our study illustrated that the synaptic strength and timing of inhibition relative to excitation can be configured differently for neurons within a defined neural ensemble, to segregate their selectivity. It provides new insights into coexisting heterogeneous local circuits.
J Neurosci, 2019; 39


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BOARD NUMBER: S03-073

COGNITIVE AND EMOTIONAL FUNCTIONS OF MICE LACKING THE MINERALOCORTICOID RECEPTOR IN GLUTAMATERGIC OR GABAERGIC NEURONS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Huanqing Yang[1], Juan Pablo Lopez[2], Carlo De Donno[2], Lotte Van Doeselaar[1], Joeri Bordes[1], Lea Brix[1], Sowmya Narayan[1], Margherita Springer[1], Clara Engelhardt[1], Shiladitya Mitra[1], Mattias Eder[1], Jan Deussing[3], Mathias Schmidt[1]
[1]Max Planck Institute of Psychiatry, Neurobiology Of Stress Resilience, München, Germany, [2]Max Planck Institute of Psychiatry, Stress Neurobiology And Neurogenetics, München, Germany, [3]Max Planck Institute of Psychiatry, Stress Neurogenetics, München, Germany

Aims: To examine whether deletion of Mineralocorticoid receptor (MR) in glutamatergic pyramidal cells and GABA-ergic cells has differential effects on behavior and cognition. Methods: We generated mice lacking the MR in glutamatergic neurons (MRlox/lox-Nex-Cre) or GABAergic neurons (MRlox/lox-Dlx-Cre). First, we performed basal phenotyping of knockout or wild-type littermates on anxiety-related behaviour and cognition. We then tested how their behaviour changes after exposure to chronic social defeat stress (CSDS). In addition, electrophysiological experiments and Golgi staining were used to observe the changes of function and structure in MRlox/lox-Nex-Cre mice. Blood corticosterone and the adrenal weights of the animals were measured to test the reactivity of the hypothalamic-pituitary-adrenal axis following the stress and harvested the brains for further analysis of gene expression. Results: Under baseline conditions, only MRlox/lox-Nex-Cre mice displayed increased anxiety in the open field and elevated plus-maze test, the learning and memory ability of MRlox/lox-Dlx-Cre mice was improved under stressful test conditions. Following CSDS, there were few genotype x chronic stress interactions in either male MRlox/lox-Nex-Cre or MRlox/lox-Dlx-Cre mice and CSDS showed mostly genotype-independent effects. Electrophysiological recordings indicate that MRlox/lox-Nex-Cre mice release less glutamate neurotransmitters in CA3 presynaptic vesicles and have less receptors in the CA1 postsynaptic membrane. The preliminary structural analysis found that the number of dendritic branches of MRlox/lox-Nex-Cre mice is significantly decreased compared to wild-type. Conclusions: MR plays a specific role in regulating anxiety-related behaviour in glutamatergic neurons, while it affects memory function in GABAergic neurons.

Pubmed:
34872938: Engelhardt C, Tang F, Elkhateib R, Bordes J, Brix LM, van Doeselaar L, Häusl AS, Pöhlmann ML, Schraut K, Yang H, Chen A, Deussing JM, Schmidt MV
FKBP51 in the Oval Bed Nucleus of the Stria Terminalis Regulates Anxiety-Like Behavior.
The cochaperone FKBP51, encoded by the gene, has been identified as central risk factor for anxiety-related disorders and stress system dysregulation. In the brain, the oval bed nucleus of the stria terminalis (ovBNST) has been implicated in stress-induced anxiety. However, the role of in the ovBNST and its impact on anxiety-like behavior have remained unknown. Here, we show in mice that in the ovBNST is reactive to acute stress and coexpressed with the stress-regulated neuropeptides and Subsequently, results obtained from viral-mediated manipulation indicate that overexpression (OE) in the ovBNST results in an anxiolytic-like tendency regarding behavior and endocrinology, whereas a knock-out (KO) exposed a clear anxiogenic phenotype, indicating that native ovBNST expression and regulation is necessary for normal anxiety-related behavior. Notably, our data suggests that a stress-induced increase of in the ovBNST may in fact have a protective role, leading to a transient decrease in anxiety and suppression of a future stress-induced hypothalamic-pituitary-adrenal (HPA) axis activation. Together, our findings provide a first insight into the previously unknown relationship and effects of and the ovBNST on anxiety-like behavior and HPA axis functioning.
eNeuro, 2021 Nov-Dec; 8
34366343: He H, Liu A, Zhang W, Yang H, Zhang M, Xu H, Liu Y, Hong B, Yan F, Yue L, Wang J, Xiao S, Xie Z, Wang T
Novel Plasma miRNAs as Biomarkers and Therapeutic Targets of Alzheimer's Disease at the Prodromal Stage.
Amnestic mild cognitive impairment (aMCI) is a prodromal stage of Alzheimer's disease (AD) involving imbalanced beta-site amyloid precursor protein cleaving enzyme 1 (BACE1). MicroRNAs (miRNAs) are associated with AD.
J Alzheimers Dis, 2021; 83
33322989: van Doeselaar L, Yang H, Bordes J, Brix L, Engelhardt C, Tang F, Schmidt MV

Chronic social defeat stress in female mice leads to sex-specific behavioral and neuroendocrine effects.
Over the years, it has become increasingly clear that males and females respond differently towards environmental stressors, highlighting the importance of including both sexes when studying the effects of stress. This study aims to provide further insight into the detailed consequences of exposing female mice to 21 days of chronic social defeat stress (CSDS). We used a protocol that relies on the ability of odorants and pheromones in male urine to trigger male mouse aggressive behavior. Collected male C57Bl/6n urine was applied to female C57Bl/6n mice who were then attacked by a novel male CD1 mouse each day according to the CDSD protocol. Control females were pair-housed and handled daily. Physiological, neuroendocrine and behavioral changes were evaluated during the experiment. CSDS exposure resulted in number of physiological changes, such as body weight gain, enlarged adrenals and reduced thymus weight, exaggerated HPA-axis negative feedback and increased anxiety-like behavior. However, no generalized social avoidance behavior was observed. This study provides important insights in the physiological, neuroendocrine and behavioral responses of female mice to CSDS, which are partially dependent on estrous cycle stage. This protocol will allow direct comparison of male and female responses to CSDS and enable sex-specific study of mechanisms underlying individual stress resilience.Lay summaryIn this study we found that there are differences in the way that female and male mice respond towards chronic social stress conditions when it comes to behavior and hormonal changes.
Stress, 2021; 24
31179429: Yang H, Xu H, Li Q, Jin Y, Jiang W, Wang J, Wu Y, Li W, Yang C, Li X, Xiao S, Shi F, Wang T
Study of brain morphology change in Alzheimer's disease and amnestic mild cognitive impairment compared with normal controls.
With an aggravated social ageing level, the number of patients with Alzheimer's disease (AD) is gradually increasing, and mild cognitive impairment (MCI) is considered to be an early form of Alzheimer's disease. How to distinguish diseases in the early stage for the purposes of early diagnosis and treatment is an important topic.
Gen Psychiatr, 2019; 32

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BOARD NUMBER: S03-074

THE ROLE OF ENDGONOUS OPOID SIGNALING IN SOCIAL RECOGNITION

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Here we investigate how endogenous opioid signaling controls social recognition mice. We have used two strains of genetically modified mice with deletion of the genes encoding the two main precursors of endogenous opioid peptides prodynorphin (*Pdyn*) and preproenkephalin (*Penk*). Male and female mice of both strains had no apparent impairments, showed normal activity in the open field test and no change in anxiety-like behaviors. Interestingly, both strains showed normal preference of saccharine-sweetened water, and did not differ in the volume of liquids consumed. The mutations had no appreciable effects on the amount of time spent in interaction in the open field with a novel partner, thus indicating normal social anxiety and/or sociability. However, in the test for social memory, animals lacking the *Penk* gene showed significantly increased social interaction time with a previously encountered partner. Conversely, social memory appeared intact in mice with the *Pdyn* mutation. Taken together, these data replicate some of the previously reported phenotypes in *Penk* and *Pdyn* KO mice, though we would like to note that, contrary to previous findings, no evidence was found for altered-anxiety like behaviors or preference for sweet taste. The potential change in social memory could be in line with the proposed role of opioid signaling in social behaviors, though this requires further validation.

Pubmed:
32941853: Harda Z, Spyrka J, Jastrzębska K, Szumiec Ł, Bryksa A, Klimczak M, Polaszek M, Gołda S, Zajdel J, Misiołek K, Błasiak A, Rodriguez Parkitna J
Loss of mu and delta opioid receptors on neurons expressing dopamine receptor D1 has no effect on reward sensitivity. Opioid signaling controls the activity of the brain's reward system. It is involved in signaling the hedonic effects of rewards and has essential roles in reinforcement and motivational processes. Here, we focused on opioid signaling through mu and delta receptors on dopaminoceptive neurons and evaluated the role these receptors play in reward-driven behaviors. We generated a genetically modified mouse with selective double knockdown of mu and delta opioid receptors in neurons expressing dopamine receptor D1. Selective expression of the transgene was confirmed using immunostaining. Knockdown was validated by measuring the effects of selective opioid receptor agonists on neuronal membrane currents using whole-cell patch clamp recordings. We found that in the nucleus accumbens of control mice, the majority of dopamine receptor D1-expressing neurons were sensitive to a mu or delta opioid agonist. In mutant mice, the response to the delta receptor agonist was blocked, while the effects of the mu agonist were strongly attenuated. Behaviorally, the mice had no obvious impairments. The mutation did not affect the sensitivity to the rewarding effects of morphine injections or social contact and had no effect on preference for sweet taste. Knockdown had a moderate effect on motor activity in some of the tests performed, but this effect did not reach statistical significance. Thus, we found that knocking down mu and delta receptors on dopamine receptor D1-expressing cells does not appreciably affect some of the reward-driven behaviors previously attributed to opioid signaling.
Neuropharmacology, 2020; 180

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BOARD NUMBER: S03-075

SOCIAL ISOLATION IMPACT ON BEHAVIOUR AND NEUROINFLAMMATORY CONTEXT – A STUDY IN A MICE MODEL

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Daniela Magalhães[1,2,3], Myrthe Mampay[3], Ana Maria Sebastião[1,2], Graham Sheridan[3,4], Cláudia Valente[1,2]
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Social isolation is classified as a chronic mild stressor, which can lead to the development of neuropsychiatric disorders, including anxiety and depression. Chronic stress can induce activation of neuroinflammatory mediators, being this molecular response also linked with depressive disorders. NLRP3 inflammasome (NLRP3) activation integrates the stress-associated signals and the consequent release of Interleukin-1β. Currently, there is a knowledge gap regarding glial cells and neuroinflammatory alterations induced by social isolation. **Aim:** This work uses a rodent model of isolation to unveil its impact over the neuroinflammatory context, focusing on the NLRP3 pathway. **Methods:** Young and Aged C57BL7/J male mice were divided into 2 groups: group housed (GH) and socially isolated (SI) for 3 weeks. Depression and anxiety were evaluated through Force Swim (FST) and Open Field (OFT) tests, respectively. Morris Water Maze (MWM) was performed to assess spatial memory. In the hippocampus, glial cell reactivity and components of NLRP3 pathway were evaluated by western blotting, while proinflammatory cytokines were quantified by ELISA. **Results:** Aged-SI mice showed a depressive and anxiety-like behaviour during FST and OFT, respectively. Moreover, social isolation induced spatial memory impairment in Aged mice. Microglia activation analyses demonstrated an increase of Iba-1 expression in Aged-SI group and a decrease of anti-inflammatory microglia marker (Ym1) in Young-SI. Also, SI groups had a higher NLRP3 protein expression. Pro-inflammatory cytokines levels were higher in Aged groups. **Conclusions:** Depressive- and anxiety-like behaviour and cognitive impairment were promoted, together with a neuroinflammatory response, by social isolation in Aged mice.

Pubmed:
29996878: Magalhães DM, Pereira N, Rombo DM, Beltrão-Cavacas C, Sebastião AM, Valente CA
Ex vivo model of epilepsy in organotypic slices-a new tool for drug screening.
Epilepsy is a prevalent neurological disorder worldwide. It is characterized by an enduring predisposition to generate seizures and its development is accompanied by alterations in many cellular processes. Organotypic slice cultures represent a multicellular environment with the potential to assess biological mechanisms, and they are used as a starting point for refining molecules for in vivo studies. Here, we investigated organotypic slice cultures as a model of epilepsy.
J Neuroinflammation, 2018; 15

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BOARD NUMBER: S03-076

HYBRIDIZATION-BASED IN SITU SEQUENCING IN THE NUCLEUS ACCUMBENS REVEALS PERIPUBERTAL STRESS-INDUCED CHANGES IN SEVERAL CELL TYPES

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Exposure to stress in early life constitutes a crucial risk factor for the development of psychopathologies in adult stages. A peripubertal stress (PPS) paradigm has been established as a mouse model for such stress-induced psychopathologies. To investigate the effect of peripubertal stress on the cells that constitute the nucleus accumbens (NAc) and dorsal striatum (DS), we utilized hybridization-based in situ sequencing (HybISS), a state-of-the-art spatial transcriptomics technique. High resolution of HybISS allowed to check the expression levels of 65 genes in a cell-type specific manner. Stress resulted in a downregulation of several genes involved in NAD^+ metabolism and circadian clock. D2 medium spiny neurons of the NAc showed most of the significant gene expression changes, while transcript levels in cells of the DS remained largely unchanged. Interestingly, overexpression of nicotinamide phosphoribosyltransferase (Nampt), which is reduced by PPS, in the adipose tissue was sufficient to revert some of the stress-associated changes in the NAc. Hence, this study identifies PPS-associated changes in the cells of the NAc and unveils a link between the adipose tissue regulation and stress.

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BOARD NUMBER: S03-077

THE ROLE OF PTH2 NEUROPEPTIDE IN THE CONTROL OF SOCIAL BEHAVIOUR – A STUDY USING PTH2 RECEPTOR KO MICE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Social behaviour is important to reproduce and survive in rodents as well as human. The role of parathormon 2 neuropeptide (PTH2) in the recognition of conspecifics was recently proven in zebrafish (Anneser et al., 2020, Nature). Here, we aim to demonstrate the role of the TIP39-PTH2R neuromodulator system in rodent social behaviour. Therefore, we compared PTH2R knock-out (KO) mice and wild type (WT) littermates in different tests of sociability. We found significant differences in social novelty preference test. WT mice spent more time with an unfamiliar mouse than their cagemates. This expected behaviour disappeared in KO mice. Furthermore, KO mice has an increased latency of sniffing in social environment, an introductory behaviour between mice. In turn, there was no detectable difference between WT and KO mice in the elevated plus maze test. Using c-Fos immunohistochemistry, we found a reduced number of activated cells in KO compared to WT mice in medial prefrontal cortex, in the ventral part of the lateral septum and in the medial preoptic area, which regions are known to be connected to social behaviour. These data suggest that TIP39 contribute to the neuronal activation in these brain regions via the PTH2R. In conclusion, TIP39 may be involved in the control of some aspects of social behaviours. The brain regions with reduced social activation in the absence of PTH2R are likely to be involved in the social brain network affected by TIP39. Grant support: 2017-1.2.1-NKP-2017-00002, OTKA K134221, and TKP2020-IKA-05

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BOARD NUMBER: S03-078

PRENATAL CHLORPYRIFOS EXPOSURE AFFECTS SOCIAL BEHAVIORS ON C57BL/6 AND APOE TRANSGENIC MICE DEPENDING UPON SEX AND GENOTYPE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Developmental exposure to pesticides such as chlorpyrifos (CPF), has been shown to cause adverse effects. Although, the EU has banned the use of CPF since 2020, it is still in use in several developing countries. The massive use of CPF in the lasts years had been linked to the increased prevalence of Autism Spectrum Disorder (ASD). A growing body of literature, hypothesizes that excitatory and inhibitory (E/I) neurotransmitters imbalance are present in autism patients. For this reason, the aims of this study are to assess autistic-like behaviors and to identify associations between autism, apoE genotype and CPF exposure, in both sexes. C57BL/6 and humanized apoE3 and apoE4 homozygous mice were exposed to 0 or 1 mg/kg/day of CPF through the diet, between gestational day (GD) 12 and 18. Moreover, as a positive control of autism, C57BL/6 were exposed to 300 mg/kg/day of valproic acid (VPA) on GD 12 and 13 by a subcutaneous injection. During adolescence, social behaviors were assessed by a Three-chamber test. Then, at postnatal day (PND) 46 mice were sacrificed and hippocampal samples were collected to study gene expression related to GABA and glutamate signaling. Results showed a general preference for the social stimulus, even thought, social memory was altered in treated C57BL/6 male mice, as well as apoE3 and apoE4 CPF-treated females. Regarding molecular pathway, CPF increase GABA expression in females, whereas in males, pesticide increase glutamate expression, generating an E/I imbalance. Thus, our finding suggests that CPF could modulate social behaviors according to genotype and sex.

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BOARD NUMBER: S03-079

DISSECTING THE CONTRIBUTION OF HOST GENETICS AND THE MICROBIOME IN COMPLEX BEHAVIORS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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The core symptoms of many neurological disorders have traditionally been thought to be caused by genetic variants affecting brain development and function. However, the gut microbiome, another important source of variation, can also influence specific behaviors. Thus, it is critical to unravel the contributions of host genetic variation, the microbiome, and their interactions to complex behaviors. Unexpectedly, we discovered that different maladaptive behaviors are interdependently regulated by the microbiome and host genes in the *Cntnap2*$^{-/-}$ model for neurodevelopmental disorders. The hyperactivity phenotype of *Cntnap2*$^{-/-}$ mice is caused by host genetics, whereas the social behavior phenotype is mediated by the gut microbiome. Interestingly, specific microbial intervention with *Lactobacillus (L.) reuteri* selectively rescued the social deficits in *Cntnap2*$^{-/-}$mice through upregulation of metabolites in the tetrahydrobiopterin synthesis pathway. Our findings that behavioral abnormalities could have distinct origins (host genetic vs microbial) may change the way we think about neurological disorders and how to treat them.

Pubmed:
33705688: Buffington SA, Dooling SW, Sgritta M, Noecker C, Murillo OD, Felice DF, Turnbaugh PJ, Costa-Mattioli M
Dissecting the contribution of host genetics and the microbiome in complex behaviors.
The core symptoms of many neurological disorders have traditionally been thought to be caused by genetic variants affecting brain development and function. However, the gut microbiome, another important source of variation, can also influence specific behaviors. Thus, it is critical to unravel the contributions of host genetic variation, the microbiome, and their interactions to complex behaviors. Unexpectedly, we discovered that different maladaptive behaviors are interdependently regulated by the microbiome and host genes in the Cntnap2 model for neurodevelopmental disorders. The hyperactivity phenotype of Cntnap2 mice is caused by host genetics, whereas the social-behavior phenotype is mediated by the gut microbiome. Interestingly, specific microbial intervention selectively rescued the social deficits in Cntnap2 mice through upregulation of metabolites in the tetrahydrobiopterin synthesis pathway. Our findings that behavioral abnormalities could have distinct origins (host genetic versus microbial) may change the way we think about neurological disorders and how to treat them.
Cell, 2021; 184
31727829: Zhu PJ, Khatiwada S, Cui Y, Reineke LC, Dooling SW, Kim JJ, Li W, Walter P, Costa-Mattioli M
Activation of the ISR mediates the behavioral and neurophysiological abnormalities in Down syndrome.
Down syndrome (DS) is the most common genetic cause of intellectual disability. Protein homeostasis is essential for normal brain function, but little is known about its role in DS pathophysiology. In this study, we found that the integrated stress response (ISR)-a signaling network that maintains proteostasis-was activated in the brains of DS mice and individuals with DS, reprogramming translation. Genetic and pharmacological suppression of the ISR, by inhibiting the ISR-inducing double-stranded RNA-activated protein kinase or boosting the function of the eukaryotic translation initiation factor eIF2-eIF2B complex, reversed the changes in translation and inhibitory synaptic transmission and rescued the synaptic plasticity and long-term memory deficits in DS mice. Thus, the ISR plays a crucial role in DS, which suggests that tuning of the ISR may provide a promising therapeutic intervention.
Science, 2019; 366
30522820: Sgritta M, Dooling SW, Buffington SA, Momin EN, Francis MB, Britton RA, Costa-Mattioli M
Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder.
Currently, there are no medications that effectively treat the core symptoms of Autism Spectrum Disorder (ASD). We recently

found that the bacterial species Lactobacillus (L.) reuteri reverses social deficits in maternal high-fat-diet offspring. However, whether the effect of L. reuteri on social behavior is generalizable to other ASD models and its mechanism(s) of action remains unknown. Here, we found that treatment with L. reuteri selectively rescues social deficits in genetic, environmental, and idiopathic ASD models. Interestingly, the effects of L. reuteri on social behavior are not mediated by restoring the composition of the host's gut microbiome, which is altered in all of these ASD models. Instead, L. reuteri acts in a vagus nerve-dependent manner and rescues social interaction-induced synaptic plasticity in the ventral tegmental area of ASD mice, but not in oxytocin receptor-deficient mice. Collectively, treatment with L. reuteri emerges as promising non-invasive microbial-based avenue to combat ASD-related social dysfunction.
Neuron, 2019; 101
30001522: Dooling SW, Costa-Mattioli M
Gut Bacteria Seize Control of the Brain to Prevent Epilepsy.
Why ketogenic diet (KD) effectively controls seizures in some people with epilepsy is unclear. In a recent issue of Cell, Olson et al. (2018) showed that KD prevents seizures by upregulating key bacterial species (Akkermansia muciniphila and Parabacteroides merdae). These bacteria synergize to decrease gammaglutamylation of amino acids, increase hippocampal GABA/Glutamate ratios, and, ultimately, prevent seizures.
Cell Host Microbe, 2018; 24

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BOARD NUMBER: S03-080

A DECREASE OF REWARDING EFFECTS OF SOCIAL CONTACT IN MALE MICE DURING EARLY ADOLESCENCE IS REVERSED BY A MU OPIOID RECEPTOR ANTAGONIST

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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In humans, adolescence is characterized by complex changes is social behaviors, among them a decrease in affect experienced when interacting with family members. Here, we test whether similar phenotypic changes occur in mice. To model the behavior we have used the social conditioned place preference task, where interaction with siblings causes an increase in the time spent in a previously neutral context. We found that pre-adolescent male C57BL/6 mice (i.e., 33 to 34 days) acquired a robust preference for the context paired with the social contact, however, the preference was significantly decreased in adolescent animals (35-40 days), and then again rose upon reaching sexual maturity (41-42 days). To test if the decrease in preference was specific to social reward, mice of corresponding age were tested for cocaine-induced place preference and opposite effects were observed, with highest mean preference observed in mice aged 35 to 37 days. Next, we tested the hypothesis that endogenous opioids are essential in signaling social reward in adolescence. We found that cyprodime, a selective mu opioid receptor antagonist, administered 1h before the post-test (1 mg/kg i.p.), restored the preference for socially conditioned context during the early-adolescent drop in rewarding effects of social interactions. Taken together, these data show an interesting parallel in the developmental changes of sensitivity to social reward in humans and mice during adolescence. Furthermore, the results reveal specific involvement of mu opioid receptors in the expression of social-conditioned behaviors during early adolescence.

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BOARD NUMBER: S03-081

DEVELOPMENT OF A NOVEL BEHAVIORAL FRAMEWORK TO STUDY SOCIAL MOTIVATION NEUROBIOLOGY IN MICE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Social behaviors are crucial for one's environmental fitness, and alterations of social interactions are observed in psychiatric disorders. Despite intense research in social neurosciences, the neurobiology underlying social interactions, and their pathological impairments are loosely described. Social motivation is a crucial component of conspecific interactions and refers to the need to interact with others. Recently, a growing body of evidence substantiated theories linking impairments in social motivation with psychiatric disorders such as autism. Research on the neurophysiological mechanisms underlying motivation in general has been a hot topic for decades, leading to the discovery of the major involvement of the mesolimbic dopaminergic system. However, *the neurobiological ground specifically underlying social motivation, namely which brain processes drive animals to engage socially, and its impairments in psychiatric disorders, remains elusive.* Several factors can explain this gap in knowledge among which (i) the absence of a unified framework to study social motivation, and (ii) therefore an incomplete description of its neurobiology. ***The main aim of our work was to address these shortcomings and pursue the characterization of the neurobiology underlying social motivation*** through (i) the development of a novel behavioral paradigm suitable to dissect social motivation feature allowing (ii) the characterization of the underlying neurocircuit using cutting-edge genetic and molecular tools, and also (iii) to evaluate the face validity of current animal models of neuropsychiatric disorders.

Pubmed:

33770505: Soria-Gomez E, Pagano Zottola AC, Mariani Y, Desprez T, Barresi M, Bonilla-Del Río I, Muguruza C, Le Bon-Jego M, Julio-Kalajzić F, Flynn R, Terral G, Fernández-Moncada I, Robin LM, Oliveira da Cruz JF, Corinti S, Amer YO, Goncalves J, Varilh M, Cannich A, Redon B, Zhao Z, Lesté-Lasserre T, Vincent P, Tolentino-Cortes T, Busquets-García A, Puente N, Bains JS, Hebert-Chatelain E, Barreda-Gómez G, Chaouloff F, Lohman AW, Callado LF, Grandes P, Baufreton J, Marsicano G, Bellocchio L
Subcellular specificity of cannabinoid effects in striatonigral circuits.
Recent advances in neuroscience have positioned brain circuits as key units in controlling behavior, implying that their positive or negative modulation necessarily leads to specific behavioral outcomes. However, emerging evidence suggests that the activation or inhibition of specific brain circuits can actually produce multimodal behavioral outcomes. This study shows that activation of a receptor at different subcellular locations in the same neuronal circuit can determine distinct behaviors. Pharmacological activation of type 1 cannabinoid (CB) receptors in the striatonigral circuit elicits both antinociception and catalepsy in mice. The decrease in nociception depends on the activation of plasma membrane-residing CB receptors (pmCB), leading to the inhibition of cytosolic PKA activity and substance P release. By contrast, mitochondrial-associated CB receptors (mtCB) located at the same terminals mediate cannabinoid-induced catalepsy through the decrease in intra-mitochondrial PKA-dependent cellular respiration and synaptic transmission. Thus, subcellular-specific CB receptor signaling within striatonigral circuits determines multimodal control of behavior.
Neuron, 2021; 109
33021007: Medrano MC, Hurel I, Mesguich E, Redon B, Stevens C, Georges F, Melis M, Marsicano G, Chaouloff F
Exercise craving potentiates excitatory inputs to ventral tegmental area dopaminergic neurons.
Physical exercise, which can be addictogenic on its own, is considered a therapeutic alternative for drug craving. Exercise might thus share with drugs the ability to strengthen excitatory synapses onto ventral tegmental area (VTA) dopaminergic neurones, as assessed by the ratio of AMPA receptor (AMPAR)-mediated excitatory postsynaptic currents (EPSCs) to NMDA receptor (NMDAR)-mediated EPSCs. As did acute cocaine, amphetamine, or Δ -tetrahydrocannabinol (THC) pretreatments, an acute 1-h wheel-running session increased the AMPAR/NMDAR ratio in VTA dopaminergic neurones. To dissect the respective influences of wheel-running seeking and performance, mice went through an operant protocol wherein wheel-running was conditioned by nose poking under fixed ratio schedules of reinforcement. Conditioned wheel-running increased

the AMPAR/NMDAR ratio to a higher extent than free wheel-running, doing so although running performance was lower in the former paradigm than in the latter. Thus, the cue-reward association, rather than reward consumption, played a major role in this increase. The AMPAR/NMDAR ratio returned to baseline levels in mice that had extinguished the cued-running motivated task, but it increased after a cue-induced reinstatement session. The amplitude of this increase correlated with the intensity of exercise craving, as assessed by individual nose poke scores. Finally, cue-induced reinstatement of running seeking proved insensitive to acute cocaine or THC pretreatments. Our study reveals for the first time that the drive for exercise bears synaptic influences on VTA dopaminergic neurones which are reminiscent of drug actions. Whether these influences play a role in the therapeutic effects of exercise in human drug craving remains to be established.
Addict Biol, 2021; 26
32971218: Hurel I, Muguruza C, Redon B, Marsicano G, Chaouloff F
Cannabis and exercise: Effects of Δ-tetrahydrocannabinol on preference and motivation for wheel-running in mice.
Recent surveys have revealed close links between cannabis and exercise. Specifically, cannabis usage before and/or after exercise is an increasingly common habit primarily aimed at boosting exercise pleasure, motivation, and performance whilst facilitating post-exercise recovery. However, whether these beliefs reflect the true impact of cannabis on these aspects of exercise is unknown. This study has thus examined the effects of cannabis' main psychoactive ingredient, namely Δ-tetrahydrocannabinol (THC), on (i) mouse wheel-running preference and performance and (ii) running motivation and seeking behaviour. Wheel-running preference and performance were investigated using a T-maze with free and locked wheels located at the extremity of either arm. Running motivation and seeking were assessed by a cued-running operant task wherein wheel-running was conditioned by nose poking. Moreover, because THC targets cannabinoid type 1 (CB) receptors, i.e. receptors previously documented to control running motivation, this study also assessed the role of these receptors in running preference, performance, and craving-like behaviour. Whilst acute blockade or genetic deletion of CB receptors decreased running preference and performance in the T-maze, THC proved ineffective on either variable. The failure of THC to affect running variables in the T-maze extended to running motivation, as assessed by cued-running under a progressive ratio (PR) reinforcement schedule. This ineffectiveness of THC was not related to the treatment protocol because it successfully increased motivation for palatable food. Although craving-like behaviour, as indexed by a cue-induced reinstatement of running seeking, was found to depend on CB receptors, THC again proved ineffective. Neither running motivation nor running seeking were affected when CB receptors were further stimulated by increasing the levels of the endocannabinoid 2-arachidonoylglycerol. These results, which suggest that the drive for running is insensitive to the acute stimulation of CB receptors, raise the hypothesis that cannabis is devoid of effect on exercise motivation. Future investigation using chronic administration of THC, with and without other cannabis ingredients (e.g. cannabidiol), is however required before conclusions can be drawn.
Prog Neuropsychopharmacol Biol Psychiatry, 2021; 105
31706198: Redon B, Violleau C, Georges F, Marsicano G, Chaouloff F
The ergogenic impact of the glucocorticoid prednisolone does not translate into increased running motivation in mice.
Glucocorticoids, such as prednisolone, are considered sport doping agents owing to their ergogenic properties. These are accounted for by peripheral mechanisms associated with energetic and anti-inflammatory processes. However, because glucocorticoids target brain tissues, it is likely that these ergogenic impacts are associated with central effects. One of these might be reward motivation, which relies on glucocorticoid receptor-expressing mesocorticolimbic dopaminergic neurons. In keeping with this possibility, this study has explored in mice whether repeated prednisolone administration (5 or 15 µg/ml of drinking water for 10 days) affected intrinsic motivation for running, a strong reinforcer in rodents. Running motivation was assessed by means of a cued-reward motivated instrumental task wherein wheel-running was conditioned by prior nose poke responses under fixed (FR), and then progressive (PR), ratio reinforcement schedules. Sub-chronic ingestion of prednisolone decreased the running distance covered during each rewarded sequence under FR schedules. This finding did not extend to wheel-running performances in mice provided free (i.e. unconditioned) wheel-running opportunities. Running motivation, as estimated under a PR reinforcement schedule, was found to be decreased (lowest concentration) or to remain unaffected (highest concentration) by prednisolone concentration. Lastly, an inter-individual analysis of the respective effects of prednisolone on muscular endurance (as assessed in the wire grid-hanging test) and on running motivation indicated that the former was not predictive of the latter. This observation suggests that prednisolone ergogenic impacts might occur without any concomitant increase in intrinsic exercise motivation.
Psychoneuroendocrinology, 2020; 111
33654877: Redon B, Hurel I, Marsicano G, Chaouloff F
An Operant Conditioning Task to Assess the Choice between Wheel Running and Palatable Food in Mice.
Wheel running, especially in the homecage, has been widely used to study the neurobiology of exercise because animal tends to use it voluntarily. However, as for each reward, its consumption (in the present case, running performance) does not specifically provide information on its incentive value, , the extent to which animals are motivated to run independently from

their consumption of that reward. This is a major drawback, especially when focusing on the neurobiology governing the pathological imbalances between exercise and , feeding (obesity, anorexia nervosa). Yet, few studies have shown that operant conditioning wherein wheel-running is used as a reinforcer that can be "consumed" after nose-poking or lever-pressing allows to distinguish motivation from consumption. Thus, nose-poking or lever-pressing under a progressive ratio schedule of reinforcement in animals trained under fixed ratio reinforcement schedules provides, through the so-called breakpoint, an index of running motivation. As compared to wheel-running, numerous studies have used food as a reinforcer, which helped to uncover the neurobiology of feeding. However, to our knowledge, there is no paradigm allowing the assessment of the choice between running and feeding when presented in concurrence, with the possibility to measure the motivation for each reward. Herein, we describe a protocol that first permits to measure the drive for each of these two rewards before it allows to measure the preference for one over the other in a reward choice setting. This paradigm could help to better characterize the neurobiology underlying pathological imbalances between physical activity and feeding, which is the core feature of eating disorders.
Bio Protoc, 2019; 9
31164828: Hurel I, Redon B, Scocard A, Malezieux M, Marsicano G, Chaouloff F
Beyond the Activity-Based Anorexia Model: Reinforcing Values of Exercise and Feeding Examined in Stressed Adolescent Male and Female Mice.
Anorexia nervosa (AN), mostly observed in female adolescents, is the most fatal mental illness. Its core is a motivational imbalance between exercise and feeding in favor of the former. The most privileged animal model of AN is the "activity-based anorexia" (ABA) model wherein partly starved rodents housed with running wheels exercise at the expense of feeding. However, the ABA model bears face and construct validity limits, including its inability to specifically assess running motivation and feeding motivation. As infant/adolescent trauma is a precipitating factor in AN, this study first analyzed post-weaning isolation rearing (PWIR) impacts on body weights and wheel-running performances in female mice exposed to an ABA protocol. Next, we studied through operant conditioning protocols i) whether food restriction affects in a sex-dependent manner running motivation before ii) investigating how PWIR and sex affect running and feeding drives under fed conditions and food restriction. Besides amplifying ABA-elicited body weight reductions, PWIR stimulated wheel-running activities in anticipation of feeding in female mice, suggesting increased running motivation. To confirm this hypothesis, we used a cued-reward motivated instrumental task wherein wheel-running was conditioned by prior nose poke responses. It was first observed that food restriction increased running motivation in male, but not female, mice. When fed grouped and PWIR mice were tested for their running and palatable feeding drives, all mice, excepted PWIR males, displayed increased nose poke responses for running over feeding. This was true when rewards were proposed alone or within a concurrent test. The increased preference for running over feeding in fed females did not extend to running performances (time, distance) during each rewarded sequence, confirming that motivation for, and performance during, running are independent entities. With food restriction, mice displayed a sex-independent increase in their preference for feeding over running in both group-housed and PWIR conditions. This study shows that the ABA model does not specifically capture running and feeding drives, i.e. components known to be affected in AN.
Front Pharmacol, 2019; 10
30843884: Muguruza C, Redon B, Fois GR, Hurel I, Scocard A, Nguyen C, Stevens C, Soria-Gomez E, Varilh M, Cannich A, Daniault J, Busquets-Garcia A, Pelliccia T, Caillé S, Georges F, Marsicano G, Chaouloff F
The motivation for exercise over palatable food is dictated by cannabinoid type-1 receptors.
The lack of intrinsic motivation to engage in, and adhere to, physical exercise has major health consequences. However, the neurobiological bases of exercise motivation are still unknown. This study aimed at examining whether the endocannabinoid system (ECS) is involved in this process. To do so, we developed an operant conditioning paradigm wherein mice unlocked a running wheel with nose pokes. Using pharmacological tools and conditional mutants for cannabinoid type-1 (CB1) receptors, we provide evidence that CB1 receptors located on GABAergic neurons are both necessary and sufficient to positively control running motivation. Conversely, this receptor population proved dispensable for the modulation of running duration per rewarded sequence. Although the ECS mediated the motivation for another reward, namely palatable food, such a regulation was independent from CB1 receptors on GABAergic neurons. In addition, we report that the lack of CB1 receptors on GABAergic neurons decreases the preference for running over palatable food when mice were proposed an exclusive choice between the two rewards. Beyond providing a paradigm that enables motivation processes for exercise to be dissected either singly or in concurrence, this study is the first to our knowledge to identify a neurobiological mechanism that might contribute to sedentary behavior.
JCI Insight, 2019; 4
30174119: Busquets-Garcia A, Oliveira da Cruz JF, Terral G, Pagano Zottola AC, Soria-Gómez E, Contini A, Martin H, Redon B, Varilh M, Ioannidou C, Drago F, Massa F, Fioramonti X, Trifilieff P, Ferreira G, Marsicano G
Hippocampal CB Receptors Control Incidental Associations.

By priming brain circuits, associations between low-salience stimuli often guide future behavioral choices through a process known as mediated or inferred learning. However, the precise neurobiological mechanisms of these incidental associations are largely unknown. Using sensory preconditioning procedures, we show that type 1 cannabinoid receptors (CBR) in hippocampal GABAergic neurons are necessary and sufficient for mediated but not direct learning. Deletion and re-expression of CBR in hippocampal GABAergic neurons abolishes and rescues mediated learning, respectively. Interestingly, paired presentations of low-salience sensory cues induce a specific protein synthesis-dependent enhancement of hippocampal CBR expression and facilitate long-term synaptic plasticity at inhibitory synapses. CBR blockade or chemogenetic manipulations of hippocampal GABAergic neurons upon preconditioning affect incidental associations, as revealed by impaired mediated learning. Thus, CBR-dependent control of inhibitory hippocampal neurotransmission mediates incidental associations, allowing future associative inference, a fundamental process for everyday life, which is altered in major neuropsychiatric diseases. VIDEO ABSTRACT.
Neuron, 2018; 99

28220044: Busquets-Garcia A, Soria-Gómez E, Redon B, Mackenbach Y, Vallée M, Chaouloff F, Varilh M, Ferreira G, Piazza PV, Marsicano G

Pregnenolone blocks cannabinoid-induced acute psychotic-like states in mice.

Cannabis-induced acute psychotic-like states (CIAPS) represent a growing health issue, but their underlying neurobiological mechanisms are poorly understood. The use of antipsychotics and benzodiazepines against CIAPS is limited by side effects and/or by their ability to tackle only certain aspects of psychosis. Thus, safer wide-spectrum treatments are currently needed. Although the blockade of cannabinoid type-1 receptor (CB1) had been suggested as a therapeutical means against CIAPS, the use of orthosteric CB1 receptor full antagonists is strongly limited by undesired side effects and low efficacy. The neurosteroid pregnenolone has been recently shown to act as a potent endogenous allosteric signal-specific inhibitor of CB1 receptors. Thus, we tested in mice the potential therapeutic use of pregnenolone against acute psychotic-like effects of Δ-tetrahydrocannabinol (THC), the main psychoactive component of cannabis. We found that pregnenolone blocks a wide spectrum of THC-induced endophenotypes typically associated with psychotic-like states, including impairments in cognitive functions, somatosensory gating and social interaction. In order to capture THC-induced positive psychotic-like symptoms (e.g. perceptual delusions), we adapted a behavioral paradigm based on associations between different sensory modalities and selective devaluation, allowing the measurement of mental sensory representations in mice. Acting at hippocampal CB1 receptors, THC impaired the correct processing of mental sensory representations (reality testing) in an antipsychotic- and pregnenolone-sensitive manner. Overall, this work reveals that signal-specific inhibitors mimicking pregnenolone effects can be considered as promising new therapeutic tools to treat CIAPS.
Mol Psychiatry, 2017; 22

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BOARD NUMBER: S03-082

SOCIAL INTERACTION MODULATES NOXIOUS THRESHOLDS IN ZEBRAFISH

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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We cope better when we are not alone. Supportive social environments are known to have an analgesic effect, significantly reducing our perception of pain. Functional and anatomical studies in humans have indicated that the nociceptive and social circuits share overlapping areas of activity in the brain. However, the underlying circuitry as well as its molecular mechanisms are poorly understood. To better understand how pain and social circuits can modulate one another, we study juvenile zebrafish. An ideal model for this study, zebrafish are highly social, show stereotyped responses to noxious stimuli, and allow whole brain imaging with single cell resolution. We developed a new behavioural assay where fish are exposed to both social and noxious heat stimuli simultaneously. This assay has provided evidence that the mere sight of other, conspecific zebrafish is enough to increase heat tolerance, strongly suggesting the presence of a descending pain modulatory pathway that is activated by social context, similar to humans. Several genes of the descending pathway have already been identified in humans and other animal models. However, they have only been characterised individually, limiting our understanding of their functional role. Zebrafish offer the unique advantage of studying the system as a whole. By using a combination of anatomical, functional and pharmacological tools, we aim to identify key brain areas and genes involved in modulating pain responses.

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BOARD NUMBER: S03-083

CHARACTERIZATION OF PARENTAL CAREGIVING OF SICK OFFSPRING IN MICE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Most organisms respond to infection and injury with physiological changes and an array of recovery-focused 'sickness behaviors' which include fatigue, appetite loss, and altered social behavior. These behaviors may thus act as social signals allowing for detection and response to sickness. While most investigations of inflammatory-induced sickness focus on social withdrawal and avoidance behaviors, there is evidence that sickness can instead promote familiarity-dependent prosociality. **Aims**
Here we identify how a mouse dam responds with directed caregiving and maternal behaviors toward sick juvenile offspring. We further test whether dams display a preference for sick vs. healthy offspring. **Methods**
Using a multi-camera longitudinal behavioral monitoring system coupled with semi-automated analysis of spontaneous behaviors, we characterized and compared maternal and caregiving behaviors of a mouse dam toward juvenile offspring injected with lipopolysaccharides (LPS) vs. saline controls over the course of 48-hours. Behaviors of interest included pup retrieval, nursing, grooming, nestbuilding, sniffing, huddling, and allo-grooming. Dams were also tested in a social preference assay and time spent in a chamber with LPS- vs. saline-injected offspring and non-offspring was measured. **Results**
Our results show that dams display increased approach and huddling behavior toward LPS-injected offspring vs. saline controls over the course of 48 hours. Dams also spend more time in the chamber with LPS-injected offspring than saline-injected offspring in the social preference test. We do not see this effect with non-offspring. **Conclusion**
These data indicate that dams exhibit increased caregiving behavior toward sick vs. healthy offspring and display a familiarity-dependent preference toward sick juveniles.

Pubmed:
28359729: Zhao Y, Lee JH, Chen D, Gu X, Caslin A, Li J, Yu SP, Wei L
DL-3-n-butylphthalide induced neuroprotection, regenerative repair, functional recovery and psychological benefits following traumatic brain injury in mice.
Previous investigations suggest that DL-3-n-butylphthalide (NBP) is a promising multifaceted drug for the treatment of stroke. It is not clear whether NBP can treat traumatic brain injury (TBI) and what could be the mechanisms of therapeutic benefits. To address these issues, TBI was induced by a controlled cortical impact in adult male mice. NBP (100 mg/kg) or saline was intraperitoneally administered within 5 min after TBI. One day after TBI, apoptotic events including caspase-3/9 activation, cytochrome c release from the mitochondria, and apoptosis-inducing factor (AIF) translocation into the nucleus in the pericontusion region were attenuated in NBP-treated mice compared to TBI-saline controls. In the assessment of the nuclear factor kappa-light-chain-enhancer of activated B (NF-κB) pathway, NBP ameliorated the p65 expression and the p-IκB-α/IκB-α ratio, indicating reduced NF-κB activation. Consistently, NBP reduced the upregulation of proinflammatory cytokines such as tumor necrotizing factor-alpha (TNF-α) and interleukin-1beta (IL-1β) after TBI. In sub-acute treatment experiments, NBP was intranasally delivered once daily for 3 days. At 3 days after TBI, this repeated NBP treatment significantly reduced the contusion volume and cell death in the pericontusion region. In chronic experiments up to 21 days after TBI, continues daily intranasal NBP treatment increased neurogenesis, angiogenesis, and arteriogenesis in the post-TBI brain, accompanied with upregulations of regenerative genes including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), endothelial-derived nitric oxide synthase (eNOS), and matrix metallopeptidase 9 (MMP-9). The NBP treatment significantly improved sensorimotor functional recovery and reduced post-TBP depressive behavior. These new findings demonstrate that NBP shows multiple therapeutic benefits after TBI.
Neurochem Int, 2017; 111
27184741: Lee JH, Espinera AR, Chen D, Choi KE, Caslin AY, Won S, Pecoraro V, Xu GY, Wei L, Yu SP

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Neonatal inflammatory pain and systemic inflammatory responses as possible environmental factors in the development of autism spectrum disorder of juvenile rats.

Autism spectrum disorder (ASD) affects many children and juveniles. The pathogenesis of ASD is not well understood. Environmental factors may play important roles in the development of ASD. We examined a possible relationship of inflammatory pain in neonates and the development of ASD in juveniles.

J Neuroinflammation, 2016; 13

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BOARD NUMBER: S03-084

EXPOSURE TO AIR POLLUTION NANOPARTICLES: DEPRESSIVE-LIKE BEHAVIORS, LEARNING AND MEMORY IMPAIRMENT AND ALTERS OF HIPPOCAMPAL CYTOKINES EXPRESSION

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Air pollution particulate matter (PM) is a world risk factor that the effects of long-term exposure to these factors in terms of damage to cardiovascular and pulmonary function are well known, but little is known comparatively about the effects of PM on emotional and cognitive processes. Exposure to ultrafine particulate matter (UFPs<100 nm (PM0.1)), can be adversely impacted the central nervous system (CNS) by the activation of proinflammatory pathways and reactive oxygen species associated with air pollution particulate matter. Therefore, we investigated whether prolonged exposure to diesel exhaust particles (DEPs) affects hippocampal inflammatory cytokines, emotional responses, learning and memory, cognition, and neuronal morphology. Male mice were DEPs exposed for 6 and 14 weeks. Mice exposed to DEPs showed more disorders in spatial memory and learning and depressive-like responses than control mice. Expression of hippocampal pro-inflammatory cytokine was increased among DEPs exposure mice. The density of neurons in hippocampus CA1, CA3, and dentate gyrus (DG) regions decreased in DEPs mice, respectively. Overall, these findings show that prolonged exposure to DEPs in the world's major cities can alter behavior and impair cognition. **Keywords:** Depression; Air pollution Nano-particles; Learning and Memory Neuroinflammation; Hippocampal morphology

Pubmed:

34302822: Ehsanifar M
Airborne aerosols particles and COVID-19 transition.
With the outbreak of Coronavirus (2019) (COVID-19), as of late March 2020, understanding how the cause of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) transmitted is one of the most important questions that researchers are seeking to answer; because this effort helps to reduce the spread of disease. The COVID-19 is highly transmissible and deadly. Despite "tracking the call" and carefully examining patient contact, it is not yet clear how the virus is transmitted from one sick person to another. Why it is so transmissible? Can viruses be transmitted through speech and exhalation aerosols? How far can these aerosols go? How long can an aerosol containing a virus stay in the air? Is the virus amount in these aerosols enough to lead to an infection? There is no consensus on aerosols' role in the transmission of SARS-CoV-2. Findings show that SARS-CoV-2 aerosol transmission is possible. Therefore, to effectively reduce SARS-CoV-2, precautionary control strategies for aerosol transfer should be considered. Our aim is to review the evidence of the aerosol transmission containing SARS-CoV-2.
Environ Res, 2021; 200
34246879: Guo M, Fang Y, Zhu J, Chen C, Zhang Z, Tian X, Xiang H, Manyande A, Ehsanifar M, Jafari AJ, Xu F, Wang J, Peng M
Investigation of metabolic kinetics in different brain regions of awake rats using the [H-C]-NMR technique.
Energy metabolism and neurotransmission are necessary for sustaining normal life activities. Hence, neurological or psychiatric disorders are always associated with changes in neurotransmitters and energy metabolic states in the brain. Most studies have only focused on the most important neurotransmitters, particularly GABA and Glu, however, other metabolites such as NAA and aspartate which are also very important for cerebral function are rarely investigated. In this study, most of the metabolic kinetics information of different brain regions was investigated in awake rats using the [H-C]-NMR technique. Briefly, rats (n = 8) were infused [1-C] glucose through the tail vein for two minutes. After 20 min of glucose metabolism, the animals were sacrificed and the brain tissue was extracted and treated. Utilizing the H observed/C-edited nuclear magnetic resonance (POCE-NMR), the enrichment of neurochemicals was detected which reflected the metabolic changes in different brain regions and the metabolic connections between neurons and glial cells in the brain. The results suggest that the distribution of every metabolite differed from every brain region and the metabolic rate of NAA was relatively low at 8.64 ± 2.37 μmol/g/h. In addition, there were some correlations between several C enriched metabolites, such as Glu-Gln ($p = 0.062$), Glu-GABA ($p < 0.01$), Glx-Glx ($p < 0.001$), Asp-NAA ($p < 0.001$). This correlativity reflects the signal transmission

between astrocytes and neurons, as well as the potential interaction between energy metabolism and neurotransmission. In conclusion, the current study systematically demonstrated the metabolic kinetics in the brain which shed light on brain functions and the mechanisms of various pathophysiological states.
J Pharm Biomed Anal, 2021; 204
34150234: Ehsanifar M, Jafari AJ, Montazeri Z, Kalantari RR, Gholami M, Ashtarinezhad A
Learning and memory disorders related to hippocampal inflammation following exposure to air pollution.
It has been demonstrated that sub-chronic exposure to air pollution containing nanoscale (<100 nm) diesel exhaust particles (DEPs) may lead to excessive oxidative stress and neuro-inflammation in adult male mice. Hereby, we investigated the effects of DEPs on hippocampus-dependent spatial learning and neuro-inflammation and memory-related gene expression in male mice. In this study, we divided 48 adult NMRI male mice into control group VS. three exposure groups. Mice were exposed to 300-350 μg/m DEPs for 2, 5, and 7 h daily for 12 weeks. The Morris Water Maze (MWM) and Elevated Plus Maze device were used to examine anxiety, spatial memory and learning, respectively. The mRNAs expression of pro-inflammatory cytokines, N-methyl-D-aspartate (NMDA) receptor subunits, and glutaminase were studied in hippocampus (HI) by real-time RT-PCR. Besides, malondialdehyde (MDA) tests were used to determine the state of oxidative stress. After 5 and 7 h. of DEPs exposure, mRNA expression of NR2A and NR3B IL1α, IL1β, TNFα, NMDA receptor subunits and MDA levels increased significantly (< 0.05). Also, DEPs exposed mice for 2, 5, and 7 h. showed diminished entrance into open arms with short time spent there. Indeed, 5 and 7 h/day exposed mice required a longer time to reach the hidden platform. Sub-chronic exposure to DEPs increased oxidative stress markers, neuroinflammation, anxiety, impaired spatial learning and memory.
J Environ Health Sci Eng, 2021; 19
33582162: Ehsanifar M, Montazeri Z, Taheri MA, Rafati M, Behjati M, Karimian M
Hippocampal inflammation and oxidative stress following exposure to diesel exhaust nanoparticles in male and female mice.
Air pollution exposure is among the most prevalent reasons for environmentally-induced oxidative stress and inflammation, both of which are involved in the development and progression of central nervous system (CNS) diseases. Ultrafine particles (UFPs) plays an important role in global air pollution and the diesel exhaust particles (DEPs) are the most important component in this regard. There are more than 40 toxic air pollutants in diesel exhaust (DE), which is one of the main constituents of an environmental pollutant and including particulate matter (PM) especially UFPs. Thus, in this study, adult female and male NMRI mice were exposed to DEPs (350-400 μg/m) for 14 weeks (6 h per day and 5 days per week). After 14 weeks of exposure, expression of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, TNF-α), nNOS, HO1, NR2A, and NR2B and malondialdehyde (MDA) level were analyzed in various brain regions such as the hippocampus (HI) and olfactory bulb (OB). Exposure to DEPs caused neuroinflammation and oxidative stress in female and male mice. That these effects observed in females were less pronounced than in male mice. The male mice emerged to be more susceptible significantly than the female mice to induced neuroinflammation following DEPs exposure. Also, our findings indicate that long term exposure to DEPs results in altered expression of hippocampal NMDA receptor subunits, and suggests that gender can play important role in the modulating susceptibility to neurotoxicity induced by DEPs exposure.
Neurochem Int, 2021; 145
30921694: Ehsanifar M, Jafari AJ, Nikzad H, Zavareh MS, Atlasi MA, Mohammadi H, Tameh AA
Prenatal exposure to diesel exhaust particles causes anxiety, spatial memory disorders with alters expression of hippocampal pro-inflammatory cytokines and NMDA receptor subunits in adult male mice offspring.
Air pollution by Diesel exhaust (DE) consists of gaseous compounds and diesel exhaust particles (DEPs). Previous studies show associations between prenatal exposure to diesel exhaust affects the central nervous system (CNS). However, there was not reported that these effects were caused by gaseous compounds, diesel exhaust particles, or both. A limited number of studies in rodent models have shown that exposure to DEPs can result in CNS. Here, we explored the effects of prenatal exposure to DEPs on anxiety and learning and memory in NMRI mice male offspring. Three groups of pregnant mice were exposed to 350-400 μg DEPs/m for 2, 4 and 6 h daily in a closed system room. We examined anxiety and learning and memory in 8-to-9-week-old male offspring using the Elevated plus maze and Morris water maze (MWM) test. Hippocampi were isolated after the behavioral tests and measured pro-inflammatory cytokines and N-methyl-D-aspartate (NMDA) receptor expression by quantitative RT-PCR analysis. Mice exposed to DEPs in utero showed deficits in the Elevated plus maze and Morris water maze test. In addition, DEPs exposed mice exhibited decreased hippocampal NR2A and NR3B expression. Taken together, our data suggest that maternal DEP exposure is associated with anxiety, disrupts learning and memory and reduction hippocampal NR2A and NR3B expression in male offspring.
Ecotoxicol Environ Saf, 2019; 176
30391838: Ehsanifar M, Tameh AA, Farzadkia M, Kalantari RR, Zavareh MS, Nikzaad H, Jafari AJ
Exposure to nanoscale diesel exhaust particles: Oxidative stress, neuroinflammation, anxiety and depression on adult male mice.
Exposure to nanoscale diesel engines exhausted particles (DEPs) is a well-recognized risk factor for respiratory and

cardiovascular diseases. Rodents as commonly used models for urban air pollution in health effect studies demonstrate constant stimulation of inflammatory responses in the main areas of the brain. Nevertheless, the primary effect of diesel exhaust particulate matter on some of the brain regions and relation by behavioral alterations still remains untouched. We evaluated the brain regional inflammatory responses to a nanosized subfraction of diesel engines exhaust particulate matter (DEPs < 200 nm) in an adult male mice brain. Adult male mice were exposed to DEPs for 3, 6, and 8 h per day, 12 weeks and five days per week. Degree of anxiety and the depression by elevated plus maze and Forced Swimming Test respectively (FST) did measurement. After behavior tests, the plasma and some of the brain regions such as olfactory bulb (OB) and hippocampus (HI) were analyzed for oxidative stress and inflammatory responses. The inflammation and oxidative stress changes in OB and HI, markedly coincides with the results of behavioral alterations. These responses corresponded with rapid induction of MDA and nitrite oxide (NO) in brain regions and neuronal nitric oxide synthase (nNOS) mRNA followed by IL6, IL1α, and TNFα in OB and HI. The different times of DEPs exposure, leads to oxidative stress and inflammatory in plasma and brain regions. That this cumulative transport of inhaled nanoscale DEPs into the brain and creating to inflammation responses of brain regions may cause problems of brain function and anxiety and depression.
Ecotoxicol Environ Saf, 2019; 168

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BOARD NUMBER: S03-085

EARLY LIFE ADOPTION SHOWS REARING ENVIRONMENT SUPERSEDES TRANSGENERATIONAL EFFECTS OF PATERNAL STRESS ON AGGRESSIVE TEMPERAMENT IN THE OFFSPRING.

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Ipshita Zutshi[1], Sonakshi Gupta[2], Olivia Zanoletti[3], Carmen Sandi[3], Guillaume Poirier[3]
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Prenatal experience and transgenerational influences are increasingly recognized as critical for defining the socio-emotional system, through the development of social competences and of their underlying neural circuitries. Here, we used an established rat model of social stress resulting from male partner aggression induced by peripubertal (P28-42) exposure to unpredictable fearful experiences. Using this model, we aimed to first, characterize adult emotionality in terms of the breadth of the socio-emotional symptoms and second, to determine the relative impact of prenatal vs postnatal influences. For this purpose, male offspring of pairs comprising a control or a peripubertally stressed male were cross-fostered at birth and tested at adulthood on a series of socio-emotional tests. In the offspring of peripubertally stressed males, the expected antisocial phenotype was observed, as manifested by increased aggression towards a female partner and a threatening intruder, accompanied by lower sociability. This negative outcome was yet accompanied by better social memory as well as enhanced active coping, based on more swimming and longer latency to immobility in the forced swim test, and less immobility in the shock probe test. Furthermore, the cross-fostering manipulation revealed that these adult behaviors were largely influenced by the post- but not the prenatal environment, an observation contrasting with both pre- and postnatal effects on attacks during juvenile play behavior. Adult aggression, other active coping behaviors, and social memory were determined by the predominance at this developmental stage of postnatal over prenatal influences. Together, our data highlight the relative persistence of early life influences.

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BOARD NUMBER: S03-086

MITOCHONDRIAL ASTROCYTIC CB1 ARE NECESSARY FOR THE AMNESIC EFFECTS OF SOCIALLY TRANSMITTED STRESS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Paula Gomez-Sotres[1], Urszula Skupio[2], Astrid Cannich[2], Francisca Julio-Kalajzic[2], Doriane Gisquet[1], Jaideep Bains[3], Giovanni Marsicano[2]
[1]INSERM U1215, Universite de Bordeaux, Neurocentre Magendie, Bordeaux Cedex, France, [2]Inserm U1215, Universite de Bordeaux, Neurocentre Magendie, Bordeaux Cedex, France, [3]University of Calgary, Physiology & Pharmacology, Calgary, Canada

AIMS: Stress impacts cognitive processes like memory. Stress is also transmitted to others, but whether transmitted stress affects memory is not known. Here we explored mechanisms responsible for the detection of stress and then used this information to ask whether transmitted stress affects memory. We focused on olfactory signaling, and specifically, the role of type-1 cannabinoid receptors (CB1R) in astrocytes of the olfactory bulb in these processes. METHODS: We investigated social interactions between a naive observer mouse (OBS) and a stressed demonstrator (DEM) and then assessed memory performance in a novel object recognition (NOR) long-term memory test. We used several conditional mutant lines and viral approaches to understand the contribution of CB1 receptors to this behavior. RESULTS: Here we show that socially-transmitted stress impairs NOR in the OBS mouse, an effect that phenocopies the deficit observed in the stressed DEM. Interestingly, genetic deletion of CB1R in astrocytes decreased time OBS spent exploring the anogenital region of the stressed DEM and did not show any impairment of memory. An identical phenotype was observed in mice expressing a mutated form of the CB1R, which excludes localization in mitochondria. Finally, loss of mtCB1R specifically in olfactory bulb astrocytes produced deficits in anogenital investigation and abolished amnesia in OBS mice. CONCLUSION: These findings indicate that mtCB1Rs in astrocytes of the olfactory bulb are a critical hub for guiding behaviors related to detection of negative affective states and for linking the consequent emotional reciprocity to memory alterations.

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BOARD NUMBER: S03-087

RFAMIDE-RELATED PEPTIDE (RFRP) NEURONS DRIVE ANXIETY-LIKE AND DEPRESSION-LIKE BEHAVIOURS IN MALE MICE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

India Sawyer, Greg Anderson
University of Otago, Centre For Neuroendocrinology And Department Of Anatomy, Dunedin, New Zealand

Activation of RFRP neurons using designer receptors increases corticosterone and central infusion of the peptide RFRP-3 increases anxiety-like behaviours in female mice[1,2]. Additionally, RFRP neurons express glucocorticoid receptors and are activated by stressor exposure and corticosterone[3]. This suggests RFRP neurons are involved in driving various stress responses, and amplifying them following glucocorticoid feedback. This research aimed to elucidate the role of RFRP neurons in anxiety-like and depression-like behaviours in mice. Mice expressing the stimulatory hM3Dq-DREADD receptor in RFRP-Cre neurons, through breeding or viral transfection, were treated with clozapine-n-oxide (CNO; 1-2 mg/kg) before testing in an elevated-plus maze followed by a forced-swim test and one week later before testing in a light-dark box followed by a tail-suspension test. There was a significant ~40% increase in immobility in depressive-like behavioural tests in bred RFRP-Cre-hM3Dq male mice vs control mice ($p<0.05$), whereas in AAV-hM3Dq transfected male mice there was a significant two-fold reduction in open space exploration in the elevated plus maze, showing increased anxiety-like behaviours, vs control mice ($p<0.05$). RFRP neuronal activation did not markedly alter female behaviours. Acute activation of RFRP neurons increases anxiety-like and depressive-like behaviours in male mice, and elicits a corticosterone response, indicating RFRP neurons are involved in the relay of stressor information to both the neuroendocrine and behavioural stress responses. This implicates RFRP neurons as potential therapeutic targets for treating HPA axis dysfunction and mental health disorders. 1 Mamgain et al., 2020, JNeurosci.
2 Kim et al., 2015, Endocrinology.
3 Kirby et al., 2009, PNAS.

Pubmed:
33219002: Mamgain A, Sawyer IL, Timajo DAM, Rizwan MZ, Evans MC, Ancel CM, Inglis MA, Anderson GM
RFamide-Related Peptide Neurons Modulate Reproductive Function and Stress Responses.
RF-amide related peptide 3 (RFRP-3) is a neuropeptide thought to inhibit central regulation of fertility. We investigated whether alterations in RFRP neuronal activity led to changes in puberty onset, fertility, and stress responses, including stress and glucocorticoid-induced suppression of pulsatile luteinizing hormone secretion. We first validated a novel RFRP-Cre mouse line, which we then used in combination with Cre-dependent neuronal ablation and DREADD technology to selectively ablate, stimulate, and inhibit RFRP neurons to interrogate their physiological roles in the regulation of fertility and stress responses. Chronic RFRP neuronal activation delayed male puberty onset and female reproductive cycle progression, but RFRP-activated and ablated mice exhibited apparently normal fertility. When subjected to either restraint- or glucocorticoid-induced stress paradigms. However, we observed a critical sex-specific role for RFRP neurons in mediating acute and chronic stress-induced reproductive suppression. Female mice exhibiting RFRP neuron ablation or silencing did not exhibit the stress-induced suppression in pulsatile luteinizing hormone secretion observed in control mice. Furthermore, RFRP neuronal activation markedly stimulated glucocorticoid secretion, demonstrating a feedback loop whereby stressful stimuli activate RFRP neurons, which in turn further activate the stress axis. These data provide evidence for a neuronal link between the stress and reproductive axes.
J Neurosci, 2021; 41

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BOARD NUMBER: S03-088

AUTOPHAGY AND NEURODEVELOPMENTAL DISORDERS 1 : SENSORY MOTOR DEVELOPMENT IN PREWEANING IRGM1-KO MICE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Loïc Angrand[1,2,3], Alexandra Chrétien[1,2,4], Gaël Grannec[3], Marika Nosten-Bertrand[3], Guillemette Crépeaux[1,2]
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Aims Autophagy is a catabolic process that degrades damaged macromolecules and organelles to preserve cellular homeostasis. Neuronal autophagy is also crucial during the development of the CNS and its refinement through synaptic pruning. Autophagy is a highly conserved process involving more than thirty autophagy-related (ATG) genes. In humans, ATG mutations and dysregulations of autophagy pathways have been reported in neurodegenerative and neuropsychiatric disorders. Our project aims to explore the role of the IRGM1 protein - involved in autophagy and related to innate immunity - during brain development and function. We thus initiate the study of neurodevelopmental impacts of *Irgm1* invalidation during preweaning stage (see also poster “Ultrasonic vocalization (USV) and social interaction from preweaning up to adult *Irgm1*-ko mice”). **Methods** The study is performed in C57BL/6NCrl *Irgm1*-ko mice (gift from Taylor, Duke University, USA). Sensorimotor battery of tests (surface righting, negative geotaxis, grasping, front-limb suspension, acoustic startle and eyelid opening) are performed between PND4 and PND17. USV are recorded in a maternal separation context at PND9 and analyzed with Avisoft software. **Results** To date, 4 litters of 4 to 9 pups were obtained from HzxHz mattings. Preliminary results indicate no premature death since pups of all genotypes and both sexes survived up two months. Experiments and data analysis are in progress. **Conclusions** Together, these behavioral studies with neurodevelopmental, -histological, -molecular and biochemical studies will be detrimental to evaluate the potential role of Irgm autophagic pathway in brain disorders. The model will subsequently be used for gene x environment interaction studies.

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BOARD NUMBER: S03-089

AUTOPHAGY AND NEURODEVELOPMENTAL DISORDERS 2 : ULTRASONIC VOCALIZATION (USV) AND SOCIAL INTERACTION FROM PREWEANING UP TO ADULT IRGM1-KO MICE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Alexandra Chrétien[1,2,3], Loïc Angrand[1,2,4], Gaël Grannec[3], Marika Nosten-Bertrand[3], Guillemette Crépeaux[1,2]
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Aims Autophagy is a catabolic process that degrades damaged macromolecules and organelles to preserve cellular homeostasis. Neuronal autophagy is also crucial during the development of the CNS and its refinement through synaptic pruning. Autophagy is a highly conserved process involving more than thirty autophagy-related (ATG) genes. In humans, ATG mutations and dysregulations of autophagy pathways have been reported in neurodegenerative and neuropsychiatric disorders. Our project aims to explore the role of the IRGM1 protein - involved in autophagy and closely related to innate immunity - during brain development and function. We thus initiate the study of USV during social interaction in *Irgm1*-ko young and adult mice (see poster “Sensory motor development in preweaning *Irgm1*-ko mice”). **Methods** The study is performed in C57BL/6NCrl *Irgm1*-ko mice (gift from Taylor, Duke University, USA). Same-sex dyadic social interaction paradigm are performed at PND20, PND33 and PND85. The sessions are video tracked, along with USV recording. In addition, locomotion and rearing activity are tested and automatically recorded at 9 and 12 weeks with Imetronic software. **Results** To date, 5 litters of 4 to 9 pups were obtained from HzxHz mattings.. Preliminary results indicate no premature death since pups of all genotypes and both sexes survived up two months. Experiments and data analysis are in progress. **Conclusions** Together, these behavioral studies with neurodevelopmental, -histological, -molecular and biochemical studies will be detrimental to evaluate the potential role of Irgm autophagic pathway in brain disorders. The model will subsequently be used for gene x environment interaction studies.

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BOARD NUMBER: S03-090

MOTHERHOOD CHANGES THE PROCESSING AND RESPONSE TO SOCIAL CUES OF FEMALE MICE.

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Sandra Sanahuja Irene, Rafael Goterris-Cerisuelo, María De La Cinta Navarro-Moreno, María José Sánchez Catalán, Fernando Martínez-García
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Motherhood entails changes in how females detect, process and react to social stimuli. After parturition, the social life of the dam is restricted to maternal care of the offspring and maternal aggression, e.g. furious attacks to adults approaching the nest. Therefore, male-derived pheromones change their significance during motherhood, from attractive to aggression-eliciting. The aim of this work is to understand the neural basis of this change. To do so, we performed two different experiments. First, we exposed virgin and late-pregnant female mice to pups or buttons (control). Second, virgin and postpartum females were exposed to clean or male soiled-bedding. Ninety minutes afterwards, females' brains were processed for immunohistochemistry of cFos, a proxy of neural activity. We quantitatively analysed the pattern of activity in three functional systems in the brain of females: a) nasal chemosensory systems (olfactory and vomeronasal); b) sociosexual brain network; c) brain circuit for motivation. The results indicate important changes in the processing of social cues in all three systems, with sharp differences relative to the activity of the motivation circuitry, which likely reflect hormone-induced changes in salience of the different social cues. Our results reinforce the view that a single neural network governs the expression of different social behaviours, instead of individual linear pathways controlling specific behaviours (maternal, aggressive, sexual, etc). Our data also stress the importance of motivation in the expression of social behaviours, which are also modulated by motherhood hormones. Funding: Generalitat Valenciana GV/2020/173; Spanish Ministry of Science and Innovation PID2019-107322GB-C21; Universitat Jaume I UJI-A2019-14

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BOARD NUMBER: S03-091

STRAIN AND SEX DIFFERENCES IN ANXIETY AND DEPRESSION-LIKE BEHAVIOR FOLLOWING EXPOSITION TO CHRONIC MILD STRESS IN MICE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Anzu G. Zevallos-Arias, Carlos Medina-Saldivar, Sergio R. Cruz-Visalaya, Grace E. Pardo, Luis F. Pacheco-Otalora
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Stress-related mood disorders such as anxiety and depression are highly prevalent in the population and affect the ability of individuals to function and live a rewarding life. Anxiety and depressive disorders are higher in women, and susceptibility is affected by multiple genetic, environmental stressors, and psychological factors. Research using inbreed mouse strain provides a relatively stable and narrow range of genetic and environmental variability that is valuable for unraveling interactions between genetic and environmental stressors factors. Here, we assess the effects of chronic mild stress (CMS) on anxiety and depressive-like behaviors of males and females of two inbred mice (C57BL/6NCrl and BALB/c) that differ in their sensitivity to stress. While exposure to CMS resulted in a consistent reduction of weight gain of males but not females in C57BL/6NCrl, BALB/c mice of both sexes were little affected at the end of CMS protocol. CMS increased anxiety-like behavior in female but not male BALB/c strain mice with no effect on C57 BL/6NCrl mice of both sexes. Exposure to CMS increased depressive-like behaviors in males but not females of C57BL/6NCrl strain mice. On the contrary, males but not females of the BALB/c strain reduced depressive-like behaviors phenotypes. These findings suggest that C57/6NCrl mice strains are more vulnerable to CMS than BALB/c mice, being male mice more susceptible to CMS than females in terms of the severity of depressive behaviors.

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MATERNAL HIGH-FAT DIET-INDUCED MICROBIAL CHANGES ARE ASSOCIATED WITH ALTERED FOETAL BRAIN METABOLOME AND ADOLESCENT BEHAVIOUR

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

Anna Ratsika[1,2], Martin Codagnone[1,2], Thomaz Bastiaanssen[1,2], Caoimhe Lynch[1,2], Ana Paula Ventura Silva[1,2], Valentina Caputi[1,2], Christine Fülling[1,2], John Cryan[1,2]
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Maternal overweight and obesity perinatally has been linked to changes in neurodevelopment, plasticity and affective disorders in the offspring, with implications for microbial input from maternal intestines. Here, we investigate how maternal microbial signals imprint foetal brain metabolomics and adolescent behaviour. Adult female C57/Bl6 mice were fed either a control diet (CD: 10% fat) or a high-fat diet (HFD: 60% fat) from 8 weeks prior to mating throughout lactation. Whole brain metabolomic analysis in embryos and maternal caecal microbiota composition were assessed at embryonic day 18 (E18). Locomotor activity and anxiety-like behaviour were assessed in the adolescent offspring. HFD induced weight gain (p=0.0098 HFDvsCD) and altered caecal microbial composition (p=0.0022 HFDvsCD) in dams at E18. Maternal HFD led to upregulation of microbial genes linked to quinolinic acid synthesis (p=0.005 HFDvsCD), a neuroplasticity mediator linked to glutamate metabolism. Metabolomic analysis of embryo brains detected molecules linked to glutamate-glutamine cycle mediated by diet, such as alanine (p=0.0011 HFDvsCD), glutamate (p=0.0029 HFDvsCD) and glutathione disulphide (p=0.0001 HFDvsCD). Pathway enrichment analysis of the differentially abundant metabolites revealed 35 times higher glutamine and glutamate metabolism in the foetal brains than expected (p= 0.00115 HFDvsCD). Female adolescent offspring from HFD-fed dams led to increased locomotor activity (total distance travelled p=0.004 HFDvsCD in open field) and anxiety-like behaviour (p=0.017 HFDvsLFD number of transitions in closed arms of the elevated plus maze). Our results suggest that maternal microbiota-mediated behavioural imprinting in the offspring is sex-specific, and might be linked to altered brain metabolites during critical developmental windows perinatally.

Pubmed:

33525617: Ratsika A, Codagnone MC, O'Mahony S, Stanton C, Cryan JF
Priming for Life: Early Life Nutrition and the Microbiota-Gut-Brain Axis.
Microbes colonize the human body during the first moments of life and coexist with the host throughout the lifespan. Intestinal microbiota and their metabolites aid in the programming of important bodily systems such as the immune and the central nervous system during critical temporal windows of development, with possible structural and functional implications throughout the lifespan. These critical developmental windows perinatally (during the first 1000 days) are susceptible timepoints for insults that can endure long lasting effects on the microbiota-gut-brain axis. Environmental and parental factors like host genetics, mental health, nutrition, delivery and feeding mode, exposure to antibiotics, immune activation and microbiota composition antenatally, are all factors that are able to modulate the microbiota composition of mother and infant and may thus regulate important bodily functions. Among all these factors, early life nutrition plays a pivotal role in perinatal programming and in the modulation of offspring microbiota from birth throughout lifespan. This review aims to present current data on the impact of early life nutrition and microbiota priming of important bodily systems and all the factors influencing the microbial coexistence with the host during early life development.
Nutrients, 2021; 13
35122781: Cabré S, Ratsika A, Rea K, Stanton C, Cryan JF
Animal models for assessing impact of C-section delivery on biological systems.
There has been a significant increase in Caesarean section (C-section) births worldwide over the past two decades and although it can be a life-saving procedure, the enduring effects on host physiology are now undergoing further scrutiny. Indeed, epidemiological data have linked C-section birth with multiple immune, metabolic and neuropsychiatric diseases. Birth by C-section is known to alter the colonisation of the neonatal gut microbiota (with C-section delivered infants lacking vaginal microbiota associated with passing along the birth canal), which in turn can impact the development and maintenance of many important biological systems. Appropriate animal models are key to disentangling the role of missing microbes in

brain health and disease in C-section births. In this review of preclinical studies, we interrogate the effects of C-section birth on the development (and maintenance) of several biological systems and we discuss the involvement of the gut microbiome on C-section-related alterations.
Neurosci Biobehav Rev, 2022; 135

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BOARD NUMBER: S03-093

A THALAMO-PREOPTIC PATHWAY PROMOTING SOCIAL TOUCH

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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We previously identified the posterior intralaminar thalamic nucleus (PIL) as a relay station of socially relevant sensory information innervating and activating oxytocin-secreting neurons upon social encounter. Here, we addressed to characterize the exact role of the PIL neurons and their projections to the preoptic area of the hypothalamus in the control of the social behavior. First, we determined the effect of chemogenetic stimulation of PIL neurons on social interactions between familiar adult female rats. The projections of PIL neurons were analyzed using anterograde tract-tracing. The selective chemogenetic stimulation of the preoptic area-projecting PIL neurons was performed using double viral injections and also by CNO administration directly into the preoptic area. PIL projects to several socially implicated brain regions, such as the medial amygdala, the medial preoptic area and the infralimbic cortex. Chemogenetic stimulation of the PIL resulted in the activation of previously anatomically identified target areas and increased the duration of social grooming. Direct contact during social interaction caused the largest increase in the activity in the medial preoptic area. Specific chemogenetic stimulation of the PIL-preoptic pathway led to elevated direct social contact. The results suggest that posterior thalamic PIL neurons convey socially relevant information to a variety of different forebrain centers, among which the preoptic area is involved in the processing of physical contact. Support: Doctoral Student Scholarship Program of the Co-operative Doctoral Program of the Ministry of Innovation and Technology, Excellence Program of the Semmelweis University, Gedeon Richter Plc. Centenary Foundation, EFOP-3.6.3-VEKOP-16-2017-00009, NKFIH-4300-1/2017-NKP_17-0002 and OTKA K134221.

Pubmed:
33546359: Lékó AH, Kumari R, Dóra F, Keller D, Udvari EB, Csikós V, Renner É, Dobolyi A
Transcriptome Sequencing in the Preoptic Region of Rat Dams Reveals a Role of Androgen Receptor in the Control of Maternal Behavior.
(1) Background: Preoptic region of hypothalamus is responsible to control maternal behavior, which was hypothesized to be associated with gene expressional changes. (2) Methods: Transcriptome sequencing was first applied in the preoptic region of rat dams in comparison to a control group of mothers whose pups were taken away immediately after parturition and did not exhibit caring behavior 10 days later. (3) Results: Differentially expressed genes were found and validated by quantitative RT-PCR, among them NACHT and WD repeat domain containing 1 (Nwd1) is known to control androgen receptor (AR) protein levels. The distribution of Nwd1 mRNA and AR was similar in the preoptic area. Therefore, we focused on this steroid hormone receptor and found its reduced protein level in rat dams. To establish the function of AR in maternal behavior, its antagonist was administered intracerebroventricularly into mother rats and increased pup-directed behavior of the animals. (4) Conclusions: AR levels are suppressed in the preoptic area of mothers possibly mediated by altered Nwd1 expression in order to allow sustained high-level care for the pups. Thus, our study first implicated the AR in the control of maternal behaviors.

Int J Mol Sci, 2021; 22
32612510: Dobolyi A, Oláh S, Keller D, Kumari R, Fazekas EA, Csikós V, Renner É, Cservenák M
Secretion and Function of Pituitary Prolactin in Evolutionary Perspective.
The hypothalamo-pituitary system developed in early vertebrates. Prolactin is an ancient vertebrate hormone released from the pituitary that exerts particularly diverse functions. The purpose of the review is to take a comparative approach in the description of prolactin, its secretion from pituitary lactotrophs, and hormonal functions. Since the reproductive and osmoregulatory roles of prolactin are best established in a variety of species, these functions are the primary subjects of discussion. Different types of prolactin and prolactin receptors developed during vertebrate evolution, which will be described in this review. The signal transduction of prolactin receptors is well conserved among vertebrates enabling us to describe the whole subphylum. Then, the review focuses on the regulation of prolactin release in mammals as we have the most knowledge on this class of vertebrates. Prolactin secretion in response to different reproductive stimuli, such as estrogen-induced release, mating, pregnancy and suckling is detailed. Reproduction in birds is different from that in mammals in several aspects. Prolactin is released during incubation in avian species whose regulation and functional significance are discussed. Little information is available on prolactin in reptiles and amphibians; therefore, they are mentioned only in specific cases to explain certain evolutionary aspects. In turn, the osmoregulatory function of prolactin is well established in fish. The different types of pituitary prolactin in fish play particularly important roles in the adaptation of eutherian species to fresh water environments. To achieve this function, prolactin is released from lactotrophs in hyposmolarity, as they are directly osmosensitive in fish. In turn, the released prolactin acts on branchial epithelia, especially ionocytes of the gill to retain salt and excrete water. This review will highlight the points where comparative data give new ideas or suggest new approaches for investigation in other taxa.
Front Neurosci, 2020; 14
29802523: Oláh S, Cservenák M, Keller D, Fazekas EA, Renner É, Lőw P, Dobolyi A
Prolactin-induced and neuronal activation in the brain of mother mice.
Nursing has important consequences on mothers. To separate the prolactin-mediated and the neuronally-mediated actions of nursing, neurons directly affected by prolactin were visualized using pSTAT5 immunohistochemistry in relation to Fos-expressing neurons in suckled mother mice. In response to pup exposure following 22-h pup deprivation, we found a markedly elevated number of pSTAT5-containing neurons in several brain regions, including the lateral septum, medial amygdaloid nucleus, subparafascicular area, caudal periaqueductal gray, dorsal raphe, lateral parabrachial nucleus, nucleus of the solitary tract, and the periventricular, medial preoptic, paraventricular, arcuate and ventromedial nuclei of the hypothalamus. Pup exposure also induced Fos expression in all of these brain regions except the arcuate and ventromedial hypothalamic nuclei. Bromocriptine treatment known to reduce prolactin levels eliminated pSTAT5 from most brain regions while it did not affect Fos activation following suckling. The degree of colocalization for pSTAT5 and Fos ranged from 8 to 80% in the different brain regions suggesting that most neurons responding to pup exposure in mother mice are driven either by prolactin or direct neuronal input from the pups, while the number of neurons affected by both types of inputs depends on the examined brain area. In addition, both pSTAT5 and Fos were also double-labeled with estrogen receptor alpha (ERα) in mother mice, which revealed a very high degree of colocalization between pSTAT5 and ERα with much less potential interaction between Fos- and ERα-containing neurons suggesting that estrogen-sensitive neurons are more likely to be affected by prolactin than by direct neuronal activation.
Brain Struct Funct, 2018; 223
27841935: Cservenák M, Keller D, Kis V, Fazekas EA, Öllös H, Lékó AH, Szabó ÉR, Renner É, Usdin TB, Palkovits M, Dobolyi Á
A Thalamo-Hypothalamic Pathway That Activates Oxytocin Neurons in Social Contexts in Female Rats.
Oxytocin is released from neurons in the paraventricular hypothalamic nucleus (PVN) in mothers upon suckling and during adult social interactions. However, neuronal pathways that activate oxytocin neurons in social contexts are not yet established. Neurons in the posterior intralaminar complex of the thalamus (PIL), which contain tuberoinfundibular peptide 39 (TIP39) and are activated by pup exposure in lactating mothers, provide a candidate projection. Innervation of oxytocin neurons by TIP39 neurons was examined by double labeling in combination with electron microscopy and retrograde tract-tracing. Potential classic neurotransmitters in TIP39 neurons were investigated by in situ hybridization histochemistry. Neurons activated after encounter with a familiar conspecific female in a familiar environment were mapped with the c-Fos technique. PVN and the supraoptic nucleus oxytocin neurons were closely apposed by an average of 2.0 and 0.4 TIP39 terminals, respectively. Asymmetric (presumed excitatory) synapses were found between TIP39 terminals and cell bodies of oxytocin neurons. In lactating rats, PIL TIP39 neurons were retrogradely labeled from the PVN. TIP39 neurons expressed vesicular glutamate transporter 2 but not glutamic acid decarboxylase 67. PIL contained a markedly increased number of c-Fos-positive neurons in response to social encounter with a familiar conspecific female. Furthermore, the PIL received ascending input from the spinal cord and the inferior colliculus. Thus, TIP39 neurons in the PIL may receive sensory input in

response to social interactions and project to the PVN to innervate and excite oxytocin neurons, suggesting that the PIL-PVN projection contributes to the activation of oxytocin neurons in social contexts.
Endocrinology, 2017; 158
27300187: Cservenák M, Kis V, Keller D, Dimén D, Menyhárt L, Oláh S, Szabó ÉR, Barna J, Renner É, Usdin TB, Dobolyi A
Maternally involved galanin neurons in the preoptic area of the rat.
Recent selective stimulation and ablation of galanin neurons in the preoptic area of the hypothalamus established their critical role in control of maternal behaviors. Here, we identified a group of galanin neurons in the anterior commissural nucleus (ACN), and a distinct group in the medial preoptic area (MPA). Galanin neurons in ACN but not the MPA co-expressed oxytocin. We used immunodetection of phosphorylated STAT5 (pSTAT5), involved in prolactin receptor signal transduction, to evaluate the effects of suckling-induced prolactin release and found that 76 % of galanin cells in ACN, but only 12 % in MPA were prolactin responsive. Nerve terminals containing tuberoinfundibular peptide 39 (TIP39), a neuropeptide that mediates effects of suckling on maternal motivation, were abundant around galanin neurons in both preoptic regions. In the ACN and MPA, 89 and 82 % of galanin neurons received close somatic appositions, with an average of 2.9 and 2.6 per cell, respectively. We observed perisomatic innervation of galanin neurons using correlated light and electron microscopy. The connection was excitatory based on the glutamate content of TIP39 terminals demonstrated by post-embedding immunogold electron microscopy. Injection of the anterograde tracer biotinylated dextran amine into the TIP39-expressing posterior intralaminar complex of the thalamus (PIL) demonstrated that preoptic TIP39 fibers originate in the PIL, which is activated by suckling. Thus, galanin neurons in the preoptic area of mother rats are innervated by an excitatory neuronal pathway that conveys suckling-related information. In turn, they can be topographically and neurochemically divided into two distinct cell groups, of which only one is affected by prolactin.
Brain Struct Funct, 2017; 222

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BOARD NUMBER: S03-094

A RANDOMIZED CONTROLLED TRIAL ON THE EFFECT OF PARENTING INTERVENTION THROUGH "CARE FOR CHILD DEVELOPMENT GUIDELINE" ON EARLY CHILD DEVELOPMENT AND BEHAVIORS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Background It is suggested that parenting intervention programs can play a core component in early child development. Given the limited healthcare resources in developing countries, a group design intervention might be cost-effective. **Methods** This randomized controlled trial was conducted in a public Pediatrics clinic in Isfahan, Iran. We included 210 pregnant women in their third trimester, and then followed their children for 18 months. The intervention group underwent 5 educational group sessions. The main outcomes were the children's development and behaviors based on Bayley Scales of Infant and Toddler Development-III (BSCID-III) at 12 months and Children Behavior Checklist (CBCL) at 18-month of age. **Results** Data of 181 children were analyzed (80 in the intervention group and 101 controls. The adjusted median differences were significantly lower in the intervention group than in controls for attention problems (-3.38; SE=1.59; P=0.035), anxiety problems (-2.28; SE=1.03; P=0.007) and pervasive developmental problems (-5; SE= 1.16; P<0.001) based on CBCL results. However, the difference of proportions was not significant in none of the BSCID-III domains in the intervention and control groups. **Conclusion** We found that parenting interventions through CCD group sessions were significantly effective on several child's behavior domains, but not on children's development. Future longitudinal studies are necessary in this field.

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IN VIVO FIBRE PHOTOMETRY REVEALS INCREASED NEURAL ACTIVITY IN THE LATERAL SEPTUM IS ASSOCIATED WITH FLEEING SOCIAL CONTACT IN MICE

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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AIMS Social avoidance is a core feature of numerous disorders. The lateral septum (LS) modulates social avoidance in mice. However, the specific behaviours affected by the LS in this context are not yet characterised. Our aim, therefore, was to examine the relationship between LS dynamics and specific social approach and avoidance behaviours with high temporal precision using fibre photometry and social fear conditioning (SFC). **METHOD** C57BL/6 mice underwent surgery to infuse a fluorescent calcium sensor and implant a fibre optic cannula within the LS. Three weeks post-surgery mice were socially isolated for one week, then presented with a series of social stimuli (caged novel mice) for 3 min each with a 3 min ITI (SFC-). One week later, mice underwent SFC, and the next day were again presented with a series of social stimuli (SFC+). Using fibre photometry and frame-locked video recordings, LS dynamics during exposure to SFC- and SFC+ social stimuli were correlated with specific behaviours. **RESULTS** Peaks in LS activity immediately preceded instances of the mouse rapidly fleeing the social stimulus. These peaks were observed when mice were unconditioned and when they were socially fear conditioned. However, peaks were most pronounced during the first social stimulus exposure during the SFC+ testing, and showed a reduction over successive social stimulus exposures, alongside the extinction of social fear. No other clear associations between LS activity and specific behaviours were identified. **CONCLUSION** These findings suggest that neuronal firing in the LS may promote social avoidance by driving social fleeing behaviour.

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STUDY OF BOTTLENOSE DOLPHIN (TURSIOPS TRUNCATUS) ACOUSTIC COMMUNICATION DURING HUMAN-DOLPHIN INTERACTION USING AI METHODS

POSTER SESSION 03 - SECTION: THE SOCIAL BRAIN: FROM GENES TO BEHAVIOR

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Bottlenose dolphins (*Tursiops truncatus*) are known for their highly social complexity and associated cognitive abilities such as communication. The animal's acoustic repertoire is manifold, spanning from echolocation clicks for navigation to burst pulsed sounds and -signature and non-signature- whistles for social interaction. As gregarious animals, voluntary interactions with other species such as humans is not innocuous and vocal interaction may often result. However, this complex exchange remains understudied due to the strenuous experimental conditions that are faced when studying marine mammals in the wild. Our project proposes to overcome this challenge by investigating dolphin acoustic communication in a semi-captive environment through the growing use of novel computational methods such as automated clustering of sounds. At Eilat reef in Israel, an habituated group of cetaceans freely comes and leaves the pod while spontaneously interacting with the local care-takers. Daily activity is recorded with underwater hydrophones and video cameras, therefore providing us with unprecedented data. To elicit human-dolphin interaction, specific bells are rang at random times during the day to which dolphins respond, or not, by approaching and interacting with the "ringers". Recurrence of dolphin sounds are then clustered, classified and assessed with AI. Preliminary results have found that dolphins sometimes display vocal mimicry during inter-species interaction. Hence, we formulate the double hypothesis that these sounds produced by dolphins are non-random and that this repertoire may vary when interacting with humans. We hope with this unique approach that this study will contribute and disclose new queries in the field of animal social communication.

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FUNCTIONAL CHARACTERIZATION OF AFFERENT INPUTS FROM THE VENTRAL MIDBRAIN TO THE ZONA INCERTA

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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In nature, animals are constantly exposed to variable environmental stimuli relevant to their survival, such as food or threat-related stimuli. When animals perceive reward-related or dangerous stimuli, they show appropriate responses to retrieve the reward or avoid the threat. Recent studies have shown that the ventral tegmental area (VTA) and substantia nigra (SN) in the ventral midbrain can regulate defensive behaviors. Although both regions send projections to the ZI, which is an integrative node for global modulation of a variety of behaviors, such as feeding, defensive or investigating behavior, the functional roles of midbrain-to-ZI connections remain elusive. As the first step to investigate their functions, we combined dual viral retrograde targteting approach with *in situ* RNAscope technique to study the neurochemical phenotypes of midbrain cells projecting to the ZI. We found that ZI-projecting VTA neurons consisted mostly of glutamatergic and a small population of GABAergic neurons, whereas ZI-projecting SN neurons consisted mostly of GABAergic and some dopaminergic neurons. To characterize the behavioral function of ZI-projecting midbrain neurons, we adapted a mouse model of stress and performed neural activity-dependent marker c-Fos immunostaining. We found that ZI-projecting midbrain neurons were significantly involved in restraint stress. Further causal manipulation experiments are ongoing to further examine the function roles of the midbrain-to-ZI connections.

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SILENCING OF ASCENDING PAIN PATHWAYS WITH BOTULINUM-SUBSTANCE P PERSISTS FOR OVER 100DAYS AND CAN BE RESTORED WITH A SECOND INJECTION OF BOTULINUM CONJUGATE.

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Aims. We have shown that a single intrathecal injection of substance P conjugated to the light chain of botulinum toxin (SP-BOT) silenced neurons in the dorsal horn of the spinal cord and alleviated persistent pain states in mice (Maiáru et al., 2018). The SP-BOT construct selectively silenced neurokinin 1 receptor positive (NK1R+) neurons in the superficial dorsal horn. A subset of these NK1R+ neurons are nociceptive projection neurons that convey pain-related information to the brainstem. Here we investigated how long botulinum-based neuronal silencing persisted in the dorsal horn following a single injection of conjugate. **Methods.** a) Peripheral spared nerve injury (SNI) was carried out at 30d, 60d, 90d and 120d after injection of SP-BOT or b) ankle injection of CFA complete Freund's adjuvant (CFA), a model of inflammatory pain, was followed by intrathecal injection of SP-BOT. Affective (attending behaviour, response to cold, elevated plus maze) and reflex (von Frey hairs) measures of pain behaviour were measured. **Results.** By monitoring both affective and reflex pain behaviours after SNI or CFA we established that NK1R+ spinal neurons in the superficial lamina of the dorsal horn were silenced for 100days following a single intrathecal injection of the botulinum construct without toxicity. We also show that behavioural alleviation of neuropathic pain symptoms could be reinstated by a second injection of SP-BOT at 128d. **Conclusions.** Botulinum toxin conjugates provide a powerful new way of providing long term pain relief following a single injection of the conjugate with the potential for repeated dosing when pain returns.

Pubmed:
28570669: Silva-Hucha S, Hernández RG, Benítez-Temiño B, Pastor ÁM, de la Cruz RR, Morcuende S
Extraocular motoneurons of the adult rat show higher levels of vascular endothelial growth factor and its receptor Flk-1 than other cranial motoneurons.
Recent studies show a relationship between the deficit of vascular endothelial growth factor (VEGF) and motoneuronal degeneration, such as that occurring in amyotrophic lateral sclerosis (ALS). VEGF delivery protects motoneurons from cell death and delayed neurodegeneration in animal models of ALS. Strikingly, extraocular motoneurons show lesser vulnerability to neurodegeneration in ALS compared to other cranial or spinal motoneurons. Therefore, the present study investigates possible differences in VEGF and its main receptor VEGFR-2 or Flk-1 between extraocular and non-extraocular brainstem motoneurons. We performed immunohistochemistry and Western blot to determine the presence of VEGF and Flk-1 in rat motoneurons located in the three extraocular motor nuclei (abducens, trochlear and oculomotor) and to compare it to that observed in two other brainstem nuclei (hypoglossal and facial) that are vulnerable to degeneration. Extraocular motoneurons presented higher amounts of VEGF and its receptor Flk-1 than other brainstem motoneurons, and thus these molecules could be participating in their higher resistance to neurodegeneration. In conclusion, we hypothesize that differences in VEGF availability and signaling could be a contributing factor to the different susceptibility of extraocular motoneurons, when compared with other motoneurons, in neurodegenerative diseases.
PLoS One, 2017; 12
28744196: Hernández RG, Silva-Hucha S, Morcuende S, de la Cruz RR, Pastor AM, Benítez-Temiño B
Extraocular Motor System Exhibits a Higher Expression of Neurotrophins When Compared with Other Brainstem Motor Systems.
Extraocular motoneurons resist degeneration in diseases such as amyotrophic lateral sclerosis. The main objective of the present work was to characterize the presence of neurotrophins in extraocular motoneurons and muscles of the adult rat. We also compared these results with those obtained from other cranial motor systems, such as facial and hypoglossal, which indeed suffer neurodegeneration. Immunocytochemical analysis was used to describe the expression of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 in oculomotor, trochlear, abducens, facial, and hypoglossal nuclei of

adult rats, and Western blots were used to describe the presence of neurotrophins in extraocular, facial (buccinator), and tongue muscles, which are innervated by the above-mentioned motoneurons. In brainstem samples, brain-derived neurotrophic factor was present both in extraocular and facial motoneuron somata, and to a lesser degree, in hypoglossal motoneurons. Neurotrophin-3 was present in extraocular motor nuclei, while facial and hypoglossal motoneurons were almost devoid of this protein. Finally, nerve growth factor was not present in the soma of any group of motoneurons, although it was present in dendrites of motoneurons located in the neuropil. Neuropil optical density levels were higher in extraocular motoneuron nuclei when compared with facial and hypoglossal nuclei. Neurotrophins could be originated in target muscles, since Western blot analyses revealed the presence of the three molecules in all sampled muscles, to a larger extent in extraocular muscles when compared with facial and tongue muscles. We suggest that the different neurotrophin availability could be related to the particular resistance of extraocular motoneurons to neurodegeneration.
Front Neurosci, 2017; 11
30050409: Acosta L, Morcuende S, Silva-Hucha S, Pastor AM, de la Cruz RR
Vascular Endothelial Growth Factor (VEGF) Prevents the Downregulation of the Cholinergic Phenotype in Axotomized Motoneurons of the Adult Rat.
Vascular endothelial growth factor (VEGF) was initially characterized by its activity on the vascular system. However, there is growing evidence indicating that VEGF also acts as a neuroprotective factor, and that its administration to neurons suffering from trauma or disease is able to rescue them from cell death. We questioned whether VEGF could also maintain damaged neurons in a neurotransmissive mode by evaluating the synthesis of their neurotransmitter, and whether its action would be direct or through its well-known angiogenic activity. Adult rat extraocular motoneurons were chosen as the experimental model. Lesion was performed by monocular enucleation and immediately a gelatine sponge soaked in VEGF was implanted intraorbitally. After 7 days, abducens, trochlear, and oculomotor nuclei were examined by immunohistochemistry against choline acetyltransferase (ChAT), the biosynthetic enzyme of the motoneuronal neurotransmitter acetylcholine. Lesioned motoneurons exhibited a noticeable ChAT downregulation which was prevented by VEGF administration. To explore whether this action was mediated via an increase in blood vessels or in their permeability, we performed immunohistochemistry against laminin, glucose transporter-1 and the plasmatic protein albumin. The quantification of the immunolabeling intensity against these three proteins showed no significant differences between VEGF-treated, axotomized and control animals. Therefore, the present data indicate that VEGF is able to sustain the cholinergic phenotype in damaged motoneurons, which is a first step for adequate neuromuscular neurotransmission, and that this action seems to be mediated directly on neurons since no sign of angiogenic activity was evident. These data reinforces the therapeutical potential of VEGF in motoneuronal diseases.
Front Mol Neurosci, 2018; 11
32189115: Silva-Hucha S, Carrero-Rojas G, Fernández de Sevilla ME, Benítez-Temiño B, Davis-López de Carrizosa MA, Pastor AM, Morcuende S
Sources and lesion-induced changes of VEGF expression in brainstem motoneurons.
Motoneurons of the oculomotor system show lesser vulnerability to neurodegeneration compared to other cranial motoneurons, as seen in amyotrophic lateral sclerosis (ALS). The overexpression of vascular endothelial growth factor (VEGF) is involved in motoneuronal protection. As previously shown, motoneurons innervating extraocular muscles present a higher amount of VEGF and its receptor Flk-1 compared to facial or hypoglossal motoneurons. Therefore, we aimed to study the possible sources of VEGF to brainstem motoneurons, such as glial cells and target muscles. We also studied the regulation of VEGF in response to axotomy in ocular, facial, and hypoglossal motor nuclei. Basal VEGF expression in astrocytes and microglial cells of the cranial motor nuclei was low. Although the presence of VEGF in the different target muscles for brainstem motoneurons was similar, the presynaptic element of the ocular neuromuscular junction showed higher amounts of Flk-1, which could result in greater efficiency in the capture of the factor by oculomotor neurons. Seven days after axotomy, a clear glial reaction was observed in all the brainstem nuclei, but the levels of the neurotrophic factor remained low in glial cells. Only the injured motoneurons of the oculomotor system showed an increase in VEGF and Flk-1, but such an increase was not detected in axotomized facial or hypoglossal motoneurons. Taken together, our findings suggest that the ocular motoneurons themselves upregulate VEGF expression in response to lesion. In conclusion, the low VEGF expression observed in glial cells suggests that these cells are not the main source of VEGF for brainstem motoneurons. Therefore, the higher VEGF expression observed in motoneurons innervating extraocular muscles is likely due either to the fact that this factor is more avidly taken up from the target muscles, in basal conditions, or is produced by these motoneurons themselves, and acts in an autocrine manner after axotomy.
Brain Struct Funct, 2020; 225
33467517: Silva-Hucha S, Pastor AM, Morcuende S
Neuroprotective Effect of Vascular Endothelial Growth Factor on Motoneurons of the Oculomotor System.
Vascular endothelial growth factor (VEGF) was initially characterized as a potent angiogenic factor based on its activity on the

vascular system. However, it is now well established that VEGF also plays a crucial role as a neuroprotective factor in the nervous system. A deficit of VEGF has been related to motoneuronal degeneration, such as that occurring in amyotrophic lateral sclerosis (ALS). Strikingly, motoneurons of the oculomotor system show lesser vulnerability to neurodegeneration in ALS compared to other motoneurons. These motoneurons presented higher amounts of VEGF and its receptor Flk-1 than other brainstem pools. That higher VEGF level could be due to an enhanced retrograde input from their target muscles, but it can also be produced by the motoneurons themselves and act in an autocrine way. By contrast, VEGF's paracrine supply from the vicinity cells, such as glial cells, seems to represent a minor source of VEGF for brainstem motoneurons. In addition, ocular motoneurons experiment an increase in VEGF and Flk-1 level in response to axotomy, not observed in facial or hypoglossal motoneurons. Therefore, in this review, we summarize the differences in VEGF availability that could contribute to the higher resistance of extraocular motoneurons to injury and neurodegenerative diseases.
Int J Mol Sci, 2021; 22

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-099

TARGETING CHOLINERGIC BASAL FOREBRAIN NEURONS TO MODULATE INFLAMMATORY PAIN IN MICE

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Cholinergic neurons in the basal forebrain (BFB) project widely to the cerebral cortex and influence attention, arousal and cognition via cholinergic modulation of cortical network activity. As attention is diminished in chronic pain patients our aim was to examine the role of the BFB cholinergic activity in mouse models of inflammatory pain. Using the expression of cFos as a surrogate marker of neuronal activity we found that cholinergic neurons in BFB nuclei that project to the cerebral cortex are activated in excess by mechanical stimuli when applied to the inflamed paw compared to the naïve state. Using *in vivo* electrical recordings in behaving mice we found that beta and gamma local field potential activity is evoked in the BFB by noxious stimuli in naïve mice and by otherwise non-noxious stimuli in mice with an inflamed paw. On enhancing the activity of cholinergic neurons in the BFB, and more specifically BFB cholinergic terminals in the medial PFC (mPFC) with optogenetic techniques, we found that paw withdrawals to mechanical and thermal stimuli in inflamed conditions were reduced. Attentional performance in the 5-CSRT task was enhanced in mice when activating BFB cholinergic neurons with a chemogenetic technique. Optogenetic stimulation of the cholinergic terminals in the mPFC enhanced neuronal cFos expression but did not alter attentional performance in the 5-CSRT task. These findings suggest that the concept of enhancing prefrontal attention networks in brain regions that are linked to descending pain control may facilitate the development of therapeutic strategies.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-100

LOCOMOTOR CONTROL BY THETA-RANGE ACTIVATION OF GLUTAMATERGIC MSDB TO VTA PROJECTIONS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Subcortical circuits modulate and select the appropriate locomotor responses based on the external demands and the internal state of the animal. However, how subcortical circuitry regulates the onset and vigor of locomotion is still unresolved. Here, we show that a glutamatergic pathway from the Medial Septum and Diagonal Band of Broca (MSDB) to neurons in the Ventral Tegmental Area (VTA) controls locomotor behavior. When stimulated in the theta frequency range mice invariably increased speed and locomotor activity in a frequency-dependent manner. Moreover, using a self-supervised machine learning approach we observed a specific change in their behavioral repertoire, leading to an overrepresentation of the walking and rearing motifs upon activation of the circuit. Finally, we mapped the VTA output cells of this glutamatergic MSDB projection and discovered that it monosynaptically targets both glutamatergic and dopaminergic VTA neurons. Taken together, we identify a previously unknown glutamatergic basal forebrain to midbrain pathway that initiates and modulates locomotor activity and contributes to the selection of locomotion-associated motifs.

Pubmed:
34366795: Mocellin P, Mikulovic S
The Role of the Medial Septum-Associated Networks in Controlling Locomotion and Motivation to Move.
The Medial Septum and diagonal Band of Broca (MSDB) was initially studied for its role in locomotion. However, the last several decades were focussed on its intriguing function in theta rhythm generation. Early studies relied on electrical stimulation, lesions and pharmacological manipulation, and reported an inconclusive picture regarding the role of the MSDB circuits. Recent studies using more specific methodologies have started to elucidate the differential role of the MSDB's specific cell populations in controlling both theta rhythm and behaviour. In particular, a novel theory is emerging showing that different MSDB's cell populations project to different brain regions and control distinct aspects of behaviour. While the majority of these behaviours involve movement, increasing evidence suggests that MSDB-related networks govern the motivational aspect of actions, rather than locomotion . Here, we review the literature that links MSDB, theta activity, and locomotion and propose open questions, future directions, and methods that could be employed to elucidate the diverse roles of the MSDB-associated networks.
Front Neural Circuits, 2021; 15

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BOARD NUMBER: S03-101

MEDIAN RAPHE GLUTAMATERGIC CELLS ENCODE AVERSIVE EXPERIENCE

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Acquisition of negative experience is essential for the survival. Negative stimuli immediately and simultaneously activate the main aversive centers, the lateral habenula (LHb) and medial ventral tegmental area (mVTA) and the memory processing septo-hippocampal system. However, it is still unknown, which neurons coordinate these processes during negative experience. Here, we found that the brainstem median raphe region (MRR) harbors a new type of excitatory population of neurons that expresses vesicular glutamate transporter 2 (vGluT2). With anatomical tracing experiments, we found that these neurons innervate LHb, mVTA and the septo-hippocampal system and they receive inputs from negative experience-related brain centers. With in vivo optical tagging method, we found that MRR vGluT2 neurons are rapidly and selectively activated during aversive, but not rewarding events. Optogenetic stimulation of MRR vGluT2 neurons induced acute and conditioned place aversion, suppressed reward seeking behavior and created memory acquisition-promoting hippocampal theta-oscillations. By contrast, optogenetic inhibition of MRR vGluT2-neurons during an aversive foot-shock impaired both contextual and cued fear memory-formation and prevented fear generalization. Our results suggest that MRR vGluT2-neurons are both necessary and sufficient to acquire negative experience and they may play an important role in several types of mood disorders. **Conflict of interest:** The authors have no conflict of interest. **Funding:** This project was supported by the ÚNKP-18-2-I-SE-22 and ÚNKP-21-3-SE-9 New National Excellence Program of the Ministry of Innovation and by the EFOP-3.6.3-VEKOP-16-2017-00009 Semmelweis 250+ Excellence PhD Fellowship.

Pubmed:
31780530: Szőnyi A, Zichó K, Barth AM, Gönczi RT, Schlingloff D, Török B, Sipos E, Major A, Bardóczi Z, Sos KE, Gulyás AI, Varga V, Zelena D, Freund TF, Nyiri G
Median raphe controls acquisition of negative experience in the mouse.
Adverse events need to be quickly evaluated and memorized, yet how these processes are coordinated is poorly understood. We discovered a large population of excitatory neurons in mouse median raphe region (MRR) expressing vesicular glutamate transporter 2 (vGluT2) that received inputs from several negative experience-related brain centers, projected to the main aversion centers, and activated the septohippocampal system pivotal for learning of adverse events. These neurons were selectively activated by aversive but not rewarding stimuli. Their stimulation induced place aversion, aggression, depression-related anhedonia, and suppression of reward-seeking behavior and memory acquisition-promoting hippocampal theta oscillations. By contrast, their suppression impaired both contextual and cued fear memory formation. These results suggest that MRR vGluT2 neurons are crucial for the acquisition of negative experiences and may play a central role in depression-related mood disorders.
Science, 2019; 366

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BOARD NUMBER: S03-102

MGLU5 RECEPTORS MODULATE SOMATOSTATIN INTERNEURON CONTROL OF EMOTIONAL BEHAVIORS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Aims: Metabotropic glutamate receptors subtype 5 (mGlu5 receptors) are known to play an important role in regulating functioning of cognitive, social and negative valence system domains. However, it remains largely unknown at which neuronal circuits and neuronal subtypes do mGlu5 receptors act to influence behavioral phenotypes relevant for neuropsychiatric disorders. Methods: Here, we examined the contribution of these receptors in somatostatin (SST+) interneuron activity function, plasticity, brain synchrony modulation and behavior. Results: Loss of mGlu5 receptors in SST+ neurons elicited excitatory synaptic dysfunction in a region and sex-specific manner and a range of emotional imbalances, which included diminished social novelty preference, anxiety and fear responses. A key finding of our study comes from the observation that mGlu5 receptors in SST+ neurons modulate the facilitation of theta oscillations during negative valence processing in both mPFC and vHPC. Conclusions: Deficits in SSTCre-mGlu5-/- mice did not arise from inability to generate theta frequency or a shift in dominant frequency, but rather as a weakened signal power, suggesting a lower engagement of neuronal ensembles encompassing an internal fearful state in those animals. Similarly, our study also points towards a necessity of mGlu5 receptors in SST+ neurons to enable cooperative neuronal spiking dynamics between the mPFC and vHPC. Altogether, our findings reveal a critical role of mGlu5 receptors in regulating SST+ neurons excitability necessary for emotional behavior homeostasis.

Pubmed:
34360592: Zangrandi L, Schmuckermair C, Ghareh H, Castaldi F, Heilbronn R, Zernig G, Ferraguti F, Ramos-Prats A
Loss of mGluR5 in D1 Receptor-Expressing Neurons Improves Stress Coping.
The metabotropic glutamate receptor type 5 (mGluR5) has been proposed to play a crucial role in the selection and regulation of cognitive, affective, and emotional behaviors. However, the mechanisms by which these receptors mediate these effects remain largely unexplored. Here, we studied the role of mGluR5 located in D1 receptor-expressing (D1) neurons in the manifestation of different behavioral expressions. Mice with conditional knockout (cKO) of mGluR5 in D1 neurons (mGluR5 cKO) and littermate controls displayed similar phenotypical profiles in relation to memory expression, anxiety, and social behaviors. However, mGluR5 cKO mice presented different coping mechanisms in response to acute escapable or inescapable stress. mGluR5 cKO mice adopted an enhanced active stress coping strategy upon exposure to escapable stress in the two-way active avoidance (TWA) task and a greater passive strategy upon exposure to inescapable stress in the forced swim test (FST). In summary, this work provides evidence for a functional integration of the dopaminergic and glutamatergic system to mediate control over internal states upon stress exposure and directly implicates D1 neurons and mGluR5 as crucial mediators of behavioral stress responses.
Int J Mol Sci, 2021; 22
31473288: Alonso L, Peeva P, Ramos-Prats A, Alenina N, Winter Y, Rivalan M
Inter-individual and inter-strain differences in cognitive and social abilities of Dark Agouti and Wistar Han rats.
Healthy animals displaying extreme behaviours that resemble human psychiatric symptoms are relevant models to study the natural psychobiological processes of maladapted behaviours. Using a Rat Gambling Task, healthy individuals spontaneously making poor decisions (PDMs) were found to co-express a combination of other cognitive and reward-based characteristics similar to symptoms observed in human patients with impulse-control disorders. The main goals of this study were to 1) confirm the existence of PDMs and their unique behavioural phenotypes in Dark Agouti (DA) and Wistar Han (WH) rats, 2) to extend the behavioural profile of the PDMs to probability-based decision-making and social behaviours and 3) to extract key discriminative traits between DA and WH strains, relevant for biomedical research. We have compared cognitive abilities, natural behaviours and physiological responses in DA and WH rats at the strain and at the individual level. Here we found that the naturally occurring PDM's profile was consistent between both rat lines. Then, although the PDM individuals

did not take more risks in probability discounting task, they seemed to be of higher social ranks. Finally and despite their similarities in performance, WH and DA lines differed in degree of reward sensitivity, impulsivity, locomotor activity and open space-occupation. The reproducibility and conservation of the complex phenotypes of PDMs and GDMs (good decision makers) in these two genetically different strains support their translational potential. Both strains, present large phenotypic variation in behaviours pertinent for the study of the underlying mechanisms of poor decision making and associated disorders.
Behav Brain Res, 2020; 377

30873001: Ramos-Prats A, Kölldorfer J, Paolo E, Zeidler M, Schmid G, Ferraguti F
An Appraisal of the Influence of the Metabotropic Glutamate 5 (mGlu5) Receptor on Sociability and Anxiety.
Amongst the many neurotransmitter systems causally linked to the expression of social behavior, glutamate appears to play a pivotal role. In particular, metabotropic glutamate 5 (mGlu5) receptors have received much attention as its altered function has been reported in several mouse models of autism spectrum disorders and mental retardation. Inhibition of the activity of mGlu5 receptors by means of genetic or pharmacological manipulations improved social deficits in some of these animal models. However, in normal wild-type (WT) mice, pharmacological blockade of mGlu5 receptors yielded inconsistent results. The aim of our study was to investigate the actual contribution of decreased or absent mGlu5 receptor function in sociability and anxiety-like behavior as well as to explore the impact of mGlu5 receptor ablation on the pattern of brain activation upon social exposure. Here we show that -/- mice display higher social preference indexes compared to age-matched WT mice in the three-chambered social task. However, this effect was accompanied by a decreased exploratory activity during the test and increased anxiety-like behavior. Contrary to mGlu5 receptor ablation, the mGlu5 receptor negative allosteric modulator 3-((2-methyl-1,4-thiazolyl)ethynyl)pyridine (MTEP) induced anxiolytic effects without affecting social preference in WT mice. By mapping c-Fos expression in 21 different brain regions known to be involved in social interaction, we detected a specific activation of the prefrontal cortex and dorsolateral septum in -/- mice following social interaction. C-Fos expression correlation-based network and graph theoretical analyses further suggested dysfunctional connectivity and disruption of the functional brain network generated during social interaction in -/- mice. The lack of mGlu5 receptors resulted in profound rearrangements of the functional impact of prefrontal and hippocampal regions in the social interaction network. In conclusion, this work reveals a complex contribution of mGlu5 receptors in sociability and anxiety and points to the importance of these receptors in regulating brain functional connectivity during social interaction.
Front Mol Neurosci, 2019; 12

30816288: Sanchís-Ollé M, Fuentes S, Úbeda-Contreras J, Lalanza JF, Ramos-Prats A, Armario A, Nadal R
Controllability affects endocrine response of adolescent male rats to stress as well as impulsivity and behavioral flexibility during adulthood.
Exposure to stress during adolescence exerts a long-term impact on behavior and might contribute to the development of several neuropsychiatric disorders. In adults, control over stress has been found to protect from the negative consequences of stress, but the influence of controllability at early ages has not been extensively studied. Here, we evaluated in a rodent model the effects of repeated exposure in adolescent male rats to controllable versus uncontrollable foot-shock stress (CST or UST, respectively). Rats were assigned to three groups: non-stress (stress-naïve), CST (exposed to 8 sessions of a two-way shuttle active avoidance task over a period of 22 days) and UST (receiving the same amount of shocks as CST, regardless of their actual behavior). During adulthood, different cohorts were tested in several tasks evaluating inhibitory control and cognitive flexibility: 5-choice serial reaction time, delay-discounting, gambling test and probabilistic reversal learning. Results showed that the hypothalamic-pituitary-adrenal response to the first shock session was similar in CST and UST animals, but the response to the 8 session was lower in CST animals. In adulthood, the UST animals presented impaired motor (but not cognitive) impulsivity and more perseverative behavior. The behavioral effects of UST were associated with increased number of D2 dopamine receptors in dorsomedial striatum, but not in other striatal regions. In summary, UST exposure during adolescence induced long-term impairments in impulsivity and compulsivity, whereas CST had only minor effects. These data support a critical role of stress uncontrollability on the long-lasting consequences of stress, as a risk factor for mental illnesses.
Sci Rep, 2019; 9

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BOARD NUMBER: S03-103

GAT1-SAPORIN-INDUCED GABAERGIC LESION IN THE VENTRAL PALLIDUM/SUBSTANTIA INNOMINATA REDUCES BEHAVIORAL DESPAIR AND PROMOTES ACTIVE COPING STRATEGIES

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Aim: The ventral pallidum (VP) and substantia innominata (SI) send substantial GABAergic projections to the amygdala and the bed nucleus of the stria terminalis (BNST), two limbic structures that respectively orchestrate acute and chronic fear response. We did two set of experiments to reveal the functional connectivity between the amygdaloid complex and VP/SI (anatomy), and elucidate the role of VP/SI GABAergic neurons in various cognitive and affective processes (behavior). Methods: We made bilateral retrograde tracer (RetroBeads, Lumafluor) injections into the VP/SI of adult male Wistar rats to reveal and characterize its limbic afferents by means of immunohistochemistry. For behavioral testing, we used bilateral GAT1-saporin (or PBS) injections to silence GABAergic neurons of the VP/SI, and assessed the animals in the forced swim test (FST), open field test (OFT), elevated plus maze (EPM), Morris water maze (MWM) and Pavlovian fear conditioning. Results: We observed several amygdaloid neuronal groups that target the VP/SI, with distinct types of projections originating from the central and lateral nuclei (anatomy). GAT1-saporin lesions in the VP/SI reduced behavioral despair without altering general locomotor activity. These animals also showed reduced freezing and increased darting behavior during the acquisition day of fear conditioning (behavior). Conclusions: Silencing the VP/SI GABAergic neurons produce an antidepressant effect while promoting active coping strategies. Long-range basal forebrain GABAergic neurons that target different nuclei of the amygdala and the BNST may constitute therapeutic targets for treating clinical depression as well as anxiety and fear-related disorders.

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BOARD NUMBER: S03-104

A MIDBRAIN-EXTENDED AMYGDALA PATHWAY CONTROLS CONTEXTUAL FEAR MEMORY AND PREDATOR ODOR AVOIDANCE

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Neuronal circuits located in the midbrain play a critical role in controlling defensive behavior. However, it is still elusive how different neuron types contribute to distinct behavioral outcomes during the presence of threat. In this study, we investigated a group of neurons in the ventral periaqueductal gray and dorsal raphe nucleus that express vasoactive intestinal polypeptide (VIP). Using viral tracing conducted in Vip-Cre mice, we observed that these VIP neurons innervated exclusively the bed nucleus of stria terminalis (BNST) and central amygdala (CeA), the two main regions of the extended amygdala. Interestingly, neurons in these two brain regions contributed to the innervation of midbrain VIP neurons to a large extent revealed by monosynaptic rabies tracing. In vitro electrophysiological recordings combined with optogenetics revealed that light stimulation of VIP afferents activated ionotropic glutamate receptors on their postsynaptic partners in the extended amygdala. Electron microscopy determined that spines were the major targets of VIP immunoreactive boutons both in the BNST and CeA. To clarify the role of the midbrain VIP neurons in defensive behavior, we inhibited their activity using chemogenetics and observed that inhibition of these midbrain neurons during fear conditioning impaired the contextual, but not cued fear memory tested on subsequent days. In addition, inhibiting the VIP neuronal activity reduced avoidance behavior in a predator odor avoidance test. These results collectively show that the excitatory midbrain-extended amygdala pathway expressing VIP plays a critical role in the regulation of a set of defensive behaviors.

Pubmed:
27013983: Vereczki VK, Veres JM, Müller K, Nagy GA, Rácz B, Barsy B, Hájos N
Synaptic Organization of Perisomatic GABAergic Inputs onto the Principal Cells of the Mouse Basolateral Amygdala.
Spike generation is most effectively controlled by inhibitory inputs that target the perisomatic region of neurons. Despite the critical importance of this functional domain, very little is known about the organization of the GABAergic inputs contacting the perisomatic region of principal cells (PCs) in the basolateral amygdala. Using immunocytochemistry combined with in vitro single-cell labeling we determined the number and sources of GABAergic inputs of PCs at light and electron microscopic levels in mice. We found that the soma and proximal dendrites of PCs were innervated primarily by two neurochemically distinct basket cell types expressing parvalbumin (PVBC) or cholecystokinin and CB1 cannabinoid receptors (CCK/CB1BC). The innervation of the initial segment of PC axons was found to be parceled out by PVBCs and axo-axonic cells (AAC), as the majority of GABAergic inputs onto the region nearest to the soma (between 0 and 10 μm) originated from PVBCs, while the largest portion of the axon initial segment was innervated by AACs. Detailed morphological investigations revealed that the three perisomatic region-targeting interneuron types significantly differed in dendritic and axonal arborization properties. We found that, although individual PVBCs targeted PCs via more terminals than CCK/CB1BCs, similar numbers (15-17) of the two BC types converge onto single PCs, whereas fewer (6-7) AACs innervate the axon initial segment of single PCs. Furthermore, we estimated that a PVBC and a CCK/CB1BC may target 800-900 and 700-800 PCs, respectively, while an AAC can innervate 600-650 PCs. Thus, BCs and AACs innervate ~10 and 20% of PC population, respectively, within their axonal cloud. Our results collectively suggest, that these interneuron types may be differently affiliated within the local amygdalar microcircuits in order to fulfill specific functions in network operation during various brain states.
Front Neuroanat, 2016; 10
31636080: Rovira-Esteban L, Gunduz-Cinar O, Bukalo O, Limoges A, Brockway E, Müller K, Fenno L, Kim YS, Ramakrishnan C, Andrási T, Deisseroth K, Holmes A, Hájos N

Excitation of Diverse Classes of Cholecystokinin Interneurons in the Basal Amygdala Facilitates Fear Extinction.
There is growing evidence that interneurons (INs) orchestrate neural activity and plasticity in corticoamygdala circuits to regulate fear behaviors. However, defining the precise role of cholecystokinin-expressing INs (CCK INs) remains elusive due to the technical challenge of parsing this population from CCK-expressing principal neurons (CCK PNs). Here, we used an intersectional genetic strategy in CCK-Cre;Dlx5/6-Flpe double-transgenic mice to study the anatomical, molecular and electrophysiological properties of CCK INs in the basal amygdala (BA) and optogenetically manipulate these cells during fear extinction. Electrophysiological recordings confirmed that this strategy targeted GABAergic cells and that a significant proportion expressed functional cannabinoid CB1 receptors; a defining characteristic of CCK-expressing basket cells. However, immunostaining showed that subsets of the genetically-targeted cells expressed either neuropeptide Y (NPY; 29%) or parvalbumin (PV; 17%), but not somatostatin (SOM) or Ca/calmodulin-dependent protein kinase II (CaMKII)-α. Further morphological and electrophysiological analyses showed that four IN types could be identified among the EYFP-expressing cells: CCK/cannabinoid receptor type 1 (CB1R)-expressing basket cells, neurogliaform cells, PV+ basket cells, and PV+ axo-axonic cells. At the behavioral level, optogenetic photostimulation of the targeted population during extinction acquisition led to reduced freezing on a light-free extinction retrieval test, indicating extinction memory facilitation; whereas photosilencing was without effect. Conversely, non-selective (i.e., inclusive of INs and PNs) photostimulation or photosilencing of CCK-targeted cells, using CCK-Cre single-transgenic mice, impaired extinction. These data reveal an unexpectedly high degree of phenotypic complexity in a unique population of extinction-modulating BA INs.
eNeuro, 2019 Nov/Dec; 6
33837051: Vereczki VK, Müller K, Krizsán É, Máté Z, Fekete Z, Rovira-Esteban L, Veres JM, Erdélyi F, Hájos N
Total Number and Ratio of GABAergic Neuron Types in the Mouse Lateral and Basal Amygdala.
GABAergic neurons are key circuit elements in cortical networks. Despite growing evidence showing that inhibitory cells play a critical role in the lateral (LA) and basal (BA) amygdala functions, neither the number of GABAergic neurons nor the ratio of their distinct types has been determined in these amygdalar nuclei. Using unbiased stereology, we found that the ratio of GABAergic neurons in the BA (22%) is significantly higher than in the LA (16%) in both male and female mice. No difference was observed between the right and left hemispheres in either sex. In addition, we assessed the ratio of the major inhibitory cell types in both amygdalar nuclei. Using transgenic mice and a viral strategy for visualizing inhibitory cells combined with immunocytochemistry, we estimated that the following cell types together compose the vast majority of GABAergic cells in the LA and BA: axo-axonic cells (5.5%-6%), basket cells expressing parvalbumin (17%-20%) or cholecystokinin (7%-9%), dendrite-targeting inhibitory cells expressing somatostatin (10%-16%), NPY-containing neurogliaform cells (14%-15%), VIP and/or calretinin-expressing interneuron-selective interneurons (29%-38%), and GABAergic projection neurons expressing somatostatin and neuronal nitric oxide synthase (5.5%-8%). Our results show that these amygdalar nuclei contain all major GABAergic neuron types as found in other cortical regions. Furthermore, our data offer an essential reference for future studies aiming to reveal changes in GABAergic cell number and in inhibitory cell types typically observed under different pathologic conditions, and to model functioning amygdalar networks in health and disease. GABAergic cells in cortical structures, as in the lateral and basal nucleus of the amygdala, have a determinant role in controlling circuit operation. In this study, we provide the first estimate for the total number of inhibitory cells in these two amygdalar nuclei. In addition, our study is the first to define the ratio of the major GABAergic cell types present in these cortical networks. Taking into account that hyperexcitability in the amygdala, arising from the imbalance between excitation and inhibition typifies many altered brain functions, including anxiety, post-traumatic stress disorder, schizophrenia, and autism, uncovering the number and ratio of distinct amygdalar inhibitory cell types offers a solid base for comparing the changes in inhibition in pathologic brain states.
J Neurosci, 2021; 41

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BOARD NUMBER: S03-105

THE CONTRIBUTION OF DISTINCT NEURONAL POPULATIONS TO THE PREFRONTAL CORTEX ENCODING OF THREAT-RELATED INFORMATION

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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In face of a threat, animals must select a response among a range of defensive behaviors, and the selection of a response suitable to each situation is essential for their survival. This response selection relies not only on the characteristics of the threat itself, but also on its multimodal integration with environmental features that constrain the repertoire of strategies that can be used to cope with the threatening encounter. The dorsomedial prefrontal cortex (dmPFC) is a key structure in the regulation of defensive behaviors, but how its excitatory and inhibitory neuronal populations differentially contribute to the selection of the most adaptive defensive response in each threatening condition remains unexplored. To investigate the contribution of the main dmPFC neuronal types - namely pyramidal, parvalbumin- (PV+) and somatostatin-expressing (SST+) neurons –, to threat evaluation and defensive response selection, *in vivo* calcium imaging is coupled to a novel behavioral paradigm that allows the execution of different defensive behaviors in response to distinct threatening situations. This approach allows us to identify the encoding of threat-related information in distinct neuronal populations and to investigate the role of the activity of dmPFC interneurons in the population representation of threats and in the mice selection of defensive behaviors.

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BOARD NUMBER: S03-106

HYPOTHALAMIC-HABENULA PATHWAYS HETEROGENEITY REVEALS DISTINCT AVERSIVE SATES.

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The lateral hypothalamic area (LHA) controls negative valence through glutamatergic projections to lateral habenula (LHb). The complex nature of negative emotional signaling suggest a specialized functional organization able to carry distinct negative values signals present in complex behavioral responses. However, basic knowledge exploring the diversity of glutamatergic LHA-LHb neurons is lacking, precluding detailed functional investigations using genetic strategies. To elucidate the organization of the LHA-LHb pathway we here characterized by Patch-seq the electrophysiological, neuroanatomical, and molecular diversity of Vglut2+ LHA-LHb neurons. While the glutamatergic LHA-LHb neurons have been considered a homogeneous population, we find that this projection is instead composed of several discrete neuron types characterized by their respective intrinsic properties, molecular markers, and spatial projection patterns. To reveal the functional relevance of such diversity, we further combined genetic targeting of identified neuron types with high-density electrophysiological recordings and optogenetic manipulations during a set of emotion-related behavior tasks. Cell-type specific optogenetic manipulations, guided by classification of LHA-LHb subtypes using Patch-seq, revealed cell-type specific negative signals, distinct immediate aversive behaviors as well as region-specific negative neural state in the prefrontal cortex (PFC) upon LHA-LHb pathways manipulation. Interestingly, we could detail a cell-type specific LHA-LHb persistent shift in the intrinsic properties upon stress. Altogether, through multimodal classification we detailed an unexpected molecular heterogeneity reflected by functional organization of parallel LHA-LHb circuits.

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BOARD NUMBER: S03-107

NEUROANATOMY OF PATHWAYS FOR INTEGRATION OF CARDIO-BEHAVIOURAL DEFENSIVE REPONSES

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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In the face of threat, higher organisms exhibit various defensive responses, of which the behavioral component has extensively been investigated. However, the defense reaction also comprises diverse physiological responses such as cardiac and endocrine adjustments, as well as analgesia. The individual defensive responses have been used to define specific defensive states, and much research was dedicated to exploration of the neuroanatomical substrates of defensive behavioral responses in a neuronal type-specific manner. However, the circuit mechanisms underlying integration of individual defensive responses to orchestrate the overall defensive state remain elusive. We used intersectional viral approaches to better understand neuroarchitecture of forebrain-to-brainstem pathways involved in behavioral as well as cardiac defensive responses. Tracing of inputs to the dorsal vagal complex (DVC), a central regulator of cardiovascular functions revealed glutamatergic projections from the ventrolateral column of the periaqueductal gray (vlPAG) as well as GABAergic, non-somatostatin (SOM-) inputs from neurons of the medial subdivision of the central amygdala (CeM). Interestingly, projections from both vlPAG and the CeM, send collaterals to common hypothalamic nuclei. Furthermore, the identified glutamatergic pathway innervates the freezing-related magnocellular nucleus of the medulla (Mc), while vlPAG and the bed nucleus of the stria terminalis (BNST), both implicated in anxiety-related defensive states, receive GABAergic collaterals from SOM- neurons in the CeM. Optogenetic interference with the GABAergic projection from the CeM to the DVC and the vlPAG suggests a modulatory function of this pathway during threat conditioning. Our results add to our understanding of the circuit basis mediating a multi-modal, integrated defense reaction.

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BOARD NUMBER: S03-108

RESPONSES OF LATERAL HABENULA NEURONS TO AN AVERSIVE STIMULUS ACROSS ALTERNATING BRAIN STATES OF URETHANE ANAESTHETISED RAT.

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The lateral habenula (LHb), a glutamatergic structure of the epithalamus, is known to signal negative value of events to the dopaminergic neurons, playing a pivotal role in processes related to learning and motivation. LHb indirectly inhibits dopaminergic neurons in the ventral tegmental area (VTA) via excitation of local GABAergic neurons or via rostromedial tegmental nucleus. Two subpopulations of LHb neurons were distinguished based on their opposite responses (excitation or inhibition) to aversive stimuli (AS). Additionally, differences in the response of LHb neurons to AS are correlated with the pattern and level of their basal activity as well as their spatial location. Our previous study described a subpopulation of VTA dopaminergic neurons that responds to AS in brain state-dependent manner under urethane anaesthesia. It is not known, however, whether LHb neurons may also undergo such changes and potentially account for the variability of VTA responses to AS. To address this question, we recorded LHb neurons' activity *in vivo*, using multi electrode arrays, and observed their responses to the AS in urethane anesthetized rats. In agreement with literature, we observed AS-excited and AS-inhibited LHb neurons. However, we also encountered a subpopulation of LHb neurons that changes the type of response to AS and the basal level of activity between REM-like and non-REM-like brain states. This study extends the knowledge about LHb neurons' responses to the aversive stimuli, and may shed new light on the relationship between the general state of brain and neuronal processing of aversion.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-109

INSULIN-LIKE GROWTH FACTOR I MITIGATES POST-TRAUMATIC STRESS BY INHIBITING AMP-KINASE IN OREXIN NEURONS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Maladaptive coping behaviors are probably involved in post-traumatic stress disorders (PTSD), but underlying mechanisms are incompletely understood. We documented that insulin-like growth factor I (IGF-I) is associated to vulnerability to stress in mice and humans. Since orexin neurons express IGF-I receptors and are involved in stress responses, we analyzed their role in modulatory actions of IGF-I on stress. Anxiolytic actions of IGF-I were measured after predator exposure using osmotic minipumps in mice lacking IGF-I receptors in orexin neurons (Firoc). Firoc mice were submitted to fear conditioning and thereafter c-Fos immunostaining in orexin and locus coeruleus nucleus was performed. Similar experiments were carried out with optogenetic activation in orexin neurons. Chemogenetic inhibition of orexin neurons was performed in fear conditioning, context recall and anhedonia tests. PTSD-related molecular changes were qPCR determined. Excitatory/inhibitory (E/I) balance in orexin neurons was analyzed. We found that Firoc mice are unresponsive to the anxiolytic actions of IGF-I and develop PTSD-like behavior that is ameliorated by inhibition of orexin neurons. Further, systemic IGF-I treatment ameliorated PTSD-like behavior in a wild type mouse model of PTSD. In addition, systemic IGF-I increased the E/I ratio in orexin neurons of wild type mice by increasing the dephosphorylation of GABA(B) receptor subunit through inhibition of AMP-kinase (AMPK). Significantly, pharmacological inhibition of AMPK mimicked IGF-I, normalizing fear in PTSD mice. Collectively, these results suggest that IGF-I enables coping behaviors by balancing E/I input onto orexin neurons in a context-dependent manner. This provides a novel therapeutic approach to PTSD through modulation of AMPK.

Pubmed:

34539376: Zegarra-Valdivia JA, Chaves-Coira I, Fernandez de Sevilla ME, Martinez-Rachadell L, Esparza J, Torres-Aleman I, Nuñez A
Reduced Insulin-Like Growth Factor-I Effects in the Basal Forebrain of Aging Mouse.
It is known that aging is frequently accompanied by a decline in cognition. Furthermore, aging is associated with lower serum IGF-I levels that may contribute to this deterioration. We studied the effect of IGF-I in neurons of the horizontal diagonal band of Broca (HDB) of young (≤6 months old) and old (≥20-month-old) mice to determine if changes in the response of these neurons to IGF-I occur along with aging. Local injection of IGF-I in the HDB nucleus increased their neuronal activity and induced fast oscillatory activity in the electrocorticogram (ECoG). Furthermore, IGF-I facilitated tactile responses in the primary somatosensory cortex elicited by air-puffs delivered in the whiskers. These excitatory effects decreased in old mice. Immunohistochemistry showed that cholinergic HDB neurons express IGF-I receptors and that IGF-I injection increased the expression of c-fos in young, but not in old animals. IGF-I increased the activity of optogenetically-identified cholinergic neurons in young animals, suggesting that most of the IGF-I-induced excitatory effects were mediated by activation of these neurons. Effects of aging were partially ameliorated by chronic IGF-I treatment in old mice. The present findings suggest that reduced IGF-I activity in old animals participates in age-associated changes in cortical activity.
Front Aging Neurosci, 2021; 13
33352990: Herrero-Labrador R, Trueba-Saiz A, Martinez-Rachadell L, Fernandez de Sevilla ME, Zegarra-Valdivia JA, Pignatelli J, Diaz-Pacheco S, Fernandez AM, Torres Aleman I
Circulating Insulin-Like Growth Factor I is Involved in the Effect of High Fat Diet on Peripheral Amyloid β Clearance.
Obesity is a risk factor for Alzheimer's disease (AD), but underlying mechanisms are not clear. We analyzed peripheral clearance of amyloid β (Aβ) in overweight mice because its systemic elimination may impact brain Aβ load, a major landmark of AD pathology. We also analyzed whether circulating insulin-like growth factor I (IGF-I) intervenes in the effects of overweight as this growth factor modulates brain Aβ clearance and is increased in the serum of overweight mice. Overweight

mice showed increased Aβ accumulation by the liver, the major site of elimination of systemic Aβ, but unaltered brain Aβ levels. We also found that Aβ accumulation by hepatocytes is stimulated by IGF-I, and that mice with low serum IGF-I levels show reduced liver Aβ accumulation-ameliorated by IGF-I administration, and unchanged brain Aβ levels. In the brain, IGF-I favored the association of its receptor (IGF-IR) with the Aβ precursor protein (APP), and at the same time, stimulated non-amyloidogenic processing of APP in astrocytes, as indicated by an increased sAPPα/sAPPβ ratio after IGF-I treatment. Since serum IGF-I enters into the brain in an activity-dependent manner, we analyzed in overweight mice the effect of brain activation by environmental enrichment (EE) on brain IGF-IR phosphorylation and its association to APP, as a readout of IGF-I activity. After EE, significantly reduced brain IGF-IR phosphorylation and APP/IGF-IR association were found in overweight mice as compared to lean controls. Collectively, these results indicate that a high-fat diet influences peripheral clearance of Aβ without affecting brain Aβ load. Increased serum IGF-I likely contributes to enhanced peripheral Aβ clearance in overweight mice, without affecting brain Aβ load probably because its brain entrance is reduced.
Int J Mol Sci, 2020; 21

33070417: Zegarra-Valdivia JA, Pignatelli J, Fernandez de Sevilla ME, Fernandez AM, Munive V, Martinez-Rachadell L, Nuñez A, Torres Aleman I
Insulin-like growth factor I modulates sleep through hypothalamic orexin neurons.
Although sleep disturbances are common co-morbidities of metabolic diseases, the underlying processes linking both are not yet fully defined. Changes in the duration of sleep are paralleled by changes in the levels of insulin-like growth factor-I (IGF-I), an anabolic hormone that shows a circadian pattern in the circulation and activity-dependent entrance in the brain. However, the specific role, if any, of IGF-I in this universal homeostatic process remains poorly understood. We now report that the activity of orexin neurons, a discrete cell population in the lateral hypothalamus that is involved in the circadian sleep/wake cycle and arousal, is modulated by IGF-I. Furthermore, mice with blunted IGF-I receptor activity in orexin neurons have lower levels of orexin in the hypothalamus, show altered electro-corticographic patterns with predominant slow wave activity, and reduced onset-sleep latency. Collectively, these results extend the role in the brain of this pleiotropic growth factor to shaping sleep architecture through the regulation of orexin neurons. We speculate that poor sleep quality associated to diverse conditions may be related to disturbed brain IGF-I input to orexin neurons.
FASEB J, 2020; 34

32189115: Silva-Hucha S, Carrero-Rojas G, Fernández de Sevilla ME, Benítez-Temiño B, Davis-López de Carrizosa MA, Pastor AM, Morcuende S
Sources and lesion-induced changes of VEGF expression in brainstem motoneurons.
Motoneurons of the oculomotor system show lesser vulnerability to neurodegeneration compared to other cranial motoneurons, as seen in amyotrophic lateral sclerosis (ALS). The overexpression of vascular endothelial growth factor (VEGF) is involved in motoneuronal protection. As previously shown, motoneurons innervating extraocular muscles present a higher amount of VEGF and its receptor Flk-1 compared to facial or hypoglossal motoneurons. Therefore, we aimed to study the possible sources of VEGF to brainstem motoneurons, such as glial cells and target muscles. We also studied the regulation of VEGF in response to axotomy in ocular, facial, and hypoglossal motor nuclei. Basal VEGF expression in astrocytes and microglial cells of the cranial motor nuclei was low. Although the presence of VEGF in the different target muscles for brainstem motoneurons was similar, the presynaptic element of the ocular neuromuscular junction showed higher amounts of Flk-1, which could result in greater efficiency in the capture of the factor by oculomotor neurons. Seven days after axotomy, a clear glial reaction was observed in all the brainstem nuclei, but the levels of the neurotrophic factor remained low in glial cells. Only the injured motoneurons of the oculomotor system showed an increase in VEGF and Flk-1, but such an increase was not detected in axotomized facial or hypoglossal motoneurons. Taken together, our findings suggest that the ocular motoneurons themselves upregulate VEGF expression in response to lesion. In conclusion, the low VEGF expression observed in glial cells suggests that these cells are not the main source of VEGF for brainstem motoneurons. Therefore, the higher VEGF expression observed in motoneurons innervating extraocular muscles is likely due either to the fact that this factor is more avidly taken up from the target muscles, in basal conditions, or is produced by these motoneurons themselves, and acts in an autocrine manner after axotomy.
Brain Struct Funct, 2020; 225

31156175: Zegarra-Valdivia JA, Santi A, Fernández de Sevilla ME, Nuñez A, Torres Aleman I
Serum Insulin-Like Growth Factor I Deficiency Associates to Alzheimer's Disease Co-Morbidities.
Increasing evidence supports the notion that Alzheimer's disease (AD), a condition that presents heterogeneous pathological disturbances, is also associated to perturbed metabolic function affecting insulin and insulin-like growth factor I (IGF-I). While impaired insulin activity leading to insulin resistance has been associated to AD, whether altered IGF-I function affects the disease is not entirely clear. Despite the limitations of mouse models to mimic AD pathology, we took advantage that serum IGF-I deficient mice (LID mice) present many functional perturbations present in AD, most prominently cognitive loss, which is reversed by treatment with systemic IGF-I. We analyzed whether these mice display other pathological traits that are usual

co-morbidities of AD. We found that LID mice not only display cognitive disturbances, but also show altered mood and sociability, increased susceptibility to epileptiform activity, and a disturbed sleep/wake cycle. Collectively, these data suggest that reduced IGF-I activity contributes to heterogeneous deficits commonly associated to AD. We suggest that impaired IGF-I activity needs to be taken into consideration when modeling this condition.
J Alzheimers Dis, 2019; 69

35115701: Fernández de Sevilla ME, Pignatelli J, Zegarra-Valdivia JA, Mendez P, Nuñez A, Torres Alemán I
Insulin-like growth factor I mitigates post-traumatic stress by inhibiting AMP-kinase in orexin neurons.
Maladaptive coping behaviors are probably involved in post-traumatic stress disorders (PTSD), but underlying mechanisms are incompletely understood. We now report that mice lacking functional insulin-like growth factor I (IGF-I) receptors in orexin neurons of the lateral hypothalamus (Firoc mice) are unresponsive to the anxiolytic actions of IGF-I and develop PTSD-like behavior that is ameliorated by inhibition of orexin neurons. Conversely, systemic IGF-I treatment ameliorated PTSD-like behavior in a wild-type mouse model of PTSD (PTSD mice). Further, systemic IGF-I modified the GABA/Glutamate synaptic structure in orexin neurons of naïve wild-type mice by increasing the dephosphorylation of GABA(B) receptor subunit through inhibition of AMP-kinase (AMPK). Significantly, pharmacological inhibition of AMPK mimicked IGF-I, normalizing fear behavior in PTSD mice. Thus, we suggest that IGF-I enables coping behaviors by balancing E/I input onto orexin neurons in a context-dependent manner. These observations provide a novel therapeutic approach to PTSD through modulation of AMPK.
Mol Psychiatry, 2022; 27

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-110

CONTROL OF THE ACTIVITY OF MIDBRAIN DOPAMINERGIC NEURONS BY THE NUCLEUS INCERTUS OF THE BRAIN STEM – ELECTROPHYSIOLOGICAL, ANATOMICAL AND BEHAVIOURAL STUDIES IN RATS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Midbrain dopaminergic neurons, located in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), control animals' motivation, experience-based learning and motor functions. Our preliminary observations suggested that one of the inputs controlling the dopaminergic system may arise from the brainstem nucleus incertus (NI) involved in aversive stimuli processing and stress response generation. Therefore, our study was aimed to further investigate the hitherto unknown neuronal pathway connecting NI and the dopaminergic system in the Sprague Dawley rats. For this purpose, anatomical, electrophysiological and behavioural experiments were performed. Before electrophysiological and behavioural experiments, two viral vectors, one retrograde, carrying Cre recombinase gene and the other carrying Cre-dependent genes for the red light-sensitive cationic channel and fluorescent protein, were injected into animal's VTA/SNc and NI respectively. To visualise the anatomy of the studied pathway retrogradely transported viral vector carrying genes for fluorescent protein was injected to VTA/SNc. Our anatomical data confirmed the existence of a monosynaptic input from NI to VTA/SNc neurons. Furthermore, electrophysiological observations showed a subpopulation of VTA/SNc neurons that respond to NI optogenetic activation with fast, short duration inhibition. Juxtacellular recording-labelling combined with immunostaining proved that those inhibited neurons are DA. Finally, behavioural data revealed the aversive nature of NI to VTA input. Overall, our study shows that NI-originating innervation of the midbrain DA neurons is inhibitory and that its activation is sufficient to induce conditioned aversion. Funding: grant OPUS UMO-2019/33/B/NZ4/03127 from Polish National Science Center. The authors declare no conflict of interest.

Pubmed:
33741724: Pradel K, Drwięga G, Błasiak T
Superior Colliculus Controls the Activity of the Rostromedial Tegmental Nuclei in an Asymmetrical Manner.
Dopaminergic (DA) neurons of the midbrain are involved in controlling orienting and approach of animals toward relevant external stimuli. The firing of DA neurons is regulated by many brain structures; however, the sensory input is provided predominantly by the ipsilateral superior colliculus (SC). It is suggested that SC also innervates the contralateral rostromedial tegmental nucleus (RMTg)-the main inhibitory input to DA neurons. Therefore, this study aimed to describe the physiology and anatomy of the SC-RMTg pathway. To investigate the anatomic connections within the circuit of interest, anterograde, retrograde, and transsynaptic tract-tracing studies were performed on male Sprague Dawley rats. We have observed that RMTg is monosynaptically innervated predominantly by the lateral parts of the intermediate layer of the contralateral SC. To study the physiology of this neuronal pathway, we conducted electrophysiological experiments combined with optogenetics; the activity of RMTg neurons was recorded using silicon probes, while either contralateral or ipsilateral SC was optogenetically stimulated. Obtained results revealed that activation of the contralateral SC excites the majority of RMTg neurons, while stimulation of the ipsilateral SC evokes similar proportions of excitatory or inhibitory responses. Consequently, single-unit recordings showed that the activation of RMTg neurons innervated by the contralateral SC, or stimulation of contralateral SC-originating axon terminals within the RMTg, inhibits midbrain DA neurons. Together, the anatomy and physiology of the discovered brain circuit suggest its involvement in the orienting and motivation-driven locomotion of animals based on the direction of external sensory stimuli. Dopaminergic neurons are the target of predominantly ipsilateral, excitatory innervation originating from the superior colliculus. However, we demonstrate in our study that SC inhibits the activity of dopaminergic neurons on the contralateral side of the brain via the rostromedial tegmental nucleus. In this way, sensory information received by the animal from one hemifield could induce opposite effects on both sides of the dopaminergic system. It was shown that the side to which an animal directs its behavior is a manifestation of asymmetry in dopamine

release between left and right striatum. Animals tend to move oppositely to the hemisphere with higher striatal dopamine concentration. This explains how the above-described circuit might guide the behavior of animals according to the direction of incoming sensory stimuli.
J Neurosci, 2021; 41
31478293: Solecki W, Wilczkowski M, Pradel K, Karwowska K, Kielbinski M, Drwięga G, Zajda K, Blasiak T, Soltys Z, Rajfur Z, Szklarczyk K, Przewłocki R
Effects of brief inhibition of the ventral tegmental area dopamine neurons on the cocaine seeking during abstinence.
Preclinical studies strongly suggest that cocaine seeking depends on the neuronal activity of the ventral tegmental area (VTA) and phasic dopaminergic (DA) signaling. Notably, VTA pharmacological inactivation or dopamine receptor blockade in the forebrain may induce behavioral inhibition in general and acute aversive states in particular, thus reducing cocaine seeking indirectly. Such artifacts hinder successful translation of these findings in clinical studies and practice. Here, we aimed to evaluate if dynamic VTA manipulations effectively reduce cocaine seeking. We used male tyrosine hydroxylase (TH) IRES-Cre rats along with optogenetic tools to inhibit directly and briefly VTA DA neurons during conditioned stimulus (CS)-induced cocaine seeking under extinction conditions. The behavioral effects of optogenetic inhibition were also assessed in the real-time dynamic place aversion, conditioned place aversion, and CS-induced food-seeking tests. We found that brief and nondysphoric/nonsedative pulses of VTA photo-inhibition (1 s every 9 s, ie, for 10% of time) attenuated CS-induced cocaine seeking under extinction conditions in rats expressing archaerhodopsin selectively on the TH neurons. Furthermore, direct inhibition of the VTA DA activity reduced CS-induced cocaine seeking 24 hours after photo-modulation. Importantly, such effect appears to be selective for cocaine seeking as similar inhibition of the VTA DA activity had no effect on CS-induced food seeking. Thus, briefly inhibiting VTA DA activity during CS-induced cocaine seeking drastically and selectively reduces seeking without behavioral artifacts such as sedation or dysphoria. Our results point to the therapeutic possibilities of coupling nonpharmacologic treatments with extinction training in reducing cocaine addiction.
Addict Biol, 2020; 25

FENS Forum 2022
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BOARD NUMBER: S03-111

NEUROKININ B NEURONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS IN EMOTION AND FEEDING BEHAVIOR

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Objective: The bed nucleus of stria terminalis (BNST) is a limbic fore brain structure involved in stress-regulation and related to human psychiatric diseases. Studies suggest that neurons within the BNST are capable of modulating both, aversive and appetitive behaviors. Neurokinin B (NKB) is an abundantly expressed yet little explored neuropeptide in the BNST. Our aim is to investigate whether NKB supports the integrative function of BNST neurons. **Methods:** Histochemistry and viral vector mediated neuronal tract tracing were used to identify, characterize and map the elaborated network of BNST NKB neurons. The modulatory role of NKB neurons on anxiety, fear and feeding behavior was studied using chemogenetic activation and inactivation followed by behavioral testing in transgenic Tac2cre mice. **Results:** NKB was abundantly expressed in calretinin positive neurons of the BNST and dominant projections were found in amygdala, hypothalamus and periaqueductal grey. Chemogenetic stimulation of BNST NKB neurons increased anxiety-related behavior and sustained fear expression but reduced food intake, while inhibition of these neuronal population did not alter these behaviors. Furthermore, activation of BNST NKB neurons in the BNST did not result in altered pain sensation nor promote an aversive phenotype. **Conclusion:** Our results suggest that NKB neurons of the BNST play a substantial role in coordinating sustained fear and feeding-related behaviors, probably by interacting with distinct amygdala, hypothalamic and brainstem nuclei.

Pubmed:

34122036: Comeras LB, Hörmer N, Mohan Bethuraj P, Tasan RO
NPY Released From GABA Neurons of the Dentate Gyrus Specially Reduces Contextual Fear Without Affecting Cued or Trace Fear.
Disproportionate, maladapted, and generalized fear are essential hallmarks of posttraumatic stress disorder (PTSD), which develops upon severe trauma in a subset of exposed individuals. Among the brain areas that are processing fear memories, the hippocampal formation exerts a central role linking emotional-affective with cognitive aspects. In the hippocampus, neuronal excitability is constrained by multiple GABAergic interneurons with highly specialized functions and an extensive repertoire of co-released neuromodulators. Neuropeptide Y (NPY) is one of these co-transmitters that significantly affects hippocampal signaling, with ample evidence supporting its fundamental role in emotional, cognitive, and metabolic circuitries. Here we investigated the role of NPY in relation to GABA, both released from the same interneurons of the dorsal dentate gyrus (DG), in different aspects of fear conditioning. We demonstrated that activation of dentate GABA neurons specifically during fear recall reduced cue-related as well as trace-related freezing behavior, whereas inhibition of the same neurons had no significant effects. Interestingly, concomitant overexpression of NPY in these neurons did not further modify fear recall, neither under baseline conditions nor upon chemogenetic stimulation. However, potentially increased co-release of NPY substantially reduced contextual fear, promoted extinction learning, and long-term suppression of fear in a foreground context-conditioning paradigm. Importantly, NPY in the dorsal DG was not only expressed in somatostatin neurons, but also in parvalbumin-positive basket cells and axoaxonic cells, indicating intense feedback and feedforward modulation of hippocampal signaling and precise curtailing of neuronal engrams. Thus, these findings suggest that co-release of NPY from specific interneuron populations of the dorsal DG modifies dedicated aspects of hippocampal processing by sharpening the activation of neural engrams and the consecutive fear response. Since inappropriate and generalized fear is the major impediment in the treatment of PTSD patients, the dentate NPY system may be a suitable access point to ameliorate PTSD symptoms and improve the inherent disease course.
Front Synaptic Neurosci, 2021; 13
28867896: Singh K, Patro N, Pradeepa M, Patro I
Neonatal Lipopolysaccharide Infection Causes Demyelination and Behavioral Deficits in Adult and Senile Rat Brain.
Neonatal bacterial infections have been reported to cause white matter loss, although studies concerning the influence of

infection on the expression of myelin and aging are still in their emerging state.
Ann Neurosci, 2017; 24

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-112

CORTICO-THALAMIC PRINCIPLES DEFINE THE COMPLEXITY OF THE INTRA-AMYGDALAR CONNECTIVITY

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The amygdala and the neurocircuitry it is embedded in have long been implicated in a wide range of emotional processes. During associative learning - according to the well-accepted model -, conditioned and unconditioned stimuli reach the basolateral complex of the amygdala (BLA) from various cortical and thalamic sources, which then, conveyed to the central amygdala (CeA). Finally, CeA outputs directly control behavioral responses via its subcortical targets. However, numerous anatomical and functional inconsistencies in the amygdala literature question this model. Therefore, our main goal was to provide a comprehensive anatomical map with connectivity principles of the amygdalar subregions. First, we delineated the different amygdala subnuclei using several molecular markers. Then, we performed antero- and retrograde tracer microinjections to selectively label all major amygdala subnuclei in mice. We mapped the intra-amygdalar connections between these territories and discovered the existence of several, rather non-overlapping pathways. This intra-amygdalar connectivity pattern showed remarkable overlap with the selectivity of the main cortical and thalamic innervations. These results were further supported by in-vivo electrophysiological data showing that stimulation of different thalamic inputs elicits distinct intra-amygdalar activation patterns. Taken together, our findings highlight the existence of parallel (rather than serial) information flow within the amygdala defined by their intra-amygdalar and cortical/thalamic innervation that could provide a framework for future studies on understanding amygdalar function.

Pubmed:
32284608: Barsy B, Kocsis K, Magyar A, Babiczky Á, Szabó M, Veres JM, Hillier D, Ulbert I, Yizhar O, Mátyás F
Associative and plastic thalamic signaling to the lateral amygdala controls fear behavior.
Decades of research support the idea that associations between a conditioned stimulus (CS) and an unconditioned stimulus (US) are encoded in the lateral amygdala (LA) during fear learning. However, direct proof for the sources of CS and US information is lacking. Definitive evidence of the LA as the primary site for cue association is also missing. Here, we show that calretinin (Calr)-expressing neurons of the lateral thalamus (CalrLT neurons) convey the association of fast CS (tone) and US (foot shock) signals upstream from the LA in mice. CalrLT input shapes a short-latency sensory-evoked activation pattern of the amygdala via both feedforward excitation and inhibition. Optogenetic silencing of CalrLT input to the LA prevents auditory fear conditioning. Notably, fear conditioning drives plasticity in CalrLT neurons, which is required for appropriate cue and contextual fear memory retrieval. Collectively, our results demonstrate that CalrLT neurons provide integrated CS-US representations to the LA that support the formation of aversive memories.
Nat Neurosci, 2020; 23
30349105: Mátyás F, Komlósi G, Babiczky Á, Kocsis K, Barthó P, Barsy B, Dávid C, Kanti V, Porrero C, Magyar A, Szűcs I, Clasca F, Acsády L
A highly collateralized thalamic cell type with arousal-predicting activity serves as a key hub for graded state transitions in the forebrain.
Sleep cycles consist of rapid alterations between arousal states, including transient perturbation of sleep rhythms, microarousals, and full-blown awake states. Here we demonstrate that the calretinin (CR)-containing neurons in the dorsal medial thalamus (DMT) constitute a key diencephalic node that mediates distinct levels of forebrain arousal. Cell-type-specific activation of DMT/CR cells elicited active locomotion lasting for minutes, stereotyped microarousals, or transient disruption of sleep rhythms, depending on the parameters of the stimulation. State transitions could be induced in both slow-wave and rapid eye-movement sleep. The DMT/CR cells displayed elevated activity before arousal, received selective subcortical inputs, and innervated several forebrain sites via highly branched axons. Together, these features enable DMT/CR cells to

summate subcortical arousal information and effectively transfer it as a rapid, synchronous signal to several forebrain regions to modulate the level of arousal.
Nat Neurosci, 2018; 21
33424439: Pomothy JM, Barna RF, Pászti EA, Babiczky Á, Szóládi Á, Jerzsele Á, Gere EP
Beneficial Effects of Rosmarinic Acid on IPEC-J2 Cells Exposed to the Combination of Deoxynivalenol and T-2 Toxin.
Mycotoxin contamination in feedstuffs is a worldwide problem that causes serious health issues both in humans and animals, and it contributes to serious economic losses. Deoxynivalenol (DON) and T-2 toxin (T-2) are major trichothecene mycotoxins and are known to challenge mainly intestinal barrier functions. Polyphenolic rosmarinic acid (RA) appeared to have antioxidant and anti-inflammatory properties . The aim of this study was to investigate protective effects of RA against DON and T-2 or combined mycotoxin-induced intestinal damage in nontumorigenic porcine cell line, IPEC-J2. It was ascertained that simultaneous treatment of DON and T-2 (DT2: 1 mol/L DON + 5 nmol/L T - 2) for 48 h and 72 h reduced transepithelial electrical resistance of cell monolayer, which was restored by 50 mol/L RA application. It was also found that DT2 for 48 h and 72 h could induce oxidative stress and elevate interleukin-6 (IL-6) and interleukin-8 (IL-8) levels significantly, which were alleviated by the administration of RA. DT2 administration contributed to the redistribution of claudin-1; however, occludin membranous localization was not altered by combined mycotoxin treatment. In conclusion, beneficial effect of RA was exerted on DT2-deteriorated cell monolayer integrity and on the perturbated redox status of IPEC-J2 cells.
Mediators Inflamm, 2020; 2020
34252445: Pomothy JM, Szabó O, Czimmermann ÁE, Babiczky Á, Jerzsele Á, Pászti-Gere E
Investigation of the inflammatory and oxidative stress-inducing effects of deoxynivalenol and T-2 toxin exposure in non-tumorigenic human intestinal cell model.
Fungi in the Fusarium genus produce trichothecene mycotoxins including deoxynivalenol (DON) and T-2 toxin which may elicit their damaging effects on the gastrointestinal tract following the consumption of contaminated cereal-based foods. The aim of our study was to evaluate the effects of these commonly occurring fusarotoxins alone and in combination using the human, non-cancerous intestinal epithelial cell line HIEC-6. Based on our experimental data, 24 h after treatment with fusarotoxins, hydrogen peroxide levels, intracellular oxidative stress and the amounts of inflammatory interleukins IL-6 and IL-8 significantly increased. Cell membrane localization of the tight junction protein claudin-1 decreased, whereas distribution of occludin remained unchanged. Taken together, the HIEC-6 cell line appears to be a suitable experimental model for monitoring the combined effects of mycotoxins at the cellular level including changes in the redox states of cells.
Toxicon, 2021; 200
34963932: Sebák F, Horváth LB, Kovács D, Szolomájer J, Tóth GK, Babiczky Á, Bősze S, Bodor A
Novel Lysine-Rich Delivery Peptides of Plant Origin ERD and Human S100: The Effect of Carboxyfluorescein Conjugation, Influence of Aromatic and Proline Residues, Cellular Internalization, and Penetration Ability.
The need for novel drug delivery peptides is an important issue of the modern pharmaceutical research. Here, we test K-rich peptides from plant dehydrin ERD14 (ERD-A, ERD-B, and ERD-C) and the C-terminal CPP-resembling region of S100A4 (S100) using the 5(6)-carboxyfluorescein (Cf) tag at the N-terminus. Via a combined pH-dependent NMR and fluorescence study, we analyze the effect of the Cf conjugation/modification on the structural behavior, separately investigating the (5)-Cf and (6)-Cf forms. Flow cytometry results show that all peptides internalize; however, there is a slight difference between the cellular internalization of (5)- and (6)-Cf-peptides. We indicate the possible importance of residues with an aromatic sidechain and proline. We prove that ERD-A localizes mostly in the cytosol, ERD-B and S100 have partial colocalization with lysosomal staining, and ERD-C mainly localizes within vesicle-like compartments, while the uptake mechanism mainly occurs through energy-dependent paths.
ACS Omega, 2021; 6

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BOARD NUMBER: S03-113

A FUNCTIONAL CHARACTERIZATION OF SOMATOSTATIN EXPRESSING NEURONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Aims: The Bed Nucleus of the Stria Terminalis (BNST) is a sexually dimorphic forebrain structure and part of the extended amygdala. Widely acknowledged for its involvement in anxiety, a growing body of evidence also suggest for a role in fear-related behaviors. The diversity of the roles that it presents calls for a better understanding of the subpopulations that compose the BNST and their specific roles. Indeed, the BNST is divided in distinct subnuclei and composed of various, mostly GABAergic neuronal populations. Among these subpopulations, inhibitory somatostatin neurons ($BNST^{SOM}$) remain elusive in their role and projection targets. We therefore aim to better characterize these neurons and their actions. **Methods :** In our study, we used a Somatostatin-IRES-Cre mouse line to selectively transfect $BNST^{SOM}$ neurons with chemogenetic activating Designer Receptors Exclusively Activated by Designer Drug (DREADD) or neuronal tracers. Mice were tested for feeding and anxiety-like behavior as well as phasic and sustained fear expression upon activation of these neurons. **Results :** We identified projections notably to the Periaqueductal Grey, a region linked to both anxiety and fear-like disorders. Chemogenetic activation of the $BNST^{SOM}$ neurons led to enhanced states of sustained fear in male mice. Interestingly, similar activation had an anxiogenic effect in female mice, and enhanced both phasic and sustained states of fear. **Conclusion :** Together, our findings point towards a potential role of $BNST^{SOM}$ neurons in the modulation of anxious states and fear expression.

Pubmed:

30756361: Francardo V, Geva M, Bez F, Denis Q, Steiner L, Hayden MR, Cenci MA
Pridopidine Induces Functional Neurorestoration Via the Sigma-1 Receptor in a Mouse Model of Parkinson's Disease. Pridopidine is a small molecule in clinical development for the treatment of Huntington's disease. It was recently found to have high binding affinity to the sigma-1 receptor, a chaperone protein involved in cellular defense mechanisms and neuroplasticity. Here, we have evaluated the neuroprotective and neurorestorative effects of pridopidine in a unilateral 6-hydroxydopamine (6-OHDA) lesion model of parkinsonism in mice. By 5 weeks of daily administration, a low dose of pridopidine (0.3 mg/kg) had significantly improved deficits in forelimb use (cylinder test, stepping test) and abolished the ipsilateral rotational bias typical of hemiparkinsonian animals. A higher dose of pridopidine (1 mg/kg) significantly improved only the rotational bias, with a trend towards improvement in forelimb use. The behavioral recovery induced by pridopidine 0.3 mg/kg was accompanied by a significant protection of nigral dopamine cell bodies, an increased dopaminergic fiber density in the striatum, and striatal upregulation of GDNF, BDNF, and phosphorylated ERK1/2. The beneficial effects of pridopidine 0.3 mg/kg were absent in 6-OHDA-lesioned mice lacking the sigma-1 receptor. Pharmacokinetic data confirmed that the effective dose of pridopidine reached brain concentrations sufficient to bind S1R. Our results are the first to show that pridopidine promotes functional neurorestoration in the damaged nigrostriatal system acting via the sigma-1 receptor. Neurotherapeutics, 2019; 16

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BOARD NUMBER: S03-114

AVERSIVE STIMULUS CODING REVISITED – BRAIN STATE-DEPENDENT RESPONSES OF MIDBRAIN DOPAMINERGIC NEURONS TO ELECTRICAL FOOTSHOCK

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Dopaminergic neurons of the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) form the core of reward and motivation system in the mammalian brain. Midbrain dopaminergic neurons have long been known to code information about either value or salience of aversive stimuli by responding with decrease or increase of their activity, respectively. Given that both level and pattern of VTA and SNc dopaminergic neurons' activity depend on alternating brain states under urethane anaesthesia, we hypothesized that midbrain dopaminergic neurons' responses to the aversive stimuli are also brain state-dependent.
We carried out in vivo recordings of dopaminergic neurons' responses to the electrical footshocks in urethane anaesthetized rats. Dopaminergic nature of recorded neurons was confirmed using juxtacellular labelling, combined with immunohistochemical staining against TH, or optotagging (TH-Cre rats). Consistently with previous studies, we observed two subpopulations of VTA and SNc dopaminergic neurons – one excited and one inhibited by the footshocks. However, we also discovered a third, previously undescribed, population of dopaminergic neurons that is characterized by dynamic, brain state-dependent changes in the type of response to electrical footshocks. Most of these dopaminergic neurons are inhibited by an aversive stimulus in a low theta-frequency brain state, but after the brain goes into slow wave state these neurons begin be excited by footshock. By describing a new population of midbrain dopaminergic neurons that likely encode both stimulus value and salience, our results broaden the current understanding of aversive event coding and processing in the brain.
Funding: National Science Centre, PRELUDIUM 2019/33/N/NZ4/03011

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BOARD NUMBER: S03-115

SIMULTANEOUS INVESTIGATION OF NEUROMODULATORY SYSTEMS DURING OPERANT CONDITIONING BY FIBER PHOTOMETRY AND ELECTROPHYSIOLOGY

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The key role of different neuromodulatory systems in learning and memory has long been known. However, the relationship between different neuromodulatory systems and possible synergistic or antagonistic effects are almost completely unknown. In addition, the known association of the degeneration of cholinergic and dopaminergic neurons with neurodegenerative diseases such as Alzheimer's and Parkinson's lends special importance to studying these systems. According to recent findings, reward prediction error previously known only in the dopaminergic system may also be encoded by the cholinergic system. It is not yet clear what similarities and differences the information represented by the two systems show. In this work, we investigated the two systems simultaneously in an auditory operant psychometric learning task using fiber photometry and electrophysiological methods. We found that dopaminergic neurons responded earlier than cholinergic cells after reward-predicting tones, while neither of them responded to punishment-predicting tones. We observed more activity after the delivery of unexpected than expected reward in both cell-types. Cholinergic neurons responded earlier than dopaminergic neurons to both expected and unexpected reward. Dopaminergic neurons were heterogeneous in their responses to punishment, being either suppressed or activated. A group of cholinergic neurons responded to punishment with an extremely precise and fast activation, while some neurons did not respond. Our results point to the similarities and differences between the two systems and how they work simultaneously. Crosscorrelation analysis revealed fast within system and slow across system temporal correlations, which suggest a complex relationship between the two neuromodulatory systems.

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BOARD NUMBER: S03-116

IN VIVO EXAMINATION OF AGE-DEPENDENT CHANGES IN THE ACTIVITY OF BASAL FOREBRAIN CHOLINERGIC NEURONS DURING A PAVLOVIAN CONDITIONING TASK

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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By releasing acetylcholine, the basal forebrain cholinergic neurons and their widespread projections to the cortical mantle play a key role in the control of cognitive functions, like sensory processing, attention, arousal and reinforcement expectation. Several age-related changes were observed in these neurons, such as degeneration of dendrites, axons and synapsis; nonetheless the connection between cholinergic activity during learning and the age-related neurodegeneration is still not completely clear. To address this, we combined fluorescent in vivo recording techniques and optogenetic manipulations in headfixed mice during an auditory cued Pavlovian conditioning task. By using fiber photometry, we measured acetylcholine release in the basolateral nucleus of the amygdala (BLA) using a newly developed acetylcholine sensor. As another approach, we optogenetically inhibited the cholinergic neurons of the horizontal diagonal band of Broca (HDB) during the presentation of conditioned stimuli (CS). Our results suggest that cholinergic cells respond to the reward-predicting CS and also to the unconditioned stimuli with an increase of activity and acetylcholine release. It seems that acetylcholine release in the BLA occurs only during the reward-predicting but not the punishment-predicting sensory stimuli. In old mice, acetylcholine levels in the BLA showed no response to the CS. In the optogenetic manipulation experiments, we found that the animals which received the inhibitory virus in their HDB had difficulties learning the task compared to the animals which received the control virus. According to our results acetylcholine release from basal forebrain neurons is required during the acquisition of the CS-US association during Pavlovian conditioning.

Pubmed:
34522902: Hegedüs P, Velencei A, Belval CH, Heckenast J, Hangya B
Training protocol for probabilistic Pavlovian conditioning in mice using an open-source head-fixed setup.
High throughput, temporally controlled, reproducible quantitative behavioral assays are important for understanding the neural mechanisms underlying behavior. Here, we provide a step-by-step training protocol for a probabilistic Pavlovian conditioning task, where two auditory cues predict probabilistic outcomes with different contingencies. This protocol allows us to study the differential behavioral and neuronal correlates of expected and surprising outcomes. It has been tested in combination with chronic electrophysiological recordings and optogenetic manipulations in ChAT-Cre and PV-Cre mouse lines. For complete details on the use and execution of this protocol, please refer to Hegedüs et al. (2021).
STAR Protoc, 2021; 2

FENS Forum 2022
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BOARD NUMBER: S03-117

THALAMO-SEPTAL-HYPOTHALAMIC CIRCUIT INVOLVED IN MATERNAL BEHAVIOURAL CONTROL IN RODENTS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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[1]Hungarian Academy of Sciences, Eötvös Loránd Research Network, and Eötvös Loránd University, Department Of Physiology And Neurobiology, Budapest, Hungary, [2]University of Veterinary Medicine Budapest, Department Of Ecology, Budapest, Hungary, [3]Indiana University, Graduate School Of The Department Of Psychological And Brain Sciences, Bloomington, United States of America, [4]Institute of Experimental Medicine, Eötvös Loránd Research Network, Department Of Behavioral And Stress Studies, Budapest, Hungary, [5]Centre for Neuroscience, Szentágothai Research Centre, Institute of Physiology, Medical School, University Of Pécs, Pécs, Hungary, [6]Eötvös Loránd University, Department Of Physiology And Neurobiology, Budapest, Hungary

Maternal care is a special form of social behaviour regulated by both hormones and neuronal inputs. Our research group previously described a thalamo-hypothalamic pathway containing the maternally induced neuropeptide, parathyroid hormone-2 (PTH2) expressed by neurons of the posterior intralaminar thalamic nucleus (PIL). Additional to the hypothalamic medial preoptic area (MPOA), the central regulator of maternal behaviour, PIL neurons also project to the lateral septum (LS). The LS is known to show pup-induced activation in rodent mothers and to have reciprocal connection with the MPOA. Therefore, we hypothesize that LS participates in the PIL-MPOA circuit controlling maternal care. Here, we demonstrate that LS receives input from maternally-activated PIL neurons and fibres arising from the PIL are PTH2+. Furthermore, we found elevated number of pup-induced activated calbindin LS neurons closely apposed by PTH2-terminals. We also revealed, all calbindin and maternally-activated LS neurons are GABAergic. Synaptic connection of GABAergic LS neurons with PTH2-terminals was confirmed by electron microscopy. We showed that LS GABAergic and calbindin neurons send prominent input to the MPOA. Since MPOA also receives dense PTH2+ projection from PIL neurons, we provided evidence that LS-projecting PIL neurons send axon collaterals to the MPOA by identifying double labelled PIL neurons following injection of different retrograde neuronal tracers into the LS and MPOA simultaneously. We conclude that pup directed information is conveyed to the LS and the MPOA via the PTH2+ input from the PIL. PTH2-innervated calbindin LS neurons may regulate maternal-related neurons of the MPOA via inhibitory signal. Support: NKFIH-4300-1/2017-NKP_17-0002, OTKA K134221, TKP2020-IKA-05

FENS Forum 2022
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BOARD NUMBER: S03-118

INHIBITION MEDIATED BY GROUP III MGLURS REGULATES HABENULAR ACTIVITY AND DEFENSIVE BEHAVIORS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Habenula is an evolutionary conserved brain region that was shown to play important roles in adaptive and defensive behavior. Notably, it receives both, excitation and inhibitory inputs from the forebrain and relays this information to monoaminergic brain nuclei. We observed that several members of group III metabotropic glutamate receptors (mGluRs) are expressed in the zebrafish habenula. Targeting those group III mGluRs pharmacologically, alters the membrane potential and the connectivity of habenular neurons. In addition, perturbing group III mGluRs with both, genetic and pharmacological methods directly modulates the ongoing and sensory driven activity in habenula and the forebrain of juvenile zebrafish. Our preliminary results suggest that perturbing group III mGluRs also alters defensive and adaptive behaviors. Altogether, these findings propose an important role for group III mGluRs for regulating habenular circuits and animal behavior.

Pubmed:
34416179: Bartoszek EM, Ostenrath AM, Jetti SK, Serneels B, Mutlu AK, Chau KTP, Yaksi E
Ongoing habenular activity is driven by forebrain networks and modulated by olfactory stimuli.
Ongoing neural activity, which represents internal brain states, is constantly modulated by the sensory information that is generated by the environment. In this study, we show that the habenular circuits act as a major brain hub integrating the structured ongoing activity of the limbic forebrain circuitry and the olfactory information. We demonstrate that ancestral homologs of amygdala and hippocampus in zebrafish forebrain are the major drivers of ongoing habenular activity. We also reveal that odor stimuli can modulate the activity of specific habenular neurons that are driven by this forebrain circuitry. Our results highlight a major role for the olfactory system in regulating the ongoing activity of the habenula and the forebrain, thereby altering brain's internal states.
Curr Biol, 2021; 31

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-119

RXFP3-INDUCED MODULATION OF AMYGDALA NEURONAL NETWORK ACTIVITY.

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The relaxin-3 (rln3) is a neuropeptide that has been shown to be involved in several physiological functions. It is synthetized in GABAergic neurons of the *nucleus incertus*. Relaxin-3 mainly acts through RXFP3, a G-protein coupled receptor that once activated, lead to a neuronal inhibition. Both RXFP3-expressing neurons and rln3 positive projections are found throughout many brain structures. Among them, the basal and central amygdala which are known to be involved in emotions processing, including pain and its comorbidities. Preliminary experiments have shown that amygdala RXFP3 activation triggers mechanical analgesia in a mice model of pain-induced inflammatory sensitization. Hence, our project aims to decipher the effect of RXFP3 activation on the amygdala neuronal network activity. To do so, we will take advantage of newly developed specific agonists of RXFP3 to characterize the RXFP3 effects using *ex vivo* patch clamp recording of both GABAergic interneurons and projection neurons. Finally, we will assess the contribution of *nucleus incertus* Rln3 neurons to the modulation of the amygdala using an optogenetic-based circuit deciphering approach.

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BOARD NUMBER: S03-120

BIOPHYSICAL PROPERTIES AND GENE EXPRESSION PROFILE OF SINGLE PERIAQUEDUCTAL GRAY NEURONS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The midbrain periaqueductal gray (PAG) is a longitudinal columnar structure where instinctive behaviours as diverse as escaping from predators, vocalising, and pup grooming segregate onto distinct anatomical subdivisions. This parallel between behaviour and brain circuit anatomy provides a unique opportunity for investigating how neural mechanisms support the computation of different adaptive actions. Here, we characterised the biophysical properties and gene expression profile of single neurons across PAG subdivisions. First, we measured the intrinsic firing properties of PAG neurons using loose-seal cell-attached recordings in acute midbrain slices of transgenic mice. We found that, even in the absence of synaptic inputs, inhibitory ($VGAT^+$) neurons fire action potentials spontaneously, whereas excitatory ($VGluT2^+$) neurons do not. Next, to link the expression of ion channels, receptors, and molecular effectors to specific PAG subdivisions, we performed cell-type specific single-cell RNA-sequencing while preserving the anatomical origin of each neuron. We individually isolated fluorescently labelled neurons from acute midbrain slices of transgenic mice and processed them with Smart-seq2 and a sequencing depth of 4 million reads per sample. Graph-based clustering of the resulting data revealed putative subtypes of neurons that map onto different PAG subdivisions, whereas differential expression analysis of these data identified candidate ion channel subunit and neuromodulator genes for setting and regulating key biophysical properties of PAG neurons. By leveraging the unique relationship between PAG circuit anatomy and behavioural output, our work uses anatomical location as an anchor to provide a framework for studying how molecularly defined biophysical properties might underpin behavioural control by the PAG.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-121

ANATOMICAL AND FUNCTIONAL CHARACTERIZATION OF AMYGDALA-STRIATAL CIRCUITS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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In humans and animals, changes in emotional states are known to modify posture, fine motor control, and/or coordination, inducing either beneficial or detrimental effects on motor performance. This suggests an overlap between neural circuits underlying emotions (limbic system) and motor control (basal ganglia). We thus performed an extensive review of the anatomical limbic-to-basal ganglia direct connections and chose to focus on the amygdala to caudate-putamen (CPu) projections in mice. The CPu is the gate of entry of the basal ganglia and is involved in action selection and movement. The amygdala, comprised of different subnuclei, is a key limbic structure involved in emotional processing such as valence-related signals, fear- and anxiety-related behavior. We hypothesize that amygdala-CPu neurons can modulate motor behavior during emotional states. Anatomical tracing tools, combined with *in vivo* and *ex-vivo* electrophysiology, show that the basolateral amygdala (BLA) complex is the main amygdala input to the CPu, with the densest projections to the medial CPu. These excitatory projections preferentially target medium-spiny neurons and parvalbumin interneurons. To define this neuronal sub-population, we mapped the inputs and outputs of the BLA-CPu neurons and determined the sources of their neuromodulators. Calcium imaging *in vivo* in anesthetized mice further confirms the functional connectivity of the main inputs to BLA-CPu neurons and shows that they respond to different sensory challenges, such as footshocks and air puffs. Our study shows that the BLA-CPu pathway connects limbic structures to the basal ganglia and can also relay sensory, cognitive and interoceptive to the motor system.

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BOARD NUMBER: S03-122

DOPAMINERGIC MODULATION OF NUCLEUS INCERTUS TO INTERPEDUNCULAR NUCLEUS INPUT – A POSSIBLE NEURONAL MECHANISM FOR STRESS-INDUCED NOVELTY PREFERENCE DEFICIENCIES

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Appropriate novelty/familiarity discrimination and adequate responses to novelty are crucial for proper functioning and behaviour. Many neuropsychiatric disorders manifest in atypical reactions to novelty, and stress plays a key role in the development of this deficiency. Here we investigated the nucleus incertus (NI) to interpeduncular nucleus (IPN) innervation. NI is a stress-sensitive brainstem nucleus, and directly innervates the IPN, a neuroanatomical substrate for familiarity signalling. Concomitantly, dopamine (DA) has an established role in motivation-related processes and novelty preference. Aim: Investigate the functional NI to IPN connectivity, along with possible interactions of this innervation and DA/D1R signalling at the level of IPN neurons. Methods: Whole-cell patch-clamp recordings of IPN neuron activity were combined with optogenetic-stimulation of NI-originating fibres and D1R agonist application. Viral vector-based retrograde tract-tracing was combined with immunofluorescent staining. Results: Upon stimulation of NI-originating fibres, mostly light-evoked inhibitory postsynaptic currents (Ie iPSCs) were observed in the IPN. Among the neurons sensitive to optogenetic stimulation, 61.5% was excited by D1R activation, revealing that the same neurons are sensitive to novelty-related dopamine signalling and stress-related signals from the brainstem. Notably, analysis of Ie iPSC shape revealed that D1R agonist application decreased their amplitude. Neural tract-tracing revealed that the ventral tegmental area (VTA) is the main source of DA terminals in the IPN. Conclusions: These electrophysiological and anatomical data suggest a role for an NI–IPN–VTA pathway in the control of novelty preference and attentional processes, with DA/D1R signalling diminishing the negative influence of brainstem-originating stress signals on novelty preference. Funding: UMO-2018/30/E/NZ4/00687

Pubmed:
35078925: Trenk A, Walczak M, Szlaga A, Pradel K, Blasiak A, Blasiak T
Bidirectional Communication between the Pontine Nucleus Incertus and the Medial Septum Is Carried Out by Electrophysiologically-Distinct Neuronal Populations.
Theta oscillations are key brain rhythm involved in memory formation, sensorimotor integration, and control of locomotion and behavioral states. Generation and spatiotemporal synchronization of theta oscillations rely on interactions between brain nuclei forming a large neural network, which includes pontine nucleus incertus (NI). Here we identified distinct populations of NI neurons, based on the relationship of their firing to hippocampal waves, with a special focus on theta oscillations, and the direction and type of interaction with the medial septum (MS) in male, urethane-anesthetized rats. By recording NI neuronal firing and hippocampal LFP, we described NI neurons that fire action potentials in a theta phase-independent or theta phase-locked and delta wave-independent or delta wave-locked manner. Among hippocampal activity-independent NI neurons, irregular, slow-firing, and regular, fast-firing cells were observed, while hippocampal oscillation-/wave-locked NI neurons were of a bursting or nonbursting type. By projection-specific optotagging, we revealed that only fast-firing theta phase-independent NI neurons innervate the MS, rarely receiving feedback information. In contrast, the majority of theta-bursting NI neurons were inhibited by MS stimulation, and this effect was mediated by direct GABAergic input. Described NI neuronal populations differ in reciprocal connections with the septohippocampal system, plausibly forming separate neuronal loops. Our results suggest that theta phase-independent NI neurons participate in theta rhythm generation through direct innervation of the MS, while theta-bursting NI neurons further transmit the rhythmic signal received from the MS to stabilize and/or strengthen rhythmic activity in other structures. The generation and spatiotemporal synchronization of theta oscillations rely on interactions between nuclei forming a large neural network, part of which is the pontine nucleus incertus (NI). Here we describe that within NI there are populations of neurons that can be distinguished based on the relationship of their firing to hippocampal theta oscillations and delta waves. We show that these neuronal populations largely do not have reciprocal connections with the septohippocampal system, but form separate neuronal loops. Our results suggest that medial septum (MS)-projecting, fast-firing, theta phase-independent NI neurons may participate in theta rhythm generation through direct

innervation of the MS, while theta-bursting NI neurons may further transmit the rhythmic signal received from the MS to other structures.
J Neurosci, 2022; 42
33095902: Grzesiak M, Burzawa G, Kurowska P, Blaszczyk K, Szlaga A, Blasiak A, Sechman A, Rak A
Altered vitamin D metabolism in the ovary and periovarian adipose tissue of rats with letrozole-induced PCOS.
Vitamin D (VD) plays an important role in the ovary and its deficiency is associated with ovarian pathologies, including polycystic ovary syndrome (PCOS). However, there is no data related to VD metabolism in the ovary during PCOS. Herein, we investigated differences in the expression of VD receptor (VDR) and key VD metabolic enzymes, 1α-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24A1), in the ovary and periovarian adipose tissue (POAT) of control (proestrus and diestrus) and PCOS induced by letrozole rats. Vdr, Cyp27b1 and Cyp24a1 mRNA expression was determined, their protein abundance was examined and immunolocalized. Furthermore, VD metabolite concentrations in plasma (25OHD) and tissues (ovary and POAT; 1,25(OH)D), and plasma calcium level were determined. 25OHD concentration decreased markedly in letrozole-treated rats in comparison with controls, whereas calcium concentration did not vary among the examined groups. The amount of 1,25(OH)D decreased in both ovary and POAT of PCOS rats. In the ovary, we found decreased Cyp27b1 and increased Vdr mRNA expression in letrozole-treated and diestrus control group. Corresponding protein abundances were down-regulated and up-regulated, respectively but only following letrozole treatment. In POAT, only Cyp27b1 transcript level and CYP27B1 protein abundance were decreased in letrozole-treated rats. VDR was immunolocalized in healthy and cystic follicles, while CYP27B1 and CYP24A1 were found exclusively in healthy ones. Concluding, our results provide the first evidence of disrupted VD metabolism in the ovary and POAT of PCOS rats. The reduced 1,25(OH)D concentration in those tissues suggests their contribution to VD deficiency observed in PCOS and might implicate in PCOS pathogenesis.
Histochem Cell Biol, 2021; 155
32532885: Kania A, Szlaga A, Sambak P, Gugula A, Blasiak E, Micioni Di Bonaventura MV, Hossain MA, Cifani C, Hess G, Gundlach AL, Blasiak A
RLN3/RXFP3 Signaling in the PVN Inhibits Magnocellular Neurons via M-like Current Activation and Contributes to Binge Eating Behavior.
Binge-eating disorder is the most common eating disorder. Various neuropeptides play important roles in the regulation of feeding behavior, including relaxin-3 (RLN3), which stimulates food intake in rats through the activation of the relaxin-family peptide-3 receptor (RXFP3). Here we demonstrate that a likely mechanism underlying the orexigenic action of RLN3 is RXFP3-mediated inhibition of oxytocin- and arginine-vasopressin-synthesizing paraventricular nucleus (PVN) magnocellular neurosecretory cells. Moreover, we reveal that, in male and female rats, this action depends on M-like potassium conductance. Notably, higher intra- and peri-PVN RLN3 fiber densities were observed in females, which may constitute an anatomic substrate for observed sex differences in binge-eating disorder. Finally, in a model of binge-eating in female rats, RXFP3 blockade within the PVN prevented binge-eating behavior. These data demonstrate a direct RLN3/RXFP3 action in the PVN of male and female rats, identify the associated ionic mechanisms, and reveal that hypothalamic RLN3/RXFP3 signaling regulates binge-eating behavior. Binge-eating disorder is the most common eating disorder worldwide, affecting women twice as frequently as men. Various neuropeptides play important roles in the regulation of feeding behavior, including relaxin-3, which acts via the relaxin-family peptide-3 receptor (RXFP3). Using a model of binge-eating, we demonstrated that relaxin-3/RXFP3 signaling in the hypothalamic paraventricular nucleus (PVN) is necessary for the expression of binge-eating behavior in female rats. Moreover, we elucidated the neuronal mechanism of RLN3/RXFP3 signaling in PVN in male and female rats and characterized sex differences in the RLN3 innervation of the PVN. These findings increase our understanding of the brain circuits and neurotransmitters involved in binge-eating disorder pathology and identify RXFP3 as a therapeutic target for binge-like eating disorders.
J Neurosci, 2020; 40
32505354: Kalamon N, Błaszczyk K, Szlaga A, Billert M, Skrzypski M, Pawlicki P, Górowska-Wójtowicz E, Kotula-Balak M, Błasiak A, Rak A
Levels of the neuropeptide phoenixin-14 and its receptor GRP173 in the hypothalamus, ovary and periovarian adipose tissue in rat model of polycystic ovary syndrome.
Phoenixin (PNX) is a newly discovered peptide produced by proteolytic cleavage of a small integral membrane protein 20 (Smim20), which acts as an important regulator of energy homeostasis and reproduction. Since dysfunction of reproduction is characteristic in polycystic ovarian syndrome (PCOS), the role of PNX in pathogenesis of PCOS needs further investigation. The objective of this study was to determine expression of Smim20, PNX-14 and its receptor GRP173 in the hypothalamus, ovary and periovarian adipose tissue (PAT) of letrozole induced PCOS rats. Phosphorylation of extracellular signal-regulated kinase (ERK1/2), protein kinases A (PKA) and B (Akt) were also estimated. We observed that PCOS rats had high weight gain and a number of ovarian cyst, high levels of testosterone, luteinizing hormone and PNX-14, while low estradiol. Smim20 mRNA expression was higher in the ovary and PAT, while PNX-14 peptide production was higher only in the ovary of PCOS

rat. Moreover, in PCOS rats Gpr173 level was lower in PAT but at the protein level increased only in the ovary. Depending on the tissues, kinases phosphorylation were significantly differ in PCOS rats. Our results showed higher levels of PNX-14 in PCOS rats and indicated some novel findings regarding the mechanisms of PCOS pathophysiology.
Biochem Biophys Res Commun, 2020; 528
31820102: Kania A, Sambak P, Gugula A, Szlaga A, Soltys Z, Blasiak T, Hess G, Rajfur Z, Blasiak A
Electrophysiology and distribution of oxytocin and vasopressin neurons in the hypothalamic paraventricular nucleus: a study in male and female rats.
Magnocellular neurosecretory cells (MNCs) clustered in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus constitute a major source of oxytocin (OXT) and arginine vasopressin (AVP) peptides, and are among the best described peptidergic neurons in the brain. OXT and AVP are involved in a range of homeostatic processes, social behaviours, emotional processes, and learning. Notably, their actions can be sex-specific, and several sex differences in the anatomies of the OXT and AVP systems have been reported. Nonetheless, possible sex differences in the detailed distributions of MNCs and in their intrinsic electrical properties ex vivo have not been extensively examined. We addressed these issues utilizing immunostaining and patch-clamp ex vivo recordings. Here, we showed that Sprague-Dawley rat PVN AVP neurons are more numerous than OXT cells and that more neurons of both types are present in males. Furthermore, we identified several previously unreported differences between putative OXT and AVP MNC electrophysiology contributing to their partially unique profiles. Notably, elucidation of the highly specific action potential (AP) shapes, with AVP MNCs having a narrower AP and faster hyperpolarizing after-potential (HAP) kinetics than OXT MNCs, allowed unambiguous discrimination between OXT and AVP MNCs ex vivo for the first time. Moreover, the examined electrophysiological properties of male and female MNCs generally overlapped with the following exceptions: higher membrane resistance in male MNCs and HAP kinetics in putative OXT MNCs, which was slower in males. These reported observations constitute a thorough addition to the knowledge of MNC properties shaping their diverse physiological actions in both sexes.
Brain Struct Funct, 2020; 225

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BOARD NUMBER: S03-123

NOXIOUS STIMULUS-RESPONSIVE NEURONS IN THE VENTRAL-VENTROLATERAL PERIAQUEDUCTAL GRAY AND DORSAL RAPHE NUCLEUS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The ventral-ventrolateral periaqueductal gray (v/vlPAG) and dorsal raphe nucleus (DRN) play a critical role in controlling anxiety, fear memory formation and most particularly, it is largely involved in descending modulation of pain perception. It has been shown that different neuron types, such as dopaminergic and serotonergic cells are part of this circuitry, however their exact functions remained unclear. Using *in vivo* juxtacellular recording technique in urethane-anesthetized mice, we successfully recorded from more than 40 neurons. After monitoring the baseline activity of blindly targeted neurons in the v/vlPAG and DRN, we applied mild electrical shocks to both paws, sequentially. After the recordings we filled the neurons with Neurobiotin and perfused the animals. We performed post hoc immunostaining on the brain sections (n=18), visualizing tyrosine-hydroxylase (TH)- and serotonin-expressing neurons in addition to the Neurobiotin-content of the recorded neurons. Based on the spiking response upon noxious stimulation, we found neurons that showed excitation (34%), excitation followed by inhibition (14%), inhibition (18%), inhibition followed by excitation (7%). The remaining neurons did not show any significant change in their activity. All the identified TH+ neurons showed excitation. Our current results show that the firing of neurons in the v/vlPAG and DRN circuitries is distinctly modified by noxious stimuli, and support the previous observations, showing that dopaminergic neurons convey pain-related information to their downstream regions. The various responsiveness of neurons in the dorsal tegmentum upon noxious stimulation may have an important role in salient information processing.

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BOARD NUMBER: S03-124

PATTERNS OF COLLATERALIZATION OF MIDLINE BRAINSTEM RAPHE AND INCERTUS NUCLEI TO THE SEPTOHIPPOCAMPAL SYSTEM.

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The relaxin 3 (RLN3) rich nucleus incertus (NI) and the serotonergic (5HT) raphe nuclei are midline brain stem units that modulates contextual related behaviours through the septo-hippocampal system. This work aims to characterize the pattern of collateralization from the NI to the 5HT groups and the supramammillary (SuM) nucleus. To this end, retrograde tracers fluorogold and cholera toxin B subunit (CTB) were injected into the medial septum (MS) and hippocampus, combined with 5HT, RLN3 and CTB immunofluorescence. Our results showed a higher density of RLN3 fibers in both median and dorsal raphe regions. In this area, RLN3 boutons were seen in close proximity from the 5HT somata and some of them contain synaptic markers. Few 5HT neurons from the MS were observed in the raphe nuclei, and a consistent density of relaxin3 boutons was observed around those neurons. Lastly, a population of SuM's neurons send collateral projections to the entorhinal/hippocampal system and the MS. Furthermore, this same population is innervated by RLN3 fibers. These findings revealed mapping of the modulator circuitry between the NI's RLN3 and Raphe's 5HT, and the interactions with the septo-hippocampus, which receives projections from the SuM. Based on this core of evidence, we can postulate that the SuM is a key component of the ascending NI's projections to the entorhinal-hippocampal circuit that is involved in the generation, coding and recall of spatial memories. Supported by the PN Drogas, Spanish Ministry of Health (2020I012); Ministerio de Ciencia, Innovación (RTI2018095698-B-I00); Generalitat Valenciana (AICO21I376)

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BOARD NUMBER: S03-125

LINKING EMOTIONAL VALENCE AND ANXIETY IN A MOUSE INSULA-AMYGDALA CIRCUIT

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The response of the insular cortex (IC) and amygdala to stimuli of positive and negative valence were found to be altered in patients with anxiety disorders. However, the coding properties of neurons controlling anxiety and valence remain unknown. Combining photometry recordings and chemogenetics in mice, we uncover the anxiogenic control of projection neurons in the anterior IC (aIC), independently of their projection target. Using viral tracing and *ex vivo* electrophysiology, we characterize the monosynaptic aIC to the basolateral amygdala (BLA) connection, and employed projection-specific optogenetics, to reveal anxiogenic properties of aIC-BLA neurons in anxiety-related behaviors. Finally, using photometry recordings, we identified that aIC-BLA neurons are active in anxiogenic spaces, and in response to aversive stimuli. Together, these findings show that negative valence, as well as anxiety-related information and behaviors, are encoded by aIC-BLA glutamatergic neurons, providing a starting point to understand how alterations of this pathway contribute to psychiatric disorders.

Pubmed:
34987089: Nicolas C, Zlebnik NE, Farokhnia M, Leggio L, Ikemoto S, Shaham Y
Sex Differences in Opioid and Psychostimulant Craving and Relapse: A Critical Review.
A widely held dogma in the preclinical addiction field is that females are more vulnerable than males to drug craving and relapse. Here, we first review clinical studies on sex differences in psychostimulant and opioid craving and relapse. Next, we review preclinical studies on sex differences in psychostimulant and opioid reinstatement of drug seeking after extinction of drug self-administration, and incubation of drug craving (time-dependent increase in drug seeking during abstinence). We also discuss ovarian hormones' role in relapse and craving in humans and animal models and speculate on brain mechanisms underlying their role in cocaine craving and relapse in rodent models. Finally, we discuss imaging studies on brain responses to cocaine cues and stress in men and women.The results of the clinical studies reviewed do not appear to support the notion that women are more vulnerable to psychostimulant and opioid craving and relapse. However, this conclusion is tentative because most of the studies reviewed were correlational, not sufficiently powered, and not a priori designed to detect sex differences. Additionally, imaging studies suggest sex differences in brain responses to cocaine cues and stress. The results of the preclinical studies reviewed provide evidence for sex differences in stress-induced reinstatement and incubation of cocaine craving but not cue- or cocaine-induced reinstatement of cocaine seeking. These sex differences are modulated in part by ovarian hormones. In contrast, the available data do not support the notion of sex differences in craving and relapse/reinstatement for methamphetamine or opioids in rodent models. SIGNIFICANCE STATEMENT: This systematic review summarizes clinical and preclinical studies on sex differences in psychostimulant and opioid craving and relapse. Results of the clinical studies reviewed do not appear to support the notion that women are more vulnerable to psychostimulant and opioid craving and relapse. Results of preclinical studies reviewed provide evidence for sex differences in reinstatement and incubation of cocaine seeking but not for reinstatement or incubation of methamphetamine or opioid seeking.
Pharmacol Rev, 2022; 74
33768375: Nicolas C, Hofford RS, Dugast E, Lardeux V, Belujon P, Solinas M, Bardo MT, Thiriet N
Prevention of relapse to methamphetamine self-administration by environmental enrichment: involvement of glucocorticoid receptors.
In rodents, environmental enrichment (EE) produces both preventive and curative effects on drug addiction, and this effect is believed to depend at least in part on EE's actions on the stress system.
Psychopharmacology (Berl), 2022; 239
33388819: Sanchez-Hernandez A, Nicolas C, Gil-Miravet I, Guarque-Chabrera J, Solinas M, Miquel M
Time-dependent regulation of perineuronal nets in the cerebellar cortex during abstinence of cocaine-self administration.
The probability of structural remodeling in brain circuits may be modulated by molecules of perineuronal nets (PNNs) that restrict neuronal plasticity to stabilize circuits. Animal research demonstrates that addictive drugs can remodel PNNs in

different brain regions, including the cerebellum.
Psychopharmacology (Berl), 2021; 238
30846301: Nicolas C, Russell TI, Pierce AF, Maldera S, Holley A, You ZB, McCarthy MM, Shaham Y, Ikemoto S
Incubation of Cocaine Craving After Intermittent-Access Self-administration: Sex Differences and Estrous Cycle.
Studies using continuous-access drug self-administration showed that cocaine seeking increases during abstinence (incubation of cocaine craving). Recently, studies using intermittent-access self-administration showed increased motivation to self-administer and seek cocaine. We examined whether intermittent cocaine self-administration would potentiate incubation of craving in male and female rats and examined the estrous cycle's role in this incubation.
Biol Psychiatry, 2019; 85
29288750: Sikora M, Nicolas C, Istin M, Jaafari N, Thiriet N, Solinas M
Generalization of effects of environmental enrichment on seeking for different classes of drugs of abuse.
Addiction is a chronic disease characterized by persistent vulnerability to relapse during abstinence. In animal models of addiction, accumulating evidence suggests that exposure to environmental enrichment (EE) during periods of abstinence can have curative effects on addiction and reduce the risks of relapse. However, until present most studies have mainly focused on cocaine. In this study, we investigated whether EE could have beneficial effects on cue-induced seeking for several psychoactive drugs belonging to different pharmacological classes such as methamphetamine (METH), heroin (HER) and nicotine (NIC).
Behav Brain Res, 2018; 341
28553833: Nicolas C, Tauber C, Lepelletier FX, Chalon S, Belujon P, Galineau L, Solinas M
Longitudinal Changes in Brain Metabolic Activity after Withdrawal from Escalation of Cocaine Self-Administration.
The chronic and relapsing nature of addiction suggests that drugs produce persistent adaptations in the brain that make individuals with drug addiction particularly sensitive to drug-related cues and stress and incapable of controlling drug-seeking and drug-taking behavior. In animal models, several long-lasting neuroadaptations have been described. However, few studies have used brain-imaging techniques to provide a complete picture of brain functioning in the course of withdrawal from cocaine. In this study, we allowed rats to self-administer cocaine under short-access (1-h/day) or long-access (6-h/day) conditions and used 2-deoxy-2-(F)fluoro-d-glucose (FDG) positron emission tomography scanning to investigate the longitudinal changes in metabolic activity 1 and 4 weeks after discontinuation of cocaine self-administration. We found that compared to naive rats, both long-access and short-access rats showed significant disruptions in basal brain metabolic activity. However, compared to short-access, long-access rats showed more intense, and long-lasting neuroadaptations in a network of brain areas. In particular, abstinence from extended access to cocaine was associated with decreased metabolic activity in the anterior cingulate cortex, the insular cortex, and the dorsolateral striatum, and increased metabolic activity in the mesencephalon, amygdala, and hippocampus. This pattern is strikingly similar to that described in humans that has led to the proposal of the Impaired Response Inhibition and Salience Attribution model of addiction. These results demonstrate that extended access to cocaine leads to persistent neuroadaptations in brain regions involved in motivation, salience attribution, memory, stress, and inhibitory control that may underlie increased risks of relapse.
Neuropsychopharmacology, 2017; 42
28391507: Talishinsky AD, Nicolas C, Ikemoto S
Interaction of chronic food restriction and methylphenidate in sensation seeking of rats.
It is necessary to understand better how chronic food restriction (CFR) and psychostimulant drugs interact in motivated behavior unrelated to food or energy homeostasis.
Psychopharmacology (Berl), 2017; 234
26997496: Nicolas C, Lafay-Chebassier C, Solinas M
Exposure to sucrose during periods of withdrawal does not reduce cocaine-seeking behavior in rats.
Concomitant access to drugs of abuse and alternative rewards such as sucrose has been shown to decrease addiction-related behaviors in animals. Here we investigated whether access to sucrose during abstinence in contexts that are temporally and physically distinct from drug-related contexts could reduce subsequent drug seeking. In addition, we investigated whether a history of cocaine self-administration would alter the rewarding effects of sucrose. Rats self-administered cocaine for ten sessions, while yoked-saline rats received only saline injections, and then we subjected them to a 30-day withdrawal period during which they had access to water and sucrose continuously or intermittently according to a schedule that induces binge-drinking behavior. At the end of the withdrawal period, rats were tested for cocaine seeking behavior during a single 6 h session. We found that exposure to cocaine increased sucrose consumption only when rats had intermittent access to sucrose, but exposure to sucrose did not alter drug seeking regardless of the schedule of access. These results suggest that exposure to cocaine cross-sensitizes to the rewarding effects of sucrose, but exposure to sucrose during abstinence, temporally and physically distinct from drug-related environments, does not to reduce drug seeking.
Sci Rep, 2016; 6

26466819: Chauvet C, Nicolas C, Lafay-Chebassier C, Jaber M, Thiriet N, Solinas M
Statins Reduce the Risks of Relapse to Addiction in Rats.
Statins are drugs that have been used for decades in humans for the treatment of hypercholesterolemia. More recently, several lines of evidence demonstrate that statins, in addition to their peripheral effects, produce a wide variety of effects in the brain and may be beneficial in neurological and psychiatric conditions. In this study, we allowed rats to self-administer cocaine for several weeks and, at the end of self-administration training, we treated them with low doses of statins daily for a 21-day period of abstinence. Chronic administration of brain-penetrating statins, simvastatin (1 mg/kg) and atorvastatin (1 mg/kg), reduced cocaine seeking compared with vehicle, whereas administration of pravastatin (2 mg/kg), a statin with low brain penetrability, did not. Importantly, the effects of brain-penetrating statins persisted even after discontinuation of the treatment and were specific for drug seeking because drug taking was not altered by simvastatin treatment. Finally, the effects of simvastatin were found to generalize to another drug of abuse such as nicotine, but not to food reward, and to reinstatement of cocaine seeking induced by stress. These results demonstrate that brain-penetrating statins can reduce risks of relapse to addiction. Given their well-known safety profile in humans, statins could be a novel effective treatment for relapse to cocaine and nicotine addiction and their use could be implemented in clinical settings without major health risks.
Neuropsychopharmacology, 2016; 41
25522382: Chauvet C, Nicolas C, Thiriet N, Lardeux MV, Duranti A, Solinas M
Chronic stimulation of the tone of endogenous anandamide reduces cue- and stress-induced relapse in rats.
The endogenous cannabinoid system plays an important role in motivation, stress, and drug abuse. Pharmacologically, the endocannabinoid system can be stimulated by either agonists of CB1 receptors or inhibition of metabolic degradation of endogenous cannabinoids and consequent increases in their brain levels.
Int J Neuropsychopharmacol, 2014; 18

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BOARD NUMBER: S03-126

THE CONNECTION OF MC3R NEURONS AND THEIR ROLE IN STRESS RESPONSES.

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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In modern society, stress has become a common trigger for inappropriate eating behaviors, yet the mechanism behind is not well established. The central melanocortin system is known to be the key regulator of energy homeostasis, it is mediated through a family of five related G protein–coupled melanocortin receptors, MC1R through MC5R, whereas only MC3R and MC4R are expressed in the brain. Mice lacking MC3R have been shown to have abnormal responses to fasting, shown by inappropriate refeeding behavior and altered corticosterone levels. However, where these MC3R neurons are acting to mediate effects of nutritional stresses such as fasting is unknown. In addition, recent work shows a role for MC3R in social behaviors as well. Therefore, we studied the role of MC3R modulation in two different stress paradigms (nutritional and non-nutritional). Further, we identified innervation of MC3R neurons to target sites using the MC3R-Cre; ROSA26-LSL-Synaptophysin-TdTomato mouse model. One site identified as a target of MC3R neurons was the amygdala. Interestingly, we found out a distinct pattern of MC3R projections to the central Amygdala and completely absent of projection to the basolateral amygdala (BLA). The amygdala is responsible for fear response, reward mechanisms and promoting anxiety-like behavior. We hypothesize, MC3R regulates neuronal circuits projecting to the AMY to mediate food intake under stress situations.

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OXYTOCIN AND VASOPRESSIN NEURONS IN THE ANTERO-LATERAL PREOPTIC (ALPO) REGION MODULATE THE REWARD SYSTEM.

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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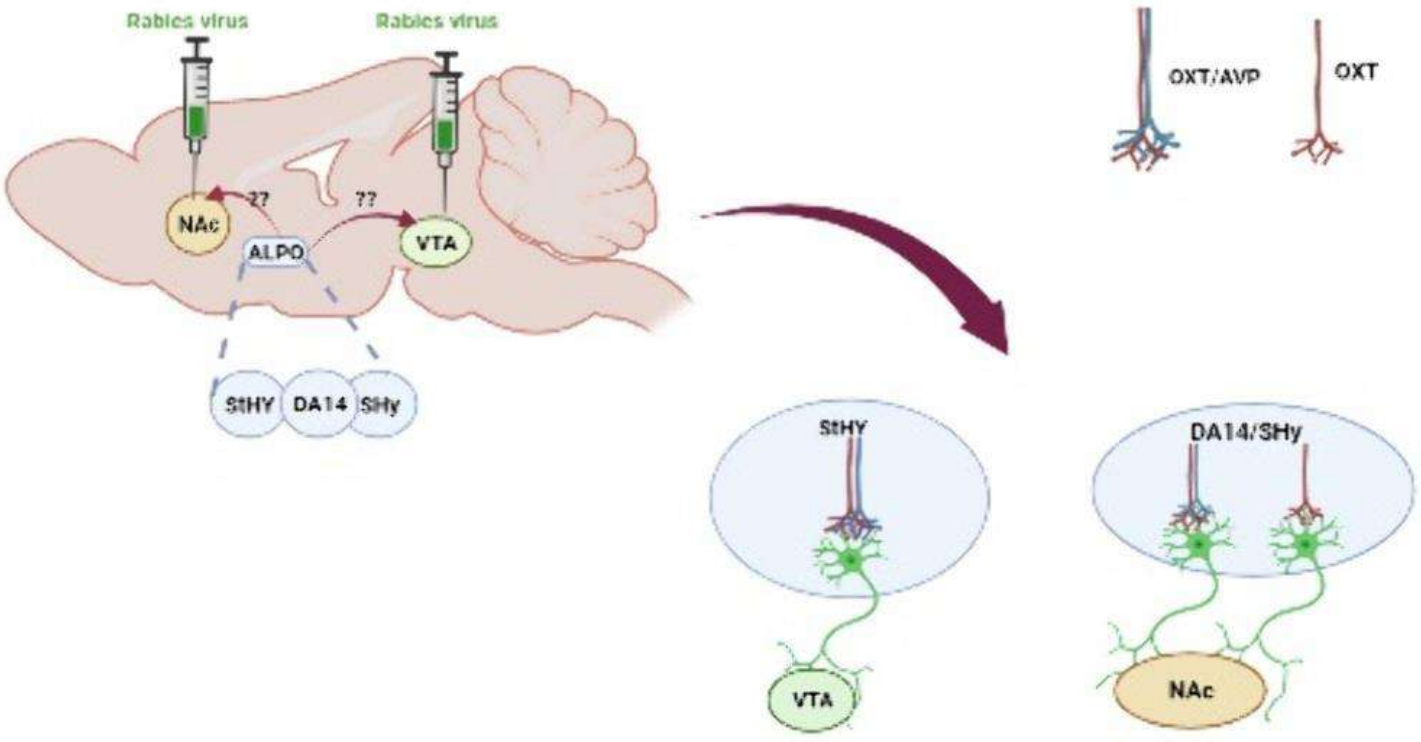

The hypothalamic neuropeptide oxytocin (OXT) and vasopressin (AVP) are two hormones known to modulate body homeostasis and social behavior. Studies have shown the action of oxytocin in enhancing complex social activities such as pair bonding, affiliative preferences, and parental behaviors. OXT is known to impact the activity of mesolimbic regions such as the ventral tegmental area (VTA) and nucleus accumbens (NAc) which play a crucial role not only in reward and motivation, but also in the expression of affiliative behaviors. The precise way in which oxytocin and vasopressin neural pathways interact with the reward system remains underinvestigated. We examined the anatomical and functional connectivity between the reward circuit regions, namely the VTA and NAc, and the ALPO, an area rich in oxytocin neurons and subdivided in different nuclei namely the striohypothamic (StHY), the Septohypothamic (SHy) and the Dopaminergic 14

(DA14). Next, we characterized the neurochemical identity of these networks by coupling rabies virus tracing method with RNAscope fluorescent in situ hybridization and immunohistoflourescence. Preliminary findings showed a direct projection between StHY and VTA and between SHy and DA14 towards NAc in addition to OXT neurons in StHY, SHy and DA14 sending an indirect path to VTA and NAc. Interestingly, two populations of OXT neurons in StHY and SHy nuclei co-express OXT and AVP in the pathways innervating VTA and NAc whereas another population expresses only OXT projected to NAc. We will discuss the role of these circuits from ALPO region and their potential role in coloring social contexts as rewarding

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DEFENSIVE BEHAVIORS BETWEEN NATURE AND NURTURE: FUNCTIONAL CHARACTERIZATION OF THE DEFENSIVE BEHAVIORS CIRCUIT IN THE MOUSE BRAIN

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Understanding the neural circuits underlying defensive behaviors is crucial in elucidating survival mechanisms. Defensive behaviors can be triggered by learned and innately aversive threats. In a learning paradigm, a neutral stimulus (CS) triggers a defensive response only after being paired with an unconditioned aversive stimulus (US). On the other hand, an innately aversive threat induces defensive responses from the first presentation. The amygdala has been studied extensively and it is known to be essential in emotional behavioral responses. Among other functions, it is well known that the amygdala is required to elicit defensive responses in a wide range of threats. However, it remains unclear whether the learned and innate defensive responses share a common node in the amygdala. Combining circuit mapping, chemogenetics, and fiberphotometry, we tested our hypothesis that the basolateral amygdala (BLA) processes learned and innately aversive threats. With a loss-of-function approach, we show that bilateral silencing of the lateral amygdala suppresses the defensive responses induced by the looming stimulus as well as aversive conditioning. Fiber photometry of the BLA pyramidal neurons reveals an increase in the calcium signal linked to the defensive behaviors induced by the looming stimulus. Repeated exposure to the stimulus, reduces the defensive response as well as the calcium signal activity. Together, these findings support the hypothesis that the amygdala is a shared node in the neural circuit that regulates the defensive responses to an innately and learned aversive threat.

Pubmed:

32499680: Krauth N, Khalil V, Jariwala M, Mermet-Joret N, Vestergaard AK, Capogna M, Nabavi S
TRACE: An Unbiased Method to Permanently Tag Transiently Activated Inputs.
A fundamental interest in circuit analysis is to parse out the synaptic inputs underlying a behavioral experience. Toward this aim, we have devised an unbiased strategy that specifically labels the afferent inputs that are activated by a defined stimulus in an activity-dependent manner. We validated this strategy in four brain circuits receiving known sensory inputs. This strategy, as demonstrated here, accurately identifies these inputs.
Front Cell Neurosci, 2020; 14
31844154: Torromino G, Autore L, Khalil V, Mastrorilli V, Griguoli M, Pignataro A, Centofante E, Biasini GM, De Turris V, Ammassari-Teule M, Rinaldi A, Mele A
Offline ventral subiculum-ventral striatum serial communication is required for spatial memory consolidation.
The hippocampal formation is considered essential for spatial navigation. In particular, subicular projections have been suggested to carry spatial information from the hippocampus to the ventral striatum. However, possible cross-structural communication between these two brain regions in memory formation has thus far been unknown. By selectively silencing the subiculum-ventral striatum pathway we found that its activity after learning is crucial for spatial memory consolidation and learning-induced plasticity. These results provide new insight into the neural circuits underlying memory consolidation and establish a critical role for off-line cross-regional communication between hippocampus and ventral striatum to promote the storage of complex information.
Nat Commun, 2019; 10

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BOARD NUMBER: S03-129

DEVELOPMENTAL DISRUPTION OF ERBB4 IN PET1+ NEURONS IMPAIRS SEROTONERGIC SUB-SYSTEM CONNECTIVITY AND MEMORY FORMATION

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The serotonergic system of mammals innervates virtually all the central nervous system and regulates a broad spectrum of behavioral and physiological functions. In mammals, serotonergic neurons located in the rostral raphe nuclei encompass diverse sub-systems characterized by specific circuitry and functional features. Substantial evidence suggest that functional diversity of serotonergic circuits has a molecular and connectivity basis. However, the landscape of intrinsic developmental mechanisms guiding the formation of serotonergic sub-systems is unclear. Here, we employed developmental disruption of gene expression specific to serotonergic subsets to probe the contribution of the tyrosine kinase receptor ErbB4 to serotonergic circuit formation and function. Through an *in vivo* loss-of-function approach, we found that ErbB4 expression occurring in a subset of serotonergic neurons, is necessary for axonal arborization of defined long-range projections to the forebrain but is dispensable for the innervation of other targets of the serotonergic system. We also found that *Erbb4*-deletion does not change the global excitability or the number of neurons with serotonin content in the dorsal raphe nuclei. In addition, ErbB4-deficiency in serotonergic neurons leads to specific behavioral deficits in memory processing that involve aversive or social components. Altogether, our work unveils a developmental mechanism intrinsically acting through ErbB4 in subsets of serotonergic neurons to orchestrate a precise long-range circuit and ultimately involved in the formation of emotional and social memories.

Pubmed:
34957103: Barettino C, Ballesteros-Gonzalez Á, Aylón A, Soler-Sanchis X, Ortí L, Díaz S, Reillo I, García-García F, Iborra FJ, Lai C, Dehorter N, Leinekugel X, Flames N, Del Pino I
Developmental Disruption of in + Neurons Impairs Serotonergic Sub-System Connectivity and Memory Formation.
The serotonergic system of mammals innervates virtually all the central nervous system and regulates a broad spectrum of behavioral and physiological functions. In mammals, serotonergic neurons located in the rostral raphe nuclei encompass diverse sub-systems characterized by specific circuitry and functional features. Substantial evidence suggest that functional diversity of serotonergic circuits has a molecular and connectivity basis. However, the landscape of intrinsic developmental mechanisms guiding the formation of serotonergic sub-systems is unclear. Here, we employed developmental disruption of gene expression specific to serotonergic subsets to probe the contribution of the tyrosine kinase receptor ErbB4 to serotonergic circuit formation and function. Through an loss-of-function approach, we found that ErbB4 expression occurring in a subset of serotonergic neurons, is necessary for axonal arborization of defined long-range projections to the forebrain but is dispensable for the innervation of other targets of the serotonergic system. We also found that -deletion does not change the global excitability or the number of neurons with serotonin content in the dorsal raphe nuclei. In addition, ErbB4-deficiency in serotonergic neurons leads to specific behavioral deficits in memory processing that involve aversive or social components. Altogether, our work unveils a developmental mechanism intrinsically acting through ErbB4 in subsets of serotonergic neurons to orchestrate a precise long-range circuit and ultimately involved in the formation of emotional and social memories.
Front Cell Dev Biol, 2021; 9

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-130

EXCITATORY OXYTOCIN RECEPTOR SIGNALLING IN THE RAT BRAINSTEM NUCLEUS INCERTUS – IMPACT ON AROUSAL AND RELATED BEHAVIOURS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Aims Oxytocin (OT)/oxytocin receptor (OTR) signalling exerts pleiotropic physiological and behavioural effects, including the modulation of food and water intake, reproduction, memory, emotional processing, and social interaction. A possible effector of these actions are the OTR-expressing neurons in the *nucleus incertus* (NI) that modulate arousal and other processes that overlap with known OT functions. Therefore, our aim was to characterise the anatomy, neuronal and behavioural effects of OT/OTR signalling in the rat NI. Methods In these studies, we employed single-cell-resolution, fluorescent *in situ* hybridisation, immunostaining, viral tract-tracing tools, and extracellular multielectrode and whole cell patch-clamp recordings *ex vivo*. Finally, using a newly generated OTR-IRES-Cre knock-in rat, we chemogenetically activated NI OTR-expressing neurons and examined the effects on behaviour. Results A majority (88%) of OTR mRNA-positive NI neurons are GABAergic (expressing vGAT mRNA). Exogenous OT dose- and OTR-dependently excited 70% of NI neurons, via a postsynaptic action. The NI lacks a substantial OT innervation, but the juxtaposition of the NI to the fourth ventricle suggests endogenous OT may reach the NI via the cerebrospinal fluid. Chemogenetic activation of OTR NI neurons promoted locomotor activity, reduced anxiety, altered social interaction, and improved social recognition. Conclusions These studies identify the brainstem *nucleus incertus* as a site of action of OT/OTR signalling. Notably, our reasults reveal a possible neuronal mechanism underlying OT-mediated modulation of arousal, and provide evidence for a key role of extrahypothalamic OT actions in governing behaviour. *Funding:* Alexander von Humboldt Foundation, Germany: Humboldt Research Fellowship; NSC, Poland: UMO-2018/30/E/NZ4/00687.

Pubmed:
33804563: Jeż M, Martyniak A, Andrysiak K, Mucha O, Szade K, Kania A, Chrobok Ł, Palus-Chramiec K, Sanetra AM, Lewandowski MH, Pośpiech E, Stępniewski J, Dulak J
Role of Heme-Oxygenase-1 in Biology of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells.
Heme oxygenase-1 (HO-1, encoded by) is a cytoprotective enzyme degrading heme into CO, Fe, and biliverdin. HO-1 was demonstrated to affect cardiac differentiation of murine pluripotent stem cells (PSCs), regulate the metabolism of murine adult cardiomyocytes, and influence regeneration of infarcted myocardium in mice. However, the enzyme's effect on human cardiogenesis and human cardiomyocytes' electromechanical properties has not been described so far. Thus, this study aimed to investigate the role of HO-1 in the differentiation of human induced pluripotent stem cells (hiPSCs) into hiPSC-derived cardiomyocytes (hiPSC-CMs). hiPSCs were generated from human fibroblasts and peripheral blood mononuclear cells using Sendai vectors and subjected to CRISPR/Cas9-mediated knock-out. After confirming lack of HO-1 expression on the protein level, isogenic control and HO-1-deficient hiPSCs were differentiated into hiPSC-CMs. No differences in differentiation efficiency and hiPSC-CMs metabolism were observed in both cell types. The global transcriptomic analysis revealed, on the other hand, alterations in electrophysiological pathways in hiPSC-CMs devoid of HO-1, which also demonstrated increased size. Functional consequences in changes in expression of ion channels genes were then confirmed by patch-clamp analysis. To the best of our knowledge, this is the first report demonstrating the link between HO-1 and electrophysiology in human cardiomyocytes.
Cells, 2021; 10
33333517: de Ávila C, Chometton S, Calvez J, Guèvremont G, Kania A, Torz L, Lenglos C, Blasiak A, Rosenkilde MM, Holst B, Conrad CD, Fryer JD, Timofeeva E, Gundlach AL, Cifani C

Estrous Cycle Modulation of Feeding and Relaxin-3/Rxfp3 mRNA Expression: Implications for Estradiol Action.
Food intake varies during the ovarian hormone/estrous cycle in humans and rodents, an effect mediated mainly by estradiol. A potential mediator of the central anorectic effects of estradiol is the neuropeptide relaxin-3 (RLN3) synthetized in the nucleus incertus (NI) and acting via the relaxin family peptide-3 receptor (RXFP3).
Neuroendocrinology, 2021; 111
32532885: Kania A, Szlaga A, Sambak P, Gugula A, Blasiak E, Micioni Di Bonaventura MV, Hossain MA, Cifani C, Hess G, Gundlach AL, Blasiak A
RLN3/RXFP3 Signaling in the PVN Inhibits Magnocellular Neurons via M-like Current Activation and Contributes to Binge Eating Behavior.
Binge-eating disorder is the most common eating disorder. Various neuropeptides play important roles in the regulation of feeding behavior, including relaxin-3 (RLN3), which stimulates food intake in rats through the activation of the relaxin-family peptide-3 receptor (RXFP3). Here we demonstrate that a likely mechanism underlying the orexigenic action of RLN3 is RXFP3-mediated inhibition of oxytocin- and arginine-vasopressin-synthesizing paraventricular nucleus (PVN) magnocellular neurosecretory cells. Moreover, we reveal that, in male and female rats, this action depends on M-like potassium conductance. Notably, higher intra- and peri-PVN RLN3 fiber densities were observed in females, which may constitute an anatomic substrate for observed sex differences in binge-eating disorder. Finally, in a model of binge-eating in female rats, RXFP3 blockade within the PVN prevented binge-eating behavior. These data demonstrate a direct RLN3/RXFP3 action in the PVN of male and female rats, identify the associated ionic mechanisms, and reveal that hypothalamic RLN3/RXFP3 signaling regulates binge-eating behavior. Binge-eating disorder is the most common eating disorder worldwide, affecting women twice as frequently as men. Various neuropeptides play important roles in the regulation of feeding behavior, including relaxin-3, which acts via the relaxin-family peptide-3 receptor (RXFP3). Using a model of binge-eating, we demonstrated that relaxin-3/RXFP3 signaling in the hypothalamic paraventricular nucleus (PVN) is necessary for the expression of binge-eating behavior in female rats. Moreover, we elucidated the neuronal mechanism of RLN3/RXFP3 signaling in PVN in male and female rats and characterized sex differences in the RLN3 innervation of the PVN. These findings increase our understanding of the brain circuits and neurotransmitters involved in binge-eating disorder pathology and identify RXFP3 as a therapeutic target for binge-like eating disorders.
J Neurosci, 2020; 40
31820102: Kania A, Sambak P, Gugula A, Szlaga A, Soltys Z, Blasiak T, Hess G, Rajfur Z, Blasiak A
Electrophysiology and distribution of oxytocin and vasopressin neurons in the hypothalamic paraventricular nucleus: a study in male and female rats.
Magnocellular neurosecretory cells (MNCs) clustered in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus constitute a major source of oxytocin (OXT) and arginine vasopressin (AVP) peptides, and are among the best described peptidergic neurons in the brain. OXT and AVP are involved in a range of homeostatic processes, social behaviours, emotional processes, and learning. Notably, their actions can be sex-specific, and several sex differences in the anatomies of the OXT and AVP systems have been reported. Nonetheless, possible sex differences in the detailed distributions of MNCs and in their intrinsic electrical properties ex vivo have not been extensively examined. We addressed these issues utilizing immunostaining and patch-clamp ex vivo recordings. Here, we showed that Sprague-Dawley rat PVN AVP neurons are more numerous than OXT cells and that more neurons of both types are present in males. Furthermore, we identified several previously unreported differences between putative OXT and AVP MNC electrophysiology contributing to their partially unique profiles. Notably, elucidation of the highly specific action potential (AP) shapes, with AVP MNCs having a narrower AP and faster hyperpolarizing after-potential (HAP) kinetics than OXT MNCs, allowed unambiguous discrimination between OXT and AVP MNCs ex vivo for the first time. Moreover, the examined electrophysiological properties of male and female MNCs generally overlapped with the following exceptions: higher membrane resistance in male MNCs and HAP kinetics in putative OXT MNCs, which was slower in males. These reported observations constitute a thorough addition to the knowledge of MNC properties shaping their diverse physiological actions in both sexes.
Brain Struct Funct, 2020; 225
29981758: Sabetghadam A, Grabowiecka-Nowak A, Kania A, Gugula A, Blasiak E, Blasiak T, Ma S, Gundlach AL, Blasiak A
Melanin-concentrating hormone and orexin systems in rat nucleus incertus: Dual innervation, bidirectional effects on neuron activity, and differential influences on arousal and feeding.
The rat nucleus incertus (NI) contains GABA/peptide-projection neurons responsive to orexin (hypocretin)/orexin receptor-2 (OX) signalling. Melanin-concentrating hormone (MCH) and orexin neurons often innervate and influence common target areas. Therefore, we assessed the relationship between these hypothalamic peptidergic systems and rat NI, by investigating the presence of an MCH innervation and MCH receptor-1 (MCH) expression, and neurophysiological and behavioural effects of MCH c.f. orexin-A (OXA), within the NI. We identified lateral hypothalamus (LH), perifornical and sub-zona incerta MCH neurons that innervate NI, and characterised the rostrocaudal distribution of MCH-containing fibres in NI. Single-cell RT-PCR

detected MCH and OX mRNA in NI, and multiplex, fluorescent in situ hybridisation revealed distinct co-expression patterns of MCH and OX mRNA in NI neurons expressing vesicular GABA transporter (vGAT) mRNA. Patch-clamp recordings revealed 34% of NI neurons tested were hyperpolarised by MCH (1 μM), representing a distinct population from OXA-sensitive NI neurons (35%). Intra-NI OXA infusion (600 pmol) in satiated rats during the light/inactive phase produced increased locomotor activity and food (standard chow) intake, whereas intra-NI MCH infusion (600 pmol) produced only a trend for decreased locomotor activity and no effect on food intake. Furthermore, in satiated or pre-fasted rats tested during the dark/active phase, intra-NI infusion of MCH did not alter the elevated locomotor activity or higher food intake observed. However, quantification of neuropeptide-immunostaining revealed differential diurnal fluctuations in orexin and MCH trafficking to NI. Our findings identify MCH and orexin inputs onto divergent NI populations which may differentially influence arousal and motivated behaviours.
Neuropharmacology, 2018; 139
28748319: Kula J, Gugula A, Blasiak A, Bobula B, Danielewicz J, Kania A, Tylko G, Hess G
Diverse action of repeated corticosterone treatment on synaptic transmission, neuronal plasticity, and morphology in superficial and deep layers of the rat motor cortex.
One of the adverse effects of prolonged stress in rats is impaired performance of skilled reaching and walking tasks. The mechanisms that lead to these abnormalities are incompletely understood. Therefore, we compared the effects of twice daily repeated corticosterone injections for 7 days on miniature excitatory postsynaptic currents (mEPSCs), as well as on synaptic plasticity and morphology of layers II/III and V pyramidal neurons of the primary motor cortex (M1) of male Wistar rats. Corticosterone treatment resulted in increased frequency, but not amplitude, of mEPSCs in layer II/III neurons accompanied by increased complexity of the apical part of their dendritic tree, with no changes in the density of dendritic spines. The frequency and amplitude of mEPSCs as well as the parameters characterizing the complexity of the dendritic tree were not changed in layer V cells; however, their dendritic spine density was increased. While corticosterone treatment resulted in an increase in the amplitude of field potentials evoked in intralaminar connections within layer II/III, it did not influence field responses in layer V intralaminar connections, as well as the extent of chemically induced layer V long-term potentiation (chemLTP) by the application of tetraethylammonium (TEA, 25 mM). However, chemLTP induction in layer II/III was impaired in slices prepared from corticosterone-treated animals. These data indicate that repeated 7-day administration of exogenous corticosterone induces structural and functional plasticity in the M1, which occurs mainly in layer II/III pyramidal neurons. These findings shed light on potential sites of action and mechanisms underlying stress-induced impairment of motor functions.
Pflugers Arch, 2017; 469
28098344: Kania A, Gugula A, Grabowiecka A, de Ávila C, Blasiak T, Rajfur Z, Lewandowski MH, Hess G, Timofeeva E, Gundlach AL, Blasiak A
Inhibition of oxytocin and vasopressin neuron activity in rat hypothalamic paraventricular nucleus by relaxin-3-RXFP3 signalling.
Relaxin-3 is a stress-responsive neuropeptide that acts at its cognate receptor, RXFP3, to alter behaviours including feeding. In this study, we have demonstrated a direct, RXFP3-dependent, inhibitory action of relaxin-3 on oxytocin and vasopressin paraventricular nucleus (PVN) neuron electrical activity, a putative cellular mechanism of orexigenic actions of relaxin-3. We observed a Gα -protein-dependent inhibitory influence of selective RXFP3 activation on PVN neuronal activity in vitro and demonstrated a direct action of RXFP3 activation on oxytocin and vasopressin PVN neurons, confirmed by their abundant expression of RXFP3 mRNA. Moreover, we demonstrated that RXFP3 activation induces a cadmium-sensitive outward current, which indicates the involvement of a characteristic magnocellular neuron outward potassium current. Furthermore, we identified an abundance of relaxin-3-immunoreactive axons/fibres originating from the nucleus incertus in close proximity to the PVN, but associated with sparse relaxin-3-containing fibres/terminals within the PVN.
J Physiol, 2017; 595
26265304: Blasiak A, Siwiec M, Grabowiecka A, Blasiak T, Czerw A, Blasiak E, Kania A, Rajfur Z, Lewandowski MH, Gundlach AL
Excitatory orexinergic innervation of rat nucleus incertus--Implications for ascending arousal, motivation and feeding control.
Orexin/hypocretin peptides play a central role in the integrated control of feeding/reward and behavioural activation, principally via interactions with other neural systems. A brainstem area involved in behavioural activation is the nucleus incertus (NI), located in the posterior ventromedial central grey. Several studies have implicated NI in control of arousal/stress and reward/feeding responses. Orexin receptor mRNA expression identifies NI as a putative target of orexin modulation. Therefore, in this study we performed neural tract-tracing and immunofluorescence staining to characterise the orexinergic innervation of NI. Our results indicate a convergent innervation of the NI area by different orexin neuron populations, with an abundance of orexin-A-containing axons making putative synaptic contacts with relaxin-3-positive NI neurons. The influence of orexin-A on NI neuron activity was investigated using patch-clamp recordings. Orexin-A depolarised the majority (64%) of

recorded neurons and this effect was maintained in the presence of tetrodotoxin and glutamate and GABA receptor antagonists, indicating a likely postsynaptic action. Voltage-clamp experiments revealed that in 'type I' NI neurons comprising relaxin-3-positive cells, orexin-A acted via L-type calcium channels, whereas in 'type II' relaxin-3-negative neurons, activation of a sodium/calcium exchanger was involved. A majority of the orexin-A sensitive neurons tested for the presence of orexin receptor mRNA, were OX2 mRNA-positive. Immunohistochemical staining for putative orexin receptors on NI neurons, confirmed stronger expression of OX2 than OX1 receptors. Our data demonstrate a strong influence of orexin-A on NI neurons, consistent with an important role for this hypothalamic/tegmental circuit in the regulation of arousal/vigilance and motivated behaviours.
Neuropharmacology, 2015; 99
25474700: Czyzewska MM, Chrobok L, Kania A, Jatczak M, Pollastro F, Appendino G, Mozrzymas JW
Dietary acetylenic oxylipin falcarinol differentially modulates GABAA receptors.
The dietary oxylipins falcarinol (1a) and falcarindiol (1b) trap thiols by direct nucleophilic addition to their diyne system, but despite this, only falcarinol (1a) is a reversible agonist of cannabinoid receptors, providing a rationale for comparing their activity also on other neuronal targets. Because GABAA receptors (GABAARs) are exquisitely sensitive to polyacetylenic oxylipins in terms of either potentiation (falcarindiol, 1b) or inhibition (oenanthotoxin, 2a), the activity of 1a was investigated on synaptic (α1β2γ2L) and extrasynaptic (α1β2δ and α1β2) subtypes of GABAARs. Falcarinol (1a) significantly enhanced the amplitude of currents mediated by α1β2γ2L receptors, but this effect was associated with a use-dependent block. Conversely, α1β2 receptors were inhibited without any sign of use-dependent block for the entire range of concentrations tested (1-10 μM). Interestingly, responses mediated by α1β2δ receptors, showing no or very little macroscopic desensitization, were strongly potentiated by 1a, exhibiting a fading reminiscent of macroscopic desensitization. When compared to the activity of falcarindiol (1b), falcarinol (1a) showed a higher affinity for GABAARs and, overall, a substantially different profile of pharmacological action. Taken together, the present data support the view that modulation of GABAARs might underlie the insecticidal and sedative activity of falcarinol (1a).
J Nat Prod, 2014; 77
24988606: Kania A, Lewandowski MH, Błasiak A
[Relaxin-3 and relaxin family peptide receptors--from structure to functions of a newly discovered mammalian brain system].
Relaxin-3, a member of the relaxin peptide family, was discovered in 2001 as a homologue of relaxin--a well-known reproductive hormone. However, it is the brain which turned out to be a major expression site of this newly discovered peptide. Both its molecular structure and expression pattern were shown to be very conserved among vertebrates. Extensive research carried out since the discovery of relaxin-3 contributed to the significant progress in our knowledge regarding this neuropeptide. The endogenous relaxin-3 receptor (RXFP3) was identified and the anatomy of the yet uncharacterized mammalian brain system was described, with nucleus incertus as the main center of relaxin-3 expression. Not only its diffusive projections throughout the whole brain, which reach various brain structures such as the hippocampus, septum, intergeniculate leaflet or amygdala, but also functional studies of the relaxin-3/RXFP3 signaling system, allowed this brain network to be classified as one of the ascending nonspecific brain systems. Thus far, research depicts the connection of relaxin-3 with phenomena such as feeding behavior, spatial memory, sleep/wake cycle or modulation of pituitary gland hormone secretion. Responsiveness of relaxin-3 neurons to stress factors and the strong orexigenic effect exerted by this peptide suggest its participation in modulation of feeding by stress, in particular of the chronic type. The discovery of relaxin-3 opened a new research field which will contribute to our better understanding of the neurobiological basis of feeding disorders.
Postepy Hig Med Dosw (Online), 2014; 68

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-131

PROBING THE NEURAL CIRCUIT MEDIATING THE ANXIOLYTIC EFFECTS OF A SEROTONERGIC PSYCHEDELIC

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Psychedelics, since time immemorial, have been known to alter sensory perceptions, and have been thought to have positive effects on mood. Over the last few decades, experimental findings have shown that serotonergic psychedelics modulate the areas in the brain that are involved in mood regulation, and can be possibly used as therapeutics against mood disorders. However, it is not well known how these drugs influence different regions of the brain to drive positive effects on mood-behaviours. We found that acute administration of a serotonergic psychedelic DOI (2,5-dimethoxy-4-iodoamphetamine; a 5-HT_{2A} receptor agonist) exhibits anxiolytic response in rodents on the EPM, and this effect is driven via the CA1/subiculum field of the ventral hippocampus. Furthermore, we note that 5-HT_{2A} receptors on the Parvalbumin-positive interneurons in the CA1/subiculum region of the ventral hippocampus may be sufficient to drive the decreased anxiety-like response on DOI administration. This work not only explores the therapeutic potential of serotonergic psychedelics, but also tries to understand how information is relayed in the brain to harness their potential against anxiety-like behaviour.

Pubmed:

35115382: Tiwari P, Kapri D, Pradhan A, Balakrishnan A, Chaudhari PR, Vaidya VA
Chronic hM4Di-DREADD-Mediated Chemogenetic Inhibition of Forebrain Excitatory Neurons in Postnatal or Juvenile Life Does Not Alter Adult Mood-Related Behavior.
G-protein-coupled receptors (GPCRs) coupled to G signaling, in particular downstream of monoaminergic neurotransmission, are posited to play a key role during developmental epochs (postnatal and juvenile) in shaping the emergence of adult anxiodepressive behaviors and sensorimotor gating. To address the role of G signaling in these developmental windows, we used a CaMKIIα-tTA::TRE hM4Di bigenic mouse line to express the hM4Di-DREADD (designer receptor exclusively activated by designer drugs) in forebrain excitatory neurons and enhanced G signaling via chronic administration of the DREADD agonist, clozapine--oxide (CNO) in the postnatal window (postnatal days 2-14) or the juvenile window (postnatal days 28-40). We confirmed that the expression of the HA-tagged hM4Di-DREADD was restricted to CaMKIIα-positive neurons in the forebrain, and that the administration of CNO in postnatal or juvenile windows evoked inhibition in forebrain circuits of the hippocampus and cortex, as indicated by a decline in expression of the neuronal activity marker c-Fos. hM4Di-DREADD-mediated inhibition of CaMKIIα-positive forebrain excitatory neurons in postnatal or juvenile life did not impact the weight profile of mouse pups, and also did not influence the normal ontogeny of sensory reflexes. Further, postnatal or juvenile hM4Di-DREADD-mediated inhibition of CaMKIIα-positive forebrain excitatory neurons did not alter anxiety- or despair-like behaviors in adulthood and did not impact sensorimotor gating. Collectively, these results indicate that chemogenetic induction of G signaling in CaMKIIα-positive forebrain excitatory neurons in postnatal and juvenile temporal windows does not appear to impinge on the programming of anxiodepressive behaviors in adulthood.
eNeuro, 2022 Jan-Feb; 9
33622703: Mukhopadhyay S, Chatterjee A, Tiwari P, Ghai U, Vaidya VA
Postnatal Fluoxetine Treatment Alters Perineuronal Net Formation and Maintenance in the Hippocampus.
Elevation of serotonin via postnatal fluoxetine (PNFlx) treatment during critical temporal windows is hypothesized to perturb the development of limbic circuits thus establishing a substratum for persistent disruption of mood-related behavior. We examined the impact of PNFlx treatment on the formation and maintenance of perineuronal nets (PNNs), extracellular matrix (ECM) structures that deposit primarily around inhibitory interneurons, and mark the closure of critical period plasticity. PNFlx treatment evoked a significant decline in PNN number, with a robust reduction in PNNs deposited around parvalbumin (PV) interneurons, within the CA1 and CA3 hippocampal subfields at postnatal day (P)21 in Sprague Dawley rat pups. While the reduction in CA1 subfield PNN number was still observed in adulthood, we observed no change in colocalization of PV-

positive interneurons with PNNs in the hippocampi of adult PNFlx animals. PNFlx treatment did not alter hippocampal PV, calretinin (CalR), or Reelin-positive neuron numbers in PNFlx animals at P21 or in adulthood. We did observe a small, but significant increase in somatostatin (SST)-positive interneurons in the DG subfield of PNFlx-treated animals in adulthood. This was accompanied by altered GABA-A receptor subunit composition, increased dendritic complexity of apical dendrites of CA1 pyramidal neurons, and enhanced neuronal activation revealed by increased c-Fos-positive cell numbers within hippocampi of PNFlx-treated animals in adulthood. These results indicate that PNFlx treatment alters the formation of PNNs within the hippocampus, raising the possibility of a disruption of excitation-inhibition (E/I) balance within this key limbic brain region.
eNeuro, 2021 Mar-Apr; 8
33523596: Tiwari P, Fanibunda SE, Kapri D, Vasaya S, Pati S, Vaidya VA
GPCR signaling: role in mediating the effects of early adversity in psychiatric disorders.
Early adversity is a key risk factor for the development of several psychiatric disorders, including anxiety and depression. During early life, neurocircuits that regulate emotionality undergo substantial structural remodeling and functional maturation, and are thus particularly susceptible to modification by environmental experience. Preclinical evidence indicates that early stress enhances adult anxio-depressive behaviors. A commonality noted across diverse early stress models is life-long alterations in neuroendocrine stress responses and monoaminergic neurotransmission in key limbic circuits. Dysregulation of G protein-coupled receptor (GPCR) signaling is noted across multiple early stress models and is hypothesized to be an important player in the programming of aberrant emotionality. This raises the possibility that disruption of GPCR signaling in key limbic regions during critical temporal windows could establish a substrate for enhanced risk of adult psychopathology. Here, we review literature, predominantly from preclinical models, which supports the building hypothesis that a disruption of GPCR signaling could play a central role in programming persistent molecular, cellular, functional, and behavioral changes as a consequence of early adversity.
FEBS J, 2021; 288
32955432: Pati S, Saba K, Salvi SS, Tiwari P, Chaudhari PR, Verma V, Mukhopadhyay S, Kapri D, Suryavanshi S, Clement JP, Patel AB, Vaidya VA
Chronic postnatal chemogenetic activation of forebrain excitatory neurons evokes persistent changes in mood behavior.
Early adversity is a risk factor for the development of adult psychopathology. Common across multiple rodent models of early adversity is increased signaling via forebrain Gq-coupled neurotransmitter receptors. We addressed whether enhanced Gq-mediated signaling in forebrain excitatory neurons during postnatal life can evoke persistent mood-related behavioral changes. Excitatory hM3Dq DREADD-mediated chemogenetic activation of forebrain excitatory neurons during postnatal life (P2-14), but not in juvenile or adult windows, increased anxiety-, despair-, and schizophrenia-like behavior in adulthood. This was accompanied by an enhanced metabolic rate of cortical and hippocampal glutamatergic and GABAergic neurons. Furthermore, we observed reduced activity and plasticity-associated marker expression, and perturbed excitatory/inhibitory currents in the hippocampus. These results indicate that Gq-signaling-mediated activation of forebrain excitatory neurons during the critical postnatal window is sufficient to program altered mood-related behavior, as well as functional changes in forebrain glutamate and GABA systems, recapitulating aspects of the consequences of early adversity.
Elife, 2020; 9
31736725: Salvi SS, Pati S, Chaudhari PR, Tiwari P, Banerjee T, Vaidya VA
Acute Chemogenetic Activation of CamKIIα-Positive Forebrain Excitatory Neurons Regulates Anxiety-Like Behaviour in Mice.
Anxiety disorders are amongst the most prevalent mental health disorders. Several lines of evidence have implicated cortical regions such as the medial prefrontal cortex, orbitofrontal cortex, and insular cortex along with the hippocampus in the top-down modulation of anxiety-like behaviour in animal models. Both rodent models of anxiety, as well as treatment with anxiolytic drugs, result in the concomitant activation of multiple forebrain regions. Here, we sought to examine the effects of chemogenetic activation or inhibition of forebrain principal neurons on anxiety and despair-like behaviour. We acutely activated or inhibited Ca/calmodulin-dependent protein kinase II α (CamKIIα)-positive forebrain excitatory neurons using the hM3Dq or the hM4Di Designer Receptor Exclusively Activated by Designer Drug (DREADD) respectively. Circuit activation was confirmed via an increase in expression of the immediate early gene, c-Fos, within both the hippocampus and the neocortex. We then examined the influence of DREADD-mediated activation of forebrain excitatory neurons on behavioural tests for anxiety and despair-like behaviour. Our results indicate that acute hM3Dq DREADD activation of forebrain excitatory neurons resulted in a significant decline in anxiety-like behaviour on the open field, light-dark avoidance, and the elevated plus maze test. In contrast, hM3Dq DREADD activation of forebrain excitatory neurons did not alter despair-like behaviour on either the tail suspension or forced swim tests. Acute hM4Di DREADD inhibition of CamKIIα-positive forebrain excitatory neurons did not modify either anxiety or despair-like behaviour. Taken together, our results demonstrate that chemogenetic activation of excitatory neurons in the forebrain decreases anxiety-like behaviour in mice.
Front Behav Neurosci, 2019; 13

31394058: Tiwari P, Fanibunda SE, Vaidya VA
The Ministry of Fear: 'The Conjuring' of Fright in the Amygdala by the Raphe.
In this issue of Neuron, Sengupta and Holmes (2019) characterize a distinct serotonergic circuit from the dorsal raphe nucleus to the basal amygdala that facilitates fear conditioning and memory.
Neuron, 2019; 103
31072928: Fanibunda SE, Deb S, Maniyadath B, Tiwari P, Ghai U, Gupta S, Figueiredo D, Weisstaub N, Gingrich JA, Vaidya ADB, Kolthur-Seetharam U, Vaidya VA
Serotonin regulates mitochondrial biogenesis and function in rodent cortical neurons via the 5-HT receptor and SIRT1-PGC-1α axis.
Mitochondria in neurons, in addition to their primary role in bioenergetics, also contribute to specialized functions, including regulation of synaptic transmission, Ca homeostasis, neuronal excitability, and stress adaptation. However, the factors that influence mitochondrial biogenesis and function in neurons remain poorly elucidated. Here, we identify an important role for serotonin (5-HT) as a regulator of mitochondrial biogenesis and function in rodent cortical neurons, via a 5-HT receptor-mediated recruitment of the SIRT1-PGC-1α axis, which is relevant to the neuroprotective action of 5-HT. We found that 5-HT increased mitochondrial biogenesis, reflected through enhanced mtDNA levels, mitotracker staining, and expression of mitochondrial components. This resulted in higher mitochondrial respiratory capacity, oxidative phosphorylation (OXPHOS) efficiency, and a consequential increase in cellular ATP levels. Mechanistically, the effects of 5-HT were mediated via the 5-HT receptor and master modulators of mitochondrial biogenesis, SIRT1 and PGC-1α. SIRT1 was required to mediate the effects of 5-HT on mitochondrial biogenesis and function in cortical neurons. In vivo studies revealed that 5-HT receptor stimulation increased cortical mtDNA and ATP levels in a SIRT1-dependent manner. Direct infusion of 5-HT into the neocortex and chemogenetic activation of 5-HT neurons also resulted in enhanced mitochondrial biogenesis and function in vivo. In cortical neurons, 5-HT enhanced expression of antioxidant enzymes, decreased cellular reactive oxygen species, and exhibited neuroprotection against excitotoxic and oxidative stress, an effect that required SIRT1. These findings identify 5-HT as an upstream regulator of mitochondrial biogenesis and function in cortical neurons and implicate the mitochondrial effects of 5-HT in its neuroprotective action.
Proc Natl Acad Sci U S A, 2019; 116

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BOARD NUMBER: S03-132

NEURAL CORRELATES OF SERIAL DEPENDENCE ACROSS VISUAL HEMIFIELDS AND BILATERAL PREFRONTAL CORTEX

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Previously perceived working memory (WM) items have been shown to attract current WM reports. This so-called serial dependence was shown to rely on the interaction of active neural representations and long-lasting activity-silent mechanisms in prefrontal cortex (PFC) [Barbosa, Stein et al., 2020]. Furthermore, WM representations have been shown to be more frequent for contralateral than ipsilateral memorized locations in PFC [Funahashi et al., 1989], and can transfer between hemispheres when midline-crossing saccades occur in the delay [Brincat et al., 2021]. This indicates the consistent specialization of each hemisphere for the corresponding visual hemifield in WM. However, serial dependence challenges this view as it is unclear how it can emerge when consecutive stimuli appear in different hemifields, which engage independent neural substrates. Here, we investigate the transfer of serial dependence between visual hemifields and the associated prefrontal correlates across hemispheres, in order to shed light on the mechanisms of integration of lateralized WM storage. We collected simultaneous multi-unit recordings in monkey bilateral PFC during an oculomotor visuospatial delayed response task. We analyzed behavioral responses, and population coding in neural data in relation to serial dependence. We found that serial dependence of stimuli presented across hemifields was diminished in comparison to trials within the same hemifield. Furthermore, the correlation of decoded neural traces between hemispheres reflected these differences. We conclude that serial dependence in WM is partly supported by lateralized mechanisms, showing an incomplete continuity of the effect, which is in line with the activity-silent theory for serial dependence.

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FOCUS ON THE MOUSE INSULAR CORTEX: IN VIVO SINGLE-UNIT RECORDINGS DURING ANXIETY ASSAYS AND MAPPING OF THE DOPAMINERGIC SYSTEM

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Aim. The insular cortex (insula) is over-activated in patients with anxiety disorders and preclinical observation showed that dysfunctions of dopamine (DA) transmission can alter anxiety level. DA receptors are widely expressed across the brain, but their role within the insula on anxiety remains unknown. ***Methods and results***. By performing extracellular recordings in freely-moving mice, we provide the first *in vivo* electrophysiological characterization of anterior insula neurons during anxiety assays. We found heterogeneity in single-unit firing profiles, depending on the location of the mice in safe or anxiogenic spaces, with a majority of units more active in anxiogenic spaces. Then, as an initial step to define the function of insula DA transmission in anxiety, we quantified dopamine 1 receptor expression in the insula using a transgenic D1:Ai14 mouse line. We found that **[1]** D1+ cells density is the highest in layer 2-3, **[2]** ~30% of inhibitory neurons are D1+, and **[3]** only 10% of excitatory neurons are D1+. Finally, by combining tyrosine hydroxylase immunohistochemistry and retrograde tracing, we mapped the source of dopaminergic inputs to the insula, and by using anterograde viral tracing in D1-cre mice, we mapped the outputs of the 10% of D1+ projection neurons. We evidenced that, as for the whole projection neurons of the insula, D1+ insula projection neurons mainly target the amygdala and controlateral insula. ***Conclusion***. Together, this preliminary study provides an electrophysiological and anatomical characterization of insula neurons as a starting point to identify the potential role of dopaminergic modulation of the insula in anxiety.

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EARLY LIFE CHRONIC STRESS ALTERS ZEBRAFISH DORSAL RAPHE SEROTONERGIC NEURON RESPONSES TO SUBSEQUENT STRESSOR EXPOSURE

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Stress elicits variable systemic and neural changes in vertebrates, resulting in adaptive to pathological outcomes. Several studies have implicated the dorsal raphe nucleus (DRN), a brainstem nucleus containing a heterogeneous population of forebrain-projecting serotonergic neurons, in the adaptive stress response and the pathological changes resulting from chronic stress. However, it is not known whether early life chronic stress affects the developing DRN and its response to subsequent stressors, or whether the stress-induced changes affect subpopulations of serotonergic DRN neurons in a region- or phenotype-specific manner. To answer these questions, we used *in vivo* 2-photon calcium imaging of DRN neurons in two weeks old zebrafish exposed to early life chronic unpredictable stress, which subsequently displayed increased anxiety-like behaviors. We found that early life chronic stress blunted the normal serotonergic DRN response to an acute stressor. This was partly supported by a heterogeneous subpopulation of stress-inhibited neurons, which were preferentially located in the rostral DRN. Early life chronic stress also prevented the normal habituation of serotonergic DRN responses to a repeated acute stressor. Finally, repeated acute stress in control fish rapidly produced a response profile similar to that observed in chronically stressed fish, with an increased proportion of inhibitory responses preferentially in the rostral DRN. Together, our results indicate that early life chronic stress robustly alters DRN serotonergic neurons' functional responses to subsequent stressors and highlight the persistent effect of early life stress on DRN serotonergic neuron subpopulations.

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NEUROCHEMICAL PROFILES OF NUCLEUS INCERTUS NEURONS AND THEIR CONNECTIONS WITH THE MEDIAL SEPTUM IN THE RAT BRAIN

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The nucleus incertus (NI) has been described as a GABAergic structure and the main source of relaxin-3 (RLN3) projections to the forebrain. The NI is involved in arousal, stress responses and theta rhythm control, and is strongly linked to the septohippocampal pathway, mainly via direct connections with the medial septum (MS). However, the precise neurochemical nature of specific NI neuron populations remains elusive, and therefore, this study further explored the characteristics of NI neurons directly innervating the MS. Male, adult Sprague-Dawley rats were injected in the MS with an mCherry viral vector and RNAscope HiPlex *in situ* hybridization was subsequently performed on coronal NI sections. NI neurons innervating MS were counted, mapped and assigned to specific cell types, depending on their mRNA expression profile. Cell counting revealed that >50% of NI neurons expressed vGAT1 mRNA, ~40% was vGlut2-mRNA-positive and ~10% expressed both vGAT1 and vGlut2 mRNA. These populations expressed relaxin-3 or cholecystokinin mRNA. 38% of mCherry mRNA-expressing (i.e., MS innervating) neurons was vGlut2 mRNA-positive and ~20% was RLN3 mRNA-positive. Notably, 40% of RLN3 mRNA-positive cells expressed vGAT1/vGlut2 mRNA. Differences in neurochemical profile translated into differential localizations within the NI. Our results indicate that the rat NI should be considered as a neurochemically heterogeneous structure, comprising equivalent populations of GABAergic and glutamatergic neurons. As demonstrated for MS-innervating cells, these NI neurons are differentially located, and potentially linked to multiple connections and functions and diverse electrophysiological effects on target structures. Funding: NSC-Poland UMO-2017/27/N/NZ4/01545; UMO-2018/30/E/NZ4/00687.

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BOARD NUMBER: S03-136

NOVELTY RESPONSES AND SEROTONIN SIGNALING IN MICE

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Novelty plays important roles for learning and decision-making. The exploration of novel stimuli is crucial to form efficient maps of one's environment. Yet, because novelty can also entail exposure to threats, novelty-seeking behaviors are dependent on a variety of affective and cognitive factors. Here, we combined optogenetics and calcium imaging with in-depth behavioral analysis to elucidate the involvement of serotonin (5-HT) signaling in novelty processing in several behavioral assays. We found that 5-HT neurons in the dorsal raphe nucleus (DRN) activate while animals approach novel stimuli and that short- and long-term manipulations of DRN 5-HT activity impact novelty-seeking behaviors in different ways. Going further, we compared serotonin and dopamine responses to novelty and we tested how novelty- and exploration-related 5-HT signals relate to behavioral plasticity and learning. Taken together, our findings reveal the unexpected role of DRN 5-HT activity in novelty detection and downstream neurobehavioral adaptations.

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VTA DOPAMINE NEURONS ORTHOGONALLY ENCODE MOTIVATED BEHAVIOURS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Adaptive regulation of innate behaviours, such as feeding and locomotion, is crucial for survival. The ventral tegmental area (VTA) forms the core of the reward system driving these behaviours. Dopamine neurons not only encode reward, but multiplex non-reward variables. Also, individual dopamine neurons do not signal reward anticipation uniformly, but each encode a different possible reward outcome from a distribution. However, it remains unclear whether subpopulations of dopamine neurons distinguish between different reward types. Therefore, the aim of this study was to uncover the role of VTA dopamine neurons in driving the interaction with distinct rewards. We performed 1-photon calcium imaging in DAT-cre mice expressing GCaMP6m in the VTA, while they freely explored a paradigm with multiple rewards, including food, water, a conspecific, a running wheel and object. We recorded mice in sated state and after food deprivation. We found that VTA dopamine neurons do not respond uniformly to distinct rewards, but encode food proximity and locomotion in an orthogonal manner, with neurons excited by food proximity being inhibited in the running wheel and vice versa. Dopamine neuron activity selectively changed following food deprivation and increased wheel running. These results suggest that VTA dopamine neuron subpopulations distinguish between different rewards and can shift these responses according to changes in motivational state. We gratefully acknowledge support by the ERC Consolidator Grant (772994, FeedHypNet, to T.K.) and DFG (431549029–SFB1451 and EXC2030-CECAD, to T.K., 233886668-GRK1960, to V.M.).

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OREXIN NEUROMODULATION OF THE DOPAMINERGIC SYSTEM: BEHAVIORAL CORRELATES AND MECHANISTIC INSIGHTS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Orexin neurons are located in the Lateral Hypothalamus (LH) and they have been established as critical modulators of sleep, wakefulness, and other motivated behaviors. There are two orexin peptides, A and B, that act at two different receptor subtypes, orexin receptor-1 (OX1R) and orexin receptor-2 (OX2R). Hypothalamic orexin neurons project to multiple brain areas including mesolimbic dopamine (DA) pathway regions, such as the ventral tegmental area (VTA) and the nucleus accumbens. In fact, the projection from the LH to the VTA is a major monosynaptic pathway among the basal forebrain and hypothalamic areas. Nevertheless, the functional consequences of DA neuron modulation by orexin inputs are not well understood. Using the Cre/lox technology and a *dopamine transporter* (*Dat*) Cre driver line, we generated two lines of mice harboring cell type-specific inactivation of the *Ox1R* or *Ox2R* genes in DA neurons. Assessing these mouse lines, along with their respective control littermates, we could show that orexin receptors in DA neurons play an important role in exploration/locomotion, reactivity to novelty and anxiety-like behavior. Moreover, our results indicate distinct contributions of OX1R and OX2R neuromodulation of the dopaminergic system to these different behavioral domains.

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BOARD NUMBER: S03-139

DIFFERENTIAL CODING OF EMOTIONAL EXPERIENCE IN VENTRAL AND DORSAL HIPPOCAMPUS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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The dorsal (d) and ventral (v) parts of the hippocampus (HPC) are considered to support complementary cognitive functions: spatial and emotional processing, respectively. Here, we aimed to compare their responses to emotional changes with simultaneous recordings of multiple single units in four male rats during a fear conditioning protocol. We studied the evolution of dHPC and vHPC coding over the course of fear conditioning and control sessions. Both dHPC and vHPC population activity exhibited significant drifts relative to baseline in the PCA space following fear conditioning. Crucially, this drift was significantly greater for vHPC than dHPC, and was accompanied by stable changes in firing rate of individual neurons. Interestingly, dHPC activity decreased during freezing episodes, with transient peaks at the offset and onset of freezing, perhaps related to motor correlates. These preliminary results therefore suggest that fear conditioning induces stable firing rate changes specifically in vHPC which may therefore underpin emotional learning.

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BOARD NUMBER: S03-140

VICARIOUS REWARD SIGNALS IN THE VTA DRIVE PROSOCIAL CHOICES IN RATS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Prosocial behaviours are those actions that benefit others. They are thought to be evolutionary conserved across different mammal species however, the neural mechanisms that explain this type of actions are yet poorly understood. In this work we first developed a new behavioural task to measure GCaMP6s activity using fiber-photometry on an observer rat, while being either rewarded or witnessing its cage-mate obtaining rewards. As expected, the reception of self-rewards induced activity in the ventral tegmental area (VTA). Furthermore, we observed increases in VTA GCaMP signals time-locked to the observation of a cage-mate receiving rewards, suggesting that observation of conspecifics in rewarding states is self-rewarding. Then, we assessed whether these reinforcing signals would guide social decisions. We tested pairs of rats in a social decision-making task, where we have previously demonstrated that rats display prosocial tendencies. In this task, a decision-maker could choose between the option that provided food to itself and its conspecific (prosocial choice) or the option that provided food only to itself (selfish choice). Using closed-loop wireless optogenetic tools, we silenced VTA activity specifically while the decision-maker was observing their partner being rewarded, after a prosocial choice. Silencing VTA activity during these specific moments blocked the emergence of prosocial choices over the testing sessions, remaining animals at chance levels. In conclusion, we found that witnessing the rewarding states from others induces vicarious reinforcing responses in rats, in the form of increased activity in VTA, being these the necessary signal that drives the motivation to help others.

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BOARD NUMBER: S03-141

KISSPEPTIN-8 SUPPRESSES LOCOMOTION AND MODULATES NUCLEUS ACCUMBENS ACTIVITY IN RATS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Aims: Neuropeptide FF receptors are expressed in the ventral tegmental area (VTA)- nucleus accumbens (NAc) dopaminergic system and have been implicated in the regulation of locomotion. We aimed to investigate how an N-terminally truncated RF-amide octapeptide, kisspeptin-8 (Kp-8) affects locomotor activity and whether it modulates neurotransmitter release and gene expression in the NAc, a key structure in locomotion, motivation and addiction. **Methods:** Male Wistar rats were injected intracerebroventricularly with 0.1 or 1 µg of Kp-8, then computerized open field (OF) and marble burying tests (MB) were performed. Moreover, fractional GABA release was measured in the NAc using *ex vivo* superfusion and the expressions of *Fos*, *DRD1R, DRD2R* (dopamine receptor D1, D2), *GAD65* (glutamic acid decarboxylase 65kD*)*, and *NPFFR1, NPFFR2* genes were determined. **Results:** After 50 minutes in the OF arena, 1 µg of Kp-8 decreased locomotion and rearing activity. In MB, the 1 µg dose reduced the number and time of interactions with marbles. There was an increase in GABA release and a downregulation of *DRD1R* (1 µg Kp-8)*, DRD2R* (0.1 and 1 µg Kp-8) and *NPFF2R* (0.1 µg Kp-8) in the NAc*.* **Conclusions:** In our study, Kp-8 caused downregulation of D1 and D2 receptors. As these receptors are involved in locomotion and motivation, respectively, their downregulation might be in the background of hypolocomotion and decreased exploratory activity. Kp-8 possibly modulates the activity of the VTA-NAc system by acting on NPFF receptors expressed on GABAergic interneurons, thus it might also suppress the dopaminergic input from the VTA.

Pubmed:
33503835: Ibos KE, Bodnár É, Bagosi Z, Bozsó Z, Tóth G, Szabó G, Csabafi K
Kisspeptin-8 Induces Anxiety-Like Behavior and Hypolocomotion by Activating the HPA Axis and Increasing GABA Release in the Nucleus Accumbens in Rats.
Kisspeptins (Kp) are RF-amide neuropeptide regulators of the reproductive axis that also influence anxiety, locomotion, and metabolism. We aimed to investigate the effects of intracerebroventricular Kp-8 (an N-terminally truncated octapeptide) treatment in Wistar rats. Elevated plus maze (EPM), computerized open field (OF), and marble burying (MB) tests were performed for the assessment of behavior. Serum LH and corticosterone levels were determined to assess kisspeptin1 receptor (Kiss1r) activation and hypothalamic-pituitary-adrenal axis (HPA) stimulation, respectively. GABA release from the nucleus accumbens (NAc) and dopamine release from the ventral tegmental area (VTA) and NAc were measured via ex vivo superfusion. Kp-8 decreased open arm time and entries in EPM, and also raised corticosterone concentration, pointing to an anxiogenic effect. Moreover, the decrease in arm entries in EPM, the delayed increase in immobility accompanied by reduced ambulatory activity in OF, and the reduction in interactions with marbles show that Kp-8 suppressed exploratory and spontaneous locomotion. The increase in GABA release from the NAc might be in the background of hypolocomotion by inhibiting the VTA-NAc dopaminergic circuitry. As Kp-8 raised LH concentration, it could activate Kiss1r and stimulate the reproductive axis. As Kiss1r is associated with hyperlocomotion, it is more likely that neuropeptide FF receptor activation is involved in the suppression of locomotor activity.
Biomedicines, 2021; 9

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BOARD NUMBER: S03-142

BRAIN-BODY INTERACTIONS IN EMOTIONS: PERSPECTIVE MATTERS

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Experiencing an emotion oneself and observing emotions in others share substantial physiological and neural similarities. Still, under normal circumstances we do not 'loose' our sense of self, and are able to distinguish our own emotions from someone else's. Neural monitoring of cardiac signals has recently been proposed as a mechanism for such self-other distinction and self-specification in the domains of perception and cognition. Here, we probe whether the same mechanism is at play for emotions – in other words, whether there is a domain-general mechanism of self-other distinction, valid across perception, cognition and emotion. We tackled this question in a paradigm where participants had to rate valence and arousal in response to affective social scenes. Before each trial, a cue indicated whether they would have to report the emotional experience they felt (Self condition), or the emotion expressed by people in the scene (Other condition). During cue, when participants prepared to adopt the Self or Other perspective, we found that Heartbeat Evoked Responses (HERs) distinguished between the Self and Other condition. During affective scene presentation, we found that in addition to expected covariations with valence and arousal, physiological measures (SCR and facial EMG) were sensitive to the perspective from which the image was being evaluated. Finally, we found that changes in bodily signals related to emotions significantly contributed to the ratings of valence and arousal of the participant irrespective of adopted perspective. The HER, however, specifically contributed to the experienced subjective valence when assessing self, but not someone else's emotions.

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BOARD NUMBER: S03-143

THE TEMPORAL STUDY OF TRAUMATIC STRESS RELATED FEAR MEMORY RETRIEVAL IN A RAT MODEL OF POST-TRAUMATIC STRESS DISORDER: BRAIN WAVE AND TRANSCRIPTOME-LEVEL GENE PROFILING

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Post-traumatic stress disorder (PTSD) is a complex syndrome, which may occur after life-threatening event exposure. Fear memory abnormalities have been indicated as a key factor in the pathological course of PTSD. It was indicated that fear memories are not rigid; the retrieval of fear memory may change over time. Furthermore, previous studies have suggested that fear expression is strongly correlated with the theta (4Hz) activities. However, the relation between pathological fears and potential brain wave features after traumatic stress exposure still remains largely unknown. Here, we hypothesize after traumatic stress exposure, the longitudinal dynamic changes of abnormal fears in PTSD animal models could be reflected by local field potentials (LFPs) measurements and transcriptome-level gene profiling in the early and late phases. In our study, we use a well-established modified single prolonged stress (SPS&FS) PTSD rat model. Temporal brain wave activities were characterized in relation to fear expression after traumatic stress exposure. We found the SPS&FS rats showed sustained fear responses and heterogeneous PTSD phenotypes (significantly higher anxiety, depression and anhedonic levels). Time-dependent higher theta (5-8 Hz) and gamma (>30 Hz) activities were observed in early phases and started to shift to continuous lower delta (0.5-4Hz) from 2-4 hrs to day 1, 3 ,7 and 14 after SPS&FS. Besides, we also observed temporal differences in gene and protein levels. The longitudinal profiling of abnormal fears may help illustrate the time-dependent pathological fear memory retrieval in PTSD and may also help find better intervention strategies for this complex syndrome.

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BOARD NUMBER: S03-144

SOCIAL FEAR AFFECTS LIMBIC SYSTEM NEURONAL ACTIVITY AND GENE EXPRESSION

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Among the spectrum of psychiatric disorders, social anxiety disorder (SAD) is a highly prevalent and comorbid anxiety disorder with rather unclear underlying mechanisms. We aimed to characterize neurobiological changes occurring in a mouse model of SAD to identify possible therapeutic targets. Social fear was induced via social fear conditioning (SFC), a validated animal model of SAD. We investigated expression levels of the immediate early genes (IEGs) cFos, Fosl2 and Arc as markers of neuronal activity and the expression levels of several genes of the GABAergic, serotoninergic, oxytocinergic, vasopressinergic and neuropeptide Y (NPY)-ergic systems. Target brain regions are involved in social- as well as fear-related behavior, the paraventricular nucleus of the hypothalamus, basolateral amygdala, septum, hippocampus and dorsal raphe nucleus. The setup investigated SFC+ and SFC- mice 2h after exposure to a conspecific. SFC+ mice showed a decreased number and density of cFos-positive cells and decreased expression levels of IEGs in the anterior hippocampus. SFC+ mice also showed alterations in the expression of NPY and serotonin system-related genes in all investigated brain regions except the hippocampus. Our results describe neuronal alterations occurring during the expression of social fear and identify the NPY and serotonergic system as possible targets in the treatment of SAD.

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BOARD NUMBER: S03-145

CONSTITUTIVE 5-HT2C RECEPTOR KNOCK-OUT FACILITATES FEAR EXTINCTION THROUGH ALTERED ACTIVITY OF A DORSAL RAPHE–BED NUCLEUS OF THE STRIA TERMINALIS PATHWAY

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Serotonin 2C receptors (5-HT2CRs) are widely distributed throughout the brain and are strongly implicated in the pathophysiology of anxiety disorders such as post-traumatic stress disorder (PTSD). Although in recent years, a considerable amount of evidence supports 5-HT2CRs facilitating effect on anxiety behavior, the involvement in learned fear responses and fear extinction is rather unexplored. Here we used a 5-HT2CR knock-out mouse line (2CKO) to gain new insights into the involvement of 5-HT2CRs in the neuronal fear circuitry. Using a cued fear conditioning paradigm, our results revealed that global loss of 5-HT2CRs exclusively accelerates fear extinction, without affecting fear acquisition and fear expression. To investigate the neuronal substrates underlying the extinction enhancing effect, we mapped the immediate-early gene product cFos, a marker for neuronal activity, in the dorsal raphe nucleus (DRN), amygdala and bed nucleus of the stria terminalis (BNST). Surprisingly, besides extinction-associated changes, our results revealed alterations in neuronal activity even under basal home cage conditions in specific subregions of the DRN and the BNST in 2CKO mice. Neuronal activity in the dorsal BNST was shifted in an extinction-supporting direction due to 5-HT2CR knock-out. Finally, the assessment of DRN–BNST connectivity using antero- and retrograde tracing techniques uncovered a discrete serotonergic DRC–BNSTad pathway showing increased activity in 2CKO mice. Thus, our results provide new insights for the fear extinction network by revealing a specific serotonergic DRC–BNSTad pathway underlying a 5-HT2CR-sensitive mechanism with high significance in the treatment of PTSD.

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BOARD NUMBER: S03-146

THE BASAL FOREBRAIN AS A MEDIATOR OF INFRALIMBIC-AMYGDALA COMMUNICATION IN FEAR EXTINCTION

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Aims: Projections from the infralimbic region (IL) of the medial prefrontal cortex to the basolateral amygdala (BLA) are needed to suppress fear responses during extinction. However, which IL afferents serve as a teaching signal during extinction remains unknown. Given the importance of the basal forebrain (BF) in learning, we investigated whether BF-IL communication contributes to extinction and to IL-BLA physiology. **Methods:** We used multisite local field potential recordings in the IL, BF, and BLA during behavior to identify patterns of communication during extinction learning and recall. Retrograde neuroanatomical tracers were then used to study IL projections to the BLA and BF, and combined with functional immunohistochemistry to assess the activity of IL-BF versus IL-BLA projectors during fear and extinction recall. **Results:** During extinction learning and recall, cue-evoked IL theta power (4-8Hz) changes resembled the BF more than the BLA. Directionality analyses showed that IL-BF theta communication was bidirectional during extinction acquisition, but IL-to-BF directionality prevailed during extinction recall, whereas the IL-to-BLA directionality remained throughout. Furthermore, partial correlations showed that IL-BLA theta power correlations dropped dramatically when the BF signal was removed. Anatomical data showed IL-BLA projections arising from LII-III and IL-BF projections from LII-III and LIV-V. Immunohistochemical analyses showed that the IL-BLA projectors didn't exhibit preferred activation during fear vs extinction recall, whereas IL-BF projectors from LIV-V were more active during extinction recall. **Conclusions**: BF input to the IL is a teaching signal during extinction acquisition that influences IL-BLA communication, and IL-to-BF signaling during extinction recall.

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BOARD NUMBER: S03-147

COMPULSIVE SEXUAL BEHAVIOR DISORDER IMPACT ON STRIATUM AND AMYGDALA FUNCTIONAL RESPONSES DURING APPETITIVE CONDITIONING AND EXTINCTION

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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Compulsive Sexual Behavior Disorder (CSBD) was recently (2022) recognized in International Classification of Diseases (ICD-11). Its mechanisms are still unclear which compromises the development of effective therapies. In our study, we aimed to verify appetitive conditioning and extinction processes in CSBD using brain functional magnetic resonance imaging (fMRI). Age-matched CSBD patients and healthy control subjects (n=32 in each group) participated in the learning tasks with erotic and monetary rewards. We analyzed fMRI regions of interest (ROIs), reaction times (RT), and participants' subjective ratings of arousal and valence toward abstract cues paired with rewards before and after conditioning. Statistical analyzes were conducted using 3-way ANOVA: (2)Group x (2)Condition x (2)Task Phase. During conditioning, CSBD subjects showed increased value attribution (subjective) and motivation (RT) for both rewarding cues suggesting general alteration of action-reward learning in patients. During the conditioning phase, fMRI results suggest hypoactivity of the ventral striatum and amygdala in CSBD for both rewarding cues compared to healthy control. The extinction process in CSBD differentiated cue types in both brain structures - ventral striatum exhibited stronger deactivation from the early to the late phase of the task specifically for erotic cues, whereas amygdala showed a tendency for hypoactivity in the late phase for erotic cues compared to healthy control. Our results suggest that altered conditioning and extinction may underlay the development and persistence of Compulsive Sexual Behavior Disorder. (NCN-Grant-2016/21/N/HS6/02635)

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BOARD NUMBER: S03-148

EYE-TRACKING EVIDENCE FOR A MOOD CONGRUENCY BIAS IN DEPRESSION

POSTER SESSION 03 - SECTION: MIDBRAIN AND FOREBRAIN FUNCTION IN STRESS AND EMOTION

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In imaging genetics/psychiatry the emotional face recognition task by Hariri et al. (2002) is one of the most commonly employed tasks to examine amygdala activity. Scant work has employed eye-tracking to detect how various subjects are processing the stimuli. Here, we aim to explore fixation patterns and their neural correlates during this task to better understand emotional processing in depression. The analyses included patients with a depressive disorder (n=84) and healthy controls (n=114) from the BeCOME (the biological classification of mental disorders) study. They performed the task to match 6 types of emotion pairs in the MR-scanner while eye-tracking was recorded simultaneously. We split the 6s trials into two stages (before and after the behavioral response (i.e. free viewing stage)) and extracted dwelling times separately. Additionally, we examined amygdala activation to emotional faces by general linear models in SPM12 modeling in the face stimuli, the baseline geometric forms (control condition), and unspecific nuisance regressors. Depressive patients spent longer dwelling times on sad faces than other negative faces but only in the free viewing stage, while healthy controls did not show this differential pattern. In fMRI analyses, both groups showed significant whole-brain corrected activation clusters in the amygdala, whereas there were no significant differences between the groups at this significance level. Taken together, individuals with depression indicated a mood congruency bias manifest in eye-tracking patterns but not in other neurobehavioral readouts, indicating that eye-tracking is a sensitive readout for emotional processing in depression.

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TEMPORAL CONTROLS OVER INTER-AREAL CORTICAL PROJECTION NEURON FATE DIVERSITY

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

Esther Klingler[1], Ugo Tomasello[1], Julien Prados[2], Justus Kebschull[3], Alessandro Contestabile[1], Gregorio Galinanes[1], Sabine Fièvre[1], Antonio Santinha[4], Randall Platt[4], Daniel Huber[1], Alexandre Dayer[2], Camilla Bellone[1], Denis Jabaudon[1]
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Interconnectivity between neocortical areas is critical for sensory integration and sensorimotor transformations. These functions are mediated by heterogeneous inter-areal cortical projection neurons (ICPN), which send axon branches across cortical areas as well as to subcortical targets. Although ICPN are anatomically diverse, they are molecularly homogeneous, and how the diversity of their anatomical and functional features emerge during development remains largely unknown. Here we address this question by linking the connectome and transcriptome in developing single ICPN of the mouse neocortex using a combination of multiplexed analysis of projections by sequencing (MAPseq, to identify single-neuron axonal projections) and single-cell RNA sequencing (to identify corresponding gene expression). Focusing on neurons of the primary somatosensory cortex (S1), we reveal a protracted unfolding of the molecular and functional diferentiation of motor cortex-projecting (M) ICPN compared with secondary somatosensory cortex-projecting (S2) ICPN. We identify SOX11 as a temporally diferentially expressed transcription factor in M versus S2 ICPN. Postnatal manipulation of SOX11 expression in S1 impaired sensorimotor connectivity and disrupted selective exploratory behaviours in mice. Together, our results reveal that within a single cortical area, diferent subtypes of ICPN have distinct postnatal paces of molecular diferentiation, which are subsequently refected in distinct circuit connectivities and functions. Dynamic diferences in the expression levels of a largely generic set of genes, rather than fundamental diferences in the identity of developmental genetic programs, may thus account for the emergence of intra-type diversity in cortical neurons.

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ORBITOFRONTAL ACTIVITY SHAPES THE OSCILLATORY ENTRAINMENT OF PRIMARY SENSORY CORTICES IN NEONATAL MICE

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The orbitofrontal cortex (OFC) encodes the subjective value of outcome-guided behaviour, through extensive communication with other brain areas, such as primary sensory cortices. However, the developmental dynamics of orbitofrontal-sensory coupling in relationship with the maturation of behavioural abilities remain largely unexplored. Here we combine *in vivo* multi-site extracellular recordings and optogenetic manipulation with projection tracing in neonatal mice (P1-12) to monitor the developmental profile of orbitofrontal-sensory communication. We show that already at neonatal age the OFC targets the primary sensory areas via monosynaptic projections. The density of these projections is higher for OFC-V1 and sparser for OFC-S1, yet the monosynaptic efferent connections from both primary sensory areas targeting OFC are almost entirely lacking. The patterns of neuronal and network activity are similar in all three brain areas; discontinuous events with sparse firing progressively transform into continuous activity and higher firing rates. In line with the monosynaptic projections, the functional coupling differs between OFC-S1 and OFC-V1, as directed and undirected communication is stronger between OFC-V1. These data reveal that during early development the OFC impacts the coordinated activity of primary sensory cortices.

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BOARD NUMBER: S03-151

INTERPLAY BETWEEN EXCITATORY AND GABAERGIC CORTEX-WIDE ACTIVITY PATTERNS ACROSS EARLY POSTNATAL DEVELOPMENT

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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GABAergic neurons contribute to key aspects of the cortical functional maturation during early postnatal development. Particularly, the timing of GABAergic maturation seems to be aligned to the different developmental trajectories of each cortical region. Although various studies have looked at the local connectivity of these microcircuits, little is known about the dynamics of the functional maturation of GABAergic cortical networks at the large-scale level. In this project, we aimed to define how the activity of GABAergic neurons shapes the maturation of the activity dynamics across the entire neonatal cortex. We used dual-colour widefield calcium imaging to chronically examine the early postnatal cortex-wide dynamics of GABAergic interneurons, as well as excitatory neurons. We focused on the interplay between the spatial and temporal activity dynamics of these two neuronal populations, before and after active sensing. The activity maps of inhibitory networks show a complex mixture of small events confined to a single brain region, and larger events of simultaneous patterns across distant areas. These postnatal activity patterns vary with age and cortical region, becoming rapidly more complex at later stages of development. We found dynamic spatiotemporal fluctuations in the relative activation of excitatory and GABAergic neuronal populations during bouts of spontaneous cortical activity. In vivo manipulation of inhibition disrupts these fluctuations affecting the local and the wider cortical functional network. The study of cortex-wide dynamics,and particularly the role of local and long-range GABAergic signalling in the control of cortex-wide neural activity maturation, is key to understand normal brain development.

Pubmed:

34555313: Cuntz H, Bird AD, Mittag M, Beining M, Schneider M, Mediavilla L, Hoffmann FZ, Deller T, Jedlicka P
A general principle of dendritic constancy: A neuron's size- and shape-invariant excitability.
Reducing neuronal size results in less membrane and therefore lower input conductance. Smaller neurons are thus more excitable, as seen in their responses to somatic current injections. However, the impact of a neuron's size and shape on its voltage responses to dendritic synaptic activation is much less understood. Here we use analytical cable theory to predict voltage responses to distributed synaptic inputs in unbranched cables, showing that these are entirely independent of dendritic length. For a given synaptic density, neuronal responses depend only on the average dendritic diameter and intrinsic conductivity. This remains valid for a wide range of morphologies irrespective of their arborization complexity. Spiking models indicate that morphology-invariant numbers of spikes approximate the percentage of active synapses. In contrast to spike rate, spike times do depend on dendrite morphology. In summary, neuronal excitability in response to distributed synaptic inputs is largely unaffected by dendrite length or complexity.
Neuron, 2021; 109
31459948: Piotrowska DG, Mediavilla L, Cuarental L, Głowacka IE, Marco-Contelles J, Hadjipavlou-Litina D, López-Muñoz F, Oset-Gasque MJ
Synthesis and Neuroprotective Properties of N-Substituted -Dialkoxyphosphorylated Nitrones.
Herein, we report the synthesis and neuroprotective power of some N-substituted -(dialkoxy)phosphorylated nitrones -, by studying their ability to increase the cell viability, as well as their capacity to reduce necrosis and apoptosis. We have identified ()---butyl-1-(diethoxyphosphoryl)methanimine oxide () as the most potent, nontoxic, and neuroprotective agent, with a high activity against neuronal necrotic cell death, a result that correlates very well with its great capacity for the inhibition of the superoxide production (72%), as well as with the inhibition of lipid peroxidation (62%), and the 5-lipoxygenase activity (45%) at 100 μM concentrations. Thus, nitrone could be a convenient promising compound for further investigation.
ACS Omega, 2019; 4
28733362: Savchenko E, Singh Y, Konttinen H, Lejavova K, Mediavilla Santos L, Grubman A, Kärkkäinen V, Keksa-Goldsteine V, Naumenko N, Tavi P, White AR, Malm T, Koistinaho J, Kanninen KM

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Loss of causes altered neurogenesis in a mouse model of a childhood neurodegenerative disorder.

Neural stem/progenitor cells (NPCs) generate new neurons in the brain throughout an individual's lifetime in an intricate process called neurogenesis. Neurogenic alterations are a common feature of several adult-onset neurodegenerative diseases. The neuronal ceroid lipofuscinoses (NCLs) are the most common group of inherited neurodegenerative diseases that mainly affect children. Pathological features of the NCLs include accumulation of lysosomal storage material, neuroinflammation and neuronal degeneration, yet the exact cause of this group of diseases remains poorly understood. The function of the CLN5 protein, causative of the CLN5 disease form of NCL, is unknown. In the present study, we sought to examine neurogenesis in the neurodegenerative disorder caused by loss of Our findings demonstrate a newly identified crucial role for CLN5 in neurogenesis. We report for the first time that neurogenesis is increased in -deficient mice, which model the childhood neurodegenerative disorder caused by loss of Our results demonstrate that, in deficiency, proliferation of NPCs is increased, NPC migration is reduced and NPC differentiation towards the neuronal lineage is increased concomitantly with functional alterations in the NPCs. Moreover, the observed impairment in neurogenesis is correlated with increased expression of the pro-inflammatory cytokine IL-1β. A full understanding of the pathological mechanisms that lead to disease and the function of the NCL proteins are critical for designing effective therapeutic approaches for this devastating neurodegenerative disorder.

Dis Model Mech, 2017; 10

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OLFACTORY-DRIVEN BETA BAND ENTRAINMENT OF CORTICAL-HIPPOCAMPAL NETWORKS DURING NEONATAL DEVELOPMENT

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Center for Molecular Neurobiology, University Medical Center Hamburg-Eppendorf, Institute Of Developmental Neurophysiology, Hamburg, Germany

Cognitive processing relies on the functional refinement of the limbic circuitry during the first two weeks of life. During this developmental period, when most sensory systems are still immature, the sense of olfaction acts as “door to the world”, providing the main source of environmental inputs. However, it is unknown whether early olfactory processing shapes the activity in cortical-hippocampal networks during neonatal development. Here, we address this question by combining simultaneous *in vivo* recordings from the olfactory bulb (OB), lateral entorhinal cortex (LEC), hippocampus (HP), and prefrontal cortex (PFC) with olfactory stimulation and opto- and chemogenetic manipulations of mitral/tufted cells (M/TCs) in the OB of non-anesthetized neonatal mice. We show that the neonatal OB synchronizes the LEC-HP-PFC circuity in beta frequency range. Moreover, M/TC activity drives neuronal and network activity in LEC, as well as subsequently, HP and PFC via long-range projections from mitral cells (MCs) to HP-projecting LEC neurons. Thus, OB activity shapes the communications within cortical-hippocampal networks during neonatal development.

Pubmed:
21384484: Papp I, Sieben C, Sisson AL, Kostka J, Böttcher C, Ludwig K, Herrmann A, Haag R
Inhibition of influenza virus activity by multivalent glycoarchitectures with matched sizes.
We describe the synthesis of a series of sialic acid-conjugated, polyglycerol-based nanoparticles with diameters in the range of 1-100 nm. Particle sizes were varied along with the degree of functionalization to match the corresponding virus size and receptor multiplicity in order to achieve maximum efficiency. To build up these architectures, we used biocompatible, hyperbranched polyglycerols as scaffolds and recently developed polyglycerol-based nanogels, the sizes of which can be varied between 2-4 nm and 40-100 nm, respectively. We demonstrate here that such multivalent nanoparticles inhibit influenza A virus cell binding and fusion and consequently infectivity. The potential of multivalency is evident from larger particles showing very efficient inhibition of viral infection up to 80 %. Indeed, both the size of the nanoparticle and the amount of ligand density are important determinants of inhibition efficiency. The inhibitory activity of the tested polymeric nanoparticles drastically increased with size. Particles with similar dimensions to the virus (50-100 nm) are exceedingly effective. We also observed a saturation point in degree of surface functionalization (i.e. ligand density), above which inhibition was not significantly improved. Our study emphasizes the importance of matching particle sizes and ligand densities to mimic biological surfaces and improve interactions; this is a vital concept underlying multivalent interactions.
Chembiochem, 2011; 12
30703080: Gretenkord S, Kostka JK, Hartung H, Watznauer K, Fleck D, Minier-Toribio A, Spehr M, Hanganu-Opatz IL
Coordinated electrical activity in the olfactory bulb gates the oscillatory entrainment of entorhinal networks in neonatal mice.
Although the developmental principles of sensory and cognitive processing have been extensively investigated, their synergy has been largely neglected. During early life, most sensory systems are still largely immature. As a notable exception, the olfactory system is functional at birth, controlling mother-offspring interactions and neonatal survival. Here, we elucidate the structural and functional principles underlying the communication between olfactory bulb (OB) and lateral entorhinal cortex (LEC)-the gatekeeper of limbic circuitry-during neonatal development. Combining optogenetics, pharmacology, and electrophysiology in vivo with axonal tracing, we show that mitral cell-dependent discontinuous theta bursts in OB drive network oscillations and time the firing in LEC of anesthetized mice via axonal projections confined to upper cortical layers. Acute pharmacological silencing of OB activity diminishes entorhinal oscillations, whereas odor exposure boosts OB-entorhinal coupling at fast frequencies. Chronic impairment of olfactory sensory neurons disrupts OB-entorhinal activity. Thus, OB activity shapes the maturation of entorhinal circuits.
PLoS Biol, 2019; 17
31191258: Chini M, Gretenkord S, Kostka JK, Pöpplau JA, Cornelissen L, Berde CB, Hanganu-Opatz IL, Bitzenhofer SH

Neural Correlates of Anesthesia in Newborn Mice and Humans.
Monitoring the hypnotic component of anesthesia during surgeries is critical to prevent intraoperative awareness and reduce adverse side effects. For this purpose, electroencephalographic (EEG) methods complementing measures of autonomic functions and behavioral responses are in use in clinical practice. However, in human neonates and infants existing methods may be unreliable and the correlation between brain activity and anesthetic depth is still poorly understood. Here, we characterized the effects of different anesthetics on brain activity in neonatal mice and developed machine learning approaches to identify electrophysiological features predicting inspired or end-tidal anesthetic concentration as a proxy for anesthetic depth. We show that similar features from EEG recordings can be applied to predict anesthetic concentration in neonatal mice and humans. These results might support a novel strategy to monitor anesthetic depth in human newborns.
Front Neural Circuits, 2019; 13
32926437: Kostka JK, Gretenkord S, Spehr M, Hanganu-Opatz IL
Bursting mitral cells time the oscillatory coupling between olfactory bulb and entorhinal networks in neonatal mice.
During early postnatal development, mitral cells show either irregular bursting or non-bursting firing patterns Bursting mitral cells preferentially fire during theta bursts in the neonatal olfactory bulb, being locked to the theta phase Bursting mitral cells preferentially fire during theta bursts in the neonatal lateral entorhinal cortex and are temporally related to both respiration rhythm- and theta phase Bursting mitral cells act as a cellular substrate of the olfactory drive that promotes the oscillatory entrainment of entorhinal networks ABSTRACT: Shortly after birth, the olfactory system provides not only the main source of environmental inputs to blind, deaf, non-whisking and motorically-limited rodents, but also the drive boosting the functional entrainment of limbic circuits. However, the cellular substrate of this early communication remains largely unknown. Here, we combine in vivo and in vitro patch-clamp and extracellular recordings to reveal the contribution of mitral cell (MC) firing to early patterns of network activity in both the neonatal olfactory bulb (OB) and the lateral entorhinal cortex (LEC), the gatekeeper of limbic circuits. We show that MCs predominantly fire either in an irregular bursting or non-bursting pattern during discontinuous theta events in the OB. However, the temporal spike-theta phase coupling is stronger for bursting than non-bursting MCs. In line with the direct OB-to-LEC projections, both bursting and non-bursting discharge augments during co-ordinated patterns of entorhinal activity, albeit with higher magnitude for bursting MCs. For these neurons, temporal coupling to the discontinuous theta events in the LEC is stronger. Thus, bursting MCs might drive the entrainment of the OB-LEC network during neonatal development.
J Physiol, 2020; 598

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INVESTIGATING ACTIVITY-DEPENDENT PROCESSES DURING CORTICAL NEURONAL ASSEMBLY IN DEVELOPMENT AND DISEASES

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

Sara Mancinelli[1], Filippo Mirabella[2], Marco Erreni[3], Francesca D'Autilia[3], Christel Depienne[4], Roberto Rusconi[5], Paolo Kunderfranco[6], Davide Pozzi[7], Simona Lodato[1]
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The cerebral cortex contains an extraordinary diversity of excitatory projection neuron (PN) and inhibitory interneurons (IN), wired together to form complex circuits. Spatiotemporally coordinated execution of intrinsic molecular programs defines both PN and IN subtype diversity. Spontaneous neuronal activity in the early stages of development, as well as evoked activity by external stimuli can contribute to the formation of balanced microcircuits. Alterations of these delicate processes, indeed, have often been associated to neurological/neurodevelopmental disorders. However, it is still unclear how cortical neuronal diversity influences early spontaneous activity, and whether subtype-specific molecular features (i.e. ion channel repertoire) can correlate with distinctive functional roles in the early assembly. In this study, we combined in utero genetic perturbations via CRISPR/Cas9 system and pharmacological inhibition of selected ion channels (i.e HCN1Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1) with RNA-sequencing and live imaging technologies to identify the activity-regulated processes controlling the development of different cortical PN classes, their wiring and the acquisition of subtype specific features. Our preliminary results suggests that HCN1 channel, which is associated with infantile genetic epilepsy, is a possible modulator of spontaneous electrical activity in developing cerebral cortex and proposes HCN-current as a fundamental signal to drive cortical wave propagation during development. Further validation of this finding would support HCN1 as potential intervention targets for developmental epilepsies.

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DIVERGENT PATTERNS OF SPONTANEOUS ACTIVITY IN DISTINCT SENSORY CORTICES DURING EARLY DEVELOPMENT

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Patterns of neuronal activity emerge spontaneously in the developing brain and play a pivotal role in the maturation of sensory circuits. However, it remains unclear to what extent different immature sensory cortices exhibit specific patterns of activity. Using meso-scale functional imaging in embryonic and early postnatal mice and classification algorithms for the data analysis, we show that specific patterns of spontaneous activity could be associated to distinct sensory cortical territories. These patterns of spontaneous activity are influenced by the pattern of activity from peripheral structures. We showed that embryonic removal of the eyes modifies the normal pattern of activity in V1 with the appearance of larger and more correlated waves and bilateral co-activations. These changes in spontaneous activity in V1 were also evident at later postnatal life as shown by multielectrode recordings and predict a perturbed sensory processing in the adult.

Pubmed:
30941394: Torres D, Makarova J, Ortuño T, Benito N, Makarov VA, Herreras O
Local and Volume-Conducted Contributions to Cortical Field Potentials.
Brain field potentials (FPs) can reach far from their sources, making difficult to know which waves come from where. We show that modern algorithms efficiently segregate the local and remote contributions to cortical FPs by recovering the generator-specific spatial voltage profiles. We investigated experimentally and numerically the local and remote origin of FPs in different cortical areas in anesthetized rats. All cortices examined show significant state, layer, and region dependent contribution of remote activity, while the voltage profiles help identify their subcortical or remote cortical origin. Co-activation of different cortical modules can be discriminated by the distinctive spatial features of the corresponding profiles. All frequency bands contain remote activity, thus influencing the FP time course, in cases drastically. The reach of different FP patterns is boosted by spatial coherence and curved geometry of the sources. For instance, slow cortical oscillations reached the entire brain, while hippocampal theta reached only some portions of the cortex. In anterior cortices, most alpha oscillations have a remote origin, while in the visual cortex the remote theta and gamma even surpass the local contribution. The quantitative approach to local and distant FP contributions helps to refine functional connectivity among cortical regions, and their relation to behavior.
Cereb Cortex, 2019; 29

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MATURATION OF ELECTROPHYSIOLOGICAL PROPERTIES OF MOTOR CORTEX NEUROS ALLOWS A FINER MOTOR CONTROL IN ADULT RATS

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

Patricia Pérez-García[1,2], Ricardo Pardillo-Diaz[1,2], Carmen Castro Gonzalez[1,2], Pedro Nunez-Abades[1,3], Livia Carrascal[1,3]
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Introduction: Rodents are mainly sessile during the first postnatal week, produce primitive hindlimb-based ambulation during the second week and perform complex locomotor behaviors as in adult animals by third week. Achieving these distinctive movements require the maturation of a great variety of specialized neural circuits that are refined with age. **Aim:** The aim of this study is to understand how the neuronal membrane properties mature to achieve an efficient motor control in the adult population. **Methods:** Using whole cell patch clamp recordings, we have studied the changes in the electrophysiological properties of layer V pyramidal neurons of the rat primary motor cortex during postnatal development. Neurons were divided into 3 groups: newborn (P2-P7), infantile (P11-P17) and adult (>P22). **Results:** Newborn neurons are recruited at very low currents and in a narrow range (10-70 pA) compared to the other groups (infantile: 40-240 pA; adult: 60-400 pA). Maturation also produced a wider distribution of the data in parameters such as maximum frequency (12-27 AP/s; 11-37 AP/s; 12-60 AP/s), cancellation current (100-295 pA; 200-960 pA; 400-2000 pA), working range (45-275 pA; 60-845 pA; 340-1855 pA) and capacitance (16-57 pF; 20-97 pF; 23-140 pF). **Conclusions**: The narrower ranges found in newborn animals may support the rapid, ballistic movements observed prior to the beginning of the accurate motor control. The diversification among motor neurons properties with age may allow more adaptable schemes as the complexity of the motor behaviors increases during later stages of postnatal maturation.

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DISSECTING THE ROLE OF HCN1 IN DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY (DEE) BY EXPLOITING PATIENT-SPECIFIC MODELS OF CEREBRAL CORTEX DEVELOPMENT IN VIVO

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Epilepsy is the most common neurological condition in childhood (1% prevalence), and constitutes a public health burden worldwide. Within the broad classification of infantile/pediatric epilepsies, particularly devastating are the developmental and epileptic encephalopathies (DEE), a group of heterogeneous conditions characterized by very early onset and spontaneous, recurrent and pharmaco-resistant seizures. Recent advancements in genome sequencing have expanded our knowledge on DEE, revealing a complex genetic architecture. Interestingly, the Hyperpolarization-activated Cyclic Nucleotide-gated (HCN1) gene has been recently linked to a particularly devastating subtype of DEE, namely DEE24. Indeed, growing evidence now supports a critical role for ion channels (classically studied exclusively in mature neurons) in controlling immature neuronal behavior and in brain pathology. In this work, we aim at understanding how developmental processes are altered by HCN1 pathogenic variants associated with DEE24. We selected 3 *de novo* HCN1 variants (M153I, G391D, A387S) showing association with both epileptic, autistic/behavioral and morphological traits and evaluated their effect on electrophysiological properties in both heterologous cell lines and neurons. Moreover, we generated chimeric brain harboring the patient-specific HCN1 variants ($HCN1^{mut}$) via *in utero* electroporation and analyzed cell intrinsic (e.g. migration, differentiation, dendritogenesis) as well as not-cell autonomous effects of the mutations on early cortical development. Our preliminary results suggest that the generation of personalized (patient-specific) in vivo models allows to identify the specific endophenotypes of distinct genetic HCN1 variants and their effects on cortical assembly.These valuable models, bridging genetic and clinical data with cellular and molecular neurodevelopment, will potentially inspire tailored therapeutic avenues.

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BOARD NUMBER: S03-157

PRETERM BIRTH IMPAIRS CROSS-AREAL PLASTICITY OF THALAMOCORTICAL AXONS

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Primary sensory areas of the neocortex are determined by their connectivity with the thalamus, receiving specific projections from distinct first-order (FO) thalamic nuclei. FO thalamocortical axons (TCA) are oriented during embryogenesis towards their cortical target and display a perinatal waiting period before innervating specific area. If TCA are initially misguided, they show an extraordinary capacity to reorient after birth within the neocortex, thereby reaching their proper cortical target. The roles of such sequential targeting and adaptation steps onto areal map formation in normal and pathological conditions remains to be fully understood. Here, using a mutant mouse model in which the trajectory of embryonic TCA is shifted, we found that TCA postnatal rewiring is preceded by a prenatal apoptosis of FO thalamic nuclei, together enabling the formation of sharp, albeit drastically reduced, primary somatosensory (S1) and visual (V1) areas. We furthermore showed that the remarkable postnatal TCA plasticity occurs within a short time-window and is drastically impaired by preterm birth, leading to a maintenance of misguided axons and a blurring of V1 molecular border. At the molecular level, TCA plasticity was regulated by levels of serotonin, which are reduced by preterm birth. Overall, our study reveals sequential embryonic and postnatal checkpoints that enable concerted adaptations of TCA and cortical areas. It also demonstrated that preterm birth impairs a serotonin-dependent plastic time-window which is essential for the rescue of prenatal miswiring and adaptation of cortical map to incoming sensory inputs. Garel S. and Lokmane L., contributed equally to this work

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OPTOGENETIC ACTIVATION OF XENOTRANSPLANTED HUMAN NEURONS IN MOUSE VISUAL CORTEX MIMICS VISUAL PERCEPTION

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Transplanting stem cell derived cortical neurons to restore higher function in diseased brains, no longer seems like a distant dream, but many hurdles remain. What are the optimal conditions under which transplanted cells can restore function? Using our recently developed xenotransplantation technique, we study how juvenile human pluripotent stem cell derived cortical pyramidal neurons integrate in the adult mouse brain. We show that human transplanted cortical neurons develop at their own intrinsic pace; nevertheless they integrate robustly and gain detailed sensory responses. It remains unclear to what extent transplanted neurons can to form meaningful output connections with the host circuit. We address this question by expressing the red-shifted opsin ChRmine in the human cells we transplant and engaging the animal in a visual behavioural paradigm. Mice are trained to detect the appearance of visual stimuli with multiple contrasts (range [3 - 100]%). To isolate the contribution of transplanted human neurons in driving decisions, we optogenetically activate a small number of human neurons in the visual cortex of the mouse. Our results indicate that detection performance levels for optogenetic stimulation and weak visual stimuli are indistinguishable and above chance level. Using identical optogenetic stimulation parameters in an animal where human cells were not detected yields chance level performance. These findings suggest that young human neurons can integrate into mature mouse cortical circuit and take up a functional role. In future experiments, we will use circuit mapping techniques to establish how the activity of transplanted human cells propagate to the host circuit.

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AREA-SPECIFIC ABNORMAL DEVELOPMENT OF CORTICAL CIRCUIT IN A 22Q11 DELETION MOUSE MODEL

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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DiGeorge syndrome (or 22q11 deletion syndrome) is a human developmental disorder caused by a 1.5 to 3.0 MB microdeletion on chromosome 22 characterized by cardiovascular and craniofacial malformations and cognitive impairment. It is the genetic neurodevelopmental disorder with the highest association with schizophrenia, making it an interesting condition to identify genes underlying neuropsychiatric disorders. “LgDel” mice have a microdeletion of chromosome 16, which recapitulates the human chromosomic deletion as most gene homologs are located in one portion of this chromosome; little is known about the impact of the deletion on distinct cell types and distinct brain regions during circuit development. Here, we investigated the transcriptional trajectories of distinct cortical cell types in wild-type and LgDel mice during postnatal development using single-cell RNA sequencing in various cortical areas. Our results suggest cell-type and area-specific anomalies that may contribute to behavioural phenotypes of this disorder.

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BOARD NUMBER: S03-160

DEVELOPMENT OF THALAMOCORTICAL CONNECTIVITY AND CORTICAL REPRESENTATION OF FACIAL WHISKERS IN MOUSE MODELS OF GREY MATTER HETEROTOPIA

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Aims: Cortical representations of the sensory periphery – or somatosensory maps – are topographically organized sub-fields of the murine somatosensory cortex. Among these, the so-called barrel field is composed of neuronal aggregates (barrels) in layer 4 (L4) that receive thalamocortical afferents (TCAs) and form a somatotopic map of facial whiskers. Here, we investigated the development of barrels and TCAs in two murine models of grey matter heterotopia (GMH), in which masses of ectopic neurons (or heterotopia) accumulate below the cortex and create an apparent duplicated cortex. Our aim is to determine if whisker maps are properly formed, altered or duplicated in this context. **Methods:** We used viral tracing approaches and immunohistochemistry to characterize the development of whisker maps and TCAs in two GMH mouse models resulting from the conditional, telencephalon-specific knockout (cKO) of Eml1 or RhoA. **Results:** In the two models, aggregates of neurons with a L4 identity are present at the expected location in the cortex, forming topographically organized barrels receiving TCAs. Unexpectedly, we also observed in the heterotopia similar barrel-like aggregates of ectopic L4 neurons that receive TCAs navigating either ventrally to the heterotopia in Eml1-cKO or dorsally in RhoA-cKO. This duplicated, ectopic whisker map is aligned with the normally positioned one, denoting an areal conservation between the heterotopia and cortex. **Conclusions:** Ectopic L4 neurons maintain their ability to instruct TCA connectivity, suggesting that intrinsic cortical programs are sufficient for areal specification of the barrel field.

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BOARD NUMBER: S03-161

STUDY OF THE INFLUENCE OF THE CEREBELLUM IN THE THALAMOCORTICAL NETWORK

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The cerebral cortex is involved in multiple functions, such as cognition, motion, language, emotion and receives afferent inputs from several regions, including the cerebellum. The cerebellum is connected with the cerebral cortex through the thalamus. The cerebellum is one of the most consistently altered brain regions reported in Autism Spectrum Disorders (ASD). According to the developmental diaschisis, meaning that one brain region could affect a remote one because they are connected, we are interested in studying how the cerebellum influences the thalamocortical network. Under this light, the closed-loop cerebello-thalamo-cortical circuit could explain the possible influence of the cerebellum in cortical development and its complex functions.
First of all, we aim at unraveling how the cerebello-thalamo-cortical circuit develops, in particular, when and where the cerebellum is connected with the cortex through the thalamus. After having assessed which thalamic nuclei receive the cerebellar afferences, we want to find out specifically how the thalamocortical network is established during development. We show which cortical regions are receiving the cerebellar inputs through the thalamus using viral and CTB tracing injections strategies. In particular, we describe that thalamic axons coming from cerebellum connect with the Front associative cortical region (Fra) and motor cortical areas such as M1 and M2.
Investigating how the cerebello-thalamo-cortical circuit emerges during development is essential for understanding cerebellar origin of neurodevelopmental disorders.

Pubmed:
35170728: Torre-Muruzabal T, Van der Perren A, Coens A, Gelders G, Barber Janer A, Camacho-Garcia S, Klingstedt T, Nilsson P, Stefanova N, Melki R, Baekelandt V, Peelaerts W
Host oligodendrogliopathy and α-synuclein strains dictate disease severity in multiple system atrophy.
Multiple system atrophy is a progressive neurodegenerative disease with prominent autonomic and motor features. During early stages different subtypes of multiple system atrophy are distinguished by their predominant parkinsonian or cerebellar symptoms reflecting the heterogeneous nature of the disease. The pathognomonic feature of multiple system atrophy is the presence of α-synuclein (αSyn) protein deposits in oligodendroglial cells. αSyn can assemble in specific cellular or disease environments and form αSyn strains with unique structural features but the ability of αSyn strains to propagate in oligodendrocytes remains elusive. More recently, it was shown that multiple multiple system atrophy strains with related conformations exist in the brain of patients. Here, we investigated if different αSyn strains can influence multiple system atrophy progression in a strain-dependent manner. To this aim, we injected two recombinant αSyn strains (fibrils and ribbons) in multiple system atrophy transgenic mice and found that αSyn protein strains determine disease severity in multiple system atrophy via host-restricted and cell-specific pathology in vivo. αSyn strains significantly impact disease progression in a strain-dependent way via oligodendroglial, neurotoxic and immune-related mechanisms. Neurodegeneration and brain atrophy were accompanied by unique microglial and astroglial responses and the recruitment of central and peripheral immune cells. The differential activation of microglial cells correlated with the structural features of αSyn strains both in vitro and in vivo. Spectral analysis showed that ribbons propagate oligodendroglial inclusions that are structurally distinct from those of fibrils, with resemblance to oligodendroglial inclusions in multiple system atrophy patient brain. This study therefore shows that the multiple system atrophy phenotype is governed by both the αSyn strain nature and the host environment and that by injecting αSyn strains in a multiple system atrophy animal model a more comprehensive phenotype can be established.
Brain, 2022;

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BOARD NUMBER: S03-162

THE DEVELOPMENT OF THE CEREBELLO-THALAMIC TRACT

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Classically, the cerebellum has been considered a pure motor brain structure. However, mounting evidence has suggested that it also has essential contributions to nonmotor functions, such as cognition and emotion. The cerebellum is well poised to contribute to these complex behaviors because it is connected to the cerebral cortex which controls these functions. The main efferent connections from the cerebellum to the cortex are made through the thalamus. Importantly, early abnormalities of this circuit have been related to diverse neurodevelopmental disorders, such as autism spectrum disorders. Thus, understanding how the cerebello-thalamic pathway develops is the required step to understanding the cerebellar contribution to high-order neurodevelopmental disorders. Here we have performed different strategies, including tracing methods and transgenic approaches, to specifically target the cerebellum and its long-range projections. By combining these techniques together with clearing methods and 3D light-sheet microscopy, we have defined the cerebellothalamic connectivity. Our data shows that the cerebellar axons invade the contralateral thalamus as early as embryonic day (E)17. At E18, cerebellar projections mainly contact to the ventromedial and the ventral anterior-lateral complex, but also to the mediodorsal and parafascicular thalamic nuclei. The cerebellothalamic innervation continues evolving during postnatal stages innervating several motor and non-motor thalamic nuclei. Our data reveal a remarkable complexity of the cerebellothalamic connectivity during late embryonic and early postnatal stages. These results suggest a potential role of the cerebellum in impacting complex and immature thalamocortical networks.

Pubmed:
35103859: Domínguez-Sala E, Andreu-Cervera A, Martín-Climent P, Murcia-Ramón R, Martínez S, Geijo-Barrientos E
Properties of the epileptiform activity in the cingulate cortex of a mouse model of LIS1 dysfunction.
Dysfunction of the LIS1 gene causes lissencephaly, a drastic neurological disorder characterized by a deep disruption of the cortical structure. We aim to uncover alterations of the cortical neuronal networks related with the propagation of epileptiform activity in the Lis1/sLis1 mouse, a model lacking the LisH domain in heterozygosis. We did extracellular field-potential and intracellular recordings in brain slices of the anterior cingulate cortex (ACC) or the retrosplenial cortex (RSC) to study epileptiform activity evoked in the presence of bicuculline (10 µM), a blocker of GABA receptors. The sensitivity to bicuculline of the generation of epileptiform discharges was similar in wild type (WT) and Lis1/sLis1 cortex (EC 1.99 and 2.24 µM, respectively). In the Lis1/sLis1 cortex, we observed a decreased frequency of the oscillatory post-discharges of the epileptiform events; also, the propagation of epileptiform events along layer 2/3 was slower in the Lis1/sLis1 cortex (WT 47.69 ± 2.16 mm/s, n = 25; Lis1/sLis1 37.34 ± 2.43 mm/s, n = 15; p = 0.004). The intrinsic electrophysiological properties of layer 2/3 pyramidal neurons were similar in WT and Lis1/sLis1 cortex, but the frequency of the spontaneous EPSCs was lower and their peak amplitude higher in Lis1/sLis1 pyramidal neurons. Finally, the propagation of epileptiform activity was differently affected by AMPA receptor blockers: CNQX had a larger effect in both ACC and RSC while GYKI53655 had a larger effect only in the ACC in the WT and Lis1/sLis1 cortex. All these changes indicate that the dysfunction of the LIS1 gene causes abnormalities in the properties of epileptiform discharges and in their propagation along the layer 2/3 in the anterior cingulate cortex and in the restrosplenial cortex.
Brain Struct Funct, 2022; 227
34722541: Company V, Moreno-Cerdá A, Andreu-Cervera A, Murcia-Ramón R, Almagro-García F, Echevarría D, Martínez S, Puelles E
Role in the Development of the Habenula and the Fasciculus Retroflexus.
is one of the morphogenes that controls the specification and differentiation of neuronal populations in the developing central nervous system. The habenula is a diencephalic neuronal complex located in the most dorsal aspect of the thalamic prosomere. This diencephalic neuronal population is involved in the limbic system and its malfunction is related with several psychiatric disorders. Our aim is to elucidate the role in the habenula and its main efferent tract, the fasciculus retroflexus,

development. In order to achieve these objectives, we analyzed these structures development in a lack of function mouse model. The habenula was generated in our model, but it presented an enlarged volume. This alteration was due to an increment in habenular neuroblasts proliferation rate. The fasciculus retroflexus also presented a wider and disorganized distribution and a disturbed final trajectory toward its target. The mid-hindbrain territories that the tract must cross were miss-differentiated in our model. The specification of the habenula is independent. Nevertheless, it controls its precursors proliferation rate. expressed in the isthmic organizer is vital to induce the midbrain and rostral hindbrain territories. The alteration of these areas is responsible for the fasciculus retroflexus axons misroute.
Front Cell Dev Biol, 2021; 9

33145610: Murcia-Ramón R, Company V, Juárez-Leal I, Andreu-Cervera A, Almagro-García F, Martínez S, Echevarría D, Puelles E

Neuronal tangential migration from Nkx2.1-positive hypothalamus.

During the development of the central nervous system, the immature neurons suffer different migration processes. It is well known that Nkx2.1-positive ventricular layer give rise to critical tangential migrations into different regions of the developing forebrain. Our aim was to study this phenomenon in the hypothalamic region. With this purpose, we used a transgenic mouse line that expresses the tdTomato reporter driven by the promotor of Nkx2.1. Analysing the Nkx2.1-positive derivatives at E18.5, we found neural contributions to the prethalamic region, mainly in the zona incerta and in the mes-diencephalic tegmental region. We studied the developing hypothalamus along the embryonic period. From E10.5 we detected that the Nkx2.1 expression domain was narrower than the reporter distribution. Therefore, the Nkx2.1 expression fades in a great number of the early-born neurons from the Nkx2.1-positive territory. At the most caudal positive part, we detected a thin stream of positive neurons migrating caudally into the mes-diencephalic tegmental region using time-lapse experiments on open neural tube explants. Late in development, we found a second migratory stream into the prethalamic territory. All these tangentially migrated neurons developed a gabaergic phenotype. In summary, we have described the contribution of interneurons from the Nkx2.1-positive hypothalamic territory into two different rostrocaudal territories: the mes-diencephalic reticular formation through a caudal tangential migration and the prethalamic zona incerta complex through a dorsocaudal tangential migration.
Brain Struct Funct, 2020; 225

32581730: Ádám Á, Kemecsei R, Company V, Murcia-Ramón R, Juarez I, Gerecsei LI, Zachar G, Echevarría D, Puelles E, Martínez S, Csillag A

Gestational Exposure to Sodium Valproate Disrupts Fasciculation of the Mesotelencephalic Dopaminergic Tract, With a Selective Reduction of Dopaminergic Output From the Ventral Tegmental Area.

Gestational exposure to valproic acid (VPA) is known to cause behavioral deficits of sociability, matching similar alterations in human autism spectrum disorder (ASD). Available data are scarce on the neuromorphological changes in VPA-exposed animals. Here, we focused on alterations of the dopaminergic system, which is implicated in motivation and reward, with relevance to social cohesion. Whole brains from 7-day-old mice born to mothers given a single injection of VPA (400 mg/kg b.wt.) on E13.5 were immunostained against tyrosine hydroxylase (TH). They were scanned using the iDISCO method with a laser light-sheet microscope, and the reconstructed images were analyzed in 3D for quantitative morphometry. A marked reduction of mesotelencephalic (MT) axonal fascicles together with a widening of the MT tract were observed in VPA treated mice, while other major brain tracts appeared anatomically intact. We also found a reduction in the abundance of dopaminergic ventral tegmental (VTA) neurons, accompanied by diminished tissue level of DA in ventrobasal telencephalic regions (including the nucleus accumbens (NAc), olfactory tubercle, BST, substantia innominata). Such a reduction of DA was not observed in the non-limbic caudate-putamen. Conversely, the abundance of TH+ cells in the substantia nigra (SN) was increased, presumably due to a compensatory mechanism or to an altered distribution of TH+ neurons occupying the SN and the VTA. The findings suggest that defasciculation of the MT tract and neuronal loss in VTA, followed by diminished dopaminergic input to the ventrobasal telencephalon at a critical time point of embryonic development (E13-E14) may hinder the patterning of certain brain centers underlying decision making and sociability.
Front Neuroanat, 2020; 14

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BOARD NUMBER: S03-163

EFFECTS OF LIS1 GENE LOSS IN PARVALBUMIN EXPRESSING CELLS ON THE MOUSE CEREBELLAR CORTEX

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The formation of cerebellar neuronal circuits and the development of the physiological functions of the cerebellar cortex require a precise sequence of neural differentiation and migration steps. The *Lis1* gene regulates the dynein dependent axonal transport, mediates neuronal migration in the developing brain, and supports synaptic integrity in the mature brain. In human, defects in *LIS1* expression produce lissencephaly type I. *Lis1* is expressed throughout the entire lifespan of the central nervous system. We have studied the cerebellar alterations caused by *Lis1* selective inactivation in parvalbumin (PV) expressing cells; PV is highly expressed in cerebellar Purkinje cell layer during development of the cerebellar cortex. For this we have created a conditional *Lis1* knockout mouse in PV^{+} neurons ($Lis1^{cKO\text{-}PV+}$). During prepuberal stages (P15-P21), $Lis1^{cKO\text{-}PV+}$ mice presented a strong ataxic locomotor phenotype, reflecting possible dysfunctions in the cerebellum. Purkinje cell layer (PCL) displayed a clear impaired organization. Purkinje cells presented an abnormal soma with a rudimentary and atrophic dendritic arborization. Moreover, staining against V-Glut1, a glutamatergic neuronal terminal marker, showed an abnormal organization of the excitatory basket nets generated by these terminals around the Purkinje cell soma. Altogether, these data suggest that *Lis1* expression in Purkinje cells is not only required for their proper development and therefore the PCL organization but also for the normal synaptogenetic mechanisms leading to the organisation of the glutamatergic presynaptic terminals. These results also indicate the presence of non-cell autonomous effects of *Lis1* dysfunction. Funding: MCIN grant PID2020-118171RB-I00 and GVA-Prometeo Grant 2018/041. Severo Ochoa Program grant SEV-2017-0723.

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BOARD NUMBER: S03-164

POSTNATAL GABAERGIC TRANSMISSION SCULPTS CALLOSAL CONNECTIVITY

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Neuronal circuits are developmentally shaped by genetic and activity-dependent mechanisms. Activity results from two opposing forces, excitation and inhibition (E/I), which depend on pyramidal cells and interneurons (INs). Cortical INs are born in the ventral telencephalon and only subsequently migrate into the cortex. Defects in INs are implicated in diverse neurodevelopmental disorders (NDDs). However, whether the integration of INs influences the assembly of excitatory networks is unknown. Here, we pharmacologically and genetically altered E/I balance to characterize its impact on the interhemispheric connections that constitute the corpus callosum (CC). This structure coordinates higher-order sensory, motor, and cognitive functions often affected in NDD. After postnatal treatments with the GABA agonist diazepam, stereotaxic injections of the retrograde tracer cholera toxin B (CTB) were performed directly into the CC to label the somatosensory and visual callosal circuits. GABAergic enhancement rearranges the adult interhemispheric circuit. Such rewiring potential is restricted to an early window, progressively diminishing with time. The most striking result is found in layer (L) 4 neurons –the paradigm of local connectivity– which exhibit a fourfold increase in callosal projections. The reprogrammed neurons receive fewer thalamic afferents and show smaller amplitude responses to intracolumnar stimulation. Aiming to pinpoint the physiological regulator of this rewiring, we genetically ablated somatostatin (SST) INs, one of the earliest cortical inhibitory subpopulations. SST deletion reproduces the L4 callosal phenotype. Hence, INs integration into the emerging excitatory circuits determines the choice between interhemispheric and local connectivity. Moreover, we describe a temporal window to pharmacologically manipulate callosal circuits.

Pubmed:
32988974: De León Reyes NS, Bragg-Gonzalo L, Nieto M
Development and plasticity of the corpus callosum.
The corpus callosum (CC) connects the cerebral hemispheres and is the major mammalian commissural tract. It facilitates bilateral sensory integration and higher cognitive functions, and is often affected in neurodevelopmental diseases. Here, we review the mechanisms that contribute to the development of CC circuits in animal models and humans. These species comparisons reveal several commonalities. First, there is an early period of massive axonal projection. Second, there is a postnatal temporal window, varying between species, in which early callosal projections are selectively refined. Third, sensory-derived activity influences axonal refinement. We also discuss how defects in CC formation can lead to mild or severe CC congenital malformations.
Development, 2020; 147
34030948: Bragg-Gonzalo L, De León Reyes NS, Nieto M
Genetic and activity dependent-mechanisms wiring the cortex: Two sides of the same coin.
The cerebral cortex is responsible for the higher-order functions of the brain such as planning, cognition, or social behaviour. It provides us with the capacity to interact with and transform our world. The substrates of cortical functions are complex neural circuits that arise during development from the dynamic remodelling and progressive specialization of immature undefined networks. Here, we review the genetic and activity-dependent mechanisms of cortical wiring focussing on the importance of their interaction. Cortical circuits emerge from an initial set of neuronal types that engage in sequential forms of embryonic and postnatal activity. Such activities further complement the cells' genetic programs, increasing neuronal diversity and modifying the electrical properties while promoting selective connectivity. After a temporal window of enhanced plasticity, the main features of mature circuits are established. Failures in these processes can lead to neurodevelopmental disorders whose treatment remains elusive. However, a deeper dissection of cortical wiring will pave the way for innovative therapies.
Semin Cell Dev Biol, 2021; 118

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BOARD NUMBER: S03-165

DEVELOPMENTAL TARGETS OF CALLOSAL PROJECTION NEURONS (CPNS)

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The mammalian cortex contains multiple classes of neurons distributed across and within cortical layers. CPNs connect the hemispheres of the brain, increasing the complexity of cortical processing. Many cortical neurons send transient axons during development, which endow them with the capacity of forming different circuits. In the lab, we try to identify the significance, targets, and limits of this plasticity. By combining stereotaxic injections of the retrotracer CTB into the corpus callosum (CC) with immunolabeling of the neuronal marker NeuN, we determined the percentage of CPNs at different postnatal days. We observed that a considerable number of cortical neurons send transient projections to the contralateral hemisphere, which are then gradually eliminated during development in layer-specific manners. Most of them are upper-layer neurons, the ones with a more recent evolutionary origin; but some reside in the deep layers. Thereafter, we evaluated if developing CPNs explore subcortical territories as well. Simultaneous injections of CTB in the CC and the thalamus or the cerebral peduncle at sequential postnatal stages showed that callosal and subcortical labeling was always segregated into different cells, indicating that CPNs do not explore long-range subcortical targets. However, we detected short-range callosal branches exploring the dorsal region of the basal ganglia, which were confirmed by in utero electroporation. These findings demonstrate that, although callosal axons undergo massive remodeling during development, the plasticity of their long-range projections is restricted to cortical territories. This helps us to define the orchestrated freedom of the cortex.

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ADVANCED ENGINEERING APPROACHES FOR RECAPITULATION OF STRUCTURE-FUNCTION DYNAMICS IN NEURAL NETWORKS

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The topology of neuronal networks is decisive for how information is processed, transferred and stored in the human brain. Engineered neural networks can be used to recapitulate key aspects of this topology in a confined and controlled environment *in vitro*, providing an invaluable tool to understand how the underlying structural motifs shape the dynamics of neural circuitries in healthy and perturbed conditions. Such modelling platforms thus enable the recapitulation and study of micro- and mesoscale network reconfigurations and how they impact a network´s functional output. Here we show that a microfluidic device with axon guiding channels with a geometry inspired by a Tesla valve can be used to engineer hierarchical neural networks *in vitro* with controllable afferent and efferent connectivity. In this way we can directly influence the network´s functional attributes, as demonstrated electrophysiologically by interfacing the platforms with nanoporous microelectrode arrays. This approach supports advanced comparative studies of network information processing capabilities in anatomically relevant circuitries in healthy versus pathological states. The relevant micro- and mesoscale investigations are per definition not possible *in vivo*. As such, these models have the potential to provide novel insights into neuroplasticity mechanisms in healthy and perturbed neural circuitries.

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BOARD NUMBER: S03-167

MOLECULAR BASIS AND BEHAVIOURAL SIGNIFICANCE OF A SEX SPECIFIC CIRCUIT SWITCH IN DROSOPHILA

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Sex-specific behavioural differences are driven by differences in circuit architecture established through development and genetically controlled. However finding a causal link between genetic, circuit and behavioural differences is challenging. The circuit responding to the male pheromone 11-cis-vaccenyl acetate (cVA) in Drosophila melanogaster offers a powerful entry point to this question. cVA has been shown to promote courtship in females and aggression in males (Kurtovic A, et al, 2007, Wang L & Anderson DJ, 2010). cVA information flow is redirected by a switch formed by two types of interneurons, aSP-g and aSP-f, with sexually dimorphic dendritic projections (Cachero S et al, 2010; Kohl J et al, 2013), whose sex is cell-autonomously controlled by the master regulator of sex-differentiation fruitless. We investigated the molecular underpinnings of sex-specific connectivity by transcriptionally profiling aSP-gs and aSP-fs in females, males and masculinised females during pupation. We identified a set of differentially expressed genes, whose function was addressed by a loss-of-function screen. We also found cell-type specific molecular markers, which gave us genetic access to these neurons. Through optogenetic activation experiments we showed that aSP-gs increase female sexual receptivity, while aSP-fs reduce male copulation success, demonstrating that the circuit switch has the hypothesised impact on behaviour. Additionally, in line with the idea that cVA induces attraction towards males by activating aSP-g neurons, their masculinisation resulted in reduced female receptivity. Together our work is revealing causal links between sex-specifc circuit architecture and behaviour, as well as the molecular genetic basis of these circuit differences.

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MOLECULAR HETEROGENEITY OF CAJAL-RETZIUS NEURONS IN THE DEVELOPING CORTEX UNDERLIES THEIR CIRCUIT CONNECTIVITY AND SENSORY DRIVEN RESPONSES

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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A central question in brain development concerns how neuronal diversity guides the formation of neural connections. In the developing cortex, there exists a transient class of glutamatergic neurons known as Cajal-Retzius cells (CRs). CRs populate layer 1 of the cortex and arise from at least three distinct ontogenetic origins. The conundrum is that all three populations are thought to share common features, however recent studies have begun to identify some molecular heterogeneity among them. We sought to systematically profile CRs and ask whether neurons of distinct origin are differentially embedded in early cortical circuits. To address this, we first captured the distribution of CRs at postnatal (P) ages by utilizing genetic fate mapping, combined with CLARITY and mesoSPIM lightsheet microscopy. We find that, unlike at embryonic time points, postnatal CRs do not segregate in the cortex but rather intermingle. By performing single cell mRNA sequencing of CRs at P6 we revealed that this class of cells displays unappreciated genetic heterogeneity, also at the level of synaptic genes. Using monosynaptic pseudotyped-rabies virus tracings, we identified shared dense layer 1 presynaptic partners, but also distinct connections from subplate neurons and subcortical structures depending on CRs origin. These connectivity differences produce CRs specific sensory driven responses *in vivo*, identified via wide-field calcium imaging. This study uncovers the diversity of ontogenetically distinct CRs at the molecular, anatomical, and functional level and paves the way for revealing their role in the formation and function of cortical circuits.

Pubmed:
34380763: Stachniak TJ, Kastli R, Hanley O, Argunsah AÖ, van der Valk EGT, Kanatouris G, Karayannis T
Postmitotic Prox1 Expression Controls the Final Specification of Cortical VIP Interneuron Subtypes.
Throughout development, neuronal identity is controlled by key transcription factors that determine the unique properties of a cell. During embryogenesis, the transcription factor Prox1 regulates VIP-positive cortical interneuron migration, survival, and connectivity. Here, we explore the role of Prox1 as a regulator of genetic programs that guide the final specification of VIP interneuron subtypes in early postnatal life. Synaptic electrophysiology in male and female mice shows that postnatal Prox1 removal differentially affects the dynamics of excitatory inputs onto VIP bipolar and multipolar subtypes. RNA sequencing reveals that one of the downstream targets of Prox1 is the postsynaptic protein Elfn1, a constitutive regulator of presynaptic release probability. Further genetic, pharmacological, and electrophysiological experiments demonstrate that removing Prox1 reduces Elfn1 function in VIP multipolar but not in bipolar cells. Finally, overexpression experiments and analysis of native mRNA expression reveal that Elfn1 levels are differentially controlled at the post-transcriptional stage. Thus, in addition to activity-dependent processes that contribute to the developmental trajectory of VIP cells, genetic programs engaged by Prox1 control the final differentiation of multipolar and bipolar subtypes. The transcription factor Prox1 generates functional diversification of cortical VIP interneuron subtypes in early postnatal life, thus expanding the inhibitory repertoire of the cortex.
J Neurosci, 2021; 41
29379870: Edmond M, Hanley O, Philippidou P
Topoisomerase IIβ Selectively Regulates Motor Neuron Identity and Peripheral Connectivity through Hox/Pbx-Dependent Transcriptional Programs.
Vital motor functions, such as respiration and locomotion, rely on the ability of spinal motor neurons (MNs) to acquire stereotypical positions in the ventral spinal cord and to project with high precision to their peripheral targets. These key properties of MNs emerge during development through transcriptional programs that dictate their subtype identity and connectivity; however, the molecular mechanisms that establish the transcriptional landscape necessary for MN specification are not fully understood. Here, we show that the enzyme topoisomerase IIβ (Top2β) controls MN migration and connectivity. Surprisingly, Top2β is not required for MN generation or survival but has a selective role in columnar specification. In the

absence of , phrenic MN identity is eroded, while other motor columns are partially preserved but fail to cluster to their proper position. In -/- mice, peripheral connectivity is impaired as MNs exhibit a profound deficit in terminal branching. These defects likely result from the insufficient activation of Hox/Pbx-dependent transcriptional programs as and genes are downregulated in the absence of . mutants recapitulate many aspects of mutant mice, such as MN disorganization and defects in medial motor column (MMC) specification. Our findings indicate that , a gene implicated in neurodevelopmental diseases such as autism spectrum disorders, plays a critical, cell-specific role in the assembly of motor circuits.
eNeuro, 2017 Nov-Dec; 4
27568519: Hanley O, Zewdu R, Cohen LJ, Jung H, Lacombe J, Philippidou P, Lee DH, Selleri L, Dasen JS
Parallel Pbx-Dependent Pathways Govern the Coalescence and Fate of Motor Columns.
The clustering of neurons sharing similar functional properties and connectivity is a common organizational feature of vertebrate nervous systems. Within motor networks, spinal motor neurons (MNs) segregate into longitudinally arrayed subtypes, establishing a central somatotopic map of peripheral target innervation. MN organization and connectivity relies on Hox transcription factors expressed along the rostrocaudal axis; however, the developmental mechanisms governing the orderly arrangement of MNs are largely unknown. We show that Pbx genes, which encode Hox cofactors, are essential for the segregation and clustering of neurons within motor columns. In the absence of Pbx1 and Pbx3 function, Hox-dependent programs are lost and the remaining MN subtypes are unclustered and disordered. Identification of Pbx gene targets revealed an unexpected and apparently Hox-independent role in defining molecular features of dorsally projecting medial motor column (MMC) neurons. These results indicate Pbx genes act in parallel genetic pathways to orchestrate neuronal subtype differentiation, connectivity, and organization.
Neuron, 2016; 91
24746670: Jung H, Mazzoni EO, Soshnikova N, Hanley O, Venkatesh B, Duboule D, Dasen JS
Evolving Hox activity profiles govern diversity in locomotor systems.
The emergence of limb-driven locomotor behaviors was a key event in the evolution of vertebrates and fostered the transition from aquatic to terrestrial life. We show that the generation of limb-projecting lateral motor column (LMC) neurons in mice relies on a transcriptional autoregulatory module initiated via transient activity of multiple genes within the HoxA and HoxC clusters. Repression of this module at thoracic levels restricts expression of LMC determinants, thus dictating LMC position relative to the limbs. This suppression is mediated by a key regulatory domain that is specifically found in the Hoxc9 proteins of appendage-bearing vertebrates. The profile of Hoxc9 expression inversely correlates with LMC position in land vertebrates and likely accounts for the absence of LMC neurons in limbless species such as snakes. Thus, modulation of both Hoxc9 protein function and Hoxc9 gene expression likely contributed to evolutionary transitions between undulatory and ambulatory motor circuit connectivity programs.
Dev Cell, 2014; 29

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THE DNA METHYLTRANSFERASE 1 IS IMPORTANT FOR THE MIGRATION OF MGE-DERIVED INTERNEURONS

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Inhibitory gamma-aminobutyric (GABA)-positive interneurons are indispensable for information processing in the mammalian neocortex. Defects in interneuron function lead to an imbalance of neuronal excitation and inhibition associated with severe neuropsychiatric diseases and syndromes. In this context, dysregulated critical milestones of interneuron development are suggested to cause these neurodevelopmental disorders and manifest into severe pathologies such as schizophrenia, epilepsy or autism spectrum disorders. Proper interneuron development is crucially determined by epigenetic mechanisms such as DNA methylation, histone modifications or non-coding RNAs emerged as regulators of various neurodevelopmental processes. In a previous study, we found an essential implication of the DNA methyltransferase 1 (DNMT1) in promoting the long-range migration of inhibitory cortical interneurons generated in the embryonic pre-optic area (POA). By crosstalk with histone modifications, DNMT1 maintains the migratory morphology and the survival of these cells.
Here, we show that DNMT1 is likewise involved in regulating the migration of interneurons generated in the medial ganglionic eminence (MGE), achieved by its canonical DNA methylation function. Decreased *Dnmt1* expression resulted in altered cellular motility and directionality of cellular migration. In contrast to POA-derived interneurons, the motility of MGE-derived cells was not regulated via DNMT1-dependent modulation of their migratory morphology. Instead, we found that DNMT1 affects the endocytic capacity of embryonic MGE-interneurons. Endocytosis in turn is a critical process guaranteeing the controlled internalization of adhesive complexes for proper migration. Conclusively, we hypothesize that DNMT1-dependent regulation of MGE interneuron migration essentially relies on modifying endocytic rate in these cells during their development.

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EMERGING TRANSCRIPTIONAL IDENTITIES OF DEVELOPING THALAMOCORTICAL NEURONS.

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The thalamus is an essential gateway for peripheral sensory signals on their way to the cortex. It organizes into nuclei containing molecularly distinct cell types with specific inputs and projection targets; however, how this diversity emerges during development remains poorly understood. To address this question, here we use single-cell transcriptomics to investigate intrinsic and extrinsic transcriptional controls over thalamic neuron diversity during embryogenesis and early postnatal life. Working in mice, we identify sequential transcriptional waves underlying differentiation into different types of thalamic neurons and their requirement for sensory input. Our study provides insights into the developmental transcriptional trajectories of input-(in)dependent differentiation and diversification of thalamocortical neurons.

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SPONTANEOUS ACTIVITY REGULATES THALAMIC CIRCUITRY SPECIFICATION DURING DEVELOPMENT

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The presence of spontaneous patterns of neuronal activity is a distinctive feature of the developing thalamus. However, the underlying gene programs regulating these activity patterns remain largely unknown. To this aim, we performed longitudinal transcriptional profiling of sensory thalamic neurons in control animals and in mice lacking synchronous neuronal activity (*Th*^{Kir} mice). This approach revealed gene programs related to thalamocortical connectivity and neural cell proliferation. Moreover, we found that spontaneous activity is necessary for the expression of nuclei-specific gene signatures. Genes related with ion channel activity and neural cell differentiation were deregulated in *Th*^{Kir} mice. In sum, our data reveals the molecular logic underlying the patterns of spontaneous activity in the developing thalamus. These results deepen our understanding of the developmental regulatory mechanisms of the distinct sensory-modality nuclei.

Pubmed:
33827819: Herrero-Navarro Á, Puche-Aroca L, Moreno-Juan V, Sempere-Ferràndez A, Espinosa A, Susín R, Torres-Masjoan L, Leyva-Díaz E, Karow M, Figueres-Oñate M, López-Mascaraque L, López-Atalaya JP, Berninger B, López-Bendito G
Astrocytes and neurons share region-specific transcriptional signatures that confer regional identity to neuronal reprogramming.
Neural cell diversity is essential to endow distinct brain regions with specific functions. During development, progenitors within these regions are characterized by specific gene expression programs, contributing to the generation of diversity in postmitotic neurons and astrocytes. While the region-specific molecular diversity of neurons and astrocytes is increasingly understood, whether these cells share region-specific programs remains unknown. Here, we show that in the neocortex and thalamus, neurons and astrocytes express shared region-specific transcriptional and epigenetic signatures. These signatures not only distinguish cells across these two brain regions but are also detected across substructures within regions, such as distinct thalamic nuclei, where clonal analysis reveals the existence of common nucleus-specific progenitors for neurons and astrocytes. Consistent with their shared molecular signature, regional specificity is maintained following astrocyte-to-neuron reprogramming. A detailed understanding of these regional-specific signatures may thus inform strategies for future cell-based brain repair.
Sci Adv, 2021; 7

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CELF1 IS INVOLVED IN THE EARLY LAMINAR ORGANIZATION IN HUMAN FETAL BRAIN

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Expression and activity of molecular networks involved in cell proliferation, migration and neuronal specification can be seen during late “preplate” and early cortical plate phase (7.5-10 PCW) in the human brain. Spatio-temporal overlapping of these neurogenetic processes leads to proper positioning of the future deep cortical layer neurons and subplate neurons. Laminar rhythm of future projection neurons is established by the expression of molecular markers which determine their identity and final destination [1]. RNA binding protein CELF1, as marker of future projection neurons is present in the mouse brain during early neurogenesis [2], but its role in formation of early laminar structure in the human brain, during the “preplate” phase remains unreveild. Thus, our aim was to analyze expression pattern of CELF1 and TBR1 (subplate neurons marker) utilizing immunofluorescence on prenatal postmortem human brain to reveal CELF1 spatio-temporal expressin pattern during the late “preplate” and early cortical plate phase. According to our results, CELF1 shows slight positivity in proliferative zones where intermediate progenitor cells reside, as well as in the “preplate” neurons. TBR1 is positive in “preplate” neurons as well as in transient neurons of the future subplate. CELF1 and TBR1 staining, alongside with the deeper cortical layer-enriched markers (TLE4, CTIP2, SOX5) showed that majority of CELF1+ and TBR1+ cells colocalize with those markers. Our results confirm that studing an early laminar organization is esential for understanding specification of neuronal identity and establishment of functional cortical connectivity.

Pubmed:
33375093: Šimić G, Vukić V, Kopić J, Krsnik Ž, Hof PR
Molecules, Mechanisms, and Disorders of Self-Domestication: Keys for Understanding Emotional and Social Communication from an Evolutionary Perspective.
The neural crest hypothesis states that the phenotypic features of the domestication syndrome are due to a reduced number or disruption of neural crest cells (NCCs) migration, as these cells differentiate at their final destinations and proliferate into different tissues whose activity is reduced by domestication. Comparing the phenotypic characteristics of modern and prehistoric man, it is clear that during their recent evolutionary past, humans also went through a process of self-domestication with a simultaneous prolongation of the period of socialization. This has led to the development of social abilities and skills, especially language, as well as neoteny. Disorders of neural crest cell development and migration lead to many different conditions such as Waardenburg syndrome, Hirschsprung disease, fetal alcohol syndrome, DiGeorge and Treacher-Collins syndrome, for which the mechanisms are already relatively well-known. However, for others, such as Williams-Beuren syndrome and schizophrenia that have the characteristics of hyperdomestication, and autism spectrum disorders, and 7dupASD syndrome that have the characteristics of hypodomestication, much less is known. Thus, deciphering the biological determinants of disordered self-domestication has great potential for elucidating the normal and disturbed ontogenesis of humans, as well as for the understanding of evolution of mammals in general.
Biomolecules, 2020; 11
33613193: Rincic M, Rados M, Kopic J, Krsnik Z, Liehr T
7p21.3 Together With a 12p13.32 Deletion in a Patient With Microcephaly-Does 12p13.32 Locus Possibly Comprises a Candidate Gene Region for Microcephaly?
Front Mol Neurosci, 2021; 14
34255828: Žunić Išasegi I, Kopić J, Smilović D, Krsnik Ž, Kostović I
Transient Subplate Sublayer Forms Unique Corridor for Differential Ingrowth of Associative Pulvinar and Primary Visual Projection in the Prospective Visual Cortical Areas of the Human Fetal Occipital Lobe.
Cytoarchitectonical parcellation of the visual cortex into the striate and extrastriate cortex requires complex histogenetic events within a precise spatio-temporal frame to attain the specification of areal domains and associated thalamocortical

connections during the fetal brain development. We analyzed a deep subplate cellular monolayer (subplate "corridor" cells) present during a restricted period of 13-15 postconceptional weeks, showing the 3D caudo-ventro-medial position in the human fetal occipital lobe, corresponding to the segregation point of pulvinocortical and geniculocortical fibers at the prospective area 17/18 border. Immunofluorescence stainings revealed subplate "corridor" cells as the specific class of the deepest subplate neurons (NeuN+, Tbr1+, Cplx3+) expressing axon guidance molecules (Sema-3A+, EphA6+), presumably for the attraction of pulvinocortical axons and the repulsion of geniculocortical axons growing at that time (SNAP25+, Syn+, FN+). Furthermore, quantitative analysis of the subplate "corridor" region of interest, considering cell number, immunofluorescence signal intensity per cell and per region, revealed significant differences to other regions across the tangential circumference of the developing cerebral wall. Thus, our study sheds new light on the deepest subplate sublayer, strategically aligned along the growing axon systems in the prospective visual system, suggesting the establishment of the area 17/18 border by differential thalamocortical input during the fetal brain development.
Cereb Cortex, 2021; 32

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NEURONAL LAMINAR IDENTITY MARKERS DELINEATE REGIONAL DIFFERENCES DURING THE PRIMATE-SPECIFIC SUBPLATE EXPANSION PERIOD IN THE HUMAN FETAL CORTEX

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Development of the cortical anlage begins with the formation of the preplate (mantle by His), which is split by the first cohort of cortical plate (CP) cells in that way the specific marginal zone (MZ) containing Cajal-Retzius neurons (CRN) and presubplate (P-SP) are formed. The period between 13 and 15 postconceptional weeks (PCW) is characterized by a transformation (cell spread-down) of the deep portion of the CP. During this human-characteristic event, thalamocortical and basal forebrain afferent fibers penetrate tangentially and obliquely in the CP. In that way, cells of the deep portion of the CP are secondarily displaced downwards („second“ CP) forming the future expanded subplate (SP). Future SP neurons are born in the proliferative zones simultaneously as prospective cortical layer VI neurons. Importantly, future layer VI neurons are not secondarily dispersed during the SP formation period. Thus, our study aimed to show regional expression patterns of specific transcription factors, i.e. projection neurons markers, as well as synaptic markers with the special emphasis on the SP expansion period utilizing immunofluorescence. Our results show that markers of projection neurons demonstrate characteristic regional differences in the neocortical part of the cerebral wall during the process of SP expansion. We concluded that projection neurons markers of cortical layers V, VI, and projection neuronal population of prospective glutamatergic SP neurons are the key players in the establishment of early regional differences of the neocortex during the SP formation period.

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CHARACTERIZATION OF THE EARLIEST THALAMOCORTICAL INTERACTIONS IN THE HUMAN FETAL BRAIN

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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While there is evidence for early control of patterning that is intrinsic to the neocortex, a major extrinsic source of patterning is provided by the early thalamocortical afferents (TCAs). In the human brain, the thalamocortical interactions start from very early stages and they continue over a prolonged period, thus potentially affecting corticogenesis in a crucial manner. To understand these interactions, we traced early projections from the thalamus with carbocyanine dyes in fixed post-mortem human brains at midgestational stages (16-17 post-conception weeks, PCW). By 17 PCW, the TCAs already reached their target cortical area, where they innervate the subplate zone, a well-known transient target of these axons. Surprisingly, TCAs also develop projections towards the germinal compartments underlying them, in particular the outer subventricular zone (OSVZ). Although both the subplate and the OSVZ are massively expanded in the fetal human brain, and they are associated with its increase in size and complexity, these two compartments contain different cell types (postmitotic neurons and mitotic progenitors, respectively). This suggests that the early-arriving TCAs might influence intrinsic neurogenic events by modulating the activity of different cortical cell populations. In particular, we selected VGF (non-acronymic) as a candidate thalamic factor that might account for the simultaneous modulation of both cortical neurons and progenitors, and we are currently validating this pathway by using available transcriptomic datasets of the human developing brain and immunohistochemical analysis. This characterization of the early thalamocortical interactions shall unravel some unique aspects of the specialization and the evolution of the human cerebral cortex.

Pubmed:
32877696: Braga A, Bandiera S, Verheyen J, Hamel R, Rutigliani C, Edenhofer F, Smith JA, Pluchino S
Combination of In Situ Lcn2 pRNA-RNAi Nanotherapeutics and iNSC Transplantation Ameliorates Experimental SCI in Mice.
Spinal cord injury (SCI) is a debilitating neurological condition characterized by different cellular and molecular mechanisms that interplay in exacerbating the progression of the pathology. No fully restorative therapies are yet available, and it is thus becoming recognized that combinatorial approaches aimed at addressing different aspects of SCI will likely results in greater functional outcomes. Here we employed packaging RNA-mediated RNA interference (pRNA-RNAi) nanotherapeutics to downregulate in situ the expression of lipocalin 2 (Lcn2), a known mediator of neuroinflammation and autocrine mediator of reactive astrogliosis, and to create a more amenable niche for the subsequent transplantation of induced neural stem cells (iNSCs). To our knowledge, this is the first approach that takes advantage of the modular and multifunctional pRNA three-way junction platform in the SCI niche, while also exploiting the therapeutic potential of immune-compatible and feasible iNSC transplants. We show the combination of such treatments in a mouse model of contusion thoracic SCI leads to significant improvement of locomotor function, albeit not better than single pRNA-RNAi treatment, and results in synergistic histopathological effects, such as reduction of glial scar volume, diminished pro-inflammatory response, and promotion of neuronal survival. Our results provide evidence for a novel combinatorial approach for treating SCI.
Mol Ther, 2020; 28
29499926: Smith JA, Braga A, Verheyen J, Basilico S, Bandiera S, Alfaro-Cervello C, Peruzzotti-Jametti L, Shu D, Haque F, Guo P, Pluchino S
RNA Nanotherapeutics for the Amelioration of Astroglial Reactivity.
In response to injuries to the CNS, astrocytes enter a reactive state known as astrogliosis, which is believed to be deleterious in some contexts. Activated astrocytes overexpress intermediate filaments including glial fibrillary acidic protein (GFAP) and vimentin (Vim), resulting in entangled cells that inhibit neurite growth and functional recovery. Reactive astrocytes also secrete inflammatory molecules such as Lipocalin 2 (Lcn2), which perpetuate reactivity and adversely affect other cells of the CNS. Herein, we report proof-of-concept use of the packaging RNA (pRNA)-derived three-way junction (3WJ) motif as a platform for the delivery of siRNAs to downregulate such reactivity-associated genes. In vitro, siRNA-3WJs induced a significant knockdown of Gfap, Vim, and Lcn2 in a model of astroglial activation, with a concomitant reduction in protein expression. Knockdown of Lcn2 also led to reduced protein secretion from reactive astroglial cells, significantly impeding the

perpetuation of inflammation in otherwise quiescent astrocytes. Intralesional injection of anti-Lcn2-3WJs in mice with contusion spinal cord injury led to knockdown of Lcn2 at mRNA and protein levels in vivo. Our results provide evidence for siRNA-3WJs as a promising platform for ameliorating astroglial reactivity, with significant potential for further functionalization and adaptation for therapeutic applications in the CNS.
Mol Ther Nucleic Acids, 2018; 10

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SPECIFICATION AND FUNCTIONAL MATURATION OF CORTICAL LONG-RANGE SST+ NEURONS

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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In the mammalian neocortex, inhibition is driven mainly by local interneurons to gate the excitation of projections. However, one key exception is somatostatin (SST+) expressing cortical long-range inhibitory neurons, a unique population that connects different cortical and subcortical areas. While their synaptic connections have been well established, less in known about the molecular mechanisms that drive their fate specification and maintenance – an important first step to decipher the cellular logic of long-range inhibitory circuit assembly and to design novel cell replacement therapies for brain disorders. In this work, we characterized the developmental trajectories of SST+ long-range inhibitory neurons and SST+ interneurons by single cell RNA sequencing and found that the factors driving their maturation are completely distinctive. Specifically, we identify *POU3f2/Brn2*, a homeobox POU class III transcription factor, to be highly enriched in developing cortical long-range inhibitory neurons and in the progenitors that generate these cells. Using two conditional mutant mouse lines that lack *POU3f2* in either post-mitotic long-range inhibitory neurons or their progenitors, we found that the conditional removal of *POU3f2* leads to an exclusive and strong reduction of long-range inhibitory neurons in the cortex. Interestingly, the temporal dynamics of long-range cell loss in post-mitotic removal of *POU3f2* only occur at second postnatal week, highlighting that *POU3f2* have both roles in neuronal specification and fate maintenances. Our work is the first to provide molecular insights toward the development and assembly of this specialized brain circuits.

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CELL-TYPE-SPECIFIC PLASTICITY OF CORTICAL NEURON FATE

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The somatosensory cortex is composed of a diversity of specialized neurons with specific connectivities, location, morphologies and molecular identities. Following their birth, these distinct neuronal identities emerge from interactions between intrinsic genetic programs and environmental cues. A proper balance between intrinsic developmental programs and external signals is essential for the differentiation and assembly of neurons into circuits, yet the cell-type specific contribution of these two types of processes during cortical development remains unclear. To determine to which extent the acquisition of distinct neuronal identity is driven by environmental determinants during the early postnatal critical period, we followed the developmental trajectories of newborn somatosensory neurons *in vivo* and compared them with *in vitro* models, where newborn neurons are deprived from their original environment. To do so, we microdissected and cultured mouse somatosensory cortex at E16, and compared transcriptional signatures, electrophysiological and connectivity features between *in vivo* neurons and their *in vitro* counterparts. We took advantage of high throughput single nucleus RNA sequencing technology and deep neuronal network-based machine learning approaches to classify and follow the identity of *in vivo* and *in vitro* neurons. Together, our results provide a cell-type specific account of environmental sensitivity in different types of cortical neurons.

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BOARD NUMBER: S03-177

CORTICAL HETEROTOPIAS IMPACT ON SINGLE NEURON IDENTITY AND CONNECTIVITY

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The mammalian brain develops along a well-defined timeline. Neocortical neurons are born sequentially during embryonic development after the division of apical progenitors (APs) located in germinal zones, close to the ventricles. Migrating neurons use AP radial processes as a scaffold in order to reach the cortical plate, where they organise in six layers and form functional circuits. Neuronal migration defects can lead to adverse consequences, ranging from minor displacement of neurons to an important remodelling of the cortical cyto-architecture, such as cortical heterotopias. Beside structural impairments, defects also trigger connectivity alterations, leading to epilepsy and intellectual disability. Recently, in humans but also mouse and rat models, mutations in genes coding for the microtubule associated protein Eml1 and the GTPase RhoA were shown to give rise to heterotopias. In these cases, AP primary cilia and radial processes were identified as key alterations in the development of such major anatomical perturbations. How cortical malformations affect neuronal maturation, identity and connectivity remains largely unknown. Here, by combining single nuclei RNA sequencing, viral tracings and high-throughput single-cell connectivity analysis, we observed an overall conservation of neuronal molecular identities but specific alterations of superficial layer neuron circuits. These findings highlight neuron-type specific alterations in the connectivity of neurons in cortical malformations.

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BOARD NUMBER: S03-178

MAP OF DOPAMINE 1- AND 2-RECEPTOR POSITIVE CELLS IN THE DEVELOPING MOUSE FOREBRAIN

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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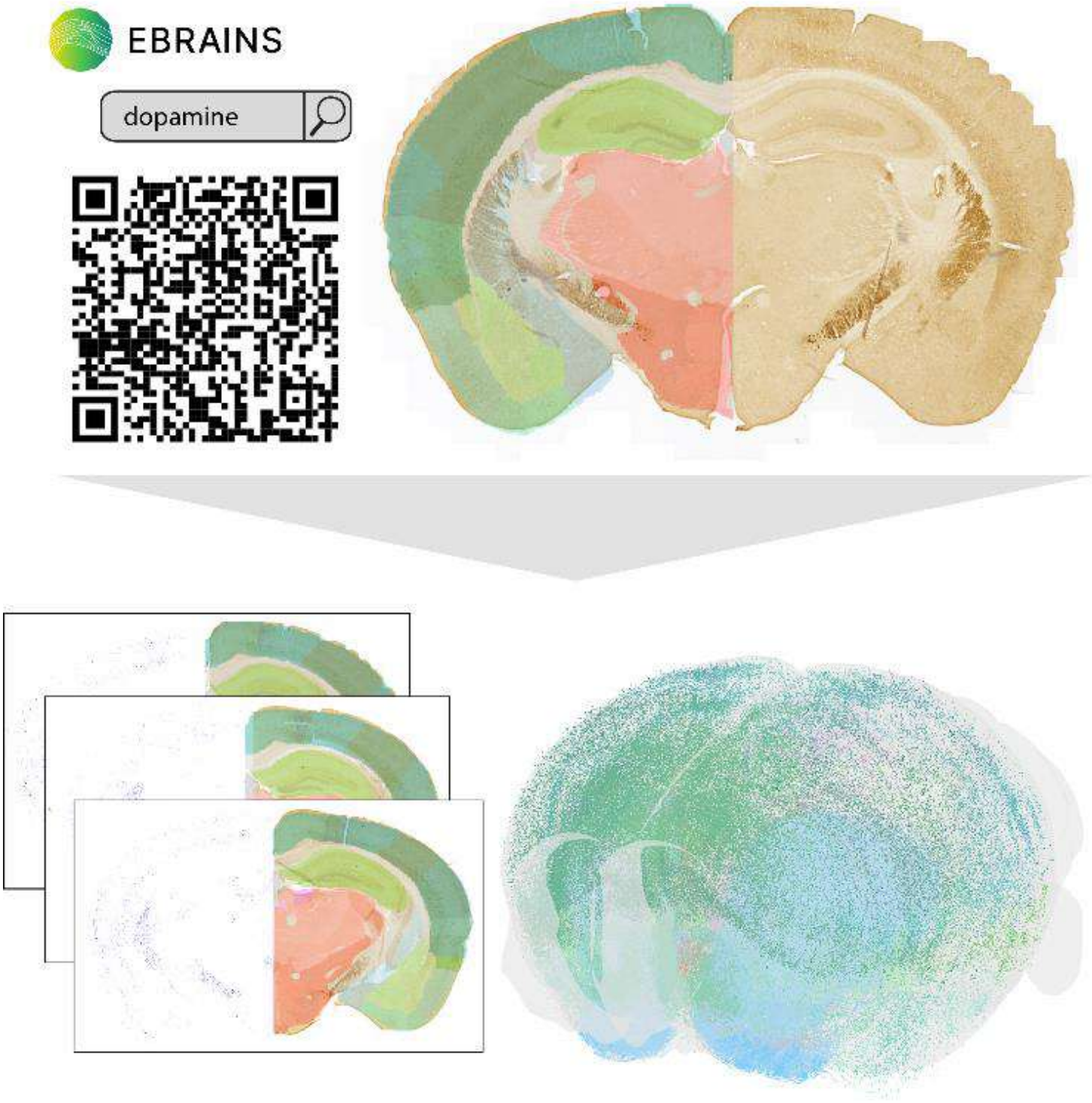
The dopaminergic system undergoes major developments during adolescence, a period especially vulnerable to mental disorders. Dopaminergic cells are primarily located in the substantia nigra and ventral tegmental area, reaching parts of the forebrain through axonal projections. Forebrain neurons expressing dopamine 1 and 2 receptors (D1R and D2R, respectively) are particularly sensitive to dopaminergic input, and contribute to higher cognitive functions such as attention, goal-directed behaviour, and reward processing. Knowledge about the typical development of the dopaminergic system is required to understand its impact on the ontogeny of different mental disorders, but such information is sparse and scattered across publications investigating one or at most a few brain regions. We have digitized and organized microscopic images of immunostained sections from 152 male and female mice at five stages of development (P17, P25, P35, P49, and adult), showing D1R and D2R expressing neurons across the forebrain. All images are registered to the Allen Mouse brain Common Coordinate Framework, for the P17-P35 age groups spatially modified to match the morphology of young brains. The high-resolution images are shared via an interactive web-microscopy viewer and as downloadable files via the EBRAINS Knowledge Graph. Spanning five age groups and including both males and females, the DOPAMAP data enables quantitative analysis of cells across sex, age, and genotype. The collection is an important resource for researchers interested in studying forebrain regions with D1R and D2R positive cells. Funded from EU Horizon 2020, Specific Grant Agreement No. 945539 (Human Brain Project SGA3) and the HBP voucher

program.



FENS Forum 2022
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BOARD NUMBER: S03-179

EARLY POSTNATAL DISRUPTION OF NEUROKININ RECEPTOR 3 FUNCTION LEADS TO IRREGULAR STRIATAL CHOLINERGIC ACTIVITY AND AUTISTIC-LIKE BEHAVIOURS.

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Molecular control of interneuron activity is critical during striatal development and perturbing this can lead to neurodevelopmental conditions, such as autism spectrum disorder (ASD). One molecule that has been functionally linked to ASD is the neuromodulator Neurokinin receptor 3 (NK3R). While NK3R is known to be activity-dependent and to increase firing in some adult striatal cholinergic interneurons (CINs), its developmental function is unknown. To address this, we aimed to understand how the expression and activity of NK3R shapes striatal maturation. Using western blotting, qRT-PCR and immunohistochemistry, we revealed high expression of NK3R in CINs during postnatal days 6-10 (P6-10), an important period for striatal cellular and network development. Concurrently, we performed in-vitro patch clamp recordings and found that compared to adult, P6-10 acute NK3R activation with the specific agonist Senktide exacerbates spontaneous CIN firing. We hypothesised that altering NK3R activity through this period would significantly modify CIN physiology. Following blockade of NK3R with the specific antagonist Osanetant, we revealed retention of immature firing patterns and altered synaptic inputs onto adult CINs. Accompanying this were striatum-related autistic-like behaviours including deficits in social interactions and increased repetitive behaviours. To further investigate the specific cellular underpinnings of these alterations, we have engineered CRISPR-edited NK3R conditional knockout mice which we are currently investigating. Overall, our results identify for the first time a novel critical period for NK3R function to shape CIN maturation and striatum-related behaviours. Further exploration of the early role of NK3R will provide important insights into ASD pathophysiology.

Pubmed:
33867115: Al Abed AS, Reynolds NJ, Dehorter N
A Second Wave for the Neurokinin Tac2 Pathway in Brain Research.
Despite promising advances in basic research of the neurokinin B/Tac2 pathway in both animals and humans, clinical applications are yet to be implemented. This is likely because of our limited understanding of the action of the pathway in the brain. While this system controls neuronal activity in multiple regions, the precise impact of Tac2-induced cellular responses on behavior remains unclear. Recently, elegant studies revealed a key contribution to stress-related behaviors and memory. Here, we discuss the crucial importance of bridging the gap between the Tac2 pathway's involvement in cell physiology and cognition to comprehend its role in health and disease. We propose that a better understanding of the Tac2 pathway in the brain could provide an essential perspective for basic investigations, which in turn will feed clinical research.
Biol Psychiatry, 2021; 90

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-180

REGIONAL CHANGES IN DENSITY AND SPATIAL DISTRIBUTION OF CALBINDIN- AND PARVALBUMIN EXPRESSING NEURONS IN THE DEVELOPING MOUSE BRAIN

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The brain undergoes major reorganization and development during adolescence, involving dynamic changes in the number and distribution of various cell types. Neurons expressing the calcium binding proteins parvalbumin and calbindin are widely distributed across the adult brain, and during development they contribute to the maturation of neural networks underlying a range of functions, e.g. fear memory processing and social behavior. However, information about the number and distribution of calbindin and parvalbumin cells in the developing mouse brain is fragmented, with most studies investigating only one or a few brain regions. We here present a comprehensive collection of microscopic image data from the developing mouse brain (postnatal days 9, 14, 21, and 35), immunohistochemically stained for calbindin and parvalbumin. Images were spatially registered to age-specific volumetric brain atlases, allowing quantification of calbindin and parvalbumin cell numbers using the QuickNII-ilastik-Nutil (QUINT) workflow provided by the EBRAINS research infrastructure (https://ebrains.eu/services/atlases). Our study focuses on areas of the mouse brain associated with social behavior (including the hypothalamus and insular cortex) and fear memory (including the basolateral amygdala, pre-, and infralimbic cortex). However, all data resulting from the project will be publicly shared through the EBRAINS research infrastructure, thus allowing researchers to investigate regional changes in mouse brain architecture through adolescence across the entire brain. Funded from EU Horizon 2020, Specific Grant Agreement No. 945539 (Human Brain Project SGA3). Rogulina and Overdevest contributed equally to this work and are co-presenting authors.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-181

DISTRIBUTION AND DEVELOPMENTAL-BASED CLASSIFICATION OF CRF NEURONS IN THE CHICKEN CENTRAL EXTENDED AMYGDALA

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Understanding the neural regulation of stress is essential to know how different animals respond to stressors and should be considered to improve animal welfare. In mammals, the central extended amygdala (EAce) is a major controller of the activation of the hypothalamic-pituitary-adrenal axis and the autonomic nervous system during stress response. Corticotropin releasing factor (CRF) is a key neuropeptide of this network, being involved in anxiety responses, and is present in a neuron subpopulation of mammalian EAce. Our group used an evolutionary developmental biology approach to study EAce in the chicken, identifying several areas and cell populations likely homologous to those of mammalian EAce. We aimed to understand the CRF cells of chicken EAce within this developmental context. We study their distribution at prehatching stages. To know their embryonic origin, we performed triple labelling studies, combining fluorescent in situ hybridization (CRF mRNA) with immunofluorescence using different region-specific transcription factors. We found that CRF neurons are spread through several EAce areas, with a significant population in lateral bed nucleus of the stria terminalis (BSTL) and smaller groups more laterally in EAce. All these neurons co-express Foxg1 meaning that they are of telencephalic origin. In addition, CRF cells of EAce included different subpopulations, such as cells co-expressing Pax6 derived from the dorsal striatal division, cells co-expressing Islet1 derived from the ventral striatal division and cells co-expressing Nkx2.1 derived from the pallidal division. Our results open new venues to study the implication of CRF cells with different embryonic origins in stress.
(Funding: H2020-MSCA-ITN No.812777)

Pubmed:
31114510: Lorenzi E, Pross A, Rosa-Salva O, Versace E, Sgadò P, Vallortigara G
Embryonic Exposure to Valproic Acid Affects Social Predispositions for Dynamic Cues of Animate Motion in Newly-Hatched Chicks.
Early predispositions to preferentially orient toward cues associated with social partners have been documented in several vertebrate species including human neonates and domestic chicks. Human newborns at high familiar risk of Autism Spectrum Disorder (ASD) show differences in their attention toward these predisposed stimuli, suggesting potential impairments in the social-orienting mechanisms in ASD. Using embryonic exposure to valproic acid (VPA) we modeled ASD behavioral deficits in domestic chicks. To investigate social predispositions toward animate motion in domestic chicks, we focused on self-propulsion, using two video-animations representing a simple red circle moving at constant speed (speed-constant) or one that was changing its speed (accelerating and decelerating; speed-change). Using a spontaneous choice test for the two stimuli, we compared spontaneous preferences for stimuli that autonomously change speed between VPA- and vehicle-injected chicks. We found that the preference for speed changes was abolished in VPA-injected chicks compared to vehicle-injected controls. These results add to previous findings indicating similar impairments for static social stimuli and suggest a specific effect of VPA on the development of mechanisms that enhance orienting toward animate stimuli. These findings strengthen the hypothesis of an early impairment of predispositions in the early development of ASD. Hence, early predispositions are a potentially useful tool to detect early ASD symptoms in human neonates and to investigate the molecular and neurobiological mechanisms underlying the onset of this neurodevelopmental disorder.
Front Physiol, 2019; 10

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BOARD NUMBER: S03-182

DYNAMICS AND MECHANISMS OF PROJECTION OF BIFURCATING NEURONS IN DROSOPHILA VISUAL SYSTEM

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Drosophila optic lobe (OL) is one of the largest structures in the *Drosophila* brain. Interestingly, a large number of specialized neurons innervating the OL terminate in the appropriate ganglia in a specific layer, following a consistent neuronal topology- a process known as retinotopy. However, the molecular cues involved in the proper targeting of neurons to their final site remain poorly known. Here we focused our studies on a particular cell type called transmedullary (TmY) neuron. TmYs neurons innervate the medulla ganglia to then project to two other OL ganglia (lobula and lobula plate) through a characteristic neurite bifurcation. Interestingly, their morphology is very similar to Tm neurons with the exception that Tms only target the Lobula. Very little is known about TmY neurons and our goal is to characterize in detail their morphology, their dynamic of projection throughout development as well as the molecular mechanisms underlying their dendritic and axonal bifurcation and pathfinding. We are also studying a specific visual projection neuron termed TmY14, which projects to the central brain in addition to the OL ganglia. To address these questions, we take advantage of the available scRNAseq dataset of the *Drosophila* optic lobe from which we identified several TmY clusters as well as Gal4 and LexA drivers specifically expressed in sub-classes of TmYs. Here we show data on TmY's dynamic of projection during early developmental stages on fixed tissue and live-imaging. We also present results aiming to test the requirement of the lobula plate in TmY's bifurcating process.

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DEVELOPMENTAL DEFICITS IN MGE-DERIVED INTERNEURONS UNDERLIE CIRCUIT MALFORMATION IN A CNTNAP2 KNOCKOUT MOUSE MODEL OF AUTISM SPECTRUM DISORDER

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Interneurons have a fundamental role in maintaining the excitation-inhibition balance in brain structures such as the prefrontal cortex (PFC) and striatum. While interneurons have been shown to play a key role in autism spectrum disorder (ASD) pathophysiology in adults, little is known about their contribution in the developing autistic brain. Taking advantage of the specific expression of the transcription factor Lhx6 (LIM Homeobox domain 6) in interneurons derived from the medial ganglionic eminence (MGE), we aimed to track developing interneurons and elucidate their molecular and physiological alterations in the Cntnap2 knockout mouse model of ASD. Using immunohistochemical staining at various developmental time-points, we observed a temporal shift in the canonical window of developmental cell death in the PFC and striatum, indicating that the developmental trajectory of Lhx6-expressing interneurons in ASD is perturbed. This was associated with changes in markers of cell maturation and activity dependent factors, such as NMDA receptor subunits, metabotropic receptors, and transcription factors, assessed using qRT-PCR and immunohistochemistry. In addition, we explored the resulting intrinsic perturbations to interneuron properties and network activity with electrophysiology and calcium imaging recordings. Overall, this study describes specific physiological, cellular, and molecular alterations in MGE derived interneurons in autism during development. Uncovering the shifted state of this neuronal population will aid in designing new therapeutic strategies targeting these specific cells early in development.

Pubmed:
33849945: Ahmed NY, Ranjbar-Slamloo Y, Al Abed AS, Gao L, Sontani Y, RCom-H'cheo-Gauthier A, Arabzadeh E, Dehorter N
Er81 Transcription Factor Fine-Tunes Striatal Cholinergic Interneuron Activity and Drives Habit Formation.
The molecular mechanisms tuning cholinergic interneuron (CIN) activity, although crucial for striatal function and behavior, remain largely unexplored. Previous studies report that the Etv1/Er81 transcription factor is vital for regulating neuronal maturation and activity. While Er81 is known to be expressed in the striatum during development, its specific role in defining CIN properties and the resulting consequences on striatal function is unknown. We report here that Er81 is expressed in CINs and its specific ablation leads to prominent changes in their molecular, morphologic, and electrophysiological features. In particular, the lack of Er81 amplifies intrinsic delayed-rectifier and hyperpolarization-activated currents, which subsequently alters the tonic and phasic activity of CINs. We further reveal that Er81 expression is required for normal CIN pause and time-locked responses to sensorimotor inputs in awake mice. Overall, this study uncovers a new cell type-specific control of CIN function in the striatum which drives habit formation in adult male mice. Although previous studies have shown that cholinergic interneurons drive striatal activity and habit formation, the underlying molecular mechanisms controlling their function are unknown. Here we reveal that key cholinergic interneuron physiological properties are controlled by Er81, a transcription factor regulating neuronal activity and development in a cell-specific manner. Moreover, our findings uncover a link between the Er81-dependent molecular control of cholinergic interneuron function and habit formation in mice. These insights will contribute to the future enhancement of our understanding of disorders that involve behavioral inflexibility, such as autism and addiction.
J Neurosci, 2021; 41
31551706: Ahmed NY, Knowles R, Dehorter N
New Insights Into Cholinergic Neuron Diversity.
Cholinergic neurons comprise a small population of cells in the striatum but have fundamental roles in fine tuning brain function, and in the etiology of neurological and psychiatric disorders such as Parkinson's disease (PD) or schizophrenia. The

process of developmental cell specification underlying neuronal identity and function is an area of great current interest. There has been significant progress in identifying the developmental origins, commonalities in molecular markers, and physiological properties of the cholinergic neurons. Currently, we are aware of a number of key factors that promote cholinergic fate during development. However, the extent of cholinergic cell diversity is still largely underestimated. New insights into the biological basis of their specification indicate that cholinergic neurons may be far more diverse than previously thought. This review article, highlights the physiological features and the synaptic properties that segregate cholinergic cell subtypes. It provides an accurate picture of cholinergic cell diversity underlying their organization and function in neuronal networks. This review article, also discusses current challenges in deciphering the logic of the cholinergic cell heterogeneity that plays a fundamental role in the control of neural processes in health and disease.
Front Mol Neurosci, 2019; 12

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-184

ANALYSIS OF THE REGULATION OF CORTICAL INTERNEURON MIGRATION BY THE D1 RECEPTOR IN THE MOUSE

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Neurodevelopmental disorders are believed to play a role in the etiology of schizophrenia. Abnormalities in parvalbumin (PV) interneurons have been reported in schizophrenic patients. Although the *Drd1* gene that encodes D1 receptor (D1R) is highly expressed in the developing forebrain, the role of dopamine signaling in cortical interneuron (cIN) development is poorly understood, especially in the context of schizophrenia. To address this question, we cultured wild type (WT) and *Drd1-KO* explants from the region of origin of PV(+) cINs, the medial ganglionic eminence (MGE), on a synthetic permissive substrate of migration (Ncadherin + laminin). We observed that D1R ablation impaired the migration of MGE cells. We then characterized the pattern of expression of D1R during development in a D1-GFP transgenic mouse strain that expresses GFP under the control of the *Drd1* promoter. Because of the strong GFP expression in the developing cortex, we investigated the role of Drd1 expressed in the cortical substrate on the migration of interneurons in co-cultures. Live cell imaging and analyses of fixed material indeed revealed a significant role of D1R expressed in the cortical substrate on the migration of MGE cells. Results identify D1R as a receptor able to regulate the migration and final distribution of MGE-derived cINs to the cortex in both cell autonomous and non-cell autonomous ways and invite to analyze the number and distribution of cIN at both embryonic and adult stages, in WT and Drd1-KO animals.

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BOARD NUMBER: S03-185

PROBABILITY-DEPENDENT ANTICIPATORY EYE MOVEMENTS ACROSS DEVELOPMENT

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Humans exploit regularities in their environment to efficiently anticipate and react to expected events. In healthy adults, regularities in a visual object's motion can elicit anticipatory smooth eye movements (ASEM) towards the expected movement direction, with ASEM velocity proportional to direction probability. Such exquisite adaptive behaviour relies on the efficient integration of probabilistic information across different time scales, and on the transformation of this memory signal into the appropriate motor command. Both these functions are plausibly dependent on the cortical pre-frontal network, which is known to complete its maturation around 25 years. Here we investigated whether probability-dependent ASEM are already present during childhood and adolescence. We recorded eye movements in a large group of participants (N=109, 7-21 years old), instructed to accurately track a small target moving horizontally. The probability *p* of target motion direction (Right/Left) was changed at different moments during the session. We report robust probability-dependent ASEM for all subjects, including at the youngest age. However, the *sensitivity* to the probability bias (as quantified by the slope of the linear regression of ASEM-velocity upon *p*) increases significantly with age. Importantly, this difference cannot be attributed to a general lower performance in smooth eye tracking at early stages of development, as all the parameters of visually-guided eye movements (with the exception of latency) were overall comparable across participants. We speculate that the children's lower sensitivity to the environment's regularities for visuomotor adaptation might be related to a less precise (*noisier*) information-processing pipeline, rather than to oculomotor control deficits.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-186

LONG-TERM FUNCTIONAL AND CYTOARCHITECTONIC EFFECTS OF THE SYSTEMIC ADMINISTRATION OF THE HISTAMINE H1 RECEPTOR ANTAGONIST/INVERSE AGONIST CHLORPHENIRAMINE DURING GESTATION IN THE RAT OFFSPRING MOTOR CORTEX

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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[1]Centro de Estudios Avanzados del Instituto Politécnico Nacional, Fisiología Biofísica Y Neurociencias, Mexico City, Mexico, [2]Instituto Nacional de Perinatología, Fisiología Y Desarrollo Celular, Mexico City, Mexico, [3]Centro de Estudios Avanzados del Instituto Politécnico Nacional. Sede Sur, Farmacobiología, Mexico City, Mexico

The transient histaminergic system is among the first neurotransmitter systems to appear during development in the rat brain. Histamine increases FOXP2 deep-layer neuron differentiation of cortical neural stem cells through H1R activation *in vitro*. The *in utero* or systemic administration of chlorpheniramine (H1R antagonist/inverse agonist) during deep-layer cortical neurogenesis decreases FOXP2 neurons in the developing cortex. Due to the role of H1R in cortical neurogenesis, the purpose of this study was to evaluate the postnatal impact of the systemic administration of chlorpheniramine during deep-layer cortical neuron differentiation (E12–14) in the primary motor cortex of neonates (P0) and 21-day-old pups (P21). Chlorpheniramine or vehicle was systemically administered (5 mg/kg, i.p.) to pregnant Wistar rats at gestational days 12–14, and the expression and distribution of deep (FOXP2 and TBR1) and superficial-layer (SATB2) neuronal cortical markers were analyzed in neonates from both groups. The qRT-PCR analysis revealed a reduction in the expression of Satb2 and FoxP2. However, Western blot and immunofluorescence showed increased protein levels in the chlorpheniramine-treated group. In P21 pups, the three markers showed impaired distribution and increased immunofluorescence in the experimental group. The Sholl analysis evidenced altered dendritic arborization of deep-layer neurons, with lower excitability in response to histamine, as evaluated by whole-cell recordings, as well as diminished depolarization-evoked [^{3}H]-glutamate release from striatal slices. Overall, these results suggest long-lasting effects of blocking H1Rs during early neurogenesis that may impact the pathways involved in voluntary motor activity and cognition.

Pubmed:
35140581: Valle-Bautista R, Márquez-Valadez B, Herrera-López G, Griego E, Galván EJ, Díaz NF, Arias-Montaño JA, Molina-Hernández A
Long-Term Functional and Cytoarchitectonic Effects of the Systemic Administration of the Histamine H Receptor Antagonist/Inverse Agonist Chlorpheniramine During Gestation in the Rat Offspring Primary Motor Cortex.
The transient histaminergic system is among the first neurotransmitter systems to appear during brain development in the rat mesencephalon/rhombencephalon. Histamine increases FOXP2-positive deep-layer neuron differentiation of cortical neural stem cells through H receptor activation . The or systemic administration of chlorpheniramine (H receptor antagonist/inverse agonist) during deep-layer cortical neurogenesis decreases FOXP2 neurons in the developing cortex, and HR- or histidine decarboxylase-knockout mice show impairment in learning and memory, wakefulness and nociception, functions modulated by the cerebral cortex. Due to the role of HR in cortical neural stem cell neurogenesis, the purpose of this study was to evaluate the postnatal impact of the systemic administration of chlorpheniramine during deep-layer cortical neuron differentiation (E12-14) in the primary motor cortex (M1) of neonates (P0) and 21-day-old pups (P21). Chlorpheniramine or vehicle were systemically administered (5 mg/kg, i.p.) to pregnant Wistar rats at gestational days 12-14, and the expression and distribution of deep- (FOXP2 and TBR1) and superficial-layer (SATB2) neuronal cortical markers were analyzed in neonates from both groups. The qRT-PCR analysis revealed a reduction in the expression of Satb2 and FoxP2. However, Western blot and immunofluorescence showed increased protein levels in the chlorpheniramine-treated group. In P21 pups, the three markers showed impaired distribution and increased immunofluorescence in the experimental group. The Sholl analysis evidenced altered dendritic arborization of deep-layer neurons, with lower excitability in response to histamine, as evaluated by whole-cell patch-clamp recording, as well as diminished depolarization-evoked [H]-glutamate release from striatal slices. Overall, these results suggest long-lasting effects of blocking HRs during early neurogenesis that may impact the pathways involved in voluntary motor activity and cognition.

Front Neurosci, 2021; 15
33042999: Valle-Bautista R, Márquez-Valadez B, Fragoso-Cabrera AD, García-López G, Díaz NF, Herrera-López G, Griego E, Galván EJ, Arias-Montaño JA, Molina-Hernández A
Impaired Cortical Cytoarchitecture and Reduced Excitability of Deep-Layer Neurons in the Offspring of Diabetic Rats.
Maternal diabetes has been related to low verbal task scores, impaired fine and gross motor skills, and poor performance in graphic and visuospatial tasks during childhood. The primary motor cortex is important for controlling motor functions, and embryos exposed to high glucose show changes in cell proliferation, migration, and differentiation during corticogenesis. However, the existing studies do not discriminate between embryos with or without neural tube defects, making it difficult to conclude whether the reported changes are related to neural tube defects or other anomalies. Furthermore, postnatal effects on central nervous system cytoarchitecture and function have been scarcely addressed. Through molecular, biochemical, morphological, and electrophysiological approaches, we provide evidence of impaired primary motor cerebral cortex lamination and neuronal function in pups from diabetic rats, showing an altered distribution of SATB2, FOXP2, and TBR1, impaired cell migration and polarity, and decreased excitability of deep-layer cortical neurons, suggesting abnormalities in cortico-cortical and extra-cortical innervation. Furthermore, phase-plot analysis of action potentials suggests changes in the activity of potassium channels. These results indicate that high-glucose insult during development promotes complex changes in migration, neurogenesis, cell polarity establishment, and dendritic arborization, which in turn lead to reduced excitability of deep-layer cortical neurons.
Front Cell Dev Biol, 2020; 8
30483218: Márquez-Valadez B, Valle-Bautista R, García-López G, Díaz NF, Molina-Hernández A
Maternal Diabetes and Fetal Programming Toward Neurological Diseases: Beyond Neural Tube Defects.
The purpose of this review was to search for experimental or clinical evidence on the effect of hyperglycemia in fetal programming to neurological diseases, excluding evident neural tube defects. The lack of timely diagnosis and the inadequate control of diabetes during pregnancy have been related with postnatal obesity, low intellectual and verbal coefficients, language and motor deficits, attention deficit with hyperactivity, problems in psychosocial development, and an increased predisposition to autism and schizophrenia. It has been proposed that several childhood or adulthood diseases have their origin during fetal development through a phenomenon called fetal programming. However, not all the relationships between the outcomes mentioned above and diabetes during gestation are clear, well-studied, or have been related to fetal programming. To understand this relationship, it is imperative to understand how developmental processes take place in health, in order to understand how the functional cytoarchitecture of the central nervous system takes place; to identify changes prompted by hyperglycemia, and to correlate them with the above postnatal impaired functions. Although changes in the establishment of patterns during central nervous system fetal development are related to a wide variety of neurological pathologies, the mechanism by which several maternal conditions promote fetal alterations that contribute to impaired neural development with postnatal consequences are not clear. Animal models have been extremely useful in studying the effect of maternal pathologies on embryo and fetal development, since obtaining central nervous system tissue in humans with normal appearance during fetal development is an important limitation. This review explores the state of the art on this topic, to help establish the way forward in the study of fetal programming under hyperglycemia and its impact on neurological and psychiatric disorders.
Front Endocrinol (Lausanne), 2018; 9

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BOARD NUMBER: S03-187

EYE MOVEMENTS BUT NOT VISUAL EXPERIENCE DRIVES THE DEVELOPMENT OF PALISADE ENDINGS

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Aims: Palisade endings are a peculiar nerve structure, which are regularly present in extraocular muscles (EOMs) of frontal-eyed species (human, monkey, cat, dog, ferret). In a previous study, we have shown in cat that palisade endings develop after birth in a heterochronic sequence. Here we tested whether external influences like visual experience and/or eye movements guide the development of palisade endings. **Methods**: Both eyes of newborn cats (n=3) were covered for a period of 45 days and the right eye of newborn cats (n=3) was covered for 30 days. Seven cats received a Botulinum toxin-A (BoNT-A) injection at birth into the retrobulbar space of the left orbit. The survival time was 45 or 95 days. EOM whole mount preparations were triple-immunolabeled with anti-neurofilament, anti-synaptophysin or anti-growth associated protein 43 (GAP43), and phalloidin. Analyses were done in the confocal laser-scanning microscope. **Results:** After bilateral/unilateral light deprivation, palisade endings were present in all rectus muscles and they were qualitatively and quantitatively equal to control palisade endings. After BoNT-A treatment for a period of 45 days, palisade endings were only present in the inferior and medial rectus, and not until the age of 95 days, palisade endings were observed in all rectus muscles. Additionally, following BoNT-A administration the number of palisade endings was reduced in all rectus muscles. There was no difference in the GAP43 expression between palisade endings after BoNT-A treatment and untreated palisade endings. **Conclusions:** Our study demonstrates that eye immobilization but not dark rearing affects the development of palisade endings.

Pubmed:
34675089: Carrero-Rojas G, Hernández RG, Blumer R, de la Cruz RR, Pastor AM
MIF versus SIF Motoneurons, What Are Their Respective Contribution in the Oculomotor Medial Rectus Pool?
Multiply-innervated muscle fibers (MIFs) are peculiar to the extraocular muscles as they are non-twitch but produce a slow build up in tension on repetitive stimulation. The motoneurons innervating MIFs establish en grappe terminals along the entire length of the fiber, instead of the typical en plaque terminals that singly-innervated muscle fibers (SIFs) motoneurons establish around the muscle belly. MIF motoneurons have been proposed to participate only in gaze holding and slow eye movements. We aimed to discern the function of MIF motoneurons by recording medial rectus motoneurons of the oculomotor nucleus. Single-unit recordings in awake cats demonstrated that electrophysiologically-identified medial rectus MIF motoneurons participated in different types of eye movements, including fixations, rapid eye movements or saccades, convergences, and the slow and fast phases of the vestibulo-ocular nystagmus, the same as SIF motoneurons did. However, MIF medial rectus motoneurons presented lower firing frequencies, were recruited earlier and showed lower eye position (EP) and eye velocity (EV) sensitivities than SIF motoneurons. MIF medial rectus motoneurons were also smaller, had longer antidromic latencies and a lower synaptic coverage than SIF motoneurons. Peristimulus time histograms (PSTHs) revealed that electrical stimulation to the myotendinous junction, where palisade endings are located, did not recurrently affect the firing probability of medial rectus motoneurons. Therefore, we conclude there is no division of labor between MIF and SIF motoneurons based on the type of eye movement they subserve. In addition to the common singly-innervated muscle fiber (SIF), extraocular muscles also contain multiply-innervated muscle fibers (MIFs), which are non-twitch and slow in contraction. MIF motoneurons have been proposed to participate only in gaze holding and slow eye movements. In the present work, by single-unit extracellular recordings in awake cats, we demonstrate, however, that both SIF and MIF motoneurons, electrophysiologically-identified, participate in the different types of eye movements. However, MIF motoneurons showed lower firing rates (FRs), recruitment thresholds, and eye-related sensitivities, and could thus contribute to the fine adjustment of eye movements. Electrical stimulation of the myotendinous junction activates antidromically MIF motoneurons but neither MIF nor SIF motoneurons receive a synaptic reafferentation that modifies their discharge probability.
J Neurosci, 2021; 41

33369640: Blumer R, Streicher J, Carrero-Rojas G, Calvo PM, de la Cruz RR, Pastor AM
Palisade Endings Have an Exocytotic Machinery But Lack Acetylcholine Receptors and Distinct Acetylcholinesterase Activity.
The purpose of this work was to test whether palisade endings express structural and molecular features of exocytotic machinery, and are associated with acetylcholine receptors, and enzymes for neurotransmitter breakdown.
Invest Ophthalmol Vis Sci, 2020; 61
32197508: Carrero-Rojas G, Benítez-Temiño B, Pastor AM, Davis López de Carrizosa MA
Muscle Progenitors Derived from Extraocular Muscles Express Higher Levels of Neurotrophins and their Receptors than other Cranial and Limb Muscles.
Extraocular muscles (EOMs) show resistance to muscle dystrophies and sarcopenia. It has been recently demonstrated that they are endowed with different types of myogenic cells, all of which present an outstanding regenerative potential. Neurotrophins are important modulators of myogenic regeneration and act promoting myoblast proliferation, enhancing myogenic fusion rates and protecting myotubes from inflammatory stimuli. Here, we adapted the pre-plate cell isolation technique to obtain myogenic progenitors from the rat EOMs, and quantified their in vitro expression of neurotrophins and their receptors by RT-qPCR and immunohistochemistry, respectively. The results were compared with the expression on progenitors isolated from buccinator, tongue and limb muscles. Our quantitative analysis of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin-3 (NT-3) transcripts showed, for the first time, that EOMs-derived cells express more of these factors and that they expressed TrkA, but not TrkB and TrkC receptors. On the contrary, the immunofluorescence analysis demonstrated high expression of p75 on all myogenic progenitors, with the EOMs-derived cells showing higher expression. Taken together, these results suggest that the intrinsic trophic differences between EOMs-derived myogenic progenitors and their counterparts from other muscles could explain why those cells show higher proliferative and fusion rates, as well as better regenerative properties.
Cells, 2020; 9
32189115: Silva-Hucha S, Carrero-Rojas G, Fernández de Sevilla ME, Benítez-Temiño B, Davis-López de Carrizosa MA, Pastor AM, Morcuende S
Sources and lesion-induced changes of VEGF expression in brainstem motoneurons.
Motoneurons of the oculomotor system show lesser vulnerability to neurodegeneration compared to other cranial motoneurons, as seen in amyotrophic lateral sclerosis (ALS). The overexpression of vascular endothelial growth factor (VEGF) is involved in motoneuronal protection. As previously shown, motoneurons innervating extraocular muscles present a higher amount of VEGF and its receptor Flk-1 compared to facial or hypoglossal motoneurons. Therefore, we aimed to study the possible sources of VEGF to brainstem motoneurons, such as glial cells and target muscles. We also studied the regulation of VEGF in response to axotomy in ocular, facial, and hypoglossal motor nuclei. Basal VEGF expression in astrocytes and microglial cells of the cranial motor nuclei was low. Although the presence of VEGF in the different target muscles for brainstem motoneurons was similar, the presynaptic element of the ocular neuromuscular junction showed higher amounts of Flk-1, which could result in greater efficiency in the capture of the factor by oculomotor neurons. Seven days after axotomy, a clear glial reaction was observed in all the brainstem nuclei, but the levels of the neurotrophic factor remained low in glial cells. Only the injured motoneurons of the oculomotor system showed an increase in VEGF and Flk-1, but such an increase was not detected in axotomized facial or hypoglossal motoneurons. Taken together, our findings suggest that the ocular motoneurons themselves upregulate VEGF expression in response to lesion. In conclusion, the low VEGF expression observed in glial cells suggests that these cells are not the main source of VEGF for brainstem motoneurons. Therefore, the higher VEGF expression observed in motoneurons innervating extraocular muscles is likely due either to the fact that this factor is more avidly taken up from the target muscles, in basal conditions, or is produced by these motoneurons themselves, and acts in an autocrine manner after axotomy.
Brain Struct Funct, 2020; 225

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ABERRANT SURVIVAL OF CAJAL-RETZIUS CELLS LEADS TO MEMORY DEFICITS AND SUSCEPTIBILITY TO EPILEPTIC SEIZURES

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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Cajal-Retzius cells (CRs) are transient neurons playing key roles during cortical development. Unlike most neurons, they almost completely disappear by programmed cell death (PCD) during the first postnatal weeks in mice. Abnormal survival of CRs is detected in pathological conditions exhibiting epilepsy, suggesting that their persistence at postnatal stages might hamper cortical functions. Here, using a mouse model preventing PCD of a CR subtype, we evaluate the functional impact of CRs persistence in the establishment of adult cognitive and neuronal functions. While no differences were detected in juvenile animals, we observed an impairment of hippocampus-dependent behaviors in adults. Both male and female mutants showed deficits in novel object recognition and spatial learning but not in associative memory formation. At the cellular level, we detected enhanced survival of CRs in both juvenile and adult mice, as well as an increased number of NPY^+ GABAergic cells and spine density of CA1 pyramidal neurons. At the synaptic level, this was associated with an enhanced frequency of inhibitory currents in adult animals. Moreover, adult mutants displayed an increased susceptibility to develop lethal tonic-clonic seizures upon kainate injection and a switch of the activity pattern from bursting to seizures in cortico-hippocampal slices. Finally, multi-site recordings from the dorsal hippocampus confirmed stronger gamma activity in mutants, together with the sporadic presence of sharp (10-15 ms) interictal-like spikes confined to the CA1 region. These results show that aberrant survival of even a small proportion of CRs results in alteration of behaviour and epilepsy prone phenotypes in adults.

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FUNCTIONAL DEVELOPMENT OF THE MOUSE FRONTO-STRIATAL NETWORK

POSTER SESSION 03 - SECTION: DEVELOPMENT OF CORTICAL CIRCUITRY

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The prefrontal cortex (PFC) acts as a hub of complex cortico-subcortical networks accounting for higher cognitive processing. For example, behavioral flexibility has been reported to relay on fronto-striatal communication. The PFC monosynaptically projects to the striatum (STR) which, in turn, is connected to the PFC through the thalamus, thereby forming a multi-synaptic loop. Despite the critical function of this circuit in adult brain, little is known about its structural and functional development. To fill this knowledge gap, we combined retrograde neural tracing and in vivo extracellular recordings from the PFC and STR of mouse pups of 5 to 12 days of age. Already during the first postnatal week, prefrontal afferents target the STR. In both areas, single unit spiking activity (SUA) and the broadband local field potential (LFP) power exponentially increased over age. The fronto-striatal coupling through synchrony was tight as mirrored by the high values of imaginary coherence in theta frequency range. Directionality analysis revealed that, in contrast to the ingrowth of projections, the STR drives the PFC during early development and the driving force decreases with age. These results give first insights into the maturation of fronto-striatal communication and represent the substrate for further dissection of circuits in relationship to the behavioral development.

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LEARNING-EVOKED CHANGES IN INTRINSIC EXCITABILITY OF GABAERGIC INTERNEURONS IN THE SOMATOSENSORY CORTEX OF MICE.

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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The GABAergic interneurons can be divided into three subclasses: somatostatin- (SST), parvalbumin- (PV), and ionotropic serotonin 5HT3a receptor-expressing (5HT3aR) cells. The last group is further divided into vasoactive intestinal polypeptide-expressing (VIP) and non-VIP-expressing cells. Several areas of evidence suggest that GABAergic interneurons are essential for basic information flow in the neuronal networks, as well as for learning and memory processing. **Aim** Here, we asked a question whether a simple model of learning in mice changes the intrinsic excitability of SST-, PV- and VIP-interneurons. **Methods** To answer this question we subjected mice to a conditioning paradigm where whiskers tactical stimulation was paired with electric tail shocks or to pseudoconditioning where these stimulations were applied randomly. After the learning procedure, we performed *in vitro* whole-cell patch-clamp recordings in the somatosensory cortex. **Results** We found that conditioning in mice leads to a rise of intrinsic excitability in low-threshold spiking SST-expressing interneurons in layer 4 of the cortical representation of stimulated whiskers. In contrast, we discovered that pseudoconditioning causes a decrease in intrinsic excitability of fast-spiking PV-expressing cells. However, changes in the intrinsic excitability of VIP-expressing interneurons are more complex and are characteristic for the learning paradigm and cell firing phenotype. **Conclusions** Our results indicate that changes in intrinsic excitability of the GABAergic interneurons are a common form of plastic alternations in the inhibitory circuit after various paradigms of learning. These changes may be critical for proper memory function and precision in information streaming through the cortical net. Polish Science Centre grant: UMO-2015/18/E/NZ4/00721

Pubmed:
34599984: Dobrzanski G, Lukomska A, Zakrzewska R, Posluszny A, Kanigowski D, Urban-Ciecko J, Liguz-Lecznar M, Kossut M
Learning-induced plasticity in the barrel cortex is disrupted by inhibition of layer 4 somatostatin-containing interneurons. Gaba-ergic neurons are a diverse cell class with extensive influence over cortical processing, but their role in experience-dependent plasticity is not completely understood. Here we addressed the role of cortical somatostatin- (SOM-INs) and vasoactive intestinal polypeptide- (VIP-INs) containing interneurons in a Pavlovian conditioning where stimulation of the vibrissae is used as a conditioned stimulus and tail shock as unconditioned one. This procedure induces a plastic change observed as an enlargement of the cortical functional representation of vibrissae activated during conditioning. Using layer-targeted, cell-selective DREADD transductions, we examined the involvement of SOM-INs and VIP-INs activity in learning-related plastic changes. Under optical recordings, we injected DREADD-expressing vectors into layer IV (L4) barrels or layer II/III (L2/3) areas corresponding to the activated vibrissae. The activity of the interneurons was modulated during all conditioning sessions, and functional 2-deoxyglucose (2DG) maps were obtained 24 h after the last session. In mice with L4 but not L2/3 SOM-INs suppressed during conditioning, the plastic change of whisker representation was absent. The behavioral effect of conditioning was disturbed. Both L4 SOM-INs excitation and L2/3 VIP-INs inhibition during conditioning did not affect the plasticity or the conditioned response. We found the activity of L4 SOM-INs is indispensable in the formation of learning-induced plastic change. We propose that L4 SOM-INs may provide disinhibition by blocking L4 parvalbumin interneurons, allowing a flow of information into upper cortical layers during learning.
Biochim Biophys Acta Mol Cell Res, 2022; 1869

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CHARACTERIZATION OF CONDITIONAL BISTABLE NEURONS, THE PUTATIVE SUBSTRATE UNDERLYING PARAMETRIC WORKING MEMORY IN THE PREFRONTAL CORTEX.

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Parametric working memory (PWM) represents the ability to maintain and manipulate transient quantitative information such as a number, the frequency of a sound or the size of an object. The mechanisms underlying PWM are still poorly understood. *In vivo* experiments in humans, monkeys and rats indicate that PWM involves graded persistent activity (GPA) in the prefrontal cortex (PFC). GPA scales with and outlasts remembered information. Models of recurrent neural networks such as found in the PFC suggest that GPA requires conditional bistability (CB). CB is a form of cellular memory that depends on sustained sub-threshold network inputs to memorize a transient input. In a biophysical model, we previously demonstrated that layer V PFC pyramidal neurons may underlie CB. However, the existence and mechanisms of CB still remain unknown in rat PFC circuits. To address these gaps, we used patch-clamp electrophysiology in acute PFC slices from adult rats. We show that **(1)** a subset of PFC layer V pyramidal neurons exhibits CB, **(2)** CB is fine-tuned by acetylcholine (97% of CB neurons in presence of carbachol vs. 14% in control conditions), **(3)** carbachol-induced CB relies on changes in fast and slow afterpolarization potentials mediated through calcium-activated non-selective cationic channels, and **(4)** CB is not modulated by other common neuromodulators such as dopamine, serotonin and noradrenaline. **Uncovering CB, a novel PFC intrinsic neuronal property, and its ionic mechanisms, will be crucial to assess its effective role in the emergence of PWM and other executive functions in frontal cortical areas.**

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ACCELERATED SIGNAL PROPAGATION SPEED IN HUMAN NEOCORTICAL MICROCIRCUITS

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Human-specific cognitive abilities depend on information processing in the cerebral cortex, where neurons are significantly larger and sparser compared to rodents. We found that, in synaptically connected layer 2/3 pyramidal cells, the soma-to-soma signal propagation delay is similar in humans and rodents. Thus, to compensate for the increase in neurons' size, membrane potential changes must propagate faster in human axons and/or dendrites. Using dual somato-dendritic and somato-axonal patch recordings, we found that the propagation speed of somatically-evoked action potentials is similar in human and rat axons but is 1.7-fold faster in human dendrites.

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COOPERATIVE MODULES OF IONIC CURRENTS AND THEIR EFFECT ON ELECTRICAL FEATURES OF BIOPHYSICALLY DETAILED MODELS.

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Neuronal membrane potential is sculptured by numerous ionic currents present in the cell. Different values of neuronal electrical features can be observed from the combination of various numbers of ionic current densities. While electrophysiological experiments provide us with a general understanding of the contribution of currents to certain electrical features, we lack a systematic exploration of the effect of the interplay between ionic currents on neuronal electrical behaviour. However, detailed biophysical models allow us to tackle this issue by unravelling the inner mechanisms of the interactions between currents. In this study, we identify modules of ionic currents and their role in shaping neuronal electrical features using statistical methods and information theory.
Using Monte Carlo Markov Chain methods, we generated thousands of non-identical electrical models with experimentally plausible electrophysiological characteristics for cells with various firing patterns. Based on the obtained parameter and feature value set we used information theory methods to detect interactions between model parameters and features at all orders, beyond pairwise correlations. We show that most features depend on the synergetic or redundant combinations of several parameters. Only a small fraction of the features was influenced by one to two ionic currents while most depended on more than 5 model parameters. In our models, we were able to map the cooperative modules of ionic currents and their relation to the electrical features. Further we aim to assess the stability of the identified modules and their possible relation to the gene expression levels and morphological properties of the cells.

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HIPPOCAMPAL-MEDIAL ENTORHINAL CIRCUIT IS DIFFERENTLY ORGANIZED ALONG THE DORSOVENTRAL AXIS IN RODENTS

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Layer V of the medial entorhinal cortex (MEC) is considered to form the major target of hippocampal outputs. Recent studies show that MEC layer V is divided into two sub-layers: LVa neurons project to telencephalic structures, whereas LVb neurons project locally, forming a recurrent entorhinal-hippocampal loop. Recent studies have shown that in dorsal MEC hippocampal input mainly targets neurons in LVb that have only sparse connections to LVa. This suggests that dorsal MEC might not efficiently convey hippocampal information to the neocortex, challenging current concepts on the hippocampal-neocortical dialogue. In this study, we aimed to identify the main source of hippocampal inputs to MEC LVa neurons by reexamining the hippocampal-MEC projections along the dorsoventral axis in rodents. By using anterograde tracing and in vitro electrophysiology, we show that the organization of hippocampal projections to MEC is markedly different along the dorsoventral axis: dorsal hippocampal projections target mainly LVb neurons in dorsal MEC, whereas ventral projections target LVa neurons in both ventral and dorsal MEC. This contrasts with previous anatomical concepts suggesting that the organization of the entorhinal-hippocampal circuits is relatively identical along the dorsoventral axis. Our findings indicate that ventral hippocampus may play an important role in regulating LVa-mediated entorhinal-neocortical outputs from both dorsal and ventral MEC. These findings may be relevant to understanding the neuronal basis of memory consolidation in hippocampal-entorhinal-cortical networks.

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POSITION DEPENDENT DIFFERENCES IN RESPONSE FEATURES OF INDIVIDUAL CELLS WITHIN THE SAME TYPE- INTRACELLULAR DOUBLE RECORDINGS OF MECHANOSENSORY T CELLS OF THE LEECH

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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University of Oldenburg, Computational Neuroscience, Oldenburg, Germany

The medical leech is known for its precise localization of tactile input to its skin, e.g. in the local bend reflex, a behavior with similar precision as the human fingertip. This behavior is elicited by only a few mechanosensory neurons, including three bilateral pairs of T (touch) cells. All T-cells in each ganglion are coupled via polysynaptic chemical and electrical connections. They are distinguished by their different receptive fields in the skin, and by their soma position within the ganglion, but are usually treated as one homogeneous cell type. We tested whether cells at two different soma positions (T2 and T3) differ in their responses to intracellular electrical stimulation and their mutual synaptic influence. In intracellular double recordings, we recorded and stimulated all possible ipsi- and contralateral combinations of T2 and T3 within the isolated ganglia. We found significant differences: Cells at the position T2 are more excitable than at the position T3. The pre-synaptic T2 cell has a higher spike count, and the postsynaptic T2 cell has a higher integral, amplitude, and length of post synaptic potentials. Additionally, only for cells at soma position T3 the postsynaptic potential features are significantly larger for ipsilateral than for contralateral inputs. Hence, this well-studied cell type falls into two different classes correlated with the soma position, indicating potential differences in ion channel properties and in synaptic interactions with the network via interneurons. The mutual feedback by these network interactions could play a major role in the leech's surprisingly precise sensory information processing.

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THE SOUND OF SILENCE – ELECTROPHYSIOLOGICAL COMPARISON OF THREE CELL TYPES

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Some neuronal cell types react to external stimuli in a flexible way by changing their response properties over time. This was recently shown for the mechanosensory touch (T-) cells in the medicinal leech. Previous studies reported that repeated intracellular current injections in T-cells lead to a hyperpolarization of the resting membrane potential over time and an increase in spike count. Here, we investigated if these changes require high spike activity and if they also occur in other leech neurons, the mechanosensory pressure (P-) cell and the neurosecretory Retzius (Rz) cell. For this purpose, an intracellular current stimulation protocol was established, consisting of a single 1 nA current pulse of 500 ms duration that was applied every 5 minutes. During this extended time without stimulation, the spike activity developed in a cell-type specific way. In P- and T-cells, the spike count elicited by the repeated current pulse raised unexpectedly, even though no spikes were elicited during the long absence of stimulation between the pulses. In contrast, the spontaneously active Rz-cells decreased the number of spikes in response to the repeated current pulses. From that, we can conclude two things: First, the changes in excitability are cell-type specific with the two mechanosensory cell types (T and P) behaving similarly, but different from the Rz cells. Second, the increase in spike count in T- and P-cells over extended recording periods does not require spike activity between the test pulses.

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NEUROPLASTIN EXPRESSION AND SUBMEMBRANE LOCALIZATION ARE AFFECTED BY MEMBRANE GANGLIOSIDE COMPOSITION

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Synaptic glycoprotein neuropastin (Np) is involved in synaptic plasticity and complex molecular events underlying learning and memory formation. Np exists in two isoforms, Np55 and Np65. Being a membrane protein, its function and localization within specific membrane subdomains like lipid rafts (LR) are most probably affected by specific lipid microenvironment. This study aimed to investigate the effect of membrane ganglioside composition on total and brain regional protein expression and submembrane localization of Np using *St8sia1 null* mice with impaired synthesis of gangliosides. Brains of adult wild type (WT) and *null* mice littermates were used. Protein expression of both Np isoforms in homogenates was analyzed by Western blotting. Immunohistochemical staining was performed on frozen brain tissue slices using an antibody specific for both isoforms. LR and non-raft (nLR) fractions from cortices and cerebella were isolated by ultracentrifugation in discontinuous sucrose gradients, and submembrane localization of Np isoforms analyzed by Western blotting. Results revealed statistically lower amounts of Np55 and Np65 in both cortices and cerebella of *null* mice compared to their WTs. Immunohistochemical staining revealed lower intensity of Np staining in the cortex and cerebellar molecular layer of *null* mice compared to WT mice. The amount of both isoforms positioned within cortical LR of *null* mice was statistically lower than those positioned within LR derived from WT animals. Results show that proper ganglioside environment is critical for specific Np functions, indicating novel roles of gangliosides as co-players in molecular basis of cognitive processes.

Pubmed:
34948386: Ilic K, Lin X, Malci A, Stojanović M, Puljko B, Rožman M, Vukelić Ž, Heffer M, Montag D, Schnaar RL, Kalanj-Bognar S, Herrera-Molina R, Mlinac-Jerkovic K
Plasma Membrane Calcium ATPase-Neuroplastin Complexes Are Selectively Stabilized in GM1-Containing Lipid Rafts.
The recent identification of plasma membrane (Ca)-ATPase (PMCA)-Neuroplastin (Np) complexes has renewed attention on cell regulation of cytosolic calcium extrusion, which is of particular relevance in neurons. Here, we tested the hypothesis that PMCA-Neuroplastin complexes exist in specific ganglioside-containing rafts, which could affect calcium homeostasis. We analyzed the abundance of all four PMCA paralogs (PMCA1-4) and Neuroplastin isoforms (Np65 and Np55) in lipid rafts and bulk membrane fractions from GM2/GD2 synthase-deficient mouse brains. In these fractions, we found altered distribution of Np65/Np55 and selected PMCA isoforms, namely PMCA1 and 2. Cell surface staining and confocal microscopy identified GM1 as the main complex ganglioside co-localizing with Neuroplastin in cultured hippocampal neurons. Furthermore, blocking GM1 with a specific antibody resulted in delayed calcium restoration of electrically evoked calcium transients in the soma of hippocampal neurons. The content and composition of all ganglioside species were unchanged in Neuroplastin-deficient mouse brains. Therefore, we conclude that altered composition or disorganization of ganglioside-containing rafts results in changed regulation of calcium signals in neurons. We propose that GM1 could be a key sphingolipid for ensuring proper location of the PMCA-Neuroplastin complexes into rafts in order to participate in the regulation of neuronal calcium homeostasis.
Int J Mol Sci, 2021; 22
32956659: Puljko B, Stojanović M, Ilic K, Maček Hrvat N, Zovko A, Damjanović V, Mlinac-Jerkovic K, Kalanj-Bognar S
Redistribution of gangliosides accompanies thermally induced Na, K-ATPase activity alternation and submembrane localisation in mouse brain.
Biochim Biophys Acta Biomembr, 2021; 1863


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BOARD NUMBER: S03-198

HIPPOCAMPAL OUTPUT PROCESSING IN LAYER VI OF THE MEDIAL ENTORHINAL CORTEX

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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The entorhinal cortex (EC) constitutes a major interface between the hippocampus and neocortex. Sensory information enters the hippocampal formation via superficial layers (II/III) of the EC. In turn, deep layers (V/VI) receive a substantial part of the hippocampal output. Recently, we investigated the processing of hippocampal output signals within layer V of the medial EC (mEC). It is still, however, unknown how hippocampal activity patterns propagate to layer VI (LVI). Here, we performed whole-cell patch-clamp recordings from mEC LVI neurons combined with field potential recordings from the hippocampal CA1 area in horizontal mouse brain slices. Location and morphology of recorded neurons were confirmed by labeling of biocytin-filled cells and immunostaining for Ctip2 (marker for LVb neurons). Morphologically, LVI was mainly found to comprise horizontal pyramidal or multipolar neurons. The axons of LVI neurons travelled towards the subiculum and to superficial layers of the mEC, indicating far-reaching innervation. In the vast majority of LVI glutamatergic neurons electrophysiological recordings revealed a characteristic delayed firing pattern. Reciprocal connections between excitatory LVI neurons in paired recordings were relatively sparse (6/105). To study the propagation of hippocampal output signals to mEC LVI, we injected a ChR2-expressing AAV into the ventral hippocampus (CA1/subiculum) and activated the axons of infected neurons by illuminating LVI with blue light. Most LVI neurons showed strong monosynaptic responses from the ventral hippocampus. Correspondingly, a naturally occurring hippocampal activity pattern, the sharp wave-ripple complex, propagated efficiently to LVI neurons. These findings suggest a critical involvement of mEC LVI in hippocampal-neocortical signal propagation.

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BOARD NUMBER: S03-199

ANATOMICAL AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF HYPOTHALAMIC NEURONS INVOLVED IN FEMALE SEXUAL BEHAVIOR.

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Throughout the reproductive cycle, fluctuating levels of sex hormones coordinate female behavior with their reproductive capacity by modulating the activity of neuronal circuits expressing their specific receptors. The ventrolateral division of the ventromedial hypothalamus (VMHvl) is rich in neurons expressing receptors for sex hormones and its function is intimately linked to female sexual receptivity. However, recent findings suggest that the VMHvl is functionally heterogeneous. To gain insight in the possible underlying causes of such functional heterogeneity, we used whole electrophysiological recordings, viral tracing, and neuronal reconstructions tools to characterize the electrophysiological and anatomical properties of individual VMHvl neurons in naturally cycling females. We found that the properties of progesterone receptor expressing (PR+) neurons, but not PR– neurons, depended systematically on the neuron's location along the anterior-posterior (AP) axis of the VMHvl and the phase within the reproductive cycle. The observations reveal the existence of phenotypes within the PR+ subpopulation, supporting the anatomic and functional subdivision of the VMHvl and its possible role in the orchestration of different aspects of female socio-sexual behavior.

Pubmed:
33879568: Dias IC, Gutierrez-Castellanos N, Ferreira L, Lima SQ
The Structural and Electrophysiological Properties of Progesterone Receptor-Expressing Neurons Vary along the Anterior-Posterior Axis of the Ventromedial Hypothalamus and Undergo Local Changes across the Reproductive Cycle.
Sex hormone levels continuously fluctuate across the reproductive cycle, changing the activity of neuronal circuits to coordinate female behavior and reproductive capacity. The ventrolateral division of the ventromedial hypothalamus (VMHvl) contains neurons expressing receptors for sex hormones and its function is intimately linked to female sexual receptivity. However, recent findings suggest that the VMHvl is functionally heterogeneous. Here, we used whole recordings and intracellular labeling to characterize the electrophysiological and morphologic properties of individual VMHvl neurons in naturally cycling females and report the existence of multiple electrophysiological phenotypes within the VMHvl. We found that the properties of progesterone receptor expressing (PR+) neurons, but not PR- neurons, depended systematically on the neuron's location along the anterior-posterior (AP) axis of the VMHvl and the phase within the reproductive cycle. Prominent among this, the resting membrane potential of anterior PR+ neurons decreased during the receptive phase, while the excitability of medial PR+ neurons increased during the non-receptive phase. During the receptive phase of the cycle, posterior PR+ neurons simultaneously showed an increase in dendritic complexity and a decrease in spine density. These findings reveal an extensive diversity of local rules driving structural and physiological changes in response to fluctuating levels of sex hormones, supporting the anatomic and functional subdivision of the VMHvl and its possible role in the orchestration of different aspects of female socio-sexual behavior.
eNeuro, 2021 May-Jun; 8

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A REALISTIC MULTI-COMPARTMENTAL MODEL OF CEREBELLAR BASKET NEURONS PREDICTS INTRINSIC AND SYNAPTIC RESPONSES

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Aims: cerebellar basket cells (BC), located in the bottom 1/3 of molecular layer, play an important role in controlling the activity of Purkinje cells (PC) via inhibitory synaptic transmission. BCs receive excitatory synaptic inputs from parallel fibers (pf) and transmit inhibitory synaptic inputs to BCs and PCs. We reconstructed a multi-compartmental biophysically realistic BC model in Python-NEURON to investigate the intrinsic and synaptic electrophysiological properties. Methods: 3D morphologies of mouse BC were reconstructed from fluorescent images obtained with a confocal microscope and analyzed with Neurolucida. Ionic channels were located according to immunohistochemistry. The maximum ionic conductances ($G_{i\text{-}max}$) were tuned to match the firing pattern revealed by whole-cell patch-clamp recordings from mice cerebellar slices. BC discharges elicited by step current injections were used as templates to extract the features needed to assess the optimization fitness function. $G_{i\text{-}max}$ tuning was performed using the multi-objective genetic algorithm in the BluePyOpt Library. Results: BC detailed models were generated starting from morphological reconstructions and automatically fitted using electrophysiological recordings as templates. Validated models reproduced whole-cell and loose cell-attached patch-clamp experimental results, showing (1) autorhythmic activity, (2) correct spike shape amplitude, (3) a linear I/O relationship following positive current injections, (4) Afterhyperpolarization (AHP) duration after positive current injections, (5) Sag following negative current injections, (6) Excitatory postsynaptic potentials (EPSPs) following pf inputs. Conclusions: optimization technique gave satisfactory results, reproducing the BC electrophysiological behaviors. The model provided a valuable tool to investigate the molecular layer interneurons function in cerebellar network activity.

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BOARD NUMBER: S03-201

EXPLORING THE DYNAMICS OF NEURONAL EXCITABILITY BY OPTOGENETICS IN EX VIVO CORTICAL CULTURES

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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It is currently still unclear how the excitability of cortical neurons changes dynamically, over distinct timescales and across cell types. We designed a medium-throughput method, combining multisite extracellular recordings with wide-field photoactivation, and targeted specific cortical genetic cell types. We focused on *ShChR/CaMKIIα*-expressing neurons (i.e., putative glutamatergic cells) and asked how they intrinsically respond to very long trains of photo stimuli repeated at different frequencies. We then analyzed spike probability and spike latency as proxies of excitability. In accordance with previous reports (Gal et al., 2010), we observed a rich temporal dynamics of neuronal responses, across a wide range of stimulation frequencies. A transient and an intermittent/chaotic phase occurred, with the latter key for our quantification of a considerable heterogeneity of neuronal responses. Our data challenge the homogeneity often conceptually assumed for the *glutamatergic cell type*, its computation and role within a circuit. However, we found the macroscopic statistical features of evoked responses to be identical, across different stimulation regimes when above a critical stimulation rate. Indeed, the very long-term fluctuations in the spike probability all resemble a *fractional* stochastic process, indicating that intrinsic neuronal activity is significantly affected by past stimuli over unexpectedly broad timescales. All in all, our work is extremely relevant to the development of better quantitative models which are capable of more accurately capturing *in silico* the biological excitability, a) across spatiotemporally diverse electrophysiological types and b) over behaviorally relevant, extended timescales.

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BOARD NUMBER: S03-202

IN VIVO IMPACT OF LOW-INTENSITY REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION ON CORTICAL NEURON EXCITABILITY AND INTEGRATIVE PROPERTIES

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

Manon Boyer[1], Paul Baudin[2], Chloé Stengel[2], Antoni Valero-Cabre[2], Ann Lohof[1], Stéphane Charpier[3], Rachel Sherrard[4], Séverine Mahon[3]
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Repetitive Transcranial Magnetic Stimulation (rTMS), either at classically-used high intensity (in Teslas, T) or sub-excitation low intensity (mT; LI-rTMS), can increase neural circuit plasticity and shows promise in treating human neurological dysfunction. However, the processes activated by magnetic fields at the single neuron level remain largely unknown, preventing the optimization of potentially therapeutic rTMS protocols.
We investigated the impact of continuous focal 10-Hz LI-rTMS on neuronal excitability with in vivo intracellular and electrocorticographic (ECoG) recordings from the primary somatosensory cortex in sufentanil-sedated rats of either sex. Neuronal electrical properties were compared before and after 10 minutes LI-rTMS, so that each neuron was its own control. All experiments were in accord with the European Union directive.
In pyramidal cortical neurons (n=16), this LI-rTMS produced: (1) large hyperpolarization of membrane potential, (2) near complete abolition of spontaneous firing and (3) decreased amplitude of ongoing synaptic events. This altered spontaneous activity and significantly changed neuronal membrane input resistance. Also, the relation linking firing frequency to intensity of injected current (F-I curves) before and after LI-rTMS was modified in all tested neurons. There was a gradual and persistent increase in the threshold of injected current required to fire cortical neurons.
These findings indicate that a short period of continuous 10-Hz LI-rTMS can strongly, and durably, reduce the membrane and synaptic properties of somatosensory cortical neurons. We are currently further investigating the functional outcome of this time-dependent modulation of cortical excitability by analyzing the ECoG patterns and whisker-evoked sensory responses before and after LI-rTMS.

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BOARD NUMBER: S03-203

MICRO-CIRCUITRY AND OUTPUT CONNECTIVITY OF THE STRIOSOME NETWORK

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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AIMS: The striatum constitutes the main input structure of the basal ganglia and is mainly composed of GABAergic medium spiny neurons (MSNs) giving rise to the direct and indirect pathways. An additional compartmentalization of the striatum, consisting in the striosomes and the matrix was described decades ago. The respective network organization and functions of these compartments are much less understood along with the differences in their MSNs intrinsic properties. Our aim is therefore, to assess the intrinsic properties of the striosomal and matrix neurons and to characterize the functional connectivity of these compartments. METHODS: Using a BAC-Cre transgenic mouse combined with stereotactic injections of Cre-dependent viral constructs and patch clamp experiments, we measured the passive and active membrane properties of the striosomal and matrix neurons. Using optogenetics to assess functional connectivity, we obtained light induced post-synaptic responses in several striosome target structures. RESULTS: Striosomal neurons displayed increased spiking and a substantial shift in spike threshold and rheobase when compared to matrix neurons without differences in other properties. Light stimulation induced short term synaptic plasticity of striosomal MSN-GPe synapses. CONCLUSIONS: These results indicate a greater excitability of striosome MSNs compared with matrix MSNs. Previous studies of characterization of striatal MSN-GPe synapses, without specific identification of striosome and matrix MSNs, have reported short term facilitation as a signature in these types of synapses. Our results may suggest the existence of subtle differences in the synaptic dynamic when stimulating exclusively striosomal MSN-GPe synapses.

Pubmed:
29164079: Torres Nupan MM, Velez Van Meerbeke A, López Cabra CA, Herrera Gomez PM
Cognitive and Behavioral Disorders in Children with Neurofibromatosis Type 1.
The last systematic review of research on the behavior of children with neurofibromatosis type 1 (NF1) was in 2012. Since then, several important findings have been published. Therefore, the study aim was to synthesize recent relevant work related to this issue.
Front Pediatr, 2017; 5
28977149: Palacios-Sánchez L, Torres Nupan M, Botero-Meneses JS
James Parkinson and his essay on "shaking palsy", two hundred years later.
In 1817, British physician James Parkinson published a 66-page document entitled "Essay on the Shaking Palsy". This brief text became a classical and fundamental piece in the history of Medicine and, in particular, of neurology. The authors of this article wish to pay tribute to this great pioneer of neurology, 200 years after the publication of his findings, which would, in turn, immortalize his name and give rise to the renaming on the entity in 1860 by Professor Jean Martin Charcot, father of neurology. It would be known, henceforth as Parkinson's disease.
Arq Neuropsiquiatr, 2017; 75

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BOARD NUMBER: S03-204

DIFFERENT FACES OF NEURONS EXPRESSING DOPAMINE RECEPTORS IN MOTOR CORTEX – THEIR LAMINAR DISTRIBUTION, ELECTROPHYSIOLOGICAL PROPERTIES AND ROLE IN SKILLED FORELIMB REACHING

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Motor cortex comprises the primary descending circuits for flexible control of voluntary movements and is critically involved in motor skill learning. However, due to the complexity of motor cortex circuits, precise mechanisms of motor control and skill learning are still not well understood. Here we have used transgenic mice, electrophysiology and neural tract-tracing methods to target genetically defined cell types expressing D1 and D2 dopamine receptors. We observed that D1+ and D2+ neurons are organized in a separate, largely non-overlapping populations, as evidenced by the laminar distribution of their cell bodies, colocalization and projection patterns. Moreover, based on ex vivo patch-clamp recordings we shown that D1+ and D2+ cells have distinct electrophysiological properties. Furthermore, we traced D1+ and D2+ cells dendritic trees and based on prepared 3D models, we compared their complexity using Sholl analysis. Finally, we observed that chemogenetic inhibition of D2+, but not D1+ neurons disrupt skilled forelimb reaching in adult mice. These results suggest that dopamine receptor-expressing cells in motor cortex are organized into separate, non-overlapping circuits and that they play specialized roles in fine motor control. We believe that a better understanding of the function of these dopamine-sensitive circuits can be a key to the development of new and more effective therapies for people suffering from neurological disorders and stroke. Funding: The National Science Centre, grant SONATINA 2018/28/C/NZ4/00102 A disclosure of conflicts of interest: The authors declare no conflict of interest.

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BOARD NUMBER: S03-205

OXYTOCIN ACTS ON ASTROCYTES IN THE CENTRAL AMYGDALA TO PROMOTE A POSITIVE EMOTIONAL STATE

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Oxytocin is a hypothalamic neuropeptide with a large range of functions. From its crucial role in delivery to its implication in maternal behaviour, the functions of oxytocin have been widely studied for decades. Many studies have recently highlighted its implication in complex behaviours, such as individual recognition and preference, pain control, or anxiety modulation. However, oxytocin effect on glia have been left behind. Therefore, the actual dogma is that oxytocin, like other neuropeptides, acts exclusively via neuronal receptors in the central nervous system. Because the anxiolytic effect of oxytocin in the central amygdala is well described, we have investigated the effect of this neuropeptide on the central amygdalar circuit. Using patch clamp recording and calcium imaging, we have shown that astrocytes respond before neurons to an endogenous oxytocin release. Moreover, blocking or stimulating those glial cells can respectively decrease or mimic the effect of oxytocin on neuronal activity. Having these elements in mind, we decided to test whether astrocyte activity can affect the effect of oxytocin *in vivo*. Thanks to the deletion of the oxytocin receptor on central amygdalar astrocytes, we have shown that oxytocin-induced astrocyte activity is essential to the effect of this neuropeptide in the amygdala. On the other hand, we demonstrate that an optogenetic activation of those glial cells is sufficient to mimic the behavioural effects of oxytocin. Unveiling this neuro-astro-neuronal circuit, this work aims to highlight that neuropeptides, like other neurotransmitters, can modulate astrocyte calcium activity, modulating *in fine* the neuronal networks.

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EFFECT OF ANTIDEPRESSANT MIRTAZAPINE INTAKE DURING GESTATION ON THE EXCITABILITY OF HIPPOCAMPAL NEURONS OBSERVED IN THE OFFSPRING

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Aims: Maternal depression experienced during pregnancy endangers both mother and her offspring. Antidepressant treatment during pregnancy relieves maternal depression and prevents its negative effects on offspring. However, antidepressants themselves may affect directly offspring development. *Methods:* Adult female Sprague-Dawley rats were used. Rats were subjected to randomly alternating stressors to create a model of depression. Antidepressant mirtazapine was administered to pregnant rats from the 10th-20th days of pregnancy at the dose of 10 mg/kg/day. Animals from the control group were administered with a vehicle. Hippocampal neurons were isolated from pups (P0) of mothers which were: 1) unstressed, 2) unstressed and treated with mirtazapine, 3) stressed, and 4) stressed and treated with mirtazapine. Hippocampal excitability was measured in neuronal culture (DIV8-10) using a whole-cell patch clamp in the current-clamp configuration. *Results:* Pyramidal hippocampal neurons were visually identified by their morphology. Resting membrane potential (V_{rest}), input resistance (R_{input}), and the generation and parameters (threshold, amplitude, rise time, width) of action potentials (AP) were monitored. V_{rest} in the neurons of the stress + mirtazapine group was significantly hyperpolarized compared to the other groups. We also observed a significant decrease in R_{inp} in both stressed groups compared to the non-stressed groups. Individual AP parameters were not significantly altered in any group. Higher depolarization was required to initiate an AP firing in the stressed group. This activation shift was compensated by mirtazapine. *Conclusions:* Administration of the antidepressant mirtazapine during gestation has only a minor effect on hippocampal excitability of offspring. *Acknowledgment:* Supported by APVV-19-0435 grant.

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BOARD NUMBER: S03-207

SIMILAR LEVELS OF ENERGY POWER FIRING ACTIVITY IN EXCITATORY AND INHIBITORY NEURONS OF DIFFERENT SIZES IN MOUSE AND HUMAN

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Across species, neurons have higher diversity in cell sizes than non-neuronal brain cells and most other cell types in the body, despite strong evolutionary conservation of neuron-specific protein-coding and regulatory DNA sequences. Enrichment of highly conserved neuron-specific genes related to transport and signal transduction, as well as similarly highly conserved musculature and heart-specific genes, led us to hypothesize that the diversity in neuronal cell size is a response to conserved molecular pathways associated with firing activities through trade-offs between cell size and excitability in neurons. In this study, we examine whether larger neurons have progressively lower firing rates by analyzing the relationships between electrophysiological and morphological features of human and mouse cortical neurons in the Allen Cell Type Database. We find that larger excitatory neurons do have progressively lower levels of firing activities, while firing rates in inhibitory neurons show no correlation with cell size. Inhibitory neurons, however, show lower membrane capacitance than excitatory neurons of similar cell sizes, and the membrane capacitance of inhibitory neurons increases much more slowly with cell size than in excitatory neurons. As a result, we find that both excitatory and inhibitory neurons, in both human and mouse cortices, maintain a fairly constant estimated power towards recharging the membrane capacitance independently of neuronal cell size or firing rate. The finding that the reported relationships apply equally to mouse and human species suggests an evolutionarily conserved trade-off between firing activity and neuronal cell volume that might originate from physical constraints to energy supply and neuronal activity.

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BOARD NUMBER: S03-208

CANNABIDIOL REGULATES HUMAN DORSAL ROOT GANGLION NEURONAL EXCITABILITY.

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Chronic pain is a debilitating disease, which affects more than a third of the world's population and has a significant cost to society. Current therapeutic approaches, which include opioids, do not provide definitive relief to patients, and are often limited by harmful side effects. A research effort is therefore necessary to identify new pharmacological targets that are more effective but also safer. Recent results show that dorsal root ganglion (DRG) primary sensory neurons, which convey sensory information to the central nervous system, carry out active signal processing involving the cannabinoid system. Normalization of aberrant nociceptive processing in DRG neurons via cannabinoid-based treatment should be explored as an emerging strategy to manage chronic pain. In this work, we report for the first time the effect of cannabidiol (CBD), an FDA-approved non-psychoactive cannabinoid, on the intrinsic excitability of human small diameter DRG neurons. CBD significantly reduces the rheobase of human sensory neurons. We also show that CBD produces a significant depolarizing shift in the action potential threshold. Phase plane plot analysis of the AP shape also revealed kinetic differences in rise and decay time constants without affecting the peak amplitude. Taken together, our results suggest that CBD dampen nociceptor excitability in human, demonstrating an effect potentially relevant to chronic pain treatment. This research was funded in part by Desert Harvest Inc.

FENS Forum 2022
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BOARD NUMBER: S03-209

ACTIVATION OF REGENERATION-ASSOCIATED PATHWAYS IN NEURONS FOLLOWING PHOTOCAPACITIVE STIMULATION

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Aims: Neuronal stimulation exerts beneficial effects on restorative processes following many debilitating neurological disorders. Current drive to create lightweight and wireless devices for electrical stimulation aims to improve quality of life of patients. Organic photocapacitors (photocaps), rapidly charging upon illumination, create electric field used for stimulation of cells in their vicinity. The aim of the study was to elicit molecular changes in stimulated neurons. Methods: We cultivated primary neurons from postnatal rats on photocaps (round shape, 11 mm ø p-n layer of H2PC-PTCDI, 30 mm ø back electrode of Au-ITO). Afterwards, the cells were stimulated by light pulses for 30 minutes with an LED red light source (10 W). Neuronal activation was assessed by c-fos immunoreactivity with fluorescent staining. Neurotrophin (BDNF, NGF, NT-3, NT-4) expression was analysed with real-time qRT-PCR Results: Cells exposed to light pulses showed increased expression of c-fos protein compared to the controls without light stimulation, proving neuronal activation on the molecular level. Whilst a broad c-fos positivity was observed in these cells, the signal intensity was lower than that of positive controls exposed to glutamate. Three hours post-stimulation no significant change in the neurotrophin mRNA expression was detectable. Conclusion: Translation of c-fos was increased in light-stimulated cells grown on photocaps as compared to the non-stimulated group, indicating a widespread activation of neuronal networks *in vitro.* Whether lack of change in the neurotrophin levels lies on the stimulation protocol, photocap design or intrinsic temporal dynamics of the gene transcription will be addressed by future experiments.

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BOARD NUMBER: S03-210

DYSFUNCTIONAL POTASSIUM-CHANNEL ACTIVATION IN A GENETIC MOUSE MODEL OF SCHIZOPHRENIA

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Microdeletion of chromosome 22q11.2 is one of the most well-known genetic risk factors for schizophrenia. 22q11 deletion syndrome (22q11DS) is a neurodevelopmental disorder with neural circuit and behavioural disruptions as key features. Recent studies have shown that network synchronization is disturbed in patients with schizophrenia. Parvalbumin (PV) interneurons have shown function deficits in animal models harbouring the 22q11 microdeletion. These interneurons are crucial for maintaining proper excitation-inhibition balance in the neocortex, for which neuropsychiatric disease-related impairments of network activity have often been linked to aberrant PV neuron activity. However, the molecular mechanism behind such PV interneuron deficits remain largely unknown. In this study, we aimed to investigate the electrophysiological properties of PV interneurons in Df1 mice, a mouse model of the 22q11DS. Using whole-cell patch-clamp recordings in hippocampal PV interneurons, we found a significant increase in their excitability. Next, in order to restore PV interneuron excitability, we applied a selective Kv1.1 activator. We found that Kv1.1 activation did not induce any discernible change in the excitability of PV interneurons in Df1 mice, despite robustly decreasing PV interneuron excitability in control mice. Furthermore, we observed a decrease in the number of PV cells that were positive for Kv1. Our findings suggest that increased excitability of PV interneurons in the CA1 region of Df1 mice might result from decreased expression or function of Kv1 channels.

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INFLUENCE OF DOPAMINE ON THETA RHYTHM: ROLE OF D2 RECEPTORS IN SST AND PV INTERNEURONS

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Aims: The hippocampus is essential for memory and novelty processing. These functions are underpinned by synchronous network activity. Recent evidence suggest that dopamine facilitates the encoding of novel spatial memories. While the role of the hippocampal D1 receptor in mnesic functions has been extensively studied, the contribution of the D2 receptor (D2R) remains elusive. We characterize the functional role of D2R in the hippocampus network by combining anatomo-functional approaches. Methods: *In situ* hybridization – Patch clamp Results: *In situ* hybridization analysis indicated that parvalbumin (PV) and somatostatin (Sst) interneurons of the hippocampal CA1 subfield expressed *Drd2* mRNAs. These interneuron subtypes entrain pyramidal cells to orchestrate information processing and are instrumental in generating hippocampal oscillations. Using electrophysiological recordings of pyramidal cells, we observed that application of D2R-like agonist quinpirole (10µM) in the presence of methacholine (muscarinic agonist, 50 nM) increases the number of theta oscillation periods by acting through the D2R on the Sst cells (Sst-D2R-Cko). To get insights into the mechanism of how D2Rs alter hippocampal theta rhythms, we next examined the intrinsic excitability of D2R-expressing cells. Our data suggest that pharmacological D2R activation alters the intrinsic excitability of these hippocampal interneurons, by increasing the firing of action potential in Sst cells and decreasing the firing of PV cells by using different intracellular signaling pathway. Conclusion: These data show that activation of D2R of hippocampal interneurons modulates the activity of the hippocampal network. Ultimately this work should provide a better understanding of mnesic deficiency observed in dopaminergic transmission dysfunctions.

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BOARD NUMBER: S03-212

ELUCIDATING HOW NON-NEURONAL ACTION POTENTIALS GENERATED BY MIDLINE RADIAL GLIA INITIATE MOTOR BEHAVIOUR

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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In the vertebrate spinal cord, waves of spontaneous neuronal activity (SNA) trigger the first motor behavior - around 12 days of embryonic development in mice - and play a critical role in the development of the neuromuscular system. However, it remains unclear how these waves of electrical activity are generated and propagate along the spinal cord at a stage when the first synapses are just starting to form. Our team recently demonstrated that midline radial glia located in the floor-plate have the unique ability to generate calcium action potentials (AP) via T-type voltage-gated calcium channels and to propagate them through gap-junctions along the spinal cord. In order to study the influence of radial glia APs on neurons during SNA, we visualized neuronal activity by calcium imaging using the Rhod2-AM indicator. We discovered that SNA events not only propagate rostro-caudally but also along a medio-lateral sequence starting from the midline. Using a transgenic line that expresses the genetically encoded calcium indicator GCaMP6f in spinal motoneurons, we showed that motoneuron columns were sequentially activated from the midline during SNA. Application of TTA-P2, a T-type calcium channel blocker, or β18-GA, a gap-junction blocker, abolished this medio-lateral activation of motor columns and strongly decrease SNA propagation speed along the rostro-caudal axis. Finally, we found that specific optogenetic stimulation of midline radial glia was sufficient to trigger SNA events in spinal neurons. In conclusion, our results suggest that radial glia excitability is responsible for patterning motoneuron activity and initiating the first motor behavior in vertebrates.

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BOARD NUMBER: S03-213

THERAPEUTIC EFFECT OF HIF PROLYL HYDROXYLASE (PHD) INHIBITION IN AN IN VITRO HYPOXIA MODEL

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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It is well known that hypoxia is crucial to stroke and contributes to neurodegeneration in traumas, Alzheimer's, etc. HIF-1 dimerization is a trigger for adaptation and survival nervous cells in hypoxia. Under normal conditions, the dimerization does not occur because of the work of the PHD. The aim of the work was studying the effects of PHD inhibition on the neuron-glial network calcium activity under hypoxia modelling in vitro. The research was carried out on primary hippocampal cultures obtained from mouse embryos (E18). Modeling of hypoxia in vitro was performed on 14 DIV. In vitro treatment of the of the PHD low molecular weight inhibitor D014-0030 (1 μM) was performed 2 hours after hypoxia modeling and then daily. The calcium imaging was executed on 21 DIV. We analyzed both individual parameters of calcium events (duration, frequency of calcium oscillations) and general network parameters (signal speed, the degree of calcium signal correlation etc.). Intact cultures activity are highly correlated (correlation 0.51±0.04). This is consistent with the data on the functioning of normal neuron-glial cultures on the 21st day cultivation. There is a significant degradation of network effects in the "Hypoxia" group (correlation level 1.45±0.71). The PHD inhibitor treatment cause the effect of mainteined the degree of correlation of calcium dynamics in neighboring cells but not in distantly located cells and increased the number of working cells, suggesting a potential for network recovery. This research was funded by the Ministry of Science and Higher Education of the Russian Federation (0729-2020-0061).

Pubmed:
34395432: Mitroshina EV, Savyuk MO, Ponimaskin E, Vedunova MV
Hypoxia-Inducible Factor (HIF) in Ischemic Stroke and Neurodegenerative Disease.
Hypoxia is one of the most common pathological conditions, which can be induced by multiple events, including ischemic injury, trauma, inflammation, tumors, etc. The body's adaptation to hypoxia is a highly important phenomenon in both health and disease. Most cellular responses to hypoxia are associated with a family of transcription factors called hypoxia-inducible factors (HIFs), which induce the expression of a wide range of genes that help cells adapt to a hypoxic environment. Basic mechanisms of adaptation to hypoxia, and particularly HIF functions, have being extensively studied over recent decades, leading to the 2019 Nobel Prize in Physiology or Medicine. Based on their pivotal physiological importance, HIFs are attracting increasing attention as a new potential target for treating a large number of hypoxia-associated diseases. Most of the experimental work related to HIFs has focused on roles in the liver and kidney. However, increasing evidence clearly demonstrates that HIF-based responses represent an universal adaptation mechanism in all tissue types, including the central nervous system (CNS). In the CNS, HIFs are critically involved in the regulation of neurogenesis, nerve cell differentiation, and neuronal apoptosis. In this mini-review, we provide an overview of the complex role of HIF-1 in the adaptation of neurons and glia cells to hypoxia, with a focus on its potential involvement into various neuronal pathologies and on its possible role as a novel therapeutic target.
Front Cell Dev Biol, 2021; 9
34063823: Loginova M, Mishchenko T, Savyuk M, Guseva S, Gavrish M, Krivonosov M, Ivanchenko M, Fedotova J, Vedunova M
Double-Edged Sword of Vitamin D3 Effects on Primary Neuronal Cultures in Hypoxic States.
The use of vitamin D3 along with traditional therapy opens up new prospects for increasing the adaptive capacity of nerve cells to the effects of a wide range of stress factors, including hypoxia-ischemic processes. However, questions about prophylactic and therapeutic doses of vitamin D3 remain controversial. The purpose of our study was to analyze the effects of vitamin D3 at different concentrations on morpho-functional characteristics of neuron-glial networks in hypoxia modeling in vitro. We showed that a single administration of vitamin D3 at a high concentration (1 μM) in a normal state has no significant effect on the cell viability of primary neuronal cultures; however, it has a pronounced modulatory effect on the functional calcium activity of neuron-glial networks and causes destruction of the network response. Under hypoxia, the use of vitamin

D3 (1 μM) leads to total cell death of primary neuronal cultures and complete negation of functional neural network activity. In contrast, application of lower concentrations of vitamin D3 (0.01 μM and 0.1 μM) caused a pronounced dose-dependent neuroprotective effect during the studied post-hypoxic period. While the use of vitamin D3 at a concentration of 0.1 μM maintained cell viability, preventive administration of 0.01 μM not only partially preserved the morphological integrity of primary neuronal cells but also maintained the functional structure and activity of neuron-glial networks in cultures. Possible molecular mechanisms of neuroprotective action of vitamin D3 can be associated with the increased expression level of transcription factor HIF-1α and maintaining the relationship between the levels of BDNF and TrkB expression in cells of primary neuronal cultures.
Int J Mol Sci, 2021; 22
33672819: Mitroshina EV, Loginova MM, Savyuk MO, Krivonosov MI, Mishchenko TA, Tarabykin VS, Ivanchenko MV, Vedunova MV
Neuroprotective Effect of Kinase Inhibition in Ischemic Factor Modeling In Vitro.
The contribution of many neuronal kinases to the adaptation of nerve cells to ischemic damage and their effect on functional neural network activity has not yet been studied. The aim of this work is to study the role of the four kinases belonging to different metabolic cascades (SRC, Ikkb, eEF2K, and FLT4) in the adaptive potential of the neuron-glial network for modeling the key factors of ischemic damage. We carried out a comprehensive study on the effects of kinases blockade on the viability and network functional calcium activity of nerve cells under ischemic factor modeling in vitro. Ischemic factor modelling was performed on day 14 of culturing primary hippocampal cells obtained from mouse embryos (E18). The most significant neuroprotective effect was shown in the blockade of FLT4 kinase in the simulation of hypoxia. The studies performed revealed the role of FLT4 in the development of functional dysfunction in cerebrovascular accidents and created new opportunities for the study of this enzyme and its blockers in the formation of new therapeutic strategies.
Int J Mol Sci, 2021; 22
33114758: Mitroshina EV, Krivonosov MI, Burmistrov DE, Savyuk MO, Mishchenko TA, Ivanchenko MV, Vedunova MV
Signatures of the Consolidated Response of Astrocytes to Ischemic Factors In Vitro.
Whether and under what conditions astrocytes can mount a collective network response has recently become one of the central questions in neurobiology. Here, we address this problem, investigating astrocytic reactions to different biochemical stimuli and ischemic-like conditions in vitro. Identifying an emergent astrocytic network is based on a novel mathematical approach that extracts calcium activity from time-lapse fluorescence imaging and estimates the connectivity of astrocytes. The developed algorithm represents the astrocytic network as an oriented graph in which the nodes correspond to separate astrocytes, and the edges indicate high dynamical correlations between astrocytic events. We demonstrate that ischemic-like conditions decrease network connectivity in primary cultures in vitro, although calcium events persist. Importantly, we found that stimulation under normal conditions with 10 μM ATP increases the number of long-range connections and the degree of corresponding correlations in calcium activity, apart from the frequency of calcium events. This result indicates that astrocytes can form a large functional network in response to certain stimuli. In the post-ischemic interval, the response to ATP stimulation is not manifested, which suggests a deep lesion in functional astrocytic networks. The blockade of Connexin 43 during ischemic modeling preserves the connectivity of astrocytes in the post-hypoxic period.
Int J Mol Sci, 2020; 21
32722310: Savyuk M, Krivonosov M, Mishchenko T, Gazaryan I, Ivanchenko M, Khristichenko A, Poloznikov A, Hushpulian D, Nikulin S, Tonevitsky E, Abuzarova G, Mitroshina E, Vedunova M
Neuroprotective Effect of HIF Prolyl Hydroxylase Inhibition in an In Vitro Hypoxia Model.
A novel potent analog of the branched tail oxyquinoline group of hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors, neuradapt, has been studied in two treatment regimes in an in vitro hypoxia model on murine primary hippocampal cultures. Neuradapt activates the expression of HIF1 and HIF2 target genes and shows no toxicity up to 20 μM, which is more than an order of magnitude higher than its biologically active concentration. Cell viability, functional activity, and network connectivity between the elements of neuronal networks have been studied using a pairwise correlation analysis of the intracellular calcium fluctuations in the individual cells. An immediate treatment with 1 μM and 15 μM neuradapt right at the onset of hypoxia not only protects from the death, but also maintains the spontaneous calcium activity in nervous cells at the level of the intact cultures. A similar neuroprotective effect in the post-treatment scenario is observed for 15 μM, but not for 1 μM neuradapt. Network connectivity is better preserved with immediate treatment using 1 μM neuradapt than with 15 μM, which is still beneficial. Post-treatment with neuradapt did not restore the network connectivity despite the observation that neuradapt significantly increased cell viability at 1 μM and functional activity at 15 μM. The preservation of cell viability and functional activity makes neuradapt promising for further studies in a post-treatment scenario, since it can be combined with other drugs and treatments restoring the network connectivity of functionally competent cells.
Antioxidants (Basel), 2020; 9


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BOARD NUMBER: S03-214

VISUALIZATION AND QUANTITATIVE ANALYSIS OF NANOSCALE PHOSPHOINOSITIDE DISTRIBUTION ON NEURONAL CELL MEMBRANES OF MOUSE CEREBELLUM AT ELECTRON MICROSCOPIC LEVEL

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Phosphoinositides (PIs) play essential roles in many aspects of neuronal functions. Although it is important to know how PIs distribute on neuronal membranes to understand their roles, this has not yet been investigated at the electron microscopic level. In this study, we aimed to observe the nanoscale distribution of a PI stereoisomer, phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$), on neuronal cell membranes and to address their potential roles in neuronal activities using SDS-digested freeze-fracture replica labeling method with a GST-tagged pleckstrin homology domain of phospholipase Cδ1 as a specific probe for $PI(4,5)P_2$. Using replicas of mouse cerebellar tissues, we found that gold particles for $PI(4,5)P_2$ make clusters broadly distributed on the cytoplasmic side of Purkinje cells (PCs) and parallel fiber (PF) bouton membranes. $PI(4,5)P_2$ was concentrated at active zones and co-localized with P/Q-type Ca^{2+} channels in PF boutons, indicating that $PI(4,5)P_2$ may contribute to the regulation of synaptic vesicle release. In the PC dendrites, $PI(4,5)P_2$ was co-localized with a metabotropic glutamate receptor mGluR1α and a G protein-gated inward-rectifying potassium channel subunit GIRK3. The co-localization of $PI(4,5)P_2$ with mGluR1α is selective to spines and may be necessary for the effective second messenger production upon the receptor activation and its coupling to PLC. The $PI(4,5)P_2$ distribution close to GIRK3 also suggests its potential role in the regulation of PC excitability, as PIP_2 has been reported to induce conformational changes in GIRKs. Manipulation of the co-localization between $PI(4,5)P_2$ and those key molecules would be useful to elucidate the potential implications of our present findings.

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BOARD NUMBER: S03-215

CATECHOLAMINERGIC MODULATION OF PERSISTENT NEURONAL ACTIVITY IN THE MOUSE AUDITORY CORTEX

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Accurate working memory performance depends on neuromodulatory systems functioning within a narrow range of concentrations. In the case of dopamine and noradrenaline, for example, both excessive activation and suppression of these systems are linked to working memory deficits. These deficits are accompanied by suppression of persistent firing, a potential mechanism of working memory in which a cell retains task relevant information by continuing to fire during the delay period between the stimulus and the learned response. However, the effect of neuromodulation on persistent firing at the single cell level is not yet fully understood. Additionally, these neuromodulatory systems have predominantly been studied in the prefrontal cortex, a brain region long thought to play the role of central executive in complex cognitive function. However, they are relatively sparsely investigated in sensory cortex, despite evidence that persistent firing also occurs in sensory cortices and may play an important role in working memory. In this study, we used whole cell patch clamp electrophysiology in ex vivo mouse auditory cortex preparations to analyze the effects of dopaminergic and noradrenergic modulation of persistent neuronal activity. The effects of these neurotransmitters were tested both alone, and in the presence of the cholinergic agonist carbachol, which is known to support long lasting persistent firing. Our results indicate that while dopamine may not have a direct effect on persistent firing in the mouse auditory cortex, norepinephrine can both enhance low levels of carbachol-supported persistent firing, and support persistent firing itself in a subset of auditory cortex cells.

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BOARD NUMBER: S03-216

THE EFFECT OF RADIOFREQUENCY ELECTROMAGNETIC FIELDS ON NEURONAL ACTIVITY: IMPLICATION OF MICROTUBULES

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Tubulin post-modifications (PTMs) play an important role in the functional diversity of microtubules (MTs). PTMs affect MT dynamics/stability, organization, and interactions with other cellular components. We previously showed that exposure to radiofrequency (RF) waves tuned to 3.0 GHz frequency altered intracellular MTs distribution in cultured neuronal cells and caused changes in neuronal excitability. Additionally, changes in the assembly and polymerization rates of MTs have been observed due to exposure to electromagnetic (EM) fields. In this study, we investigated if the observed effect on MT dynamics could be associated with changes in PTMs of tubulin and MTs. We specifically examined the effect of RF exposures at different frequencies on tubulin detyrosination, phosphorylation, and acetylation in neuronal cells. We exposed neuronal cells in an environmentally controlled EM exposure setup at three different RF frequencies for 1 hour at a constant E-field. We investigated changes following RF exposures using Western blotting, fluorescence imaging, and current- and voltage-clamp electrophysiology techniques. The exposure of neuronal cells to three tested RF frequencies showed a clear difference in the distribution of endogenous MTs. Specifically, fluorescent MTs were primarily localized within the cytoplasm compared to membrane localization in the unexposed neuronal cells. Furthermore, RF exposure affected the morphology of the cells, which exhibited reduced action potential (AP) amplitude, increased resting membrane potential, and increased spontaneous AP firing. The results also showed reduced acetylated tubulin following RF exposure, which suggest that reduced acetylation following RF exposure could be related to changes in MT dynamics and localization.

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BOARD NUMBER: S03-217

GUANOSINE MODULATES K+ MEMBRANE CURRENTS IN SH-SY5Y CELLS: INVOLVEMENT OF ADENOSINE RECEPTORS

POSTER SESSION 03 - SECTION: NEURONS AND GLIA: CELL EXCITABILITY

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Background: Guanosine (GUO) is implicated in the regulation of numerous cellular processes and mediates several effects within the central nervous system. While its interaction with neural membranes has been described, GUO still is an orphan neuromodulator. Within the context of excitability control, it has been hypothesized that, among the possible cellular targets of GUO, potassium channels and adenosine (ADO) receptors (ARs) could play a major role. **Aim:** In this light, we aim to investigate the effects of GUO on the bioelectric activity of human neuroblastoma SH-SY5Y cells, which have been considered an invaluable experimental model for studying the effects of GUO in the attempt to fully uncover its role within purinergic signaling. **Methods:** We apply whole-cell patch-clamp recordings to first explore the contribution of voltage-dependent K^+ channels and, besides this, the role of ARs in the regulation of GUO-dependent cellular electrophysiology. **Results:** Our data support that GUO is able to specifically modulate K^+-dependent outward currents over cell membranes. The present findings suggest that K^+ outward membrane channels may be targeted by GUO with an implication of adenosine receptors in SH-SY5Y cells, but also support the hypothesis of a functional interaction with ADO. **Conclusions:** The present research runs through the leitmotif of the deorphanization of GUO, adding insight into the interplay with adenosinergic signaling and suggesting GUO as a powerful modulator of SH-SY5Y excitability.

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BOARD NUMBER: S03-218

CSPα/DNAJC5 IN GLUTAMATERGIC SYNAPTIC FUNCTION AND MAINTENANCE

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Cysteine String Protein (CSPα/DNAJC5) is a synaptic co-chaperone that prevents activity-dependent degeneration of synapses. CSPα/DNAJC5 knock-out mice suffer from a neurological phenotype and early postnatal lethality soon after the first month of age. CSPα/DNAJC5 is critical to maintain the levels of the SNARE protein SNAP25, especially in highly active synapses. Indeed, the decrease in SNAP25 levels is thought to be a key event leading to presynaptic neurodegeneration. We are interested in studying the synaptic effects of removing CSPα/DNAJC5 from adult glutamatergic neurons that operate at a low activity regime. For this purpose we have generated CaMK^{CreERT2}:Ai27D:*Dnajc5*^{flox} mice to conditionally target *Dnajc5* in adult hippocampal glutamatergic neurons. This line also expresses channelrhodopsin2 fused to the fluorescent reporter td-tomato (Ai27D) in targeted neurons. CSPα/DNAJC5 conditional knock-out mice survive and do not develop an evident neurological phenotype. We have analyzed synaptic transmission in two hippocampal synapses: (1) synapses formed by mossy fibers at CA3 pyramidal neurons (MF-CA3 synapse) and (2) synapses formed by Schaffer collaterals at CA1 pyramidal neurons (SC-CA1 synapse). Interestingly, those synapses show different phenotypes in the absence of CSPα/DNAJC5. We are investigating the molecular mechanisms of such a phenotype to understand why those apparently similar synapses have different requirement of CSPα/DNAJC5 and how those differences might be related to SNAP25. We are grateful to Prof. Angel Barco (INA) and Prof. G. Schütz (DKFZ) for kindly providing CaMK^{CreERT2} mice. Supported by: MICINN (BFU2016-76050-P, BES-2017-082324, PID2019-105530GB-I00), ISCIII (CIBERNED) and FEDER.

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BOARD NUMBER: S03-219

ACUTE GENETIC ELIMINATION OF A SYNAPTIC CO-CHAPERONE TO STUDY AND TO REVERT PRESYNAPTIC DYSFUNCTION AND NEURODEGENERATION

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Synapses operate throughout life, but synaptic proteins last only for several days or weeks. The mechanisms by which synaptic proteins are maintained are not well understood yet. We are interested in the role of the synaptic co-chaperone CSPα/DNAJC5. Conventional KO mice lacking CSPα/DNAJC5 develop presynaptic degeneration and die soon after birth, preventing studies in adulthood. We have bred mice bearing the *Dnajc5* floxed allele (Nieto Gonzalez et al. PNAS. 2019) against UBC-Cre-ERT2 mice (Ruzankina et al. Cell Stem Cell. 2007) to target *Dnajc5* ubiquitously in a time-controlled manner. CSPα/DNAJC5 is almost undetected in hippocampal cultures of *UBC-Cre-ERT2;Dnajc5*$^{flox/flox}$ mice after 13 days in tamoxifen. To investigate the function of CSPα/DNAJC5 in adulthood, we fed 2 months old *UBC-Cre-ERT2;Dnajc5*$^{flox/flox}$ mice with tamoxifen during 30 days. Two weeks after tamoxifen, they exhibited weight loss and a neurological phenotype characterized by loss of spontaneous activity, strength and motor coordination. This lead, two weeks later, to early death. The time-controlled induction of the phenotype has opened potential opportunities to rescue the phenotype that we are investigating by using viral vectors engineered to cross the blood brain-barrier to deliver CSPα/DNAJC5 to the central nervous system. We aim to define the time-window in which the rescue is feasible to unveil which dysfunctional mechanisms are amenable for restorative intervention. Support: MICINN (PID2019-105530GB-I00), MICIU (FPU18/01700), Andalusian CTEICU (P18-FR-2144), Fundación Tatiana Pérez de Guzmán El Bueno, ISCIII (CIBERNED) and FEDER. Thanks to M.C. Rivero for previous technical assistance and to Dr. Eric Brown (Univ of Penn) for sharing UBC-Cre-ERT2 mice.

FENS Forum 2022
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BOARD NUMBER: S03-220

CORTICAL WIRING BY SYNAPSE-SPECIFIC CONTROL OF LOCAL PROTEIN SYNTHESIS

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Compared to other cell types, neurons have a highly arborised morphology, and form and receive an astonishing number of synapses. It is thought that local protein synthesis has evolved in neurons as a mechanism to support this morphological complexity which requires independent control across multiple subcellular compartments. Local translation is ubiquitous in neuronal pre- and postsynaptic compartments. However, to what extent local translation is differentially regulated at the level of specific synapses during wiring of neural circuits is not known. Here we identify a dedicated signalling pathway that regulates synaptic protein synthesis for the formation of excitatory synapses on parvalbumin (PV+) interneurons in the mouse cerebral cortex. We first show that mTOR hyperactivity in interneurons leads to wiring defects in a cell type-specific and synapse-specific manner. We then find that the interneuron-specific receptor tyrosine kinase ErbB4 regulates the synaptic activity of mTOR at excitatory synapses on PV+ interneurons. By using a RiboTRAP strategy, we identify a set of synaptic proteins regulated by ErbB4. We further show that ErbB4 enhances the local synthesis of these proteins and that they are necessary for the correct wiring of cortical interneurons. Multiple lines of evidence suggest that deficits in the excitation/inhibition balance are underlying several neurodevelopmental disorders. In particular, an impaired excitatory drive on PV+ interneurons seems to be a potential hub for autism and schizophrenia. Our work deciphers a molecular mechanism by which these synapses are formed and maintained, through a synapse-specific control of local translation.

Pubmed:
22764251: Beurdeley M, Spatazza J, Lee HH, Sugiyama S, Bernard C, Di Nardo AA, Hensch TK, Prochiantz A
Otx2 binding to perineuronal nets persistently regulates plasticity in the mature visual cortex.
Specific transfer of (orthodenticle homeobox 2) Otx2 homeoprotein into GABAergic interneurons expressing parvalbumin (PV) is necessary and sufficient to open, then close, a critical period (CP) of plasticity in the developing mouse visual cortex. The accumulation of endogenous Otx2 in PV cells suggests the presence of specific Otx2 binding sites. Here, we find that perineuronal nets (PNNs) on the surfaces of PV cells permit the specific, constitutive capture of Otx2. We identify a 15 aa domain containing an arginine-lysine doublet (RK peptide) within Otx2, bearing prototypic traits of a glycosaminoglycan (GAG) binding sequence that mediates Otx2 binding to PNNs, and specifically to chondroitin sulfate D and E, with high affinity. Accordingly, PNN hydrolysis by chondroitinase ABC reduces the amount of endogenous Otx2 in PV cells. Direct infusion of RK peptide similarly disrupts endogenous Otx2 localization to PV cells, reduces PV and PNN expression, and reopens plasticity in adult mice. The closure of one eye during this transient window reduces cortical acuity and is specific to the RK motif, as an Alanine-Alanine variant or a scrambled peptide fails to reactivate plasticity. Conversely, this transient reopening of plasticity in the adult restores binocular vision in amblyopic mice. Thus, one function of PNNs is to facilitate the persistent internalization of Otx2 by PV cells to maintain CP closure. The pharmacological use of the Otx2 GAG binding domain offers a novel, potent therapeutic tool with which to restore cortical plasticity in the mature brain.
J Neurosci, 2012; 32
23154924: Despras G, Bernard C, Perrot A, Cattiaux L, Prochiantz A, Lortat-Jacob H, Mallet JM
Toward libraries of biotinylated chondroitin sulfate analogues: from synthesis to in vivo studies.
Chondroitin sulfate-E (CS-E) oligosaccharidic analogues (di to hexa) were prepared from lactose. In these compounds, the 2-acetamido group was replaced by a hydroxyl group. This modification speeded up the synthesis, and large oligosaccharides were constructed in a few steps from a lactose-originated block. The protecting groups used were as follows; Fmoc for hydroxyl groups to be glycosylated, allyl group for anomeric position protection, and trichoroacetimidate leaving groups were used to prepare up to octasaccharides. We took advantage of the presence of allyl group to develop a click biotinylation, through its transformation into a 3-azido-2-hydroxyl propyl group in two steps (epoxidation and sodium azide epoxide opening). The biotinylating agent was a water-soluble propargylated and biotinylated triethylene glycol (PEG). By using

surface plasmon resonance (SPR), it was shown that the di-, tetra-, and hexasaccharides display a binding affinity and selectivity toward HSF/GSF and CXCL12 similar to that of CS-E. A parallel study confirmed their mimicry of natural compounds, based on the hexasaccharide interaction with Otx2, a homeodomain protein involved in brain maturation, thus validating our simplification approach to synthesize bioactive GAG.
Chemistry, 2013; 19
24234651: Bernard C, Kim HT, Torero Ibad R, Lee EJ, Simonutti M, Picaud S, Acampora D, Simeone A, Di Nardo AA, Prochiantz A, Moya KL, Kim JW
Graded Otx2 activities demonstrate dose-sensitive eye and retina phenotypes.
In the human, mutations of OTX2 (Orthodenticle homeobox 2 transcription factor) translate into eye malformations of variable expressivity (even between the two eyes of the same individual) and incomplete penetrance, suggesting the existence of subtle thresholds in OTX2 activity. We have addressed this issue by analyzing retinal structure and function in six mutant mice with graded Otx2 activity: Otx2(+/+), Otx2(+/AA), Otx2(+/GFP), Otx2(AA/AA), Otx2(AA/GFP) and Otx2(GFP/GFP). Null mice (Otx2(GFP/GFP)) fail to develop the head and are embryonic lethal, and compound heterozygous Otx2(AA/GFP) mice show a truncated head and die at birth. All other genotypes develop until adulthood. We analyzed eye structure and visual physiology in the genotypes that develop until adulthood and report that phenotype severity parallels Otx2 activity. Otx2(+/AA) are only mildly affected whereas Otx2(+/GFP) are more affected than Otx2(+/AA) but less than Otx2(AA/AA) mice. Otx2(AA/AA) mice later manifest the most severe defects, with variable expressivity. Electrophysiological and histological analyses of the mouse retina revealed progressive death of bipolar cells and cone photoreceptors that is both Otx2 activity- and age-dependent with the same ranking of phenotypic severity. This study demonstrates the importance of gene dosage in the development of age-dependent pathologies and underscores the fact that small gene dosage differences can cause significant pathological states.
Hum Mol Genet, 2014; 23
26881132: Bernard C, Prochiantz A
Otx2-PNN Interaction to Regulate Cortical Plasticity.
The ability of the environment to shape cortical function is at its highest during critical periods of postnatal development. In the visual cortex, critical period onset is triggered by the maturation of parvalbumin inhibitory interneurons, which gradually become surrounded by a specialized glycosaminoglycan-rich extracellular matrix: the perineuronal nets. Among the identified factors regulating cortical plasticity in the visual cortex, extracortical homeoprotein Otx2 is transferred specifically into parvalbumin interneurons and this transfer regulates both the onset and the closure of the critical period of plasticity for binocular vision. Here, we review the interaction between the complex sugars of the perineuronal nets and homeoprotein Otx2 and how this interaction regulates cortical plasticity during critical period and in adulthood.
Neural Plast, 2016; 2016
27171438: Bernard C, Vincent C, Testa D, Bertini E, Ribot J, Di Nardo AA, Volovitch M, Prochiantz A
A Mouse Model for Conditional Secretion of Specific Single-Chain Antibodies Provides Genetic Evidence for Regulation of Cortical Plasticity by a Non-cell Autonomous Homeoprotein Transcription Factor.
During postnatal life the cerebral cortex passes through critical periods of plasticity allowing its physiological adaptation to the environment. In the visual cortex, critical period onset and closure are influenced by the non-cell autonomous activity of the Otx2 homeoprotein transcription factor, which regulates the maturation of parvalbumin-expressing inhibitory interneurons (PV cells). In adult mice, the maintenance of a non-plastic adult state requires continuous Otx2 import by PV cells. An important source of extra-cortical Otx2 is the choroid plexus, which secretes Otx2 into the cerebrospinal fluid. Otx2 secretion and internalization requires two small peptidic domains that are part of the DNA-binding domain. Thus, mutating these "transfer" sequences also modifies cell autonomous transcription, precluding this approach to obtain a cell autonomous-only mouse. Here, we develop a mouse model with inducible secretion of an anti-Otx2 single-chain antibody to trap Otx2 in the extracellular milieu. Postnatal secretion of this single-chain antibody by PV cells delays PV maturation and reduces plasticity gene expression. Induced adult expression of this single-chain antibody in cerebrospinal fluid decreases Otx2 internalization by PV cells, strongly induces plasticity gene expression and reopens physiological plasticity. We provide the first mammalian genetic evidence for a signaling mechanism involving intercellular transfer of a homeoprotein transcription factor. Our single-chain antibody mouse model is a valid strategy for extracellular neutralization that could be applied to other homeoproteins and signaling molecules within and beyond the nervous system.
PLoS Genet, 2016; 12
28194008: Lee HHC, Bernard C, Ye Z, Acampora D, Simeone A, Prochiantz A, Di Nardo AA, Hensch TK
Genetic Otx2 mis-localization delays critical period plasticity across brain regions.
Accumulation of non-cell autonomous Otx2 homeoprotein in postnatal mouse visual cortex (V1) has been implicated in both the onset and closure of critical period (CP) plasticity. Here, we show that a genetic point mutation in the glycosaminoglycan recognition motif of Otx2 broadly delays the maturation of pivotal parvalbumin-positive (PV+) interneurons not only in V1 but

also in the primary auditory (A1) and medial prefrontal cortex (mPFC). Consequently, not only visual, but also auditory plasticity is delayed, including the experience-dependent expansion of tonotopic maps in A1 and the acquisition of acoustic preferences in mPFC, which mitigates anxious behavior. In addition, Otx2 mis-localization leads to dynamic turnover of selected perineuronal net (PNN) components well beyond the normal CP in V1 and mPFC. These findings reveal widespread actions of Otx2 signaling in the postnatal cortex controlling the maturational trajectory across modalities. Disrupted PV+ network function and deficits in PNN integrity are implicated in a variety of psychiatric illnesses, suggesting a potential global role for Otx2 function in establishing mental health.
Mol Psychiatry, 2017; 22
29771284: Apulei J, Kim N, Testa D, Ribot J, Morizet D, Bernard C, Jourdren L, Blugeon C, Di Nardo AA, Prochiantz A
Non-cell Autonomous OTX2 Homeoprotein Regulates Visual Cortex Plasticity Through Gadd45b/g.
The non-cell autonomous transfer of OTX2 homeoprotein transcription factor into juvenile mouse cerebral cortex regulates parvalbumin interneuron maturation and critical period timing. By analyzing gene expression in primary visual cortex of wild-type and Otx2+/GFP mice at plastic and nonplastic ages, we identified several putative genes implicated in Otx2-dependent visual cortex plasticity for ocular dominance. Cortical OTX2 infusion in juvenile mice induced Gadd45b/g expression through direct regulation of transcription. Intriguingly, a reverse effect was found in the adult, where reducing cortical OTX2 resulted in Gadd45b/g upregulation. Viral expression of Gadd45b in adult visual cortex directly induced ocular dominance plasticity with concomitant changes in MeCP2 foci within parvalbumin interneurons and in methylation states of several plasticity gene promoters, suggesting epigenetic regulation. This interaction provides a molecular mechanism for OTX2 to trigger critical period plasticity yet suppress adult plasticity.
Cereb Cortex, 2019; 29
32737182: Torero Ibad R, Mazhar B, Vincent C, Bernard C, Dégardin J, Simonutti M, Lamonerie T, Di Nardo AA, Prochiantz A, Moya KL
OTX2 Non-Cell Autonomous Activity Regulates Inner Retinal Function.
OTX2 is a homeoprotein transcription factor expressed in photoreceptors and bipolar cells in the retina. OTX2, like many other homeoproteins, transfers between cells and exerts non-cell autonomous effects such as promoting the survival of retinal ganglion cells that do not express the protein. Here we used a genetic approach to target extracellular OTX2 in the retina by conditional expression of a secreted single-chain anti-OTX2 antibody. Compared with control mice, the expression of this antibody by parvalbumin-expressing neurons in the retina is followed by a reduction in visual acuity in 1-month-old mice with no alteration of the retinal structure or cell type number or aspect. The a-waves and b-waves measured by electroretinogram were also indistinguishable from those of control mice, suggesting no functional deficit of photoreceptors and bipolar cells. Mice expressing the OTX2-neutralizing antibody did show a significant doubling in the flicker amplitude and a reduction in oscillatory potential, consistent with a change in inner retinal function. Our results show that interfering with OTX2 non-cell autonomous activity in the postnatal retina leads to an alteration in inner retinal cell functions and causes a deficit in visual acuity.
eNeuro, 2020 Sep/Oct; 7
33320083: Exposito-Alonso D, Osório C, Bernard C, Pascual-García S, Del Pino I, Marín O, Rico B
Subcellular sorting of neuregulins controls the assembly of excitatory-inhibitory cortical circuits.
The assembly of specific neuronal circuits relies on the expression of complementary molecular programs in presynaptic and postsynaptic neurons. In the cerebral cortex, the tyrosine kinase receptor ErbB4 is critical for the wiring of specific populations of GABAergic interneurons, in which it paradoxically regulates both the formation of inhibitory synapses as well as the development of excitatory synapses received by these cells. Here, we found that Nrg1 and Nrg3, two members of the neuregulin family of trophic factors, regulate the inhibitory outputs and excitatory inputs of interneurons in the mouse cerebral cortex, respectively. The differential role of Nrg1 and Nrg3 in this process is not due to their receptor-binding EGF-like domain, but rather to their distinctive subcellular localization within pyramidal cells. Our study reveals a novel strategy for the assembly of cortical circuits that involves the differential subcellular sorting of family-related synaptic proteins.
Elife, 2020; 9
34445655: Planques A, Oliveira Moreira V, Benacom D, Bernard C, Jourdren L, Blugeon C, Dingli F, Masson V, Loew D, Prochiantz A, Di Nardo AA
OTX2 Homeoprotein Functions in Adult Choroid Plexus.
The choroid plexus is an important blood barrier that secretes cerebrospinal fluid, which essential for embryonic brain development and adult brain homeostasis. The OTX2 homeoprotein is a transcription factor that is critical for choroid plexus development and remains highly expressed in adult choroid plexus. Through RNA sequencing analyses of constitutive and conditional knockdown adult mouse models, we reveal putative functional roles for OTX2 in adult choroid plexus function, including cell signaling and adhesion, and show that OTX2 regulates the expression of factors that are secreted into the cerebrospinal fluid, notably transthyretin. We also show that expression impacts choroid plexus immune and stress

responses, and affects splicing, leading to changes in the mRNA isoforms of proteins that are implicated in the oxidative stress response and DNA repair. Through mass spectrometry analysis of OTX2 protein partners in the choroid plexus, and in known non-cell-autonomous target regions, such as the visual cortex and subventricular zone, we identify putative targets that are involved in cell adhesion, chromatin structure, and RNA processing. Thus, OTX2 retains important roles for regulating choroid plexus function and brain homeostasis throughout life.
Int J Mol Sci, 2021; 22

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CODING THE IDENTITY OF A SINGLE SYNAPSE TYPE: THE CLIMBING FIBER/PURKINJE CELL SYNAPSE

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Collège de France, Cirb, Paris, France

Brain function is based on the establishment of highly stereotyped neuronal networks through precise and diverse synaptic contacts. While Sperry postulated the chemo-affinity hypothesis in the 1960s, implying a molecular combination for each synapse type, the existence and nature of this combination has not been demonstrated. We searched for the molecular combination underlying a synapse key for computation in the cerebellum and its associated motor and cognitive functions: the climbing fiber/Purkinje cell synapse. Combining neuron-specific gene expression profiling and quantitative transcriptomics in mice and bioinformatics analyses, we have identified a combination of four genes with a developmental profile and coding for proteins with structural properties coherent with their role in the specification of the climbing fiber/Purkinje cell synapse. Functional screening of these genes during postnatal development is currently performed to confirm their synaptogenic role. Our work will demonstrate the instructive role of this unique combination of presynaptic proteins for the specification of the climbing fiber/Purkinje cell synapse. This work will shed light on the rules controlling the establishment of proper network connectivity and brain function.

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INVESTIGATING THE MOLECULAR DIVERSITY OF COPII-DEPENDENT TRANSPORT IN CORTICAL NEURONS

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Neurons are the most highly compartmentalized and morphologically complex cells of the body. In particular, synapses are dynamic nanomachines composed by a unique repertoire of molecules arranged in multimolecular complexes. For this reason, their correct development and function require sophisticated mechanisms to target proteins and lipids to their site of action in the right amount at the right time. However, how this daunting task is achieved is largely unknown. Here, we focus on the role of the first stations of the secretory pathway, namely the Endoplasmic Reticulum (ER) and the Golgi Apparatus (GA), in the transport of newly synthetized neuronal proteins to their final destination. In particular, we investigate the role of the molecular diversity of SEC24, a component of the inner coat of COPII vesicles involved in cargo selection. To this aim, we developed a proteomic screen based on proximity-dependent biotinylation to identify the protein interaction networks of distinct SEC24 isoforms. Preliminary data obtained from heterologous cells and primary cultures of cortical neurons indicate that SEC24 proteins fused to the biotin ligase are enriched at the ER exit sites, interact with protein cargoes and, upon biotin administration, efficiently biotinylate proteins in an isoform-specific manner. Second, we are also investigating the subcellular localization of SEC24 isoforms in neuronal compartments to assess their potential role in directing cargoes to specific transport pathways. Together, our approaches may shed the light on the contribution of the early secretory pathway to neurodevelopment and unravel novel mechanisms underlying the formation and function of synaptic connections.

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DISSECTING THE CONTRIBUTION OF ASTROCYTES AND UPPER LAYER NEURONS TO HUMAN CORTICAL CIRCUIT DYNAMICS IN DOWN SYNDROME

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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[1]Imperial College London, Department Of Brain Sciences, London, United Kingdom, [2]The Blizard Institute, Barts and The London School of Medicine, Queen Mary University of London, Genomics And Child Health, London, United Kingdom, [3]Faculty of Veterinary Medicine of University of Zagreb, Anatomy, Histology And Embriology, Zagreb, Croatia, [4]Imperial College London, Institute Of Clinical Sciences, London, United Kingdom, [5]Duke-NUS Medical School, Neuroscience And Behavioural Disorders, Singapore, Singapore

Human cortical circuit assembly is critical to neurodevelopment and higher-order cognition, but our understanding of this process is limited *in vivo*. Much remains unknown about cortical circuit assembly failure in neurodevelopmental conditions such as Down Syndrome (DS). We recently used transplanted donor-derived induced pluripotent stem cells and longitudinal imaging to find that DS grafts have increased synaptic structural stability and decreased neuronal oscillatory activity. These changes could help explain some DS cognitive symptoms. Here, we investigate the mechanisms of such alterations, focusing on cell-type composition. Immunohistochemistry indicated that DS and isogenic control cortical grafts contain equivalent distributions of immature neurons (Pax6+, Ki67+) and deep layer excitatory neurons (Tbr1+, Ctip2+). However, we found an overproduction of astrocytes (GFAP+) and a reduction in upper layer cortical neurons (SATB2+) in DS grafts. We reasoned that differences in cell-type proportions could underlie previously detected changes in synaptic turnover and neural network activity. Therefore, we normalised the contribution of SATB2+ and GFAP+ cells by modifying induction and differentiation protocols. We monitored subsequent neural network activity and synaptic stability with 2-photon microscopy. Importantly, the turnover rate of axonal en-passant boutons at 3-5 months post-transplantation (mpt) and the frequency and amplitude of somatic calcium fluctuations at 1-3-5-7 mpt were not different between genotypes when comparable proportions of GFAP+ and SATB2+ cells were present. Future work will attempt to isolate individual contributions of GFAP+ and SATB2+ cells. Rescue experiments will be performed on each cell type separately, and effects on synaptic stability and neural activity will be analyzed.

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PSYCHOSIS RISK CANDIDATE ZNF804A - A KEY PLAYER IN SYNAPTOGENESIS BY REGULATING PROTEIN SYNTHESIS?

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Genome wide association studies identified a robust link for schizophrenia and variants of ZNF804A. Previous literature implicated ZNF804A in multiple cellular processes including protein synthesis, synapse maintenance and formation, and activity-dependent signalling. Further, gene expression peaks during the second trimester of foetal development indicating a critical role for the susceptibility gene during neurodevelopment. However, the exact mode of action mediating these processes remains to be elucidated. In this study, human induced pluripotent stem cell-derived immature neurons combined with a CRISPR/Cas9 approach were used to evaluate ZNF804A functioning during synaptogenesis. Bulk RNA sequencing of immature neurons carrying a mutation in the gene revealed a strong association with cell adhesion molecules. Interestingly, a high-throughput imaging approach showed that not only pre- and postsynaptic protein expression was affected by ZNF804A mutations but also expression of ribosomal proteins. Furthermore, translational efficiency is decreased in mutation lines indicating a crucial role for ZNF804A in translational control. This seems to be governed by disruptions of the Erk1/2 pathway leading to dysregulation of translation initiation factors such as ribosomal protein S6 and initiation factor 4E binding protein (4EBP). These findings indicate a critical role for ZNF804A in protein synthesis initiation by mediating Erk1/2 signalling cascades downstream of cell adhesion molecules. Determining specific neurobiological functioning of genetic risk factors may help to decipher the underlying aetiology of complex disorders such as schizophrenia and may provide targets for future therapeutic interventions.

Pubmed:
30172593: Sichlinger L, Cibelli E, Goldrick M, Mittal VA
Clinical correlates of aberrant conversational turn-taking in youth at clinical high-risk for psychosis.
Schizophr Res, 2019; 204

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THE HUMAN SPECIFIC GENE FRMPD2B REGULATES DENDRITIC BRANCHING AND SYNAPTIC DENSITY IN CORTICAL PYRAMIDAL NEURONS

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Human neurons of the neocortex are morphologically more complex than those of other species including great apes. Their dendrites are more ramified, they form more synaptic connections and develop over longer time scales. These features reflect human-specific regulations of neuronal morphogenesis and synaptic development. Yet their molecular underpinning is poorly understood. Here, we characterize the role of *FRMPD2B* (FERM and PDZ Domain containing 2B), a human-specific gene almost exclusively expressed in excitatory neurons of the neocortex. *FRMDP2B* is the product of a partial gene duplication that occurred during the transition between *Autralopithecus* and *Homo*. Using whole-length transcript nanopore sequencing, we show that *FRMPD2B* encodes a 320 amino-acids protein corresponding to the C-terminus of the ancestral copy FRMPD2A. When sparsely expressed in mouse layer 3 cortical pyramidal neurons, FRMPD2B increases dendritic branching and spine density. FRMPD2B localizes to and regulates endolysosomes, organelles that play a key role in proteostasis. Together, our results suggest that *FRMPD2B* has contributed to human neuron and cortical circuit evolution through unique regulations of the endolysosomal pathway.

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A MICROFLUIDIC BASED IN VITRO MODEL TO RECONSTRUCT THE CORTICOSTRIATAL SYNAPSE IN THE STUDY OF HUNTINGTON'S DISEASE

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Aim Huntington's disease (HD) is a severe neurodegenerative characterized by the selective loss of Medium Spiny Neurons (MSNs) within the Caudate/Putamen brain nuclei. These neurons promotes initiation of movements and are highly interconnected with several areas of the brain as the cortex. To better understand how this connection is degraded in HD and enable testing of potential new treatments, we have combined the advantages of microfluidic devices and human pluripotent stem cell (hPSC) differentiation, building a model that allows the *in vitro* study of the human pathology, by modelling the cortico-striatal circuit affected in HD. **Methods:** Our new microfluidic device was built by culturing hPSC-derived cortical cells and MSNs in different compartments interconnected by microchannels for axon isolation. **Results:** We showed that cortical and striatal neurons remain isolated in their respective compartments. Furthermore, we demonstrated that the two cultures can be seeded and maintained in parallel with two different differentiation protocols during a time long enough to allow the completion of hPSC differentiation into mature neurons and stablish synaptic connections. Moreover, the device correctly allows MSN differentiation from HD patient-derived hPSCs which survived and were targeted by glutamatergic projections, coming from the cortical compartment and passing through the microfluidic channels. **Conclusions:** Our microfluidic devices represent a suitable model for uncovering mechanisms of altered development in HD would allow a better understanding of early phases of the pathology and contribute to find out new therapeutic targets that could be addressed in its very premature stages to prevent the neurodegeneration of MSNs.

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INTERFERON GAMMA EXPOSURE OF HUMAN IPSC-DERIVED NEURONS ALTERS MAJOR HISTOCOMPATIBILITY COMPLEX I AND SYNAPSIN I PROTEIN EXPRESSION

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Inflammatory cytokines such as interferon gamma (IFNg) are thought to be an important link between maternal immune activation and disrupted brain and behavioral development. Previously, we showed that acute exposure of cultured human neurons and neuroprogenitors to IFNg results in altered cellular morphology and gene expression phenotypes that resemble those in neurodevelopmental disorders, including downregulated expression of presynaptic genes associated with synaptic vesicles. We further extend this work using a neurogenin 2 (*NGN2*) optimized inducible overexpression ioGlutamatergic cell line, which allows accelerated differentiation of glutamatergic neurons. We studied the effects of IFNg on synaptic gene and protein expression at a very early phase of synaptogenesis, finding multiple effects even in the absence of glial cells. We found using quantitative PCR that acute IFNg exposure results in significantly increased major histocompatibility complex I (*MHCI*) gene expression. Consistent with this, immunocytochemistry showed there was increased MHCI protein expression. MHCI is thought to regulate synapse number and plasticity. We also observed increased expression of complement component 4A (*C4A*) mRNA. In parallel, there was decreased synapsin I protein in the neurons, but no effect on expression of select synaptic genes. IFNg is known to act through JAK-STAT signaling, although several signaling pathways are thought to be activated in parallel. We have thus tested whether JAK-STAT signaling mediates these various protein and RNA-level changes. The *NGN2* inducible expression line could thus be used to further understand the intrinsic effects of IFNg on glutamatergic neurons and how this may contribute to neurodevelopmental disorder-associated phenotypes.

Pubmed:
32880858: Filova I, Dvorakova M, Bohuslavova R, Pavlinek A, Elliott KL, Vochyanova S, Fritzsch B, Pavlinkova G
Combined Atoh1 and Neurod1 Deletion Reveals Autonomous Growth of Auditory Nerve Fibers.
Ear development requires the transcription factors ATOH1 for hair cell differentiation and NEUROD1 for sensory neuron development. In addition, NEUROD1 negatively regulates Atoh1 gene expression. As we previously showed that deletion of the Neurod1 gene in the cochlea results in axon guidance defects and excessive peripheral innervation of the sensory epithelium, we hypothesized that some of the innervation defects may be a result of abnormalities in NEUROD1 and ATOH1 interactions. To characterize the interdependency of ATOH1 and NEUROD1 in inner ear development, we generated a new Atoh1/Neurod1 double null conditional deletion mutant. Through careful comparison of the effects of single Atoh1 or Neurod1 gene deletion with combined double Atoh1 and Neurod1 deletion, we demonstrate that NEUROD1-ATOH1 interactions are not important for the Neurod1 null innervation phenotype. We report that neurons lacking Neurod1 can innervate the flat epithelium without any sensory hair cells or supporting cells left after Atoh1 deletion, indicating that neurons with Neurod1 deletion do not require the presence of hair cells for axon growth. Moreover, transcriptome analysis identified genes encoding axon guidance and neurite growth molecules that are dysregulated in the Neurod1 deletion mutant. Taken together, we demonstrate that much of the projections of NEUROD1-deprived inner ear sensory neurons are regulated cell-autonomously.
Mol Neurobiol, 2020; 57

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SYNAPTIC RELEASE POTENTIATION AT AGING AUDITORY HAIR CELL RIBBON SYNAPSES

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Age-related hearing loss (ARHL or presbycusis), manifested by a decrease in speech intelligibility in noisy environment, is the most prevalent form of sensory disability in human populations. ARHL is often found associated with hyperacusis, a highly debilitating condition that can be defined as an exacerbated noise sensitivity. The mechanisms of ARHL-associated hyperacusis remain essentially unknown, in particular we don't know whether it finds its origin at peripheral or central synapses. Here, we characterized this aging process in inner hair cell (IHCs) ribbon synapses of C57BL/6J mice, a strain which is known to carry a cadherin23 mutation and experiences early hearing loss with age. These mice, while displaying a large increase in auditory thresholds due to 50 % loss of IHC afferent synaptic ribbons at middle age (postnatal day 365), paradoxically show enhanced acoustic startle reflex suggesting a hyperacusis-like response. Ca^{2+} imaging in aging IHCs indicated larger presynaptic Ca^{2+} microdomains and an increased capacity to sustain high rate of exocytosis. This exocytotic potentiation was linked to larger presynaptic ribbon structures, higher density of Ca^{2+} channels and larger postsynaptic AMPA GLUA2 receptor clusters. In addition, the fast repolarizing BK currents of IHCs, known to negatively control transmitter release, was severely decreased. In conclusions, our study brings novel evidence that points to the origin of ARHL and hyperacusis at the peripheral cochlear ribbon synapses, the very first synaptic afferent relay from cochlear hair cells whose function become abnormally potentiated with aging. This work is supported by the Fondation Pour l'Audition and Entendre SAS

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PHYSICAL ACTIVITY ADAPTS THE BDNF/TRKB SIGNALLING TOWARDS A MOLECULAR FATIGUE-RESISTANT PHENOTYPE AT THE NMJ

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Several motor units innervate the set of myocytes in one muscle to generate a complete contraction in accordance with its functional requirements. Exercise improves resistance to fatigue and reinforces neuroprotective mechanisms in central and peripheral nervous system. In particular, TrkB signalling is a retrograde neurotrophic signalling tightly related with this process at the neuromuscular junction (NMJ), as it is involved in ACh release control, influencing both the neurotrophic and synaptic control. This study analyses the adaptations of the BDNF/TrkB signalling in the skeletal muscle as an intermediate step between physical activity and morphological changes. To do it, B6SJLF1/J mice were trained between 70 and 115 days of age following either running or swimming protocols, 20 minutes, 5 days/week. Western blotting of plantaris muscle was used to characterize the full pathway and immunofluorescence served to morphometrically analyse NMJ and characterise motor neurons size and number in lumbar spinal cord sections. Results report that TrkB signalling shifts from a fast to a slow molecular phenotype in the plantaris muscle, proportionally to the intensity of the training protocol. In accordance, NMJ area also increased proportionally to the intensity of the training protocol. On the other hand, exercise did not modify soma size with exercise. Thus, TrkB signalling seems to mediate for the beneficial effects of exercise at the NMJ due to the triggering of a molecular shift towards a resistant phenotype in fatigable muscles that affects NMJ morphology and that can be useful to prevent neuromuscular atrophy in diseases like Amyotrophic Lateral Sclerosis.

Pubmed:

33925507: Just-Borràs L, Cilleros-Mañé V, Hurtado E, Biondi O, Charbonnier F, Tomàs M, Garcia N, Tomàs J, Lanuza MA
Running and Swimming Differently Adapt the BDNF/TrkB Pathway to a Slow Molecular Pattern at the NMJ.
Physical exercise improves motor control and related cognitive abilities and reinforces neuroprotective mechanisms in the nervous system. As peripheral nerves interact with skeletal muscles at the neuromuscular junction, modifications of this bidirectional communication by physical activity are positive to preserve this synapse as it increases quantal content and resistance to fatigue, acetylcholine receptors expansion, and myocytes' fast-to-slow functional transition. Here, we provide the intermediate step between physical activity and functional and morphological changes by analyzing the molecular adaptations in the skeletal muscle of the full BDNF/TrkB downstream signaling pathway, directly involved in acetylcholine release and synapse maintenance. After 45 days of training at different intensities, the BDNF/TrkB molecular phenotype of trained muscles from male B6SJLF1/J mice undergo a fast-to-slow transition without affecting motor neuron size. We provide further knowledge to understand how exercise induces muscle molecular adaptations towards a slower phenotype, resistant to prolonged trains of stimulation or activity that can be useful as therapeutic tools.
Int J Mol Sci, 2021; 22
31646358: Just-Borràs L, Hurtado E, Cilleros-Mañé V, Biondi O, Charbonnier F, Tomàs M, Garcia N, Tomàs J, Lanuza MA
Running and swimming prevent the deregulation of the BDNF/TrkB neurotrophic signalling at the neuromuscular junction in mice with amyotrophic lateral sclerosis.
Nerve-induced muscle contraction regulates the BDNF/TrkB neurotrophic signalling to retrogradely modulate neurotransmission and protect the neuromuscular junctions and motoneurons. In muscles with amyotrophic lateral sclerosis, this pathway is strongly misbalanced and neuromuscular junctions are destabilized, which may directly cause the motoneuron degeneration and muscular atrophy observed in this disease. Here, we sought to demonstrate (1) that physical exercise, whose recommendation has been controversial in amyotrophic lateral sclerosis, would be a good option for its therapy, because it normalizes and improves the altered neurotrophin pathway and (2) a plausible molecular mechanism underlying its positive effect. SOD1-G93A mice were trained following either running or swimming-based protocols since the beginning of

the symptomatic phase (day 70 of age) until day 115. Next, the full BDNF pathway, including receptors, downstream kinases and proteins related with neurotransmission, was characterized and motoneuron survival was analysed. The results establish that amyotrophic lateral sclerosis-induced damaging molecular changes in the BDNF/TrkB pathway are reduced, prevented or even overcompensated by precisely defined exercise protocols that modulate TrkB isoforms and neurotransmission regulatory proteins and reduce motoneuron death. Altogether, the maintenance of the BDNF/TrkB signalling and the downstream pathway, particularly after the swimming protocol, adds new molecular evidence of the benefits of physical exercise to reduce the impact of amyotrophic lateral sclerosis. These results are encouraging since they reveal an improvement even starting the therapy after the onset of the disease.
Cell Mol Life Sci, 2020; 77
30929165: Just-Borràs L, Hurtado E, Cilleros-Mañé V, Biondi O, Charbonnier F, Tomàs M, Garcia N, Lanuza MA, Tomàs J
Overview of Impaired BDNF Signaling, Their Coupled Downstream Serine-Threonine Kinases and SNARE/SM Complex in the Neuromuscular Junction of the Amyotrophic Lateral Sclerosis Model SOD1-G93A Mice.
Amyotrophic lateral sclerosis (ALS) is a chronic neurodegenerative disease characterized by progressive motor weakness. It is accepted that it is caused by motoneuron degeneration leading to a decrease in muscle stimulation. However, ALS is being redefined as a distal axonopathy, in that neuromuscular junction dysfunction precedes and may even influence motoneuron loss. In this synapse, several metabotropic receptor-mediated signaling pathways converge on effector kinases that phosphorylate targets that are crucial for synaptic stability and neurotransmission quality. We have previously shown that, in physiological conditions, nerve-induced muscle contraction regulates the brain-derived neurotrophic factor/tropomyosin-related kinase B (BDNF/TrkB) signaling to retrogradely modulate presynaptic protein kinases PKC and PKA, which are directly involved in the modulation of acetylcholine release. In ALS patients, the alteration of this signaling may significantly contribute to a motor impairment. Here, we investigate whether BDNF/TrkB signaling, the downstream PKC (cPKCβI, cPKCα, and nPKCε isoforms), and PKA (regulatory and catalytic subunits) and some SNARE/SM exocytotic machinery proteins (Munc18-1 and SNAP-25) are altered in the skeletal muscle of pre- and symptomatic SOD1-G93A mice. We found that this pathway is strongly affected in symptomatic ALS mice muscles including an unbalance between (I) BDNF and TrkB isoforms, (II) PKC isoforms and PKA subunits, and (III) Munc18-1 and SNAP-25 phosphorylation ratios. Changes in TrkB.T1 and cPKCβI are precociously observed in presymptomatic mice. Altogether, several of these molecular alterations can be partly associated with the known fast-to-slow motor unit transition during the disease process but others can be related with the initial disease pathogenesis.
Mol Neurobiol, 2019; 56
34199823: Garcia N, Lanuza MA, Tomàs M, Cilleros-Mañé V, Just-Borràs L, Duran M, Polishchuk A, Tomàs J
PKA and PKC Balance in Synapse Elimination during Neuromuscular Junction Development.
During the development of the nervous system, synaptogenesis occurs in excess though only the appropriate connections consolidate. At the neuromuscular junction, competition between several motor nerve terminals results in the maturation of a single axon and the elimination of the others. The activity-dependent release of transmitter, cotransmitters, and neurotrophic factors allows the direct mutual influence between motor axon terminals through receptors such as presynaptic muscarinic ACh autoreceptors and the tropomyosin-related kinase B neurotrophin receptor. In previous studies, we investigated the synergistic and antagonistic relations between these receptors and their downstream coupling to PKA and PKC pathways and observed a metabotropic receptor-driven balance between PKA (stabilizes multinnervation) and PKC (promotes developmental axonal loss). However, how much does each kinase contribute in the developmental synapse elimination process? A detailed statistical analysis of the differences between the PKA and PKC effects in the synapse elimination could help to explore this point. The present short communication provides this analysis and results show that a similar level of PKA inhibition and PKC potentiation would be required during development to promote synapse loss.
Cells, 2021; 10
34133802: Cilleros-Mañé V, Just-Borràs L, Polishchuk A, Durán M, Tomàs M, Garcia N, Tomàs JM, Lanuza MA
M and M mAChRs activate PDK1 and regulate PKC βI and ε and the exocytotic apparatus at the NMJ.
Neuromuscular junctions (NMJ) regulate cholinergic exocytosis through the M and M muscarinic acetylcholine autoreceptors (mAChR), involving the crosstalk between receptors and downstream pathways. Protein kinase C (PKC) regulates neurotransmission but how it associates with the mAChRs remains unknown. Here, we investigate whether mAChRs recruit the classical PKCβI and the novel PKCε isoforms and modulate their priming by PDK1, translocation and activity on neurosecretion targets. We show that each M and M mAChR activates the master kinase PDK1 and promotes a particular priming of the presynaptic PKCβI and ε isoforms. M recruits both primed-PKCs to the membrane and promotes Munc18-1, SNAP-25, and MARCKS phosphorylation. In contrast, M downregulates PKCε through a PKA-dependent pathway, which inhibits Munc18-1 synthesis and PKC phosphorylation. In summary, our results discover a co-dependent balance between muscarinic autoreceptors which orchestrates the presynaptic PKC and their action on ACh release SNARE-SM mechanism. Altogether, this molecular signaling explains previous functional studies at the NMJ and guide toward potential therapeutic

targets.
FASEB J, 2021; 35
32052889: Cilleros-Mañé V, Just-Borràs L, Tomàs M, Garcia N, Tomàs JM, Lanuza MA
The M muscarinic receptor, in association to M , regulates the neuromuscular PKA molecular dynamics.
Muscarinic acetylcholine receptor 1 subtype (M) and muscarinic acetylcholine receptor 2 subtype (M) presynaptic muscarinic receptor subtypes increase and decrease, respectively, neurotransmitter release at neuromuscular junctions. M involves protein kinase A (PKA), although the muscarinic regulation to form and inactivate the PKA holoenzyme is unknown. Here, we show that M signaling inhibits PKA by downregulating Cβ subunit, upregulating RIIα/β and liberating RIβ and RIIα to the cytosol. This promotes PKA holoenzyme formation and reduces the phosphorylation of the transmitter release target synaptosome-associated protein 25 and the gene regulator cAMP response element binding. Instead, M signaling, which is downregulated by M , opposes to M by recruiting R subunits to the membrane. The M and M reciprocal actions are performed through the anchoring protein A kinase anchor protein 150 as a common node. Interestingly, M modulation on protein expression needs M signaling. Altogether, these results describe the dynamics of PKA subunits upon M muscarinic signaling in basal and under presynaptic nerve activity, uncover a specific involvement of the M receptor and reveal the M /M balance to activate PKA to regulate neurotransmission. This provides a molecular mechanism to the PKA holoenzyme formation and inactivation which could be general to other synapses and cellular models.
FASEB J, 2020; 34
31817487: Lanuza MA, Just-Borràs L, Hurtado E, Cilleros-Mañé V, Tomàs M, Garcia N, Tomàs J
The Impact of Kinases in Amyotrophic Lateral Sclerosis at the Neuromuscular Synapse: Insights into BDNF/TrkB and PKC Signaling.
Brain-derived neurotrophic factor (BDNF) promotes neuron survival in adulthood in the central nervous system. In the peripheral nervous system, BDNF is a contraction-inducible protein that, through its binding to tropomyosin-related kinase B receptor (TrkB), contributes to the retrograde neuroprotective control done by muscles, which is necessary for motor neuron function. BDNF/TrkB triggers downstream presynaptic pathways, involving protein kinase C, essential for synaptic function and maintenance. Undeniably, this reciprocally regulated system exemplifies the tight communication between nerve terminals and myocytes to promote synaptic function and reveals a new view about the complementary and essential role of pre and postsynaptic interplay in keeping the synapse healthy and strong. This signaling at the neuromuscular junction (NMJ) could establish new intervention targets across neuromuscular diseases characterized by deficits in presynaptic activity and muscle contractility and by the interruption of the connection between nervous and muscular tissues, such as amyotrophic lateral sclerosis (ALS). Indeed, exercise and other therapies that modulate kinases are effective at delaying ALS progression, preserving NMJs and maintaining motor function to increase the life quality of patients. Altogether, we review synaptic activity modulation of the BDNF/TrkB/PKC signaling to sustain NMJ function, its and other kinases' disturbances in ALS and physical and molecular mechanisms to delay disease progression.
Cells, 2019; 8
31652775: Garcia N, Balañà C, Lanuza MA, Tomàs M, Cilleros-Mañé V, Just-Borràs L, Tomàs J
Opposed Actions of PKA Isozymes (RI and RII) and PKC Isoforms (cPKCβI and nPKCε) in Neuromuscular Developmental Synapse Elimination.
During neuromuscular junction (NMJ) development, synapses are produced in excess. By sensing the activity-dependent release of ACh, adenosine, and neurotrophins, presynaptic receptors prompt axonal competition and loss of the unnecessary axons. The receptor action is mediated by synergistic and antagonistic relations when they couple to downstream kinases (mainly protein kinases A and C (PKA and PKC)), which phosphorylate targets involved in axonal disconnection. Here, we directly investigated the involvement of PKA subunits and PKC isoforms in synapse elimination.
Cells, 2019; 8
30607888: Simó A, Cilleros-Mañé V, Just-Borràs L, Hurtado E, Nadal L, Tomàs M, Garcia N, Lanuza MA, Tomàs J
nPKCε Mediates SNAP-25 Phosphorylation of Ser-187 in Basal Conditions and After Synaptic Activity at the Neuromuscular Junction.
Protein kinase C (PKC) and substrates like SNAP-25 regulate neurotransmission. At the neuromuscular junction (NMJ), PKC promotes neurotransmitter release during synaptic activity. Thirty minutes of muscle contraction enhances presynaptic PKC isoform levels, specifically cPKCβI and nPKCε, through retrograde BDNF/TrkB signaling. This establishes a larger pool of these PKC isoforms ready to promote neuromuscular transmission. The PKC phosphorylation site in SNAP-25 has been mapped to the serine 187 (Ser-187), which is known to enhance calcium-dependent neurotransmitter release in vitro. Here, we localize SNAP-25 at the NMJ and investigate whether cPKCβI and/or nPKCε regulate SNAP-25 phosphorylation. We also investigate whether nerve and muscle cell activities regulate differently SNAP-25 phosphorylation and the involvement of BDNF/TrkB signaling. Our results demonstrate that nPKCε isoform is essential to positively regulate SNAP-25 phosphorylation on Ser-187 and that muscle contraction prevents it. TrkB and cPKCβI do not regulate SNAP-25 protein level

or its phosphorylation during neuromuscular activity. The results provide evidence that nerve terminals need both pre- and postsynaptic activities to modulate SNAP-25 phosphorylation and ensure an accurate neurotransmission process.
Mol Neurobiol, 2019; 56
29946239: Simó A, Just-Borràs L, Cilleros-Mañé V, Hurtado E, Nadal L, Tomàs M, Garcia N, Lanuza MA, Tomàs J
BDNF-TrkB Signaling Coupled to nPKCε and cPKCβI Modulate the Phosphorylation of the Exocytotic Protein Munc18-1 During Synaptic Activity at the Neuromuscular Junction.
Munc18-1, a neuron-specific member of the Sec1/Munc18 family, is involved in neurotransmitter release by binding tightly to syntaxin. Munc18-1 is phosphorylated by PKC on Ser-306 and Ser-313 which reduces the amount of Munc18-1 able to bind syntaxin. We have previously identified that PKC is involved in neurotransmitter release when continuous electrical stimulation imposes a moderate activity on the NMJ and that muscle contraction through TrkB has an important impact on presynaptic PKC isoforms levels, specifically cPKCβI and nPKCε. Therefore, the present study was designed to understand how Munc18-1 phosphorylation is affected by (1) synaptic activity at the neuromuscular junction, (2) nPKCε and cPKCβI isoforms activity, (3) muscle contraction , and (4) the BDNF/TrkB signaling in a neuromuscular activity-dependent manner. We performed immunohistochemistry and confocal techniques to evidence the presynaptic location of Munc18-1 in the rat diaphragm muscle. To study synaptic activity, we stimulated the phrenic nerve (1 Hz, 30 min) with or without contraction (abolished by μ-conotoxin GIIIB). Specific inhibitory reagents were used to block nPKCε and cPKCβI activity and to modulate the tropomyosin receptor kinase B (TrkB). Main results obtained from Western blot experiments showed that phosphorylation of Munc18-1 at Ser-313 increases in response to a signaling mechanism initiated by synaptic activity and directly mediated by nPKCε. Otherwise, cPKCβI and TrkB activities work together to prevent this synaptic activity-induced Munc18-1 phosphorylation by a negative regulation of cPKCβI over nPKCε. Therefore, a balance between the activities of these PKC isoforms could be a relevant cue in the regulation of the exocytotic apparatus. The results also demonstrate that muscle contraction prevents the synaptic activity-induced Munc18-1 phosphorylation through a mechanism that opposes the TrkB/cPKCβI/nPKCε signaling.
Front Mol Neurosci, 2018; 11

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BOARD NUMBER: S03-230

THE NEURONAL VARIANT OF LYSINE SPECIFIC DEMETHYLASE 1 MODULATES DOPAMINERGIC RESPONSE TO PSYCHOSTIMULANT

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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LSD1 (KDM1a) is an enzyme that, associated with co-repressors such as CoREST (RCOR1), erases activating epigenetic marks to repress transcription of a plethora of neuronal genes. LSD1 has a neuronal variant, neuroLSD1, which differs from the ubiquitous LSD1 (uLSD1) by a 4-amino acid microdomain. neuroLSD1 exerts less transcriptional repressive capacity than uLSD1, allowing it to be proposed as a dominant-negative for the actions of uLSD1. neuroLSD1 and uLSD1 coexist in a ratio of about 50%, which is rapidly and transiently modified by neuronal activity. NeuroLSD1-null mice were shown to be less responsive to stress and exhibit altered synaptic plasticity, suggesting that neuroLSD1 might regulate dopaminergic neurotransmission. To test this idea, we studied basal and amphetamine (AMPH)-induced dopamine efflux in the nucleus accumbens (NAc) of wild-type (WT) and neuroLSD1-null mice. Fast scan cyclic voltammetry (FSCV) and microdialysis were used to assess dopamine efflux. Basal synaptic dopamine levels in the NAc were similar in WT mice and null for neuroLSD1. However, AMPH-induced dopamine efflux was lower in NAc from neuroLSD1-null mice than in WT mice, as studied with FSCV and microdialysis. The tissue content of dopamine in the striatum was similar in both genotypes. Taken together, our data show that neuroLSD1 is involved in the dopaminergic response to psychostimulants.

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THE ROLE OF ACTIVE ZONE-ATTACHED SYNAPTIC RIBBONS IN VESICLE RELEASE DURING CONE PHOTORECEPTOR DEVELOPMENT

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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The presence of synaptic ribbons at photoreceptor terminal active zones (AZ) was first shown more than half a century ago, but their exact function in regulating synaptic vesicle (SV) release is still unsolved. Here, we have investigated the effect of the attachment of the synaptic ribbon to the AZ on the physiological properties of SV release during postnatal cone photoreceptor development. We used a combination of patch-clamp recordings of mouse cone photoreceptors and postsynaptic horizontal cells alongside electrical stimulation and pharmacological interventions. The developmental switch from free-floating to AZ-attached cone photoreceptor synaptic ribbons was measured using a combination of electron microscopy and immunocytochemistry. The main change occurred between the day when synaptic ribbons first appear at the AZ and the eye-opening period. Our measurements suggest that the increase in the number of AZ-attached synaptic ribbons increases the readily releasable SV pool in cone photoreceptors. We also found that SV release kinetics, SV replenishment rate and Ca^{2+} sensitivity of evoked SV release were significantly altered through the developmental appearance of AZ-attached synaptic ribbons. These results suggest that the synaptic ribbon has an important role in shaping the physiological parameters of evoked SV release, which probably help to satisfy the high demand for continuous and fast signaling to postsynaptic neurons during visual information processing.

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BOARD NUMBER: S03-232

MOLECULAR AND ULTRASTRUCTURAL ANALYSIS OF DOPAMINE SYNAPSES CONTACTING CORTICO-STRIATAL GLUTAMATERGIC SYNAPSES

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

Paul Lapios[1], Vincent Paget-Blanc[1], Robin Anger[2], Etienne Herzog[1], Rémi Fronzes[2], David Perrais[1]
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Dopamine is an essential neuromodulator involved in reward and motor control in the striatum which are altered in Parkinson's disease and addiction. Dopamine contained in vesicles is released by axons and binds to metabotropic receptors to modulate glutamate and GABA transmission onto Spiny Projection Neurons (SPNs). However, the basic features of dopamine release sites, e.g. their location relative to receptors and other neuronal processes, are still largely unknown. We have shown recently with fluorescent tagging of dopaminergic neurons, sorting of synaptosomes with flow cytometry (FASS) and quantitative analysis with immunocytochemistry, that a vast proportion of dopaminergic varicosities contact specifically glutamatergic terminals, which are themselves contacting the SPNs. We named these multipartite structures **Hub synapses**. (https://doi.org/10.1101/2020.02.18.952978) Here, our goal is to characterize the ultrastructure of hub synapses. First, we have used super-resolution microscopy (**Tau-STED**) to show that the presence of dopaminergic terminals next to cortico-striatal glutamatergic synapses correlates with an increase of the presynaptic markers VGLUT1, Bassoon and Rim1a and an altered composition of the post-synaptic proteins PSD95, Homer1a and Synaptopodin. Second, we used cryo-correlative light electron microscopy (**Cryo-CLEM**) to unravel the ultrastructure of dopaminergic-cortico-striatal hub synapses and quantify fundamental parameters, such as vesicle density, vesicle size, size of adhesion between the various hub elements. Our findings will help understanding the basic mechanisms of dopaminergic modulation in the basal ganglia.

Pubmed:
34410010: Blumenstock S, Schulz-Trieglaff EK, Voelkl K, Bolender AL, Lapios P, Lindner J, Hipp MS, Hartl FU, Klein R, Dudanova I
Fluc-EGFP reporter mice reveal differential alterations of neuronal proteostasis in aging and disease.
The cellular protein quality control machinery is important for preventing protein misfolding and aggregation. Declining protein homeostasis (proteostasis) is believed to play a crucial role in age-related neurodegenerative disorders. However, how neuronal proteostasis capacity changes in different diseases is not yet sufficiently understood, and progress in this area has been hampered by the lack of tools to monitor proteostasis in mammalian models. Here, we have developed reporter mice for in vivo analysis of neuronal proteostasis. The mice express EGFP-fused firefly luciferase (Fluc-EGFP), a conformationally unstable protein that requires chaperones for proper folding, and that reacts to proteotoxic stress by formation of intracellular Fluc-EGFP foci and by reduced luciferase activity. Using these mice, we provide evidence for proteostasis decline in the aging brain. Moreover, we find a marked reaction of the Fluc-EGFP sensor in a mouse model of tauopathy, but not in mouse models of Huntington's disease. Mechanistic investigations in primary neuronal cultures demonstrate that different types of protein aggregates have distinct effects on the cellular protein quality control. Thus, Fluc-EGFP reporter mice enable new insights into proteostasis alterations in different diseases.
EMBO J, 2021; 40

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BOARD NUMBER: S03-233

CHLORIDE-MEDIATED MODULATION OF GLUTAMATE RELEASE FROM PRESYNAPTIC NERVE TERMINALS ON HYPOTHALAMIC NEURONS.

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Regulation of synaptic neurotransmission is essential for neural information processing but the complex mechanisms that control neurotransmitter release from presynaptic terminals are only partially understood. Here we show that spontaneous release of glutamate onto preoptic hypothalamic neurons from rat is, paradoxically, potentiated by activation of presynaptic $GABA_A$- and glycine receptors, which are known as main mediators of neuronal inhibition in the CNS. Application of selective agonists of $GABA_A$- and glycine receptors (GABA, muscimol and glycine, correspondingly) significantly increased the frequency of glutamate-mediated spontaneous excitatory postsynaptic currents (EPSCs) recorded *in vitro* with the patch-clamp technique. A depolarizing efflux of Cl^- via presynaptic $GABA_A$- and glycine receptors was best explaining this effect, suggesting a Cl^- concentration in the presynaptic terminals that is higher than previously estimated for the preoptic neuronal cell bodies. The $GABA_A$- and glycine-receptor induced potentiation of EPSC frequency was sensitive to the Na^+-channel blocker tetrodotoxin, suggesting that GABA- and glycine-evoked presynaptic depolarization *per se* was insufficient, but depended on local presynaptic action potentials, to trigger glutamate release. Blockade of Cl^- extrusion using VU 0463271, a selective antagonist of potassium-chloride cotransporter type 2 (KCC2), further augmented glutamate release in response to presynaptic activation of $GABA_A$- and glycine receptors, suggesting that KCC2 is present in presynaptic terminals and that KCC2-mediated regulation of presynaptic Cl^- homeostasis may play an important role in the control of neurotransmitter release.

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BOARD NUMBER: S03-234

ACTIVITY-DRIVEN SYNAPTIC TRANSLOCATION OF LGI1 CONTROLS EXCITATORY NEUROTRANSMISSION.

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Physiological function of the mammalian brain relies on the activity of excitatory and inhibitory synapses in neural circuits to precisely encode information driving cognition and behavior. Genetic alterations in proteins involved in sustaining neuronal function and structure alter the equilibrium, driving an imbalance between excitation and inhibition causing epilepsy. Leucine Rich Glioma Inactivated 1 (LGI1) protein is a secreted protein forming a synaptic bridge, and when dysfunctional causes epilepsy. Here we sought to understand how LGI1 is secreted in neurons and how its abundance at the synapse affects neuronal function. Our hypothesis is that in healthy neurons LGI1 abundance in the synapse could be controlled and modulated by neuronal activity, impacting neuronal function. To approach this long-standing question, we developed a novel optical tool named LGI1-pHluorin, which allows monitoring LGI1 surface localization and trafficking in firing synapses. Using this tool, we found that neuronal activity drives LGI1 translocation at presynaptic terminals, leading to an increased accumulation of LGI1 at the synaptic cleft of firing synapses. Increased surface LGI1 levels at single synapses result in synapse-specific reductions of action potential (AP)-driven Ca2+ entry and glutamate release, suggesting that activity-driven molecular rearrangement of LGI1 trans-synaptic bridges modulates excitatory transmission. We discovered that LGI1 vesicles had distinct properties and were not synaptic vesicles and are likely different organelles. These experiments reveal a critical role for neuronal activity in shaping the molecular architecture of trans-synaptic connections, framing future investigations into the molecular control of neurotransmission by LGI1 and other trans-synaptic molecules.

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PARALLEL PROCESSING OF QUICKLY AND SLOWLY MOBILIZED RESERVE VESICLES IN HIPPOCAMPAL SYNAPSES

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Synaptic vesicles within presynaptic terminals are thought to be contained within multiple readily releasable and reserve pools. A widespread premise has been that quickly and slowly mobilized reserves are connected in series. However, results from our electrophysiological studies of short-term synaptic plasticity at excitatory hippocampal synapses were not consistent with the first principles, and suggested instead that quickly and slowly mobilized reserves instead undergo exocytosis in parallel. Using FM-dyes, we now confirm the basic concept that synaptic vesicles are segregated into quickly and slowly mobilized reserves, and the prediction that the two types of reserves are mobilized in parallel. No intermixing was seen over minutes during heavy or light stimulation, providing additional constraints on future models. And, we observed extensive heterogeneity among synapses in the relative sizes of quickly and slowly mobilized reserves that may be relevant to understanding the role of synapses in information processing and storage..

Pubmed:
33462194: Mejias R, Rodriguez-Gotor JJ, Niwa M, Krasnova IN, Adamczyk A, Han M, Thomas GM, Xi ZX, Huganir RL, Pletnikov MV, Sawa A, Cadet JL, Wang T
Increased novelty-induced locomotion, sensitivity to amphetamine, and extracellular dopamine in striatum of Zdhhc15-deficient mice.
Novelty-seeking behaviors and impulsivity are personality traits associated with several psychiatric illnesses including attention deficits hyperactivity disorders. The underlying neural mechanisms remain poorly understood. We produced and characterized a line of knockout mice for zdhhc15, which encodes a neural palmitoyltransferase. Genetic defects of zdhhc15 were implicated in intellectual disability and behavioral anomalies in humans. Zdhhc15-KO mice showed normal spatial learning and working memory but exhibited a significant increase in novelty-induced locomotion in open field. Striatal dopamine content was reduced but extracellular dopamine levels were increased during the habituation phase to a novel environment. Administration of amphetamine and methylphenidate resulted in a significant increase in locomotion and extracellular dopamine levels in the ventral striatum of mutant mice compared to controls. Number and projections of dopaminergic neurons in the nigrostriatal and mesolimbic pathways were normal. No significant change in the basal palmitoylation of known ZDHHC15 substrates including DAT was detected in striatum of zdhhc15 KO mice using an acyl-biotin exchange assay. These results support that a transient, reversible, and novelty-induced elevation of extracellular dopamine in ventral striatum contributes to novelty-seeking behaviors in rodents and implicate ZDHHC15-mediated palmitoylation as a novel regulatory mechanism of dopamine in the striatum.
Transl Psychiatry, 2021; 11

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-236

HYDROXYNORKETAMINE AND KETAMINE CONVERGE ON REGULATION OF SYNAPTIC VESICLE RELEASE COMPETENCE VIA INDEPENDENT MECHANISMS

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Major depressive disorder (MDD) is a highly prevalent and severe psychiatric disorder. Conventional medication with classical antidepressants targeting the monoaminergic system have varying degrees of efficacy in ameliorating disease symptoms, as it requires prolonged treatment and leave many patients treatment resistant. Ketamine emerged as a potent and rapidly acting antidepressant recently. It acts by modulating glutamatergic neurotransmission, via its action on NMDA and AMPA receptors. However, psychotomimetic and addictive properties of ketamine cannot be disregarded. Hydroxynorketamine (HNK) is the major metabolite of ketamine and was also shown to successfully mitigate depressive symptoms in rodent models. HNK lacks the psychotic side effects of ketamine, making it a promising candidate in neuropsychiatric research. Nonetheless, the know-how regarding effects of HNK and ketamine on critical presynaptic events that regulate neurotransmitter release remain ambiguous. In the present study, we monitored effects of HNK and ketamine on synaptic vesicle (SV) recycling at the level of individual synapses using optical imaging of electrically active mature cultured cortical neurons. We employed pharmacological and genetic tools to dissect the ketamine and HNK-induced presynaptic signalling. Our experiments revealed that ketamine and HNK, albeit acting on specific receptors and downstream signalling, converge on identical regulation of synaptic vesicle pools, directly controlling their release competence in glutamatergic and GABAergic synapses.These data provide an important mechanistic understanding of the cellular signaling that mediates antidepressant effect of ketamine and its metabolite HNK.

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INDUCTION OF PHASIC NEUROTRANSMITTER RELEASE VIA PRESYNAPTIC GABAB RECEPTORS ON MEDIAL HABENULA TERMINALS

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Presynaptic $GABA_B$ receptors reduce neurotransmitter release at most synapses. The only known exception is the synaptic connection from the medial habenula (MHb) to the interpeduncular nucleus (IPN), where presynaptic $GABA_B$ receptors induce a massive increase in neurotransmitter release. To identify the mechanisms underlying this unusual effect of $GABA_B$ receptors, we performed whole-cell patch clamp recordings in IPN neurons in mouse brain slices and measured postsynaptic currents in response to electrical stimulation of MHb axons at room temperature. To study the structural correlate of $GABA_B$ receptor-mediated potentiation, we performed timed high-pressure freezing after optogenetic stimulation of MHb terminals (“Flash and Freeze”) in ChAT-ChR2-EYFP mice. Using freeze-fracture replication after “Flash and Freeze”, we immunolabeled vesicle-associated proteins in the presynaptic active zone. Our electrophysiological results indicate that application of the $GABA_B$ receptor agonist baclofen (1 µM) induces a transition in the mode of neurotransmitter release from tonic to phasic release. This transition was mediated by a 3.5 ± 0.6-fold increase in the readily releasable vesicle pool size, as well as a 2.3 ± 0.3-fold increase in release probability (n=17 cells; 7 mice). Structurally, phasic release was associated with a 3.5 ± 0.4-fold increase in the density of docked synaptic vesicles. Molecularly, we identified two vesicle-associated proteins selectively involved in tonic and phasic release, with synaptoporin mediating tonic release augmentation and Ca^{2+}-dependent activator for protein secretion 2 (CAPS2) mediating the priming of phasic vesicles. **Conclusions:** We identified an unexpected two-pool mechanism underlying the potentiation of neurotransmitter release following $GABA_B$ receptor activation on MHb terminals.

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AGEING-ASSOCIATED ALTERATIONS IN BDNF/TRKB PATHWAY INVOLVING PKC AND SYNAPTIC TARGETS OF THE NEUROMUSCULAR JUNCTION.

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

Marta Balanyà Segura, Erica Hurtado, Laia Just Borràs, Aleksandra Polishchuk, Víctor Cilleros Mañé, Marta Tomàs, Josep Tomàs, Maria Angel Lanuza, Neus García
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Ageing of the population in many countries around the world is one of the important challenges of modern society. Age-related conditions, such as sarcopenia or the impaired neuromuscular transmission are, currently, among the leading causes of morbidity and death worldwide. The bidirectional communication between the nervous system and muscles is fundamental for their health. Despite that the molecular mechanisms behind its regulation remain largely unknown, several signaling pathways that control neurotransmission, are crucial for this interaction. The muscle-derived Brain-derived-neurotrophic-factor (BDNF) acting through its receptor, Tropomyosin-related-kinase-B (TrkB) is well known for its neuroprotective functions and has been demonstrated that enhances presynaptic downstream effectors PKC isoforms and exocytotic proteins of synaptic vesicles (SNAP25 and Munc18-1) at the neuromuscular junction (NMJ). However, whether this signaling pathway is compromised in the aged neuromuscular system has not been analysed yet. To address it, we analyze by Western Blot how BDNF/TrkB pathway could be altered in aged Extensor digitorum longus (EDL) rat muscles. Results obtained show that in EDL muscles this signaling cascade is strongly perturbed in aging. Phosphorylation status of PKC isoforms and their exocytotic targets of synaptic vesicles that directly regulate neuromuscular activity are decreased in aged muscles. These results are in concordance with the age-related impairment of the neurotransmission. Thus, considering these results, therapeutic strategies recovering this signaling pathway function should improve NMJ functionality, slowing down the aging of the neuromuscular system and, thus, improving quality of life of aged people. Funding: PID2019-106332GB-I00,2017PFR-URV-B2-85,2017SGR704, PRE2020-092084,2021-FI-B00755,LE1511314-2014PEJ-04,LE1911587-2019PEJ-04.

Pubmed:
33684132: Zandawala M, Nguyen T, Balanyà Segura M, Johard HAD, Amcoff M, Wegener C, Paluzzi JP, Nässel DR
A neuroendocrine pathway modulating osmotic stress in Drosophila.
Environmental factors challenge the physiological homeostasis in animals, thereby evoking stress responses. Various mechanisms have evolved to counter stress at the organism level, including regulation by neuropeptides. In recent years, much progress has been made on the mechanisms and neuropeptides that regulate responses to metabolic/nutritional stress, as well as those involved in countering osmotic and ionic stresses. Here, we identified a peptidergic pathway that links these types of regulatory functions. We uncover the neuropeptide Corazonin (Crz), previously implicated in responses to metabolic stress, as a neuroendocrine factor that inhibits the release of a diuretic hormone, CAPA, and thereby modulates the tolerance to osmotic and ionic stress. Both knockdown of Crz and acute injections of Crz peptide impact desiccation tolerance and recovery from chill-coma. Mapping of the Crz receptor (CrzR) expression identified three pairs of Capa-expressing neurons (Va neurons) in the ventral nerve cord that mediate these effects of Crz. We show that Crz acts to restore water/ion homeostasis by inhibiting release of CAPA neuropeptides via inhibition of cAMP production in Va neurons. Knockdown of CrzR in Va neurons affects CAPA signaling, and consequently increases tolerance for desiccation, ionic stress and starvation, but delays chill-coma recovery. Optogenetic activation of Va neurons stimulates excretion and simultaneous activation of Crz and CAPA-expressing neurons reduces this response, supporting the inhibitory action of Crz. Thus, Crz inhibits Va neurons to maintain osmotic and ionic homeostasis, which in turn affects stress tolerance. Earlier work demonstrated that systemic Crz signaling restores nutrient levels by promoting food search and feeding. Here we additionally propose that Crz signaling also ensures osmotic homeostasis by inhibiting release of CAPA neuropeptides and suppressing diuresis. Thus, Crz ameliorates stress-associated physiology through systemic modulation of both peptidergic neurosecretory cells and the fat body in Drosophila.
PLoS Genet, 2021; 17


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PKA-DEPENDENT SNAP-25 AND SYN-1 PHOSPHORYLATION ARE DIFFERENTLY REGULATED BY THE NEUROMUSCULAR ACTIVITY.

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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At the neuromuscular junction (NMJ), PKA enhances ACh release maybe phosphorylating targets from the synaptic vesicle (SV) exocytotic cycle, although this is unknown. Synaptosomal associated protein (SNAP-25), which is part of the SNARE complex, and Synapsin-1 (Syn-1), which controls the release of the SV from the cytoskeleton to promote their docking, are PKA targets that highly influence the SV exocytosis. Although ACh release mechanism is regulated by presynaptic stimulus and retrogradely by the resulting muscle contraction, PKA regulation by the pre- and postsynaptic activities had not been studied until now. To separate the effect of presynaptic activity from that of the resulting muscle contraction on PKA subunits and its activity, the rat phrenic nerve was stimulated (1Hz, 30min) with and without contraction (abolished by μ-conotoxin GIIIB). PKA was pharmacologicaly inhibitited (H-89) to assess the interactions of PKA and its targets (SNAP-25 and Syn-1). We used Western blotting and cytosol/membrane translocation by subcellular fractionation. We demonstrate that the pre- and postsynaptic activities differentially regulate the PKA subunit dynamics to be catalytic active at the NMJ and to phosphorylate SNAP-25 and Syn-1. Synaptic Cβ subunit regulated by RIIβ or RIIα subunits controls activity-dependent phosphorylation of SNAP-25 and Syn-1 respectively. Muscle contraction retrogradly downregulates presynaptic activity-induced pSyn-1 while that enhances pSNAP-25 T138. We hypothesize that both actions could coordinately contribute to decrease the neurotransmitter release at the NMJ. These results provide a molecular mechanism of the bidirectional communication between nerve terminals and muscle cell to balance the optimal process of ACh release. Funding:PID2019-106332GB-I00,2017SGR704,PRE2020-092084,2021-FI-B00755,LE1511314-2014PEJ-04,LE1911587-2019PEJ-04.

Pubmed:

29894840: Ukraintseva YV, Liaukovich KM, Polishchuk AA, Martynova OV, Belov DA, Simenel ES, Meira E Cruz M, Nizhnik AN
Slow-wave sleep and androgens: selective slow-wave sleep suppression affects testosterone and 17α-hydroxyprogesterone secretion.
Levels of steroid hormones such as androgens and cortisol exhibit circadian variation, and their fluctuations are related to the sleep-wake cycle. Currently, the functional role of different stages of sleep in steroid hormone secretion remains unclear. The present study aims to explore the effect of slow-wave sleep (SWS) suppression on morning levels of cortisol and androgens.
Sleep Med, 2018; 48
34199823: Garcia N, Lanuza MA, Tomàs M, Cilleros-Mañé V, Just-Borràs L, Duran M, Polishchuk A, Tomàs J
PKA and PKC Balance in Synapse Elimination during Neuromuscular Junction Development.
During the development of the nervous system, synaptogenesis occurs in excess though only the appropriate connections consolidate. At the neuromuscular junction, competition between several motor nerve terminals results in the maturation of a single axon and the elimination of the others. The activity-dependent release of transmitter, cotransmitters, and neurotrophic factors allows the direct mutual influence between motor axon terminals through receptors such as presynaptic muscarinic ACh autoreceptors and the tropomyosin-related kinase B neurotrophin receptor. In previous studies, we investigated the synergistic and antagonistic relations between these receptors and their downstream coupling to PKA and PKC pathways and observed a metabotropic receptor-driven balance between PKA (stabilizes multinnervation) and PKC (promotes developmental axonal loss). However, how much does each kinase contribute in the developmental synapse elimination process? A detailed statistical analysis of the differences between the PKA and PKC effects in the synapse elimination could help to explore this point. The present short communication provides this analysis and results show that a similar level of PKA inhibition and PKC potentiation would be required during development to promote synapse loss.
Cells, 2021; 10

34133802: Cilleros-Mañé V, Just-Borràs L, Polishchuk A, Durán M, Tomàs M, Garcia N, Tomàs JM, Lanuza MA
M and M mAChRs activate PDK1 and regulate PKC βI and ε and the exocytotic apparatus at the NMJ.
Neuromuscular junctions (NMJ) regulate cholinergic exocytosis through the M and M muscarinic acetylcholine autoreceptors (mAChR), involving the crosstalk between receptors and downstream pathways. Protein kinase C (PKC) regulates neurotransmission but how it associates with the mAChRs remains unknown. Here, we investigate whether mAChRs recruit the classical PKCβI and the novel PKCε isoforms and modulate their priming by PDK1, translocation and activity on neurosecretion targets. We show that each M and M mAChR activates the master kinase PDK1 and promotes a particular priming of the presynaptic PKCβI and ε isoforms. M recruits both primed-PKCs to the membrane and promotes Munc18-1, SNAP-25, and MARCKS phosphorylation. In contrast, M downregulates PKCε through a PKA-dependent pathway, which inhibits Munc18-1 synthesis and PKC phosphorylation. In summary, our results discover a co-dependent balance between muscarinic autoreceptors which orchestrates the presynaptic PKC and their action on ACh release SNARE-SM mechanism. Altogether, this molecular signaling explains previous functional studies at the NMJ and guide toward potential therapeutic targets.
FASEB J, 2021; 35

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TWO DISEASE-CAUSING MUTATIONS IN SNAREOPATHY GENE SNAP25 CAUSE DISTINCT NEUROTRANSMISSION IMPAIRMENTS IN HUMAN NEURONS

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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The SNARE proteins and additional regulatory proteins make up the fusion machinery responsible for precisely timed fusion of synaptic vesicles containing neurotransmitter. To date, human pathogenic *de novo* mutations have been reported for all eight core components of this fusion machinery – the so called SNAREopathy genes – giving rise to diverse neurodevelopmental syndromes, including neurodevelopmental delay, intellectual disability and epileptic encephalopathy. Despite major progress in genetics, the diagnosis and prognosis for patients carrying a SNAREopathy gene mutation are still at a rudimentary stage. There is an urgent need to establish experimental systems, ideally using human neurons, which can help unravel the underlying disease mechanisms and improve patient stratification and prognosis, and be used as a tool to develop successful treatments. In this study we generated human glutamatergic neurons by lentiviral NGN2-expression in CRISPR/Cas9-engineered iPSC lines carrying patient mutations, V48F or I67N in the SNAREopathy gene *Snap25.* Performing whole-cell patch-clamp recordings on these neurons we were able to detect disease-relevant changes in synaptic transmission and provide insight into the underlying disease mechanisms. Our results demonstrate that these pathogenic mutations lead to distinct synaptic phenotypes in human excitatory neurons. The V48F mutation leads to an increase in the frequency of spontaneous release events while impairing Ca^{2+}-triggered release, whereas the I67N mutation causes a decrease in both spontaneous and evoked fusion of synaptic vesicles and affects short-term synaptic plasticity. This indicates that disease-causing mutations in the same SNAREopathy gene can have distinct effects on neurotransmission, which can be detected in iPSC-derived human glutamatergic neurons.

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SNARING A SNAREOPATHY – CHARACTERIZING THE RELEASE PHENOTYPE OF PATHOGENIC I192T AND I192N SNAP25 VARIANTS

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Pathogenic mutations in genes encoding the proteins of the neuronal synaptic transmission machinery cause diseases referred to as “SNAREopathies”, which lead to neurodevelopmental disorders. Such a SNAREopathy gene is *Snap25* coding for the **syn**aptosomal-**a**ssociated **p**rotein of **25**kDa (SNAP25). Two disease-associated SNAP25 variants are I192T and I192N, which are substitutions of the same hydrophobic layer +5 residue that is critical for SNARE complex formation and stability. According to clinical reports, I192N is lethal within the first year of life whereas I192T-patients are currently in their early adolescence. To investigate these differences in terms of disease severity, the two SNAP25 variants were expressed in self-innervating glutamatergic hippocampal neurons from E18 SNAP25-knockout (KO) mice using a lentiviral expression-system. Their release phenotype was characterized by measuring synaptic transmission using patch-clamp electrophysiology. Homozygous and heterozygous conditions were mimicked by expressing the SNAP25 variants alone or in a 1:1 ratio with wild-type (WT) SNAP25, respectively. When co-expressed with the WT protein, I192N-expressing neurons, but not I192T, displayed significantly reduced evoked EPSC amplitudes. In SNAP25 KO neurons only expressing the mutation, both variants exhibited a reduced evoked EPSC amplitudes with I192N being the most severely affected. Assessing protein expression levels of I192T and I192N revealed no apparent difference compared to WT SNAP25. This indicates that the I192T exhibits mainly a loss-of-function phenotype whereas I192N exerts a dominant-negative effect, without affecting expression levels. These data show that different mutations of the same amino acid position in SNAP25 can lead to phenotypes that vary both in severity and dominance.

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NEUROPLASTIC EFFECTS OF SEROTONERGIC PSYCHEDELICS AT THE PRESYNAPSE

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Serotonergic psychedelics, including psilocybin, LSD or DMT, have been widely used to induce altered states of consciousness both in traditional rites of indigenous people as well as recreational drugs in modern societies. Recent studies suggest these substances pose significant potential for treatment of psychiatric disorders including depression, post-traumatic stress disorder or addiction. The present study focuses on the molecular mechanisms involved in the acute and persistent effects of serotonergic psychedelics on neuronal networks. We used rat cortical cultures to evaluate changes in synaptic transmission and neuroplasticity induced by these drugs. To visualize neurotransmitter release at the level of individual synapses, we monitored synaptic vesicle recycling using live-cell imaging of electrically stimulated cells expressing SypHy (reporter created by fusion of vesicle protein synaptophysin with pH-sensitive fluorescent protein). Next, we used pharmacological approach to dissect the receptors and signalling pathways involved in the drug induced regulations. Our results show that psychedelics induce time-dependent changes in synaptic transmission and suggest a converging mechanism with the serotonergic neuromodulatory effect. Taken together, this study further unravels the underlying mechanisms of psychedelics-induced neuroplasticity and underscores the importance of the presynaptic component in its action.

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SPONTANEOUS NEUROTRANSMISSION IS REGULATED BY NETRIN SIGNALLING

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Spontaneous neurotransmitter release events, also known as miniature neurotransmission or ‘minis’, occur when synaptic vesicles are released in the absence of an action potential. Since their discovery over 70 years ago, miniature events have been confirmed as a universal feature of fast chemical synapses. The functional requirement for spontaneous release events however has been more recalcitrant, but recent studies have established the singular and essential necessity of miniature neurotransmission for the structural maturation and maintenance of synapses. Here, we now demonstrate that the frequency of spontaneous release events can be regulated autonomously of evoked neurotransmission. We establish that a novel presynaptic Netrin signalling pathway bidirectionally regulates the rate of miniature events and influences adult synaptic structural maintenance. Our results challenge the historical view of miniature neurotransmission as spontaneous noise and support that miniature events are an independently regulated parallel mode of transsynaptic chemical communication with unique activites.

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STUDYING SNARE MUTATIONS UNDERLYING BRAIN DYSFUNCTION AND DEVELOPMENTAL DISORDERS USING ZEBRAFISH

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Neurotransmission is a complex process involving the generation, endocytosis and exocytosis and recycling of vesicles. Exocytosis depends on soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins located on the vesicular and presynaptic membranes forming a complex that drives membrane fusion. At synapses, the vesicular SNARE mediating neurotransmitter release is a Vesicle Associated Membrane Protein (VAMP) homologue - VAMP2 in most neurons. Human mutations in *VAMP2* have been recently discovered and lead to autistic and epileptic phenotypes among others. The effect of *VAMP2* mutations on individual cell and circuit development and function, leading to such phenotypes, is unclear. We employ zebrafish, owing to their highly conserved SNAREs and to their transparency, which enables non-invasive, high resolution live imaging of the intact vertebrate nervous-system. Here, using time-lapse imaging, we analyse vamp2-mediated exocytosis in individual neurons as well as glia by sparsely expressing a wildtype or mutant vamp2-pHluorin reporter. In this reporter, pH-sensitive GFP pHluorin is fused to the C-terminus of *vamp2* and localized in acidic vesicle lumen which fluoresces upon pH neutralization on exocytosis. Using this reporter, we are able to measure how the frequency, amplitude, and duration of exocytosis is affected by the three mutations causing the most severe phenotypes in humans. Our in vivo approach enables elucidation of role of *VAMP2*, linking molecular defects to cellular and circuit dysfunction. Understanding the pathophysiology of neurological conditions driven by SNARE mutations in the zebrafish in vivo model may lead to the identification of therapeutic targets to ameliorate these conditions in the future.

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THE ROLE OF ACTIVITY-DEPENDENT PHOSPHORYLATION IN THE PRESYNAPTIC FUNCTION OF α-SYNUCLEIN

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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Repeated rounds of synaptic vesicle exocytosis and endocytosis play a critical role in the maintenance of neurotransmission. α-Synuclein, a protein extensively studied for its role in Parkinson's disease, is a known modulator of this synaptic vesicle cycle. However, its exact function remains unclear. This has been elucidated by examining post-translational modifications as a regulatory mechanism of the protein's presynaptic function. Through a phosphoproteomic screen of primary hippocampal neuron lysates we identified two α-synuclein residues that become more highly phosphorylated with neuronal activity. These novel activity-dependent phosphorylation sites were studied by transfecting α-synuclein knockout hippocampal neuron cultures with variants of α-synuclein that mimicked or abolished phosphorylation at these residues. Neurons were subjected to live cell imaging and changes in fluorescence intensity quantified synaptic vesicle and protein trafficking in real time. The presence of different α-synuclein phosphomutants impacted the distribution between plasma membrane and synaptic vesicles of the SNARE protein synaptobrevin-2, a known binding partner of α-synuclein crucial for efficient synaptic vesicle fusion. We found that the phosphorylation state of one of the identified residues specifically affected the endocytic retrieval of synaptobrevin-2 during neuronal activity. This constitutes the first evidence that dynamic, activity-dependent phosphorylation of α-synuclein modulates synaptic vesicle protein trafficking. Loss of this form of synaptic vesicle cycle regulation in disease may interfere with efficient neurotransmission, especially considering that α-synuclein is sequestered into Lewy bodies in Parkinson's disease and Lewy body dementia. As such, disruption of activity-dependent α-synuclein phosphorylation has the potential to contribute to the underlying pathogenesis of these synucleinopathies.

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QUANTIFYING THE SYNAPTIC CALCIUM-BINDING KINETICS OF SYNAPTOTAGMIN-1, THE CALCIUM SENSOR FOR TRANSMITTER RELEASE IN THE FOREBRAIN

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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The Ca^{2+} sensitivity of transmitter release is a major determinant of synaptic fidelity and plasticity. Two Synaptotagmin (Syt) isoforms, Syt1 and Syt2, are the main Ca^{2+} sensors triggering fast release in the brain; however, only Ca^{2+} binding to Syt2, the dominant isoform in the hindbrain, has been studied in detail. For Syt1, the dominating sensor in the forebrain, similar quantitative detail from brain synapses is not available at present. To quantify the Ca^{2+}-binding kinetics of Syt1 in the context of the intact release machinery we adapted a method combining Ca^{2+}-uncaging, two-photon G/R Ca^{2+}-imaging and patch-clamp electrophysiology to pairs of connected layer 5 pyramidal neurons in the S1 somatosensory cortex of mature mice. To define the local intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) at the release sensor, $[Ca^{2+}]_i$ was uniformly elevated in the presynaptic terminal from a caged Ca^{2+} compound by brief UV-flashes. Changes in the presynaptic G/R fluorescence were measured from individual boutons by point-mode two-photon imaging and converted to $d[Ca^{2+}]_i$ based on cuvette calibrations. The corresponding EPSCs were recorded and synaptic delays and deconvolution-based release rates (RR) were quantified. Release started at $d[Ca^{2+}]_i$ above ~1.5 µM and peak RR increased until $d[Ca^{2+}]_i$ of ~25 µM, saturating thereafter with no substantial further increase up to $d[Ca^{2+}]_i$ of ~120 µM. Synaptic delays decreased concomitantly. In comparison to Syt2, the slope of the $[Ca^{2+}]_i$-dependency of Syt1-triggered release was shallower and saturated at higher $[Ca^{2+}]_i$. A model with reduced forward-binding rates is capable of predicting our experimental results.

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AREA-SPECIFIC DIFFERENTIATION OF NEOCORTICAL SYNAPTIC COUPLING DISTANCES

POSTER SESSION 03 - SECTION: SYNAPTIC FUNCTION AND NEUROTRANSMISSION

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The physical coupling distance between presynaptic Ca^{2+} channels and transmitter-filled synaptic vesicles is a fundamental determinant of their release probability (p_v). Investigations at different excitatory synapses in different parts of the matured brain, including brainstem, cerebellum and neocortex, indicate that highly reliable synapses processing sensory information operate with tight coupling, while highly plastic excitatory synapses in the hippocampus were found to use loose coupling. Hence, it appears that in the mature brain tight coupling is favored by reliable synapses and loose coupling by highly plastic synapses. To probe this hypothesis at the same type of synapses within the same part of the matured brain, we focused on two functionally distinct areas of the neocortex, the prefrontal (PFC) and the somatosensory (S1) cortex and analyzed coupling distances at synapses between pyramidal neurons in layer 2/3 (L2/3PNs) and layer 5 (L5PNs). We preformed whole-cell recordings from L5PNs in acute slices and stimulated connected L2/3PNs extracellularly. Synapses in PFC showed paired-pulse facilitation, while synapses in S1 possessed paired-pulse depression, indicating that p_v in PFC is lower than in S1. The Ca^{2+} chelator EGTA-AM decreased EPSCs in PFC significantly to 66% of the control value (P=0.03), whereas EPSCs in S1 remained unchanged (102%; P=0.9). These findings suggest that L2/3PN to L5PN synapses in PFC operate with loose coupling as opposed to tight coupling in S1. Our results show that coupling distances of the same synapses differ between cortical areas, thus, providing evidence that the presynaptic nanostructure is adapted to its cortex-area related function.

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OPTICAL MANIPULATION AND INTERROGATION OF GLUN2B-NMDA RECEPTORS IN THE BRAIN

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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NMDA receptors (NMDARs) are glutamate-gated ion channel receptors broadly expressed in the brain playing a crucial role in synaptic transmission and plasticity. They are tetrameric complexes usually incorporating two GluN1 and two GluN2 subunits. There are 4 types of GluN2 subunits (GluN2A-D), leading to multiple subpopulations of NMDARs with unique biophysical, pharmacological and signaling properties. Understanding the physiological role of each of these subpopulations is of great interest to develop specific therapies to counteract the deleterious effects of their dysfunction, since both their hyper- and hypo-activation are linked to neurological and psychiatric diseases. However, *in vivo* targeting of specific NMDAR subpopulations is currently limited by the weak target specificity and spatio-temporal resolution of classical pharmacological agents. By combining the power of light, genetics and molecular pharmacology, optogenetic pharmacology can overcome these limitations. Using this approach, we developed an innovative photoswitchable tool (Opto-2B) to selectively and reversibly potentiate (up to 3-fold) the activity of GluN2B-NMDARs with light. Using this tool, we investigated the subcellular and temporal regulation of synaptic and extrasynaptic GluN2B-NMDAR expression during development in hippocampal brain slices, as well as the role of these receptors in hippocampal synaptic plasticity. By allowing the control of GluN2B-NMDARs with unrivaled molecular, cellular and sub-cellular specificity, the Opto2B tool should allow unraveling the long-debated roles of GluN2B receptors in synapse and brain function, and offer novel strategies for the treatment of pathologies linked to NMDAR hypofunction such as schizophrenia or age-dependent cognitive decline.

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OPTICAL CONTROL OF GLUN2B-NMDA RECEPTORS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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NMDA receptors (NMDARs) are glutamate-gated ion channels playing a central role in synaptic transmission and plasticity but also involved in many neuropsychiatric disorders. NMDARs are tetramers usually composed of two GluN1 and two GluN2 subunits. There are four GluN2 subunits (GluN2A-D), resulting in many receptor subtypes having distinct anatomical, biophysical, pharmacological and signaling properties. Understanding the functional role of these individual subtypes is fundamental to develop strategies to counteract the deleterious effects of NMDAR deregulation. NMDARs containing the GluN2B subunit (GluN2B-NMDARs) play key roles in the brain, with evidence for both pro-cognitive and pro-excitotoxic actions. Yet these roles are still under debate. Methodologies commonly used to study GluN2B-NMDARs, either pharmacological or genetic, have important shortcomings, limiting their use in native tissues. Taking advantage of the unrivaled spatio-temporal resolution of light, optopharmacology, a technique based on the use of photosensitive ligands, allows overcoming these barriers. We developed OptoNAM-3, a photoswitchable negative allosteric modulator selective for GluN2B-NMDARs that can reversibly alternate between a *trans* and a *cis* configuration with light. We found that *trans*-OptoNAM-3 is a potent inhibitor of GluN2B-NMDARs, while *cis*-OptoNAM-3 is inactive, allowing fast and robust modulation of GluN2B-NMDAR activity with light. We furthermore demonstrate a photosensitive action of OptoNAM-3 on glutamate-induced neuronal toxicity in cultured neurons as well as *in vivo* on the locomotion of Xenopus tadpoles. By acting as a light-operated drug enabling precise and reversible photocontrol of endogenous GluN2B-NMDARs, OptoNAM-3 should advance our understanding of the role of this NMDAR class in brain function and dysfunction.

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BOARD NUMBER: S03-250

PHOTOCONTROL OF NMDA RECEPTORS WITH SUBUNIT STOICHIOMETRY RESOLUTION

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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A current challenge lies in understanding the molecular determinants of neurotransmitter receptor functional diversity in the brain. NMDA receptors (NMDARs) are a class of glutamate−gated channels involved in synaptic transmission and plasticity, but also in several neurological and psychiatric diseases. NMDARs are tetramers usually composed of two GluN1 and two GluN2 subunits encoded by four different genes (GluN2A-D), resulting in a large number of receptor subtypes having distinct anatomical, biophysical, pharmacological and signaling properties. The three major NMDAR populations in the adult forebrain are 2A/2A and 2B/2B di-heteromers (containing two identical GluN2 subunits) and 2A/2B tri-heteromers (containing one GluN2A and one GluN2B subunit), whose relative involvement in processes like synaptic plasticity or neurotoxicity are still highly debated. Current genetic and pharmacological approaches, limited in specificity and spatio-temporal resolution, cannot target each NMDAR population in isolation. Optogenetic pharmacology, on the other hand, which combines the power of light, genetics and pharmacology, allows controlling receptors and ion channels with unrivaled spatio-temporal, cellular and molecular specificity. We have developed in the lab the first optopharmacological tool to selectively potentiate 2B/2B di-heteromers with light (Opto2B). The specificity of Opto2B even goes beyond NMDAR molecular nature, since it allowed us to independently probe the synaptic and extrasynaptic populations of 2B/2B NMDARs in cortical brain slices. By allowing control of an NMDAR subpopulation with the most exquisite molecular and subcellular resolution so far, the Opto2B tool should bring light to the debated role of 2B/2B NMDARs in the brain and the translational potential of their potentiation.

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BOARD NUMBER: S03-251

CONTRIBUTION OF AMBIENT GLUTAMATE AND GLUTAMATE TRANSPORTERS TO RETINAL GANGLION CELL VULNERABILITY IN EXPERIMENTAL MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Glutamate transporter (GluT)-mediated neurotransmitter uptake allows for low, but relevant, ambient glutamate levels in neuronal tissue which can activate extrasynaptic NMDARs (esNMDARs). In pathological situations, esNMDARs have been shown to be major initiators of neurodegeneration. Here we analysed the regulation of ambient glutamate around retinal ganglion cells (RGCs). These cells express consistent pools of esNMDARs, and are vulnerable during early stages of multiple sclerosis, possibly triggered by tonic excitation of esNMDARs. Patch clamp electrophysiology was performed on rat retinal whole-mount preparations to study αON- and αOFF-RGC subtypes, since we previously demonstrated αOFF-RGCs to be more vulnerable than αON-RGCs to degeneration during experimental autoimmune encephalomyelitis (EAE), a disease model of multiple sclerosis. αRGCs were classified according to their ON/OFF light responses and ambient glutamate-induced tonic currents were measured in both healthy and EAE conditions. Under these conditions, tonic currents were observed in both αON and αOFF RGC subtypes, which were partially blocked by application of the NMDAR antagonist MK-801. Perfusion of the retina with TBOA, a pan-GluT inhibitor, induced an increase in the tonic current of between 50-150 fold. However, both the magnitude of the TBOA-induced currents and the relative esNMDAR-mediated components were different between αON/OFF-RGC subtypes in healthy and EAE conditions. These data suggest the physiological presence of ambient glutamate around αRGCs. Impaired GluT function under neuroinflammatory conditions may lead to an increase of ambient glutamate causing over-activation of esNMDARs. A differential proportion of activated NMDARs between the αON/OFF-RGC subtypes could underlie their type-specific vulnerability in multiple sclerosis.

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BOARD NUMBER: S03-252

INVESTIGATION OF GLUTAMATE RECEPTOR MODULATION BY INTEGRAL MEMBRANE PROTEINS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Ionotropic glutamate receptors are tetrameric ligand-gated cation channels that are responsible for synaptic signal transmission. They are known to be modulated via auxiliary subunits and various chaperone proteins. The investigation of new potential auxiliary subunits is therefore very interesting. It has been observed in our lab that some members of the claudin protein family are able to interact with homomeric α-amino-3-hydroxy-4-methylisoxazole-5-propionic acid receptors (AMPARs) and alter their current amplitudes. However, AMPARs in vivo mostly form heteromers. Therefore, the goal of the study was to investigate the potential modulatory effects of claudins and characterize their possible properties as auxiliary subunits for heteromeric AMPARs. Besides their effects on receptor steady-state current amplitudes, channel properties such as desensitization were also addressed. cRNAs of AMPARs and claudins were injected into *Xenopus laevis* oocytes in different combinations, and electrophysiological measurements using the two-electrode voltage clamp method were performed to compare the modulation of homomeric and heteromeric receptors when coexpressed with varying amounts of auxiliary proteins. Our data indicate that in some of the heteromeric receptor subunit combinations higher potentiation is observable in comparison to homomeric subunits. However, some of the investigated claudins elicit higher potentiation when the expression level of the receptor is low and obtained currents are moderate in size. Our results illustrate that the current amplitudes of heteromeric AMPARs are distinctly affected by several of the investigated claudins and depend on the level of expressed receptor. The latter finding hints at the importance of a defined stoichiometric expression of claudins and AMPARs.

Pubmed:
35046772: Becic A, Leifeld J, Shaukat J, Hollmann M
Tetraspanins as Potential Modulators of Glutamatergic Synaptic Function.
Tetraspanins (Tspans) comprise a membrane protein family structurally defined by four transmembrane domains and intracellular N and C termini that is found in almost all cell types and tissues of eukaryotes. Moreover, they are involved in a bewildering multitude of diverse biological processes such as cell adhesion, motility, protein trafficking, signaling, proliferation, and regulation of the immune system. Beside their physiological roles, they are linked to many pathophysiological phenomena, including tumor progression regulation, HIV-1 replication, diabetes, and hepatitis. Tetraspanins are involved in the formation of extensive protein networks, through interactions not only with themselves but also with numerous other specific proteins, including regulatory proteins in the central nervous system (CNS). Interestingly, recent studies showed that Tspan7 impacts dendritic spine formation, glutamatergic synaptic transmission and plasticity, and that Tspan6 is correlated with epilepsy and intellectual disability (formerly known as mental retardation), highlighting the importance of particular tetraspanins and their involvement in critical processes in the CNS. In this review, we summarize the current knowledge of tetraspanin functions in the brain, with a particular focus on their impact on glutamatergic neurotransmission. In addition, we compare available resolved structures of tetraspanin family members to those of auxiliary proteins of glutamate receptors that are known for their modulatory effects.
Front Mol Neurosci, 2021; 14

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-253

PROTEINS OF THE TETRASPANIN FAMILY AS POTENTIAL MODULATORS OF AMPA RECEPTORS.

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Glutamate is the major excitatory neurotransmitter in the brain where it acts via glutamate-gated ion channels. Dysfunction of glutamate receptors is connected to various disorders such as Parkinson´s and Alzheimer´s disease. This work is focused on one subfamily of ionotropic glutamate receptors, the so-called AMPA receptors. These tetrameric receptor complexes are functionally modulated by several auxiliary proteins, including TARPs (transmembrane AMPA receptor regulatory proteins). Based on the structural similarity of TARPs and proteins from the large tetraspanin family, as well as arising evidence of tetraspanin importance in synapses, five different tetraspanins were tested for their potential modulatory effects on AMPA receptors. Experiments were performed using the *Xenopus* oocyte heterologous expression system and two-electrode voltage clamp electrophysiology. Tetraspanin cDNAs, isolated from human brain tissue were in vitro-transcribed to obtain cRNAs that were injected into oocytes. Whole-cell glutamate-activated steady-state current amplitudes of eight different AMPA receptor subunit combinations were recorded and compared to currents in oocytes where additionally tetraspanin cRNAs were expressed. Further, tetraspanins and glutamate receptors were tagged with green and red fluorescent proteins to observe their subcellular localization in the oocyte using confocal microscope. The strongest modulatory effects observed were that Tspan28 and Tspan30 significantly increase glutamate-activated currents of GluA4(Q)flip, while Tspan28 decreases the currents of GluA1(Q)flip and GluA2(Q)i+GluA2(R)flip channels. Furthermore, Tspan7 increases the currents of GluA1(Q)flip up to 3-fold. Our electrophysiological data demonstrates that the investigated tetraspanins clearly modulate AMPA receptor amplitudes, while our confocal microscopy results confirm colocalization of tetraspanins and receptors in the plasma membrane.

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35046772: Becic A, Leifeld J, Shaukat J, Hollmann M
Tetraspanins as Potential Modulators of Glutamatergic Synaptic Function.
Tetraspanins (Tspans) comprise a membrane protein family structurally defined by four transmembrane domains and intracellular N and C termini that is found in almost all cell types and tissues of eukaryotes. Moreover, they are involved in a bewildering multitude of diverse biological processes such as cell adhesion, motility, protein trafficking, signaling, proliferation, and regulation of the immune system. Beside their physiological roles, they are linked to many pathophysiological phenomena, including tumor progression regulation, HIV-1 replication, diabetes, and hepatitis. Tetraspanins are involved in the formation of extensive protein networks, through interactions not only with themselves but also with numerous other specific proteins, including regulatory proteins in the central nervous system (CNS). Interestingly, recent studies showed that Tspan7 impacts dendritic spine formation, glutamatergic synaptic transmission and plasticity, and that Tspan6 is correlated with epilepsy and intellectual disability (formerly known as mental retardation), highlighting the importance of particular tetraspanins and their involvement in critical processes in the CNS. In this review, we summarize the current knowledge of tetraspanin functions in the brain, with a particular focus on their impact on glutamatergic neurotransmission. In addition, we compare available resolved structures of tetraspanin family members to those of auxiliary proteins of glutamate receptors that are known for their modulatory effects.
Front Mol Neurosci, 2021; 14
28684664: Börger V, Bremer M, Ferrer-Tur R, Gockeln L, Stambouli O, Becic A, Giebel B
Mesenchymal Stem/Stromal Cell-Derived Extracellular Vesicles and Their Potential as Novel Immunomodulatory Therapeutic Agents.
Extracellular vesicles (EVs), such as exosomes and microvesicles, have been identified as mediators of a newly-discovered intercellular communication system. They are essential signaling mediators in various physiological and pathophysiological processes. Depending on their origin, they fulfill different functions. EVs of mesenchymal stem/stromal cells (MSCs) have been found to promote comparable therapeutic activities as MSCs themselves. In a variety of in vivo models, it has been observed that they suppress pro-inflammatory processes and reduce oxidative stress and fibrosis. By switching pro-

inflammatory into tolerogenic immune responses, MSC-EVs very likely promote tissue regeneration by creating a pro-regenerative environment allowing endogenous stem and progenitor cells to successfully repair affected tissues. Accordingly, MSC-EVs provide a novel, very promising therapeutic agent, which has already been successfully applied to humans. However, the MSC-EV production process has not been standardized, yet. Indeed, a collection of different protocols has been used for the MSC-EV production, characterization and application. By focusing on kidney, heart, liver and brain injuries, we have reviewed the major outcomes of published MSC-EV in vivo studies.
Int J Mol Sci, 2017; 18

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BOARD NUMBER: S03-254

MOLECULAR MECHANISMS UNDERLYING PLASTICITY OF GLUA3-CONTAINING AMPA-RECEPTORS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Background: Pyramidal neurons in the CA1 region of the hippocampus mainly express two types of AMPARs: GluA1/2s and GluA2/3s. Unlike GluA1/2s, GluA2/3s are predominantly in a closed state under basal conditions. However, upon a strong increase in intracellular cAMP levels, the GluA2/3s can convert to an open state. We previously found that this effect of cAMP on GluA2/3s is dependent on Ras. **Aim:** Investigate which signaling cascade(s) are responsible for GluA3-dependent plasticity. **Methods**: After ≥7 days *in vitro* electrophysiology was performed in the CA1 of mouse organotypic hippocampal cultures. Sindbis virus was used to (over)express various proteins (e.g. GFP-PTEN, SEP-GluA3) for 48 hours before patching/imaging. **Results:** The two main signaling cascades downstream of Ras are Erk and PI3K. Inhibition of the Erk pathway did not prevent the opening of GluA2/3s, while inhibiting PI3K did, suggesting that the PI3K pathway is important for the opening of GluA2/3s. Overexpression of PTEN, an inhibitor of the PI3K pathway, decreased synaptic currents in a GluA3-dependent manner and prevented the opening of GluA2/3s. PTEN does so without affecting the synaptic fraction of GluA2/3s, suggesting that PTEN affects the opening of the channel, rather than trafficking. **Conclusions**: Together these experiments demonstrate that activation of the PI3K pathway leads to GluA3-dependent plasticity, while activation of PTEN blocks this potentiation. Understanding how PI3K and PTEN affect GluA2/3s is important for unraveling the mechanisms behind GluA3 plasticity, and is especially relevant for Alzheimer's disease research, as both GluA2/3s and PTEN have been implicated in this disease.

Pubmed:
34077732: Dore K, Carrico Z, Alfonso S, Marino M, Koymans K, Kessels HW, Malinow R
PSD-95 protects synapses from β-amyloid.
Beta-amyloid (Aβ) depresses excitatory synapses by a poorly understood mechanism requiring NMDA receptor (NMDAR) function. Here, we show that increased PSD-95, a major synaptic scaffolding molecule, blocks the effects of Aβ on synapses. The protective effect persists in tissue lacking the AMPA receptor subunit GluA1, which prevents the confounding synaptic potentiation by increased PSD-95. Aβ modifies the conformation of the NMDAR C-terminal domain (CTD) and its interaction with protein phosphatase 1 (PP1), producing synaptic weakening. Higher endogenous levels or overexpression of PSD-95 block Aβ-induced effects on the NMDAR CTD conformation, its interaction with PP1, and synaptic weakening. Our results indicate that increased PSD-95 protects synapses from Aβ toxicity, suggesting that low levels of synaptic PSD-95 may be a molecular sign indicating synapse vulnerability to Aβ. Importantly, pharmacological inhibition of its depalmitoylation increases PSD-95 at synapses and rescues deficits caused by Aβ, possibly opening a therapeutic avenue against Alzheimer's disease.
Cell Rep, 2021; 35

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BOARD NUMBER: S03-255

IMMUNOCYTOCHEMICAL LOCALIZATION OF AMPA GLUTAMATE RECEPTORS SUBTYPE GLUR2&3 IN THE SQUID OPTIC LOBE

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Glutamate is a major excitatory neurotransmitter in the cephalopod visual system. The α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptors are known to mediate relatively fast ionotropic glutamate signaling and have four different subtypes. In cephalopod central nervous systems, optic lobes (OL) process visual inputs and are involved in learning and memory. The purpose of present study is to identify immunocytochemical localization of AMPA glutamate receptor subtype 2 and 3 (GluR2&3)-immunoreactive (IR) neurons in the OL of pacific flying squid, *Tordarodes pacificus*. GluR2&3-IR neurons were predominantly located in the tangential zone of medulla of OL. Morphologically diverse GluR2&3-IR neurons were observed, including multipolar stellate, multipolar round/oval, small unipolar round/oval, and horizontal types. Medium-to-large sized GluR2&3-IR neurons were prevalently observed. The diameter and area of the neuron ranged between 13.63–29.74 μm (20.18 ± 3.22 μm) and 145.83–694.72 μm^2 (328.39 ± 106.98 μm^2), respectively (mean ± standard deviation). The distribution patterns and cell morphologies of calcium binding proteins (CBPs), calbindin-D28K, calretinin, and parvalbumin-IR neurons were similar to those of GluR2&3-IR neurons. However, none of these CBPs-IR neurons were colocalized with GluR2&3-IR neurons. The result showed specific localization and diverse types of GluR2&3-IR neurons in squid OL. The present findings further complemented glutamatergic visual systems in cephalopod and provide some insights for understanding their vision.

Pubmed:

35052772: Kwon KM, Lee MJ, Chung HS, Pak JH, Jeon CJ
The Organization of Somatostatin-Immunoreactive Cells in the Visual Cortex of the Gerbil.
Somatostatin (SST) is widely expressed in the brain and plays various, vital roles involved in neuromodulation. The purpose of this study is to characterize the organization of SST neurons in the Mongolian gerbil visual cortex (VC) using immunocytochemistry, quantitative analysis, and confocal microscopy. As a diurnal animal, the Mongolian gerbil provides us with a different perspective to other commonly used nocturnal rodent models. In this study, SST neurons were located in all layers of the VC except in layer I; they were most common in layer V. Most SST neurons were multipolar round/oval or stellate cells. No pyramidal neurons were found. Moreover, 2-color immunofluorescence revealed that only 33.50%, 24.05%, 16.73%, 0%, and 64.57% of SST neurons contained gamma-aminobutyric acid, calbindin-D28K, calretinin, parvalbumin, and calcium/calmodulin-dependent protein kinase II, respectively. In contrast, neuropeptide Y and nitric oxide synthase were abundantly expressed, with 80.07% and 75.41% in SST neurons, respectively. Our immunocytochemical analyses of SST with D and D dopamine receptors and choline acetyltransferase, α and β nicotinic acetylcholine receptors suggest that dopaminergic and cholinergic fibers contact some SST neurons. The results showed some distinguishable features of SST neurons and provided some insight into their afferent circuitry in the gerbil VC. These findings may support future studies investigating the role of SST neurons in visual processing.
Biomedicines, 2022; 10
32490536: Lee MJ, Kwon KM, Lee WT, Kim GH, Jeon CJ
Immunocytochemical localization of tyrosine hydroxylase in the visual cortex of the microbat, Rhinolophus ferrumequinum.
In order to enhance our understanding of bat vision, we investigated tyrosine hydroxylase (TH)-immunoreactive (IR) fibers in the visual cortex of the microbat.
Folia Histochem Cytobiol, 2020; 58

FENS Forum 2022
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BOARD NUMBER: S03-256

INVESTIGATIONS ON THE MODULATION OF GLUTAMATE RECEPTOR FUNCTION BY ACCESSORY PROTEINS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Ionotropic glutamate receptors (iGluRs) are ligand-gated cation channels and key players of central nervous system (CNS) synaptic function. They include, among others, AMPA and NMDA receptors, whose interplay provides the molecular basis for higher-level brain functions such as learning and memory formation. For AMPARs in particular, many interacting proteins are already known, which often share a similar transmembrane topology, and which have a significant impact on physiological receptors. Connexins and pannexins have a topology very similar to many of these proteins. Canonical connexins are involved in the formation of gap junctions, although many members of this family have not yet been functionally characterized. Even less is known about pannexins. Given the similar topology of known auxiliary proteins compared to connexins and pannexins, it is conceivable that some members of these two families may also function as modulatory subunits of iGluRs. Hence, the aim of this project is to identify and subsequently characterize possible new accessory proteins of iGluRs in the connexin and pannexin families. Therefore, all genes of the connexin and pannexin families expressed in the CNS have been isolated and will subsequently be electrophysiologically investigated for possible receptor-modulating properties, by co-expression with AMPARs and NMDARs using the two-electrode voltage-clamp technique. Although preliminary data from the two pannexins and connexins tested so far showed only minor changes of receptor-mediated currents, the insights gained from further experiments may enrich our knowledge not only in the field of iGluRs, but also with respect to potentially novel functions of certain connexins and pannexins.

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Tetraspanins as Potential Modulators of Glutamatergic Synaptic Function.
Tetraspanins (Tspans) comprise a membrane protein family structurally defined by four transmembrane domains and intracellular N and C termini that is found in almost all cell types and tissues of eukaryotes. Moreover, they are involved in a bewildering multitude of diverse biological processes such as cell adhesion, motility, protein trafficking, signaling, proliferation, and regulation of the immune system. Beside their physiological roles, they are linked to many pathophysiological phenomena, including tumor progression regulation, HIV-1 replication, diabetes, and hepatitis. Tetraspanins are involved in the formation of extensive protein networks, through interactions not only with themselves but also with numerous other specific proteins, including regulatory proteins in the central nervous system (CNS). Interestingly, recent studies showed that Tspan7 impacts dendritic spine formation, glutamatergic synaptic transmission and plasticity, and that Tspan6 is correlated with epilepsy and intellectual disability (formerly known as mental retardation), highlighting the importance of particular tetraspanins and their involvement in critical processes in the CNS. In this review, we summarize the current knowledge of tetraspanin functions in the brain, with a particular focus on their impact on glutamatergic neurotransmission. In addition, we compare available resolved structures of tetraspanin family members to those of auxiliary proteins of glutamate receptors that are known for their modulatory effects.
Front Mol Neurosci, 2021; 14
34414407: Hamad MIK, Petrova P, Daoud S, Rabaya O, Jbara A, Melliti N, Leifeld J, Jakovčevski I, Reiss G, Herz J, Förster E
Reelin restricts dendritic growth of interneurons in the neocortex.
Reelin is a large secreted glycoprotein that regulates neuronal migration, lamination and establishment of dendritic architecture in the embryonic brain. Reelin expression switches postnatally from Cajal-Retzius cells to interneurons. However, reelin function in interneuron development is still poorly understood. Here, we have investigated the role of reelin in interneuron development in the postnatal neocortex. To preclude early cortical migration defects caused by reelin deficiency, we employed a conditional reelin knockout (RelncKO) mouse to induce postnatal reelin deficiency. Induced reelin deficiency caused dendritic hypertrophy in distal dendritic segments of neuropeptide Y-positive (NPY+) and calretinin-positive (Calr+) interneurons, and in proximal dendritic segments of parvalbumin-positive (Parv+) interneurons. Chronic recombinant Reelin

treatment rescued dendritic hypertrophy in Relncko interneurons. Moreover, we provide evidence that RelncKO interneuron hypertrophy is due to presynaptic GABABR dysfunction. Thus, GABABRs in RelncKO interneurons were unable to block N-type (Cav2.2) Ca2+ channels that control neurotransmitter release. Consequently, the excessive Ca2+ influx through AMPA receptors, but not NMDA receptors, caused interneuron dendritic hypertrophy. These findings suggest that reelin acts as a 'stop-growth-signal' for postnatal interneuron maturation.
Development, 2021; 148

FENS Forum 2022
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BOARD NUMBER: S03-257

CALCIUM REGULATION AND MULTI-KINASE SIGNALING CASCADES CONTRIBUTE TO OUABAIN NEUROPROTECTION IN GLUTAMATE- AND HOMOCYSTEINE-INDUCED NEUROTOXICITY.

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Ouabain is a cardiac glycoside, which was previously shown to induce neuroprotection in excitotoxic stress caused by ionotropic glutamate receptor agonists. It is widely accepted that glutamate and homocysteine-induced neurodegeneration is caused by the permanent plasma membrane depolarization and cytosolic Ca2+ overload of neurons. Pathological homocysteine accumulation in the human plasma, known as hyperhomocysteinemia, exacerbates neurodegenerative diseases because this amino acid acts as an inherent agonist of ionotropic NMDA receptors in the brain. We studied the effects of 0.1–1 nM ouabain on intracellular Ca2+ signaling, mitochondrial inner membrane voltage (φmit), and cell viability in primary cultures of rat cortical neurons during glutamate and homocysteine neurotoxic insults. In addition, apoptosis-related protein expression and the involvement of some kinases in ouabain-mediated effects were evaluated. In short insults, homocysteine was less potent than glutamate as a neurotoxic agent and induced a 20% loss of φmit, whereas glutamate caused a 70% decrease of this value. Subnanomolar ouabain exhibited immediate and postponed neuroprotective effects on neurons. For instance, ouabain rapidly abolished the Ca2+ overload of neurons and loss of φmit evoked by glutamate and homocysteine that rescued neurons in short insults.In prolonged (24h) excitotoxic insults, ouabain prevented neuronal apoptosis, triggering proteinkinase A and proteinkinase C dependent intracellular neuroprotective cascades for homocysteine, but not for glutamate. We, therefore, demonstrated here the role of PKC and PKA involving pathways in neuronal survival caused by ouabain in excitotoxicity, which suggests existence of different appropriate pharmacological treatment for hyperhomocysteinemia and glutamate excitotoxicity. Supported with Russian Science Foundation grant #21-15-00403.

Pubmed:

32722349: Ivanova MA, Kokorina AD, Timofeeva PD, Karelina TV, Abushik PA, Stepanenko JD, Sibarov DA, Antonov SM
Calcium Export from Neurons and Multi-Kinase Signaling Cascades Contribute to Ouabain Neuroprotection in Hyperhomocysteinemia.
Pathological homocysteine (HCY) accumulation in the human plasma, known as hyperhomocysteinemia, exacerbates neurodegenerative diseases because, in the brain, this amino acid acts as a persistent -methyl-d-aspartate receptor agonist. We studied the effects of 0.1-1 nM ouabain on intracellular Ca signaling, mitochondrial inner membrane voltage (φ), and cell viability in primary cultures of rat cortical neurons in glutamate and HCY neurotoxic insults. In addition, apoptosis-related protein expression and the involvement of some kinases in ouabain-mediated effects were evaluated. In short insults, HCY was less potent than glutamate as a neurotoxic agent and induced a 20% loss of φ, whereas glutamate caused a 70% decrease of this value. Subnanomolar ouabain exhibited immediate and postponed neuroprotective effects on neurons. (1) Ouabain rapidly reduced the Ca overload of neurons and loss of φ evoked by glutamate and HCY that rescued neurons in short insults. (2) In prolonged 24 h excitotoxic insults, ouabain prevented neuronal apoptosis, triggering proteinkinase A and proteinkinase C dependent intracellular neuroprotective cascades for HCY, but not for glutamate. We, therefore, demonstrated here the role of PKC and PKA involving pathways in neuronal survival caused by ouabain in hyperhomocysteinemia, which suggests existence of different appropriate pharmacological treatment for hyperhomocysteinemia and glutamate excitotoxicity.
Biomolecules, 2020; 10
32933545: Belaya I, Ivanova M, Sorvari A, Ilicic M, Loppi S, Koivisto H, Varricchio A, Tikkanen H, Walker FR, Atalay M, Malm T, Grubman A, Tanila H, Kanninen KM
Astrocyte remodeling in the beneficial effects of long-term voluntary exercise in Alzheimer's disease.
Increased physical exercise improves cognitive function and reduces pathology associated with Alzheimer's disease (AD). However, the mechanisms underlying the beneficial effects of exercise in AD on the level of specific brain cell types remain

poorly investigated. The involvement of astrocytes in AD pathology is widely described, but their exact role in exercise-mediated neuroprotection warrant further investigation. Here, we investigated the effect of long-term voluntary physical exercise on the modulation of the astrocyte state.
J Neuroinflammation, 2020; 17

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-258

HUMAN METABOTROPIC GLUTAMATE RECEPTOR 5 (MGLUR5) ANTIBODIES INDUCE MEMORY LOSS, INCREASED ANXIETY AND MOLECULAR CHANGES IN MICE

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Aims To determine the antibody pathogenicity in patients with encephalitis associated with antibodies against the metabotropic glutamate receptors 5 (mGluR5) in a mouse model of cerebroventricular transfer of antibodies. **Methods** Mice received continuous cerebroventricular infusion of patients' or controls' IgG for 14 days, followed by a 15-day wash-out. Visuospatial memory, anxiety-like behavior, locomotor activity and hippocampal mGluR5 cluster density were assessed. **Results** Animals infused with patients' IgG, but not controls' IgG, showed memory impairment and increased anxiety. Brain tissue studies showed brain-bound human antibodies predominantly in the hippocampus. Confocal microscopy showed decreased total and synaptic neuronal surface mGluR5 clusters. After antibody clearance, both behavioral and molecular changes reversed to baseline conditions. **Conclusions** These findings support the pathogenicity of the antibodies associated with anti-mGluR5 encephalitis, establishing a link between behavioral deficits and antibody-mediated reduction of mGluR5 levels, and offer a model for testing experimental therapies.

Pubmed:

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Clinical, Neuroimmunologic, and CSF Investigations in First Episode Psychosis.
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To determine in a mouse model whether neonatal Fc receptor (FcRn) blockade prevents the placental transfer of class G immunoglobulin (IgG) derived from patients with anti-NMDA receptor (NMDAR) encephalitis and their pathogenic effects on the fetuses and offspring.
Neurol Neuroimmunol Neuroinflamm, 2021; 8
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Clinical significance of anti-NMDAR concurrent with glial or neuronal surface antibodies.
To determine the frequency and significance of concurrent glial (glial-Ab) or neuronal-surface (NS-Ab) antibodies in patients with anti-NMDA receptor (NMDAR) encephalitis.
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Allosteric modulation of NMDA receptors prevents the antibody effects of patients with anti-NMDAR encephalitis.
Anti-N-methyl-d-aspartate receptor (NMDAR) encephalitis is an immune-mediated disease characterized by a complex neuropsychiatric syndrome in association with an antibody-mediated decrease of NMDAR. About 85% of patients respond to immunotherapy (and removal of an associated tumour if it applies), but it often takes several months or more than 1 year for patients to recover. There are no complementary treatments, beyond immunotherapy, to accelerate this recovery. Previous studies showed that SGE-301, a synthetic analogue of 24(S)-hydroxycholesterol, which is a potent and selective positive allosteric modulator of NMDAR, reverted the memory deficit caused by phencyclidine (a non-competitive antagonist of NMDAR), and prevented the NMDAR dysfunction caused by patients' NMDAR antibodies in cultured neurons. An advantage of SGE-301 is that it is optimized for systemic delivery such that plasma and brain exposures are sufficient to modulate NMDAR activity. Here, we used SGE-301 to confirm that in cultured neurons it prevented the antibody-mediated reduction of receptors, and then we applied it to a previously reported mouse model of passive cerebroventricular transfer of patient's CSF antibodies. Four groups were established: mice receiving continuous (14-day) infusion of patients' or controls' CSF, treated with daily subcutaneous administration of SGE-301 or vehicle (no drug). The effects on memory were examined with the novel object location test at different time points, and the effects on synaptic levels of NMDAR (assessed with confocal microscopy) and plasticity (long-term potentiation) were examined in the hippocampus on Day 18, which in this model corresponds to the last day of maximal clinical and synaptic alterations. As expected, mice infused with patient's CSF antibodies, but not those infused with controls' CSF, and treated with vehicle developed severe memory deficit without locomotor alteration, accompanied by a decrease of NMDAR clusters and impairment of long-term potentiation. All antibody-mediated pathogenic effects (memory, synaptic NMDAR, long-term potentiation) were prevented in the animals treated with SGE-301, despite this compound not antagonizing antibody binding. Additional investigations on the potential mechanisms related to these SGE-301 effects showed that (i) in cultured neurons SGE-301 prolonged the decay time of NMDAR-dependent spontaneous excitatory postsynaptic currents suggesting a prolonged open time of the channel; and (ii) it significantly decreased, without fully preventing, the internalization of antibody-bound receptors suggesting that additional, yet unclear mechanisms, contribute in keeping unchanged the surface NMDAR density. Overall, these findings suggest that SGE-301, or similar NMDAR modulators, could potentially serve as complementary treatment for anti-NMDAR encephalitis and deserve future investigations.
Brain, 2020; 143


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EXCITATORY GLYCINE GLUN1/GLUN3A RECEPTORS IN THE ADULT HIPPOCAMPUS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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The unconventional glycine-binding GluN3A subunit confers to N-methyl-D-aspartate (NMDA) receptors atypical biophysical and signaling properties. GluN3A containing NMDARs are highly expressed during postnatal development, when they are thought to modulate synapse maturation and stabilization. GluN3A expression declines in the transition to adulthood but is maintained at significant levels in several specific brain areas. Recent evidence suggest that GluN3A operates in the adult brain by associating with the glycine-binding subunit GluN1, to form excitatory GluN1/GluN3A receptors activated by glycine only (eGlyRs) which control neuronal excitability and emotional behaviors. Here we investigate eGlyRs in the adult mouse hippocampus and disclose regional differences, cell specificity and endogenous mode of activation with the purpose of unveiling eGlyR cellular physiology and implication in behavior. We show that eGlyRs reside in specific neuronal populations including CA1 pyramidal cells and SST-positive interneurons but not CA3 pyramidal cells and granule cells of the dentate gyrus. Within CA1 pyramidal neurons, eGlyR functional expression follows a marked dorso-ventral gradient, with no expression in the dorsal region but high expression in the ventral one. Unlike CA1 pyramidal neurons, SST-positive interneurons express eGlyRs in both the dorsal and the ventral region. eGlyRs are tonically active, sensing endogenous glycine levels and controlling neuronal excitability in pyramidal cells and SST-positive interneurons. Thus, eGlyRs establish a signaling system in the adult hippocampus with unique functional properties and expression that strikingly differ from conventional NMDARs. Our work opens new perspectives on the exploration of eGlyRs in hippocampal function with an emphasis on ventral hippocampus related behaviors.

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WIDESPREAD EXPRESSION AND ACTION OF GLUN1/GLUN3A EXCITATORY GLYCINE RECEPTORS IN THE ADULT BRAIN

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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NMDA receptors (NMDARs) constitute key components of the excitatory neurotransmission and play central roles in brain development and function. Classical GluN1/GluN2 NMDARs are specialized in detection of the excitatory neurotransmitter glutamate using glycine or D-serine as a co-agonist. The exclusivity of such a ubiquitous working model was challenged by the finding that GluN1 subunits could co-assemble with GluN3 subunits to form 'unconventional' GluN1/GluN3 receptors gated by glycine only. The existence of GluN1/GluN3 NMDARs in native neuronal tissue has long been controversial. Recent work revealed that GluN1/GluN3A receptors are genuine neuronal receptors, constituting a new type of excitatory glycine receptors (eGlyRs). However, how this unusual receptor controls neuronal function and circuits in the adult brain is still mostly unknown. Combining multi-disciplinary approaches, we now provide evidence that GluN1/GluN3A receptors control the function of adult cortical and amygdalar circuits. In the primary somatosensory cortex (S1) eGlyRs are selectively expressed at high levels in somatostatin interneurons while in basolateral amygdala (BLA), GluN1/GluN3A mediated currents are detected in glutamatergic principal neurons. In both cell types, eGlyRs are mostly extrasynaptic and control neuronal excitability through the generation of tonic excitatory currents driven by extracellular glycine, which can be modulated by the dopaminergic system. Finally, we report that eGlyRs are important for behavior-dependent modulation of spontaneous pyramidal cell activity in the S1, as well as consolidation of fear memories in the BLA. Overall, our results reveal unique molecular, anatomical and functional attributes of eGlyRs, opening new vistas on the diversity of NMDAR signaling and glycinergic neurotransmission.

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STUDYING THE SPECIFICITY OF AUTOANTIBODIES AGAINST GLUTAMATE RECEPTORS AND AUXILIARY PROTEINS IN RASMUSSEN'S ENCEPHALITIS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Rasmussen's Encephalitis (RE) is a chronic inflammatory disease of the brain. Several studies have found autoantibodies against certain subtypes of ionotropic glutamate receptors (iGluRs) of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type in blood sera of RE-patients. Plasmapheresis mitigated the RE-symptoms, supporting the assumption that autoantibodies may have an impact on the development of the disease. Since experimental studies produced inconsistent results regarding the involvement of AMPAR autoantibodies in RE, we aimed at optimising experimental procedures to improve the reliability of RE-autoantibody detection in blood sera. We employ immunofluorescent double staining (human immunoglobulin and transfection marker tag antibodies) of HEK293T cells cotransfected with myc-tagged GluA2 and GluA3 to check for autoantibodies against iGluRs in RE-sera. Preabsorbing the used blood sera on untransfected HEK cells decreased background staining and thus improved the signal-to-noise ratio. Using this approach, we detected autoantibodies only of the IgG type, confirming published data in the literature. Next we tested the RE-sera for autoantibodies against transmembrane AMPAR regulatory proteins (TARPs), which are frequently associated with AMPARs. Here, we show that some RE-sera appear to contain IgG autoantibodies against several TARPs. These preliminary results provide further hints to the processes involved in RE.

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SECRETED OLFACTOMEDIN1-3 CONTROL SYNAPTIC INCORPORATION OF AMPA-TYPE GLUTAMATE RECEPTORS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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AMPA-type glutamate receptors (AMPARs) are key players for excitatory synaptic transmission in the central nervous system. In particular, the fine-tuned synaptic incorporation of receptors is responsible for activity-dependent synaptic plasticity. Here we show that secreted Olfactomedin 1-3 (Olfm1-3), which are established constituents of macromolecular AMPAR complexes, are important determinants for availability and dynamics of functional receptors at the cell surface. By unbiased quantitative proteomic analysis of affinity isolated Olfm1 protein from native brain we gained molecular insights which provide a firm basis for understanding their role at synaptic and extra-synaptic sites. They interact with core subunits of AMPARs and form contacts to secreted ligands, extracellular matrix, presynaptic adhesion proteins and a GPI-anchored neurotrophic factor. The latter has a regulatory function by stabilizing Olfm1 at the plasma membrane. We studied the consequences of a complete removal of Olfm1-3 proteins on AMPAR physiology in the hippocampus formation by freeze-fracture electron microscopy and the measure of electrophysiological properties of neurons in acute brain slices. As a result, we show that in the absence of Olfm1-3 the number of receptors at dendritic surfaces and postsynaptic densities of CA1 pyramidal cells are reduced by about 70%. Respectively, AMPAR mediated synaptic transmission as well as synaptic plasticity (LTP) is strongly impaired. Together, we present molecular details and mechanisms important for recruitment and positioning of AMPARs at the synapse. Olfm1-3 proteins are therefore essential by building a multi-protein complex with AMPARs and guide their synaptic incorporation and trans-synaptic tethering.

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HEMPHASIS 2 : A PHARMACOLOGICAL SIGNATURE TO SELECTIVELY DETERMINE MGLU7 CONTAINING HETERODIMERS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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ABSTRACT mGlu7 homodimers have an apparent very low affinity for glutamate much lower than that of the other mGluRs. We hypothese that mGlu7 is mainly present on the brain as heterodimer. It was already highlighted that mGlu7 subunit is able to heterodimerize, especially with the mGlu2 subunit. Here we determined the pharmacological in order properties of mGlu7/2 and compared it to those of mGlu7 and mGlu2 homodimers. Our final aim is to be able to recognize the imprint of mGlu7/2 in vivo. We used the IPOne assay to study the pharmacological properties of mGlu2, mGlu7 and mGlu7/2 transiently expressed in HEK cells together with the chimeric G protein GqTop. For the mGlu7/2, we used the optimized quality control system based on the GABAB receptor to make sure that only the heterodimer reached the cell surface. Both orthosteric and allosteric ligands for mGlu2 and mGlu7 was tested on each homodimer, and then tested on mGlu7/2 heterodimers. We identified a specific combination of ligands that can be used as a pharmacological signature of this heterodimer relative to the homodimers, as already found for the mGlu2-4 heterodimer. Functionnally, we have a symmetric activation of the heterodimer mGlu7/2 with an mGlu7 G protein coupling. The assays on mGlu2-7 permit us to identified ligands that act differently on mGlu7 whether in a homo or heterodimeric form. This pharmacological signature will be used to identify mGlu7/2 heterodimers in the brain. **Keywords:** Neuropharmacology, Metabotropic glutamate receptor, Heterodimer, FRET

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FRAGILE X MENTAL RETARDATION PROTEIN MEDIATES BDNF-INDUCED INCREASE OF SYNAPTIC NMDA RECEPTORS CONTENT

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Brain-Derived Neurotrophic Factor (BDNF) is a mediator of synaptic plasticity and long-term synaptic potentiation. The facilitatory effects of BDNF on glutamatergic synapses depend on local translation. A master regulator of local protein synthesis at the synapse is Fragile X Mental Retardation Protein (FMRP), that exerts translational control on a subset of neuronal transcripts. However, it is still unclear whether FMRP mediates the effects of BDNF on protein synthesis at the synapse. In this work we investigated whether FMRP regulates the synaptic content of GluN2A- and GluN2B-containing NMDA receptors (NMDAR) and acts downstream of the BDNF-TrkB signalling pathway. We knocked down (KD) *Fmr1* expression in cultured hippocampal neurons using two different sets of shRNAs and treated the cells with BDNF. Surface GluN2A and GluN2B were stained with antibodies against an extracellular epitope. We also evaluated the phosphorylation (activation) of TrkB on tyrosine 816 by immunocytochemistry. Synaptic localisation of surface GluN2A and GluN2B, as well as phosphorylated TrkB, were assessed by colocalization with the post- and pre-synaptic markers PSD95 and vGlut. Fmr1 KD had no effect on the surface synaptic content of GluN2A and GluN2B but abolished BDNF-induced upregulation of synaptic NMDAR. Finally, we observed that TrkB phosphorylation is not affected by Fmr1 KD. In conclusion, our data show a role for FMRP in BDNF-induced facilitation at the glutamatergic synapse. Since KD of FMRP was without effect of BDNF-induced activation of TrkB, the RNA-binding protein is likely to act downstream of the receptors to mediate BDNF-induced upregulation of glutamatergic synapses.

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DELETION OF THE DOPAMINE TRANSPORTER DEPOTENTIATES THE GLUTAMATERGIC NEUROTRANSMISSION IN THE RAT STRIATUM

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Dopaminergic and glutamatergic pathways interact at dendritic spines of medium spiny neurons in the striatum. Notably, NMDA and AMPA glutamatergic receptors colocalize with dopaminergic receptors influencing neural excitability and promoting synaptic plasticity. This interaction is critical for mediating top-down control, reward processing, and depressive mood; however, little is known about the molecular mechanisms underlying the crosstalk between dopamine and glutamate. The aim of our work was to unveil the effect of the deletion of the dopamine transporter (DAT) on the homeostasis of the glutamate synapse in the striatum and its role in electrophysiological transmission to further deepening features of dopamine-glutamate interaction. Taking advantage of targeted inactivation of the dopamine transporter in rats (DATko), which caused a hyperdopaminergic condition, we found a reduced expression of the main subunits of both NMDA and AMPA receptors, as well as of their specific glutamatergic scaffolding proteins in the post-synaptic density of the striatum. These changes were paralleled by an increased trafficking at the extra-synaptic sites of both NMDA and AMPA receptors, suggesting a lateral diffusion toward the extra-synapse. Moreover, we found an upregulation of the glial antiporter xCT, suggesting an increased extra-synaptic release of glutamate. Interestingly, such reduced retention of glutamate receptors in the membrane was accompanied by an impairment in both LTP and LTD post-synaptic transmission in the striatum of DATko animals. Taken together, these data suggest that a condition of hyperdopaminergia has reorganized the glutamatergic synapse in the striatum, causing an overall destabilization and depotentiation of the glutamatergic neurotransmission.

Pubmed:
32721055: Caffino L, Verheij MMM, Roversi K, Targa G, Mottarlini F, Popik P, Nikiforuk A, Golebiowska J, Fumagalli F, Homberg JR
Hypersensitivity to amphetamine's psychomotor and reinforcing effects in serotonin transporter knockout rats: Glutamate in the nucleus accumbens.
Amphetamine (AMPH) use disorder is a serious health concern, but, surprisingly, little is known about the vulnerability to the moderate and compulsive use of this psychostimulant and its underlying mechanisms. Previous research showed that inherited serotonin transporter (SERT) down-regulation increases the motor response to cocaine, as well as moderate (as measured during daily 1-h self-administration sessions) and compulsive (as measured during daily 6-h self-administration sessions) intake of this psychostimulant. Here, we sought to investigate whether these findings generalize to AMPH and the underlying mechanisms in the nucleus accumbens.
Br J Pharmacol, 2020; 177
33880773: Caffino L, Mottarlini F, Targa G, Verheij MMM, Homberg J, Fumagalli F
Long access to cocaine self-administration dysregulates the glutamate synapse in the nucleus accumbens core of serotonin transporter knockout rats.
It is well established that the nucleus accumbens and glutamate play a critical role in the motivation to take drugs of abuse. We have previously demonstrated that rats with ablation of the serotonin (5-HT) transporter (SERT rats) show increased cocaine intake reminiscent of compulsivity.
Br J Pharmacol, 2021;
34299015: Caffino L, Mottarlini F, Bilel S, Targa G, Tirri M, Maggi C, Marti M, Fumagalli F
Single Exposure to the Cathinones MDPV and α-PVP Alters Molecular Markers of Neuroplasticity in the Adult Mouse Brain.
Synthetic cathinones have gained popularity among young drug users and are widely used in the clandestine market. While the cathinone-induced behavioral profile has been extensively investigated, information on their neuroplastic effects is still

rather fragmentary. Accordingly, we have exposed male mice to a single injection of MDPV and α-PVP and sacrificed the animals at different time points (i.e., 30 min, 2 h, and 24 h) to have a rapid readout of the effect of these psychostimulants on neuroplasticity in the frontal lobe and hippocampus, two reward-related brain regions. We found that a single, low dose of MDPV or α-PVP is sufficient to alter the expression of neuroplastic markers in the adult mouse brain. In particular, we found increased expression of the transcription factor , increased ratio between the vesicular GABA transporter and the vesicular glutamate transporter together with changes in the expression of the neurotrophin , confirming the widespread impact of these cathinones on brain plasticity. To sum up, exposure to low dose of cathinones can impair cortical and hippocampal homeostasis, suggesting that abuse of these cathinones at much higher doses, as it occurs in humans, could have an even more profound impact on neuroplasticity.
Int J Mol Sci, 2021; 22

35174489: Caffino L, Mottarlini F, Targa G, Verheij MMM, Fumagalli F, Homberg JR
Responsivity of serotonin transporter knockout rats to short and long access to cocaine: Modulation of the glutamate signalling in the nucleus accumbens shell.
It has been well established that glutamate in the nucleus accumbens (NAc) plays a critical role in the motivation to take drugs of abuse. We have previously demonstrated that rats with ablation of the serotonin transporter (SERT rats) show increased cocaine intake reminiscent of compulsivity.
Br J Pharmacol, 2022;

33511707: Caffino L, Moro F, Mottarlini F, Targa G, Di Clemente A, Toia M, Orrù A, Giannotti G, Fumagalli F, Cervo L
Repeated exposure to cocaine during adolescence enhances the rewarding threshold for cocaine-conditioned place preference in adulthood.
Previous studies have shown that adolescent exposure to cocaine increases drug use in adulthood, albeit incubation of cocaine seeking was found to be attenuated in rats trained to self-administer cocaine during adolescence. We here hypothesize that adolescent exposure to cocaine could alter the rewarding properties of the psychostimulant in adulthood. By employing two of the most widely used animal-experimental-preclinical models to investigate drug addiction, we evaluated whether contingent versus non-contingent cocaine self-administration during adolescence modulates its rewarding threshold in adulthood evaluated by conditioned place preference (CPP). Cocaine self-administration during adolescence increases the rewarding threshold in adulthood; CPP for cocaine was observed at the higher (20 mg/kg), but not at the lower (10 mg/kg), dose employed. Rats exposed to either contingent or non-contingent cocaine during adolescence exhibited the same behavior in the CPP paradigm suggesting that, under our experimental conditions, cocaine rewarding properties are shaped by the psychostimulant itself and not by its motivational effects. From a mechanistic standpoint, the preference for the 20 mg/kg cocaine-paired side in a CPP paradigm appears to depend, at least partially, upon the formation of GluA2-lacking Ca -permeable AMPA receptors and the consequent increase of αCaMKII activity in the NAc, both of which are instead reduced when the 10 mg/kg dose was used. In conclusion, contingent or non-contingent cocaine exposure during adolescence desensitizes adult animals to a rewarding dose of cocaine (10 mg/kg) elevating the rewarding threshold necessary (20 mg/kg) to drive conditioned place preference, an effect that may predispose to higher consumption of cocaine during adulthood.
Addict Biol, 2021; 26

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MGLU5 RECEPTOR FUNCTION DEPENDS ON MEMBRANE VOLTAGE

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Objectives G-Protein Coupled Receptors (GPCR) are seven transmembrane domain receptors found in a large variety of organs where they transduce extracellular signals such as hormones or neurotransmitters. In neurons they are exposed to wide membrane voltage variations recently shown to be an important direct modulator of some GPCRs activity but still remains unwell characterized. Metabotropic glutamate receptor 5 (mGlu5) is a class C GPCR localized on the glutamatergic post-synapse where it modulates synaptic transmission and plasticity. We investigated the sensitivity of mGlu5 to the neuronal membrane voltage. Methods We worked on Human Embryonic Kidney 293T (HEK 293T) cell lines stably expressing human mGlu5. We used a FRET-based sensor to report conformational changes (activation / inactivation) of mGlu5 triggered by agonist and antagonists following membrane depolarization by K^+. Moreover, thanks to a fluorescent calcium sensor and microscopy imaging we studied mGlu5 induced calcium oscillations. Results We show that membrane depolarization potentiates the inactive-like conformation of mGlu5 triggered by LY341495 antagonist. Consistently, mGlu5 agonist-induced intracellular calcium increase was impaired by depolarization of the cell membrane. This global inhibition is the result of a reduced number of cells responding to the stimulation combined with a decreased mGlu5-induced calcium spiking activity in cells responding to the stimulation. Put together, these results reflect a disturbance of the mGlu5 functionality during depolarization. Conclusion Our findings support a role of membrane voltage on mGlu5 pharmacological activation and signaling in HEK 293T. This singular property of mGlu5 is of interest for glutamatergic synaptic transmission understanding.

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OPTICAL BIOSENSORS OF NATIVE MEMBRANE PROTEIN COMPLEXES REVEAL A HIGH PROPORTION OF MGLU HETERODIMERS IN THE BRAIN

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Metabotropic Glutamate receptor play key roles in regulating many synapses in the brain. Eight mGluRs have been identified, sub-classified into group-I (mGlu1 & 5), group-II (mGlu2 & 3) and group-III (mGlu4, 6, 7 & 8)[1]. These receptors are mandatory dimers[2]. Although assumed to be exclusively homodimers, we reported 10 years ago that mGlu subunits can associate into heterodimers[3]. While both group-I subunits can associate, any group-II and -III subunits can form heterodimers, generating 16 possible additional mGluRs with specific properties[4-6]. In this study, we developed a method for detecting mGlu homo and heterodimers in native tissues. Our method relies on nanobody-based biosensors which enable the proximity detection between subunits by FRET. Additionally, given conformation-specific nanobodies, activation of these native complexes can be recorded. Applied to the metabotropic glutamate receptors 2 and 4 subunits, this approach revealed the clear existence of functional mGlu2-4 heterodimers in addition to mGlu2 and mGlu4 homodimers. Strikingly, the mGlu4 subunits appear to be mainly in heterodimers in the brain. Overall, these data confirm the existence of mGlu heterodimers in the brain, and provide innovative tools to determine their specific properties in their native environment. [1] Conn, P.J. & Pin, J.-P. *Ann. Rev. Pharmacol. Toxicol.* 37, 205-237, 1997 [2] Pin, J.-P. & Bettler, B. *Nature* 540, 60-68, 2016 [3] Doumazane, E. *et al. FASEB J* 25, 66-77, 2011 [4] Scholler, P. *et al. Nat Chem Biol* 13, 372-380, 2017 [5] Moreno Delgado, D. *et al. eLife* 6, 2017 [6] Liu, J. *et al. eLife* 6, 2017

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BOARD NUMBER: S03-268

A NANOBODY ACTIVATING METABOTROPIC GLUTAMATE RECEPTOR 4 DISCRIMINATES BETWEEN HOMO AND HETERODIMERS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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There is growing interest in developing biologics as pharmacological tools due to their high target selectivity. The G protein-coupled homo and heterodimeric metabotropic glutamate (mGlu) receptors regulate many synapses and are promising targets for the treatment of numerous brain diseases. Although subtype selective allosteric small molecules have been reported, their effects on the recently discovered heterodimeric receptors are often not known. Here we describe a nanobody that specifically and fully activates homodimeric human mGlu4 receptors. Our studies revealed that the nanobody acts by stabilizing the closed active state of the glutamate binding domain by interacting with both lobes. In contrast, this nanobody does not activate the heterodimeric mGlu2-4, but acts as a pure positive allosteric modulator. These data further demonstrate that nanobodies can be useful tools to discover the specific functional roles of all mGlu subtypes, including the heterodimeric ones.

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GROUP III METABOTROPIC GLUTAMATE RECEPTORS AT THE INNER HAIR CELL RIBBON SYNAPSE IN THE COCHLEA

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Hearing impairment is often preceded by neurodegeneration at inner hair-cell (IHC) ribbon synapses in the cochlea. It was suggested that the primary cause of synapse loss during noise and aging in the cochlea is caused by glutamate overexposure. Pre-synaptically localized metabotropic glutamate receptors (mGluRs) are well suited to protect neurons from glutamate excitotoxicity, since they can limit pre-synaptic glutamate release. Interestingly, mGluR7 has been correlated with age-related and noise-induced hearing deficits. Recently, we analysed expression and localization of the mGluR7 isoforms mGluR7a and mGluR7b in mouse cochlear wholemounts, using confocal microscopy and 3D reconstructions. We observed a pre-synaptic localization of both mGluR7 isoforms at the IHC ribbon synapse and a reduced amount of these receptors at synapses encoding higher frequencies and in older animals. We also found a pre-synaptic localization of mGluR4 and mGluR8b at IHC synapses. This enables the possibility to form homo- and/or heterodimeric receptors composed of mGluR4, mGluR7a, mGluR7b and mGluR8b at IHC ribbon synapses. Various studies described the formation of heterodimers of mGluR types that revealed distinct characteristics. The ability of heterodimerization between mGluR4, mGluR7a/b and mGluR8b are unknown. Here, we analyzed the formation of potential heterodimers using co-staining's on cochlear wholemounts and immunoprecipitations from HEK-293 cells and from native tissue. Our data show that all receptor combinations formed heterodimers when co-expressed in HEK-293 cells, while only some of them were detectable in the brain. These receptor complexes might represent new molecular targets suited for pharmacological concepts to protect the cochlea against noxious stimuli and excitotoxicity.

Pubmed:
34644430: Klotz L, Enz R
MGluR7 is a presynaptic metabotropic glutamate receptor at ribbon synapses of inner hair cells.
Glutamate is the most pivotal excitatory neurotransmitter in the central nervous system. Metabotropic glutamate receptors (mGluRs) dimerize and can couple to inhibitory intracellular signal cascades, thereby protecting glutamatergic neurons from excessive excitation and cell death. MGluR7 is correlated with age-related hearing deficits and noise-induced hearing loss; however its exact localization in the cochlea is unknown. Here, we analyzed the expression and localization of mGluR7a and mGluR7b in mouse cochlear wholemounts in detail, using confocal microscopy and 3D reconstructions. We observed a presynaptic localization of mGluR7a at inner hair cells (IHCs), close to the synaptic ribbon. To detect mGluR7b, newly generated antibodies were characterized and showed co-localization with mGluR7a at IHC ribbon synapses. Compared to the number of synaptic ribbons, the numbers of mGluR7a and mGluR7b puncta were reduced at higher frequencies (48 to 64 kHz) and in older animals (6 and 12 months). Previously, we reported a presynaptic localization of mGluR4 and mGluR8b at this synapse type. This enables the possibility for the formation of homo- and/or heterodimeric receptors composed of mGluR4, mGluR7a, mGluR7b and mGluR8b at IHC ribbon synapses. These receptor complexes might represent new molecular targets suited for pharmacological concepts to protect the cochlea against noxious stimuli and excitotoxicity.
FASEB J, 2021; 35
31585509: Klotz L, Wendler O, Frischknecht R, Shigemoto R, Schulze H, Enz R
Localization of group II and III metabotropic glutamate receptors at pre- and postsynaptic sites of inner hair cell ribbon synapses.
Glutamate is the major excitatory neurotransmitter in the CNS binding to a variety of glutamate receptors. Metabotropic glutamate receptors (mGluR1 to mGluR8) can act excitatory or inhibitory, depending on associated signal cascades. Expression and localization of inhibitory acting mGluRs at inner hair cells (IHCs) in the cochlea are largely unknown. Here, we analyzed expression of mGluR2, mGluR3, mGluR4, mGluR6, mGluR7, and mGluR8 and investigated their localization with respect to the presynaptic ribbon of IHC synapses. We detected transcripts for mGluR2, mGluR3, and mGluR4 as well as for mGluR7a, mGluR7b, mGluR8a, and mGluR8b splice variants. Using receptor-specific antibodies in cochlear

wholemounts, we found expression of mGluR2, mGluR4, and mGluR8b close to presynaptic ribbons. Super resolution and confocal microscopy in combination with 3-dimensional reconstructions indicated a postsynaptic localization of mGluR2 that overlaps with postsynaptic density protein 95 on dendrites of afferent type I spiral ganglion neurons. In contrast, mGluR4 and mGluR8b were expressed at the presynapse close to IHC ribbons. In summary, we localized in detail 3 mGluR types at IHC ribbon synapses, providing a fundament for new therapeutical strategies that could protect the cochlea against noxious stimuli and excitotoxicity.-Klotz, L., Wendler, O., Frischknecht, R., Shigemoto, R., Schulze, H., Enz, R. Localization of group II and III metabotropic glutamate receptors at pre- and postsynaptic sites of inner hair cell ribbon synapses.
FASEB J, 2019; 33

28295323: Mascia F, Klotz L, Lerch J, Ahmed MH, Zhang Y, Enz R
CRIP1a inhibits endocytosis of G-protein coupled receptors activated by endocannabinoids and glutamate by a common molecular mechanism.
The excitability of the central nervous system depends largely on the surface density of neurotransmitter receptors. The endocannabinoid receptor 1 (CB R) and the metabotropic glutamate receptor mGlu R are expressed pre-synaptically where they reduce glutamate release into the synaptic cleft. Recently, the CB R interacting protein cannabinoid receptor interacting protein 1a (CRIP1a) was identified and characterized to regulate CB R activity in neurons. However, underlying molecular mechanisms are largely unknown. Here, we identified a common mechanism used by CRIP1a to regulate the cell surface density of two different types of G-protein coupled receptors, CB R and mGlu R. Five amino acids within the CB R C-terminus were required and sufficient to reduce constitutive CB R endocytosis by about 72% in the presence of CRIP1a. Interestingly, a similar sequence is present in mGlu R and consistently, endocytosis of mGlu R depended on CRIP1a, as well. Docking analysis and molecular dynamics simulations identified a conserved serine in CB R (S468) and mGlu R (S894) that forms a hydrogen bond with the peptide backbone of CRIP1a at position R82. In contrast to mGlu R, the closely related mGlu R splice-variant carries a lysine (K894) at this position, and indeed, mGlu R endocytosis was not affected by CRIP1a. Chimeric constructs between CB R, mGlu R, and mGlu R underline the role of the identified five CRIP1a sensitive amino acids. In summary, we suggest that CRIP1a negatively regulates endocytosis of two different G-protein coupled receptor types, CB R and mGlu R.
J Neurochem, 2017; 141

34333057: Bachert W, Klotz L, Sticht H, Enz R
Homodimerization of a proximal region within the C-terminus of the orphan G-protein coupled receptor GPR179.
G-protein coupled receptors exhibit numerous biological functions. The orphan G-protein coupled receptor GPR179 is a central component of a 1 Megadalton large signalling complex in the ON-pathway of the mammalian retina that assembles multiple proteins, including the metabotropic glutamate receptor mGluR6. Dimer formation is a hallmark of G-protein coupled receptors and some use intracellular C-termini for dimerization. Here we tested the dimerization properties of the intracellular C-terminal domains of mGluR6 and GPR179. While the C-termini of GPR179 and mGluR6 did not interact, we detected a robust homodimerization of a proximal region in the GPR179 C-terminus. Mapping studies defined a linear stretch of 64 amino acids as dimerization region. Bioinformatic analysis indicated that this dimerization region might adopt an α-helical structure that is predicted to dimerize by forming a coiled-coil. Based on these data, we speculate that homodimerization of GPR179 might contribute to the formation of large signalling complexes in the mammalian retina.
Neurochem Int, 2021; 149

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ENDOGENOUS NMDA RECEPTOR MODULATORS ALTER DENDRITIC ARBOR COMPLEXITY IN CULTURED CORTICAL NEURONS

POSTER SESSION 03 - SECTION: GLUTAMATE RECEPTORS AND THEIR MODULATION

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Schizophrenia is a chronic developmental neuropsychiatry disorder affecting 1% of the population, manifesting through an array of severely disabling symptoms. Hypofunction of NMDA receptor (NMDAR) is one of the main hypothesis for the neurobiology of schizophrenia, as the dissociative anesthetics ketamine and phencyclidine (non-competitive NMDAR antagonists) induce schizophrenia-like symptoms in healthy humans. Evidences from postmortem studies show an alteration in morphology and density of postsynaptic elements in cortical tissue in schizophrenia. While ketamine, phencyclidine and another exogenous antagonist of NMDAR have been used as pharmacological approach for inducing NMDAR hypofunction, little attention has been payed to the endogenous modulators of NMDAR, which emerge to be dysregulated in patients with schizophrenia. We studied the effects of the endogenous NMDAR modulators kynurenic acid, pregnenolone sulfate, spermidine and zinc on neuronal morphology and synaptic density in cortical culture. We found that those modulators did not alter cell viability and glutamate release. In addition, dendritic branching measurements of total dendritic length, number of branches, number of primary branches, number of branch points, and soma size were not different when cultures were treated for 5 days. However, spermidine and pregnenolone sulfate altered dendritic arbor complexity. Finally, pregnenolone sulfate decrease the number of postsynaptic marker PSD-95 and a trend to decrease synapse number. Importantly, these modulators are also ligands for other receptors, so future studies need to assess whether these effects on morphology and synaptic density are by NMDAR-related mechanisms to elucidate the impact of the NMDAR hypofunction by endogenous modulators in schizophrenia.

Pubmed:
33336545: Jorratt P, Hoschl C, Ovsepian SV
Endogenous antagonists of N-methyl-d-aspartate receptor in schizophrenia.
Schizophrenia is a chronic neuropsychiatric brain disorder that has devastating personal impact and rising healthcare costs. Dysregulation of glutamatergic neurotransmission has been implicated in the pathobiology of the disease, attributed largely to the hypofunction of the N-methyl-d-aspartate (NMDA) receptor. Currently, there is a major gap in mechanistic analysis as to how endogenous modulators of the NMDA receptors contribute to the onset and progression of the disease. We present a systematic review of the neurobiology and the role of endogenous NMDA receptor antagonists in animal models of schizophrenia, and in patients. We discuss their neurochemical origin, release from neurons and glia with action mechanisms, and functional effects, which might contribute toward the impairment of neuronal processes underlying this complex pathological state. We consider clinical evidence suggesting dysregulations of endogenous NMDA receptor in schizophrenia, and highlight the pressing need in future studies and emerging directions, to restore the NMDA receptor functions for therapeutic benefits.
Alzheimers Dement, 2021; 17
32926322: Zaitsev AV, Smolensky IV, Jorratt P, Ovsepian SV
Neurobiology, Functions, and Relevance of Excitatory Amino Acid Transporters (EAATs) to Treatment of Refractory Epilepsy.
Epilepsy is one of the most prevalent and devastating neurological disorders characterized by episodes of unusual sensations, loss of awareness, and reoccurring seizures. The frequency and intensity of epileptic fits can vary to a great degree, with almost a third of all cases resistant to available therapies. At present, there is a major unmet need for effective and specific therapeutic intervention. Impairments of the exquisite balance between excitatory and inhibitory synaptic processes in the brain are considered key in the onset and pathophysiology of the disease. As the primary excitatory neurotransmitter in the central nervous system, glutamate has been implicated in the process, with the glutamatergic system holding center stage in the pathobiology as well as in developing disease-modifying therapies. Emerging data pinpoint impairments of glutamate clearance as one of the key causative factors in drug-resistant disease forms. Reinstatement of

glutamate homeostasis using pharmacological and genetic modulation of glutamate clearance is therefore considered to be of major translational relevance. In this article, we review the neurobiological and clinical evidence suggesting complex aberrations in the activity and functions of excitatory amino acid transporters (EAATs) in epilepsy, with knock-on effects on glutamate homeostasis as a leading cause for the development of refractory forms. We consider the emerging data on pharmacological and genetic manipulations of EAATs, with reference to seizures and glutamate dyshomeostasis, and review their fundamental and translational relevance. We discuss the most recent advances in the EAATs research in human and animal models, along with numerous questions that remain open for debate and critical appraisal. Contrary to the widely held view on EAATs as a promising therapeutic target for management of refractory epilepsy as well as other neurological and psychiatric conditions related to glutamatergic hyperactivity and glutamate-induced cytotoxicity, we stress that the true relevance of EAAT2 as a target for medical intervention remains to be fully appreciated and verified. Despite decades of research, the emerging properties and functional characteristics of glutamate transporters and their relationship with neurophysiological and behavioral correlates of epilepsy challenge the current perception of this disease and fit unambiguously in neither EAATs functional deficit nor in reversal models. We stress the pressing need for new approaches and models for research and restoration of the physiological activity of glutamate transporters and synaptic transmission to achieve much needed therapeutic effects. The complex mechanism of EAATs regulation by multiple factors, including changes in the electrochemical environment and ionic gradients related to epileptic hyperactivity, impose major therapeutic challenges. As a final note, we consider the evolving views and present a cautious perspective on the key areas of future progress in the field towards better management and treatment of refractory disease forms.
CNS Drugs, 2020; 34

29163062: Jorratt P, Delano PH, Delgado C, Dagnino-Subiabre A, Terreros G
Difference in Perseverative Errors during a Visual Attention Task with Auditory Distractors in Alpha-9 Nicotinic Receptor Subunit Wild Type and Knock-Out Mice.
The auditory efferent system is a neural network that originates in the auditory cortex and projects to the cochlear receptor through olivocochlear (OC) neurons. Medial OC neurons make cholinergic synapses with outer hair cells (OHCs) through nicotinic receptors constituted by α9 and α10 subunits. One of the physiological functions of the α9 nicotinic receptor subunit (α9-nAChR) is the suppression of auditory distractors during selective attention to visual stimuli. In a recent study we demonstrated that the behavioral performance of alpha-9 nicotinic receptor knock-out (KO) mice is altered during selective attention to visual stimuli with auditory distractors since they made less correct responses and more omissions than wild type (WT) mice. As the inhibition of the behavioral responses to irrelevant stimuli is an important mechanism of the selective attention processes, behavioral errors are relevant measures that can reflect altered inhibitory control. Errors produced during a cued attention task can be classified as premature, target and perseverative errors. Perseverative responses can be considered as an inability to inhibit the repetition of an action already planned, while premature responses can be considered as an index of the ability to wait or retain an action. Here, we studied premature, target and perseverative errors during a visual attention task with auditory distractors in WT and KO mice. We found that α9-KO mice make fewer perseverative errors with longer latencies than WT mice in the presence of auditory distractors. In addition, although we found no significant difference in the number of target error between genotypes, KO mice made more short-latency target errors than WT mice during the presentation of auditory distractors. The fewer perseverative error made by α9-KO mice could be explained by a reduced motivation for reward and an increased impulsivity during decision making with auditory distraction in KO mice.
Front Cell Neurosci, 2017; 11

27383594: Terreros G, Jorratt P, Aedo C, Elgoyhen AB, Delano PH
Selective Attention to Visual Stimuli Using Auditory Distractors Is Altered in Alpha-9 Nicotinic Receptor Subunit Knock-Out Mice.
During selective attention, subjects voluntarily focus their cognitive resources on a specific stimulus while ignoring others. Top-down filtering of peripheral sensory responses by higher structures of the brain has been proposed as one of the mechanisms responsible for selective attention. A prerequisite to accomplish top-down modulation of the activity of peripheral structures is the presence of corticofugal pathways. The mammalian auditory efferent system is a unique neural network that originates in the auditory cortex and projects to the cochlear receptor through the olivocochlear bundle, and it has been proposed to function as a top-down filter of peripheral auditory responses during attention to cross-modal stimuli. However, to date, there is no conclusive evidence of the involvement of olivocochlear neurons in selective attention paradigms. Here, we trained wild-type and α-9 nicotinic receptor subunit knock-out (KO) mice, which lack cholinergic transmission between medial olivocochlear neurons and outer hair cells, in a two-choice visual discrimination task and studied the behavioral consequences of adding different types of auditory distractors. In addition, we evaluated the effects of contralateral noise on auditory nerve responses as a measure of the individual strength of the olivocochlear reflex. We demonstrate that KO mice have a reduced olivocochlear reflex strength and perform poorly in a visual selective attention paradigm. These results confirm that an intact medial olivocochlear transmission aids in ignoring auditory distraction during selective attention to visual

stimuli.
J Neurosci, 2016; 36

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BOARD NUMBER: S03-271

THE INVOLVEMENT OF ASTROCYTE CALCIUM-DEPENDENT SIGNALING IN BEHAVIOR

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Astrocytes are critical players in the regulation of brain development and function. They sense and respond to neuronal activity by elevating intracellular calcium levels, which derive from different sources and display complex spatiotemporal properties. Calcium elevations appear spatially distributed in global (soma and main processes) and focal regions (microdomains). Such astrocytic calcium activity is expected to underlie the astrocyte involvement in synaptic transmission, metabolism, and brain homeostasis. In this work, we studied the IP3 receptor type 2 knockout (IP3R2 KO) mouse model that lacks global calcium elevations in astrocytes to disclose its implications in cognitive function. We found an influence of global astrocyte calcium in long-term memory performance. Thus, we performed a structural and molecular analysis of cortico-limbic regions that revealed a shift to immature spines in pyramidal neurons of the dorsal hippocampus that could support the changes in synaptic plasticity underlying our behavioral observations. The characterization of the IP3R2 KO mouse model provided new insights into the importance of astrocytic calcium-dependent signaling in the modulation of neural activity. These findings broaden the scope of astrocytic modulation of brain circuits.

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ASTROCYTES OF NUCLEUS ACCUMBENS CONTROL THE IMPAIRMENTS DERIVED FROM CHRONIC EXPOSURE OF THC

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Drug use is a growing problem in actual society. Usually, the first experience with drugs takes place during adolescence, being cannabis the most used illicit drug[1]. Although cannabis could be considered a harmless drug, we are beginning to appreciate its consequences. Chronic exposure to addictive drugs has shown to imbalance glutamate homeostasis in Nucleus Accumbens (NAc), altering plasticity mechanisms such as long-term depression. Therefore, it is crucial to elucidate the mechanisms underlying these alterations and how to reverse them. It is known the activation of cannabinoid receptors in astrocytes modulate synaptic plasticity[2,3] and could be involved in glutamate homeostasis. However, the functional role of astrocytes in alterations derived from chronic drug exposure is not fully understood. In this study, we analyzed how astrocytes contribute to alterations produced by tetrahydrocannabinol (THC). Using fiber photometry in vivo we analyzed astrocytic activity (Ca^{2+} and glutamate dynamics) in NAc after 1mg/kg THC chronic administration in wildtype and p38αMAPK$^{-/-}$ (Astrop38α) mice[4] and we performed electrophysiology experiments to analyze synaptic plasticity. Moreover, we performed behavioral tests to assess whether THC had reinforcing properties or affected learning and memory. Furthermore, using chemogenetic approaches (DREADDs) we activated NAc astrocytes to analyze their behavioral implications. We observed: 1)THC increases astrocytic calcium activity; 2)THC induces glutamate release in NAc in wildtype, but not Astrop38α; 3)NAc astrocytes are involved in learning; 4)Removal of p38αMAPK in NAc astrocytes restores THC-related impairments. Altogether, our results reveal astrocytes as critical elements for the maintenance of glutamate signaling, with a significant role in drug-use-related alterations.

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NOVEL MECHANISM OF HYPOXIC NEURONAL DAMAGE MEDIATED BY NON-EXCITATORY AMINO ACIDS AND ASTROGLIAL SWELLING

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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AIMS. Bleeding into cerebral parenchyma during head trauma and hemorrhagic stroke leads to tissue anoxia and release of plasmatic content, including amino acids (AA). Although excitotoxic AA have been extensively studied, little is known about non-excitatory AA during hypoxic injury. Hypoxia-induced synaptic depression becomes irreversible with non-excitatory AA in hippocampal slices, corresponding with their intracellular accumulation and increased tissue electrical resistance. METHODS. A combination of four AA (AGQS: L-alanine, glycine, L-glutamine, L-serine) at plasmatic concentrations were applied (30-min) to brain slices from mice expressing EGFP in pyramidal neurons (Thy1/EGFP) or astrocytes (GFAP/EGFP) during normoxia or hypoxia. Simultaneous 2-photon imaging, changes in light transmittance (LT) and electrophysiological field recordings followed by electron microscopy in hippocampal CA1 *st. radiatum* were used to monitor cell swelling and injury, and synaptic function. RESULTS. During normoxia, AGQS-induced increase in LT is due to astroglial but not neuronal swelling. Fast LT increment during hypoxia and AGQS manifests neuronal and astroglial swelling, which was not accompanied by spreading depolarization. AGQS-induced permanent loss of synaptic transmission during hypoxia develops alongside dendritic beading, indicating acute structural damage, as revealed by 2-photon and electron microscopy. Hypoxia without AGQS does not cause cell swelling, leaving dendrites intact. Inhibition of NMDAR prevents neuronal damage and irreversible loss of field potentiaIs. Deleterious effects of AGQS during hypoxia are avoided by alanine-serine-cysteine transporters (ASCT2) and volume-regulated anion channels (VRAC) blockers. CONCLUSIONS. Swelling induced by intracellular accumulation of non-excitatory AA and release of excitotoxins through antiporters and VRAC may exacerbate hypoxia-induced neuronal injury. NIHGrant:N

BOARD NUMBER: S083858(SAK), FPU16/06368,MCIU,Spain(IAM).

Pubmed:
31428912: Luengo JG, Muñoz MD, Álvarez-Merz I, Herranz AS, González JC, Martín Del Río R, Hernández-Guijo JM, Solís JM
Intracellular accumulation of amino acids increases synaptic potentials in rat hippocampal slices.
The application of high concentrations of taurine induces long-lasting potentiation of synaptic responses and axon excitability. This phenomenon seems to require the contribution of a transport system with a low affinity for taurine. The prototypic taurine transporter TauT (SLC6A6) was discarded by experimental evidence obtained in TauT-KO mice. The purpose of the present study was to determine whether the proton-coupled amino acid transporter 1 (PAT1; SLC36A1) which is a transport system with low affinity and high capacity for a great variety of amino acids including taurine, contributes to the taurine-induced synaptic potentiation. In rat hippocampal slices, the application of several amino acids (L- and D-alanine, L-glutamine, β-guanidinopropionic acid, glycine, L-histidine, L- and D-serine, sarcosine, L- and D-threonine) imitated the synaptic potentiation induced by taurine. The magnitude of the potentiation caused by some of these amino acids was even greater than that induced by taurine. By contrast, the application of other amino acids (L-arginine, betaine, L-leucine, L-methionine, L- and D-proline, and L-valine) did not induce potentiation. The behaviour of these different amino acids on synaptic potentiation is not compatible with a role of PAT1 in synaptic potentiation. There was a positive correlation between the accumulation of the different amino acids in the slice and the magnitude of synaptic potentiation induced by them. Some of the amino acids inducing synaptic potentiation, like taurine and L-threonine, also increased electrical resistance of the slice, whereas L-

leucine did not modify this parameter. Modifications induced by either taurine or L-threonine in synaptic potentiation, slice resistance and amino acid accumulation were dependent on extracellular chloride concentration. These findings support the idea that the accumulation of amino acids throughout the action of transporters causes cell swelling enhancing the electrical resistance of the slice, which by itself could be sufficient to increase field synaptic potentials.
Amino Acids, 2019; 51
33848510: Álvarez-Merz I, Luengo JG, Muñoz MD, Hernández-Guijo JM, Solís JM
Hypoxia-induced depression of synaptic transmission becomes irreversible by intracellular accumulation of non-excitatory amino acids.
The intracellular accumulation of some amino acids (AAs), mainly glutamine, can contribute to brain edema observed during liver failure. We recently demonstrated that individual applications of high concentrations (10 mM) of some non-excitatory AAs increase the electrical resistance of hippocampal slices, indicating cell swelling. Therefore, we pondered whether an AA mixture's application might cause cell swelling at a physiological concentration range. In rat hippocampal slices, we carried out extra- and intracellular electrophysiological recordings and AAs analysis to address this question. We applied a mixture of 19 AAs at their plasmatic concentrations (Plasma solution: Ala, Gly, Gln, His, Ser, Tau, Thr, Arg, Leu, Met, Pro, Val, Asn, Cys, Phe, Ile, Lys, Tyr, and Trp). This solution was afterward divided into two according to the individual AAs at 10 mM concentration inducing synaptic potentiation (Plasma1, containing the first seven AAs of Plasma) or not (Plasma2, with the remaining AAs). Plasma application increased evoked field potentials requiring extracellular chloride. This effect was mimicked by the Plasma1 but not the Plasma2 solution. Plasma1-induced potentiation was independent of changes in release probability, basic electrophysiological membrane properties, and NMDAR activation. AAs in Plasma1 act cooperatively to accumulate intracellularly and to induce synaptic potentiation. In the presence of Plasma1, the reversible synaptic depression caused by a 40-min hypoxia period turned into an irreversible disappearance of synaptic potentials through an NMDAR-dependent mechanism. The presence of a system A transport inhibitor did not block Plasma1-mediated effects. These results indicate that cell swelling, induced by the accumulation of non-excitotoxic AAs through unidentified transporters, might foster deleterious effects produced by hypoxia-ischemia episodes.
Neuropharmacology, 2021; 190
34356384: Ramos E, López-Muñoz F, Gil-Martín E, Egea J, Álvarez-Merz I, Painuli S, Semwal P, Martins N, Hernández-Guijo JM, Romero A
The Coronavirus Disease 2019 (COVID-19): Key Emphasis on Melatonin Safety and Therapeutic Efficacy.
Viral infections constitute a tectonic convulsion in the normophysiology of the hosts. The current coronavirus disease 2019 (COVID-19) pandemic is not an exception, and therefore the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, like any other invading microbe, enacts a generalized immune response once the virus contacts the body. Melatonin is a systemic dealer that does not overlook any homeostasis disturbance, which consequently brings into play its cooperative triad, antioxidant, anti-inflammatory, and immune-stimulant backbone, to stop the infective cycle of SARS-CoV-2 or any other endogenous or exogenous threat. In COVID-19, the corporal propagation of SARS-CoV-2 involves an exacerbated oxidative activity and therefore the overproduction of great amounts of reactive oxygen and nitrogen species (RONS). The endorsement of melatonin as a possible protective agent against the current pandemic is indirectly supported by its widely demonstrated beneficial role in preclinical and clinical studies of other respiratory diseases. In addition, focusing the therapeutic action on strengthening the host protection responses in critical phases of the infective cycle makes it likely that multi-tasking melatonin will provide multi-protection, maintaining its efficacy against the virus variants that are already emerging and will emerge as long as SARS-CoV-2 continues to circulate among us.
Antioxidants (Basel), 2021; 10

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BOARD NUMBER: S03-274

PHYSIOLOGICAL SYNAPTIC ACTIVITY AND RECOGNITION MEMORY REQUIRE ASTROGLIAL GLUTAMINE

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Presynaptic glutamate replenishment is fundamental to brain function. In high activity regimes, such as epileptic episodes, this process is thought to rely on the glutamate-glutamine cycle between neurons and astrocytes. However the presence of an astroglial glutamine supply, as well as its functional relevance in vivo in the healthy brain remain controversial, partly due to a lack of tools that can directly examine glutamine transfer. Here, we generated a fluorescent probe that tracks glutamine in live cells, which provides direct visual evidence of an activity-dependent glutamine supply from astroglial networks to presynaptic structures under physiological conditions. This mobilization is mediated by connexin43, an astroglial protein with both gap-junction and hemichannel functions, and is essential for synaptic transmission and object recognition memory. Our findings uncover an indispensable recruitment of astroglial glutamine in physiological synaptic activity and memory via an unconventional pathway, thus providing an astrocyte basis for cognitive processes.

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SPATIOTEMPORAL ATP RELEASE IN CORTICAL ASTROCYTES

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

Yoshiki Hatashita[1], Zhaofa Wu[2], Yulong Li[2], Takafumi Inoue[1]
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Astrocytes participate in information processing by modulating synaptic activity through the release of ATP as a gliotransmitter. Since there are approximately 140,000 synapses in the territory of a single astrocyte in rodents, knowledge about the spatiotemporal dynamics of ATP gliotransmission, which is not understood well, will help to understand how astrocytes are involved in the information process at the neuronal circuit-level. Here, we introduced a novel genetically encoded extracellular ATP sensor, $GRAB_{ATP1.0}$, to murine cortical astrocytes by *in utero* electroporation and monitored extracellular ATP changes by two-photon microscopy. In acute slice, tetrodotoxin insensitive spontaneous ATP release events were observed, which were suppressed by an astrocyte specific toxin, fluorocitrate. Typical ATP release events were localized to sub-cellular 50–200 μm^2 areas at concentration of 0.5–5 µM, implying that a single ATP release event can activate purinergic receptors in hundreds of nearby synapses. Dual color imaging of $GRAB_{ATP1.0}$ and a calcium sensor Lck-REX-GECO1 showed that most of the spontaneous ATP release occurred independently of Ca^{2+}elevation. Inhibition of vesicular release with bafilomycin A1 did not suppress ATP release, indicating that astrocytes regulate ATP gliotransmission through non-vesicular Ca^{2+} independent ATP release mechanisms. Classification of ATP release according to the temporal waveforms suggests that astrocytes release ATP in multiple fashions. In conclusion, our findings suggest that astrocyte may locally modulate hundreds of nearby synapses by spontaneously releasing ATP via multiple Ca^{2+} independent mechanisms.

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BOARD NUMBER: S03-276

UNRAVELLING THE ROLE OF SPINAL ASTROCYTES IN NOCICEPTION AND PAIN

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Center For Brain Research (Medical University Vienna), Neurophysiology, Vienna, Austria

Aim Changes in synaptic plasticity at nociceptive synapses are strongly implicated in the pathogenesis of chronic pain. Astrocytes can profoundly affect synaptic transmission by releasing gliotransmitters and studies highlighted how altered astrocytic activity may contribute to pathogenic changes in nociception. Here, we tested whether the selective activation of astrocytes in the lumbar spinal cord dorsal horn is sufficient to induce synaptic plasticity at nociceptive synapses. **Methods** Male Sprague Dawley rats were used in all experiments. The astrocytes were targeted selectively via cell-specific *G_qDREADDs* (*Designer Receptors Exclusively Activated by Designer Drug*s) which were activated via Clozapine-N-oxide (CNO). To investigate the effect of astrocyte activation on the cellular and synaptic levels, we performed whole cell patch-clamp recordings from dorsal horn neurons in acute spinal cord slices with dorsal roots attached. Additionally, we recorded C-fibre-evoked field potentials in intact, deeply anaesthetized rats to assess synaptic strength in vivo. **Results** The selective activation of spinal astrocytes by CNO induced a robust long-term depression at nociceptive synapses in laminae I/II, both in vitro and in vivo. Furthermore, activation of spinal astrocytes increased spontaneous inhibitory currents in neurons in superficial laminae in the acute slice preparation. **Conclusions** Our study demonstrates that a selective activation of spinal astrocytes using G_q-DREADDs is sufficient to depress synaptic strength at nociceptive synapses. Our findings substantiate the role of astrocytes as active players in nociception, and can provide a rationale for the development of novel curative therapeutic strategies for pain patients . *Support: FWF Austrian Science Fund Medical Neuroscience, FWF fund(DOC 33-B27)*

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BOARD NUMBER: S03-277

RAPID MODULATION OF CHOLINERGIC INTERNEURONS AND DOPAMINE RELEASE BY SATELLITE ASTROCYTES

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Astrocytes are key supporters of brain functioning and have been shown to influence neuronal circuits at slow timescales of multiple seconds to minutes to hours. Here, we show that striatal astrocytes can rapidly modulate dopamine release at subsecond timescales. Brief optogenetic manipulation of astrocytes in mouse *ex vivo* slices directly modulates dopamine release, dependent upon activation of nicotinic acetylcholine receptors (nAChRs) on dopamine axons. We identify that striatal astrocytes in mouse are typically found in close apposition to cholinergic interneuron somata in a satellite configuration, a feature rarely observed for striatal projection neurons. As a result, satellite astrocytes potently regulate cholinergic interneuron excitability through tight regulation of extracellular ion concentrations, leading to transient increases in spiking and activation of downstream nAChRs. These rapid and reversible effects from satellite astrocytes on cholinergic interneurons are synapse-independent and not mediated by gap junction coupling, fast ionotropic glutamatergic or GABAergic transmission. Preliminary findings show that cholinergic interneurons also present satellite astrocytes in human *post mortem* striatal tissue. Together, these findings show striatal satellite astrocytes can rapidly modulate cholinergic interneurons and dopamine release at subsecond timescales.

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IMPLICATION OF GLIAL CELLS IN ACTIVITY DEPENDENT PLASTICITY OF SPINAL INHIBITION IN THE DORSAL HORN

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

Louise Vial-Markiewicz, Benjamin Léonardon, Rémy Schlichter, Perrine Inquimbert
University of Strasbourg-CNRS, Insitut Des Neurosciences Cellulaires Et Intégratives, Strasbourg, France

The dorsal horn of the spinal cord plays a pivotal role in the reception, integration and transmission of nociceptive information from the periphery to supra-spinal centers. The dorsal horn interneurons, comprised of excitatory glutamatergic neurons and inhibitory glycinergic and GABAergic neurons function as a balance, allowing normal processing of nociceptive information. In some pathological cases, plasticity in the dorsal horn network can lead to a rupture of the excitation/inhibition balance. One of the known mechanisms leading to this rupture is the recruitment of NMDA receptors, by an increase of synaptic excitation. Their role in modulation and plasticity of excitatory transmission in the dorsal horn has been largely studied, but their potential role in modulation of inhibitory GABAergic transmission has not. Our aim is to characterize the effect of NMDA receptor activation on inhibitory synaptic transmission in the dorsal horn and the implication of glial cells. To study a potential target specificity, we used GAD65eGFP mice, differentiating between GAD+ (assumed GABAergic) and GAD- (assumed glutamatergic) cells. We have shown an NMDA-dependent facilitation of synaptic GABAergic transmission in two thirds of recorded cells. Using the gliotoxin fluorocitrate to determine the role of astrocytes in NMDA-dependent facilitation, we showed a smaller proportion of neurons presenting NMDA-dependent facilitation. Interestingly, we found that astrocytes are involved in mediation specifically when recording GAD- cells, underlining a cell-specific involvement of astrocytes in the NMDA effect. Altogether, our results bring new insights in the cross-talk between excitation and inhibition in the dorsal horn, involving NMDA receptors and astrocytes.

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BOARD NUMBER: S03-279

MORPHOLOGICAL ANALYSIS OF ASTROCYTES IN DIFFERENT PREPARATIONS

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

João Luís Machado[1], João Filipe Viana[2], Daniela Sofia Abreu[1], Jérôme Wahis[1,3], Chen Liu[1], Matthew Holt[4], João Filipe Oliveira[2]
[1]ICVS, School of Medicine, Neuroscience, Braga, Portugal, [2]ICVS, School of Medicine - University of Minho, Neuroscience, Braga, Portugal, [3]VIB-KU Leuven, Center For Brain And Disease Research, Leuven, Belgium, [4]KU Leuven, Department Of Neuroscience, Laboratory Of Glia Biology, Leuven, Belgium

Astrocytes are the most abundant glial cells in the brain, presenting a typical star-shaped morphology. Their extended morphology is pivotal to allow their dynamic interactions with neighboring neurons and glia. Therefore, assessing astrocytic morphology is crucial for further insights into their regional distribution and integration in neuronal networks. For that, we used a semi-automatic tool called SNT to evaluate the morphological structure of astrocytes and gather numerous morphometric parameters such as total length, number of processes, and arbor complexity (Sholl and Convex Hull analyses). In this work, we performed a 3D reconstruction of the backbone of hippocampal astrocytes in brain slices of C57BL6/J mice using immunohistochemistry staining of the Glial Fibrillary Acidic Protein (GFAP), the astrocytic cytoskeleton protein. Furthermore, we also studied brain slices from astrocyte-specific RiboTag mice allowing the more detailed identification of the astrocytic structure. Our results show that both preparations allow the detailed reconstruction of the astrocytic backbone, despite the considerable increase in detail provided by the RiboTag approach. Nevertheless, the simpler and more flexible GFAP-immunostaining might be enough to reveal intra-hippocampal heterogeneity.

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BOARD NUMBER: S03-280

HETEROGENEITY OF THE ASTROCYTE MORPHOLOGY WITHIN THE MOUSE HIPPOCAMPUS

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

João Filipe Viana[1], João Luís Machado[1], Daniela Sofia Abreu[1], Gabriela Tavares[1], Manuella Martins[1], Vanessa Morais Sardinha[1], Sónia Guerra-Gomes[1], Cátia Domingos[2], Alberto Pauletti[2], Jérôme Wahis[3], Chen Li[4], Christian Henneberger[5], Matthew Holt[6], João Filipe Oliveira[1]
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Astrocytes integrate brain circuits, and they can sense, process, and respond to incoming signals, giving rise to the concept of “tripartite synapse”. These close interactions are due to the complex morphological structure that allows them to extend their processes to cover several synapses. Therefore, assessing astrocytic morphology is pivotal for further insights into their regional distribution and its importance for brain functioning. Here, we evaluated the morphological structure of astrocytes from several layers of the hippocampus, namely CA1 oriens, radiatum, lacunosum moleculare layers, and DG molecular and hilus layers. Furthermore, we also assessed the relevance of astrocyte exocytosis for maintaining the astrocytic backbone in the CA1 radiatum and DG molecular layers. We used the SNT Fiji tool, which allowed the tridimensional reconstruction of the main astrocytic structure. This analysis obtained several morphometric parameters such as total length, number of processes, and arbor complexity (Sholl analysis). Briefly, the results showed that astrocytes from different hippocampal layers display distinct morphological profiles, and these morphological differences are conserved throughout the dorsalventral axis of the hippocampus. Moreover, we also showed that astrocyte exocytosis is also involved in the maintenance of astrocytes structure in the hippocampus.

Pubmed:
32166822: Guerra-Gomes S, Cunha-Garcia D, Marques Nascimento DS, Duarte-Silva S, Loureiro-Campos E, Morais Sardinha V, Viana JF, Sousa N, Maciel P, Pinto L, Oliveira JF
IP R2 null mice display a normal acquisition of somatic and neurological development milestones.
Astrocytes are key players in the regulation of brain development and function. They sense and respond to the surrounding activity by elevating their intracellular calcium (Ca) levels. These astrocytic Ca elevations emerge from different sources and display complex spatio-temporal properties. Ca elevations are spatially distributed in global (soma and main processes) and/or focal regions (microdomains). The inositol 1,4,5-trisphosphate receptor type 2 knockout (IP R2 KO) mouse model lacks global Ca elevations in astrocytes, and it has been used by different laboratories. However, the constitutive deletion of IP R2 during development may trigger compensating phenotypes, which could bias the results of experiments using developing or adult mice. To address this issue, we performed a detailed neurodevelopmental evaluation of male and female IP R2 KO mice, during the first 21 days of life, as well as an evaluation of motor function, strength and neurological reflexes in adult mice. Our results show that male and female IP R2 KO mice display a normal acquisition of developmental milestones, as compared with wild-type (WT) mice. We also show that IP R2 KO mice display normal motor coordination, strength and neurological reflexes in adulthood. To exclude a potential compensatory overexpression of other IP Rs, we quantified the relative mRNA levels of all 3 subtypes, in brain tissue. We found that, along with the complete deletion of Itpr2, there is no compensatory expression of Itpr1 or Itrp3. Overall, our results show that the IP R2 KO mouse is a reliable model to study the functional impact of global IP R2-dependent astrocytic Ca elevations.
Eur J Neurosci, 2021; 54
32139688: Batiuk MY, Martirosyan A, Wahis J, de Vin F, Marneffe C, Kusserow C, Koeppen J, Viana JF, Oliveira JF, Voet T, Ponting CP, Belgard TG, Holt MG
Identification of region-specific astrocyte subtypes at single cell resolution.
Astrocytes, a major cell type found throughout the central nervous system, have general roles in the modulation of synapse formation and synaptic transmission, blood-brain barrier formation, and regulation of blood flow, as well as metabolic support

of other brain resident cells. Crucially, emerging evidence shows specific adaptations and astrocyte-encoded functions in regions, such as the spinal cord and cerebellum. To investigate the true extent of astrocyte molecular diversity across forebrain regions, we used single-cell RNA sequencing. Our analysis identifies five transcriptomically distinct astrocyte subtypes in adult mouse cortex and hippocampus. Validation of our data in situ reveals distinct spatial positioning of defined subtypes, reflecting the distribution of morphologically and physiologically distinct astrocyte populations. Our findings are evidence for specialized astrocyte subtypes between and within brain regions. The data are available through an online database (https://holt-sc.glialab.org/), providing a resource on which to base explorations of local astrocyte diversity and function in the brain.
Nat Commun, 2020; 11
30455631: Guerra-Gomes S, Viana JF, Nascimento DSM, Correia JS, Sardinha VM, Caetano I, Sousa N, Pinto L, Oliveira JF
The Role of Astrocytic Calcium Signaling in the Aged Prefrontal Cortex.
Aging is a lifelong process characterized by cognitive decline putatively due to structural and functional changes of neural circuits of the brain. Neuron-glial signaling is a fundamental component of structure and function of circuits of the brain, and yet its possible role in aging remains elusive. Significantly, neuron-glial networks of the prefrontal cortex undergo age-related alterations that can affect cognitive function, and disruption of glial calcium signaling has been linked with cognitive performance. Motivated by these observations, we explored the possible role of glia in cognition during aging, considering a mouse model where astrocytes lacked IPR2-dependent Ca signaling. Contrarily to aged wild-type animals that showed significant impairment in a two-trial place recognition task, aged IPR2 KO mice did not. Consideration of neuronal and astrocytic cell densities in the prefrontal cortex, revealed that aged IPR2 KO mice present decreased densities of NeuN neurons and increased densities of S100β astrocytes. Moreover, aged IPR2 KO mice display refined dendritic trees in this region. These findings suggest a novel role for astrocytes in the aged brain. Further evaluation of the neuron-glial interactions in the aged brain will disclose novel strategies to handle healthy cognitive aging in humans.
Front Cell Neurosci, 2018; 12

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BOARD NUMBER: S03-281

THE ROLE OF ASTROCYTES IN SOMATOSENSORY SYNAPTIC PLASTICITY DURING DEVELOPMENT: INTEGRATION OF DATA AND COMPUTATIONAL MODELING

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Astrocytes have been shown to be important regulators of neural development and circuit maturation, including control of the number of synapses and synaptic connectivity. Recent evidence also indicates that astrocytes modulate synaptic plasticity during postnatal development, at least in somatosensory and prefrontal cortices as well as in hippocampus. Synaptic plasticity, defined as the activity-dependent change in the strength of the synaptic connection between neurons, is expressed in the brain in diverse forms across multiple timescales and orchestrated through complex biophysical and biochemical mechanisms. We have previously analyzed published computational models that involve some of the suggested mechanisms (Manninen et al., 2018). In addition, we have developed a model for a layer 4 to layer 2/3 synapse based on more than 70 references on experimental data in somatosensory cortex during postnatal development and on computational modeling (Manninen et al., 2020). The model links neuronal and astrocytic signaling pathways to the time window of long-term depression (LTD) induction. In the present study, we use our model to address the natural spike pattern-induced LTD by computational means. The modeling results provide insights into the influence of spike frequency on the dynamics of the cellular pathways. Continuous cross-talk between wet-lab experiments and computational modeling is needed to decipher the roles of astrocytes in synaptic computations in developing sensory systems. **Acknowledgements:** The work was supported by Academy of Finland (Nos. 326494, 326495, 345280) and European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3).

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-282

NOVEL CA2+-MODULATED PHOTOACTIVATABLE IMAGING REVEALS NEURON-ASTROCYTE GLUTAMATERGIC CIRCUITRIES WITHIN THE NUCLEUS ACCUMBENS

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Astrocytes have been traditionally studied as a homogeneous group, however, recent research has started to evidence their heterogeneity. Our hypothesis is that specialized astrocyte subsets are responsible for the modulation of specific neuronal circuits. We focused on the Nucleus Accumbens (NAc), an important integrator center in which converges different glutamatergic signals coming primarily from the medial prefrontal cortex (mPFC), basolateral amygdala (Amyg), and ventral hippocampus (vHip). In this work, we analyze whether astrocytes establish segregated populations in the NAc with intrinsic properties and functional consequences for the circuit. To this end, we have used optogenetic manipulations to afferent-specific synaptic stimulation to the NAc combining with a new adapted technique (calcium-modulated photoactivatable ratiometric integrator under GFAP promoter, CaMPARI$_{GFAP}$) to specifically dissect the active astrocyte circuits with spatio-temporal precision. We demonstrate that NAc astrocytes show pathway-specific interactions with the glutamatergic afferents and that this activity does not correlate with the glutamatergic innervation patterns, suggesting unexpected astrocytic connectivity, i.e. activation of precise astrocytic populations in response to specific glutamatergic inputs. Moreover, the spatial activation of these astrocytic networks is not defined by alterations in astrocyte density or uneven expression of mGluR5. Finally, we show that different subpopulations of astrocytes in both NAc regions receive and integrate signals arising from all the excitatory afferents. Our work reveals different neuron-astrocyte glutamatergic circuits in the NAc, showing pathway-specific astrocytic responses mediated by mGluR5. These results highlight the astrocytic contribution to NAc functionality, providing a potential explanation to comprehend how NAc integrates information from multiple glutamatergic inputs.

Pubmed:

32034128: Domingo-Rodriguez L, Ruiz de Azua I, Dominguez E, Senabre E, Serra I, Kummer S, Navandar M, Baddenhausen S, Hofmann C, Andero R, Gerber S, Navarrete M, Dierssen M, Lutz B, Martín-García E, Maldonado R
A specific prelimbic-nucleus accumbens pathway controls resilience versus vulnerability to food addiction.
Food addiction is linked to obesity and eating disorders and is characterized by a loss of behavioral control and compulsive food intake. Here, using a food addiction mouse model, we report that the lack of cannabinoid type-1 receptor in dorsal telencephalic glutamatergic neurons prevents the development of food addiction-like behavior, which is associated with enhanced synaptic excitatory transmission in the medial prefrontal cortex (mPFC) and in the nucleus accumbens (NAc). In contrast, chemogenetic inhibition of neuronal activity in the mPFC-NAc pathway induces compulsive food seeking. Transcriptomic analysis and genetic manipulation identified that increased dopamine D2 receptor expression in the mPFC-NAc pathway promotes the addiction-like phenotype. Our study unravels a new neurobiological mechanism underlying resilience and vulnerability to the development of food addiction, which could pave the way towards novel and efficient interventions for this disorder.
Nat Commun, 2020; 11
31273206: Navarrete M, Cuartero MI, Palenzuela R, Draffin JE, Konomi A, Serra I, Colié S, Castaño-Castaño S, Hasan MT, Nebreda ÁR, Esteban JA
Astrocytic p38α MAPK drives NMDA receptor-dependent long-term depression and modulates long-term memory.
NMDA receptor-dependent long-term depression (LTD) in the hippocampus is a well-known form of synaptic plasticity that has been linked to different cognitive functions. The core mechanism for this form of plasticity is thought to be entirely neuronal. However, we now demonstrate that astrocytic activity drives LTD at CA3-CA1 synapses. We have found that LTD induction enhances astrocyte-to-neuron communication mediated by glutamate, and that Ca signaling and SNARE-dependent vesicular release from the astrocyte are required for LTD expression. In addition, using optogenetic techniques, we show that low-frequency astrocytic activation, in the absence of presynaptic activity, is sufficient to induce postsynaptic AMPA receptor removal and LTD expression. Using cell-type-specific gene deletion, we show that astrocytic p38α MAPK is required for the increased astrocytic glutamate release and astrocyte-to-neuron communication during low-frequency

stimulation. Accordingly, removal of astrocytic (but not neuronal) p38α abolishes LTD expression. Finally, this mechanism modulates long-term memory in vivo.
Nat Commun, 2019; 10
34246770: Navarro-Gonzalez C, Carceller H, Benito Vicente M, Serra I, Navarrete M, Domínguez-Canterla Y, Rodríguez-Prieto Á, González-Manteiga A, Fazzari P
Nrg1 haploinsufficiency alters inhibitory cortical circuits.
Neuregulin 1 (NRG1) and its receptor ERBB4 are schizophrenia (SZ) risk genes that control the development of both excitatory and inhibitory cortical circuits. Most studies focused on the characterization ErbB4 deficient mice. However, ErbB4 deletion concurrently perturbs the signaling of Nrg1 and Neuregulin 3 (Nrg3), another ligand expressed in the cortex. In addition, NRG1 polymorphisms linked to SZ locate mainly in non-coding regions and they may partially reduce Nrg1 expression. Here, to study the relevance of Nrg1 partial loss-of-function in cortical circuits we characterized a recently developed haploinsufficient mouse model of Nrg1 (Nrg1). These mice display SZ-like behavioral deficits. The cellular and molecular underpinnings of the behavioral deficits in Nrg1 mice remain to be established. With multiple approaches including Magnetic Resonance Spectroscopy (MRS), electrophysiology, quantitative imaging and molecular analysis we found that Nrg1 haploinsufficiency impairs the inhibitory cortical circuits. We observed changes in the expression of molecules involved in GABAergic neurotransmission, decreased density of Vglut1 excitatory buttons onto Parvalbumin interneurons and decreased frequency of spontaneous inhibitory postsynaptic currents. Moreover, we found a decreased number of Parvalbumin positive interneurons in the cortex and altered expression of Calretinin. Interestingly, we failed to detect other alterations in excitatory neurons that were previously reported in ErbB4 null mice suggesting that the Nrg1 haploinsufficiency does not entirely phenocopies ErbB4 deletions. Altogether, this study suggests that Nrg1 haploinsufficiency primarily affects the cortical inhibitory circuits in the cortex and provides new insights into the structural and molecular synaptic impairment caused by NRG1 hypofunction in a preclinical model of SZ.
Neurobiol Dis, 2021; 157

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BOARD NUMBER: S03-283

QUANTIFICATION OF REGIONAL AND INTERSPECIES ASTROCYTE INVOLVEMENT IN SYNAPSES

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Evidence suggests that astrocytes play key roles in the structural and functional organization of neuronal circuits. Astrocytes seem to be an integral part of the synapse, according to the tripartite synapse concept. Because recent evidence has demonstrated that astrocytes integrate and process synaptic information and control synaptic transmission, astrocytes could be cellular elements involved in the processing and transfer of information by the nervous system. To investigate tripartite synapse involvement in neural circuit complexity, we wanted to see the regional differences how many synapses astrocytes are involved in and if there is a difference between species.. We performed immunofluorescent staining on human and murine brain sections of different brain areas. Synapses were marked by anti-synaptophysin and the astrocytes were marked with anti-GFAP antibodies. Image acquisition was done by confocal microscope and images were analyzed by ImageJ software. In our preliminary results, we have found that the human brain has much more astrocyte density and involvement in synapses than that of a mouse. This difference in astrocyte and synapse overlap can be a possible explanation of the difference between organisms of higher and lower cognitive function. Acknowledgments: Research was funded by the Centre of Excellence for Basic, Clinical and Translational Neuroscience (project “Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain”; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund) and by the project of the Croatian Science Foundation FURNACE (IP-2018-01-7416).

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-284

MECHANISM OF NMDA RECEPTOR POTENTIATION BY LACTATE

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Astrocyte-derived lactate fuels high-energy demands of neurons and acts as a signal promoting synaptic plasticity and memory consolidation. Recent findings have shown that lactate regulates NMDA receptor activity and modulates gene expression related to synaptic plasticity and neuroprotection. Here, we used patch-clamp recordings in cultured cortical neurons to elucidate further the mechanism of NMDA receptor potentiation by lactate. We found that lactate rapidly enhanced both the amplitude and inactivation time-constant of NMDA receptor currents (I_{NMDAR}) evoked by brief applications of glutamate and glycine. These effects were not observed by agonists of the HCAR1/gpr81 receptor while the lactate-mediated increase in I_{NMDAR} amplitude was prevented by monocarboxylate transporters and lactate dehydrogenase inhibitors. Intracellular infusion of specific CaMKII peptide inhibitors also abolished the potentiation of peak I_{NMDAR} responses by lactate, indicating a mechanism requiring entry of lactate into neurons, its oxidation to pyruvate, and involvement of CaMKII. Moreover, interfering with the binding between CaMKII and GluN2B, using a pharmaco-genetic model consisting of CaMKIIα-expressing HEK293 cells transiently transfected with functional NMDA receptors containing mutant GluN2B subunits, prevented the potentiation of I_{NMDAR} responses by lactate. Proximity ligation assays between GluN2B and the postsynaptic density marker PSD-95 in cultured neurons revealed that lactate induced an accumulation of GluN2B in dendritic spines, an effect that was prevented by a CaMKII peptide inhibitor. Together these observations indicate that lactate uptake stimulates synaptic plasticity by increasing the distribution of NMDA receptors in spines by a mechanism promoting the interaction between CaMKII and GluN2b subunits.

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BOARD NUMBER: S03-285

PHARMACOLOGICALLY COMPROMISING CENTRAL AMYGDALA ASTROCYTES PREVENTS THE BENEFICIAL EFFECTS OF OXYTOCIN ON PAIN-RELATED BEHAVIORS

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

Alexandre Charlet[1], Damien Kerspern[2], Jérôme Wahis[3], Angel Baudon[4], Benjamin Boury-Jamot[5], Marie Pertin[6], Perrine Inquimbert[7], Isabelle Decosterd[8]
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Over the past decade studies have started to focus on the role of the astrocytes as a key modulator of the neuronal network activity. Importantly, the scientific community has now access to advanced tools to study the astrocytic contribution to the modulation of the neuronal network: genetically modified mice or engineered viral vectors. However, those tools remain expensive and therefore not affordable for many laboratories. Taking advantage of a highly studied circuit in which the crucial involvement of astrocytes has recently been described, namely the modulation of central amygdala circuit by the neuropeptide oxytocin, we aimed at providing evidence that pharmacological metabolic silencing of astrocytes can be of interest to modern science. Here, we demonstrated that fluorocitrate is an efficient inhibitor of OT-evoked astroglial calcium activity. We further found that metabolic silencing of astrocytes disturbs astro-neuronal communication without impairing the neuronal network basal activity. Finally, we showed that in vivo local infusion of fluorocitrate efficiently and safely impaired the OT-induced modulation of CeA-related behavior.

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THE PROXIMITY OF ASTROCYTE LEAFLETS TO THE SYNAPSE DETERMINES MEMORY STRENGTH

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Astrocyte distal processes, known as leaflets or perisynaptic astrocyte processes (PAPs), fine-tune synaptic activity by clearing neurotransmitters and limiting extrasynaptic glutamate diffusion. While learning and memory depend on orchestrated synaptic activity of neuronal ensembles within the hippocampus, it is becoming increasingly evident that astrocytes residing in the environment of these synapses play a central role in shaping memories. However, how astroglial synaptic coverage contributes to mnemonic processing remains largely unknown. Here, we targeted astrocyte leaflet structure *in vivo* by depleting Ezrin, an integral leaflet-structural protein, in astrocytes of the adult hippocampal CA1 using a CRISPR-Cas9 genetic approach. This resulted in significantly smaller astrocyte territories and reduced astroglial synaptic coverage. In addition, using genetically encoded glutamate sensors and whole-cell patch-clamp recordings from pyramidal neurons, we found that Ezrin deletion and the resultant manipulation of leaflet structure boosted extrasynaptic glutamate diffusion and NMDA-receptor activation. Importantly, these cellular phenotypes translated to enhanced fear memory expression that was accompanied by increased activation of CA1 pyramidal neurons in the days after learning occurred. We show that Ezrin is critical for astrocyte morphology as well as for adult hippocampal synapse integrity and function. Our data show that astrocyte leaflets structure gates memory strength by regulating glutamate spillover in the vicinity of memory-related synaptic activity.

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BOARD NUMBER: S03-287

ASTROCYTE SIGNALLING TO NEURONS THROUGH EXOSOMES

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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In the CNS, astrocyte processes regulate synaptic transmission and plasticity. They have also a role in the extra-synaptic signalling, participating with the release of extracellular vesicles. Exosomes are non-classical signal transmission and can indeed participate in volume transmission. ***Aims.*** It is known that cultured astrocytes can release exosomes, but these cells can only marginally mimic the behaviour of astrocytes *in situ*. Here we assess if isolated astrocyte processes could send messages through exosomes. ***Methods*.** We prepared freshly isolated astrocyte processes from the adult rat cerebral cortex and collected extracellular vesicles. By WB, we proved the presence of glial and exosomal markers; the vesicle size was measured with DLS; by EM, we investigated the presence of multivesicular bodies in the processes and the vesicle features. By IF, we assessed the exosome cell target when we added exosomes to a primary astrocyte-neuron co-culture. ***Results*.** Astrocyte processes expressed Alix and TSG101, and presented multivesicular bodies, consistent with their ability to release exosomes. The astrocyte-released extracellular vesicles had the typical cup-shaped appearance, the size, and markers for their parental astrocytic origin and for exosomes. These exosomes were able to selectively target neuronal cells and to be internalized by neurons. ***Conclusions*.** Isolated astrocyte processes, acutely prepared from astrocytes matured in a neuron-astrocyte network in the CNS, might participate to signal transmission by releasing exosomes. Astrocyte-released exosomes might target near or long-distance targets by volume transmission. Moreover, these exosomes might transfer NGB of astrocytic origin to target neurons.

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BOARD NUMBER: S03-288

CX3CR1 SIGNALING INVOLVEMENT IN SLEEP-INDUCED MICROGLIAL MORPHODYNAMICS CHANGES

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Microglial cells, the resident immune cells of the brain, have particularly dynamic processes. Various studies suggested that beyond a possible role in surveillance, microglial dynamics could be linked to synaptic mechanisms or, at least, to neuronal activity. However, signaling pathways modulating neuronal control of microglial motility remain largely unknown. As vigilance states are characterized by distinct neuronal activities, we first assessed whether and how microglial morphodynamics is modulated during sleep/wake alternation. Our second aim was to identify neuronal signaling pathways involved in the regulation of microglial dynamic. We focused on the fractalkine signaling pathway, a chemokine released by neurons that binds to CX3CR1 expressed by microglia. Microglial morphodynamic changes were monitored through *in vivo* transcranial imaging using two-photon microscopy, while electroencephalogram and electromyogram were recorded to monitor vigilance states. Transgenic CX3CR1-GFP mice were used to observe and analyze fluorescent microglial cells. We evaluated the impact of sleep-wake cycles and fractalkine receptor depletion to figure out their role in microglial dynamic. Consequently, we performed morphodynamic analysis to evaluate process motility and cell complexity. Our results indicate a decrease in microglial morphodynamics during slow wave sleep depending on daytime. We also found that depletion of the fractalkine receptor abolished these sleep-induced morphodynamics changes, indicating that fractalkine could be involved in microglia's detection and/or response to neuronal activity changes. In conclusion, this work identifies fractalkine as potent modulator of microglial dynamics in physiological conditions. This study will lead to a better understanding of microglial functions in the context of synaptic transmission and plasticity.

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BOARD NUMBER: S03-289

OPPOSITE EFFECTS OF NEURONAL AND GLIAL A2A RECEPTORS ON GLUTAMATERGIC SYNAPSE REMODELING DURING SYNAPTOGENESIS

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Adenosine acts as a fine regulator of synaptic plasticity in the adult brain. However, its action during development remains poorly understood. We recently showed that a transient increase of the A_{2A} receptor ($A_{2A}R$) expression during hippocampal synaptogenesis matched with a role in the stabilization of nascent inhibitory GABAergic synapses of the hippocampus (Gomez-Castro et al., Science 374 2021). We asked here if this regulation extends to glutamatergic excitatory synapses. In mixed neuron-glia cultures undergoing synaptogenesis, the suppression of neuronal $A_{2A}R$ with a shRNA approach led to the loss of ~30% of glutamatergic synapses identified with the pre- and post- synaptic markers VGlut1 and PSD95. This suggests that the activation of neuronal $A_{2A}R$ stabilizes nascent glutamatergic synapses. In contrast, blocking $A_{2A}R$ in the mixed neuron-glia cultures with a selective antagonist (SCH58261) tended to increase the density of VGlut1/PSD95 synapses, and significantly reduced the mobility of the AMPA receptor GluR1 subunit within synapses, which was trapped within synapses. On the contrary, activating $A_{2A}R$ in these cultures with a selective agonist (CGS21680) led to synapse loss. Since this effect was blocked upon depletion of glial cells with arabinofuranoside or microglia with L-methylester or PLX or upon application of C1q neutralizing antibodies, we proposed that the activation of microglial $A_{2A}R$ would lead to synaptic pruning through complement-based mechanism. Therefore, neuronal and microglial $A_{2A}R$ have opposite effects on the stabilization of nascent glutamatergic synapses. We are now using conditional approaches to study the contribution of neuronal and microglial $A_{2A}R$ in vitro and in vivo.

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BOARD NUMBER: S03-290

IMPAIRED 5-HT SIGNALING TO MICROGLIA IN THE DEVELOPPING BRAIN IMPACTS CIRCUITS REFINEMENT, ADULT SOCIABILITY AND FLEXIBILITY, AND THE RESPONSE TO INFLAMMATION.

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Background: Microglia are key factors of brain development, notably through their role in synaptic refinement. Serotonin (5-HT), besides its role of neuromodulator in adulthood, is a neurodevelopmental factor involved in the postnatal formation of somatosensory and emotional circuits. Microglial cells express serotonin receptors, mainly the 5-HT_{2B} subtype, and we have shown that 5-HT can induce an oriented growth of their processes. Aim: Considering the involvement of microglia in normal brain development as well as in neurodevelopmental psychiatric disorders, and their sensitivity to 5-HT, we investigated how 5-HT regulates microglia in the postnatal period and the consequences of abrogating this control. Methods: To this aim, we investigated microglia morphology and synapses in mice conditionally ablated for 5-HT2B receptors in microglia specifically, and the behavioral consequences of this ablation, either since birth or P30. Results: We observed that a deletion of *Htr2b* since birth impacts on microglia development and on synapse density and axonal refinement during adolescence. Absence of this early serotonin-microglia interaction has also detrimental consequences on sociability and behavioral flexibility, and on the response to a systemic inflammation in adulthood. In contrast, invalidation of microglial *Htr2b* at P30 onward has no effect on these behaviors. Conclusion: Our results indicate that a neonatal instruction of microglia by 5-HT, before P30, is required to allow their normal functions in brain development, and that a perturbation of this early control can be a risk factor for neurodevelopmental psychiatric disorders.

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BOARD NUMBER: S03-291

MICROGLIAL REMOVAL OF INHIBITORY SYNAPSES UNLEASH THE MULTI-SENSORY POTENTIAL IN THE ASSOCIATION CORTEX

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Sensory inputs are essential to detect the external environment, but a part of them is disturbed in the blind and deaf. Traditionally, the concept of cross-modal plasticity has been raised, which an impaired sensory input is compensated by the other sensory systems. Previous study showed whisker-dependent activation of visual cortex in the eye enucleated mice. However, the mechanism of cross-modal plasticity has not been shown yet. In this research, we unravel the effect of early visual deprivation on the activation of the visual cortex with whisker stimulation. We first visualized the axonal projection from S1 (primary somatosensory cortex) to V2 (extrastriate cortex), which has whisker-evoked responses both in normal sighted and monocular deprived mice (MD). Then we detected clear differences of neuronal activity of V2 between MD and control when touched with sandpaper; normal sighted mice showed strong suppression while MD did not. This suggests that visual deprivation triggers the removal of the inherited system that the other modality suppress the cortex. We hypothesized that microglia, mediators for experience-depended synaptic plasticity, can play a part in remodeling of the circuits of V2. Actually, the depletion of microglia with pexidartinib reduced the effect of visual deprivation. We discovered microglia in MD wrap the soma of pyramidal neurons and cut the inputs from parvalbumin-expressing interneurons with immunohistological and electrophysiological methods. Furthermore, interrupting the remodeling of extracellular matrix by inhibiting matrix metalloproproteinase-9 lessens the cross-modal effect. This study will be an important clue to understand the microglial physiological function in experience-depended synaptic plasticity.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-292

MODULATION OF NEURONAL ACTIVITY BY WHISKER STIMULATION CONTROLS MICROGLIAL DYNAMICS TOWARDS DENDRITIC SPINES

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Microglia are the resident immune cells of the central nervous system. They widely contribute to the neuroinflammatory process, but their exact function in the non-pathologic brain remains elusive. In non-pathologic conditions, microglia are characterized by highly ramified and dynamic processes that constantly probe the parenchyma and contact synapses. However, the exact purpose of microglia processes motility and its potential regulation by neuronal activity remain to be determined. Recent data from the lab indicate that neuronal activity was associated with changes in microglial morphodynamics. The aim of the present study was to better characterize the interactions between synaptic activity and microglial dynamics. To that end, we used whisker stimulations (WS) *in vivo* to manipulate neuronal activity while performing two-photon imaging. We monitored both microglial morphodynamics and calcium activity of dendritic spines of the corresponding barrel cortex. Our results indicated that WS induced various dendritic spines calcium responses due to the polysynaptic nature of the network. Increase of activity in spines triggered by WS induced a shortening of the distance between the spine and the closest microglial process, an increased probability of contact, and a longer duration of contact in average. Conversely, decrease of activity in spines triggered by WS induced a shortening of the contact duration and remote location of microglial processes in respect to their initial distance. Overall, the present model provides a causal link between neuronal activity and microglial contacts in physiological condition. Our results indicate that microglial processes' proximity with spines is directly controlled by change of activity.

FENS Forum 2022
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BOARD NUMBER: S03-293

MICROGLIA MEDIATE SYNAPTIC PLASTICITY INDUCED BY TRANSCRANIAL MAGNETIC STIMULATION

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Aims: Microglia are the resident immune cells of the brain. They play an important role in neuroinflammatory processes. However, in recent years their role in physiological processes such as neural excitability and plasticity has been identified. Here we investigated the role of microglia in synaptic plasticity induced by 10 Hz repetitive magnetic stimulation (rMS), a clinically established non-invasive brain stimulation technique. **Methods:** Microglia were depleted from mouse organotypic tissue cultures with PLX3397 (Pexidartinib). Using whole-cell patch-clamp recordings, confocal microscopy, immunohistochemistry, protein and transcriptome analyses, we assessed structural and functional properties of CA1 pyramidal neurons in the presence or absence of microglia, and investigated the effects of 10 Hz rMS on excitatory neurotransmission and microglia. **Results:** The presence of microglia was required for the expression of excitatory synaptic plasticity in CA1 pyramidal neurons after 10 Hz rMS. Although rMS did not alter the morphology or the dynamics of microglia, an increased production and secretion of microglia-related cytokines was observed 3 h after stimulation. Indeed, substitution of these cytokines in microglia-depleted tissue cultures rescued the expression of rMS-induced synaptic plasticity. **Conclusion:** We conclude that clinically employed non-invasive electromagnetic stimulation affects synaptic plasticity by modulating the release of cytokines from microglia. Supported by DFG (SFB/TRR 167) and the MOTI-VATE Program, Faculty of Medicine, University of Freiburg.

Pubmed:
34723795: Lenz M, Eichler A, Kruse P, Muellerleile J, Deller T, Jedlicka P, Vlachos A
All-trans retinoic acid induces synaptopodin-dependent metaplasticity in mouse dentate granule cells.
Previously we showed that the vitamin A metabolite all-trans retinoic acid (atRA) induces synaptic plasticity in acute brain slices prepared from the mouse and human neocortex (Lenz et al., 2021). Depending on the brain region studied, distinct effects of atRA on excitatory and inhibitory neurotransmission have been reported. Here, we used intraperitoneal injections of atRA (10 mg/kg) in adult C57BL/6J mice to study the effects of atRA on excitatory and inhibitory neurotransmission in the mouse fascia dentata-a brain region implicated in memory acquisition. No major changes in synaptic transmission were observed in the ventral hippocampus while a significant increase in both spontaneous excitatory postsynaptic current frequencies and synapse numbers were evident in the dorsal hippocampus 6 hr after atRA administration. The intrinsic properties of hippocampal dentate granule cells were not significantly different and hippocampal transcriptome analysis revealed no essential neuronal changes upon atRA treatment. In light of these findings, we tested for the metaplastic effects of atRA, that is, for its ability to modulate synaptic plasticity expression in the absence of major changes in baseline synaptic strength. Indeed, in vivo long-term potentiation (LTP) experiments demonstrated that systemic atRA treatment improves the ability of dentate granule cells to express LTP. The plasticity-promoting effects of atRA were not observed in synaptopodin-deficient mice, therefore, extending our previous results regarding the relevance of synaptopodin in atRA-mediated synaptic strengthening in the mouse prefrontal cortex. Taken together, our data show that atRA mediates synaptopodin-dependent metaplasticity in mouse dentate granule cells.
Elife, 2021; 10
33781382: Lenz M, Kruse P, Eichler A, Straehle J, Beck J, Deller T, Vlachos A

All-trans retinoic acid induces synaptic plasticity in human cortical neurons.
A defining feature of the brain is the ability of its synaptic contacts to adapt structurally and functionally in an experience-dependent manner. In the human cortex, however, direct experimental evidence for coordinated structural and functional synaptic adaptation is currently lacking. Here, we probed synaptic plasticity in human cortical slices using the vitamin A derivative all-trans retinoic acid (atRA), a putative treatment for neuropsychiatric disorders such as Alzheimer's disease. Our experiments demonstrated that the excitatory synapses of superficial (layer 2/3) pyramidal neurons underwent coordinated structural and functional changes in the presence of atRA. These synaptic adaptations were accompanied by ultrastructural remodeling of the calcium-storing spine apparatus organelle and required mRNA translation. It was not observed in synaptopodin-deficient mice, which lack spine apparatus organelles. We conclude that atRA is a potent mediator of synaptic plasticity in the adult human cortex.
Elife, 2021; 10
33652912: Lenz M, Eichler A, Vlachos A
Monitoring and Modulating Inflammation-Associated Alterations in Synaptic Plasticity: Role of Brain Stimulation and the Blood-Brain Interface.
Inflammation of the central nervous system can be triggered by endogenous and exogenous stimuli such as local or systemic infection, trauma, and stroke. In addition to neurodegeneration and cell death, alterations in physiological brain functions are often associated with neuroinflammation. Robust experimental evidence has demonstrated that inflammatory cytokines affect the ability of neurons to express plasticity. It has been well-established that inflammation-associated alterations in synaptic plasticity contribute to the development of neuropsychiatric symptoms. Nevertheless, diagnostic approaches and interventional strategies to restore inflammatory deficits in synaptic plasticity are limited. Here, we review recent findings on inflammation-associated alterations in synaptic plasticity and the potential role of the blood-brain interface, i.e., the blood-brain barrier, in modulating synaptic plasticity. Based on recent findings indicating that brain stimulation promotes plasticity and modulates vascular function, we argue that clinically employed non-invasive brain stimulation techniques, such as transcranial magnetic stimulation, could be used for monitoring and modulating inflammation-induced alterations in synaptic plasticity.
Biomolecules, 2021; 11
33391287: Lenz M, Eichler A, Kruse P, Strehl A, Rodriguez-Rozada S, Goren I, Yogev N, Frank S, Waisman A, Deller T, Jung S, Maggio N, Vlachos A
Interleukin 10 Restores Lipopolysaccharide-Induced Alterations in Synaptic Plasticity Probed by Repetitive Magnetic Stimulation.
Systemic inflammation is associated with alterations in complex brain functions such as learning and memory. However, diagnostic approaches to functionally assess and quantify inflammation-associated alterations in synaptic plasticity are not well-established. In previous work, we demonstrated that bacterial lipopolysaccharide (LPS)-induced systemic inflammation alters the ability of hippocampal neurons to express synaptic plasticity, i.e., the long-term potentiation (LTP) of excitatory neurotransmission. Here, we tested whether synaptic plasticity induced by repetitive magnetic stimulation (rMS), a non-invasive brain stimulation technique used in clinical practice, is affected by LPS-induced inflammation. Specifically, we explored brain tissue cultures to learn more about the direct effects of LPS on neural tissue, and we tested for the plasticity-restoring effects of the anti-inflammatory cytokine interleukin 10 (IL10). As shown previously, 10 Hz repetitive magnetic stimulation (rMS) of organotypic entorhino-hippocampal tissue cultures induced a robust increase in excitatory neurotransmission onto CA1 pyramidal neurons. Furthermore, LPS-treated tissue cultures did not express rMS-induced synaptic plasticity. Live-cell microscopy in tissue cultures prepared from a novel transgenic reporter mouse line [] confirms that LPS administration triggers microglial tumor necrosis factor alpha (TNFα) expression, which is ameliorated in the presence of IL10. Consistent with this observation, IL10 hampers the LPS-induced increase in TNFα, IL6, IL1β, and IFNγ and restores the ability of neurons to express rMS-induced synaptic plasticity in the presence of LPS. These findings establish organotypic tissue cultures as a suitable model for studying inflammation-induced alterations in synaptic plasticity, thus providing a biological basis for the diagnostic use of transcranial magnetic stimulation in the context of brain inflammation.
Front Immunol, 2020; 11

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BOARD NUMBER: S03-294

MICROGLIA MOTILITY DEPENDS ON NEURONAL ACTIVITY AND IS ASSOCIATED WITH STRUCTURAL PLASTICITY OF DENDRITIC SPINES IN THE HIPPOCAMPUS

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Microglia, the resident immune cells of the brain, play a complex role in health and disease. They actively survey the brain parenchyma by physically interacting with other cells and structurally shaping the brain. Yet, the mechanisms underlying microglia motility and their significance for synapse stability, especially during adulthood, remain widely unresolved. Here we investigated the impact of neuronal activity on microglia motility and its implication for synapse formation and survival. We used repetitive two-photon *in vivo* imaging in the hippocampus of awake mice to simultaneously study microglia motility and their interaction with synapses. By applying a pharmacological and chemogenetic approach we found that microglia process motility depended on neuronal activity. Simultaneously, more dendritic spines emerged in awake compared to anesthetized mice. Interestingly, microglia contact rates with individual dendritic spines were associated with their stability. These results suggest that microglia are not only sensing neuronal activity, but participate in synaptic rewiring of the hippocampus during adulthood, which has profound relevance for learning and memory processes.

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BOARD NUMBER: S03-295

A ROLE OF MICROGLIA ACTIVATION IN TNFα-MEDIATED SYNAPTIC PLASTICITY

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Aims: The pro-inflammatory cytokine tumor necrosis factor-α (TNFα) has been implicated in different forms of synaptic plasticity. Even though a wealth of literature suggests that TNFα affects synaptic transmission, the role of TNFα-mediated activation of microglia has not been addressed in this context. Here, we assessed concentration-dependent effects of TNFα on synaptic strength and the activation of microglia. **Methods:** We employed three-week old organotypic entorhino-hippocampal tissue cultures prepared from wild type mice of either sex. Whole-cell patch-clamp recordings of CA1 pyramidal neurons, transcriptomic analysis, immunohistochemistry and live-cell microscopy were used to study the effects of TNFα on synaptic transmission and microglial activation. **Results:** Low concentrations of TNFα promoted synaptic plasticity by enhancing excitatory synaptic strength and not affecting inhibitory neurotransmission. An increase in synaptic GluA1-content was evident 24 h after exposure to TNFα. At higher concentrations TNFα strongly activated microglia and increased inhibitory neurotransmission without affecting excitatory synaptic strength. The passive properties of CA1 pyramidal neurons were not altered by TNFα. Interestingly, microglia-depleted tissue cultures treated with a high concentration of TNFα showed enhanced excitatory neurotransmission comparable to what we observed in non-depleted tissue cultures treated with a low concentration of TNFα. **Conclusions:** These findings extend previous results on the role of TNFα in synaptic plasticity by demonstrating concentration-dependent effects of TNFα on excitatory and inhibitory neurotransmission. They suggest a role of activated microglia in homeostasis of excitatory neurotransmission.

Pubmed:
30401642: Lenz M, Galanis C, Kleidonas D, Fellenz M, Deller T, Vlachos A
Denervated mouse dentate granule cells adjust their excitatory but not inhibitory synapses following in vitro entorhinal cortex lesion.
Neurons adjust their synaptic strength in a homeostatic manner following changes in network activity and connectivity. While this form of plasticity has been studied in detail for excitatory synapses, homeostatic plasticity of inhibitory synapses remains not well-understood. In the present study, we employed entorhinal cortex lesion (ECL) of organotypic entorhino-hippocampal tissue cultures to test for homeostatic changes in GABAergic neurotransmission onto partially denervated dentate granule cells. Using single and paired whole-cell patch-clamp recordings, as well as immunostainings for synaptic markers, we find that excitatory synaptic strength is robustly increased 3 days post lesion (dpl), whereas GABAergic neurotransmission is not changed after denervation. Even under conditions of pharmacological inhibition of glutamatergic neurotransmission, which prevents neurons to compensate for the loss of input via excitatory synaptic scaling, down-scaling of GABAergic synapses does not emerge 3 days after denervation. We conclude that granule cells maintain structural and functional properties of GABAergic synapses even in the face of substantial changes in network connectivity. Hence, alterations in inhibitory neurotransmission, as seen in pathological brain states, may not simply reflect a homeostatic response to disconnection.
Exp Neurol, 2019; 312
34831454: Kleidonas D, Vlachos A
Scavenging Tumor Necrosis Factor α Does Not Affect Inhibition of Dentate Granule Cells Following In Vitro Entorhinal Cortex Lesion.
Neurons that lose part of their afferent input remodel their synaptic connections. While cellular and molecular mechanisms of denervation-induced changes in excitatory neurotransmission have been identified, little is known about the signaling

pathways that control inhibition in denervated networks. In this study, we used mouse entorhino-hippocampal tissue cultures of both sexes to study the role of the pro-inflammatory cytokine tumor necrosis factor α (TNFα) in denervation-induced plasticity of inhibitory neurotransmission. In line with our previous findings in vitro, an entorhinal cortex lesion triggered a compensatory increase in the excitatory synaptic strength of partially denervated dentate granule cells. Inhibitory synaptic strength was not changed 3 days after the lesion. These functional changes were accompanied by a recruitment of microglia in the denervated hippocampus, and experiments in tissue cultures prepared from TNF-reporter mice [] showed increased TNFα expression in the denervated zone. However, inhibitory neurotransmission was not affected by scavenging TNFα with a soluble TNF receptor. In turn, a decrease in inhibition, i.e., decreased frequencies of miniature inhibitory postsynaptic currents, was observed in denervated dentate granule cells of microglia-depleted tissue cultures. We conclude from these results that activated microglia maintain the inhibition of denervated dentate granule cells and that TNFα is not required for the maintenance of inhibition after denervation.
Cells, 2021; 10

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BOARD NUMBER: S03-296

IN VIVO TWO-PHOTON STED IMAGING OF MICROGLIA-SYNAPSE INTERACTIONS

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Microglia-synapse interactions have been examined in a variety of contexts, *in vitro* and *in vivo*. Our approach goes beyond the current state-of-the-art and for the first time yields microglia-synapse interactions in super-resolution time-lapse imaging of awake mice. We established two-photon (2P) stimulated emission depletion (STED) microscopy in the hippocampus *in vivo*. This is feasible by implantation of a cranial window and head-fixation of the mice during imaging. 2P imaging enables us to increase imaging depth, decrease light scattering and restrict excitation to a smaller focal volume. At the same time the additional 3D-STED application further minimizes the illumination of the focal point and improves the point spread function not only in the x, y- dimensions but also in the z-dimension. Extensive research has been done on microglia in disease and under inflammatory conditions. However, information how microglia influence synapse stability under normal physiological conditions and related to learning and memory is still scarce. Therefore, our aim is to determine the molecular mechanisms of how microglia maintain synapse stability under normal physiological conditions in relation to learning and memory. Our results show improved resolution of 2P 3D-STED imaging of microglia-synapse interactions and a relationship between dendritic spine formation and previous contact frequency by microglia. These findings will pave the way to visualize nanoscale anatomical structures during microglia-synapse interactions *in vivo* and therefore allows longitudinal and correlative studies in combination with behavioral experiments.

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THE ROLE OF MICROGLIA IN SYNAPTIC ADAPTATIONS IN THE NUCLEUS ACCUMBENS AFTER COCAINE-INDUCED CONDITIONED PLACE PREFERENCE

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Exposure to cocaine generates silent synapses in the nucleus accumbens. During drug withdrawal, those synapses progressively maturate by recruiting calcium permeable-AMPA receptors (CP-AMPAR). This form of plasticity has been linked to the emergence of drug-sensitization and cue-induced drug-seeking. The study of the molecular substrates that underlie these synaptic adaptations has long focused on neuronal signaling molecules. Recently microglia have emerged as a critical player for experience-dependent synaptic plasticity in the adult brain. We investigated the role of microglia in the maturation of silent synapses by the incorporation of CP-AMPAR in the nucleus accumbens after withdrawal from cocaine-conditioned place preference (CPP). We trained mice for cocaine-induced conditioned place preference. We then depleted microglia with PLX-supplemented diet for three weeks after CPP training. On day 21 we tested the mice for place preference and then performed whole-cell patch clamp recordings to determine the presence of silent synapses and CP-AMPAR.

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POSSIBLE ROLES OF AMYLOID-β IN MICROGLIA-MEDIATED SYNAPSE REMODELING

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Microglia, the resident immune cells of the central nervous system, are critical players in neuronal plasticity and function. They are highly dynamic, constantly interacting with neighboring cells and synaptic structures. Experimental evidence indicates that close contacts with synapses are driven by synaptic activity, and are important for microglia-mediated synapse remodeling. A growing literature shows a link between synaptic activity and amyloid-β (Aβ) peptide. Aβ has been widely studied in Alzheimer's disease (AD), being the major component of the extracellular plaques associated with the pathology. Interestingly, intracellular Aβ also correlates with synaptic function. Our data, supportive of high Aβ levels in the postnatal brain, led us to hypothesize that intrasynaptic Aβ might play a role in microglia-mediated synapse remodeling.
Using an AD mouse model (ArcAβ mice) and a pharmacological approach to modulate intraneuronal Aβ load, we aim at investigating the involvement of Aβ in microglial synaptic pruning during brain development. First, we characterized the lipidomic profiles of synaptosomes isolated from ArcAβ and wildtype littermates at postnatal day 15 (P15), identifying candidate molecules that could promote engulfment by microglia. We also found that the synaptic profile in the ArcAβ hippocampus displayed alterations in both pre- and post-synaptic markers already at early time points (P15-P30). Furthermore, microglial density was reduced at P15, indicating that Aβ/humanAPP overexpression is associated with early changes in microglia. Overall, these findings suggest that synaptic and microglial alterations are present at early stages in the brain of an AD mouse model, likely contributing to neurodegeneration later in life.

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ROLE OF PV+ BC MYELINATION IN INHIBITORY PRECISION IN THE MOUSE CA1 HIPPOCAMPAL MICROCIRCUIT.

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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[1]Netherlands Institute for Neuroscience, Axonal Signalling Group, Amsterdam, Netherlands, [2]Utrecht University, Cell Biology, Neurobiology And Biophysics, Department Of Biology, Utrecht, Netherlands

The hippocampus is of fundamental importance for the storage and retrieval of episodic memories. In the process of neuronal computations, the reliability of information transfer between neurons is critical. One mechanism to optimise precision and speed within neuronal circuits is the wrapping of neuronal axons with multi-lamellar membrane sheaths of compact myelin, reducing the capacitive load of internodes and thereby increasing conduction velocity. While the speed advantage applies to long-range axonal projections, previous studies in the neocortex suggest that myelination of locally projecting parvalbumin-positive basket cells (PV^+BC) may impact release characteristics. PV^+BC axons allow reliable propagation of high frequency trains of action potentials (APs) and are characterized by synchronous transmitter release at elevated release probabilities. These features allow PV^+BCs to function as clockworks driving network oscillations in the gamma frequency range. Here, to better understand how myelination of hippocampal PV^+BC axons affects synaptic physiology and microcircuit function, we made targeted paired patch-clamp recordings of tdTomato expressing PV^+BCs and CA1 pyramidal neurons and used the unitary inhibitory postsynaptic currents to estimate basal release and vesicle pool dynamics. Control myelinated connections were characterized by a fast onset latency of ~0.8 ms, rise- and decay time of 0.47 ms and ~6 ms, respectively. Furthermore, synapses showed a high P_r of 0.6 together with a readily-releasable-pool of ~460 pA (n = 18 pairs). Presently, using a hypomyelinated- (*Shiverer*) and toxic demyelination (cuprizone) mouse model, we are investigating the direct contribution of compact myelin to the features of synaptic precision in hippocampal area CA1.

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BOARD NUMBER: S03-300

ACETYLCHOLINE AND LATERAL HABENULA PARTNERSHIP IN SHAPING PUNISHMENT ANTICIPATION

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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The ability to associate a neutral environmental stimulus with an aversive experience is fundamental for animal's survival since it allows to anticipate negative outcomes. The lateral habenula (LHb) is instrumental to compute aversive events and guides actions to avoid them. Recent evidence indicates that LHb neurons express functional acetylcholine (ACh) receptors whose inactivation interferes with optimal decision-making. Here we examine whether and how ACh release modulates LHb neuronal dynamics potentially dictating punishment anticipatory actions. We study neuronal and ACh dynamics in the LHb in head-restrained mice undergoing a discriminatory Pavlovian aversive conditioning task. We employ two-photon calcium imaging and fiber photometry recordings to assess LHb neuronal and ACh dynamics during the conditioning procedure, and perturb LHb function through optogenetic approaches. We find two populations of LHb neurons developing opposite dynamics to a punishment predictive cue (CS+) when mice display punishment anticipatory behaviors (i.e. eye blinking). Optogenetic manipulation of LHb neurons disrupt mouse ability to discriminate between neutral (CS-) and airpuff predictive (CS+) cues. Finally, using an ACh biosensor (GRAB-ACh), we report that ACh-dependent signal develops in the LHb in response to the CS+ after conditioning. Overall, our results indicate that two sub-populations of LHb neurons develop opposite responses to punishment predictive cues, which are relevant for mouse optimal performance. Lastly, increased ACh release arises in the LHb after learning, likely representing the underlying mechanistic substrate for LHb activity changes and mouse ability to anticipate threats appropriately.

Pubmed:
35013094: Mondoloni S, Mameli M, Congiu M
Reward and aversion encoding in the lateral habenula for innate and learned behaviours.
Throughout life, individuals experience a vast array of positive and aversive events that trigger adaptive behavioural responses. These events are often unpredicted and engage actions that are likely anchored on innate behavioural programs expressed by each individual member of virtually all animal species. In a second step, environmental cues, that are initially neutral, acquire value through the association with external sensory stimuli, and become instrumental to predict upcoming positive or negative events. This process ultimately prompts learned goal-directed actions allowing the pursuit of rewarding experience or the avoidance of a danger. Both innate and learned behavioural programs are evolutionarily conserved and fundamental for survival. Among the brain structures participating in the encoding of positive/negative stimuli and contributing to innate and learned behaviours is the epithalamic lateral habenula (LHb). The LHb provides top-down control of monoaminergic systems, responds to unexpected appetitive/aversive stimuli as well as external cues that predict the upcoming rewards or punishments. Accordingly, the LHb controls a number of behaviours that are innate (originating from unpredicted stimuli), and learned (stemming from predictive cues). In this review, we will discuss the progresses that rodent's experimental work made in identifying how LHb activity governs these vital processes, and we will provide a view on how these findings integrate within a complex circuit connectivity.
Transl Psychiatry, 2022; 12
34553321: Congiu M, Micheli L, Santoni M, Sagheddu C, Muntoni AL, Makriyannis A, Malamas MS, Ghelardini C, Di Cesare Mannelli L, Pistis M
N-Acylethanolamine Acid Amidase Inhibition Potentiates Morphine Analgesia and Delays the Development of Tolerance.
Opioids are essential drugs for pain management, although long-term use is accompanied by tolerance, necessitating dose escalation, and dependence. Pharmacological treatments that enhance opioid analgesic effects and/or attenuate the development of tolerance (with a desirable opioid-sparing effect in treating pain) are actively sought. Among them, N-palmitoylethanolamide (PEA), an endogenous lipid neuromodulator with anti-inflammatory and neuroprotective properties, was shown to exert anti-hyperalgesic effects and to delay the emergence of morphine tolerance. A selective augmentation in endogenous PEA levels can be achieved by inhibiting N-acylethanolamine acid amidase (NAAA), one of its primary

hydrolyzing enzymes. This study aimed to test the hypothesis that NAAA inhibition, with the novel brain permeable NAAA inhibitor AM11095, modulates morphine's antinociceptive effects and attenuates the development of morphine tolerance in rats. We tested this hypothesis by measuring the pain threshold to noxious mechanical stimuli and, as a neural correlate, we conducted in vivo electrophysiological recordings from pain-sensitive locus coeruleus (LC) noradrenergic neurons in anesthetized rats. AM11095 dose-dependently (3-30 mg/kg) enhanced the antinociceptive effects of morphine and delayed the development of tolerance to chronic morphine in behaving rats. Consistently, AM11095 enhanced morphine-induced attenuation of the response of LC neurons to foot-shocks and prevented the attenuation of morphine effects following chronic treatment. Behavioral and electrophysiological effects of AM11095 on chronic morphine were paralleled by a decrease in glial activation in the spinal cord, an index of opioid-induced neuroinflammation. NAAA inhibition might represent a potential novel therapeutic approach to increase the analgesic effects of opioids and delay the development of tolerance.
Neurotherapeutics, 2021; 18

34019906: Clerke JA, Congiu M, Mameli M

Neuronal adaptations in the lateral habenula during drug withdrawal: Preclinical evidence for addiction therapy.
The epithalamic lateral habenula (LHb) regulates monoaminergic systems and contributes to the expression of both appetitive and aversive behaviours. Over the past years, the LHb has emerged as a vulnerable brain structure in mental illnesses including addiction. Behavioural and functional evidence in humans and rodents provide substantial support for a role of LHb in the negative affective symptoms emerging during withdrawal from addictive substances. Multiple forms of cellular and synaptic adaptations that take hold during drug withdrawal within the LHb are causally linked with the emergence of negative affective symptoms. These results indicate that targeting drug withdrawal-driven adaptations in the LHb may represent a potential strategy to normalize drug-related behavioural adaptations. In the current review we describe the mechanisms leading to functional alterations in the LHb, as well as the existing interventions used to counteract addictive behaviours. Finally, closing this loop we discuss and propose new avenues to potentially target the LHb in humans in light of the mechanistic understanding stemming from pre-clinical studies. Altogether, we provide an overview on how to leverage cellular-level understanding to envision clinically-relevant approaches for the treatment of specific aspects in drug addiction.
Neuropharmacology, 2021; 192

33535028: Nuno-Perez A, Trusel M, Lalive AL, Congiu M, Gastaldo D, Tchenio A, Lecca S, Soiza-Reilly M, Bagni C, Mameli M

Stress undermines reward-guided cognitive performance through synaptic depression in the lateral habenula.
Weighing alternatives during reward pursuit is a vital cognitive computation that, when disrupted by stress, yields aspects of neuropsychiatric disorders. To examine the neural mechanisms underlying these phenomena, we employed a behavioral task in which mice were confronted by a reward and its omission (i.e., error). The experience of error outcomes engaged neuronal dynamics within the lateral habenula (LHb), a subcortical structure that supports appetitive behaviors and is susceptible to stress. A high incidence of errors predicted low strength of habenular excitatory synapses. Accordingly, stressful experiences increased error choices while decreasing glutamatergic neurotransmission onto LHb neurons. This synaptic adaptation required a reduction in postsynaptic AMPA receptors (AMPARs), irrespective of the anatomical source of glutamate. Bidirectional control of habenular AMPAR transmission recapitulated and averted stress-driven cognitive deficits. Thus, a subcortical synaptic mechanism vulnerable to stress underlies behavioral efficiency during cognitive performance.
Neuron, 2021; 109

33031862: Sagheddu C, Traccis F, Serra V, Congiu M, Frau R, Cheer JF, Melis M

Mesolimbic dopamine dysregulation as a signature of information processing deficits imposed by prenatal THC exposure.
Cannabis is the illicit drug most widely used by pregnant women worldwide. Its growing acceptance and legalization have markedly increased the risks of child psychopathology, including psychotic-like experiences, which lowers the age of onset for a first psychotic episode. As the majority of patients with schizophrenia go through a premorbid condition long before this occurs, understanding neurobiological underpinnings of the prodromal stage of the disease is critical to improving illness trajectories and therapeutic outcomes. We have previously shown that male rat offspring prenatally exposed to Δ-tetrahydrocannabinol (THC), a rat model of prenatal cannabinoid exposure (PCE), exhibit extensive molecular and synaptic changes in dopaminergic neurons of the ventral tegmental area (VTA), converging on a hyperdopaminergic state. This leads to a silent psychotic-like endophenotype that is unmasked by a single exposure to THC. Here, we further characterized the VTA dopamine neuron and sensorimotor gating functions of PCE rats exposed to acute stress or a challenge of the D2 receptor agonist apomorphine, by using in vivo single-unit extracellular recordings and Prepulse Inhibition (PPI) analyses. At pre-puberty, PCE male rat offspring display a reduced population activity of VTA dopamine neurons in vivo, the majority of which are tonically active. PCE male progeny also exhibit enhanced sensitivity to dopamine D2 (DAD2) receptor activation and a vulnerability to acute stress, which is associated with compromised sensorimotor gating functions. This data extends our knowledge of the multifaceted sequelae imposed by PCE in the mesolimbic dopamine system of male pre-adolescent rats, which renders a neural substrate highly susceptible to subsequent challenges that may trigger psychotic-like outcomes.

Prog Neuropsychopharmacol Biol Psychiatry, 2021; 105
31611707: Frau R, Miczán V, Traccis F, Aroni S, Pongor CI, Saba P, Serra V, Sagheddu C, Fanni S, Congiu M, Devoto P, Cheer JF, Katona I, Melis M
Prenatal THC exposure produces a hyperdopaminergic phenotype rescued by pregnenolone.
The increased legal availability of cannabis has led to a common misconception that it is a safe natural remedy for, among others, pregnancy-related ailments such as morning sickness. Emerging clinical evidence, however, indicates that prenatal cannabis exposure (PCE) predisposes offspring to various neuropsychiatric disorders linked to aberrant dopaminergic function. Yet, our knowledge of how cannabis exposure affects the maturation of this neuromodulatory system remains limited. Here, we show that male, but not female, offspring of Δ-tetrahydrocannabinol (THC)-exposed dams, a rat PCE model, exhibit extensive molecular and synaptic changes in dopaminergic neurons of the ventral tegmental area, including altered excitatory-to-inhibitory balance and switched polarity of long-term synaptic plasticity. The resulting hyperdopaminergic state leads to increased behavioral sensitivity to acute THC exposure during pre-adolescence. The neurosteroid pregnenolone, a US Food and Drug Administration (FDA) approved drug, rescues synaptic defects and normalizes dopaminergic activity and behavior in PCE offspring, thus suggesting a therapeutic approach for offspring exposed to cannabis during pregnancy.
Nat Neurosci, 2019; 22
30860301: Congiu M, Trusel M, Pistis M, Mameli M, Lecca S
Opposite responses to aversive stimuli in lateral habenula neurons.
Appropriate behavioural strategies to cope with unexpected salient stimuli require synergistic neuronal responses in diverse brain regions. Among them, the epithalamic lateral habenula (LHb) plays a pivotal role in processing salient stimuli of aversive valence. Integrated in the complex motivational circuit, LHb neurons are indeed excited by aversive stimuli, including footshock (Fs). However, whether such excitation is a common feature represented throughout the LHb remains unclear. Here, we combined single-unit extracellular recordings in anaesthetized mice with juxtacellular labelling to describe the nature, location and pharmacological properties of Fs-driven responses within the LHb. We find that, along with Fs-excited cells, about 10% of LHb neurons display Fs-mediated inhibitory responses. Such inhibited neuronal population, in contrast to Fs-excited neurons, display regular and high frequency activity at baseline and is clustered in the medial portion of the LHb. Juxtacellular labelling of Fs-excited and inhibited neurons unravels that both populations are of glutamatergic type, as they co-localized with the EAAC1 glutamatergic transporter but not with the GAD67 GABAergic marker. Moreover, while the excitatory responses to Fs require both AMPA and NMDA receptors, the inhibitory responses rely instead on GABA channels. Taken together, our results indicate that two functionally and partly segregated LHb neuronal ensembles encode Fs in an opposite fashion. This highlights the neuronal complexity in the LHb for processing aversive external stimuli.
Eur J Neurosci, 2019; 50
30765165: Trusel M, Nuno-Perez A, Lecca S, Harada H, Lalive AL, Congiu M, Takemoto K, Takahashi T, Ferraguti F, Mameli M
Punishment-Predictive Cues Guide Avoidance through Potentiation of Hypothalamus-to-Habenula Synapses.
Throughout life, individuals learn to predict a punishment via its association with sensory stimuli. This process ultimately prompts goal-directed actions to prevent the danger, a behavior defined as avoidance. Neurons in the lateral habenula (LHb) respond to aversive events as well as to environmental cues predicting them, supporting LHb contribution to cue-punishment association. However, whether synaptic adaptations at discrete habenular circuits underlie such associative learning to instruct avoidance remains elusive. Here, we find that, in mice, contingent association of an auditory cue (tone) with a punishment (foot shock) progressively causes cue-driven LHb neuronal excitation during avoidance learning. This process is concomitant with the strengthening of LHb AMPA receptor-mediated neurotransmission. Such a phenomenon occludes long-term potentiation and occurs specifically at hypothalamus-to-habenula synapses. Silencing hypothalamic-to-habenulainputs or optically inactivating postsynaptic AMPA receptors within the LHb disrupts avoidance learning. Altogether, synaptic strengthening at a discrete habenular circuit transforms neutral stimuli into salient punishment-predictive cues to guide avoidance.
Neuron, 2019; 102
30439418: Sagheddu C, Scherma M, Congiu M, Fadda P, Carta G, Banni S, Wood JT, Makriyannis A, Malamas MS, Pistis M
Inhibition of N-acylethanolamine acid amidase reduces nicotine-induced dopamine activation and reward.
Tobacco smoke is the leading preventable cause of death in the world and treatments aimed to increase success rate in smoking cessation by reducing nicotine dependence are sought. Activation of peroxisome proliferator-activated receptor-alpha (PPARα) by synthetic or endogenous agonists was shown to suppress nicotine-induced activation of mesolimbic dopamine system, one of the major neurobiological substrates of nicotine dependence, and nicotine-seeking behavior in rats and monkeys. An alternative indirect way to activate PPARα is inhibition of N-acylethanolamine acid amidase (NAAA), one of the major hydrolyzing enzyme for its endogenous agonists palmitoylethanolamide (PEA) and oleoylethanolamide (OEA). We synthetized a novel specific brain permeable NAAA inhibitor, AM11095. We administered AM11095 to rats and carried out

brain lipid analysis, a functional observational battery (FOB) to assess toxicity, in vivo electrophysiological recording from dopamine cells in the ventral tegmental area, brain microdialysis in the nucleus accumbens shell and behavioral experiments to assess its effect on nicotine -induced conditioned place preference (CPP). AM11095 (5 and 25 mg/kg, i.p.) was devoid of neurotoxic and behavioral effects and did not affect motor behavior and coordination. This NAAA inhibitor (5 mg/kg i.p.) increased OEA and PEA levels in the hippocampus and cortex, prevented nicotine-induced activation of mesolimbic dopamine neurons in the ventral tegmental area, nicotine-induced elevation of dopamine levels in the nucleus accumbens shell and decreased the expression of nicotine CPP. Our results indicate that NAAA inhibitors represent a new class of pharmacological tools to modulate brain PEA/PPARα signalling and show potential in the treatment of nicotine dependence.
Neuropharmacology, 2019; 144

28623385: Pisu MG, Boero G, Biggio F, Garau A, Corda D, Congiu M, Concas A, Porcu P, Serra M
Juvenile social isolation affects maternal care in rats: involvement of allopregnanolone.
Social isolation of rats immediately after weaning is thought to represent an animal model of anxiety-like disorders. Socially isolated virgin females showed a significant decrease in allopregnanolone levels, associated with increased anxiety-related behavior compared with group-housed rats.
Psychopharmacology (Berl), 2017; 234

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FUNCTIONAL AND MOLECULAR ANALYSES OF NG2 GLIA IN THE CEREBELLUM

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Aims. Grey matter NG2 glia constitute a heterogeneous glial population whose functions remain incompletely understood. In the hippocampus, Schaffer collaterals activate AMPA receptors (AMPARs) in NG2 glia, giving rise to small post-synaptic currents (PSCs). Cerebellar climbing fibers can also form synapses with NG2 glia, producing much larger PSCs. We aim to assess mechanisms underlying these regional differences and better understand the role of NG2 glia AMPARs in influencing the activity of neuronal networks. **Methods.** Combined patch-clamp and RT-PCR analyses allowed for determining properties and functions of AMPARs expressed by cerebellar NG2 glia, and comparing them with their hippocampal counterpart. Field potential recordings (fEPSPs) were conducted in slices from mice with inducible deletion of AMPARs GluA1-4 in NG2 glia (GluAflox). **Results.** RT-PCR data suggested selective expression of the auxiliary AMPAR subunit TARP-γ2 in cerebellar NG2 glia, which is required for translocation of Ca^{2+}-permeable (CP) AMPARs to the plasma membrane. Comparing excitatory PSCs in NG2 glia of both regions while applying CP-AMPARs antagonists revealed a higher expression of those receptors in the cerebellum. fEPSPs recordings in slices from GluAflox mice unraveled impaired long term potentiation (LTP) in both regions. **Conclusions.** These findings help explaining divergent properties of NG2 glia AMPARs in the hippocampus and cerebellum. Currently, we are investigating whether the impaired plasticity in GluAflox mice can be rescued pharmacologically, to identify potential mechanisms by which NG2 glia influence neuronal networks. Supported by DFG SPP1757 (STE 552/5, SE 774/6)

Pubmed:
34177466: Hardt S, Tascio D, Passlick S, Timmermann A, Jabs R, Steinhäuser C, Seifert G
Auxiliary Subunits Control Function and Subcellular Distribution of AMPA Receptor Complexes in NG2 Glia of the Developing Hippocampus.
Synaptic and axonal glutamatergic signaling to NG2 glia in white matter is critical for the cells' differentiation and activity dependent myelination. However, in gray matter the impact of neuron-to-NG2 glia signaling is still elusive, because most of these cells keep their non-myelinating phenotype throughout live. Early in postnatal development, hippocampal NG2 glia express AMPA receptors with a significant Ca permeability allowing for plasticity of the neuron-glia synapses, but whether this property changes by adulthood is not known. Moreover, it is unclear whether NG2 glia express auxiliary transmembrane AMPA receptor related proteins (TARPs), which modify AMPA receptor properties, including their Ca permeability. Through combined molecular and functional analyses, here we show that hippocampal NG2 glia abundantly express TARPs γ4, γ7, and γ8 as well as cornichon (CNIH)-2. TARP γ8 undergoes profound downregulation during development. Receptors of adult NG2 glia showed an increased sensitivity to blockers of Ca permeable AMPA receptors, but this increase mainly concerned receptors located close to the soma. Evoked synaptic currents of NG2 glia were also sensitive to blockers of Ca permeable AMPA receptors. The presence of AMPA receptors with varying Ca permeability during postnatal maturation may be important for the cells' ability to sense and respond to local glutamatergic activity and for regulating process motility, differentiation, and proliferation.
Front Cell Neurosci, 2021; 15

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BOARD NUMBER: S03-302

A COMPREHENSIVE PROTEOMIC ANALYSIS OF THE NEUREXIN INTERACTOME IN THE MAMMALIAN BRAIN

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Neurons are polarized cells, which build functional networks of communication, through synapses. These are specialized junctions between neurons and their formation is mediated by cell adhesion proteins. Among those, Neurexins are widely recognized as key proteins for synapse organization and loss-off-function mutations are associated with neurodevelopmental disorders as autism or schizophrenia. Three coding genes and extensive alternative splicing generate Neurexin proteins with various binding motifs, a flexibility which may determine synapse specification. A comprehensive understanding of the underlying molecular mechanisms is however largely missing. Our aim is to unravel the molecular composition of Neurexin networks in the brain by using unbiased quantitative proteomic approaches. Here we show results from targeted proteomics, combining affinity isolation of endogenous Neurexin complexes from mouse brain with high-resolution quantitative mass spectrometry. The identified interactome of Neurexin 1-3 contains members of different classes of secreted, adhesion or integral membrane proteins. This includes known binding partners (such as Cerebellin, Neuroligin and LRRTM proteins), but also quite a number of proteins that lack any annotation of primary function(s) and relation to synaptic physiology. We present in detail the common protein building blocks of Neurexins at the pre-synapse, the repertoire of ligands and trans-synaptic bridges and the differences among the isoforms. Furthermore, the impact of heparan sulfate modifications on the interactome will be shown. Together, we provide insights into the complex molecular environment of Neurexins in the brain and therefore open a possibility for future targeted analysis of molecular mechanisms involved in synapse formation, maintenance and function.

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BOARD NUMBER: S03-303

SELECTIVE OPTICAL CONTROL OF CALCIUM SIGNALLING IN ASTROCYTES BY AZOBENZENE PHOTOSWITCHES IN VITRO AND EX-VIVO.

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

Diletta Spennato[1], Massimo Baroncini[2], Emanuela Saracino[1], Roberta Fabbri[1], Francesco Formaggio[3], Denisa Belov Kirdajova[4], Barbara Barile[5], Marco Caprini[3], Grazia Paola Nicchia[5], Miroslava Anderova[4], Roberto Zamboni[1], Alberto Credi[2], Valentina Benfenati[1]
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Several studies emphasize the major role of astrocytic intracellular calcium signaling in brain homeostasis and physiology. Among membrane ion Ca^{2+} channels, transient receptor potential channels (TRP), called TRPA1 and TRPV4 are of interest of astrocytic Ca^{2+} signaling. Azobenzene (Azo) is a molecule that can be switched from *trans* to *cis* configuration by light stimuli. Recent evidence indicated that Azo-derived molecular photoswitches can modulate the functionality of neurons. However, it is unknown whether Azo also specifically affects astrocytes' functions. **AIMS** Novel tools to modulate astrocytic Ca^{2+} signaling might be relevant to elucidate the mechanisms underpinning astrocytic role in information processing and in pathologies. Here, we investigate the effect of Azo in primary cultured astrocytes, heterologous expression systems and brain slices. **METHODS** Ca^{2+} imaging technique. **RESULTS** We found that photostimulation of Azo elicits Ca^{2+} signalling in astrocytes in a dose-dependent manner. Pharmacological analyses revealed that extracellular Ca^{2+} influx, intracellular Ca^{2+} release and the function of TRPV4, TRPA1 are critical for the Azo-induced Ca^{2+} signal. Experiments in heterologous expression confirmed that TRPA1 and TRPV4 are activated by Azo. We used a set of Azo derivatives to describe mechanisms beyond the observed effect. The results were confirmed also in brain slices from GFAP-eGFP mice, clearly defining a Ca^{2+}response induced by Azo photostimulation in rodent brain astrocytes. **CONCLUSIONS** Collectively the results demonstrated that the photoswitching of Azo could represent a powerful tool to trigger and modulate TRP Ca^{2+} signalling in astrocytes, thereby opening a new perspective to study astrocytes' role in brain function and dysfunction.

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BOARD NUMBER: S03-304

GLYCOGEN-BINDING FLUORESCENT PROBE IN ASTROCYTES DISPLAYS SIGNAL TRANSLOCATION IN RESPONSE TO METABOLIC OR NEURONAL ACTIVITY MANIPULATION IN VIVO

POSTER SESSION 03 - SECTION: ASTROCYTES AND MICROGLIA

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Glycogen is predominantly stored in astrocytes in the brain, and glycogen metabolism has been implicated in synaptic plasticity and memory. Nonetheless, cellular glycogen changes have not been visualized in live animal's brain. Here we attempted to label glycogen clusters by expressing a fluorescent protein-fused glycogen-binding protein. We expressed such a glycogen-tag probe in astrocytes of the mouse somatosensory cortex using an adeno-associated viral vector. *In vivo* two-photon microscopy through a cranial window showed a punctate distribution in astrocytic processes resembling glycogen immunohistochemistry and was distinct from the cytosolic morphology by GFP expression. Systemic administration of 20% glucose resulted in significant increases of fluorescence in the soma. Attenuating neuronal activity by Gi-DREADD also resulted in fluorescent signal increases in somas and processes after an hour. Methionine sulfoximime (LMSO, i.p. 50 mg/kg, a subconvulsive low dosage), a known reagent to increase brain glycogen content, increased fluorescence signals in somas and processes after one hour. Since cAMP elevation induces glycogen breakdown, we co-expressed Gs-DREADD and the glycogen-binding fluorescent protein. However, we found that fluorescence signal distribution was no longer punctate even before Gs-DREADD activation by CNO. Instead, the fluorescence signal had a cytosolic appearance, which is possibly due to a constitutive activation of Gs-DREADD. This first-generation glycogen probe appears to indicate glycogen localization in astrocytes. Future studies shall address glycogen dynamics in distinct brain states and during disease development.

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BOARD NUMBER: S03-305

THE S100B PROTEIN AS A THERAPEUTIC TARGET FOR MULTIPLE SCLEROSIS PROCESSES

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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S100B is a calcium-binding protein mainly concentrated in astrocytes. Its levels in biological fluids are recognized as a reliable, even predictive, biomarker of active neural distress. Mounting evidence now points to S100B as a Damage-Associated Molecular Pattern protein which, when released at high concentration, triggers tissue reaction to damage in various neural disorders (1). In particular, the inhibitor of S100B activity pentamidine has been shown to ameliorate clinical scores and neuropathologic-biomolecular parameters in the relapsing-remitting experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis (MS) (2). Also arundic acid (AA), a known inhibitor of astrocytic S100B synthesis, in the chronic EAE,, which is another mouse model of MS usually studied. by the daily evaluation of clinical scores and neuropathologic-molecular analysis, induced lower severity compared to the vehicle-treated mice, particularly in the early phase of disease onset. A significant reduction of astrocytosis, demyelination, immune infiltrates, proinflammatory cytokines expression and enzymatic oxidative reactivity in the AA-treated group was also observed, indicating the participation of S100B in processes leading to the activated neuroinflammatory phenotype, reasonably as an astrocytic activity. The active participation of astrocytes in S100B-induced MS processes is currently studied. This scenario supports the perspective that S100B may be regarded as a therapeutic target for MS, as for different neural disorders appearing to share some common pathogenic features, reasonably attributable to neuroinflammation (1). Nando Elsa Peretti Foundation and FISM are acknowledged for support 1.Michetti F et al, *Neurosci Biobehav Rev* 2021, 127: 446-58 2.DiSante G et al *Cells* 2020, 9, 748

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-306

IRF5 IS ESSENTIAL FOR PROPER MYELIN DEGRADATION AND REMYELINATION

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Aims: Understanding the mechanisms of myeloid cells-specific responses during neurodegenerative pathologies is vital to promote regenerative processes. IRF5, a transcription factor highly involved in innate immunity, drives microglia/macrophage towards a pro-inflammatory state and has been associated to multiple sclerosis (MS) susceptibility. On this basis, we assessed the specific role of IRF5 in the development of MS pathogenesis. Methods and results: We analyzed the role of Irf5 in autoimmune inflammation. *Irf5*$^{-/-}$ showed exacerbated damage in the chronic phase of experimental autoimmune encephalomyelitis (EAE) mice and an excess of myelin debris in microglia/macrophages. To specifically analyze the role of Irf5 in remyelination, we induced lesions in the spinal cord of WT and *Irf5*$^{-/-}$ mice by lysolecithin injection. Interestingly, *Irf5*$^{-/-}$ animals showed larger lesions and an impairment in oligodendrocyte migration into the core of the lesion, probably as a result of extracellular matrix alterations. Moreover, *knock-out* mice animals showed a higher accumulation of myelin debris as well as an aberrant aggregation and accumulation of lipids into lipid droplets, both in the EAE and the LPC-demyelinating lesions. Transcriptomic and lipidomic analysis of WT and *Irf5*$^{-/-}$ microglia evidence a role of this transcription factor in myelin metabolism and cholesterol homeostasis. Indeed, *Irf5*$^{-/-}$ microglia showed increased accumulation of cholesterol esters and formation of cholesterol crystals. Conclusion: We demonstrated that the pro-inflammatory transcription factor Irf5 modulates lipid metabolism, mechanistic link that could be crucial for myelin regeneration in MS.

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BOARD NUMBER: S03-307

VAGUS NERVE STIMULATION REDUCES DISEASE IN RODENT MODEL OF MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Background: Despite multiple approved therapies for treating multiple sclerosis (MS), there remains a need for new therapeutic options. Vagus nerve stimulation (VNS) can activate neuro-immune reflexes that attenuate inflammation, increase T_{reg} populations, and is neuroprotective in the central nervous system (Immunol Rev 2012; 248(1):188). We hypothesized that VNS would be ameliorative in an experimental autoimmune encephalomyelitis (EAE) model of MS. **Aims**: To explore the effect of cervical VNS on disease severity in a standard rodent model of MS. **Methods**: VNS devices (SetPoint Medical, CA) were fully implanted in 6-week-old female Lewis rats. Following recovery, EAE was induced with myelin basic protein (0.1 mg/rat) and complete Freund's adjuvant (Hooke Laboratories, CT). Conscious rats were treated with VNS (1mA, 10 Hz, 60 second train, TID) from 7 days post-immunization (DPI), the day prior to the typical onset of clinical symptoms, through 21 DPI. Clinical scores were recorded daily in a treatment-blinded manner (0-5 scale). **Results** Cervical VNS decreased disease severity compared to the sham treatment. Maximum clinical score was significantly reduced in the VNS group (mean±SEM: 3.4±0.1 vs. 2.4±0.3, $p<0.005$) as were the number of symptomatic days (7.9±0.2 vs. 6.3±0.3, $p <0.0001$). Total AUC of clinical score vs. DPI was also significantly reduced (30%, $p <0.01$). **Conclusions**: These data indicate that daily cervical VNS reduced disease severity in a rat model of semi-established EAE, comparable to treatment with 3mg/kg/day teriflunomide. To further investigate the underlying mechanisms of these neuroprotective effects, glial and immunocyte targets are being investigated.

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BOARD NUMBER: S03-308

THE EMERGING ROLE OF MICRORNAS IN EXPERIMENTAL AND CLINICAL MULTIPLE SCLEROSIS: IMPLICATIONS FOR INFLAMMATION-DRIVEN SYNAPTIC DYSFUNCTIONS AND DISEASE COURSE.

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Aim. MicroRNAs (miRs) have come up as pleiotropic determinants in the CNS-immune system crosstalk. We aim at investigating their role in the course of multiple sclerosis (MS) especially linked to the inflammatory synaptopathy, a crucial hallmark of the disease. **Methods.** We screened, by qPCR and Bio-plex system, 24 selected miRs and 27 inflammation-related proteins circulating cerebrospinal fluid (CSF) in a large cohort of MS patients, and we correlated them with clinical, cognitive and transcranial magnetic stimulation parameters assessed at the diagnosis (T0) and after follow-up periods (Tf). Multiple statistical and bioinformatics analyses were confirmed by preclinical studies in MOG_{35-55} EAE model and transgenic mice. **Results.** We identified let-7b-5p and miR-142-3p as two main miRs with opposite functions in neuroinflammation and MS prognosis. Let-7b-5p is a potential protective factor for MS course, with anti-inflammatory and neuroprotective properties from the earliest stages of the disease. Moreover, CSF let-7b-5p levels are reduced in progressive MS and negatively correlate with disease severity at both T0 and Tf. On the contrary, miR-142-3p emerged as an adverse biomarker of the synaptopathy-driven disease progression and a promising tool for identifying personalized therapies. Indeed, we demonstrated in MS and in EAE that miR-142-3p is an essential effector of IL-1ß-induced synaptic alterations and low miR-142-3p levels associate with a more effective response to dimethyl fumarate, an established MS treatment. **Conclusions.** Our results lay the basis for an important advance in MS diagnosis and prognosis related to synaptopathy-driven detrimental outcomes, with possible implication in the therapeutic decision-making strategy.

Pubmed:
28100738: Mandolesi G, De Vito F, Musella A, Gentile A, Bullitta S, Fresegna D, Sepman H, Di Sanza C, Haji N, Mori F, Buttari F, Perlas E, Ciotti MT, Hornstein E, Bozzoni I, Presutti C, Centonze D
miR-142-3p Is a Key Regulator of IL-1β-Dependent Synaptopathy in Neuroinflammation.
MicroRNAs (miRNA) play an important role in post-transcriptional gene regulation of several physiological and pathological processes. In multiple sclerosis (MS), a chronic inflammatory and degenerative disease of the CNS, and in its mouse model, the experimental autoimmune encephalomyelitis (EAE), miRNA dysregulation has been mainly related to immune system dysfunction and white matter (WM) pathology. However, little is known about their role in gray matter pathology. Here, we explored miRNA involvement in the inflammation-driven alterations of synaptic structure and function, collectively known as synaptopathy, a neuropathological process contributing to excitotoxic neurodegeneration in MS/EAE. Particularly, we observed that miR-142-3p is increased in the CSF of patients with active MS and in EAE brains. We propose miR-142-3p as a molecular mediator of the IL-1β-dependent downregulation of the glial glutamate-aspartate transporter (GLAST), which causes an enhancement of the glutamatergic transmission in the EAE cerebellum. The synaptic abnormalities mediated by IL-1β and the clinical and neuropathological manifestations of EAE disappeared in miR-142 knock-out mice. Furthermore, we observed that in vivo miR-142-3p inhibition, either by a preventive and local treatment or by a therapeutic and systemic strategy, abolished IL-1β- and GLAST-dependent synaptopathy in EAE wild-type mice. Consistently, miR-142-3p was responsible for the glutamatergic synaptic alterations caused by CSF of patients with MS, and CSF levels of miR-142-3p correlated with prospective MS disease progression. Our findings highlight miR-142-3p as key molecular player in IL-1β-mediated synaptic dysfunction, possibly leading to excitotoxic damage in both EAE and MS diseases. Inhibition of miR-142-3p could be neuroprotective in MS.

J Neurosci, 2017; 37
26307611: Capitano F, Camon J, Ferretti V, Licursi V, De Vito F, Rinaldi A, Vincenti S, Mannironi C, Fragapane P, Bozzoni I, Oliverio A, Negri R, Presutti C, Mele A
microRNAs Modulate Spatial Memory in the Hippocampus and in the Ventral Striatum in a Region-Specific Manner.
MicroRNAs are endogenous, noncoding RNAs crucial for the post-transcriptional regulation of gene expression. Their role in spatial memory formation, however, is poorly explored. In this study, we analyzed learning-induced microRNA expression in the hippocampus and in the ventral striatum. Among miRNAs specifically downregulated by spatial training, we focused on the hippocampus-specific miR-324-5p and the ventral striatum-specific miR-24. In vivo overexpression of the two miRNAs demonstrated that miR-324-5p is able to impair memory if administered in the hippocampus but not in the ventral striatum, while the opposite is true for miR-24. Overall, these findings demonstrate a causal relationship between miRNA expression changes and spatial memory formation. Furthermore, they provide support for a regional dissociation in the post-transcriptional processes underlying spatial memory in the two brain structures analyzed.
Mol Neurobiol, 2016; 53
27566665: Gentile A, Musella A, Bullitta S, Fresegna D, De Vito F, Fantozzi R, Piras E, Gargano F, Borsellino G, Battistini L, Schubart A, Mandolesi G, Centonze D
Siponimod (BAF312) prevents synaptic neurodegeneration in experimental multiple sclerosis.
Data from multiple sclerosis (MS) and the MS rodent model, experimental autoimmune encephalomyelitis (EAE), highlighted an inflammation-dependent synaptopathy at the basis of the neurodegenerative damage causing irreversible disability in these disorders. This synaptopathy is characterized by an imbalance between glutamatergic and GABAergic transmission and has been proposed to be a potential therapeutic target. Siponimod (BAF312), a selective sphingosine 1-phosphate1,5 receptor modulator, is currently under investigation in a clinical trial in secondary progressive MS patients. We investigated whether siponimod, in addition to its peripheral immune modulation, may exert direct neuroprotective effects in the central nervous system (CNS) of mice with chronic progressive EAE.
J Neuroinflammation, 2016; 13
26585978: Mandolesi G, Gentile A, Musella A, Fresegna D, De Vito F, Bullitta S, Sepman H, Marfia GA, Centonze D
Synaptopathy connects inflammation and neurodegeneration in multiple sclerosis.
Multiple sclerosis (MS) has long been regarded as a chronic inflammatory disease of the white matter that leads to demyelination and eventually to neurodegeneration. In the past decade, several aspects of MS pathogenesis have been challenged, and degenerative changes of the grey matter, which are independent of demyelination, have become a topic of interest. CNS inflammation in MS and experimental autoimmune encephalomyelitis (EAE; a disease model used to study MS in rodents) causes a marked imbalance between GABAergic and glutamatergic transmission, and a loss of synapses, all of which leads to a diffuse 'synaptopathy'. Altered synaptic transmission can occur early in MS and EAE, independently of demyelination and axonal loss, and subsequently causes excitotoxic damage. Inflammation-driven synaptic abnormalities are emerging as a prominent pathogenic mechanism in MS-importantly, they are potentially reversible and, therefore, represent attractive therapeutic targets. In this Review, we focus on the connection between inflammation and synaptopathy in MS and EAE, which sheds light not only on the pathophysiology of MS but also on that of primary neurodegenerative disorders in which inflammatory processes contribute to disease progression.
Nat Rev Neurol, 2015; 11
24023867: Mannironi C, Camon J, De Vito F, Biundo A, De Stefano ME, Persiconi I, Bozzoni I, Fragapane P, Mele A, Presutti C
Acute stress alters amygdala microRNA miR-135a and miR-124 expression: inferences for corticosteroid dependent stress response.
The amygdala is a brain structure considered a key node for the regulation of neuroendocrine stress response. Stress-induced response in amygdala is accomplished through neurotransmitter activation and an alteration of gene expression. MicroRNAs (miRNAs) are important regulators of gene expression in the nervous system and are very well suited effectors of stress response for their ability to reversibly silence specific mRNAs. In order to study how acute stress affects miRNAs expression in amygdala we analyzed the miRNA profile after two hours of mouse restraint, by microarray analysis and reverse transcription real time PCR. We found that miR-135a and miR-124 were negatively regulated. Among in silico predicted targets we identified the mineralocorticoid receptor (MR) as a target of both miR-135a and miR-124. Luciferase experiments and endogenous protein expression analysis upon miRNA upregulation and inhibition allowed us to demonstrate that mir-135a and mir-124 are able to negatively affect the expression of the MR. The increased levels of the amygdala MR protein after two hours of restraint, that we analyzed by western blot, negatively correlate with miR-135a and miR-124 expression. These findings point to a role of miR-135a and miR-124 in acute stress as regulators of the MR, an important effector of early stress response.
PLoS One, 2013; 8

23864696: Mandolesi G, Musella A, Gentile A, Grasselli G, Haji N, Sepman H, Fresegna D, Bullitta S, De Vito F, Musumeci G, Di Sanza C, Strata P, Centonze D
Interleukin-1β alters glutamate transmission at purkinje cell synapses in a mouse model of multiple sclerosis.
Cerebellar deficit contributes significantly to disability in multiple sclerosis (MS). Several clinical and experimental studies have investigated the pathophysiology of cerebellar dysfunction in this neuroinflammatory disorder, but the cellular and molecular mechanisms are still unclear. In experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, proinflammatory cytokines, together with a degeneration of inhibitory neurons, contribute to impair GABAergic transmission at Purkinje cells (PCs). Here, we investigated glutamatergic transmission to gain insight into the pathophysiology of cerebellar dysfunction in EAE. Electrophysiological recordings from PCs showed increased duration of spontaneous excitatory postsynaptic currents (EPSCs) during the symptomatic phase of EAE, suggesting an alteration of glutamate uptake played by Bergmann glia. We indeed observed an impaired functioning of the glutamate-aspartate transporter/excitatory amino acid transporter 1 (GLAST/EAAT1) in EAE cerebellum caused by protein downregulation and in correlation with prominent astroglia activation. We have also demonstrated that the proinflammatory cytokine interleukin-1β (IL-1β), released by a subset of activated microglia/macrophages and infiltrating lymphocytes, was involved directly in such synaptic alteration. In fact, brief incubation of IL-1β in normal cerebellar slices replicated EAE modifications through a rapid GLAST/EAAT1 downregulation, whereas incubation of an IL-1 receptor antagonist (IL-1ra) in EAE slices reduced spontaneous EPSC alterations. Finally, EAE mice treated with intracerebroventricular IL-1ra showed normal glutamatergic and GABAergic transmissions, along with GLAST/EAAT1 normalization, milder inflammation, and reduced motor deficits. These results highlight the crucial role played by the proinflammatory IL-1β in triggering molecular and synaptic events involved in neurodegenerative processes that characterize neuroinflammatory diseases such as MS.
J Neurosci, 2013; 33
33562569: Mandolesi G, Rizzo FR, Balletta S, Stampanoni Bassi M, Gilio L, Guadalupi L, Nencini M, Moscatelli A, Ryan CP, Licursi V, Dolcetti E, Musella A, Gentile A, Fresegna D, Bullitta S, Caioli S, Vanni V, Sanna K, Bruno A, Buttari F, Castelli C, Presutti C, De Santa F, Finardi A, Furlan R, Centonze D, De Vito F
The microRNA let-7b-5p Is Negatively Associated with Inflammation and Disease Severity in Multiple Sclerosis.
The identification of microRNAs in biological fluids for diagnosis and prognosis is receiving great attention in the field of multiple sclerosis (MS) research but it is still in its infancy. In the present study, we observed in a large sample of MS patients that let-7b-5p levels in the cerebrospinal fluid (CSF) were highly correlated with a number of microRNAs implicated in MS, as well as with a variety of inflammation-related protein factors, showing specific expression patterns coherent with let-7b-5p-mediated regulation. Additionally, we found that the CSF let-7b-5p levels were significantly reduced in patients with the progressive MS compared to patients with relapsing-remitting MS and were negatively correlated with characteristic hallmark processes of the two phases of the disease. Indeed, in the non-progressive phase, let-7b-5p inversely associated with both central and peripheral inflammation; whereas, in progressive MS, the CSF levels of let-7b-5p negatively correlated with clinical disability at disease onset and after a follow-up period. Overall, our results uncovered, by the means of a multidisciplinary approach and multiple statistical analyses, a new possible pleiotropic action of let-7b-5p in MS, with potential utility as a biomarker of MS course.
Cells, 2021; 10
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MiR-142-3p regulates synaptopathy-driven disease progression in multiple sclerosis.
We recently proposed miR-142-3p as a molecular player in inflammatory synaptopathy, a new pathogenic hallmark of multiple sclerosis (MS) and of its mouse model experimental autoimmune encephalomyelitis (EAE), that leads to neuronal loss independently of demyelination. MiR-142-3p seems to be unique among potential biomarker candidates in MS, since it is an inflammatory miRNA playing a dual role in the immune and central nervous systems. Here, we aimed to verify the impact of miR-142-3p circulating in the cerebrospinal fluid (CSF) of MS patients on clinical parameters, neuronal excitability and its potential interaction with disease modifying therapies (DMTs).
Neuropathol Appl Neurobiol, 2022; 48
31125471: Gentile A, De Vito F, Fresegna D, Rizzo FR, Bullitta S, Guadalupi L, Vanni V, Buttari F, Stampanoni Bassi M, Leuti A, Chiurchiù V, Marfia GA, Mandolesi G, Centonze D, Musella A
Peripheral T cells from multiple sclerosis patients trigger synaptotoxic alterations in central neurons.
The crucial step in the pathogenic events that lead to the development and the progression of multiple sclerosis (MS) is the infiltration of autoreactive T cells in the brain. Data from experimental autoimmune encephalomyelitis (EAE) mice indicate that, together with microglia, T cells are responsible for the enhancement of the glutamatergic transmission in central

neurons, contributing to glutamate-mediated excitotoxicity, a pathological hallmark of both EAE and MS brains. Here, we addressed the synaptic role of T cells taken from MS patients.
Neuropathol Appl Neurobiol, 2020; 46
19913057: Rinaldi A, Vincenti S, De Vito F, Bozzoni I, Oliverio A, Presutti C, Fragapane P, Mele A
Stress induces region specific alterations in microRNAs expression in mice.
Several studies have demonstrated that exposure to both acute and chronic aversive stimuli can affect neural activity in different brain areas. In particular it has been shown that stressful events can induce not only short-term changes in neural transmission and gene regulation, but also long-term changes that can lead to structural modification. In this study we investigated, in CD1 mice, the effects of single or repeated exposures to restraint stress (2h for 1 or 5 consecutive days) in the frontal cortex on a crucial class of gene expression regulators, the microRNAs (miRs).First we performed a microarray profiling on RNA extracted from the frontal cortex of mice exposed to acute or repeated restraint stress. The results indicated a prominent increase in the expression levels of different miRs after acute stress while only minor changes were observed after repeated restraint. The Northern blot analysis on selected miRs confirmed an increase after acute restraint for let-7a, miR-9 and miR 26-a/b. Finally, Northern blot analysis of the selected miRs on RNA extracted from the hippocampus of stressed mice demonstrated that such changes were region specific, as no differences were observed in the hippocampus. These data suggest that control of mRNA translation through miRs is an additional mechanism by which stressful events regulates protein expression in the frontal cortex.
Behav Brain Res, 2010; 208

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CHEMOGENETIC LOCUS COERULEUS ACTIVATION ALLEVIATES EXPERIMENTAL MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Multiple Sclerosis (MS) is a chronic autoimmune neurologic disease that affects the central nervous system and produces a progressive irreversible demyelination with severe immunologic, inflammatory and neurodegenerative consequences. Alterations in the noradrenergic system have been described in MS patients and animal models. Moreover, the lesion of Locus Coeruleus (LC) noradrenergic neurons worsen the symptomatology of a MS animal model. Additionally, evidence reported beneficial effects using several experimental approaches to increase noradrenaline availability. Therefore, we hypothesize that chronic activation of LC noradrenergic neurons improves the symptomatology of an experimental autoimmune encephalomyelitis (EAE) model. Hence, designer receptor exclusively activated by designer drugs (DREADD) was bilaterally administered into the LC of transgenic C57BL/6(TH:cre) female mice. Immunization with myelin oligodendrocyte glycoprotein peptide$_{35–55}$ was used as an EAE model and oral Clozapine N-oxide (DREADD ligand) was administered to activate LC neurons since the onset or the peak of motor symptomatology. Chronic LC activation reduced the motor deficit and sickness behavior in both treatments. However, only treating since the onset of motor symptomatology reduced the anxiety and improved motor activity in EAE animals. Furthermore, both treatments reduced demyelination, glial activation and perivascular infiltration in the spinal cord (SC) of EAE animals. Interestingly, treatments improved the reduced dopamine beta-hydroxylase expression we demonstrated in the SC of EAE mice. However, the treatments effect on these processes was lower in prefrontal and motor cortices. These results support the role of noradrenergic system in MS development and highlight it as a potential target to alleviate its severe clinical course.

Pubmed:
34373893: Llorca-Torralba M, Camarena-Delgado C, Suárez-Pereira I, Bravo L, Mariscal P, Garcia-Partida JA, López-Martín C, Wei H, Pertovaara A, Mico JA, Berrocoso E
Pain and depression comorbidity causes asymmetric plasticity in the locus coeruleus neurons.
There is strong comorbidity between chronic pain and depression, although the neural circuits and mechanisms underlying this association remain unclear. By combining immunohistochemistry, tracing studies and western blotting, with the use of different DREADDS (designer receptor exclusively activated by designer drugs) and behavioural approaches in a rat model of neuropathic pain (chronic constriction injury), we explore how this comorbidity arises. To this end, we evaluated the time-dependent plasticity of noradrenergic locus coeruleus neurons relative to the site of injury: ipsilateral (LCipsi) or contralateral (LCcontra) locus coeruleus at three different time points: short (2 days), mid (7 days) and long term (30-35 days from nerve injury). Nerve injury led to sensorial hypersensitivity from the onset of injury, whereas depressive-like behaviour was only evident following long-term pain. Global chemogenetic blockade of the LCipsi system alone increased short-term pain sensitivity while the blockade of the LCipsi or LCcontra relieved pain-induced depression. The asymmetric contribution of locus coeruleus modules was also evident as neuropathy develops. Hence, chemogenetic blockade of the LCipsi→spinal cord projection, increased pain-related behaviours in the short term. However, this lateralized circuit is not universal as the bilateral chemogenetic inactivation of the locus coeruleus-rostral anterior cingulate cortex pathway or the intra-rostral anterior cingulate cortex antagonism of alpha1- and alpha2-adrenoreceptors reversed long-term pain-induced depression. Furthermore, chemogenetic locus coeruleus to spinal cord activation, mainly through LCipsi, reduced sensorial hypersensitivity irrespective of the time post-injury. Our results indicate that asymmetric activation of specific locus coeruleus modules promotes early restorative analgesia, as well as late depressive-like behaviour in chronic pain and depression comorbidity.
Brain, 2022; 145

35164940: Suárez-Pereira I, Llorca-Torralba M, Bravo L, Camarena-Delgado C, Soriano-Mas C, Berrocoso E
The Role of the Locus Coeruleus in Pain and Associated Stress-Related Disorders.
The locus coeruleus (LC)-noradrenergic system is the main source of noradrenaline in the central nervous system and is involved intensively in modulating pain and stress-related disorders (e.g., major depressive disorder and anxiety) and in their comorbidity. However, the mechanisms involving the LC that underlie these effects have not been fully elucidated, in part owing to the technical difficulties inherent in exploring such a tiny nucleus. However, novel research tools are now available that have helped redefine the LC system, moving away from the traditional view of LC as a homogeneous structure that exerts a uniform influence on neural activity. Indeed, innovative techniques such as DREADDs (designer receptors exclusively activated by designer drugs) and optogenetics have demonstrated the functional heterogeneity of LC, and novel magnetic resonance imaging applications combined with pupillometry have opened the way to evaluate LC activity in vivo. This review aims to bring together the data available on the efferent activity of the LC-noradrenergic system in relation to pain and its comorbidity with anxiodepressive disorders. Acute pain triggers a robust LC stress response, producing spinal cord-mediated endogenous analgesia while promoting aversion, vigilance, and threat detection through its ascending efferents. However, this protective biological system fails in chronic pain, and LC activity produces pain facilitation, anxiety, increased aversive memory, and behavioral despair, acting at the medulla, prefrontal cortex, and amygdala levels. Thus, the activation/deactivation of specific LC projections contributes to different behavioral outcomes in the shift from acute to chronic pain.
Biol Psychiatry, 2022; 91
35025190: Camarena-Delgado C, Llorca-Torralba M, Suárez-Pereira I, Bravo L, López-Martín C, Garcia-Partida JA, Mico JA, Berrocoso E
Nerve injury induces transient locus coeruleus activation over time: role of the locus coeruleus-dorsal reticular nucleus pathway.
The transition from acute to chronic pain results in maladaptive brain remodeling, as characterized by sensorial hypersensitivity and the ensuing appearance of emotional disorders. Using the chronic constriction injury of the sciatic nerve as a model of neuropathic pain in male Sprague-Dawley rats, we identified time-dependent plasticity of locus coeruleus (LC) neurons related to the site of injury, ipsilateral (LCipsi) or contralateral (LCcontra) to the lesion, hypothesizing that the LC→dorsal reticular nucleus (DRt) pathway is involved in the pathological nociception associated with chronic pain. LCipsi inactivation with lidocaine increased cold allodynia 2 days after nerve injury but not later. However, similar blockade of LCcontra reduced cold allodynia 7 and 30 days after inducing neuropathy but not earlier. Furthermore, lidocaine blockade of the LCipsi or LCcontra reversed pain-induced depression 30 days after neuropathy. Long-term pain enhances phosphorylated cAMP-response element binding protein expression in the DRtcontra but not in the DRtipsi. Moreover, inactivation of the LCcontra→DRtcontra pathway using dual viral-mediated gene transfer of designer receptor exclusively activated by designer drugs produced consistent analgesia in evoked and spontaneous pain 30 days postinjury. This analgesia was similar to that produced by spinal activation of α2-adrenoreceptors. Furthermore, chemogenetic inactivation of the LCcontra→DRtcontra pathway induced depressive-like behaviour in naïve animals, but it did not modify long-term pain-induced depression. Overall, nerve damage activates the LCipsi, which temporally dampens the neuropathic phenotype. However, the ensuing activation of a LCcontra→DRtcontra facilitatory pain projection contributes to chronic pain, whereas global bilateral LC activation contributes to associated depressive-like phenotype.
Pain, 2022; 163
32437745: Bravo L, Llorca-Torralba M, Suárez-Pereira I, Berrocoso E
Pain in neuropsychiatry: Insights from animal models.
Pain is the most common symptom reported in clinical practice, meaning that it is associated with many pathologies as either the origin or a consequence of other illnesses. Furthermore, pain is a complex emotional and sensorial experience, as the correspondence between pain and body damage varies considerably. While these issues are widely acknowledged in clinical pain research, until recently they have not been extensively considered when exploring animal models, important tools for understanding pain pathophysiology. Interestingly, chronic pain is currently considered a risk factor to suffer psychiatric disorders, mainly stress-related disorders like anxiety and depression. Conversely, pain appears to be altered in many psychiatric disorders, such as depression, anxiety and schizophrenia. Thus, pain and psychiatric disorders have been linked in epidemiological and clinical terms, although the neurobiological mechanisms involved in this pathological bidirectional relationship remain unclear. Here we review the evidence obtained from animal models about the co-morbidity of pain and psychiatric disorders, placing special emphasis on the different dimensions of pain.
Neurosci Biobehav Rev, 2020; 115
30987747: Llorca-Torralba M, Suárez-Pereira I, Bravo L, Camarena-Delgado C, Garcia-Partida JA, Mico JA, Berrocoso E
Chemogenetic Silencing of the Locus Coeruleus-Basolateral Amygdala Pathway Abolishes Pain-Induced Anxiety and Enhanced Aversive Learning in Rats.

Pain affects both sensory and emotional aversive responses, often provoking anxiety-related diseases when chronic. However, the neural mechanisms underlying the interactions between anxiety and chronic pain remain unclear.
Biol Psychiatry, 2019; 85
32568562: Perez-Caballero L, Perez V, Berrocoso E
What ketamine can teach us about the opioid system in depression?
Expert Opin Drug Discov, 2020; 15
30130302: Alba-Delgado C, Llorca-Torralba M, Mico JA, Berrocoso E
The onset of treatment with the antidepressant desipramine is critical for the emotional consequences of neuropathic pain.
Neuropathic pain is a chronic condition that is challenging to treat. It often produces considerable physical disability and emotional distress. Patients with neuropathic pain often experience depression and anxiety both of which are known to be temporally correlated with noradrenergic dysfunction in the locus coeruleus (LC) as pain becomes chronic. Antidepressants are the first-line drug therapy for neuropathic pain, and the LC represents a potential target for such therapy. In this study, we evaluated the efficacy of the tricyclic antidepressant desipramine (DMI, a noradrenaline reuptake inhibitor) in preventing or relieving the noradrenergic impairment induced by neuropathic pain. The treatment started before or after the onset of the anxiodepressive phenotype ("early or late treatment") in adult rats subjected to chronic sciatic constriction. Electrophysiological and western blotting assays showed LC dysfunction (increased bursting activity, alpha2-adrenoceptor sensitivity, tyrosine hydroxylase, and noradrenaline transporter expression) in chronic constriction injury at long term. These noradrenergic changes were concomitant to the progression of anxiety and despair-like features. Desipramine induced efficient analgesia, and it counteracted the despair-like behavior in chronic constriction injury-DMI animals, reducing the burst rate and tyrosine hydroxylase expression. Surprisingly, "early" DMI treatment did not modify pain-induced anxiety, and it dampened pain aversion, although these phenomena were abolished when the treatment commenced after noradrenaline impairment had been established. Hence, DMI seems to produce different outcomes depending when the treatment commences, indicating that the balance between the benefits and adverse effects of DMI therapy may shift as neuropathy progresses.
Pain, 2018; 159
30001902: Perez-Caballero L, Soto-Montenegro ML, Hidalgo-Figueroa M, Mico JA, Desco M, Berrocoso E
Deep brain stimulation electrode insertion and depression: Patterns of activity and modulation by analgesics.
An initial antidepressant effect when using deep brain stimulation (DBS) of the subcallosal area of the cingulate cortex (Cg25) to treat resistant depression that could be the result of electrode insertion has been described. We previously showed that electrode insertion into the infralimbic cortex (ILC; the Cg25 rodent correlate) provokes a temporally limited antidepressant-like effect that is counteracted by non-steroidal anti-inflammatory drugs, such as those routinely used for pain relief.
Brain Stimul, 2018 Nov - Dec; 11
29074339: Torres-Sanchez S, Perez-Caballero L, Mico JA, Celada P, Berrocoso E
Effect of Deep Brain Stimulation of the ventromedial prefrontal cortex on the noradrenergic system in rats.
Deep Brain Stimulation (DBS) of the subgenual cingulate cortex (SCC) is a promising therapeutic alternative to treat resistant major depressive disorder. In preclinical studies, DBS of the ventromedial prefrontal cortex (vmPFC, the rodent SCC correlate) provokes an antidepressant-like effect, along with changes in noradrenaline levels at the site of stimulation. Hence, DBS appears to activate the noradrenergic-locus coeruleus (LC) system.
Brain Stimul, 2018 Jan - Feb; 11
21903816: Emery EC, Young GT, Berrocoso EM, Chen L, McNaughton PA
HCN2 ion channels play a central role in inflammatory and neuropathic pain.
The rate of action potential firing in nociceptors is a major determinant of the intensity of pain. Possible modulators of action potential firing include the HCN ion channels, which generate an inward current, I(h), after hyperpolarization of the membrane. We found that genetic deletion of HCN2 removed the cyclic adenosine monophosphate (cAMP)-sensitive component of I(h) and abolished action potential firing caused by an elevation of cAMP in nociceptors. Mice in which HCN2 was specifically deleted in nociceptors expressing Na(V)1.8 had normal pain thresholds, but inflammation did not cause hyperalgesia to heat stimuli. After a nerve lesion, these mice showed no neuropathic pain in response to thermal or mechanical stimuli. Neuropathic pain is therefore initiated by HCN2-driven action potential firing in Na(V)1.8-expressing nociceptors.
Science, 2011; 333

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BOARD NUMBER: S03-310

OSTEOPONTIN IS A BIOMARKER FOR EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND UVEITIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Osteopontin as a biomarker has been used for the diagnosis of diseases as well as therapeutic targets. The biological role of osteopontin remain to be evaluated in an animal model of autoimmune central nervous system diseases. The present study was aimed to evaluate the involvement of OPN in the eyes of experimental autoimmune uveitis (EAU) and experimental autoimmune encephalomyelitis (EAE) models. EAU model was developed by immunization of interphotoreceptor retinoid-binding protein in Lewis rats, while EAE was developed by immunization of MOG peptides in C57BL mice. In EAU, OPN level has been found to be significantly upregulated in the serum and eyes of EAU afftected rats on day 9 post-immunization. The level of CD44, a ligand of OPN, was increased in the ciliary body in EAU rats. Furthermore, OPN was also detected in the ciliary body and activated microglia/macrophages in the EAU retina. The results of EAE were largely matched with those of EAU cases. These results suggest that OPN is significantly increased in the eyes of both EAE mice and EAU rats, and that it may be useful as an early biomarker of ocular autoimmune diseases. Funding: This study was supported by the National Research Foundation of Korea, No. NRF-2019R1A2C1087753.

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PERIPHERAL MYELOID DERIVED SUPPRESSOR CELLS ARE GOOD BIOMARKERS OF RESPONSE AND EFFICACY FOR FINGOLIMOD TREATMENT IN MULTIPLE SCLEROSIS.

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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The increasing number of available treatments for multiple sclerosis (MS) highlights the need for biomarkers for individualized medicine. Fingolimod is a disease-modifying treatment that acts as a sphingosine-1-phosphate receptor modulator. It promotes the immunosuppressive activity of Myeloid-Derived Suppressor Cells (MDSCs), a cell type which can be used as biomarker of disease severity and of the degree of demyelination and axonal damage extent in MS. In the present study, we interrogate whether the abundance of peripheral MDSCs could be a useful biomarker of Fingolimod efficacy. For this purpose, blood immune cells were analyzed at disease onset of the MS model, experimental autoimmune encephalomyelitis (EAE), in an individualized manner. Fingolimod treated animals presented a milder EAE course with a less demyelination and axonal damage. However, few animals that did not respond to Fingolimod were invariably associated with a lower number of MDSCs prior to treatment initiation. Remarkably, higher MDSC abundance also revealed to be an important parameter to distinguish EAE mice prone to higher Fingolimod efficacy. Cox regression analysis showed that Fingolimod effectiveness was related to MDSC abundance and independent of other clinical or immunological variables. Lastly, MDSCs were quantified in peripheral blood mononuclear cells from MS patients at baseline and after 6 and 12 months of Fingolimod treatment. Our preliminary data showed that a higher MDSC abundance at baseline seemed to be related with lower disease activity at 12 months of follow-up. In sum, our data indicate that MDSCs may be good biomarkers for Fingolimod responsiveness and treatment efficacy in MS.

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REVISITING THE ROLE OF ANDROGENS IN DEMYELINATION MODELS OF THE CENTRAL NERVOUS SYSTEM

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Multiple sclerosis is currently considered as the most common cause of disability in the young adults. This autoimmune, demyelinating and neurodegenerative pathology of the central nervous system (CNS) classically progress from a relapsing-remitting form in which a spontaneous regeneration of the lost myelin (also called remyelination) occurs, towards a progressive form. The latter is characterized by remyelination failure and the subsequent onset of irreversible neurological disabilities related to axonal and neuronal damage and loss (Thompson et al, 2018). The male hormones or androgens have been well-studied in animal models of CNS demyelination (Hussain et al, 2013 ; Bielecki et al, 2016 ; Laouarem et al, 2021). Their neuroprotective, anti-inflammatory and remyelinating properties have been mainly shown in male animals. However, females also produce these hormones nevertheless at much lower levels than males. The aim of the present work was to investigate if androgens display specific activities in females compared to males. In this purpose, we used different models of CNS demyelination and analyzed the brain and spinal cord from the demyelinated animals by using specific immunostainings as well as fluorescence-activated cell sorting of the peripheral immune cells. Our data indicate the repairing properties of androgens in the female tissues including neuroprotective, remyelinating and anti-inflammatory effects. They also show the regulation of peripheral immune cells in both the lymphoid organs and neural tissue. The male hormones thus contribute to CNS regeneration in females with nevertheless several specific effects compared to those previously characterized in males.

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DECIPHERING THE IMPACT OF MYELOID-DERIVED SUPPRESSOR CELL FUNCTION ON DISEASE PROGRESSION AND NEURAL TISSUE DAMAGE IN MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells with a regulatory role in multiple sclerosis (MS). Our group has recently described the presence of MDSCs in the CNS of MS patients, being mainly circumscribed to areas with a high inflammatory activity. Interestingly, the abundance of MDSCs in the peripheral blood at the onset of the symptoms in the MS model experimental autoimmune encephalomyelitis is inversely correlated with clinical course severity and tissue damage extent, pointing to MDSCs as feasible bioindicators of disease progression. We investigate whether functional differences of MDSCs are involved in the severity of MS in both human samples and the EAE model. In the CNS from MS patients, the higher abundance of MDSCs in active lesions was related to a lower density of T lymphocytes. Moreover, the apoptotic T cell density increased in MS patients with milder clinical courses. After the identification of two groups of mice with different clinical course based on the clustering analysis of clinical and immunological variables at EAE onset, we corroborated that the differences in the abundance of MDSCs in both groups were not only numerical, but also functional showing different immunosuppressive activity. RNAseq analysis of MDSCs from mild EAE mice pointing to a notable MDSC immunosuppressive phenotype in less severe EAE mice. Our results suggest that disease severity is related not only to MDSC abundance but also to a better immunoregulatory function, suggesting that the modulation of MDSCs should be a promising strategy to control the disease progression in MS.

Pubmed:

33830504: Rosa JM, Farré-Alins V, Ortega MC, Navarrete M, Lopez-Rodriguez AB, Palomino-Antolín A, Fernández-López E, Vila-Del Sol V, Decouty C, Narros-Fernández P, Clemente D, Egea J
TLR4 pathway impairs synaptic number and cerebrovascular functions through astrocyte activation following traumatic brain injury.
Activation of astrocytes contributes to synaptic remodelling, tissue repair and neuronal survival following traumatic brain injury (TBI). The mechanisms by which these cells interact to resident/infiltrated inflammatory cells to rewire neuronal networks and repair brain functions remain poorly understood. Here, we explored how TLR4-induced astrocyte activation modified synapses and cerebrovascular integrity following TBI.
Br J Pharmacol, 2021; 178
33217041: Melero-Jerez C, Fernández-Gómez B, Lebrón-Galán R, Ortega MC, Sánchez-de Lara I, Ojalvo AC, Clemente D, de Castro F
Myeloid-derived suppressor cells support remyelination in a murine model of multiple sclerosis by promoting oligodendrocyte precursor cell survival, proliferation, and differentiation.
The most frequent variant of multiple sclerosis (MS) is the relapsing-remitting form, characterized by symptomatic phases followed by periods of total/partial recovery. Hence, it is possible that these patients can benefit from endogenous agents that control the inflammatory process and favor spontaneous remyelination. In this context, there is increasing interest in the role of myeloid-derived suppressor cells (MDSCs) during the clinical course of experimental autoimmune encephalomyelitis (EAE). MDSCs speed up infiltrated T-cell anergy and apoptosis. In different animal models of MS, a milder disease course is related to higher presence/density of MDSCs in the periphery, and smaller demyelinated lesions in the central nervous system (CNS). These observations lead us to wonder whether MDSCs might not only exert an anti-inflammatory effect but might also have direct influence on oligodendrocyte precursor cells (OPCs) and remyelination. In the present work, we reveal for the first time the relationship between OPCs and MDSCs in EAE, relationship that is guided by the distance from the

inflammatory core. We describe the effects of MDSCs on survival, proliferation, as well as potent promoters of OPC differentiation toward mature phenotypes. We show for the first time that osteopontin is remarkably present in the analyzed secretome of MDSCs. The ablation of this cue from MDSCs-secretome demonstrates that osteopontin is the main MDSC effector on these oligodendroglial cells. These data highlight a crucial pathogenic interaction between innate immunity and the CNS, opening ways to develop MDSC- and/or osteopontin-based therapies to promote effective myelin preservation and repair in MS patients.
Glia, 2021; 69
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Tissue plasminogen activator worsens experimental autoimmune encephalomyelitis by complementary actions on lymphoid and myeloid cell responses.
Tissue plasminogen activator (tPA) is a serine protease involved in fibrinolysis. It is released by endothelial cells, but also expressed by neurons and glial cells in the central nervous system (CNS). Interestingly, this enzyme also contributes to pathological processes in the CNS such as neuroinflammation by activating microglia and increasing blood-brain barrier permeability. Nevertheless, its role in the control of adaptive and innate immune response remains poorly understood.
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27435092: Macrez R, Ortega MC, Bardou I, Mehra A, Fournier A, Van der Pol SM, Haelewyn B, Maubert E, Lesept F, Chevilley A, de Castro F, De Vries HE, Vivien D, Clemente D, Docagne F
Neuroendothelial NMDA receptors as therapeutic targets in experimental autoimmune encephalomyelitis.
Multiple sclerosis is among the most common causes of neurological disability in young adults. Here we provide the preclinical proof of concept of the benefit of a novel strategy of treatment for multiple sclerosis targeting neuroendothelial N-methyl-D-aspartate glutamate receptors. We designed a monoclonal antibody against N-methyl-D-aspartate receptors, which targets a regulatory site of the GluN1 subunit of N-methyl-D-aspartate receptor sensitive to the protease tissue plasminogen activator. This antibody reverted the effect of tissue plasminogen activator on N-methyl-D-aspartate receptor function without affecting basal N-methyl-D-aspartate receptor activity ($n = 21$, $P < 0.01$). This antibody bound N-methyl-D-aspartate receptors on the luminal surface of neurovascular endothelium in human tissues and in mouse, at the vicinity of tight junctions of the blood-spinal cord barrier. Noteworthy, it reduced human leucocyte transmigration in an in vitro model of the blood-brain barrier ($n = 12$, $P < 0.05$). When injected during the effector phase of MOG-induced experimental autoimmune encephalomyelitis ($n = 24$), it blocked the progression of neurological impairments, reducing cumulative clinical score ($P < 0.001$) and mean peak score ($P < 0.001$). This effect was observed in wild-type animals but not in tissue plasminogen activator knock-out animals ($n = 10$). This therapeutic effect was associated to a preservation of the blood-spinal cord barrier ($n = 6$, $P < 0.001$), leading to reduced leucocyte infiltration ($n = 6$, $P < 0.001$). Overall, this study unveils a critical function of endothelial N-methyl-D-aspartate receptor in multiple sclerosis, and highlights the therapeutic potential of strategies targeting the protease-regulated site of N-methyl-D-aspartate receptor.
Brain, 2016; 139
26527182: Melero-Jerez C, Ortega MC, Moliné-Velázquez V, Clemente D
Myeloid derived suppressor cells in inflammatory conditions of the central nervous system.
The knowledge of the immune system elements and their relationship with other tissues, organs and systems are key approximations for the resolution of many immune-related disorders. The control of the immune response and/or its modulation from the pro-inflammatory to the anti-inflammatory response is being deeply studied in the field. In the last years, the study of myeloid-derived suppressor cells (MDSCs), a group of immature myeloid cells with a high suppressive activity on T cells has been extensively addressed in cancer. In contrast, their role in neuroimmune diseases is far from being totally understood. In this review, we will summarize data about MDSCs coming from the study of neuroinflammatory diseases in general and their potential role in multiple sclerosis, in order to introduce the putative use of this extraordinary promising cell type for future cell-based therapies. This article is part of a Special Issue entitled: Neuro Inflammation edited by Helga E. de Vries and Markus Schwaninger.
Biochim Biophys Acta, 2016; 1862
24709559: Moliné-Velázquez V, Ortega MC, Vila del Sol V, Melero-Jerez C, de Castro F, Clemente D
The synthetic retinoid Am80 delays recovery in a model of multiple sclerosis by modulating myeloid-derived suppressor cell fate and viability.
Relapsing-remitting multiple sclerosis (RR-MS) is an inflammatory and demyelinating disease of the central nervous system (CNS). It is characterized by relapsing phases with ongoing neurological affectation that are followed by a remitting period in which inflammatory events are controlled and the patients partially recover. Experimental Autoimmune Encephalomyelitis (EAE) is the animal model most often used to study the inflammatory component of MS. Several cell types are involved in controlling the immune response in EAE and immature myeloid-derived suppressor cells (MDSCs) have emerged as

important actors in the immunomodulation that occurs in EAE due to their ability to suppress inflammatory responses by inducing T cell apoptosis. In this study, we assessed whether MDSC differentiation may have consequences on the clinical course of EAE by treating mice around the peak of the clinical course EAE with the MDSC-differentiating agent Am80, an analogue of retinoid acid. Am80 administration abrogates the immunomodulation that occurs in EAE mice through different MDSC-related mechanisms: i) induction of MDSC apoptosis; ii) polarization of MDSCs to mature subsets of myeloid cells (dendritic cells/macrophages/neutrophils); and iii) altering their immunosuppressor phenotype. Consequently, T cell density increases and their viability is promoted, delaying the animal's recovery. Therefore, our data point to MDSC behaviour as a crucial factor in facilitating the transition from the relapsing to the remission phase in EAE, which should be considered for future immune-related therapies for MS.
Neurobiol Dis, 2014; 67
24391545: Clemente D, Ortega MC, Melero-Jerez C, de Castro F
The effect of glia-glia interactions on oligodendrocyte precursor cell biology during development and in demyelinating diseases.
Oligodendrocyte precursor cells (OPCs) originate in specific areas of the developing central nervous system (CNS). Once generated, they migrate towards their destinations where they differentiate into mature oligodendrocytes. In the adult, 5-8% of all cells in the CNS are OPCs, cells that retain the capacity to proliferate, migrate, and differentiate into oligodendrocytes. Indeed, these endogenous OPCs react to damage in demyelinating diseases, like multiple sclerosis (MS), representing a key element in spontaneous remyelination. In the present work, we review the specific interactions between OPCs and other glial cells (astrocytes, microglia) during CNS development and in the pathological scenario of MS. We focus on: (i) the role of astrocytes in maintaining the homeostasis and spatial distribution of different secreted cues that determine OPC proliferation, migration, and differentiation during CNS development; (ii) the role of microglia and astrocytes in the redistribution of iron, which is crucial for myelin synthesis during CNS development and for myelin repair in MS; (iii) how microglia secrete different molecules, e.g., growth factors, that favor the recruitment of OPCs in acute phases of MS lesions; and (iv) how astrocytes modify the extracellular matrix in MS lesions, affecting the ability of OPCs to attempt spontaneous remyelination. Together, these issues demonstrate how both astroglia and microglia influence OPCs in physiological and pathological situations, reinforcing the concept that both development and neural repair are complex and global phenomena. Understanding the molecular and cellular mechanisms that control OPC survival, proliferation, migration, and differentiation during development, as well as in the mature CNS, may open new opportunities in the search for reparative therapies in demyelinating diseases like MS.
Front Cell Neurosci, 2013; 7
22354480: Ortega MC, Cases O, Merchán P, Kozyraki R, Clemente D, de Castro F
Megalin mediates the influence of sonic hedgehog on oligodendrocyte precursor cell migration and proliferation during development.
Oligodendrocyte precursor cells (OPCs) of the optic nerve are generated in the preoptic area, from where they migrate to colonize it entirely. Sonic hedgehog (Shh) induces the proliferation of these cells as well as influencing their migration, acting through its canonical receptor (Ptc-1). However, the multiligand receptor megalin (or LRP-2) is also involved in Shh-induced OPC proliferation and migration, and thus, we have evaluated the relevance of this interaction. During the stages at which Shh influences OPC development, we found megalin to be selectively expressed by optic nerve astrocytes, whereas Ptc-1 and Gli1 were found in OPCs. Indeed, this pattern of expression paralleled the rostral-caudal expression of the three Shh-related molecules during the time course of plp-dm20(+) -OPC colonization. The blockage of megalin partially abolished OPC chemoattraction and fully impaired Shh-induced proliferation. Using in vitro co-cultures of dissociated optic nerve cells, we demonstrated that Shh was internalized by astrocytes via megalin, and sufficient Shh was subsequently released to produce the biological effects on OPCs observed in the nerve. Together, these data indicate that at least part of the influence of Shh on OPCs is mediated by megalin during optic nerve development, and that astrocytes expressing megalin transiently capture Shh to present it to OPCs and/or to control the gradient of this molecule during development.
Glia, 2012; 60
21507122: Moliné-Velázquez V, Cuervo H, Vila-Del Sol V, Ortega MC, Clemente D, de Castro F
Myeloid-derived suppressor cells limit the inflammation by promoting T lymphocyte apoptosis in the spinal cord of a murine model of multiple sclerosis.
Multiple Sclerosis (MS) is a demyelinating/inflammatory disease of the central nervous system. Relapsing-remitting MS is characterized by a relapsing phase with clinical symptoms and the production of inflammatory cell infiltrates, and a period of remission during which patients recover partially. Myeloid-derived suppressor cells (MDSCs) are immature cells capable of suppressing the inflammatory response through Arginase-I (Arg-I) activity, among other mechanisms. Here, we have identified Arg-I(+) -MDSCs in the spinal cord during experimental autoimmune encephalomyelitis (EAE), cells that were largely restricted to the demyelinating plaque and that always exhibited the characteristic MDSC surface markers Arg-

I/CD11b/Gr-1/M-CSF1R. The presence and density of Arg-I(+) -cells, and the proportion of apoptotic but not proliferative T cells, were correlated with the EAE time course: peaked in parallel with the clinical score, decreased significantly during the remitting phase and completely disappeared during the chronic phase. Spinal cord-isolated MDSCs of EAE animals augmented the cell death when co-cultured with stimulated control splenic CD3 T cells. These data point to an important role for MDSCs in limiting inflammatory damage in MS, favoring the relative recovery in the remitting phase of the disease. Thus, the MDSC population should be considered as a potential therapeutic target to accelerate the recovery of MS patients.
Brain Pathol, 2011; 21

22016523: Clemente D, Ortega MC, Arenzana FJ, de Castro F
FGF-2 and Anosmin-1 are selectively expressed in different types of multiple sclerosis lesions.
Multiple sclerosis is a demyelinating disease that affects ≈ 2,000,000 people worldwide. In the advanced stages of the disease, endogenous oligodendrocyte precursors cannot colonize the lesions or differentiate into myelinating oligodendrocytes. During development, both FGF-2 and Anosmin-1 participate in oligodendrocyte precursor cell migration, acting via the FGF receptor 1 (FGFR1). Hence, we performed a histopathological and molecular analysis of these developmental modulators in postmortem tissue blocks from multiple sclerosis patients. Accordingly, we demonstrate that the distribution of FGF-2 and Anosmin-1 varies between the different types of multiple sclerosis lesions: FGF-2 is expressed only within active lesions and in the periplaque of chronic lesions, whereas Anosmin-1 is upregulated within chronic lesions and is totally absent in active lesions. We show that the endogenous oligodendrocyte precursor cells recruited toward chronic-active lesions express FGFR1, possibly in response to the FGF-2 produced by microglial cells in the periplaque. Also in human tissue, FGF-2 is upregulated in perivascular astrocytes in regions of the normal-appearing gray matter, where the integrity of the blood-brain barrier is compromised. In culture, FGF-2 and Anosmin-1 influence adult mouse oligodendrocyte precursor cell migration in the same manner as at embryonic stages, providing an explanation for the histopathological observations: FGF-2 attracts/enhances its migration, which is hindered by Anosmin-1. We propose that FGF-2 and Anosmin-1 are markers for the histopathological type and the level of inflammation of multiple sclerosis lesions, and that they may serve as novel pharmacogenetic targets to design future therapies that favor effective remyelination and protect the blood-brain barrier.
J Neurosci, 2011; 31

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BOARD NUMBER: S03-314

OLFACTORY DYSFUNCTION AND PRONOUNCED GLIOSIS IN THE OLFACTORY BULB PRECEDE MOTOR IMPAIRMENT IN THE RAT MODEL OF MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Background: Olfactory dysfunction is a common symptom in several neurodegenerative disorders, including Parkinson's and Alzheimer's disease, which appear decades before motor disability and cognitive decline. Clinical studies have shown that a certain percentage of multiple sclerosis (MS) patients manifest reduced olfaction in the prodromal phase of the disease. The underlying mechanism of olfactory dysfunction and the changes in the olfactory pathway may be central to understanding the autoimmune disease onset and progression. **Aim**: The objective of this study was to examine the occurrence of olfactory dysfunction and dynamics of neuroinflammatory activation that may underlie the sensory deterioration in experimental autoimmune encephalomyelitis (EAE), a rat model of MS. **Methods**: Two-month-old male *Dark agouti* rats were used in the study. Animals were tested for olfactory latency by Buried food test before and after the induction of EAE until motor symptoms began to interfere with the test performance. EAE was induced by injection of encephalitogenic emulsion (rat spinal cord homogenate in a complete Freund's adjuvant). Animals were sacrificed in the paralytic stage of the disease and olfactory bulbs (OB) were bilaterally dissected and processed for immunohistochemistry. **Results**: The first signs of olfactory dysfunction appeared at 2-dpi and exhibited a relapsing-remitting-like dynamic, while motor disabilities first began at 7-dpi. Immunohistochemical analysis demonstrated prominent gliosis in OB, reflected as massively increased GFAP and Iba1 immunoreactivity. **Conclusion:** Olfactory dysfunction and associated reactive gliosis precede the onset of neurological disabilities in EAE and may be used as an early diagnostic tool and/or marker of disease progression.

Pubmed:
34205965: Dragić M, Zeljković M, Stevanović I, Adžić M, Stekić A, Mihajlović K, Grković I, Ilić N, Ilić TV, Nedeljković N, Ninković M
Downregulation of CD73/AR-Mediated Adenosine Signaling as a Potential Mechanism of Neuroprotective Effects of Theta-Burst Transcranial Magnetic Stimulation in Acute Experimental Autoimmune Encephalomyelitis.
Multiple sclerosis (MS) is a chronic neurodegenerative disease caused by autoimmune-mediated inflammation in the central nervous system. Purinergic signaling is critically involved in MS-associated neuroinflammation and its most widely applied animal model-experimental autoimmune encephalomyelitis (EAE). A promising but poorly understood approach in the treatment of MS is repetitive transcranial magnetic stimulation. In the present study, we aimed to investigate the effect of continuous theta-burst stimulation (CTBS), applied over frontal cranial bone, on the adenosine-mediated signaling system in EAE, particularly on CD73/AR/AR in the context of neuroinflammatory activation of glial cells. EAE was induced in two-month-old female DA rats and in the disease peak treated with CTBS protocol for ten consecutive days. Lumbosacral spinal cord was analyzed immunohistochemically for adenosine-mediated signaling components and pro- and anti-inflammatory factors. We found downregulated IL-1β and NF- κB- and upregulated IL-10 pointing towards a reduction in the neuroinflammatory process in EAE animals after CTBS treatment. Furthermore, CTBS attenuated EAE-induced glial eN/CD73 expression and activity, while inducing a shift in AR expression from glia to neurons, contrary to EAE, where tight coupling of eN/CD73 and AR on glial cells is observed. Finally, increased glial AR expression following CTBS supports anti-inflammatory adenosine actions and potentially contributes to the overall neuroprotective effect observed in EAE animals after CTBS treatment.
Brain Sci, 2021; 11

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BOARD NUMBER: S03-315

EPENDYMA: A NEW TARGET FOR AUTOANTIBODIES FROM NEUROMYELITIS OPTICA PATIENTS?

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Background: Ependyma maintains crucial functions such as the regulation of cerebrospinal fluid (CSF) circulation by synchronous ciliary beating, the monitoring of molecular exchanges between CSF and parenchyma and the regulation of neural stem cells (NSCs) proliferation and survival present in the subventricular zone (SVZ). Neuromyelitis Optica (NMO) is a severe neurological disease associated with autoantibodies (NMO-IgG), directed against aquaporin 4 (AQP4). NMO-IgG are known to trigger astrocyte dysfunction leading to demyelination and axonal loss. Interestingly, ependymal cells also express AQP4 and evidences of ependymal alteration are reported in NMO. **Aims:** To evaluate morphological changes and dysfunctions of ependyma induced by NMO-IgG. **Methods:** NMO-IgG were purified from AQP4 antibodies positive patients' plasma. IgG from healthy donors (CTRL-IgG) and non-treated conditions were used as controls. Primary ependymal cell cultures and cultured wholemount dissections ("en-face" view of the entire ependyma) from adult rat lateral ventricular were exposed to NMO-IgG or control conditions. Immunolabeling and ependymal flow assay with fluorescent microbeads were used to evaluate morphological and functional changes. **Results:** We showed that NMO-IgG: 1) induced agglomeration of AQP4 and the gap junction connexin-43 at the lateral membrane of ependymocyte, and increased cell size; 2) induced planar apical depolarization of cilia tufts and altered the speed of beads injected on the surface of wholemounts; 3) activated the subventricular NSCs by increasing the number of proliferating cells and the number of quiescent NSCs processes contacting the CSF. **Conclusions:** NMO-IgG directly induce alterations of ependymal cells' functions and suggest an involvement of ependyma in NMO physiopathology.

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MYELIN ALTERATION AND COGNITIVE IMPAIRMENT

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Patients suffering from relapsing remitting form of multiple sclerosis go through successive episodes of demyelination/remyelination with sensory-motor loss followed by functional recovery. Cognitive impact of such episodes is rarely addressed, although 40-60% of MS patients suffer from cognitive impairment. Yet, growing amount of studies suggest that myelin alteration is a common feature of psychiatric disorders and neurodegenerative diseases such as Alzheimer's disease (AD). In this project, we address in mouse models the link between myelin alteration and cognitive deficits by evaluating the long-term effects of a transient demyelination at the cognitive, histological, ultrastructural and electrophysiological levels. Mice fed with cuprizone for 5 weeks undergo massive demyelination, followed by spontaneous remyelination. While sensory-motor and cognitive deficits have largely been studied during the demyelination/remyelination process, few studies have questioned the possible long-term effects. We followed up cognitive performances of mice at late time points when remyelination is supposed to be complete, and evidenced persistent signs of anxiety, together with working memory and flexibility deficits. We hypothesize that although gross myelin quantification shows efficient remyelination, subtle alterations of the myelin pattern in the cortex and/or in the hippocampus may underlie network dysfunction. To test this hypothesis, we are starting to perform electrophysiological recordings and ultrastructural analyses with optical and electronic microscopy in the medial prefrontal-hippocampus network of remyelinated mice. To further address the role of myelin in cognitive deficits observed in neurodegenerative diseases, we also study AD pathogenesis and evaluate weather myelin dysfunction may underlie or contribute to AD development.

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PROTEOMIC ALTERATION AND MORPHOLOGICAL CHANGES OF SIBLING NG2 GLIAL CELLS IN RESPONSE TO TWO EXPERIMENTAL MODELS OF MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Multiple sclerosis (MS) is an autoimmune and neurodegenerative disorder of the central nervous system (CNS). The variability in the MS pathophysiology makes difficult its study, although the use of animal models gives a more holistic comprehension of this disease. Here we used two murine experimental models, the autoimmune encephalomyelitis (EAE) and the toxin-induced demyelination by cuprizone (CPZ). Those models reflect different MS pathophysiological mechanisms to respectively simulate the relapsing-remitting and the primary progressive MS forms in human. NG2-glia, also referred as oligodendrocyte precursor cells (OPC), are of great interest in demyelinating diseases due to their remyelination potential. Here, we investigate the morphological differential response, to CPZ and EAE cortical brain lesions, of clonally related NG2-glia using an *in vivo* genetic multicolor lineage-tracing tool, StarTrack, along with a single-cell morphometric cluster analysis. Data from the morphometric analysis allowed us to unravel different NG2-glia cell clusters categorized by morphological parameters related, not only to their ontogenic origin, but also to their changes due to the different demyelinating scenarios. In addition, to address the differential altered neural pathways, we compared the proteomic profile of EAE brains (at the peak stage of acute EAE), and CPZ brains (during the acute phase) in both somatosensory cortex and spinal cord. Our analysis revealed altered proteins in both regions and MS murine models.

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INHIBITION OF CHONDROITIN SULFATE PROTEOGLYCANS (CSPGS) TO PROMOTE REGENERATION IN THE CNS IN MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Background: CSPGs are key components of the extracellular matrix involved in forming a glial scar around injuries in the central nervous system (CNS). An injury in the CNS causes astrocytes to produce CSPGs in excess, which prevents spontaneous remyelination, facilitated by the interactions between CSPGs and their receptors from the protein tyrosine phosphatase receptor (PTPRS,F) and Nogo receptor families (NgR1,3). **Aim:** The objective of this investigation was to study the potential regenerative effect created by the blockage of the interaction of CSPGs and their inhibitory receptors by a proliferation inducing ligand from the TNF superfamily (APRIL, TNFSF13), a natural ligand of CSPGs recently identified in the laboratory. **Methods:** Competitive ELISA was performed to test the effect of APRIL on the interaction between CSPGs and their receptors PTPRS and NgR1. To see the functional effect of these interactions, *in vitro* neurite outgrowth assays were performed, by culturing neurons on CSPG coated surfaces with a subsequent rescue condition using APRIL. These were followed by an *ex vivo* myelination assay, carried out with chemically demyelinated organotypic cerebellar tissues, to observe the effect of APRIL on remyelination. **Results:** Binding of APRIL to various CSPGs impaired their interactions with PTPRS and NgR1. APRIL also restored the neurite outgrowth suppressed by CSPGs *in vitro,* and showed a marked increase of remyelination in the *ex vivo* demyelinated cerebellar slices. **Conclusion:** CSPG antagonism by APRIL shows great promise in terms of neuroregeneration. Our future investigations will explore the possibility to translate this trait of APRIL's to Multiple Sclerosis.

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PLASMA CONCENTRATION OF IFNα2, MCP-3, IL-6 AND IL-8 IS DIFFERENTIALLY DECREASED IN NON-ACTIVE PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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AIMS: Disease presentation in multiple sclerosis (MS) is heterogeneous, making diagnosis sometimes challenging. Descriptors such as activity and progression have been applied to relapsing-remitting (RRMS) and primary progressive (PPMS) phenotypes. PPMS can also be further characterized as non-active or active according to the ongoing disease process. However, biomarkers that facilitate distinction of MS phenotypes and prediction of the disease course might improve patient care. We aim to identify differential patterns in biomarkers of inflammation and oxidative stress in cerebrospinal fluid (CSF) and blood which could be used to distinguish between these two MS phenotypes. METHODS: Plasma and cell-free CSF from RRMS, PPMS, and control individuals were collected. Eight pro-inflammatory cytokines were assessed in plasma using a Milliplex multiplex assay. CSF levels of two biomarkers for oxidative stress, H_2O_2 and 8-iso-prostaglandin-F2α, were measured by luminometry and ELISA respectively. RESULTS: In plasma, IFNα2 and MCP-3 levels were decreased in non-active PPMS and controls compared to RRMS. IL-8 concentration was significantly lower in non-active PPMS compared to RRMS while IL-6 levels were reduced in non-active PPMS compared to active PPMS. H_2O_2 concentration was higher in non-active PPMS CSF than in RRMS. No differences between groups have been observed in 8-iso-prostaglandin-F2α levels in CSF. CONCLUSIONS: Our results suggest that IFNα2, MCP-3, IL-6, and IL-8 concentration in plasma and H_2O_2 concentration in CSF could be a helpful tool to distinguish between active and non-active PPMS. These results may contribute, after a validation process, to change our treatment approach for people diagnosed with this debilitating disease.

Pubmed:
34206241: Muñoz-San Martín M, Gomez I, Miguela A, Belchí O, Robles-Cedeño R, Quintana E, Ramió-Torrentà L
Description of a CSF-Enriched miRNA Panel for the Study of Neurological Diseases.
The study of circulating miRNAs in CSF has gained tremendous attention during the last years, as these molecules might be promising candidates to be used as biomarkers and provide new insights into the disease pathology of neurological disorders.
Life (Basel), 2021; 11

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IMPACT OF THE NEW POMEGRANATE-PEELS EXTRACT FORMULATION IN MICE SUFFERING FROM EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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In recent years, dietary supplementations are attracting the attention of researchers to support therapy and ameliorate life quality in patients suffering from multiple sclerosis (MS). Among foods, pomegranate recently gained huge popularity as a nutraceutical source, thanks to its high content in bioactive compounds, such as ellagic acid (EA). However, the efficacy of pomegranate extracts is questioned because of the low solubility of this component. To increase its bioavailability, a new formulation of a pomegranate-peel extract (PEm) has been proposed. The extract was administered to mice suffering from the experimental autoimmune encephalomyelitis (EAE), and its potential health properties were investigated and compared to those elicited by a formulation containing EA (EAm).
Control and EAE mice were chronically administered with EAm and PEm (50mg/kg) dissolved in the drinking water for 14 days starting from the onset of the clinical signs (11 d.p.i). Then, animals were sacrificed, and the spinal cords were collected for immunohistochemical and western blot analyses.
The treatment with both EAm and PEm elicted a significant amelioration of the clinical symptoms as well as a reduction of microgliosis, astrocytosis, and lymphocyte infiltration in the spinal cord of EAE mice since western blot and immunohistochemical studies unveiled a reduction of CD45, GFAP, and Iba1 immunopositivity.
These findings support the potential use of both EAm and PEm as a dietary supplement and therapeutic support in MS patients.

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NUTRITIONAL OVERLOAD WORSENS EAE SEVERITY BY PROMOTING NEUROINFLAMMATION AND SYNAPTIC DAMAGE

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Aim. Chronic inflammation promoted by nutritional overload contributes to Multiple Sclerosis (MS) susceptibility and disease severity. In MS and in its mouse model experimental autoimmune encephalomyelitis (EAE) inflammatory molecules and downstream mechanisms cause 'synaptopathy', a reversible synaptic dysfunction that can cause excitotoxic damage and neuronal death. We aim at further understanding the relationship of nutritional overload with neuroinflammation and synaptic damage participating in EAE and MS course.
Methods. We explored the impact of high fat diet (HFD) compared to standard diet (SD) in EAE and control mice (n=25 for each experimental group) by monitoring clinical score and by performing behavioural, electrophysiological and molecular experiments. A clinical retrospective study on MS patients was performed to support our preclinical observations.
Results. HFD caused significant increase of both striatal excitatory transmission and neuroinflammation in control mice. HFD obesity prompted a worsen EAE clinical deficits by increasing clinical score and weight loss dependent on EAE induction. Interestingly, during the acute phase of the disease the HFD exacerbated the EAE striatal synaptopathy, strongly increasing the duration and the frequency of glutamatergic currents. In parallel, the striatal neuroinflammatory status of EAE mice fed with HFD was significantly enhanced compared to EAE mice fed with SD. Finally, statistical analyses in MS patients showed that an altered lipid profile correlates with disease severity and cerebrospinal fluid glutamate levels in MS patients.
Conclusions. Our results indicate that HFD strongly contributes to the pathophysiology of EAE/MS by enhancing neuroinflammation and glutamate-mediated synaptopathy, both reversible mechanisms that control MS severity.

Pubmed:
33020408: Dolcetti E, Bruno A, Guadalupi L, Rizzo FR, Musella A, Gentile A, De Vito F, Caioli S, Bullitta S, Fresegna D, Vanni V, Balletta S, Sanna K, Buttari F, Stampanoni Bassi M, Centonze D, Mandolesi G
Emerging Role of Extracellular Vesicles in the Pathophysiology of Multiple Sclerosis.
Extracellular vesicles (EVs) represent a new reality for many physiological and pathological functions as an alternative mode of intercellular communication. This is due to their capacity to interact with distant recipient cells, usually involving delivery of the EVs contents into the target cells. Intensive investigation has targeted the role of EVs in different pathological conditions, including multiple sclerosis (MS). MS is a chronic inflammatory and neurodegenerative disease of the nervous system, one of the main causes of neurological disability in young adults. The fine interplay between the immune and nervous systems is profoundly altered in this disease, and EVs seems to have a relevant impact on MS pathogenesis. Here, we provide an overview of both clinical and preclinical studies showing that EVs released from blood-brain barrier (BBB) endothelial cells, platelets, leukocytes, myeloid cells, astrocytes, and oligodendrocytes are involved in the pathogenesis of MS and of its rodent model experimental autoimmune encephalomyelitis (EAE). Most of the information points to an impact of EVs on BBB damage, on spreading pro-inflammatory signals, and altering neuronal functions, but EVs reparative function of brain damage deserves attention. Finally, we will describe recent advances about EVs as potential therapeutic targets and tools for

therapeutic intervention in MS.
Int J Mol Sci, 2020; 21
33066433: Fresegna D, Bullitta S, Musella A, Rizzo FR, De Vito F, Guadalupi L, Caioli S, Balletta S, Sanna K, Dolcetti E, Vanni V, Bruno A, Buttari F, Stampanoni Bassi M, Mandolesi G, Centonze D, Gentile A
Re-Examining the Role of TNF in MS Pathogenesis and Therapy.
Multiple sclerosis (MS) is a common neurological disorder of putative autoimmune origin. Clinical and experimental studies delineate abnormal expression of specific cytokines over the course of the disease. One major cytokine that has been shown to play a pivotal role in MS is tumor necrosis factor (TNF). TNF is a pleiotropic cytokine regulating many physiological and pathological functions of both the immune system and the central nervous system (CNS). Convincing evidence from studies in human and experimental MS have demonstrated the involvement of TNF in various pathological hallmarks of MS, including immune dysregulation, demyelination, synaptopathy and neuroinflammation. However, due to the complexity of TNF signaling, which includes two-ligands (soluble and transmembrane TNF) and two receptors, namely TNF receptor type-1 (TNFR1) and type-2 (TNFR2), and due to its cell- and context-differential expression, targeting the TNF system in MS is an ongoing challenge. This review summarizes the evidence on the pathophysiological role of TNF in MS and in different MS animal models, with a special focus on pharmacological treatment aimed at controlling the dysregulated TNF signaling in this neurological disorder.
Cells, 2020; 9
32655374: Bruno A, Dolcetti E, Rizzo FR, Fresegna D, Musella A, Gentile A, De Vito F, Caioli S, Guadalupi L, Bullitta S, Vanni V, Balletta S, Sanna K, Buttari F, Stampanoni Bassi M, Centonze D, Mandolesi G
Inflammation-Associated Synaptic Alterations as Shared Threads in Depression and Multiple Sclerosis.
In the past years, several theories have been advanced to explain the pathogenesis of Major Depressive Disorder (MDD), a neuropsychiatric disease that causes disability in general population. Several theories have been proposed to define the MDD pathophysiology such as the classic "monoamine-theory" or the "glutamate hypothesis." All these theories have been recently integrated by evidence highlighting inflammation as a pivotal player in developing depressive symptoms. Proinflammatory cytokines have been indeed claimed to contribute to stress-induced mood disturbances and to major depression, indicating a widespread role of classical mediators of inflammation in emotional control. Moreover, during systemic inflammatory diseases, peripherally released cytokines circulate in the blood, reach the brain and cause anxiety, anhedonia, social withdrawal, fatigue, and sleep disturbances. Accordingly, chronic inflammatory disorders, such as the inflammatory autoimmune disease multiple sclerosis (MS), have been associated to higher risk of MDD, in comparison with overall population. Importantly, in both MS patients and in its experimental mouse model, Experimental Autoimmune Encephalomyelitis (EAE), the notion that depressive symptoms are reactive epiphenomenon to the MS pathology has been recently challenged by the evidence of their early manifestation, even before the onset of the disease. Furthermore, in association to such mood disturbance, inflammatory-dependent synaptic dysfunctions in several areas of MS/EAE brain have been observed independently of brain lesions and demyelination. This evidence suggests that a fine interplay between the immune and nervous systems can have a huge impact on several neurological functions, including depressive symptoms, in different pathological conditions. The aim of the present review is to shed light on common traits between MDD and MS, by looking at inflammatory-dependent synaptic alterations associated with depression in both diseases.
Front Cell Neurosci, 2020; 14
34490928: De Vito F, Musella A, Fresegna D, Rizzo FR, Gentile A, Stampanoni Bassi M, Gilio L, Buttari F, Procaccini C, Colamatteo A, Bullitta S, Guadalupi L, Caioli S, Vanni V, Balletta S, Sanna K, Bruno A, Dolcetti E, Furlan R, Finardi A, Licursi V, Drulovic J, Pekmezovic T, Fusco C, Bruzzaniti S, Hornstein E, Uccelli A, Salvetti M, Matarese G, Centonze D, Mandolesi G
MiR-142-3p regulates synaptopathy-driven disease progression in multiple sclerosis.
We recently proposed miR-142-3p as a molecular player in inflammatory synaptopathy, a new pathogenic hallmark of multiple sclerosis (MS) and of its mouse model experimental autoimmune encephalomyelitis (EAE), that leads to neuronal loss independently of demyelination. MiR-142-3p seems to be unique among potential biomarker candidates in MS, since it is an inflammatory miRNA playing a dual role in the immune and central nervous systems. Here, we aimed to verify the impact of miR-142-3p circulating in the cerebrospinal fluid (CSF) of MS patients on clinical parameters, neuronal excitability and its potential interaction with disease modifying therapies (DMTs).
Neuropathol Appl Neurobiol, 2022; 48
34391817: Rizzo FR, Guadalupi L, Sanna K, Vanni V, Fresegna D, De Vito F, Musella A, Caioli S, Balletta S, Bullitta S, Bruno A, Dolcetti E, Stampanoni Bassi M, Buttari F, Gilio L, Mandolesi G, Centonze D, Gentile A
Exercise protects from hippocampal inflammation and neurodegeneration in experimental autoimmune encephalomyelitis.
Exercise is increasingly recommended as a supportive therapy for people with Multiple Sclerosis (pwMS). While clinical research has still not disclosed the real benefits of exercise on MS disease, animal studies suggest a substantial beneficial

effect on motor disability and pathological hallmarks such as central and peripheral dysregulated immune response. The hippocampus, a core area for memory formation and learning, is a brain region involved in MS pathophysiology. Human and rodent studies suggest that the hippocampus is highly sensitive to the effects of exercise, the impact of which on MS hippocampal damage is still elusive. Here we addressed the effects of chronic voluntary exercise on hippocampal function and damage in experimental autoimmune encephalomyelitis (EAE), animal model of MS. Mice were housed in standard or wheel-equipped cages starting from the day of immunization and throughout the disease course. Although running activity was reduced during the symptomatic phase, exercise significantly ameliorated motor disability. Exercise improved cognition that was assessed through the novel object recognition test and the nest building in presymptomatic and acute stages of the disease, respectively. In the acute phase exercise was shown to prevent EAE-induced synaptic plasticity abnormalities in the CA1 area, by promoting the survival of parvalbumin-positive (PV+) interneurons and by attenuating inflammation. Indeed, exercise significantly reduced microgliosis in the CA1 area, the expression of tumour necrosis factor (TNF) in microglia and, to a lesser extent, the hippocampal level of interleukin 1 beta (IL-1β), previously shown to contribute to aberrant synaptic plasticity in the EAE hippocampus. Notably, exercise exerted a precocious and long-lasting mitigating effect on microgliosis that preceded its neuroprotective action, likely underlying the improved cognitive function observed in both presymptomatic and acute phase EAE mice. Overall, these data provide evidence that regular exercise improves cognitive function and synaptic and neuronal pathology that typically affect EAE/MS brains.
Brain Behav Immun, 2021; 98

33562569: Mandolesi G, Rizzo FR, Balletta S, Stampanoni Bassi M, Gilio L, Guadalupi L, Nencini M, Moscatelli A, Ryan CP, Licursi V, Dolcetti E, Musella A, Gentile A, Fresegna D, Bullitta S, Caioli S, Vanni V, Sanna K, Bruno A, Buttari F, Castelli C, Presutti C, De Santa F, Finardi A, Furlan R, Centonze D, De Vito F
The microRNA let-7b-5p Is Negatively Associated with Inflammation and Disease Severity in Multiple Sclerosis.
The identification of microRNAs in biological fluids for diagnosis and prognosis is receiving great attention in the field of multiple sclerosis (MS) research but it is still in its infancy. In the present study, we observed in a large sample of MS patients that let-7b-5p levels in the cerebrospinal fluid (CSF) were highly correlated with a number of microRNAs implicated in MS, as well as with a variety of inflammation-related protein factors, showing specific expression patterns coherent with let-7b-5p-mediated regulation. Additionally, we found that the CSF let-7b-5p levels were significantly reduced in patients with the progressive MS compared to patients with relapsing-remitting MS and were negatively correlated with characteristic hallmark processes of the two phases of the disease. Indeed, in the non-progressive phase, let-7b-5p inversely associated with both central and peripheral inflammation; whereas, in progressive MS, the CSF levels of let-7b-5p negatively correlated with clinical disability at disease onset and after a follow-up period. Overall, our results uncovered, by the means of a multidisciplinary approach and multiple statistical analyses, a new possible pleiotropic action of let-7b-5p in MS, with potential utility as a biomarker of MS course.
Cells, 2021; 10

32455907: Musella A, Gentile A, Guadalupi L, Rizzo FR, De Vito F, Fresegna D, Bruno A, Dolcetti E, Vanni V, Vitiello L, Bullitta S, Sanna K, Caioli S, Balletta S, Nencini M, Buttari F, Stampanoni Bassi M, Centonze D, Mandolesi G
Central Modulation of Selective Sphingosine-1-Phosphate Receptor 1 Ameliorates Experimental Multiple Sclerosis.
Future treatments of multiple sclerosis (MS), a chronic autoimmune neurodegenerative disease of the central nervous system (CNS), aim for simultaneous early targeting of peripheral immune function and neuroinflammation. Sphingosine-1-phosphate (S1P) receptor modulators are among the most promising drugs with both "immunological" and "non-immunological" actions. Selective S1P receptor modulators have been recently approved for MS and shown clinical efficacy in its mouse model, the experimental autoimmune encephalomyelitis (EAE). Here, we investigated the anti-inflammatory/neuroprotective effects of ozanimod (RPC1063), a S1P modulator recently approved in the United States for the treatment of MS, by performing ex vivo studies in EAE brain. Electrophysiological experiments, supported by molecular and immunofluorescence analysis, revealed that ozanimod was able to dampen the EAE glutamatergic synaptic alterations, through attenuation of local inflammatory response driven by activated microglia and infiltrating T cells, the main CNS-cellular players of EAE synaptopathy. Electrophysiological studies with selective S1P (AUY954) and S1P (A971432) agonists suggested that S1P modulation is the main driver of the anti-excitotoxic activity mediated by ozanimod. Accordingly, in vivo intra-cerebroventricular treatment of EAE mice with AUY954 ameliorated clinical disability. Altogether these results strengthened the relevance of S1P agonists as immunomodulatory and neuroprotective drugs for MS therapy.
Cells, 2020; 9

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CXCR2 increases in ALS cortical neurons and its inhibition prevents motor neuron degeneration in vitro and improves neuromuscular function in SOD1G93A mice.
Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by depletion of motor neurons

(MNs), for which effective medical treatments are still required. Previous transcriptomic analysis revealed the up-regulation of C-X-C motif chemokine receptor 2 (CXCR2)-mRNA in a subset of sporadic ALS patients and SOD1G93A mice. Here, we confirmed the increase of CXCR2 in human ALS cortex, and showed that CXCR2 is mainly localized in cell bodies and axons of cortical neurons. We also investigated the effects of reparixin, an allosteric inhibitor of CXCR2, in degenerating human iPSC-derived MNs and SOD1G93A mice. In vitro, reparixin rescued MNs from apoptotic cell death, preserving neuronal morphology, mitochondrial membrane potential and cytoplasmic membrane integrity, whereas in vivo it improved neuromuscular function of SOD1G93A mice. Altogether, these data suggest a role for CXCR2 in ALS pathology and support its pharmacological inhibition as a candidate therapeutic strategy against ALS at least in a specific subgroup of patients. Neurobiol Dis, 2021; 160

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ASSESSMENT OF IL-38 LEVELS IN MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Multiple Sclerosis (MS) is chronic inflammatory disease of the central nervous system with an increasing prevalence last years. Interleukin 38 (IL-38) is the most recently discovered cytokine of the IL-1 family with reported anti-inflammatory effects. However, little is known about the changes in IL-38 expression in MS. **Aims** In this work we aimed to investigate the changes in IL-38 levels in serum samples from MS patients, as well as, in the spinal cord of experimental autoimmune encephalomyelitis mice, a murine model of MS **Methods** Human Serum samples of patients with MS and healthy individuals were collected and IL-38 levels were quantified by ELISA. Spinal cords from EAE mice were harvested at different stages of the disease and IL-38 levels were assessed by QPCR. **Results** Despite IL-38 has potent anti-inflammatory actions, the protein levels of this cytokine were not increased in MS patients. Similarly, IL-38 transcripts were not detected in the spinal cord of EAE mice at the onset and peak of the disease. However, we observed that IL-38 mRNA levels were induced in the spinal cord of EAE mice at the remission phase of the disease, coinciding with the attenuation of the neuroinflammatory response. **Conclusions** These results suggest that IL-38 could be involved in attenuating inflammation in EAE mice, and that the induction of this cytokine is defective in MS patients.

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APTAMERS TO REMYELINATE MULTIPLE SCLEROSIS: EFFECTS OF APTOLL© TREATMENT IN PRECLINICAL MODELS AND DETECTION OF MECHANISMS IN HUMAN SAMPLES

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Multiple sclerosis (MS) is a neurodegenerative, autoimmune and chronic disease of the central nervous system (CNS) and the second cause of neurological disability in young adults. The main histopathogenic feature is the patched death of oligodendrocytes and the subsequent loss of myelin, both in the white and in the grey matters. Demyelination is mainly accepted to derive from autoimmune-triggered inflammation, where the Toll-like receptor 4 (TLR4) plays a crucial role. ApTOLL© has been developed with aptamer technology to antagonize TLR4. The molecular nature and mechanism of action of this single chain DNA aptamer represents a novel strategy to treat diseases with an important inflammatory component, such as MS. In our present research, we have determined the optimal dose for the in vivo use of ApTOLL© to treat murine Experimental Autoimmune Encephalitis (0.91 mg/kg). Clinical score was significantly lower in ApTOLL©-treated animals, where myelin and axons were better preserved, too. Furthermore, this molecule seems to directly promote the proliferation of oligodendrocytes precursors cells (OPCs) and its differentiation towards myelin-forming phenotypes. Finally, we have determined the presence of TLR4 in different cell types from human tissue of MS patients and controls. Altogether, our data point to ApTOLL© as an innovative candidate to be incorporated to the pharmaceutical arsenal to treat MS as (re)myelinating and neuroprotector.

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IMPACT OF VAGAL NERVE STIMULATION ON THE PROGRESSION OF DEMYELINATED LESIONS IN A MURINE MODEL OF MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Multiple Sclerosis (MS) is a neurodegenerative disease, characterized by the loss of myelin, a specialized oligodendrocytes (OL)-derived membrane. At early stages, demyelinated lesions are characterized by the presence of OLs precursor cells (OPCs), reactive astrocytes and proinflammatory microglia (‘M1-like’ phenotype). One intervention that has shown promising results in the treatment of peripheral and central inflammation is the electrical stimulation of vagus nerve (VNS). In murine models of neuroinflammation the evidence indicates that VNS reduces proinflammatory cytokines, as well as microglia and macrophages infiltration, promoting the anti-inflammatory phenotype of microglia (‘M2-like’). However, the effect of VNS in MS-animal models have not been systematically studied. Our aim was to study the effect of *in vivo* VNS in the neuroinflammation and the progression of demyelination/remyelination in a murine MS model. We induced our MS model with focal stereotaxic injections of lysolecithin (LPC) in C57BL/6 mice (PN45). Mice were treated with VNS after 7-to-9 days post injection, when the peak of OPCs in the lesion is reached. Afterwards, brain tissue was analyzed by immunostaining of myelin, glial and neuronal markers, as well as cytokines expression. Interestingly, we observed an increase in the myelinated area, a reduction in the population of astrocytes and M1-like microglia, along with changes in the TGFß-1 levels in the VNS treated group. Our preliminary results suggest that VNS improves remyelination by reducing the proinflammatory environment in demyelinating MS-like lesions. *Funded by fondecyt#1210940 to FCO, VRAID-DUIA #210-2021 to RV*

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IN VIVO IMAGING OF OLIGODENDROCYTE INJURY IN AN NMO MOUSE MODEL

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Neuromyelitis optica (NMO) is an autoimmune disease predominantly affecting spinal cord and optic nerve. The majority of NMO patients have serum antibodies (IgG) against the water channel aquaporin4 (AQP4), which is expressed on astrocytes in the CNS. Despite this primary astrocytic target, demyelination is prominent in AQP4-IgG+-NMO. However, how NMO pathology spreads beyond astrocytes remains elusive. We investigated early signs of oligodendrocyte damage in a mouse model of acute NMO induced by spinal application of patient-derived AQP4-IgG and human complement followed by in vivo imaging. Morphological assessment and calcium imaging of astrocytes and oligodendrocytes revealed the relative time-course of injury: Within an hour of AQP4-IgG application, astrocytes showed a global calcium rise and membrane rupture confirmed by uptake of a cell-impermeable dye and cellular fragmentation. Concurrent to astrocytic calcium rise, oligodendrocyte processes also showed calcium overload, which however reached the soma comparatively later. In contrast to pervasive lytic death of astrocytes, only some oligodendrocytes were lost at later time points. While dye exclusion experiments negated overt membrane rupture, expression of the MAC-inhibitor protein CD59 on oligodendrocytes protected these cells after AQP4-IgG-mediated astrocyte injury. These results imply that oligodendrocyte pathology in NMO is not driven by the loss of astrocytes per se, but rather evolves from MAC-dependent ‘bystander’ targeting of oligodendrocytes as suggested by prior in vitro and fixed tissue observations. Our dynamic observations suggest that despite the similar starting point of injury, executive phase might differ and activate distinct cell death pathways in the two major glial target cells of NMO.

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VALIDATION OF IHC MARKERS ANTIBODY PANEL IN RAT EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) MODEL OF MULTIPLE SCLEROSIS

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Animal models allow for greater understanding of underlying mechanisms of CNS disorders. Highly validated specific antibodies further enable generation of high-quality results, also in translational research. The aim of the present study was to validate an antibody panel developed by Atlas Antibodies using Experimental Autoimmune Encephalomyelitis (EAE) rat models of multiple sclerosis (MS). Forty-nine antibodies developed against human epitopes, including markers for neurons, astrocytes, oligodendrocytes, and microglia were evaluated in IHC experiments using human and rodent tissues. Normal human tissues were obtained from a commercial biobank (Asterand®, BioIVT, West Sussex, UK). EAE rodent tissues were provided by Redoxis (Redoxis, Sweden). Tissue sections were stained using a standard IHC protocol with DAB visualization. Multiplexed immunofluorescence IHC was performed with selected number of antibodies. All 49 antibodies showed expected staining in human tissues. 26 antibodies (59%) displayed expected immunoreactivity in normal rat brain. These antibodies were further validated in EAE models (SCH- and MOG-induced), including multiplexed IHC-IF analysis to confirm cell type specificity. Both SCH and MOG groups displayed decreased expression of MBP, indicative of demyelination/loss of myelinated axons. Increased number of P2RX7+ reactive astrocytes was observed in both SCH and MOG, especially in the areas of demyelination. CRYAB expression was increased in SCH- and decreased in MOG within areas of demyelination. Fewer GPR17+ cells were detected in SCH respect normal brain, while a greater numbers in MOG, indicating oligodendrocyte aberration. The data confirm the usability of EAE rat model and the antibody panel for studying the underlying cellular mechanisms of MS.

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EXPLORING THE MECHANISM OF ACTION OF THE NOVEL REMYELINATION THERAPY NEFIRACETAM

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Aims: Multiple sclerosis (MS) is a neurodegenerative disorder involving immune-mediated damage to myelin. Initially, damage is repaired through the activation of a repair process called remyelination. However, as MS progresses, remyelination fails leaving axons vulnerable to damage. Loss of chronically demyelinated axons causes a decline in neuronal activity, resulting in progressive disability. Current therapies target the immune component of MS but do not address the remyelination deficits underlying progression. Thus, there is a need for therapies which encourage remyelination to improve treatment outcomes. We have found that nefiracetam accelerates remyelination; our aim is to elucidate its mechanism of action. **Methods:** C57/Bl6j mice were fed a cuprizone diet for 6 weeks to induce a demyelinating insult. Mice were given daily injections of 15mg/kg nefiracetam for 3 days from day 38. Corpus callosum and cortical tissue samples were taken for molecular analysis. Primary oligodendrocyte precursor cells (OPCs) derived from neonatal rodent cortices were used to study the direct effect of nefiracetam on OPCs. **Results:** Omics-based analyses of the tissue identified several signalling pathways which may be central to nefiracetam's therapeutic effect. The data also highlights genes and pathways which are regulated in response to the model in these brain regions. The *in vitro* studies on OPCs revealed that nefiracetam directly modulates a key phase of the remyelination process. In particular, the data implicates the regulation of glutamate signalling in nefiracetam's remyelinating action. **Conclusion:** We have identified a novel remyelination therapy which has the potential to be used in the treatment of MS.

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IMMUNOREGULATORY NEUTROPHILS IN NEUROINFLAMMATION

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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The onset and progression of neurological disorders have been recently linked to neutrophils. The neutrophil-to-lymphocyte ratio has been proposed as a clinical marker both for ischemic stroke and Multiple Sclerosis (MS). It is known that CD66b+ neutrophils can exert either a pro-inflammatory or an anti-inflammatory function, but their role in Multiple Sclerosis is still unclear. Khorooshi R, and colleagues stated that a subgroup of neutrophils might have a protective function in the first phases of EAE (Experimental Autoimmune Encephalitis). Moreover, the PD-1 axis seems to have a key role in this population and its functions. Our group recently observed a group of neutrophils expressing PD-1 ligand 2 (CD273) both in patients with an active form of MS and in mice with EAE. We characterized CD273+ neutrophils and performed functional studies on both human and murine neutrophils. We also confirmed that CD273+ neutrophils release EVs and tested whether they could exert an immunosuppressive function. Finally, we validated the role of CD273+ neutrophils in the murine model of EAE. We speculate that CD273 might identify a novel subpopulation of regulatory neutrophils in MS patients, paving the way for innovative approaches in terms of diagnosis, cell therapies, or new drug delivery tools through vesicles.

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ROSTRAL-TO-CAUDAL INCREASE IN METABOLIC ACTIVITY OF CULTURED EX VIVO SLICES OF NEONATAL RAT BRAIN

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Ex vivo organotypic slice models are optimum for investigation of cell types in their natural 3D orientation, as well as maintaining endogenous signalling pathways and cell:cell interactions. Here, we examined metabolic activity in slice cultures from different anatomical areas of the cortex. Sequential coronal slices (12-13) were dissected, along the rostral-caudal axis, from each of four neonatal rats (P 10/11) and cultured in Millicell Culture Plate Inserts (0.4µm pore size). Slices were maintained in serum-free medium for 7 days and metabolic activity was determined by daily testing of supernatant with alamarBlue™ reagent (biological replicates). This approach showed a pattern: the metabolic activity of slices dropped over time, with a slight recovery on the 4th day. The impact of anatomical location, as well as the presence of connected subcortical regions, on slice metabolic activity was determined. Results from the average viability over the 7 days of sampling indicate that the more caudal slices (Bregma point -1,10mm to -3,50mm) had 29.6% higher metabolic activity than rostral regions ($p<0,001$, $n=50$). Additionally, slices with cortex alone showed 38.5% lower metabolic activity than those with all connections between structures intact ($p<0,001$, $n=26$). When those connections got interrupted, a 25.5% ($p<0,02$, $n=20$) drop-in metabolic activity was observed. This finding emphasises the importance of tracking the anatomical origin and connectivity between regions, when designing and executing cortex-focused ex vivo experiments. We recommend use of caudal region slices, connected with all structures within the slice, as an optimal platform for mimicking cortical physiology under control and diseased conditions.

Pubmed:
34438094: Simons E, Labro A, Saenen J, Nijak A, Sieliwonczyk E, Vandendriessche B, Dąbrowska M, Van Craenenbroeck EM, Schepers D, Van Laer L, Loeys BL, Alaerts M
Molecular autopsy and subsequent functional analysis reveal de novo DSG2 mutation as cause of sudden death.
Sudden cardiac death (SCD) is a common cause of death in young adults. In up to 80% of cases a genetic cause is suspected. Next-generation sequencing of candidate genes can reveal the cause of SCD, provide prognostic management, and facilitate pre-symptomatic testing and prevention in relatives. Here we present a proband who experienced SCD in his sleep for which molecular autopsy was performed. We performed a post-mortem genetic analysis of a 49-year-old male who died during sleep after competitive kayaking, using a Cardiomyopathy and Primary Arrhythmia next-generation sequencing panel, each containing 51 candidate genes. Autopsy was not performed. Genetic testing of the proband resulted in missense variants in KCNQ1 (c.1449C > A; p.(Asn483Lys)) and DSG2 (c.2979G > T; p.(Gln993His)), both absent from the gnomAD database. Familial segregation analysis showed de novo occurrence of the DSG2 variant and presence of the KCNQ1 variant in the proband's mother and daughter. KCNQ1 p.(Asn483Lys) was predicted to be pathogenic by MutationTaster. However, none of the KCNQ1 variant carrying family members showed long QTc on ECG or Holter. We further functionally analysed this variant using patch-clamp in a heterologous expression system (Chinese Hamster Ovary (CHO) cells) expressing the KCNQ1 mutant in combination with KCNE1 wild type protein and showed no significant changes in electrophysiological function of Kv7.1. Based on the above evidence, we concluded that the DSG2 p.(Gln993His) variant is the most likely cause of SCD in the presented case, and that there is insufficient evidence that the identified KCNQ1 p.(Asn483Lys) variant would confer risk for SCD in his mother and daughter. Fortunately, the DSG2 variant was not inherited by the proband's two children. This case report indicates the added value of molecular autopsy and the importance of subsequent functional study of variants to inform patients and family members about the risk of variants they might carry.
Eur J Med Genet, 2021; 64

FENS Forum 2022
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BOARD NUMBER: S03-330

FAILED REMYELINATION OF THE NON-HUMAN PRIMATE OPTIC NERVE LEADS TO AXON DEGENERATION, RETINAL DAMAGES AND VISUAL DYSFUNCTION.

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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White matter disorders of the CNS such as MS, lead to failure of nerve conduction and long-lasting neurological disabilities affecting a variety of sensory and motor systems including vision. While most disease-modifying therapies target the immune and inflammatory response, the promotion of remyelination has become a new therapeutic avenue, to prevent neuronal degeneration and promote recovery. Most of these strategies are developed in short-lived rodent models of demyelination, which spontaneously repair and do not reflect the size, organization, and biology of the human CNS. Thus, well-defined non-human primate models are required to efficiently advance therapeutic approaches for patients. Here, we followed the consequence of long-term toxin-induced demyelination of the macaque optic nerve on remyelination and axon preservation, as well as its impact on visual functions. Findings from oculo-motor behavior, ophthalmic examination, electrophysiology, and retinal imaging indicate visual impairment involving the optic nerve and retina. These visual dysfunctions fully correlated at the anatomical level, with sustained optic nerve demyelination, axonal degeneration, and alterations of the inner retinal layers. This non-human primate model of chronic optic nerve demyelination associated with axonal degeneration and visual dysfunction, recapitulates several key features of MS lesions and should be instrumental in providing the missing link to translate emerging repair pro-myelinating/neuroprotective therapies to the clinic for myelin disorders such as MS.

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BOARD NUMBER: S03-331

THE PREVALENCE AND TOPOGRAPHY OF DEMYELINATION AND INFLAMMATORY ACTIVITY IN THE MULTIPLE SCLEROSIS SPINAL CORD

POSTER SESSION 03 - SECTION: MULTIPLE SCLEROSIS: BIOMARKERS, PATHOGENESIS AND THERAPIES

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Background: The demyelinated lesion is a cardinal feature of progressive multiple sclerosis (MS) pathology that commonly affects the spinal cord. The study of lesion topography in the spinal cord and the extent of inflammation may provide important clues about disease pathogenesis. Aims: We aimed to characterise the prevalence and distribution of demyelination in the progressive MS spinal cord. Methods: Immunohistochemistry was used to detect demyelination (proteolipid protein) and classify its inflammatory activity (CD68) in the cervical, thoracic, and lumbar spinal cord of 119 MS cases. Lesions were standardised onto anatomical templates before mixed models and permutation-based cluster analysis were used to identify patterns in the prevalence and topography of demyelination. Results: Spinal cord lesions were observed in 76.5% of cases. When stratified by histological classification, inflammatory activity was a common feature observed in 87.9% of cases. Lesions consistently affected the dorsal columns, lateral corticospinal tracts, and central canal. Interestingly, the subpial surface was commonly spared. However, when present, subpial demyelination only affected a limited circumference (less than 15%). The presence of lesions also exhibited a strong relationship with clinical disease milestones. Conclusions: Our findings demonstrate that 1) inflammatory spinal cord lesions are an unappreciated feature of progressive MS pathology, 2) white matter tracts and the central canal are common lesion predilection sites, 3) subpial sparing is a salient feature of spinal cord lesional pathology which argues against an outside-in gradient of spinal cord demyelination in MS, and 4) spinal cord demyelination is a substrate underpinning a more aggressive disease course.

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BOARD NUMBER: S03-332

CONTRIBUTION OF MUSCLE-INTRINSIC TOXICITY OF ALS-MUTANT FUS TO MOTOR NEURON DEGENERATION

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is the most frequent adult-onset motor neurodegenerative disease characterized by relatively selective degeneration of upper and lower motor neurons. Nevertheless, non-cell autonomous contributions of cell types other than motor neurons have been implicated in ALS pathogenesis. However, whether skeletal muscles also contribute to motor neuron degeneration is quite controversial. We gained evidence that skeletal muscles contribute to neuromuscular junction pathology in ALS caused by heterozygous mutations in the gene encoding the RNA-binding protein FUS. FUS-ALS accounts for the majority of the most aggressive forms of ALS with juvenile onset and rapid disease progression. In FUS-ALS patients, mutations are commonly clustered in the nuclear localization signal (NLS). The $Fus^{\Delta NLS/+}$ mouse expresses a truncated FUS protein lacking the NLS, which leads to FUS-ALS symptoms from 10 months of age onward. Newborn $Fus^{\Delta NLS/\Delta NLS}$ mice and patient iPSC-derived motor neuron-myotube co-cultures showed impaired maturation of neuromuscular junctions, which was attributable to toxicity of ALS-mutant FUS in motor neurons, but also skeletal muscle/myotubes. These findings raise the question whether skeletal muscle intrinsic toxicity of ALS-mutant FUS is an active contributor to motor neuron degeneration and motor deficits in adult $Fus^{\Delta NLS/+}$ mice. We tackle this question by genetically manipulating ALS-mutant FUS specifically in skeletal muscle of $Fus^{\Delta NLS}$ mice. We assess the neuromuscular function of these mice in a longitudinal motor behavior study and by histological and molecular analyses. Our study indicates that the skeletal muscle may constitute a novel therapeutic target for FUS-ALS.

Pubmed:
34516840: Zuko A, Mallik M, Thompson R, Spaulding EL, Wienand AR, Been M, Tadenev ALD, van Bakel N, Sijlmans C, Santos LA, Bussmann J, Catinozzi M, Das S, Kulshrestha D, Burgess RW, Ignatova Z, Storkebaum E
tRNA overexpression rescues peripheral neuropathy caused by mutations in tRNA synthetase.
Heterozygous mutations in six transfer RNA (tRNA) synthetase genes cause Charcot-Marie-Tooth (CMT) peripheral neuropathy. CMT mutant tRNA synthetases inhibit protein synthesis by an unknown mechanism. We found that CMT mutant glycyl-tRNA synthetases bound tRNA but failed to release it, resulting in tRNA sequestration. This sequestration potentially depleted the cellular tRNA pool, leading to insufficient glycyl-tRNA supply to the ribosome. Accordingly, we found ribosome stalling at glycine codons and activation of the integrated stress response (ISR) in affected motor neurons. Moreover, transgenic overexpression of tRNA rescued protein synthesis, peripheral neuropathy, and ISR activation in and mouse CMT disease type 2D (CMT2D) models. Conversely, inactivation of the ribosome rescue factor GTPBP2 exacerbated peripheral neuropathy. Our findings suggest a molecular mechanism for CMT2D, and elevating tRNA levels may thus have therapeutic potential.
Science, 2021; 373

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BOARD NUMBER: S03-333

C9ORF72 AND GRN MUTATIONS SENSITIZE MICROGLIA TO PRO-INFLAMMATORY ACTIVATION BY EXTRACELLULAR TDP-43

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Frontotemporal dementia (FTD) and Amyotrophic lateral sclerosis (ALS) are two neurodegenerative diseases of a common clinical, genetic and neuropathological spectrum but common biological pathways leading to these two diseases remain elusive. In this study, we show that TDP-43, the most common form of aggregated protein in ALS and FTD, activates microglial NLRP3 inflammasome pathway through recognition by TLR receptors and its endocytosis. By stimulating C9orf72-/- and GrnR493X/R493X deficient murine microglia with TDP-43, we were able to show that these cells were hyperreactive to this stimulus. This effect was reproducible using monocyte-derived-microglia-like cells obtained from FTD patients with C9ORF72 and GRN mutations. Our data highlight for the first time a similar dysregulation by mutations in the two main genes responsible for TDP-43 proteinopathy and causing, respectively, FTD and FTD/ALS. It points to neuroinflammation and microglia endolysosomal dynamics as key players in FTD and ALS and open new avenues for therapeutic developement in these diseases.

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BOARD NUMBER: S03-334

ROLE OF METABOLISM IN PATHOLOGICAL AGGREGATION OF TDP-43 AND ITS DOWN-STREAM TOXICITY

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Background: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are two fatal neurodegenerative disorders with considerable molecular overlap. Cytoplasmic aggregation of transactive response DNA binding protein of 43 kDa (TDP-43) in neurons and glia is a consistent feature of the majority of ALS and FTLD cases. Certain metabolic conditions that are conventionally considered risk factors for cardiovascular and cerebrovascular diseases; such as type-2 diabetes mellitus, high body mass index, and dyslipidaemia are intriguingly associated with delayed onset, slower disease progression, and/or longer survival in both ALS and FTLD. **Aims:** This study aims to investigate the molecular mechanisms underlying the disease-modifying effects of metabolic disorders in ALS and FTLD with a particular focus on the role of TDP-43 in the regulation of neuronal metabolism. **Results:** Our preliminary investigations performed in NSC-34 mouse motor neuron-like cells reveals a regulatory role of TDP-43 in neuronal glucose metabolism. Notably, TDP-43 knock-down alters expression of key rate-limiting enzymes involved in glycolysis and the citric acid cycle. At the functional level, these changes correlate with increased utilization of glucose by the NSC-34 cells and over-reliance on anaerobic respiration. Furthermore, TDP-43 depletion renders NSC-34 cells more vulnerable to the effects of glucose depletion. **Conclusion:** Our current findings underscore an interplay between TDP-43 and neuronal glucose metabolism that may have important implications for neuronal survival under conditions of metabolic stress. Our current focus is on validating the findings via modelling TDP-43 aggregation in NSC -34 cells and identifying the underlying molecular cascade. **Keywords:** TDP-43, metabolism, neurodegeneration, ALS, FTLD

Pubmed:
30918455: Omotoso GO, Olajide OJ, Gbadamosi IT, Rasheed MA, Izuogu CT
Kolaviron Protects the Prefrontal Cortex and Hippocampus against Histomorphological and Neurobehavioural Changes in Cuprizone Model of Multiple Sclerosis.
This study explored the efficacy of kolaviron-a biflavonoid complex isolated from the seeds of -in protecting against cuprizone (CPZ)-induced demyelination in both the prefrontal cortex and the hippocampus of Wistar rats.
Malays J Med Sci, 2018; 25
33420680: Olajide OJ, Gbadamosi IT, Yawson EO, Arogundade T, Lewu FS, Ogunrinola KY, Adigun OO, Bamisi O, Lambe E, Arietarhire LO, Oluyomi OO, Idowu OK, Kareem R, Asogwa NT, Adeniyi PA
Hippocampal Degeneration and Behavioral Impairment During Alzheimer-Like Pathogenesis Involves Glutamate Excitotoxicity.
The hallmarks of Alzheimer's disease (AD) pathology include senile plaques accumulation and neurofibrillary tangles, which is thought to underlie synaptic failure. Recent evidence however supports that synaptic failure in AD may instead be instigated by enhanced N-methyl-D-aspartate (NMDA) activity, via a reciprocal relationship between soluble amyloid-β (Aβ) accumulation and increased glutamate agonist. While previous studies have shown Aβ-mediated alterations to the glutamatergic system during AD, the underlying etiology of excitotoxic glutamate-induced changes has not been explored. Here, we investigated the acute effects of stereotaxic dentate gyrus (DG) glutamate injection on behavior and molecular expression of specific proteins and neurochemicals modulating hippocampal functions. Dependence of glutamate-mediated effects on NMDA receptor (NMDAR) hyperactivation was tested using NMDARs antagonist memantine. DG of Wistar rats (12-weeks-old) were bilaterally microinjected with glutamate (500 mM) with or without daily intraperitoneal (i.p.) memantine injection (20 mg/kg) for 14 days, while controls received either intrahippocampal/i.p. PBS or i.p. memantine. Behavioral characterization in open field and Y-maze revealed that glutamate evoked anxiogenic responses and perturbed spatial memory were inhibited by memantine. In glutamate-treated rats, increased NO expression was accompanied by marked reduction in profiles of glutathione-s-transferase and glutathione peroxidase. Similarly, glutamate-mediated increase in acetylcholinesterase expression corroborated downregulation of synaptophysin and PSD-95, coupled with initiation of reactive astrogliosis (GFAP). While neurofilament immunolocalization/immunoexpression was unperturbed, we found

glutamate-mediated reduction in neurogenic markers Ki67 and PCNA immunoexpression, with a decrease in NR2B protein expression, whereas mGluR1 remains unchanged. In addition, increased expression of apoptotic regulatory proteins p53 and Bax was seen in glutamate infused rats, corroborating chromatolytic degeneration of granule neurons in the DG. Interestingly, memantine abrogated most of the degenerative changes associated with glutamate excitotoxicity in this study. Taken together, our findings causally link acute glutamate dyshomeostasis in the DG with development of AD-related behavioral impairment and molecular neurodegeneration.
J Mol Neurosci, 2021; 71
33643554: Omotoso GO, Arietarhire LO, Ukwubile II, Gbadamosi IT
The Protective Effect of Kolaviron on Molecular, Cellular, and Behavioral Characterization of Cerebellum in the Rat Model of Demyelinating Diseases.
This study aimed at assessing the protective mechanisms of Kolaviron (KV) on the cerebellum in a rat model of demyelination.
Basic Clin Neurosci, 2020 Sep-Oct; 11
29984057: Omotoso GO, Gbadamosi IT, Afolabi TT, Abdulwahab AB, Akinlolu AA
Ameliorative effects of on cuprizone-induced memory decline in rat model of multiple sclerosis.
Cuprizone is a neurotoxin with copper-chelating ability used in animal model of multiple sclerosis in which oxidative stress has been documented as one of the cascade in the pathogenesis. is a phytomedicinal plant with antioxidant and neuroprotective properties. This study aimed at evaluating the ameliorative capability of in cuprizone-induced behavioral and histopathological alterations in the prefrontal cortex and hippocampus of Wistar rats. Four groups of rats were treated with normal saline, cuprizone, and a combination of and cuprizone, for five weeks. The rats were subjected to Morris water maze and Y-maze to assess long and short-term memory respectively. The animals were sacrificed, and brain tissues were removed for histochemical and enzyme lysate immunosorbent assay for catalase, superoxide dismutase, and nitric oxide. Cuprizone significantly induced oxidative and nitrosative stress coupled with memory decline and cortico-hippocampal neuronal deficits; however, administration of significantly reversed the neuropathological deficits induced by cuprizone.
Anat Cell Biol, 2018; 51
29307662: Omotoso GO, Gbadamosi IT, Olajide OJ, Dada-Habeeb SO, Arogundade TT, Yawson EO
Moringa oleifera phytochemicals protect the brain against experimental nicotine-induced neurobehavioral disturbances and cerebellar degeneration.
Nicotine is a neuro-stimulant that has been implicated in the pathophysiology of many brain diseases. The need to prevent or alleviate the resulting dysfunction is therefore paramount, which has also given way to the use of medicinal plants in the management of brain conditions. This study was designed to determine the histomorphological and neurobehavioural changes in the cerebellum of Wistar rats following nicotine insult and how such injuries respond to Moringa intervention. Twenty-four adult male Wistar rats were divided into 4 groups. Group A and B were orally treated with normal saline and Moringa oleifera respectively for twenty-eight days; Group C was treated with nicotine while group D was treated orally with Moringa oleifera and intraperitoneally with nicotine for twenty-eight days. Animals were subjected to the open field test on the last day of treatment. 24 h after last day treatment, the animals were anesthetized and perfusion fixation was carried out. The cerebellum was excised and post-fixed in 4% paraformaldehyde and thereafter put through routine histological procedures. Results revealed cytoarchitectural distortion and extreme chromatolysis in neuronal cells of the cerebellar cortical layers in the nicotine-treated group. The Purkinje cells of the cerebellum of animals in this group were degenerated. There were also reduced locomotor activities in the group. Moringa was able to prevent the chromatolysis, distortion of the cerebellar cortical cells and neurobehavioural deficit. Our result suggests that Moringa oleifera could prevent nicotine-induced cerebellar injury in Wistar rats, with the possibility of ameliorating the clinical features presented in associated cerebellar pathology.
Pathophysiology, 2018; 25
28192749: Olajide OJ, Yawson EO, Gbadamosi IT, Arogundade TT, Lambe E, Obasi K, Lawal IT, Ibrahim A, Ogunrinola KY
Ascorbic acid ameliorates behavioural deficits and neuropathological alterations in rat model of Alzheimer's disease.
Exploring the links between neural pathobiology and behavioural deficits in Alzheimer's disease (AD), and investigating substances with known therapeutic advantages over subcellular mechanisms underlying these dysfunctions could advance the development of potent therapeutic molecules for AD treatment. Here we investigated the efficacy of ascorbic acid (AA) in reversing aluminium chloride (AlCl)-induced behavioural deficits and neurotoxic cascades within prefrontal cortex (PFC) and hippocampus of rats. A group of rats administered oral AlCl (100mg/kg) daily for 15days showed degenerative changes characterised by significant weight loss, reduced exploratory/working memory, frontal-dependent motor deficits, cognitive decline, memory dysfunction and anxiety during behavioural assessments compared to control. Subsequent analysis showed that oxidative impairment-indicated by depleted superoxide dismutase and lipid peroxidation (related to glutathione-S-transferase activity), cholinergic deficits seen by increased neural acetylcholinesterase (AChE) expression and elevated lactate dehydrogenase underlie behavioural alterations. Furthermore, evidences of proteolysis were seen by reduced Nissl

profiles in neuronal axons and dendrites which correspond to apoptotic changes observed in H&E staining of PFC and hippocampal sections. Interestingly, AA (100mg/kg daily for 15days) significantly attenuated behavioural deficits in rats through inhibition of molecular and cellular stressor proteins activated by AlCl Our results showed that the primary mechanisms underlying AA therapeutic advantages relates closely with its abilities to scavenge free radicals, prevent membrane lipid peroxidation, modulate neuronal bioenergetics, act as AChE inhibitor and through its anti-proteolytic properties. These findings suggest that supplementing endogenous AA capacity through its pharmacological intake may inhibit progression of AD-related neurodegenerative processes and behavioural alterations.
Environ Toxicol Pharmacol, 2017; 50

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FUNCTIONAL ROLE OF AMYOTROPHIC LATERAL SCLEROSIS-ASSOCIATED OPTINEURIN VARIANT IN SH-SY5Y NEURONAL CELLS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Aim: To study the cell death mechanisms in SH-SY5Y neuronal cells expressing wild-type and mutant optineurin protein (OPTN) associated with Amyotrophic Lateral Sclerosis (ALS) and carry out a comparative functional analysis to understand its relative participation in apoptosis, autophagy, necroptosis and inflammation. **Methods:** We performed sanger sequencing for confirming the mutation, MTT assay to measure the cell viability, real-time PCR to measure the mRNA expression, immunoblotting to check the protein expression, Confocal microscopy to check the localisation of all optineurin linked proteins, and pulldown assay to check all binding partners of optineurin. **Results:** The mutation identified in Indian patients is novel and occurs in the ubiquitin-binding domain of OPTN. From immunoblotting, RT-PCR, and confocal microscopy data, we observed that this mutant altered mRNA and protein expression of miR9, REST, CoREST, BDNF, RIPK1, RIPK3 and MLKL in SH-SY5Y neuronal cells. A time-course MTT assay showed an increase in the death of SH-SY5Y cells harbouring the mutant OPTN. **Conclusion:** We found that OPTN mutant causes a notable increase in cell death and is most likely pathogenic. From our study, we observed that this variant of optineurin causes severe cell damage via apoptosis and necroptosis in SH-SY5Y neuronal cells. Our results show that the optineurin novel mutant affects miR9 and BDNF mediated apoptosis, and RIPK1 and MLKL mediated necroptosis in SH-SY5Y cells. ***Corresponding Author:** Prof. James Gomes, Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, New Delhi, India **Acknowledgements:** Indian Institute of Technology Delhi for the infrastructure facility, scholarship and funding.

Pubmed:
31432357: Narain P, Padhi AK, Dave U, Mishra D, Bhatia R, Vivekanandan P, Gomes J
Identification and characterization of novel and rare susceptible variants in Indian amyotrophic lateral sclerosis patients.
Rare missense variants play a crucial role in amyotrophic lateral sclerosis (ALS) pathophysiology. We report rare/novel missense variants from 154 Indian ALS patients, identified through targeted sequencing of 25 ALS-associated genes. As pathogenic variants could explain only a small percentage of ALS pathophysiology in our cohort, we investigated the frequency of tolerated and benign novel/rare variants, which could be potentially ALS susceptible. These variants were identified in 5.36% (8/149) of sporadic ALS (sALS) cases; with one novel variant each in ERBB4, SETX, DCTN1, and MATR3; four rare variants, one each in PON2 and ANG and two different rare variants in SETX. Identified variants were either absent or present at extremely rare frequencies (MAF < 0.01) in large population databases and were absent in 50 healthy controls sequenced through Sanger method. Furthermore, an oligogenic basis of ALS was observed in three sALS, with co-occurrence of intermediate-length repeat expansions in ATXN2 and a rare/novel variant in DCTN1 and SETX genes. Additionally, molecular dynamics and biochemical functional analysis of an angiogenin variant (R21G) identified from our cohort demonstrated loss of ribonucleolytic and nuclear translocation activities. Our findings suggest that rare variants could be potentially pathogenic and functional studies are warranted to decisively establish the pathogenic mechanisms associated with them.
Neurogenetics, 2019; 20

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ROS SCAVENGERS ALLEVIATE INCREASED ROS AND DNA DAMAGE IN ANIMAL MODEL OF ALS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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[1]Ruhr University Bochum, Department Of Cytology, Bochum, Germany, [2]Institute of Biochemistry and Pathobiochemistry, Medical Faculty, Bochum, Germany, [3]Institute of Cell Biology, Cancer Research, Essen, Germany

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, which is characterized by motor neuron degeneration in the central nervous system. Wobbler mice (WR) show similar symptoms and cellular defects as ALS patients and therefore can be used to study ALS *in vivo*. One of these impairments that is commonly seen in ALS is the production of increased levels of reactive oxygen species (ROS). Under pathological conditions increased ROS level and impairments in the cell defense mechanisms can be detrimental, and lead to cell death. In this study, we aim to investigate the underlying mechanisms for increased ROS levels in the WR spinal cord. We demonstrated increased ROS levels in the WR primary motor neurons by using live cell imaging with dihydroethidium *ex vivo*. Additionally, we have shown elevated levels of DNA damage response proteins, 53BP1 and γH2AX, in WR by qPCR and IF analyses. Furthermore, several ROS detoxification molecules were evaluated, of all, glutathione peroxidase 4 was found to be upregulated in WR by qPCR and WB analyses. Concordantly, WR showed reduced total glutathione, in addition to reduced ratio of glutathione-to-glutathione disulfide. Lastly, primary motor neurons were treated with several ROS scavenger molecules and stained for γH2AX to compare the DNA damage. All used ROS scavenger molecules decreased the number of γH2AX spots in WR. Overall, this study suggests that there is an increased ROS production, impairment in the antioxidant system, and DNA damage in the spinal cord tissue of WR.

Pubmed:
33498186: Cihankaya H, Theiss C, Matschke V
Little Helpers or Mean Rogue-Role of Microglia in Animal Models of Amyotrophic Lateral Sclerosis.
Amyotrophic lateral sclerosis (ALS) is one of the most common neurodegenerative diseases, causing degeneration of both upper and lower motor neurons in the central nervous system (CNS). ALS patients suffer from hyperreflexia, spasticity, paralysis and muscle atrophy and typically die due to respiratory failure 1-5 years after disease onset. In addition to the degeneration of motor neurons on the cellular level, ALS has been associated with neuroinflammation, such as microgliosis. Microglial activation in ALS can either be protective or degenerative to the neurons. Among others, mutations in superoxide dismutase 1 (SOD1), chromosome 9 open reading frame 72 (C9Orf72), transactive response DNA binding protein (TDP) 43 and vacuolar protein sorting-associated protein 54 (VPS54) genes have been associated with ALS. Here, we describe the dual role and functionality of microglia in four different in vivo ALS models and search for the lowest common denominator with respect to the role of microglia in the highly heterogeneous disease of ALS.
Int J Mol Sci, 2021; 22
34558526: Cihankaya H, Theiss C, Matschke V
Significance of intercellular communication for neurodegenerative diseases.
Neural Regen Res, 2022; 17
34691359: Stein J, Walkenfort B, Cihankaya H, Hasenberg M, Bader V, Winklhofer KF, Röderer P, Matschke J, Theiss C, Matschke V
Increased ROS-Dependent Fission of Mitochondria Causes Abnormal Morphology of the Cell Powerhouses in a Murine Model of Amyotrophic Lateral Sclerosis.
Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in humans and remains to have a fatal prognosis. Recent studies in animal models and human ALS patients indicate that increased reactive oxygen species (ROS) play an important role in the pathogenesis. Considering previous studies revealing the influence of ROS on mitochondrial physiology, our attention was focused on mitochondria in the murine ALS model, wobbler mouse. The aim of this study was to investigate morphological differences between wild-type and wobbler mitochondria with aid of superresolution structured illumination fluorescence microscopy, TEM, and TEM tomography. To get an insight into mitochondrial dynamics, expression

studies of corresponding proteins were performed. Here, we found significantly smaller and degenerated mitochondria in wobbler motor neurons at a stable stage of the disease. Our data suggest a ROS-regulated, Ox-CaMKII-dependent Drp1 activation leading to disrupted fission-fusion balance, resulting in fragmented mitochondria. These changes are associated with numerous impairments, resulting in an overall self-reinforcing decline of motor neurons. In summary, our study provides common pathomechanisms with other ALS models and human ALS cases confirming mitochondria and related dysfunctions as a therapeutic target for the treatment of ALS.
Oxid Med Cell Longev, 2021; 2021

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BOARD NUMBER: S03-337

INVESTIGATION OF THE FUNCTIONAL INTERACTION BETWEEN TDP-43 AND SMN IN THE GENETIC ZEBRAFISH MODEL OF SMA AND IN PATIENT DERIVED MOTOR NEURONS.

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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The two most prominent motor neuron diseases, **Spinal Muscular Atrophy (SMA)** and **Amyotrophic Lateral Sclerosis (ALS)**, are both linked to defects in RNA-processing factors. SMA, currently the most common genetic cause of infant mortality, results from the loss of function of the **survival motor neuron** (**SMN***)* protein, an essential factor in the assembly of spliceosomes in the nuclear bodies gems and Cajal Bodies. For ALS, an adult onset fatal motor neuron degeneration, the RNA-binding protein **TDP-43** is found in cytoplasmic aggregates of affected regions, representing the pathological hallmark of the disease. TDP-43 and SMN have convergent functions, with both ALS and SMA sharing disrupted spliceosome assembly and perturbed RNA metabolism. In this study we investigate the functional interaction between TDP-43 and SMN in the genetic zebrafish model of SMA and in patient-derived motor neurons. We show that knock-down of the zebrafish orthologue of SMN causes locomotor defects accompanied by spinal motor neuron axonopathy in zebrafish larvae. Overexpression of human TDP-43 significantly improves these features, while a TPD-43 construct bearing the ALS-linked mutation G93A is unable to ameliorate the SMN knock-down phenotype. To test this effect in patient derived cells, we have differentiated motor neurons from SMA patient iPSCs. Motor neurons were analysed with the live cell imaging and analysis system Incucyte, observing an early phenotype consisting of reduced neurite extension in SMA mutant vs. control motor neurons. Currently, this screenable assay is used to further determine the functional interaction between TDP-43 and SMN in a patient-relevant context.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-338

CHARACTERIZATION OF A THERAPEUTIC APPROACH TO TARGET INTRACELLULAR TDP-43 AGGREGATES IN CELLULAR AND ANIMAL MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Aims: Amyotrophic Lateral Sclerosis (ALS) is an incurable progressive motor neuron disease. Two pharmaceutical drugs, Riluzole® and Edaravone®, are available for treatment but exhibit very modest effects on average. Hence, the search for novel, therapeutic alternatives is necessary. One hallmark of ALS is the presence of toxic cytoplasmic aggregates of the protein TDP-43 in ≥97% of patients, rendering this protein a major therapeutic target. Thus, the aim of our study is to develop biotherapeutics targeting TDP-43. **Methods/Results:** Using phage display to screen a library of single-chain variable fragments (scFv) against purified, human, wild-type TDP-43, we identified 4 clones exhibiting TDP-43-specific affinity. Their overexpression/colocalization with TDP-43 and safety in HEK293T cells were confirmed via immunofluorescence and MTT reduction, respectively. Surface plasmon resonance (SPR) suggested high affinity for TDP-43, reflected by a K_D of 5.59 nM. Immunoblots of lysates of co-transfected cells revealed that the scFv decreased the expression and aggregation of TDP-43. Moreover, *in silico* binding prediction revealed relevant, potential binding sites that are known to have roles in the aggregation of TDP-43. We have recently succeeded in conjugating the scFv candidates to PEG-SPION complexes to facilitate their vectorization through the blood-brain-barrier for pre-clinical trials. **Conclusion:** Overall, our results confirm the binding of our scFv's to TDP-43 and suggest a potential role in counteracting TDP-43 pathology. We will further evaluate their effect on processes altered by TDP-43 proteinopathy including energy metabolism, RNA metabolism, and cell viability in ALS patient-derived fibroblasts and a mouse TDP-43 model.

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BOARD NUMBER: S03-339

INCREASED SURFACE P2X4 RECEPTORS BY MUTANT SOD1 PROTEINS CONTRIBUTE TO ALS PATHOGENESIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by the motor neurons death. The aggregation of misfolded proteins such as SOD1 or TDP-43 is the pathological hallmark of ALS and has been associated with neuroinflammation and ultimately, cellular degeneration. P2X4 receptor (P2X4), a non-selective cationic ATP-gated channel is involved in various diseases such as chronic pain and neurodegenerative diseases including ALS. In this study, we observed a surface upregulation of P2X4 in peripheral macrophages of SOD1G93A ALS mouse model (SOD1) at presymptomatic stages and within microglial spinal cord during disease progression. We demonstrated that this pathological increase of surface P2X4 density is due to the interference of misfolded mutant SOD1 or TDP-43 proteins with P2X4 endocytosis machinery. To address the role of P2X4 in ALS pathogenesis, we generate double transgenic SOD1 mice expressing either internalization-defective P2X4mCherryIN knock-in gene (SOD1:P2X4KI) or lacking the P2X4 gene (SOD1:P2X4KO). Our results showed that the absence of P2X4 improves motor performances and survival of SOD1 mice. Interestingly, increased P2X4 surface density has also a positive impact on ALS, a paradoxical output that may be the result of complex time and cell-dependent roles of P2X4. We are currently developing new transgenic mice expressing conditional either knock-in non-internalized P2X4 (cP2X4KI) or knock-out (cP2X4KO) selectively in macrophage/microglia or neurons to unravel the cell-specific function of P2X4 in ALS pathogenesis of SOD1 mice. Overall, this work will determine whether manipulating P2X4 is a promising therapeutic strategy for fighting against ALS.

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NANOSTRING MOLECULAR BARCODING OF PATIENT TISSUE IDENTIFIES MOLECULAR SIGNATURES OF HETEROGENEITY IN C9ORF72-ALS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Aims: Amyotrophic lateral sclerosis (ALS) exists on a pathogenetic disease spectrum with frontotemporal dementia (FTD), with patients sometimes experiencing elements of both conditions (ALS-FTSD). For mutations associated with ALS-FTSD, such as the C9orf72 hexanucleotide repeat expansion, the factors influencing where an individual may lie on this spectrum require further characterisation. As such, our study aimed to investigate molecular signatures underlying heterogeneity in C9orf72-associated disease. Methods: Using digital pathology analysis and random forest modelling we have previously shown that microglial staining is predictive of C9orf72-ALS disease status, and that microglial activation is elevated in the language region (BA39) for language-impaired cases. Here we used NanoString molecular barcoding with a panel of 770 neuroinflammatory genes to interrogate this dysregulation at the level of gene expression. Validation was performed using BaseScope(TM) *in situ* hybridisation and immunohistochemistry. Results: We identified 21 top hits for dysregulated neuroinflammatory genes in the motor cortex, with enrichment of microglial and inflammatory response gene sets in disease. Our analyses also revealed two distinct C9orf72-ALS subtypes, with inflammatory signatures that segregate with glial TDP-43 burden and language impairment. Conclusions: These data imply that distinct molecular signatures can be detected within well curated and deeply clinically phenotyped cohorts that could hold promise for future targeted therapies.

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BOARD NUMBER: S03-341

BODY COMPLEXION AND CIRCULATING LIPIDS IN THE RISK OF FRONTOTEMPORAL DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Frontotemporal dementia (FTD) and Amyotrophic lateral sclerosis (ALS) are neurodegenerative diseases that seems to be the two ends of a spectrum due to clinical and genetic overlap. Nearly 50% of FTD and 98% of all ALS patients have histopathological TDP-43 aggregates. In this work we aim to evaluate whether different body lipid metabolic traits are causally associated with the risk of FTD TDP43 subtype, and compare it to their causal role in the risk of ALS, and identify genetic variants shared between these two TDP-43 related disorders in relation to lipid metabolic traits. Two-sample mendelian randomization analyses (2SMR) were performed to evaluate the causal association of 9 body complexion and 9 circulating lipids traits with the risk of FTD TDP-43 subtype, and the risk of ALS. The inverse-variance weighted method (IVW) was the primary analysis followed by secondary sensitive analyses. Genetically increased trunk predicted mass and fat-free mass were suggestively associated with the risk of FTD TDP-43 subtype. We corroborated that genetically predicted high levels of LDL cholesterol are causally associated with higher risk of ALS, and found suggestive causal associations with other circulating fatty acids. This work provides evidence that body complexion and circulating lipids traits impact differentially on the risk of FTD TDP-43 subtype and ALS, advocating for new and specific interventional approaches in the control of body lipid metabolism for FTD and ALS.

Pubmed:
34502460: Fernández-Beltrán LC, Godoy-Corchuelo JM, Losa-Fontangordo M, Williams D, Matias-Guiu J, Corrochano S
A Transcriptomic Meta-Analysis Shows Lipid Metabolism Dysregulation as an Early Pathological Mechanism in the Spinal Cord of SOD1 Mice.
Amyotrophic lateral sclerosis (ALS) is a multifactorial and complex fatal degenerative disorder. A number of pathological mechanisms that lead to motor neuron death have been identified, although there are many unknowns in the disease aetiology of ALS. Alterations in lipid metabolism are well documented in the progression of ALS, both at the systemic level and in the spinal cord of mouse models and ALS patients. The origin of these lipid alterations remains unclear. This study aims to identify early lipid metabolic pathways altered before systemic metabolic symptoms in the spinal cord of mouse models of ALS. To do this, we performed a transcriptomic analysis of the spinal cord of mice at an early disease stage, followed by a robust transcriptomic meta-analysis using publicly available RNA-seq data from the spinal cord of SOD1 mice at early and late symptomatic disease stages. The meta-analyses identified few lipid metabolic pathways dysregulated early that were exacerbated at symptomatic stages; mainly cholesterol biosynthesis, ceramide catabolism, and eicosanoid synthesis pathways. We present an insight into the pathological mechanisms in ALS, confirming that lipid metabolic alterations are transcriptionally dysregulated and are central to ALS aetiology, opening new options for the treatment of these devastating conditions.
Int J Mol Sci, 2021; 22

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-342

LIPIDS AS A NOVEL BIOMARKER FOR AMYOTROPHIC LATERAL SCLEROSIS - EVIDENCE FROM THE SOD1 MOUSE.

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Diagnosis of Amyotrophic lateral sclerosis (ALS) can be a lengthy process, mainly due to the lack of accurate biomarkers that are easily accessible. To date, blood-based biomarkers lack specificity and are unable to distinguish MND from other neurodegenerative disorders. It has increasingly been acknowledged that lipid metabolism plays a crucial role in ALS. Therefore, the aim of this study is to discover novel biomarkers that are specific to ALS, based on the blood lipidome of mutant SOD1 mice. We performed targeted lipidomic analysis on plasma of $SOD1^{G93A}$ and wildtype littermates at metabolomics Australia. Analysis was conducted pre-symptomatically at postnatal day (P) 60, symptomatic – P90 and end stage ~P150. Further, we treated a separate group of mice with ambroxol, an inhibitor of a key enzyme in the glycosphingolipid pathway and a novel candidate for treating MND symptoms. Treatment started at P60 and blood was collected at P90 and end stage. We discovered a distinct lipid dysregulation found in the blood of $SOD1^{G93A}$ mice compared to their wildtype littermates at P60. Next, we sought how lipids change with disease progression. Here, we compared targeted lipidomics of P60, P90 and ~P150 $SOD1^{G93A}$ mice. We found that lipids have a dynamic abundance, changing with disease progression. Lastly, we show that ambroxol can significantly restructure lipid composition, possibly explaining extension in lifespan of mutant SOD1 mice. Together, we are the first to show that blood lipids have a strong potential to function as reliable biomarkers, which may improve diagnostic interval in ALS.

Pubmed:
34857917: Wang T, Tomas D, Perera ND, Cuic B, Luikinga S, Viden A, Barton SK, McLean CA, Samson AL, Southon A, Bush AI, Murphy JM, Turner BJ
Ferroptosis mediates selective motor neuron death in amyotrophic lateral sclerosis.
Amyotrophic lateral sclerosis (ALS) is caused by selective degeneration of motor neurons in the brain and spinal cord; however, the primary cell death pathway(s) mediating motor neuron demise remain elusive. We recently established that necroptosis, an inflammatory form of regulated cell death, was dispensable for motor neuron death in a mouse model of ALS, implicating other forms of cell death. Here, we confirm these findings in ALS patients, showing a lack of expression of key necroptotic effector proteins in spinal cords. Rather, we uncover evidence for ferroptosis, a recently discovered iron-dependent form of regulated cell death, in ALS. Depletion of glutathione peroxidase 4 (GPX4), an anti-oxidant enzyme and central repressor of ferroptosis, occurred in post-mortem spinal cords of both sporadic and familial ALS patients. GPX4 depletion was also an early and universal feature of spinal cords and brains of transgenic mutant superoxide dismutase 1 (SOD1), TDP-43 and C9orf72 mouse models of ALS. GPX4 depletion and ferroptosis were linked to impaired NRF2 signalling and dysregulation of glutathione synthesis and iron-binding proteins. Novel BAC transgenic mice overexpressing human GPX4 exhibited high GPX4 expression localised to spinal motor neurons. Human GPX4 overexpression in SOD1 mice significantly delayed disease onset, improved locomotor function and prolonged lifespan, which was attributed to attenuated lipid peroxidation and motor neuron preservation. Our study discovers a new role for ferroptosis in mediating motor neuron death in ALS, supporting the use of anti-ferroptotic therapeutic strategies, such as GPX4 pathway induction and upregulation, for ALS treatment.
Cell Death Differ, 2022; 29
29128447: Luikinga SJ, Kim JH, Perry CJ
Developmental perspectives on methamphetamine abuse: Exploring adolescent vulnerabilities on brain and behavior.
Most people that experience illicit drugs do so for the first time during adolescence, and methamphetamine (meth) is no exception. Therefore, research into the effects of meth should highlight the adolescent period. Despite this, the vast majority of current literature has mainly focused on meth exposure during adulthood. In this review, we first describe existing literature that compares the behavioral effects of meth where exposure occurs in adolescence compared to adulthood. Given that there

are actually very few such studies, we also look at what is known about neural effects of meth in the adult brain, and relate these to normal neural development occurring during the adolescent period to establish how meth may target maturing regions and related neurochemistry. What emerges overall is that adolescents appear to be more vulnerable to the rewarding and reinforcing effects of meth, and that meth indeed has effects on areas that are in flux during adolescence. However, there is some evidence for a paradoxical resistance to the neurotoxic effects during this period. We highlight the need for further age-related research to better understand, treat, and prevent meth use disorders and addiction in general.
Prog Neuropsychopharmacol Biol Psychiatry, 2018; 87

33798740: Perera ND, Tomas D, Wanniarachchillage N, Cuic B, Luikinga SJ, Rytova V, Turner BJ
Stimulation of mTOR-independent autophagy and mitophagy by rilmenidine exacerbates the phenotype of transgenic TDP-43 mice.
Autophagy, which mediates the delivery of cytoplasmic substrates to the lysosome for degradation, is essential for maintaining proper cell homeostasis in physiology, ageing, and disease. There is increasing evidence that autophagy is defective in neurodegenerative disorders, including motor neurons affected in amyotrophic lateral sclerosis (ALS). Restoring impaired autophagy in motor neurons may therefore represent a rational approach for ALS. Here, we demonstrate autophagy impairment in spinal cords of mice expressing mutant TDP-43 or co-expressing TDP-43 transgenes. The clinically approved anti-hypertensive drug rilmenidine was used to stimulate mTOR-independent autophagy in double transgenic TDP-43 mice to alleviate impaired autophagy. Although rilmenidine treatment induced robust autophagy in spinal cords, this exacerbated the phenotype of TDP-43 mice, shown by truncated lifespan, accelerated motor neuron loss, and pronounced nuclear TDP-43 clearance. Importantly, rilmenidine significantly promoted mitophagy in spinal cords TDP-43 mice, evidenced by reduced mitochondrial markers and load in spinal motor neurons. These results suggest that autophagy induction accelerates the phenotype of this TDP-43 mouse model of ALS, most likely through excessive mitochondrial clearance in motor neurons. These findings also emphasise the importance of balancing autophagy stimulation with the potential negative consequences of hyperactive mitophagy in ALS and other neurodegenerative diseases.
Neurobiol Dis, 2021; 154

32666555: Guerin AA, Zbukvic IC, Luikinga SJ, Drummond KD, Lawrence AJ, Madsen HB, Kim JH
Extinction and drug-induced reinstatement of cocaine seeking following self-administration or conditioned place preference in adolescent and adult rats.
Adolescence marks a particularly vulnerable period to developing substance use disorders, and people who start using drugs in adolescence are more likely to relapse. A limited number of studies have investigated age difference in relapse following re-exposure to the drug after a period of abstinence. Using a cocaine self-administration paradigm, we showed no age difference in acquisition or extinction of self-administration. Interestingly, adolescent rats displayed impaired cocaine-primed reinstatement of cocaine seeking. Using the same dose as that self-administered in the first experiment, we then investigated age differences in acquisition and extinction of conditioned place preference, as well as locomotor sensitization. While there were no differences in locomotor activity or acquisition of preference, adolescents failed to extinguish their preference, even when the number of extinction sessions was doubled from what adults received. Taken together, these results suggest that while cocaine has similar rewarding and reinforcing effects regardless of age, adolescents may attribute stronger salience to the drug-associated context. In addition, re-exposure to cocaine itself may not be a strong relapse trigger in adolescence. Overall, these findings suggest that we should focus more on alleviating drug-context salience compared to re-exposure to substance in order to reduce relapse of drug seeking in adolescents.
Dev Psychobiol, 2021; 63

31338719: Luikinga SJ, Perry CJ, Madsen HB, Lawrence AJ, Kim JH
Effects of Methamphetamine Exposure on Fear Learning and Memory in Adult and Adolescent Rats.
Methamphetamine (meth) use is often comorbid with anxiety disorders, with both conditions predominant during adolescence. Conditioned fear extinction is the most widely used model to study the fear learning and regulation that are relevant for anxiety disorders. The present study investigates how meth binge injections or meth self-administration affect subsequent fear conditioning, extinction and retrieval in adult and adolescent rats. In experiment 1, postnatal day 35 (P35-adolescent) and P70 (adult) rats were intraperitoneally injected with increasing doses of meth across 9 days. At P50 or P85, they underwent fear conditioning followed by extinction and test. In experiments 2a-c, P35 or P70 rats self-administered meth for 11 days then received fear conditioning at P50 or P85, followed by extinction and test. We observed that meth binge exposure caused a significant disruption of extinction retrieval in adult but not adolescent rats. Interestingly, meth self-administration in adolescence or adulthood disrupted acquisition of conditioned freezing in adulthood. Meth self-administration in adolescence did not affect conditioned freezing in adolescence. These results suggest that intraperitoneal injections of high doses of meth and meth self-administration have dissociated effects on fear conditioning and extinction during adulthood, while adolescent fear conditioning and extinction are unaffected.
Neurochem Res, 2019; 44

28536511: Ganella DE, Lee-Kardashyan L, Luikinga SJ, Nguyen DLD, Madsen HB, Zbukvic IC, Coulthard R, Lawrence AJ, Kim JH
Aripiprazole Facilitates Extinction of Conditioned Fear in Adolescent Rats.
Anxiety disorders are the most common type of mental disorder during adolescence, which is at least partly due to the resistance to extinction exhibited at this age. The dopaminergic system is known to be dysregulated during adolescence; therefore, we aimed to facilitate extinction in adolescent rats using the dopamine receptor 2 partial agonist aripiprazole (Abilify™), and examine the behavioral and neural outcomes. Adolescent rats were conditioned to fear a tone. The next day, rats received extinction 30 min after a systemic injection of either 5 mg/kg aripiprazole or vehicle, and then were tested the following day. For the immunohistochemistry experiment, naïve and "no extinction" conditions were added and rats were perfused either on the extinction day or test day. To assess the activation of neurons receiving dopaminergic input, c-Fos, and dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32) labeled neurons were quantified in the amygdala and the medial prefrontal cortex (mPFC). Systemic treatment with aripiprazole at the time of extinction significantly reduced freezing at test the next day. This effect was not observed in rats that were fear conditioned but did not receive any extinction. Aripiprazole's facilitation of extinction was accompanied by increased activation of neurons in the mPFC. Taken together, aripiprazole represents a novel pharmacological adjunct to exposure therapy worthy of further examination. The effect of aripiprazole is related to enhanced activation of mPFC neurons receiving dopaminergic innervation.
Front Behav Neurosci, 2017; 11
31819151: Charlton AJ, May C, Luikinga SJ, Burrows EL, Hyun Kim J, Lawrence AJ, Perry CJ
Chronic voluntary alcohol consumption causes persistent cognitive deficits and cortical cell loss in a rodent model.
Chronic alcohol use is associated with cognitive decline that impedes behavioral change during rehabilitation. Despite this, addiction therapy does not address cognitive deficits, and there is poor understanding regarding the mechanisms that underlie this decline. We established a rodent model of chronic voluntary alcohol use to measure ensuing cognitive effects and underlying pathology. Rats had intermittent access to alcohol or an isocaloric solution in their home cage under voluntary 2-bottle choice conditions. In Experiments 1 and 2 cognition was assessed using operant touchscreen chambers. We examined performance in a visual discrimination and reversal task (Experiment 1), and a 5-choice serial reaction time task (Experiment 2). For Experiment 3, rats were perfused immediately after cessation of alcohol access period, and volume, cell density and microglial populations were assessed in the prefrontal cortex and striatum. Volume was assessed using the Cavalieri probe, while cell and microglial counts were estimated using unbiased stereology with an optical fractionator. Alcohol-exposed and control rats showed comparable acquisition of pairwise discrimination; however, performance was impaired when contingencies were reversed indicating reduced behavioral flexibility. When tested in a 5-choice serial reaction time task alcohol-exposed rats showed increased compulsivity and increased attentional bias towards a reward associated cue. Consistent with these changes, we observed decreased cell density in the prefrontal cortex. These findings confirm a detrimental effect of chronic alcohol and establish a model of alcohol-induced cognitive decline following long-term voluntary intake that may be used for future intervention studies.
Sci Rep, 2019; 9
27614140: Madsen HB, Zbukvic IC, Luikinga SJ, Lawrence AJ, Kim JH
Extinction of conditioned cues attenuates incubation of cocaine craving in adolescent and adult rats.
Relapse to drug use is often precipitated by exposure to drug associated cues that evoke craving. Cue-induced drug craving has been observed in both animals and humans to increase over the first few weeks of abstinence and remain high over extended periods, a phenomenon known as 'incubation of craving'. As adolescence represents a period of vulnerability to developing drug addiction, potentially due to persistent reactivity to drug associated cues, we first compared incubation of cocaine craving in adolescent and adult rats. Adolescent (P35) and adult (P70) rats were trained to lever press to obtain intravenous cocaine, with each drug delivery accompanied by a light cue that served as the conditioned stimulus (CS). Following acquisition of stable responding, rats were tested for cue-induced cocaine-seeking after either 1 or 30days of abstinence. Additional groups of rats were also tested after 30days of abstinence, however these rats were subjected to a cue extinction session 1week into the abstinence period. Rats were injected with aripiprazole, a dopamine 2 receptor (D2R)-like partial agonist, or vehicle, 30min prior to cue extinction. We found that adolescent and adult rats acquired and maintained a similar level of cocaine self-administration, and rats of both ages exhibited a higher level of cue-induced cocaine-seeking if they were tested after 30days of abstinence compared to 1day. Incubation of cocaine craving was significantly reduced to 1day levels in both adults and adolescents that received cue extinction training. Administration of aripiprazole prior to cue extinction did not further reduce cue-induced drug-seeking. These results indicate that cue extinction training during abstinence may effectively reduce cue-induced relapse at a time when cue-induced drug craving is usually high.
Neurobiol Learn Mem, 2017; 143
24712397: Kim JH, Perry C, Luikinga S, Zbukvic I, Brown RM, Lawrence AJ
Extinction of a cocaine-taking context that protects against drug-primed reinstatement is dependent on the metabotropic

glutamate 5 receptor.
We investigated the effects of extinguishing action-reward versus context-reward associations on drug-primed reinstatement, and the potential role of the metabotropic glutamate 5 receptor (mGlu5) in these different types of extinction in rats that self-administer cocaine. We observed that daily context extinction (non-reinforced exposures to the cocaine-taking context with retracted levers) was just as effective as daily lever extinction in reducing cocaine-primed reinstatement compared with passive abstinence. Additionally, systemic injections of the mGlu5 negative allosteric modulator MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine) following each extinction session significantly impaired the ability of context extinction to reduce cocaine-primed reinstatement, without affecting reinstatement after lever extinction or passive abstinence.
Addict Biol, 2015; 20
23894375: Seemann I, Te Poele JA, Luikinga SJ, Hoving S, Stewart FA
Endoglin haplo-insufficiency modifies the inflammatory response in irradiated mouse hearts without affecting structural and mircovascular changes.
It is now widely recognized that radiotherapy of thoracic and chest wall tumors increases the long-term risk of cardiovascular damage although the underlying mechanisms are not fully elucidated. There is increasing evidence that microvascular damage is involved. Endoglin, an accessory receptor for TGF-β1, is highly expressed in damaged endothelial cells and may play a crucial role in cell proliferation and revascularization of damaged heart tissue. We have therefore specifically examined the role of endoglin in microvascular damage and repair in the irradiated heart.
PLoS One, 2013; 8

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BOARD NUMBER: S03-343

THE MOTONEURONAL RECEPTOROME IN ALS REVEALS ADRENERGIC ENTRY POINTS TO MODULATE MN EXCITABILITY AND FIRING

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Modulation of motoneuron (MN) excitability and synaptic excitation constitutes an important entry point to affect MN degeneration in several MN diseases. We have previously demonstrated that chemogenetic interventions on excitability and PKA signaling exert beneficial effects on synaptic integrity and disease burden in ALS MN. To achieve a similar upregulation of PKA signaling and MN firing through natural receptor, we explored the PKA-coupled motoneuronal receptorome in ALS. Among the receptors prioritized by screening available databases (Allen Spinal Cord Atlans, GPCR database), adenosinergic, histaminergic, cholinergic and several peptidergic receptors are downregulated in G93A SOD1 mice, whereas beta-1 adrenergic receptor is distinctively upregulated and the expression of dopaminergic D5 and beta-2 and beta-3 adrenergic receptors are preserved. Importantly, in vivo intracellular recordings of MN in G93A SOD1 mice show that acute activation of Dopaminergic D5 and beta2/3 adrenergic receptors by selective agonists increase MN excitability, through a decreased recruitment current for D5 agonist, and through an increased gain of the frequency-current relationship for beta2/3 agonists, indicating a role for D5 and beta2/3 receptors in the regulation of MN excitability in ALS. In addition, intracellular iontophoretic injection of cAMP-sP increases the gain of the frequency-current relationship, suggesting that beta2/3 receptors increase MN excitability through the PKA pathway. The ALS MN receptorome is nevertheless highly dynamic and all studied receptors are downregulated in advanced stages of disease. Our data show that MN display extensive entry points for modulation of their electrophysiological properties, which can be accessed with small molecules with translational potential.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-344

ASSESSING THE ROLE OF CORTICAL NETWORK DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS THROUGH CHEMOGENETIC SILENCING OF THE CORTICOSPINAL NEURONS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) arises from the combined degeneration of both corticospinal neurons (CSNs) and motorneurons (MNs). Transcranial magnetic stimulation studies revealed that ALS patients display early cortical hyperexcitability, while various ALS mouse models display altered excitability of cortical excitatory neurons, including CSNs and inhibitory interneurons, from development to adulthood. “Designer Receptors Exclusively Activated by Designer Drugs” (DREADD)-mediated inhibition of Parvalbumin-positive cortical interneurons was demonstrated as beneficial in a mouse model of ALS, while DREADD-mediated activation of CSNs was deleterious to MNs in wildtype mice. We recently demonstrated that in the context of ALS, CSNs are toxic to their downstream targets. Thus, we hypothesize that this toxicity may result from their adaptation to their hyperexcitable environment leading to their aberrant neuronal activity and deleterious impacts on their spinal targets. To test this hypothesis, we aimed to evaluate the consequences of CSN silencing on disease onset and progression in a mouse model of ALS. We crossed Sod1G86R mice with CrymCreERT2 mice that selectively express Cre in layer V cortical neurons, for selective expression of the hM4Di DREADD silencing receptor, or control reporter gene upon AAV injection in the motor cortex. Four groups were generated (Sod1G86R or control, injected either with hM4Di or control), chronically treated with Clozapine-N-Oxide (CNO), the DREADD ligand, in drinking water from the pre-symptomatic ages of 30 or 60 days and are currently under evaluation using adapted clinical and motor tests. The study intends to evaluate whether cortical hyperexcitability could represent a new therapeutic target in ALS.

Pubmed:

27717098: Maduna T, Lelievre V
Neuropeptides shaping the central nervous system development: Spatiotemporal actions of VIP and PACAP through complementary signaling pathways.
Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) are neuropeptides with wide, complementary, and overlapping distributions in the central and peripheral nervous systems, where they exert important regulatory roles in many physiological processes. VIP and PACAP display a large range of biological cellular targets and functions in the adult nervous system including regulation of neurotransmission and neuroendocrine secretion and neuroprotective and neuroimmune responses. As the main focus of the present review, VIP and PACAP also have been long implicated in nervous system development and maturation through their interaction with the seven transmembrane domain G protein-coupled receptors, PAC1, VPAC1, and VPAC2, initiating multiple signaling pathways. Compared with PAC1, which solely binds PACAP with very high affinity, VPACs exhibit high affinities for both VIP and PACAP but differ from each other because of their pharmacological profile for both natural accessory peptides and synthetic or chimeric molecules, with agonistic and antagonistic properties. Complementary to initial pharmacological studies, transgenic animals lacking these neuropeptides or their receptors have been used to further characterize the neuroanatomical, electrophysiological, and behavioral roles of PACAP and VIP in the developing central nervous system. In this review, we recapitulate the critical steps and processes guiding/driving neurodevelopment in vertebrates and superimposing the potential contribution of PACAP and VIP receptors on the given timeline. We also describe how alterations in VIP/PACAP signaling may contribute to both (neuro)developmental and adult pathologies and suggest that tuning of VIP/PACAP signaling in a spatiotemporal manner may represent a novel avenue for preventive therapies of neurological and psychiatric disorders. © 2016 Wiley Periodicals, Inc.
J Neurosci Res, 2016; 94
32154159: Gabel F, Aubry AS, Hovhannisyan V, Chavant V, Weinsanto I, Maduna T, Darbon P, Goumon Y
Unveiling the Impact of Morphine on Tamoxifen Metabolism in Mice .
Tamoxifen is used to treat breast cancer and cancer recurrences. After administration, tamoxifen is converted into two more potent antitumor compounds, 4OH-tamoxifen and endoxifen by the CYP3A4/5 and 2D6 enzymes in human. These active

compounds are inactivated by the same UDP-glucuronosyltransferase isoforms as those involved in the metabolism of morphine. Importantly, cancer-associated pain can be treated with morphine, and the common metabolic pathway of morphine and tamoxifen suggests potential clinically relevant interactions. Mouse liver microsomes were used to determine the impact of morphine on 4OH-tamoxifen metabolism . For experiments, female mice were first injected with tamoxifen alone and then with tamoxifen and morphine. Blood was collected, and LC-MS/MS was used to quantify tamoxifen, 4OH-tamoxifen, N-desmethyltamoxifen, endoxifen, 4OH-tamoxifen-glucuronide, and endoxifen-glucuronide. , we found increased values for the production of 4OH-tamoxifen-glucuronide in the presence of morphine, suggesting an inhibitory effect on 4OH-tamoxifen glucuronidation. Conversely, morphine treatment decreased 4OH-tamoxifen levels in the blood while dramatically increasing the formation of inactive metabolites 4OH-tamoxifen-glucuronide and endoxifen-glucuronide. Our findings emphasize the need for caution when extrapolating results from metabolic assays to drug metabolism interactions. Importantly, morphine strongly impacts tamoxifen metabolism in mice. It suggests that tamoxifen efficiency could be reduced when both drugs are co-administered in a clinical setting, e.g., to relieve pain in breast cancer patients. Further studies are needed to assess the potential for tamoxifen-morphine metabolic interactions in humans.
Front Oncol, 2020; 10

32253777: Reiss D, Maduna T, Maurin H, Audouard E, Gaveriaux-Ruff C
Mu opioid receptor in microglia contributes to morphine analgesic tolerance, hyperalgesia, and withdrawal in mice.
A major challenge in medicine is developing potent pain therapies without the adverse effects of opiates. Neuroinflammation and in particular microglial activation have been shown to contribute to these effects. However, the implication of the microglial mu opioid receptor (MOR) is not known. We developed a novel conditional knockout (cKO) mouse line, wherein MOR is deleted in microglia. Morphine analgesic tolerance was delayed in both sexes in cKO mice in the hot plate assay. Opioid-induced hyperalgesia (OIH) as measured in the tail immersion assay was abolished in male cKO mice, and physical dependence to morphine as assessed by naloxone-induced withdrawal was attenuated in female cKO mice. Our results show a sex-dependent contribution of microglial MOR in morphine analgesic tolerance, OIH, and physical dependence. In conclusion, our data suggest that blockade of microglial MOR could represent a therapeutic target for opiate analgesia without the opiate adverse effects.
J Neurosci Res, 2022; 100

29353538: Weinsanto I, Mouheiche J, Laux-Biehlmann A, Aouad M, Maduna T, Petit-Demoulière N, Chavant V, Poisbeau P, Darbon P, Charlet A, Giersch A, Parat MO, Goumon Y
Lithium reverses mechanical allodynia through a mu opioid-dependent mechanism.
Background Lithium is widely used to treat bipolar disorders and displays mood stabilizing properties. In addition, lithium relieves painful cluster headaches and has a strong analgesic effect in neuropathic pain rat models. Objectives To investigate the analgesic effect of lithium on the cuff model of neuropathic pain. Methods We used behavioral and pharmacological approaches to study the analgesic effect of a single injection of lithium in wild-type and mu opioid receptor (MOR) null cuffed neuropathic mice. Mass spectrometry and enzyme-linked immunosorbent assay allowed to measure the levels of endogenous MOR agonist beta-endorphin as well as monoamines in brain and plasma samples 4 h after lithium administration. Results A single injection of lithium chloride (100 mg/kg, ip) alleviated mechanical allodynia for 24 h, and this effect was absent in MOR null neuropathic mice. Biochemical analyses highlight a significant increase in beta-endorphin levels by 30% in the brain of lithium-treated mice compared to controls. No variation of beta-endorphin was detected in the blood. Conclusions Together, our results provide evidence that lithium induces a long-lasting analgesia in neuropathic mice presumably through elevated brain levels of beta-endorphin and the activation of MORs.
Mol Pain, 2018 Jan-Dec; 14

30662412: Maduna T, Audouard E, Dembélé D, Mouzaoui N, Reiss D, Massotte D, Gaveriaux-Ruff C
Microglia Express Mu Opioid Receptor: Insights From Transcriptomics and Fluorescent Reporter Mice.
Microglia activation contributes to chronic pain and to the adverse effects of opiate use such as analgesic tolerance and opioid-induced hyperalgesia. Both mu opioid receptor (MOR) encoded by gene and toll like receptor 4 (TLR4) have been reported to mediate these morphine effects and a current question is whether microglia express the Oprm1 transcript and MOR protein. The aim of this study was to characterize -MOR expression in naive murine and human microglia, combining transcriptomics datasets previously published by other groups with our own imaging study using the Cx3cr1-eGFP-MOR-mCherry reporter mouse line. We analyzed microglial expression obtained from transcriptomics datasets, focusing on studies from adult wild-type animals and adult post-mortem human cerebral cortex. , as well as co-regulated gene sets were examined. The expression of MOR in microglia was also investigated using our novel fluorescent Cx3cr1-eGFP-MOR-mcherry reporter mouse line. We determined whether CX3cR1-eGFP positive microglial cells expressed MOR-mCherry protein by imaging various brain areas including the Frontal Cortex, Nucleus Accumbens, Ventral Tegmental Area, Central Amygdala, and Periaqueductal Gray matter, as well as spinal cord. expression was found in all 12 microglia datasets from mouse whole brain, in 7 out of 8 from cerebral cortex, 3 out of 4 from hippocampus, 1 out of 1 from striatum, and 4 out of 5

from mouse or rat spinal cord. was expressed in 16 out of 17 microglia transcriptomes from human cerebral cortex. In Cx3cr1-eGFP-MOR-mCherry mice, the percentage of MOR-positive microglial cells ranged between 35.4 and 51.6% in the different brain areas, and between 36.8 and 42.4% in the spinal cord. The comparative analysis of the microglia transcriptomes indicates that transcripts are expressed in microglia. The investigation of Cx3cr1-eGFP-MOR-mCherry mice also shows microglial expression of MOR proteinin the brain and spine. These results corroborate functional studies showing the actions of MOR agonists on microglia and suppression of these effects by MOR-selective antagonists or MOR knockdown.
Front Psychiatry, 2018; 9
30051501: Weinsanto I, Laux-Biehlmann A, Mouheiche J, Maduna T, Delalande F, Chavant V, Gabel F, Darbon P, Charlet A, Poisbeau P, Lamshöft M, Van Dorsselaer A, Cianferani S, Parat MO, Goumon Y
Stable isotope-labelled morphine to study in vivo central and peripheral morphine glucuronidation and brain transport in tolerant mice.
Chronic administration of medication can significantly affect metabolic enzymes leading to physiological adaptations. Morphine metabolism in the liver has been extensively studied following acute morphine treatment, but such metabolic processes in the CNS are poorly characterized. Long-term morphine treatment is limited by the development of tolerance, resulting in a decrease of its analgesic effect. Whether or not morphine analgesic tolerance affects in vivo brain morphine metabolism and blood-brain barrier (BBB) permeability remains a major question. Here, we have attempted to characterize the in vivo metabolism and BBB permeability of morphine after long-term treatment, at both central and peripheral levels.
Br J Pharmacol, 2018; 175

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-345

DYSREGULATION OF SPINAL INTERNEURON SUBPOPULATIONS IN THE SOD1G93A ALS MOUSE MODEL

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

Roser Montañana-Rosell[1], Raghavendra Selvan[2], Pablo Hernández-Varas[3], Dana Ahlmark[1], Jan Kaminski[4], Ole Kiehn[1], Ilary Allodi[1]
[1]University of Copenhagen, Department Of Neuroscience, Copenhagen N, Denmark, [2]University of Copenhagen, Department Of Computer Science & Department Of Neuroscience, Copenhagen N, Denmark, [3]University of Copenhagen, Core Facility For Integrated Microscopy, Copenhagen N, Denmark, [4]University of Copenhagen, Department Of Computer Science, Copenhagen Ø, Denmark

ALS is a neurodegenerative disease characterized by the progressive loss of motor neurons and premature death. Given their central role in the disease, major focus has been directed to motor neuron intrinsic properties as a cause for degeneration. However, our previous data demonstrated that V1 inhibitory interneurons degenerate and lose their connectivity to vulnerable motor neurons, possibly playing a role in disease initiation. V1 interneurons are a heterogeneous population which can be identified by specific marker co-expression. Using *in situ* multiplexing techniques, we have performed a comprehensive study on the expression of several markers in the $SOD1^{G93A}$ mouse model with the aim to elucidate the contribution of specific interneuron subpopulations to ALS pathology. We first validated the expression of 24 interneuron markers, typically described at embryonic stages, in adult mouse tissue by *in situ* sequencing. Then, we quantified transcript expression by RNAscope HiPlexUp, an *in situ* hybridization quantitative technique allowing for co-detection of multiple transcripts, at three different timepoints. Our data show downregulation of different V1 interneuron clade-defining transcription factors starting at early stages of disease (from day 63) and preceding motor neuron degeneration. Notable is the early dysregulation of V1 Renshaw cells, defined by Calbindin 1. Additionally, we investigated other interneuron populations. Here, we describe substantial downregulation of the Chx10 transcription factor, marker for the excitatory V2a interneuron subpopulation, at later stages (from day 112). Overall, our study evidences a pronounced dysregulation of interneuron motor networks throughout ALS progression and identifies several potential sources for non-autonomous motor neuron degeneration.

Pubmed:
34059686: Allodi I, Montañana-Rosell R, Selvan R, Löw P, Kiehn O
Locomotor deficits in a mouse model of ALS are paralleled by loss of V1-interneuron connections onto fast motor neurons.
ALS is characterized by progressive inability to execute movements. Motor neurons innervating fast-twitch muscle-fibers preferentially degenerate. The reason for this differential vulnerability and its consequences on motor output is not known. Here, we uncover that fast motor neurons receive stronger inhibitory synaptic inputs than slow motor neurons, and disease progression in the SOD1 mouse model leads to specific loss of inhibitory synapses onto fast motor neurons. Inhibitory V1 interneurons show similar innervation pattern and loss of synapses. Moreover, from postnatal day 63, there is a loss of V1 interneurons in the SOD1 mouse. The V1 interneuron degeneration appears before motor neuron death and is paralleled by the development of a specific locomotor deficit affecting speed and limb coordination. This distinct ALS-induced locomotor deficit is phenocopied in wild-type mice but not in SOD1 mice after appearing of the locomotor phenotype when V1 spinal interneurons are silenced. Our study identifies a potential source of non-autonomous motor neuronal vulnerability in ALS and links ALS-induced changes in locomotor phenotype to inhibitory V1-interneurons.
Nat Commun, 2021; 12
34090131: Khorooshi R, Marczynska J, Dubik M, Dieu RS, Sørensen SF, Montanana-Rosell R, Limburg HL, Tygesen C, Asgari N, Steckelings UM, Owens T
The protective effect of Angiotensin AT2-receptor stimulation in Neuromyelitis optica spectrum disorder is independent of astrocyte-derived BDNF.
Neuromyelitis optica spectrum disorder (NMOSD) is an antibody-mediated autoimmune inflammatory disease of the central nervous system (CNS), resulting in primary astrocytopathy. We have previously shown that Angiotensin AT2-receptor (AT2R) stimulation with the specific agonist Compound 21 (C21) attenuated NMOSD-like pathology. Recent studies have proposed that the mechanism behind protective effects of AT2R includes induction of brain derived neurotrophic factor (BDNF).

Astrocytes are a major cellular source of BDNF. In this study we used mice with conditional BDNF deficiency in astrocytes (GfapF) to examine the involvement of astrocyte-derived BDNF in NMOSD-like pathology and in mediating the protective effect of AT2R stimulation.
Mult Scler Relat Disord, 2021; 53
31287367: Khorooshi R, Tofte-Hansen EU, Tygesen C, Montanana-Rosell R, Limburg HL, Marczynska J, Asgari N, Steckelings UM, Owens T
Angiotensin AT2 receptor-induced interleukin-10 attenuates neuromyelitis optica spectrum disorder-like pathology.
Neuromyelitis optica spectrum disorder (NMOSD) is a relapsing inflammatory central nervous system (CNS) disease for which there is no cure. Immunoglobulin G autoantibodies specific for the water channel aquaporin-4 are a serum biomarker, believed to induce complement-dependent astrocyte damage with secondary demyelination.
Mult Scler, 2020; 26

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-346

DEFINING THE FUNCTIONAL ROLE OF TBK1 USING A NOVEL ZEBRAFISH MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS).

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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ALS is a progressive neurodegenerative disorder affecting motor neurons that leads to muscle paralysis and denervation of the respiratory muscle through the degeneration of cortical and spinal motor neurons. In 2015, mutations leading to haploinsufficiency of TBK1 were first described in ALS patients. Tightly linked to both autophagy and inflammation, two impaired cellular pathways in ALS, it has become of major interest to understand TBK1 functional role in the pathogenesis. Therefore, we utilized zebrafish, an optimal vertebrate model to study ALS related genes, including Tbk1. To analyze the mechanisms underlying *TBK1* deletion in motor neurons degeneration, we designed and characterized novel *tbk1* KO and KD zebrafish models. We observed a decrease in the locomotor ability associated with tbk1 loss of function and we correlated it to a decrease in the number of spinal motor neurons. The KO model is also characterized by a high lethality early in development. To elucidate pathways that could be responsible for motor neuron degeneration and early lethality, we have performed age-dependent metabolic profiles in these models. We demonstrate that tbk1 loss of function leads to an increase production of antioxidant molecules followed by dysregulation in the NAD metabolism. These two profiles suggest a harmful oxidative stress followed by a decrease in the NAD pool; two hallmarks of the pathogenesis observed in ALS patients attesting the validity of our model to study this disease. Finally, we are in the process of targeting these dysregulated pathways using prioritized compounds to identify novel therapeutic strategies for ALS.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-347

PRECLINICAL EVALUATION OF CK-1δ AND TTBK1 INHIBITORS IN A TDP-43-RELATED FRONTOTEMPORAL DEMENTIA MOUSE MODEL

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

Claudia Gonzalo-Consuegra[1], Adrián Parrilla Mesas[1], Carmen Rodríguez-Cueto[1], Loreto Martínez-González[2], Javier Fernández Ruiz[1], Carmen Gil[2], Ana Martínez[2], Eva De Lago[1]
[1]Instituto Universitario de Investigación en Neuroquímica, School of Medicine, Department Of Biochemistry And Molecular Biology, Ucm, Ciberned, Irycis, Madrid, Spain, [2]Centro de Investigaciones Biológicas, Csic, Ciberned, Madrid, Spain

Aims: Frontotemporal dementia (FTD) characterizes by progressive degeneration of frontal and temporal lobes which results in cognitive, behavioral, and language dysfunction. Almost half of the FTD patients present toxic, cytoplasmic aggregates of the ribonucleoprotein TDP-43 as a consequence, among others, of its post-translational hyperphosphorylation. Given that CK-1δ and TTBK1 kinases have been involved in TDP-43 phosphorylation, we hypothesize that their pharmacological inhibition could be beneficial against TDP-43-based FTD. **Methods**: Presymptomatic CaMKII-TDP-43 transgenic mice (at post-natal day (PND) 45) were chronically treated with IGS2.7 (CK-1δ inhibitor), VNG1.47 (TTBK1 inhibitor) or vehicle for 45 days. Novel object recognition (NOR) test was performed at PND60 and PND90. Animals were euthanized at PND90, and brain hemispheres were processed for immunohistochemistry and biochemical analysis. **Results:** As early as PND60, CaMKII-TDP-43 transgenic mice treated with IGS2.7 or VNG1.47 showed higher discrimination index in the NOR test than CaMKII-TDP-43 mice treated with vehicle. This finding was also observed at PND90. At biochemical level, both inhibitors decreased TDP-43 phosphorylation in the prefrontal cortex (PFC) and the hippocampus compared to non-treated CaMKII-TDP-43 transgenic mice. These benefits were accompanied, at histological level, by neuronal preservation in the PFC and the hippocampus, as well as by reduced astrogliosis in the PFC and a subtle attenuation in hippocampal microgliosis. **Conclusion:** Our data suggest that inhibition of TDP-43 phosphorylation could be a beneficial disease-modifying strategy to tackle FTD with TDP-43 proteinopathy and propose CK-1δ and TTBK1 as promising targets.

Pubmed:
34978799: Nozal V, Martínez-González L, Gomez-Almeria M, Gonzalo-Consuegra C, Santana P, Chaikuad A, Pérez-Cuevas E, Knapp S, Lietha D, Ramírez D, Petralla S, Monti B, Gil C, Martín-Requero A, Palomo V, de Lago E, Martinez A
TDP-43 Modulation by Tau-Tubulin Kinase 1 Inhibitors: A New Avenue for Future Amyotrophic Lateral Sclerosis Therapy.
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease without any effective treatment. Protein TDP-43 is a pathological hallmark of ALS in both sporadic and familiar patients. Post-translational modifications of TDP-43 promote its aggregation in the cytoplasm. Tau-Tubulin kinase (TTBK1) phosphorylates TDP-43 in cellular and animal models; thus, TTBK1 inhibitors emerge as a promising therapeutic strategy for ALS. The design, synthesis, biological evaluation, kinase-ligand complex structure determination, and molecular modeling studies confirmed novel pyrrolopyrimidine derivatives as valuable inhibitors for further development. Moreover, compound revealed good brain penetration and was able to reduce TDP-43 phosphorylation not only in cell cultures but also in the spinal cord of transgenic TDP-43 mice. A shift to M2 anti-inflammatory microglia was also demonstrated . Both these activities led to motor neuron preservation in mice, proposing pyrrolopyrimidine as a valuable lead compound for future ALS therapy.
J Med Chem, 2022; 65
34946726: Burgaz S, García C, Gonzalo-Consuegra C, Gómez-Almería M, Ruiz-Pino F, Unciti JD, Gómez-Cañas M, Alcalde J, Morales P, Jagerovic N, Rodríguez-Cueto C, de Lago E, Muñoz E, Fernández-Ruiz J
Preclinical Investigation in Neuroprotective Effects of the GPR55 Ligand VCE-006.1 in Experimental Models of Parkinson's Disease and Amyotrophic Lateral Sclerosis.
Cannabinoids act as pleiotropic compounds exerting, among others, a broad-spectrum of neuroprotective effects. These effects have been investigated in the last years in different preclinical models of neurodegeneration, with the cannabinoid type-1 (CB) and type-2 (CB) receptors concentrating an important part of this research. However, the issue has also been extended to additional targets that are also active for cannabinoids, such as the orphan G-protein receptor 55 (GPR55). In the present study, we investigated the neuroprotective potential of VCE-006.1, a chromenopyrazole derivative with biased orthosteric and positive allosteric modulator activity at GPR55, in murine models of two neurodegenerative diseases. First, we

proved that VCE-006.1 alone could induce ERK1/2 activation and calcium mobilization, as well as increase cAMP response but only in the presence of lysophosphatidyl inositol. Next, we investigated this compound administered chronically in two neurotoxin-based models of Parkinson's disease (PD), as well as in some cell-based models. VCE-006.1 was active in reversing the motor defects caused by 6-hydroxydopamine (6-OHDA) in the pole and the cylinder rearing tests, as well as the losses in tyrosine hydroxylase-containing neurons and the elevated glial reactivity detected in the substantia nigra. Similar cytoprotective effects were found in vitro in SH-SY5Y cells exposed to 6-OHDA. We also investigated VCE-006.1 in LPS-lesioned mice with similar beneficial effects, except against glial reactivity and associated inflammatory events, which remained unaltered, a fact confirmed in BV2 cells treated with LPS and VCE-006.1. We also analyzed GPR55 in these in vivo models with no changes in its gene expression, although GPR55 was down-regulated in BV2 cells treated with LPS, which may explain the lack of efficacy of VCE-006.1 in such an assay. Furthermore, we investigated VCE-006.1 in two genetic models of amyotrophic lateral sclerosis (ALS), mutant SOD1, or TDP-43 transgenic mice. Neither the neurological decline nor the deteriorated rotarod performance were prevented with this compound, and the same happened with the elevated microglial and astroglial reactivities, albeit modest spinal motor neuron preservation was achieved in both models. We also analyzed GPR55 in these in vivo models and found no changes in both TDP-43 transgenic and mSOD1 mice. Therefore, our findings support the view that targeting the GPR55 may afford neuroprotection in experimental PD, but not in ALS, thus stressing the specificities for the development of cannabinoid-based therapies in the different neurodegenerative disorders.
Molecules, 2021; 26

34445680: Martínez-González L, Gonzalo-Consuegra C, Gómez-Almería M, Porras G, de Lago E, Martín-Requero Á, Martínez A

Tideglusib, a Non-ATP Competitive Inhibitor of GSK-3β as a Drug Candidate for the Treatment of Amyotrophic Lateral Sclerosis.

Amyotrophic Lateral Sclerosis (ALS) is the most common degenerative motor neuron disease in adults. About 97% of ALS patients present TDP-43 aggregates with post-translational modifications, such as hyperphosphorylation, in the cytoplasm of affected cells. GSK-3β is one of the protein kinases involved in TDP-43 phosphorylation. Up-regulation of its expression and activity is reported on spinal cord and cortex tissues of ALS patients. Here, we propose the repurposing of Tideglusib, an in-house non-ATP competitive GSK-3β inhibitor that is currently in clinical trials for autism and myotonic dystrophy, as a promising therapeutic strategy for ALS. With this aim we have evaluated the efficacy of Tideglusib in different experimental ALS models both in vitro and in vivo. Moreover, we observed that GSK-3β activity is increased in lymphoblasts from sporadic ALS patients, with a simultaneous increase in TDP-43 phosphorylation and cytosolic TDP-43 accumulation. Treatment with Tideglusib decreased not only phospho-TDP-43 levels but also recovered its nuclear localization in ALS lymphoblasts and in a human TDP-43 neuroblastoma model. Additionally, we found that chronic oral treatment with Tideglusib is able to reduce the increased TDP-43 phosphorylation in the spinal cord of Prp-hTDP-43 mouse model. Therefore, we consider Tideglusib as a promising drug candidate for ALS, being proposed to start a clinical trial phase II by the end of the year.
Int J Mol Sci, 2021; 22

33486755: Rodríguez-Cueto C, García-Toscano L, Santos-García I, Gómez-Almería M, Gonzalo-Consuegra C, Espejo-Porras F, Fernández-Ruiz J, de Lago E

Targeting the CB receptor and other endocannabinoid elements to delay disease progression in amyotrophic lateral sclerosis.

Cannabinoids form a singular group of plant-derived compounds, endogenous lipids and synthetic derivatives with multiple therapeutic effects exerted by targeting different elements of the endocannabinoid system. One of their therapeutic applications is the preservation of neuronal integrity exerted by attenuating the multiple neurotoxic events that kill neurons in neurodegenerative disorders. In this review, we will address the potential of cannabinoids as neuroprotective agents in amyotrophic lateral sclerosis (ALS), a devastating neurodegenerative disorder characterized by muscle denervation, atrophy and paralysis, and progressive deterioration in upper and/or lower motor neurons. The emphasis will be paid on the cannabinoid type 2 (CB) receptor, whose activation limits glial reactivity, but the potential of additional endocannabinoid-related targets will be also addressed. The evidence accumulated so far at the preclinical level supports the need to soon move towards the patients and initiate clinical trials to confirm the potential of cannabinoid-based medicines as disease modifiers in ALS. LINKED ARTICLES: This article is part of a themed issue on Neurochemistry in Japan. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v178.6/issuetoc.
Br J Pharmacol, 2021; 178

33338634: Burgaz S, García C, Gómez-Cañas M, Navarrete C, García-Martín A, Rolland A, Del Río C, Casarejos MJ, Muñoz E, Gonzalo-Consuegra C, Muñoz E, Fernández-Ruiz J

Neuroprotection with the cannabigerol quinone derivative VCE-003.2 and its analogs CBGA-Q and CBGA-Q-Salt in Parkinson's disease using 6-hydroxydopamine-lesioned mice.

The quinone derivative of the non-psychotropic cannabinoid cannabigerol (CBG), so-called VCE-003.2, has been recently investigated for its neuroprotective properties in inflammatory models of Parkinson's disease (PD) in mice. Such potential

derives from its activity at the peroxisome proliferator-activated receptor-γ (PPAR-γ). In the present study, we investigated the neuroprotective properties of VCE-003.2 against the parkinsonian neurotoxin 6-hydroxydopamine (6-OHDA), in comparison with two new CBG-related derivatives, the cannabigerolic acid quinone (CBGA-Q) and its sodium salt CBGA-Q-Salt, which, similarly to VCE-003.2, were found to be active at the PPAR-γ receptor, but not at the cannabinoid CB and CB receptors. First, we investigated their cytoprotective properties in vitro by analyzing cell survival in cultured SH-SY5Y cells exposed to 6-OHDA. We found an important cytoprotective effect of VCE-003.2 at a concentration of 20 μM, which was not reversed by the blockade of PPAR-γ receptors with GW9662, supporting its activity at an alternative site (non-sensitive to classic antagonists) in this receptor. We also found CBGA-Q and CBGA-Q-Salt being cytoprotective in this cell assay, but their effects were completely eliminated by GW9662, thus indicating that they are active at the canonical site in the PPAR-γ receptor. Then, we moved to in vivo testing using mice unilaterally lesioned with 6-OHDA. Our data confirmed that VCE-003.2 administered orally (20 mg/kg) preserved tyrosine hydroxylase (TH)-positive nigral neurons against 6-OHDA-induced damage, whereas it completely attenuated the astroglial (GFAP) and microglial (CD68) reactivity found in the substantia nigra of lesioned mice. Such neuroprotective effects caused an important recovery in the motor deficiencies displayed by 6-OHDA-lesioned mice in the pole test and the cylinder rearing test. We also investigated CBGA-Q, given orally (20 mg/kg) or intraperitoneally (10 mg/kg, i.p.), having similar benefits compared to VCE-003.2 against the loss of TH-positive nigral neurons, glial reactivity and motor defects caused by 6-OHDA. Lastly, the sodium salt of CBGA-Q, given orally (40 mg/kg) to 6-OHDA-lesioned mice, also showed benefits at behavioral and histopathological levels, but to a lower extent compared to the other two compounds. In contrast, when given i.p., CBGA-Q-Salt (10 mg/kg) was poorly active. We also analyzed the concentrations of dopamine and its metabolite DOPAC in the striatum of 6-OHDA-lesioned mice after the treatment with the different compounds, but recovery in the contents of both dopamine and DOPAC was only found after the treatment with VCE-003.2. In summary, our data confirmed the neuroprotective potential of VCE-003.2 in 6-OHDA-lesioned mice, which adds to its previous activity found in an inflammatory model of PD (LPS-lesioned mice). Additional phytocannabinoid derivatives, CBGA-Q and CBGA-Q-Salt, also afforded neuroprotection in 6-OHDA-lesioned mice, but their effects were lower compared to VCE-003.2, in particular in the case of CBGA-Q-Salt. In vitro studies confirmed the relevance of PPAR-γ receptors for these effects.
Mol Cell Neurosci, 2021; 110

33139113: Rojas-Prats E, Martinez-Gonzalez L, Gonzalo-Consuegra C, Liachko NF, Perez C, Ramírez D, Kraemer BC, Martin-Requero Á, Perez DI, Gil C, de Lago E, Martinez A

Targeting nuclear protein TDP-43 by cell division cycle kinase 7 inhibitors: A new therapeutic approach for amyotrophic lateral sclerosis.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with no known cure. Aggregates of the nuclear protein TDP-43 have been recognized as a hallmark of proteinopathy in both familial and sporadic cases of ALS. Post-translational modifications of this protein, include hyperphosphorylation, cause disruption of TDP-43 homeostasis and as a consequence, promotion of its neurotoxicity. Among the kinases involved in these changes, cell division cycle kinase 7 (CDC7) plays an important role by directly phosphorylating TDP-43. In the present manuscript the discovery, synthesis, and optimization of a new family of selective and ATP-competitive CDC7 inhibitors based on 6-mercaptopurine scaffold are described. Moreover, we demonstrate the ability of these inhibitors to reduce TDP-43 phosphorylation in both cell cultures and transgenic animal models such as C. elegans and Prp-hTDP43 (A315T) mice. Altogether, the compounds described here may be useful as versatile tools to explore the role of CDC7 in TDP-43 phosphorylation and also as new drug candidates for the future development of ALS therapies.
Eur J Med Chem, 2021; 210

32510591: Palomares B, Garrido-Rodriguez M, Gonzalo-Consuegra C, Gómez-Cañas M, Saen-Oon S, Soliva R, Collado JA, Fernández-Ruiz J, Morello G, Calzado MA, Appendino G, Muñoz E

Δ -Tetrahydrocannabinolic acid alleviates collagen-induced arthritis: Role of PPARγ and CB receptors.

Δ -Tetrahydrocannabinolic acid (Δ -THCA-A), the precursor of Δ -THC, is a non-psychotropic phytocannabinoid that shows PPARγ agonist activity. Here, we investigated the ability of Δ -THCA-A to modulate the classic cannabinoid CB and CB receptors and evaluated its anti-arthritis activity in vitro and in vivo.
Br J Pharmacol, 2020; 177

FENS Forum 2022
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BOARD NUMBER: S03-348

THE SERUM RESPONSE FACTOR (SRF) REGULATES MOTONEURON VULNERABILITY IN ALS THROUGH THE REGULATION OF AUTOPHAGY FLUX

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

Natalie Dikwella[1], Jialei Song[2], Daniela Sinske[2], Francesco Roselli[1], Bernd Knoell[2]
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Neuronal activity plays a crucial role in motoneurons vulnerability in amyotrophic lateral sclerosis (ALS). Enhanced motoneurons excitability has been shown to promote neuroprotection while reduced excitability has been shown to accelerate disease progression. However, the molecular basis of neuronal activity impact in ALS has not been identified yet. In this study, the impact of the activity-dependent transcription factor Serum Response Factor (SRF) was investigated in vivo and in vitro. Conditional motoneuron-selective SRF ablation in the context of the SOD1(G93A) ALS mouse model caused an early disease onset as revealed by anticipated start of body weight loss, earlier appearance of advanced clinical stages and faster decline in grip-strength. However, overall survival was not affected, indicating a major role of SRF only for some motoneuron subpopulations. At P50, loss of SRF in motoneurons caused a more pronounced neuromuscular junctions' denervation and expanded neuroinflammatory response. Increased vulnerability of motoneurons corresponded to fewer Beclin+ inclusions in histology and reduced induction of autophagy genes assessed in laser-microdissected motoneurons. We complemented these studies assessing autophagy genes induction in HEK cells expressing the C9-Orf72-associated polyGA polypeptide: the overexpression of SRF resulted in a decreased burden of polyGA inclusions. Live-imaging microscopy with the autophagy sensor P62-GFP-RFP revealed a faster autophagy flux upon overexpression of SRF compared to an inactive SRF mutant protein. Thus, in vitro data further confirmed a role of SRF in the transcriptional regulation of cellular proteostasis. In conclusion, a novel link between neuronal activity, synaptic input and autophagy imbalance was provided in this project.

FENS Forum 2022
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BOARD NUMBER: S03-349

THE NEGATIVE ALLOSTERIC MODULATOR CTEP AMELIORATES THE REACTIVE PHENOTYPE OF I-ASTROCYTES FROM PATIENTS AFFECTED BY AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

Carola Torazza[1], Mandeep Kumar[1], Sara Tessitore[1], Allan Shaw[2], Pamela Shaw[3], Laura Ferraiuolo[2], Giambattista Bonanno[1,4], Marco Milanese[1]
[1]University of Genoa, Department Of Pharmacy-pharmacology And Toxicology Unit, Genoa, Italy, [2]The University of Sheffield, Department Of Neuroscience, Sheffield, United Kingdom, [3]Royal Hallamshire Hospital, Department Of Neuroscience, Sheffield, United Kingdom, [4]IRCCS, San Martino Polyclinic Hospital, Genoa, Italy

AIMS: Amyotrophic Lateral Sclerosis (ALS) is a non-cell-autonomous neurodegenerative disease. One major cause of motor neuron degeneration is glutamate-mediated excitotoxicity, and metabotropic glutamate receptor type 5 (mGluR5) seems highly involved. Here, we investigated the *in-vitro* effects of the negative mGluR5 allosteric modulator 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine (CTEP) exposure on the phenotype of inducible neural progenitor cell (iNPC)-derived astrocytes from ALS patients and healthy donors. **METHODS**: iNPCs were directly converted from human skin fibroblasts of C9orf72 and SOD1^{A4V} ALS patients and healthy individuals. After differentiation, i-astrocytes were treated with 100nM CTEP or vehicle for three days and then used for western blot (WB), immunofluorescence (IF) and enzymatic assays. **RESULTS:** CTEP reduced the expression of mGluR5 and the reactive astrocyte markers GFAP, S100-beta, and C3 in C9orf72 and SOD1^{A4V} i-astrocytes, as detected by WB and IF. CTEP also reduced the expression of NLRP3 and increase the expression of Nrf2, two markers of inflammation and oxidative stress response, respectively. We tested the activity of glucose-6-phosphate dehydrogenase, glutathione reductase, glutathione peroxidase and catalase, four enzymes associated with antioxidant cellular defenses, which were higher in C9orf72 and SOD1^{A4V} iNPC-derived i-astrocytes than in controls. CTEP reduced the enzyme activity, suggesting amelioration of the redox state. Accordingly, CTEP-treated ALS i-astrocytes showed a reduced lipid peroxidation compared to untreated cells. We detected no functional alterations in CTEP-treated i-astrocytes from healthy donors. **CONCLUSIONS:** These results suggest that mGluR5 modulation by CTEP positively impacts on the i-astrocyte reactive phenotype of ALS patients, expanding *in-vitro* and *in-vivo* results previously obtained with SOD1^{G93A} mouse astrocytes.

Pubmed:
34830115: Bonifacino T, Zerbo RA, Balbi M, Torazza C, Frumento G, Fedele E, Bonanno G, Milanese M
Nearly 30 Years of Animal Models to Study Amyotrophic Lateral Sclerosis: A Historical Overview and Future Perspectives.
Amyotrophic lateral sclerosis (ALS) is a fatal, multigenic, multifactorial, and non-cell autonomous neurodegenerative disease characterized by upper and lower motor neuron loss. Several genetic mutations lead to ALS development and many emerging gene mutations have been discovered in recent years. Over the decades since 1990, several animal models have been generated to study ALS pathology including both vertebrates and invertebrates such as yeast, worms, flies, zebrafish, mice, rats, guinea pigs, dogs, and non-human primates. Although these models show different peculiarities, they are all useful and complementary to dissect the pathological mechanisms at the basis of motor neuron degeneration and ALS progression, thus contributing to the development of new promising therapeutics. In this review, we describe the up to date and available ALS genetic animal models, classified by the different genetic mutations and divided per species, pointing out their features in modeling, the onset and progression of the pathology, as well as their specific pathological hallmarks. Moreover, we highlight similarities, differences, advantages, and limitations, aimed at helping the researcher to select the most appropriate experimental animal model, when designing a preclinical ALS study.
Int J Mol Sci, 2021; 22
34573024: Marini C, Cossu V, Kumar M, Milanese M, Cortese K, Bruno S, Bellese G, Carta S, Zerbo RA, Torazza C, Bauckneht M, Venturi C, Raffa S, Orengo AM, Donegani MI, Chiola S, Ravera S, Castellani P, Morbelli S, Sambuceti G, Bonanno G
The Role of Endoplasmic Reticulum in the Differential Endurance against Redox Stress in Cortical and Spinal Astrocytes from the Newborn SOD1 Mouse Model of Amyotrophic Lateral Sclerosis.
Recent studies reported that the uptake of [18F]-fluorodeoxyglucose (FDG) is increased in the spinal cord (SC) and

decreased in the motor cortex (MC) of patients with ALS, suggesting that the disease might differently affect the two nervous districts with different time sequence or with different mechanisms. Here we show that MC and SC astrocytes harvested from newborn B6SJL-Tg (SOD1) 1Gur mice could play different roles in the pathogenesis of the disease. Spectrophotometric and cytofluorimetric analyses showed an increase in redox stress, a decrease in antioxidant capacity and a relative mitochondria respiratory uncoupling in MC SOD1 astrocytes. By contrast, SC mutated cells showed a higher endurance against oxidative damage, through the increase in antioxidant defense, and a preserved respiratory function. FDG uptake reproduced the metabolic response observed in ALS patients: SOD1 mutation caused a selective enhancement in tracer retention only in mutated SC astrocytes, matching the activity of the reticular pentose phosphate pathway and, thus, of hexose-6P dehydrogenase. Finally, both MC and SC mutated astrocytes were characterized by an impressive ultrastructural enlargement of the endoplasmic reticulum (ER) and impairment in ER-mitochondria networking, more evident in mutated MC than in SC cells. Thus, SOD1 mutation differently impaired MC and SC astrocyte biology in a very early stage of life.
Antioxidants (Basel), 2021; 10
33931856: Milanese M, Bonifacino T, Torazza C, Provenzano F, Kumar M, Ravera S, Zerbo AR, Frumento G, Balbi M, Nguyen TPN, Bertola N, Ferrando S, Viale M, Profumo A, Bonanno G
Blocking glutamate mGlu receptors with the negative allosteric modulator CTEP improves disease course in SOD1 mouse model of amyotrophic lateral sclerosis.
The pathogenesis of amyotrophic lateral sclerosis (ALS) is not fully clarified, although excessive glutamate (Glu) transmission and the downstream cytotoxic cascades are major mechanisms for motor neuron death. Two metabotropic glutamate receptors (mGlu and mGlu) are overexpressed in ALS and regulate cellular disease processes. Expression and function of mGlu receptors are altered at early symptomatic stages in the SOD1 mouse model of ALS and knockdown of mGlu5 receptors in SOD1 mice improved disease progression.
Br J Pharmacol, 2021; 178
32638178: Marini C, Cossu V, Bonifacino T, Bauckneht M, Torazza C, Bruno S, Castellani P, Ravera S, Milanese M, Venturi C, Carlone S, Piccioli P, Emionite L, Morbelli S, Orengo AM, Donegani MI, Miceli A, Raffa S, Marra S, Signori A, Cortese K, Grillo F, Fiocca R, Bonanno G, Sambuceti G
Mechanisms underlying the predictive power of high skeletal muscle uptake of FDG in amyotrophic lateral sclerosis.
We recently reported that enhanced [18F]-fluorodeoxyglucose (FDG) uptake in skeletal muscles predicts disease aggressiveness in patients with amyotrophic lateral sclerosis (ALS). The present experimental study aimed to assess whether this predictive potential reflects the link between FDG uptake and redox stress that has been previously reported in different tissues and disease models.
EJNMMI Res, 2020; 10
31540330: Bonifacino T, Rebosio C, Provenzano F, Torazza C, Balbi M, Milanese M, Raiteri L, Usai C, Fedele E, Bonanno G
Enhanced Function and Overexpression of Metabotropic Glutamate Receptors 1 and 5 in the Spinal Cord of the SOD1 Mouse Model of Amyotrophic Lateral Sclerosis during Disease Progression.
Glutamate (Glu)-mediated excitotoxicity is a major cause of amyotrophic lateral sclerosis (ALS) and our previous work highlighted that abnormal Glu release may represent a leading mechanism for excessive synaptic Glu. We demonstrated that group I metabotropic Glu receptors (mGluR1, mGluR5) produced abnormal Glu release in SOD1 mouse spinal cord at a late disease stage (120 days). Here, we studied this phenomenon in pre-symptomatic (30 and 60 days) and early-symptomatic (90 days) SOD1 mice. The mGluR1/5 agonist ()-3,5-Dihydroxyphenylglycine (3,5-DHPG) concentration dependently stimulated the release of [H]d-Aspartate ([H]d-Asp), which was comparable in 30- and 60-day-old wild type mice and SOD1 mice. At variance, [H]d-Asp release was significantly augmented in 90-day-old SOD1 mice and both mGluR1 and mGluR5 were involved. The 3,5-DHPG-induced [H]d-Asp release was exocytotic, being of vesicular origin and mediated by intra-terminal Ca release. mGluR1 and mGluR5 expression was increased in Glu spinal cord axon terminals of 90-day-old SOD1 mice, but not in the whole axon terminal population. Interestingly, mGluR1 and mGluR5 were significantly augmented in total spinal cord tissue already at 60 days. Thus, function and expression of group I mGluRs are enhanced in the early-symptomatic SOD1 mouse spinal cord, possibly participating in excessive Glu transmission and supporting their implication in ALS. Please define all abbreviations the first time they appear in the abstract, the main text, and the first figure or table caption.
Int J Mol Sci, 2019; 20
31282572: Ravera S, Torazza C, Bonifacino T, Provenzano F, Rebosio C, Milanese M, Usai C, Panfoli I, Bonanno G
Altered glucose catabolism in the presynaptic and perisynaptic compartments of SOD1 mouse spinal cord and motor cortex indicates that mitochondria are the site of bioenergetic imbalance in ALS.
Amyotrophic lateral sclerosis is an adult-onset neurodegenerative disease that develops because of motor neuron death. Several mechanisms occur supporting neurodegeneration, including mitochondrial dysfunction. Recently, we demonstrated that the synaptosomes from the spinal cord of SOD1 mice, an in vitro model of presynapses, displayed impaired

mitochondrial metabolism at early pre-symptomatic stages of the disease, whereas perisynaptic astrocyte particles, or gliosomes, were characterized by mild energy impairment only at symptomatic stages. This work aimed to understand whether mitochondrial impairment is a consequence of upstream metabolic damage. We analyzed the critical pathways involved in glucose catabolism at presynaptic and perisynaptic compartments. Spinal cord and motor cortex synaptosomes from SOD1 mice displayed high activity of hexokinase and phosphofructokinase, key glycolysis enzymes, and of citrate synthase and malate dehydrogenase, key Krebs cycle enzymes, but did not display high lactate dehydrogenase activity, the key enzyme in lactate fermentation. This enhancement was evident in the spinal cord from the early stages of the disease and in the motor cortex at only symptomatic stages. Conversely, an increase in glycolysis and lactate fermentation activity, but not Krebs cycle activity, was observed in gliosomes from the spinal cord and motor cortex of SOD1 mice although only at the symptomatic stages of the disease. The cited enzymatic activities were enhanced in spinal cord and motor cortex homogenates, paralleling the time-course of the effect observed in synaptosomes and gliosomes. The observed metabolic modifications might be considered an attempt to restore altered energetic balance and indicate that mitochondria represent the ultimate site of bioenergetic impairment.
J Neurochem, 2019; 151
31102766: Bonifacino T, Provenzano F, Gallia E, Ravera S, Torazza C, Bossi S, Ferrando S, Puliti A, Van Den Bosch L, Bonanno G, Milanese M
In-vivo genetic ablation of metabotropic glutamate receptor type 5 slows down disease progression in the SOD1 mouse model of amyotrophic lateral sclerosis.
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease due to motor neuron (MN) loss. The mechanisms causing selective MN death are largely unknown, thus prejudicing successful pharmacological treatments. Major causes of MN damage are effects downstream of the abnormal glutamate (Glu) neurotransmission. Group I metabotropic Glu receptors (mGluR1, mGluR5) actively contribute to the excitotoxicity in ALS and represent druggable molecular targets. We previously demonstrated that halving mGluR1 or mGluR5 expression in the widely studied SOD1 mouse model of ALS had a positive impact on disease onset, clinical progression and survival, as well as on cellular and biochemical parameters altered in ALS. Whereas these effects were similar in female and male mGluR1 heterozygous SOD1mice, only male mGluR5 heterozygous SOD1 mice showed improved motor skills during disease progression. To further validate the role of Group I mGluRs in ALS, we generated in this study mGluR1 or mGluR5 null mice expressing the SOD1 mutation (SOD1Grm1 or SOD1Grm5, respectively). SOD1Grm1 mice showed early and progressive motor impairments and died even before SOD1 mice, while SOD1Grm5 mice exhibited delayed disease onset, longer survival, and ameliorated motor skills than SOD1 mice. No difference between female and male SOD1Grm5 mice were observed. These effects were associated with enhanced MN preservation and decreased astrocytic and microglial activation. Our results strongly support the assumption that constitutively lowering of mGluR5 expression has a positive impact in mice with ALS by counteracting the abnormal Glu transmission and this could be a potentially effective pharmacological target in ALS.
Neurobiol Dis, 2019; 129

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IN-VIVO TREATMENT WITH THE GPR17 ANTAGONIST MONTELUKAST AMELIORATED THE LIFESPAN AND DELAYED THE DISEASE PROGRESSION IN THE SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

T.P. Nhung Nguyen[1], S. Raffaele[2], G. Frumento[1], M. Milanese[1], M.P. Abbracchio[3], G. Bonanno[1], M. Fumagalli[2], T. Bonifacino[1]
[1]University of Genoa, Department Of Pharmacy, Genoa, Italy, [2]University of Milan, Department Of Pharmacological And Biomolecular Sciences, Milan, Italy, [3]University of Milan, Department Of Pharmaceutical Sciences, Milan, Italy

Aims. Amyotrophic lateral sclerosis (ALS) is a non-cell-autonomous neurodegenerative disease involving oligodendrocyte (OL) damage. We reported that the function of the GPR17 receptor, involved in oligodendrocyte precursor cell (OPC) maturation, is altered in the SOD1^{G93A} mouse model of ALS. Interestingly, primary cultured SOD1^{G93A} OPCs displayed differentiation defects compared to wild-type cells, which were significantly restored by *in vitro* exposure to the already marketed non-selective GPR17 antagonist montelukast (MTK). Here we translated the *in-vitro* data to *in-vivo* studies assessing the effects of the MTK treatment in SOD1^{G93A} mice. **Methods.** MTK (10 or 30 mg/kg/day; gavage) was chronically administered to SOD1^{G93A} mice from day 90 of life until death. Vehicle-treated SOD1^{G93A} mice were used as controls. We evaluated survival probability and body weight loss; motor functions by Rotarod, beam balance, gait, and extension reflex tests. Immunohistochemistry (IHC) was performed to assess oligodendrocyte and microglia markers. **Results.** MTK (10 mg/kg/day) neither increased survival probability nor ameliorated disease progression. Accordingly, IHC did not highlight MTK-induced modifications of OL differentiation and microglia activation state. On the contrary, 30 mg/kg/day MTK ameliorated survival probability and delayed weight loss in SOD1^{G93A} female but not male mice. Motor abilities also improved in MTK-treated SOD1^{G93A} female mice only. IHC is currently ongoing to evaluate the MTK treatment impact on OL dysfunction, neuroinflammation, and MN degeneration. **Conclusion.** Our results suggest that *in-vivo* targeting GPR17 by the antagonist MTK gender-dependently improves ALS progression and highlights GPR17 as a novel promising pharmacological target. Supported by Fondazione AriSLA, GPR17ALS-1 to MF and TB.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-351

EARLY REVERSIBLE STRUCTURAL AND FUNCTIONAL IMPAIRMENTS OF EXCITATORY SYNAPSES ON ALS MOTONEURONS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

Marcin Bączyk[1], Najwa Ouali Alami[2], Kamil Grycz[3], Nicolas Delestrée[1], Clémence Martinot[1], Tang Linyun[1], Barbara Commisso[2], Nicolas Doisne[1], Marin Manuel[1], Francesco Roselli[2,4], Daniel Zytnicki[1]
[1]Université de Paris, Sppin, Paris, France, [2]Ulm University, Department Of Neurology, Ulm, Germany, [3]Poznan University of Physical Education, Department Of Neurobiology, Poznan, Poland, [4]Ulm University, German Center For Neurodegenerative Diseases, Ulm, Germany

Excessive excitation is hypothesized to cause motoneuron (MN) degeneration in amyotrophic lateral sclerosis (ALS), but actual proof of hyperexcitation in vivo is missing: how are synaptic inputs to MN affected by the disease, and are they increased or decreased? We demonstrate, by in vivo intracellular MN electrophysiology, that, contrary to expectations, excitatory post-synaptic potentials evoked by electrical or mechanical stimulation of Ia sensory fibers are reduced in MNs of adult presymptomatic mutSOD1 mice. This synaptic impairment correlates with disrupted postsynaptic clustering of Homer1b, Shank, and GluR4 subunits. Moreover, this impairment has a deep impact on the whole MN biology since mechanically-induced Ia inputs translate in a reduced phosphorylation of the CREB transcription factor in MNs. Interestingly, a similar functional impairment is observed in synapses on MN originating from the brainstem descending medial longitudinal fasciculus, indicating a widespread phenomenon. Restoration of excitatory synapses can be achieved by activation of the cAMP/PKA pathway, by either intracellular injection of cAMP or DREADD-Gs stimulation. Furthermore, we reveal, through independent control of signaling and excitability in MN allowed by multiplexed DREADD/PSAM chemogenetics, that PKA-induced restoration of synapses triggers an excitation-dependent decrease in misfolded SOD1 burden and autophagy overload. In turn, increased MN excitability contributes to restoring synaptic structures. Thus, the decrease of excitation to MN is an early but reversible event in ALS. Failure of the postsynaptic site, rather than hyperexcitation, drives disease pathobiochemistry at this stage of the disease evolution. Fundings: Thierry Latran Foundation, Radala Foundation for ALS research, ANR/DFG (SynaptALS), NIH-NINDS 5R01NS110953, NCN 2019/35/B/NZ4/02058

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BOARD NUMBER: S03-352

CHARACTERIZATION OF EXTRACELLULAR VESICLES RELEASED FROM SPINAL CORD ASTROCYTES OF LATE SYMPTOMATIC SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

Roberta Arianna Zerbo[1], Fabrizio Fabbiano[2], Carola Torazza[1], Matilde Balbi[1], Marco Milanese[1], Vito Giuseppe D'Agostino[2], Giambattista Bonanno[1,3]
[1]University of Genoa, Department Of Pharmacy-pharmacology And Toxicology Unit, Genoa, Italy, [2]University of Trento, Department Of Cellular, Computational And Integrative Biology, Trento, Italy, [3]IRCCS, San Martino Polyclinic Hospital, Genoa, Italy

Aims Amyotrophic Lateral Sclerosis (ALS) is a non-cell-autonomous neurodegenerative disease, and astrocytes play a crucial role in motor neuron (MN) damage, possibly trough paracrine mechanisms mediated by secretion of soluble factors or extracellular vesicles (EVs). Here, we compared the characteristics of EVs secreted by spinal cord astrocytes from $SOD1^{G93A}$ mice, a model of ALS, and WT mice. **Methods** Astrocytes were cultured (20 DIV) from the spinal cord of 120-day-old $SOD1^{G93A}$ and WT mice. EVs were isolated by the nickel-based isolation (NBI) procedure from the astrocyte supernatant. MNs were purified by a gradient from the spinal cord of E13,5 WT mouse embryos, cultured, and counted for viability from day 8 and for 14 days after seeding. EV-RNA was extracted using the Single Cell RNA extraction kit (Norgen-Biotek) and analyzed by RNA sequencing. **Results** We obtained more astrocytes from $SOD1^{G93A}$ mice ($6.37x10^5$/spinal cord) than from WT mice ($1.61x10^5$/spinal cord). Moreover, $SOD1^{G93A}$ astrocytes secreted more EVs ($6,73x10^5$ EV/cell) than WT astrocytes ($1,78x10^6$ EV/cell). We did not detect differences in EV size or Z potential. We now completed the EV RNA collection and sequencing to define the RNA and microRNA repertoire of $SOD1^{G93A}$ and WT astrocyte-derived EVs. Bioinformatic analysis is ongoing. Finally, we measured MN viability as a functional readout, which appeared significantly reduced when exposing MNs to EV from $SOD1^{G93A}$ astrocytes. **Conclusions** Our results indicate that $SOD1^{G93A}$ astrocytes influence MN viability in ALS through EV secretion and suggest that EVs play a key role in spreading their neurotoxic properties.

Pubmed:

34830115: Bonifacino T, Zerbo RA, Balbi M, Torazza C, Frumento G, Fedele E, Bonanno G, Milanese M
Nearly 30 Years of Animal Models to Study Amyotrophic Lateral Sclerosis: A Historical Overview and Future Perspectives.
Amyotrophic lateral sclerosis (ALS) is a fatal, multigenic, multifactorial, and non-cell autonomous neurodegenerative disease characterized by upper and lower motor neuron loss. Several genetic mutations lead to ALS development and many emerging gene mutations have been discovered in recent years. Over the decades since 1990, several animal models have been generated to study ALS pathology including both vertebrates and invertebrates such as yeast, worms, flies, zebrafish, mice, rats, guinea pigs, dogs, and non-human primates. Although these models show different peculiarities, they are all useful and complementary to dissect the pathological mechanisms at the basis of motor neuron degeneration and ALS progression, thus contributing to the development of new promising therapeutics. In this review, we describe the up to date and available ALS genetic animal models, classified by the different genetic mutations and divided per species, pointing out their features in modeling, the onset and progression of the pathology, as well as their specific pathological hallmarks. Moreover, we highlight similarities, differences, advantages, and limitations, aimed at helping the researcher to select the most appropriate experimental animal model, when designing a preclinical ALS study.
Int J Mol Sci, 2021; 22
34573024: Marini C, Cossu V, Kumar M, Milanese M, Cortese K, Bruno S, Bellese G, Carta S, Zerbo RA, Torazza C, Bauckneht M, Venturi C, Raffa S, Orengo AM, Donegani MI, Chiola S, Ravera S, Castellani P, Morbelli S, Sambuceti G, Bonanno G
The Role of Endoplasmic Reticulum in the Differential Endurance against Redox Stress in Cortical and Spinal Astrocytes from the Newborn SOD1 Mouse Model of Amyotrophic Lateral Sclerosis.
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Antioxidants (Basel), 2021; 10

33931856: Milanese M, Bonifacino T, Torazza C, Provenzano F, Kumar M, Ravera S, Zerbo AR, Frumento G, Balbi M, Nguyen TPN, Bertola N, Ferrando S, Viale M, Profumo A, Bonanno G

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Br J Pharmacol, 2021; 178

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DECREASED NORADRENALINE LEVELS CONTRIBUTE TO CORTICAL HYPEREXCITABILITY IN MOUSE MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS), the most common adult-onset motor neuron disease, relies on the detection of the combined degeneration of upper motor neurons in the cerebral cortex, and lower motor neurons in the brain stem and spinal cord. Cortical hyperexcitability was demonstrated in ALS patients using paired-pulse transcranial magnetic stimulation (ppTMS). Cortical hyperexcitability precedes motoneuron signs, is detrimental to motoneuron function, and negatively correlates with patients' survival. This earliness could make cortical hyperexcitability a powerful feature to promote early diagnosis of ALS. To unravel the dynamics of cortical network impairment in ALS mouse models, we used electrocorticography (ECoG) and analyses of phase-amplitude frequency coupling (PAC), a specific pattern of oscillatory activity corresponding to the modulation of the amplitude of a fast oscillation (gamma) by the phase of a slow oscillation (theta), as a proxi for cortical excitation/inhibition imbalance. We demonstrate that Sod1G86R and FusΔNLS mouse models of ALS present early and constant alteration of cortical network functions, and propose EEG and PAC analyses as an alternative to ppTMS in ALS patients. To gain insights into the molecular and cellular origins of this cortical dysfunction, we analysed the cortical levels of different neurotransmitters and unravelled decreased noradrenaline levels, common to Sod1G86R and FusΔNLS mice. Experimental cortical noradrenaline depletion was sufficient to significantly decrease PAC in WT animals, while noradrenaline supplementation was sufficient to significantly improved PAC in ALS mouse models. Together, the data pave the way to the development of new diagnostic and therapeutic approaches targeting cortical network dysfunction in ALS.

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A MIRNA FINGERPRINT IN PLASMA-DERIVED EXTRACELLULAR VESICLES OF HSOD1G93A TRANSGENIC SWINE

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Two goals of Amyotrophic Lateral Sclerosis (ALS) research are: a) validation of new experimental models b) identification of diagnostic biomarkers to speed up the diagnosis. Extracellular vesicles (EVs) and their content may be reliable clinical biomarkers for ALS, as they have been used for the diagnosis and prognosis of various diseases. In this context, we developed a hSOD1G93A transgenic swine characterized by a reproducible preclinical and clinical phase to clarify certain ALS etiopathogenetic aspects. Therefore, this study aimed at evaluating of miRNA into EVs isolated from hSOD1G93A transgenic swine plasma, in preclinical and clinical phase. EVs were isolated from plasma of hSOD1G93A and wild type pigs by a modified precipitation method. EVs were characterized by Nanosight, flow cytometry and Western blotting. miRNAs were sequenced on a NextSeq 500/550. Phenotype characterization confirmed that the majority of EVs were exosomes expressing the typical exosome markers (CD63, TSG101, Flotillin 1, Alix). As regard miRNA analysis, few miRNAs were deregulated in transgenic swine, representing a specific expression pattern in the model. Also, an evident up-regulation of miR-206 was observed. This miRNA is involved in the proper formation and regeneration of the mature neuromuscular junction. Interestingly, its increased exosomal expression was also detected comparing hSOD1G93A samples at clinical phase with their corresponding preclinical samples. Nonetheless, miR-206 was up-regulated in homozygous transgenic swine compared to heterozygous ones. In conclusion, these data show that in the swine model, biomarkers already associated to ALS are expressed and that miRNAs may be used as monitoring tools for disease severity.

FENS Forum 2022
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BOARD NUMBER: S03-355

PASSIVE TRANSFER MOUSE MODEL OF ALS SHOWS DIFFERENTLY ELEVATED CALCIUM LEVELS AND LOSS OF LUMBAR MOTOR NEURONS ACCORDING TO THE GENETIC ALTERATIONS OF THE PATIENTS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Increasing evidence support that neuroinflammation has a key role in the pathobiological processes of ALS. Immunoglobulins from ALS patients showed anti-motoneuronal properties and their presence resulted in increased proinflammatory factors in the spinal cord of mice after intraperitoneal injection. Furthermore, these autoantibodies can bind and modify the function of calcium channels, which leads to disturbance in the calcium homeostasis and eventually neuronal loss. Utilizing these degenerative effects, blood sera from ALS patients without known genetic alterations or with identified mutations were injected into Balb/c mice to create an immune-based experimental motoneuron (MN) degeneration model. Previously we demonstrated increased calcium content in the axon terminals but the effect of such treatment in the perikaryon reminded unknown. Similarly to the axon terminals, significant increase in the calcium level of the MN cell bodies accompanied with remarkable motor neuronal loss was observed in the ALS serum treated group compared to the untreated and the healthy serum treated groups. Interestingly, we found that mice treated with ALS sera from patients with C9ORF72 mutation showed the most prominent elevation in the calcium level also the most robust motoneuronal loss. Expectation-Maximization cluster analysis based on the correlation of calcium elevation and motoneuronal loss is capable of separating controls and ALS patients, furthermore, C9ORF72 mutation sorted into a more progressive subgroup. Since this mutation has a significant role in numerous immune-mediated processes, in the future, we would like to examine these events which might lead to novel therapeutic targets for ALS.

Pubmed:
34576165: Polgár TF, Meszlényi V, Nógrádi B, Körmöczy L, Spisák K, Tripolszki K, Széll M, Obál I, Engelhardt JI, Siklós L, Patai R
Passive Transfer of Blood Sera from ALS Patients with Identified Mutations Results in Elevated Motoneuronal Calcium Level and Loss of Motor Neurons in the Spinal Cord of Mice.
Previously, we demonstrated the degeneration of axon terminals in mice after repeated injections of blood sera from amyotrophic lateral sclerosis (ALS) patients with identified mutations. However, whether a similar treatment affects the cell body of motor neurons (MNs) remained unresolved. Sera from healthy individuals or ALS patients with a mutation in different ALS-related genes were intraperitoneally injected into ten-week-old male Balb/c mice (= 3/serum) for two days. Afterward, the perikaryal calcium level was measured using electron microscopy. Furthermore, the optical disector method was used to evaluate the number of lumbar MNs. The cytoplasmic calcium level of the lumbar MNs of the ALS-serum-treated mice, compared to untreated and healthy-serum-treated controls, was significantly elevated. While injections of the healthy serum did not reduce the number of MNs compared to the untreated control group, ALS sera induced a remarkable loss of MNs. Similarly to the distant motor axon terminals, the injection of blood sera of ALS patients has a rapid degenerative effect on MNs. Analogously, the magnitude of the evoked changes was specific to the type of mutation; furthermore, the degeneration was most pronounced in the group treated with sera from ALS patients with a mutation in the gene.
Int J Mol Sci, 2021; 22
32389588: Nogradi B, Meszlenyi V, Patai R, Polgar TF, Spisak K, Kristof R, Siklos L
Diazoxide blocks or reduces microgliosis when applied prior or subsequent to motor neuron injury in mice.
Diazoxide (DZX), an anti-hypertonic and anti-hypoglycemic drug, was shown to have anti-inflammatory effects in several injured cell types outside the central nervous system. In the brain, the neuroprotective potential of DZX is well described, however, its anticipated anti-inflammatory effect after acute injury has not been systematically analyzed. To disclose the anti-

inflammatory effect of DZX in the central nervous system, an injury was induced in the hypoglossal and facial nuclei and in the oculomotor nucleus by unilateral axonal transection and unilateral target deprivation (enucleation), respectively. On the fourth day after surgery, microglial analysis was performed on tissue in which microglia were DAB-labeled and motoneurons were labeled with immunofluorescence. DZX treatment was given either prophylactically, starting 7 days prior to the injury and continuing until the animals were sacrificed, or postoperatively only, with daily intraperitoneal injections (1.25 mg/kg; in 10 mg/ml dimethyl sulfoxide in distilled water). Prophylactically + postoperatively applied DZX completely eliminated the microglial reaction in each motor nuclei. If DZX was applied only postoperatively, some microglial activation could be detected, but its magnitude was still significantly smaller than the non-DZX-treated controls. The effect of DZX could also be demonstrated through an extended period, as tested in the hypoglossal nucleus on day 7 after the operation. Neuronal counts, determined at day 4 after the operation in the hypoglossal nucleus, demonstrated no loss of motor neurons, however, an increased Feret's diameter of mitochondria could be measured, suggesting increased oxidative stress in the injured cells. The increase of mitochondrial Feret's diameter could also be prevented with DZX treatment.
Brain Res, 2020; 1741

32756522: Meszlényi V, Patai R, Polgár TF, Nógrádi B, Körmöczy L, Kristóf R, Spisák K, Tripolszki K, Széll M, Obál I, Engelhardt JI, Siklós L

Passive Transfer of Sera from ALS Patients with Identified Mutations Evokes an Increased Synaptic Vesicle Number and Elevation of Calcium Levels in Motor Axon Terminals, Similar to Sera from Sporadic Patients.
Previously, we demonstrated increased calcium levels and synaptic vesicle densities in the motor axon terminals (MATs) of sporadic amyotrophic lateral sclerosis (ALS) patients. Such alterations could be conferred to mice with an intraperitoneal injection of sera from these patients or with purified immunoglobulin G. Later, we confirmed the presence of similar alterations in the superoxide dismutase 1 G93A transgenic mouse strain model of familial ALS. These consistent observations suggested that calcium plays a central role in the pathomechanism of ALS. This may be further reinforced by completing a similar analytical study of the MATs of ALS patients with identified mutations. However, due to the low yield of muscle biopsy samples containing MATs, and the low incidence of ALS patients with the identified mutations, these examinations are not technically feasible. Alternatively, a passive transfer of sera from ALS patients with known mutations was used, and the MATs of the inoculated mice were tested for alterations in their calcium homeostasis and synaptic activity. Patients with 11 different ALS-related mutations participated in the study. Intraperitoneal injection of sera from these patients on two consecutive days resulted in elevated intracellular calcium levels and increased vesicle densities in the MATs of mice, which is comparable to the effect of the passive transfer from sporadic patients. Our results support the idea that the pathomechanism underlying the identical manifestation of the disease with or without identified mutations is based on a common final pathway, in which increasing calcium levels play a central role.
Int J Mol Sci, 2020; 21

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BOARD NUMBER: S03-356

EFFECTS OF EARLY-ONSET ALS PATHOLOGY ON NEURAL NETWORK DYNAMICS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease causing gradual paralysis of voluntary muscles due to the progressive degeneration of upper and lower motor neurons. There is no cure and patients usually die 3-5 years after the onset of symptoms. A crucial aspect for developing effective treatment is early intervention, but this remains a challenge due to the lack of biomarkers. Evolving pathological processes in ALS can cause changes in the dynamical interplay between structural and functional connectivity, and we hypothesize that such changes emerge before the manifestation of symptoms. We aim to identify how ALS affects the underlying structural organization and the accompanying functional connectivity of *in vitro* neural networks. We study neural networks using human induced pluripotent stem cell (hiPSC)-derived motor neurons from ALS patients and healthy donors on micro-electrode arrays (MEAs) and high-density CMOS MEAs. We apply graph theoretical and topological methods to infer differences between healthy and ALS conditions. We hypothesize that subtle changes in the underlying network structure are accompanied by abnormal network activity which may compromise the global network efficiency. Such changes could be a result of intrinsic cellular responses attempting to preserve normal function in an increasingly impaired network, effectively masking overt signs of pathology at early stages. A better understanding of early-onset network configurations could contribute to the improvement of diagnostic tools, as well as the identification of critical phases for therapeutic intervention.

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BOARD NUMBER: S03-357

ALTERATIONS IN WWOX PROTEIN AND GENE LEAD TO MITOCHONDRIAL DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Aims. To date, there are only two modestly beneficial treatments for amyotrophic lateral sclerosis (ALS), highlighting the importance of unraveling the mechanisms leading to motor neuron loss. One potential pathway is mediated by the WW domain-containing oxidoreductase (WWOX), a protein involved in neurodegeneration. ***Methods.*** Western blots were used to assess the levels of WWOX, and proteins involved in the mitochondrial electron transport chain in ALS and control post-mortem motor cortex (mCTX). Project MinE was analyzed to identify genetic variants in *WWOX*. Cell viability, ATP, and reactive oxygen species (ROS) were measured in SH-SY5Y cells following WWOX knock down using small interfering RNA (siWWOX) or treatment with wild-type and mutant recombinant WWOX proteins. siWWOX was used in a fly model and alterations in behavior were assessed. ***Results.*** WWOX levels were decreased in ALS mCTX and correlated with disease duration, suggesting a role for WWOX loss in ALS. Accordingly, siWWOX increased ROS levels in SH-SY5Y cells indicating a link between loss of WWOX and increases in oxidative stress. Similarly, siWWOX decreased sleep in a fly model, further supporting a pathogenic role. Additionally, we identified several rare and ALS specific variants in WWOX. Among these variants, the 261E stop codon mutation decreased cell viability and ATP levels, and increased ROS *in vitro*, consistent with decreases in the mitochondrial ATP synthase of complex V and the cytochrome c oxidase of complex IV in ALS mCTX. ***Conclusions.*** Together, our findings suggest that loss of WWOX or mutations in the gene exacerbate mitochondrial dysfunction in ALS.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-358

BENEFICIAL AND SEXUALLY DIMORPHIC RESPONSE TO COMBINED HDAC INHIBITOR VALPROATE AND AMPK/SIRT1 PATHWAY ACTIVATOR RESVERATROL IN THE TREATMENT OF ALS MICE

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder. Epigenetic modulation in the acetylation state of NF-kB RelA and histone 3 (H3) protein, involved in the development of neurodegeneration, is a drugable target for the class-I histone deacetylases (HDAC) inhibitors, valproate, and the AMP-activated kinase (AMPK)-sirtuin 1 pathway activator, resveratrol. Aim: In this study, we demonstrated that the combination of valproate and resveratrol can restore the acetylation state of RelA in the SOD1(G93A) murine model of ALS. We also investigated the sexually dimorphic development of the disease, as well as the sex-sensibility to the treatment administered. Method: Animals where subjected to behavioural tests to examine motor function. They were sacrificed at the end stage, and immunohistochemistry and molecular analysis were carried out on the spinal cord of the mice. Results: The combined drugs, which rescued RelA and the histone 3 acetylation state, reduced the motor deficit and the disease pathology associated with motor neuron loss and microglial reactivity, Brain-Derived Neurotrophic Factor (BDNF) and B-cell lymphoma-extra large (Bcl-xL) level decline. Specifically, the treatment administered at 50 days of life, postponed the time of onset in the male by 22 days, but not significantly in females. Nevertheless, in females, the drugs significantly reduced symptom severity of the later phase of the disease and prolonged the mice's survival. Only minor beneficial effects were produced in the latter stage in males. Conclusion: Overall, this study shows a beneficial and sexually dimorphic response to valproate and resveratrol treatment in ALS mice.

Pubmed:

26446938: Laoye BJ, Okurumeh OA, Obagaye OV, Olagunju MO, Bankole OO, Olubiyi OO, Ogundele OM
Dopamine binds calmodulin during autoregulation of dopaminergic D2 receptor signaling through CaMKIIα-calmodulin complex.
The role of dopaminergic D2 receptor (D2R) autoregulation in dopamine (DA) neurotransmission cannot be overemphasized in cause and progression of disorders associated with complex behaviors. Although previous studies have shown that D2R is structurally and physiologically linked with calcium/calmodulin-dependent kinase II (CaMKIIα), however, the role of calmodulin in the CaMKIIα complex in D2R regulation remains elusive. In this study, using structural biology modeling softwares (iGEMDOCK and CueMol), we have shown the interaction between D2R, CaMKIIα, calmodulin, and DA under varying conditions. The outcomes of this study suggest that CaMKIIα causes a change in DA binding affinity to the D2R receptive site while the detached DA binds to calmodulin to stop the activity of D2R in the D2R-dopaminergic D1 receptor (D1R) heteromer. Ultimately, we concluded that D2R autoregulates to stop its heteromeric combination with D1R. D2R interacts with D1R to facilitate calcium movement that activates calmodulin, then CaMKIIα. The CaMKIIα-calmodulin complex changes the affinity of DA-D2R causing DA to break free and bind with calmodulin.
J Recept Signal Transduct Res, 2016; 36
25916484: Olajide OJ, Enaibe BU, Bankole OO, Akinola OB, Laoye BJ, Ogundele OM
Kolaviron was protective against sodium azide (NaN3) induced oxidative stress in the prefrontal cortex.
Kolaviron is a phytochemical isolated from Garcina kola (G. kola); a common oral masticatory agent in Nigeria (West Africa). It is a bioflavonoid used--as an antiviral, anti-inflammatory and antioxidant--in relieving the symptoms of several diseases and infections. In this study we have evaluated the neuroprotective and regenerative effect of kolaviron in neurons of the prefrontal cortex (Pfc) before or after exposure to sodium azide (NaN3) induced oxidative stress. Separate groups of animals were treated as follows; kolaviron (200 mg/Kg) for 21 days; kolaviron (200 mg/Kg for 21 days) followed by NaN3 treatment (20 mg/Kg for 5 days); NaN3 treatment (20 mg/Kg for 5 days) followed by kolaviron (200 mg/Kg for 21 days); 1 ml of corn-oil (21 days-vehicle); NaN3 treatment (20 mg/Kg for 5 days). Exploratory activity associated with Pfc function was assessed in the open field test (OFT) following which the microscopic anatomy of the prefrontal cortex was examined in histology

(Haematoxylin and Eosin) and antigen retrieval Immunohistochemistry to show astroglia activation (GFAP), neuronal metabolism (NSE), cytoskeleton (NF) and cell cycle dysregulation (p53). Subsequently, we quantified the level of Glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) in the brain tissue homogenate as a measure of stress-related glucose metabolism. Kolaviron (Kv) and Kolaviron/NaN3 treatment caused no prominent change in astroglia density and size while NaN3 and NaN3/Kv induced astroglia activation and scar formation (astrogliosis) in the Pfc when compared with the control. Similarly, Kolaviron and Kv/NaN3 did not alter NSE expression (glucose metabolism) while NaN3 and NaN3/Kv treatment increased cortical NSE expression; thus indicating stress related metabolism. Further studies on enzymes of glucose metabolism (G6PDH and LDH) showed that NaN3 increased LDH while kolaviron reduced LDH in the brain tissue homogenate ($P < 0.001$). In addition kolaviron treatment before ($P < 0.001$) or after ($P < 0.05$) NaN3 treatment also reduced LDH expression; thus supporting its role in suppression of oxidative stress. Interestingly, NF deposition increased in the Pfc after kolaviron treatment while Kv/NaN3 showed no significant change in NF when compared with the control. In furtherance, NaN3 and NaN3/Kv caused a decrease in NF deposition (degeneration). Ultimately, the protective effect of KV administered prior to NaN3 treatment was confirmed through p53 expression; which was similar to the control. However, NaN3 and NaN3/Kv caused an increase in p53 expression in the Pfc neurons (cell cycle dysregulation). We conclude that kolaviron is not neurotoxic when used at 200 mg/Kg BW. Furthermore, 200 mg/Kg of kolaviron administered prior to NaN3 treatment (Kv/NaN3) was neuroprotective when compared with Kolaviron administered after NaN3 treatment (NaN3/Kv). Some of the observed effects of kolaviron administered before NaN3 treatment includes reduction of astroglia activation, absence of astroglia scars, antioxidation (reduced NSE and LDH), prevention of neurofilament loss and cell cycle regulation.
Metab Brain Dis, 2016; 31

26088184: Adeniyi PA, Ishola AO, Laoye BJ, Olatunji BP, Bankole OO, Shallie PD, Ogundele OM
Neural and behavioural changes in male periadolescent mice after prolonged nicotine-MDMA treatment.
The interaction between MDMA and Nicotine affects multiple brain centres and neurotransmitter systems (serotonin, dopamine and glutamate) involved in motor coordination and cognition. In this study, we have elucidated the effect of prolonged (10 days) MDMA, Nicotine and a combined Nicotine-MDMA treatment on motor-cognitive neural functions. In addition, we have shown the correlation between the observed behavioural change and neural structural changes induced by these treatments in BALB/c mice. We observed that MDMA (2 mg/Kg body weight; subcutaneous) induced a decline in motor function, while Nicotine (2 mg/Kg body weight; subcutaneous) improved motor function in male periadolescent mice. In combined treatment, Nicotine reduced the motor function decline observed in MDMA treatment, thus no significant change in motor function for the combined treatment versus the control. Nicotine or MDMA treatment reduced memory function and altered hippocampal structure. Similarly, a combined Nicotine-MDMA treatment reduced memory function when compared with the control. Ultimately, the metabolic and structural changes in these neural systems were seen to vary for the various forms of treatment. It is noteworthy to mention that a combined treatment increased the rate of lipid peroxidation in brain tissue.
Metab Brain Dis, 2016; 31

35162978: Bankole O, Scambi I, Parrella E, Muccilli M, Bonafede R, Turano E, Pizzi M, Mariotti R
Beneficial and Sexually Dimorphic Response to Combined HDAC Inhibitor Valproate and AMPK/SIRT1 Pathway Activator Resveratrol in the Treatment of ALS Mice.
Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disorder. There is no cure and current treatments fail to slow the progression of the disease. Epigenetic modulation in the acetylation state of NF-kB RelA and the histone 3 (H3) protein, involved in the development of neurodegeneration, is a drugable target for the class-I histone deacetylases (HDAC) inhibitors, entinostat or valproate, and the AMP-activated kinase (AMPK)-sirtuin 1 pathway activator, resveratrol. In this study, we demonstrated that the combination of valproate and resveratrol can restore the normal acetylation state of RelA in the SOD1(G93A) murine model of ALS, in order to obtain the neuroprotective form of NF-kB. We also investigated the sexually dimorphic development of the disease, as well as the sex-sensibility to the treatment administered. We showed that the combined drugs, which rescued AMPK activation, RelA and the histone 3 acetylation state, reduced the motor deficit and the disease pathology associated with motor neuron loss and microglial reactivity, Brain-Derived Neurotrophic Factor (BDNF) and B-cell lymphoma-extra large (Bcl-xL) level decline. Specifically, vehicle-administered males showed earlier onset and slower progression of the disease when compared to females. The treatment, administered at 50 days of life, postponed the time of onset in the male by 22 days, but not in a significant way in females. Nevertheless, in females, the drugs significantly reduced symptom severity of the later phase of the disease and prolonged the mice's survival. Only minor beneficial effects were produced in the latter stage in males. Overall, this study shows a beneficial and sexually dimorphic response to valproate and resveratrol treatment in ALS mice.
Int J Mol Sci, 2022; 23


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BOARD NUMBER: S03-359

SLEEP AND OREXINERGIC PATHWAY ALTERATIONS IN MICE MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease inexorably leading to an early death. Sleep disturbances have been described and appear at a later stage of the disease. Those disturbances can be triggered by muscle cramps, spasticity, or restless legs syndrome, all leading to increased wakefulness. However, sleep changes were rarely studied in the context of ALS. We used two murine models of ALS, Superoxide Dismutase 1 G86R ($Sod1^{G86R}$) and Fused in Sarcoma ($Fus^{\Delta NLS}$), which both represent 25% of the familial cases of ALS, to underpin the structural and cellular mechanism involved. A recent pathological study in ALS patients observed decreased neurons immunoreactive for orexin, a neuropeptide highly involved in sleep regulation. In both $Sod1^{G86R}$ and $Fus^{\Delta NLS}$ mice, there was no change in the number of Orexin-positive neurons in the lateral hypothalamus. However, electroencephalograms showed an increase in wakefulness and a decrease in rapid eye movement (REM) episodes in both mouse models before the onset of major motor troubles, while Suvorexant® - a dual Orexin receptor antagonist - was able to increase the REM episodes and decrease the wakefulness in both $Sod1^{G86R}$ and $Fus^{\Delta NLS}$ mice. Thus, our results show that two mouse models of ALS models display an impaired sleep pattern, that is, at least partially, dependent upon orexinergic neurons. This study is a starting point for a better comprehension of sleep defects observed in ALS patients and the potential benefit from pharmacological manipulation.

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BOARD NUMBER: S03-360

INVOLVEMENT OF INHIBITORY NEURONS IN AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA LINKED TO FUSED IN SARCOMA PROTEIN

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Mutations in the *Fused in Sarcoma (FUS)* gene, encoding for a ubiquitous and multifunctional DNA/RNA-binding protein, cause severe forms of amyotrophic lateral sclerosis (ALS), particularly when the nuclear localisation signal (NLS) of FUS is truncated. This truncation leads to the cytoplasmic mislocalisation of the protein, which is also observed in ALS and frontotemporal dementia (FTD) patients devoid of mutations. Our laboratory generated a mouse model displaying a constitutive and ubiquitous NLS deletion, and effectively induced the cytoplasmic delocalisation of FUS. This led to a cortical hyperactivity associated with molecular and ultrastructural alterations of GABAergic synapses, ALS-like motor impairments and FTD-like behavioural dysfunctions. The aim of my PhD is then to understand the contribution of inhibitory neurons in *FUS*-ALS and *FUS*-FTD. To conduct my project, we developed two new mouse models using a Cre-Lox recombination technology and mice in which the Cre recombinase expression is restricted to vesicular GABA transporter-positive neurons. By crossing these mice with mice in which *Fus* can be truncated upon recombination, we mutated *Fus* solely in inhibitory neurons. In parallel, by using mice displaying a constitutive NLS deletion which can be rescued to the wild type situation upon recombination, we truncated *Fus* in every cell type except inhibitory neurons. With histological studies we validated the recombination efficiency and are investigating the potential effects on neurons degeneration and pathological hallmarks appearance. These models are now being characterised for behavioural outcomes to determine if *Fus* truncation in inhibitory neurons is sufficient and/or necessary to induce ALS and FTD-like symptoms.

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RESCUING LOCOMOTOR DEFICITS IN AN ALS MOUSE MODEL BY EXTENDED SYNAPTOTAGMIN 1 (ESYT1) OVEREXPRESSION

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by a progressive inability to execute movement. Although the disease is characterized by the loss of spinal motor neurons (MNs), novel evidence from our laboratory has pointed towards the implication of interneurons (INs) in the progression of the disease. Specifically, V1 spinal inhibitory INs (positive for Engrailed 1 – En1) lose their connections to MNs at early pre-symptomatic stages, which might lead to a MN hyperexcitability and cell death. The present study aimed to investigate whether forced overexpression of the presynaptic protein Extended Synagtotagmin 1 (ESYT1) in INs could stabilize MN-IN connectivity and ameliorate associated behavioral deficits. ESYT1, which is downregulated in spinal neurons early in disease, has been shown to enhance neurotransmission and modulate membrane trafficking. Here, intraspinal lumbar injections of the *cre*-dependent AAV8-hSyn-DIO-hESYT1-W3SL viral construct were performed in SOD1^{G93A} mice crossed with En1^{cre} mice. Four genotypes were investigated: SOD1, SOD1;En1^{cre}, En1^{cre} and wild-type. Locomotor behavior assessment between P49 and P112 showed that viral injections attenuated the deficits in SOD1;En1^{cre} compared to SOD1 animals, in parameters such as speed, peak acceleration, step frequency, and stride length. MN quantification showed that SOD1;En1^{cre} exhibited a significantly higher number of MN compared to SOD1 littermates and wildtype mice. Together, our results suggest that interneurons can be a potential therapeutic target to delay the development of ALS motor symptoms and point to a relevant role of ESYT1 in promoting MN survival.

Pubmed:

35142515: Abreu AC, Mora S, Tristán AI, Martín-González E, Prados-Pardo Á, Moreno M, Fernández I
NMR-based Metabolomics and Fatty Acid Profiles to Unravel Biomarkers in Preclinical Animal Models of Compulsive Behavior.
Compulsivity is a key manifestation of inhibitory control deficit and a cardinal symptom of psychopathological conditions such as obsessive-compulsive and attention-deficit hyperactivity disorders, in which metabolic alterations have raised attention as putative biomarkers for early identification. The present study assessed the metabolic profile in a preclinical model of a compulsive phenotype of rats. We used the schedule-induced polydipsia (SIP) method to classify male Wistar rats into high drinkers (HDs) or low drinkers (LDs) according to their compulsive drinking rate developed by exposure to a fixed-time 60 s (FT-60) schedule of reinforcement with water available during 20 sessions. Before and after SIP, blood samples were collected for subsequent serum analysis by nuclear magnetic resonance spectroscopy coupled to multivariate analysis. Although no differences existed in the pre-SIP set, the compulsive drinking behavior induced remarkable metabolic alterations: HD rats selected by SIP exhibited a hyperlipidemic, hypoglycemic, and hyperglutaminergic profile compared with their low-compulsive counterparts. Interestingly, these alterations were not attributable to the mere exposure to reward pellets because a control experiment did not show differences between HDs and LDs after 20 sessions of pellet consumption without intermittent reinforcement. Our results shed light toward the implication of dietary and metabolic factors underpinning the vulnerability to compulsive behaviors.
J Proteome Res, 2022; 21
34066570: Sánchez-Salvador L, Prados-Pardo Á, Martín-González E, Olmedo-Córdoba M, Mora S, Moreno M
The Role of Social Stress in the Development of Inhibitory Control Deficit: A Systematic Review in Preclinical Models.
Inhibitory control deficit and impulsivity and compulsivity behaviours are present in different psychopathological disorders such as addiction, obsessive-compulsive disorders and schizophrenia, among others. Social relationships in humans and animals are governed by social organization rules, which modulate inhibitory control and coping strategies against stress. Social stress is associated with compulsive alcohol and drug use, pointing towards a determining factor in an increased

vulnerability to inhibitory control deficit. The goal of the present review is to assess the implication of social stress and dominance on the vulnerability to develop impulsive and/or compulsive spectrum disorders, with the aid of the information provided by animal models. A systematic search strategy was carried out on the PubMed and Web of Science databases, and the most relevant information was structured in the text and tables. A total of 34 studies were recruited in the qualitative synthesis. The results show the role of social stress and dominance in increased drug and alcohol use, aggressive and impulsive behaviour. Moreover, the revised studies support the role of Dopaminergic (DA) activity and the alterations in the dopaminergic D1/D2 receptors as key factors in the development of inhibitory control deficit by social stress.
Int J Environ Res Public Health, 2021; 18
33924858: Mora S, Martín-González E, Prados-Pardo Á, Flores P, Moreno M
Increased Compulsivity in Adulthood after Early Adolescence Immune Activation: Preclinical Evidence.
Immune activation during early developmental stages has been proposed as a contributing factor in the pathogenesis of neuropsychiatric conditions such as obsessive-compulsive disorder, attention-deficit/hyperactivity disorder, and autism in both human and animal studies. However, its relationship with the vulnerability to inhibitory control deficit, which is a shared feature among those conditions, remains unclear. The present work studied whether postnatal immune activation during early adolescence, combined with exposure to early-life adverse events, could lead to adult vulnerability to impulsive and/or compulsive behaviors. Male Wistar rats were exposed to lipopolysaccharide (LPS) in early adolescence at postnatal day 26 (PND26). During peripuberal period, half of the animals were exposed to a mild stress protocol. In adulthood, behavioral assessment was performed with the aid of the sustained attentional 5-choice serial reaction time (5-CSRT) task, schedule-induced polydipsia (SIP), and open-field locomotor activity and novelty reactivity. Rats exposed to LPS showed more compulsive responses than their control counterparts on 5-CSRT task, although no differences were observed in SIP or locomotor responses. Our study contributes to the knowledge of the relationship between immune activation and inhibitory control deficit. Future studies should aim to disentangle how, and to what extent, immune activation impacts behavior, and to understand the role of early life mild stress.
Int J Environ Res Public Health, 2021; 18
32798664: Mora S, Martín-González E, Prados-Pardo Á, Moreno J, López MJ, Pilar-Cuellar F, Castro E, Díaz Á, Flores P, Moreno M
Increased vulnerability to impulsive behavior after streptococcal antigen exposure and antibiotic treatment in rats.
The inflammation induced by Group A Streptococcus (GAS) infection has been viewed as a vulnerability factor in mental disorders characterized by inhibitory control deficits, such as attention-deficit/hyperactivity disorder or obsessive-compulsive disorder. Antibiotic treatment reduces GAS symptoms; however, its effects on impulsivity have not been fully assessed.
Brain Behav Immun, 2020; 89
32417273: Mora S, Merchán A, Aznar S, Flores P, Moreno M
Increased amygdala and decreased hippocampus volume after schedule-induced polydipsia in high drinker compulsive rats.
Fronto-limbic structures and serotonin 2A receptors (5-HT) have been implicated in the pathophysiology and treatment of compulsive spectrum disorders. Schedule-Induced Polydipsia (SIP), characterized by the development of excessive drinking under intermittent food reinforcement schedules, is a valid preclinical model for studying the compulsive phenotype. In the present study, we explored the individual differences and effect of SIP in brain volume and 5-HT receptor binding in fronto-limbic structures in rats selected according to their compulsive drinking behavior. Rats were divided into high (HD) and low drinkers (LD) by SIP (20 sessions); later, we analyzed the brains of HD and LD selected rats, in two different conditions: non-re-exposure (NRE) or re-exposure to SIP (RE), with four groups: LD-NRE, LD-RE, HD-NRE and HD-RE. Histological analyses were carried out for volumetric (stereology) and receptor binding (autoradiography) in the prelimbic and infralimbic cortex, dorsal hippocampus and basolateral amygdala. After SIP re-exposure, HD-RE showed an increased basolateral amygdala and a reduced hippocampus volume compared to HD-NRE rats, and also compared to LD-RE rats. No differences were found between HD and LD in NRE condition. Moreover, HD rats exhibit a lower 5-HT receptor binding in the basolateral amygdala, independently of SIP re-exposure, compared to LD rats. However, LD-RE showed a decreased 5-HT receptor binding in basolateral amygdala compared to LD-NRE. No differences were found in the remaining structures. These findings suggest that SIP might be differentially impacting HD and LD brains, pointing towards a possible explanation of how the latent vulnerability to compulsivity is triggered.
Behav Brain Res, 2020; 390
30818033: Mora S, Martín-González E, Flores P, Moreno M
Neuropsychiatric consequences of childhood group A streptococcal infection: A systematic review of preclinical models.
In recent years, clinical studies have shown strong epidemiological evidence of an increased risk of developing neuropsychiatric disorders after childhood exposure to streptococcal infection, including the Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infection (PANDAS). New preclinical studies on group A streptococcus (GAS) exposure investigate how to disentangle the influences of immune activation to induce long-term

neurobehavioral effects associated with neuropsychiatric disorders such as obsessive-compulsive disorder, schizophrenia or autism. The present systematic review collects neurobehavioral evidence regarding the use of GAS exposure in animal models to study the vulnerability to different neuropsychiatric disorders, improving our understanding of its possible causes and consequences, and compares its contribution with other preclinical models of immune activation in a variety of paradigms. Specifically, we reviewed the effects of postnatal GAS exposure, in comparison with post- and prenatal exposure to Lipopolysaccharide (LPS) and Polyinosinic:polycytidylic acid (Poly I:C), on the long-term effects concerning psychomotor, cognition and socioemotional outcomes in rodents. GAS exposure in animal models has revealed different behavioral alterations such as reduced locomotion and motor coordination, a deficit in sensorimotor gating, learning, working memory, altered social behavior, and increased anxiety and stereotyped behavior. Most of the results found are in accordance with other immune activation models -LPS and Poly I:C-, with some discrepancies. The systematic review of the literature supports the preclinical model of GAS exposure as a valid model for studying the neurobehavioral consequences of streptococcal infections. Future studies on streptococcal infection could contribute increasing our knowledge on preventive actions or treatments for neuropsychiatric disorders.
Brain Behav Immun, 2020; 86

31133835: Prados-Pardo Á, Martín-González E, Mora S, Merchán A, Flores P, Moreno M
Increased Fear Memory and Glutamatergic Modulation in Compulsive Drinker Rats Selected by Schedule-Induced Polydipsia.
Compulsive behavior is observed in several neuropsychiatric disorders such as obsessive-compulsive disorder (OCD), anxiety, depression, phobia, and schizophrenia. Thus, compulsivity has been proposed as a transdiagnostic symptom with a highly variable pharmacological treatment. Recent evidence shows that glutamate pharmacotherapy may be of benefit in impaired inhibitory control. The purpose of the present study was: first, to test the comorbidity between compulsivity and other neuropsychiatric symptoms on different preclinical behavioral models; second, to assess the therapeutic potential of different glutamate modulators in a preclinical model of compulsivity. Long Evans rats were selected as either high (HD) or low (LD) drinkers corresponding with their water intake in schedule-induced polydipsia (SIP). We assessed compulsivity in LD and HD rats by marble burying test (MBT), depression by forced swimming test (FST), anxiety by elevated plus maze (EPM) and fear behavior by fear conditioning (FC) test. After that, we measured the effects of acute administration (i.p.) of glutamatergic drugs: N-Acetylcysteine (NAC; 25, 50, 100 and 200 mg/kg), memantine (3.1 and 6.2 mg/kg) and lamotrigine (15 and 30 mg/kg) on compulsive drinking on SIP. The results obtained showed a relation between high compulsive drinking on SIP and a higher number of marbles partially buried in MBT, as well as a higher percentage of freezing on the retrieval day of FC test. We did not detect any significant differences between LD and HD rats in FST, nor in EPM. The psychopharmacological study of glutamatergic drugs revealed that memantine and lamotrigine, at all doses tested, decreased compulsive water consumption in HD rats compared to LD rats on SIP. NAC did not produce any significant effect on SIP. These results indicate that the symptom clusters of different forms of compulsivity and phobia might be found in the compulsive phenotype of HD rats selected by SIP. The effects of memantine and lamotrigine in HD rats point towards a dysregulation in the glutamatergic signaling as a possible underlying mechanism in the vulnerability to compulsive behavior on SIP. Further studies on SIP, could help to elucidate the therapeutic role of glutamatergic drugs as a pharmacological strategy on compulsive spectrum disorders.
Front Behav Neurosci, 2019; 13

29877027: Merchán A, Mora S, Gago B, Rodriguez-Ortega E, Fernández-Teruel A, Puga JL, Sánchez-Santed F, Moreno M, Flores P
Excessive habit formation in schedule-induced polydipsia: Microstructural analysis of licking among rat strains and involvement of the orbitofrontal cortex.
Schedule-induced polydipsia (SIP) is an animal model of compulsive drinking that selects for individual differences and varies across rat strains. The aim of this study was to investigate excessive habit formation by analyzing the SIP licking microstructure among rat strains, and to compare the brain areas activated by SIP in different populations. Wistar, Long Evans and Roman High- and Low-Avoidance rat strains were compared using a cluster analysis of 2 main variables, that is, frequency of licking (percentage of interpellet intervals with drinking episodes) and intensity of licking (mean number of licks per interpellet interval), and were found to exhibit high intensity and frequent licking (compulsive drinkers, CD), low intensity but frequent licking (habitual drinkers, HD), and low intensity and low-frequency licking (low drinkers, LD). The Wistar strain showed a higher frequency and intensity of licking, and had the largest group of CD rats when compared with the other strains. Regarding the acquisition of SIP, CD rats showed a higher intensity of licking when compared with the HD and LD rats. Moreover, c-Fos quantification revealed that rats in the CD group showed hyperactivity in the lateral orbitofrontal cortex and basolateral amygdala when compared with the LD group. Analyzing the SIP microstructure could be a valuable tool for understanding the role of excessive habit formation in the development of compulsive drinking and its underpinning neurobiological mechanisms.

Genes Brain Behav, 2019; 18
30201211: Mora S, Merchán A, Vilchez O, Aznar S, Klein AB, Ultved L, Campa L, Suñol C, Flores P, Moreno M
Reduced cortical serotonin 5-HT receptor binding and glutamate activity in high compulsive drinker rats.
Serotonin receptors and glutamate signaling have been implicated in the pathophysiology and treatment of compulsive spectrum disorders. Schedule-Induced Polydipsia (SIP), characterized by excessive drinking under intermittent food reinforcement schedules, is a valid model for studying the compulsive phenotype in rats. We explored the expression, function, and neurochemistry of 5-HT receptors in the frontal cortex (FC) of rats with individual differences to compulsivity. Rats were selected for high (HD) versus low (LD) drinking on SIP. First, we measured 5-HT, 5-HT, and mGlu receptors and serotonin transporter binding in different brain regions. Second, we assessed the effect of microinfusion into the medial prefrontal cortex (mPFC) of the 5-HT receptor agonist DOI, the mGlu agonist LY379268, and the combination of DOI with the 5-HT receptor antagonist M100907 and the 5-HT receptor antagonist SB242084. Finally, we measured the serotonin and glutamate efflux in mPFC in basal condition and after DOI local application. The compulsive HD rats showed a specific reduction of 5-HT receptor binding in FC compared to LD rats. The highest dose of DOI reduced compulsive drinking in HD rats on SIP, whereas LY379268 did not induce any significant effect. The 5-HT receptor antagonist M100907 reversed the DOI induced reduction on compulsive drinking in HD rats while blocking the 5-HT receptor did not affect SIP. Compulsive HD rats showed increased serotonin and decreased glutamate efflux in basal conditions that were modified by the DOI application. These findings indicate that reduced 5-HT receptor binding and glutamate neurochemical mechanisms may underlie compulsive behavior vulnerability.
Neuropharmacology, 2018; 143
29313138: Martín-González E, Prados-Pardo Á, Mora S, Flores P, Moreno M
Do psychoactive drugs have a therapeutic role in compulsivity? Studies on schedule-induced polydipsia.
Clinical studies have shown that some psychoactive recreational drugs have therapeutic applications in anxiety, depression, and schizophrenia. However, to date, there are few studies on the therapeutic potential efficacy of recreational drugs in compulsive neuropsychiatric disorders.
Psychopharmacology (Berl), 2018; 235

FENS Forum 2022
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MOTOR AND COGNITIVE BEHAVIORAL ALTERATIONS IN A NOVEL RAT MODEL OF TDP-43 OVEREXPRESSION

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Aims: Overexpression of TarDNA-binding protein-43(TDP-43) has recently been identified as one of the reasons of proteinopathy seen in various neurodegenerative diseases. In this study, we aimed to investigate behavioral alterations of rats following TDP-43 transduction via viral vectors. **Methods:** Sprague-Dawley male rats(n=8) were injected with Adeno-Associative Virus serotype 9 ($1.5X10^{12}$ gc/ml) containing the native TDP-43 with GFP under CMV promoter(AAV9-pCMV-TDP43-GFP). Control groups(n=6/each group) were received IV injections of either saline (SF) or vectors encoding only GFP (AAV9-pCMV-GFP) at postnatal day 30. After 15 days, motor skills of animals were assessed using the horizontal ladder rung walking test(HLRWT), attention and retention abilities via the novel object recognition (NOR) test, and spatial learning, memory performances via the 8-arm radial maze test(RA). **Results**: In HLRWT, motor performances of control animals were better and foot fault scores were significantly ($p<0.01$) lower than those of the experimental group. NOR test evaluated three different cognitive indexes; discrimination, recognition, and preference. Control groups received higher scores ($p<0.05$) in all of them. The time elapsed until all food rewards were consumed and the time spent in the center area of RA were significantly ($p< 0.05$) lower in the control groups. **Conclusion:** AAV9-based TDP-43 overexpression rat model induced motor, retention, and attention deficits, perhaps due to the natural neurotropism of viral capsid. This relatively low-cost and rapidly induced animal model might be used to investigate TDP-43 mediated disease mechanisms whose spectrum spans both FTLD and ALS (Supported by TUBITAK-Grant#1919B012004851). **Keywords**: AAV, TDP-43, CMV, motor behavior

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-363

PERIPHERAL INFLAMMATORY CHANGES AROUND MOTOR AXON TERMINALS IN THE TDP-43 MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Mutations in TDP-43 are only present in 2-3% of ALS patients, but TDP-43 pathology is described in 95% of the cases, suggesting a prominent role in the pathogenesis. TDP-43 mislocalization induces a pro-inflammatory response around the spinal motoneurons, but similar alterations might also present around neuromuscular junctions (NMJs). We aimed to characterize the inflammatory changes around NMJs and their role in the propagation of TDP-43 pathology. Transgenic mice with neuron-specific TDP-43 pathology and age-matched hTDP-43 -/- control animals were used at postnatal day 20, representing a late-stage pathology. The tibialis anterior (TA) and gastrocnemius (GC) muscles were used for immunostaining and quantification of CD45+ leukocytes, CD3+ T-cells, and CD68+ macrophages. Cell counting was carried out both in the innervation zone (IZ) and outside of the innervation zone (OIZ). In both muscles, the number of CD45+ and CD3+ cells was elevated, but the elevation was more pronounced in the IZ. Furthermore, these changes were more prominent in the GC, than in the TA muscle. The CD68+ cell count was only elevated in the IZ. Protein microarray revealed increased pro-inflammatory factors in both muscles. Our results showed extensive immunoreaction in the muscle samples of hTDP-43 +/+ animals. Interestingly, the higher leukocyte density and chemotactic activity in the GC muscle was inversely proportional to the rate of NMJ degeneration, since denervation is known to be more prominent in the TA, than in the GC muscle. Such elevated immunoactivity around the degenerating NMJs, suggests NMJ-specific processes.

Pubmed:
27545602: Patai R, Nógrádi B, Engelhardt JI, Siklós L
Calcium in the pathomechanism of amyotrophic lateral sclerosis - Taking center stage?
Amyotrophic lateral sclerosis is an incurable, relentlessly progressive disease primarily affecting motor neurons. The cause of the disease, except for the mutations identified in a small fraction of patients, is unknown. The major mechanisms contributing to the degeneration of motor neurons have already been disclosed and characterized, including excitotoxicity, oxidative stress, mitochondrial dysfunction, and immune/inflammatory processes. During the progression of the disease these toxic processes are not discrete, but each facilitates the deleterious effect of the other. However, due to their common reciprocal calcium dependence, calcium ions may act as a common denominator and through a positive feedback loop may combine the individual pathological processes into a unified escalating mechanism of neuronal destruction. This mini-review provides an overview of the mutual calcium dependence of the major toxic mechanisms associated with amyotrophic lateral sclerosis.
Biochem Biophys Res Commun, 2017; 483
29870639: Patai R, Nógrádi B, Meszlényi V, Obál I, Engelhardt J, Siklós L
[Calcium ion is a common denominator in the pathophysiological processes of amyotrophic lateral sclerosis].
Amyotrophic lateral sclerosis (ALS), the most frequent motor neuron disease is characterized by progressive muscle weakness caused by the degeneration of the motor neurons in the spinal cord and motor cortex. However, according to the recent observations, ALS is a rather complex syndrome which frequently involves symptoms of cognitive impairment. Therefore, ALS cases can be interpreted in a clinico-pathological spectrum spanning from the classical ALS involving only the motor system to the fronto-temporal dementia. The progression of the disease, however, manifested in the degeneration of the upper and lower motor neurons, is based on the same complex pathobiology. The main elements of the pathomechanism, such as oxidative stress, excitotoxicity, immune/inflammatory processes and mitochondrial dysfunction are well described already, which operate in orchestrated way and amplify the deleterious effect of each other. It is assumed that calcium ions act as a catalyst in this interaction, hence each of the individual mechanisms has strong, positive and reciprocal

calcium dependence thus may combine the individual pathological processes into a unified escalating mechanism of neuronal destruction. This review provides an overview of the role of calcium in connecting and amplifying the major mechanisms which lead to degeneration of the motor neurons in ALS.
Ideggyogy Sz, 2017; 70
31426859: Haskó J, Fazakas C, Molnár K, Mészáros Á, Patai R, Szabó G, Erdélyi F, Nyúl-Tóth Á, Győri F, Kozma M, Farkas AE, Krizbai IA, Wilhelm I
Response of the neurovascular unit to brain metastatic breast cancer cells.
Therapeutic resistance of cerebral secondary tumours largely depends on unique aspects linked to the neurovascular unit, especially cerebral endothelial cells and astrocytes. By using advanced microscopy techniques, here we explored novel mechanisms related to the neurovascular unit during extravasation and proliferation of triple negative breast cancer cells in the brain. Metastatic mammary carcinoma cells arrested and elongated within one hour in cerebral microvessels, but their number decreased by almost 80% in the first two days. Interestingly, malignant cells induced vasoconstriction and development of intraluminal endothelial plugs, which isolated invading cells from the circulation. During diapedesis - which usually took place on day four and five after inoculation of the tumour cells - continuity of cerebral endothelial tight junctions remained intact, indicating migration of cancer cells through the transcellular pathway. In addition, metastatic cells induced formation of multiluminal vessels and claudin-5-positive endothelial blebs. However, even severe endothelial blebbing could be reversed and the vessel morphology was restored shortly after the tumour cells completed transendothelial migration. Similar to neuro-inflammatory leukocytes, tumour cells migrated not only through the endothelial layer, but through the glia limitans perivascularis as well. Nevertheless, along with the growth of metastatic lesions by co-option of pre-existing capillaries, astrocytes and astrocyte end-feet were gradually expelled from the vessels to the border of the tumour. Taken together, we identified previously unknown mechanisms involved in the reaction of brain resident cells to invading breast cancer cells. Our results contribute to a better understanding of the complex cross-talk between tumour cells and host cells in the brain, which is essential for the identification of new therapeutic targets in this devastating disease.
Acta Neuropathol Commun, 2019; 7
34576165: Polgár TF, Meszlényi V, Nógrádi B, Körmöczy L, Spisák K, Tripolszki K, Széll M, Obál I, Engelhardt JI, Siklós L, Patai R
Passive Transfer of Blood Sera from ALS Patients with Identified Mutations Results in Elevated Motoneuronal Calcium Level and Loss of Motor Neurons in the Spinal Cord of Mice.
Previously, we demonstrated the degeneration of axon terminals in mice after repeated injections of blood sera from amyotrophic lateral sclerosis (ALS) patients with identified mutations. However, whether a similar treatment affects the cell body of motor neurons (MNs) remained unresolved. Sera from healthy individuals or ALS patients with a mutation in different ALS-related genes were intraperitoneally injected into ten-week-old male Balb/c mice (= 3/serum) for two days. Afterward, the perikaryal calcium level was measured using electron microscopy. Furthermore, the optical disector method was used to evaluate the number of lumbar MNs. The cytoplasmic calcium level of the lumbar MNs of the ALS-serum-treated mice, compared to untreated and healthy-serum-treated controls, was significantly elevated. While injections of the healthy serum did not reduce the number of MNs compared to the untreated control group, ALS sera induced a remarkable loss of MNs. Similarly to the distant motor axon terminals, the injection of blood sera of ALS patients has a rapid degenerative effect on MNs. Analogously, the magnitude of the evoked changes was specific to the type of mutation; furthermore, the degeneration was most pronounced in the group treated with sera from ALS patients with a mutation in the gene.
Int J Mol Sci, 2021; 22
32389588: Nogradi B, Meszlenyi V, Patai R, Polgar TF, Spisak K, Kristof R, Siklos L
Diazoxide blocks or reduces microgliosis when applied prior or subsequent to motor neuron injury in mice.
Diazoxide (DZX), an anti-hypertonic and anti-hypoglycemic drug, was shown to have anti-inflammatory effects in several injured cell types outside the central nervous system. In the brain, the neuroprotective potential of DZX is well described, however, its anticipated anti-inflammatory effect after acute injury has not been systematically analyzed. To disclose the anti-inflammatory effect of DZX in the central nervous system, an injury was induced in the hypoglossal and facial nuclei and in the oculomotor nucleus by unilateral axonal transection and unilateral target deprivation (enucleation), respectively. On the fourth day after surgery, microglial analysis was performed on tissue in which microglia were DAB-labeled and motoneurons were labeled with immunofluorescence. DZX treatment was given either prophylactically, starting 7 days prior to the injury and continuing until the animals were sacrificed, or postoperatively only, with daily intraperitoneal injections (1.25 mg/kg; in 10 mg/ml dimethyl sulfoxide in distilled water). Prophylactically + postoperatively applied DZX completely eliminated the microglial reaction in each motor nuclei. If DZX was applied only postoperatively, some microglial activation could be detected, but its magnitude was still significantly smaller than the non-DZX-treated controls. The effect of DZX could also be demonstrated through an extended period, as tested in the hypoglossal nucleus on day 7 after the operation. Neuronal counts, determined at day 4 after the operation in the hypoglossal nucleus, demonstrated no loss of motor neurons, however,

an increased Feret's diameter of mitochondria could be measured, suggesting increased oxidative stress in the injured cells. The increase of mitochondrial Feret's diameter could also be prevented with DZX treatment.
Brain Res, 2020; 1741
31130623: Obál I, Nógrádi B, Meszlényi V, Patai R, Ricken G, Kovacs GG, Tripolszki K, Széll M, Siklós L, Engelhardt JI
Experimental Motor Neuron Disease Induced in Mice with Long-Term Repeated Intraperitoneal Injections of Serum from ALS Patients.
In an earlier study, signs of commencing degeneration of spinal motor neurons were induced in mice with short-term intraperitoneal injections of immunoglobulin G (IgG) taken from patients with amyotrophic lateral sclerosis (ALS). Since in that study, neither weakness nor loss of motor neurons was noted, to test whether the ALS IgG in this paradigm has the potential to evoke relentless degeneration of motor neurons, treatment with repeated injections over a longer period was carried out. Mice were systematically injected intraperitoneally with serum taken from ALS patients over a 75-day period. At selected time points, the isometric force of the limbs, number of spinal motor neurons and their intracellular calcium levels were determined. Furthermore, markers of glial activation and the motoneuronal uptake of human IgG were monitored. During this period, gliosis and progressive motoneuronal degeneration developed, which led to gradual loss of spinal motor neurons, more than 40% at day 21, along with decreasing muscle strength in the limbs. The inclusion-like accumulation of IgG appeared in the perikarya with the increase of intracellular calcium in the cell bodies and motor nerve terminals. Our results demonstrate that ALS serum can transfer motor neuron disease to mice.
Int J Mol Sci, 2019; 20
33328988: Nógrádi B, Nyúl-Tóth Á, Kozma M, Molnár K, Patai R, Siklós L, Wilhelm I, Krizbai IA
Upregulation of Nucleotide-Binding Oligomerization Domain-, LRR- and Pyrin Domain-Containing Protein 3 in Motoneurons Following Peripheral Nerve Injury in Mice.
Neuronal injuries are accompanied by release and accumulation of damage-associated molecules, which in turn may contribute to activation of the immune system. Since a wide range of danger signals (including endogenous ones) are detected by the nucleotide-binding oligomerization domain-, LRR- and pyrin domain-containing protein 3 (NLRP3) pattern recognition receptor, we hypothesized that NLRP3 may become activated in response to motor neuron injury. Here we show that peripheral injury of the oculomotor and the hypoglossal nerves results in upregulation of NLRP3 in corresponding motor nuclei in the brainstem of mice. Although basal expression of NLRP3 was observed in microglia, astroglia and neurons as well, its upregulation and co-localization with apoptosis-associated speck-like protein containing a caspase activation and recruitment domain, suggesting inflammasome activation, was only detected in neurons. Consequently, increased production of active pro-inflammatory cytokines interleukin-1β and interleukin-18 were detected after hypoglossal nerve axotomy. Injury-sensitive hypoglossal neurons responded with a more pronounced NLRP3 upregulation than injury-resistant motor neurons of the oculomotor nucleus. We further demonstrated that the mitochondrial protector diazoxide was able to reduce NLRP3 upregulation in a post-operative treatment paradigm. Our results indicate that NLRP3 is activated in motoneurons following acute nerve injury. Blockade of NLRP3 activation might contribute to the previously observed anti-inflammatory and neuroprotective effects of diazoxide.
Front Pharmacol, 2020; 11
32756522: Meszlényi V, Patai R, Polgár TF, Nógrádi B, Körmöczy L, Kristóf R, Spisák K, Tripolszki K, Széll M, Obál I, Engelhardt JI, Siklós L
Passive Transfer of Sera from ALS Patients with Identified Mutations Evokes an Increased Synaptic Vesicle Number and Elevation of Calcium Levels in Motor Axon Terminals, Similar to Sera from Sporadic Patients.
Previously, we demonstrated increased calcium levels and synaptic vesicle densities in the motor axon terminals (MATs) of sporadic amyotrophic lateral sclerosis (ALS) patients. Such alterations could be conferred to mice with an intraperitoneal injection of sera from these patients or with purified immunoglobulin G. Later, we confirmed the presence of similar alterations in the superoxide dismutase 1 G93A transgenic mouse strain model of familial ALS. These consistent observations suggested that calcium plays a central role in the pathomechanism of ALS. This may be further reinforced by completing a similar analytical study of the MATs of ALS patients with identified mutations. However, due to the low yield of muscle biopsy samples containing MATs, and the low incidence of ALS patients with the identified mutations, these examinations are not technically feasible. Alternatively, a passive transfer of sera from ALS patients with known mutations was used, and the MATs of the inoculated mice were tested for alterations in their calcium homeostasis and synaptic activity. Patients with 11 different ALS-related mutations participated in the study. Intraperitoneal injection of sera from these patients on two consecutive days resulted in elevated intracellular calcium levels and increased vesicle densities in the MATs of mice, which is comparable to the effect of the passive transfer from sporadic patients. Our results support the idea that the pathomechanism underlying the identical manifestation of the disease with or without identified mutations is based on a common final pathway, in which increasing calcium levels play a central role.
Int J Mol Sci, 2020; 21

28528135: Patai R, Paizs M, Tortarolo M, Bendotti C, Obál I, Engelhardt JI, Siklós L
Presymptomatically applied AMPA receptor antagonist prevents calcium increase in vulnerable type of motor axon terminals of mice modeling amyotrophic lateral sclerosis.
Increased intracellular calcium (Ca), which might be the consequence of an excess influx through Ca-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, plays a crucial role in degeneration of motor neurons. Previously we demonstrated that the presymptomatic application of AMPA receptor antagonist, talampanel, could reduce Ca elevation in spinal motor neurons of mice carrying the G93A mutation of superoxide dismutase 1 (SOD1), modeling amyotrophic lateral sclerosis (ALS). It remained to be examined whether the remote, functionally semi-autonomous motor axon terminals could be rescued from the Ca overload, or if the terminals, where the degeneration possibly starts, already experience intractable changes at early time points. Thus using electron microscopic techniques, we measured the Ca level of motor axon terminals in the interosseus muscle of the SOD1 mutant animals, which are prototypes of vulnerable nerve endings in ALS. In line with the results obtained in the perikarya, talampanel treatment could reduce Ca increase evoked by the presence of mutant SOD1 in the axon terminals if the treatment was started presymptomatically but not at an early symptomatic stage. We also tested the Ca level in the cell bodies and axon terminals of the oculomotor neurons, which are resistant to the disease. Neither Ca increase, nor talampanel effect could be demonstrated at either time point. This is consistent with the observations that oculomotor neurons contain increased level of Ca buffer, which could reduce excess Ca load, and they also express glutamate receptor subunit type 2, which renders AMPA receptors impermeable to Ca.
Biochim Biophys Acta Mol Basis Dis, 2017; 1863
28017131: Paizs M, Patai R, Engelhardt JI, Katarova Z, Obal I, Siklos L
Axotomy Leads to Reduced Calcium Increase and Earlier Termination of CCL2 Release in Spinal Motoneurons with Upregulated Parvalbumin Followed by Decreased Neighboring Microglial Activation.
Motoneurons with naturally elevated calcium binding protein content, such as parvalbumin, are more resistant against injury. Furthermore, increase of intracellular calcium, which plays a pivotal role in injury of neurons, could be moderated by elevating their calcium binding proteins.
CNS Neurol Disord Drug Targets, 2017; 16

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CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MICROGLIA FROM A FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS PATIENT

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Microglia has helpful and detrimental actions in amyotrophic lateral sclerosis (ALS). The development of humanized mouse models with human microglia might be a promising tool to better understand the role of microglia in ALS and overcoming the limitations of the current ALS mouse models. **Aims:** We aimed to characterize *in vitro* human induced pluripotent stem cell (iPSC)-derived ALS microglia and, in an *in vivo* context, assess its cytotoxic effects to motoneurons (MN), as well as, to evaluate the differentiation of human iPSC-ALS microglial precursors in the mouse spinal cord. **Methods:** Human SOD^{I114T} iPSC were derived to microglia-like cells and their phenotype was confirmed by flow cytometry and immunofluorescence. We then, stimulated microglia with lipopolysaccharide and analysed cytokine expression by qPCR. Conditioned medium from ALS microglia was injected in the lumbar mouse spinal cord and MN counting was performed to assess its cytotoxicity. Finally, human SOD^{I114T} iPSC-derived microglial precursors were transplanted in the lumbar mouse spinal cord and studied their integration and differentiation. **Results:** *In vitro* human SOD^{I114T} iPSC-derived microglia-like cells expressed the main microglial markers (CD45^{low}, CD11b, Iba1, P2RY12), exhibited a ramified morphology and responded to a proinflammatory stimulus. Additionally, human SOD1^{I114T} iPSC-derived microglia released neurotoxic factors that induced MN degeneration in the mouse spinal cord. Transplantation of human SOD1^{I114T} iPSC-derived microglial precursors integrated and differentiated into microglia in the mouse spinal cord. **Conclusions:** This work recapitulates the preliminary data to further optimize a chimeric mouse with human ALS microglia.

Pubmed:
34815787: Amo-Aparicio J, Garcia-Garcia J, Francos-Quijorna I, Urpi A, Esteve-Codina A, Gut M, Quintana A, Lopez-Vales R
Interleukin-4 and interleukin-13 induce different metabolic profiles in microglia and macrophages that relate with divergent outcomes after spinal cord injury.
Microglia and macrophages adopt a pro-inflammatory phenotype after spinal cord injury (SCI), what is thought to contribute to secondary tissue degeneration. We previously reported that this is due, in part, to the low levels of anti-inflammatory cytokines, such as IL-4. Since IL-13 and IL-4 share receptors and both cytokines drive microglia and macrophages towards an anti-inflammatory phenotype , here we studied whether administration of IL-13 and IL-4 after SCI leads to beneficial effects. We injected mice with recombinant IL-13 or IL-4 at 48 h after SCI and assessed their effects on microglia and macrophage phenotype and functional outcomes. We also performed RNA sequencing analysis of macrophages and microglia sorted from the injured spinal cords of mice treated with IL-13 or IL-4 and evaluated the metabolic state of these cells by using Seahorse technology. We observed that IL-13 induced the expression of anti-inflammatory markers in microglia and macrophages after SCI but, in contrast to IL-4, it failed to mediate functional recovery. We found that these two cytokines induced different gene signatures in microglia and macrophages after SCI and that IL-4, in contrast to IL-13, shifted microglia and macrophage metabolism from glycolytic to oxidative phosphorylation. These findings were further confirmed by measuring the metabolic profile of these cells. Importantly, we also revealed that macrophages stimulated with IL-4 or IL-13 are not deleterious to neurons, but they become cytotoxic when oxidative metabolism is blocked. This suggests that the metabolic shift, from glycolysis to oxidative phosphorylation, is required to minimize the cytotoxic responses of microglia and macrophages. These results reveal that the metabolic fitness of microglia and macrophages after SCI contributes to secondary damage and that strategies aimed at boosting oxidative phosphorylation might be a novel approach to minimize the deleterious actions of microglia and macrophages in neurotrauma.
Theranostics, 2021; 11
34624330: Amo-Aparicio J, Garcia-Garcia J, Puigdomenech M, Francos-Quijorna I, Skouras DB, Dinarello CA, Lopez-Vales R

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Inhibition of the NLRP3 inflammasome by OLT1177 induces functional protection and myelin preservation after spinal cord injury.

Spinal cord injury (SCI) leads to irreversible functional deficits due to the disruption of axons and the death of neurons and glial cells. The inflammatory response that occurs in the injured spinal cord results in tissue degeneration; thus, targeting inflammation after acute SCI is expected to ameliorate histopathological evidence indicative of damage and, consequently, reduce functional disabilities. Interleukin 1 beta (IL-1β) and interleukin 18 (IL-18) are pro-inflammatory cytokines members of the IL-1 family that initiate and propagate inflammation. Here, we report that protein levels of IL-1β and IL-18 were increased in spinal cord parenchyma after SCI, but with different expression profiles. Whereas levels of IL-1β were rapidly increased reaching peak levels at 12 h after the injury, levels of IL-18 did not increase until 7 days after the injury. Since activation of the NLRP3 inflammasome is required for the processing and release of IL-1β and IL-18, we intraperitoneally administered OLT1177, a selective inhibitor of the NLRP3 inflammasome, to reduce the contribution of these cytokines to SCI. At a dose of 200 mg/kg, OLT1177 protected against neurological deficits and histological evidence of damage. OLT1177 also reduced the levels of IL-1β in the spinal cord after contusion injury and diminished the accumulation of neutrophils and macrophages at later time points. These data suggest that targeting the NLRP3 inflammasome with OLT1177 could be a novel therapeutic strategy to arrest neuroinflammation and reduce functional impairments after acute SCI in humans.

Exp Neurol, 2022; 347

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SIMULTANEOUS ACTIVATION OF TWO COMPLEMENTARY TARGETS, KV7.2/3 AND TSPO: A PROMISING AND NOVEL TREATMENT FOR AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterised by the death of upper and lower motoneurons (MNs). The mechanisms underlying MN degeneration in ALS are not fully known, but it might be a combination of multiple pathogenic mechanisms that culminate in a larger network disruption. Current studies are mostly focused on treating a single pathogenic pathway involved, leading to lack of effective cure for ALS. Therefore, it is pursued to find novel and combinational therapeutic strategies. In this project, we evaluate whether the simultaneous activation of two complementary targets, the voltage-gated potassium channels 7.2/3 (Kv7.2/3) and the mitochondrial translocator protein (TSPO), by the novel synthetized GRTX chemical compound could be effective for neuroprotection and gliosis reduction in MN degenerative models. GRTX effectively preserved spinal cord neurons and modulated glial reactivity in spinal cord organotypic cultures under exposure to astrocyte conditioned medium (ACM) derived from $SOD1^{G93A}$ mice astrocytes and $SOD1^{D90A}$ primary human astrocytes derived from SOD1 patients. Similar results are shown when cultures are exposed to ACM derived from $TDP43^{A90V}$ astrocytes differentiated from human induced pluripotent stem cells. *In vivo* toxicological studies indicated the safety profile of GRTX and its capacity to cross the blood-brain barrier. Daily oral administration of GRTX compound in $SOD1^{G93A}$ mice improved motor function determined by electrophysiologic, rotarod and treadmill tests. Histological studies proved that GRTX preserves motoneuron degeneration, modulates glial reactivity and maintains neuromuscular junction innervation. All these data suggest GRTX is a good candidate to be explored for the treatment of ALS.

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PARTIAL DELETION OF MGLUR5 AFFECTS MICROGLIA INFLAMMATORY PHENOTYPE, BIOENERGETIC CHARACTERISTICS, AND RED-OX STATE DURING ALS PROGRESSION IN SOD1G93A MICE.

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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AIMS In amyotrophic lateral sclerosis (ALS), glial cells contribute to disease progression and motoneuron damage. Although ALS etiology has not been entirely elucidated, the role of glutamate-mediated excitotoxicity and the involvement of metabotropic glutamate receptor type-5 (mGluR5) is widely accepted. We aimed to investigate the impact of in-vivo partial deletion of mGluR5 in $SOD1^{G93A}$ mice ($SOD1^{G93A}mGluR5^{+/-}$) on microglia inflammatory, bioenergetic, and oxidative characteristics and sex influence. METHODS Spinal cord microglia was acutely isolated from 30-and 120-days-old WT, $SOD1^{G93A}$, $WTmGluR5^{+/-}$ and $SOD1^{G93A}mGluR5^{+/-}$ mice by CD11b-linked magnetic MicroBeads. Microglia expression of pro-(CD40, CD86, MHCII, iNOS) and anti-inflammatory (CD16-32, CD206, CD163, Arg1) markers was detected by flow cytometry. Oxygen consumption, ATP synthesis, aerobic/anaerobic glucose metabolism, glutathione reductase and peroxidase, catalase and malondialdehyde were also studied. RESULTS 120-days-old microglia displayed a significantly different inflammatory pattern with respect to 30-days-old ones, showing increased MHCII, CD16-32, ARG1 and CD86. The two WT phenotypes showed high expression of CD206, CD163, and iNOS, while the $SOD1^{G93A}$ phenotypes were characterized by a higher expression of CD40. Deletion of mGluR5 did not affect the inflammatory WT or $SOD1^{G93A}$ patterns. Bioenergetic studies highlighted increased oxygen consumption and decreased ATP synthesis, accompanied by increased anaerobic glycolysis in $SOD1^{G93A}$ mice. Also, antioxidant responses and lipid peroxidation augmented in $SOD1^{G93A}$ mice. All the above changes were restored in $SOD1^{G93A}mGluR5^{+/-}$mice. Finally, sex always induced no significant differences. CONCLUSIONS Pre- and late-symptomatic microglia exhibited a different inflammatory pattern. Deleting mGluR5 in $SOD1^{G93A}$ micerestored the energetic metabolism/red-ox failure while not affecting the inflammatory status.

Pubmed:

34830115: Bonifacino T, Zerbo RA, Balbi M, Torazza C, Frumento G, Fedele E, Bonanno G, Milanese M
Nearly 30 Years of Animal Models to Study Amyotrophic Lateral Sclerosis: A Historical Overview and Future Perspectives. Amyotrophic lateral sclerosis (ALS) is a fatal, multigenic, multifactorial, and non-cell autonomous neurodegenerative disease characterized by upper and lower motor neuron loss. Several genetic mutations lead to ALS development and many emerging gene mutations have been discovered in recent years. Over the decades since 1990, several animal models have been generated to study ALS pathology including both vertebrates and invertebrates such as yeast, worms, flies, zebrafish, mice, rats, guinea pigs, dogs, and non-human primates. Although these models show different peculiarities, they are all useful and complementary to dissect the pathological mechanisms at the basis of motor neuron degeneration and ALS progression, thus contributing to the development of new promising therapeutics. In this review, we describe the up to date and available ALS genetic animal models, classified by the different genetic mutations and divided per species, pointing out their features in modeling, the onset and progression of the pathology, as well as their specific pathological hallmarks. Moreover, we highlight similarities, differences, advantages, and limitations, aimed at helping the researcher to select the most appropriate experimental animal model, when designing a preclinical ALS study.
Int J Mol Sci, 2021; 22
33931856: Milanese M, Bonifacino T, Torazza C, Provenzano F, Kumar M, Ravera S, Zerbo AR, Frumento G, Balbi M, Nguyen TPN, Bertola N, Ferrando S, Viale M, Profumo A, Bonanno G
Blocking glutamate mGlu receptors with the negative allosteric modulator CTEP improves disease course in SOD1 mouse model of amyotrophic lateral sclerosis.

The pathogenesis of amyotrophic lateral sclerosis (ALS) is not fully clarified, although excessive glutamate (Glu) transmission and the downstream cytotoxic cascades are major mechanisms for motor neuron death. Two metabotropic glutamate receptors (mGlu and mGlu) are overexpressed in ALS and regulate cellular disease processes. Expression and function of mGlu receptors are altered at early symptomatic stages in the SOD1 mouse model of ALS and knockdown of mGlu5 receptors in SOD1 mice improved disease progression.
Br J Pharmacol, 2021; 178
31540330: Bonifacino T, Rebosio C, Provenzano F, Torazza C, Balbi M, Milanese M, Raiteri L, Usai C, Fedele E, Bonanno G
Enhanced Function and Overexpression of Metabotropic Glutamate Receptors 1 and 5 in the Spinal Cord of the SOD1 Mouse Model of Amyotrophic Lateral Sclerosis during Disease Progression.
Glutamate (Glu)-mediated excitotoxicity is a major cause of amyotrophic lateral sclerosis (ALS) and our previous work highlighted that abnormal Glu release may represent a leading mechanism for excessive synaptic Glu. We demonstrated that group I metabotropic Glu receptors (mGluR1, mGluR5) produced abnormal Glu release in SOD1 mouse spinal cord at a late disease stage (120 days). Here, we studied this phenomenon in pre-symptomatic (30 and 60 days) and early-symptomatic (90 days) SOD1 mice. The mGluR1/5 agonist ()-3,5-Dihydroxyphenylglycine (3,5-DHPG) concentration dependently stimulated the release of [H]d-Aspartate ([H]d-Asp), which was comparable in 30- and 60-day-old wild type mice and SOD1 mice. At variance, [H]d-Asp release was significantly augmented in 90-day-old SOD1 mice and both mGluR1 and mGluR5 were involved. The 3,5-DHPG-induced [H]d-Asp release was exocytotic, being of vesicular origin and mediated by intra-terminal Ca release. mGluR1 and mGluR5 expression was increased in Glu spinal cord axon terminals of 90-day-old SOD1 mice, but not in the whole axon terminal population. Interestingly, mGluR1 and mGluR5 were significantly augmented in total spinal cord tissue already at 60 days. Thus, function and expression of group I mGluRs are enhanced in the early-symptomatic SOD1 mouse spinal cord, possibly participating in excessive Glu transmission and supporting their implication in ALS. Please define all abbreviations the first time they appear in the abstract, the main text, and the first figure or table caption.
Int J Mol Sci, 2019; 20
29265673: Rebosio C, Balbi M, Passalacqua M, Ricciarelli R, Fedele E
Presynaptic GLP-1 receptors enhance the depolarization-evoked release of glutamate and GABA in the mouse cortex and hippocampus.
Glucagon-like peptide-1 receptors (GLP-1Rs) have been shown to mediate cognitive-enhancing and neuroprotective effects in the central nervous system. However, little is known about their physiological roles on central neurotransmission, especially at the presynaptic level. Using purified synaptosomal preparations and immunofluorescence techniques, here we show for the first time that GLP-1Rs are localized on mouse cortical and hippocampal synaptic boutons, in particular on glutamatergic and GABAergic nerve terminals. Their activation by the selective agonist exendin-4 (1-100 nM) was able to increase the release of either [H]d-aspartate or [H]GABA. These effects were abolished by 10 nM of the selective GLP1-R antagonist exendin-3 (9-39) and were prevented by the selective adenylyl cyclase inhibitor 2',5'-dideoxyadenosine (10 µM), indicating the involvement of classic GLP-1Rs coupled to G protein stimulating cAMP synthesis. Our data demonstrate the existence and activity of presynaptic receptors for GLP-1 that could represent additional mechanisms by which this neurohormone exerts its effects in the CNS. © 2017 BioFactors, 44(2):148-157, 2018.
Biofactors, 2018; 44
28402318: Ricciarelli R, Brullo C, Prickaerts J, Arancio O, Villa C, Rebosio C, Calcagno E, Balbi M, van Hagen BT, Argyrousi EK, Zhang H, Pronzato MA, Bruno O, Fedele E
Memory-enhancing effects of GEBR-32a, a new PDE4D inhibitor holding promise for the treatment of Alzheimer's disease.
Memory loss characterizes several neurodegenerative disorders, including Alzheimer's disease (AD). Inhibition of type 4 phosphodiesterase (PDE4) and elevation of cyclic adenosine monophosphate (cAMP) has emerged as a promising therapeutic approach to treat cognitive deficits. However, PDE4 exists in several isoforms and pan inhibitors cannot be used in humans due to severe emesis. Here, we present GEBR-32a, a new PDE4D full inhibitor that has been characterized both in vitro and in vivo using biochemical, electrophysiological and behavioural analyses. GEBR-32a efficiently enhances cAMP in neuronal cultures and hippocampal slices. In vivo pharmacokinetic analysis shows that GEBR-32a is rapidly distributed within the central nervous system with a very favourable brain/blood ratio. Specific behavioural tests (object location and Y-maze continuous alternation tasks) demonstrate that this PDE4D inhibitor is able to enhance memory in AD transgenic mice and concomitantly rescues their hippocampal long-term potentiation deficit. Of great relevance, our preliminary toxicological analysis indicates that GEBR-32a is not cytotoxic and genotoxic, and does not seem to possess emetic-like side effects. In conclusion, GEBR-32a could represent a very promising cognitive-enhancing drug with a great potential for the treatment of Alzheimer's disease.
Sci Rep, 2017; 7


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ALTERED ELECTROPHYSIOLOGICAL PROPERTIES AND EXCITATORY NETWORK FUNCTION OF CORTICOMOTOR NEURONS IN C9ORF72 LOSS-OF-FUNCTION MICE

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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ALS is the most common motor neuron disease in humans, whereby upper and lower motor neurons degenerate, eventually resulting in death. A major hypothesis underlying the mechanistic origin of neurodegeneration in ALS postulates that cortical hyperexcitability facilitates cell death. Previous research has identified the G_4C_2 hexanucleotide repeat expansion in the *C9orf72* gene as the most common genetic cause of ALS; however, little is known about the contribution of the *C9orf72* gene to neuronal excitability in the primary motor cortex. Thus, using a *C9orf72* knockout loss-of-function (C9-KO LOF) mouse model, we assessed the intrinsic firing excitability of corticomotor neurons using whole-cell patch-clamp recordings made from acute brain slices. We have found that after disease onset, the action potential firing frequency is significantly higher in the C9-KO LOF mice compared to wildtype mice, highlighting cortical excitability at this timepoint. Moreover, we have found a significant reduction in spontaneous and miniature excitatory postsynaptic current frequency, as well as a significant increase in miniature excitatory postsynaptic current amplitude in the C9-KO LOF mice compared to wildtype mice, suggesting impaired basal excitatory network function. Further investigation into the local inhibitory circuitry will reveal essential information about the neurophysiological mechanisms underlying neurodegeneration in *C9orf72* ALS patients, which could contribute to the development of future therapeutic strategies.

Pubmed:
31967536: Hunt C, de Saint-Rome M, Di Salle C, Michalak A, Wilcock R, Baker A
Mapping Stakeholder Perspectives on Engagement in Concussion Research to Theory.
Involving stakeholders has been acknowledged as a way to improve quality and relevance in health research. The mechanisms that support effective research engagement with stakeholders have not been studied in the area of concussion. Concussion is a large public health concern worldwide with billions of dollars spent on health care services and research with improvements in care and service delivery not moving forward as quickly as desired. Enabling effective stakeholder engagement could improve concussion research and care.
Can J Neurol Sci, 2020; 47

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NOVEL INSIGHTS ON THE ROLE AND THERAPEUTIC POTENTIAL OF GLYCOPROTEIN NONMETASTATIC MELANOMA PROTEIN B (GPNMB) IN AMYOTROPHIC LATERAL SCLEROSIS.

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

Mauro Giuseppe Spatafora[1], Paolo Cabras[1], Gianluca Di Nolfi[1], Andrea Gazzano[1], Loris Bandirali[1], Bruno Maria Custode[1], Agnese Dimartino[1], Daniela Curti[1], Alessandra Biffi[2], Tetua Domi[3], Nilo Riva[3], Marco Peviani[1]
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Background. Increased levels of a peptide derived from Gpnmb in the cerebrospinal fluid (CSF) were recently associated with a poor prognosis in patients affected by Amyotrophic Lateral Sclerosis (ALS). On the other hand, other studies highlighted that upregulation of Gpnmb could play a neuroprotective and immunomodulatory role. **Aims.** In this study we engaged an in-depth characterization of Gpnmb alterations in SOD1.G93A transgenic (TG) rat model of ALS and in patients, to clarify the value of Gpnmb as prognostic biomarker and to identify a precise time-window, during the disease process, suitable for successful therapeutic intervention. **Methods.** We applied in-situ hybridization (ISH) and immunohistochemistry (IHC) in the central and peripheral nervous system, coupled to the assessment of Gpnmb ectodomain ($Gpnmb^{E}$) in the CSF and blood of TG rats. In parallel, $Gpnmb^{E}$ was assessed in a small cohort of ALS patients. **Results and discussion.** Gpnmb is mainly expressed in MNs in healthy conditions. However, in TG animals there is an early decrease of Gpnmb mRNA and protein levels in MNs and upregulation in reactive microglia after symptom onset. ISH and IHC highlighted a critical role for glial cells in the synthesis and release of $Gpnmb^{E}$. In parallel, we spotted a significant increase of $Gpnmb^{E}$ in the CSF and blood of TG rats, as well as in ALS patients, when the pathology is more severe. We are currently running a preclinical proof of concept study to verify the therapeutic potential of early administration of recombinant Gpnmb while monitoring $Gpnmb^{E}$ as biomarker of target engagement.

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NOVEL FUNCTIONALIZED NANOPARTICLES TARGETED TO 18KDA TRANSLOCATOR PROTEIN (TSPO) TO TRACK AND MODULATE NEUROINFLAMMATION IN ANIMAL MODELS OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Background. Neuroinflammation is recognized as a pathological hallmark and potential therapeutic target for many neurodegenerative diseases including Amyotrophic Lateral Sclerosis (ALS). However, neuroinflammatory responses are heterogeneous and reflect not only the extent of neuronal demise but also variable engagement glial cells in the attempt to cope with the neuronal damage. Thus, new pharmacological tools targeting specific cell subpopulations are warranted. We hypothesized that TSPO ligands, already widely used in the clinic to track neuroinflammation through PET, could be exploited to achieve selective cell targeting via a novel theranostic platform based on MRI/PET traceable nanoparticles (NPs). **Aims.** In this work we aimed at: i) obtaining an in-depth analysis of TSPO distribution and correlation with disease stage in ALS animal models; ii) validating TSPO-targeted nanoparticles as potential novel cell-specific pharmacological tool. **Methods.** We performed in-situ hybridization (ISH) and immunohistochemistry (IHC) experiments to investigate the expression and distribution of TSPO in the CNS of transgenic SOD1(G93A) rat model of ALS, which recapitulates the heterogeneous disease manifestations observed in patients. In parallel, we developed and validated novel polymeric NPs functionalized with TSPO-ligands. **Results, conclusions.** We confirmed by ISH/IHC a clearcut upregulation of TSPO in microglia cells in the CNS areas most severely affected by the disease. Functionalization of NPs with two TSPO-selective PET tracers (PBR-28, PK11195) determined a TSPO-dependent NPs internalization in microglia cells both in vitro and in vivo. Based on these results, we launched a proof-of-concept preclinical study (in progress) to test the therapeutic potential of NPs targeting the NF-kB proinflammatory pathway.

Pubmed:
33113845: Cipollina G, Davari Serej A, Di Nolfi G, Gazzano A, Marsala A, Spatafora MG, Peviani M
Heterogeneity of Neuroinflammatory Responses in Amyotrophic Lateral Sclerosis: A Challenge or an Opportunity?
Amyotrophic Lateral Sclerosis (ALS) is a complex pathology: (i) the neurodegeneration is chronic and progressive; it starts focally in specific central nervous system (CNS) areas and spreads to different districts; (ii) multiple cell types further than motor neurons (i.e., glial/immune system cells) are actively involved in the disease; (iii) both neurosupportive and neurotoxic neuroinflammatory responses were identified. Microglia cells (a key player of neuroinflammation in the CNS) attracted great interest as potential target cell population that could be modulated to counteract disease progression, at least in preclinical ALS models. However, the heterogeneous/multifaceted microglia cell responses occurring in different CNS districts during the disease represent a hurdle for clinical translation of single-drug therapies. To address this issue, over the past ten years, several studies attempted to dissect the complexity of microglia responses in ALS. In this review, we shall summarize these results highlighting how the heterogeneous signature displayed by ALS microglia reflects not only the extent of neuronal demise in different regions of the CNS, but also variable engagement in the attempts to cope with the neuronal damage. We shall discuss novel avenues opened by the advent of single-cell and spatial transcriptomics technologies, underlining the potential for discovery of novel therapeutic targets, as well as more specific diagnostic/prognostic not-invasive markers of neuroinflammation.
Int J Mol Sci, 2020; 21

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MODELLING ALS WITH GENOME ENGINEERED HUMANISED DROSOPHILA

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder which induces motor system degeneration causing paralysis and death within 3-5 years of diagnosis. While the underlying cause of the majority of ALS cases is unknown, over 25 different genes have been linked to inherited familial forms of the disease (fALS). Among these is Fused in Sarcoma (FUS) which is mutated in 5% of fALS cases. FUS is an RNA and DNA binding nuclear protein that regulates gene expression and mRNA splicing. In fALS patients mutations in the *FUS* gene can cause disruption of FUS nuclear localization and induce cytoplasmic aggregation. We have used novel CRISPR/Cas9 based genome engineering technology to replace the *Drosophila* FUS ortholog Caz with wildtype or ALS-associated mutant human FUS, creating strains where human FUS is expressed in the correct pattern and level for the host animal. We will present data on the motor system and other phenotypes of these models which contrasts with existing overexpression and misexpression FUS models. We envision these new high fidelity humanised strains will enable interrogation of the molecular mechanisms of ALS in a genetically tractable animal model.

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BOARD NUMBER: S03-371

MOLECULAR EFFECTS OF B-RAF AND 14-3-3 ζ/δ ON EMBRYONIC SPINAL CORD CELLS IN A SEVERE MODEL FOR SPINAL MUSCULAR ATROPHY (SMA)

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Aims: Spinal Muscular Atrophy (SMA) is an autosomal-recessive neurodegenerative disease. Mutations of the Survival of Motoneuron 1 (*SMN1*) gene cause SMA. Low levels of the SMN protein cause death of motoneurons in the spinal cord and brain stem resulting in muscular atrophy. Approved therapies focus on enhancing SMN levels. However, not all dysregulated pathways in SMA are restored by those treatments. ERK1/2, a member of the MAPK-signaling cascade, and AKT are both hyper-phosphorylated in SMA. Furthermore B-RAF, an upstream factor of ERK, and 14-3-3 ζ/δ, an upstream regulator of B-Raf, are both downregulated. B-Raf functions as a central hub between altered AKT and 14-3-3 ζ/δ signaling clusters (Hensel et al. 2021; PNAS). In this study, we aimed to evaluate the effects of upregulation of both factors on pathway regulation. Methods: Embryonic spinal cord cells of a severe mouse model of SMA were transfected with an Adeno-associated virus-vector comprising either the cDNA of B-Raf or 14-3-3 ζ/δ. Protein levels were evaluated by Western blot analyses. Results: A significant upregulation of phosphorylated ERK was observed in control cells, but not in SMA cells. No influence on B-Raf or 14-3-3 ζ/δ was observed in control and SMA cells after transduction. Conclusion: We conclude from those experiments that ERK phosphorylation is not only regulated by B-Raf, but instead by additional mechanisms. Therefore, additional strategies on top of B-Raf restauration have to be taken into consideration to achieve a more pronounced rescue of the SMA phenotype.

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BOARD NUMBER: S03-372

MITOCHONDRIAL MORPHO-FUNCTIONAL DYSFUNCTIONS IN SPINAL MUSCULAR ATROPHY

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Spinal Muscular Atrophy (SMA) is a paediatric neuromuscular disease caused by survival motor neuron (*SMN*) 1 gene mutation, leading to degeneration of lower alpha motor neurons, muscular atrophy and premature death. Despite the genetic causes of SMA are known, many aspects of its pathogenesis are still poorly understood. In the last years, mitochondrial dysfunction is emerging as a key factor in several diseases, including SMA. Here, we deepened the role of mitochondrial morpho-functional dysfunctions in SMA both at central and peripheral level in lumbar spinal cord and mouse embryonic fibroblasts (MEFs) from delta7 SMA mouse model, and in human fibroblasts derived from patients. TEM analysis highlighted dramatic ultrastructural mitochondrial alterations in SMA compared to healthy controls. Mitochondrial Network Analysis in MEFs underlined an increased mitochondrial network fragmentation and the presence of giant and swollen organelles in SMA. Then, by 2-DE and MALDI-TOF mass spectrometry on pure mitochondria isolated from the spinal cord, we identified m-Aco as differentially expressed between SMA and controls. Co-immunoprecipitation and enzymatic tests revealed Aconitase ubiquitination and loss of function both in murine and human fibroblasts. Overall, these observations suggest mitochondria as possible new targets for SMA treatment: indeed the maintenance of mitochondria integrity and mitochondrial turnover could potentially become one major site of pharmacological intervention, in the effort of identifying innovative SMN-independent therapies.

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BOARD NUMBER: S03-373

STORE-OPERATED CA2+ ENTRY IS ALTERED IN SPASTIN-LINKED HEREDITARY SPASTIC PARAPLEGIA

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Pathogenic variants in SPAST, the gene coding for spastin are the single most common cause of Hereditary spastic paraplegia (HSP), a progressive motor neuron disorder which mainly affects the axons of corticospinal motor neurons in a “dying-back” manner. Spastin is a microtubule-severing protein that in addition contributes to the ER-morphogenesis. How spastin dysregulation leads to axonal degeneration is still unclear. Increasing evidence shows that the endoplasmic reticulum (ER) and its interplay with the microtubule network is crucial for effective store-operated calcium entry (SOCE), a cellular process which is triggered by ER-Ca2+ store depletion, and requires reshaping of ER and of the ER-resident protein STIM1. Here, we link the dysregulation of spastin, to alterations in the ER structure and dynamic transport of STIM1, and show that Ca2+ regulation via SOCE is compromised in neurons derived from the induced pluripotent stem cells (iPSCs) of patients with pathogenic mutations in SPAST. Genome editing using CRISPR/Cas9 technology to correct the pathogenic variants in spastin, successfully restored spastin expression levels, Ca2+ regulation, and axonal integrity. Our results show that spastin is a key component in the regulation of Ca2+ homeostasis via SOCE and implicates SOCE dysregulation as a pathogenic mechanism in spastin-linked motor neuron disease

Pubmed:
32009903: Simmnacher K, Lanfer J, Rizo T, Kaindl J, Winner B
Modeling Cell-Cell Interactions in Parkinson's Disease Using Human Stem Cell-Based Models.
Parkinson's disease (PD) is the most frequently occurring movement disorder, with an increasing incidence due to an aging population. For many years, the post-mortem brain was regarded as the gold standard for the analysis of the human pathology of this disease. However, modern stem cell technologies, including the analysis of patient-specific neurons and glial cells, have opened up new avenues for dissecting the pathologic mechanisms of PD. Most data on morphological changes, such as cell death or changes in neurite complexity, or functional deficits were acquired in 2D and few in 3D models. This review will examine the prerequisites for human disease modeling in PD, covering the generation of midbrain neurons, 3D organoid midbrain models, the selection of controls including genetically engineered lines, and the study of cell-cell interactions. We will present major disease phenotypes in human models of PD, focusing on those phenotypes that have been detected in genetic and sporadic PD models. An additional point covered in this review will be the use of induced pluripotent stem cell (iPSC)-derived technologies to model cell-cell interactions in PD.
Front Cell Neurosci, 2019; 13
29481671: Newton T, Allison R, Edgar JR, Lumb JH, Rodger CE, Manna PT, Rizo T, Kohl Z, Nygren AOH, Arning L, Schüle R, Depienne C, Goldberg L, Frahm C, Stevanin G, Durr A, Schöls L, Winner B, Beetz C, Reid E
Mechanistic basis of an epistatic interaction reducing age at onset in hereditary spastic paraplegia.
Many genetic neurological disorders exhibit variable expression within affected families, often exemplified by variations in disease age at onset. Epistatic effects (i.e. effects of modifier genes on the disease gene) may underlie this variation, but the mechanistic basis for such epistatic interactions is rarely understood. Here we report a novel epistatic interaction between SPAST and the contiguous gene DPY30, which modifies age at onset in hereditary spastic paraplegia, a genetic axonopathy. We found that patients with hereditary spastic paraplegia caused by genomic deletions of SPAST that extended into DPY30 had a significantly younger age at onset. We show that, like spastin, the protein encoded by SPAST, the DPY30 protein controls endosomal tubule fission, traffic of mannose 6-phosphate receptors from endosomes to the Golgi, and lysosomal ultrastructural morphology. We propose that additive effects on this pathway explain the reduced age at onset of hereditary spastic paraplegia in patients who are haploinsufficient for both genes.
Brain, 2018; 141

28389476: Allison R, Edgar JR, Pearson G, Rizo T, Newton T, Günther S, Berner F, Hague J, Connell JW, Winkler J, Lippincott-Schwartz J, Beetz C, Winner B, Reid E
Defects in ER-endosome contacts impact lysosome function in hereditary spastic paraplegia.
Contacts between endosomes and the endoplasmic reticulum (ER) promote endosomal tubule fission, but the mechanisms involved and consequences of tubule fission failure are incompletely understood. We found that interaction between the microtubule-severing enzyme spastin and the ESCRT protein IST1 at ER-endosome contacts drives endosomal tubule fission. Failure of fission caused defective sorting of mannose 6-phosphate receptor, with consequently disrupted lysosomal enzyme trafficking and abnormal lysosomal morphology, including in mouse primary neurons and human stem cell-derived neurons. Consistent with a role for ER-mediated endosomal tubule fission in lysosome function, similar lysosomal abnormalities were seen in cellular models lacking the WASH complex component strumpellin or the ER morphogen REEP1. Mutations in , , or cause hereditary spastic paraplegia (HSP), a disease characterized by axonal degeneration. Our results implicate failure of the ER-endosome contact process in axonopathy and suggest that coupling of ER-mediated endosomal tubule fission to lysosome function links different classes of HSP proteins, previously considered functionally distinct, into a unifying pathway for axonal degeneration.
J Cell Biol, 2017; 216
26200799: Johann S, Heitzer M, Kanagaratnam M, Goswami A, Rizo T, Weis J, Troost D, Beyer C
NLRP3 inflammasome is expressed by astrocytes in the SOD1 mouse model of ALS and in human sporadic ALS patients.
Amyotrophic lateral sclerosis (ALS) is characterized by the degeneration of motoneurons in the cerebral cortex, brainstem and spinal cord. Neuroinflammation plays an important role in the pathogenesis of ALS and involves the activation of microglia and astrocytes. Intracellular inflammasome complexes are part of the innate immunity as they sense and execute host inflammatory responses. The best characterized component is the NLRP3 inflammasome comprised of the NLR protein NLRP3, the adaptor ASC and pro-caspase 1. The NLRP3 inflammasome is critical for the activation of caspase 1 and the processing and release of IL1β and IL18. In this study, we investigated the expression, activation and co-localization of the NLRP3 inflammasome in the spinal cord of male SOD1(G93A) mice carrying a mutant human superoxide dismutase 1 (SOD1) variant and regarded as an animal model for ALS as well as in post-mortem tissue of ALS patients. NLRP3 and its molecular components as well as IL1β were already detectable in SOD1 mice at a pre-symptomatic stage after 9 weeks and further increased in 14 week old animals. Spinal cord astrocytes were identified as the major cell type expressing NLRP3 components. In human ALS tissue, we also found increased NLRP3, ASC, IL18 and active caspase 1 levels compared to control patients. Our findings suggest that astroglial NLRP3 inflammasome complexes are critically involved in neuroinflammation in ALS.
Glia, 2015; 63

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BOARD NUMBER: S03-374

EFFECT OF HIGH-FAT DIET ON HIPPOCAMPAL SYNAPTIC TRANSMISSION AND PLASTICITY AND NEUROINFLAMMATION IN A MURINE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is the most prevalent motor neuron disease characterized by the progressive degeneration of motor functions and other non-motor symptoms, including cognitive deficits. Interestingly, it has been described a positive correlation between body mass index and delayed symptoms in ALS patients suggesting that high levels of leptin of obese patients could be underlying the increased survival rate. Thus, the aim of this study was to evaluate, in a murine model of ALS, the effect of a chronic high fat diet (HFD) intake, capable to increase leptin levels, on hippocampal synaptic transmission and plasticity and neuroinflammation markers in the hippocampus and motor cortex. Transgenic TDP43^{A315T} (Tg) and wildtype five-week old male mice were fed *ad libitum* with HFD and standard rodent chow for 8 weeks. Long-Term Potentiation (LTP) and basal synaptic transmission (BST) were measured using extracellular field potential recordings in hippocampal slices. The changes in microglia and astrocytes of hippocampus (CA1, CA2, CA3 and dentate gyrus) and motor cortex (M1 and M2) were examined by inmmunofluorescence. Our results showed that hippocampal BST and LTP were impaired in Tg regardless of the diet. Related to immunofluorescence assay, we found an increase of inflammation markers (microgliosis and astrocytosis) for most of the areas studied in Tg mice, both in the hippocampus and in the motor cortex. These effects are not modulated by HFD intake, indicating that this diet, at least in our conditions, is not enough to revert ALS effects in both areas.

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ASTROCYTIC IMPACT IN SYNAPTIC PLASTICITY AND TRANSMISSION DEFICITS IN SOD1G93A MICE

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Aims: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease, affecting mainly motor function, as well as, in some cases, cognitive function. The relevance of astrocytes in excitotoxicity and neurodegeneration has recently been recognized. Thus, we aimed to study the astrocytic contribution for synaptic activity and plasticity in the hippocampus and motor cortex of SOD1^{G93A} and wild-type (wt) mice. **Methods:** Astrocytic metabolism was selectively reduced using fluorocitrate (FC), and synaptic plasticity and transmission was assessed by eliciting long-term potentiation (LTP) protocols and recording input/output curves, respectively, in the CA1 area of hippocampal slices and layer II of primary motor cortex slices. **Results:** In the presence of FC, hippocampal synaptic responses were significantly lower in pre-symptomatic (4-6w) SOD1^{G93A} mice, when compared with wt mice. In the symptomatic phase (14-18w), SOD1^{G93A} mice exhibited higher post-tetanic potentiation and LTP magnitudes when compared with wt mice. However, astrocytic inhibition impaired significantly LTP, as well as synaptic responses, in both wt and SOD1^{G93A} mice. Regarding the motor cortex, pre-symptomatic SOD1^{G93A} mice showed an impairment in LTP magnitude and basal synaptic transmission. Interestingly, presence of FC led to an impairment of LTP only in wt mice, to similar levels that of SOD1^{G93A} mice, in both stages of disease. **Conclusions:** These findings suggest that, in the hippocampus, astrocytes are essential for the maintenance of LTP in healthy and ALS conditions. More importantly, in the motor cortex of SOD1^{G93A} mice, astrocytes seem to be impaired even before the onset of symptoms. SFRH/BD/147277/2019, PTDC/BTM-SAL/32147/2017 and 2021 ISN Career Development Grant.

Pubmed:
28567000: Pinto S, Cunha C, Barbosa M, Vaz AR, Brites D
Exosomes from NSC-34 Cells Transfected with hSOD1-G93A Are Enriched in miR-124 and Drive Alterations in Microglia Phenotype.
Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disorder affecting motor neurons (MNs). Evidences indicate that ALS is a non-cell autonomous disease in which glial cells participate in both disease onset and progression. Exosomal transfer of mutant copper-zinc superoxide dismutase 1 (mSOD1) from cell-to-cell was suggested to contribute to disease dissemination. Data from our group and others showed that exosomes from activated cells contain inflammatory-related microRNAs (inflamma-miRNAs) that recapitulate the donor cell. While glia-derived exosomes and their effects in neurons have been addressed by several studies, only a few investigated the influence of motor neuron (MN)-derived exosomes in other cell function, the aim of the present study. We assessed a set of inflamma-miRs in NSC-34 MN-like cells transfected with mutant SOD1(G93A) and extended the study into their derived exosomes (mSOD1 exosomes). Then, the effects produced by mSOD1 exosomes in the activation and polarization of the recipient N9 microglial cells were investigated. Exosomes in coculture with N9 microglia and NSC-34 cells [either transfected with either wild-type (wt) human SOD1 or mutant SOD1(G93A)] showed to be transferred into N9 cells. Increased miR-124 expression was found in mSOD1 NSC-34 cells and in their derived exosomes. Incubation of mSOD1 exosomes with N9 cells determined a sustained 50% reduction in the cell phagocytic ability. It also caused a persistent NF-kB activation and an acute generation of NO, MMP-2, and MMP-9 activation, as well as upregulation of IL-1β, TNF-α, MHC-II, and iNOS gene expression, suggestive of induced M1 polarization. Marked elevation of IL-10, Arginase 1, TREM2, RAGE, and TLR4 mRNA levels, together with increased miR-124, miR-146a, and miR-155, at 24 h incubation, suggest the switch to mixed M1 and M2 subpopulations in the exosome-treated N9 microglial cells. Exosomes from mSOD1 NSC-34 MNs also enhanced the number of senescent-like positive N9 cells. Data suggest that miR-124 is translocated from the mSOD1 MNs to exosomes, which determine early and late phenotypic alterations in the recipient N9-microglial cells. In conclusion, modulation of the inflammatory-associated miR-124, in mSOD1 NSC-34 MNs, with potential benefits in the cargo of their exosomes may reveal a promising therapeutic strategy in halting microglia activation and associated effects in MN degeneration.

Front Neurosci, 2017; 11
34572073: Garcia G, Pinto S, Cunha M, Fernandes A, Koistinaho J, Brites D
Neuronal Dynamics and miRNA Signaling Differ between SH-SY5Y and Mutant iPSC-Derived AD Models upon Modulation with miR-124 Mimic and Inhibitor.
Neuronal miRNA dysregulation may have a role in the pathophysiology of Alzheimer's disease (AD). miRNA(miR)-124 is largely abundant and a critical player in many neuronal functions. However, the lack of models reliably recapitulating AD pathophysiology hampers our understanding of miR-124's role in the disease. Using the classical human SH-SY5Y-neuroblastoma cells (SH-) and the mutant iPSC-derived neurons (iNEU-), we observed a sustained upregulation of miR-124/miR-125b/miR-21, but only miR-124 was consistently shuttled into their exosomes. The miR-124 mimic reduced gene expression in both AD models. While miR-124 mimic in SH- neurons led to neurite outgrowth, mitochondria activation and small Aβ oligomer reduction, in iNEU- cells it diminished Tau phosphorylation, whereas miR-124 inhibitor decreased dendritic spine density. In exosomes, cellular transfection with the mimic predominantly downregulated miR-125b/miR-21/miR-146a/miR-155. The miR-124 inhibitor upregulated miR-146a in the two experimental cell models, while it led to distinct miRNA signatures in cells and exosomes. In sum, though miR-124 function may be dependent on the neuronal AD model, data indicate that keeping miR-124 level strictly controlled is crucial for proper neuronal function. Moreover, the iNEU- cellular model stands out as a useful tool for AD mechanistic studies and perhaps for the development of personalized therapeutic strategies.
Cells, 2021; 10
31024256: Vaz AR, Pinto S, Ezequiel C, Cunha C, Carvalho LA, Moreira R, Brites D
Phenotypic Effects of Wild-Type and Mutant SOD1 Expression in N9 Murine Microglia at Steady State, Inflammatory and Immunomodulatory Conditions.
Accumulation of mutated superoxide dismutase 1 (mSOD1) in amyotrophic lateral sclerosis (ALS) involves injury to motor neurons (MNs), activation of glial cells and immune unbalance. However, neuroinflammation, besides its detrimental effects, also plays beneficial roles in ALS pathophysiology. Therefore, the targeting of microglia to modulate the release of inflammatory neurotoxic mediators and their exosomal dissemination, while strengthening cell neuroprotective properties, has gained growing interest. We used the N9 microglia cell line to identify phenotype diversity upon the overexpression of wild-type (WT; hSOD1) and mutated G93A (hSOD1) protein. To investigate how each transduced cell respond to an inflammatory stimulus, N9 microglia were treated with lipopolysaccharide (LPS). Glycoursodeoxycholic acid (GUDCA) and dipeptidyl vinyl sulfone (VS), known to exert neuroprotective properties, were tested for their immunoregulatory properties. Reduced Fizz1, IL-10 and TLR4 mRNAs were observed in both transduced cells. However, in contrast with hSOD1-induced decreased of inflammatory markers, microglia transduced with hSOD1 showed upregulation of pro-inflammatory (TNF-α/IL-1β/HMGB1/S100B/iNOS) and membrane receptors (MFG-E8/RAGE). Importantly, their derived exosomes were enriched in HMGB1 and SOD1. When inflammatory-associated miRNAs were evaluated, increased miR-146a in cells with overexpressed hSOD1 was not recapitulated in their exosomes, whereas hSOD1 triggered elevated exosomal miR-155/miR-146a, but no changes in cells. LPS stimulus increased M1/M2 associated markers in the naïve microglia, including MFG-E8, miR-155 and miR-146a, whose expression was decreased in both hSOD1 and hSOD1 cells treated with LPS. Treatment with GUDCA or VS led to a decrease of TNF-α, IL-1β, HMGB1, S100B and miR-155 in hSOD1 microglia. Only GUDCA was able to increase cellular IL-10, RAGE and TLR4, together with miR-21, while decreased exosomal miR-155 cargo. Conversely, VS reduced MMP-2/MMP-9 activation, as well as upregulated MFG-E8 and miR-146a, while producing miR-21 shuttling into exosomes. The current study supports the powerful role of overexpressed hSOD1 in attenuating M1/M2 activation, and that of hSOD1 in switching microglia from the steady state into a reactive phenotype with low responsiveness to stimuli. This work further reveals GUDCA and VS as promising modulators of microglia immune response by eliciting common and compound-specific molecular mechanisms that may promote neuroregeneration.
Front Cell Neurosci, 2019; 13

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BOARD NUMBER: S03-376

DOES REGULAR CAFFEINE CONSUMPTION IMPACT COGNITION IN AMYOTROPHIC LATERAL SCLEROSIS?

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Caffeine, the most consumed psychoactive substance worldwide, positively impacts the risk to develop Parkinson's disease and Alzheimer's disease. Compelling epidemiological evidence also support that regular caffeine consumption modulates synaptic plasticity and reduces cognitive decline in ageing, Alzheimer's disease and other neurological/neuropsychiatric conditions. Amyotrophic Lateral Sclerosis (ALS), a fatal and devastating neurodegenerative disease, is characterized by the loss of corticospinal neurons and motor neurons within the brainstem and spinal cord leading to death within 2-5 years following diagnosis. About 50% of ALS patients also develop non-motor symptoms encompassing cognitive and behavioral changes that appear before or after motor onset. While few studies addressed the impact of habitual caffeine on ALS risk, its impact on the progression of the cognitive disorders has been overlooked. To address this question we used the Pulse cohort, a prospective multi-centric and multi-modal French cohort following ALS patients from diagnosis up to end of life. Among the 463 patients included, 358 filled out a detail consumption caffeine survey. The link to ALS phenotype, ALSFRS scale, cognitive (ECAS) scores was then investigated. We observed a significative correlation between regular caffeine consumption and ECAS ($p<0.01$, $r=0.19$) within the 358 patients' population that was more pronounced within the population presenting with bulbar phenotype ($n=78$, $p<0.01$, $r=0.43$). In the later, we also observed a trend for a longer survival linked to the caffeine consumption ($p=0.08$). We demonstrate for the first time a positive impact of the regular caffeine consumption on cognitive function in ALS patients, predominantly on the bulbar form.

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BOARD NUMBER: S03-377

ALTERED EXPRESSION OF PEROXISOMAL MARKERS IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a degenerative disorder affecting both upper and lower motor neurons, leading to skeletal muscle atrophy and weakness. Increased energy expenditure, lipid metabolism alterations and oxidative stress, related to mitochondrial dysfunction, play crucial roles in ALS onset and progression. The interplay between mitochondria and peroxisomes, mainly in controlling lipid and reactive oxygen species (ROS) metabolism, is receiving growing attention, as (dys)-functions in either of the two determine altered metabolism of the other. Importantly, peroxisomes are highly dynamic, responding to physiopathological changes, by altering their enzyme content, morphology, size and abundance. These features are modulated by peroxisome-proliferator activated receptors (PPARs), a class of ligand-activated transcription factors, and by their coactivator PGC1α, which also controls mitochondrial biogenesis. We addressed the role played by peroxisomes in ALS, by analyzing the expression of peroxisomal membrane protein of 70 kDa, acyl-CoA oxidase, acetyl-CoA acyltransferase and catalase, in the spinal cord and striated muscle of a transgenic mouse model overexpressing the human mutation SOD1-G93A. The expression of mitochondrial and cytosolic antioxidant enzymes were also studied. Immunoblotting analysis of peroxisomal proteins and antioxidant enzymes shows an increase concomitant with pathology progression, suggesting an enhancement in the number of peroxisomes and in fatty acyl β-oxidation. Consistent results were obtained by qRT-PCR analysis of the same markers. These data correlate with the increase of PGC1-α that, stimulating peroxisomal and mitochondrial biogenesis, elicits an antioxidant response to redox imbalance. Overall, we suggest an involvement of peroxisomes in ALS onset and progression, possibly coping with mitochondrial dysfunction.

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BOARD NUMBER: S03-377a

ROLE OF THE CHRONIC STRESS ON THE TDP-43 SUBCELLULAR LOCALIZATION IN FIBROBLASTS OF THREE DIFFERENT ALS-RELATED TARDBP MUTATIONS

POSTER SESSION 03 - SECTION: AMYOTROPHIC LATERAL SCLEROSIS

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Aims. TAR-DNA-Binding-Protein 43 (TDP-43) forms cytoplasmic aggregates in ALS, thought to play a role in the pathogenesis of the disease. Here, we analyzed the subcellular localization of TDP-43 after chronic stress in fibroblasts of three patients, each affected by a specific TARDBP gene mutation.

Methods. Skin fibroblasts were purified from three ALS patients, each carrying a pathogenic TARDBP variant (G376D,familial; G294V, sporadic; A382T,sporadic). The G376D variant associated to a more aggressive phenotype. Control fibroblasts were from a sporadic ALS, without known mutations. Chronic stress was induced by arsenite for 30 hours. Immunofluorescence was performed at rescue, and 24,48,72 hours later. We used antibodies to TDP-43 and TIA-R (a marker for SG). The co-expression of TDP-43 and G3BP1 in each mutant fibroblast was analyzed with the confocal microscope Nikon A1.

Results. Chronic stress induced the formation of TIA-R-positive SGs that persisted up to 48h after rescue. In all three cell types, TDP-43 formed cytoplasmic inclusions, which co-localized with TIA-R. The TDP-43 inclusions disappeared at 48h after rescue. Note that TDP-43 was diffusely expressed in the cytoplasm of the mutant G376D fibroblasts, but not in the other mutant cells.

Conclusions. Chronic stress induced TDP-43 translocation to the cytoplasm of all mutant cells, where it forms transient aggregates. Therefore, these inclusions are probably not related to the persistent aggregates found in the disease. Furthermore, the diffuse cytoplasm mislocalization of the G376D TDP-43 protein after stress might represent a biological correlate of the more aggressive phenotype that characterizes this mutation.

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HIDDEN TARGETS OF AUTISM SPECTRUM DISORDERS: DISSECTING THE PATHOPHYSIOLOGY OF WAC IN THE UBIQUITIN-PROTEASOME SYSTEM

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Aims: In order to enable and maintain the physiological organization and functioning of the cerebral cortex, an internal balance of necessary elements, including protein synthesis and breakdown, are crucial. A prevailing hypothesis suggests that protein levels are altered upon mutations in the ubiquitin-proteasome system (UPS), leading to an accumulation of protein substrates and thus interfering with an usually balanced system. Unsurprisingly, our current understanding supports an involvement of ubiquitin-dependent degradation in the development of autism spectrum disorders (ASDs). Therefore, we aim to decipher effects and consequences of proteasome dysfunction by analyzing the molecular and behavioral changes of a mouse model haploinsufficient for the high risk ASD gene *Wac.* **Methods:** We first assessed *Wac* regional and temporal expression throughout mouse development and adulthood by western blot and qPCR analyses. Furthermore, we employed a broad array of histological, molecular and behavioral assays in *Wac*$^{+/-}$ mice. Specific attention is drawn onto neurogenesis, neuronal proliferation and migration, but also other processes mutually affected. **Results and Conclusion:** *Wac* haploinsufficient animals display hyperactivity, mild sociability defects and aberrant freezing behavior in the contextual fear conditioning task. In addition, *Wac* animals present slight brain abnormalities at juvenile and adult stages. To investigate effects of Wac loss on brain protein composition and its direct interaction partners, quantitative proteomic analyses are ongoing. The multidisciplinary combination of cellular, molecular und quantitative techniques aims to tackle the role of UPS function in brain development from multiple angles. Our findings indicate a pivotal role of *Wac* for normal brain development and function.

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IMPAIRMENT OF OLFACTORY HABITUATION AND DISRUPTED AFFECTIVE DISCRIMINATION IN NEGR1 MOUSE MODEL FOR AUTISM

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Olfaction is the most prominent sensory input for affective recognition in animals. Although it is well-known that both olfactory and social dysfunctions are observed simultaneously in autism specturm disroder (ASD), little is known about the neural mechanism that explains both impairments are interrelated. Neuronal growth regulator 1 (Negr1) belongs to the the immunoglobulin superfamily of cell adhesion molecules, which has been reported as one of the autism candidate genes. Here we showed Negr1$^{-/-}$ mice impaired in discrminating between affective and control demonstrator mice (i.e., stressful or painful vs neutral), meaning that they did not approach or avoid the affective demonstrator in a preferential way. Besides, Negr1$^{-/-}$ mice, although, had intact olfactory sensitivity on motivational state, were not (dis)habituated to replaced or repetitive odors. Next, when newly generated olfactory neurons fail to integrate into the olfactory bulb, olfactory functions such as odor discrimination are dysregulated. Furthermore, we reported that hippocampal neurogenesis was reduced in Negr1$^{-/-}$ mouse in our previous work. So we measured the number of proliferative cells (Ki67^{+}) using immunohistochemstry along the rostral migratory stream (RMS) known as the migratory pathway for the olfactory neuronal precursor cells. We found the number of Ki67^{+} cells along the RMS of Negr1$^{-/-}$ mouse was decreased. These results suggested, in Negr1$^{-/-}$ autism model mouse, the capacity for neurogenesis along the RMS is limited, which in turn would diminish the integration of newly generated inhibitory neurons into the olfactory bulb. This fail of integration would lead to dysregulated olfactory response, followed by maladaptive affective recognition.

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CHARACTERISING SMALL INTESTINE MOTILITY PATTERNS IN THE NEUROLIGIN-3R451C MOUSE MODEL OF AUTISM

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Gastrointestinal (GI) motility is regulated by the intrinsic nervous system of the gut; the enteric nervous system (ENS). GI dysfunction is commonly reported in individuals with autism spectrum disorder (ASD; autism). We previously reported colonic dysfunction in mice expressing the autism-associated R451C mutation in neuroligin-3 ($NL3^{R451C}$ mice) and increased numbers of neuronal nitric oxide synthase (NOS)-expressing myenteric neurons in the jejunum of the small intestine, however whether small intestine motility is altered is unknown. Here we aimed to characterise small intestine function in $NL3^{R451C}$ mice. Mouse jejunum and ileum segments were cannulated and perfused with physiological saline in an organ bath. Neurally-mediated gut contractions were recorded in control, NOLA (Nω-Nitro-L-arginine; an inhibitor of Nitric Oxide Synthase (NOS)) and washout conditions for 1 hour each. We utilized customised MATLAB based software (“GutMap”) to convert video recordings to spatiotemporal heatmaps to analyse motility patterns. Propagating contractile clusters (PCCs) in the jejunum decreased in response to NOLA, suggesting PCCs are NOS-dependent. Compared to wild-type mice, the proximal, mid, and distal jejunal diameter of $NL3^{R451C}$ mice was constricted in control conditions. In NOLA and washout conditions, the diameter of the distal jejunum was also constricted compared to wild-type mice. High frequency (0.5-0.7 Hz) contractions were increased in $NL3^{R451C}$ jejunum in control and washout conditions. Ileal motility patterns were similar across genotypes and in response to NOLA. We show that small intestinal (jejunal) motility patterns are altered in $NL3^{R451C}$ mice suggesting that alterations in the ENS may contribute to GI dysfunction in autism.

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IMPACT OF OMEGA-3 DIET ON BEHAVIOR AND HISTOLOGY IN AN ENVIRONMENTAL MOUSE MODEL OF AUTISM SPECTRUM DISORDER

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Autism spectrum disorder (ASD) is characterized by deficits in social communication as well as increased stereotyped and repetitive behavior. Motor and gait impairments are amongst the earliest and most consistent signs of this neurodevelopmental disorder but remain excluded from the diagnosis criteria of the disease. Recently, omega-3 long chain polyunsaturated fatty acids (PUFA) have been shown to decrease motor symptoms in ASD patients and prevent behavioral deficits in maternal immune activation mouse models of ASD**.** Our main aim is to determine omega-3 long chain PUFA impact on both motor and social behaviors as well as cellular correlates in the valproic acid mouse model (VPA). Pregnant female mice (C57BL/6J) were injected i.p. either with valproic acid (450mg/kg) or saline solution at embryonic day E12.5. Both dams and offspring were subjected to omega-3 long chain PUFA supplemented diet or omega-3 precursor PUFA balanced diet from the first day of gestation to the end of the experiment. From P45 on, male and female offspring underwent social and motor behavioral tests before being sacrificed at P60 for further biological analysis. Stereological analysis of Purkinje cell number was performed, cerebellum and liver lipid composition were investigated, and feces from all animal groups were analyzed to determine microbiota and metabolic profiles. Our results indicate that a balanced diet with omega-3 PUFA precursor may be sufficient to alleviate ASD motor and social symptoms and that omega-3 long chain PUFA supplementation brings only limited benefits in these conditions.

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METABOLIC DEFECTS IN 16P11.2-DEFICIENT PRIMARY MOUSE BRAIN ENDOTHELIAL CELLS

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Brain development and function are highly reliant on adequate development and maintenance of vascular networks. As such, early impairments in vascular health can lead to neurodevelopmental defects. Despite a wealth of knowledge on neuronal mechanisms of autism spectrum disorders (ASD), very few studies have considered the role of the brain vasculature in ASD. ASD are neurodevelopmental conditions associated with genetic origins such as the common 16p11.2 deletion, which leads to a haploinsufficiency of ~30 genes. We recently revealed that the 16p11.2 deletion induced endothelial-dependent structural and functional vascular abnormalities in the mouse brain, establishing a novel link between vascular impairments and ASD. While we now know that the 16p11.2 deletion leads to endothelial dysfunction, the mechanisms leading to these dysfunctions remain unknown. To address this knowledge gap, we used metabolomics and assessed mitochondrial parameters to decipher core features of brain endothelial cells (ECs) isolated from 14-day-old 16p11.2-deficient and wild-type (WT) mice. We identified a lower concentration of metabolites in 16p11.2-deficient ECs compared to WT ECs. Notably, 16p11.2-deficient ECs displayed an energetic failure with a 50% reduction of high energy metabolites including ATP, CTP, GTP, and UTP, which are critical energy fuels required for the maintenance of cellular processes. Currently, we are attempting to rescue these phenotypes by targeting various pathways. This study will provide new insight into key players of ASD pathogenesis, an essential pre-requisite for the development of transformative therapeutic strategies.

Pubmed:
32661394: Ouellette J, Toussay X, Comin CH, Costa LDF, Ho M, Lacalle-Aurioles M, Freitas-Andrade M, Liu QY, Leclerc S, Pan Y, Liu Z, Thibodeau JF, Yin M, Carrier M, Morse CJ, Dyken PV, Bergin CJ, Baillet S, Kennedy CR, Tremblay MÈ, Benoit YD, Stanford WL, Burger D, Stewart DJ, Lacoste B
Vascular contributions to 16p11.2 deletion autism syndrome modeled in mice.
While the neuronal underpinnings of autism spectrum disorder (ASD) are being unraveled, vascular contributions to ASD remain elusive. Here, we investigated postnatal cerebrovascular development in the 16p11.2 mouse model of 16p11.2 deletion ASD syndrome. We discover that 16p11.2 hemizygosity leads to male-specific, endothelium-dependent structural and functional neurovascular abnormalities. In 16p11.2 mice, endothelial dysfunction results in impaired cerebral angiogenesis at postnatal day 14, and in altered neurovascular coupling and cerebrovascular reactivity at postnatal day 50. Moreover, we show that there is defective angiogenesis in primary 16p11.2 mouse brain endothelial cells and in induced-pluripotent-stem-cell-derived endothelial cells from human carriers of the 16p11.2 deletion. Finally, we find that mice with an endothelium-specific 16p11.2 deletion (16p11.2) partially recapitulate some of the behavioral changes seen in 16p11.2 syndrome, specifically hyperactivity and impaired motor learning. By showing that developmental 16p11.2 haploinsufficiency from endothelial cells results in neurovascular and behavioral changes in adults, our results point to a potential role for endothelial impairment in ASD.
Nat Neurosci, 2020; 23
34744690: Ouellette J, Lacoste B
From Neurodevelopmental to Neurodegenerative Disorders: The Vascular Continuum.
Structural and functional integrity of the cerebral vasculature ensures proper brain development and function, as well as healthy aging. The inability of the brain to store energy makes it exceptionally dependent on an adequate supply of oxygen and nutrients from the blood stream for matching colossal demands of neural and glial cells. Key vascular features including a dense vasculature, a tightly controlled environment, and the regulation of cerebral blood flow (CBF) all take part in brain health throughout life. As such, healthy brain development and aging are both ensured by the anatomical and functional interaction between the vascular and nervous systems that are established during brain development and maintained

throughout the lifespan. During critical periods of brain development, vascular networks remodel until they can actively respond to increases in neural activity through neurovascular coupling, which makes the brain particularly vulnerable to neurovascular alterations. The brain vasculature has been strongly associated with the onset and/or progression of conditions associated with aging, and more recently with neurodevelopmental disorders. Our understanding of cerebrovascular contributions to neurological disorders is rapidly evolving, and increasing evidence shows that deficits in angiogenesis, CBF and the blood-brain barrier (BBB) are causally linked to cognitive impairment. Moreover, it is of utmost curiosity that although neurodevelopmental and neurodegenerative disorders express different clinical features at different stages of life, they share similar vascular abnormalities. In this review, we present an overview of vascular dysfunctions associated with neurodevelopmental (autism spectrum disorders, schizophrenia, Down Syndrome) and neurodegenerative (multiple sclerosis, Huntington's, Parkinson's, and Alzheimer's diseases) disorders, with a focus on impairments in angiogenesis, CBF and the BBB. Finally, we discuss the impact of early vascular impairments on the expression of neurodegenerative diseases.
Front Aging Neurosci, 2021; 13
34950893: Ouellette J, Lacoste B
Isolation and functional characterization of primary endothelial cells from mouse cerebral cortex.
Endothelial cells (ECs) lining blood vessels are implicated in organ development, function, and maintenance. We present a detailed protocol enabling isolation and characterization of primary mouse brain ECs, including quality controls and functional assays. These procedures promote survival of primary brain ECs for the assessment of endothelial health. Since alterations in brain ECs are involved in the onset and progression of neurological disorders, this protocol represents a valuable tool to better understand the roles of ECs in brain health. For complete details on the use and execution of this profile, please refer to Ouellette et al. (2020).
STAR Protoc, 2021; 2

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CHARACTERIZATION OF THE NITRERGIC SYSTEM IN THE VPA MURINE MODEL OF AUTISM

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Although progresses have been made in the knowledge of autism, there is still a long way to understand its cellular, neuroanatomical, and behavioural bases. One of the main neuronal alterations observed in autism is an imbalance between excitatory and inhibitory activity due to GABAergic system defects. It has also been shown that nitric oxide (NO) participates in the modulation of excitatory *versus* inhibitory neurotransmission by interacting with both glutamate and GABA. Until now, little is known about how the nitrergic system works in the autistic brain. In this study we investigated the nitrergic system in the valproic acid-induced rodent model of autism (VPA). We focused on olfactory bulb, since recent studies report the importance of sensorial dysregulations in this neurodevelopmental disorder, highlighting olfaction. We carried out histochemistry for nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) and immunohistochemistry for the different isoforms of nitric oxide synthase (NOS) to analyse the expression of enzymes involved in NO production. Finally, we determined the NO levels through quantification of its metabolites using the Griess technique. Our preliminary results indicate a relevant decrease in both NADPH-d activity and neuronal NOS expression. This leads us to think that NO production could be significantly reduced in the VPA model. Examining the nitrergic system will shed light on the research field of autism spectrum disorders, as it is known that alterations in this system, as a regulator of neuronal excitability, could be participating in or aggravating the important excitatory/inhibitory imbalance observed. E-mail: cabedonavarro.valeria@usal.es; ddiaz@usal.es; jralonso@usal.es Support: MICINN, JCyL, USAL

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BOARD NUMBER: S03-384

IMMUNE ACTIVATION DURING EARLY-LIFE DEVELOPMENT CHANGES THE PSYCHOSOCIAL BEHAVIOR OF ADULT MICE

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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The development of the nervous system is a complex and fragile process. Any aberration may lead to abnormal brain functioning. Collectively, disorders that affect the nervous system development are called neurodevelopmental disorders (NDDs). NDDs characterized by altered psychosocial behavior, such as Autism spectrum disorder or schizophrenia are considered to have a multifactorial etiology. It is believed that besides genetic predispositions inflammatory reactions may be one of the causes. Aim The aim of this study was to evaluate changes in psychosocial behavior of adult mice treated with lipopolysaccharide (LPS) in early life. Methods LPS injections are commonly used to mimic bacterial infection in animal models. In terms of onset of exuberant synaptogenesis, postnatal day 7 (P7) in mice corresponds with the second trimester of pregnancy in humans. On P7, mice pups of both sexes were injected either with LPS or physiological saline. After 3 weeks, a wide range of behavioral tests evaluating activity, sociability, and learning abilities was conducted. Results Mice after LPS treatment were less anxious and were less active. The LPS-treated group spent more time together in the course of the social test. Interestingly, males avoided social stimulus from unknown animals, whilst females were more interested in social stimulus than a control group. Additionally, animals injected with LPS needed more time to learn new conditions. Conclusions In aggregate the presented results suggest, that immune activation during the critical stage of neurodevelopment induces alterations of behavior that resemble symptoms observed in human NDD patients.

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LOW INTENSITY REPETITIVE MAGNETIC STIMULATION (LI RTMS) EFFECTS ON A MODEL OF PATHOLOGICAL CEREBELLAR DEVELOPMENT

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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The etiology of neurodevelopmental disorders (NDD) e.g. autism spectrum disorder (ASD) is still poorly understood because of their complexity, and wide range of symptoms. However, there is increasing interest in the contribution of the cerebellum to NDDs** because of its protracted period of development making it vulnerable to injury, and its extensive connections to the forebrain showing its involvement in motor, emotional and cognitive functions. To understand the contribution of the cerebellum to NDDs, we evaluate cerebellar development in a mouse haplodeficient for a transcription factor essential for normal cerebellar development, Retinoic acid Orphan related Receptor alpha (RORα+/-), and seek to improve its pathology at behavioral, cellular and molecular levels with LI rTMS (low intensity repetitive transcranial magnetic stimulation). We stimulated mouse pup's cerebellum between 2 and 14 postnatal days with either cTBS, iTBS (continuous or intermittent theta burst stimulation) or SHAM and assessed motor, social and cognitive development as well as cerebellar Purkinje cell (PC) morphology at different ages. We found developmental delay in RORα+/- pups compared to wild-type (WT) littermates, at both behavioral and PCs levels. We found that cTBS stimulation did not change development in either group but iTBS improves behavioral performances in RORα+/- mice compared to SHAM treated ones. These results indicate that the RORα-mediated cerebellar pathological development may contribute to the emergence of NDDs and could be a therapeutic target in young population with LI-rTMS. **Tsai PT et al, (2018) Cell Rep 25:357-367. doi:10.1016/j.celrep.2018.09.039.

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SELECTIVE NKCC1 INHIBITORS FOR THE TREATMENT OF AUTISM, DOWN SYNDROME AND BRAIN DISORDERS WITH DEFECTIVE NKCC1/KCC2 RATIO

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Proper intracellular Cl concentration is fundamental for physiological brain development and function. Accordingly, the aberrant expression-ratio of Cl importer NKCC1 and exporter KCC2 is implicated in several brain conditions, including autism spectrum disorders (ASD) and Down syndrome (DS). Interestingly, NKCC1 inhibition rescues core symptoms of these brain conditions in rodent models and/or clinical trials. However, current NKCC1 inhibitors have diuretic effects inhibiting the kidney Cltransporter NKCC2. These effects create critical issues with health concerns, strongly jeopardizing them from becoming a viable therapy for long-term chronic treatment. Selective NKCC1 inhibitors would be devoid of diuretic effects, thus solving these problems. Here, we present the discovery of a new class of selective NKCC1 inhibitors (lead compound IAMA-6 and backup compound IAMA-92) obtained by an extensive drug discovery effort. IAMA-6 and IAMA-92 have a favourable drug-like profile. IAMA-6 was able to rescue social/repetitive behaviours in ASD mice. IAMA-6 and IAMA-92 were able to recover cognitive deficits in DS mice. Both compounds did not show any diuretic effect in animals. IAMA-6 neither showed any significant off-target *in vitro* nor induced histopathological alterations *in vivo*. Thus, IAMA-6 represents a solid lead compound ready for advanced preclinical studies toward its development into a clinically relevant drug for unprecedented sustainable therapeutics in ASD and DS. IAMA-92 further strengthens the potential of our class of compounds. Currently in the discovery phase, new classes of selective NKCC1 inhibitors will expand our pipeline of compounds to treat additional and diverse brain conditions characterized by defective NKCC1/KCC2 expression-ratio.

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BEHAVIORAL CHANGES AND FUNCTIONAL, MORPHOLOGICAL ALTERATIONS IN THE RAT VALPROATE MODEL OF AUTISM

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Autism is a neurodevelopmental disorder associated with loss of excitation-inhibition balance and increased risk of developing epilepsy. The valproate rat model is commonly used to study autism-like behaviors. In our study, pregnant Wistar rats received 500 mg/bwkg valproate i.p. on gestation day 12.5. Pups were tested for various motor functions and behaviors. Electrophysiological and immunohistochemical studies were performed in 6 weeks and 3 months. Entorhinal and prefrontal cortex slices were prepared for field potential measurements and parallel detection of intrinsic optical signals (IOS). Excitability changes were tested with spontaneous bursts evoked by magnesium-free solution and afterdischarges evoked by high-frequency stimulation. Intracellular functions were observed with whole-cell patch clamp method, using standard current clamp and dynamic clamp stimulation. Behavioral tests indicate that VPA-treatment delays the appearance of surface righting reflex, negative geotaxis and visual placing reflex significantly. In slices, the pattern of electrical activity and the extent of IOS changes depended on the stimulation method. Valproate treatment increased epileptiform activity, suggesting an increased tendency to epilepsy. Regarding patch clamp recordings, resting membrane potential, membrane resistance, rheobase, and spike amplitude altered according to VPA treatment, sex and age. Alterations in glutamate receptor density were also observed. After prenatal VPA exposure, developmental delay and disturbed social skills confirm the presence of autistic traits in treated animals. There are significant differences in cortical network excitability between sexes and age groups, which VPA treatment may enhance. The present study was supported by CELSA (grant no. 874735) and Gedeon Richter Plc. Centenarial Foundation.

Pubmed:
35185478: Bódi V, Májer T, Kelemen V, Világi I, Szűcs A, Varró P
Alterations of the Hippocampal Networks in Valproic Acid-Induced Rat Autism Model.
Autism Spectrum Disorder (ASD) is one of the most frequently diagnosed neurodevelopmental disorders, characterized among others by impairments in social interactions and repetitive behavior. According to one of the leading hypotheses about its origin, ASD is caused by the imbalance of excitatory and inhibitory circuit activity. ASD-related morphological and functional changes can be observed in several brain regions i.e., in the prefrontal cortex and the hippocampus. It is well-established that prenatal valproic-acid (VPA) exposure of rats on day 12.5 leads to neurodevelopmental alterations with autism-like clinical and behavioral symptoms. The aim of this study was to investigate potential changes in the excitability of neuronal networks and individual neurons of the hippocampus elicited by prenatal VPA treatment. As there are marked sex differences in ASD, offspring of both sexes were systematically tested, using two different age groups, to elucidate eventual differences in neurodevelopment after VPA treatment. Excitatory connections and long-term synaptic plasticity as well as intrinsic excitability of CA1 pyramidal cells were examined. Pregnant female Wistar rats received saline or 500 mg/kg VPA i. p. on gestation day 12.5. Brain slices of 6-week-old and 3-month-old offspring were investigated using extra- and intracellular electrophysiological techniques. Field potential- and whole-cell patch clamp recordings were carried out to measure network excitability and single cell activity in the CA1 region hippocampus. Enhanced excitability of hippocampal networks was detected in the 6-week-old VPA-treated male rats; however, this change could not be observed in 3-month-old males. Intrinsic excitability of single neurons, however, was increased in 3-month-old males. In 6-week-old treated females, the most prominent effect of VPA was an increase in voltage sag, to a similar degree to the neurons of the older age group. In 3-month-old females, a network excitability increase could be demonstrated, in a lesser degree than in younger males. It can be concluded, that VPA treatment had diverse effects on hippocampal excitability depending on the sex and the age of the animals. We found that certain alterations manifested in 6-week-old rats were compensated later, on the other hand, other

changes persisted until the age of 3 months.
Front Neural Circuits, 2022; 16

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INFLUENCE OF PERINATAL INFLAMMATION ON MENINGEAL IMMUNITY AND NEURODEVELOPMENT

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Aims Early-life inflammation correlates with an increased risk of developing neurological disorders, including autism. Studies in rodents have shown that perinatal inflammation can lead to cytokine signaling in brains of neonates, abnormal neurodevelopment and social behavior. The meninges represent an immunologically active site, at the interface between the brain and the periphery. Hence, because the meninges can nurture the brain but also relay and amplify inflammation, we hypothesize that they could play a major role in inflammation-induced neurodevelopmental disorders. In particular, the meninges are inhabited by a dense population of immune sentinels which are absent from the brain parenchyma itself. Two meningeal macrophage (MM) populations have been identified: an MHC-II- MM population abundant in neonates, and a post-natal MHC-II+ MM population increasing over time. Methods We have investigated their response to peripheral inflammation, using single-cell transcriptomics and imaging approaches. Results Both populations of MM responded to compounds injected intravascularly such as viruses (LCMV), LPS and cytokines (IFNs, …) although in different manners. Neonatal MHC-II- macrophages decreased pathways linked with tissue growth and development while post-natal MHC-II+ macrophages up-regulated pathways linked with cytokine production. Using innovative transgenic mice models and pharmacological approaches, we are depleting MM and their cytokine signaling pathway to know if this influences 1/brain development (myelination, neurogenesis) and 2/behavior in neonates and in adult mice. Conclusions We hope that this work will provide a new link between peripheral inflammation and neurodevelopmental disorders, that could provide a rationale to target meningeal macrophages in the clinic.

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EXPLORING PAIN AND NOCICEPTION IN A VALPROATE-INDUCED MOUSE MODEL OF AUTISM SPECTRUM DISORDER

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Autism Spectrum Disorders (ASD) are neurodevelopmental disorders in which abnormalities in sensory information processing have been described. Among sensory functions, nociception and its potential result, pain, are of a critical importance due to their crucial role for survival and the high prevalence of potentially painful comorbidities in ASD. However, there is no consensus among studies in humans and hypo-, hyper- as well as normal sensitivity to pain have been described. We decided to use an animal model of ASD to try to answer this question, allowing us to do standardize measures of pain sensitivity as well as study of the underlying cellular and molecular mechanisms. Using an environmental model of ASD taking advantage of teratogenic drug sodium valproate (VPA) in CD1 mice, we explored behavioral response to nociceptive and painful situations and tried to elucidate underlying molecular and cellular mechanisms using electrophysiology, calcium imaging and immunohistochemistry. Our model showed differences in non-nociceptive comfort temperature, nociceptive heat, as well as a reduced sensitivity to capsaicin- and formalin-induced pain models. These differences are mostly present in males and only appears at a post-puberty age. Our results showed reduced responses to nociceptive and potentially painful situations in VPA animals, affecting mostly adult males. Given the predominant phenotype on heat modality and capsaicin, these could potentially involve modifications in heat-related nociceptive tracts.

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BOARD NUMBER: S03-390

ASSESSMENT OF VASCULARIZATION AND NEUROGENESIS IN AN IPSC-DERIVED 16P11.2 DELETION ORGANOID MODEL

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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This project aims at expanding upon previous Lacoste lab findings to better understand the effect of the 16p11.2 deletion (16pDel) specific to endothelial cells (EC) on angiogenesis and neural development by using Human-induced-Pluripotent-Stem-Cells and microfluidic plates, through the creation of vascularized cerebral organoids. HiPSCs with 16pDel and non-isogenic control lines were differentiated into human induced-ECs. HiECs underwent positive selection after which cell identity was confirmed using immunocytochemistry. A Matrigel-based network-formation assay is used to quantify network length and sprouting, comparing control and 16pDel lines. Angiogenic activity of 16pDel and control hiECs will be compared using Microfluidic 3-Lane OrganoPlate. Vascular tube formed in the perfusable channel, sprouts through a collagen channel towards angiogenic factors. Sprouts are quantified for length, diameter, and branching. The microfluidic Graft OrganoPlate will be used to embed a cerebral organoid in growing vascular networks using 16pDel or control hiECs. This is expected to create vascularized organoids which will then be characterized using immunohistochemistry and transcriptomics. Complexity of vascular incorporation into cerebral organoids will be investigated once optimal experimental conditions have been established. Neuronal, vascular and glial cell types identified in the control versus 16pDel organoids will be compared at select time points in order to detect neurodevelopmental delays resulting from defective vascularization as seen in 16pDel mice. Early timepoints will focus on the presence of precursor cells and later timepoints will focus on differences in neuronal layering. Overall, this will further the understanding of the altered function of ECs associated with the 16pDel and its neurodevelopmental consequences.

FENS Forum 2022
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BOARD NUMBER: S03-391

EFFECTS OF EARLY-LIFE SODIUM BUTYRATE SUPPLEMENTATION ON AUTISM-LIKE BEHAVIORAL PHENOTYPE, NEUROINFLAMMATORY PROFILE AND GUT MICROBIOTA ALTERATIONS INDUCED BY MATERNAL IMMUNE ACTIVATION IN MOUSE OFFSPRING

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Aims
Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental condition characterized by deficits in social communication and interaction, and repetitive/stereotyped behaviors. In recent years, the role of microbiota-gut-brain axis (MBGA) in ASD pathogenesis received growing attention, appearing as an attractive therapeutic target. A key mediator in MBGA is butyrate, a short-chain fatty acid produced by bacterial fermentation of dietary fibers, involved in the regulation of gut permeability and immune system, found impaired in ASD. The present study evaluated the role of early-life sodium butyrate (NaB) supplementation in the modulation of ASD-like behavioral phenotype, neuroinflammatory response, and gut microbiota alterations in the maternal immune activation (MIA) mouse model. **Methods**
We modeled MIA by treating C57BL6/J female mice with a single dose of polyinosinic:polycytidylic acid (Poly I:C, 20 mg/kg, i.p.) at gestational day 12.5. After delivery, dams received NaB (200 mg/kg) administered in drinking water through the whole duration of lactation. In order to detect ASD-like behavioral deficits in offspring, we assessed their behavioral phenotype from early neonatal stage to adulthood. At the end of behavioral testing, we analyzed i) gut microbiota composition by 16S rRNA sequencing and ii) neuroinflammatory markers in prefrontal cortex, hippocampus and cerebellum by Real-Time PCR. **Results**
Early-life NaB supplementation attenuated some of the MIA-induced behavioral alterations (i.e. stereotyped behavior), and exerted modulatory effects on neuroinflammation and gut microbiota composition in MIA adult offspring. **Conclusions**
Our data support the development of therapeutic interventions targeting gut microbiota to modulate brain and behavioral changes in ASD.

Pubmed:
34520749: Garí M, Grzesiak M, Krekora M, Kaczmarek P, Jankowska A, Król A, Kaleta D, Jerzyńska J, Janasik B, Kuraś R, Tartaglione AM, Calamandrei G, Hanke W, Polańska K
Prenatal exposure to neurotoxic metals and micronutrients and neurodevelopmental outcomes in early school age children from Poland.
Exposure to environmental factors, such as neurotoxic metals and micronutrients, during critical periods of development can contribute to long-term consequences in offspring's health, including neurodevelopmental outcomes. The aim of this study was to evaluate the association between simultaneous prenatal exposure to metals [lead (Pb), cadmium (Cd), mercury (Hg)] and micronutrients [selenium (Se), zinc (Zn), copper (Cu)] and neurodevelopmental outcomes in school-age children from the Polish Mother and Child Cohort (REPRO_PL). Metals and micronutrients concentrations were measured in cord blood (Pb, Cd, Se, Zn, Cu) and in maternal hair (Hg) collected during the 3rd trimester of pregnancy. Behavioral and emotional problems, as well as children's cognitive and psychomotor development, were assessed in 436 school-age children using the Strengths and Difficulties Questionnaire (SDQ, filled in by the mothers) and the Polish adaptation of the Intelligence and Development Scales (IDS, administered by trained psychologists). Multivariate regression models were applied after imputation of missing values, using two approaches: (i) a joint analysis taking into account all metals and micronutrients simultaneously, and (ii) an ExWAS study (single-exposure model). In the SDQ, Hyperactivity/Inattention problems and Total difficulties were associated with higher Hg concentrations in maternal hair (0.18, 95% CI: 0.05; 0.3; and 0.14, 95% CI: 0.01; 0.3, respectively), whereas Emotional symptoms were inversely associated with Se and Zn levels in cord blood (-0.13, 95% CI: -0.3; 0.004; and -0.10, 95% CI: -0.2; 0.02, respectively). In the IDS, cord blood Pb levels were found to be negatively associated with Fluid and Crystallized IQ (-0.12, 95% CI: -0.3; 0.02; and -0.14, 95% CI: -0.3; 0.007, respectively) as well as

Mathematical skills (-0.15, 95% CI: -0.3; 0.01). The current research has been able to simultaneously assess the exposure to various interacting chemicals during the prenatal period. We demonstrate that prenatal co-exposures to Pb, Hg, Zn and Se have long-term influences on the neuropsychological outcome of school-age children.
Environ Res, 2022; 204
34320234: Chiodi V, Domenici MR, Biagini T, De Simone R, Tartaglione AM, Di Rosa M, Lo Re O, Mazza T, Micale V, Vinciguerra M
Systemic depletion of histone macroH2A1.1 boosts hippocampal synaptic plasticity and social behavior in mice.
Gene expression and epigenetic processes in several brain regions regulate physiological processes such as cognitive functions and social behavior. MacroH2A1.1 is a ubiquitous variant of histone H2A that regulates cell stemness and differentiation in various organs. Whether macroH2A1.1 has a modulatory role in emotional behavior is unknown. Here, we employed macroH2A1.1 knock-out ( ) mice to perform a comprehensive battery of behavioral tests, and an assessment of hippocampal synaptic plasticity (long-term potentiation) accompanied by whole hippocampus RNA sequencing. MacroH2A1.1 mice exhibit a stunningly enhancement both of sociability and of active stress-coping behavior, reflected by the increased social behavior in social activity tests and higher mobility time in the forced swim test, respectively. They also display an increased hippocampal synaptic plasticity, accompanied by significant neurotransmission transcriptional networks changes. These results suggest that systemic depletion of histone macroH2A1.1 supports an epigenetic control necessary for hippocampal function and social behavior.
FASEB J, 2021; 35
32290408: Tartaglione AM, Serafini MM, Raggi A, Iacoponi F, Zianni E, Scalfari A, Minghetti L, Ricceri L, Cubadda F, Calamandrei G, Viviani B
Sex-Dependent Effects of Developmental Lead Exposure in Wistar Rats: Evidence from Behavioral and Molecular Correlates.
Lead (Pb) exposure in early life affects brain development resulting in cognitive and behavioral deficits. Epidemiologic and experimental evidence of sex as an effect modifier of developmental Pb exposure is emerging. In the present study, we investigated Pb effects on behavior and mechanisms of neuroplasticity in the hippocampus and potential sex differences. To this aim, dams were exposed, from one month pre-mating to offspring weaning, to Pb via drinking water at 5 mg/kg body weight per day. In the offspring of both sexes, the longitudinal assessment of motor, emotional, and cognitive end points was performed. We also evaluated the expression and synaptic distribution of N-methyl-D-Aspartate receptor (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits at post-natal day (pnd) 23 and 70 in the hippocampus. Neonatal motor patterns and explorative behavior in offspring were affected in both sexes. Pb effects in emotional response and memory retention were observed in adult females only, preceded by increased levels of GluN2A and GluA1 subunits at the post-synapse at pnd 23. These data suggest that Pb exposure during development affects glutamatergic receptors distribution at the post-synaptic spine in females. These effects may contribute to alterations in selected behavioral domains.
Int J Mol Sci, 2020; 21
31706595: Calamandrei G, Ricceri L, Meccia E, Tartaglione AM, Horvat M, Tratnik JS, Mazej D, Špirić Z, Prpić I, Vlašić-Cicvarić I, Neubauer D, Kodrič J, Stropnik S, Janasik B, Kuraś R, Mirabella F, Polańska K, Chiarotti F
Pregnancy exposome and child psychomotor development in three European birth cohorts.
Characterization of the exposome, the totality of all environmental factors that one is exposed to from conception onwards, has been recommended to better evaluate the role of environmental influences on developmental programming and life-course vulnerability to major chronic diseases. In the framework of the Health and Environment-wide Associations based on Large population Surveys (HEALS) project we considered the pregnancy exposome exploiting two databases (PHIME and REPRO_PL) that include birth cohorts from three EU countries (Croatia, Slovenia and Poland). The databases contained information on several chemical exposures, socio-demographic, lifestyle and health related factors from conception to child birth, and neuropsychological scores assessed by the Bayley Scales of Infant and Toddler Development in the first two years of life. Our main goal was to assess consistency of environmental influences on neurodevelopment, if any, across European countries differing for geographical, socio-demographic characteristics and levels of chemical exposures to metals such as lead (Pb), mercury (Hg), cadmium (Cd) and trace elements, including micronutrients such as zinc (Zn) and selenium (Se). To this aim, we first selected variables common to the different databases, then applied univariate and multivariate regression analyses to identify factors linked to neurodevelopment, and finally performed meta-analysis to detect potential heterogeneity among cohorts and pooled estimates. Significant differences in exposure levels among the three sub-cohorts were observed as for Hg and Se; exposure levels under study were relatively low and within the range described in existing EU biomonitoring studies. The univariate analyses did not show any common pattern of association as only in the Polish cohort chemical exposure had an impact on neuropsychological outcome. In the meta-analysis, some consistent trends were evident, relative to the adverse influence of Pb on children's language and cognition and the positive influence of Se on language abilities. The effects of the neurotoxic metal Hg positively influenced the motor scores in the Polish cohorts, while it decreased the

motor scores in the Slovenia and Croatian sub-cohorts. The only socio-demographic factor consistently associated to the outcome among cohorts was child's sex, with females performing better than males on cognitive and language scores. These findings point to the need of harmonizing existing cohorts or creating prospective study designs that facilitate comparisons in the exposome over time, places and kind of environmental exposures.
Environ Res, 2020; 181
31419718: Jankowska A, Polańska K, Hanke W, Wesołowska E, Ligocka D, Waszkowska M, Stańczak A, Tartaglione AM, Mirabella F, Chiarotti F, Garí M, Calamandrei G
Prenatal and early postnatal phthalate exposure and child neurodevelopment at age of 7 years - Polish Mother and Child Cohort.
Phthalates are among the most frequently investigated environmental chemicals influencing children's health and particularly their neuropsychological development. However, the reported effects of these compounds on child behavior, cognitive and psychomotor outcomes are not fully consistent. The aim of this study is to evaluate the associations between prenatal and early postnatal phthalate exposures and child neurodevelopment at age of 7 years. A total of 134 mother-child pairs from Polish Mother and Child Cohort (REPRO_PL) constitute the basis for current analysis. Eleven phthalate metabolites were measured in urine samples collected from mothers in the 3rd trimester of pregnancy and from children at the age of 2 years. Child neuropsychological development at early school age (7 years) was assessed by both the Strengths and Difficulties Questionnaire (SDQ) filled by mothers and the Polish adaptation of the Intelligence and Development Scales (IDS) performed by psychologists. Mono-ethyl phthalate (MEP) concentration during pregnancy was significantly associated with increased risk of peer relationship problems in SDQ (OR = 2.7, p = 0.03). The results of the IDS analyses focused on child's cognitive and psychomotor development are not fully conclusive. Negative associations were evident between some phthalates in early childhood period and fluid intelligence and cognition (MEP: β = -5.2; p = 0.006; β = -4.2; p = 0.006; mono-n-butyl phthalate (MnBP): β = -4.9; p = 0.03; β = -4.0; p = 0.03; respectively), while positive associations have been found in the prenatal period (mono-2-ethyl-5-oxo-hexyl phthalate (oxo-MEHP): β = 3.6; p = 0.03 for fluid intelligence; β = 2.9; p = 0.03 for cognition). Further studies are required in order to elucidate which are the most critical periods of phthalate exposure on children's neurodevelopmental outcomes.
Environ Res, 2019; 177
30639388: Tartaglione AM, Schiavi S, Calamandrei G, Trezza V
Prenatal valproate in rodents as a tool to understand the neural underpinnings of social dysfunctions in autism spectrum disorder.
Impairments in social interaction and verbal and non verbal communication are among the main features of Autism Spectrum Disorder (ASD). The causes of ASD are still unknown but the research efforts of the last decade have identified a number of factors (rare gene mutations, gene variations and adverse environmental events) that, interacting in complex ways, affect early brain development. The clinical evidence that prenatal exposure to the antiepileptic drug valproate (VPA) is associated with increased risk of neurodevelopmental delay, cognitive deficits and autism in children, has drawn the attention of scientists on VPA as a tool to unravel the environment contribution to ASD risk in children. In agreement with the clinical evidence, rodents prenatally exposed to VPA display behavioral anomalies resembling ASD symptoms. The mechanisms by which administration of VPA in pregnancy increases the risk of autism are still far to be clear as are still undetermined the specific targets of VPA in the developing brain both in humans and rodents. However, the robustness of the behavioral alterations, mainly in the social domain, and the neural/molecular changes revealed so far support the VPA model as a reliable instrument to investigate the neural underpinnings of social impairment. Here we provide an update of preclinical studies on prenatal exposure to VPA in rodents with a focus on the social and communication deficits induced by VPA, discussing potential pitfalls and future directions in this research field and corroborating the potential of the VPA model to identify new pharmacological targets for ASD. This article is part of the Special Issue entitled 'The neuropharmacology of social behavior: from bench to bedside'.
Neuropharmacology, 2019; 159
30194517: Tartaglione AM, Cipriani C, Chiarotti F, Perrone B, Balestrieri E, Matteucci C, Sinibaldi-Vallebona P, Calamandrei G, Ricceri L
Early Behavioral Alterations and Increased Expression of Endogenous Retroviruses Are Inherited Across Generations in Mice Prenatally Exposed to Valproic Acid.
Prenatal treatment with the antiepileptic drug valproic acid (VPA) is associated with a significant risk of somatic anomalies, neurodevelopmental delays, and 7-10× increase in the incidence of autism spectrum disorders (ASD) in children. Rodents exposed to VPA in pregnancy show birth defects, deficits in neurodevelopment, and cognitive/social anomalies resembling those of ASD children. Mechanisms of VPA neurobehavioral toxicity are still unclear but as VPA is a non-selective inhibitor of histone deacetylases, epigenetic modifications are likely involved. This study was aimed to evaluate the transgenerational impact of prenatal VPA exposure on mouse early behavioral development, studying F, F, and F generations after VPA

challenge on gestational day (GD) 10.5. We also analyzed in brain and in peripheral blood mononuclear cells the expression levels of different endogenous retrovirus (ERV) families, potential biomarkers of derailed brain development, since human ERVs have been implicated in the pathogenesis of neurodevelopmental disorders (NDDs) such as ASD. Somatic effects of VPA were evident only in F generation and more markedly in the female sex. Across F and F generations, VPA delayed righting reflex, increased motor activity, and reduced ultrasonic vocalizations. The behavioral changes in F are milder though in the same direction. VPA increased expression of most ERVs across the three generations in brain and blood. In utero VPA induced neurodevelopmental alterations more marked in the maternal lineage that persisted also in F, suggesting ERVs as possible downstream effectors of the VPA epigenetic alterations.
Mol Neurobiol, 2019; 56
29330412: Cipriani C, Ricceri L, Matteucci C, De Felice A, Tartaglione AM, Argaw-Denboba A, Pica F, Grelli S, Calamandrei G, Sinibaldi Vallebona P, Balestrieri E
High expression of Endogenous Retroviruses from intrauterine life to adulthood in two mouse models of Autism Spectrum Disorders.
Retroelements, such as Human Endogenous Retroviruses (HERVs), have been implicated in many complex diseases, including neurological and neuropsychiatric disorders. Previously, we demonstrated a distinctive expression profile of specific HERV families in peripheral blood mononuclear cells from Autistic Spectrum Disorders (ASD) patients, suggesting their involvement in ASD. Here we used two distinct ASD mouse models: inbred BTBR T+tf/J mice and CD-1 outbred mice prenatally exposed to valproic acid. Whole embryos, blood and brain samples from the offspring were collected at different ages and the expression of several ERV families (ETnI, ETnII-α, ETnII-β, ETnII-γ, MusD and IAP), proinflammatory cytokines (IL-1β, IL-6 and TNF-α) and Toll-like receptors (TLR3 and TLR4) was assessed. In the two distinct mouse models analysed, the transcriptional activity of the ERV families was significant higher in comparison with corresponding controls, in whole embryos, blood and brain samples. Also the expression levels of the proinflammatory cytokines and TLRs were significantly higher than controls. Current results are in agreement with our previous findings in ASD children, supporting the hypothesis that ERVs may serve as biomarkers of atypical brain development. Moreover, the changes in ERVs and proinflammatory cytokines expression could be related with the autistic-like traits acquisition in the two mouse models.
Sci Rep, 2018; 8
27374158: Tartaglione AM, Armida M, Potenza RL, Pezzola A, Popoli P, Calamandrei G
Aberrant self-grooming as early marker of motor dysfunction in a rat model of Huntington's disease.
In the study of neurodegenerative diseases, rodent models provide experimentally accessible systems to study multiple pathogenetic aspects. The identification of early and robust behavioural changes is crucial to monitoring disease progression and testing potential therapeutic strategies in animals. Consistent experimental data support the translational value of rodent self-grooming as index of disturbed motor functions and perseverative behaviour patterns in different rodent models of brain disorders. Huntington's disease (HD) is a progressive neurodegenerative disorder, characterized by severe degeneration of basal ganglia, cognitive and psychiatric impairments and motor abnormalities. In the rat species, intrastriatal injection of the excitotoxin quinolinic acid (QA) mimics some of the neuroanatomical and behavioural changes found in HD, including the loss of GABAergic neurons and the appearance of motor and cognitive deficits. We show here that striatal damage induced by unilateral QA injection in dorsal striatum of rats triggers aberrant grooming behaviour as early as three weeks post-lesion in absence of other motor impairments: specifically, both quantitative (frequency and duration) and qualitative (the sequential pattern of movements) features of self-grooming behaviour were significantly altered in QA-lesioned rats placed in either the elevated plus-maze and the open-field. The consistent abnormalities in self-grooming recorded in two different experimental contexts support the use of this behavioural marker in rodent models of striatal damage such as HD, to assess the potential effects of drug and cell replacement therapy in the early stage of disease.
Behav Brain Res, 2016; 313
26695168: Tartaglione AM, Venerosi A, Calamandrei G
Early-Life Toxic Insults and Onset of Sporadic Neurodegenerative Diseases-an Overview of Experimental Studies.
The developmental origin of health and disease hypothesis states that adverse fetal and early childhood exposures can predispose to obesity, cardiovascular, and neurodegenerative diseases (NDDs) in adult life. Early exposure to environmental chemicals interferes with developmental programming and induces subclinical alterations that may hesitate in pathophysiology and behavioral deficits at a later life stage. The mechanisms by which perinatal insults lead to altered programming and to disease later in life are still undefined. The long latency between exposure and onset of disease, the difficulty of reconstructing early exposures, and the wealth of factors which the individual is exposed to during the life course make extremely difficult to prove the developmental origin of NDDs in clinical and epidemiological studies. An overview of animal studies assessing the long-term effects of perinatal exposure to different chemicals (heavy metals and pesticides) supports the link between exposure and hallmarks of neurodegeneration at the adult stage. Furthermore, models of maternal immune activation show that brain inflammation in early life may enhance adult vulnerability to environmental toxins, thus

supporting the multiple hit hypothesis for NDDs' etiology. The study of prospective animal cohorts may help to unraveling the complex pathophysiology of sporadic NDDs. In vivo models could be a powerful tool to clarify the mechanisms through which different kinds of insults predispose to cell loss in the adult age, to establish a cause-effect relationship between "omic" signatures and disease/dysfunction later in life, and to identify peripheral biomarkers of exposure, effects, and susceptibility, for translation to prospective epidemiological studies.
Curr Top Behav Neurosci, 2016; 29

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9-13 July 2022 | Paris, France

BOARD NUMBER: S03-392

MITOCHONDRIAL MORPHOLOGICAL REMODELING IN RESPONSE TO MITOCHONDRIAL STRESS IN A NEURONAL CELL MODEL

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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The significance of mitochondrial function in neuronal differentiation is well established and is important for neurodevelopmental disorders like Autism Spectrum Disorder (ASD). We identified differentially methylated genes, including a gene that results in propionate accumulation, associated with mitochondrial function in a South African ASD cohort. Sodium propionate (NaP) has been used to study ASD in rat models. **Aims:** (i) Induce mitochondrial dysfunction in SH-SY5Y cells using NaP treatment and (ii) Investigate the effects of NaP on mitochondrial biogenesis, dynamics and morphology. **Methods:** We set-up a mitochondrial stress model using NaP (1.5mM, 3mM, 5mM) to induce mitochondrial dysfunction in SH-SY5Y cells. PGC1α expression and mitochondrial copy number confirmed mitochondrial dysfunction using RT-qPCR. Microscopy (cryogenic electron and fluorescent) measured changes in mitochondrial dynamics and morphology. Bright-field microscopy and immunocytochemistry measured neurodifferentiation after NaP treatment. Significance was determined using one-way ANOVA ($p<0.05$). **Results:** NaP treatment at all concentrations altered mitochondrial biogenesis and dynamics in undifferentiated and differentiated SH-SY5Y cells. PGC1α expression and mitochondrial copy number (1.5mM and 3mM NaP) indicated changes in biogenesis. Changes in mitochondrial morphology were seen by differences between mitochondrial length, area and aspect ratio at different NaP concentrations. Higher NaP concentrations (5mM) inhibited neurodifferentation with decreases in neurite extensions being observed. **Conclusions:** These data are consistent with the link between mitochondrial dysfunction and fluctuations in biogenesis and dynamics. This study offers insight into how mitochondrial induced stress impacts neurogenesis, and thus may potentially play a role in ASD aetiology.

FENS Forum 2022
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BOARD NUMBER: S03-393

CAECAL DYSFUNCTION IN THE NEUROLIGIN-3 R451C MOUSE MODEL OF AUTISM

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Individuals with autism often experience gastrointestinal symptoms alongside core behavioural traits which may indicate a role for the gut-brain axis and neuro-immune cross-talk. The gastrointestinal tract houses both the largest immune organ and the enteric nervous system (ENS), which regulates gut function. The mouse caecum corresponds to the human appendix and serves as a fermentation site and reservoir for commensal bacteria, and is thus an ideal region to study neuro-immune interactions. Here we assessed for changes in caecal function in mice expressing an autism-associated missense mutation encoding the post-synaptic protein neuroligin-3 ($NL3^{R451C}$ mice). These mice have reduced caecal weight, altered caecal ENS architecture, and changes in immune cell morphology in the lymphoid tissue, known as the caecal patch. To characterise neuro-immune interactions in autism, caecal motility was assessed using an *ex vivo* video imaging technique. Changes in caecal secretion of fluids and mucosal permeability were also investigated using an Ussing chamber, and immune cell morphology was studied using immunofluorescence for the dendritic cell marker, CD11c. NL3 mouse caecal motility showed a higher frequency and shorter duration of contractile patterns compared to wildtypes. Neurally-evoked secretion was decreased in NL3 mice, whereas mucosal permeability was unchanged. NL3 mice had less caecal content and more lymphoid patches, with no morphological difference observed in $CD11c^{+}$ dendritic cells. These findings indicate that the R451C nervous system mutation alters caecal structure, motility and secretion. These findings provide a foundation for future investigations into how caecal function contributes to health and neurodevelopmental disorders via the gut-brain axis.

Pubmed:
34946105: Cheok YY, Lee CYQ, Cheong HC, Vadivelu J, Looi CY, Abdullah S, Wong WF
An Overview of Survival Tactics in the Hostile Human Stomach Environment.
is well established as a causative agent for gastritis, peptic ulcer, and gastric cancer. Armed with various inimitable virulence factors, this Gram-negative bacterium is one of few microorganisms that is capable of circumventing the harsh environment of the stomach. The unique spiral structure, flagella, and outer membrane proteins accelerate movement within the viscous gastric mucosal layers while facilitating its attachment to the epithelial cells. Furthermore, secretion of urease from eases the acidic pH within the stomach, thus creating a niche for bacteria survival and replication. Upon gaining a foothold in the gastric epithelial lining, bacterial protein CagA is injected into host cells through a type IV secretion system (T4SS), which together with VacA, damage the gastric epithelial cells. does not only establishes colonization in the stomach, but also manipulates the host immune system to permit long-term persistence. Prolonged infection causes chronic inflammation that precedes gastric cancer. The current review provides a brief outlook on survival tactics, bacterial-host interaction and their importance in therapeutic intervention as well as vaccine development.
Microorganisms, 2021; 9
34707599: Cheok YY, Tan GMY, Fernandez KC, Chan YT, Lee CYQ, Cheong HC, Looi CY, Vadivelu J, Abdullah S, Wong WF
Podoplanin Drives Motility of Active Macrophage Regulating Filamin C During Infection.
Podoplanin (Pdpn) is a mucin-type transmembrane protein that has been implicated in multiple physiological settings including lymphangiogenesis, platelet aggregation, and cancer metastasis. Here, we reported an absence of Pdpn transcript expression in the resting mouse monocytic macrophages, RAW264.7 cells; intriguingly, a substantial upregulation of Pdpn was observed in activated macrophages following or lipopolysaccharide stimulation. Pdpn-knockout macrophages demonstrated intact phagocytic and intracellular bactericidal activities comparable to wild type but exhibited impaired migration due to attenuated filopodia formation. In contrast, an ectopic expression of Pdpn augmented filopodia protrusion in activated macrophages. NanoString analysis uncovered a close dependency of Filamin C gene on the presence of Pdpn, highlighting an involvement of Filamin C in modulation of actin polymerization activity, which controls cell filopodia formation and migration. In addition, interleukin-1β production was significantly declined in the absence of Pdpn, suggesting a role of

Pdpn in orchestrating inflammation during infection besides cellular migration. Together, our findings unravel the Pdpn network that modulates movement of active macrophages.
Front Immunol, 2021; 12
33125061: Balasuriya GK, Mohsenipour M, Brassington K, Dobric A, De Luca SN, Mou K, Seow HJ, Lee CYQ, Herath M, Chan SMH, Vlahos R, Hill-Yardin EL
Ebselen prevents cigarette smoke-induced gastrointestinal dysfunction in mice.
Gastrointestinal (GI) dysfunction is a common comorbidity of chronic obstructive pulmonary disease (COPD) for which a major cause is cigarette smoking (CS). The underlying mechanisms and precise effects of CS on gut contractility, however, are not fully characterised. Therefore, the aim of the present study was to investigate whether CS impacts GI function and structure in a mouse model of CS-induced COPD. We also aimed to investigate GI function in the presence of ebselen, an antioxidant that has shown beneficial effects on lung inflammation resulting from CS exposure. Mice were exposed to CS for 2 or 6 months. GI structure was analysed by histology and immunofluorescence. After 2 months of CS exposure, ex vivo gut motility was analysed using video-imaging techniques to examine changes in colonic migrating motor complexes (CMMCs). CS decreased colon length in mice. Mice exposed to CS for 2 months had a higher frequency of CMMCs and a reduced resting colonic diameter but no change in enteric neuron numbers. Ten days cessation after 2 months CS reversed CMMC frequency changes but not the reduced colonic diameter phenotype. Ebselen treatment reversed the CS-induced reduction in colonic diameter. After 6 months CS, the number of myenteric nitric-oxide producing neurons was significantly reduced. This is the first evidence of colonic dysmotility in a mouse model of CS-induced COPD. Dysmotility after 2 months CS is not due to altered neuron numbers; however, prolonged CS-exposure significantly reduced enteric neuron numbers in mice. Further research is needed to assess potential therapeutic applications of ebselen in GI dysfunction in COPD.
Clin Sci (Lond), 2020; 134
32485290: Lee CYQ, Franks AE, Hill-Yardin EL
Autism-associated synaptic mutations impact the gut-brain axis in mice.
Interactions between the gut microbiome and the brain affect mood and behaviour in health and disease. Using preclinical animal models, recent discoveries begin to explain how bacteria in the gut influence our mood as well as highlighting new findings relevant to autism. Autism-associated gene mutations known to alter synapse function in the CNS also affect inflammatory response and modify the enteric nervous system resulting in abnormal gastrointestinal motility and structure. Strikingly, these mutations additionally affect the gut microbiome in mice. This review describes the changes in gut physiology and microbiota in mouse models of autism with modified synapse function. The rationale for different regions of the gastrointestinal tract having variable susceptibility to dysfunction is also discussed. To dissect underlying biological mechanisms involving gut-brain axis dysfunction in preclinical models, a range of multidisciplinary approaches are required. This research will provide insights into the role of the gut-brain axis in health and neurodevelopmental disorders including autism.
Brain Behav Immun, 2020; 88
31963395: Cheok YY, Lee CYQ, Cheong HC, Looi CY, Wong WF
Chronic Inflammatory Diseases at Secondary Sites Ensuing Urogenital or Pulmonary Infections.
and are members of the family of obligate intracellular bacteria. The former causes diseases predominantly at the mucosal epithelial layer of the urogenital or eye, leading to pelvic inflammatory diseases or blindness; while the latter is a major causative agent for pulmonary infection. On top of these well-described diseases at the respective primary infection sites, are notoriously known to migrate and cause pathologies at remote sites of a host. One such example is the sexually acquired reactive arthritis that often occurs at few weeks after genital infection. , on the other hand, has been implicated in an extensive list of chronic inflammatory diseases which include atherosclerosis, multiple sclerosis, Alzheimer's disease, asthma, and primary biliary cirrhosis. This review summarizes the infection associated diseases at the secondary sites of infection, and describes the potential mechanisms involved in the disease migration and pathogenesis.
Microorganisms, 2020; 8
31738795: Cheong HC, Yap PSX, Chong CW, Cheok YY, Lee CYQ, Tan GMY, Sulaiman S, Hassan J, Sabet NS, Looi CY, Gupta R, Arulanandam B, AbuBakar S, Teh CSJ, Chang LY, Wong WF
Diversity of endocervical microbiota associated with genital Chlamydia trachomatis infection and infertility among women visiting obstetrics and gynecology clinics in Malaysia.
The cervical microbiota constitutes an important protective barrier against the invasion of pathogenic microorganisms. A disruption of microbiota within the cervical milieu has been suggested to be a driving factor of sexually transmitted infections. These include Chlamydia trachomatis which frequently causes serious reproductive sequelae such as infertility in women. In this study, we profiled the cervical microbial composition of a population of 70 reproductive-age Malaysian women; among which 40 (57.1%) were diagnosed with genital C. trachomatis infection, and 30 (42.8%) without C. trachomatis infection. Our findings showed a distinct compositional difference between the cervical microbiota of C. trachomatis-infected subjects and

subjects without C. trachomatis infection. Specifically, significant elevations of mostly strict and facultative anaerobes such as Streptococcus, Megasphaera, Prevotella, and Veillonella in the cervical microbiota of C. trachomatis-positive women were detected. The results from the current study highlights an interaction of C. trachomatis with the environmental microbiome in the endocervical region.
PLoS One, 2019; 14
31137741: Cheong HC, Lee CYQ, Cheok YY, Tan GMY, Looi CY, Wong WF
: Diseases in Primary Hosts and Zoonosis.
Bacteria of the family are a type of Gram-negative microorganism typified by their obligate intracellular lifestyle. The majority of the members in the family are known pathogenic organisms that primarily infect the host mucosal surfaces in both humans and animals. For instance, is a well-known etiological agent for ocular and genital sexually transmitted diseases, while has been implicated in community-acquired pneumonia in humans. Other chlamydial species such as , , , , , and are important pathogens that are associated with high morbidities in animals. Importantly, some of these animal pathogens have been recognized as zoonotic agents that pose a significant infectious threat to human health through cross-over transmission. The current review provides a succinct recapitulation of the characteristics as well as transmission for the previously established members of the family and a number of other recently described chlamydial organisms.
Microorganisms, 2019; 7
30477893: Cheong HC, Lee CYQ, Cheok YY, Shankar EM, Sabet NS, Tan GMY, Movahed E, Yeow TC, Sulaiman S, Wong WF, Looi CY, Gupta R, Hassan J, Arulanandam B, AbuBakar S
CPAF, HSP60 and MOMP antigens elicit pro-inflammatory cytokines production in the peripheral blood mononuclear cells from genital Chlamydia trachomatis-infected patients.
Persistent inflammation caused by Chlamydia trachomatis in the female genital compartment represents one of the major causes of pelvic inflammatory disease (PID), ectopic pregnancy and infertility in females. Here, we examined the pro-inflammatory cytokine response following stimulation with three different types of C. trachomatis antigens, viz. chlamydial protease-like factor (CPAF), heat shock protein 60 (HSP60) and major outer membrane protein (MOMP).
Immunobiology, 2019; 224
30473697: Saeidi A, Zandi K, Cheok YY, Saeidi H, Wong WF, Lee CYQ, Cheong HC, Yong YK, Larsson M, Shankar EM
T-Cell Exhaustion in Chronic Infections: Reversing the State of Exhaustion and Reinvigorating Optimal Protective Immune Responses.
T-cell exhaustion is a phenomenon of dysfunction or physical elimination of antigen-specific T cells reported in human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infections as well as cancer. Exhaustion appears to be often restricted to CD8+ T cells responses in the literature, although CD4+ T cells have also been reported to be functionally exhausted in certain chronic infections. Although our understanding of the molecular mechanisms associated with the transcriptional regulation of T-cell exhaustion is advancing, it is imperative to also explore the central mechanisms that control the altered expression patterns. Targeting metabolic dysfunctions with mitochondrion-targeted antioxidants are also expected to improve the antiviral functions of exhausted virus-specific CD8+ T cells. In addition, it is crucial to consider the contributions of mitochondrial biogenesis on T-cell exhaustion and how mitochondrial metabolism of T cells could be targeted whilst treating chronic viral infections. Here, we review the current understanding of cardinal features of T-cell exhaustion in chronic infections, and have attempted to focus on recent discoveries, potential strategies to reverse exhaustion and reinvigorate optimal protective immune responses in the host.
Front Immunol, 2018; 9
30409128: Movahed E, Cheok YY, Tan GMY, Lee CYQ, Cheong HC, Velayuthan RD, Tay ST, Chong PP, Wong WF, Looi CY
Lung-infiltrating T helper 17 cells as the major source of interleukin-17A production during pulmonary Cryptococcus neoformans infection.
IL-17A has emerged as a key player in the pathologies of inflammation, autoimmune disease, and immunity to microbes since its discovery two decades ago. In this study, we aim to elucidate the activity of IL-17A in the protection against Cryptococcus neoformans, an opportunistic fungus that causes fatal meningoencephalitis among AIDS patients. For this purpose, we examined if C. neoformans infection triggers IL-17A secretion in vivo using wildtype C57BL/6 mice. In addition, an enhanced green fluorescence protein (EGFP) reporter and a knockout (KO) mouse models were used to track the source of IL-17A secretion and explore the protective function of IL-17A, respectively.
BMC Immunol, 2018; 19

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BOARD NUMBER: S03-394

BLOCKING THE P2X7-NLRP3-IL-1β PATHWAY IN THE MATERNAL IMMUNE ACTIVATION MODEL PREVENTS AUTISM-LIKE PHENOTYPE IN MALE MOUSE OFFSPRING

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Autism spectrum disorder (ASD) is a complex neurodevelopmental condition caused by environmental and genetic factors. Mimicking a perinatal infection, treating pregnant mice with a double-stranded RNA (poly(I:C)) activates an antiviral immune response. Elevation of extracellular ATP activates P2X7 resulting in the formation of active NLRP3 and caspase-1, leading to the synthesis of IL-1β. Our aim was to identify downstream signaling pathways coupled to P2X7, converting maternal immune activation (MIA) to ASD-like features. To induce MIA dams were challenged with poly(I:C) injection on E12.5 and E17.5. A cohort of pregnant mice was pretreated with NLRP3 antagonist MCC950 or with neutralizing anti-mouse IL-1β antibody on E12. Behavioral experiments were performed on male offspring in the following order: social preference, self-grooming, marble-burying, and rotarod test followed by immunostained Purkinje cell counting and electron microscopic synaptosome integrity examination. Concentrations of inflammatory cytokines and chemokines were measured in fetal brain, and maternal plasma samples collected 0,1,6,24 or 48 hours following MIA induction. Maternal treatment with MCC950 and IL-1β antibodies counteracted the development of autistic behavioral and morphological characteristics in the offspring. We also explored time-dependent changes of a widespread cytokine and chemokine profile in maternal blood and fetal brain samples of poly(I:C)/saline-treated dams. MIA-induced increases in plasma RANTES, MCP-1, and fetal brain IL-1β, IL-2, IL-6, MCP-1 concentrations have been shown to be the effect of P2X7/NLRP3 regulation. Our results highlight that in addition to P2X7, NLRP3 and inflammatory cytokines may also be potential biomarkers and therapeutic targets of repetitive behavioral and social deficits observed in ASD.

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BOARD NUMBER: S03-395

JANUS KINASE AND MICROTUBULE-INTERACTING PROTEIN 1 (JAKMIP1), A NOVEL REGULATOR OF NUCLEAR RNA EXPORT IN NEURONS: COUPLING TRANSCRIPTION TO CYTOPLASMIC TRANSLATION THROUGH THE MICROTUBULE CYTOSKELETON

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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JAKMIP1 was first identified as an Autism spectrum disorder (ASD) candidate gene due to its downregulated expression in both idiopathic and syndromic forms of ASD. A later study then demonstrated that Jakmip1 deficiency results in core ASD-associated behaviours in knockout mice. This suggests that JAKMIP1 may play important roles in nervous system development and that its dysregulation may underlie the development of ASD-related behaviours. However, the molecular functions of JAKMIP1 are not well understood. To investigate its molecular function, we utilised truncated JAKMIP1 constructs tagged with fluorescent proteins. Interestingly, we observed predominantly nuclear localisation of the JAKMIP1 C-terminus, in stark contrast to the microtubular localisation of the N-terminus. To further understand its nuclear role, we employed a dual transcriptomic-proteomic approach utilising RNA-sequencing and immunoprecipitation-mass spectrometry. Our results reveal that JAKMIP1 interacts with cytoskeletal proteins through its N-terminus, and with proteins regulating mRNA splicing and nuclear tRNA export through its C-terminus. Furthermore, JAKMIP1 interacts with various ribosomal and translational regulatory proteins in the cytoplasm. We also identify novel pathways impacted by JAKMIP1 deficiency involved in extracellular matrix organisation and cytokine signalling. These findings suggest that JAKMIP1 may mediate the splicing and processing of mRNAs and tRNAs, facilitate their nuclear export and couple this with the assembly of ribonucleoprotein complexes for cytoplasmic translation through the microtubule network. How these processes affect broader cellular behaviours, such as neuronal responses to cytokines and the composition of the extracellular matrix, will require further investigation.

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BOARD NUMBER: S03-396

THE EFFECT OF L. REUTERI ON SOCIAL BEHAVIOR IS INDEPENDENT OF THE ADAPTIVE IMMUNE SYSTEM.

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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Gut microbes can modulate almost all aspects of host physiology throughout life. As a result, specific microbial interventions are attracting considerable attention as potential therapeutic strategies for treating a variety of conditions. Nonetheless, little is known about the mechanisms through which many of these microbes work. Recently, we and others have found that the commensal bacteria *L. reuteri* reverses social deficits in several mouse models (genetic, environmental and idiopathic) for neurodevelopmental disorders in a vagus nerve-, oxytocin- and biopterin-dependent manner. Given that gut microbes can signal to the brain through the immune system and *L. reuteri* promotes wound healing via the adaptive immune response, we asked whether the prosocial effect mediated by *L. reuteri* also depends on adaptive immunity. Here, we found that the effects of *L. reuteri* on social behavior and related changes in synaptic function are independent of the mature adaptive immune system. Interestingly, these finding indicate that the same microbe (*L. reuteri*) can affect different host phenotypes through distinct mechanisms.

Pubmed:
28181561: Wang IC, Chung CY, Liao F, Chen CC, Lee CH
Peripheral sensory neuron injury contributes to neuropathic pain in experimental autoimmune encephalomyelitis.
Multiple sclerosis (MS)-induced neuropathic pain deteriorates quality of life in patients but is often refractory to treatment. In experimental autoimmune encephalomyelitis (EAE), a rodent model of MS, animals develop neuropathy and inflammation-induced tissue acidosis, which suggests the involvement of acid-sensing ion channels (ASICs). Also, peripheral neuropathy is reported in MS patients. However, the involvement of the peripheral nervous system (PNS) in MS neuropathic pain remains elusive. This study investigated the contribution of ASICs and peripheral neuropathy in MS-induced neuropathic pain. Elicited pain levels were as high in Asic1a, Asic2 and Asic3 mice as wild-type mice even though only Asic1a mice showed reduced EAE disease severity, which indicates that pain in EAE was independent of disease severity. We thus adopted an EAE model without pertussis toxin (EAEnp) to restrain activated immunity in the periphery and evaluate the PNS contribution to pain. Both EAE and EAEnp mice showed similar pain behaviors and peripheral neuropathy in nerve fibers and DRG neurons. Moreover, pregabalin significantly reduced neuropathic pain in both EAE and EAEnp mice. Our findings highlight the essential role of the PNS in neuropathic pain in EAE and pave the way for future development of analgesics without side effects in the CNS.
Sci Rep, 2017; 7
31636454: Chen CJ, Sgritta M, Mays J, Zhou H, Lucero R, Park J, Wang IC, Park JH, Kaipparettu BA, Stoica L, Jafar-Nejad P, Rigo F, Chin J, Noebels JL, Costa-Mattioli M
Therapeutic inhibition of mTORC2 rescues the behavioral and neurophysiological abnormalities associated with Pten-deficiency.
Dysregulation of the mammalian target of rapamycin (mTOR) signaling, which is mediated by two structurally and functionally distinct complexes, mTORC1 and mTORC2, has been implicated in several neurological disorders. Individuals carrying loss-of-function mutations in the phosphatase and tensin homolog (PTEN) gene, a negative regulator of mTOR signaling, are prone to developing macrocephaly, autism spectrum disorder (ASD), seizures and intellectual disability. It is generally believed that the neurological symptoms associated with loss of PTEN and other mTORopathies (for example, mutations in the tuberous sclerosis genes TSC1 or TSC2) are due to hyperactivation of mTORC1-mediated protein synthesis. Using molecular genetics, we unexpectedly found that genetic deletion of mTORC2 (but not mTORC1) activity prolonged lifespan, suppressed seizures, rescued ASD-like behaviors and long-term memory, and normalized metabolic changes in the brain of mice lacking Pten. In a more therapeutically oriented approach, we found that administration of an antisense oligonucleotide (ASO) targeting mTORC2's defining component Rictor specifically inhibits mTORC2 activity and reverses the behavioral and

neurophysiological abnormalities in adolescent Pten-deficient mice. Collectively, our findings indicate that mTORC2 is the major driver underlying the neuropathophysiology associated with Pten-deficiency, and its therapeutic reduction could represent a promising and broadly effective translational therapy for neurological disorders where mTOR signaling is dysregulated.
Nat Med, 2019; 25

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ASSESSMENT OF TOXICITY CAUSED BY EXPOSURE TO MICRO/NANOPLASTICS DURING ZEBRAFISH (DANIO RERIO) EARLY STAGES DEVELOPMENT

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

Luiza Kist[1], Lilian Teodoro[1], Kaue Pelegrini[1], Thuany Maraschin[1], Camilo Jablonski[1], Talita Pereira[1], Nara Basso[1], Mauricio Bogo[2]
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Inappropriate disposal of plastics and plastic particles has been contributed significantly to accumulation in the environment. Under these conditions, a remarkable amount of plastics end up in the aquatic environment where, by natural processes, they are continually fragmented into smaller particles called micro/nanoplastics that can be ingested/absorbed by aquatic organisms and accumulate in different tissues. Once accumulated, these particles can induce several toxic effects. Although several good studies have linked toxic effects with exposure to plastic particles, the whole process is far from be completely understood and almost nothing is known regarding the molecular mechanisms involved. Thus, this study evaluated the effects of PET (polyethylene terephthalate) micro/nanoplastics exposure (at 0.5, 1, 5,10 and 20 mg/L) during six consecutive days on mortality, hatching, spontaneous movement, heart rate, morphology and locomotor behavior of zebrafish embryos/larvae. There was no significant change in the mortality and hatching rate. In the 20 mg/L, spontaneous movement significantly decrease in comparison to the control group. Heart rate was increase at the four highest concentrations when compared to the control group. No significant changes were found in either body length or ocular surface area at any of the concentrations tested. On the other hand, there was a significant decrease in the ocular distance at all concentrations when compared to the control group. No significant alteration were found in the parameters used to assess locomotor behavioral. Additional studies are in course for deeper understanding of the toxic effects of PET micro/nanoplastics exposure.

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BOARD NUMBER: S03-398

ACUTE TOXICITY OF METHOMYL COMMERCIAL FORMULATION INDUCES MORPHOLOGICAL AND BEHAVIORAL CHANGES IN LARVAL ZEBRAFISH (DANIO RERIO)

POSTER SESSION 03 - SECTION: NEUROIMMUNE MECHANISMS MEDIATING DYSFUNCTION IN AUTISM

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The use of pesticides has continue grown over recent years, leading to several environmental and health concerns, such as the contamination of surface and groundwater resources and associated biota, potentially affecting populations that are not primary targets of these complex chemical mixtures. In this work, we investigate lethal and sublethal effects of acute exposure of methomyl commercial formulation in zebrafish embryo and larvae. Methomyl is a broad-spectrum carbamate insecticide and acaricide that acts primarily in acetylcholinesterase inhibition (AChE). Methomyl formulation 96h-LC_{50} was determined through the *Fish Embryo Acute Toxicity Test* (FET) and resulted in 1.2 g/L ± 0.04. Sublethal 6-day exposure was performed in six methomyl formulation concentrations (0.5; 1.0; 2.2; 4.8; 10.6; 23.3 mg/L) to evaluate developmental, physiological, morphological, behavioral, biochemical, and molecular endpoints of zebrafish early-development. Methomyl affected embryo hatching and larva morphology and behavior, especially in higher concentrations; resulting in smaller body and eyes size, failure in swimming bladder inflation, hypolocomotor activity, and concentration-dependent reduction of AChE activity; demonstrating methomyl strong acute toxicity and neurotoxic effect.

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BOARD NUMBER: S03-399

MODULATION OF GUT MICROBIOTA BY ANTIBIOTICS DID NOT AFFECT ANHEDONIA IN A HIGH-FAT DIET-INDUCED MODEL OF DEPRESSION IN MALE MICE.

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Long-term consumption of a high-fat diet (HFD) causes obesity and is a risk factor for depression. HFD has a significant impact on the gut microbiota, and dysbiosis of the microbiota is now associated with certain psychiatric disorders such as anxiety and depression. We aimed to investigate whether modulation by antibiotic treatment of the composition of the gut microbiota in diet-induced obese (DIO) mice has an impact on depressive-like behavior. In this study, we analyzed the effects of a 15 weeks HFD consumption by male mice on their depressive-like behavior assessed in the forced swim and sucrose preference tests. Two weeks before beginning the behavioral tests, a group of DIO mice were given a combination of two non-absorbable antibiotics, neomycin and polymyxin B. HFD induced anhedonia, as revealed by the sucrose preference test, and significant changes in gut microbiota composition at the phyla and family levels. On the other hand, there was no significant effect of HFD on the peripheral and brain inflammatory profiles. In DIO mice treated with the antibiotics, an even more pronounced alteration in the composition of the gut microbiota occurred, without any change in anhedonia behavior. Only four families of bacteria were not affected in their abundance by the antibiotic treatment, the *Rikenellaceae*, *Streptococcaceae*, *Erysipelotrichaceae* and *Bifidobacteriaceae*. This stability concomitant with that of anhedonia may suggest that these families were involved in this depression-like behavior.

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LOCOMOTOR AND EXPLORATIVE BEHAVIOUR OF JUVENILE MALE MOUSE OFFSPRING WERE ALTERED BY MATERNAL HIGH FAT DIET DURING THE PREIMPLANTATION

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Introduction: Both human and rodent studies have demonstrated that altered maternal environment influences foetal development, causing impairments in offspring brain. We hypothesise, in the absence of obesity, a maternal high-fat diet (HFD) during gestation/lactation or only during the preimplantation period affects offspring locomotor, explorative and anxiety behaviour. **Methods**: After mating, female-MF1 were allocated to one of three groups: Embryonic-HFD (EHFD: HFD up to E3.5, chow diet after); HFD and control group (NFD), consuming HFD and chow diet throughout pregnancy/lactation periods, respectively. Open field test (OFT) was performed for 10min at 4 (OFT1) and 10 weeks old (OFT2). Comparisons were made between HFD(n=9), EHFD(n=8), and NFD(n=9) groups using a hierarchical linear regression model. **Results:** In OFT1, EHFD males but not females, significantly travelled less (p=0.050), rested more (p=0.043), and had fewer ambulatory (p=0.037) and jump counts (p=0.026) than NFD. HFD males had fewer jump counts (p=0.016) while HFD females spent less time rearing compared to NFD (p=0.014), and EHFD (p=0.009). However, none of these differences were observed in OFT2 (p>0.05). EHFD females spent significantly less time than NFD (p=0.006) and HFD (p=0.007) females in the periphery area in the OFT1, but less time than NFD in the centre area in OFT2 (p=0.044). **Conclusions**: Maternal HFD exposure during only the preimplantation period particularly decreased juvenile male offspring locomotor and exploratory behaviour. This confirms that diet changes during the preimplantation period can affect offspring future health, while also suggesting that exposure to a normal diet can rescue the phenotype in adulthood.

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BOARD NUMBER: S03-401

BEHAVIORAL LANDSCAPE OF MICE UNDERGOING SICKNESS

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Most people face at some point of their life an unpleasant experience of feeling sick, a state that usually combines body aches, fever, lack of appetite, depressed mood and a need to seclude in a warm place. These symptoms are part of an evolutionarily conserved response among vertebrates known as sickness syndrome. The sickness syndrome is triggered by mediators produced by the immune system during systemic inflammation. Among the best studied mediators of sickness is prostaglandin E2 (PGE_2). It remains unclear, though, how different responses to PGE_2 develop over time. To address this question, we delivered PGE_2 intracerebroventricularly in mice and used a supervised machine learning approach to classify mouse behavioral motifs during sickness. Our data show that PGE_2 acting within the central nervous system can induce a rapid modification of both physiology and behavior, and elicit a state that recapitulates many of the symptoms observed during natural sicknesses. We envision that this detailed profiling of sickness-associated behavioral changes will identify key time windows on the action of PGE_2 and guide our future experiments to clarify neuronal mechanisms underlying sickness syndrome.

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IMPACT OF CALORIE-RESTRICTED CAFETERIA FEEDING AND TREADMILL EXERCISE ON SUCROSE INTAKE, SENSITIVITY AND REACTIVITY IN DIET-INDUCED OBESE MALE AND FEMALE RATS

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

Adam Alvarez[1,2], Alex Subias-Gusils[1,3], Noemí Boqué[4], Antoni Caimari[5], Josep Del Bas[4], Roger Mariné-Casadó[4], Rosa Escorihuela[1,3], Montserrat Solanas[1,2]
[1]Universitat Autònoma de Barcelona, Institut De Neurociències, Bellatera, Barcelona, Spain, [2]Autonomous University of Barcelona, Department Of Cell Biology, Physiology And Immunology, Faculty Of Medicine, Bellatera, Barcelona, Spain, [3]Autonomous University of Barcelona, Dept. Psychiatry And Forensic Medicine, Bellaterra, Spain, [4]Eurecat, Centre Tecnològic de Catalunya, Technological Unit Of Nutrition And Health, Reus, Spain, [5]Eurecat, Centre Tecnològic de Catalunya, Biotechnology Area And Technological Unit Of Nutrition And Health, Reus, Spain

We aimed to study how a previously characterized calorie-restricted cafeteria diet (CAFR) and moderate treadmill exercise (12-17 m/min) affects the sweet taste and biometrical and metabolic parameters in obese male and female rats. Animals were fed standard chow (STD) or cafeteria (CAF) diet for 8 weeks to induce obesity. Afterwards, animals were either maintained with *ad libitum* CAF or switched to CAFR diet. Dietary groups were subdivided into control (C) and exercise (E) groups. Biometric measures (body weight, Body mass index and abdominal perimeter) were recorded periodically. After 8 weeks of treatment, animals performed a two-bottle preference test, a brief-access licking test and a taste reactivity test with sucrose (0.01M-1M). CAFR feeding decreased all biometrical parameters measured compared with CAF only in females. CAF diet in both sexes decreased sucrose intake compared with STD, while CAFR diet partially reverted this effect in females. Exercise in CAF fed males decreased sucrose intake compared with the CAF-C group. In the brief-access test, CAF feeding decreased sucrose sensitivity and CAFR feeding reverted this effect in both sexes. Exercise also decreased sucrose sensitivity in CAF fed females. Hedonic behaviour elicited by sucrose was decreased in CAF fed males compared to the STD fed. These results indicate that the cafeteria diet-induced obesity decreases intake and sensitivity to sucrose, possibly through the development of an anhedonic state associated with obesity. This effect can be partially reverted by CAFR feeding. Additionally, treadmill exercise might decrease sucrose intake and its rewarding value in CAF-induced obese rats.

Pubmed:
29953904: García-Brito S, Aldavert-Vera L, Huguet G, Álvarez A, Kádár E, Segura-Torres P
Increased training compensates for OX1R blockage-impairment of spatial memory and c-Fos expression in different cortical and subcortical areas.
It has been suggested that the orexin system modulates learning and memory-related processes. However, the possible influence that training could have on the effect of the blockade of orexin-A selective receptor (OX1R) on a spatial memory task has not been explored. Therefore, the present study attempts to compare the effects of OX1R antagonist SB-334867 infusion on spatial memory in two different conditions in the Morris Water Maze (MWM). This experiment evaluated the animals' performance in weak training (2 trials per session) vs strong training (6 trials per session) protocols in a spatial version of the MWM. We found that in the 2-trial condition the post-training SB-334867 infusion had a negative effect on consolidation as well as on the retention and reversal learning of the task 72 h later. This effect was not apparent in the 6-trial condition. In addition, while the strong training groups showed a general increase in c-Fos expression in several brain areas of the hippocampal-thalamic-cortical circuit, SB-334867 administration had the opposite effect in areas that have been previously reported to have a high density of OX1R. Specifically, the SB-infused group in the 2-trial condition showed a decrease in c-Fos immunoreactivity in the dentate gyrus, granular retrosplenial and prelimbic cortices, and centrolateral thalamic nucleus. This was not observed for subjects in the 6-trial condition. The activation of these areas could constitute a neuroanatomical substrate involved in the compensatory mechanisms of training upon SB-334867 impairing effects on a MWM spatial task.
Behav Brain Res, 2018; 353
34960026: Subias-Gusils A, Álvarez-Monell A, Boqué N, Caimari A, Del Bas JM, Mariné-Casadó R, Solanas M, Escorihuela RM
Behavioral and Metabolic Effects of a Calorie-Restricted Cafeteria Diet and Oleuropein Supplementation in Obese Male Rats.

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Diet-induced obesity models are widely used to investigate dietary interventions for treating obesity. This study was aimed to test whether a dietary intervention based on a calorie-restricted cafeteria diet (CAF-R) and a polyphenolic compound (Oleuropein, OLE) supplementation modified sucrose intake, preference, and taste reactivity in cafeteria diet (CAF)-induced obese rats. CAF diet consists of high-energy, highly palatable human foods. Male rats fed standard chow (STD) or CAF diet were compared with obese rats fed CAF-R diet, alone or supplemented with an olive tree leaves extract (25 mg/kg*day) containing a 20.1% of OLE (CAF-RO). Biometric, food consumption, and serum parameters were measured. CAF diet increased body weight, food and energy consumption and obesity-associated metabolic parameters. CAF-R and CAF-RO diets significantly attenuated body weight gain and BMI, diminished food and energy intake and improved biochemical parameters such as triacylglycerides and insulin resistance which did not differ between CAF-RO and STD groups. The three cafeteria groups diminished sucrose intake and preference compared to STD group. CAF-RO also diminished the hedonic responses for the high sucrose concentrations compared with the other groups. These results indicate that CAF-R diet may be an efficient strategy to restore obesity-associated alterations, whilst OLE supplementation seems to have an additional beneficial effect on sweet taste function.
Nutrients, 2021; 13

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BOARD NUMBER: S03-403

EFFECTS OF A CAFETERIA RESTRICTED DIET AND OLEUROPEIN SUPPLEMENTATION ON SWEET TASTE MODIFICATIONS IN A CAFETERIA DIET-INDUCED OBESITY RODENT MODEL

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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[1]Autonomous University of Barcelona, Dept. Psychiatry And Forensic Medicine, Bellaterra, Spain, [2]Universitat Autònoma de Barcelona, Institut De Neurociències, Bellatera, Barcelona, Spain, [3]Universitat Autònoma de Barcelona, Department Of Cell Biology, Physiology And Immunology, Faculty Of Medicine, Bellatera, Barcelona, Spain, [4]Eurecat, Centre Tecnològic de Catalunya, Technological Unit Of Nutrition And Health, Reus, Tarragona, Spain, [5]Eurecat, Centre Tecnològic de Catalunya, Biotechnology Area And Technological Unit Of Nutrition And Health, Reus, Tarragona, Spain

AIMS: Diet-induced obesity models are widely used to investigate dietary interventions for treating obesity. This study was aimed to test whether a dietary intervention based on a calorie-restricted cafeteria diet (CAF-R) and a polyphenolic compound (Oleuropein, OLE) supplementation modified sucrose intake, preference and taste reactivity in a cafeteria diet (CAF)-induced obese rats. **METHODS:** Male Sprague-Dawley rats fed standard chow (STD) or CAF diet were compared with obese rats fed CAF-R diet, alone or supplemented with an olive tree leaves extract (25 mg/kg*day) containing a 20.1% of OLE (CAF-RO). Two-bottle preference and taste reactivity tests were performed to evaluate the sweet preference and the hedonic responses to sucrose solutions, respectively. Also, biometric, food consumption, and serum parameters were measured. **RESULTS:** As expected, CAF diet increased body weight, food and energy consumption and obesity-associated metabolic parameters. Interestingly, CAF-R and CAF-RO diets significantly attenuated body weight gain and BMI, diminished food and energy intake and improved biochemical parameters such as triacylglycerides and insulin resistance, which did not differ between CAF-RO and STD groups. The three cafeteria groups diminished sucrose intake and preference compared to STD group. CAF-RO also diminished the hedonic responses for the high sucrose concentrations compared with the other groups. **CONCLUSIONS:** These results indicate that CAF-R diet may be an efficient strategy to restore obesity-associated alterations, whilst OLE supplementation seems to have an additional beneficial effect on sweet taste function.

Pubmed:
33427533: Subias-Gusils A, Boqué N, Caimari A, Del Bas JM, Mariné-Casadó R, Solanas M, Escorihuela RM
A restricted cafeteria diet ameliorates biometric and metabolic profile in a rat diet-induced obesity model.
The administration of anti-obesity bioactive compounds and/or functional foods in rodents fed energy restriction diets based on chow food can be difficult to interpret. We propose an energy restricted cafeteria (CAF) diet as a dietetic intervention to be combined with other therapies. Postweaning male rats were fed standard chow, CAF diet or 30% energy restricted CAF diet (CAF-R) for 8 weeks. The CAF-R diet lowered energy intake and the increase of body weight and body mass index due to the CAF diet, lead to an intermediate feed efficiency, and dampened the CAF diet-induced alterations on body composition, serum levels of triacylglycerides and NEFAs, and insulin resistance. These effects were associated with diminished , and gene expression in brown adipose tissue. In conclusion, the CAF-R diet ameliorated obesity and related metabolic disorders induced by a regular CAF diet, turning it in a useful tool to study anti-obesity compounds.
Int J Food Sci Nutr, 2021; 72
34960026: Subias-Gusils A, Álvarez-Monell A, Boqué N, Caimari A, Del Bas JM, Mariné-Casadó R, Solanas M, Escorihuela RM
Behavioral and Metabolic Effects of a Calorie-Restricted Cafeteria Diet and Oleuropein Supplementation in Obese Male Rats.
Diet-induced obesity models are widely used to investigate dietary interventions for treating obesity. This study was aimed to test whether a dietary intervention based on a calorie-restricted cafeteria diet (CAF-R) and a polyphenolic compound (Oleuropein, OLE) supplementation modified sucrose intake, preference, and taste reactivity in cafeteria diet (CAF)-induced obese rats. CAF diet consists of high-energy, highly palatable human foods. Male rats fed standard chow (STD) or CAF diet were compared with obese rats fed CAF-R diet, alone or supplemented with an olive tree leaves extract (25 mg/kg*day) containing a 20.1% of OLE (CAF-RO). Biometric, food consumption, and serum parameters were measured. CAF diet increased body weight, food and energy consumption and obesity-associated metabolic parameters. CAF-R and CAF-RO

diets significantly attenuated body weight gain and BMI, diminished food and energy intake and improved biochemical parameters such as triacylglycerides and insulin resistance which did not differ between CAF-RO and STD groups. The three cafeteria groups diminished sucrose intake and preference compared to STD group. CAF-RO also diminished the hedonic responses for the high sucrose concentrations compared with the other groups. These results indicate that CAF-R diet may be an efficient strategy to restore obesity-associated alterations, whilst OLE supplementation seems to have an additional beneficial effect on sweet taste function.
Nutrients, 2021; 13

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-404

CHARACTERISING 'THE MUNCHIES'; EFFECTS OF TETRAHYDROCANNABINOL (THC) VAPOUR INHALATION ON RAT FEEDING BEHAVIOURS AND HOMEOSTATIC APPETITE-REGULATING PATHWAYS

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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There is an urgent drive to assess if cannabis has potential health-related effects. It's known that cannabis promotes food intake ('the munchies') and that tetrahydrocannabinol (THC), the main psychoactive cannabis component, is responsible for driving these effects. The aim of this study is to characterise the effects of THC on feeding patterns and investigate the mechanisms underlying THC-driven feeding. This research uses a THC vapour inhalation rat model which produces a translationally relevant THC dose. Rats are exposed to THC or vehicle vapour for 15-minutes each day, and subsequent food intake measured. We show that THC robustly drives food intake, and this is not dependent on what foods are available, or whether rats are satiated or not. THC also alters food choices, and the preferred food is dependent on the rat's state, where high-fat food is preferred in normal conditions and high-sugar food preferred in satiated conditions. Furthermore, once daily THC exposure does not affect total daily energy intake, suggesting that acute THC-driven feeding does not cause overeating. Our findings also imply that THC acts directly on the brain to drive feeding, as THC does not alter peripheral appetite hormone profiles and THC-driven feeding is not blocked by a peripherally restricted cannabinoid 1 receptor antagonist. Work is underway to assess how THC affects the activity of appetite-regulating brain circuits, to decipher the mechanisms by which THC alters feeding behaviour. Overall, this research sheds light on the physiological effects of THC, information which is critical for the health of cannabis users.

Pubmed:

34916909: Vecchiarelli HA, Aukema RJ, Hume C, Chiang V, Morena M, Keenan CM, Nastase AS, Lee FS, Pittman QJ, Sharkey KA, Hill MN
Genetic Variants of Fatty Acid Amide Hydrolase Modulate Acute Inflammatory Responses to Colitis in Adult Male Mice. Cannabinoids, including derived phytocannabinoids and endogenous cannabinoids (endocannabinoids), are typically considered anti-inflammatory. One such endocannabinoid is -arachidonoylethanolamine (anandamide, AEA), which is metabolized by fatty acid amide hydrolase (FAAH). In humans, there is a loss of function single nucleotide polymorphism (SNP) in the FAAH gene (C385A, rs324420), that leads to increases in the levels of AEA. Using a mouse model with this SNP, we investigated how this SNP affects inflammation in a model of inflammatory bowel disease. We administered 2,4,6-trinitrobenzene sulfonic acid (TNBS) intracolonically, to adult male FAAH SNP mice and examined colonic macroscopic tissue damage and myeloperoxidase activity, as well as levels of plasma and amygdalar cytokines and chemokines 3 days after administration, at the peak of colitis. We found that mice possessing the loss of function alleles (AC and AA), displayed no differences in colonic damage or myeloperoxidase activity compared to mice with wild type alleles (CC). In contrast, in plasma, colitis-induced increases in interleukin (IL)-2, leukemia inhibitory factor (LIF), monocyte chemoattractant protein (MCP)-1, and tumor necrosis factor (TNF) were reduced in animals with an A allele. A similar pattern was observed in the amygdala for granulocyte colony stimulating factor (G-CSF) and MCP-1. In the amygdala, the mutant A allele led to lower levels of IL-1α, IL-9, macrophage inflammatory protein (MIP)-1β, and MIP-2 independent of colitis-providing additional understanding of how FAAH may serve as a regulator of inflammatory responses in the brain. Together, these data provide insights into how FAAH regulates inflammatory processes in disease.
Front Cell Neurosci, 2021; 15
34907248: Baglot SL, Hume C, Petrie GN, Aukema RJ, Lightfoot SHM, Grace LM, Zhou R, Parker L, Rho JM, Borgland SL, McLaughlin RJ, Brechenmacher L, Hill MN
Pharmacokinetics and central accumulation of delta-9-tetrahydrocannabinol (THC) and its bioactive metabolites are influenced by route of administration and sex in rats.

Up to a third of North Americans report using cannabis in the prior month, most commonly through inhalation. Animal models that reflect human consumption are critical to study the impact of cannabis on brain and behaviour. Most animal studies to date utilize injection of delta-9-tetrahydrocannabinol (THC; primary psychoactive component of cannabis). THC injections produce markedly different physiological and behavioural effects than inhalation, likely due to distinctive pharmacokinetics. The current study directly examined if administration route (injection versus inhalation) alters metabolism and central accumulation of THC and metabolites over time. Adult male and female Sprague-Dawley rats received either an intraperitoneal injection or a 15-min session of inhaled exposure to THC. Blood and brains were collected at 15, 30, 60, 90 and 240-min post-exposure for analysis of THC and metabolites. Despite achieving comparable peak blood THC concentrations in both groups, our results indicate higher initial brain THC concentration following inhalation, whereas injection resulted in dramatically higher 11-OH-THC concentration, a potent THC metabolite, in blood and brain that increased over time. Our results provide evidence of different pharmacokinetic profiles following inhalation versus injection. Accordingly, administration route should be considered during data interpretation, and translational animal work should strongly consider using inhalation models.
Sci Rep, 2021; 11
34882838: Baglot SL, VanRyzin JW, Marquardt AE, Aukema RJ, Petrie GN, Hume C, Reinl EL, Bieber JB, McLaughlin RJ, McCarthy MM, Hill MN
Maternal-fetal transmission of delta-9-tetrahydrocannabinol (THC) and its metabolites following inhalation and injection exposure during pregnancy in rats.
Cannabis use during pregnancy has increased over the past few decades, with recent data indicating that, in youth and young adults especially, up to 22% of people report using cannabis during pregnancy. Animal models provide the ability to study prenatal cannabis exposure (PCE) with control over timing and dosage; however, these studies utilize both injection and inhalation approaches. While it is known that Δ9-tetrahydrocannabinol (THC; primary psychoactive component of cannabis) can cross the placenta, examination of the transmission and concentration of THC and its metabolites from maternal blood into the placenta and fetal brain remains relatively unknown, and the influence of route of administration has never been examined. Pregnant female rats were exposed to either vaporized THC-dominant cannabis extract for pulmonary consumption or subcutaneous injection of THC repeatedly during the gestational period. Maternal blood, placenta, and fetal brains were collected following the final administration of THC for analysis of THC and its metabolites, as well as endocannabinoid concentrations, through mass spectrometry. Both routes of administration resulted in the transmission of THC and its metabolites in placenta and fetal brain. Repeated exposure to inhaled THC vapor resulted in fetal brain THC concentrations that were about 30% of those seen in maternal blood, whereas repeated injections resulted in roughly equivalent concentrations of THC in maternal blood and fetal brain. Neither inhalation nor injection of THC during pregnancy altered fetal brain endocannabinoid concentrations. Our data provide the first characterization of maternal-fetal transmission of THC and its metabolites following both vaporized delivery and injection routes of administration. These data are important to establish the maternal-fetal transmission in preclinical injection and inhalation models of PCE and may provide insight into predicting fetal exposure in human studies.
J Neurosci Res, 2022; 100
31925973: Plaisier F, Hume C, Menzies J
Neural connectivity between the hypothalamic supramammillary nucleus and appetite- and motivation-related regions of the rat brain.
The supramammillary nucleus (SuM) has an emerging role in appetite control. We have shown that the rat SuM is activated during hunger or food anticipation, or by ghrelin administration. In the present study, we characterised the connectivity between the SuM and key appetite- and motivation-related nuclei in the rat. In adult wild-type rats, or rats expressing Cre recombinase under the control of the tyrosine hydroxylase (TH) promoter (TH-Cre rats), we used c-Fos immunohistochemistry to visualise and correlate the activation of medial SuM (SuMM) with activation in the lateral hypothalamic area (LH), the dorsomedial hypothalamus (DMH) or the ventral tegmental area (VTA) after voluntary consumption of a high-sugar, high-fat food. To determine neuroanatomical connectivity, we used retrograde and anterograde tracing methods to specifically investigate the neuronal inputs and outputs of the SuMM. After consumption of the food there were positive correlations between c-Fos expression in the SuMM and the LH, DMH and VTA ($P = 0.0001$, 0.01 and 0.004). Using Fluoro-Ruby as a retrograde tracer, we demonstrate the existence of inputs from the LH, DMH, VTA and ventromedial hypothalamus (VMH) to the SuMM. The SuMM showed reciprocal inputs to the LH and DMH, and we identified a TH-positive output from SuMM to DMH. We co-labelled retrogradely-labelled sections for TH in the VMH, or for TH, orexin and melanin-concentrating hormone in the LH and DMH. However, we did not observe any colocalisation of immunoreactivity with any retrogradely-labelled cells. Viral mapping in TH-Cre rats confirms the existence of a reciprocal SuMM-DMH connection and shows that TH-positive cells project from the SuMM and VTA to the lateral septal area and cingulate cortex, respectively. These data provide evidence for the connectivity of the SuMM to brain regions involved in appetite control, and form the

foundation for functional and behavioural studies aiming to further characterise the brain circuitry controlling eating behaviours.
J Neuroendocrinol, 2020; 32
31677199: Hume C, Allchorne A, Grinevich V, Leng G, Ludwig M
Effects of optogenetic stimulation of vasopressinergic retinal afferents on suprachiasmatic neurones.
Physiological circadian rhythms are orchestrated by the hypothalamic suprachiasmatic nucleus (SCN). The activity of SCN cells is synchronised by environmental signals, including light information from retinal ganglion cells (RGCs). We recently described a population of vasopressin-expressing RGCs (VP-RGC) that send axonal projections to the SCN. To determine how these VP-RGCs influence the activity of cells in the SCN, we used optogenetic tools to specifically activate their axon terminals within the SCN. Rats were intravitreally injected with a recombinant adeno-associated virus to express the channelrhodopsin-2 and the red fluorescent protein mCherry under the vasopressin promoter (VP-ChR2mCherry). In vitro recordings in acute brain slices showed that approximately 30% of ventrolateral SCN cells responded to optogenetic stimulation with an increase in firing rate that progressively increased during the first 200 seconds of stimulation and which persisted after the end of stimulation. Finally, application of a vasopressin V1A receptor antagonist dampened the response to optogenetic stimulation. Our data suggest that optogenetic stimulation of VP-RGC axons within the SCN influences the activity of SCN cells in a vasopressin-dependent manner.
J Neuroendocrinol, 2019; 31
30580497: Le May MV, Hume C, Sabatier N, Schéle E, Bake T, Bergström U, Menzies J, Dickson SL
Activation of the rat hypothalamic supramammillary nucleus by food anticipation, food restriction or ghrelin administration.
The circulating orexigenic hormone ghrelin targets many brain areas involved in feeding control and signals via a dedicated receptor, the growth hormone secretagogue receptor 1A. One unexplored target area for ghrelin is the supramammillary nucleus (SuM), a hypothalamic area involved in motivation and reinforcement and also recently linked to metabolic control. Given that ghrelin binds to the SuM, we explored whether SuM cells respond to ghrelin and/or are activated when endogenous ghrelin levels are elevated. We found that peripheral ghrelin injection activates SuM cells in rats, reflected by an increase in the number of cells expressing c-Fos protein in this area, as welll as by the predominantly excitatory response of single SuM cells recorded in in vivo electrophysiological studies. Further c-Fos mapping studies reveal that this area is also activated in rats in situations when circulating ghrelin levels are known to be elevated: in food-restricted rats anticipating the consumption of food and in fed rats anticipating the consumption of an energy-dense food. We also show that intra-SuM injection of ghrelin induces a feeding response in rats suggesting that, if peripheral ghrelin is able to access the SuM, it may have direct effects on this brain region. Collectively, our data demonstrate that the SuM is activated when peripheral ghrelin levels are high, further supporting the emerging role for this brain area in metabolic and feeding control.
J Neuroendocrinol, 2019; 31
28430937: Hume C, Sabatier N, Menzies J
High-Sugar, but Not High-Fat, Food Activates Supraoptic Nucleus Neurons in the Male Rat.
Oxytocin is a potent anorexigen and is believed to have a role in satiety signaling. We developed rat models to study the activity of oxytocin neurons in response to voluntary consumption or oral gavage of foods using c-Fos immunohistochemistry and in vivo electrophysiology. Using c-Fos expression as an indirect marker of neural activation, we showed that the percentage of magnocellular oxytocin neurons expressing c-Fos increased with voluntary consumption of sweetened condensed milk (SCM). To model the effect of food in the stomach, we gavaged anesthetized rats with SCM. The percentage of supraoptic nucleus and paraventricular nucleus magnocellular oxytocin-immunoreactive neurons expressing c-Fos increased with SCM gavage but not with gastric distention. To further examine the activity of the supraoptic nucleus, we made in vivo electrophysiological recordings from SON neurons, where anesthetized rats were gavaged with SCM or single cream. Pharmacologically identified oxytocin neurons responded to SCM gavage with a linear, proportional, and sustained increase in firing rate, but cream gavage resulted in a transient reduction in firing rate. Blood glucose increased after SCM gavage but not cream gavage. Plasma osmolarity and plasma sodium were unchanged throughout. We show that in response to high-sugar, but not high-fat, food in the stomach, there is an increase in the activity of oxytocin neurons. This does not appear to be a consequence of stomach distention or changes in osmotic pressure. Our data suggest that the presence of specific foods with different macronutrient profiles in the stomach differentially regulates the activity of oxytocin neurons.
Endocrinology, 2017; 158
27543760: Hume C, Jachs B, Menzies J
Homeostatic responses to palatable food consumption in satiated rats.
Energy intake is regulated by overlapping homeostatic and hedonic systems. Consumption of palatable foods has been implicated in weight gain, but this assumes that homeostatic control systems do not accurately detect this hedonically driven energy intake. This study tested this assumption, hypothesizing that satiated rats would reduce their voluntary food intake

and maintain a stable body weight after consuming a palatable food.
Obesity (Silver Spring), 2016; 24

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THE LACK OF GHSR SIGNALING ENHANCES THE ANOREXIGENIC AND HYPOGLYCEMIC EFFECTS OF LIRAGLUTIDE IN MICE

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Liraglutide is a GLP-1 receptor agonist that reduces glycemia and appetite and is used as an anti-diabetic medication to treat type 2 diabetes, obesity, and chronic weight management. Ghrelin is an orexigenic peptide hormone that acts via the growth hormone secretagogue receptor (GHSR) and upregulates mechanisms that increase glycemia. Aim: we tested here if the genetic deficiency of GHSR signaling in mice affects the anorexigenic and hypoglycemic effects of Liraglutide. Methods: we treated WT and GHSR-deficient mice with vehicle or Liraglutide (400 microg/Kg) under different experimental conditions, and assessed glycemia and food intake. Results: In *ad libitum* fed condition, Liraglutide did not acutely (0-90 min) affect glycemia of GHSR-deficient vs. WT mice, but induced a stronger reduction of overnight food intake in GHSR-deficient mice than WT mice. In fasted condition, Liraglutide did not acutely affect glycemia of GHSR-deficient vs. WT mice. After refeeding, Liraglutide reduced both fasting-induced hyperphagia and post-prandial increase of glycemia in GHSR-deficient mice and in WT mice but both effects were significantly more pronounced in GHSR-deficient mice. In vehicle-treated WT mice that were refed after fasting with the same amount of food eaten by Liraglutide-treated WT mice, postprandial glycemia was similar as detected in unrestrictedly refed WT mice suggesting that the liraglutide-induced reduction of postprandial glycemia was independent of food intake. After glucose infusion, Liraglutide similarly reduced hyperglycemia in GHSR-deficient vs. WT mice. Conclusion: The genetic deficiency of GHSR enhances the anorexigenic and hypoglycemic effects of liraglutide in mice via independent mechanisms.

Pubmed:

34721302: Hassouna R, Fernandez G, Lebrun N, Fiquet O, Roelfsema F, Labarthe A, Zizzari P, Tomasetto C, Epelbaum J, Viltart O, Chauveau C, Perello M, Tolle V
Ghrelin Gene Deletion Alters Pulsatile Growth Hormone Secretion in Adult Female Mice.
Using preproghrelin-deficient mice (), we previously observed that preproghrelin modulates pulsatile growth hormone (GH) secretion in post-pubertal male mice. However, the role of ghrelin and its derived peptides in the regulation of growth parameters or feeding in females is unknown. We measured pulsatile GH secretion, growth, metabolic parameters and feeding behavior in adult and male and female mice. We also assessed GH release from pituitary explants and hypothalamic growth hormone-releasing hormone (GHRH) expression and immunoreactivity. Body weight and body fat mass, linear growth, spontaneous food intake and food intake following a 48-h fast, GH pituitary contents and GH release from pituitary explants , fasting glucose and glucose tolerance were not different among adult and male or female mice. , pulsatile GH secretion was decreased, while approximate entropy, that quantified orderliness of secretion, was increased in adult females only, defining more irregular GH pattern. The number of neurons immunoreactive for GHRH visualized in the hypothalamic arcuate nucleus was increased in adult females, as compared to females, whereas the expression of GHRH was not different amongst groups. Thus, these results point to sex-specific effects of preproghrelin gene deletion on pulsatile GH secretion, but not feeding, growth or metabolic parameters, in adult mice.
Front Endocrinol (Lausanne), 2021; 12
34559253: Cornejo MP, Denis RGP, García Romero G, Fernández G, Reynaldo M, Luquet S, Perello M
Ghrelin treatment induces rapid and delayed increments of food intake: a heuristic model to explain ghrelin's orexigenic effects.
Ghrelin is a stomach-derived peptide hormone with salient roles in the regulation of energy balance and metabolism. Notably, ghrelin is recognized as the most powerful known circulating orexigenic hormone. Here, we systematically investigated the effects of ghrelin on energy homeostasis and found that ghrelin primarily induces a biphasic effect on food intake that has indirect consequences on energy expenditure and nutrient partitioning. We also found that ghrelin-induced biphasic effect on

food intake requires the integrity of Agouti-related peptide/neuropeptide Y-producing neurons of the hypothalamic arcuate nucleus, which seem to display a long-lasting activation after a single systemic injection of ghrelin. Finally, we found that different autonomic, hormonal and metabolic satiation signals transiently counteract ghrelin-induced food intake. Based on our observations, we propose a heuristic model to describe how the orexigenic effect of ghrelin and the anorectic food intake-induced rebound sculpt a timely constrain feeding response to ghrelin.
Cell Mol Life Sci, 2021; 78
34478806: Uriarte M, De Francesco PN, Fernández G, Castrogiovanni D, D'Arcangelo M, Imbernon M, Cantel S, Denoyelle S, Fehrentz JA, Praetorius J, Prevot V, Perello M
Circulating ghrelin crosses the blood-cerebrospinal fluid barrier via growth hormone secretagogue receptor dependent and independent mechanisms.
Ghrelin is a peptide hormone mainly secreted from gastrointestinal tract that acts via the growth hormone secretagogue receptor (GHSR), which is highly expressed in the brain. Strikingly, the accessibility of ghrelin to the brain seems to be limited and restricted to few brain areas. Previous studies in mice have shown that ghrelin can access the brain via the blood-cerebrospinal fluid (CSF) barrier, an interface constituted by the choroid plexus and the hypothalamic tanycytes. Here, we performed a variety of in vivo and in vitro studies to test the hypothesis that the transport of ghrelin across the blood-CSF barrier occurs in a GHSR-dependent manner. In vivo, we found that the uptake of systemically administered fluorescent ghrelin in the choroid plexus epithelial (CPE) cells and in hypothalamic tanycytes depends on the presence of GHSR. Also, we detected lower levels of CSF ghrelin after a systemic ghrelin injection in GHSR-deficient mice, as compared to WT mice. In vitro, the internalization of fluorescent ghrelin was reduced in explants of choroid plexus from GHSR-deficient mice, and unaffected in primary cultures of hypothalamic tanycytes derived from GHSR-deficient mice. Finally, we found that the GHSR mRNA is detected in a pool of CPE cells, but is nearly undetectable in hypothalamic tanycytes with current approaches. Thus, our results suggest that circulating ghrelin crosses the blood-CSF barrier mainly by a mechanism that involves the GHSR, and also possibly via a GHSR-independent mechanism.
Mol Cell Endocrinol, 2021; 538
32882134: Haj Salah KB, Maingot M, Blayo AL, M'Kadmi C, Damian M, Mary S, Cantel S, Neasta J, Oiry C, Péraldi-Roux S, Fernandez G, Romero GG, Perello M, Marie J, Banères JL, Fehrentz JA, Denoyelle S
Development of Nonpeptidic Inverse Agonists of the Ghrelin Receptor (GHSR) Based on the 1,2,4-Triazole Scaffold.
GHSR controls, among others, growth hormone and insulin secretion, adiposity, feeding, and glucose metabolism. Therefore, an inverse agonist ligand capable of selectively targeting GHSR and reducing its high constitutive activity appears to be a good candidate for the treatment of obesity-related metabolic diseases. In this context, we present a study that led to the development of several highly potent and selective inverse agonists of GHSR based on the 1,2,4-triazole scaffold. We demonstrate that, depending on the nature of the substituents on positions 3, 4, and 5, this scaffold leads to ligands that exert an intrinsic inverse agonist activity on GHSR-catalyzed G protein activation through the stabilization of a specific inactive receptor conformation. Thanks to an in vivo evaluation, we also show that one of the most promising ligands not only exerts an effect on insulin secretion in rat pancreatic islets but also affects the orexigenic effects of ghrelin in mice.
J Med Chem, 2020; 63
32029231: Cabral A, Fernandez G, Tolosa MJ, Rey Moggia Á, Calfa G, De Francesco PN, Perello M
Fasting induces remodeling of the orexigenic projections from the arcuate nucleus to the hypothalamic paraventricular nucleus, in a growth hormone secretagogue receptor-dependent manner.
Arcuate nucleus (ARC) neurons producing Agouti-related peptide (AgRP) and neuropeptide Y (NPY; ARC neurons) are activated under energy-deficit states. ARC neurons innervate the hypothalamic paraventricular nucleus (PVH), and ARC→PVH projections are recognized as key regulators of food intake. Plasma ghrelin levels increase under energy-deficit states and activate ARC neurons by acting on the growth hormone secretagogue receptor (GHSR). Here, we hypothesized that activation of ARC neurons in fasted mice would promote morphological remodeling of the ARC→PVH projections in a GHSR-dependent manner.
Mol Metab, 2020; 32
30582239: Perello M, Cabral A, Cornejo MP, De Francesco PN, Fernandez G, Uriarte M
Brain accessibility delineates the central effects of circulating ghrelin.
Ghrelin is a hormone produced in the gastrointestinal tract that acts via the growth hormone secretagogue receptor. In the central nervous system, ghrelin signalling is able to recruit different neuronal targets that regulate the behavioural, neuroendocrine, metabolic and autonomic effects of the hormone. Notably, several studies using radioactive or fluorescent variants of ghrelin have found that the accessibility of circulating ghrelin into the mouse brain is both strikingly low and restricted to some specific brain areas. A variety of studies addressing central effects of systemically injected ghrelin in mice have also provided indirect evidence that the accessibility of plasma ghrelin into the brain is limited. Here, we review these previous observations and discuss the putative pathways that would allow plasma ghrelin to gain access into the brain

together with their physiological implications. Additionally, we discuss some potential features regarding the accessibility of plasma ghrelin into the human brain based on the observations reported by studies that investigate the consequences of ghrelin administration to humans.
J Neuroendocrinol, 2019; 31
30276663: Uriarte M, De Francesco PN, Fernandez G, Cabral A, Castrogiovanni D, Lalonde T, Luyt LG, Trejo S, Perello M
Evidence Supporting a Role for the Blood-Cerebrospinal Fluid Barrier Transporting Circulating Ghrelin into the Brain.
The stomach-derived hormone ghrelin mainly acts in the brain. Studies in mice have shown that the accessibility of ghrelin into the brain is limited and that it mainly takes place in some circumventricular organs, such as the median eminence. Notably, some known brain targets of ghrelin are distantly located from the circumventricular organs. Thus, we hypothesized that ghrelin could also access the brain via the blood-cerebrospinal fluid (CSF) barrier, which consists of the choroid plexus and the hypothalamic tanycytes. Using systemic injection of ghrelin or fluorescent-ghrelin in mice, we found that cells of the blood-CSF barrier internalize these molecules. In time-response studies, we found that peripherally injected fluorescent-ghrelin quickly reaches hypothalamic regions located in apposition to the median eminence and more slowly reaches the periventricular hypothalamic parenchyma, adjacent to the dorsal part of the third ventricle. Additionally, we found that CSF ghrelin levels increase after the systemic administration of ghrelin, and that central infusions of either an anti-ghrelin antibody, which immuno-neutralizes CSF ghrelin, or a scrambled version of ghrelin, which is also internalized by cells of the blood-CSF barrier, partially impair the orexigenic effect of peripherally injected ghrelin. Thus, current evidence suggests that the blood-CSF barrier can transport circulating ghrelin into the brain, and that the access of ghrelin into the CSF is required for its full orexigenic effect.
Mol Neurobiol, 2019; 56
29300858: Fernandez G, Cabral A, Andreoli MF, Labarthe A, M'Kadmi C, Ramos JG, Marie J, Fehrentz JA, Epelbaum J, Tolle V, Perello M
Evidence Supporting a Role for Constitutive Ghrelin Receptor Signaling in Fasting-Induced Hyperphagia in Male Mice.
Ghrelin is a potent orexigenic peptide hormone that acts through the growth hormone secretagogue receptor (GHSR), a G protein-coupled receptor highly expressed in the hypothalamus. In vitro studies have shown that GHSR displays a high constitutive activity, whose physiological relevance is uncertain. As GHSR gene expression in the hypothalamus is known to increase in fasting conditions, we tested the hypothesis that constitutive GHSR activity at the hypothalamic level drives the fasting-induced hyperphagia. We found that refed wild-type (WT) mice displayed a robust hyperphagia that continued for 5 days after refeeding and changed their food intake daily pattern. Fasted WT mice showed an increase in plasma ghrelin levels, as well as in GHSR expression levels and ghrelin binding sites in the hypothalamic arcuate nucleus. When fasting-refeeding responses were evaluated in ghrelin- or GHSR-deficient mice, only the latter displayed an ∼15% smaller hyperphagia, compared with WT mice. Finally, fasting-induced hyperphagia of WT mice was significantly smaller in mice centrally treated with the GHSR inverse agonist K-(D-1-Nal)-FwLL-NH2, compared with mice treated with vehicle, whereas it was unaffected in mice centrally treated with the GHSR antagonists D-Lys3-growth hormone-releasing peptide 6 or JMV2959. Taken together, genetic models and pharmacological results support the notion that constitutive GHSR activity modulates the magnitude of the compensatory hyperphagia triggered by fasting. Thus, the hypothalamic GHSR signaling system could affect the set point of daily food intake, independently of plasma ghrelin levels, in situations of negative energy balance.
Endocrinology, 2018; 159
28204197: Cabral A, Cornejo MP, Fernandez G, De Francesco PN, Garcia-Romero G, Uriarte M, Zigman JM, Portiansky E, Reynaldo M, Perello M
Circulating Ghrelin Acts on GABA Neurons of the Area Postrema and Mediates Gastric Emptying in Male Mice.
Ghrelin is known to act on the area postrema (AP), a sensory circumventricular organ located in the medulla oblongata that regulates a variety of important physiological functions. However, the neuronal targets of ghrelin in the AP and their potential role are currently unknown. In this study, we used wild-type and genetically modified mice to gain insights into the neurons of the AP expressing the ghrelin receptor [growth hormone secretagogue receptor (GHSR)] and their role. We show that circulating ghrelin mainly accesses the AP but not to the adjacent nucleus of the solitary tract. Also, we show that both peripheral administration of ghrelin and fasting induce an increase of c-Fos, a marker of neuronal activation, in GHSR-expressing neurons of the AP, and that GHSR expression is necessary for the fasting-induced activation of AP neurons. Additionally, we show that ghrelin-sensitive neurons of the AP are mainly γ-aminobutyric acid (GABA)ergic, and that an intact AP is required for ghrelin-induced gastric emptying. Overall, we show that the capacity of circulating ghrelin to acutely induce gastric emptying in mice requires the integrity of the AP, which contains a population of GABA neurons that are a target of plasma ghrelin.
Endocrinology, 2017; 158
26661382: Fernandez G, Cabral A, Cornejo MP, De Francesco PN, Garcia-Romero G, Reynaldo M, Perello M

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Des-Acyl Ghrelin Directly Targets the Arcuate Nucleus in a Ghrelin-Receptor Independent Manner and Impairs the Orexigenic Effect of Ghrelin.

Ghrelin is a stomach-derived octanoylated peptide hormone that plays a variety of well-established biological roles acting via its specific receptor known as growth hormone secretagogue receptor (GHSR). In plasma, a des-octanoylated form of ghrelin, named des-acyl ghrelin (DAG), also exists. DAG is suggested to be a signalling molecule that has specific targets, including the brain, and regulates some physiological functions. However, no specific receptor for DAG has been reported until now, and, consequently, the potential role of DAG as a hormone has remained a matter of debate. In the present study, we show that DAG specifically binds to and acts on a subset of arcuate nucleus (ARC) cells in a GHSR-independent manner. ARC cells labelled by a DAG fluorescent tracer include the neuropeptide Y (NPY) and non-NPY neurones. Given the well-established role of the ARC in appetite regulation, we tested the effect of centrally administered DAG on food intake. We found that DAG failed to affect dark phase feeding, as well as food intake, after a starvation period; however, it impaired the orexigenic actions of peripherally administered ghrelin. Thus, we conclude that DAG directly targets ARC neurones and antagonises the orexigenic effects of peripherally administered ghrelin.

J Neuroendocrinol, 2016; 28

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BOARD NUMBER: S03-406

BINGE-EATING ALTERS DOPAMINERGIC SYSTEM, REDUCES ANXIETY, AND INCREASES IMPULSIVITY IN PERIADOLESCENT RATS

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

Elena Alvarez, Cinthia García-Luna, Patricia De Gortari, Paulina Soberanes
National Institute of Psychiatry, Neuroscience Research, Mexico City, Mexico

Binge-eating (BE) is a behavior characterized by repeated and intermittent overconsumption of palatable food during a short period of time, accompanied by dopaminergic disfunction. Such alterations perpetuate BE and promote obesity, impulsivity, and anxiety-like behaviors. Early-age dietary manipulations alter brain development, modifying feeding behavior and emotional states, increasing animal susceptibility to mood or eating disorders. We aim to describe BE impact in weaning rats' food intake, body and fat depots weight, prefrontal cortex (PFC) and accumbal (NAc) dopaminergic systems, impulsivity, and anxiety-like behavior, during periadolescence. Twenty-one-day old Wistar male rats were subjected to elevated plus maze test weekly. Chow and water were available throughout the study, access to Oreo was limited to two hours, three times per week (BED-group), during 4 weeks. Compared to control rats, body weight and daily chow intake of BED-group were reduced, while Oreo intake escalated until the end of the experiment. Higher caloric intake of BED-group increased brown adipose tissue (BAT) weight. Intermittent access to palatable food induced a gradual reduction in anxiety-like behaviors and more impulsivity. Accumbal dopamine and DOPAC levels decreased in BED-group. Dopamine receptor type 2 expression was decreased in PFC and increased in NAc. Intermittent access to palatable food altered dopaminergic signaling in PFC and NAc which might be responsible for the less anxious and more impulsive behaviors. The increase in BAT depot could be related to the lack of overweight despite high energy intake, nevertheless, other metabolic adaptations in BED-group remain to be elucidated.

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BOARD NUMBER: S03-407

OBESITY-DRIVEN MICROGLIAL ACTIVATION – FUNCTIONAL ROLE OF CEREBRAL STEROL METABOLISM

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Microglia are resident tissue macrophages, long-lived and show signs of aging. Their activation state is affected by obesity and diet, both associated with cognitive decline, impaired learning, memory loss and neurodegeneration. We hypothesize that the accumulation of certain nutrition-derived fatty acids, biologically active lipids and their metabolites in the brain has impact on aging and microglial cell loss. We isolated brain regions from C57BL6/N mice divided into two groups, one on `normal diet´ and one on `high fat diet´ (HFD) for 4, 12 and 24 weeks. RNA was isolated and sequenced using the Illumina NextSeq 2000 sequencing system; lipid content was studied using LC-MS/MS. The main findings are that phytosterols accumulate in the brain with age, moreover phytosterol content in brain and adipose tissue correlates with dietary phytosterol content. As shown by the increase in Iba1-positive cells and an activated morphological phenotype, HFD-related obesity is associated with microglia activation. Moreover, our data show a negative correlation between the amount of phytosterols and the level of pro-inflammatory COX-dependent lipid mediators in the brain. ***Thus, phytosterols may inhibit microglial activation and thus act in an anti-inflammatory way.***

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IDENTIFICATION OF A NOVEL HYPOTHALAMIC SYSTEM CONTROLING FEEDING BEHAVIOR AND INVESTIGATION OF ITS THERAPEUTIC POTENTIAL IN OBESITY.

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

Eleni Siopi[1], Mathieu Galerne[1], Audrey Rousseau[1], Manon Rivagorda[1], François Mailliet[1], Christiane Schreiweiss[1], Valérie Boitez[1], Mélissa Glatigny[1], Stéphanie Moriceau[1], Serge Luquet[2], Cyrille Confavreux[3], Alexandre Benani[4], Franck Oury[1]
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Objective. Obesity is a global health challenge with no effective treatment. Understanding the complex regulation of energy metabolism by the hypothalamus (HpT) is essential to provide new therapeutics. Our team has identified a well-known paracrine system with unknown roles in the brain as a key element of the HpT regulation of energy metabolism. Materials & Methods. Using multiple *in vitro* and *in vivo* models of gain and loss of function, we assessed the functional, cellular and molecular traits of this hypothalamic system, as well as it's therapeutic interest in the paradigm of high-fat diet-induced obesity. Results. We found that our system of interest is predominantly expressed in orexigenic AgRP – neurons in the HpT and that it harnesses the neurons' autophagy machinery to induce AgRP neuropeptide release and promote hunger. We then revealed that selective manipulation of this system protects from various symptoms of HFD-induced obesity, namely fat accumulation. Conclusion. We identified a novel hypothalamic system playing a key role in energy metabolism, generated results with promising clinical applications and open a plethora of perspectives pertaining to the role of this system in other HpT-driven functions.

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BOARD NUMBER: S03-409

SYMPATHETIC ASSOCIATED PERINEURIAL CELLS (SAPCS) ORCHESTRATE NEUROENDOCRINE LOOP OF LEPTIN ACTION TO MAINTAIN METABOLIC HOMEOSTASIS

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Sympathetically-innervated adipose tissue (AT) is the primary site of both energy storage and hormone production. Leptin is one such hormone released from AT which suppresses appetite and drives AT lipolysis by activating sympathetic neurons, providing a neuroendocrine feedback loop that controls energy balance. A novel sympathetic associated macrophage (SAM) has recently been implicated in the modulation of extracellular norepinephrine availability at the neuro-adipose junction in obesity, indicating a complex neuro-immune interaction in energy balance. Like SAM, other cellular players present in the neuro-adipose junction may also contribute to energy homeostasis, which is largely unknown. Here, using single-cell RNA sequencing, we transcriptionally profiled heterogenous immune and non-immune cell populations in the mouse sympathetic ganglia and nerve fibers innervating the AT. We identified a novel sympathetic neuron-associated population that is endothelial in nature and is uniquely marked by the expression of leptin receptor ($LepR^+$). We discovered that this population of sympathetic-associated $LepR^+$ cells constitutes the perineurial barrier surrounding sympathetic nerve bundles in fat and ganglia. Moreover, we found that these Sympathetic Associated $LepR^+$Perineurial Cells (SAPCs) express immunomodulatory cytokine, Il-33 which has previously been linked to reducing AT inflammation associated with obesity. SAPCs specific ablation of Il33 results in excessive weight gain and reduction of brown AT activity in mice fed a high-fat diet, suggesting Il33 in SAPCs are essential to protect obesity. Collectively, our study reveals that SAPCs play a crucial role to maintain metabolic homeostasis by sensing the variation at leptin levels and inducing an immune response via Il33.

Pubmed:
31900463: Sarker G, Larabee CM, Domingos AI
ILC3s gut rhythm.
Nat Immunol, 2020; 21
31754139: Sarker G, Litwan K, Kastli R, Peleg-Raibstein D
Maternal overnutrition during critical developmental periods leads to different health adversities in the offspring: relevance of obesity, addiction and schizophrenia.
Maternal overnutrition during sensitive periods of early development increases the risk for obesity and neuropsychiatric disorders later in life. However, it still remains unclear during which phases of early development the offspring is more vulnerable. Here, we investigate the effects of maternal high-fat diet (MHFD) at different stages of pre- or postnatal development and characterize the behavioral, neurochemical and metabolic phenotypes. We observe that MHFD exposure at pre-conception has no deleterious effects on the behavioral and metabolic state of the offspring. Late gestational HFD exposure leads to more prominent addictive-like behaviors with reduced striatal dopamine levels compared to early gestational HFD. Conversely, offspring exposed to MHFD during lactation display the metabolic syndrome and schizophrenia-like phenotype. The latter, is manifested by impaired sensory motor gating, and latent inhibition as well as enhanced sensitivity to amphetamine. These effects are accompanied by higher striatal dopamine levels. Together, our data suggest that MHFD exposure during specific stages of development leads to distinct neuropathological alterations that determine the severity and nature of poor health outcome in adulthood, which may provide insight in identifying effective strategies for early intervention.

Sci Rep, 2019; 9
31061112: Sarker G, Sun W, Rosenkranz D, Pelczar P, Opitz L, Efthymiou V, Wolfrum C, Peleg-Raibstein D
Maternal overnutrition programs hedonic and metabolic phenotypes across generations through sperm tsRNAs.
There is a growing body of evidence linking maternal overnutrition to obesity and psychopathology that can be conserved across multiple generations. Recently, we demonstrated in a maternal high-fat diet (HFD; MHFD) mouse model that MHFD induced enhanced hedonic behaviors and obesogenic phenotypes that were conserved across three generations via the paternal lineage, which was independent of sperm methylome changes. Here, we show that sperm tRNA-derived small RNAs (tsRNAs) partly contribute to the transmission of such phenotypes. We observe increased expression of sperm tsRNAs in the F1 male offspring born to HFD-exposed dams. Microinjection of sperm tsRNAs from the F1-HFD male into normal zygotes reproduces obesogenic phenotypes and addictive-like behaviors, such as increased preference of palatable foods and enhanced sensitivity to drugs of abuse in the resultant offspring. The expression of several of the differentially expressed sperm tsRNAs predicted targets such as and GRIN3A, which have been implicated in addiction pathology, are altered in the mesolimbic reward brain regions of the F1-HFD father and the resultant HFD-tsRNA offspring. Together, our findings demonstrate that sperm tsRNA is a potential vector that contributes to the transmission of MHFD-induced addictive-like behaviors and obesogenic phenotypes across generations, thereby emphasizing its role in diverse pathological outcomes.
Proc Natl Acad Sci U S A, 2019; 116
30577472: Sarker G, Peleg-Raibstein D
Maternal Overnutrition Induces Long-Term Cognitive Deficits across Several Generations.
Ample evidence from epidemiological studies has linked maternal obesity with metabolic disorders such as obesity, cardiovascular disease, and diabetes in the next generation. Recently, it was also shown that maternal obesity has long-term effects on the progeny's central nervous system. However, very little is known regarding how maternal overnutrition may affect, in particular, the cognitive abilities of the offspring. We reported that first-generation offspring exposed to a maternal high-fat diet (MHFD) displayed age-dependent cognitive deficits. These deficits were associated with attenuations of amino acid levels in the medial prefrontal cortex and the hippocampus regions of MHFD offspring. Here, we tested the hypothesis that MHFD in mice may induce long-term cognitive impairments and neurochemical dysfunctions in the second and third generations. We found that MHFD led to cognitive disabilities and an altered response to a noncompetitive receptor antagonist of the N-Methyl-D-aspartic acid (NMDA) receptor in adult MHFD offspring in both second and third generations in a sex-specific manner. Our results suggest that maternal overnutrition leads to an increased risk of developing obesity in subsequent generations as well as to cognitive impairments, affecting learning and memory processes in adulthood. Furthermore, MHFD exposure may facilitate pathological brain aging which is not a consequence of obesity. Our findings shed light on the long-term effects of maternal overnutrition on the development of the central nervous system and the underlying mechanisms which these traits relate to disease predisposition.
Nutrients, 2018; 11
30315171: Sarker G, Berrens R, von Arx J, Pelczar P, Reik W, Wolfrum C, Peleg-Raibstein D
Transgenerational transmission of hedonic behaviors and metabolic phenotypes induced by maternal overnutrition.
Maternal overnutrition has been associated with increased susceptibility to develop obesity and neurological disorders later in life. Most epidemiological as well as experimental studies have focused on the metabolic consequences across generations following an early developmental nutritional insult. Recently, it has been shown that maternal high-fat diet (HFD) affects third-generation female body mass via the paternal lineage. We showed here that the offspring born to HFD ancestors displayed addictive-like behaviors as well as obesity and insulin resistance up to the third generation in the absence of any further exposure to HFD. These findings, implicate that the male germ line is a major player in transferring phenotypic traits. These behavioral and physiological alterations were paralleled by reduced striatal dopamine levels and increased dopamine 2 receptor density. Interestingly, by the third generation a clear gender segregation emerged, where females showed addictive-like behaviors while male HFD offspring showed an obesogenic phenotype. However, methylome profiling of F1 and F2 sperm revealed no significant difference between the offspring groups, suggesting that the sperm methylome might not be the major carrier for the transmission of the phenotypes observed in our mouse model. Together, our study for the first time demonstrates that maternal HFD insult causes sustained alterations of the mesolimbic dopaminergic system suggestive of a predisposition to develop obesity and addictive-like behaviors across multiple generations.
Transl Psychiatry, 2018; 8
30087436: Sun W, Dong H, Becker AS, Dapito DH, Modica S, Grandl G, Opitz L, Efthymiou V, Straub LG, Sarker G, Balaz M, Balazova L, Perdikari A, Kiehlmann E, Bacanovic S, Zellweger C, Peleg-Raibstein D, Pelczar P, Reik W, Burger IA, von Meyenn F, Wolfrum C
Publisher Correction: Cold-induced epigenetic programming of the sperm enhances brown adipose tissue activity in the offspring.
In the version of this article originally published, the bars in the mean temperature graph in Fig. 1a were incorrectly aligned.

The left-most bar should have been aligned with the Apr label on the projected month of conception axis. The error has been corrected in the print, PDF and HTML versions of this article.
Nat Med, 2018; 24
30087435: Sun W, Dong H, Becker AS, Dapito DH, Modica S, Grandl G, Opitz L, Efthymiou V, Straub LG, Sarker G, Balaz M, Balazova L, Perdikari A, Kiehlmann E, Bacanovic S, Zellweger C, Peleg-Raibstein D, Pelczar P, Reik W, Burger IA, von Meyenn F, Wolfrum C
Author Correction: Cold-induced epigenetic programming of the sperm enhances brown adipose tissue activity in the offspring.
In the version of this article originally published, the months on the axis labeled projected month of conception in Fig. 1a were out of order. April and March should have been the first and last months listed, respectively. The error has been corrected in the print, PDF and HTML versions of this article.
Nat Med, 2018; 24
29988127: Sun W, Dong H, Becker AS, Dapito DH, Modica S, Grandl G, Opitz L, Efthymiou V, Straub LG, Sarker G, Balaz M, Balazova L, Perdikari A, Kiehlmann E, Bacanovic S, Zellweger C, Peleg-Raibstein D, Pelczar P, Reik W, Burger IA, von Meyenn F, Wolfrum C
Cold-induced epigenetic programming of the sperm enhances brown adipose tissue activity in the offspring.
Recent research has focused on environmental effects that control tissue functionality and systemic metabolism. However, whether such stimuli affect human thermogenesis and body mass index (BMI) has not been explored. Here we show retrospectively that the presence of brown adipose tissue (BAT) and the season of conception are linked to BMI in humans. In mice, we demonstrate that cold exposure (CE) of males, but not females, before mating results in improved systemic metabolism and protection from diet-induced obesity of the male offspring. Integrated analyses of the DNA methylome and RNA sequencing of the sperm from male mice revealed several clusters of co-regulated differentially methylated regions (DMRs) and differentially expressed genes (DEGs), suggesting that the improved metabolic health of the offspring was due to enhanced BAT formation and increased neurogenesis. The conclusions are supported by cell-autonomous studies in the offspring that demonstrate an enhanced capacity to form mature active brown adipocytes, improved neuronal density and more norepinephrine release in BAT in response to cold stimulation. Taken together, our results indicate that in humans and in mice, seasonal or experimental CE induces an epigenetic programming of the sperm such that the offspring harbor hyperactive BAT and an improved adaptation to overnutrition and hypothermia.
Nat Med, 2018; 24

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BOARD NUMBER: S03-410

LATE, BUT NOT EARLY ACTIVE-PHASE FORCED WHEEL RUNNING PREVENTED VISCERAL ADIPOSE TISSUE GAIN WITHOUT CHRONIC HYPOTHALAMIC CHANGES DURING THE ADOLESCENCE OF SPRAGUE-DAWLEY RATS

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

Yevheniy Kutsenko[1,2], Luis Iñiguez[3], Alberto Barreda[2], José Ángel Toval[4], Daniel Garrigos[1,2], Marta Martínez-Morga[1,2], Bruno Ribeiro Do Couto[5], Marta Wiśniewska[6], Marta Garaulet[7], Manuel Irimia[3], José Luis Ferrán[1,2]
[1]University of Murcia, Faculty of Medicine, Department Of Human Anatomy And Psychobiology, Murcia, Spain, [2]Institute of Biosanitary Research of Murcia, Department Of Human Anatomy And Psychobiology, Murcia, Spain, [3]Institute of Science and Technology, Centre For Genomic Regulation, Barcelona, Spain, [4]University of Granada, Physical And Sports Education, Granada, Spain, [5]University of Murcia, Department Of Psychology, Murcia, Spain, [6]Centre of New Technologies, Laboratory Of Molecular Neurobiology, Warsaw, Poland, [7]Institute of Biosanitary Research of Murcia, Department Of Physiology, Murcia, Spain

Changes in body composition during adolescence can increase the appearance of diseases such as obesity or diabetes later in life. In a previous work we found that male but not female rats showed decreased adipose tissue contents after a forced running with “early” (ZT14) and “late” (ZT20) active phase sessions (2 sessions a day). The suprachiasmatic nucleus is a region located in the anterior part of the hypothalamus and is the responsible for most of the circadian rythms of the body. Here, were analyzed the effects of forced running during either “early” (ZT13) or “late” (ZT23) sessions in the adipose and hypothalamic tissues. Adolescent rats were trained by forced running. On P24 and P57, the whole body of the rats was analyzed through computerized tomography. Food and water intake were measured every 24 hours. On P60, the hypothalamic region, the inguinal and the retro-renal fat were removed, and stored at -80C until further analysis. Only the late exercise group showed lower adipose tissue content ($p<0.05$). No differences were observed in the food intake or the orexigenic/anorexigenic genes expression of Pomc, Agrp, Npy, Cartpt ($p>0.05$) from the hypothalamic region. The KEGG pathway analysis revealed the steroid biosynthesis as a potential candidate of the transcriptomic differences between the exercised and sedentary PM rats in the inguinal fat. Our results suggest that the differences could be attributed to the steroid biosynthesis pathway, specifically the cholesterol synthesis. Further studies are required to elucidate the circadian mechanisms responsible for these differential effects of exercise.

Pubmed:
33980961: Kutsenko Y, Barreda A, Toval A, Garrigos D, Martínez-Morga M, Ribeiro Do Couto B, Ferran JL
Sex-dependent effects of forced exercise in the body composition of adolescent rats.
Determining the body composition during adolescence can predict diseases such as obesity, diabetes, and metabolic syndromes later in life; and physical activity became an effective way to restore changes in body composition. However, current available literature assessing the body composition before, during and after adolescence in female and male rodents by in vivo techniques is scarce. Thus, by using computerized tomography, we aimed to define the baseline of the weight and body composition during the adolescence and young adulthood of female and male Sprague-Dawley rats (on P30, P60 and P90) under standard diet. Then, we determined the effect of 18 days of forced exercise on the body weight and composition during the early adolescence (P27-45). The highest percentual increments in weight, body volume and relative adipose contents occurred during the female and male adolescence. Forced running during the early adolescence decreased weight, body volume and relative adipose delta and increment values in males only. The adolescence of rats is a period of drastic body composition changes, where exercise interventions have sex-dependent effects. These results support a model that could open new research windows in the field of adolescent obesity.
Sci Rep, 2021; 11
34040580: Garrigos D, Martínez-Morga M, Toval A, Kutsenko Y, Barreda A, Do Couto BR, Navarro-Mateu F, Ferran JL
A Handful of Details to Ensure the Experimental Reproducibility on the FORCED Running Wheel in Rodents: A Systematic Review.
A well-documented method and experimental design are essential to ensure the reproducibility and reliability in animal research. Experimental studies using exercise programs in animal models have experienced an exponential increase in the last decades. Complete reporting of forced wheel and treadmill exercise protocols would help to ensure the reproducibility of

training programs. However, forced exercise programs are characterized by a poorly detailed methodology. Also, current guidelines do not cover the minimum data that must be included in published works to reproduce training programs. For this reason, we have carried out a systematic review to determine the reproducibility of training programs and experimental designs of published research in rodents using a forced wheel system. Having determined that most of the studies were not detailed enough to be reproducible, we have suggested guidelines for animal research using FORCED exercise wheels, which could also be applicable to any form of forced exercise.
Front Endocrinol (Lausanne), 2021; 12
33394335: Toval A, Garrigos D, Kutsenko Y, Popović M, Do-Couto BR, Morales-Delgado N, Tseng KY, Ferran JL
Dopaminergic Modulation of Forced Running Performance in Adolescent Rats: Role of Striatal D1 and Extra-striatal D2 Dopamine Receptors.
Improving exercise capacity during adolescence impacts positively on cognitive and motor functions. However, the neural mechanisms contributing to enhance physical performance during this sensitive period remain poorly understood. Such knowledge could help to optimize exercise programs and promote a healthy physical and cognitive development in youth athletes. The central dopamine system is of great interest because of its role in regulating motor behavior through the activation of D1 and D2 receptors. Thus, the aim of the present study is to determine whether D1 or D2 receptor signaling contributes to modulate the exercise capacity during adolescence and if this modulation takes place through the striatum. To test this, we used a rodent model of forced running wheel that we implemented recently to assess the exercise capacity. Briefly, rats were exposed to an 8-day period of habituation in the running wheel before assessing their locomotor performance in response to an incremental exercise test, in which the speed was gradually increased until exhaustion. We found that systemic administration of D1-like (SCH23390) and/or D2-like (raclopride) receptor antagonists prior to the incremental test reduced the duration of forced running in a dose-dependent manner. Similarly, locomotor activity in the open field was decreased by the dopamine antagonists. Interestingly, this was not the case following intrastriatal infusion of an effective dose of SCH23390, which decreased motor performance during the incremental test without disrupting the behavioral response in the open field. Surprisingly, intrastriatal delivery of raclopride failed to impact the duration of forced running. Altogether, these results indicate that the level of locomotor response to incremental loads of forced running in adolescent rats is dopamine dependent and mechanistically linked to the activation of striatal D1 and extra-striatal D2 receptors.
Mol Neurobiol, 2021; 58
32499715: Toval A, Vicente-Conesa F, Martínez-Ortega P, Kutsenko Y, Morales-Delgado N, Garrigos D, Alonso A, Ribeiro Do Couto B, Popović M, Ferran JL
Hypothalamic /, Plasmatic Glucose and Lactate Remain Unchanged During Habituation to Forced Exercise.
It has been demonstrated that physical activity contributes to a healthier life. However, there is a knowledge gap regarding the neural mechanisms producing these effects. One of the keystones to deal with this problem is to use training programs with equal loads of physical activity. However, irregular motor and stress responses have been found in murine exercise models. Habituation to forced exercise facilitates a complete response to a training program in all rodents, reaching the same load of physical activity among animals. Here, it was evaluated if glucose and lactate - which are stress biomarkers - are increased during the habituation to exercise. Sprague-Dawley rats received an 8-days habituation protocol with progressive increments of time and speed of running. Then, experimental and control (non-habituated) rats were subjected to an incremental test. Blood samples were obtained to determine plasmatic glucose and lactate levels before, immediately after and 30 min after each session of training. and mRNA expression was determined by two-step qPCR. Our results revealed that glucose and lactate levels are not increased during the habituation period and tend to decrease toward the end of the protocol. Also, and were not chronically activated by the habituation program. Lactate and glucose, determined after the incremental test, were higher in control rats without previous contact with the wheel, compared with habituated and wheel control rats. These results suggest that the implementation of an adaptive phase prior to forced exercise programs might avoid non-specific stress responses.
Front Physiol, 2020; 11

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EXPRESSION OF BDNF IN ANOREXIA NERVOSA MOUSE MODEL, A BIOMARKER OF DIAGNOSIS AND PROGNOSIS?

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

Jingxian Cao[1], Chloé Tezenas Du Montcel[2], Julia Mattioni[3], Nicolas Lebrun[3], Virginie Tolle[3], Philip.Gorwood Gorwood[3], Odile Viltart[2], Nicolas Ramoz[3]
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Aims: Anorexia nervosa (AN) is a complex mental disorder mainly characterized by a voluntary food restriction and excessive physical activity resulting in dramatic weight loss. Changes in the brain-derived neurotropic factor (BDNF) have been described in AN. Besides its function on neuronal survival, synaptic plasticity and mood, BDNF was also reported to have a metabolic effect via both central nervous system (CNS) and peripheral organs, which makes BDNF a candidate for AN diagnosis biomarkers Our goal is to investigate changes in BDNF using mouse AN-like model by measuring BDNF levels in specific brain areas and blood in food-restricted and refeed animals. Methods: We used a mouse model combining a phase of chronic food restriction (50%) followed by an *ad libitum* refeeding period. Female mice have or not access to a running with wheel to create a similar metabolic environment that those of AN patients during restriction and recovery once hospitalised. The BDNF mRNA and protein levels were measured in both blood and brain samples (prefrontal cortex, hippocampus, hypothalamus, dorsal striatum, NAcc, VTA and amygdala) using qPCR and ELISA methods. Results: we expect significant differences of BDNF expression in blood and brain region in the fast animal compared to the levels in the refeed animals. Conclusions: The BDNF could represent a possible biomarker of AN for the diagnostic and the evolution to the remission when weight recover and thus will allow a better understanding of the aetiology of AN. This study is supported by Fédération pour la Recherche sur le Cerveau.

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MELATONIN AS MODULATOR OF MELATONIN RECEPTORS EXPRESSION AND ENDOGENOUS SYNTHESIS IN OBESE MICE BRAIN

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

Eduardo Antuña[1,2], Yaiza Potes[1,2], Juan Carlos Bermejo-Millo[1,2], Cristina Cachán-Vega[1,2], Beatriz Caballero[1,2], Ignacio Vega-Naredo[1,2], Ana Coto-Montes[1,2]
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Aims: Nowadays, obesity is one of the most incidence disease in the worldwide and its growth seems to be sharpening in the last decade. Our work has revealed substancial molecular alterations triggered by obesity in the brain of leptin-deficient mice. **Methods**: The present work has used six-week-old male mice (*Mus musculus*) purchased from Charles River Laboratories: eight Wild-type mice (C57BL/6J) and eight leptin-deficient ob/ob mice (B6.V-Lepob/J). Animals were randomly divided into four groups: two untreated control groups, and another two groups treated with 500µg melatonin per kg body weigth during 4 weeks. Controls groups received vehicle at comparable amount, route and treatment duration. The brain tissue processing of each mouse was homogenized following PARIS kit guidelines. The protein expression analysis was carried out using PCR and Western Blot immunoassay techniques. **Results**: Our results showed that leptin-deficience does not alter the melatonin membrane receptor (MT1) neither nuclear receptor (RORα) levels. However, melatonin treatment was able to modulate their expression. The two most important enzymes of the melatonin synthesis pathway were modified by leptin-deficiency, while exogenous melatonin was able to reverse those effects, decreasing protein expression, in the obese mice brain. **Conclusions**: Melatonin administration could be an efficient treatment to modify the melatonin receptors expression in brain and to reduce the impairment in melatonin synthesis induced by leptin deficiency. **Acknowledgments**: PAPI-21-PF-28, FI18/00149, IDI/2021/000033, PI17/02009, PI21/01596.

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BOARD NUMBER: S03-413

NASAL ADMINISTRATION OF MENTHAE HERBA EXTRACT IMPROVES LIPID METABOLISM IN OBESE MICE

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

Mi Ryeo Kim, So Young Kim, Moon-Yeol Choi, Mi Hyeong Kim, Kyung A Ko
Daegu Haany University, Physiology, Daegu, Korea, Republic of

Objective: This study was investigated the effect of Menthae Herba volatile extract (MEO) on peripheral and central changes in *ob/ob* mice. **Method**: Mice(7W) were divided into four groups: wild type (WT), WT-MEO and *ob/ob* (OB) and OB-MEO groups. Both the normal lean and OB mice were provided with a standard diet. After MEO treatment for 7 weeks, weight gain, feeding efficiency ratio, blood lipid profiles, abdominal fat weight, histopathological changes, and neurotransmitters in hypothalamus were measured using LC-MS/MS. **Results**: The MEO groups showed a tendency to increase weight and decrease body fat compared to the control group. The levels of serum TG, ALT and AST were decreased in the OB-MEO compared to the OB group. Consistent with the effects on weight gain and fat mass, the levels of TG, TC, and LDL decreased in the MEO compared to the control group. The size of adipocytes was reduced with MEO treatment compared to the control group. As a result of measuring GABA, 5-HT, DA, glutamate, Leu-enkephalin, and Met-enkephalin related to appetite control in the hypothalamus, the WT-MEO group showed a tendency to increase compared to the WT group, and the OB group showed an increase compared to the WT group, but the OB-MEO group showed a tendency to recover to the level of the WT group. **Conclusion**: Our data demonstrate that treatment of MEO volatile extract has anti-obesity effect through changes in markers related to lipid metabolism in peripheral tissue and hypothalamus. Supported by : NRF grant funded by MSIT (2021R1A2C201471711, 2018R1A2B600903613).

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BOARD NUMBER: S03-414

THE NEUROPROTECTIVE EFFECT PROMOTED BY THE SUPPLEMENTATION WITH SPRAY-DRIED PORCINE PLASMA INVOLVES THE MICROBIOTA-GUT-BRAIN AXIS.

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

Cristina Rosell-Cardona[1], Lluïsa Miró[1], Concepció Amat[1], Javier Polo[2], Miquel Moretó[1], Anna Pérez Bosque[1]
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Alterations on gut microbiota are associated to Alzheimer's disease (AD) progression via the microbiota-gut-brain axis. Dietary supplementation with spray-dried porcine plasma (SDP) prevents neuropathological AD hallmarks in SAMP8 mice. The aim of this work was to study whether changes in the gut microbiota can exert a role in the neuroprotective effects of SDP supplementation. Experiments were performed in 2- and 6-month-old SAMP8 mice fed a control or 8% SDP supplemented diets for 4 months. Cognitive performance was evaluated using the Novel Object Recognition test, BDNF abundance was determined by Western Blot, cytokines were quantified by Real-Time PCR and a commercial kit. Faecal microbiota analysis was performed using Illumina MiSeq platform. Senescence reduced short- and long-term memory as well as cortical BDNF abundance, while SDP supplementation prevented these effects (all, $p<0.05$). Senescence increased the expression of the pro-inflammatory cytokine *Il-1*β and reduced the concentration of the anti-inflammatory IL-10 in the cortex tissue (both, $p<0.05$). Aging also augmented serum concentration of IL-1β and TNF-α and colonic expression of these two cytokines (all, $p<0.05$). SDP prevented these aging effects on brain, systemic and colon inflammation (all, $p<0.05$). Furthermore, aging reduced the abundance of health-promoting bacteria such as *Lactobacillus*, and *Pediococcus*, and increased inflammation-associated bacteria like *Erysipelhotrix* (all, $q<0.05$), while SDP prevented these effects (all, $q<0.05$). In conclusion, the neuroprotective effects of SDP could be exerted by promoting the abundance of probiotic species and enhancing mucosal and systemic anti-inflammatory pathways, thus involving the microbiota-gut-brain axis.

Pubmed:

34836320: Rosell-Cardona C, Griñan-Ferré C, Pérez-Bosque A, Polo J, Pallàs M, Amat C, Moretó M, Miró L
Reply to Nifli, A.-P. Comment on "Rosell-Cardona et al. Dietary Spray-Dried Porcine Plasma Reduces Neuropathological Alzheimer's Disease Hallmarks in SAMP8 Mice. 2021, , 2369".
Thank you for your comments on our recent work of the effects of supplementation with spray-dried porcine plasma (SDP) on neuropathological markers of Alzheimer's disease (AD) [...].
Nutrients, 2021; 13
34371878: Rosell-Cardona C, Griñan-Ferré C, Pérez-Bosque A, Polo J, Pallàs M, Amat C, Moretó M, Miró L
Dietary Spray-Dried Porcine Plasma Reduces Neuropathological Alzheimer's Disease Hallmarks in SAMP8 Mice.
Alzheimer's disease (AD) is characterized by the aberrant processing of amyloid precursor protein (APP) and the accumulation of hyperphosphorylated tau, both of which are accompanied by neuroinflammation. Dietary supplementation with spray-dried porcine plasma (SDP) has anti-inflammatory effects in inflammation models. We investigated whether dietary supplementation with SDP prevents the neuropathological features of AD. The experiments were performed in 2- and 6-month-old SAMP8 mice fed a control diet, or a diet supplemented with 8% SDP, for 4 months. AD brain molecular markers were determined by Western blot and real-time PCR. Senescent mice showed reduced levels of p-GSK3β (Ser9) and an increase in p-CDK5, p-tau (Ser396), sAPPβ, and the concentration of Aβ (all < 0.05). SDP prevented these effects of aging and reduced levels (all < 0.05). Senescence increased the expression of and and pro-inflammatory cytokines (and ; all < 0.05); these changes were prevented by SDP supplementation. Moreover, SDP increased expression (< 0.05). Furthermore, in aged mice, the gene expression levels of the microglial activation markers , , and were increased, and SDP prevented these increases (all < 0.05). Thus, dietary SDP might delay AD onset by reducing its hallmarks in senescent mice.
Nutrients, 2021; 13

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THE GUT MICROBIOTA REGULATES THE CATECHOLAMINE BIOSYNTHETIC PATHWAY IN THE ADRENAL GLANDS OF STRESSED RATS.

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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It is now well established that the gut microbiota contributes to the regulation of the hypothalamo-pituitary-adrenal axis. Several studies showed that, following an acute or chronic stress, the plasma concentration of corticosterone is higher in germ-free (GF) rodents than in their conventional (SPF) counterparts. Additionally, the expression of genes encoding the corticotropin releasing hormone in the hypothalamus and the glucocorticoid receptors in several brain regions is altered in GF animals. The possible influence of the gut microbiota on other adrenal stress hormones, *i.e.* the catecholamines (epinephrine and norepinephrine), has comparatively received little attention. We addressed this issue by comparing the expression of genes encoding the enzymes involved in the catecholamine biosynthetic pathway in the adrenal glands of GF and SPF rats, subjected or not to a chronic stress (unpredictable chronic mild stress for 2 weeks) and/or an acute stress (insulin injection). In chronically stressed rats, the adrenal weight is significantly higher (+22% in SPF and +19% in GF rats). Reflecting the contribution of the gut microbiota to the stimulus-secretion coupling in chromaffin cells is the finding that the expression of the gene encoding phenylethanolamine N-methyltransferase (PNMT, enzyme converting norepinephrine to epinephrine) is significantly higher in GF stressed rats compared with SPF stressed counterparts. No significant change was observed for genes encoding tyrosine hydroxylase (TH) and dopamine beta hydroxylase (DbH). We also confirmed the ability of chronic stress to disrupt the composition of the gut microbiota. Collectively, our results unveil the gut microbiota as a new regulator of the adrenomedullary tissue.

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SOCIAL ISOLATION IN ADOLESCENCE: CHANGES IN THE GUT MICROBIOTA COMPOSITION AND IN THE HIPPOCAMPAL INFLAMMATION

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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[1]IRCCS Fatebenefratelli S. Giovanni di Dio, Laboratory Of Biological Psychiatry, Brescia, Italy, [2]University of Milan, Pharmacological And Biomolecular Sciences, Milan, Italy, [3]Free University of Bozen/Bolzano, Faculty Of Science And Technology, Bolzano, Italy

The principal aim of our work was to evaluate, in a preclinical model, changes in the gut microbiota in physiological condition and in response to stress during adolescence. We aimed to correlate the stressed microbiota composition to the inflammatory status in the brain. We used the paradigm of social deprivation in adolescent rats for four weeks after weaning, followed by re-socialization until adulthood. We collected fecal samples at different post-natal days to investigate the short- and long-lasting effects of social isolation on gut microbiota composition and we collected different brain areas at killing to measure a panel of inflammatory and microglia activation mediators. We performed 16S metagenomics sequencing for analyzing the gut microbiota composition and Real-Time PCR to evaluate the expression levels of selected markers. Principal coordinate analysis revealed that microbial changes were influenced by age in both isolated and control rats, regardless of sex, whereas social isolation impacted the microbial composition in a sex-dependent manner. Indeed, a multivariate analysis showed that social isolation induced short-term gut microbiota alterations in females but not in males. LEfSe analysis identified several stress-related bacterial taxa affected by social isolation, whose effect was maintained only for few of them. In the brain we found a specific inflammatory pattern, especially in dorsal hippocampus, significantly correlated with gut microbiota composition. We reported a novel association between gut microbiota community structure and inflammatory status related to social isolation during adolescence, suggesting that stress exposure during this sensitive period of development produces long-lasting effects on different biological systems.

Pubmed:
30057098: Cattane N, Mora C, Lopizzo N, Borsini A, Maj C, Pedrini L, Rossi R, Riva MA, Pariante CM, Cattaneo A
Identification of a miRNAs signature associated with exposure to stress early in life and enhanced vulnerability for schizophrenia: New insights for the key role of miR-125b-1-3p in neurodevelopmental processes.
Epidemiological and clinical studies have provided evidence for a role of both genetic and environmental factors, such as stressful experiences early in life, in the pathogenesis of Schizophrenia (SZ) and microRNAs (miRNAs) have been suggested to play a key role in the interplay between the environment and our genome. In this study, we conducted a miRNOme analysis in different samples (blood of adult subjects exposed to childhood trauma, brain (hippocampus) of rats exposed to prenatal stress and human hippocampal progenitor cells treated with cortisol) and we identified miR-125b-1-3p as a down-regulated miRNA in all the three datasets. Interestingly, a significant down-regulation was observed also in SZ patients exposed to childhood trauma. To investigate the biological systems targeted by miR-125b-1-3p and also involved in the effects of stress, we combined the list of biological pathways modulated by predicted and validated target genes of miR-125b-1-3p, with the biological systems significantly regulated by cortisol in the in vitro model. We found, as common pathways, the CXCR4 signaling, the G-alpha signaling, and the P2Y Purigenic Receptor Signaling Pathway, which are all involved in neurodevelopmental processes. Our data, obtained from the combining of miRNAs datasets across different tissues and species, identified miR-125b-1-3p as a key marker associated with the long-term effects of stress early in life and also with the enhanced vulnerability of developing SZ. The identification of such a miRNA biomarker could allow the early detection of vulnerable subjects for SZ and could provide the basis for the development of preventive therapeutic strategies.
Schizophr Res, 2019; 205
31350592: Lopizzo N, Zonca V, Cattane N, Pariante CM, Cattaneo A
miRNAs in depression vulnerability and resilience: novel targets for preventive strategies.
The exposure to stressful experiences during the prenatal period and through the first years of life is known to affect the brain developmental trajectories, leading to an enhanced vulnerability for the development of several psychiatric disorders later in

life. However, not all the subjects exposed to the same stressful experience develop a pathologic condition, as some of them, activating coping strategies, become more resilient. The disclosure of mechanisms associated with stress vulnerability or resilience may allow the identification of novel biological processes and potential molecules that, if properly targeted, may prevent susceptibility or potentiate resilience. Over the last years, miRNAs have been proposed as one of the epigenetic mechanisms mediating the long-lasting effects of stress. Accordingly, they are associated with the development of stress vulnerability or resilience-related strategies. Moreover, miRNAs have been proposed as possible biomarkers able to identify subjects at high risk to develop depression and to predict the response to pharmacological treatments. In this review, we aimed to provide an overview of findings from studies in rodents and humans focused on the involvement of miRNAs in the mechanisms of stress response with the final goal to identify distinct sets of miRNAs involved in stress vulnerability or resilience. In addition, we reviewed studies on alterations of miRNAs in the context of depression, showing data on the involvement of miRNAs in the pathogenesis of the disease and in the efficacy of pharmacological treatments, discussing the potential utility of miRNAs as peripheral biomarkers able to predict the treatment response.
J Neural Transm (Vienna), 2019; 126
32194233: Horowitz MA, Cattaneo A, Cattane N, Lopizzo N, Tojo L, Bakunina N, Musaelyan K, Borsini A, Zunszain PA, Pariante CM
Glucocorticoids prime the inflammatory response of human hippocampal cells through up-regulation of inflammatory pathways.
Increased pro-inflammatory cytokines and an overactive hypothalamic-pituitary-adrenal (HPA) axis have both been implicated in the pathogenesis of depression. However, these explanations appear contradictory because glucocorticoids are well recognised for their anti-inflammatory effects. Two hypotheses exist to resolve this paradox: the mediating presence of glucocorticoid receptor resistance, or the possibility that glucocorticoids can potentiate inflammatory processes in some circumstances. We sought to investigate these hypotheses in a cell model with significant relevance to depression: human hippocampal progenitor cells. We demonstrated that dexamethasone in vitro given for 24 hours and followed by a 24 hours rest interval before an immune challenge potentiates inflammatory effects in these neural cells, that is, increases the IL-6 protein secretion induced by stimulation with IL-1β (10 ng/mL for 24 hours) by + 49% ($P < 0.05$) at a concentration of 100 nM and by + 70% ($P < 0.01$) for 1 μM. These effects are time- and dose-dependent and require activation of the glucocorticoid receptor. Gene expression microarray assays using Human Gene 2.1st Array Strips demonstrated that glucocorticoid treatment up-regulated several innate immune genes, including chemokines and Nod-like receptor, NLRP6; using transcription factor binding motifs we found limited evidence that glucocorticoid resistance was induced in the cells. Our data suggests a mechanism by which stress may prime the immune system for increased inflammation and suggests that stress and inflammation may be synergistic in the pathogenesis of depression.
Brain Behav Immun, 2020; 87
32636817: Marizzoni M, Gurry T, Provasi S, Greub G, Lopizzo N, Ribaldi F, Festari C, Mazzelli M, Mombelli E, Salvatore M, Mirabelli P, Franzese M, Soricelli A, Frisoni GB, Cattaneo A
Comparison of Bioinformatics Pipelines and Operating Systems for the Analyses of 16S rRNA Gene Amplicon Sequences in Human Fecal Samples.
Amplicon high-throughput sequencing of 16S ribosomal RNA (rRNA) gene is currently the most widely used technique to investigate complex gut microbial communities. Microbial identification might be influenced by several factors, including the choice of bioinformatic pipelines, making comparisons across studies difficult. Here, we compared four commonly used pipelines (QIIME2, Bioconductor, UPARSE and mothur) run on two operating systems (OS) (Linux and Mac), to evaluate the impact of bioinformatic pipeline and OS on the taxonomic classification of 40 human stool samples. We applied the SILVA 132 reference database for all the pipelines. We compared phyla and genera identification and relative abundances across the four pipelines using the Friedman rank sum test. QIIME2 and Bioconductor provided identical outputs on Linux and Mac OS, while UPARSE and mothur reported only minimal differences between OS. Taxa assignments were consistent at both phylum and genus level across all the pipelines. However, a difference in terms of relative abundance was identified for all phyla (< 0.013) and for the majority of the most abundant genera (< 0.028), such as (QIIME2: 24.5%, Bioconductor: 24.6%, UPARSE-linux: 23.6%, UPARSE-mac: 20.6%, mothur-linux: 22.2%, mothur-mac: 21.6%, < 0.001). The use of different bioinformatic pipelines affects the estimation of the relative abundance of gut microbial community, indicating that studies using different pipelines cannot be directly compared. A harmonization procedure is needed to move the field forward.
Front Microbiol, 2020; 11
32699209: Cattaneo A, Ferrari C, Turner L, Mariani N, Enache D, Hastings C, Kose M, Lombardo G, McLaughlin AP, Nettis MA, Nikkheslat N, Sforzini L, Worrell C, Zajkowska Z, Cattane N, Lopizzo N, Mazzelli M, Pointon L, Cowen PJ, Cavanagh J, Harrison NA, de Boer P, Jones D, Drevets WC, Mondelli V, Bullmore ET, , Pariante CM
Whole-blood expression of inflammasome- and glucocorticoid-related mRNAs correctly separates treatment-resistant depressed patients from drug-free and responsive patients in the BIODEP study.

The mRNA expression signatures associated with the 'pro-inflammatory' phenotype of depression, and the differential signatures associated with depression subtypes and the effects of antidepressants, are still unknown. We examined 130 depressed patients (58 treatment-resistant, 36 antidepressant-responsive and 36 currently untreated) and 40 healthy controls from the BIODEP study, and used whole-blood mRNA qPCR to measure the expression of 16 candidate mRNAs, some never measured before: interleukin (IL)-1-beta, IL-6, TNF-alpha, macrophage inhibiting factor (MIF), glucocorticoid receptor (GR), SGK1, FKBP5, the purinergic receptor P2RX7, CCL2, CXCL12, c-reactive protein (CRP), alpha-2-macroglobulin (A2M), acquaporin-4 (AQP4), ISG15, STAT1 and USP-18. All genes but AQP4, ISG15 and USP-18 were differentially regulated. Treatment-resistant and drug-free depressed patients had both increased inflammasome activation (higher P2RX7 and proinflammatory cytokines/chemokines mRNAs expression) and glucocorticoid resistance (lower GR and higher FKBP5 mRNAs expression), while responsive patients had an intermediate phenotype with, additionally, lower CXCL12. Most interestingly, using binomial logistics models we found that a signature of six mRNAs (P2RX7, IL-1-beta, IL-6, TNF-alpha, CXCL12 and GR) distinguished treatment-resistant from responsive patients, even after adjusting for other variables that were different between groups, such as a trait- and state-anxiety, history of childhood maltreatment and serum CRP. Future studies should replicate these findings in larger, longitudinal cohorts, and test whether this mRNA signature can identify patients that are more likely to respond to adjuvant strategies for treatment-resistant depression, including combinations with anti-inflammatory medications.
Transl Psychiatry, 2020; 10
33074224: Marizzoni M, Cattaneo A, Mirabelli P, Festari C, Lopizzo N, Nicolosi V, Mombelli E, Mazzelli M, Luongo D, Naviglio D, Coppola L, Salvatore M, Frisoni GB
Short-Chain Fatty Acids and Lipopolysaccharide as Mediators Between Gut Dysbiosis and Amyloid Pathology in Alzheimer's Disease.
Metagenomic data support an association between certain bacterial strains and Alzheimer's disease (AD), but their functional dynamics remain elusive.
J Alzheimers Dis, 2020; 78
33429258: Lopizzo N, Mazzelli M, Zonca V, Begni V, D'Aprile I, Cattane N, Pariante CM, Riva MA, Cattaneo A
Alterations in 'inflammatory' pathways in the rat prefrontal cortex as early biological predictors of the long-term negative consequences of exposure to stress early in life.
Early life stress, especially when experienced during the first period of life, affects the brain developmental trajectories leading to an enhanced vulnerability for stress-related psychiatric disorders later in life. Although both clinical and preclinical studies clearly support this association, the biological pathways deregulated by such exposure, and the effects in shaping the neurodevelopmental trajectories, have so far been poorly investigated. By using the prenatal stress (PNS) model, a well-established rat model of early life stress, we performed transcriptomic analyses in the prefrontal cortex of rats exposed or not to PNS and sacrificed at different postnatal days (PNDs 21, 40, 62). We first investigated the long-lasting mechanisms and pathways affected in the PFC. We have decided to focus on the prefrontal cortex because we have previously shown that this brain region is highly sensitive to PNS exposure. We found that adult animals exposed to PNS show alterations in 389 genes, mainly involved in stress and inflammatory signalling. We then wanted to establish whether PNS exposure could also affect the neurodevelopmental trajectories in order to identify the most critical temporal window. We found that PNS rats show the most significant changes during adolescence (between PND 40 versus PND 21), with alterations of several pathways related to stress, inflammation and metabolism, which were maintained until adulthood.
Psychoneuroendocrinology, 2021; 124
34054596: Tosato S, Bonetto C, Lopizzo N, Cattane N, Barcella M, Turco G, Ruggeri M, Provasi S, Tomassi S, Dazzan P, Cattaneo A
Childhood and Adulthood Severe Stressful Experiences and Biomarkers Related to Glucose Metabolism: A Possible Association?
No study investigated the association between stress exposure in different stages of life and metabolic dysfunction. We explore the association between stress exposure and several biomarkers related to glucose metabolism (insulin, c-peptide, GIP, GLP-1, glucagon) in a group of 72 healthy individuals. We used the Childhood Experience of Care and Abuse-Questionnaire (CECA-Q) and a modified version of the Life Events Scale to define exposure to stress, according to four categories: no exposure to childhood trauma (CT) nor to stressful life events (SLEs) (46%), only to CT (25%), only to SLEs (21%), to both (8%). We found that c-peptide (= 0.006) and insulin (= 0.002) levels differed among the four categories: 0.77 ng/ml (SD 0.27) and 0.21 ng/ml (SD 0.06) for none, 0.77 (SD 0.37) and 0.20 (SD 0.08) for only SLEs, 0.88 (SD 0.39) and 0.27 (SD 0.12) for only CT, 1.33 (SD 0.57) and 0.40 (SD 0.28) for both, respectively. The highest levels of biomarkers were found in subjects exposed to both CT and SLEs. Our preliminary results seem to suggest that CT might be specifically associated with a dysfunction of glucose metabolism, which might increase the risk of poorer health outcomes in adulthood. This association seems to be even stronger in individuals additionally exposed to SLEs in adulthood. In conclusion, if

confirmed in other studies, subjects exposed to both CT and SLEs appear the most vulnerable individuals, for whom preventative interventions, such as healthy lifestyle education programs, might ameliorate the risk of developing metabolic abnormalities.
Front Psychiatry, 2021; 12
34593267: Lopizzo N, Marizzoni M, Begni V, Mazzelli M, Provasi S, Borruso L, Riva MA, Cattaneo A
Social isolation in adolescence and long-term changes in the gut microbiota composition and in the hippocampal inflammation: Implications for psychiatric disorders - Dirk Hellhammer Award Paper 2021.
Exposure to early adverse experiences induces persistent changes in physiological, emotional and behavioural functions predisposing the individual to an enhanced vulnerability to develop different disorders during lifespan. The adverse outcomes depend upon the timing of the stressful experiences, and in this contest, adolescence represents a key sensitive period for brain development. Among the biological systems involved, gut microbiota has recently been proposed to act on the interplay between the stress response, brain functions and immune system, through the gut-brain axis communication. In the current study we aimed to evaluate, in a preclinical model, changes over time in the microbiota community structure in physiological condition and in response to stress during adolescence. We also aimed to correlate the microbiota composition to the inflammatory status in brain. We used the preclinical model of social deprivation in rats during adolescence, based on the lack of all social contacts, for four weeks after weaning, followed by re-socialization until adulthood. We collected fecal samples at different post-natal days to investigate the short- and long-lasting effects of social isolation on gut microbiota composition and we collected brain areas (dorsal and ventral hippocampus) samples at killing to measure a panel of inflammatory and microglia activation markers. 16 S metataxonomic sequencing analysis revealed that microbial changes were influenced by age in both isolated and controls rats, regardless of sex, whereas social isolation impacted the microbial composition in a sex-dependent manner. A multivariate analysis showed that social isolation induced short-term gut microbiota alterations in females but not in males. We also identified several stress-related genera associated with social isolation condition. In brain areas we found a specific inflammatory pattern, in dorsal and ventral hippocampus, that significantly correlated with gut microbiota composition. Overall, in this study we reported a novel sex-specific association between gut microbiota composition and inflammatory response related to social isolation paradigm during adolescence, suggesting that stressful experiences during this sensitive period could have a long-lasting impact on the development of different biological systems that could in turn influence the vulnerability to develop mental disorders later in life.
Psychoneuroendocrinology, 2021; 133
34819883: Scassellati C, Marizzoni M, Cattane N, Lopizzo N, Mombelli E, Riva MA, Cattaneo A
The Complex Molecular Picture of Gut and Oral Microbiota-Brain-Depression System: What We Know and What We Need to Know.
Major depressive disorder (MDD) is a complex mental disorder where the neurochemical, neuroendocrine, immune, and metabolic systems are impaired. The microbiota-gut-brain axis is a bidirectional network where the central and enteric nervous systems are linked through the same endocrine, immune, neural, and metabolic routes dysregulated in MDD. Thus, gut-brain axis abnormalities in MDD patients may, at least in part, account for the symptomatic features associated with MDD. Recent investigations have suggested that the oral microbiome also plays a key role in this complex molecular picture of relationships. As on one hand there is a lot of what we know and on the other hand little of what we still need to know, we structured this review focusing, in the first place, on putting all pieces of this complex puzzle together, underlying the endocrine, immune, oxidative stress, neural, microbial neurotransmitters, and metabolites molecular interactions and systems lying at the base of gut microbiota (GM)-brain-depression interphase. Then, we focused on promising but still under-explored areas of research strictly linked to the GM and potentially involved in MDD development: (i) the interconnection of GM with oral microbiome that can influence the neuroinflammation-related processes and (ii) gut phageome (bacteria-infecting viruses). As conclusions and future directions, we discussed potentiality but also pitfalls, roadblocks, and the gaps to be bridged in this exciting field of research. By the development of a broader knowledge of the biology associated with MDD, with the inclusion of the gut/oral microbiome, we can accelerate the growth toward a better global health based on precision medicine.
Front Psychiatry, 2021; 12

FENS Forum 2022
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BOARD NUMBER: S03-417

A NEW GUT-BRAIN COMMUNICATION PATHWAY IN WHICH BACTERIAL SENSING VIA NEURONAL NOD2 REGULATES APPETITE AND BODY TEMPERATURE

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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This work uncovered a new microbiota-gut-brain axis involved in metabolism control. In homeostatic conditions, bacterial products are directly sensed by brain neurons, regulating their activity and impacting satiety. MDP, a muropeptide present in all bacteria cell walls, affects hypothalamic inhibitory neurons, and is implicated in feeding and body temperature control. The MDP receptor Nod2 is expressed in neurons from several brain regions, including the hypothalamus. Older female mice lacking Nod2 expression in inhibitory neurons displayed increased weight gain and food consumption. These older females also had reduced propensity to build nests, and, altered temperature regulation. MDP reduced food intake, only when Nod2 was present in inhibitory neurons. Muropeptides, given orally, or produced by intestinal bacteria, reached the brain and regulated neurons of diverse brain areas. Remarkably, the affected regions were distinct, depending on the age and sex of the mice. Only in older females, the arcuate nucleus of the hypothalamus, a main regulator of feeding behavior, was affected by MDP. The activity of inhibitory neurons of the ARC is depressed upon feeding, and it was similarly depressed upon oral administration of MDP. Infusion of MDP into single neurons, demonstrated that MDP-mediated effects were cell-autonomous. Furthermore, the ablation of Nod2 in specific hypothalamic regions, reproduced the phenotype observed in the conditional knockout mice. Oral antibiotic treatment abrogated the Nod2-mediated control over feeding and weight gain, suggesting that the intestinal microbiota is the Nod2 ligand source. Our work reveals a sex- and age-dependent pathway of gut–brain crosstalk, highlighting additional avenues for the treatment of neurological and metabolic disorders

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EFFECT OF GUT MICROBIOTA FROM CHILDREN WITH AUTISM SPECTRUM DISORDER ON BEHAVIOR AND ASD-RELATED BIOLOGICAL MARKERS IN GERM-FREE MICE

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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The involvement of the microbiota-gut-brain axis has recently been taken into account in the study of the pathophysiology of autism spectrum disorders (ASD). Preclinical studies have shown that interventions on the gut microbiota, including fecal microbiota transplantations, can modulate behavior in animal models of ASD. The germ-free mouse is a potential model, as it shows impairments in social behavior and increased repetitive behaviors compared to conventional mice. Therefore, our project aimed to test the effect of fecal transplantation of ASD children on behavior and several biological markers in germ-free mice. We hypothesized that behavioral phenotypes impaired in germ-free mice would not be improved, or even be worsened, by fecal microbiota from children with ASD, compared with mice receiving the microbiota from their neurotypical siblings. The experiment was carried out with fecal microbiota from children with or without gastro-intestinal (GI) symptoms as a co-morbidity. We analyzed social behavior, repetitive behavior, anxiety and spatial memory. The latter was impaired in the group receiving microbiota from children with ASD and GI symptoms. We also saw differences between groups in the composition and metabolic activity of the gut microbiota as well as in inflammatory markers. These results show that the microbiota of ASD children, particularly those with GI symptoms, can cause behavioral and biochemical differences in germ-free recipient mice. The current stage of our analyses is to determine whether correlations exist between those differences and the microbial composition and activity of the transferred microbiota.

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BOARD NUMBER: S03-419

A GUT-BRAIN CONNECTION: GUT MICROBIOME COMPOSITION IS DIFFERENTIALLY ALTERED AFTER REPETITIVE MILD TRAUMATIC BRAIN INJURY IN ADOLESCENT AND ADULT RATS

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Introduction: The adolescent period has recently been identified as a critical window in brain, immune, and microbiome development. However, there has been no investigation into the effects that the microbiome has on repetitive mild traumatic brain injury (RmTBI) pathophysiology and microbiome composition at this age. **Methods:** Eighty adolescent and 80 adult rats were used. To deplete the microbiome, half of the rats were administered an antibiotic cocktail in their drinking water for 14 days (80 Female: 80 Male). Microbiome depletion was confirmed via fecal samples. Rats were then randomized to either RmTBI or sham injuries. Fecal samples collected 11 days post-injury were analysed via 16s rRNA sequencing. **Results:** In adolescents, PCoA plots demonstrated clear separations between samples at 11 days post-injury. Analysis of inferred amplicon sequence variants (ASVs) demonstrated differences in composition between placebo+RmTBI and Antibiotic+RmTBI we identified increases in *muribaculaceae* and *lachnospiraceae* in antibiotic+RmTBI, both of which are involved in reducing inflammatory processes. A further 46 significant ASVs were also identified. In adults, PCoA plots demonstrated less separation between groups, with increases reductions in *bacteroides*, *rhodospirillales,* and *bacteroidales* in the Antibiotic+RmTBI which are beneficial bacteria. However, as only 4 ASVs were significantly different in the adult antibiotic+RmTBI compared to placebo+RmTBI, the adult microbiome appears to be more resilient to RmTBI, exhibiting quicker recolonization when compared to adolescents. **Conclusions:** Microbiome recolonization and influences on RmTBI differed between adolescents and adults, which may provide insight as to why adolescents are often more susceptible to poorer mTBI outcomes than adults.

Pubmed:
34757429: Sgro M, Kodila ZN, Brady RD, Reichelt AC, Mychaisuk R, Yamakawa GR
Synchronizing our clocks as we age: the influence of the brain-gut-immune axis on the sleep-wake cycle across the lifespan.
The microbes that colonize the small and large intestines, known as the gut microbiome, play an integral role in optimal brain development and function. The gut microbiome is a vital component of the bidirectional communication pathway between the brain, immune system, and gut, also known as the brain-gut-immune axis. To date, there has been minimal investigation into the implications of improper development of the gut microbiome and the brain-gut-immune axis on the sleep-wake cycle, particularly during sensitive periods of physical and neurological development, such as childhood, adolescence, and senescence. Therefore, this review will explore the current literature surrounding the overlapping developmental periods of the gut microbiome, brain, and immune system from birth through to senescence, while highlighting how the brain-gut-immune axis affects the maturation and organization of the sleep-wake cycle. We also examine how a dysfunction to either the microbiome or the sleep-wake cycle negatively affects the bidirectional relationship between the brain and gut, and subsequently the overall health and functionality of this complex system. Additionally, this review integrates therapeutic studies to demonstrate when dietary manipulations, such as supplementation with probiotics and prebiotics, can modulate the gut microbiome to enhance the health of the brain-gut-immune axis and optimize our sleep-wake cycle.
Sleep, 2022; 45
34553007: Wong KR, Sgro M, Yamakawa GR, Li C, McDonald SJ, Sun M, Shultz SR, Brady RD, Mychasiuk R
Gut microbiome depletion and repetitive mild traumatic brain injury differentially modify bone development in male and female adolescent rats.
Dysregulation of the gut microbiome has been shown to disrupt both bone formation and bone resorption in several preclinical and clinical models. However, the role of microbiome in adolescent bone development remains poorly understood. This effect of disrupted bone development may be more pronounced during adolescence, when bone development is vulnerable to environmental stimuli and external insults (e.g., antibiotic treatment and traumatic brain injury), as this is a critical window of development. Therefore, in this study, we sought to investigate the effect of repetitive mild traumatic brain

injury (RmTBI) and gut microbiome depletion by antibiotic treatment on femur length and bone density in male and female adolescent Sprague Dawley rats. Rats were randomly assigned to receive standard or antibiotic autoclaved drinking water and to receive sham or RmTBIs injuries. Using micro-computed tomography (μCT), we found sexually dimorphic changes in adolescent bone development in response to microbiome depletion and RmTBI. Specifically, gut microbiome depletion stunted femur growth in males and altered cross sectional bone area (CSA), bone area fraction, and the bone volume of low and mid density bone in the distal metaphyseal region of the femur. Conversely, RmTBI and antibiotic treatment individually disrupted bone growth, bone area fraction, and bone volume of high-density bone within the distal metaphyseal region of the femur in females, but not when combined. Therefore, findings from this study indicate that gut microbiome and RmTBI may alter bone development in a sex-dependent manner during adolescence.
Bone Rep, 2021; 15
32653883: Salberg S, Sgro M, Brady RD, Noel M, Mychasiuk R
The Development of Adolescent Chronic Pain following Traumatic Brain Injury and Surgery: The Role of Diet and Early Life Stress.
Pain is evolutionarily necessary for survival in that it reduces tissue damage by signaling the body to respond to a harmful stimulus. However, in many circumstances, acute pain becomes chronic, and this is often dysfunctional. Adolescent chronic pain is a growing epidemic with an unknown etiology and limited effective treatment options. Given that the relationship between acute pain and chronic pain is not straightforward, there is a need to better understand the factors that contribute to the chronification of pain. Since early life factors are critical to a variety of outcomes in the developmental and adolescent periods, they pose promise as potential mechanisms that may underlie the transition from acute to chronic pain. This review examines two early life factors: poor diet and adverse childhood experiences (ACEs); they may increase susceptibility to the development of chronic pain following surgical procedures or traumatic brain injury (TBI). Beyond their high prevalence, surgical procedures and TBI are ideal models to prospectively understand mechanisms underlying the transition from acute to chronic pain. Common themes that emerged from the examination of poor diet and ACEs as mechanisms underlying this transition included: prolonged inflammation and microglia activation leading to sensitization of the pain system, and stress-induced alterations to hypothalamic-pituitary-adrenal axis function, where cortisol is likely playing a role in the development of chronic pain. These areas provide promising targets for interventions, the development of diagnostic biomarkers, and suggest that biological treatment strategies should focus on regulating the neuroinflammatory and stress responses in an effort to modulate and prevent the development of chronic pain.
Dev Neurosci, 2020; 42
32954298: Bhatt D, Hazari A, Yamakawa GR, Salberg S, Sgro M, Shultz SR, Mychasiuk R
Investigating the cumulative effects of Δ9-tetrahydrocannabinol and repetitive mild traumatic brain injury on adolescent rats.
The prevalence of mild traumatic brain injury is highest amongst the adolescent population and can lead to complications including neuroinflammation and excitotoxicity. Also pervasive in adolescents is recreational cannabis use. Δ9-Tetrahydrocannabinol, the main psychoactive component of cannabis, is known to have anti-inflammatory properties and serves as a neuroprotective agent against excitotoxicity. Thus, we investigated the effects of Δ9-tetrahydrocannabinol on recovery when administered either prior to or following repeated mild brain injuries. Male and female Sprague-Dawley rats were randomly assigned to receive Δ9-tetrahydrocannabinol or vehicle either prior to or following the repeated injuries. Rats were then tested on a behavioural test battery designed to measure post-concussive symptomology. The hippocampus, nucleus accumbens and prefrontal cortex were extracted from all animals to examine mRNA expression changes (, , , , and). We hypothesized that, in both experiments, Δ9-tetrahydrocannabinol administration would provide neuroprotection against mild injury outcomes and confer therapeutic benefit. Δ9-Tetrahydrocannabinol administration following repeated mild traumatic brain injury was beneficial to three of the six behavioural outcomes affected by injury (reducing anxiety and depressive-like behaviours while also mitigating injury-induced deficits in short-term working memory). Δ9-Tetrahydrocannabinol administration following injury also showed beneficial effects on the expression of , and in the hippocampus, nucleus accumbens and prefrontal cortex. There were no notable benefits of Δ9-tetrahydrocannabinol when administered prior to injury, suggesting that Δ9-tetrahydrocannabinol may have potential therapeutic benefit on post-concussive symptomology when administered post-injury, but not pre-injury.
Brain Commun, 2020; 2

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TARGETING THE GUT MICROBIOTA FOR POSSIBLE BIOMARKERS IN ALZHEIMER'S DISEASE

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Neurodegenerative disorders such as Alzheimer's disease (AD) have become a critical public health. Although AD research has made important breakthroughs, the pathogenesis of this disease remains unclear, and specific AD diagnostic biomarkers and therapeutic strategies are still lacking. Meanwhile, the gut microbiota participates in pathogenesis in diseases such as AD, diabetes, obesity and has an impact on aging process. Moreover, the endocannabinoid system (ECS) and the gut microbiota are increasingly emerging as important players in maintaining the general homeostasis and the health status of the host. To understand the role of the gut microbiota and the ECS in AD, induced by diabetes mellitus type 2, we used males and females mice that were subjected to a different diets: control, control & prebiotics, high-fat and high-fat & prebiotics. During 15 months with diets and several behaviour tests, faecal microbiota analysis were performed on particular time points. Additionally, we investigated gene of interest in selected 11 different genus of bacteria. The components of the ECS were examined in different brain and gastrointestinal tract. We showed that specific high-fat diets triggered obesity, leading to diabetes mellitus type 2 and caused Alzheimer‘s disease, which is clinically diagnosed by observing declining cognitive functions and changes in different components of the ECS. In searching for a connection between gut microbiota and AD, we also noticed changes in the gut microbiota throughout the experimental period and observed a different detection of gene, encoding an enzyme involved in the metabolism of nucleotides in different diets and time points.

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BOARD NUMBER: S03-421

IMPACT OF THE GUT MICROBIOTA ON NICOTINE EFFECTS AND GLIA WITHIN THE REWARD SYSTEM IN MICE

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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The gut hosts trillions of microbes, which are key players of the host physiology, such as nutrient metabolism and immunomodulation. Over the past decade, it has become very clear that the microorganisms that inhabit the gastrointestinal tract have also a critical role in orchestrating brain functioning and behavior. Many studies have identified a link between dysbiosis and the pathogenesis of several neuropsychiatric disorders. However, studies on the impact of the gut microbiota on brain and behavioral responses to drugs of abuse have been very limited so far. In this context, our objective was to assess their influence on nicotine addiction-like processes, as tobacco addiction is still responsible for more than 8 million deaths each year. Here we demonstrate that a lack of gut microbiota in mice potentiates nicotine-induced activation of the reward system and reinforcing properties. These effects were not associated with gross behavioral alterations. By contrast, nicotine withdrawal syndrome was not impacted by gut microbiota depletion. Importantly, glial activity has a crucial role in shaping the reward system and its response to drugs. Here we further show that gut microbiota depletion modulates the resident immune cells in the reward system. Notably, it increases astrocyte density in the ventral tegmental area (VTA). Finally, we identify several sub-populations of microglia in the VTA that differ between its sub-parts, and show that they are re-organized in conditions of gut microbiota depletion. The present study suggests that probiotics modulating brain immune cells may be a promising therapeutic strategy for nicotine addiction.

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BOARD NUMBER: S03-422

GLOBAL PROTEOME PROFILING OF THE TEMPORAL CORTEX OF FEMALE RATS EXPOSED TO CHRONIC STRESS AND A WESTERN DIET

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Introduction: Animal studies have confirmed that long-term intake of obesogenic diets and stress exposure impair cognition, especially with regard to aspects that are dependent on the hippocampus, including memory processes and reversal learning. The main aim of our study was to verify the hypothesis that 12-week exposure to stress modifies alterations in the brain proteome induced by western diet in the female rats. Methods: Female Long Evans rats with or without exposure to social stress received standard chow diet, or obesogenic rodent diet (a western diet with human snacks). The cerebrocortical samples from each rat were immediately frozen on dry ice and stored for liquid chromatography-tandem mass spectrometry (LC-MS/ MS) analysis. Results: Based on the discovery analysis, a total of 2,793 proteins were identified and quantified, of which 239 were changed significantly between the temporal cortices across the four animal groups. Statistical analysis showed that western diet consumption alone and in combination with stress exposure resulted in changes only in 5.4% and 3.8% proteins, respectively. Whereas 48% of proteins were affected statistically by chronic stress exposure and this exposition down-regulated 86.3% of them. The most overrepresented biological processes were related to generation of precursor metabolites and energy, synapse transport, and regulation of neurotransmitter levels. Conclusion: Observed changes may contribute to the understanding of functional and morphological brain alterations described in literature as well as behavioral disturbances induced by exposure to the western diet and stress. Grant Support: This research is supported by National Science Center grant no. 2015/19/D/NZ7/02408.

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BOARD NUMBER: S03-423

THE IMPACT OF MATERNAL HIGH-FAT DIET ON OFFSPRING NEURODEVELOPMENT

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Aims: A typical Western diet is excessively fatty, leading to a rapid increase in human obesity worldwide, including the women of reproductive age. There is growing evidence that maternal high-fat diet (mHFD) may cause neurodevelopmental disorders in the offspring, in part due to the changes in the microbiota. Our aim was to determine how mHFD alters the microbiota in the mothers and their offspring and how these changes affect the behavior in offspring using a mouse model. *Methods:* Female C57Bl/6 mice were fed a control diet (CD, 10% fat) or high-fat diet (HFD, 60%) from weaning to lactation. Before mating, the metabolic status of the dams was evaluated by the body mass and glucose and insulin tolerance tests. The offspring were weaned to CD. We investigated the behavioral phenotype of the offspring in the open field, three-chamber, novel object recognition, marble burying, and Barnes maze tests. Microbiota composition of the cecum of the dams and their offspring were analyzed using 16S rRNA gene sequencing. *Results:* We determined that the consumption of HFD causes metabolic dysfunction in the dams. mHFD changed gut microbiota diversity and composition in both dams and offspring. Microbiota alterations at the phylum, genus, and species level were more prominent in mHFD female versus male offspring. In contrast, mHFD disturbed the behavior more in male offspring than in females. *Conclusions:* Our findings show, that mHFD altered the composition of the offspring gut microbiota which may contribute to abnormal behavior in a sex-specific manner.

Pubmed:
31050961: Morkunas V, Urbonaite G, Gabryte-Butkiene E, Sobutas S, Vengris M, Danielius R, Ruksenas O
DNA-Damaging Effect of Different Wavelength (206 and 257 nm) Femtosecond Laser Pulses.
The purpose of this study was to investigate the possible cytotoxic and genotoxic impact of new-generation 206 nm femtosecond solid-state laser irradiation on murine skin cells in vitro, and to compare the cell and DNA damage caused by different wavelength (206 vs. 257 nm) femtosecond laser pulses. The first attempts to evaluate the possible genotoxic impact of ultrashort laser pulses on the murine bone marrow cells in vitro revealed the unlooked-for DNA-damaging effect. However, the impact of far-ultraviolet (UV) radiation on genetic material of internal and external organs' cells may differ due to differences in size, structure, and biochemical composition of the cells. Mouse skin cells were exposed to different doses of 206 and 257 nm wavelength femtosecond laser, and 254 nm UV lamp irradiation. Comet assay in two versions-the standard alkaline and the enzyme-linked-was used for the evaluation of DNA damage. The irradiation determined by different parameters demonstrated intensity-dependent genotoxic impact. The pyrimidine dimers made up the greater part of DNA photodamage, but with rising exposure dose the increase of relative amount of more energy-consuming primary damage-DNA strand breaks-was detected. The 206 nm femtosecond laser irradiation was much more cytotoxic but caused less primary DNA damage than the same pulse duration longer wavelength (257 nm) laser irradiation. DNA-damaging effect of 206 nm femtosecond laser pulses with extremely low penetration force may highly depend on the size, structure, and biochemical composition of the cells of organ or tissue targets.
Photobiomodul Photomed Laser Surg, 2019; 37

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BOARD NUMBER: S03-424

REGULATION OF FEEDING BY OPTOGENETIC ACTIVATION AND INHIBITION OF LILLIPUTIAN GENE IN DROSOPHILA MELANOGASTER LARVAE

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Optogenetics is a tool that allows controlling cells with light. Here, we studied the effect of optogenetic activation and inhibition in *lilli*^{189Y} transgenic larvae. The *lilliputian* gene encodes for a DNA binding transcription factor. It is involved in several different biological processes.

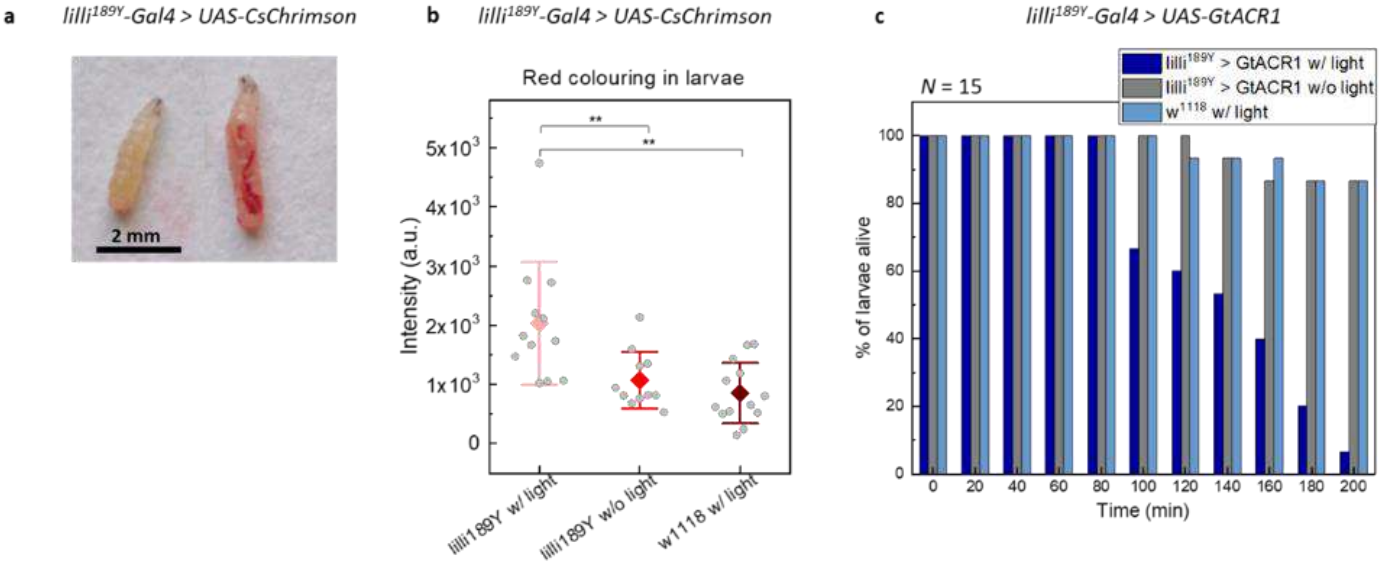

Figure 1. Behavioral response of *lilli*^{189Y} upon optogenetic stimulation. a) Comparison of apple agarose uptake (colored with red food dye) between w^{1118} (left) and *lilli*^{189Y}*-Gal4 > UAS-CsChrimson* (right) upon optogenetic activation. b) Intensity of ingested red color of larvae from a) after 200 min with and without optogenetic activation. c) Percentage of larvae alive over time during optogenetic inhibition. We observed that the optogenetic stimulation of *lilli*^{189Y}*-Gal4 > UAS-CsChrimson* larvae caused aggregation of larvae when located on agarose. In the absence of agarose, the larvae became aggressive showing cannibalistic behavior. We noticed a significant increase in food uptake due to activation with red light (Fig. 1a,b). On the other hand, inhibition of *lilli*^{189Y}*-Gal4 > UAS-GtACR1* caused larvae to die after 100 min of stimulation suggesting optogenetically induced dehydration (Fig. 1c). Fluorescence imaging of *lilli*^{189Y}*-Gal4* larvae showed expression in the salivary glands and in a few neurons of the larval central nervous system, pronounced in a few pairs of antennal lobe interneurons. We hypothesized that optogenetic control of the salivary glands caused the described feeding regulation. Repetition of our experiments with *SGS-Gal4*, a driver with sole expression in the salivary glands, led to the same results. In conclusion, our observations showed that the salivary glands contribute to regulating food uptake and may lead to collective behavior in *D. melanogaster* larvae.

Pubmed:
33077824: Meloni I, Sachidanandan D, Thum AS, Kittel RJ, Murawski C
Controlling the behaviour of Drosophila melanogaster via smartphone optogenetics.
Invertebrates such as Drosophila melanogaster have proven to be a valuable model organism for studies of the nervous system. In order to control neuronal activity, optogenetics has evolved as a powerful technique enabling non-invasive stimulation using light. This requires light sources that can deliver patterns of light with high temporal and spatial precision.

Currently employed light sources for stimulation of small invertebrates, however, are either limited in spatial resolution or require sophisticated and bulky equipment. In this work, we used smartphone displays for optogenetic control of Drosophila melanogaster. We developed an open-source smartphone app that allows time-dependent display of light patterns and used this to activate and inhibit different neuronal populations in both larvae and adult flies. Characteristic behavioural responses were observed depending on the displayed colour and brightness and in agreement with the activation spectra and light sensitivity of the used channelrhodopsins. By displaying patterns of light, we constrained larval movement and were able to guide larvae on the display. Our method serves as a low-cost high-resolution testbench for optogenetic experiments using small invertebrate species and is particularly appealing to application in neuroscience teaching labs.
Sci Rep, 2020; 10

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BOARD NUMBER: S03-425

THE IMPACT OF INHIBITORY TRAINING ON IMPLICIT ATTITUDE AND FOOD CONSUMPTION IN RESTRICTIVE EATERS

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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Restrained eaters are people that regularly limit their food consumption to control their weightγ They are at risk of developing eating disorders. Behaviorally, they showed inconsistent inhibition patterns, e.g., better inhibition when they were exposed to food stimuli and worsened inhibition when stimuli were neutral. Cognitively, restrained eaters show more negative thoughts about eating, weight, and shape than healthy patients. The implications of those beliefs help them to avoid all "threatening" stimuli, like high-calorie food, that could disturb them to achieve their goal that is weight loss. The current study used the stop-signal task as a training task and implicit association task and food intake test as measures to evaluate the effectiveness of the training. The participants were divided into 3 training conditions. In the first group, food stimuli were paired with the "stop" sign (stop-condition), in the second they attached to the "go" sign (go-condition) and in the third, they attached evenly for both signs (control-condition). The results of the study showed that food consumption was similar in the three groups. However, in the stop condition, participants endorsed more positive attitudes toward high-calorie food, and they reported the highest improvement of food-anxiety levels (in comparison to both other conditions). It could be that the training with stop condition creates a coupling between inhibition and food, in the broadest sense of the word. That is, the training helps participants to inhibit their intrusive thoughts about food, and thus to decrease their anxiety and to increase their positive attitude toward high-calorie food.

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COMPARATIVE IN SILICO ANALYSIS OF MICROBIAL DYSBIOSIS DISCERN POTENTIAL METABOLIC LINK IN NEURODEGENERATIVE DISEASES

POSTER SESSION 03 - SECTION: GUT-BRAIN AXIS AND THE MODULATION OF APPETITE

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A healthy gut flora contains a diverse and stable commensal group of microorganisms, whereas, in disease conditions, there is a shift towards pathogenic microbes, termed microbial dysbiosis. Many studies associate microbial dysbiosis with neurodegenerative disorders, including Alzheimer's, Parkinson's, Multiple sclerosis and Amyloid lateral sclerosis. Although several studies document microbial dysbiosis in neurodegenerative diseases, an overall comparative analysis of microbes and their metabolic involvement in these diseases is lacking. In this study, we performed a comparative analysis of microbial composition changes occurring in these 4 neurodegenerative disorders. Our analysis showed a high resemblance of microbial dysbiosis signatures between AD, PD and MS. However ALS appeared dissimilar in microbial dysbiosis signatures. The functional analysis of these dysbiotic microbes showed several potential metabolic links which can be involved in the altered microbiome-gut-brain axis in neurodegenerative diseases. The microbes with elevated populations lack pathways for synthesizing SCFA, butyrate, and Propionate. Also, these microbes have a high capacity for producing L-glutamate, an excitatory neurotransmitter and precursor of GABA. On the other hand, Tryptophan, a precursor for serotonin and other neuroactive molecules has a lower representation in the annotated genome of elevated microbes. Similarly, histamine, a crucial neuro-immuno-modulatory compound, was also less expressed in microbes found in elevated conditions. Finally, the neuroprotective compound spermidine was also less represented in the genome of elevated microbes. Together, our study showed the potential metabolic involvement of dysbiotic microbes in neurodegenerative disorders. Further, this can lead to microbiome-based therapeutic and diagnostic approaches in neurodegenerative diseases including AD, PD, MS and ALS.

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BOARD NUMBER: S03-427

A NON-INVASIVE BRAIN-MACHINE INTERFACE SYSTEM FOR THE CONTROL OF HAND PROSTHESES

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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[1]Otto Bock, Otto Bock France, Courtaboeuf, France, [2]Université de Paris, CNRS, Integrative Neuroscience And Cognition Center, Paris, France, [3]Institut Robert Merle d'Aubigné, Pôle Appareillage Du Membre Supérieur, Valenton, France

In this work we present the current results we obtained in our ongoing project aiming at the development of a non-invasive Brain-Machine Interface (BMI) for the sensorimotor control of a commercially available myoelectric prosthesis (Myobock©, Ottobock) in collaboration with its manufacturer (Ottobock France) and the rehabilitation center IRMA. The new system uses as input the ElectroEncephaloGraphy (EEG) signals of the user as well as vibrations produced by a bracelet containing vibrating motors whose frequencies are proportional to the forces measured by the force sensors installed on the fingertips of the prosthesis. EEG signals were collected by two different recording systems with different number of electrodes during the experiments performed by two groups composed of able-bodied and amputee subjects, in which they were asked to grasp and displace different objects, either performing or mimicking the grip of the prosthesis. A combination of the Common Spatial Patterns and Wavelet Decomposition techniques have been used to construct the feature vector's content. The classification/prediction performances of three machine-learning algorithms (Artificial Neural Network, Support Vector Machine with two different kernels) were first tested off-line for a preliminary glimpse at the system performance and for different system parameters adjustment. The real-time performance was then computed during the online tests done with both groups using the whole system; the able bodied subjects using the prosthesis with the help of a 3D printed apparatus. The obtained results advocate for a proof of concept regarding the use of a wireless BMI dedicated to the control of a myoelectric prosthesis.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-428

A CALCIUM IMAGING BASED BRAIN-MACHINE INTERFACE FOR VIRTUAL NAVIGATION

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Modern calcium-imaging techniques allow for the simultaneous recording of large populations of neurons over extended periods of time. Using these techniques, scientists can decode task relevant variables from recorded neural activity. By further providing continuous feedback of decoded neural activity, we can ask more probing questions about learning, plasticity, and function in the brain. This can be done by implementing a brain-machine interface (BMI) that decodes neural activity and provides feedback of the output to the subject in real-time. We present a calcium-imaging based BMI for whole body navigation of mice through a virtual T-maze task. We recorded neural signals from the posterior parietal cortex of mice using 2-photon calcium imaging, and decoded continuous kinematic variables from these signals using linear decoding. We provided visual feedback in real-time by updating the kinematics of the mice in the virtual environment using the decoder output. Segmentation of individual neurons from images was not required, and we decoded directly from downsampled and filtered images. Mice successfully completed trials using this interface, and continued to do so with a fixed decoder over several days. We propose that such an interface could be used to bypass standard neural pathways, and make direct connections between neural signals and control outputs, to explore new questions about the brain.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-429

M/EEG NETWORKS INTEGRATION TO ELICIT PATTERNS OF MOTOR IMAGERY-BASED BCI TRAINING

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Despite its clinical application, voluntarily modulating brain activity appears to be a learned skill that affects the usability of brain-computer interfaces (BCIs) systems. The involved learning process induces a brain network reorganization that remains poorly understood. We hypothesized that integrating information from electroencephalography (EEG) and magnetoencephalography (MEG) data, provides a better description of the network changes occurring during the BCI training. We performed experiments on a group of 20 naive healthy subjects during a BCI training consisting of four sessions over two weeks in which both EEG and MEG signals were recorded. The task consisted in controlling the vertical position of a moving cursor through the α/β modulation to reach a target displayed on a screen. We adopted a multilayer approach to integrate brain network properties from EEG and MEG data. Regardless the chosen modality, a progressive increase in the integration of somatosensory areas in the α band was paralleled by a decrease of the integration of visual processing and working memory areas in the β band. Only brain network properties in multilayer network correlated with future BCI scores in the α_2 band ($p < 0.01$): positively in somatosensory and decision-making related areas (gyrus rectus & subcentral gyrus) and negatively in associative areas (superior occipital gyrus). Our results constitute the first proof of a learning process from the integration of M/EEG networks properties. Integrating multimodal brain network properties could be considered as a potential marker of BCI learning.

Pubmed:
29768971: Corsi MC, Chavez M, Schwartz D, Hugueville L, Khambhati AN, Bassett DS, De Vico Fallani F
Integrating EEG and MEG Signals to Improve Motor Imagery Classification in Brain-Computer Interface.
We adopted a fusion approach that combines features from simultaneously recorded electroencephalogram (EEG) and magnetoencephalogram (MEG) signals to improve classification performances in motor imagery-based brain-computer interfaces (BCIs). We applied our approach to a group of 15 healthy subjects and found a significant classification performance enhancement as compared to standard single-modality approaches in the alpha and beta bands. Taken together, our findings demonstrate the advantage of considering multimodal approaches as complementary tools for improving the impact of noninvasive BCIs.
Int J Neural Syst, 2019; 29
30010553: Labyt E, Corsi MC, Fourcault W, Palacios Laloy A, Bertrand F, Lenouvel F, Cauffet G, Le Prado M, Berger F, Morales S
Magnetoencephalography With Optically Pumped He Magnetometers at Ambient Temperature.
In this paper, we present the first proof of concept confirming the possibility to record magnetoencephalographic (MEG) signals with optically pumped magnetometers (OPMs) based on the parametric resonance of He atoms. The main advantage of this kind of OPM is the possibility to provide a tri-axis vector measurement of the magnetic field at room-temperature (the He vapor is neither cooled nor heated). The sensor achieves a sensitivity of 210 fT/ √ Hz in the bandwidth [2-300 Hz]. MEG simulation studies with a brain phantom were cross-validated with real MEG measurements on a healthy subject. For both studies, MEG signal was recorded consecutively with OPMs and superconducting quantum interference devices (SQUIDs) used as reference sensors. For healthy subject MEG recordings, three MEG proofs of concept were carried out: auditory evoked fields, visual evoked fields, and spontaneous activity. M100 peaks have been detected on evoked responses recorded by both OPMs and SQUIDs with no significant difference in latency. Concerning spontaneous activity, an attenuation of the signal power between 8-12 Hz (alpha band) related to eyes opening has been observed with OPM similarly to SQUID. All these results confirm that the room temperature vector He OPMs can record MEG signals and provide reliable information on brain activity.

IEEE Trans Med Imaging, 2019; 38
31211359: Gaubert S, Raimondo F, Houot M, Corsi MC, Naccache L, Diego Sitt J, Hermann B, Oudiette D, Gagliardi G, Habert MO, Dubois B, De Vico Fallani F, Bakardjian H, Epelbaum S,
EEG evidence of compensatory mechanisms in preclinical Alzheimer's disease.
Early biomarkers are needed to identify individuals at high risk of preclinical Alzheimer's disease and to better understand the pathophysiological processes of disease progression. Preclinical Alzheimer's disease EEG changes would be non-invasive and cheap screening tools and could also help to predict future progression to clinical Alzheimer's disease. However, the impact of amyloid-β deposition and neurodegeneration on EEG biomarkers needs to be elucidated. We included participants from the INSIGHT-preAD cohort, which is an ongoing single-centre multimodal observational study that was designed to identify risk factors and markers of progression to clinical Alzheimer's disease in 318 cognitively normal individuals aged 70-85 years with a subjective memory complaint. We divided the subjects into four groups, according to their amyloid status (based on 18F-florbetapir PET) and neurodegeneration status (evidenced by 18F-fluorodeoxyglucose PET brain metabolism in Alzheimer's disease signature regions). The first group was amyloid-positive and neurodegeneration-positive, which corresponds to stage 2 of preclinical Alzheimer's disease. The second group was amyloid-positive and neurodegeneration-negative, which corresponds to stage 1 of preclinical Alzheimer's disease. The third group was amyloid-negative and neurodegeneration-positive, which corresponds to 'suspected non-Alzheimer's pathophysiology'. The last group was the control group, defined by amyloid-negative and neurodegeneration-negative subjects. We analysed 314 baseline 256-channel high-density eyes closed 1-min resting state EEG recordings. EEG biomarkers included spectral measures, algorithmic complexity and functional connectivity assessed with a novel information-theoretic measure, weighted symbolic mutual information. The most prominent effects of neurodegeneration on EEG metrics were localized in frontocentral regions with an increase in high frequency oscillations (higher beta and gamma power) and a decrease in low frequency oscillations (lower delta power), higher spectral entropy, higher complexity and increased functional connectivity measured by weighted symbolic mutual information in theta band. Neurodegeneration was associated with a widespread increase of median spectral frequency. We found a non-linear relationship between amyloid burden and EEG metrics in neurodegeneration-positive subjects, either following a U-shape curve for delta power or an inverted U-shape curve for the other metrics, meaning that EEG patterns are modulated differently depending on the degree of amyloid burden. This finding suggests initial compensatory mechanisms that are overwhelmed for the highest amyloid load. Together, these results indicate that EEG metrics are useful biomarkers for the preclinical stage of Alzheimer's disease.
Brain, 2019; 142
31927130: Corsi MC, Chavez M, Schwartz D, George N, Hugueville L, Kahn AE, Dupont S, Bassett DS, De Vico Fallani F
Functional disconnection of associative cortical areas predicts performance during BCI training.
Brain-computer interfaces (BCIs) have been largely developed to allow communication, control, and neurofeedback in human beings. Despite their great potential, BCIs perform inconsistently across individuals and the neural processes that enable humans to achieve good control remain poorly understood. To address this question, we performed simultaneous high-density electroencephalographic (EEG) and magnetoencephalographic (MEG) recordings in a motor imagery-based BCI training involving a group of healthy subjects. After reconstructing the signals at the cortical level, we showed that the reinforcement of motor-related activity during the BCI skill acquisition is paralleled by a progressive disconnection of associative areas which were not directly targeted during the experiments. Notably, these network connectivity changes reflected growing automaticity associated with BCI performance and predicted future learning rate. Altogether, our findings provide new insights into the large-scale cortical organizational mechanisms underlying BCI learning, which have implications for the improvement of this technology in a broad range of real-life applications.
Neuroimage, 2020; 209
32369802: Stiso J, Corsi MC, Vettel JM, Garcia J, Pasqualetti F, De Vico Fallani F, Lucas TH, Bassett DS
Learning in brain-computer interface control evidenced by joint decomposition of brain and behavior.
Motor imagery-based brain-computer interfaces (BCIs) use an individual's ability to volitionally modulate localized brain activity, often as a therapy for motor dysfunction or to probe causal relations between brain activity and behavior. However, many individuals cannot learn to successfully modulate their brain activity, greatly limiting the efficacy of BCI for therapy and for basic scientific inquiry. Formal experiments designed to probe the nature of BCI learning have offered initial evidence that coherent activity across spatially distributed and functionally diverse cognitive systems is a hallmark of individuals who can successfully learn to control the BCI. However, little is known about how these distributed networks interact through time to support learning.
J Neural Eng, 2020; 17
33147577: Gonzalez-Astudillo J, Cattai T, Bassignana G, Corsi MC, De Vico Fallani F
Network-based brain-computer interfaces: principles and applications.
Brain-computer interfaces (BCIs) make possible to interact with the external environment by decoding the mental intention of

individuals. BCIs can therefore be used to address basic neuroscience questions but also to unlock a variety of applications from exoskeleton control to neurofeedback rehabilitation. In general, BCI usability depends on the ability to comprehensively characterize brain functioning and correctly identify the user's mental state. To this end, much of the efforts have focused on improving the classification algorithms taking into account localized brain activities as input features. Despite considerable improvement BCI performance is still unstable and, as a matter of fact, current features represent oversimplified descriptors of brain functioning. In the last decade, growing evidence has shown that the brain works as a networked system composed of multiple specialized and spatially distributed areas that dynamically integrate information. While more complex, looking at how remote brain regions functionally interact represents a grounded alternative to better describe brain functioning. Thanks to recent advances in network science, i.e. a modern field that draws on graph theory, statistical mechanics, data mining and inferential modeling, scientists have now powerful means to characterize complex brain networks derived from neuroimaging data. Notably, summary features can be extracted from brain networks to quantitatively measure specific organizational properties across a variety of topological scales. In this topical review, we aim to provide the state-of-the-art supporting the development of a network theoretic approach as a promising tool for understanding BCIs and improve usability.
J Neural Eng, 2021; 18
33725682: Corsi MC, Chavez M, Schwartz D, George N, Hugueville L, Kahn AE, Dupont S, Bassett DS, De Vico Fallani F
BCI learning induces core-periphery reorganization in M/EEG multiplex brain networks.
Brain-computer interfaces (BCIs) constitute a promising tool for communication and control. However, mastering non-invasive closed-loop systems remains a learned skill that is difficult to develop for a non-negligible proportion of users. The involved learning process induces neural changes associated with a brain network reorganization that remains poorly understood.To address this inter-subject variability, we adopted a multilayer approach to integrate brain network properties from electroencephalographic and magnetoencephalographic data resulting from a four-session BCI training program followed by a group of healthy subjects. Our method gives access to the contribution of each layer to multilayer network that tends to be equal with time.We show that regardless the chosen modality, a progressive increase in the integration of somatosensory areas in theband was paralleled by a decrease of the integration of visual processing and working memory areas in theband. Notably, only brain network properties in multilayer network correlated with future BCI scores in theband: positively in somatosensory and decision-making related areas and negatively in associative areas.Our findings cast new light on neural processes underlying BCI training. Integrating multimodal brain network properties provides new information that correlates with behavioral performance and could be considered as a potential marker of BCI learning.
J Neural Eng, 2021; 18
34102381: Gaubert S, Houot M, Raimondo F, Ansart M, Corsi MC, Naccache L, Sitt JD, Habert MO, Dubois B, De Vico Fallani F, Durrleman S, Epelbaum S,
A machine learning approach to screen for preclinical Alzheimer's disease.
Combining multimodal biomarkers could help in the early diagnosis of Alzheimer's disease (AD). We included 304 cognitively normal individuals from the INSIGHT-preAD cohort. Amyloid and neurodegeneration were assessed on F-florbetapir and F-fluorodeoxyglucose PET, respectively. We used a nested cross-validation approach with non-invasive features (electroencephalography [EEG], APOE4 genotype, demographic, neuropsychological and MRI data) to predict: 1/ amyloid status; 2/ neurodegeneration status; 3/ decline to prodromal AD at 5-year follow-up. Importantly, EEG was most strongly predictive of neurodegeneration, even when reducing the number of channels from 224 down to 4, as 4-channel EEG best predicted neurodegeneration (negative predictive value [NPV] = 82%, positive predictive value [PPV] = 38%, 77% specificity, 45% sensitivity). The combination of demographic, neuropsychological data, APOE4 and hippocampal volumetry most strongly predicted amyloid (80% NPV, 41% PPV, 70% specificity, 58% sensitivity) and most strongly predicted decline to prodromal AD at 5 years (97% NPV, 14% PPV, 83% specificity, 50% sensitivity). Thus, machine learning can help to screen patients at high risk of preclinical AD using non-invasive and affordable biomarkers.
Neurobiol Aging, 2021; 105
34115589: Cattai T, Colonnese S, Corsi MC, Bassett DS, Scarano G, De Vico Fallani F
Phase/Amplitude Synchronization of Brain Signals During Motor Imagery BCI Tasks.
In the last decade, functional connectivity (FC) has been increasingly adopted based on its ability to capture statistical dependencies between multivariate brain signals. However, the role of FC in the context of brain-computer interface applications is still poorly understood. To address this gap in knowledge, we considered a group of 20 healthy subjects during an EEG-based hand motor imagery (MI) task. We studied two well-established FC estimators, i.e. spectral- and imaginary-coherence, and we investigated how they were modulated by the MI task. We characterized the resulting FC networks by extracting the strength of connectivity of each EEG sensor and we compared the discriminant power with respect to standard power spectrum features. At the group level, results showed that while spectral-coherence based network features were increasing in the sensorimotor areas, those based on imaginary-coherence were significantly decreasing. We demonstrated that this opposite, but complementary, behavior was respectively determined by the increase in amplitude and phase

synchronization between the brain signals. At the individual level, we eventually assessed the potential of these network connectivity features in a simple off-line classification scenario. Taken together, our results provide fresh insights into the oscillatory mechanisms subserving brain network changes during MI and offer new perspectives to improve BCI performance.
IEEE Trans Neural Syst Rehabil Eng, 2021; 29

34892456: Venot T, Corsi MC, Saint-Bauzel L, Vico Fallani F

Towards multimodal BCIs: the impact of peripheral control on motor cortex activity and sense of agency.

In the recent years, brain computer interfaces (BCI) using motor imagery have shown some limitations regarding the quality of control. In an effort to improve this promising technology, some studies intended to develop hybrid BCI with other technologies such as eye tracking which shows more reliability. However, the use of an eye tracker in the control of a robot might affect by itself the sense of agency (SoA) and the brain activity in the regions used for motor imagery (MI). Here, we explore the link between the sense of agency and the activity of the motor cortex. For this purpose, we used of a virtual arm projected on a surface which is either controlled by motion capture or controlled by gaze using an eye tracker. We found out that there is an activity in the motor cortex during the task of control by gaze and that having control over a projected robotic arm presents significant differences with the situation of observing the robot moving.
Annu Int Conf IEEE Eng Med Biol Soc, 2021; 2021

FENS Forum 2022
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BOARD NUMBER: S03-430

CHRONIC STABILITY OF 1024-CHANNEL UTAH-ARRAY-BASED NEUROPROSTHESIS IN MONKEYS

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Electrical stimulation of visual cortex via a neuroprosthesis generates the perception of dots of light known as 'phosphenes,' allowing perception of simple shapes, even after decades of blindness. We evaluated the efficacy and stability of a 1024-channel neuroprosthesis system in non-human primates (NHPs) over >3 years to assess its suitability for long-term vision restoration. We implanted two rhesus macaques (M. mulatta) with 16 Utah arrays in primary visual cortex (V1) and visual area 4 (V4). The monkeys performed a stimulus detection task and were perfused after 3-3.5 years for histological analysis. We monitored the animals' health and measured electrode impedances, neuronal signal quality, and current thresholds for phosphene generation. The monkeys remained healthy throughout the implantation period and the 1024-channel device retained its mechanical integrity and electrical conductivity. Over time, however, we observed decreases in 1) signal-to-noise ratios (SNR) of visually evoked responses, 2) peak-to-peak voltages of recorded neuronal signals, 3) the number of high-amplitude channels (peak-to-peak voltage >100 uV) and 4) the number of channels through which phosphenes could be evoked, as well as impaired visual perception at locations corresponding to implanted areas. Tissue dissections and histological analysis revealed encapsulation of the arrays, and associated cortical degeneration. In conclusion, long-term implantation of a high-channel-count device in NHP visual cortex was accompanied by deformation of cortical tissue and decreased stimulation efficacy and signal quality over time, implying that improvement of device biocompatibility and/or refinement of implantation techniques is needed before future clinical use is feasible.

Pubmed:
33273097: Chen X, Wang F, Fernandez E, Roelfsema PR
Shape perception via a high-channel-count neuroprosthesis in monkey visual cortex.
Blindness affects 40 million people across the world. A neuroprosthesis could one day restore functional vision in the blind. We implanted a 1024-channel prosthesis in areas V1 and V4 of the visual cortex of monkeys and used electrical stimulation to elicit percepts of dots of light (called phosphenes) on hundreds of electrodes, the locations of which matched the receptive fields of the stimulated neurons. Activity in area V4 predicted phosphene percepts that were elicited in V1. We simultaneously stimulated multiple electrodes to impose visible patterns composed of a number of phosphenes. The monkeys immediately recognized them as simple shapes, motions, or letters. These results demonstrate the potential of electrical stimulation to restore functional, life-enhancing vision in the blind.
Science, 2020; 370
34730515: Klink PC, Chen X, Vanduffel W, Roelfsema PR
Population receptive fields in nonhuman primates from whole-brain fMRI and large-scale neurophysiology in visual cortex.
Population receptive field (pRF) modeling is a popular fMRI method to map the retinotopic organization of the human brain. While fMRI-based pRF maps are qualitatively similar to invasively recorded single-cell receptive fields in animals, it remains unclear what neuronal signal they represent. We addressed this question in awake nonhuman primates comparing whole-brain fMRI and large-scale neurophysiological recordings in areas V1 and V4 of the visual cortex. We examined the fits of several pRF models based on the fMRI blood-oxygen-level-dependent (BOLD) signal, multi-unit spiking activity (MUA), and local field potential (LFP) power in different frequency bands. We found that pRFs derived from BOLD-fMRI were most similar to MUA-pRFs in V1 and V4, while pRFs based on LFP gamma power also gave a good approximation. fMRI-based pRFs thus reliably reflect neuronal receptive field properties in the primate brain. In addition to our results in V1 and V4, the whole-brain fMRI measurements revealed retinotopic tuning in many other cortical and subcortical areas with a consistent increase in pRF size with increasing eccentricity, as well as a retinotopically specific deactivation of default mode network nodes similar to previous observations in humans.
Elife, 2021; 10

30315163: Sanayei M, Chen X, Chicharro D, Distler C, Panzeri S, Thiele A
Perceptual learning of fine contrast discrimination changes neuronal tuning and population coding in macaque V4.
Perceptual learning, the improvement in perceptual abilities with training, is thought to be mediated by an alteration of neuronal tuning. It remains poorly understood how tuning properties change as training progresses, whether improved stimulus tuning directly links to increased behavioural readout of sensory information, or how population coding mechanisms change with training. Here, we recorded continuously from multiple neuronal clusters in area V4 while macaque monkeys learned a fine contrast categorization task. Training increased neuronal coding abilities by shifting the steepest point of contrast response functions towards the categorization boundary. Population coding accuracy of difficult discriminations resulted largely from an increased information coding of individual channels, particularly for those channels that in early learning had larger ability for easy discriminations, but comparatively small encoding abilities for difficult discriminations. Population coding was also enhanced by specific changes in correlations. Neuronal activity became more indicative of upcoming choices with training.
Nat Commun, 2018; 9
28512008: Chen X, Possel JK, Wacongne C, van Ham AF, Klink PC, Roelfsema PR
3D printing and modelling of customized implants and surgical guides for non-human primates.
Primate neurobiologists use chronically implanted devices such as pedestals for head stabilization and chambers to gain access to the brain and study its activity. Such implants are skull-mounted, and made from a hard, durable material, such as titanium.
J Neurosci Methods, 2017; 286
25340335: Chen X, Sanayei M, Thiele A
Stimulus roving and flankers affect perceptual learning of contrast discrimination in Macaca mulatta.
'Stimulus roving' refers to a paradigm in which the properties of the stimuli to be discriminated vary from trial to trial, rather than being kept constant throughout a block of trials. Rhesus monkeys have previously been shown to improve their contrast discrimination performance on a non-roving task, in which they had to report the contrast of a test stimulus relative to that of a fixed-contrast sample stimulus. Human psychophysics studies indicate that roving stimuli yield little or no perceptual learning. Here, we investigate how stimulus roving influences perceptual learning in macaque monkeys and how the addition of flankers alters performance under roving conditions. Animals were initially trained on a contrast discrimination task under non-roving conditions until their performance levels stabilized. The introduction of roving contrast conditions resulted in a pronounced drop in performance, which suggested that subjects initially failed to heed the sample contrast and performed the task using an internal memory reference. With training, significant improvements occurred, demonstrating that learning is possible under roving conditions. To investigate the notion of flanker-induced perceptual learning, flanker stimuli (30% fixed-contrast iso-oriented collinear gratings) were presented jointly with central (roving) stimuli. Presentation of flanker stimuli yielded substantial performance improvements in one subject, but deteriorations in the other. Finally, after the removal of flankers, performance levels returned to their pre-flanker state in both subjects, indicating that the flanker-induced changes were contingent upon the continued presentation of flankers.
PLoS One, 2014; 9
24259674: Chen X, Sanayei M, Thiele A
Perceptual learning of contrast discrimination in macaca mulatta.
Rhesus monkeys underwent training in a contrast discrimination task, in which grating stimuli were presented at parafoveal and peripheral visual field locations. Subjects had to compare a sample stimulus that had a fixed contrast of 30% to a test stimulus that varied in contrast from trial to trial. Extensive practice yielded improvements in contrast discrimination that were observed across the full range of test stimulus contrasts. These improvements occurred across multiple sessions, as well as across trials within individual sessions. The finer the contrast discriminations required, the longer it took for subjects to improve. Improvements in psychophysical performance resulted in the steepening of psychometric functions and/or shifts in the point of subjective equality towards the contrast of the sample stimulus. Enhancement in discrimination was especially pronounced around the contrast level of the sample stimulus, to which the subject was consistently exposed. The changes resulted in increased accuracy overall, lower discrimination thresholds, and faster response times. Partial transfer of learning, from vertically oriented training stimuli to horizontally oriented testing stimuli, was observed, while transfer to stimuli with different spatial frequencies was less pronounced. The results demonstrate the existence of perceptual learning in the contrast domain, whereby learning affects multiple performance-related psychophysical metrics.
J Vis, 2013; 13
22170961: Chen X, Hoffmann KP, Albright TD, Thiele A
Effect of feature-selective attention on neuronal responses in macaque area MT.
Attention influences visual processing in striate and extrastriate cortex, which has been extensively studied for spatial-, object-, and feature-based attention. Most studies exploring neural signatures of feature-based attention have trained animals

to attend to an object identified by a certain feature and ignore objects/displays identified by a different feature. Little is known about the effects of feature-selective attention, where subjects attend to one stimulus feature domain (e.g., color) of an object while features from different domains (e.g., direction of motion) of the same object are ignored. To study this type of feature-selective attention in area MT in the middle temporal sulcus, we trained macaque monkeys to either attend to and report the direction of motion of a moving sine wave grating (a feature for which MT neurons display strong selectivity) or attend to and report its color (a feature for which MT neurons have very limited selectivity). We hypothesized that neurons would upregulate their firing rate during attend-direction conditions compared with attend-color conditions. We found that feature-selective attention significantly affected 22% of MT neurons. Contrary to our hypothesis, these neurons did not necessarily increase firing rate when animals attended to direction of motion but fell into one of two classes. In one class, attention to color increased the gain of stimulus-induced responses compared with attend-direction conditions. The other class displayed the opposite effects. Feature-selective activity modulations occurred earlier in neurons modulated by attention to color compared with neurons modulated by attention to motion direction. Thus feature-selective attention influences neuronal processing in macaque area MT but often exhibited a mismatch between the preferred stimulus dimension (direction of motion) and the preferred attention dimension (attention to color).
J Neurophysiol, 2012; 107

34665780: Fernández E, Alfaro A, Soto-Sánchez C, Gonzalez-Lopez P, Lozano AM, Peña S, Grima MD, Rodil A, Gómez B, Chen X, Roelfsema PR, Rolston JD, Davis TS, Normann RA
Visual percepts evoked with an intracortical 96-channel microelectrode array inserted in human occipital cortex.
BACKGROUNDA long-held goal of vision therapy is to transfer information directly to the visual cortex of blind individuals, thereby restoring a rudimentary form of sight. However, no clinically available cortical visual prosthesis yet exists.METHODSWe implanted an intracortical microelectrode array consisting of 96 electrodes in the visual cortex of a 57-year-old person with complete blindness for a 6-month period. We measured thresholds and the characteristics of the visual percepts elicited by intracortical microstimulation.RESULTSImplantation and subsequent explantation of intracortical microelectrodes were carried out without complications. The mean stimulation threshold for single electrodes was 66.8 ± 36.5 μA. We consistently obtained high-quality recordings from visually deprived neurons and the stimulation parameters remained stable over time. Simultaneous stimulation via multiple electrodes was associated with a significant reduction in thresholds ($P < 0.001$, ANOVA) and evoked discriminable phosphene percepts, allowing the blind participant to identify some letters and recognize object boundaries.CONCLUSIONSOur results demonstrate the safety and efficacy of chronic intracortical microstimulation via a large number of electrodes in human visual cortex, showing its high potential for restoring functional vision in the blind.TRIAL REGISTRATIONClinicalTrials.gov identifier NCT02983370.FUNDINGThe Spanish Ministerio de Ciencia Innovación y Universidades, the Generalitat Valenciana (Spain), the Europan Union's Horizon 2020 programme, the Bidons Egara Research Chair of the University Miguel Hernández (Spain), and the John Moran Eye Center of the University of Utah.
J Clin Invest, 2021; 131

22081989: Bartolo MJ, Gieselmann MA, Vuksanovic V, Hunter D, Sun L, Chen X, Delicato LS, Thiele A
Stimulus-induced dissociation of neuronal firing rates and local field potential gamma power and its relationship to the resonance blood oxygen level-dependent signal in macaque primary visual cortex.
The functional magnetic resonance imaging (fMRI) blood oxygenation level-dependent (BOLD) signal is regularly used to assign neuronal activity to cognitive function. Recent analyses have shown that the local field potential (LFP) gamma power is a better predictor of the fMRI BOLD signal than spiking activity. However, LFP gamma power and spiking activity are usually correlated, clouding the analysis of the neural basis of the BOLD signal. We show that changes in LFP gamma power and spiking activity in the primary visual cortex (V1) of the awake primate can be dissociated by using grating and plaid pattern stimuli, which differentially engage surround suppression and cross-orientation inhibition/facilitation within and between cortical columns. Grating presentation yielded substantial V1 LFP gamma frequency oscillations and significant multi-unit activity. Plaid pattern presentation significantly reduced the LFP gamma power while increasing population multi-unit activity. The fMRI BOLD activity followed the LFP gamma power changes, not the multi-unit activity. Inference of neuronal activity from the fMRI BOLD signal thus requires detailed a priori knowledge of how different stimuli or tasks activate the cortical network.
Eur J Neurosci, 2011; 34

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BOARD NUMBER: S03-431

EMBODIMENT OF A FORELIMB NEUROPROSTHESIS IN THE MOUSE MODEL

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Significant research is made towards the restoration of upper limb function through prostheses controlled by brain-machine interfaces (BMI). This research calls for tractable experimental models. Although the mouse model is increasingly used for BMI developments, a mouse upper limb prosthesis with the correct anatomy and scale is lacking. Also, it is still unclear if mice would be able to embody a limb prosthesis, even though encouraging results have been obtained by testing the embodiment of a fake tail (Wada et al., 2016, J Neurosci). Here, we aimed to develop a mouse forelimb neuroprosthesis to explore different BMI-based control and feedback strategies, as well as an assay to probe its embodiment in the mouse body representation. Our 3D-printed forelimb prosthesis includes four degrees of freedom, a closed-loop control to ensure accuracy and robustness, and provides position and contact feedback to the closed-loop BMI. To explore the induction of an embodiment-like behaviour towards the prosthesis, we have reproduced the rubber hand illusion (Botvinick & Cohen, 1998, Nature) in the mouse. Tactile stimulations were applied to both a visible static forelimb prosthesis and the hidden real paw, either synchronously or asynchronously. At the end of the stimulation, we threatened the prosthesis and quantified the animal's reaction through the analysis of its facial expressions. Overall, these developments will enable us to better understand the role of somatosensory feedback on embodiment. Moreover, these tools will consolidate the mouse model in the field of neuroprosthetic research.

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BOARD NUMBER: S03-432

BRAIN-MACHINE INTERFACE LEARNING IS FACILITATED BY DISTRIBUTED CORTICAL FEEDBACK THAT IS SPATIALLY AND TEMPORALLY STRUCTURED

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Neuroprosthetics offer great hope for motor-impaired human patients. One obstacle is that fine motor control requires near-instantaneous, rich somatosensory feedback. Such distributed touch feedback may be recreated in a brain-machine interface using distributed artificial stimulation across the primary somatosensory cortex surface. Here, we hypothesized that this neuronal stimulation must be contiguous in its spatial organization and temporal dynamics in order to be efficiently integrated by sensorimotor circuits. Using a closed-loop brain-machine interface, we trained head-fixed mice to control a virtual cursor by modulating the activity of motor cortex neurons. We provided artificial feedback in real time, consisting of distributed optogenetic stimulation patterns in the primary somatosensory cortex. We found that the mice only developed a specific motor strategy and sustained task performance when the optogenetic feedback pattern was contiguous while it moved across the topography of the somatosensory cortex. These results reveal new properties of cortical integration, and set new constraints on the design of neuroprosthetics.

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BOARD NUMBER: S03-433

A BRAIN-MACHINE INTERFACE BASED ON COTICAL MESOSCALE DYNAMICS

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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In the cerebral cortex, activity dynamics measured at a mesoscopic (0.1 to 1 mm) scale are characterized by both spontaneous and task-related dynamical waves of neuronal synchrony that travel through the cortical network. The dynamics of these waves include the spread of neuronal depolarization from a stationary bump to traveling waves that can follow complex trajectories. The function of these traveling waves, which are thought to participate in information propagation and processing in primary sensory areas, remains poorly understood. To test whether cortical waves can be actively generated and controlled, we are implementing a goal-directed task for head-fixed mice based on the online processing of wide-field calcium imaging signals. Transgenic mice expressing GCaMP6f in excitatory cortical neurons (Ai-95 x EMX-Cre) are implanted with 6 mm optical windows covering the left primary somatosensory and motor cortex. Calcium-dependent optical signals are analyzed in real time in order to track the trajectory of propagating mesoscale waves, which allows to condition the delivery of water rewards to the detection of waves depending of their specific spatiotemporal features. This methodological framework provides a unique opportunity to better understand how neuronal assemblies can orchestrate the flow of information within cortical networks while an animal is actively performing a task. Our preliminary experiments suggest that mice can be trained to voluntary control the emergence of waves of activity. If confirmed, this will represent an important proof-of-concept of the feasibility of using mesoscale cortical dynamics for building brain-machine interfaces.

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BOARD NUMBER: S03-434

NOVEL GRAPHENE-BASED ELECTRODE FOR INTERFACING THE PERIPHERAL NERVOUS SYSTEM

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Neuroprostheses aim to restore the lost function after a limb amputation or severe neural injuries. Within a peripheral neuroprosthesis, the interface between nerve and machine is intended to record neural signals and stimulate selective populations of nerve fibers, constituting a bidirectional interface. In this work, a new generation of neural interfaces that replaces metals by engineered graphene has been tested. This new device is based on modified reduced-graphene oxide, named EGNITE. The biocompatibility of EGNITE devices was tested *in vitro* and *in vivo*. *In vitro*, cell viability of dorsal root ganglia and cortical neurons seed on top of the material was analyzed. *In vivo*, polyimide devices coated with EGNITE were longitudinally implanted in the sciatic nerve of rats for 8 weeks. EGNITE had no impact on cell viability and did not produce any significant functional deficit or axonal damage in the implanted animals. To test the functionality of EGNITE devices, transverse intrafascicular multichannel electrodes containing 16 EGNITE active sites were implanted in the sciatic nerve of rats for 90 days. EGNITE devices were able to stimulate nerve fascicles to produce selective muscle activation. Compared with standard electrodes using metal conductors, EGNITE devices needed about 3 times less current to produce the same muscle activation. The biocompatibility of EGNITE together with its superior electrical capabilities may have relevant implications in the field of neuroprostheses by reducing potential tissue damage and lowering the energy consumption of the devices, thus extending the window of effective stimulation after implantation.

Pubmed:
33644015: Ferrari LM, Rodríguez-Meana B, Bonisoli A, Cutrone A, Micera S, Navarro X, Greco F, Del Valle J
All-Polymer Printed Low-Cost Regenerative Nerve Cuff Electrodes.
Neural regeneration after lesions is still limited by several factors and new technologies are developed to address this issue. Here, we present and test in animal models a new regenerative nerve cuff electrode (RnCE). It is based on a novel low-cost fabrication strategy, called "Print and Shrink", which combines the inkjet printing of a conducting polymer with a heat-shrinkable polymer substrate for the development of a bioelectronic interface. This method allows to produce miniaturized regenerative cuff electrodes without the use of cleanroom facilities and vacuum based deposition methods, thus highly reducing the production costs. To fully proof the electrodes performance we assessed functional recovery and adequacy to support axonal regeneration after section of rat sciatic nerves and repair with RnCE. We investigated the possibility to stimulate the nerve to activate different muscles, both in acute and chronic scenarios. Three months after implantation, RnCEs were able to stimulate regenerated motor axons and induce a muscular response. The capability to produce fully-transparent nerve interfaces provided with polymeric microelectrodes through a cost-effective manufacturing process is an unexplored approach in neuroprosthesis field. Our findings pave the way to the development of new and more usable technologies for nerve regeneration and neuromodulation.
Front Bioeng Biotechnol, 2021; 9

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OPTOGENETIC AND MACHINE LEARNING STRATEGIES FOR AN AUDITORY CORTICAL IMPLANT.

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Aim: Cochlear implants are among the most successful sensory restoration devices, drastically improving hearing in impaired patients via auditory nerve stimulation. However, they still have limitations and require a functional auditory nerve. To explore new ways of solving these limits, we are currently developing methodologies to demonstrate, in mice, the feasibility of auditory rehabilitation using a cortical optogenetic implant. Methods: We first established benchmarks of mouse hearing using a Go/NoGo discrimination for 5 important features of hearing. Frequency discrimination, amplitude modulation, robustness to noise, frequency modulation and harmonics discrimination. These benchmarks will be compared to perceptions obtained with the implant. Concomitantly, we implemented algorithms to convert sounds into tonotopic activation patterns which we can test in behavioral discrimination tasks using a video projector system through cranial chronic windows before implementing them on the 2D implant prototype. Results: Behavioral experiments allowed us to measure frequency perception accuracy for 16 different stimuli of tone frequencies and 16 AM frequencies. Also, background noise diminishes discrimination capacities. Regarding encoding models, we use tonotopic maps of the auditory cortex obtained through intrinsic imaging, to construct a linear model, which computes projections of the sound spectrum onto a tonotopic axis. Alternatively, we've also developed an autoencoder network model that heavily compresses sound representations while keeping nearly all spectro-temporal information. Conclusions: These results pave the way for generating artificial auditory perception via cortical stimulation and benchmark them against auditory discrimination performances of normally-hearing mice.

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BOARD NUMBER: S03-436

THE ROLE OF AFFERENT INPUT IN NEUROPROSTHETIC LEARNING

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Brain-Machine-Interfaces can potentially provide powerful means to replace impaired motor functions. To improve currently available devices, it is important to gain a better understanding of the neuronal mechanisms underlying neuroprosthetic control. We have demonstrated that learning-related changes in neuronal firing can be highly specific to the conditioned neuron. Independent of the type of plasticity involved, what is the origin and nature of the synaptic input driving the conditioned neuron? To identify the afferent activity driving neuroprosthetic learning, we designed a novel two-photon microscope capable of imaging multiple cortical layers simultaneously while we condition a chosen neuron. By labeling the conditioned neurons (L2/3) and afferent incoming axons (L1) with spectrally different calcium indicators, this optical approach allows us to image the in- and output of specific synaptic interactions simultaneously. Our first goal was to characterize the role of the motor thalamus (VM) and premotor areas in the learning process. Both regions show high correlations with the conditioned neuron, and with their signals we can easily reconstruct the activity patterns of conditioned neurons and its neighbors. However, calcium dynamics of buttons from the two regions also show differences. Thalamic activity is slowly evolving over a learning session suggesting a stable input contribution, which could be important for long-term memory consolidation. In contrast we observed that input from premotor areas showed more transient changes which might be related to early phases of learning. Taken togther, these experiments suggest a differential role of the two afferent input sources in the process of neuroprosthetic learning.

Pubmed:
34580478: Kathe C, Michoud F, Schönle P, Rowald A, Brun N, Ravier J, Furfaro I, Paggi V, Kim K, Soloukey S, Asboth L, Hutson TH, Jelescu I, Philippides A, Alwahab N, Gandar J, Huber D, De Zeeuw CI, Barraud Q, Huang Q, Lacour SP, Courtine G
Wireless closed-loop optogenetics across the entire dorsoventral spinal cord in mice.
Optoelectronic systems can exert precise control over targeted neurons and pathways throughout the brain in untethered animals, but similar technologies for the spinal cord are not well established. In the present study, we describe a system for ultrafast, wireless, closed-loop manipulation of targeted neurons and pathways across the entire dorsoventral spinal cord in untethered mice. We developed a soft stretchable carrier, integrating microscale light-emitting diodes (micro-LEDs), that conforms to the dura mater of the spinal cord. A coating of silicone-phosphor matrix over the micro-LEDs provides mechanical protection and light conversion for compatibility with a large library of opsins. A lightweight, head-mounted, wireless platform powers the micro-LEDs and performs low-latency, on-chip processing of sensed physiological signals to control photostimulation in a closed loop. We use the device to reveal the role of various neuronal subtypes, sensory pathways and supraspinal projections in the control of locomotion in healthy and spinal-cord injured mice.
Nat Biotechnol, 2022; 40

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S03-437

ERROR-RELATED POTENTIALS DETECTION WITH DRY AND WET EEG ELECTRODES

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Electroencephalography (EEG) is a non-invasive technique for measuring brain electrical activity from electrodes placed on the scalp surface. Improvements in this technology are particularly relevant because they also boost brain-machine interfaces (BMI) development. Commonly, gel-based electrodes are used since they guarantee a high-quality signal. Alternatively, dry electrodes have been introduced, more suitable for daily use. In this work, we compare conventional dry and wet electrode systems specifically for the detection of error-related potentials (ErrPs). ErrPs are elicited as a reaction to both self-made and external errors. There has been increased interest in the integration of these signals into BMIs to improve their performance since they provide a convenient source of feedback to the system with no extra workload for the subject. These signals can be used, e.g., to correct errors or even for system adaptation. ErrP-based BMIs in the literature have consistently used wet electrodes. Therefore, even though both electrodes types have been compared for other event-related potentials (e.g., P300), it is relevant to know whether the signal quality for the detection of ErrPs is comparable among them. In this work, we implement a simple game to elicit ErrPs and compare the quality of the measured signals. We tested the feasibility of the experimental protocol to elicit ErrP and the measured ErrP displayed a similar waveshape in terms of observed peaks. However, differences exist in both latencies as well as in their amplitude. These variations and other relevant characteristics have to be further verified with more subjects.

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BOARD NUMBER: S03-438

INVESTIGATING PERFORMANCE IN MULTIMODAL BCI THROUGH MOTOR IMAGERY SPATIOTEMPORAL EEG PATTERNS

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Investigating the keys of performance in Brain Computer interface is like falling down the rabbit hole, a huge number of relevant parameters to study covering completely different fields such as semantic (instructions given), psychology (subject's profiling), machine learning (classification) or interface ergonomics (feedback representation). Each of those domains interact to ease the subject to achieve of demanding tasks such as motor imagery (MI). Following the trend of multi-modality in MI BCI context, we propose to put the subject into an engaging environment where it will control a robotic arm by its eyes and brain to grasp real objects. We aim to study the importance of ergonomics as a key of performances in MI BCI. Our method has different strategies regarding the timing of the task in a new enriched environment where the task is oriented towards a target. We compare between strategies the spatiotemporal EEG features used in MI BCI (Power spectrum between condition, R^2 statistical test) and classification accuracy to assess the overall experimentation's performance. We found out encouraging results for our three preliminary test-pilots (1F, 25 ± 1 year) who all presented good desynchronization in the alpha or beta bands that translated into high statistical differences between resting and motor imagery states and into good classification performances. In the Testing session, two strategies seem to give better results in terms both of statistical differences and classification accuracy. To conclude, the engaging environment with embodiment provides good constant performance and timing seems to be a relevant parameter to study.

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BOARD NUMBER: S03-439

SPATIALLY AND TEMPORALLY CONTINUOUS OPTOGENETIC FEEDBACK IN A CLOSED-LOOP CORTICAL BRAIN-MACHINE INTERFACE

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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Distributed microstimulations at the cortical surface can efficiently communicate feedback to a subject during control of a prosthesis through a brain-machine interface. Such feedback can potentially convey vast amounts of information, although the ability of the targeted cortical circuits to integrate arbitrary neuronal activations may constrain the dynamical patterns that can be used to convey this feedback. Here, we explore in mice a novel class of closed-loop brain-machine interface where distributed cortical feedback on the primary somatosensory cortex encodes the instantaneous angular position of a prosthesis joint in a spatially continuous way. We tested the ability of the mice to decode the rotary joint angle and optimize its trajectory based on a patterned rotary bar microstimulation applied online through optogenetics on the primary somatosensory cortex of mice. We showed that mice are able to use this continuous, rotating feedback in an active behaving context to increase the rate of rewards. Mice achieved this outcome by efficiently detecting reward opportunities, but also by moving the joint faster towards the rewarded angular zone while maintaining the cursor in a rewarded area comparatively longer than in a no-feedback condition. These findings indicate a path towards providing distributed cortical feedback with arbitrary shapes and topology, with a direct application to the control of a rotary joint — a frequent occurrence in many robotic posthesis.

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SPONTANEOUS CORRELATION STRUCTURE IN THE VISUAL CORTEX OF A BLIND HUMAN PATIENT

POSTER SESSION 03 - SECTION: BRAIN COMPUTER INTERFACE

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The development of penetrating cortical visual prostheses has recently advanced by the successful induction of visual percepts with various shapes in a blind human patient [1]. This achievement raises the natural question of how to control the evoked shape by stimulating a particular subset of electrodes from the micro-electrode array. However, such ideas rely on the assumption that the functional structure of the cortical circuit is still at least to some extent preserved despite the long-lasting blindness of the patient. As no visually evoked activity can be recorded from the primary visual cortex of a blind patient, we test the idea whether its functional structure can be inferred from spontaneous activity. As shown by imaging studies in several animal species [2,3], cells with similar orientation tuning tend to be more correlated during spontaneous activity than average and the orientation preference map can be retrieved from the spontaneous activity. However, so far it is unclear whether the Utah array used as the cortical visual prosthesis is sufficiently dense to permit such inference and whether the correlation structure is present after long-lasting blindness. We developed an algorithm based on a self-organizing map that retrieves a relatively robust correlation structure from the spontaneous activity of a blind patient recorded with a Utah array, with a longer reach of correlations compared to a sighted monkey. [1] Fernández, E. et al. Journal of Clinical Investigation 131 (2021)
[2] Kenet, T. et al. Nature 425 (2003)
[3] Omer, D.B. et al. Cerebral Cortex 29 (2018)

Pubmed:
23214816: Penington CJ, Korvasová K, Hughes BD, Landman KA
Collective motion of dimers.
We consider a discrete agent-based model on a one-dimensional lattice and a two-dimensional square lattice, where each agent is a dimer occupying two sites. Agents move by vacating one occupied site in favor of a nearest-neighbor site and obey either a strict simple exclusion rule or a weaker constraint that permits partial overlaps between dimers. Using indicator variables and careful probability arguments, a discrete-time master equation for these processes is derived systematically within a mean-field approximation. In the continuum limit, nonlinear diffusion equations that describe the average agent occupancy of the dimer population are obtained. In addition, we show that multiple species of interacting subpopulations give rise to advection-diffusion equations. Averaged discrete simulation data compares very well with the solution to the continuum partial differential equation models. Since many cell types are elongated rather than circular, this work offers insight into population-level behavior of collective cellular motion.
Phys Rev E Stat Nonlin Soft Matter Phys, 2012; 86
25484005: Korvasová K, Gaffney EA, Maini PK, Ferreira MA, Klika V
Investigating the Turing conditions for diffusion-driven instability in the presence of a binding immobile substrate.
Turing's diffusion-driven instability for the standard two species reaction-diffusion system is only achievable under well-known and rather restrictive conditions on both the diffusion rates and the kinetic parameters, which necessitates the pairing of a self-activator with a self-inhibitor. In this study we generalize the standard two-species model by considering the case where the reactants can bind to an immobile substrate, for instance extra-cellular matrix, and investigate the influence of this dynamics on Turing's diffusion-driven instability. Such systems have been previously studied on the grounds that binding of the self-activator to a substrate may effectively reduce its diffusion rate and thus induce a Turing instability for species with equal diffusion coefficients, as originally demonstrated by Lengyel and Epstein (1992) under the assumption that the bound state dynamics occurs on a fast timescale. We, however, analyse the full system without any separation of timescales and demonstrate that the full system also allows a relaxation of the standard constraints on the reaction kinetics for the Turing instability, increasing the type of interactions that could give rise to spatial patterning. In particular, we show that two self-

activators can undertake a diffusively driven instability in the presence of a binding immobile substrate, highlighting that the interactions required of a putative biological Turing instability need not be associated with a self-activator-self-inhibitor morphogen pair.
J Theor Biol, 2015; 367
23327443: Diekmann O, Korvasová K
A didactical note on the advantage of using two parameters in Hopf bifurcation studies.
In order to maximize the information that a linearized stability analysis provides, one should work with two free parameters rather than one. Moreover, it is recommended to first consider coefficients in the characteristic equation as parameters and in a second step (try to) invert the map that defines the coefficients in terms of the parameters as they occur in the original equation. Our aim is to substantiate these claims by way of a delay equation example taken from the literature.
J Biol Dyn, 2013; 7 Suppl 1

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BOARD NUMBER: S03-441

A CONTINUUM OF BIOPHYSICAL TIME SCALES OF NEURONS IN THE INTERMEDIATE NUCLEUS OF THE LATERAL LEMNISCUS

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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The intermedial nucleus of the lateral lemniscus (INLL) integrates auditory information from the cochlear and superior olivary nuclei and feeds output to the inferior colliculus. This connectivity suggests that INLL neurons process complex auditory information, potentially including frequency, temporal pattern content and sound source location, prior to the midbrain processing. The tonotopic pattern of the INLL has been suggested to be patchy or organized along a dorso-ventral axis. Besides possible cross-frequency integration, little is known about the functional role of the INLL or the biophysical characteristics of its neuronal population. Here we describe the biophysical and synaptic properties of INLL neurons. Patch-clamp recordings in acute brain slices showed that nearly all membrane properties correlate with the membrane time constant (τ_{mem}), which spans over three magnitudes to generate a large continuum of cellular integration times. The postsynaptic current size and kinetic increase with τ_{mem}, and thereby amplify the continuum of integration times. The lack of correlation between τ_{mem} and cell morphology suggests that varying intrinsic membrane properties underlie this heterogeneity. Recovery of the location of each recorded neuron did not reveal an organizational principle. Therefore, the biophysical heterogeneity seems to be independent of the INLLs tonotopy. Overall, INLL neurons process inputs over a broad range of timescales, supported by their biophysical and synaptic properties. Thus, INLL neurons might be able to process multiple features of auditory information over a broad range of timescales, and possibly serve as a temporal filterbank across the range of perceived frequencies.

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BOARD NUMBER: S03-442

IMPAIRED PROCESSING OF AMPLITUDE-MODULATED TONES IN THE INFERIOR COLLICULUS IN CACNA2D3 MICE - A RISK GENE FOR AUTISM SPECTRUM DISORDERS IN HUMANS

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Temporal processing of complex sounds is a fundamental and intricate task in hearing and a prerequisite for processing and understanding vocalization, speech, and prosody. Here we examined response properties of neurons of the inferior colliculus (IC) in mice lacking *Cacna2d3*, a risk gene for autism spectrum disorders. The $\alpha_2\delta3$ auxiliary Ca^{2+} channel subunit encoded by *Cacna2d3* is essential for proper function of the endbulb of Held synapse in the auditory brainstem (Pirone et al., 2014). Recent evidence has shown that much of auditory feature extraction is being performed in the auditory brainstem and the auditory midbrain, the IC, including processing of amplitude modulation. We determined spectral and temporal response properties of single and multi-unit responses in IC neurons of anesthetized mice. IC units of $\alpha_2\delta3^{-/-}$ mice showed normal tuning properties yet increased spontaneous rates compared with wildtype. When stimulated with amplitude-modulated (AM) tones, $\alpha_2\delta3^{-/-}$ units exhibited less precise temporal coding and reduced evoked rates to higher modulation frequencies (f_m). Population peak latencies were increased for f_m up to 100 Hz in $\alpha_2\delta3^{-/-}$ compared with wildtype units. The loss of precision of temporal coding with increasing f_m from 70 Hz to 160 Hz was characterized using a normalized offset-corrected (Pearson-like) correlation coefficient, which appeared more appropriate than the metrics of vector strength. The processing deficits of AM sounds that we analyzed at the level of the IC indicate that $\alpha_2\delta3^{-/-}$ mice exhibit a subcortical auditory processing disorder. Similar deficits may be present in other mouse models for autism spectrum disorders.

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BOARD NUMBER: S03-443

SINGLE CELL TRANSCRIPTOMIC ATLAS OF THE MURINE COCHLEA

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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[1]Institut Pasteur, Institut De L'audition, Paris, France, [2]Institut Pasteur, Flow Cytometry Platform, Paris, France, [3]Institut Pasteur, 3cytometry And Biomarkers (cb Utechs), Translational Research Center, Paris, France

Aims The functional molecular data available to date in the cochlea has been obtained through the identification of the causal genes for deafness, a total of 140 genes responsible for isolated deafness and 300-400 genes for syndromic deafness. These advances open the way for curing deafness by gene therapy approaches, a clinical domain in which only prostheses can be presently proposed to patients. However, progress in the development of gene therapy requires a complete inventory of the cochlear cell types and an exhaustive characterisation of the genes expressed by each cochlear cell type with their developmental dynamics. To this purpose, we constructed a transcriptomic atlas including more than 110,000 cochlear cells and nuclei. **Methods** The cochleae were extracted from C57bl6J mice before and upon hearing onset, and on P20 when the cochlea is mature. After tissue dissociation, the cochlear cells and nuclei were sorted by flow cytometry and underwent RNA-sequencing. **Results** Our dataset provides a comprehensive map of deafness genes and transcription factors expressed in all cochlear cell types, the identification of new markers for poorly characterized cell types, and evidence for a yet unidentified cell type based on transcriptomic signatures and hybridization assays. In addition, overlooked candidate diseased cochlear cell types for several monogenic forms of deafness were also revealed. **Conclusion** Our study provides an exhaustive and highly detailed map of gene expression in the mouse cochlea in a timeframe corresponding to the postnatal development of the human ear compatible with gene therapy.

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BOARD NUMBER: S03-444

PROBING THE SPECTRAL RESOLUTION AND RESPONSE CHARACTERISTICS OF RED FIBER BASED OPTICAL STIMULATION OF SPIRAL GANGLION NEURONS FROM INFERIOR COLLICULUS RECORDINGS

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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The optogenetic cochlear implants strives to overcome the problems of large current spread and limited dynamic range of electrical cochlear implants (Dieter et al., 2020). F-Chrimson is a promising Channelrhodopsin(ChR) for this technology, combining fast kinetics and less phototoxic red-shifted light absorption. We investigated the effect of emitter size and pulse properties on the inferior colliculus (IC) activity elicited by f-Chrimson mediated optogenetic stimulation of the auditory nerve following prior experiments on blue-light sensitive CatCh (Dieter et al., 2019). We injected adeno-associated virus carrying f-Chrimson (AAV2/6-hSyn-f-Chrimson-EYFP) into the cochlea of neonatal Mongolian gerbils. Eight to fifteen weeks after injection, we inserted laser (594 nm)-coupled optical fibers (50/200µm diameter) into the basal, medial and/or apical turns of cochlea via a cochleostomy. We recorded optically evoked auditory brainstem responses verifying functional f-Chrimson expression. Following placement of a 32-channel electrode array in the contralateral IC, we drove optogenetic stimulation of the auditory nerve by light pulses of varying intensities, durations, and stimulation frequencies. Preliminary results from five gerbils show shorter latencies of IC responses of f-Chrimson mediated optogenetic stimulation of the auditory nerve than reported for CatCh-injected animals. IC responses followed optogenetic stimulation up to frequencies of 180 Hz (n=2) and the response strength increased with energy input. Preliminary analysis of cochlear spread of excitation suggests more spectrally selective stimulation with the 50µm diameter optical fiber (NA 0.22) than with the 200µm fiber (NA 0.39) (n=2). These results indicate that f-Chrimson is a promising ChR for optogenetic stimulation of the auditory nerve.

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BOARD NUMBER: S03-445

MULTIPLE PDZ DOMAIN CONTAINING PROTEIN DELETION HAS SENSORY AND COGNITIVE CONSEQUENCES

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Multiple PDZ domain containing protein (MPDZ or Mupp1) is a large cytosolic polarity protein with thirteen PDZ domains present in neurons and epithelial cells. Mutations in *MPDZ* gene have been reported in patients with autosomal recessive congenital hydrocephalus (HYC2, OMIM #603785) with brain and eye anomalies and with some patients displaying sensorineural hearing loss and mild Intellectual Disability (*Shaheen et al.,* 2017). Also, genetic variations within *Mpdz* have been associated with alcohol and sedative dependence in both mice and humans, which suggest that it may regulate responses to multiple drug abuse (PMID35095607). MPDZ is found at the tight junction of epithelial cells (*Marivin et al., 2019*; *Jarysta et al.,* 2021), but also at the postsynaptic density of synapses in the brain (*Krapivinsky et al*., 2004; *Jones et al.,* 2009). In both systems it functions as a scaffolding protein, interacting with different partners to control the development and function of the cell and tissues. Here we characterized a conditional *Mpdz* mouse mutant for inner ear and cognitive deficits. We identified both epithelial and synaptic disruptions behind the hearing deficits of our model, supporting a role for the protein in both compartments. To evaluate the role of MPDZ on cognitive (notably learning and memory), we developed brain-specific mutants and evaluated reference and working memory, so far never assessed in absence of *Mpdz* in a rodent model. Our result show both brain and sensory organ dysfunctions in absence of MPDZ, supporting a peripheral and a central function for MPDZ in hearing and cognition.

Pubmed:
30061390: Honda A, Kita T, Seshadri SV, Misaki K, Ahmed Z, Ladbury JE, Richardson GP, Yonemura S, Ladher RK
FGFR1-mediated protocadherin-15 loading mediates cargo specificity during intraflagellar transport in inner ear hair-cell kinocilia.
The mechanosensory hair cells of the inner ear are required for hearing and balance and have a distinctive apical structure, the hair bundle, that converts mechanical stimuli into electrical signals. This structure comprises a single cilium, the kinocilium, lying adjacent to an ensemble of actin-based projections known as stereocilia. Hair bundle polarity depends on kinociliary protocadherin-15 (Pcdh15) localization. Protocadherin-15 is found only in hair-cell kinocilia, and is not localized to the primary cilia of adjacent supporting cells. Thus, Pcdh15 must be specifically targeted and trafficked into the hair-cell kinocilium. Here we show that kinocilial Pcdh15 trafficking relies on cell type-specific coupling to the generic intraflagellar transport (IFT) transport mechanism. We uncover a role for fibroblast growth factor receptor 1 (FGFR1) in loading Pcdh15 onto kinociliary transport particles in hair cells. We find that on activation, FGFR1 binds and phosphorylates Pcdh15. Moreover, we find a previously uncharacterized role for clathrin in coupling this kinocilia-specific cargo with the anterograde IFT-B complex through the adaptor, DAB2. Our results identify a modified ciliary transport pathway used for Pcdh15 transport into the cilium of the inner ear hair cell and coordinated by FGFR1 activity.
Proc Natl Acad Sci U S A, 2018; 115

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BOARD NUMBER: S03-446

PURINERGIC RECEPTOR AGONISTS ACTIVATED CA2+ SIGNALING IN THE DEITERS' CELLS IN THE ORGAN OF CORTI IN DIFFERENT POSTNATAL DEVELOPMENTAL STAGES FROM PREHEARING TO MATURED

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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The supporting cells of the organ of Corti and their ATP activated Ca^{2+} signaling are studied extensively in cochlear explants of newborn mice (1-2 days old (P1-2)) and the fundamental role of the ATP-dependent spontaneous activity in cochlear development has been shown. However, information on purinergic Ca^{2+} signaling during cochlear development and in mature stage are much sparse. We have investigated the ATP and UTP-evoked Ca^{2+} signals in Deiters' cells in the hemicochlea preparation in different postnatal developmental-stages from P5, deaf mice to > P18, hearing mice. Single-cell electroporation of Deiters' cells with Ca^{2+} sensitive dyes and their fluorescent imaging showed that the ATP and UTP-induced Ca^{2+} transients have different amplitude, duration and area under the curve characteristics. For mathematical modeling the intracellular Ca^{2+} dynamics of the Deiters' cells we set up a closed cell model to investigate the UTP induced Ca^{2+} responses, and an open cell model to simulate the ATP induced ones. Our results showed that extracellular Ca^{2+} dependent P2X and intracellular Ca^{2+} store dependent P2Y receptors were involved in the ATP and UTP-evoked Ca^{2+} transients in each developmental stages. The UTP induced responses were smaller than ATP induced ones. This was also predicted by our models.

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CHARACTERIZATION OF PROMOTER EXPRESSION IN TYPE 1 AND TYPE 2 SPIRAL GANGLION NEURONS IN VITRO AND IN VIVO

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Aims: Spiral ganglion neurons (SGNs) inside the cochlea play a pivotal role in hearing and are subdivided in type 1 (T1) and type 2 (T2) SGNs. Optogenetic tools to modulate SGN activity display a promising approach to decipher their exact role in hearing and adaptation to varying sound intensities. However, strong and SGN type specific expression of a transgene is still demanding. Therefore, the characterization of potential promoters that drive target gene expression in either T1 or T2 SGNs is essential to specifically investigate the function of these cell types. **Methods:** In order to achieve this goal, RNAseq data (umgear.org) were screened for genes specifically and highly expressed in T1 or T2 SGNs, corresponding promoter elements were cloned and their *in vitro* activity was analyzed by luciferase assay. Successfully evaluated promoters were used to drive transgene expression of f-Chrimson, a channelrhodopsin, in murine cochlea after early postnatal adenovirus associated virus injection. Promotor function was examined *in vivo*, by recording functional responses to optogenetic stimulation (optically evoked auditory brainstem responses, oABR) followed by analysis of cochlear f-Chrimson expression by immunohistology. **Results:** Preliminary data shows that the selected promoters are capable to drive transgene expression sufficiently to enable oABRs. Additionally, histological analysis revealed cell type specific expression of the transgene in SGNs. **Conclusions:** Taken together, our data indicates that we can specifically target SGNs *in vivo* and this will further support research to elucidate the specific role of T1 and T2 SGNs.

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BOARD NUMBER: S03-448

NEURAL CIRCUITRY FOR OXYTOCIN RELEASE AND MATERNAL BEHAVIOR

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Parenting behaviors emerge from complex neural circuits conferring sensitivity to infant needs to ensure survival of the species. One important molecular signal for the maternal brain is the hypothalamic neuropeptide oxytocin. Oxytocin is believed to powerfully enhance parental behaviors by increasing the salience of sensory cues from the offspring but it remains unexplored what sensory cues from infants can activate oxytocin neurons in new mothers. Here we describe a neural circuit routing auditory information about infant vocalizations to the oxytocin system in maternal mice (dams). We found that oxytocin neurons, but not other hypothalamic cells, are activated following playback of pup distress vocalizations. Using anatomical tracing approaches and slice physiology, we identified the a subcortical pathway relaying auditory information to oxytocin neurons. In hypothalamic brain slices, we found that optogenetic activation of PIL fibers led to long-term depression of synaptic inhibition in OT neurons mediated by postsynaptic internalization of GABAARs via dynamin signaling. Using a genetically-encoded oxytocin sensor, we demonstrated that pup calls, but not pure tones, were efficient in triggering central oxytocin release. Finally, inhibiting PIL projections to hypothalamus with chemogenetics, perturbed pup retrieval behavior in dams by decreasing the amount of pups retrieved and increasing the latency of retrieval over multiple trials. These findings suggest that the thalamus-hypothalamus noncanonical auditory pathway may be a specific circuit for the detection of social sounds, important for disinhibiting oxytocin neurons, gating oxytocin release in downstream brain areas, and sustaining maternal performance over time.

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ELUCIDATING THE NEURONAL POPULATION CODE OF SOUND LOCATION AT THE INFERIOR COLLICULUS OF AWAKE MICE USING A FAST VOLUMETRIC CALCIUM IMAGING APPROACH.

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Sound location is proposed to be encoded by neuronal population activity patterns at the mammalian inferior colliculus (IC). However, simultaneous recordings of IC population responses are lacking to capture these population dynamics and characterize the underlying neural code. Using a bespoke scanned temporal focusing two-photon microscope, we performed fast volumetric calcium imaging in awake mice, and simultaneously recorded the activity of unprecedentedly large populations of IC neurons in response to sounds delivered from 13 different frontal horizontal locations (azimuths). The responses of IC neurons showed marked trial-to-trial variability. Nevertheless, our information theoretic analysis identified that individual IC neurons carried a small but significant amount of information about stimulus azimuth, suggesting that the azimuth information is distributed over IC populations. To better understand a potential population code, we compared the performance of theoretical decoding models on the simultaneously recorded IC population responses. We found that a decoding model based on an ensemble of binary classifiers outperformed previously proposed single-architecture models when operating on simultaneously recorded single-trial responses. These findings support the existence of IC activity patterns that encode sound location on the single-trial basis. Our simultaneous recordings showed significant noise correlations, but they did not impact the decoder accuracy. Finally, our volumetric imaging approach revealed the presence of azimuth tuned neurons in a previously unexplored anatomical subdivision of IC: the dorsal cortex. Altogether these findings point out a role of IC as a key relay of the mammalian auditory pathway in sound source localization via parallel distributed processing.

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INVESTIGATING THE ROLE OF THE CEREBELLUM IN BIRDSONG SENSORIMOTOR LEARNING

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Aims: Vocal communication relies on the precise timing of vocalizations. In particular, songbirds learn to produce vocalization with tight temporal precision. The cerebellum is thought to be involved in sensorimotor learning and particularly in controlling and learning the timing of motor actions. However, its possible contribution to song timing and its acquisition in songbird remains unknown. Here we investigate the role of the cerebellum in the control and plasticity of song production in zebra finches. **Methods**: We recorded extracellular neuronal activity in the cerebellum of zebra finches during singing and auditory stimulation. We induced song plasticity through a closed-loop auditory perturbation protocol. Chemical lesions are realised through ibotenic acid injections. Finally, we performed anatomical tracing (dextran dye injections) and electrical brain stimulation. **Results**: Anatomical and electrophysiological data support the existence of a connection between the deep cerebellar nuclei (DCN) and the pallial nuclei involved in song timing via the thalamus. Neurons in the lobules IV/V of the cerebellum modulate their firing rate during singing and are sensitive to auditory stimulation during rest and auditory perturbation during singing. However, lesions of the lateral DCN do not affect the plasticity of syllable durations in adult birds. **Conclusions**: Our data show that the lobule IV/V of the cerebellum displays auditory and singing-related activity in zebrafinches. However, the lateral cerebellum doesn't seem to be involved in adult song plasticity.

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KV3.3 SUBUNITS CONTROL PRESYNAPTIC WAVEFORM AND IMPROVE TIMING AT A CENTRAL EXCITATORY SYNAPSE

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Aims It has been shown that Medial Nucleus of the Trapezoid Body 'MNTB' neurons possess functional Kv3 channels that are composed of either Kv3.1 and/or Kv3.3, and the deletion of either subunit caused a small increase in postsynaptic AP duration, consistent with functional redundancy of either subunit in the postsynaptic MNTB cell body. Here we test for Kv3 subunit-specific roles at the presynaptic calyx of Held terminal innervating MNTB neurons using *in vivo* single unit recordings in Kv3.3 knockout mice. Methods Adult Kv3.3 KO mice of both sexes (n=5) and 5 age-matched CBA wild-type (WT) mice were anaesthetised and stabilized in a sound-proof chamber, in a custom stereotaxic device. Single unit recordings were acquired while presenting pure tone stimuli (100ms duration, 5ms rise/fall, at varying intensities 0-90dB SPL) to the contralateral ear. Results Extracellular recordings from MNTB neurons exhibited a typical complex waveform, comprised of a presynaptic and a postsynaptic component. The time between the peak and trough of extracellular action potentials (APs) are compelling markers for AP half-width and showed that the presynaptic APs and synaptic delays were significantly longer in Kv3.3 knockouts compared to WT recordings. Conclusion These results suggest that Kv3.3 is the presynaptic ‘delayed rectifier', enabling fast presynaptic APs and precisely timed synaptic delays. The changes in presynaptic AP duration and synaptic delay in the Kv3.3 knockout are likely to affect temporal processing in the MNTB output, like first-spike latency and jitter. However, the longer presynaptic APs will also affect transmitter release and hence spontaneous and sound driven firing rates.

Pubmed:
30233328: Călin A, Stancu M, Zagrean AM, Jefferys JGR, Ilie AS, Akerman CJ
Chemogenetic Recruitment of Specific Interneurons Suppresses Seizure Activity.
Current anti-epileptic medications that boost synaptic inhibition are effective in reducing several types of epileptic seizure activity. Nevertheless, these drugs can generate significant side-effects and even paradoxical responses due to the broad nature of their action. Recently developed chemogenetic techniques provide the opportunity to pharmacologically recruit endogenous inhibitory mechanisms in a selective and circuit-specific manner. Here, we use chemogenetics to assess the potential of suppressing epileptiform activity by enhancing the synaptic output from three major interneuron populations in the rodent hippocampus: parvalbumin (PV), somatostatin (SST), and vasoactive intestinal peptide (VIP) expressing interneurons. To target different neuronal populations, promoter-specific cre-recombinase mice were combined with viral-mediated delivery of chemogenetic constructs. Targeted electrophysiological recordings were then conducted in an model of chronic, drug-resistant epilepsy. In addition, behavioral video-scoring was performed in an model of acutely triggered seizure activity. Pre-synaptic and post-synaptic whole cell recordings in brain slices revealed that each of the three interneuron types increase their firing rate and synaptic output following chemogenetic activation. However, the interneuron populations exhibited different effects on epileptiform discharges. Recruiting VIP interneurons did not change the total duration of epileptiform discharges. In contrast, recruiting SST or PV interneurons produced robust suppression of epileptiform synchronization. PV interneurons exhibited the strongest effect per cell, eliciting at least a fivefold greater reduction in epileptiform activity than the other cell types. Consistent with this, we found that chemogenetic recruitment of PV interneurons suppressed convulsive behaviors by more than 80%. Our findings support the idea that selective chemogenetic enhancement of inhibitory synaptic pathways offers potential as an anti-seizure strategy.
Front Cell Neurosci, 2018; 12

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BOARD NUMBER: S03-452

GOOD INTERAURAL TIME DIFFERENCE (ITD) SENSITIVITY WITH BILATERAL COCHLEAR IMPLANTS REQUIRES ITDS IN PULSE TIMING, NOT ENVELOPES

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Interaural time difference (ITD) discrimination is a major challenge for bilateral cochlear implant (biCIs) patients, especially after early deafness. Our recent work demonstrating that neonatally deafened (ND) rats with synchronized biCIs develop excellent ITD sensitivity may therefore come as surprise. Today's clinical CI processors employ asynchronous pulsatile stimulation in each ear, so that biCI users only experience ITDs in pulse train envelopes (envITD). It seems possible that uninformative pulse-timing ITDs (ptITDs) will swamp envITDs. This motivated us to investigate the relative effectiveness of ptITDs and envITDs in well-controlled behavioral animal experiments. ND biCI rats were trained to lateralize sinusoidally enveloped pulse trains in which envITD and ptITDs co-varied. Animals were tested with envITDs and ptITDs, which were drawn independently from {-100, 0, +100} µs. Various combinations of two pulse rates {900, 4500} pps and three envelope modulation rates {5, 20, 100} Hz were tested. Probit analysis was used to quantify how strongly animals based their lateralization judgments on envITDs or ptITDs at each condition. Animals learned to lateralize ITDs with high accuracy when envITDs and ptITDs were congruent. When envITDs and ptITDs conflicted, ptITDs completely dominated the lateralization judgments. At none of the conditions tested did envITDs significantly influence lateralization behavior. Our results suggest that the auditory pathway cannot process envITDs in biCI stimulation effectively. Healthy ears can be highly sensitive to acoustic envITDs but the situation in biCI stimulation is radically different. To make rich binaural hearing available to biCI patients, pulse timing has to be considered more strongly.

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SOUND LOCALIZATION TUNING OF THE MEDIAL SUPERIOR OLIVE IN MONGOLIAN GERBILS AFTER HEARING ONSET

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Sound sources can be localized in the horizontal plane by comparing their arrival times at both ears. A specialized brainstem circuit that converges on the medial superior olive (MSO) computes these interaural time differences (ITDs) in arrival. This computation depends on the unique biophysical properties of MSO neurons and on the time it takes the signals to travel to the MSO from both ears. How the ITD tuning of MSO neurons develops is still unknown. Aim. We compared ITD tuning and monaural travel delays for MSO neurons in anesthetized, two to four weeks old Mongolian gerbils. Methods. We made juxtacellular or single-unit recordings of principal MSO neurons using glass pipettes. ITD tuning and monaural delays were measured using sound stimulation with an in-ear speaker. Results. We observed that young MSO neurons received excitatory inputs from both ears. The firing of MSO neurons was facilitated by binaural stimulation and showed the highest firing rate ('best ITD') when sounds were presented much earlier at the contralateral ear. This excess delay became smaller during the fourth week. First spike latencies to monaural clicks decreased during the third and fourth week. The difference in monaural first spike latencies was in line with the excess contralateral delay, and was strongly correlated with the best ITD of the same neuron ($r = 0.8$). Conclusions. We conclude that, unlike the rapid development of other features, the MSO's ITD tuning and the monaural travel delays to the MSO mature beyond the third week in Mongolian gerbils.

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HOW DO INTERAURAL TIME AND INTERAURAL LEVEL DIFFERENCES INTERACT IN SPATIAL HEARING WITH COCHLEAR IMPLANTS?

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Sound localization in the horizontal plane is based on two binaural cues: interaural level differences (ILDs) and interaural time differences (ITDs). For bilateral cochlear implant (biCI) users, sound localization and spatial hearing are major challenges, and ITD sensitivity is particularly poor, with most biCI patients relying almost exclusively on ILDs. However, our recent work demonstrates that neonatally deafened (ND) rats with synchronized biCIs can develop excellent ITD sensitivity. Here, we investigate how the sensitivities to ILDs and ITDs compare and interact in ND rats which are supplied with synchronized biCI input from the outset. ND biCI rats were trained to lateralize pulse trains at a pulse rate of 900 pps with either solitary ILDs (range of ±6 dB) or co-varying ITDs and ILDs of {+/- 100, 80, 60, 0} µs and {+/-6, 4, 1, 0.5, 0} dB. Trials in which ITDs and ILDs varied independently from each other were used to determine the relative strength and interaction of these two spatial cues. Our biCI animals developed excellent ILD as well as ITD sensitivities, and the two types of cues interacted additively. Importantly, very small pulse timing ITDs of ~80 µs could influence an animal's lateralization judgment as powerfully as relatively very large electrical pulse amplitude ILDs of ~4 dB. These results add to the growing evidence that the inability of CI processing strategies in current clinical use to encode auditory information in precise pulse timing constitute a very significant technical limitation.

Pubmed:
34911002: Weltin A, Kieninger J, Urban GA, Buchholz S, Arndt S, Rosskothen-Kuhl N
Standard cochlear implants as electrochemical sensors: Intracochlear oxygen measurements in vivo.
Cochlear implants are the most successful neural prostheses worldwide and routinely restore sensorineural hearing loss by direct electrical stimulation of the auditory nerve. Enhancing this standard implant by chemical sensor functionality opens up new possibilities, ranging from access to the biochemical microenvironment of the implanted electrode array to the long-term study of the electrode status. We developed an electrochemical method to turn the platinum stimulation microelectrodes of cochlear implants into electrochemical sensors. The electrodes showed excellent and stable chemical sensor properties, as demonstrated by in vitro characterizations with combined amperometric and active potentiometric dissolved oxygen and hydrogen peroxide measurements. Linear, stable and highly reproducible sensor responses within the relevant concentration ranges with negligible offset were shown. This approach was successfully applied in vivo in an animal model. Intracochlear oxygen dynamics in rats upon breathing pure oxygen were reproducibly and precisely measured in real-time from the perilymph. At the same time, correct implant placement and its functionality was verified by measurements of electrically evoked auditory brainstem responses with clearly distinguishable peaks. Acute measurements indicated no adverse influence of electrical stimulation on electrochemical measurements and vice versa. Our work is ground-breaking towards advanced implant functionality, future implant lifetime monitoring, and implant-life-long in situ investigation of electrode degradation in cochlear implant patients.
Biosens Bioelectron, 2022; 199

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BOARD NUMBER: S03-455

NEUROPATHOLOGICAL ALTERATIONS IN INDIVIDUALS WITH TINNITUS: A POST-MORTEM STUDY

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Introduction Tinnitus is a phantom perception of sound. Degenerative or inflammatory processes as well as changes in the monoaminergic systems have been suggested as potential underlying mechanisms. To our knowledge however, no post-mortem study was conducted on tinnitus patients. Here, we aimed to study the pathological changes in the auditory and non-auditory brain regions of tinnitus patients. **Methods** Brain specimens were obtained from tinnitus patients and matched controls. The paraffin-embedded tissue blocks contained the medial geniculate body (MGB), inferior colliculus (IC) and dorsal raphe nucleus (DRN). Nissl staining was performed to access cell density and cell/area measurements. The number of glial cells was assessed using anti-GFAP and iba-1 antibodies. In addition, the DRN was stained using antibodies raised against phenylalanine hydroxylase-8 (PH8) and tyrosine-hydroxylase (TH) to detect serotonergic and dopaminergic neurons, respectively. **Results** Quantitative analysis of Nissl stained sectioned revealed a significant reduction in neuronal cell density in the MGB and IC of tinnitus subjects. Although cell size was not changed between groups. Moreover, a lower number of astrocytes were found in the IC of tinnitus cases. No significant cell loss was found in the DRN in Nissl stained sections. However, the number of PH8 positive cells was significantly reduced in tinnitus cases, while the number of TH positive neurons did not differ between groups. **Conclusion** This study suggests that both neurodegenerative and inflammatory processes may be involved in the neuropathophysiology of tinnitus, and point towards a potential role for raphe serotonergic system in this complex neuro-otologic disorder.

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EFFECT OF GROUP I MGLUR ACTIVATION ON SYNAPTIC TRANSMISSION AND NEURON EXCITABILITY IN THE AUDITORY MIDBRAIN OF FXS MOUSE MODEL

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Metabotropic glutamate receptors (mGluRs) play a critical role in the developmental shaping of sensory pathways. In spite of combined efforts to understand their role, the exact involvement in this process remains elusive. Dysfunctional overactivity of pathways activated by mGluRs may contribute to multiple debilitating conditions, an example of which is Fragile X Syndrome (FXS) - the most inherited cause of autism. In this work, the effects of acute mGluR1/5 activation on neural circuit function are dissected and compared between genotypes of the FXS mouse model in the central nucleus of the inferior colliculus - a major integratory hub of the auditory pathway - around the critical period of hearing development. Our results obtained using whole-cell patch-clamp in acute murine brain slices indicate a range of group I mGluR-related phenomena, affecting both excitatory and inhibitory synaptic transmission in a cell-type-specific manner. Local mGluR agonist administration (3,5-DHPG) also led to temporary changes in the neuronal membrane properties, increasing input resistance and lowering the firing threshold in a majority of recorded neurons. Additionally, an increase in rebound spiking and changes in neuronal firing patterns were observed. The effects of mGluR activation seem to be less profound in animals missing the fragile X mental retardation protein (FMRP) and increase with age, persisting even after a critical period of auditory circuit development. These phenomena may underlie the behavioural and sensory processing deficits for which the loss of FMRP is a prerequisite and give new insight into factors shaping the ontogenesis of the auditory pathway.

Pubmed:

33762969: Chrobok L, Wojcik M, Klich JD, Pradel K, Lewandowski MH, Piggins HD
Phasic Neuronal Firing in the Rodent Nucleus of the Solitary Tract .
Phasic pattern of neuronal activity has been previously described in detail for magnocellular vasopressin neurons in the hypothalamic paraventricular and supraoptic nuclei. This characteristic bistable pattern consists of alternating periods of electrical silence and elevated neuronal firing, implicated in neuropeptide release. Here, with the use of multi-electrode array recordings , we aimed to study the firing pattern of neurons in the nucleus of the solitary tract (NTS) - the brainstem hub for homeostatic, cardio-vascular, and metabolic processes. Our recordings from the mouse and rat hindbrain slices reveal the phasic activity pattern to be displayed by a subset of neurons in the dorsomedial NTS subjacent to the area postrema (AP), with the inter-spike interval distribution closely resembling that reported for phasic magnocellular vasopressin cells. Additionally, we provide interspecies comparison, showing higher phasic frequency and firing rate of phasic NTS cells in mice compared to rats. Further, we describe daily changes in their firing rate and pattern, peaking at the middle of the night. Last, we reveal these phasic cells to be sensitive to adrenergic receptors activation and to respond to electrical stimulation of the AP. This study provides a comprehensive description of the phasic neuronal activity in the rodent NTS and identifies it as a potential downstream target of the AP noradrenergic system.
Front Physiol, 2021; 12
32997815: Chrobok L, Jeczmien-Lazur JS, Pradel K, Klich JD, Bubka M, Wojcik M, Kepczynski M, Lewandowski MH
Circadian actions of orexins on the retinorecipient lateral geniculate complex in rat.
Rhythmic processes in living organisms are controlled by biological clocks. The orexinergic system of the lateral hypothalamus carries circadian information to provide arousal for the brain during the active phase. Here, we show that orexins exert an excitatory action in three parts of the lateral geniculate nucleus (LGN), in particular upon directly retinorecipient neurons in the non-image forming visual structures. We provide evidence for the high nocturnal levels of orexins with stable circadian expression of predominant orexin receptor 2 in the LGN. Our data additionally establish the convergence of orexinergic and pituitary adenylate cyclase (PAC)-activating peptide/PAC1 receptor systems (used by melanopsin-expressing retinal ganglion cells), which directly regulates responses to the retinal input. These results help us better understand circadian orexinergic control over the non-image forming subcortical visual system, forming the animal's preparedness for the behaviourally active night.

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J Physiol, 2021; 599
30448422: Potasiewicz A, Holuj M, Piotrowska D, Zajda K, Wojcik M, Popik P, Nikiforuk A
Evaluation of ultrasonic vocalizations in a neurodevelopmental model of schizophrenia during the early life stages of rats.
In an animal neurodevelopmental model of schizophrenia, we investigated ultrasonic communication and social behavior in male and female rats. Pregnant dams were treated with methylazoxymethanol acetate (MAM; 22 mg/kg) at 17 days of gestation. First, we examined the ultrasonic vocalizations (USVs) emitted by 8-day-old pups isolated from their mothers and placed in a familiar or an unfamiliar environment. Second, we assessed tickling-induced USVs, social play (SP) behavior and accompanying USVs in 30-day-old juveniles. Independent of the prenatal treatment, sex differences were noted at both ages. In the pups isolated from their mothers, compared to the females, the males produced flatter calls with a lower frequency. Compared to the females, the tickling-induced male USVs were characterized by a higher frequency, and the male SP-induced USVs showed a broader bandwidth and more modulated structure. Additionally, the numbers of both SP-induced USVs and SP episodes in the males were higher than those in the females. In contrast, the MAM exposure reduced the ultrasonic communication and social behavior independent of age almost equally in the male and female rats. The MAM-exposed isolated pups and juveniles experiencing tickling and social interaction displayed lower USV bandwidths, suggesting that the complexity of their ultrasonic communication was reduced. In addition, the MAM-exposed juveniles demonstrated a lower number of 50-kHz "happy calls" and decreased SP duration, which is suggestive of social withdrawal or negative-like symptoms. These data demonstrate that young MAM-exposed rats display an atypical repertoire of USVs and reduced play behavior suggestive of communication deficits associated with schizophrenia.
Neuropharmacology, 2019; 146

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BOARD NUMBER: S03-457

SODIUM SALICYLATE IMPROVES DETECTION OF AMPLITUDE MODULATED SOUND IN MICE

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Aims: Sodium salicylate is commonly used as a pharmacological model for tinnitus in laboratory animals. The effects of salicylate on the detection of amplitude modulated (AM) sound are unknown. Here, we assessed the salicylate-induced effect on behavioural AM detection thresholds and neuronal AM coding in the inferior colliculus (IC). **Methods:** Six B6CBAF1/J mice were trained and tested in a Go-No-Go AM detection task. The detection thresholds for 16 Hz and 512 Hz AM noise were assessed in blocks of 3 consecutive days both before, during and after injections with salicylate (daily 250 mg/kg i.p., 2 hours before first session). Acute electrophysiological recordings were performed in the IC of 10 mice under ketamine/xylazine anaesthesia using multichannel silicon arrays. Spike sorting was performed using Kilosort2 to isolate single units. Pure tone, AM noise and dynamic random chord (DRC) stimuli were presented. After a baseline recording period, sodium salicylate was injected, and its effect on the responses was evaluated in the same units. DiO-stained electrode tracks were retrieved in histology to estimate subregional localization of recorded units. Plasma salicylate concentrations were measured. **Results:** Salicylate improved the behavioural sensitivity of mice to amplitude modulations. Upon salicylate, most IC neurons showed lower spontaneous activity, elevated pure-tone thresholds and improved responses to AM stimuli as measured by spike rate and vector strength; spectral-temporal receptive fields became more predictive of the response to DRCs. **Conclusion:** Salicylate unexpectedly improved AM sensitivity in mice, reflected both in neuronal activity in the auditory midbrain and in lower behavioural detection thresholds.

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BOARD NUMBER: S03-458

SOUND REPRESENTATIONS IN THE AUDITORY BRAINSTEM OF AWAKE MICE

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Sound information is shaped along the auditory pathway to generate sound perception. Yet, even the transformation occurring at the first processing steps remain incompletely understood due to the difficulty to record large number of neurons in the auditory brainstem areas in awake freely listening animals. Here, we used the length and high contact density of Neuropixels 1.0 probes to record neurons from the cochlear nucleus (CN), superior olivary complex (SOC) and inferior colliculus (IC) regions together in head-fixed, awake mice listening passively to a set of 307 short (<500ms) artificial sounds and 10 long (30s) sounds from natural environments. The set of sounds was designed to include sounds of increasing complexity: diverse categories of simple sounds, complex sounds (bird songs, music), as well as partial and complete reconstructions of the complex sounds. We compared the responses recorded with known properties in anesthetized animals and confirmed the presence of primary-like and onset responses. While the responses to more complex sounds remain to be analyzed, we also developed models to capture the transformations from a precise biophysical model of the cochlea to brainstem.

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INTRA-MODAL AND CROSS-MODAL REORGANIZATION OF THALAMOCORTICAL AXONS AFTER THE PRENATAL ABLATION OF THE AUDITORY NUCLEUS OF THE THALAMUS

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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[1] Instituto de Neurociencias de Alicante, UMH-CSIC, Sant Joan d´ Alacant, Spain [2] Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA The majority of sensory and motor information is conveyed to the cerebral cortex through the thalamocortical axons (TCAs). Thalamocortical connectivity is very precise and formed prenatally, thus it plays a crucial role in several aspects of cortical development such as cortical area specification, identity and wiring. However, the mechanisms by which TCAs regulate the establishment and plasticity of these cortical features remain largely unknown. To this end, our aim is to determine the capacity of TCAs to compete for neocortical space and impose a sensory input when the thalamus has been genetically manipulated. To achieve this, we have developed a genetic approach to selectively ablate the medial geniculate body (MGB) of the auditory thalamus very early in embryonic development before thalamic axons reach the cortical plate. As a result, these mice develop normally receiving peripheral sensory input, but lacking a specific thalamic relay station. We found a rewired auditory pathway with misrouted auditory TCAs passing through the somatosensory thalamic nucleus as well as an increased primary somatosensory cortex. Furthermore, we observed additional inter-modal and intra-modal changes occurring in our ablated model. This study will provide essential information not only for the development of thalamocortical projections, but also for the capacity of spared sensory circuits to rewire following an early thalamic manipulation.

Pubmed:
35078267: Giasafaki C, Grant E, Hoerder-Suabedissen A, Hayashi S, Lee S, Molnár Z
Cross-hierarchical plasticity of corticofugal projections to dLGN after neonatal monocular enucleation.
Perception is the result of interactions between the sensory periphery, thalamus, and cerebral cortex. Inputs from the retina project to the first-order dorsal lateral geniculate nucleus (dLGN), which projects to the primary visual cortex (V1). In return, the cortex innervates the thalamus. While layer 6 projections innervate all thalamic nuclei, cortical layer 5 neurons selectively project to the higher order lateral posterior nucleus (LP) and not to dLGN. It has been demonstrated that a subpopulation of layer 5 (Rbp4-Cre+) projections rewires to dLGN after monocular or binocular enucleation in young postnatal mice. However, the exact cortical regional origin of these projections was not fully determined, and it remained unclear whether these changes persisted into adulthood. In this study, we report gene expression changes observed in the dLGN after monocular enucleation at birth using microarray, qPCR at P6, and in situ hybridization at P8. We report that genes that are normally enriched in dLGN, but not LP during development are preferentially downregulated in dLGN following monocular enucleation. Comparisons with developmental gene expression patters in dLGN suggest more immature and delayed gene expression in enucleated dLGN. Combined tracing and immuno-histochemical analysis revealed that the induced layer 5 fibers that innervate enucleated dLGN originate from putative primary visual cortex and they retain increased VGluT1+ synapse formation into adulthood. Our results indicate a new form of plasticity when layer 5 driver input takes over the innervation of an originally first-order thalamic nucleus after early sensory deficit.
J Comp Neurol, 2022; 530

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BOARD NUMBER: S03-460

CORTICO-FUGAL REGULATION OF PREDICTIVE CODING

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Sensory cues are differentially encoded depending on the contextual stream in which they are embedded. This adaptation can be understood through a predictive coding framework, in which responses to predictable stimuli become attenuated (repetition suppression), while unexpected cues elicit a prediction error response. In the auditory system, prediction error first appears at the level of the auditory midbrain, or inferior colliculus (IC), and is most prominent in the auditory cortex (AC). To determine the role of descending connections from AC to IC in predictive coding, we selectively suppressed the cortico-collicular (CC) pathway while recording responses to predictable and unpredictable stimuli in IC. To selectively target auditory CC cells, we made bilateral injections of a retro AAV-Cre construct in IC, while injecting an AAV-Flex-ArchT construct in AC. We performed extracellular recordings in IC in awake mice while playing tone sequences designed to parse prediction error and repetition suppression effects, suppressing CC cells on a fraction of trials. We found that suppression of the CC pathway led to a decrease in prediction error but did not affect repetition suppression. We also discovered populations of IC neurons that exhibit repetition enhancement, an increase in firing with stimulus repetition, and negative prediction error, a stronger response to a tone in a predictable rather than unpredictable context, both of which were suppressed during CC inactivation. Neurons in IC responded more similarly to each context in the absence of cortical input, suggesting that AC provides cues about the statistical context of sound to subcortical brain regions.

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BRAIN-WIDE MAPPING OF AUDITORY-EVOKED RESPONSES IN DANIONELLA

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Charite, Judkewitz Lab, Berlin, Germany

Our understanding of how auditory information is transformed along the auditory pathway is still limited and would greatly benefit from dense recordings of neuronal population activity across brain areas. Yet, the size and opacity of vertebrate brains make it technically challenging to monitor brain-wide activity at a temporally and spatially high resolution. To fill this gap, we recently introduced a novel model organism, the teleost fish Danionella cerebrum. Danionella combines small size, genetic tractability, and brain transparency with the complexity of a mature vertebrate and thereby enables new ways to explore distributed neuronal circuits. At the same time our data show that Dc exhibit complex behaviours including acoustic communication. Here, we developed a custom-tailored microscope (image transfer oblique plane microscope, ITOPM) to enable the recording of quasi-synchronous neural activity across the majority of the Danionella brain with single-cell resolution at 1 Hz volume rate during auditory stimulation. Using whole-brain oblique plane microscopy we identify Danionella's major auditory processing areas spanning hindbrain, midbrain and forebrain. Our data demonstrates that the acoustic processing of pure tones and pulses is already segregated at the level of the acoustic nuclei in the hindbrain. Finally, we show how tuning specificity and sensitivity of cells increases with the transition from hindbrain to midbrain and how reponses to vocalisation mimics systematically vary along the whole acoustic pathway.

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GABAERGIC NEURONS OF THE POSTERIOR NUCLEUS BASALIS MODULATE INFORMATION PROCESSING IN THE AUDITORY PATHWAY

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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The posterior Nucleus Basalis (pNB) is a subset of the basal forebrain (BF) regulating the auditory pathway, that contains extensively documented cholinergic projections, but also GABAergic neurons projecting to cortex as well as thalamus. Here, we study how optogenetic manipulation of pNB GABAergic neurons impacts auditory neural circuit responses and auditory based behavior. We designed an auditory detection paradigm in which animals had to detect a relevant target stimulus in the presence of auditory noise. Freely moving rats were trained to hold down a lever and release it upon presentation of a narrow bandpass-filtered auditory stimulus in the presence of a broad-band masking noise. When inhibiting the pNB- parvalbumin expressing (PV) neurons only, we observed robust impairment of stimulus detection. While activating the same population did not modify detection abilities, more general activation of the entire pNB-GABAergic population using mDlx improved detection. In parallel, we recorded LFP and single-unit activity under isoflurane anesthesia in pNB, primary auditory cortex (A1), and the auditory responsive thalamic medial geniculate nucleus (MGN), during similar optogenetics manipulations. We document suppression of neural responses to the target stimulus in the presence of masking noise in MGN and A1. PV specific 40Hz stimulation elicited frequency-specific modulation of neural activity in MGN and A1. In addition, we observed 40Hz entrainment in A1 following pNB-GABAergic population activation using mDlx. Our studies suggest that distinct populations of pNB-GABAergic neurons may exert important influences on auditory information processing, complementing neuromodulatory effects due to cholinergic projections.

Pubmed:
32244254: Azimi H, Klaassen AL, Thomas K, Harvey MA, Rainer G
Role of the Thalamus in Basal Forebrain Regulation of Neural Activity in the Primary Auditory Cortex.
Many studies have implicated the basal forebrain (BF) as a potent regulator of sensory encoding even at the earliest stages of or cortical processing. The source of this regulation involves the well-documented corticopetal cholinergic projections from BF to primary cortical areas. However, the BF also projects to subcortical structures, including the thalamic reticular nucleus (TRN), which has abundant reciprocal connections with sensory thalamus. Here we present naturalistic auditory stimuli to the anesthetized rat while making simultaneous single-unit recordings from the ventral medial geniculate nucleus (MGN) and primary auditory cortex (A1) during electrical stimulation of the BF. Like primary visual cortex, we find that BF stimulation increases the trial-to-trial reliability of A1 neurons, and we relate these results to change in the response properties of MGN neurons. We discuss several lines of evidence that implicate the BF to thalamus pathway in the manifestation of BF-induced changes to cortical sensory processing and support our conclusions with supplementary TRN recordings, as well as studies in awake animals showing a strong relationship between endogenous BF activity and A1 reliability. Our findings suggest that the BF subcortical projections that modulate MGN play an important role in auditory processing.
Cereb Cortex, 2020; 30

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BOARD NUMBER: S03-463

HIDDEN HEARING-LOSS AND INFORMATION TRANSMISSION IN THE AUDITORY MIDBRAIN

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

Juan Fuentes[1], Roland Schaette[2], David Mcalpine[1]
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Hidden hearing-loss (HHL) refers to listening difficulties in noisy environments, suggested to arise from over-exposure to loud sounds that spares hearing sensitivity but damages high-threshold auditory nerve fibres. Perceptual and physiological effects haven't been fully understood, and as there's no measure to quantify them, our goal is to investigate primitives for an electrophysiological measure of HHL and its consequences to perception. Here we adapt a recently developed framework that combines normative theories and population spike-train data to predict the optimal state in which neural populations of the inferior colliculus (IC) are inferred (via maximum likelihood estimation) to operate. We assessed single- and multi-neuron IC recordings of mice and gerbils, and explored the sample of fitted neural parameters (thresholds and slopes of input-output functions) through a maximum-entropy continuum of models. Relative to unexposed animals, animals exposed to loud sounds sufficient to generate HHL showed increased average coding utility (defined over mutual information) only for quiet acoustic contexts, but the reverse this effect for louder contexts, i.e., unexposed animals achieving increased average coding utility, among other stable differences in more population parameters. This is consistent with exposed animals having an overrepresentation of low-threshold fibres and a compensatory change in neural gain following selective damage to high-threshold auditory fibres. The normative model employed here, the way it is combined with spike-train data, and the consistent effect across different types of auditory stimuli, suggests a potential means of developing an information-theoretic approach to hearing problems not evident in standard hearing tests.

Pubmed:
32205896: Mucarquer JA, Prado P, Escobar MJ, El-Deredy W, Zañartu M
Improving EEG Muscle Artifact Removal With an EMG Array.
Removal of artifacts induced by muscle activity is crucial for analysis of the electroencephalogram (EEG), and continues to be a challenge in experiments where the subject may speak, change facial expressions, or move. Ensemble empirical mode decomposition with canonical correlation analysis (EEMD-CCA) has been proven to be an efficient method for denoising of EEG contaminated with muscle artifacts. EEMD-CCA, likewise the majority of algorithms, does not incorporate any statistical information of the artifact, namely, electromyogram (EMG) recorded over the muscles actively contaminating the EEG. In this paper, we propose to extend EEMD-CCA in order to include an EMG array as information to aid the removal of artifacts, assessing the performance gain achieved when the number of EMG channels grow. By filtering adaptively (recursive least squares, EMG array as reference) each component resulting from CCA, we aim to ameliorate the distortion of brain signals induced by artifacts and denoising methods. We simulated several noise scenarios based on a linear contamination model, between real and synthetic EEG and EMG signals, and varied the number of EMG channels available to the filter. Our results exhibit a substantial improvement in the performance as the number of EMG electrodes increase from 2 to 16. Further increasing the number of EMG channels up to 128 did not have a significant impact on the performance. We conclude by recommending the use of EMG electrodes to filter components, as it is a computationally inexpensive enhancement that impacts significantly on performance using only a few electrodes.
IEEE Trans Instrum Meas, 2020; 69

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BOARD NUMBER: S03-464

CELL TYPE SPECIFIC AUDITORY RESPONSES IN THE AUDITORY STRIATUM

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Despite its importance in health and disease, we know remarkably little about sensorimotor transformation (i.e how we assign meaning to and use sensory stimuli to guide behavior). The dorsal striatum is thought to be particularly important for the formation of sensorimotor associations during reinforcement learning due to the dopaminergic inputs it receives, as well as a diverse array of cortical and subcortical inputs. There are two cell types that make up the two output pathways of the dorsal striatum, direct pathway striatal projection neurons (dSPNs) and indirect pathway striatal neurons (iSPNs). While much work has focused on how these pathways might function to initiate movements, very little is known about how sensory learning influences the neuronal activity of these neurons and what effect this has on behavior. In this study, we focus on a the posterior, or auditory striatum (AudStr), that receives dense auditory inputs from both the medial geniculate body (MGB) of the thalamus and primary auditory cortex (A1). We first investigated the sensory response of dSPNs and iSPNs in AudStr by examining the membrane potential (Vm) fluctuation *in vivo* in awake head-fixed animal in response to auditory cues. Interestingly, our results reveal cell-type specific differences: dSPNs show significant subthreshold responses more frequently than iSPNs. We hypothesized these differences are the result of differential connectivity from MGB or A1, and are currently assaying the probability and strength of these connections. In future work, we plan to understand how auditory learning shapes auditory responses onto dSPNs and iSPNs.

Pubmed:
31496960: Druart M, Le Magueresse C
Emerging Roles of Complement in Psychiatric Disorders.
The complement system consists of more than 30 proteins that have long been known to participate to the immune defence against pathogens and to the removal of damaged cells. Their role, however, extends beyond immunity and clearance of altered "self" components in the periphery. In particular, complement proteins can be induced by all cell types in the brain. Recent discoveries highlight the role of complement in normal and pathological brain development. Specifically, the complement system mediates synaptic pruning, a developmental process whereby supernumerary synapses are eliminated in the immature brain. The complement system has been implicated in pathological synapse elimination in schizophrenia, West Nile virus infection, and lupus, all of which are associated with psychiatric manifestations. Complement also contributes to synapse loss in neurodegenerative conditions. This review provides a brief overview of the well-studied role of complement molecules in immunity. The contribution of complement to embryonic and adult neurogenesis, neuronal migration, and developmental synaptic elimination in the normal brain is reviewed. We discuss the role of complement in synapse loss in psychiatric and neurological diseases and evaluate the therapeutic potential of complement-targeting drugs for brain disorders.
Front Psychiatry, 2019; 10
33020214: Druart M, Groszer M, Le Magueresse C
An Etiological Foxp2 Mutation Impairs Neuronal Gain in Layer VI Cortico-Thalamic Cells through Increased GABA/GIRK Signaling.
A rare mutation affecting the Forkhead-box protein P2 (FOXP2) transcription factor causes a severe monogenic speech and language disorder. Mice carrying an identical point mutation to that observed in affected patients (Foxp2 mice) display motor deficits and impaired synaptic plasticity in the striatum. However, the consequences of the mutation on neuronal function, in particular in the cerebral cortex, remain little studied. Foxp2 is expressed in a subset of Layer VI cortical neurons. Here, we used Ntsr1-EGFP mice to identify Foxp2+ neurons in the mouse auditory cortex We studied the functional impact of the R552H mutation on the morphologic and functional properties of Layer VI cortical neurons from Ntsr1-EGFP; Foxp2 male and female mice. The complexity of apical, but not basal dendrites was significantly lower in Foxp2 cortico-thalamic neurons than in control Foxp2 neurons. Excitatory synaptic inputs, but not inhibitory synaptic inputs, were decreased in Foxp2 mice. In response, homeostatic mechanisms would be expected to increase neuronal gain, i.e., the conversion of a synaptic input into

a firing output. However, the intrinsic excitability of Foxp2+ cortical neurons was lower in Foxp2 neurons. A-type and delayed-rectifier (DR) potassium currents, two putative transcriptional targets of Foxp2, were not affected by the mutation. In contrast, GABA/GIRK signaling, another presumed target of Foxp2, was increased in mutant neurons. Blocking GIRK channels strongly attenuated the difference in intrinsic excitability between wild-type (WT) and Foxp2 neurons. Our results reveal a novel role for Foxp2 in the control of neuronal input/output homeostasis. Mutations of the Forkhead-box protein 2 (FOXP2) gene in humans are the first known monogenic cause of a speech and language disorder. The Foxp2 mutation may directly affect neuronal development and function in neocortex, where Foxp2 is expressed. Brain imaging studies in patients with a heterozygous mutation in FOXP2 showed abnormalities in cortical language-related regions relative to the unaffected members of the same family. However, the role of Foxp2 in neocortical neurons is poorly understood. Using mice with a Foxp2 mutation equivalent to that found in patients, we studied functional modifications in auditory cortex neurons We found that mutant neurons exhibit alterations of synaptic input and GABAB/GIRK signaling, reflecting a loss of neuronal homeostasis.
J Neurosci, 2020; 40

33837272: Druart M, Nosten-Bertrand M, Poll S, Crux S, Nebeling F, Delhaye C, Dubois Y, Mittag M, Leboyer M, Tamouza R, Fuhrmann M, Le Magueresse C

Elevated expression of complement C4 in the mouse prefrontal cortex causes schizophrenia-associated phenotypes. Accumulating evidence supports immune involvement in the pathogenesis of schizophrenia, a severe psychiatric disorder. In particular, high expression variants of C4, a gene of the innate immune complement system, were shown to confer susceptibility to schizophrenia. However, how elevated C4 expression may impact brain circuits remains largely unknown. We used in utero electroporation to overexpress C4 in the mouse prefrontal cortex. We found reduced glutamatergic input to pyramidal cells of juvenile and adult, but not of newborn C4-overexpressing (C4-OE) mice, together with decreased spine density, which mirrors spine loss observed in the schizophrenic cortex. Using time-lapse two-photon imaging in vivo, we observed that these deficits were associated with decreased dendritic spine gain and elimination in juvenile C4-OE mice, which may reflect poor formation and/or stabilization of immature spines. In juvenile and adult C4-OE mice, we found evidence for NMDA receptor hypofunction, another schizophrenia-associated phenotype, and synaptic accumulation of calcium-permeable AMPA receptors. Alterations in cortical GABAergic networks have been repeatedly associated with schizophrenia. We found that functional GABAergic transmission was reduced in C4-OE mice, in line with diminished GABA release probability from parvalbumin interneurons, lower GAD67 expression, and decreased intrinsic excitability in parvalbumin interneurons. These cellular abnormalities were associated with working memory impairment. Our results substantiate the causal relationship between an immunogenetic risk factor and several distinct cortical endophenotypes of schizophrenia and shed light on the underlying cellular mechanisms.
Mol Psychiatry, 2021; 26

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BOARD NUMBER: S03-465

RESPONSES TO ACOUSTIC STIMULI IN THE VENTRAL TEGMENTAL AREA OF FREELY-MOVING MICE

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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In humans, the neuronal substrates of pleasure-inducing effects of music were described as an interaction between ancient reward mechanisms, believed to be related to dopamine release, and cortical systems which are highly modifiable by individual experience and culture. However, little is known about the encoding of both simple and complex auditory stimuli in the reward system in rodents. Here, we recorded the neuronal activity by using fiber photometry in an important relay of the reward system, the ventral tegmental area (VTA), in freely-moving mice. We compared these responses to the well-understood responses in auditory cortex in anesthetized mice. We compared the temporal patterns of calcium responses in both structures at the presentation of broadband noise, pure tones and music excerpts. We found that VTA neurons responded to all acoustic stimuli but with clear differences compared to auditory cortical responses. In response to broadband noise and pure tones, VTA responses seemed to have somewhat longer latencies than A1 responses. Moreover, A1 responses were often biphasic, with a short-latency component that corresponds to the onset responses in A1 and a later component that peaks a few hundreds of ms after sound onset. In contrast, the responses in VTA consisted of a single component whose duration was significantly longer than the early responses in A1. These data indicate that non-music acoustic stimuli can activate the reward system in awake mice but with different temporal properties compared to the auditory cortex.

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BOARD NUMBER: S03-466

UNRAVELLING THE FUNCTIONAL HETEROGENEITY OF RIBBON SYNAPSES FROM COCHLEAR INNER HAIR CELLS

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Synaptic heterogeneity can expand the encoding capacity of individual neurons or sensory cells. In the auditory system, the ribbon synapses between cochlear inner hair cells (IHCs) and spiral ganglion neurons (SGNs) exhibit a high degree of heterogeneity. At the presynaptic level, one individual IHC contains 10 to 30 active zones that differ in their size as well as in the number and function of Ca^{2+} channels. This presynaptic heterogeneity follows a particular gradient along the neural/abneural axis of the IHC. Synapses from the neural side have larger active zones and Ca^{2+} channel clusters, yet require stronger depolarizations to elicit Ca^{2+} influx. At the postsynaptic level, SGNs exhibit differences in their spontaneous activity, spike rate adaptation and operating range. However, the connection between presynaptic IHC's heterogeneity and postsynaptic SGN's response diversity has remained elusive. Here, we studied the *synaptic* heterogeneity of murine IHC ribbon synapses using paired pre- and postsynaptic bouton recordings at near physiological conditions. Our results emphasize that IHC synapses have differences in the spontaneous rate (SR) of release that depend on the topographical position of the synapses. High SR synapses were located predominantly at the abneural side of the IHC and displayed spontaneous EPSCs of higher amplitude and more compact waveform. High SR synapses also had lower thresholds for evoked neurotransmitter release, shorter response latencies and stronger initial release rates. This study reveals that IHCs employ heterogeneous synapses to provide different input into SGNs that collectively code the wide range of audible sound intensities.

Pubmed:
30733243: Kroll J, Jaime Tobón LM, Vogl C, Neef J, Kondratiuk I, König M, Strenzke N, Wichmann C, Milosevic I, Moser T
Endophilin-A regulates presynaptic Ca influx and synaptic vesicle recycling in auditory hair cells.
Ribbon synapses of cochlear inner hair cells (IHCs) operate with high rates of neurotransmission; yet, the molecular regulation of synaptic vesicle (SV) recycling at these synapses remains poorly understood. Here, we studied the role of endophilins-A1-3, endocytic adaptors with curvature-sensing and curvature-generating properties, in mouse IHCs. Single-cell RT-PCR indicated the expression of endophilins-A1-3 in IHCs, and immunoblotting confirmed the presence of endophilin-A1 and endophilin-A2 in the cochlea. Patch-clamp recordings from endophilin-A-deficient IHCs revealed a reduction of Ca influx and exocytosis, which we attribute to a decreased abundance of presynaptic Ca channels and impaired SV replenishment. Slow endocytic membrane retrieval, thought to reflect clathrin-mediated endocytosis, was impaired. Otoferlin, essential for IHC exocytosis, co-immunoprecipitated with purified endophilin-A1 protein, suggestive of a molecular interaction that might aid exocytosis-endocytosis coupling. Electron microscopy revealed lower SV numbers, but an increased occurrence of coated structures and endosome-like vacuoles at IHC active zones. In summary, endophilins regulate Ca influx and promote SV recycling in IHCs, likely via coupling exocytosis to endocytosis, and contributing to membrane retrieval and SV reformation.
EMBO J, 2019; 38
29328020: Jean P, Lopez de la Morena D, Michanski S, Jaime Tobón LM, Chakrabarti R, Picher MM, Neef J, Jung S, Gültas M, Maxeiner S, Neef A, Wichmann C, Strenzke N, Grabner C, Moser T
The synaptic ribbon is critical for sound encoding at high rates and with temporal precision.
We studied the role of the synaptic ribbon for sound encoding at the synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) in mice lacking RIBEYE (RBE). Electron and immunofluorescence microscopy revealed a lack of synaptic ribbons and an assembly of several small active zones (AZs) at each synaptic contact. Spontaneous and sound-

evoked firing rates of SGNs and their compound action potential were reduced, indicating impaired transmission at ribbonless IHC-SGN synapses. The temporal precision of sound encoding was impaired and the recovery of SGN-firing from adaptation indicated slowed synaptic vesicle (SV) replenishment. Activation of Ca-channels was shifted to more depolarized potentials and exocytosis was reduced for weak depolarizations. Presynaptic Ca-signals showed a broader spread, compatible with the altered Ca-channel clustering observed by super-resolution immunofluorescence microscopy. We postulate that RIBEYE disruption is partially compensated by multi-AZ organization. The remaining synaptic deficit indicates ribbon function in SV-replenishment and Ca-channel regulation.
Elife, 2018; 7

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OPTIMIZING STIMULUS PARAMETERS FOR ULTRAFAST COCHLEAR OPTOGENETIC ENCODING

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Spatially confined optogenetic stimulation of the spiral ganglion neurons (SGNs) represents a prospective alternative to electrical stimulation currently used in cochlear implants (CI) and opens up new perspective to study neural networks in the auditory pathway. Identifying the optimal stimulation parameters (light pulse duration and repetition rate) is critical to optogenetically control the SGNs firing, to develop the sound coding strategies of the future optical cochlear implant, and to computationally model the biophysical parameters of optogenetically driven SGN firing *in vivo*. Those parameters can be empirically measured by *in vivo* single SGN juxta-cellular recordings in response to optical stimulation delivered into the cochlea. Nonetheless, those recordings are limited in time and, hence, measuring all combinations of interests per SGN using deterministic stimulus is challenging. Therefore, we developed and validated an optical stochastic stimulus paradigm containing the combination of pulse duration and repetition rate of interest. The validation was done by recording *in vivo* from mice single SGNs expressing targeting optimized Chronos (Keppeler et al., 2018) and transduced using a viral approach (AAV-PHP.B, human synapsin promoter, titer = 3.3-8.4 x 10^{12} GC/ml, early postnatal injection). This stimulus paradigm allowed us to identify optimal illumination parameters of SGNs in minutes. Our results will be used to analyze neural network function in the auditory brainstem and to design coding strategies for the future optical CI.

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EVOLUTIONARY DEVELOPMENT OF EXCITATORY PROJECTION NEURONS IN MAMMALIAN SOUND LOCALIZATION CIRCUITS

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Three neuronal populations in the mammalian auditory brainstem enable sound localization: the Dorsal Cochlear Nucleus, the Lateral Superior Olive and the Medial Superior Olive. Fusiform VGluT2-positive excitatory neurons make up the major projection neurons in these populations and simultaneously emerge from the rhombic lip of rhombomere 5 at embryonic day 10. This study aims to determine the evolutionary and developmental relationship between these subpopulations. By single nucleus mRNA sequencing of these Vglut2-positive cells the underlying gene regulatory networks are to be reconstructed. To ensure unambiguous identification of the cells of interest the *VGluT2::Cre;H2BmCherry* mouse line is utilized which expresses the fluorescent histone-bound protein *H2BmCherry* specifically in all VGluT2-positive cells. Starting with established, publicly available protocols the procedures were adjusted and scaled to suit our particular needs and to obtain high quality single nuclei of all three neuronal subpopulations. Many steps of the initial sample processing were adjusted to gain a higher yield or a better quality of nuclei from fresh, unfixed mouse brain tissue; from the preparation of the tissue to dissection, digestion, isolation and storage of the single nuclei towards final protocol modifications to fit size and age of the input material. At this step of the project I conclude that murine brain tissue is best stored in a high-salt solution at 4°C for a maximum of two days. Further, gradient centrifugation gently and efficiently separates nuclei from debris. Any alteration of the centrifugation times or -forces during these steps can cause severe loss or damage to the sample.

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THE INTERPLAY BETWEEN SENSORY AND COGNITIVE NEURODEGENERATION: COCHLEAR VULNERABILITY TO NOISE EXPOSURE IN A MODEL OF ALZHEIMER'S DISEASE.

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Aim: Recent epidemiological evidence suggests a strong association between hearing loss and cognitive decline with increased risk for the onset of neurodegenerative disorders, including Alzheimer's disease (AD). Several hypotheses have been proposed to explain the relationship between auditory deprivation and cognitive impairment, but such association remains still controversial. We previously reported that noise exposure can accelerate cognitive decline in an AD animal model (3×Tg-AD mice). Here, we wondered if cochlear structures in AD mice could be most vulnerable to noise-induced hearing loss. **Methods:** Wild type (WT) and 3×Tg-AD mice were exposed to noise (100 dB, 10 kHz, 60 min/day, 10 days) at 2 months of age (M), before the cognitive phenotype is manifested. Functional and morphological evaluations were performed to assess auditory threshold and cochlear structure alterations (hair cells, spiral ganglion neurons, afferent nerve fibers). Analyses of AD hallmarks (increased Tau phosphorylation, inflammatory and oxidative stress makers) were carried out. **Results:** Morphological analyses showed a significant neuronal damage (reduction of spiral ganglion neurons and afferent fibers) in noise-exposed AD mice. Western Blot results displayed an increase of phosphorylation levels of Tau (ser396) following noise, probably mediated by Cdk5 up-regulation. Also, an increase of oxidative stress and inflammatory markers expression (TNFα and NF-κB) confirmed the high susceptibility of cochlear tissue to noise-insult in the neurodegenerative model. **Conclusion:** Our findings demonstrates that 3×Tg-AD mice are more vulnerable to cochlear damage induced by noise exposure, and supports the hypothesis that hearing loss can affect both sensory and cognitive neurodegenerative processes, exacerbating AD pathology.

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BOARD NUMBER: S03-470

ROLE OF THE FERRITIN LIGHT-CHAIN IN THE LOGIC OF SOUND CODING

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Hearing relies on the recruitment of two populations of cochlear sensory cells, the outer hair cells (OHCs) and the inner hair cells (IHCs). While the pool of outer hair cells amplifies incoming sound-stimulation to achieve exquisite frequency selectivity, the inner hair cells transduce the mechanical pressure into release of glutamate onto the postsynaptic receptors of the auditory nerve fibers. Hair cells active zones harbor a synaptic ribbon, an electron-dense organelle surrounded by a monolayer of glutamate-filled vesicles. Using yeast-two hybrid assay, we isolated the ferritin light-chain (FTL1), known to store intracellular iron, as an interacting partner of RIBEYE, the major component of the ribbon body. Here, our objective is to probe the function of FTL1 in the logic of sound coding. In the FTL1 knock-out mouse, we found-out an auditory threshold shift in 20% of the homozygous mice associated with the loss of the distortion product of otoacoustic emissions, as a proxy of the OHC's activity. Consistently, light and electron microscopy show a progressive massive degeneration of the OHCs. In addition, we found-out splayed hair bundle in IHCs in the fraction of $FTL1^{-/-}$ with threshold shift. However, the loss of FTL1 did not change the number and size of the hair cell's synaptic ribbons. We propose that ferritin may protect up to some extent the hair cells against iron-induced oxidative stress. Moreover, the interaction between FTL1 and RIBEYE might suggest that ribbon synapse is vulnerable to free radicals.

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BOARD NUMBER: S03-471

KV4.2 KNOCKOUT MICE HAVE INCREASED AUDITORY SUPRATHRESHOLD RESPONSES BUT POOR GAP DETECTION

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Aims. Type I spiral ganglion neurons transmit acoustic information from cochlear inner hair cells to the auditory brainstem. An important regulator of their function are potassium channels. We set out to determine the influence of the potassium channel Kv4.2 on hearing, susceptibility to noise-induced hearing loss, and auditory temporal processing using measurements of auditory brainstem responses and gap detection in Kv4.2 knockout (KO) and wildtype (WT) animals. Methods. Auditory brainstem responses (ABR) were collected from C57BL/6 and Kcnd2-/- animals at baseline (6 weeks old) and one week following acoustic overexposure. Auditory temporal acuity was assessed by gap prepulse inhibition of the acoustic startle reflex (GPIAS). Results. KO animals showed greater wave I amplitudes at most tested frequencies. Following acoustic overexposure, KO animals lost these advantages and showed no difference in either thresholds or wave I amplitudes from WT animals. Preliminary GPIAS results indicate poor temporal acuity in Kv4.2 KO animals based on an inability to supress startle responses by silent gaps, in spite of otherwise normal prepulse inhibition. Conclusion. The lack of Kv4.2 leads to increased wave I suprathreshold responses without greatly affecting auditory thresholds, presumably as a result of spiral ganglion neuron hyperexcitability. This increased excitability rendered KO animals more susceptible to noise-induced hearing loss. However, the increased suprathreshold responses did not lead to improved temporal acuity; in contrary, KO animals appear to have poor gap detection. Further experiments should resolve whether this deficit is arising from peripheral or central hyperexcitability.

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BOARD NUMBER: S03-471a

FUNCTIONAL CHARACTERIZATION OF THE BASAL COCHLEAR INNER HAIR CELLS UPON NOISE EXPOSURE

POSTER SESSION 03 - SECTION: COCHLEAR & SUBCORTICAL AUDITORY PROCESSING

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Acoustic overexposures are the primary cause of hearing loss nowadays. In animal models, histological analysis of the cochlea after exposure to acoustic trauma revealed damages of cellular structures, such as stereocilia and ribbon synapses of the inner hair cells (IHCs).
Unlike the extensive knowledge on the anatomical aspects, much less is known about the noise-induced functional changes on the IHC ribbon synapses and synaptic transmission to the spiral ganglion neurons (SGNs). Despite the basal region of the cochlea being recognized as the most vulnerable to noise overexposure, the only data on the effects of noise exposure on the IHC synaptic function so far come from patch-clamp recordings of the apical end of the cochlea.
Here we show for the first time patch-clamp recordings of basal IHCs (bIHCs) after noise trauma protocols that caused a permanent threshold shift (PTS) of the auditory brainstem response (ABR). Anaesthetized mice were exposed to 8-16 kHz noise at different sound pressure levels and used for both patch-clamp recordings and immunohistochemistry of ribbon synapses immediately after and two weeks after exposure. While significant increase in ABR thresholds was observed after exposure across all tested noise intensities and lasted for at least 2 weeks afterwards, this was accompanied by no IHC synaptic loss. In line with this notion, no functional alterations have been found either in the Ca^{2+} -dependent exocytosis of bIHCs or in the voltage-dependent whole-cell calcium influx. Further experiments are needed to better investigate the functional properties of the bIHCs synapses after noise trauma.

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BOARD NUMBER: S03-472

INFORMATION CONTENT AND ROUTING OF VISUOMOTOR SIGNALS IN THE CORTEX

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Flexible sensorimotor transformations are a hallmark of complex cognitive behaviors. It remains unclear if specific sensory information gets routed to motor areas of the cortex, or if motor areas dispense with detailed sensory representations. By simultaneously measuring neural activity in both sensory and premotor areas during a flexible sensorimotor transformation, we can begin to identify principles governing the information content and routing of signals relevant for sensorimotor behaviors. We developed a novel behavioral task where head-fixed mice report detection of different visual stimuli with different actions (N = 5 mice). Mice licked left to report stimuli detected in the central (binocular) visual field, and licked right when detecting stimuli in the right (monocular) visual field. Stimuli were presented in blocks of 15-35 trials in the same location before switching. Behavioral performance varied psychometrically with contrast at each stimulus location. We next performed acute recordings with Neuropixels probes in primary visual cortex (V1) and anterolateral motor cortex (ALM). Peristimulus time histograms from both areas showed clear short-latency (<0.2 s) effects of stimulus location and trial outcome (hit vs miss). To assess the specificity of visual information routed to ALM, we leveraged simultaneous recordings from V1 and ALM. ALM and V1 neurons whose stimulus preference matched showed stronger and more temporally reliable [HB1] pairwise correlations than ALM and V1 neurons with opposite stimulus preferences. These trial-by-trial relationships exhibited both location and visual contrast specificity[HB2] . We are currently investigating whether these functional relationships flexibly change in the context of updated stimulus-reward contingencies.

Pubmed:
34446296: Speed A, Haider B
Probing mechanisms of visual spatial attention in mice.
The role of spatial attention for visual perception has been thoroughly studied in primates, but less so in mice. Several behavioral tasks in mice reveal spatial attentional effects, with similarities to observations in primates. Pairing these tasks with large-scale, cell-type-specific techniques could enable deeper access to underlying mechanisms, and help define the utility and limitations of resolving attentional effects on visual perception and neural activity in mice. In this Review, we evaluate behavioral and neural evidence for visual spatial attention in mice; assess how specializations of the mouse visual system and behavioral repertoire impact interpretation of spatial attentional effects; and outline how several measurement and manipulation techniques in mice could precisely test and refine models of attentional modulation across scales.
Trends Neurosci, 2021; 44
34314675: Williams B, Del Rosario J, Muzzu T, Peelman K, Coletta S, Bichler EK, Speed A, Meyer-Baese L, Saleem AB, Haider B
Spatial modulation of dark versus bright stimulus responses in the mouse visual system.
A fundamental task of the visual system is to respond to both increases and decreases of luminance with action potentials (ON and OFF responses). OFF responses are stronger, faster, and more salient than ON responses in primary visual cortex (V1) of both cats and primates, but in ferrets and mice, ON responses can be stronger, weaker, or balanced in comparison to OFF responses. These discrepancies could arise from differences in species, experimental techniques, or stimulus properties, particularly retinotopic location in the visual field, as has been speculated; however, the role of retinotopy for ON/OFF dominance has not been systematically tested across multiple scales of neural activity within species. Here, we measured OFF versus ON responses across large portions of visual space with silicon probe and whole-cell patch-clamp recordings in mouse V1 and lateral geniculate nucleus (LGN). We found that OFF responses dominated in the central visual field, whereas ON and OFF responses were more balanced in the periphery. These findings were consistent across local field potential (LFP), spikes, and subthreshold membrane potential in V1, and were aligned with spatial biases in ON and OFF responses in LGN. Our findings reveal that retinotopy may provide a common organizing principle for spatial modulation of

OFF versus ON processing in mammalian visual systems.
Curr Biol, 2021; 31
33677512: Del Rosario J, Speed A, Arrowood H, Motz C, Pardue M, Haider B
Diminished Cortical Excitation and Elevated Inhibition During Perceptual Impairments in a Mouse Model of Autism.
Sensory impairments are a core feature of autism spectrum disorder (ASD). These impairments affect visual perception and have been hypothesized to arise from imbalances in cortical excitatory and inhibitory activity. There is conflicting evidence for this hypothesis from several recent studies of transgenic mouse models of ASD; crucially, none have measured activity from identified excitatory and inhibitory neurons during simultaneous impairments of sensory perception. Here, we directly recorded putative excitatory and inhibitory population spiking in primary visual cortex (V1) while simultaneously measuring visual perceptual behavior in CNTNAP2-/- knockout (KO) mice. We observed quantitative impairments in the speed, accuracy, and contrast sensitivity of visual perception in KO mice. During these perceptual impairments, stimuli evoked more firing of inhibitory neurons and less firing of excitatory neurons, with reduced neural sensitivity to contrast. In addition, pervasive 3-10 Hz oscillations in superficial cortical layers 2/3 (L2/3) of KO mice degraded predictions of behavioral performance from neural activity. Our findings show that perceptual deficits relevant to ASD may be associated with elevated cortical inhibitory activity along with diminished and aberrant excitatory population activity in L2/3, a major source of feedforward projections to higher cortical regions.
Cereb Cortex, 2021; 31
31980628: Speed A, Del Rosario J, Mikail N, Haider B
Spatial attention enhances network, cellular and subthreshold responses in mouse visual cortex.
Internal brain states strongly modulate sensory processing during behaviour. Studies of visual processing in primates show that attention to space selectively improves behavioural and neural responses to stimuli at the attended locations. Here we develop a visual spatial task for mice that elicits behavioural improvements consistent with the effects of spatial attention, and simultaneously measure network, cellular, and subthreshold activity in primary visual cortex. During trial-by-trial behavioural improvements, local field potential (LFP) responses to stimuli detected inside the receptive field (RF) strengthen. Moreover, detection inside the RF selectively enhances excitatory and inhibitory neuron responses to task-irrelevant stimuli and suppresses noise correlations and low frequency LFP fluctuations. Whole-cell patch-clamp recordings reveal that detection inside the RF increases synaptic activity that depolarizes membrane potential responses at the behaviorally relevant location. Our study establishes that mice display fundamental signatures of visual spatial attention spanning behavioral, network, cellular, and synaptic levels, providing new insight into rapid cognitive enhancement of sensory signals in visual cortex.
Nat Commun, 2020; 11
30865879: Speed A, Del Rosario J, Burgess CP, Haider B
Cortical State Fluctuations across Layers of V1 during Visual Spatial Perception.
Many factors modulate the state of cortical activity, but the importance of cortical state variability for sensory perception remains debated. We trained mice to detect spatially localized visual stimuli and simultaneously measured local field potentials and excitatory and inhibitory neuron populations across layers of primary visual cortex (V1). Cortical states with low spontaneous firing and correlations in excitatory neurons, and suppression of 3- to 7-Hz oscillations in layer 4, accurately predicted single-trial visual detection. Our results show that cortical states exert strong effects at the initial stage of cortical processing in V1 and can play a prominent role for visual spatial behavior in mice.
Cell Rep, 2019; 26
30256741: Williams B, Speed A, Haider B
A novel device for real-time measurement and manipulation of licking behavior in head-fixed mice.
The mouse has become an influential model system for investigating the mammalian nervous system. Technologies in mice enable recording and manipulation of neural circuits during tasks where they respond to sensory stimuli by licking for liquid rewards. Precise monitoring of licking during these tasks provides an accessible metric of sensory-motor processing, particularly when combined with simultaneous neural recordings. There are several challenges in designing and implementing lick detectors during head-fixed neurophysiological experiments in mice. First, mice are small, and licking behaviors are easily perturbed or biased by large sensors. Second, neural recordings during licking are highly sensitive to electrical contact artifacts. Third, submillisecond lick detection latencies are required to generate control signals that manipulate neural activity at appropriate time scales. Here we designed, characterized, and implemented a contactless dual-port device that precisely measures directional licking in head-fixed mice performing visual behavior. We first determined the optimal characteristics of our detector through design iteration and then quantified device performance under ideal conditions. We then tested performance during head-fixed mouse behavior with simultaneous neural recordings in vivo. We finally demonstrate our device's ability to detect directional licks and generate appropriate control signals in real time to rapidly suppress licking behavior via closed-loop inhibition of neural activity. Our dual-port detector is cost effective and easily replicable, and it should enable a wide variety of applications probing the neural circuit basis of sensory perception, motor

action, and learning in normal and transgenic mouse models. NEW & NOTEWORTHY Mice readily learn tasks in which they respond to sensory cues by licking for liquid rewards; tasks that involve multiple licking responses allow study of neural circuits underlying decision making and sensory-motor integration. Here we design, characterize, and implement a novel dual-port lick detector that precisely measures directional licking in head-fixed mice performing visual behavior, enabling simultaneous neural recording and closed-loop manipulation of licking.
J Neurophysiol, 2018; 120

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BOARD NUMBER: S03-473

REORGANIZATION OF CENTRAL VISUAL POPULATION RECEPTIVE FIELDS IN RESPONSE TO AN INHALED PSYCHEDELIC TRYPTAMINE

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Introduction: Dimetiltriptamina (DMT) is an indole alkaloid widely found in nature, best known by its presence in ayahuasca. It is a psychedelic tryptamine associated with intense visual hallucinatory phenomena, perceptual changes and profound spiritual experiences. **Aims:** In this work we questioned if populations of visual neurons do reorganize along eccentricity in response to inhaled DMT. **Methods:** In order to study population receptive field (pRF) properties of visual neurons, whose alterations can be indicative of visual neuronal reorganization in response to inhaled DMT, a cross-sectional study investigating control and DMT condition was conducted at ICNAS/CIBIT. We acquired anatomical and functional MRI data from two groups of individuals 14 naïve for the control condition and 7 DMT experienced individuals for the active condition. Experienced individuals inhaled DMT immediately before acquisition. We used a visual technique called population receptive field (pRF) mapping, an optimized alternative to traditional retinotopy, to estimate the average sizes of pRF in V1 visual area along eccentricity for each participant. **Results:** Since normality assumption was not assured we performed the non parametric Mann-whitney U test. We found statistically significant differences for the central bins of eccentricity, between 0º and 5º (see Table 1 for details). **Discussion and Conclusion:** The average pRF size of V1 was statistically significantly higher for the central bins of eccentricity in active condition (inhaled DMT) which suggests that there is a early level visual reorganization due to inhaled DMT and this reorganization differs for central and peripheral locations.

Table 1 *- Test Statistics (group variable: naive vs DMT).*

	0º and 5º of eccentricity (central)	5º and 10º of eccentricity (peripheral)
Mann-Whitney U	10	33
Wilcoxon W	115	138
Z	-2,910	-1,194
Asymp. Sig. (2-tailed)	0,004	0,233
Exact Sig. [2*(1-tailed Sig.)]	0,002[b]	0,255[b]

[b]Not corrected for ties.

Pubmed:
34658876: Castelhano J, Lima G, Teixeira M, Soares C, Pais M, Castelo-Branco M
The Effects of Tryptamine Psychedelics in the Brain: A meta-Analysis of Functional and Review of Molecular Imaging

Studies.
There is an increasing interest in the neural effects of psychoactive drugs, in particular tryptamine psychedelics, which has been incremented by the proposal that they have potential therapeutic benefits, based on their molecular mimicry of serotonin. It is widely believed that they act mainly through 5HT2A receptors but their effects on neural activation of distinct brain systems are not fully understood. We performed a quantitative meta-analysis of brain imaging studies to investigate the effects of substances within this class (e.g., LSD, Psilocybin, DMT, Ayahuasca) in the brain from a molecular and functional point of view. We investigated the question whether the changes in activation patterns and connectivity map into regions with larger 5HT1A/5HT2A receptor binding, as expected from indolaemine hallucinogens (in spite of the often reported emphasis only on 5HT2AR). We did indeed find that regions with changed connectivity and/or activation patterns match regions with high density of 5HT2A receptors, namely visual BA19, visual fusiform regions in BA37, dorsal anterior and posterior cingulate cortex, medial prefrontal cortex, and regions involved in theory of mind such as the surpramarginal gyrus, and temporal cortex (rich in 5HT1A receptors). However, we also found relevant patterns in other brain regions such as dorsolateral prefrontal cortex. Moreover, many of the above-mentioned regions also have a significant density of both 5HT1A/5HT2A receptors, and available PET studies on the effects of psychedelics on receptor occupancy are still quite scarce, precluding a metanalytic approach. Finally, we found a robust neuromodulatory effect in the right amygdala. In sum, the available evidence points towards strong neuromodulatory effects of tryptamine psychedelics in key brain regions involved in mental imagery, theory of mind and affective regulation, pointing to potential therapeutic applications of this class of substances.
Front Pharmacol, 2021; 12

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THE ORIENTATION COLUMN AS A WHOLE PROCESSING UNIT : A FALSE IDEA ?

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Numerous studies have shown that the cat's secondary visual cortex (V2) is functionally organized, following an orientation column structure similar to V1. This structure is characterized by a uniform orientation selectivity throughout cortical layers, where supragranular and infragranular neurons share the same preferred orientation. This organization relies heavily on the existing functional connections between superficial and deep layers within V2, with the emergence of a stimulus-evoked connectome. This interlayer connectome is the basis of the orientation column, as communications between layers play a large role in unifying the tuning properties. Previous investigations in our lab have shown that the neurons' orientation tuning can be changed using visual adaptation, with a differential effect between superficial and deep layers of V2 (Lussiez, 2020). Moreover, it has been demonstrated that connectomes can change depending on the presented stimulus (Bharmauria,2015). Using electrophysiological recordings in V2, this study aims to observe the effects of adaptation on stimulus-evoked connectomes within and between cortical layers. Our study shows that before adaptation, functional connections between supragranular and infragranular layers are largely dominant compared to intralayer connections, in perfect accordance with the orientation column concept. However, as neurons have changed their orientation preference after adaptation, interlayers connections are greatly reduced, to the benefice of intralayer communication. We hypothesize that while the orientation column may function as a whole processing unit in control conditions, it is comprised of two different « tissues » that can function independently after adaptation, and consequently, it seems that adaptation ruptures the orientation column.

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BOARD NUMBER: S03-475

BEHAVIOURAL AND IN VITRO STUDIES ON PROCESSING OF BISTABLE STIMULI IN THE MOUSE VISUAL SYSTEM

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Visual decision mechanisms are often studied using bistable stimuli. In a bistable visual stimulus the evidence for one visual percept is as strong as the evidence for the other percept, but only one of the percepts appears. We studied the basic mechanisms how the brain decides what percept arises. In behavioural experiments we placed mice head-fixed on an air-floating ball at the center of a large dome on the inside of which we projected moving random dot patterns for two seconds. Rightward or leftward moving random dot patterns causes the mice to change their running direction in the direction of the moving pattern. We investigated the behavioural response under conditions with motion patterns in two different motion directions at the same time (’transparency”) that evoke bistable perception. The results show that the mice follow the average motion directions. In an in-vitro set-up we further investigated the fundamental mechanisms of bistable perception. We developed a “hybrid” system where two pyramidal neurons in a mouse visual cortical brain slice interact through a computer-simulated mutual inhibition circuit. Simultaneous activation of the mutually inhibiting pyramidal neurons leads to bi-stable activity. We show that this in-vitro circuit exhibits characteristics strikingly similar to the properties of bistable visual perception.

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BOARD NUMBER: S03-476

KETAMINE AFFECTS ADAPTATION-INDUCED CHANGES IN PREFERRED ORIENTATION AND NETWORK DYNAMICS

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Ketamine affects adaptation-induced changes in preferred orientation and network dynamics Ouelhazi Afef, Lussiez Rudy, Molotchnikoff Stéphane **Abstract** Primary visual cortex features a well demonstrated selectivity by virtue of which neurons respond maximally to specific attributes of stimulus characteristics. However, the imposition of a non-preferred stimulus for many minutes (visual adaptation), or the application of an antidepressant, such as ketamine, shifts the preferred orientation. That is, the neuron acquires a new orientation selectivity. The mechanism of the change of stimulus selectivity is not yet ascertained. This investigation explores, in mouse and cat, the modification of orientation selectivities and its outcome on correlations between neurons. Electrophysiological recordings of monocular stimulations in control, post-adaptation, and post-ketamine conditions revealed that ketamine impacts post-adaptation effects by altering neuronal tuning curves and segregating them into two neuronal groups with distinct effects: it facilitates recovery in cells exhibiting large shifts after adaptation, whereas in units displaying small shifts, the drug potentiates the shifts. Moreover, pair-wise cross correlogram analyses show that ketamine disrupts post-adaptation neuronal circuits, potentiating functional connectivity between neurons in cats, but not mice. In cat, ketamine significantly increases the number of connections and their strengths and enhances neuronal synchrony. We conclude that ketamine affects adaptation-induced changes in preferred orientation and network correlations by reorganizing pre-existing neuronal networks.

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BOARD NUMBER: S03-477

INVOLVEMENT OF THE TRANSCRIPTION FACTOR REST IN VISUAL CORTEX PLASTICITY

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Aims: Repressor Element 1-Silencing Transcription Factor (REST) regulates the expression of a wide array of neuronal genes implicated in differentiation, development and plasticity. We set out to investigate the effect of REST inhibition on primary visual cortex (V1b) plasticity in the adult mouse. **Methods:** Employing the monocular deprivation paradigm in a $REST^{lox/lox}$ conditional knockout (cKO) model, we studied the effects of stereotaxically delivering Cre recombinase into V1b via adeno-associated virus vectors (AAVs) on ocular dominance plasticity *in vivo.* To this end, we recorded visual evoked potentials (VEPs) with chronic multi-electrode implants in the awake animal, vision-correlated behavior via the optomotor response (OMR) and molecular changes in *ex vivo* brain tissue with immunofluorescence and RT-PCR. We also employed these techniques in wild type C57BL/6 mice to study the effects of engineered chimeric REST inhibitors based on the photo-activated *Avena sativa* light-oxygen-voltage-sensing 2-phototrophin 1 (*As*LOV2). **Results:** Electrophysiology shows that 3 days of monocular deprivation in REST-inhibited animals produce a significant shift in ocular dominance towards the non-deprived eye in the V1b, mirrored by a lowered OMR score. RT-PCR and immunofluorescence demonstrate a concurrent increase in the expression of plasticity-related genes BDNF, Nav1.2, SYN1 and NPAS4, normally targeted by REST repression. **Conclusions:** Our data demonstrates that inhibition of REST activity *in vivo* can result in reactivation of ocular dominance plasticity in the adult visual cortex after the end of the critical period, paving the way for new therapeutic strategies for the correction of amblyopia in adults.

Pubmed:
28250420: Maya-Vetencourt JF, Ghezzi D, Antognazza MR, Colombo E, Mete M, Feyen P, Desii A, Buschiazzo A, Di Paolo M, Di Marco S, Ticconi F, Emionite L, Shmal D, Marini C, Donelli I, Freddi G, Maccarone R, Bisti S, Sambuceti G, Pertile G, Lanzani G, Benfenati F
A fully organic retinal prosthesis restores vision in a rat model of degenerative blindness.
The degeneration of photoreceptors in the retina is one of the major causes of adult blindness in humans. Unfortunately, no effective clinical treatments exist for the majority of retinal degenerative disorders. Here we report on the fabrication and functional validation of a fully organic prosthesis for long-term in vivo subretinal implantation in the eye of Royal College of Surgeons rats, a widely recognized model of retinitis pigmentosa. Electrophysiological and behavioural analyses reveal a prosthesis-dependent recovery of light sensitivity and visual acuity that persists up to 6-10 months after surgery. The rescue of the visual function is accompanied by an increase in the basal metabolic activity of the primary visual cortex, as demonstrated by positron emission tomography imaging. Our results highlight the possibility of developing a new generation of fully organic, highly biocompatible and functionally autonomous photovoltaic prostheses for subretinal implants to treat degenerative blindness.
Nat Mater, 2017; 16
32601447: Maya-Vetencourt JF, Manfredi G, Mete M, Colombo E, Bramini M, Di Marco S, Shmal D, Mantero G, Dipalo M, Rocchi A, DiFrancesco ML, Papaleo ED, Russo A, Barsotti J, Eleftheriou C, Di Maria F, Cossu V, Piazza F, Emionite L, Ticconi F, Marini C, Sambuceti G, Pertile G, Lanzani G, Benfenati F
Subretinally injected semiconducting polymer nanoparticles rescue vision in a rat model of retinal dystrophy.
Inherited retinal dystrophies and late-stage age-related macular degeneration, for which treatments remain limited, are among the most prevalent causes of legal blindness. Retinal prostheses have been developed to stimulate the inner retinal network; however, lack of sensitivity and resolution, and the need for wiring or external cameras, have limited their application. Here we show that conjugated polymer nanoparticles (P3HT NPs) mediate light-evoked stimulation of retinal

neurons and persistently rescue visual functions when subretinally injected in a rat model of retinitis pigmentosa. P3HT NPs spread out over the entire subretinal space and promote light-dependent activation of spared inner retinal neurons, recovering subcortical, cortical and behavioural visual responses in the absence of trophic effects or retinal inflammation. By conferring sustained light sensitivity to degenerate retinas after a single injection, and with the potential for high spatial resolution, P3HT NPs provide a new avenue in retinal prosthetics with potential applications not only in retinitis pigmentosa, but also in age-related macular degeneration.
Nat Nanotechnol, 2020; 15

32015505: DiFrancesco ML, Lodola F, Colombo E, Maragliano L, Bramini M, Paternò GM, Baldelli P, Serra MD, Lunelli L, Marchioretto M, Grasselli G, Cimò S, Colella L, Fazzi D, Ortica F, Vurro V, Eleftheriou CG, Shmal D, Maya-Vetencourt JF, Bertarelli C, Lanzani G, Benfenati F
Neuronal firing modulation by a membrane-targeted photoswitch.
Optical technologies allowing modulation of neuronal activity at high spatio-temporal resolution are becoming paramount in neuroscience. In this respect, azobenzene-based photoswitches are promising nanoscale tools for neuronal photostimulation. Here we engineered a light-sensitive azobenzene compound (Ziapin2) that stably partitions into the plasma membrane and causes its thinning through trans-dimerization in the dark, resulting in an increased membrane capacitance at steady state. We demonstrated that in neurons loaded with the compound, millisecond pulses of visible light induce a transient hyperpolarization followed by a delayed depolarization that triggers action potential firing. These effects are persistent and can be evoked in vivo up to 7 days, proving the potential of Ziapin2 for the modulation of membrane capacitance in the millisecond timescale, without directly affecting ion channels or local temperature.
Nat Nanotechnol, 2020; 15

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HOW DOES OPTOGENETIC RESTORATION OF RETINAL LIGHT SENSITIVITY AFFECT VISUAL PROCESSING IN MICE?

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Leber congenital amaurosis (LCA) is an inherited retinal disease that causes early-onset blindness in infants and children. A possible strategy to recover vision in late stages of disease is the introduction of light-sensitive channel proteins in bipolar cells to compensate for photoreceptor cell loss. However, it is largely unknown whether the newly-generated signal from light-sensitive bipolar cells is sufficient to re-establish behaviorally relevant visual perception. Therefore, the aim of our study is to assess how intraocular expression of an engineered light-sensitive protein (Opto-mGluR6) affects primary visual cortex function in a mouse model of early-onset retinal degeneration (*Lca5*$^{-/-}$). To answer this objective, three groups of mice (untreated *Lca5*$^{-/-}$, *Lca5*$^{-/-}$ intraocularly treated with Opto-mGluR6 and wild type C57BL/6J mice) were chronically implanted with high-density silicon probes targeting primary visual cortex. Mice were head-fixed and trained to perform a virtual-reality visual discrimination task by running on a spherical treadmill. Local field potentials and single unit responses were recorded throughout task performance. Our initial analyses of behavioral data indicate lower learning performance in treated and untreated *Lca5*$^{-/-}$ mice compared to C57BL/6J control mice. We are currently analyzing the electrophysiological data to assess whether this trend is also reflected in primary visual cortex activity. Overall, we currently do not find recovery of visually-guided behavior in Lca5-/- mice that underwent treatment with an optogenetic construct to produce light-sensitivity in retinal bipolar cells.

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HIGH-DIMENSIONAL GEOMETRY OF POPULATION RESPONSES IN SECONDARY VISUAL CORTEX

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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[Aims] Population responses in the mouse primary visual cortex follow a high-dimensional geometry characterized by a covariance eigenspectrum with a power law coefficient around 1.04. This structure allows codes to be decorrelated, optimizing efficiency while maintaining smoothness (Stringer et al., *Nature* 2019). Is this structure maintained across the visual hierarchy? [Methods] We used 2-photon imaging to record simultaneously from 4000-6000 neurons in visual cortical areas AM and V1 of awake mice on a spherical treadmill. 1800 natural image stimuli were presented twice each. We calculated the eigenspectra of stimulus responses with cross-validated PCA. [Results] Replicating the original finding, the V1 power law coefficient was 1.12 (n=2: 1.09, 1.16). AM population response eigenspectra are also well-approximated by a power law with a coefficient of 1.27 (n=3: 1.30, 1.27, 1.25), higher than in V1 ($P < 0.05$, unpaired t-test). Additionally, the distribution of the fraction of neural variance that is stimulus-related in AM is lower than in V1, with a larger fraction of AM neurons having lower stimulus responsiveness. AM population responses also perform worse at decoding stimulus identity (9 +/- 1% stimulus decoding accuracy) compared to V1 neurons (15 +/- 2%). Furthermore, a larger fraction of neural activity is predictable by behavioral data from face videos of mice in AM than in V1. [Conclusions] Our results suggest less reliable, lower-dimensional population responses to natural images in AM than in V1, possibly due to larger behavioral influences in higher visual areas.

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BOARD NUMBER: S03-480

A NOVEL AND HIGHLY SENSITIVE STATISTICAL TEST FOR CALCIUM IMAGING

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Aims In neurophysiological studies, such as those using calcium imaging data, a standard approach is to test all cells for stimulus responsiveness and include only those that are significantly modulated. We recently proposed a statistical test for spiking data: the ZETA-test. This test outperforms model-based approaches, t-tests, and ANOVAs, but unfortunately can only be applied to point events, such as spike times. Here, we present a new method that solves this drawback: the time-series (TS)-ZETA test. **Methods** We construct a reference time vector over the window of interest, using stochastic across-trial delay variations to achieve a temporal resolution higher than the frame acquisition speed. For each trial, we interpolate the values to this reference vector. We average across all trials, calculate the cumulative sum, and subtract a linear null-hypothesis distribution. We jitter the stimulus onsets and repeat the above procedure to sample null-hypothesis curves. Applying extreme value theory to these samples, we calculate the statistical significance of the observed neuronal response. **Results** We quantified the sensitivity of the TS-ZETA test on V1 neuronal responses to drifting gratings recorded with calcium imaging (fig. 1). We compared this to commonly used approaches, such as t-tests and ANOVAs using an ROC analysis (TS-ZETA AUC=0.946; ANOVA AUC=0.923; T-test AUC=0.841; Mann-Whitney AUC tests, all comparisons, $p<10^{-9}$).

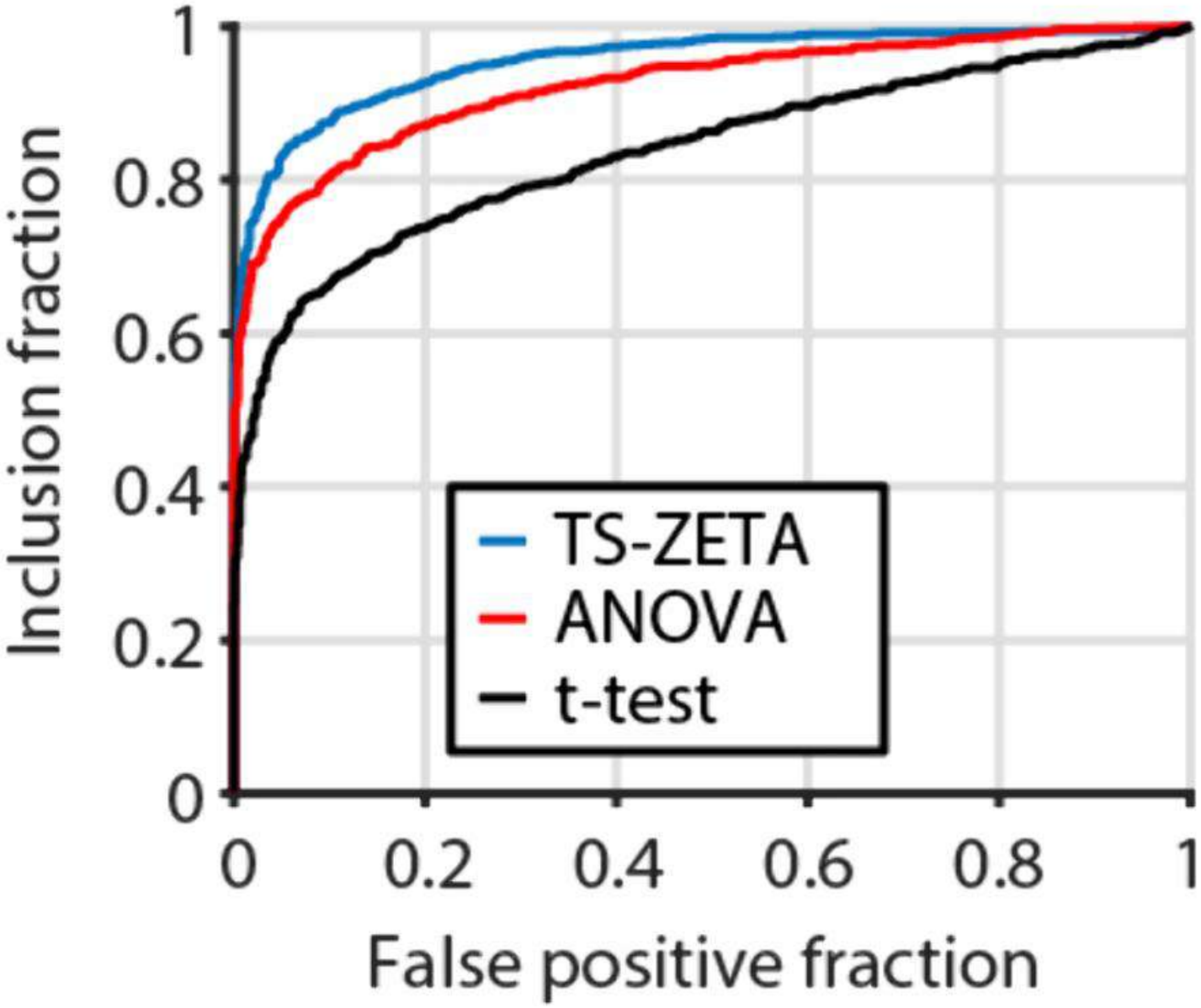

Figure 1. Benchmarking of the performance of TS-ZETA, ANOVA, and t-test on 1055 V1 cells. **Conclusions** The binning-free and parameter-free TS-ZETA test includes more cells than a t-test or ANOVA for similar false positive rates.

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BOARD NUMBER: S03-481

CONTEXTUAL REPRESENTATIONS OF NATURAL SCENES IN MONKEY AND MOUSE V1 NEURONS

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Neuronal activity in the primary visual cortex (V1) is governed by feedforward information processing in each neuron's receptive field (RF) and by contextual information in surrounding regions. Ongoing feedforward responses are rapidly affected by contextual inputs, so it is challenging to dissociate their functional roles. We used full and occluded views of natural scenes to reveal the impact of contextual mechanisms on V1 representations in the absence of information in the RF, as well as the effect of training on the selectivity and response dynamics of V1 neurons. We presented partially occluded and full (non-occluded) natural scenes while recording V1 activity from monkeys using electrophysiology (24 images per condition; 211 sites in two macaques) and from mice using chronic widefield and two-photon calcium imaging (6 images per condition; ~900 neurons in six animals). In mice, V1 activity was recorded both before and after perceptual training to detect 4 full natural scenes. We successfully decoded stimulus identity from V1 activity in both species without visual stimulation in neuronal RFs. In monkeys, decoding was significant in the sustained phase of the response, from ~75ms after stimulus onset. In mice, we found significant decoding both before and after perceptual training (all *p*s < 0.05, permutation test), and training mice on fullscreen images weakened V1 responses to these images but strengthened responses to occluded images. Our results support predictive coding theories of neuronal activity and lay the groundwork for investigating the precise contextual features that drive and modify V1 responses.

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SHAPE- AND MOTION-BASED RESPONSES TO BODIES IN MACAQUE ANTERIOR INFERIOR TEMPORAL CORTEX.

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Aims: Nonverbal social communication relies heavily on visual analysis of body movements. fMRI studies have identified body patches in the macaque inferotemporal (IT) cortex. However, the organization of "dynamic" body patches activated by dynamic bodies, and their feature selectivity at the single-cell level, are unknown. Here we investigated the neural representations underlying the visual processing of dynamic bodies in macaque anterior IT. **Methods**: We used a set of 1 s long videos of dynamic monkey bodies, dynamic monkey faces, and dynamic artificial objects to localize the dynamic body patches with fMRI in two monkeys. We found several dynamic body patches along and ventral to the Superior Temporal Sulcus (STS). Then, we recorded body-selective single cells identified with the same videos used for fMRI mapping in anterior IT, ventral to the STS, in the same two monkeys. **Results**: The majority of neurons responded only during a specific segment of the video and demonstrated high selectivity for different body videos. Most neurons responded similarly to the original videos and a version in which the body was reduced to a silhouette. Many neurons responded to both static snapshots and the original videos, while others only responded to the videos, requiring motion. The latter cells were also sensitive about the order in which video frames were displayed. **Conclusion**: These findings suggest that the majority of anterior IT body-selective cells are shape-selective, and that some of these cells, located ventral to the STS, also encode the snapshot sequence.

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WHEN THE VISUAL CORTEX STOPS CARING ABOUT ORIENTATION: FEATURE AND CATEGORY REPRESENTATION IN V1 DURING ORIENTATION DISCRIMINATION

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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A prerequisite to visual discrimination is that two different sensory stimuli should evoke distinct population activities, or representations. However, the mechanisms by which sensory processing is adapted to a visual discrimination task computational constraints remains poorly understood. Here, we investigated the relationship between orientation discrimination and orientation representations in the primary visual cortex (V1) using calcium imaging in mice performing a Go/NoGo task with oriented drifting gratings throughout six consecutive days. Each day, the orientation of the NoGo stimulus was made closer to the Go orientation (from +90° to +15°), increasing the task's difficulty. As the angle between the Go and NoGo cue decreased, the response profiles in the orientation space stopped corresponding to the presented orientations. NoGo cues activated the same 'NoGo region' of the orientation space day after day, despite the change of presented orientation. Additionally, the NoGo cues activated neurons tuned for the Go orientation, creating a bimodal activation in the orientation space. The ratio between the activation of the Go and NoGo regions of the orientation space was correlated to the behavioral choice probability. Additionally, presenting pure tones simultaneously to the visual stimuli improved the orientation discrimination performance, and changed the Go/NoGo activation ratio accordingly. Our results therefore show that V1 can abandon feature representation in favor of categorical representation (Go or NoGo) to improve discrimination performance. This categorical representation seems to be generated by attractors in the orientation space that are activated by the sensory inputs.

Pubmed:
29354679: Corbo J, Zennou-Azogui Y, Xerri C, Catz N
Cortical Merging in S1 as a Substrate for Tactile Input Grouping.
Perception is a reconstruction process guided by rules based on knowledge about the world. Little is known about the neural implementation of the rules of object formation in the tactile sensory system. When two close tactile stimuli are delivered simultaneously on the skin, subjects feel a unique sensation, spatially centered between the two stimuli. Voltage-sensitive dye imaging (VSDi) and electrophysiological recordings [local field potentials (LFPs) and single units] were used to extract the cortical representation of two-point tactile stimuli in the primary somatosensory cortex of anesthetized Long-Evans rats. Although layer 4 LFP responses to brief costimulation of the distal region of two digits resembled the sum of individual responses, approximately one-third of single units demonstrated merging-compatible changes. In contrast to previous intrinsic optical imaging studies, VSD activations reflecting layer 2/3 activity were centered between the representations of the digits stimulated alone. This merging was found for every tested distance between the stimulated digits. We discuss this laminar difference as evidence that merging occurs through a buildup stream and depends on the superposition of inputs, which increases with successive stages of sensory processing. These findings show that layers 2/3 are involved in the grouping of sensory inputs. This process that could be inscribed in the cortical computing routine and network organization is likely to promote object formation and implement perception rules.
eNeuro, 2018 Jan-Feb; 5
29924709: Corbo J, Caron-Guyon J
Sensory-evoked propagating waves of activity in the primary sensory cortices: poorly understood, yet ubiquitous.
Any sensory stimulus evokes a propagating wave of activity in the corresponding sensory cortex that exceeds its topographical boundaries within the primary sensory map. Hama and colleagues (Hama N, Kawai M, Ito S-I, Hirota A. J Neurophysiol 119: 1934-1946, 2018) provided a first study, in the tactile modality, of the interactions between two successively evoked waves. We argue that the difficulty in finding a simple rule to account for all the various observed interactions calls for an effort to clarify the mechanisms and substrates of the propagating waves and their role in sensory processing.

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J Neurophysiol, 2018; 120
32494803: Caron-Guyon J, Corbo J, Zennou-Azogui Y, Xerri C, Kavounoudias A, Catz N
Neuronal Encoding of Multisensory Motion Features in the Rat Associative Parietal Cortex.
Motion perception is facilitated by the interplay of various sensory channels. In rodents, the cortical areas involved in multisensory motion coding remain to be identified. Using voltage-sensitive-dye imaging, we revealed a visuo-tactile convergent region that anatomically corresponds to the associative parietal cortex (APC). Single unit responses to moving visual gratings or whiskers deflections revealed a specific coding of motion characteristics strikingly found in both sensory modalities. The heteromodality of this region was further supported by a large proportion of bimodal neurons and by a classification procedure revealing that APC carries information about motion features, sensory origin and multisensory direction-congruency. Altogether, the results point to a central role of APC in multisensory integration for motion perception.
Cereb Cortex, 2020; 30

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ONGOING ACTIVATION OF VISUAL CORTEX AND SUPERIOR COLLICULUS IN THE RD10 MOUSE MODEL OF RETINITIS PIGMENTOSA

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Restoring vision in patients suffering from retinal degeneration has been an ongoing effort in the last decades. Retinal implants have been shown to be a promising approach, but the fidelity of evoked visual impression is still very limited and general success is not guaranteed. While technical limitations might be one of the reasons for these ongoing issues, we investigated whether functional changes in visual cortex (V1) e.g., based on cross-modal plasticity upon loss of visual input might lead to an impairment in the processing of retinally evoked stimuli. First, we used optomotor reflex testing to ascertain complete blindness of experimental animals and found that behavioural responses to visual stimulation were completely abolished at up to 30 weeks of age, much later than previously suggested by histological investigations. Next, we performed high-density Neuropixels recordings in V1 and superior colliculus (SC) of awake and behaviourally blind *rd10* mice while presenting auditory, tactile, and visual stimuli. As expected, meaningful responses to visual stimulation were non-existent in V1 as well as SC of *rd10* mice. However, auditory and tactile neuronal responses were either slightly enhanced (SC) or unchanged (V1), showing no evidence for cross-modal plasticity in these visual areas. Furthermore, retinally degenerated mice showed characteristic oscillatory activity in V1 and SC very similar to activity previously found in extracted *rd10* retinae. This pathological input might prevent visual areas from enhancing their responses to other modalities and likely retain their responsivity to stimulation by artificial retinal implants.

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ATTENTION-DEPENDENT MODULATION OF MONKEY V1 EPIDURAL FIELD POTENTIALS IS CLOSELY LINKED TO BEHAVIORAL PERFORMANCE

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Epidural multielectrode arrays constitute a key technique for future clinical applications and offer interesting options for basic science research, because of their reduced invasiveness and high coverage density. Visual epidural field potentials (EFPs) were recently shown to possess high selectivity regarding basic visual stimulus features, but selectivity during cognitive processing has only rarely been studied. We here investigate neural signatures of selective visual attention in EFPs and their correlation to signatures of attention in behavioral data by recording EFPs from monkey primary visual cortex (V1) during a covert-attention task. Monkeys were allowed to respond to a slight orientation change in both the cued and uncued object. A non-binary rewarding scheme (Fischer & Wegener, 2019) was used to support either more distributed or more selective attention. We hypothesized that attentional modulation of the EFP should follow the strength of behavioral effects in the two reward regimes. Reaction time (RT) differences for responses to cued and uncued changes were found to be significantly larger in the regime supporting more selective attention. Accordingly, gamma-band power of EFPs showed an attentional modulation of about 50% with selective attention but only a weak modulation with distributed attention. Attentional modulation was strongest in trials with fast responses as compared to trials with medium or slow responses. The strength of attentional modulation significantly decreased with increasing distance from the attended object, consistent with a spatial attention field. The results show that EFPs from monkey V1 are strongly modulated by attention and closely linked to behavioral performance.

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HISTOLOGICAL VALIDATION OF THE ACCURACY OF DIFFUSION TENSOR IMAGING FOR TRACING FIBRE TRACTS IN MACAQUE EXTRASTRIATE VISUAL CORTEX.

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Diffusion tensor methods in magnetic resonance imaging (DTI) are increasingly used with probabilistic tractography to plot white matter pathways in human and animal brains. Here we compare the use of DTI against histological visualization of neuronal connections with the aim of validating DTI plus tractography against the 'gold standard' methods of neuronal tract tracing. BDA tracer was injected into the left hemispheric extrastriate cortical area V5/MT+ of two anaesthetized rhesus macaque monkeys, for which DTI measurements had been previously made under anaesthesia. The path of the tracer was hand-drawn and the digital output was aligned and overlaid onto the fractional anisotropy (FA) image using FSL software. Tractography was conducted on the FA images of both brains using ProbtrackX from the FSL FDT toolbox starting from seed points at the injection site. Tracer and tractography data were normalised and spatially cross-correlated slice by slice in MATLAB to compare the extent to which DTI reveals brain connectivity between regions compared with histology. The highest correlations were found in the neighbourhood of the injection site. Errors in alignment could be categorised as systematic (revealed by a non-zero peak of the correlogram) or random (revealed by the width of the correlogram), but in addition, there were brain sites where only one of the tracing methods reported any connectivity. We conclude that over short distances the results of the two methods are reasonably concordant but longer-range trajectories are harder to assess.

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BOARD NUMBER: S03-487

BROADBAND VISUAL STIMULI ENGAGE NEW NEURONAL POPULATIONS IN THE MOUSE VISUAL CORTEX AND FACILITATE VISUAL DISCRIMINATION

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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The visual system has evolved in congruence to its core function of making useful representations of the visual features of an animal's environment. Many of these visual features (e.g. orientation of edges) are present in natural sceneries at a broad variability of distributions. Identifying what computations are implemented for coping with this variability, is, therefore, pertinent in acquiring a better understanding of signal processing in the visual system and how natural statistics ultimately affects visual perception of the environment. This study aimed to explore this effect in mice in respect to their capability to discriminate between orientations and spatial frequencies in bandwidth-enriched naturalistic stimuli. For this purpose, we designed a mouse touchscreen-chamber-based 2AFC visual discrimination task, whereby we tested the effect of different spatial frequency bandwidths on the capability of mice to discriminate orientations of the presented visual stimuli and the effect of varying orientation bandwidths on visual stimulus spatial frequency discrimination capability of these mice. We observed that the spatial frequency discrimination capability of our mice was significantly improved by broadening the orientation bandwidth. Next, we used *in vivo* multiphoton imaging to address how bandwidth-enriched naturalistic stimuli activate neurons in visual cortex. Pseudo-random sequences of orientation bandwidth-enriched and spatial frequency bandwidth-enriched stimuli were presented to a passively viewing animal. Upon enrichment of stimulus bandwidth in the orientation domain, new cohorts of neurons of different orientation preferences were engaged. We conclude that the additional computational power provided by these newly engaged channels facilitates the perception of the visual stimulus.

Pubmed:
30141080: Körfer G, Novoa C, Kern J, Balla E, Grütering C, Davari MD, Martinez R, Vojcic L, Schwaneberg U
Directed evolution of an acid Yersinia mollaretii phytase for broadened activity at neutral pH.
Phytases are phosphohydrolases that initiate the sequential hydrolysis of phosphate from phytate, which is the main storage form of phosphorous in numerous plant seeds, especially in cereals and grains. Phytate is indigestible for most monogastric animals, such as poultry, swine, fish, and humans; therefore, microbial phytases have been widely used in plant (specially soy)-based animal feeding to improve nutrition by enhanced phosphorus, mineral, and trace element absorption, and reducing phosphorus pollution by animal waste. Most phytases used as animal feed additives have an acid pH optimum (pH 2.5 and 5.5 for Aspergillus and pH 4.5 for E. coli phytases) and show a sharp decrease in performance at neutral pH, correlating with intestinal digestion. Directed evolution of phytases has been previously reported to improve enzyme thermostability, pH, or specific activity. In this manuscript, we report a directed evolution campaign of the highly active bacterial phytase from Yersinia mollaretii (YmPh) towards a broadened pH activity spectrum. Directed evolution identified the key positions T44 and K45 for increased YmPh activity at neutral pH. Both positions are located in the active site loop of the phytase and have a synergistic effect on activity with a broadened pH spectrum. Kinetic characterization of the improved variants, YmPh-M10 and -M16, showed up to sevenfold increased specific activity and up to 2.2-fold reduced K at pH 6.6 under screening conditions compared to Yersinia mollaretii phytase wild type (YmPhWT).
Appl Microbiol Biotechnol, 2018; 102

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BOARD NUMBER: S03-488

THE EFFECT OF MOTION IN THE BODY AND FACE PATCH SYSTEMS OF MACAQUES

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Aims: Visual information from body movements and facial expressions are essential elements of non-verbal social communication and action recognition. To understand the neural substrate underlying the visual processing of dynamic monkey bodies (DB) and dynamic monkey faces (DF) we conducted fMRI studies in two macaques. Methods & Results: First, 1 s long videos of DB, DF, dynamic artificial objects (DO) and phase scrambled (P) versions of these videos were presented on a dynamic white noise background during passive fixation. Nine body patches were localized along and ventral to the STS with the contrast [(DB > PB) & (DB > DF) & (DB > DO)]. Using the contrast [(DF > PF) & (DF > DO)] we localized 9 face patches in both animals. In a follow-up scan session, we presented the original movies and 2 static images from each movie for 500 ms in a random order, using a block design. Comparing the percent signal change in the previously defined 9 body patches, we found a higher activation for dynamic than static stimuli and a preserved category selectivity for the static stimuli. When comparing the activations in the 9 face patches, the category selectivity was preserved for the static stimuli, whereas the activation for dynamic and static stimuli was similar. Conclusion: These results suggest that movements have a stronger effect on the body patch system. To understand how the shape and motion information is coded in dynamic body patches single-unit recordings are ongoing (Raman et al FENS 2022).

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LINEAR DECODING APPLIED TO V5/MT NEURONAL ACTIVITY ON PAST TRIALS PREDICTS CURRENT SENSORY CHOICES

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Perceptual decisions about sequences of sensory stimuli often show serial dependence. The behavioural choice on one trial is often affected by the choice on previous trials. We investigated whether neuronal signals in extrastriate visual area V5/MT on preceding trials might influence choice on the current trial and thereby reveal the neuronal mechanisms of sequential choice effects. We analysed data from 30 single neurons recorded from V5/MT in three Rhesus monkeys making sequential choices about the rotation direction of a three-dimensional cylinder. We focused on the responses of neurons that showed significant choice-related firing (mean choice probability=0.73) while the monkey viewed perceptually ambiguous stimuli. Application of wavelet analysis to the early choice-related firing (80–500 ms) revealed differences in the frequency bands of neuronal activity. When the previous unambiguous trial had resulted in a correct choice that was the same as the current choice, there was a pattern of high theta, low alpha & low beta, whereas when the choices were different the opposite pattern was seen. To probe this in further detail, we applied a regularized linear decoder to predict the choice for an ambiguous trial by referencing the neuronal activity of the preceding unambiguous trial. Neuronal activity on a previous trial significantly predicted the current choice (61% correct, 95%CI ~52%), even when limiting analysis to preceding trials that were correct and rewarded. These findings provide a potential neuronal signature of sequential choice strategy (win-stay; win-switch) in primate visual cortex with differences in the spectral profile for persistent responses.

Pubmed:
34734977: Di Bello F, Ben Hadj Hassen S, Astrand E, Ben Hamed S
Prefrontal Control of Proactive and Reactive Mechanisms of Visual Suppression.
In everyday life, we are continuously struggling at focusing on our current goals while at the same time avoiding distractions. Attention is the neuro-cognitive process devoted to the selection of behaviorally relevant sensory information while at the same time preventing distraction by irrelevant information. Distraction can be prevented proactively, by strategically prioritizing task-relevant information at the expense of irrelevant information, or reactively, by suppressing the ongoing processing of distractors. The distinctive neuronal signature of these suppressive mechanisms is still largely unknown. Thanks to machine-learning decoding methods applied to prefrontal cortical activity, we monitor the dynamic spatial attention with an unprecedented spatial and temporal resolution. We first identify independent behavioral and neuronal signatures for long-term (learning-based spatial prioritization) and short-term (dynamic spatial attention) mechanisms. We then identify distinct behavioral and neuronal signatures for proactive and reactive suppression mechanisms. We find that while distracting task-relevant information is suppressed proactively, task-irrelevant information is suppressed reactively. Critically, we show that distractor suppression, whether proactive or reactive, strongly depends on the implementation of both long-term and short-term mechanisms of selection. Overall, we provide a unified neuro-cognitive framework describing how the prefrontal cortex deals with distractors in order to flexibly optimize behavior in dynamic environments.
Cereb Cortex, 2021;
32066740: Gaillard C, Ben Hadj Hassen S, Di Bello F, Bihan-Poudec Y, VanRullen R, Ben Hamed S
Prefrontal attentional saccades explore space rhythmically.
Recent studies suggest that attention samples space rhythmically through oscillatory interactions in the frontoparietal network. How these attentional fluctuations coincide with spatial exploration/displacement and exploitation/selection by a dynamic attentional spotlight under top-down control is unclear. Here, we show a direct contribution of prefrontal attention selection mechanisms to a continuous space exploration. Specifically, we provide a direct high spatio-temporal resolution prefrontal population decoding of the covert attentional spotlight. We show that it continuously explores space at a 7-12 Hz rhythm. Sensory encoding and behavioral reports are increased at a specific optimal phase w/ to this rhythm. We propose that this prefrontal neuronal rhythm reflects an alpha-clocked sampling of the visual environment in the absence of eye

movements. These attentional explorations are highly flexible, how they spatially unfold depending both on within-trial and across-task contingencies. These results are discussed in the context of exploration-exploitation strategies and prefrontal top-down attentional control.
Nat Commun, 2020; 11
30876931: Reynaud AJ, Froesel M, Guedj C, Ben Hadj Hassen S, Cléry J, Meunier M, Ben Hamed S, Hadj-Bouziane F
Atomoxetine improves attentional orienting in a predictive context.
The role of norepinephrine (NE) in visuo-spatial attention remains poorly understood. Our goal was to identify the attentional processes influenced by atomoxetine (ATX) injections, a NE-reuptake inhibitor that boosts the level of NE in the brain, and to characterize these influences. We tested the effects of ATX injections, on seven monkeys performing a saccadic cued task in which cues and distractors were used to manipulate spatial attention. We found that when the cue accurately predicted the location of the upcoming cue in 80% of the trials, ATX consistently improved attentional orienting, as measured from reaction times (RTs). These effects were best accounted for by a faster accumulation rate in the valid trials, rather than by a change in the decision threshold. By contrast, the effect of ATX on alerting and distractor interference was more inconsistent. Finally, we also found that, under ATX, RTs to non-cued targets were longer when these were presented separately from cued targets. This suggests that the impact of NE on visuo-spatial attention depends on the context, such that the adaptive changes elicited by the highly informative value of the cues in the most frequent trials were accompanied by a cost in the less frequent trials.
Neuropharmacology, 2019; 150

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DECODING RAPIDLY PRESENTED VISUAL STIMULI FROM PREFRONTAL ENSEMBLES WITHOUT REPORT NOR POST-PERCEPTUAL PROCESSING

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Two leading theories, the Global Neural Workspace (GNW) and the Integrated Information Theory (IIT), make diverging predictions concerning the role of the prefrontal cortex (PFC) in consciousness. PFC is activated when reporting conscious perception but it has been argued that PFC is only involved in an introspective process occurring after consciousness (post-perception). In the present study, we measured the timing of visual information available to the ventrolateral-lateral PFC (vlPFC) using Utah arrays in macaque monkeys instructed to passively watch a stream of flashing images. Linear classifiers could predict above chance the identity of some images as early as 60 milliseconds after stimulus onset. Moreover, after 150 milliseconds the classifiers reached a peak accuracy when all images could be discriminated from each other. To test whether the vlPFC activity was reflecting introspection, we also let the monkeys watch streams of images quickly succeeding each other at a pace of 10 Hz (RSVP condition). At this rate, post-perception was unlikely for every image in the stream but classifiers accuracy continued to peak at the same time and as high as in the condition where each image was presented at a slow pace. Our results suggest that vlPFC is involved in visual processing earlier than, and independently from post-perception. Such early decoding of stimulus identity in the PFC might be related to phenomenal consciousness. We also show that the RSVP condition alters the dynamics of sensory representations in vlPFC. Our results challenge and might refine both the IIT and GNW theory of consciousness.

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FACE-BODY INTEGRATION IN ANTERIOR INFEROTEMPORAL CORTEX

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Aims: Socially relevant stimuli recruit different brain areas but little is known about the integrative mechanisms allowing for whole-agent perception. Therefore, we conducted fMRI and electrophysiological experiments in two macaque monkeys, by employing greyscale images and comparing responses to natural (head at its anatomical position) and unnatural (head attached to a different part of the upper body) monkey composites with the sum of their corresponding parts (faces and bodies, presented separately). Methods & Results: First, we localized the areas activated more by monkeys than manmade objects. In these areas in anterior inferotemporal (IT) cortex, activations to the natural composites were higher than to the unnatural ones, and that difference could not be explained by the summed activations of their corresponding parts. Importantly, the activation to the natural composites equaled the summed face-body activations, while the activation to the unnatural ones was subadditive. Second, we localized the IT patches activated more by the natural than the unnatural composites while accounting for differences in stimulus eccentricity. In these patches, we recorded single neurons in several tests, changing the position of the stimuli (monkey- or face-centered, face or body left or right of fixation) or face-body distance (0°, 0.2°, 0.4°). In all tests, responses to the natural composites were higher and approximated the face-body sums, while responses to the unnatural ones were subadditive. Detaching the head from the body resulted in comparable natural-unnatural responses at the longest distance. Conclusion: These results suggest that face-body integration depends on the configuration of the two parts.

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NEURONAL BASES OF EFFICIENT CODING OF MULTIPOINT CORRELATIONS IN RAT VISUAL CORTEX

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Efficient processing of sensory data requires adapting the neuronal encoding strategy to the statistics of natural stimuli. One area where this concept has been applied is the perceptual saliency of textures: multiple works have shown that humans are most sensitive to textures containing multipoint correlations that vary the most across natural images. The neuronal mechanisms underlying such adaptation are not understood but investigating them in depth requires invasive experiments that are impossible in humans. We have shown that rats can be trained to discriminate between binary textures containing unstructured random noise and textures defined by single- to four-point correlations among nearby pixels. We observed a decrease in sensitivity from 2- to 4-point correlations and a further decrease from 4- to 3-point, fully reproducing results obtained on humans. Building on this result, we started performing extracellular recordings of neural activity from primary visual cortex (V1) and extrastriate areas of anaesthetized rats passively viewing textures containing multi-point correlations or unstructured noise. We found that, in V1, the highest fraction of selective units (p-value<0.05, ANOVA) was tuned for 1-point correlations, followed by 2-,4- and 3-point. In extrastriate cortex, we found the largest fraction of units to be selective for 1-point, followed by 4-, 2- and 3-point correlations. Finally, we confirmed a difference in neural representations of texture statistics between striate and extrastriate cortex with a population decoding analysis. These results provide an early indication of the relative contribution to this process by distinct visual cortical areas.

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CNN CLASSIFIES VISUAL STIMULI FROM PRIMARY VISUAL CORTEX IN MOUSE

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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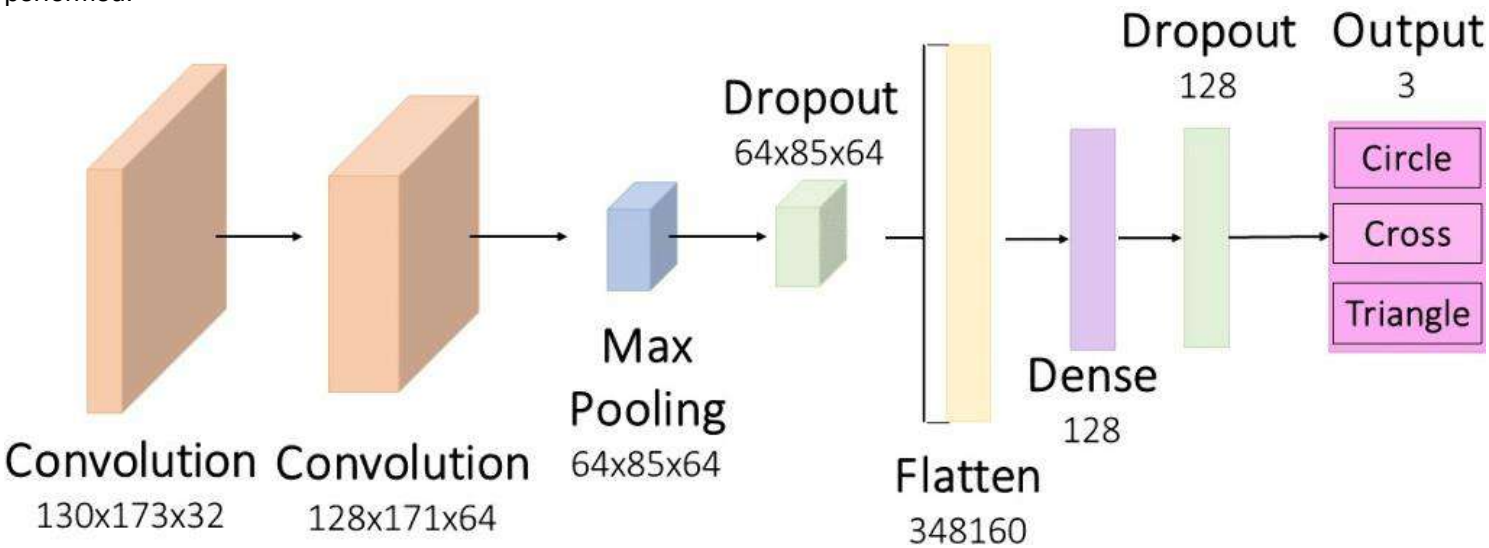
Optic nerve stimulation holds great potential for visual prostheses. Its effectiveness depends on the stimulation protocol, which can be optimized to achieve cortical activation similar to that recorded in response to visual stimuli. To identify a target cortical activation, it is necessary to characterize the cortical response. We here propose a convolutional neural network (CNN) to do it exploiting widefield calcium brain images. A mouse was presented with 3 types of visual stimuli (circle, cross, triangle) for one second each, and the activity from its primary visual cortex (V1) was recorded using widefield calcium imaging. This technique allows large-scale visualization of cortical activity with a high signal-to-noise ratio. A dataset of 45 images (15 per stimulus) with a size of 656x875 pixels was collected. Each image was classified into the 3 stimuli by a CNN with 9 layers (Fig). The CNN was trained minimizing categorical cross-entropy with Adadelta for 100 epochs. Offline data augmentation was applied to the training set. The best model among epochs was selected according to the highest accuracy on the validation set. For robust testing, 5-fold stratified cross-validation was performed.



The CNN achieved an accuracy of 86.67%±9.30%. Although very preliminary, this interesting result shows that a shallow CNN can decode a visual stimulus from V1 neural activity of a mouse, and that deep learning can associate cortical activation to visual perception. This can be leveraged not only to properly drive optic nerve stimulation but also as a useful insight for applications that exploit visual cortex activity.

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VARIATION OF CONTRAST INDUCES MUSCARINIC-DEPENDENT CHANGES IN OSCILLATORY ACTIVITY OF THE PRIMARY VISUAL CORTEX

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Aims. Cholinergic/ muscarinic stimulation can enhance oscillatory activity in the primary visual cortex (V1). How this modulation mediates oscillatory processing associated with visual signals in V1 is still unknown. Therefore, in the present study, cortical oscillatory responses to variation of visual contrast were examined and the effect of acetylcholine and muscarinic receptors on such evoked rhythms was analyzed. **Methods**. Eight long Evans Rats (300-350g) were used. Evoked local field potentials were acquired at 30 kHz using linear 16-channel multiunit electrodes implanted in V1. A luminance based sinusoidal grating (0.12cpd, 3Hz drift in the preferred direction, 0%, 6%, 12%, 25%, 50%, 60% and 100% contrast) was displayed (2sec on/2sec off, 20 repetitions) on a CRT screen. Wavelet analysis was used to calculate spectral estimates normalized by condition with Matlab (*Cortes Hernandez et al., Vision, 2021*). The response under scopolamine (0.3 mg/kg. i.p.), a muscarinic antagonist, was also measured. **Results**. Cortical alpha- and gamma-band oscillations were increased as a function of contrast, as shown by spectral time-frequency analysis in layers 2/3, 4, and 5. The scopolamine shifted linearly gamma-waves responses associated with contrast in supragranular and granular layers. It also shifted linearly alpha rhythms in supragranular layer, but responses to visual contrast in the granular layer were abolished by scopolamine. **Conclusions**. In the gamma-band range, the acetylcholine produces a contrast gain control in layers 2/3 and 4, which might influence perceptual sensitivity to contrast.

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EXTENSIVE DIMENSIONALITY OF NEURAL CIRCUIT MANIFOLDS ASSOCIATED WITH A SALT-AND-PEPPER ORGANIZATION OF CORTICAL STIMULUS PREFERENCES

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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In rodent sensory cortex, cortical circuits include a dense blanket of inhibition (Bopp et al. 2014). If cortical principal cells acquire their stimulus preferences by selecting afferent connections through Hebbian mechanisms. Then strong feedback inhibition can force neurons to adopt maximally dissimilar selectivities (Rubner&Schulten1990). Here we introduce and examine a set of idealized models to investigate manifolds of stable network configurations in inhibition dominated circuits. Biological neural circuits are expected to converge to one of many stable network configurations. For the form vision core circuit of primate/carnivore V1 prior work indicates that stable network configurations form high-dimensional continua (Wolf 2005, Kaschube et al. 2010). Similar results for network configurations in rodents V1, called salt-and-pepper organizations (SaP), are currently not available. We utilize techniques from the study of spin liquid states (Chalker2015) to construct and examine mathematically tractable models with SaP optimal states. We demonstrate that these models can exhibit ground state manifolds with extensive dimensionality for uniform, range-dependent and selectivity-dependent neuronal connectivity. This result is consistent with the general expectation that there are a very high number of equivalent SaP configurations. Disordered SaP states result even for dense and uniform connectivity patterns and do not require any structural source of disorder. These studies expand the toolbox for analyzing the multiplicity of stable cortical circuit configurations. Our results suggest that the evolutionary transition from a rodent ancestral circuit configurations of V1 to a primate/carnivore V1 architecture was accompanied by a radical reduction of the dimensionality of the cortical circuit state manifold.

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ORIENTATION PREFERENCE MAPS IN THE DUNNART (SMINTHOPSIS CRASSICAUDATA) V1 REVEALS NOVEL V1 FUNCTIONAL ORGANISATION

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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The primary visual cortex, V1, of mammals contains orientation selective neurons. Previously only two types of spatial organisation was observed: orientation preference pinwheels and salt-and-pepper patterns. Furthermore a recent analysis in primates including the smallest living primate, the mouse lemur (Microcebus murinus), revealed that primate orientation domains are nearly incompressible, scaling weakly with brain and body size. Thus it has been argued that brains with small visual cortices such as the approximately 5 mm^2 V1 of the mouse and ancestral mammals (Keil et al. 2012, Kaschube 2014) should not be able to contain orientation domains. Here we present evidence for the relatively large orientation domains in an extremely small V1. We performed intrinsic signal imaging in the visual cortex of the fat-tailed dunnart, Sminthopsis crassicaudata, a marsupial with a V1 size of 5.6 mm2 (Tyler et.al. 1998). Despite this, we observed well expressed orientation domains. This makes the dunnart the smallest mammal studied to possess orientation domains. Dunnart orientation domains seemingly differ in layout substantially from those found in primates and carnivores. In particular the dunnart maps lack a quasi-periodic structure and exhibit no pinwheel singularities. Dunnart orientation domains are variable in size with the largest domains considerably larger than column sizes found in the mouse lemur (Ho 2021). Our studies suggest that a third type of V1 functional architecture emerged in marsupial V1. Moreover, the novel arrangement and small brain size of the dunnart means key principles of sensory cortex theories, such as the coverage-continuity-compromise, can be critically tested.

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FUNCTIONAL PARCELLATION OF THE HUMAN FACE-SELECTIVE AREAS: A RESTING-STATE CONNECTIVITY HOMOGENEITY ANALYSIS.

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Although different functional parcellations of human face-selective areas in the ventral visual stream have been proposed, the extent to which such regions share similar properties is still unclear[1]. Using a subset of 165 subjects from the HCP1200 functional magnetic resonance imaging dataset, we combined task-based definition of face-selective areas and resting-state connectivity homogeneity analysis to reach a finer-grained parcellation of face-selective areas. We segmented individual surface-based statistical maps of activation during vision of faces vs places through a watershed algorithm, identifying three parcels lying between the fusiform and the middle occipital gyrus. We then computed a connectivity fingerprint of each face-selective surface vertex, defined as the correlation between its resting-state timeseries and that of all vertices outside the face parcels. Finally, we computed a homogeneity measure between the connectivity fingerprints of each pair of vertices within and between the face parcels. The connectivity homogeneity between face vertices was inversely proportional to the anatomical distance between them measured on the individual cortical surface. Having accounted for distance effects, homogeneity was higher within than between face parcels. This result shows that three face regions are distinguishable based on their resting-state functional connectivity fingerprints. Despite needing validation, our method shows the possibility of segregating parcels with similar functional properties using the profile of their functional connectivity at rest. 1. Grill-Spector, K., Weiner, K. S., Kay, K., & Gomez, J. (2017). The functional neuroanatomy of human face perception. Annual review of vision science, 3, 167-196.

Pubmed:
33524574: Bencivenga F, Sulpizio V, Tullo MG, Galati G
Assessing the effective connectivity of premotor areas during real vs imagined grasping: a DCM-PEB approach.
The parieto-frontal circuit underlying grasping, which requires the serial involvement of the anterior intraparietal area (aIPs) and the ventral premotor cortex (PMv), has been recently extended enlightening the role of the dorsal premotor cortex (PMd). The supplementary motor area (SMA) has been also suggested to encode grip force for grasping actions; furthermore, both PMd and SMA are known to play a crucial role in motor imagery. Here, we aimed at assessing the dynamic couplings between left aIPs, PMv, PMd, SMA and primary motor cortex (M1) by comparing executed and imagined right-hand grasping, using Dynamic Causal Modelling (DCM) and Parametrical Empirical Bayes (PEB) analyses. 24 subjects underwent an fMRI exam (3T) during which they were asked to perform or imagine a grasping movement visually cued by photographs of commonly used objects. We tested whether the two conditions a) exert a modulatory effect on both forward and feedback couplings among our areas of interest, and b) differ in terms of strength and sign of these parameters. Results of the real condition confirmed the serial involvement of aIPs, PMv and M1. PMv also exerted a positive influence on PMd and SMA, but received an inhibitory feedback only from PMd. Our results suggest that a general motor program for grasping is planned by the aIPs-PMv circuit; then, PMd and SMA encode high-level features of the movement. During imagery, the connection strength from aIPs to PMv was weaker and the information flow stopped in PMv; thus, a less complex motor program was planned. Moreover, results suggest that SMA and PMd cooperate to prevent motor execution. In conclusion, the comparison between execution and imagery reveals that during grasping premotor areas dynamically interplay in different ways, depending on task demands.
Neuroimage, 2021; 230
33821379: Boccia M, Sulpizio V, Bencivenga F, Guariglia C, Galati G
Neural representations underlying mental imagery as unveiled by representation similarity analysis.
It is commonly acknowledged that visual imagery and perception rely on the same content-dependent brain areas in the high-level visual cortex (HVC). However, the way in which our brain processes and organizes previous acquired knowledge to allow the generation of mental images is still a matter of debate. Here, we performed a representation similarity analysis of

three previous fMRI experiments conducted in our laboratory to characterize the neural representation underlying imagery and perception of objects, buildings and faces and to disclose possible dissimilarities in the neural structure of such representations. To this aim, we built representational dissimilarity matrices (RDMs) by computing multivariate distances between the activity patterns associated with each pair of stimuli in the content-dependent areas of the HVC and HC. We found that spatial information is widely coded in the HVC during perception (i.e. RSC, PPA and OPA) and imagery (OPA and PPA). Also, visual information seems to be coded in both preferred and non-preferred regions of the HVC, supporting a distributed view of encoding. Overall, the present results shed light upon the spatial coding of imagined and perceived exemplars in the HVC.
Brain Struct Funct, 2021; 226

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BOARD NUMBER: S03-498

TOWARDS AN OPTIMIZATION OF FUNCTIONAL LOCALIZERS IN NON-HUMAN PRIMATE IMAGING WITH (FMRI) FREQUENCY-TAGGING

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

Marie-Alphée Laurent[1], Pauline Audurier[2], Vanessa De Castro[2], Xiaoqing Gao[3], Jean-Baptiste Durand[2], Jacques Jonas[4], Bruno Rossion[1], Benoit R. Cottereau[2]
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Aims Non-human primate (NHP) neuroimaging can provide essential insights into the neural basis of human cognitive functions. While functional fMRI localizers can play an essential role in reaching this objective (Russ et al., 2021), they often differ substantially across species in terms of paradigms, measured signals and data analysis, biasing the comparisons. Here we introduce a functional frequency-tagging face localizer for NHP imaging, successfully developed in humans and outperforming standard “face localizers” (Gao et al., 2018). **Methods** FMRI recordings were performed in two awake macaques. Within a rapid 6Hz stream of natural images of non-face objects, 7 human or monkey face stimuli were presented in bursts every 9s during a 243s run. We also included control conditions with phase-scrambled versions of all images. As in humans, runs were analyzed in the frequency domain where face-selective responses were objectively identified and quantified at the peak of the face-stimulation frequency (0.111Hz) and its second harmonic (0.222Hz). **Results** Focal activations with high signal-to-noise ratio were observed in regions previously described as face-selective, mainly in the STS (clusters PL, ML, MF; also, AL, AF), both for human and monkey faces. Robust activations were also found in the prefrontal cortex of one monkey in the PVL and PO clusters. **Conclusions** Face-selective responses were highly reliable, excluding all contributions from low-level visual cues contained in the amplitude spectrum of the images. These observations indicate that fMRI frequency-tagging provides a valid approach to objectively compare human and monkey neural face recognition systems within the same framework.

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BOARD NUMBER: S03-499

IMPAIRMENT OF EVIDENCE ACCUMULATION UNDERLIES VISUOMOTOR DEFICITS IN MOUSE MODELS OF AUTISM.

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Individuals with autism spectrum disorders (ASDs) often exhibit impairments in gaze fixation, visual attention, and hyper- or hyposensitivity to visual stimuli. However, the neural mechanisms underlying these problems remain unclear. Here we show that the mechanism of evidence accumulation required to initiate efficient threat responses is disrupted in Setd5(+/-) mice, a model of ASD. Although mutant animals can quickly detect visual threats, on average they require longer to evaluate and respond to them compared to their wild-type (WT) siblings. Other behavioural dynamics, as well as visual and threat evoked responses in the medial superior colliculus (mSC) remain unaffected, indicating cognitive rather than motor or sensory impairments. These behavioural deficits were recapitulated following optogenetic activation of excitatory vGlut2+ mSC neurons, known to initiate threat responses by exciting the dorsal periaqueductal grey (dPAG). In vitro patch-clamp recordings of Setd5(+/-) dPAG neurons revealed a stark reduction in firing rates in response to high, but not low, current injections. This change appears to be mediated by misregulation of Kv1.1 channels, suggesting a disruption in homeostatic mechanisms. In accordance with these intrinsic firing changes, a low saliency threat stimuli known to drive dPAG cells weakly, elicits WT behavioural responses in Setd5(+/-) mice. Finally, we observed similar phenotypic changes across two other independent ASD models, Cul3(+/-) and Ptchd1(-/y), suggesting that SC to PAG pathway might be a key node in ASD-related visuomotor deficits. Overall, our results provide a mechanistic model of cognitive dysfunction from gene to behaviour.

Pubmed:
31306639: Hao MM, Bergner AJ, Nguyen HTH, Dissanayake P, Burnett LE, Hopkins CD, Zeng K, Young HM, Stamp LA
Role of JNK, MEK and adenylyl cyclase signalling in speed and directionality of enteric neural crest-derived cells.
Cells derived from the neural crest colonize the developing gut and give rise to the enteric nervous system. The rate at which the ENCC population advances along the bowel will be affected by both the speed and directionality of individual ENCCs. The aim of the study was to use time-lapse imaging and pharmacological activators and inhibitors to examine the role of several intracellular signalling pathways in both the speed and the directionality of individual enteric neural crest-derived cells in intact explants of E12.5 mouse gut. Drugs that activate or inhibit intracellular components proposed to be involved in GDNF-RET and EDN3-ETB signalling in ENCCs were used.
Dev Biol, 2019; 455
28671186: McCann CJ, Cooper JE, Natarajan D, Jevans B, Burnett LE, Burns AJ, Thapar N
Transplantation of enteric nervous system stem cells rescues nitric oxide synthase deficient mouse colon.
Enteric nervous system neuropathy causes a wide range of severe gut motility disorders. Cell replacement of lost neurons using enteric neural stem cells (ENSC) is a possible therapy for these life-limiting disorders. Here we show rescue of gut motility after ENSC transplantation in a mouse model of human enteric neuropathy, the neuronal nitric oxide synthase (nNOS) deficient mouse model, which displays slow transit in the colon. We further show that transplantation of ENSC into the colon rescues impaired colonic motility with formation of extensive networks of transplanted cells, including the development of nNOS neurons and subsequent restoration of nitrergic responses. Moreover, post-transplantation non-cell-autonomous mechanisms restore the numbers of interstitial cells of Cajal that are reduced in the nNOS colon. These results provide the first direct evidence that ENSC transplantation can modulate the enteric neuromuscular syncytium to restore function, at the organ level, in a dysmotile gastrointestinal disease model.
Nat Commun, 2017; 8
28472654: Beattie R, Postiglione MP, Burnett LE, Laukoter S, Streicher C, Pauler FM, Xiao G, Klezovitch O, Vasioukhin V, Ghashghaei TH, Hippenmeyer S
Mosaic Analysis with Double Markers Reveals Distinct Sequential Functions of Lgl1 in Neural Stem Cells.
The concerted production of neurons and glia by neural stem cells (NSCs) is essential for neural circuit assembly. In the developing cerebral cortex, radial glia progenitors (RGPs) generate nearly all neocortical neurons and certain glia lineages.

RGP proliferation behavior shows a high degree of non-stochasticity, thus a deterministic characteristic of neuron and glia production. However, the cellular and molecular mechanisms controlling RGP behavior and proliferation dynamics in neurogenesis and glia generation remain unknown. By using mosaic analysis with double markers (MADM)-based genetic paradigms enabling the sparse and global knockout with unprecedented single-cell resolution, we identified Lgl1 as a critical regulatory component. We uncover Lgl1-dependent tissue-wide community effects required for embryonic cortical neurogenesis and novel cell-autonomous Lgl1 functions controlling RGP-mediated glia genesis and postnatal NSC behavior. These results suggest that NSC-mediated neuron and glia production is tightly regulated through the concerted interplay of sequential Lgl1-dependent global and cell intrinsic mechanisms.
Neuron, 2017; 94

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BOARD NUMBER: S03-500

IMPLICIT LEARNING OF VISUAL TRACKING IS SPECIFIC TO RESPONSE MODALITY AND RELIES ON IMPROVEMENT OF SIGNAL PREDICTION

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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There is no consensus on the conditions under which implicit learning of visual tracking occurs. Here, we assessed the occurrence of such learning in eye and hand tracking tasks .We used an information-theoretic framework to gain better insight into the nature of the learning process. In experiment 1, 20 volunteers tracked a signal (order-2 autoregressive) composed of five segments, two of which repeated across trials . Individuals tracked with the eye or with a joystick in counter-balanced blocks. We found that tracking performance was better on the repeated than on the random segments but only in the joystick condition. This learning was improved on the next day. Subjects had no conscious awareness of the repetition. Through information-theoretic measures, we showed that improved performance in the joystick condition was caused by a better capacity to predict future positions of the signal. In Experiment 2 (N= 24), we replicated these findings and accounted for confounding factors. We also assessed the capacity of the subjects to generate the signal spontaneously when the target disappeared during five seconds. Preliminary results suggest that volunteers were not able to generate the signal during the blanks, despite their capacity to use their implicit knowledge of the signal to improve tracking performance. These findings show clearly that visual tracking signals can be learned implicitly, allowing individuals to improve their capacity to predict future signal values. The motor specificity of the learning process suggests that learning takes place at the level of the motor control circuit.

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BOARD NUMBER: S03-501

FLEXIBLE, CONTEXT-DEPENDENT, HIERARCHY OF EYE-HEAD COORDINATION

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Visual orienting responses had traditionally been studied in head-fixed conditions. It had been shown that when the head is free to move, eye movements exhibit complex behavior, such as multisaccadic gaze-shifts. Here we show that head-eye coordination exhibits a flexible hierarchy, in which either the eye or the head can lead the orienting movement, depending on the context. We tested 20 human subjects in a virtual reality (VR) environment consisting of a neutral background and a ball. The subjects were asked to track the ball with their gaze and were free to move their head and body. Each trial started with the ball appearing at the same (virtual) allocentric position. After 2 seconds the ball jumped 15-55 deg horizontally, and the subject's gaze followed it ("exogenous orientation"). After 4 seconds the ball disappeared. The subjects, then, typically oriented their gaze back to the approximate area of trial initiation (without explicit instructions, "endogenous orientation"). We found that the hierarchy of head-eye coordination switched between these two orienting movements: the eye was leading during exogenous orientation (29±10ms), and the head was leading during endogenous orientation (16±10ms). Endogenous orientations involved 57±6% more saccades than exogenous orientations, with the first saccade being smaller by 29±7%. We model orientation control by a system of eye and head sensory-motor closed-loops, competing for action, based on visual, proprioceptive and context information. The model replicates our data, can replicate an opto-kinetic reflex and suggests that both saccades and smooth-pursuit movements are controlled by the same loop.

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BOARD NUMBER: S03-502

LOCUS COERULEUS BROADCASTS VISUOMOTOR PREDICTION ERRORS ACROSS THE NEOCORTEX

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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Friedrich Miescher Institute for Biomedical Research, Neurobiology, Basel, Switzerland

We have a remarkable ability to distinguish sensory inputs caused by our own actions, and those caused by the external environment. To achieve this, the brain must continually learn the relationships between motor actions and their sensory consequences. One way the brain may do this is to calculate prediction errors – deviations between predicted and actual sensory outcomes – in order to drive learning. If such prediction errors activate widely-projecting neuromodulatory systems, this would enable a coordinated and global modulation of learning rates by prediction errors. The noradrenergic locus coeruleus is one system that is known to promote cortical plasticity and has been shown to be responsive to unexpected sensory stimuli. To test if the mouse locus coeruleus responds to visuomotor prediction errors, we use a virtual reality system to generate visuomotor mismatches. Using two-photon calcium imaging and widefield imaging of noradrenaline sensors, we find that cortical locus coeruleus axons respond to visuomotor mismatches, of both the positive (more visual flow than expected) and negative (less visual flow than expected) type. These responses are widespread and found in different cortical regions, including visual cortex, somatosensory cortex, and motor cortex. Our results are consistent with the locus coeruleus broadcasting unsigned sensorimotor prediction errors.

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BOARD NUMBER: S03-503

DENDRITIC SIGNALING IN CORTICAL PYRAMIDAL CELLS DURING VISUAL DISCRIMINATION

POSTER SESSION 03 - SECTION: CODING IN VISUAL CORTEX

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The role of dendritic calcium spikes in the neocortex is unsettled as yet, not least because dendritic arborization is extensive and branches can reach several hundred micrometers, causing recordings in vivo to stay technically challenging. It is demonstrated that dendritic signaling takes part in the formation of perception by associating feed-forward and feedback information streams arriving to different cortical layers. A different hypothesis suggests that dendrites could also participate in associative learning by carrying information – an 'error signal' – backwards. Here we look into how stimulus properties and behavioral performance affect dendritic signaling, and perhaps by doing so also gain further insight on the current theories regarding the functional involvements of dendritic activity. We used 3D two-photon laser scanning microscopy with acousto-optical deflectors to examine calcium activity in the somata and apical dendrites of pyramidal neurons in V1 of mice performing visual discrimination tasks. Controlled water-access animals were trained to discriminate between two types of drifting grating visual stimuli, one followed by a drop of water as a reward while the other was unrewarded. Learning progression was assessed on the ratio of anticipatory lick rates during stimulation. The discrepancy between the expected and the actual outcomes offers an opportunity to investigate prediction error signaling. Calcium measurements were obtained with 3-dimensional ribbon scanning, a composite imaging field covering the whole length of neurons. Here we present the signals recorded on the pyramidal neurons' dendrites and somata, as well as an observed functional grouping of pyramidal cells based on signal types.

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BOARD NUMBER: S03-504

GABAERGIC MECHANISMS OF LOCALIZED FAST NEURONAL OSCILLATIONS IN THE MOUSE HIPPOCAMPAL CA1 AREA DURING ACTIVE EXPLORATION

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

Ece Sakalar, Alexander Wallerus, Thomas Klausberger, Balint Lasztoczi
Center for Brain Research, Medical University of Vienna, Division Of Cognitive Neurobiology, Vienna, Austria

During exploratory behaviour the brain processes diverse information streams, integrating multiple sensory modalities with mnemonic and motivational cues to guide adaptive behaviour. In the CA1 area of hippocampus online brain states are characterized by continuous 8 - 12 Hz theta oscillations. Layer-specific slow (30 - 50 Hz), mid-frequency (60 - 90 Hz) and fast (100 - 150 Hz) gamma oscillations occur at distinct phases of theta waves, and together they provide a temporal framework that orchestrates network operations such as integration and segregation of information streams. Here we explore the contribution of identified GABA*ergic* cell types to this complex temporal framework. We recorded local field potentials from the hippocampal CA1 area of head fixed mice with multisite linear silicon probes, while animals performed unidirectional runs in a virtual corridor for a small water reward. Layer specific gamma oscillations were separated by applying current source density analysis. Simultaneously, we recorded spike timing of individual pyramidal cells and interneurons using multi shank silicon probes and firing of GABA*ergic* cells by glass microelectrodes filled with neurobiotin. A subset of recorded cells was subsequently labelled with neurobiotin using the juxtacellular method, and their affiliation to identified cell types was established based on their axonal and dendritic distribution and protein content. We found that the spike timing of GABA*ergic* cells is selectively modulated by different gamma oscillations contingent on cell type affiliations. Our data suggest that GABA*ergic* cells make cell-type specific contributions in establishing the temporal framework that orchestrates hippocampal network operations. (Funding: FWF P29744-B27).

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BOARD NUMBER: S03-505

WHAT IS THE MECHANICAL BASIS OF BETA OSCILLATIONS AND WAVES OF NEURAL ACTIVITY OBSERVED IN THE MOTOR CORTEX?

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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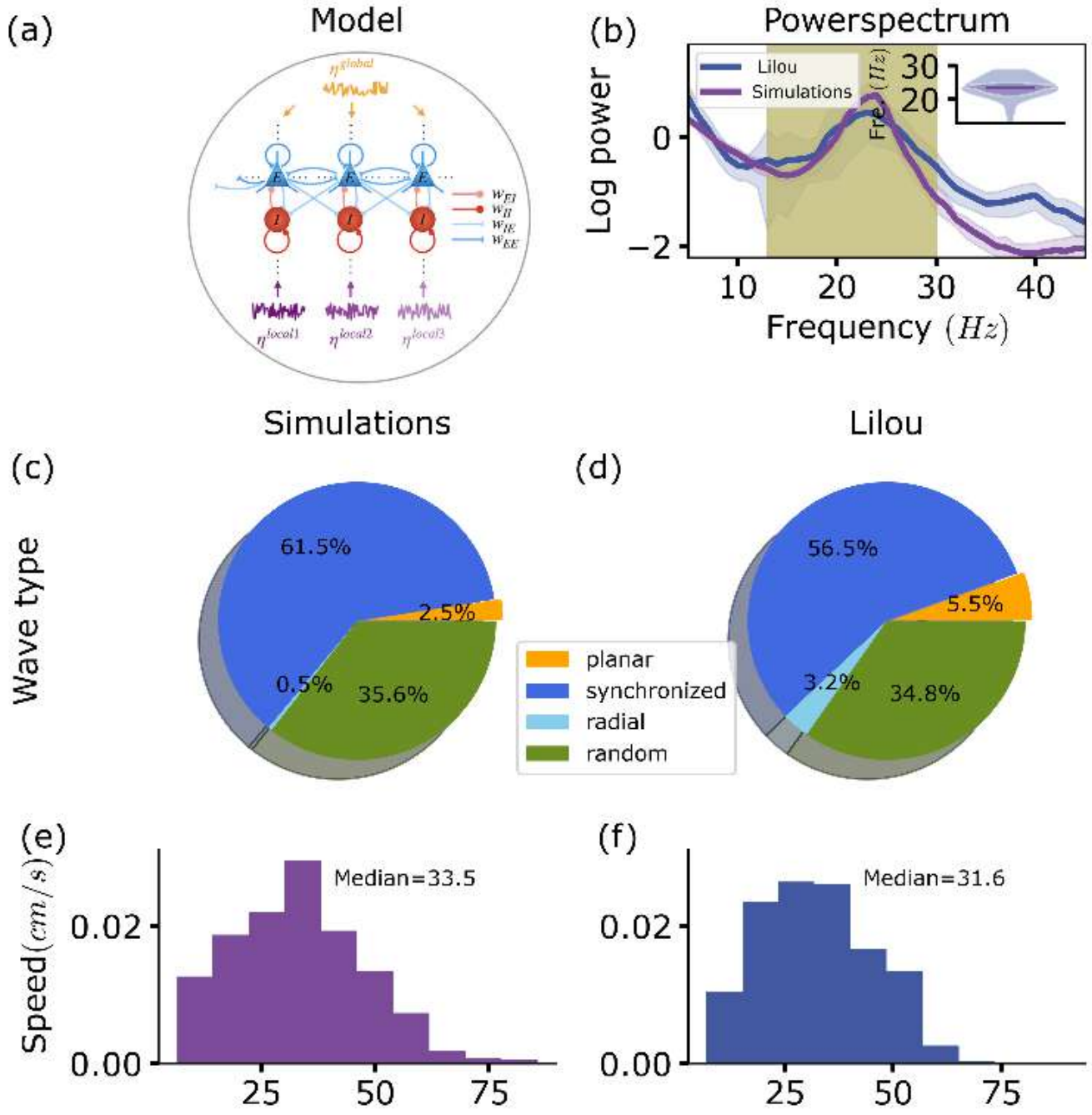
Ecole Normale Superieure, Biophysique Et Neuroscience Theoriques, Paris, France

Modeling the nonlinear dynamics of neural networks can help to make sense of phenomena observed in neural recordings. We focus on beta frequency (~20Hz) oscillations that are observed in motor cortex during movement preparation. In experiments, local field potentials (LFPs) recorded with multi-electrode arrays have been observed to display transient oscillations and organize into a variety of traveling waves (planar, radial, rotating,..). We model beta oscillation as arising from reciprocal interactions between randomly connected excitatory (E) pyramidal cells and local inhibitory interneurons (I). We use a rate model (mean-field) description of the local neural activity that has been shown to provide an accurate population-level description of network simulations based on coupled spiking neurons. Using distance-dependent interactions and delays matching those reported from experiments, we study this model in 2D to investigate possible origins of transient bursts of beta oscillations and of the observed spatial waves. Stochastic local entries are introduced to mimic inputs to the motor cortex from other neural areas. We compare our simulation results to electrophysiological datasets recorded in motor cortex of macaque monkey during an instructed delayed reach-to-grasp task [4]. We find that our model closely agrees with the recordings. It reproduces the observed power spectrum of the local field potential, beta oscillation burst statistics and produces a variety of traveling waves of speed and types similar to those seen in experiments. Our results suggest that both time-varying external entries and intrinsic network architecture shape the LFP dynamics of motor

cortex.


(a)
Model
(b)
Powerspectrum
Lilou
Simulations
Log power
Frequency (Hz)
Simulations
Lilou
(c)
(d)
Wave type
61.5%
2.5%
0.5%
35.6%
56.5%
5.5%
3.2%
34.8%
planar
synchronized
radial
random
(e)
(f)
Speed(cm/s)
Median=33.5
Median=31.6

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DEND MUTATION DISRUPTS HIPPOCAMPAL NETWORK ACTIVITY AND NOCTURNAL γ SHIFT

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Aims ATP-sensitive potassium (K_{ATP}) channels mediate cell metabolism and electrical activity by coupling intracellular ATP levels to membrane K^+-conductance. The activating mutation V59M in the $K_{ir}6.2$ subunit of the K_{ATP} channel causes developmental **d**elay and **e**pilepsy with **n**eonatal **d**iabetes, i.e., DEND syndrome. While the origin of neonatal diabetes is well understood, the pathophysiology of the neurological symptoms remains to be elucidated. Inhibitory parvalbumin$^+$-interneurons (PV-INs) play a pivotal role in generating cognition-associated hippocampal sharp-wave ripples (SWRs) and gamma oscillations (30-100 Hz) as well as epileptogenesis. Therefore, we asked whether constitutively open K_{ATP} channels in PV-INs may contribute to the neurological symptoms of the DEND syndrome. **Methods** We performed *in vivo* and *in vitro* local field potential as well as patch-clamp recordings of PV-INs in the hippocampal area CA1 in control mice and mice with the V59M mutation expressed in PV-INs (PV-V59M). **Results** We found that *in vitro* SWRs and gamma oscillations are similarly impaired in PV-V59M mice and control mice with pharmacologically opened K_{ATP} channels. Patch-clamp recordings of PV-INs in PV-V59M mice revealed a decreased power of intrinsic gamma oscillations and fewer PV-INs with gamma resonance behaviour. Moreover, PV-V59M mice showed seizures and a reduced power of gamma oscillations during wakefulness in *in vivo* LFP recordings. **Conclusions** Our findings provide evidence that the K_{ATP} channel mutation Kir6.2-V59M, expressed only in PV-INs, leads to altered network oscillations and seizures that potentially underly the neurological symptoms of DEND syndrome and highlight the crucial role of PV-INs in oscillatory activity and network inhibition.

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THE MEDIAL SEPTUM MODULATES HIPPOCAMPAL OSCILLATIONS BEYOND THE THETA RHYTHM

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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[1]Institute of Experimental Medicine, Lendület Laboratory Of Systems Neuroscience, Budapest, Hungary, [2]Institute of Experimental Medicine, Department Of Cerebral Cortex Research, Budapest, Hungary, [3]University of Oxford, Nuffield Department Of Clinical Neurosciences, Budapest, Hungary, [4]Research Centre for Natural Sciences, Institute Of Cognitive Neuroscience And Psychology, Budapest, Hungary

Hippocampal activity is organized by a collection of network oscillators on multiple time scales. Of these, theta oscillations reflect rhythmic inputs, occurring typically during exploratory or memory-guided behaviour or REM sleep. Recent studies revealed an unexpected diversity of theta cycles. In rodents performing memory tasks, cycle-by-cycle variations were found based on beta-gamma frequency theta-nested spectral components (tSCs), likely related to distinct spiking dynamics with different contributions to memory processes. The medial septum (MS) has been identified as responsible for generation of theta oscillations while gamma rhythms mostly have been associated with entorhinal cortex (EC) and CA3 inputs or local CA1 networks. To examine the possible contribution of the MS theta generator to the wave-by-wave spectral heterogeneity of theta, we recorded septal neurons simultaneously with the hippocampal local field potential in mice moving freely in a linear track and in anesthetized mice and rats, and used optogenetics to stimulate parvalbumin-expressing MS neurons in PV-IRES-Cre mice. We found that MS neurons' firing rate and phase-locking to theta waves were correlated to the presence of different CA1 tSCs. Furthermore, most MS neurons not only showed strong phase-coupling to tSCs but also preceded them. Optogenetic activation of MS parvalbumin-expressing neurons with theta-modulated stimulation bursts proved that the MS is capable of eliciting theta-nested beta-gamma oscillations in the CA1. In conclusion, these results suggest that septal firing is not only involved in the generation of the hippocampal theta oscillations but also modulate the higher frequency spectral components nested in individual theta cycles.

Pubmed:
30788253: Kiraly L, Kiraly B, Szigeti K, Tamas CZ, Daranyi S
Virtual museum of congenital heart defects: digitization and establishment of a database for cardiac specimens.
Education and training of morphology for medical students, and professionals specializing in pediatric cardiology and surgery has traditionally been based on hands-on encounter with congenitally malformed cardiac specimens. Large international archives are no longer widely available due to stricter data protection rules, a reduced number of autopsies, attrition rate of existing specimens, and most importantly due to a higher survival rate of patients. Our Cardiac Archive houses about 400 cardiac specimens with congenital heart disease. The collection spans almost 60 years and thus goes back to pre-surgical era. Unfortunately, attrition rate due to desiccation has led to an increased natural decay in recent years. The present multi-institutional project focuses on saving the collection by digitization. Specimens are scanned by high-resolution micro-CT/MRI. Virtual 3D-models are segmented and a comprehensive database is built. We now report an initial feasibility study with six test specimens that provided promising results, however, adequate presentation of the intracardiac anatomy, including septa and cardiac valves requires further refinements. Computer assisted design methods are necessary to overcome consequences of pathological examination, shrinkage and/or distortion of the specimens. For a next step, we anticipate an expandable web-based virtual museum with interactive reference and training tools. Web access for professional third parties will be provided by registration/subscription. In a future phase, segmental wall motion data could be added to virtual models. 3D-printed models may replace actual specimens and serve as hands-on surgical training to elucidate complex morphologies, promote surgical emulation, and extract more accurate procedural knowledge based on such a collection.
Quant Imaging Med Surg, 2019; 9
32191855: Király B, Hangya B
Cartographers of the Cognitive Map: Locus Coeruleus Is Part of the Guild.
Noradrenergic cells of the locus coeruleus were associated with aversive learning and arousal. In this issue of Neuron, Kaufman et al. (2020) show that they also shape the spatial map after translocation of reward.

Neuron, 2020; 105
32885586: Tőkési N, Kozák E, Fülöp K, Dedinszki D, Hegedűs N, Király B, Szigeti K, Ajtay K, Jakus Z, Zaworski J, Letavernier E, Pomozi V, Váradi A
Pyrophosphate therapy prevents trauma-induced calcification in the mouse model of neurogenic heterotopic ossification.
Trauma-induced calcification is the pathological consequence of complex injuries which often affect the central nervous system and other parts of the body simultaneously. We demonstrated by an animal model recapitulating the calcification of the above condition that adrenaline transmits the stress signal of brain injury to the calcifying tissues. We have also found that although the level of plasma pyrophosphate, the endogenous inhibitor of calcification, was normal in calcifying animals, it could not counteract the acute calcification. However, externally added pyrophosphate inhibited calcification even when it was administered after the complex injuries. Our finding suggests a potentially powerful clinical intervention of calcification triggered by polytrauma injuries which has no effective treatment.
J Cell Mol Med, 2020; 24
32943633: Király B, Balázsfi D, Horváth I, Solari N, Sviatkó K, Lengyel K, Birtalan E, Babos M, Bagaméry G, Máthé D, Szigeti K, Hangya B
In vivo localization of chronically implanted electrodes and optic fibers in mice.
Electrophysiology provides a direct readout of neuronal activity at a temporal precision only limited by the sampling rate. However, interrogating deep brain structures, implanting multiple targets or aiming at unusual angles still poses significant challenges for operators, and errors are only discovered by post-hoc histological reconstruction. Here, we propose a method combining the high-resolution information about bone landmarks provided by micro-CT scanning with the soft tissue contrast of the MRI, which allowed us to precisely localize electrodes and optic fibers in mice in vivo. This enables arbitrating the success of implantation directly after surgery with a precision comparable to gold standard histology. Adjustment of the recording depth with micro-drives or early termination of unsuccessful experiments saves many working hours, and fast 3-dimensional feedback helps surgeons avoid systematic errors. Increased aiming precision enables more precise targeting of small or deep brain nuclei and multiple targeting of specific cortical or hippocampal layers.
Nat Commun, 2020; 11

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BOARD NUMBER: S03-508

BASAL GANGLIA FEEDBACK LOOPS AS POSSIBLE CANDIDATES FOR GENERATION OF BETA OSCILLATION

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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An increase in beta-band frequency (13-30 Hz) activity has been observed in different nuclei of the basal ganglia in Parkinson's disease (PD). However, which neural circuits give rise to these oscillations remains unclear. In this study, we computationally investigate the mechanisms responsible for the generations of beta oscillations that happen persistently in Dopamine depletion and also transiently in healthy rats. Our results show that using a rate model with realistic time constants, the classical STN-GPe recurrent network produces higher frequencies than the beta range and consequently is unlikely to be the source of these oscillations alone. We then propose two alternative circuits made of pallidostriatal feedback loops that can generate network oscillations close to this range. The dynamics of the loops are modeled using both the rate model as well as a spiking neural network with more precise time constants derived from experiments. We explore how each of these loops behaves around the point of transition between stable fixed point to the oscillatory regime depending on synaptic connection strengths. Then, one by one, we add the aforementioned loops together and explore how the frequency of the neural oscillations changes as a function of relative synaptic gains of the loops. Moreover, we show that with the pathophysiological changes due to Dopamine depletion in PD, the network can transition from a steady-state firing rate to the state of oscillating in beta-band frequencies. Our results show that it is plausible that the oscillations are a product of the interactions between multiple feedback loops.

Pubmed:
33306949: Aristieta A, Barresi M, Azizpour Lindi S, Barrière G, Courtand G, de la Crompe B, Guilhemsang L, Gauthier S, Fioramonti S, Baufreton J, Mallet NP
A Disynaptic Circuit in the Globus Pallidus Controls Locomotion Inhibition.
The basal ganglia (BG) inhibit movements through two independent circuits: the striatal neuron-indirect and the subthalamic nucleus-hyperdirect pathways. These pathways exert opposite effects onto external globus pallidus (GPe) neurons, whose functional importance as a relay has changed drastically with the discovery of two distinct cell types, namely the prototypic and the arkypallidal neurons. However, little is known about the synaptic connectivity scheme of different GPe neurons toward both motor-suppressing pathways, as well as how opposite changes in GPe neuronal activity relate to locomotion inhibition. Here, we optogenetically dissect the input organizations of prototypic and arkypallidal neurons and further define the circuit mechanism and behavioral outcome associated with activation of the indirect or hyperdirect pathways. This work reveals that arkypallidal neurons are part of a novel disynaptic feedback loop differentially recruited by the indirect or hyperdirect pathways and that broadcasts inhibitory control onto locomotion only when arkypallidal neurons increase their activity.
Curr Biol, 2021; 31
24125356: Montazeri Namin R, Azizpour Lindi S, Amjadi A, Jafari N, Irajizad P
Experimental investigation of the stability of the floating water bridge.
When a high voltage is applied between two beakers filled with deionized water, a floating bridge of water is formed in between exceeding the length of 2 cm when the beakers are pulled apart. Currently two theories regarding the stability of the floating water bridge exist, one suggesting that the tension caused by electric field in the dielectric medium is holding the bridge and the other suggesting surface tension to be responsible for the vertical equilibrium. We construct experiments in which the electric field and the geometry of the bridge are measured and compared with predictions of theories of the floating water bridge stability. We use a numerical simulation for estimation of the electric field. Our results indicate that the two forces of dielectric and surface tensions hold the bridge against gravity simultaneously and, having the same order of magnitude, neither of the two forces are negligible. In bridges with larger diameters, the effect of dielectric tension is slightly more in the vertical equilibrium than surface tension. Results show that the stability can be explained by macroscopic forces, regardless

of the microscopic changes in the water structure.
Phys Rev E Stat Nonlin Soft Matter Phys, 2013; 88

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TRANSVERS-PLANE-SNAPSHOT OF SIGNAL PROPAGATING NEURONAL PATHWAYS REVEALS RELATIONSHIP BETWEEN SPONTANEOUS AND EVOKED ACTIVITY IN A CULTURED NEURONAL CIRCUIT.

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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In cultured neuronal networks, a single current stimulus often evokes a relatively reproducible spike pattern. In the neuronal network, not only evoked response activity but also spontaneous electrical activity is expressed. The physical neural pathways expressing this spontaneous activity are thought to include multiple neuronal pathways expressing evoked responses. Therefore, the spatial pattern of spontaneous activity is a slice of the state in which several neural pathways are propagating signals in a fixed time window. In this study, rat hippocampal neurons from 18-day old Wistar Rat embryos were cultivated over 30 days on a MED probe provided with 64-multi-electrodes array. Firstly, we summarized the reproducible response activity, then all responses evoked by repeated electro stimulation were sliced into 1-ms-width-time-windows, which cannot include one or more transmitted signals between neurons. As a result, spikes were detected simultaneously at several channels in a 1-ms-width-timewindow, indicating that the signals evoked by a stimulus were propagating in a branched manner, and were transmitted along parallel pathways. The spatial pattern of spontaneous activity was snapshot of the signals propagating the neuronal pathways and composed of several transverse plane of branched and parallels pathways undertaking evoked activity. It suggests the possibility that evoked activity pattern based on spontaneous activity pattern.

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BOARD NUMBER: S03-510

A DIURNAL RHYTHM OF INTRACELLULAR CHLORIDE IN PYRAMIDAL NEURONS AFFECTS CORTICAL DYNAMICS

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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The main inhibitory synaptic currents, gated by gamma-aminobutyric acid (GABA), are mediated by Cl^--conducting channels, and are therefore sensitive to changes in the chloride electrochemical gradient. As GABAergic activity dictates neuronal firing, the intracellular chloride concentration ($[Cl^-]_i$) plays a major role in the regulation of neuronal activity. We measured $[Cl^-]_i$ with 2-photon LSSmClopHensor imaging in anaesthetized young adult mice, and we found a large physiological diurnal fluctuation of baseline $[Cl^-]_i$ in pyramidal neurons. This equates to a ~15mV positive shift in chloride equilibrium potential at times when mice are typically active (midnight), relative to their sleep phase (midday). Accordingly, the KCC2 chloride extruder shows reduced membrane expression and increased inactivating phosphorylation at night, when the NKCC1 chloride importer shows increased activating phosphorylation. We then demonstrated that at midnight: 1) optogenetic activation of parvalbumin interneurons in the visual cortex displays a more variable effect compared to the stable inhibiting activity observed at midday; 2) visually evoked gamma-band oscillations are reduced, and this can be rescued by the NKCC1 blocker bumetanide; 3) the brain cortex is more sensitive to the pro-epileptic drug 4-ammino pyridine, and this raised epileptogenicity is rescued by bumetanide.
These results shed light on a new scenario in which GABAergic transmission changes its efficacy according to $[Cl]_i$ also in the cortex of adult mammals following diurnal oscillations. As GABA exerts plenty of physiological actions, further analysis will be carried out to assess the implications of this finding.

Pubmed:
31381467: Pasquini G, Kunej T
A Map of the microRNA Regulatory Networks Identified by Experimentally Validated microRNA-Target Interactions in Five Domestic Animals: Cattle, Pig, Sheep, Dog, and Chicken.
Domestic animals are members of the broader ecological context, in which humans are situated. Yet, genomics and systems science research have lagged behind and been relatively underappreciated in domestic animals compared to human genetics/genomics. Harnessing big data calls for omics data mapping studies in a broad range of mammals. To this end, microRNAs (miRNAs) regulate posttranscriptional expression of target genes, hence, governing different biological pathways and physiological processes. The knowledge of miRNA regulatory networks and maps is important for understanding regulation of gene expression and functions in both humans and domestic animals. However, complete miRNA regulatory networks have not yet been described in all species, particularly in domestic animals. We report here an original analysis so as to map the miRNA regulatory networks in domestic animals based on miRNA-target interactions (MTIs). Validated MTIs for five species; cattle, pig, sheep, dog, and chicken were extracted from the miRTarBase. miRNA regulomes were visualized using the Cytoscape software. The data in cattle, chicken, and pig were sufficient to visualize networks, identify central molecules, and subnetworks associated with the same phenotype; however, the MTI data in dog and sheep are still limited. We found several hub genes with large number of interactions, for example, 1 miRNA (bta-miR-17-5p) interacting with 27 genes and 7 miRNAs interacting with the same gene (tumor necrosis factor []) in cattle. In addition, two single-nucleotide polymorphisms were identified within the seed region of a previously demonstrated MTI, namely, between (high mobility group box 3) gene and bta-miR-17-5p. In summary, this miRNA regulome mapping study will enable and guide further studies of genome function in mammals with a view to applications in human as well as veterinary medicine. Furthermore, these miRNA regulomes can help to clarify fundamental pathways in cell biology and reveal molecular insights on phenotypic trait variability in common complex diseases and response phenotypes of drugs or other health interventions for precision

medicine in the future.
OMICS, 2019; 23

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BOARD NUMBER: S03-511

GENERATING SLEEP OSCILLATIONS USING PRIMARY AND HIPSC-DERIVED THALAMO-CORTICAL CULTURES

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Slow oscillations, delta waves, and spindles are the major brain oscillations during non-rapid eye movement (NREM) sleep, arising from the thalamo-cortical circuitries. Whether the generation of these oscillatory patterns requires subcortical neuromodulatory inputs remain unknown. Using a high-density microelectrode array with 26400 electrodes, we recorded both single-unit activity and local field potential (LFP) of primary and hiPSC-derived cortical, thalamic, and thalamo-cortical co-cultures. We found that cell assemblies of these *in vitro* networks mimic oscillatory patterns of slow oscillations (<1Hz), delta waves (2-4 Hz), and spindles (9-16 Hz), respectively, similar to NREM sleep. Interestingly, spindles of thalamo-cortical co-cultures show strong coupling to the slow waves as *in vivo*, and slow waves travel across the cortical culture and are accompanied with fast gamma oscillations, as in the intact brain. Addition to cortical cultures of an antagonist of calcium-activated potassium channels suppressed the slow oscillation, suggesting a subcellular pathway for travelling of these oscillations. Furthermore, stimulation of these networks using wake-promoting neuromodulators induced desynchronized wake-like states in cultures. Our results demonstrate that NREM sleep with its oscillatory characteristics is the default state of the cortical and thalamo-cortical networks and highlight the potential applications of developing *in vitro* sleep models to answer basic questions in the field of sleep research.

Pubmed:
33087472: Facchin L, Schöne C, Mensen A, Bandarabadi M, Pilotto F, Saxena S, Libourel PA, Bassetti CLA, Adamantidis AR
Slow Waves Promote Sleep-Dependent Plasticity and Functional Recovery after Stroke.
Functional recovery after stroke is associated with a remapping of neural circuits. This reorganization is often associated with low-frequency, high-amplitude oscillations in the peri-infarct zone in both rodents and humans. These oscillations are reminiscent of sleep slow waves (SW) and suggestive of a role for sleep in brain plasticity that occur during stroke recovery; however, direct evidence is missing. Using a stroke model in male mice, we showed that stroke was followed by a transient increase in NREM sleep accompanied by reduced amplitude and slope of ipsilateral NREM sleep SW. We next used 5 ms optical activation of Channelrhodopsin 2-expressing pyramidal neurons, or 200 ms silencing of Archeorhodopsin T-expressing pyramidal neurons, to generate local cortical UP, or DOWN, states, respectively, both sharing similarities with spontaneous NREM SW in freely moving mice. Importantly, we found that single optogenetically evoked SW (SW) in the peri-infarct zone, randomly distributed during sleep, significantly improved fine motor movements of the limb corresponding to the sensorimotor stroke lesion site compared with spontaneous recovery and control conditions, while motor strength remained unchanged. In contrast, SW during wakefulness had no effect. Furthermore, chronic SW during sleep were associated with local axonal sprouting as revealed by the increase of anatomic presynaptic and postsynaptic markers in the peri-infarct zone and corresponding contralesional areas to cortical circuit reorganization during stroke recovery. These results support a role for sleep SW in cortical circuit plasticity and sensorimotor recovery after stroke and provide a clinically relevant framework for rehabilitation strategies using neuromodulation during sleep. Brain stroke is one of the leading causes of death and major disabilities in the elderly worldwide. A better understanding of the pathophysiological mechanisms underlying spontaneous brain plasticity after stroke, together with an optimization of rehabilitative strategies, are essential to improve stroke treatments. Here, we investigate the role of optogenetically induced sleep slow waves in an animal model of ischemic stroke and identify sleep as a window for poststroke intervention that promotes neuroplasticity and facilitates sensorimotor recovery.
J Neurosci, 2020; 40
33067436: Bandarabadi M, Herrera CG, Gent TC, Bassetti C, Schindler K, Adamantidis AR
A role for spindles in the onset of rapid eye movement sleep.
Sleep spindle generation classically relies on an interplay between the thalamic reticular nucleus (TRN), thalamo-cortical (TC) relay cells and cortico-thalamic (CT) feedback during non-rapid eye movement (NREM) sleep. Spindles are hypothesized to stabilize sleep, gate sensory processing and consolidate memory. However, the contribution of non-sensory thalamic nuclei

in spindle generation and the role of spindles in sleep-state regulation remain unclear. Using multisite thalamic and cortical LFP/unit recordings in freely behaving mice, we show that spike-field coupling within centromedial and anterodorsal (AD) thalamic nuclei is as strong as for TRN during detected spindles. We found that spindle rate significantly increases before the onset of rapid eye movement (REM) sleep, but not wakefulness. The latter observation is consistent with our finding that enhancing spontaneous activity of TRN cells or TRN-AD projections using optogenetics increase spindle rate and transitions to REM sleep. Together, our results extend the classical TRN-TC-CT spindle pathway to include non-sensory thalamic nuclei and implicate spindles in the onset of REM sleep.
Nat Commun, 2020; 11
32732431: Oesch LT, Gazea M, Gent TC, Bandarabadi M, Gutierrez Herrera C, Adamantidis AR
REM sleep stabilizes hypothalamic representation of feeding behavior.
During rapid eye movement (REM) sleep, behavioral unresponsiveness contrasts strongly with intense brain-wide neural network dynamics. Yet, the physiological functions of this cellular activation remain unclear. Using in vivo calcium imaging in freely behaving mice, we found that inhibitory neurons in the lateral hypothalamus (LH) show unique activity patterns during feeding that are reactivated during REM, but not non-REM, sleep. REM sleep-specific optogenetic silencing of LH cells induced a reorganization of these activity patterns during subsequent feeding behaviors accompanied by decreased food intake. Our findings provide evidence for a role for REM sleep in the maintenance of cellular representations of feeding behavior.
Proc Natl Acad Sci U S A, 2020; 117
32426821: Pace M, Colombi I, Falappa M, Freschi A, Bandarabadi M, Armirotti A, Encarnación BM, Adamantidis AR, Amici R, Cerri M, Chiappalone M, Tucci V
Loss of Snord116 alters cortical neuronal activity in mice: a preclinical investigation of Prader-Willi syndrome.
Prader-Willi syndrome (PWS) is a neurodevelopmental disorder that is characterized by metabolic alteration and sleep abnormalities mostly related to rapid eye movement (REM) sleep disturbances. The disease is caused by genomic imprinting defects that are inherited through the paternal line. Among the genes located in the PWS region on chromosome 15 (15q11-q13), small nucleolar RNA 116 (Snord116) has been previously associated with intrusions of REM sleep into wakefulness in humans and mice. Here, we further explore sleep regulation of PWS by reporting a study with PWScrm+/p- mouse line, which carries a paternal deletion of Snord116. We focused our study on both macrostructural electrophysiological components of sleep, distributed among REMs and nonrapid eye movements. Of note, here, we study a novel electroencephalography (EEG) graphoelements of sleep for mouse studies, the well-known spindles. EEG biomarkers are often linked to the functional properties of cortical neurons and can be instrumental in translational studies. Thus, to better understand specific properties, we isolated and characterized the intrinsic activity of cortical neurons using in vitro microelectrode array. Our results confirm that the loss of Snord116 gene in mice influences specific properties of REM sleep, such as theta rhythms and, for the first time, the organization of REM episodes throughout sleep-wake cycles. Moreover, the analysis of sleep spindles present novel specific phenotype in PWS mice, indicating that a new catalog of sleep biomarkers can be informative in preclinical studies of PWS.
Hum Mol Genet, 2020; 29
31749757: Bandarabadi M, Gast H, Rummel C, Bassetti C, Adamantidis A, Schindler K, Zubler F
Assessing Epileptogenicity Using Phase-Locked High Frequency Oscillations: A Systematic Comparison of Methods.
High frequency oscillations (HFOs) are traditional biomarkers to identify the epileptogenic tissue during presurgical evaluation in pharmacoresistant epileptic patients. Recently, the resection of brain tissue exhibiting coupling between the amplitude of HFOs and the phase of low frequencies demonstrated a more favorable surgical outcome. Here we compare the predictive value of ictal HFOs and four methods for quantifying the ictal phase-amplitude coupling, namely mean vector length, phase-locked high gamma, phase locking value, and modulation index (MI). We analyzed 32 seizures from 16 patients to identify the channels that exhibit HFOs and phase-locked HFOs during seizures. We compared the resection ratio, defined as the percentage of channels exhibiting coupling located in the resected tissue, with the postsurgical outcome. We found that the MI is the only method to show a significant difference between the resection ratios of patients with good and poor outcomes. We further show that the whole seizure, not only the onset, is critical to assess epileptogenicity using the phase-locked HFOs. We postulate that the superiority of MI stems from its capacity to assess coupling of discrete HFO events and its independence from the HFO power. These results confirm that quantitative analysis of HFOs can boost presurgical evaluation and indicate the paramount importance of algorithm selection for clinical applications.
Front Neurol, 2019; 10
31410477: Bandarabadi M, Boyce R, Gutierrez Herrera C, Bassetti CL, Williams S, Schindler K, Adamantidis A
Dynamic modulation of theta-gamma coupling during rapid eye movement sleep.
Theta phase modulates gamma amplitude in hippocampal networks during spatial navigation and rapid eye movement (REM) sleep. This cross-frequency coupling has been linked to working memory and spatial memory consolidation; however, its

spatial and temporal dynamics remains unclear. Here, we first investigate the dynamics of theta-gamma interactions using multiple frequency and temporal scales in simultaneous recordings from hippocampal CA3, CA1, subiculum, and parietal cortex in freely moving mice. We found that theta phase dynamically modulates distinct gamma bands during REM sleep. Interestingly, we further show that theta-gamma coupling switches between recorded brain structures during REM sleep and progressively increases over a single REM sleep episode. Finally, we show that optogenetic silencing of septohippocampal GABAergic projections significantly impedes both theta-gamma coupling and theta phase coherence. Collectively, our study shows that phase-space (i.e. cross-frequency coupling) coding of information during REM sleep is orchestrated across time and space consistent with region-specific processing of information during REM sleep including learning and memory.
Sleep, 2019; 42
30998681: Miladinović Đ, Muheim C, Bauer S, Spinnler A, Noain D, Bandarabadi M, Gallusser B, Krummenacher G, Baumann C, Adamantidis A, Brown SA, Buhmann JM
SPINDLE: End-to-end learning from EEG/EMG to extrapolate animal sleep scoring across experimental settings, labs and species.
Understanding sleep and its perturbation by environment, mutation, or medication remains a central problem in biomedical research. Its examination in animal models rests on brain state analysis via classification of electroencephalographic (EEG) signatures. Traditionally, these states are classified by trained human experts by visual inspection of raw EEG recordings, which is a laborious task prone to inter-individual variability. Recently, machine learning approaches have been developed to automate this process, but their generalization capabilities are often insufficient, especially across animals from different experimental studies. To address this challenge, we crafted a convolutional neural network-based architecture to produce domain invariant predictions, and furthermore integrated a hidden Markov model to constrain state dynamics based upon known sleep physiology. Our method, which we named SPINDLE (Sleep Phase Identification with Neural networks for Domain-invariant LEearning) was validated using data of four animal cohorts from three independent sleep labs, and achieved average agreement rates of 99%, 98%, 93%, and 97% with scorings from five human experts from different labs, essentially duplicating human capability. It generalized across different genetic mutants, surgery procedures, recording setups and even different species, far exceeding state-of-the-art solutions that we tested in parallel on this task. Moreover, we show that these scored data can be processed for downstream analyzes identical to those from human-scored data, in particular by demonstrating the ability to detect mutation-induced sleep alteration. We provide to the scientific community free usage of SPINDLE and benchmarking datasets as an online server at https://sleeplearning.ethz.ch. Our aim is to catalyze high-throughput and well-standardized experimental studies in order to improve our understanding of sleep.
PLoS Comput Biol, 2019; 15
30158846: Luca G, Bandarabadi M, Konofal E, Lecendreux M, Ferrié L, Figadère B, Tafti M
Lauflumide (NLS-4) Is a New Potent Wake-Promoting Compound.
Psychostimulants are used for the treatment of excessive daytime sleepiness in a wide range of sleep disorders as well as in attention deficit hyperactivity disorder or cognitive impairment in neuropsychiatric disorders. Here, we tested in mice the wake-promoting properties of NLS-4 and its effects on the following sleep as compared with those of modafinil and vehicle. C57BL/6J mice were intraperitoneally injected with vehicle, NLS-4 (64 mg/kg), or modafinil (150 mg/kg) at light onset. EEG and EMG were recorded continuously for 24 h after injections and vigilance states as well as EEG power densities were analyzed. NLS-4 at 64 mg/kg induced significantly longer wakefulness duration than modafinil at 150 mg/kg. Although no significant sleep rebound was observed after sleep onset for both treatments as compared with their vehicles, modafinil-treated mice showed significantly more NREM sleep when compared to NLS-4. Spectral analysis of the NREM EEG after NLS-4 treatment indicated an increased power density in delta activity (0.75-3.5 Hz) and a decreased power in theta frequency range (6.25-7.25 Hz), while there was no differences after modafinil treatment. Also, time course analysis of the delta activity showed a significant increase only during the first 2 time intervals of sleep after NLS-4 treatment, while delta power was increased during the first 9 time intervals after modafinil. Our results indicate that NLS-4 is a highly potent wake-promoting drug with no sign of hypersomnia rebound. As opposed to modafinil, recovery sleep after NLS-4 treatment is characterized by less NREM amount and delta activity, suggesting a lower need for recovery despite longer drug-induced wakefulness.
Front Neurosci, 2018; 12
29892048: Gent TC, Bandarabadi M, Herrera CG, Adamantidis AR
Thalamic dual control of sleep and wakefulness.
Slow waves (0.5-4 Hz) predominate in the cortical electroencephalogram during non-rapid eye movement (NREM) sleep in mammals. They reflect the synchronization of large neuronal ensembles alternating between active (UP) and quiescent (Down) states and propagating along the neocortex. The thalamic contribution to cortical UP states and sleep modulation remains unclear. Here we show that spontaneous firing of centromedial thalamus (CMT) neurons in mice is phase-advanced to global cortical UP states and NREM-wake transitions. Tonic optogenetic activation of CMT neurons induces NREM-wake

transitions, whereas burst activation mimics UP states in the cingulate cortex and enhances brain-wide synchrony of cortical slow waves during sleep, through a relay in the anterodorsal thalamus. Finally, we demonstrate that CMT and anterodorsal thalamus relay neurons promote sleep recovery. These findings suggest that the tonic and/or burst firing pattern of CMT neurons can modulate brain-wide cortical activity during sleep and provides dual control of sleep-wake states.
Nat Neurosci, 2018; 21

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BOARD NUMBER: S03-512

STATE-SPECIFIC ACTIVITY OF PYRAMIDAL CELLS AND INTERNEURONS IN SUPRAGRANULAR NEOCORTICAL LAYERS DURING NATURAL SLEEP AND WAKEFULNESS

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Rhythmic population activity of neocortical neurons is linked to behavioral states. We applied a drug-free technique of juxtacellular recording/labeling to determine contributions of neocortical pyramidal cells (PYR) regular spiking interneurons (RSIs) and fast spiking interneurons (FSIs) in neural oscillations in freely behaving rats during natural sleep and awake states. In general, layer2 and 3 PYRs (n=40) showed sporadic activity in non-REM sleep with moderate spindle phase coupled firing detected in layer3 PYRs. Sporadic and phase unrelated firing was characteristic of layer2 PYRs during theta oscillations in REM sleep with a moderate increase in firing in theta oscillations of quiet wakefulness. Layer3 PYRs showed phase related packets of sporadic firing in REM theta with a decreased activity in quiet wakefulness. Rhythmic activity was observed in all FSIs (n=32) and most RSIs (n=27 out of 34) during spindles with FSI and RSI firing locked to different phases of spindle cycles, respectively. RSIs (n=14) showed elevated firing during REM sleep theta relative to nonREM episodes and theta phase relatedness in REM was preserved during awake theta in n=4 cells. Most interneurons (both FSI, n=18 and RSI, n=20) showed a shift in phase preference in spindle vs. theta oscillations, but a minority of individual FSIs (n=3) and RSIs (n=5) fired at the same phase of spindle and theta. In conclusion, the sporadic firing of layer2 and 3 PYRs is accompanied by heterogeneous involvement of FSIs and RSIs during nonREM, REM sleep and revealed a prominent contribution of RSIs to theta periods relative to FSIs.

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BOARD NUMBER: S03-513

GABA-A RHYTHMIC INHIBITION IN THE CLAUSTRUM DRIVES NETWORK OSCILLATION

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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The claustrum and the endopiriform nucleus (DEN) form a sheet-like structure below the insular cortex. Thanks to a variety of interneurons and far-projecting excitatory claustrocortical neurons, it is the most connected structure in the forebrain. This brain hub is not well understood yet, despite its presence in amniotes, including reptiles and mammals (Norimoto et al., 2020). It modulates neocortical sleep slow-wave synchronization (Narikiyo et al., 2020) and may mediate the unified sense of cognition (Crick and Koch, 2005) and attention (Atlan et al., 2018; Fodoulian et al., 2021). In mouse brain slices containing portions of the claustrum/DEN and insula, we found that claustral principal cells undergo a 0,3-1 Hz network oscillation under cholinergic modulation. To examine the mechanisms underlying this oscillation we performed voltage-clamp protocols in neurons from wild-type and transgenic mice. In putative principal claustral neurons, we found a clear rhythmicity of inhibitions, while excitatory post-synaptic currents distribution remained mostly non-rhythmic. Rhythmic inhibitions were driven through chloride and not potassium conductances, suggesting the involvement of GABA-A receptors. In current-clamp, rhythmic burst firing of action potentials in principal cells was generated by excitatory inputs and terminated by inhibitions. These experiments suggest a pacemaker role for inhibitory neurons under cholinergic modulation of the network. We also recorded a subset of claustral neurons with endogenous rhythmic behavior. Whether these (inter)neurons play a pacemaker role in the network oscillation, and what kinds of synaptic loops there are within the claustrum and/or between the claustrum and the adjacent insular cortex, remains to be examined.

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INVESTIGATION OF THE ROLE OF MIDLINE THALAMIC NEURONS IN HIPPOCAMPO-CORTICAL COUPLING DURING SLOW WAVE SLEEP

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Memory consolidation is a process of stabilisation of information in two stages: the fast synaptic consolidation and the gradual system consolidation. The latter consists in the reorganisation of information and its transfer from temporary storage in the hippocampus to long-term storage in the neocortex. Sleep, and in particular its deepest stage called slow wave sleep, is thought to play a crucial role in memory consolidation. Evidence suggest that information transfer could occur through the temporal coupling of hippocampal ripples and cortical spindles. We recently showed that midline thalamic neurons synchronise neocortical slow oscillations during slow wave sleep. Here, we hypothesise that through synchronising cortical activity, midline thalamus mediates coordination of spindles and ripples. To investigate the role of midline NECAB-1 thalamic neurons in hippocampo-cortical coupling, and its role in memory consolidation during slow wave sleep, we conducted a spatial memory task called displaced object recognition. After exposing the mice to a set of objects, we optogenetically or chemogenetically inhibited NECAB-1 thalamic neurons during the first hours of sleep, while recording the local field potential signals in multiple cortices and hippocampus. We hypothesize that inhibiting NECAB-1 thalamic neurons will impede spindles-ripples coordination and thus hindering the memory consolidation of spatial memory.

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BOARD NUMBER: S03-515

ARE SPONTANEOUS SPIKES OF THE MITRAL CELLS OF THE RODENT OLFACTORY BULB MODULATED BY INTRINSIC THETA OSCILLATIONS?

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Both spontaneous and odor-evoked network activity in rodent olfactory bulbs (OB) are patterned by respiration, resulting in oscillations within the theta regime. Previously, we observed theta rhythms in local field potential recordings (LFP) in semi-intact nose-brain preparations of rats that are uncoupled from respiration (Perez et al. 2015). Thus, we hypothesize that the respiratory theta rhythm taps into an intrinsic theta resonance of the bulbar network.
Here we investigate the properties of these intrinsic theta rhythms. First, we frequently observed harmonics of theta, as recently reported from hippocampal LFPs . We also observed that both frequency and power of theta rhythms were stable, persisting for at least one hour (average frequency 2.1 ± 0.3 Hz, n=10).
Moreover, theta frequency did not change across all bulbar locations recorded from (up to nine), suggesting that the frequency is homogenous within the entire OB network (n=14). Next, we asked if the spontaneous spiking activity recorded near the mitral cell layer is correlated with the ongoing theta rhythm. We developed an algorithm to isolate the spikes to determine their phase relative to the LFP theta, and applied circular statistical tests to check for preferred phases of firing. We observed significant correlations between spike-phase distributions and LFP theta within subsets of one-minute recording intervals. Moreover, our results align well with in vivo recordings showing a correlation between mitral cell spontaneous activity and breathing (Fukunaga et al., 2012) and prove that the preparation allows to investigate rhythmic bulbar activity at the single cell level.

Pubmed:
29561201: Jammal L, Whalley B, Barkai E
Learning-induced modulation of the effect of neuroglial transmission on synaptic plasticity.
Training rats in a complex olfactory discrimination task results in acquisition of "rule learning" (learning how to learn), a term describing the capability to perform the task superbly. Such rule learning results in strengthening of both excitatory and inhibitory synaptic connections between neurons in the piriform cortex. Moreover, intrinsic excitability is also enhanced throughout the pyramidal neuron population. Surprisingly, the cortical network retains its stability under these long-term modifications. In particular, the susceptibility for long-term potentiation (LTP) induction, while decreased for a short time window, returns to almost its pretraining value, although significant strengthening of AMPA receptor-mediated glutamatergic transmission remains. Such network balance is essential for maintaining the single-cell modifications that underlie long-term memory while preventing hyperexcitability that would result in runaway synaptic activity. However, the mechanisms underlying the long-term maintenance of such balance have yet to be described. In this study, we explored the role of astrocyte-mediated gliotransmission in long-term maintenance of learning-induced modifications in susceptibility for LTP induction and control of the strength of synaptic inhibition. We show that blocking connexin 43 hemichannels, which form gap junctions between astrocytes, decreases significantly the ability to induce LTP by stimulating the excitatory connections between piriform cortex pyramidal neurons after learning only. In parallel, spontaneous miniature inhibitory postsynaptic current amplitude is reduced in neurons from trained rats only, to the level of prelearning. Thus gliotransmission has a key role in maintaining learning-induced cortical stability by a wide-ranged control on synaptic transmission and plasticity. NEW & NOTEWORTHY We explore the role of astrocyte-mediated gliotransmission in maintenance of olfactory discrimination learning-induced modifications. We show that blocking gap junctions between astrocytes decreases significantly the ability to induce long-term potentiation in the piriform cortex after learning only. In parallel, synaptic inhibition is reduced in neurons from trained rats only, to the level of prelearning. Thus gliotransmission has a key role in maintaining learning-induced cortical stability by a wide-ranged control on synaptic transmission and plasticity.
J Neurophysiol, 2018; 119
24598518: Kfir A, Ohad-Giwnewer N, Jammal L, Saar D, Golomb D, Barkai E
Learning-induced modulation of the GABAB-mediated inhibitory synaptic transmission: mechanisms and functional

significance.
Complex olfactory-discrimination (OD) learning results in a series of intrinsic and excitatory synaptic modifications in piriform cortex pyramidal neurons that enhance the circuit excitability. Such overexcitation must be balanced to prevent runway activity while maintaining the efficient ability to store memories. We showed previously that OD learning is accompanied by enhancement of the GABAA-mediated inhibition. Here we show that GABAB-mediated inhibition is also enhanced after learning and study the mechanism underlying such enhancement and explore its functional role. We show that presynaptic, GABAB-mediated synaptic inhibition is enhanced after learning. In contrast, the population-average postsynaptic GABAB-mediated synaptic inhibition is unchanged, but its standard deviation is enhanced. Learning-induced reduction in paired pulse facilitation in the glutamatergic synapses interconnecting pyramidal neurons was abolished by application of the GABAB antagonist CGP55845 but not by blocking G protein-gated inwardly rectifying potassium channels only, indicating enhanced suppression of excitatory synaptic release via presynaptic GABAB-receptor activation. In addition, the correlation between the strengths of the early (GABAA-mediated) and late (GABAB-mediated) synaptic inhibition was much stronger for each particular neuron after learning. Consequently, GABAB-mediated inhibition was also more efficient in controlling epileptic-like activity induced by blocking GABAA receptors. We suggest that complex OD learning is accompanied by enhancement of the GABAB-mediated inhibition that enables the cortical network to store memories, while preventing uncontrolled activity.
J Neurophysiol, 2014; 111
27449811: Jammal L, Whalley B, Ghosh S, Lamrecht R, Barkai E
Physiological expression of olfactory discrimination rule learning balances whole-population modulation and circuit stability in the piriform cortex network.
Once trained, rats are able to execute particularly difficult olfactory discrimination tasks with exceptional accuracy. Such skill acquisition, termed "rule learning", is accompanied by a series of long-lasting modifications to three cellular properties which modulate pyramidal neuron activity in piriform cortex; intrinsic excitability, synaptic excitation, and synaptic inhibition. Here, we explore how these changes, which are seemingly contradictory at the single-cell level in terms of their effect on neuronal excitation, are manifested within the piriform cortical neuronal network to store the memory of the rule, while maintaining network stability. To this end, we monitored network activity via multisite extracellular recordings of field postsynaptic potentials (fPSPS) and with single-cell recordings of miniature inhibitory and excitatory synaptic events in piriform cortex slices. We show that although 5 days after rule learning the cortical network maintains its basic activity patterns, synaptic connectivity is strengthened specifically between spatially proximal cells. Moreover, while the enhancement of inhibitory and excitatory synaptic connectivity is nearly identical, strengthening of synaptic inhibition is equally distributed between neurons while synaptic excitation is particularly strengthened within a specific subgroup of cells. We suggest that memory for the acquired rule is stored mainly by strengthening excitatory synaptic connection between close pyramidal neurons and runaway synaptic activity arising from this change is prevented by a nonspecific enhancement of synaptic inhibition.
Physiol Rep, 2016; 4

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TEMPORAL DISPARITY OF ACTION POTENTIALS TRIGGERED IN AXON INITIAL SEGMENTS AND DISTAL AXONS IN THE NEOCORTEX

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Neural population activity determines the timing of synaptic inputs, which arrive to dendrites, cell bodies and axon initial segments (AISs) of cortical neurons. Action potentials in the AIS (AIS-APs) are driven by input integration, and the phase preference of AIS-APs during network oscillations is characteristic to cell classes. Distal regions of cortical axons do not receive synaptic inputs, yet experimental induction protocols can trigger reatroaxonal action potentials (RA-APs) in axons distal from the soma. We report spontaneously occurring RA-APs in human and rodent cortical interneurons that appear uncorrelated to inputs and population activity. Network linked triggering of AIS-APs versus network independent timing of RA-APs of the same interneurons result in disparate temporal contribution of a single cell to in vivo network operation through perisomatic and distal axonal firing.

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BOARD NUMBER: S03-517

SIMULTANEOUS LFP RECORDING AND CALCIUM IMAGING OF CORTICAL LAYER I NDNF+ INTERNEURONS IN FREELY-MOVING MICE DURING SLEEP

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Cortical layer I (L1) contains a sparse population of interneurons expressing NDNF. They receive long-range projections from association cortices, higher-order thalamic nuclei and neuromodulatory nuclei and control the local cortical activity through extensive projections on the distal dendrites of pyramidal cells located in deeper layers. Previous studies have identified L1 NDNF+ interneurons as key for long-term associative memory. Because oscillations in the Local Field Potential (LFP) during sleep – namely Sharp-Wave Ripples, thalamic spindles and slow oscillations – are thought to play a causal role in memory consolidation, we hypothesise that L1 interneurons could take part in coordinating these oscillations. To do so, we use a miniaturised wide-field fluorescence microscope to image calcium activity of L1 NDNF+ interneurons in the parietal and retrosplenial cortices while simultaneously recording LFP in CA1 hippocampus, prefrontal and somatosensory cortices in freely-moving mice during sleep. We aim to study the calcium activity of L1 NDNF+ interneurons relative to SWR and spindles, and how the relationships between their calcium activity and sleep oscillations may evolve throughout a progressive learning task.

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BOARD NUMBER: S03-518

A5-CONTAINING NICOTINIC ACETYLCHOLINE RECEPTOR REGULATION OF FAST GABAA-MEDIATED INHIBITION DURING UP AND DOWN STATES

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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AIMS: Cortical “slow oscillations”, a form of spontaneous network activity, observed during quiescent brain states, are successive epochs of a depolarized “Up state” and a hyperpolarized “Down state”. Up and Down state (UDS) activity is a feature of both developing and mature cortical networks and is considered the default activity of the cerebral cortex. The aim of this study was to investigate the role of a5-containing nicotinic acetylcholine receptor (nAChR) in regulating GABAa-mediated inhibition during UDS in young mice. METHODS: Our study was conducted in acute brain slices (Layer II/III, S1bf cortex) of wild type (WT) and ACNA5 (a5-nAChR knock out) mice (18-25 days old) and UDSs were assessed by simultaneous local field potential (LFP) and intracellular (IC) recordings. 200nM of gabazine was used for the blockade of GABAa receptors, and voltage clamp (VC) recordings of pyramidal neurons were conducted to further investigate GABAa-mediated currents (sIPSCs and mIPSCs). RESULTS: We show that the effect of gabazine was genotype-dependent; under GABAa blockade, the presence of a5-nAChR in WT mice leads to shrinkage of UDSs and transition of network activity into epileptiform spike-and-wave discharges (SWDs); however ACNA5 mice retain UDS activity and exhibit a low occurrence of SWDs. Our VC recordings showed that pyramidal neurons of layer II/III receive differential inhibitory input, which depends on the presence (WT) or absence (ACNA5) of a5-nAChR. CONCLUSIONS: These results indicate that a5-subunit-mediated cholinergic regulation of GABAa receptors is crucial for the maintenance of UDS.

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NORADRENERGIC MEDIATION OF HIPPOCAMPAL THETA RHYTHM INDUCED BY VAGAL NERVE STIMULATION

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Noradrenergic mediation of hippocampal theta rhythm induced by vagal nerve stimulation Aim: Vagal nerve stimulation (VNS) is recognized as a low risk therapy for patients suffering from a broad spectrum of central nervous system disturbances. We demonstrated that theta rhythm can be an effective biomarker of central VNS disorders. Specifically, VNS was found to produce hippocampal (HPC) theta rhythm. We also provided data demonstrating that VNS-induced theta is mediated by cholinergic and GABAergic nature. The noradrenergic mediation of VNS-induced theta rhythm has never been tested. Hence, the present study was undertaken to identify the noradrenergic receptors profile mediating the production of VNS-induced theta in anesthetized rats. **Methods:** The experiments were performed on urethanized male Wistar rats. All animals were implanted with a VNS cuff electrode on the left vagal nerve, a tungsten microelectrode for recording the hippocampal field activity, and a cannula for the drug injections. **Results:** The intrahimpocampal microinjection of selective $alpha_1$-, $alpha_2$-, $beta_1$-adrenergic agonists (phenylephrine, tizanidine and dobutamine respectively) diminish VNS-induced theta rhythm in HPC. Injection of fenoterol and BRL37344 ($beta_2$-and $beta_3$-adrenergic agonists) did not affect theta field activity. **Conclusion**: These data provide evidences that only $alpha_1$, $alpha_2$, $beta_1$-adrenergic receptors localized in HPC are involved in the pharmacological mechanisms responsible for the production of VNS-induced theta rhythm. The remaining hippocampal noradrenergic receptors ($beta_2$- and $beta_3$) do not seem to be directly involved in the mechanism responsible for VNS-induced hippocampal theta rhythm. *This research project was supported by Neuromedical Ltd.*

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MULTIELECTRODE RECORDINGS IN THE POSTERIOR HYPOTHALAMUS OF FREELY MOVING RATS: THETA RHYTHM IN THE SUPRAMAMMILLARY AND POSTERIOR HYPOTHALAMIC NUCLEI

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Rodent theta rhythm is best known for its appearance in the hippocampus (HPC). The presence of HPC theta is a result of the activation of the supramammillary (SuM) and posterior hypothalamic (PH) nuclei. They both send projections to the HPC and facilitate the appearance of theta rhythm in the structure. Additionally, theta rhythm can also be recorded locally from both the SuM and PH. However, very little is known about this posterior hypothalamic theta rhythm, its role and how the SuM and PH differently contribute to its local or hippocampal oscillation genesis. AIMS Investigation of the differences in theta oscillations between the SuM and PH nuclei in freely moving rats. METHODS Eight adult Wistar rats were implanted with a 16-channel multielectrode for chronic local field potential recordings. The recording sites were the supramammillary and posterior hypothalamic nuclei. Basic parameters of theta oscillations, i.e. frequency, amplitude, power of dominant frequency, were later analyzed and compared between the SuM and PH nuclei. RESULTS Clear oscillations in theta range (3-12 Hz) were observed in all rats tested in both the SuM and PH nuclei. No evident differences were observed in terms of theta rhythm parameters depending on the recording site. CONCLUSIONS Lack of clear differences between SuM and PHa local theta characteristics raises the question about the mechanism of posterior hypothalamic theta rhythm generation and its role in driving the HPC theta rhythm. *Supported by National Science Centre Poland: UMO-2017/25/B/NZ4/01476.*

Pubmed:
34948401: Kazmierska-Grebowska P, Siwiec M, Sowa JE, Caban B, Kowalczyk T, Bocian R, MacIver MB
Lamotrigine Attenuates Neuronal Excitability, Depresses GABA Synaptic Inhibition, and Modulates Theta Rhythms in Rat Hippocampus.
Theta oscillations generated in hippocampal (HPC) and cortical neuronal networks are involved in various aspects of brain function, including sensorimotor integration, movement planning, memory formation and attention. Disruptions of theta rhythms are present in individuals with brain disorders, including epilepsy and Alzheimer's disease. Theta rhythm generation involves a specific interplay between cellular (ion channel) and network (synaptic) mechanisms. HCN channels are theta modulators, and several medications are known to enhance their activity. We investigated how different doses of lamotrigine (LTG), an HCN channel modulator, and antiepileptic and neuroprotective agent, would affect HPC theta rhythms in acute HPC slices (in vitro) and anaesthetized rats (in vivo). Whole-cell patch clamp recordings revealed that LTG decreased GABA-fast transmission in CA3 cells, in vitro. In addition, LTG directly depressed CA3 and CA1 pyramidal neuron excitability. These effects were partially blocked by ZD 7288, a selective HCN blocker, and are consistent with decreased excitability associated with antiepileptic actions. Lamotrigine depressed HPC theta oscillations in vitro, also consistent with its neuronal depressant effects. In contrast, it exerted an opposite, enhancing effect, on theta recorded in vivo. The contradictory in vivo and in vitro results indicate that LTG increases ascending theta activating medial septum/entorhinal synaptic inputs that over-power the depressant effects seen in HPC neurons. These results provide new insights into LTG actions and indicate an opportunity to develop more precise therapeutics for the treatment of dementias, memory disorders and epilepsy.
Int J Mol Sci, 2021; 22
34271089: Kowalczyk T, Staszelis A, Kaźmierska-Grębowska P, Tokarski K, Caban B
The Role of the Posterior Hypothalamus in the Modulation and Production of Rhythmic Theta Oscillations.
Theta rhythm recorded as an extracellular synchronous field potential is generated in a number of brain sites including the hippocampus. The physiological occurrence of hippocampal theta rhythm is associated with the activation of a number of structures forming the ascending brainstem-hippocampal synchronizing pathway. Experimental evidence indicates that the supramammillary nucleus and posterior hypothalamic nuclei, considered as the posterior hypothalamic area, comprise a critical node of this ascending pathway. The posterior hypothalamic area plays an important role in movement control, place-learning, memory processing, emotion and arousal. In the light of multiplicity of functions of the posterior hypothalamic area and the influence of theta field oscillations on a number of neural processes, it is the authors' intent to summarize the data

concerning the involvement of the supramammillary nucleus and posterior hypothalamic nuclei in the modulation of limbic theta rhythmicity as well as the ability of these brain structures to independently generate theta rhythmicity.
Neuroscience, 2021; 470
33739386: Salamian A, Legutko D, Nowicka K, Badyra B, Kaźmierska-Grębowska P, Caban B, Kowalczyk T, Kaczmarek L, Beroun A
Inhibition of Matrix Metalloproteinase 9 Activity Promotes Synaptogenesis in the Hippocampus.
Information coding in the hippocampus relies on the interplay between various neuronal ensembles. We discovered that the application of a cholinergic agonist, carbachol (Cch), which triggers oscillatory activity in the gamma range, induces the activity of matrix metalloproteinase 9 (MMP-9)-an enzyme necessary for the maintenance of synaptic plasticity. Using electrophysiological recordings in hippocampal organotypic slices, we show that Cch potentiates the frequency of miniature inhibitory and excitatory postsynaptic currents (mIPSCs and mEPSCs, respectively) in CA1 neurons and this effect is MMP-9 dependent. Interestingly, though MMP-9 inhibition prevents the potentiation of inhibitory events, it further boosts the frequency of excitatory mEPSCs. Such enhancement of the frequency of excitatory events is a result of increased synaptogenesis onto CA1 neurons. Thus, the function of MMP-9 in cholinergically induced plasticity in the hippocampus is to maintain the fine-tuned balance between the excitatory and the inhibitory synaptic transmission.
Cereb Cortex, 2021; 31
30502054: Urbanska M, Kazmierska-Grebowska P, Kowalczyk T, Caban B, Nader K, Pijet B, Kalita K, Gozdz A, Devijver H, Lechat B, Jaworski T, Grajkowska W, Sadowski K, Jozwiak S, Kotulska K, Konopacki J, Van Leuven F, van Vliet EA, Aronica E, Jaworski J
GSK3β activity alleviates epileptogenesis and limits GluA1 phosphorylation.
Glycogen synthase kinase-3β (GSK3β) is a key regulator of cellular homeostasis. In neurons, GSK3β contributes to the control of neuronal transmission and plasticity, but its role in epilepsy remains to be defined.
EBioMedicine, 2019; 39
30027636: Caban B, Staszelis A, Kazmierska P, Kowalczyk T, Konopacki J
Postnatal Development of the Posterior Hypothalamic Theta Rhythm and Local Cell Discharges in Rat Brain Slices.
Theta rhythms have been recorded from rat brain slices of the posterior hypothalamic area (PHa), including the supramammillary and posterior hypothalamic nuclei. Additionally, in numerous studies theta-related neurons were identified in the PHa according to the classification of Bland and Colom (Progress in Neurobiology, 41, 157-208, 1993). It is currently widely accepted that the PHa contributes to the process of HPC theta frequency programming at least in certain behavioral states. The postnatal development of the HPC and its ability to generate theta has also been a subject of studies. Specifically, it was found that theta oscillations are present in the HPC of 8-10 days old rat pups and turn into a well-synchronized and high-amplitude activity in the following few days. In our current study, we therefore focused on the postnatal development of cholinergically-induced theta rhythm and theta-related neuronal activity in PHa slices obtained from 8 to 24 days old rat pups. Theta activity was observed in the PHa preparations at the age of 8-10 days and then progressively increased its probability of occurrence, amplitude and synchrony up to the age of 22-24 days when it reached a plateau phase. A steady increase in the number of recorded neurons correlated with local theta oscillations was also observed.
Dev Neurobiol, 2018; 78
27421240: Bocian R, Caban B, Kłos-Wojtczak P, Konopacki J, Kowalczyk T
Is electrical coupling involved in the generation of posterior hypothalamic theta rhythm?
Data obtained in in vitro experiments and urethane anaesthetized animals have revealed that the mechanisms responsible for the generation of hippocampal cholinergic theta rhythm are specifically affected by the administration of broad spectrum gap junctions (GJs) blocker - carbenoxolone (CBX). The aim of this study was to examine the effect of GJs modulation on the production of posterior hypothalamic theta. Specifically, we were interested in evaluating whether CBX could attenuate the theta rhythm recorded from the supramammillary nucleus and posterior hypothalamic nuclei, in both in vitro and in vivo preparations. The data we obtained from in vitro and in vivo preparations demonstrated that the administration of CBX did not suppress cholinergically induced theta in posterior hypothalamic area (PHa) slices nor the theta rhythm observed in the PHa of urethane anaesthetized rats. Moreover, the application of trimethylamine, while very effective in the enhancement of hippocampal theta rhythm, did not produce any changes in theta oscillations observed in either in vitro or in vivo posterior hypothalamic area preparations. These data show that electrical coupling via GJs is not involved in theta rhythm generation in the PHa. Surprisingly, we observed a significant enhancement of theta activity in response to the carbenoxolone administration in both in vitro and in vivo PHa preparations. The theta rhythm enhancement detected in those experiments was attenuated by the application of spironolactone (mineralocorticoid receptors antagonist). We suggest that the observed excitatory effects of CBX on posterior hypothalamic oscillatory activity in the theta band could be mediated by mineralocorticoid receptors.
Eur J Neurosci, 2016; 44

27326660: Bocian R, Kłos-Wojtczak P, Caban B, Kowalczyk T, Kaźmierska P, Konopacki J
Cell discharge correlates of posterior hypothalamic theta rhythm recorded in anesthetized rats and brain slices.
Kowalczyk et al. (Hippocampus 2014; 24:7-20) were probably the first to conduct a systemic study of posterior hypothalamic area (PHa) theta rhythm in anesthetized rats. They demonstrated that local PHa theta field potentials were tail-pinch resistant and could be generated in urethane-anesthetized rats independently of ongoing hippocampal formation theta rhythm. These in vivo data were also confirmed in PHa slice preparations perfused with cholinergic agonist, carbachol. In the current experiments we extend our earlier observations concerning PHa theta rhythm. Specifically, PHa field potentials were analyzed in relation to the ongoing local cell firing repertoire. Single-unit discharge patterns of cells localized in the posterior hypothalamic and supramammillary nuclei were characterized according to the criteria that was developed previously to classify theta-related cells in the hippocampal formation. The present study demonstrated that in addition to the earlier described theta-related cells (theta-on, theta-off and gating cells) the PHa also contains cells discharging in a very regular manner, which were labelled "timing cells". This type of neuron has not been previously documented. We suggest that "timing cells" form a part of the ascending brainstem synchronizing pathway, provideing a regular rhythmic signal which facilitates the transduction of tonic discharges of cells localized in the brain stem into theta-frequency rhythmic discharges. © 2016 Wiley Periodicals, Inc.
Hippocampus, 2016; 26
23836546: Kowalczyk T, Bocian R, Caban B, Konopacki J
Atropine-sensitive theta rhythm in the posterior hypothalamic area: in vivo and in vitro studies.
Theta rhythm is the largest, most prominent, and well-documented electroencephalography activity present in a number of mammals, including humans. Spontaneous theta activity recorded locally in the posterior hypothalamic area (PHa) has never been the subject of detailed studies. The authors have shown that local theta field potentials could be generated in urethane-anesthetized rats in the supramammillary (SuM) nuclei and posterior hypothalamic (PH) nuclei. Theta recorded in the PHa was produced independently of simultaneously occurring hippocampal theta. These data were confirmed in the PHa maintained in vitro. Local theta field activity was recorded in the SuM and PH nuclei of PHa slice preparations perfused with cholinergic agonist carbachol. Both in vivo and in vitro recorded PHa theta rhythmicity had a cholinergic-muscarinic profile, that is, it was antagonized by muscarinic antagonist atropine sulfate.
Hippocampus, 2014; 24
23724051: Macias M, Blazejczyk M, Kazmierska P, Caban B, Skalecka A, Tarkowski B, Rodo A, Konopacki J, Jaworski J
Spatiotemporal characterization of mTOR kinase activity following kainic acid induced status epilepticus and analysis of rat brain response to chronic rapamycin treatment.
Mammalian target of rapamycin (mTOR) is a protein kinase that senses nutrient availability, trophic factors support, cellular energy level, cellular stress, and neurotransmitters and adjusts cellular metabolism accordingly. Adequate mTOR activity is needed for development as well as proper physiology of mature neurons. Consequently, changes in mTOR activity are often observed in neuropathology. Recently, several groups reported that seizures increase mammalian target of rapamycin (mTOR) kinase activity, and such increased activity in genetic models can contribute to spontaneous seizures. However, the current knowledge about the spatiotemporal pattern of mTOR activation induced by proconvulsive agents is rather rudimentary. Also consequences of insufficient mTOR activity on a status epilepticus are poorly understood. Here, we systematically investigated these two issues. We showed that mTOR signaling was activated by kainic acid (KA)-induced status epilepticus through several brain areas, including the hippocampus and cortex as well as revealed two waves of mTOR activation: an early wave (2 h) that occurs in neurons and a late wave that predominantly occurs in astrocytes. Unexpectedly, we found that pretreatment with rapamycin, a potent mTOR inhibitor, gradually (i) sensitized animals to KA treatment and (ii) induced gross anatomical changes in the brain.
PLoS One, 2013; 8
22733788: Kowalczyk T, Konopacki J, Bocian R, Caban B
Theta-related gating cells in hippocampal formation: in vivo and in vitro study.
In this study we extended our earlier in vitro findings concerning the discovery of a novel type of theta-related cells, which we have termed gating cells. There were two main objectives of our present investigations. The first was to determine the distribution of theta gating cells in the separated CA1 and CA3 generators in three different pharmacological conditions: (i) the presence of a cholinergic agonist-carbachol, (ii) the presence of carbachol and GABA(A) ergic antagonist-bicuculline, (iii) the presence of carbachol and GABA(B) ergic antagonist-2-hydroxysaclofen. The second objective of our studies was to verify our earlier in vitro findings and to demonstrate, for the first time, gating cells in intact hippocampus during the generation of Type II theta in urethane anaesthetized rats. Two hundred ninety-nine theta-related cells were isolated and recorded from in vivo and in vitro hippocampal formation. Twenty out of all 299 neurons (6.6%) were classified as gating cells. The neuron was classified as a gating cell if it met one of the following criteria: (i) the cell discharges occurred precisely in the beginning and at the end of each theta epoch (gating cell A); (ii) the cell began to discharge just before the transition from

non-theta interval/LIA into the theta epoch (gating cell B); (iii) the cell began to discharge just after the transition from the theta epoch into non-theta interval/LIA (gating cell C). Our data demonstrates that the appearance of theta epochs and their length, as well as the appearance of non-theta states (in vivo recorded LIA or in vitro recorded intervals between theta epochs) and their length, may require the existence of a specific population of hippocampal neurons which we termed gating cells.

Hippocampus, 2013; 23

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MULTIELECTRODE RECORDINGS IN THE POSTERIOR HYPOTHALAMUS OF FREELY MOVING RATS: MOVEMENT-RELATED AND IMMOBILITY-RELATED THETA RHYTHM

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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It is well-established that hippocampal theta rhythms can be divided into two distinct subtypes according to their pharmacological profile and relation to different animal's behavior. Our recent studies have revealed that well-synchronized theta rhythm can also successfully be recorded from the posterior hypothalamic area (PHa) in anesthetized rats and isolated brain slices of PHa maintained in vitro. AIMS: The aim of the present study was to investigate the possible differences in theta band oscillations recorded locally in the posterior hypothalamic area of freely-moving rats during different types of behavior. METHODS: The experiments were performed in eight adult rats. Animals were anesthetized with ketamine and 16-channel multielectrodes were implanted into the region of posterior hypothalamus. After recovery period animals were tested in the open field. Radio-transmitted EEG recordings form supramammillary and posterior hypothalamic nuclei were taken simultaneously with video recordings. RESULTS: Multielectrode recordings performed in freely moving rats have shown that both posterior hypothalamic and supramammillary nuclei are capable of generating movement-related theta activity. It was shown that PHa theta activity recorded during voluntary movements differs in frequency and amplitude parameters from theta rhythm observed during immobility or automatic activities. CONCLUSIONS: Two subtypes of theta rhythmic oscillations can be recorded from the posterior hypothalamic area of freely moving rats expressing different locomotor activities. Supported by National Science Centre, Poland, No. 2017/25/B/NZ4/01476

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SILENT JUXTACELLULAR FIELD POTENTIALS CORRESPOND TO NEURONAL CELL TYPES

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Extracellular recording techniques characterize single neurons based on firing characteristics of monitored cells in relation to field potentials generated by population activity. Thus, silent network states or subthreshold periods of individual cells make cell identification difficult if not impossible with extracellular approaches. Juxtacelluar recordings provide means to record the suprathreshold activity of individual neurons together with local field potentials while allowing anatomical labeling of the recorded cells. In freely behaving rats, we juxtacellularly recorded pyramidal cells (PYRs), fast spiking interneurons (FSIs) and regular spiking interneurons (RSIs). The three cell types were distinguished based on morphology and firing characteristics during nonREM sleep packets, sleep spindles and down to up (DU) state transitions. We observed that down states (DSs) preceding DU transitions were different in FSIs than those in RSI and PYR cells and hypothesized that silent periods of local field potentials might express complex cell type specific characteristics. Indeed, simultaneous triple juxtacellular recordings confirmed heterogeneous DSs in neighboring cells. We developed a workflow for clustering individually recorded silent DSs (n>23000) based on principal component analysis, unsupervised learning algorithms and self-organizing maps (SOMs). Cell groups of PYRs, FSIs and RSIs showed significant difference in SOM profiles constructed from silent DSs. We conclude that local field potentials recorded juxtacellular to nonspiking individual cells are cell type specific. This suggests that field potentials in the network are highly compartmentalized and retain identities of cellular units in space and time even if neuronal populations are in a silent state.

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BOARD NUMBER: S03-523

PATHWAY SPECIFIC REGULATION OF CONVERGENT INFORMATION STREAMS BY HIPPOCAMPAL NEUROGLIAFORM CELLS

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Synchronized activation of distributed neuronal assemblies is key for communication in brain circuits. In broadcasting areas synchrony emerges as gamma oscillations (30 - 150 Hz) that entrain spiking in recipient networks when information transfer is successful. In hippocampal CA1 area, temporoammonic pathway from entorhinal cortex and Schaffer collaterals from hippocampal CA3 carrying sensory and mnemonic information converge onto pyramidal cells. The information flow into and within the CA1 networks is regulated by co-existing nested electrical oscillations. We aimed to find out how cortico-hippocampal communication is regulated by local CA1 interneuron types in association with mid-frequency gamma oscillations (50-90 Hz) that occur in stratum lacunosum-moleculare, where cortical fibers terminate. We recorded electrical signals from the hippocampi of awake, head-fixed mice performing unidirectional runs along a corridor in a virtual reality environment. Using current source density analysis, we localized slow, mid-frequency and fast gamma oscillations. We simultaneously recorded the spike timing of multiple units and single GABA*ergic* neurons in CA1, using silicon probes and extracellular glass microelectrodes, respectively. The glass electrode recorded neurons were filled with neurobiotin and their cell type was identified by their axonal arborisation and protein content. We discovered that spike timing of a small population of inhibitory interneurons, which were identified as neurogliaform cells, were selectively and strongly phase-locked to mid-frequency gamma oscillations associated with cortical afferents, indicating their selective engagement. We will present data on how the neurogliaform cells regulate information transfer of converging inputs by selectively rebalancing contributions from specific pathways. (Funding: FWF P29744-B27).

FENS Forum 2022
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BOARD NUMBER: S03-524

THE IMPACT OF NMDA RECEPTOR SUBUNIT GLUN3A DELETION ON THE BRAIN ACTIVITY OF YOUNG AND ADULT MICE

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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The maturation of functional sensory circuits with the capacity to process information is a highly orchestrated process that takes place during postnatal stages. It involves the interplay of progressive and regressive events that will stabilize some synapses and remove others. Problems during this critical period contribute to a wide spectrum of neurological disorders in later life. The pruning of axons and dendrites is controlled by multiple factors, one of them being the activity sensed and transmitted by N-methyl-D-Aspartate receptors (NMDARs). Here we focus on a new class of NMDARs that are characterized by the presence of GluN3A subunits and play an essential role in the activity-dependent refinement of synaptic connections. In mouse primary somatosensory cortex (S1) GluN3A expression peaks around postnatal days 6-9 and declines until reaching very low adult levels, with temporal differences between cortical layers (Murillo et al., Cereb Cortex 2020). Results from our collaborators show that GluN3A-containing NMDARs participate in axonal refinement in such a way that knocking out the gene encoding GluN3A (*Grin3a*) results in an aberrant pattern of callosal innervation of S1. Therefore, in this study, we investigated the impact of GluN3A deletion on the communication between hemispheres, and analyzed the spontaneous activity and sensory processing in S1 of P20 and P40 mice. We performed multi-site bilateral recordings of spontaneous activity in S1 of awake GluN3A knockout mice (*Grin3a*-/-). Preliminary results reveal changes in the power of several frequency bands at P20. We are currently characterizing the ipsilateral responses, which are mediated by callosal inputs.

FENS Forum 2022
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BOARD NUMBER: S03-525

STATE-DEPENDENT FUNCTIONAL INTERACTIONS BETWEEN PONTINE WAVES AND HIPPOCAMPAL OSCILLATIONS DURING SLEEP

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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Pontine (P) or ponto-geniculo-occipital (PGO) waves are a prominent electrophysiological marker of rapid-eye-movement (REM) sleep. Although P-waves have long been recognized, they have been less studied compared to other sleep-related neural events. Recent studies in mice and macaques uncovered state-dependent functional coupling of P-waves with hippocampal oscillations including sharp wave-ripples (SWRs) during non-REM (NREM) sleep and theta oscillations during REM sleep. However, the detailed characteristics of this functional coupling remain unclear. In addition, since P-waves in mice have been reported rarely, it is also important to describe their detailed electrophysiological properties, given the importance of the animal model. Here, we address these issues by performing multiple types of electrophysiological recordings in mice. We found that while P-waves appear more frequently during REM sleep and synchronously in two hemispheres, the frequency of P-waves during REM sleep can predict REM sleep episode duration. Brainstem neural ensembles underlying P-waves consist of diverse functional classes. The duration of hippocampal SWRs was associated with the coupling with P-waves. In addition to phase coupling between P-waves and hippocampal theta oscillations during REM sleep, P-waves led to increased excitability of hippocampal populations. These results suggest that P-waves play a role in systems memory consolidation by functionally coupling with hippocampal ensembles in a state-dependent fashion.

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BOARD NUMBER: S03-526

COUPLING DURING NREM SLEEP BETWEEN THE PRELIMBIC CORTEX, NUCLEUS REUNIENS, AND HIPPOCAMPUS REMAINS STABLE UNDER COGNITIVE AND HO-MEOSTATIC DEMANDS

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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The interplay between the medial prefrontal cortex and hippocampus during non-REM (NREM) sleep is important for the consolidation of contextual memories. To assess the role of the thalamic nucleus reuniens (Nre) in this interaction, we investigated the coupling of neuro-oscillatory activity between prelimbic cortex, Nre, and hippocampus across sleep states and their role in the consolidation of contextual memories using multi-site electrophysiological recordings and optogenetic manipulations. We showed that ripples are time-locked to the Up state of cortical slow waves, the transition to the negative slope after the Up state in thalamic slow waves, the troughs of cortical spindles, and the peaks of thalamic spindles in unperturbed baseline sleep, sleep rebound after fear conditioning. In addition, spiking activity in Nre increases before hippocampal ripples and the phase-locking of hippocampal ripples and thalamic spindles during NREM sleep was stronger after acquisition of a fear memory. We showed that optogenetic inhibition of Nre neurons reduced a clear phase-locking ripples to cortical slow waves in the ventral hippocampus while their activation altered the preferred phase of ripples to slow waves in ventral and dorsal hippocampi. However, these optogenetic manipulations of Nre during sleep after acquisition of a fear conditioning did not alter sleep-dependent memory consolidation. Collectively, these results showed that Nre is central in modulating hippocampus and cortical rhythms during NREM sleep homeostasis and congnition.

FENS Forum 2022
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BOARD NUMBER: S03-527

LARGE-SCALE SINGLE UNIT RECORDINGS FROM OPTOGENETICALLY IDENTIFIED NEURONS IN THE DORSAL MIDLINE THALAMUS IN FREELY BEHAVING MICE

POSTER SESSION 03 - SECTION: OSCILLATIONS IN THE BRAIN

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The dorsal midline thalamus (dMT) has long been implicated in behaviors associated with high arousal state, such as motivated behaviors. We recently showed that activity of calretinin (CR) containing neurons in the dMT (CR-dMT) correlates with arousal level and their selective stimulation reliably leads to behavioral arousal. Recent advances of optogenetics have helped us extend our understanding of how dMT interacts with its input-output structures at the behavioral level. However, a detailed characterization of how dMT interacts with its synaptically connected counterparts at the neuronal level is still lacking. We sought to fill this gap by performing large-scale unit recordings from optogenetically identified CR-dMT neurons during unrestrained sleep-wake behavior, while simultaneously recorded population activity from the neocortex and the ventral subiculum; two regions reciprocally connected with the dMT. To discriminate CR-dMT neurons from neighboring non-CR expressing neurons, we transduced neurons in the dMT of CR-Cre mice with Channelrhodopsin-2 and implanted optic fibers both into the somatic and into the efferent regions of the CR-dMT neurons (prelimbic cortex and ventral subiculum). Using brief optical stimulation in either structure while monitoring neuronal activity within the dMT enabled us to further discriminate CR-dMT neurons in a projection-specific manner. We analyzed the relationship between the firing characteristics of CR-dMT neurons and various patterns of cortical and subicular network activity during sleep and wakefulness. Identifying how dMT interacts with the neocortex and the subiculum at the neuronal level might help clarify its role in motivated behaviors. (P*roject was funded from EU-Horizon 2020 MSCA grant 892957.)*

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Poster Session 04

FENS Forum 2022
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BOARD NUMBER: S04-001

CHEMOGENETIC ORBITO FRONTAL CORTEX INHIBITION AND CHEMOGENETIC AMYGDALA ACTIVATION IN HIGH COMPULSIVE RATS.

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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Introduction: Compulsivity is associated with the loss of inhibitory control over a broad range of behaviours that are prone to excess. Preclinical and clinical studies on compulsivity have demonstrated that fronto-limbic structures are implicated in the pathophysiology of compulsive spectrum disorders. Aims: The present study was designed to assess the effects of chemogenetic stimulation or inhibition of orbitofrontal cortex (OFC)-amygdala circuit in a preclinical model of compulsivity. Methods: male Wistar rats (approx. 300 g) were selected as either high compulsive (HD) or low (LD) drinkers according to their level acquisition of water intake (ml) on schedule-induced polydipsia (SIP, fixed time schedule of 60s) after 20 sessions. In a first experiment, we used designer receptor–mediated inhibition (hM4Di) in HD and LD rats' OFC. In a second experiment, we used designer receptor-mediated activation (hM3Dq) in HD and LD rats' amygdala. Finally, we re-exposed rats to SIP. Results: Repeated measured ANOVA revealed no significant effects nor by the chemogenetic OFC inhibition, nor by the chemogenetic amygdala activation in the water intake of HD and LD rats on SIP. Conclusions: The chemogenetic inhibition or activation of the OFC-amygdala circuit did not have effect on the compulsive behaviour observed on SIP. Future studies should explore other brain areas implicated in the inhibitory control brain circuit with chemogenetic stimulation on preclinical models of compulsivity to identify the underlying mechanisms related to obsessive-compulsive disorder. Funded by Gobierno de España MCIN/ AEI /10.13039/501100011033/ grant number PGC2018-099117-B-C21 and UAL2020-CTSD2068 FEDER I+D+i "Una manera de hacer Europa".

FENS Forum 2022
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BOARD NUMBER: S04-002

PERIORBITAL HYPERPIGMENTATION'S RELATION TO NEUROLOGY AND MENTAL HEALTH: MALADAPTIVE DAYDREAMING ACCOMPANYING PSYCHOSIS AND ITS TREATMENT METHODS

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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Objective: It was observed on a 17 yo female individual experiencing Psychosis accompanying Maladaptive Daydreaming (MD) the Peri-Orbital Hyperpigmentation (POH) symptom which is characterized by the darkness and thin lids. The self diagnosed girl noticed that the POH increases after having an MD episode. it is supposed that excess amount of Oxygen resulted from over-reaction of Dopamine synthesis in Psychosis with MD, due to high activity in SN, leads to the formation of pigmented eyelids (POH). It is suggested too that the activity of DMN and SCN in the brain has a role in developing psychotic episodes which are triggered by MD. Methods: We have studied the relationship between Psychosis accompaying MD and POH to a 17 yo female individual. We established some physical exercises to strengthen the eye and the eyelid muscles and to develop and construct new dopaminergic and excitatory neurotransmitters pathways in new neural networks; to enhance the effort of the visual cortex and the primary motor cortex to see the results if they would support the hypothesis. A 22-inquiries questionnaire was conducted on 22 participants about Maladaptive daydreaming accompanying mental health issues and Peri-Orbital Hyperpigmentation. Results: In brain stimulation, POH decreased which means the individual has developed new circuits participating the visual cortex neurons in them. The muscles strength increased increasing the eyes' plasticity in addition to increasing the plasticity/action potential of the frontal cortex. 68% of the individuals said they had POH which support the hypothesis' consistency. p-value = .06. p< .10.

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BOARD NUMBER: S04-003

A PRELIMINARY INVESTIGATION ON THE EFFECT OF ACUTE TRIGEMINAL NERVE STIMULATION ON COGNITIVE FUNCTION.

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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Aims: Neuromodulation techniques based on peripheral nerve stimulation are used in clinical practice as a supportive treatment for different neurologic and neuropsychiatric disorders, and in basic neuroscience research to investigate brain functions. Recent research suggests that transcutaneous trigeminal nerve stimulation (TNS) may positively affect cognitive function. However, no clear-cut evidence is available yet, since the majority of the evidence derives from clinical studies, and the few data on healthy subjects show inconsistent results. In this preliminary study we report on the effects of short-term TNS on event-related potentials (ERP) recorded during the administration of a simple visual discrimination task and a paired-click paradigm, both considered useful for studying cognitive processing functions. Methods: Thirty-two healthy subjects (15 males and 17 females; mean age 25.03±4.80 years) were assigned to two groups: real-TNS (20-min, cyclic modality 30s ON and 30s OFF, 120 Hz, 0.25ms, intensity below pain threshold) and sham-TNS. Subjects underwent EEG recording before and after sham- or real-TNS, amplitude and latency of P200 and P300 waves (visual discrimination task), and P50, N100 and P200 waves (paired-click paradigm), were analysed using repeated-measures ANOVAs. Results: TNS did not affect subjects' performance in both protocols, as shown by the non-significant differences in amplitude and latency after sham- or real-TNS interventions. Conclusions: Data demonstrate that in healthy young adults short-term TNS does not affect cognitive functions such as pre-attentional processes, early allocation of attention and immediate memory.

FENS Forum 2022
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BOARD NUMBER: S04-005

VENTRAL PALLIDO-SUBTHALAMIC ADAPTATIONS UNDERLYING PATHOLOGICAL DECISION MAKING IN ALCOHOL USE DISORDERS

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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Alcohol use disorder is a chronic relapsing disorder characterized by impulsive and compulsive decision-making processes driven by a disinhibition of behavior control. Impaired decision-making happens during acute alcohol use, which drives a disinhibitory effect on behavior control, yet it can persist for years of abstinent people and can also actively drive relapse. The subthalamic nucleus (STN) is of particular importance for appropriate decision making, especially its ventromedial portion (vmSTN), a limbic territory involved in mood and motivated behavior. Behavioral inhibition requires burst firing of the STN while impulsivity correlates with decreased activity. The ventral Pallidum (VP), a limbic structure mainly inhibitory, projects to the STN and is theoretically the main inhibitory input of the vmSTN. VP activity reflect perceived reward, is affected by ethanol consumption and is increased in impulsive patients. We hypothesized that VP GABAergic projections to the vmSTN become persistently strengthened following alcohol intake, promoting impaired decision making and compulsivity. In a mouse model of ethanol drinking, we combined behavioral assays to highlight maladaptive decision making, tracing approaches to characterize the VP to vmSTN pathway, *ex vivo* electrophysiology patch clamp coupled with pharmacology and optogenetic modulation to elucidate how ethanol drinking alters the VP to vmSTN synapse strength, and *in vivo* optogenetic experiments to modulate the VP to vmSTN activity during decision making. Together, these results will elucidate neural substrates of inhibitory controls and how they are altered in alcohol use disorders.

Pubmed:

34663957: Markovic T, Pedersen CE, Massaly N, Vachez YM, Ruyle B, Murphy CA, Abiraman K, Shin JH, Garcia JJ, Yoon HJ, Alvarez VA, Bruchas MR, Creed MC, Morón JA
Pain induces adaptations in ventral tegmental area dopamine neurons to drive anhedonia-like behavior.
The persistence of negative affect in pain leads to co-morbid symptoms such as anhedonia and depression-major health issues in the United States. The neuronal circuitry and contribution of specific cellular populations underlying these behavioral adaptations remains unknown. A common characteristic of negative affect is a decrease in motivation to initiate and complete goal-directed behavior, known as anhedonia. We report that in rodents, inflammatory pain decreased the activity of ventral tegmental area (VTA) dopamine (DA) neurons, which are critical mediators of motivational states. Pain increased rostromedial tegmental nucleus inhibitory tone onto VTA DA neurons, making them less excitable. Furthermore, the decreased activity of DA neurons was associated with reduced motivation for natural rewards, consistent with anhedonia-like behavior. Selective activation of VTA DA neurons was sufficient to restore baseline motivation and hedonic responses to natural rewards. These findings reveal pain-induced adaptations within VTA DA neurons that underlie anhedonia-like behavior.
Nat Neurosci, 2021; 24
33495635: Vachez YM, Tooley JR, Abiraman K, Matikainen-Ankney B, Casey E, Earnest T, Ramos LM, Silberberg H, Godynyuk E, Uddin O, Marconi L, Le Pichon CE, Creed MC
Ventral arkypallidal neurons inhibit accumbal firing to promote reward consumption.
The nucleus accumbens shell (NAcSh) and the ventral pallidum (VP) are critical for reward processing, although the question of how coordinated activity within these nuclei orchestrates reward valuation and consumption remains unclear. Inhibition of NAcSh firing is necessary for reward consumption, but the source of this inhibition remains unknown. Here, we report that a subpopulation of VP neurons, the ventral arkypallidal (vArky) neurons, project back to the NAcSh, where they inhibit NAcSh neurons in vivo in mice. Consistent with this pathway driving reward consumption via inhibition of the NAcSh, calcium activity of vArky neurons scaled with reward palatability (which was dissociable from reward seeking) and predicted the subsequent drinking behavior during a free-access paradigm. Activation of the VP-NAcSh pathway increased ongoing reward consumption while amplifying hedonic reactions to reward. These results establish a pivotal role for vArky neurons in the promotion of reward consumption through modulation of NAcSh firing in a value-dependent manner.

Nat Neurosci, 2021; 24
33328933: Vachez YM, Creed MC
Deep Brain Stimulation of the Subthalamic Nucleus Modulates Reward-Related Behavior: A Systematic Review.
Deep brain stimulation of the subthalamic nucleus (STN-DBS) is an effective treatment for the motor symptoms of movement disorders including Parkinson's Disease (PD). Despite its therapeutic benefits, STN-DBS has been associated with adverse effects on mood and cognition. Specifically, apathy, which is defined as a loss of motivation, has been reported to emerge or to worsen following STN-DBS. However, it is often challenging to disentangle the effects of STN-DBS from concurrent reduction of dopamine replacement therapy, from underlying PD pathology or from disease progression. To this end, pre-clinical models allow for the dissociation of each of these factors, and to establish neural substrates underlying the emergence of motivational symptoms following STN-DBS. Here, we performed a systematic analysis of rodent studies assessing the effects of STN-DBS on reward seeking, reward motivation and reward consumption across a variety of behavioral paradigms. We find that STN-DBS decreases reward seeking in the majority of experiments, and we outline how design of the behavioral task and DBS parameters can influence experimental outcomes. While an early hypothesis posited that DBS acts as a "functional lesion," an analysis of lesions and inhibition of the STN revealed no consistent pattern on reward-related behavior. Thus, we discuss alternative mechanisms that could contribute to the amotivational effects of STN-DBS. We also argue that optogenetic-assisted circuit dissection could yield important insight into the effects of the STN on motivated behavior in health and disease. Understanding the mechanisms underlying the effects of STN-DBS on motivated behavior-will be critical for optimizing the clinical application of STN-DBS.
Front Hum Neurosci, 2020; 14
32562462: Vachez Y, Carcenac C, Magnard R, Goff LK, Salin P, Savasta M, Carnicella S, Boulet S
Reply to: Letter to the Editor by Martínez-Fernández.
Mov Disord, 2020; 35
31930749: Vachez Y, Carcenac C, Magnard R, Kerkerian-Le Goff L, Salin P, Savasta M, Carnicella S, Boulet S
Subthalamic Nucleus Stimulation Impairs Motivation: Implication for Apathy in Parkinson's Disease.
Apathy is one of the most disabling neuropsychiatric symptoms in Parkinson's disease (PD) patients and has a higher prevalence in patients under subthalamic nucleus deep brain stimulation. Indeed, despite its effectiveness for alleviating PD motor symptoms, its neuropsychiatric repercussions have not yet been fully uncovered. Because it can be alleviated by dopaminergic therapies, especially D and D dopaminergic receptor agonists, the commonest explanation proposed for apathy after subthalamic nucleus deep brain stimulation is a too-strong reduction in dopaminergic treatments. The objective of this study was to determine whether subthalamic nucleus deep brain stimulation can induce apathetic behaviors, which remains an important matter of concern. We aimed to unambiguously address this question of the motivational effects of chronic subthalamic nucleus deep brain stimulation.
Mov Disord, 2020; 35
30618665: Magnard R, Vachez Y, Carcenac C, Boulet S, Houeto JL, Savasta M, Belin D, Carnicella S
Nigrostriatal Dopaminergic Denervation Does Not Promote Impulsive Choice in the Rat: Implication for Impulse Control Disorders in Parkinson's Disease.
Impulse control disorders (ICDs) are frequent behavioral complications of dopaminergic (DA) replacement therapies (DRTs) in Parkinson's disease (PD). Impulsive choice, which refers to an inability to tolerate delays to reinforcement, has been identified as a core pathophysiological process of ICDs. Although impulsive choices are exacerbated in PD patients with ICDs under DRTs, some clinical and preclinical studies suggest that the DA denervation of the dorsal striatum induced by the neurodegenerative process as well as a pre-existing high impulsivity trait, may both contribute to the emergence of ICDs in PD. We therefore investigated in a preclinical model in rats, specifically designed to study PD-related non-motor symptoms, the effect of nigrostriatal DA denervation on impulsive choice, in relation to pre-existing levels of impulsivity, measured in a Delay Discounting Task (DDT). In this procedure, rats had the choice between responding for a small sucrose reinforcer delivered immediately, or a larger sucrose reinforcer, delivered after a 0, 5, 10 or 15 s delay. In two different versions of the task, the preference for the large reinforcer decreased as the delay increased. However, and in contrast to our initial hypothesis, this discounting effect was neither exacerbated by, or related to, the extent of the substantia nigra pars compacta (SNc) DA lesion, nor it was influenced by pre-existing variability in impulsive choice. These results therefore question the potential implication of the nigrostriatal DA system in impulsive choice, as well as the DA neurodegenerative process as a factor contributing significantly to the development of ICDs in PD.
Front Behav Neurosci, 2018; 12
28134302: Favier M, Carcenac C, Drui G, Vachez Y, Boulet S, Savasta M, Carnicella S
Implication of dorsostriatal D3 receptors in motivational processes: a potential target for neuropsychiatric symptoms in Parkinson's disease.
Beyond classical motor symptoms, motivational and affective deficits are frequently observed in Parkinson's disease (PD),

dramatically impairing the quality of life of patients. Using bilateral 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra pars compacta (SNc) in rats, we have been able to reproduce these neuropsychiatric/non-motor impairments. The present study describes how bilateral 6-OHDA SNc lesions affect the function of the main striatal dopaminergic (DA) receptor subtypes. Autoradiography was used to measure the levels of striatal DA receptors, and operant sucrose self-administration and neuropharmacological approaches were combined to investigate the causal implication of specific DA receptors subtypes in the motivational deficits induced by a dorsostriatal DA denervation. We found that D3 receptors (DR) exclusively are down-regulated within the dorsal striatum of lesioned rats. We next showed that infusion of a DR antagonist (SB-277011A) in non-lesioned animals specifically disrupts preparatory, but not consummatory behaviors. Our findings reveal an unexpected involvement of dorsostriatal DR in motivational processes. They strongly suggest an implication of dorsostriatal DR in the neuropsychiatric symptoms observed in PD, highlighting this receptor as a potential target for pharmacological treatment.
Sci Rep, 2017; 7

25588931: Carcenac C, Favier M, Vachez Y, Lacombe E, Carnicella S, Savasta M, Boulet S
Subthalamic deep brain stimulation differently alters striatal dopaminergic receptor levels in rats.
High-frequency stimulation (HFS) of the subthalamic nucleus (STN) is recognized as an effective treatment for the motor symptoms of Parkinson's disease (PD), but its mechanisms, particularly as concern dopaminergic transmission, remain unclear. The aim of this study was to evaluate changes in the expression of dopaminergic receptors (D1, D2, and D3 receptors) after prolonged (4 h) unilateral STN-HFS in anesthetized intact rats and rats with total dopaminergic denervation. We used [(3)H]SCH 23390, [(125)I]iodosulpride, and [(125)I]OH-PIPAT to assess the densities of D1R, D2R, and D3R, respectively, within different areas of the striatum-a major input structure of the basal ganglia-including the nucleus accumbens. We found that STN-HFS increased D1 R levels in almost all of the striatal areas examined, in both intact and denervated rats. By contrast, STN-HFS led to a large decrease in D2 R and D3R levels, limited to the nucleus accumbens and independent of the dopaminergic state of the animals. These data suggest that the influence of STN-HFS on striatal D1 R expression may contribute to its therapeutic effects on motor symptoms, whereas its impact on D2R/D3 R levels in the nucleus accumbens may account for the neuropsychiatric side effects often observed in stimulated PD patients, such as postoperative apathy.
Mov Disord, 2015; 30

24260200: Ducarouge B, Pelissier-Rota M, Lainé M, Cristina N, Vachez Y, Scoazec JY, Bonaz B, Jacquier-Sarlin M
CRF2 signaling is a novel regulator of cellular adhesion and migration in colorectal cancer cells.
Stress has been proposed to be a tumor promoting factor through the secretion of specific neuromediators, such as Urocortin2 and 3 (Ucn2/3), however its role in colorectal cancer (CRC) remains elusive. We observed that Ucn2/3 and their receptor the Corticotropin Releasing Factor receptor 2 (CRF2) were up-regulated in high grade and poorly differentiated CRC. This suggests a role for CRF2 in the loss of cellular organization and tumor progression. Using HT-29 and SW620 cells, two CRC cell lines differing in their abilities to perform cell-cell contacts, we found that CRF2 signals through Src/ERK pathway to induce the alteration of cell-cell junctions and the shuttle of p120ctn and Kaiso in the nucleus. In HT-29 cells, this signaling pathway also leads to the remodeling of cell adhesion by i) the phosphorylation of Focal Adhesion Kinase and ii) a modification of actin cytoskeleton and focal adhesion complexes. These events stimulate cell migration and invasion. In conclusion, our findings indicate that CRF2 signaling controls cellular organization and may promote metastatic potential of human CRC cells through an epithelial-mesenchymal transition like process. This contributes to the comprehension of the tumor-promoting effects of stress molecules and designates Ucn2/3-CRF2 tandem as a target to prevent CRC progression and aggressiveness.
PLoS One, 2013; 8

26954980: Magnard R, Vachez Y, Carcenac C, Krack P, David O, Savasta M, Boulet S, Carnicella S
What can rodent models tell us about apathy and associated neuropsychiatric symptoms in Parkinson's disease?
In addition to classical motor symptoms, Parkinson's disease (PD) patients display incapacitating neuropsychiatric manifestations, such as apathy, anhedonia, depression and anxiety. These hitherto generally neglected non-motor symptoms, have gained increasing interest in medical and scientific communities over the last decade because of the extent of their negative impact on PD patients' quality of life. Although recent clinical and functional imaging studies have provided useful information, the pathophysiology of apathy and associated affective impairments remains elusive. Our aim in this review is to summarize and discuss recent advances in the development of rodent models of PD-related neuropsychiatric symptoms using neurotoxin lesion-based approaches. The data collected suggest that bilateral and partial lesions of the nigrostriatal system aimed at inducing reliable neuropsychiatric-like deficits while avoiding severe motor impairments that may interfere with behavioral evaluation, is a more selective and efficient strategy than medial forebrain bundle lesions. Moreover, of all the different classes of pharmacological agents, D2/D3 receptor agonists such as pramipexole appear to be the most efficient treatment for the wide range of behavioral deficits induced by dopaminergic lesions. Lesion-based rodent models, therefore, appear to be relevant tools for studying the pathophysiology of the non-motor symptoms of PD. Data accumulated

so far confirm the causative role of dopaminergic depletion, especially in the nigrostriatal system, in the development of behavioral impairments related to apathy, depression and anxiety. They also put forward D2/D3 receptors as potential targets for the treatment of such neuropsychiatric symptoms in PD.
Transl Psychiatry, 2016; 6

FENS Forum 2022
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BOARD NUMBER: S04-006

NEUROSTRUCTURAL ABNORMALITIES IN HIGH DRINKERS COMPULSIVE RATS SELECTED BY SCHEDULE-INDUCED POLYDIPSIA

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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Introduction: Compulsivity has been considered a transdiagnostic dimension in compulsive spectrum disorders, characterized by a heterogeneous cognitive and behavioral phenotype associated with different alterations in the cortico-striatal-thalamic-cortical loop. Objective: We investigated the morphological alterations in different brain areas associated with inhibitory control deficit in a preclinical model of compulsivity. Methods: Brain volumetric differences were assessed in rats selected as high or low compulsive drinking (HD, LD), on Schedule-Induced Polydipsia (SIP), using high-resolution magnetic resonance imaging (MRI). Results: Voxel-based morphometry of segmented MRI images revealed that compulsive HD rats showed a significantly increased volume of white matter areas (Corpus Callosum and Anterior Commissure), cortical structures (Motor Cortex and dorsolateral Orbitofrontal Cortex), subcortical structures (Striatum, Preoptic Area, Amygdala, Dentate Gyrus, Subthalamic Nucleus, Periaqueductal Gray, Midbrain and Parasubiculum) and Cerebellum relative to LD animals. However, HD rats showed a decreased volume of mPFC compared to LD rats. Finally, no differences were observed between HD and LD groups in the whole brain nor in cerebrospinal fluid (CSF) volume. Conclusions: These results highlight and extend the knowledge about brain morphological differences in a compulsive phenotype of rats; pointing towards the pathogenesis of inhibitory control deficit, such as those related to goal-directed behavior, habit learning, cognitive flexibility and motor inhibition. Moreover, these findings demonstrate the importance of mapping different structural patterns to enhancing the knowledge about the vulnerability to compulsive spectrum disorders. Funded by Gobierno de España MCIN/ AEI /10.13039/501100011033/ grant number PGC2018-099117-B-C21 and UAL2020-CTSD2068 FEDER I+D+i "Una manera de hacer Europa"

Pubmed:
29313138: Martín-González E, Prados-Pardo Á, Mora S, Flores P, Moreno M
Do psychoactive drugs have a therapeutic role in compulsivity? Studies on schedule-induced polydipsia.
Clinical studies have shown that some psychoactive recreational drugs have therapeutic applications in anxiety, depression, and schizophrenia. However, to date, there are few studies on the therapeutic potential efficacy of recreational drugs in compulsive neuropsychiatric disorders.
Psychopharmacology (Berl), 2018; 235

FENS Forum 2022
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BOARD NUMBER: S04-007

INVESTIGATING THE MECHANISMS UNDERLYING THE BENEFICIAL EFFECTS OF EXERCISE ON COGNITIVE IMPAIRMENT ASSOCIATED WITH SCHIZOPHRENIA - FOCUS ON PARVALBUMIN INTERNEURONS AND PERINEURONAL NETS

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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Background: NMDA receptor antagonist models have been widely used to mimic the cognitive and patholigcal deficits associated with schizophrenia. Increasing evidence indicates parvalbumin interneurons (PVI) and associated perineuronal nets (PNN) play a crucial role in cognition. In the current study we investigated changes to prefrontal PVI and PNN in the subchronic phencyclidine (scPCP) model. The second study aimed to explore PNN density changes in the model after chronic aerobic exercise, which has rescued PVI reductions in animal models and improved cognition in patients. **Methods:** 100 female lister hooded rats received vehicle or PCP (2mg/Kg, bidaily, 7 days). In study one, 60 brains were taken 8 weeks after dosing for mRNA quantification using qPCR and protein quantification using both simple western analysis and immunohistochemistry (IHC) (n = 10 per analysis and treatment). In study two vehicle and scPCP groups were subdivided into exercise and sedentary groups. Exercise involved access to a running wheel for thirty one-hour bouts over 6 weeks (n=10 per group). **Results:** Parvalbumin mRNA and protein were increased in scPCP-treated rats relative to controls; however, IHC showed reduced PVI number. There was no change to mRNA expression of PNN components, though IHC revealed reductions of total PNN density in scPCP-treated rats. After chronic exercise, PVI density increased, and PNN density decreased within treatments. **Conclusion:** These results indicate scPCP dosing results in a reduced number of dysfunctional PVI in the PFC. After exercise, decreased PNN may contribute to a more plastic PVI, increasing PVI density and improving cognitive function.

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BOARD NUMBER: S04-008

INDIVIDUAL DIFFERENCES IN DOPAMINE NEURAL DYNAMICS PREDICT VOLUNTARY ALCOHOL DRINKING

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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Context: Innate variability in response to reward is a striking but understudied phenomenon. This heterogeneity is most prominently seen amongst those that consume alcohol ; some drink casually while others drink in an uncontrolled manner. Dopamine dynamics are critical in encoding the reinforcing properties of rewarding and drug stimuli, yet, how these dynamics contribute to the phenotypic divergence seen in drug consumers remains unknown. Aims: Here, we aim to isolate the pre-existing neural dynamics that may explain behavioral individuation in response to natural reward and thus, future propensity to consume alcohol. Methods: Following measurement of behavioral responses to natural reward, we electrophysiologically record *in vivo* dopamine neurons' activity and their response to alcohol (i.v.) . We then utilize *in vivo* fiber photometry in freely behaving mice to record projection-specific dopamine dynamics elicited by natural rewards and investigate how voluntary alcohol drinking is predicted by these dopamine dynamics. Finally, using chemogenetics, we manipulate these activity patterns, subsequently controlling alcohol consumption in individuals. Results: We identify that innate behavioral responses to reward are mirrored by heterogeneous yet distinct dopamine firing profiles. We then establish that individual alcohol profiles are associated with pre-existing dopamine dynamics and find that individuals with a hyperdopaminergic activity profile have a lower neuronal response to alcohol and develop lower a lower preference for alcohol in the future. Conclusion: By assessing innate variability in response to rewards- natural and drug- this project will provide novel insights into the neural dynamics and mechanisms driving the phenotypic divergence we see among alcohol consumers.

Pubmed:
27151970: Bajo M, Montgomery SE, Cates LN, Nadav T, Delucchi AM, Cheng K, Yin H, Crawford EF, Roberts AJ, Roberto M
Evaluation of TLR4 Inhibitor, T5342126, in Modulation of Ethanol-Drinking Behavior in Alcohol-Dependent Mice.
Several lines of evidence support a critical role of TLR4 in the neuroimmune responses associated with alcohol disorders and propose inhibitors of TLR4 signaling as potential treatments for alcoholism. In this work, we investigated the effect of T5342126 compound, a selective TLR4 inhibitor, on excessive drinking and microglial activation associated with ethanol dependence.
Alcohol Alcohol, 2016; 51
32680583: Warden AS, Wolfe SA, Khom S, Varodayan FP, Patel RR, Steinman MQ, Bajo M, Montgomery SE, Vlkolinsky R, Nadav T, Polis I, Roberts AJ, Mayfield RD, Harris RA, Roberto M
Microglia Control Escalation of Drinking in Alcohol-Dependent Mice: Genomic and Synaptic Drivers.
Microglia, the primary immune cells of the brain, are implicated in alcohol use disorder. However, it is not known if microglial activation contributes to the transition from alcohol use to alcohol use disorder or is a consequence of alcohol intake.
Biol Psychiatry, 2020; 88
32497591: Liu Y, Montgomery SE, Juarez B, Morel C, Zhang S, Kong Y, Calipari ES, Nestler EJ, Zhang L, Han MH
Different adaptations of dopamine release in Nucleus Accumbens shell and core of individual alcohol drinking groups of mice.
Alcohol use disorder (AUD) places a tremendous burden on society, with approximately two billion alcohol users in the world. While most people drink alcohol recreationally, a subpopulation (3-5%) engages in reckless and compulsive drinking, leading to the development of AUD and alcohol dependence. The Ventral Tegmental Area (VTA)-Nucleus Accumbens (NAc) circuit has been shown to encode rewarding stimuli and drive individual alcohol drinking behavior. Our previous work successfully separated C57BL/6J isogenic mice into high or low alcohol drinking subgroups after a 12-day, two-bottle choice voluntary alcohol access paradigm. Electrophysiological studies revealed that low alcohol drinking mice exhibited elevated spontaneous and burst firing properties of their VTA dopamine (DA) neurons and specifically mimicking this pattern of activity in VTA-NAc neurons in high alcohol drinking mice using optogenetics decreased their alcohol preference. It is also known

that VTA DA neurons encode the salience and rewarding properties of external stimuli while also regulating downstream dopamine concentrations. Here, as a follow-up to this study, we utilized Fast Scan Cyclic Voltammetry (FSCV) to examine dopamine release in the NAc shell and core between alcohol drinking groups. We observed dynamic changes of dopamine release in the core of high drinking mice, but failed to see widely significant differences of dopamine release in the shell of both groups, when compared with ethanol-naive controls. Overall, the present data suggest subregion-specific differences of evoked dopamine release in the NAc of low and high alcohol drinking mice, and may provide an anatomical substrate for individual alcohol drinking behavior. This article is part of the special issue on Stress, Addiction and Plasticity.
Neuropharmacology, 2020; 175
32302586: Olson JM, Li JK, Montgomery SE, Nitz DA
Secondary Motor Cortex Transforms Spatial Information into Planned Action during Navigation.
Fluid navigation requires constant updating of planned movements to adapt to evolving obstacles and goals. For that reason, a neural substrate for navigation demands spatial and environmental information and the ability to effect actions through efferents. The secondary motor cortex (M2) is a prime candidate for this role given its interconnectivity with association cortices that encode spatial relationships and its projection to the primary motor cortex. Here, we report that M2 neurons robustly encode both planned and current left/right turning actions across multiple turn locations in a multi-route navigational task. Comparisons within a common statistical framework reveal that M2 neurons differentiate contextual factors, including environmental position, route, action sequence, orientation, and choice availability. Despite significant modulation by environmental factors, action planning, and execution are the dominant output signals of M2 neurons. These results identify the M2 as a structure integrating spatial information toward the updating of planned movements.
Curr Biol, 2020; 30
31427770: Lorsch ZS, Hamilton PJ, Ramakrishnan A, Parise EM, Salery M, Wright WJ, Lepack AE, Mews P, Issler O, McKenzie A, Zhou X, Parise LF, Pirpinias ST, Ortiz Torres I, Kronman HG, Montgomery SE, Loh YE, Labonté B, Conkey A, Symonds AE, Neve RL, Turecki G, Maze I, Dong Y, Zhang B, Shen L, Bagot RC, Nestler EJ
Stress resilience is promoted by a Zfp189-driven transcriptional network in prefrontal cortex.
Understanding the transcriptional changes that are engaged in stress resilience may reveal novel antidepressant targets. Here we use gene co-expression analysis of RNA-sequencing data from brains of resilient mice to identify a gene network that is unique to resilience. Zfp189, which encodes a previously unstudied zinc finger protein, is the highest-ranked key driver gene in the network, and overexpression of Zfp189 in prefrontal cortical neurons preferentially activates this network and promotes behavioral resilience. The transcription factor CREB is a predicted upstream regulator of this network and binds to the Zfp189 promoter. To probe CREB-Zfp189 interactions, we employ CRISPR-mediated locus-specific transcriptional reprogramming to direct CREB or G9a (a repressive histone methyltransferase) to the Zfp189 promoter in prefrontal cortex neurons. Induction of Zfp189 with site-specific CREB is pro-resilient, whereas suppressing Zfp189 expression with G9a increases susceptibility. These findings reveal an essential role for Zfp189 and CREB-Zfp189 interactions in mediating a central transcriptional network of resilience.
Nat Neurosci, 2019; 22
30336931: Zhang H, Chaudhury D, Nectow AR, Friedman AK, Zhang S, Juarez B, Liu H, Pfau ML, Aleyasin H, Jiang C, Crumiller M, Calipari ES, Ku SM, Morel C, Tzavaras N, Montgomery SE, He M, Salton SR, Russo SJ, Nestler EJ, Friedman JM, Cao JL, Han MH
α- and β-Adrenergic Receptor-Mediated Mesolimbic Homeostatic Plasticity Confers Resilience to Social Stress in Susceptible Mice.
Homeostatic plasticity in mesolimbic dopamine (DA) neurons plays an essential role in mediating resilience to social stress. Recent evidence implicates an association between stress resilience and projections from the locus coeruleus (LC) to the ventral tegmental area (VTA) (LC→VTA) DA system. However, the precise circuitry and molecular mechanisms of the homeostatic plasticity in mesolimbic DA neurons mediated by the LC→VTA circuitry, and its role in conferring resilience to social defeat stress, have not been described.
Biol Psychiatry, 2019; 85
30038282: Ayata P, Badimon A, Strasburger HJ, Duff MK, Montgomery SE, Loh YE, Ebert A, Pimenova AA, Ramirez BR, Chan AT, Sullivan JM, Purushothaman I, Scarpa JR, Goate AM, Busslinger M, Shen L, Losic B, Schaefer A
Epigenetic regulation of brain region-specific microglia clearance activity.
The rapid elimination of dying neurons and nonfunctional synapses in the brain is carried out by microglia, the resident myeloid cells of the brain. Here we show that microglia clearance activity in the adult brain is regionally regulated and depends on the rate of neuronal attrition. Cerebellar, but not striatal or cortical, microglia exhibited high levels of basal clearance activity, which correlated with an elevated degree of cerebellar neuronal attrition. Exposing forebrain microglia to apoptotic cells activated gene-expression programs supporting clearance activity. We provide evidence that the polycomb repressive complex 2 (PRC2) epigenetically restricts the expression of genes that support clearance activity in striatal and

cortical microglia. Loss of PRC2 leads to aberrant activation of a microglia clearance phenotype, which triggers changes in neuronal morphology and behavior. Our data highlight a key role of epigenetic mechanisms in preventing microglia-induced neuronal alterations that are frequently associated with neurodegenerative and psychiatric diseases.
Nat Neurosci, 2018; 21
29263389: Juarez B, Morel C, Ku SM, Liu Y, Zhang H, Montgomery S, Gregoire H, Ribeiro E, Crumiller M, Roman-Ortiz C, Walsh JJ, Jackson K, Croote DE, Zhu Y, Zhang S, Vendruscolo LF, Edwards S, Roberts A, Hodes GE, Lu Y, Calipari ES, Chaudhury D, Friedman AK, Han MH
Midbrain circuit regulation of individual alcohol drinking behaviors in mice.
Alcohol-use disorder (AUD) is the most prevalent substance-use disorder worldwide. There is substantial individual variability in alcohol drinking behaviors in the population, the neural circuit mechanisms of which remain elusive. Utilizing in vivo electrophysiological techniques, we find that low alcohol drinking (LAD) mice have dramatically higher ventral tegmental area (VTA) dopamine neuron firing and burst activity. Unexpectedly, VTA dopamine neuron activity in high alcohol drinking (HAD) mice does not differ from alcohol naive mice. Optogenetically enhancing VTA dopamine neuron burst activity in HAD mice decreases alcohol drinking behaviors. Circuit-specific recordings reveal that spontaneous activity of nucleus accumbens-projecting VTA (VTA-NAc) neurons is selectively higher in LAD mice. Specifically activating this projection is sufficient to reduce alcohol consumption in HAD mice. Furthermore, we uncover ionic and cellular mechanisms that suggest unique neuroadaptations between the alcohol drinking groups. Together, these data identify a neural circuit responsible for individual alcohol drinking behaviors.
Nat Commun, 2017; 8
30251377: Morel C, Montgomery S, Han MH
Nicotine and alcohol: the role of midbrain dopaminergic neurons in drug reinforcement.
Nicotine and alcohol addiction are leading causes of preventable death worldwide and continue to constitute a huge socio-economic burden. Both nicotine and alcohol perturb the brain's mesocorticolimbic system. Dopamine (DA) neurons projecting from the ventral tegmental area (VTA) to multiple downstream structures, including the nucleus accumbens, prefrontal cortex, and amygdala, are highly involved in the maintenance of healthy brain function. VTA DA neurons play a crucial role in associative learning and reinforcement. Nicotine and alcohol usurp these functions, promoting reinforcement of drug taking behaviors. In this review, we will first describe how nicotine and alcohol individually affect VTA DA neurons by examining how drug exposure alters the heterogeneous VTA microcircuit and network-wide projections. We will also examine how coadministration or previous exposure to nicotine or alcohol may augment the reinforcing effects of the other. Additionally, this review briefly summarizes the role of VTA DA neurons in nicotine, alcohol, and their synergistic effects in reinforcement and also addresses the remaining questions related to the circuit-function specificity of the dopaminergic system in mediating nicotine/alcohol reinforcement and comorbidity.
Eur J Neurosci, 2019; 50
31046935: Morel C, Montgomery S, Han MH
Small-Conductance, Calcium-Activated Potassium Channels: A Key Circuit Determinant for Stress-Induced Amygdala Dysfunction.
Biol Psychiatry, 2019; 85

FENS Forum 2022
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BOARD NUMBER: S04-009

EFFECTS OF TRANSCRANIAL DIRECT-CURRENT STIMULATION ON MEDIAL PREFRONTAL CORTEX IN A PRECLINICAL MODEL OF COMPULSIVITY

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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Compulsivity is a failure to stop an ongoing behavior that is becoming inappropriate to the situation. Clinical research indicates that compulsive spectrum disorders often are refractory to current treatments. Transcranial direct current stimulation (tDCS), a non-invasive neurostimulation, has been proposed as a safe and effective treatment for obsessive-compulsive disorder, schizophrenia, depression and addiction. The aim of the present study has been to assess the therapeutical effectivity of anodal tDCS on a preclinical model of compulsivity. For that purpose, male Wistar rats were selected as either high (HD) or low (LD) drinkers according to their rate of drinking behavior on Schedule-Induced Polydipsia (SIP). Subsequently, an electrode was implanted by stereotaxic surgery in the left medial prefrontal cortex of HD and LD animals. After one week of recovery, animals were re-exposed to SIP. Next, HD and LD animals received anodal tDCS or sham stimulation over the medial prefrontal cortex for five consecutive days. There were four experimental groups (HD-tDCS; HD-Sham; LD-tDCS; LD-sham). One day after receiving the last stimulation, animals were re-exposed to SIP. The results will be discussed in terms of the possible effects of neurostimulation by anodal tDCS on medial prefrontal cortex to reduce compulsive behavior on SIP. Neuromodulation using anodal tDCS might induce neuroplastic changes in the inhibitory control brain circuit, pointing towards a potential treatment for compulsive spectrum disorders. Funded by Gobierno de España MCIN/ AEI /10.13039/501100011033/ grant number PGC2018-099117-B-C21 and UAL2020-CTSD2068 FEDER I+D+i "Una manera de hacer Europa"

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BOARD NUMBER: S04-010

MELATONIN REDUCES ALCOHOL DRINKING IN RATS WITH DISRUPTED FUNCTION OF THE SEROTONERGIC SYSTEM

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

Ieva Pocevičiūtė[1], Rokas Buišas[2], Osvaldas Rukšėnas[3], Valentina Vengeliene[2]
[1]Vilnius university, Life Sciences Center, Vilnius, Lithuania, [2]Life Sciences Center, Vilnius University, Neurobiology And Biophysics, Institute Of Bioscience, Vilnius, Lithuania, [3]Vilnius University, Life Sciences Center, Vilnius, Lithuania

The reason for the limited treatment success of substance-use-related problems may be a causal heterogeneity of this disorder, which, at least partly, is manifested as differences in substance-use motives between individuals. Aim: The aim of the present study was to assess if rats with pharmacologically induced differences in the function of the serotonergic system would respond differently to melatonin treatment compared to their control counterpart rats with respect to voluntary alcohol consumption. Method: To achieve this goal, we treated rats neonatally with the selective serotonin transporter SERT inhibitor escitalopram. This procedure has been reported to cause long-lasting sleep abnormalities in rodents. To measure the effects of neonatal escitalopram treatment on voluntary home-cage alcohol consumption rats were given intermittent access to tap water and alcohol solution. During melatonin treatment, rats had access to continuous water and alcohol solutions. Results: The study demonstrated that during adulthood, rats that had been treated with escitalopram tended to drink higher amounts of alcohol compared to control rats. Further, administration of melatonin significantly decreased alcohol intake in escitalopram-treated animals but caused only a slight, nonsignificant reduction in alcohol consumption in control rats. In conclusion, our data support the therapeutic potential of melatonin as a treatment for alcohol use disorder. However, interindividual differences between alcohol users may considerably modify the outcome of the melatonin treatment, whereby patients that manifest lower sleep quality due to disruption of serotonergic activity are more likely to benefit from this treatment.

Pubmed:
34718391: Poceviciute I, Buisas R, Danelius T, Dulinskas R, Ruksenas O, Vengeliene V
The Anticonvulsant Lamotrigine Reduces Bout-Like Alcohol Drinking in Rats.
We used an optical lickometer system to study drinking microstructure and effect of lamotrigine in voluntary alcohol-drinking rats. We showed that, similar to humans, animals differ by their drinking microstructure where some consume alcohol exclusively in a bout-like patterns. The study suggests that anticonvulsants, such as lamotrigine, may be one treatment strategy specifically affecting this type of drinking.
Alcohol Alcohol, 2022; 57

FENS Forum 2022
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BOARD NUMBER: S04-011

PAY ATTENTION TO THIS CHANGE: LATERAL HYPOTHALAMIC GABAERGIC NEURONS IN ATTENTION AND ALCOHOL MEMORIES.

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

Isis Alonso-Lozares[1], Pelle Wilbers[2], Dustin Schetters[1], Phillip Jean-Richard-Dit-Bressel[3], Tim Heistek[4], Allison Mcdonald[2], Huibert Mansvelder[4], Taco De Vries[1], Nathan Marchant[1]
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In alcohol use disorders, drug memories can persist throughout abstinence, and re-exposure to the cues that signal alcohol can lead to relapse. Recent studies suggest GABAergic neurons in Lateral Hypothalamus (LH-GABA) are key players in memory processes, although the specific mechanisms through which LH-GABA encode and express memories are still understudied. In this study, we aim to describe how alcohol reward memories are encoded and expressed in LH GABA neurons. Using fibre photometry, we monitored LH-GABA calcium transients during acquisition and expression of cue-alcohol associations in rats. Then, we inhibited these neurons during acquisition of the cue-alcohol association with optogenetics. We first trained the rats on an alcohol conditioning task in which they learned to associate one conditioned stimulus (CS+) with alcohol availability in a magazine (20% EtOH in water), while a different conditioned stimulus (CS-) was presented without consequence. We then tested expression of the memory by presenting the CS+ and CS- alone. Our results show LH-GABA initially respond to all incoming stimuli regardless of their valence. This increased activity is maintained to alcohol-predictive stimuli and decreases to behaviorally irrelevant stimuli. In addition, we also found that LH-GABA activity decreases to below baseline during alcohol consumption. Moreover, in extinction, this decrease is no longer present and activity remains above baseline, potentially indicating that these neurons might be signalling reward prediction error. Finally, we also show that LH-GABA are functionally involved in the acquisition of cue-alcohol associations, as inhibiting these neurons during acquisition of such cues reduces alcohol seeking.

Pubmed:
34744653: McDonald AJ, Alonso-Lozares I, Rauh V, van Mourik Y, Schetters D, De Vries TJ, Marchant NJ
Alcohol Seeking Under Risk of Punishment Is Associated With Activation of Cortical and Subcortical Brain Regions.
In humans, stimuli associated with alcohol availability can provoke relapse during abstinence. In this study, we investigated the role of discriminative stimuli (DS) in the control of alcohol seeking in two types of behavioral tests. The first test examined the ability of an alcohol-associated DS to promote alcohol seeking (relapse) after punishment-imposed abstinence in the presence of a different DS. Following this, we tested whether the differentially associated DS can promote and suppress alcohol self-administration in a within-session discrimination task. During the within-session discrimination task, we also tested the rate of alcohol self-administration when two DS are presented in a compound. We first trained Long-Evans male rats (= 24) to self-administer alcohol in the presence of one DS (reward-associated discriminative stimulus, rewDS) and then punished that behavior in the presence of a different DS (punishment-associated discriminative stimulus, punDS). On the test, we found that rats tested with the rewDS showed higher alcohol seeking than rats tested with the punDS. This result shows that a single Cue DS can promote alcohol seeking in a manner comparable to contexts. Subsequently, we trained 16 of these rats in a within-session trial-based discrimination task, comprised of intervening 2-min trials of rewDS, punDS, or conflict with rewDS and punDS in compound and a reduced probability of punishment. We found that alcohol self-administration is bi-directionally regulated by the rewDS and punDS. In conflict trials, alcohol self-administration was at a rate that was intermediate between the rewDS and punDS trials. In a final test, rats were presented with one of the three trial conditions and perfused for Fos immunohistochemistry. We found Fos expression was higher in the rats tested in the conflict condition in three interconnected sub-cortical brain regions. This study demonstrated the important role that alcohol-associated DS plays an important role in promoting relapse to alcohol seeking after punishment-imposed abstinence. We also implemented a within-session discrimination task that allows for the study of alcohol seeking under motivational conflict, which may be relevant for alcohol use despite negative consequences. The results from the Fos data suggest that higher

alcohol seeking in approach-avoidance motivational conflict is associated with activation of sub-cortical regions but not cortical regions.
Front Behav Neurosci, 2021; 15

FENS Forum 2022
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BOARD NUMBER: S04-012

ALPHA7 NICOTINIC RECEPTORS IN VENTRAL, BUT NOT DORSAL, HIPPOCAMPUS REGULATE NEURONAL ACTIVATION DURING REINSTATEMENT OF HEROIN-CONDITIONED PLACE PREFERENCE

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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AIMS: We previously reported the alpha7 nicotinic acetylcholine receptor (α7nAChR) antagonist, methyllycaconitine (MLA) selectively attenuated reinstatement of morphine-conditioned place preference (CPP) when injected into the ventral hippocampus (vHIP)[1]. The vHIP contributes to the processing of drug-context associations critical for promoting relapse. This study explores the role of α7nAChRs in heroin-CPP and their location and contribution to neuronal activation in vHIP. **METHODS:** Male C57BL/6J mice (7-8 weeks old) acquired heroin-induced CPP (heroin: 2mg/kg, intraperitoneal). After extinction, mice were pretreated with MLA (4mg/kg, subcutaneous) or saline and reinstatement was induced by heroin-priming (1mg/kg, intraperitoneal), followed by perfusion-fixation to detect c-Fos expression. Whole-cell patch-clamp experiments were conducted on vHIP and dHIP CA1 pyramidal neurons in brain slices from naïve male C57BL/6J mice (4 weeks old). **RESULTS:** MLA decreased heroin-primed reinstatement of heroin-CPP ($p<0.05$ Student's t-test) and post-CPP c-Fos expression in vHIP CA1 pyramidal neurons ($p<0.05$ Student's t-test), but not in dHIP. A combination of α7 positive allosteric modulator and agonist (PNU-120596 (10μM) and PNU-282987 (300nM)) induced inward currents in vHIP and dHIP ($p<0.001$ one-way ANOVA, with post-hoc Tukey's test), confirming α7nAChRs in both regions. **CONCLUSIONS:** MLA blockade of reinstatement reinforces the importance of α7nAChRs in vHIP in circuitry governing associative memories. Although α7nAChRs are expressed in CA1 pyramidal neurons in vHIP and dHIP, MLA only affected c-Fos expression after heroin CPP in vHIP. It suggests the region-specific effect of MLA to inhibit opiate CPP is due to differences in networks involving vHIP and dHIP rather than α7nAChR expression. [1]Addiction Biology 24:590–603.

Pubmed:
32479855: Latagliata EC, Coccia G, Chiacchierini G, Milia C, Puglisi-Allegra S
Concomitant D1 and D2 dopamine receptor agonist infusion in prelimbic cortex is required to foster extinction of amphetamine-induced conditioned place preference.
Dopamine (DA) in medial prefrontal cortex is crucial in extinction of aversive or appetitive experiences. Although attention has been mostly focused on the infralimbic area of prefrontal cortex, a role of the prelimbic (PL) area has been envisaged pointing to DA transmission in the extinction of drug conditioned behavior. Evidence shows that DA exerts its action also via both D1 and D2 receptor subtypes. Here we investigated the effects of D1 and D2 receptor agonist microinfusion in the PL cortex of C57BL/6J mice on expression and extinction of amphetamine-induced conditioned place preference (CPP), in order to ascertain the effects of selective vs concomitant receptor subtypes stimulation. SKF38393 and Quinpirole were used at doses not impairing expression of amphetamine-induced CPP on the day of infusion. Acute infusion of each agonist alone did not affect extinction in subsequent days in comparison with Vehicle-treated mice, while concomitant infusion of both agonists produced a clear-cut advance of extinction of preference for the compartment previously paired with amphetamine. These results show that concomitant stimulation of D1 and D2 receptors in PL is required to foster extinction suggesting a synergic action between receptors or a heteromeric receptor involvement.
Behav Brain Res, 2020; 392

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BOARD NUMBER: S04-013

FRONTO-STRIATAL CONNECTIVITY PATTERNS ACCOUNT FOR THE IMPACT OF METHYLPHENIDATE ON CHOICE IMPULSIVITY AMONG HEALTHY ADULTS

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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Choice impulsivity (CI) depicts a preference towards smaller-sooner rewards over larger-delayed rewards, as revealed in delay discounting (DD) tasks. Previous research uncovered the prominent role of dopaminergic signaling within fronto-striatal circuits in mediating CI. Administration of methylphenidate (MPH), an indirect dopaminergic agonist, was shown to reduce CI in animals and pathological populations, although significant inter-individual variability in these effects was reported. Whether MPH impacts CI among healthy individuals, and whether variability in the impact of MPH is related to neural activation and connectivity patterns, has yet to be assessed. In order to address this gap, fifty-seven healthy young adults completed the DD task twice during fMRI scans, after acute administration of either MPH (20mg) or placebo, in a randomized double-blind placebo-controlled design. Acute MPH administration was found to reduce DD rates at the group level, yet substantial variability in this behavioral response was observed. MPH was also found to increase activation in the bilateral putamen and the right caudate, and to enhance functional connectivity between the left putamen and medial prefrontal cortex (mPFC), particularly during non-impulsive choices. Notably, the more putamen-mPFC functional connectivity increased during non-impulsive choices following MPH administration, the less an individual was likely to make impulsive choices. Our findings reveal, for the first time in healthy adults, that acute MPH administration is associated with reduced CI and increased striatal activation and fronto-striatal connectivity, and furthermore, that the magnitude of MPH-induced change in fronto-striatal connectivity may account for individual differences in the impact of MPH on impulsive behavior.

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BOARD NUMBER: S04-014

PREDICTING LOCAL BRAIN ACTIVITY FROM CONVERSATIONAL BEHAVIOURS: A NEW EXPERIMENTAL APPROACH TO INVESTIGATE THE NEURAL BASES OF NATURAL SOCIAL INTERACTIONS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims: “Second-person neuroscience” (Schilbach, 2013) requires new paradigms to study the neurophysiology of natural social interactions. fMRI was recorded while participants (n=24) discussed freely with a robot or a human. Acquired audio, video, eye tracking and physiological time-series synchronized with BOLD time-series (Rauchbauer, 2019) were used to identify which behavioural features are associated with brain responses across individually unique trials. Methods: Discretized local brain activity is predicted based on the behavioural recordings of each trial. Analysis included two steps, a feature extraction step to derive high-level features from raw signals, and the application of a dynamic prediction model to predict brain activity from the extracted features. 9-fold-cross-validation is performed on training data comprising eighteen participants to find the appropriate parameters of the Random Forest classifier based on the F-score, then tested on the six remaining participants. Results: We focused on a limited set of brain regions from the Brainnetome atlas (Fan, 2016) to validate the analysis, using behavioural features automatically extracted from linguistic exchanges, participants’ eye movements and conversational agents’ head and facial movements. Significantly accurate (p<0.001) predictions were found for the superior temporal sulcus, temporoparietal junction and precuneus bilaterally for Human-Human Interactions, while prediction was not significant for a white matter control region. Speech features dominated predictions for the temporal areas, while the interlocutor’s head and facial movements played an important role for prediction of the precuneus activity. Conclusions: This analysis demonstrates the possibility of associating complex combinations of behavioural features to local brain activity in unconstrained social interactions.

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BOARD NUMBER: S04-015

VISUAL SPATIAL PERCEPTION WITH DESCARTE'S THEORY IN THE VISUAL AIDS TEACHING CLASSROOM.

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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With the support of Visual-Spatial Perception with Descarte's Theory and Gardners's Visual-Spatial Intelligence Theory following narrative inquiry as to the research method, I collected the data through a series of interview sessions, casual conversations and class observations with four participants from one secondary school based on the North-eastern part of the Kathmandu Valley. I also observed their activities in the tutorial room, library, schoolroom, audio-visual laboratory, and computer test centre. For interpretation, analysis and discussion of the data, I read and reread them, searched patterns and saw the emerging themes and discussed them within the theoretical framework of Howard Gardner's 'Visual-Spatial Intelligence' theory of multiple intelligences. From the interpretation, analysis, and discussion of the data, I learned that learning a language like English through visual aids enables the learners to develop positive social skills, new techniques of reading habits in visual aids supporting the words of books, and learning constructing knowledge for life. Correspondingly, this new way of learning habits in P-Model Classroom changes the practices of students into visual literacy through technology-rich-environment scenarios in the classroom. In addition, the learning method creates a creative embellishment and fascination of students' creativity, making their classroom well-structured and engaging them in different techniques of creative works. So, this new way of learning habits supports students' efforts to understand content taught in the classroom. These findings also indicate the sturdy practice for secondary school students, which reduces the uncertain activities at the school by saving the teacher's time.

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BOARD NUMBER: S04-016

OXYTOCINERGIC MODULATION OF SPEECH PRODUCTION – A DOUBLE-BLIND PLACEBO CONTROLLED FMRI STUDY

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Many socio-affective behaviors, such as speech, are modulated by oxytocin. While oxytocin modulates speech perception, it is not known whether it also affects speech production. Here we investigated effects of oxytocin administration and interactions with the functional rs53576 oxytocin receptor (OXTR) polymorphism on produced speech and its underlying brain activity. During fMRI, 50 healthy male participants read sentences with either neutral or happy intonation, a covert reading condition served as a common baseline. Participants were studied once under the influence of intranasal oxytocin and in another session under placebo. Participants were grouped based on their polymorphism (AA/AG vs. GG). In carriers of the A allele, the OXTR is thought to be less effective compared to GG homozygotes. Oxytocin administration increased the second formant of produced vowels, a phonetic feature associated with speech valence. When preparing to speak, oxytocin enhanced brain activity in sensorimotor cortices of both dorsal and right ventral speech streams, as well as limbic and executive control regions. In some of these regions, oxytocin administration helped carriers of the A allele to pre-activate similar to GG homozygotes. In the remaining regions, oxytocin increased preparatory activity in all participants. Oxytocin also gated cortico-basal loops involved in the generation of happy prosody. Our findings suggest several neural processes underlying speech production are gated by oxytocin, including control of affective intonation but also of sensorimotor aspects during emotionally neutral speech.

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BOARD NUMBER: S04-017

COGNITIVE STATUS OF INDIVIDUALS WITH APHASIA- ELECTROPHYSIOLOGICAL MARKERS.

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aphasia is an acquired impairment of language functions resulting from a brain lesion. It is also accompanied by deficits in non-language cognitive functions, such as working memory, executive functions and temporal resolution. Assessment of cognitive capacity in people with aphasia (PWA) with standard neuropsychological methods may be problematic due to their language difficulties. To avoid the language difficulties bias, the objective electrophysiological methods may be applied to assess the cognitive capacity of PWA. The study was aimed to verify the utility of P300 and mismatch negativity (MMN) parameters as measures of cognitive function efficiency in PWA. A total of 26 PWA after left-hemispheric stroke participated in the study. Several language and non-language cognitive functions were assessed behaviourally. Two electrophysiological procedures were applied: visual Go/No-Go task as well as auditory syllable discrimination task. Shorter P300 latencies were associated with better temporal resolution, psychomotor speed, planning ability, spatial working memory, as well as better word and sentence comprehension and verbal fluency. On the other hand, higher MMN amplitudes corresponded to better psychomotor speed, spatial working memory, phoneme hearing, word comprehension and verbal fluency. The results suggest that studied ERP components might be objective indices of non-verbal and verbal cognitive functions in PWA and can be potentially applicable to monitor cognitive status of these patients. Supported by National Science Centre, Poland, grant number: 2016/21/B/HS6/03775.

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BOARD NUMBER: S04-018

ESM AND FMRI EVIDENCE OF L1 AND L2 LANGUAGE SITES SPATIALLY DISSOCIATED WITHIN THE TEMPORAL LOBE IN BILINGUAL BRAIN TUMOR PATIENTS AND HEALTHY SUBJECTS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Bilingualism has raised concern about how languages are represented in the brain. Neuroimaging and electrocortical stimulation studies have shown identical cortical representations for the native (L1) and second language (L2), variable overlap or no overlap at all. Our aim was to identify and compare cortical representations for L1 and L2 within the temporal cortex considering its relation with language. We tested 5 Spanish-Basque bilingual low-grade glioma patients with left temporal tumours through intraoperative mapping and 20 healthy bilingual subjects with neuroimaging methods using a standardised multilingual picture naming test (MULTIMAP). Healthy participants showed a common system for L1 and L2, with the left middle and superior temporal areas responding differently in terms of activation. While the middle temporal gyrus showed higher activation for L1 than for L2, the superior temporal gyrus was recruited only for L1. Patients showed language specific sites for L1 in the superior temporal gyrus and for L2 in the middle temporal gyrus. Evidence combined suggests representations for both languages may involve functionally independent populations within the left temporal territory explored and underlies the importance of testing multiple languages to prevent possible deficits, moving towards individually tailored interventions.

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BOARD NUMBER: S04-019

THE EMBODIMENT OF ADJECTIVES

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims: Language embodiment theories typically propose that processing language is built upon experiences of the actions, objects, and events to which the language refers (Marino et al., 2011). Generally, language embodiment experiments have tested motor responses to verbs and nouns only; therefore, knowledge of the topic is constructed upon a limited set of word categories. The current experiment used three different tasks requiring different levels of processing to test the embodiment of ***adjectives.*** **Methods:** Three behavioural experiments were conducted – a GoSignal task (*N* = 40), a lexical decision task (online) (*N* = 150), and a semantic decision task (*N* = 40). Each experiment tested the effect of adjective type (negative vs. non-negative vs. pseudo) on the expected affordance effect of noun type (i.e. quicker hand responses to hand-related vs. non-hand related nouns). **Results:** The GoSignal task revealed a significant main effect for noun type only (quicker for hand-related nouns). However, the semantic decision task revealed a main effect for noun type in the opposite direction (slower responses) and a main effect for adjective type (slower for pseudo) – but no interaction effect between adjective/noun type. The lexical decision task is ongoing. **Conclusions**: In a passive GoSignal task, there was no interaction between adjective/noun type. Similarly, there was no interaction in the semantic decision task; however, responses to adjective and noun types were significantly influenced by task demands. The lexical decision task will allow us to further test the embodiment of adjectives and the influence of task demands on embodiment.

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BOARD NUMBER: S04-020

A DEEP HIERARCHY OF PREDICTIONS ENABLES DYNAMIC ASSIGNMENT OF SEMANTIC ROLES IN SPEECH COMPREHENSION

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Understanding speech requires mapping fleeting and often ambiguous soundwaves to meaning. Humans are known to exploit their capacity to contextualize to facilitate this process, but how internal knowledge is used and deployed in real time remains an open question. Existing models of speech processing focus on either word recognition irrespective of meaning or interactions among abstract linguistic representations without time constraints, providing only partial insights into the dynamics of speech comprehension. Here, we present a model that incrementally extracts multiple levels of information from continuous speech signals in real time, based on the inversion of a generative model that represents the listener's internal knowledge of linguistic and non-linguistic processing levels in a nested temporal hierarchy. In each hierarchy, the model periodically incorporates bottom-up incoming evidence to update its internal representations and generate new top-down predictions. We show that a context level, beyond linguistic representations, can provide the model with semantic predictions informed by sensory inputs, crucial for the disambiguation among multiple meanings of the same word. We also show that hierarchical predictions can reduce peripheral processing effort via minimizing uncertainty and prediction error, especially when sensory precisions become degraded. With this proof-of-concept model, we demonstrate that the deployment of hierarchical predictions is a possible strategy for the brain to utilize structured knowledge dynamically for speech comprehension.

Pubmed:
33891217: Su Y, Chung Y, Goodman DFM, Hancock KE, Delgutte B
Rate and Temporal Coding of Regular and Irregular Pulse Trains in Auditory Midbrain of Normal-Hearing and Cochlear-Implanted Rabbits.
Although pitch is closely related to temporal periodicity, stimuli with a degree of temporal irregularity can evoke a pitch sensation in human listeners. However, the neural mechanisms underlying pitch perception for irregular sounds are poorly understood. Here, we recorded responses of single units in the inferior colliculus (IC) of normal hearing (NH) rabbits to acoustic pulse trains with different amounts of random jitter in the inter-pulse intervals and compared with responses to electric pulse trains delivered through a cochlear implant (CI) in a different group of rabbits. In both NH and CI animals, many IC neurons demonstrated tuning of firing rate to the average pulse rate (APR) that was robust against temporal jitter, although jitter tended to increase the firing rates for APRs ≥ 1280 Hz. Strength and limiting frequency of spike synchronization to stimulus pulses were also comparable between periodic and irregular pulse trains, although there was a slight increase in synchronization at high APRs with CI stimulation. There were clear differences between CI and NH animals in both the range of APRs over which firing rate tuning was observed and the prevalence of synchronized responses. These results suggest that the pitches of regular and irregular pulse trains are coded differently by IC neurons depending on the APR, the degree of irregularity, and the mode of stimulation. In particular, the temporal pitch produced by periodic pulse trains lacking spectral cues may be based on a rate code rather than a temporal code at higher APRs.
J Assoc Res Otolaryngol, 2021; 22
31996454: Su Y, Delgutte B
Robust Rate-Place Coding of Resolved Components in Harmonic and Inharmonic Complex Tones in Auditory Midbrain.
Harmonic complex tones (HCTs) commonly occurring in speech and music evoke a strong pitch at their fundamental frequency (F0), especially when they contain harmonics individually resolved by the cochlea. When all frequency components of an HCT are shifted by the same amount, the pitch of the resulting inharmonic tone (IHCT) can also shift, although the envelope repetition rate is unchanged. A rate-place code, whereby resolved harmonics are represented by local maxima in firing rates along the tonotopic axis, has been characterized in the auditory nerve and primary auditory cortex, but little is known about intermediate processing stages. We recorded single-neuron responses to HCT and IHCT with varying F0 and

sound level in the inferior colliculus (IC) of unanesthetized rabbits of both sexes. Many neurons showed peaks in firing rate when a low-numbered harmonic aligned with the neuron's characteristic frequency, demonstrating "rate-place" coding. The IC rate-place code was most prevalent for F0 > 800 Hz, was only moderately dependent on sound level over a 40 dB range, and was not sensitive to stimulus harmonicity. A spectral receptive-field model incorporating broadband inhibition better predicted the neural responses than a purely excitatory model, suggesting an enhancement of the rate-place representation by inhibition. Some IC neurons showed facilitation in response to HCT relative to pure tones, similar to cortical "harmonic template neurons" (Feng and Wang, 2017), but to a lesser degree. Our findings shed light on the transformation of rate-place coding of resolved harmonics along the auditory pathway. Harmonic complex tones are ubiquitous in speech and music and produce strong pitch percepts when they contain frequency components that are individually resolved by the cochlea. Here, we characterize a "rate-place" code for resolved harmonics in the auditory midbrain that is more robust across sound levels than the peripheral rate-place code and insensitive to the harmonic relationships among frequency components. We use a computational model to show that inhibition may play an important role in shaping the rate-place code. Our study fills a major gap in understanding the transformations in neural representations of resolved harmonics along the auditory pathway.
J Neurosci, 2020; 40
31664871: Su Y, Delgutte B
Pitch of harmonic complex tones: rate and temporal coding of envelope repetition rate in inferior colliculus of unanesthetized rabbits.
Harmonic complex tones (HCTs) found in speech, music, and animal vocalizations evoke strong pitch percepts at their fundamental frequencies. The strongest pitches are produced by HCTs that contain harmonics resolved by cochlear frequency analysis, but HCTs containing solely unresolved harmonics also evoke a weaker pitch at their envelope repetition rate (ERR). In the auditory periphery, neurons phase lock to the stimulus envelope, but this temporal representation of ERR degrades and gives way to rate codes along the ascending auditory pathway. To assess the role of the inferior colliculus (IC) in such transformations, we recorded IC neuron responses to HCT and sinusoidally modulated broadband noise (SAMN) with varying ERR from unanesthetized rabbits. Different interharmonic phase relationships of HCT were used to manipulate the temporal envelope without changing the power spectrum. Many IC neurons demonstrated band-pass rate tuning to ERR between 60 and 1,600 Hz for HCT and between 40 and 500 Hz for SAMN. The tuning was not related to the pure-tone best frequency of neurons but was dependent on the shape of the stimulus envelope, indicating a temporal rather than spectral origin. A phenomenological model suggests that the tuning may arise from peripheral temporal response patterns via synaptic inhibition. We also characterized temporal coding to ERR. Some IC neurons could phase lock to the stimulus envelope up to 900 Hz for either HCT or SAMN, but phase locking was weaker with SAMN. Together, the rate code and the temporal code represent a wide range of ERR, providing strong cues for the pitch of unresolved harmonics. Envelope repetition rate (ERR) provides crucial cues for pitch perception of frequency components that are not individually resolved by the cochlea, but the neural representation of ERR for stimuli containing many harmonics is poorly characterized. Here we show that the pitch of stimuli with unresolved harmonics is represented by both a rate code and a temporal code for ERR in auditory midbrain neurons and propose possible underlying neural mechanisms with a computational model.
J Neurophysiol, 2019; 122
30613198: Su Y, Delgutte B
Pitch of Harmonic Complex Tones: Rate Coding of Envelope Repetition Rate in the Auditory Midbrain.
Envelope repetition rate (ERR) is an important cue for the pitch of harmonic complex tones (HCT), especially when the tone consists entirely of unresolved harmonics. Neural synchronization to the stimulus envelope provides a prominent cue for ERR in the auditory periphery, but this temporal code becomes degraded and gives way to rate codes in higher centers. The inferior colliculus (IC) likely plays a key role in this temporal-to-rate code transformation. Here we recorded single IC neuron responses to HCT at varying fundamental frequencies ( ). ERR was manipulated by applying different inter-harmonic phase relationships. We identified a subset of neurons that showed a 'non-tonotopic' rate tuning to ERR between 160 and 1500 Hz. A comparison of neural responses to HCT and sinusoidally amplitude modulated (SAM) noise suggests that this tuning is dependent on the shape of stimulus envelope. A phenomenological model is able to reproduce the non-tonotopic tuning to ERR, and suggests it arises in the IC via synaptic inhibition.
Acta Acust United Acust, 2018 Sep-Oct; 104
28061857: Aquino P, Honda B, Jaini S, Lyubetskaya A, Hosur K, Chiu JG, Ekladious I, Hu D, Jin L, Sayeg MK, Stettner AI, Wang J, Wong BG, Wong WS, Alexander SL, Ba C, Bensussen SI, Bernstein DB, Braff D, Cha S, Cheng DI, Cho JH, Chou K, Chuang J, Gastler DE, Grasso DJ, Greifenberger JS, Guo C, Hawes AK, Israni DV, Jain SR, Kim J, Lei J, Li H, Li D, Li Q, Mancuso CP, Mao N, Masud SF, Meisel CL, Mi J, Nykyforchyn CS, Park M, Peterson HM, Ramirez AK, Reynolds DS, Rim NG, Saffie JC, Su H, Su WR, Su Y, Sun M, Thommes MM, Tu T, Varongchayakul N, Wagner TE, Weinberg BH, Yang R, Yaroslavsky A, Yoon C, Zhao Y, Zollinger AJ, Stringer AM, Foster JW, Wade J, Raman S, Broude N, Wong WW, Galagan JE
Coordinated regulation of acid resistance in Escherichia coli.

Enteric Escherichia coli survives the highly acidic environment of the stomach through multiple acid resistance (AR) mechanisms. The most effective system, AR2, decarboxylates externally-derived glutamate to remove cytoplasmic protons and excrete GABA. The first described system, AR1, does not require an external amino acid. Its mechanism has not been determined. The regulation of the multiple AR systems and their coordination with broader cellular metabolism has not been fully explored.
BMC Syst Biol, 2017; 11

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BOARD NUMBER: S04-021

READING NEGATIVE ACTION VERBS: ONE OR TWO-STEP PROCESSING IN THE PRIMARY MOTOR CORTEX?

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Action language comprehension automatically activates distributed semantic motor representations. However, controversy persists regarding the representation of negated actions, specifically concerning the activation and inhibitory mechanisms in the motor system, and whether this occurs in one or two-steps. We conducted two experiments probing corticospinal excitability (CSE) and short intracortical inhibition (SICI) in the primary motor cortex at different timings while reading affirmative and negative action sentences. Twenty-six participants silently read manual action and non-action sentences in the affirmative or negative form (The potato is cooked, I peel / don't peel it). Using transcranial magnetic stimulation, we probed CSE in hand muscles by means of the peak-to-peak amplitude of motor-evoked potentials at rest and at several latencies (200, 300, 400, 500 and 600ms) after verb presentation. We observed a main effect of action, with greater CSE for action sentences compared to non-action sentences, regardless of verb form. In experiment two, nineteen participants silently read affirmative and negative action sentences. We measured CSE and SICI at short and long latencies after verb presentation. CSE was greater for affirmative and negative action verbs at both latencies compared to rest. SICI did not change at the short latency but increased at long ones, regardless of verb form. Our results are broadly consistent with a two-step model, as negated actions showed the same motor excitability as affirmed actions with no additional inhibition at early latencies. Neural differences between affirmative and negative verb forms in a second processing step may occur outside the primary motor cortex.

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BOARD NUMBER: S04-022

THE CONTRIBUTION OF THE SENSORY-MOTOR INTERACTIONS TO IMAGINED SPEECH

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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The neural activity responsible for imagined speech has mainly been studied by comparing overt with covert speech. Although very informative, the results of these studies are limited in scope as they focus on the motor articulation of speech. However, imagined speech does not only involve the articulatory system, but also the activation of language representations. We propose here to address this issue by comparing two covert speech conditions: imagined speech and silent reading. Our hypothesis is that the coupling between the sensory-motor systems is at the origin of the activation of syllabic and phonemic representations of silent speech. We recorded magnetoencephalography data from 21 adults in two experimental conditions: the same sentence was first read silently and then imagined silently. We mapped the spatiotemporal flow of information across the cortex, characterizing the distribution and timing of evoked activity and the frequency composition of the induced activity. Temporal and motor areas show an earlier evoked response, and a stronger early information transfer in the Imagine Saying condition compared to the Silent Reading condition. Our data also show that the production of covert speech is associated with bursts of low-beta (12-15 Hz) neural oscillations in the sensory and motor areas. Finally, we observed that information propagation between motor and sensory areas relies on a phase-amplitude coupling between low-beta and low gamma.

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BOARD NUMBER: S04-023

CONTROLLING A BRAIN-COMPUTER INTERFACE TO DECODE INTERNALLY SPOKEN SYLLABLES

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Real-time decoding of covert speech from Electroencephalography (EEG) could provide non-invasive solutions for people with compromised speech, such as patients affected by aphasia. We designed an EEG-based Brain-Computer Interface (BCI) to classify two syllables having different articulatory and phonetic features. In order to investigate whether operating a BCI can be learnt, participants were trained to control the BCI daily for 5 consecutive days. We used a random forest classifier based on power spectrum density between 1-70Hz to 'train' (offline, i.e. without any feedback) and then to 'test' (online) the data with real-time visual feedback. Results show that the gamma band (30-70Hz) from the bilateral temporal electrodes largely contribute to the offline classification accuracy. Further, participants show maximum control during online BCI training on day-4, through a progressive performance improvement from day-1, followed by a decrease on day-5. Corresponding neural data showed a significant power increase on day-4 compared to day-1 in left and fronto-temporal electrodes in theta, beta, and high-gamma bands that might underpin the acquisition of BCI-control skills. EEG response related to covert speech indicate Event-Related Desynchronization and Synchronization in the frequency bands related to overt speech, namely theta, beta, and gamma bands. The location and power difference in the neural data corresponding to the two class of syllables support the classifier preference of using gamma band to distinguish between the two class of syllable. Overall, our results indicate that EEG gamma band power is effective in classifying syllable imagery and that training can improve BCI performance.

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BOARD NUMBER: S04-024

PASSIVE LEARNING OF SPEECH SOUNDS ASSOCIATED WITH MICROSTRUCTURE OF FRONTO-TEMPORO-PARIETAL BUT NOT FRONTO-STRIATAL WHITE MATTER TRACTS: POSSIBLE IMPLICATIONS FOR IMPLICIT LANGUAGE LEARNING TASKS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Language learners must become able to perceive and process new speech sounds in order to segment words and sentences. The process of obtaining these skills is, to a high degree, implicit. However, research attempting to find the neuroanatomical correlates of such an implicit learning system has mostly used artificial grammar or serial reaction time tasks that are lacking in linguistic relevance. In this study, we investigated the relationship between aptitude for implicit learning of novel speech sounds and tissue microstructure in fronto-temporo-parietal and fronto-striatal white matter tracts using the LLAMA D (phonetic memory) test and diffusion-weighted magnetic resonance imaging. We found positive correlations between phonetic memory scores and axial kurtosis in the left arcuate (AF) and superior longitudinal fasciculus (SLF) III ($r=-0.518$, $p=8.53e-05$ and $r=-0.440$, $p=0.0011$, respectively) as well as mean and radial kurtosis in the left SLF III ($r=-0.356$, $p=0.0095$ and $r=-0.378$, $p=0.0058$, respectively). Diffusion parameters in fronto-striatal tracts showed no significant association with phonetic memory score (all $p>0.05$). These results indicate that left AF and SLF III are important for passive memorisation of novel speech sounds, possibly linked to auditory-motor and sensorimotor associations involved in articulation. The lack of significant correlations in fronto-striatal tracts might be due to the absence of sequence learning in the LLAMA D test. This highlights the need for learning tasks that target more specific aspects of the neural bases of implicit learning.

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BOARD NUMBER: S04-025

NEURAL DYNAMICS UNDERLYING HUMAN VOCALIZATION

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Human speech allows to convey information via different execution modalities. On the behavioral level, speech content is independent from production and execution modalities. However, it remains unclear whether such a content dimension can be found on the neural level and whether it is possible to dissociate it from the motor dimension. To address this, we recorded magnetoencephalography (MEG) in human subjects that performed a rule-based vocalization task. Content (one of two vowels) and production (overt or covert) were instructed separately and in random order. Applying multivariate pattern analysis (MVPA), we found robust neural information about the content and production of vocalization several seconds before vocalization behavior. Source analysis revealed neural information in speech areas of the left hemisphere. The strength of both types of neural information correlated with the degree of motor involvement. When isolated, both types of information overlapped. Later in the trial, the neural format of production information transformed depending on the content, whereas content information remained stable independent of production type. Our results provide new insights into the neural dynamics underlying basic human vocalization and open a new window for non-invasive speech research in humans.

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BOARD NUMBER: S04-026

IDENTIFICATION OF EARLY NEUROCOGNITIVE AND NEUROANATOMICAL PREDICTORS OF LANGUAGE AND LEARNING DISORDERS: SYSTEMATIC REVIEW AND META-ANALYSIS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Objective: To identify the significant early neurophysiological, neuroanatomical, and neurocognitive predictors of developmental-associated language and learning disabilities (LLDs) in children. **Data Sources:** We searched three electronic databases (PubMed, Scopus, and WOS) for studies published in English between January 2000 and November 2021. **Study Selection:** Eighty-four studies (Randomized controlled trials (RCT) n =12; non-RCT n=74) involves neurophysiological, neuroanatomical, and neurocognitive predictors of dyslexia, dyscalculia, and dysgraphia among children aged between 3–16-year-old were included. **Data Extraction:** Two reviewers independently assessed full-text articles, one reviewer extracted the data, the second reviewer checked the reliability, then narrative methods of synthesis were used, and for sufficiently similar studies, a meta-analysis of effect size was carried out (using a random-effects meta-analysis model). **Data Synthesis:** An electroencephalogram (EEG) finding revealed dyslexia-related neurophysiological predictors such as altered mismatch response, amplitude, and latency changes in event-related potentials. From functional magnetic resonance imaging (fMRI) results children with dyslexia showed decreased activity in the left parietotemporal, left occipitotemporal, left superior temporal gyrus, bilateral middle frontal gyrus, left inferior temporal gyrus, and left hemisphere prefrontal areas during reading, visual scanning, and listening tasks. Moreover, magnetoencephalography (MEG) revealed increased activity in the right inferior and superior parietal areas during arithmetic tasks in children with dyscalculia. From neurocognitive findings, a combined group of dyslexia and dysgraphia showed deficits in verbal working memory, semantic memory, quantitative reasoning, visuospatial reasoning, and phonological processing. **Conclusion:** Early predictors of neurodevelopmental-associated LLDs would be helpful as targets for specific prevention and intervention programs to be applied at very young ages.

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BOARD NUMBER: S04-027

EVENT-RELATED CAUSALITY IN STEREO-EEG DECODES SYNTACTIC CATEGORY OF PERCEIVED SENTENCES

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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In recent years, there have been important technological and methodological advancements in perceived and imagined speech decoding. The most advanced online decoding techniques rely heavily on the articulatory representation of syllables and words in the motor and supplementary motor cortices. However, this approach can only be applied to patients with intact motor commands. Thus, other decoding strategies that rely on the brain regions that encode speech are needed. Here, we decoded the syntactic category of perceived stimuli exploiting 29 different speech-encoding cortical areas spanning the entire brain. Using a protocol able to separate syntactic information from sound information we investigated the neural causal connections evoked by the processing of homophonous phrases, either verb phrases (VP) or noun phrases (NP). Each stimulus (a sentence) was composed of three segments where only the intermediate segment was an homophonous phrase. We used event-related causality from stereo-electroencephalographic recordings in 10 epileptic patients. We identified the different networks involved in the processing of the two syntactic operations. We extracted time-varying features on the identified significant connections and we used a Support Vector Machine to decode the syntactic content of the homophonous phrase (NP or VP) in a time-dependent fashion. The trial by trial accuracy was significantly above chance during the homophonous phrase presentation. The signals that drove the decoding were directly entangled to the syntactic representation of the stimuli rather than their articular components. A decoding strategy that relies on multiple speech-encoding cortical areas will drastically improve the performance of speech prostheses.

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BOARD NUMBER: S04-028

CEREBELLAR TDCS HAS NO EFFECT ON SEMANTIC PREDICTION

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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The cerebellum is considered to regulate behavioral output in multiple domains (motor, affective, and cognitive). While growing evidence indicates that the cerebellum supports speech and language, its exact role in language processing is yet poorly understood. It has been proposed that an important aspect of the cerebellar function is the capacity to generate predictions in perception and action. Semantic prediction importantly supports speech production and comprehension. The aim of our study was to investigate the engagement of the cerebellum in semantic prediction using transcranial direct current stimulation (tDCS). For this purpose, we administered a sentence completion task to 136 healthy adults prior and immediately after sham (n = 45), anodal (n = 45) or cathodal (n = 46) tDCS of the cerebellum (20 min, 2 mA). We found, however, that the cerebellar tDCS had no significant effect on completing predictable or unpredictable sentences. These results argue against a significant role of the cerebellum in predictive language processing. This study was supported by grants no. VEGA 2/0059/20 and APVV-19-0570.

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BOARD NUMBER: S04-029

CEREBELLAR TDCS MODULATES RETRIEVAL FROM LEXICAL-SEMANTIC MEMORY

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Increasing evidence suggests that the cerebellum plays a role in language functions. Our study focused on lexical-semantic memory and tested the contribution of the cerebellum to automatic vs. controlled retrieval using transcranial direct current stimulation (tDCS). Our sample included 136 healthy adults, who underwent either sham (*n* = 45), anodal (*n* = 45) or cathodal tDCS (*n* = 46). The stimulating electrode (5x7 cm) was placed over the cerebellum (with its top border 1-2 cm below the inion) and the reference electrode on the right arm. Stimulation lasted for 20 minutes; the current intensity was 2 mA. Lexical-semantic retrieval was assessed prior to and after stimulation by the associative chain test (ACT), in which participants generate word chains according to specific rules (continuous retrieval of semantically related words, continuous retrieval of unrelated words or retrieval of related and unrelated words in alternation). We found that anodal tDCS facilitated continuous retrieval of semantically related words but had no effect in other retrieval conditions. Cathodal tDCS yielded no significant changes in retrieval performance. These findings indicate that the cerebellum is involved in automatic retrieval from lexical-semantic memory, rather than retrieval control. This study was supported by the project VEGA 2/0059/20 and APVV-19-0570.

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BOARD NUMBER: S04-030

EXAMINING THE ASSOCIATION BETWEEN EXECUTIVE FUNCTIONS AND DECODING IN FRENCH AND ITALIAN SCHOOL-AGED CHILDREN

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Executive functions (EFs) are related to reading, as regards both decoding and comprehension of print. Few studies examined in one single model the contribution of multiple EFs to decoding. The present study aims to investigate whether visual working memory (WM), verbal WM, planning, and visual attention uniquely contribute to reading speed and reading accuracy in school-aged children. It further compares those influences across orthographically opaque (French) and transparent languages (Italian). 201 French-speaking and 148 Italian-speaking 7-to-13-years-old children took part in our study. Participants performed a verbal WM task (Backward Digit Span), a visual span task (Corsi backward), a visual attentional task (barrage test) and a planning task (Tower of London). We also estimated fluid intelligence and reading (word and pseudo-words reading lists). Combining multiple regression and factor analysis, we used Structural Equation Models to test whether our theoretical model based on multiple EFs correctly describes empirical data. Results show that EFs differently contribute to reading speed and accuracy in French and Italian. Both languages show an association between verbal WM and reading accuracy and speed. However, for the French model, we report a relationship between fluid intelligence and reading accuracy, while the Italian model shows an association between visual attention and both reading speed and accuracy. Our models confirm the importance of EFs in reading and their different contribution to decoding across orthographically distinct languages, in line with previous literature.

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BOARD NUMBER: S04-031

THE ROLE OF THE POSTERIOR CEREBELLUM IN DYSFUNCTIONAL SOCIAL SEQUENCING

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims: Recent advances in social neuroscience have highlighted the critical role of the cerebellum in social cognition, and especially the posterior cerebellum. Studies have shown that the posterior cerebellum is recruited when identifying and automatizing sequences of social actions involving mentalizing (i.e., inferring others' beliefs, intentions, traits, …), and inconsistencies in these sequences or their social implications. This supports the view that the posterior cerebellum predicts how other people's actions will unfold in an automatic and intuitive way. **Hypothesis:** We argue that the central role of the posterior cerebellum in social sequencing provides a fruitful starting point for investigating social dysfunctions in a variety of clinical pathologies, such as autism, obsessive–compulsive and bipolar disorder, depression, and addiction. Our key hypothesis is that dysfunctions of the posterior cerebellum lead to under- or overuse of inflexible social routines and lack of plasticity for learning new, more adaptive, social automatisms. **Methods & Results:** A brief review of meta-analyses shows that the posterior cerebellum is involved in most of these pathologies. Recent research applying cerebellar TMS or tDCS showed improvements in identifying social and non-social sequences, and a novel narrative training program with individuals with autism focusing on identifying social sequences in stories is currently being investigated (Van Overwalle et al, 2021). **Conclusions:** We conclude that this novel cerebellar perspective might aid to alleviate a variety of mental problems related to inflexible automatizations, by increasing posterior cerebellar plasticity using noninvasive neurostimulation or neuro-guided training programs.

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BOARD NUMBER: S04-032

SPATIAL EMBODIMENT OF TIME AND EMOTION DURING A MOVEMENT ALONG THE FRONTAL AND SAGITTAL AXIS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims: The literature evidencing spatial embodiment of time (Boroditsky, 2000; Flumini and Santiago 2013) or emotional valence (Bradley & Lang, 2006; Spatola et al, 2018) used push button responses without hand movements along a given axis of response. Here, we tested if hand movements would be facilitated or impaired in response to verbs, depending on whether these movements are congruent or incongruent with respect to the spatial metaphor of abstract concepts. **Methods :** Thirty-five participants indicated either the tense or the emotional of verbs using a hand movement (about 15 cm) toward a response button located along either the frontal or sagittal axis, depending on group. Each participant performed two blocks of trials: either congruent (rightward/forward: future or positive; leftward/backward: past or negative), or incongruent (rightward/forward: past or negative; leftward/backward: future or positive) with the spatial metaphor of concepts. In order to control for the fact that leftward/rightward or forward/backward movements may be asymmetric, in a preliminary control participants performed each possible movement in response to an arrow. We then subtracted these times to the reaction times in response to the verbs in order to compute a processing time (PT). **Results**: We conducted a RM-ANOVA on PTs that evidenced spatial embodiment of concepts in both group ($M_{congruent}$ = 467ms; $M_{incongruent}$ = 564ms), p = 0.016, d = 0.42, BF_{10} = 2.66 > BF_{01} = 0.38. **Conclusion:** The present study provides evidence of an influence on hand movement of abstract concepts such as time and emotional valence.

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BRAIN STRUCTURE RELATES TO SELF-REPORT EMPATHY AND CLINICAL PSYCHOPATHY IN INCARCERATED MALES

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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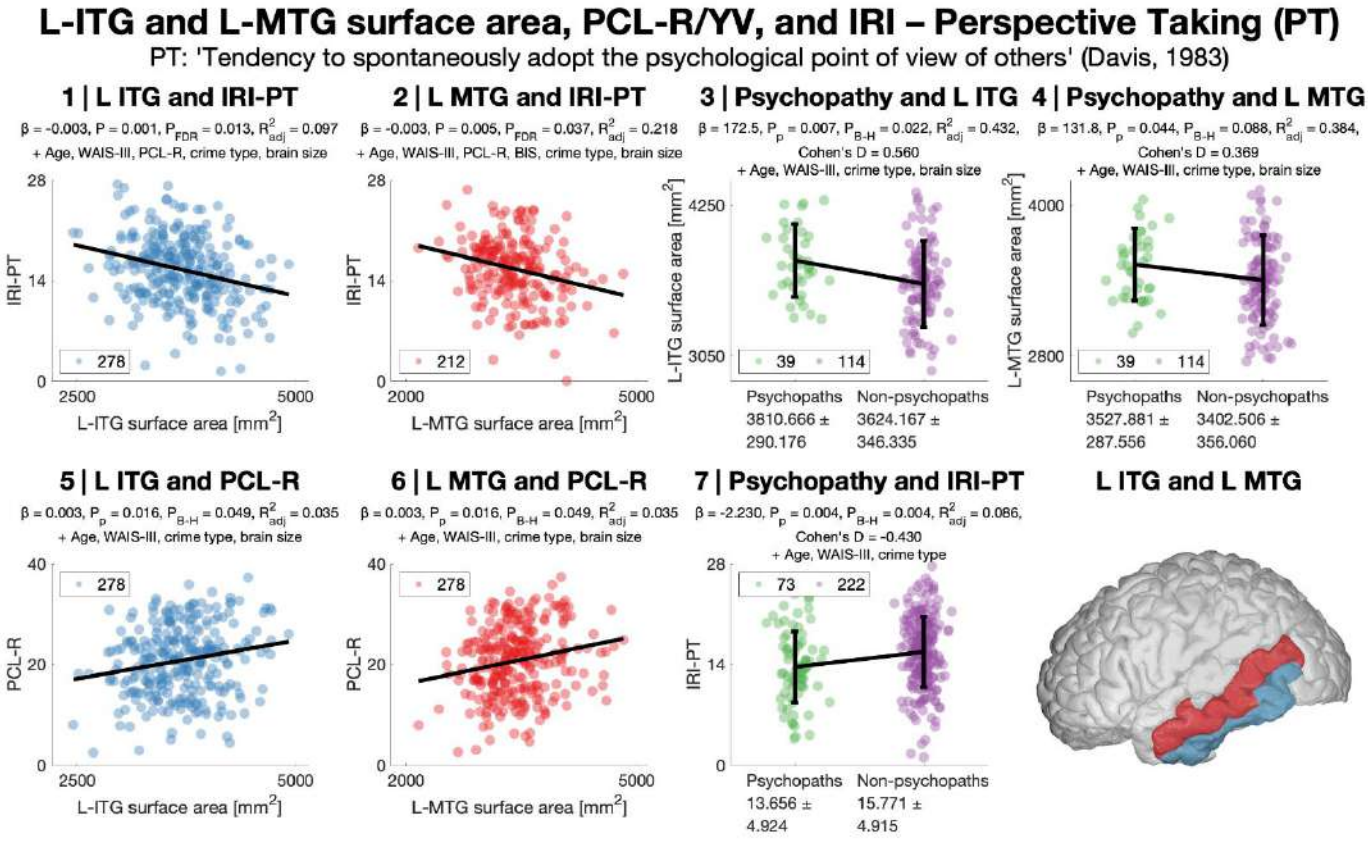
AIMS: We examined relationships between self-report cognitive and affective empathy, clinical psychopathy and brain structure in incarcerated males. **METHODS:** Perspective Taking (PT) and Empathic Concern (EC) subscales of the Interpersonal Reactivity Index were used to measure cognitive and affective empathy, respectively, and the Hare Psychopathy Checklist-Revised to measure psychopathic traits (N=550). Brain measures included FreeSurfer-derived surface area for the 68 cortical Desikan-Killiany regions following high-resolution T1-weighted MRI (N=278, all White). We also measured IQ using the WAIS-III and impulsivity using the Barratt Impulsiveness Scale. With robust multiple linear regression (P<0.05, corrected), we tested whether: brain structure relates to empathy; identified brain regions relate to psychopathic traits; psychopathy diagnosis relates to both empathy and identified brain regions. **RESULTS:** Left inferior temporal gyrus (LITG) related negatively to PT when covarying for age, IQ, psychopathic traits, crime type (non-violent/violent/homicide) and brain size. Left middle temporal gyrus (LMTG) related negatively to PT when also covarying for impulsivity. Right medial orbitofrontal cortex (RmOFC) related negatively to EC when controlling for the first five covariates. LITG and LMTG – but not RmOFC – related positively to psychopathic traits when covarying for age, IQ, crime type and brain size. Psychopathy diagnosis related to reduced PT when covarying for age, IQ and crime type as well as enlarged LITG when also covarying for brain size. **CONCLUSIONS:** Cognitive and affective empathy related negatively to the surface area of temporal and prefrontal regions, respectively. These findings provide novel insights into the brain correlates of callous-unemotional traits among antisocial

populations.



Pubmed:
30570276: Radecki MA, Cox SR, MacPherson SE
Theory of mind and psychosocial characteristics in older men.
The extent to which early-life cognitive ability shapes individuals' social functioning throughout life, in the context of later-life factors, is unknown. We investigated performance on the Faux Pas test (FP) in relation to psychosocial characteristics and childhood intelligence scores in 90 healthy older men. FP performance was associated with close social network size but not social contact, social support, or loneliness when accounting for both childhood and later-life intelligence, affect, personality, and sociodemography. We add to a growing literature on associations between theory of mind and intelligence, affect, and personality. (PsycINFO Database Record (c) 2019 APA, all rights reserved).
Psychol Aging, 2019; 34

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BOARD NUMBER: S04-034

MUSIC ELICITS A PRIMING EFFECT IN MOTOR RELATED AREAS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aim To analyze the effect of listening to a popular and motivating song in brain activity at rest. *Methods* A resting-state functional magnetic resonance imaging (rs-fMRI) study was conducted. Thirty-four subjects (17 women) were included. None of the participants had received formal musical training. Two rs-fMRI runs separated by a period of four minutes where participants listened to the song “Viva la Vida” from Coldplay (2008) were performed on a 3T MRI scanner. After preprocessing, images were analyzed to identify changes in the amplitude of low frequency fluctuations (ALFF), regional homogeneity (ReHo) and functional connectivity (FC). FC analysis was performed using the seed to voxel approach. Seeds were in primary motor area (BA4), primary somatosensory area (BA1,2,3) and premotor area (BA6, including the supplementary motor area). *Results* Higher ALFF in parietal regions and cerebellum was identified in post- than pre-song listening rs-fMRI. The opposite contrast (i.e., Pre>Post) showed a higher ALFF in left frontal basal regions. Regarding ReHo changes, higher ReHo was identified in the left cerebellum (mainly in the posterior lobe) for post-song listening ReHo map. The opposite contrast (i.e., Pre>Post) showed higher ReHo in the right cerebellum, involving voxels of the occipital lobe. In the FC analysis, all selected regions of interest presented an increase in FC with the cerebellum after listening. *Conclusion* Music modulates brain’s resting-state activity. This can be interpreted as a priming effect of music that may explain (at least in part) some of the ergogenic effects of music.

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BOARD NUMBER: S04-035

SELF-RELEVANT FACES ATTENUATE THETA RHYTHMS IN OCCIPITO-TEMPORAL AND MEDIO-PREFRONTAL AREAS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims Self-relevant stimuli tend to capture attention. Enhancements of own name or face processing have been hypothesized and often observed in previous studies. Recent findings suggest a pivotal role of occipital theta rhythms in visual attention, whereas medio-frontal area has been implicated in top-down control of self-reference processing. Thus, we examined the self-related effects in terms of theta-band activity (4-8 Hz), focusing on the visual processing and its functional connections with the medio-prefrontal area, in an attempt to identify a self-relevance processing network. **Methods** Twenty-five participants performed a task of simple detection of faces from four categories: own face (Self), own face from the past (Past Self), friend's face, famous face, and unknown face. **Results** Self-reference modulated theta-band power and phase in both visual and prefrontal areas. The results showed that the theta activity was smallest for own face, and was rising gradually with the diminishing self-relevance. The self-unrelated stimuli (famous, unknown) induced the strongest theta responses. In comparison, the amplitude of P3b component of the ERP was largest for the self-related stimuli, replicating previous findings. **Conclusions**
The study provided a coherent pattern of self-related modulations of face processing in theta band. Two interpretations are possible. (1) Own face processing is over-learned and automatized to such extent that it captures attention through a direct visual-prefrontal processing pathway, thus needs less local processing than other stimuli. (2) Attention-grabbing capacity of own face may be realized in part through attenuation of lower (theta-band) rate of sampling/gating of sensory information.
(Funding: NCN 2018/29/N/HS6/01082).

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BOARD NUMBER: S04-036

PHYSIOLOGICAL RESPONSE TO IMAGES OF THE THIRD PLACE ACCORDING TO THE PRESENCE OR ABSENCE OF CAMPUS LIFE EXPERIENCE BEFORE COVID-19

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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This study quantitatively analyzed the physiological responses of subjects in order to plan a university campus lounge that will encourage user interaction after COVID-19. The subject's Prefrontal Alpha EEG Asymmetry was measured with five images and five color combination palettes. The subjects of the experiment were university students, who were divided into those who experienced campus life before COVID-19 (Group A) and who did not (Group B). The five images correspond to the representative 'Third Places': a cafe, a coworking space, a bookstore, an office, and a public rest area. Each image's color palette was converted into a 60% mosaic in a graphic program. Subjects were exposed to 10 images at an interval of 20 seconds, and their physiological preferences were compared and analyzed. Both groups showed the most positive response to the image of the café. The mean values of Group B were significantly lower than those of group A in images of coworking space, bookstore, and public rest area ($p<0.05$). Both groups also showed the most positive response in DL tones of GY, the color combination palette of bookstore. The mean values of group B were lower than those of group A ($p<0.05$), except for LGR tone of BG, color combination palette of public rest area. In conclusion, Group A and Group B responded differently to the images of 'Third Places.' Group B who did not have campus life before COVID-19 responded positively only to the image of café and showed lower sensitivity to the color scheme.

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BOARD NUMBER: S04-037

OBJECTIVE AND SUBJECTIVE FEATURES MODULATE THE AESTHETIC PERCEPTION OF INTERACTING BODY DYADS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Dance represents an ideal framework for studies on body and action aesthetic perception and evaluation. Several investigations provided evidence for a strong link between aesthetic appreciation, previous expertise, and objective features of observed movements. Most studies on dance focused on single body stimuli and only recently on group aesthetics. Therefore, interacting body dyads stands out as a novelty in the neuroaesthetics research field; also, the possible interplay between objective and subjective features in the aesthetics of dancing dyads requires further deepening. The current project investigates aesthetic, emotional, and semantic responses to non-symbolic postures of body dyads of varying visuospatial attributes in non-expert observers. A dance-inspired training was used to create a set of virtual stimuli by digitalizing dancers' movement kinematics via motion capture. Several visuospatial features were quantified via custom MATLAB scripts and included the shared interpersonal space, distance, dyad's orientation, and symmetry. Dance allowed an ecological investigation of human action perception and avoided eventual incongruency effects due to the combination of body postures. In four studies, subjective evaluations were collected with observers' empathic and cognitive dispositional traits and preferred interpersonal distance. Our evidence indicates that the aesthetic appreciation of interacting body dyads critically depends on the level of shared interpersonal space between bodies of a dyad. Such an aesthetic experience is modulated by the observer's focus on dyads' low-level (e.g., symmetry) and high-level (e.g., emotional and semantic content) features. Crucially, the likability of body dyads is strongly linked to both observer's subjective attributes and the stimuli' visuospatial features.

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BOARD NUMBER: S04-038

INTERSECTING KINEMATIC ENCODING AND READOUT OF INTENTION IN AUTISM

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Observers with autism spectrum disorders (ASDs) find it difficult to read intentions from movements. Here, we combined motion tracking, psychophysics and statistical analyses to investigate the computational bases of these difficulties. Eight- to thirteen-year-old typically developing (TD) children (n=35) and ASD children without intellectual impairment (n=35) watched a hand reaching for a bottle, either to pour or to place, and judged on the intention of the observed grasp. In a within-subjects counterbalanced order, participants watched videos of actions performed by TD and ASD children. Using a logistic regression-based model fitted to the experimental data, we analysed how TD and ASD observers read the intention information encoded in TD and ASD actions with single-subject, single-trial level resolution. By applying this approach, we were able to reveal that both TD and ASD observers read variations in movement kinematics. TD readers were better able to identify intention-informative kinematic features during observation of TD actions; conversely, ASD readers were better able to identify informative features when observing ASD actions. Crucially, while TD observers were generally able to extract the correct intention information, autistic observers were unable to do so. These findings suggest that intention reading difficulties in ASD reflect both an interaction failure in feature identification, rooted in kinematic dissimilarities between TD and ASD movements, and an individual deficit in information extraction, accompanied by an overall reduced sensitivity to kinematic variations. Initial data from a training program aimed at improving feature identification and information extraction are discussed.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-039

ASSOCIATIONS OF DEPRESSIVE SYMPTOMATOLOGY, SOCIAL ENGAGEMENT AND SUPPORT, AND LIFESTYLE BEHAVIORS AMONG NON-HISPANIC BLACK AND HISPANIC MEN WITH CHRONIC CONDITIONS IN THE UNITED STATES

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims. Self-management of depressive symptomatology is influenced by co-morbidity, social supports, and health-related behaviors. Men are less likely to discuss depressive moods and seek healthcare. This study examines factors associated with depression among non-Hispanic Black and Hispanic men ages ≥40 years with ≥1 chronic condition in the U.S. **Methods.** Using an internet-delivered survey, data were analyzed from 1,907 non-Hispanic Black and Hispanic males with chronic conditions. A logistic regression model was fitted to assess factors associated with depressive symptomatology, identified as a Patient Health Questionnaire-2 score ≥3. The model adjusted for sociodemographics, disease characteristics, health status, social engagement and support, and lifestyle behaviors. **Results.** Twenty-four percent of participants had depressive symptomatology. Men who were Hispanic (OR=1.39, P=0.017) and took more medications daily (OR=1.10, P=0.010) were more likely to have depressive symptomatology. Men who felt socially disconnected (OR=1.65, P<0.001), relied on others to improve their health and manage problems (OR=1.04, P<0.001), and were limited from doing usual activities because of their health (OR=3.15, P<0.001) were more likely to have depressive symptomatology. Those who had barriers to self-care (OR=1.16, P<0.001), felt frustrated about their healthcare situation (OR=1.13, P<0.001), sat for longer periods each week (OR=1.01, P=0.030), and used tobacco (OR=1.56, P=0.002) were more likely to have depressive symptomatology. **Conclusion.** Findings highlight the dynamic interplay of depression, social engagement, and lifestyle behaviors among non-Hispanic Black and Hispanic men with complex disease profiles. Efforts are needed to address depressive symptomatology through self-managing conditions, strengthening supportive networks, and alleviating burdens associated with healthcare interactions.

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BOARD NUMBER: S04-040

WATCH MY MOVES AT THE SILENT DISCO: EFFECTS OF MUSIC AND SOCIAL CONTEXT ON SPONTANEOUS MOVEMENT AND INTERPERSONAL SYNCHRONY

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Spontaneous movement in response to music is a universal human behaviour that generally takes place in social and communicative contexts. Yet, studies of this phenomenon traditionally focus on individual listeners in non-interactive scenarios. The present (currently ongoing) study investigates the behavioural and neural underpinnings of music-induced movements during joint music listening. In particular, we aim to understand how specific movements are recruited during social music-listening and whether they may serve social communicative purposes. To this aim, full-body kinematics, EEG activity and eye movements are measured from pairs of listeners in a “silent disco” setting, where participants jointly listen to *groove* music through earphones. Using this novel experimental setup, we test whether neural and behavioural responses are modulated by (1) joint listening (whether participants listen to the same or different music) and (2) social context (whether participants see each other or not). Results are expected to shed light on the cognitive and neurobiological origins of the human capacity to spontaneously communicate through music and movement.

Pubmed:

34714862: Bigand F, Prigent E, Berret B, Braffort A
Decomposing spontaneous sign language into elementary movements: A principal component analysis-based approach.
Sign Language (SL) is a continuous and complex stream of multiple body movement features. That raises the challenging issue of providing efficient computational models for the description and analysis of these movements. In the present paper, we used Principal Component Analysis (PCA) to decompose SL motion into elementary movements called principal movements (PMs). PCA was applied to the upper-body motion capture data of six different signers freely producing discourses in French Sign Language. Common PMs were extracted from the whole dataset containing all signers, while individual PMs were extracted separately from the data of individual signers. This study provides three main findings: (1) although the data were not synchronized in time across signers and discourses, the first eight common PMs contained 94.6% of the variance of the movements; (2) the number of PMs that represented 94.6% of the variance was nearly the same for individual as for common PMs; (3) the PM subspaces were highly similar across signers. These results suggest that upper-body motion in unconstrained continuous SL discourses can be described through the dynamic combination of a reduced number of elementary movements. This opens up promising perspectives toward providing efficient automatic SL processing tools based on heavy mocap datasets, in particular for automatic recognition and generation.
PLoS One, 2021; 16
34368103: Bigand F, Prigent E, Berret B, Braffort A
Machine Learning of Motion Statistics Reveals the Kinematic Signature of the Identity of a Person in Sign Language.
Sign language (SL) motion contains information about the identity of a signer, as does voice for a speaker or gait for a walker. However, how such information is encoded in the movements of a person remains unclear. In the present study, a machine learning model was trained to extract the motion features allowing for the automatic identification of signers. A motion capture (mocap) system recorded six signers during the spontaneous production of French Sign Language (LSF) discourses. A principal component analysis (PCA) was applied to time-averaged statistics of the mocap data. A linear classifier then managed to identify the signers from a reduced set of principal components (PCs). The performance of the model was not affected when information about the size and shape of the signers were normalized. Posture normalization decreased the performance of the model, which nevertheless remained over five times superior to chance level. These findings demonstrate that the identity of a signer can be characterized by specific statistics of kinematic features, beyond information related to size, shape, and posture. This is a first step toward determining the motion descriptors necessary to account for the human ability to identify signers.
Front Bioeng Biotechnol, 2021; 9


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BOARD NUMBER: S04-041

INCREASED FRACTIONAL ANISOTROPY (FA) IN THE LEFT ANTERIOR CORONA RADIATA AFTER A 31-DAY WEB-BASED MINDFULNESS TRAINING

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Diffusion-tensor-imaging(DTI) of long-term meditators have shown increased fractional anisotropy (FA) values in major projection (i.e., corona radiata), association[i.e., cingulum and superior longitudinal fasciculus(SLF)], and commissural white matter tracts(i.e., corpus callosum)(1,2). Longitudinal studies done on naïve meditators following a short-term mindfulness training confirmed these findings as a result of a mindfulness training(2,3,4). Our aim was to investigate if a 31-day web-based mindfulness training can replicate these effects.44 right-handed normal adults(21-61 years) divided into two groups(control and experimental) were scanned twice(before and after completion of the training or completion of a control intervention) in a 3T MRI Philipps scanner using a DTI sequence for imaging of the white matter tracts. The experimental group completed an online mindfulness training course whereas the control group participated in a course on ‘everyday health’.Results of the two-way rmANOVAs showed a trend in training-associated increase in FA in the left anterior corona radiata[F(1,42)=3.281,p=0.07] of the experimental group compared to the control group. No trends were seen in the FA of the SLF, corpus callosum, and cingulum, probably due by the shortness of our training; however, increased FA values in the left corona radiata might indicate the start of neuroplasticity changes as a consequence of mindfulness practice[i.e., increased connectivity between the anterior cingulate cortex(ACC) and other brain structures]. 1.Zsadanyi et al.The Effects of Mindfulness and Meditation on the CC in the Healthy Human-Brain.*Mindfulness.*2021. 2.Tang et al.The neuroscience of mindfulness meditation.*NatRevNeurosci.*2015. 3.Kral et al.MBSR-related changes in posterior cingulate resting brain connectivity.*SocCognAffectNeurosci.*2019. 4.Yoon et al.Plastic-Changes in White-Matter Induced by Templestay.*Mindfulness.*2019.

Pubmed:
31022257: Mora Álvarez MG, Stobbe RW, Beaulieu C
High resolution continuous arterial spin labeling of human cerebral perfusion using a separate neck tagging RF coil.
For standard clinical applications, ASL images are typically acquired with 4-8 mm thick slices and 3-4 mm in-plane resolution. However, in this paper we demonstrate that high-resolution continuous arterial spin labeling (CASL) perfusion images can be acquired in a clinically relevant scan time using current MRI technology. CASL was implemented with a separate neck coil for labeling the arterial blood on a 4.7T MRI using standard axial 2D GE-EPI. Typical-resolution to high-resolution (voxels of 95, 60, 45, 27, or 7 mm3) images were compared for qualitative and quantitative cerebral blood flow analysis (CBF) in nine healthy volunteers (ages: 24-32 years). The highest resolution (1.5x1.5x3 = 7 mm3) CASL implementation yielded perfusion images with improved cortex depiction and increased cortical CBF measurements (53 ± 8 ml/100g/min), consistent with reduced partial volume averaging. The 7 mm3 voxel images were acquired with 6 cm brain coverage in a clinically relevant scan of 6 minutes. Improved spatial resolution facilitates CBF measurement with reduced partial volume averaging and may be valuable for the detection of perfusion deficits in small lesions and perfusion measurement in small brain regions.
PLoS One, 2019; 14

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BOARD NUMBER: S04-042

INFLUENCE OF SHARED PERCEPTION ON SENSORY EVIDENCE ACCUMULATION IN THE HUMAN BRAIN

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Audience effects show that performance can change by being observed. Yet does having shared access to the same sensory information with others, rather than just being observed, change perception? Present work aimed to investigate whether observing the same stimuli with others would change visual perception. Human participants (N=15) performed a random-dot-motion task while being watched by an observer who sat either right or left to them. There were 640 stimuli with varying levels of motion coherence that appeared either on the left or right side of the screen. The screen was separated by an opaque divider, allowing participants to see both sides, but the observer could only see stimuli presented on their own side. Thus, participants responded to stimuli that were either privately or commonly seen, while social observation was kept constant. Accuracy, reaction time (RT) and confidence were recorded. EEG data were collected using a 64-channel system. RTs were significantly slower under shared vs private conditions. Neither accuracy nor confidence were different, thus excluding a possible speed-accuracy trade-off. Consistent with previous works, a stimulus-locked (300-600 ms post-stimulus) centro-parietal positivity (CPP) was observed whose slope of rise correlated with motion coherence. Importantly, CPP slope was larger in shared vs private trials. Current results suggest that, besides social presence, dynamics of sensory evidence accumulation and corresponding behavioral reports differ when we see the same objects with others. We are currently working on an attractor network model that could explain our findings and propose a neuronal mechanism for shared vs. private perception.

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BOARD NUMBER: S04-043

EARLY LIFE ATTACHMENT IN TERM AND PRETERM BORN INFANTS.

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims: Preterm birth affects infant attention, which could impact social cognition and attachment to caregivers. Therefore, we aimed to test the hypothesis that preterm birth leads to differences in infant attachment, and to identify variables that explain variance in attachment. To avoid predefined attention thresholds to categorise attachment, we used a data-driven approach on infant behaviours coded from the Still-Face Paradigm (SFP). **Methods**: 68 preterm and 68 term infants with mean (range) gestational age at birth 29.7 (22.1-32.9) and 39.6 (36.4-42.1) weeks, respectively, completed the SFP at nine months of corrected age. Attachment dimensions and categories were obtained from infant responses to the SFP using a published coding scheme, principal component (PC) and cluster analyses. Clinicodemographic and neurodevelopmental data (Vineland Adaptive Behaviour Scales) were collected at nine months and included in analyses. **Results**: Preterm and term infants did not differ in attachment dimensions (distress, fretfulness, attentiveness to caregivers/PCs; p-value > 0.07), or the distribution of attachment categories (styles/clusters, p-value > 0.28). In the whole sample, fretfulness correlated with socioeconomic deprivation (r_s = -0.23, p-value < 0.01), and attentiveness correlated with motor development (r_s = 0.24, p-value < 0.01) and birth weight (r_s = 0.17, p-value = 0.04). **Conclusion**: The data do not support differences in attachment between preterm and term infants at nine months, suggesting that atypical attention reported in preterm infants does not relate to differences in caregiver-infant attachment relationships. The data highlight putative links between socioeconomic deprivation and infant attachment, and suggest attentiveness associates with neuromotor development.

Pubmed:
31050830: González-Arnay E, Jiménez-Sánchez L, García-Simón D, Valdés-Vilches L, Salazar-Zamorano CH, Boada-Pié S, Aguirre JA, Eichenberger U, Fajardo-Pérez M
Ultrasonography-guided anterior approach for axillary nerve blockade: An anatomical study.
Combined ultrasound (US)-guided blockade of the suprascapular and axillary nerves (ANs) has been proposed as an alternative to interscalene blockade for pain control in shoulder joint pathology or postsurgical care. This technique could help avoid respiratory complications and/or almost total upper limb palsy. Nowadays, the AN blockade is mostly performed using an in-plane caudal-to-cephalic approach from the posterior surface of the shoulder, reaching the nerve immediately after it exits the neurovascular quadrangular space (part of the spatium axillare). Despite precluding most respiratory complications, this approach has not made postsurgical pain relief any better than an interscalene blockade, probably because articular branches of the AN are not blocked.Cephalic-to-caudal methylene blue injections were placed in the first segment of the AN of six Thiel-embalmed cadavers using an US-guided anterior approach in order to compare the distribution with that produced by a posterior approach to the contralateral AN in the same cadaver. Another 21 formalin-fixed cadavers were bilaterally dissected to identify the articular branches of the AN.We found a good spread of the dye on the AN and a constant relationship of this nerve with the subscapularis muscle. The dye reached the musculocutaneous nerve, which also contributes to shoulder joint innervation. We describe the anatomical landmarks for an ultrasonography-guided anterior AN blockade and hypothesize that this anterior approach will provide better pain control than the posterior approach owing to complete blocking of the joint nerve. Clin. Anat. 33:488-499, 2020. © 2019 Wiley Periodicals, Inc.
Clin Anat, 2020; 33
31589886: Jiménez-Sánchez L, Blesa J, Del Rey NL, Monje MHG, Obeso JA, Cavada C
Serotonergic innervation of the striatum in a nonhuman primate model of Parkinson's disease.
Parkinson's disease (PD) is characterized by dopaminergic neurodegeneration in the substantia nigra and dopamine depletion in the striatum. Non-dopaminergic systems are also affected, including the serotonergic system. Enhanced striatal

serotonergic innervation is a proposed compensatory mechanism for the dopaminergic deficit. Meanwhile a serotonergic deficit has been suggested as preceding the nigrostriatal dopaminergic pathology in PD. Our aim was to assess the serotonergic innervation of the striatum in a model of progressive experimental parkinsonism in macaques, from pre-symptomatic to symptomatic stages. The neurotoxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) was administered to adult macaque monkeys using a slow intoxication protocol. The intoxicated animals were classified into asymptomatic, recovered, moderate and severe parkinsonian, based on their motor behavior. The serotonergic innervation was studied by immunohistochemistry against serotonin (5-HT). In the striatum, the density of 5-HT-immunoreactive (5-HT+) axons was estimated with stereology. Images of the striatum in the immunostained sections were taken to compare the distribution patterns of the serotonergic innervation between groups. These patterns were apparently similar among the groups. Axonal density estimations showed no differences in striatal 5-HT+ innervation between the intoxicated groups and the control group. Accordingly, this study fails to find significant changes in the striatal serotonergic axonal innervation in MPTP-treated monkeys, coinciding with previous biochemical findings in our model. However, it is possible that alterations in the serotonergic system in PD could be independent of axonal density changes. Consequently, the proposed role for striatal serotonin serving as a compensatory mechanism for dopaminergic denervation merits further study. This article is part of the special issue entitled 'Serotonin Research: Crossing Scales and Boundaries'.
Neuropharmacology, 2020; 170

34107350: Sewell MDE, Jiménez-Sánchez L, Shen X, Edmondson-Stait AJ, Green C, Adams MJ, Rifai OM, McIntosh AM, Lyall DM, Whalley HC, Lawrie SM
Associations between major psychiatric disorder polygenic risk scores and blood-based markers in UK biobank.
Major depressive disorder (MDD), schizophrenia (SCZ), and bipolar disorder (BD) have both shared and discrete genetic risk factors, and are associated with peripheral abnormalities. The relationships between such genetic architectures and blood-based markers are, however, unclear. We investigated relationships between polygenic risk scores (PRS) for these disorders and peripheral markers in the UK Biobank cohort. We calculated polygenic risk scores for n = 367,329 (MDD PRS), n = 366,465 (SCZ PRS), and n = 366,383 (BD PRS) UK Biobank cohort subjects. We then examined associations between disorder PRS and 58 inflammatory/immune, hematological, bone, cardiovascular, hormone, liver, renal and diabetes-associated blood markers using two generalized linear regression models: 'minimally adjusted' controlling for variables such as age and sex, and 'fully adjusted' including additional lifestyle covariates: BMI, alcohol and smoking status, and medication intake. There were 38/58 MDD PRS, 32/58 SCZ PRS, and 20/58 BD PRS-blood marker associations detected for our minimally adjusted model. Of these, 13/38 (MDD PRS), 14/32 (SCZ PRS), and 10/20 (BD PRS) associations remained significant after controlling for lifestyle factors. Many were disorder-specific, with 8/13 unique MDD PRS associations identified. Several disorder-specific associations for MDD and SCZ were immune-related, with mostly positive and negative associations identified for MDD and SCZ PRS respectively. This study suggests that MDD, SCZ and BD have both shared and distinct peripheral markers associated with disorder-specific genetic risk. The results also implicate inflammatory dysfunction in MDD and SCZ, albeit with differences in patterns between the two conditions, and enrich our understanding of potential underlying pathophysiological mechanisms in major psychiatric disorders.
Brain Behav Immun, 2021; 97

34777227: Jiménez-Sánchez L, Hamilton OKL, Clancy U, Backhouse EV, Stewart CR, Stringer MS, Doubal FN, Wardlaw JM
Sex Differences in Cerebral Small Vessel Disease: A Systematic Review and Meta-Analysis.
Cerebral small vessel disease (SVD) is a common cause of stroke, mild cognitive impairment, dementia and physical impairments. Differences in SVD incidence or severity between males and females are unknown. We assessed sex differences in SVD by assessing the male-to-female ratio (M:F) of recruited participants and incidence of SVD, risk factor presence, distribution, and severity of SVD features. We assessed four recent systematic reviews on SVD and performed a supplementary search of MEDLINE to identify studies reporting M:F ratio in covert, stroke, or cognitive SVD presentations (registered protocol: CRD42020193995). We meta-analyzed differences in sex ratios across time, countries, SVD severity and presentations, age and risk factors for SVD. Amongst 123 relevant studies (= 36,910 participants) including 53 community-based, 67 hospital-based and three mixed studies published between 1989 and 2020, more males were recruited in hospital-based than in community-based studies [M:F = 1.16 (0.70) vs. M:F = 0.79 (0.35), respectively; < 0.001]. More males had moderate to severe SVD [M:F = 1.08 (0.81) vs. M:F = 0.82 (0.47) in healthy to mild SVD; < 0.001], and stroke presentations where M:F was 1.67 (0.53). M:F did not differ for recent (2015-2020) vs. pre-2015 publications, by geographical region, or age. There were insufficient sex-stratified data to explore M:F and risk factors for SVD. Our results highlight differences in male-to-female ratios in SVD severity and amongst those presenting with stroke that have important clinical and translational implications. Future SVD research should report participant demographics, risk factors and outcomes separately for males and females. [PROSPERO], identifier [CRD42020193995].
Front Neurol, 2021; 12

34536540: González-Arnay E, Galluccio F, Pérez-Santos I, Merlano-Castellanos S, Bañón-Boulet E, Jiménez-Sánchez L,

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Rivier-Julien C, Barrueco-Fernández M, Olea MS, Yamak-Altinpulluk E, Teles AS, Fajardo-Pérez M

Permeable spaces between glenohumeral ligaments as potential gateways for rapid regional anesthesia of the shoulder.

Shoulder pain is a highly prevalent condition, often resulting in major life limitations, and requiring effective treatments. In this work, we explore the anatomical basis of a proposed approach to the regional anesthesia of the shoulder through a single injection under the subscapularis muscle. Bilateral experimental injections in shoulders from body donors (Radiolar ® and Methylene-Blue) under the subscapular muscle (n = 11) and cadaveric systematic dissections of other 35 shoulders from body donors were performed. Injectate spread was then qualitatively assessed. Long axis of permeable foramina in the anterior aspect of the shoulder joint capsule was measured in centimeters using a digital caliper. More than 40% of specimens had at least one permeable space (Weitbrech and/or Rouvière foramina) communicating the subscapular bursa and the articular space. We further demonstrate that an ultrasonography-guided injection under the subscapularis muscle allows the spread of the injectate through the anterior, inferior and posterodorsal walls of the articular capsule, the subacromial bursa, and the bicipital groove, as well as into the articular space for some injections. The odds of accidental intraarticular injection decrease when injecting with low volumes. This anatomical study provides a detailed description of foramina between glenohumeral ligaments. Furthermore, the data shown in this work supports, as a proof of concept, a safe alternative for rapid and specific blockade of terminal sensory branches innervating the shoulder joint capsule.

Ann Anat, 2022; 239

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BOARD NUMBER: S04-044

EMPATHIC REACTION FOR PAIN IN HIGH SENSORY PROCESSING SENSITIVITY PERSONS (HSP) – EEG STUDY.

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims. The main goal of this study was to characterize the mechanism of the empathic reaction to pain in people characterized by high sensory processing sensitivity (HSP), who show deep processing of both external and internal stimuli, with a positive and negative affect, including pain. **Methods.** The study was carried out on 31 people (16 HSP, 15 NHSP) using the EEG method of potentials associated with the ERP event. When viewing pictures presenting pain and no pain of others, the subjects were asked to imagine what others might feel. **Results.** At the level of early processing of the stimulus (N1) and late processing associated with conscious perception (P3), there were no differences in responses to the pain of others between the HSP and NHSP groups. These differences were seen in the amplitude of the mid-latency (P2, N2) waves: an empathic response to the pain of others was seen in the NHSP group, but not in the HSP group. Differences between HSP and NHSP in the amplitudes of the N1 and P3 waves, independent of the stimulus type, were observed, which may indicate the specificity of stimulus processing by HSP, and therefore should be further investigated. **Conclusions**: The results obtained initially indicate that HSP displays a higher level of emotional empathy in the empathic reaction and may give emotional significance to both painful and neutral stimuli. The HSP persons compared to NHSP persons may be characterized by different dynamics of the empathic reaction to pain.

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BOARD NUMBER: S04-045

DISSOCIATION BETWEEN OBSERVATIONAL LEARNING AND SOCIAL COMPARISON NEURAL SIGNALS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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[1]University of Plymouth, School Of Psychology, Plymouth, United Kingdom, [2]Charité Universitätsmedizin Berlin, Bernstein Center For Computational Neuroscience, Berlin, Germany, [3]IMT School for Advanced Studies, Momilab, Lucca, Italy, [4]Inserm, Stem Cell And Brain Research Institute, Bron, France, [5]Univeristy of Oxford, Department Of Experimental Psychology, Oxford, United Kingdom

Aims. When making decisions in the presence of our peers, several signals can shape learning. A first signal, elicited by social comparison, concerns the relative skill or welfare of an individual compared to our own and is important for learning dominance relationships. By contrast, observational learning signals enable us to learn from the successes and mistakes of others. In most studies, social comparison and observational learning signals are confounded. Our aim was to disentangle their patterns of neural activity. **Methods.** Thirty participants performed a probabilistic instrumental learning task. The learning task was designed to manipulate both the learning context - they learned either from the same or different sets of cues as their counterpart – and the outcome valence. We used a reinforcement learning model and multi-voxel pattern analysis on fMRI data to investigate latent behavioural variables estimated and their supporting neural activity. **Results**. Participants benefited more from observation in the same than in the different cues condition, suggesting an effect of observational learning. Interestingly, they also learned better from rewards than from punishments, especially in the different cues conditions and when observing a competitive, high-performing counterpart. **Conclusions.** Our results show that both observational learning and social comparison affect learning performance in our task. Further analysis will be performed to dissociate the neural basis of both processes. We expect the vmPFC, striatum and ACC to integrate information from different sources of learning (direct experience vs. observation) and the dmPFC to support social comparison. Data analysis is currently ongoing.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S04-046

THE PHYSIOLOGICAL CORRELATES OF SOCIAL SPACE IN CHILDHOOD AUTISM SPECTRUM DISORDERS

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Background Interpersonal space (IPS) is the area that individuals maintain between themselves and others and that, when it is violated, cause discomfort. The IPS's extent depends on others' behaviour, and the closeness with an unknown person increases the skin conductance response (SCR), linking between behavioral and autonomic components of IPS. **Aim** We investigate if the deficit in IPS regulation reported in children with Autism Spectrum Disorders (ASD), is associated to an altered autonomic response to social proximity. **Methods** Children with typical development (TD) and children with ASD performed two Experiments. In Experiment 1, participants stopped an unknown adult (confederate), who moved toward to or away from them, when they felt comfortable with other's distance (comfort-distancetask). In Experiment 2, participants' SCR was recorded when the confederate moved and briefly stopped at five spatial positions, simulating an approach/withdrawal movement. **Results** We found that ASD children preferred a larger IPS and exhibited higher SCR than controls. Interestingly, in TD and ASD children, the SCR increased when the confederate was closer to them, reflecting the ability to detect a possible threat around the body. However, in TD children this effect was observed in Approaching but not in Withdrawal condition, whereas in the ASD children the SCR was not modulated by the confederate's movement direction. This result can be ascribed to a deficit in ASD children in anticipating the movement direction performed by the confederate. **Conclusion** We reveal that impairment of both physiological responses and predictive mechanisms contribute to abnormal IPS regulation found in autism.

Pubmed:
35115914: Candini M, D'Angelo M, Frassinetti F
Time Interaction With Two Spatial Dimensions: From Left/Right to Near/Far.
In this study, we explored the time and space relationship according to two different spatial codings, namely, the left/right extension and the reachability of stimulus along a near/far dimension. Four experiments were carried out in which healthy participants performed the time and spatial bisection tasks in near/far space, before and after short or long tool-use training. Stimuli were prebisected horizontal lines of different temporal durations in which the midpoint was manipulated according to the Muller-Lyer illusion. The perceptual illusory effects emerged in spatial but not temporal judgments. We revealed that temporal and spatial representations dynamically change according to the action potentialities of an individual: temporal duration was perceived as shorter and the perceived line's midpoint was shifted to the left in far than in near space. Crucially, this dissociation disappeared following a but not tool-use training. Finally, we observed age-related differences in spatial attention which may be crucial in building the memory temporal standard to categorize durations.
Front Hum Neurosci, 2021; 15
33511981: Massaccesi C, Groessing A, Rosenberger LA, Hartmann H, Candini M, di Pellegrino G, Frassinetti F, Silani G
Neural Correlates of Interpersonal Space Permeability and Flexibility in Autism Spectrum Disorder.
Previous research indicates that the size of interpersonal space at which the other is perceived as intrusive (permeability) and the ability to adapt interpersonal distance based on contextual factors (flexibility) are altered in Autism Spectrum Disorder (ASD). However, the neurophysiological basis of these alterations remains poorly understood. To fill this gap, we used fMRI and assessed interpersonal space preferences of individuals with ASD before and after engaging in cooperative and non-cooperative social interactions. Compared to matched controls, ASDs showed lower comfort in response to an approaching confederate, indicating preference for larger interpersonal space in autism (altered permeability). This preference was accompanied by reduced activity in bilateral dorsal intraparietal sulcus (dIPS) and left fusiform face area (FFA), regions previously shown to be involved in interpersonal space regulation. Furthermore, we observed differences in effective connectivity among dIPS, FFA, and amygdala in ASDs compared to controls, depending on the level of experienced comfort. No differences between groups were observed in interpersonal space regulation after an experienced social interaction (flexibility). Taken together, the present findings suggest that a dysregulation of the activity and connectivity of brain areas

involved in interpersonal space processing may contribute to avoidance of physical proximity and social impairments in ASD.
Cereb Cortex, 2021; 31
33510396: Candini M, Battaglia S, Benassi M, di Pellegrino G, Frassinetti F
The physiological correlates of interpersonal space.
Interpersonal space (IPS) is the area around the body that individuals maintain between themselves and others during social interactions. When others violate our IPS, feeling of discomfort rise up, urging us to move farther away and reinstate an appropriate interpersonal distance. Previous studies showed that when individuals are exposed to closeness of an unknown person (a confederate), the skin conductance response (SCR) increases. However, if the SCR is modulated according to participant's preferred IPS is still an open question. To test this hypothesis, we recorded the SCR in healthy participants when a confederate stood in front of them at various distances simulating either an approach or withdrawal movement (Experiment 1). Then, the comfort-distance task was adopted to measure IPS: participants stop the confederate, who moved either toward or away from them, when they felt comfortable with other's proximity (Experiment 2). We found higher SCR when the confederate stood closer to participants simulating an IPS intrusion, compared to when the confederate moved farther away. Crucially, we provide the first evidence that SCR, acting as a warning signal, contributes to interpersonal distance preference suggesting a functional link between behavioral components of IPS regulation and the underlying physiological processes.
Sci Rep, 2021; 11
32827540: Candini M, di Pellegrino G, Frassinetti F
The plasticity of the interpersonal space in autism spectrum disorder.
In recent years, there has been a dramatic increase in research examining interpersonal space, i.e., the sector of space immediately around the body in which we interact with other people. These studies have consistently revealed impairments of interpersonal space regulation in psychopathological disorders characterized by social disability, such as autism, schizophrenia and social anxiety. The primary goal of this review is to discuss several key points that have emerged in research on interpersonal space regulation in autism spectrum disorders. Particularly, we review recent behavioral evidence revealing that individuals with autism prefer abnormally larger or shorter interpersonal distance than healthy controls, indicating a deficit in regulating the size of interpersonal space (permeability). Then, we focus on how individuals with autism fail to modify their interpersonal space following a brief cooperative interaction with an unfamiliar adult, suggesting a deficit in adapting interpersonal space to the social context (plasticity). Moreover, we discuss evidence indicating that space regulation deficits primarily affect interpersonal (i.e., social), but not peripersonal (i.e., action), space in autism. Finally, we take into consideration the variables influencing interpersonal space plasticity such as person's perspective and severity of social impairment as well as its neural underpinnings. These findings may provide a critical contribution to understanding of the functional mechanisms underlying interpersonal space regulation and its rehabilitation in autism spectrum disorders.
Neuropsychologia, 2020; 147
30663321: Candini M, Giuberti V, Santelli E, di Pellegrino G, Frassinetti F
When social and action spaces diverge: A study in children with typical development and autism.
The space around the body has been defined as action space () and a social space (). Within the current debate about the characteristics of these spaces, here we investigated the functional properties and plasticity of action and social space in developmental age. To these aims, children with typical development and autism spectrum disorders were submitted to Reaching- and Comfort-distance tasks, to assess peripersonal and interpersonal space, respectively. Participants approached a person (confederate) or an object and stopped when they thought they could reach the stimulus (Reaching-distance task), or they felt comfortable with stimulus' proximity (Comfort-distance task). Both tasks were performed before and after a cooperative tool-use training, in which participant and confederate actively cooperated to reach tokens by using either a long (Experiment 1) or a short (Experiment 2) tool. Results showed that in both groups, peripersonal space extended following long-tool-use but not short-tool-use training. Conversely, in typical development, but not in autism spectrum disorders children, interpersonal space toward confederate reduced following the cooperative tool-use training. These findings reveal that action and social spaces are functionally dissociable both in typical and atypical development, and that action but not social space regulation is intact in children with autism.
Autism, 2019; 23
27630550: Candini M, Farinelli M, Ferri F, Avanzi S, Cevolani D, Gallese V, Northoff G, Frassinetti F
Implicit and Explicit Routes to Recognize the Own Body: Evidence from Brain Damaged Patients.
Much research suggested that recognizing our own body-parts and attributing a body-part to our physical self-likely involve distinct processes. Accordingly, facilitation for self-body-parts was found when an implicit, but not an explicit, self-recognition was required. Here, we assess whether implicit and explicit bodily self-recognition is mediated by different cerebral networks and can be selectively impaired after brain lesion. To this aim, right- (RBD) and left- (LBD) brain damaged patients and age-matched controls were presented with rotated pictures of either self- or other-people hands. In the Implicit task participants were submitted to hand laterality judgments. In the Explicit task they had to judge whether the hand belonged, or not, to

them. In the Implicit task, controls and LBD patients, but not RBD patients, showed an advantage for self-body stimuli. In the Explicit task a disadvantage emerged for self-compared to others' body stimuli in controls as well as in patients. Moreover, when we directly compared the performance of patients and controls, we found RBD, but not LBD, patients to be impaired in both the implicit and explicit recognition of self-body-part stimuli. Conversely, no differences were found for others' body-part stimuli. Crucially, 40% RBD patients showed a selective deficit for implicit processing of self-body-part stimuli, whereas 27% of them showed a selective deficit in the explicit recognition of their own body. Additionally, we provide anatomical evidence revealing the neural basis of this dissociation. Based on both behavioral and anatomical data, we suggest that different areas of the right hemisphere underpin implicit and explicit self-body knowledge.
Front Hum Neurosci, 2016; 10
27157094: Candini M, Giuberti V, Manattini A, Grittani S, di Pellegrino G, Frassinetti F
Personal space regulation in childhood autism: Effects of social interaction and person's perspective.
Studies in children with Typical Development (TD) and with Autism Spectrum Disorder (ASD) revealed that autism affects the personal space regulation, influencing both its size (permeability) and its changes depending on social interaction (flexibility). Here, we investigate how the nature of social interaction (Cooperative vs. Uncooperative) and the person perspective influence permeability and flexibility of interpersonal distance. Moreover, we tested whether the deficit observed in ASD children, reflects the social impairment (SI) in daily interactions. The stop-distance paradigm was used to measure the preferred distance between the participant and an unfamiliar adult (first-person perspective, Experiment 1), and between two other people (third-person perspective, Experiment 2). Interpersonal distance was measured before and after the interaction with a confederate. The Wing Subgroups Questionnaire was used to evaluate SI in everyday activities, and each ASD participant was accordingly assigned either to the lower (children with low social impairment [low-SI ASD]), or to the higher SI group (children with high social impairment [high-SI ASD]). We observed larger interpersonal distance (permeability) in both ASD groups compared to TD children. Moreover, depending on the nature of social interaction, a modulation of interpersonal distance (flexibility) was observed in TD children, both from the first- and third-person perspective. Similar findings were found in low-SI but not in high-SI ASD children, in Experiment 1. Conversely, in Experiment 2, no change was observed in both ASD groups. These findings reveal that SI severity and a person's perspective may account for the deficit observed in autism when flexibility, but not permeability, of personal space is considered. Autism Res 2017, 10: 144-154. © 2016 International Society for Autism Research, Wiley Periodicals, Inc.
Autism Res, 2017; 10
26717521: Anelli F, Candini M, Cappelletti M, Oliveri M, Frassinetti F
The Remapping of Time by Active Tool-Use.
Multiple, action-based space representations are each based on the extent to which action is possible toward a specific sector of space, such as near/reachable and far/unreachable. Studies on tool-use revealed how the boundaries between these representations are dynamic. Space is not only multidimensional and dynamic, but it is also known for interacting with other dimensions of magnitude, such as time. However, whether time operates on similar action-driven multiple representations and whether it can be modulated by tool-use is yet unknown. To address these issues, healthy participants performed a time bisection task in two spatial positions (near and far space) before and after an active tool-use training, which consisted of performing goal-directed actions holding a tool with their right hand (Experiment 1). Before training, perceived stimuli duration was influenced by their spatial position defined by action. Hence, a dissociation emerged between near/reachable and far/unreachable space. Strikingly, this dissociation disappeared after the active tool-use training since temporal stimuli were now perceived as nearer. The remapping was not found when a passive tool-training was executed (Experiment 2) or when the active tool-training was performed with participants' left hand (Experiment 3). Moreover, no time remapping was observed following an equivalent active hand-training but without a tool (Experiment 4). Taken together, our findings reveal that time processing is based on action-driven multiple representations. The dynamic nature of these representations is demonstrated by the remapping of time, which is action- and effector-dependent.
PLoS One, 2015; 10
25463145: Candini M, Zamagni E, Nuzzo A, Ruotolo F, Iachini T, Frassinetti F
Who is speaking? Implicit and explicit self and other voice recognition.
In the domain of self-recognition, voice is a critical feature for self/other distinction. The aim of this study was to explore if people have an implicit and/or explicit knowledge of their voice. A group of healthy participants were submitted to an implicit and an explicit self-voice recognition task. They listened to pairs of pre-recorded auditory stimuli (words or pseudowords) pronounced by themselves, by a familiar or an unfamiliar person. Afterwards, in the "Implicit task" participants had to judge whether the pair of stimuli were pronounced by same or different speakers; in the "Explicit task" they had to identify if one of the stimuli was or not their own voice. Results showed a difference between Implicit and Explicit tasks since participants were more accurate in implicit than explicit self voice-recognition. Moreover, in the Implicit task, participants had the same level of accuracy when they had to judge stimuli pronounced with self or others' voice, whereas when an explicit voice-recognition

was required, they were less accurate with self than with others' voice.
Brain Cogn, 2014; 92C

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BOARD NUMBER: S04-047

HUMAN BRAIN RESPONSES TO CANNIBALISTIC IMAGES

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Cannibalism is a major cultural taboo and its prevalence has been widely debated. Whether cannibalism is widespread in the animal kingdom used to be controversial, but is now firmly established, even in our closest relatives, the Chimpanzees. Anthropology faced a debate regarding presence of cannibalism in indigenous populations. Novel evidence has established cannibalism in some indigenous people and also in prehistoric populations. While it is clear that cannibalistic information evokes strong reactions in humans, little is known about how it is processed in the human brain. To address this issue, we investigate the neural processing of images showing cannibalistic material using functional Magnetic Resonance Imaging. Participants passively viewed images taken from three categories, selected in preliminary psychophysical pilot studies: “Cannibalism”, “Body Mutilation”, and “Disgust”. For each participant, 6 runs of 30 pictures were presented, each run with 10 pictures per category. Pictures were shown for 2 s followed by an 8 s break. A fixation cross was shown throughout. Preliminary data indicate that images representing “Cannibalism” evoked stronger activity in bilateral occipital and left parietal cortex than the control conditions. The “Body Mutilation” and “Disgust” signals also activated the fusiform body area and the right occipital region. The increased occipital brain activity evoked by cannibalistic stimuli might reflect increased attention. Further work is needed to understand if such differential processing of cannibalistic images reflects their cultural taboo or instead a specific biological “preparedness” to react to cannibalism.

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BOARD NUMBER: S04-048

SOCIAL TASK MANAGEMENT: SWITCHING BETWEEN HUMANIZED AND DEHUMANIZED PERCEPTION - AN EXPLORATORY EEG STUDY

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Recent behavioral and neuroimaging studies suggest that people are constantly required to transition between a humanized and dehumanized mode of perception to adapt to changing contexts and navigate their complex environment. However, the temporal dynamics underlying the controlled switch between these two perceptual and behavioral orientations towards others remain unclear. Thus, in this paper we examined the switch between humanized and dehumanized perception using EEG. We recorded EEG activity from 19 (30.24 ± 11.98 years; 8 men) neurotypical participants who were presented with 80 color-images of individuals. To operationalize humanized and dehumanized perception, participants were asked to perform a social (warmth) and a financial (competence) decision task respectively, based on the images. The social and non-social task were presented in form of 'stay' or 'switch' trials. In the ‚switch' condition trials alternated between the two tasks, in the ‚stay' condition participants repeated the same task. As hypothesized, N170 amplitudes were larger to the social compared to the financial task. However, unexpectedly, P300 amplitudes did not differ between stay and switch trials. Supporting prior social cognition research, differences in N170 amplitudes indicate that a dehumanized mode of perception begins when early, exogenous, perceptual processes are employed. However, contradicting prior task-switching studies, findings related to P300 amplitudes suggest that task switching in social contexts is not implemented by endogenous, cognitive processes but by an alternative, yet unexplored, control mechanism. Thus, overall, we assume the control of social cognition to be distinct to the control of other previously researched cognitive systems.

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BOARD NUMBER: S04-049

STRATEGIES THAT ACTIVATE THE SOOTHING AND THREAT SYSTEM DURING A BODY IMAGE STRESSOR ARE ASSOCIATED WITH INTENTIONS TO ENGAGE IN DISORDERED EATING BEHAVIOUR AMONG WOMEN

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims: Self-compassion encompasses being kind to oneself with non-judgemental acceptance and awareness, while acknowledging that others may face similar circumstances. Self-compassion is thought to deactivate the stress system by activating the soothing system, mitigating stress reactivity. For those with high eating disorder symptoms, threats to body image, such as mirror exposure, elicits a stress response and compensatory behaviours (i.e., disordered eating). It has yet to be investigated whether the link between stress responses and disordered eating is mediated by strategies that activate the self-soothing system, the threat system or attentional-emotional interoceptive regulation. This study examined whether self-compassion, attention and emotion regulation, and appearance fixing mediate the relationship between eating disorder symptoms and intent for disordered eating after a body image stressor. Methods: 55 undergraduate women spoke about their bodies while exposed to a mirror in the presence of female confederates. Trait eating disorder symptoms were measured prior to the task. Self-compassion, attention and emotion regulation, appearance fixing, and intentions to engage in disordered eating were measured following the task. Results: Self-compassion and one individual component of self-compassion, self-kindness, and appearance fixing significantly mediated the relationship between eating disorder symptoms and intentions for disordered eating. Neither attention nor emotion regulation were mediators in the relationship between eating disorder symptoms and intentions for disordered eating. Conclusions: Activating the self-soothing system by being self-compassionate may reduce compensatory eating behaviour in those with higher eating disorder symptoms, whereas fixating on appearance may increase compensatory behaviours by activating the threat response system during a stressor.

Pubmed:
35026685: Barbeau K, Carbonneau N, Pelletier L
Family members and peers' negative and positive body talk: How they relate to adolescent girls' body talk and eating disorder attitudes.
Research examining the interpersonal correlates of body talk has primarily focused on the negative consequences of perceiving fat talk on women's own body talk and eating behaviours; however, little is known about the correlates and effects of positive body talk. This study examined the associations between perceived family and peer negative and positive body talk and adolescent girls' body talk and eating disorder attitudes, and the mediating role of adolescents' fear of negative evaluation and self-compassion in these relationships. Influences of family members and peers were compared to examine the uniformity of these interpersonal processes. Adolescent girls (N = 331, M age = 15.7, SD = 1.0) completed a survey. Path analysis models suggested that in the peer and family models, perceived negative body talk was associated with more self-related negative talk through fear of negative evaluation and lower self-compassion, whereas perceived positive body talk was associated with more self-related positive talk through self-compassion. Additionally, adolescents' fear of negative evaluation and self-related negative talk were mediators between perceived negative body talk and eating disorder attitudes. Results suggest that negative and positive body talk are cultivated interpersonally by increasing evaluative concerns and decreasing self-compassion.
Body Image, 2022; 40
34579003: Carbonneau N, Cantin M, Barbeau K, Lavigne G, Lussier Y
Self-Compassion as a Mediator of the Relationship between Adult Women's Attachment and Intuitive Eating.
Despite growing interest in intuitive eating-a non-dieting approach to eating that is based on feeding the body in accordance with physiological and satiety cues-research on its determinants is scarce. The present study aimed to examine the associations between dimensions of adult attachment (i.e., anxiety and avoidance) and intuitive eating, and the mediating role of self-compassion in these relationships. The sample comprised 201 French-Canadian young adult women (M = 25.1, SD = 4.6). Participants completed self-report questionnaires through an online survey. Results of the structural equation model demonstrated that attachment-related anxiety and avoidance were negatively associated with intuitive eating, and

these relationships were at least partially mediated by self-compassion. Findings suggest that women who have high levels of attachment anxiety or avoidance engage in less intuitive eating partly because they are less self-compassionate. Results highlight the importance of self-compassion in facilitating adaptive eating behaviors in adult women, especially if they have an insecure attachment style to romantic partners.
Nutrients, 2021; 13
32702399: Sparling JE, Barbeau K, Boileau K, Konkle ATM
Environmental enrichment and its influence on rodent offspring and maternal behaviours, a scoping style review of indices of depression and anxiety.
Environmental enrichment is a widely used experimental manipulation that consistently shows measurable effects on rodent behaviour across the lifespan. This scoping review assesses and thematically summarizes the literature of the past decade concerning the effects of environmental enrichment applied during sensitive developmental periods in rodent mothers and offspring. Maternal behaviours as well as maternal and offspring anxiety- and depressive-like behaviours are considered. Relevant terms were searched across five databases (Embase, MEDLINE, PsycINFO, PubMed, Web of Science) and articles were screened with inclusion and exclusion criteria. The remaining articles were thematically analysed. Our results suggest that a greater number of articles reviewed the impacts of environmental enrichment on offspring anxiety-like behaviour (n = 23) rather than on depressive-like behaviour (n = 11) or maternal caregiving behaviour (n = 12). Maternal anxiety- (n = 4) or depressive-like (n = 2) behaviours are not often evaluated for in enrichment studies. The main behavioural tests of anxiety that were reviewed include the elevated plus-maze, the open field test, and the light-dark box whereas those for depression included the forced swim test and the sucrose preference test. Our results yielded mixed findings and significant variation in behavioural responses across all tests. In mothers, trends of increased maternal care behaviours and decreased maternal depressive-like behaviours in enriched mothers were appreciated. Enrichment during the gestational period was identified as pivotal to creating behavioural change in mother subjects. In enriched offspring rodents, a trend towards decreased anxiety-like behaviours was observed most often. Potential confounds inherent in enrichment paradigms and relevant theories of enrichment and their relation to rodent behavioural tests are discussed.
Pharmacol Biochem Behav, 2020; 197
32539730: Rhodes RE, Guerrero MD, Vanderloo LM, Barbeau K, Birken CS, Chaput JP, Faulkner G, Janssen I, Madigan S, Mâsse LC, McHugh TL, Perdew M, Stone K, Shelley J, Spinks N, Tamminen KA, Tomasone JR, Ward H, Welsh F, Tremblay MS
Development of a consensus statement on the role of the family in the physical activity, sedentary, and sleep behaviours of children and youth.
Children and youth who meet the physical activity, sedentary, and sleep behaviour recommendations in the Canadian 24-Hour Movement Guidelines are more likely to have desirable physical and psychosocial health outcomes. Yet, few children and youth actually meet the recommendations. The family is a key source of influence that can affect lifestyle behaviours. The purpose of this paper is to describe the process used to develop the Consensus Statement on the Role of the Family in the Physical Activity, Sedentary, and Sleep Behaviours of Children and Youth (0-17 years) and present, explain, substantiate, and discuss the final Consensus Statement.
Int J Behav Nutr Phys Act, 2020; 17
31250487: Boileau K, Barbeau K, Sharma R, Bielajew C
Ethnic differences in diurnal cortisol profiles in healthy adults: A meta-analysis.
Cortisol is a well-known biomarker of the physiological stress system; atypical cortisol patterns have been linked to many psychological and physiological illnesses. Previous studies have found vast health disparities among ethnic groups; however, studies examining the relationship between cortisol and ethnicity have found mixed results. This meta-analysis investigated whether there are differences in diurnal cortisol outcomes among ethnic groups, while considering the moderating roles of various individual factors and methodological approaches.
Br J Health Psychol, 2019; 24
29944012: Guertin C, Barbeau K, Pelletier L
Examining fat talk and self-compassion as distinct motivational processes in women's eating regulation: A self-determination theory perspective.
This study examined whether pursuing intrinsic versus extrinsic goals was associated with distinct motivational processes in eating regulation and with healthy versus unhealthy eating. Path analysis demonstrated that appearance goals were associated with fat talk, whereas health goals were associated with self-compassion. Fat talk was positively associated with non-self-determined motivation and unhealthy eating, whereas self-compassion was positively associated with self-determined motivation and healthy eating, and negatively associated with unhealthy eating. Findings emphasize the negative effects of pursuing appearance goals and engaging in fat talk and the benefits of pursuing health goals and being self-compassionate.

J Health Psychol, 2020; 25
27842290: Guertin C, Barbeau K, Pelletier L, Martinelli G
Why do women engage in fat talk? Examining fat talk using Self-Determination Theory as an explanatory framework.
This study used Self-Determination Theory to examine the motivational processes involved in individuals' engagement in fat talk and its association with unhealthy eating behaviors. Female undergraduate students (N=453) completed an online questionnaire, which assessed general and contextual motivation, importance placed on goals, fat talk, and unhealthy eating behaviors. Structural equation modeling revealed that being generally non-self-determined and placing more importance on extrinsic goals, such as thinness, was associated with fat talk. Fat talk was further associated with non-self-determined motivation for eating regulation, which in turn was associated with unhealthy eating. General self-determination and placing more importance on intrinsic goals, such as health, were not associated with fat talk, but instead, were associated with more adaptive forms of eating regulation and diet quality. Findings further current knowledge on the respective roles of motivation and goals on the engagement in fat talk, and its consequences on eating regulation and behavior.
Body Image, 2017; 20

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BOARD NUMBER: S04-050

VOICE DISCRIMINATION IN MARMOSET MONKEYS USING BEHAVIORAL TESTING

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Marmosets can perform vocalization labeling tasks or identity discrimination using playbacks. In this study, we wanted to train marmosets in an operant sound discrimination task and determine the acoustic parameters involved in sound discrimination. We adapted an in-cage automated test system, allowing ad libitum access and individualized learning. The first task is a Same/Different task, the purpose is to train monkeys to differentiate between two calls and indicate whether they are equal or different. To do this, the subject hears two successive sounds and then touches the touch screen on the left if the sounds are equal or on the right if they are different. The second task is a Go/Nogo like task, the goal is to train the monkeys to discriminate between two calls from different individuals. The monkey successively hears a random number of Nogo stimuli (the same sound) before a Go stimulus (different sound). The monkey succeeds if it touches the touch screen when the go stimulus is played. The results obtained in the first task show that even if 12 marmosets, out of 14 tested, understood the sound-touch-reward association, most of them were unable to perform the full task and discriminate the difference in the sounds. For the second task, the preliminary results indicate that the task is more suitable for marmosets to discriminate sounds. Ultimately, this task will allow us to test the discrimination among voice parameters and compare the performance in comparable tasks in other primates.

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ELECTROPHYSIOLOGICAL INVESTIGATION OF FMRI-IDENTIFIED VOICE PATCHES IN THE MACAQUE TEMPORAL CORTEX

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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The ability to process voice information is a crucial aspect of our social life. Neuroimaging studies have shown the existence of temporal and frontal cortical regions selective for conspecific vocalizations in both humans and non-human primates, supporting the hypothesis of a homology on the voice processing between humans and their closest relatives. But how the voice information is treated at the neuronal level in these areas is still not clear. To tackle this issue, we implanted two macaque monkeys with multiple high density multi-electrode arrays in fMRI-localized voice patches of the superior temporal gyrus. The neuronal activity was recorded during an auditory stimulation task in which a large set of stimuli from different categories (human voices, macaque vocalizations, marmoset vocalizations, non-vocal sounds) was presented. A total of 1550 single and multi-units was recorded from 5 arrays in the 2 monkeys. Preliminary results showed complex and heterogeneous patterns of neural response among stimulus categories. At the population level, the activity was dynamically modulated along the stimulus presentation time, representing different aspects of voice information. Moreover, decoding analysis shows that anterior patches were able to better discriminate between different call types of conspecific (macaque) vocalizations than more posterior patches. These results are bringing new knowledge on the neural substrates of voice information processing in fMRI-localized voice patches, probing for the first time spatial and temporal specificities of activation.

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BOARD NUMBER: S04-052

MULTIMODAL SENSORY INTEGRATION FOR FLY SEXUAL BEHAVIOUR

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims: The brain integrates sensory streams to create a coherent representation of external events. During courtship, a female fly receives multiple male-specific sensory inputs, including olfactory, visual, auditory, tactile and others. We study how separate streams are combined in the female brain to evaluate the male, and decide whether to accept him. **Methods**: we used a combination of connectomics, optogenetic activation of neuronal populations during social interaction, and functional imaging in the *Drosophila* female brain, to study sensory pathways responding to male-specific signals. **Results:** We identified sensory pathways responding to male taste or pheromone smell (cis-Vaccenyl acetate, cVA). Optogenetic activation of either pathway alone has no effect on female sexual receptivity, but simultaneous activation of both pathways increased female receptivity. Using the female connectome, we identified a third order neuronal population that receives synaptic input from both sensory streams, the aSP-g neurons. Manipulating aSP-g neurons during behaviour bi-directionally modulate female receptivity. We additionally identified lvPN neurons, a novel, parallel cVA-responsive pathway, which bi-directionally modulate female receptivity, and signal pheromone information to a central receptivity hub, pC1 neurons. In a behavioural epistasis experiment, we found that aSP-g neurons are able to partially restore female receptivity when pC1 neurons are ablated. **Conclusions:** We describe parallel pheromone-responsive channels; both control female receptivity but with distinct logic. While the lvPN->pC1 channel directly regulates receptivity, multisensory integration of taste and smell at aSP-g dendrites is required for that pathway to promote receptivity. Thus two separate pathways with overlapping sensory information cooperate to regulate female receptivity.

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NEURAL CORRELATES OF THIRD-PARTY PUNISHMENT: EEG STUDY

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Aims: Current study focused on the role of the neural salience network in third-party punishment of violation of the distribution norm. The recent study showed that the medial frontal negativity (MFN), that is generated by the salience network, is implicated into the altruistic tendencies (Sun et al., 2015). But this study failed to find a link between the MFC and third-party punishment behavior. We aimed to further investigate the relationship between third-party behavior and MFN. Methods: Seventeen right-handed adults were instructed to play the third-party dictator game. We analyzed EEG the time window when third-parties observed the distribution of 40 monetary units (MU) between the dictator and the recipient (-200 to 1000 ms). We analyzed the MFN (200-230 ms) at Fz and Cz electrodes using the permutation test with FDR correction. For behavioral data, we calculated the *fairness sensitivity* index as a ratio of MUs invested in third-party punishment of the nearly fair splits (25:15) and MUs invested in third-party punishment of unfair splits (30:10, 35:5, 40:0): MU(unfair splits)+MU(nearly fair splits)/MU(unfair splits)-MU(nearly fair splits). Results: Similar to Sun and colleagues (2015), we observed the MFN evoked by the unfair splits in comparison to fair splits. But in contrast to Sun and colleagues (2015), we found a significant correlation between *fairness sensitivity* index and MFN amplitude: r = -0,516; p = 0,034. Conclusions: Our findings suggest that people with the larger MFN evoked by unfair dictators' behavior also imposed stronger altruistic punishments of norm violations.

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BOARD NUMBER: S04-054

ROBUST INFERENCE AND MODELING OF SOCIAL EFFECTS ON MICE LEARNING IN INTELLICAGES

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Learning by imitating others in a group allows to acquire and reuse knowledge learned by peers. It is beneficial to the group as a whole as it allows to predict expected consequences of decisions without experiencing them.
Here, we attempt to measure the ability to learn from others in mice using Intellicages, a system that tracks access to drinking bottles of animals housed in a group. In our proposed paradigm, reward (access to sweetened water) is offered conditionally depending on random assignment of each animal to either “majority” or “minority” group. The two groups are assigned different reward locations. To assess the effect of mutual influence we developed a formalism and a model-based approach to analysis of Intellicage data. The proposed methods combine point process techniques known from spike train analysis with reinforcement learning and are validated with simulated data. Using data from Intellicage experiments with different expected social effects and different reward protocols we show a range of analytical approaches and present their effectiveness. Our results show that the proposed stochastic models capture the mice behaviour well and can be used to estimate the social effects of learning in different situations, adding extra information for quantitative description of different strains or modified animals. Corresponding generative models can be useful for prediction of group behaviour, for example, at early planning stages of experiments in which Intellicages are employed.

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BOARD NUMBER: S04-055

ON THE ENVIRONMENTAL AND BEHAVIOURAL VARIABLES THAT INFLUENCE EMPATHY AND PROSOCIAL BEHAVIOUR IN MICE

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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Increasing research evidence convincingly demonstrates the presence of empathy and prosocial behaviours in rodents, and the modulation of these behaviours is based on social experiences. However, these experiments have been conducted mostly on rodents housed in pairs, in laboratory conditions, and monitored only during the experimental period. This leaves us with limited knowledge of the effect of the environment and behavioural variables on empathy and prosocial behaviours. To get a better understanding of the personality traits and the effect of different environments on these behaviours, we created ‘Social Cities’. In these ‘Social Cities’, groups of mice can comfortably live together for extended periods of time. After they were habituated in the cities, we established a helping behaviour experiment during which one individual was in need. Our main question was to investigate the social relationship between the “helpers” and the “victim”. Analysing the video data recorded from multiple cameras on several days, we first determined single animal identities and their body poses using a computer vision approach. We then extracted a large set of behavioural features and performed regression analysis to determine which variables affect the outcome of the helping behaviour experiment. For further work, we seek to modify environmental variables of the experiment and determine how they modify the social dynamics and the willingness to help during the behavioural paradigm.

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BOARD NUMBER: S04-056

ENCODING SOCIAL EXPLORATION IN THE AMYGDALA

POSTER SESSION 04 - SECTION: NEURAL CORRELATES OF SOCIAL INTERACTIONS

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An animal's behavior is determined by metabolic, emotional and social factors. Depending on its state, an animal will focus on avoiding threats, foraging for food or on social interactions, and will display the appropriate behavioral repertoire. Moreover, survival and reproduction depend on the ability to adapt to changes in the environment by prioritizing the appropriate state. Although these states are thought to be associated with particular functional configurations of large-brain systems, the underlying principles are poorly understood. Using deep-brain calcium imaging of mice engaged in spatial or social exploration, we investigate the basolateral amygdala (BLA), a region that integrates emotional, social and metabolic information. We demonstrate that the BLA encodes animals' engagement in exploratory behavior by means of two large, functionally anticorrelated ensembles exhibiting slow dynamics. These findings reveal that the basolateral amygdala acts as a low-dimensional, but context-dependent, hierarchical classifier that encodes state-dependent behavioral repertoires. This computational function may have a fundamental role in the regulation of internal states in health and disease.

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BOARD NUMBER: S04-057

OBJECT DECODING WITH SPATIAL ATTENTION IN THE HUMAN LATERAL OCCIPITAL COMPLEX

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Spatial attention can selectively enhance responses of sensory neurons. According to invasive electrophysiological studies performed in non-human primates (NHPs), the main effect of spatial attention is to resolve competition between objects in the same image in such a way that the neural population response to the object at the attended location resembles the response to the same object when presented in isolation. Until now, this hypothesis remained untested at the single unit activity level in human visual cortex. We had the unique opportunity to record intracortical neuronal activity (96-electrodes Utah arrays) from the lateral occipital complex (LOC) in 2 epilepsy surgery patients during a spatial attention task. In this task, we presented two objects at a time, and cued subjects to covertly attend to a particular location in the visual field to detect a specific object. We then decoded information related to the identity of each of the objects present in the image from the recorded population using linear classifiers. The results show that we could only decode the object at the cued position above chance level, suggesting that spatial attention works similarly in NHPs and human visual cortex. Finally, we studied the dynamics of spatial attention by decoding object identity every 50 ms. The amount of information related the identity of the object at the cued location became significant at 125 ms after stimulus onset but increased markedly after this initial visual response. This indicates that spatial attention not only biases bottom-up processing but may also involve recurrent computations.

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BOARD NUMBER: S04-058

TASK-INDEPENDENT ENCODING OF ABSTRACT BEHAVIORAL CATEGORIES IN FERRET PREMOTOR CORTEX

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Multiple regions of frontal cortex have been shown to encode a variety of task-related signals, including task-relevant sensory features and stimulus behavioral categories, possibly with major inter-species differences (Mante et al, 2013, Rogers & DeWeese 2014, Siegel et al, 2015, Fritz et al 2010, Lui et al 2020, Reinert et al 2021). To date, there are conflicting results about the extent to which specific frontal regions preserve low-level sensory information, or are restricted to high-level behavioral categories. To tackle this question, we trained ferrets on a Go/NoGo auditory task, alternating in blocks requiring flexible attention to different acoustic dimensions. Using the burgeoning technology of functional Ultrasound Imaging (fUS), we recorded hemodynamic responses of multiple functional areas of ferret frontal cortex while the animals performed the alternating task, with sub-millimeter spatial resolution, up to 6 mm below the cortical surface. We have observed stimulus-evoked responses sparsely throughout this cortex, but have additionally observed robust differences in the response to Go and NoGo sounds during behavior in contained regions including lateral premotor cortex (lPMC) and the paraorbital gyrus (pOBG), as well as motor and sensorimotor cortex. In each case, these contrasts are independent of the sensory dimension the ferret attended to, suggesting that at this stage in processing and at this spatial resolution, the evoked responses no longer strongly encode specific stimulus identity. This result is not dependent on choice, as Go/NoGo differences are similar in these locations whether the ferret correctly rejects the NoGo or if they mistakenly lick.

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BOARD NUMBER: S04-059

CATEGORY-DEPENDENT TASK-LOAD EFFECTS AT CATEGORY-SELECTIVE AREAS

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Brain imaging studies by functional MRI (fMRI) revealed primary and high cognitive functional areas. For visual stimuli, categorically selective brain areas were found consistently in relation with visual object processing. However, effects of other tasks not related with stimulus attributes on fMRI responses at those stimulus-selective areas are unclear yet. In this study we examined the dependency of the functional specificity to visual stimuli on a load task at face and visual language processing areas. We examined whether the category selective areas at ventral region selectively respond to task-load levels or not. Face and characters images were used as visual stimulation for fMRI experiments. The load levels were two of simple discrimination and complex. One was simple discrimination task and the other was an associative task with stimuli. 3T MRI scanner with 12 channel head matrix coil was used for fMRI experiments. Four-teen subjects participated in the study. The study was approved by the Institutional Review Board of Tohoku Fukushi University. Stimulus-selective areas were identified by localization scans for face and visual language areas. Responses in the areas were compared. We found that the task-load level affected on the fMRI response selectively to its preferred stimulation at each categorically specific area, that is, non-preferred stimulus was not affected by the task-load level. This indicates that the effects of applied task on category-selective areas were from top-down modulation or associative interaction with stimulus processing. Further study will elucidate the mechanism.

FENS Forum 2022
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BOARD NUMBER: S04-060

EFFECTS OF ACUTE EXERCISE ON INHIBITORY CONTROL AND FRONTAL THETA OSCILLATIONS IN PREADOLESCENT CHILDREN

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Aims: To determine the neural underpinnings of acute exercise-induced changes in inhibitory control, this study investigated the acute effects of aerobic exercise on neural oscillations during an inhibitory control task in preadolescent children. **Methods:** Thirty-three preadolescent children between 8-11 years old were recruited into this randomized, within-subjects, crossover study. Participants engaged in an aerobic exercise condition (20 minutes of moderate intensity treadmill walking; heart rate during exercise = 61 ± 4% of pre-determined maximal heart rate) and a 20-minute seated rest condition in a counterbalanced order. After both experimental conditions, participants completed a modified Eriksen flanker task to assess inhibitory control, with concurrent electroencephalography (EEG) data collection. **Results:** Our data indicated that, during incongruent trials requiring greater amounts of inhibitory control, participants had higher response accuracy following aerobic exercise relative to seated rest. There was also a congruency effect on response accuracy, wherein higher accuracy was observed during congruent trials relative to incongruent trials, only following seated rest; an effect not found following aerobic exercise. Neuroelectric analyses revealed a similar congruency effect on task-related frontal theta synchronization following seated rest, wherein theta synchronization was larger during congruent relative to incongruent trials. Lastly, during incongruent trials, there was a trend (p = .07) for theta synchronization, wherein greater theta synchronization was found following aerobic exercise relative to seated rest. **Conclusions:** Acute exercise may transiently enhance performance on tasks requiring greater inhibitory control in preadolescent children, which are accompanied by flexible modulation of top-down cortical control signified by frontal theta oscillations.

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BOARD NUMBER: S04-061

EFFECT OF THE VOCAL AND EMOTIONAL PROSODIC CONTENT ON THE NEURAL ADAPTATION TO SOUNDS.

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Adaptation to sensory information is essential to detect, anticipate and respond to changes in the environment, especially for the adaptation of behavior in response to others' voices and emotions. Whether social content and emotional prosody impact the process of neural adaptation to sounds remains to be investigated. At the brain level, adaptation is characterized by a decrease of the response amplitude to a repeated stimulus. This phenomenon is referred to as Repetition Suppression (RS) and is measured using ERPs through the modulation of P1 at about 100ms in response to sounds. We compared RS and P1 between vocal and non-vocal as well as emotional and non-emotional sounds by recording auditory evoked potentials in a group of adults. Results showed that in a changing context (roving paradigm), regularity encoding is possible for both vocal and non-vocal sounds, but with different dynamics according to the nature of the sounds. In fact, fewer repetitions are required for the stabilization of the P1 amplitude to non-vocal sounds compared to vocal ones. Moreover, results suggest that participants adapt more rapidly to sounds involving emotional prosody than to neutral sounds, consistently with the social and empathetic nature of human beings. The presence of Repetition Positivity (RP) in all conditions indicates that adaptation operates for sounds of different nature. However, the number of repetitions required to reach regularity encoding depends on the vocal and emotional content of the sounds.

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BOARD NUMBER: S04-062

40HZ-TRANSCRANIAL ALTERNATING CURRENT STIMULATION (TACS) MODULATES ILLUSORY PERCEPTION: SHEDDING LIGHT ON PAREIDOLIA

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Aims: The study aims to investigate the physiological substrate of face Pareidolia, that is the perception of faces in non-existent entities. Transcranial Alternating Current Stimulation (tACS) at gamma (γ) frequency was adopted to modulate face Pareidolia phenomena.

Methods: 40Hz-tACS was delivered over the right lateral occipito-temporal cortex of 75 participants during or after the administration of 5 tasks for illusory perception, including the 'Toast test' for faces, and a 'Mooney task' (Faces and Objects) with both validated and ad-hoc generated stimuli. Participants were divided into three groups receiving 35 minutes of either Sham, Online, or Offline stimulation.

Results: Results from the Toast Test showed significant effects on RTs: Pareidolia responses (erroneous face detections) were significantly slower than non-Pareidolia detections in the Sham ($p < .0001$) and Online ($p < .001$) groups, but not in the Offline one. Results from the Mooney (Faces) task showed Pareidolia responses' accuracy in the Offline group was significantly lower than in the Sham ($p < .01$) and Online ($p < .05$) ones. A similar but weaker pattern emerged for Objects.

Conclusions: This is the first study investigating face Pareidolia via a tACS protocol. Our study showed an aftereffect of 40Hz-tACS over the right lateral occipito-temporal cortex on visual perception, which was modulated toward a higher proneness to Pareidolia (i.e., change in RTs and a higher rate of illusory face detections). It sheds some light on γ-tACS potential to modulate brain plasticity for future clinical applications in abnormal visual perception.

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LATERALIZED OCCIPITAL TMS ELICITS DIFFERENTIAL PHOSPHENE EEG ACTIVITY BETWEEN LEFT AND RIGHT HEMISPHERES

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Aims: This study aims to shed light on the neural dynamics leading to visual perception, looking for differences between the hemispheres. To do so, we used TMS over left and right primary visual cortex to trigger phosphenes while recording EEG signal, to detect hemispheric differences in conscious and unconscious visual processing. **Methods:** Eighteen participants were tested. Firstly, we determined an individual stimulation intensity at which participants could perceive a phosphene on 50% of trials, per each stimulation site (i.e. electrodes O1 and O2). We then administered single-pulse TMS and participants reported the presence or absence of a phosphene by pressing respectively two keyboard keys. Participants received 360 TMS pulses per stimulation site, which were counterbalanced. **Results:** A significant effect of stimulated hemisphere and phosphene awareness was found on recorded TEPs. The interaction was also significant: analysis of positive and negative answers separately for the two sites revealed that stimulating O1 determined early differential activity located over occipital and right frontal electrodes, while O2 stimulation elicited later activations on centro-parietal electrodes as a function of awareness. **Conclusions:** Phosphene perception seems to elicit differential activity depending on the stimulated hemisphere, with earlier activations occurring after left stimulation compared to right stimulation. These differential patterns may suggest a different role for the two hemispheres in processing visual information.

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BOARD NUMBER: S04-064

EXOGENOUS ATTENTION INTERACTS WITH CONSCIOUS PERCEPTION: EVIDENCE FROM HUMAN INTRACEREBRAL RECORDINGS

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Competing theories of consciousness stress the importance of reverberating activity in sensory areas, of integrated information in the posterior cortex, or of global broadcasting of information through frontoparietal (FP) networks. Here, we recorded neural activity from 727 intracranial contacts in 13 patients with drug-resistant epilepsy, while they detected near-threshold peripheral targets preceded by supra-threshold, non-predictive exogenous cues. Valid cues increased the number of seen targets. K-means clustering grouped electrode activities according to their temporal trajectories across experimental conditions. Three patterns emerged: 1) validly cued seen targets elicited stronger neural activity than invalidly cued ones; after fast transient target-related visual activity, there was sustained activity in FP and temporal regions, followed by late accumulation activity in bilateral PFC. 2) Invalidly cued seen targets elicited early, sustained activity in the right hemisphere temporoparietal junction / inferior frontal gyrus (reorienting network). 3) Seen, but not unseen, targets elicited late sustained activity in the left dorsolateral PFC, independent of attention. White matter tractography showed that the superior longitudinal fasciculus (SLF) II/III connected the FP network with sustained activity; SLF I/III connected the FP network with late accumulation activity; SLF III connected the reorienting network. Simulation of the task with recurrent neural networks supported the causal contribution of these FP networks to conscious perception. Our evidence from direct brain activity recording, white matter tractography, and computational modeling specifies the different roles of FP networks in conscious processing, resulting from orienting and reorienting of spatial attention. Left dorsolateral PFC activity can instead reflect post-orienting perceptual decisions.

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BOARD NUMBER: S04-065

TWO INDEPENDENT MOVEMENT STATE RELATED INFLUENCES ON SENSORY PROCESSING

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Walking influences visual processing related neural responses, however, the underlying mechanism remains underexplored. Based on previous findings, we hypothesised that walking leads to a processing change that can be predicted by a movement state related decrease in alpha power. The influence of the state dependent alpha power on sensory processing and the resulting neural activity should therefore be only minorly affected by the stimulus type. In the current study, we investigated the neural nature of those walking-induced changes (ERP and alpha power) and behavioural performance in a discrimination task while participants were standing or freely walking. Mobile EEG was combined with AR glasses for mobile visual stimulation. The results showed enhanced early N1 and decreased P3 responses to stimulus onset during walking. This amplitude modulation was correlated with pre-stimulus alpha power, and alpha power was reduced during walking. As predicted further, the walking-induced N1/P3 amplitude modulation was independent of the exact stimulus nature, such as eccentricity, hemifield and distractor presence. Stimulus-induced alpha power on the other hand showed a modulation by movement state specifically for certain stimulus feature, namely a reduction during walking for eccentricity of 1 degree without distractor. These findings indicate two movement-related influences on sensory processing. One is based on an ongoing reduction in alpha power due to walking which will increase early responses to sensory input such as N1. The second influence is specific for the central visual field and when no distracting input is present, indicating attention modulation at the central visual field during walking.

Pubmed:
35182897: Chen X, Cao L, Haendel BF
Differential effects of walking across visual cortical processing stages.
Perceptual processes are almost exclusively investigated and understood under marked movement restriction, while natural behaviour includes pronounced movements. Recent human studies have indicated a profound influence of body movement on early visual responses (e.g., evoked components around 100 msec in EEG, electroencephalogram). However, very little is known about the influence of free walking on later visual responses (e.g., responses related to visual selective attention in a later time window than the stimulus evoked N1 component). In the current study, we measured neural signals (EEG) and behavioural performance in a visual selective attention task while participants were standing or freely walking. The results showed that walking was associated with an amplification of early sensory-evoked potential as indicated by the N1 component. Interestingly, neural indexes of the succeeding processing stages of stimulus discrimination and identification, namely the N2pc component and alpha oscillations, and the eventual behavioural measures were comparable between standing and walking. Additionally, in both standing and walking conditions, an overall advantage in target processing for the right visual field was observed. Our work provides evidence that the early sensory processing is enhanced during locomotion while the succeeding processing steps in a later time window are not modulated by locomotion. We conclude that walking has differential effects across visual cortical processing stages.
Cortex, 2022; 149
33414709: Cao L, Chen X, Haendel BF
Overground Walking Decreases Alpha Activity and Entrains Eye Movements in Humans.
Experiments in animal models have shown that running increases neuronal activity in early visual areas in light as well as in darkness. This suggests that visual processing is influenced by locomotion independent of visual input. Combining mobile electroencephalography, motion- and eye-tracking, we investigated the influence of overground free walking on cortical alpha activity (~10 Hz) and eye movements in healthy humans. Alpha activity has been considered a valuable marker of inhibition of sensory processing and shown to negatively correlate with neuronal firing rates. We found that walking led to a decrease in alpha activity over occipital cortex compared to standing. This decrease was present during walking in darkness as well as

during light. Importantly, eye movements could not explain the change in alpha activity. Nevertheless, we found that walking and eye related movements were linked. While the blink rate increased with increasing walking speed independent of light or darkness, saccade rate was only significantly linked to walking speed in the light. Pupil size, on the other hand, was larger during darkness than during light, but only showed a modulation by walking in darkness. Analyzing the effect of walking with respect to the stride cycle, we further found that blinks and saccades preferentially occurred during the double support phase of walking. Alpha power, as shown previously, was lower during the swing phase than during the double support phase. We however could exclude the possibility that the alpha modulation was introduced by a walking movement induced change in electrode impedance. Overall, our work indicates that the human visual system is influenced by the current locomotion state of the body. This influence affects eye movement pattern as well as neuronal activity in sensory areas and might form part of an implicit strategy to optimally extract sensory information during locomotion.
Front Hum Neurosci, 2020; 14
33104691: Zaikauskaite L, Chen X, Tsivrikos D
The effects of idealism and relativism on the moral judgement of social vs. environmental issues, and their relation to self-reported pro-environmental behaviours.
Many studies have demonstrated that moral philosophies, such as idealism and relativism, could be used as robust predictors of judgements and behaviours related to common moral issues, such as business ethics, unethical beliefs, workplace deviance, marketing practices, gambling, etc. However, little consideration has been given to using moral philosophies to predict environmentally (un)friendly attitudes and behaviours, which could also be classified as moral. In this study, we have assessed the impact of idealism and relativism using the Ethics Position Theory. We have tested its capacity to predict moral identity, moral judgement of social vs. environmental issues, and self-reported pro-environmental behaviours. The results from an online MTurk study of 432 US participants revealed that idealism had a significant impact on all the tested variables, but the case was different with relativism. Consistently with the findings of previous studies, we found relativism to be a strong predictor of moral identity and moral judgement of social issues. In contrast, relativism only weakly interacted with making moral judgements of environmental issues, and had no effects in predicting pro-environmental behaviours. These findings suggest that Ethics Position Theory could have a strong potential for defining moral differences between environmental attitudes and behaviours, capturing the moral drivers of an attitude-behaviour gap, which continuously stands as a barrier in motivating people to become more pro-environmental.
PLoS One, 2020; 15
31545320: Chen J, Chen X, Lv S, Zhang Y, Long H, Yang K, Qi S, Zhang W, Wang J
Application of 3D Printing in the Construction of Burr Hole Ring for Deep Brain Stimulation Implants.
3D printing has been widely applied in the medical field since the 1980s, especially in surgery, such as preoperative simulation, anatomical learning and surgical training. This raises the possibility of using 3D printing to construct a neurosurgical implant. Our previous works took the construction of the burr hole ring as an example, described the process of using softwares like computer aided design (CAD), Pro/Engineer (Pro/E) and 3D printer to construct physical products. That is, a total of three steps are required, the drawing of 2D-image, the construction of 3D-image of burr hole ring, and using a 3D printer to print the physical model of burr hole ring. This protocol shows that the burr hole ring made of carbon fiber can be rapidly and accurately molded by 3D printing. It indicated that both CAD and Pro/E softwares can be used to construct the burr hole ring via integrating with the clinical imaging data and further applied 3D printing to make the individual consumables.
J Vis Exp, 2019;

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BOARD NUMBER: S04-066

PLEASE DON'T STOP THE MUSIC: AUDITORY SPATIAL CUEING REDUCES NEGLECT AFTER RIGHT-HEMISPHERIC STROKE - A PROOF OF CONCEPT STUDY

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

Brigitte Kaufmann[1,2,3], Paolo Bartolomeo[2], Julia Frey[1], Tobias Pflugshaupt[1], Samuel Knobel[4], Tobais Nef[4], René Müri[3,4], Thomas Nyffeler[1,3,4]
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Neglect after stroke is a supramodal syndrome affecting not only the visual but also auditory modality. Preliminary studies used this audio-visual cross-modal effect to show short-lasting effects on attention towards the neglected space. Here we aimed to investigate the therapeutic potential of auditory stimulation combining the unspecific effect of music (i.e. listen to preferred music) with the effects of auditory spatial cueing (i.e. dynamic presentation of music as moving from right to left). The effect of this technique was investigated in two proof-of-concept experiments using repeated-measures, cross-over designs in 21 patients with visual neglect after right-hemispheric stroke. In experiment I (n=9), patients showed a significant larger improvement in Letter Cancellation after listening to preferred music *with* than *without* auditory spatial cueing. After granting its feasibility, the long-term effects of this technique were investigated in experiment II (n=12), using video-oculography, a sensitive tool to assess spatial attention over time. Listening to music *with* auditory spatial cueing –compared to *without*– significantly improved neglect in terms of free visual exploration behaviour for up to 3h. Hence, the positive effects of auditory stimulation with preferred music can be significantly enhanced and prolonged when combined with a spatial cueing component. Additional Voxel-based-lesion-symptom-mapping analysis revealed that the response variability of this effect is determined by the integrity of the IPL with its intra-and inter-hesmipheric connections (SLF_II, parieto-parietal Callosum). Finally, the patients experienced listening to music with auditory spatial cueing as pleasant. Thus, supporting the potential of this technique as an add-on in neurorehabilitative neglect therapy.

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BOARD NUMBER: S04-067

NEURAL DYNAMICS OF TASK-SWITCHING IN SENSORIMOTOR DECISION-MAKING

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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The ability to flexibly switch between tasks or rules is crucial for intelligent behaviour. Psychological studies show lower accuracy and longer reaction times after switching from one task to another, yet the source of these switch costs and the neural switch mechanisms remain poorly understood. We used magnetoencephalography (MEG) to resolve the neural dynamics of task-switching in human perceptual decision-making. In a visuomotor decision-making task (Pape & Siegel 2016), two task features randomly alternated or repeated from trial to trial: the order of the visual stimulus and choice-response cue ("order"), as well as the choice-response mapping itself ("mapping"). Using multivariate pattern analysis (MVPA), we found weaker neural order and mapping information after a switch. Furthermore, we found switch-specific neural signals for both types of task-switches, independent of the switch direction, about half a second after presentation of the corresponding switch cue. There was little cross-information between the different types of switches, suggesting that the switch signals are domain-dependent. Our results provide new insights into the large-scale neural dynamics underlying different types of task-switching in human sensorimotor decision-making. **References**: Pape AA and Siegel M (2016) Motor cortex activity predicts response alternation during sensorimotor decisions. Nature Communications 7:13098

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BOARD NUMBER: S04-068

ANALYSIS OF INFORMATION CODING AND EXCHANGE IN THE MULTIPLE DEMAND NETWORK USING FMRI-MEG FUSION

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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The multiple-demand network (MDN) encodes information across a variety of cognitive tasks. It is hypothesised to select and integrate information from across neural systems (Duncan et al. 2020) but evidence for the timing and potential exchange of information across the MDN is lacking. To fill this gap, we fused the time courses of information coding (from MEG) to the information contained in different MDN regions (from fMRI) using Representational Similarity Analysis. We then developed a novel fusion-based connectivity analysis to evaluate information exchange across the MDN. On each trial, one of four visual stimuli appeared (white squares on grey background; two in top-right and two in top-left hemi-fields), and participants (MEG: n=24; fMRI: n=30) had to press one of four buttons. They learned two orthogonal stimulus-button mappings (rules; indicated by fixation colour) in a training phase. We fused four aspects of information: coarse stimulus (left vs. right stimuli), fine stimulus (inner vs. outer stimuli), rule and response information. Both coarse and fine stimulus information appeared first in posterior followed by anterior regions of the MDN. Rule information appeared in the same order. Parietal MDN showed the response information immediately before the response. Connectivity analysis showed information transfer from occipital to MDN and its circulation within the MDN most dominantly for the fine stimulus information. These results provide evidence for differential timing of information processing across nodes of the MDN. The new connectivity method opens new opportunities for accurate study of information exchange with high spatial and temporal resolution.

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BOARD NUMBER: S04-069

THE INFLUENCE OF RETINAL AND REAL-WORLD SPEEDS ON SPEED PERCEPTION FOR MOTION IN DEPTH

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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For motion in depth, retinal motion accelerates as the target approaches the observer when the target moves at a constant speed in the world. The purpose of this study was to determine whether the difference between retinal and real-world speeds affects speed perception for motion in depth. Therefore, we used a two-alternative forced-choice paradigm and implemented two experimental tasks (retinal constant and world constant tasks) to measure psychometric functions for speed discrimination. For the retinal constant task, a visual stimulus moved at a constant speed on the retina and randomly moved 4 to 8 deg (far condition), 8 to 12 deg (middle condition), and 12 to 16 deg (near condition) as vergence angle. For the world constant task, a visual stimulus moved at a constant speed in the real-world and randomly moved 80 to 60 cm (far condition), 60 to 40 cm (middle condition), and 40 to 20 cm (near condition) from the observer. The results of the retinal constant task showed that the stimulus speed was perceived faster in the order of the far, middle, and near conditions. In contrast, the results of the world constant task represented that the stimulus speed was perceived faster for the near condition than for the middle and far conditions. Therefore, our results suggest that target speed moving far to near is not always perceived depending on the retinal speed. It is also suggested that the retinal speed of motion influences the speed perception when the target position is close to the observer.

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BOARD NUMBER: S04-070

OSCILLATORY BRAIN ACTIVITY IN INFANTS DURING VISUAL ATTENTION TO DYNAMIC FACES AND OBJECTS AT THE AGE OF 11 MONTHS.

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Infants learn a lot about their social world from observing faces. Previous studies have demonstrated, attenuation of the alpha power spectral density (PSD) in the frontal, central and posterior electrodes, during sustained visual attention(Orekhova, 2001; Stroganova, 1999). Additionally, children with better inhibitory control and more mature attention shifting abilities, had higher gamma power density (PSD) functions (Benasich, 2008).Thus, this study aimed to test, replicability of these results while an infant is watching dynamic social stimuli involving rhyming faces and rotating toys on the screen. 51 typically developing infant's EEG data of 11 months-old were assessed for changes in power in the Alpha (6 to 9 Hz), Lower Gamma (21 to 30Hz) and Upper Gamma (31 to 44Hz) bands. Results showed that infants had lower alpha PSD in frontal, central and left temperoparietal electrodes ($t(50)=-3.080, p<.003, t(50)=-2.535$ $p<.014$, $t(50)=-3.862$ $p<.000$) and lower PSD in lower and upper gamma frequencies in central,occipital, left temperoparietal and right temperoparietal electrodes ($t(50)=-1.888, p<.065, t(50)=-3.175, p<.003, t(50)=-2.760. p<.008, t(50)=-2.178, p<.034$ $t(50)=-2.242$, $p=.029$, $t(50)=-3.282, p=.002$, $t(50)=-2.920, p=.005, t(50)= -2.351, p=.023$) during viewing of the dynamic actress in comparison to dynamic toys. The results imply that infants find dynamic facial movement more engaging and exhibit better attentional control to moving toys stimuli.

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BOARD NUMBER: S04-071

VISUAL AND SEMANTIC NOVELTY IN MOVIES DRIVE PROMINENT RAPID NEURAL RESPONSES IN HUMAN

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

Maximilian Nentwich[1], Marcin Leszczynski[2,3], Brian Russ[3,4,5], Noah Markowitz[6], Kaustubh Sapru[1], Charles Schroeder[2,3], Ashesh Mehta[6], Stephan Bickel[6], Lucas Parra[1]
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Our continuous visual experience in daily life is frequently interrupted by rapid changes of visual dynamics and semantics of unfolding events. Research on neural responses to change in naturalistic stimuli has focused on the individual effects of visual movement and event boundaries in film. Here we investigate the relative importance of neural responses in human intracranial recordings to rapid visual change caused by motion, scene cuts, event boundaries and eye movements. We analyzed intracranial recordings from a total of 6328 electrodes, as well as eyetracking data, from 26 patients who passively watched up to 50 minutes of videos. We extracted high-frequency broadband activity (BHA, 70-150Hz) neural responses to optic flow, saccades, and scene cuts using a system identification approach. Responses to optic flow were much weaker than responses to saccades or scene cuts and mostly localized to the occipital lobe. Responses to both saccades and scene cuts are distributed widely across the brain, beyond traditional visual processing areas. While responses to saccades are rapid (~200ms), responses to scene cuts last up to 1000 ms. Scene cuts associated with semantic event boundaries elicit stronger responses as compared to other cuts with matched low-level visual properties. They also elicited more selective responses in higher level cortical areas. In summary, we suggest that novelty in the visual input is the dominant driver of immediate neural responses, with increasing reach and duration for semantically complex novelty.

Pubmed:
32492509: Nentwich M, Ai L, Madsen J, Telesford QK, Haufe S, Milham MP, Parra LC
Functional connectivity of EEG is subject-specific, associated with phenotype, and different from fMRI.
A variety of psychiatric, behavioral and cognitive phenotypes have been linked to brain "functional connectivity" -- the pattern of correlation observed between different brain regions. Most commonly assessed using functional magnetic resonance imaging (fMRI), here, we investigate the connectivity-phenotype associations with functional connectivity measured with electroencephalography (EEG), using phase-coupling. We analyzed data from the publicly available Healthy Brain Network Biobank. This database compiles a growing sample of children and adolescents, currently encompassing 1657 individuals. Among a variety of assessment instruments we focus on ten phenotypic and additional demographic measures that capture most of the variance in this sample. The largest effect sizes are found for age and sex for both fMRI and EEG. We replicate previous findings of an association of Intelligence Quotient (IQ) and Attention Deficit Hyperactivity Disorder (ADHD) with the pattern of fMRI functional connectivity. We also find an association with socioeconomic status, anxiety and the Child Behavior Checklist Score. For EEG we find a significant connectivity-phenotype relationship with IQ. The actual spatial patterns of functional connectivity are quite different between fMRI and source-space EEG. However, within EEG we observe clusters of functional connectivity that are consistent across frequency bands. Additionally we analyzed reproducibility of functional connectivity. We compare connectivity obtained with different tasks, including resting state, a video and a visual flicker task. For both EEG and fMRI the variation between tasks was smaller than the variability observed between subjects. We also found an increase of reliability with increasing frequency of the EEG, and increased sampling duration. We conclude that, while the patterns of functional connectivity are distinct between fMRI and phase-coupling of EEG, they are nonetheless similar in their robustness to the task, and similar in that idiosyncratic patterns of connectivity predict individual phenotypes. Neuroimage, 2020; 218


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THE NEXT TRIAL WILL BE CONFLICTING! EEG SIGNATURES OF PROACTIVE CONTROL IN THE FLANKER TASK.

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Aims. The study was set to investigate the neural basis of proactive adjustments of executive control, and their modulatory effects on online processing of response conflict. **Methods.** In two experiments, participants (N 59) performed the flanker task in with conflict trials were signaled by predictive cues. The electroencephalogram (EEG) was recorded in both experiments. **Results and Conclusions.** The following questions were addressed: *Does conflict-cueing improve performance?* We observed improved behavioral performance in the predictive condition, suggesting that participants proactively utilized the cues to prepare for the upcoming demands. *How is conflict processing affected by predictive cueing?* Conflict-related modulations of midfrontal N2, theta power, and theta phase synchrony were smaller in the predictive than in the neutral condition. This suggests that proactive control suppressed the impact of incongruent flankers, so that the conflict was reduced, and so was the online control involvement. *Is proactive control implemented through pre-activation of online control?* Conflict-cueing also increased midfrontal theta power and connectivity before target onset, suggesting pre-activation of the control processes beforehand. *Do proactive and reactive control depend on common or unique processes?* Unlike the online control, the proactive control triggered a burst of theta power in the right hemisphere's dorsal and ventral lateral prefrontal cortices. In other words, two separate components of frontal theta power were observed during the proactive adjustments of control. This indicates that the two modes of control involve partially unique neural processes.
(Funding: NCN 2016/22/E/HS6/00139)

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BOARD NUMBER: S04-073

THE PROBABILISTIC INTEGRATION OF SOMATOSENSORY INFORMATION INTO THE MOTOR RESPONSE USING EEG AS BIOMARKER IN PSYCHOSIS

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

Anaelle Alouit[1], Anton Iftimovici[1,2], Macarena Cuenca[1,3], Céline Ramdani[4], Martine Gavaret[1,2], Marie-Odile Krebs[1,2], Pâvel Lindberg[1], Lucile Dupin[1]
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People with a first episode of psychosis may show cognitive deficits, sensorimotor impairments, and neurological soft signs. In schizophrenia, an imbalance in cortical excitation / inhibition has been shown to be related to sensorimotor and attentional deficits. Since motor actions are facilitated by somatosensory information and attention, we hypothesized that altered motor response in psychosis could be due to impaired processing and integration of somatosensory information. The overall goal of this study is to identify brain biomarkers of psychosis, in order to provide early predictive markers on the risk of developing psychiatric pathology, such as schizophrenia. Specifically, we aim to study (i) the modulation of cortical excitability and inhibition, (ii) the temporal and spatial dynamics of sensorimotor integration, and (iii) to correlate the cortical processing to the prodromal clinical symptoms. To do so, we developed a visuo-haptic attentional task where the participant is instructed to press one key or another with left or right hand, based on a visual signal preceded by a spatially congruent or incongruent tactile cue, in order to modulate spatial attention in a varying probabilistic context. Our method utilizes 64-channel electroencephalography, and is based on the recording and analysis of the somatosensory evoked potentials. This technique allows us to measure primary somatosensory cortex activity, related alpha and beta oscillations known to quantify cortical excitability and attentional adaptation. The preliminary data indicate that our protocol can measure spatial attention modulation, and shows how the integration of somatosensation, as an attentional cue, influences behavioral and neurophysiological measures.

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BOARD NUMBER: S04-074

LARGE-SCALE CORTICAL NETWORK MODULATIONS DURING DYNAMIC BODY PERCEPTION

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

Baichen Li[1], Marta Poyo Solanas[1], Giuseppe Marrazzo[1], Rajani Raman[2,3], Rufin Vogels[2,3], Nick Taubert[4], Martin Giese[4], Beatrice De Gelder[1]
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AIMS: Body recognition is a fundamental survival skill for many social animals. Several body-selective regions have already been identified in both humans and nonhuman primates. However, there is no comprehensive comparison of the species selectivity or of the network organization of those body patches. The present 7T fMRI study investigated the neural correlates of dynamic human and monkey bodies and faces in human participants. METHODS: The study used a block design with videos of dynamic faces, bodies, and objects from both monkey and human recording. Scramble stimuli were created for systematic control of low-level features. Univariate analysis and ICA-based network analysis were conducted in parallel to find body/species modulations at both voxel and network levels. RESULTS: Widely distributed body patches were found by univariate analysis including extrastriate body area (EBA), superior temporal sulcus (STS), medial frontal gyrus (MFG), temporoparietal junction (TPJ), amygdala, etc. Among the body patches, the highest species-selectivity was found in MFG and amygdala. Two networks, with major involvement of lateral occipital cortex and right STS respectively, were found sensitive to human bodies. The rSTS network showed higher species selectivity for bodies than for faces, and within the network, significant human-body-induced connectivity was found around EBA, TPJ, and inferior frontal gyrus (IFG). CONCLUSION: By using dynamic stimuli and multiple analysis methodologies, we found widely distributed body patches covered by two large-scale cortical networks. Furthermore, the results suggested that the network connectivity of EBA, TPJ, and IFG may play an important role in human body perception.

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SPATIAL AND FEATURE-SELECTIVE ATTENTION INTERACT MULTIPLICATIVELY IN MULTIPLE-DEMAND NETWORK

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Aim: Distinct neural effects are associated with attention deployed to a location (spatial attention) or to a particular object feature (feature attention), but it is less clear how these types of attention interact to affect information processing. To examine this, we compared the effects of spatial and feature attention on stimulus representations within a frontoparietal "multiple-demand" (MD) network posited to play a critical role in attentional control. **Method:** Participants (N=30, 16 female, 14 male, mean age=27.2) underwent fMRI while they covertly attended to one of two objects, presented left and right of a fixation cross (spatial attention manipulation), and reported the attended object's colour ("red" or "green") or shape ("X-shaped" or "flat") (feature attention manipulation) via button press (Fig1, Panel A). We used multivariate pattern analysis to measure coding of attended and unattended stimulus information. **Results:** We found a significant multiplicative interaction between spatial and feature attention in MD ($F_{(1,30)}$=69.924, p<.001) and visual cortices ($F_{(1,30)}$=17.304, p<.001). Moreover, stimulus decoding was only above chance for the attended feature of the attended object (BF_{10}>100)(Fig1, Panel B). We additionally found these task-relevant representations were organised along two major dimensions, reflecting physical stimulus properties and task
difficulty.

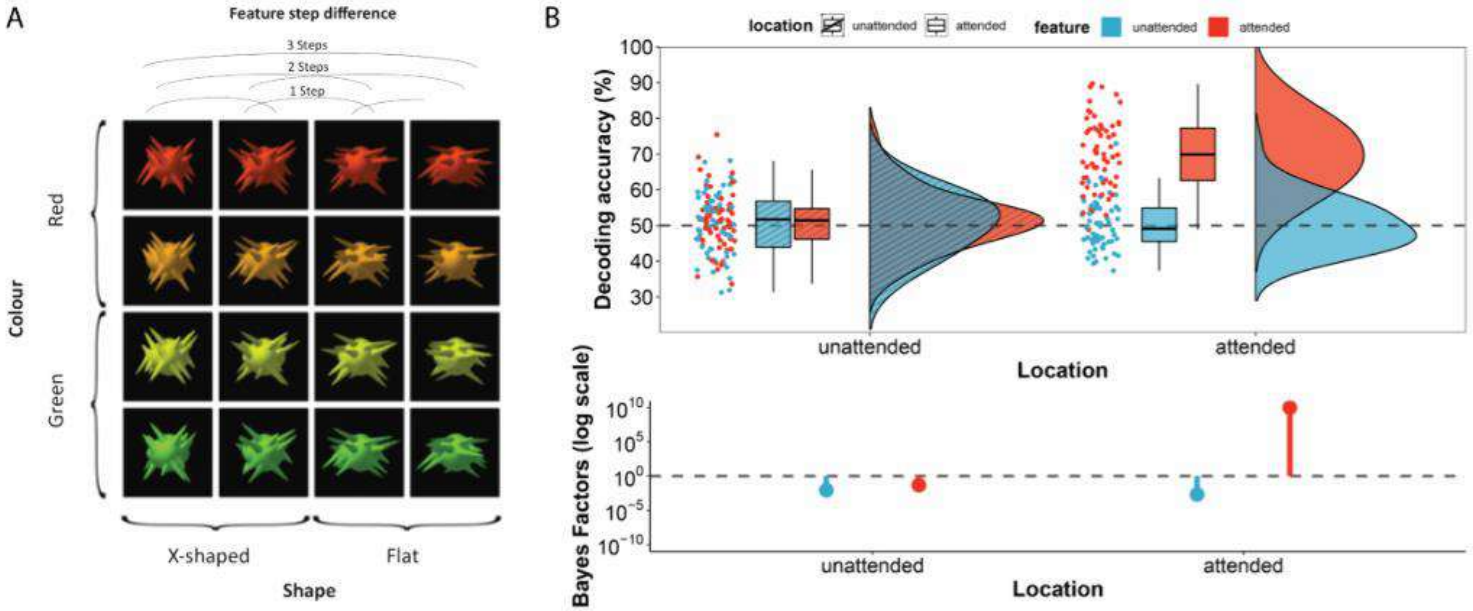

Fig 1. **Panel A:** Visual stimuli used in the experiment, adapted from Goddard et al (*J Cog Neurosci*, 2022) . Each object varied across two dimensions, colour and shape. In the colour task, participants categorised the attended object as either "red" or "green". In the shape task, participants categorised the attended object as either "X-shaped" or "flat", based on the orientations of the object's spikes. For each colour and shape, objects were either close or far from the decision boundary (e.g., "weakly red" and "strongly red", respectively) making some discriminations easier than others. Feature step size difference for decoding is shown for shape, with those one step apart reflecting a "small" discrimination, two steps "medium", and three steps "large". **Panel B:** Average decoding accuracy for unattended and attended feature information for the object at the attended or unattended location, averaged across MD ROIs. Decoding accuracy here reflects average accuracy in decoding colour or shape information in the object that was in the attended (solid colour) or unattended (lined colour) location, when the feature in question was attended (red) or unattended (blue). Scatter at left depicts decoding accuracy for each participant for the given condition. Boxplots indicate median decoding accuracy, first and third quartiles, and largest and smallest decoding accuracy value. Half violin plots indicate data distribution. The strength of evidence for above chance (H1) or at chance (H0) decoding was tested using Bayesian t-tests and is shown here as Bayes Factors for each of these conditions on a logarithmic scale.

Conclusion: Our results suggest spatial and feature attention interact multiplicatively, selectively enhancing coding of the attended feature of the attended object. Rather than boosting processing of whole objects or relevant features across space, selective attention in the MD system – at least in this difficult perceptual task – appears to reflect all-or-nothing tuning to behaviourally relevant information.

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ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION TARGETING TEMPOROPARIETAL JUNCTION INCREASES SUSTAINED ATTENTION AND VISUOMOTOR ABILITIES VIA EMBODIMENT MODULATION.

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

Valentina Cesari, Danilo Menicucci, Sergio Frumento, Graziella Orrù, Angelo Gemignani
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AIMS The sense of embodiment allows to experience non-bodily objects as part of the body (ownership), to feel the author of observed actions (agency), and to know the positioning of the body in space (location). The aim of this study was to investigate the effect of the modulation of the embodiment on cognitive performance, as previous works scarcely investigated the relationship between them. **METHODS** We modulated the sense of embodiment during a sustained attention driving task in virtual reality *via* anodal transcranial direct current stimulation (tDCS) targeting temporo-parietal junction (a pivotal area in bodily self-processing) using within-subject design with sham-controlled stimulation. Twenty-four healthy volunteers underwent two randomized and balanced sessions. Changes in sustained attentional performance were evaluated by studying the series of visuomotor adjustments during the task; the components of the embodiment were assessed using psychometric questionnaires. Also, correlations between variations from active to sham of task indices with those of the embodiment were computed. **RESULTS** After anodal tDCS, participants showed greater accuracy, less attentional lapses, and higher long-range correlations of the series of visuomotor adjustments during task, and these results paralleled with greater sense of agency, location, and total embodiment. Correlations between variations in task indices and changes in embodiment indices showed that greater agency and total embodiment were associated with less attentional lapses. **CONCLUSIONS** Embodiment might positively affect attentional and dexterous performance; this relationship might be explained by greater confidence toward the non-bodily objects that could facilitate the attentional and visuomotor control over the task.

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BOARD NUMBER: S04-077

DECODING THE NEURAL CORRELATES OF MUSICAL PREDICTIONS WITH DEEP LEARNING

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Our acoustic environment is full of statistical regularities. According to the predictive coding (PC) theory, the human brain uses such predictable features to generate expectations about future stimuli to optimize perception. Music is a complex naturalistic stimulus containing rhythmic and harmonic regularities and constitutes an ideal framework for studying the neural underpinnings of PC. To study neural correlates of temporal predictions in music listening, we capitalize on deep learning models to extract relevant features of predictive processing. We collected magnetoencephalography (MEG) data on 27 normal-hearing subjects listening to piano performances repeated throughout the experiment. We then assessed the same musical pieces in terms of continuous predictive features, namely surprisal and entropy. Those mathematical quantities were estimated by a recurrent neural network originally designed to generate piano performances. This model is particularly promising as compared with methods commonly used in the field: (i) it relies on minimal prior musical knowledge and (ii) it efficiently works on polyphonic music. Validating this approach on behavioral data confirms that this model efficiently predicts continuous human surprisal ratings to the same music. Using a temporal response function – a linear model mapping the continuous stimulus features to the MEG data – we quantify the relative influence of exogenous (stimulus driven) and endogenous (prediction driven) neural signals underlying processing of musical sequences. Finally, comparing the signatures of ongoing (probabilistic) versus memory-based (experiential) expectations, we distinguish two categories of commonly confounded priors to further delineate the spatial and spectrotemporal architecture of predictive processing in the human brain.

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BOARD NUMBER: S04-078

LET'S FACE IT: LATERALIZATION OF THE FACE PERCEPTION NETWORK IS CHARACTERIZED BY LARGE INTERINDIVIDUAL VARIABILITY AND NOT BY A CLEAR RIGHT HEMISPHERIC DOMINANCE

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Aims: In face perception research, the dominant role is almost unanimously attributed to the right hemisphere. However, large scale functional magnetic resonance imaging (fMRI) studies that systematically assess hemispheric lateralization in the core system of face perception are missing. Thus, we aimed to thoroughly investigate hemispheric lateralization in the core system in a large cohort, decribing the distribution of lateralization results across subjects and analyzing effects of area, handedness, and sex. **Methods:** 108 healthy subjects (23 left-handers) were included in an fMRI study. Lateralization was assessed with a lateralization index (LI) that compares the brain activation strength between homologous brain areas in the left and right hemisphere. Effects of brain area, handedness, and sex were tested with a generalized linear mixed effects regression model. **Results:** We found no main effect of area, sex, or handedness and no significant interactions. In contrast to our expectations, there was no clear right-hemispheric dominance, neither on the single subject level nor on the group-level. On the contrary, our results clearly show substantial variability between subjects. LIs ranged from strong left to strong right dominance. Only approximately 50% of subjects could be classified as right dominant. **Conclusion:** Strictly speaking, we do not question the dominance of the right hemisphere per se. However – based on fMRI activations patterns – it might be more suitable to describe the face perception network as "bilateral with a slight tendency towards right-dominance at the group level and a large interindividual variability".

Pubmed:
34676995: Sahraei I, Hildesheim FE, Thome I, Kessler R, Rusch KM, Sommer J, Kamp-Becker I, Stark R, Jansen A
Developmental changes within the extended face processing network: A cross-sectional functional magnetic resonance imaging study.
In the field of face processing, the so-called "core network" has been intensively researched. Its neural activity can be reliably detected in children and adults using functional magnetic resonance imaging (fMRI). However, the core network's counterpart, the so-called "extended network," has been less researched. In the present study, we compared children's and adults' brain activity in the extended system, in particular in the amygdala, the insula, and the inferior frontal gyrus (IFG). Using fMRI, we compared the brain activation pattern between children aged 7-9 years and adults during an emotional face processing task. On the one hand, children showed increased activity in the extended face processing system in relation to adults, particularly in the left amygdala, the right insula, and the left IFG. On the other hand, lateralization indices revealed a "leftward bias" in children's IFG compared to adults. These results suggest that brain activity associated with face processing is characterized by a developmental decrease in activity. They further show that the development is associated with a rightward migration of face-related IFG activation, possibly due to the competition for neural space between several developing brain functions ("developmental competition hypothesis").
Dev Neurobiol, 2022; 82
34322712: Thome I, Hohmann DM, Zimmermann KM, Smith ML, Kessler R, Jansen A
"I Spy with my Little Eye, Something that is a Face…": A Brain Network for Illusory Face Detection.
The most basic aspect of face perception is simply detecting the presence of a face, which requires the extraction of features that it has in common with other faces. Putatively, it is caused by matching high-dimensional sensory input with internal face templates, achieved through a top-down mediated coupling between prefrontal regions and brain areas in the occipito-temporal cortex ("core system of face perception"). Illusory face detection tasks can be used to study these top-down influences. In the present functional magnetic resonance imaging study, we showed that illusory face perception activated just as real faces the core system, albeit with atypical left-lateralization of the occipital face area. The core system was coupled with two distinct brain regions in the lateral prefrontal (inferior frontal gyrus, IFG) and orbitofrontal cortex (OFC). A dynamic causal modeling (DCM) analysis revealed that activity in the core system during illusory face detection was upregulated by a modulatory face-specific influence of the IFG, not as previously assumed by the OFC. Based on these

findings, we were able to develop the most comprehensive neuroanatomical framework of illusory face detection until now.
Cereb Cortex, 2021; 32
33123034: Hildesheim FE, Debus I, Kessler R, Thome I, Zimmermann KM, Steinsträter O, Sommer J, Kamp-Becker I, Stark R, Jansen A
The Trajectory of Hemispheric Lateralization in the Core System of Face Processing: A Cross-Sectional Functional Magnetic Resonance Imaging Pilot Study.
Face processing is mediated by a distributed neural network commonly divided into a "core system" and an "extended system." The core system consists of several, typically right-lateralized brain regions in the occipito-temporal cortex, including the occipital face area (OFA), the fusiform face area (FFA) and the posterior superior temporal sulcus (pSTS). It was recently proposed that the face processing network is initially bilateral and becomes right-specialized in the course of the development of reading abilities due to the competition between language-related regions in the left occipito-temporal cortex (e.g., the visual word form area, VWFA) and the FFA for common neural resources. In the present pilot study, we assessed the neural face processing network in 12 children (aged 7-9 years) and 10 adults with functional magnetic resonance imaging (fMRI). The hemispheric lateralization of the core face regions was compared between both groups. The study had two goals: First, we aimed to establish an fMRI paradigm suitable for assessing activation in the core system of face processing in young children at the single subject level. Second, we planned to collect data for a power analysis to calculate the necessary group size for a large-scale cross-sectional imaging study assessing the ontogenetic development of the lateralization of the face processing network, with focus on the FFA. It was possible to detect brain activity in the core system of 75% of children at the single subject level. The average scan-to-scan motion of the included children was comparable to adults, ruling out that potential activation differences between groups are caused by unequal motion artifacts. Hemispheric lateralization of the FFA was 0.07 ± 0.48 in children (indicating bilateral activation) and -0.32 ± 0.52 in adults (indicating right-hemispheric dominance). These results thus showed, as expected, a trend for increased lateralization in adults. The estimated effect size for the FFA lateralization difference was = 0.78 (indicating medium to large effects). An adequately powered follow-up study (sensitivity 0.8) testing developmental changes of FFA lateralization would therefore require the inclusion of 18 children and 26 adults.
Front Psychol, 2020; 11
32872536: Thome I, Lacle R, Voß A, Bortolussi G, Pantazis G, Schmidt A, Conrad C, Jacob R, Timmesfeld N, Bartsch JW, Pagenstecher A
Neoplastic Cells are the Major Source of MT-MMPs in -Mutant Glioma, Thus Enhancing Tumor-Cell Intrinsic Brain Infiltration.
Tumor-cell infiltration is a major obstacle to successful therapy for brain tumors. Membrane-type matrix metalloproteinases (MT-MMPs), a metzincin subfamily of six proteases, are important mediators of infiltration. The cellular source of MT-MMPs and their role in glioma biology, however, remain controversial. Thus, we comprehensively analyzed the expression of MT-MMPs in primary brain tumors. All MT-MMPs were differentially expressed in primary brain tumors. In diffuse gliomas, MT-MMP1, -3, and -4 were predominantly expressed by IDH1 tumor cells, while macrophages/microglia contributed significantly less to MT-MMP expression. For functional analyses, individual MT-MMPs were expressed in primary mouse p53 astrocytes. Invasion and migration potential of MT-MMP-transduced astrocytes was determined via scratch, matrigel invasion, and novel organotypic porcine spinal slice migration (OPoSSM) and invasion assays. Overall, MT-MMP-transduced astrocytes showed enhanced migration compared to controls. MMP14 was the strongest mediator of migration in scratch assays. However, in the OPoSSM assays, the glycosylphosphatidylinositol (GPI)-anchored MT-MMPs MMP17 and MMP25, not MMP14, mediated the highest infiltration rates of astrocytes. Our data unequivocally demonstrate for the first time that glioma cells, not microglia, are the predominant producers of MT-MMPs in glioma and can act as potent mediators of tumor-cell infiltration into CNS tissue. These proteases are therefore promising targets for therapeutic interventions.
Cancers (Basel), 2020; 12
30640899: Zimmermann KM, Stratil AS, Thome I, Sommer J, Jansen A
Illusory face detection in pure noise images: The role of interindividual variability in fMRI activation patterns.
Illusory face detection tasks can be used to study the neural correlates of top-down influences on face perception. In a typical functional magnetic resonance imaging (fMRI) study design, subjects are presented with pure noise images, but are told that half of the stimuli contain a face. The illusory face perception network is assessed by comparing blood oxygenation level dependent (BOLD) responses to images in which a face has been detected against BOLD activity related to images in which no face has been detected. In the present study, we highlight the existence of strong interindividual differences of BOLD activation patterns associated with illusory face perception. In the core system of face perception, 4 of 9 subjects had highly significant ($p<0.05$, corrected for multiple comparisons) activity in the bilateral occipital face area (OFA) and fusiform face area (FFA). In contrast, 5 of 9 subjects did not show any activity in these regions, even at statistical thresholds as liberal as $p = 0.05$, uncorrected. At the group level, this variability is reflected by non-significant activity in all regions of the core system. We argue that these differences might be related to individual differences in task execution: only some participants really detected faces in the noise images, while the other subjects simply responded in the desired way. This has several

implications for future studies on illusory face detection. First, future studies should not only analyze results at the group level, but also for single subjects. Second, subjects should be explicitly queried after the fMRI experiment about whether they really detected faces or not. Third, if possible, not only the overt response of the subject, but also additional parameters that might indicate the perception of a noise stimulus as face should be collected (e.g., behavioral classification images).
PLoS One, 2019; 14

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BOARD NUMBER: S04-079

ALPHA POWER IS COUPLED TO THE INFRA-SLOW GASTRIC RHYTHM IN DIFFERENT VISUAL TASKS

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Rhythmic bodily signals influence cortical activity as well as perception in animals and humans. In addition to influences from cardiac and respiration cycles, the infra-slow gastric rhythm (0.05 Hz) has recently been shown to be coupled to brain activity in humans. Alpha power in occipitoparietal areas and the insula, as well as BOLD activity in a large network cutting across classical resting-state networks, are coupled to the phase of the gastric rhythm. So far, the coupling has only been investigated during resting-state. Does gastric-brain coupling also occur when participants are engaged in a task? Here, we address this issue in two experiments where participants were either passively presented with different natural images or were performing a visual perceptual task at threshold. We measured the gastric basal rhythm using electrogastrography and brain activity with magnetoencephalography and analyzed gastric-alpha coupling during fixation. We find that alpha rhythm power was significantly coupled to the phase of the gastric rhythm in both experiments. Source analysis and parcellation into resting state networks revealed that coupling consistently occurred within the default and visual network, while differences between the experiments appeared for the somatomotor network. Our results show that alpha power is coupled to the gastric rhythm while participants are engaged in a more active behaviour, which opens the possibility that gastric-alpha coupling plays a functional role.

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BOARD NUMBER: S04-080

EFFORT AND ATTENTION MEASUREMENT IN NATURAL SPEECH PERCEPTION USING EEG AND PUPILLOMETRY

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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City College of New York, Biomedical Engineering, New York, United States of America

Aims: This study aims to demonstrate a novel paradigm for measuring speech intelligibility for subjects in adverse listening conditions. Additionally, we use a combination of EEG and pupillometry to measure the subject's attention and effort levels in various listening conditions. These are compared among conditions in order to show a contrast between effort and attention in naturalistic listening tasks. **Methods**: Subjects are asked to listen to narrative speech in a variety of noisy listening conditions while responding with button presses if certain target words are heard. This method allows for granular measurement of behavioral performance which is then compared with biometric signals. Inter-subject correlation (ISC) of brain signals and pupillometry provide objective measures of subject effort and attention. **Results**: We find that performance on the behavioral task is linked with ISC of both pupil and neural signals. Surprisingly, we find that most subjects showed overall pupillary contraction in more adverse listening conditions. Additionally, we find a pupillary dilation present when target words are detected that is mirrored in EEG responses, but not present when the subject does not recognize the target. **Conclusions**: We show a relationship between effort and attention during a naturalistic listening task using pupillometric and EEG signals. Both of these signals are predictive of subject performance on a word-identification listening task. We also demonstrate cases in various listening conditions where effort and attention are modulated differently from one another.

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BOARD NUMBER: S04-081

IS THERE A LINK BETWEEN RHYTHMIC AND ATTENTIONAL ABILITIES?

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Aims. Sensorimotor synchronization (SMS) is a natural healthy human skill and is particularly accurate and stable with auditory or audio-visual compared to visual rhythmic stimuli. However, inter-individual differences exist in rhythmic abilities, probably due to the intervention of cognitive functions such as attention. In the present study, we aimed to test the link between attentional capacities of participants and their rhythmic abilities measured by SMS to rhythmic auditory, visual and audio-visual stimuli. **Methods.** To date, sixteen right-handed participants (age 23,8 ± 2 years) performed a SMS task by tapping on a button with their right thumb in synchrony with stimuli delivered in 4 Modalities (Congruent Audio-Visual CAV, Auditory A, Visual V, Incongruent Audio-Visual IAV) presented in a pseudo-random order. Using circular analyses, we computed the mean angle (θ) and length (L) of the resultant vector for each Modality. We also collected the median reaction time and its standard deviation during auditory, visual and audio-visual attentional tasks. Correlations will be performed between each variable of the SMS and attentional tasks for each Modality. Significance level will be corrected for multiple comparisons. **Results.** We expect that SMS to rhythmic A, V and CAV stimuli will be respectively correlated with auditory, visual and audio-visual attentional variables. Results are being processed to verify these assumptions. **Conclusion.** Results will provide a better understanding of the modality-specific link between rhythmic and attentional abilities. Behavioral results will be completed by electroencephalographic analyses related to attentional level during SMS (alpha frequency band over parietal regions).

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BOARD NUMBER: S04-082

SYNCHRONIZATION OF PUPIL SIZE DURING AUDITORY NARRATIVES PREDICTS MEMORY

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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The pupil of the eye is said to be the window into the human mind. Yet a great number of factors influence the size of the pupil. The luminance changes in our field of view, sudden changes in our environment or cognitive effort during task solving among others. In past research we have found the pupil size to synchronize between people when they watch short audiovisual stimuli. However to which extent this is cognitive processing of the stimuli or simply the luminance fluctuations in the video that is causing this synchronization is largely unknown. In this study we show that pupil size synchronizes between people when they listen to auditory narratives. This intersubject correlation (ISC) is modulated by attention on a time scale of 5-10 seconds and it is predictive of memory performance across subjects. One might expect that the modulation by attention is due to a change in the brain-pupil coupling, however we don't find the coupling between EEG and pupils to depend on attentional state. We therefore investigate which features of the auditory narratives are predictive of the pupil size. Surprisingly we find pupil size is correlated with the envelope of the speech signal at slow time scales, and the strength of that correlation depends on whether subjects are paying attention. We show that the pupil is responsive to word onset and semantic novelty in auditory narratives. This work opens up the possibility to track attention to speech and its semantics just by looking at the eyes.

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BOARD NUMBER: S04-083

ANATOMICAL CHARACTERIZATION OF THE FRONTAL VOICE AREAS BASED ON THE INDIVIDUAL SULCAL ANATOMY

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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In communication, two aspects are very important to maintain a conversation: voice production and voice perception. In voice perception, vocal stimuli carry a wealth of information on the interlocutor: age, gender, identity, mood, etc. The existence of a specialized voice processing cortical network has been well established in humans using fMRI: in particular, the “Temporal Voice Areas” (TVAs, Belin et al., 2000; Pernet et al., 2015) are organized bilaterally in three clusters arranged along the anterior, middle and posterior parts of the superior temporal sulcus and gyrus (STS/STG). More recently, voice selective regions have also been found in the frontal lobe (Aglieri et al., 2018). A group analysis led to the characterization of three bilateral clusters in the frontal lobe: the anterior, middle and posterior "Frontal Voice Areas" (a, m and pFVAs). Here we perform an anatomo-functional characterization of the FVAs based on individual sulcal anatomy: voice-selective regions were identified as those showing a significantly greater activity for vocal versus non-vocal sounds. For each subject, functional peaks were identified in volume space, and then visualised on the cortical surface mesh, in order to characterize their individual anatomical locations, and to compare them across individuals. We found that FVAs at the individual level could be identified bilaterally in a large proportion of individuals, although with a variable number of peaks. Importantly, despite substantial variations in their cortical locations across participants, the closest sulci to the six FVAs remained largely consistent across individuals, revealing critical sulci-function links for FVAs localisations in the human brain.

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BOARD NUMBER: S04-084

FMRI ATTENTION-BASED NEUROFEEDBACK CORRELATES WITH SPECIFIC CHANGES IN ATTENTIONAL BEHAVIOURAL PERFORMANCE AND ATTENTIONAL BRAIN FUNCTIONS

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Neurofeedback aims at training targeted brain functions, by converting ongoing brain activity in selected brain regions into a sensory feedback to help subjects learn regulate their brain activity. Although neurofeedback is emerging as an important cognitive training tool offering a non-pharmacological solution to brain disorders, this effort has still not translated into clear advances in clinical brain-based attention training protocols. In this study, we deploy a functional magnetic resonance imaging (fMRI) real-time neurofeedback protocol to enhance covert spatial attention in healthy participants by directly showing them, on every trial, how informative was their brain signal in response to a cued instruction. We show that neurofeedback triggers participant dependent behavioral changes as well as specific changes in the spatial attentional cortical network. The reported behavioral effects are not specifically linked to the task used during the neurofeedback but also reflect onto a range of other attentional tasks, indicating a potential for generalization. Brain changes in the main task manifest as an enhanced fronto-parietal top-down attentional control associated with a functional reorganization of the occipital cortex. Mirroring this effect, we identify, after cognitive training, in resting state data, specific brain plasticity changes characterized by a perduring functional disconnection between the fronto-parietal and the visual network. Overall, these results indicate that neurofeedback re-organizes the balance between top-down and bottom-up attentional control and can thus become an efficient non-invasive tool to enhance spatial attention and mitigate specific attention deficits such as observed in Attention Deficit and Hyperactivity Disorder patients.

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BOARD NUMBER: S04-085

RESTING EEG THETA ACTIVITY AS AN ELECTROPHYSIOLOGICAL MARKER OF INDIVIDUAL DIFFERENCES IN EFFICIENCY OF TEMPORAL INFORMATION PROCESSING

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Abstract Temporal Information Processing (TIP) constitutes an essential component of human cognition because many cognitive functions like language, attention, memory, motor control, planning, etc. are characterized by the specific temporal dynamics. Because the *theta* band activity has been suggested to be involved in processes of cognitive control, we tested if it is also related to individual differences in TIP. The main goal of this study was to find electrophysiological markers of TIP efficiency in resting EEG. Thirty seven young healthy participants (M_{age} = 25 years) completed: (1) Temporal-Order Judgement task (TOJ) which measured the efficiency of TIP on the millisecond level, and (2) EEG resting state procedure. The Fast Fourier Transform was used for assessment of frontal and central *theta* spectral amplitudes from 2-s epochs. To analyse the relationship between resting *theta* activity and TOJ efficiency, Pearson's correlation analyses were performed. These analyses showed positive moderate significant correlations between the indices of TOJ and resting *theta* both frontal ($r = .345$, $p = .037$), and central activity ($r = .332$, $p = .039$). These results showed that more efficient millisecound timing is associated with lower both frontal and central *theta* power. This suggests that the *theta* rhythm may be a good electrophysiological marker of TIP efficiency. Such new knowledge may provide more insights into the dynamic flow of information in our brains, as well as to relationship '*time-cognition'*. Supported by National Science Centre, Poland, grant no. 2018/29/B/HS6/02038

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BOARD NUMBER: S04-086

IMPLICIT AND EXPLICIT TIMING – DO THEY SHARE A REPRESENTATION OF TIME?

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Human observers efficiently extract temporal regularities from sensory environments to form implicit temporal predictions. Also, time provides the structure to our conscious experience, for instance as the perceived duration between two events. Thus, time is implicit and essential for cognition, but also explicit, i.e. consciously represented. Here, we asked whether implicit and explicit timing rely on shared vs. separate mechanisms, using an auditory foreperiod paradigm in a magnetoencephalography experiment. Implicit temporal predictability was induced by a manipulation of the foreperiod. Participants received two consecutive task instructions: discriminate pitch (indirect measure of implicit timing) or duration (direct measure of explicit timing). The results show that the human brain uses the implicit temporal statistics of sensory environments, to enhance the behavioral and neural responses to auditory stimuli. Surprisingly however, temporal predictability did not improve explicit timing. In both tasks, attentional orienting in time during predictive foreperiods was indexed by an increase in alpha power over visual and parietal areas. Furthermore, pre-target induced beta power in sensorimotor and parietal areas increased during implicit compared to explicit timing, in line with the suggested role for beta oscillations in temporal prediction. Yet, no distinct neural dynamics emerged when participants explicitly paid attention to time. In conclusion, these results show that implicit timing automatically shapes behavior and sensory processing, and is reflected in oscillatory responses, but does not necessarily translate to explicit duration judgements.

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BOARD NUMBER: S04-087

DOES TEMPORAL PREDICTABILITY ENHANCE AUDITORY TEMPORAL RESOLUTION ? BEHAVIORAL RESULTS FROM A GAP DETECTION PARADIGM

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Perception takes advantage of the dynamic structure of our sensory environment. Temporal predictions of "when" an event will occur are built from temporal regularities, and used to orient attention in time. These temporal predictions improve motor responses to, and perceptual processing of, predicted inputs. Here, we studied whether the perceptual benefits from temporal predictions can be explained by an enhanced temporal resolution of the sensory system. Studies in the visual modality indeed showed that temporal resolution was improved by non-rhythmic temporal predictions, constructed from a predictive time interval separating two discrete events. To extend this finding to the auditory literature, we built a behavioral experiment where we coupled a gap detection task, assessing temporal resolution, and a foreperiod paradigm, inducing non-rhythmic temporal predictions. 26 healthy adult human participants were behaviorally tested on a 2-alternative-forced-choice version of the gap detection task. We measured performance for varying gap durations by means of psychometric curves. To induce temporal predictability, we kept the foreperiod intervals between the onset of the stimulus and the gap constant in half of the blocks. In the other blocks, the foreperiod intervals were randomly assigned. Our analyses showed that temporal predictions did not improve the performance in the gap detection task. However, response time improvements did indicate the use of temporal predictions, and suggested a possible decrease of decision times by temporal predictability. Hence, non-rhythmic temporal predictions did not benefit auditory temporal resolution as we operationalized it. Further research is needed to assess the generality of these findings.

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BOARD NUMBER: S04-088

SELECTIVE ATTENTION AND ADAPTATION IN AN AUDIO-VISUAL TEMPORAL ORDER JUDGMENT TASK

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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The encoding of event timing in the brain is crucial to build a coherent and unified representation of the external world. While audio-visual events can be perceived as 'one' coherent event, differences in the speeds of light and sound, local transduction and coding circuitry delays, and selective attention contribute to temporal delays that can be captured by asking participants to estimate the temporal order of sensory events. We know that the repeated exposures of a participant to a pair of desynchronized audio-visual stimuli shift individuals' simultaneity towards the exposed delay e.g. adaptation with a sound followed by a visual event will shift perceived simultaneity towards the sound. In this study, we asked whether such temporal recalibration is valid if the exposed delay is re-referenced to the individual's subjective simultaneity. Second, we tested whether selective attention impacted the direction of the shift. We conducted a behavioral experiment (N = 32) using temporal order judgment and lag-adaptation paradigms. Results did not replicate the literature and an additional experiment will be reported.

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BOARD NUMBER: S04-089

NEUROCOGNITIVE EVIDENCE OF ENHANCED IMPLICIT TEMPORAL PROCESSING IN VIDEO GAME PLAYERS

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Winning in action video games requires to predict timed events in order to react fast enough. In these games, the temporal structure of events is repetitive enough to develop implicit (automatic) preparation mechanisms. We compared action video game players (VGPs) and non-VGPs in a reaction time task involving both implicit time preparations and explicit (conscious) temporal attention cues. Participants were immersed in virtual reality and instructed to respond to a visual target appearing at variable delays after a warning signal (WS). In half of the trials, an explicit cue indicated when the target would occur after the WS. Behavioral, oculomotor and EEG data consistently indicate that, compared with NVGPs, VGPs better prepare in time using implicit mechanisms. This sheds light on the neglected role of implicit timing in VGPs and related electrophysiological mechanisms. The results further suggest that game-based interventions may help remediate timing alterations found in psychiatric populations.

FENS Forum 2022
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BOARD NUMBER: S04-090

PREFRONTAL THETA OSCILLATIONS SHAPE V4 GAMMA MODULATION AND INTERAREAL COHERENCE DURING SPATIAL ATTENTION

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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The Frontal Eye Field is considered a source of spatial attention signals to visual areas but little is known about how other prefrontal regions contribute to this process. To examine the role of ventrolateral prefrontal cortex (vlPFC) in spatial attention, we performed simultaneous extracellular recordings from vlPFC and V4 in two macaques engaged in a covert spatial attention task. Attention modulated spiking activity in both areas but attention effects emerged significantly earlier in vlPFC compared to V4. Within V4, LFP power and spike-LFP coherence were significantly enhanced by spatial attention in gamma frequencies (>30Hz) and they were reduced in low frequencies (4-30Hz). Within vlPFC, theta power and coherence (4-8Hz) were significantly enhanced with attention. Across the two areas, phase coupling was found both in the theta and gamma bands with attention. Results from spike-LFP coherence between V4 and vlPFC and a Granger causality analysis between LFPs indicated a vlPFC origin for theta interactions and a V4 origin for gamma interactions. Notably, attentional effects in theta and gamma V4-vlPFC coherence showed a significant positive correlation indicating a theta–gamma band interdependence and PFC theta phase modulated V4 gamma amplitude and V4-vlPFC gamma coherence. Effects in theta band coherence emerged significantly earlier compared to gamma band coherence. These results indicate that vlPFC theta provides a possible mechanism for the attentional modulation of gamma synchrony in V4. Work was co-financed by Greece and the European Union (European Regional Development Fund) through the Operational Programme “Competitiveness Entrepreneurship Innovation 2014-2020” (MIS 5070462).

Pubmed:

30154164: Sapountzis P, Paneri S, Gregoriou GG
Distinct roles of prefrontal and parietal areas in the encoding of attentional priority.
When searching for an object in a crowded scene, information about the similarity of stimuli to the target object is thought to be encoded in spatial priority maps, which are subsequently used to guide shifts of attention and gaze to likely targets. Two key cortical areas that have been described as holding priority maps are the frontal eye field (FEF) and the lateral intraparietal area (LIP). However, little is known about their distinct contributions in priority encoding. Here, we compared neuronal responses in FEF and LIP during free-viewing visual search. Although saccade selection signals emerged earlier in FEF, information about the target emerged at similar latencies in distinct populations within the two areas. Notably, however, effects in FEF were more pronounced. Moreover, LIP neurons encoded the similarity of stimuli to the target independent of saccade selection, whereas in FEF, encoding of target similarity was strongly modulated by saccade selection. Taken together, our findings suggest hierarchical processing of saccade selection signals and parallel processing of feature-based attention signals within the parietofrontal network with FEF having a more prominent role in priority encoding. Furthermore, they suggest discrete roles of FEF and LIP in the construction of priority maps.
Proc Natl Acad Sci U S A, 2018; 115
29033784: Paneri S, Gregoriou GG
Top-Down Control of Visual Attention by the Prefrontal Cortex. Functional Specialization and Long-Range Interactions.
The ability to select information that is relevant to current behavioral goals is the hallmark of voluntary attention and an essential part of our cognition. Attention tasks are a prime example to study at the neuronal level, how task related information can be selectively processed in the brain while irrelevant information is filtered out. Whereas, numerous studies have focused on elucidating the mechanisms of visual attention at the single neuron and population level in the visual cortices, considerably less work has been devoted to deciphering the distinct contribution of higher-order brain areas, which are known to be critical for the employment of attention. Among these areas, the prefrontal cortex (PFC) has long been considered a source of top-down signals that bias selection in early visual areas in favor of the attended features. Here, we review recent experimental data that support the role of PFC in attention. We examine the existing evidence for functional

specialization within PFC and we discuss how long-range interactions between PFC subregions and posterior visual areas may be implemented in the brain and contribute to the attentional modulation of different measures of neural activity in visual cortices.
Front Neurosci, 2017; 11
25712615: Gregoriou GG, Paneri S, Sapountzis P
Oscillatory synchrony as a mechanism of attentional processing.
The question of how the brain selects which stimuli in our visual field will be given priority to enter into perception, to guide our actions and to form our memories has been a matter of intense research in studies of visual attention. Work in humans and animal models has revealed an extended network of areas involved in the control and maintenance of attention. For many years, imaging studies in humans constituted the main source of a systems level approach, while electrophysiological recordings in non-human primates provided insight into the cellular mechanisms of visual attention. Recent technological advances and the development of sophisticated analytical tools have allowed us to bridge the gap between the two approaches and assess how neuronal ensembles across a distributed network of areas interact in visual attention tasks. A growing body of evidence suggests that oscillatory synchrony plays a crucial role in the selective communication of neuronal populations that encode the attended stimuli. Here, we discuss data from theoretical and electrophysiological studies, with more emphasis on findings from humans and non-human primates that point to the relevance of oscillatory activity and synchrony for attentional processing and behavior. These findings suggest that oscillatory synchrony in specific frequencies reflects the biophysical properties of specific cell types and local circuits and allows the brain to dynamically switch between different spatio-temporal patterns of activity to achieve flexible integration and selective routing of information along selected neuronal populations according to behavioral demands. This article is part of a Special Issue entitled SI: Prediction and Attention.
Brain Res, 2015; 1626

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BOARD NUMBER: S04-091

LATENCY PEAK OF EARLY VISUAL EVOKED POTENTIAL IS MODIFIED BY AGE AND NOVELTY DURING VISUAL MEMORY TASK

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

Marie Pieron, Elsie Moukarzel, Valentin Ritou, Cendra Agulhon
CNRS, Umr8002 Incc, Paris, France

Encoding of a sensory stimulus is believed to be the first step in perceptual learning. Detection of novel events is strongly related to memory, as it requires a comparison between incoming events and memory representations. In the mouse model, the plasticity dynamic measured by the visual evoked potential (VEP) latency modulation has not yet been studied during a visual memory task. To understand visual maturation, we recorded VEP in non-anaesthetized mice *in vivo* at two developmental stages during a visual memory task. We also examined if a novel stimulus has an impact on the speed of sensory processing, as indicated by latency modulations of early visual ERP components. N1 and N2 peak latencies are shorter for adult than for juvenile mice. The repetition of the familiar stimuli for 6 days led to a reduction of latency with the acquisition of the familiarity only for the juvenile group. Moreover, for juvenile mice, shorter peak latencies for N2 and P2 were observed in response to a novel *versus* a familiar stimulus but also an increase of latency variability. Our results indicate developmental differences in processing the acquisition of the familiarity of a visual stimulus. As age affects N1 and N2 latency values during the visual recognition memory task, they could be a potential measure of cortical maturation. In the juvenile group, the novelty effect on the latency of earlier peak (N2, P2) could result from a pre-attentional phenomenon. New stimuli capture better attention than a familiar one.

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BOARD NUMBER: S04-092

THE STRUCTURE OF NEURONAL INFORMATION MULTIPLEXING IN THE FRONTAL EYE FIELD ACCOUNTS FOR BOTH NEURONAL AND BEHAVIORAL VARIABILITY.

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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[1]UMR5229 Institut des sciences cognitives Marc Jeannerod, Neural Bases Of Spatial Cognition And Action, BRON CEDEX, France, [2]CNRS, Institut Des Sciences Cognitives, Bron Cedex, France

The frontal eye field (FEF) is a cortical area classically associated with spatial attention, perception and oculomotor functions. However, in any given task, these different functions do not account for all the observed neuronal variability. This “unaccounted for” neuronal variability can be considered as noise or associated to covert functions. Here, we show that the major part of FEF neuronal non-attention related variability is associated with specific task-and behavior-related sources that predict the structure of neuronal information multiplexing in the FEF. To do so, we trained two macaques to perform a visual attention task while recording FEF neuronal activity using multi-contact electrodes. As previously described, the neural population encoded the position of the attentional locus. Concurrently, FEF neurons encoded task-related (time in trial; CTOA) and behavior-related (reaction time, RT; focus of attention, TA) parameters prior to target onset. Using demixed principal component analysis, we characterized the functional relationship between the neural populations associated with each of these parameters and we investigated how this functional relationship predicts behavior. All parameters were encoded in the FEF by orthogonal components by pairs, except for CTOA and RT and CTOA and TA. This overlap correlated with the behavioral interaction between these two pairs of parameters. We thus present evidence that different sources of information can be extracted simultaneously from the FEF, and we describe how FEF neurons mix this information accounting for behavioral variability. This has major implications for the understanding of prefrontal cortex functional organization and for the real-time access to cognitive information.

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DISTINCT NEURONAL STATES ENCODE TASK IDENTITY IN FRONTAL EYE FIELD AND INTERACT WITH ITS CORE SPATIAL PROPERTIES

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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[1]Institut des Sciences Cognitive Marc Jeannerod, Neuroscience Cognitive, bron, France, [2]Otto-von-Guericke university Magdeburg, Sensorische Physiologie, Magdeburg, Germany, [3]Malardalen University, Division Of Intelligent Future Technologies, Västerås, Sweden, [4]Université de Tours, Umr 1253, Ibrain, Inserm, Tours, France, [5]UMR5229 Institut des sciences cognitives Marc Jeannerod, Neural Bases Of Spatial Cognition And Action, BRON CEDEX, France

Human cognition relies on the coordinated activity of multiple neuronal populations dynamically interacting together and shaping our behavior. Interestingly, single-neuron activity in the prefrontal cortex displays mixed selectivity, showing simultaneous tuning to different task-related processes. For example, prefrontal populations can jointly represent the position of a visual spatial information, spatial attention and working memory content. This feature is a hallmark of high-dimensionality, which provides the ability to encode different types of information simultaneously. In this context, the question we tackle is whether the prefrontal cortex population also represents task identity and how this impacts on its core specific functional computations. We trained two monkeys to perform three different task: a memory guided saccade task and two cued target detection tasks: peripherally cued (cue at expected target location, exogenous) and centrally cued (central cue, endogenous). During their performance, multi-unit activity (MUA) was recorded in both Frontal eye fields (FEF). Using demixed Principal Component Analysis, we found a two-dimensional neural states that fully characterized each of these tasks. This result indicates a task-related neural state in the recorded population. Furthermore, we observed that encoding of the spatial information was task-dependent. Such interaction between task and position coding indicates that task and spatial information are non-linearly mixed which is considered as a signature of a high-dimensional neuronal representation. Overall, this indicates that the FEF encodes ongoing task-identity in an identifiable neuronal dimension that interacts with its core spatial computations.

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BOARD NUMBER: S04-094

COVERT SPATIAL ATTENTION-BASED NEUROFEEDBACK CHANGES THE NEURONAL POPULATION CODE OF THE FRONTAL EYE FIELDS IN AN OSCILLATORY MANNER: A NON-HUMAN PRIMATE STUDY

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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[1]Institut des Sciences Cognitives Marc Jeannerod, Cnrs Umr 5229, Bron, France, [2]Institute of Biology, Otto-von-Guericke-Universitaet, Department Of Sensory Physiology, Magdeburg, Germany, [3]Malardalen University, Division Of Intelligent Future Technologies, Västerås, Sweden

A brain-computer interface (BCI) system is a synthetic interface with the brain allowing direct communication between the subject's neuronal activity and the external world. In the context of cognitive BCIs, the goal is to enhance or restore behavior through changes in the neural code. However, little is known about how cognitive BCIs impact the neural code. To improve attention, we thus conceived a closed-loop, invasive, adaptive, cognitive, and synchronous BCI system based on neurofeedback (NF). Two 24-contact electrodes were implanted in each Frontal Eye Field to record multiunit activity (MUA) from two macaques during a cued visuospatial attentional task. The position of the attentional spotlight (AS) was decoded in real-time. Its location relative to the expected target was indicated as auditory feedback. Subjects were rewarded for keeping decoded AS in the cued quadrant. To investigate the impact of NF on the spatial attention neuronal population code over time, cross-temporal decoding maps (CTDM) were computed either within or across pre-NF and post-NF neuronal data. When computing the CTDM using pre-NF and post-NF trials as training and test sets, after NF, the prefrontal population spatial code for attention changed dynamically during the cue-to-target interval in an oscillatory manner, but not within the pre-NF nor the post-NF CTDM. This indicates an alternation between a new code generated by the NF and the pre-existent pre-NF code, at two distinct non-harmonic frequencies: 4.5Hz and 7.6Hz (average estimates). These changes in neural code are correlated with complex rhythmic changes in behavioural performance.

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INFORMATION ROUTING BETWEEN CORTICAL LAYERS IN MACAQUE AREA V1 DEPENDS ON THE PHASE-RELATION BETWEEN THEIR GAMMA-OSCILLATIONS

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Local neuronal populations cannot process concurrently arriving information in parallel. Selective attention resolves this conflict, resulting in preferential processing of vital information. An explanatory approach for this preferential processing is "Routing-by-synchronization". Its driving concept is that receiving neurons should selectively synchronize their oscillatory activity with those subsets of inputs delivering the vital information. The synchronization allows sender neurons to provide information at the most effective period of the receiving neurons' oscillatory cycle. However, selective attention might also influence oscillatory activity on the input side to facilitate the synchronization with upstream neurons. To test this hypothesis, we investigate 1.) if information transmission depends on phase-specific synchronization and 2.) whether attention modulates this phase-specific synchronization. Therefore, we recorded with multi-contact-probes in area V1 of two monkeys (*Macaca mulatta*) performing a demanding shape-tracking task. The visual stimuli randomly and quickly changed their luminance. This task-irrelevant flicker allowed the ongoing estimation of stimulus-specific representation by calculating the spectral coherence between neuronal signals and the flicker signal. We found that the level of stimulus-specific information arriving in superficial layers depended on the phase difference between the oscillatory activity in granular- and supragranular layers. Furthermore, the phase-difference promoting most information to superficial V1 occurred more frequently when animals attended the respective stimulus. These results show: (1) that phase-relations between neurons of different layers have an optimal mode for routing information and (2) that dynamic adjustments of phase-relations between neurons located in different layers of V1 support preferential routing and processing of the behaviorally relevant information.

Pubmed:
30809117: Drebitz E, Schledde B, Kreiter AK, Wegener D
Optimizing the Yield of Multi-Unit Activity by Including the Entire Spiking Activity.
Neurophysiological data acquisition using multi-electrode arrays and/or (semi-) chronic recordings frequently has to deal with low signal-to-noise ratio (SNR) of neuronal responses and potential failure of detecting evoked responses within random background fluctuations. Conventional methods to extract action potentials (spikes) from background noise often apply thresholds to the recorded signal, usually allowing reliable detection of spikes when data exhibit a good SNR, but often failing when SNR is poor. We here investigate a threshold-independent, fast, and automated procedure for analysis of low SNR data, based on fullwave-rectification and low-pass filtering the signal as a measure of the entire spiking activity (ESA). We investigate the sensitivity and reliability of the ESA-signal for detecting evoked responses by applying an automated receptive field (RF) mapping procedure to semi-chronically recorded data from primary visual cortex (V1) of five macaque monkeys. For recording sites with low SNR, the usage of ESA improved the detection rate of RFs by a factor of 2.5 in comparison to MUA-based detection. For recording sites with medium and high SNR, ESA delivered 30% more RFs than MUA. This significantly higher yield of ESA-based RF-detection still hold true when using an iterative procedure for determining the optimal spike threshold for each MUA individually. Moreover, selectivity measures for ESA-based RFs were quite compatible with MUA-based RFs. Regarding RF size, ESA delivered larger RFs than thresholded MUA, but size difference was consistent over all SNR fractions. Regarding orientation selectivity, ESA delivered more sites with significant orientation-dependent responses but with somewhat lower orientation indexes than MUA. However, preferred orientations were similar for both signal types. The results suggest that ESA is a powerful signal for applications requiring automated, fast, and reliable response detection, as e.g., brain-computer interfaces and neuroprosthetics, due to its high sensitivity and its independence from user-dependent intervention. Because the full information of the spiking activity is preserved, ESA also constitutes a valuable alternative for offline analysis of data with limited SNR.
Front Neurosci, 2019; 13
30210309: Drebitz E, Haag M, Grothe I, Mandon S, Kreiter AK
Attention Configures Synchronization Within Local Neuronal Networks for Processing of the Behaviorally Relevant Stimulus.

The need for fast and dynamic processing of relevant information imposes high demands onto the flexibility and efficiency of the nervous system. A good example for such flexibility is the attention-dependent selection of relevant sensory information. Studies investigating attentional modulations of neuronal responses to simultaneously arriving input showed that neurons respond, as if only the attended stimulus would be present within their receptive fields (RF). However, attention also improves neuronal representation and behavioral performance, when only one stimulus is present. Thus, attention serves for selecting relevant input and changes the neuronal processing of signals representing selected stimuli, ultimately leading to a more efficient behavioral performance. Here, we tested the hypothesis that attention configures the strength of functional coupling between a local neuronal network's neurons specifically for effective processing of signals representing attended stimuli. This coupling is measured as the strength of γ-synchronization between these neurons. The hypothesis predicts that the pattern of synchronization in local networks should depend on which stimulus is attended. Furthermore, we expect this pattern to be similar for the attended stimulus presented alone or together with irrelevant stimuli in the RF. To test these predictions, we recorded spiking-activity and local field potentials (LFP) with closely spaced electrodes in area V4 of monkeys performing a demanding attention task. Our results show that the γ-band phase coherence (γ-PhC) between spiking-activity and the LFP, as well as the spiking-activity of two groups of neurons, strongly depended on which of the two stimuli in the RF was attended. The γ-PhC was almost identical for the attended stimulus presented either alone or together with a distractor. The functional relevance of dynamic γ-band synchronization is further supported by the observation of strongly degraded γ-PhC before behavioral errors, while firing rates were barely affected. These qualitatively different results point toward a failure of attention-dependent top-down mechanisms to correctly synchronize the local neuronal network in V4, even though this network receives the correctly selected input. These findings support the idea of a flexible, demand-dependent dynamic configuration of local neuronal networks, for performing different functions, even on the same sensory input.
Front Neural Circuits, 2018; 12

31837345: Drebitz E, Rausch LP, Kreiter AK
A novel approach for removing micro-stimulation artifacts and reconstruction of broad-band neuronal signals.
Electrical stimulation is a widely used method in the neurosciences with a variety of application fields. However, stimulation frequently induces large and long-lasting artifacts, which superimpose on the actual neuronal signal. Existing methods were developed for analyzing fast events such as spikes, but are not well suited for the restoration of LFP signals.
J Neurosci Methods, 2020; 332

21801439: Koliwer-Brandl H, Gbem TT, Waespy M, Reichert O, Mandel P, Drebitz E, Dietz F, Kelm S
Biochemical characterization of trans-sialidase TS1 variants from Trypanosoma congolense.
Animal African trypanosomiasis, sleeping sickness in humans and Nagana in cattle, is a resurgent disease in Africa caused by Trypanosoma parasites. Trans-sialidases expressed by trypanosomes play an important role in the infection cycle of insects and mammals. Whereas trans-sialidases of other trypanosomes like the American T. cruzi are well investigated, relatively little research has been done on these enzymes of T. congolense.
BMC Biochem, 2011; 12

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IMPACT OF TASK-IRRELEVANT AUDITORY INFORMATION ON A VISUAL RATE CATEGORIZATION TASK

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Successful discrimination of sensory information often depends on the ability to integrate inputs across sensory modalities. In the brain, recent evidence demonstrated that primary sensory regions, traditionally considered unisensory, participate in multisensory processing. For instance, mouse V1 neurons were shown to encode the temporal congruency of audiovisual stimuli. Yet, the perceptual role of multisensory interactions in the sensory cortex remains unexplored at the behavioral level. We thus designed an audiovisual task and trained rats to make a decision based on the temporal frequency (TF) of outward-moving circular gratings, paired with a fixed-amplitude sound. To explore the impact of auditory information on visual perception, we then introduced amplitude modulation to the sounds, delivering both temporally congruent and incongruent audiovisual stimuli. Although rats were not explicitly trained to judge the rate of the acoustic feature, the psychometric curves revealed higher sensitivity to the TF of the visual stimuli when paired with rate-changing sounds, compared to fixed-amplitude sounds. Surprisingly, this sharpening was independent of the temporal coherence of the visual and the auditory features. By contrast, rats reacted faster when visual stimuli were paired with fixed-amplitude compared to temporally-modulated sounds. Our data suggest that information about task-irrelevant but behaviorally-salient sounds are reflexively routed to early visual areas, where it spontaneously facilitates the detection of visual events. Our experimental approach can thus pave the way to the investigation of the functional impact of auditory inputs over visual cortical representations, mediated by well-established cortico-cortical connections.

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ROLE OF THE ANTERIOR AND POSTERIOR PARAVENTRICULAR NUCLEUS OF THE THALAMUS ON SIGN-TRACKING IN INBRED C57BL/6J AND DBA/2J MICE

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

Valeria Tarmati[1], Cristina Orsini[1], Simona Cabib[2]
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Aims: Sign-tracking (ST) behavior is a model of overwhelming attraction to rewards-associated stimuli due to incentive salience attribution as measured by the Pavlovian Conditioned Approach (PCA) paradigm. Previous studies suggested different strain-specific neurobiological substrates underlying ST behavior. This study was aimed at investigating the genotype-dependent networks mediating ST phenotype in C57BL/6J (C57) and DBA/2J (DBA) mice. **Methods**: In experiment 1 quantification of the c-fos protein was a marker of neural activation of strain-specific circuits during expression of ST behavior. After training, Paired (CS-food presentation) and Unpaired (random CS and food presentation) mice underwent a short exposure only to the lever-CS and then sacrificed for the immunohistochemistry. According to the results, in experiment 2 an excitotoxic lesion by NMDA infusion within the anterior and posterior Paraventricular Nucleus of the Thalamus (PVT) was performed before PCA training. **Results**: PVT was the only area expressing c-fos immunostaining in both strains though with opposite direction. Indeed, when compared to the respective Unpaired mice, c-fos expression was increased in C57 Paired and decreased in DBA Paired mice. Moreover, while the aPVT showed to be more involved in ST expression for DBA mice, any antero-posterior gradient was found relative to the activation for C57 mice. However, lesion of the aPVT selectively reduced ST behavior in DBA mice, while the pPVT lesion selectively reduced ST behavior in C57 mice. **Conclusions**: This strain-dependent functional role of the PVT and its anatomical division confirm the hypothesis that ST phenotype may be mediated by different networks in these strains.

Pubmed:
34940109: Cabib S, Campus P, Latagliata EC, Orsini C, Tarmati V
Repetitive and Inflexible Active Coping and Addiction-like Neuroplasticity in Stressed Mice of a Helplessness-Resistant Inbred Strain.
Dysfunctional coping styles are involved in the development, persistence, and relapse of psychiatric diseases. Passive coping with stress challenges (helplessness) is most commonly used in animal models of dysfunctional coping, although active coping strategies are associated with generalized anxiety disorder, social anxiety disorder, panic, and phobias as well as obsessive-compulsive and post-traumatic stress disorder. This paper analyzes the development of dysfunctional active coping strategies of mice of the helplessness-resistant DBA/2J (D2) inbred strain, submitted to temporary reduction in food availability in an uncontrollable and unavoidable condition. The results indicate that food-restricted D2 mice developed a stereotyped form of food anticipatory activity and dysfunctional reactive coping in novel aversive contexts and acquired inflexible and perseverant escape strategies in novel stressful situations. The evaluation of FosB/DeltaFosB immunostaining in different brain areas of food-restricted D2 mice revealed a pattern of expression typically associated with behavioral sensitization to addictive drugs and compulsivity. These results support the conclusion that an active coping style represents an endophenotype of mental disturbances characterized by perseverant and inflexible behavior.
Behav Sci (Basel), 2021; 11
33709985: Maiolati M, Tarmati V, Latagliata C, Cabib S, Orsini C
Opposite genotype-specific effects of serotoninergic treatments on Pavlovian Conditioned Approach in mice of two inbred strains C57 BL/6J and DBA/2J.
Individual variability in the response to pharmacological therapies is a major problem in the treatment of psychiatric disorders. Comparative studies of phenotypes expressed by mice of the C57BL/6J (C57) and DBA/2J (DBA) inbred strains can help identify neurobiological determinants of this variability at preclinical levels. We have recently demonstrated that whereas young adult mice of both strains develop sign-tracking in a Pavlovian Conditioned Approach (PCA), a trait associated with dysfunctional behavior in rat models, in full adult C57 mice acquisition of this phenotype is inhibited by pre-frontal cortical (PFC) serotonin (5HT) transmission. These findings suggest a different role of 5HT transmission on sign-tracking

development in mice of the two genotypes. In the present experiments, we tested the effects of the 5-HT synthesis booster 5-hydroxytryptophan (5-HTP) and of the selective 5HT reuptake inhibitor (SSRI) fluoxetine on the development and expression of sign-tracking in young adult mice from both inbred strains. In mice of the C57 strain, administration of 5-HTP before each training session blocked the training-induced shift to positive PCA scores which indicates the development of sign-tracking, whereas the same treatment was ineffective in mice of DBA strain. On the other hand, a single administration of fluoxetine was ineffective in unhandled saline- and 5-HTP-treated C57 mice, whereas it enhanced the expression of positive PCA scores by mice of DBA strain treated with 5-HTP during training. These findings confirm the strain-specific inhibitory role of PFC 5-HT transmission on sign-tracking development by mice of the C57 strain and support the hypothesis that different genotype-specific neurobiological substrates of dysfunctional phenotypes contribute to variable effects of pharmacotherapies.
Behav Pharmacol, 2021; 32

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TEMPORAL SURPRISE REDUCES AROUSAL AND THE CONTINGENT NEGATIVE VARIATION

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

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Université Catholique de Louvain, Institute Of Neuroscience (ions), Bruxelles, Belgium

Temporal expectation builds up before predicted sensory stimuli. However, events do not always occur at the predicted time, potentially creating temporal surprise. Psychological theory suggests that stimulus-bound surprise could alter subjective expectation and affect levels of arousal. First, we hypothesised that low temporal predictability could decrease arousal-related oculomotor responses. Secondly, we tested the influence of temporal surprise on the contingent negative variation (CNV) that reflects subjective expectation. Finally, we assumed that lowered temporal expectation could prolong the saccadic reaction times. Eye movements and EEG were recorded in 42 subjects performing a simple variable foreperiod task. A central target was briefly presented on a display followed by an eccentric one after a variable delay (or foreperiod). The task was to gaze at the central target and wait for the eccentric one to make a saccadic eye movement. In different blocks of trials, the variance of the FP distribution (μ = 1600 ms or 2400 ms) was either 5 ms or 120 ms. Pupil size and centrally located CNV preceding the visually-guided saccade were reduced in the high surprise condition. The maximum velocity of visually-guided saccades decreased with increasing temporal surprise but their latency was only minimally affected by stimulus predictability. Results suggest that temporal surprise decreased arousal and expectation. This effect could be mediated by a reduced CNV.

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BOARD NUMBER: S04-098a

RESEARCH OF THE READING FATIGUE AND THE SIGNIFICANCE OF THIS PARAMETER FOR ASSESSING THE COGNITIVE STATE OF A READER

POSTER SESSION 04 - SECTION: ATTENTION AND PERCEPTION

Victor Anisimov, Ksenia Babanova, Andrey Pikunov, Sergey Zuev, Alexandra Latyshkova, Konstantin Chernozatonskiy
Oken Technologies Inc, NY, United States of America

For many years fatigue has been a topic of scientific and applied research, especially in relation to evaluating a person's cognitive state in occupations settings such as factory work, client services, education and other areas where a work routine tends to lead to fatigue. Reading and education are also topics of great scientific interest in their broader sense. A person who is tired is hardly capable of perceiving new information; tiredness reduces cognitive abilities including attention, concentration and a capacity for work. A reader becomes distracted when fatigue develops.

According to the available literature, fatigue is associated with physiological parameters that reflect the human body's functional state. Eye movements are great indicators of mental state and cognitive processes that are represented in the brain at that moment. The eyes develop ontogenetically from telencephalon, and highly reflect the processes of human's exploration and search behavior. While these statements seem at first glance to be purely theoretical, they are nevertheless directly related to almost every psychological process, activity or mental state.

In this study the authors developed algorithms for estimating fatigue during reading based on eye tracking data collected from modern smartphone or tablet PC. The described method could prove useful for evaluating reading and comprehension effectiveness in order to make predictions about the state of a person's cognitive capabilities.

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BOARD NUMBER: S04-099

MODULATION OF MOTOR SEQUENCE LEARNING WITH TDCS AT 4MA

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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[1]City College of New York, Biomedical Engineering, New York, United States of America, [2]The Graduate Center, City University of New York, Advanced Science Research Center, New York, United States of America

In vivo human trials of transcranial direct current stimulation (tDCS) on motor learning have seen mixed results in the literature at the typical intensities of up to 2mA. We hypothesize that, provided sufficiently high field intensity is delivered to the motor cortex, tDCS can have a reliable behavioral effect on motor sequence learning. In a single-blind design, 72 healthy right-handed adult participants received anodal (N = 36) HD-tDCS while they performed a sequential finger tapping task (FTT) using their non-dominant hand for 12 minutes. A cathodal (N = 36) group served as an active control for sensation and an additional bllinded sham control group (N = 36) was used to establish baseline levels of learning. Functional magnetic resonance imaging (fMRI) of healthy participants (N = 10) was used in a current flow model to formulate a generalized 4+4 focal high-definition tDCS (HD-tDCS) electrode montage with 4 anterior and 4 parietal electrodes each drawing 1mA, targeting the hand knob on the contralateral primary motor cortex (M1). We find a significant gain in task performance in in anodal stimulation over cathodal stimulation (Cohen's d = 0.7), as well as over no stimulation. This effect persisted for at least one hour and carried over to the untrained hand as well as an untrained sequence. Sensation levels of 4mA tDCS were similar in both active groups and did not exceed moderate levels of discomfort. These results demonstrate that 4mA HD-tDCS distributed over a 4+4 montage yields a reliable boost of motor sequence learning and is well tolerated.

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OPTIMIZING THE MONTAGE FOR CEREBELLAR TRANSCRANIAL ALTERNATING CURRENT STIMULATION (TACS): A COMBINED COMPUTATIONAL AND EXPERIMENTAL STUDY

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Fatemeh Sadeghi Hassanabadi[1], Jonas Misselhorn[2], Simone Zittel-Dirks[1], Christian Gerloff[1]
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Objective. The application of cerebellar transcranial alternating current stimulation (tACS) is limited by the absence of commonly agreed montages and also the presence of unpleasant side effects. We aimed to find the most effective cerebellar tACS montage with minimum side effects (skin sensations and phosphenes). *Approach.* We first simulated cerebellar tACS with four montages (return electrode on forehead, buccinator, jaw, and neck positions) to compare induced cerebellar current, then stimulated healthy participants and evaluated side effects for each montage and different stimulation frequencies. *Main results.* The simulation revealed a descending order of current density in the cerebellum from forehead to buccinator, jaw, and neck respectively. Montages inducing higher current intensity in the eyeballs during the simulation resulted in stronger and broader phosphenes during tACS sessions. Strong co-stimulation of the brainstem was observed for the neck. Skin sensations did not differ between montages or frequencies. We propose the jaw montage as an optimal choice for maximizing cerebellar stimulation while minimizing unwanted side effects. *Significance.* These findings contribute to adopting a standard cerebellar tACS protocol. The combination of computational modelling and experimental data offers improved experimental control, safety, effectiveness, and reproducibility to all brain stimulation practices.

Pubmed:
33017272: Rostami R, Kazemi R, Mozaffarinejad F, Nasiri Z, Rostami M, L Hadipour A, Sadeghihassanabadi F
6 Hz transcranial alternating current stimulation of mPFC improves sustained attention and modulates alpha phase synchronization and power in dorsal attention network.
Transcranial alternating current stimulation (tACS) is a noninvasive brain stimulation tool appropriate to modulate cortical oscillations and activity via the application of weak currents. The major goal of this study was to investigate the effects of medial Prefrontal Cortex (mPFC) stimulation on sustained attention task performance measured by Rapid Visual Information Processing (RVIP) task and the brain networks assumed to be critical to sustained attention. mPFC has been shown to be involved in sustained attention performance and as a main hub in default mode network (DMN). mPFC activity modulation via theta tACS was implemented in this study. This was a single blind study with 21 participants receiving active and sham stimulation with the electrodes on FPz and the Inion. tACS was able to impact different RVIP measures (total hits, A' (sensitivity to target), total correct rejection, etc.). Relative power spectrum density (PSD) analysis yielded significant increases in theta frequency mostly in the fronto-central regions after active tACS and current source density (CSD) analysis yielded significant power modulations in theta frequency band in post-central gyrus. Furthermore, phase locking value (PLV) analysis showed that there were significant changes in cortical connections in the Dorsal Attention Network (DAN) in alpha frequency band. This study showed that theta frequency tACS over mPFC, was able to produce significant modulations in an RVIP task and its associated brain networks in healthy participants.
Cogn Neurosci, 2021; 12

FENS Forum 2022
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BOARD NUMBER: S04-101

TOWARDS STRETCHABLE HYBRID NEUROELECTRONIC IMPLANTS: UNIDIRECTIONAL AXONAL LONG-DISTANCE SIGNAL TRANSMISSION WITHIN PDMS GUIDANCE STRUCTURES

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Tobias Ruff[1], Léo Sifringer[1], Stephan Ihle[1], Simon Steffens[1], Tuğçe Delipinar[1], Anna Beltraminelli[1], Tao Zhang[1], Eylul Ceylan[1], Jens Duru[1], Sean Weaver[1], Sophie Girardin[1], Blandine Clément[1], Giulia Amos[1], Srinivas Madduri[2], Botond Roska[3]
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Current deep brain stimulation electrodes have a very limited spatial resolution which highly limits their application for targeted single neuron stimulation. To overcome this limitation, we propose a living biohybrid electrode in which we exploit real neurons as relays to transmit information from a stretchable microelectrode array (MEA[1]) to a neuronal target structure. To achieve this goal we are establishing a PDMS microstructure based *in vitro* co-culture system mounted on conventional glass MEAs in which we can seed embryonic rat retinal and thalamus spheroids at defined locations within the PDMS microstructure and guide the axons unidirectionally[2] across electrodes[3] through few µm sized channels towards the target. We optimize the design for reliable unidirectional multichannel stimulation of the target tissue. We show that retinal neurons grow several mm long axons within the PDMS guidance structures to innervate the thalamic target spheroids. We compare how different structural elements enhance unidirectional axon merging, growth speed, channel crosstalk and efficiency to stimulate thalamic target structures. *References* 1. 1 Renz, A. et al. Opto-E-Dura: A Soft, Stretchable ECoG Array for Multimodal, Multiscale Neuroscience. Adv. Healthc. Mater. 2000814, 1–11 (2020) 2. C. Forró, et al. Modular microstructure design to build neural structures with defined functional connectivity. Biosensors and Bioelectronics, 10.1016, 75-78 (2018) 3. Duru et al. Engineered biological neural networks on high density CMOS microelectrode arrays. Front. Neurosci., 2022.829884 (2022)

Pubmed:
28475893: Del Toro D, Ruff T, Cederfjäll E, Villalba A, Seyit-Bremer G, Borrell V, Klein R
Regulation of Cerebral Cortex Folding by Controlling Neuronal Migration via FLRT Adhesion Molecules.
The folding of the mammalian cerebral cortex into sulci and gyri is thought to be favored by the amplification of basal progenitor cells and their tangential migration. Here, we provide a molecular mechanism for the role of migration in this process by showing that changes in intercellular adhesion of migrating cortical neurons result in cortical folding. Mice with deletions of FLRT1 and FLRT3 adhesion molecules develop macroscopic sulci with preserved layered organization and radial glial morphology. Cortex folding in these mutants does not require progenitor cell amplification but is dependent on changes in neuron migration. Analyses and simulations suggest that sulcus formation in the absence of FLRT1/3 results from reduced intercellular adhesion, increased neuron migration, and clustering in the cortical plate. Notably, FLRT1/3 expression is low in the human cortex and in future sulcus areas of ferrets, suggesting that intercellular adhesion is a key regulator of cortical folding across species.
Cell, 2017; 169
25374360: Seiradake E, del Toro D, Nagel D, Cop F, Härtl R, Ruff T, Seyit-Bremer G, Harlos K, Border EC, Acker-Palmer A, Jones EY, Klein R
FLRT structure: balancing repulsion and cell adhesion in cortical and vascular development.
FLRTs are broadly expressed proteins with the unique property of acting as homophilic cell adhesion molecules and as heterophilic repulsive ligands of Unc5/Netrin receptors. How these functions direct cell behavior and the molecular mechanisms involved remain largely unclear. Here we use X-ray crystallography to reveal the distinct structural bases for FLRT-mediated cell adhesion and repulsion in neurons. We apply this knowledge to elucidate FLRT functions during cortical development. We show that FLRTs regulate both the radial migration of pyramidal neurons, as well as their tangential spread. Mechanistically, radial migration is controlled by repulsive FLRT2-Unc5D interactions, while spatial organization in the tangential axis involves adhesive FLRT-FLRT interactions. Further, we show that the fundamental mechanisms of FLRT adhesion and repulsion are conserved between neurons and vascular endothelial cells. Our results reveal FLRTs as powerful

guidance factors with structurally encoded repulsive and adhesive surfaces.
Neuron, 2014; 84
31928845: Del Toro D, Carrasquero-Ordaz MA, Chu A, Ruff T, Shahin M, Jackson VA, Chavent M, Berbeira-Santana M, Seyit-Bremer G, Brignani S, Kaufmann R, Lowe E, Klein R, Seiradake E
Structural Basis of Teneurin-Latrophilin Interaction in Repulsive Guidance of Migrating Neurons.
Teneurins are ancient metazoan cell adhesion receptors that control brain development and neuronal wiring in higher animals. The extracellular C terminus binds the adhesion GPCR Latrophilin, forming a trans-cellular complex with synaptogenic functions. However, Teneurins, Latrophilins, and FLRT proteins are also expressed during murine cortical cell migration at earlier developmental stages. Here, we present crystal structures of Teneurin-Latrophilin complexes that reveal how the lectin and olfactomedin domains of Latrophilin bind across a spiraling beta-barrel domain of Teneurin, the YD shell. We couple structure-based protein engineering to biophysical analysis, cell migration assays, and in utero electroporation experiments to probe the importance of the interaction in cortical neuron migration. We show that binding of Latrophilins to Teneurins and FLRTs directs the migration of neurons using a contact repulsion-dependent mechanism. The effect is observed with cell bodies and small neurites rather than their processes. The results exemplify how a structure-encoded synaptogenic protein complex is also used for repulsive cell guidance.
Cell, 2020; 180
33205564: Llerena Zambrano B, Renz AF, Ruff T, Lienemann S, Tybrandt K, Vörös J, Lee J
Soft Electronics Based on Stretchable and Conductive Nanocomposites for Biomedical Applications.
Research on the field of implantable electronic devices that can be directly applied in the body with various functionalities is increasingly intensifying due to its great potential for various therapeutic applications. While conventional implantable electronics generally include rigid and hard conductive materials, their surrounding biological objects are soft and dynamic. The mechanical mismatch between implanted devices and biological environments induces damages in the body especially for long-term applications. Stretchable electronics with outstanding mechanical compliance with biological objects effectively improve such limitations of existing rigid implantable electronics. In this article, the recent progress of implantable soft electronics based on various conductive nanocomposites is systematically described. In particular, representative fabrication approaches of conductive and stretchable nanocomposites for implantable soft electronics and various in vivo applications of implantable soft electronics are focused on. To conclude, challenges and perspectives of current implantable soft electronics that should be considered for further advances are discussed.
Adv Healthc Mater, 2021; 10
35032845: Ihle SJ, Girardin S, Felder T, Ruff T, Hengsteler J, Duru J, Weaver S, Forró C, Vörös J
An experimental paradigm to investigate stimulation dependent activity in topologically constrained neuronal networks.
We present a stimulate and record paradigm to examine the behavior of multiple neuronal networks with controlled topology in vitro. Our approach enabled us to electrically induce and record neuronal activity from 60 independent networks in parallel over multiple weeks. We investigated the network performance of neuronal networks of primary hippocampal neurons until 29 days in vitro. We introduced a systematic stimulate and record protocol during which well-defined 4-node neural networks were stimulated electrically 4 times per second (4Hz) and their response was recorded over many days. We found that the network response pattern to a stimulus remained fairly stable for at least 12 h. Moreover, continuous stimulation over multiple weeks did not cause a significant change in the stimulation-induced mean spiking frequency of a circuit. We investigated the effect of stimulation amplitude and stimulation timing on the detailed network response. Finally, we could show that our setup can apply complex stimulation protocols with 125 different stimulation patterns. We used these patterns to perform basic addition tasks with the network, revealing the highly non-linear nature of biological networks' responses to complex stimuli.
Biosens Bioelectron, 2022; 201
34955746: Ruff T, Peters C, Matsumoto A, Ihle SJ, Morales PA, Gaitanos L, Yonehara K, Del Toro D, Klein R
FLRT3 Marks Direction-Selective Retinal Ganglion Cells That Project to the Medial Terminal Nucleus.
The mammalian retina extracts a multitude of diverse features from the visual scene such as color, contrast, and direction of motion. These features are transmitted separately to the brain by more than 40 different retinal ganglion cell (RGC) subtypes. However, so far only a few genetic markers exist to fully characterize the different RGC subtypes. Here, we present a novel genetic Flrt3-CreERT2 knock-in mouse that labels a small subpopulation of RGCs. Using single-cell injection of fluorescent dyes in Flrt3 positive RGCs, we distinguished four morphological RGC subtypes. Anterograde tracings using a fluorescent Cre-dependent Adeno-associated virus (AAV) revealed that a subgroup of Flrt3 positive RGCs specifically project to the medial terminal nucleus (MTN), which is part of the accessory optic system (AOS) and is essential in driving reflex eye movements for retinal image stabilization. Functional characterization using patch-clamp recordings showed that the MTN-projecting Flrt3 RGCs preferentially respond to downward motion in an ON-fashion. These neurons distribute in a regular pattern and most of them are bistratified at the level of the ON and OFF bands of cholinergic starburst amacrine cells where they express the known ON-OFF direction-selective RGC marker CART. Together, our results indicate that MTN-projecting

Flrt3 RGCs represent a new functionally homogeneous AOS projecting direction-selective RGC subpopulation. Front Mol Neurosci, 2021; 14

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CLOSED-LOOP NEUROMODULATION OF SPINAL CIRCUITS FOR THE TREATMENT OF GAIT DEFICITS IN ANIMAL MODELS OF PARKINSON'S DISEASE.

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Elisa Lilly Garulli, Burçe Kabaoglu, Christoph Harms, Nikolaus Wenger
Charité – Universitätsmedizin Berlin, Experimental Neurology, Berlin, Germany

Around one-third of parkinsonian patients suffer from gait impairments. Given the poor response to dopaminergic medication and standard DBS therapies, this poses an unmet clinical need. Our project focuses on establishing a closed-loop, epidural electrical stimulation system to treat gait disorder in Parkinson's disease, specifically freezing of gait (FOG) episodes. Using a rat model of Parkinson's disease (6-OHDA unilateral injection in the Medial Forebrain Bundle), we are characterizing neural biomarkers of movement and pathological episodes. During motor tasks we record from the cortex using ECoG screws and from the Subthalamic Nucleus using bipolar electrodes. Simultaneously, we record kinematic data using a marker-based video capturing system.
We established analysis pipelines that allow us to explore the power spectrum at the time of voluntary initiation of movement, as well as during gait cycles. This sets the basis for the detection of neural biomarkers of involuntary, pathological movement interruptions.
Following a comprehensive characterization of the neural correlates of movement, we will close the loop by monitoring changes in supraspinal motor networks in real-time and adjusting the stimulation parameters accordingly.
Detecting pathological patterns will allow us to deliver timely epidural stimulation in the spinal cord, aimed at preventing FOG episodes, or, in the best-case scenario, avoid them altogether.

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BOARD NUMBER: S04-103

ELECTRICAL STIMULATION OF SPINAL CIRCUITS FOR GAIT RECOVERY IN PARKINSON'S DISEASE

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Burçe Kabaoglu, Elisa Lilly Garulli, Christoph Harms, Nikolaus Wenger
Charité – Universitätsmedizin Berlin, Experimental Nerurology, Berlin, Germany

Gait impairments in Parkinson's disease (PD) are a scientific and therapeutic challenge as freezing of gait (FOG) shows a limited response to dopamine replacement and standard STN-DBS therapies. Recently, epidural electrical stimulation (EES) of the spinal cord was proposed as an alternative treatment for gait impairments in PD. However, due to limited understanding of the underlying neurobiology of FOG, the efficacy of EES for gait recovery remains an open question. Animal models of PD offer us the opportunity to unravel the mechanisms of gait network disorders. To this extent, we will investigate the specificity of EES, then characterize the effect of stimulation parameters on pathological oscillations in the locomotor network; finally, we will test an EES paradigm to retune the pathological oscillations and improve the motor deficits that accompany them.
To achieve this healthy rats will be implanted with EES electrodes over the cervical, thoracic and lumbar segments of the spinal cord and the response will be recorded in the supraspinal motor network. Then, the neural activity will be recorded in synchrony with refined movement tracking, to identify biomarkers to predict FOG. Finally, we will combine the gathered knowledge to implant neural electrodes, EES implants, and electromyography probes in PD model rats to assess the effects of segment-specific EES on gait deficit amelioration and retuning of pathological network oscillations. Our approach will be benchmarked against dopamine replacement therapy.
This project will help evaluate the impact of segment-specific EES on network modulation, to treat motor deficits in animal models of PD.

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SINGLE PULSE ELECTRICAL STIMULATION EVOKED RESPONSES: A PIONEERING PRECLINICAL TOOL TO HIGHLIGHT NEW SPECIFIC EEG SIGNATURES

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Eloïse Gronlier[1,2], Julien Volle[2], Chloé Habermacher[2], Véronique Coizet[1], Venceslas Duveau[2], Olivier David[1,3]
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Aims Brain excitability can be defined as the ability of the cortex to produce a response following a brief direct stimulation. According to standard systems theory, the best way to infer the state of a system is to record and characterize its impulse response to a brief event, producing much more informative data than simply recording spontaneous brain activity. This unique technique allows to infer brain connectivity and dynamics which can be altered in several neurological disorders such as Parkinson's disease (PD). **Methods** During the first step of this project, we have been able to transfer a technology used in clinic, based on brain excitability, to the preclinical stage. This methodological approach was developed in freely moving rodents. A proof-of-concept study was realized in naive animals. In a second step, we applied this technique in an animal model of PD, the unilateral 6-OHDA lesioned rat, to identify a specific EEG signature. We focused our effort on PD because of its well-known altered connectivity within the cortico-basal ganglia-thalamo-cortical circuits. **Results** We successfully transferred this methodology to naïve rats and obtained highly reproducible responses with site-specificities. By comparing evoked responses between 6-OHDA and control groups, we identified a specific electroencephalographic signature. **Conclusions** In this study, we demonstrated that inferring brain excitability using electrical stimulation could be a relevant tool to discover new signatures in PD and could be extended to other pathologies involving excitability dysfunctions (e.g: Alzheimer, Multiple Sclerosis). Such unique methodological approach would play a key role in drug development process.

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BOARD NUMBER: S04-105

ELECTRIC-DIPOLE INTERACTIONS EXPLAIN THE EFFECTS OF ENDOGENOUS AND EXOGENOUS ELECTRIC FIELDS

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Joana Covelo[1], Jaume Colom Hernandez[1,2], Alain Destexhe[2], Maria V. Sanchez-Vives[1,3]
[1]Institut d'Investigacions Biomediques August Pi i Sunyer, Systems Neuroscience Group, Barcelona, Spain, [2]CNRS, Paris-Saclay University, Neuropsi, Saclay, France, [3]ICREA, Catalan Institution For Research And Advanced Studies, Barcelona, Spain

Cortical activity can be modulated by endogenous and exogenous electric fields (EF). In a previous study (Rebollo *et al.*, 2021), experimental and computational data suggested that endogenous EF-mediated effects are compatible with electric dipoles, which contribute to the synchronization of neighbouring cortical columns. Moreover, tDCS models have shown that the orientation of the current flow determines the effect of the intervention (Rawji *et al.*, 2019). In the current study, we investigated the rotation of exogenous EFs to assess the hypothesis that electric dipoles aligned with the cortical columns are responsible for the impact of EF direction on cortical modulation. In cortical slices expressing spontaneous slow oscillations (ca. 0.3 Hz), we applied constant exogenous EFs (-/+ 3 V/m) with different orientations (0º, 45º and 90º between the cortical surface and the DC electrodes). While DC fields orthogonal to the brain surface had a maximum modulatory effect, the effectivity decreased with the rotation of the EF, having a null effect when parallel to the cortical surface. These results were successfully reproduced in a computational model with interacting dipoles. The mean-field model suggested that the membrane potential shift created by an external EF is proportional to the cosine of the EF angle being applied and to the coefficient of ephaptic coupling. Therefore, we present experimental and computational data supporting the role of electric dipoles on the impact of both endogenous and exogenous electric fields in the cerebral cortex. Funded by CORTICOMOD PID2020-112947RB-I00 and MSCA-ITN-ETN H2020-860563.

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BOARD NUMBER: S04-106

WIRELESS CONTROL OF INSECT-MACHINE HYBRID

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Francisco Zurita, Lukas Hiendlmeier, Bernhard Wolfrum
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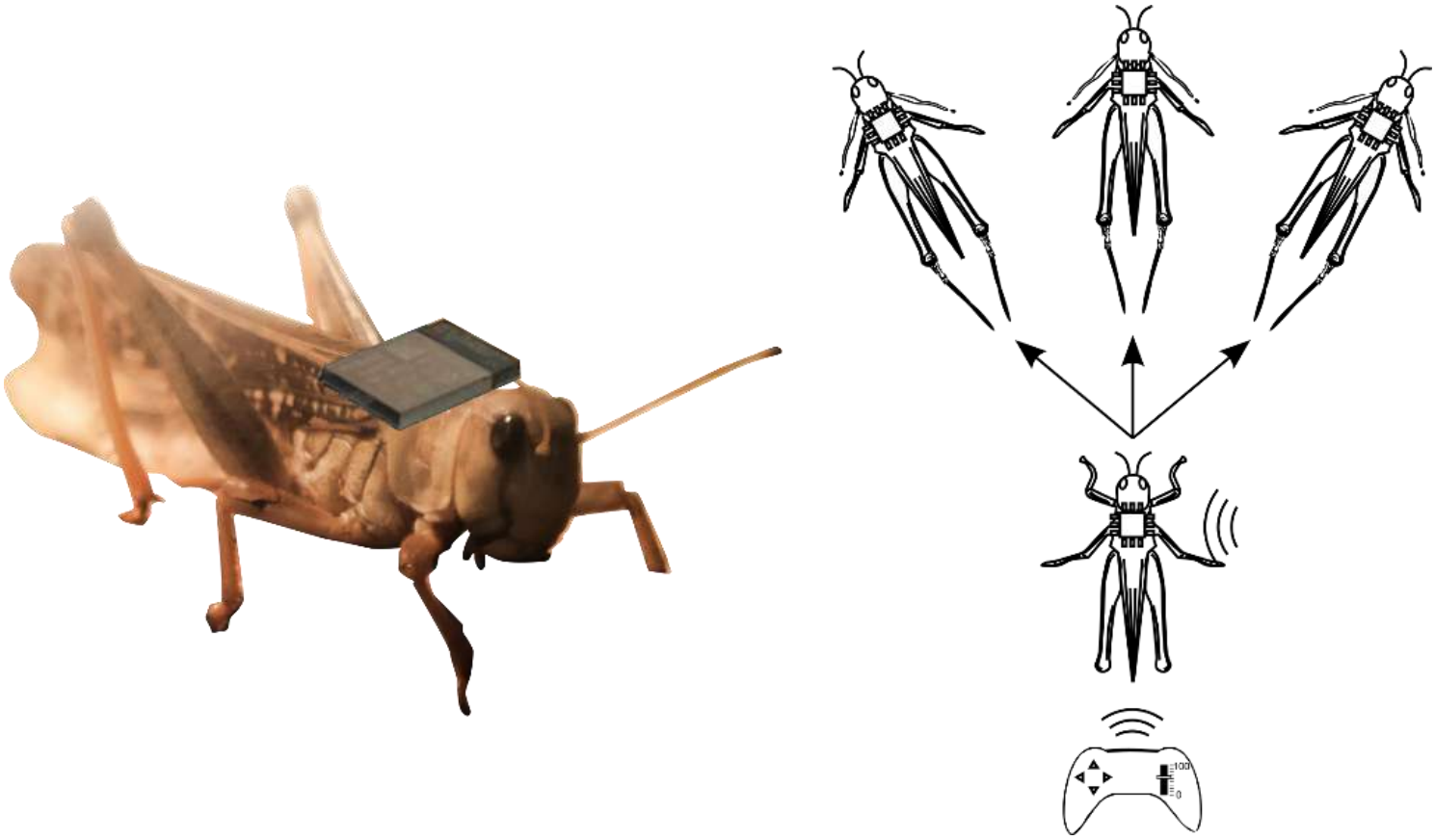

Insects are powerful animal models for neuroscience research. Normally, studies involving them are conducted in tissue preparations or fixed positions of the animal. However, more insight can be obtained from studies in freely moving animals. The aim of this work is to trigger the jumping mechanism of the locust artificially and wirelessly, to better understand the underlying neurophysiological mechanisms involved in an untethered subject. Both legs of the locust are interfaced at the muscle and motor nerves. Self-folding electrodes are implanted into the chest of the animal to interface with the extensor nerve fibers, and Ag wire is used to interface with the flexor muscle of the locust. Finally, these electrodes are connected to a microcontroller, which is set on the back of the insect. The microcontroller triggers the jumping, by stimulating nerve and muscles with frequency-modulated pulse trains. We successfully interfaced the extensor nerve and flexor muscle to elicit controlled extension and flexion movements of the legs after the surgery. Furthermore, we mounted the microcontroller on the back of the animal and observed that it does not prevent it from performing its regular activities. The work shows how to use electrical stimulation to modulate the jump of the locust in an untethered way. We expect to be able to modulate the length and direction of the jump with the stimulation signal's frequency and dephasing.

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BOARD NUMBER: S04-107

HIPPOCAMPAL SYNAPTIC PLASTICITY MEDIATES VAGUS NERVE STIMULATION INDUCED COGNITIVE ENHANCEMENT IN HEALTHY MALE RATS

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Laura Olsen[1], Raquel Moore[1], Naomi Bechmann[1], Victoria Ethridge[2], Nathan Gargas[2], Sylvia Cunningham[1], Zhanpeng Kuang[1], Joshua Whicker[1], Joyce Rohan[2], Candice Hatcher-Solis[1]
[1]Air Force Research Laboratory, Cognitive Neuroscience, Fairborn, United States of America, [2]Air Force Materiel Command, Naval Medical Research Unit, Fairborn, United States of America

Aims Vagus nerve stimulation (VNS) improves cognition in humans and rodents, but the underlying mechanisms of this enhancement are not understood. Behavioral performance and hippocampal (HC) electrophysiology/neurotrophin expression were measured in healthy adult rats after VNS paired training to investigate changes in cognition and synaptic plasticity. **Methods** All animal activities were approved under an IACUC protocol. Platinum/iridium electrodes were surgically implanted around the left VN of anesthetized male Sprague-Dawley rats (N=47). VNS (100 μs biphasic pulses, 30 Hz, 0.8 mA) paired Novel Object Recognition (NOR)/Passive Avoidance Task (PAT) were assessed 24 h after training and post-mortem tissue was collected 48 h after VNS (N=28). Electrophysiology recordings were collected using a microelectrode array system to assess function effects on HC slices 90 min after VNS (N=19). Sham received the same treatment without VNS and experimenters were blinded. **Results** Stimulated rats exhibited improved performance in NOR ($p<0.05$, n=12) and PAT ($p<0.05$, n=14). VNS enhanced long-term potentiation ($p<0.05$, n=7-12) and spike amplitude ($p<0.05$, n=7-12), while reducing paired pulse facilitation ($p<0.05$, n=7-12) in the CA1. Immunohistochemical analysis found increased brain derived neurotrophic factor expression in the CA1 ($p<0.05$, n=8-9) and CA2 ($p<0.01$, n=7-8). **Conclusions** These findings suggest that our VNS parameters promote synaptic plasticity and target the CA1, which may mediate the positive cognitive effects of VNS. This study significantly contributes to a better understanding of VNS mediated HC synaptic plasticity, which may improve clinical utilization of VNS for cognitive enhancement.

Pubmed:
32714585: Olsen LK, Dowd E, McKernan DP
A role for viral infections in Parkinson's etiology?
Despite over 200 years since its first description by James Parkinson, the cause(s) of most cases of Parkinson's disease (PD) are yet to be elucidated. The disparity between the current understanding of PD symptomology and pathology has led to numerous symptomatic therapies, but no strategy for prevention or disease cure. An association between certain viral infections and neurodegenerative diseases has been recognized, but largely ignored or dismissed as controversial, for decades. Recent epidemiological studies have renewed scientific interest in investigating microbial interactions with the central nervous system (CNS). This review examines past and current clinical findings and overviews the potential molecular implications of viruses in PD pathology.
Neuronal Signal, 2018; 2
31029796: Olsen LK, Cairns AG, Ådén J, Moriarty N, Cabre S, Alamilla VR, Almqvist F, Dowd E, McKernan DP
Viral mimetic priming enhances α-synuclein-induced degeneration: Implications for Parkinson's disease.
Evidence is accumulating to suggest that viral infections and consequent viral-mediated neuroinflammation may contribute to the etiology of idiopathic Parkinson's disease. Moreover, viruses have been shown to influence α-synuclein oligomerization as well as the autophagic clearance of abnormal intra-cellular proteins aggregations, both of which are key neuropathological events in Parkinson's disease pathogenesis. To further investigate the interaction between viral-mediated neuroinflammation and α-synuclein aggregation in the context of Parkinson's disease, this study sought to determine the impact of viral neuroinflammatory priming on α-synuclein aggregate-induced neuroinflammation and neurotoxicity in the rat nigrostriatal pathway. To do so, male Sprague-Dawley rats were intra-nigrally injected with a synthetic mimetic of viral dsRNA (poly I:C) followed two weeks later by a peptidomimetic small molecule which accelerates α-synuclein fibril formation (FN075). The impact of the viral priming on α-synuclein aggregation-induced neuroinflammation, neurodegeneration and motor dysfunction was assessed. We found that prior administration of the viral mimetic poly I:C significantly exacerbated or precipitated the α-synuclein aggregate induced neuropathological and behavioral effects. Specifically, sequential exposure to the two

challenges caused a significant increase in nigral microgliosis ($p < 0.001$) and astrocytosis ($p < 0.01$); precipitated a significant degeneration of the nigrostriatal cell bodies ($p < 0.05$); and precipitated a significant impairment in forelimb kinesis ($p < 0.01$) and sensorimotor integration ($p < 0.01$). The enhanced sensitivity of the nigrostriatal neurons to pathological α-synuclein aggregation after viral neuroinflammatory priming further suggests that viral infections may contribute to the etiology and pathogenesis of Parkinson's disease.
Brain Behav Immun, 2019; 80

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BOARD NUMBER: S04-108

THE NEURAL CIRCUIT DYNAMICS EVOKED BY TEMPORAL INTERFERENCE (TI) ELECTRICAL NEUROSTIMULATION IN VIVO

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Temporal interference (TI) is a novel electrical brain stimulation technique that can target deep brain structures noninvasively. It is based on the simultaneous application of two kHz electric fields, which generate a field with an amplitude-modulation (AM) oscillating at the difference frequency where the applied fields overlap. Previous work suggested that the TI stimulation area corresponds to the area of maximum AM. However, there has been no experimental evidence up to date to confirm this hypothesis. Herein, we set to examine for the first time the direct response of large-scale neural circuits to TI stimulation *in vivo*. We used widefield calcium imaging to track the activity of large cortical populations of Thy1-GCaMP6s mice undergoing TI and control (i.e., traditional AC) stimulation. We found that TI stimulation reliably evoked neuronal oscillations at the difference frequency of the applied fields. The strength of the evoked response was inversely proportional to the carrier frequency used. The locus of the oscillatory response was located between the stimulating electrodes, as was predicted by our FEM modelling of the TI fields. This is the first demonstration of the direct effects of TI on neural circuits *in vivo*, which is an essential step in uncovering the mechanism of action and facilitating the translation of the technique.

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NON-INVASIVE VAGUS NERVE STIMULATION DECREASES HEART RATE VARIABILITY IN A HUNGRY STATE

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Vagus nerve signals from the gut to brain are disrupted by consuming large amounts of high-calorie foods, neccessitating greater food intake to elicit a similar neural response. Non-invasive vagus nerve stimulation (nVNS) via a branch innervating the ear is a candidate treatment for obesity. We aimed to test the optimal ear position(s) for nVNS and explore the effects of nVNS during hungry and full states. In a within-subject design, each participant received nVNS in four different locations (cymba conchae, tragus, lobe, or tragus AND cymba conchae (CT)) on separate days. In each session, participants were asked to drink a chocolate flavored milk. rMSSD (measure of heart-rate variability) was measured for 15 minutes before and at least 35 minutes after consumption. We observed a significant effect of time ($F[1,13] = 8.157$, $p=.014$), such that rMSSD decreases after drink consumption. We also observed an interaction between location and time ($F[3,39] = 3.845$, $p=.017$). There was no main effect of location. The pre-drink effect of cymba nVNS was to lower rMSSD compared to CT (post-hoc $p_{corr}=0.03$), and unlike nVNS in the other locations, cymba does not decrease in rMSSD post drink consumption. Before drink consumption, rMSSD is lower with nVNS in cymba conchae, which remains similarly low after drink consumption. rMSSD decreases for all other conditions after drink consumption. These results suggest that nVNS in the cymba conchae in a hungry state has a similar acute effect on vagal tone as food consumption: to decrease heart rate variability. Funded by TÜBİTAK project no 118C299

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BOARD NUMBER: S04-110

NON-INVASIVE VAGUS NERVE STIMULATION REDUCES WANTING OF A PALATABLE CHOCOLATE DRINK

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Berçem Yar[1], Ilkim Buyukguduk[2], Zeynep Altınkaya[3], Dilan Koyuncu[3], Uğur Dal[3], Lina Öztürk[4], Hüseyin Yanık[5], Evren Değirmenci[5], Maria Veldhuizen[6]
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The vagus nerve plays an important role in the regulation of food intake. Non-invasive vagus nerve stimulation via the branch innervating the ear (nVNS) is considered as an alternative approach to treatment of obesity. The aim of this study was to determine the optimal ear position(s) for nVNS is the outer ear as well as to explore the effect of nVNS on hedonic and perceptual response to food. In a within-subject design (10 women, on average 29.4 +/- 6.7 years old), with 4 sessions per subject, each on separate days. In a session participants were asked to arrive fasted, then they received 15 minutes of stimulation (4 different locations: lobe (control), cymba conchae, tragus, or tragus and cymba conchae). Then they sample and rated a palatable chocolate drink (10% fat, 10% sugar, w/v). The drink was rated for liking, wanting ('how much do you want to consume this drink?'), intensity, sweetness, sourness, fattiness, creaminess, calorie-richness, healthiness, and familiarity on visual analog scales. Wanting after tVNS in cymba conchae is lower (main effect of location $F[3,33]=3.721$, $p=0.021$) compared to the other locations. There was a trend for liking to be decreased after tVNS in cymba conchae too (main effect of location $F(3,33)=2.689$, $p = 0.062$). Other ratings did not show an effect of stimulation location. We conclude that only nVNS in cymba conchae location affects motivation to consume foods, in line with recent reports of an increased drive to work for rewards in tVNS. Funded by TÜBİTAK project no 118C299

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BOARD NUMBER: S04-111

BIOPHYSICAL INVESTIGATION OF TEMPORAL INTERFERENCE STIMULATION MECHANISM

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Temporal Interference (TI) stimulation is a non-invasive deep brain stimulation technique. During TI, two electric fields are applied to the brain at kHz frequencies that differ in frequency by a small amount. The two fields overlap at the target brain location to drive neural activity at the difference frequency of the applied fields. The mechanism by which TI stimulation drives activity at the difference frequency, despite no (or minute) depolarisation at the applied kHz frequencies, is unknown. Herein we report the discovery that TI stimulation is mediated by a nonlinear frequency mixing phenomenon at the neural cell membrane. We show, using *ex-vivo* whole-cell patch-clamp recordings and extracellular stimulation, that subthreshold transmembrane depolarisation at two different frequencies reliably evokes polarisation at their difference frequency, across a broad range of frequencies, spanning endogenous to kHz frequencies. The frequency mixing product at the difference frequency is sustained under synaptic input block, but attenuated under voltage-gated ion channel block, indicating a cell membrane origin involving voltage-gated ion channels. The strength of the nonlinear frequency mixing process, and thus the strength of TI stimulation, is dependent on the applied frequency but not on the difference frequency. Overall, our work demonstrates how nonlinear biophysical properties of the cell membrane support novel electrical neurostimulation paradigms such as TI. Additionally, this insight into the mechanism of action, and parameter space, of TI may aid in the development and clinical adoption of the technique.

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BOARD NUMBER: S04-112

STIMULATION OF VAGAL AFFERENTS IN DIFFERENT EAR LOCATIONS DOES NOT INFLUENCE EFFERENT METABOLISM PRE- OR POST CONSUMPTION OF A PALATABLE DRINK

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Maria Veldhuizen[1], Ilkim Buyukguduk[2], Berçem Yar[3], Lina Öztürk[4], Zeynep Altınkaya[5], Dilan Koyuncu[5], Hüseyin Yanık[6], Evren Değirmenci[6], Uğur Dal[5]
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The vagus nerve forms a connection between nutrient-sensing cells in the gut and the brain. Stimulation of vagal cell bodies in the nodose ganglion is capable of conditioning flavor preferences and releasing dopamine in the dorsal striatum. This makes the vagus nerve an interesting stimulation target for modulating responses to food reward in humans. Non-invasive vagus nerve stimulation via the auricular branch in the ear has successfully been used to influence processes related to vagus nerve activity. There is disagreement on the optimal ear location for nVNS. The aim of this study was to compare the effect of nVNS in four different locations of the ear on efferent metabolism before and after consumption of a palatable chocolate drink (10% fat, 10% sugar, w/v). In a randomized cross-over design with 14 participants (10 women, on average 29.4 +/- 6.7 years old), we applied transcutaneous VNS in earlobe (control), cymba conchae, tragus and cymba concha + tragus and measured gastric frequency (GF) and diet-induced thermogenesis (DIT). We observed no differential effect of nVNS location on GF or DIT (p's >.182). We did observe an effect of time on DIT, such that DIT always increased post-drink consumption, regardless of ear location ($F[1,13] = 96.026$, $p<.001$). We conclude that efferent metabolism as reflected in GF and DIT is not sensitive to acute shifts in vagal tone pre- or post-consumption of a palatable drink. Funded by TÜBİTAK project no 118C299

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BOARD NUMBER: S04-113

INVASIVE AND NON-INVASIVE TEMPORARY INTERFERING ELECTRIC FIELDS STIMULATION (TIEF) OF THE RAT BRAIN: A C-FOS IMMUNOCYTOCHEMICAL QUANTITATIVE ANALYSIS

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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We reported here the effects of TIEF in the rat brain using both transcranial and epidural approaches for stimulation. For non-invasive stimulation, a 0.015 mm silver wires were placed inside two 0.2 mm polyamide tubes filled with saline solution and glued on the skull surface along with two 1 cm autoadhesive electrodes placed on the chest. Dorsal electrodes were paired with ventral ones and activated at 2000 Hz and 2010 Hz, with an intensity of 0.12 mA, in a 30-min single session. For epidural stimulation protocol, we implanted four 1 mm silver ball electrodes on the left surface of the skull fixed in specific stereotaxic coordinates. Paired electrodes were activated by 2000 Hz and 2005 Hz, 0.60 mA, in 30 min sessions in four consecutive days. In both experimental groups, 40 min after the end of the stimulation protocol, animals were perfused and processes for C-fos (activation maps) and GFAP (tissular damage) immunocytochemistry. Three serial sections per case at specific coordinates were analyzed densitometrical- and morphometrically after density thresholding segmentation (ImageJ). MATLAB full section maps performed after codification of the collected data from ImageJ analysis allowed to compare changes in number, size, and optical density distribution of immunoreactive neurons. These types of analysis allowed us to define the activated brain areas in the different experimental groups. Present results support the presence of cortical, instead of deep brain activation areas, by the two types of interfering fields stimulation protocols. Grant from Spanish Ministry of Science and Innovation PID2020-117266RB-C21

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REMOTE DEEP BRAIN STIMULATION BY TRANSGENE-FREE MAGNETOMECHANICAL APPROACH

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Deep brain stimulation (DBS) has been used to treat neurological diseases, especially related to movement disorders like Parkinson's disease, essential tremor and other diseases. However, the conventional DBS treatment is an invasive treatment method that requires electrodes implanted in the deep brain area. Although other minimally invasive treatment methods have been developed, including optical, acoustic, and electromagnetic for neuronal manipulation. Only the magnetic field can penetrate the brain without absorption or scattering. Wireless DBS with magnetogenetics were developed rapidly in the last decade. However, overexpression of exogenous ion channels and gene delivery with viral vectors might raise the concern of potential side effects. Here, we used a low-frequency and weak alternating magnetic field driving the magnetic nanodiscs to generate mechanical force for activating the transgene-free neurons. In our study, iron oxide magnetic nanodiscs were synthesized with diameter at ~270 nm. The torque of magnetic nanodiscs generated by applying alternative magnetic field in low frequency could repeatedly induce neuronal activity. Moreover, we revealed that the intrinsic mechanosensitive ion channel transient receptor potential canonical (TRPC), which were widely expressed in the brain, played crucial roles in this magnetomechanical stimulation. Finally, we injected the magnetic nanodiscs into the subthalamic nucleus and applied the magnetic stimulation in awake mice after recovery. The magnetomechanical force triggered neuronal activities in the target and downstream areas. Therefore, we developed a wireless DBS without transgene or hardware implantations by using magnetomechanical approach.

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THE EFFICACY OF NON-INVASIVE BRAIN STIMULATION TECHNIQUES ON CHRONIC PRIMARY PAIN DISORDERS: A SYSTEMATIC REVIEW AND META-ANALYSIS

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Background: Chronic Primary Pain (CPP) is characterized by an intense pain, not explained by lesions/damage to the nervous system, lasting for more than three months. Not all CPP patients benefit from standard pharmacological therapies nor are suitable for invasive neurosurgery treatments. Non-invasive brain stimulation (NIBS) uses cortical stimulation to reduce pain by directly altering brain activity, and its effects in treating CPP is still unclear. **Objectives:** We aimed at evaluating NIBS effects on CPP patients. **Methods:** A PRISMA systematic review was performed in PubMed, Embase, Scopus and Web of Science in May 2021. English-written, original studies measuring the effects of NIBS on pain perception were considered. The quality of studies was assessed with the Cochrane Risk of Bias and the Robins-I. The meta-analysis was conducted considering the pain intensity measured through visual or numerical rating scales as outcome of interest. **Results:** 134 studies were included, comprising 2444 patients and 13 different Primary Pain conditions. The meta-analysis was run on a preliminary cohort of 39 studies. When compared to sham effects, real NIBS induced a significant decrease of pain intensity (SMD −0.58 [CI: −0.83, −0.32];$p < 0.001$). **Conclusions:** Our findings showed a reduction of the perceived pain following real vs. sham stimulation, suggesting that NIBS can be used as an alternative or as a combined approach in CPP treatment.

Pubmed:
32696342: Consonni M, Telesca A, Dalla Bella E, Bersano E, Lauria G
Amyotrophic lateral sclerosis patients' and caregivers' distress and loneliness during COVID-19 lockdown.
J Neurol, 2021; 268
33205374: Consonni M, Telesca A, Grazzi L, Cazzato D, Lauria G
Life with chronic pain during COVID-19 lockdown: the case of patients with small fibre neuropathy and chronic migraine.
We aimed at investigating the impact of COVID-19-related distress on patients with chronic pain, highlighting the effects of changes in individual habits and public health care reconfiguration on physical and psychological health.
Neurol Sci, 2021; 42
33263428: Consonni M, Dalla Bella E, Bersano E, Telesca A, Lauria G
Cognitive reserve is associated with altered clinical expression in amyotrophic lateral sclerosis.
: Long-term life experiences, such as education, occupational attainment, leisure activities, and bilingualism, have been considered proxies of cognitive reserve (CR). In neurodegenerative disease, CR is considered as a modulator of a more favorable cognitive trajectory and motor functions. Our study investigated the role of CR on cognitive and motor involvement in a large cohort of incident patients with amyotrophic lateral sclerosis (ALS). : Cognition assessment and clinical and demographic information were obtained in 101 incident ALS patients. CR was measured based on years of education, occupational attainment, amount of leisure activities, and bilingualism. Correlation and regression analyses were performed to test the association between CR and the clinical expression of ALS. : We found that all proxies of CR were positively associated with executive functions, verbal fluency, and memory domains. Motor impairment was inversely related to educational level and occupational attainment. Regression analysis documented the association between CR and cognitive performances in all patients and the predictive role of CR in modulating motor functional disability in patients with bulbar-onset. : Our findings showed that CR mediates the extent of cognitive decline and that of functional bulbar impairment, suggesting that the concept of reserve applied to ALS should encompass cognitive and motor domains.
Amyotroph Lateral Scler Frontotemporal Degener, 2021; 22
34932161: Grazzi L, Telesca A, Rizzoli P
Management of chronic migraine with medication overuse by web-based behavioral program during the COVID-19 emergency: results at 12 months.
The study had been initiated because of restrictions put in place to control the spread of coronavirus in Milan in March 2020

that impacted clinical activities at our tertiary headache center in Milan (Foundation IRCSS Carlo Besta Neurological Institute). Treatment efforts were modified to make use of telephonic and internet communication to maintain care of our patients.

Neurol Sci, 2022; 43

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BRAIN DERIVED NEUROTROPHIC FACTOR NEGATIVELY RESPONDED TO TRANSCRANIAL DIRECT CURRENT STIMULATION: RANDOMIZED CONTROLLED TRAIL

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Background Brain-derived neurotrophic factor (BDNF) levels could objectively indicate synaptic plasticity, it has also been suggested that modulation of BDNF might be a part of mechanisms involved in transcranial direct current stimulation tDCS effects on synaptic connectivity. The aim of this study is to investigate associated change within BDNF level in response to tDCS in subacute stroke patients. The trial registration in the clinical trial ID is NCT04770363.

Methods 36 sub-acute ischemic stroke survivors participated, randomly assigned to one of three groups receiving tDCS, bihemispheric (Anodal over affected M1, and cathodal over healthy M1) or unihemispheric (Anodal over affected M1, and cathodal stimulation over supraorbital bone) or sham (No current). ActivaDose tDCS (USA) used, consisted of 20 minutes of 2 mA intensity; in each session for 12 sessions three sessions per week. A 3ml blood sample was withdrawn. First sample was withdrawn in first session and second sample by last session. BDNF were determined using commercially available ELISA kits.

Results there was a statistically significant difference (Negative change) within groups for bihemispheric (P-value = .011), and unihemispheric stimulation (P-value = .003). For sham group, no significant difference (P-value = .492). Significant difference between groups (P-value = .005). Post-hoc test by pairwise revealed both bihemispheric & unihemispheric stimulation significantly decreased BDNF levels more than sham (P = .001), (P = .021), respectively and with no significant difference between both experimental groups (P = .217).
Conclusion BDNF has showed significant decrease after tDCS in stroke patients, even motor measures positively improved.

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DOSE DEPENDENT EFFECTS OF TMS ON THE MODULATION OF FRONTO-STRIATAL CONNECTIVITY. A 18F-DMFP PET STUDY.

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Introduction: Transcranial Magnetic Stimulation (TMS) can modulate fronto-striatal connectivity in the human brain. Positron Emission Tomography (PET) and TMS were combined to investigate the fronto-striatal connectivity in the human brain. We employed ^{18}F-DesmethoxyFallypride (DMFP) - a high affinity Dopamine receptor-antagonist - to measure the release of endogenous dopamine in the striatum in response to repeated blocks of excitatory, intermittent theta burst stimulation (iTBS) of the Prefrontal Cortex (PFC). **Methods:** 23 healthy subjects participated in two PET sessions, each one with four blocks of iTBS separated by 30 minutes: *sham* (control) and *verum* (90% of individual resting motor threshold). Binding Potentials (BPs) were collected for sham and verum sessions across 28 time frames (about 130 minutes) in striatal regions (Nucleus Caudate and Putamen). **Results:** Verum iTBS increased the dopamine release in striatal regions, as compared to sham iTBS. Dopamine levels in the verum session increased progressively across the time frames within about 75 minutes (after three blocks of iTBS) and then eventually remained stable until the end of the session. **Conclusion:** Results suggest that the short iTBS protocol performed in repeated blocks can effectively induce a dose dependent increase in dopaminergic fronto-striatal connectivity. This scheme could present an alternative to painful, long stimulation protocols in experimental and theurapeutic settings. Specifically, it was demonstrated that three repeated iTBS, spaced by short intervals, achieve larger effects than one single stimulation. This finding has implications for the planning of therapeutic interventions, for example, treatment of major

depression.

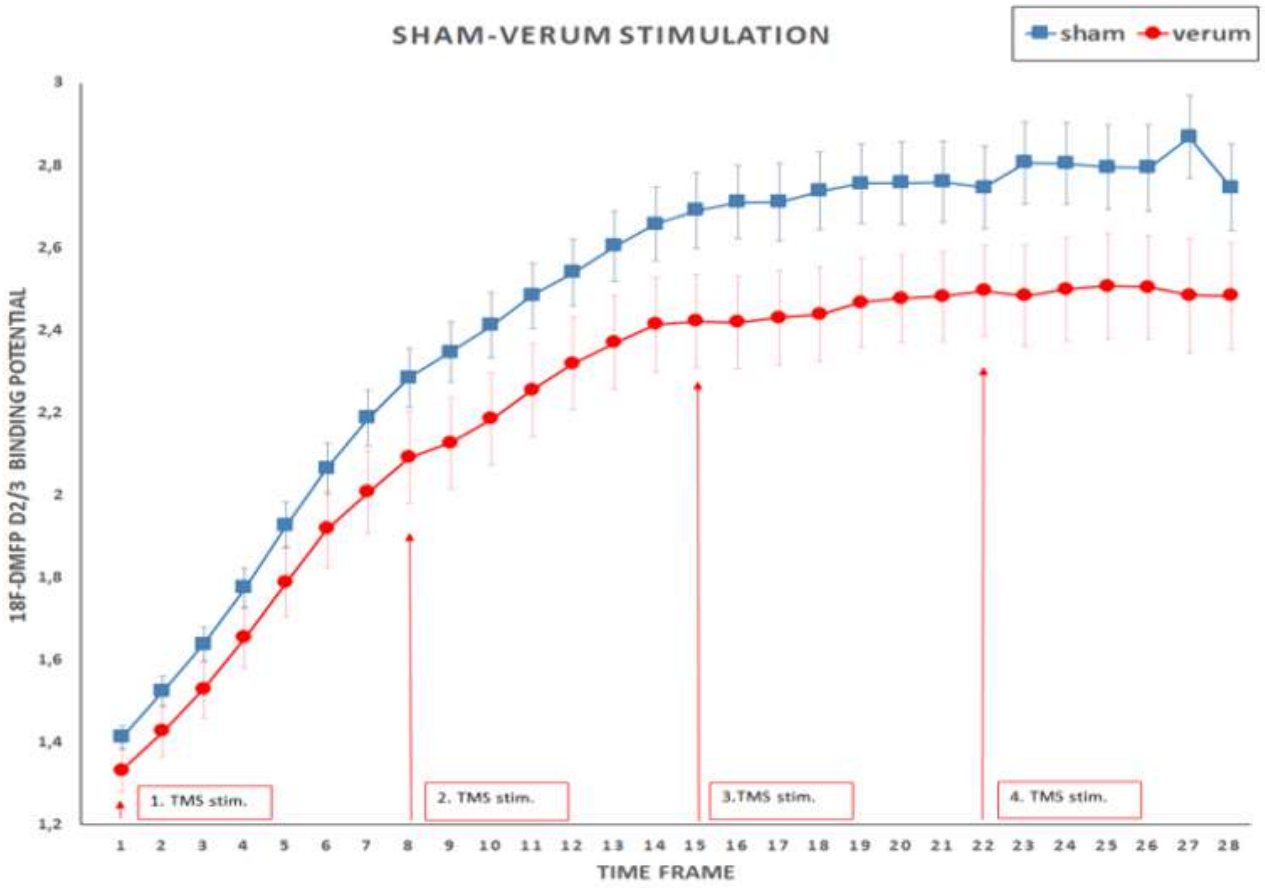

Figure 1: Mean Binding Potentials (BPs) at sham and verum TMSs across 28 time frames. We observed lower BPs in the verum stimulation compared to sham stimulation. Moreover, BPs in the verum condition showed a progressive decrease from time frame 1 to time frame 19 (about 75 mins) and then essentially stabilized until the end of the session, frame 28.

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REPEATED ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION (RA-TDCS) INCREASES HIPPOCAMPAL CELL PROLIFERATION IN YOUNG-ADULT MICE

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Repeated anodal transcranial direct current stimulation (RA-tDCS) is a neuromodulatory technique consisting in stimulating the cerebral cortex with a weak constant electric current in a non-invasive manner. RA-tDCS has antidepressant-like properties and improves memory both in human and laboratory animals. However, the mechanisms of action of RA-tDCS remain poorly understood. Since adult hippocampal neurogenesis is involved in the pathophysiology of depression and in cognitive processes, our aim was to study the impact of RA-tDCS on hippocampal neurogenesis levels in mice. RA-tDCS was applied 20 minutes per day for five consecutive days over the frontal cortex of 2-month-old and 10-month-old female mice (young-adults and adults, respectively). Then, animals received 3 intraperitoneal injections of bromodeoxyuridine (BrdU) and the brains were collected 1 or 21 days later. Cell proliferation, survival and differentiation were evaluated using simple or double staining immunohistochemistry. RA-tDCS increased cell proliferation specifically in young-adult animals, preferentially in the dorsal part of the dentate gyrus. Whatever the age, RA-tDCS had no significant effect on cell survival or differentiation. We conclude that the behavioural effects of RA-tDCS in mice previously described by our team (antidepressant and pro-cognitive properties) might be independent of an effect of RA-tDCS on neurogenesis levels in hippocampus.

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RETEST-RELIABILITY OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION OVER THE HEALTHY HUMAN MOTOR CORTEX: A SYSTEMATIC REVIEW

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Background: Repetitive transcranial magnetic stimulation (rTMS) is a standard tool to investigate neuronal plasticity. Yet, underlying mechanisms are not fully understood and heuristics about inhibitory and facilitatory cortical changes lack of evidence. Hence, identifying reliable protocols is necessary to derive validity of rTMS induced plastic changes. **Aims:** The present work systematically reviews studies on healthy subjects to extract and compare retest-reliability of rTMS effects, assessed by single-pulse TMS measures of the human primary motor cortex, i.e. motor evoked potentials of hand muscles. By structured methodological quality assessment, we wanted to ensure comparability of these experiments. **Methods:** Out of 742 database entries published until January 2022 in PubMed, fourteen articles were judged as eligible from two independent authors according to PRISMA guidelines. Inclusion criteria were English language and repeated application of rTMS on primary motor cortex of healthy subjects. Retest-reliability measures were compared. Two independent authors rated the quality of experiments with a standardized assessment tool and calculated sum scores. For inter-rater reliability we conducted Cohen's Kappa. **Results:** More recent protocols like paired associative stimulation (PAS) and theta burst stimulation (TBS) reveal moderate values of reliability whereas standard continuous rTMS procedures, for example 1 Hz, 10 Hz or 20 Hz, were less reliable. Methodological quality was similar and Cohen's Kappas strong to almost perfect. **Conclusions:** Some repetitive transcranial magnetic stimulation protocols are repeatable over time. Still, results are heterogeneous and depict existing inconsistencies across the field of TMS. Continuous improvements in quality of recent studies reveal promising outlooks to future research.

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MAGNETIC FIELD IN THE EXTREME LOW FREQUENCY BAND PROTECTS NEURONAL CELLS FROM OXYGEN-GLUCOSE DEPRIVATION IN-VITRO

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Aims Ischemic stroke consists of a rapid loss of cerebral function as consequence of the obstruction of a brain vessel, followed by subsequent damage to neighbouring tissue. Cerebral tissue quickly becomes irreversibly damaged in the vicinity of the closed vessel, but this tissue is potentially salvageable towards the periphery of the lesion during the initial phases after stroke, a region called the ischemic penumbra. This study is an in-vitro approach to understand the effect of Extreme Low Frequency Electromagnetic Stimulation (ELF-EMS) on oxygen-glucose deprivation (OGD) induced neuronal cell death in vitro. Methods Primary neuronal cultures derived from cerebral cortex of embryonic E18 pups were subjected to OGD in the presence of an inhibitor of glycolysis, iodoacetic acid (IAA 20 µM or 50 µM). After OGD, neurons were magnetically stimulated in a solenoid designed and characterized with SIM4LIFE (ZMT Zurich MedTech AG) to generate a homogenous magnetic field (MF) on the cell plate. Neuronal death was measured 24h after OGD using fluormetry and calcein-AM as the survival dye. Results We tested different protocols of magnetic stimulation (f=10-100 Hz and B=300 µT -10 mT). None of the protocols applied for 24 h induce any affect in neuronal survival under basal conditions (without OGD). Importantly, magnetic stimulation significantly reduced neuronal cell death secondary to OGD. Conclusions This preliminary study shows the potential use of MF in the ELF band for decreasing neuronal cell death after OGD and points to MF as a possible therapeutic strategy to reduce ischemic penumbra damage.

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NEURAL NONLINEARITIES REVEAL ONLINE EFFECTS OF TACS

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Despite the widespread use of transcranial alternating current stimulation (tACS) as a non-invasive brain stimulation technique, it remains challenging to study online neural effects of tACS due to stimulation artifacts. Here, we exploitneural nonlinearities to account for artifacts and reveal online neural effects of tACS. We first provide a linear model that predicts brain response to tACS. Then, we show that magnetoencephalography (MEG) measurements during tACS deviate from the predictions of this model. More specifically, we measured brain activity in human subjects either during 10 Hz visual flicker stimulation, during 10 Hz tACS, or during simultaneous visual stimulation and tACS. We found that brain activity during the simultaneous condition deviated from the linear combination of responses to theseparate stimulation conditions. Furthermore, this interaction depended on the relative phase of visual stimulation and tACS. Our work provides a new approach to study online neural effects of tACS, and adds novel evidence for aninteraction between tACS and ongoing neural activity.

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RELIABILITY OF MOTOR EVOKED POTENTIALS IN HAND MUSCLES OF HEALTHY INDIVIDUALS: A SYSTEMATIC REVIEW

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Aims: Motor evoked potentials elicited by transcranial magnetic stimulation over the primary motor cortex are used as a neurophysiological marker of cortical excitability in daily clinical and scientific practice. Tough, the reliability of this outcome parameter has not been clarified. Using a systematic approach, the present work reviews and critically appraises studies on the reliability of motor evoked potential amplitudes and input-output curves derived from hand muscles of healthy subjects. **Methods**: A systematic literature research was performed in PubMed, according to the PRISMA guidelines. Articles published up to July 2021 were included. Two authors conducted the search independently and rated the articles in terms of eligibility and methodical criteria with standardized instruments. Reliability parameters were extracted and summarized in a structured way. Included studies had to be written in English, conducted repeated measures on the hand muscles of healthy individuals and reported statistical reliability parameter. **Results**: Twenty-eight articles testing short- and long-term reliability fulfilled the inclusion requirements. Critical appraisal of the studies reveals methodological heterogeneity and partly contradictory results regarding the reliability of the outcome parameters. Reliability is influenced by the number of applied stimuli, muscle target, stimulation intensity, inter-pulse interval and waveform. **Conclusion**: The methodology of transcranial magnetic studies is still subject to heterogeneity, which could contribute to the partly contradictory results. According to the current knowledge status, the reliability of the outcome parameters can be increased by adjusting the experimental setup.

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CONTINUOUS THETA BURST STIMULATION DECREASES STRIATAL DOPAMINE RELEASE ACUTELY BUT NOT CHRONICALLY: AN IN VIVO AND POSTMORTEM STUDY

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Aim: To evaluate the immediate and chronic effects of theta burst stimulation (TBS) on the dopaminergic system. **Method:** Using healthy non-human primates and positron emission tomography (PET), we scanned animals with [^{11}C]raclopride (a $D_{2/3}$ receptor antagonist) before and immediately after the first session and 24hrs after a 3-week course of daily TBS delivery to the awake animals. Subjects received either sham, continuous (cTBS) or intermittent TBS (iTBS). The animals were then euthanized, and immunofluorescence staining was carried out with antibodies targeting the D_2 receptor to further evaluate chronic TBS effects. **Results:** CTBS (an inhibitory form of rTMS) over the left primary motor cortex acutely decreased dopamine release bilaterally in the putamen as shown by an increase in [^{11}C]raclopride binding. No significant changes in tracer binding were noted after chronic stimulation. ITBS (an excitatory form of rTMS) produced mixed results (both increase and decrease in tracer binding) leading to non-significant effects on dopamine release, acutely or chronically. Similarly, sham stimulation produced no effects. Post-mortem evaluation showed no significant difference in D_2 receptor immunoreactivity between active TBS and sham stimulation. **Conclusions:** In healthy brains, acute cTBS had a more consistent effect on DA release than iTBS but both seemed to induce a transient effect with no long-term changes in D_2 receptors as measured in vivo and post-mortem. Taken together this suggests that the DA system may not be directly responsible for long-term TBS clinical therapeutic efficacy or that compensatory homeostatic responses in a healthy brain obscures the possible effects in disease conditions.

Pubmed:
34622422: Aceves-Serrano L, Sossi V, Doudet DJ
Comparison of Invasive and Non-invasive Estimation of [C]PBR28 Binding in Non-human Primates.
To identify a reliable alternative to the full blood [C]PBR28 quantification method that would be easily replicated in multiple research and clinical settings.
Mol Imaging Biol, 2022; 24

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BOARD NUMBER: S04-124

EFFECTIVENESS OF INTERMITTENT THETA BURST STIMULATION OVER THE MEDIAL PREFRONTAL CORTEX COMBINED WITH ATTENTION MODIFICATION TRAINING ON EMOTION REGULATION

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Literature supports the critical role of medial prefrontal cortex (mPFC) in facilitating and managing emotions. Aim of the current study was the development of a treatment program to enhance emotion regulation. The treatment included intermittent Theta Burst Stimulation (iTBS) over the mPFC combined with Attention Modification Training (AMT). Eighty-seven participants (Mage = 21.02, SD = 1.89, males 50,6%) were recruited from a community sample in Cyprus. The duration of the treatment was 8 sessions and participants were randomized in three groups: iTBS - AMT, iTBS - control AMT, and sham iTBS - AMT. Prefrontal hemoglobin activation during emotional scripts (fear, sad and happy) was recorded prior and after the treatment using functional near-infrared spectroscopy. Repeated measures ANOVA was conducted for the average hemoglobin concentration in mPFC to examine the effectiveness of the treatment. As expected higher mPFC hemoglobin activation was observed during sad scripts and results shown a significant interaction between the types of emotional stimuli and the treatment. The two groups who received iTBS – AMT and sham iTBS - AMT shown decreased mPFC hemoglobin activation after treatment in all emotions. The group that received iTBS - control AMT shown increased mPFC hemoglobin activation in sad and happy scripts, but the opposite was found for fearful scripts. The goal of AMT was to direct attention to empathetic cues and current findings suggest that by stimulating prefrontal areas associated with emotion regulation, iTBS improved the effectiveness of AMT compared to sham stimulation on reducing mPFC hemoglobin activation.

Pubmed:
27176499: Kyranides MN, Fanti KA, Sikki M, Patrick CJ
Triarchic dimensions of psychopathy in young adulthood: Associations with clinical and physiological measures after accounting for adolescent psychopathic traits.
This study examined associations of psychopathy facets of boldness, meanness, and disinhibition with clinically relevant variables and physiological reactivity to affective stimuli. These associations were examined after accounting for developmental associations with adolescent psychopathic traits, namely callous-unemotional traits, narcissism, and impulsivity. Psychopathic traits were assessed during adolescence using the Antisocial Process Screening Device and the Inventory of Callous Unemotional traits and during young adulthood via the Triarchic Psychopathy Measure. Clinical variables (N = 99, Mage = 15.91, 53% female), as well as affective and physiological responses (heart rate, skin conductance, startle modulation) to violent and erotic videos (N = 88, Mage = 19.92, 50% female) were also assessed during adulthood. After accounting for adolescent psychopathic traits, boldness was associated with high cognitive reappraisal and low anxiety, fear, and hostility, and meanness was related to callous-unemotional traits, hostility, less sympathy to victims, and less use of cognitive reappraisal. Disinhibition, by contrast, was associated with impulsivity, increased anxiety, and hostile and aggressive tendencies, as well as conduct disorder, antisocial personality disorder symptoms, and cognitive suppression. In addition, evidence was found for different physiological measures operating as biological indicators of these distinctive dimensions, with reduced resting heart rate and cardiac reactivity to violent stimuli indicative of boldness, above and beyond adolescent psychopathic traits, and low startle potentiation for violent stimuli indicative of callous-unemotional traits and meanness. These findings provide evidence for the value of a multidomain approach for clarifying neurobiological mechanisms of psychopathic tendencies that can inform prevention and treatment efforts. (PsycINFO Database Record Personal Disord, 2017; 8
27046166: Fanti KA, Colins OF, Andershed H, Sikki M
Stability and change in callous-unemotional traits: Longitudinal associations with potential individual and contextual risk and protective factors.
This longitudinal study examines developmental heterogeneity in callous-unemotional (CU) traits in a large sample of school-age children in Cyprus. Latent Class Growth Analysis revealed 4 trajectory groups of CU traits across 3 time points: stable

high, increasing, decreasing, and low. Findings suggested that children in the stable high CU trajectory were more likely to (a) exhibit high and stable levels of conduct problems, attention-deficit/hyperactivity disorder symptoms, impulsivity and narcissism, (b) experience low parental involvement and high parental distress, (c) report low peer support and school connectedness, and (d) score lower on academic performance, executive functioning, social competence, and self-regulation compared to children with low, decreasing, and increasing CU traits. These findings were verified by both parent and child reports. Repeated analysis of variance suggested that increases and decreases in CU traits were associated with similar changes in conduct problems, narcissism, impulsivity, and maternal involvement. Further, children in the decreasing trajectory group were not differentiated from children in the low risk group on measures of executive functioning, academic performance, school connectedness, and peer social support at the last wave of measurement. These findings provide evidence for the importance of taking longitudinal change into account for understanding developmental heterogeneity in CU traits and the association of these traits with possible protective (e.g., stable high maternal involvement) and risk (e.g., decreases in maternal involvement and increases in conduct problems, impulsivity and narcissism) variables. (PsycINFO Database Record
Am J Orthopsychiatry, 2017; 87

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BOARD NUMBER: S04-125

THE EFFECTS OF PREFRONTAL VS PARIETAL CORTEX TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS) ON INHIBITION AND MEASURES OF SELF-ESTEEM

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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We examined the effects of transcranial direct current stimulation (tDCS), dorsolateral prefrontal cortex (DLPFC), and inferior parietal lobule (IPL) on inhibition and subjective psychological aspects like general body image and self-esteem state and trait. tDCS was administered at 1.5 mA for five days, 20 min each day (two rounds of 10 min divided by a 10 min break during which subjects engaged in mindfulness task) to DLPFC, IPL, or as SHAM stimulation. To decompose immediate and cumulative effects, we measured inhibition through the Go/no-go task together with self-esteem trait and state and body appreciation through a battery of questionnaires on days 1 and 5. We found that false alarm errors decreased in the DLPFC group, increased in IPL, and remained the same in the SHAM group. Correct hits and reaction time to Go signals were not altered. Only at day 5, there was a trend for DLPFC stimulation to increase the false alarms between pre and post-stimulation, possibly mirroring immediate and cumulative effects. Self-esteem and body perception were not affected by stimulation. Instead, there was a significant improvement in self-esteem trait over time in all groups, possibly due to the mindfulness exercise. These preliminary results indicate that tDCS modulates inhibition in fronto-parietal areas with opposite effects, enhancing it in DLPFC and impairing it in IPL. Judgments of self and body seem to be not affected by neuromodulation but may rely on broader regions as more complex constructs.

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BOARD NUMBER: S04-126

ENHANCING STANDARD ADDICTION TREATMENT PROTOCOLS EFFICACY THROUGH NON-INVASIVE BRAIN STIMULATION: A TDCS STUDY

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Lilia Del Mauro, Albert Matoshi, Susana Vicente, Nicoletta Piccitto, Andrea Silva, Laura Cappelletti, Pietro Farneti, Leonor Romero Lauro
University of Milano-Bicocca, Psychology, Milan, Italy

Aims: Substance abuse stands as a major health concern, severely affecting the well-being of addicted individuals. However, a gold standard treatment able to successfully reduce the high rate of relapses typically associated with this pathology has not been identified yet. Neuroimaging investigations highlighted diffuse brain alterations involving the dorsolateral prefrontal cortex (DLPFC). In this randomized sham-controlled study we tested whether transcranial direct current stimulation (tDCS) applied over the DLPFC could improve the recovery of addiction. Methods: 60 patients enrolled in a residential (n=39) or outpatient (n=21) intensive rehab program received either 6 active (n=34) bilateral (2 mA for 20 min) or sham (n=26) tDCS over the DLPFC (anodal right/cathodal left) over a period of two weeks. During stimulation drugs or alcohol related cues were presented to increase the desire of consumption. Measures of craving, depression, impulsiveness, general psychopathology and quality of life were collected before and after the treatment. A 3- and 6-months follow-ups were also included. Results: For each measure of outcome a mixed model was run, showing significant improvements in craving scores, impulsiveness, depression, psychopathology and overall perception of quality of life over time. No statistically significant differences between active and sham stimulation emerged. Conclusions: Our results, although still preliminary, suggest the feasibility of successfully applying tDCS within clinical settings to boost standard addiction rehabilitation protocols. Once collected the entire sample and follow-ups, this study will provide powerful insight on the therapeutic role of tDCS in the recovery of addiction.

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EFFECTS OF TRANSCRANIAL PULSE STIMULATION (TPS) ON YOUNG ADULTS WITH SYMPTOMS OF DEPRESSION – A PILOT RANDOMIZED CONTROLLED TRIAL

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Teris Cheung[1], Janice Ho[1], Calvin Cheng[2], Jerry Wing-Fai Yeung[1], Georg S Kranz[3], Kenneth Fong[3], Roland Beisteiner[4], Sau Fong Leung[1]
[1]the Hong Kong Polytechnic University, Hong Kong SAR China, School Of Nursing, Hung Hom, Hong Kong PRC, [2]University of Hong Kong, Department Of Psychiatry, Pok Ful Lam, Hong Kong PRC, [3]The Hong Kong Polytechnic University, Department Of Rehabilitation Sciences, Hung Hom, Hong Kong PRC, [4]Medical University of Vienna, Department Of Neurology, Vienna, Austria

Abstract Background Transcranial Pulse Stimulation (TPS) is a recent development of Non-invasive Brain Stimulation (NIBS) that has been proven effective to significantly improve Alzheimer patients' cognition, memory and execution function. There is no trial evaluating the efficacy of TPS on Major Depressive Disorder (MDD) nationwide. We aimed to evaluate the efficacy of TPS on young adults with MDD in Hong Kong. **Methods** In this single-blinded, randomized controlled trial, participants received a 2-week TPS treatment comprised six 30-min TPS sessions. Thirty participants were recruited and randomized into either the TPS group or the Waitlist Control (WC) group. Randomization was stratified by gender and age by an independent statistician on a 1: 1 ratio. Our primary outcome was determined by whether participants' depressive symptom severity demonstrated significant reduction, compared with the WC group, using Hamilton Depression Rating Scale-17 ($HDRS_{17}$). Clin.Trials.gov, number NCT05006365. **Findings** We recruited 30 participants aged between 18-54 years and were predominantly female (73%), ethnic Chinese from 1 August – 31 Oct 2021. There was a significant group x time interaction ($F(1, 28) = 18.8$, $p < .001$)[y1] . Compared with the WC group, there was a significant reduction in the depressive symptom severity in the TPS group (mean difference = -6.60, $p = 0.02$, Cohen's $d = -0.93$). Results showed a significant intervention effect and the effect was large. **Interpretation** TPS is safe and effective to reduce depressive symptoms among young individuals with MDD in this trial. **Funding** DGRF, the Hong Kong Polytechnic University, Hong Kong SAR, China

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BOARD NUMBER: S04-128

NON-INVASIVE NEUROMODULATION APPROACHES IN THE TREATMENT OF ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Noa Peeleman[1], Hannes Heylen[1], Ahmad Khatoum[1], Tom Theys[2], Myles Mclaughlin[1], Nick Van Helleputte[3], Mathieu Vandenbulcke[1], Peter Janssen[1]
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To develop a minimally invasive way to electrically stimulate the brain in order to preserve neurons in brain regions involved in memory by means of epicranial electrical stimulation (ECS), we implanted two adult rhesus monkeys with two platinum stimulation electrodes on the temporal skull on both sides. Animals were trained in a non-navigational spatial memory task. We observed a significant memory improvement (percent correct) with ECS at 40 Hz and 3 mA, but stimulation at 10 Hz induced a significant decrease in performance. We measured the changes in neuronal activity in the upper bank of the lateral sulcus in the same animal. Overall, 16 out of 25 cells recorded showed significant changes. When comparing pre and post stimulation spike rate, 10 Hz stimulation caused a significant decrease in spike rate when considering a 500 ms interval post stimulation, while 40 Hz stimulation increased the spike rate around 450 ms above the level before stimulation. To chart the effects of ECS at the network level, we performed functional Magnetic Resonance Imaging (fMRI) during ECS in one monkey, while we alternated stimulation blocks with no-stimulation blocks. ECS at 40 Hz and at 3 mA resulted in significant activation of the hippocampus bilaterally, and of lateral temporal cortex on one side underneath the electrode. Interestingly, ECS at 10 Hz and at 3 mA showed no significant activations in the hippocampus and only weak activations in lateral temporal cortex. Our results suggest a promising role for ECS for improving cognition and activating deeper brain regions.

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INVESTIGATING THE ELECTRICAL STIMULATION OF SUBTHALAMIC NUCLEUS FOR THE TREATMENT OF CORTICAL STROKE

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Zhengdao Deng[1], Boateng Asamoah[2], Ugur Kilic[1], Myles Mc Laughlin[2], Bart Nuttin[1]
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Stroke remains a leading cause of long-term disability in developed countries and the second leading cause of death worldwide. Recent decades have witnessed substantial progress in understanding the pathophysiology of ischemic stroke and the treatment of stroke-related motor deficit. However, current therapies fail to offer patients satisfactory improvements. Therefore, a more effective approach for treating stroke-related motor deficit is still needed. Here we investigated the effect of electrical stimulation of the subthalamic nucleus (ES-STN) on motor deficit induced by photothrombotic lesion in the motor cortex (MC). We hypothesized that ES-STN can reduce motor deficit in stroke rats. First, 1 Sprague-Dawley rat was included in this pilot study. He was trained with the single pellet reaching task (SFPRT) and determined the baseline performance. We then induced a photothrombotic ischemic lesion in the forelimb region of the MC. After stroke induction we implanted a stimulation electrode into the STN. 4 days after stroke we performed ES-STN while evaluating SFPRT performance. We performed 100 Hz stimulation where rat showed improved performance during stimulation. At 130 Hz rat showed even more improvement. We then performed histology to confirm that the stimulation electrode was in the STN. Our pilot study indicates that ES-STN may be a promising approach for treating cortical stroke-related motor deficit. A larger scale study with more subjects is necessary to further investigate the effect of ES-STN on the motor deficit after cortical stroke.

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A NOVEL TDCS RAT MODEL TO STUDY LEARNING & MEMORY

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Transcranial direct current stimulation (tDCS) is a popular non-invasive neuromodulation method that passes a weak current through two electrodes on the scalp. It is currently assumed that the technique's underlying modulatory mechanisms involve the direct (de)polarization of brain regions, but evidence is scarce. Recent research even suggests a potential role for peripheral nerve activation in mediating tDCS's modulatory effects. In order to understand tDCS's working mechanism, it is important to study the effects of the technique's cerebral and peripheral electric fields at a behavioral and neurophysiological level. To this end, we developed an awake rat model in which DC electric fields can be generated respectively in brain regions or the skin. For cerebral stimulation, this model uses multiple skull electrodes, while peripheral stimulation is achieved with subcutaneous carbon electrodes. These electrodes do not immobilize the animal and allow for isolated stimulation of the cerebrum and skin in the freely moving rat. We investigated whether DC stimulation over the skull (over the hippocampus) or skin (around the occipital nerve) could effectively enhance learning. Preliminary results indicate that 30 minutes of stimulation (0.25 mA) of the skull, but not of the skin, can enhance passive avoidance learning in this rat model. Future experiments are necessary to clarify amplitude thresholds at which learning can be enhanced through the skull and skin. By determining the applied electric field strengths in brain regions and skin, we obtain an estimate of the tDCS electrode configuration and amplitude required in humans to achieve similar modulatory effects.

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INVESTIGATE TDCS NEUROPHYSIOLOGICAL MECHANISMS IN HEALTHY VOLUNTEERS USING A NOVEL TDCS CONDITION

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Transcranial direct current stimulation (tDCS) is a noninvasive neuromodulation method that can increase cortical excitability by applying direct current over stimulation electrodes on the scalp. Until recently, it was accepted tDCS causes neuromodulation via direct polarization of cortical neurons. However, recent studies show the electric field in the brain is relatively weak and suggest that the stronger field in the scalp may directly stimulate cranial nerves. Interestingly, direct stimulation of cranial nerves can also affect cortical excitability and plasticity. In a healthy volunteer study, we are investigating the neurophysiological mechanism behind tDCS: are observed effects caused by direct polarization of cortical neurons, or by indirect cranial nerve stimulation or a combination of both? We developed a novel tDCS stimulation condition in which the peripheral input is blocked by applying topical anesthetics on the scalp underneath the stimulation electrodes, referred to as transcranial-only tDCS. We will compare the effects of tDCS on motor sequence learning to this novel stimulation condition and standard sham stimulation in a double blinded study including 99 healthy volunteers, pre-registered at the Open Science Framework platform. Data collection is ongoing. After data collection and analysis, if our results indicate tDCS significantly improves motor sequence learning compared to sham but the effect disappears for transcranial-only tDCS, we could conclude the observed learning effect are mainly caused by co-stimulation of peripheral nerves in the scalp. If not, we could conclude the effects are caused by cortical polarization.

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USING A PUPIL DILATION METRIC TO CHARACTERIZE THE EFFECT OF TRIGEMINAL NERVE STIMULATION

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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External stimulation of the trigeminal nerve is thought to activate certain nuclei in the brainstem, such as the locus coeruleus (LC), providing a means to indirectly stimulate noradrenergic pathways in the central nervous system. Trigeminal nerve stimulation (TNS) is already used in multiple clinical settings, however healthy volunteer studies are limited and the working mechanism underlying TNS effects are poorly understood. Studies showed that LC activity correlated positively with norepinephrine (NE) concentrations in the brain and that pupil size increases when LC increases. Taken together, the LC-NE system might play a role in TNS effects. In the current study, the effect of TNS on pupil dilation was investigated in healthy volunteers. Participants (n=4, data collection ongoing) underwent three different stimulation conditions (TNS, sham and median nerve stimulation - MNS) while pupil diameter was recorded. In addition to sham stimulation, MNS was applied as a control condition as it is thought to not activate the LC. TNS, sham and MNS were applied alternatingly with 4000 ms between each stimulation condition. The results showed that TNS elicited a pupil response approximately 1.5 ms after stimulation onset, while sham elicited small to no pupil responses. MNS also elicited a pupil response, however compared to TNS, pupil responses were smaller with MNS stimulation. The preliminary results suggest that the LC-NE system might contribute to the working mechanisms underpinning TNS effects. However, more participants need to be included to confirm these results.

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TOLERABILITY AND BLINDING EFFICACY OF PERSONALIZED THETA-MODULATED TRANSCRANIAL ELECTRICAL CURRENT STIMULATION

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Marija Stanković[1], Marko Živanović[2], Uroš Konstantinović[1], Dunja Paunovic[1], Katarina Vulić[1], Jovana Bjekić[1], Saša Filipović[1]
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Transcranial electrical stimulation (tES) is widely used in neuromodulation research. Numerous studies show that the conventional type of tES – transcranial direct current stimulation (tDCS) is a safe and well-tolerated technique. An increasingly popular line of research focuses on implementing alternative tES protocols aimed at the entrainment of brain oscillations. Thus, there is a need for a detailed assessment of the side effects profile and blinding efficacy of such protocols. Here we evaluate the tolerability and blinding efficacy of two personalized oscillatory tES protocols: Transcranial alternating current stimulation (tACS) and oscillatory tDCS (otDCS), applied in individual theta frequency for each participant (4-8Hz). Forty-two young, healthy individuals took part in a within-subject experiment in which they went through four stimulation conditions in a counterbalanced order: TACS (2mA peak-to-peak), otDCS (1.5mA±0.5mA), constant tDCS (1.5mA), and sham. The participants rated discomfort level at four timepoints during the 20-minute stimulation. Before and after each stimulation condition, they completed the standardized side effects questionnaire. The successfulness of blinding was assessed with the end-of-study guess of the sham condition. Overall, the reported discomfort level was low and comparable across all stimulation conditions, with otDCS being slightly more unpleasant than sham. Mild tingling and itching sensations were the most common side effects. No serious side effects were detected. Participant blinding was successful, and sham-guessing was not related to the reported discomfort in any of the stimulation conditions. The data has shown that personalized theta otDCS and tACS are well-tolerated and safe, with adequate blinding success.

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DESIGN AND IMPLEMENTATION OF AN AT-HOME EEG-NEUROFEEDBACK PROTOCOL FOR DECREASING VISUAL MOTION SENSITIVITY

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Aims Patients who suffer from motion-sickness, vertigo and other conditions relating to visually induced dizziness (VID) will often feel dizzy when exposed to intense visual stimuli. This study aims to show how a visual neurofeedback system may be used for treating motion-sickness and VID. **Methods** We constructed a neurofeedback system, synchronizing a virtual spinning maze-like pattern where the velocity was proportional to theta wave activity as recorded by a wearable EEG (Muse 2). Eight participants (5M and 3F, aged 28.6 ± 8.6) had their balance, EEG, eye-movements and overall neurofeedback proficiency measured, after which they were issued a 7-day neurofeedback training regimen before repeating the trials. **Results** One subject was excluded due to achieving the highest level of neurofeedback-control at baseline. A 19.3% drop in theta wave activity was seen from the first day of training to last ($n = 7$, $p < 0.001$). Mean theta wave activity during in-lab testing dropped by 9.4% ± 0.9% ($p = 0.048$), eye-tracking showed that torsional slow-phase velocities dropped by 18.5% ± 3.9% ($p = 0.005$) while balance was unaffected. **Conclusions** The neurofeedback regimen was successful in allowing participants to lower their levels of attention, as shown by both EEG data as well as the decreased eye movement response, both reflecting attentional levels. While the training had no effect on balance in healthy participants, it may be interesting to repeat the procedure on VID patients. This study suggests that neurofeedback training may serve a clinical function in decreasing participants' sensitivity to visual motion.

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CHANGES IN INTEROCEPTIVE ABILTIES FOLLOWING HDTDCS

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

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Interoception characterizes the processing of ongoing bodily changes as well as their perception and is usually subdivided into different facets such as interoceptive accuracy (IAcc), sensibility (IS) or emotional interoceptive evaluation (IE). Interoceptive abilities are related to various psychological phenomena (emotions, psychopathology), playing a crucial role in many theories of emotional processing. Changing interoceptive abilities by the use of neurostimulation is an innovative way to examine its importance and to find new methods to implement in therapeutic interventions. In a pilot study we used high-definition transcranial direct current stimulation (HD-tDCS) to improve interoceptive abilities targeting underlying neural networks. 52 participants were included in two conditions (stimulating protocol, sham) in randomized order and different interoceptive facets (IAcc, IS, IE) were examined during the experimental conditions. In addition, subjective report and questionnaire data were assessed. Main results revealed that using the HD-tDCS protocol targeting interoceptive network structures such as the anterior insula did not significantly improve interoceptive accuracy as measured by a heartbeat perception task or respiratory resistance task, while first findings referred to effects for other interoceptive dimensions. Our study suggests that stimulating the interoceptive network using HD-tDCS could benefit from a more intense or repetitive protocol or the combination to other methods known to be efficient in a short-term interval application. Further research is needed to better exploit this promising research avenue.

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THE EFFECTS OF MAGNETOELECTRIC DEEP BRAIN STIMULATION ON THE MONOAMINERGIC SYSTEMS IN MICE

POSTER SESSION 04 - SECTION: NEUROSTIMULATION AND NEUROFEEDBACK

Faisal Alosaimi[1], Rick Knoben[1], David Dominguez-Paredes[1], Faris Almasabi[1], Sarah Hescham[1], Kristen Kozielski[2], Yasin Temel[1], Ali Jahanshahi[1]
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Aim: Deep brain stimulation (DBS) is used to alleviate motor disabilities in neurological disorders. However, the current approach requires invasive brain surgery. Recently, we have shown that injectable magnetoelectric nanoelectrodes may offer an alternative approach to conventional DBS. However, this method is in its infancy, and research is required to characterize its mechanisms of action. Herein, we aimed to investigate the effect of magnetoelectric stimulation on main neurotransmitter systems that have implications for DBS and movement disorders. Methods: Mice were injected with either magnetoelectric nanoparticles (MENPs) or magnetostrictive nanoparticles (MSNPs) in the subthalamic nucleus (STN). Mice underwent magnetic stimulation, and their motor behavior was assessed. In addition, post-mortem brains were processed for immunohistochemistry (IHC) to assess the co-expression of c-Fos with either tyrosine hydroxylase (TH), or tryptophan hydroxylase-2 (TPH2). *Results*: The stimulated animals exhibited an increased distance traveled in the open field test. In IHC we found a significant increase in c-Fos expression in the motor cortex (MC) and paraventricular region of the thalamus (PV-thalamus) after magnetic stimulation. Stimulated animals showed fewer TPH2/c-Fos double-labeled cells in the dorsal raphe nucleus (DRN), as well as TH/c-Fos double-labeled cells in the ventral tegmental area (VTA). *Conclusion:* These findings suggest that magnetic stimulation of MENPs in mice enables selective modulation of the deep brain areas and animal behavior. The measured behavioral responses are associated with changes in relevant neurotransmitter systems. These changes are somewhat similar to those that have been observed in conventional DBS, suggesting that magnetic DBS could replace electrical DBS.

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BOARD NUMBER: S04-137

FLEXIBLE POLYMER-BASED NEURAL PROBES DEVELOPED FOR LAMINAR RECORDINGS FROM THE HUMAN NEOCORTEX

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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In this study, we developed and validated a single-shank polymer-based neural probe designed for human intracortical laminar recordings. The flexible probe has a 3.9-mm-long, 75 to 300-µm-wide and 10-µm-thick tapered, implantable shank which contains 24 linearly placed gold microelectrodes with a diameter of 20 µm and center-to-center distance of 150 µm. Two probe variants with two different connector types were developed to compare their electrophysiological performance as well as their usability under different experimental conditions. To test the probes, first we performed impedance spectroscopy *in vitro*, in physiological saline solution. The impedance magnitude of recording sites was 221 ± 130 kΩ at 1 kHz (mean ± s.d. of 117 sites). Electrophysiological performance of the probes was validated in *in vivo* experiments by acute implantations into neocortical and hippocampal areas of anesthetized rats and mice. We recorded good-quality local field potentials, single- and multi-unit activity from the investigated brain regions. We were able to monitor cortical slow waves as well as hippocampal gamma activity with the implant. Furthermore, we could detect spike amplitudes over 100 µV and record the activity of multiple well-isolated single units simultaneously. Our future plans are to chronically implant the flexible probes in rodents to evaluate their long-term electrophysiological performance as well as the brain tissue response in the vicinity of the probe shank. Our long-term goal is to test the devices in human cortical tissue, for example, by implanting them into the brain of drug-resistant epileptic patients who are potential candidates for brain surgery.

Pubmed:

34267214: Horváth C, Tóth LF, Ulbert I, Fiáth R
Dataset of cortical activity recorded with high spatial resolution from anesthetized rats.
Publicly available neural recordings obtained with high spatial resolution are scarce. Here, we present an electrophysiological dataset recorded from the neocortex of twenty rats anesthetized with ketamine/xylazine. The wideband, spontaneous recordings were acquired with a single-shank silicon-based probe having 128 densely-packed recording sites arranged in a 32 × 4 array. The dataset contains the activity of a total of 7126 sorted single units extracted from all layers of the cortex. Here, we share raw neural recordings, as well as spike times, extracellular spike waveforms and several properties of units packaged in a standardized electrophysiological data format. For technical validation of our dataset, we provide the distributions of derived single unit properties along with various spike sorting quality metrics. This large collection of in vivo data enables the investigation of the high-resolution electrical footprint of cortical neurons which in turn may aid their electrophysiology-based classification. Furthermore, the dataset might be used to study the laminar-specific neuronal activity during slow oscillation, a brain rhythm strongly involved in neural mechanisms underlying memory consolidation and sleep.
Sci Data, 2021; 8
33479289: Fiáth R, Meszéna D, Somogyvári Z, Boda M, Barthó P, Ruther P, Ulbert I
Recording site placement on planar silicon-based probes affects signal quality in acute neuronal recordings.
Multisite, silicon-based probes are widely used tools to record the electrical activity of neuronal populations. Several physical features of these devices are designed to improve their recording performance. Here, our goal was to investigate whether the position of recording sites on the silicon shank might affect the quality of the recorded neural signal in acute experiments. Neural recordings obtained with five different types of high-density, single-shank, planar silicon probes from anesthetized rats were analyzed. Wideband data were filtered to extract spiking activity, then the amplitude distribution of samples and quantitative properties of the recorded brain activity (single unit yield, spike amplitude and isolation distance) were compared between sites located at different positions of the silicon shank, focusing particularly on edge and center sites. Edge sites outperformed center sites: for all five probe types there was a significant difference in the signal power computed from the

amplitude distributions, and edge sites recorded significantly more large amplitude samples both in the positive and negative range. Although the single unit yield was similar between site positions, the difference in spike amplitudes was noticeable in the range corresponding to high-amplitude spikes. Furthermore, the advantage of edge sites slightly decreased with decreasing shank width. Our results might aid the design of novel neural implants in enhancing their recording performance by identifying more efficient recording site placements.
Sci Rep, 2021; 11
32839617: Yuste R, Hawrylycz M, Aalling N, Aguilar-Valles A, Arendt D, Armañanzas R, Ascoli GA, Bielza C, Bokharaie V, Bergmann TB, Bystron I, Capogna M, Chang Y, Clemens A, de Kock CPJ, DeFelipe J, Dos Santos SE, Dunville K, Feldmeyer D, Fiáth R, Fishell GJ, Foggetti A, Gao X, Ghaderi P, Goriounova NA, Güntürkün O, Hagihara K, Hall VJ, Helmstaedter M, Herculano-Houzel S, Hilscher MM, Hirase H, Hjerling-Leffler J, Hodge R, Huang J, Huda R, Khodosevich K, Kiehn O, Koch H, Kuebler ES, Kühnemund M, Larrañaga P, Lelieveldt B, Louth EL, Lui JH, Mansvelder HD, Marin O, Martinez-Trujillo J, Chameh HM, Mohapatra AN, Munguba H, Nedergaard M, Němec P, Ofer N, Pfisterer UG, Pontes S, Redmond W, Rossier J, Sanes JR, Scheuermann RH, Serrano-Saiz E, Staiger JF, Somogyi P, Tamás G, Tolias AS, Tosches MA, García MT, Wozny C, Wuttke TV, Liu Y, Yuan J, Zeng H, Lein E
A community-based transcriptomics classification and nomenclature of neocortical cell types.
To understand the function of cortical circuits, it is necessary to catalog their cellular diversity. Past attempts to do so using anatomical, physiological or molecular features of cortical cells have not resulted in a unified taxonomy of neuronal or glial cell types, partly due to limited data. Single-cell transcriptomics is enabling, for the first time, systematic high-throughput measurements of cortical cells and generation of datasets that hold the promise of being complete, accurate and permanent. Statistical analyses of these data reveal clusters that often correspond to cell types previously defined by morphological or physiological criteria and that appear conserved across cortical areas and species. To capitalize on these new methods, we propose the adoption of a transcriptome-based taxonomy of cell types for mammalian neocortex. This classification should be hierarchical and use a standardized nomenclature. It should be based on a probabilistic definition of a cell type and incorporate data from different approaches, developmental stages and species. A community-based classification and data aggregation model, such as a knowledge graph, could provide a common foundation for the study of cortical circuits. This community-based classification, nomenclature and data aggregation could serve as an example for cell type atlases in other parts of the body.
Nat Neurosci, 2020; 23
30643182: Fiáth R, Márton AL, Mátyás F, Pinke D, Márton G, Tóth K, Ulbert I
Slow insertion of silicon probes improves the quality of acute neuronal recordings.
Neural probes designed for extracellular recording of brain electrical activity are traditionally implanted with an insertion speed between 1 μm/s and 1 mm/s into the brain tissue. Although the physical effects of insertion speed on the tissue are well studied, there is a lack of research investigating how the quality of the acquired electrophysiological signal depends on the speed of probe insertion. In this study, we used four different insertion speeds (0.002 mm/s, 0.02 mm/s, 0.1 mm/s, 1 mm/s) to implant high-density silicon probes into deep layers of the somatosensory cortex of ketamine/xylazine anesthetized rats. After implantation, various qualitative and quantitative properties of the recorded cortical activity were compared across different speeds in an acute manner. Our results demonstrate that after the slowest insertion both the signal-to-noise ratio and the number of separable single units were significantly higher compared with those measured after inserting probes at faster speeds. Furthermore, the amplitude of recorded spikes as well as the quality of single unit clusters showed similar speed-dependent differences. Post hoc quantification of the neuronal density around the probe track showed a significantly higher number of NeuN-labelled cells after the slowest insertion compared with the fastest insertion. Our findings suggest that advancing rigid probes slowly (~1 μm/s) into the brain tissue might result in less tissue damage, and thus in neuronal recordings of improved quality compared with measurements obtained after inserting probes with higher speeds.
Sci Rep, 2019; 9
30144495: Fiáth R, Raducanu BC, Musa S, Andrei A, Lopez CM, Welkenhuysen M, Ruther P, Aarts A, Ulbert I
Fine-scale mapping of cortical laminar activity during sleep slow oscillations using high-density linear silicon probes.
The cortical slow (~1 Hz) oscillation (SO), which is thought to play an active role in the consolidation of memories, is a brain rhythm characteristic of slow-wave sleep, with alternating periods of neuronal activity and silence. Although the laminar distribution of cortical activity during SO is well-studied by using linear neural probes, traditional devices have a relatively low (20-100 μm) spatial resolution along cortical layers.
J Neurosci Methods, 2019; 316
29478038: Fiáth R, Hofer KT, Csikós V, Horváth D, Nánási T, Tóth K, Pothof F, Böhler C, Asplund M, Ruther P, Ulbert I
Long-term recording performance and biocompatibility of chronically implanted cylindrically-shaped, polymer-based neural interfaces.
Stereo-electroencephalography depth electrodes, regularly implanted into drug-resistant patients with focal epilepsy to

localize the epileptic focus, have a low channel count (6-12 macro- or microelectrodes), limited spatial resolution (0.5-1 cm) and large contact area of the recording sites (~mm2). Thus, they are not suited for high-density local field potential and multiunit recordings. In this paper, we evaluated the long-term electrophysiological recording performance and histocompatibility of a neural interface consisting of 32 microelectrodes providing a physical shape similar to clinical devices. The cylindrically-shaped depth probes made of polyimide (PI) were chronically implanted for 13 weeks into the brain of rats, while cortical or thalamic activity (local field potentials, single-unit and multi-unit activity) was recorded regularly to monitor the temporal change of several features of the electrophysiological performance. To examine the tissue reaction around the probe, neuron-selective and astroglia-selective immunostaining methods were applied. Stable single-unit and multi-unit activity were recorded for several weeks with the implanted depth probes and a weak or moderate tissue reaction was found around the probe track. Our data on biocompatibility presented here and in vivo experiments in non-human primates provide a strong indication that this type of neural probe can be applied in stereo-electroencephalography recordings of up to 2 weeks in humans targeting the localization of epileptic foci providing an increased spatial resolution and the ability to monitor local field potentials and neuronal spiking activity.
Biomed Tech (Berl), 2018; 63
29414094: Fiáth R, Raducanu BC, Musa S, Andrei A, Lopez CM, van Hoof C, Ruther P, Aarts A, Horváth D, Ulbert I
A silicon-based neural probe with densely-packed low-impedance titanium nitride microelectrodes for ultrahigh-resolution in vivo recordings.
In this study, we developed and validated a single-shank silicon-based neural probe with 128 closely-packed microelectrodes suitable for high-resolution extracellular recordings. The 8-mm-long, 100-µm-wide and 50-µm-thick implantable shank of the probe fabricated using a 0.13-µm complementary metal-oxide-semiconductor (CMOS) metallization technology contains square-shaped (20 × 20 µm), low-impedance (~ 50 kΩ at 1 kHz) recording sites made of rough and porous titanium nitride which are arranged in a 32 × 4 dense array with an inter-electrode pitch of 22.5 µm. The electrophysiological performance of the probe was tested in in vivo experiments by implanting it acutely into neocortical areas of anesthetized animals (rats, mice and cats). We recorded local field potentials, single- and multi-unit activity with superior quality from all layers of the neocortex of the three animal models, even after reusing the probe in multiple (> 10) experiments. The low-impedance electrodes monitored spiking activity with high signal-to-noise ratio; the peak-to-peak amplitude of extracellularly recorded action potentials of well-separable neurons ranged from 0.1 mV up to 1.1 mV. The high spatial sampling of neuronal activity made it possible to detect action potentials of the same neuron on multiple, adjacent recording sites, allowing a more reliable single unit isolation and the investigation of the spatiotemporal dynamics of extracellular action potential waveforms in greater detail. Moreover, the probe was developed with the specific goal to use it as a tool for the validation of electrophysiological data recorded with high-channel-count, high-density neural probes comprising integrated CMOS circuitry.
Biosens Bioelectron, 2018; 106
27535370: Fiáth R, Beregszászi P, Horváth D, Wittner L, Aarts AA, Ruther P, Neves HP, Bokor H, Acsády L, Ulbert I
Large-scale recording of thalamocortical circuits: in vivo electrophysiology with the two-dimensional electronic depth control silicon probe.
Recording simultaneous activity of a large number of neurons in distributed neuronal networks is crucial to understand higher order brain functions. We demonstrate the in vivo performance of a recently developed electrophysiological recording system comprising a two-dimensional, multi-shank, high-density silicon probe with integrated complementary metal-oxide semiconductor electronics. The system implements the concept of electronic depth control (EDC), which enables the electronic selection of a limited number of recording sites on each of the probe shafts. This innovative feature of the system permits simultaneous recording of local field potentials (LFP) and single- and multiple-unit activity (SUA and MUA, respectively) from multiple brain sites with high quality and without the actual physical movement of the probe. To evaluate the in vivo recording capabilities of the EDC probe, we recorded LFP, MUA, and SUA in acute experiments from cortical and thalamic brain areas of anesthetized rats and mice. The advantages of large-scale recording with the EDC probe are illustrated by investigating the spatiotemporal dynamics of pharmacologically induced thalamocortical slow-wave activity in rats and by the two-dimensional tonotopic mapping of the auditory thalamus. In mice, spatial distribution of thalamic responses to optogenetic stimulation of the neocortex was examined. Utilizing the benefits of the EDC system may result in a higher yield of useful data from a single experiment compared with traditional passive multielectrode arrays, and thus in the reduction of animals needed for a research study.
J Neurophysiol, 2016; 116
27177594: Fiáth R, Kerekes BP, Wittner L, Tóth K, Beregszászi P, Horváth D, Ulbert I
Laminar analysis of the slow wave activity in the somatosensory cortex of anesthetized rats.
Rhythmic slow waves characterize brain electrical activity during natural deep sleep and under anesthesia, reflecting the synchronous membrane potential fluctuations of neurons in the thalamocortical network. Strong evidence indicates that the neocortex plays an important role in the generation of slow wave activity (SWA), however, contributions of individual cortical

layers to the SWA generation are still unclear. The anatomically correct laminar profiles of SWA were revealed under ketamine/xylazine anesthesia, with combined local field potential recordings, multiple-unit activity (MUA), current source density (CSD) and time-frequency analyses precisely co-registered with histology. The up-state related negative field potential wave showed the largest amplitude in layer IV, the CSD was largest in layers I and III, whereas MUA was maximal in layer V, suggesting spatially dissociated firing and synaptic/transmembrane processes in the rat somatosensory cortex. Up-state related firing could start in virtually any layers (III-VI) of the cortex, but were most frequently initiated in layer V. However, in a subset of experiments, layer IV was considerably active in initiating up-state related MUA even in the absence of somatosensory stimulation. Somatosensory stimulation further strengthened up-state initiation in layer IV. Our results confirm that cortical layer V firing may have a major contribution to the up-state generation of ketamine/xylazine-induced SWA, however, thalamic influence through the thalamorecipient layer IV can also play an initiating role, even in the absence of sensory stimulation.
Eur J Neurosci, 2016; 44

29048396: Raducanu BC, Yazicioglu RF, Lopez CM, Ballini M, Putzeys J, Wang S, Andrei A, Rochus V, Welkenhuysen M, Helleputte NV, Musa S, Puers R, Kloosterman F, Hoof CV, Fiáth R, Ulbert I, Mitra S
Time Multiplexed Active Neural Probe with 1356 Parallel Recording Sites.
We present a high electrode density and high channel count CMOS (complementary metal-oxide-semiconductor) active neural probe containing 1344 neuron sized recording pixels (20 µm × 20 µm) and 12 reference pixels (20 µm × 80 µm), densely packed on a 50 µm thick, 100 µm wide, and 8 mm long shank. The active electrodes or pixels consist of dedicated in-situ circuits for signal source amplification, which are directly located under each electrode. The probe supports the simultaneous recording of all 1356 electrodes with sufficient signal to noise ratio for typical neuroscience applications. For enhanced performance, further noise reduction can be achieved while using half of the electrodes (678). Both of these numbers considerably surpass the state-of-the art active neural probes in both electrode count and number of recording channels. The measured input referred noise in the action potential band is 12.4 µVrms, while using 678 electrodes, with just 3 µW power dissipation per pixel and 45 µW per read-out channel (including data transmission).
Sensors (Basel), 2017; 17

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-138

PLASTICITY AND MICROORGANIZATION OF SYNAPTIC TRANSMISSION IN THE HUMAN CORTEX

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Aims: Homeostatic plasticity is an intrinsic property of neurons that keeps synaptic transmission between a physiological range, and therefore it is of great relevance for the functional activity and survival of neurons, however, so far has only been described in rodents. This project focuses on the characterization of homeostatic plasticity in the human brain. Methods: Human organotypic cortical slice cultures enable the investigation of dynamic properties of human neurons over a long time period, allowing to implement protocols to investigate long term changes on human neuronal networks that developed physiologically. After extracting brain tissue from the temporal pole and culturing it, we silence synaptic transmission via tetrodotoxin administration and wash out the drug after 48h. We then record the electrophysiological changes suffered by the network via Multielectrode Array Recordings. Finally, with confocal and volumetric electron microscopy we image that same tissue and reconstruct the spines and synapses, correlating the changes in synaptic activity with the morphological alterations that those same synapses show. Results and Conclusions: With this ongoing project we will determine whether homeostatic plasticity takes place in the human brain. Finding out how it acts is of great relevance for the development of new drugs to treat diseases that seem to arise as a consequence of the misfunction of some of the proteins involved in this regulation, such as schizophrenia, autism spectrum disorder o depression.

Pubmed:
33374598: Eltokhi A, Santuy A, Merchan-Perez A, Sprengel R
Glutamatergic Dysfunction and Synaptic Ultrastructural Alterations in Schizophrenia and Autism Spectrum Disorder: Evidence from Human and Rodent Studies.
The correlation between dysfunction in the glutamatergic system and neuropsychiatric disorders, including schizophrenia and autism spectrum disorder, is undisputed. Both disorders are associated with molecular and ultrastructural alterations that affect synaptic plasticity and thus the molecular and physiological basis of learning and memory. Altered synaptic plasticity, accompanied by changes in protein synthesis and trafficking of postsynaptic proteins, as well as structural modifications of excitatory synapses, are critically involved in the postnatal development of the mammalian nervous system. In this review, we summarize glutamatergic alterations and ultrastructural changes in synapses in schizophrenia and autism spectrum disorder of genetic or drug-related origin, and briefly comment on the possible reversibility of these neuropsychiatric disorders in the light of findings in regular synaptic physiology.
Int J Mol Sci, 2020; 22
32814795: Santuy A, Tomás-Roca L, Rodríguez JR, González-Soriano J, Zhu F, Qiu Z, Grant SGN, DeFelipe J, Merchan-Perez A
Estimation of the number of synapses in the hippocampus and brain-wide by volume electron microscopy and genetic labeling.
Determining the number of synapses that are present in different brain regions is crucial to understand brain connectivity as a whole. Membrane-associated guanylate kinases (MAGUKs) are a family of scaffolding proteins that are expressed in excitatory glutamatergic synapses. We used genetic labeling of two of these proteins (PSD95 and SAP102), and Spinning Disc confocal Microscopy (SDM), to estimate the number of fluorescent puncta in the CA1 area of the hippocampus. We also used FIB-SEM, a three-dimensional electron microscopy technique, to calculate the actual numbers of synapses in the same area. We then estimated the ratio between the three-dimensional densities obtained with FIB-SEM (synapses/µm) and the bi-dimensional densities obtained with SDM (puncta/100 µm). Given that it is impractical to use FIB-SEM brain-wide, we used previously available SDM data from other brain regions and we applied this ratio as a conversion factor to estimate the minimum density of synapses in those regions. We found the highest densities of synapses in the isocortex, olfactory areas, hippocampal formation and cortical subplate. Low densities were found in the pallidum, hypothalamus, brainstem and

cerebellum. Finally, the striatum and thalamus showed a wide range of synapse densities.
Sci Rep, 2020; 10
32054677: Rodriguez-Moreno J, Porrero C, Rollenhagen A, Rubio-Teves M, Casas-Torremocha D, Alonso-Nanclares L, Yakoubi R, Santuy A, Merchan-Pérez A, DeFelipe J, Lübke JHR, Clasca F
Area-Specific Synapse Structure in Branched Posterior Nucleus Axons Reveals a New Level of Complexity in Thalamocortical Networks.
Thalamocortical posterior nucleus (Po) axons innervating the vibrissal somatosensory (S1) and motor (MC) cortices are key links in the brain neuronal network that allows rodents to explore the environment whisking with their motile snout vibrissae. Here, using fine-scale high-end 3D electron microscopy, we demonstrate in adult male C57BL/6 wild-type mice marked differences between MC versus S1 Po synapses in (1) bouton and active zone size, (2) neurotransmitter vesicle pool size, (3) distribution of mitochondria around synapses, and (4) proportion of synapses established on dendritic spines and dendritic shafts. These differences are as large, or even more pronounced, than those between Po and ventro-posterior thalamic nucleus synapses in S1. Moreover, using single-axon transfection labeling, we demonstrate that the above differences actually occur on the MC versus the S1 branches of individual Po cell axons that innervate both areas. Along with recently-discovered divergences in efficacy and plasticity, the synaptic structure differences reported here thus reveal a new subcellular level of complexity. This is a finding that upends current models of thalamocortical circuitry, and that might as well illuminate the functional logic of other branched projection axon systems. Many long-distance brain connections depend on neurons whose branched axons target separate regions. Using 3D electron microscopy and single-cell transfection, we investigated the mouse Posterior thalamic nucleus (Po) cell axons that simultaneously innervate motor and sensory areas of the cerebral cortex involved in whisker movement control. We demonstrate significant differences in the size of the boutons made in each area by individual Po axons, as well as in functionally-relevant parameters in the composition of their synapses. In addition, we found similarly large differences between the synapses of Po versus ventral posteromedial thalamic nucleus axons in the whisker sensory cortex. Area-specific synapse structure in individual axons implies a new, unsuspected level of complexity in long-distance brain connections.
J Neurosci, 2020; 40
31665237: Turegano-Lopez M, Santuy A, DeFelipe J, Merchan-Perez A
Size, Shape, and Distribution of Multivesicular Bodies in the Juvenile Rat Somatosensory Cortex: A 3D Electron Microscopy Study.
Multivesicular bodies (MVBs) are membrane-bound organelles that belong to the endosomal pathway. They participate in the transport, sorting, storage, recycling, degradation, and release of multiple substances. They interchange cargo with other organelles and participate in their renovation and degradation. We have used focused ion beam milling and scanning electron microscopy (FIB-SEM) to obtain stacks of serial sections from the neuropil of the somatosensory cortex of the juvenile rat. Using dedicated software, we have 3D-reconstructed 1618 MVBs. The mean density of MVBs was 0.21 per cubic micron. They were unequally distributed between dendrites (39.14%), axons (18.16%), and nonsynaptic cell processes (42.70%). About one out of five MVBs (18.16%) were docked on mitochondria, representing the process by which the endosomal pathway participates in mitochondrial maintenance. Other features of MVBs, such as the presence of tubular protrusions (6.66%) or clathrin coats (19.74%) can also be interpreted in functional terms, since both are typical of early endosomes. The sizes of MVBs follow a lognormal distribution, with differences across cortical layers and cellular compartments. The mean volume of dendritic MVBs is more than twice as large as the volume of axonic MVBs. In layer I, they are smaller, on average, than in the other layers.
Cereb Cortex, 2020; 30
30060007: Santuy A, Turégano-López M, Rodríguez JR, Alonso-Nanclares L, DeFelipe J, Merchán-Pérez A
A Quantitative Study on the Distribution of Mitochondria in the Neuropil of the Juvenile Rat Somatosensory Cortex.
Mitochondria play a key role in energy production and calcium buffering, among many other functions. They provide most of the energy required by neurons, and they are transported along axons and dendrites to the regions of higher energy demands. We have used focused ion beam milling and scanning electron microscopy (FIB/SEM) to obtain stacks of serial sections from the somatosensory cortex of the juvenile rat. We have estimated the volume fraction occupied by mitochondria and their distribution between dendritic, axonal, and nonsynaptic processes. The volume fraction of mitochondria increased from layer I (4.59%) to reach its maximum in layer IV (7.74%) and decreased to its minimum in layer VI (4.03%). On average, 44% of mitochondrial volume was located in dendrites, 15% in axons and 41% in nonsynaptic elements. Given that dendrites, axons, and nonsynaptic elements occupied 38%, 23%, and 39% of the neuropil, respectively, it can be concluded that dendrites are proportionally richer in mitochondria with respect to axons, supporting the notion that most energy consumption takes place at the postsynaptic side. We also found a positive correlation between the volume fraction of mitochondria located in neuronal processes and the density of synapses.
Cereb Cortex, 2018; 28

29387782: Santuy A, Rodríguez JR, DeFelipe J, Merchán-Pérez A
Study of the Size and Shape of Synapses in the Juvenile Rat Somatosensory Cortex with 3D Electron Microscopy.
Changes in the size of the synaptic junction are thought to have significant functional consequences. We used focused ion beam milling and scanning electron microscopy (FIB/SEM) to obtain stacks of serial sections from the six layers of the rat somatosensory cortex. We have segmented in 3D a large number of synapses (= 6891) to analyze the size and shape of excitatory (asymmetric) and inhibitory (symmetric) synapses, using dedicated software. This study provided three main findings. Firstly, the mean synaptic sizes were smaller for asymmetric than for symmetric synapses in all cortical layers. In all cases, synaptic junction sizes followed a log-normal distribution. Secondly, most cortical synapses had disc-shaped postsynaptic densities (PSDs; 93%). A few were perforated (4.5%), while a smaller proportion (2.5%) showed a tortuous horseshoe-shaped perimeter. Thirdly, the curvature was larger for symmetric than for asymmetric synapses in all layers. However, there was no correlation between synaptic area and curvature.
eNeuro, 2018 Jan-Feb; 5
28968773: Rodriguez-Moreno J, Rollenhagen A, Arlandis J, Santuy A, Merchan-Pérez A, DeFelipe J, Lübke JHR, Clasca F
Quantitative 3D Ultrastructure of Thalamocortical Synapses from the "Lemniscal" Ventral Posteromedial Nucleus in Mouse Barrel Cortex.
Thalamocortical synapses from "lemniscal" neurons of the dorsomedial portion of the rodent ventral posteromedial nucleus (VPMdm) are able to induce with remarkable efficacy, despite their relative low numbers, the firing of primary somatosensory cortex (S1) layer 4 (L4) neurons. To which extent this high efficacy depends on structural synaptic features remains unclear. Using both serial transmission (TEM) and focused ion beam milling scanning electron microscopy (FIB/SEM), we 3D-reconstructed and quantitatively analyzed anterogradely labeled VPMdm axons in L4 of adult mouse S1. All VPMdm synapses are asymmetric. Virtually all are established by axonal boutons, 53% of which contact multiple (2-4) elements (overall synapse/bouton ratio = 1.6). Most boutons are large (mean 0.47 µm3), and contain 1-3 mitochondria. Vesicle pools and postsynaptic density (PSD) surface areas are large compared to others in rodent cortex. Most PSDs are complex. Most synapses (83%) are established on dendritic spine heads. Furthermore, 15% of the postsynaptic spines receive a second, symmetric synapse. In addition, 13% of the spine heads have a large protrusion inserted into a membrane pouch of the VPMdm bouton. The unusual combination of structural features in VPMdm synapses is likely to contribute significantly to the high efficacy, strength, and plasticity of these thalamocortical synapses.
Cereb Cortex, 2018; 28
28721455: Santuy A, Rodriguez JR, DeFelipe J, Merchan-Perez A
Volume electron microscopy of the distribution of synapses in the neuropil of the juvenile rat somatosensory cortex.
Knowing the proportions of asymmetric (excitatory) and symmetric (inhibitory) synapses in the neuropil is critical for understanding the design of cortical circuits. We used focused ion beam milling and scanning electron microscopy (FIB/SEM) to obtain stacks of serial sections from the six layers of the juvenile rat (postnatal day 14) somatosensory cortex (hindlimb representation). We segmented in three-dimensions 6184 synaptic junctions and determined whether they were established on dendritic spines or dendritic shafts. Of all these synapses, 87-94% were asymmetric and 6-13% were symmetric. Asymmetric synapses were preferentially located on dendritic spines in all layers (80-91%) while symmetric synapses were mainly located on dendritic shafts (62-86%). Furthermore, we found that less than 6% of the dendritic spines establish more than one synapse. The vast majority of axospinous synapses were established on the spine head. Synapses on the spine neck were scarce, although they were more common when the dendritic spine established multiple synapses. This study provides a new large quantitative dataset that may contribute not only to the knowledge of the ultrastructure of the cortex, but also towards defining the connectivity patterns through all cortical layers.
Brain Struct Funct, 2018; 223
25203994: Corenthy L, Garcia M, Bayona S, Santuy A, Martin JS, Benavides-Piccione R, DeFelipe J, Pastor L
Haptically assisted connection procedure for the reconstruction of dendritic spines.
Dendritic spines are thin protrusions that cover the dendritic surface of numerous neurons in the brain and whose function seems to play a key role in neural circuits. The correct segmentation of those structures is difficult due to their small size and the resulting spines can appear incomplete. This paper presents a four-step procedure for the complete reconstruction of dendritic spines. The haptically driven procedure is intended to work as an image processing stage before the automatic segmentation step giving the final representation of the dendritic spines. The procedure is designed to allow both the navigation and the volume image editing to be carried out using a haptic device. A use case employing our procedure together with a commercial software package for the segmentation stage is illustrated. Finally, the haptic editing is evaluated in two experiments; the first experiment concerns the benefits of the force feedback and the second checks the suitability of the use of a haptic device as input. In both cases, the results shows that the procedure improves the editing accuracy.
IEEE Trans Haptics, 2014 Oct-Dec; 7


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BOARD NUMBER: S04-139

LOW-AMPLITUDE ELECTRICAL MICROSTIMULATION OF A CORTICAL COLUMN IN RAT BARREL CORTEX WITH HIGH-DENSITY SILICON PROBES

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Aims We tested the efficiency of the newly developed 512-channel stimulation/recording system in evoking neuronal responses within a barrel column of a rat. Methods The system is based on a custom-designed 64-channel CMOS chip that provides independent microstimulation and recording in every channel. It can control up to four probes with a total of 512 electrodes. For the tests, we used gold-plated Neuronexus and Masmanidis lab probes (0.1-0.2 MΩ). Probes were inserted into the barrel cortex of an adult, anaesthetized rat. Stimulation pulses with amplitudes of 1-4 μA were applied simultaneously to clusters of 1-5 electrodes in up to 80 locations in the barrel column. Results In response to the microstimulation, we recorded action potentials (AP) and electrically evoked local field potentials (EEP). APs with latencies as low as 1 ms characterized individual activated neurons. Slow EEPs waves, similar in shape to the LFP evoked by tactile whisker stimulation, were visible on multiple shanks of the stimulating probe. Their amplitude scaled with the intensity of the applied current. Stimulation in lower cortical layers induced high-frequency oscillations typical for strong multi-whisker responses. Conclusions Using a custom-built 512-channel system and standard commercial silicon probes for patterned low-amplitude stimulation in the rat barrel cortex, we successfully evoked subcomponents of natural sensory responses. **Funding: This work was supported by the Polish National Science Centre grant DEC-2013/10/M/NZ4/00268 (PH). P.J. has been partially supported by the EU Project POWR.03.02.00-00-I004/16.**

Pubmed:
34203305: Jurgielewicz P, Fiutowski T, Kublik E, Skoczeń A, Szypulska M, Wiącek P, Hottowy P, Mindur B
Modular Data Acquisition System for Recording Activity and Electrical Stimulation of Brain Tissue Using Dedicated Electronics.
In this paper, we present a modular Data Acquisition (DAQ) system for simultaneous electrical stimulation and recording of brain activity. The DAQ system is designed to work with custom-designed Application Specific Integrated Circuit (ASIC) called Neurostim-3 and a variety of commercially available Multi-Electrode Arrays (MEAs). The system can control simultaneously up to 512 independent bidirectional i.e., input-output channels. We present in-depth insight into both hardware and software architectures and discuss relationships between cooperating parts of that system. The particular focus of this study was the exploration of efficient software design so that it could perform all its tasks in real-time using a standard Personal Computer (PC) without the need for data precomputation even for the most demanding experiment scenarios. Not only do we show bare performance metrics, but we also used this software to characterise signal processing capabilities of Neurostim-3 (e.g., gain linearity, transmission band) so that to obtain information on how well it can handle neural signals in real-world applications. The results indicate that each Neurostim-3 channel exhibits signal gain linearity in a wide range of input signal amplitudes. Moreover, their high-pass cut-off frequency gets close to 0.6Hz making it suitable for recording both Local Field Potential (LFP) and spiking brain activity signals. Additionally, the current stimulation circuitry was checked in terms of the ability to reproduce complex patterns. Finally, we present data acquired using our system from the experiments on a living rat's brain, which proved we obtained physiological data from non-stimulated and stimulated tissue. The presented results lead us to conclude that our hardware and software can work efficiently and effectively in tandem giving valuable insights into how information is being processed by the brain.
Sensors (Basel), 2021; 21

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BOARD NUMBER: S04-140

BOTTOM-UP NEUROSCIENCE ON HIGH DENSITY CMOS BASED MICROELECTRODE ARRAYS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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The fundamental mechanisms of information processing in the brain are still poorly understood. Despite the vast amount of technical possibilities, we still have very little understanding about basic neuroscience concepts such as memory formation and learning. Due to the high complexity of the brain a functional isolation of single circuits is not possible *in vivo*. Thus, further progress in neuroscience requires a fundamentally new approach in which we can employ intelligent circuit design to test fundamental neural network hypotheses. We aim to tackle this challenge using the bottom-up neuroscience approach, in which we engineer biological neuronal networks *in vitro* with precise architectures and connectivity using polydimethylsiloxane (PDMS) microstructures. Such a system enables the analysis of neuronal information processing at a much more simplified level with only a few neurons[1,2]. We have developed methods to ahere PDMS microstructures to complementary metal-oxide-semiconductor (CMOS) microelectrode arrays, that provide up to 26'400 microelectrodes[3]. These platforms allow to study engineered neuronal networks with unprecedented spatial resolution. We show that we can reliably adhere PDMS microstructures to CMOS MEAs and guide axonal growth with high precision. We demonstrate that we can maintain viable rat primary neuron cultures for several weeks and obtain signals with high signal-to-noise ratio across the whole microstructure[4]. **References**:
[1] Forró et al. Biosensors and Bioelectronics, 122, 75–87 (2018)
[2] Ihle et al. Biosensors and Bioelectronics, 201: 113896 (2021)
[3] Müller et al. Lab Chip, 15, 2767-2780.3 (2015)
[4] Duru et al. Frontiers in Neuroscience, 16:829884 (2022)

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BOARD NUMBER: S04-141

MAXLAB LIVE AXONTRACKING ASSAY: LABEL-FREE IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF AXONS IN NEURONAL NETWORKS AT HIGH-THROUGHPUT

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Axons enable neuronal communication by propagating electrical signals within neuronal networks. Its dysfunction plays a central role in deliberating pathologies such as Parkinson's Disease and Amyotrophic Lateral Sclerosis. Therefore, access to axonal physiology is crucial for studying information processing within neuronal networks and accelerating drug development for neurological disorders. High-density microelectrode array (HD-MEA) technology enables chronic label-free *in-vitro* extracellular recordings of axonal action potentials in neurons. MaxOne (single-) and MaxTwo (multi-well) HD-MEA Systems (MaxWell Biosystems, Switzerland) simultaneously capture fast propagating action potentials along multiple axons. Here, we present the MaxLab Live AxonTracking Assay, a software which automatically detects and functionally characterizes axonal signals across hundreds of neurons within a network. We reliably identified and measured from axonal arbors in primary neuronal cultures as well as human iPSC-derived glutamatergic and motor neurons over multiple weeks. We tracked the signal propagation to deduce the conduction velocity, axonal length, and number of axonal branches. We found that the neuronal and branch propagation velocity significantly increased between DIV 13 and 20, corresponding to the maturation of the neuronal network. In conclusion, MaxLab Live AxonTracking Assay combined with HD-MEA technology enables reliable access to electrophysiological recordings of axons, providing a novel functional phenotype for neurological disease modelling and drug screening studies.

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BOARD NUMBER: S04-142

THIN FLEXIBLE ARRAYS FOR LONG-TERM MULTI-ELECTRODE RECORDINGS IN MACAQUE PRIMARY VISUAL CORTEX

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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The need for large-scale invasive recordings has increased with the increasing interest in brain-machine interfaces (BMI). Chronically implanted multi-electrode arrays (MEAs) are used because hundreds of neurons can be recorded for a long period of time. However, most MEAs are made from rigid materials, which are less compliant with the brain tissue, with short shafts and containing only 1 electrode, making it impossible to reach deeper cortical areas and increase the spatial resolution of the recordings. Here, we developed and tested thin (typically 20 by 80 microns diameter) flexible MEAs with one to three electrodes per shaft, inserted in the primary visual cortex of 2 macaque monkeys (92 in monkey 1 and 103 electrodes in monkey 2). To allow manual insertion of the floating arrays, we temporarily strengthened the shafts with a bio-degradable polylactic-co-glycolic acid (PLGA) coating. A high-resolution Magnetic Resonance Imaging (MRI) scan demonstrated the MRI compatibility of the arrays. We observed clear single-unit activity on 40% of the electrodes, and multi-unit activity (MUA) on 60-100% of the electrodes. Even after 5 months, we measured significant MUA responses on 60-90% of the electrodes, with highly stable receptive fields. Histological analysis 3 months after implantation in monkey 1 showed minimal scar tissue formation around the implantation site. In conclusion, we achieved long-term recordings of neural responses with thin, MRI compatible, flexible arrays that can reach deeper cortical areas. These new implants offer essential advantages for future BMIs in terms of reduced scar tissue formation, high scalability and large brain coverage.

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BOARD NUMBER: S04-143

TRANSCRANIAL DIRECT CURRENT STIMULATION EFFECTS ACROSS MOTOR CORTEX LAYERS IN AWAKE MICE

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Previous studies have demonstrated the clinical value of transcranial direct current stimulation (tDCS) for modulating sensory, motor, and cognitive functions, nevertheless, knowledge about how external electric fields affect different components of neuronal networks is still not completely clear, and in vivo animal models are not fully developed. For this reason, we aimed to establish a mouse model of M1-tDCS that replicates acute-effects and after-effects of stimulation observed in humans. To evaluate the impact of tDCS, we recorded electrical activity in M1 of alert mice during and after M1-tDCS. Neuronal activity was recorded from layers 2-3, 5 and 6, evoked by stimulation of the ventral-lateral-nucleus of the thalamus (VAL). M1-tDCS was applied at 50,100,200 and 300 µA for 5s to test the acute-effects on neuronal excitability, and during 15min to analyze after-effects. Acute M1-tDCS increased and decreased the amplitude of the VAL-evoked-potentials in a polarity- and intensity-dependent manner. We observed an increase of evoked-potentials for anodal stimulation and a decrease for cathodal stimulation. For 15min of anodal or cathodal tDCS, there was a similar polarity- and intensity-dependent modulation of the VAL-evoked-potential amplitude during the 15-min of stimulation for all intensities tested, but when tDCS was switched off, only protocols with higher intensities induced a robust after-effect during one hour after stimulation. The current study demonstrates the feasibility of a mouse model of M1-tDCS to accomplish similar modulatory effects of tDCS as observed in human experiments. A proper adjustment of tDCS parameters is required to obtain these translational effects.

Pubmed:
34593517: Fernández M, Sánchez-León CA, Llorente J, Sierra-Arregui T, Knafo S, Márquez-Ruiz J, Peñagarikano O
Altered Cerebellar Response to Somatosensory Stimuli in the Mouse Model of Autism.
Atypical sensory processing is currently included within the diagnostic criteria of autism. The cerebellum is known to integrate sensory inputs of different modalities through its connectivity to the cerebral cortex. Interestingly, cerebellar malformations are among the most replicated features found in postmortem brain of individuals with autism. We studied sensory processing in the cerebellum in a mouse model of autism, knock-out (KO) for the gene. is widely expressed in Purkinje cells (PCs) and has been recently reported to regulate their morphology. Further, individuals with mutations display cerebellar malformations and CNTNAP2 antibodies are associated with a mild form of cerebellar ataxia. Previous studies in the mouse model show an altered cerebellar sensory learning. However, a physiological analysis of cerebellar function has not been performed yet. We studied sensory evoked potentials in cerebellar Crus I/II region on electrical stimulation of the whisker pad in alert mice and found striking differences between wild-type and KO mice. In addition, single-cell recordings identified alterations in both sensory-evoked and spontaneous firing patterns of PCs. These changes were accompanied by altered intrinsic properties and morphologic features of these neurons. Together, these results indicate that the mouse model could provide novel insight into the pathophysiological mechanisms of autism core sensory deficits.
eNeuro, 2021 Sep-Oct; 8
34167661: Sánchez-León CA, Sánchez-López Á, Gómez-Climent MA, Cordones I, Cohen Kadosh R, Márquez-Ruiz J
Impact of chronic transcranial random noise stimulation (tRNS) on GABAergic and glutamatergic activity markers in the prefrontal cortex of juvenile mice.
Transcranial random noise stimulation (tRNS), a non-invasive neuromodulatory technique capable of altering cortical activity, has been proposed to improve the signal-to-noise ratio at the neuronal level and the sensitivity of the neurons following an inverted U-function. The aim of this study was to examine the effects of tRNS on vGLUT1 and GAD 65-67 and its safety in terms of pathological changes. For that, juvenile mice were randomly distributed in three different groups: "tRNS 1×" receiving tRNS at the density current used in humans (0.3A/m, 20min), "tRNS 100×" receiving tRNS at two orders of magnitude higher (30.0A/m, 20min) and "sham" (0.3A/m, 15s). Nine tRNS sessions during 5 weeks were administered to the prefrontal cortex of awake animals. No detectable tissue macroscopic lesions were observed after tRNS sessions. Post-stimulation immunohistochemical analysis of GAD 65-67 and vGLUT1 immunoreactivity showed reduced GAD 65-67

immunoreactivity levels in the region directly beneath the electrode for tRNS 1× group with no significant effects in the tRNS 100× nor sham group. The observed results suggest an excitatory effect associated with a decrease in GABA levels in absence of major histopathological alterations providing a novel mechanistic explanation for tRNS effects.
Prog Brain Res, 2021; 264
33542338: Sánchez-León CA, Cordones I, Ammann C, Ausín JM, Gómez-Climent MA, Carretero-Guillén A, Sánchez-Garrido Campos G, Gruart A, Delgado-García JM, Cheron G, Medina JF, Márquez-Ruiz J
Immediate and after effects of transcranial direct-current stimulation in the mouse primary somatosensory cortex.
Transcranial direct-current stimulation (tDCS) is a non-invasive brain stimulation technique consisting in the application of weak electric currents on the scalp. Although previous studies have demonstrated the clinical value of tDCS for modulating sensory, motor, and cognitive functions, there are still huge gaps in the knowledge of the underlying physiological mechanisms. To define the immediate impact as well as the after effects of tDCS on sensory processing, we first performed electrophysiological recordings in primary somatosensory cortex (S1) of alert mice during and after administration of S1-tDCS, and followed up with immunohistochemical analysis of the stimulated brain regions. During the application of cathodal and anodal transcranial currents we observed polarity-specific bidirectional changes in the N1 component of the sensory-evoked potentials (SEPs) and associated gamma oscillations. On the other hand, 20 min of cathodal stimulation produced significant after-effects including a decreased SEP amplitude for up to 30 min, a power reduction in the 20-80 Hz range and a decrease in gamma event related synchronization (ERS). In contrast, no significant changes in SEP amplitude or power analysis were observed after anodal stimulation except for a significant increase in gamma ERS after tDCS cessation. The polarity-specific differences of these after effects were corroborated by immunohistochemical analysis, which revealed an unbalance of GAD 65-67 immunoreactivity between the stimulated versus non-stimulated S1 region only after cathodal tDCS. These results highlight the differences between immediate and after effects of tDCS, as well as the asymmetric after effects induced by anodal and cathodal stimulation.
Sci Rep, 2021; 11
30272042: Sánchez-León CA, Sánchez-López Á, Ammann C, Cordones I, Carretero-Guillén A, Márquez-Ruiz J
Exploring new transcranial electrical stimulation strategies to modulate brain function in animal models.
Transcranial electrical stimulation (tES) refers to a group of non-invasive brain stimulation techniques to induce changes in the excitability of cortical neurons in humans. In recent years, studies in animal models have been shown to be essential for disentangling the neuromodulatory effects of tES, defining safety limits, and exploring potential therapeutic applications in neurological and neuropsychiatric disorders. Testing in animal models is valuable for the development of new unconventional protocols intended to improve tES administration and optimize the desired effects by increasing its focality and enabling deep-brain stimulation. Successful and controlled application of tES in humans relies on the knowledge acquired from studies meticulously performed in animal models.
Curr Opin Biomed Eng, 2018; 8
30013890: Sánchez-León CA, Ammann C, Medina JF, Márquez-Ruiz J
Using animal models to improve the design and application of transcranial electrical stimulation in humans.
Transcranial electrical stimulation (tES) is a non-invasive stimulation technique used for modulating brain function in humans. To help tES reach its full therapeutic potential, it is necessary to address a number of critical gaps in our knowledge. Here, we review studies that have taken advantage of animal models to provide invaluable insight about the basic science behind tES.
Curr Behav Neurosci Rep, 2018; 5

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CHARACTERISATION OF SEIZURE-SPREADING DEPOLARISATION INTERACTIONS IN AWAKE-HEADFIXED MICE USING MULTISITE GRAPHENE SOLUTION-GATED FIELD EFFECT TRANSISTOR ARRAYS COMBINED WITH CA2+ IMAGING.

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Multiple clinical and experimental studies have reported concurrent cortical seizures and spreading depolarisation (SD). Nonetheless, the relationship between these phenomena remains enigmatic. During epileptic paroxysms, SD-induced activity suppression has been observed to result in seizure termination. However, other studies have observed an increase in epileptiform discharges immediately following SD. To dissect interactions between ictal discharges, seizures and SD waves, we developed a method to characterise the spatial propagation and interaction of these events in the cortex of awake mice. We expressed GCaMP7f bilaterally, and recorded the propagation of picrotoxin-induced seizures and SDs through different cortical regions using widefield imaging. Moreover, we simultaneously recorded from novel multisite graphene solution-gated field effect transistor (gSGFET) arrays; which allowed recording of full-bandwidth electrophysiological signals. To examine the temporal relationship between seizures and SD, we developed an automated analysis pipeline to characterize events based on origin and propagation. For each event, we extracted multiple properties and performed unbiased analysis. We resolve the full-bandwidth gSGFET recordings into physiological frequency bands and investigate the power of each during different events. This allowed correlation of cortical oscillations with activity throughout both hemispheres. Using these methods, we were able to perform high-throughput event analyses to examine the diversity of ictal-associated DC-events. The methodology presented in this study provides a novel approach to investigate the relationship between SD and seizures. We envision that combined calcium imaging and gSGFET recordings will provide unparalleled insight into seizure initiation and termination; and will facilitate the development of novel therapeutic avenues and technology.

Pubmed:
33690187: Masvidal-Codina E, Smith TM, Rathore D, Gao Y, Illa X, Prats-Alfonso E, Corro ED, Calia AB, Rius G, Martin-Fernandez I, Guger C, Reitner P, Villa R, Garrido JA, Guimerà-Brunet A, Wykes RC
Characterization of optogenetically-induced cortical spreading depression in awake mice using graphene micro-transistor arrays.
The development of experimental methodology utilizing graphene micro-transistor arrays to facilitate and advance translational research into cortical spreading depression (CSD) in the awake brain.CSDs were reliably induced in awake nontransgenic mice using optogenetic methods. High-fidelity DC-coupled electrophysiological mapping of propagating CSDs was obtained using flexible arrays of graphene soultion-gated field-effect transistors (gSGFETs).Viral vectors targetted channelrhopsin expression in neurons of the motor cortex resulting in a transduction volume ⩾1 mm. 5-10 s of continous blue light stimulation induced CSD that propagated across the cortex at a velocity of 3.0 ± 0.1 mm min. Graphene micro-transistor arrays enabled high-density mapping of infraslow activity correlated with neuronal activity suppression across multiple frequency bands during both CSD initiation and propagation. Localized differences in the CSD waveform could be detected and categorized into distinct clusters demonstrating the spatial resolution advantages of DC-coupled recordings. We exploited the reliable and repeatable induction of CSDs using this preparation to perform proof-of-principle pharmacological interrogation studies using NMDA antagonists. MK801 (3 mg kg) suppressed CSD induction and propagation, an effect mirrored, albeit transiently, by ketamine (15 mg kg), thus demonstrating this models' applicability as a preclinical drug

screening platform. Finally, we report that CSDs could be detected through the skull using graphene micro-transistors, highlighting additional advantages and future applications of this technology.CSD is thought to contribute to the pathophysiology of several neurological diseases. CSD research will benefit from technological advances that permit high density electrophysiological mapping of the CSD waveform and propagation across the cortex. We report anassay that permits minimally invasive optogenetic induction, combined with multichannel DC-coupled recordings enabled by gSGFETs in the awake brain. Adoption of this technological approach could facilitate and transform preclinical investigations of CSD in disease relevant models.
J Neural Eng, 2021; 18

34937934: Bonaccini Calia A, Masvidal-Codina E, Smith TM, Schäfer N, Rathore D, Rodríguez-Lucas E, Illa X, De la Cruz JM, Del Corro E, Prats-Alfonso E, Viana D, Bousquet J, Hébert C, Martínez-Aguilar J, Sperling JR, Drummond M, Halder A, Dodd A, Barr K, Savage S, Fornell J, Sort J, Guger C, Villa R, Kostarelos K, Wykes RC, Guimerà-Brunet A, Garrido JA
Full-bandwidth electrophysiology of seizures and epileptiform activity enabled by flexible graphene microtransistor depth neural probes.
Mapping the entire frequency bandwidth of brain electrophysiological signals is of paramount importance for understanding physiological and pathological states. The ability to record simultaneously DC-shifts, infraslow oscillations (<0.1 Hz), typical local field potentials (0.1-80 Hz) and higher frequencies (80-600 Hz) using the same recording site would particularly benefit preclinical epilepsy research and could provide clinical biomarkers for improved seizure onset zone delineation. However, commonly used metal microelectrode technology suffers from instabilities that hamper the high fidelity of DC-coupled recordings, which are needed to access signals of very low frequency. In this study we used flexible graphene depth neural probes (gDNPs), consisting of a linear array of graphene microtransistors, to concurrently record DC-shifts and high-frequency neuronal activity in awake rodents. We show here that gDNPs can reliably record and map with high spatial resolution seizures, pre-ictal DC-shifts and seizure-associated spreading depolarizations together with higher frequencies through the cortical laminae to the hippocampus in a mouse model of chemically induced seizures. Moreover, we demonstrate the functionality of chronically implanted devices over 10 weeks by recording with high fidelity spontaneous spike-wave discharges and associated infraslow oscillations in a rat model of absence epilepsy. Altogether, our work highlights the suitability of this technology for in vivo electrophysiology research, and in particular epilepsy research, by allowing stable and chronic DC-coupled recordings.
Nat Nanotechnol, 2022; 17

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BOARD NUMBER: S04-145

A NOVEL MULTIMODAL APPROACH FOR PROBING SYNAPTIC CONNECTIVITY AND FUNCTION

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Numerous synaptic processes, such as long-term synaptic plasticity and short-term modulations of transmission efficacy, can depend on the spiking patterns of the corresponding pre- and postsynaptic cells. Some progress has been made towards revealing the underlying principles of such regulations by identifying and monitoring monosynaptic connectivity. However, existing techniques do not provide both the precise spiking sequence of the presynaptic cell and additional information on structural (e.g., accurate location of the synapse, synapse size) or functional properties (e.g., transmission) of the synapse. Here, we introduce a novel multimodal approach to overcome this limitation by combining high-resolution dendritic spine Ca^{2+} imaging with simultaneous large-scale recording of extracellular spiking by means of high-density microelectrode arrays (HD-MEAs). Primary rat cortical neurons, sparsely expressing jGCaMP7b, were plated on a CMOS-based HD-MEA that features 26’400 electrodes at 17.5 µm pitch and allows for simultaneous recording from up to 1’024 electrodes. A spinning-disc upright confocal microscope was employed to perform simultaneous Ca^{2+} imaging from target dendritic spines of the postsynaptic cell. We developed an open-source analysis pipeline to identify the presynaptic unit of a specific dendritic spine based on correlation of pre- and postsynaptic activity. Moreover, we introduce a surrogate-based statistical test to identify only trustworthy connections. Finally, we used data-driven simulations to validate and optimize this approach and demonstrate that it can successfully infer connections in *in vitro* recordings. The presented method represents a versatile tool to characterize synaptic properties and processes, including activity-dependent synaptic development, spatial synaptic organization, and synaptic interactions.

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BOARD NUMBER: S04-146

MAGNESIUM FLUORIDE THIN FILMS AS COVER LAYER FOR MULTI-ELECTRODE ARRAY TECHNOLOGY AIMING TO COMBINE NEURONAL NETWORK RECORDING AND SUPER-RESOLUTION MICROSCOPY

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Aims: Combination of functional and optical analysis becomes increasingly important in neuroscience. Here, we present an approach for fabrication of reproducible, chemically and mechanically robust functionalized multi-electrode array (MEA) based on indium tin oxide (ITO) and magnesium fluoride (MgF_2) thin films on thin glass substrates. These new MEAs are especially suited for combination with a new super-resolution microscopy (SRM) technique also shown here. **Methods:** The MgF_2 layers were deposited using electron beam evaporation. The ITO layers using sputtering. The structuring of films was performed by photolithography and ion beam etching. The film properties of these MEAs were analysed by atomic-force microscopy, scanning-electron microscopy, X-ray diffraction, electrical 4-point- and spectral transmission measurements. Finally, we tested the functionalization of the MgF_2 layer by using an immunostaining of 10 µm brain slices for direct stochastic optical reconstruction microscopy (*d*STORM) recording. **Results:** We found that the transmittance of the coated substrates is adjustable by the layer thickness. Brain slices were adherent to the MgF2 layers. By analysis of synaptic structures with *d*STORM we found similar localization properties compared to regularly coated cover slips. The conductivity measurements showed no isolating defects or leakage currents for the 110 nm MgF_2 layer. **Conclusions:** In summary, this approach may serve as a basis for further developments with respect to the simultaneous use of optical and electrophysiological *in-vitro* experiments. The possibility to correlate microscopy with electrophysiology is an important step towards understanding the molecular mechanisms of neuronal and synaptic function and of molecular pathophysiology in neurological diseases.

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BOARD NUMBER: S04-147

ASTROCYTES REGULATE DRUG-INDUCED HYPERACTIVITY IN NEURON-ASTROCYTE CO-CULTURES ON MICROELECTRODE ARRAYS.

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Astrocytes regulate neuronal network maturation and function and control neuronal electrochemical activity by secreting and circulating excitatory and inhibitory transmitters. Therefore, astrocyte dysfunctions and resulting abnormal neuronal function and homeostasis can be linked to diseases such as epilepsy. We studied the regulatory effects of astrocytes in drug-induced epileptic-like activity. We cultured rat cortical neuron-astrocyte co-cultures with different neuron-astrocyte ratios on microelectrode arrays. The cell ratios ranged from 'pure' neuronal cultures without separately added astrocytes up to 50/50 percent of neuron/astrocyte co-cultures. Neuronal activity was recorded weekly, and cultures were stimulated with gamma-aminobutyric acid type A receptor ($GABA_AR$) blocker gabazine at 28 days *in vitro*. Astrocytes enhanced neuronal maturation during the first weeks *in vitro* and significantly affected neuronal connectivity and drug efficacy depending on the astrocyte numbers in the culture. The drug showed its efficacy later in time with the increasing ratio of astrocytes in the culture; the 50/50 co-culture presented a delay of drug action of about 200s. Furthermore, the effect size of the drug on the connectivity weights appeared higher for co-cultures with higher numbers of astrocytes. Different neuron/astrocyte ratios could model different brain areas and, as our results suggest, different seizure susceptibilities in the brain. The diverse responses to drug-induced hyper-synchronization in the co-cultures elucidates the importance of considering the different proportions of astrocytes in *in vitro* epilepsy models. Acknowledgment: The project has received funding from the European Union's Horizon 2020 research and innovation programme, grant agreement No. 824164.

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BOARD NUMBER: S04-148

A 3D-PRINTED NEURAL IMPLANT FOR EXTRACELLULAR RECORDINGS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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The main factors for a successful electrode implantation and a successful electrophysiological recording are the precise positioning of the electrode, the stability of the implant and a fast operation procedure. Here we introduce a novel 3D-printed modular implant. The implant consists of a base that is individually designed using an MRI of the subject to achieve exact fit to the skull. This base can contain multiple sites where electrodes, connectors or reference screws can be attached. All components can be placed before, during or after the initial implantation to allow flexible change of recording site or electrode after the initial operation. Our approach also allows the minimally invasive replacement or repositioning of electrodes. Breaking up complicated procedures into multiple shorter procedures can also be a feasible way to reduce duration of anesthesia. Our new design also offers an easy system to securely attach wired and wireless headstages. All in all, this novel approach can reduce the duration of surgeries by bringing fully assembled implants to the OR and it allows adjustments in minimally invasive procedures including the partial explantation between experiments. Keywords: Implant, 3D printing, extracellular recording, operation

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-149

NOVEL MULTI-SENSOR ORIGAMI PLATFORM FOR IN-VITRO BRAIN MODELS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Advances in the field of tissue engineering enables better emulation of the human body by creating multi-cellular 3D in-vitro models. To better understand brain functionality and find therapeutic solutions, the neuro-vascular-unit (NVU) is being investigated worldwide. While complex 3D brain structures are fabricated by bio-printing and other methods; the monitoring of these models is very challenging and there is a lack of sensing solutions. To address this need we developed a multi-sensory platform that could be customized for each brain model. In our platform we combine flexible chip structure, a permeability sensor: trans-endothelial electrical resistance (TEER) and electrical activity sensors: 3D multi electrode array (MEA); all to advance brain research. To establish this platform we designed and fabricated an assembled chip which its hinges facilitates the folding of the structure around the printed NVU, like origami. This design allows minimum harm to the tissue. The placing of the electrodes is designed in advance in a complementary way to the biological structure, enabling exact location in relation to the blood vessel and the parenchymal tissue. The platform is easy to handle with common tissue culture equipment and the model is visually accessible via microscope. To make the platform more useful and meaningful for the scientific community the 3D electrical sensing electrodes are connected to a commercial MEA system. By giving access to the molecular brain this platform could advance basic physiological research, disease investigation as well as drug testing.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-150

AN EIGHTEEN-CHANNEL MULTIELECTRODE ARRAY FOR COMBINING EPIDURAL RECORDINGS AND OPTOGENETIC STIMULATION IN THE MOUSE BRAIN.

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Because of their low invasiveness and high spatiotemporal resolution, high-density epidural multielectrode arrays (hd-EMAs) constitute a key technology for future clinical applications. In combination with cortical stimulation, monitoring ongoing brain activity with chronically implanted arrays can serve to manipulate pathological brain states in targeted manner. We here introduce a new multielectrode array with eighteen electrode contacts on a small polyimide patch with 3 mm diameter, arranged around a central opening for an optical fibre for combining chronic epidural recordings with optogenetic stimulation in the mouse model. The hd-EMA was fabricated on top of a 100 mm oxidized silicon wafer by applying a thin layer of the polyimide U-Varnish S (UBE Europe GmbH, Germany) onto the wafer's surface, followed by sputtering a 300 nm gold layer on top of it. Electrode thickness was increased by 2.7 µm via electroplating, resulting in a total thickness of 3 µm. After structuring the design of the connector tracks, a quick oxygen plasma treatment via reactive ion etching (RIE) served to improve the adhesion between both polyimide layers. Finally, a layer of polyimide U-Varnish S was applied and structured, resulting in a flexible, polyimide-encapsulated multielectrode array. Each electrode has a diameter of 280 µm. Electrodes are arranged around a central opening of 300 µm diameter for the optical fibre and have an inter-electrode, center-to-center spacing of 565 – 1390 µm. We coated half of all electrodes with the electrically conductive polymer PEDOT:PSS to further analyze its influence on signal quality. Supported by DFG WE 5469/3-1.

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BOARD NUMBER: S04-151

MICROPORED ELECTRODES FOR IMPROVED BIOCOMPATIBILITY AND NEURONAL ATTACHMENT IN IMPLANTABLE BRAIN ELECTRODE ARRAYS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Thin-film flexible microelectrode arrays gained attention in neurophysiology for their minimal tissue displacement and their high compliance compared to silicon probes, which minimize subsequent tissue inflammation. However, their flat structure disrupts the 3D distribution of cells and hinders transport of molecules. In this work, we design and fabricate flexible multielectrode arrays with micropored torus-shaped electrodes, with the aim of allowing interpenetration and adhesion of neurons inside the pored electrode. Further, we aim to promote the natural diffusion of important molecules and signaling species through the pores, improving biocompatibility. The fabrication of these electrodes involves chemical vapor deposition of parylene-C, photolithographic patterning of gold, and dry etching to open the through-hole in the active sites. The pored electrodes are further galvanized into a torus shape to increase surface area. As a proof of principle, we test the devices for biocompatibility both with cardiomyocyte-like cells in-vitro and in locusts in-vivo. The resulting device is 8 µm thin and compliant with neural tissue. The electrode openings are through-hole circular gold structures with an inner diameter of 15 µm, comparable to neuronal cells. In-vitro, cardiomyocyte-like cells adhere to the electrode surface and inside the gold torus pores. We further demonstrate successful in-vivo, long-term implantation of the probes in locusts' neural tissue. In conclusion, the micropored electrodes allow interpenetration of cells and diffusion of molecules. Micropore-patterning of the electrode's substrate will further yield a mesh-like structure, which we expect will integrate seamlessly with the brain tissue.

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BOARD NUMBER: S04-152

ON-DEMAND OPTOGENETIC-INDUCTION OF SEIZURES AND ITS CHARACTERISATION IN A MOUSE MODEL OF FOCAL CONDITIONAL KNOCKOUT OF KIR4.1 CHANNELS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Kir4.1 inwardly-rectifying potassium channels play a key role in maintaining physiological levels of extracellular potassium ions in the brain and are exclusively expressed in glial cells in CNS. Kir4.1 channel levels are reduced in TLE patients and loss-of-function mutations in Kir4.1 encoding *KCNJ10* gene were reported to cause EAST/SeSAME epileptic disorders. Global knockout of Kir4.1 in mice were shown to be lethal with lifespan of only 3-4 post-natal weeks. This hampers our ability to understand the activity and properties of an epileptic network lacking Kir4.1. Furthermore, the shorter lifespan restricts the ability to record and modulate multiple seizures. To overcome the above problems, we have investigated targeted regional knockout in hippocampus of adult *kcnj10*-floxed mice. 24x7 telemetry recordings indicate that these animals develop spontaneous seizures 7-14 days after injection of cre-expressing viral constructs under astrocyte-specific promoters. To examine the Kir4.1 knockout effects on ultraslow potential shifts associated with seizures, i.e., active DC-shifts and post-ictal spreading depolarisation, we used an awake head-fixed setup, with graphene transistors capable of wideband recordings. Recorded spontaneous and optogenetically-evoked seizures and epileptiform activity. Optogenetic-activation of pyramidal neurons induced seizures in a stimulation frequency- and duration-dependent manner. Furthermore, almost all seizures induced were observed to be associated with large ultraslow potential shifts and their metrics were analysed. Here, we report a novel method to record spontaneous seizures and optogenetically induce seizures on-demand in a focal conditional Kir4.1 knockout mouse model that will provide insights into the role Kir4.1 plays in seizure and seizure and spreading depolarisation susceptibility.

Pubmed:

33769452: Hristova K, Martinez-Gonzalez C, Watson TC, Codadu NK, Hashemi K, Kind PC, Nolan MF, Gonzalez-Sulser A
Medial septal GABAergic neurons reduce seizure duration upon optogenetic closed-loop stimulation.
Seizures can emerge from multiple or large foci in temporal lobe epilepsy, complicating focally targeted strategies such as surgical resection or the modulation of the activity of specific hippocampal neuronal populations through genetic or optogenetic techniques. Here, we evaluate a strategy in which optogenetic activation of medial septal GABAergic neurons, which provide extensive projections throughout the hippocampus, is used to control seizures. We utilized the chronic intrahippocampal kainate mouse model of temporal lobe epilepsy, which results in spontaneous seizures and as is often the case in human patients, presents with hippocampal sclerosis. Medial septal GABAergic neuron populations were immunohistochemically labelled and were not reduced in epileptic conditions. Genetic labelling with mRuby of medial septal GABAergic neuron synaptic puncta and imaging across the rostral to caudal extent of the hippocampus, also indicated an unchanged number of putative synapses in epilepsy. Furthermore, optogenetic stimulation of medial septal GABAergic neurons consistently modulated oscillations across multiple hippocampal locations in control and epileptic conditions. Finally, wireless optogenetic stimulation of medial septal GABAergic neurons, upon electrographic detection of spontaneous hippocampal seizures, resulted in reduced seizure durations. We propose medial septal GABAergic neurons as a novel target for optogenetic control of seizures in temporal lobe epilepsy.
Brain, 2021; 144
31587522: Codadu NK, Graham RT, Burman RJ, Jackson-Taylor RT, Raimondo JV, Trevelyan AJ, Parrish RR
Divergent paths to seizure-like events.
Much debate exists about how the brain transitions into an epileptic seizure. One source of confusion is that there are likely to be critical differences between experimental seizure models. To address this, we have compared the evolving activity patterns in two widely used in vitro models of epileptic discharges. Brain slices from young adult mice were prepared in the same way and bathed either in 0 Mg or 100 µmol/L 4AP artificial cerebrospinal fluid. We have found that while local field

potential recordings of epileptiform discharges in the two models appear broadly similar, patch-clamp analysis reveals an important difference in the relative degree of glutamatergic involvement. 4AP affects parvalbumin-expressing interneurons more than other cortical populations, destabilizing their resting state and inducing spontaneous bursting behavior. Consequently, the most prominent pattern of transient discharge ("interictal event") in this model is almost purely GABAergic, although the transition to seizure-like events (SLEs) involves pyramidal recruitment. In contrast, interictal discharges in 0 Mg are only maintained by a very large glutamatergic component that also involves transient discharges of the interneurons. Seizure-like events in 0 Mg have significantly higher power in the high gamma frequency band (60-120Hz) than these events do in 4AP, and are greatly delayed in onset by diazepam, unlike 4AP events. We, therefore, conclude that the 0 Mg and 4AP models display fundamentally different levels of glutamatergic drive, demonstrating how ostensibly similar pathological discharges can arise from different sources. We contend that similar interpretative issues will also be relevant to clinical practice.
Physiol Rep, 2019; 7
31553050: Burman RJ, Selfe JS, Lee JH, van den Berg M, Calin A, Codadu NK, Wright R, Newey SE, Parrish RR, Katz AA, Wilmshurst JM, Akerman CJ, Trevelyan AJ, Raimondo JV
Excitatory GABAergic signalling is associated with benzodiazepine resistance in status epilepticus.
Status epilepticus is defined as a state of unrelenting seizure activity. Generalized convulsive status epilepticus is associated with a rapidly rising mortality rate, and thus constitutes a medical emergency. Benzodiazepines, which act as positive modulators of chloride (Cl-) permeable GABAA receptors, are indicated as first-line treatment, but this is ineffective in many cases. We found that 48% of children presenting with status epilepticus were unresponsive to benzodiazepine treatment, and critically, that the duration of status epilepticus at the time of treatment is an important predictor of non-responsiveness. We therefore investigated the cellular mechanisms that underlie acquired benzodiazepine resistance, using rodent organotypic and acute brain slices. Removing Mg2+ ions leads to an evolving pattern of epileptiform activity, and eventually to a persistent state of repetitive discharges that strongly resembles clinical EEG recordings of status epilepticus. We found that diazepam loses its antiseizure efficacy and conversely exacerbates epileptiform activity during this stage of status epilepticus-like activity. Interestingly, a low concentration of the barbiturate phenobarbital had a similar exacerbating effect on status epilepticus-like activity, while a high concentration of phenobarbital was effective at reducing or preventing epileptiform discharges. We then show that the persistent status epilepticus-like activity is associated with a reduction in GABAA receptor conductance and Cl- extrusion capability. We explored the effect on intraneuronal Cl- using both gramicidin, perforated-patch clamp recordings and Cl- imaging. This showed that during status epilepticus-like activity, reduced Cl- extrusion capacity was further exacerbated by activity-dependent Cl- loading, resulting in a persistently high intraneuronal Cl-. Consistent with these results, we found that optogenetic stimulation of GABAergic interneurons in the status epilepticus-like state, actually enhanced epileptiform activity in a GABAAR dependent manner. Together our findings describe a novel potential mechanism underlying benzodiazepine-resistant status epilepticus, with relevance to how this life-threatening condition should be managed in the clinic.
Brain, 2019; 142
31483698: Salar S, Guhathakurta D, Marx Hofmann L
Differential contribution of pyramidal cells and interneurons to activity-dependent gene transcription changes.
The type of neuronal activity determines the outcome of gene expression. Hence, the characterization of underlying mechanisms in transcriptome alterations may serve as a biomarker and provide new intervention methods for the treatment of pathologic conditions. Parrish et al. (Parrish RR, Codadu NK, Racca C, Trevelyan AJ. 120: 2358-2367, 2018) show that the changes in interneuronal gene transcription are correlated with the type of the activated neuronal population and that the initiation route of Ras/ERK MAPK pathway determines the polarity of the gene expression.
J Neurophysiol, 2019; 122
30784081: Parrish RR, Codadu NK, Mackenzie-Gray Scott C, Trevelyan AJ
Feedforward inhibition ahead of ictal wavefronts is provided by both parvalbumin- and somatostatin-expressing interneurons. There is a rapid interneuronal response to focal activity in cortex, which restrains laterally propagating activity, including spreading epileptiform activity. The interneuronal response involves intense activation of both parvalbumin- and somatostatin-expressing interneurons. Interneuronal bursting is time-locked to glutamatergic barrages in the pre-ictal period. Ca imaging using conditional expression of GCaMP6f provides an accurate readout of the evolving firing patterns in both types of interneuron. The activation profiles of the two interneuronal classes are temporally offset, with the parvalbumin population being activated first, and typically, at higher rates.
J Physiol, 2019; 597
30681139: Codadu NK, Parrish RR, Trevelyan AJ
Region-specific differences and areal interactions underlying transitions in epileptiform activity.
Local neocortical and hippocampal territories show different and sterotypical patterns of acutely evolving, epileptiform activity.

Neocortical and entorhinal networks show tonic-clonic-like events, but the main hippocampal territories do not, unless it is relayed from the other areas. Transitions in the pattern of locally recorded epileptiform activity can be indicative of a shift in the source of pathological activity, and may spread through both synaptic and non-synaptic means. Hippocampal epileptiform activity is promoted by 4-aminopyridine and inhibited by GABA receptor agonists, and appears far more sensitive to these drugs than neocortical activity. These signature features of local epileptiform activity can provide useful insight into the primary source of ictal activity, aiding both experimental and clinical investigation.
J Physiol, 2019; 597
30148192: Parrish RR, Grady J, Codadu NK, Racca C, Trevelyan AJ
Graphical user interface for simultaneous profiling of activity patterns in multiple neuronal subclasses.
We provide notes on how to use a graphical user interface (GUI), implemented with MATLAB, for aligning imaging datasets of biological tissue. The original use was for matching two imaging data sets, where one set was taken of the living preparation and another was taken post-fixation and following immunohistochemical processing. This technique is described in detail in an accompanying paper (Parrish et al., [1], where we also include information about the experimental procedures, and examples of the usage of the GUI.
Data Brief, 2018; 20
30110232: Parrish RR, Codadu NK, Racca C, Trevelyan AJ
Pyramidal cell activity levels affect the polarity of activity-induced gene transcription changes in interneurons.
Changes in gene expression are an important mechanism by which activity levels are regulated in the nervous system. It is not known, however, how network activity influences gene expression in interneurons; since they themselves provide negative feedback in the form of synaptic inhibition, there exists a potential conflict between their cellular homeostatic tendencies and those of the network. We present a means of examining this issue, utilizing simple in vitro models showing different patterns of intense network activity. We found that the degree of concurrent pyramidal activation changed the polarity of the induced gene transcription. When pyramidal cells were quiescent, interneuronal activation led to an upregulation of glutamate decarboxylase 1 (GAD1) and parvalbumin (Pvalb) gene transcriptions, mediated by activation of the Ras/extracellular signal-related kinase mitogen-activated protein kinase (Ras/ERK MAPK) pathway. In contrast, coactivation of pyramidal cells led to an ionotropic glutamate receptor N-methyl-d-aspartate 2B-dependent decrease in transcription. Our results demonstrate a hitherto unrecognized complexity in how activity-dependent gene expression changes are manifest in cortical networks. NEW & NOTEWORTHY We demonstrate a novel feedback mechanism in cortical networks, by which glutamatergic drive, mediated through the Ras/ERK MAPK pathway, regulates gene transcription in interneurons. Using a unique feature of certain in vitro epilepsy models, we show that without this glutamatergic feedback, intense activation of interneurons causes parvalbumin and glutamate decarboxylase 1 mRNA expression to increase. If, on the other hand, pyramidal cells are coactivated with interneurons, this leads to a downregulation of these genes.
J Neurophysiol, 2018; 120
29588195: Parrish RR, Grady J, Codadu NK, Trevelyan AJ, Racca C
Simultaneous profiling of activity patterns in multiple neuronal subclasses.
Neuronal networks typically comprise heterogeneous populations of neurons. A core objective when seeking to understand such networks, therefore, is to identify what roles these different neuronal classes play. Acquiring single cell electrophysiology data for multiple cell classes can prove to be a large and daunting task. Alternatively, Ca network imaging provides activity profiles of large numbers of neurons simultaneously, but without distinguishing between cell classes.
J Neurosci Methods, 2018; 303
28899919: Pirttimaki TM, Sims RE, Saunders G, Antonio SA, Codadu NK, Parri HR
Astrocyte-Mediated Neuronal Synchronization Properties Revealed by False Gliotransmitter Release.
Astrocytes spontaneously release glutamate (Glut) as a gliotransmitter (GT), resulting in the generation of extrasynaptic NMDAR-mediated slow inward currents (SICs) in neighboring neurons, which can increase local neuronal excitability. However, there is a deficit in our knowledge of the factors that control spontaneous astrocyte GT release and the extent of its influence. We found that, in rat brain slices, increasing the supply of the physiological transmitter Glut increased the frequency and signaling charge of SICs over an extended period. This phenomenon was replicated by exogenous preexposure to the amino acid D-aspartate (D-Asp). Using D-Asp as a "false" GT, we determined the extent of local neuron excitation by GT release in ventrobasal thalamus, CA1 hippocampus, and somatosensory cortex. By analyzing synchronized neuronal NMDAR-mediated excitation, we found that the properties of the excitation were conserved in different brain areas. In the three areas, astrocyte-derived GT release synchronized groups of neurons at distances of >;200 μm. Individual neurons participated in more than one synchronized population, indicating that individual neurons can be excited by more than one astrocyte and that individual astrocytes may determine a neuron's synchronized network. The results confirm that astrocytes can act as excitatory nodes that can influence neurons over a significant range in a number of brain regions. Our findings further suggest that chronic elevation of ambient Glut levels can lead to increased GT Glut release, which may be

relevant in some pathological states. Astrocytes spontaneously release glutamate (Glut) and other gliotransmitters (GTs) that can modify neuronal activity. Exposing brain slices to Glut and D-aspartate (D-Asp) before recording resulted in an increase in frequency of GT-mediated astrocyte-neuron signaling. Using D-Asp, it was possible to investigate the effects of specific GT release at neuronal NMDARs. Calcium imaging showed synchronized activity in groups of neurons in cortex, hippocampus, and thalamus. The size of these populations was similar in all areas and some neurons were involved in more than one synchronous group. The findings show that GT release is supply dependent and that the properties of the signaling and activated networks are largely conserved between different brain areas.
J Neurosci, 2017; 37

FENS Forum 2022
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BOARD NUMBER: S04-153

LARGE-SCALE RECORDINGS IN THE FOREBRAIN-BASAL-GANGLIA CIRCUIT OF SINGING ZEBRA FINCHES USING CHRONICALLY IMPLANTED NEUROPIXELS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Songbirds have evolved with a network of brain areas dedicated to one specific task – learning and producing complex song. This close link between structure and behavior is a rare trait in the animal kingdom, thus presenting them as an excellent model for the study of neural motor learning and control. Research in this field, however, still lags behind technical advances of high-yield electrophysiology because songbirds are particularly small and sensitive to the weight of a chronic implant. We designed and implemented a lightweight and reusable tool that allows for chronic Neuropixels recordings in freely moving zebra finches (*Taeniopygia guttata*). We tested the implant targeting both the cortical premotor region LMAN and the basal-ganglia-like striatal area including Area X and recorded neural activity during singing from eight animals. Our preliminary data show that the birds were able to cope with the weight of the implant (1.7g) and returned to a singing rate of up to several hundreds of motif renditions per hour within a few days after the implantation surgery. Furthermore, the device enabled us to acquire a large data set with several hundred spiking units after spike sorting and manual curation, including dozens of units from LMAN and Area X. As such, our device provides the opportunity for large-scale recordings that have become a critical tool to gain insight into neural population dynamics.

Pubmed:
32776943: Yamahachi H, Zai AT, Tachibana RO, Stepien AE, Rodrigues DI, Cavé-Lopez S, Lorenz C, Arneodo EM, Giret N, Hahnloser RHR
Undirected singing rate as a non-invasive tool for welfare monitoring in isolated male zebra finches.
Research on the songbird zebra finch (Taeniopygia guttata) has advanced our behavioral, hormonal, neuronal, and genetic understanding of vocal learning. However, little is known about the impact of typical experimental manipulations on the welfare of these birds. Here we explore whether the undirected singing rate can be used as an indicator of welfare. We tested this idea by performing a post hoc analysis of singing behavior in isolated male zebra finches subjected to interactive white noise, to surgery, or to tethering. We find that the latter two experimental manipulations transiently but reliably decreased singing rates. By contraposition, we infer that a high-sustained singing rate is suggestive of successful coping or improved welfare in these experiments. Our analysis across more than 300 days of song data suggests that a singing rate above a threshold of several hundred song motifs per day implies an absence of an acute stressor or a successful coping with stress. Because singing rate can be measured in a completely automatic fashion, its observation can help to reduce experimenter bias in welfare monitoring. Because singing rate measurements are non-invasive, we expect this study to contribute to the refinement of the current welfare monitoring tools in zebra finches.
PLoS One, 2020; 15
29042433: Seillier L, Lorenz C, Kawaguchi K, Ott T, Nieder A, Pourriahi P, Nienborg H
Serotonin Decreases the Gain of Visual Responses in Awake Macaque V1.
Serotonin, an important neuromodulator in the brain, is implicated in affective and cognitive functions. However, its role even for basic cortical processes is controversial. For example, in the mammalian primary visual cortex (V1), heterogenous serotonergic modulation has been observed in anesthetized animals. Here, we combined extracellular single-unit recordings with iontophoresis in awake animals. We examined the role of serotonin on well-defined tuning properties (orientation, spatial frequency, contrast, and size) in V1 of two male macaque monkeys. We find that in the awake macaque the modulatory effect of serotonin is surprisingly uniform: it causes a mainly multiplicative decrease of the visual responses and a slight increase in the stimulus-selective response latency. Moreover, serotonin neither systematically changes the selectivity or variability of the response, nor the interneuronal correlation unexplained by the stimulus ("noise-correlation"). The modulation by serotonin has qualitative similarities with that for a decrease in stimulus contrast, but differs quantitatively from decreasing contrast. It can be captured by a simple additive change to a threshold-linear spiking nonlinearity. Together, our results show that

serotonin is well suited to control the response gain of neurons in V1 depending on the animal's behavioral or motivational context, complementing other known state-dependent gain-control mechanisms. Serotonin is an important neuromodulator in the brain and a major target for drugs used to treat psychiatric disorders. Nonetheless, surprisingly little is known about how it shapes information processing in sensory areas. Here we examined the serotonergic modulation of visual processing in the primary visual cortex of awake behaving macaque monkeys. We found that serotonin mainly decreased the gain of the visual responses, without systematically changing their selectivity, variability, or covariability. This identifies a simple computational function of serotonin for state-dependent sensory processing, depending on the animal's affective or motivational state.
J Neurosci, 2017; 37
24671172: Butz MV, Kutter EF, Lorenz C
Rubber hand illusion affects joint angle perception.
The Rubber Hand Illusion (RHI) is a well-established experimental paradigm. It has been shown that the RHI can affect hand location estimates, arm and hand motion towards goals, the subjective visual appearance of the own hand, and the feeling of body ownership. Several studies also indicate that the peri-hand space is partially remapped around the rubber hand. Nonetheless, the question remains if and to what extent the RHI can affect the perception of other body parts. In this study we ask if the RHI can alter the perception of the elbow joint. Participants had to adjust an angular representation on a screen according to their proprioceptive perception of their own elbow joint angle. The results show that the RHI does indeed alter the elbow joint estimation, increasing the agreement with the position and orientation of the artificial hand. Thus, the results show that the brain does not only adjust the perception of the hand in body-relative space, but it also modifies the perception of other body parts. In conclusion, we propose that the brain continuously strives to maintain a consistent internal body image and that this image can be influenced by the available sensory information sources, which are mediated and mapped onto each other by means of a postural, kinematic body model.
PLoS One, 2014; 9

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BOARD NUMBER: S04-154

FEASIBILITY AND FUTURE ROLE OF HIGH-DENSITY TRANSCRANIAL MAGNETIC STIMULATION (HD-TMS) IN AMYOTROPHIC LATERAL SCLEROSIS (ALS): A PILOT STUDY IN HEALTHY VOLUNTEERS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder that causes progressive paralysis and death on average within three years of symptom onset. There is currently no effective treatment, and the discovery of novel therapies is hindered by the lack of early disease progression biomarkers. Transcranial magnetic stimulation (TMS) combined with single-channel EMG has demonstrated that cortical-spinal hyperexcitability is an early pathogenic mechanism preceding irreversible muscular atrophy in ALS. However, it is still unclear how well different TMS parameters of abnormal cortico-spinal excitability track disease progression. This study piloted the novel combination of TMS with the 64-channel high-density surface EMG (HDSEMG) for the first time. Established TMS protocols probing cortical inhibition through the duration of the cortical silent period (CSP), the magnitude of short interval intracortical inhibition (SICI) and intracortical facilitation (ICF), were measured during simultaneous HDSEMG registration from the first dorsal interosseous (FDI) muscle of the dominant hand in 15 healthy volunteers (9 males, 6 females, mean age 69.3). This study indicated high data quality and the methodological validity of our novel approach, which allowed for the characterisation of CSP, SICI and ICF with better spatial resolution into a 3D anatomical map of the FDI firing. The application of these findings has the potential to improve our understanding of the topographical distribution of disinhibition or excess facilitation that has been postulated to underlie cortical hyperexcitability in ALS. Precisely determining how excitability abnormalities evolve over time may help the progression of a detailed anatomical map of disease trajectory in ALS.

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BOARD NUMBER: S04-155

LIGHTWEIGHT, REUSABLE CHRONIC IMPLANTS FOR NEUROPIXELS 2.0 PROBES

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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[Aims] Neuropixels probes have dramatically increased the number of neurons that can be acquired in a single experiment. With chronic recordings, these neurons can be tracked across days, but this typically requires the experimenter to permanently cement the probe to the skull. There is thus substantial interest in developing chronic implants that are recoverable while being light enough to be used in mice. [Methods] Here, we present the "Apollo Implant", a device for reversible chronic implantation of Neuropixels 2.0 probes. The implant comprises two modules which are combined before implantation. The payload module accommodates two probes, for a maximum of 8 shanks in the brain, and is recoverable (~€8). The docking module is cemented to the skull during implantation and is not recoverable (~€2). The implant is made of Formlabs Rigid Resin and weighs ~2.0 g with two probes. Its design is open source and can be readily adjusted to change the angle of insertion or the distance between the probes. [Results] We used the Apollo Implant to insert the same probes across at least 4 mice with no noticeable reduction in recording quality. We successfully tracked the same neurons across weeks, allowing us to explore changes of sensory responses and correlation patterns over time, and during a behavioural task. [Conclusions] Thus, the Apollo Implant provides a cheap, lightweight, and flexible solution for chronic recordings in head-fixed mice. We are currently developing versions of the implant optimised for Neuropixels 1.0 probes and freely moving animals.

FENS Forum 2022
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BOARD NUMBER: S04-156

SPATIO-TEMPORAL MEMBRANE POTENTIAL AND RESISTIVE CURRENT RECONSTRUCTION FROM PARALLEL MULTIELECTRODE AND INTRACELLULAR MEASUREMENTS IN SINGLE NEURONS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Here we show, that based on parallel multichannel extracellular and single-channel intracellular potential recordings, it is possible to reconstruct the spatio-temporal distribution of membrane potential with the spatial resolution of the extracellular recordings in a single neuron. Moreover, we show, that reconstruction of intracellular membrane potential made possible the distinction between two components of the current source density (CSD): the resistive and the capacitive currents. This distinction would provide a clue to the proper interpretation of the CSD distribution, as the resistive component corresponds to the active channel currents, both synaptic and voltage-sensitive channel membrane currents, while capacitive current corresponds to the passive counter currents. The importance of this distinction is further emphasized by different features of the resistive membrane current distribution compared to the CSD. As the CSD is a net membrane current, the sum of the CSD along a whole intact cell should be zero at each time moment, according to the charge conservation law. In contrast to this, the sum of the resistive current should not be necessarily zero since it governs the membrane potential dynamics. Thus, estimation of the spatial distribution of the resistive membrane current makes possible the distinction between active and passive sinks and sources of the CSD map and localization of the synaptic input currents, which makes the neuron fire. We validate our reconstruction approach on simulations and demonstrate its application on simultaneous and co-localized extra- and intracellular *in vitro* recordings in the hippocampal CA1 region using *in vitro* rat brain slice preparations.

Pubmed:

33479289: Fiáth R, Meszéna D, Somogyvári Z, Boda M, Barthó P, Ruther P, Ulbert I
Recording site placement on planar silicon-based probes affects signal quality in acute neuronal recordings.
Multisite, silicon-based probes are widely used tools to record the electrical activity of neuronal populations. Several physical features of these devices are designed to improve their recording performance. Here, our goal was to investigate whether the position of recording sites on the silicon shank might affect the quality of the recorded neural signal in acute experiments. Neural recordings obtained with five different types of high-density, single-shank, planar silicon probes from anesthetized rats were analyzed. Wideband data were filtered to extract spiking activity, then the amplitude distribution of samples and quantitative properties of the recorded brain activity (single unit yield, spike amplitude and isolation distance) were compared between sites located at different positions of the silicon shank, focusing particularly on edge and center sites. Edge sites outperformed center sites: for all five probe types there was a significant difference in the signal power computed from the amplitude distributions, and edge sites recorded significantly more large amplitude samples both in the positive and negative range. Although the single unit yield was similar between site positions, the difference in spike amplitudes was noticeable in the range corresponding to high-amplitude spikes. Furthermore, the advantage of edge sites slightly decreased with decreasing shank width. Our results might aid the design of novel neural implants in enhancing their recording performance by identifying more efficient recording site placements.
Sci Rep, 2021; 11
32409039: Márton G, Tóth EZ, Wittner L, Fiáth R, Pinke D, Orbán G, Meszéna D, Pál I, Győri EL, Bereczki Z, Kandrács Á, Hofer KT, Pongrácz A, Ulbert I, Tóth K
The neural tissue around SU-8 implants: A quantitative in vivo biocompatibility study.
The use of SU-8 material in the production of neural sensors has grown recently. Despite its widespread application, a detailed systematic quantitative analysis concerning its biocompatibility in the central nervous system is lacking. In this immunohistochemical study, we quantified the neuronal preservation and the severity of astrogliosis around SU-8 devices implanted in the neocortex of rats, after a 2 months survival. We found that the density of neurons significantly decreased up

to a distance of 20 µm from the implant, with an averaged density decrease to 24 ± 28% of the control. At 20 to 40 µm distance from the implant, the majority of the neurons was preserved (74 ± 39% of the control) and starting from 40 µm distance from the implant, the neuron density was control-like. The density of synaptic contacts - examined at the electron microscopic level - decreased in the close vicinity of the implant, but it recovered to the control level as close as 24 µm from the implant track. The intensity of the astroglial staining significantly increased compared to the control region, up to 560 µm and 480 µm distance from the track in the superficial and deep layers of the neocortex, respectively. Electron microscopic examination revealed that the thickness of the glial scar was around 5-10 µm thin, and the ratio of glial processes in the neuropil was not more than 16% up to a distance of 12 µm from the implant. Our data suggest that neuronal survival is affected only in a very small area around the implant. The glial scar surrounding the implant is thin, and the presence of glial elements is low in the neuropil, although the signs of astrogliosis could be observed up to about 500 µm from the track. Subsequently, the biocompatibility of the SU-8 material is high. Due to its low cost fabrication and more flexible nature, SU-8 based devices may offer a promising approach to experimental and clinical applications in the future.
Mater Sci Eng C Mater Biol Appl, 2020; 112
31648240: Orbán G, Meszéna D, Tasnády KR, Rózsa B, Ulbert I, Márton G
Correction: Method for spike detection from microelectrode array recordings contaminated by artifacts of simultaneous two-photon imaging.
[This corrects the article DOI: 10.1371/journal.pone.0221510.].
PLoS One, 2019; 14
31430357: Orbán G, Meszéna D, Tasnády KR, Rózsa B, Ulbert I, Márton G
Method for spike detection from microelectrode array recordings contaminated by artifacts of simultaneous two-photon imaging.
The simultaneous utilization of electrophysiological recordings and two-photon imaging allows the observation of neural activity in a high temporal and spatial resolution at the same time. The three dimensional monitoring of morphological features near the microelectrode array makes the observation more precise and complex. In vitro experiments were performed on mice neocortical slices expressing the GCaMP6 genetically encoded calcium indicator for monitoring the neural activity with two-photon microscopy around the implanted microelectrodes. A special filtering algorithm was used for data analysis to eliminate the artefacts caused by the imaging laser. Utilization of a special filtering algorithm allowed us to detect and sort single unit activities from simultaneous two-photon imaging and electrophysiological measurement.
PLoS One, 2019; 14
29148974: Cserpán D, Meszéna D, Wittner L, Tóth K, Ulbert I, Somogyvári Z, Wójcik DK
Revealing the distribution of transmembrane currents along the dendritic tree of a neuron from extracellular recordings.
Revealing the current source distribution along the neuronal membrane is a key step on the way to understanding neural computations; however, the experimental and theoretical tools to achieve sufficient spatiotemporal resolution for the estimation remain to be established. Here, we address this problem using extracellularly recorded potentials with arbitrarily distributed electrodes for a neuron of known morphology. We use simulations of models with varying complexity to validate the proposed method and to give recommendations for experimental applications. The method is applied to in vitro data from rat hippocampus.
Elife, 2017; 6

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MULTIPLEXED CELL-BASED ASSAY OF NEURONAL STRUCTURE-FUNCTION FOR NEUROTOXICITY AND DISEASE MODELLING

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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The flexibility and accessibility of induced pluripotent stem cell technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Rapid advances in the technology have led to widespread adoption for the development of in vitro models of neuron electrophysiology to be used in drug discovery and safety. Furthermore, advanced cells preparations, such as spheroids or organoids, are under intense investigation with aims towards establishing mature human phenotypes in vitro. For the establishment of in vitro neuronal models, it is critical to evaluate the structure and function of neurons, synapses, and networks. Here we develop and validate a multiplexed structure-function assay as an efficient approach for evaluating neuronal models in vitro. A planar grid of microelectrodes embedded in the substrate of each well interfaces with cultured cellular networks to continuously monitor both the electrophysiological function and cell structural viability. At the cell-electrode interface, electrical activity from the cells is detected identifying changes in function, while changes in impedance allow the detection of structural effects, such as morphological changes and cell viability. We characterized and validated this multiplexed assay using control compounds known to differentially affect the cell structure and function. All compounds tested altered functional spiking activity. Only tributyltin, glutamate, and Triton X-100 also affected neuronal viability, with Triton X-100 serving as the positive control for complete loss of membrane integrity. These results support the continued development and use of human iPSC-derived neural assays on the Maestro Pro for high throughput drug discovery and safety assessment.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S04-158

STANDARDIZED QUALITY ASSESSMENTS FOR CHRONIC NEURAL PROBES

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Neural probes are essential for diagnosis and treatment of neurological diseases and for mapping of neural circuits in basic research. Their long-term performance, however, is limited through deterioration of the conductive interfaces, scarring, and consequently a successive decrease in signal to noise ratio. To overcome this, aspects such as choice of materials, flexibility, coatings, pre- and postoperative care have been addressed, but there exists no systematic process for the *in vivo* characterization comparing novel probes and materials. Implementation of standardized methods for material and device characterization, probe implantation and explantation, and histological tissue analysis as well as protocols for electrical recording and stimulation, would deeply improve our understanding of the long-term functionality. It would also enable implant developers to ensure long-term performance beyond biocompatibility assessments and reduce animal testing. Consequently, we develop standardized quality assessments suitable for neural implants over long time periods > 30 days, from surgical procedures up to data analyses. We employed methods identified already for a new Michigan-style multi-electrode probe design on which the recording electrodes are located on flexible polyimide wings, combining the benefits of rigid silicone backbones to reach deep brain regions with the advantage of soft materials to minimize the foreign body response. Our results show stable recording quality over long periods of time, but for generalization of quality assessment we require additional data sets and method development to ensure comparability between different probes and materials. This work was supported by the EU Horizon 2020 programme (GA 814654) and by the BMBF.

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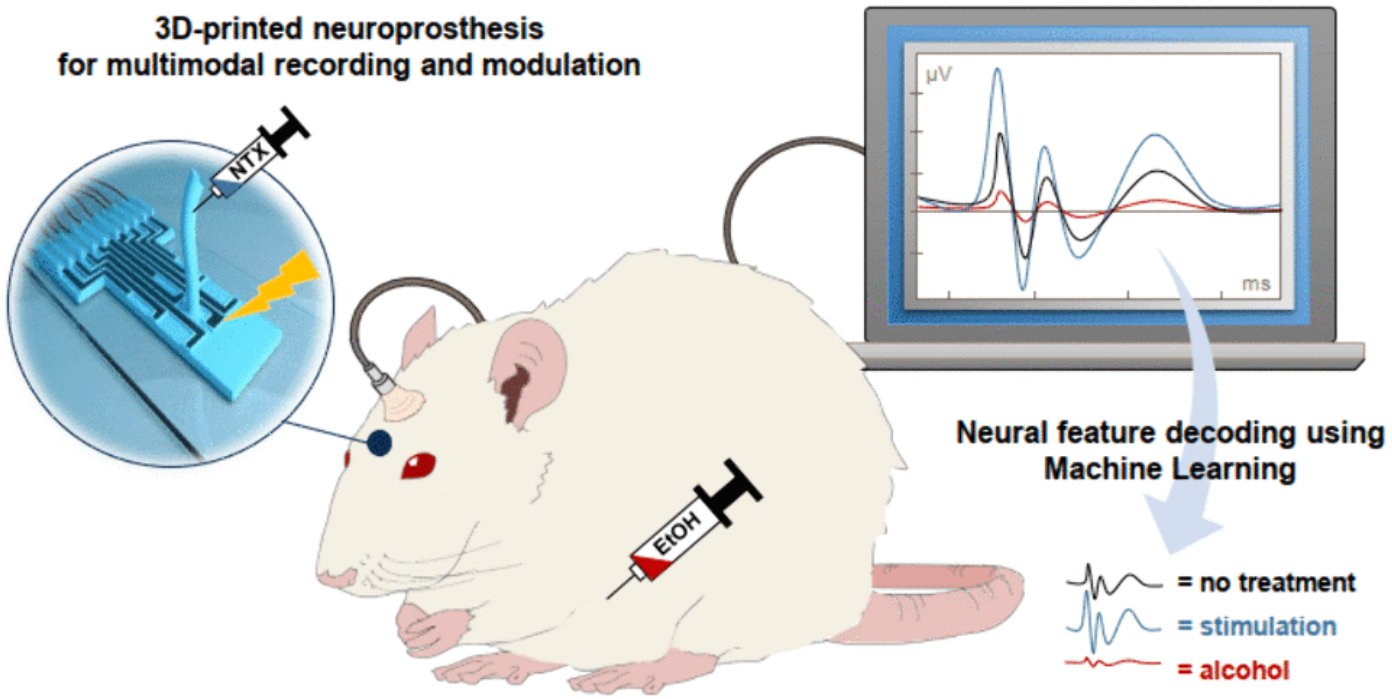
BOARD NUMBER: S04-159

FLEXIBLE NEUROPROSTHETICS FOR RECORDING, MODULATION AND IDENTIFICATION OF ELECTROPHYSIOLOGICAL BIOMARKERS OF COGNITIVE FUNCTION

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

Bettina Habelt[1], Christopher Wirth[2], Dzmitry Afanasenkau[3], Lyudmila Mihaylova[2], Christine Winter[4], Mahnaz Arvaneh[2], Ivan Minev[2], Nadine Bernhardt[1]
[1]Technische Universität Dresden, Department Of Psychiatry And Psychotherapy, Medical Faculty Carl Gustav Carus, Dresden, Germany, [2]University of Sheffield, Department Of Automatic Control And Systems Engineering, Sheffield, United Kingdom, [3]Technische Universität Dresden, Biotechnology Center (biotec), Center For Molecular And Cellular Bioengineering (cmcb), Dresden, Germany, [4]Charite University Medicine Berlin, Department Of Psychiatry And Psychotherapy, Berlin, Germany



Introduction Neuropsychiatric conditions such as substance use disorders are associated with behavioural and cognitive impairments arising from disturbed prefrontal neural circuits. Advanced treatments, including brain stimulation, aim at counteracting their chronically recurring courses. While these techniques operate in unidirectional mode, not allowing feedback control, we here developed soft neural interfaces offering multimodal functionality by combining electrocorticographic measurements, electrical/pharmacological stimulation and computational modelling. **Methods** Using 3D-bioprinting of soft silicones and a conductive platinum composite, we fabricated flexible multielectrode arrays for neural recording and stimulation. A microfluidic channel further allowed the cortical application of pharmacological substances. Implanted epidurally above the medial prefrontal cortex of rats, the neuroprosthesis aquired neural activity patterns indicating

stimulus perception, attentive processing and decision making known to be affected in neuropsychiatric disorders. In awake animals, we obtained auditory event-related brain potentials (ERPs) under treatment-naïve conditions and following alcohol intake, cortical electrical stimulation and local application of the anti-relapse medication naltrexone. Machine learning algorithms based on stepwise linear discriminant analysis further allowed advanced identification of treatment effects in single-trial ERP data. **Results** The neuroprosthesis recorded sound-specific neural responses and captured alcohol-induced neural depression indicated by diminished ERP components. Implant-driven electrical stimulation and naltrexone application resulted in enhanced ERP amplitudes. These treatment-induced neural modifications were also accurately identified through the applied computational algorithms. **Conclusions** These findings demonstrate the potential of multifunctional, multimodal neuroprosthetics to monitor and modulate higher-order cognitive function. The combination with computational modelling enables closed-loop feedback control, providing an innovative, comprehensive approach for diagnosing and treating neuropsychiatric symptoms.

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BOARD NUMBER: S04-160

GOLD COATED SILICON NANOWIRE FORMATION OF A FUNCTIONAL NETWORK OF DORSAL ROOT GANGLIA NEURONS AND SATELLITE GLIAL CELLS.

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Aims: Over the last decades, glial cells' role in neural communication is considered critical in Central Nervous System. In Peripheral Nervous System (PNS), Satellite Glial Cells (SGCs) are emerging as important players in sensory ganglia homeostasis modulation and in inflammation. Our study goal is to develope and validate new technologies to stimulate and record glial cells' functions in vitro. We previously demonstrated that a forest of gold coated silicon nanowires (AuSi/NWs) allows the recording of extracellular activity from in-vivo-like primary astrocyte culture. Here, we translate this approach to PNS using primary co-culture of Dorsal Root Ganglia (DRG) neurons and SGCs on AuSi/NWs devices. **Methods:** DRG co-culture was plated on AuSi/NWs and Flat gold samples were used as control. Cell viability was assessed by fluorescein diacetate/Hoechst staining. Morphological features were evaluated through Scanning electron microscopy. Protein and mRNA expression were measured through quantitative RT-PCR, western blot densitometric analysis and immunofluorescence. **Results:** Cell viability assay at 2 and 5 days in vitro showed that the device enabled the formation of a DRG glia-neuron network without laminin or Matrigel extra coating. mRNA and protein expression analysis demonstrated typical structural features of DRG co-culture in cells grown on AuSi/NWs. GAP43 immunoreactivity underlined an outgrowth regeneration process occurring on AuSi/NWs plated cells. We performed a full characterization of the device's electronic and surface proprieties. **Conclusions:** AuSi/NWs device allows the growing of a DRG glia-neuron network with molecular and morphological characteristics resembling the ones observed in vivo, therefore, representing a powerful tool to study neuron-glia interaction in PNS.

Pubmed:
34962289: Beamer E, Morgan J, Alves M, Menéndez Méndez A, Morris G, Zimmer B, Conte G, de Diego-Garcia L, Alarcón-Vila C, Yiu Ng NK, Madden S, Calzaferri F, de Los Ríos C, García AG, Hamacher M, Dinkel K, Pelegrín P, Henshall DC, Nicke A, Engel T
Increased expression of the ATP-gated P2X7 receptor reduces responsiveness to anti-convulsants during status epilepticus in mice.
Refractory status epilepticus is a clinical emergency associated with high mortality and morbidity. Increasing evidence suggests neuroinflammation contributes to the development of drug-refractoriness during status epilepticus. Here, we have determined the contribution of the ATP-gated P2X7 receptor, previously linked to inflammation and increased hyperexcitability, to drug-refractory status epilepticus and its therapeutic potential.
Br J Pharmacol, 2022; 179
34572093: Conte G, Menéndez-Méndez A, Bauer S, El-Naggar H, Alves M, Nicke A, Delanty N, Rosenow F, Henshall DC, Engel T
Circulating P2X7 Receptor Signaling Components as Diagnostic Biomarkers for Temporal Lobe Epilepsy.
Circulating molecules have potential as biomarkers to support the diagnosis of epilepsy and to assist with differential diagnosis, for example, in conditions resembling epilepsy, such as in psychogenic non-epileptic seizures (PNES). The P2X7 receptor (P2X7R) is an important regulator of inflammation and mounting evidence supports its activation in the brain during epilepsy. Whether the P2X7R or P2X7R-dependent signaling molecules can be used as biomarkers of epilepsy has not been reported. P2X7R levels were analyzed by quantitative ELISA using plasma samples from controls and patients with temporal lobe epilepsy (TLE) or PNES. Moreover, blood cell P2X7R expression and P2X7R-dependent cytokine signature was measured following status epilepticus in P2X7R-EGFP reporter, wildtype, and P2X7R-knockout mice. P2X7R plasma levels were higher in TLE patients when compared with controls and patients with PNES. Plasma levels of the broad inflammatory marker protein C-Reactive protein (CRP) were similar between the three groups. Using P2X7R-EGFP reporter mice, we

identified monocytes as the main blood cell type expressing P2X7R after experimentally evoked seizures. Finally, cytokine array analysis in P2X7R-deficient mice identified KC/GRO as a potential P2X7R-dependent plasma biomarker following status epilepticus and during epilepsy. Our data suggest that P2X7R signaling components may be a promising subclass of circulating biomarkers to support the diagnosis of epilepsy.
Cells, 2021; 10
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Elevated blood purine levels as a biomarker of seizures and epilepsy.
There is a major unmet need for a molecular biomarker of seizures or epilepsy that lends itself to fast, affordable detection in an easy-to-use point-of-care device. Purines such as adenosine triphosphate and adenosine are potent neuromodulators released during excessive neuronal activity that are also present in biofluids. Their biomarker potential for seizures and epilepsy in peripheral blood has, however, not yet been investigated. The aim of the present study was to determine whether blood purine nucleoside measurements can serve as a biomarker for the recent occurrence of seizures and to support the diagnosis of epilepsy.
Epilepsia, 2021; 62
33199725: Ramírez-Fernández A, Urbina-Treviño L, Conte G, Alves M, Rissiek B, Durner A, Scalbert N, Zhang J, Magnus T, Koch-Nolte F, Plesnila N, Deussing JM, Engel T, Kopp R, Nicke A
Deviant reporter expression and P2X4 passenger gene overexpression in the soluble EGFP BAC transgenic P2X7 reporter mouse model.
The ATP-gated P2X7 receptor is highly expressed in microglia and has been involved in diverse brain diseases. P2X7 effects were also described in neurons and astrocytes but its localisation and function in these cell types has been challenging to demonstrate in situ. BAC transgenic mouse lines have greatly advanced neuroscience research and two BAC-transgenic P2X7 reporter mouse models exist in which either a soluble EGFP (sEGFP) or an EGFP-tagged P2X7 receptor (P2X7-EGFP) is expressed under the control of a BAC-derived P2rx7 promoter. Here we evaluate both mouse models and find striking differences in both P2X expression levels and EGFP reporter expression patterns. Most remarkably, the sEGFP model overexpresses a P2X4 passenger gene and sEGFP shows clear neuronal localisation but appears to be absent in microglia. Preliminary functional analysis in a status epilepticus model suggests functional consequences of the observed P2X receptor overexpression. In summary, an aberrant EGFP reporter pattern and possible effects of P2X4 and/or P2X7 protein overexpression need to be considered when working with this model. We further discuss reasons for the observed differences and possible caveats in BAC transgenic approaches.
Sci Rep, 2020; 10
33070315: Conte G, Parras A, Alves M, Ollà I, De Diego-Garcia L, Beamer E, Alalqam R, Ocampo A, Mendez R, Henshall DC, Lucas JJ, Engel T
High concordance between hippocampal transcriptome of the mouse intra-amygdala kainic acid model and human temporal lobe epilepsy.
Pharmacoresistance and the lack of disease-modifying actions of current antiseizure drugs persist as major challenges in the treatment of epilepsy. Experimental models of chemoconvulsant-induced status epilepticus remain the models of choice to discover potential antiepileptogenic drugs, but doubts remain as to the extent to which they model human pathophysiology. The aim of the present study was to compare the molecular landscape of the intra-amygdala kainic acid model of status epilepticus in mice with findings in resected brain tissue from patients with drug-resistant temporal lobe epilepsy (TLE).
Epilepsia, 2020; 61
32982684: Conte G, Nguyen NT, Alves M, de Diego-Garcia L, Kenny A, Nicke A, Henshall DC, Jimenez-Mateos EM, Engel T
P2X7 Receptor-Dependent microRNA Expression Profile in the Brain Following Status Epilepticus in Mice.
The ionotropic ATP-gated P2X7 receptor is an important contributor to inflammatory signaling cascades the release of Interleukin-1β, as well as having roles in cell death, neuronal plasticity and the release of neurotransmitters. Accordingly, there is interest in targeting the P2X7 receptor for the treatment of epilepsy. However, the signaling pathways downstream of P2X7 receptor activation remain incompletely understood. Notably, recent studies showed that P2X7 receptor expression is controlled, in part, by microRNAs (miRNAs). Here, we explored P2X7 receptor-dependent microRNA expression by comparing microRNA expression profiles of wild-type (wt) and P2X7 receptor knockout mice before and after status epilepticus. Genome-wide microRNA profiling was performed using hippocampi from wt and P2X7 receptor knockout mice following status epilepticus induced by intra-amygdala kainic acid. This revealed that the genetic deletion of the P2X7 receptor results in distinct patterns of microRNA expression. Specifically, we found that in vehicle-injected control mice, the lack of the P2X7 receptor resulted in the up-regulation of 50 microRNAs and down-regulation of 35 microRNAs. Post-status epilepticus, P2X7 receptor deficiency led to the up-regulation of 44 microRNAs while 13 microRNAs were down-regulated. Moreover, there was only limited overlap among identified P2X7 receptor-dependent microRNAs between control conditions

and post-status epilepticus, suggesting that the P2X7 receptor regulates the expression of different microRNAs during normal physiology and pathology. Bioinformatic analysis revealed that genes targeted by P2X7 receptor-dependent microRNAs were particularly overrepresented in pathways involved in intracellular signaling, inflammation, and cell death; processes that have been repeatedly associated with P2X7 receptor activation. Moreover, whereas genes involved in signaling pathways and inflammation were common among up- and down-regulated P2X7 receptor-dependent microRNAs during physiological and pathological conditions, genes associated with cell death seemed to be restricted to up-regulated microRNAs during both physiological conditions and post-status epilepticus. Taken together, our results demonstrate that the P2X7 receptor impacts on the expression profile of microRNAs in the brain, thereby possibly contributing to both the maintenance of normal cellular homeostasis and pathological processes.
Front Mol Neurosci, 2020; 13
32895896: Morgan J, Alves M, Conte G, Menéndez-Méndez A, de Diego-Garcia L, de Leo G, Beamer E, Smith J, Nicke A, Engel T
Characterization of the Expression of the ATP-Gated P2X7 Receptor Following Status Epilepticus and during Epilepsy Using a P2X7-EGFP Reporter Mouse.
Mounting evidence suggests that the ATP-gated P2X7 receptor contributes to increased hyperexcitability in the brain. While increased expression of P2X7 in the hippocampus and cortex following status epilepticus and during epilepsy has been repeatedly demonstrated, the cell type-specific expression of P2X7 and its expression in extra-hippocampal brain structures remains incompletely explored. In this study, P2X7 expression was visualized by using a transgenic mouse model overexpressing P2X7 fused to the fluorescent protein EGFP. The results showed increased P2X7-EGFP expression after status epilepticus induced by intra-amygdala kainic acid and during epilepsy in different brain regions including the hippocampus, cortex, striatum, thalamus and cerebellum, and this was most evident in microglia and oligodendrocytes. Co-localization of P2X7-EGFP with cell type-specific markers was not detected in neurons or astrocytes. These data suggest that P2X7 activation is a common pathological hallmark across different brain structures, possibly contributing to brain inflammation and neurodegeneration following acute seizures and during epilepsy.
Neurosci Bull, 2020; 36
32594159: Parras A, de Diego-Garcia L, Alves M, Beamer E, Conte G, Jimenez-Mateos EM, Morgan J, Ollà I, Hernandez-Santana Y, Delanty N, Farrell MA, O'Brien DF, Ocampo A, Henshall DC, Méndez R, Lucas JJ, Engel T
Polyadenylation of mRNA as a novel regulatory mechanism of gene expression in temporal lobe epilepsy.
Temporal lobe epilepsy is the most common and refractory form of epilepsy in adults. Gene expression within affected structures such as the hippocampus displays extensive dysregulation and is implicated as a central pathomechanism. Post-transcriptional mechanisms are increasingly recognized as determinants of the gene expression landscape, but key mechanisms remain unexplored. Here we show, for first time, that cytoplasmic mRNA polyadenylation, one of the post-transcriptional mechanisms regulating gene expression, undergoes widespread reorganization in temporal lobe epilepsy. In the hippocampus of mice subjected to status epilepticus and epilepsy, we report >25% of the transcriptome displays changes in their poly(A) tail length, with deadenylation disproportionately affecting genes previously associated with epilepsy. Suggesting cytoplasmic polyadenylation element binding proteins (CPEBs) being one of the main contributors to mRNA polyadenylation changes, transcripts targeted by CPEBs were particularly enriched among the gene pool undergoing poly(A) tail alterations during epilepsy. Transcripts bound by CPEB4 were over-represented among transcripts with poly(A) tail alterations and epilepsy-related genes and CPEB4 expression was found to be increased in mouse models of seizures and resected hippocampi from patients with drug-refractory temporal lobe epilepsy. Finally, supporting an adaptive function for CPEB4, deletion of Cpeb4 exacerbated seizure severity and neurodegeneration during status epilepticus and the development of epilepsy in mice. Together, these findings reveal an additional layer of gene expression regulation during epilepsy and point to novel targets for seizure control and disease-modification in epilepsy.
Brain, 2020; 143
31048325: Alves M, De Diego Garcia L, Conte G, Jimenez-Mateos EM, D'Orsi B, Sanz-Rodriguez A, Prehn JHM, Henshall DC, Engel T
Context-Specific Switch from Anti- to Pro-epileptogenic Function of the P2Y Receptor in Experimental Epilepsy.
Extracellular ATP activates inflammatory responses to tissue injury. It is also implicated in establishing lasting network hyperexcitability in the brain by acting upon independent receptor systems. Whereas the fast-acting P2X channels have well-established roles driving neuroinflammation and increasing hyperexcitability, the slower-acting metabotropic P2Y receptors have received much less attention. Recent studies of P2Y receptor function in seizures and epilepsy have produced contradictory results, suggesting that the role of this receptor during seizure pathology may be highly sensitive to context. Here, by using male mice, we demonstrate that the metabotropic P2Y receptor mediates either proconvulsive or anticonvulsive responses, dependent on the time point of activation in relation to the induction of status epilepticus. P2Y deficiency or a P2Y antagonist (MRS2500) administered before a chemoconvulsant, exacerbates epileptiform activity,

whereas a P2Y agonist (MRS2365) administered at this time point is anticonvulsant. When these drugs are administered after the onset of status epilepticus, however, their effect on seizure severity is reversed, with the antagonist now anticonvulsant and the agonist proconvulsant. This result was consistent across two different mouse models of status epilepticus (intra-amygdala kainic acid and intraperitoneal pilocarpine). Pharmacologic P2Y blockade during status epilepticus reduces also associated brain damage, delays the development of epilepsy and, when applied during epilepsy, suppresses spontaneous seizures, in mice. Our data show a context-specific role for P2Y during seizure pathology and demonstrate that blocking P2Y after status epilepticus and during epilepsy has potent anticonvulsive effects, suggesting that P2Y may be a novel candidate for the treatment of drug-refractory status epilepticus and epilepsy. This is the first study to fully characterize the contribution of a metabotropic purinergic P2Y receptor during acute seizures and epilepsy. The findings suggest that targeting P2Y may offer a potential novel treatment strategy for drug-refractory status epilepticus and epilepsy. Our data demonstrate a context-specific role of P2Y activation during seizures, switching from a proconvulsive to an anticonvulsive role depending on physiopathological context. Thus, our study provides a possible explanation for seemingly conflicting results obtained between studies of different brain diseases where P2Y targeting has been proposed as a potential treatment strategy and highlights that the timing of pharmacological interventions is of critical importance to the understanding of how receptors contribute to the generation of seizures and the development of epilepsy.
J Neurosci, 2019; 39

30765791: Bernareggi A, Conte G, Constanti A, Borelli V, Vita F, Zabucchi G

On the mechanism of the electrophysiological changes and membrane lesions induced by asbestos fiber exposure in Xenopus laevis oocytes.

The so-called amphibole asbestos fibers are enriched with mineral iron ions, able to stimulate ROS production. We recently reported that crocidolite asbestos was able to interact with the cell membranes of Xenopus laevis oocytes, to alter their electrical membrane properties. Here, we found that applied iron ions (Fe) or HO (for ROS generation) mimicked these effects, suggesting that at least one effect of iron-containing asbestos fiber exposure was mediated by ROS production. Furthermore, combined Fe and HO acted synergistically, producing a membrane effect stronger than that induced by these factors alone. Similar to crocidolite, these changes peaked within 30 minutes of incubation and vanished almost completely after 120 min. However, in the presence of cytochalasin D, which inhibits membrane actin repair mechanisms, crocidolite or applied Fe/HO invariably produced oocyte cell death. While the electrophysiological modifications induced by crocidolite suggested a modification of an intrinsic chloride ion channel, the morphological appearance of the treated oocytes also indicated the formation of membrane "pores"; the effects of asbestos exposure may therefore consist of multiple (not necessarily exclusive) underlying mechanisms. In conclusion, using Xenopus oocytes allowed us for the first time, to focus on a specific membrane effect of crocidolite asbestos exposure, which deserves to be tested also on human lung cell lines. Much available evidence suggests that asbestos fibers damage cells through the production of ROS. Our present data confirm that crocidolite fibers can indeed trigger ROS-mediated damaging effects in the oocyte cell membrane, provided iron ions and HO are available for ROS production.
Sci Rep, 2019; 9

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WIRE INSERTION SYSTEM FOR ULTRA-FLEXIBLE HIGH-DENSITY ELECTRODE ARRAYS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Understanding how communication between different brain areas supports behavior and cognition is a tremendous neuroscience challenge. Monitoring the electrical activity of neurons is a key technology for advancing the understanding of the brain. Neural implants have evolved at high pace over the last decades with state-of-the-art silicon-based probes like Neuropixels[1] and SiNaps[2]. Despite their success, long-term brain interfacing remains questionable due to the mechanical mismatch between the probe and brain. Micromotions often lead to tissue damage and neural loss. The bending stiffness can be drastically lowered by reducing the Young's modulus and implant size. Advanced cleanroom processes were used to fabricate a high-density 128ch ultra-flexible polyimide probe with <100µm shank width and <3µm thickness. The probe includes low impedance iridiumoxide electrodes and features a modified tip to facilitate the implantation by a wire. Due to the probe's ultra-flexibility a dedicated insertion method had to be developed. The insertion technique is based on a stiff tungsten wire that catches the probe through the modified tip by moving an XYZ-manipulator and uses a camera for visual feedback. The probe is then precisely implanted in the brain by controlling a stepper motor. After implantation the wire is retracted leaving the flexible probe in place. Implantation depths up to 4cm can be reached and the entire system can be mounted on a standard stereotaxic frame. The implantation technique was successfully validated in a phantom brain gel-model and in-vivo in freely moving rodents. [1]Steinmetz N.A. et al. Science._372_(2021)_6539. [2]Angotzi G.N. et al. Biosensors and Bioelectronics._126_(2019)_355-364.

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BOARD NUMBER: S04-162

AN ORGAN-ON-CHIP PLATFORM TO EVALUATE NEURO-IMMUNE SIGNAL TRANSMISSION USING HUMAN CELLS

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Microfluidic devices are a valuable tool for modeling *in vitro* neuronal networks allowing neuronal connectivity in physiological and pathological conditions. Here, we present a human Brain-on-Chip of well-characterized human cortical Glutamatergic Neurons in an asymmetric-shape microfluidic device. Our objective is to evaluate the integration and transduction of an immunological signal from monocytes-derived dendritic cells (MoDC) to human cortical glutamatergic neurons. We designed a device composed of three compartments with an asymmetric channel allowing isolation of soma and neurites thanks to microchannels to create an *in vitro* synaptic communication. Human-induced pluripotent stem cell-derived Glutamatergic Neurons are maintained in soma compartments for 21 days. We performed a localized exposition of dendritic cells (MoDC), obtained from human blood isolated monocytes, and differentiated using cytokines and primed with LPS, on either soma or synaptic compartment for 3 hours. The microfluidic device was coupled with MicroElectrode Arrays (MEAs) to confirm the transduction of an immunological signal. We show that this model can be used for quantitative analysis of functional activity according to immuno-neuronal communication. We also analyze the release of neurotransmitters and immune factors. Our data highlight that an electrophysiologic signal was transmitted between two compartments of Glutamatergic Neurons linked by synapses in a bottom-up way when soma is exposed to primed MoDC. In conclusion, this model of BoC opens the field of pharmacological compound screening under physiological conditions. This model could be used totest the effects of compounds on immuno-neuronal signal transduction implicated in a lot of pathology with neurologic and behavioral manifestations.

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NOVEL FIELD-EFFECT-TRANSISTOR NANOELECTRODE PROBES FOR ACTIVE INTRACELLULAR ELECTROPHYSIOLOGY: A SIMULATION STUDY

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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Aims. Miniaturization of silicon technology-based nanoelectrode probes for electrophysiology enables high spatial resolution of one or more electrode(s) per neuron while facilitating intracellular access for superior sensitivity to subthreshold signals. To overcome most of the common miniaturization drawbacks of passive electrodes (notably: the reduced signal-to-noise ratio), active electrodes with integrated field-effect-transistors (FETs) need to be used instead. Technology-CAD (TCAD) and multiscale-multiphysics simulation can support the improvement of the electrode device design, yielding complete control of their performance. *Methods.* Based on a finite element modelling (FEM) TCAD, two representative nanoelectrode FET-based devices (i.e., the vertical nanowire-FET (VNW-FET), and the extended-gate silicon-on-insulator FET (EG-SOI-FET)) in intracellular contact with neurons for in-vitro studies are examined. The FEM simulation domain includes a patch of an excitable membrane, confining the cytosol, and surrounded by the electrolyte. The remaining portion of the neuron is accounted for at the circuit level via mixed device-circuit TCAD simulations. *Results.* The drain current transient responses to action potentials (APs) are calculated for nanoelectrodes partly or fully coated and for different neuron insertions. To this end, the FET's threshold voltage is adjusted to maximize the transconductance (gm,MAX) in the middle of the typical AP voltage range. The neuron-device transfer function, for all structures, configurations, and insertions, is essentially frequency independent over the entire AP spectrum and close to the gm,MAX, but quite dependent on the coating design. *Conclusion.* A partial nanoelectrode coating makes the transduction gain dependent on the insertion depth for both devices, resulting in fluctuations of the signal amplitudes.

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MODULATING AND MONITORING THE FUNCTIONALITY OF CORTICOSTRIATAL CIRCUITS USING AN ELECTROSTIMULABLE MICROFLUIDIC DEVICE

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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The central nervous system is organized into different neural circuits, each with particular functions and properties. Studying neural circuits is essential to understanding brain function and neuronal diseases. Microfluidic systems are widely used for reconstructing and studying neural circuits but still need improvement to allow modulation and monitoring of the physiological properties of circuits. In this study, we developed and built a microfluidic device that supports reassembly and electrical modulation of neural circuits. We demonstrated that our microfluidic device system provides a structural platform for corticostriatal (CStr) circuits and a system for modulating and monitoring the physiological function of these circuits. In particular, our microfluidic device measures activity-driven Ca^{2+} dynamics using Ca^{2+} indicators (synaptophysin-GCaMP6f and Fluo5F-AM), as well as activity-driven synaptic transmission using vGlut-pHluorin. Overall, our findings indicate that the advanced microfluidic platform described here is an invaluable tool for studying the physiological properties of specific neural circuits.

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ENGINEERING TOOLSET PLATFORM FOR NEUROBIOLOGICAL INTERFACES

POSTER SESSION 04 - SECTION: NOVEL NEURAL INTERFACES FOR STIMULATION & TRACKING

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To understand the neural mechanisms and ultimately develop effective intervention approaches for neurological and psychiatric disorders, it is essential to reveal the causal link between behavioral output and neural cellular activity. Engineering approaches provide methodological assistance to establish a toolset platform. Ideally, the toolset platform will be featured with minimal invasiveness to biological tissue, functional longevity, widespread coverage of neural circuits, the capability to manipulate neurons at multiple scales, ranging from individual synapse to broad neural circuits, and the specificity to identify targeted neural populations. We have been developing effective engineering tools at the neurobiological interfaces to investigate neural dynamics. This toolset platform includes two main research directions: precise interventional tools for remotely controlled neuromodulation and real-time recording techniques to monitor neural dynamics. Firstly, a magnetic toolkit for remote neuromodulation, which allows remotely controlled release of pharmacological compounds to modulate targeted neural circuits. This chemomagnetic technique combines magnetic tools and behavioral neuroscience to enable temporally precise modulation of specific neural circuits underlying motivation and social interactions. Secondly, an optical recording system to monitor neural dynamics from multiple sites across the central nervous system in freely behaving mice with simultaneous behavioral output.

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POSSIBLE INTERACTION BETWEEN VOLTAGE GATED SODIUM CHANNEL NAV1.2 AND TRANSCRIPTION FACTOR CTBP1

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Voltage-gated sodium channels (Na_V) are known for their fundamental role in action potential firing in excitable cells, such as muscle cells and neurons. However, evidence accumulated in recent years indicates that Na_V's also participate in a variety of cellular processes such as phagocytosis, motility and metastatic activity, although the mechanisms underlying such activities are unknown. Studies from our laboratory indicate that transcription factor mSin3B can interact with sodium channels $Na_V1.2$ and $Na_V1.6$. Here we show that another transcription factor, CTBP1, can also bind these channels in Y2H. We confirm this interaction by co-IP of CTBP1 and $Na_V1.2$ channel from mouse brain proteins extracted with RIPA buffer, using either antibodies raised against the C-terminus of $Na_V1.2$ or antibodies against CtBP1, and testing by western blot. Our results indicate that CTBP1 co-IP with a fragment of >63 kDa, identified by two anti-$Na_V1.2$ antibodies, from nuclear and cytosolic fractions. These antibodies recognize a 250 kDa band (full length channel) and a >63 kDa band in whole lystes, but only the 63 kDa isoform CoIP with CTPB1 from nuclear and cytosolic fractions. Interaction between this $Na_V1.2$ fragment with CTPB1 raises the question about the impact on CtBP1 activity as a transcription factor and as a Golgi-scaffold protein, as well as if this interaction can modify the activity of $Na_V1.2$ at the membrane. Further experiments are needed to better understand this interaction physiological role in neurons and other non excitable cells. **Supported by Conacyt Grant A1-S-36984**

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BRIEF ULTRASOUND STIMULATION INDUCES SUSTAINED, REVERSIBLE MODIFICATION TO NEURONAL POTASSIUM CHANNEL FUNCTION.

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Aims: We previously demonstrated that ultrasound stimulation increases intrinsic excitability of cultured cortical rat neurons, and this effect lasted for at least 8 hours (Clennell et al. 2021. Brain Stimul. 14(2):217-225). We subsequently investigated the effect on whole-cell ion currents, neuronal proteome, and excitatory synaptic transmission.
Methods: Primary rat cortical neurons were stimulated with a 40 second, 200kHz pulsed ultrasound- or sham-stimulation. Whole-cell ion currents were measured under voltage clamp at 0-2, 6-8, 12-14 and 24-26 hours post-stimulation. Total protein and phosphopeptide abundance was analysed by nanoLC MS/MS. Excitatory synaptic transmission was analysed by recording of mEPSCs.
Results: We observed statistically significant increases in whole-cell potassium currents at 0-2hr (p = 0.004), 6-8hr (p = 0.001) and 12-14hr (p < 0.001) post-stimulation. These changes were absent by 24-hours. Conversely, we did not find any significant differences in whole-cell sodium currents at any timepoint. Proteomic analysis revealed no change to total levels of voltage-gated ion channels but did indicate differential phosphorylation specific to $K_v2.1$. Ingenuity Pathway Analysis indicated induction of synaptogenic signalling pathways. This was corroborated by mEPSC recordings indicating 243% increase in mEPSC frequency following stimulation (p < 0.001).
Conclusion: Ultrasound stimulation induces sustained, reversible increases in whole-cell potassium currents suggesting changes to voltage-gated potassium channel function. Proteomic analysis indicates a role for $K_v2.1$ phosphorylation in mediating the effects and suggests induction of synaptogenesis by ultrasound. This is supported by observation of increased excitatory synaptic transmission. These results can inform the application of transcranial ultrasound in experimental and therapeutic settings.

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CHANNEL NOISE LEADS TO LARGER STOCHASTIC VOLTAGE FLUCTUATIONS IN THE AXON THAN IN THE SOMA

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Neuronal excitability is mediated by voltage-gated ion channels which biophysics are usually described as deterministic phenomena. However, it is well accepted that individual ion channels undergo thermodynamic fluctuations which provoke stochastic transitions between closed and open states. This so-called “channel-noise” leads to stochastic fluctuations of the membrane potential (Vm) and to unpredictability of the neuronal output. Channel noise has been mostly studied through somatic recordings which may underestimate it for two reasons: 1) the somatic capacitance makes individual channels stochastic transitions unlikely to entail observable Vm fluctuations, 2) the large somatic channels amount provokes an averaging of the individual channel stochasticity, leading to a global deterministic behavior. Computational studies have suggested that thinner compartments such as axons express strong voltage fluctuations due to channel noise but this has not been experimentally verified. To answer this question, we performed dual soma-axon whole-cell recordings in cortical layer 5 pyramidal neurons (L5PC). We observed, both in soma and axon, the presence of stochastic fluctuations of subthreshold Vm, presenting larger amplitudes in axon than in the soma. These fluctuations increased with voltage depolarization and decreased following TTX application, strongly suggesting that they were due to channel noise. Moreover, the channel noise was negatively correlated with the diameter of the recorded compartment. Using a detailed L5PC model, we showed that axonal channel noise may have a big impact on both spike initiation and spike propagation dynamics.

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OPTICAL INVESTIGATION OF EXCITABILITY AND ION CHANNELS IN LAYER-5 NEOCORTICAL PYRAMIDAL NEURONS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Electrical excitability is based on the action potential defined by sequences of voltage changes across the membrane, and its monitoring is critical for understanding the neuronal behavior that underpins brain function. Pyramidal neurons of the layer–5 of the neocortex are a major site of signal integration from different areas of the brain, underlying high-level functions. In particular, ion channels are the main contributors to the initiation, propagation and back-propagation of action potentials (AP) in neurons and given their critical role, genetically defined abnormalities altering their function result in a large set of human diseases known as "channelopathies". Thus, imaging action potentials and ion currents from dendritic and axonal areas at submillisecond time scales and with a precision of a few microns, in combination with selective pharmacological manipulations, is fundamental to investigate the multiple channel isoforms that occupy different parts of the neuron, the activity of an individual ion channel, and how they interact within their native environment to define AP characteristics and shape AP waveforms. Here, we present some examples of novel measurements that are advancing our detailed understanding on the mechanisms underlying excitability in this important neuron. We compare signals in the axon and in the apical dendrite showing how the same channel can contribute to the action potential in different ways in different compartments.

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HEAVY DRINKING DURING ADOLESCENCE AFFECTS ETHANOL RESPONSE OF DENTATE GYRUS GRANULE CELLS IN ADULT MOUSE HIPPOCAMPUS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Excessive ethanol consumption during adolescence is regarded as a risk factor for development of alcoholism later in life, but the pathophysiological mechanisms that render adult brain susceptible to ethanol are largely unknown. $GABA_A$ receptors and inwardly rectifying K^+ (IRK) channels are prime targets of ethanol to regulate neural activity. Work from our lab has demonstrated that activin, a member of TGF-β family, controls ethanol potentiation of $GABA_A$ receptors and IRK currents in mouse hippocampus. Elaborating on the latter, we asked here, if adolescent drinking would affect IRK current responses to acute alcohol in hippocampal slices from adult mice, and if activin plays a role as an adaptive factor. Whole-cell voltage-clamp recordings were performed on dentate gyrus granule cells from wild-type mice and transgenic mice, expressing a dominant-negative mutant of activin receptor IB (dnActRIB), which disrupts signaling. We found that, compared to control mice, heavy drinking in dark during postnatal days 32-45 produced a long-lasting sensitization so that, in adulthood, IRK current response to alcohol (30-150 mM) were consistently enhanced. IRK currents were identified by their reversal potential near -90 mV and their suppression by low Ba^{2+}. Granule cells from alcohol-naïve dnActRIB mice responded stronger to acute alcohol than their wild-type counterparts. Moreover, they exhibited pronounced potentiation of IRK response when examined after adolescent drinking paradigm. Our results show that heavy adolescent drinking has a long-lasting impact on how IRK channels of granule cells react upon alcohol re-exposure later in life, and that this process might possibly involve activin receptor signaling.

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ACTIVIN REGULATION OF GIRK CURRENT RESPONSE TO ETHANOL IN DENTATE GYRUS GRANULE CELLS REVERSES AFTER ADOLESCENCE

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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G protein-gated inwardly rectifying potassium (GIRK) channels play an essential role in regulating neuronal excitability. While many neurotransmitters/-modulators use Gi/o protein as intermediary to open the channels, GIRKs can also be directly activated by alcohol. In previous work, we have shown that activin, a member of the TGF-β family, modulates $GABA_B$ receptor-evoked GIRK current. To explore if activin also modulates GIRK response to ethanol, we performed whole-cell voltage-clamp recordings from dentate gyrus granule cells (GCs) in dorsal hippocampal slices from adolescent (P37-42) and adult wild-type (wt) mice and transgenic mice, which express a dominant-negative mutant of activin receptors IB (dnActRIB). In wt GCs from both age groups, ethanol induced outward current responses (V_h -70 mV) in a dose-dependent fashion, with juvenile cells exhibiting significantly stronger responses to low ethanol (15 – 30 mM) than adult cells. Ethanol-mediated currents had a reversal potential near -90 mV, were diminished to a large extent by the GIRK-blocker tertiapin Q, and were fully suppressed by low Ba^{2+} (200 µM). Unexpectedly, we found that the effect of activin A (50 ng/ml, pre-incubated for 3-5 h) on ethanol responses was opposite in the two age groups: Whereas activin A enhanced the ethanol-induced current in juvenile slices, the response was decreased in adult slices. The dichotomous effect of activin was completely reversed when re-examined in GCs from adolescent and adult dnActRIB mice, underscoring the essential role of endogenous activin signaling in determining the neural impact of alcohol consumption at different stages of life.

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TREK CHANNELS AND THEIR PHYSIOLOGICAL ROLE IN THE INTRACARDIAC NEURONS: FOCUSING ON TEMPERATURE AND INTRACELLULAR ACIDIFICATION

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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The intrinsic activity of the intracardiac nervous system is determined by cardiac pacemarkers and is strongly modulated by the sympathetic and parasympathetic branches of the autonomic nervous system. Although general mechanisms controlling cardiac activity have been extensively studied, little is known about the electrical properties and function of parasympathetic neurons in the intracardiac ganglion (ICG). TREK channels, a subfamily of two-pore domain potassium (K2P) channels, are proteins with a crucial role in maintaining resting membrane potential and controlling excitability, but their function in the ICG remains unclear. In this work we investigated the effect of changes in physiological parameters such as increased temperature and intracellular acidification and how these variations affect to cultured mouse ICG neurons behaviour. First, the presence of TREK channels in the ICG was assessed by RT-qPCR and western-blot analysis. Using electrophysiological patch-clamp technique (perforated-patch), passive and active properties were examined at 24 and 37 °C and before and after cytosolic acidification. In the "current-clamp" experiments, the excitability of ICG neurons was clearly reduced when the temperature was increased from 24 to 37 °C, the neurons resulted hyperpolarized, the action potential firing rate decreased, and some action potential characteristics were also affected. The same phenomenon occurred when the cytosolic pH was diminished. Consistently, in "voltage-clamp" both physiological temperature and intracellular acidification induced outward currents and an increase in conductance through K+channels. Altogether, these results highlight the contribution of TREK channels in establishing neuronal excitability properties at physiological temperature and their role as neuroprotective channels.

Pubmed:
34690704: Rueda-Ruzafa L, Herrera-Pérez S, Campos-Ríos A, Lamas JA
Are TREK Channels Temperature Sensors?
Internal human body normal temperature fluctuates between 36.5 and 37.5°C and it is generally measured in the oral cavity. Interestingly, most electrophysiological studies on the functioning of ion channels and their role in neuronal behavior are carried out at room temperature, which usually oscillates between 22 and 24°C, even when thermosensitive channels are studied. We very often forget that if the core of the body reached that temperature, the probability of death from cardiorespiratory arrest would be extremely high. Does this mean that we are studying ion channels in dying neurons? Thousands of electrophysiological experiments carried out at these low temperatures suggest that most neurons tolerate this aggression quite well, at least for the duration of the experiments. This also seems to happen with ion channels, although studies at different temperatures indicate large changes in both, neuron and channel behavior. It is known that many chemical, physical and therefore physiological processes, depend to a great extent on body temperature. Temperature clearly affects the kinetics of numerous events such as chemical reactions or conformational changes in proteins but, what if these proteins constitute ion channels and these channels are specifically designed to detect changes in temperature? In this review, we discuss the importance of the potassium channels of the TREK subfamily, belonging to the recently discovered family of two-pore domain channels, in the transduction of thermal sensitivity in different cell types.
Front Cell Neurosci, 2021; 15
34357968: Campos-Ríos A, Rueda-Ruzafa L, Herrera-Pérez S, Rivas-Ramírez P, Lamas JA
Tetrodotoxin: A New Strategy to Treat Visceral Pain?
Visceral pain is one of the most common symptoms associated with functional gastrointestinal (GI) disorders. Although the origin of these symptoms has not been clearly defined, the implication of both the central and peripheral nervous systems in visceral hypersensitivity is well established. The role of several pathways in visceral nociception has been explored, as well

as the influence of specific receptors on afferent neurons, such as voltage-gated sodium channels (VGSCs). VGSCs initiate action potentials and dysfunction of these channels has recently been associated with painful GI conditions. Current treatments for visceral pain generally involve opioid based drugs, which are associated with important side-effects and a loss of effectiveness or tolerance. Hence, efforts have been intensified to find new, more effective and longer-lasting therapies. The implication of VGSCs in visceral hypersensitivity has drawn attention to tetrodotoxin (TTX), a relatively selective sodium channel blocker, as a possible and promising molecule to treat visceral pain and related diseases. As such, here we will review the latest information regarding this toxin that is relevant to the treatment of visceral pain and the possible advantages that it may offer relative to other treatments, alone or in combination.
Toxins (Basel), 2021; 13

34205717: Herrera-Pérez S, Campos-Ríos A, Rueda-Ruzafa L, Lamas JA
Contribution of K2P Potassium Channels to Cardiac Physiology and Pathophysiology.
Years before the first two-pore domain potassium channel (K2P) was cloned, certain ion channels had already been demonstrated to be present in the heart with characteristics and properties usually attributed to the TREK channels (a subfamily of K2P channels). K2P channels were later detected in cardiac tissue by RT-PCR, although the distribution of the different K2P subfamilies in the heart seems to depend on the species analyzed. In order to collect relevant information in this regard, we focus here on the TWIK, TASK and TREK cardiac channels, their putative roles in cardiac physiology and their implication in coronary pathologies. Most of the RNA expression data and electrophysiological recordings available to date support the presence of these different K2P subfamilies in distinct cardiac cells. Likewise, we show how these channels may be involved in certain pathologies, such as atrial fibrillation, long QT syndrome and Brugada syndrome.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S04-173

HOW KV2.1 CONTRIBUTES TO DENDRITE ACTIVE PROPERTIES?

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Kv2 channels, which underlie most of the “delayed-rectifier” K^+ current, were shown to sustain high frequency firing by avoiding depolarization block. Interestingly, the voltage dependence (Vhalf) of Kv2.1 may vary in a ~40mV range depending on phosphorylation of its C-ter, which also affects the formation of patches (clusters) of Kv2.1 at the soma, proximal dendrites and axonal initial segment. Several mutations of KCNB1, coding for Kv2.1, have been described in patients with epileptic encephalopathy. We focused on two mutated forms that introduce a stop codon (Y533* and R583*) before or within the C-ter region responsible for clustering. In cultured pyramidal neurons transfected to express WT or mutated Kv2.1, mutated channels displayed a non-clustered distribution at the cell surface. Single Particle Tracking experiments confirmed the loss of scaffolding interactions that immobilize the channel in clusters. Importantly, both mutations redistributed Kv2.1 throughout the dendritic arbor. As a shift in voltage dependence was reported for the Y533* mutant, we used a detailed biophysical model of a pyramidal CA1 neuron in order to disentangle the consequences of changes in voltage dependence or a modified distribution in dendrites alone. More negative values of Vhalf of a WT Kv2-like conductance (gkdr2) not only decreased excitability but also reduced the impact of backpropagated action potentials (bAP) in the apical dendrite. Redistributing gkdr2 in all dendritic segments also reduced excitability and strongly affected bAPs. Thus, modelling proposes Kv2 as an important actor of the active properties of dendrites, role that may be modified by mutations or phosphorylation.

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BOARD NUMBER: S04-174

VOLTAGE-GATED $Ca_v2.2$ CALCIUM ION CHANNELS IN TRPV1 NERVE ENDINGS IN SKIN CONTRIBUTE TO THE RELEASE OF PROINFLAMMATORY MEDIATORS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Voltage-gated calcium ion channels (Ca_v) are essential for the transmission of noxious stimuli detected at peripheral nerve endings and the central perception of pain. Recently, the Lipscombe lab showed that capsaicin-induced heat hypersensitivity in peripheral Trpv1 nociceptor nerve endings in skin depends on $Ca_v2.2$ channel activity (DuBreuil et al., 2021). The precise mechanism by which $Ca_v2.2$ channels mediate heat hypersensitivity in peripheral nerve endings is not fully understood, but it is likely that they are the source of calcium that triggers vesicular release of inflammatory signaling molecules from nerve endings. We tested the hypothesis that $Ca_v2.2$ channel activation is necessary for the release of early inflammatory mediators linked to behavioral hypersensitivity to heat and mechanical stimuli. Within 15 minutes of intraplantar capsaicin injections mice exhibit maximal heat and mechanical hypersensitivity and, at the same time point, we observe an associated spike in interleukin-1α (IL-1α) detectable in interstitial fluid of the injected hindpaw. Interleukin IL-1α is an early proinflammatory mediator released from non-neuronal cells. We show that recombinant IL-1α injected directly into the hindpaw of wild type mice induces heat hypersensitivity when compared to vehicle controls (n= 6 per group, p =0.0050). In $Ca_v2.2$ KO mice, capsaicin-induced heat hypersensitivity and IL-1α levels in hindpaw were reduced by 60% as compared to wild type controls (6-8 animals per group; p =0.0400). Our data suggest that $Ca_v2.2$ channels in peripheral nerve endings trigger downstream neuroimmune signaling underlying transient heat hypersensitivity.

Pubmed:

30064314: Ho AL, Salib AN, Pendharkar AV, Sussman ES, Giardino WJ, Halpern CH
The nucleus accumbens and alcoholism: a target for deep brain stimulation.
Alcohol use disorder (AUD) is a difficult to treat condition with a significant global public health and cost burden. The nucleus accumbens (NAc) has been implicated in AUD and identified as an ideal target for deep brain stimulation (DBS). There are promising preclinical animal studies of DBS for alcohol consumption as well as some initial human clinical studies that have shown some promise at reducing alcohol-related cravings and, in some instances, achieving long-term abstinence. In this review, the authors discuss the evidence and concepts supporting the role of the NAc in AUD, summarize the findings from published NAc DBS studies in animal models and humans, and consider the challenges and propose future directions for neuromodulation of the NAc for the treatment of AUD.
Neurosurg Focus, 2018; 45
29843426: Salib AN, Ho AL, Sussman ES, Pendharkar AV, Halpern CH
Neuromodulatory Treatments for Alcohol Use Disorder: A Review.
Alcohol use disorder (AUD) is a prevalent condition characterized by chronic alcohol-seeking behaviors and has become a significant economic burden with global ramifications on public health. While numerous treatment options are available for AUD, many are unable to sustain long-term sobriety. The nucleus accumbens (NAcc) upholds an integral role in mediating reward behavior and has been implicated as a potential target for deep brain stimulation (DBS) in the context of AUD. DBS is empirically thought to disrupt pathological neuronal synchrony, a hallmark of binge behavior. Pre-clinical animal models and pilot human clinical studies utilizing DBS for the treatment of AUD have shown promise for reducing alcohol-related cravings and prolonging abstinence. In this review, we outline the various interventions available for AUD, and the translational potential DBS has to modulate functionality of the NAcc as a treatment for AUD.
Brain Sci, 2018; 8

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-175

SST-POSITIVE GABAERGIC INTERNEURONS COUNTERBALANCE CORTICAL HYPEREXCITABILITY AFTER TRAUMATIC BRAIN INJURY IN MICE BY A SWITCH OF α-SUBUNITS IN L-TYPE VOLTAGE-GATED CALCIUM CHANNELS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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[1]University Medical Center of the Johannes Gutenberg-University, Institute Of Physiology, Mainz, Germany, [2]University Medical Center of the Johannes Gutenberg-University Mainz, Institute For Immunology, Mainz, Germany, [3]UMC of the Johannes Gutenberg-University Mainz, Clinics For Anaesthesiology, Mainz, Germany

Traumatic brain injury (TBI) often causes cell death and cortical dysfunction in the neighboring area. Interestingly, functional changes also spread to primarily undamaged tissue causing neuronal hyperactivity in the contralateral hemisphere already 24 hours after injury (Le Prieult et al. 2017). In search for the underlying mechanisms here we investigated the role of somatostatin-positive (SST)-GABAergic interneurons in mice. Unilateral TBI was induced under anesthesia by use of a controlled mechanical impact in vivo. We isolated the contralateral cortex after a survival time of 72 h and performed Fluorescence-Activated Cell Sorting (FACS) and Mass Spectrometry (MS) in GFP+-interneurons. Proteomic data showed a TBI-induced putative reciprocal regulation of α subunits of pore-forming L-Type Voltage-gated calcium channel (VGCCs), represented by an expression of CaV1.3 and simultaneous ablation of CaV1.2. Next, we tested whether this switch in α-subunits supported a recovery of the early (24h after TBI) contralateral hyperexcitability, therefore we performed electrophysiological recordings in acute brain slices in presence of a CaV1.3-selective antagonist. Isradipine stabilized the extracellular cortical network activity back to the level of controls at 72 h post-TBI. Furthermore, patch-clamp recordings from somatostatin-positive (SST) interneurons confirmed this switch in VGCC α-subunits. F/I-curves revealed an isradipine sensitive increase in excitability through expression of CaV1.3. In conclusion, the TBI-induced expression of CaV1.3 in VGCCs in SST-interneurons supports the adaptive processes of functional reorganization in the contralateral network after the injury. These mechanisms might affect the later expression of post-traumatic-epilepsy (PTE). This work was supported by a grant to TM (DFG, CRC 1080, C02).

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-176

ON THE KINETICS OF CALCIUM CURRENTS AND THE ACTIVATION OF CALCIUM-ACTIVATED POTASSIUM CHANNELS IN LAYER-5 PYRAMIDAL NEURONS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Calcium influx via voltage-gated calcium channels (VGCCs) contributes to the shaping of the action potential (AP) by regulating the potassium conductance during the decaying phase. Here we optically analysed the kinetics of the calcium current associated with the AP in the proximal part of the apical dendrite of the layer-5 pyramidal neuron of the somato-sensory cortex of the mouse. We found that this current is mediated by L-type, P/Q-type, N-type, R-type and T-type VGCCs. The selective block of each individual channel decreases the amplitude of the calcium current, except for the N-type VGCC where inhibition produces the opposite effect. We then correlated the optical recording of the AP, using membrane potential imaging, with the calcium current finding that the peak of the AP precedes the peak of the calcium current by 0.5-1 ms. The effect of blocking N-type or L-type VGCCs, as well as the effect of blocking BK or SK calcium-activated potassium channels, was a widening of the AP shape preceding the peak of the calcium current. We concluded that calcium-activated potassium channels are activated by the early calcium influx and we formulate the hypothesis that each potassium channel is specifically coupled to a calcium channel. In particular, N-type VGCCs, although less expressed with respect to other VGCCs, substantially contribute to the potassium conductance associated with the AP repolarisation. More generally, the results of this study may shed new light on functional channel-channel interactions and how the synergy of diverse channels result in the shaping of the AP.

FENS Forum 2022
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BOARD NUMBER: S04-177

DIFFERENTIAL MODULATION OF GABAA AND GLYCINE RECEPTORS BY GELSEMIUM ALKALOIDS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

Ana María Marileo[1], César Lara[1], Victoria San Martín[1], Anggelo Sazo[1], Jessica Panes[2], Carlos Felipe Burgos[2], Gustavo Moraga-Cid[2], Gonzalo Yévenes[2]
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The extracts of the *Gelsemium* genus plants have been used in traditional medicine to treat several diseases. Nevertheless, they also have shown an intrinsic high toxicity. Chemical characterizations of *Gelsemium* extracts have determined that the major active components of these plants are alkaloids, such as gelsemine, koumine, humantenmine, and gelsevirine. Our previous studies have shown that gelsemine can modulate the activity of glycine receptors (GlyRs), a pentameric inhibitory ligand-gated ion channel of the nervous system. Whether other *Gelsemium* alkaloids also modulate GlyRs or other inhibitory channels, such as $GABA_A$ receptors ($GABA_ARs$), is currently unknown. Here, we have explored the effects of gelsemine, koumine, humantenmine, and gelsevirine on $GABA_A$ and glycine receptors using electrophysiological recordings performed on recombinant and native receptors. We have found that these alkaloids differentially modulated $GABA_A$ and glycine receptors. Our preliminary results have shown that gelsevirine has a potentiating effect on the GABA-evoked currents of native $GABA_ARs$. In contrast, we didn't observe any significant effect on glycine-evoked currents on recombinant GlyRs. We found that Gelsemine and koumine have an inhibitory effect over both kinds of receptors. Experiments with humentenmine didn't find a significant effect on the $GABA_ARs$. Finally, molecular modeling suggests that all these alkaloids can bind to the orthosteric site of both receptors. These ongoing experiments will let us explore the alkaloids' effects on different conformations of these receptors, which let us explain some of the toxic effects of these alkaloids and contribute to expanding the pharmacological studies of inhibitory receptors.

Pubmed:
32179786: Moraga-Cid G, San Martín VP, Lara CO, Muñoz B, Marileo AM, Sazo A, Muñoz-Montesino C, Fuentealba J, Castro PA, Guzmán L, Burgos CF, Zeilhofer HU, Aguayo LG, Corringer PJ, Yévenes GE
Modulation of glycine receptor single-channel conductance by intracellular phosphorylation.
Glycine receptors (GlyRs) are anion-permeable pentameric ligand-gated ion channels (pLGICs). The GlyR activation is critical for the control of key neurophysiological functions, such as motor coordination, respiratory control, muscle tone and pain processing. The relevance of the GlyR function is further highlighted by the presence of abnormal glycinergic inhibition in many pathophysiological states, such as hyperekplexia, epilepsy, autism and chronic pain. In this context, previous studies have shown that the functional inhibition of GlyRs containing the α3 subunit is a pivotal mechanism of pain hypersensitivity. This pathway involves the activation of EP2 receptors and the subsequent PKA-dependent phosphorylation of α3GlyRs within the intracellular domain (ICD), which decrease the GlyR-associated currents and enhance neuronal excitability. Despite the importance of this mechanism of glycinergic dis-inhibition associated with dysfunctional α3GlyRs, our current understanding of the molecular events involved is limited. Here, we report that the activation of PKA signaling pathway decreases the unitary conductance of α3GlyRs. We show in addition that the substitution of the PKA-targeted serine with a negatively charged residue within the ICD of α3GlyRs and of chimeric receptors combining bacterial GLIC and α3GlyR was sufficient to generate receptors with reduced conductance. Thus, our findings reveal a potential biophysical mechanism of glycinergic dis-inhibition and suggest that post-translational modifications of the ICD, such as phosphorylation, may shape the conductance of other pLGICs.
Sci Rep, 2020; 10
31024303: San Martín VP, Burgos CF, Marileo AM, Lara CO, Sazo A, Fuentealba J, Guzmán L, Castro PA, Aguayo LG, Moraga-Cid G, Yévenes GE
Inhibitory Actions of Tropeines on the α3 Glycine Receptor Function.
Glycine receptors (GlyRs) are chloride-permeable pentameric ligand-gated ion channels. The inhibitory activity of GlyRs is essential for many physiological processes, such as motor control and respiration. In addition, several pathological states, such as hyperekplexia, epilepsy, and chronic pain, are associated with abnormal glycinergic inhibition. Recent studies have

pointed out that positive allosteric modulators targeting the GlyR α3 subunit (α3GlyR) displayed beneficial effects in chronic pain models. Interestingly, previous electrophysiological studies have shown that tropeines, which are a family of synthetic antagonists of the serotonin type 3 receptors (5-HTRs), potentiate the activity of GlyRs conformed by α1 subunits. However, despite its importance as a pharmacological target in chronic pain, it is currently unknown whether the α3GlyR function is modulated by tropeines. Using electrophysiological techniques and molecular docking simulations, here we show that tropeines are inhibitors of the α3GlyR function. Tropisetron, a prototypical tropeine, exerted concentration-dependent inhibitory effects on α3GlyRs at the low micromolar range. In addition, three other tropeines showed similar effects. Single-channel recordings show that tropisetron inhibition is associated with a decrease in the open probability of the ion channel. Molecular docking assays suggest that tropeines preferentially bind to an agonist-free, closed state of the ion channel. The tropeine binding occurs in a discrete pocket around the vicinity of the orthosteric site within the extracellular domain of α3GlyR. Thus, our results describe the pharmacological modulation of tropeines on α3GlyRs. These findings may contribute to the development of GlyR-selective tropeine derivatives for basic and/or clinical applications.
Front Pharmacol, 2019; 10
27128379: Lara CO, Murath P, Muñoz B, Marileo AM, Martín LS, San Martín VP, Burgos CF, Mariqueo TA, Aguayo LG, Fuentealba J, Godoy P, Guzman L, Yévenes GE
Functional modulation of glycine receptors by the alkaloid gelsemine.
Gelsemine is one of the principal alkaloids produced by the Gelsemium genus of plants belonging to the Loganiaceae family. The extracts of these plants have been used for many years, for a variety of medicinal purposes. Coincidentally, recent studies have shown that gelsemine exerts anxiolytic and analgesic effects on behavioural models. Several lines of evidence have suggested that these beneficial actions were dependent on glycine receptors, which are inhibitory neurotransmitter-gated ion channels of the CNS. However, it is currently unknown whether gelsemine can directly modulate the function of glycine receptors.
Br J Pharmacol, 2016; 173

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-178

COMPARING THE FUNCTIONAL EFFECTS OF TWO DIFFERENT POSITIVE ALLOSTERIC MODULATORS OF GLYCINE RECEPTORS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

César Lara[1,2], Gustavo Moraga-Cid[1,2], Ana María Marileo[1,2], Victoria San Martín[1,2], Anggelo Sazo[1,2], Jeremías Corradi[3], Jorge Fuentealba[1], Leonardo Guzmán[1], Patricio Castro[1], Luis Aguayo[1], Cecilia Bouzat[3], Gonzalo Yévenes[1,2]
[1]University of Concepción, Department Of Physiology, Concepción, Chile, [2]Millennium Nucleus for the Study of Pain, (minuspain), Santiago, Chile, [3]Universidad Nacional del Sur/CONICET, Instituto De Investigaciones Bioquímicas De Bahía Blanca, Bahía Blanca, Argentina

Glycine Receptors (GlyRs) are pentameric anion-permeable ligand-gated ion channels. Hypo-functional GlyRs have been related to several pathological states, such as chronic pain and hyperekplexia. The modulation of GlyRs by positive allosteric modulators (PAMs) has emerged as a promising therapeutic approach against chronic pain. However, only few studies have explored the action mechanisms of glycinergic PAMs at the functional level. Here, we studied the mechanisms of modulation of two different types of glycinergic PAMs, which displayed analgesic actions in behavioral studies. By using single-channel and whole-cell electrophysiology of recombinant GlyRs, we focus our functional analyses on the effects of 2,6-di-tert-butylphenol (2,6-DTBP) and of the tricyclic sulfonamide AM-1488. Whole-cell recordings show that both 2,6-DTBP and AM-1488 exerted concentration-dependent potentiation of homomeric GlyRs composed of α1 and α3 subunits. While previous structural data showed that AM-1488 bound to the extracellular domain of the receptor, our preliminary mutagenesis studies combined with molecular modeling showed that the actions of 2,6-DTBP are linked to two binding sites localized on both transmembrane and intracellular domains. Single-channel recordings revealed that the effects of 2,6-DTBP and AM-1488 are associated with different biophysical mechanisms of modulation. Dwell analysis showed that 2,6-DTBP generated longer burst time and longer channel openings, whereas AM-1488 also produced longer burst time but without modifications on the open time. Altogether, our results suggest that different biophysical mechanisms of modulation for PAMs targeting GlyRs are likely related with independent allosteric sites. These findings may contribute to the development of novel glycinergic PAMs with differential modulatory profiles.

Pubmed:
32903667: Muñoz-Montesino C, Burgos CF, Lara CO, Riquelme CR, Flaig D, San Martin VP, Aguayo LG, Fuentealba J, Castro PA, Guzmán L, Yévenes GE, Moraga-Cid G
Inhibition of the Glycine Receptor alpha 3 Function by Colchicine.
Colchicine is a plant alkaloid that is widely used as a therapeutic agent. It is widely accepted that colchicine reduces the production of inflammatory mediators mainly by altering cytoskeleton dynamics due to its microtubule polymerization inhibitory activity. However, other lines of evidence have shown that colchicine exerts direct actions on the function of ion channels, which are independent of cytoskeleton alterations. Colchicine is able to modify the function of several pentameric ligand-gated ion channels, including glycine receptors (GlyRs). Previous electrophysiological studies have shown that colchicine act as an antagonist of GlyRs composed by the subunit. In addition, it was recently demonstrated that colchicine directly bind to the subunit of GlyRs. Interestingly, other studies have shown a main role of GlyRs on chronic inflammatory pain. Nevertheless, the functional effects of colchicine on the GlyR function are still unknown. Here, by using electrophysiological techniques and bioinformatics, we show that colchicine inhibited the function of the GlyRs. Colchicine elicited concentration-dependent inhibitory effects on GlyRs at micromolar range and decreased the apparent affinity for glycine. Single-channel recordings show that the colchicine inhibition is associated with a decrease in the open probability of the ion channel. Molecular docking assays suggest that colchicine preferentially bind to the orthosteric site in the closed state of the ion channel. Altogether, our results suggest that colchicine is a competitive antagonist of the GlyRs.
Front Pharmacol, 2020; 11
32218730: Lara CO, Burgos CF, Moraga-Cid G, Carrasco MA, Yévenes GE
Pentameric Ligand-Gated Ion Channels as Pharmacological Targets Against Chronic Pain.
Chronic pain is a common detrimental condition that affects around 20% of the world population. The current drugs to treat chronic pain states, especially neuropathic pain, have a limited clinical efficiency and present significant adverse effects that

complicates their regular use. Recent studies have proposed new therapeutic strategies focused on the pharmacological modulation of G-protein-coupled receptors, transporters, enzymes, and ion channels expressed on the nociceptive pathways. The present work intends to summarize recent advances on the pharmacological modulation of pentameric ligand-gated ion channels, which plays a key role in pain processing. Experimental data have shown that novel allosteric modulators targeting the excitatory nicotinic acetylcholine receptor, as well as the inhibitory GABA and glycine receptors, reverse chronic pain-related behaviors in preclinical assays. Collectively, these evidences strongly suggest the pharmacological modulation of pentameric ligand-gated ion channels is a promising strategy towards the development of novel therapeutics to treat chronic pain states in humans.
Front Pharmacol, 2020; 11
32179786: Moraga-Cid G, San Martín VP, Lara CO, Muñoz B, Marileo AM, Sazo A, Muñoz-Montesino C, Fuentealba J, Castro PA, Guzmán L, Burgos CF, Zeilhofer HU, Aguayo LG, Corringer PJ, Yévenes GE
Modulation of glycine receptor single-channel conductance by intracellular phosphorylation.
Glycine receptors (GlyRs) are anion-permeable pentameric ligand-gated ion channels (pLGICs). The GlyR activation is critical for the control of key neurophysiological functions, such as motor coordination, respiratory control, muscle tone and pain processing. The relevance of the GlyR function is further highlighted by the presence of abnormal glycinergic inhibition in many pathophysiological states, such as hyperekplexia, epilepsy, autism and chronic pain. In this context, previous studies have shown that the functional inhibition of GlyRs containing the α3 subunit is a pivotal mechanism of pain hypersensitivity. This pathway involves the activation of EP2 receptors and the subsequent PKA-dependent phosphorylation of α3GlyRs within the intracellular domain (ICD), which decrease the GlyR-associated currents and enhance neuronal excitability. Despite the importance of this mechanism of glycinergic dis-inhibition associated with dysfunctional α3GlyRs, our current understanding of the molecular events involved is limited. Here, we report that the activation of PKA signaling pathway decreases the unitary conductance of α3GlyRs. We show in addition that the substitution of the PKA-targeted serine with a negatively charged residue within the ICD of α3GlyRs and of chimeric receptors combining bacterial GLIC and α3GlyR was sufficient to generate receptors with reduced conductance. Thus, our findings reveal a potential biophysical mechanism of glycinergic dis-inhibition and suggest that post-translational modifications of the ICD, such as phosphorylation, may shape the conductance of other pLGICs.
Sci Rep, 2020; 10
31024303: San Martín VP, Burgos CF, Marileo AM, Lara CO, Sazo A, Fuentealba J, Guzmán L, Castro PA, Aguayo LG, Moraga-Cid G, Yévenes GE
Inhibitory Actions of Tropeines on the α3 Glycine Receptor Function.
Glycine receptors (GlyRs) are chloride-permeable pentameric ligand-gated ion channels. The inhibitory activity of GlyRs is essential for many physiological processes, such as motor control and respiration. In addition, several pathological states, such as hyperekplexia, epilepsy, and chronic pain, are associated with abnormal glycinergic inhibition. Recent studies have pointed out that positive allosteric modulators targeting the GlyR α3 subunit (α3GlyR) displayed beneficial effects in chronic pain models. Interestingly, previous electrophysiological studies have shown that tropeines, which are a family of synthetic antagonists of the serotonin type 3 receptors (5-HTRs), potentiate the activity of GlyRs conformed by α1 subunits. However, despite its importance as a pharmacological target in chronic pain, it is currently unknown whether the α3GlyR function is modulated by tropeines. Using electrophysiological techniques and molecular docking simulations, here we show that tropeines are inhibitors of the α3GlyR function. Tropisetron, a prototypical tropeine, exerted concentration-dependent inhibitory effects on α3GlyRs at the low micromolar range. In addition, three other tropeines showed similar effects. Single-channel recordings show that tropisetron inhibition is associated with a decrease in the open probability of the ion channel. Molecular docking assays suggest that tropeines preferentially bind to an agonist-free, closed state of the ion channel. The tropeine binding occurs in a discrete pocket around the vicinity of the orthosteric site within the extracellular domain of α3GlyR. Thus, our results describe the pharmacological modulation of tropeines on α3GlyRs. These findings may contribute to the development of GlyR-selective tropeine derivatives for basic and/or clinical applications.
Front Pharmacol, 2019; 10
30893555: Lara CO, Burgos CF, Silva-Grecchi T, Muñoz-Montesino C, Aguayo LG, Fuentealba J, Castro PA, Guzmán JL, Corringer PJ, Yévenes GE, Moraga-Cid G
Large Intracellular Domain-Dependent Effects of Positive Allosteric Modulators on Glycine Receptors.
Glycine receptors (GlyRs) are members of the pentameric ligand-gated ionic channel family (pLGICs) and mediate fast inhibitory neurotransmission in the brain stem and spinal cord. The function of GlyRs can be modulated by positive allosteric modulators (PAMs). So far, it is largely accepted that both the extracellular (ECD) and transmembrane (TMD) domains constitute the primary target for many of these PAMs. On the other hand, the contribution of the intracellular domain (ICD) to the PAM effects on GlyRs remains poorly understood. To gain insight about the role of the ICD in the pharmacology of GlyRs, we examined the contribution of each domain using a chimeric receptor. Two chimeras were generated, one consisting of the

ECD of the prokaryotic homologue Gloeobacter violaceus ligand-gated ion channel (GLIC) fused to the TMD of the human αGlyR lacking the ICD (Lily) and a second with the ICD (Lily-ICD). The sensitivity to PAMs of both chimeric receptors was studied using electrophysiological techniques. The Lily receptor showed a significant decrease in the sensitivity to four recognized PAMs. Remarkably, the incorporation of the ICD into the Lily background was sufficient to restore the wild-type αGlyR sensitivity to these PAMs. Based on these data, we can suggest that the ICD is necessary to form a pLGIC having full sensitivity to positive allosteric modulators.
ACS Chem Neurosci, 2019; 10
27270175: Acuña MA, Yévenes GE, Ralvenius WT, Benke D, Di Lio A, Lara CO, Muñoz B, Burgos CF, Moraga-Cid G, Corringer PJ, Zeilhofer HU
Phosphorylation state-dependent modulation of spinal glycine receptors alleviates inflammatory pain.
Diminished inhibitory neurotransmission in the superficial dorsal horn of the spinal cord is thought to contribute to chronic pain. In inflammatory pain, reductions in synaptic inhibition occur partially through prostaglandin E2- (PGE2-) and PKA-dependent phosphorylation of a specific subtype of glycine receptors (GlyRs) that contain α3 subunits. Here, we demonstrated that 2,6-di-tert-butylphenol (2,6-DTBP), a nonanesthetic propofol derivative, reverses inflammation-mediated disinhibition through a specific interaction with heteromeric αβGlyRs containing phosphorylated α3 subunits. We expressed mutant GlyRs in HEK293T cells, and electrophysiological analyses of these receptors showed that 2,6-DTBP interacted with a conserved phenylalanine residue in the membrane-associated stretch between transmembrane regions 3 and 4 of the GlyR α3 subunit. In native murine spinal cord tissue, 2,6-DTBP modulated synaptic, presumably αβ heteromeric, GlyRs only after priming with PGE2. This observation is consistent with results obtained from molecular modeling of the α-β subunit interface and suggests that in α3βGlyRs, the binding site is accessible to 2,6-DTBP only after PKA-dependent phosphorylation. In murine models of inflammatory pain, 2,6-DTBP reduced inflammatory hyperalgesia in an α3GlyR-dependent manner. Together, our data thus establish that selective potentiation of GlyR function is a promising strategy against chronic inflammatory pain and that, to our knowledge, 2,6-DTBP has a unique pharmacological profile that favors an interaction with GlyRs that have been primed by peripheral inflammation.
J Clin Invest, 2016; 126
27128379: Lara CO, Murath P, Muñoz B, Marileo AM, Martín LS, San Martín VP, Burgos CF, Mariqueo TA, Aguayo LG, Fuentealba J, Godoy P, Guzman L, Yévenes GE
Functional modulation of glycine receptors by the alkaloid gelsemine.
Gelsemine is one of the principal alkaloids produced by the Gelsemium genus of plants belonging to the Loganiaceae family. The extracts of these plants have been used for many years, for a variety of medicinal purposes. Coincidentally, recent studies have shown that gelsemine exerts anxiolytic and analgesic effects on behavioural models. Several lines of evidence have suggested that these beneficial actions were dependent on glycine receptors, which are inhibitory neurotransmitter-gated ion channels of the CNS. However, it is currently unknown whether gelsemine can directly modulate the function of glycine receptors.
Br J Pharmacol, 2016; 173

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-179

ENLIGHTENING THE GLYCINE RECEPTOR α2 AS A KEY REGULATOR IN THE BRAIN REWARD PATHWAY

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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[1]Hasselt University, Neuroscience, Diepenbeek, Belgium, [2]Hasselt University, Advanced Optical Microscopy, Diepenbeek, Belgium

The glycine receptor alpha 2 subunit (GlyRα2) is functionally expressed in the striatal projection neurons (SPNs). SPNs are a convergence point for glutamatergic and dopaminergic input and this signal integration coordinates reward-motivated behavior. Data from our laboratory show that the GlyRα2 modulates SPN activity, thus, targeting GlyRα2 offers great potential to modulate striatum-orchestrated behavior. However, it is unclear how the subcellular localization of GlyRα2 affects its role in regulating SPN activity. We aim to investigate the subcellular localization and dynamics of the GlyRα2 by fusing a genetically encoded yellow fluorescent protein-based chloride-sensor (Zhong S, et al. (2014). PLoS One 9(6): e99095) to a human GlyRα2 (hGlyRα-mClYFP). Using patch clamp, whole-cell glycine induced currents (10, 30, 100, 300, 1000 μM glycine, holding potential -60 mV) of hGlyRα-mClYFP transfected into HEK293 cells were obtained. The EC50 of the hGlyRα-mClYFP current (~400 μM) corresponds with previously published EC50 of the GlyRα2 (~200 μM), indicating normal glycine sensitivity of the chimeric receptor. Chloride induced fluorescent quenching was measured in epifluorescence microscopy and indicated that the fluorescence signal is directly linked to the glycine induced chloride current, witnessed by an EC50 of 170 μM. These experiments suggest that the hGlyRα-mClYFP retains normal whole cell current activity whilst possessing mClYFP sensor properties to detect chloride concentration changes.

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BOARD NUMBER: S04-180

OXIDATION SENSITIZES TRPV2 TO CHEMICAL AND HEAT STIMULATION, BUT NOT MECHANICAL STIMULATION

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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The transient receptor potential vanilloid 2 (TRPV2) ion channel is activated by a chemical ligand (2-aminoethoxydiphenyl borate; 2-APB), noxious heat and mechanical stimulation. In a heterologous mammalian cell expression system, the oxidant chloramine T (ChT) sensitizes TRPV2 activation in response to 2-APB and heat by oxidation of methionine residues at positions 528 and 607 in rat TRPV2. Here, we used a *Xenopus* oocyte expression system to determine whether ChT-mediated oxidation can also sensitize TRPV2 to mechanical stimulation. In this system, we confirmed that ChT sensitized TRPV2 activation in response to 2-APB and heat, but we detected no sensitization to mechanical stimulation. This result suggests that the activation mechanism of TRPV2 by a chemical ligand and heat differs from that for mechanical stimulation. Further, we demonstrated that two-electrode voltage clamp recording in the *Xenopus* oocyte expression system is an excellent format for high throughput analysis of oxidization of redox-sensitive TRP channels.

FENS Forum 2022
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BOARD NUMBER: S04-181

COMPUTATIONAL STUDY OF THE EFFECTS OF HCN CHANNEL MODULATION ON L5PC EXCITABILITY

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Dendrites of cortical pyramidal cells are densely populated by hyperpolarization-activated cyclic nucleotide-gated channels, a.k.a. I_h channels. I_h channels are targeted by multiple neuromodulatory pathways and thus are one of the key ion-channel populations regulating the pyramidal cell activity. Previous observations and theories attribute opposing effects of the I_h channels on neuronal excitability due to their mildly hyperpolarized reversal potential. These effects are difficult to measure experimentally due to the fine spatiotemporal landscape of the I_h activity in the dendrites, but computational models provide an efficient tool for studying this question in a reduced but generalizable setting. In this work, we build upon existing biophysically detailed models of thick-tufted layer V pyramidal cells (L5PC) and model the effects of over- and under-expression of I_h channels as well as their neuromodulation by dopamine (gain of I_h function) and acetylcholine (loss of I_h function). We show that I_h channel activity facilitates the action potentials of L5PCs in response to proximal dendritic stimulus while it hinders the action potentials in response to distal dendritic stimulus at the apical dendrite. We also show that the inhibitory action of the I_h channels in L5PCs is due to the interactions between I_h channels and a hot zone of low voltage-activated Ca^{2+}channels at the apical dendrite. Our simulations suggest that a combination of dopaminergic neuromodulation of I_h channels at the proximal apical dendrite and cholinergic modulation at the distal apical dendrite can increase the L5PC excitability more than any of the two neuromodulators alone.

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BOARD NUMBER: S04-182

CHRFAM7A: A HUMAN SPECIFIC α7- NICOTINIC ACETYLCHOLINE RECEPTOR GENE

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Genome-wide association studies suggest a critical role of the *CHRNA7* (coding for α7 nAChR) and/or *CHRFAM7A* genes in cognitive and psychiatric disorders including schizophrenia and many others. *CHRFAM7A* occurs only in humans and encodes a partially duplicated version of the α7 nAChR (dupα7) subunit lacking the agonist binding site. Our hypothesis is that dupα7 subunit acts as a functional dominant negative regulator and may reduce the α7 nAChR function. In our project, we aim to investigate the functional consequences of *CHRFAM7A* on α7 nAChR in human neurons. To study human specific *CHRFAM7A,* cortical neurons are differentiated from human induced pluripotent stem cells (hiPSCs) with either overexpressing or lacking *CHRFAM7A*. hiPSC-neurons are stained by using fluorescently labelled neuronal markers for molecular characterization and analysed functionally by Fura-2 Ca^{2+}-Imaging and Patch-Clamp electrophysiology. With patch-clamp recordings in the voltage-clamp mode, hiPSC-neurons lacking *CHRFAM7A* responded to depolarizing voltage steps by inward-directed sodium currents and outward-directed potassium currents. After at least 4 weeks of differentiation, these currents formed the basis for trains of action potentials recorded in current-clamp mode. We observed an increase in the frequency of miniature excitatory post-synaptic currents upon application of the α7 nAChR specific agonist PNU-282987 when combined with the PAMII PNU-120596. Fura-2 Ca^{2+}-Imaging revealed that in the presence of TTX, the combined application of PNU-282987 and PNU-120596 increased intracellular Ca^{2+}. This increase was significantly reduced in hiPSC-neurons overexpressing *CHRFAM7A.* In conclusion, we were able to confirm that dupα7 subunit reduces the α7 nAChR function in hiPSC-neurons.

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MODULATION BY NEUROSTEROIDS OF THE GABAA RECEPTORS AND KIR CHANNELS EXPRESSED IN OLIGODENDROGLIA

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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In the central nervous system, the myelination of axons carried out by oligodendrocytes (OLs) is a fundamental phenomenon for the proper functioning of the system. OLs are differentiated from OPCs through a process that is controlled by several factors. Neuron-OPC communication through distinct neurotransmitters has been shown important for the process, and, for example it is known that OPCs differentiation, as well as OLs maturation, and myelination per se, are phenomena regulated by Ɣ-amino butyric acid (GABA) acting on GABA receptors type A (GABAAR) , and by neurosteroids (NEs), which are GABAAR modulators. The specific effect(s)of NEs on membrane proteins of OLs is unknown, thus, in here our main goal was to characterize the possible effects on two membrane currents that play an important role in the maturation of the oligodendrocytes and the subsequent myelination process, the response generated by GABAAR opening and the activity of Kir channels. For this, we characterized electrophysiologically the current response elicited by GABA (3-10 µM) in cultured rat OPCs with or without Ganaxolone (Gx), a synthetic allopregnanolone analogue, as well as recorded Kir currents in cells which presented characteristics of premyelinating cells from the same cultures. The results show that Gx (100 nM — 1 µM) promotes a robust enhancement of 350 ± 50 % (n=31) of the GABA response and inhibited the Kir current by 90 ± 12 % (n=14). We suggest that NEs modulation of the GABA response and Kir channels might be of importance during oligodendrocyte differentiation and/or maturation.

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PATHOGENIC MUTATION GLUN1-N650K IN COMBINATION WITH GLUN2A SUBUNIT CHANGES KINETIC PARAMETERS AND CONDUCTANCE OF NMDA RECEPTORS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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AIMS: This study investigated the effect of the pathogenic GluN1-N650K mutation, associated with developmental delay and seizures (doi:10.1111/epi.12987), on the functional and pharmacological properties of NMDARs. **METHODS:** Whole-cell voltage-clamp recordings performed in HEK293 cells transfected with the WT and mutated hGluN1-4a/hGluN2A receptors and hippocampal neurons infected with lentiviruses expressing the WT and mutated YFP-tagged hGluN1 subunits. Immunofluorescence was performed 24 hours after transfection (for HEK293 cells) and 10 days after lentiviral infection (for hippocampal neurons) using an anti-GFP antibody. Excitotoxicity experiments performed in hippocampal neurons (DIV14) by calculating the proportion of pyknotic/non-pyknotic nuclei (staining with Hoechst 33342). **RESULTS:** Our microscopic experiments showed that the GluN1-N650K mutation increased surface expression of GluN1/GluN2A receptors in HEK293 calls and in the hippocampal neurons. Our electrophysiological experiments showed that the GluN1-N650K mutation increased the potency of GluN1/GluN2A receptors for glutamate and glycine. In the presence of physiological concentrations of Mg^{2+}, GluN1-N650K/GluN2A receptors showed stronger potency to memantine but not to ketamine. Moreover, the hGluN1-N650K mutation significantly slowed the binding and unbinding kinetics of memantine and ketamine. Finally, we found that the presence of the GluN1-N650K subunit reduces the level of excitotoxic damage to hippocampal neurons and memantine shows promising neuroprotective effects in the presence of WT and GluN1-N650K subunits. **CONCLUSIONS:** This study indicate that pathogenic GluN1-N650K mutation in the M3 domain of the GluN1 subunit affect the trafficking and functional properties of NMDARs. *This work was supported by a project from the Czech Science Foundation (20-12420S) and the Grant Agency of Charles University (GAUK:320521).*

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PATHOGENIC MUTATION GLUN1-N650K IN COMBINATION WITH GLUN2A SUBUNIT CHANGES KINETIC PARAMETERS AND CONDUCTANCE OF NMDA RECEPTORS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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AIMS: To describe how the pathogenic mutation N650K in the GluN1 subunit, associated with developmental delay and seizures, affects kinetic parameters and conductivity of GluN1/GluN2A NMDA receptors. **METHODS:** HEK293 cells expressing wild-type (WT) or mutated human GluN1-4a/GluN2A receptors were measured using the patch-clamp technique. For the calculation of the probability of opening (Po), single-channel steady-state activity was recorded in cell-attached patches, whereas NMDAR conductivity measurements were performed in pulled outside-out patches. **RESULTS:** The analysis of cell-attached recordings containing WT receptors showed that the distribution of dwell times was best fitted with an average kinetic scheme consisting of two open and five closed states. In contrast, recordings containing mutated receptors were described using only one open state and just four closed states. From the calculated time constants and their areas, we observed ~165-fold reduction of Po of mutated receptors compared to WT receptors. This robust reduction of Po results from a ~10-fold decrease in mean open time and a ~32-fold increase of the mean closed time. From the cell-attached recording, we also observed reduced amplitude for mutated receptors. We confirmed this observation with our conductivity measurements, which showed that the conductance significantly decreased from 52.1±0.8 pS for WT receptors to 36.3±1.0 pS for mutated receptors. **CONCLUSIONS**: This study showed robust changes in kinetic parameters and conductivity in receptors containing pathogenic mutation GluN1-N650K associated with developmental delay and seizures. *This work was supported by a project from the Czech Science Foundation (*20-12420S*) and the Grant Agency of Charles University (GAUK: 306221).*

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MECHANOSENSITIVITY OF URINARY BLADDER SMOOTH MUSCLES: THE ROLE OF TREK-1/TRPV4/PIEZO1 CHANNELS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Urinary bladder mechanosensivity is realized not only via neurogenic and urothelium-dependent mechanisms, but may also rely directly on detrusor smooth muscle cells (DSMC). Here we have investigated the role of mechano-gated ion channels, TREK1, TRPV4, and Piezo1, as possible candidates for mechanoreceptors in DSMC. Membrane currents (I_m) and $[Ca^{2+}]_i$ signals in rat DSMC in response to mechanostimulation (shear stress at 5 nl/s) or application of selective TREK-1, Piezo1, and TRPV4 activators, arachidonic acid (AA, 50 μM), Yoda1 (20 μM) and GSK1016790A (50 μM), respectively, were studied by whole-cell patch-clamp and fluorescence calcium imaging with Fluo-4AM. TREK-1-mediated current was also probed with specific inhibitor, L-methionine (3 mM). DSMC in terms of I_m generated in response to mechanostimulation could be separated onto three groups: 1) nonresponsive (46%), 2) generating outwardly-rectifying, L-methionine-sensitive current with reversal potential V_r~-70 mV (19%), and 3) generating current with quasilinear I-V relationship and V_r~0 mV. Yoda1 application evoked $[Ca^{2+}]_i$ elevation but, surprisingly, inhibited outward component of the I_m rather than activated inward one. GSK1016790A application appeared ineffective in either activating I_m or elevating $[Ca^{2+}]_i$. Application of AA caused drastic increase of inward currents. We conclude that rat DSMC functionally express TREK-1 and Piezo1, but not TRPV4 channels which may shape up myogenic bladder mechanosensitivity. Heterogeneity of DSMC population with respect to the size and type of mechanoactivated I_m may reflect either their origin from different bladder parts or differences in their functional state (degree of contraction). Supported by 2020.02/0189 grant from National Research Foundation of Ukraine.

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BOARD NUMBER: S04-187

LIPID ENVIRONMENT IS ESSENTIAL FOR OPTIMAL ACTIVITY OF PLASMA MEMBRANE CA2+ ATPASE IN MURINE BRAIN TISSUE

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Plasma membrane calcium ATPase (PMCA) is an integral resident across eukaryotic membrane systems, implicated in miscellaneous brain functions such as equilibrating cellular Ca^{2+} levels and signal transduction. Our preliminary data indicate that Toll-like receptor 2 deficient mice (TLR2D) exhibit differential expression of PMCA isoforms across nervous tissue. Due to firm evidence that P-type ATPases generally require a specialized lipid environment to manage assigned functions, we aimed to track specific PMCA activity and its submembrane distribution in TLR2D compared to wild-type (WT) mice. For that purpose, we used cortical, hippocampal, and cerebellar samples dissected from the TRL2D and WT brains. PMCA activity was measured by spectrophotometry, while PMCA positioning within membranes was investigated in isolated lipid raft and non-raft fractions. The results strongly imply probable interactions between PMCA and membrane lipids. The subfractionation of the membrane gave us a clue that PMCA is shifting through different lipid microenvironments with specific membrane properties. Secondly, the measured variations in the specific PMCA activity between TLR2 KO and the control animal group reflect the fact that P-type ATPases activity correlates with a different lipid-residing environment characterized by different membrane properties. These findings argue that specific PMCA arrangements in a lipid environment fine-tune differential expression of PMCA, thus possibly changing Ca^{2+} signaling and influencing physiological functions of the nervous system in TLR2D mice. Further study will help us understand the exact role and neurophysiological outcome of the interplay between PMCA isoforms and its neighboring membrane lipids in mammalian brain tissue.

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HIGH-RESOLUTION PROTEOMICS UNRAVEL A NATIVE FUNCTIONAL UNIT MADE UP OF CAV1.3, SK3 AND HCN2 CHANNELS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Aims Pacemaking activity is critical to the function of *substantia nigra pars compacta* (SNc) dopaminergic neurons and is intrinsically driven by several voltage-and Ca^{2+}-dependent channels. However, spontaneous activity pattern was found to be robust to knock-down of Cav1.3 voltage-gated calcium, and calcium activated SK3 channels, suggesting that compensatory mechanisms must occur. Furthermore, co-variation of voltage dependences of I_A (Kv4) and I_H (HCN) was characterized in SNc neurons. Thus, pacemaking stability may potentially be generated by ion channel coordinated regulations taking place in large protein complexes. Methods We performed immunoprecipitation using magnetic bead-coupled antibody against Cav1,2, Cav1.3, HCN2, HCN4, Kv4.3, and SK3 channels from solubilized midbrain wild-type mice membranes or antigen-deleted knock out mice as negative control. After tandem mass spectrometry analysis, quantitative proteomic data were statistically analyzed using the MaxQuant/Perseus framework to decipher significant interactants. Results The already known multiprotein assembly of Kv4.3, kchip4 and DPP6/10 was found, validating the technique. Unexpectedly, we uncovered distinctive interactors for channels with functional and structural similarities such as Cav1.2, Cav1.3 and HCN2, HCN4. The following new potential binding partners were found: Cav1.3 –RiMBP2; Pex5-Cav1.2; HCN4-Sarm1, SK3-TSC1-TSc2; HCN2-AMPAR. Interestingly a Cav1.3 -SK3 and HCN2 super-channel complex was characterized and further confirmed by *in situ* Proximity Ligation Assay. Conclusion We could decipher a calcium-sensing nanodomain where voltage-dependent calcium channels and calcium-sensitive potassium channels would physically interact with cAMP responsive HCN channels themselves closely modulated by calcium-activated adenylate cyclase producing cAMP. This interacting structure could be a molecular basis for pacemaking activity robustness.

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RNAS EXPRESSION PROFILE OF N1E-115 CELLS IN RESPONSE TO HETEROLOGOUS EXPRESSION OF VOLTAGE-DEPENDENT SODIUM CHANNEL NAV1.2 C-TERMINUS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Voltage-gated sodium channels (Na_V) best known function is the initiation and propagation of the action potential. However, the expression of different isotypes of this family of proteins in non excitable cells has been documented and even cancer cells. Our research group has found a number of transcription factors (like Sin3B) that interact with the C-terminus of $Na_V1.2$, interestingly, we have found that this region of the channel exhibits a functional nuclear location signal (NLS). This has led us to ask if the interaction with transcription factors may affect the transcription of their targets. Here we evaluated the expression profile of neuroblastoma cells transfected with a plasmid encoding a $Na_V1.2$ CT (229 amino acid peptide tagged with EGFP), and analyzed the mRNA profile using microarray technology. Thus, we found change in the expression of a group of 13 genes involved in the intracellular signaling pathway JAK-STAT. We are currently exploring five up- or down-regulated messengers, IL11, IL6R, STAM, TYK2 and SOCS. Since JAK-STAT pathway is linked to different biological processes such as differentiation, cell proliferation, apoptosis and immune regulation, regulation of these messenger may link the presence of sodium channels to non-canonical roles observed in non-excitable cells, as has been reported from other researchers. **Supported by CONACyT, grant A1-S-36984**

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STRUCTURE AND BEHAVIOR OF NAV1.2 AND NAV1.6 C-TERMINUS. A MOLECULAR DYNAMICS ANALYSIS.

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Voltage-regulated sodium channels (NaV's) are transmembrane proteins that allow the influx of sodium ions into excitable and non-excitable cells. In the central nervous system (CNS) we find mainly two isoforms, NaV's 1.2 and 1.6, where they key to the action potential initiation and propagation and, in a less understood manner, they are involved in cell motility and proliferation. Multiple proteins can be anchored to different parts of NaV's, however, the terminal carboxyl (C-terminus) is the anchor site that binds more partners, yet very little is known about its structure. Here we used an in silico approach to obtain a molecular model of C-Terminus NaV1.2 and 1.6, where a 3D structure of NaV 1.2 (229 aa) and 1.6 (214 aa). To analyze conformational changes and their biochemical significance we ran molecular dynamics simulations (MDs) at 310 K. Different conformations of the proteins were evidenced during 100 ns. Although both sequences become more compact throughout the MD, NaV1.2 gradually denatures and loses its conformation, while NaV 1.6 remains more stable. Unfolding of the structure may be relevant to regulate C-Terminus' NaV's binding with other proteins. This description helps us to better understand the functionality and stability of NaV's C-terminus and will help to further models of binding and interaction with other proteins. **Supported by CONACyT Grant A1-S-36984, 287002 and IPN-SIP-20220704**

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THE DEAFNESS CAUSING PITCH AND AUDIO-1 MUTATIONS AFFECT THE NEUROPLASTIN AND PLASMA MEMBRANE CA2+ ATPASE COMPLEX FUNCTION

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Aims : Neuroplastin (*Nptn*) is essential for hearing functions and for the expression of its binding partner the plasma membrane Ca^{2+} ATPase (PMCA) in hair cells. Mouse mutants carrying either of the single amino acid changes in the *Nptn* sequence *pitch* or *audio1* are deaf. We investigated the effect of the neuroplastin mutations on the expression and function of PMCA. **Methods :** The *pitch* (C315S) and *audio-1* (I122N) neuroplastin protein structures and the interactions with PMCA were predicted by bioinformatic means. Plasmids encoding the two variants were constructed for the two neuroplastin isoforms (Np55 and Np65) and transfected into HEK 293T cells. Expression levels and subcellular location of neuroplastin and PMCA were investigated by Western blot and immuno-histochemistry. Calcium imaging was used to evaluate the effect of the kinetics of electrically-evoked cytosolic calcium transients in cultured primary hippocampal neurons. **Results :** The *pitch* and *audio-1* mutations affect the structure of neuroplastin differently. Transfection of the mutated *Nptn* variants in HEK 293T cells revealed a tendency towards decreased PMCA. Expression of the mutant variants in hippocampal neurons showed the slower restoration of calcium levels after stimulation determined by *in vitro* calcium imaging indicating the impaired function of PMCA. **Conclusions :** We show that the *pitch* and *audio-1* mutations in *Nptn* impair structurally and functionally the association with PMCA. Therefore, in addition to the identified role of neuroplastin in outer hair cell amplification and inner hair cell synaptogenesis, our results indicate that PMCA-dependent calcium dynamic imbalance underlies deafness by the *Nptn* mutations. ***Keywords:*** neuroplastin, PMCA, hearing, hair cells, deafness, calcium

Pubmed:
34680901: Lin X, Liang Y, Herrera-Molina R, Montag D
Neuroplastin in Neuropsychiatric Diseases.
Molecular mechanisms underlying neuropsychiatric and neurodegenerative diseases are insufficiently elucidated. A detailed understanding of these mechanisms may help to further improve medical intervention. Recently, intellectual abilities, creativity, and amnesia have been associated with neuroplastin, a cell recognition glycoprotein of the immunoglobulin superfamily that participates in synapse formation and function and calcium signaling. Data from animal models suggest a role for neuroplastin in pathways affected in neuropsychiatric and neurodegenerative diseases. Neuroplastin loss or disruption of molecular pathways related to neuronal processes has been linked to various neurological diseases, including dementia, schizophrenia, and Alzheimer's disease. Here, we review the molecular features of the cell recognition molecule neuroplastin, and its binding partners, which are related to neurological processes and involved in learning and memory. The emerging functions of neuroplastin may have implications for the treatment of diseases, particularly those of the nervous system.
Genes (Basel), 2021; 12

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BOARD NUMBER: S04-192

CLONING THE PUTATIVE VOLTAGE-GATED CALCIUM CHANNEL GENE IN ASTACUS LEPTODACTYLUS AND DETERMINATION OF THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF THE RELATED PROTEIN

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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The majority of the cellular functions depends on the concentration of intercellular Ca^{++}. L-type voltage-gated calcium channels (CaV-L) initiate the transfer of calcium into the cell as a result of the potential changes in the membrane, and forms temporary calcium currents necessary for cellular mechanisms. An analysis of the amino acid sequences of the alpha subunits predicts that Ca^{++} channels consist of four similar domains (repeat I-IV) with 6 putative transmembrane segments that assemble to form a central pore. Although *Astacus leptodactylus* (crayfish), is a widely used model animal in neuroscience, information about its genetic properties is rather limited. In this study we have cloned the putative L-type voltage-gated calcium channel mRNA from muscle tissue cDNA libraries of *Astacus leptodactylus*. In order to reveal the sequence, Sanger sequencing and NGS methods were used after following PCR and RACE experiments. The complete sequence was 7060 bp for CaV-L and 5550 bp for the ORF. When the sequence was analyzed in NCBI Conserved Domain Database, the domains relevant to ion selectivity and voltage gating were observed. Besides, the sequence was submitted into the BLASTn platform to examine its similarities with the L-type calcium channel sequences of closely related species. Crayfish putative CaV-L channel is going to be expressed in *Xenopus laevis* oocytes to obtain the expected Ca^{++} currents for functional analyses. The study was supported by Hacettepe University Research Foundation (ref. HU Research Project #15403) and The Scientific and Technological Research Council of Turkey (ref. TUBITAK Project #218S553).

Pubmed:

33820917: Kayman Kürekçi G, Kural Mangit E, Koyunlar C, Unsal S, Saglam B, Ergin B, Gizer M, Uyanik I, Boustanabadimaralan Düz N, Korkusuz P, Talim B, Purali N, Hughes SM, Dincer PR
Knockout of zebrafish desmin genes does not cause skeletal muscle degeneration but alters calcium flux.
Desmin is a muscle-specific intermediate filament protein that has fundamental role in muscle structure and force transmission. Whereas human desmin protein is encoded by a single gene, two desmin paralogs (desma and desmb) exist in zebrafish. Desma and desmb show differential spatiotemporal expression during zebrafish embryonic and larval development, being similarly expressed in skeletal muscle until hatching, after which expression of desmb shifts to gut smooth muscle. We generated knockout (KO) mutant lines carrying loss-of-function mutations for each gene by using CRISPR/Cas9. Mutants are viable and fertile, and lack obvious skeletal muscle, heart or intestinal defects. In contrast to morphants, knockout of each gene did not cause any overt muscular phenotype, but did alter calcium flux in myofibres. These results point to a possible compensation mechanism in these mutant lines generated by targeting nonsense mutations to the first coding exon.
Sci Rep, 2021; 11

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BOARD NUMBER: S04-193

CLONING OF ASTACUS LEPTODACTYLUS RYANODINE RECEPTOR GENE

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Cytoplasmic Ca2+ concentration plays an essential role in many types of cellular function including electro-mechanical coupling in striated muscle fibers. Ryanodine receptor channels (RyR), mediating Ca2+ release from sarcoplasmic reticulum (SR), has a homotetrameric structure. It is the largest ion channel with a size of 2.2 MDa. Vertebrate and invertebrate RyR channels are structurally and functionally similar. Although *Astacus leptodactylus,* narrow-clawed crayfish, is a widely used model animal in neuroscience, information about genetic properties of the animal is rather limited. The present study is focused onto *de novo* cloning of the mRNA of the crayfish RyR channel which encodes the largest ion channel. A hybrid cloning method has been used, referring to the homology between RyR mRNA molecules and the computational assembly of the next generation sequencing data. A complete mRNA molecule of 17562 bp in size has originally been cloned. The putative RyR protein, with 5042 amino acids, has a significant similarity to the sequences reported in other species. Furthermore, the putative sequence possessed many of the conserved domains specific to the RyR channel. Thus, it has been proposed that a mRNA coding RyR channel has originally been cloned in the present study. The 3D protein structure can also be determined by the help of this revealed genetic information, or future mutation studies can be designed. The study was supported by Hacettepe University Research Foundation (HU research project #15403) and The Scientific and Technological Research Council of Turkey (TUBITAK project #218S553).

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BOARD NUMBER: S04-194

ELUCIDATING THE INTERACTION BETWEEN GABA-A RECEPTORS AND PHOSPHOLIPASE-C DELTA-1

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Benzodiazepines are amongst the most widely prescribed medications for the management of anxiety and the relief of insomnia. They produce their effects through positive allosteric modulation of the GABA-A receptors, the main inhibitory receptors in the brain. Previous studies have demonstrated that dissociation of phospholipase-C delta-1 (PLCD1) from the GABA-A receptor upon chronic administration of diazepam *in-vitro*, triggers a cascade of events leading to the loss of GABAergic inhibitory synapses and the development of pharmacological tolerance to this drug. In this study, we aim to define the specific amino acid residues involved in the interaction between GABA-A receptors and PLCD1. Using Modeller 9v21, computational homology modelling was employed to assign structure to the intracellular loop (ICL) of the beta-3 subunits TM3-4 domain where this is lacking in existing GABA-A receptor crystal structures including beta-3 homopentamer (PDB ID: 4COF), heteropentamer (PDB ID: 6HUG), as well as refining a PLCD1 crystal structure (PDB ID: 2ISD). Protein-protein docking approaches using HADDOCK and ClusPro determined five residues (Q716, K717, Q778, Y779, R780) within the ICL likely to directly mediate the interaction with PLCD1. These residues were mutated to alanine using site-directed mutagenesis and individual beta-3 subunit mutants were co-immunoprecipitated with PLCD1 to determine which of these residues had the most prominent effect. The functional implications of these mutations are currently being tested with cell-surface ELISA and electrophysiology. Determining the specific sites of interaction of PLCD1 with GABA-A receptors, will help direct the ongoing research efforts aimed at the prevention of tolerance development to benzodiazepines.

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BOARD NUMBER: S04-195

A CONSERVED REGION AT THE END OF THE N-TERMINAL EXTRACELLULAR DOMAIN OF GABAA RECEPTOR SUBUNITS IS CRUCIAL FOR THE RECEPTOR FORWARD TRAFFICKING

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Type A γ-aminobutyric acid receptors ($GABA_ARs$) represent a family of pentameric GABA-gated Cl^-/HCO_3^- ion channels which mediate inhibitory transmission in the central nervous system (CNS). Cell surface expression of $GABA_ARs$, a prerequisite for their function, is dependent on the appropriate assembly of the receptor subunits and their transient interactions with molecular chaperones within the endoplasmic reticulum (ER) and Golgi apparatus. Here we describe a highly conserved amino acid sequence within the extracellular N-terminal domain of the receptor subunits adjoining the first transmembrane domain as a region important for $GABA_AR$ processing within the ER. Structural modelling indicated that this positively charged region is located on the outer surface of the $GABA_AR$ heteropentamer. It's modifications in the α1, β3 and γ2 subunits using insertion or site-directed mutagenesis impair $GABA_AR$ trafficking to the cell surface in heterologous cell systems although they have no effect on subunit assembly. Mutated receptors accumulate in the ER where they associate with calnexin, BiP and Grp94, as shown by co-immunoprecipitation. When compared to the wt receptors, mutated receptors show decreased interaction with calnexin, similar binding to BiP and increased association with Grp94, changes that could not be reversed by treatments with chemical chaperones, protease and proteasome inhibitors. Structural modelling through protein docking revealed close proximity between calnexin and $GABA_ARs$ in this region suggesting that structural alterations caused by mutations may impair their interaction. Thus, this previously uncharacterised region plays an important role in $GABA_AR$ processing in the ER by regulating at least in part their interaction with calnexin.

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BOARD NUMBER: S04-196

CLONING OF A PUTATIVE TRANSMEMBRANE CHANNEL LIKE PROTEIN AND A CALCIUM PERMEABLE STRESS GATED CATION CHANNEL LIKE PROTEIN IN ASTACUS LEPTODACTYLUS

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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In this study we have cloned the putative transmembrane channel-like protein(TMC) mRNA and the putative calcium stress gated cation channel like protein (CSC1-like) mRNA from *Astacus leptodactylus* ganglia cDNA libraries with a series of PCR, RACE – PCR experiments, using a set of specific and degenerate primers, and consequent Sanger and next-generation sequencing methods. The target proteins were chosen with refer to their proposed mechanosensitive properties in different species. The complete sequence for TMC was 3133 base-pairs and the ORF was 2497 base-pairs. For the CSC1-like mRNA the complete sequence was 3039 base-pairs and the ORF was 2662 base-pairs. A CDS search in NCBI platform resulted in the respective conserved domains of the related genes. Protein sequences were highly similar to those in the related species. Currently there isn't any crystallographic data for the TMC. However, there are two crystallographic structures with substantial similarity to CSC1-like proteins. Submission of the protein sequences to Alphafold collaborative environment resulted in similar structures to that of a monomer of the OSCA proteins for CSC1-like protein. The TMC protein sequence resulted in a similar structure to that of the mouse and human TMC-1 proteins obtained in the same platform. The study was supported by Hacettepe University Research Foundation (HU research project 15403) and The Scientific and Technological Research Council of Turkey (TUBITAK project 218S553)

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BOARD NUMBER: S04-197

POTENTIATION OF THE RAT P2X2, P2X4 AND P2X7 RECEPTORS BY STEROIDAL AMIDES

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Aims: Purinergic P2X receptors are ubiquitously expressed and play a role in many physiological and pathological processes including inflammation, pain, and cancer. We tested a hypothesis that a new class of neurosteroids that contain amide structural motifs act as allosteric modulators of P2X. **Methods:** We designed, synthesized and examined 16 synthetic steroidal amides. Whole-cell recordings from HEK293 cells transfected with rat P2X2, P2X4, or P2X7 receptors, and from pituitary cells or hypothalamic neurons endogenously expressing P2X receptors was used. To localize steroid-binding site, alanine scanning mutagenesis of P2X7-TM1 was performed, and the effect of steroids on mutated receptors was examined. **Results:** We found that 8 compounds potentiated equally P2X2, P2X4, and P2X7, while 5 compounds potentiated P2X2 and P2X4 without having a significant effect on P2X7. The remaining 3 compounds were inactive. The most effective compound, 5β-androstran 3α-yl-β-aspartate (10 μM), potentiated P2X receptors to about 350 %., its effect was concentration-dependent, and the agonist concentration-response curves were shifted to the left. The potentiating effect of steroid was dependent on (i) metabolically stable amide moiety at position C-3 of steroidal ring, (ii) on the length of the C-3 dicarboxylic acid substituent and (iii) the presence of free NH_2 which is important determinant for P2X7 potentiation. Significantly lower effect was observed for P2X7-V41A mutant, indicating that it binds possibly in the middle of TM. **Conclusions:** Our work explains the mechanism and site of action for a series of new P2X allosteric modulators and establishes critical TM1 mutants for P2X7 binding-mode characterization.

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BOARD NUMBER: S04-198

ROLE OF TRPC-4 AND -5 CHANNELS IN PERSISTENT FIRING IN MEDIAL ENTORHINAL CORTEX LAYER II

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Path integration (PI) is a fundamental self-motion-based navigation strategy that is highly conserved in mammals. PI involves the integration of self-motion information and is facilitated by the cholinergic system to maintain a consistent representation of the location of self in the environment. Grid cells, mostly found in the medial entorhinal cortex (MEC) Layer II, are believed to support PI by providing an invariant metric system through their spatially tuned hexagonal firing fields. One of the proposed cellular mechanisms of grid cell firing is intrinsic cholinergic-dependent persistent firing that has been shown to be graded up or down in MEC LV/LIII cells following multiple stimuli. To date, however, evidence of temporal integration through persistent firing in LII is still lacking. Besides, the cellular mechanism of persistent firing in MEC LII pyramidal and stellate cells is still controversial. Here, using *in-vitro* whole-cell patch recording, we show that pyramidal and stellate cells similarly support persistent firing via intrinsic mechanisms involving the transient receptor potential canonical (TRPC) 1/4/5 channels. We tested novel and highly specific TRPC channel blockers in cholinergically induced persistent firing in mice MEC LII. The TRPC-4 blocker ML204 and TRPC-4 and -5 blocker Pico145 both significantly inhibited persistent firing while the TRPC5 blocker Clemizole hydrochloride didn't reduce persistent firing. Similarly, AAVshRNA-mediated knockdown of MEC TRPC-4 channels showed a clear inhibition of persistent firing. Furthermore, we evaluated the expression of TRPC-1, -4, and -5 in pyramidal and stellate cells using in-situ hybridization.

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BOARD NUMBER: S04-199

DELETION OF THE BACKGROUND POTASSIUM CHANNEL TWIK-1 INCREASES SUSCEPTIBILITY TO KAINIC ACID-INDUCED SEIZURE

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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TWIK-1 channel works as a heteromer with other channels in the two-pore potassium family. We recently reported that TWIK-1/TREK-1 heteromer mediates astrocytic passive conductance using a knockdown system. However, contrary to expectations, there was no change in the only reported TWIK-1 KO mice. Here we studied in detail why there is no change in the passive conductance of astrocytes in TWIK-1 KO mice. As a result, we found that the deletion of exon2 in the TWIK-1 gene made an in-framed connection between exon1 and exon3 of TWIK-1 and that the truncated TWIK-1 proteins, ΔEx2 TWIK-1, remained stable. When we targeted other undeleted exons of TWIK-1 with shRNA or CRISPR/Cas9 system, the passive conductance of the TWIK-1 KO mice was prominently reduced. In addition, we confirmed through molecular tools and 3D protein modeling that the ΔEx2 TWIK-1 protein heteromers with TREK-1 exhibit a linear IV relationship like the full-length TWIK-1 protein. Therefore, we generated new TWIK-1 KO mice that deleted a portion of the CDS of exon1 and confirmed that passive conduction was significantly reduced in hippocampal astrocytes, as expected. In addition, we found that this new TWIK-1 KO mouse had significantly increased seizure susceptibility in the KA-induced seizure. This result was similar in mice in which the expression of TWIK-1 was selectively reduced only in hippocampal astrocytes. Taken together, these data suggest that the results of previous TWIK-1 KO studies should be reinterpreted, suggesting that the passive conductance of hippocampal astrocytes is strongly related to seizure susceptibility.

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BOARD NUMBER: S04-200

NHERF-1 IS A KEY REGULATOR OF THE ASTROCYTIC PASSIVE CONDUCTANCE

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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Mature hippocampal astrocytes exhibit a linear current-to-voltage (*I-V*) K^+ membrane conductance, which is called a passive conductance. It is estimated to enable astrocytes to keep potassium homeostasis in the brain. We previously reported that the TWIK-1/TREK-1 heterodimeric channels are crucial for astrocytic passive conductance. However, the regulatory mechanism of these channels still remains elusive. Here, we identified Na+/H+ exchange regulator-1 (NHERF-1), a protein highly expressed in astrocytes, as a candidate interaction partner for these channels. NHERF-1 endogenously bound to TWIK-1/TREK-1 in hippocampal cultured astrocytes. When NHERF-1 is overexpressed or silenced, surface expression and activity of TWIK-1/TREK-1 heterodimeric channels were inhibited or enhanced, respectively. Furthermore, we confirmed astrocytic passive conductance was increased in hippocampal slices of NHERF-$1^{+/-}$ mice. Taken together, these finding suggests that NHERF-1 is a key regulator of TWIK-1/TREK-1 heterodimeric channels-mediated astrocytic passive conductance.

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BOARD NUMBER: S04-201

PROGRESSIVE HYPERPOLARIZATION-ACTIVATED CATION CURRENT (IH) REDUCTION: A RESPONSE MECHANISM TO DECREASE COCAINE-INDUCED EXCITABILITY IN VTA DA NEURONS.

POSTER SESSION 04 - SECTION: ION CHANNELS & EXCITABILITY

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The hyperpolarization-activated cation current (I_h) is a contributing factor of intrinsic excitability in dopaminergic neurons (DA) of the ventral tegmental area (VTA). I_h is activated by hyperpolarization and produces a time-dependent depolarizing current. Our laboratory demonstrated that cocaine sensitization, significantly reduces I_h amplitude in VTA DA neurons. However, the role of I_h in controlling VTA DA excitability is still poorly understood. VTA DA cell's spontaneous activity remains unchanged when compared to the control groups after sensitization. Our hypothesis is that I_h reduction serves as a homeostatic regulator compensating for the cocaine-induced alteration in excitability. Using *in vivo* single-unit extracellular electrophysiology in anesthetized rats, we explored the I_h contribution on acute cocaine-induced spontaneous activity. We perfused an I_h blocker (ZD7288) and evaluated its effect on VTA DA spontaneous firing. I_h blockade significantly reduced acute cocaine-induced firing rate, bursting frequency, and percent of spikes within a burst while significantly increasing the mean interspike interval (ISI). This suggest that I_h blockade can reduce cocaine-induced firing activity in VTA DA neurons through an increase in the mean ISI. Furthermore, using whole-cell patch-clamp, we found a significant reduction in I_h only after 24hours of acute cocaine (15mg/kg). Using a rebound spiking protocol, we determined at different intervals of acute cocaine administration (2hrs, 24hrs and, 24hrs following 7-days cocaine administration) that there was an increase in rebound action potentials. I_h blockade significantly reduced rebound firing. These results suggest that progressive I_h reduction could serve as a homeostatic regulator of cocaine-induced spontaneous firing in VTA DA excitability.

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CHARACTERIZATION OF ASTROCYTE REACTIVITY IN A MODEL OF ENCEPHALOPATHY OF PREMATURITY

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Premature birth caused by maternal infection represent an increased risk factor of brain lesions affecting both developing gray and white matter, known as encephalopathy of prematurity (EoP), and long-term neurodevelopmental disorders. It has been suggested that the set-up of a pro-inflammatory environment with the secretion of cytokines and chemokines might initiate an inappropriate inflammatory response driven by reactive microglia and astrocytes, which participate to neurodevelopmental disruption. Astrocytes, located at the interface between the brain parenchyma and the blood brain barrier, preserve homeostasis. They also participate in the inflammatory response and go through morphological and functional changes called astrogliosis. However, little is known about astrocytic reactivity during perinatal inflammation. Our team has developed a mouse model of EoP based on systemic injections of IL-1beta (a pro-inflammatory cytokine) reproducing the deficits seen in premature infants. Using this model, we aim to precisely characterize astrocyte reactivity in EoP. Astrocyte subpopulations are highlighted using flow cytometry, emphasizing their heterogeneity. Bulk RNA sequencing of purified astrocytes showed a transcriptomic signature of astrocytes during perinatal inflammation. Analysis of A1/A2 phenotypes by quantitative RT-PCR revealed a pro-inflammatory phenotype of the astrocytic response along time. Significant morphological changes of GFAP+ astrocytes in the subventricular zone have been shown by immunohistochemistry. Functional differences have been studied by quantitative RT-PCR and revealed a decrease of synaptogenesis factors secreted by astrocytes. This in-depth characterization of astrocytes will pave the way for designing new strategies to restore the homeostatic functions of astrocytes and protect the brain of preterm infants.

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BOARD NUMBER: S04-203

RACK1 REGULATES LOCAL TRANSLATION IN ASTROCYTE PERISYNAPTIC PROCESSES

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Astrocytes are the most numerous glial cells in the brain and display long processes contacting both synapses and blood vessels. Perisynaptic astrocytic processes (PAP) form the tripartite synapse with the neuronal compartments and participate in gliotransmission, neurotransmitters recycling and ion homeostasis among others. Astrocytes are highly polarized but how these polarities are set is unknown. Local translation is one of the most conserved mechanism for cell polarity and has been recently characterized in PAPs. However, mechanisms setting local translation in astrocytes remain largely unknown. We aimed at identifying proteins associated with polysomes in astrocytes to study their role in local translation in astrocytes. We combined an astrocyte-specific translating ribosome affinity purification approach with mass Spectrometry. Among identified proteins, we focused on “Receptor for activated protein kinase C” (RACK1), a protein involved in the regulation of translation. We showed that RACK1 is specifically associated with mRNAs such as *kcnj10,* coding for the potassium channel KIR4.1 and enriched in PAPs. *In vitro,* we found that RACK1 targets the kcnj10 5’UTR. In a mouse model deleted for RACK1 in astrocytes, we observed a higher level of KIR4.1 in synaptogliosomes. In a previous analysis, we showed that RACK1 polysomal mRNAs are enriched in PAPs compared to the soma. Thus together with our present results, we propose that RACK1 by binding specifically to Kcnj10 mRNAs may regulate the translation of KIR4.1 in PAPs to control potassium homeostasis at the neuroglial interface and electrophysiological properties of neuronal networks.

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BOARD NUMBER: S04-204

ATP STIMULATION REGULATES ASTROCYTE-DERIVED EXTRACELLULAR VESICLE SECRETION AND MIRNA CONTENT

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Cerebral function depends on the transmission of chemical signals between neuronal elements at synapses. Over the last two decades, accumulating data have shown that this fundamental process is regulated by astrocytes. Among other functions astrocytes participate in the clearance of ions and neurotransmitters. In addition, these glial cells release gliotransmitters to regulate synaptic functions such as basal synaptic transmission and long-term synaptic plasticity. Clearance and gliotransmission are probably not the only ways through which astrocytes influence neuronal communication. An interesting but under-investigated pathway would be through the release of extracellular vesicles (EVs). EVs are small, double membrane bound vesicles containing proteins, lipids and nucleic acids. To understand the role of EVs during synaptic transmission, it is important to first investigate whether acute stimulation of astrocytes can impact the quantity, size and content of secreted EV. By combining calcium imaging, EVs quantification techniques, electronic microscopy and RNAseq analysis, our data indicate that exposing primary mouse astrocyte cultures to 50µM ATP for 30 min is sufficient to modify the number of EVs released and their miRNA content.

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BOARD NUMBER: S04-205

IMPACT OF ASTROCYTE REACTIVITY IN COCAINE ADDICTION

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Drug addiction is a biomedical challenge in which molecular and cellular mechanisms underlying drug-seeking behavior are not fully elucidated. However, some evidence suggests that astrocytes are implicated in cocaine addiction. Here, we analyzed the effect of cocaine on astrocytes in the nucleus accumbens, a structure in the ventral striatum implicated in the reward circuit and addictive behavior, and we tested their impact on cocaine-induced behavior. Mice were sensitized to cocaine by daily i.p. injection of cocaine. The locomotion was recorded in an open-field arena and the expression of astrocytic reactivity markers was assessed by immunohistochemistry. We manipulated the astrocyte activity by inhibiting the JAK/STAT signaling pathway by inducing the expression of SOCS3. We found that cocaine sensitization induces morphological changes and triggers the expression of immune-markers of astroglial reactivity. Exploring the potential cause of astrocyte reactivity, we identified the JAK/STAT signaling pathway as instrumental in behavior induced by cocaine sensitization. Our results suggest that blocking the astrocyte reactivity by inhibiting the JAK/STAT signaling pathway might ameliorate drug-seeking behavior, representing a promising novel treatment strategy. ***Keywords:*** *astrocytes, addiction, cocaine, reactivity, JAK/STAT signaling pathway*

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BOARD NUMBER: S04-206

A ROLE FOR ASTROCYTES IN THE VISUAL CORTEX CRITICAL PERIOD

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

Rachel Breton[1,2,3], Jérôme Ribot[1], Charles-Félix Calvo[1], Julien Moulard[1,4], Pascal Ezan[1], Jonathan Zapata[1], Kevin Samama[1], Matthieu Moreau[5], Alexis Bemelmans[6], Valentin Sabatet[7], Dingli Florent[7], Damarys Loew[7], Milleret Chantal[1], Pierre Billuart[8], Glenn Dallérac[1], Nathalie Rouach[1]
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Information processing in the brain is generally considered as a neuronal performance. Yet, astrocytes play a crucial role in controlling synapse formation, maturation, activity and elimination, and thus shape synaptic circuits during development. Remarkably, astrocytes regulate critical developmental periods, defined as temporal windows of early postnatal development during which neuronal networks are highly plastic and sensitive to experience. Pioneer data have shown that immature astrocytes are able to re-open a period of strong plasticity when re-implanted in adult visual cortex. However how astrocytes control critical developmental period remains unknown. Here, using a multidisciplinary approach involving molecular biology, immunohistochemistry, intrinsic optical imaging, and electrophysiology, we uncovered a mechanism by which astrocytes maturation control the timing of critical period termination. We indeed discovered an unconventional astroglial pathway controlled by connexin 30 gap junction protein allowing for maturation of inhibitory circuits in the visual cortex, well-known to induce closure of the critical period. Our results provide a novel astroglial mechanism controlling termination of the critical period, which is associated with settling of neuronal circuits, essential for efficient information processing. These data indicate that astrocytes play a major role in the experience-dependent plasticity of brain developing circuits, and point to a novel cellular target to treat neurological disorders involving defects in the closure of critical periods as well as new strategies to re-induce enhanced plasticity in adults and favors rehabilitation after brain damage.

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BOARD NUMBER: S04-207

A NOREPINEPHRINE-DEPENDENT GLIAL CALCIUM WAVE TRAVELS IN THE SPINAL CORD UPON ACOUSTOVESTIBULAR STIMULI

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Although calcium waves have been widely observed in glial cells, their occurrence *in vivo* during behavior remains less understood. Here, we investigated the recruitment of glial cells in the hindbrain and spinal cord after acousto-vestibular (AV) stimuli triggering escape responses using *in vivo* population calcium imaging in larval zebrafish. We observed that gap-junction-coupled spinal glial network exhibits large and homogenous calcium increases that rose in the rostral spinal cord and propagated bi-directionally towards the spinal cord and towards the hindbrain. Spinal glial calcium waves were driven by the recruitment of neurons and in particular, of noradrenergic signaling acting through α-adrenergic receptors. Noradrenergic neurons of the medulla-oblongata (NE-MO) were revealed in the vicinity of where the calcium wave started. NE-MO were recruited upon AV stimulation and sent dense axonal projections in the rostro-lateral spinal cord, suggesting these cells could trigger the glial wave to propagate down the spinal cord. Altogether, our results revealed that a simple AV stimulation is sufficient to recruit noradrenergic neurons in the brainstem that trigger in the rostral spinal cord two massive glial calcium waves, one travelling caudally in the spinal cord and another rostrally into the hindbrain.

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BOARD NUMBER: S04-208

HIV-1 NEUROPATHOGENESIS IS MEDIATED BY EPHRINA3 EXPRESSED ON ASTROCYTES

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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HIV infection of the central nervous system (CNS) causes dementia and cognitive disorders, termed as HIV-associated neurocognitive disorder (HAND). HIV infects the brain at an early stage, which is corroborated by the presence of HIV-1 Tat protein in the CSF of these patients. Astrocytes being the most abundant cells in the CNS are actively infected by the virus and also serve as reservoir to latent virus. Astrocytes and neurons are in dynamic crosstalk for homeostatic brain functioning and the principle reason behind neuronal death in case of HIV is glutamate excitotoxicity. As astrocytes are bestowed with the responsibility to clear excess glutamate from the synapses through glutamate transporters EAAT1/EAAT2, their inefficiency to do so in case of HIV leads to the accumulation of glutamate, which eventually causes neuronal death. EphrinA3, a ligand expressed on astrocytes, has been known to regulate glutamate transporters via reverse signaling by EphA4, its receptor expressed on neurons. As both the receptor and ligand are present on different cell types, neuron-glia co-culture was adopted as the model system for this study. There was an upregulation observed in EphrinA3 in case of HIV-1 Tat B, with downregulation seen in glutamate transporters EAAT1/EAAT2 as compared to the control. Knockdown of EphrinA3 in presence of HIV-1 Tat B reversed the neurotoxic effects of HIV-1 Tat B transfection. This study highlights the importance of EphrinA3 in HIV-1 Tat B mediated neuronal death, through the regulation the glutamate transporters and extracellular glutamate concentration.

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BOARD NUMBER: S04-209

CHOLESTEROL METABOLISM IS MODULATED BY NGF IN AN ASTROCYTE-DERIVED CELL LINE AND EXHIBITS A NEUROPROTECTIVE ROLE AGAINST OXIDATIVE STRESS

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Introduction Cholesterol derived from astrocytes is essential for proper neuronal functioning. Recent evidence showed that nerve growth factor (NGF) influences several metabolic processes in brain cells, including astrocytes. Nevertheless, the activity of NGF on glial cholesterol homeostasis is not fully elucidated. **Aim** The aim of this project is to evaluate whether NGF administration could affect cholesterol metabolism in glial cells, and whether this event could influence neuronal survival under oxidative stress conditions. **Methods** U373 astrocyte-derived cell line and N1E-115, a neuroblastoma cell line, were used as experimental models. Fully differentiated N1E-115 were treated with rotenone for 16 hours to induce oxidative stress. Subsequently, N1E-115 were co-cultured with U373 cells, previously treated with NGF for 48 hours. Results were obtained by means of immunological, biochemical and morphological analysis. **Results** NGF administration in U373 cells increases the expression of different proteins and enzymes involved in cholesterol homeostasis network. Furthermore, NGF significantly increases extracellular cholesterol levels and also promotes ApoE secretion in the culture medium. Morphological evaluation demonstrated that NGF-treated astrocytic cells significantly protect N1E-115 neuronal cells from rotenone-induced oxidative stress. Conversely, the effect promoted by NGF stimulation was prevented when neuronal cells were co-cultured with ApoE-silenced U373 cells. **Conclusions** The obtained data suggest that NGF modulates cholesterol metabolism in U373 cells. Moreover, NGF displays a putative neuroprotective role, which is likely mediated by the enhancement of glial ApoE secretion in the culture medium.

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APPLICATION OF THE CEFTRIAXONE CHANGES THE GLT-1 DISTRIBUTION AFTER TRAUMATIC BRAIN INJURY

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Traumatic brain injury (TBI) is a serious problem whereas its consequences are now considered irreversible. The morphofunctional changes that follow TBI increase the risk of delayed development of neurodegenerative pathologies which can only be slowed down by known treatments. Nevertheless, the beta-lactam antibiotic ceftriaxone has been shown to have a positive effect on neuronal preservation as well as reducing glial reactivity and excitotoxicity. Previous studies have found that ceftriaxone affects the expression of glutamate transporter 1 (GLT-1) which is mainly located on the astrocytic membranes. The effect of the ceftriaxone on the localization of GLT-1on the astrocytes are still unknown. **Aim:** We studied the localization of the GLT-1 on astrocytic membranes in the CA1 area of hippocampus at different time points (3, 7, and 14 days) after mild TBI and application of ceftriaxone. **Methods:** We have used closed head weight drop model of mild TBI, immunohistochemical approaches with GLT-1 and GFAP markers, confocal microscopy, quantitative analysis. **Result and conclusions:** We found that localization of GLT-1 on astrocytic membranes changes due to injury: clusters of the receptor become less compacted, more scattered along the astrocytic branches which possibly affects glutamate reuptake. We also have shown that application of the ceftriaxone changes the GLT-1 distribution after TBI, approaching those in control.

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BOARD NUMBER: S04-211

INVESTIGATING THE EFFECTIVENESS OF KEAP1-NRF2 PROTEIN-PROTEIN INTERACTION DISRUPTORS IN PROTECTING HUMAN NEURONAL MODELS OF ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Introduction: Classical Nrf2 activators prevent memory-loss in animal models of Alzheimer's Disease (AD), but have adverse effects in humans. Our work has identified a new class of Nrf2 activators, Keap1-Nrf2 disruptors, that prevent Aβ toxicity in mouse neurons. By developing human AD models, we now **aim** to test their relevance to human systems and to investigate the cell-specific mechanisms of their neuroprotective properties. **Methods:** Lund Human Mesencephalic (LUHMES) cells were differentiated to neurons, alone or in co-culture with primary human cortical astrocytes. Astrocytic responses to Keap1-Nrf2 disruptors, 22h and 18e, or classical activators, DMF and CDDO-me were determined by measuring activity of the Nrf2 target NQO1. Neurons or co-cultures were treated with synthetic $A\beta_{42}$ oligomers (Aβ42-O), with or without DMF, at differentiation day 6, and neurite morphology, cell viability and ΔΨm were measured by confocal imaging, CellTitre-Glo and JC-10 assays respectively. **Results:** Nrf2 activation was confirmed in response to 18e, DMF and CDDO-me, but not 22h, in astrocytes. Efficacy was lost in co-cultures, due to high basal astrocytic Nrf2 activity in LUHMES differentiation media. Aβ42-O (2.5-10μM) disrupted neurite morphology, reduced cell viability and increased ΔΨm in neurons alone, but this was not protected by the Nrf2 activator DMF. Preliminary studies suggest that DMF may be neuroprotective in co-cultures depending on the duration of exposure. **Conclusion:** Our studies demonstrate varying potency of Nrf2 activators in astrocytes, and indicate that DMF cannot protect neurons alone against Ab42-O toxicty. Further studies are required to confirm whether this depends on the presence of astrocytes.

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DREBRIN CONTROLS SCAR FORMATION AND ASTROCYTE REACTIVITY UPON TRAUMATIC BRAIN INJURY BY REGULATING MEMBRANE TRAFFICKING

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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The brain of mammals lacks a significant ability to regenerate neurons and is thus particularly vulnerable. To protect the brain from injury and disease, damage control by astrocytes through astrogliosis and scar formation is essential. We recently showed that brain injury in mice triggers an immediate upregulation of the actin-binding protein Drebrin (DBN) in astrocytes, which is essential for scar formation and maintenance of astrocyte reactivity. In turn, DBN loss leads to defective astrocyte scar formation and excessive neurodegeneration following brain injuries. At the cellular level, we show that DBN switches actin homeostasis from ARP2/3-dependent arrays to microtubule-compatible scaffolds, facilitating the formation of RAB8-positive membrane tubules. This injury-specific RAB8 tubular endosomal compartment serves as a hub for the trafficking of surface proteins involved in astrogliosis and adhesion mediators, such as β1-integrin. DBN-mediated RAB8-dependent polarized membrane trafficking controls reactive astrogliosis required to form astrocyte scars and to protect the CNS from traumatic brain injury. We use an *in vitro* scratch injury model of cultured astrocytes to further characterize RAB8-positive tubular endosome trafficking dynamic. The injury-induced RAB8+ tubules increase further in number when astrocytes are briefly cultured under serum starvation conditions. Subsequent refeeding with serum causes the immediate disintegration of membrane tubules and cytoplasmic localization of RAB8, suggesting a critical role of growth factors supply, as well as signaling molecules, in regulating RAB8+ tubular endosome dynamics in injury conditions.

Pubmed:

33674568: Schiweck J, Murk K, Ledderose J, Münster-Wandowski A, Ornaghi M, Vida I, Eickholt BJ
Drebrin controls scar formation and astrocyte reactivity upon traumatic brain injury by regulating membrane trafficking.
The brain of mammals lacks a significant ability to regenerate neurons and is thus particularly vulnerable. To protect the brain from injury and disease, damage control by astrocytes through astrogliosis and scar formation is vital. Here, we show that brain injury in mice triggers an immediate upregulation of the actin-binding protein Drebrin (DBN) in astrocytes, which is essential for scar formation and maintenance of astrocyte reactivity. In turn, DBN loss leads to defective astrocyte scar formation and excessive neurodegeneration following brain injuries. At the cellular level, we show that DBN switches actin homeostasis from ARP2/3-dependent arrays to microtubule-compatible scaffolds, facilitating the formation of RAB8-positive membrane tubules. This injury-specific RAB8 membrane compartment serves as hub for the trafficking of surface proteins involved in astrogliosis and adhesion mediators, such as β1-integrin. Our work shows that DBN-mediated membrane trafficking in astrocytes is an important neuroprotective mechanism following traumatic brain injury in mice.
Nat Commun, 2021; 12
34458253: Murk K, Ornaghi M, Schiweck J
Profilin Isoforms in Health and Disease - All the Same but Different.
Profilins are small actin binding proteins, which are structurally conserved throughout evolution. They are probably best known to promote and direct actin polymerization. However, they also participate in numerous cell biological processes beyond the roles typically ascribed to the actin cytoskeleton. Moreover, most complex organisms express several profilin isoforms. Their cellular functions are far from being understood, whereas a growing number of publications indicate that profilin isoforms are involved in the pathogenesis of various diseases. In this review, we will provide an overview of the profilin family and "typical" profilin properties including the control of actin dynamics. We will then discuss the profilin isoforms of higher animals in detail. In terms of cellular functions, we will focus on the role of Profilin 1 (PFN1) and Profilin 2a (PFN2a), which are co-expressed in the central nervous system. Finally, we will discuss recent findings that link PFN1 and PFN2a to neurological diseases, such as amyotrophic lateral sclerosis (ALS), Fragile X syndrome (FXS), Huntington's disease and spinal muscular atrophy (SMA).
Front Cell Dev Biol, 2021; 9


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BOARD NUMBER: S04-213

MACROPHAGE-DERIVED AMPHIREGULIN MODULATES ASTROCYTE NETWORK REACTIVITY

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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During neurodegeneration, astrocytes alter their gap junctional networks to redistribute resources and maintain neuronal function. How is this beneficial reactivity induced? To answer this, we looked at similar mechanisms in other organs and applied them to the nervous system. In the heart, macrophage-derived amphiregulin (AREG) signals upon epidermal growth factor receptor (EGFR) to induce connexin 43 (cx43) phosphorylation and form additional gap junctions during arrhythmia. Like cardiac macrophages, cultured primary microglia (serum-free; n=3 isolations) release AREG in response to stress (LPS, 1ug/ml; p<0.001 vs baseline). Cultured primary astrocytes (serum-free; mRNA n=3 isolations; protein n=5 isolations) respond to AREG (100ng/mL) like cardiac myocytes: they phosphorylate cx43, with a 3-fold increase 9 minutes after AREG administration (p<0.001). Three minutes after AREG administration, cx43 mRNA increases 2-fold; protein levels increase 2-fold by 11 minutes (mRNA p=0.007; protein p≤0.01). AREG also increases membrane-localized astrocyte EGFR 2-fold within a minute (p=0.006) and 3-fold in 7 minutes (p < 0.001). To measure changes in functional network size, astrocytes were cut-loaded with biotin (244Da) and dextran (10kDa). We found a 3-fold increase in astrocyte connectivity 10 min after AREG (p<0.001). Intriguingly, higher levels of AREG (1000ng/mL) have the opposite effect – cx43 mRNA is reduced 2-fold in 3 minutes (p = 0.02), and cx43 protein declines 2-fold within 30 minutes (p=0.01; n=3 isolations). This differential response may dictate whether astrocytes enlarge or contract their network in response to different types of stress.

Pubmed:
34199470: Boal AM, Risner ML, Cooper ML, Wareham LK, Calkins DJ
Astrocyte Networks as Therapeutic Targets in Glaucomatous Neurodegeneration.
Astrocytes are intimately involved in the response to neurodegenerative stress and have become an attractive target for the development of neuroprotective therapies. However, studies often focus on astrocytes as single-cell units. Astrocytes are densely interconnected by gap junctions that are composed primarily of the protein connexin-43 (Cx43) and can function as a broader network of cells. Such networks contribute to a number of important processes, including metabolite distribution and extracellular ionic buffering, and are likely to play an important role in the progression of neurodegenerative disease. This review will focus on the pro-degenerative and pro-survival influence of astrocyte Cx43 in disease progression, with a focus on the roles of gap junctions and hemichannels in the spread of degenerative stress. Finally, we will highlight the specific evidence for targeting these networks in the treatment of glaucomatous neurodegeneration and other optic neuropathies.
Cells, 2021; 10
34090501: Risner ML, Pasini S, McGrady NR, D'Alessandro KB, Yao V, Cooper ML, Calkins DJ
Neuroprotection by Wld depends on retinal ganglion cell type and age in glaucoma.
Early challenges to axonal physiology, active transport, and ultrastructure are endemic to age-related neurodegenerative disorders, including those affecting the optic nerve. Chief among these, glaucoma causes irreversible vision loss through sensitivity to intraocular pressure (IOP) that challenges retinal ganglion cell (RGC) axons, which comprise the optic nerve. Early RGC axonopathy includes distal to proximal progression that implicates a slow form of Wallerian degeneration. In multiple disease models, including inducible glaucoma, expression of the slow Wallerian degeneration (Wld) allele slows axon degeneration and confers protection to cell bodies.
Mol Neurodegener, 2021; 16
32690710: Cooper ML, Pasini S, Lambert WS, D'Alessandro KB, Yao V, Risner ML, Calkins DJ
Redistribution of metabolic resources through astrocyte networks mitigates neurodegenerative stress.
In the central nervous system, glycogen-derived bioenergetic resources in astrocytes help promote tissue survival in response to focal neuronal stress. However, our understanding of the extent to which these resources are mobilized and utilized during neurodegeneration, especially in nearby regions that are not actively degenerating, remains incomplete. Here

we modeled neurodegeneration in glaucoma, the world's leading cause of irreversible blindness, and measured how metabolites mobilize through astrocyte gap junctions composed of connexin 43 (Cx43). We elevated intraocular pressure in one eye and determined how astrocyte-derived metabolites in the contralateral optic projection responded. Remarkably, astrocyte networks expand and redistribute metabolites along distances even 10 mm in length, donating resources from the unstressed to the stressed projection in response to intraocular pressure elevation. While resource donation improves axon function and visual acuity in the directly stressed region, it renders the donating tissue susceptible to bioenergetic, structural, and physiological degradation. Intriguingly, when both projections are stressed in a WT animal, axon function and visual acuity equilibrate between the two projections even when each projection is stressed for a different length of time. This equilibration does not occur when Cx43 is not present. Thus, Cx43-mediated astrocyte metabolic networks serve as an endogenous mechanism used to mitigate bioenergetic stress and distribute the impact of neurodegenerative disease processes. Redistribution ultimately renders the donating optic nerve vulnerable to further metabolic stress, which could explain why local neurodegeneration does not remain confined, but eventually impacts healthy regions of the brain more broadly.
Proc Natl Acad Sci U S A, 2020; 117
31354422: Bernardo-Colón A, Vest V, Cooper ML, Naguib SA, Calkins DJ, Rex TS
Progression and Pathology of Traumatic Optic Neuropathy From Repeated Primary Blast Exposure.
Indirect traumatic optic neuropathy (ITON) is a condition that is often associated with traumatic brain injury and can result in significant vision loss due to degeneration of retinal ganglion cell (RGC) axons at the time of injury or within the ensuing weeks. We used a mouse model of eye-directed air-blast exposure to characterize the histopathology of blast-induced ITON. This injury caused a transient elevation of intraocular pressure with subsequent RGC death and axon degeneration that was similar throughout the length of the optic nerve (ON). Deficits in active anterograde axon transport to the superior colliculus accompanied axon degeneration and first appeared in peripheral representations of the retina. Glial area in the ON increased early after injury and involved a later period of additional expansion. The increase in area involved a transient change in astrocyte organization independent of axon degeneration. While levels of many cytokines and chemokines did not change, IL-1α and IL-1β increased in both the ON and retina. In contrast, glaucoma shows distal to proximal axon degeneration with astrocyte remodeling and increases in many cytokines and chemokines. Further, direct traumatic optic neuropathies have a clear site of injury with rapid, progressive axon degeneration and cell death. These data show that blast-induced ITON is a distinct neuropathology from other optic neuropathies.
Front Neurosci, 2019; 13
30367086: Bernardo-Colón A, Vest V, Clark A, Cooper ML, Calkins DJ, Harrison FE, Rex TS
Antioxidants prevent inflammation and preserve the optic projection and visual function in experimental neurotrauma.
We investigated the role of oxidative stress and the inflammasome in trauma-induced axon degeneration and vision loss using a mouse model. The left eyes of male mice were exposed to over-pressure air waves. Wild-type C57Bl/6 mice were fed normal, high-vitamin-E (VitE), ketogenic or ketogenic-control diets. Mice lacking the ability to produce vitamin C (VitC) were maintained on a low-VitC diet. Visual evoked potentials (VEPs) and retinal superoxide levels were measured in vivo. Tissue was collected for biochemical and histological analysis. Injury increased retinal superoxide, decreased SOD2, and increased cleaved caspase-1, IL-1α, IL-1β, and IL-18 levels. Low-VitC exacerbated the changes and the high-VitE diet mitigated them, suggesting that oxidative stress led to the increase in IL-1α and activation of the inflammasome. The injury caused loss of nearly 50% of optic nerve axons at 2 weeks and astrocyte hypertrophy in mice on normal diet, both of which were prevented by the high-VitE diet. The VEP amplitude was decreased after injury in both control-diet and low-VitC mice, but not in the high-VitE-diet mice. The ketogenic diet also prevented the increase in superoxide levels and IL-1α, but had no effect on IL-1β. Despite this, the ketogenic diet preserved optic nerve axons, prevented astrocyte hypertrophy, and preserved the VEP amplitude. These data suggest that oxidative stress induces priming and activation of the inflammasome pathway after neurotrauma of the visual system. Further, blocking the activation of the inflammasome pathway may be an effective post-injury intervention.
Cell Death Dis, 2018; 9
30203289: Fardone E, Celen AB, Schreiter NA, Thiebaud N, Cooper ML, Fadool DA
Loss of odor-induced c-Fos expression of juxtaglomerular activity following maintenance of mice on fatty diets.
Diet-induced obesity (DIO) decreases the number of OMP+ olfactory sensory neurons (OSN) in the olfactory epithelium by 25% and reduces correlate axonal projections to the olfactory bulb (OB). Whether surviving OSNs have equivalent odor responsivity is largely unknown. Herein, we utilized c-fos immediate-early gene expression to map neuronal activity and determine whether mice weaned to control (CF), moderately-high fat (MHF), or high-fat (HF) diet for a period of 6 months had changes in odor activation. Diet-challenged M72-IRES-tau-GFP mice were exposed to either a preferred M72 (Olfr160) ligand, isopropyl tiglate, or clean air in a custom-made Bell-jar infusion chamber using an alternating odor exposure pattern generated by a picosprizer™. Mice maintained on fatty diets weighed significantly more and cleared glucose less efficiently

as determined by an intraperitoneal glucose tolerance test (IPGTT). The number of juxtaglomerular cells (JGs) decreased following maintenance of the mice on the MHF diet for cells surrounding the medial but not lateral M72 glomerulus within a 4 cell-column distance. The percentage of c-fos + JGs surrounding the lateral M72 glomerulus decreased in fat-challenged mice whereas those surrounding the medial glomerulus were not affected by diet. Altogether, these results show an asymmetry in the responsiveness of the 'mirror image' glomerular map for the M72 receptor that shows greater sensitivity of the lateral vs. medial glomerulus upon exposure to fatty diet.
J Bioenerg Biomembr, 2019; 51
29747701: Cooper ML, Collyer JW, Calkins DJ
Astrocyte remodeling without gliosis precedes optic nerve Axonopathy.
Astroyctes serve myriad functions but are especially critical in white matter tracts, where energy-demanding axons propagate action potentials great distances between neurons. Axonal dependence on astrocytes for even normal function accentuates the critical role astrocytes serve during disease. In glaucoma, the most common optic neuropathy, sensitivity to intraocular pressure (IOP) challenges RGC axons early, including degradation of anterograde transport to the superior colliculus (SC). Astrocyte remodeling presages overt axon degeneration in glaucoma and thus may present a therapeutic opportunity. Here we developed a novel metric to quantify organization of astrocyte processes in the optic nerve relative to axon degeneration in the DBA/2 J hereditary mouse model of glaucoma. In early progression, as axons expand prior to loss, astrocyte processes become more parallel with migration to the nerve's edge without a change in overall coverage of the nerve. As axons degenerate, astrocyte parallelism diminishes with increased glial coverage and reinvasion of the nerve. In longitudinal sections through aged DBA/2 J nerve, increased astrocyte parallelism reflected elevated levels of the astrocyte gap-junction protein connexin 43 (Cx43). In the distal nerve, increased Cx43 also indicated with a higher level of intact anterograde transport from retina to SC. Our results suggest that progression of axonopathy in the optic nerve involves astrocyte remodeling in two phases. In an early phase, astrocyte processes organize in parallel, likely through gap-junction coupling, while a later phase involves deterioration of organization as glial coverage increases and axons are lost.
Acta Neuropathol Commun, 2018; 6
29463759: Risner ML, Pasini S, Cooper ML, Lambert WS, Calkins DJ
Axogenic mechanism enhances retinal ganglion cell excitability during early progression in glaucoma.
Diseases of the brain involve early axon dysfunction that often precedes outright degeneration. Pruning of dendrites and their synapses represents a potential driver of axonopathy by reducing activity. Optic nerve degeneration in glaucoma, the world's leading cause of irreversible blindness, involves early stress to retinal ganglion cell (RGC) axons from sensitivity to intraocular pressure (IOP). This sensitivity also influences survival of RGC dendrites and excitatory synapses in the retina. Here we tested in individual RGCs identified by type the relationship between dendritic organization and axon signaling to light following modest, short-term elevations in pressure. We found dendritic pruning occurred early, by 2 wk of elevation, and independent of whether the RGC responded to light onset (ON cells) or offset (OFF cells). Pruning was similarly independent of ON and OFF in the DBA/2J mouse, a chronic glaucoma model. Paradoxically, all RGCs, even those with significant pruning, demonstrated a transient increase in axon firing in response to the preferred light stimulus that occurred on a backdrop of generally enhanced excitability. The increased response was not through conventional presynaptic signaling, but rather depended on voltage-sensitive sodium channels that increased transiently in the axon. Pruning, axon dysfunction, and deficits in visual acuity did not progress between 2 and 4 wk of elevation. These results suggest neurodegeneration in glaucoma involves an early axogenic response that counters IOP-related stress to excitatory dendritic architecture to slow progression and maintain signaling to the brain. Thus, short-term exposure to elevated IOP may precondition the neural system to further insult.
Proc Natl Acad Sci U S A, 2018; 115
28153739: Calkins DJ, Pekny M, Cooper ML, Benowitz L,
The challenge of regenerative therapies for the optic nerve in glaucoma.
This review arose from a discussion of regenerative therapies to treat optic nerve degeneration in glaucoma at the 2015 Lasker/IRRF Initiative on Astrocytes and Glaucomatous Neurodegeneration. In addition to the authors, participants included Jonathan Crowston, Andrew Huberman, Elaine Johnson, Richard Lu, Hemai Phatnami, Rebecca Sappington, and Don Zack. Glaucoma is a neurodegenerative disease of the optic nerve, and is the leading cause of irreversible blindness worldwide. The disease progresses as sensitivity to intraocular pressure (IOP) is conveyed through the optic nerve head to distal retinal ganglion cell (RGC) projections. Because the nerve and retina are components of the central nervous system (CNS), their intrinsic regenerative capacity is limited. However, recent research in regenerative therapies has resulted in multiple breakthroughs that may unlock the optic nerve's regenerative potential. Increasing levels of Schwann-cell derived trophic factors and reducing potent cell-intrinsic suppressors of regeneration have resulted in axonal regeneration even beyond the optic chiasm. Despite this success, many challenges remain. RGC axons must be able to form new connections with their appropriate targets in central brain regions and these connections must be retinotopically correct. Furthermore, for new axons

penetrating the optic projection, oligodendrocyte glia must provide myelination. Additionally, reactive gliosis and inflammation that increase the regenerative capacity must be outweigh pro-apoptotic processes to create an environment within which maximal regeneration can occur.
Exp Eye Res, 2017; 157

26646560: Cooper ML, Crish SD, Inman DM, Horner PJ, Calkins DJ

Early astrocyte redistribution in the optic nerve precedes axonopathy in the DBA/2J mouse model of glaucoma.

Glaucoma challenges the survival of retinal ganglion cell axons in the optic nerve through processes dependent on both aging and ocular pressure. Relevant stressors likely include complex interplay between axons and astrocytes, both in the retina and optic nerve. In the DBA/2J mouse model of pigmentary glaucoma, early progression involves axonopathy characterized by loss of functional transport prior to outright degeneration. Here we describe novel features of early pathogenesis in the DBA/2J nerve. With age the cross-sectional area of the nerve increases; this is associated generally with diminished axon packing density and survival and increased glial coverage of the nerve. However, for nerves with the highest axon density, as the nerve expands mean cross-sectional axon area enlarges as well. This early expansion was marked by disorganized axoplasm and accumulation of hyperphosphorylated neurofilamants indicative of axonopathy. Axon expansion occurs without loss up to a critical threshold for size (about 0.45-0.50 μm(2)), above which additional expansion tightly correlates with frank loss of axons. As well, early axon expansion prior to degeneration is concurrent with decreased astrocyte ramification with redistribution of processes towards the nerve edge. As axons expand beyond the critical threshold for loss, glial area resumes an even distribution from the center to edge of the nerve. We also found that early axon expansion is accompanied by reduced numbers of mitochondria per unit area in the nerve. Finally, our data indicate that both IOP and nerve expansion are associated with axon enlargement and reduced axon density for aged nerves. Collectively, our data support the hypothesis that diminished bioenergetic resources in conjunction with early nerve and glial remodeling could be a primary inducer of progression of axon pathology in glaucoma.
Exp Eye Res, 2016; 150

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BOARD NUMBER: S04-214

ULTRAMICRONIZED PALMITOYLETHANOLAMIDE REGULATES MAST CELL-ASTROCYTE CROSSTALK: A NEW POTENTIAL MECHANISM UNDERLYING THE INHIBITION OF MORPHINE TOLERANCE

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Aim. Tolerance to analgesia represents a huge limit for the use of opioids in treating chronic pain. Previous *in vivo* studies have shown that ultramicronized palmitoylethanolamide (um-PEA) was able to delay morphine tolerance and increase opioid effectiveness. Since glial cells are involved in tolerance and mast cells are one of the pivotal targets of PEA, we hypothesized that um-PEA counteracts morphine tolerance by controlling the crosstalk between these cell populations. **Methods.** A rat mast cell line, RBL-2H3, and a primary culture of cortical astrocytes were used. Mast cell degranulation was analyzed through β-hexosaminidase, tryptase and histamine release. Gene expression was evaluated by RT-PCR. **Results.** Morphine (30 μM for 30 minutes) induced RBL-2H3 cell degranulation, which was significantly reduced by 18-hour pre-treatment with um-PEA (100 μM). Conditioned media of RBL-2H3 cells highly increased the expression of genes associated with inflammation and pain from morphine-activated astrocytes. Conversely, conditioned media of um-PEA treated RBL-2H3 cells significantly reduced the expression of inflammatory genes (IL-1β and IL-6) and genes associated with pain modulation (ccl2, Serpin 3N and glutamate transporter EAAT2), while upregulating GFAP gene expression. **Conclusion.** Ultramicronized PEA inhibited morphine-induced mast cell degranulation and down-regulated the expression of inflammatory and pain-related genes from astrocytes challenged with mast cell conditioned media, while stimulating protective pathways (i.e., GFAP upregulation). The findings suggest um-PEA may delay morphine tolerance through regulating mast cell-astrocyte crosstalk.

Pubmed:
34021474: Toti A, Santi A, Pardella E, Nesi I, Tomasini R, Mello T, Paoli P, Caselli A, Cirri P
Activated fibroblasts enhance cancer cell migration by microvesicles-mediated transfer of Galectin-1.
Cancer-associated fibroblasts (CAFs) are one of the main components of the stromal compartment in the tumor microenvironment (TME) and the crosstalk between CAFs and cancer cells is essential for tumor progression and aggressiveness. Cancer cells mediate an activation process, converting normal fibroblasts into CAFs, that are characterized by modified expression of many proteins and increased production and release of microvesicles (MVs), extracellular vesicles generated by outwards budding from the cell membrane. Recent evidence underlined that the uptake of CAF-derived MVs changes the overall protein content of tumor cells. In this paper, we demonstrate that tumor activated fibroblasts overexpress Galectin-1 (Gal-1) and consequently release MVs containing increased levels of this protein. The uptake of Gal-1 enriched MVs by tumor cells leads to the upregulation of its intracellular concentration, that strongly affects cancer cell migration, while neither proliferation nor adhesion are altered. Accordingly, tumor cells co-cultured with fibroblasts silenced for Gal-1 have a reduced migratory ability. The present work reveals the key role of an exogenous protein, Gal-1, derived from activated fibroblasts, in cancer progression, and contributes to clarify the importance of MVs-mediated protein trafficking in regulating tumor-stroma crosstalk.
J Cell Commun Signal, 2021; 15
34829900: Lucarini E, Seguella L, Vincenzi M, Parisio C, Micheli L, Toti A, Corpetti C, Del Re A, Squillace S, Maftei D, Lattanzi R, Ghelardini C, Di Cesare Mannelli L, Esposito G
Role of Enteric Glia as Bridging Element between Gut Inflammation and Visceral Pain Consolidation during Acute Colitis in Rats.
Acute inflammation is particularly relevant in the pathogenesis of visceral hypersensitivity associated with inflammatory bowel diseases. Glia within the enteric nervous system, as well as within the central nervous system, contributes to neuroplasticity during inflammation, but whether enteric glia has the potential to modify visceral sensitivity following colitis is still unknown. This work aimed to investigate the occurrence of changes in the neuron-glial networks controlling visceral perception along the gut-brain axis during colitis, and to assess the effects of peripheral glial manipulation. Enteric glia activity was altered by

the poison fluorocitrate (FC; 10 µmol kg i.p.) before inducing colitis in animals (2,4-dinitrobenzenesulfonic acid, DNBS; 30 mg in 0.25 mL EtOH 50%), and visceral sensitivity, colon damage, and glia activation along the pain pathway were studied. FC injection significantly reduced the visceral hyperalgesia, the histological damage, and the immune activation caused by DNBS. Intestinal inflammation is associated with a parallel overexpression of TRPV1 and S100β along the gut-brain axis (colonic myenteric plexuses, dorsal root ganglion, and periaqueductal grey area). This effect was prevented by FC. Peripheral glia activity modulation emerges as a promising strategy for counteracting visceral pain induced by colitis.
Biomedicines, 2021; 9

34649573: Micheli L, Parisio C, Lucarini E, Vona A, Toti A, Pacini A, Mello T, Boccella S, Ricciardi F, Maione S, Graziani G, Lacal PM, Failli P, Ghelardini C, Di Cesare Mannelli L
VEGF-A/VEGFR-1 signalling and chemotherapy-induced neuropathic pain: therapeutic potential of a novel anti-VEGFR-1 monoclonal antibody.
Neuropathic pain is a clinically relevant adverse effect of several anticancer drugs that markedly impairs patients' quality of life and frequently leads to dose reduction or therapy discontinuation. The poor knowledge about the mechanisms involved in neuropathy development and pain chronicization, and the lack of effective therapies, make treatment of chemotherapy-induced neuropathic pain an unmet medical need. In this context, the vascular endothelial growth factor A (VEGF-A) has emerged as a candidate neuropathy hallmark and its decrease has been related to pain relief. In the present study, we have investigated the role of VEGF-A and its receptors, VEGFR-1 and VEGFR-2, in pain signalling and in chemotherapy-induced neuropathy establishment as well as the therapeutic potential of receptor blockade in the management of pain.
J Exp Clin Cancer Res, 2021; 40

34393197: Lucarini E, Di Pilato V, Parisio C, Micheli L, Toti A, Pacini A, Bartolucci G, Baldi S, Niccolai E, Amedei A, Rossolini GM, Nicoletti C, Cryan JF, O'Mahony SM, Ghelardini C, Di Cesare Mannelli L
Visceral sensitivity modulation by faecal microbiota transplantation: the active role of gut bacteria in pain persistence.
Recent findings linked gastrointestinal disorders characterized by abdominal pain to gut microbiota composition. The present work aimed to evaluate the power of gut microbiota as a visceral pain modulator and, consequently, the relevance of its manipulation as a therapeutic option in reversing postinflammatory visceral pain persistence. Colitis was induced in mice by intrarectally injecting 2,4-dinitrobenzenesulfonic acid (DNBS). The effect of faecal microbiota transplantation from viscerally hypersensitive DNBS-treated and naive donors was evaluated in control rats after an antibiotic-mediated microbiota depletion. Faecal microbiota transplantation from DNBS donors induced a long-lasting visceral hypersensitivity in control rats. Pain threshold trend correlated with major modifications in the composition of gut microbiota and short chain fatty acids. By contrast, no significant alterations of colon histology, permeability, and monoamines levels were detected. Finally, by manipulating the gut microbiota of DNBS-treated animals, a counteraction of persistent visceral pain was achieved. The present results provide novel insights into the relationship between intestinal microbiota and visceral hypersensitivity, highlighting the therapeutic potential of microbiota-targeted interventions.
Pain, 2022; 163

34312766: Micheli L, Rajagopalan R, Lucarini E, Toti A, Parisio C, Carrino D, Pacini A, Ghelardini C, Rajagopalan P, Di Cesare Mannelli L
Pain Relieving and Neuroprotective Effects of Non-opioid Compound, DDD-028, in the Rat Model of Paclitaxel-Induced Neuropathy.
Chemotherapy-induced neuropathy (CIN) is a major dose-limiting side effect of anticancer therapy that can compel therapy discontinuation. Inadequate analgesic efficacy of current pharmacological approaches requires the identification of innovative therapeutics and, hence, the purpose of this study is to conduct a preclinical evaluation of the efficacy of DDD-028, a versatile pentacyclic pyridoindole derivative, against paclitaxel-induced neuropathic pain. In two separate experiments, DDD-028 was administered per os acutely (1-25 mg kg) or repeatedly (10 mg kg) in paclitaxel-treated rats. The response to mechanical noxious stimulus (paw pressure) as well as to non-noxious mechanical (von Frey) and thermal (cold plate) stimuli was investigated. Acute administration of DDD-028 induced a dose-dependent anti-neuropathic pain effect in all tests performed. Further, repeated daily treatment for 18 consecutive days (starting the first day of paclitaxel administration) significantly reduced the development of pain over time without the development of tolerance to the anti-hyperalgesic effect. Ex vivo analysis showed that DDD-028 was able to reduce oxidative damage of dorsal root ganglia as evidenced by the increase in the level of carbonylated proteins and the decrease in catalase activity. In the lumbar spinal cord, periaqueductal gray matter, thalamus, and somatosensory cortex 1, DDD-28 significantly prevented the activation of microglia and astrocytes. The pharmacodynamic study revealed that the pain-relieving effects of DDD-028 were fully blocked by both the non-selective nicotinic receptor (nAChR) antagonist mecamylamine and by the selective α7 nAChR antagonist methyllycaconitine. In conclusion, DDD-028 was active in reducing paclitaxel-induced neuropathic pain after single or repeated administrations without tolerance development and displaying a double symptomatic and neuroprotective profile. DDD-028 could represent a valuable candidate for the treatment of CIN.

Neurotherapeutics, 2021; 18
33071790: Micheli L, Di Cesare Mannelli L, Lucarini E, Parisio C, Toti A, Fiorentino B, Rigamonti MA, Calosi L, Ghelardini C
Intranasal Low-Dose Naltrexone Against Opioid Side Effects: A Preclinical Study.
Opioids are broad spectrum analgesics that are an integral part of the therapeutic armamentarium to combat pain in the clinical practice. Unfortunately, together with analgesia, a number of adverse effects can occur such as nausea, vomiting, constipation, gastrointestinal alterations and cognitive impairments. Naltrexone is a competitive antagonist of opioid receptors commonly used to treat opioid addiction; its oral use against agonists side effects is limited by the decrease of opioids-therapeutic efficacy and own adverse effects. The intranasal delivery of naltrexone could offer a quick and effective achievement of CNS based on extracellular mechanisms including perineural and perivascular transport. The aim of the study was to test the efficacy of intranasal low-dose naltrexone in reducing intraperitoneal morphine and oxycodone side effects in rodents. In mice, 1 µg naltrexone intranasally administered 30 min before opioids reduced cognitive impairments and motor alteration induced by 10 mg kg morphine and 60 mg kg oxycodone in the Passive avoidance and Rota rod tests, respectively. Moreover, naltrexone rebalanced opioid-induced reduction of the intestinal transit and latency of feces expulsion as well as food intake inhibition. Importantly, 1 µg naltrexone instillation did not block analgesia as demonstrated by the Hot plate test. In rats, intranasal naltrexone counteracted the opioid-induced pica phenomenon related to emesis and increased water and palatable food intake. The effects were comparable to that achieved by metoclopramide used as reference drug. Treatments did not influence body weight. Lastly, the safety of the intranasal delivery has been checked by hematoxylin-eosin staining that did not show histological alterations of the nasal cavity. In conclusion, intranasal low-dose naltrexone counteracted morphine and oxycodone induced gastrointestinal and CNS side effects without impairing opioid analgesia. It is a candidate to be a valid clinical strategy deserving deep analysis.
Front Pharmacol, 2020; 11
27421795: Caselli A, Paoli P, Santi A, Mugnaioni C, Toti A, Camici G, Cirri P
Low molecular weight protein tyrosine phosphatase: Multifaceted functions of an evolutionarily conserved enzyme.
Originally identified as a low molecular weight acid phosphatase, LMW-PTP is actually a protein tyrosine phosphatase that acts on many phosphotyrosine-containing cellular proteins that are primarily involved in signal transduction. Differences in sequence, structure, and substrate recognition as well as in subcellular localization in different organisms enable LMW-PTP to exert many different functions. In fact, during evolution, the LMW-PTP structure adapted to perform different catalytic actions depending on the organism type. In bacteria, this enzyme is involved in the biosynthesis of group 1 and 4 capsules, but it is also a virulence factor in pathogenic strains. In yeast, LMW-PTPs dephosphorylate immunophilin Fpr3, a peptidyl-prolyl-cis-trans isomerase member of the protein chaperone family. In humans, LMW-PTP is encoded by the ACP1 gene, which is composed of three different alleles, each encoding two active enzymes produced by alternative RNA splicing. In animals, LMW-PTP dephosphorylates a number of growth factor receptors and modulates their signalling processes. The involvement of LMW-PTP in cancer progression and in insulin receptor regulation as well as its actions as a virulence factor in a number of pathogenic bacterial strains may promote the search for potent, selective and bioavailable LMW-PTP inhibitors.
Biochim Biophys Acta, 2016; 1864
34685520: Lucarini E, Nocentini A, Bonardi A, Chiaramonte N, Parisio C, Micheli L, Toti A, Ferrara V, Carrino D, Pacini A, Romanelli MN, Supuran CT, Ghelardini C, Di Cesare Mannelli L
Carbonic Anhydrase IV Selective Inhibitors Counteract the Development of Colitis-Associated Visceral Pain in Rats.
Persistent pain affecting patients with inflammatory bowel diseases (IBDs) is still very difficult to treat. Carbonic anhydrase (CA) represents an intriguing pharmacological target considering the anti-hyperalgesic efficacy displayed by CA inhibitors in both inflammatory and neuropathic pain models. The aim of this work was to evaluate the effect of inhibiting CA IV, particularly when expressed in the gut, on visceral pain associated with colitis induced by 2,4-di-nitrobenzene sulfonic acid (DNBS) in rats. Visceral sensitivity was assessed by measuring animals' abdominal responses to colorectal distension. Repeated treatment with the selective CA IV inhibitors AB-118 and NIK-67 effectively counteracted the development of visceral pain induced by DNBS. In addition to pain relief, AB-118 showed a protective effect against colon damage. By contrast, the anti-hyperalgesic activity of NIK-67 was independent of colon healing, suggesting a direct protective effect of NIK-67 on visceral sensitivity. The enzymatic activity and the expression of CA IV resulted significantly increased after DNBS injection. NIK-67 normalised CA IV activity in DNBS animals, while AB-118 was partially effective. None of these compounds influenced CA IV expression through the colon. Although further investigations are needed to study the underlying mechanisms, CA IV inhibitors are promising candidates in the search for therapies to relieve visceral pain in IBDs.
Cells, 2021; 10
35149388: Micheli L, Parisio C, Lucarini E, Carrino D, Ciampi C, Toti A, Ferrara V, Pacini A, Ghelardini C, Di Cesare Mannelli L
Restorative and pain-relieving effects of fibroin in preclinical models of tendinopathy.

The term tendinopathy indicates a wide spectrum of conditions characterized by alterations in tendon tissue homeostatic response and damage to the extracellular matrix. The current pharmacological approach involves the use of nonsteroidal anti-inflammatory drugs and corticosteroids often with unsatisfactory results, making essential the identification of new treatments. In this study, the pro-regenerative and protective effects of an aqueous fibroin solution (0.5-500 µg/mL) against glucose oxidase (GOx)-induced damage in rat tenocytes were investigated. Then, fibroin anti-hyperalgesic and protective actions were evaluated in two models of tendinopathy induced in rats by collagenase or carrageenan injection, respectively. In vitro, 5-10 µg/mL fibroin per se increased cell viability and reverted the morphological alterations caused by GOx (0.1 U/mL). Fibroin 10 µg/mL evoked proliferative signaling upregulating the expression of decorin, scleraxin, tenomodulin ($p < 0.001$), FGF-2, and tenascin-C ($p < 0.01$) genes. Fibroin enhanced the basal FGF-2 and MMP-9 protein concentrations and prevented their GOx-mediated decrease. Furthermore, fibroin positively modulated the production of collagen type I. In vivo, the peri-tendinous injection of fibroin (5 mg) reduced the development of spontaneous pain and hypersensitivity ($p < 0.01$) induced by the intra-tendinous injection of collagenase; the efficacy was comparable to that of triamcinolone. The pain-relieving action of fibroin (peri-tendinous) was confirmed in the model of tendinopathy induced by carrageenan (intra-tendinous) where this fibrous protein was also able to improve tendon matrix organization, normalizing the orientation of collagen fibers. In conclusion, the use of fibroin in tendinopathies is suggested taking advantage of its excellent mechanical properties, pain-relieving effects, and ability to promote tissue regeneration processes.
Biomed Pharmacother, 2022; 148

27266957: Peppicelli S, Toti A, Giannoni E, Bianchini F, Margheri F, Del Rosso M, Calorini L
Metformin is also effective on lactic acidosis-exposed melanoma cells switched to oxidative phosphorylation.
Low extracellular pH promotes in melanoma cells a malignant phenotype characterized by an epithelial-to-mesenchymal transition (EMT) program, endowed with mesenchymal markers, high invasiveness and pro-metastatic property. Here, we demonstrate that melanoma cells exposed to an acidic extracellular microenvironment, 6.7±0.1, shift to an oxidative phosphorylation (Oxphos) metabolism. Metformin, a biguanide commonly used for type 2 diabetes, inhibited the most relevant features of acid-induced phenotype, including EMT and Oxphos. When we tested effects of lactic acidosis, to verify whether sodium lactate might have additional effects on acidic melanoma cells, we found that EMT and Oxphos also characterized lactic acid-treated cells. An increased level of motility was the only gained property of lactic acidic-exposed melanoma cells. Metformin treatment inhibited both EMT markers and Oxphos and, when its concentration raised to 10 mM, it induced a striking inhibition of proliferation and colony formation of acidic melanoma cells, both grown in protons enriched medium or lactic acidosis. Thus, our study provides the first evidence that metformin may target either proton or lactic acidosis-exposed melanoma cells inhibiting EMT and Oxphox metabolism. These findings disclose a new potential rationale of metformin addition to advanced melanoma therapy, e.g. targeting acidic cell subpopulation.
Cell Cycle, 2016; 15

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LOW TEMPERATURES DELAY THE EFFECTS OF ISCHEMIA IN CEREBELLAR SLICES

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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University Paris-Saclay, Neuropsi, Cnrs Umr9197, Saclay, France

Cerebral ischemia results in oxygen and glucose deprivation that most commonly occurs after a reduction or interruption of blood supply into the brain. The consequences of cerebral ischemia are complex and involve the loss of metabolic ATP, excessive K+ and glutamate accumulation in the extracellular space, electrolytes imbalance and brain oedema formation. So far, several treatments have been proposed to ameliorate ischemia effects, however only few are effective. Here we focused on the neuroprotective role of lowering the temperature on ischemia mimicked by an episode of Oxygen and Glucose Deprivation (OGD) in cerebellar slices. Our results suggest that lowering the temperature of the extracellular 'milieu' delays both [K+]e raises and tissue swelling, two dreaded consequences of cerebellar ischemia. Moreover, radial glial cells (Bergmann glia) are also sensitive to temperature lowering during OGD. In fact, both membrane depolarisation and morphological change of these glial cells appeared significantly delayed than those observed at more physiological temperatures.

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THE APP A673T VARIANT AND THE APOE GENOTYPE AFFECT ASTROCYTE MORPHOLOGY AND CHOLESTEROL METABOLISM IN A MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

Pinja Kettunen[1], Johanna Kuusisto[2], Markku Laakso[2], Jari Koistinaho[1], Taisia Rolova[1]
[1]University of Helsinki, Hilife, Helsinki, Finland, [2]University of Eastern Finland, Institude Of Clinical Medicine, Kuopio, Finland

Aims Alzheimer's disease (AD) is the most common cause of dementia worldwide. The A673T mutation in the amyloid precursor protein (APP) reduces beta-amyloid (Aβ) production in neurons and protects its carriers from AD. According to Oksanen et. al. 2017 a familial presenilin-1 mutation increases astrocytic $A\beta_{(1-42)}$ production to the level of neurons and induces functional deficits in astrocytes. Thus, we postulated that the APP A673T variant may excert some its protective effects through altered astrocyte function. Methods We compared APP A673T APOE3/3 astrocytes to APOE3/3 controls and astrocytes carrying the AD risk genotype APOE4/4. First, we differentiated astrocytes from human induced pluripotent stem cells (hIPSCs) and characterized them using immunocytochemistry and qPCR. Then, we analysed astrocyte morphology and cytokine production. Finally, we measured astrocytic cholesterol accumulation with or without docosahexaenoic acid (DHA) and choline supplementation. These compounds are anti-inflammatory and modulate cholesterol metabolism in the brain. Results Our hIPSC-derived astrocytes expressed astrocyte markers, displayed the characteristic astrocyte morphology and secreted cytokines in response to pro-inflammatory stimulation, as expected. Astrocytes carrying the APP A67T variant displayed altered morphology compared to controls. Docosahexaenoic acid and choline supplementation reduced cholesterol accumulation in astrocytes carrying the APOE4/4 genotype or the APP A673T variant but not in APOE3/3 controls. Conclusions Astrocytes carrying the APP variant A673T display altered morphology and responsivity to anti-inflammatory and cholesterol-modulating treatments compared to controls. DHA and choline supplementation also reduces cholesterol accumulation in APOE4/4, which may modulate Aβ production, uptake, and inflammation.

Pubmed:
33794144: Groussin M, Poyet M, Sistiaga A, Kearney SM, Moniz K, Noel M, Hooker J, Gibbons SM, Segurel L, Froment A, Mohamed RS, Fezeu A, Juimo VA, Lafosse S, Tabe FE, Girard C, Iqaluk D, Nguyen LTT, Shapiro BJ, Lehtimäki J, Ruokolainen L, Kettunen PP, Vatanen T, Sigwazi S, Mabulla A, Domínguez-Rodrigo M, Nartey YA, Agyei-Nkansah A, Duah A, Awuku YA, Valles KA, Asibey SO, Afihene MY, Roberts LR, Plymoth A, Onyekwere CA, Summons RE, Xavier RJ, Alm EJ
Elevated rates of horizontal gene transfer in the industrialized human microbiome.
Industrialization has impacted the human gut ecosystem, resulting in altered microbiome composition and diversity. Whether bacterial genomes may also adapt to the industrialization of their host populations remains largely unexplored. Here, we investigate the extent to which the rates and targets of horizontal gene transfer (HGT) vary across thousands of bacterial strains from 15 human populations spanning a range of industrialization. We show that HGTs have accumulated in the microbiome over recent host generations and that HGT occurs at high frequency within individuals. Comparison across human populations reveals that industrialized lifestyles are associated with higher HGT rates and that the functions of HGTs are related to the level of host industrialization. Our results suggest that gut bacteria continuously acquire new functionality based on host lifestyle and that high rates of HGT may be a recent development in human history linked to industrialization.
Cell, 2021; 184
33270954: Rõlova T, Lehtonen Š, Goldsteins G, Kettunen P, Koistinaho J
Metabolic and immune dysfunction of glia in neurodegenerative disorders: Focus on iPSC models.
The research on neurodegenerative disorders has long focused on neuronal pathology and used transgenic mice as disease models. However, our understanding of the chronic neurodegenerative process in the human brain is still very limited. It is increasingly recognized that neuronal loss is not caused solely by intrinsic degenerative processes but rather via impaired interactions with surrounding glia and other brain cells. Dysfunctional astrocytes do not provide sufficient nutrients and antioxidants to the neurons, while dysfunctional microglia cannot efficiently clear pathogens and cell debris from extracellular space, thus resulting in chronic inflammatory processes in the brain. Importantly, human glia, especially the astrocytes, differ significantly in morphology and function from their mouse counterparts, and therefore more human-based disease models are needed. Recent advances in stem cell technology make it possible to reprogram human patients' somatic cells to induced

pluripotent stem cells (iPSC) and differentiate them further into patient-specific glia and neurons, thus providing a virtually unlimited source of human brain cells. This review summarizes the recent studies using iPSC-derived glial models of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis and discusses the applicability of these models to drug testing. This line of research has shown that targeting glial metabolism can improve the survival and function of cocultured neurons and thus provide a basis for future neuroprotective treatments.
Stem Cells, 2021; 39

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ROLE OF ASTROCYTIC CA2+ SIGNALING IN BRAIN OSCILLATORY ACTIVITY AND MEMORY: IMPLICATIONS FOR NEURODEGENERATIVE DISEASES.

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Astrocytes are the most abundant glial cells in the central nervous system and are in close contact with synapses. In response to neurotransmitters, their intracellular Ca^{2+} levels are increased, leading to the release of neuroactive molecules. In addition, astrocytic Ca^{2+} signaling is dysregulated in Alzheimer's and Huntington's neurodegenerative diseases. Patients' brain neural oscillations and memory are also altered in both diseases. Our main hypothesis is that chronic modulation of astrocytic Ca^{2+} signaling leads to altered release of glutamate or pro-inflammatory factors from astrocytes, and consequent deficits in brain oscillations and memory formation. We have tested this hypothesis *in vivo* using the mouse primary visual cortex as a model system, combined with electrophysiology, chemogenetics, genetically-modified mouse models, and behavior. Data are currently being processed.
Our study could have profound implications in the clinic, as it may lead to a better understanding of the astrocyte role in memory deficits and in the etiology of neurodegenerative diseases.

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THE ASTROCYTE NEURON LACTATE SHUTTLE (ANLS) FUELS THE NEURON ASTROCYTE LIPID SHUTTLE (NALS) IN PATHOPHYSIOLOGICAL CONDITIONS

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

Pierre Magistretti[1], Hubert Fiumelli[2], Arnaud Tauffenmberger[2], Siharei Laptenok[2], Vijayakumar Rajamanickam[2], Nadia Steiner[2], Carlo Liberale[2]
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The tight metabolic cooperation between neurons and astrocytes maintains brain activity and homeostasis. When neuronal activity is increased, astrocytes provide neurons with lactate and different factors to protect them from oxidative stress produced during mitochondrial respiration. However upon neuronal hyperactivation and in response to the increase of reactive oxygen species, neurons produce lipid particles that are then transferred to astrocytes where they accumulate into lipid droplets. Lipid droplets have been linked to many neurodegenerative diseases and a better understanding of the mechanism involved in their formation and utilization is important for the development of therapeutic strategies. In the current study, the metabolic cooperation between astrocytes and neurons during oxidative stress was investigated with a special interest in lipid droplet formation and composition. Here, primary cultures of neurons and astrocytes grown together or in co-culture of primary neurons and astrocytes seeded separately were treated with rotenone. The number of lipid droplets, as well as their size and composition have been evaluated using confocal microscopy and Stimulated Raman Spectroscopy. Our preliminary observations indicate that lactate may be used not only as a signaling molecule but also as a building block of the fatty acids stored in lipid droplets.

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THE ELEPHANT IN THE ROOM: A TISSUE-ENGINEERED 3D MODEL TO EXPLORE ASTROCYTE ROLE IN NEURODEGENERATION

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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One accepted reason for neuro-regeneration failure in central nervous system (CNS) neurodegenerative diseases is the formation of the scar tissue containing reactive astrocytes. These cells change their phenotype from quiescent to reactive, overexpressing extracellular matrix (ECM) proteins and strongly modifying the biophysical properties of the lesion environment. Although the process is inexorable, it is mutable in time. Our working hypothesis is that the alterations in the matrix caused by reactive astrocytes are a barrier to neuro-regeneration. Here we propose a tissue-engineered model to dynamically recreate the astrogliosis environment. Alginate (ALG) was chosen to mimic the ECM due to its biocompatibility and tunable mechanical properties. ALG modifications with cell adhesive (RGD) and MMP-sensitive (PVGLIG) peptides allowed astrocytes to extend long processes and form a network in a 3D environment (Figure 1), highlighting the biologically relevance of the model. Activation with LPS/IFN-γ led to an increase of *Lcn2* and *Aqp4*, indicating that astrocytes acquired an astrogliosis-like phenotype, with no significant changes in cellular viability, metabolic activity and morphology. To model temporal changes in ECM stiffness, ALG hydrogels were submitted to an external crosslinking stimulus (barium). Barium treatment led to a six-fold increase in the stiffness of the hydrogels. Astrocytic metabolic activity slightly decreased, but the viability remained unchanged. A dramatic alteration in cellular morphology was observed, along with an increased expression of astrogliosis (*Gfap* and *Aqp4)* and mechanosensing (*Piezo1)* related

genes.

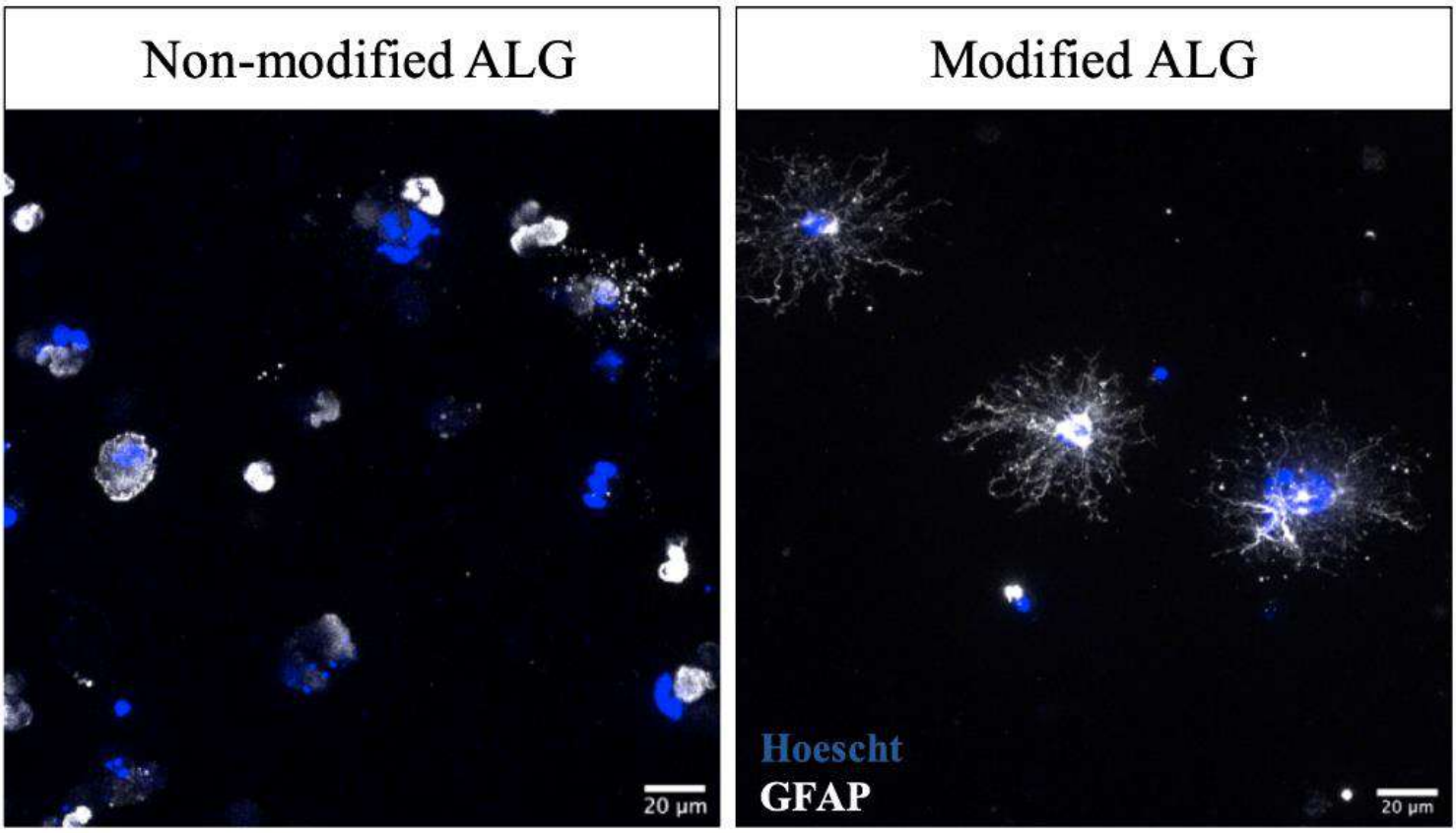

Figure 1 - Astrocytes embedded in non-modified and modified alginate matrices (DIV 3).

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ADRENERGIC MODULATION OF AQUAPORIN-4 NANOSCALE DISTRIBUTION AND DYNAMICS IN PRIMARY MOUSE ASTROCYTES

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Adrenergic signaling has been shown to regulate the volume of the extracellular space of the brain via aquaporin-4 (AQP4) localized at the end-feet of astrocytes. The mechanisms by which AQP4 regulate changes in the extracellular space volume and cerebrospinal fluid inflow and outflow in the brain remain elusive. This is partly due to the complex dynamics of AQP4 nanoscale cluster assembly and disassembly at the end-feet of astrocytes. Our study aims to evaluate the effect of adrenergic modulation on the localization, mobility and cluster-size of AQP4 in primary astrocytes, both in dissociated cultures and brain slices. To achieve the necessary level of spatial and temporal resolution we used a combination of super-resolution imaging and single-molecule tracking techniques to evaluate changes in localization and mobility of AQP4M23. Our results suggest that adrenergic modulation affects the mobility, localization and cluster size of AQP4 in primary astrocytes. This work will contribute to understand the fundamental mechanisms underlying the volume change of the extracellular space of the brain during sleep.

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EPILEPTOGENIC FEATURES OF NEURAL PROGENITORS DERIVED FROM CEREBRAL BIOPSIES OF FCDS PATIENTS IN CHIMERIC MICE

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Focal cortical dysplasia (FCD), a malformation of cortical development, is a primary cause of refractory epilepsy. Neural progenitors from FCD harbor specific mutations in the mTOR pathway of cell activation, leading to a disrupted cerebral cortex assemblage during development. Here we tested whether neural progenitors isolated from brain specimens of epileptic patients suffering from FCDs, integrate into neuronal networks after transplantation in immunodeficient mice and can generate abnormal activity. Following dissociation of brain resections from epileptic patients, we evidence *in vitro* these progenitors in neurosphere cultures. Moreover, we developed a chimeric mice model of FCD by transplanting human cultured human epileptic progenitors in immunodeficient pups. Interestingly, using different electrophysiological *in vivo* and *in vitro* recordings, we found that the xenografted cells integrate in the network of the host cells and were able to recapitulate the abnormal activity patterns recorded in the patient before surgery. Taken together these results highlight a new paradigm to better understand the physiopathological mechanisms underlying genetic epilepsy.

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BOARD NUMBER: S04-222

THE CORRELATION BETWEEN CALCIUM ACTIVITY IN ASTROCYTES AND MOUSE BEHAVIOR

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Non-neuronal cells, including astrocytes, contribute to brain function and, together with neurons, form the 'brain active milieu.' We investigated how the astrocytic calcium activity pattern is organized and changes during mouse behavior. The genetically encoded calcium indicator, GCaMP6f, was expressed under the astrocyte-specific promoter in C57BL/6 mice. Two-photon calcium imaging was done either in acute hippocampal slices or on the somatosensory cortex of awake head-fixed mice. Fiber photometry was performed from mouse hippocampi during behavioral tests. We observed spontaneous fluctuations of calcium activity in the astrocytic population in slices. These fluctuations were linked to an increase in areas and spatial density of the individual calcium events. In head-fixed mice, an increase of calcium event areas correlated with an initial phase of animal running, while spatial density changed with running speed. Photometry data revealed that calcium activity in hippocampal astrocytes changes during animal navigation in the open field and social novelty tests. Our findings suggest that properties of astrocytic calcium activity patterns change specifically with animal behavior, prompting a possibility of a causal link. The work is supported by the Russian Science Foundation (grant 20-14-00241).

Pubmed:
32258690: Fedotova A, Slavutskaya M
Voluntary inhibition of saccadic eye movements: EEG study: .
Porto Biomed J, 2017 Sep-Oct; 2

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-223

NITRIC OXIDE PARTICIPATES TO METABOLIC CHANGES IN THE ASTROCYTIC-MICROGLIAL CROSSTALK DURING HYPOTHALAMIC INFLAMMATION

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Aims: Cellular adaptation to low oxygen is mediated by hypoxia inducible factors (HIFs), whose stabilization affects the expression of several target genes involved in different processes such as glycolysis, angiogenesis, cell proliferation and survival. Therefore, we aim to investigate how the physiological oxygen concentrations (3-5% in the brain) are affecting HIFs activity on metabolism in the hypothalamic inflammation. **Methods:** Primary hypothalamic murine glial cells have been cultured for three weeks before treatments at different oxygen conditions. Expression of HIF-1 and its target genes has been assessed by Western blot and qPCR. **Results:** In hypothalamic primary mixed glial culture, HIF-1 is stable and active at hypoxia (1%) as expected but also at physioxia (3%). Following pro-inflammatory stimuli, such as lipopolysaccharide (LPS) or interleukin-1β (IL-1β) HIF-1 expression greatly increased at both physioxia and hypoxia, thereby differentially regulating HIF-1 target genes involved in lactate production and release. Besides the lactate transporter MCT4, the inducible nitric oxide (NO) synthase displayed the most evident upregulation. By using the NO chelating agent cPTIO we confirmed the involvement of NO, known for stabilizing HIF-1 protein, in shifting the cellular metabolism from oxidative phosphorylation to glycolysis after pro-inflammatory stimulation. Furthermore, we evidenced by microglial ablation that HIF-1 activity is also depending on the presence of this cellular population, hinting at a crosstalk with astrocytes. **Conclusion:** We demonstrate the capacity of inflammation to activate HIF-1 in hypothalamic glia and suggest a role for NO in maintaining a positive feedback loop between astrocytes and microglia.

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MORPHOLOGICAL AND FUNCTIONAL FAñANAS CELLS CHARACTERIZATION IN THE MOUSE CEREBELLUM.

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Fañanas cells or “feathered” cells are cerebellar glia of unknown function. Described more than 100 years ago, they could only made visible through either the gold sublimate impregnation technique of Ramón y Cajal or tedious silver staining methods. The investigation of Fañanas cells with modern techniques has become possible by the recent identification in rat of two potassium channel-related polypeptides, Kv2.2 and Calsenilin (KChIP3), as potential marker proteins for Fañanas cells. While we confirmed the findings of this study, a similar approach in mouse failed. Therefore, in the current work, we used the combination of immunofluorescence and whole-cell patch-clamp recordings for characterizing the morphology and basic electrophysiological properties of these enigmatic cerebellar glia. In transverse slices, we reliably recognized Fañanas cells through the expression of pan-glial markers together with their atypical cell-body localization. Finally, extracellular stimulation in molecular and granular cell layers led to a slow and long-lasting inward current largely resulting from AMPA-receptor activation. Although we are at the beginning of the Fañanas cells investigation, this study cover a new face of the glial organization in the cerebellum.

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VASOCONSTRICTOR ENDOTHELIN B RECEPTOR IS PROLIFERATED IN REACTIVE ASTROCYTES IN APP KNOCK-IN MOUSE MODEL OF ALZHEIMER'S DISEASE.

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Aims: Alzheimer's disease (AD) is associated with cognitive impairment, and is correlated with proliferation of reactive astrocytes, the most abundant glial cells, important for providing neuronal support and regulating cerebral blood flow. The endothelin B receptor (ET_B) is a G protein-coupled receptor (GPCR) for endothelin-1, a potent vasoconstrictor, which is thought to be upregulated due to brain injury. This project aimed to investigate whether there was a change in the ET_B expression levels in reactive astrocytes during the progression of AD. **Method:** The alteration and distribution of reactive astrocytes, indicated by glial fibrillary acidic protein (GFAP) and ET_B in the lateral entorhinal cortex (LEC) and hippocampus CA1 region, were investigated by immunoperoxidase and immunofluorescence using a knock-in mouse model of AD ($APP^{NL-F/NL-F}$) that expresses a mutant form of human amyloid-β (Aβ) precursor protein, age-matched (12-16 months) to wild-type control mice. **Results:** ET_B was shown to be differentially expressed in the wild-type mice, age-matched to $APP^{NL-F/NL-F}$ mice. There was a proliferation of GFAP and ET_B in the LEC and CA1 region in the $APP^{NL-F/NL-F}$ mouse model at 12-16 months, compared with wild-type mice. Furthermore, confocal microscopy revealed a high degree of colocalization of ET_B expression in reactive astrocytes in the AD model compared to the wild-type control mice. **Conclusions:** Our data suggest a direct correlation of reactive astrocytes with ET_B that increased during the pathogenesis of AD astrocytes in cortical regions and could potentially be further investigated as a new drug target for treating AD by improving cerebral blood flow.

Pubmed:

30773830: Liu Y, Chen D, Li J, Xia D, Yu M, Tao J, Zhang X, Li L, Gan Y
NPC1L1-Targeted Cholesterol-Grafted Poly(β-Amino Ester)/pDNA Complexes for Oral Gene Delivery.
Gene vectors for oral delivery encounter harsh conditions throughout the gastrointestinal tract, and the continuous peristaltic activity can quickly remove the vectors, leading to inefficient intestinal permeation. Therefore, vectors have demanding property requirements, such as stability under various pH and, more importantly, efficient uptake in different intestinal segments. In this study, a functional polymer, cholesterol-grafted poly(β-amino ester) (poly[hexamethylene diacrylate-β-(5-amino-1-pentanol)] (CH-PHP)), is synthesized and electrostatically interacted with plasmid DNA to form a CH-PHP/DNA complex (CPNC). This complex is designed to target the Niemann-Pick C1-like receptor, a cholesterol receptor, to improve oral gene delivery efficacy. With the presence of cholesterol, CH-PHP shows mitigated cytotoxicity, enhanced enzyme resistance, and improved gene condensing ability. CPNC further contributes to ≈43.1- and 2.3-fold increases in luciferase expression in Caco-2 cells compared with PNC and Lipo 2000/DNA complexes, respectively. In addition, the in vivo transfection efficacy of CPNC is ≈4.1-, 2.1-, and 1.6-fold higher than that of Lipo 2000/DNA complexes in rat duodenum, jejunum, and ileum, respectively. Therefore, CPNC may be a promising delivery vector for gene delivery, and using a cholesterol-specific endocytic pathway can be a novel approach to achieve efficient oral gene transfection.
Adv Healthc Mater, 2019; 8
31671946: Nie D, Dai Z, Li J, Yang Y, Xi Z, Wang J, Zhang W, Qian K, Guo S, Zhu C, Wang R, Li Y, Yu M, Zhang X, Shi X, Gan Y
Cancer-Cell-Membrane-Coated Nanoparticles with a Yolk-Shell Structure Augment Cancer Chemotherapy.
Despite rapid advancements in antitumor drug delivery, insufficient intracellular transport and subcellular drug accumulation are still issues to be addressed. Cancer cell membrane (CCM)-camouflaged nanoparticles (NPs) have shown promising potential in tumor therapy due to their immune escape and homotypic binding capacities. However, their efficacy is still limited due to inefficient tumor penetration and compromised intracellular transportation. Herein, a yolk-shell NP with a mesoporous silica nanoparticle (MSN)-supported PEGylated liposome yolk and CCM coating, CCM@LM, was developed for chemotherapy and exhibited a homologous tumor-targeting effect. The yolk-shell structure endowed CCM@LM with moderate rigidity, which might contribute to the frequent transformation into an ellipsoidal shape during infiltration, leading to facilitated penetration throughout multicellular spheroids (up to a 23.3-fold increase compared to the penetration of

membrane vesicles). CCM@LM also exhibited a cellular invasion profile mimicking an enveloped virus invasion profile. CCM@LM was directly internalized by membrane fusion, and the PEGylated yolk (LM) was subsequently released into the cytosol, indicating the execution of an internalization pathway similar to that of an enveloped virus. The incoming PEGylated LM further underwent efficient trafficking throughout the cytoskeletal filament network, leading to enhanced perinuclear aggregation. Ultimately, CCM@LM, which co-encapsulated low-dose doxorubicin and the poly(ADP-ribose) polymerase inhibitor, mefuparib hydrochloride, exhibited a significantly stronger antitumor effect than the first-line chemotherapeutic drug Doxil. Our findings highlight that NPs that can undergo facilitated tumor penetration and robust intracellular trafficking have a promising future in cancer chemotherapy.
Nano Lett, 2020; 20

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BOARD NUMBER: S04-226

NEURON TO ASTROCYTE EXOCYTOTIC CROSS-TALK OR DO NEURONS CAN REGULATE ASTROCYTIC GLIOTRANSMISSION?

POSTER SESSION 04 - SECTION: ASTROCYTE FUNCTION AND DYSFUNCTION

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Cross-talk between neuronal connections and astrocytic processes was described by tripartite synapse concept which assumes that neurotransmission can be modulated by mechanisms of gliotransmitters secretion from astrocytes. A lot of controversies arose regarding the process of gliotransmission therefore the aim of our work is take a closer look on astrocytic exocytosis in the presence of neurons. To get an insight into the mechanisms controlling gliotransmission, we use Synaptobrevin2 fused with pH-dependent GFP (pHluorin) to observe astrocytic exocytosis in primary mixed hippocampal cultures as well as in pure astrocytic cultures. Our results indicate that the frequency of vesicular gliotransmission in astrocytes is higher in pure astrocytic cultures than in neuron/astrocyte co-cultures, where blocking neuronal transmission with TTX does not significantly reduce the rate of exocytosis. Nonetheless, we have shown that electrical stimulation of mixed culture increases the frequency of gliotransmitters release even when we block Ca^{2+} release from the endoplasmic reticulum using 2-APB and Ryanodine. Furthermore, activation of group I metabotropic glutamate receptors with DHPG significantly increases exocytosis rate but only in pure astrocytic culture. On the other hand, our results present that incubating cells with Ca^{2+} chelator (BAPTA) decreases the rate of vesicles released in both types of cultures justifying importance of extracellular Ca^{2+} in regulating process of astrocytic exocytosis. To summarize our results there is a correspondence between presence of neurons, Ca^{2+} signal localisation and the efficiency of gliotransmitters release in astrocytes. This study is supported by National Science Centre, Poland grant 2017/26/D/N23/01017.

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BOARD NUMBER: S04-227

EVOLUTION INCREASES STRESS-RESPONSE COMPLEXITY IN HIGHER PRIMATES EXTENDING RBFOX1 SPLICING ACTIVITY TO LSD1 MODULATION

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Recent branching (100 MYA) of the mammalian evolutionary tree has enhanced brain complexity and functions at the putative cost of increased emotional circuitry vulnerability. Thus, to better understand psychopathology, a burden for the modern society, novel approaches should exploit evolutionary aspects of psychiatric-relevant molecular pathways. A handful of genes is nowadays tightly associated to psychiatric disorders. Among them, neuronal-enriched RbFOX1 modifies the activity of synaptic regulators in response to neuronal activity, keeping excitability within healthy domains. Thanks to the combination of transcriptional approaches, minigene splicing assay and post-mortem human brain samples analyses, we dissected a higher primates-restricted interaction between RbFOX1 and the transcriptional corepressor Lysine Specific Demethylase 1 (LSD1/KDM1A). A single nucleotide variation (AA to AG) in LSD1 gene appeared in higher primates and humans, endowing RbFOX1 with the ability to promote the alternative usage of a novel 3' AG splice site, which extends LSD1 exon E9 in the upstream intron (E9-long). Exon E9-long regulates LSD1 levels by Nonsense-Mediated mRNA Decay. As reintroduction of the *archaic* LSD1 variant (AA) abolishes E9-long splicing, the novel 3' AG splice site is necessary for RbFOX1 to control LSD1 levels. LSD1 is a homeostatic Immediate Early Genes (IEGs) regulator playing a relevant part in environmental stress-response. In primates and humans, inclusion of LSD1 as RbFOX1 target provides RbFOX1 with the additional ability to regulate the IEGs. These data, together with extensive RbFOX1 involvement in psychiatric disorders and its stress-dependent regulation in male mice, suggest the RbFOX1-LSD1-IEGs axis as an evolutionary recent psychiatric-relevant pathway.

Pubmed:

33050350: Italia M, Forastieri C, Longaretti A, Battaglioli E, Rusconi F
Rationale, Relevance, and Limits of Stress-Induced Psychopathology in Rodents as Models for Psychiatry Research: An Introductory Overview.
Emotional and cognitive information processing represent higher-order brain functions. They require coordinated interaction of specialized brain areas via a complex spatial and temporal equilibrium among neuronal cell-autonomous, circuitry, and network mechanisms. The delicate balance can be corrupted by stressful experiences, increasing the risk of developing psychopathologies in vulnerable individuals. Neuropsychiatric disorders affect twenty percent of the western world population, but therapies are still not effective for some patients. Elusive knowledge of molecular pathomechanisms and scarcity of objective biomarkers in humans present complex challenges, while the adoption of rodent models helps to improve our understanding of disease correlate and aids the search for novel pharmacological targets. Stress administration represents a strategy to induce, trace, and modify molecular and behavioral endophenotypes of mood disorders in animals. However, a mouse or rat model will only display one or a few endophenotypes of a specific human psychopathology, which cannot be in any case recapitulated as a whole. To override this issue, shared criteria have been adopted to deconstruct neuropsychiatric disorders, i.e., depression, into specific behavioral aspects, and inherent neurobiological substrates, also recognizable in lower mammals. In this work, we provide a rationale for rodent models of stress administration. In particular, comparing each rodent model with a real-life human traumatic experience, we intend to suggest an introductive guide to better comprehend and interpret these paradigms.
Int J Mol Sci, 2020; 21
31364026: Gerosa L, Grillo B, Forastieri C, Longaretti A, Toffolo E, Mallei A, Bassani S, Popoli M, Battaglioli E, Rusconi F
SRF and SRFΔ5 Splicing Isoform Recruit Corepressor LSD1/KDM1A Modifying Structural Neuroplasticity and Environmental Stress Response.
Ten to 20% of western countries population suffers from major depression disorder (MDD). Stressful life events represent the main environmental risk factor contributing to the onset of MDD and other stress-related neuropsychiatric disorders. In this

regard, investigating brain physiology of stress response underlying the remarkable individual variability in terms of behavioral outcome may uncover stress-vulnerability pathways as a source of candidate targets for conceptually new antidepressant treatments. Serum response factor (SRF) has been addressed as a stress transducer via promoting inherent experience-induced Immediate Early Genes (IEGs) expression in neurons. However, in resting conditions, SRF also represents a transcriptional repressor able to assemble the core LSD1/CoREST/HDAC2 corepressor complex, including demethylase and deacetylase activities. We here show that dominant negative SRF splicing isoform lacking most part of the transactivation domain, namely SRFΔ5, owes its transcriptional repressive behavior to the ability of assembling LSD1/CoREST/HDAC2 corepressor complex meanwhile losing its affinity for transcription-permissive cofactor ELK1. SRFΔ5 is highly expressed in the brain and developmentally regulated. In the light of its activity as negative modulator of dendritic spine density, SRFΔ5 increase along with brain maturation suggests a role in synaptic pruning. Upon acute psychosocial stress, SRFΔ5 isoform transiently increases its levels. Remarkably, when stress is chronically repeated, a different picture occurs where SRF protein becomes stably upregulated in vulnerable mice but not in resilient animals. These data suggest a role for SRFΔ5 that is restricted to acute stress response, while positive modulation of SRF during chronic stress matches the criteria for stress-vulnerability hallmark.
Mol Neurobiol, 2020; 57

32141088: Longaretti A, Forastieri C, Gabaglio M, Rubino T, Battaglioli E, Rusconi F
Termination of acute stress response by the endocannabinoid system is regulated through lysine-specific demethylase 1-mediated transcriptional repression of 2-AG hydrolases ABHD6 and MAGL.
Acute environmental stress rarely implies long-lasting neurophysiological and behavioral alterations. On the contrary, chronic stress exerts a potent toxic effect at the glutamatergic synapse whose altered physiology has been recognized as a core trait of neuropsychiatric disorders. The endocannabinoid system (ECS) plays an important role in the homeostatic response to acute stress. In particular, stress induces synthesis of endocannabinoid (eCB) 2-arachidonyl glycerol (2-AG). 2-AG stimulates presynaptic cannabinoid 1 (CB1) receptor contributing to stress response termination through inhibition of glutamate release, restraining thereafter anxiety arousal. We employ mouse models of stress response coupled to gene expression analyses, unravelling that in response to acute psychosocial stress in the mouse hippocampus, ECS-mediated synaptic modulation is enhanced via transcriptional repression of two enzymes involved in 2-AG degradation: α/β-hydrolase domain containing 6 (ABHD6) and monoacylglycerol lipase (MAGL). Such a process is orchestrated by the epigenetic corepressor LSD1 who directly interacts with promoter regulatory regions of Abhd6 and Magl. Remarkably, negative transcriptional control of Abhd6 and Magl is lost in the hippocampus upon chronic psychosocial stress, possibly contributing to trauma-induced drift of synapse physiology toward uncontrolled glutamate transmission. We previously showed that in mice lysine-specific demethylase 1 (LSD1) increases its hippocampal expression in response to psychosocial stress preventing excessive consolidation of anxiety-related plasticity. In this work, we unravel a nodal epigenetic modulation of eCB turn over, shedding new light on the molecular substrate of converging stress-terminating effects displayed by ECS and LSD1.
J Neurochem, 2020; 155

33457471: Longaretti A, Forastieri C, Toffolo E, Caffino L, Locarno A, Misevičiūtė I, Marchesi E, Battistin M, Ponzoni L, Madaschi L, Cambria C, Bonasoni MP, Sala M, Perrone D, Fumagalli F, Bassani S, Antonucci F, Tonini R, Francolini M, Battaglioli E, Rusconi F
LSD1 is an environmental stress-sensitive negative modulator of the glutamatergic synapse.
Along with neuronal mechanisms devoted to memory consolidation -including long term potentiation of synaptic strength as prominent electrophysiological correlate, and inherent dendritic spines stabilization as structural counterpart- negative control of memory formation and synaptic plasticity has been described at the molecular and behavioral level. Within this work, we report a role for the epigenetic corepressor Lysine Specific Demethylase 1 (LSD1) as a negative neuroplastic factor whose stress-enhanced activity may participate in coping with adverse experiences. Constitutively increasing LSD1 activity via knocking out its dominant negative splicing isoform neuroLSD1 (neuroLSD1 mice), we observed extensive structural, functional and behavioral signs of excitatory decay, including disrupted memory consolidation. A similar LSD1 increase, obtained with acute antisense oligonucleotide-mediated neuroLSD1 splicing knock down in primary neuronal cultures, dampens spontaneous glutamatergic transmission, reducing mEPSCs. Remarkably, LSD1 physiological increase occurs in response to psychosocial stress-induced glutamatergic signaling. Since this mechanism entails neuroLSD1 splicing downregulation, we conclude that LSD1/neuroLSD1 ratio modulation in the hippocampus is instrumental to a negative homeostatic feedback, restraining glutamatergic neuroplasticity in response to glutamate. The active process of forgetting provides memories with salience. With our work, we propose that softening memory traces of adversities could further represent a stress-coping process in which LSD1/neuroLSD1 ratio modulation may help preserving healthy emotional references.
Neurobiol Stress, 2020; 13


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CBP IS REQUIRED FOR ESTABLISHING ADAPTIVE EXPERIENCE-INDUCED GENE PROGRAMS IN ADULT EXCITATORY NEURONS

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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The paralogous transcriptional co-activators and lysine acetyltransferases CBP and p300 have been involved in neuronal activity-dependent transcription and plasticity, but their specific roles in adult neurons are not fully understood. Here we investigated the impact in transcription, chromatin acetylation and behavior of eliminating either one of these proteins in excitatory neurons of the adult forebrain. We used inducible forebrain-specific knockout mice for CBP or p300 and studied the role of each one in normal and challenging tasks paradigms. With this aim, we analyzed the effect on the transcriptome and epigenome and studied behavioral response differences in the models. The elimination of CBP, reduced the expression of plasticity genes, dampened activity-driven transcription, and caused memory deficits in different tasks that rely on spontaneous exploratory behavior. In contrary, p300 did not present any abnormality. The importance of CBP became more prominent in paradigms that involved a chronic or recurrent change in transcription, including kindling and neuroadaptation to environmental enrichment, in which CBP loss interfered with the establishment of activity-induced transcriptional and epigenetic changes in response to a stimulus or experience. CBP is required for experience-driven gene programs in adult excitatory neurons. Moreover, comparative analyses in mice lacking the paralog p300 underscored the specificity of CBP function. These new findings on the epigenetic regulation of plasticity-related gene programs by CBP contribute to clarify the strong links between CBP deficiency and the etiology of different brain disorders.

Pubmed:
29452236: Guitart-Mampel M, Gonzalez-Tendero A, Niñerola S, Morén C, Catalán-Garcia M, González-Casacuberta I, Juárez-Flores DL, Ugarteburu O, Matalonga L, Cascajo MV, Tort F, Cortés A, Tobias E, Milisenda JC, Grau JM, Crispi F, Gratacós E, Garrabou G, Cardellach F
Cardiac and placental mitochondrial characterization in a rabbit model of intrauterine growth restriction.
Intrauterine growth restriction (IUGR) is associated with cardiovascular remodeling persisting into adulthood. Mitochondrial bioenergetics, essential for embryonic development and cardiovascular function, are regulated by nuclear effectors as sirtuins. A rabbit model of IUGR and cardiovascular remodeling was generated, in which heart mitochondrial alterations were observed by microscopic and transcriptomic analysis. We aimed to evaluate if such alterations are translated at a functional mitochondrial level to establish the etiopathology and potential therapeutic targets for this obstetric complication.
Biochim Biophys Acta Gen Subj, 2018; 1862
34787081: Conde-Dusman MJ, Dey PN, Elía-Zudaire Ó, Rabaneda LG, García-Lira C, Grand T, Briz V, Velasco ER, Andero R, Niñerola S, Barco A, Paoletti P, Wesseling JF, Gardoni F, Tavalin SJ, Perez-Otaño I
Control of protein synthesis and memory by GluN3A-NMDA receptors through inhibition of GIT1/mTORC1 assembly.
De novo protein synthesis is required for synapse modifications underlying stable memory encoding. Yet neurons are highly compartmentalized cells and how protein synthesis can be regulated at the synapse level is unknown. Here, we characterize neuronal signaling complexes formed by the postsynaptic scaffold GIT1, the mechanistic target of rapamycin (mTOR) kinase, and Raptor that couple synaptic stimuli to mTOR-dependent protein synthesis; and identify NMDA receptors containing GluN3A subunits as key negative regulators of GIT1 binding to mTOR. Disruption of GIT1/mTOR complexes by enhancing GluN3A expression or silencing GIT1 inhibits synaptic mTOR activation and restricts the mTOR-dependent translation of specific activity-regulated mRNAs. Conversely, GluN3A removal enables complex formation, potentiates mTOR-dependent protein synthesis, and facilitates the consolidation of associative and spatial memories in mice. The memory enhancement becomes evident with light or spaced training, can be achieved by selectively deleting GluN3A from excitatory neurons during adulthood, and does not compromise other aspects of cognition such as memory flexibility or extinction. Our findings provide

mechanistic insight into synaptic translational control and reveal a potentially selective target for cognitive enhancement. Elife, 2021; 10
28571526: López-González MJ, Luis E, Fajardo O, Meseguer V, Gers-Barlag K, Niñerola S, Viana F
TRPA1 Channels Mediate Human Gingival Fibroblast Response to Phenytoin.
Drug-induced gingival enlargement (GE) is a frequent adverse effect observed in patients treated with anticonvulsant, immunosuppressant, and some antihypertensive medications-the antiepileptic phenytoin being the main drug associated with GE due to its high incidence (around 50%). The molecular mechanisms behind drug-induced gingival overgrowth are still unknown. By reverse transcription polymerase chain reaction, we demonstrate that the calcium-permeable ion channels TRPA1, TRPV1, and its capsaicin-insensitive isoform TRPV1b are expressed in human gingival fibroblasts (HGFs), the most abundant cellular type in periodontal tissue. Cultured HGFs responded with intracellular calcium elevations to phenytoin and to the canonical TRPA1 agonist allyl isothiocyanate. Application of phenytoin activated a nonselective cationic current in HGFs with a typical signature for TRPA1 channels. Moreover, this activation was blocked by HC030031, a specific TRPA1 blocker. Similarly, the use of shRNAs against hTRPA1 in HGFs reduced TRPA1 expression and activation by phenytoin. In addition, we show that phenytoin increased intracellular calcium levels in cells transfected with mouse or human TRPA1 channels. Responses to phenytoin were not observed in untransfected cells or cells expressing TRPM8 or TRPV1. The activation of HGFs by phenytoin was markedly reduced in the presence of antioxidant vitamins: ascorbic acid, folic acid, and α-tocopherol. By performing cell proliferation assays, we found that phenytoin did not augment the proliferation rate of HGFs. In contrast, alcian blue and picrosirius red staining of long-term HGFs cultures indicated that phenytoin induces extracellular matrix accumulation of collagen. Collectively, these findings support an important role of TRPA1 channels in phenytoin-induced GE, provide insight into the pathophysiologic mechanism, and offer novel therapeutic opportunities for its treatment. J Dent Res, 2017; 96

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CELL-TYPE SPECIFIC CHROMATIN PROFILING OF HUMAN MDD DISEASE SIGNATURE IDENTIFIES NOVEL EPIGENETIC MECHANISMS OF ASTROCYTE PLASTICITY DRIVING BIDIRECTIONAL STRESS RESPONSE

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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The overall risk for developing major depressive disorder (MDD) is determined by a complex interaction between heritable genetic variants and adverse experiences such as stress exposure. Mechanistic understanding of this gene-environment interface requires characterization of epigenomic processes within the unique regulatory context of complex human disease-relevant cell types, but to date this approach has not been applied to MDD. Although relatively understudied in MDD, the orbitofrontal cortex (OFC) integrates homeostatic states, emotion, and reward-stimuli to flexibly guide motivated behavior. Functional imaging studies consistently find significant OFC dysfunction in MDD patients, however, the molecular and cellular substrates underlying these neuroplastic changes in the OFC are not well understood. Here, we use cell population-specific chromatin accessibility profiling to capture the chromatin regulatory signature in MDD orbitofrontal cortex (OFC). We mapped genetic risk for MDD to open chromatin regions (OCRs) in non-neuronal OFC cell-types. Characterization of MDD-specific OCRs revealed a key role for the chromatin remodeling protein ZBTB7A, which facilitates transcription of inflammation genes, and is enriched in astrocytes, though its role in neuropsychiatric disease is unknown. In a series of astrocyte-specific bidirectional viral manipulation studies in preclinical mouse models, we further show that ZBTB7A chromatin remodeling activity specifically in OFC astrocytes is both necessary and sufficient to alter behavioral, transcriptional, and neuronal activity responses to stress. Together, these data demonstrate that epigenetic regulation of inflammatory signaling in astrocytes mediates both maladaptive and neuroprotective responses to stress, with direct implications for the central role of astrocyte plasticity in OFC dysfunction and MDD pathology.

Pubmed:
32273471: Lepack AE, Werner CT, Stewart AF, Fulton SL, Zhong P, Farrelly LA, Smith ACW, Ramakrishnan A, Lyu Y, Bastle RM, Martin JA, Mitra S, O'Connor RM, Wang ZJ, Molina H, Turecki G, Shen L, Yan Z, Calipari ES, Dietz DM, Kenny PJ, Maze I
Dopaminylation of histone H3 in ventral tegmental area regulates cocaine seeking.
Vulnerability to relapse during periods of attempted abstinence from cocaine use is hypothesized to result from the rewiring of brain reward circuitries, particularly ventral tegmental area (VTA) dopamine neurons. How cocaine exposures act on midbrain dopamine neurons to precipitate addiction-relevant changes in gene expression is unclear. We found that histone H3 glutamine 5 dopaminylation (H3Q5dop) plays a critical role in cocaine-induced transcriptional plasticity in the midbrain. Rats undergoing withdrawal from cocaine showed an accumulation of H3Q5dop in the VTA. By reducing H3Q5dop in the VTA during withdrawal, we reversed cocaine-mediated gene expression changes, attenuated dopamine release in the nucleus accumbens, and reduced cocaine-seeking behavior. These findings establish a neurotransmission-independent role for nuclear dopamine in relapse-related transcriptional plasticity in the VTA.
Science, 2020; 368
35094023: Fulton SL, Mitra S, Lepack AE, Martin JA, Stewart AF, Converse J, Hochstetler M, Dietz DM, Maze I
Histone H3 dopaminylation in ventral tegmental area underlies heroin-induced transcriptional and behavioral plasticity in male rats.
Persistent transcriptional events in ventral tegmental area (VTA) and other reward relevant brain regions contribute to enduring behavioral adaptations that characterize substance use disorder. Recent data from our laboratory indicate that aberrant accumulation of the newly discovered histone post-translational modification (PTM), H3 dopaminylation at glutamine 5 (H3Q5dop), contributes significantly to cocaine-seeking behavior following prolonged periods of abstinence. It remained unclear, however, whether this modification is important for relapse vulnerability in the context of other drugs of abuse, such

as opioids. Here, we showed that H3Q5dop plays a critical role in heroin-mediated transcriptional plasticity in midbrain regions, particularly the VTA. In rats undergoing abstinence from heroin self-administration (SA), we found acute and persistent accumulation of H3Q5dop in VTA. Attenuation of H3Q5dop during abstinence induced persistent changes in gene expression programs associated with neuronal signaling and dopaminergic function in heroin abstinence and led to reduced heroin-seeking behavior. Interestingly, the observed changes in molecular pathways after heroin SA showed significant yet reversed overlap with the same genes altered in cocaine SA. These findings establish an essential role for H3Q5dop, and its downstream transcriptional consequences, in heroin-induced functional plasticity in VTA.
Neuropsychopharmacology, 2022;
32513670: Stewart AF, Fulton SL, Maze I
Epigenetics of Drug Addiction.
Substance use disorders (SUDs) are chronic brain diseases characterized by transitions from recreational to compulsive drug use and aberrant drug craving that persists for months to years after abstinence is achieved. The transition to compulsive drug use implies that plasticity is occurring, altering the physiology of the brain to precipitate addicted states. Epigenetic phenomena represent a varied orchestra of transcriptional tuning mechanisms that, in response to environmental stimuli, create and maintain gene expression-mediated physiological outcomes. Therefore, epigenetic mechanisms represent a convergent regulatory framework through which the plasticity required to achieve an addicted state can arise and then persist long after drug use has ended. In the first section, we will introduce basic concepts in epigenetics, such as chromatin architecture, histones and their posttranslational modifications, DNA methylation, noncoding RNAs, and transcription factors, along with methods for their investigation. We will then examine the implications of these mechanisms in SUDs, with a particular focus on cocaine-mediated neuroepigenetic plasticity across multiple behavioral models of addiction.
Cold Spring Harb Perspect Med, 2021; 11
31628598: Fulton SL, Maze I
Translational Molecular Approaches in Substance Abuse Research.
Excessive abuse of psychoactive substances is one of the leading contributors to morbidity and mortality worldwide. In this book chapter, we review translational research strategies that are applied in the pursuit of new and more effective therapeutics for substance use disorder (SUD). The complex, multidimensional nature of psychiatric disorders like SUD presents difficult challenges to investigators. While animal models are critical for outlining the mechanistic relationships between defined behaviors and genetic and/or molecular changes, the heterogeneous pathophysiology of brain diseases is uniquely human, necessitating the use of human studies and translational research schemes. Translational research describes a cross-species approach in which findings from human patient-based data can be used to guide molecular genetic investigations in preclinical animal models in order to delineate the mechanisms of reward circuitry changes in the addicted state. Results from animal studies can then inform clinical investigations toward the development of novel treatments for SUD. Here we describe the strategies that are used to identify and functionally validate genetic variants in the human genome which may contribute to increased risk for SUD, starting from early candidate gene approaches to more recent genome-wide association studies. We will next examine studies aimed at understanding how transcriptional and epigenetic dysregulation in SUD can persistently alter cellular function in the disease state. In our discussion, we then focus on examples from the literature illustrating molecular genetic methodologies that have been applied to studies of different substances of abuse - from alcohol and nicotine to stimulants and opioids - in order to exemplify how these approaches can both delineate the underlying molecular systems driving drug addiction and provide insights into the genetic basis of SUD.
Handb Exp Pharmacol, 2020; 258

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CONTRIBUTION OF C-FOS EXPRESSION IN THE ARCUATE NUCLEUS TO THE DEVELOPMENT OF OBESITY IN MIRNA-DEFICIENT MICE

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

Joanna Przybys, Ali Jawaid, Witold Konopka
Nencki Institute of Experimental Biology, PAS, Braincity, Warsaw, Poland

Conditional/inducible miRNAs deletion from adult forebrain neurons in mice lacking the *Dicer1* gene (Dicer cKO) leads to transient hyperphagia and obesity. The arcuate nucleus (Arc) of the hypothalamus plays a key role in regulation of hunger and satiety via its AgRP and POMC neurons respectively. Here, we show that miRNAs and immediate early gene (IEG) c-*Fos* are intricately involved in this regulation. Fasting of wild-type mice for 24 hours induces a wide-spread expression of *c-Fos* in the Arc indicating its relevance to the activity of Arc. However, contrary to the usual rapid turnover properties of IEGs, c-*Fos* exhibits a strong and persistent expression throughout the fasting period indicating its sensitivity to the state of hunger in the study. To investigate the identity of *c-Fos* as an IEG, we performed optogenetic experiment in well-fed mice. Optogenetic activation of the AgRP neurons in the Arc induced feeding along with increased expression of c-*Fos* that returned to baseline after canonical 6 hours. Furthermore, we detected upregulated c-Fos in the resting phase in hyperphagic Dicer cKO mice. Thus, dysregulation of c-Fos in the Arc could underlie the hyperphagia and resultant obesity in Dicer cKO mice. Overall, our findings indicate an intimate association between miRNAs and the *c-Fos* gene in regulating the activity/plasticity of neurons involved in controlling the body's energy balance. Our ongoing investigations focus on silencing c-Fos in Dicer cKO mice to fully establish its role in mediating the effects of deficient miRNA biogenesis in the Arc on feeding behaviors and whole body metabolism in mice.

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REGION-SPECIFIC MICRORNA ALTERATIONS IN MARMOSETS CARRYING SLC6A4 POLYMORPHISMS ARE ASSOCIATED WITH ANXIETY-LIKE BEHAVIOR

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Psychiatric diseases such as depression and anxiety are multifactorial conditions, highly prevalent in western societies. Human studies have identified a number of high-risk genetic variants for these diseases. Among them, polymorphisms in the promoter region of the serotonin transporter gene (SLC6A4) have attracted much attention. However, due to the paucity of experimental models, molecular alterations induced by these genetic variants and how they correlate to behavioral deficits have not been examined. Marmosets have emerged as a powerful model in translational neuroscience to investigate molecular underpinnings of complex behaviors. Here, we took advantage of naturally occurring genetic polymorphisms in marmoset *SLC6A4* gene that have been linked to anxiety-like behaviors. Using FACS-sorted cells from different brain regions, we revealed that marmosets bearing different *SLC6A4* variants exhibit distinct microRNAs signatures in a region of the prefrontal cortex whose activity has been consistently altered in patients with depression/anxiety. We also identified DCC, a gene previously linked to these diseases, as a downstream target of the dysregulated microRNAs. Significantly, we showed that levels of both microRNAs and DCC in this region were highly correlated to anxiety-like behaviors as well as to the response to citalopram, a selective serotonin re-uptake inhibitor and widely prescribed anti-depressant. Our findings establish links between genetic variants, molecular modifications in specific cortical regions and complex behavioral/pharmacological responses, providing new insights into gene-behavior relationships underlying human psychopathology.

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OPTIMIZED REPORTERS UNCOVER DIFFERENCES IN MIR-124 EXPRESSION AMONG NEURONAL POPULATIONS IN THE BRAIN

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Although intensively studied in the last decades, how microRNAs are expressed across different cell types in the brain remains largely unknown. To address this issue, we sought to develop optimized fluorescence reporters that could be expressed in precise cellular subsets and used to accurately quantify miR contents in vivo. Focusing on miR-124, we tested different reporter designs whose efficiency was initially confirmed in vitro. Then, we packed them into AAV vectors and test them in cultured neurons as well in different in vivo settings. Unlike previous reporters, we provide experimental evidence that our optimized designs can faithfully translate miR levels in vitro and in vivo. More importantly, we show that these reporters can be used not only for mapping miRNA expression but also for the prospective identification in vivo of different neuronal subsets. Together, such tools would enable assessing miRNA expression at the single cell resolution and are expected to significantly contribute to future miRNA research.

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INDIVIDUAL DIFFERENCES IN DNA METHYLATION ASSOCIATED WITH SENSITIVITY AND RESISTANCE TO THE EXTINCTION OF COCAINE MEMORIES

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Cocaine addiction is a chronic condition in which craving and drug seeking can be elicited by drug-associated cues. In rodents, this can be modeled using conditioned place preference (CPP) wherein animals acquire a preference for a chamber where they receive cocaine. Once place preference is acquired, the drug-associated memory can be extinguished by repeated exposure to the previously cocaine-paired environment. However, some mice fail to extinguish, continuing to prefer the previously cocaine-paired side. Animals that fail to extinguish the previously drug-associated memory could provide insight into individual differences in vulnerability to drug dependence. To identify a possible underlying epigenetic mechanism, we analyzed the DNA methylomes of the dorsal dentate gyrus of Extinction and Failed Extinction mice. These animals showed differentially methylated regions (DMRs; genomic regions with significantly different methylation between groups). Genome-wide methylation analysis showed that Intermediate Methylated Regions (IMRs; methylation between 15-85%) were hypo- or hyper-methylated after successfully extinguishing the drug-paired memory. An Ingenuity Pathway Analysis of extinction DMRs showed enrichment for genes within an activity-dependent calcium and glutamate pathway. One differentially methylated gene in this pathway is *cacna1c*, which encodes the $Ca_v1.2$ isoform of the L-type voltage-gated calcium channel, a gene implicated in drug dependence. Supporting the role of this methylation data, there was an increase in *cacna1c* mRNA and protein only in animals with successful extinction. Taken together, this preliminary work suggests differences in DNA methylation within the dorsal dentate gyrus could dictate underlying differences that lead to a propensity to develop drug dependence.

Pubmed:

33600828: Baker MR, Sciortino RK, So VM, Romeo RD
Prepubertal and adult male rats differ in the degree and pattern of stress reactive neurons in brain regions that project to the paraventricular nucleus of the hypothalamus.
The hormonal stress response, mediated by the hypothalamic-pituitary-adrenal (HPA) axis, shows greater responsiveness to various stressors in prepubertal compared to adult animals. Though the implications of this age-related change are unclear, this heightened reactivity might contribute to the increase in stress-related dysfunctions observed during adolescence. Interestingly, prepubertal animals show greater stress-induced neural activation compared to adults in the paraventricular nucleus of the hypothalamus (PVN), the area responsible for initiating the hormonal stress response. Thus, it is possible that direct afferents to the PVN, such as the anterior bed nucleus of the stria terminalis (aBST), nucleus of the solitary tract (NTS), posterior BST (pBST), medial preoptic area (MPOA), and dorsomedial nucleus (DMN), contribute to this age-dependent change in reactivity. To investigate these possibilities, two separate experiments were conducted in prepubertal (30 days old) and adult (70 days old) male rats using the retrograde tracer, Fluoro-Gold (FG), and FOS immunohistochemistry to study neural connectivity and activation, respectively. Though there was no difference in the number or size of FG-positive cells in the PVN afferents we examined, we found a significantly greater number of stress-induced FOS-like-positive cells in the aBST and significantly fewer in the DMN in prepubertal compared to adult animals. Together these data suggest that functional, instead of structural, changes in nuclei that project to the PVN may lead to the greater PVN stress responsiveness observed prior to adolescence. Furthermore, these data indicate that nuclei known to directly modulate HPA stress responsiveness show differential activation patterns before and after adolescent development.
Brain Res, 2021; 1760
28435085: Pham L, Baker MR, Shahanoor Z, Romeo RD
Adolescent changes in hindbrain noradrenergic A2 neurons in male rats.
During adolescence, the increased susceptibility to stress-related dysfunctions (e.g., anxiety, drug use, obesity) may be

influenced by changes in the hormonal stress response mediated by the hypothalamic-pituitary-adrenal (HPA) axis. We have previously reported that restraint stress leads to significantly prolonged HPA responses in pre-adolescent compared to adult rats. Further, pre-adolescent animals exposed to restraint show greater levels of neural activation than adults in the paraventricular nucleus of the hypothalamus (PVN), a key nucleus integrating information from brain regions that coordinate HPA responses. Here, we examined the potential contribution of the noradrenergic A2 region of the nucleus of the solitary tract (NST) as a contributor to these age-dependent shifts in HPA reactivity. Specifically, we used double-labeled immunohistochemistry for FOS and dopamine-β-hydroxylase (DβH) to measure cellular activation and noradrenergic cells, respectively, before or after restraint stress in pre-adolescent (30days old) and adult (70days old) male rats. We also measured the density of DβH-immunoreactive fibers in the PVN as an index of noradrenergic inputs to this area. We found that pre-adolescent animals have a greater number of DβH-positive cells in the A2 region compared to adults, yet the number and percentage of double-labeled DβH/FOS cells were similar between these two ages. We found no differences between the ages in the staining intensity of DβH-immunoreactive fibers in the PVN. These data indicate there are adolescent-related changes in the number of noradrenergic cells in the A2 region, but no clear association between the increased stress reactivity prior to pubertal maturation and activation of A2 noradrenergic afferents to the PVN.
Brain Res, 2017; 1666

29020649: Shahanoor Z, Sultana R, Baker MR, Romeo RD

Neuroendocrine stress reactivity of male C57BL/6N mice following chronic oral corticosterone exposure during adulthood or adolescence.

Adolescence is associated with the maturation of the hypothalamic-pituitary-adrenal (HPA) axis, the major neuroendocrine axis mediating the hormonal stress response. Adolescence is also a period in development marked by a variety of stress-related vulnerabilities, including psychological and physiological dysfunctions. Many of these vulnerabilities are accompanied by a disrupted HPA axis. In adult mice, a model of disrupted HPA function has been developed using oral chronic corticosterone administration via the drinking water, which results in various physiological and neurobehavioral abnormalities, including changes in stress reactivity and anxiety-like behaviors. In an effort to further complement and extend this model, we tested the impact of HPA disruption in adolescent mice. We also examined whether this disruption led to different outcomes depending on whether the treatment happened during adolescence or adulthood. In the current set of experiments, we exposed adult (70days of age) or adolescent (30days of age) male C57BL/6N mice to 4 weeks of either 0 or 25µg/ml oral corticosterone via their drinking water. We measured body weight during treatment and plasma corticosterone levels and activation of the paraventricular nucleus (PVN), as indexed by FOS immunohistochemistry, before and after a 30min session of restraint stress. Our data indicate that adolescent animals exposed to chronic corticosterone showed weight loss during treatment, an effect not observed in adults. Further, we found stress failed to elevate plasma corticosterone levels in treated mice, regardless of whether exposure occurred in adulthood or adolescence. Despite this reduced hormonal responsiveness, we found significant neural activation in the PVN of both adult- and adolescent-treated mice, indicating a dissociation between stress-induced peripheral and central stress responses following chronic corticosterone exposure. Moreover, stress-induced neural activation in the PVN was unaffected by chronic corticosterone treatment in adult animals, but led to a hyper-responsive PVN in the corticosterone-treated adolescent animals, suggesting an age-specific effect of corticosterone treatment on later PVN stress reactivity. Together, these experiments highlight the influence of developmental stage on somatic and neuroendocrine outcomes following chronic HPA disruption by noninvasive, oral corticosterone treatment. Given the substantial vulnerabilities to HPA dysfunctions during adolescence this model may prove useful in better understanding these vulnerabilities.
Psychoneuroendocrinology, 2017; 86

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A BIOPHYSICAL MECHANISM FOR EPIGENETIC INHERITANCE OF ENHANCED COMPLEX LEARNING CAPABILITIES

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Rule learning is mediated by enhanced intrinsic neuronal excitability throughout the neuronal population of the relevant brain areas,which results from decreased conductance of a calcium-dependent potassium current(s), which is induced by metabotropic activation of the GluK2 subtype glutamatergic receptor, mediated by well identified second messenger systems. Here we show that rats trained in a complex olfactory-discrimination pass on trans-generationally to their offspring superb learning capabilities. Such inheritance is also evident when only one of the parents is trained, if fostered by non-trained females and if the F2 or F3 generation is trained,without any training of the F1 generation.Notably, offspring excel also in other, novel tasks. At the cellular level, intracellular neurophysiological recordings show that the intrinsic excitability of CA1 pyramidal neurons of trained rat`s offspring is higher compared to control, as result of reduction in the slow potassium current(s), the same change induced in the brains of the F0 rats after they acquire the rule. Thus, offspring are superb learners since they are born with neurons that show the same biophysical change induced in parents' brains by rule learning. Analysis of mRNA expression levels show that the hippocampi of trained offspring differs from the controls offspring hippocampi in more than 500 genes. In particular,we found downregulation of genes that code for channels that suppress intrinsic excitability, as well as downregulation of genes that code for synaptic receptors. We speculate that these changes create a favorable set point for future increased plasticity, thereby granting trained rats' offspring with superb learning capabilities.

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SIRTUINS MODULATORS COUNTERACT MITOCHONDRIAL DYSFUNCTION IN CHEMICAL AND NEONATAL HYPOXIA: IMPLICATION TO NEURODEVELOPMENTAL DISORDERS

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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The hypoxia is an environmental factor correlated with complications in neurodevelopment because of mitochondrial dysfunction. Once sirtuins are involved in the regulation of cellular metabolism we investigated whether sirtuins modulators could be a neuroprotective strategy against hypoxia. We used primary neurons of Wistar (control), and Spontaneous Hypertensive Rats (model of neonatal hypoxia and schizophrenia) exposed to chemical hypoxia ($CoCl_2$). The cells were treated with $CoCl_2$ (800 µM and 2 mM) for 24h previously and simultaneously stimulated with Nicotinamide (50 µM), Resveratrol (0.5 µM) and Sirtinol (5 µM) (48h). We measured: calcium levels with Fluo-4-AM (10 µM), mitochondrial membrane potential with TMRE (500 nM), and redox homeostasis. Moreover, genes related to mitochondrial metabolism, dynamics, and biogenesis, as well high-energy compounds levels were investigated. The chemical and neonatal hypoxia induced mitochondrial depolarization, disruption in Ca^{2+} handling, and raising in ROS, *Nfe2l2* and mitochondrial biogenesis and fission gene expression. Hypoxia also induced a decrease in ATP levels and an increase in pyruvate and lactate levels. The previous exposure to all sirtuins modulators reduced ROS, boosted *Nfe2l2* expression, and increased mitochondrial fusion genes expression and pyruvate levels. Altogether, our results suggest that sirtuins modulators could be used as a neuroprotective strategy. These data contribute to a better comprehension of hypoxia effects in neuronal function, adding new bricks to mitochondrial function knowledge and its relation to neurodevelopmental disorders. 2022 - 2018/13814-0 - Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Pubmed:

31792231: E Silva LFS, Brito MD, Yuzawa JMC, Rosenstock TR
Mitochondrial Dysfunction and Changes in High-Energy Compounds in Different Cellular Models Associated to Hypoxia: Implication to Schizophrenia.
Schizophrenia (SZ) is a multifactorial mental disorder, which has been associated with a number of environmental factors, such as hypoxia. Considering that numerous neural mechanisms depends on energetic supply (ATP synthesis), the maintenance of mitochondrial metabolism is essential to keep cellular balance and survival. Therefore, in the present work, we evaluated functional parameters related to mitochondrial function, namely calcium levels, mitochondrial membrane potential, redox homeostasis, high-energy compounds levels and oxygen consumption, in astrocytes from control (Wistar) and Spontaneously Hypertensive Rats (SHR) animals exposed both to chemical and gaseous hypoxia. We show that astrocytes after hypoxia presented depolarized mitochondria, disturbances in Ca handling, destabilization in redox system and alterations in ATP, ADP, Pyruvate and Lactate levels, in addition to modification in NAD/NADH ratio, and Nfe2l2 and Nrf1 expression. Interestingly, intrauterine hypoxia also induced augmentation in mitochondrial biogenesis and content. Altogether, our data suggest that hypoxia can induce mitochondrial deregulation and a decrease in energy metabolism in the most prevalent cell type in the brain, astrocytes. Since SHR are also considered an animal model of SZ, our results can likewise be related to their phenotypic alterations and, therefore, our work also allow an increase in the knowledge of this burdensome disorder.
Sci Rep, 2019; 9

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DECIPHERING THE ROLE OF A NON-NEURONAL LNCRNA IN AGE-ASSOCIATED CONGNITIVE DISEASES

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Long non-coding RNAs (lncRNAs) are emerging as important regulators of neuronal plasticity and have recently been linked to the onset and progression of neurodegenerative diseases. However, our knowledge about the role of CNS-specific lncRNAs in neuronal and non-neuronal cells is still limited. The aim of this project was to identify cell type specific expression changes of non-neuronal lncRNAs in the context of age-associated neurodegenerative diseases and to characterize their function. To this end we performed an RNA sequencing-based screening in the hippocampus of young and cognitively impaired aged mice. We identified multiple candidate lncRNAs for further analysis and studied their role in the relevant cell types via gain and loss of function approaches. Our data reveals that the candidate lncRNAs regulate several key cellular pathways and could serve as biomarker for cognitive decline and therapeutic interventions via RNA-based medicines.

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INFLUENCE OF THE APOLIPOPROTEIN E4 ALLELE (APOE4) ON ASTROCYTIC AND NEURONAL ACETYLATION LANDSCAPES IN PHYSIOLOGICAL AND α-SYNUCLEIN-INDUCED PATHOLOGICAL CONTEXTS

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Aim: The ApoE4 gene has been established as the strongest genetic risk factor for Alzheimer's disease and Dementia with Lewy bodies (DLB). However, the mechanisms by which ApoE4 contributes to aetipathogenesis of either of these diseases remain unclear. ApoE is the major lipoprotein in the CNS, predominately produced by astrocytes, playing an important role in transport and neuronal uptake of cholesterol. We hypothesized that the presence of its e4 allelic variant could impact cellular epigenetic regulations, hence modifying cellular functions. To that aim, we established neuronal and astrocytic acetylation-related epigenomes of ApoE4 and ApoE3 transgenic models with or without inclusion of pathological α-synuclein fibrils – causative agents of DLB neuropathology. **Methods:** The genome-wide epigenomic distribution of two acetylated histone marks (H3K27ac and H3K9ac) was assessed by CUT&Tag followed by deep sequencing, on magnetically isolated hippocampal neurons and astrocytes from ApoE4 and ApoE3 (control) KI murine models. Same methodology was conducted on the aforementioned models 2 months following hippocampal stereotaxic injections of α-synuclein preformed fibrils (PFF) or PBS (control). **Results:** Epigenomic data revealed deacetylation of astrocytic and hyperacetylation of neuronal epigenomic landscapes with matrisome and lipid metabolism pathways as most dysregulated solely under the ApoE4 influence. Introduction of PFFs displayed additional upregulation of neuronal inflammatory pathways regardless of the ApoE genotype, while genes comprising phosphorylation processes and synapse-related functions were found specifically dysregulated by the e4 allelic variant of ApoE. **Conclusion:** Overall, our findings provide differences in cell-type specific epigenetic profiles influenced by either the ApoE4 risk factor and/or α-synuclein PFFs.

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UNRAVELLING MOLECULAR EPIGENETIC MECHANISMS IN HUMAN BRAIN DEVELOPMENT

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Allowing for a dynamic expression of genes, epigenetic regulation plays a key role in human brain development. Thus, identifying mechanisms of epigenetic regulation has an immense importance in developmental neurobiology, where conservation of gene expression patterns is crucial. Dysregulation of the epigenome can lead to devastating consequences by altering the cell's expression profile, both during embryonic and adult neurogenesis. In particular, the aberrant expression of transposable elements (TEs) has been shown to cause neuroinflammation. TEs have contributed to human evolution by acting as non-classical transcription regulators and gene promoters, displaying their ability to cause neuronal variations. This suggests that proper epigenetic regulation of TEs is necessary for healthy brain development. This project investigates the contributions and the mechanistic model of the human silencing hub (HUSH complex) in the regulation of TEs in human neural stem cells. Differential expression analyses was employed to compare control and CRISPRi cells, revealing an upregulation of 295 individual elements (p-adj < 0.05; LFC >=1). Furthermore, analysis of the histone methylation patterns supported the model of HUSH regulating H3K9me3 over its targets, supporting the importance of HUSH in neurogenesis. Future outlooks of this project involve expanding similar strategies to general cell regulators that also control TE activity such as DNMT1, DNMT3A/B and SETDB1. With this project we aim to reach greater understanding of the connection between cell regulators and TEs in human brain development

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DYSREGULATION OF THE CHOLESTEROL PATHWAY ALTERS EPIGENOMIC SIGNATURES OF HIPPOCAMPAL NEURONS IN ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Objectives: Alzheimer's disease (AD), the most common form of dementia, is characterized by the presence of β-amyloid plaques and neurofibrillary tangles (containing hyperphosphorylated Tau protein). Aging is suggested to be the main AD risk factor. Dysregulated epigenetic mechanisms such as histone acetylation have been associated with both aging and AD. However, whether/how aging-deregulated events contribute to AD is unknown. We aimed to establish epigenetic/transcriptomic signatures in physiological and pathological AD/Tau aging. **Methods:** We investigated the marker of active transcription H3K27ac at genome-wide level (ChIP-seq) and transcriptomics (RNA-seq) in the hippocampus of 12-month-old THY-Tau22 mice (tauopathy) as well as 18-*versus* 3-month-old WT mice (aging). **Results:** Epigenomic data revealed opposite H3K27ac enrichments between aging and tauopathy: synaptic transmission and glutamate receptor genes were found enriched in tauopathic hippocampi whereas these regions were depleted in aged ones. At transcriptomic level, while aging and tauopathy shared a common inflammatory signature, cholesterol metabolism-genes were found exclusively decreased in tauopathic mice. Excitingly, epigenetic treatment using the histone acetyltransferase activator CSP-TTK21 restored recognition memory in tauopathic mice. Additionally, this treatment rescued the expression of cholesterol metabolism genes and proper epigenomic signatures, suggesting a link between these two events. **Conclusion:** Overall, our findings suggest that tauopathy is not an exacerbation of the epigenetic drift observed during the aging process but rather an epigenetic/histone acetylation upsurge resulting from the dysregulation of the cholesterol pathway. Further, CSP-TTK21 revealed to be a promising new "epidrug" to counteract memory impairment, through modulation of brain cholesterol biosynthesis, in the pathological condition of AD.

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BOARD NUMBER: S04-240

EFFECTS OF ADOLESCENT ETHANOL EXPOSURE ON HIPPOCAMPAL MICRORNA EXPRESSION.

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Ethanol has been shown to induce epigenetic changes with increasing interest in its effect on small non-coding RNAs (about 19-24 nucleotides), known as microRNAs (miRNAs). They can regulate the expression of a wide variety of genes interfering with brain development and function. Despite the fact that alcohol voluntary consumption begins in adolescence and is widely spread at this stage, research on the miRNAs changes produced by alcohol consumption has focused on prenatal exposure. The aim of this study was to explore if the repeated exposure to alcohol during adolescence would alter normal miRNA hippocampal expression patterns that undergo reorganization during this neurodevelopmental period as well as the potential long-term persistence into adulthood. Twenty-two adolescent male rats received intermittent 2g/kg alcohol (AIE) or volume-matched saline (AIS) by intraperitoneal injections on a 2 day on/off schedule from PND-28 to PND-41. Half of the subjects were sacrificed on the last day of AIE/AIS and we extracted the brains to test short-term on the hippocampus. The other half were sacrificed on PND-98 to assess long-term effects in the same area. The design allows us to explore the hippocampal expression patterns of miRNAs related to both alcohol consumption and adolescent brain development. The results are relevant to understanding the epigenetic short- and long-term impact of alcohol intake during adolescence on brain development. Funded by PID2020-114269GB-I00 (MICIU, Spain), BSEJ.514.UGR20 (FEDER, Junta de Andalucía, Spain) and FPU18/05012 (MIU, Spain).

Pubmed:

33937370: Perea C, Vázquez-Ágredos A, Ruiz-Leyva L, Morón I, Zúñiga JM, Cendán CM
Caloric Restriction in Group-Housed Mice: Littermate and Sex Influence on Behavioral and Hormonal Data.
Much of the research done on aging, oxidative stress, anxiety, and cognitive and social behavior in rodents has focused on caloric restriction (CR). This often involves several days of single housing, which can cause numerous logistical problems, as well as cognitive and social dysfunctions. Previous results in our laboratory showed the viability of long-term CR in grouped rats. Our research has studied the possibility of CR in grouped female and male littermates and unrelated CB6F1/J (C57BL/6J × BALBc/J hybrid strain) mice, measuring: (i) possible differences in body mass proportions between mice in and CR conditions (at 70% of), (ii) aggressive behavior, using the number of and as an indicator and social behavior using the as indicator, and (iii) difference in serum adrenocorticotropic hormone (ACTH) concentrations (stress biomarker), under and CR conditions. Results showed the impossibility of implementing CR in unrelated male mice. In all other groups, CR was possible, with a less aggressive behavior (measured only with the number of) observed in the unrelated female mice under CR conditions. In that sense, the ACTH levels measured on the last day of CR showed no difference in stress levels. These results indicate that implementantion of long-term CR in mice can be optimized technically and also related to their well-being by grouping animals, in particular, related mice.
Front Vet Sci, 2021; 8
33654997: Jiménez-García AM, Ruiz-Leyva L, Vázquez-Ágredos A, Torres C, Papini MR, Cendán CM, Morón I
Consummatory Successive Negative Contrast in Rats.
Using animal models in addiction and pain research is pivotal to unravel new pathways and mechanisms for the treatment of these disorders. Reward devaluation through a consummatory successive negative contrast (cSNC) task has shown the ability to reduce physical pain sensitivity (hypoalgesia) and increase oral ethanol consumption in rats. The procedure is based on exposing the experimental animals to a 32% sucrose solution during several sessions (preshift sessions) followed by a devaluation to 4% sucrose during the next few sessions (postshift sessions). The cSNC effect can be monitored by comparing the experimental group to an unshifted control that had access to 4% sucrose throughout the entire experiment (preshift and postshift sessions). The cSNC phenomenon is defined by lower consumption of sucrose in the downshifted group than in the unshifted group during postshfit sessions.
Bio Protoc, 2019; 9


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UNCOVERING THE SIGNALING PATHWAYS TO COGNITIVE IMPAIRMENTS AND NEURODEGENERATION IN DOWN SYNDROME BY CELL PROFILING OF THE LOCUS COERULEUS IN TRISOMIC MICE

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Alzheimer's disease is frequent and occurs earlier in individuals with Down syndrome (DS) as compared to sporadic AD. The locus coeruleus (LC), the region containing the neurons producing the noradrenaline, degenerates in diseases associated with dementia, such as AD, DS, and Parkinson's disease (PD). Although LC cell loss is associated with aging, DS demented cases exhibit pathology similar to AD brains despite their younger age.
We hypothesized that the risk for dementia in DS could rely on a defective noradrenergic system, partially due to their genetic condition. Using a DS mouse model, here we showed impaired behavior in tasks as compared to control mice. To uncover the signaling pathways that may underlie some of the cognitive deficits, we performed a bulk RNA-sequencing analysis in the LC from individual mice using laser microdissection. This experiment revealed differential expression patterns in LC between genotypes for different biological processes related to subcellular transport.
In parallel, we optimized a protocol for the cell dissociation of fresh LC from adult mice, and performed single cell RNA-sequencing (scRNAseq). Our preliminary analysis revealed more than 5,000 common expressed genes between Bulk-RNAseq and scRNAseq experiments. Moreover, cells clustered in 12 different groups, which suggest a rich cell diversity despite the reduced size of the brain region.
Single-cell profiling of the LC in trisomic mice offers exciting opportunities to reveal molecular basis for neurodegeneration in DS. We hope our work will be useful in understanding dementia in DS but also in AD, and PD, all conditions with LC pathology.

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TRANSPOSABLE ELEMENT-MEDIATED LOCAL HETEROCHROMATIN IN X-LINKED DYSTONIA PARKINSONISM

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Neurodegenerative disorders affect millions of people worldwide, but in most cases the genetic causes remain poorly understood. In recent years transposable elements (TEs) have been suggested as novel players in the development of neurodegenerative disorders, and the discovery of a polymorphic TE allele causing X-linked Dystonia Parkinsonism (XDP) strengthened this hypothesis. The XDP specific TE was identified in intron 32 of transcriptional regulator gene *TAF*1 and has been associated with its downregulation and the retention of intron 32. We hypothesize that these alterations in transcription and splicing are caused by the formation of a local heterochromatin as a result of the recruitment of TRIM28 to the TE insertion through a KRAB-Zinc Finger protein (KZFP). To study this, we developed a novel, XDP patient derived neuroepithelial like stem (NES) cell model. By using our XDP cell model and the CRISPRi technique first we inhibited the expression of TRIM28, to see its role in *TAF1* gene regulation. We found no effect on the *TAF1* gene expression nor on intron 32 retention, however nearly 300 TE families were up-regulated in the TRIM28 CRIPSRi NES cells. When we inhibited the expression of the KZFP putatively binding the TE insertion, we found recovered *TAF1* expression, but no effect on the intron retention nor on TEs. Our results suggest that the interplay of the TRIM28/KZFP complex is influencing the alterations in *TAF1* expression level while keeping TEs under control. Overall, this exciting finding points to an important role for polymorphic TE insertions in influencing human health.

Pubmed:
34936701: Pircs K, Drouin-Ouellet J, Horváth V, Gil J, Rezeli M, Garza R, Grassi DA, Sharma Y, St-Amour I, Harris K, Jönsson ME, Johansson PA, Vuono R, Fazal SV, Stoker T, Hersbach BA, Sharma K, Lagerwall J, Lagerström S, Storm P, Hébert SS, Marko-Varga G, Parmar M, Barker RA, Jakobsson J
Distinct subcellular autophagy impairments in induced neurons from Huntington's disease patients.
Huntington's disease (HD) is a neurodegenerative disorder caused by CAG expansions in the huntingtin (HTT) gene. Modelling Huntington's disease is challenging, as rodent and cellular models poorly recapitulate the disease as seen in aging humans. To address this, we generated induced neurons (iNs) through direct reprogramming of human skin fibroblasts, which retain age-dependent epigenetic characteristics. HD-iNs displayed profound deficits in autophagy, characterised by reduced transport of late autophagic structures from the neurites to the soma. These neurite-specific alterations in autophagy resulted in shorter, thinner and fewer neurites specifically in HD-iNs. CRISPRi-mediated silencing of HTT did not rescue this phenotype but rather resulted in additional autophagy alterations in ctrl-iNs, highlighting the importance of wild type HTT in normal neuronal autophagy. In summary, our work identifies a distinct subcellular autophagy impairment in adult patient derived Huntington's disease neurons and provides a new rational for future development of autophagy activation therapies. Brain, 2021;
34469576: Kapun M, Nunez JCB, Bogaerts-Márquez M, Murga-Moreno J, Paris M, Outten J, Coronado-Zamora M, Tern C, Rota-Stabelli O, Guerreiro MPG, Casillas S, Orengo DJ, Puerma E, Kankare M, Ometto L, Loeschcke V, Onder BS, Abbott JK, Schaeffer SW, Rajpurohit S, Behrman EL, Schou MF, Merritt TJS, Lazzaro BP, Glaser-Schmitt A, Argyridou E, Staubach F, Wang Y, Tauber E, Serga SV, Fabian DK, Dyer KA, Wheat CW, Parsch J, Grath S, Veselinovic MS, Stamenkovic-Radak M, Jelic M, Buendía-Ruíz AJ, Gómez-Julián MJ, Espinosa-Jimenez ML, Gallardo-Jiménez FD, Patenkovic A, Eric K, Tanaskovic M, Ullastres A, Guio L, Merenciano M, Guirao-Rico S, Horváth V, Obbard DJ, Pasyukova E, Alatortsev VE, Vieira CP, Vieira J, Torres JR, Kozeretska I, Maistrenko OM, Montchamp-Moreau C, Mukha DV, Machado HE, Lamb K, Paulo T, Yusuf L, Barbadilla A, Petrov D, Schmidt P, Gonzalez J, Flatt T, Bergland AO
Drosophila Evolution over Space and Time (DEST): A New Population Genomics Resource.
Drosophila melanogaster is a leading model in population genetics and genomics, and a growing number of whole-genome data sets from natural populations of this species have been published over the last years. A major challenge is the integration of disparate data sets, often generated using different sequencing technologies and bioinformatic pipelines, which hampers our ability to address questions about the evolution of this species. Here we address these issues by developing a

bioinformatics pipeline that maps pooled sequencing (Pool-Seq) reads from D. melanogaster to a hologenome consisting of fly and symbiont genomes and estimates allele frequencies using either a heuristic (PoolSNP) or a probabilistic variant caller (SNAPE-pooled). We use this pipeline to generate the largest data repository of genomic data available for D. melanogaster to date, encompassing 271 previously published and unpublished population samples from over 100 locations in >20 countries on four continents. Several of these locations have been sampled at different seasons across multiple years. This data set, which we call Drosophila Evolution over Space and Time (DEST), is coupled with sampling and environmental metadata. A web-based genome browser and web portal provide easy access to the SNP data set. We further provide guidelines on how to use Pool-Seq data for model-based demographic inference. Our aim is to provide this scalable platform as a community resource which can be easily extended via future efforts for an even more extensive cosmopolitan data set. Our resource will enable population geneticists to analyze spatiotemporal genetic patterns and evolutionary dynamics of D. melanogaster populations in unprecedented detail.
Mol Biol Evol, 2021; 38
31175824: Villanueva-Cañas JL, Horvath V, Aguilera L, González J
Diverse families of transposable elements affect the transcriptional regulation of stress-response genes in Drosophila melanogaster.
Although transposable elements are an important source of regulatory variation, their genome-wide contribution to the transcriptional regulation of stress-response genes has not been studied yet. Stress is a major aspect of natural selection in the wild, leading to changes in the transcriptional regulation of a variety of genes that are often triggered by one or a few transcription factors. In this work, we take advantage of the wealth of information available for Drosophila melanogaster and humans to analyze the role of transposable elements in six stress regulatory networks: immune, hypoxia, oxidative, xenobiotic, heat shock, and heavy metal. We found that transposable elements were enriched for caudal, dorsal, HSF, and tango binding sites in D. melanogaster and for NFE2L2 binding sites in humans. Taking into account the D. melanogaster population frequencies of transposable elements with predicted binding motifs and/or binding sites, we showed that those containing three or more binding motifs/sites are more likely to be functional. For a representative subset of these TEs, we performed in vivo transgenic reporter assays in different stress conditions. Overall, our results showed that TEs are relevant contributors to the transcriptional regulation of stress-response genes.
Nucleic Acids Res, 2019; 47
30753202: Rech GE, Bogaerts-Márquez M, Barrón MG, Merenciano M, Villanueva-Cañas JL, Horváth V, Fiston-Lavier AS, Luyten I, Venkataram S, Quesneville H, Petrov DA, González J
Stress response, behavior, and development are shaped by transposable element-induced mutations in Drosophila.
Most of the current knowledge on the genetic basis of adaptive evolution is based on the analysis of single nucleotide polymorphisms (SNPs). Despite increasing evidence for their causal role, the contribution of structural variants to adaptive evolution remains largely unexplored. In this work, we analyzed the population frequencies of 1,615 Transposable Element (TE) insertions annotated in the reference genome of Drosophila melanogaster, in 91 samples from 60 worldwide natural populations. We identified a set of 300 polymorphic TEs that are present at high population frequencies, and located in genomic regions with high recombination rate, where the efficiency of natural selection is high. The age and the length of these 300 TEs are consistent with relatively young and long insertions reaching high frequencies due to the action of positive selection. Besides, we identified a set of 21 fixed TEs also likely to be adaptive. Indeed, we, and others, found evidence of selection for 84 of these reference TE insertions. The analysis of the genes located nearby these 84 candidate adaptive insertions suggested that the functional response to selection is related with the GO categories of response to stimulus, behavior, and development. We further showed that a subset of the candidate adaptive TEs affects expression of nearby genes, and five of them have already been linked to an ecologically relevant phenotypic effect. Our results provide a more complete understanding of the genetic variation and the fitness-related traits relevant for adaptive evolution. Similar studies should help uncover the importance of TE-induced adaptive mutations in other species as well.
PLoS Genet, 2019; 15
28947157: Horváth V, Merenciano M, González J
Revisiting the Relationship between Transposable Elements and the Eukaryotic Stress Response.
A relationship between transposable elements (TEs) and the eukaryotic stress response was suggested in the first publications describing TEs. Since then, it has often been assumed that TEs are activated by stress, and that this activation is often beneficial for the organism. In recent years, the availability of new high-throughput experimental techniques has allowed further interrogation of the relationship between TEs and stress. By reviewing the recent literature, we conclude that although there is evidence for a beneficial effect of TE activation under stress conditions, the relationship between TEs and the eukaryotic stress response is quite complex.
Trends Genet, 2017; 33
26295476: Beleznai O, Tholt G, Tóth Z, Horváth V, Marczali Z, Samu F

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Cool Headed Individuals Are Better Survivors: Non-Consumptive and Consumptive Effects of a Generalist Predator on a Sap Feeding Insect.

Non-consumptive effects (NCEs) of predators are part of the complex interactions among insect natural enemies and prey. NCEs have been shown to significantly affect prey foraging and feeding. Leafhopper's (Auchenorrhyncha) lengthy phloem feeding bouts may play a role in pathogen transmission in vector species and also exposes them to predation risk. However, NCEs on leafhoppers have been scarcely studied, and we lack basic information about how anti-predator behaviour influences foraging and feeding in these species. Here we report a study on non-consumptive and consumptive predator-prey interactions in a naturally co-occurring spider-leafhopper system. In mesocosm arenas we studied movement patterns during foraging and feeding of the leafhopper Psammotettix alienus in the presence of the spider predator Tibellus oblongus. Leafhoppers delayed feeding and fed much less often when the spider was present. Foraging movement pattern changed under predation risk: movements became more frequent and brief. There was considerable individual variation in foraging movement activity. Those individuals that increased movement activity in the presence of predators exposed themselves to higher predation risk. However, surviving individuals exhibited a 'cool headed' reaction to spider presence by moving less than leafhoppers in control trials. No leafhoppers were preyed upon while feeding. We consider delayed feeding as a "paradoxical" antipredator tactic, since it is not necessarily an optimal strategy against a sit-and-wait generalist predator.
PLoS One, 2015; 10

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EVALUATION OF REPEAT EXPRESSION OVER LIFESPAN REVEALS AGE CONTRIBUTES TO INCREASED HERV-K EXPRESSION IN THE HUMAN, NEUROTYPICAL BRAIN

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Repetitive sequences, or repeats, comprise over 50% of the human genome and influence it through a variety of mechanisms. Repeat expression is highly regulated by cells, therefore, dysregulated repeat expression can alter a tissue's transcriptome. The neurotypical brain has the highest detectable level of repeat expression, and dysregulated repeat expression has been linked to aging and several neurodegenerative disorders, including multiple sclerosis and Alzheimer's disease. It is unclear how repeat dysregulation contributes to disease etiology, as our current understanding relies on limited data from non-human models, peripheral tissues, and post-mortem tissue from limited timepoints. Consequently, the field has struggled to contextualize repeat expression in neurological disease without a comprehensive understanding of repeat expression over the lifespan of the neurotypical brain. Here, we leverage our large cohort spanning the human lifespan, containing post-mortem brain samples obtained from 395 unique individuals across caudate nucleus, dorsolateral prefrontal cortex, and hippocampus, to catalogue repeat expression across the lifespan of the neurotypical brain. Using differential expression, we identify differentially expressed repeats (DERs) dysregulated with brain age. We find HERV-K is a DER, upregulated from 0-15 to 60+ years across all three brain regions, making it a potential transcriptional signature of neurotypical aging. Importantly, we find expression of select repeats downregulated with age. In addition to serving as a resource to the scientific community, this work proves global assessment of repeat behavior across age, tissue, and repeat class is necessary to contextualize disease-specific observations.

Pubmed:
33229216: Evans TA, Erwin JA
Retroelement-derived RNA and its role in the brain.
Comprising ~40% of the human genome, retroelements are mobile genetic elements which are transcribed into RNA, then reverse-transcribed into DNA and inserted into a new site in the genome. Retroelements are referred to as "genetic parasites", residing among host genes and relying on host machinery for transcription and evolutionary propagation. The healthy brain has the highest expression of retroelement-derived sequences compared to other somatic tissue, which leads to the question: how does retroelement-derived RNA influence human traits and cellular states? While the functional importance of upregulating retroelement expression in the brain is an active area of research, RNA species derived from retroelements influence both self- and host gene expression by contributing to chromatin remodeling, alternative splicing, somatic mosaicism and translational repression. Here, we review the emerging evidence that the functional importance of RNA derived from retroelements is multifaceted. Retroelements can influence organismal states through the seeding of epigenetic states in chromatin, the production of structured RNA and even catalytically active ribozymes, the generation of cytoplasmic ssDNA and RNA/DNA hybrids, the production of viral-like proteins, and the generation of somatic mutations. Comparative sequencing suggests that retroelements can contribute to intraspecies variation through these mechanisms to alter transcript identity and abundance. In humans, an increasing number of neurodevelopmental and neurodegenerative conditions are associated with dysregulated retroelements, including Aicardi-Goutieres syndrome (AGS), Rett syndrome (RTT), Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), multiple sclerosis (MS), schizophrenia (SZ), and aging. Taken together, these concepts suggest a larger functional role for RNA derived from retroelements. This review aims to define retroelement-derived RNA, discuss how it impacts the mammalian genome, as well as summarize data supporting phenotypic consequences of this unique RNA subset in the brain.

Semin Cell Dev Biol, 2021; 114
33085659: Joynt AT, Evans TA, Pellicore MJ, Davis-Marcisak EF, Aksit MA, Eastman AC, Patel SU, Paul KC, Osorio DL, Bowling AD, Cotton CU, Raraigh KS, West NE, Merlo CA, Cutting GR, Sharma N
Evaluation of both exonic and intronic variants for effects on RNA splicing allows for accurate assessment of the effectiveness of precision therapies.
Elucidating the functional consequence of molecular defects underlying genetic diseases enables appropriate design of therapeutic options. Treatment of cystic fibrosis (CF) is an exemplar of this paradigm as the development of CFTR modulator therapies has allowed for targeted and effective treatment of individuals harboring specific genetic variants. However, the mechanism of these drugs limits effectiveness to particular classes of variants that allow production of CFTR protein. Thus, assessment of the molecular mechanism of individual variants is imperative for proper assignment of these precision therapies. This is particularly important when considering variants that affect pre-mRNA splicing, thus limiting success of the existing protein-targeted therapies. Variants affecting splicing can occur throughout exons and introns and the complexity of the process of splicing lends itself to a variety of outcomes, both at the RNA and protein levels, further complicating assessment of disease liability and modulator response. To investigate the scope of this challenge, we evaluated splicing and downstream effects of 52 naturally occurring CFTR variants (exonic = 15, intronic = 37). Expression of constructs containing select CFTR intronic sequences and complete CFTR exonic sequences in cell line models allowed for assessment of RNA and protein-level effects on an allele by allele basis. Characterization of primary nasal epithelial cells obtained from individuals harboring splice variants corroborated in vitro data. Notably, we identified exonic variants that result in complete missplicing and thus a lack of modulator response (e.g. c.2908G>A, c.523A>G), as well as intronic variants that respond to modulators due to the presence of residual normally spliced transcript (e.g. c.4242+2T>C, c.3717+40A>G). Overall, our data reveals diverse molecular outcomes amongst both exonic and intronic variants emphasizing the need to delineate RNA, protein, and functional effects of each variant in order to accurately assign precision therapies.
PLoS Genet, 2020; 16
31967360: Taylor-Cousar JL, Evans TA, Cutting GR, Sharma N
Potentially lethal cystic fibrosis gene variant in the orangutan.
A syndrome of chronic upper and lower airway disease leading to increased morbidity and mortality occurs primarily in captive orangutans. Similarities in symptoms to the inherited human respiratory disease, cystic fibrosis, led us to hypothesize that orangutan respiratory disease is a result of variants in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. We identified the nonsense variant, c.484A>T (p.Lys162X), in heterozygosity in an unaffected orangutan. Analysis of the pedigree of this orangutan confirmed that both his sire and deceased fetus also harbored the c.484A>T allele. An expression minigene harboring c.484A>T produced no full-length CFTR protein in HEK293 cells. Finally, the c.484A>T CFTR messenger RNA abundance was severely reduced in primary nasal epithelial cells of the orangutan indicating that c.484A>T (p.Lys162X) is potentially lethal. Genetic screening of the captive orangutan population could be used to prevent transmission of this potentially lethal variant, and thus aid in the conservation of this critically endangered species.
Am J Primatol, 2021; 83
30888834: McCague AF, Raraigh KS, Pellicore MJ, Davis-Marcisak EF, Evans TA, Han ST, Lu Z, Joynt AT, Sharma N, Castellani C, Collaco JM, Corey M, Lewis MH, Penland CM, Rommens JM, Stephenson AL, Sosnay PR, Cutting GR
Correlating Cystic Fibrosis Transmembrane Conductance Regulator Function with Clinical Features to Inform Precision Treatment of Cystic Fibrosis.
The advent of precision treatment for cystic fibrosis using small-molecule therapeutics has created a need to estimate potential clinical improvements attributable to increases in cystic fibrosis transmembrane conductance regulator (CFTR) function. To derive CFTR function of a variety of genotypes and correlate with key clinical features (sweat chloride concentration, pancreatic exocrine status, and lung function) to develop benchmarks for assessing response to CFTR modulators. CFTR function assigned to 226 unique genotypes was correlated with the clinical data of 54,671 individuals enrolled in the Clinical and Functional Translation of CFTR (CFTR2) project. Cross-sectional FEV% predicted measurements were plotted by age at which measurement was obtained. Shifts in sweat chloride concentration and lung function reported in CFTR modulator trials were compared with function-phenotype correlations to assess potential efficacy of therapies. CFTR genotype function exhibited a logarithmic relationship with each clinical feature. Modest increases in CFTR function related to differing genotypes were associated with clinically relevant improvements in cross-sectional FEV% predicted over a range of ages (6-82 yr). Therapeutic responses to modulators corresponded closely to predictions from the CFTR2-derived relationship between CFTR genotype function and phenotype. Increasing CFTR function in individuals with severe disease will have a proportionally greater effect on outcomes than similar increases in CFTR function in individuals with mild disease and should reverse a substantial fraction of the disease process. This study provides reference standards for clinical outcomes that may be achieved by increasing CFTR function.
Am J Respir Crit Care Med, 2019; 199

30803905: Aksit MA, Bowling AD, Evans TA, Joynt AT, Osorio D, Patel S, West N, Merlo C, Sosnay PR, Cutting GR, Sharma N
Decreased mRNA and protein stability of W1282X limits response to modulator therapy.
Cell-based studies have shown that W1282X generates a truncated protein that can be functionally augmented by modulators. However, modulator treatment of primary cells from individuals who carry two copies of W1282X generates no functional CFTR. To understand the lack of response to modulators, we investigated the effect of W1282X on CFTR RNA transcript levels.
J Cyst Fibros, 2019; 18
30444886: Sharma N, Evans TA, Pellicore MJ, Davis E, Aksit MA, McCague AF, Joynt AT, Lu Z, Han ST, Anzmann AF, Lam AN, Thaxton A, West N, Merlo C, Gottschalk LB, Raraigh KS, Sosnay PR, Cotton CU, Cutting GR
Capitalizing on the heterogeneous effects of CFTR nonsense and frameshift variants to inform therapeutic strategy for cystic fibrosis.
CFTR modulators have revolutionized the treatment of individuals with cystic fibrosis (CF) by improving the function of existing protein. Unfortunately, almost half of the disease-causing variants in CFTR are predicted to introduce premature termination codons (PTC) thereby causing absence of full-length CFTR protein. We hypothesized that a subset of nonsense and frameshift variants in CFTR allow expression of truncated protein that might respond to FDA-approved CFTR modulators. To address this concept, we selected 26 PTC-generating variants from four regions of CFTR and determined their consequences on CFTR mRNA, protein and function using intron-containing minigenes expressed in 3 cell lines (HEK293, MDCK and CFBE41o-) and patient-derived conditionally reprogrammed primary nasal epithelial cells. The PTC-generating variants fell into five groups based on RNA and protein effects. Group A (reduced mRNA, immature (core glycosylated) protein, function <1% (n = 5)) and Group B (normal mRNA, immature protein, function <1% (n = 10)) variants were unresponsive to modulator treatment. However, Group C (normal mRNA, mature (fully glycosylated) protein, function >1% (n = 5)), Group D (reduced mRNA, mature protein, function >1% (n = 5)) and Group E (aberrant RNA splicing, mature protein, function > 1% (n = 1)) variants responded to modulators. Increasing mRNA level by inhibition of NMD led to a significant amplification of modulator effect upon a Group D variant while response of a Group A variant was unaltered. Our work shows that PTC-generating variants should not be generalized as genetic 'nulls' as some may allow generation of protein that can be targeted to achieve clinical benefit.
PLoS Genet, 2018; 14
30046002: Han ST, Rab A, Pellicore MJ, Davis EF, McCague AF, Evans TA, Joynt AT, Lu Z, Cai Z, Raraigh KS, Hong JS, Sheppard DN, Sorscher EJ, Cutting GR
Residual function of cystic fibrosis mutants predicts response to small molecule CFTR modulators.
Treatment of individuals with cystic fibrosis (CF) has been transformed by small molecule therapies that target select pathogenic variants in the CF transmembrane conductance regulator (CFTR). To expand treatment eligibility, we stably expressed 43 rare missense CFTR variants associated with moderate CF from a single site in the genome of human CF bronchial epithelial (CFBE41o-) cells. The magnitude of drug response was highly correlated with residual CFTR function for the potentiator ivacaftor, the corrector lumacaftor, and ivacaftor-lumacaftor combination therapy. Response of a second set of 16 variants expressed stably in Fischer rat thyroid (FRT) cells showed nearly identical correlations. Subsets of variants were identified that demonstrated statistically significantly higher responses to specific treatments. Furthermore, nearly all variants studied in CFBE cells (40 of 43) and FRT cells (13 of 16) demonstrated greater response to ivacaftor-lumacaftor combination therapy than either modulator alone. Together, these variants represent 87% of individuals in the CFTR2 database with at least 1 missense variant. Thus, our results indicate that most individuals with CF carrying missense variants are (a) likely to respond modestly to currently available modulator therapy, while a small fraction will have pronounced responses, and (b) likely to derive the greatest benefit from combination therapy.
JCI Insight, 2018; 3
29805046: Raraigh KS, Han ST, Davis E, Evans TA, Pellicore MJ, McCague AF, Joynt AT, Lu Z, Atalar M, Sharma N, Sheridan MB, Sosnay PR, Cutting GR
Functional Assays Are Essential for Interpretation of Missense Variants Associated with Variable Expressivity.
Missense DNA variants have variable effects upon protein function. Consequently, interpreting their pathogenicity is challenging, especially when they are associated with disease variability. To determine the degree to which functional assays inform interpretation, we analyzed 48 CFTR missense variants associated with variable expressivity of cystic fibrosis (CF). We assessed function in a native isogenic context by evaluating CFTR mutants that were stably expressed in the genome of a human airway cell line devoid of endogenous CFTR expression. 21 of 29 variants associated with full expressivity of the CF phenotype generated <10% wild-type CFTR (WT-CFTR) function, a conservative threshold for the development of life-limiting CF lung disease, and five variants had moderately decreased function (10% to ~25% WT-CFTR). The remaining three variants in this group unexpectedly had >25% WT-CFTR function; two were higher than 75% WT-CFTR. As expected, 14 of

19 variants associated with partial expressivity of CF had >25% WT-CFTR function; however, four had minimal to no effect on CFTR function (>75% WT-CFTR). Thus, 6 of 48 (13%) missense variants believed to be disease causing did not alter CFTR function. Functional studies substantially refined pathogenicity assignment with expert annotation and criteria from the American College of Medical Genetics and Genomics and Association for Molecular Pathology. However, four algorithms (CADD, REVEL, SIFT, and PolyPhen-2) could not differentiate between variants that caused severe, moderate, or minimal reduction in function. In the setting of variable expressivity, these results indicate that functional assays are essential for accurate interpretation of missense variants and that current prediction tools should be used with caution.
Am J Hum Genet, 2018; 102

28475858: Lee M, Roos P, Sharma N, Atalar M, Evans TA, Pellicore MJ, Davis E, Lam AN, Stanley SE, Khalil SE, Solomon GM, Walker D, Raraigh KS, Vecchio-Pagan B, Armanios M, Cutting GR

Systematic Computational Identification of Variants That Activate Exonic and Intronic Cryptic Splice Sites.

We developed a variant-annotation method that combines sequence-based machine-learning classification with a context-dependent algorithm for selecting splice variants. Our approach is distinctive in that it compares the splice potential of a sequence bearing a variant with the splice potential of the reference sequence. After training, classification accurately identified 168 of 180 (93.3%) canonical splice sites of five genes. The combined method, CryptSplice, identified and correctly predicted the effect of 18 of 21 (86%) known splice-altering variants in CFTR, a well-studied gene whose loss-of-function variants cause cystic fibrosis (CF). Among 1,423 unannotated CFTR disease-associated variants, the method identified 32 potential exonic cryptic splice variants, two of which were experimentally evaluated and confirmed. After complete CFTR sequencing, the method found three cryptic intronic splice variants (one known and two experimentally verified) that completed the molecular diagnosis of CF in 6 of 14 individuals. CryptSplice interrogation of sequence data from six individuals with X-linked dyskeratosis congenita caused by an unknown disease-causing variant in DKC1 identified two splice-altering variants that were experimentally verified. To assess the extent to which disease-associated variants might activate cryptic splicing, we selected 458 pathogenic variants and 348 variants of uncertain significance (VUSs) classified as high confidence from ClinVar. Splice-site activation was predicted for 129 (28%) of the pathogenic variants and 75 (22%) of the VUSs. Our findings suggest that cryptic splice-site activation is more common than previously thought and should be routinely considered for all variants within the transcribed regions of genes.
Am J Hum Genet, 2017; 100

26911677: Lee M, Vecchio-Pagán B, Sharma N, Waheed A, Li X, Raraigh KS, Robbins S, Han ST, Franca AL, Pellicore MJ, Evans TA, Arcara KM, Nguyen H, Luan S, Belchis D, Hertecant J, Zabner J, Sly WS, Cutting GR

Loss of carbonic anhydrase XII function in individuals with elevated sweat chloride concentration and pulmonary airway disease.

Elevated sweat chloride levels, failure to thrive (FTT), and lung disease are characteristic features of cystic fibrosis (CF, OMIM #219700). Here we describe variants in CA12 encoding carbonic anhydrase XII in two pedigrees exhibiting CF-like phenotypes. Exome sequencing of a white American adult diagnosed with CF due to elevated sweat chloride, recurrent hyponatremia, infantile FTT and lung disease identified deleterious variants in each CA12 gene: c.908-1 G>A in a splice acceptor and a novel frameshift insertion c.859_860insACCT. In an unrelated consanguineous Omani family, two children with elevated sweat chloride, infantile FTT, and recurrent hyponatremia were homozygous for a novel missense variant (p.His121Gln). Deleterious CFTR variants were absent in both pedigrees. CA XII protein was localized apically in human bronchiolar epithelia and basolaterally in the reabsorptive duct of human sweat glands. Respiratory epithelial cell RNA from the adult proband revealed only aberrant CA12 transcripts and in vitro analysis showed greatly reduced CA XII protein. Studies of ion transport across respiratory epithelial cells in vivo and in culture revealed intact CFTR-mediated chloride transport in the adult proband. CA XII protein bearing either p.His121Gln or a previously identified p.Glu143Lys missense variant localized to the basolateral membranes of polarized Madin-Darby canine kidney (MDCK) cells, but enzyme activity was severely diminished when assayed at physiologic concentrations of extracellular chloride. Our findings indicate that loss of CA XII function should be considered in individuals without CFTR mutations who exhibit CF-like features in the sweat gland and lung.
Hum Mol Genet, 2016; 25

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BOARD NUMBER: S04-244

LINE-1 EXPRESSION AND ORF1P INTERACTOME IN THE BRAIN UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Aims. The expression and regulation of transposable elements (TEs) in the brain remain poorly characterized. We are exploring the potential contribution of LINE-1 (L1) retrotransposon and encoded proteins (ORF1p/ORF2p) in aging-associated neurodegenerative diseases (NDs) such as Parkinson disease (PD) and Alzheimer's disease (AD). **Methods**. We use bioinformatic, biochemical and imaging analyses to quantify the transcript and protein levels of L1 (including at the locus level) and other TEs in the brains of healthy and PD/AD patients, correlating these with established disease-markers. The distribution of ORF1p in mouse and human brain was investigated by immunohistochemistry with cutting-edge analysis methods on large-scale image acquisition and by cell fractionation. Mass-Spectrometry quantifications and co-immunoprecipitation experiments were performed to decipher the interactome of ORF1p in neurons. **Results and Conclusion**. We found widespread expression of ORF1p in neurons of mice and humans. Several TEs including specific L1 elements are disease-specifically dysregulated in AD and PD and correlations with markers of these NDs and known TE regulators are being characterized. In subcellular fractionation of human neurons, ORF1p was detected in the cytosolic but also, stress-dependently, in the chromatin fraction, suggesting that ORF1p translocates to the nucleus during aging and possibly in PD/AD. In human dopaminergic neurons in culture, ORF1p protein partners form a clustered network linked to mRNA processing, ribosome biogenesis, and nucleocytoplasmic transport. Following stress, additional clusters emerge which concern nuclear envelope and transcription factor binding. These results point to novel so far unsuspected roles of ORF1p in the nucleus under physiological and pathological conditions.

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BOARD NUMBER: S04-245

DOPAMINE-DEPENDENT HDAC REGULATION AND ITS ROLE IN POSTSYNAPTIC NEURODEGENERATION

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Aims Neurotransmitter dopamine is critically involved in many physiological functions in the brain and its dysregulation is linked to several pathological states. A loss of dopaminergic innervations is a key feature of Parkinson's disease, while enhanced dopaminergic tone has been speculated to contribute to the pathogenesis of schizophrenia and Huntington's disease. The aim of this study was to perform an unbiased identification of protein network related to dopamine signaling. **Methods** We applied proteomic 2D-DIGE analysis of striatal tissue from genetic mouse model of persistent hyperdopaminergia, the dopamine transporter knock-out (DAT-KO) mice, and from pharmacogenetic model of dopamine deficiency, the dopamine-deficient DAT-KO (DDD) mice. **Results** By DIGE analysis, we identified 11 proteins that were significantly altered in DAT and/or in DDD mice. Intriguingly, DAVID analysis revealed that several proteins involved in acetylation processes were changed and, generally, DAT-KO mice presented a pattern of acetylated proteins that was significantly different from one found in DDD mice. We observed an up-regulation of several subtypes of histone deacetylase (HDAC) in DAT-KO mice, but not in DDD-mice. An acute increase of dopamine induced by amphetamine administration was able to induce a similar pattern of HDAC expression. Strikingly, pharmacological inhibition of HDAC activity in DAT-KO mice decreased the level of phosphorylated tau and prevented the development of neurodegenerative phenotype and mortality spontaneously present in DAT-KO mice. **Conclusions** Our data suggest an unappreciated role for dopamine in the regulation of HDAC and a contribution of this process to dopamine-dependent postsynaptic neurodegeneration.

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BOARD NUMBER: S04-246

GENETIC VARIABILITY OF THE NERVE GROWTH FACTOR RECEPTOR (NGFR/P75NTR) GENE AND RISK OF SPORADIC ALZHEIMER'S DISEASE: A CASE-CONTROL ASSOCIATION STUDY

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Background. Alzheimer's Disease (AD) is the most widespread neurodegenerative disorder. To date the exact cause of sporadic AD (sAD) is still not fully known. It has been shown that Single Nucleotide Polymorphisms (SNPs) of the Nerve Growth Factor Receptor (*NGFR/p75NTR*) gene could represent risk factors for sAD. However, only a few studies have investigated this relationship with conflicting results.
Aims. The general aim of this study was to better characterize the association between *NGFR/p75NTR* SNPs and sAD.
Methods. This case-control association study was conducted in a Southern Italian cohort consisting of sAD patients and 119 age- and sex-matched controls. Nineteen tag-SNPs were selected and genotyped TaqMan SNP genotyping assays. The associations between tag-SNPs and the risk of sAD were assessed by logistic regression models.
Results. Some variants, such as the functional *rs2072446*, were found associated to sAD, with a significant decrease/increase of the minor allele frequency compared to controls, suggesting either a protective or a risk-increasing effect in the developing of sAD.
Conclusion. Our results reveal a new role of *NGFR/p75NTR* in sAD from a genetic perspective. *NGFR/p75NTR* SNPs analysis should be considered for the genetic screening of sAD.

Pubmed:
34917136: Bruno F, Conidi ME, Puccio G, Frangipane F, Laganà V, Bernardi L, Smirne N, Mirabelli M, Colao R, Curcio S, Di Lorenzo R, Maletta R, Bruni AC
A Novel Mutation (D395A) in Valosin-Containing Protein Gene Is Associated With Early Onset Frontotemporal Dementia in an Italian Family.
Inclusion body myopathy (IBM) with Paget's disease of bone (PDB) and/or frontotemporal dementia (FTD) (IBMPFD) was recently identified as rare autosomal dominant disorder due to mutations in gene. However, mutations have also been documented in patients with amyotrophic lateral sclerosis (ALS), Charcot-Marie-Tooth type 2 (CMT2) disease, and hereditary spastic paraplegia (HSP), underlining the heterogeneity of the phenotypes due to mutations. In this study, we reported a novel missense heterozygous variant c.1184A > C (.D395A) in exon 10 of gene identified in three patients (two sisters and one brother) belonging to an Italian family. The patients underwent a detailed clinical evaluation including medical history, neurological examination, and neuropsychological assessment. Brain's morphologic and functional analysis was also performed. The whole picture was consistent with the criteria of behavioral variant frontotemporal dementia (bvFTD) without IBM and PBD. Our report confirms the high degree of heterogeneity of disease. A analysis should be considered for the genetic screening of familial bvFTD with an early onset also in absence of IBM or PDB signs.
Front Genet, 2021; 12
27586038: Caporali P, Bruno F, Palladino G, Dragotto J, Petrosini L, Mangia F, Erickson RP, Canterini S, Fiorenza MT
Developmental delay in motor skill acquisition in Niemann-Pick C1 mice reveals abnormal cerebellar morphogenesis.
Niemann-Pick type C1 (NPC1) disease is a lysosomal storage disorder caused by defective intracellular trafficking of exogenous cholesterol. Purkinje cell (PC) degeneration is the main sign of cerebellar dysfunction in both NPC1 patients and animal models. It has been recently shown that a significant decrease in Sonic hedgehog (Shh) expression reduces the proliferative potential of granule neuron precursors in the developing cerebellum of Npc1 (-/-) mice. Pursuing the hypothesis that this developmental defect translates into functional impairments, we have assayed Npc1-deficient pups belonging to the milder mutant mouse strain Npc1 (nmf164) for sensorimotor development from postnatal day (PN) 3 to PN21. Npc1 (nmf164) / Npc1 (nmf164) pups displayed a 2.5-day delay in the acquisition of complex motor abilities compared to wild-type (wt) littermates, in agreement with the significant disorganization of cerebellar cortex cytoarchitecture observed between PN11

and PN15. Compared to wt, Npc1 (nmf164) homozygous mice exhibited a poorer morphological differentiation of Bergmann glia (BG), as indicated by thicker radial shafts and less elaborate reticular pattern of lateral processes. Also BG functional development was defective, as indicated by the significant reduction in GLAST and Glutamine synthetase expression. A reduced VGluT2 and GAD65 expression also indicated an overall derangement of the glutamatergic/GABAergic stimulation that PCs receive by climbing/parallel fibers and basket/stellate cells, respectively. Lastly, Npc1-deficiency also affected oligodendrocyte differentiation as indicated by the strong reduction of myelin basic protein. Two sequential 2-hydroxypropyl-β-cyclodextrin administrations at PN4 and PN7 counteract these defects, partially preventing functional impairment of BG and fully restoring the normal patterns of glutamatergic/GABAergic stimulation to PCs.These findings indicate that in Npc1 (nmf164) homozygous mice the derangement of synaptic connectivity and dysmyelination during cerebellar morphogenesis largely anticipate motor deficits that are typically observed during adulthood.
Acta Neuropathol Commun, 2016; 4
24969023: Nusca S, Canterini S, Palladino G, Bruno F, Mangia F, Erickson RP, Fiorenza MT
A marked paucity of granule cells in the developing cerebellum of the Npc1(-/-) mouse is corrected by a single injection of hydroxypropyl-β-cyclodextrin.
In this study we show that postnatal development of cerebellar granule neurons (GNs) is defective in Npc1(-/-) mice. Compared to age-matched wild-type littermates, there is an accelerated disappearance of the external granule layer (EGL) in these mice. This is due to a premature exit from the cell cycle of GN precursors residing at the level of the EGL. As a consequence, the size of cerebellar lobules of these mice displays a 20%-25% reduction compared to that of age-matched wild-type mice. This size reduction is detectable at post-natal day 28 (PN28), when cerebellar GN development is completed while signs of neuronal atrophy are not yet apparent. Based on the analysis of EGL thickness and the determination of proliferating GN fractions at increasing developmental times (PN8-PN14), we trace the onset of this GN developmental defect during the second postnatal week. We also show that during this developmental time Shh transcripts undergo a significant reduction in Npc1(-/-) mice compared to age-matched wild-type mice. In light of the mitogenic activity of Shh on GNs, this observation further supports the presence of defective GN proliferation in Npc1(-/-) mice. A single injection of hydroxypropyl-β-cyclodextrin at PN7 rescues this defect, restoring the normal patterns of granule neuron proliferation and cerebellar lobule size. To our knowledge, these findings identify a novel developmental defect that was underappreciated in previous studies. This defect was probably overlooked because Npc1 loss-of-function does not affect cerebellar foliation and causes the internal granule layer and molecular layer to decrease proportionally, giving rise to a normally appearing, yet harmoniously smaller, cerebellum.
Neurobiol Dis, 2014; 70
34845503: Chirico A, Vizza D, Valente M, Iacono ML, Campagna MR, Palombi T, Alivernini F, Lucidi F, Bruno F
Assessing the fear of recurrence using the Cancer Worry Scale in a sample of Italian breast cancer survivors.
The fear of cancer recurrence (FCR) is one of the most reported problems by cancer survivors. A valid instrument to detect this issue could be useful to identify cancer survivors who are more vulnerable to developing FCR and related adverse outcomes (e.g., anxiety). The present study aimed to evaluate FCR in a sample of Italian breast cancer survivors using an Italian version of the 8-item Cancer Worry Scale (CWS) in order to establish a cut-off for the use in clinical settings.
Support Care Cancer, 2022; 30
33862348: Chiesi F, Bruno F
Mean differences and individual changes in nursing students' attitudes toward statistics: The role of math background and personality traits.
Nursing students have compulsory statistics courses in their degree program, but they usually have negative attitudes toward statistics that may hinder their learning. The present study aims: (i) to investigate nurse students' attitudes toward statistics and the relationships with mathematical background and personality traits; (ii) to stress individual changes in attitude that occur during the course; and (iii) to explore if mathematical background and personality traits influence these changes. We adopted a one-group pre-post survey study. Fifty nursing students were enrolled in an introductory statistics course at the Sapienza University of Rome in Italy during the 2018-2019 academic year. Participants were surveyed at the beginning and end of the course administering a multidimensional measure of attitude toward statistics. Multiple regression analyses were run to establish the relative impact of mathematical background and Big Five personality factors on attitude components, as well as the changes in these attitude components. Results confirmed the predictive role of mathematic competence on some attitude dimensions and showed that also personality traits influenced attitudes toward statistics. However, the observed changes in attitudes during the course were minimally influenced by these factors. Findings suggest that course pedagogy can enhance students' attitudes regardless their mathematical background and personality.
Nurse Educ Pract, 2021; 52
34864668: Altomari N, Bruno F, Laganà V, Smirne N, Colao R, Curcio S, Di Lorenzo R, Frangipane F, Maletta R, Puccio G, Bruni AC

A Comparison of Behavioral and Psychological Symptoms of Dementia (BPSD) and BPSD Sub-Syndromes in Early-Onset and Late-Onset Alzheimer's Disease.
Behavioral and psychological symptoms of dementia (BPSD) have a large impact on the quality of life of patients with Alzheimer's disease (AD). Few studies have compared BPSD between early-onset (EOAD) and late-onset (LOAD) patients, finding conflicting results.
J Alzheimers Dis, 2022; 85
35052700: Abondio P, Sarno S, Giuliani C, Laganà V, Maletta R, Bernardi L, Bruno F, Colao R, Puccio G, Frangipane F, Borroni B, Van Broeckhoven C, Luiselli D, Bruni A
Amyloid Precursor Protein A713T Mutation in Calabrian Patients with Alzheimer's Disease: A Population Genomics Approach to Estimate Inheritance from a Common Ancestor.
Mutation A713T in the amyloid precursor protein (APP) has been linked to cases of Alzheimer's disease (AD), cerebral amyloid angiopathy (CAA) and cerebrovascular disease. Despite its rarity, it has been observed in several families from the same geographical area, in the Calabria region in Southern Italy. Genotyping of 720,000 genome-wide SNPs with the HumanOmniExpress BeadChip was performed for six patients that were representative of apparently unrelated Calabrian families, as well as a Belgian subject of Italian descent (all with the same A713T mutation and disease). Their genomic structure and genetic relationships were analyzed. Demographic reconstruction and coalescent theory were applied to estimate the time of the most recent common ancestor (tMRCA) among patients. Results show that all A713T carriers fell into the genetic variability of Southern Italy and were not more closely related to each other than to any other healthy Calabrian individual. However, five out of seven patients shared a 1.7 Mbp-long DNA segment centered on the A713T mutation, making it possible to estimate a tMRCA for its common origin in the Calabrian region dating back over 1000 years. The analysis of affected individuals with methodologies based on human population genomics thus provides informative insights in support of clinical observations and biomedical research.
Biomedicines, 2021; 10

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FUNCTIONAL CHARACTERIZATION OF MITOCHONDRIAL BETA-OXIDATION GENES IN DROSOPHILA NERVOUS SYSTEM

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Lipid metabolic dysregulation in the brain is a commonality between the aging process and the onset of neurodegenerative disorders. Despite the emerging evidence linking altered lipostasis with pre-symptomatic stages of neurodegeneration, so far no molecular mechanism has been associated with these observations. Furthermore, the mitochondrial beta-oxidation has not been fully studied in the context of degenerative processes of the nervous system. Here we perform a comprehensive in silico analysis of *Drosophila melanogaster* genes involved in beta-oxidation, and we functionally characterize their role in the nervous system. Using publicly available databases, we explored sequence homology, interaction networks and expression maps to filter 75 candidate genes, and obtained a *bonafide* group including the most relevant proteins for each enzymatic step of beta-oxidation. We then analyzed their expression in *Drosophila* brains and identified that the whole pathway is down-regulated in aged flies. To study a functional role of these genes in the nervous system, we down-regulated their expression in neurons and confirmed that they are required not only for neuronal integrity as observed through immunofluorescence, but also for functional adult flies after measuring lifespan and motor performance. These phenotypes correlated with an altered distribution and concentration of neuronal lipids, as observed with neutral lipid dyes in brains down-regulating selected genes. Finally, we observed that these mutant brains also carried altered mitochondria number and morphology compared to controls. These data suggest a non-canonical role of beta-oxidation in neurons that could provide clues on pathomechanisms of neurodegenerative diseases.

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BOARD NUMBER: S04-248

ELUCIDATING THE ROLE OF FUS AND FMRP IN REGULATING SYNGAP1 EXPRESSION IN HUMAN IPSC-DERIVED NEURONS

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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SYNGAP1 is a major dendritic signalling protein that regulates synaptic plasticity and neuronal maturation during a specific developmental window. Loss of-function mutations in SYNGAP1 result in Intellectual Disability, Autism Spectrum Disorder, and epilepsy. So far, SYNGAP1 has been mainly characterized in mouse models, however species-specific regulatory mechanisms lead to the production of functionally different transcript isoforms. Thus, it is necessary to study this gene in the context of a human model system. The aim is to elucidate the regulation of SYNGAP1 expression and its function in synaptic plasticity using human induced pluripotent stem cell (hiPSC)-derived neurons. Previous studies in murine models have shown that FUS, an RNA binding protein (RBP) involved in amyotrophic lateral sclerosis, positively regulates the expression of SYNGAP1 by 3'UTR binding, thus promoting dendritic spine maturation and cognitive function. However, PAR-CLIP data produced in our lab on hiPSC-derived motoneurons show that FUS binds the translated region of SYNGAP1 mRNA while a mutant FUS protein (P525L) binds the 3'UTR. Moreover, we found that the Fragile X Syndrome-linked RBP FMRP negatively regulates SYNGAP1 expression in hiPSC-derived cortical neurons. A recent model proposes that FUS and FMRP share common targets, which they regulate in opposite way, and that their balance is crucial for proper neuronal protein expression. Thus, investigation of the role of these RBPs in regulating SYNGAP1 expression would allow to have a better understanding of SYNGAP1 physiological roles and how dysregulation of these circuits in nervous system diseases possibly leads to altered dendritic spine and neuronal network formation.

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MIRNAS NETWORKS MEDIATE A COMPENSATORY RESPONSE IN HEART FAILURE INDUCED COGNITIVE IMPAIRMENT

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Heart failure patients often suffer from cognitive dysfunctions. The underlying molecular mechanisms are not well understood. In a recent study from our group, we have employed 3 months old CamKIIδC-overexpressing transgenic mice (CamKIIδCTG) as a model for heart failure and showed that these mice develop an impairment of hippocampus-dependent memory function which is accompanied by specific changes of epigenetic gene expression. Interestingly, at 6 months of age memory impairment was diminished, despite the fact that cardiac phenotypes persisted and massive gene-expression changes were detected in the hippocampus. However, the hippocampal gene-expression changes were fundamentally different to the data obtained from 3 months old mice and suggest the induction of a compensatory response. Further analysis point to a key role of a compensatory microRNA network in mediating this effect. In conclusion, our results suggest that better understanding of these microRNA networks may provide novel therapeutic targets to manage heart failure related cognitive dysfunctions.

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BOARD NUMBER: S04-250

PERINATAL EXPOSURE TO TITANIUM NANOPARTICLES INDUCES ABNORMAL BREATHING IN THE OFFSPRING OF MICE

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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The use of titanium dioxide nanoparticles (TiO_2 NPs) has increase significantly over the last decades, and these particles are now present in many daily consumer products. Recent studies in rodents have shown that perinatal exposure to TiO_2 NPs has deleterious consequences on the development of the offspring. In addition to these, epigenetic modifications of newborns' DNA have also been reported suggesting trans-generational effects. However, whether the development of postnatal respiration is altered by TiO_2 NPs perinatal exposure had still to be determined. Here, F0 dams were perinatally exposed to TiO_2 NPs at 100 and 200 mg/kg via voluntary food intake. We show that F1 newborns' breathing rate was slower in comparison to non-exposed animals. This difference in breathing rhythmicity was observed both in females and males during at least 11 days after birth. We also report an increase of the proportion of F1 newborns that produced apneas during this time window. Similar observations were made when the exposure was limited to the postnatal period, identifying lactation as a critical time period for TiO_2 NPs adverse effects on postnatal breathing development. Finally, TiO_2 NPs-induced breathing deficits were transmitted from F1 females to the F2 generation. In addition, perinatal re-exposure of F1 females to TiO_2 NPs aggravated breathing deficits in F2 males. To conclude, perinatal exposure to TiO_2 NPs alters postnatal respiration of the offspring over several generations.

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BOARD NUMBER: S04-251

COORDINATION OF HEAD DIRECTION REPRESENTATIONS IN MOUSE ANTERODORSAL THALAMIC NUCLEUS AND RETROSPLENIAL CORTEX

POSTER SESSION 04 - SECTION: EPIGENETICS OF THE NERVOUS SYSTEM

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Aim: Our sense of orientation is critical for our survival in changing environments. It depends on flexibly integrating self-motion signals with external sensory cues. However, the circuit dynamics and mechanisms that update head direction (HD) representations with incoming visual information remain poorly understood. Retrosplenial cortex (RSC) is a key region for flexible encoding of variables that guide spatial navigation, including HD. We hypothesized that updates for HD representation first appear in RSC, due to its dense connections with visual areas, and are then conveyed to the anterodorsal nucleus of thalamus (ADn), the obligatory thalamic relay of HD to the rest of the brain. **Methods:** We simultaneously recorded single units in RSC and ADn in freely behaving mice navigating a circular arena, where a prominent visual cue was manipulated. We implemented a GLM to decode HD from the recorded units and determined the temporal correlation between the two representations. Finally, we assessed anatomical connectivity through monosynaptic rabies tracing and functional connectivity through spike cross correlation. **Results:** We observed that, despite differences in HD coding, HD representations in the two regions were nearly synchronous during rotation of a visual cue and during locomotion in darkness. Anatomical and functional monosynaptic connectivity were consistent with a strong feedforward drive of HD information from ADn to RSC, with surprisingly little top-down feedback in the corticothalamic direction. **Conclusions:** Our results provide direct evidence for a coordinated global HD reference update across cortex and thalamus and of the underlying functional circuit connectivity that supports this coordination.

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BOARD NUMBER: S04-252

STRIATAL ARGININE HOMEOSTASIS IS CONTROLLED BY NEURONAL ARGINASE 2

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

Martyna Podgajna[1], Katarzyna Skowrońska[2], Agata Dalka[1], Michalina Graczyk[1], Elżbieta Ziemińska[3], Beata Toczyłowska[4], Magdalena Zielińska[2], Michał Węgrzynowicz[1]
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Aims: Arginase converts arginine (Arg) to ornithine (Orn). Brain distribution of arginase isoenzymes (Arg1 and Arg2) is poorly defined, although striatum is highly enriched in Arg2. Nevertheless, the role of this enzyme in striatal functioning is not understood. The aim of this study was to determine detailed Arg2 distribution within the striatum and to establish the importance of Arg2 for striatal Arg metabolism. **Methods:** Protein levels were analyzed by western blot, and their localization by immunohistochemistry. The concentrations of amino acids were measured with HPLC, and nitrates and nitrites (NOx; cellular nitric oxide (NO) indicator) with fluorimetric assay. The experiments were performed on wild-type and Arg2 knock-out (Arg2 KO) mice. **Results:** Striatal Arg2 expression appears to be specific for some fraction of medium spiny neurons (MSNs; mostly in cell bodies), and is lacking in interneurons, glia and other subpopulations of MSNs. As expected, Arg2 is present in the mitochondria. The absence of Arg2 leads to the accumulation of Arg, however it doesn't affect the content of other related amino acids. No effect of Arg2 loss on NOx levels is observed. **Conclusions:** This study shows neuronal Arg2 localization in the striatum, limited to a defined population of MSNs. Arg2 controls levels of striatal Arg, however the absence of effect of Arg2 loss on Orn content may indicate that a compensatory mechanism is activated to maintain physiological levels of Orn. Arginase may potentially compete with NO synthase for their common substrate, Arg, although striatal Arg2 appears not to affect NO synthesis.

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BOARD NUMBER: S04-253

WE AGE WHAT WE EAT: EXACERBATED MEMORY DECLINE AND DISRUPTED SYNAPTIC PLASTICITY PROMPTED BY A CHRONIC HIGH-CALORIC DIET

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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The alarming increase in the consumption of fat and sugar over the last decades, leading to overweight and obesity, is associated with a higher risk of metabolic and cardiovascular diseases. Recent evidence links this type of diet with a deleterious effect on cognition and synaptic function. However, whether obesity presents an additional risk factor for cognitive impairment in the elderly remains to be determined. Thus, we aimed at unravelling the effects of a chronic high caloric diet (HCD) upon synaptic plasticity and memory performance of aged rats. Male rats were fed an HCD from 1 to 24 months of age. All analyses were performed at 24 months old. HCD-fed aged rats were obese (***p<0.001, n=6-7), and behaviour assessment revealed significant long-term memory impairment in the novel object recognition test, compared to age-matched controls (*p<0.05, n=6). Field excitatory postsynaptic potentials, recorded from hippocampal slices, showed decreased magnitude of long-term potentiation in HCD aged rats (*p<0.05, n=5-6). This was accompanied by a reduction in the hippocampal levels of the brain-derived neurotrophic factor receptors TrkB full-length (TrkB-FL) (*p<0.05, n=7). In contrast, no changes were observed in the number of immature doublecortin immune-positive cells (p>0.05, n=3). These results highlight that a chronic HCD leading to obesity can exacerbate age-associated cognitive decline, possibly due to impaired synaptic plasticity, which might in turn be associated with deficits related to TrkB-FL signalling.

Pubmed:
30959794: Rodrigues RS, Lourenço DM, Paulo SL, Mateus JM, Ferreira MF, Mouro FM, Moreira JB, Ribeiro FF, Sebastião AM, Xapelli S
Cannabinoid Actions on Neural Stem Cells: Implications for Pathophysiology.
With the increase of life expectancy, neurodegenerative disorders are becoming not only a health but also a social burden worldwide. However, due to the multitude of pathophysiological disease states, current treatments fail to meet the desired outcomes. Therefore, there is a need for new therapeutic strategies focusing on more integrated, personalized and effective approaches. The prospect of using neural stem cells (NSC) as regenerative therapies is very promising, however several issues still need to be addressed. In particular, the potential actions of pharmacological agents used to modulate NSC activity are highly relevant. With the ongoing discussion of cannabinoid usage for medical purposes and reports drawing attention to the effects of cannabinoids on NSC regulation, there is an enormous, and yet, uncovered potential for cannabinoids as treatment options for several neurological disorders, specifically when combined with stem cell therapy. In this manuscript, we review in detail how cannabinoids act as potent regulators of NSC biology and their potential to modulate several neurogenic features in the context of pathophysiology.
Molecules, 2019; 24
32723008: Rodrigues RS, Paulo SL, Moreira JB, Tanqueiro SR, Sebastião AM, Diógenes MJ, Xapelli S
Adult Neural Stem Cells as Promising Targets in Psychiatric Disorders.
The development of new therapies for psychiatric disorders is of utmost importance, given the enormous toll these disorders pose to society nowadays. This should be based on the identification of neural substrates and mechanisms that underlie disease etiopathophysiology. Adult neural stem cells (NSCs) have been emerging as a promising platform to counteract brain damage. In this perspective article, we put forth a detailed view of how NSCs operate in the adult brain and influence brain homeostasis, having profound implications at both behavioral and functional levels. We appraise evidence suggesting that adult NSCs play important roles in regulating several forms of brain plasticity, particularly emotional and cognitive flexibility, and that NSC dynamics are altered upon brain pathology. Furthermore, we discuss the potential therapeutic value of utilizing

adult endogenous NSCs as vessels for regeneration, highlighting their importance as targets for the treatment of multiple mental illnesses, such as affective disorders, schizophrenia, and addiction. Finally, we speculate on strategies to surpass current challenges in neuropsychiatric disease modeling and brain repair.
Stem Cells Dev, 2020; 29
34151790: Paulo SL, Ribeiro-Rodrigues L, Rodrigues RS, Mateus JM, Fonseca-Gomes J, Soares R, Diógenes MJ, Solá S, Sebastião AM, Ribeiro FF, Xapelli S
Sustained Hippocampal Neural Plasticity Questions the Reproducibility of an Amyloid-β-Induced Alzheimer's Disease Model.
The use of Alzheimer's disease (AD) models obtained by intracerebral infusion of amyloid-β (Aβ) has been increasingly reported in recent years. Nonetheless, these models may present important challenges.
J Alzheimers Dis, 2021; 82
34940136: Paulo SL, Miranda-Lourenço C, Belo RF, Rodrigues RS, Fonseca-Gomes J, Tanqueiro SR, Geraldes V, Rocha I, Sebastião AM, Xapelli S, Diógenes MJ
High Caloric Diet Induces Memory Impairment and Disrupts Synaptic Plasticity in Aged Rats.
The increasing consumption of sugar and fat seen over the last decades and the consequent overweight and obesity, were recently linked with a deleterious effect on cognition and synaptic function. A major question, which remains to be clarified, is whether obesity in the elderly is an additional risk factor for cognitive impairment. We aimed at unravelling the impact of a chronic high caloric diet (HCD) on memory performance and synaptic plasticity in aged rats. Male rats were kept on an HCD or a standard diet (control) from 1 to 24 months of age. The results showed that under an HCD, aged rats were obese and displayed significant long-term recognition memory impairment when compared to age-matched controls. Ex vivo synaptic plasticity recorded from hippocampal slices from HCD-fed aged rats revealed a reduction in the magnitude of long-term potentiation, accompanied by a decrease in the levels of the brain-derived neurotrophic factor receptors TrkB full-length (TrkB-FL). No alterations in neurogenesis were observed, as quantified by the density of immature doublecortin-positive neurons in the hippocampal dentate gyrus. This study highlights that obesity induced by a chronic HCD exacerbates age-associated cognitive decline, likely due to impaired synaptic plasticity, which might be associated with deficits in TrkB-FL signaling.
Curr Issues Mol Biol, 2021; 43

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BOARD NUMBER: S04-254

REGULATION OF THE APOPTOSIS/AUTOPHAGY SWITCH BY PROPIONIC ACID IN VENTROMEDIAL HYPOTHALAMUS OF RATS WITH TYPE 2 DIABETES MELLITUS

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Aim: The balance between autophagy and apoptosis in hypothalamus may be disrupted upon type 2 diabetes mellitus (T2DM). Since propionic acid (PA) exerts neuroprotective effects, the aim was to investigate its effects on apoptosis/autophagy switch in the ventromedial hypothalamus (VMH) in a T2DM rat model. **Methods:** Male Wistar rats were divided into untreated control rats and rats in which T2DM was induced. The untreated T2DM rats were compared to a group that received metformin, a group that received sodium salt of PA and a group that got PA+metformin. Western blotting, RT-PCR, transmission electron microscopy and immunohistochemical staining were from VMH tissue were performed. **Results:** T2DM-induced apoptosis and mitoptosis, enlarged ER tubules/cisterns were observed in VMH, andaccompanied by imbalance of pro- and anti-apoptotic factors. Elevation of pro-apoptotic Bax and caspase-3, decrease in autophagy marker LC3 and anti-apototic Bcl-xl were observed in VMH of T2DM rats. Metformin and PA partially improved VMH ultrastructural changes by reducing mitochondrial swelling and diminishing the number of apoptotic neurons. Metformin inhibited neuronal apoptosis, however accumulation of lipofuscin. Elevated number of autophagosomes was associated with LC3, Beclin-1 and Bcl-xl increase and decrease in Bax and caspase-3 in VMH of T2DM rats. PA switched cell fate from apoptosis to autophagy by elevating LC3 and Beclin-1 levels and increasing Bcl-xl that altogether may represent adaptive response to T2DM-induced apoptosis. **Conclusions:** T2DM was associated with apoptosis activation leading to impairments in VMH. PA+metformin may be effective against diabetes-induced cell death by switching apoptosis to autophagy in VMH.

Pubmed:
35103058: Natrus LV, Osadchuk YS, Lisakovska OO, Labudzinskyi DO, Klys YG, Chaikovsky YB
Effect of Propionic Acid on Diabetes-Induced Impairment of Unfolded Protein Response Signaling and Astrocyte/Microglia Crosstalk in Rat Ventromedial Nucleus of the Hypothalamus.
The aim was to investigate the influence of propionic acid (PA) on the endoplasmic reticulum (ER), unfolded protein response (UPR) state, and astrocyte/microglia markers in rat ventromedial hypothalamus (VMH) after type 2 diabetes mellitus (T2DM).
Neural Plast, 2022; 2022

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BOARD NUMBER: S04-255

STRUCTURAL ORGANIZATION OF SUBFORNICAL ORGAN'S NEUROTRANSMITTER SYSTEMS IN ADULT RATS

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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The subfornical organ (SFO) is located in the roof of the third ventricle and, as one of the circumventricular organs (CVOs) of the brain, is involved in cardiovascular regulation and body fluid homeostasis regulation by blood-brain communication. Although researchers demonstrate interest in the SFO, a comprehensive analysis of its neurotransmitter systems has not been conducted. The aim of this study was to examine SFO transmitter systems using immunohistochemical methods. The brain of adult (4-6 months) male Wistar rats approximately at the 1.4 mm behind Bregma was examined (n=3). Antibodies against glutamate decarboxylase 67 (GAD67), tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT) were used in order to visualize GABA-, catecholamine- and cholinergic structures respectively. It was established that SFO harbors both GABA- and catecholaminergic structures within. GABAergic neurons tend to locate in the peripheral regions near ventricle and GABAergic terminals are ubiquitous within SFO. In contrast, no TH-positive cell bodies were observed there. Catecholaminergic fibers mostly occur in the medial area of SFO and also found to contact its fenestrated capillaries and ependymal cells. However, SFO seems to completely lack ChAT-immunopositive neurons and projections. Thus, our observations allow further description of the SFO connectivity and offer great promise for understanding integrative complexity of its organization and functions. The reported study was funded by RSF according to the research project № 22-25-00105, https://rscf.ru/project/22-25-00105/.

Pubmed:

33132188: Efimova EV, Kozlova AA, Razenkova V, Katolikova NV, Antonova KA, Sotnikova TD, Merkulyeva NS, Veshchitskii AS, Kalinina DS, Korzhevskii DE, Musienko PE, Kanov EV, Gainetdinov RR
Increased dopamine transmission and adult neurogenesis in trace amine-associated receptor 5 (TAAR5) knockout mice. Trace amine-associated receptors (TAARs) are a class of sensory G protein-coupled receptors that detect biogenic amines, products of decarboxylation of amino acids. The majority of TAARs (TAAR2-TAAR9) have been described mainly in the olfactory epithelium and considered to be olfactory receptors sensing innate odors. However, there is recent evidence that one of the members of this family, TAAR5, is expressed also in the limbic brain areas receiving projection from the olfactory system and involved in the regulation of emotions. In this study, we further characterized a mouse line lacking TAAR5 (TAAR5 knockout, TAAR5-KO mice) that express beta-galactosidase mapping TAAR5 expression. We found that in TAAR5-KO mice the number of dopamine neurons, the striatal levels of dopamine and its metabolites, as well as striatal levels of GDNF mRNA, are elevated indicating a potential increase in dopamine neuron proliferation. Furthermore, an analysis of TAAR5 beta-galactosidase expression revealed that TAAR5 is present in the major neurogenic areas of the brain such as the subventricular zone (SVZ), the subgranular zone (SGZ) and the less characterized potentially neurogenic zone surrounding the 3rd ventricle. Direct analysis of neurogenesis by using specific markers doublecortin (DCX) and proliferating cell nuclear antigen (PCNA) revealed at least 2-fold increase in the number of proliferating neurons in the SVZ and SGZ of TAAR5-KO mice, but no such markers were detected in mutant or control mice in the areas surrounding the 3rd ventricle. These observations indicate that TAAR5 involved not only in regulation of emotional status but also adult neurogenesis and dopamine transmission. Thus, future TAAR5 antagonists may exert not only antidepressant and/or anxiolytic action but may also provide new treatment opportunity for neurodegenerative disorders such as Parkinson's disease.
Neuropharmacology, 2021; 182

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BOARD NUMBER: S04-256

FLEXIBILITY WITHIN THE STIMULATION OF NEURONAL METABOLISM BY CA2+

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Aims: Ca^{2+} influx during neuronal excitation stimulates energy production by increasing NADH shuttling through Malate aspartate shuttle (MAS) and Glycerol-3-phosphate shuttle (G3PS) or by acting on the TCA cycle enzymes after entering the mitochondria via the mitochondrial calcium uniporter (MCU). Our experiments aimed to investigate the significance of these components of neuronal metabolic machinery under different intensities of electrical activation. **Methods**: Hippocampal neuronal cultures derived from newborn rats were subjected to electric field stimulation at DIV14-21. The induced electrical activity and the metabolic changes in individual neurons were measured via live-cell imaging using fluorescent biosensors. Pharmacological agents were used to modulate various components of the neuronal metabolic machinery. **Results**: Our results show that neuronal bioenergetics is modulated by the intensity of induced neuronal activity. Stimulation of NADH shuttling by cytosolic Ca^{2+} rises sustained the energy demand at low workload, while mitochondrial calcium uptake and TCA cycle stimulation was observed only during higher workload. Moreover, neurons can compensate for the loss of any one Ca^{2+}-dependent pathways by increasing the activity of remaining pathways. Our data demonstrate that in the worst case G3PS alone can suffice to maintain cellular ATP levels during neuronal stimulation, because its activity increases during pharmacological inhibition of MAS and MCU. **Conclusions**: Metabolic flexibility in neurons is achieved by a dynamic interplay between various components of the neural metabolic machinery. Unexpectedly, much of this flexibility arises from the activity of G3PS, which appears to provide a back-up system activated during neuronal energetic crisis. **Acknowledgements:** FWF,Project P-33797

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BOARD NUMBER: S04-257

INVESTIGATION OF NEURONAL METABOLISM BY DEVELOPING A NOVEL TECHNIQUE TO ISOLATE MITOCHONDRIA

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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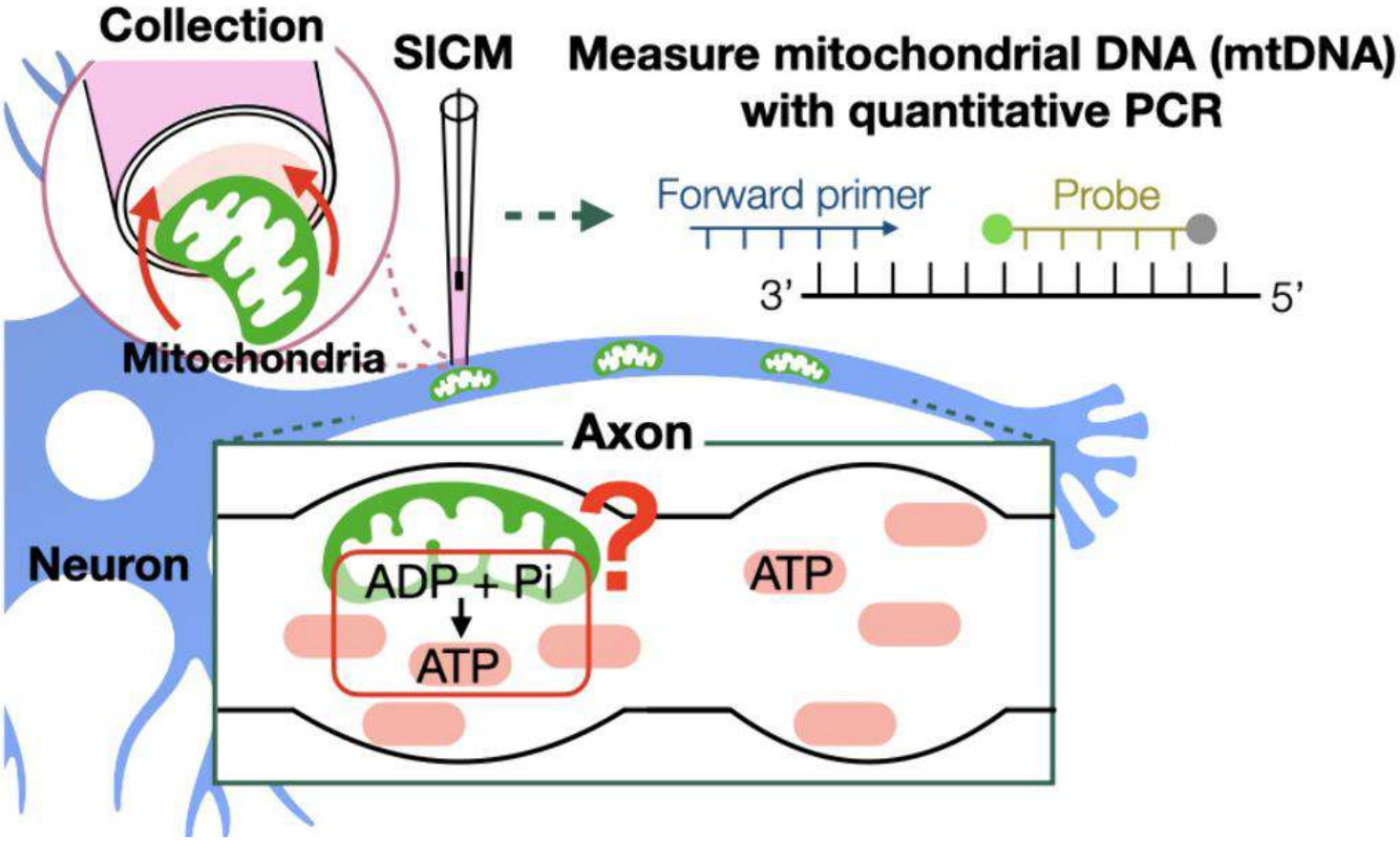
The human brain weighs only 2% of the total body mass, yet it uses about 20% of our energy. Most of the energy is consumed by neuronal activity at presynaptic boutons in axons. Mitochondria have been believed to play a main role in the production of this energy, ATP. However, it has been shown that only 50% of presynaptic boutons are associated with the mitochondria in cortical excitatory neurons. Moreover, recently, it has been reported that glycolysis, which is independent of mitochondria, could maintain presynaptic ATP levels. Therefore, it remains unclear to what extent the axonal mitochondria contribute to the ATP supply in neuronal activity. To examine the ATP production capacity of axonal mitochondria, we focused on the amount of mitochondrial DNA (mtDNA), which encodes parts of essential respiratory chain complex proteins, in each axonal mitochondrion. We aimed to determine the amount of mtDNA in single mitochondria by using quantitative PCR. To selectively isolate single axonal mitochondria from primary neuron culture, we developed a novel microscope by combining the scanning ion conductance microscope (SICM) with the fluorescence microscope. In this conference, I would like to report the characteristics of axonal mitochondria isolated by the SICM-Fluorescence

microscope.


Collection
SICM
Measure mitochondrial DNA (mtDNA)
with quantitative PCR
Forward primer
Probe
3'
5'
Mitochondria
Axon
Neuron
ADP + Pi
ATP
ATP
?

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BOARD NUMBER: S04-258

CHOLESTEROL HOMEOSTASIS IN BRAIN FROM SAMP8 MICE: AGE-DEPENDENT EFFECT OF RESVERATROL IN CHOLESTEROL REGULATION

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Cholesterol displays a large variety of biological functions. In brain it represents ca. 23% of the total body cholesterol. Its presence and ability to regulate the fluidity of plasma membrane is crucial for the maintaining of correct neurotransmission. Cholesterol homeostasis has been reported to be dysregulated in neurodegeneration, but underlying mechanisms involved are poorly known. On the other hand, resveratrol (RSV) is an antioxidant polyphenol with neuroprotective properties. Therefore, the aim of this work was to study the age-related molecular implications of cholesterol uptake, efflux, transport and *de novo* synthesis and the effect of RSV on the cholesterol homeostasis in brain from SAMP8 mice, an animal model of aging and Alzheimer's disease. Results clearly showed an age-related increase of free-cholesterol in plasma membrane and ApoE, whereas LDL receptor, HMG-CoA-Reductase, HMG-CoA-C1 Synthase and ABCA1 transporter remained unaltered with age. On the other side, RSV treatment exhibited a different effect on cholesterol metabolism depending on age. While levels of free-cholesterol were not affected by RSV, mechanisms related to cholesterol uptake, efflux, transport and *de novo* synthesis were differently affected. In younger RSV-treated mice, LDL receptor, HMG CoA-Reductase and HMG-CoA-C1 Synthase were significantly decreased and increased, respectively, whereas ApoE and ABCA1 transporter remain unchanged. However, ApoE and HMG CoA-C1 Synthase were reduced, whereas LDL receptor, ABCA1 transporter and HMG CoA-Reductase remained unchanged in older RSV-treated mice. In conclusion, age is a prominent factor that causes cholesterol homeostasis dysregulation in brain and RSV treatment exhibited a different effect on cholesterol metabolism depending on age.

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BOARD NUMBER: S04-259

AXONAL MITOCHONDRIAL CALCIUM EFFLUX MODULATES PRESYNAPTIC BIOENERGETICS

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

Anjali Amrapali Vishwanath[1,2], Abhi Aggarwal[3,4], Kaspar Podgorski[3,4], Jaime De Juan-Sanz[1,2]
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Synaptic communication is a highly energy consuming process which is central to the functioning of the brain. It is highly plastic and thus subject to variations in firing intensity and timing. Hence, energy production must also be flexible and coordinated with synaptic activity. In the pre-synapse, this process of on-demand energy production takes place mainly in the mitochondria and is driven by calcium entry into the mitochondrial matrix during firing. Although the mechanism and importance of mitochondrial calcium influx in controlling synaptic Adenosine triphosphate (ATP) production has been established, how mitochondria ensure that matrix calcium (Ca^{2+}) levels are rapidly restored to normal levels remains poorly understood. Our project aims to understand the mechanisms and importance of calcium export in axonal mitochondria. Combining genetic and optical tools with electrophysiological stimulation, we show that the protein ***Letm1*** (Leucine Zipper and EF-Hand Containing Transmembrane Protein 1), located on the inner mitochondrial matrix, regulates calcium efflux. Knockdown of Letm1 leads to increased mitochondrial calcium dwell-time, subsequently leading to excessive activity-dependent ATP production and the accumulation of presynaptic ATP in firing synapses. With respect to its effect on overall presynaptic function, knockdown of Letm1 does not change the vesicle fusion or endocytosis rates but leads to an excessive release of the neurotransmitter glutamate. Indeed, mutations in Letm1 have been linked to the occurrence of epilepsy in human patients. In the future, we aim to characterize and establish the link between mitochondrial calcium retention and the production of the neurotransmitter glutamate.

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BOARD NUMBER: S04-261

DOSE-DEPENDENT DIFFERENTIAL EFFECTS OF INTERMITTENT KETOSIS ESTABLISHED BY MEDIUM-CHAIN TRIGLYCERIDE SUPPLEMENTATION ON COGNITIVE PARAMETERS IN RATS

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

Alexander Trofimov[1], Veronika Nikitina[1], Irina Ivleva[1], Alexander Schwarz[2], Darya Krytskaya[1], Sergey Apryatin[1], Marina Karpenko[1], Ksenia Shcherbakova[1]
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Aims: Medium-chain triglycerides (MCT) demonstrated a range of neuroprotective effects, mostly attributed to their ketogenic properties. However, the exact mechanisms of the MCT effects on the brain and the scope of targetable deficits and cognitive functions are largely unknown. Long-term metabolic consequences of MCT supplementation in cognition-enhancing doses have not been rigorously investigated. **Methods:** We administered MCT oil (1, 3, or 6 g/kg/day) or water (control) orogastrically to 2.5-m.o. male Wistar rats for 28 days, without limiting access to standard chow, and assessed working memory in Y-maze, behavior in the Open Field, spatial learning in Morris water maze (MWM), and weight of internal organs. In a separate experiment, we evaluated the effects of MCT (3 g/kg) or lard (3 g/kg, control) supplementation for 28 days on blood biochemical parameters. **Results:** In Y-maze, MCT-1 and MCT-3 treatment improved working memory. In MWM, MCT-6 treatment improved spatial memory. The MCT-6 treatment increased liver weight and the brown/white adipose tissue ratio. Chronic MCT treatment (3 g/kg) did not affect the blood levels of glucose, lactate, pyruvate, acetoacetate, β-hydroxybutyrate, total and HDL cholesterol, triglycerides, malondialdehyde, and aspartate transaminase and alanine transaminase activities. **Conclusions:** Chronic MCT supplementation of regular chow demonstrated a non-uniform dose-dependent effect on the measured cognitive parameters. For spatial memory, the effect disappeared at the highest dose, which was also associated with changes in organ weight and adipose tissue metabolism. Further detailed studies are needed to better understand the neuroprotective effects of MCT. Supported by Russian Science Foundation (RSF, project no. 19-75-10076).

Pubmed:
27184538: Couch Y, Trofimov A, Markova N, Nikolenko V, Steinbusch HW, Chekhonin V, Schroeter C, Lesch KP, Anthony DC, Strekalova T
Low-dose lipopolysaccharide (LPS) inhibits aggressive and augments depressive behaviours in a chronic mild stress model in mice.
Aggression, hyperactivity, impulsivity, helplessness and anhedonia are all signs of depressive-like disorders in humans and are often reported to be present in animal models of depression induced by stress or by inflammatory challenges. However, chronic mild stress (CMS) and clinically silent inflammation, during the recovery period after an infection, for example, are often coincident, but comparison of the behavioural and molecular changes that underpin CMS vs a mild inflammatory challenge and impact of the combined challenge is largely unexplored. Here, we examined whether stress-induced behavioural and molecular responses are analogous to lipopolysaccharide (LPS)-induced behavioural and molecular effects and whether their combination is adaptive or maladaptive.
J Neuroinflammation, 2016; 13
28421528: Trofimov A, Strekalova T, Mortimer N, Zubareva O, Schwarz A, Svirin E, Umriukhin A, Svistunov A, Lesch KP, Klimenko V
Postnatal LPS Challenge Impacts Escape Learning and Expression of Plasticity Factors Mmp9 and Timp1 in Rats: Effects of Repeated Training.
Bacterial intoxication associated with inflammatory conditions during development can impair brain functions, in particular evolutionarily novel forms of memory, such as explicit learning. Little is known about the dangers of early-life inflammation on more basic forms of learning, for example, the acquisition of motor escape abilities, which are generally better preserved under pathological conditions. To address this limitation in knowledge, an inflammatory response was elicited in Wistar pups by lipopolysaccharide (LPS) injections (25 μg/kg) on postnatal days P15, P18 and P21. The acquisition of escape behaviour

was tested from P77 by active avoidance footshock model and water maze. Open-field behaviour and blood corticosterone levels were also measured. Rat brain tissue was collected from pups 2 h post-injection and from adult rats which either underwent escape training on P77-P81 or remained untrained. mRNA levels of developmental brain plasticity factors MMP-9 and TIMP-1 were investigated in the medial prefrontal cortex and ventral/dorsal hippocampus. LPS-challenged rats displayed moderately deficient escape responses in both memory tests, increased freezing behaviour and, surprisingly, reduced blood cortisol levels. Mmp9 and Timp1, and their ratio to one another, were differentially altered in pups versus adult untrained rats but remained unchanged overall in rats trained in either learning task. Together, our data indicate that systemic pro-inflammatory response during early postnatal development has long-lasting effects, including on the acquisition of motor escape abilities and plasticity factor expression, into adulthood. Our data suggest that altered stress response could possibly mediate these deviations and repeated training might generate positive effects on plasticity under the employed conditions.
Neurotox Res, 2017; 32
28673768: Schwarz AP, Trofimov AN, Zubareva OE, Lioudyno VI, Kosheverova VV, Ischenko AM, Klimenko VM
Prefrontal mRNA expression of long and short isoforms of D2 dopamine receptor: Possible role in delayed learning deficit caused by early life interleukin-1β treatment.
Long (D2L) and short (D2S) isoform of the D2 dopamine receptor are believed to play different roles in behavioral regulation. However, little is known about differential regulation of these isoforms mRNA expression during the process of learning in physiological and pathological states. In this study, we have investigated the combined effect of training in active avoidance (AA) paradigm and chronic early life treatment with pro-inflammatory cytokine interleukin (IL)-1β (1μg/kg i.p., P15-21) on D2S and D2L dopamine receptor mRNA expression in the medial prefrontal cortex (mPFC) of adult rats. We have shown differential regulation of D2 short and long mRNA isoform expression in the mPFC. There was no effect of AA-training on D2S mRNA expression, while D2L mRNA was downregulated in AA-trained control (intact and saline-treated) animals, and this effect was not observed in rats treated with IL-1β. D2S mRNA expression level negatively correlated with learning ability within control (saline-treated and intact) groups but not in IL-1β-treated animals. Thus, prefrontal expression of distinct D2 dopamine receptor splice variants is supposed to be implicated in cognitive decline caused by early life immune challenge.
Behav Brain Res, 2017; 333
28856531: Strekalova T, Bahzenova N, Trofimov A, Schmitt-Böhrer AG, Markova N, Grigoriev V, Zamoyski V, Serkova T, Redkozubova O, Vinogradova D, Umriukhin A, Fisenko V, Lillesaar C, Shevtsova E, Sokolov V, Aksinenko A, Lesch KP, Bachurin S
Pro-neurogenic, Memory-Enhancing and Anti-stress Effects of DF302, a Novel Fluorine Gamma-Carboline Derivative with Multi-target Mechanism of Action.
A comparative study performed in mice investigating the action of DF302, a novel fluoride-containing gamma-carboline derivative, in comparison to the structurally similar neuroprotective drug dimebon. Drug effects on learning and memory, emotionality, hippocampal neurogenesis and mitochondrial functions, as well as AMPA-mediated currents and the 5-HT6 receptor are reported. In the step-down avoidance and fear-conditioning paradigms, bolus administration of drugs at doses of 10 or 40 mg/kg showed that only the higher dose of DF302 improved long-term memory while dimebon was ineffective at either dosage. Short-term memory and fear extinction remained unaltered across treatment groups. During the 5-day predation stress paradigm, oral drug treatment over a period of 2 weeks at the higher dosage regimen decreased anxiety-like behaviour. Both compounds supressed inter-male aggression in CD1 mice, the most eminent being the effects of DF302 in its highest dose. DF302 at the higher dose decreased floating behaviour in a 2-day swim test and after 21-day ultrasound stress. The density of Ki67-positive cells, a marker of adult neurogenesis, was reduced in the dentate gyrus of stressed dimebon-treated and non-treated mice, but not in DF302-treated mice. Non-stressed mice that received DF302 had a higher density of Ki67-positive cells than controls unlike dimebon-treated mice. Similar to dimebon, DF302 effectively potentiated AMPA receptor-mediated currents, bound to the 5-HT6 receptor, inhibited mitochondrial permeability transition and displayed cytoprotective properties in cellular models of neurodegeneration. Thus, DF302 exerts multi-target effects on the key mechanisms of neurodegenerative pathologies and can be considered as an optimized novel analogue of the neuroprotective agent dimebon.
Mol Neurobiol, 2018; 55
29550264: Shavva VS, Bogomolova AM, Efremov AM, Trofimov AN, Nikitin AA, Babina AV, Nekrasova EV, Dizhe EB, Oleinikova GN, Missyul BV, Orlov SV
Insulin downregulates C3 gene expression in human HepG2 cells through activation of PPARγ.
C3 is an acute phase protein, and thus its plasma concentration increases quickly and drastically during the onset of inflammation. Insulin plays a complex role in inflammation. Elevated level of plasma C3 was shown to correlate with heightened fasting insulin levels and insulin resistance and appears to be a risk factor for the cardiovascular disease and atherosclerosis. The main source of plasma C3 is liver. Nothing is known about effects of insulin on C3 gene expression and protein secretion by hepatocytes. In light of these data we asked if insulin is capable of regulating C3 production in

hepatocytes. Here we show that insulin downregulates C3 gene expression in human hepatoma cells HepG2 through activation of PI3K, mTORC1, p38 and MEK1/2 signaling pathways. Transcription factors PPARα, PPARγ, HNF4α and NF-κB are important contributors to this process. Insulin activates PPARγ through PI3K/Akt/mTORC1 pathway, which results in PPARγ binding to DR4 and DR0 cis-acting elements within the C3 promoter and subsequent displacement of HNF4α and PPARα from these sites. As a result PPARα/NF-κB complex, which exists on C3 promoter, is broken down and C3 gene expression is downregulated. The data obtained can potentially be used to explain the molecular mechanism underlying the correlation between heightened level of plasma C3 and insulin resistance in humans.
Eur J Cell Biol, 2018; 97
30092312: Schwarz AP, Rotov AY, Chuprina OI, Krytskaya DU, Trofimov AN, Kosheverova VV, Ischenko AM, Zubareva OE
Developmental prefrontal mRNA expression of D2 dopamine receptor splice variants and working memory impairments in rats after early life Interleukin-1β elevation.
Long (D2L) and Short (D2S) isoforms of D2 dopamine receptor differ in their biochemical and physiological properties, which could affect functioning of prefrontal cortex. Contribution of distinct D2 dopamine receptor isoforms to cognitive dysfunctions and its developmental regulation are currently not fully elucidated. In the present study, we evaluated developmental mRNA expression of D2S/D2L dopamine receptor isoforms within the rat medial prefrontal cortex (mPFC) in the model of neurodevelopmental cognitive dysfunction. Working memory performance (Y-maze spontaneous alternations) and D2S/D2L mRNA expression in the mPFC (by qRT-PCR) were evaluated in juvenile (P27), adolescent (P42-47) and adult (P75-90) rats after chronic early life treatment with proinflammatory cytokine interleukin (IL)-1β (1 μg/kg i.p. daily P15-21). It was shown that IL-1β elevation during the 3rd week of life leads to working memory deficit originating in juvenile animals and persisting into adulthood. D2S mRNA expression was strongly downregulated during adolescence, and such downregulation was exaggerated in animals injected with IL-1β during P15-21. Early life IL-1β administrations influenced developmental changes in the D2S/D2L mRNA ratio. This measure was found to be decreased in adolescent and adult control (intact and vehicle-treated) rats compared to juvenile control, while in the case of IL-1β-treated animals, the decrease in D2S/D2L ratio was observed only in adulthood but not in adolescence compared to juvenile rats. During the adolescence, D2S mRNA expression was downregulated and D2S/D2L ratio was upregulated in the mPFC of rats treated with IL-1β during the 3rd week of life compared to controls. Based on these data we conclude that changes in the developmental expression of D2 dopamine receptor splice variants within mPFC may underlie long-lasting cognitive deficit associated with neonatal pathology.
Neurobiol Learn Mem, 2018; 155
34225597: Nikitina VA, Zakharova MV, Trofimov AN, Schwarz AP, Beznin GV, Tsikunov SG, Zubareva OE
Neonatal Exposure to Bacterial Lipopolysaccharide Affects Behavior and Expression of Ionotropic Glutamate Receptors in the Hippocampus of Adult Rats after Psychogenic Trauma.
According to the two-hit hypothesis of psychoneuropathology formation, infectious diseases and other pathological conditions occurring during the critical periods of early ontogenesis disrupt normal brain development and increase its susceptibility to stress experienced in adolescence and adulthood. It is believed that these disorders are associated with changes in the functional activity of the glutamatergic system in the hippocampus. Here, we studied expression of NMDA (GluN1, GluN2a, GluN2b) and AMPA (GluA1, GluA2) glutamate receptor subunits, as well as glutamate transporter EAAT2, in the ventral and dorsal regions of the hippocampus of rats injected with LPS during the third postnatal week and then subjected to predator stress (contact with a python) in adulthood. The tests were performed 25 days after the stress. It was found that stress altered protein expression in the ventral, but not in the dorsal hippocampus. Non-stressed LPS-treated rats displayed lower levels of the GluN2b protein in the ventral hippocampus vs. control animals. Stress significantly increased the content of GluN2b in the LPS-treated rats, but not in the control animals. Stress also affected differently the exploratory behavior of LPS-injected and control rats. Compared to the non-stressed animals, stressed control rats demonstrated a higher locomotor activity during the 1st min of the open field test, while the stressed LPS-injected rats displayed lower locomotor activity than the non-stressed rats. In addition, LPS-treated stressed and non-stressed rats spent more time in the open arms of the elevated plus maze and demonstrated reduced blood levels of corticosterone. To summarize the results of our study, exposure to bacterial LPS in the early postnatal ontogenesis affects the pattern of stress-induced changes in the behavior and hippocampal expression of genes coding for ionotropic glutamate receptor subunits after psychogenic trauma suffered in adulthood.
Biochemistry (Mosc), 2021; 86
25697011: Trofimov AN, Zubareva OE, Shvarts AP, Ishchenko AM, Klimenko VM
[The administration of interleukin-1beta during early postnatal develop ment impairs FGF2, but not TIMP1, mRNA expression in brain structures of adult rats].
According to the Neurodevelopmental hypothesis, the long-lasting cognitive deficit in schizophrenia and other types of neuropathology may occur by injurious factors, such as hypoxia, traumas, infections that take place during pre- and postnatal development, at least at early stages. These pathological conditions are often associated with the high production of pro-inflammatory cytokine interleukin-1B (IL-1B) by the cells of immune and nervous systems. We investigated the expression of

genes involved in the neuroplastic regulation (Fgf2 and Timp2) in medial prefrontal cortex and dorsal and ventral regions of hippocampus of adult rats that were treated with IL-1beta between P15 and P21. The learning impairment in IL-1beta-treated rats is accompanied by lower FGF-2 mRNA levels in medial prefrontal cortex and ventral (not dorsal) hippocampus, but TIMP-1 was not affected. No differences in TIMP-1 and FGF-2 mRNA expressions were observed in untrained IL-1beta-treated when compared to control rats.
Ross Fiziol Zh Im I M Sechenova, 2014; 100

23013016: Trofimov AN, Zubareva OE, Simbirtsev AS, Klimenko VM
[The influence of neonatal interleukin-1beta increase on the formation of adult rats' spatial memory].
Children's and adults' cognitive dysfunctions are frequently caused by various types of pathology such as birth injuries, hypoxias, and infections suffered in prenatal and early postnatal periods of ontogenesis. These abnormal conditions trigger high production of proinflammatory cytokines by the cells of nervous and immune systems. The role of interleukin-1 beta (IL-1beta), one of such proteins, in the formation of cognitive deficit in early ontogenesis is not sufficiently studied. In present research it was revealed that administration of IL-1beta during the third week of postnatal ontogenesis impaired the learning of adult rats in Morris Water Maze. The differences between rats of control and experimental groups were observed during the training of searching for hidden platform and during the alteration of formed reflex (when the platform was in a different place). Meanwhile the spatial extinction has not been disrupted. The nature of experimental rats' learning abnormalities allows us to assume that the mechanisms of long-term but not short-term spatial memory are damaged in this experimental situation.
Ross Fiziol Zh Im I M Sechenova, 2012; 98

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BOARD NUMBER: S04-262

ELECTROPHYSIOLOGICAL CHARACTERIZATION AND COMPUTATIONAL MODELING OF INSULIN PRODUCING CELLS IN DROSOPHILA

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

Federico Cascino Milani[1], Lorenzo Fontolan[2], Sabine Fischer[3], Jan Ache[1]
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Insulin Producing Cells (IPCs) are an important population of modulatory neurons in the insect brain that release Insulin-like Peptides (ILPs). ILPs have been demonstrated to play a key role in metabolic homeostasis, aging, development, and the modulation of behavior in different species, including *Drosophila melanogaster*, our model of choice. Quantifying IPC activity dynamics is crucial to developing an understanding of insulin signaling, but this task is quite challenging: IPCs are interconnected with a complex network of neuromodulatory neurons that have a wide variety of effects on IPCs and form numerous direct and indirect reciprocal connections. Due to the complexity of this system, an experimental approach is insufficient to develop a comprehensive understanding of the neural mechanisms that underlie the modulation of IPC activity, and thus insulin release. Theoretical models are powerful tools to study the dynamics of such interactions in a coherent framework. Hence, we decided to use a combined approach of electrophysiological characterization and computational modeling of IPCs and the complex network they are part of. Our aim is to develop a conductance-based neuron model of IPCs and some of their key pre- and postsynaptic partners. To feed our model, we use data collected in patch-clamp recordings of individual IPCs quantifying their intrinsic electrophysiological properties combined with optogenetic functional connectivity experiments. This data-driven model will allow us to analyze how neuromodulators produce circuit-level changes that affect the IPC network. These results will contribute to broaden our understanding of neuromodulation and metabolism in general.

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DIETARY EFFECTS ON THE ACTIVITY OF INSULIN PRODUCING CELLS IN DROSOPHILA

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Insulin signaling plays a key role in controlling metabolic homeostasis. In addition, insulin is heavily implicated in processes underlying reproduction, aging and stress resistance. Insulin producing cells (IPCs) in *Drosophila* are functionally analogous to mammalian pancreatic beta cells and produce different *Drosophila* insulin like peptides (DILPs) during different stages of a fly's lifespan. In the adult fly, release of these DILPs is dependent on nutrient availability. Nutrient sensing is vital for metabolic control. Fuel substrates such as glucose serve immediate energy demanding processes such as locomotion, mating, etc. *Drosophila* IPCs are hypothesized to play a role in sensing hemolymph glucose levels cell-autonomously. Therefore, we set out to perform an *in-vivo* electrophysiological study of nutrient sensing in Drosophila IPCs. To label IPCs for patch-clamp recordings, we used a Dilp2-Gal4 driver line to express GFP in IPCs of the adult *Drosophila* brain. Using these flies, we performed targeted *in-vivo* patch-clamp recordings from IPCs while perfusing the brain with artificial hemolymph containing different concentrations of glucose and other sugars. Furthermore, we exposed flies to different diets and quantified their effects on IPC activity. In addition to quantifying the different dietary effects, we explored the effects of aging on IPC activity. We found that nutritional state and aging strongly affected IPC activity. However, varying extracellular glucose levels caused no significant changes in their activity. This indicates that, although dietary sugars have strong effects on IPC activity, IPCs are not sensitive to local changes in extracellular glucose levels *in-vivo*.

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BEHAVIORAL STATE-DEPENDENT MODULATION OF INSULIN-PRODUCING CELLS IN DROSOPHILA

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Insulin plays a pivotal role in metabolic control, neuromodulation, and aging, but its release dynamics are not well understood. We used *Drosophila melanogaster* to study effects of locomotion on the activity of insulin-producing cells (IPCs). Using a combination of *in-vivo* patch-clamp recordings, calcium imaging, and optogenetics, we found that IPCs were inhibited during walking and flight. This modulation was graded, such that the inhibition was stronger during flight – the more energy-demanding behavior. A resulting decrease in insulin levels would support the mobilization of fuel stores and the suppression of anabolic processes during locomotion. IPC activity was increased immediately after cessation of locomotion. This rebound could contribute to replenishing muscle glycogen stores. Surprisingly, IPC modulation preceded the onset of locomotion, suggesting a feedforward mechanism impinging on IPCs. This was further supported by *ex-vivo* recordings combined with optogenetic activation of motor circuits, which revealed that IPC inhibition neither requires actual behavior, nor decreased blood sugar levels – a simple motor command was sufficient to inhibit IPCs. In a nutshell, we add the behavioral state to the list of factors regulating IPC activity in *Drosophila*. These rapid changes in IPC activity precede locomotion and may serve an increased metabolic demand. Moreover, high insulin levels are known to decrease the sensitivity of olfactory sensory neurons in flies. Hence, the inhibition of IPCs could lead to a disinhibition of olfactory sensory neurons, which could increase the likelihood of locating food sources during locomotion. Thus, behavioral state-dependent IPC modulation might enable differential sensorimotor processing.

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THE EXPRESSION OF PYRUVATE CARBOXYLASE IN HUMAN BRAIN AND IN CELL LINES OF ASTROCYTES

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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[1]Comenius University Bratislava, Jessenius Faculty of Medicine in Martin, Department Of Medical Biochemistry, Martin, Slovak Republic, [2]Jessenius faculty of Medicine, Comenius University Bratislava, Jessenius Faculty of Medicine in Martin, Department Of Medical Biochemistry, Martin, Slovak Republic, [3]Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Biomedical Centre Martin, Martin, Slovak Republic

Pyruvate carboxylase (PC) is a mitochondrial, biotin-containing enzyme catalyzing the ATP-dependent synthesis of oxaloacetate from pyruvate and bicarbonate. PC has an important anaplerotic role in sustaining the brain metabolism because it compensate for TCA cycle intermediates loss and prevent a collapse of the mitochondrial metabolism. Based on the studies performed on animal models, the expression of PC was assigned to be glia-specific. We probed the cultured human astrocytes and brain sections with antibodies against PC to study PC distribution among human neural cells. Additionally, we tested the importance of PC for the viability of cultured human astrocytes by applying the PC inhibitor 3-chloropropane-1,2-diol (CPD). Our results establish the presence of PC in cultured astrocytes and brain tissue. In addition to astrocytes, a subpopulation of the neurons in the cerebral cortex appeared to bear PC. CPD negatively affected the viability of astrocytes in culture, which could be reversed by supplementation of media with citrate, malate, and other metabolites from the TCA cycle. Our results confirmed the expected expression of PC by human astrocytes in culture and brain parenchyma. In addition to astrocytes, a subpopulation of the neurons in the cerebral cortex appeared to express PC. Furthermore, the enzymatic activity of PC is crucial for sustaining the viability of cultured astrocytes. This work was supported by the Slovak Research and Development Agency under Contract No. APVV-18-0088 and VEGA 1/0255/20.

Pubmed:

35158853: Gondáš E, Kráľová Trančíková A, Baranovičová E, Šofranko J, Hatok J, Kowtharapu BS, Galanda T, Dobrota D, Kubatka P, Busselberg D, Murín R
Expression of 3-Methylcrotonyl-CoA Carboxylase in Brain Tumors and Capability to Catabolize Leucine by Human Neural Cancer Cells.
Leucine is an essential, ketogenic amino acid with proteinogenic, metabolic, and signaling roles. It is readily imported from the bloodstream into the brain parenchyma. Therefore, it could serve as a putative substrate that is complementing glucose for sustaining the metabolic needs of brain tumor cells. Here, we investigated the ability of cultured human cancer cells to metabolize leucine. Indeed, cancer cells dispose of leucine from their environment and enrich their media with the metabolite 2-oxoisocaproate. The enrichment of the culture media with a high level of leucine stimulated the production of 3-hydroxybutyrate. When C-leucine was offered, it led to an increased appearance of the heavier citrate isotope with a molar mass greater by two units in the culture media. The expression of 3-methylcrotonyl-CoA carboxylase (MCC), an enzyme characteristic for the irreversible part of the leucine catabolic pathway, was detected in cultured cancer cells and human tumor samples by immunoprobing methods. Our results demonstrate that these cancer cells can catabolize leucine and furnish its carbon atoms into the tricarboxylic acid (TCA) cycle. Furthermore, the release of 3-hydroxybutyrate and citrate by cancer cells suggests their capability to exchange these metabolites with their milieu and the capability to participate in their metabolism. This indicates that leucine could be an additional substrate for cancer cell metabolism in the brain parenchyma. In this way, leucine could potentially contribute to the synthesis of metabolites such as lipids, which require the withdrawal of citrate from the TCA cycle.
Cancers (Basel), 2022; 14
33880999: Gondáš E, Kráľová Trančíková A, Majerčíková Z, Pokusa M, Baranovičová E, Bystrický P, Dobrota D, Murín R
Expression of pyruvate carboxylase in cultured human astrocytoma, glioblastoma and neuroblastoma cells.
Pyruvate carboxylase (PC) is an enzyme catalyzing the conversion of pyruvate to oxaloacetate, which possesses anaplerotic role in cellular metabolism. The expression of PC was confirmed in cells of several cancer types, in which it ensures several cellular functions, such as growth and division. To investigate the expression of PC in human astrocytoma, glioblastoma and neuroblastoma cells we applied the immunodetection methods. The results of the Western blot analysis and

immunocytochemical detection revealed the presence of PC in human astrocytoma, glioblastoma and neuroblastoma cells. Furthermore, application of PC inhibitor, 3-chloro-1,2-dihydroxypropane (CDP), negatively impacts the viability of astrocytoma cells. The cytotoxic effect of CDP could be partially reversed by application of citrate, 2-oxoglutarate and malate in incubation media. Our results revealed that astrocytoma, glioblastoma and neuroblastoma cells are equipped with PC, which might significantly contribute by its anaplerotic activity to sustain the metabolism of cancer cells.
Gen Physiol Biophys, 2021; 40

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DIETARY METHYLGLYOXAL IMPACTS METABOLISM AND BRAIN INFLAMMATION

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Background: Methylglyoxal (MGO), a by-product of glycolysis, is a major precursor in the formation of advanced glycation end products (AGEs) and are associated with cognitive impairment. MGO can be formed endogenously during diabetes and degraded to D-lactate by the glyoxalase (Glo) system. MGO can also be increased by dietary intake. The impact of dietary MGO on the brain is, however, unknown. Method: Adult male C57Bl/6J mice were treated with MGO (1mmol/L, drinking water) or not (control) for 3 months (n=16/group). Glo-1 protein activity was quantified as conversion rate of MGO into S-D-lactoylglutathione. MGO was measured using UHPLC-MS/MS in plasma and brain. Brain gene expression was analysed with RT-PCR. Plasma inflammatory markers were measured by ELISA. Results: Dietary MGO led to a reduction in non-fasting glucose ($p<0.0001$) and an increase in plasma levels of insulin ($p<0.0002$), leptin ($p<0.02$), CRP ($p=0.005$), IL-10 ($p=0.03$) and TNFα ($p=0.02$). Plasma MGO was unchanged, but in the subcortical region we found a 50% decrease in MGO content ($p<0.0001$). Expression and activity of Glo-1 and Glo-2 mRNA expression was unchanged, while Glo-1 activity was decreased by 9% in subcortical regions ($p=0.0016$). In cortical and subcortical regions we found a decrease in MCP-1 mRNA ($p=0.005$ and $p=0.02$) and an increase in the cortical synaptophysin mRNA ($p=0.03$). Conclusion: MGO intake decreased brain MGO content, which was associated with a reduction of the expression of inflammatory marker MCP-1 in the brain. The reduction of MGO in the brain may be indirectly due to dietary MGO induced metabolic changes.

Pubmed:
33461408: Kylkilahti TM, Berends E, Ramos M, Shanbhag NC, Töger J, Markenroth Bloch K, Lundgaard I
Achieving brain clearance and preventing neurodegenerative diseases-A glymphatic perspective.
Age-related neurodegenerative diseases are a growing burden to society, and many are sporadic, meaning that the environment, diet and lifestyle play significant roles. Cerebrospinal fluid (CSF)-mediated clearing of brain waste products via perivascular pathways, named the glymphatic system, is receiving increasing interest, as it offers unexplored perspectives on understanding neurodegenerative diseases. The glymphatic system is involved in clearance of metabolic by-products such as amyloid-β from the brain, and its function is believed to lower the risk of developing some of the most common neurodegenerative diseases. Here, we present magnetic resonance imaging (MRI) data on the heart cycle's control of CSF flow in humans which corroborates findings from animal studies. We also review the importance of sleep, diet, vascular health for glymphatic clearance and find that these factors are also known players in brain longevity.
J Cereb Blood Flow Metab, 2021; 41

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BRAIN HISTONE BETA-HYDROXY-BUTYRYLATION COUPLES METABOLISM WITH GENE EXPRESSION

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Metabolic status has a well-documented influence on peripheral organs' physiology and pathology; however mounting evidence suggests that it can also affect brain function. For example, brain resilience to aging is enhanced by caloric restriction, and ketogenic diets have been used to treat neurological diseases. Unfortunately, little is known about the impact of metabolic stimuli on brain tissue at a molecular level. Recent data obtained in liver tissue suggest that beta-hydroxybutyrate (BHB) can be a key signaling molecule regulating gene transcription. Thus, we adopted a ketogenic metabolic challenge, 48 hrs of fasting, and then assessed lysine beta-hydroxybutyrylation (K-bhb) levels in proteins extracted from the cerebral cortex. Fasting enhanced K-bhb in a variety of proteins and on histone H3. ChIP-seq experiments showed that K9 beta-hydroxybutyrylation of H3 (H3K9-bhb) was significantly enriched by fasting on more than 8000 DNA loci. Transcriptomic analysis showed that H3K9-bhb on enhancers and promoters correlated with active gene expression. Since one of the most enriched functional annotations both at the epigenetic and transcriptional level was “circadian rhythms", we studied the expression of core-clock genes in the cortex during fasting. We found that the diurnal oscillation of specific transcripts was modulated at distinct times of the day along the circadian cycle. Moreover, fasting caused long-lasting changes in diurnal locomotro behaviour. Thus, our results suggest that fasting dramatically impinges on the cerebral cortex transcriptional and epigenetic landscape, and BHB acts as a powerful epigenetic molecule in the brain through direct and specific histone marks remodelling in neural tissue cells.

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THE INHIBITION OF DREAM PROTEIN AS A POTENTIAL TREATMENT AGAINST METABOLIC SYNDROME AND ITS ASSOCIATED NEUROLOGIC SIGNS.

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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High fat diet (HFD) chronic intake induces metabolic syndrome in mice, characterized by a body weight increase and insulin resistance (IR). Obesity is a risk factor in the development of neurodegenerative diseases, neuropsychiatric disorders and is associated with cognitive decline. DREAM/kchip3/calsenilin (DREAM) is a multifunctional protein that belong to neuronal Ca2+ sensors family. Previous studies have demonstrated the relevance of DREAM in nociception and in learning and memory processes. In the present study we characterize the consequences of DREAM, genetic or pharmacologic, inhibition in the metabolic and behavioural alterations caused by HFD intake in mice. Our results showed that chronic pharmacological and genetic DREAM inhibition block the metabolic syndrome development, and both of its neurologic comorbidity symptoms: the anxiety-related behaviour, and the cognition deficiency. Also, pharmacologic DREAM inhibition when metabolic syndrome is established did not affect metabolic parameter but improved metabolic syndrome-related neurologic alterations. Therefore, in this study we demonstrate: DREAM inhibition may be a potential treatment to restore neurological symptoms related with metabolic syndrome; and to block metabolic syndrome induced by HFD intake.

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ASTROCYTIC GLUT1 ABLATION IMPROVES SYSTEMIC GLUCOSE METABOLISM AND PRESERVES MEMORY THROUGH ENHANCED INSULIN-STIMULATED ATP RELEASE

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Aims: Brain glucose supply is controlled by glucose transporter GLUT1, highly expressed in astrocytes. Ablating vascular GLUT1 leads to brain hypometabolism and impaired cognition, but this approach cannot discriminate between insufficient glucose supply and vascular breakdown-derived effects. Such question is the focus of the present work, which aims to elucidate the relevance of astrocytic GLUT1 to glucose metabolism and cognition. **Methods:** *In vitro*, cellular metabolism was examined using an extracellular flux analyzer (Seahorse). *In vivo*, astrocyte-specific gene ablation was performed using tamoxifen-inducible Cre/LoxP approaches. ^{18}F-FDG PET, glucose tolerance, insulin secretion and fasting-induced hyperphagia were characterized. Memory was assessed using the Novel Object Recognition and Morris Water Maze tasks. Purinergic agonists or antagonists were intracerebroventricularly administered before each task. ATP levels were determined using microdialysis. **Results:** GLUT1-ablated astrocytes featured reduced glucose uptake and glycolysis, but intact ATP production. Unexpectedly, mice subjected to astrocytic GLUT1 deletion (GLUT1$^{\Delta GFAP}$) showed increased CNS glucose utilization and improved metabolic status, being more efficient at suppressing hyperphagia and readjusting systemic glucose levels after hyperglycemia. Moreover, GLUT1$^{\Delta GFAP}$ mice performed memory tasks adequately. Remarkably, GLUT1-ablated astrocytes showed enhanced insulin receptor (IR) expression and insulin-stimulated ATP release. Blocking brain purinergic signaling was sufficient to prevent GLUT1$^{\Delta GFAP}$-induced metabolic and cognitive abilities. Concomitant ablation of GLUT1 and IR in astrocytes exerted the same effect, which could be rescued upon brain purinergic stimulation. **Conclusion:** Astrocytic GLUT1 ablation drives brain and systemic glucose metabolism towards a more efficient glucose-handling phenotype and promotes memory resilience, requiring enhanced astrocytic IR-dependent ATP release for these features. .

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SULPIRIDE, ANTAGONIST OF D2 DOPAMINE RECEPTOR IMPROVES GLUCOSE ABSORPTION AND INSULIN TOLERANCE, AND ALTERS THE METABOLIC RATE OF MALE C57BL/6 MICE.

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Sulpiride, a selective antagonist of the dopamine D2 receptors, is a second-generation antipsychotic commonly used in the treatment of schizophrenia, depression, and gastroparesis. However, second-generation antipsychotics have been linked to detrimental effects on metabolism, such as increased body weight and hyperglycemia in humans. In male rats on a control chow diet, long term administration of sulpiride induced fatty liver and insulin resistance, and when combined with a high fat diet (HFD), sulpiride treatment resulted in exacerbated hyperglycemia and hyperinsulinemia, with unaltered body weight compared with controls fed an HFD. Here, we evaluated whether the metabolic effects of sulpiride involve actions of this drug on respiratory quotient and energy expenditure in mice. We used 8 week-old C57BL/6 male mice, treated with sulpiride (30 mg/kg/day) during 30 days and evaluated indirect calorimetry with metabolic cages during the last week of sulpiride administration. Interestingly, we found that sulpiride neither altered body weight gain nor caloric intake, but improved insulin tolerance, decreased glucose levels in fasting and posprandial conditions, and diminished serum triglyceride levels. Moreover, sulpiride decreased the respiratory quotient at daytime indicating the use of other sources of energy beyond carbohydrates, and decreased energy expenditure during the night. Together, these results suggest that sulpiride improves metabolic parameters, which is in contradiction to the notion of sulpiride being deleterious for metabolism. These differences may relate to the high doses of sulpiride used as antipsychotic. More research is needed to elucidate the mechanisms that trigger the opposite metabolic effects of sulpiride.

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METABOLIC SYNDROME STATUS AND FITNESS DETERMINE THE ASSOCIATION OF INSULIN RESISTANCE WITH ABNORMAL BRAIN FUNCTIONAL DYNAMICS AND COGNITION IN PRE-DIABETES

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Altered brain functional dynamics in type-2 diabetes is associated with insulin resistance (IR). Here we study whether this relationship can be evidenced in earlier stages such as pre-diabetes, whether it depends on metabolic syndrome status (MS) and/or cardiorespiratory fitness (CRF), and whether it explains variability in cognitive performance. The cross-sectional study included 144 cognitively healthy adults (79 females, 55-78 years), 71 of whom were pre-diabetic. We applied vertex-level linear regression analysis of the entire cortex to test if IR, assessed by the Homeostasis Model Assessment (HOMA-IR), was associated with abnormalities in short- and long-range functional connectivity density (srFCD and lrFCD) hubs and whether these hubs showed altered functional coupling in pre-diabetes as a function of CRF and MS. HOMA-IR and pre-diabetes were associated with reduced srFCD and lrFCD in the left and right medial orbitofrontal cortices (mOFC), respectively. The former was mainly evident in individuals with higher CRF and the latter in individuals with better MS, but neither of these anomalies was related to cognition. Pre-diabetics with lower CRF showed grater coupling between the right mOFC and superior frontal gyrus with increased HOMA-IR that was associated with better cognition; whereas pre-diabetics with worse MS showed greater lrFCD in the left cuneus with increased HOMA-IR that was associated with worse cognition. Results suggests that pre-diabetes and IR may alter functional dynamics within vulnerable networks in Alzheimer’s disease, which might be necessary for maintaining cognitive functioning in individuals with worse CRF and MS.

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RENAL AND CEREBRAL SMALL VESSEL DISEASE: AN INFLAMMATORY TANGLE

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Background - Both kidney and brain are highly susceptible to vascular injury as a result of exposure to vascular risk factors such as hypertension, diabetes, and obesity. Patients with kidney dysfunction are known to have a higher prevalence of cerebrovascular disease. Microvascular dysfunction, subsequent vessel rarefaction, and inflammation are suggested to be involved in this cerebro-renal connection. However, the underlying mechanisms and causative pathways are yet unknown. Our group has demonstrated the presence of cognitive dysfunction and diabetic nephropathy in Obese ZSF1 rats, making this model suitable to study renal and cerebral small vessel disease. **Aim -** To study the sequential relationship between renal and cerebral microvascular dysfunction and rarefaction induced by common risk factors in Obese vs Lean ZSF1 rats. **Methods –** Microvascular dysfunction and expression of inflammatory mediators will be characterized in renal and cerebral tissues from ZSF1 rats at three different time points. The 3D architecture and density of the microcirculation in whole brains and kidneys will be studied using tissue clearing technique combined with 2-photon and light sheet microscopies. Furthermore, mass spectrometry imaging with molecular histology will be used to investigate the pro-inflammatory lipid mediators associated with microvascular dysfunction. **Expected results -** We expect to show that capillary rarefaction and inflammation occur conjointly in renal tissue first and in brain tissue thereafter, related to the progressive expression of a set of pro-inflammatory mediators. The results of this ongoing study will contribute to identifying new inflammatory drivers which wire kidney dysfunction and cerebral vascular pathology together.

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EFFECT OF GRAPE STEMS EXTRACT ON BEHAVIOR AND BIOCHEMICAL MARKERS IN ADULT MALE MICE.

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Aims The winemaking procedure results in the production of stems, a by-product that is harmful for the environment. Simultaneously, grape stems are rich in polyphenols and, therefore, they are putatively beneficial for human health. The aim of the present study was to investigate the effect of the grape stems extract on behavior, metabolism (plasma biomarkers), redox status (liver and brain regions) and on the activity of two isoforms of acetylcholinesterase (brain regions) of adult male mice. **Methods** The grape stem extract was derived from a native Greek vine, namely Mavrodaphne and was rich in polyphenols (205.2 mg/g extract). The stem extract was administrated orally (gavage, 100 mg/Kg body weight) for 28 days (long-term). Behavior analysis was assessed by using the open-field test, followed by video-tracking software (Any-maze 6.3) analysis. Metabolism parameters were determined with a clinical chemistry analyzer, while antioxidant analysis was performed by determining the SOD activity. Acetylcholinesterase activity was determined in both salt-soluble and detergent-soluble in the brain, by using Ellman's colorimetric method. **Results** Anxiolytic-like behavior was observed after the stem extract administration. This long-term administration had no effect on mice's metabolism. Stem extract treatment showed antioxidant effect and reduced acetylcholinesterase activity of both isoforms in various brain regions. **Conclusion** In conclusion, long term administration of grape stem extract, rich in polyphenols, appears to have anxiolytic, antioxidant as well as acetylcholinesterase inhibitory activity.

Pubmed:

31756277: El Mubarak MA, Danika C, Cachon C, Korovila C, Atsopardi K, Panagopoulos N, Margarity M, Poulas K, Sivolapenko GB
In vivo quantification and pharmacokinetic studies of cotinine in mice after smoke exposure by LC-MS/MS.
A sensitive analytical method was developed and validated for the quantification of cotinine in mouse plasma after exposure to smoke of 0.5, 1.0, and 1.5 commercially available cigarettes, using liquid chromatography tandem mass spectrometry. The method was validated over a linear concentration range of 0.075-20.0 ng/mL with the R value being higher than 0.99. Both the precision (coefficient of variation; %) and accuracy (relative error; %) were within acceptable criteria of <15%. The lower limit of quantification (LLOQ) for cotinine was 0.075 ng/mL with sufficient specificity, accuracy, and precision. Following exposure to 0.5, 1.0, and 1.5 cigarette smoke, it was observed that the AUC and the C increased linearly as the doses increased. The pharmacokinetics of cotinine was found linear for the range of 0.5-1.5 commercial cigarette smoke. The quantification of the concentration of cotinine in mouse plasma after smoke exposure will facilitate future behavioral and toxicological experiments in animals and may prove useful in predicting cotinine levels in humans during smoking.
Biomed Chromatogr, 2020; 34
31747631: Anesti M, Stavropoulou N, Atsopardi K, Lamari FN, Panagopoulos NT, Margarity M
Effect of rutin on anxiety-like behavior and activity of acetylcholinesterase isoforms in specific brain regions of pentylenetetrazol-treated mice.
The aim of the present study was to investigate the effect of rutin administration (100 mg/kg/day) to pentylenetetrazol (PTZ)-treated Balb-c mice (60 mg/kg/day), with respect to anxiety-like behavior using both open-field and elevated plus-maze (EPM) tests, and acetylcholinesterase (AChE) activity in salt-soluble (SS) fraction and detergent-soluble (DS) fraction of the cerebral cortex, hippocampus, striatum, midbrain, and diencephalon. Our results demonstrated that the administration of PTZ in 3 doses and the induction of seizures increased significantly anxiety behavior of mice and reduced significantly DS-AChE activity in all brain regions examined, while the reduction in the SS fraction was brain region-specific. Rutin administration to

normal mice did not affect their behavior, while it induced a brain region-specific reduction in SS-AChE and a significant decrease in DS-AChE in all brain regions. We demonstrated for the first time that pretreatment of PTZ-mice with rutin (PTZ + Rutin group) prevented the manifestation of anxiety and induced interestingly a further significant reduction on the SS- and DS-AChE activities only in the cerebral cortex and striatum, in comparison with PTZ group. Our results show that rutin exhibits an important anxiolytic effect and an anticholinesterase activity in specific brain areas in the seizure model of PTZ.
Epilepsy Behav, 2020; 102

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ABLATION OF CAROTID BODY ACTIVITY PREVENTS COGNITIVE DYSFUNCTION AND DECREASES ALPHA-SYNUCLEIN LEVELS IN THE BRAIN OF AN ANIMAL MODEL OF DYSMETABOLISM

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Type 2 diabetes (T2D) is an important risk factor for neurodegenerative disorders. Importantly, the overactivation of the carotid bodies (CBs) was identified in both prediabetes and T2D animal models, and the abolishment of CB activity was shown to prevent and reverse dysmetabolic states. We hypothesize that the abolishment of CBs activity, via resection of carotid sinus nerve (CSN), can ameliorate the neurodegenerative processes associated to dysmetabolism. Male Wistar rats were fed a high fat-high sucrose (HFHSu), or normal chow (NC) diet. Fourteen weeks post-diet, animals were divided and submitted to CSN ablation or sham surgery. Animals were kept under diet for more 7 weeks. Metabolic and behavioural parameters were evaluated. After sacrifice, brains were collected for protein analysis. HFHSu animals decreased the time spent in the novel arm in the y-maze test in comparison with the NC group. CSN denervated HFHSu animals spend similar time in the novel arm when compared to the NC animals.In block test at 20 weeks of diet, HFHSu animals sniff less time the novel block when compared to NC animals, and CSN resection did not modify this effect.Considering that the brain regions most related to cognitive functions are the prefrontal cortex and the hippocampus we evaluated the levels of some proteins herein. Both in the prefrontal cortex and in the hippocampus, the HFHSu animals exhibit increased levels of alpha-synuclein and APP when compared to NC, and CSN resection was able to restore it. Ablation of CBs activity prevent neurodegenerative processes by improving metabolic function.

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CYTOPROTECTIVE EFFECTS OF CORDYCEPIN AGAINST OXIDATIVE STRESS-INDUCED DNA DAMAGE AND APOPTOSIS IN C6 GLIAL CELLS

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Aim: Cordycepin is one of the active ingredients extracted from the fungi of genus *Cordyceps*, which have been used for traditional herbal remedies. In this study, we examined the effect of cordycepin on the proliferation and apoptosis of H_2O_2-induced oxidative stress in C6 glial cells and its mechanism of action. **Methods**: To investigate the antioxidant effects of cordycepin in H_2O_2 oxidative stressed C6 cells, we measured the levels of ROS and apoptosis by flow cytometry, and also DNA damage by DNA fragmentation assay, comet assay and 8-OHdG analysis. To elucidate the mechanism underlying the effect of cordycepin the expression of genes involved in the expression of antioxidative regulators was also investigated by immune blotting. **Results and Conclusions**: The results demonstrated that preincubation of C6 glial cells with cordycepin prior to H_2O_2 stimulation inhibited cytotoxicity, comet tail formation, chromatin condensation, phospho-histone γH2AX and 8-OHdG expressions, suggesting that it prevents H_2O_2-induced ROS accumulation, cellular DNA damage and apoptotic cell death. Cordycepin also restored H_2O_2-induced mitochondrial dysfunction and prevented apoptosis by Bax/Bcl-2 ratio. Interestingly, the expression of Nrf2 was significantly increased by cordycepin in the presence of H_2O_2, which was associated with a marked increase in expression of HO-1. Additionally, the preventive effects of cordycepin were significantly abolished by zinc protoporphyrin, a HO-1 specific inhibitor, indicating that cordycepin activates the Nrf2/HO-1 axis in C6 glial cells to protect against oxidative stress. These findings suggest that cordycepin may protect glial cells against oxidative stress-induced injury through activation of the Nrf2/HO-1 signaling pathway.

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FUSDELTA14 MUTATION IMPAIRS NORMAL NEURONAL DEVELOPMENT AND CAUSES SYSTEMIC LIPID METABOLIC ALTERATIONS

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Aims: Metabolic alterations have been reported in neurodegenerative disorders as a consequence of disease. However, research suggests that these alterations occur before disease onset and may be integral to early disease mechanisms. Fused in sarcoma (FUS) is an RNA binding protein that is ubiquitously expressed. *FUS* mutations are implicated in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). FUSDelta14 mutation causes an aggressive form of juvenile ALS. Heterozygosity in the partially humanized FUSDelta14 mouse causes late onset motor neuron degeneration with minimal phenotypes. Homozygous mice die at birth. We aim to generate homozygous mice and evaluate the effect of *FUS* mutations in disease and its role in lipid dysregulation as a pathomechanism. **Methods:** FUSDelta14 homozygous mice were generated in a defined F1 hybrid background of C57Bl/6JxDBA/2J. Comprehensive behavioural tests (metabolic, motor and cognitive) and RNA-sequencing on brain, spinal cord, white and brown adipose tissue, liver and tibialis muscle were conducted at a pre-symptomatic disease stage. **Results:** Homozygous FUSDelta14 mice are smaller than heterozygous and wild-type littermates. The brain is the most transcriptionally altered tissue with dysregulation in neuronal activity and function. At 9-weeks, homozygous mice showed structural brain changes and cognitive impairment. These mice also displayed early systemic metabolic alterations, from increases in fat depots to a fat fuel preference. Targeted transcriptomic analysis revealed widespread lipid metabolism dysregulation. **Conclusions:** The FUSDelta14 mutation causes early central lipid metabolic alterations that have an impact on phenotypes, placing lipid dysregulation as one of the main pathological mechanisms in the ALS-FTD spectrum of disorders.

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BOARD NUMBER: S04-277

AMPK INVOLVEMENT IN THE CONTROL OF TANYCYTIC LEPTIN SHUTTLE

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Introduction/Aim: The anorexigenic adipokine leptin controls energy homeostasis through its hypothalamic action, however in a context of diet-induced obesity (DIO) circulating leptin quickly fails to reach its target neurons in the mediobasal hypothalamus (MBH). Interestingly it has been shown that tanycytes, ependymoglial cells lining the floor of the third ventricle, endocytose blood-borne leptin from the external zone of the median eminence and shuttle it into the MBH. AMPK-activated protein kinase (AMPK) is a known regulator of both cellular and whole-body energy homeostasis. Interestingly, in the hypothalamus, it has been demonstrated that AMPK mediates peripheral hormones-actions over their central control of metabolism. Considering the relevant role of AMPK in the control of cellular energy balance and its implication in energy metabolism, here we investigate whether pharmacological AMPK modulation plays a role in tanycyte-mediated shuttling of blood-borne leptin into the MBH. **Methods/Results:** Using tanycyte primary cultures from rats, we tested if leptin uptake and secretion by tanycytes is regulated by AMPK. Pharmacological activation of AMPK potentiates tanycytic leptin transcytosis while its inhibition reduces it. Since it has been previously described that ERK-pathway is involved in the release of the leptin taken up by tanycytes, we also assessed whether AMPK modulation induces ERK activation, but neither AMPK activation nor its inhibition had any effects on ERK phosphorylation. **Conclusions:** Taking into account the promising *in vitro* results, we are next investigating whether pharmacological and genetically modulation of AMPK alters circulating leptin transport into the MBH of DIO mice. Acknowledgments: ERC WATCH No 810331

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COMPARTMENTATION OF POLYAMINE METABOLISM IN HIPPOCAMPAL SUBREGIONS

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Aims: Polyamines are polyvalent cations involved, among others, in neurotransmission and neuroprotection. Hippocampus contains high levels of polyamines, but their distribution and roles in this structure are not well understood. The aim of this study was to determine the precise localization of polyamine metabolic machinery within the hippocampus in order to establish the potential involvement of polyamines in specific functions and phenotypes of selected hippocampal subregions. A special attention was given to arginase 2 (Arg2), an enzyme supplying ornithine (Orn), a major substrate for polyamine synthesis.
Methods: Allen Brain Atlas of mouse gene expression was used to explore the spatial distribution of mRNA of polyamine metabolism-related genes. Next, the experiments were performed using WT mice and mouse line with Arg2 gene knockout. Localization of selected proteins was studied using immunohistochemistry. The protein levels were determined with western blotting. The amino acid content was measured by HPLC.
Results: According to the Allen Brain Atlas, CA2 subregion appears to display a unique profile of expression of polyamine-related genes. As confirmed by immunohistochemistry, Arg2 protein is specifically expressed in pyramidal neurons of CA2, and absent in other cell types and other regions. Loss of Arg2 results in accumulation of arginine (Arg) and reduction of Orn.
Conclusions: These results suggest strict compartmentation of polyamine metabolism in the hippocampal subregions. Neuronal Arg2 in CA2 appears to be a key source of Orn for local polyamine synthesis, what may support specific phenotypes of this region, including high synaptic stability and decreased sensitivity to neurotoxic insults.

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MYELIN LIPIDS AS NERVOUS SYSTEM ENERGY RESERVES

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Neuronal functions and impulse propagation depend on the continuous supply of glucose. Surprisingly, the mammalian brain has no obvious energy stores, except for astroglial glycogen granules. Oligodendrocytes make myelin for rapid axonal impulse conduction and also support axons metabolically with lactate. Here, we show that myelin itself, a lipid-rich membrane compartment, becomes a local energy reserve when glucose is lacking. In the mouse optic nerve, a model white matter tract, myelinating oligodendrocytes survive glucose deprivation far better than astrocytes. Surviving starvation required oxygen and fatty acid beta-oxidation. Importantly, fatty acid oxidation also contributes to axonal ATP generation and nerve conductivity. This metabolic support by fatty acids is an oligodendrocyte function, involving mitochondria and myelin-associated peroxisomes, as shown with mice lacking Mfp2. To study reduced glucose availability *in vivo* without physically starving mice, we deleted the Slc2a1 gene from mature oligodendrocytes. This caused a significant decline of the glucose transporter GLUT1 from the myelin compartment leading to myelin sheath thinning. We suggest a model of myelin dynamics in which normal lipid turnover can buffer axonal energy metabolism under low glucose conditions. This may explain the gradual loss of myelin in a range of neurodegenerative diseases with underlying hypometabolism.

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L-SERINE-MEDIATED PKM2 ALLOSTERIC REGULATION COORDINATES L-SERINE SYNTHESIS, GLYCOLYTIC RATE AND LACTATE RELEASE

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Lactate and L-serine are both 3-carbon metabolites produced from the glycolytic processing of glucose. Whereas lactate corresponds to the end product of glycolysis, L-serine is derived from 3-phosphoglycerate, a glycolytic intermediate. In the brain, both compounds are mainly produced by astrocytes, glial cells endowed with the ability to regulate their glycolytic flux. Interestingly, both metabolites are also signalling molecules. Neuronal activity stimulates astrocytic glycolysis and results in the production of lactate that supports neurons' energetic needs, increases their excitability and favours memory consolidation by potentiating neuronal NMDA receptors through still unknown mechanisms. L-serine is a direct precursor for the NMDA receptor co-agonists D-serine and glycine and, as such, indirectly contributes to synaptic plasticity and memory. Together, the converging inputs of both L-serine and lactate on NMDA receptor activity highlights the potential relevance of a tight coordination between lactate and L-serine production. Interestingly, in astrocytes, the last irreversible step of glycolysis is catalysed by the M2 isoform of pyruvate kinase (PKM2), which was previously reported to be allosterically activated by L-serine in cancer cell lines, thus suggesting a potential mechanism for coordinating L-serine with lactate production. Here, by using a combination of intracellular and extracellular lactate and pyruvate nanosensors expressed in primary astrocytes, we show that the allosteric activation of PKM2 by L-serine stimulates glycolysis and triggers the release of lactate. Those preliminary results suggest that the allosteric regulation of PKM2 by L-serine may provide a mean to match the NMDA receptor activity of neurons with their appropriate energy supply.

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REFERENCE GENE SELECTION WITHIN THE RAT BRAIN UNDER MILD INTERMITTENT KETOSIS INDUCED BY SUPPLEMENTATION WITH MEDIUM-CHAIN TRIGLYCERIDES

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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RT-qPCR has become the gold standard in mRNA expression analysis. It requires an accurate choice of reference genes (RGs) for adequate normalization. The aim of this study was to validate the RGs for qPCR experiments in the brain of adult Wistar rats in the model of mild ketosis established through intermittent fasting (6 h a day) and supplementation with medium chain triglycerides (MCT) oil (2 ml/kg, i.g.; equivolume volume in controls) for 1 month. The mRNA expression of 9 RGs (*Actb, B2m, Gapdh, Hprt1, Pgk1, Ppia, Rpl13a, Sdha, Ywhaz*) was measured in the medial prefrontal cortex (mPFC), dorsal (DH) and ventral (VH) hippocampus. We used the RefFinder® online tool to asses RG stability. The most stably expressed RGs were found to be *Ppia, Actb,* and *Rpl13a* in the mPFC; *Rpl13a, Ywhaz* , and *Pgk1* in the DH; *Ywhaz, Sdha* , and *Ppia* in the VH. The stabilities of the examined RGs were lower in the DH compared to the VH and the mPFC. When normalized to the three most stably expressed RGs, the *Gapdh* mRNA was upregulated, while the *Sdha* mRNA was downregulated in the mPFC of MCT-fed animals. Thus, the RGs expression stability strongly depends on the examined brain regions. The dorsal and ventral hippocampal areas differ in RG stability rankings, which should be taken into account in the RT-qPCR experimental design. The study was funded by the Russian Science Foundation (RSF), project no. 19-75-10076.

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BOARD NUMBER: S04-282

THE HEPATIC ESTROGEN RECEPTOR ALPHA POTENTIAL ROLE IN THE IN MEDIOBASAL HYPOTHALAMUS PLASTICITY

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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The maintenance of energy homeostasis requires communication between the hypothalamus and peripheral organs. The latter send messages about their metabolic status to the hypothalamic arcuate nucleus, which integrates and respond according to these signals. The modulation of this access is done in part via the blood-arcuate nucleus interface whose permeability is regulated after a 24-hour fast, linking oestrogens, energy metabolism and the access of peripheral signals to the arcuate nucleus. While many studies focus on the dialogue between adipose tissue and the hypothalamus via leptin access to the arcuate nucleus, few studies have focused on the liver-hypothalamus axis. In this study we are interested in the action of oestrogens on hepatic metabolism and the consequences on the hypothalamic regulation of energy metabolism. Indeed, in the liver, the expression of the alpha receptor (ERα) regulates lipid and carbohydrate metabolism as well as the secretion of hepatokines. We hypothesize that hepatic ERα is involved in energy metabolism regulated by the liver-brain axis and that among the different molecules secreted by the liver under the influence of oestrogens, there is a factor allowing the modulation of the permeability of the blood-arcuate nucleus interface. The preliminary studies indicate that the absence of the hepatic receptor alters the increase in permeability of the blood-arcuate nucleus interface normally induced by fasting. Thus, the hepatic ERα would be involved in regulating the access of peripheral signals to their brain targets and would contribute to the regulation of energy metabolism by the hypothalamus.

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BOARD NUMBER: S04-283

EARLY LIFE ADVERSITY AND A SEX-SPECIFIC POLYGENIC RISK SCORE FOR ALTERED FASTING INSULIN ARE ASSOCIATED WITH EXECUTIVE FUNCTIONING

POSTER SESSION 04 - SECTION: THE BRAIN AND METABOLISM

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Considering the high prevalence of co-morbidity between altered metabolism and executive function problems (e.g. obesity and ADHD), we hypothesized that 1) the genetic background associated with altered fasting insulin (FI) and ADHD could be shared; 2) if (1) is rejected, interactive models between the genetic background associated with altered FI and childhood environment would better predict executive functions. Using conjunctional false discovery rate, we found no shared SNPs between the FI GWAS and ADHD GWAS. (2) We calculated polygenic risk scores (PRS) from the sex-specific FI GWAS and identified the threshold that best predicted insulin levels in male and female in ALSPAC children [N_{males}=1,901, $N_{females}$=1,834; $p_{t\text{-}intial\text{-}males}$= 0.05 (11,121 SNPs), $p_{t\text{-}intial\text{-}females}$=0.15 (27,202 SNPs)], further refining it to only include SNPs significantly associated with insulin levels [$p_{t\text{-}refined\text{-}males}$=635 SNPs, $p_{t\text{-}refined\text{-}females}$=1,449 SNPs]. Phewas was run to identify executive functions predicted by the interaction between the refined PRS (rPRS) and early adversity at different ages and attention-related outcomes were highlighted in multiple cohorts (MAVAN, GUSTO, ABCD, UKB). To assess whether there is direct causal relationship between fasting insulin levels and attention, we used mendelian randomization (MR). Two-sample MR analysis using an inverse-variance-weighted fixed-effects approach between the sex-specific FI GWAS and the sex-specific ADHD GWAS suggests that there is no causal effect between the traits in either males [p=0.06] or females [p=0.18]. This solidifies our hypothesis that the relationship between altered fasting insulin and executive function is not direct but especially relevant after early life adversity exposure, which we plan to inspect in our future work.

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EFFECTS OF THE CHRONIC TREATMENT WITH AN A2A/A2B RECEPTOR MIXED AGONIST, MRS3997, ON CEREBRAL INJURY IN A RAT MODEL OF TRANSIENT BRAIN ISCHEMIA

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Cerebral ischemia is a multifactorial pathology characterized by different events evolving in time. The acute injury, characterized by a massive increase of extracellular glutamate levels, is followed by a secondary brain injury that develops from hours to days after ischemia. Previous data obtained by our laboratory demonstrated that both adenosine A_{2A} and A_{2B} receptor agonists, CGS21680 and BAY60-6583, chronically administered, are protective after transient cerebral ischemia in the rat, by contrasting the secondary inflammatory damage. Aim of our study was to assess the putative neuroprotective effects of the newly synthetized A_{2A}/A_{2B} receptor mixed agonist, MRS3997, chronically administered (0.1 mg/kg, i.p., twice/day for 7 days) after transient (1 hour) focal cerebral ischemia induced by middle cerebral artery occlusion (MCAo) in the rat. Behavioural, histological and immunohistochemical experiments were performed to evaluate damage parameters after ischemia. Chronic treatment with MRS3997 significantly improved neurological deficit, up to 7 days after tMCAo ($P<0.03$). Seven days after ischemia, A_{2A}/A_{2B} receptor mixed agonist has significantly reduced the volume of ischemic brain damage in cortex and striatum ($P<0.04$) and reduced the activation of microglial cells and the astrogliosis in perischemic areas. These preliminary results show that synergic stimulation of A_{2A} and A_{2B} receptors reduces the ischemic brain damage and improves the neurological deficit. Further experiments are needed to confirm the hypothesis that the simultaneous activation of A_{2A} and A_{2B} receptors located both on brain cells and on blood cells could potentially be more protective than A_{2A} and A_{2B} receptor agonists administered individually.

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BRAIN DAMAGE IN APOE -/- MICE WITH CHRONIC CEREBRAL HYPOPERFUSION: PARTICIPATION OF SIRT1, SIRT3 AND IGF1

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Chronic cerebral hypoperfusion (CCH) is the cause of most cerebrovascular diseases. Apolipoprotein E (ApoE) is an important risk factor for atherosclerosis, myocardial ischemia, and Alzheimer's disease. The aim of present study was to investigate sirtuin-dependent mechanisms underlying CCH-induced brain damage in ApoE-/- mice. Methods: The experiments were performed on 6-weeks old C57 wild type (WT) and ApoE-/- male mice. A model of chronic cerebral hypoperfusion by permanent unilateral occlusion of the common carotid artery was used to mimic chronic hypoxic/ischemic states. In 8 weeks after occlusion, histological changes in the hippocampus were observed by hematoxylin and eosin staining. DNA neurons damages were assessed using the DNA comet assay. SIRT1, SIRT3, IGF1 were detected by RT-PCR assay. Results: ApoE-/- animals displayed increased of injured hippocampal neurons and DNA neurons damage (due to 3 classes of DNA comets) after CCH, as compared to controls. WT mice were severely affected: we observed increased damage of the hippocampus of the CCH and 3-4 classes of DNA comets as compared to controls. Moreover, in the brain of WT mice exposed to CCH sharply reduced levels of SIRT1 by 89%, SIRT3 by 91%, IGF1 by 90% expressions respectively, and these levels were reduced by 15%, 31%, 29% respectively in ApoE-/- mice under the same conditions. Conclusions: We conclude that ApoE exerts an injurious action in CCH-induced brain damage affecting SIRT1, SIRT3, and IGF1 expression; it may be a target of therapeutic strategies in hypoxic/ischemic states treatment.
Keywords: chronic cerebral hypoperfusion, ApoE, SIRT1, SIRT3, IGF1, brain damage.

Pubmed:
34331429: Dytiatkovskyi V, Drevytska T, Lapikova-Bryhinska T, Dosenko V, Abaturov O
Genotype Associations with the Different Phenotypes of Atopic Dermatitis in Children.
This study deals with detecting the associations of atopic dermatitis' (AD) phenotypes in children: alone or combined with seasonal allergic rhino-conjunctivitis (SARC) and/or perennial allergic rhinitis (PAR), and/or with bronchial asthma (BA) with single nucleotide polymorphisms (SNP) of filaggrin (FLG), thymic stromal lymphopoietin (TSLP) and orsomucoid-like-1 protein 3 (ORMDL3) genes. Male and female pediatric patients aged from 3 to 18 years old were recruited into the main (AD in different combinations with SARC, PAR, BA) and control groups (disorders of digestives system, neither clinical nor laboratory signs of atopy). Patients were genotyped for SNP of rs_7927894 FLG, rs_11466749 TSLP, rs_7216389 ORMDL3 variants. Statistically significant associations of the increased risk were detected of AD combined with SARC and/or PAR and AD combined with BA (possibly, SARC and/or PAR) with C/T rs_7927894 FLG and T/T rs_7216389 ORMDL3 genotypes. Genotype C/C rs_7927894 FLG significantly decreases the risk of AD combined with SARC and/or PAR by 2.56 fold. Several genotypes' associations had a trend to significance: C/C rs_7216389 ORMDL3 decreases and C/T rs_7216389 ORMDL3 increases the risk for developing AD alone phenotype; A/G rs_11466749 TSLP decreases the risk of AD combined with BA (possibly, SARC and/or PAR) phenotype development.
Acta Medica (Hradec Kralove), 2021; 64
31104265: Lapikova-Bryhinska T, Zhukovska A, Nagibin V, Tumanovska L, Portnichenko G, Goncharov S, Portnychenko A, Dosenko V
Altered biogenesis of microRNA-1 is associated with cardiac dysfunction in aging of spontaneously hypertensive rats.
Currently we face the issues of aging-associated pathologies, particularly those leading to heart failure. With that in mind, in current research we focus on aging and hypertension combination as a widely spread threating problem. In a row with

functional and morphological characterization of these aging- and hypertension-associated cardiac changes, we evaluate biogenesis of microRNA-1 being one of major microRNAs in the heart. The aim of this study was to check the hypothesis if dysregulation of microRNA-1 biogenesis is associated with heart failure in aged and especially aged hypertensive rats. The experiments were carried out on male SHR and Wistar rats of age 6 months (young) and 18 months (old). The evaluation of hemodynamic parameters was performed in heart left ventricles of narcotized rats using the ultra-small 2F catheter. The development of fibrosis was determined using light and electron microscopy. Levels of mature and immature forms of microRNA-1 and mRNA encoding the proteins involved in its biogenesis were determined using reverse transcription and quantitative PCR. Aging of both Wistar and SHRs is accompanied with altered hemodynamic parameters compared with correspondent younger mates. SHRs, especially old ones, demonstrated significant heart fibrosis. In aged animals, the level of primary microRNA-1 in Wistar rats were 7 times higher ($p < 0.05$) and in SHR 17 times higher ($p < 0.05$) in comparison with young rats of the same strain. We also observed 22 times higher level of immature microRNA-1 in the heart of Wistar and 5.9 times higher level for aged hypertensive rats ($p < 0.05$) compared to young rats. At the same time, the level of mature microRNA-1 occurred 2.5 and 3.2 times lower in respective groups ($p < 0.05$). In the current study, we observe the significant dysregulation of microRNA-1 processing in the heart associated with aging and arterial hypertension.
Mol Cell Biochem, 2019; 459

30530891: Drevytska T, Morhachov R, Tumanovska L, Portnichenko G, Nagibin V, Boldyriev O, Lapikova-Bryhinska T, Gurianova V, Dons'koi B, Freidin M, Ivanisenko V, Bragina EY, Hofestädt R, Dosenko V
shRNA-Induced Knockdown of a Bioinformatically Predicted Target IL10 Influences Functional Parameters in Spontaneously Hypertensive Rats with Asthma.
One of the most common comorbid pathology is asthma and arterial hypertension. For experimental modeling of comorbidity we have used spontaneously hypertensive rats with ovalbumin (OVA)-induced asthma. Rats were randomly divided into three groups: control group, OVA-induced asthma group; OVA-induced asthma + IL10 shRNA interference group. Target gene (IL10) was predicted by ANDSystem. We have demonstrated that RNA-interference of IL10 affected cardiovascular (tested using Millar microcatheter system) as well as respiratory functions (tested using force-oscillation technique, Flexivent) in rats. We have shown that during RNA-interference of IL10 gene in vivo there were changes in both cardiac and lung function parameters. These changes in the cardiovascular parameters can be described as positive. But the more intensive heart workload can lead to exhaust and decompensation of the heart functions. Knockdown of IL10 gene in asthma modeling induces some positive changes in respiratory functions of asthmatic animals such as decreased elastance and increased compliance of the lungs, as well as less pronounced pathomorphological changes in the lung tissue. Thus, we provide the data about experimentally confirmed functionality changes of the target which was in silico predicted to be associated with both asthma and hypertension - in our new experimental model of comorbid pathology.
J Integr Bioinform, 2018; 15

29971601: Balatskyi VV, Macewicz LL, Gan AM, Goncharov SV, Pawelec P, Portnichenko GV, Lapikova-Bryginska TY, Navrulin VO, Dosenko VE, Olichwier A, Dobrzyn P, Piven OO
Correction to: Cardiospecific deletion of αE-catenin leads to heart failure and lethality in mice.
The original version of this article unfortunately contained a mistake. The published paper presented an incorrect version of Table 1. The corrected Table is given here.
Pflugers Arch, 2018; 470

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OXYGEN AND GLUCOSE DEPRIVATION INDUCES UNCONVENTIONAL TRANSLOCATION OF ER-RESIDENT PROTEIN TO THE NEURONAL SURFACE.

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Aims: Disruption of proteostasis is a major consequence of ischemic stroke, causing a disruption of calcium homeostasis and an endoplasmic reticulum (ER) stress. This latter promotes the translocation of the ER resident chaperone GRP78 to the neuronal surface where it appears to act as a receptor for unsuspected proteins. For example, we shown that tissue-type plasminogen activator (tPA), binds to cell surface GRP78 to promote neuroprotection during stroke. The aim of this study is to understand the mechanism of translocation of GRP78 to the neuronal surface. Methods: Both PC12 cells and mouse primary cortical neurons have been subjected to various stresses including ER stress inducers, autophagy modulators, and oxygen and glucose deprivation (OGD, an in vitro model of ischemia). Proteostasis (including ER stress and autophagy), GRP78 translocation and secretory pathways have been investigated in these conditions by western blot, cross-linking and immunoprecipitation, and confocal imagery following lentiviral transduction. Results: Biotinylation of total cell surface protein and avidin pull-down showed that both ER stress and autophagy inducers as well as OGD increase chaperone proteins (GRP78 and GRP94) and their translocation to the cell surface. Treatment with blockers of the classic secretory pathway (ER to Golgi) did not affect the translocation of chaperones. Conclusions: Our results indicate that pharmacological dysregulation of proteostasis and OGD induce a translocation of chaperones from the ER to the cell surface (mainly GRP78 and GRP94) through an unconventional secretory pathway.

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THE ROLE OF THE HYPOXIA-INDUCIBLE FACTOR 2α IN FOCAL CEREBRAL ISCHEMIA

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Insufficient blood flow in stroke interferes the brain from proper oxygenation, leading to the stabilization of hypoxia-inducible factors (HIFs). To determine the role of HIFs in stroke, particularly HIF-2α, we use an *in vivo* mouse model called tMCAO (transient middle cerebral artery occlusion) and an *in vitro* model with human SH-SY5Y cells. On both, *Hif-2α* deficient and wild type mice, tMCAO was performed for two hours and protein and RNA samples were collected of ischemic and control brains. To simulate stroke in SY5Y cell culture, oxygen-glucose-deprivation (OGD) experiments were performed under almost anoxic conditions (0.2% oxygen) and without glucose for up to 4 hours following reperfusion. Prior to reperfusion, either Roxadustat or a selective HIF-2α inhibitor (PT-2358) were administered. First results show a significant increased expression of HIF-2α regulated neurotrophic factors already after one hour of hypoxia, while the inhibition of HIF-2α during reperfusion leads to significantly less cell death. Our preliminary results lead us to the conclusion that HIF-2α might influence the outcome during hypoxia and reperfusion in a time-dependent manner. In future experiments we want to figure out the effects of HIF-2α during stroke and reperfusion by analyzing more HIF-2α target genes via qPCR, as well as getting more insights of the HIF-2α regulation by Western Blot analysis.

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PARTIAL PRESSURE OF OXYGEN AND CEREBRAL BLOOD FLOW IN THE BRAINS OF CONSCIOUS, FREELY MOVING RATS DURING HYPOXEMIA.

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

Rawan Barakat, Ghanim Al-Khaledi, Marian Turcani, Kamal Matar, Al-Sarraf, Zoran Redzic
Kuwait University, Physiology, Kuwait, Kuwait

Background: Low pressure of O_2 in inspired air (PiO_2) causes CNS dysfunction, however, it is unclear how PO_2 in the brain (PtO_2) and cerebral blood flow (CBF) change when PiO_2 is low. **Aim**: To explore PtO_2, CBF and lactate in the brains of conscious, freely moving rats during hypoxemia caused by low PiO_2. **Methods** PtO_2 or CBF sensors were implanted in the associated motor cortices of SD rats, and, after a recovery period, measurements were recorded before and during 48h hypoxemia (8% O_2 in N_2 in O_2-Control *In-Vivo* Cabinet equipped with gas analyzer), with or without CO_2 scrubbing protocol being applied. Blood, CSF and brain samples were collected at various time points to estimate lactate concentrations. Data was compared by paired or unpaired t-test; $p<0.05$ was set as a level of significance. **Results:** PtO_2 was 49.5±0.52 mmHg when PiO_2 was normal. Reduced PiO_2 caused hypoxia in the brains, which was evident from a sharp decline in PtO_2, reaching ≤10±2.09 mmHg after 2.5h, and from an increase in lactate concentrations in the brain and CSF ($p<0.05$). However, PtO_2 increased steadily after 3h, reaching 27.6±0.15 and 16.5±0.55 mmHg ($p<0.05$) after 48h, without and with CO_2 scrubbing protocol being applied, respectively. Lactate in the brain and CSF remained elevated, while pial and capillary CBF did not change during the time course of hypoxemia. **Conclusion:** The ability of the rat brain to adapt to hypoxemia was largely related to the degree of hyperventilation, which depended on P_{CO2}, rather than the increase in the CBF.

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EVALUATION OF P53 EXPRESSION AND INTERACTION WITH HDAC2 IN THE ACUTE PERIOD AFTER PHOTOTHROMBOTIC STROKE IN RATS

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Aims. The aim of our work was to study the change in the level of the apoptosis regulator p53 protein and its interaction with histone deacetylase 2 (HDAC2) in the perifocal region of photothrombotic stroke (PTS) 4 hours and 7 days after injury. **Methods.** Western blotting was used to study the level of p53 in the nuclear and cytoplasmic fractions of the penumbra after PTS. Colocalization of p53 with class I HDACs was assessed by immunofluorescence microscopy. We also assessed the interaction between p53 and HDAC2 using Duolink Proximity Ligation Assay (PLA) followed by fluorescence microscopy. **Results.** The level of p53 increased in the nuclear fraction of the penumbra by 1.5 times 4 hours after PTS. However, 7 days after PTS, its content in the nuclei did not differ from that in sham-operated animals. P53 increased in the cytoplasmic fraction by 3 times on the first day and did not change on the 7th day after PTS. P53 was found in neurons 4 hours and 7 days later, but not in astrocytes or macrophages. Immunofluorescence microscopy showed that the cellular and intracellular localization of p53 coincided with HDAC2. Interaction between p53 and HDAC2 was detected in the perifocal region 4 hours after photothrombotic stroke in rats. **Conclusions.** Our results indicate the increased p53 expression and its interaction with HDAC2 in the acute period after photothrombotic stroke in rats. The study was supported by the Russian Science Foundation (grant No. 21-15-00188).

Pubmed:
35011655: Demyanenko SV, Pitinova MA, Dzreyan VA, Kalyuzhnaya YN, Eid MA, Abramov AY, Evgen'ev MB, Garbuz DG
The Role of p53 Protein in the Realization of the Exogenous Heat Shock Protein 70 Anti-Apoptotic Effect during Axotomy.
The search for effective neuroprotective agents for the treatment of neurotrauma has always been of great interest to researchers around the world. Extracellular heat shock protein 70 (eHsp70) is considered a promising agent to study, as it has been demonstrated to exert a significant neuroprotective activity against various neurodegenerative diseases. We showed that eHsp70 can penetrate neurons and glial cells when added to the incubation medium, and can accumulate in the nuclei of neurons and satellite glial cells after axotomy. eHsp70 reduces apoptosis and necrosis of the glial cells, but not the neurons. At the same time, co-localization of eHsp70 with p53 protein, one of the key regulators of apoptosis, was noted. eHsp70 reduces the level of the p53 protein apoptosis promoter both in glial cells and in the nuclei and cytoplasm of neurons, which indicates its neuroprotective effect. The ability of eHsp70 to reverse the proapoptotic effect of the p53 activator WR1065 may indicate its ability to regulate p53 activity or its proteosome-dependent degradation.
Cells, 2021; 11

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THE ROLE OF α-KETOGLUTARATE/MTOR-MEDIATED SIGNALING PATHWAYS IN MAINTAINING THE VIABILITY OF BRAIN CELLS IN NORMAL AND ISCHEMIC CONDITIONS

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Most brain diseases are accompanied by circulatory failure and subsequent ischemia/reperfusion injury. The pathogenesis involves such processes as glutamate excitotoxicity, ion imbalance, oxidative stress, apoptosis, autophagy, etc. Investigation of the role of the mTOR-mediated signaling pathway (mammalian target of rapamycin (mTOR) is a key regulator of cellular metabolism including autophagy) in neuroprotective mechanisms has been the subject of numerous studies. In the context of ischemia-induced autophagy, the relationship between mTOR- and AKG-mediated signaling (α-ketoglutarate (AKG)) is assumed, but many aspects of this interaction have not yet been elucidated. This study presents an analysis of AKG/mTOR-mediated neuroprotection in ischemia model in vitro using transient oxygen-glucose deprivation (OGD) or glutamate excitotoxicity in hippocampal cell cultures. Using LDH/MTS assays, we monitored cell viability and mitochondrial activity with/without AKG and rapamycin (mTOR inhibitor) treatment. It was found that treatment with AKG and rapamycin, both separately and together, increased the viability of hippocampal cell cultures, which was reduced under conditions of OGD or glutamate. This was accompanied by an increase in PSD95 immunoreactivity at an early stage of the study (1 h after damage to OGD or glutamate), which may indicate the activation of synaptogenesis. In addition, an increase in LC3 (marker of autophagy) immunoreactivity was detected at 4 hours, indicating a delayed activation of autophagy. In our experimental model, AKG and rapamycin caused unidirectional effects, with the action of AKG being comparable to mTOR blocking. Thus, our results point to the important role of AKG/mTOR-mediated signaling pathways in maintaining neuronal viability under ischemic conditions.

Pubmed:
26075640: Goncharova K, Skibo G, Kovalenko T, Osadchenko I, Ushakova G, Vovchanskii M, Pierzynowski SG
Diet-induced changes in brain structure and behavior in old gerbils.
Aging is associated with many physiological alterations such as changes in metabolism, food intake and brain dysfunction. Possible ways to correct age-related brain dysfunction using dietary treatments still remains undeveloped. The aim of our research was to investigate whether long-term dietary treatment with 2-oxoglutarate (2-OX), which is involved in many regulatory pathways, together with pancreatic-like enzymes of microbial origin (PLEM), which ensure appropriate digestion and absorption of nutrients, affects age-related changes in the brain morphology and cognitive function in old Mongolian gerbils.
Nutr Diabetes, 2015; 5

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BOARD NUMBER: S04-291

MODULATING REDUCED FOLATE CARRIER 1 GENE EXPRESSION CHANGES BLOOD-RETINA BARRIER IN HEALTHY AND ISCHEMIC MICE RETINA

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Background: Retina has similar microcirculation characteristics like the brain, and harbors inner blood-retina barrier (iBRB) resembling blood-brain barrier including pericytes. Reduced Folate Carrier-1 (RFC1) is an abundant folate transporter in pericytes as shown by a few transcriptomic studies. However, RFC1 protein presence and its functions in retinal microvessels and pericytes have never been investigated. Here, after detecting RFC1 protein in retinal microvessels, we genetically modified RFC1 in vivo, to examine its roles in healthy and ischemic mice retina. **Method:** We used whole mount retinas, and trypsin digest preparations of adult Swiss albino mice. RFC1 was overexpressed by intravitreally delivered RFC1-expressing(n=6) and control(n=6) lentivirus. To knockdown RFC1, we delivered RFC1-targeted custom designed Accell short interfering RNA (RFC1-siRNA)(n=6) and scrambled(control)(n=6) siRNA intravitreally. Retinal ischemia was induced for 1-hour by applying $FeCl_3$ to central retinal artery for control(n=6) and genetically modified groups(n=6). We used RT-qPCR and Western blotting to determine RFC1. Endothelia(CD31), pericytes(PDGFR-beta, CD13, NG2), tight-junctions(occludin), major basement membrane protein(collagen-4) and RFC1 were determined immunohistochemically. Vessels(Lectin) and nuclei(Hoechst-33258) were also visualized. **Results:** We demonstrated that RFC1 protein is abundant in retinal endothelia and pericytes. RFC1-expressing lentivirus upregulated RFC1 levels resulting in upregulation of occludin and collagen-4, and the pre-treatment rescued decreased occludin and collagen-4 in retinal ischemia. RFC1-siRNA mediated knock-down caused occludin and collagen-4 reduction. Besides, knocking down RFC1 before retinal ischemia demolished iBRB integrity compared to control ischemic group, observed by IgG immunostainings. **Conclusion:** Thus, we suggest that RFC1 is pivotal to stabilize iBRB in health and ischemia. Supported by TÜBİTAK(120N690), HacettepeUniversity(TDK-2020-18590)

Pubmed:
31299535: Serdaroglu E, Konuskan B, Karli Oguz K, Gurler G, Yalnizoglu D, Anlar B
Epilepsy in neurofibromatosis type 1: Diffuse cerebral dysfunction?
Neurofibromatosis type 1 (NF1) is accompanied by epileptic seizures in 4-7% of patients. We examined clinical, electrophysiological, and radiological features associated with epilepsy in our NF1 series in order to identify risk factors. Epilepsy Behav, 2019; 98

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BOARD NUMBER: S04-292

BRAIN NATRIURETIC PEPTIDE EXPRESSION IN ACUTE ISCHAEMIC STROKE CLOTS IS NOT ASSOCIATED WITH STROKE AETIOLOGY, BUT HEIGHTENED S100B EXPRESSION IS ASSOCIATED WITH POST-THROMBECTOMY INTRACRANIAL HAEMORRHAGE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

Rosanna Rossi[1], Andrew Douglas[1], Adaobi Okolo[1], Klearchos Psychogios[2], Istvan Szikora[3], John Thornton[4], Turgut Tatlisumak[5], Alexandros Rentzos[6], Jesus Jeuga Morino[7], Karen Doyle[1]
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Aims: We investigated the expression of the glial protein S100b, Brain Natriuretic Peptide (BNP) and its precursor proBNP in clots removed by mechanical thrombectomy from 160 patients with Acute Ischemic Stroke (AIS). We studied the association of S100b, BNP and proBNP with stroke etiologies (atheroembolic versus cardioembolic) and with post-thrombectomy intracranial haemorrhage (PTIH). **Methods:** Mechanically extracted thrombi were collected by endovascular thrombectomy at collaborating stroke centres in Ireland, Sweden, Hungary and Greece, formalin-fixed and shipped to NUI Galway for analysis. Gross photos of each clot were taken and extracted clot area (ECA) was measured using ImageJ. Clots were analysed by histology (Martius Scarlet Blue; red and white blood cells, fibrin, platelets) and immunohistochemistry (S100b, BNP and proBNP). Orbit Image Analysis was used for quantification of whole slide scans. The non-parametric Kruskal-Wallis test was used for statistical analysis. **Results:** We did not find any difference in BNP or proBNP expression in atheroembolic versus cardioembolic clots (BNP: 0.19[0.03-1.27]mm2 vs 0.17[0.02-1.14]mm2, P=0.751; proBNP: 0.07[0.03-0.23]mm2 vs 0.04[0.01-0.11]mm2, P=0.081). Similarly, S100b expression was not affected by aetiology, however S100b was significantly higher in clots from patients with PTIH compared to the non-PTIH group (0.33[0.08-0.85]mm2 vs 0.07[0.02-0.27]mm2, P=0.014*). **Conclusion:** BNP, proBNP and S100b were expressed at similar levels in atheroembolic and cardioembolic clots and were not indicative of stroke aetiology. However, higher expression of S100b occurred with PTIH reflecting blood brain barrier dysfunction. Funded by Science Foundation Ireland (Grant number 13/RC/2073_2) and Cerenovus

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CONTRIBUTION OF CIRCULATING TISSUE-TYPE PLASMINOGEN ACTIVATOR (TPA) TO CEREBRAL PHYSIOPATHOLOGY.

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Aims. Intravenous injection of recombinant tissue-type plasminogen activator (tPA) as a thrombolytic agent has revolutionized the management of ischemic stroke patients. Several observations suggest that tPA, either endogenous (neuronal and endothelial) or exogenous may also worsen the loss of integrity of nerve cells and of the blood/brain interface during stroke, thus limiting the benefit of thrombolysis. However, the respective role of the neuronal tPA versus blood tPA in the cerebrovascular physiopathology is not well known. **Methods**. We sought to develop a tool to either selectively remove tPA in the circulation or in the brain parenchyma. We performed parabiosis between tPA deficient mice (tPA NULL) and/or wild-type mice (tPA WT). Then, neurovascular coupling (NVC) and stroke damages were investigated by measuring cerebral blood flow in response to whiskers stimulation and measuring infarct volumes by MRI in thrombin-induced stroke, respectively. **Results**. Our data reveal a reduced NVC in Null/Null parabionts compared to WT/WT parabionts. The deficit was absent in Null/WT couples. In the stroke model, observed in Null/Null parabionts displayed larger infarct volumes compared to WT/WT littermates. In heterotypic Null/WT parabionts, stroke damages in the WT partner were similar to those in WT/WT couples. Interestingly, stroke damages in the Null partner were lower than in the Null/Null parabionts, but bigger than in the WT/WT parabionts. **Conclusions.** All together, these data suggest that circulating tPA (but not parenchymal tPA) participates in NVC. On the other hand, our findings show that parenchymal tPA has deleterious effects during stroke, while circulating tPA is beneficial.

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METABOTROPIC GLUTAMATE RECEPTORS GROUP II (MGLUR2/3) AGONISTS REDUCED APOPTOSIS AND REGULATED BDNF, GDNF, AND TGF – BETA LEVELS IN HYPOXIC-ISCHEMIC INJURY IN NEONATAL RATS.

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Birth asphyxia occurs when the brain blood flow is impaired (ischemia) and oxygen supply is arrested (hypoxia). Hypoxia-ischemia (HI) results in damage of the central nervous system leading to neonatal death or developmental disorders. A recent study has shown that group II metabotropic glutamate receptors (mGluR2/3) activation can provide neuroprotection against HI but the mechanism of this effect is still not clear. **Aims:** This study aimed to establish the anti-apoptotic effect of mGluR2/3 agonists in an experimental model of birth asphyxia. **Methods:** The animal model of HI on 7-day old rat pups was used. Specific agonists of mGluR2 (LY 379268) and mGluR3 (NAAG) were injected intraperitoneally 1 h or 6 h after HI. The weight deficit of the ischemic brain hemisphere was measured and the expression of Bax, Bcl-2, HTR/OMI was examined. The expression of trophic factors GDNF, BDNF, TGF-beta was also measured. **Results:** The application of each agonist decreased brain tissue weight loss in the ischemic hemisphere independently on the time of application (from 40% in HI to 15 - 20% in treated). Both agonists applied 1h or 6h after HI increased expression of Bcl-2 and decreased expression of Bax and HtrA2/OMI proteins compared to untreated HI. mGluR2/3 agonists decreased expression of TGF-beta and GDNF and increased BDNF in the ischemic hemisphere compared to HI. **Conclusions:** The results show that activation of mGluR2 or mGluR3 in a short time after H-I insult triggered neuroprotective mechanisms and reduced apoptotic processes initiated by HI in the developing brain.

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INTRA-ARTERIAL MESENCHYMAL STEM CELL THERAPY REGULATES AQUAPORIN 4 TO ALLEVIATE PERIFOCAL VASOGENIC EDEMA POST-STROKE IN ANIMAL MODEL OF ISCHEMIC STROKE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Background: Post-stroke vasogenic edema following blood brain barrier (BBB) disruption and upregulation of aquaporin 4 (AQP4) water transport channels are well reported. Previous studies from our lab have demonstrated that intra-arterial (IA) administration of $1*10^5$ mesenchymal stem cell (MSCs) is neuroprotective. Protein kinase C δ (PKCδ) plays an important role to exacerbate ischemic reperfusion injury. Therefore, our study aims to decipher the possible molecular mechanism of post-stroke vasogenic edema alleviation by regulating AQP4 expression via modulation of PKCδ following IA MSCs administration. **Materials and Methods:** Ovariectomized female SD rats were infused $1*10^5$ IA MSCs at 6 h post-middle cerebral artery occlusion. Following 24 h of stroke, neurodeficit scores and motor functions were evaluated and animals were sacrificed. Brains were harvested for estimation of infarct size, edema and BBB damage. Cortical brain regions were harvested for further biochemical and molecular studies. **Results:** Administration of IA MSCs exhibited neuroprotection as evident by the reduction of infarct size, edema and improvement in functional outcome. Reduction in gene and consequent protein expression of AQP4 and PKCδ were observed within cortical brain region following IA MSCs administration. **Discussion and conclusion:** Vasogenic edema is one of the factors that contributes to high post-stroke mortality. Our study provides a preliminary evidence of IA MSCs mediated alleviation of post-stroke vasogenic edema by regulating AQP4 via modulation of PKCδ, thereby rendering neuroprotection. The novelty of our study lies in using IA MSCs intervention post-stroke to resolve vasogenic edema which is clinically relevant.

Pubmed:

31820348: Vats K, Sarmah D, Datta A, Saraf J, Kaur H, Pravalika K, Wanve M, Kalia K, Borah A, Dave KR, Yavagal DR, Bhattacharya P
Intra-arterial Stem Cell Therapy Diminishes Inflammasome Activation After Ischemic Stroke: a Possible Role of Acid Sensing Ion Channel 1a.
Studies from our lab demonstrated that 1 × 10 intra-arterial mesenchymal stem cells (IA MSCs) at 6 h following ischemic stroke are efficacious owing to its maximum homing due to elevated stromal derived factor 1 (SDF1) in the tissue. Further, IA MSCs could abate the infarct progression, improve functional outcome, and decrease expression of calcineurin by modifying neuronal Ca channels following ischemic stroke. Since stroke pathology also encompasses acidosis that worsens the condition; hence, the role of acid sensing ion channels (ASICs) in this context could not be overlooked. ASIC1a being the major contributor towards acidosis triggers Ca ions overload which progressively contributes towards exacerbation of neuronal injury following ischemic insult. Inflammasome involvement in ischemic stroke is well reported as activated ASIC1a increases the expression of inflammasome in a pH-dependent manner to trigger inflammatory cascade. Hence, the current study aimed to identify if IA MSCs can decrease the production of inflammasome by attenuating ASIC1a expression to render neuroprotection. Ovariectomized Sprague Dawley (SD) rats exposed to middle cerebral artery occlusion (MCAo) for 90 min were treated with phosphate-buffered saline (PBS) or 1 × 10 MSCs IA at 6 h to check for the expression of ASIC1a and inflammasome in different groups. Inhibition studies were carried out to explore the underlying mechanism. Our results demonstrate that IA MSCs improves functional outcome and oxidative stress parameters, and decreases the expression of ASIC1a and inflammasomes in the cortical brain region after ischemic stroke. This study offers a preliminary evidence of the role of IA MSCs in regulating inflammasome by modulating ASIC1a.
J Mol Neurosci, 2021; 71
34553602: Kaur H, Sarmah D, Veeresh P, Datta A, Kalia K, Borah A, Yavagal DR, Bhattacharya P
Endovascular Stem Cell Therapy Post Stroke Rescues Neurons from Endoplasmic Reticulum Stress-Induced Apoptosis by Modulating Brain-Derived Neurotrophic Factor/Tropomyosin Receptor Kinase B Signaling.
Ischemic stroke is devastating, with serious long-term disabilities affecting millions of people worldwide. Growing evidence

has shown that mesenchymal stem cells (MSCs) administration after stroke provides neuroprotection and enhances the quality of life in stroke patients. Previous studies from our lab have shown that 1 × 10 MSCs administered intra-arterially (IA) at 6 h post stroke provide neuroprotection through the modulation of inflammasome and calcineurin signaling. Ischemic stroke induces endoplasmic reticulum (ER) stress, which exacerbates the pathology. The current study intends to understand the involvement of brain-derived neurotrophic factor/tropomyosin receptor kinase B (BDNF/TrkB) signaling in preventing apoptosis induced by ER stress post stroke following IA MSCs administration. Ischemic stroke was induced in ovariectomized female Sprague Dawley rats. The MSCs were administered IA, and animals were sacrificed at 24 h post stroke. Infarct area, neurological deficit score, motor coordination, and biochemical parameters were evaluated. The expression of various genes and proteins was assessed. An inhibition study was also carried out to confirm the involvement of BDNF/TrkB signaling in ER stress-induced apoptosis. IA-administered MSCs improved functional outcomes, reduced infarct area, increased neuronal survival, and normalized biochemical parameters. mRNA and protein expression of ER stress markers were reduced, while those of BDNF and TrkB were increased. Reduction in ER stress-mediated apoptosis was also observed. The present study shows that IA MSCs administration post stroke provides neuroprotection and can modulate ER stress-mediated apoptosis via the BDNF/TrkB signaling pathway.
ACS Chem Neurosci, 2021; 12
35112234: Sarmah D, Datta A, Kaur H, Kalia K, Borah A, Rodriguez AM, Yavagal DR, Bhattacharya P
Sirtuin-1 - Mediated NF-κB Pathway Modulation to Mitigate Inflammasome Signaling and Cellular Apoptosis is One of the Neuroprotective Effects of Intra-arterial Mesenchymal Stem Cell Therapy Following Ischemic Stroke.
Stroke results in long term serious disability that affect millions across the globe. Several clinical and preclinical studies have reinforced the therapeutic use of stem cells in stroke patients to enhance their quality of life. Previous studies from our lab have demonstrated that 1*10 allogeneic bone marrow-derived mesenchymal stem cells (BM-MSCs) when given intraarterially (IA) render neuroprotection by modulating the expression of inflammasomes. Sirtuins are a class of important deacylases having a significant role in cellular functioning. Sirtuin-1 (SIRT-1) is an important enzyme essential for regulating cellular metabolism, which is reduced following an ischemic episode. The present study aims to unviel the role of MSCs in regulating the brain SIRT-1 levels following stroke and the involvement of SIRT-1 in regulating inflammasome signaling to reduce cellular apoptosis towards rendering neuroprotection.
Stem Cell Rev Rep, 2022; 18
32939758: Baidya F, Bohra M, Datta A, Sarmah D, Shah B, Jagtap P, Raut S, Sarkar A, Singh U, Kalia K, Borah A, Wang X, Dave KR, Yavagal DR, Bhattacharya P
Neuroimmune crosstalk and evolving pharmacotherapies in neurodegenerative diseases.
Neurodegeneration is characterized by gradual onset and limited availability of specific biomarkers. Apart from various aetiologies such as infection, trauma, genetic mutation, the interaction between the immune system and CNS is widely associated with neuronal damage in neurodegenerative diseases. The immune system plays a distinct role in disease progression and cellular homeostasis. It induces cellular and humoral responses, and enables tissue repair, cellular healing and clearance of cellular detritus. Aberrant and chronic activation of the immune system can damage healthy neurons. The pro-inflammatory mediators secreted by chief innate immune components, the complement system, microglia and inflammasome can augment cytotoxicity. Furthermore, these inflammatory mediators accelerate microglial activation resulting in progressive neuronal loss. Various animal studies have been carried out to unravel the complex pathology and ascertain biomarkers for these harmful diseases, but have had limited success. The present review will provide a thorough understanding of microglial activation, complement system and inflammasome generation, which lead the healthy brain towards neurodegeneration. In addition to this, possible targets of immune components to confer a strategic treatment regime for the alleviation of neuronal damage are also summarized.
Immunology, 2021; 162
32348103: Raut S, Singh U, Sarmah D, Datta A, Baidya F, Shah B, Bohra M, Jagtap P, Sarkar A, Kalia K, Borah A, Dave KR, Yavagal DR, Bhattacharya P
Migraine and Ischemic Stroke: Deciphering the Bidirectional Pathway.
Migraine and stroke are common, disabling neurological conditions with several theories being proposed to explain this bidirectional relationship. Migraine is considered as a benign neurological disorder, but research has revealed a connection between migraine and stroke, predominantly those having migraine with aura (MA). Among migraineurs, females with MA are more susceptible to ischemic stroke and may have a migrainous infarction. Migrainous infarction mostly occurs in the posterior circulation of young women. Although there are several theories about the potential relationship between MA and stroke, the precise pathological process of migrainous infarction is not clear. It is assumed that cortical spreading depression (CSD) might be one of the essential factors for migrainous infarction. Other factors that may contribute to migrainous infarction may be genetic, hormonal fluctuation, hypercoagulation, and right to left cardiac shunts. Antimigraine drugs, such as ergot alkaloids and triptans, are widely used in migraine care. Still, they have been found to cause severe

vasoconstriction, which may result in the development of ischemia. It is reported that patients with stroke develop migraines during the recovery phase. Both experimental and clinical data suggest that cerebral microembolism can act as a potential trigger for MA. Further studies are warranted for the treatment of migraine, which may lead to a decline in migraine-related stroke. In this present article, we have outlined various potential pathways that link migraine and stroke.
ACS Chem Neurosci, 2020; 11
33242696: Sarmah D, Banerjee M, Datta A, Kalia K, Dhar S, Yavagal DR, Bhattacharya P
Nanotechnology in the diagnosis and treatment of stroke.
Increasing developments in the field of nanotechnology have ignited its use in stroke diagnosis and treatment. The benefits of structural modification, ease of synthesis, and biocompatibility support the use of nanomaterials in the clinic. The pathophysiology of stroke is complex, involving different brain regions; hence, therapeutic agents are required to be delivered to specific regions. Nanoparticles (NPs) can be engineered to help improve the delivery and release of therapeutic agents in a localized manner, especially in the penumbra. This contributes not only to therapy, but also to neurosurgery and neuroimaging. Nanomaterials also offer high efficacy with few adverse effects. In this review, we provide a concise summary of the caveats associated with nanotechnology with respect to stroke therapy and diagnosis.
Drug Discov Today, 2021; 26
33359639: Sarkar A, Sarmah D, Datta A, Kaur H, Jagtap P, Raut S, Shah B, Singh U, Baidya F, Bohra M, Kalia K, Borah A, Wang X, Dave KR, Yavagal DR, Bhattacharya P
Post-stroke depression: Chaos to exposition.
Cerebral ischemia contributes to significant disabilities worldwide, impairing cognitive function and motor coordination in affected individuals. Stroke has severe neuropsychological outcomes, the major one being a stroke. Stroke survivors begin to show symptoms of depression within a few months of the incidence that overtime progresses to become a long-term ailment. As the pathophysiology for the progression of the disease is multifactorial and complex, it limits the understanding of the disease mechanism completely. Meta-analyses and randomized clinical trials have shown that intervening early with tricyclic antidepressants and selective serotonin receptor inhibitors can be effective. However, these pharmacotherapies possess several limitations that have given rise to newer approaches such as brain stimulation, psychotherapy and rehabilitation therapy, which in today's time are gaining attention for their beneficial results in post-stroke depression (PSD). The present review highlights numerous factors like lesion location, inflammatory mediators and genetic abnormalities that play a crucial role in the development of depression in stroke patients. Further, we have also discussed various mechanisms involved in post-stroke depression (PSD) and strategies for early detection and diagnosis using biomarkers that may revolutionize treatment for the affected population. Towards the end, along with the preclinical scenario, we have also discussed the various treatment approaches like pharmacotherapy, traditional medicines, psychotherapy, electrical stimulation and microRNAs being utilized for effectively managing PSD.
Brain Res Bull, 2021; 168
35066715: Jadhav P, Karande M, Sarkar A, Sahu S, Sarmah D, Datta A, Chaudhary A, Kalia K, Sharma A, Wang X, Bhattacharya P
Glial Cells Response in Stroke.
As the second-leading cause of death, stroke faces several challenges in terms of treatment because of the limited therapeutic interventions available. Previous studies primarily focused on metabolic and blood flow properties as a target for treating stroke, including recombinant tissue plasminogen activator and mechanical thrombectomy, which are the only USFDA approved therapies. These interventions have the limitation of a narrow therapeutic time window, the possibility of hemorrhagic complications, and the expertise required for performing these interventions. Thus, it is important to identify the contributing factors that exacerbate the ischemic outcome and to develop therapies targeting them for regulating cellular homeostasis, mainly neuronal survival and regeneration. Glial cells, primarily microglia, astrocytes, and oligodendrocytes, have been shown to have a crucial role in the prognosis of ischemic brain injury, contributing to inflammatory responses. They play a dual role in both the onset as well as resolution of the inflammatory responses. Understanding the different mechanisms driving these effects can aid in the development of therapeutic targets and further mitigate the damage caused. In this review, we summarize the functions of various glial cells and their contribution to stroke pathology. The review highlights the therapeutic options currently being explored and developed that primarily target glial cells and can be used as neuroprotective agents for the treatment of ischemic stroke.
Cell Mol Neurobiol, 2022;
32297583: Datta A, Sarmah D, Kalia K, Borah A, Wang X, Dave KR, Yavagal DR, Bhattacharya P
Advances in Studies on Stroke-Induced Secondary Neurodegeneration (SND) and Its Treatment.
The occurrence of secondary neurodegeneration has exclusively been observed after the first incidence of stroke. In humans and rodents, post-stroke secondary neurodegeneration (SND) is an inevitable event that can lead to progressive neuronal loss at a region distant to initial infarct. SND can lead to cognitive and motor function impairment, finally causing dementia.

The exact pathophysiology of the event is yet to be explored. It is seen that the thalami, in particular, are susceptible to cause SND. The reason behind this is because the thalamus functioning as the relay center and is positioned as an interlocked structure with direct synaptic signaling connection with the cortex. As SND proceeds, accumulation of misfolded proteins and microglial activation are seen in the thalamus. This leads to increased neuronal loss and worsening of functional and cognitive impairment.
Curr Top Med Chem, 2020; 20
32219729: Datta A, Sarmah D, Mounica L, Kaur H, Kesharwani R, Verma G, Veeresh P, Kotian V, Kalia K, Borah A, Wang X, Dave KR, Yavagal DR, Bhattacharya P
Cell Death Pathways in Ischemic Stroke and Targeted Pharmacotherapy.
Ischemic stroke is one of the significant causes of morbidity and mortality, affecting millions of people across the globe. Cell injury in the infarct region is an inevitable consequence of focal cerebral ischemia. Subsequent reperfusion exacerbates the harmful effect and increases the infarct volume. These cellular injuries follow either a regulated pathway involving tightly structured signaling cascades and molecularly defined effector mechanisms or a non-regulated pathway, also known as accidental cell death, where the process is biologically uncontrolled. Classical cell death pathways are long established and well reported in several articles that majorly define apoptotic cell death. A recent focus on cell death study also considers investigation on non-classical pathways that are tightly regulated, may or may not involve caspases, but non-apoptotic. Pathological cell death is a cardinal feature of different neurodegenerative diseases. Although ischemia cannot be classified as a neurodegenerative disease, it is a cerebrovascular event where the infarct region exhibits aberrant cell death. Over the past few decades, several therapeutic options have been implicated for ischemic stroke. However, their use has been hampered owing to the number of limitations that they possess. Ischemic penumbral neurons undergo apoptosis and become dysfunctional; however, they are salvageable. Thus, understanding the role of different cell death pathways is crucial to aid in the modern treatment of protecting apoptotic neurons.
Transl Stroke Res, 2020; 11

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BOARD NUMBER: S04-296

INHIBITION OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3) – A PROMISING NEUROPROTECTION STRATEGY FOR NEONATAL HYPOXIC-ISCHAEMIC BRAIN DAMAGE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Hypoxic-ischaemic encephalopathy (HIE) is a leading cause of child mortality and morbidity. The only currently available treatment for neonatal hypoxia-ischaemia (HI) is therapeutic hypothermia which has limited effectiveness and application. Thus, there is a need for alternative therapies for neonatal HI brain damage. HI strongly up-regulates Signal Transducer and Activator of Transcription 3 (STAT3) in the immature brain. We have previously shown that neuronal or astroglial STAT3-deletion reduce cell death, tissue loss, glial activation in a postnatal day 7 (P7) HI mouse model, suggesting detrimental effect of STAT3 in neonatal HI. Moreover, pre-insult STAT3-blockade at tyrosine 705 (Y705) with JAK2-inhibitor WP1066 reduces microglial and astroglial activation to a more moderate degree, but similarly to the cell-specific deletions. The P7 mouse is considered slightly pre-term, while the P9 corresponds to term when compared to the human fetus. Therefore, we aimed to investigate whether inhibition of STAT3 phosphorylation at Y705 will provide neuroprotection in the P9 mouse similarly to the P7 model. We hypothesized that pharmacological STAT3 Y705 inhibition immediately post-HI will provide neuroprotection in the neonatal P9 mouse. We subjected P9 mice to unilateral carotid artery ligation and 60min hypoxia, and then treated them with different doses of WP1066, compared with non-treated controls.WP1066 treatment reduced brain tissue loss, cell death, microglial and astroglial activation at a dose of 80µg/g body weight, however doses of 40µg/g and 160µg/g were not neuroprotective. In conclusion, the application of WP1066 as a neuroprotective agent in the regulation of STAT3 phosphorylation may be a promising new strategy in neonatal HIE.

Pubmed:
34504417: Tetorou K, Sisa C, Iqbal A, Dhillon K, Hristova M
Current Therapies for Neonatal Hypoxic-Ischaemic and Infection-Sensitised Hypoxic-Ischaemic Brain Damage.
Neonatal hypoxic-ischaemic brain damage is a leading cause of child mortality and morbidity, including cerebral palsy, epilepsy, and cognitive disabilities. The majority of neonatal hypoxic-ischaemic cases arise as a result of impaired cerebral perfusion to the foetus attributed to uterine, placental, or umbilical cord compromise prior to or during delivery. Bacterial infection is a factor contributing to the damage and is recorded in more than half of preterm births. Exposure to infection exacerbates neuronal hypoxic-ischaemic damage thus leading to a phenomenon called infection-sensitised hypoxic-ischaemic brain injury. Models of neonatal hypoxia-ischaemia (HI) have been developed in different animals. Both human and animal studies show that the developmental stage and the severity of the HI insult affect the selective regional vulnerability of the brain to damage, as well as the subsequent clinical manifestations. Therapeutic hypothermia (TH) is the only clinically approved treatment for neonatal HI. However, the number of HI infants needed to treat with TH for one to be saved from death or disability at age of 18-22 months, is approximately 6-7, which highlights the need for additional or alternative treatments to replace TH or increase its efficiency. In this review we discuss the mechanisms of HI injury to the immature brain and the new experimental treatments studied for neonatal HI and infection-sensitised neonatal HI.
Front Synaptic Neurosci, 2021; 13

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BOARD NUMBER: S04-297

CANNABINOIDS AND CEREBRAL ISCHEMIA: EXPERIMENTAL STUDIES IN AN IN VITRO MODEL.

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Introduction: Cannabinoids are implicated in a number of physiological and pathological mechanisms in the central nervous system. However, their role in mechanisms leading to neurodegeneration following cerebral ischemia is yet unclear. Many studies have produced conflicting results on cannabinoids putative protective and/or toxic effects. Aim: We examined the effects of natural Cannabis extracts or selected cannabinoids and their possible pharmacological mechanism in an *in vitro* model of cerebral ischemia. Methods: We investigated the effects of two Cannabis extracts (Bedrocan and FM2) or selected cannabinoids, Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol (CBG) in rat organotypic hippocampal slices exposed to Oxygen and Glucose Deprivation (OGD), an *in vitro* model of forebrain global ischemia. Cell death in the CA1 subregion of slices was quantified by propidium iodide fluorescence. To gain deeper the possible mechanism of action underlying THC toxicity and/or CBD protection in our model, we used specific antagonists of CB1, CB2, TRPV1, TRPV2, 5-HT1A and PPAR receptors, at concentrations that displayed no effect when used alone. Results: When present in the incubation medium the Bedrocan extract or THC exacerbated, whereas the FM2 extract or cannabidiol attenuated CA1 injury induced by OGD. CB1 receptor antagonists prevented Δ9-THC toxicity; TRPV2, 5-HT1A, and PPAR antagonists blocked the neuroprotective effect of cannabidiol. Conclusions: Our results suggest that cannabinoids play different roles in the mechanisms of post-ischemic neuronal death. In particular, appropriate concentrations of CBD or CBD/THC ratios may represent a valid therapeutic intervention in the treatment of post-ischemic neuronal death.

Pubmed:
34959374: Landucci E, Bonomolo F, De Stefani C, Mazzantini C, Pellegrini-Giampietro DE, Bilia AR, Bergonzi MC
Preparation of Liposomal Formulations for Ocular Delivery of Thymoquinone: In Vitro Evaluation in HCEC-2 e HConEC Cells.
Thymoquinone (TQ) is the main constituent of L. essential oil. In vitro studies have shown its protective effect against HO-induced oxidative stress in human retinal pigment epithelium cells, and in vivo experiments have demonstrated its effect in decreasing corneal neovascularization and reducing the inflammation in an experimental dry eye model in mice. Its therapeutic use is limited by poor bioavailability, low solubility, and scarce permeability. In this study, two liposomal formulations have been developed, both of which consist of phosphatidylcholine and Plurol Oleique, a liquid lipid, and one of which is coated with 0.1% / hyaluronic acid (HA) to increase both TQ solubility and its ocular therapeutic potential. Each formulation has a size <200 nm and an EE% around 70%, determined by scattering techniques and the HPLC-DAD analytical method, respectively, and they result in a 2-fold increase in TQ solubility. HA-coated liposomes are stable over 2 months at +4 °C, and coated and uncoated liposomes present a gradual and prolonged release of TQ. Two cell lines, human corneal epithelial cells (HCEC-2) and human conjunctival epithelial cells (HConEC) were used to investigate the safety of the liposomal formulations. Uptake studies were also performed using fluorescent liposomes. Both liposomes and, in particular, HA-coated liposomes reduce the TQ toxicity observed at high doses in both HCEC-2 and HConEC cells, and both formulations increase the absorption at the cellular level and especially at the nucleus level, with a more pronounced effect for HA-coated liposomes.
Pharmaceutics, 2021; 13
34575932: Landucci E, Mazzantini C, Lana D, Davolio PL, Giovannini MG, Pellegrini-Giampietro DE
Neuroprotective Effects of Cannabidiol but Not Δ-Tetrahydrocannabinol in Rat Hippocampal Slices Exposed to Oxygen-Glucose Deprivation: Studies with Extracts and Selected Cannabinoids.
(1) Background: Over the past 10 years, a number of scientific studies have demonstrated the therapeutic potential of cannabinoid compounds present in the and plants. However, their role in mechanisms leading to neurodegeneration following cerebral ischemia is yet unclear. (2) Methods: We investigated the effects of extracts (Bedrocan, FM2) or selected cannabinoids (Δ-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabigerol) in rat organotypic hippocampal slices exposed to oxygen-glucose deprivation (OGD), an in vitro model of forebrain global ischemia. Cell death in the CA1 subregion of slices was quantified by propidium iodide fluorescence, and morphological analysis and tissue organization were

examined by immunohistochemistry and confocal microscopy. (3) Results: Incubation with the Bedrocan extract or THC exacerbated, whereas incubation with the FM2 extract or cannabidiol attenuated CA1 injury induced by OGD. Δ-THC toxicity was prevented by CB1 receptor antagonists, the neuroprotective effect of cannabidiol was blocked by TRPV2, 5-HT1A, and PPARγ antagonists. Confocal microscopy confirmed that CBD, but not THC, had a significant protective effect toward neuronal damage and tissue disorganization caused by OGD in organotypic hippocampal slices. (4) Conclusions: Our results suggest that cannabinoids play different roles in the mechanisms of post-ischemic neuronal death. In particular, appropriate concentrations of CBD or CBD/THC ratios may represent a valid therapeutic intervention in the treatment of post-ischemic neuronal death.
Int J Mol Sci, 2021; 22
33805696: Landucci E, Mazzantini C, Buonvicino D, Pellegrini-Giampietro DE, Bergonzi MC
Neuroprotective Effects of Thymoquinone by the Modulation of ER Stress and Apoptotic Pathway in In Vitro Model of Excitotoxicity.
Experimental evidence indicates that the activation of ionotropic glutamate receptors plays an important role in neurological disorders' models such as epilepsy, cerebral ischemia and trauma. The glutamate receptor agonist kainic acid (KA) induces seizures and excitotoxic cell death in the CA3 region of the hippocampus. Thymoquinone (TQ) is the most important component of the essential oil obtained from black cumin (L.) seeds. It has many pharmacological actions including antioxidant, anti-inflammatory, and anti-apoptotic effects. TQ was used in an in vitro experimental model of primary cultures where excitotoxicity was induced. Briefly, rat organotypic hippocampal slices were exposed to 5 μM KA for 24 h. Cell death in the CA3 subregions of slices was quantified by measuring propidium iodide fluorescence. The cross-talk between TQ, ER stress and apoptotic pathways was investigated by Western blot. In untreated slices TQ (10 μM) induced a significant increase on the PSD95 levels and it decreased the excitotoxic injury induced by KA. Additionally, TQ was able to ameliorate the KA-induced increase in unfolded proteins GRP78 and GRP94 expression. Finally, TQ was able to partially rescue the reduction of the KA-induced apoptotic pathway activation. Our results suggest that TQ modulates the processes leading to post-kainate neuronal death in the CA3 hippocampal area.
Molecules, 2021; 26
33667466: Mencucci R, Favuzza E, Becatti M, Tani A, Mazzantini C, Vignapiano R, Fiorillo C, Pellegrini-Giampietro D, Manetti M, Marini M, Landucci E
Co-expression of the SARS-CoV-2 entry receptors ACE2 and TMPRSS2 in healthy human conjunctiva.
The purpose of this study was to evaluate the expression of the SARS-CoV-2 receptors ACE2 and TMPRSS2 in an immortalized human conjunctival epithelial cell line and in healthy human conjunctiva excised during ocular surgery, using Western blot, confocal microscopy and immunohistochemistry. The Western blot showed that ACE2 and TMPRSS2 proteins were expressed in human immortalized conjunctival cells, and this was confirmed by confocal microscopy images, that demonstrated a marked cellular expression of the viral receptors and their co-localization on the cell membranes. Healthy conjunctival samples from 11 adult patients were excised during retinal detachment surgery. We found the expression of ACE2 and TMPRSS2 in all the conjunctival surgical specimens analyzed and their co-localization in the superficial conjunctival epithelium. The ACE2 Western blot levels and immunofluorescence staining for ACE2 were variable among specimens. These results suggest the susceptibility of the conjunctival epithelium to SARS-CoV-2 infection, even though with a possible interindividual variability.
Exp Eye Res, 2021; 205
32980315: Mencucci R, Favuzza E, Bottino P, Mazzantini C, Zanotto E, Pellegrini-Giampietro DE, Landucci E
A new ophthalmic formulation containing antiseptics and dexpanthenol: In vitro antimicrobial activity and effects on corneal and conjunctival epithelial cells.
Antibiotic resistance is increasing even in ocular pathogens, therefore the interest towards antiseptics in Ophthalmology is growing. The aim of this study was to analyze the in vitro antimicrobial efficacy and the in vitro effects of an ophthalmic formulation containing hexamidine diisethionate 0.05%, polyhexamethylene biguanide (PHMB) 0.0001% disodium edetate (EDTA) 0.01%, dexpanthenol 5% and polyvinyl alcohol 1.25% (Keratosept, Bruschettini, Genova, Italy) on cultured human corneal and conjunctival cells. The in vitro antimicrobial activity was tested on Staphylococcus aureus, Methicillin-Resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Streptococcus pneumoniae, Streptococcus pyogenes and Streptococcus mitis. For each microbial strain 10 μL of a 0.5 MacFarland standardized bacterial inoculum were incubated at 25 °C with 100 μL of ophthalmic solution for up to 6 h. After different periods of time, samples were inoculated on blood agar with 5% sheep blood. Moreover, a 0.5 MacFarland bacterial inoculum was seeded in triplicate on Mueller-Hinton Agar or on Mueller-Hinton Fastidious Agar; then a cellulose disc soaked with 50 μL of ophthalmic solution was applied on the surface of agar and plates were incubated for 18 h at 37 °C, in order to evaluate the inhibition of bacterial growth around the disc. Human corneal and conjunctival epithelial cells in vitro were incubated for 5, 10 and 15 min with Keratosept or its components. The cytotoxicity was assessed through the release of cytoplasmic enzyme lactate dehydrogenase (LDH) into

the medium immediately after exposure to the drugs; the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to evaluate the metabolic cell activity. Our results show that Keratosept ophthalmic solution gave an average logarithmic (log) reduction of bacterial load of 2.14 ± 0.35 within 6 h of exposure (p-value < 0.05 versus control saline solution). On agar plates, all microbial strains, excluding P. Aeruginosa, showed an inhibition zone of growth around the Keratosept-soaked discs. Keratosept and its components after 5 and 10 min did not show any cytotoxic effect on cultured corneal and conjunctival cells, and only after 15 min a significant reduction of cell viability and an increase of cytotoxicity compared to control (vehicle) was seen; dexpanthenol 5% and polyvinyl alcohol accelerated the wounding of corneal cells in vitro. In conclusion, Keratosept showed good antimicrobial activity on the tested strains; the ophthalmic solution and its components were safe and non-toxic for the corneal and conjunctival epithelial cells for 5 and 10 min at the concentrations analyzed, and dexpanthenol 5% and polyvinyl alcohol promoted the wounding of corneal cells.
Exp Eye Res, 2020; 201

32375297: Gencarelli M, Laurino A, Landucci E, Buonvicino D, Mazzantini C, Chiellini G, Raimondi L
3-Iodothyronamine Affects Thermogenic Substrates' Mobilization in Brown Adipocytes.
We investigated the effect of 3-iodothyronamine (T1AM) on thermogenic substrates in brown adipocytes (BAs). BAs isolated from the stromal fraction of rat brown adipose tissue were exposed to an adipogenic medium containing insulin in the absence (M) or in the presence of 20 nM T1AM (M+T1AM) for 6 days. At the end of the treatment, the expression of p-PKA/PKA, p-AKT/AKT, p-AMPK/AMPK, p-CREB/CREB, p-P38/P38, type 1 and 3 beta adrenergic receptors (β1-β3AR), GLUT4, type 2 deiodinase (DIO2), and uncoupling protein 1 (UCP-1) were evaluated. The effects of cell conditioning with T1AM on fatty acid mobilization (basal and adrenergic-mediated), glucose uptake (basal and insulin-mediated), and ATP cell content were also analyzed in both cell populations. When compared to cells not exposed, M+T1AM cells showed increased p-PKA/PKA, p-AKT/AKT, p-CREB/CREB, p-P38/P38, and p-AMPK/AMPK, downregulation of DIO2 and β1AR, and upregulation of glycosylated β3AR, GLUT4, and adiponectin. At basal conditions, glycerol release was higher for M+T1AM cells than M cells, without any significant differences in basal glucose uptake. Notably, in M+T1AM cells, adrenergic agonists failed to activate PKA and lipolysis and to increase ATP level, but the glucose uptake in response to insulin exposure was more pronounced than in M cells. In conclusion, our results suggest that BAs conditioning with T1AM promote a catabolic condition promising to fight obesity and insulin resistance.
Biology (Basel), 2020; 9

31075293: Landucci E, Gencarelli M, Mazzantini C, Laurino A, Pellegrini-Giampietro DE, Raimondi L
N-(3-Ethoxy-phenyl)-4-pyrrolidin-1-yl-3-trifluoromethyl-benzamide (EPPTB) prevents 3-iodothyronamine (T1AM)-induced neuroprotection against kainic acid toxicity.
Thyroid hormone and thyroid hormone metabolites, including 3-iodothyronamine (T1AM) and 3-iodothyroacetic acid (TA1), activate AKT signaling in hippocampal neurons affording protection from excitotoxic damage. We aim to explore whether the mechanism of T1AM neuroprotection against kainic acid (KA)-induced excitotoxicity included the activation of the trace amine associated receptor isoform 1 (TAAR1), one of T1AM targets. Rat organotypic hippocampal slices were exposed to vehicle (Veh) or to 5 μM kA for 24 h in the absence or presence of 0.1, 1 and 10 μM T1AM or to 0.1, 1 and 10 μM T1AM and 1 μM N-(3-Ethoxy-phenyl)-4-pyrrolidin-1-yl-3-trifluoromethyl-benzamide (EPPTB), the only available TAAR1 antagonist, or to 1 μM T1AM in the absence or in the presence of 10 μM LY294002, an inhibitor of phosphoinositide 3-kinases (PI3Ks). Cell death was evaluated by measuring propidium iodide (PI) levels of fluorescence 24 h after treatment. In parallel, the expression levels of p-AKT and p-PKA were evaluated by Western blot analysis of slice lysates. The activity of mitochondrial monoamine oxidases (MAO) was assayed fluorimetrically. 24 h exposure of slices to T1AM resulted in the activation of AKT and PKA. KA exposure induced cell death in the CA3 region and significantly reduced p-AKT and p-PKA levels. The presence of 1 and 10 μM T1AM significantly protected neurons from death and conserved both kinase levels with the essential role of AKT in neuroprotection. Furthermore, EPPTB prevented T1AM-induced neuroprotection, activation of PKA and AKT. Of note, in the presence of EPPTB T1AM degradation by MAO was reduced. Our results indicate that the neuroprotection offered by T1AM depends, as for TA1, on AKT activation but do not allow to conclusively indicate TAAR1 as the target implicated.
Neurochem Int, 2019; 129

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BOARD NUMBER: S04-298

MILD HYPOTHERMIA ALLEVIATES REDUCTIVE STRESS, A ROOT CAUSE OF ISCHEMIA REPERFUSION INJURY

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Over the past 15 years, therapeutic hypothermia has proven to have potential to be one of the most attractive therapies for ischemic stroke, however, uncertainty around why and how hypothermia (32°C) provides protection is still a challenge. In a recent study our group has demonstrated for the first time that 32°C activates Nrf2, a major regulator of antioxidant gene transcription, in normoxic cells and provides protection from oxidative stress presumably by orchestrating adaptive responses to redox stress. The aim of the current study is to understand whether hypothermia mitigates the dangers posed by reductive equivalents. To study hypothermia in cell culture, cells were incubated at 32°C or kept at 37°C under normoxia or hypoxia (1% O2) followed by different downstream applications like biochemistry, molecular biology, and novel cellular gas flux system for measuring CO2 and O2 flux. We found that hypoxia reduces ATP/ADP ratio at 37°C while 32°C increased ATP/ADP ratio in both normoxic and hypoxic cells. In line with previous observations, we found that lipid content was increased by hypoxia, which are chemically more stable depos of reductive power. However, mild hypothermia prevented hypoxia-dependent increase in lipid content. In addition, hypothermia introduced during anoxia enhanced the recovery of respiration. This study addresses an important question on why hypothermia is effective in reducing hypoxic tissue damage. It suggests that hypothermia alleviates reductive stress by improving recovery of respiration after anoxia, maintaining ATP/ADP ratio, reducing lipid content, a conceptually novel and largely overlooked phenomenon at the root of ischemia reperfusion injury.

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BOARD NUMBER: S04-299

THE IMPACT OF GENERAL ANAESTHESIA ON THE DEVELOPING BRAIN PREVIOUSLY EXPOSED TO PERINATAL ASPHYXIA

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Aims: General anaesthesia (A) in paediatric patients is extensively used nowadays, although it still represents a debatable concern, especially in the context of its co-occurrence with other clinical conditions, such as perinatal asphyxia (PA). Our experimental study investigates hippocampal glial activation and inflammation secondary to PA or A alone, and to combined exposure (PAA). Methods: On postnatal day (PND) 6, we exposed Wistar rats to 90-minute of either asphyxia (PA) or normoxia, and on PND-15 to 180-minute of either sevoflurane anaesthesia (A) or normoxia. Hippocampal tissue was immediately harvested thereafter from each of the 4 groups (control, PA, A, PAA; 15 animals/group), and S-100B and IL-1B were assessed by ELISA. Results: Our results revealed an increased hippocampal level of S-100B consecutive to PA, A, and combined PAA exposure, showing that both PA and A significantly contribute to glial activation, however with no cumulative effect. The hippocampal level of IL-1B was increased consecutive to PA, was not impacted by A alone when compared to control, and was decreased in PAA group when compared to PA group. Conclusions: Our study pointed out that PA has a deleterious effect on the immature hippocampus by increasing glial activation and neuroinflammation, while A alone produces only glial activation. When combined, PA and A do not potentiate reciprocally. Further studies are needed to better understand the controversial general anaesthesia impact in pathophysiological conditions like PA, to ultimately increase the patients' safety. This research was supported by the Romanian Minister of Education, Project No. PN-III-P1-1.2-PD-2019-1219.

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BOARD NUMBER: S04-300

THE EFFECT OF MINOCYCLINE ON THE ISCHEMIC AREA AND IMPROVED MOTOR FUNCTION IN CORTICAL MODEL OF PHOTO THROMBOTIC ISCHEMIC STROKE IN RATS.

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Background And Aims Still stroke is a leading cause of death worldwide. In survivors, it can result in long-term disabilityin with various severity ranges. Strokes account for great amount of mortality and morbidity worldwide. Minocycline, by launching plethora of neuroprotective mechanisms may be beneficial as the treatment. Therefore, it is important to search for neuroprotection mechanisms that would allow to extend the therapeutic window and develop new strategies for treating ischemic strokes. The first aim was to investigate the effect of minocycline on penumbra and nesrosis. Our second goal was to examine the effect of treatment on the condition of ischemic rats
Methods Photothrombotic focal ischemia of motor cortex was produced in 72 male Long-Evans rats. We tested different time windows: 24h, 48h and 7 days after stroke induction. Half of the experimental groups received an intravenous dose of minocycline (1 mg / 1 kg b.w / 1ml solution, 10 minutes after stroke). CatWalk XT, Grip Strength-test and elevated runway-tests were performed. These functional tests were applied before and after ischemic stroke.
Results In groups with minocycline we observed statistically significant improvement of speed of walking, correctness of the stepping pattern and fewer foot slips compared to the untreated groups. Necrosis were localized by immunohistochemical techniques and staining by Nissl technique. The size, shape and area of the necrosis were measured. The greatest changes were observed in the time groups 7 days. **Conclusions** Minocycline improves motor function in ischemic rats. Minocycline action also correlates with size and shape of necrosis.

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EFFECTS OF NUCLEAR RECEPTOR REV-ERBα/β ON ISCHEMIC BRAIN INJURY IN MICE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Nuclear receptor Rev-Erbα/β plays an essential role not only in the regulation of the circadian rhythm but also in the regulation of many cellular functions such as metabolism, glial activation, and inflammation. In addition, recent studies show that Rev-Erbα/β may have an important role in neurodegenerative diseases. Thus, we explored the role of nuclear receptor Rev-Erbα/β in neuronal injury after focal cerebral ischemia in mice. For this aim, we produced lentivirus to overexpress or inhibit the Rev-Erbα/β protein expression. Viral particles were intrastriatally administered to male 10-12 weeks old C57BL/6j mice. Ten days after, mice were subjected to 30 min or 90 min of intraluminal middle cerebral artery occlusion (MCAO) and 72 h or 24 h reperfusion, respectively. Overexpression of Rev-Erbα/β decreased brain infarct volume, brain swelling, blood-brain barrier leakage, which was evaluated by serum IgG extravasation. However, inhibition of Rev-Erbα/β only increased infarct volume after 90 min of MCAO. In addition, neuronal survival and disseminated neuronal injury were evaluated by NeuN staining and TUNEL staining, respectively. It was observed that the number of surviving neurons increased, and the DNA fragmented cells decreased in the overexpression groups. However, in the inhibition groups, neuronal survival decreased, and DNA fragmentation increased after 30 min of MCAO. We provide evidence that the regulation of nuclear receptor Rev-Erbα/β straightforwardly affects brain infarct volume, brain swelling, neurological deficits, neuronal survival, and DNA fragmentation after ischemic brain injury. This study is supported by TUBITAK (219S913).

Pubmed:
34735672: Beker MC, Caglayan AB, Altunay S, Ozbay E, Ates N, Kelestemur T, Caglayan B, Kilic U, Doeppner TR, Hermann DM, Kilic E
Phosphodiesterase 10A Is a Critical Target for Neuroprotection in a Mouse Model of Ischemic Stroke.
Phosphodiesterase 10A (PDE10A) hydrolyzes adenosine 3',5'-cyclic monophosphate (cAMP) and guanosine 3',5'-cyclic monophosphate (cGMP). It is highly expressed in the striatum. Recent evidence implied that PDE10A may be involved in the inflammatory processes following injury, such as ischemic stroke. Its role in ischemic injury was unknown. Herein, we exposed mice to 90 or 30-min middle cerebral artery occlusion, followed by the delivery of the highly selective PDE10A inhibitor TAK-063 (0.3 mg/kg or 3 mg/kg) immediately after reperfusion. Animals were sacrificed after 24 or 72 h, respectively. Both TAK-063 doses enhanced neurological function, reduced infarct volume, increased neuronal survival, reduced brain edema, and increased blood-brain barrier integrity, alongside cerebral microcirculation improvements. Post-ischemic neuroprotection was associated with increased phosphorylation (i.e., activation) of pro-survival Akt, Erk-1/2, GSK-3α/β and anti-apoptotic Bcl-xL abundance, decreased phosphorylation of pro-survival mTOR, and HIF-1α, MMP-9 and pro-apoptotic Bax abundance. Interestingly, PDE10A inhibition reduced inflammatory cytokines/chemokines, including IFN-γ and TNF-α, analyzed by planar surface immunoassay. In addition, liquid chromatography-tandem mass spectrometry revealed 40 proteins were significantly altered by TAK-063. Our study established PDE10A as a target for ischemic stroke therapy.
Mol Neurobiol, 2022; 59
31836786: Beker MC, Caglayan B, Caglayan AB, Kelestemur T, Yalcin E, Caglayan A, Kilic U, Baykal AT, Reiter RJ, Kilic E
Interaction of melatonin and Bmal1 in the regulation of PI3K/AKT pathway components and cellular survival.
The circadian rhythm is driven by a master clock within the suprachiasmatic nucleus which regulates the rhythmic secretion of melatonin. Bmal1 coordinates the rhythmic expression of transcriptome and regulates biological activities, involved in cell metabolism and aging. However, the role of Bmal1 in cellular- survival, signaling, its interaction with intracellular proteins, and how melatonin regulates its expression is largely unclear. Here we observed that melatonin increases the expression of Bmal1 and both melatonin and Bmal1 increase cellular survival after oxygen glucose deprivation (OGD) while the inhibition of Bmal1 resulted in the decreased cellular survival without affecting neuroprotective effects of melatonin. By using a planar surface immunoassay for PI3K/AKT signaling pathway components, we revealed that both melatonin and Bmal1 increased phosphorylation of AKT, ERK-1/2, PDK1, mTOR, PTEN, GSK-3αβ, and p70S6K. In contrast, inhibition of Bmal1 resulted in decreased phosphorylation of these proteins, which the effect of melatonin on these signaling molecules was not affected by

the absence of Bmal1. Besides, the inhibition of PI3K/AKT decreased Bmal1 expression and the effect of melatonin on Bmal1 after both OGD in vitro and focal cerebral ischemia in vivo. Our data demonstrate that melatonin controls the expression of Bmal1 via PI3K/AKT signaling, and Bmal1 plays critical roles in cellular survival via activation of survival kinases.
Sci Rep, 2019; 9
28421530: Beker MC, Caglayan B, Yalcin E, Caglayan AB, Turkseven S, Gurel B, Kelestemur T, Sertel E, Sahin Z, Kutlu S, Kilic U, Baykal AT, Kilic E
Time-of-Day Dependent Neuronal Injury After Ischemic Stroke: Implication of Circadian Clock Transcriptional Factor Bmal1 and Survival Kinase AKT.
Occurrence of stroke cases displays a time-of-day variation in human. However, the mechanism linking circadian rhythm to the internal response mechanisms against pathophysiological events after ischemic stroke remained largely unknown. To this end, temporal changes in the susceptibility to ischemia/reperfusion (I/R) injury were investigated in mice in which the ischemic stroke induced at four different Zeitgeber time points with 6-h intervals (ZT0, ZT6, ZT12, and ZT18). Besides infarct volume and brain swelling, neuronal survival, apoptosis, ischemia, and circadian rhythm related proteins were examined using immunohistochemistry, Western blot, planar surface immune assay, and liquid chromatography-mass spectrometry tools. Here, we present evidence that midnight (ZT18; 24:00) I/R injury in mice resulted in significantly improved infarct volume, brain swelling, neurological deficit score, neuronal survival, and decreased apoptotic cell death compared with ischemia induced at other time points, which were associated with increased expressions of circadian proteins Bmal1, Perl, and Clock proteins and survival kinases AKT and Erk-1/2. Moreover, ribosomal protein S6, mTOR, and Bad were also significantly increased, while the levels of PRAS40, negative regulator of AKT and mTOR, and phosphorylated p53 were decreased at this time point compared to ZT0 (06:00). Furthermore, detailed proteomic analysis revealed significantly decreased CSKP, HBB-1/2, and HBA levels, while increased GNAZ, NEGR1, IMPCT, and PDE1B at midnight as compared with early morning. Our results indicate that nighttime I/R injury results in less severe neuronal damage, with increased neuronal survival, increased levels of survival kinases and circadian clock proteins, and also alters the circadian-related proteins.
Mol Neurobiol, 2018; 55
26416428: Beker MC, Caglayan AB, Kelestemur T, Caglayan B, Yalcin E, Yulug B, Kilic U, Hermann DM, Kilic E
Effects of normobaric oxygen and melatonin on reperfusion injury: role of cerebral microcirculation.
In order to protect the brain before an irreversible injury occurs, penumbral oxygenation is the primary goal of current acute ischemic stroke treatment. However, hyperoxia treatment remains controversial due to the risk of free radical generation and vasoconstriction. Melatonin is a highly potent free radical scavenger that protects against ischemic stroke. Considering its anti-oxidant activity, we hypothesized that melatonin may augment the survival-promoting action of normobaric oxygen (NBO) and prevent brain infarction. Herein, we exposed mice to 30 or 90 min of intraluminal middle cerebral artery occlusion (MCAo) and evaluated the effects of NBO (70% or 100% over 90 min), administered either alone or in combination with melatonin (4 mg/kg, i.p.), on disseminate neuronal injury, neurological deficits, infarct volume, blood-brain barrier (BBB) permeability, cerebral blood flow (CBF) and cell signaling. Both NBO and particularly melatonin alone reduced neuronal injury, neurological deficits, infarct volume and BBB permeability, and increased post-ischemic CBF, evaluated by laser speckle imaging (LSI). They also improved CBF significantly in the ischemic- core and penumbra, which was associated with reduced IgG extravasation, DNA fragmentation, infarct volume, brain swelling and neurological scores. Levels of phosphorylated Akt, anti-apoptotic Bcl-xL, pro-apoptotic Bax and endothelial nitric oxide synthase (NOS) were re-regulated after combined oxygen and melatonin delivery, whereas neuronal and inducible NOS, which were increased by oxygen treatment, were not influenced by melatonin. Our present data suggest that melatonin and NBO are promising approaches for the treatment of acute-ischemic stroke, which encourage proof-of-concept studies in human stroke patients.
Oncotarget, 2015; 6

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BOARD NUMBER: S04-302

NIR LASER PHOTOBIOMODULATION AS PROMISING THERAPY FOR BRAIN HYPOXIA/ISCHEMIA: IN VITRO STUDY IN ORGANOTYPIC HIPPOCAMPAL SLICES

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Background: Brain photobiomodulation (PBM) is an innovative treatment for a variety of neurological conditions, including cerebral ischemia. However, the capability of PBM for ischemic stroke needs to be further explored and its mechanisms of action remain currently unclear. **Aim**: to identify a treatment protocol capable of inducing neuroprotection and to investigate the molecular mechanisms activated by a dual-wavelength near infrared (NIR) laser source in an organotypic hippocampal slice model of hypoxia/ischemia. **Methods**: Hippocampal slices were exposed to oxygen and glucose deprivation (OGD) for 30 min followed by NIR laser treatment (fluence 3.71, 7.42, or 14.84 J/cm2; wavelengths 808 nm and 905 nm) delivered immediately, 30 min or 60 min after OGD. Neuronal injury, CA1 confocal microscopy and western blot analysis were assessed 24 h later. **Results**: NIR laser treatment attenuates OGD neurotoxicity once applied immediately or 30 min after OGD, suggesting a therapeutic window. Western blot analysis of proteins involved in neuroinflammation (iNOS, COX-2, NFkB subunit p65, and Bcl-2) and in glutamatergic-mediated synaptic activity (vGluT1, EAAT2, GluN1, and PSD95) showed that the protein modifications induced by OGD were reverted by NIR laser application. Moerover, CA1 confocal microscopy revealed that the profound morphological changes induced by OGD were reverted by NIR laser radiation **Conclusions**: NIR laser radiation reduces OGD neurotoxicity in organotypic hippocampal slices through attenuation of inflammatory mechanisms. These findings shed light on molecular definition of NIR neuroprotective mechanisms, thus underlining the potential benefit of this technique for the treatment of cerebral ischemia.

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BOARD NUMBER: S04-303

SPREADING DEPOLARIZATION DISRUPTS NEUROVASCULAR COUPLING AFTER GLOBAL CEREBRAL ISCHEMIA IN MICE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Introduction: Neurovascular coupling (NVC) is weak or absent upon acute ischemic stroke (AIS). The mechanistic basis of NVC dysfunction might be the evolution of spreading depolarization (SD), that causes vasoconstriction and lesion progression in AIS. Here, we show that SD occurrence disrupts functional hyperemia during NVC despite reperfusion after AIS. **Methods:** Male C57BL/6 mice (n=11) were anesthetized with isoflurane (0.6-1.2%). A baseline of 10 min was followed by a transient (45 min) bilateral common carotid artery occlusion (2VO) and a subsequent 60 min reperfusion. Cerebral blood flow (CBF) variations were captured using laser speckle contrast imaging (LASCA). After 60 min reperfusion, NVC function was evaluated under isoflurane (0.1%)-medetomidine (0.1 mg/kg) anesthesia by whisker stimulation (~2Hz). SHAM operated animals served as control. **Results:** Low CBF (<20%) early under ischemia favored spontaneous SD evolution (CBF <20% vs. >30%, SD vs. no SD; 16 SDs in 9 mice). SDs occurred in both hemispheres (bilateral) in 7, and in one hemisphere (unilateral) in 2 mice. In concert, functional hyperemia was diminished in 7, and was unilaterally intact in 2 mice (hyperemia amplitude: 8.73±3.01 vs. 17.16±6.21 %; bilateral vs. unilateral). The amplitude of unilaterally intact functional hyperemia was comparable to SHAM mice (17.16±6.21 vs. 16.71 %, unilateral vs. SHAM). **Discussion:** Our data demonstrate that SD evolution impairs NVC after AIS. SD is known to trigger vasoconstriction, called spreading oligemia that might diminish NVC function. We propose the pharmacological attenuation of spreading oligemia to improve NVC function after AIS.

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NEUROPROTECTION MEDIATED BY REMOTE ISCHEMIC POSTCONDITIONING IS IMPAIRED IN ZUCKER DIABETIC FATTY RATS MODEL

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Obesity is associated with increasing risk of ischemic stroke. Accordingly, the project aimed to investigate the neuroprotective effect of remote ischemic postconditioning (RIPostC) in obese rats. Male lean and obese Zucker diabetic fatty (ZDF) rats were subjected to 90 minutes of right middle artery occlusion (MCAO) followed by 24 h of reperfusion. Animals were then randomized into 2 groups: hind-limb ischemia treated (ischemic tolerance) and untreated. Although the infarct volume in obese rats was about 113 % higher compared to lean rats in the ischemia group, there were no significant differences among ischemic and tolerance group. Since oxidative stress have substantial role in the expansion of poststroke brain injury, total fragmented DNA in peripheral lymphocytes was measured by Comet Assay. Our results did not confirm the incidence of DNA strand breaks elevation in cell exposed to ischemia of both lean and obese groups, neither after the RIPostC intervention. However, a significantly higher level of oxidative stress was determined in the obese animals, even before the surgery. Assessment of oxidative stress between control and ischemic group revealed down-regulation of SOD activity in both groups without any significant differences between lean and obese group. We did not detect changes in CAT activity among all groups. In contrast, we observed a rapid increase of glutamate levels in peripheral blood in obese group compared to lean group. However, the glutamate level did not change in ischemic groups, neither after the RIPostC. This project was supported by research grants: VEGA 2/0073/21, 2/0096/22 and IMTS: 313011V344

Pubmed:
33809098: Petrova K, Kello M, Kuruc T, Backorova M, Petrovova E, Vilkova M, Goga M, Rucova D, Backor M, Mojzis J
Potential Effect of (L.) Zopf Extract and Metabolite Physodic Acid on Tumour Microenvironment Modulation in MCF-10A Cells.
Lichens comprise a number of unique secondary metabolites with remarkable biological activities and have become an interesting research topic for cancer therapy. However, only a few of these metabolites have been assessed for their effectiveness against various in vitro models. Therefore, the aim of the present study was to assess the effect of extract (L.) Zopf (PSE) and its metabolite physodic acid (Phy) on tumour microenvironment (TME) modulation, focusing on epithelial-mesenchymal transition (EMT), cancer-associated fibroblasts (CAFs) transformation and angiogenesis. Here, we demonstrate, by using flow cytometry, Western blot and immunofluorescence microscopy, that tested compounds inhibited the EMT process in MCF-10A breast cells through decreasing the level of different mesenchymal markers in a time- and dose-dependent manner. By the same mechanisms, PSE and Phy suppressed the function of Transforming growth factor beta (TGF-β)-stimulated fibroblasts. Moreover, PSE and Phy resulted in a decreasing level of the TGF-β canonical pathway Smad2/3, which is essential for tumour growth. Furthermore, PSE and Phy inhibited angiogenesis in a quail embryo chorioallantoic model, which indicates their potential anti-angiogenic activity. These results also provided the first evidence of the modulation of TME by these substances.
Biomolecules, 2021; 11
31947708: Kello M, Takac P, Kubatka P, Kuruc T, Petrova K, Mojzis J
Oxidative Stress-Induced DNA Damage and Apoptosis in Clove Buds-Treated MCF-7 Cells.
In recent decades, several spices have been studied for their potential in the prevention and treatment of cancer. It is documented that spices have antioxidant, anti-inflammatory, immunomodulatory, and anticancer effects. The main mechanisms of spices action included apoptosis induction, proliferation, migration and invasion of tumour inhibition, and sensitization of tumours to radiotherapy and chemotherapy. In this study, the ability of clove buds extract (CBE) to induce oxidative stress, DNA damage, and stress/survival/apoptotic pathways modulation were analysed in MCF-7 cells. We demonstrated that CBE treatment induced intrinsic caspase-dependent cell death associated with increased oxidative stress mediated by oxygen and nitrogen radicals. We showed also the CBE-mediated release of mitochondrial pro-apoptotic factors,

signalling of oxidative stress-mediated DNA damage with modulation of cell antioxidant SOD (superoxide dismutase) system, and modulation activity of the Akt, p38 MAPK, JNK and Erk 1/2 pathways.
Biomolecules, 2020; 10
33802621: Kuruc T, Kello M, Petrova K, Kudlickova Z, Kubatka P, Mojzis J
The Newly Synthetized Chalcone L1 Is Involved in the Cell Growth Inhibition, Induction of Apoptosis and Suppression of Epithelial-to-Mesenchymal Transition of HeLa Cells.
Over the past decades, natural products have emerged as promising agents with multiple biological activities. Many studies suggest the antioxidant, antiangiogenic, antiproliferative and anticancer effects of chalcones and their derivatives. Based on these findings, we decided to evaluate the effects of the newly synthetized chalcone L1 in a human cervical carcinoma cell (HeLa) model. Presented results were obtained by western blot and flow cytometric analyses, live cell imaging and antimigratory potential of L1 in HeLa cells was demonstrated by scratch assay. In the present study, we proved the role of L1 as an effective agent with antiproliferative activity supported by G2/M cell cycle arrest and apoptosis. Moreover, we proved that L1 is involved in modulating Transforming Growth Factor-β1 (TGF-β) signal transduction through Smad proteins and it also modulates other signalling pathways including Akt, JNK, p38 MAPK, and Erk1/2. The involvement of L1 in epithelial-to-mesenchymal transition was demonstrated by the regulation of N-cadherin, E-cadherin, and MMP-9 levels. Here, we also evaluated the effect of conditioned medium from BJ-5ta human foreskin fibroblasts in HeLa cell cultures with subsequent L1 treatment. Taken together, these data suggest the potential role of newly synthesized chalcone L1 as an anticancer-tumour microenvironment modulating agent.
Molecules, 2021; 26
31426865: Goga M, Kello M, Vilkova M, Petrova K, Backor M, Adlassnig W, Lang I
Oxidative stress mediated by gyrophoric acid from the lichen Umbilicaria hirsuta affected apoptosis and stress/survival pathways in HeLa cells.
Lichens produce a huge diversity of bioactive compounds with several biological effects. Gyrophoric acid (GA) is found in high concentrations in the common lichen Umbilicaria hirsuta, however evidence for biological activity was limited to anti-proliferative activity described on several cancer cell lines.
BMC Complement Altern Med, 2019; 19
34959454: Kello M, Kuruc T, Petrova K, Goga M, Michalova Z, Coma M, Rucova D, Mojzis J
Pro-Apoptotic Potential of (L.) Extract and Isolated Physodic Acid in Acute Lymphoblastic Leukemia Model In Vitro.
Acute lymphoblastic leukemia (ALL) is the most frequently diagnosed type of leukemia among children. Although chemotherapy is a common treatment for cancer, it has a wide range of serious side effects, including myelo- and immunosuppression, hepatotoxicity and neurotoxicity. Combination therapies using natural substances are widely recommended to attenuate the adverse effects of chemotherapy. The aim of the present study was to investigate the anti-leukemic potential of extract from the lichen (L.) (PSE) and isolated physodic acid (Phy) in an in vitro ALL model. A screening assay, flow cytometry and Western blotting were used to analyze apoptosis occurrence, oxidative stress, DNA damage and stress/survival/apoptotic pathway modulation induced by the tested substances in Jurkat cells. We demonstrate for the first time that PSE and Phy treatment-induced intrinsic caspase-dependent cell death was associated with increased oxidative stress, DNA damage and cell cycle arrest with the activation of cell cycle checkpoint proteins p53, p21 and p27 and stress/survival kinases p38 MAPK, JNK and PI3K/Akt. Moreover, using peripheral T lymphocytes, we confirmed that PSE and Phy treatment caused minimal cytotoxicity in normal cells, and therefore, these naturally occurring lichen secondary metabolites could be promising substances for ALL therapy.
Pharmaceutics, 2021; 13
29973576: Fáber L, Kováč I, Mitrengová P, Novotný M, Varinská L, Vasilenko T, Kello M, Čoma M, Kuruc T, Petrová K, Miláčková I, Kuczmannová A, Peržeľová V, Mižáková Š, Dosedla E, Sabol F, Luczy J, Nagy M, Majerník J, Koščo M, Mučaji P, Gál P
Genistein Improves Skin Flap Viability in Rats: A Preliminary In Vivo and In Vitro Investigation.
Selective estrogen receptor modulators (SERMs) have been developed to achieve beneficial effects of estrogens while minimizing their side effects. In this context, we decided to evaluate the protective effect of genistein, a natural SERM, on skin flap viability in rats and in a series of in vitro experiments on endothelial cells (migration, proliferation, antioxidant properties, and gene expression profiling following genistein treatment). Our results showed that administration of genistein increased skin flap viability, but importantly, the difference is only significant when treatment is started 3 days prior the flap surgery. Based on our in vitro experiments, it may be hypothesized that the underlying mechanism may rather by mediated by increasing SOD activity and Bcl-2 expression. The gene expression profiling further revealed 9 up-regulated genes (angiogenesis/inflammation promoting: CTGF, CXCL5, IL-6, ITGB3, MMP-14, and VEGF-A; angiogenesis inhibiting: COL18A1, TIMP-2, and TIMP-3). In conclusion, we observed a protective effect of genistein on skin flap viability which could be potentially applied in plastic surgery to women undergoing a reconstructive and/or plastic intervention. Nevertheless,

further research is needed to explain the exact underlying mechanism and to find the optimal treatment protocol.
Molecules, 2018; 23

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PROTECTIVE EFFECT OF SYNTHETIC MACAMIDE AGAINST MORPHOLOGICAL AND NEUROLOGICAL DEFICIT INDUCED BY FOCAL BRAIN ISCHEMIC STROKE INJURY

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Stroke is one of the leading causes of death and disability worldwide. Previous studies have demonstrated the neuroprotective effect of pentane extract of *Lepidium meyenii* (maca) as well as its isolate macamides. Along these lines, this study aims to ascertain the protective effect of macamide N-benciloctadecanamida on morphological conservation and neurological deficits. For this purpose, we use the MCAO model to induce the stroke on Sprague Dawley rats, during surgery an intraperitoneal dose of 3 mg/ kg macamide (MCH1) was administered before the occlusion; this dose was repeated every 24 hours for 10 days. A battery of neurological evaluations was applied on day 3 and day 10 after the stroke, showing significantly different results both times between rats treated with MCH1 and vehicle (VH). On day 3, motor functions were affected more severely than sensitivity functions, this was observed more so in forelimbs than hindlimbs. On day 10, the VH group was the only one still manifesting a significant sensitive hindlimbs asymmetry. Our results obtained from the coronal section of brain stain with 2% of 2, 3, 5-triphenyltetrazolium-chloride solution showed a significant difference in the conservation of sensorimotor areas between the VH and MCH1 group. These findings suggest the protective effect of macamide against brain damage during the stroke, as well as the conservation of sensorimotor function

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MODULATING MICROGLIA PHAGOCYTOSIS

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Ischemic stroke is one of the leading causes of death and disability in adults. Currently, there are no effective drugs to promote the
functional recovery from stroke. Adequate healing of the damaged brain area depends on clearance of cell and myelin debris, but this
process is slow and perturbs with neuronal regeneration. Thus, enhancing phagocytosis could improve removal of cell debris. We have created a small library of lentiviral vectors encoding different genes related to phagocytosis or recruitment of microglia/macrophages, namely Monocyte chemoattractant protein 1 (MCP1), three isoforms of Macrophage colony-stimulating factor (M-CSF), Complement Component 3 (C3), Complement Component 3a (C3a), Adhesion G protein-coupled receptor E1 (Emr1/ADGRE1/F4/80), MER receptor tyrosine kinase (MerTK) and Mesencephalic astrocyte-derived neurotrophic factor (MANF). Their effect on phagocytosis and induction of inflammation are tested in microglia (BV2) after transient transfection *in vitro* by phagocytosis assay and cytokine (TNFa, IL-6 and IL10) ELISAs. The highest effect on phagocytosis was deteced with LV-MerTK, LV-MCSF32-E and LV-MCSF1-E while LV-C3a and LV-Emr1 transfections enhanced phagocytosis over 80% of the induction of the positive control. In parallel, LV-MANF and LV-MerTK were equally potent in enhancing TNFa and IL-6. In summary, this is the first study to compare the effect of different chemotactic and phagocytosis related proteins enhancing phagocytosis and changing the inflammatory profile of microglia.

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TARGETING OXIDATIVE STRESS AND PROLYL HYDROXYLASE DOMAIN INHIBITION AS NEUROPROTECTIVE STRATEGIES AGAINST HYPOXIA IN ISOLATED RAT HIPPOCAMPAL SLICES.

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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The contribution of hypoxia and oxidative stress to the pathophysiology of acute ischemic stroke (AIS) are well established and can lead to disruptions in synaptic signaling. Hypoxia and oxidative stress lead to the neurotoxic overproduction of reactive oxygen species (ROS) and the stabilization of hypoxia inducible factors. Antioxidant compounds and prolyl-4-hydroxylase domain enzyme inhibitors (PHDI) have been shown to have a preconditioning and neuroprotective effect against an ischemic insult. Therefore, this study explores the effects of the ROS scavenger, MnTMPyP, and the PHDI, DMOG, on synaptic transmission post hypoxia in isolated rat hippocampal slices using electrophysiological techniques and organotypic hippocampal slice cultures. We report for the first time an acute deleterious effect of MnTMPyP (25µM) on synaptic transmission post hypoxia, an effect specific to the CA1 region of the hippocampus. This effect was attenuated through the co-application of the adenosine A1 receptor antagonist, DPCPX (200nM), and the NMDA receptor antagonist, AP5 (10µM), suggesting pre- and post-synaptic effects. Interestingly, our organotypic data demonstrates a protective role for MnTMPyP, where slices had significantly less cell death post hypoxia compared to controls. Additionally, we provide evidence for a protective role for DMOG (1mM) on synaptic signaling post hypoxia, an effect that appears to be post-synaptic. Taken together, our results suggest a crucial role for ROS and PHDs in maintaining synaptic integrity during episodes of hypoxic stress. These new findings may help to disambiguate the complex interaction between synaptic signaling and hypoxia informing novel therapies for the treatment of AIS.

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EARLY APPLICATION OF CATHODAL-TDCS IN A MOUSE MODEL OF BRAIN ISCHEMIA RESULTS IN FUNCTIONAL IMPROVEMENT AND PERILESIONAL MICROGLIA MODULATION

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Aims. Treatment of acute ischemic stroke is mostly limited to thrombolysis, which unfortunately can be applied only to a minority of cases because of its narrow therapeutic window (<6 hrs). Hence, new therapeutic strategies capable of modulating the ischemic pathophysiology (including the inflammatory response) that can be applied beyond the time window for thrombolysis, are urgently needed. In the last few decades, non-invasive neuromodulatory techniques, such as transcranial direct current stimulation (tDCS), have emerged as an effective treatment for chronic stroke and could be a promising tool for acute/subacute stroke as well. The purpose of this project is to study the effects of early cathodal tDCS (C-tDCS) on motor functionality and microglia activation in a mouse model of ischemic stroke. *Methods.* Photothrombotic stroke was induced in Cx3CR1$^{GFP/+}$ mice and C-tDCS applied 6 hours after unilateral M1 ischemia. Motor functionality was tested with the cylinder and foot-fault tests, while the density and activation state of microglia cells were evaluated via 3D imaging quantifications. *Results.* Though tDCS application did not decrease the lesion volume at 48 hours, the functionality of the affected forelimb was significantly higher in the C-tDCS group *vs* sham treated mice. C-tDCS treatment also reduced microglia density in the perilesional area, and induced a resting microglial phenotype with ramified morphology, increased total processes length, process branch points and terminal process branches, *vs* the sham group. *Conclusions.* C-tDCS applied beyond the conventional time window for thrombolysis can significantly ameliorate functional deficits and reduce microglia activation in the perilesional ischemic area.

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NEUROPROTECTIVE EFFECT OF SODIUM BUTYRATE – THE HDAC INHIBITOR - ON THE ACTIVATION OF THE COMPLEMENT SYSTEM IN RAT MODEL OF NEONATAL ASPHYXIA

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Background: One of the key pathogenic factors in hypoxic-ischemic (HI) brain injury is the complement system. Complement usually protects against infection and promotes tissue repair, but can cause tissue damage if over-activated. Histone deacetylase inhibitor (HDACi) - sodium butyrate (SB), provides protection associated with reduction of inflammation. Therefore pharmacological modifications of complement activation could potentially create new therapeutic approach to reduce brain damage after neonatal HI. **Aim:** The main purpose of this study was to examine the effect of SB treatment on complement activation and synapse elimination after neonatal hypoxia-ischemia. **Methods:** Cerebral HI was induced in seven-day-old Wistar rats by permanent unilateral ligation of the common carotid artery followed by 60 min hypoxia (7.6% O2). HDACi - Sodium butyrate (SB) (300 mg/kg bw) was administered on a 5-day regimen with the first injection administered immediately after hypoxia exposure. **Results:** We observed increased expression some of the complement system genes after HI, which decreased significantly after SB treatment. Additionally, expression some of the synaptic proteins decreased after HI, and returned to the control level after SB administration. HI induced brain tissue damage and a disturbance in synaptic morphology. In animals treated with SB, the tissue had normal morphology of neurons and synaptic connections. **Conclusion:** The results obtained in this study suggested the neuroprotective effect of SB by reduction the activity of complement system proteins as well as the protection of synaptic proteins and synaptic connections. Supported by: NSC, Poland, grant no 2017/27/B/NZ300582 and ESF, POWR.03.02.00-00-1028/17-00

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REMOTE THALAMIC IRON ACCUMULATION: A NEW PREDICTING TOOL FOR LONG TERM VOLUME AND NEURONAL LOSS, INFLAMMATION ACTIVATION AND THALAMO-CORTICAL TRACTS IMPAIRMENT IN STROKE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

Marion Tible[1], Ismail Koubiyr[1], Nadège Dubourdieu[1], Marlène Maitre[2], Thierry Lesté-Lasserre[3], Chloé Galmiche[1], Laurent Petit[4], Thomas Tourdias[1]
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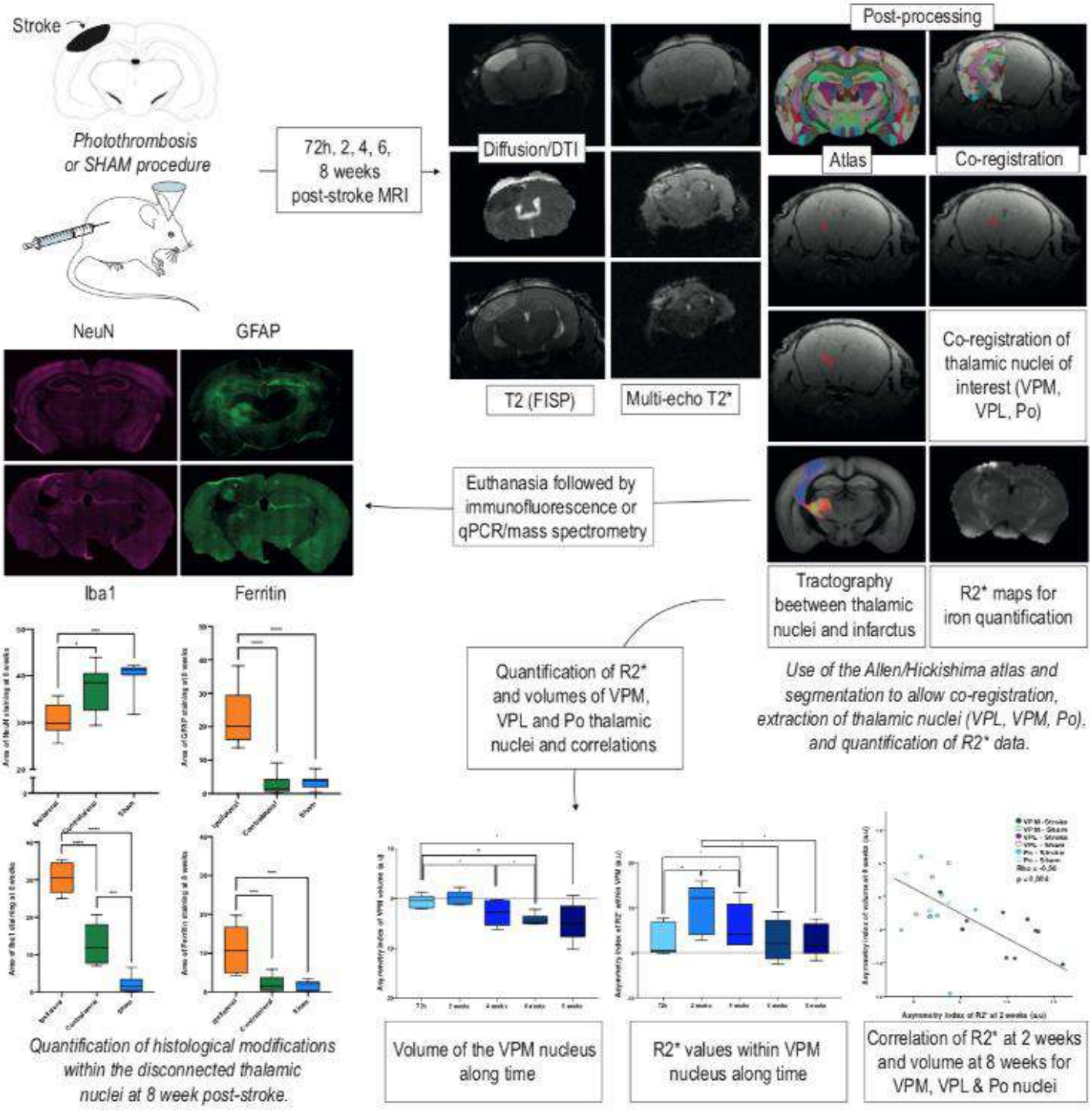
Aims It has been shown that infarcts can exert effects remotely from the initial lesion, notably through disconnections. We hypothesise that progressive iron accumulation within disconnected thalamic nuclei precedes and is associated with long term neurodegeneration. Methods Six weeks-old mice (n=56 total) were submitted to photo-thrombosis surgery (or sham procedure) to induce a cortical stroke and *in vivo* MRI was acquired at 72h, 2, 4, 6 and 8 weeks. R2*, a proxy of iron, was computed from a multi-echo T2* sequence. Images of each timepoint were co-registered and wrapped to the Allen/Hikishima atlas to allow R2* and volume quantification within nuclei known to project toward the infarcted region (posterior, ventro-postero-medial and ventro-postero-lateral). Tractography was also computed from post-mortem MRI to confirm such projections and quantify fiber degenerations. After euthanasia, immunofluorescence, iron mass spectrometry (MS) and qPCR analyses were performed to assess iron-linked processes, neuronal death and glial and inflammatory responses. Results R2* values in disconnected thalamic nuclei displayed a significant peak at 2/4 weeks, preceding and significantly correlating to long term volumetric loss. Histological analyses, qPCR and MS also suggested a fluctuant thalamic response in terms of neuronal death, inflammation, iron uptake and ferroptosis. Conclusion These data support our hypothesis that iron dynamically accumulates in disconnected areas, and may accelerate cell death, promoting remote neurodegeneration in a vicious circle involving one or more processes. Iron might serve as a future predictive tool and as a potential therapeutic target for

neuroprotection.



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UROTENSIN II RECEPTOR MEDIATES MENINGES INFLAMMATION, MACROPHAGE-INDUCED VASOSPASM AND COGNITIVE DEFICITS POST-SUBARACHNOID HEMORRHAGE IN MICE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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In aneurysmal subarachnoid hemorrhage (SAH), cerebral vasospasm (CVS) and neuroinflammation are known associated with delayed ischemia, but addressing these symptoms does not improve the bad neurological outcome of patients. Urotensin II (UII) is a vasoactive peptide activating the UT receptor, involved in brain vascular pathologies and inflammation. We first established that high plasma level of UII in SAH patients is a predictive factor of CVS. Here we investigated the critical role of UT, especially brain border-associated macrophages (BAMs), on the CVS and neuroinflammation leading to cognitive impairment in a mouse model of SAH. We used a double intracisternal blood injection SAH procedure in $UT^{+/+}$ and KO-$UT^{-/-}$ or humanized $UT^{h+/h+}$ C57Bl/6 mice. Increased expression of UT and UII were neural and myeloid cells as in meninges and inflamed endothelial compartments with mouse brain slices in 2D and 3D immunofluorescence. We highlighted early and delayed vascular hypoperfusion (fUS) and CVS or large arteries, neurovascular inflammation (MPIO-VCAM-1 MRI) and outcome (cognitive, depressive and sensitivomotor behaviors). Mechanistically, we studied meninges plasticity *via in vivo* siRNA injection, involvement of BAM in CVS by depletion (clodronate)/replacement (BMDMs) strategies, and prevention of cognitive deficits by using UT antagonists/biased ligands. We identified UT as a critical regulator of SAH-driven CVS, neuroinflammation and behavioral disorders. UT activation by meningeal and perivascular macrophages worsens vascular defects and subsequent neurological deficits and UT expressed in meningeal fibroblasts contributes to cognitive impairment after SAH. Importantly, pharmacological UT antagonism in a humanized UT mouse model entirely prevents CVS, neuroinflammation and behavioral disorders post-SAH.

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THE EFFECTS OF THE SOD MIMETIC, MNTMPYP, ON SYNAPTIC SIGNALLING AND VIABILITY IN AN IN VITRO RAT OGD MODEL.

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Oxidative stress plays an important role in ischemia-reperfusion injury (IRI). Antioxidants have been shown to have beneficial effects during increased levels of reactive oxygen species in ischemic stroke. Therefore, this study investigated the effects of the antioxidant and SOD mimetic, MnTMPyP on synaptic transmission and neuronal viability in an oxygen-glucose deprivation (OGD) rat stroke model. Field excitatory post-synaptic potentials (fEPSPs) were elicited by stimulation of the Schaffer collateral pathway in young rat CA1 hippocampal slices. During OGD hippocampal slices were perfused with glucose-free aCSF bubbled with 95% N_2/5% CO_2 for 20 min. Organotypic hippocampal slices were obtained from P7-11 rat pups. Slices were exposed to 1 hr of OGD with a glucose-free media before staining with 2µM Propidium Iodide (PI). Images were taken 40 min later. All data are presented as mean value±SEM. 25µM MnTMPyP had no effect on the OGD-induced depression of synaptic transmission (23.0±10% vs control 21.2±3.0%) but significantly impaired recovery post OGD (15.2±2.1% compared to controls 70.0±11.3%, n=5). Interestingly, application of a lower concentration of MnTMPyP (2.5µM), did not attenuated the depression (24.4±6.6% vs controls 21.2±3.0%) but gave rise to a faster and full recovery of synaptic transmission after OGD (89.0±9.0% compared to controls 70.0±11.3%, n=5). Furthermore, in the organotypic slice cultures, MnTMPyP improved the viability of hippocampal cells after 1hr OGD compared to untreated slices (25.0±5.4% vs controls 60.0±9.0%). In conclusion, MnTMPyP protected neuronal viability and synaptic signalling in hippocampal slices. Whether MnTMPyP will play an important role in IRI remains to be determined.

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GSK-3β INHIBITOR, VP3.15, RESTORES COGNITIVE IMPAIRMENT AND NEURONAL COMPROMISE IN A MURINE MODEL OF INTRAVENTRICULAR HEMORRHAGE OF THE PRETERM NEWBORN

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Introduction: Germinal matrix-intraventricular hemorrhage (GM-IVH) is one of the most severe neurological pathologies affecting preterm newborns (PTNB) and causing serious alterations in their neurodevelopment. To date, there are no effective treatments for GM-IVH. **Objectives**: We proposed to study the short- and long-term effects of VP3.15, an inhibitor of the glucogen sintase 3-β (GSK3-β) enzyme that has shown neuroprotective effects in other neurodegenerative and inflammatory diseases. **Methods**: GM-IVH is induced by unilateral intracerebroventricular injection of collagenase to P7 CD1 mice. Animals are treated with VP3.15 (10 mg/kg/day) i.p., or vehicle for 7 days. Short (P14) and long-term (P110) postmortem studies are performed to evaluate brain and cerebellar atrophy by cresyl violet and calbindin staining. Neuronal density using NeuN-DAPI immunostaining is also evaluated. Behavioral studies are performed prior to sacrifice at P110 and spatial learning and memory, as well as episodic memory are assessed in the Morris water maze and new object discrimination test. **Results**: VP3.15 treatment reduces brain atrophy, reducing cortical thinning and ventricular enlargement. It also improves neuronal density in the cortex, the subventricular zone and the ipsilateral cerebellar trunk in our murine model of GM-IVH. Interestingly, our results show that spatial and episodic memory are also improved after VP3.15 treatment. **Conclusion**: VP3.15 treatment limits brain atrophy and cognitive impairment in our model, supporting further studies on the neuroprotective role of VP3.15 in GM-IVH of PTNB. **Funding**: I + D + i Programa operativo del Fondo Europeo de Desarrollo Regional FEDER Andalucía 2014–2020 (FEDER-UCA18–107189).

Pubmed:
33172205: Atienza-Navarro I, Alves-Martinez P, Lubian-Lopez S, Garcia-Alloza M
Germinal Matrix-Intraventricular Hemorrhage of the Preterm Newborn and Preclinical Models: Inflammatory Considerations.
The germinal matrix-intraventricular hemorrhage (GM-IVH) is one of the most important complications of the preterm newborn. Since these children are born at a critical time in brain development, they can develop short and long term neurological, sensory, cognitive and motor disabilities depending on the severity of the GM-IVH. In addition, hemorrhage triggers a microglia-mediated inflammatory response that damages the tissue adjacent to the injury. Nevertheless, a neuroprotective and neuroreparative role of the microglia has also been described, suggesting that neonatal microglia may have unique functions. While the implication of the inflammatory process in GM-IVH is well established, the difficulty to access a very delicate population has lead to the development of animal models that resemble the pathological features of GM-IVH. Genetically modified models and lesions induced by local administration of glycerol, collagenase or blood have been used to study associated inflammatory mechanisms as well as therapeutic targets. In the present study we review the GM-IVH complications, with special interest in inflammatory response and the role of microglia, both in patients and animal models, and we analyze specific proteins and cytokines that are currently under study as feasible predictors of GM-IVH evolution and prognosis.
Int J Mol Sci, 2020; 21
34975451: Carranza-Naval MJ, Del Marco A, Hierro-Bujalance C, Alves-Martinez P, Infante-Garcia C, Vargas-Soria M, Herrera M, Barba-Cordoba B, Atienza-Navarro I, Lubian-Lopez S, Garcia-Alloza M
Liraglutide Reduces Vascular Damage, Neuronal Loss, and Cognitive Impairment in a Mixed Murine Model of Alzheimer's

Disease and Type 2 Diabetes.
Alzheimer's disease is the most common form of dementia, and epidemiological studies support that type 2 diabetes (T2D) is a major contributor. The relationship between both diseases and the fact that Alzheimer's disease (AD) does not have a successful treatment support the study on antidiabetic drugs limiting or slowing down brain complications in AD. Among these, liraglutide (LRGT), a glucagon-like peptide-1 agonist, is currently being tested in patients with AD in the Evaluating Liraglutide in Alzheimer's Disease (ELAD) clinical trial. However, the effects of LRGT on brain pathology when AD and T2D coexist have not been assessed. We have administered LRGT (500 μg/kg/day) to a mixed murine model of AD and T2D (APP/PS1xdb/db mice) for 20 weeks. We have evaluated metabolic parameters as well as the effects of LRGT on learning and memory. analysis included assessment of brain amyloid-β and tau pathologies, microglia activation, spontaneous bleeding and neuronal loss, as well as insulin and insulin-like growth factor 1 receptors. LRGT treatment reduced glucose levels in diabetic mice (db/db and APP/PS1xdb/db) after 4 weeks of treatment. LRGT also helped to maintain insulin levels after 8 weeks of treatment. While we did not detect any effects on cortical insulin or insulin-like growth factor 1 receptor m-RNA levels, LRGT significantly reduced brain atrophy in the db/db and APP/PS1xdb/db mice. LRGT treatment also rescued neuron density in the APP/PS1xdb/db mice in the proximity (= 0.008) far from amyloid plaques (< 0.001). LRGT reduced amyloid plaque burden in the APP/PS1 animals (< 0.001), as well as Aβ aggregates levels (= 0.046), and tau hyperphosphorylation (= 0.009) in the APP/PS1xdb/db mice. Spontaneous bleeding was also ameliorated in the APP/PS1xdb/db animals (= 0.012), and microglia burden was reduced in the proximity of amyloid plaques in the APP/PS1 and APP/PS1xdb/db mice (< 0.001), while microglia was reduced in areas far from amyloid plaques in the db/db and APP/PS1xdb/db mice (< 0.001). This overall improvement helped to rescue cognitive impairment in AD-T2D mice in the new object discrimination test (< 0.001) and Morris water maze (< 0.001). Altogether, our data support the role of LRGT in reduction of associated brain complications when T2D and AD occur simultaneously, as regularly observed in the clinical arena.
Front Aging Neurosci, 2021; 13

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PROFOUND ALTERATIONS IN BRAIN TISSUE LINKED TO HYPOXIC EPISODE AFTER DEVICE IMPLANTATION.

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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To enable authentic interfacing with neuronal structures in the brain, preventing alterations of tissue during implantation of devices is critical. By transiently implanting oxygen microsensors into rat cortex cerebri for 2 h, substantial and long lasting (>1 h) hypoxia is routinely generated in surrounding tissues; this hypoxia is linked to implantation generated compressive forces. Preferential loss of larger neurons and reduced metabolic components in surviving neurons indicates decreased viability one week after such hypoxic, compressive implantations. By devising an implantation method that relaxes compressive forces; magnitude and duration of hypoxia generated following such an implantation are ameliorated and neurons appear similar to naïve tissues. In line with these observations, astrocyte proliferation was significantly more pronounced for more hypoxic, compressive implantations. Surprisingly, astrocyte processes were frequently found to traverse cellular boundaries into nearby neuronal nuclei, indicating injury induction of a previously not described astrocyte-neuron interaction. Found more frequently in less hypoxic, force-relaxed insertions and thus correlating to a more beneficial outcome, this finding may suggest a novel protective mechanism. In conclusion, substantial and long lasting insertion induced hypoxia around brain implants, a previously overlooked factor, is linked to significant
adverse alterations in nervous tissue.

Pubmed:
34653937: Kumosa LS, Schouenborg J
Profound alterations in brain tissue linked to hypoxic episode after device implantation.
To enable authentic interfacing with neuronal structures in the brain, preventing alterations of tissue during implantation of devices is critical. By transiently implanting oxygen microsensors into rat cortex cerebri for 2 h, substantial and long lasting (>1 h) hypoxia is routinely generated in surrounding tissues; this hypoxia is linked to implantation generated compressive forces. Preferential loss of larger neurons and reduced metabolic components in surviving neurons indicates decreased viability one week after such hypoxic, compressive implantations. By devising an implantation method that relaxes compressive forces; magnitude and duration of hypoxia generated following such an implantation are ameliorated and neurons appear similar to naïve tissues. In line with these observations, astrocyte proliferation was significantly more pronounced for more hypoxic, compressive implantations. Surprisingly, astrocyte processes were frequently found to traverse cellular boundaries into nearby neuronal nuclei, indicating injury induction of a previously not described astrocyte-neuron interaction. Found more frequently in less hypoxic, force-relaxed insertions and thus correlating to a more beneficial outcome, this finding may suggest a novel protective mechanism. In conclusion, substantial and long lasting insertion induced hypoxia around brain implants, a previously overlooked factor, is linked to significant adverse alterations in nervous tissue.
Biomaterials, 2021; 278

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BOARD NUMBER: S04-315

INHIBITION OF NEURONAL AUTOPHAGY CONTRIBUTES TO REDUCED ISCHEMIC BRAIN DAMAGE IN RATS

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Introduction: Emerging evidence suggests a strong correlation between apoptosis, autophagy and their pathological processes in cerebral ischemic injury particularly in diabetes. Oxidative stress and blood brain barrier dysfunction are one of the important factors causing disability and mortality in stroke patients, which have a significant impact on diabetic induced stroke progression. Citral have been reported to antioxidant and anti-inflammatory activity. The aim of this study was to investigated the therapeutic effects of citral against ischemic stroke in hyperglycemic rats and its role in modulating autophagy. **Methods**: Hyperglycemia was induced by intraperitoneal (i.p) injection of streptozotocin (35 mg/kg) in male Sprague Dawley (SD) rats and rats were subjected to middle cerebral artery occlusion (MCAO) for 1 hour. Citral was administered at 3 hours after the induction of MCAO. Lipid profile, blood glucose, Neurological deficit, oxidative stress makers (MDA & GSH), blood brain barrier (BBB) permeability and brain edema, were measured. Additionally, RT-PCR and western blot analysis of Bcl-2, Beclin-1 and LC3 were examined. **Result:** Citral significantly reduced brain edema, BBB integrity, oxidative damage and ameliorated neurologic outcome in rats. Citral treatment significantly decreased serum glucose level, serum TG, TC and serum LDL. Citral decreased apoptosis and autophagy via down-regulation of the LC3 and Beclin-1 expression. **Conclusion:** Our finding suggests that citral attenuates cerebral ischemic injury in hyperglycaemic rats and promotes functional recovery via its antioxidant, anti-apoptosis, and anti-autophagy properties, may have a therapeutic potential for stroke prevention in diabetic settings.

Pubmed:

30944708: Mishra C, Khalid MA, Fatima N, Singh B, Tripathi D, Waseem M, Mahdi AA
Effects of citral on oxidative stress and hepatic key enzymes of glucose metabolism in streptozotocin/high-fat-diet induced diabetic dyslipidemic rats.
Phytochemicals such as polyphenols, alkaloids, and terpenoids, protect against the development of early stages and complications of diabetes mellitus according to various reports. The aim of this study was to measure the anti-dyslipidemic and anti-diabetic effects of Citral on high-fat-diet (HFD) and streptozotocin (STZ) induced diabetic dyslipidemic rats and to see also its effect on carbohydrate metabolic regulatory enzymes in the liver.
Iran J Basic Med Sci, 2019; 22
29949404: Abdullahi W, Tripathi D, Ronaldson PT
Blood-brain barrier dysfunction in ischemic stroke: targeting tight junctions and transporters for vascular protection.
The blood-brain barrier (BBB) is a physical and biochemical barrier that precisely controls cerebral homeostasis. It also plays a central role in the regulation of blood-to-brain flux of endogenous and exogenous xenobiotics and associated metabolites. This is accomplished by molecular characteristics of brain microvessel endothelial cells such as tight junction protein complexes and functional expression of influx and efflux transporters. One of the pathophysiological features of ischemic stroke is disruption of the BBB, which significantly contributes to development of brain injury and subsequent neurological impairment. Biochemical characteristics of BBB damage include decreased expression and altered organization of tight junction constituent proteins as well as modulation of functional expression of endogenous BBB transporters. Therefore, there is a critical need for development of novel therapeutic strategies that can protect against BBB dysfunction (i.e., vascular protection) in the setting of ischemic stroke. Such strategies include targeting tight junctions to ensure that they maintain their correct structure or targeting transporters to control flux of physiological substrates for protection of endothelial homeostasis. In this review, we will describe the pathophysiological mechanisms in cerebral microvascular endothelial cells that lead to BBB dysfunction following onset of stroke. Additionally, we will utilize this state-of-the-art knowledge to provide insights on novel pharmacological strategies that can be developed to confer BBB protection in the setting of ischemic stroke.
Am J Physiol Cell Physiol, 2018; 315

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THROMBUS HISTOPATHOLOGY IN ACUTE ISCHEMIC STROKE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Aims: In this study, it was aimed to examine the histopathological structure of thrombus in acute ischemic stroke. **Methods**: 90 patients who underwent mechanical thrombectomy were included. Detailed clinical information were recorded. Thrombi were stained with Hematoxylin-Eosin (H&E) and Martius Scarlet Blue (MSB) for fibrin and erythrocyte contents. Fiji and Orbit programs were used for image analysis. CD3, CD20, CD45 and CD34 stainings were performed to examine T and B lymphocytes, total leukocytes and endothelial cells in thrombus. One thrombus section from each patient was used for each staining. Differences between groups were analyzed with t-test or Mann-Whitney U, correlation analyzes were performed with Pearson or Spearman tests. **Results**: From all 90 thrombi, 63 (70%) were considered fibrin-dominant, and 27 (30%) were erythrocyte-dominant.There was no difference in fibrin or erythrocyte-dominant thrombus rates between Fiji and Orbit programs and two different staining methods, H&E and MSB. CD3/CD45 and CD20/CD45 ratios were negatively correlated with the percentage of fibrin (p=0.003, p=0.001) and positively correlated with the percentage of erythrocyte (p=0.003, p=0.001). Similarly, CD3/CD45 and CD20/CD45 ratios were lower in fibrin-dominant thrombi (p=0.001, p=0.007). The number of endothelial cells stained with CD34 was positively correlated with symptom-to-door, symptom-to-puncture and symptom-to-recanalization times (p=0.022, p=0.001, p=0.001), but not with duration of procedure. **Conclusions**: It has been determined that leukocyte subtype contents of thrombi, as well as fibrin/erythrocyte contents, may be associated with the pathophysiology of stroke. Further investigation of the thrombus content in subsequent studies is important to elucidate the pathophysiology of acute ischemic stroke.

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EFFECTS OF TLR2 DEFICIENCY ON NEUROINFLAMMATION AFTER ISCHEMIC LESION IN THE MOUSE BRAIN - WORSE FUNCTIONAL OUTCOME AND MORE INFLAMMATION THAN IN WILD TYPE CONTROLS?

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

Sanja Srakočić, Paula Josić, Rok Ister, Siniša Škokić, Anton Glasnović, Marina Radmilović Dobrivojević, Srecko Gajovic
University of Zagreb School of Medicine, Croatian Institute For Brain Research, Zagreb, Croatia

Tlr2 (Toll-like receptor 2) in the brain is located in microglia, and involved in innate immunity recognising the damage-associated molecular patterns after ischemia. It is postulated that inhibition of the Tlr2-mediated signalling will reduce the neuroinflammatory response and subsequently reduce the post-ischemic brain damage. In our study we compared Tlr2 deficient (knock-out) mice to their wild type controls and analyzed the brain consequences of transient MCAO (middle cerebral artery occlusion) for 28 days using in vivo magnetic resonance and biolumescence imaging. Moreover, we created Tlr2 deficient mice with Tlr2 promoter driven bioluminescence reporter, provding an insight in neuroinflammmation in vivo. The functional outcome was provided by neurological deficit scoring, and inflammatory status was determined by flow cytometry of the mouse brain and cytokine levels in the blood. Although one would expect reduced neuroinflammation in Tlr2 deficient mice after ischemia and subsequent benefits to the animals, the results were controversial. The ischemic lesion was bigger in Tlr2 deficient animals, however their survival was better. The neurological deficit score was worse in Tlr2 deficient animals. Moroever, the Tlr2 bioluminescence being a marker of neuroinflammation was higher in Tlr2 deficient animals, which was partially confirmed by flow cytometry. Among cytokines, IL-23 and IL-1alpha were higher in the blood, and none of the analysed cytokines was reduced in Tlr2 deficient animals. The statistical modelling showed that Tlr2 deficient mice were worse than the wild type controls, however they recovered faster. In conclusion, the effects of Tlr2 deficiency are complex and indicate multiple aspects of neuroinflammation in post-ischemic brain damage and repair.

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THE NEUROPROTECTIVE EFFECTS OF PHOSPHOGLYCERATE MUTASE 5 ARE MEDIATED BY DECREASING OXIDATIVE STRESS IN HT22 HIPPOCAMPAL CELLS AND GERBIL HIPPOCAMPUS

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Phosphoglycerate mutase 5 (PGAM5), a glycolytic enzyme, plays an important role in cell death and in the regulation of mitochondrial dynamics. In this study, we investigated the effects of PGAM5 against oxidative stress in HT22 hippocampal cells and ischemic damage in the gerbil hippocampus to elucidate the roles of PGAM5 in oxidative and ischemic stress. Constructs were designed with a PEP-1 expression vector to facilitate the intracellular delivery of PGAM5 proteins. We observed time- and concentration-dependent increases in the intracellular delivery of PEP-1-PGAM5 protein, but not its control protein (PGAM5), in HT22 cells, and morphologically demonstrated the cytoplasmic localization of the transduced protein, which was stably expressed in the cytoplasm after 12 h of PEP-1-PGAM5 treatment. PEP-1-PGAM5 treatment significantly ameliorated cell death, reactive oxygen species formation, and DNA fragmentation induced by H2O2 treatment in HT22 cells. In addition, PEP-1-PGAM5 treatment significantly alleviated ischemia-induced hyperlocomotion and neuronal death in the hippocampal CA1 region 1 and 4 days after ischemia, respectively. Microglial activation induced by ischemia was also mitigated by treatment with 1.0 mg/kg PEP-1-PGAM5. At 3 h after ischemia, PEP-1-PGAM5 treatment significantly ameliorated increases in lipid peroxidation, as assessed by malondialdehyde and hydroperoxide levels, and decreases in glutathione levels (increases in glutathione disulfide, oxidized form of glutathione) in the hippocampus. The neuroprotective effects of PEP-1-PGAM5 are partially mediated by a reduction in oxidative stress, such as the formation of reactive oxygen species.

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IN-VIVO MRI BRAIN VOLUMETRIC CHANGES IN A RAT MODEL OF MODERATE PERINATAL HYPOXIA

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

Matea Drlje[1], Sara Trnski[1], Andrija Štajduhar[1,2], Barbara Nikolić[3], Mihaela Bobić-Rasonja[1,4], Dubravka Hranilović[3], Siniša Škokić[1], Nataša Jovanov Milošević[1,4]
[1]School of Medicine, University of Zagreb, Croatian Institute For Brain Research, Zagreb, Croatia, [2]"Andrija Štampar" School of Public Health, University of Zagreb, Department Of Medical Statistics, Epidemiology And Medical Informatics, Zagreb, Croatia, [3]Faculty of Science,University of Zagreb, Department Of Biology, Zagreb, Croatia, [4]University of Zagreb School of Medicine, Department Of Medical Biology And Genetics, Zagreb, Croatia

Aim The *in-vivo* MRI study of juvenile rats aims to detect neuroradiological structural and volumetric changes consequently to moderate perinatal hypoxic brain lesion. Methods Sixteen male Wistar Han (RccHan: WIST) rats were subjected to moderate hypoxia (8% O_2, 92% N_2/2h, 8 treated and 8 control) on the first postnatal day (P1). On P15, MRI T2-weighted scans were obtained *in-vivo*, and brain volume was quantified after manual delineation of regions of interest, using ITK-SNAP and custom scripts in Python 3.8. In addition,16 post-hypoxia (8 males, 8 females) and 15 control (7 males, 8 females) rats were sacrificed on P50, brains were instantly isolated and immediately weighed on a digital scale. Due to the sex-related difference in size, brain mass was compared separately in males and females by Student t-test. Results The rats subjected to hypoxia show an indicative increase in olfactory bulbs volume fraction (VF) median from the total brain volume (TBV; 4.36 ± 0.47% and 4.55 ± 0.37%, p=0.0734). We found no difference between groups in the TBV or ventricular system VF medians. Post-hypoxic adult animals displayed a slight but significant increase in the brain mass in both males (1.81 ± 0.06 g and 1.72 ± 0.03 g, respectively, p=0.0032) and females (1.67 ± 0.05 g and 1.54 ± 0.15 g, respectively, p=0.0448), in comparison to control rats. Conclusion Further investigation is needed to understand the underlying mechanisms of demonstrated brain volume and mass changes after moderate perinatal hypoxia. Funding **CSF-IP-2019-04-3182**; **GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund**

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NEUROINFLAMMATORY MODULATION OF POMALIDOMIDE AND ITS ANALOGS 3,6 & 1,6 DITHIOPOMALIDOMIDE THROUGH PYROPTOSIS AND FERROPTOSIS AFTER STROKE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Aims: According to the World Health Organization (WHO), stroke is the second global causes of death and responsible for approximately 11 % of total deaths in 2019. Currently, acute stroke have limited treatment modalities. After ischemic stroke occurs, a robust inflammation response initiates within minutes and persists for days and alters neurological prognosis. Our previous studies demonstrated pomalidomide exerted anti-neuroinflammatory effect against brain damage. In this study, we demonstrate administration of inflammatory mediators, pomalidomide and its analogs, 3,6 & 1,6 dithiopomalidomide (DP) markedly decreased the brain infract size and improved body asymmetry through pyroptosis and ferroptosis. Methods: We treated the rats after middle cerebral artery occlusion (MCAo) with pomalidomide, 3,6-DP & 1,6-DP. The cytokine interleukin-1β (IL-1β), interleukin-6 (IL-6) and Tumor necrosis factor-α (TNF-α) were assessed by multiplex assays kit. In addition, inflammasomes NLRP3, anti-oxidant enzyme SOD2 and catalase were analyzed by ELISA. Results: Post-MCAo administration of all drugs lowered pro-inflammatory TNF-α, IL-1β, and IL-6 levels, and reduced stroke-induced postural asymmetry and infarct size. Moreover, expressions of inflammasomes NLRP3 and pyroptosis-related cytokines were reduced after treatments. In contrast, levels of ferroptosis-related anti-oxidation enzymes catalase and SOD2 were enhanced. Conclusions: These results showed pyroptosis and ferroptosis are involved in the neuro-inflammatory modulation of pomalidomide and its analogs after ischemic brain injury. It implicated considerable pathways as a further potential therapeutic approach for ischemic stroke and other brain-related disorders. The understanding of the mechanisms behind effect of Pomalidomide shows beneficial outcomes to provide the current treatment of ischemia via protection of injured neurons.

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CUTAMESINE EFFECT IN A LONG-TERM MCAO RAT MODEL

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Aims
Cutamesine has been described to play a neuroprotective role in acute stroke models. Here we present for the first time that cutamesine maintains this neuroprotection in a long-term ischemia model (15-days reperfusion).
Methods
Transient middle cerebral artery occlusion (tMCAO) was carried out in 12 week-old-male Sprague Dawley rats as previously described (Santos-Galdiano et al., 2018, J. Pharmacol. Exp. Ther. 367(3):528-542). Two experimental groups underwent 60 and 45 minutes of occlusion treated with cutamesine, as well as vehicle and sham groups were compared.
Cutamesine 10 µg/kg was administered 1 hour after reperfusion, once a day for 6 days. The vehicle group received sterile saline parallely to the cutamesine-treated group. The assays included behavioral tests (cylinder, adhesive removal, hanging wire, and apomorphine-induced rotation tests), apoptosis marker transcript, and magnetic resonance imaging (MRI) studies.
Results
Cutamesine improved the outcome in behavioral tests in animals subjected to 60-minutes occlusion while this effect was not observed in 45-minutes occlusion.
Cutamesine decreased apoptosis markers in the penumbra of animals underwent 45-minutes (but not in 60-minutes) occlusion, consistently with a decreasing trend in plasma levels of C reactive protein.
MRI showed a cutamesine-trend to reduce the infarct volume from 48 hours after reperfusion in animals with a 60-minutes occlusion.
Conclusions
MRI, biochemical and behavioral assays support a neuroprotective effect of cutamesine in a long-term (15 days) tCMAO model.
This study was supported by Neural Therapies SL and Ministerio de Ciencia e Innovación (Grants DIN2018-010144 and DIN2019-010883).

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IMAGING INCREASED METABOLISM IN THE SPINAL CORD IN A MOUSE MODEL OF ISCHEMIC STROKE

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Emerging evidence indicates a crosstalk between the haematopoietic system and the brain following cerebral ischemia. Here, we investigated the metabolism and oxygenation in the spleen and spinal cord in the transient middle cerebral artery occlusion (tMCAO) mouse model that is widely used in focal cerebral ischemia research. Naïve, sham and tMCAO mice underwent positron emission tomography (PET) using [^{18}F]fluorodeoxyglucose (FDG) for assessing glucose metabolism and multispectral optoacoustic tomography (MSOT) for oxygenation in these tissues at 24 h after reperfusion in mice. We employed the non-negative model-based reconstruction and unmixing algorithm to map the MSOT oxygen saturation pattern. After *in vivo* imaging the ischemic lesion was confirmed by *ex vivo* 2,3,5-triphenyltetrazolium chloride staining. We found increased levels of [^{18}F]FDG uptake and reduced MSOT oxygen saturation indicating hypoxia in the thoracic spinal cord of tMCAO mice compared with sham-operated mice but not in the spleen. A positive correlation was observed between splenic and ipsilateral striatal [^{18}F]FDG uptake. No difference was observed in the spleen size between groups *ex vivo*. In conclusion, we demonstrated the utility of MSOT in measuring spinal cord oxygenation, and found hypoxia and increased metabolic activity in the spinal cord of tMCAO mice at 24 h after reperfusion compared to sham-operated mice.

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H-FABP AS A BIOMARKER OF VASCULAR BRAIN DAMAGE IN TRANSIENT ISCHEMIC ATTACK

POSTER SESSION 04 - SECTION: BRAIN HYPOXIA-ISCHEMIA AND INFLAMMATION

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Background and purpose
The accurate diagnosis of transient ischemic attack (TIA) appears to be a challenge in clinical practice. Our objective is to evaluate the potential value of heart fatty-acid binding protein (H-FABP) as a possible biomarker for the differential diagnosis of TIA versus TIA-mimicking conditions (mimics).
Methods
A retrospective study with data from two prospectively recruited cohorts has been conducted. One hundred seventy-nine patients from the multicentric StrokeChip Study, and ninety-one patients from a biobank of TIA patients who underwent acute DWI were included. Blood was drawn within 6 and 24 hours of symptom onset respectively. H-FABP was measured using a rapid (15´) Point-of-care-test (POCT). Biomarker levels were compared between TIA and mimics, and between patients with or without brain injury in DWI. Accuracy was evaluated with ROC curves and cut-offs were obtained using PanelomiX algorithm.
Results
H-FABP level was significantly higher in TIA patients than in mimics [2.90 ng/mL (IQR 2.00 - 4.30) vs. 1.70 ng/mL (IQR 1.25-2.30)], p<0.001, respectively. The discriminatory power was 0.74 (95% CI, 0.66 - 0.83). A trend to higher concentrations was observed in those with DWI brain injury [3.20 ng/mL (IQR 2.40-4.10) vs. 2.40 ng/mL (IQR 1.90-3.77)] p=0.17.
Conclusion
High blood concentration of H-FABP measured in patients with transient neurological symptoms may point to vascular origin and to the presence of brain lesions following TIA. The availability of a rapid POCT will allow testing its clinical significance in real life in the emergency departments.

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INFLAMMATION PATTERN AFTER RAT SPINAL CORD INJURY WITH A COMPARATIVE ANALYSIS OF CONTUSION VS TRANSECTION EXPERIMENTAL MODELS

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal cord injury (SCI) in mammals leads to a series of cellular and molecular changes that will ultimately inhibit axon regeneration and autonomous nerve tissue repair. Regarding the immune response, the breakdown of the blood-spinal cord barrier (BSBC) favors infiltration of blood-derived monocytes/macrophages that, together with activated microglia, will spread inflammation, further exacerbated by formation of free radicals and glutamate excitotoxicity. This detrimental environment will cause secondary damage through neurodegenerative lesions that extend caudally and rostrally from the initial impact, aggravating the neurological deficits. The fundamental imbalance between pro- and anti-inflammatory cytokines during the second phase of injury leads to a persistent inflammatory state. Many studies have investigated the kinetics of inflammation-associated cytokines following SCI. However, the injured spinal segment, the time post-injury analyzed, and in particular, the lesion model differed among these studies, making a conclusive interpretation difficult. Accordingly, the present study compares the inflammation profiles at acute and chronic stages for the two commonly used lesion models, contusion vs. dorsal bilateral hemisection of rat thoracic spinal cord, revealing similarities and unexpected differences. In addition, we investigated possible correlations between serum- and intramedullary cytokine levels in rats after SCI. Multiple therapeutic strategies under pre-clinical development include an evaluation of the inflammatory response, whose modulation is considered crucial for a beneficial outcome. Our study should contribute to optimize the strategies to be adapted with respect to each trauma aspect; the type of injury and time post-lesion.

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NEUROECTODERMAL STEM CELLS IMPROVE THE FUNCTIONAL AND MORPHOLOGICAL OUTCOME AFTER CHRONIC SPINAL CORD CONTUSION INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal cord contusion injury leads to severe tissue loss and subsequent deficit of motor, sensory and vegetative functions below the lesion. In this study we investigated whether transplantation of neuroectodermal stem cells into the injured rat spinal cord is able to induce morphological and functional improvement in a chronic spinal cord injury model. Mouse embryonic clonal neuroectodermal stem cells were grafted intraspinally five weeks after a thoracic spinal cord contusion injury performed in SD rats. Control animals underwent contusion injury without stem cell transplantation. Functional tests and detailed morphological analysis were performed to evaluate the effects of grafted cells. Grafted animals showed significantly better functional recovery compared with control animals. Morphologically, the contusion cavity was significantly smaller, and the amount of spared tissue was significantly higher in grafted animals than in controls. Retrograde tracing studies showed a statistically significant increase in the number of FB-labelled neurons rostral to the injury. The extent of functional improvement was related to the amount of inhibitory factors around the cavity and microglial reactions in the injured segment. Five days after transplantation the majority of grafted cells appeared to survive, formed clusters and a small proportion of the cells differentiated into neurons and astrocytes. Ten days after grafting the majority of the grafted cells appeared as nonviable fragments in microglia/macrophage cells. These data suggest that grafted neuroectodermal stem cells are able to induce morphological and functional recovery after chronic spinal cord contusion injury despite the limited survival of transplanted cells. This work was supported by grant NTP-NFTÖ-21-B-0039.

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BOARD NUMBER: S04-326

A SYNTHETIC SYNAPTIC ORGANIZER PROTEIN “CPTX” RESTORES DAMAGED NEURONAL CIRCUITS FROM SPINAL CORD INJURY : THE RECOVERY FROM THE CHRONIC-PHASE OF SPINAL CORD INJURY.

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal cord injury(SCI) causes permanent dysfunction in the body movement and sensation. It is well known that recovery becomes more difficult as time passes from injury. Even with the power of stem cell transplant, it is still hard to cure the late phase of SCI. Despite the difficulty, majority of patients suffering from SCI are clinically late phase. Thus, overcoming the late phase of SCI is an important mission. CPTX is the structurally guided synthetic peptide that joins the pre-synaptic neurexin and the post-synaptic AMPA receptors (Suzuki, Sasakura et al., *Science*, (2020)). We previously showed that CPTX recovered the early phase of SCI in mouse model. CPTX restored the behavioral deficiency of Ataxia and Alzheimer’s mouse model as well. Therefore, CPTX is expected to be a promising medical agent to cure degenerated or injured CNS with novel concept (Salines, Science, 2020). To test if CPTX is effective to the late phase of SCI, we applied CPTX to the mice 4 or 6 weeks after SCI, which correspond to subacute or chronic phase of SCI patients, respectively. CPTX restored the subacute- and chronic-phase of SCI. CPTX injection activated the hind leg movement within a few days, which otherwise showed the permanently poor movement. At 2 weeks after injection, the effect of CPTX became maximum to the extent that some animals smoothly stepped. These results suggest that CPTX boost the dormant neural pathway into the active circuit. We are attempting the more robust recovery combined with rehabilitation.

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BOARD NUMBER: S04-327

DYNAMIC ECM CONSTRUCTS FOR SPINAL CORD INJURY REPAIR

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Developing reliable therapeutic methods to treat spinal cord injuries (SCIs) has been a major challenge due to the complex and dynamic cellular microenvironment during the disease progression. While several current therapeutic approaches have aimed to restore neural signaling, and prevent subsequent damage to the injured area, to date, there is no single biological intervention that can address the physiological events that damage the spinal cord. Scaffold-based therapeutic strategies and particularly decellularized ECM (dECM) materials have shown great potential for regenerative medicine but only few studies have attempted to explore their potential in SCI repair. In this study, we test the effect of dECM constructs from different developmental stages (fetal versus adult dECM) on human iPSCs derived motor neurons maturation, which can be used to support mechanical, structural and functional aspect of damaged spinal cord tissues. Both dECM showed identical physical properties but different chemical composition in structural proteins such as laminins, collagens, Tenascins and glycoproteins. iPSCs derived motor neurons exhibited increased survival and became morphologically and electrophysiological more mature on adult dECM relative to fetal dECM and commercial laminin coatings. We also explored the effect of 3D dECM constructs into the spinal cord of rodents with a severe SCI. Overall, our technology highlights the importance of the ECM in recapitulating in vivo conditions and offers a translational platform to study the dysfunction of the CNS after injury.

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NEW STRATEGY USING ALDYNOGLIA CELLS IN COMBINATION WITH FIBRIN AND SULPHOGLYCOLIPID INHIBITOR FOR SPINAL CORD INJURY REPAIR

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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A traumatic accident involving an injury to the spinal cord does not have effective treatment and implies of a deep social significance due to the effects it has on society. Traumatic spinal cord injury (TSCI) has a complex pathophysiologic events, that start after the trauma; one of them, formation of fibroglial scar performed by fibroblasts and reactive astrocytes. This provokes inhibition of axonal growth, by actived astroglial cells by secretion of inhibitory molecules, such as chondroitin sulphate proteoglycans. The objective of the study was to use the aldynoglia cells, a neuro-glial precursor, as a growth axon promoter, together with a biomaterial (fibrin gelated under alkaline conditions) to act as a cell support and directing neural cell growth. In addition, a synthetic sulphoglycolipid inhibitor of astrocytes and microglia was added to checking its effect in axonal growth promoting function of aldynoglia precursor cells. We observed an increase of differentiation to glial phenotype and cell outgrowth of aldynoglia in the modified fibrin. In dorsal root ganglia (DRG) and aldynoglia cocultures, a promotion in axonal growth was measured for DRG neurons. We concluded that modified fibrin combining it with synthetic sulphoglycolipid, increased adhesion capacity, promoting aldynoglia differentiation and growth. We observed that precursor cells were grouped first and then make niches from which they grew and connected to adjacent cell groups. There not negative effect on behavior of aldynoglia cells after the addition of sulphoglycolipid, suggesting that a combination of aldynoglia, plus the modified fibrin and inhibitor sulphoglycolipid compound could be useful as a new strategy for repair TSCI.

Pubmed:
32210903: Buzoianu-Anguiano V, Rivera-Osorio J, Orozco-Suárez S, Vega-García A, García-Vences E, Sánchez-Torres S, Jiménez-Estrada I, Guizar-Sahagún G, Mondragon-Caso J, Fernández-Valverde F, Madrazo I, Grijalva I
Single vs. Combined Therapeutic Approaches in Rats With Chronic Spinal Cord Injury.
The regenerative capability of the central nervous system is limited after traumatic spinal cord injury (SCI) due to intrinsic and extrinsic factors that inhibit spinal cord regeneration, resulting in deficient functional recovery. It has been shown that strategies, such as pre-degenerated peripheral nerve (PPN) grafts or the use of bone marrow stromal cells (BMSCs) or exogenous molecules, such as chondroitinase ABC (ChABC) promote axonal growth and remyelination, resulting in an improvement in locomotor function. These treatments have been primarily assessed in acute injury models. The aim of the present study is to evaluate the ability of several single and combined treatments in order to modify the course of chronic complete SCI in rats. A complete cord transection was performed at the T9 level. One month later, animals were divided into five groups: original injury only (control group), and original injury plus spinal cord re-transection to create a gap to accommodate BMSCs, PPN, PPN + BMSCs, and PPN + BMSCs + ChABC. In comparison with control and single-treatment groups (PPN and BMSCs), combined treatment groups (PPN + BMSCs and PPN + BMSCs + ChABC) showed significative axonal regrowth, as revealed by an increase in GAP-43 and MAP-1B expression in axonal fibers, which correlated with an improvement in locomotor function. In conclusion, the combined therapies tested here improve locomotor function by enhancing axonal regeneration in rats with chronic SCI. Further studies are warranted to refine this promising line of research for clinical purposes.
Front Neurol, 2020; 11
34685763: Buzoianu-Anguiano V, Torres-Llacsa M, Doncel-Pérez E
Role of Aldynoglia Cells in Neuroinflammatory and Neuroimmune Responses after Spinal Cord Injury.
Aldynoglia are growth-promoting cells with a morphology similar to radial glia and share properties and markers with astrocytes and Schwann cells. They are distributed in several locations throughout the adult central nervous system, where the cells of the aldynoglia interact and respond to the signals of the immune cells. After spinal cord injury (SCI), the functions of resident aldynoglia, identified as ependymocytes, tanycytes, and ependymal stem cells (EpSCs) of the spinal cord are crucial for the regeneration of spinal neural tissue. These glial cells facilitate axonal regrowth and remyelination of injured axons. Here, we review the influence of M1 or M2 macrophage/microglia subpopulations on the fate of EpSCs during

neuroinflammation and immune responses in the acute, subacute, and chronic phases after SCI.
Cells, 2021; 10
32300328: Rodríguez-Barrera R, Flores-Romero A, Buzoianu-Anguiano V, Garcia E, Soria-Zavala K, Incontri-Abraham D, Garibay-López M, Juárez-Vignon Whaley JJ, Ibarra A
Use of a Combination Strategy to Improve Morphological and Functional Recovery in Rats With Chronic Spinal Cord Injury. Immunization with neural derived peptides (INDP), as well as scar removal (SR) and the use of matrices with bone marrow-mesenchymal stem cells (MSCs), have been studied separately and proven to induce a functional and morphological improvement after spinal cord injury (SCI). Herein, we evaluated the therapeutic effects of INDP combined with SR and a fibrin glue matrix (FGM) with MSCs (FGM-MSCs), on motor recovery, axonal regeneration-associated molecules and cytokine expression, axonal regeneration (catecholaminergic and serotonergic fibers), and the induction of neurogenesis after a chronic SCI. For this purpose, female adult rats were subjected to SCI, 60 days after lesion, rats were randomly distributed in four groups: (1) Rats immunized with complete Freund's adjuvant + PBS (vehicle; PBS-I); (2) Rats with SR+ FGM-MSCs; (3) Rats with SR+ INDP + FGM-MSCs; (4) Rats only with INDP. Afterwards, we evaluated motor recovery using the BBB locomotor test. Sixty days after the therapy, protein expression of TNFα, IL-4, IL-10, BDNF, and GAP-43 were evaluated using ELISA assay. The number of catecholaminergic and serotonergic fibers were also determined. Neurogenesis was evaluated through immunofluorescence. The results show that treatment with INDP alone significantly increased motor recovery, anti-inflammatory cytokines, regeneration-associated molecules, axonal regeneration, and neurogenesis when compared to the rest of the groups. Our findings suggest that the combination therapy (SR + INDP + FGM-MSCs) modifies the non-permissive microenvironment post SCI, but it is not capable of inducing an appropriate axonal regeneration or neurogenesis when compared to the treatment with INDP alone.
Front Neurol, 2020; 11
30762019: García E, Rodríguez-Barrera R, Buzoianu-Anguiano V, Flores-Romero A, Malagón-Axotla E, Guerrero-Godinez M, De la Cruz-Castillo E, Castillo-Carvajal L, Rivas-Gonzalez M, Santiago-Tovar P, Morales I, Borlongan C, Ibarra A
Use of a combination strategy to improve neuroprotection and neuroregeneration in a rat model of acute spinal cord injury. Spinal cord injury is a very common pathological event that has devastating functional consequences in patients. In recent years, several research groups are trying to find an effective therapy that could be applied in clinical practice. In this study, we analyzed the combination of different strategies as a potential therapy for spinal cord injury. Immunization with neural derived peptides (INDP), inhibition of glial scar formation (dipyridyl: DPY), as well as the use of biocompatible matrix (fibrin glue: FG) impregnated with bone marrow mesenchymal stem cells (MSCs) were combined and then its beneficial effects were evaluated in the induction of neuroprotection and neuroregeneration after acute SCI. Sprague-Dawley female rats were subjected to a moderate spinal cord injury and then randomly allocated into five groups: 1) phosphate buffered saline; 2) DPY; 3) INDP + DPY; 4) DPY+ FG; 5) INDP + DPY + FG + MSCs. In all rats, intervention was performed 72 hours after spinal cord injury. Locomotor and sensibility recovery was assessed in all rats. At 60 days after treatment, histological examinations of the spinal cord (hematoxylin-eosin and Bielschowsky staining) were performed. Our results showed that the combination therapy (DPY+ INDP + FG + MSCs) was the best strategy to promote motor and sensibility recovery. In addition, significant increases in tissue preservation and axonal density were observed in the combination therapy group. Findings from this study suggest that the combination theapy (DPY+ INDP + FG + MSCs) exhibits potential effects on the protection and regeneration of neural tissue after acute spinal cord injury. All procedures were approved by the Animal Bioethics and Welfare Committee (approval No. 178544; CSNBTBIBAJ 090812960) on August 15, 2016.
Neural Regen Res, 2019; 14

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LSD1 INHIBITION IMPROVES FUNCTIONAL OUTCOME AFTER SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal cord injury (SCI) is a major cause of sensorial, motor and autonomic dysfunction worldwide. The primary impact causes neuronal damage and blood-brain barrier disruption, leading to the activation of secondary neurodegenerative mechanisms such as inflammatory processes, excitotoxicity and oxidative stress. Lysine specific demethylase 1 (LSD1) is an epigenetic eraser that promotes and represses gene transcription by removing methyl groups from histone 3, being involved in a wide range of biological processes. LSD1 inhibition reduces neuronal death caused by glutamate neurotoxicity and oxidative stress and has also been related with neuroplasticity. However, studies of this demethylase after nervous system trauma are scarce. Therefore, we aimed to analyze the effects of LSD1 inhibition on neuroprotection, regeneration, and functional recovery after a SCI. Spinal cord injured mice were treated with the LSD1 inhibitor RN1. Locomotor function assessed using both the basso mouse scale and the walking track showed that the RN1-treated group had an enhancement of the functional outcome compared to vehicle-treated animals. Electrophysiological analysis suggested a better preservation of the spinal cord descending pathways after LSD1 inhibition. Injured mice treated with RN1 also presented a decrease in neuropathic pain compared to the vehicle group. However, histological analysis did not show significative changes in neuronal death nor glial reactivity. Finally, by analyzing protein levels in the spinal cord after LSD1 inhibition, we identify GLT-1 as a possible LSD1 target involved in neuroprotection after SCI. These findings suggest that LSD1 inhibition may be a therapeutic approach to enhance functional recovery after SCI.

Pubmed:
31385133: Galán-Ganga M, Del Río R, Jiménez-Moreno N, Díaz-Guerra M, Lastres-Becker I
Cannabinoid CB Receptor Modulation by the Transcription Factor NRF2 is Specific in Microglial Cells.
Nuclear factor erythroid 2-related factor 2 (NRF2) is a pleiotropic transcription factor that has neuroprotective and anti-inflammatory effects, regulating more than 250 genes. As NRF2, cannabinoid receptor type 2 (CB) is also implicated in the preservation of neurons against glia-driven inflammation. To this concern, little is known about the regulation pathways implicated in CB receptor expression. In this study, we analyze whether NRF2 could modulate the transcription of CB in neuronal and microglial cells. Bioinformatics analysis revealed an antioxidant response element in the promoter sequence of the CB receptor gene. Further analysis by chemical and genetic manipulations of this transcription factor demonstrated that NRF2 is not able to modulate the expression of CB in neurons. On the other hand, at the level of microglia, the expression of CB is NRF2-dependent. These results are related to the differential levels of expression of both genes regarding the brain cell type. Since modulation of CB receptor signaling may represent a promising therapeutic target with minimal psychotropic effects that can be used to modulate endocannabinoid-based therapeutic approaches and to reduce neurodegeneration, our findings will contribute to disclose the potential of CB as a novel target for treating different pathologies.
Cell Mol Neurobiol, 2020; 40

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GUIDANCE LANDSCAPES UNVEILED BY QUANTITATIVE PROTEOMICS TO CONTROL REINNERVATION IN ADULT VISUAL SYSTEM

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Central nervous system (CNS) long distance regeneration has been achieved through the modulation of specific molecular pathways within neurons. However, regenerating axons display severe guidance defects: many axons are lost at choice points or avoid their initial targets compromising recovery of function. Therefore, depicting the mature neuronal environment, beyond the lesion site, is essential to understand adult axon guidance to build neuronal circuits in mature CNS. To this end, we used quantitative mass spectrometry to characterize the proteome of the adult visual system major nuclei: the suprachiasmatic nucleus, ventral and dorsal lateral geniculate nucleus, and the superior colliculus as well as the optic chiasm, where axons show the most impressive guidance defects. We uncovered the expression of guidance molecules and associated factors in the mature visual targets. Moreover, upon bilateral optic nerve crush, we showed that axon lesion modifies the proteome of all brain targets. In contrast, we found that the expression of guidance remains steady after injury. We hypothesized that guidance cues expression contributes to prevent axons to reconnect their appropriate brain targets and further recovery of functions. Indeed, we showed that mature regenerating axons are still sensitive to guidance molecules. Together our results provide an extensive characterization of the molecular environment of intact and injured brain. Moreover, as guidance factors could counteract brain reinnervation, our study opens new venues to build mature neuronal circuits upon injury.

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FLEXIBLE NEUROTRANSMITTER PHENOTYPE OF SPINAL EXCITATORY INTERNEURONS REGULATES LOCOMOTOR ABILITY AFTER SPINAL CORD INJURY.

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Severe spinal cord injury to the mature nervous system leads to irreversible paralysis below the lesion. However, adult rodents receiving a complete thoracic lesion just after birth demonstrate proficient hindlimb locomotion without input from the brain. How the spinal cord achieves such striking plasticity and functionality remains unknown. Here, we used a combinatorial approach of kinematic analyses, mouse genetics, and circuit manipulation to uncover age of injury-dependent circuit signatures with an aim to steer adult injured spinal cords to such autonomous circuits. We found divergent synaptic connectivity profiles from interneurons to motor neurons. Adult injury prompts neurotransmitter phenotype switching of spatially defined excitatory interneurons to inhibitory phenotype, promoting inhibition at synapses interfacing motor neurons. In contrast, neonatal injury causes synaptic sprouting of identical populations to facilitate excitation. Ablation of proprioceptive afferents leads to gain of inhibitory neurotransmitter phenotype after neonatal injury, whereas daily locomotor training to maximize proprioceptive afferent activity leads to partial maintenance of excitatory neurotransmitter phenotype after adult injury. These findings reveal activity-dependent, flexible neurotransmitter phenotype of defined excitatory spinal interneurons. Furthermore, genetic manipulation to mimic inhibitory phenotype observed after adult injury by excitatory interneurons abrogates autonomous locomotor functionality in neonatally injured mice. In comparison, attenuating inhibitory phenotype improves locomotor recovery after adult injury. Together, our study demonstrates that flexible NT phenotype of defined excitatory interneurons as a defining signature of age of injury-dependent locomotor circuit plasticity and an entry point to regulate locomotor capacity after injury.

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BENEFICIAL EFFECTS OF ANGIOTENSIN II RECEPTOR TYPE 2 STIMULATION AFTER SEVERE SPINAL CORD COMPRESSION

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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In the present study we tested pharmacological AT_2 receptor stimulation as a therapeutic approach in an experimental model of severe spinal cord compression in adult female Wistar rats (n=15/group) using selective AT_2 receptor agonist CGP42112 (0.1 mg/kg per day) administrated systemically by osmotic minipumps (s.c.) from 14th to 28th post-injury day when the trauma-induced expression of AT_2 receptors occurs in the injured spinal cord parenchyma. During the 28-day posttraumatic period, the voiding, body weight changes, blood pressure and hind-limb locomotor function by using BBB scale and transcranial motor evoked potentials were evaluated. The motoric hind limbs function recovered rapidly and more profound after AT_2 receptor stimulation compare to spinal cord injury (BBB locomotor score: 10.4 points vs. 9 points) and strongly negatively correlates (Pearson r= -0,908) with evidently faster neural response latency (7.03 ms vs. 10.8 ms). Bladder function completely recovered on 20^{th} day of surviving period in all animals after AT_2 receptor stimulation while the dysfunction of urination persisted 26 days in animals that underwent spinal cord compression without any treatment. Obtained behavioural results clearly demonstrate that AT_2 receptor stimulation beneficially affects both the locomotor and sensory neurological functions of treated experimental animals. These beneficial effects were prevented by the AT_2 receptor blocking with specific antagonist PD123319 that confirms the neuroprotective features mediated through the stimulation. Our results suggested that the AT_2 receptor stimulation may be considered as promising therapeutic approach for the spinal cord injury that promotes the improvement of functional neurological outcomes. *Supported by grant APVV-18-0163, ERDF-IMTS:313011V344.*

Pubmed:

34410553: Fedorova J, Kellerova E, Bimbova K, Pavel J
The Histopathology of Severe Graded Compression in Lower Thoracic Spinal Cord Segment of Rat, Evaluated at Late Post-injury Phase.
Spontaneous recovery of lost motor functions is relative fast in rodent models after inducing a very mild/moderate spinal cord injury (SCI), and this may complicate a reliable evaluation of the effectiveness of potential therapy. Therefore, a severe graded (30 g, 40 g and 50 g) weight-compression SCI at the Th9 spinal segment, involving an acute mechanical impact followed by 15 min of persistent compression, was studied in adult female Wistar rats. Functional parameters, such as spontaneous recovery of motor hind limb and bladder emptying function, and the presence of hematuria were evaluated within 28 days of the post-traumatic period. The disruption of the blood-spinal cord barrier, measured by extravasated Evans Blue dye, was examined 24 h after the SCI, when maximum permeability occurs. At the end of the survival period, the degradation of gray and white matter associated with the formation of cystic cavities, and quantitative changes of glial structural proteins, such as GFAP, and integral components of axonal architecture, such as neurofilaments and myelin basic protein, were evaluated in the lesioned area of the spinal cord. Based on these functional and histological parameters, and taking the animal's welfare into account, the 40 g weight can be considered as an upper limit for severe traumatic injury in this compression model.
Cell Mol Neurobiol, 2022; 42
30648463: Fedorova J, Pavel J
An Accurate Method for Histological Determination of Neural Tissue Loss/Sparing after Compression-Induced Spinal Cord Injury with Optimal Reproducibility.
In addition to behavioral testing, the efficacy of neuroprotective therapies applied after spinal cord injury (SCI) is commonly evaluated by means of histological quantification of spared neural tissue. The primary insult itself, but mainly the pathological processes of secondary injury are the underlying causes of spinal tissue degeneration, the extent of which depends on the injury severity and post-injury time. Under-estimation of tissue loss due to spinal cord shrinkage and subjective evaluation

(impeding reproducibility) are substantial factors that negatively affect the final results. Moreover, processing large numbers of stained spinal cord sections is very time-consuming. To overcome the problem, our new quantification approach combines a modified method for predicting the cross-sectional area at the lesion site with semi-automatic measurement of spared neural tissue and cystic cavities, using freely accessible National Institutes of Health (NIH) ImageJ software, with a Java-based image processing program. Based on the histological parameters measured after differing compression-induced SCI and correlated with behavioral outcomes, we can conclude that our new method is relatively fast, accurate, and optimally reproducible.
J Neurotrauma, 2019; 36
29642434: Bimbova K, Bacova M, Kisucka A, Pavel J, Galik J, Zavacky P, Marsala M, Stropkovska A, Fedorova J, Papcunova S, Jachova J, Lukacova N
A Single Dose of Atorvastatin Applied Acutely after Spinal Cord Injury Suppresses Inflammation, Apoptosis, and Promotes Axon Outgrowth, Which Might Be Essential for Favorable Functional Outcome.
The aim of our study was to limit the inflammatory response after a spinal cord injury (SCI) using Atorvastatin (ATR), a potent inhibitor of cholesterol biosynthesis. Adult Wistar rats were divided into five experimental groups: one control group, two Th9 compression (40 g/15 min) groups, and two Th9 compression + ATR (5 mg/kg, i.p.) groups. The animals survived one day and six weeks. ATR applied in a single dose immediately post-SCI strongly reduced IL-1β release at 4 and 24 h and considerably reduced the activation of resident cells at one day post-injury. Acute ATR treatment effectively prevented the excessive infiltration of destructive M1 macrophages cranially, at the lesion site, and caudally (by 66%, 62%, and 52%, respectively) one day post-injury, whereas the infiltration of beneficial M2 macrophages was less affected (by 27%, 41%, and 16%). In addition, at the same time point, ATR visibly decreased caspase-3 cleavage in neurons, astrocytes, and oligodendrocytes. Six weeks post-SCI, ATR increased the expression of neurofilaments in the dorsolateral columns and Gap43-positive fibers in the lateral columns around the epicenter, and from day 30 to 42, significantly improved the motor activity of the hindlimbs. We suggest that early modulation of the inflammatory response via effects on the M1/M2 macrophages and the inhibition of caspase-3 expression could be crucial for the functional outcome.
Int J Mol Sci, 2018; 19
30339879: Bacova M, Bimbova K, Fedorova J, Lukacova N, Galik J
Epidural oscillating field stimulation as an effective therapeutic approach in combination therapy for spinal cord injury.
Traumatic spinal cord injury (SCI) causes partial or total loss of sensory and motor functions. Despite enormous efforts, there is still no effective treatment which might improve patients' neurological status.The application of electric current to the injured spinal cord is known to promote healing and tissue regeneration. The use of this modality in treating the injured spinal cord to improve neurological recovery has been introduced as a potential treatment.
J Neurosci Methods, 2019; 311

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TRANSCRIPTOMIC ANALYSIS' OF AXON REGENERATION-INDUCING MANIPULATIONS DISCOVER DISTINCT MOLECULAR PROGRAMS DEFINING A CELL'S FATE TO DIE, SURVIVE OR REGENERATE AFTER AN INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Aims: Neurons of the central nervous system (CNS) display only a limited ability to survive and regenerate their axons after an injury. In mice, 85% of retinal ganglion cells (RGCs) in the retina die within 2 weeks of axotomy by optic nerve crush (ONC) and only few survivors regenerate axons. However, some treatments can enhance survival and promote regeneration. Here, to elucidate mechanisms underlying these protective effects, we investigated genes regulated by three well established survival and regeneration promoting interventions – activation of the MTOR pathway via deletion of its inhibitor PTEN, activation of the Jak/Stat-pathway by deletion of its endogenous inhibitor SOCS3, and overexpression of the neurotrophic cytokine CNTF. **Methods:** We collected >125,000 single RGCs at various time points after ONC (0,2,7 and 21dpc) and established a new method to separate regenerating RGCs from those that survived but did not regenerate, followed by scRNA-sequencing (10X and SS2). **Results:** Analysis of gene expression patterns, showed that our interventions improve the survival and regeneration of RGCs by mitigating injury responses and cell death associated genes while simultaneously upregulating survival and regeneration associated programs. These programs are differentially regulated among RGCs, with distinct signatures for dying, surviving and regenerating cells. **Conclusion:** Together, this study provides insights into why neuronal cell types respond differently to neuroprotective and pro-regenerative treatments. Moreover, overexpression of some the genes associated to the identified regeneration-program promote axonal regeneration *in vivo*.

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ULTRAFAST DOPPLER IMAGING AND ULTRASOUND LOCALIZATION MICROSCOPY REVEAL VASCULAR REARRANGEMENT'S COMPLEXITY IN CHRONIC SPINAL LESION LESION

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Acute spinal cord injury (SCI) leads to severe damage to the microvascular network. The process of spontaneous repair is accompanied by formation of new blood vessels; their functionality, however, presumably very important for functional recovery, has never been clearly established, as most studies so far used fixed tissues. Here, combining ultrafast Doppler imaging and Ultrasound Localization Microscopy (ULM) on the same animals, we proceeded at a detailed analysis of structural and functional vascular alterations associated with the establishment of chronic SCI, both at macroscopic and microscopic scales. Using a standardized animal model of SCI, our results demonstrate striking hemodynamic alterations in several subparts of the spinal cord: a reduced blood velocity in the lesion site, and an asymmetrical hypoperfusion caudal but not rostral to the lesion. In addition, the worsening of many evaluated parameters at later time points suggests that the neoformed vascular network is not yet fully operational, and reveals ULM as an efficient *in vivo* readout for spinal cord vascular alterations. Finally, we show statistical correlations between the diverse biomarkers of vascular dysfunction and SCI severity. The imaging modality developed here will allow evaluating recovery of vascular function over time in pre-clinical models of SCI. Also, used on SCI patients in combination with other quantitative markers of neural tissue damage, it may help classifying lesion severity and predict possible treatment outcomes in patients.

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BOARD NUMBER: S04-335

ORAL ADMINISTRATION OF 4-METHYLUMBELLIFERONE COMBINED WITH REHABILITATION PROMOTES ANATOMICAL PLASTICITY AND FUNCTIONAL RECOVERY IN THE CHRONIC STAGE OF SPINAL CORD INJURY.

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

Katerina Stepankova[1,2], Milada Chudickova[2], Sarka Kubinova[2], Lucia Urdzikova Machova[1,2], James Fawcett[2,3], Pavla Jendelova[1,2], Jessica Kwok[2,4]
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Currently, there is no effective treatment for chronic spinal cord injury (SCI). Strong inhibitors of neural regeneration are chondroitin sulfate proteoglycans (CSPG). 4-methylumbelliferone (4MU) inhibits the synthesis of hyaluronan and CSPGs and regulates perineuronal net formation in the nervous system. Firstly, we investigated the pharmacological, biochemical, and biomechanical effects of long-term 4MU (2.5% w/w) treatment in non-injured rats. Results of haematological and serological parameters showed non-significant changes between groups. Tissue biochemistry showed significant downregulation of glycosaminoglycans (GAGs) in the 4MU-treated group suggesting attenuated PNNs, which was also confirmed by immunohistochemistry of WFA and HABP. Next, we tested 2.5% (w/w) 4MU to promote axonal plasticity and functional recovery in rat chronic contusion model of SCI. Animals were fed daily with 4MU for 8 weeks, starting 6 weeks after SCI, followed by daily treadmill training. Our results showed that oral administration of 2.5% (w/w) 4MU reduced glial scar and promoted anatomical plasticity, but not functional recovery in chronic stage of spinal cord injury. Our previous experiment has demonstrated efficacy of 4MU in enhancing recovery after acute SCI. We thus increased the dose of 4MU to 5% (w/w) to test in the chronic SCI paradigm. We observed the significant locomotor improvement of rats with SCI. Our results indicate that 5% (w/w) 4MU reopened a window of plasticity in chronic SCI, allowing rehabilitation to promote functional recovery.
Supported by: Czech Science Foundation GACR 19-10365S, by Operational Programme Research, Development and Education in the framework of the project “Center of Reconstructive Neuroscience”, registration number CZ.02.1.01/0.0./0.0/15_003/0000419.

Pubmed:
33167447: Krupa P, Stepankova K, Kwok JC, Fawcett JW, Cimermanova V, Jendelova P, Machova Urdzikova L
New Model of Ventral Spinal Cord Lesion Induced by Balloon Compression in Rats.
Despite the variety of experimental models of spinal cord injury (SCI) currently used, the model of the ventral compression cord injury, which is commonly seen in humans, is very limited. Ventral balloon compression injury reflects the common anatomical mechanism of a human lesion and has the advantage of grading the injury severity by controlling the inflated volume of the balloon. In this study, ventral compression of the SCI was performed by the anterior epidural placement of the balloon of a 2F Fogarty's catheter, via laminectomy, at the level of T10. The balloon was rapidly inflated with 10 or 15 µL of saline and rested in situ for 5 min. The severity of the lesion was assessed by behavioral and immunohistochemical tests. Compression with the volume of 15 µL resulted in severe motor and sensory deficits represented by the complete inability to move across a horizontal ladder, a final Basso, Beattie and Bresnahan (BBB) score of 7.4 and a decreased withdrawal time in the plantar test (11.6 s). Histology and immunohistochemistry revealed a significant loss of white and gray matter with a loss of motoneuron, and an increased size of astrogliosis. An inflation volume of 10 µL resulted in a mild transient deficit. There are no other balloon compression models of ventral spinal cord injury. This study provided and validated a novel, easily replicable model of the ventral compression SCI, introduced by an inflated balloon of Fogarty´s catheter. For a severe incomplete deficit, an inflated volume should be maintained at 15 µL.
Biomedicines, 2020; 8
34071245: Stepankova K, Jendelova P, Machova Urdzikova L
Planet of the AAVs: The Spinal Cord Injury Episode.
The spinal cord injury (SCI) is a medical and life-disrupting condition with devastating consequences for the physical, social, and professional welfare of patients, and there is no adequate treatment for it. At the same time, gene therapy has been

studied as a promising approach for the treatment of neurological and neurodegenerative disorders by delivering remedial genes to the central nervous system (CNS), of which the spinal cord is a part. For gene therapy, multiple vectors have been introduced, including integrating lentiviral vectors and non-integrating adeno-associated virus (AAV) vectors. AAV vectors are a promising system for transgene delivery into the CNS due to their safety profile as well as long-term gene expression. Gene therapy mediated by AAV vectors shows potential for treating SCI by delivering certain genetic information to specific cell types. This review has focused on a potential treatment of SCI by gene therapy using AAV vectors.
Biomedicines, 2021; 9

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BOARD NUMBER: S04-336

AAV-MEDIATED GENE THERAPY FOR SENSORY REGENERATION AFTER SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal cord injury (SCI), a lifelong disability, remains an unsolved problem not only due to glial scar forming a physical barrier around the lesion site, but also due to the limited intrinsic capacity of axon regeneration. One of the main ECM inhibitory molecules upregulated after spinal cord injury is Tenascin-C. The migration-inducing tenascin-binding integrin is alpha9beta1, which is not upregulated after injury. Integrin α9, needs an activator kindlin 1 which prevents an integrin inactivation by inhibitory CSPGs and NogoA. The aim of this project was to achieve sensory axon regeneration in animals with dorsal column crush lesion using viral vector delivery of the relevant genes (integrin α9 and kindlin 1 in 3:1 ratio, kindlin 1 alone or GFP) to the DRG. We addressed two different levels of SCI, C4 lesion with DRG C6 and C7 injections for forelimb sensory restoration and T10 lesion with DRG L4 and L5 injections for hindlimb sensory restoration. The animals underwent dorsal column crush injury with concurrent DRG injections followed by 12 weeks of behavioural testing. Significant improvement was observed in Von Frey test for mechanical perception and Hargreaves test for thermal sensation in treated animals with both, cervical and thoracic lesions when compared to controls. Functional improvement was confirmed by cFos staining and counting GFP axons from integrin α9 and kindlin 1 group above the lesion. In conclusion, the AAV-mediated gene therapy leads to sensory regeneration after SCI at C4 and T10 level, proved by behavioural tests and immunohistochemical staining.
Supported by NEURORECON.02.1.01/0.0./0.0/15_003/0000419 and GAUK_320421.

Pubmed:
34073791: Vargova I, Machova Urdzikova L, Karova K, Smejkalova B, Sursal T, Cimermanova V, Turnovcova K, Gandhi CD, Jhanwar-Uniyal M, Jendelova P
Involvement of mTOR Pathways in Recovery from Spinal Cord Injury by Modulation of Autophagy and Immune Response. Traumatic spinal cord injury (SCI) is untreatable and remains the leading cause of disability. Neuroprotection and recovery after SCI can be partially achieved by rapamycin (RAPA) treatment, an inhibitor of mTORC1, complex 1 of the mammalian target of rapamycin (mTOR) pathway. However, mechanisms regulated by the mTOR pathway are not only controlled by mTORC1, but also by a second mTOR complex (mTORC2). Second-generation inhibitor, pp242, inhibits both mTORC1 and mtORC2, which led us to explore its therapeutic potential after SCI and compare it to RAPA treatment. In a rat balloon-compression model of SCI, the effect of daily RAPA (5 mg/kg; IP) and pp242 (5 mg/kg; IP) treatment on inflammatory responses and autophagy was observed. We demonstrated inhibition of the mTOR pathway after SCI through analysis of p-S6, p-Akt, and p-4E-BP1 levels. Several proinflammatory cytokines were elevated in pp242-treated rats, while RAPA treatment led to a decrease in proinflammatory cytokines. Both RAPA and pp242 treatments caused an upregulation of LC3B and led to improved functional and structural recovery in acute SCI compared to the controls, however, a greater axonal sprouting was seen following RAPA treatment. These results suggest that dual mTOR inhibition by pp242 after SCI induces distinct mechanisms and leads to recovery somewhat inferior to that following RAPA treatment.
Biomedicines, 2021; 9

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BOARD NUMBER: S04-337

EARLY INTRAVENOUS INFUSION OF NEUROECTODERMAL STEM CELL EXERTS BENEFICIAL EFFECT ON NEUROPROTECTION FOLLOWING TRAUMATIC SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Traumatic spinal cord injury (SCI) is characterized by an acute mechanical insult followed by a series of secondary lesion events. In this study we investigated the effect of delayed intravenous neuroectodermal stem cell therapy which could reduce the severity of secondary injury and enhance tissue preservation and promote functional recovery. A thoracic contusion injury was induced at T5 spinal level in adult female Sprague-Dawley rats, followed by an intravenous tail vein infusion of NE-GFP-4C stem cells 30 min, 1, 2 or 3 weeks after the injury. Control animals underwent contusion injury without intravenous stem cell administration. Functional tests (BBB test, and kinematic analysis) and detailed morphological analysis (quantification of retrograde labelling and immunohistochemistry) were performed to evaluate the effect of grafted cells. One day after intravenous stem cell administration proteome profiler arrays were used to assess the cytokine expression in the spleen, blood serum and spinal cord. Significantly greater functional improvement was observed in transplanted animals treated intravenously with NE-GFP-4C cells immediately or one week after injury compared with controls. Significant neuroprotection was observed in the same treatment groups and significantly more retrogradely-labelled supra- and propriospinal neurons were counted compared with control animals. Immediate and one-week-delayed stem cell administration increased levels of the tissue inhibitor metalloproteinase-1 (TIMP-1, a potent beneficial candidate for vascular integrity) in the injured spinal cord and spleen one day after cell infusion. Our results suggest that immediate or one-week-delayed intravenous stem cell treatment is likely to induce nearly equal morphological and functional recovery through TIMP-1 modulation.

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SELECTIVE MODULATION OF A1 ASTROCYTES BY DRUG-LOADED NANO-STRUCTURED GEL IN SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Abstract: Spinal cord injury (SCI) is the most frequent disabling injury of the spine. The magnitude of SCI pathology and the related functional loss correlates with a robust astrocytes response. Astrocytes do not constitute a single uniform cell population, but comprise a family of cells with a spectrum of activation states with opposing phenotypes, some destructive (A1) and others beneficial (A2). This suggests that A1 astrocytes may be a good therapeutic target for SCI. **Aims:** In this project, we aimed to study a new therapeutic treatment to block the pro-inflammatory A1 response in SCI by acting on astrocytes with a selective drug-loaded nanovector system, in vitro and *in vivo*. **Methods:** A new functionalized nanogel-based nanovector (NG) was tested on activated primary cultures of mouse or human astrocytes. We measured: (i) uptake (ii) subcellular distribution (iii) biocompatibility and (iv) drug delivery (Rolipram). In vivo experiments were conducted to validate the NG system and therapeutic effect. **Results:** Rolipram administered by NG limits the inflammatory response of A1 astrocytes, reducing inflammatory factors (iNOS and Lcn2) and astrocytosis. Moreover, it improves motor performance in *in vivo* SCI mouse model. **Conclusion:** We demonstrated the capability of the NG system to selectively deliver drugs in astrocytes with a cell-specific therapeutic approach and demonstrated therapeutic effect in SCI. This delivery strategy could also be considered for other molecules which are able to enhance the neuroprotective A2 in SCI progression.

Pubmed:
33309836: Papa S, Veneruso V, Mauri E, Cremonesi G, Mingaj X, Mariani A, De Paola M, Rossetti A, Sacchetti A, Rossi F, Forloni G, Veglianese P
Functionalized nanogel for treating activated astrocytes in spinal cord injury.
Astrogliosis has a unique reaction during spinal cord damage, with helpful or adverse impacts on recovery. There is consequently a pressing need for treatment to target activated astrocytes and their unsafe response after injury to ensure some preservative effect during the progressive damage. We specifically developed and characterized a functionalized nanogel-based nanovector in vitro and in vivo, demonstrating its selectivity towards astrocytes, and limited uptake by macrophages when functionalized with both NH and Cy5 groups. In vitro experiments showed that the internalization was mediated by a clathrin-dependent endocytic pathway. After internalization into the cytoplasm of astrocytes, nanogels undergo lysosomal degradation and release compounds with potential therapeutic efficacy.
J Control Release, 2021; 330
31887011: Vismara I, Papa S, Veneruso V, Mauri E, Mariani A, De Paola M, Affatato R, Rossetti A, Sponchioni M, Moscatelli D, Sacchetti A, Rossi F, Forloni G, Veglianese P
Selective Modulation of A1 Astrocytes by Drug-Loaded Nano-Structured Gel in Spinal Cord Injury.
Astrogliosis has a very dynamic response during the progression of spinal cord injury, with beneficial or detrimental effects on recovery. It is therefore important to develop strategies to target activated astrocytes and their harmful molecular mechanisms so as to promote a protective environment to counteract the progression of the secondary injury. The challenge is to formulate an effective therapy with maximum protective effects, but reduced side effects. In this study, a functionalized nanogel-based nanovector was selectively internalized in activated mouse or human astrocytes. Rolipram, an anti-inflammatory drug, when administered by these nanovectors limited the inflammatory response in A1 astrocytes, reducing iNOS and Lcn2, which in turn reverses the toxic effect of proinflammatory astrocytes on motor neurons , showing advantages over conventionally administered anti-inflammatory therapy. When tested acutely in a spinal cord injury mouse model, it improved motor performance, but only in the early stage after injury, reducing the astrocytosis and preserving neuronal cells.
ACS Nano, 2020; 14
30851286: Veneruso V, Rossi F, Villella A, Bena A, Forloni G, Veglianese P
Stem cell paracrine effect and delivery strategies for spinal cord injury regeneration.
Spinal cord injury (SCI) is a complicated neuropathological condition that results in functional dysfunction and paralysis.

Various treatments have been proposed including drugs, biological factors and cells administered in several ways. Stem cell therapy offers a potentially revolutionary mode to repair the damaged spinal cord after injury. Initially, stem cells were considered promising for replacing cells and tissue lost after SCI. Many studies looked at their differentiation to replace neuronal and glial cells for a better functional outcome. However, it is becoming clear that different functional improvements recognized to stem cells are due to biomolecular activities by the transplanted stem cells rather than cell replacement. This review aimed to discuss the paracrine mechanisms for tissue repair and regeneration after stem cell transplantation in SCI. It focuses on stem cell factor production, effect in tissue restoration, and novel delivery strategies to use them for SCI therapy.
J Control Release, 2019; 300

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BOARD NUMBER: S04-339

IN VITRO MICROFLUIDIC DESIGN TO STUDY MITOCHONDRIA-MICROTUBULES INTERACTIONS AFTER AN AXONAL TRAUMATIC INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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A lesion in the nervous system induces cellular and molecular mechanisms leading to irreversible dysfunctions. Post-injured environment is non permissive for axonal regeneration and CNS neurons also exhibit a reduced intrinsic growth capacity. Nevertheless, neuronal plasticity is still possible: injured axons can degenerate or regenerate, but mechanisms governing this response remains elusive. My aim is to understand the relation between microtubules and mitochondria on axon fate after a traumatic injury, in particular by focusing on the corticospinal tract connecting to the spinal cord neurons. Corticospinal neurons are known to show poor regeneration capacities after a spinal cord injury. Axonal degeneration is associated with a fast decrease of NAD+ leading to energy drop, cytoskeletal disorganization and finally axonal degradation. Mitochondria repositioning in the axon can stimulate regeneration and branching by rescuing energy deficits. In addition, microtubules (MT) to which converge extracellular cues for intracellular trafficking are also involved in mitochondrial transport. Thereby, mitochondria and MT are essential in determining the axon fate. I am developping an *in vitro* set-up with microfluidics oriented networks of cortico-spinal neurons on which a physical injruy can be apply. This system will allow to study the influence of post-translationnal MT modifications on mitochondria dynamic during axonal regeneration or degeneration.

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MODULATION OF PRO-INFLAMMATORY M1 MICROGLIA AND A1 ASTROCYTES INTO THEIR ANTI-INFLAMMATORY M2 AND A2 PHENOTYPES IS CRUCIAL FOR SPONTANEOUS NEUROLOGICAL OUTCOME AFTER SCI

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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The goal of studies dealing with microglia/macrophages and astrocytes intracellular communication after spinal cord injury (SCI) is to improve our ability to contend this serious and incurable disorder. Both microglia and astrocytes are activated into two polarization states early after trauma: the pro-inflammatory (M1 and A1) and anti-inflammatory (M2 and A2) phenotypes, and this plays an important role in the regulation of immune responses under various pathological conditions and repair. The aim of our study was to examine the time depending behavior of reactive astrocytes and microglia/macrophages and their polarization states after Th9 compression (40g/15 min) at the lesion site and in adjacent spinal cord sections. Adult Wistar rats were divided into three experimental groups: 1) control, and 2-3) a group of animals that survived 7 and 14 days after SCI. While the gene expression of microglia/macrophages and pro-inflammatory M1 microglia was strongly upregulated one week after SCI at the lesion site and caudally from the injury epicenter and downregulated after two weeks, astrocyte gene markers and markers for A1 phenotype were significantly expressed predominantly 2 weeks after trauma at the site of injury and cranially. The positive correlation between neurological score and gene expression of anti-inflammatory M2a, M2c microglia and A2 astrocytes has been detected. The results show that the first post-injury week is critical for microglia/macrophages and astrocytes transformation into their neuroprotective states. We assume, this could be a very effective time window for treatment strategy after SCI. Supported by APVV-15-0766; APVV-19-0324; VEGA 2/0145/21; ERDF-IMTS 313011V344.

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BOARD NUMBER: S04-341

MICROGLIA-DRIVEN REGULATION OF GLIAL SCAR FORMATION AFTER SPINAL CORD INJURY: EFFECT OF Y-27632 IN REGENERATIVE PROCESSES

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

Nadezda Lukacova, Andrea Stropkovska, Alexandra Kisucka, Katarina Kiss Bimbova, Maria Bacova, Maria Ileninova, Tomas Kuruc, Jan Galik
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Spinal cord injury (SCI) activates intracellular signaling molecule Rho, leading to a cascade of events culminating in the collapse of axonal growth cones, failure of injured axons to regenerate, as well as neuronal loss. We studied acute inflammatory response (1,3,7,14d) and its effect on neuropathological changes in the rat SCI model (Th9 compression; 40g/15 min) eight weeks post-SCI. Rho-kinase inhibitor, Y-27632 (40µg/d for 14d post-SCI) was delivered intrathecally via osmotic Alzet mini-pump. Two weeks post-SCI, the TGF-ß mRNA expression was ~five-fold higher (marker of neuroprotective M2c microglia) than in naïve control at lesion site and cranially, and ~two-fold higher caudally, suggesting its role in promoting the glial scar formation by enhanced secretion (~2- to 4- fold) of CSPGs (neurocan, phosphacan and NG2). Increased expression of these CSPGs at lesion site and cranially was consistent with strong expression of GFAP, S100B and reactive A1 astrocytes. Y-27632 modulated the inhibitory milieu by specifically targeting gray and white matter integrity (LFB staining) and GFAP-immunoreactivity, and significantly improved spinal cord repair eight weeks post-SCI. Increased outgrowth of neurofilaments (0.4-1µm -35%, 1-2µm -30%, 2-3µm -17%, 3-4µm-13%) and GAP-43 immunoreactive axons was observed across the lesion area, leading to significant functional outcome from the 3th to 8th week post-SCI. The results show that microglia-astrocytes modulation is key for glial scar formation in subacute phase after SCI. Y-27632 is able to support endogeneous regenerative processes leading to better axonal regeneration and locomotor recovery. Supported by APVV-15-0766; APVV-19-0324; VEGA 2/0145/21; VEGA 2/0098/20; ERDF-IMTS 313011V344.

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BOARD NUMBER: S04-342

ACUTE INTRALESIONAL APPLICATION OF EXTRACELLULAR VESICLES IMPROVES OUTCOMES IN A RAT MODEL OF TRAUMATIC SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

Lara Bieler[1,2,3], Pasquale Romanelli[1,2,4], Patrick Heimel[3,5,6], Siniša Škokić[7], Dominika Jakubecova[1], Christina Kreutzer[1,2], Pia Zaunmair[2], Tomislav Smolčić[7], Bruno Benedetti[1,2,3], Eva Rohde[2,8,9,10], Mario Gimona[2,8,10,11], David Hercher[3,5], Marina Dobrivojević Radmilović[7], Sébastien Couillard-Després[1,2,3]
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Local inflammation is a deleterious process after spinal cord injury (SCI). Acute intravenous application of extracellular vesicles (EVs) secreted by human mesenchymal stromal cells were shown to dampen inflammation following SCI. Here, we compared the impact of intralesional vs intravenous application of EVs, on functional regeneration and structural integrity following SCI. Fischer-344 rats received a 200kdyn contusion (Th8) and were acutely treated with EVs. Expression of inflammatory cytokines in the spinal cord was determined at 24h post-injury. Locomotor recovery was assessed for 8 weeks and histological analysis was performed at 2 and 8 weeks post-SCI. In addition, DTI was performed *ex vivo* 8 weeks after injury. Locomotion recovery post-SCI was significantly improved by intralesional EV-application. Analyses 24h post-SCI revealed that acute application of EVs reduced IL-1beta and IL-6 gene expression. Two weeks post-injury, the density of microglia detected close to the lesion was significantly lower in rats treated with intralesional vs intravenous application of EVs. Furthermore, scar formation, based on astrogliosis, collagen-1 and CSPG deposition, was also more attenuated by intralesional EVs treatment. Finally, 8 weeks post-injury, DTI detected close to the lesion site more longitudinal fibre tracts after EVs-treatment compared to the vehicle, which correlated with higher degree of white matter sparing. Our observation demonstrates that acute intralesional EV-application is particularly potent to address the processes of inflammation and scarring and to promote locomotion recovery. Moreover, this improvement of the spinal microenvironment and the higher structural preservation obtained with EVs-treatment constitute valuable assets for follow-up pro-regenerative interventions.

Pubmed:
35046776: Romanelli P, Bieler L, Heimel P, Škokić S, Jakubecova D, Kreutzer C, Zaunmair P, Smolčić T, Benedetti B, Rohde E, Gimona M, Hercher D, Dobrivojević Radmilović M, Couillard-Despres S
Enhancing Functional Recovery Through Intralesional Application of Extracellular Vesicles in a Rat Model of Traumatic Spinal Cord Injury.
Local inflammation plays a pivotal role in the process of secondary damage after spinal cord injury. We recently reported that acute intravenous application of extracellular vesicles (EVs) secreted by human umbilical cord mesenchymal stromal cells dampens the induction of inflammatory processes following traumatic spinal cord injury. However, systemic application of EVs is associated with delayed delivery to the site of injury and the necessity for high doses to reach therapeutic levels locally. To resolve these two constraints, we injected EVs directly at the lesion site acutely after spinal cord injury. We report here that intralesional application of EVs resulted in a more robust improvement of motor recovery, assessed with the BBB score and sub-score, as compared to the intravenous delivery. Moreover, the intralesional application was more potent in reducing inflammation and scarring after spinal cord injury than intravenous administration. Hence, the development of EV-based therapy for spinal cord injury should aim at an early application of vesicles close to the lesion.
Front Cell Neurosci, 2021; 15
34542287: Urmann C, Bieler L, Priglinger E, Aigner L, Couillard-Despres S, Riepl HM

Neuroregenerative Potential of Prenyl- and Pyranochalcones: A Structure-Activity Study.
Loss of neuronal tissue is a hallmark of age-related neurodegenerative diseases. Since adult neurogenesis has been confirmed in the human brain, great interest has arisen in substances stimulating the endogenous neuronal regeneration mechanism based on adult neural stem cells. Medicinal plants are a valuable source of neuroactive small molecules. In the structure-activity study presented here, the activities of prenyl- and pyranochalcones were compared to each other, using a differentiation assay based on the doublecortin promoter sequences. The latter revealed that the pyrano ring is a crucial structural element for the induction of neuronal differentiation of adult neural stem cells, while compounds with a prenyl group show significantly lower activities. Furthermore, a decrease of pro-differentiation activity was observed following structural modifications, such as substitutions on the pyrano ring and on the B-ring of the chalcone. We also initiated the elucidation of the structural characteristics of the newly discovered lead substance xanthohumol C, which correlated with the activation of the doublecortin promoter during neuronal differentiation.
J Nat Prod, 2021; 84
33915732: Locker F, Bieler L, Nowack LMF, Leitner J, Brunner SM, Zaunmair P, Kofler B, Couillard-Despres S
Involvement of Neuropeptide Galanin Receptors 2 and 3 in Learning, Memory and Anxiety in Aging Mice.
The neuropeptide galanin (GAL), which is expressed in limbic brain structures, has a strong impact on the regulation of mood and behavior. GAL exerts its effects via three G protein-coupled receptors (GAL-R). Little is known about the effects of aging and loss of GAL-Rs on hippocampal-mediated processes connected to neurogenesis, such as learning, memory recall and anxiety, and cell proliferation and survival in the dorsal dentate gyrus (dDG) in mice. Our results demonstrate that loss of -R, but not -R, slowed learning and induced anxiety in older (12-14-month-old) mice. Lack of -R increased cell survival (BrdU incorporation) in the dDG of young mice. However, normal neurogenesis was observed in vitro using neural stem and precursor cells obtained from -R and -R knockouts upon GAL treatment. Interestingly, we found sub-strain differences between C57BL/6J and C57BL/6N mice, the latter showing faster learning, less anxiety and lower cell survival in the dDG. We conclude that GAL-R signaling is involved in cognitive functions and can modulate the survival of cells in the neurogenic niche, which might lead to new therapeutic applications. Furthermore, we observed that the mouse sub-strain had a profound impact on the behavioral parameters analyzed and should therefore be carefully considered in future studies.
Molecules, 2021; 26
33593735: Timotius IK, Bieler L, Couillard-Despres S, Sandner B, Garcia-Ovejero D, Labombarda F, Estrada V, Müller HW, Winkler J, Klucken J, Eskofier B, Weidner N, Puttagunta R
Combination of Defined CatWalk Gait Parameters for Predictive Locomotion Recovery in Experimental Spinal Cord Injury Rat Models.
In many preclinical spinal cord injury (SCI) studies, assessment of locomotion recovery is key to understanding the effectiveness of the experimental intervention. In such rat SCI studies, the most basic locomotor recovery scoring system is a subjective observation of animals freely roaming in an open field, the Basso Beattie Bresnahan (BBB) score. In comparison, CatWalk is an automated gait analysis system, providing further parameter specifications. Although together the CatWalk parameters encompass gait, studies consistently report single parameters, which differ in significance from other behavioral assessments. Therefore, we believe no single parameter produced by the CatWalk can represent the fully-coordinated motion of gait. Typically, other locomotor assessments, such as the BBB score, combine several locomotor characteristics into a representative score. For this reason, we ranked the most distinctive CatWalk parameters between uninjured and SC injured rats. Subsequently, we combined nine of the topmost parameters into an SCI gait index score based on linear discriminant analysis (LDA). The resulting combination was applied to assess gait recovery in SCI experiments comprising of three thoracic contusions, a thoracic dorsal hemisection, and a cervical dorsal column lesion model. For thoracic lesions, our unbiased machine learning model revealed gait differences in lesion type and severity. In some instances, our LDA was found to be more sensitive in differentiating recovery than the BBB score alone. We believe the newly developed gait parameter combination presented here should be used in CatWalk gait recovery work with preclinical thoracic rat SCI models.
eNeuro, 2021 Mar-Apr; 8
31849808: Romanelli P, Bieler L, Scharler C, Pachler K, Kreutzer C, Zaunmair P, Jakubecova D, Mrowetz H, Benedetti B, Rivera FJ, Aigner L, Rohde E, Gimona M, Strunk D, Couillard-Despres S
Extracellular Vesicles Can Deliver Anti-inflammatory and Anti-scarring Activities of Mesenchymal Stromal Cells After Spinal Cord Injury.
Spinal cord injury is characterized by initial neural tissue disruption that triggers secondary damage and extensive non-resolving inflammation, which aggravates loss of function and hinders recovery. The early onset of inflammation following traumatic spinal cord injury underscores the importance of acute intervention after the initial trauma. Injections of mesenchymal stromal cells (MSCs) can reduce inflammation following spinal cord injury. We asked if extracellular vesicles (EVs) can substitute the anti-inflammatory and anti-scarring activities of their parental MSCs in a rat model of contusion spinal cord injury. We report that MSC-EVs were as potent as the parental intact cells in reducing the level of neuroinflammation for

up to 2 weeks post-injury. Acute application of EVs after spinal cord injury was shown to robustly decrease the expression of pro-inflammatory cytokines in the spinal cord parenchyma in the very early phase of secondary damage. Moreover, the anti-scarring impact of MSC-EVs was even more efficient than the parental cells. We therefore conclude that anti-inflammatory and anti-scarring activities of MSC application can be mediated by their secreted EVs. In light of their substantial safety and druggability advantages, EVs may have a high potential in early therapeutic treatment following traumatic spinal cord injury.
Front Neurol, 2019; 10
31610603: Kirchinger M, Bieler L, Tevini J, Vogl M, Haschke-Becher E, Felder TK, Couillard-Després S, Riepl H, Urmann C
Development and Characterization of the Neuroregenerative Xanthohumol C/Hydroxypropyl-β-cyclodextrin Complex Suitable for Parenteral Administration.
The chroman-like chalcone Xanthohumol C, originally found in hops, was demonstrated to be a potent neuroregenerative and neuroprotective natural product and therefore constitutes a strong candidate for further pharmaceutical research. The bottleneck for experiments is the low water solubility of this chalcone. Consequently, we developed and validated a suitable formulation enabling administration. Cyclodextrins were used as water-soluble and nontoxic complexing agents, and the complex of Xanthohumol C and 2-hydroxypropyl--cyclodextrin was characterized using HPLC, HPLC-MS, NMR, and differential scanning calorimetry. The water solubility of Xanthohumol C increases with increasing concentrations of cyclodextrin. Using 50 mM 2-hydroxypropyl--cyclodextrin, solubility was increased 650-fold. Furthermore, bioactivity of Xanthohumol C in free and complexed form did not significantly differ, suggesting the release of Xanthohumol C from 2-hydroxypropyl--cyclodextrin. Finally, a small-scaled experiment in a rat model showed that after i.p. administration of the complex, Xanthohumol C can be detected in serum, the brain, and the cerebrospinal fluid at 1 and 6 h post-administration. Mean (± SD) Xanthohumol C serum concentrations after 1, 6, and 12 h were determined as 463.5 (± 120.9), 61.9 (± 13.4), and 9.3 (± 0.8) ng/mL upon i.v., and 294.3 (± 22.4), 45.5 (± 0.7), and 13 (± 1.0) ng/mL after i.p. application, respectively. Accordingly, the formulation of Xanthohumol C/2-hydroxypropyl--cyclodextrin is suitable for further experiments and further pharmaceutical research aiming for the determination of its neuroregenerative potential in animal disease models.
Planta Med, 2019; 85
31396054: Bieler L, Vogl M, Kirchinger M, Urmann C, Riepl H, Bandtlow C, Klimaschewski L, Aigner L, Couillard-Despres S
The Prenylflavonoid ENDF1 Overrules Central Nervous System Growth Inhibitors and Facilitates Regeneration of DRG Neurons.
Restoration of neuronal connectivity after lesion of the central nervous system, such as spinal cord injury, is one of the biggest challenges in modern medicine. In particular, the accumulation of axon growth inhibitory factors at the site of injury constitutes a major obstacle to structural and thus functional repair. We previously investigated a group of prenylflavonoids derived from hops for their capacity to promote neuroregeneration. We identified a molecule called ENDF1 that was very potent to enhance regrowth and branching of neurites from dorsal root ganglion neurons in culture on growth promoting substrates. In the present study, we investigated ENDF1's capacity to promote regeneration of rat dorsal root ganglion neurons in the presence of three main components of the extracellular matrix acting as axon growth inhibitors: Semaphorin 3A, Ephrin A4 and mixed chondroitin sulfate proteoglycans. We report that ENDF1 application significantly promoted the percentages of sensory neurons able to regrow their neurites regardless of the presence of those inhibitors, and this to an extent similar to the one obtained after NGF treatment. Moreover, ENDF1 strongly enhanced the total neurite length and the complexity of neurites extending from neurons challenged with axon growth inhibitors. Although the impact of NGF and ENDF1 on the regeneration of neurons was similar, the activity of ENDF1 was not mediated by signaling through the TrkA receptor, indicating that each molecule act through different signaling pathways. In addition, ENDF1 did not decrease the phosphorylation of cofilin, a downstream effector of the regeneration-associated RhoA/ROCK signaling pathway. Hence, ENDF1 is a potent pro-neuroregenerative factors that could help in identifying new efficient targets for regenerative therapies of the nervous system.
Front Cell Neurosci, 2019; 13
29560455: Bieler L, Grassner L, Zaunmair P, Kreutzer C, Lampe L, Trinka E, Marschallinger J, Aigner L, Couillard-Despres S
Motor deficits following dorsal corticospinal tract transection in rats: voluntary versus skilled locomotion readouts.
Following spinal cord injury, severe deficits result from damages to ascending and descending tracts, such as the corticospinal tract (CST) which is highly relevant for the motor execution in humans. Unfortunately, no curative treatment is available and intensive efforts are deployed in animal models, such as the CST transection model, to identify interventions providing functional regeneration after spinal cord injury. The CatWalk XT is a system for multi-parameter gait analysis of voluntary locomotion. In this study, the performance of the CatWalk XT for monitoring of functional deficits associated with dorsal CST lesion in rats was compared to skilled locomotion tests. Motor deficits associated with dorsal CST transection could be reliably monitored over seven weeks based on skilled locomotion testing, i.e. Horizontal Ladder Walk and Grid Walk. The collateral lesion to the overlaying gracile and cuneate funiculi occurring during dorsal CST transection resulted in slight hyposensitivity and proprioceptive deficit, which likely contributed to the lowered performance in skilled locomotion. In

contrast, parameters of voluntary locomotion were not significantly affected by dorsal CST transection. Finally, an abnormal adduction reflex was detected immediately after lesion of the CST and could be conveniently used to confirm successful CST lesion in rats of experimental groups. The functional relevance of the dorsal CST in locomotion of rats is not as prominent as compared to in humans and thus challenging the motor execution is mandatory to reliably investigate CST function. A detailed analysis of voluntary walking using the CatWalk XT is not adequate to detect deficits following dorsal CST lesion in rats.
Heliyon, 2018; 4

28834740: De La Fuente AG, Lange S, Silva ME, Gonzalez GA, Tempfer H, van Wijngaarden P, Zhao C, Di Canio L, Trost A, Bieler L, Zaunmair P, Rotheneichner P, O'Sullivan A, Couillard-Despres S, Errea O, Mäe MA, Andrae J, He L, Keller A, Bátiz LF, Betsholtz C, Aigner L, Franklin RJM, Rivera FJ
Pericytes Stimulate Oligodendrocyte Progenitor Cell Differentiation during CNS Remyelination.
The role of the neurovascular niche in CNS myelin regeneration is incompletely understood. Here, we show that, upon demyelination, CNS-resident pericytes (PCs) proliferate, and parenchymal non-vessel-associated PC-like cells (PLCs) rapidly develop. During remyelination, mature oligodendrocytes were found in close proximity to PCs. In Pdgfb mice, which have reduced PC numbers, oligodendrocyte progenitor cell (OPC) differentiation was delayed, although remyelination proceeded to completion. PC-conditioned medium accelerated and enhanced OPC differentiation in vitro and increased the rate of remyelination in an ex vivo cerebellar slice model of demyelination. We identified Lama2 as a PC-derived factor that promotes OPC differentiation. Thus, the functional role of PCs is not restricted to vascular homeostasis but includes the modulation of adult CNS progenitor cells involved in regeneration.
Cell Rep, 2017; 20

26903808: Kaser-Eichberger A, Schroedl F, Bieler L, Trost A, Bogner B, Runge C, Tempfer H, Zaunmair P, Kreutzer C, Traweger A, Reitsamer HA, Couillard-Despres S
Expression of Lymphatic Markers in the Adult Rat Spinal Cord.
Under physiological conditions, lymphatic vessels are thought to be absent from the central nervous system (CNS), although they are widely distributed within the rest of the body. Recent work in the eye, i.e., another organ regarded as alymphatic, revealed numerous cells expressing lymphatic markers. As the latter can be involved in the response to pathological conditions, we addressed the presence of cells expressing lymphatic markers within the spinal cord by immunohistochemistry. Spinal cord of young adult Fisher rats was scrutinized for the co-expression of the lymphatic markers PROX1 and LYVE-1 with the cell type markers Iba1, CD68, PGP9.5, OLIG2. Rat skin served as positive control for the lymphatic markers. PROX1-immunoreactivity was detected in many nuclei throughout the spinal cord white and gray matter. These nuclei showed no association with LYVE-1. Expression of LYVE-1 could only be detected in cells at the spinal cord surface and in cells closely associated with blood vessels. These cells were found to co-express Iba1, a macrophage and microglia marker. Further, double labeling experiments using CD68, another marker found in microglia and macrophages, also displayed co-localization in the Iba1+ cells located at the spinal cord surface and those apposed to blood vessels. On the other hand, PROX1-expressing cells found in the parenchyma were lacking Iba1 or PGP9.5, but a significant fraction of those cells showed co-expression of the oligodendrocyte lineage marker OLIG2. Intriguingly, following spinal cord injury, LYVE-1-expressing cells assembled and reorganized into putative pre-vessel structures. As expected, the rat skin used as positive controls revealed classical lymphatic vessels, displaying PROX1+ nuclei surrounded by LYVE-1-immunoreactivity. Classical lymphatics were not detected in adult rat spinal cord. Nevertheless, numerous cells expressing either LYVE-1 or PROX1 were identified. Based on their localization and overlapping expression with Iba1, the LYVE-1+ cell population likely represents a macrophage subpopulation, while a significant fraction of PROX1+ cells belong to the oligodendrocytic lineage based on their distribution and the expression of OLIG2. The response of these LYVE-1+ and PROX1+ cell subpopulations to pathological conditions, especially in spinal cord inflammatory conditions, needs to be further elucidated.
Front Cell Neurosci, 2016; 10

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BOARD NUMBER: S04-343

RESTORATION OF ER PROTEOSTASIS AUGMENTS THE AUTOPHAGIC FLUX AND MITIGATES REMOTE DEGENERATION AFTER SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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The pathogenic mechanisms underlying the progression of remote degeneration after axonal damage are not fully understood. In this study, we aimed to assess the relationship between endoplasmic reticulum (ER) stress and autophagy in remote degeneration after axonal damage due to spinal cord injury (SCI). Starting from 1 day after SCI, significant increases in markers of ER stress (activating transcription factor 4 (ATF4), glucose-regulated protein 78 (GRP78/BiP) and C/EBP homologous protein (CHOP) were found in remote axotomized neurons compared with sham-lesioned animals. In SCI animals' ER stress-mediated signaling was parallel to the blockade of the autophagic flux assessed by increase in protein levels of SQSTM1/p62, microtubule-associated protein 2 light chain 3 (LC3-II), and decrease of the lysosomal marker LAMP1, suggesting an accumulation of dysfunctional autophagosomes in axotomized neurons. Pharmacological modulation of UPR by Guanabenz significantly modulated ER stress responses and elicits autophagy machinery homeostasis in remote axotomized neurons, consistent with the detrimental effects of persistent ER stress on the autophagic flux and, consequently, on remote degeneration. These effects correlate with an increased activation of TFEB —the master regulator of autophagy/lysosomes biogenesis— and the halt of neuronal cell death as well as with an improved functional recovery. Thus, our results suggest that therapies aimed to restore ER proteostasis might attenuate the progression of remote degeneration after spinal cord injury

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BOARD NUMBER: S04-344

CENTRAL VERSUS PERIPHERAL NERVOUS INJURIES: WHAT ARE THE TRANSCRIPTOMIC DIFFERENCES AND SIMILARITIES, 24 HOURS AFTER LESION?

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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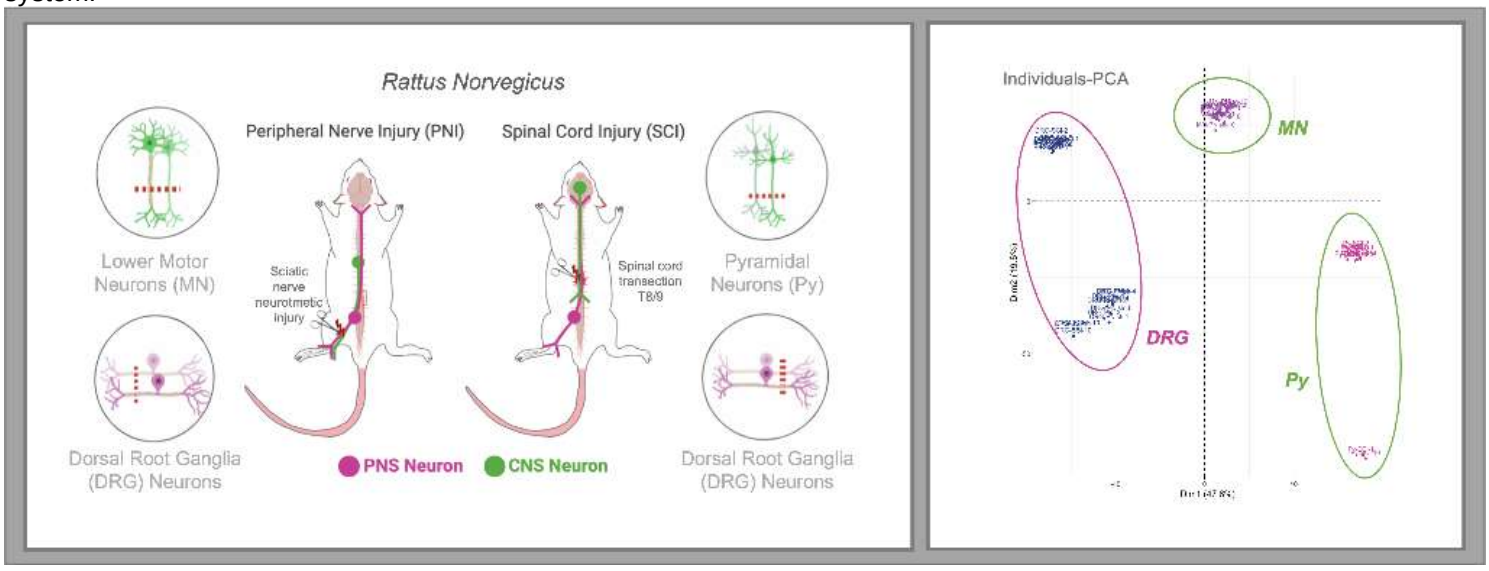
[1]SPARK Lab on Cell Differentiation and Regeneration, iBiMED - instituto de biomedicina da universidade de aveiro, Department Of Medical Sciences, Aveiro, Portugal, [2]Molecular Neurobiology & Neuroregeneration, University-Hospital Düsseldorf Laboratory, Heinrich-Heine-University, Department Of Neurology, Düsseldorf, Germany, [3]iBiGen – Genomics Facility, iBiMED - instituto de biomedicina da universidade de aveiro, Department Of Medical Sciences, Aveiro, Portugal, [4]The Discovery CTR, Universidade De Aveiro, Aveiro, Portugal

Aims: High-throughput screenings have profiled spinal cord or sciatic nerve tissue responses after lesion, to pave the discovery of new regeneration-associated genes. However, it is difficult to depict the neuronal-specific transcriptional profile from the entire tissue response. In the first hours after injury, alterations in the early transcriptome of the affected neuronal populations may trigger relevant regenerative processes. **Methods:** Rat models of Peripheral Nerve Injury (PNI) and Spinal Cord Injury (SCI), were used. Neuronal bodies of Dorsal Root Ganglia (DRG), Lower Motor Neurons (MN) and Pyramidal Neurons from Cortex Layer V (Py) were collected 24 hours after lesion, using Laser Capture Microdissection. Upon mRNA sequencing, differential expression and gene ontology analyses were performed to identify highly relevant transcripts. The 4 lists of differentially expressed genes were compared to unveil the differences and similarities between cell types and injury locations. **Results:** Principal Component Analysis (PCA) of the sequencing data shows that dimension 1 separates samples by cell-type. Py cells exhibited few transcriptional alterations at an early time-point after injury. Further, injury-induced mRNA regulation was more significant in DRG neurons after PNI (regenerative) than after SCI (non-regenerative), and also more robust than the MNs, after PNI. **Conclusions:** We observed transcriptomic differences in the same injured cell-type, depending on if it was affected its peripheral or central axonal branch. This is highly relevant to understand what is different and similar in the regeneration processes between peripheral and central nervous system.



Pubmed:
30714554: Martins SA, Correia PD, Dias RA, da Cruz E Silva OAB, Vieira SI
CD81 Promotes a Migratory Phenotype in Neuronal-Like Cells.

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9-13 July 2022 | Paris, France

Tetraspanins, such as CD81, can form lateral associations with each other and with other transmembrane proteins. These interactions may underlie CD81 functions in multiple cellular processes, such as adhesion, morphology, migration, and differentiation. Since CD81's role in neuronal cells' migration has not been established, we here evaluated effects of CD81 on the migratory phenotype of SH-SY5Y neuroblastoma cells. CD81 was found enriched at SH-SY5Y cell's membrane, co-localizing with its interactor filamentous-actin (F-actin) in migratory relevant structures of the leading edge (filopodia, stress fibers, and adhesion sites). CD81 overexpression increased the number of cells with a migratory phenotype, in a potentially phosphatidylinositol 3 kinase (PI3K)-Ak strain transforming (AKT) mediated manner. Indeed, CD81 also co-localized with AKT, a CD81-interactor and actin remodeling agent, at the inner leaflet of the plasma membrane. Pharmacologic inhibition of PI3K, the canonical AKT activator, led both to a decrease in the acquisition of a migratory phenotype and to a redistribution of intracellular CD81 and F-actin into cytoplasmic agglomerates. These findings suggest that in neuronal-like cells CD81 bridges active AKT and actin, promoting the actin remodeling that leads to a motile cell morphology. Further studies on this CD81-mediated mechanism will improve our knowledge on important physiological and pathological processes such as cell migration and differentiation, and tumor metastasis.
Microsc Microanal, 2019; 25

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THE ROLE OF MESENCHYMAL STEM CELLS (MSCS) IN SPINE CORD INJURIES: A SYSTEMATIC REVIEW OF THE LITERATURE

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Objectives Spinal cord injury (SCI) is a devastating condition that leaves patients with permanent neurological deficit as a result of biochemical impairment in neurogenic repair in the central nervous system (CNS). Mesenchymal stem cells (MSCs) have been utilised in regenerative medicine and are shown to differentiate into several lineages including neurons and has implications for potential strategies to combat challenges of CNS repair. **Design**: A systematic review was performed to assess MSCs at a basic science level and to identify further challenges with MSC use in SCI. A PRISM methodology using PubMed, Ovid, EMBASE and MEDLINE to assess available studies looking into the role of MSCs in human neuronal tissue was utilised. Two independent reviewers identified relevant studies. A third senior reviewer was utilised if any discrepancies between reviewers were identified. **Subjects and Methods** We reported the ability of neuronal cell differentiation, capacity to form scaffolding to bridge damaged tissue and capacity for functional return from studies up to November 2021. Exclusion criteria included studies reporting animal tissue, and non-English studies. **Results** This review has identified the role of MSCs in neuronal repair, scaffolding for creation of new neural networks along with paracrine and autocrine effects on neighbouring glial cells. These have proved exciting areas of research and are providing understanding of the role of these MSCs in regeneration. **Conclusion**: By reporting these studies, we highlight and guide readers from our study about future potential questions, challenges and current concepts to help steer future research within this vastly expanding field.

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BOARD NUMBER: S04-346

THE PORCINE CORTICAL MOTOR SYSTEM: ANATOMY AND RESPONSE TO SPINAL CORD INJURY (SCI).

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Despite the increasing use of pig as experimental model for neurological diseases, the anatomy of the porcine cortical motor system and its response to injury is mostly unknown. We studied the origin and termination of axons projecting from the cerebral cortex to the brainstem and spinal cord in domestic pigs, in the uninjured state and after a cervical spinal cord hemisection. Two cortical regions, namely the primary motor cortex (M1) and the premotor cortex (PM), sent numerous spinal projections. Anterograde axonal labelling with Alexa-conjugated dextrans showed a grossly similar distribution of corticospinal axons from both cortical areas in the spinal white matter funiculi and gray matter laminae, with some similarities to primates and humans. However, the corticospinal tract (CST) was limited to the cervical and mid-thoracic spinal cord, without relevant innervation of spinal motoneurons. At the brainstem, most corticorubral projections arose from M1, whereas other regions received axonal contacts from both M1 and PM. This general pattern of connectivity was unmodified after the cervical spinal cord hemisection. The lesion eliminated most corticospinal innervation to the caudal, ipsilateral spinal cord, but preserved the possibility of reactive growth from axons descending through the uninjured side and decussating through the spinal commissures. Our data support the usefulness of pigs as a translational model for SCI and other diseases involving the cortical motor system. However, the absence of corticomotoneuronal innervation, as well as the limited extension of CST axons along the spinal cord, must be considered for experiment planning and interpretation of the results.

Pubmed:
34790101: Del Cerro P, Rodríguez-De-Lope Á, Collazos-Castro JE
The Cortical Motor System in the Domestic Pig: Origin and Termination of the Corticospinal Tract and Cortico-Brainstem Projections.
The anatomy of the cortical motor system and its relationship to motor repertoire in artiodactyls is for the most part unknown. We studied the origin and termination of the corticospinal tract (CST) and cortico-brainstem projections in domestic pigs. Pyramidal neurons were retrogradely labeled by injecting aminostilbamidine in the spinal segment C1. After identifying the dual origin of the porcine CST in the primary motor cortex (M1) and premotor cortex (PM), the axons descending from those regions to the spinal cord and brainstem were anterogradely labeled by unilateral injections of dextran alexa-594 in M1 and dextran alexa-488 in PM. Numerous corticospinal projections from M1 and PM were detected up to T6 spinal segment and showed a similar pattern of decussation and distribution in the white matter funiculi and the gray matter laminae. They terminated mostly on dendrites of the lateral intermediate laminae and the internal basilar nucleus, and some innervated the ventromedial laminae, but were essentially absent in lateral laminae IX. Corticofugal axons terminated predominantly ipsilaterally in the midbrain and bilaterally in the medulla oblongata. Most corticorubral projections arose from M1, whereas the mesencephalic reticular formation, superior colliculus, lateral reticular nucleus, gigantocellular reticular nucleus, and raphe received abundant axonal contacts from both M1 and PM. Our data suggest that the porcine cortical motor system has some common features with that of primates and humans and may control posture and movement through parallel motor descending pathways. However, less cortical regions project to the spinal cord in pigs, and the CST neither seems to reach the lumbar enlargement nor to have a significant direct innervation of cervical, foreleg motoneurons.
Front Neuroanat, 2021; 15
34121450: Cerro PD, Barriga-Martín A, Vara H, Romero-Muñoz LM, Rodríguez-De-Lope Á, Collazos-Castro JE
Neuropathological and Motor Impairments after Incomplete Cervical Spinal Cord Injury in Pigs.
Humans, primates, and rodents with cervical spinal cord injury (SCI) show permanent sensorimotor dysfunction of the upper/forelimb as consequence of axonal damage and local neuronal death. This work aimed at characterizing a model of cervical SCI in domestic pigs in which hemisection with excision of 1 cm of spinal cord was performed to reproduce the loss of neural tissue observed in human neuropathology. Posture and motor control were assessed over 3 months by scales and kinematics of treadmill locomotion. Histological measurements included lesion length, atrophy of the adjacent spinal cord

segments, and neuronal death. In some animals, the retrograde neural tracer aminostilbamidine was injected in segments caudal to the lesion to visualize propriospinal projection neurons. Neuronal loss extended for 4-6 mm from the lesion borders and was more severe in the ipsilateral, caudal spinal cord stump. Axonal Wallerian degeneration was observed caudally and rostrally, associated with marked atrophy of the white matter in the spinal cord segments adjacent to the lesion. The pigs showed chronic monoplegia or severe monoparesis of the foreleg ipsilateral to the lesion, whereas the trunk and the other legs had postural and motor impairments that substantially improved during the first month post-lesion. Adaptations of the walking cycle such as those reported for rats and humans ameliorated the negative impact of focal neurological deficits on locomotor performance. These results provide a baseline of behavior and histology in a porcine model of cervical spinal cord hemisection that can be used for translational research in SCI therapeutics.
J Neurotrauma, 2021; 38
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Activation of the Cannabinoid Type 2 Receptor by a Novel Indazole Derivative Normalizes the Survival Pattern of Lymphoblasts from Patients with Late-Onset Alzheimer's Disease.
Alzheimer's disease is a multifactorial disorder for which there is no disease-modifying treatment yet. CB2 receptors have emerged as a promising therapeutic target for Alzheimer's disease because they are expressed in neuronal and glial cells and their activation has no psychoactive effects.
CNS Drugs, 2018; 32

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SPONTANEOUS REGENERATION OF CHOLECYSTOKINERGIC DESCENDING AXONS AFTER A COMPLETE SPINAL CORD INJURY IN LAMPREYS

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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In contrast to humans, lampreys spontaneously recover locomotion after a complete spinal cord injury (SCI) and the process of recovery involves the regeneration of descending axons. Axon regeneration in lampreys has been mainly studied in giant descending neurons of the brainstem. However, the regeneration of neurochemically distinct descending neuronal populations has been much less studied. Cholecystokinin (CCK) is a regulatory neuropeptide found in the brain and spinal cord that modulates several processes such as satiety, anxiety, nociception or locomotion. This neuropeptide shows high evolutionary conservation and is present in all representative species of vertebrates from agnathans to mammals. Recent work in lampreys has shown that all CCK immunoreactive (CCK-ir) spinal cord axons originate in a neuronal population located in the caudal rhombencephalon (Sobrido-Cameán et al., 2020). Here, we used immunohistochemical methods to quantify the regenerative capacity of descending CCK-ir axons of larval sea lampreys after a complete SCI at the level of the fifth gill. The degree of regeneration of CCK-ir axons was analysed at the level of the sixth gill at 10 weeks post-lesion (wpl), when behavioural analyses showed that injured animals had recovered normal appearing locomotion. The quantification of CCK-ir profiles revealed that, at 10 wpl, larvae recovered 80% of the CCK-ir innervation present at the level of sixth gill in control un-injured animals. These results indicate that descending CCKergic neurons can be used as a model to study spontaneous axonal regeneration by means of immunofluorescence methods in a neurochemically distinct neuronal population after SCI in lampreys.

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THE REGENERATIVE ABILITY OF CORTICOSPINAL NEURONS IS LOST IN A SEGMENTALLY-DISTINCT MANNER

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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The neonatal central nervous system (CNS) is known to support long-distance axon regeneration better than the adult CNS after injury. However, the precise time course of this regenerative decline through development has not been investigated to date. We investigated the long-distance regenerative ability of the corticospinal tract (CST) during development. For this, we developed a novel microsurgical approach to axotomize the developing CST, while leaving the spinal environment largely unperturbed. Using this approach, we have identified rather surprisingly that long-distance CST regenerative ability is lost at distinct times at distinct spinal levels. For example, at postnatal day 4 (P4) while the CST is still growing in the lumbar cord, the regenerative ability is robustly maintained at thoracic T11, partially maintained at thoracic T2, but completely abolished at cervical C2. This differential loss of regenerative ability is not due to differences in astrogliosis at these distinct spinal levels. Using intersectional mouse genetics, we further identify that these segmental differences are not due to heterogeneity of corticospinal neurons (CSN). Finally, we establish that long-distance regrowth does not require axons to traverse in their “normal” white matter tract; CSN axons can exhibit long-distance growth even in the dorsolateral funiculus, where only a minority of the CST normally traverses. These results indicate that the loss of long-distance regenerative ability is differentially governed, at least in time, across the length of the CST. Further, this work also suggests that distinct approaches will be required to effect CST regeneration after adult injury at distinct spinal levels.

Pubmed:

26464223: Li H, Wong C, Li W, Ruven C, He L, Wu X, Lang BT, Silver J, Wu W
Enhanced regeneration and functional recovery after spinal root avulsion by manipulation of the proteoglycan receptor PTPσ.
Following root avulsion, spinal nerves are physically disconnected from the spinal cord. Severe motoneuron death and inefficient axon regeneration often result in devastating motor dysfunction. Newly formed axons need to extend through inhibitory scar tissue at the CNS-PNS transitional zone before entering into a pro-regenerative peripheral nerve trajectory. CSPGs are dominant suppressors in scar tissue and exert inhibition via neuronal receptors including PTPσ. Previously, a small peptide memetic of the PTPσ wedge region named ISP (Intracellular Sigma Peptide) was generated, and its capabilities to target PTPσ and relieve CSPG inhibition were validated. Here, we demonstrate that after ventral root avulsion and immediate re-implantation, modulation of PTPσ by systemic delivery of ISP remarkably enhanced regeneration. ISP treatment reduced motoneuron death, increased the number of axons regenerating across scar tissue, rebuilt healthy neuromuscular junctions and enhanced motor functional recovery. Our study shows that modulation of PTPσ is a potential therapeutic strategy for root avulsion.
Sci Rep, 2015; 5
29420729: Ruven C, Badea SR, Wong WM, Wu W
Combination Treatment With Exogenous GDNF and Fetal Spinal Cord Cells Results in Better Motoneuron Survival and Functional Recovery After Avulsion Injury With Delayed Root Reimplantation.
When spinal roots are torn off from the spinal cord, both the peripheral and central nervous system get damaged. As the motoneurons lose their axons, they start to die rapidly, whereas target muscles atrophy due to the denervation. In this kind of complicated injury, different processes need to be targeted in the search for the best treatment strategy. In this study, we tested glial cell-derived neurotrophic factor (GDNF) treatment and fetal lumbar cell transplantation for their effectiveness to prevent motoneuron death and muscle atrophy after the spinal root avulsion and delayed reimplantation. Application of exogenous GDNF to injured spinal cord greatly prevented the motoneuron death and enhanced the regeneration and axonal sprouting, whereas no effect was seen on the functional recovery. In contrast, cell transplantation into the distal nerve did not affect the host motoneurons but instead mitigated the muscle atrophy. The combination of GDNF and cell graft reunited the positive effects resulting in better functional recovery and could therefore be considered as a promising strategy for nerve and spinal cord injuries that involve the avulsion of spinal roots.
J Neuropathol Exp Neurol, 2018; 77

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Transplantation of Embryonic Spinal Cord Derived Cells Helps to Prevent Muscle Atrophy after Peripheral Nerve Injury.
Injuries to peripheral nerves are frequent in serious traumas and spinal cord injuries. In addition to surgical approaches, other interventions, such as cell transplantation, should be considered to keep the muscles in good condition until the axons regenerate. In this study, E14.5 rat embryonic spinal cord fetal cells and cultured neural progenitor cells from different spinal cord segments were injected into transected musculocutaneous nerve of 200-300 g female Sprague Dawley (SD) rats, and atrophy in biceps brachii was assessed. Both kinds of cells were able to survive, extend their axons towards the muscle and form neuromuscular junctions that were functional in electromyographic studies. As a result, muscle endplates were preserved and atrophy was reduced. Furthermore, we observed that the fetal cells had a better effect in reducing the muscle atrophy compared to the pure neural progenitor cells, whereas lumbar cells were more beneficial compared to thoracic and cervical cells. In addition, fetal lumbar cells were used to supplement six weeks delayed surgical repair after the nerve transection. Cell transplantation helped to preserve the muscle endplates, which in turn lead to earlier functional recovery seen in behavioral test and electromyography. In conclusion, we were able to show that embryonic spinal cord derived cells, especially the lumbar fetal cells, are beneficial in the treatment of peripheral nerve injuries due to their ability to prevent the muscle atrophy.
Int J Mol Sci, 2017; 18
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Spinal root avulsion: an excellent model for studying motoneuron degeneration and regeneration after severe axonal injury.
Neural Regen Res, 2014; 9

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IN VIVO ADMINISTRATION OF METABOLIC PRECURSORS AMELIORATES METABOLIC IMPAIRMENT FOLLOWING SEVERE CONTUSIVE SPINAL CORD INJURY IN A MOUSE MODEL

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Neural tissue has high metabolic requirements. Following spinal cord injury (SCI), the damaged tissue suffers from a severe metabolic impairment, which aggravates axonal degeneration and neuronal loss. Impaired cellular energetic, tricarboxylic acid (TCA) cycle and oxidative phosphorylation metabolism in neuronal cells has been demonstrated to be a major cause of neural tissue death and regeneration failure following SCI. Therefore, rewiring the spinal cord cell metabolism may be an innovative therapeutic strategy for the treatment of SCI. In this study, we evaluated the therapeutic effect of the recovery of oxidative metabolism in a mouse model of severe contusive SCI following oral administration of TCA cycle intermediates, co-factors, essential amino acids, and branched-chain amino acids (α5). The α5 treatment started 3 days post-injury and last for 30 days. At the end of the experiments, animals were sacrificed, and we analyzed the injured spinal cord sections. Specifically, at 14 days post injury, we evaluated whether α5 treatment produced a metabolic modulation of the injured spinal cord through targeted metabolomic analysis. Metabolomic, immunofluorescence and western blot data, revealed that metabolic precursors i) induced TCA and OXPHOS metabolism in spinal cord tissue, ii) increased mTORC1 related proteins (p70S6K, rpS6) in neurons, iii) increased mitochondrial mass and respiration and iv) reduced oxidative stress at the site of the lesion. Lastly, treated animals showed significant, although partial, improvement of the motor functions. Overall, our data demonstrate that rewiring the cellular metabolism may represent an effective strategy to treat SCI.

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SCINFLAM: SERUM CHANGES IN INFLAMMATORY ANALYTES IN SPINAL CORD INJURY PATIENTS

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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[1]University of Aveiro, Institute Of Biomedicine – Ibimed, Department Of Medical Sciences, Aveiro, Portugal, [2]Centro de Reabilitação do Norte - Dr Ferreira Alves (CRN), Centro Hospitalar de Vila Nova de Gaia e Espinho (CHVNG/E) , Spinal Cord Injury Unit, Vila Nova de Gaia, Portugal

Aims. The inflammatory reaction following a traumatic spinal cord injury (SCI) may highly impact the patients' neurological outcome. We aimed to correlate changes in inflammatory analytes from pre- to post-rehabilitation in serum of sub-acute SCI patients, with their neurological impairment and recovery rates. Methods. Different inflammatory cytokines/chemokines were measured in serum collected at pre- and post-rehabilitation from 21 SCI patients, using the LEGENDplex™ Human Inflammation Panel-1. SCI patients were classified using gold-standard scales for impairment (AIS grade), motor and functional independence scores. Absolute analyte levels and their pre- and post-rehabilitation alterations were associated with patients' better or worse prognosis at discharge from the rehabilitation center, i.e. AIS grade, functional independence and motor scores above or below 70-75%, respectively. Results. With rehabilitation, IL-8 and IL-10 tendentially reduce within the worst prognosis group, and IL-8, IL-10 and IL-17A remain steady or increase within the best prognosis group. Accordingly, variations in IL-8, IL-10 and IL-17A significantly correlated with motor scores' variation. TNF-alpha and IL-18 remain unaltered within the worst prognosis group, while increasing within the best prognosis one, and a negative correlation occurs between both analytes' variations and the recovery rates. MCP-1 presented major increases in patients with better functional score, and pre-rehabilitation MCP-1 levels negatively correlate with functional recovery rate. Conclusions. Overall, variations from pre- to post-rehabilitation for each analyte are similar regardless of the patients' division criteria (by AIS grade, motor score or functional independence post-rehabilitation), although significant correlations were only observed for some of these division criteria.

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THE RSK PROTEIN PROMOTE CENTRAL NERVOUS SYSTEM REGENERATION AND FUNCTIONAL RECOVERY VIA TRANSLATIONAL CONTROL.

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

Charlotte Decourt[1], Julia Schaeffer[1], Béatrice Blot[1], Antoine Paccard[1], Blandine Excoffier[1], Mario Pende[2], Homaira Nawabi[1], Stephane Belin[1]
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Central Nervous System (CNS) is not able to regenerate after injury. Any insult in the CNS will lead to circuit disruption and definitive loss of cognitive and motor functions. Major effort has been done to identify molecules that can promote CNS regeneration. However, building de-novo functional circuit after a lesion remains a great challenge, mostly due to the insufficient number of axons reaching their targets. Thus, there is an urge to uncover molecular pathways critical for promoting extensive axon regeneration and functional recovery. Using the unique model of Dorsal Root Ganglia neurons, we found that the p90 S6 ribosomal kinase (RSK) is key for axon regeneration. Indeed, our work demonstrated that RSK expression is required for both sciatic nerve and spinal cord regeneration and its expression controls functional recovery in mice after injury. We characterized its downstream effectors and our results demonstrate that RSK regulates the level of ribosomal S6 phosphorylation and this modification is determinant to promote axon regeneration. Finally, our work will shed the light on specific mRNA translation control by the RSK-RPS6 axis required for axonal regeneration. Altogether, our study characterizes a new RSK-RPS6 axis that controls both peripheral and central nervous system regeneration via the modulation of specific protein translation. Our results will be determinant in the process of understanding axon regeneration in order to develop innovative therapeutic strategies.

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SELECTIVE TRANSLATION CONTROLS AXON REGENERATION IN THE CENTRAL NERVOUS SYSTEM

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

Julia Schaeffer[1], Noémie Vilallongue[1], Béatrice Blot[1], Nacera El Bakdouri[1], Anne-Marie Hesse[2], Charlotte Decourt[1], Elise Plissonnier[1], Blandine Excoffier[1], Antoine Paccard[1], Jean-Jacques Diaz[3], Sandrine Humbert[1], Frédéric Catez[3], Yohann Couté[2], Frédéric Saudou[1], Homaira Nawabi[1], Stephane Belin[1]
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Neuronal circuit formation after adult central nervous system (CNS) injury represents one of the biggest challenges in Neuroscience. Indeed, mature CNS neurons fail to regenerate spontaneously, leading to irreversible loss of sensory-motor and cognitive functions in patients affected by neurodegenerative or traumatic injuries. In the past ten years, major advances in the modulation of neuronal intrinsic properties have unlocked adult axon regeneration. However, regenerative axons fail to make functional circuits, due to lack of sensitivity of some neurons to pathways identified so far and to disorganized axon growth. In this study, we highlight that regulation of protein synthesis from specific mRNAs is a key stone to achieve CNS regeneration. Notably, we show that this process is coordinated by the association of unexpected factors to the ribosome, the functional unit of translation, leading to selective translation of a specific pool of mRNAs that control axon regeneration. Modulation of expression of these translationally-regulated genes favors axon regeneration. Thus, selective protein synthesis stands out as a novel regulatory mechanism in axon regeneration, allowing to coordinate expression of pro-regenerative proteins at the translational level to obtain a functional circuit. Our results will have major implications in the development of therapeutic strategies to trigger CNS regeneration after traumatic injury and in neurodegenerative diseases.

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THE TIMING OF ANGIOTENSIN II RECEPTOR REGULATION AFTER SEVERE SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Many scientific results in recent decades clearly demonstrated the beneficial effects of the AT1 receptor blockade and the AT2 receptor stimulation. The appropriate timing of receptor blockade or stimulation after a specific lesion is one of the crucial factors affecting the efficiency of the therapeutic approach. Therefore, circulating Angiotensin II in plasma, blood pressure, and the expression of both AT1 and AT2 receptors in spinal cord parenchyma were measured within 4 weeks after compression-induced spinal cord injury (40g lasting 15 minutes at Th9 level). Severe spinal cord compression caused a progressive reduction of the AT1 receptors in lesion epicenter and rostrocaudal extent during the first 2 weeks, which correlates with degradation of highly vascularized grey matter. Then a gradual increase to the control values was detected. Although a significant increase in circulating Angiotensin II was observed only during the first 3 days after trauma, an elevated blood pressure persisted until the 10th day. The temporary upregulated expression of the AT2 receptors was detected from two to three post-injury weeks. Surprisingly, their expression a week later was at the level of control values. Based on these results, we can assume that (a) AT1 receptor blockers can be used immediately after severe trauma and administered at least 10 days, and (b) AT2 receptor agonist after 14 days. *Supported by APVV Grant No. APVV-18-0163.*

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PRECLINICAL STUDY OF THE THERAPEUTIC EFFICACY ON FUNCTIONAL RECOVERY OF THE ADIPOSE STROMAL VASCULAR FRACTION AFTER SPINAL CONTUSION IN THE RAT

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal cord injuries lead to functional alteration with important consequences such as motor and sensory disorders. The repair strategies developed to date remain ineffective. The adipose tissue-derived stromal vascular fraction (SVF) is composed of a cocktail of cells with trophic, pro-angiogenic and immunomodulatory effects. Numerous therapeutic benefits were shown for tissue reconstitution, peripheral neuropathy and for the improvement of neurodegenerative diseases. The aim of this study is to evaluate the neuroprotective, neuritogenic and neurotrophic effects of SVF on acute spinal cord contusion in adult rats. A spinal contusion, using a force-controlled impactor, was performed at thoracic level (T_{10}) in adult male Sprague Dawley rats inducing sensory-motor deficits of hindlimbs. A sub-dural injection of NaCl (vehicle) or SVF was performed within 4 to 6 hours after surgery. Sensory-motor recovery of the animals was assessed for 90 days using behavioral tests (BBB test, ladder rung climbing test). At week 12, electrophysiological measurements were recorded. In addition, the spinal cords were collected, and histological and biochemical analyzes were performed at 7, 14, 21, and 90 days post-injury. The preliminary results show an axonal regeneration, a decrease of the inflammation and an increase of the neovascularization around the lesion. Moreover, electrophysiological measurements show i) an adaptation of the ventilation during rhythmic muscle contractions, ii) of the H reflex activity, and iii) an enhancement of the sensory-motor recovery in the SVF group. To conclude, the autograft of SVF could represent an interesting therapeutic strategy to repair spinal cord after contusion and enhance the behavioral recovery.

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DEACTIVATING MAST CELLS AND DELOCALIZING AQUAPORIN-4 AVOID EARLY FORMATION OF DIFFERENT EDEMA SUBTYPES AND PREVENTS SENSORIMOTOR IMPAIRMENTS AFTER SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Edema formation is one of the very first events to occur after spinal cord injury (SCI) leading to an increase of the intrathecal pressure and consequently to serious spinal tissue and functional impairments. Current edema treatments are still symptomatic and/or non-specific. Since edema is mainly described as vasogenic and cytotoxic, it becomes crucial to understand the interplay between these two subtypes. Acting on key targets to inhibit edema formation may reduce secondary damage and related functional impairments. In this study we characterize the edema kinetic after T10 spinal contusion using the Infinite Horizon impactor. We use trifluoperazine (TFP) to block the expression and the functional subcellular localization of aquaporin-4 supposed to be implicated in the cytotoxic edema formation. We also use sodium cromolyn (SC) to deactivate Mast cells degranulation supposed to be implicated in the vasogenic edema formation. The sensorimotor performances were assessed weekly by using the BBB score and the inclined ladder test, then the spinal reflexivity (H-reflex) was evaluated 10 weeks after SCI. The kinetic of the cytokine expression during the edema formation were determined using biochemical assays and the estimation of the tissue loss and the glial scar volumes were estimated using a stereological approach. Our results show a significant reduction of edema after both TFP and SC treatments compared to control. This reduction is correlated with limited sensorimotor impairments. Such results suggest a therapeutic potential of these two compounds and highlight the importance of complementary strategies in edema therapy after SCI.

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RESIDENT NEURAL STEM CELLS GUARANTEE THE REGENERATION PROMOTED BY BULBAR OLFACTORY ENSHEATHING CELL TRANSPLANTATION AFTER SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Both cell transplantation and modulating endogenous neural stem cells have raised great hope for regenerative medicine. Recently, clinical data showed that autologous transplantation of bulbar olfactory ensheathing cells (bOECs) enhanced substantial functional recovery in human. However, the cellular mechanisms behind the effects of transplanted bOECs on the injured spinal cord are still poorly understood, which leads to the difficulty in clinical repetitions. Ependymal cells, the endogenous spinal cord stem cells, are shown essential for tissue restriction and neuronal survival after spinal cord injury (SCI). By using FoxJ1-CreER^{T2}-YFP mice to fate-map ependymal cells, we found that bOECs transplantation enhances the stem cell potential of ependymal cells after SCI both *in vivo* and *in vitro*, and it modulates microenvironment for better axonal regrowth and neuronal survival. Surprisingly, by using FoxJ1-Rasless mice to specifically block ependymal cell proliferation, we no longer observed the regenerative effects promoted by bOECs transplantation. Altogether, our data provide a model that the bOECs transplantation therapy is dependent on the activated ependymal cells for the enrichment of the microenvironment and promoting neuronal survival after SCI.

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EFFECTS OF DORSAL ROOT AVULSION INJURY ON THE SPINAL GANGLIA AND SPINAL CORD

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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High impact vehicle accidents and sport injuries often result in avulsion of the dorsal and ventral roots of the spinal cord. The changes in the ventral horn after ventral root injury are well-known, however, there are only few studies investigating the effect of dorsal root avulsion (DRA). Here we examined the avulsion-induced changes in the affected cell populations of the dorsal root ganglia and spinal cord. The lumbar 4 and 5 (L4-5) dorsal roots were avulsed. Animals were perfused 3, 8, 21 and 90 days after the surgery. The injured and contralateral dorsal root ganglia along with the L4-5 spinal segments were collected. The expression of TrpV1 receptor, CGRP, NF-200kDa and the GSA B4 isolectin was detected. The expression of TrpV-1 increased until day 90. The CGRP expression showed a maximum in the injured ganglia at day 8 while GSA-B4 peaked 21 days after DRA in injured neurons. Significant decrease of NF-200 kDa expression could be found in the affected ganglia and in the ipsilateral gracile tract of the spinal cord 21 days after the injury. The decreased density of CGRP-positive fibers was already significant in the affected dorsal horn as early as 3 days after the injury. In contrast, GSA-B4-positive fibers remained well preserved for at least 3 weeks after the injury. Our data suggest that DRA induces unique expression pattern changes of the investigated markers not only in the injured dorsal root ganglia, but in the contralateral ones, too. Relevant changes appeared in the ipsilateral spinal cord, too.

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TRANSCRIBED MESSENGER RNA – A POTENTIAL THERAPEUTIC PLATFORM FOR SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal cord injury results in irreversible tissue damage followed by limited recovery of function. Interleukin-10 (IL-10) attenuates the effects of pro-inflammatory cytokines and reduces apoptosis. In this study lipid nanoparticle (LNP)-encapsulated human IL-10-encoding nucleoside-modified mRNA (hIL-10 mRNA-LNP) and recombinant hIL-10 loaded via osmotic pump were used to induce neuroprotection and functional recovery following spinal cord contusion injury (at the level of thoracic 10 vertebra) in a rat model. The hIL-10 mRNA LNP or recombinant hIL-10 were administrated 7 days after injury directly into the lesion cavity. The functional analysis showed that hIL-10 in both treatment groups enhanced the coordinated movement relative to controls. Similarly, administration of hIL-10 in both treatment strategies resulted in significantly smaller lesion area at the epicentre of the injury and rescued significantly greater amount of tissue. Analysis of supra and propriospinal connections with the retrograde tracer Fast Blue indicated that hIL-10 treatment enhanced the number of connections between the segments caudal to the lesion and various cranial parts of the CNS. Astrocytes, microglial cells and neurons also expressed hIL-10 protein after hIL-10 mRNA LNP injection up to 5 days in the injured spinal cord. The mRNA treatment induced time-delayed expression of TIMP-1 and CNTF in injured spinal segment. These results demonstrate that the delayed hIL-10 treatment is able to induce morphological and functional improvement after spinal cord contusion. The hIL-10 mRNA LNP provides a simple and controllable new therapeutic approach that is less-invasive than other treatments and does not integrate into the genome.

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THE GENETIC DOWNREGULATION OF CALPAIN 1 REVERTS SPINAL HYPEREXCITABILITY IN A NEONATE MOUSE MODEL OF COMPLETE SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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We previously described a spinal cord injury (SCI) model in neonate rats consisting in a low-thoracic transection. We reported the emergence of spinal hyperexcitability and signs of spasticity 4–5 days post-injury. We related these physiopathological alterations to an excitatory/inhibitory imbalance in spinal motoneurons where calpains were proved to play a primary role (Plantier et al., 2019). **Aims and Methods.** We recently translated our complete SCI model to neonate mice. We first provided model validation by confirming the appearance of spinal network hyperexcitability in isolated spinal cords. We further characterized passive and active features of lumbar motoneurons and unidentified ventral interneurons surrounding the central canal by means of patch clamp recordings. In a subset of SCI mice, we injected intramuscularly the AAV2-retro containing a shRNA against calpain 1. **Results.** We found out that SCI spinal cords showed huge spontaneous busts and hyperreflexia. Lumbar motoneurons were markedly hyperexcitable, being the resting membrane potential (RMP) notably depolarized. Furthermore, the percentage of sublesional motoneurons exhibiting plateau potentials was increased, providing a sustained excitatory drive supporting exaggerated reflex responses. Noteworthy, fictive locomotion bursts spontaneously occurred in SCI preparations, in line with the presence of autorhythmic interneurons spontaneously oscillating at 1.2 mM of extracellular calcium. The genetic downregulation of calpain 1 reduced both network and cellular hyperexcitability in the sublesional spinal cord. **Conclusion.** A general hyperexcitability was shown to take place in the neonate mouse spinal cord after SCI, which was reverted by an innovative gene therapy approach targeting calpain 1.

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BOARD NUMBER: S04-360

ALTERATION OF CEREBROSPINAL FLUID-CONTACTING NEURONS ACTIVITY AFTER A LATERAL CERVICAL SPINAL CORD HEMISECTION

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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The central canal ependymal niche of the spinal cord contains cerebrospinal fluid-contacting neurons (CSF-cNs) which are chemosensory and mechanosensory neurons. Since ependymal cells are known to react to spinal cord injury by triggering reparative processes, we assess whether CSF-cNs may also develop a post-injury (PI) reaction, especially since they appear strategically positioned to sense CSF inflammatory molecules. We analysed therefore the reaction of the CSF-cNs (identified by immunofluorescence by their specific PKD2L1 channel) at various time after lateral cervical C2 injury (1 hour to 30D PI) in mice, notably the expression of the neural activity marker c-Fos. CSF inflammatory protein analysis (IL6, IL1, TNF) shows that spinal injury, but also sham chirurgical intervention, induces a strong transient CSF inflammatory response. At this inflammatory peak (1H and 1day post-treatment), we observed a two folds increase of c-Fos+ CSF-cNs (among 15% in injured and sham mice versus 8% in controls) indicating a CSF-cNs activation. In 1H and 1D PI mice (but not in shams) c-Fos was moreover transiently induced in ependymal cells. By contrast, after chronic injury (7D, 15D and 30D PI), the percent of c-Fos+ CSF-cNs was markedly reduced (3-5% versus 8% in shams), indicating a CSF-cNs inactivation in chronic PI conditions. Altogether our results show that CSF-cNs activity may be altered by spinal injury but also by chirurgical and/or inflammatory stress. CSF-cNs and ependymal cells activation in acute conditions may putatively contribute to plasticity processes, whereas CSF-cNs deactivation after chronic injury may reinforce motor disabilities.

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BOARD NUMBER: S04-361

IMMUNOMODULATOR AND PRO-REGENERATIVE EFFECT OF FIBRIN HYDROGEL AND CARBON MICROFIBERS BASED BIOCOMPATIBLE NEUROPROSTHETIC IMPLANT TO RECONNECT INJURED SPINAL CORD

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Aims: We have recently shown the anti-inflammatory and proregenerative actions of implanted biofunctionalized carbon microfibres (MFs) in a rodent model of spinal cord injury. Based on these promising effects and to potentiate them, we here evaluated the therapeutic potential of a neuroprosthetic implant based on the embedment of several MFs inside a presumably proregenerative fibrin hydrogel matrix. **Methods:** Spinal cord injury was performed on “AMU-Neuroinflam” triple reporter mice to monitor the densities of axons, infiltrated myeloid cells and activated microglial cells by 2P intravital imaging. Density of cell types was precisely measured by automatic computational segmentation and evolution of inflammation and axonal regeneration were characterized in 3 different conditions: treatment with fibrin only, treatment with MFs+fibrin, no treatment. Fibrin bundle was obtained by polymerization of a 1:2 mixture of fibrinogen and thrombin on top of a layered MFs scaffolds. The tailored neuroprosthetic implant was implanted immediately after trauma. **Results**: MFs+fibrin implant boosted the early recruitment of inflammatory cells with an enhanced contribution of monocyte derived Dendritic Cells (moDCs) when compared to fibrin or sham treated lesions. At two weeks, opacification of the transparent fibrin bundles precluded intravital imaging in treated animals. Nevertheless, terminal histology at 3 months demonstrated the significant reduction of chronic inflammation and the enhanced axonal regeneration specifically in MF+fibrin mice. **Conclusions:** Fibrin and carbon appear as a proper combination for the development of biocompatible neuroprosthetic devices to manage spinal cord injury. Moreover, electrical conductivity of carbon is suitable to apply electrotherapeutic strategies to further promote axonal regeneration.

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BOARD NUMBER: S04-362

PRECLINICAL DEVELOPMENT OF A THERAPY FOR CHRONIC TRAUMATIC SPINAL CORD INJURY USING WHARTON'S JELLY MESENCHYMAL STROMAL CELLS: PROOF OF CONCEPT AND REGULATORY COMPLIANCE

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal Cord Injury is a devastating condition resulting in loss of sensory and/or motor function. The use of multipotent Mesenchymal Stromal Cells (MSC) in emerging therapies for the treatment of spinal cord injury may hold the potential to contribute to sensory and/or motor function improvements in the patients. However, the development of cell-based medicines is challenging and a series of preclinical studies addressing quality and safety aspects, in addition to signs of efficacy, need to be performed prior to receiving regulatory approval for clinical testing in humans. Herein we present a series of *in vitro* and *in vivo* studies addressing the characterization of the quality attributes of MSC derived from the Wharton's jelly of the umbilical cord, safety of intrathecal infusion of MSC, and their effect in a rat model of spinal cord injury by controlled impaction after single (at day 7 post-injury) and repeated dose of $1x10^6$ MSC (at days 7 and 14 post-injury). Animals were monitored for 70 days using a broad panel of tests including electrophysiological tests, motor function assessment and histology evaluation. Remarkably, recovery of locomotion was promoted at early time points and safety of repeated doses was demonstrated. The relevance of the data resulting from these studies is discussed in terms of their scientific significance as well as their suitability for being included in the Investigational Medicinal Product Dossier for further consideration by the competent Regulatory Authority.

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BOARD NUMBER: S04-363

BET PROTEIN INHIBITION IN MACROPHAGES ENHANCES DORSAL ROOT GANGLION NEURITE OUTGROWTH IN FEMALE MICE

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Peripheral nerve regeneration is limited after injury, especially in humans, due to the large distance the axons have to grow in the limbs. Currently, there is a lack of effective treatments in the clinics, subjecting patients to prolonged rehabilitation and poor quality of life. Thus, it is relevant to find novel strategies to enhance nerve regeneration after trauma. For instance, this process is highly dependent on the expression of neuroinflammatory factors produced by macrophages and glial cells. Thus, given the importance of BET proteins on inflammation, we aimed to ascertain if BET inhibition may have an effect on axonal outgrowth. BET proteins are epigenetic readers of acetylated lysine residues that recruit transcriptional complexes. We found that although administration of the BET inhibitor JQ1 in nerve crush-injured female mice provided an increase of GAP-43, IL-4 and IL-13 transcription, the treatment did not produce any enhancement of sensory and motor reinnervation. Then, we assessed the effects of JQ1 *ex vivo*. We observed that JQ1 decreased neurite outgrowth of DRG explants in culture, whereas conditioned media from JQ1-treated macrophages increased DRG neurite number and length. Thus, macrophages were responsible to counteract the negative effects of JQ1 *in vivo*, probably by enhancing anti-inflammatory cytokine production. Finally, we identified the activation of STAT6 pathway on DRG explants treated with conditioned medium from JQ1-treated macrophages. To conclude, this study demonstrates that BET inhibition on macrophages enhances axonal outgrowth. However, a specific targeting on macrophages may be needed to increase functional recovery after injury.

Pubmed:
34157407: Palomés-Borrajo G, Badia J, Navarro X, Penas C
Nerve Excitability and Neuropathic Pain is Reduced by BET Protein Inhibition After Spared Nerve Injury.
Neuropathic pain is a common disability produced by enhanced neuronal excitability after nervous system injury. The pathophysiological changes that underlie the generation and maintenance of neuropathic pain require modifications of transcriptional programs. In particular, there is an induction of pro-inflammatory neuromodulators levels, and changes in the expression of ion channels and other factors intervening in the determination of the membrane potential in neuronal cells. We have previously found that inhibition of the BET proteins epigenetic readers reduced neuroinflammation after spinal cord injury. Within the present study we aimed to determine if BET protein inhibition may also affect neuroinflammation after a peripheral nerve injury, and if this would beneficially alter neuronal excitability and neuropathic pain. For this purpose, C57BL/6 female mice underwent spared nerve injury (SNI), and were treated with the BET inhibitor JQ1, or vehicle. Electrophysiological and algesimetry tests were performed on these mice. We also determined the effects of JQ1 treatment after injury on neuroinflammation, and the expression of neuronal components important for the maintenance of axon membrane potential. We found that treatment with JQ1 affected neuronal excitability and mechanical hyperalgesia after SNI in mice. BET protein inhibition regulated cytokine expression and reduced microglial reactivity after injury. In addition, JQ1 treatment altered the expression of SCN3A, SCN9A, KCNA1, KCNQ2, KCNQ3, HCN1 and HCN2 ion channels, as well as the expression of the Na/K ATPase pump subunits. In conclusion, both, alteration of inflammation, and neuronal transcription, could be the responsible epigenetic mechanisms for the reduction of excitability and hyperalgesia observed after BET inhibition. Inhibition of BET proteins is a promising therapy for reducing neuropathic pain after neural injury. PERSPECTIVE: Neuropathic pain is a common disability produced by enhanced neuronal excitability after nervous system injury. The underlying pathophysiological changes require modifications of transcriptional programs. This study notes that inhibition of BET proteins is a promising therapy for reducing neuropathic pain after neural injury.
J Pain, 2021; 22

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BOARD NUMBER: S04-364

INVESTIGATION OF SENSORY NEURON-DERIVED CSF1 AS A POTENTIAL CHEMOATTRACTANT FACTOR FOR MACROPHAGES AFTER PERIPHERAL NERVE INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Aims: Growing evidence suggests that macrophage activation at the dorsal root ganglia (DRG) level and neuron-macrophage communication are essential mechanisms underlying the pathogenesis of peripheral neuropathic pain. The aim of this study was to investigate the expression of colony-stimulating factor 1 (CSF1) and its potential role in macrophage accumulation in the DRG after spared nerve injury (SNI) model of neuropathic pain. **Methods:** CX3CR1-GFP transgenic mice were assigned to 3 groups: Control (animals did not undergo any kind of surgery), Sham (the sciatic nerve was exposed) and SNI (the tibial and common peroneal rami of sciatic nerve were ligated and cut). Seven days after surgery, L3 and L4 DRGs were collected, frozen and cut and DRG sections were double immunostained using antibodies against NF200 or CGRP and against CSF1. **Results:** CSF1 expression is significantly increased in sensory neurons in both L3/L4 DRGs after SNI, but with a higher expression in L4. This pattern is also observed in the macrophage accumulation, with the highest degree of activation in L4, but still with a significant number of activated cells in L3. Moreover, among CSF1(+) neurons, 40% have peri-neuronal rings of macrophages in L4, while in L3 this percentage decreases to 30%. Lastly, 50% of the CSF1(+) neurons co-express NF200, a marker for large, myelinated neurons, while less than 10% of them are small, peptidergic neurons, co-expressing CGRP. **Conclusions:** The pattern of CSF1 expression after SNI is spatially correlated with macrophage accumulation, suggesting its role in attracting macrophages in DRGs after peripheral nerve injury.

Pubmed:
31950314: Gheorghe RO, Deftu A, Filippi A, Grosu A, Bica-Popi M, Chiritoiu M, Chiritoiu G, Munteanu C, Silvestro L, Ristoiu V
Silencing the Cytoskeleton Protein Iba1 (Ionized Calcium Binding Adapter Protein 1) Interferes with BV2 Microglia Functioning.
Iba1 (ionized calcium binding adapter protein 1) is a cytoskeleton protein specific only for microglia and macrophages, where it acts as an actin-cross linking protein. Although frequently regarded as a marker of activation, its involvement in cell migration, membrane ruffling, phagocytosis or in microglia remodeling during immunological surveillance of the brain suggest that Iba1 is not a simple cytoskeleton protein, but a signaling molecule involved in specific signaling pathways. In this study we investigated if Iba1 could also represent a drug target, and tested the hypothesis that its specific silencing with customized Iba1-siRNA can modulate microglia functioning. The results showed that Iba1-silenced BV2 microglia migrate less due to reduced proliferation and cell adhesion, while their phagocytic activity and P2x7 functioning was significantly increased. Our data are the proof of concept that Iba1 protein is a new microglia target, which opens a new therapeutic avenue for modulating microglia behavior.
Cell Mol Neurobiol, 2020; 40

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ANTINOCICEPTIVE EFFECT OF EHRETIA CYMOSA LEAVES IN STREPTOZOTOCIN-INDUCED DIABETIC NEUROPATHY IN SPRAGUE DAWLEY RATS

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Background: *Ehretia cymosa* is used as an antihyperglycemic agent among the Akan and Ewe communities in Ghana. Its anticonvulsant, antioxidant, and analgesic properties make it a potential remedy for painful diabetic neuropathy. This study investigated the role of *Ehretia cymosa leaves* as an antinociceptive agent for streptozotocin-induced type 2 diabetic neuropathy in rats. **Method:** The leaves were pulverized, cold macerated with 70% ethanol and rotary-evaporated to obtain a dry extract, ECE. A single intraperitoneal injection of streptozotocin (STZ) 60 mg/kg and nicotinamide 110 mg/kg induced diabetes in male Sprague Dawley rats. Onset of neuropathy was followed with a 7-day treatment with ECE (30, 100, 300) mg/kg and pregabalin (PGB) (10, 30, 100) mg/kg. Fasting blood sugar (FBS), glycated hemoglobin (HbA1c), IL-6, malondialdehyde (MDA), organ to body weight ratio, liver and kidney function, lipid profile, pancreas, kidney, sciatic nerve and hippocampus were assessed. **Results:** Diabetic rats showed reductions in tail-flick ($p<0.0001$) and paw licking ($p<0.0001$) latencies. Thermal hyperalgesia was attenuated with ECE 30mg/kg on days 3 and 4 ($p<0.05$), and with PGB 100 mg/kg on day 1 ($p<0.01$). PGB 30 mg/kg attenuated tail-flick latency. ECE increased IL-6 at 300mg/kg. Characteristic features of beta cells loss; fiber degeneration; axonopathy; schwann cell proliferation; glomerulosclerosis; and loss of granular and pyramidal cells in the dentate gyrus, and CA3 of the hippocampus were observed. **Conclusion:** The ethanolic extract of *Ehretia cymosa* demonstrated antinociceptive effects in thermal hyperalgesia at 30mg/kg ($p<0.05$) in STZ-induced diabetic rats.

Pubmed:
29754167: Amoateng P, Kukuia KKE, Mensah JA, Osei-Safo D, Adjei S, Eklemet AA, Vinyo EA, Karikari TK
An extract of Synedrella nodiflora (L) Gaertn exhibits antidepressant properties through monoaminergic mechanisms. Synedrella nodiflora (SNE) has been used traditionally for many neurological conditions and some of these neuroactive effects have been scientifically substantiated. The usefulness of SNE in depression has however not been investigated despite the availability of data in other disease models indicating it may be useful. The present study therefore examined the effect of SNE in acute murine models of depression and the possible mechanisms mediating its activities in these models. Preliminary qualitative phytochemical and high performance liquid chromatography (HPLC) screening were conducted on SNE. The behavioural effects of SNE (100, 300 and 1000 mg/kg) pre-treated mice were examined in the forced swimming (FST) and tail suspension (TST) tests. Behavioural events such as mobility (swimming, climbing, curling and climbing), and immobility, were scored. The possible involvement of monoamines in the effects of SNE was assessed in the TST by pre-treating mice with α-methyldopa, reserpine and para-chlorophenylalanine (pCPA) in separate experiments. Flavonoids, tannins, saponins, alkaloids, cardiac glycosides, coumarins, triterpenes, sterols, anthraquinones and phenolic compounds were present in SNE. HPLC analysis revealed the presence of two major constituents observed at retention times 42.56 and 46.51 min, with percentage composition of 45.72% and 36.88% respectively. SNE significantly reduced immobility scores in both FST and TST, suggesting antidepressant effects. The antidepressant properties of SNE were reversed by the pre-treatment of α-methyldopa, reserpine and pCPA, suggesting a possible involvement of monoamines (noradrenaline and serotonin) in its mechanism(s) of actions. SNE exhibits antidepressant effects, possibly mediated through an interplay of enhancement of noradrenergic and serotoninergic mechanisms.
Metab Brain Dis, 2018; 33

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OBESITY-INDUCED LEARNING DEFICITS IN THE FEMALE RAT ARE OESTROUS CYCLE-DEPENDENT AND ARE ASSOCIATED WITH IMPAIRED TRYPTOPHAN METABOLISM THROUGH THE KYNURENINE PATHWAY.

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Background: Obesity has a negative impact on memory affecting males more than females. It is believed that females are protected from cognitive decline by estrogens. The concentration of these hormones, peaks during proestrus and gradually declines through estrus, diestrus and metestrus. Estrogens enhance the expression of tryptophan hydroxylase-2, the rate-limiting enzyme in the transformation of tryptophan (TRP) to serotonin. This neurotransmitter, plays a significant role in learning and memory. However, several learning/memory-regulating compounds arise also from tryptophan metabolism through the kynurenine pathway including xanthurenic acid (XA) and NAD+. **Aim:** The aim of the present study was to determine the involvement of the kynurenine pathway of TRP metabolism in the regulation of learning in control and obese female rats. **Methods:** The learning capabilities of control and obese female rats were evaluated using the Novel Object Recognition test. Tryptophan and TRP-derived metabolites were quantified in the hippocampus and frontal cortex by Ultra-performance liquid chromatography-tandem mass spectrometry. **Results:** Control rats in proestrus/estrus performed better than their control mates in diestrus/metestrus. In contrast, while there were no differences in learning between control and obese rats in diestrus/metestrus, obese rats in proestrus/estrus displayed decreased memory capacity along with reduced production of XA and NAD+ and upregulated expression of mRNAs coding for the enzyme Indoleamine 2,3-dioxygenase, that catalyses the transformation of tryptophan to kynurenine. **Conclusions:** The deleterious effects of obesity on learning are closely linked to the oestrous cycle and are mediated, at least in part, by an impairment of the kynurenine pathway of tryptophan metabolism.

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BRAIN SIZE, GUT SIZE AND COGNITIVE ABILITIES: EXPERIMENTAL EVOLUTION OF ENERGY TRADE-OFFS

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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The enlarged brains of homeotherms bring behavioural advantages, but also incur high energy expenditures. The 'Expensive Brain' (EB) hypothesis posits that the energetic costs of the enlarged brain and the resulting increased cognitive abilities (CA) were met either by increased energy turnover or reduced allocation to other expensive organs, such as the gut. We tested the directionality of the evolutionary relationships between energy expenditures, brain, gut and CA using an experimental evolution model in which we subjected line types of laboratory mice to artificial selection on basal (BMR) or maximum (VO_{2max}) aerobic metabolism - traits that are implicated in evolution of homeothermy, having been pre-requisites for the encephalisation and exceptional CA of mammals, including humans. High-BMR mice had bigger guts, but not brains. Yet, they performed better on the cognitively demanding tasks carried out in both reward and avoidance learning contexts. Furthermore, the high BMR mice had higher neuronal plasticity (indexed as the long-term potentiation, LTP) than their counterparts. Our data indicate that the evolutionary increase of CA in mammals was initially associated with increased BMR and brain plasticity. It was also fueled by an enlarged gut, which was not traded off for brain size.

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MICROGLIA DEPLETION INHIBITS LACTATION BY THE INHIBITION OF PROLACTIN SECRETION IN RODENTS

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Microglia are tissue-resident macrophage-like innate immune cells of the central nervous system. In addition, they also contribute to neuroplastic changes of the central nervous system under physiological circumstances. Since maternal behaviour is accompanied by synaptic changes in mothers' brain, we hypothesized the role of microglia in maternal function. The role of microglia was examined in lactation, and also in the formation of maternal behaviour during pregnancy and the postpartum period. The microglial cells were eliminated from the brain by blocking colony stimulating factor 1 receptor. The intensity of maternal behaviour decreased only if mothers were treated during pregnancy, that is postpartum elimination of microglia was without effect on the maternal behaviour. In contrast, the weight gain of pups was reduced in both protocols even if poster pups were used. Suckling induced serum prolactin levels were measured in the blood obtained via jugular cannula. The level of prolactin in treated mothers was markedly reduced as compared to control animals. The results suggest that microglial cells are required for proper lactation but they are not necessary for the general motility or the postpartum emotional changes. Maternal motivation may be normal in the postpartum absence of microglia. In conclusion, microglia play important roles is specific aspects of the maternal adaptation probably by allowing proper prolactin secretion. The work was supported by the Hungarian National Research, Development and Innovation Office NKFIH-4300-1/2017-NKP_17-00002 (National Brain Program 2.0), and OTKA K134221, and also by the ÚNKP-16-3 New National Excellence Program of the Ministry of Human Capacities" program.

Pubmed:

32472169: Csikós V, Varró P, Bódi V, Oláh S, Világi I, Dobolyi A
The mycotoxin deoxynivalenol activates GABAergic neurons in the reward system and inhibits feeding and maternal behaviours.
Deoxynivalenol (DON) or vomitoxin, is a trichothecene mycotoxin produced mainly by Fusarium graminearum and culmorum. Mycotoxins or secondary metabolic products of mold fungi are micro-pollutants, which may affect human and animal health. The neuronal and behavioural actions of DON were analysed in the present study. To address, which neurons can be affected by DON, the neuronal activation pattern following intraperitoneal injection of DON (1 mg/kg) was investigated in adult male rats and the results were confirmed in mice, too. DON-induced neuronal activation was assessed by c-Fos immunohistochemistry. DON injection resulted in profound c-Fos activation in only the elements of the reward system, such as the accumbens nucleus, the medial prefrontal cortex, and the ventral tegmental area. Further double labelling studies suggested that GABAergic neurons were activated by DON treatment. To study the behavioural relevance of this activation, we examined the effect of DON on feed intake as an example of reward-driven behaviours. Following DON injection, feed consumption was markedly reduced but returned to normal the following day suggesting an inhibitory action of DON on feed intake without forming taste-aversion. To further test how general the effect of DON on goal-directed behaviours is, its actions on maternal behaviour was also examined. Pup retrieval latencies were markedly increased by DON administration, and DON-treated mother rats spent less time with nursing suggesting reduced maternal motivation. In a supplementary control experiment, DON did not induce conditioned place preference arguing against its addictive or aversive actions. The results imply that acute uptake of the mycotoxin DON can influence the reward circuit of the brain and exert inhibitory actions on goal-directed, reward-driven behaviours. In addition, the results also suggest that DON exposure of mothers may have specific implications.
Arch Toxicol, 2020; 94
32612510: Dobolyi A, Oláh S, Keller D, Kumari R, Fazekas EA, Csikós V, Renner É, Cservenák M
Secretion and Function of Pituitary Prolactin in Evolutionary Perspective.
The hypothalamo-pituitary system developed in early vertebrates. Prolactin is an ancient vertebrate hormone released from the pituitary that exerts particularly diverse functions. The purpose of the review is to take a comparative approach in the

description of prolactin, its secretion from pituitary lactotrophs, and hormonal functions. Since the reproductive and osmoregulatory roles of prolactin are best established in a variety of species, these functions are the primary subjects of discussion. Different types of prolactin and prolactin receptors developed during vertebrate evolution, which will be described in this review. The signal transduction of prolactin receptors is well conserved among vertebrates enabling us to describe the whole subphylum. Then, the review focuses on the regulation of prolactin release in mammals as we have the most knowledge on this class of vertebrates. Prolactin secretion in response to different reproductive stimuli, such as estrogen-induced release, mating, pregnancy and suckling is detailed. Reproduction in birds is different from that in mammals in several aspects. Prolactin is released during incubation in avian species whose regulation and functional significance are discussed. Little information is available on prolactin in reptiles and amphibians; therefore, they are mentioned only in specific cases to explain certain evolutionary aspects. In turn, the osmoregulatory function of prolactin is well established in fish. The different types of pituitary prolactin in fish play particularly important roles in the adaptation of eutherian species to fresh water environments. To achieve this function, prolactin is released from lactotrophs in hyposmolarity, as they are directly osmosensitive in fish. In turn, the released prolactin acts on branchial epithelia, especially ionocytes of the gill to retain salt and excrete water. This review will highlight the points where comparative data give new ideas or suggest new approaches for investigation in other taxa.
Front Neurosci, 2020; 14

30872665: Barna J, Dimén D, Puska G, Kovács D, Csikós V, Oláh S, Udvari EB, Pál G, Dobolyi Á
Complement component 1q subcomponent binding protein in the brain of the rat.
Complement component 1q subcomponent binding protein (C1qbp) is a multifunctional protein involved in immune response, energy homeostasis of cells as a plasma membrane receptor, and a nuclear, cytoplasmic or mitochondrial protein. Recent reports suggested its neuronal function, too, possibly in axon maintenance, synaptic function, and neuroplasticity. Therefore, we addressed to identify C1qbp in the rat brain using in situ hybridization histochemistry and immunolabelling at light and electron microscopic level. C1qbp has a topographical distribution in the brain established by the same pattern of C1qbp mRNA-expressing and protein-containing neurons with the highest abundance in the cerebral cortex, anterodorsal thalamic nucleus, hypothalamic paraventricular (PVN) and arcuate nuclei, spinal trigeminal nucleus. Double labelling of C1qbp with the neuronal marker NeuN, with the astrocyte marker S100, and the microglia marker Iba1 demonstrated the presence of C1qbp in neurons but not in glial cells in the normal brain, while C1qbp appeared in microglia following their activation induced by focal ischemic lesion. Only restricted neurons expressed C1qbp, for example, in the PVN, magnocellular neurons selectively contained C1qbp. Further double labelling by using the mitochondria marker Idh3a antibody suggested the mitochondrial localization of C1qbp in the brain, confirmed by correlated light and electron microscopy at 3 different brain regions. Post-embedding immunoelectron microscopy also suggested uneven C1qbp content of mitochondria in different brain areas but also heterogeneity within single neurons. These data suggest a specific function of C1qbp in the brain related to mitochondria, such as the regulation of local energy supply in neuronal cells.
Sci Rep, 2019; 9

34363844: Bódi V, Csikós V, Májer T, Tóth A, Dobolyi Á, Világi I, Varró P
Zearalenone alters the excitability of rat neuronal networks after acute in vitro exposure.
Zearalenone (ZEA) is a mycotoxin produced by Fusarium species, detectable in various cereals and processed food products worldwide. ZEA displays a significant estrogenic activity, thus its main health risk is the interference with sexual maturation and reproduction processes. However, in addition to being key hormonal regulators of reproductive function, estrogenic compounds have a widespread role in brain, as neurotrophic and neuroprotective factors, and they may influence the activity of several brain areas not directly linked to reproduction, as well. Therefore, in the present study, acute effects of ZEA were studied on certain neuronal functions in rats. Experiments were performed on rat brain slices or live rats. Slices were incubated in ZEA-containing (10-100 μM) solution for 30 min. Electrically evoked and spontaneous field potentials were studied in the neocortex and in the hippocampus. At higher concentrations, ZEA incubation of the slices altered excitability and the pattern of epileptiform activity in neocortex and inhibited the development of LTP in hippocampus. For the verification of these in vitro results, in vivo electrophysiological and immunohistochemical investigations were also performed. ZEA was administered systemically (5 mg/kg, i.p.) to male rats and somatosensory evoked potentials and neuronal activation studied by c-fos expression were analyzed. No neuronal activation could be demonstrated in the hippocampus within 2 h of the injection. In the somatosensory cortex, ZEA did not change in vivo evoked potential parameters, but the activation of a small neuronal population could be demonstrated with the c-fos technique in this brain area. This result could be associated with the ZEA-induced alteration of epileptiform activity observed in vitro. Altogether, the toxin altered the excitability and plasticity of neuronal networks after direct treatment in slices, but the effects were less prominent on the given brain areas after systemic treatment in vivo. A probable explanation for the partial lack of in vivo effects may be that after a single injection, ZEA did not cross the blood-brain barrier at sufficient rate to allow the build-up of comparable concentrations in the investigated brain areas. However, in case of compromised blood-brain barrier functions or long-term repeated exposure,

alterations in cortical and hippocampal functions cannot be ruled out.
Neurotoxicology, 2021; 86
32561249: Bódi V, Csikós V, Rátkai EA, Szűcs A, Tóth A, Szádeczky-Kardoss K, Dobolyi Á, Schlett K, Világi I, Varró P
Short-term neuronal effects of fumonisin B1 on neuronal activity in rodents.
Fumonisin B1 (FB1) is a mycotoxin produced by microscopic fungi (mostly Fusarium species), which may infect our major crops. The toxin inhibits the development of these plants and may also have harmful effects on animals and humans consuming the infected crops. FB1 inhibits sphingolipid biosynthesis which leads to altered membrane characteristics and consequently, altered cellular functions. There are some indications that the toxin has inhibitory effects on neuronal activity in case of repeated consumption, presumably due to sphingolipid depletion. However, according to new literature data, FB1 may have acute excitatory neural effects, too, via different mechanisms of action. Therefore, in the present study, we addressed the neuronal network effects of FB1 following acute treatment, using different electrophysiological techniques in vitro and in vivo. Acute treatments with FB1 (10-100 μM) were carried out on brain slices, tissue cultures and live animals. After direct treatment of samples, electrically evoked or spontaneous field potentials were examined in the hippocampus and the neocortex of rat brain slices and in hippocampal cell cultures. In the hippocampus, a short-term increase in the excitability of neuronal networks and individual cells was observed in response to FB1 treatment. In some cases, the initially enhanced excitation was reversed presumably due to overactivation of neuronal networks. Normal spontaneous activity was found to be stimulated in hippocampal cell cultures. Seizure susceptibility was not affected in the neocortex of brain slices. For the verification of the results caused by direct treatment, effects of systemic administration of FB1 (7.5 mg/kg, i.p.) were also examined. Evoked field potentials recorded in vivo from the somatosensory cortex and cell activation measured by the c-fos technique in hippocampus and somatosensory cortex were analyzed. However, the hippocampal and cortical stimulatory effect detected in vitro could not be demonstrated by these in vivo assays. Altogether, the toxin enhanced the basic excitability of neurons and neuronal networks after direct treatment but there were no effects on the given brain areas after systemic treatment in vivo. Based on the observed in vitro FB1 effects and the lack of data on the penetration of FB1 across the blood-brain barrier, we assume that in vivo consequences of FB1 administration can be more prominent in case of perturbed blood-brain barrier functions.
Neurotoxicology, 2020; 80
33546359: Lékó AH, Kumari R, Dóra F, Keller D, Udvari EB, Csikós V, Renner É, Dobolyi A
Transcriptome Sequencing in the Preoptic Region of Rat Dams Reveals a Role of Androgen Receptor in the Control of Maternal Behavior.
(1) Background: Preoptic region of hypothalamus is responsible to control maternal behavior, which was hypothesized to be associated with gene expressional changes. (2) Methods: Transcriptome sequencing was first applied in the preoptic region of rat dams in comparison to a control group of mothers whose pups were taken away immediately after parturition and did not exhibit caring behavior 10 days later. (3) Results: Differentially expressed genes were found and validated by quantitative RT-PCR, among them NACHT and WD repeat domain containing 1 (Nwd1) is known to control androgen receptor (AR) protein levels. The distribution of Nwd1 mRNA and AR was similar in the preoptic area. Therefore, we focused on this steroid hormone receptor and found its reduced protein level in rat dams. To establish the function of AR in maternal behavior, its antagonist was administered intracerebroventricularly into mother rats and increased pup-directed behavior of the animals. (4) Conclusions: AR levels are suppressed in the preoptic area of mothers possibly mediated by altered Nwd1 expression in order to allow sustained high-level care for the pups. Thus, our study first implicated the AR in the control of maternal behaviors.
Int J Mol Sci, 2021; 22
29478038: Fiáth R, Hofer KT, Csikós V, Horváth D, Nánási T, Tóth K, Pothof F, Böhler C, Asplund M, Ruther P, Ulbert I
Long-term recording performance and biocompatibility of chronically implanted cylindrically-shaped, polymer-based neural interfaces.
Stereo-electroencephalography depth electrodes, regularly implanted into drug-resistant patients with focal epilepsy to localize the epileptic focus, have a low channel count (6-12 macro- or microelectrodes), limited spatial resolution (0.5-1 cm) and large contact area of the recording sites (~mm2). Thus, they are not suited for high-density local field potential and multiunit recordings. In this paper, we evaluated the long-term electrophysiological recording performance and histocompatibility of a neural interface consisting of 32 microelectrodes providing a physical shape similar to clinical devices. The cylindrically-shaped depth probes made of polyimide (PI) were chronically implanted for 13 weeks into the brain of rats, while cortical or thalamic activity (local field potentials, single-unit and multi-unit activity) was recorded regularly to monitor the temporal change of several features of the electrophysiological performance. To examine the tissue reaction around the probe, neuron-selective and astroglia-selective immunostaining methods were applied. Stable single-unit and multi-unit activity were recorded for several weeks with the implanted depth probes and a weak or moderate tissue reaction was found around the probe track. Our data on biocompatibility presented here and in vivo experiments in non-human primates provide

a strong indication that this type of neural probe can be applied in stereo-electroencephalography recordings of up to 2 weeks in humans targeting the localization of epileptic foci providing an increased spatial resolution and the ability to monitor local field potentials and neuronal spiking activity.
Biomed Tech (Berl), 2018; 63
30384196: Vehovszky Á, Farkas A, Csikós V, Székács A, Mörtl M, Győri J
Neonicotinoid insecticides are potential substrates of the multixenobiotic resistance (MXR) mechanism in the non-target invertebrate, Dreissena sp.
Mussels are among the most frequently used invertebrate animals in aquatic toxicology to detect toxic exposure in the environment. The presence and activity of a cellular defence system, the multixenobiotic resistance (MXR) mechanism, was also established in these organisms. In isolated gill tissues of dreissenid mussels (D. bugensis) the MXR activity was assayed after treatment by commercially available insecticides (formulated products) which contain neonicotinoids as their active ingredients: Actara (thiamethoxam), Apacs (clothianidin), Calypso (thiacloprid) and Kohinor (imidacloprid), respectively. While applying the accumulation assay method, 0.5 µM rhodamine B was used as model substrate and 20 µM verapamil as model inhibitor of the MXR mechanism. In acute (in vitro) experiments when isolated gills were co-incubated in graded concentrations of insecticides and rhodamine B simultaneously, Calypso and Kohinor treatment resulted increasing rhodamine accumulation. Chemical analysis of gills in vitro incubated in insecticides demonstrated higher tissue concentrations of thiamethoxam, clothianidin and thiacloprid in the presence of verapamil suggesting that the active ingredients of Actara, Apacs and Calypso are potential substrates of the MXR mediated cellular efflux. In contrast, verapamil did significantly alter the accumulated imidacloprid concentrations in gills, suggesting that the active component of Kohinor is not transported by the MXR mechanism. Chronic (in vivo) exposures of the intact animals in lower, 1, 10 mg/L concentration of neonicotinoid products, resulted in a decreased level of both rhodamine accumulation and verapamil inhibition by the 12th-14th days of treatment. These results suggest an enhancement of MXR activity (chemostimulation), building up gradually in the animals exposed to Actara, Apacs and Kohinor, respectively. Neonicotinoid-type insecticides are generally considered as selective neurotoxins for insects, targeting the nicotinic type acetylcholine receptors (nAChRs) in their central nervous system. Our present results provide the first evidences that neonicotinoid insecticides are also able to alter the transmembrane transport mechanisms related to the MXR system.
Aquat Toxicol, 2018; 205

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BOARD NUMBER: S04-370

INHIBITION OF THE NLRP3 INFLAMMASOME BY OLT1177 INDUCES FUNCTIONAL PROTECTION AND MYELIN PRESERVATION AFTER SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal cord injury (SCI) leads to irreversible functional deficits due to the disruption of axons and the death of neurons and glial cells. The inflammatory response that occurs in the injured spinal cord results in tissue degeneration; thus, targeting inflammation after acute SCI is expected to ameliorate histopathological evidence indicative of damage and, consequently, reduce functional disabilities. Interleukin 1 beta (IL-1β) and interleukin 18 (IL-18) are pro-inflammatory cytokines members of the IL-1 family that initiate and propagate inflammation. Here, we report that protein levels of IL-1β and IL-18 were increased in spinal cord parenchyma after SCI, but with different expression profiles. Whereas levels of IL-1β were rapidly increased reaching peak levels at 12 h after the injury, levels of IL-18 did not increase until 7 days after the injury. Since activation of the NLRP3 inflammasome is required for the processing and release of IL-1β and IL-18, we intraperitoneally administered OLT1177, a selective inhibitor of the NLRP3 inflammasome, to reduce the contribution of these cytokines to SCI. At a dose of 200 mg/kg, OLT1177 protected against neurological deficits and histological evidence of damage. OLT1177 also reduced the levels of IL-1β in the spinal cord after contusion injury and diminished the accumulation of neutrophils and macrophages at later time points. These data suggest that targeting the NLRP3 inflammasome with OLT1177 could be a novel therapeutic strategy to arrest neuroinflammation and reduce functional impairments after acute SCI in humans.

Pubmed:
28993250: Lopez-Alvarez VM, Puigdomenech M, Navarro X, Cobianchi S
Monoaminergic descending pathways contribute to modulation of neuropathic pain by increasing-intensity treadmill exercise after peripheral nerve injury.
This study characterizes the impact of increasing-intensity treadmill exercise (iTR) on noradrenergic (NE) and serotonergic (5HT) modulation of neuropathic pain. Following sciatic nerve transection and repair (SNTR) rats developed significant mechanical and thermal hyperalgesia that was partially prevented by iTR performed during the first 2weeks after injury. Marked decrease in the expression of 5HT and α and β-, but not α adrenergic receptors in the spinal cord dorsal horn was associated to SNTR and recovered by iTR, particularly in lamina II. iTR significantly increased 5HT in periaqueductal grey (PAG), raphe magnus (RM) and dorsal raphe nucleus (DRN), with a pattern suggesting reorganization of serotonergic excitatory interconnections between PAG and DRN. iTR also increased the expression of α in locus coeruleus (LC) and DRN, and β in LC, indicating that exercise enhanced activity of NE neurons, likely by activating autologous projections from DRN and PAG. iTR hypoalgesia was antagonized by blockade of β and 5HT receptors with administration of butoxamine and ketanserin. The neurotoxin DSP4 was injected to induce depletion of NE projections from LC before starting iTR. DSP4 treatment worsened mechanical hyperalgesia, but iTR hypoalgesia was similarly produced. Moreover, 5HT expression in LC further increased after DSP4 injection, all these results suggesting an intrinsic regulation of 5HT and NE activity between PAG, DRN and LC neurons activated by iTR. Finally, iTR significantly reduced microglial reactivity in LC and increased non-microglial BDNF expression, an effect that was reverted by butoxamine, implicating BDNF regulation in central 5HT/NE actions on neuropathic pain.
Exp Neurol, 2018; 299
30863285: Arbat-Plana A, Puigdomenech M, Navarro X, Udina E
Role of Noradrenergic Inputs From Locus Coeruleus on Changes Induced on Axotomized Motoneurons by Physical Exercise.

Physical rehabilitation is one of the cornerstones for the treatment of lesions of the nervous system. After peripheral nerve injuries, activity dependent therapies promote trophic support for the paralyzed muscles, enhance axonal growth and also modulate the maladaptive plastic changes induced by the injury at the spinal level. We have previously demonstrated that an intensive protocol of treadmill running (TR) in rats reduces synaptic stripping on axotomized motoneurons, preserves their perineuronal nets (PNN) and attenuates microglia reactivity. However, it is not clear through which mechanisms exercise is exerting these effects. Here we aimed to evaluate if activation of the locus coeruleus (LC), the noradrenergic center in the brain stem, plays a role in these effects. Since LC is strongly activated during stressful situations, as during intensive exercise, we selectively destroyed the LC by administering the neurotoxin DPS-4 before injuring the sciatic nerve of adult rats. Animals without LC had increased microglia reactivity around injured motoneurons. In these animals, an increasing intensity protocol of TR was not able to prevent synaptic stripping on axotomized motoneurons and the reduction in the thickness of their PNN. In contrast, TR was still able to attenuate microglia reactivity in DSP-4 treated animals, thus indicating that the noradrenergic projections are important for some but not all the effects that exercise induces on the spinal cord after peripheral nerve injury. Moreover, animals subjected to treadmill training showed delayed muscle reinnervation, more evident if treated with DSP-4. However, we did not find differences in treated animals regarding the H/M amplitude ratio, which increased during the first stages of regeneration in all injured groups.
Front Cell Neurosci, 2019; 13

31790581: González-Gil I, Zian D, Vázquez-Villa H, Hernández-Torres G, Martínez RF, Khiar-Fernández N, Rivera R, Kihara Y, Devesa I, Mathivanan S, Del Valle CR, Zambrana-Infantes E, Puigdomenech M, Cincilla G, Sanchez-Martinez M, Rodríguez de Fonseca F, Ferrer-Montiel AV, Chun J, López-Vales R, López-Rodríguez ML, Ortega-Gutiérrez S
A Novel Agonist of the Type 1 Lysophosphatidic Acid Receptor (LPA), UCM-05194, Shows Efficacy in Neuropathic Pain Amelioration.
Neuropathic pain (NP) is a complex chronic pain state with a prevalence of almost 10% in the general population. Pharmacological options for NP are limited and weakly effective, so there is a need to develop more efficacious NP attenuating drugs. Activation of the type 1 lysophosphatidic acid (LPA) receptor is a crucial factor in the initiation of NP. Hence, it is conceivable that a functional antagonism strategy could lead to NP mitigation. Here we describe a new series of LPA agonists among which derivative ()- (UCM-05194) stands out as the most potent and selective LPA receptor agonist described so far (= 118%, EC = 0.24 μM, = 19.6 nM; inactive at autotaxin and LPA receptors). This compound induces characteristic LPA-mediated cellular effects and prompts the internalization of the receptor leading to its functional inactivation in primary sensory neurons and to an efficacious attenuation of the pain perception in an model of NP.
J Med Chem, 2020; 63

34624330: Amo-Aparicio J, Garcia-Garcia J, Puigdomenech M, Francos-Quijorna I, Skouras DB, Dinarello CA, Lopez-Vales R
Inhibition of the NLRP3 inflammasome by OLT1177 induces functional protection and myelin preservation after spinal cord injury.
Spinal cord injury (SCI) leads to irreversible functional deficits due to the disruption of axons and the death of neurons and glial cells. The inflammatory response that occurs in the injured spinal cord results in tissue degeneration; thus, targeting inflammation after acute SCI is expected to ameliorate histopathological evidence indicative of damage and, consequently, reduce functional disabilities. Interleukin 1 beta (IL-1β) and interleukin 18 (IL-18) are pro-inflammatory cytokines members of the IL-1 family that initiate and propagate inflammation. Here, we report that protein levels of IL-1β and IL-18 were increased in spinal cord parenchyma after SCI, but with different expression profiles. Whereas levels of IL-1β were rapidly increased reaching peak levels at 12 h after the injury, levels of IL-18 did not increase until 7 days after the injury. Since activation of the NLRP3 inflammasome is required for the processing and release of IL-1β and IL-18, we intraperitoneally administered OLT1177, a selective inhibitor of the NLRP3 inflammasome, to reduce the contribution of these cytokines to SCI. At a dose of 200 mg/kg, OLT1177 protected against neurological deficits and histological evidence of damage. OLT1177 also reduced the levels of IL-1β in the spinal cord after contusion injury and diminished the accumulation of neutrophils and macrophages at later time points. These data suggest that targeting the NLRP3 inflammasome with OLT1177 could be a novel therapeutic strategy to arrest neuroinflammation and reduce functional impairments after acute SCI in humans.
Exp Neurol, 2022; 347

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FEEDING AND EXERCISE ESSENTIAL VALUES EXAMINED IN CANNABINOID TYPE-1 (CB1) RECEPTOR MUTANT MICE LIVING IN CLOSED ECONOMY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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AIMS Pathological imbalances between feeding and exercise find their roots at the motivation level. Using an original instrumental task, we reported that cannabinoid type-1 receptors (CB_1Rs) located on GABAergic neurons are involved in the choice between palatable feeding and wheel-running (Muguruza et al., 2019). However, this task bears translational limits as mice were only daily exposed to it for a limited period (1 h) and were housed in cages without a running apparatus but with unlimited food. We thus investigated the role of CB_1Rs under closed-economy conditions wherein the prices for food and exercise were progressively increased over 12 days. **METHODS** Male/female mice bearing a constitutive (CB_1-KO) or a conditional deletion of CB_1R in GABAergic neurons (GABA-CB_1-KO) lived in operant chambers. Therein, they were able to feed a standard diet and to run, doing so under progressively increased fixed ratio (FR) reinforcement schedules (FR1 up to FR30). Reward essential values were obtained through demand curves (Hursh, 2014). **RESULTS** Compared to their respective wild-type male littermates, CB_1-KO and GABA-CB_1-KO mice displayed a decreased essential value for running, but not feeding. This decrease was amplified in females and partly reversed when CB_1Rs were selectively re-expressed in GABAergic neurons of male/female mice lacking CB_1R expression. **CONCLUSIONS** These findings indicate that CB_1Rs on GABAergic neurons are necessary, but only partly sufficient, for running motivation under closed economy conditions. Combining genetic and viral approaches, our present experiments are aimed at further dissecting the neurobiological circuits involved in running motivation.

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INVESTIGATING THE MECHANISMS OF MOTOR NEURON SUBTYPE DIFFERENTIAL VULNERABILITY IN DISTAL SPINAL MUSCULAR ATROPHY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Intracellular transport defects are observed in many neurological disorders. Analysis of animal models indicate that impaired transport can cause neurodegeneration or impinge on the development of neural circuits. In support of a similar role in human, mutations in DYNC1H1 encoding the heavy chain of Dynein and its partner BICD2, two core components of the intracellular transport machinery, are causing a rare neurodevelopmental motor neuron (MN) disease termed Spinal Muscular Atrophy with Lower Extremity Dominance (SMALED). Limb and even more leg-innervating MNs are preponderantly affected in SMALED suggesting a graded reliance on axonal transport among neuronal subtypes and that specific MN subtypes might be the most vulnerable cells to defects in this pathway. To approach the basis of this differential vulnerability, we have reprogrammed patient cells carrying a *BICD2* SMALED-inducing mutation into human induced pluripotent stem cells (hiPSC) as well as generated a mouse model with a similar mutation. Differentiating hiPSC into limb-innervating motor neurons and analysis of heterozygous and homozygous mouse embryonic spinal cords indicate that motor neuron subtype specification is not impacted in SMALED. Yet, homozygous mutant mice die at late gestation or early post-natal stages suggesting a deleterious effect of the mutation. We are now investigating in vivo and in vitro the consequences of the mutation on intracellular transport and MN subtype survival. The overarching aim of this study is to determine how mutations in core components of the transport machinery impact globally on intracellular transport but leads to the greater vulnerability of leg-innervating MNs.

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EFFECTS OF OLFACTORY MUCOSA STEM/STROMAL CELL AND OLFACTORY ENSHEATING CELLS SECRETOME ON PERIPHERAL NERVE REGENERATION

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Despite recent advances in promoting peripheral nerve regeneration after injury, it has not yet been possible to establish an alternative treatment to supplant traditional surgical methods as gold-standard approaches. Regenerative medicine, a therapeutic field alternative to conventional treatments, drew attention due to its versatility, multifactorial approach, and potential to revolutionize different medical fields, including Peripheral Nerve Injury (PNI). Among all options, the combination of cell-based therapies with biomaterials has been the most promising. In previous works, our research group has extensively studied the functional characteristics of olfactory mucosa mesenchymal stem cells (OM-MSCs), a type of MSCs with a high therapeutic potential for the promotion of nerve regeneration after PNI. In this work, the secretome produced and collected from OM-MSCs and Olfactory Ensheating Cells (OECs) was therapeutically applied to promote PNI. OM-MSCs and OECs in low passages were subjected to a conditioning process for 48h. Subsequently, the concentrated conditioned medium (CM) was applied to sciatic nerves of rats after neurotmesis, using Reaxon® as tube-guides and as containers for CM. Over 20 weeks, the animals were subjected to periodic functional assessments, with evaluation of the motor and sensory recovery, as well as gait pattern characterization. Preliminary functional results demonstrate the capacity of the two types of CM to promote an effective functional recovery over the study period, with results comparable to those observed after the application of the OM-MSCs themselves. The results are identical between OECs and OM-MSCs secretome, and it is not possible, so far, to define which is the best approach.

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COMPARATIVE MODEL OF MINIMAL SPINAL CORD INJURY REVEALS SUPERIOR REGENERATIVE POTENTIAL OF NERVOUS TISSUE DURING DEVELOPMENT COMPARED TO ADULTHOOD

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Spinal cord injury resulting from trauma essentially decreases the quality of human life. Numerous clues indicate that the limited endogenous regenerative potential is a result of interplay between inhibitory nature of mature nervous tissue and inflammatory actions of immune and glial cells. Knowledge gained from comparative studies on juvenile animals could draw attention to factors, which should be removed/added for more effective regenerative therapy of adults. In our study, we introduced the concept of minimal spinal cord injury (mSCI) with comparable impact on the spinal cord of Wistar rats at the age of full adulthood, preadolescence and neonatal period. The mechanism of injury is based on the unilateral incision of either 20 gauge (5% mSCI) needle tip according to stereotaxic coordinates into dorsal horn in lumbar spinal segment L4. The incision should harm similar amount of gray matter on coronal section (five percent) in each group of experimental animals. According to our results, impact causes mild injury with minimal adverse effects on neurological functions of animals, but still with effect on cells residing the ependymal layer, microglia and astroglia. Moreover, developing spinal cord exhibits superior regenerative potential compared to adulthood and thus indicates the importance of comparative neuroregenerative research in the future. The research was supported by Slovak Research and Development Agency under the contract No. APVV-19-0279 and VEGA grant no. 11/0706/20.

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BOARD NUMBER: S04-375

A NOVEL HYALURONIC ACID (HLA) BASED HYDROGEL FOR THE ENHANCEMENT OF PERIPHERAL NERVE REGENERATION

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Introduction: Peripheral nerve injuries present significant healthcare and economic burdens with the majority of cases occurring due to trauma in the workforce. Despite all that is known about this condition, complete functional recovery remains difficult to obtain through current surgical methods. **Aim:** The aim of this research was to modify HLA to enable the 3D printing of hydrogel nerve conduits. Also, several neurotrophic compounds were screened in conjunction with HLA to assess any potential neurotrophic benefits to their inclusion in the final formulation. **Methods:** Methacrylated HLA (MeHLA) and thiolated HLA (HLASH) were synthesized to enable hydrogel formation via thiolene click-chemistry. The degree of modification (MoD) was calculated using FTIR and H^1-NMR. The HLA derivatives and photopolymerised samples were screened in a neuronal (SH-SY5Y) and glial (RT4 D6P2T) cell line using the resazaurin reduction assay in the presence and absence of putative neurotrophic compounds. **Results:** HLA was successfully substituted as confirmed by FTIR and H^1-NMR with MoD of 100% and 27% respectively. No cytotoxicity was observed in isolation or synergistically. Screening of the neurotrophic compounds revealed significantly increased glial cell proliferation after exposure to tyrosol (2-(4-Hydroxyphenol) ethanol) (****P<0.0001). **Conclusion:** HLA was modified to allow for thiolene click-chemistry reactions to produce hydrogels with unique viscoelastic properties. Some neurotrophic compounds were identified, and these compounds will be included in the final formulation prior to stereolithography 3D printing of hydrogel nerve conduits. Modulated HLA could have future applications in the development of improved nerve conduits for enhanced peripheral nerve repair post illness or injury.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-376

HUMAN NEURAL PRECURSOR CELLS PRIMED WITH A NANOCONJUGATE OF FASUDIL, A RHO/ROCK INHIBITOR, FOR THE TREATMENT OF ACUTE SPINAL CORD INJURIES

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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In the following study we aim to develop a combinatory therapy for the treatment of acute spinal cord injury (SCI) using human fetal neural progenitor cells (hfNPCs) primed with a novel Rock inhibitor (Fasudil) nanoconjugate (PGA-SS-FAS). We have followed GMP-like procedures, which could be easily replicated in a clean room for subsequent clinical use, to isolate, expand and preserve "clinical grade" human neural progenitor cells (hfNPCs) derived from human spinal cords obtained from 19-21 week fetal abortions. *In vitro* expanded hfNPCs retained neuronal characteristics and multipotency after GMP-like procedures and 24h treatment with PGA-SS-FAS on hfNPCs induced neuronal differentiation and overcame neuritic retraction in the presence of lysophosphatidic acid (LPA), a Rho-Rock activator. For *in vivo* studies, immune-deficient female NU(*NCr)*-Foxn1^{nu} mice were subjected to SCI at the thoracic level T8 by compression and a total of 2.5x10^5 hfNPCs primed or not with PGA-SS-FAS (10% w/w; 50mm equimolecular concentration) for 24h were intramedullary injected in the lesion epicenter immediately after SCI induction. Control group of animals instead received injections of culture medium. We found that PGA-SS-FAS priming 1) enhanced hfNPCs activation and migration through the spinal cord injured tissue; 2) increased neuronal activation and 3) enhanced preservation of GABAergic inhibitory Lbx1 and glutamatergic excitatory Tlx3 somatosensory interneurons in the dorsal horns of the spinal cord. Thus, we have developed a "clinical grade" cell therapy using hfNPCs, following GMP guidelines, which in combination with PGA-SS-FAS priming for transplantation shows potential neuroprotective effects for clinical applications and treatment of acute SCIs.

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BOARD NUMBER: S04-377

EXTENSIVE OSSIFICATION OF THE PARASPINAL LIGAMENT MISDIAGNOSED AS A SUBDURAL CERVICAL HEMATOMA IN A PATIENT WITH VITAMIN D-RESISTANT RICKETS: A CASE REPORT

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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We report a rare case of extensive ossification of the paraspinal ligaments misdiagnosed as subdural hematoma at cervical levels in a patient with vitamin D-resistant rickets. A 49-year-old woman with Vitamin D-resistant rickets (VDRR) reported neck and radiating pain in both shoulder and arm after a traffic accident. Neck and shoulder pain was alleviated after trigger point injection, however, the weakness of the upper extremities and gait disturbance had gradually worsened over 1 year. Magnetic resonance imaging showed hypointensity on T2 weighted imaging chronic spinal subdural hematoma leading to cervical cord compression, which extended on C1-C7 levels with cord signal changes and was diagnosed with chronic subdural hematoma. But CT imaging showed hyperintense ectopic ossification of the spinal ligaments and she was finally diagnosed with extensive ossification of the cervical paraspinal ligaments.

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BOARD NUMBER: S04-378

OZONE THERAPY AS A MINIMALLY-INVASIVE ALTERNATIVE IN PATIENTS WITH ACUTE LUMBAR DISC HERNIATION: A RANDOMIZED CLINICAL TRIAL

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Low back pain (LBP) management via conservative therapy with intervention fails in some cases. However, there are still many challenges to choose the best choice. Minimally-invasive techniques such as ozone therapy are emerging choices for surgery. In this Randomized phase III clinical trial (2017-19), one hundred patients admitted to Imam Reza Hospital (Tabriz-Iran) for herniated disk-induced LBP were randomly divided (shape- and color-identical envelopes) into two case and control groups. Patients in the case group were treated with ozone therapy plus medical therapy. Alternatively, patients in the control group received only conventional medical therapy. Primary outcomes such as changes in pain intensity (VAS) and basal test before and after treatment and also secondary outcomes like the amount of analgesic used were evaluated in the patients for two weeks, three months, and six months after surgery. Student T-test and Chi-square were compared for comparing the data. Mean pain intensities estimated by VAS and improvement of restless leg syndrome were not significantly different between the two groups two weeks (p=0.8), three months (p=0.5), and six months (p=0.9) after the intervention. Pain intensity was found to be lower in both groups after the intervention compared with before treatment (p=0.001 for both). Moreover, significant differences were found between the two groups in the Lasegue test two weeks (p=0.02) and six months (p=0.01) after the intervention. The application of ozone therapy not only improves clinical pain syndrome in LBP patients but also leads to improved medical treatment in these patients.

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MAPPING HUMAN PROPRIOCEPTIVE PROJECTIONS OF THE UPPER LIMB MUSCLES THROUGH SPINAL CORD FMRI

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Recent advances in functional magnetic resonance imaging (fMRI) have allowed investigation of the somatosensory and motor networks in the human spinal cord (Landelle et al. 2021; Rowald et al. 2022). Maps of different upper limb myotomes have been revealed by motor tasks (Kinany et al. 2019) but no fMRI study has investigated the precise location of muscle proprioceptive projections in the cervical spinal cord. Here, we explored the rostrocaudal activation patterns during upper limb proprioceptive stimulation to better characterize the spinal proprioceptive circuits in 14 healthy volunteers. We exploited amagnetic vibrators to specifically stimulate proprioceptive afferences innervating six muscles in wrist, elbow and shoulder joints of participants' left arm. This kind of stimulation activates muscle spindles and elicits illusory sensations of movement by recruiting full sensorimotor pathways (Kavounoudias et al. 2008). Functional MR images were acquired between C2 and C8 vertebrae and preprocessed with the Spinal Cord Toolbox (De Leener et al. 2017). Group-level analysis revealed a rostrocaudal organization of proprioceptive projections from C3 to C7-C8 that matched the expected proximo-distal location of upper-limb proprioceptive neurons, although a substantial inter-subject variability was observed. Activations were primarily distributed along the extent of the left dorsal hemicord, though ventral and contralateral hemicords were also activated to a lesser extent. This study reveals muscle proprioceptive maps of the cervical spinal cord based on functional MR recordings. These maps are essential for improving our understanding of the healthy and injured spinal cord, guiding neurosurgical interventions, and helping the design of neuroprosthetic treatments.

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BOARD NUMBER: S04-380

RESPIRATION AS A CONFOUNDER IN CSF PRESSURE MONITORING: A SUBGROUP ANALYSIS OF THE COMP-CORD STUDY

POSTER SESSION 04 - SECTION: SPINAL CORD, PERIPHERAL NERVE INJURY AND REGENERATION

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Intraoperative CSF pressure (CSFP) monitoring has been previously pioneered in patients with degenerative cervical myelopathy (DCM) aiming to estimate effective cord decompression. The intraoperative setting is different from the postoperative one because of mechanical ventilation and anesthesia, which influence CSFP dynamics due to the interaction between the CSF compartment and the cardiorespiratory system. This is mediated by changes in CSF flow and the effect of variations in CO_2 levels on the vascular bed. Therefore, analyzing CSFP dynamics needs to account for ventilation and anesthesia. In this study, we investigated intrathecal CSFP metrics in 17 patients with DCM who underwent surgical decompression. Intraoperative data (i.e., patients mechanically ventilated and anesthetized) were analyzed in the time window after decompression to limit interference with cord compression. In the postoperative setting, patients were awake and breathing spontaneously. Intraoperative baseline CSFP had a median and interquartile range of 10.8 [4.3] mmHg and increased 1.6-fold to 16.3 [5.8] mmHg post-operatively. Similarly, cardiac-induced CSFP peak-to-trough amplitude (CSFPp) was 0.6 [0.9] and increased 3-fold to 1.7 [3.2]. In contrast, the breathing modulation amplitude declined from 1.9 [1.4] mmHg to 0.7 [1.1] mmHg. During weaning (N=3), there was a continuous rise of CSFP and CSFPp, up to 9.6 [4.2] and 3.9 [3.3] mmHg, respectively. Our findings highlight that intraoperative setting significantly impacts CSFP dynamics and the underlying mechanisms should be carefully considered in data recording and interpretation, in particular when comparing CSFP across different settings.

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BOARD NUMBER: S04-381

TEMPORAL DYNAMICS OF NEUROINFLAMMATION FOLLOWING CONTROLLED CORTICAL IMPACT TRAUMATIC BRAIN INJURY IN JUVENILE AND ADULT MICE

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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AIMS: Characterisation of neuroinflammation in juvenile and adult mice following Controlled Cortical Impact Traumatic Brain Injury (TBI) at 5 and 35 days post-injury (dpi), to assess differences in the injury response in the immature vs. the mature brain.
METHODS: TBI was induced using the Controlled Cortical Impact (CCI) model in juvenile postnatal day 30 (P30) and adult (P70-84) CD1 male mice. Mice were humanely killed at 5 and 35 dpi, along with control groups (naïve and craniotomy-sham). Brain tissue was analysed by immunofluorescence using markers for astrocytes (GFAP), and microglia (P2Y12 and Iba1). Expression analysis was carried out, along with morphological analysis, to identify activated microglia and astrocytes. Heat maps were produced to show GFAP and Iba1 distribution patterns, along with the incidence of cellular activation.
RESULTS: A higher P2Y12 expression was seen in juveniles compared to adults post-TBI at 5 dpi ($P= 0.0215$), whilst adults showed a higher expression than juveniles after TBI at 35 dpi ($P = 0.0160$). A reduced P2Y12 expression was seen at 35 dpi in juvenile TBI compared to 5 dpi ($P < 0.001$). Morphology analysis and heat maps showed activated astrocytes in hippocampal and cortical regions around the injury in adults post-CCI at 35 dpi. Higher activated microglia were found around the injury lesion in the adult CCI group at 5 dpi and this persisted at 35 dpi.
CONCLUSIONS: Adult mice experienced sustained neuroinflammation peri-lesionally post-injury, whilst juvenile mice appeared to experience a faster resolving local neuroinflammation reaction than adults.

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BOARD NUMBER: S04-382

DESCRIPTION OF POST-TRAUMATIC BRAIN LESION IN A PEDIATRIC MURINE MODEL OF INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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In children, traumatic brain injury (TBI) is an underestimated pathology that can generate a long-term handicap with difficulties at school, social isolation and possibly a lifelong impact. We used a model of pediatric TBI on postnatal day (P) seven male mice. We investigated microglial activation, astrogliosis, white matter lesions and behavioral disorders on P45 mice. Study of microglial phenotype by RT-qPCR showed that TBI induced a mixed population of microglial cells with pro-inflammatory, anti-inflammatory and immunomodulatory markers particularly three days after the injury; besides seven days after, the TBI microglial activation seemed to be completely resolved. Additionally, immunochemistry labelling revealed an astroglial scar and a neuronal loss in the impact region in association with white matter loss at P45; electronic microscopy showed also a decrease of myelinated axons in TBI mice. This results were also confirmed in diffusion tensor imaging with an important alteration of the anisotropic fraction, also functional ultrasound imaging with fUS showed brain cerebral connectivity alteration. Ultrasonic vocalizations recorded at P8 showed a decrease in the number and the duration of USV calls in TBI mice. Also, TBI led to social deficiency at P45 during a social interaction test adapted from the three-room test. These results are very promising, and encourage the study of the mechanisms involved in post-concussion syndrome and more particularly the involvement of microglial activation. This study could allow identification of signaling pathways and possibly new therapeutic targets in order to care for post- traumatic disorder patients.

Pubmed:
29656803: Jacquens A, Sigaut S, Degos V

Presse Med, 2018; 47
32898926: Csaba Z, Vitalis T, Charriaut-Marlangue C, Margaill I, Coqueran B, Leger PL, Parente I, Jacquens A, Titomanlio L, Constans C, Demene C, Santin MD, Lehericy S, Perrière N, Glacial F, Auvin S, Tanter M, Ghersi-Egea JF, Adle-Biassette H, Aubry JF, Gressens P, Dournaud P
A simple novel approach for detecting blood-brain barrier permeability using GPCR internalization.
Impairment of blood-brain barrier (BBB) is involved in numerous neurological diseases from developmental to aging stages. Reliable imaging of increased BBB permeability is therefore crucial for basic research and preclinical studies. Today, the analysis of extravasation of exogenous dyes is the principal method to study BBB leakage. However, these procedures are challenging to apply in pups and embryos and may appear difficult to interpret. Here we introduce a novel approach based on agonist-induced internalization of a neuronal G protein-coupled receptor widely distributed in the mammalian brain, the somatostatin receptor type 2 (SST2).
Neuropathol Appl Neurobiol, 2021; 47
28780988: Bombled C, André A, Jacquens A, Clarençon F, Degos V
Postoperative neurosurgery complication in 2017: A new window to take into account surgical ischaemic events.
Anaesth Crit Care Pain Med, 2017; 36
32992024: Debarle C, Perlbarg V, Jacquens A, Pélégrini-Issac M, Bisch M, Prigent A, Lesimple B, Caron E, Lefort M, Bayen E, Galanaud D, Pradat-Diehl P, Puybasset L, Degos V
Global mean diffusivity: A radiomarker discriminating good outcome long term after traumatic brain injury.
Traumatic brain injury (TBI) is a chronic pathology responsible for cognitive disorders impacting outcome. Global clinical outcome several years after TBI may be associated with anatomical sequelae. Anatomical lesions are not well described

because characterizing diffuse axonal injury and brain atrophy require using specific MRI sequences with quantitative measures. The best radiologic parameter to describe the lesions long term after TBI is not known.
Ann Phys Rehabil Med, 2021; 64
34634297: Premat K, Azuar C, Galanaud D, Jacquens A, Dormont D, Degos V, Clarençon F,
Pathomechanisms behind cognitive disorders following ruptured anterior communicating aneurysms: A diffusion tensor imaging study.
After the rupture of anterior communicating aneurysms, most patients experience debilitating cognitive disorders; and sometimes even without showing morphological anomaly on MRI examinations. Diffusion Tensor Imaging (DTI) may help understanding the pathomechanisms leading to such disorders in this subset of patients.
J Neuroradiol, 2022; 49
31964285: Jacquens A, Shotar E, Bombled C, Glémain B, Sourour NA, Nouet A, Premat K, Lenck S, Degos V, Clarençon F
Is Anatomical Variations a Risk Factor for Cerebral Vasospasm in Anterior Communicating Complex Aneurysms Rupture?
Background and Purpose- One-third of ruptured aneurysms are located on the anterior communicating complex with high prevalence of anatomic variations of this arterial segment. In this study, we hypothesized that anatomic variations of the anterior communicating complex increase the risk of angiographic vasospasm. Methods- Retrospective study of prospectively collected data from a monocentric subarachnoid hemorrhage cohort of patients admitted to neurointensive care between 2002 and 2018. Univariate followed by multivariate logistic regression analysis was used to identify factors associated with angiographic vasospasm. Results- One thousand three hundred seventy-four patients with aneurismal subarachnoid hemorrhage were admitted to our institution; 29.8% (n=410) were related to an anterior communicating complex aneurysm rupture; 9.2% (n=38) of them showed an anterior communicating artery variation. Angiographic vasospasm was diagnosed in 55.6% of this subgroup (vs 28.1%, =0.003). In the multivariate analysis, external ventricular drain (2.2 [1.32-3.65], =0.003) and anterior communicating artery variation (2.40 [1.2-4.9], =0.04) were independently and significantly associated with angiographic vasospasm, while age above 60 years (0.3 [0.2-0.7]; =0.002) was a protective factor. However, anterior communicating artery variation was not statistically associated with ischemic vasospasm or poor neurological outcome after anterior communicating artery aneurysm rupture. Conclusions- Anatomic variation of anterior communicating artery could be a new biomarker to identify patients at risk to develop angiographic vasospasm post-subarachnoid hemorrhage. External validation cohorts are necessary to confirm these results.
Stroke, 2020; 51
34507289: Riche M, Marijon P, Amelot A, Bielle F, Mokhtari K, Chambrun MP, Joncour AL, Idbaih A, Touat M, Do CH, Deme M, Pasqualotto R, Jacquens A, Degos V, Shotar E, Chougar L, Carpentier A, Mathon B
Severity, timeline, and management of complications after stereotactic brain biopsy.
The literature shows discrepancies in stereotactic brain biopsy complication rates, severities, and outcomes. Little is known about the timeline of postbiopsy complications. This study aimed to analyze 1) complications following brain biopsies, using a graded severity scale, and 2) a timeline of complication occurrence. The secondary objectives were to determine factors associated with an increased risk of complications and to assess complication-related management and extra costs.
J Neurosurg, 2022; 136
31136601: Kerever S, Jacquens A, Smail-Faugeron V, Gayat E, Resche-Rigon M
Methodological management of end-of-life decision data in intensive care studies: A systematic review of 178 randomized control trials published in seven major journals.
End-of-life (EOL) decisions are a serious ethical dilemma and are frequently carried out in intensive care units (ICUs). The aim of this systematic review was to investigated the different approaches used in ICUs and reported in randomized controlled trials (RCTs) to address EOL decisions and compare the impact of these different strategies regarding potential bias and mortality estimates.
PLoS One, 2019; 14
32387750: Jacquens A, Khorrami A, Rossignon MD, Rigolot R, Butel N, Rémond AL, Bonnin S, Toulemont M, Touitou V, Bodaghi B, Degos V
Safe short circuit in cataract surgery: Incidence and risk factors for intraoperative medical action.
Cataract surgery has become the most frequent surgical procedure performed every year in Western countries. Perioperative patient circuit has to be adapted to the important medical needs and progress. Hence, a secure short circuit (SSC) for surgeries of the anterior segment of the eye under topical anaesthesia was created. Patients included in the circuit are selected first by surgeons and answer a medical questionnaire, they do not have any preoperative evaluation by anaesthesiologist, are monitored during surgery by the surgical team and in case of problem an intraoperative medical action (IMA) can be performed. We conducted a retrospective observational incidence study of the occurrence of the IMA, followed by a case control study. The primary outcome was to identify risk factors of IMA among the patients' medical history. Out of 2744 screened patients, 1592 patients were included during the period of November 2015 to November 2017. The rate of

IMA was 5%, 81% of them presenting with intraoperative high blood pressure (HBP). In the case control study part, stepwise regression analysis revealed that a history of HBP and insulin-dependent diabetes (IDD) was significantly correlated with IMA (respectively, adjusted odds ratio 1.7, P=0.005 and 2.6, P=0.002). The low incidence of IMA showed that the SSC is a safe tool thanks to a selection and an optimised and secure pathway. A history of HBP and IDD was significantly associated with the occurrence of IMA. Therefore, an optimisation of the perioperative period would be beneficial in these cases.
Anaesth Crit Care Pain Med, 2020; 39
28968602: Shotar E, Pistocchi S, Haffaf I, Bartolini B, Jacquens A, Nouet A, Chiras J, Degos V, Sourour NA, Clarençon F
Early Rebleeding after Brain Arteriovenous Malformation Rupture, Clinical Impact and Predictive Factors: A Monocentric Retrospective Cohort Study.
Brain arteriovenous malformations (BAVMs) are a leading cause of intracranial hemorrhage in young adults. This study aimed to identify individual predictive factors of early rebleeding after BAVM rupture and determine its impact on prognosis.
Cerebrovasc Dis, 2017; 44

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BOARD NUMBER: S04-383

KETOGENIC DIET PROTECTS THE BRAIN AGAINST WEIGHT DECREASE AFTER TRAUMATIC BRAIN INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is associated with pathological changes in nervous tissue, related to neuronal death and gliosis. Ketogenic diet (KD) is one of the potential neuroprotective strategies, that may prevent brain posttraumatic remodelling. The aim of this study was to determine the influence of KD on animal state after TBI and glial scar formation. Rats were divided into groups obtaining standard or ketogenic diet (SD and KD respectively) from postnatal day 27 (P27). On P30 penetrating brain injury was induced in cerebral cortex in half of the animals from each group. The body weight, blood level of glucose and ketone bodies were monitored after TBI. Different cohorts of rats were perfused 2, 8, 16 and 30 days after TBI, brains were dissected and weighted. Lower body mass of injured KD-fed animals in comparison to SD-fed rats was observed in all age groups, which remains consistent with negative correlation of blood ketone bodies level and body weight. TBI resulted in lower body mass in rats on ketogenic diet on P60, in comparison to KD-fed uninjured group, whereas no such effect was observed in animals on SD. Nonetheless, in SD-fed group brain mass was significantly lower in rats subjected to TBI procedure than in control animals. Interestingly, there were no differences in brain mass between control and injured rats fed with KD. To conclude, KD, despite lowering rats' body mass, prevents brain weight decrease after TBI. Histological analysis will provide information about its influence on glial scar formation.

Pubmed:
35038032: Kosiek W, Rauk Z, Szulc P, Cichy A, Rugieł M, Chwiej J, Janeczko K, Setkowicz Z
Ketogenic diet impairs neurological development of neonatal rats and affects biochemical composition of maternal brains: evidence of functional recovery in pups.
The ketogenic diet (KD) is a type of diet in which the intake of fats significantly increases at the cost of carbohydrates while maintaining an adequate amount of proteins. This kind of diet has been successfully used in clinical therapies of drug-resistant epilepsy, but there is still insufficient evidence on its safety when used in pregnancy. To assess KD effects on the course of gestation and fetal development, pregnant females were fed with: (i) KD during pregnancy and lactation periods (KD group), (ii) KD during pregnancy replaced with ND from the day 2 postpartum (KDND group) and (iii) normal diet alone (ND group). The body mass, ketone and glucose blood levels, and food intake were monitored. In brains of KD-fed females, FTIR biochemical analyses revealed increased concentrations of lipids and ketone groups containing molecules. In offspring of these females, significant reduction of the body mass and delays in neurological development were detected. However, replacement of KD with ND in these females at the beginning of lactation period led to regainment of the body mass in their pups as early as on the postnatal day 14. Moreover, the vast majority of our neurological tests detected functional recovery up to the normal level. It could be concluded that the ketogenic diet undoubtedly affects the brain of pregnant females and impairs the somatic and neurological development of their offspring. However, early postnatal withdrawal of this diet may initiate compensatory processes and considerable functional restitution of the nervous system based on still unrecognized mechanisms.
Brain Struct Funct, 2022; 227

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-384

A NOVEL MODEL FOR MULTIPLEXED, TRIDIMENSIONAL ANALYSIS OF CORTICAL REGENERATION UPON BRAIN DAMAGE IN VIVO

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Brain damage is the major cause of adult disabilities, specifically on the elderly population. In this paradigm, there are a variety of biological events to consider in brain injury: neuronal death, neuroinflammation, loss of synapses and alteration in neuronal circuitry. The rewiring process upon brain damage is poorly understood and there is no pharmacological treatment available. Here, we designed a novel *in vivo* model to investigate cortical rewiring upon brain injury. Briefly, we trace contralateral callosal projection neurons with viral vectors to study the response upon a controlled mechanical lesion of the hindlimb motor cortex. To analyze functional motor outcome after injury, we perform a battery of motor behavioral test. Finally, we design a multiplexed and tridimensional semi-automatic analysis using coronal brain section. This allows us to analyze different labeling from the same animals: since 3D reconstruction of the brain to subcellular resolution, using synaptic markers. This novel model presents a variety of advantages to study cortical regeneration and rewiring upon injury. First, the injury protocol produces a consistent and focal damage on the cortical motor area, facilitating the study of contralateral projections and giving functional motor outcome. Secondly, combining conventional confocal microscopy with semi-automated 3D reconstruction, this methodology permits since the study of subcellular structures, such as synaptic buttons in different brain areas, to a reconstruction of the cortical innervation around the injured area. Hence, this innovative experimental approach will contribute in a deeper understand of molecular and cellular interactions in cortical regeneration upon brain damage.

Pubmed:
34369923: Rodríguez-Prieto Á, González-Manteiga A, Domínguez-Canterla Y, Navarro-González C, Fazzari P
A Scalable Method to Study Neuronal Survival in Primary Neuronal Culture with Single-cell and Real-Time Resolution.
Neuronal loss is at the core of many neuropathologies, including stroke, Alzheimer's disease, and Parkinson's disease. Different methods were developed to study the process of neuronal survival upon cytotoxic stress. Most methods are based on biochemical approaches that do not allow single-cell resolution or involve complex and costly methodologies. Presented here is a versatile, inexpensive, and effective experimental paradigm to study neuronal survival. This method takes advantage of sparse fluorescent labeling of the neurons followed by live imaging and automated quantification. To this aim, the neurons are electroporated to express fluorescent markers and co-cultured with non-electroporated neurons to easily regulate cell density and increase survival. Sparse labeling by electroporation allows a simple and robust automated quantification. In addition, fluorescent labeling can be combined with the co-expression of a gene of interest to study specific molecular pathways. Here, we present a model of stroke as a neurotoxic model, namely, the oxygen-glucose deprivation (OGD) assay, which was performed in an affordable and robust homemade hypoxic chamber. Finally, two different workflows are described using IN Cell Analyzer 2200 or the open-source ImageJ for image analysis for semi-automatic data processing. This workflow can be easily adapted to different experimental models of toxicity and scaled up for high-throughput screening. In conclusion, the described protocol provides an approachable, affordable, and effective in vitro model of neurotoxicity, which can be suitable for testing the roles of specific genes and pathways in live imaging and for high-throughput drug screening.
J Vis Exp, 2021;
34246770: Navarro-Gonzalez C, Carceller H, Benito Vicente M, Serra I, Navarrete M, Domínguez-Canterla Y, Rodríguez-Prieto Á, González-Manteiga A, Fazzari P
Nrg1 haploinsufficiency alters inhibitory cortical circuits.
Neuregulin 1 (NRG1) and its receptor ERBB4 are schizophrenia (SZ) risk genes that control the development of both excitatory and inhibitory cortical circuits. Most studies focused on the characterization ErbB4 deficient mice. However, ErbB4

deletion concurrently perturbs the signaling of Nrg1 and Neuregulin 3 (Nrg3), another ligand expressed in the cortex. In addition, NRG1 polymorphisms linked to SZ locate mainly in non-coding regions and they may partially reduce Nrg1 expression. Here, to study the relevance of Nrg1 partial loss-of-function in cortical circuits we characterized a recently developed haploinsufficient mouse model of Nrg1 (Nrg1). These mice display SZ-like behavioral deficits. The cellular and molecular underpinnings of the behavioral deficits in Nrg1 mice remain to be established. With multiple approaches including Magnetic Resonance Spectroscopy (MRS), electrophysiology, quantitative imaging and molecular analysis we found that Nrg1 haploinsufficiency impairs the inhibitory cortical circuits. We observed changes in the expression of molecules involved in GABAergic neurotransmission, decreased density of Vglut1 excitatory buttons onto Parvalbumin interneurons and decreased frequency of spontaneous inhibitory postsynaptic currents. Moreover, we found a decreased number of Parvalbumin positive interneurons in the cortex and altered expression of Calretinin. Interestingly, we failed to detect other alterations in excitatory neurons that were previously reported in ErbB4 null mice suggesting that the Nrg1 haploinsufficiency does not entirely phenocopies ErbB4 deletions. Altogether, this study suggests that Nrg1 haploinsufficiency primarily affects the cortical inhibitory circuits in the cortex and provides new insights into the structural and molecular synaptic impairment caused by NRG1 hypofunction in a preclinical model of SZ.
Neurobiol Dis, 2021; 157

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-385

OPTIMIZED PROTOCOL REVEALS MODULATION OF ANESTHETIC PRECONDITIONING BY GENETIC BACKGROUND IN A DROSOPHILA MELANOGASTER (FRUIT FLY) MODEL OF POLYTRAUMA WITH TRAUMATIC BRAIN INJURY (TBI)

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Anesthetic preconditioning (AP) with volatile general anesthetics (VGAs) prior to injury protects a variety of organs from damage caused by ischemia and subsequent reperfusion. We have previously shown that exposure of flies to the VGAs isoflurane (ISO) and sevoflurane (SEVO) prior to TBI substantially reduces early mortality and extends lifespan (J. Fischer 2018). The aim of this project is to test the hypothesis that the efficacy of AP is determined by genetic factors. Methods: We subjected different Drosophila strains to TBI with or without AP using exposure to different doses of ISO and compared mortality at 24 h after TBI (Mi_{24}). We used young adult (1-7 day old) w^{1118} and Canton-S flies cultured at 25°C on cornmeal-molasses food. TBI was induced using a High-Impact Trauma (HIT) device (R. Katzenberger 2013), anesthesia was administered using the Serial Anesthesia Array (SAA) (Z. Olufs 2018). Results: Maximal suppression of the Mi_{24} was achieved with 30 min of 2% isoflurane. AP reduced Mi_{24} by >50% in w^{1118} but had no effect in Canton-S flies. Conclusions: Our findings are significant because they demonstrate that a brief exposure to VGAs effectively suppresses mortality after TBI. Since cellular and molecular responses to damage are likely to be similar across tissues and evolutionarily conserved between flies and mammals while exposure to anesthetics is largely disregarded in most animal models of TBI, genetic background may further contribute to the difficulty in translating pharmacologic brain protection strategies into clinical practice.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-386

OCULAR MOTOR DEFICITS AND RECOVERY IN AUSTRALIAN RULES FOOTBALL FOLLOWING A SPORTS-RELATED CONCUSSION

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Georgia F Symons[1], William O'Brien[1], Larry Abel[2], Terence O'Brien[1,3], Scott Kolbe[1], Joanne Fielding[1], Sandy Shultz[1], Meaghan Clough[1]
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Aims: Identifying when a sports-related concussion (SRC) has occurred, and determining when an athlete has recovered, remains a challenge for caretakers as clinical practices remain highly subjective. To assess the utility of ocular motor (OM) assessment, a demonstrated objective marker of cognitive function, to identify cognitive abnormalities and monitor recovery after an SRC. **Methods:**18 footballers with an SRC (12 males, 6 females) completed OM tasks at 2-, 6- and 13-days post-SRC, and performance was compared to their baseline (i.e., preseason before SRC) measures. OM tasks included; visually-guided (VG), antisaccade (AS) and memory-guided saccade (MG) and require an eye movement towards, away from, and to a remembered target respectively. Processing speed and visual spatial accuracy on these tasks was compared over time and between sex using a linear mixed effect model. **Results:** AS processing speed in females was significantly worse at 2-days compared to baseline (p<0.001), improving at 7-days (p=0.004), and returning to baseline as 13-days post-SRC (p<0.001). This recovery trajectory aligned with self-reported post-SRC symptoms. However, both males and females demonstrated significantly poorer visual spatial planning at 2-, 7- and 13-days post-SRC compared to baseline (p<0.05). Findings on the VG and MG tasks were of minimal clinical relevance. **Conclusion:** While AS processing speed recovered within 2-week in females, persistent visual spatial deficits may be indicative of prolonged cognitive dysfunction even after symptom resolution. These findings are consistent with emerging literature, and suggest that the AS task may have utility to objectively monitor cognitive abnormalities and recovery post-SRC.

Pubmed:
35176905: Sun M, Symons GF, O'Brien WT, Mccullough J, Aniceto R, Lin IH, Eklund M, Brady RD, Costello D, Chen Z, O'Brien TJ, McDonald SJ, Agoston DV, Shultz SR
Serum Protein Biomarkers of Inflammation, Oxidative Stress, and Cerebrovascular and Glial Injury in Concussed Australian Football Players.
Clinical decisions related to sports-related concussion (SRC) are challenging, because of the heterogenous nature of SRC symptoms coupled with the current reliance on subjective self-reported symptom measures. Sensitive and objective methods that can diagnose SRC and determine recovery would aid clinical management, and there is evidence that SRC induces changes in circulating protein biomarkers, indicative of neuroaxonal injury. However, potential blood biomarkers related to other pathobiological responses linked to SRC are still poorly understood. Therefore, here we analyzed blood samples from concussed (male = 30; female = 9) and non-concussed (male = 74; female = 27) amateur Australian rules football players collected during the pre-season (i.e., baseline), and at 2, 6, and 13 days post-SRC to determine time-dependent changes in serum levels of biomarkers related to glial (i.e., brain lipid-binding protein [BLBP]; phosphoprotein enriched in astrocytes 15) and cerebrovascular injury (i.e., von Willebrand factor, claudin-5), inflammation (i.e., fibrinogen, high mobility group box protein 1), and oxidative stress (i.e., 4-hydroxynoneal). In females, BLBP levels were significantly decreased at 2 days post-SRC compared with their pre-season baseline; however, area under the receiver operating characteristic curve (AUROC) analysis found that BLBP was unable to distinguish between SRC and controls. In males, AUROC analysis revealed a statistically significant change at 2 days post-SRC in the serum levels of 4-hydroxynoneal, however the associated AUROC value (0.6373) indicated little clinical utility for this biomarker in distinguishing SRC from controls. There were no other statistically significant findings. These results indicate that the serum biomarkers tested in this study hold little clinical value in the management of SRC at 2, 6, and 13 days post-injury.
J Neurotrauma, 2022; 39
34595476: Symons GF, Clough M, Mutimer S, Major BP, O'Brien WT, Costello D, McDonald SJ, Chen Z, White O, Mychasiuk R, Law M, Wright DK, O'Brien TJ, Fielding J, Kolbe SC, Shultz SR

Cognitive ocular motor deficits and white matter damage chronically after sports-related concussion.
A history of concussion has been linked to long-term cognitive deficits; however, the neural underpinnings of these abnormalities are poorly understood. This study recruited 26 asymptomatic male Australian footballers with a remote history of concussion (i.e. at least six months since last concussion), and 23 non-collision sport athlete controls with no history of concussion. Participants completed three ocular motor tasks (prosaccade, antisaccade and a cognitively complex switch task) to assess processing speed, inhibitory control and cognitive flexibility, respectively. Diffusion tensor imaging data were acquired using a 3 T MRI scanner, and analysed using tract-based spatial statistics, to investigate white matter abnormalities and how they relate to ocular motor performance. Australian footballers had significantly slower adjusted antisaccade latencies compared to controls (= 0.035). A significant switch cost (i.e. switch trial error > repeat trial error) was also found on the switch task, with Australian footballers performing increased magnitude of errors on prosaccade switch trials relative to prosaccade repeat trials (= 0.023). Diffusion tensor imaging analysis found decreased fractional anisotropy, a marker of white matter damage, in major white matter tracts (i.e. corpus callosum, corticospinal tract) in Australian footballers relative to controls. Notably, a larger prosaccade switch cost was significantly related to reduced fractional anisotropy in anterior white matter regions found to connect to the prefrontal cortex (i.e. a key cortical ocular motor centre involved in executive functioning and task switching). Taken together, Australian footballers with a history of concussion have ocular motor deficits indicative of poorer cognitive processing speed and cognitive flexibility, which are related to reduce white matter integrity in regions projecting to important cognitive ocular motor structures. These findings provide novel insights into the neural mechanisms that may underly chronic cognitive impairments in individuals with a history of concussion.
Brain Commun, 2021; 3
34148076: Major B, Symons GF, Sinclair B, O'Brien WT, Costello D, Wright DK, Clough M, Mutimer S, Sun M, Yamakawa GR, Brady RD, O'Sullivan MJ, Mychasiuk R, McDonald SJ, O'Brien TJ, Law M, Kolbe S, Shultz SR
White and Gray Matter Abnormalities in Australian Footballers With a History of Sports-Related Concussion: An MRI Study.
Sports-related concussion (SRC) is a form of mild traumatic brain injury that has been linked to long-term neurological abnormalities. Australian rules football is a collision sport with wide national participation and is growing in popularity worldwide. However, the chronic neurological consequences of SRC in Australian footballers remain poorly understood. This study investigated the presence of brain abnormalities in Australian footballers with a history of sports-related concussion (HoC) using multimodal MRI. Male Australian footballers with HoC (n = 26), as well as noncollision sport athletes with no HoC (n = 27), were recruited to the study. None of the footballers had sustained a concussion in the preceding 6 months, and all players were asymptomatic. Data were acquired using a 3T MRI scanner. White matter integrity was assessed using diffusion tensor imaging. Cortical thickness, subcortical volumes, and cavum septum pellucidum (CSP) were analyzed using structural MRI. Australian footballers had evidence of widespread microstructural white matter damage and cortical thinning. No significant differences were found regarding subcortical volumes or CSP. These novel findings provide evidence of persisting white and gray matter abnormalities in Australian footballers with HoC, and raise concerns related to the long-term neurological health of these athletes.
Cereb Cortex, 2021; 31
33860291: Wright DK, Symons GF, O'Brien WT, McDonald SJ, Zamani A, Major B, Chen Z, Costello D, Brady RD, Sun M, Law M, O'Brien TJ, Mychasiuk R, Shultz SR
Diffusion Imaging Reveals Sex Differences in the White Matter Following Sports-Related Concussion.
Sports-related concussion (SRC) is a serious health concern. However, the temporal profile of neuropathophysiological changes after SRC and how these relate to biological sex are still poorly understood. This preliminary study investigated whether diffusion-weighted magnetic resonance imaging (dMRI) was sensitive to neuropathophysiological changes following SRC; whether these changes were sex-specific; and whether they persisted beyond the resolution of self-reported symptoms. Recently concussed athletes (n = 14), and age- and education-matched nonconcussed control athletes (n = 16), underwent MRI 24-48-h postinjury and again at 2-week postinjury (i.e., when cleared to return-to-play). Male athletes reported more symptoms and greater symptom severity compared with females. dMRI revealed white matter differences between athletes with SRC and their nonconcussed counterparts at 48-h postinjury. These differences were still present at 2-week postinjury, despite SRC athletes being cleared to return to play and may indicate increased cerebral vulnerability beyond the resolution of subjective symptoms. Furthermore, we identified sex-specific differences, with male SRC athletes having significantly greater white matter disruption compared with female SRC athletes. These results have important implications for the management of concussion, including guiding return-to-play decisions, and further improve our understanding regarding the role of sex in SRC outcomes.
Cereb Cortex, 2021; 31
33422120: McDonald SJ, O'Brien WT, Symons GF, Chen Z, Bain J, Major BP, Costello D, Yamakawa G, Sun M, Brady RD, Mitra B, Mychasiuk R, O'Brien TJ, Shultz SR
Prolonged elevation of serum neurofilament light after concussion in male Australian football players.

Biomarkers that can objectively guide the diagnosis of sports-related concussion, and consequent return-to-play decisions, are urgently needed. In this study, we aimed to determine the temporal profile and diagnostic ability of serum levels of neurofilament light (NfL), ubiquitin carboxy-terminal hydrolase L1 (UCHL1), glial fibrillary acidic protein (GFAP), and tau in concussed male and female Australian footballers.
Biomark Res, 2021; 9
33308001: O'Brien WT, Symons GF, Bain J, Major BP, Costello DM, Sun M, Kimpton JS, Chen Z, Brady RD, Mychasiuk R, O'Brien TJ, Monif M, Shultz SR, McDonald SJ
Elevated Serum Interleukin-1β Levels in Male, but not Female, Collision Sport Athletes with a Concussion History.
It is increasingly reported that a history of concussion may be associated with chronic deleterious consequences. While the pathophysiology that contributes to these consequences is not well understood, neuroinflammation is postulated to be critical. Activation of multi-protein complexes termed inflammasomes, a key component of this inflammatory response, has been reported in more severe TBIs; however, it has not been investigated in milder TBIs, such as concussion. This study investigated serum levels of interleukin (IL)-1β and IL-18 (key proteins activated downstream of these inflammasomes) at acute, sub-acute, and chronic time-points post-concussion. We recruited 105 Australian footballers (65 male, 40 female) during the pre-season, then prospectively followed these players for the occurrence of concussion during the season. At baseline, 58 footballers reported a previous concussion history, and 47 reported no previous concussion history. Additionally, 25 players sustained a mid-season concussion and were sampled at 2, 6, and 13 days post-concussion. Serum levels of IL-1β and IL-18 were quantified using highly sensitive Simoa HD-X Analyzer assays. At baseline, IL-1β levels were higher in male, but not female, footballers with a previous concussion history compared with footballers with no concussion history. There was also a positive correlation between years of collision sport participation and IL-18 levels in males. No evidence was found in males or females to indicate that IL-1β or IL-18 levels differed at 2, 6, or 13 days post-concussion. These findings provide novel insights into potential sex-specific physiological consequences of concussion, and suggest that neuroinflammation may be persistent chronically following concussion in male athletes.
J Neurotrauma, 2021; 38
33117257: Major BP, McDonald SJ, O'Brien WT, Symons GF, Clough M, Costello D, Sun M, Brady RD, Mccullough J, Aniceto R, Lin IH, Law M, Mychasiuk R, O'Brien TJ, Agoston DV, Shultz SR
Serum Protein Biomarker Findings Reflective of Oxidative Stress and Vascular Abnormalities in Male, but Not Female, Collision Sport Athletes.
Studies have indicated that concussive and sub-concussive brain injuries that are frequent during collision sports may lead to long-term neurological abnormalities, however there is a knowledge gap on how biological sex modifies outcomes. Blood-based biomarkers can help to identify the molecular pathology induced by brain injuries and to better understand how biological sex affects the molecular changes. We therefore analyzed serum protein biomarkers in male (= 50) and female (= 33) amateur Australian rules footballers (i.e., Australia's most participated collision sport), both with a history of concussion (HoC) and without a history of concussion (NoHoC). These profiles were compared to those of age-matched control male (= 24) and female (= 20) athletes with no history of neurotrauma or participation in collision sports. Serum levels of protein markers indicative of neuronal, axonal and glial injury (UCH-L1, NfL, tau, p-tau, GFAP, BLBP, PEA15), metabolic (4-HNE) and vascular changes (VEGF-A, vWF, CLDN5), and inflammation (HMGB1) were assessed using reverse phase protein microarrays. Male, but not female, footballers had increased serum levels of VEGF-A compared to controls regardless of concussion history. In addition, only male footballers who had HoC had increased serum levels of 4-HNE. These findings being restricted to males may be related to shorter collision sport career lengths for females compared to males. In summary, these findings show that male Australian rules footballers have elevated levels of serum biomarkers indicative of vascular abnormalities (VEGF-A) and oxidative stress (4-HNE) in comparison to non-collision control athletes. While future studies are required to determine how these findings relate to neurological function, serum levels of VEGF-A and 4-HNE may be useful to monitor subclinical neurological injury in males participating in collision sports.
Front Neurol, 2020; 11
32252777: O'Brien WT, Pham L, Symons GF, Monif M, Shultz SR, McDonald SJ
The NLRP3 inflammasome in traumatic brain injury: potential as a biomarker and therapeutic target.
There is a great clinical need to identify the underlying mechanisms, as well as related biomarkers, and treatment targets, for traumatic brain injury (TBI). Neuroinflammation is a central pathophysiological feature of TBI. NLRP3 inflammasome activity is a necessary component of the innate immune response to tissue damage, and dysregulated inflammasome activity has been implicated in a number of neurological conditions. This paper introduces the NLRP3 inflammasome and its implication in the pathogenesis of neuroinflammatory-related conditions, with a particular focus on TBI. Although its role in TBI has only recently been identified, findings suggest that priming and activation of the NLRP3 inflammasome are upregulated following TBI. Moreover, recent studies utilizing specific NLRP3 inhibitors have provided further evidence that this inflammasome is a major driver of neuroinflammation and neurobehavioral disturbances following TBI. In addition, there is emerging evidence

that circulating inflammasome-associated proteins may have utility as diagnostic biomarkers of neuroinflammatory conditions, including TBI. Finally, novel and promising areas of research will be highlighted, including the potential involvement of the NLRP3 inflammasome in mild TBI, how factors such as biological sex may affect NLRP3 activity in TBI, and the use of emerging biomarker platforms. Taken together, this review highlights the exciting potential of the NLRP3 inflammasome as a target for treatments and biomarkers that may ultimately be used to improve TBI management.
J Neuroinflammation, 2020; 17

32056505: Symons GF, Clough M, Fielding J, O'Brien WT, Shepherd CE, Wright DK, Shultz SR
The Neurological Consequences of Engaging in Australian Collision Sports.
Collision sports are an integral part of Australian culture. The most common collision sports in Australia are Australian rules football, rugby union, and rugby league. Each of these sports often results in participants sustaining mild brain traumas, such as concussive and subconcussive injuries. However, the majority of previous studies and reviews pertaining to the neurological implications of sustaining mild brain traumas, while engaging in collision sports, have focused on those popular in North America and Europe. As part of this 2020 International Neurotrauma Symposium special issue, which highlights Australian neurotrauma research, this article will therefore review the burden of mild brain traumas in Australian collision sports athletes. Specifically, this review will first provide an overview of the consequences of mild brain trauma in Australian collision sports, followed by a summary of the previous studies that have investigated neurocognition, ocular motor function, neuroimaging, and fluid biomarkers, as well as neuropathological outcomes in Australian collision sports athletes. A review of the literature indicates that although Australians have contributed to the field, several knowledge gaps and limitations currently exist. These include important questions related to sex differences, the identification and implementation of blood and imaging biomarkers, the need for consistent study designs and common data elements, as well as more multi-modal studies. We conclude that although Australia has had an active history of investigating the neurological impact of collision sports participation, further research is clearly needed to better understand these consequences in Australian athletes and how they can be mitigated.
J Neurotrauma, 2020; 37

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-387

SERUM NFL AND GFAP TRAJECTORIES AFTER SPORTS-RELATED CONCUSSION IN AUSTRALIAN FOOTBALL PLAYERS

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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[1]Monash University, Department Of Neuroscience, Melbourne, Australia, [2]The Alfred Hospital, Emergency & Trauma Centre, Melbourne, Australia, [3]The Alfred Hospital, National Trauma Research Institute, Melbourne, Australia, [4]Monash University, School Of Public Health & Preventitive Medicine, Melbourne, Australia, [5]Alfred Health, Neurology, Melbourne, Australia, [6]Monash University, Neuroscience, Melbourne, Australia

Introduction: The diagnosis and management of sports-related concussion (SRC) remains notoriously difficult due to a significant reliance on subjective signs and symptoms. Recent studies have shown that blood levels of glial fibrillary acidic protein (GFAP) and neurofilament light (NfL) are promising indicators of neuropathology after SRC. Nonetheless, greater understanding of their temporal profiles, and association with clinical and neuropathological recovery, are required to determine exactly when and how these biomarkers might be used to assist clinical decisions. **Aim:** To generate the most comprehensive profile of serum GFAP and NfL changes after SRC to date. **Methods:** Male and female Australian football players with SRC were recruited from amateur Australian football clubs in the 2021 and 2022 seasons. Players that had sustained a musculoskeletal injury, and un-injured players, were recruited as controls. Blood was collected for serum GFAP and NfL quantification with SIMOA assays at 24-hours, and 1-, 2-, 4-, 6-, 8-, 12-, and 26-weeks. SRC-related symptoms and neurocognitive measures were also assessed. **Results:** Recruitment and analysis for this study is ongoing (2021 SRC n = 35); however, group level analysis revealed an acute rise and rapid resolution of serum GFAP after SRC, and a delayed but prolonged elevation of serum NfL. Some heterogeneity was apparent, with preliminary trajectory analysis revealing the likely presence of distinct clusters of biomarker trajectories. Analysis of these profiles and associations with clinical outcomes is ongoing. **Conclusion:** This study provides further evidence of the potential of serum GFAP and NfL as sensitive indicators of neuropathology after SRC.

Pubmed:
35176905: Sun M, Symons GF, O'Brien WT, Mccullough J, Aniceto R, Lin IH, Eklund M, Brady RD, Costello D, Chen Z, O'Brien TJ, McDonald SJ, Agoston DV, Shultz SR
Serum Protein Biomarkers of Inflammation, Oxidative Stress, and Cerebrovascular and Glial Injury in Concussed Australian Football Players.
Clinical decisions related to sports-related concussion (SRC) are challenging, because of the heterogenous nature of SRC symptoms coupled with the current reliance on subjective self-reported symptom measures. Sensitive and objective methods that can diagnose SRC and determine recovery would aid clinical management, and there is evidence that SRC induces changes in circulating protein biomarkers, indicative of neuroaxonal injury. However, potential blood biomarkers related to other pathobiological responses linked to SRC are still poorly understood. Therefore, here we analyzed blood samples from concussed (male = 30; female = 9) and non-concussed (male = 74; female = 27) amateur Australian rules football players collected during the pre-season (i.e., baseline), and at 2, 6, and 13 days post-SRC to determine time-dependent changes in serum levels of biomarkers related to glial (i.e., brain lipid-binding protein [BLBP]; phosphoprotein enriched in astrocytes 15) and cerebrovascular injury (i.e., von Willebrand factor, claudin-5), inflammation (i.e., fibrinogen, high mobility group box protein 1), and oxidative stress (i.e., 4-hydroxynoneal). In females, BLBP levels were significantly decreased at 2 days post-SRC compared with their pre-season baseline; however, area under the receiver operating characteristic curve (AUROC) analysis found that BLBP was unable to distinguish between SRC and controls. In males, AUROC analysis revealed a statistically significant change at 2 days post-SRC in the serum levels of 4-hydroxynoneal, however the associated AUROC value (0.6373) indicated little clinical utility for this biomarker in distinguishing SRC from controls. There were no other statistically significant findings. These results indicate that the serum biomarkers tested in this study hold little clinical value in the management of SRC at 2, 6, and 13 days post-injury.
J Neurotrauma, 2022; 39

34929656: Mitra B, Reyes J, O'Brien WT, Surendran N, Carter A, Bain J, McEntaggart L, Sorich E, Shultz SR, O'Brien TJ, Willmott C, Rosenfeld JV, McDonald SJ
Micro-RNA levels and symptom profile after mild traumatic brain injury: A longitudinal cohort study.
Micro riboneucleic acids (miRNAs) may be transcribed after brain injury and be detectable in plasma. This study aimed to assess the discriminative ability of seven miRNAs in plasma to differentiate between patients with mild traumatic brain injury (mTBI) and healthy controls. Changes in miRNA levels over 28 days were compared to changes in self-reported symptom profile. This was a prospective cohort study with longitudinal measurements of miRNA levels and symptom self-report. The Rivermead Post-Concussion Symptom Questionnaire (RPQ) was used to determine symptom severity. Mean normalised expression ratios (NER) of miRNAs at day 0 between mTBI and healthy controls were compared. An analysis of response profiles compared the response over time of miRNA species with RPQ symptom severity. miRNA levels of subjects who were defined to have "recovered" on Day 7 and 28 were compared to "non-recovered" subjects. There were 28 mTBI patients and 30 healthy controls included for analysis. Symptom severity was significantly higher on the day of injury among mTBI subjects ($p < 0.001$), and miRNA 32-5p levels were also higher ($p = 0.009$). Change of miRNA levels were similar to RPQ change at Day 7, but significantly different at Day 28. Differences were observed among miRNA levels of recovered subjects. This study demonstrated differences in miRNA levels among mTBI subjects compared to healthy controls and different miRNA levels among those who had recovered compared to those reporting symptoms. The change in profiles of miRNAs was different to symptom severity, suggesting that the two measures reflect different aspects of brain injury and recovery.
J Clin Neurosci, 2022; 95
34720579: Wong KR, O'Brien WT, Sun M, Yamakawa G, O'Brien TJ, Mychasiuk R, Shultz SR, McDonald SJ, Brady RD
Serum Neurofilament Light as a Biomarker of Traumatic Brain Injury in the Presence of Concomitant Peripheral Injury.
Serum neurofilament light (NfL) is an emerging biomarker of traumatic brain injury (TBI). However, the effect of peripheral injuries such as long bone fracture and skeletal muscle injury on serum NfL levels is unknown. Therefore, the aim of this study was to determine whether serum NfL levels can be used as a biomarker of TBI in the presence of concomitant peripheral injuries.
Biomark Insights, 2021; 16
33860291: Wright DK, Symons GF, O'Brien WT, McDonald SJ, Zamani A, Major B, Chen Z, Costello D, Brady RD, Sun M, Law M, O'Brien TJ, Mychasiuk R, Shultz SR
Diffusion Imaging Reveals Sex Differences in the White Matter Following Sports-Related Concussion.
Sports-related concussion (SRC) is a serious health concern. However, the temporal profile of neuropathophysiological changes after SRC and how these relate to biological sex are still poorly understood. This preliminary study investigated whether diffusion-weighted magnetic resonance imaging (dMRI) was sensitive to neuropathophysiological changes following SRC; whether these changes were sex-specific; and whether they persisted beyond the resolution of self-reported symptoms. Recently concussed athletes ($n = 14$), and age- and education-matched nonconcussed control athletes ($n = 16$), underwent MRI 24-48-h postinjury and again at 2-week postinjury (i.e., when cleared to return-to-play). Male athletes reported more symptoms and greater symptom severity compared with females. dMRI revealed white matter differences between athletes with SRC and their nonconcussed counterparts at 48-h postinjury. These differences were still present at 2-week postinjury, despite SRC athletes being cleared to return to play and may indicate increased cerebral vulnerability beyond the resolution of subjective symptoms. Furthermore, we identified sex-specific differences, with male SRC athletes having significantly greater white matter disruption compared with female SRC athletes. These results have important implications for the management of concussion, including guiding return-to-play decisions, and further improve our understanding regarding the role of sex in SRC outcomes.
Cereb Cortex, 2021; 31
33727100: O'Brien WT, Pham L, Brady RD, Bain J, Yamakawa GR, Sun M, Mychasiuk R, O'Brien TJ, Monif M, Shultz SR, McDonald SJ
Temporal profile and utility of serum neurofilament light in a rat model of mild traumatic brain injury.
There is a widely recognized need for blood biomarkers to assist clinical decisions surrounding mild traumatic brain injury (mTBI). Serum neurofilament light (NfL), an indicator of neuroaxonal damage, is one such candidate, with early mTBI clinical investigations demonstrating significant promise. To facilitate the translation of pre-clinical mTBI findings, clinically relevant outcomes should be integrated into animal studies wherever possible. Despite this, the temporal profile and potential utility of NfL as a blood biomarker in pre-clinical mTBI is poorly understood. Here, we quantified serum NfL at 2-h, 1-, 3-, 7- and 14-days following mTBI in rats and compared these to pre-injury levels. We also investigated cumulative effects of repeat-mTBI by delivering 0, 1 or 5 mTBIs separated by 24 h. Sensorimotor performance was evaluated with the beam task at 1- and 4-h after mTBI, and serum was collected 1-day after the final procedure. We found that serum NfL levels were substantially elevated at all acute and sub-acute time-points after a single-mTBI, peaked at 1-day, and remained elevated 14-days post-injury. An mTBI dose-dependent effect on serum NfL levels was also observed, with substantially higher NfL levels found at

1-day post repeat-mTBI when compared to single-mTBI and sham-injured rats. Furthermore, NfL levels were found to be greatest in rats with the highest degree of sensorimotor impairment. In conclusion, these findings have described the temporal profile of serum NfL elevations following a single-mTBI in rats, and indicate a profile with some similarities and differences to that seen in the clinical condition. Moreover, we found that serum NfL levels were potentiated by repeat-mTBI, and that this biomarker may have utility as an indicator of injury severity. As such, future pre-clinical TBI studies may benefit from incorporating measures of serum NfL as an objective injury outcome.
Exp Neurol, 2021; 341
33422120: McDonald SJ, O'Brien WT, Symons GF, Chen Z, Bain J, Major BP, Costello D, Yamakawa G, Sun M, Brady RD, Mitra B, Mychasiuk R, O'Brien TJ, Shultz SR
Prolonged elevation of serum neurofilament light after concussion in male Australian football players.
Biomarkers that can objectively guide the diagnosis of sports-related concussion, and consequent return-to-play decisions, are urgently needed. In this study, we aimed to determine the temporal profile and diagnostic ability of serum levels of neurofilament light (NfL), ubiquitin carboxy-terminal hydrolase L1 (UCHL1), glial fibrillary acidic protein (GFAP), and tau in concussed male and female Australian footballers.
Biomark Res, 2021; 9
33308001: O'Brien WT, Symons GF, Bain J, Major BP, Costello DM, Sun M, Kimpton JS, Chen Z, Brady RD, Mychasiuk R, O'Brien TJ, Monif M, Shultz SR, McDonald SJ
Elevated Serum Interleukin-1β Levels in Male, but not Female, Collision Sport Athletes with a Concussion History.
It is increasingly reported that a history of concussion may be associated with chronic deleterious consequences. While the pathophysiology that contributes to these consequences is not well understood, neuroinflammation is postulated to be critical. Activation of multi-protein complexes termed inflammasomes, a key component of this inflammatory response, has been reported in more severe TBIs; however, it has not been investigated in milder TBIs, such as concussion. This study investigated serum levels of interleukin (IL)-1β and IL-18 (key proteins activated downstream of these inflammasomes) at acute, sub-acute, and chronic time-points post-concussion. We recruited 105 Australian footballers (65 male, 40 female) during the pre-season, then prospectively followed these players for the occurrence of concussion during the season. At baseline, 58 footballers reported a previous concussion history, and 47 reported no previous concussion history. Additionally, 25 players sustained a mid-season concussion and were sampled at 2, 6, and 13 days post-concussion. Serum levels of IL-1β and IL-18 were quantified using highly sensitive Simoa HD-X Analyzer assays. At baseline, IL-1β levels were higher in male, but not female, footballers with a previous concussion history compared with footballers with no concussion history. There was also a positive correlation between years of collision sport participation and IL-18 levels in males. No evidence was found in males or females to indicate that IL-1β or IL-18 levels differed at 2, 6, or 13 days post-concussion. These findings provide novel insights into potential sex-specific physiological consequences of concussion, and suggest that neuroinflammation may be persistent chronically following concussion in male athletes.
J Neurotrauma, 2021; 38
33127468: Pham L, Wright DK, O'Brien WT, Bain J, Huang C, Sun M, Casillas-Espinosa PM, Shah AD, Schittenhelm RB, Sobey CG, Brady RD, O'Brien TJ, Mychasiuk R, Shultz SR, McDonald SJ
Behavioral, axonal, and proteomic alterations following repeated mild traumatic brain injury: Novel insights using a clinically relevant rat model.
A history of mild traumatic brain injury (mTBI) is linked to a number of chronic neurological conditions, however there is still much unknown about the underlying mechanisms. To provide new insights, this study used a clinically relevant model of repeated mTBI in rats to characterize the acute and chronic neuropathological and neurobehavioral consequences of these injuries. Rats were given four sham-injuries or four mTBIs and allocated to 7-day or 3.5-months post-injury recovery groups. Behavioral analysis assessed sensorimotor function, locomotion, anxiety, and spatial memory. Neuropathological analysis included serum quantification of neurofilament light (NfL), mass spectrometry of the hippocampal proteome, and ex vivo magnetic resonance imaging (MRI). Repeated mTBI rats had evidence of acute cognitive deficits and prolonged sensorimotor impairments. Serum NfL was elevated at 7 days post injury, with levels correlating with sensorimotor deficits; however, no NfL differences were observed at 3.5 months. Several hippocampal proteins were altered by repeated mTBI, including those associated with energy metabolism, neuroinflammation, and impaired neurogenic capacity. Diffusion MRI analysis at 3.5 months found widespread reductions in white matter integrity. Taken together, these findings provide novel insights into the nature and progression of repeated mTBI neuropathology that may underlie lingering or chronic neurobehavioral deficits.
Neurobiol Dis, 2021; 148
33117257: Major BP, McDonald SJ, O'Brien WT, Symons GF, Clough M, Costello D, Sun M, Brady RD, Mccullough J, Aniceto R, Lin IH, Law M, Mychasiuk R, O'Brien TJ, Agoston DV, Shultz SR
Serum Protein Biomarker Findings Reflective of Oxidative Stress and Vascular Abnormalities in Male, but Not Female, Collision Sport Athletes.

Studies have indicated that concussive and sub-concussive brain injuries that are frequent during collision sports may lead to long-term neurological abnormalities, however there is a knowledge gap on how biological sex modifies outcomes. Blood-based biomarkers can help to identify the molecular pathology induced by brain injuries and to better understand how biological sex affects the molecular changes. We therefore analyzed serum protein biomarkers in male (= 50) and female (= 33) amateur Australian rules footballers (i.e., Australia's most participated collision sport), both with a history of concussion (HoC) and without a history of concussion (NoHoC). These profiles were compared to those of age-matched control male (= 24) and female (= 20) athletes with no history of neurotrauma or participation in collision sports. Serum levels of protein markers indicative of neuronal, axonal and glial injury (UCH-L1, NfL, tau, p-tau, GFAP, BLBP, PEA15), metabolic (4-HNE) and vascular changes (VEGF-A, vWF, CLDN5), and inflammation (HMGB1) were assessed using reverse phase protein microarrays. Male, but not female, footballers had increased serum levels of VEGF-A compared to controls regardless of concussion history. In addition, only male footballers who had HoC had increased serum levels of 4-HNE. These findings being restricted to males may be related to shorter collision sport career lengths for females compared to males. In summary, these findings show that male Australian rules footballers have elevated levels of serum biomarkers indicative of vascular abnormalities (VEGF-A) and oxidative stress (4-HNE) in comparison to non-collision control athletes. While future studies are required to determine how these findings relate to neurological function, serum levels of VEGF-A and 4-HNE may be useful to monitor subclinical neurological injury in males participating in collision sports.
Front Neurol, 2020; 11

32252777: O'Brien WT, Pham L, Symons GF, Monif M, Shultz SR, McDonald SJ
The NLRP3 inflammasome in traumatic brain injury: potential as a biomarker and therapeutic target.
There is a great clinical need to identify the underlying mechanisms, as well as related biomarkers, and treatment targets, for traumatic brain injury (TBI). Neuroinflammation is a central pathophysiological feature of TBI. NLRP3 inflammasome activity is a necessary component of the innate immune response to tissue damage, and dysregulated inflammasome activity has been implicated in a number of neurological conditions. This paper introduces the NLRP3 inflammasome and its implication in the pathogenesis of neuroinflammatory-related conditions, with a particular focus on TBI. Although its role in TBI has only recently been identified, findings suggest that priming and activation of the NLRP3 inflammasome are upregulated following TBI. Moreover, recent studies utilizing specific NLRP3 inhibitors have provided further evidence that this inflammasome is a major driver of neuroinflammation and neurobehavioral disturbances following TBI. In addition, there is emerging evidence that circulating inflammasome-associated proteins may have utility as diagnostic biomarkers of neuroinflammatory conditions, including TBI. Finally, novel and promising areas of research will be highlighted, including the potential involvement of the NLRP3 inflammasome in mild TBI, how factors such as biological sex may affect NLRP3 activity in TBI, and the use of emerging biomarker platforms. Taken together, this review highlights the exciting potential of the NLRP3 inflammasome as a target for treatments and biomarkers that may ultimately be used to improve TBI management.
J Neuroinflammation, 2020; 17

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BOARD NUMBER: S04-388

THIS IS AN INJURED BRAIN ON DRUGS: EXAMINING THE EFFECTS OF MODAFINIL ADMINISTRATION ON REPETITIVE MILD TRAUMATIC BRAIN INJURY OUTCOMES IN ADULT RATS

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Mild traumatic brain injury (mTBI) is one of the most common neurological disorders, with an exceptionally high incidence rate among males. Modafinil is a wake-promoting substance prescribed to treat hypersomnolence. Modafinil has been found to modulate the immune response while reducing neuroinflammation and neurotoxicity. Since mTBI-associated impairments are induced by these changes, modafinil may be a promising therapeutic prospect. AIM: To examine the effect of chronic modafinil administration on RmTBI-induced behavioural outcomes, and proteomic and metabolomic CSF profile changes in adult male rats. METHODS: An intracerebroventricular (ICV) cannula was implanted into the lateral ventricle of adult male Sprague Dawley rats. Animals underwent either RmTBIs or sham injuries, with injuries being 48 hours apart. An acute behavioural test battery confirmed RmTBI-induced impairments. Animals then received intermittent ICV injections of modafinil or vehicle at the beginning of the light phase. A second behavioural test battery was initiated one week post-ICV injections. CSF was collected at the end of the study to analyze proteomic and metabolomic profiles. RESULTS: Significant ($p<.05$) improvements observed in OF distance travelled, EPM time in open arms, Y maze novelty preference, brain weight, and spleen weight indicate some beneficial effects of modafinil administration for both the sham and RmTBI animals. Injury- and treatment-associated changes ($p<.05$) in the CSF profiles of several proteins and metabolites, including AQP1, thiamine, and CD5L, were also observed. CONCLUSION: Modafinil's ability to modulate the immune response and neuroinflammation likely assists in diminishing deleterious effects induced by RmTBI. Therefore, modafinil exhibits therapeutic potential in treating RmTBI.

Pubmed:

34968296: Christensen J, Beveridge JK, Wang M, Orr SL, Noel M, Mychasiuk R
A Pilot Study Investigating the Role of Gender in the Intergenerational Relationships between Gene Expression, Chronic Pain, and Adverse Childhood Experiences in a Clinical Sample of Youth with Chronic Pain.
Chronic pain is a highly prevalent and costly issue that often emerges during childhood or adolescence and persists into adulthood. Adverse childhood experiences (ACEs) increase risk for several adverse health conditions, including chronic pain. Recent evidence suggests that parental trauma (ACEs, post-traumatic stress disorder (PTSD) symptoms) confers risk of poor health outcomes in their children. Intergenerational relationships between parental trauma and child chronic pain may be mediated by epigenetic mechanisms. A clinical sample of youth with chronic pain and their parents completed psychometrically sound questionnaires assessing ACEs, PTSD symptoms, and chronic pain, and provided a saliva sample. These were used to investigate the intergenerational relationships between four epigenetic biomarkers (COMT, DRD2, GR, and SERT), trauma, and chronic pain. The results indicated that the significant biomarkers were dependent upon the gender of the child, wherein parental ACEs significantly correlated with changes in DRD2 expression in female children and altered COMT expression in the parents of male children. Additionally, the nature of the ACE (maltreatment vs. household dysfunction) was associated with the specific epigenetic changes. There may be different pathways through which parental ACEs confer risk for poor outcomes for males and females, highlighting the importance of child gender in future investigations.
Epigenomes, 2021; 5
32991958: Christensen J, Yamakawa GR, Shultz SR, Mychasiuk R
Is the glymphatic system the missing link between sleep impairments and neurological disorders? Examining the implications and uncertainties.
Until recently, both the purpose of the biological need for sleep and the mechanism by which the central nervous system eliminated metabolic waste products were unknown. The glymphatic system is the recently discovered macroscopic waste clearance system for the CNS, which predominantly functions during sleep states. Important implications for the glymphatic system exist for a significant proportion of neurological disorders, including traumatic brain injury, epilepsy, stroke, migraine,

and Alzheimer's disease. Within the limited amount of research pertaining to this novel system there exists controversy regarding several of the key structural and functional aspects of the glymphatic system. In this review we address evidence from both standpoints regarding the prominent debates surrounding the glymphatic system, including the functional differences in wakefulness vs. sleep, the role of glial aquaporin-4 water channels, and whether it reflects a convective flow or a passive diffusion process. The answers that underlie these questions will have crucial and distinct outcomes for the future of the glymphatic system and the disorders it has been implicated in. However, this review also summarizes the potential role of the glymphatic system in the development and progression of the aforementioned neurological disorders. Furthermore, the possible contribution of the orexinergic system to this relationship between the glymphatic system, sleep, and these neurological disorders is also explored. Overall, in order to develop and utilize therapeutic interventions centred around the glymphatic system we must first dedicate further investigation to elucidating these discrepancies and unanswered questions.
Prog Neurobiol, 2021; 198

32411081: Shafqat Q, Christensen J, Hamilton AM, Imhof E, Mychasiuk RM, Dunn JF
Acute Dilation of Venous Sinuses in Animal Models of Mild Traumatic Brain Injury Detected Using 9.4T MRI.
Mild traumatic brain injury (mTBI) is a debilitating but extremely common form of brain injury that affects a substantial number of people each year. mTBI is especially common in children and adolescents. Our understanding of mTBI pathophysiology is limited, and there is currently no accepted marker for disease severity. A potential marker for disease severity may be cerebrovascular dysfunction. Recent findings have implicated cerebrovascular alteration as an important component of mTBI and suggest it contributes to the development of persistent, long-term symptoms. In this paper, we conducted two studies to investigate whether mTBI affects venous drainage patterns in the central nervous system using alterations in the size of venous sinuses as a marker of changes in drainage. Using a closed head vertical weight-drop model and a lateral impact injury model of mTBI, we imaged and quantified the size of three major draining vessels in the adolescent rat brain using 9.4T MRI. Areas and volumes were quantified in the superior sagittal sinus and left and right transverse sinuses using images acquired from T2w MRI in one study and post-gadolinium T1w MRI in another. Our results indicated that the three venous sinuses were significantly larger in mTBI rats as compared to sham rats 1-day post injury but recovered to normal size 2 weeks after. Acutely enlarged sinuses post-mTBI may indicate abnormal venous drainage, and this could be suggestive of a cerebrovascular response to trauma.
Front Neurol, 2020; 11

32354109: Tabor J, Wright DK, Christensen J, Zamani A, Collins R, Shultz SR, Mychasiuk R
Examining the Effects of Anabolic-Androgenic Steroids on Repetitive Mild Traumatic Brain Injury (RmTBI) Outcomes in Adolescent Rats.
Repetitive mild traumatic brain injury (RmTBI) is increasingly common in adolescents. Anabolic-androgenic steroid (AAS) consumption among younger professional athletes is a significant risk factor for impaired neurodevelopment. Given the increased rates and overlapping symptomology of RmTBI and AAS use, we sought to investigate the behavioural and neuropathological outcomes associated with the AAS Metandienone (Met) and RmTBI on rats.
Brain Sci, 2020; 10

32277097: Christensen J, Wright DK, Yamakawa GR, Shultz SR, Mychasiuk R
Repetitive Mild Traumatic Brain Injury Alters Glymphatic Clearance Rates in Limbic Structures of Adolescent Female Rats.
The glymphatic system is the macroscopic waste clearance system for the central nervous system. Glymphatic dysfunction has been linked to several neurological conditions, including traumatic brain injury (TBI). Adolescents are at particularly high risk for experiencing a TBI, particularly mild TBI (mTBI) and repetitive mTBI (RmTBI); however, glymphatic clearance, and how it relates to behavioral outcomes, has not been investigated in this context. Therefore, this study examined glymphatic function in the adolescent brain following RmTBI. Female adolescent Sprague Dawley rats were subjected to either three mTBIs or sham injuries spaced three days apart. One-day after their final injury, the animals underwent a beam walking task to assess sensorimotor function, and contrast-enhanced MRI to visualize glymphatic clearance rate. Behavioural measures indicated that the RmTBI group displayed an increase in loss of consciousness as well as motor coordination and balance deficits consistent with our previous studies. The contrast-enhanced MRI results indicated that the female adolescent glymphatic system responds to RmTBI in a region-specific manner, wherein an increased influx but reduced efflux was observed throughout limbic structures (hypothalamus, hippocampus, and amygdala) and the olfactory bulb but neither the influx or efflux were altered in the cortical structures (primary motor cortex, insular cortex, and dorsolateral prefrontal cortex) examined. This may indicate a role for an impaired and/or inefficient glymphatic system in the limbic structures and cortical structures, respectively, in the development of post-concussive symptomology during adolescence.
Sci Rep, 2020; 10

31705690: Christensen J, Yamakawa GR, Salberg S, Wang M, Kolb B, Mychasiuk R
Caffeine consumption during development alters spine density and recovery from repetitive mild traumatic brain injury in young adult rats.

Caffeine is the most commonly used psychostimulant throughout the world, with its consumption being especially prevalent among adolescents and young adults, as over 75% of this group consumes caffeine daily. Similarly, the adolescent and young adult age group exhibit the highest incidence of traumatic brain injury (TBI). Given that both caffeine consumption and mild TBI (mTBI) are more prevalent among the late adolescent/young adult age group and that changes in dendritic spine morphology during this developmental period are poorly understood, this study sought to examine the effects of caffeine consumption during late adolescence/early adulthood on recovery from repetitive mTBI (RmTBI). The study specifically focused on changes to neuronal dendritic morphology as synaptic changes likely underlie long-term behavioral outcomes. The results demonstrate that during young adulthood caffeine consumption differentially affects the RmTBI outcomes of males and females, where the effects of caffeine and RmTBI were often additive in males while being equally detrimental, but rarely additive, in females. In general, caffeine and RmTBI induced the greatest impairments in males on cognitive and motor tasks whereas in females the most significant detriments were on pain-related tasks. Both caffeine and RmTBI increased spine density in the Cg3 (medial prefrontal cortex [mPFC]), AID (orbitofrontal cortex [OFC]), and nucleus accumbens (NAc), which is proposed to reflect an impairment in the normal pruning processes. Overall, despite caffeine's neuroprotective abilities among other age groups, this study offers concerning results regarding the detrimental effects of caffeine and RmTBI, in isolation, and especially in combination, in this susceptible population.
Synapse, 2020; 74

31418318: Christensen J, Eyolfson E, Salberg S, Bhatt D, Weerawardhena H, Tabor J, Mychasiuk R
When Two Wrongs Make a Right: The Effect of Acute and Chronic Binge Drinking on Traumatic Brain Injury Outcomes in Young Adult Female Rats.
Alcohol is the most commonly abused drug by young adults across North America. Although alcohol consumption itself incurs a risk of neurological damage, it is also a significant risk factor for traumatic brain injury (TBI). TBI among young adults is described as a modern healthcare epidemic. The drastic changes occurring within their neurological networks put young adults at greater risk for developing long-term post-traumatic deficits. Contradictory findings have been indicated regarding the effects of alcohol consumption on TBI outcomes in adults, with some studies demonstrating detrimental effects, whereas others suggest neuroprotective abilities. However, little is known about the effects of alcohol consumption on TBI outcomes during the sensitive stage of early adulthood. Young adult female Sprague-Dawley rats were randomly assigned to one of six experimental conditions: Pre-injury alcohol+TBI; Pre-injury alcohol+Sham; Pre- and Post-injury alcohol+TBI; Pre- and Post-injury alcohol+Sham; No alcohol+TBI; No alcohol+Sham. Alcohol consumption groups received an amount of 10% v/v ethanol solution based on the animals' weight. Following the injury, the rats were subjected to a behavioral test battery to assess post-concussive symptomology. Overall, chronic binge drinking significantly improved TBI outcomes related to motor coordination and balance, whereas binge drinking in general significantly decreased anxiety-like behaviors. Additionally, in many cases, chronic binge drinking appears to return the TBI animal's behavioral outcomes to levels comparable to those of the no alcohol sham animals. Thus, the results suggest that alcohol may exhibit neuroprotective abilities in the context of early adulthood TBI.
J Neurotrauma, 2020; 37

30621863: Christensen J, Noel M, Mychasiuk R
Neurobiological mechanisms underlying the sleep-pain relationship in adolescence: A review.
Adolescence characterizes a period of significant change in brain structure and function, causing the neural circuitry to be particularly susceptible to the environment and various other experiences. Chronic pain and sleep deprivation represent major health issues that plague adolescence. A bidirectional relationship exists between sleep and pain; however, emerging evidence suggests that sleep disturbances have a stronger influence on subsequent pain than vice versa. The neurobiological underpinnings of this relationship, particularly during adolescence, are poorly understood. This review examines the current literature regarding sleep and pain in adolescence, with a particular focus on the neurobiological mechanisms underlying pain, sleep problems, and the neural circuitry that potentially links the two. Finally, a research agenda is outlined to stimulate future research on this topic. Given the high prevalence of these health issues during adolescence and the debilitating effects they inflict on nearly every domain of development, it is crucial that we determine the neurobiological mechanisms fundamental to this relationship and identify potential therapeutic strategies.
Neurosci Biobehav Rev, 2019; 96

30074871: Salberg S, Christensen J, Yamakawa GR, Lengkeek C, Malik H, Tabor J, Hazari A, Mychasiuk R
A Bump on the Head or Late to Bed: Behavioral and Pathophysiological Effects of Sleep Deprivation after Repetitive Mild Traumatic Brain Injury in Adolescent Rats.
An old wives' tale, and strongly held dogma, maintains that one should be kept awake after a mild traumatic brain injury (mTBI) to prevent a coma. This, however, conflicts with the known benefits of sleep: repair and restoration. We therefore sought to examine the effects of sleep deprivation (SD) in the post-traumatic sleep period on post-concussion symptomology (PCS). Adolescent male and female rats were administered repetitive mTBIs (RmTBI) or sham injuries and were then

assigned to 5 h of SD or left undisturbed. All animals were then tested using seven behavioral tasks validated to examine PCS, followed by analysis of serum cytokines, and quantitative real-time PCR for messenger RNA (mRNA) expression. Exposure to 3 SD epochs significantly impaired behavior in 4 of 7 of the measures, while RmTBI also produced dysfunction in 5 of 7 tests, but the effects of SD and RmTBI were not cumulative. SD induced long-lasting changes in serum levels of Tnf-α, IL6, and IL-1ß. mRNA expression in the pre-frontal cortex, hippocampus, hypothalamus, and anterior cingulate cortex was modified in response to SD and RmTBI; but similar to the behavioral measures, the mRNA changes were not cumulative. Consequently, we report that SD often produced impairments similar or worse than RmTBI, and sleep hygiene should become a priority for adolescent health.
J Neurotrauma, 2018; 35
28988852: Salberg S, Yamakawa G, Christensen J, Kolb B, Mychasiuk R
Assessment of a nutritional supplement containing resveratrol, prebiotic fiber, and omega-3 fatty acids for the prevention and treatment of mild traumatic brain injury in rats.
Children and adolescents have the highest rates of traumatic brain injury (TBI), with mild TBI (mTBI) accounting for most of these injuries. Adolescents are particularly vulnerable and often suffer from post-injury symptomologies that may persist for months. We hypothesized that the combination of resveratrol (RES), prebiotic fiber (PBF), and omega-3 fatty acids (docosahexaenoic acid (DHA)) would be an effective therapeutic supplement for the mitigation of mTBI outcomes in the developing brain. Adolescent male and female Sprague-Dawley rats were randomly assigned to the supplement (3S) or control condition, which was followed by a mTBI or sham insult. A behavioral test battery designed to examine symptomologies commonly associated with mTBI was administered. Following the test battery, tissue was collected from the prefrontal cortex (PFC) and primary auditory cortex for Golgi-Cox analysis of spine density, and for changes in expression of 6 genes (Aqp4, Gfap, Igf1, Nfl, Sirt1, and Tau). 3S treatment altered the behavioral performance of sham animals indicating that dietary manipulations modify premorbid characteristics. 3S treatment prevented injury-related deficits in the longer-term behavior measures, medial prefrontal cortex (mPFC) spine density, and levels of Aqp4, Gfap, Igf1, Nfl, and Sirt1 expression in the PFC. Although not fully protective, treatment with the supplement significantly improved post-mTBI function and warrants further investigation.
Neuroscience, 2017; 365

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SERUM NEUROFILAMENT LIGHT AS A BIOMARKER OF VULNERABILITY TO REPEATED MILD TRAUMATIC BRAIN INJURY IN ADOLESCENT MALE RATS

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Introduction: There is concern regarding the effects of repeat mild traumatic brain injury (rmTBI) and premature return to play/duty. There are, however, no validated biomarkers shown to objectively monitor recovery and indicate when the brain is no longer highly vulnerable to rmTBI. One biomarker receiving interest is the neuroaxonal protein neurofilament light (NfL). **Objectives:** To assess the utility of monitoring serum NfL to prevent cumulative effects of rmTBI. **Materials and methods:** Male rats were randomly allocated to receive sham injuries, a single-mTBI, or two mTBIs separated by either 1-day (i.e. peak NfL levels in rats), 3- or 7-days (i.e. during resolution), or 14-days (i.e. following resolution). Serum was collected for NfL quantification at the time of the final injury, and at 3-, 7-, 14- and 28-days. Sensorimotor function, cognition and anxiety-like behaviours were assessed during the 28-day period, followed by *ex vivo* diffusion tensor imaging. Outcomes were compared between the injury groups, and also within rmTBI rats dichotomized into high or low NfL groups based on levels at the time of the second mTBI. **Results:** When compared to rats given single-mTBI, those with rmTBI had a prolonged elevation in serum NfL. Furthermore, rats with high NfL levels had a significantly potentiated NfL response to the second mTBI when compared to rats with low NfL levels. Behavioural and MRI analysis is ongoing. **Conclusion:** This study provides novel evidence that irrespective of recovery interval, monitoring serum NfL levels may have utility to prevent the cumulative effects associated with rmTBI.

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EVALUATION OF REPETITIVE MILD TRAUMATIC BRAIN INJURY BY FLUORESCENCE AND FDG PET IMAGING

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Background: Repetitive, mild, traumatic brain injury (TBI) can cause minor neurologic impairment, but the imaging assessment of this type of injury are not fully understood. We investigated the effect of TBI and neuroprotective drug using fluorescence and FDG PET imaging system. Methods: TBI was induced by modified Marmarou's method, which was designed to total five successive head impacts with an interval of 12 hours. The experiment was conducted in the untreated TBI and treated TBI with neuroprotective drug. Neurological prognosis was evaluated by NSS 1 hour after each strike, and cellular structure was evaluated by H&E staining. BBB integrity was evaluated after EB injection using the fluorescence imaging system. Cerebral glucose metabolic function was measured by FDG PET images in each cerebral region. Results: NSS and gross changes showed no significant differences. However, fluorescence imaging reflecting BBB integrity demonstrated more severe BBB disruption in the untreated TBI group than in the treated TBI group (p=0.004). Quantitative analysis of FDG PET demonstrated higher glucose uptake in whole brain (p=0.046), striatum (p=0.040), thalamus (p=0.012) and cerebral gray matter (p=0.036) in the untreated TBI group than in the treated TBI group. Conclusion: The fluorescence and FDG PET imaging are more sensitive to evaluate the repetitive, mild TBI, although the neurological function or histologic assessments may not be sufficiently sensitive. These results suggest that fluorescence and FDG PET imaging may be useful for assessment of the degree of severity and the effect of neuroprotective drug in the repetitive, mild, traumatic injury.

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TRAUMATIC BRAIN INJURY AND ITS EFFECTS ON THE REACTION OF GLIAL CELLS

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Despite intensive research efforts, treatment options for neurotrauma remain limited, due to the scarce regenerative capacity of the brain. The cellular response to traumatic brain injury (TBI) is primarily carried out by glial cells, namely microglia, NG2-glia -the oligodendrocyte progenitor cells- and astrocytes, whose reactions coordinately contribute to limiting the damage and recovering tissue homeostasis. While the individual reactions of microglia and astrocytes are already partially described, little is known about NG2-glia reaction to TBI and, in general, how glial cells work together to secure trauma resolution. A better understanding of this strictly regulated mechanism could bring us closer to supporting regeneration after trauma. Here, we aimed to investigate the response of NG2-glia, astrocytes, and microglia to TBI in a spatial and temporal resolution and identified extracellular and intracellular conditions that can modify their reaction. The understanding of how glial cells coordinate their global reaction and the identification of factors that could modify or predict TBI outcomes could help us to limit brain damage and to support tissue recovery.

Pubmed:
34207710: Pagliaroli L, Fothi A, Nespoli E, Liko I, Veto B, Devay P, Szeri F, Hengerer B, Barta C, Aranyi T
Riluzole Administration to Rats with Levodopa-Induced Dyskinesia Leads to Loss of DNA Methylation in Neuronal Genes.
Dyskinesias are characterized by abnormal repetitive involuntary movements due to dysfunctional neuronal activity. Although levodopa-induced dyskinesia, characterized by tic-like abnormal involuntary movements, has no clinical treatment for Parkinson's disease patients, animal studies indicate that Riluzole, which interferes with glutamatergic neurotransmission, can improve the phenotype. The rat model of Levodopa-Induced Dyskinesia is a unilateral lesion with 6-hydroxydopamine in the medial forebrain bundle, followed by the repeated administration of levodopa. The molecular pathomechanism of Levodopa-Induced Dyskinesia is still not deciphered; however, the implication of epigenetic mechanisms was suggested. In this study, we investigated the striatum for DNA methylation alterations under chronic levodopa treatment with or without co-treatment with Riluzole. Our data show that the lesioned and contralateral striata have nearly identical DNA methylation profiles. Chronic levodopa and levodopa + Riluzole treatments led to DNA methylation loss, particularly outside of promoters, in gene bodies and CpG poor regions. We observed that several genes involved in the Levodopa-Induced Dyskinesia underwent methylation changes. Furthermore, the Riluzole co-treatment, which improved the phenotype, pinpointed specific methylation targets, with a more than 20% methylation difference relative to levodopa treatment alone. These findings indicate potential new druggable targets for Levodopa-Induced Dyskinesia.
Cells, 2021; 10
32754111: Denoix N, Merz T, Unmuth S, Hoffmann A, Nespoli E, Scheuerle A, Huber-Lang M, Gündel H, Waller C, Radermacher P, McCook O
Cerebral Immunohistochemical Characterization of the HS and the Oxytocin Systems in a Porcine Model of Acute Subdural Hematoma.
The hydrogen sulfide (HS) and the oxytocin/oxytocin receptor (OT/OTR) systems interact in trauma and are implicated in vascular protection and regulation of fluid homeostasis. Acute brain injury is associated with pressure-induced edema formation, blood brain barrier disruption, and neuro-inflammation. The similarities in brain anatomy: size, gyrencephalic organization, skull structure, may render the pig a highly relevant model for translational medicine. Cerebral biomarkers for pigs for pathophysiological changes and neuro-inflammation are limited. The current study aims to characterize the localization of OT/OTR and the endogenous HS producing enzymes together with relevant neuro-inflammatory markers on available porcine brain tissue from an acute subdural hematoma (ASDH) model. In a recent pilot study, anesthetized pigs underwent ASDH by injection of 20 mL of autologous blood above the left parietal cortex and were resuscitated with neuro-intensive care measures. After 54 h of intensive care, the animals were sacrificed, the brain was removed and analyzed via immunohistochemistry. The endogenous HS producing enzymes cystathionine-ϒ-lyase (CSE) and cystathionine-β-synthase

(CBS), the OTR, and OT were localized in neurons, vasculature and parenchyma at the base of sulci, where pressure-induced injury leads to maximal stress in the gyrencephalic brain. The pathophysiological changes in response to brain injury in humans and pigs, we show here, are comparable. We additionally identified modulators of brain injury to further characterize the pathophysiology of ASDH and which may indicate future therapeutic approaches.
Front Neurol, 2020; 11
30484112: Pagliaroli L, Widomska J, Nespoli E, Hildebrandt T, Barta C, Glennon J, Hengerer B, Poelmans G
Riluzole Attenuates L-DOPA-Induced Abnormal Involuntary Movements Through Decreasing CREB1 Activity: Insights from a Rat Model.
Chronic administration of L-DOPA, the first-line treatment of dystonic symptoms in childhood or in Parkinson's disease, often leads to the development of abnormal involuntary movements (AIMs), which represent an important clinical problem. Although it is known that Riluzole attenuates L-DOPA-induced AIMs, the molecular mechanisms underlying this effect are not understood. Therefore, we studied the behavior and performed RNA sequencing of the striatum in three groups of rats that all received a unilateral lesion with 6-hydroxydopamine in their medial forebrain bundle, followed by the administration of saline, L-DOPA, or L-DOPA combined with Riluzole. First, we provide evidence that Riluzole attenuates AIMs in this rat model. Subsequently, analysis of the transcriptomics data revealed that Riluzole is predicted to reduce the activity of CREB1, a transcription factor that regulates the expression of multiple proteins that interact in a molecular landscape involved in apoptosis. Although this mechanism underlying the beneficial effect of Riluzole on AIMs needs to be confirmed, it provides clues towards novel or existing compounds for the treatment of AIMs that modulate the activity of CREB1 and, hence, its downstream targets.
Mol Neurobiol, 2019; 56
29698507: Nespoli E, Rizzo F, Boeckers T, Schulze U, Hengerer B
Altered dopaminergic regulation of the dorsal striatum is able to induce tic-like movements in juvenile rats.
Motor tics are sudden, repetitive, involuntary movements representing the hallmark behaviors of the neurodevelopmental disease Tourette's syndrome (TS). The primary cause of TS remains unclear. The initial observation that dopaminergic antagonists alleviate tics led to the development of a dopaminergic theory of TS etiology which is supported by post mortem and in vivo studies indicating that non-physiological activation of the striatum could generate tics. The striatum controls movement execution through the balanced activity of dopamine receptor D1 and D2-expressing medium spiny neurons of the direct and indirect pathway, respectively. Different neurotransmitters can activate or repress striatal activity and among them, dopamine plays a major role. In this study we introduced a chronic dopaminergic alteration in juvenile rats, in order to modify the delicate balance between direct and indirect pathway. This manipulation was done in the dorsal striatum, that had been associated with tic-like movements generation in animal models. The results were movements resembling tics, which were categorized and scored according to a newly developed rating scale and were reduced by clonidine and riluzole treatment. Finally, post mortem analyses revealed altered RNA expression of dopaminergic receptors D1 and D2, suggesting an imbalanced dopaminergic regulation of medium spiny neuron activity as being causally related to the observed phenotype.
PLoS One, 2018; 13
29487562: Rizzo F, Nespoli E, Abaei A, Bar-Gad I, Deelchand DK, Fegert J, Rasche V, Hengerer B, Boeckers TM
Aripiprazole Selectively Reduces Motor Tics in a Young Animal Model for Tourette's Syndrome and Comorbid Attention Deficit and Hyperactivity Disorder.
Tourette's syndrome (TS) is a neurodevelopmental disorder characterized primarily by motor and vocal tics. Comorbidities such as attention deficit and hyperactivity disorder (ADHD) are observed in over 50% of TS patients. We applied aripiprazole in a juvenile rat model that displays motor tics and hyperactivity. We additionally assessed the amount of ultrasonic vocalizations (USVs) as an indicator for the presence of vocal tics and evaluated the changes in the striatal neurometabolism using proton magnetic resonance spectroscopy (1H-MRS) at 11.7T. Thirty-one juvenile spontaneously hypertensive rats (SHRs) underwent bicuculline striatal microinjection and treatment with either aripiprazole or vehicle. Control groups were sham operated and sham injected. Behavior, USVs, and striatal neurochemical profile were analyzed at early, middle, and late adolescence (postnatal days 35 to 50). Bicuculline microinjections in the dorsolateral striatum induced motor tics in SHR juvenile rats. Acute aripiprazole administration selectively reduced both tic frequency and latency, whereas stereotypies, USVs, and hyperactivity remained unaltered. The striatal neurochemical profile was only moderately altered after tic-induction and was not affected by systemic drug treatment. When applied to a young rat model that provides high degrees of construct, face, and predictive validity for TS and comorbid ADHD, aripiprazole selectively reduces motor tics, revealing that tics and stereotypies are distinct phenomena in line with clinical treatment of patients. Finally, our 1H-MRS results suggest a critical revision of the striatal role in the hypothesized cortico-striatal dysregulation in TS pathophysiology.
Front Neurol, 2018; 9
27601976: Forde NJ, Kanaan AS, Widomska J, Padmanabhuni SS, Nespoli E, Alexander J, Rodriguez Arranz JI, Fan S, Houssari R, Nawaz MS, Rizzo F, Pagliaroli L, Zilhäo NR, Aranyi T, Barta C, Boeckers TM, Boomsma DI, Buisman WR,

Buitelaar JK, Cath D, Dietrich A, Driessen N, Drineas P, Dunlap M, Gerasch S, Glennon J, Hengerer B, van den Heuvel OA, Jespersgaard C, Möller HE, Müller-Vahl KR, Openneer TJ, Poelmans G, Pouwels PJ, Scharf JM, Stefansson H, Tümer Z, Veltman DJ, van der Werf YD, Hoekstra PJ, Ludolph A, Paschou P
TS-EUROTRAIN: A European-Wide Investigation and Training Network on the Etiology and Pathophysiology of Gilles de la Tourette Syndrome.
Gilles de la Tourette Syndrome (GTS) is characterized by the presence of multiple motor and phonic tics with a fluctuating course of intensity, frequency, and severity. Up to 90% of patients with GTS present with comorbid conditions, most commonly attention-deficit/hyperactivity disorder (ADHD), and obsessive-compulsive disorder (OCD), thus providing an excellent model for the exploration of shared etiology across disorders. TS-EUROTRAIN (FP7-PEOPLE-2012-ITN, Grant Agr.No. 316978) is a Marie Curie Initial Training Network (http://ts-eurotrain.eu) that aims to elucidate the complex etiology of the onset and clinical course of GTS, investigate the neurobiological underpinnings of GTS and related disorders, translate research findings into clinical applications, and establish a pan-European infrastructure for the study of GTS. This includes the challenges of (i) assembling a large genetic database for the evaluation of the genetic architecture with high statistical power; (ii) exploring the role of gene-environment interactions including the effects of epigenetic phenomena; (iii) employing endophenotype-based approaches to understand the shared etiology between GTS, OCD, and ADHD; (iv) establishing a developmental animal model for GTS; (v) gaining new insights into the neurobiological mechanisms of GTS via cross-sectional and longitudinal neuroimaging studies; and (vi) partaking in outreach activities including the dissemination of scientific knowledge about GTS to the public. Fifteen partners from academia and industry and 12 PhD candidates pursue the project. Here, we aim to share the design of an interdisciplinary project, showcasing the potential of large-scale collaborative efforts in the field of GTS. Our ultimate aims are to elucidate the complex etiology and neurobiological underpinnings of GTS, translate research findings into clinical applications, and establish Pan-European infrastructure for the study of GTS and associated disorders.
Front Neurosci, 2016; 10
27092043: Nespoli E, Rizzo F, Boeckers TM, Hengerer B, Ludolph AG
Addressing the Complexity of Tourette's Syndrome through the Use of Animal Models.
Tourette's syndrome (TS) is a neurodevelopmental disorder characterized by fluctuating motor and vocal tics, usually preceded by sensory premonitions, called premonitory urges. Besides tics, the vast majority-up to 90%-of TS patients suffer from psychiatric comorbidities, mainly attention deficit/hyperactivity disorder (ADHD) and obsessive-compulsive disorder (OCD). The etiology of TS remains elusive. Genetics is believed to play an important role, but it is clear that other factors contribute to TS, possibly altering brain functioning and architecture during a sensitive phase of neural development. Clinical brain imaging and genetic studies have contributed to elucidate TS pathophysiology and disease mechanisms; however, TS disease etiology still is poorly understood. Findings from genetic studies led to the development of genetic animal models, but they poorly reflect the pathophysiology of TS. Addressing the role of neurotransmission, brain regions, and brain circuits in TS disease pathomechanisms is another focus area for preclinical TS model development. We are now in an interesting moment in time when numerous innovative animal models are continuously brought to the attention of the public. Due to the diverse and largely unknown etiology of TS, there is no single preclinical model featuring all different aspects of TS symptomatology. TS has been dissected into its key symptomst hat have been investigated separately, in line with the Research Domain Criteria concept. The different rationales used to develop the respective animal models are critically reviewed, to discuss the potential of the contribution of animal models to elucidate TS disease mechanisms.
Front Neurosci, 2016; 10
24295619: Udvardi PT, Nespoli E, Rizzo F, Hengerer B, Ludolph AG
Nondopaminergic neurotransmission in the pathophysiology of Tourette syndrome.
A major pathophysiological role for the dopaminergic system in Tourette's syndrome (TS) has been presumed ever since the discovery that dopamine-receptor antagonists can alleviate tics. Especially recent molecular genetic studies, functional imaging studies, and some rare postmortem studies have given more and more hints that other neurotransmitter systems are involved as well. Dysfunction in the dopamine metabolism-in particular during early development-might lead to counter-regulations in the other systems or vice versa. This chapter will give an overview of the studies that prove the involvement of other neurotransmitter systems such as the major monoaminergic neurotransmitters norepinephrine, serotonin, and histamine; the most important excitatory neurotransmitter, the amino acid glutamate; the major inhibitory neurotransmitter y-aminobutyric acid, as well as acetylcholine, endocannabinoid, corticoid; and others. These studies will hopefully lead to fundamental advances in the psychopharmacological treatment of TS. While tic disorders have been previously treated mainly with dopamine antagonists, some authors already favor alpha-agonists. Clinical trials with glutamate agonists and antagonists and compounds influencing the histaminergic system are currently being conducted. Since the different neurotransmitter systems consist of several receptor subtypes which might mediate different effects on locomotor activity, patients with TS may respond differentially to selective agonists or antagonists. Effects of agonistic or antagonistic

compounds on tic symptoms might also be dose dependent. Further studies will lead to a broader spectrum of psychopharmacological treatment options in TS.
Int Rev Neurobiol, 2013; 112

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CHALLENGING THE ROLE OF OLIGODENDROCYTES UPON TRAUMATIC BRAIN INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI), an injury affecting or disabling brain function, is still a major cause of morbidity worldwide. Unlike other brain disorders, TBI involves a sudden event that triggers neuroinflammation, which is key to subsequent pathological processes following TBI. To investigate transcriptional changes related to inflammation as a consequence of TBI, we sequenced bulk and single nuclei RNA from TBI patients and used post-mortem samples with non-neurological deaths as control. We found inflammatory related genes to be upregulated in microglia as well as in oligodendrocytes in TBI patients compared to control. Interestingly, TBI-oligodendrocytes also display a clear upregulation in genes related to viral response and interferon response. This is interesting, as recent studies have linked the expression of endogenous retroviruses (ERVs) to the activation of an interferon response in the brain. Therefore, to investigate the expression of ERVs in TBI, we applied a novel bioinformatical approach to examine the transcriptional activity of these elements in the different cell types. Strikingly, our results show a clear upregulation of human specific ERVs in oligodendrocytes in TBI patients. These observations challenge the role of oligodendrocytes during neuroinflammation, and the consequences of ERV expression during such process.

Pubmed:
34936701: Pircs K, Drouin-Ouellet J, Horváth V, Gil J, Rezeli M, Garza R, Grassi DA, Sharma Y, St-Amour I, Harris K, Jönsson ME, Johansson PA, Vuono R, Fazal SV, Stoker T, Hersbach BA, Sharma K, Lagerwall J, Lagerström S, Storm P, Hébert SS, Marko-Varga G, Parmar M, Barker RA, Jakobsson J
Distinct subcellular autophagy impairments in induced neurons from Huntington's disease patients.
Huntington's disease (HD) is a neurodegenerative disorder caused by CAG expansions in the huntingtin (HTT) gene. Modelling Huntington's disease is challenging, as rodent and cellular models poorly recapitulate the disease as seen in aging humans. To address this, we generated induced neurons (iNs) through direct reprogramming of human skin fibroblasts, which retain age-dependent epigenetic characteristics. HD-iNs displayed profound deficits in autophagy, characterised by reduced transport of late autophagic structures from the neurites to the soma. These neurite-specific alterations in autophagy resulted in shorter, thinner and fewer neurites specifically in HD-iNs. CRISPRi-mediated silencing of HTT did not rescue this phenotype but rather resulted in additional autophagy alterations in ctrl-iNs, highlighting the importance of wild type HTT in normal neuronal autophagy. In summary, our work identifies a distinct subcellular autophagy impairment in adult patient derived Huntington's disease neurons and provides a new rational for future development of autophagy activation therapies. Brain, 2021;
34624206: Johansson PA, Brattås PL, Douse CH, Hsieh P, Adami A, Pontis J, Grassi D, Garza R, Sozzi E, Cataldo R, Jönsson ME, Atacho DAM, Pircs K, Eren F, Sharma Y, Johansson J, Fiorenzano A, Parmar M, Fex M, Trono D, Eichler EE, Jakobsson J
A cis-acting structural variation at the ZNF558 locus controls a gene regulatory network in human brain development.
The human forebrain has expanded in size and complexity compared to chimpanzees despite limited changes in protein-coding genes, suggesting that gene expression regulation is an important driver of brain evolution. Here, we identify a KRAB-ZFP transcription factor, ZNF558, that is expressed in human but not chimpanzee forebrain neural progenitor cells. ZNF558 evolved as a suppressor of LINE-1 transposons but has been co-opted to regulate a single target, the mitophagy gene SPATA18. ZNF558 plays a role in mitochondrial homeostasis, and loss-of-function experiments in cerebral organoids suggests that ZNF558 influences developmental timing during early human brain development. Expression of ZNF558 is controlled by the size of a variable number tandem repeat that is longer in chimpanzees compared to humans, and variable in the human population. Thus, this work provides mechanistic insight into how a cis-acting structural variation establishes a regulatory network that affects human brain evolution.

Cell Stem Cell, 2022; 29
33644903: Jönsson ME, Garza R, Sharma Y, Petri R, Södersten E, Johansson JG, Johansson PA, Atacho DA, Pircs K, Madsen S, Yudovich D, Ramakrishnan R, Holmberg J, Larsson J, Jern P, Jakobsson J
Activation of endogenous retroviruses during brain development causes an inflammatory response.
Endogenous retroviruses (ERVs) make up a large fraction of mammalian genomes and are thought to contribute to human disease, including brain disorders. In the brain, aberrant activation of ERVs is a potential trigger for an inflammatory response, but mechanistic insight into this phenomenon remains lacking. Using CRISPR/Cas9-based gene disruption of the epigenetic co-repressor protein Trim28, we found a dynamic H3K9me3-dependent regulation of ERVs in proliferating neural progenitor cells (NPCs), but not in adult neurons. In vivo deletion of Trim28 in cortical NPCs during mouse brain development resulted in viable offspring expressing high levels of ERVs in excitatory neurons in the adult brain. Neuronal ERV expression was linked to activated microglia and the presence of ERV-derived proteins in aggregate-like structures. This study demonstrates that brain development is a critical period for the silencing of ERVs and provides causal in vivo evidence demonstrating that transcriptional activation of ERV in neurons results in an inflammatory response.
EMBO J, 2021; 40
32499105: Jönsson ME, Garza R, Johansson PA, Jakobsson J
Transposable Elements: A Common Feature of Neurodevelopmental and Neurodegenerative Disorders.
The etiology of most neurological disorders is poorly understood and current treatments are largely ineffective. New ideas and concepts are therefore vitally important for future research in this area. This review explores the concept that dysregulation of transposable elements (TEs) contributes to the appearance and pathology of neurodevelopmental and neurodegenerative disorders. Despite TEs making up at least half of the human genome, they are vastly understudied in relation to brain disorders. However, recent advances in sequencing technologies and gene editing approaches are now starting to unravel the pathological role of TEs. Aberrant activation of TEs has been found in many neurological disorders; the resulting pathogenic effects, which include alterations of gene expression, neuroinflammation, and direct neurotoxicity, are starting to be resolved. An increased understanding of the relationship between TEs and pathological processes in the brain improves the potential for novel diagnostics and interventions for brain disorders.
Trends Genet, 2020; 36

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LONG-TERM ENDOSCOPIC CALCIUM IMAGING OF A NOVEL COMPRESSION TBI MOUSE MODEL

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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We have developed a new Traumatic Brain Injury (TBI) mouse model that allows long-term endoscopic calcium imaging of the impact zone. Recordings can be made prior to the injury and after, all the way until the apparition of comorbidities such as memory disorders and epileptic seizures. Our approach combines the high cellular resolution of Inscopix miniendoscopes with a novel impact methodology developed in house. Thanks to this model, we have been able to follow the cellular changes that occur within the impact zone and analyze changes occurring in a single cells during the first two months after impact. This model allows for freely moving imaging of mice and combination with several behavioral tests.

Pubmed:
33099743: Khirug S, Soni S, Saez Garcia M, Tessier M, Zhou L, Kulesskaya N, Rauvala H, Lindholm D, Ludwig A, Molinari F, Rivera C
Protective Role of Low Ethanol Administration Following Ischemic Stroke via Recovery of KCC2 and p75 Expression.
A striking result from epidemiological studies show a correlation between low alcohol intake and lower incidence for ischemic stroke and severity of derived brain injury. Although reduced apoptosis and inflammation has been suggested to be involved, little is known about the mechanism mediating this effect in vivo. Increase in intracellular chloride concentration and derived depolarizing GABAR-mediated transmission are common consequences following various brain injuries and are caused by the abnormal expression levels of the chloride cotransporters NKCC1 and KCC2. Downstream pro-apoptotic signaling through p75 may link GABA depolarization with post-injury neuronal apoptosis. Here, we show that changes in GABAergic signaling, Cl homeostasis, and expression of chloride cotransporters in the post-traumatic mouse brain can be significantly reduced by administration of 3% ethanol to the drinking water. Ethanol-induced upregulation of KCC2 has a positive impact on neuronal survival, preserving a large part of the cortical peri-infarct zone, as well as preventing the massive post-ischemic upregulation of the pro-apoptotic protein p75. Importantly, intracortical multisite in vivo recordings showed that ethanol treatment could significantly ameliorate stroke-induced reduction in cortical activity. This surprising finding discloses a pathway triggered by low concentration of ethanol as a novel therapeutically relevant target.
Mol Neurobiol, 2021; 58
33005130: Kesaf S, Khirug S, Dinh E, Saez Garcia M, Soni S, Orav E, Delpire E, Taira T, Lauri SE, Rivera C
The Kainate Receptor Subunit GluK2 Interacts With KCC2 to Promote Maturation of Dendritic Spines.
Kainate receptors (KAR) play a crucial role in the plasticity and functional maturation of glutamatergic synapses. However, how they regulate structural plasticity of dendritic spines is not known. The GluK2 subunit was recently shown to coexist in a functional complex with the neuronal K-Cl cotransporter KCC2. Apart from having a crucial role in the maturation of GABAergic transmission, KCC2 has a morphogenic role in the maturation of dendritic spines. Here, we show that local inactivation of GluK2 expression in CA3 hippocampal neurons induces altered morphology of dendritic spines and reduction in mEPSC frequency. GluK2 deficiency also resulted in a strong change in the subcellular distribution of KCC2 as well as a smaller somatodendritic gradient in the reversal potential of GABA. Strikingly, the aberrant morphology of dendritic spines in GluK2-deficient CA3 pyramidal neurons was restored by overexpression of KCC2. GluK2 silencing in hippocampal neurons significantly reduced the expression of 4.1N and functional form of the actin filament severing protein cofilin. Consistently, assessment of actin dynamics using fluorescence recovery after photobleaching (FRAP) of β-actin showed a significant increase in the stability of F-actin filaments in dendritic spines. In conclusion, our results demonstrate that GluK2-KCC2 interaction plays an important role in the structural maturation of dendritic spines. This also provides novel insights into the connection between KAR dysfunction, structural plasticity, and developmental disorders.
Front Cell Neurosci, 2020; 14
30800473: Gajdos T, Fleming SM, Saez Garcia M, Weindel G, Davranche K
Revealing subthreshold motor contributions to perceptual confidence.
Established models of perceptual metacognition, the ability to evaluate our perceptual judgements, posit that perceptual

confidence depends on the strength or quality of feedforward sensory evidence. However, alternative theoretical accounts suggest the entire perception-action cycle, and not only variation in sensory evidence, is monitored when evaluating confidence in one's percepts. Such models lead to the counterintuitive prediction that perceptual confidence should be directly modulated by features of motor output. To evaluate this proposal here we recorded electromyographic (EMG) activity of motor effectors while subjects performed a near-threshold perceptual discrimination task and reported their confidence in each response in a pre-registered experiment. A subset of trials exhibited subthreshold EMG activity in response effectors before a decision was made. Strikingly, trial-by-trial analysis showed that confidence, but not accuracy, was significantly higher on trials with subthreshold motor activation. These findings support a hypothesis that preparatory motor activity, or a related latent variable, impacts upon confidence over and above performance, consistent with models in which perceptual metacognition integrates information across the perception-action cycle.
Neurosci Conscious, 2019; 2019

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SINGLE-CELL RNA SEQUENCING REVEALS SENESCENT-LIKE NEURONS IN THE INJURED MOUSE BRAIN AND TREATMENT WITH SENOLYTIC DRUG ABT263 IMPROVES INJURY-INDUCED COGNITIVE IMPAIRMENT: IS THERE THERAPEUTIC POTENTIAL?

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Mild traumatic brain injury (mTBI) is an urgent public health issue, as it is linked to long-term neurological impairment and a propensity towards neurodegenerative disease. The underlying mechanisms driving long-term dysfunction that may be therapeutically targeted remain unclear. In this study, we induced three mild closed-skull injuries or sham procedures, 24h apart, in C57BL/6 mice followed by behavioural tests or sacrifice one week later. Injured mice showed prolonged righting reflex, neurocognitive impairment in the Morris water maze, and increased Iba1 and GFAP reactivity, indicating induction of a mild injury with no gross lesion. The cortical and hippocampal region under impact revealed extensive DNA damage in the form of double-strand breaks, oxidative damage, and R-loops, markers of senescence including p16 and p21, and markers of the senescence-associated secretory phenotype (SASP). Single-cell RNA sequencing revealed activation of the DNA damage response, senescence, and SASP in all cell types, including neurons. Cell-type specific changes were also present with evidence of innate immune activation, synaptic transmission and structural dysfunction, gliosis, and metabolic reprogramming indicative of the DNA damage response. Treatment of injured mice with the senolytic drug ABT263 significantly improved performance in the Morris water maze and reduced markers of DNA damage and senescence. This study is the first to use senolytic therapy in an impact model, providing compelling evidence that targeting senescence may be clinically beneficial. Developing more targeted strategies that do not eliminate cells, such as high capacity multifaceted nanoparticle-mediated therapies, will be critical going forward in the treatment of mTBI.

Pubmed:
34924026: Velayudhan PS, Schwab N, Hazrati LN, Wheeler AL
Temporal patterns of microglial activation in white matter following experimental mild traumatic brain injury: a systematic literature review.
Mild traumatic brain injuries (mTBIs) are a prevalent form of injury that can result in persistent neurological impairments. Microglial activation has become increasingly recognized as a key process regulating the pathology of white matter in a wide range of brain injury and disease contexts. As white matter damage is known to be a major contributor to the impairments that follow mTBI, microglia have rightfully become a common target of investigation for the development of mTBI therapies and biomarkers. Recent work has demonstrated that the efficacy of microglial manipulation as a therapeutic intervention following injury or disease is highly time-sensitive, emphasizing the importance of advancing our understanding of the dynamics of post-mTBI microglial activation from onset to resolution. Current reporting of microglial activation in experimental studies of mTBI is non-standardized, which has limited our ability to identify concrete patterns of post-mTBI microglial activation over time. In this review, we examine preclinical studies of mTBI that report on microglial activation in white matter regions to summarize our current understanding of these patterns. Specifically, we summarize timecourses of post-mTBI microglial activation in white matter regions of the brain, identify factors that influence this activation, examine the temporal relationship between microglial activation and other post-mTBI assessments, and compare the relative sensitivities of various methods for detecting microglial activation. While the lack of replicated experimental conditions has limited the extent of conclusions that can confidently be drawn, we find that microglia are activated over a wide range of timecourses following mTBI and that microglial activation is a long-lasting outcome of mTBI that may resolve after most typical post-mTBI assessments, with the exception of those measuring oligodendrocyte lineage cell integrity. We identify several understudied parameters of post-mTBI microglial activation in white matter, such as the inclusion of female subjects. This review summarizes our current understanding of the progression of microglial activation in white matter structures following experimental mTBI and offers suggestions for important future research directions.
Acta Neuropathol Commun, 2021; 9

34650425: Schwab N, Leung E, Hazrati LN
Cellular Senescence in Traumatic Brain Injury: Evidence and Perspectives.
Mild traumatic brain injury (mTBI) can lead to long-term neurological dysfunction and increase one's risk of neurodegenerative disease. Several repercussions of mTBI have been identified and well-studied, including neuroinflammation, gliosis, microgliosis, excitotoxicity, and proteinopathy - however the pathophysiological mechanisms activating these pathways after mTBI remains controversial and unclear. Emerging research suggests DNA damage-induced cellular senescence as a possible driver of mTBI-related sequalae. Cellular senescence is a state of chronic cell-cycle arrest and inflammation associated with physiological aging, mood disorders, dementia, and various neurodegenerative pathologies. This narrative review evaluates the existing studies which identify DNA damage or cellular senescence after TBI (including mild, moderate, and severe TBI) in both experimental animal models and human studies, and outlines how cellular senescence may functionally explain both the molecular and clinical manifestations of TBI. Studies on this subject clearly show accumulation of various forms of DNA damage (including oxidative damage, single-strand breaks, and double-strand breaks) and senescent cells after TBI, and indicate that cellular senescence may be an early event after TBI. Further studies are required to understand the role of sex, cell-type specific mechanisms, and temporal patterns, as senescence may be a pathway of interest to target for therapeutic purposes including prognosis and treatment.
Front Aging Neurosci, 2021; 13
33964983: Schwab N, Ju Y, Hazrati LN
Early onset senescence and cognitive impairment in a murine model of repeated mTBI.
Mild traumatic brain injury (mTBI) results in broad neurological symptoms and an increased risk of being diagnosed with a neurodegenerative disease later in life. While the immediate oxidative stress response and post-mortem pathology of the injured brain has been well studied, it remains unclear how early pathogenic changes may drive persistent symptoms and confer susceptibility to neurodegeneration. In this study we have used a mouse model of repeated mTBI (rmTBI) to identify early gene expression changes at 24 h or 7 days post-injury (7 dpi). At 24 h post-injury, gene expression of rmTBI mice shows activation of the DNA damage response (DDR) towards double strand DNA breaks, altered calcium and cell-cell signalling, and inhibition of cell death pathways. By 7 dpi, rmTBI mice had a gene expression signature consistent with induction of cellular senescence, activation of neurodegenerative processes, and inhibition of the DDR. At both timepoints gliosis, microgliosis, and axonal damage were evident in the absence of any gross lesion, and by 7 dpi rmTBI also mice had elevated levels of IL1β, p21, 53BP1, DNA2, and p53, supportive of DNA damage-induced cellular senescence. These gene expression changes reflect establishment of processes usually linked to brain aging and suggests that cellular senescence occurs early and most likely prior to the accumulation of toxic proteins. These molecular changes were accompanied by spatial learning and memory deficits in the Morris water maze. To conclude, we have identified DNA damage-induced cellular senescence as a repercussion of repeated mild traumatic brain injury which correlates with cognitive impairment. Pathways involved in senescence may represent viable treatment targets of post-concussive syndrome. Senescence has been proposed to promote neurodegeneration and appears as an effective target to prevent long-term complications of mTBI, such as chronic traumatic encephalopathy and other related neurodegenerative pathologies.
Acta Neuropathol Commun, 2021; 9
33722991: Hazrati LN, Schwab N
Embracing the Unknown in the Diagnosis of Traumatic Encephalopathy Syndrome.
Neurology, 2021; 96
33627496: Schwab N, Wennberg R, Grenier K, Tartaglia C, Tator C, Hazrati LN
Association of Position Played and Career Duration and Chronic Traumatic Encephalopathy at Autopsy in Elite Football and Hockey Players.
To determine whether an association exists between career duration or position played and the presence of chronic traumatic encephalopathy (CTE) at autopsy in a series of elite football and hockey players.
Neurology, 2021; 96
32150612: Etxeandia-Ikobaltzeta I, Zhang Y, Brundisini F, Florez ID, Wiercioch W, Nieuwlaat R, Begum H, Cuello CA, Roldan Y, Chen R, Ding C, Morgan RL, Riva JJ, Zhang Y, Charide R, Agarwal A, Balduzzi S, Morgano GP, Yepes-Nuñez JJ, Rehman Y, Neumann I, Schwab N, Baldeh T, Braun C, Rodríguez MF, Schünemann HJ
Patient values and preferences regarding VTE disease: a systematic review to inform American Society of Hematology guidelines.
Values and preferences relate to the importance that patients place on health outcomes (eg, bleeding, having a deep venous thrombosis) and are essential when weighing benefits and harms in guideline recommendations. To inform the American Society of Hematology guidelines for management of venous thromboembolism (VTE) disease, we conducted a systematic review of patients' values and preferences related to VTE. We searched Medline, Embase, Cochrane Central Register of Controlled Trials, PsycINFO, and the Cumulative Index to Nursing and Allied Health Literature from inception to April of 2018

(PROSPERO-CRD42018094003). We included quantitative and qualitative studies. We followed Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidance for rating the certainty and presenting findings for quantitative research about the relative importance of health outcomes and a grounded theory approach for qualitative thematic synthesis. We identified 14 quantitative studies (2465 participants) describing the relative importance of VTE-related health states in a widely diverse population of patients, showing overall small to important impact on patients' lives (certainty of the evidence from low to moderate). Additionally, evidence from 34 quantitative studies (6424 participants) and 15 qualitative studies (570 participants) revealed that patients put higher value on VTE risk reduction than on the potential harms of the treatment (certainty of evidence from low to moderate). Studies also suggested a clear preference for oral medication over subcutaneous medication (moderate certainty). The observed variability in health state values may be a result of differences in the approaches used to elicit them and the diversity of included populations rather than true variability in values. This finding highlights the necessity to explore the variability induced by different approaches to ascertain values.
Blood Adv, 2020; 4

31727161: Schwab N, Grenier K, Hazrati LN

DNA repair deficiency and senescence in concussed professional athletes involved in contact sports.

Mild traumatic brain injury (mTBI) leads to diverse symptoms including mood disorders, cognitive decline, and behavioral changes. In some individuals, these symptoms become chronic and persist in the long-term and can confer an increased risk of neurodegenerative disease and dementia diagnosis later in life. Despite the severity of its consequences, the pathophysiological mechanism of mTBI remains unknown. In this post-mortem case series, we assessed DNA damage-induced cellular senescence pathways in 38 professional athletes with a history of repeated mTBI and ten controls with no mTBI history. We assessed clinical presentation, neuropathological changes, load of DNA damage, morphological markers of cellular senescence, and expression of genes involved in DNA damage signaling, DNA repair, and cellular senescence including the senescence-associated secretory phenotype (SASP). Twenty-eight brains with past history of repeated mTBI history had DNA damage within ependymal cells, astrocytes, and oligodendrocytes. DNA damage burden was increased in brains with proteinopathy compared to those without. Cases also showed hallmark features of cellular senescence in glial cells including astrocytic swelling, beading of glial cell processes, loss of H3K27Me3 (trimethylation at lysine 27 of histone H3) and lamin B1 expression, and increased expression of cellular senescence and SASP pathways. Neurons showed a spectrum of changes including loss of emerin nuclear membrane expression, loss of Brahma-related gene-1 (BRG1 or SMARCA4) expression, loss of myelin basic protein (MBP) axonal expression, and translocation of intranuclear tau to the cytoplasm. Expression of DNA repair proteins was decreased in mTBI brains. mTBI brains showed substantial evidence of DNA damage and cellular senescence. Decreased expression of DNA repair genes suggests inefficient DNA repair pathways in this cohort, conferring susceptibly to cellular senescence and subsequent brain dysfunction after mTBI. We therefore suggest that brains of contact-sports athletes are characterized by deficient DNA repair and DNA damage-induced cellular senescence and propose that this may affect neurons and be the driver of brain dysfunction in mTBI, predisposing the progression to neurodegenerative diseases. This study provides novel targets for diagnostic and prognostic biomarkers, and represents viable targets for future treatments.
Acta Neuropathol Commun, 2019; 7

30760862: Schwab N, Tator C, Hazrati LN

DNA damage as a marker of brain damage in individuals with history of concussions.

Mild traumatic brain injury (mTBI) is common in many populations, including athletes, veterans, and domestic abuse victims. mTBI can cause chronic symptoms, including depression, irritability, memory problems, and attention deficits. A history of repetitive mTBI has been epidemiologically associated with developing early-onset dementia and neurodegenerative diseases and, in particular, is thought to be the underlying cause of chronic traumatic encephalopathy (CTE)-a progressive tauopathy diagnosed by the presence of perivascular hyperphosphorylated tau protein (p-tau) in the depths of cortical sulci. However, the scarce and focal pathology often seen in CTE does not correlate with the severity of symptoms experienced by patients. This paper proposes accumulation of γH2AX, a marker of double-stranded DNA damage, as a novel pathological marker to identify brain damage post-mTBI. We present two cases of men with history of mTBI. Immunohistochemistry revealed extensive DNA damage throughout the frontal cortex, hippocampus, and brainstem areas. Furthermore, gene expression profiling showed increases of ataxia telangiectasia mutated (ATM) and checkpoint kinase 2 (CHEK2), two serine/threonine kinases recruited in response to double-strand breaks in the DNA damage response pathway. These cases highlight the complex pathophysiology of head trauma, and suggest DNA damage as the molecular mechanism behind mTBI-induced pathology and symptoms.
Lab Invest, 2019; 99

30010133: Schwab N, Hazrati LN

Assessing the Limitations and Biases in the Current Understanding of Chronic Traumatic Encephalopathy.

Chronic traumatic encephalopathy (CTE) is considered to be a progressive neurodegenerative disease caused by mild

traumatic brain injury (mTBI). Recently there has been a significant amount of media attention surrounding the commonness of CTE in professional athletes, particularly American football, based on several postmortem case series. However, despite the persuasive claims made by the media about CTE, research on the disease and the effects of mTBI in general remain in its infancy. Commonly cited case series studying CTE are limited by methodological biases, pathological inconsistencies, insufficient clinical data, and a reliance on inherently biased postmortem data. These case series do not allow for the collection of any epidemiological data and are not representative of the general population. The exaggerated assumptions and assertions taken from these studies run the risk of creating a self-fulfilling prophecy for individuals who believe they are at risk and have the potential to negatively influence sports-related policymaking. This review outlines the status and limitations of recent CTE case series and calls for future prospective, longitudinal studies to further characterize the pathological and clinical hallmarks of CTE.
J Alzheimers Dis, 2018; 64
30482767: Bates SM, Rajasekhar A, Middeldorp S, McLintock C, Rodger MA, James AH, Vazquez SR, Greer IA, Riva JJ, Bhatt M, Schwab N, Barrett D, LaHaye A, Rochwerg B
American Society of Hematology 2018 guidelines for management of venous thromboembolism: venous thromboembolism in the context of pregnancy.
Venous thromboembolism (VTE) complicates ~1.2 of every 1000 deliveries. Despite these low absolute risks, pregnancy-associated VTE is a leading cause of maternal morbidity and mortality.
Blood Adv, 2018; 2

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BOARD NUMBER: S04-395

THE ACUTE EFFECTS OF PEGYLATED SINGLE-WALLED CARBON NANOTUBES ON THE PROTEIN OXIDATIVE DAMAGE AND HSP-70 LEVELS IN PRIMARY MOUSE ASTROCYTES EXPOSED TO STRETCH INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Kristina Pilipović[1], Nika Gržeta[1], Anja Harej Hrkać[1], Vladimir Parpura[2]
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Aims: We have previously shown that the application of single-walled carbon nanotubes chemically functionalized with polyethylene glycol (PEG-SWCNTs) influences the expression of some proteins and causes changes in the cytokine release from the cultured astrocytes exposed to severe *in vitro* traumatic brain injury (TBI). Still, questions remain regarding the potential toxicity of the investigated nanomaterial. Here, using the *in vitro* TBI model, we tested the effects of PEG-SWCNTs on oxidative protein damage and the expression of a stress protein, heat shock protein 70 (HSP-70) in astrocytes. Methods: Primary mouse astrocytes were grown on 6-well plates with deformable membranes and subjected to severe stretch injury. PEG-SWCNTs or vehicle were applied at 1 h post-injury and the astrocytes were collected 23 h later. The dot blot method was used to detect the levels of oxidatively damaged proteins, and the Western blot was used to determine the expression levels of HSP-70. Non-injured, vehicle-treated astrocytes were used as the control group. Results: Stretch injury caused a slight, but non-significant increase in the levels of oxidatively damaged proteins and the application of the PEG-SWCNTs to the injured astrocytes did not cause additional protein oxidation. *In vitro* trauma, with or without PEG-SWCNTs treatment, did not affect the HSP-70 levels in astrocytes. Conclusions: Results of this study suggest that PEG-SWCNTs do not cause oxidative protein damage in astrocytes exposed to *in vitro* TBI, nor do they increase the level of cell stress as determined by measuring the HSP-70 levels. This research was fully supported by the Croatian Science Foundation grant UIP-2017-05-9517 to KP.

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EXPLORING AGE-AND SEX-RELATED NEUROLOGICAL AND BEHAVIOURAL DIFFERENCES IN A RAT MODEL OF CHRONIC TRAUMATIC ENCEPHALOPATHY (CTE)

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Aim. Repetitive mild traumatic brain injury (mTBI) as occurring in contact sports can progress into chronic traumatic encephalopathy (CTE), a neurodegenerative condition leading to severe behavioural changes. Here, we explore sex and age effects on neurological and behavioural outcome parameters in a rat model of CTE. **Methods.** To induce CTE, rats aged 7-weeks (n=8 females; n=6 males) and 14-weeks (n=8, each) underwent 3 mTBIs (induced by weight drop under anaesthesia) with 5-days intervals. Time-to-right was determined immediately after each mTBI and neurological severity score (NSS) at baseline and the first day after each mTBI. Sucrose preference test (SPT) was performed 1 and 2 weeks after CTE induction. At week 12, NSS, SPT, Y-maze and open field tests (OFT) were performed. One-way and two-way ANOVA were used to analyse data pooled by sex and age. **Results.** Independent of sex and age, time-to-right and NSS showed neurological impairment at multiple time-points (up to $p<0.01$), with NSS impairment lasting until week 12 (up to $p<0.0001$), while OFT and Y-maze did not reach significance ($p>0.05$). Interestingly, no age-dependent effects were found. However, male CTE rats showed worse performance than females in the time to right after the second mTBI ($p<0.001$), SPT after 2 weeks (-41% sucrose consumption, $p<0.01$), and NSS after 12 weeks ($p<0.05$). **Conclusion.** Our results do not reveal evidence of age-related effects after CTE-induction. Sex, however seems to be a relevant factor to be included in subsequent investigations.

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NEUROPROTECTIVE INCREASE OF A COMBINED TREATMENT OF TWO OXIMES AGAINST VX-EXPOSURE IN MICE

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Alexandre Champault[1], Marilène Trancart[1], Julie Knoertzer[1], Ludovic Jean[2], Nicolas Probst[2], Anne-Sophie Hanak[1], André-Guilhem Calas[1], Gregory Dal Bo[1], Karine Thibault[1]
[1]French Armed Forces Biomedical Research Institute, Departement Of Toxicology And Chemical Risks, Brétigny-sur-orge, France, [2]Université de Rouen, Cobra, Mont-Saint-Aignan, France

Organophosphates (OP) are toxic chemical compounds initially developed as pesticides, which still cause several million poisonings and thousand deaths worldwide each year. OP nerve agents, such as sarin or VX, have been used in Syria civil war and in Kim Jong-Nam assassination respectively. Acute exposure to OP could lead to muscle paralysis and respiratory distress resulting to death in few minutes post-exposure. Antidote therapy contains a muscarinic cholinergic receptor antagonist (atropine sulfate) and one oxime, a strong nucleophile, able to reactivate OP-inhibited acetylcholinesterase (AChE). Unfortunately, oximes have limitations in their effectiveness, in particular as they poorly cross the blood brain barrier (BBB) and therefore weakly reactivate central AChE. The goal of this study is to test therapeutic potential of a new oxime, called RM048 which can cross the BBB *in vitro* and its combination with HI-6, known not to cross the BBB. At first, we focused on short term effect of HI-6, RM048 or the mix of both after supralethal doses of VX agent exposure on different physiological parameters. We assessed protective index of each treatment and the corresponding quality of recovery. Cerebral and blood ChE activity inhibition has been measured. Then, we characterized the effect of the different treatments on cerebral electrical activity and in the epileptic seizure occurrence. Finally, we focused on midterm therapy effects of each treatment, one month after VX exposure, and we evaluated behavioural impact on anxiety, cognition, locomotion, and depression.

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ROLE OF MITOCHONDRIAL FISSION-FUSION DYNAMICS IN PROGRESSIVE NEURODEGENERATION AND MEMORY DEFICIT AFTER TRAUMATIC BRAIN INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Preethy Sridharan[1], Yeojung Koh[2], Emiko Miller[1], Kathryn Franke[3], Matasha Dhar[3], Meredith Whitney[3], Edwin Vásquez-Rosa[3], Min-Kyoo Shin[3], Xin Qi[4], Andrew Pieper[3,5]
[1]Case Western Reserve University, Neurosciences, Cleveland, United States of America, [2]Case Western Reserve University, Pathology, Cleveland, United States of America, [3]University Hospitals, Psychiatry, Cleveland, United States of America, [4]Case Western Reserve University, Biophysics, Cleveland, United States of America, [5]Harrington Discovery Institute, University Hospitals, Cleveland, United States of America

Aberrant mitochondrial fission has been identified as important in the pathophysiology of traumatic brain injury (TBI), Alzheimer's disease, and related dementias. We aim to determine the nature of mitochondrial fission involvement in TBI, and to use a small peptide inhibitor of mitochondrial fission protein Drp1, called P110, to mitigate downstream neurodegeneration. We subjected C57/bl6 mice to either TBI or sham injury, and subsequently treated them with either P110 or vehicle by daily intraperitoneal injection for 2 weeks. Following treatment, mice were subjected to a battery of neurobehavioral tests, including novel object recognition (NOR), open field test, and others. Brain tissues were then processed for western blot analysis, staining, and electron microscopy. Mice subjected to TBI showed increased Drp1 expression in the hippocampus 24 after injury, which returned to normal 2 weeks later. There were also observed changes in phosphorylation of Drp1, as well as Drp1 oligomerization, indicating altered Drp1-mediated fission activity. Two weeks following TBI, mice showed a memory deficit in the NOR task, against which P110 treatment was protective. At this time, mitochondria show an elongated morphology specifically in the P110-treated TBI group, but not the sham group, suggesting injury-specificity in its therapeutic mechanism. The same mice, when retested in the NOR task 6 and 9 months post-injury, showed sustained memory protection from early P110 treatment. Our data indicate that early specific inhibition of mitochondrial fission after TBI protects mice from developing cognitive impairment at both acute and chronic stages of injury.

Pubmed:
33501899: Schroeder R, Sridharan P, Nguyen L, Loren A, Williams NS, Kettimuthu KP, Cintrón-Pérez CJ, Vázquez-Rosa E, Pieper AA, Stevens HE
Maternal P7C3-A20 Treatment Protects Offspring from Neuropsychiatric Sequelae of Prenatal Stress.
Impaired embryonic cortical interneuron development from prenatal stress is linked to adult neuropsychiatric impairment, stemming in part from excessive generation of reactive oxygen species in the developing embryo. Unfortunately, there are no preventive medicines that mitigate the risk of prenatal stress to the embryo, as the underlying pathophysiologic mechanisms are poorly understood. Our goal was to interrogate the molecular basis of prenatal stress-mediated damage to the embryonic brain to identify a neuroprotective strategy. Chronic prenatal stress in mice dysregulated nicotinamide adenine dinucleotide (NAD) synthesis enzymes and cortical interneuron development in the embryonic brain, leading to axonal degeneration in the hippocampus, cognitive deficits, and depression-like behavior in adulthood. Offspring were protected from these deleterious effects by concurrent maternal administration of the NAD-modulating agent P7C3-A20, which crossed the placenta to access the embryonic brain. Prenatal stress also produced axonal degeneration in the adult corpus callosum, which was not prevented by maternal P7C3-A20. Prenatal stress dysregulates gene expression of NAD-synthesis machinery and GABAergic interneuron development in the embryonic brain, which is associated with adult cognitive impairment and depression-like behavior. We establish a maternally directed treatment that protects offspring from these effects of prenatal stress. NAD-synthesis machinery and GABAergic interneuron development are critical to proper embryonic brain development underlying postnatal neuropsychiatric functioning, and these systems are highly susceptible to prenatal stress. Pharmacologic stabilization of NAD in the stressed embryonic brain may provide a neuroprotective strategy that preserves normal embryonic development and protects offspring from neuropsychiatric impairment. . 35, 511-530.
Antioxid Redox Signal, 2021; 35
32799605: Sridharan PS, Lu Y, Rice RC, Pieper AA, Rajadhyaksha AM
Loss of Cav1.2 channels impairs hippocampal theta burst stimulation-induced long-term potentiation.

CACNA1 C, which codes for the Ca1.2 isoform of L-type Ca channels (LTCCs), is a prominent risk gene in neuropsychiatric and neurodegenerative conditions. A role forLTCCs, and Ca1.2 in particular, in transcription-dependent late long-term potentiation (LTP) has long been known. Here, we report that elimination of Ca1.2 channels in glutamatergic neurons also impairs theta burst stimulation (TBS)-induced LTP in the hippocampus, known to be transcription-independent and dependent on N-methyl D-aspartate receptors (NMDARs) and local protein synthesis at synapses. Our expansion of the established role of Ca1.2channels in LTP broadens understanding of synaptic plasticity and identifies a new cellular phenotype for exploring treatment strategies for cognitive dysfunction.
Channels (Austin), 2020; 14

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-399

FATE MAPPING OF PERIPHERALLY DERIVED MACROPHAGES AFTER TRAUMATIC BRAIN INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Background and Aim Traumatic Brain Injury (TBI) represents a critical health problem for our society, given its increasing occurrence, complex pathophysiology, challenging diagnosis, and long-term neurological disabilities. It is well established that TBI-induced neuroinflammation engages resident microglia as well as infiltrating monocytes (CCR2+) recruited from the periphery, which both contribute to long lasting cognitive deficits. After brain engraftment, peripherally derived macrophages stop expressing their signature marker CCR2, thus making discrimination from reactive microglia cells elusive. **Methods** We took advantage of CCR2CreER:Ai14D mice, where CCR2+ cells are permanently labelled even after *in situ* reprogramming. Adult CCR2CreER:Ai14D mice were injured using the Controlled Cortical Impact (CCI) TBI model. Injury-induced cognitive deficits were measured using the Radial Arm Water Maze. Infiltrated CCR2+ macrophages were traced 7 and 30 days post injury (dpi) and analyzed using flow cytometry, brain clearing and imaging, RNA sequencing and *in vivo* synapses phagocytosis assays. **Results** We are characterizing localization, transcriptomic signatures and functionality of peripherally derived macrophages focusing on how these features change from sub-acute (7 dpi) and chronic (30 dpi) time points. We expect to observe a previously under-reported level of infiltrating monocyte-to-microglia transition after TBI. **Conclusions** Our study uses the novel CCR2CreER:Ai14D mouse model to unravel a more comprehensive understanding of the long-term role of peripherally derived macrophages in TBI-induced cognitive impairment.

Pubmed:

34654458: Feng X, Frias ES, Paladini MS, Chen D, Boosalis Z, Becker M, Gupta S, Liu S, Gupta N, Rosi S
Functional role of brain-engrafted macrophages against brain injuries.
Brain-resident microglia have a distinct origin compared to macrophages in other organs. Under physiological conditions, microglia are maintained by self-renewal from the local pool, independent of hematopoietic progenitors. Pharmacological depletion of microglia during whole-brain radiotherapy prevents synaptic loss and long-term recognition memory deficits. However, the origin or repopulated cells and the mechanisms behind these protective effects are unknown.
J Neuroinflammation, 2021; 18
33862064: Rienecker KDA, Paladini MS, Grue K, Krukowski K, Rosi S
Microglia: Ally and Enemy in Deep Space.
In 2024 the first female astronaut will land on the moon, advancing our preparations for human missions to Mars. While on Earth we are protected from space radiation by our planet's magnetic field, on such deep space voyages astronauts will be exposed to high energy particles from solar flares and galactic cosmic rays (GCR). This exposure carries risks to the central nervous system (CNS) that could jeopardize the mission and astronaut health. Earth-bound studies have employed a variety of single-beam and sequential radiation exposures to simulate the effects of GCR exposure in rodents. Multiple studies have shown that GCR simulation induces a maladaptive activation of microglia - the brain-resident immune cells. GCR simulation also induced synaptic changes resulting in lasting cognitive and behavioral defects. Female and male mice show different susceptibilities to GCR exposure, and evidence suggests this sexually dimorphic response is linked to microglia. Manipulating microglia can prevent the development of cognitive deficits in male mice exposed to components of GCR. This discovery may provide clues towards how to protect astronauts' cognitive and behavioral health both during deep space missions and upon return to Earth.
Neurosci Biobehav Rev, 2021; 126
33276101: Paladini MS, Spero V, Begni V, Marchisella F, Guidi A, Gruca P, Lason M, Litwa E, Papp M, Riva MA, Molteni R
Behavioral and molecular effects of the antipsychotic drug blonanserin in the chronic mild stress model.
Psychiatric disorders represent a critical challenge to our society, given their high global prevalence, complex symptomatology, elusive etiology and the variable effectiveness of pharmacological therapies. Recently, there has been a shift in investigating and redefining these diseases by integrating behavioral observations and multilevel neurobiological measures. Accordingly, endophenotype-oriented studies are needed to develop new therapeutic strategies, with the idea of

targeting shared symptoms instead of one defined disease. With these premises, here we investigated the therapeutic properties of chronic treatment with the second-generation antipsychotic blonanserin in counteracting the alterations caused by 7 weeks of Chronic Mild Stress (CMS) in the rat. CMS is a well-established preclinical model able to induce depressive and anxiety-like alterations, which are shared by different psychiatric disorders. Our results demonstrated that the antipsychotic treatment normalizes the CMS-induced emotionality deficits, an effect that may be due to its ability in modulating, within the prefrontal cortex, redox mechanisms, a molecular dysfunction associated with several psychiatric disorders. These evidences provide new insights into the therapeutic properties and potential use of blonanserin as well as in its mechanisms of action and provide further support for the role of oxidative stress in the pathophysiology of psychiatric disorders.
Pharmacol Res, 2021; 163
33259927: Paladini MS, Feng X, Krukowski K, Rosi S
Microglia depletion and cognitive functions after brain injury: From trauma to galactic cosmic ray.
Microglia are the resident immune cells of the central nervous system (CNS). In physiological conditions, microglia contribute to maintaining brain homeostasis by scanning the surrounding parenchyma and acting as scavenger cells. Following different insults to the CNS, microglia turn into a "reactive" state characterized by the production of inflammatory mediators that promote tissue repair to restore homeostasis. Brain insults such as traumatic brain injury, therapeutic brain irradiation and galactic cosmic ray exposure are associated with chronic microglia activation. Chronic microglia activation contributes to injury-related impairments in cognitive functions. Microglia depletion achieved either by pharmacological or genetic techniques represents not only a useful tool for more extensive investigations of microglia roles, but also a potential therapeutic approach to ameliorate or prevent cognitive dysfunctions following brain injury.
Neurosci Lett, 2021; 741
33259004: Paladini MS, Marangon D, Rossetti AC, Guidi A, Coppolino GT, Negri C, Spero V, Abbracchio MP, Lecca D, Molteni R
Prenatal Stress Impairs Spinal Cord Oligodendrocyte Maturation via BDNF Signaling in the Experimental Autoimmune Encephalomyelitis Model of Multiple Sclerosis.
One of the most substantial and established environmental risk factors for neurological and psychiatric disorders is stress exposure, whose detrimental consequences hinge on several variables including time. In this regard the gestational period is known to present an intrinsic vulnerability to environmental insults and thus stressful events during pregnancy can lead to severe consequences on the offspring's brain development with long-term repercussions throughout adulthood. On this basis, we investigated the long-lasting impact of prenatal stress exposure on the susceptibility to the experimental autoimmune encephalomyelitis (EAE), a well-established murine model of multiple sclerosis. Although stress is considered a triggering factor for this chronic, progressive, autoimmune disease, little is known about the underlying mechanisms. To this end, EAE was induced by immunization with MOG35-55/CFA and pertussis toxin administration in adult female C57BL/6 mice born from control or stressed dams exposed to restraint stress during the last days of gestation. Our results demonstrate that gestational stress induces a marked increase in the severity of EAE symptoms in adulthood. Further, we highlight an altered maturation of oligodendrocytes in the spinal cord of prenatally stressed EAE mice, as indicated by the higher levels of GPR17, a marker of immature oligodendrocyte precursor cells. These behavioral and molecular alterations are paralleled by changes in the expression and signaling of the neurotrophin BDNF, an important mediator of neural plasticity that may contribute to stress-induced impaired remyelination. Since several already marketed drugs are able to modulate BDNF levels, these results pave the way to the possibility of repositioning these drugs in multiple sclerosis.
Cell Mol Neurobiol, 2022; 42
32296859: Marchisella F, Paladini MS, Guidi A, Begni V, Brivio P, Spero V, Calabrese F, Molteni R, Riva MA
Chronic treatment with the antipsychotic drug blonanserin modulates the responsiveness to acute stress with anatomical selectivity.
Patients diagnosed with schizophrenia typically receive life-long treatments with antipsychotic drugs (APDs). However, the impact of chronic APDs treatment on neuroplastic mechanisms in the brain remains largely elusive.
Psychopharmacology (Berl), 2020; 237
32165136: Rossetti AC, Paladini MS, Riva MA, Molteni R
Oxidation-reduction mechanisms in psychiatric disorders: A novel target for pharmacological intervention.
While neurotransmitter dysfunction represents a key component in mental illnesses, there is now a wide agreement for a central pathophysiological hub that includes hormones, neuroinflammation, redox mechanisms as well as oxidative stress. With respect to oxidation-reduction (redox) mechanisms, preclinical and clinical evidence suggests that an imbalance in the pro/anti-oxidative homeostasis toward the increased production of substances with oxidizing potential may contribute to the etiology and manifestation of different psychiatric disorders. The substantial and continous demand for energy renders the brain highly susceptible to disturbances in its energy supply, especially following exposure to stressful events, which may

lead to overproduction of reactive oxygen and nitrogen species under conditions of perturbed antioxidant defenses. This will eventually induce different molecular alterations, including extensive protein and lipid peroxidation, increased blood-brain barrier permeability and neuroinflammation, which may contribute to the changes in brain function and morphology observed in mental illnesses. This view may also reconcile different key concepts for psychiatric disorders, such as the neurodevelopmental origin of these diseases, as well as the vulnerability of selective cellular populations that are critical for specific functional abnormalities. The possibility to pharmacologically modulate the redox system is receiving increasing interest as a novel therapeutic strategy to counteract the detrimental effects of the unbalance in brain oxidative mechanisms. This review will describe the main mechanisms and mediators of the redox system and will examine the alterations of oxidative stress found in animal models of psychiatric disorders as well as in patients suffering from mental illnesses, such as schizophrenia and major depressive disorder. In addition, it will discuss studies that examined the effects of psychotropic drugs, including antipsychotics and antidepressants, on the oxidative balance as well as studies that investigated the effectiveness of a direct modulation of oxidative mechanisms in counteracting the behavioral and functional alterations associated with psychiatric disorders, which supports the promising role of the redox system as a novel therapeutic target for the improved treatment of brain disorders.
Pharmacol Ther, 2020; 210
31379496: Rossetti AC, Paladini MS, Trepci A, Mallien A, Riva MA, Gass P, Molteni R
Differential Neuroinflammatory Response in Male and Female Mice: A Role for BDNF.
A growing body of evidence supports the close relationship between major depressive disorder (MDD), a severe psychiatric disease more common among women than men, and alterations of the immune/inflammatory system. However, despite the large number of studies aimed at understanding the molecular bases of this association, a lack of information exists on the potential cross-talk between systems known to be involved in depression and components of the inflammatory response, especially with respect to sex differences. Brain-derived neurotrophic factor (BDNF) is a neurotrophin with a well-established role in MDD etiopathology: it is altered in depressed patients as well as in animal models of the disease and its changes are restored by antidepressant drugs. Interestingly, this neurotrophin is also involved in the inflammatory response. Indeed, it can be secreted by microglia, the primary innate immune cells in the central nervous system whose functions may be in turn regulated by BDNF. With these premises, in this study, we investigated the reciprocal impact of BDNF and the immune system by evaluating the neuroinflammatory response in male and female BDNF-heterozygous mutant mice acutely treated with the cytokine-inducer lipopolysaccharide (LPS). Specifically, we assessed the potential onset of an LPS-induced sickness behavior as well as changes of inflammatory mediators in the mouse hippocampus and frontal cortex, with respect to both genotype and sex. We found that the increased inflammatory response induced by LPS in the brain of male mice was independent of the genotype, whereas in the female, it was restricted to the heterozygous mice with no changes in the wild-type group, suggestive of a role for BDNF in the sex-dependent effect of the inflammatory challenge. Considering the involvement of both BDNF and neuroinflammation in several psychiatric diseases and the diverse incidence of such pathologies in males and females, a deeper investigation of the mechanisms underlying their interaction may have a critical translational relevance.
Front Mol Neurosci, 2019; 12
31333079: Brivio P, Paladini MS, Racagni G, Riva MA, Calabrese F, Molteni R
From Healthy Aging to Frailty: In Search of the Underlying Mechanisms.
Population aging is accelerating rapidly worldwide, from 461 million people older than 65 years in 2004 to an estimated 2 billion people by 2050, leading to critical implications for the planning and delivery of health and social care. The most problematic expression of population aging is the clinical condition of frailty, which is a state of increased vulnerability that develops as a consequence of the accumulation of microscopic damages in many physiological systems that lead to a striking and disproportionate change in health state, even after an apparently small insult. Since little is known about the biology of frailty, an important perspective to understand this phenomenon is to establish how the alterations that physiologically occur during a condition of healthy aging may instead promote cumulative decline with subsequent depletion of homoeostatic reserve and increase the vulnerability also after minor stressor events. In this context, the present review aims to provide a description of the molecular mechanisms that, by having a critical impact on behavior and neuronal function in aging, might be relevant for the development of frailty. Moreover, since these biological systems are also involved in the coping strategies set in motion to respond to environmental challenges, we propose a role for lifestyle stress as an important player to drive frailty in aging.
Curr Med Chem, 2019; 26
29788232: Rossetti AC, Paladini MS, Colombo M, Gruca P, Lason-Tyburkiewicz M, Tota-Glowczyk K, Papp M, Riva MA, Molteni R
Chronic Stress Exposure Reduces Parvalbumin Expression in the Rat Hippocampus through an Imbalance of Redox Mechanisms: Restorative Effect of the Antipsychotic Lurasidone.

Psychiatric disorders are associated with altered function of inhibitory neurotransmission within the limbic system, which may be due to the vulnerability of selective neuronal subtypes to challenging environmental conditions, such as stress. In this context, parvalbumin-positive GABAergic interneurons, which are critically involved in processing complex cognitive tasks, are particularly vulnerable to stress exposure, an effect that may be the consequence of dysregulated redox mechanisms. Int J Neuropsychopharmacol, 2018; 21

FENS Forum 2022
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BOARD NUMBER: S04-400

CRANIECTOMY CAMOUFLAGES THE MILD FLUID PERCUSSION INJURY-INDUCED CHANGES IN NEUROGENESIS, NEUROINFLAMMATION, AND BEHAVIOR

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Craniectomy procedure is a prerequisite in several commonly used preclinical rodent models of Traumatic Brain Injury (TBI), including Fluid Percussion Injury (FPI). In preclinical TBI, injured animals are often compared with the 'sham' craniectomy. The present study conceptualized to evaluate the neurobehavioral consequences of craniectomy and its additive damaging effect with FPI. For this purpose, C57BL/6J male mice were exposed to lateral FPI and assessed for the potential changes in the structural and functional outcomes of the brain. The results showed a significant increase in immuno-reactivity of neurogenesis markers (DCX & Beta-III tubulin) in the hippocampus in both the craniectomy and mild FPI mice. Similarly, a significant increase in neuroinflammatory markers (GFAP & TMEM) in CA1 and CA3 regions were also reported. Additionally, significant axonal and dendritic structural alterations and cortical neuronal death were also reported following both the craniectomy and FPI. Moreover, both the groups also showed significant changes in neuromuscular strength and affective state. However, cognitive, motor and exploratory functions remain unaffected. In conclusion, we observed that craniectomy alone may cause substantial structural and functional changes in the brain and the magnitude of such alteration is very similar to mild TBI. Since FPI is the model for a mixed (focal & diffused) injury, it is more relevant to compare the results with naive control instead of the sham. However, for the long-term follow-up studies, the craniectomy-induced damage and time-dependent recovery have to be separately quantified. Moreover, procedural improvement is also warranted to minimize the detrimental consequences of craniectomy.

Pubmed:

32240659: Aleem M, Goswami N, Kumar M, Manda K
Low-pressure fluid percussion minimally adds to the sham craniectomy-induced neurobehavioral changes: Implication for experimental traumatic brain injury model.
Modeling experimental traumatic brain injury (TBI) in rodents is necessarily required to understand the pathophysiological and neurobehavioral consequences of neurotrauma. Numerous models have been developed to study experimental TBI. Fluid percussion injury (FPI) is the most extensively used model to represent clinical phenotypes. Nevertheless, the surgical 'sham' procedure (craniectomy), a prerequisite of FPI, is the impeding factor in experimental TBI. We hypothesized that if craniectomy causes substantial structural and functional changes in the brain, it might mimic the mild FPI-induced neurobehavioral dysfunctions. To understand the hypothesis, C57BL/6 mice were exposed to lateral FPI at 1.2 atm pressure and changes in the neuronal architecture, hippocampal neurogenesis, neuroinflammation, and behavioral functions were compared to the sham (craniectomy) and control mice at day 7 post-FPI. We observed that both the craniectomy and FPI significantly augmented the ipsilateral hippocampal neurogenesis as evaluated by DCX and Beta-III tubulin immunoreactivity. Similarly, a significant increase in GFAP and TMEM immunoreactivity in CA1 and CA3 regions showed that craniectomy mimics FPI-induced neuroinflammation. The additive damaging effect of craniectomy with FPI was also reported in the term of axonal and dendritic fragmentation, swelling and neuronal death using silver staining, Fluoro-jade, and MAP-2 immunoreactivity. Sham-exposed mice showed a significant functional decrease in grip strength. Our results indicate that sham craniectomy itself is enough to cause TBI like characteristics, and thus fluid percussion at mild pressure is minimally additive with craniectomy. Considering the method as a mixed (focal & diffused) injury model, the 'net neurotrauma severity' should be compared with naïve control instead of the sham as it is an outcome of cumulative damage due to fluid pressure and craniectomy. Nevertheless, to understand the long term consequences of neurotrauma, the extent of recovery in surgical sham may separately be quantified.
Exp Neurol, 2020; 329
33290758: Goswami N, Aleem M, Manda K
Clinical relevance of chronic neuropathic pain phenotypes in mice: A comprehensive behavioral analysis.
Despite a large number of preclinical studies performed each year, the safe and effective therapeutic interventions for chronic

pain are scant. Therefore, it appears that pre-clinical modeling requires a systematically organized behavioral test paradigm to quantify the response of animals for a specific pain state. The present study, therefore, conceptualized a test battery to evaluate the behavioral changes in mice following neuropathic pain. We employed sciatic nerve chronic constriction injury (CCI) in C57BL/6 J mice to model chronic pain state. Mice were monitored for thermal hyperalgesia and grip strength for 30 days. Subsequently, mice underwent a behavioral test battery consisting of the nociceptive threshold, the affective and cognitive functions and motor coordination, and strength. Our results showed that CCI mice are insensitive to thermal stimuli. However, nerve-injured mice showed significant changes in neuromuscular coordination, basal anxiety, and hedonic state. Such impaired neuromuscular coordination is indicative of disability rather than the actual pain phenotype. While using the digital gait analysis, our study revealed rationales for the insensitivity of CCI mice to thermal stimuli. Our results suggest that the predictive validity of the CCI model necessitates a comprehensive behavioral test battery to select the clinically relevant and measurable phenotype to quantify chronic neuropathic pain.
Behav Brain Res, 2021; 400
34306267: Goswami N, Aleem M, Manda K
Intranasal Ketamine for Acute Pain: Behavioral and Neurophysiological Safety Analysis in Mice.
Subanesthetic ketamine has been used for treatment-resistant depression and is popular as an opioid-sparing agent.
Curr Ther Res Clin Exp, 2021; 94

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BOARD NUMBER: S04-401

THE IMPACT OF A PRE-EXISTING TOXOPLASMA GONDII INFECTION FOLLOWING TRAUMATIC BRAIN INJURY IN MICE

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Introduction: Neuroinflammation is a fundamental response to traumatic brain injury (TBI) and contributes to secondary brain injury. The neurotropic parasite *Toxoplasma gondii* infects one-third globally and as no cure exists, many individuals have a chronic *T. gondii* infection when TBI occurs. Chronic *T. gondii* infection is characterised by parasitic cysts in neurons and low-grade neuroinflammation. **Aims:** To investigate the impact of chronic *T. gondii* infection on TBI outcomes in a mouse model of severe TBI, with the hypothesis that chronic *T. gondii* infection would exacerbate neuroinflammation and behavioural outcomes. **Methods:** Six-week-old male and female C57BL/6 Jax mice were intraperitoneally administered either *T. gondii* tachyzoites or PBS. At six-weeks post-injection, mice received either a controlled cortical impact or sham injury. Behavioural and neuroinflammatory changes were evaluated within 1-week or 3-4 months post-injury. Seizure susceptibility and pathology were assessed 4 months post-injury. **Results:** Gene expression analyses indicate that acutely post-injury, *T. gondii* infection intensified the neuroimmune response in the ipsilateral cortex. In the long-term, both TBI and *T. gondii* infection negatively altered spatial memory in female mice. In both sexes, TBI mice exhibited decreased anxiety levels compared to sham-injured mice. Further, data suggests *T. gondii* infection modified motor function and various gait parameters in female mice. **Conclusions:** Chronic *T. gondii* infection amplified neuroinflammation acutely post-injury; however, findings to date suggest that infection and injury did not alter long-term behavioural outcomes in a synergistic manner. Analysis of seizure susceptibility, as well as brain and peripheral tissue is ongoing.

Pubmed:
32711529: Baker TL, Sun M, Semple BD, Tyebji S, Tonkin CJ, Mychasiuk R, Shultz SR
Catastrophic consequences: can the feline parasite Toxoplasma gondii prompt the purrfect neuroinflammatory storm following traumatic brain injury?
Traumatic brain injury (TBI) is one of the leading causes of morbidity and mortality worldwide; however, treatment development is hindered by the heterogenous nature of TBI presentation and pathophysiology. In particular, the degree of neuroinflammation after TBI varies between individuals and may be modified by other factors such as infection. Toxoplasma gondii, a parasite that infects approximately one-third of the world's population, has a tropism for brain tissue and can persist as a life-long infection. Importantly, there is notable overlap in the pathophysiology between TBI and T. gondii infection, including neuroinflammation. This paper will review current understandings of the clinical problems, pathophysiological mechanisms, and functional outcomes of TBI and T. gondii, before considering the potential synergy between the two conditions. In particular, the discussion will focus on neuroinflammatory processes such as microglial activation, inflammatory cytokines, and peripheral immune cell recruitment that occur during T. gondii infection and after TBI. We will present the notion that these overlapping pathologies in TBI individuals with a chronic T. gondii infection have the strong potential to exacerbate neuroinflammation and related brain damage, leading to amplified functional deficits. The impact of chronic T. gondii infection on TBI should therefore be investigated in both preclinical and clinical studies as the possible interplay could influence treatment strategies.
J Neuroinflammation, 2020; 17
33966527: Baker TL, Agoston DV, Brady RD, Major B, McDonald SJ, Mychasiuk R, Wright DK, Yamakawa GR, Sun M, Shultz SR
Targeting the Cerebrovascular System: Next-Generation Biomarkers and Treatment for Mild Traumatic Brain Injury.
The diagnosis, prognosis, and treatment of mild traumatic brain injuries (mTBIs), such as concussions, are significant unmet medical issues. The kinetic forces that occur in mTBI adversely affect the cerebral vasculature, making cerebrovascular injury

(CVI) a pathophysiological hallmark of mTBI. Given the importance of a healthy cerebrovascular system in overall brain function, CVI is likely to contribute to neurological dysfunction after mTBI. As such, CVI and related pathomechanisms may provide objective biomarkers and therapeutic targets to improve the clinical management and outcomes of mTBI. Despite this potential, until recently, few studies have focused on the cerebral vasculature in this context. This article will begin by providing a brief overview of the cerebrovascular system followed by a review of the literature regarding how mTBI can affect the integrity and function of the cerebrovascular system, and how this may ultimately contribute to neurological dysfunction and neurodegenerative conditions. We then discuss promising avenues of research related to mTBI biomarkers and interventions that target CVI, and conclude that a clinical approach that takes CVI into account could result in substantial improvements in the care and outcomes of patients with mTBI.
Neuroscientist, 2021;

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ROAD TO RECOVERY: EXAMINING THE EFFECTS OF SHIP-1 ON NEUROIMMUNE RESPONSES AFTER PAEDIATRIC HEAD INJURIES

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Introduction: Recovery after early-life traumatic brain injury (TBI) is often hindered by secondary neuroinflammation, likely driven by chronically activated pro-inflammatory microglia. However, factors that induce aberrant microglial responses have remained elusive. Various studies indicate that loss of SH2 domain-containing inositol 5' phosphatase (SHIP-1) activity dysregulate immunological responses. SHIP-1 negatively regulates the phosphoinositide 3-kinase (PI3K) signalling pathway in immune cells, such that SHIP-1 deficiency results in chronic systemic inflammation. **Aims:** To investigate the role of SHIP-1 in microglial activation and function in a mouse model of severe paediatric TBI, with the hypothesis that SHIP-1 deficiency (-/-) would exacerbate microglial responses and worsen outcomes following severe paediatric TBI. **Methods:** SHIP-1+/- and -/- mice received a controlled cortical impact (n=17; n=11) or sham surgery (n=15; n=10) at postnatal day 21. Behavioural changes, microglial responses (immunostaining and gene expression), and tissue damage were evaluated at 7 days post-injury. **Results:** TBI mice exhibited elevated anxiety levels and altered short-term spatial memory compared to sham animals, alongside a reduction in the volume of healthy cortical tissue, independent of genotype ($p>0.05$). Gene expression analyses of microglial activation markers indicate enhanced microglial responses in SHIP-1-/- mice. However, injury-induced Iba1+ microglial immunofluorescence was similar between genotypes in the ipsilateral cortex, dentate gyrus and dorsolateral thalamus ($p>0.05$), while GFAP+ astrocyte immunoreactivity was elevated 2-fold in SHIP-1-/- mice compared to +/- controls ($p=0.01$). **Conclusion:** SHIP-1 deficiency altered acute neuroimmune responses after paediatric TBI. However, further work is required to understand how SHIP-1 may regulate recovery in this context.

Pubmed:

34838047: Chu E, Mychasiuk R, Hibbs ML, Semple BD
Dysregulated phosphoinositide 3-kinase signaling in microglia: shaping chronic neuroinflammation.
Microglia are integral mediators of innate immunity within the mammalian central nervous system. Typical microglial responses are transient, intending to restore homeostasis by orchestrating the removal of pathogens and debris and the regeneration of damaged neurons. However, prolonged and persistent microglial activation can drive chronic neuroinflammation and is associated with neurodegenerative disease. Recent evidence has revealed that abnormalities in microglial signaling pathways involving phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT) may contribute to altered microglial activity and exacerbated neuroimmune responses. In this scoping review, the known and suspected roles of PI3K-AKT signaling in microglia, both during health and pathological states, will be examined, and the key microglial receptors that induce PI3K-AKT signaling in microglia will be described. Since aberrant signaling is correlated with neurodegenerative disease onset, the relationship between maladapted PI3K-AKT signaling and the development of neurodegenerative disease will also be explored. Finally, studies in which microglial PI3K-AKT signaling has been modulated will be highlighted, as this may prove to be a promising therapeutic approach for the future treatment of a range of neuroinflammatory conditions.
J Neuroinflammation, 2021; 18
33731173: Sharma R, Zamani A, Dill LK, Sun M, Chu E, Robinson MJ, O'Brien TJ, Shultz SR, Semple BD
A systemic immune challenge to model hospital-acquired infections independently regulates immune responses after pediatric traumatic brain injury.
Traumatic brain injury (TBI) is a major cause of disability in young children, yet the factors contributing to poor outcomes in this population are not well understood. TBI patients are highly susceptible to nosocomial infections, which are mostly acquired within the first week of hospitalization, and such infections may modify TBI pathobiology and recovery. In this study,

we hypothesized that a peripheral immune challenge such as lipopolysaccharide (LPS)-mimicking a hospital-acquired infection-would worsen outcomes after experimental pediatric TBI, by perpetuating the inflammatory immune response. J Neuroinflammation, 2021; 18

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BOARD NUMBER: S04-403

DEPLETION OF GUT MICROBIOME AND EXPOSURE TO REPEAT MILD TRAUMATIC BRAIN INJURIES MODIFIES SOCIAL BEHAVIOUR AND NEUROPATHOLOGICAL CHANGES WITHIN THE ADOLESCENT BRAIN

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Zoe Kodila[1], Marissa Sgro[2], Crystal Li[3], Glenn Yamakawa[1], Richelle Mychasiuk[1]
[1]Monash University, Neuroscience, Melbourne, Australia, [2]Central Clinical School, Neuroscience, Melbourne, Australia, [3]Monash University (Central Clinical School), Neuroscience, Melbourne, Australia

Background Adolescence is associated with ongoing maturation in the brain, particularly the medial prefrontal cortex (mPFC), and systemically, whereby the brain-gut-immune axis is developing. Given that adolescents are more likely than adults to experience repetitive mild traumatic brain injuries (RmTBI), they may be at increased risk for worsened outcomes. **Aim** This study aimed to examine how depletion of the gut microbiome mediates behavioural and neuropathological recovery from RmTBI in adolescent rats. **Methods** The rat microbiota was depleted using an antibiotic cocktail which was administered for 14 days prior to receiving 3 mTBIs. Rodent temperament was assessed five days post-injury using a 15-minute video recording. Additionally, we used immunohistochemical staining to examine the number of perineuronal nets (PNNs) and parvalbumin interneurons (PVs) within the mPFC. Three-way ANOVAs with sex, injury, and treatment as factors were run. **Results** We identified significant sex, injury, and treatment effects in adolescent temperament, including modifications to social interactions such as allogrooming and playing ($p<.05$). The number of PNNs increased in males and females that experience RmTBI+Placebo ($p <.05$). Interestingly, for rats that experienced RmTBI+antibiotics, the number of PNNs increased in males but decreased in females ($p<.05$). For PVs there was an increase in RmTBI+placebo treated rats however, in the RmTBI+antibiotic treated rats there was a decrease in females, but an increase in males. **Conclusions** We found that changes within the microbiome can modify behavioural and neuropathological outcomes following RmTBI in adolescence, however contrary to our hypotheses, the effects of RmTBI and microbiome depletion were not additive.

Pubmed:
34757429: Sgro M, Kodila ZN, Brady RD, Reichelt AC, Mychaisuk R, Yamakawa GR
Synchronizing our clocks as we age: the influence of the brain-gut-immune axis on the sleep-wake cycle across the lifespan. The microbes that colonize the small and large intestines, known as the gut microbiome, play an integral role in optimal brain development and function. The gut microbiome is a vital component of the bidirectional communication pathway between the brain, immune system, and gut, also known as the brain-gut-immune axis. To date, there has been minimal investigation into the implications of improper development of the gut microbiome and the brain-gut-immune axis on the sleep-wake cycle, particularly during sensitive periods of physical and neurological development, such as childhood, adolescence, and senescence. Therefore, this review will explore the current literature surrounding the overlapping developmental periods of the gut microbiome, brain, and immune system from birth through to senescence, while highlighting how the brain-gut-immune axis affects the maturation and organization of the sleep-wake cycle. We also examine how a dysfunction to either the microbiome or the sleep-wake cycle negatively affects the bidirectional relationship between the brain and gut, and subsequently the overall health and functionality of this complex system. Additionally, this review integrates therapeutic studies to demonstrate when dietary manipulations, such as supplementation with probiotics and prebiotics, can modulate the gut microbiome to enhance the health of the brain-gut-immune axis and optimize our sleep-wake cycle.
Sleep, 2022; 45

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BOARD NUMBER: S04-404

CHRONIC INTRACEREBROVENTRICULAR ADMINISTRATION OF OREXIN-A DOES NOT MODIFY BEHAVIOURAL OUTCOMES FOLLOWING REPETITIVE MILD TRAUMATIC BRAIN INJURY IN RATS

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Crystal Li, Jennaya Christensen, Glenn Yamakawa, Sandy Shultz, Richelle Mychasiuk
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AIMS: Sleep-wake disturbances following mild traumatic brain injury (mTBI) are associated with complications to recovery, so it is essential to consider the mechanisms underlying mTBI-induced sleep-wake disturbances. Changes to hypothalamic function, specifically orexinergic function, have been established following mTBI. Considering that orexinergic function plays a dominant role in regulating the sleep-wake cycle, the aim of this study was to assess how pharmacological manipulation of orexinergic signalling influences repetitive mTBI (RmTBI) outcomes. METHODS: Adult male Sprague Dawley rats were randomly assigned to receive either RmTBIs or sham injuries. Following the injuries, intracerebroventricular (ICV) injections of orexin-A (ORXA) or vehicle were administered via cannulas previously implanted into the lateral ventricle. Behavioural testing was conducted to examine the effects of ORXA versus vehicle treatment on RmTBI-induced impairments. Brain tissue was additionally processed for immunohistochemical analysis of hypothalamic regions. RESULTS: RmTBI-driven changes were observed whereby RmTBI groups spent significantly more time in the open arms of the elevated plus maze ($p<.05$) and remained for longer on the rotarod relative to sham groups ($p<.05$). Video-based sleep monitoring confirmed that ORXA increased duration of wakefulness relative to vehicle groups ($p<.05$), but ORXA did not significantly influence behavioural outcomes when compared to vehicle groups ($p>.05$). Immunohistochemical analysis of brain tissue showed RmTBI-driven differences ($p<.05$). DISCUSSION: Sleep-wake disturbances and impairments following Rm-TBI may not be consequences of orexinergic dysregulation. To better understand the basis of these observed outcomes, additional insights into RmTBI-driven alterations in cognition and hypothalamic function are required.

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A NOVEL RAT MODEL OF HETEROTOPIC OSSIFICATION AFTER POLYTRAUMA WITH TRAUMATIC BRAIN INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Introduction: Neurological heterotopic ossification (NHO) is characterized by abnormal bone growth in soft tissue and joints that commonly occurs in severe traumatic brain injury (TBI) patients, particularly in the presence of concomitant musculoskeletal injuries (i.e. polytrauma). The ectopic bone frequently causes pain, and restricts mobility. Objectives: There is currently a lack of animal models that mimic these injury combinations, which has limited our understanding of NHO pathobiology, biomarkers, and treatments. To address this shortcoming, here we present a novel rat model that combines TBI, femoral fracture, and muscle crush injury. Materials and Methods: Young adult male Sprague Dawley rats were randomly assigned into three different injury groups: triple sham-injury, peripheral injury only (i.e., sham-TBI + fracture + muscle injury) or triple injury (i.e., TBI + fracture + muscle injury). Results and Discussion: Evidence of ectopic bone in the injured hind-limb, as confirmed by micro-computed tomography was found at 6-weeks post-injury in 70% of triple injury rats, 20% of peripheral injury rats, and 0% of the sham-injured controls. Furthermore, the triple injury rats had higher ectopic bone severity scores than the sham-injured group. We also have muscle and blood samples from rats at 2-, and 7 days post injury and at 12 weeks post injury. Analysis to identify blood biomarkers and novel mechanisms of NHO formation is ongoing. Conclusion: This model will provide a platform for future studies to identify underlying mechanisms, biomarkers, and develop evidence based pharmacological treatments to combat this debilitating long-term complication of TBI and polytrauma.

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BMX DEFICIENCY ALLEVIATES COGNITIVE AND MOTOR IMPAIRMENT AFTER TRAUMATIC BRAIN INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Kai-Yun Chen[1], Jing-Huei Lai[2], Chi-Zong Huang[3], Yen-Hwa Chen[1], Shuo-Jhen Kang[3], Yung-Hsiao Chiang[3,4], John Chung-Che Wu[3,4]
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Aims: Traumatic brain injury (TBI) is becoming not only a global health problem but also a serious socioeconomic burden. TBI patients may develop persistent cognitive impairments such as loss of attention and memory, increasing risk of neurodegenerative disorders. Our previous study has proven the correlation of BMX to severity of TBI in rat model. TBI patients with an increased level of BMX in the first 3 weeks of their injury are more prone to have a greater severity of dizziness at 6 weeks post-injury. Additionally, BMX single nucleotide polymorphism is associated with post-TBI dizziness and anxiety. However, mechanisms behind the role of BMX in TBI are not yet understood completely. Here, we aim to investigate the role of BMX in cognitive impairment after TBI. **Methods:** We induced TBI in C57BL/6 (WT) mice and BMX knockout (BMX-KO) mice using controlled cortical impact. Cognitive and memory functions were analyzed by novel object recognition (NOR) and water maze test. Motor functions were analyzed by open field and beam walking. **Results:** NOR and water maze analysis showed that cognitive and memory function in WT mice decreased 7 days post-injury but there was no significant difference in BMX-KO mice after injury. Open field analysis revealed BMX-KO mice exhibited less anxiety than WT mice. Interestingly, balance function is worse in BMX-KO mice comparing to WT mice. **Conclusions:** Depletion of BMX can improve cognitive and motor function after TBI. Targeting BMX and its downstream molecules may be a good diagnostic and therapeutic strategy for TBI.

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BOARD NUMBER: S04-407

EMULATING THE SECONDARY INJURIES OF TRAUMATIC BRAIN INJURY AND THE EXPLORATION OF NEUROTHERAPEUTICS.

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Cameron Hall[1], Koby Baranes[1,2], Monica Killen[1], Mark Kotter[1,2], Keri Carpenter[1], Peter Hutchinson[1]
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Introduction: After traumatic brain injury (TBI), evolving changes include abnormal brain metabolism, contributing to secondary injury. High brain extracellular lactate/pyruvate ratio (LPR), indicating increased glycolysis and reduced mitochondrial activity, correlates with unfavourable patient outcome. In-vitro, succinate partially protected rat glia against rotenone-induced metabolic dysfunction. Rotenone inhibits complex I of the mitochondrial electron transport chain (ETC). Succinate, a tricarboxylic acid cycle intermediate, interacts with complex II of the ETC, bypassing complex I. **Objectives:** To study metabolic dysfunction in human induced neurons (iNs) as a model for TBI and assess the putative neurotherapeutic agent succinate for metabolic protection. **Materials and Methods:** We used an established protocol Optimised Inducible Overexpression, producing iNs by overexpression of transcription factor Neurogenin-2. After differentiation, rotenone at 10- or 25-uM and disodium succinate at 5-, 10-, 25- or 50-mM were added to iNs, evaluated at 12-, 24- and 48-h. Hoechst 33342 and propidium iodide staining quantified viability. Metabolism was assessed by ISCUSflex analysis of LPR in the extracellular medium. **Results:** At 24h and 48h (but not at 12h), LPR increased significantly in rotenone-treated iNs, indicating metabolic dysfunction as expected. However, succinate did not protect iNs against rotenone at any of the concentrations and timepoints we tested. **Conclusion:** We showed that neurons' mitochondria were inhibited by rotenone but not protected by succinate, unlike our earlier study where succinate protected glia against rotenone. We postulate that a mechanism whereby succinate might rescue neurons is via interaction between glia and neurons. Future investigations will thus include co-culture of iNs and glia.

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BOARD NUMBER: S04-408

ELECTROPHYSIOLOGICAL AND BEHAVIORAL CHARACTERIZATION OF MURINE MODEL EXPOSED TO ACUTE SARIN SUBLETHAL DOSES AND ANTIDOTE THERAPY EVALUATION

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Warfare neurotoxicants such as sarin, soman or VX, are organophosphorus compounds which irreversibly inhibit cholinesterase. High-dose exposure with nerve agents (NA) is known to produce seizure activity and related brain damage, while less is known about the effects of acute sublethal dose exposure. The aim of this study is to characterize behavioral, brain activity and neuroinflammatory modifications at different time points after exposure to sublethal doses of sarin. First, we conducted a behavioral analysis of symptoms during the first hour following sarin challenge and reported the intoxication severity using a specific scoring scale. The toxidrome intensity was dose-dependent and proportional to the cholinesterase activity inhibition evaluated in mice brain. Electrocorticographic (ECoG) recording revealed an altered cerebral activity early after sarin exposure, with a disruption of brainwave power in a dose-dependent manner and persistent up to 24 hours. To evaluate long lasting changes and protective effects of emergency treatment (ET; atropine + pralidoxime), we conducted ECoG recording for 30 days on mice exposed to 0.9 LD50 of sarin with or without ET. Only mice exposed to sarin without ET showed long-lasting impairment of theta and beta rhythms 1-month post-exposure. The administration of the ET seems to restore cerebral activity in sarin-exposed mice. Our findings identified both transient and long-term ECoG alterations following exposure to sub-lethal doses of sarin, effects partially ameliorated by the administration of ET. In addition, we plan to evaluate ET effects on neuroinflammation, cognitive behavior and histopathologies in sarin-exposed mice.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-409

ACUTE MICROGLIAL INTERVENTION BY CMET INHIBITOR ELICITS NEURONAL SURVIVAL AND MOTOR RECOVERY IN MURINE MILD BLUNT TBI MODEL

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Traumatic Brain Injury (TBI) is a highly complicated pathology involving multiple events occurring simultaneously including, but not limited to, neuroinflammation, blood brain barrier disruption, apoptosis and alteration in neuronal signaling. Microglia acts as a double-edged sword in brain trauma and plays a critical role in injury-related neuroinflammation. To understand the temporal dynamics of signaling processes in traumatic Brain Injury (TBI), we performed an advanced unbiased approach using large-scale array phosphoproteomics to map receptor tyrosine kinase (RTK) mediated acute signaling in a murine mild blunt TBI model. Our results revealed that phosphorylated levels of B-cell specific Bruton's tyrosine kinase (Btk) and wound healing associated cMet (HGFR) were upregulated as early as 3h post injury in microglial cells. Small molecule inhibition of Btk failed to produce an effect in signaling pattern or behavioral assessment however cMet emerged as a selective modifier of the early microglial response. cMet blockade prevented the induction of microglial inflammatory mediators, reactive microglia morphology, and TBI-associated responses in neurons, vessels, and brain extracellular matrix. Acute or prolonged cMet inhibition altered microglial morphology to ramified (resting state), compared to amebiod (activated) in sham controls, ameliorated neuronal survival and significantly improved motor performance. Our findings identify cMet as a modulator of early neuroinflammation in TBI with translational potential and indicate several RTK families as possible targets for TBI treatment. We conclude that acute microglia inactivation by cMet (expressed in microglia) inhibition and promotion of resting microglial state mitigates pathological response as early as 1-day post TBI and ensures neuronal protection.

Pubmed:

34604393: Li Z, Zhang J, Halbgebauer S, Chandrasekar A, Rehman R, Ludolph A, Boeckers T, Huber-Lang M, Otto M, Roselli F, Heuvel FO
Differential effect of ethanol intoxication on peripheral markers of cerebral injury in murine blunt traumatic brain injury.
Blood-based biomarkers have proven to be a reliable measure of the severity and outcome of traumatic brain injury (TBI) in both murine models and patients. In particular, neuron-specific enolase (NSE), neurofilament light (NFL) and S100 beta (S100B) have been investigated in the clinical setting post-injury. Ethanol intoxication (EI) remains a significant comorbidity in TBI, with 30-40% of patients having a positive blood alcohol concentration post-TBI. The effect of ethanol on blood-based biomarkers for the prognosis and diagnosis of TBI remains unclear. In this study, we investigated the effect of EI on NSE, NFL and S100B and their correlation with blood-brain barrier integrity in a murine model of TBI.
Burns Trauma, 2021; 9
34326766: Rehman R, Tar L, Olamide AJ, Li Z, Kassubek J, Böckers T, Weishaupt J, Ludolph A, Wiesner D, Roselli F
Acute TBK1/IKK-ε Inhibition Enhances the Generation of Disease-Associated Microglia-Like Phenotype Upon Cortical Stab-Wound Injury.
Traumatic brain injury has a poorer prognosis in elderly patients, possibly because of the enhanced inflammatory response characteristic of advanced age, known as "inflammaging." Recently, reduced activation of the TANK-Binding-Kinase 1 (Tbk1) pathway has been linked to age-associated neurodegeneration and neuroinflammation. Here we investigated how the blockade of Tbk1 and of the closely related IKK-ε by the small molecule Amlexanox could modify the microglial and immune response to cortical stab-wound injury in mice. We demonstrated that Tbk1/IKK-ε inhibition resulted in a massive expansion of microglial cells characterized by the TMEM119/CD11c phenotype, expressing high levels of CD68 and CD317, and with the upregulation of Cst7a, Prgn and Ccl4 and the decrease in the expression levels of Tmem119 itself and P2yr12, thus a profile close to Disease-Associated Microglia (DAM, a subset of reactive microglia abundant in Alzheimer's Disease and other neurodegenerative conditions). Furthermore, Tbk1/IKK-ε inhibition increased the infiltration of CD3 lymphocytes, CD169 macrophages and CD11c/CD169 cells. The enhanced immune response was associated with increased expression of Il-33, Ifn-g, Il-17, and Il-19. This upsurge in the response to the stab wound was associated with the expanded astroglial scars and

increased deposition of chondroitin-sulfate proteoglycans at 7 days post injury. Thus, Tbk1/IKK-ε blockade results in a massive expansion of microglial cells with a phenotype resembling DAM and with the substantial enhancement of neuroinflammatory responses. In this context, the induction of DAM is associated with a detrimental outcome such as larger injury-related glial scars. Thus, the Tbk1/IKK-ε pathway is critical to repress neuroinflammation upon stab-wound injury and Tbk1/IKK-ε inhibitors may provide an innovative approach to investigate the consequences of DAM induction.
Front Aging Neurosci, 2021; 13
31207335: Olde Heuvel F, Holl S, Chandrasekar A, Li Z, Wang Y, Rehman R, Förstner P, Sinske D, Palmer A, Wiesner D, Ludolph A, Huber-Lang M, Relja B, Wirth T, Röszer T, Baumann B, Boeckers T, Knöll B, Roselli F
STAT6 mediates the effect of ethanol on neuroinflammatory response in TBI.
Traumatic brain injury (TBI) and ethanol intoxication (EI) frequently coincide, particularly in young subjects. However, the mechanisms of their interaction remain poorly understood. Among other pathogenic pathways, TBI induces glial activation and neuroinflammation in the hippocampus, resulting in acute and chronic hippocampal dysfunction. In this regard, we investigated the role of EI affecting these responses unfolding after TBI. We used a blunt, weight-drop approach to model TBI in mice. Male mice were pre-administered with ethanol or vehicle to simulate EI. The neuroinflammatory response in the hippocampus was assessed by monitoring the expression levels of >20 cytokines, the phosphorylation status of transcription factors and the phenotype of microglia and astrocytes. We used AS1517499, a brain-permeable STAT6 inhibitor, to elucidate the role of this pathway in the EI/TBI interaction. We showed that TBI causes the elevation of IL-33, IL-1β, IL-38, TNF-α, IFN-α, IL-19 in the hippocampus at 3 h time point and concomitant EI results in the dose-dependent downregulation of IL-33, IL-1β, IL-38, TNF-α and IL-19 (but not of IFN-α) and in the selective upregulation of IL-13 and IL-12. EI is associated with the phosphorylation of STAT6 and the transcription of STAT6-controlled genes. Moreover, ethanol-induced STAT6 phosphorylation and transcriptional activation can be recapitulated in vitro by concomitant exposure of neurons to ethanol, depolarization and inflammatory stimuli (simulating the acute trauma). Acute STAT6 inhibition prevents the effects of EI on IL-33 and TNF-α, but not on IL-13 and negates acute EI beneficial effects on TBI-associated neurological impairment. Additionally, EI is associated with reduced microglial activation and astrogliosis as well as preserved synaptic density and baseline neuronal activity 7 days after TBI and all these effects are prevented by acute administration of the STAT6 inhibitor concomitant to EI. EI concomitant to TBI exerts significant immunomodulatory effects on cytokine induction and microglial activation, largely through the activation of STAT6 pathway, ultimately with beneficial outcomes.
Brain Behav Immun, 2019; 81
29774782: Chandrasekar A, Olde Heuvel F, Wepler M, Rehman R, Palmer A, Catanese A, Linkus B, Ludolph A, Boeckers T, Huber-Lang M, Radermacher P, Roselli F
The Neuroprotective Effect of Ethanol Intoxication in Traumatic Brain Injury Is Associated with the Suppression of ErbB Signaling in Parvalbumin-Positive Interneurons.
Ethanol intoxication (EI) is a frequent comorbidity of traumatic brain injury (TBI), but the impact of EI on TBI pathogenic cascades and prognosis is unclear. Although clinical evidence suggests that EI may have neuroprotective effects, experimental support is, to date, inconclusive. We aimed at elucidating the impact of EI on TBI-associated neurological deficits, signaling pathways, and pathogenic cascades in order to identify new modifiers of TBI pathophysiology. We have shown that ethanol administration (5 g/kg) before trauma enhances behavioral recovery in a weight-drop TBI model. Neuronal survival in the injured somatosensory cortex was also enhanced by EI. We have used phospho-receptor tyrosine kinase (RTK) arrays to screen the impact of ethanol on TBI-induced activation of RTK in somatosensory cortex, identifying ErbB2/ErbB3 among the RTKs activated by TBI and suppressed by ethanol. Phosphorylation of ErbB2/3/4 RTKs were upregulated in vGlut2 excitatory synapses in the injured cortex, including excitatory synapses located on parvalbumin (PV)-positive interneurons. Administration of selective ErbB inhibitors was able to recapitulate, to a significant extent, the neuroprotective effects of ethanol both in sensorimotor performance and structural integrity. Further, suppression of PV interneurons in somatosensory cortex before TBI, by engineered receptors with orthogonal pharmacology, could mimic the beneficial effects of ErbB inhibitors. Thus, we have shown that EI interferes with TBI-induced pathogenic cascades at multiple levels, with one prominent pathway, involving ErbB-dependent modulation of PV interneurons.
J Neurotrauma, 2018; 35
29463176: Förstner P, Rehman R, Anastasiadou S, Haffner-Luntzer M, Sinske D, Ignatius A, Roselli F, Knöll B
Neuroinflammation after Traumatic Brain Injury Is Enhanced in Activating Transcription Factor 3 Mutant Mice.
Traumatic brain injury (TBI) induces a neuroinflammatory response resulting in astrocyte and microglia activation at the lesion site. This involves upregulation of neuroinflammatory genes, including chemokines and interleukins. However, so far, there is lack of knowledge on transcription factors (TFs) modulating this TBI-associated gene expression response. Herein, we analyzed activating transcription factor 3 (ATF3), a TF encoding a regeneration-associated gene (RAG) predominantly studied in peripheral nervous system (PNS) injury. ATF3 contributes to PNS axon regeneration and was shown before to regulate inflammatory processes in other injury models. In contrast to PNS injury, data on ATF3 in central nervous system

(CNS) injury are sparse. We used Atf3 mouse mutants and a closed-head weight-drop-based TBI model in adult mice to target the rostrolateral cortex resulting in moderate injury severity. Post-TBI, ATF3 was upregulated already at early time points (i.e,. 1-4 h) post-injury in the brain. Mortality and weight loss upon TBI were slightly elevated in Atf3 mutants. ATF3 deficiency enhanced TBI-induced paresis and hematoma formation, suggesting that ATF3 limits these injury outcomes in wild-type mice. Next, we analyzed TBI-associated RAG and inflammatory gene expression in the cortical impact area. In contrast to the PNS, only some RAGs (Atf3, Timp1, and Sprr1a) were induced by TBI, and, surprisingly, some RAG encoding neuropeptides were downregulated. Notably, we identified ATF3 as TF-regulating proneuroinflammatory gene expression, including CCL and CXCL chemokines (Ccl2, Ccl3, Ccl4, and Cxcl1) and lipocalin. In Atf3 mutant mice, mRNA abundance was further enhanced upon TBI compared to wild-type mice, suggesting immune gene repression by wild-type ATF3. In accord, more immune cells were present in the lesion area of ATF3-deficient mice. Overall, we identified ATF3 as a new TF-mediating TBI-associated CNS inflammatory responses.
J Neurotrauma, 2018; 35

29306704: Chandrasekar A, Aksan B, Heuvel FO, Förstner P, Sinske D, Rehman R, Palmer A, Ludolph A, Huber-Lang M, Böckers T, Mauceri D, Knöll B, Roselli F

Neuroprotective effect of acute ethanol intoxication in TBI is associated to the hierarchical modulation of early transcriptional responses.

Ethanol intoxication is a risk factor for traumatic brain injury (TBI) but clinical evidence suggests that it may actually improve the prognosis of intoxicated TBI patients. We have employed a closed, weight-drop TBI model of different severity (2cm or 3cm falling height), preceded (-30min) or followed (+20min) by ethanol administration (5g/Kg). This protocol allows us to study the interaction of binge ethanol intoxication in TBI, monitoring behavioral changes, histological responses and the transcriptional regulation of a series of activity-regulated genes (immediate early genes, IEGs). We demonstrate that ethanol pretreatment before moderate TBI (2cm) significantly reduces neurological impairment and accelerates recovery. In addition, better preservation of neuronal numbers and cFos+cells was observed 7days after TBI. At transcriptional level, ethanol reduced the upregulation of a subset of IEGs encoding for transcription factors such as Atf3, c-Fos, FosB, Egr1, Egr3 and Npas4 but did not affect the upregulation of others (e.g. Gadd45b and Gadd45c). While a subset of IEGs encoding for effector proteins (such as Bdnf, InhbA and Dusp5) were downregulated by ethanol, others (such as Il-6) were unaffected. Notably, the majority of genes were sensitive to ethanol only when administered before TBI and not afterwards (the exceptions being c-Fos, Egr1 and Dusp5). Furthermore, while severe TBI (3cm) induced a qualitatively similar (but quantitatively larger) transcriptional response to moderate TBI, it was no longer sensitive to ethanol pretreatment. Thus, we have shown that a subset of the TBI-induced transcriptional responses were sensitive to ethanol intoxication at the instance of trauma (ultimately resulting in beneficial outcomes) and that the effect of ethanol was restricted to a certain time window (pre TBI treatment) and to TBI severity (moderate). This information could be critical for the translational value of ethanol in TBI and for the design of clinical studies aimed at disentangling the role of ethanol intoxication in TBI.
Exp Neurol, 2018; 302

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BOARD NUMBER: S04-410

EFFECTS OF PIOGLITAZONE ON THE CORTICAL DAMAGE AND MOTOR PERFORMANCE FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Aims: Pioglitazone has shown some beneficial effects in various animal models of brain injury. In this study, we investigated whether pioglitazone has effects on some parameters of the parietal cortex tissue damage as well as on the motoric performance following traumatic brain injury (TBI) in rats. Methods: Pioglitazone (3 mg/kg) or vehicle were administered i.p. at 10 minutes, 12, 24 and 48 hours after the TBI induced by the lateral fluid percussion brain injury method. Motor functions were evaluated at post-injury day (PID) 1, 2 and 3 using the modified neurological severity score (mNSS) and rotarod test (RRT). Rats were sacrificed 72 h after TBI and their brains were prepared for histologic analyses. Results: In all the brain-injured animals significant decrease in the mNSS at all investigated time points was detected, with no difference between the vehicle and pioglitazone groups. At PID1, same results were detected using RRT, but on PID2, pioglitazone treated animals seemed to return to the control values while vehicle treated rats still showed motor impairment. On PID3, no difference between the groups was evident. Apoptotic cells were present in parietal cortex of both brain-injured groups but pioglitazone did not have significant effects on this parameter. Significant synaptic perturbations, using synaptophysin as a marker, were not detected. Conclusions: Preliminary results of our study imply limited beneficial effects of pioglitazone on motoric performance in traumatized rats but not on the other investigated parameters in used experimental conditions. This work was supported by project uniri-biomed-18-204 to PD.

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BOARD NUMBER: S04-411

CELLULAR SENESCENCE AND NEUROINFLAMMATION FOLLOWING CONTROLLED CORTICAL IMPACT TRAUMATIC BRAIN INJURY IN JUVENILE MICE

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is the leading cause of disability and death in young adults, and also increases the risk of neurodegeneration. The mechanisms linking moderate to severe TBI to neurodegeneration are not known. It has been proposed that cellular senescence induction post-injury could amplify neuroinflammation and induce long-term changes. The impact of these processes after injury to an immature brain has not been characterised. We carried out a controlled cortical impact injury (CCI) in juvenile 1 month-old male CD1 mice. Animals were anesthetised and received a unilateral CCI injury. The sham group received anaesthesia and had a craniotomy. A naïve group had no intervention. The brain tissue was analysed at 5 days and 35 days post-injury using immunohistochemistry and markers for microglia, astrocytes and senescence. Compared to naïve animals, injured mice showed an increased microglial and astrocytic reaction early post-injury, as reflected in Iba1 and GFAP markers, respectively; the GFAP increase persisted in the later phase. The senescence analysis showed a significant increase in γH2AX-53BP1 nuclear foci, 8-oxoguanine, $p19^{ARF}$, $p16^{INK4a}$ and p53 expressions in naïve vs. sham groups and naïve vs. CCI groups, at 5-dpi. At 35 days, the difference was no longer statistically significant. The injury induced a decrease p21 expression vs. the naïve group, at 35-dpi. These results indicate the induction of a complex senescence response after immature brain injury. Some changes occur early and may reflect the activation/proliferation of non-neuronal cells post-injury, whereas changes such as p21 downregulation, may reflect a delayed response and pro-repair processes.

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INTERACTION BETWEEN NO AND ITS DERIVATIVES WITH OGDHC IN CEREBRAL CORTEX UPON BRAIN INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Annette Vaglio[1], Adelheid Weidinger[1], Vanessa Goeschl[2], Matej Hotka[2], Helmut Kubista[2], Andrey Kozlov[1]
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Hemorrhage, cerebral ischemia, and neuro-inflammation are the major features of Trauma Brain Injury (TBI). Neuro-inflammation is accompanied by the elevated expression and the release of inflammatory mediators, such as nitric oxide (NO) synthetized by inducible NO synthase (iNOS), while upon ischemia NO can be released by the reduction of nitrite. NO has been shown to inhibit complex I and IV of the electron transport chain in the mitochondria. More recently, it has been shown that (i) NO also inhibits oxoglutarate dehydrogenase complex (OGDHC) an enzyme of the tricarboxylic acid cycle (TCA), and (ii) nitrite, an oxidative product of NO, accumulates in the brain tissue upon trauma. The aim of this study was to clarify whether NO or nitrite or both can inhibit OGDHC in the brain. We used adult Sprague Dawley cerebral cortex to analyze the OGDHC enzymatic activity. We have shown that both NO and nitrite can modulate OGDHC activity at hypoxic and normoxic conditions, but only NO has pronounced inhibitory effect on OGDHC catalisis at concentrations observed in the brain tissue. In addition, we have examined the susceptibility of OGDHC activity to nitrite at different incubation temperatures. We conclude that in vivo, OGDHC can be inhibited only by NO originating from iNOS rather than by reduction of NO from nitrite under hypoxic conditions.

Pubmed:
32098034: Retana Moreira L, Vargas Ramírez D, Linares F, Prescilla Ledezma A, Vaglio Garro A, Osuna A, Lorenzo Morales J, Abrahams Sandí E
Isolation of T5 from Water: Characterization of Its Pathogenic Potential, Including the Production of Extracellular Vesicles.
is a genus of free-living amoebae widely distributed in nature, associated with the development of encephalitis and keratitis. Despite the fact that it is common to find genotype T5 in environmental samples, only a few cases have been associated with clinical cases in humans. The wide distribution of , the characteristic of being amphizoic and the severity of the disease motivate researchers to focus on the isolation of these organisms, but also in demonstrating direct and indirect factors that could indicate a possible pathogenic potential. Here, we performed the characterization of the pathogenic potential of an T5 isolate collected from a water source in a hospital. Osmo- and thermotolerance, the secretion of proteases and the effect of trophozoites over cell monolayers were analyzed by different methodologies. Additionally, we confirm the secretion of extracellular vesicles (EVs) of this isolate incubated at two different temperatures, and the presence of serine and cysteine proteases in these vesicles. Finally, using atomic force microscopy, we determined some nanomechanical properties of the secreted vesicles and found a higher value of adhesion in the EVs obtained at 37 °C, which could have implications in the parasite´s survival and damaging potential in two different biological environments.
Pathogens, 2020; 9

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HUMAN PLATELET PROTEOME IMPROVES TRAUMATIC BRAIN INJURY IN ANIMAL MODELS

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) can lead to major brain anatomopathological damages underlined by neuroinflammation, oxidative stress and progressive neurodegeneration, ultimately leading to motor and cognitive deterioration. Thus, TBI has been described as a risk factor for neurodegenerative disorders including Alzheimer's disease and Parkinson's disease. Unfortunately, the multiple pathological events resulting from TBI can be addressed not by a single therapeutic approach, but rather by a synergistic biotherapy capable of activating a complementary set of signaling pathways that provide synergistic neuroprotective, anti-inflammatory, antioxidative, and neurorestorative activities. The human platelet lysate (HPL) might constitute a potential candidate. HPL is a reliable source of accessible human biomaterial containing biomolecules that are accessible therapeutics for TBI or neurodegenerative diseases. Here, we prepared HPL from clinical grade human platelet concentrates and tested the therapeutic potential HPL using two mouse models of TBI: the controlled cortical impact (CCI) and the in-house developed cortical brain scratch (CBS). The HPL was administrated by topical application and intranasal delivery. We demonstrated, that topical application and intranasal HPL mitigates cortical neuroinflammation, oxidative stress, and synaptic alterations, ultimately improving motor and cognitive functions. Moreover, proteomics analyses revealed that HPL attenuated multiple detrimental cortical pathways and mitigated disease-associated microglia (DAMs) and astrocytes (DAAs) changes triggered by the brain injuries. Brain administration of HPL is a therapeutical strategy that deserves serious consideration for TBI treatment or neurodegenerative diseases.

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NEUROPROTECTIVE EFFECT OF PHYSICAL EXERCISE AFTER TRAUMATIC BRAIN INJURY: INFLUENCE OF THE ONSET DELAY AND PRE-INJURY FITNESS.

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) remains one of the leading causes of morbidity and mortality worldwide and it is a risk factor of dementia later in life. Different studies demonstrated that physical exercise provides strong neuroprotection effects in an experimental animal model of TBI[MCA1]. However, when addressing physical exercise in the context of TBI, additional factors such as pre-trauma fitness, post-injury delay onset and duration of exercise will dictate whether exercise has a beneficial effect on recovery. We evaluated the long-term effects (14 days after TBI) of pre-injury exercise alone, post-injury (early and late onset) exercise and the combination of both treatments after controlled cortical impact injury, on hippocampal volume, density of NeuN+ cells and Iba1 staining (CCI). METHODS: rats were distributed into Sham and TBI condition. TBI subjects were distributed into: sedentary (TBI), pre-injury exercise (E-TBI), early (24 hours, TBI-eE) or late (6 days, TBI-lE) onset of exercise after TBI, and a combination of pre-injury and early (E-TBI-eE) or late (E-TBI-lE) post-injury exercise. RESULTS: TBI decreased hippocampal volume, and increased neuronal loss and neuroinflammation. Pre-injury exercise alone seems to slightly reduce this effect. Post-injury exercise alone did not modify the neuronal loss and neuroinflammation when initiated early, but late onset completely reverted both the neuronal loss and inflammation. However, when combined with pre-injury exercise, none of post-injury treatments had a beneficial effect. CONCLUSION: On the long-term, only post-injury exercise initiated late after injury has a strong neuroprotective and anti-inflammatory effect.

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BOARD NUMBER: S04-415

CB1 RECEPTORS REGULATE NEUROMUSCULAR JUNCTION RE-INNERVATION FOLLOWING INJURY

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Peripheral nerves show a great regenerative ability following nerve injury. Not only changes along the damaged nerve occurred but also at the neuromuscular junction (NMJ), where degeneration/re-innervation processes occur in pathological and physiological conditions. Perisynaptic Schwann cells, glial cells at the NMJ, are important for NMJ's repair. Despite evidence for their roles in axonal guidance and synapse formation, Cannabinoids' involvement in nerve injury response remains ill defined. We observed that CB1 receptors are expressed in different muscles and are upregulated immediately after nerve crush. Daily treatment with the CB1 antagonist AM251 (IP injections, 3 mg/kg) during the period of re-innervation following nerve injury caused a downregulation of the CB1 expression and greatly limited re-innervation as indicated with a significant number of denervated NMJs ($p < 0.05$). In EDL muscles, the increased percentage of denervated NMJ was accompanied by a decrease of poly-innervation, likely due to a delay in the reinnervation process. Moreover, this CB1 regulation may be mediated by a c-Jun pathway since we observed a significant decrease of c-Jun expression ($p < 0.05$). These data highlight a novel role of CB1 receptor at NMJ and in the control of NMJ reinnervation after nerve injury. A better understanding of these mechanisms could help address the inadequate NMJ maintenance observed in motor neuron-related neurodegenerative diseases.

Pubmed:
34946750: Salazar Intriago MS, Piovesana R, Matera A, Taggi M, Canipari R, Fabrizi C, Papotto C, Matera C, De Amici M, Dallanoce C, Tata AM
The Mechanisms Mediated by α7 Acetylcholine Nicotinic Receptors May Contribute to Peripheral Nerve Regeneration.
Due to the microenvironment created by Schwann cell (SC) activity, peripheral nerve fibers are able to regenerate. Inflammation is the first response to nerve damage and the removal of cellular and myelin debris is essential in preventing the persistence of the local inflammation that may negatively affect nerve regeneration. Acetylcholine (ACh) is one of the neurotransmitters involved in the modulation of inflammation through the activity of its receptors, belonging to both the muscarinic and nicotinic classes. In this report, we evaluated the expression of α7 nicotinic acetylcholine receptors (nAChRs) in rat sciatic nerve, particularly in SCs, after peripheral nerve injury. α7 nAChRs are absent in sciatic nerve immediately after dissection, but their expression is significantly enhanced in SCs after 24 h in cultured sciatic nerve segments or in the presence of the proinflammatory neuropeptide Bradykinin (BK). Moreover, we found that activation of α7 nAChRs with the selective partial agonist ICH3 causes a decreased expression of c-Jun and an upregulation of uPA, MMP2 and MMP9 activity. In addition, ICH3 treatment inhibits IL-6 transcript level expression as well as the cytokine release. These results suggest that ACh, probably released from regenerating axons or by SC themselves, may actively promote through α7 nAChRs activation an anti-inflammatory microenvironment that contributes to better improving the peripheral nerve regeneration.
Molecules, 2021; 26
34066354: Piovesana R, Salazar Intriago MS, Dini L, Tata AM
Cholinergic Modulation of Neuroinflammation: Focus on α7 Nicotinic Receptor.
All nervous system pathologies (e.g., neurodegenerative/demyelinating diseases and brain tumours) develop neuroinflammation, a beneficial process during pathological events, aimed at removing damaged cells, toxic agents, and/or pathogens. Unfortunately, excessive inflammation frequently occurs during nervous system disorders, becoming a detrimental event capable of enhancing neurons and myelinating glial cell impairment, rather than improving their survival and activity. Consequently, targeting the neuroinflammation could be relevant for reducing brain injury and rescuing neuronal and glial cell functions. Several studies have highlighted the role of acetylcholine and its receptors in the regulation of central and peripheral inflammation. In particular, α7 nicotinic receptor has been described as one of the main regulators of the "brain

cholinergic anti-inflammatory pathway". Its expression in astrocytes and microglial cells and the ability to modulate anti-inflammatory cytokines make this receptor a new interesting therapeutic target for neuroinflammation regulation. In this review, we summarize the distribution and physiological functions of the α7 nicotinic receptor in glial cells (astrocytes and microglia) and its role in the modulation of neuroinflammation. Moreover, we explore how its altered expression and function contribute to the development of different neurological pathologies and exacerbate neuroinflammatory processes.
Int J Mol Sci, 2021; 22
33334089: Pernarella M, Piovesana R, Matera C, Faroni A, Fiore M, Dini L, Reid AJ, Dallanoce C, Tata AM
Effects mediated by the α7 nicotinic acetylcholine receptor on cell proliferation and migration in rat adipose-derived stem cells.
Adipose-derived stem cells (ASCs) are an attractive source for regenerative medicine as they can be easily isolated, rapidly expandable in culture and show excellent in vitro differentiation potential. Acetylcholine (ACh), one of the main neurotransmitters in central and peripheral nervous systems, plays key roles in the control of several physiological processes also in non-neural tissues. As demonstrated in our previous studies, ACh can contribute to the rat ASCs physiology, negatively modulating ASCs proliferation and migration via M2 muscarinic receptor (mAChR) activation. In the present work we show that rat ASCs also express α7 nicotinic receptors (nAChRs). In particular, we have investigated the effects mediated by the selective activation of α7 nAChRs, which causes a reduction of ASC proliferation without affecting cell survival and morphology, and significantly promotes cell migration via upregulation of the CXCR4 expression. Interestingly, the activation of the α7 nAChR also upregulates the expression of M2 mAChR protein, indicating a cooperation between muscarinic and nicotinic receptors in the inhibition of ASC proliferation.
Eur J Histochem, 2020; 64
33269784: Piovesana R, Faroni A, Tata AM, Reid AJ
Schwann-like adipose-derived stem cells as a promising therapeutic tool for peripheral nerve regeneration: effects of cholinergic stimulation.
Neural Regen Res, 2021; 16
32933046: Piovesana R, Faroni A, Tata AM, Reid AJ
Functional Characterization of Muscarinic Receptors in Human Schwann Cells.
Functional characterization of muscarinic cholinergic receptors in myelinating glial cells has been well described both in central and peripheral nervous system. Rat Schwann cells (SCs) express different muscarinic receptor subtypes with the prevalence of the M2 subtype. The selective stimulation of this receptor subtype inhibits SC proliferation, improving their differentiation towards myelinating phenotype. In this work, we describe for the first time that human SCs are cholinoceptive as they express several muscarinic receptor subtypes and, as for rat SCs, M2 receptor is one of the most abundant. Human SCs, isolated from adult nerves, were cultured in vitro and stimulated with M2 muscarinic agonist arecaidine propargyl ester (APE). Similarly to that observed in rat, M2 receptor activation causes a decreased cell proliferation and promotes SC differentiation as suggested by increased Egr2 expression with an improved spindle-like shape cell morphology. Conversely, the non-selective stimulation of muscarinic receptors appears to promote cell proliferation with a reduction of SC average cell diameter. The data obtained demonstrate that human SCs are cholinoceptive and that human cultured SCs may represent an interesting tool to understand their physiology and increase the knowledge on how the cholinergic stimulation may contribute to address human SC development in normal and pathological conditions.
Int J Mol Sci, 2020; 21
31069117: Piovesana R, Faroni A, Magnaghi V, Reid AJ, Tata AM
M2 receptors activation modulates cell growth, migration and differentiation of rat Schwann-like adipose-derived stem cells.
Schwann cells (SCs) play a central role in peripheral nervous system physiology and in the response to axon injury. The ability of SCs to proliferate, secrete growth factors, modulate immune response, migrate and re-myelinate regenerating axons has been largely documented. However, there are several restrictions hindering their clinical application, such as the difficulty in collection and a slow in vitro expansion. Adipose-derived stem cells (ASCs) present good properties for peripheral nerve regenerative medicine. When exposed to specific growth factors in vitro, they can acquire a SC-like phenotype (dASCs) expressing key SCs markers and assuming spindle-shaped morphology. Nevertheless, the differentiated phenotype is unstable and several strategies, including pharmacological stimulation, are being studied to improve differentiation outcomes. Cholinergic receptors are potential pharmacological targets expressed in glial cells. Our previous work demonstrated that muscarinic cholinergic receptors, in particular M2 subtype, are present in SCs and are able to modulate several physiological processes. In the present work, muscarinic receptors expression was characterised and the effects mediated by M2 muscarinic receptor were evaluated in rat dASCs. M2 receptor activation, by the preferred agonist arecaidine propargyl ester (APE), caused a reversible arrest of dASCs cell growth, supported by the downregulation of proteins involved in the maintenance of cell proliferation and upregulation of proteins involved in the differentiation (i.e., c-Jun and Egr-2), without affecting cell survival. Moreover, M2 receptor activation in dASCs enhances a pronounced spindle-shaped morphology,

supported by Egr2 upregulation, and inhibits cell migration. Our data clearly demonstrate that rat dASCs express functional muscarinic receptors, in particular M2 subtype, which is able to modulate their physiological and morphological processes, as well as SCs differentiation. These novel findings could open new opportunities for the development of combined cell and pharmacological therapies for peripheral nerve regeneration, harnessing the potential of dASCs and M2 receptors.
Cell Death Discov, 2019; 5
29857516: Di Bari M, Bevilacqua V, De Jaco A, Laneve P, Piovesana R, Trobiani L, Talora C, Caffarelli E, Tata AM
Mir-34a-5p Mediates Cross-Talk between M2 Muscarinic Receptors and Notch-1/EGFR Pathways in U87MG Glioblastoma Cells: Implication in Cell Proliferation.
Glioblastoma (GBM) is the most aggressive human brain tumor. The high growth potential and decreased susceptibility to apoptosis of the glioma cells is mainly dependent on genetic amplifications or mutations of oncogenic or pro-apoptotic genes, respectively. We have previously shown that the activation of the M2 acetylcholine muscarinic receptors inhibited cell proliferation and induced apoptosis in two GBM cell lines and cancer stem cells. The aim of this study was to delve into the molecular mechanisms underlying the M2-mediated cell proliferation arrest. Exploiting U87MG and U251MG cell lines as model systems, we evaluated the ability of M2 receptors to interfere with Notch-1 and EGFR pathways, whose activation promotes GBM proliferation. We demonstrated that the activation of M2 receptors, by agonist treatment, counteracted Notch and EGFR signaling, through different regulatory cascades depending, at least in part, on p53 status. Only in U87MG cells, which mimic p53-wild type GBMs, did M2 activation trigger a molecular circuitry involving p53, Notch-1, and the tumor suppressor mir-34a-5p. This regulatory module negatively controls Notch-1, which affects cell proliferation mainly through the Notch-1/EGFR axis. Our data highlighted, for the first time, a molecular circuitry that is deregulated in the p53 wild type GBM, based on the cross-talk between M2 receptor and the Notch-1/EGFR pathways, mediated by mir-34a-5p.
Int J Mol Sci, 2018; 19
29227527: Piovesana R, Melfi S, Fiore M, Magnaghi V, Tata AM
M2 muscarinic receptor activation inhibits cell proliferation and migration of rat adipose-mesenchymal stem cells.
Mesenchymal stem cells (MSCs), also known as stromal mesenchymal stem cells, are multipotent cells, which can be found in many tissues and organs as bone marrow, adipose tissue and other tissues. In particular MSCs derived from Adipose tissue (ADSCs) are the most frequently used in regenerative medicine because they are easy to source, rapidly expandable in culture and excellent differentiation potential into adipocytes, chondrocytes, and other cell types. Acetylcholine (ACh), the most important neurotransmitter in Central nervous system (CNS) and peripheral nervous system (PNS), plays important roles also in non-neural tissue, but its functions in MSCs are still not investigated. Although MSCs express muscarinic receptor subtypes, their role is completely unknown. In the present work muscarinic cholinergic effects were characterized in rat ADSCs. Analysis by RT-PCR demonstrates that ADSCs express M1-M4 muscarinic receptor subtypes, whereas M2 is one of the most expressed subtype. For this reason, our attention was focused on M2 subtype. By using the selective M2 against Arecaidine Propargyl Ester (APE) we performed cell proliferation and migration assays demonstrating that APE causes cell growth and migration inhibition without affecting cell survival. Our results indicate that ACh via M2 receptors, may contribute to the maintaining of the ADSCs quiescent status. These data are the first evidence that ACh, via muscarinic receptors, might contribute to control ADSCs physiology.
J Cell Physiol, 2018; 233
32346125: Piovesana R, Faroni A, Taggi M, Matera A, Soligo M, Canipari R, Manni L, Reid AJ, Tata AM
Muscarinic receptors modulate Nerve Growth Factor production in rat Schwann-like adipose-derived stem cells and in Schwann cells.
Regenerative capability of the peripheral nervous system after injury is enhanced by Schwann cells (SCs) producing several growth factors. The clinical use of SCs in nerve regeneration strategies is hindered by the necessity of removing a healthy nerve to obtain the therapeutic cells. Adipose-derived stem cells (ASCs) can be chemically differentiated towards a SC-like phenotype (dASCs), and represent a promising alternative to SCs. Their physiology can be further modulated pharmacologically by targeting receptors for neurotransmitters such as acetylcholine (ACh). In this study, we compare the ability of rat dASCs and native SCs to produce NGF in vitro. We also evaluate the ability of muscarinic receptors, in particular the M2 subtype, to modulate NGF production and maturation from the precursor (proNGF) to the mature (mNGF) form. For the first time, we demonstrate that dASCs produce higher basal levels of proNGF and mature NGF compared to SCs. Moreover, muscarinic receptor activation, and in particular M2 subtype stimulation, modulates NGF production and maturation in both SCs and dASCs. Indeed, both cell types express both proNGF A and B isoforms, as well as mNGF. After M2 receptor stimulation, proNGF-B (25 kDa), which is involved in apoptotic processes, is strongly reduced at transcript and protein level. Thus, we demonstrate that dASCs possess a stronger neurotrophic potential compared to SCs. ACh, via M2 muscarinic receptors, contributes to the modulation and maturation of NGF, improving the regenerative properties of dASCs.
Sci Rep, 2020; 10


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LONGITUDINAL CHANGE IN INTERHEMISPHERIC CONNECTIVITY PREDICTS RECOVERY FROM VESTIBULAR AGNOSIA

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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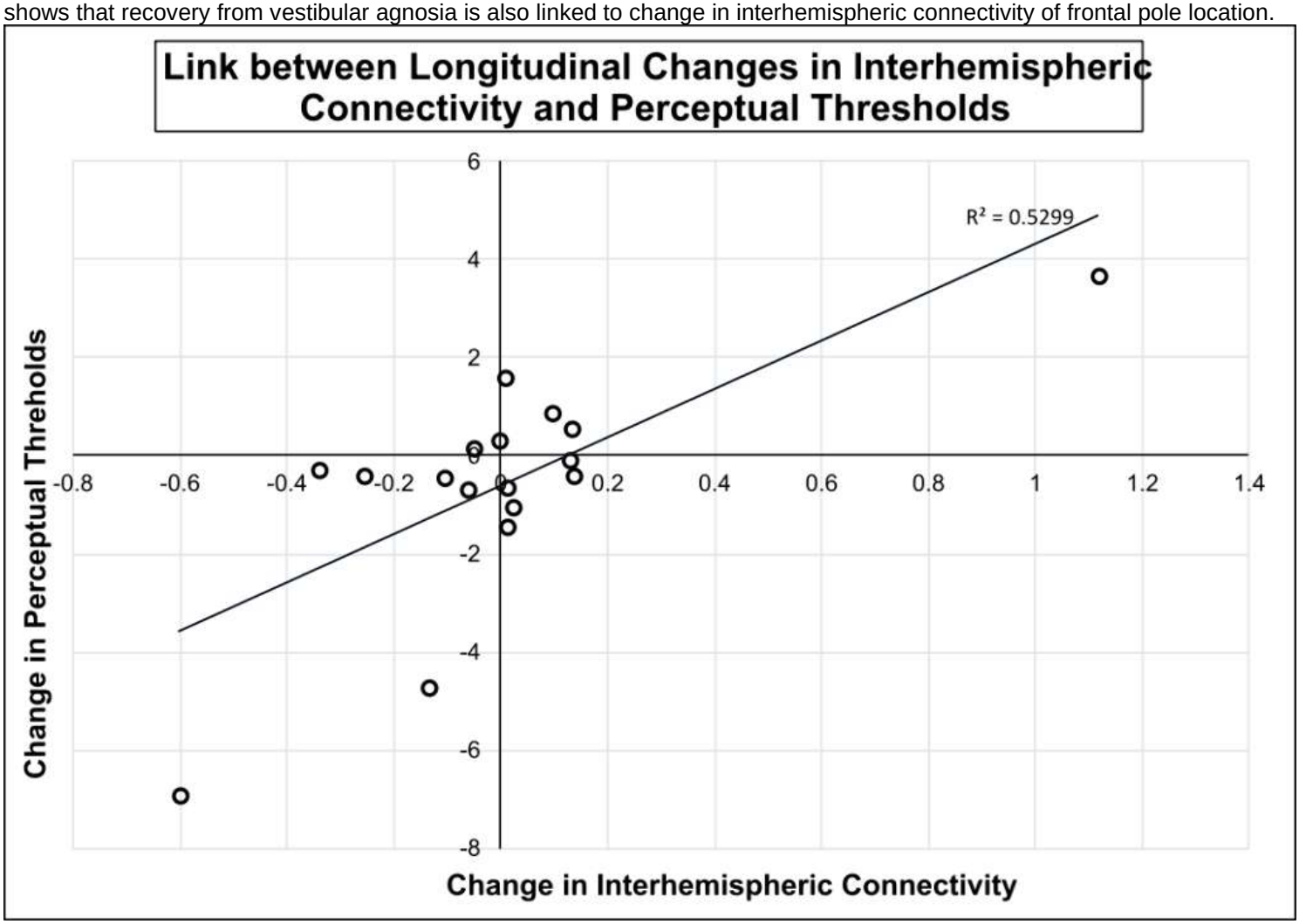
Introduction: Traumatic brain injury (TBI) commonly results in vestibular dysfunction impairing vestibular perception, this later manifesting as a vestibular agnosia (i.e. loss of vertigo sensation despite manifest peripheral and reflex vestibular activation). We recently identified brain regions predicting vestibular agnosia in acute TBI, however, it remains unknown whether longitudinal connectivity changes predict recovery from vestibular agnosia. **Methods:** The data was collected as part of an MRC-funded prospective study (Calzolari et al., 2021). Resting state fMRI was acquired from 39 acute TBI patients with preserved peripheral and reflex vestibular function during acute admission and then after 6 months. Vestibular agnosia was measured via self-motion perceptual thresholds during passive yaw rotations in dark. After accounting for missing scans at any timepoint, and quality control of rsfMRI scans, 17 subjects' longitudinal data were analyzed and using resting state fMRI we looked at the interhemispheric connectivity over time and its link with recovery from Vestibular Agnosia. **Results:** We found that large change in interhemispheric connectivity in the frontal poles (Cluster size: 30; MNI: ± 21, +54, +24) was linked to a large change in perceptual thresholds over time (shown in figure below). **Conclusion:** Frontal poles are part of the salience network, which is known for its role as brain's sensory integration center. We previously reported a link between vestibular agnosia and altered functional connectivity of salience network localized at left frontal pole location. Our data

shows that recovery from vestibular agnosia is also linked to change in interhemispheric connectivity of frontal pole location.



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LONGITUDINAL VOLUME LOSS AFTER TRAUMATIC BRAIN INJURY PREDICTS VESTIBULAR DYSFUNCTION

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

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Introduction: Traumatic brain injury (TBI) nearly always results in vestibular dysfunction impairing balance and vestibular perception. We recently identified brain regions predicting vestibular dysfunction in acute TBI, however, it remains unknown whether longitudinal structural brain changes predict recovery from vestibular dysfunction. **Methods:** The data was collected as part of an MRC-funded prospective study (Calzolari et al., 2021). Structural T1 MRI was acquired from 39 acute TBI patients with preserved peripheral and reflex vestibular function during acute admission and then after 6 months. Imbalance was measured with Posturography on soft surface with eyes closed. Vestibular perception was measured via self-motion perceptual thresholds during passive yaw rotations in dark. After accounting for missing scans at any timepoint, and quality control of MRI scans, 33 subjects' longitudinal data were analyzed and Voxel Based Morphometry we looked at the volume change over time and its link with recovery from vestibular dysfunction. **Results:** We found an interaction (FWE corrected) between recovery of balance and vestibular perception and the left Calcarine and right Supplementary Motor Area. **Conclusion:** Volume loss in left Calcarine and right Supplementary Motor Area are linked to poor recovery of balance and vestibular perception in patients with TBI. This is the first prospective acute TBI follow up study to show structural changes to be directly associated with vestibular recovery which is a core clinical function that improves quality of life and return to work.

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CELLULAR IMMUNITY IN PATIENTS WITH CONCUSSION AND POSSIBLE ASSOCIATION WITH SUBSEQUENT COGNITIVE IMPAIRMENT WITHDRAWN BY AUTHOR

POSTER SESSION 04 - SECTION: TRAUMATIC BRAIN INJURY

Anna Norka[1], Sergey Vorobjev[2], Raisa Kuznetsova[3], Igor Kudryavtsev[3], Maria Serebriakova[4], Sergey Kovalenko[5], Dmitry Monashenko[6], Anatoly Saveliev[5]
[1]Pavlov First Saint Petersburg State Medical University, Immunology, Saint Petersburg, Russian Federation, [2]V. Almazov National Medical Research Centre, Research Laboratory Of Neurology And Neurorehabilitation, Saint Petersburg, Russian Federation, [3]First St. Petersburg State I. Pavlov Medical University, Immunology, Saint Petersburg, Russian Federation, [4]Institute of Experimental Medicine, Immunology, Saint Petersburg, Russian Federation, [5]S. Kirov Military Medical Academy, Neurosurgery, Saint Petersburg, Russian Federation, [6]St. Petersburg State Budgetary Health Institution "City Hospital No. 26", Neurosurgery, Saint Petersburg, Russian Federation

Introduction. The immunological response to traumatic brain injury is a complex reaction with the involvement of cells of the cellular immunity, which determines the course of the disease. Aim. It was a study the role of immunological responses in the pathogenesis of concussion and the relationship with cognitive impairment. Materials and methods. In a trial of 22 patients aged 20-45 years with a concussion. The control group consisted of 37 healthy individuals aged 20-46 years. The examination included of complaints, medical history, assessment of neurological status, neuropsychological testing. Venous blood was used as the object of laboratory research. The main attention was paid to T-helpers of central memory (CM, CD45RA-CD62L+), which were carried out using multicolor cytometric analysis (Navios™,Beckman Coulter, USA). Results. Patients with a concussion among Th cells of the central memory decreased (p-value=0.044) the relative content of Th1 cells from 13.57% (11.50;15.91) to 11.47% (7.13;14.03). At the same time, frequency of Th17 cells was significantly higher (p-value<0.001) in patients than controls from 34.35% (31.20;42.04) to 45.03% (39.51;48.12). In addition in our study a direct correlation was established between the content of DN Th17 cells and the results of performing tests as MMSE,MoCA. At the same time, the concentration of Th1 cells had an opposite correlation with these tests. Conclusion. These findings indicate the possible involvement of T-helpers of the central memory in a concussion. Further the data can be validated to better understand the role of Th cells which might help in formulating targeted therapeutic strategy in future.

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DISTINCT SUBPOPULATIONS OF CEREBELLAR MOLECULAR LAYER INTERNEURONS IN CRUS1 ENCODE INTENDED WHISKER TRAJECTORIES

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Rodents primarily use their whiskers to explore their environment, a behaviour which requires integration of sensory information and motor commands. The cerebellum plays a key role in integrating whisking-related sensorimotor signals arising from a multitude of brain regions. In the cerebellar cortex, molecular layer interneurons (MLIs) receive excitatory drive from granule cells, as do Purkinje cells (PCs). MLIs exclusively provide inhibition to PCs, awarding them a central role for influencing cerebellar output. Previous results generated in the lab showed that individual MLIs in Crus1 linearly encode whisker position during active whisking by increasing or decreasing spiking. Here we use 2-photon imaging in adult head-fixed NOS-Cre mice expressing Cre-dependent GCaMP6f in Crus1 to explore the diversity and topographical organisation of MLI population activity during voluntary whisking. Using unsupervised clustering, a subpopulation of MLIs exhibiting increased neuronal activity upon whisking can be identified. This subpopulation is randomly interspersed with MLIs not excited by whisking. A linear transfer function calculated from MLI Ca^{2+} signals replicates momentary whisker position very reliably, but not slower changes in resting position. Whisking-evoked Ca^{2+} response properties in MLIs are unchanged when the animal is touching a pole at various locations. Furthermore, MLIs do not appear to respond to passive air puff stimulation directed at the whiskers, suggesting that MLI signals constitute primarily internally generated efferent representations of predicted movement. Taken together, these data show that MLI subpopulations in Crus1 represent intended whisker trajectories on a moment-to-moment basis. We are currently exploring the synaptic basis of this encoding motif.

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THE ROLE OF CALCIUM CURRENTS IN MAINTAINING TONIC FIRING IN LARVAL ZEBRAFISH PURKINJE NEURONS

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Purkinje cells of the cerebellum have been shown to display membrane bistability (Williams et 2002,Lowenstein et al, 2005, Sengupta and Thirumalai, 2015). In larval zebrafish the cells display an up state characterized by a depolarized membrane potential and tonic firing of simple spikes while the down state is characterized by a hyperpolarized membrane potential and burst firing. The up state is the default state of the neuron while the down state is a result of network activity (Sengupta and Thirumalai 2015). These cells also have large calcium currents (Raman and Bean, 1999; Womack et al, 2004). We thus wanted to understand the role of cellular currents in maintaining the up state of Purkinje cells. We performed in vivo whole-cell patch clamp recordings to measure these currents and their effects on spontaneous activity of Purkinje cells. By clamping the cells at various voltages, we found that these cells have a large calcium current (~350pA). Further, using various pharmacological agents, we found that the current is mainly contributed by L and T type voltage gated calcium channels. We also measured a small amount of Ih current. We then observed the effect of various channel antagonist on the spontaneous activity of these cells in current clamp. The experiments show that the main effect of calcium current is to facilitate tonic firing of simple spikes via coupling with calcium activated potassium channels. This study shows that calcium and calcium activated potassium currents are key for maintaining the up state of Purkinje cells.

Pubmed:

33291119: Balasubramanian N, Sagarkar S, Jadhav M, Shahi N, Sirmaur R, Sakharkar AJ
Role for Histone Deacetylation in Traumatic Brain Injury-Induced Deficits in Neuropeptide Y in Arcuate Nucleus: Possible Implications in Feeding Behavior.
Repeated traumatic events result in long-lasting neuropsychiatric ailments, including neuroendocrine imbalances. Neuropeptide Y (NPY) in the arcuate nucleus (Arc) is an important orexigenic peptide. However, the molecular underpinnings of its dysregulation owing to traumatic brain injury remain unknown.
Neuroendocrinology, 2021; 111
34346310: Sitaraman S, Yadav G, Agarwal V, Jabeen S, Verma S, Jadhav M, Thirumalai V
Gjd2b-mediated gap junctions promote glutamatergic synapse formation and dendritic elaboration in Purkinje neurons.
Gap junctions between neurons serve as electrical synapses, in addition to conducting metabolites and signaling molecules. During development, early-appearing gap junctions are thought to prefigure chemical synapses, which appear much later. We present evidence for this idea at a central, glutamatergic synapse and provide some mechanistic insights. Loss or reduction in the levels of the gap junction protein Gjd2b decreased the frequency of glutamatergic miniature excitatory postsynaptic currents (mEPSCs) in cerebellar Purkinje neurons (PNs) in larval zebrafish. Ultrastructural analysis in the molecular layer showed decreased synapse density. Further, mEPSCs had faster kinetics and larger amplitudes in mutant PNs, consistent with their stunted dendritic arbors. Time-lapse microscopy in wild-type and mutant PNs reveals that Gjd2b puncta promote the elongation of branches and that CaMKII may be a critical mediator of this process. These results demonstrate that Gjd2b-mediated gap junctions regulate glutamatergic synapse formation and dendritic elaboration in PNs.
Elife, 2021; 10

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UNIPOLAR BRUSH CELLS IN THE VESTIBULOCEREBELLUM.

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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In mammals, unipolar brush cells (UBCs) have been suggested to enhance the diversity in temporal patterns in the granular layer of the cerebellar cortex to facilitate performance and adaptation of motor activity by generating delayed and/or sustained cerebellar activity. UBCs are prominently distributed in the vestibulocerebellum, but their precise role during vestibular and visual stimulation remains elusive. Here, we describe the distribution of typical ON-UBCs in the flocculus of the vestibulocerebellum in mice and we elaborate on their potential role during compensatory eye movements. Using immunolabelling against Metabotropic glutamate receptor 1 alpha we found that ON-UBCs are predominantly located in the main zone of the flocculus that controls horizontal compensatory eye movements. *Moonwalker* mice, which suffer from a mutation in their cation-permeable transient receptor potential channel type 3 (TRPC3) that leads to an early-onset ablation of ON-UBCs, show significant gain deficits in their horizontal optokinetic reflex at larger amplitudes as well as in their horizontal vestibulo-ocular reflex (VOR) across a broad range of frequencies. In addition, *Moonwalker* mice are significantly impaired in phase reversal learning of the horizontal VOR. Instead, Purkinje cell specific knock-outs of TRPC3 do not show any of these deficits. These data raise the possibility that ON-UBCs in the flocculus receive not only vestibular, but also visual and/or oculomotor mossy fibre inputs, and that they may enhance the diversity of these afferent signals so as to facilitate performance and acquisition of eye movement patterns.

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OPTOGENETIC DISSECTION OF THE CEREBELLAR CORTEX OUTPUT IN THE CONTEXT OF POSTURAL MAINTENANCE.

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Posture is defined by the arrangement of body parts resulting from tonic muscle contraction. The vermal part of the cerebellar cortex plays a central role in postural muscle tone control. The cerebellar cortex is organized in parasagittal bands known as “microzones” based on the receptive fields of its inputs. However, whether Purkinje cells (PCs) are organized relative to the motor output they control is not known. Previous studies in head-restrained animals have shown that inhibiting or stimulating PC activity can produce movements either directly or in rebound. To investigate the functional organization of the cerebellar cortex ouput, we performed optogenetic stimulations of PCs in freely moving mice expressing the actuator Channelrodopsin. By using a 3D-printed implant coupled to an optic fiber, we achieve confined optogenetic stimulations at tunable locations over the vermal lobules IV/V/VI. We demonstrate through video analysis that a minimal optogenetic stimulation at the midline evokes a rapid body collapse proprotional to the stimulation, followed by a compensatory reflex movement of the limbs. The short-latency loss of antigravitational tone has never been characterized before. Using electromyograms, we observe that several axial muscles are involved in the compensatory movement but cannot resolve the tone decrease. Displacing laterally the site of stimulation did not show a clear zonation in the motor output. The amplitude of the postural collapse decreases gradually with the distance from the midline and is lateralized in lobule VI. Our results suggest a rate-control of antigravitational posture maintenance by PCs of the vermal lobules IV/V/VI.

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ROLE OF THE CEREBELLO-PREFRONTAL COMMUNICATION IN THE TEMPORAL PREDICTION OF EVENTS: IMPLICATION FOR SCHIZOPHRENIA.

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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One of the key feature of the cerebellar computation is its abilities to predict the sensory consequences of motor commands through the cerebro-cerebello-cortical loop. Prediction capabilities of the cerebellum extend to non-motor functions as attested by an extensive bidirectional connectivity between the cerebellum and the prefrontal cortex (PFC). We hypothesize that the cerebellum make predictions based on contextual and goal-directed information enabling smooth transitions from the present to the future in the PFC. An alteration of this pathway may lead to a dysfunction in action sequencing, which is one of the symptom of schizophrenia. Our aim is to understand better the communication between the cerebellum and the PFC during temporal related tasks both in rodent models of schizophrenia. We performed multiunit recordings in the PFC during an interval task paradigm in transgenic animal expressing Channelrhodopsin2 in Purkinje cells. We showed that in WT animals stable and variable delays have distinct electrophysiological signatures. Optogenetic stimulation of Purkinje cells during the task altered these activities in the PFC in a task specific manner, suggesting that cerebellar perturbation can influence the task. We then set out to assess whether the Synapsin II knock-out mice could be used as a model of schizophrenia. To do this, we will use a novel apparatus enabling simultaneous tracking of a group of mice and investigate social abnormalities in synapsin II KO mice. We will then study how KO mice are affected in the interval timing task and assess whether cerebellar stimulation can influence and compensate these alterations.

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FAITHFUL ENCODING OF LOCOMOTOR COORDINATION BY INDIVIDUAL PURKINJE CELLS

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Whole-body locomotion requires precise coordination of movements across the body. Moreover, control needs to be robust and flexible to changes in the body and environment. The cerebellum is critical for coordinating movement; during locomotion it is particularly important for interlimb coordination. Decades of recordings have consistently shown that Purkinje cell modulation (the sole output of the cerebellar cortex) is broadly correlated with the locomotor stride cycle. However, much of the firing rate variability has remained unexplained; moreover, previous analyses do not provide a clear model for how Purkinje cell activity could be read out to control coordination. Here we performed cell-attached recordings from individual Purkinje cells in head-fixed mice during locomotion, along with high-speed 3D tracking of limb and body kinematics. We find that beyond representing the locomotor stride cycle, Purkinje cells are exquisitely sensitive to stride-to-stride kinematic variation. Further, many individual Purkinje cells respond to multiple behavioral events, including movements of multiple limbs. We used several approaches, including Generalized Additive Models, to disentangle the contribution of individual body parts from the highly correlated locomotor pattern. The analyses reveal that a substantial proportion of Purkinje cells simultaneously encode movements of multiple body parts to provide precise representations of temporal coordination across diverse combinations of behavioral events. High prevalence of this non-linear mixed selectivity across the Purkinje cell population resolves long-standing controversies surrounding the role of Purkinje cells in locomotor control and could allow for efficient readouts of whole-body coordination by a simple linear decoder.

Pubmed:
28009283: Ramirez JE, Stell BM
Calcium Imaging Reveals Coordinated Simple Spike Pauses in Populations of Cerebellar Purkinje Cells.
The brain's control of movement is thought to involve coordinated activity between cerebellar Purkinje cells. The results reported here demonstrate that somatic Ca imaging is a faithful reporter of Na-dependent "simple spike" pauses and enables us to optically record changes in firing rates in populations of Purkinje cells in brain slices and in vivo. This simultaneous calcium imaging of populations of Purkinje cells reveals a striking spatial organization of pauses in Purkinje cell activity between neighboring cells. The source of this organization is shown to be the presynaptic gamma-Aminobutyric acid producing (GABAergic) network, and blocking ionotropic gamma-Aminobutyric acid receptor (GABARs) abolishes the synchrony. These data suggest that presynaptic interneurons synchronize (in)activity between neighboring Purkinje cells, and thereby maximize their effect on downstream targets in the deep cerebellar nuclei.
Cell Rep, 2016; 17

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BOARD NUMBER: S04-425

OLIVOCEREBELLAR CONTROL OF MOVEMENT SYMMETRY

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Coordination of bilateral movements is essential for a large variety of animal behaviors. The olivocerebellar system is critical for the control of movement, but its role in bilateral coordination has yet to be elucidated. Here, we examined whether Purkinje cells encode and influence synchronicity of left-right whisker movements. We found that complex spike activity is correlated with a prominent left-right symmetry of spontaneous whisker movements within parts, but not all, of Crus1 and Crus2. Optogenetic stimulation of climbing fibers in the areas with high and low correlations resulted in symmetric and asymmetric whisker movements, respectively. Moreover, when simple spike frequency prior to the complex spike was higher, the complex spike-related symmetric whisker protractions were larger. This finding alludes to a role for rebound activity in the cerebellar nuclei, which indeed turned out to be enhanced during symmetric protractions. Tracer injections suggest that regions associated with symmetric whisker movements are anatomically connected to the contralateral cerebellar hemisphere. Together, these data point toward the existence of modules on both sides of the cerebellar cortex that can differentially promote or reduce the symmetry of left and right movements in a context-dependent fashion.

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ACQUISITION OF A COMPLEX LOCOMOTOR TASK: ACTIVITY OF CEREBELLAR MOLECULAR LAYER INTERNEURONS AND GAIT DYNAMICS.

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The cerebellum is a key brain region for motor coordination and learning. By processing sensorimotor information, it predicts and refines movements. However, the contribution of the cerebellar cortex neurons in the learning of a challenging motor task remains poorly understood. To study the implication of the cerebellar cortex microcircuit in motor coordination and learning, we combined behavioral analyses and two photon calcium imaging of the molecular layer interneurons (MLI) network which exerts a strong inhibitory control over the activity of cerebellar cortex output neurons, the Purkinje cells. We implemented a challenging locomotor task where we trained the mice to walk on a motorized treadmill with rungs over multiple days while simultaneously recording the activity of the same MLI population in lobulus simplex using 2 photon calcium imaging. We used high-speed behavioral video recordings to extract individual paw and rung locations, which were subsequently divided into swing and stance phases. When studying individual paws movement patterns during the task acquisition, we found that mice performed fewer, longer strides and increased the duration of the stance phase during learning. MLIs exhibit pronounced locomotion-related activity: a global increase in the MLI population upon locomotion onset and rich dynamics during walking. A majority of recorded MLIs show paw specific swing-related transients. Those transients consist of increases or decreases from the baseline activity with a magnitude and sign that vary over days in a non-monotonous fashion. Our results shed light on the involvement of the cerebellar interneurons in generating coordinated movement in mammals.

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FUNCTIONAL DIVERSITY OF GLUTAMATE RELEASE AT INDIVIDUAL GRANULE CELL TERMINALS IN THE CEREBELLAR CORTEX

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The cerebellar cortex is composed of modules that process sensorimotor information and coordinate movements. Information are conveyed to the cerebellar cortex by mossy fibers (MFs) and relayed by granule cells (GCs) up to Purkinje cells (PCs), the sole output of the cerebellar cortex. The T-shaped axon of GCs makes parallel fibers (PF) that also contact molecular layer interneurons (MLI) providing feed-forward inhibition onto PCs and enable communication between cerebellar modules. Incoming information are conveyed as high-frequency bursts. On the MF-GC-(MLI)-PC pathway, bursts of activity trigger several forms of short-term synaptic plasticity (STP) that dynamically and differentially shape the synaptic strength in the millisecond range and shape the PC discharge. While heterogeneous STP properties have been observed at GC-MLI synapses, their organization at GC terminals along the PF is still unknown. We performed *ex-vivo* patch-clamp recordings and two-photon imaging of a genetically-encoded fluorescent glutamate reporter (iGluSnFR) expressed in GCs to monitor STP at single terminals. Using high-frequency electrical stimulation of GCs, we demonstrate that STP profiles are heterogeneous at terminals on the same parallel fiber and independent of the postsynaptic target. Although STP diversity is reduced at 4mM $[Ca^{2+}]_e$, we show that single terminals still strongly sustain glutamate release. These results suggest that GCs terminals recruit a large dynamic range of vesicles to diversify STP providing temporal signatures for PCs to differentially process information between the feed-forward inhibition and the excitatory pathway.

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THE ROLE OF UBCS IN THE DEVELOPING CEREBELLUM AND MOTOR CONTROL SYSTEM

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Medulloblastoma is one of the most common malignant tumors of the central nervous system in children and develops in the cerebellum. Four distinct molecular groups of medulloblastoma have been identified: wingless-activated (WNT), sonic hedgehog-activated (SHH), Group 3 and Group 4. Of all medulloblastoma cases, 40% of medulloblastoma patients are diagnosed with Group 4 medulloblastoma. Although Group 4 medulloblastoma is the most prevalent group, their pathogenesis remains poorly understood, mainly because of the lack of good preclinical models. Recently, it was discovered that the transcriptome of Group 4 medulloblastoma matches the unipolar brush cell (UBC) lineage, giving the hint that UBC progenitors might be the cell of origin for this tumor type. The function of UBCs is not well understood, but there are studies showing that UBCs play a role in balance and movement. To better understand UBC biology and function, we will investigate the role of UBCs in the developing cerebellum using mouse models. Based on previous studies, human single cell RNA-sequencing data and immunohistochemistry of the normal developing murine cerebellum, the transcription factors *Tbr2* and *Lmx1a* appear to be essential for UBC development, as well as *Atoh1* for UBC progenitors. We generated conditional knock-out mice where either *Lmx1a* or *Tbr2* is removed from the UBC lineage and are investigating the downstream effect on UBC biology and animal health. By studying the regular function of UBCs and their progenitors, we might get an insight about how UBCs and/or their progenitors are hijacked to give rise to Group 4 medulloblastoma.

Pubmed:
33128250: Homberg U, Hensgen R, Rieber E, Seyfarth J, Kern M, Dippel S, Dircksen H, Spänig L, Kina YP
Orcokinin in the central complex of the locust Schistocerca gregaria: Identification of immunostained neurons and colocalization with other neuroactive substances.
The central complex is a group of highly interconnected neuropils in the insect brain. It is involved in the control of spatial orientation, based on external compass cues and various internal needs. The functional and neurochemical organization of the central complex has been studied in detail in the desert locust Schistocerca gregaria. In addition to classical neurotransmitters, immunocytochemistry has provided evidence for a major contribution of neuropeptides to neural signaling within the central complex. To complement these data, we have identified all orcokinin-immunoreactive neurons in the locust central complex and associated brain areas. About 50 bilateral pairs of neurons innervating all substructures of the central complex exhibit orcokinin immunoreactivity. Among these were about 20 columnar neurons, 33 bilateral pairs of tangential neurons of the central body, and seven pairs of tangential neurons of the protocerebral bridge. In silico transcript analysis suggests the presence of eight different orcokinin-A type peptides in the desert locust. Double label experiments showed that all orcokinin-immunostained tangential neurons of the lateral accessory lobe cluster were also immunoreactive for GABA and the GABA-synthesizing enzyme glutamic acid decarboxylase. Two types of tangential neurons of the upper division of the central body were, furthermore, also labeled with an antiserum against Dip-allatostatin I. No colocalization was found with serotonin immunostaining. The data provide additional insights into the neurochemical organization of the locust central complex and suggest that orcokinin-peptides of the orcokinin-A gene act as neuroactive substances at all stages of signal processing in this brain area.
J Comp Neurol, 2021; 529

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BOARD NUMBER: S04-429

CEREBELLAR CLIMBING FIBER ACTIVITY CAN RESHAPE THE STRUCTURE OF THE OLIVOCEREBELLAR CIRCUIT

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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AIMS
Cerebellar climbing fibers (CFs) convey a teaching signal to Purkinje cells (PCs) that is crucial for learning. It is known that CFs can undergo activity-dependent synaptic plasticity and that they are able of major lesion-induced structural plasticity. However, it is still not clear whether they are able of structural modifications dependent on their activity during adulthood and how this affects the architecture and function of the olivocerebellar circuit.
Here we investigate whether CF activity controls the morphology of the fiber itself and of its target PC, as well as the underlying molecular mechanism. METHODS
We chronically reduced CF intrinsic excitability in mouse in vivo by knocking-down voltage-gated sodium channels (NaV1.1 and NaV1.2, or NaV1.6) or the growth-associated protein GAP-43 (previously shown to control CF morphology) with lentiviral vectors and used immunofluorescent staining, confocal microscopy and 3D reconstructions to assess morphological effects on CF and PC morphology. RESULTS
We observed that knocking-down NaV1.1/2 causes a CF atrophy (affecting its length and branching), inducing a compensatory increase of density of synaptic terminals as well as of PC dendritic spines. Knocking-down GAP-43 had a similar effect on CF branches but not on synaptic terminals or PC spines suggesting that it may mediate activity-dependent CF structural plasticity. CONCLUSIONS
Our study shows that CFs can undergo activity-dependent structural plasticity affecting PC morphology, that can be potentially relevant for the circuit function, and it suggests that this may be mediated by GAP-43.

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MULTIPLE WHISKER REPRESENTATIONS IN THE CEREBELLAR CRUS1 AND PARAMEDIAN LOBULES

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The whisker system has been widely used for understanding brain functioning and it is suited to clarify how the cerebellum operates sensorimotor integration. The cerebellum could play a role in active whisking because previous studies have shown that Purkinje cells from lobule Crus 1 linearly encode the whisker position, and in particular the whisker setpoint, with their simple spike activity. However, whether active whisking is encoded only in Crus 1 lobule and whether other cerebellar lobules encode other kinematic parameters has been neglected. We performed optogenetic stimulation of the right cerebellar hemisphere on mice expressing channelrhodopsin specifically in Purkinje cells to examine whether other cerebellar lobules also encode and induce whisker movement. Surprisingly, we found the shortest latency of optogenetic-induced whisker movements in the paramedian lobule. Next, we recorded extracellularly single units Purkinje cells from a large part of the right hemisphere, to investigate if whisker velocity is also encoded by simple spikes. We found that about half of the Purkinje cells are tuned more to whisker velocity than whisker position. Finally, we examined whether the cells tuned to whisker velocity were preferably located in the cerebellar area inducing faster whisker movements. It turns out that the Purkinje cells encoding velocity were in medial paramedian, but also lateral Crus1. Taken together, these results indicate that there are multiple whisker representations in the lobules of the cerebellar hemisphere, and this differential topographic organization could be related to the control of different aspects of the whisker movements.

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WIDESPREAD FUNCTIONAL CONVERGENCE FROM CEREBELLAR CORTEX TO CEREBELLAR NUCLEI

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The cerebellum powerfully influences numerous downstream regions involved in regulation of motor control, reward, and internal brain activity. The output of the cerebellum is funneled through a collection of cerebellar nuclei (CN), namely fastigial, interposed, and dentate nucleus. Purkinje cells in the cerebellar cortex are thought to be organized into parasagittal modules (medial, intermediate, and lateral) which topographically innervate specific cerebellar nuclei. Here we mapped the cerebellar cortico-nuclear connectivity using photoactivation of ChR2 expressed in Purkinje cells and silicon probe recordings in cerebellar nuclei. Interestingly, photoactivation mapping revealed only weak topography in the cerebellar cortex to nucleus connectivity. Activation of Purkinje cell clusters anywhere within a 3 mm medial-to-lateral cortical territory significantly inhibited individual nuclei. Nevertheless, the strongest Purkinje cell influence to the fastigial nucleus was observed near the vermal regions, and the strongest influence to the dentate nucleus was observed near the lateral regions. Retrograde tracing from individual CN labeled Purkinje cells across a large swatch of the cerebellar cortex, similar to the spatial maps obtained from photoactivation mapping. Our results suggest that individual CN integrates spatially broad input from all three longitudinal compartments, the vermis, the paravermis and the hemisphere. Given diffused mossy fiber input to the cerebellar cortex through pontine nucleus, the broad cerebellar output to the nucleus could allow the cerebellum to learn associations of widespread inputs related to sensory, motor and internal signals with specific teaching signals to form internal models.

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EFFECTS OF ACETYLCHOLINE RECEPTOR ACTIVATION ON PROPERTIES OF CEREBELLAR NUCLEI NEURONS

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The cerebellum receives neuromodulatory inputs including cholinergic projections from the brainstem. The aim of this work was to determine how acetylcholine influences intrinsic and firing properties of cerebellar nuclear neurons, to further our understanding of the role of acetylcholine in the modulation of cerebellar output. Cerebellar slices were obtained from adult rats, and whole cell current clamp recordings were performed on neurons of the cerebellar nuclei (CN). The spontaneous firing rate of the cells was recorded, and a hyperpolarising current injection step was applied to allow extraction of intrinsic cell properties. Properties were monitored before and after bath application of carbachol (10 uM), a cholinergic agonist. As multiple cell types are present within the CN, principal component analysis and cluster analysis were employed on baseline properties to differentiate cell types. Analysis suggests recordings were obtained from two distinct cell types, and carbachol had a differential effect on the two groups. For the first type of cell, carbachol significantly decreased membrane time constant (by 5.42 +/- 1.84 ms), sag (by 2.91 +/- 1 %), and spontaneous firing rate (by 0.87 +/- 0.34 Hz); for the second type of cells, carbachol significantly decreased input resistance (by 43 +/- 16.5 MOhm) and action potential threshold (by 2.69 +/- 0.85 mV). These results indicate that acetylcholine impacts on cerebellar processing by having distinct effects on different neurons in the CN.

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AN FN-OLIVARY FEEDBACK LOOP SHAPES CEREBELLAR OUTPUTS FOR MOVEMENT CONTROL

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Movements can be executed gracefully and effortlessly depending on optimal feedback control of the brain. The cerebello-olivary loop is known to be crucial for motor learning, but its role in the control of body movements is less understood. In this study we described a fastigial nucleus (FN)-olivary pathway that involves in the modulation of rapid body movements. We first characterized the cytoarchitectural and ultrastructural organizations, as well as the synaptic properties of this projection. Using monosynaptic anterograde and retrograde tracing strategies, we identified a closed loop for the nucleo-olivo-cortico module that could be utilized for movement control. Manipulation of the FN neurons directly affects the complex spike (CS) firing probability in vermal Purkinje cells, which could bidirectionally control the output patterns of the medial cerebellum. Activating this FN-olivary module elicited rapid oculomotor and upper body movements. Our study identifies an unique FN-olivary pathway that transmits an efferent feedback copy to the cerebellar cortex, providing a novel substrate for the cerebellar control of movements.

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CEREBELLAR CONTROL OF TARGETED MOVEMENTS

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Goal-directed movements are often caused by sequential activation of muscles and cerebellar Purkinje cells are vital for the coordination of such muscle synergies. Goal-directed movements are not restricted to the limbs, but for instance also the tongue can reach for specific targets. Of late, directional tongue movements have often been used as behavioural read-out during associative learning paradigms. As Purkinje cells are also known to orchestrate associative learning, we asked to what extent Purkinje cells can encode for the pre-motor or for the motor aspects of directional tongue movements. To this end, we recorded Purkinje cell complex spike and simple spike activity in mice during spontaneous and goal-directed licking. We found that, dependent on their location in the cerebellar cortex, Purkinje cells can encode different aspects of licking, including the direction of tongue protrusion. Optogenetic stimulation experiments confirmed that Purkinje cell activity can be causally linked to the direction of tongue protrusion. Thus, we show that anatomically separate groups of Purkinje cells can be related to differential aspects of targeted movements, with some Purkinje cells being involved in a cycle-by-cycle fashion, while others are more likely to convey a readiness to act signal.

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TOPOGRAPHICAL ORGANISATION OF WHISKER MOVEMENT WITHIN PURKINJE CELL MICROZONES

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Whisking is a well-defined paradigm to study active sensory processing and whisker movements are encoded by Crus 1 Purkinje cells (PCs) via linear changes in firing rate. The topographical organisation and functional relevance of these signals are unknown. PCs are the sole output of the cerebellum and can generate powerful depolarising Ca^{2+} events via input from climbing fibres (CFs). Previously, co-ordinated CF -evoked activity has provided insight into the topographical sensorimotor processing in the cerebellum. Here we explored how whisking motor and sensory signals are represented at a population level, by injecting AAV-FLEX-GCaMP6f into Crus 1 lobule of Pcp2-Cre mice, and using 2-photon imaging to image the Ca^{2+}-dynamics during head-fixed voluntary whisking and perturbed whisking behaviours. We observed that whisker movement dynamics are reflected in the Ca^{2+} dynamics of subpopulations of PC dendrites. These subpopulations are spatially clustered into parasagittal bands of varying width (range: 300 to 1000µm), such that synchronised activity correlated with whisking behaviour could span large proportions of Crus 1. Within these bands, PCs activity was synchronised showing a reliable increase in Ca^{2+} during voluntary whisking, while in addition, a small population of dendrites (20-60µm wide parasagittal bands) at the lateral edge of Crus 1 showed heterogeneous responses to air-puff stimulation. Responses from air-puff stimulation varied between decreases or increases in Ca^{2+}, or biphasic reductions followed by increases. These results indicate that discrete subpopulations of PC dendrites may encode information about efferent (motor) and afferent (sensory) signals relating to whisker movement.

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EVALUATING THE MODULAR HYPOTHESIS: COMPARISON OF CEREBELLAR MEDIAL NUCLEUS AND LATERAL NUCLEUS ACTIVITY IN A REWARDED SPATIAL MEMORY TASK

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The cerebellum has a highly organised structure with topographically organised connections linking rostrocaudally-orientated zones of cerebellar cortex with different parts of the inferior olive and cerebellar nuclei, forming a series of distinct olivo-cortico-nuclear modules. Different modules are predicted to preferentially subserve different aspects of behaviour across cognitive and motor domains, however there has been little interrogation of this reported to date. Moreover, while clinical and experimental evidence from humans has implicated the cerebellum in cognition in addition to sensorimotor function, little is known about the physiological correlates of cognition in cerebellar neuronal activity which can readily be investigated in the rodent. We address these two areas of enquiry by: i) investigating the neural correlates of spatial memory in single units of two cerebellar nuclei related to different modules, specifically ii) comparing medial with lateral nucleus across the cognitive and motor components of a spatial memory task. We predict that the medial nucleus (A and AX module output) preferentially relays motor-related information, while lateral nucleus (D1 and D2 module output) preferentially relays cognition-related information, thereby serving the motor and cognitive components respectively of goal-directed behaviour. Tetrode recordings were obtained from medial or lateral nucleus of freely moving rats during a T-maze-like spatial alternation task for food reward. Preliminary findings provide evidence of cells modulated at task-relevant behavioural timepoints, including acquisition of reward. We compare modulation patterns between the nuclei to evaluate the modular hypothesis of cerebellar function, and consider our results within the wider context of neural correlates of memory.

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MICROENDOSCOPIC CALCIUM IMAGING OF CEREBELLAR CORTEX DURING ASSOCIATIVE MOTOR LEARNING IN FREELY-MOVING MICE

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Understanding the cellular counterparts of learning and memory is one of the biggest challenges in neurosciences. In this scenario, the cerebellum plays a pivotal role in motor processing and learning, though the spatiotemporal dynamics and interplay among neuronal subtypes during planning and execution of movements are still largely unknown. Microendoscopic Ca^{2+} imaging through genetically encoded Ca^{2+} indicators represent a cutting-edge approach for monitoring neuronal populations activity in specific brain regions. In this work, we show a pioneering study of Ca^{2+} imaging recordings of cerebellar activity using head-mounted miniscopes in freely-moving mice performing an associative learning task on a custom-built treadmill. Neuronal Ca^{2+} dynamics in the cerebellar cortex were tracked across trial-to-trial repetition of a task in which a conditioned stimulus (CS, the treadmill rotation), was paired to an unconditioned stimulus (US, a sound). Preliminary data show that neuronal activity becomes more uniform from trial to trial and responses to the CS tends to anticipate after the US. Behavioral analysis confirmed that after few days of training, the mice learned the task showing a similar motor response each time after the US (as opposed to the trial-to-trial variability in the first presentations). Further analysis will be necessary to characterize the spatiotemporal dynamics of specific neuronal subtypes and the neuronal correlates of behavior. These preliminary data show the impressive potential of this approach in studying physiological mechanisms of learning in freely-moving rodents, and opens new perspectives in the investigation of the role of cerebellar processing across diverse motor and non-motor behaviors.

Pubmed:
30894802: Moscato L, Montagna I, De Propris L, Tritto S, Mapelli L, D'Angelo E
Long-Lasting Response Changes in Deep Cerebellar Nuclei Correlate With Low-Frequency Oscillations.
The deep cerebellar nuclei (DCN) have been suggested to play a critical role in sensorimotor learning and some forms of long-term synaptic plasticity observed have been proposed as a possible substrate. However, till now it was not clear whether and how DCN neuron responses manifest long-lasting changes . Here, we have characterized DCN unit responses to tactile stimulation of the facial area in anesthetized mice and evaluated the changes induced by theta-sensory stimulation (TSS), a 4 Hz stimulation pattern that is known to induce plasticity in the cerebellar cortex . DCN units responded to tactile stimulation generating bursts and pauses, which reflected combinations of excitatory inputs most likely relayed by mossy fiber collaterals, inhibitory inputs relayed by Purkinje cells, and intrinsic rebound firing. Interestingly, initial bursts and pauses were often followed by stimulus-induced oscillations in the peri-stimulus time histograms (PSTH). TSS induced long-lasting changes in DCN unit responses. Spike-related potentiation and suppression (SR-P and SR-S), either in units initiating the response with bursts or pauses, were correlated with stimulus-induced oscillations. Fitting with resonant functions suggested the existence of peaks in the theta-band (burst SR-P at 9 Hz, pause SR-S at 5 Hz). Optogenetic stimulation of the cerebellar cortex altered stimulus-induced oscillations suggesting that Purkinje cells play a critical role in the circuits controlling DCN oscillations and plasticity. This observation complements those reported before on the granular and molecular layers supporting the generation of multiple distributed plasticities in the cerebellum following naturally patterned sensory entrainment. The unique dependency of DCN plasticity on circuit oscillations discloses a potential relationship between cerebellar learning and activity patterns generated in the cerebellar network.
Front Cell Neurosci, 2019; 13
32260234: Prestori F, Montagna I, D'Angelo E, Mapelli L
The Optogenetic Revolution in Cerebellar Investigations.
The cerebellum is most renowned for its role in sensorimotor control and coordination, but a growing number of anatomical and physiological studies are demonstrating its deep involvement in cognitive and emotional functions. Recently, the

development and refinement of optogenetic techniques boosted research in the cerebellar field and, impressively, revolutionized the methodological approach and endowed the investigations with entirely new capabilities. This translated into a significant improvement in the data acquired for sensorimotor tests, allowing one to correlate single-cell activity with motor behavior to the extent of determining the role of single neuronal types and single connection pathways in controlling precise aspects of movement kinematics. These levels of specificity in correlating neuronal activity to behavior could not be achieved in the past, when electrical and pharmacological stimulations were the only available experimental tools. The application of optogenetics to the investigation of the cerebellar role in higher-order and cognitive functions, which involves a high degree of connectivity with multiple brain areas, has been even more significant. It is possible that, in this field, optogenetics has changed the game, and the number of investigations using optogenetics to study the cerebellar role in non-sensorimotor functions in awake animals is growing. The main issues addressed by these studies are the cerebellar role in epilepsy (through connections to the hippocampus and the temporal lobe), schizophrenia and cognition, working memory for decision making, and social behavior. It is also worth noting that optogenetics opened a new perspective for cerebellar neurostimulation in patients (e.g., for epilepsy treatment and stroke rehabilitation), promising unprecedented specificity in the targeted pathways that could be either activated or inhibited.
Int J Mol Sci, 2020; 21

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BOARD NUMBER: S04-438

RELATIONSHIPS BETWEEN GRANULE CELL LINEAGES AND FUNCTIONAL SYNAPTIC ORGANIZATION IN THE CEREBELLAR CORTEX

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The cerebellum's ability to learn multisensory contextual patterns relies on signal integration across the anatomically and functionally diverse cerebellar modules. The intermodular communication is mediated by parallel fibres, the axons of the granule cells (GCs) which integrate mossy fiber (MF) inputs from multiple sensory modalities. During postnatal cerebellar development, groups of clonally related GCs differentiate in a restricted medio-lateral zone. However, relationships between GC clones and the topography of cerebellar inputs or synaptic properties within the cerebellar cortex is unknown. Here, we describe a unique approach using brainbow technology where multicolour label expression is under control of inducible Cre in order to sparsely visualise clonally related GCs. To check wether clonally related GCs receive the same set of sensory modalities, AAV viral vectors will be injected in pre-cerebellar nuclei of Brainbow mice to label MF inputs. In addition, we describe an innovative strategy where GC clones can be identified by novel 'iON' vectors that mediate genomic integration of reporter genes in GC progenitors by DNA electroporation. This method will be used to express opsins in subsets of GC clones, which will allow studying the relationships between GC clones and synaptic organization (topography and synaptic properties of parallel fibers inputs). Together these strategies have the potential to shed light on the unknown links between GC lineages and the functional organisation in the cerebellar cortex.

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BOARD NUMBER: S04-439

MOTOR LEARNING AND TEMPORAL DYNAMICS IN THE OLIVARY-CEREBELLAR NETWORK

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Inferior olive (IO) sends Climbing Fiber (CF) axons to Purkinje cells (PC) of the cerebellar cortex. According to classical models of cerebellar learning, CF inputs act as instructive signals causing PC synaptic plasticity by reporting sensory-motor unexpected events. In the context of complex motor learning, those signals and their contribution to postural adaptation are still unknown. To investigate this question, we perform chronic in vivo multiphoton recordings in multiple PC dendrites expressing the calcium indicator GCaMP6f. High temporal resolution allows millisecond precision of calcium transients (CT) onset detection. In order to follow variations of CF firing dynamics during self-generated postural learning, head-fixed mice are placed on a small frictionless wheel and recordings are started on the 1st day of the experiments, without habituation. We find that IO activity is increased over a 200 ms period before postural adjustment onset and is followed by a long-lasting decrease. In addition, this activity comprises 10 ms synchronous population events. Unlearned postural adjustments show an excess of sequences composed of two CTs (50-70ms interval). Strikingly, the second CT is associated with 10ms-tight synchronies, higher amplitudes and is timed at the movement onset. Over the course of postural learning, movement related activity decreases together with CT-doublets occurrence, reaching a steady state the 3rd day and is shifted in time towards movement execution. Reappearance of these features after wheel position modification confirms their causal role in postural learning. These results show that IO contributes to motor learning using exquisite spatio-temporal activity patterns.

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BOARD NUMBER: S04-440

LOCOMOTOR ACTIVITY SHIFTS THE TEMPORAL WINDOW FOR CEREBELLAR MEMORY CONSOLIDATION

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Motor learning is present throughout life, from learning how to swim to riding a bicycle. Motor memories are often consolidated over time, becoming less susceptible to interference. Despite its ubiquity, mechanisms for motor memory consolidation are still poorly understood. The cerebellum is crucial for several forms of motor learning, including delay eyeblink conditioning. We previously showed that engaging in locomotor activity enhances eyeblink conditioning in mouse cerebellum. Here we report that locomotor activity also accelerates the consolidation of learned eyeblink responses. Only animals that were not actively locomoting during training sessions were susceptible to pharmacological and optogenetic perturbations of activity in cerebellar cortex immediately following each training session. In contrast, post-session manipulations of neural activity had no effect on memory consolidation in mice that were running on a motorized treadmill throughout training. Conversely, acute optogenetic perturbation of cerebellar granule cells, when presented in between trials within training sessions, impaired learning only in animals that *were* engaged in locomotion during training, but did not affect learning in quiescent animals. These results suggest that locomotor activity drives a shift in the critical time window for motor memory consolidation in the cerebellar cortex, from immediately following, to within training sessions. In ongoing work we are further investigating the mechanisms through which running facilitates consolidation on the cellular and systems levels.

Pubmed:
33077026: Albergaria C, Silva NT, Darmohray DM, Carey MR
Cannabinoids modulate associative cerebellar learning via alterations in behavioral state.
Cannabinoids are notorious and profound modulators of behavioral state. In the brain, endocannabinoids act via Type 1-cannabinoid receptors (CB1) to modulate synaptic transmission and mediate multiple forms of synaptic plasticity. CB1 knockout (CB1KO) mice display a range of behavioral phenotypes, in particular hypoactivity and various deficits in learning and memory, including cerebellum-dependent delay eyeblink conditioning. Here we find that the apparent effects of CB1 deletion on cerebellar learning are not due to direct effects on CB1-dependent plasticity, but rather, arise as a secondary consequence of altered behavioral state. Hypoactivity of CB1KO mice accounts for their impaired eyeblink conditioning across both animals and trials. Moreover, learning in these mutants is rescued by walking on a motorized treadmill during training. Finally, cerebellar granule-cell-specific CB1KOs exhibit normal eyeblink conditioning, and both global and granule-cell-specific CB1KOs display normal cerebellum-dependent locomotor coordination and learning. These findings highlight the modulation of behavioral state as a powerful independent means through which individual genes contribute to complex behaviors.
Elife, 2020; 9
29662214: Albergaria C, Silva NT, Pritchett DL, Carey MR
Locomotor activity modulates associative learning in mouse cerebellum.
Changes in behavioral state can profoundly influence brain function. Here we show that behavioral state modulates performance in delay eyeblink conditioning, a cerebellum-dependent form of associative learning. Increased locomotor speed in head-fixed mice drove earlier onset of learning and trial-by-trial enhancement of learned responses that were dissociable from changes in arousal and independent of sensory modality. Eyelid responses evoked by optogenetic stimulation of mossy fiber inputs to the cerebellum, but not at sites downstream, were positively modulated by ongoing locomotion. Substituting prolonged, low-intensity optogenetic mossy fiber stimulation for locomotion was sufficient to enhance conditioned responses. Our results suggest that locomotor activity modulates delay eyeblink conditioning through increased activation of the mossy fiber pathway within the cerebellum. Taken together, these results provide evidence for a novel role for behavioral state modulation in associative learning and suggest a potential mechanism through which engaging in movement can improve an individual's ability to learn.
Nat Neurosci, 2018; 21
25357129: Leite C, Silva NT, Mendes S, Ribeiro A, de Faria JP, Lourenço T, dos Santos F, Andrade PZ, Cardoso CM, Vieira

M, Paiva A, da Silva CL, Cabral JM, Relvas JB, Grãos M
Differentiation of human umbilical cord matrix mesenchymal stem cells into neural-like progenitor cells and maturation into an oligodendroglial-like lineage.
Mesenchymal stem cells (MSCs) are viewed as safe, readily available and promising adult stem cells, which are currently used in several clinical trials. Additionally, their soluble-factor secretion and multi-lineage differentiation capacities place MSCs in the forefront of stem cell types with expected near-future clinical applications. In the present work MSCs were isolated from the umbilical cord matrix (Wharton's jelly) of human umbilical cord samples. The cells were thoroughly characterized and confirmed as bona-fide MSCs, presenting in vitro low generation time, high proliferative and colony-forming unit-fibroblast (CFU-F) capacity, typical MSC immunophenotype and osteogenic, chondrogenic and adipogenic differentiation capacity. The cells were additionally subjected to an oligodendroglial-oriented step-wise differentiation protocol in order to test their neural- and oligodendroglial-like differentiation capacity. The results confirmed the neural-like plasticity of MSCs, and suggested that the cells presented an oligodendroglial-like phenotype throughout the differentiation protocol, in several aspects sharing characteristics common to those of bona-fide oligodendrocyte precursor cells and differentiated oligodendrocytes.
PLoS One, 2014; 9

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9-13 July 2022 | Paris, France

BOARD NUMBER: S04-441

THE ROLE OF DISTINCT CEREBELLAR CIRCUIT ELEMENTS IN LOCOMOTION AND LOCOMOTOR LEARNING

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The cerebellum is a brain structure involved in various sensorimotor and cognitive functions. For locomotor behaviors, the cerebellum is critical for keeping movements calibrated and coordinated across the body. Further, within the cerebellar circuit, various cell types have been mapped to distinct functions required for proper execution of movement. Here, we sought to ascertain how various cerebellar circuit neurons contribute to overground locomotion and locomotor learning on a split-belt treadmill in adult mice. We targeted Purkinje cells, granule cells, and a combination of granule cells and mossy fiber inputs for ablation via cell-type specific, cre-recombinase mediated expression of the diphtheria toxin receptor (DTR) and subsequent intraperitoneal administration of diphtheria toxin (DT). We found that ablations across all cell types caused an ataxic phenotype and impairments in locomotor learning, both of which scaled with the degree of cell death. Generally, the observed phenotypes in the two behaviours were similar to those previously reported for ataxic mutant mice, as forward motion of individual limbs was largely spared, while other 3D limb movements and interlimb coordination were impaired. Surprisingly, we also found that incomplete granule cell ablation led to outcomes commensurate with near/complete loss of Purkinje cells in both learning and overground locomotion. Overall, our results suggest that cell-specific manipulations within the cerebellar circuit yield overlapping phenotypes but gradations in outcome are observed in a cell-type dependent manner.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S04-442

LONGITUDINAL MULTIELECTRODE RECORDINGS OF PURKINJE CELL ACTIVITY DURING ACCELERATED EYEBLINK CONDITIONING IN MICE

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

Victor Llobet[1], Aurélien Wyngaard[1], Elie Oriol[2], Valentin Normand[3], Lina Jeantin[4], Jean-Charles Wurtz[4], Gérard Parésys[4], Jonas Ranft[1], Vincent Hakim[2], Boris Barbour[1,4,5,6]
[1]CNRS, Umr 8197, Paris, France, [2]Ecole Normale Superieure, Physics, Paris, France, [3]Normegian University of Science and Technology, Kavli Institute For System Neuroscience, Trondheim, Norway, [4]PSL, Ibens, Paris, France, [5]INSERM, U1024, Paris, France, [6]Ecole Normale Supérieure, Ibens, PARIS, France

It is broadly agreed that synaptic plasticity underlies most forms of learning and memory. There are, however, few situations in which the plastic changes have been observed in vivo and linked to alterations of behaviour. Challenges include targeting high-resolution, longitudinal recordings to causal neurones. Several cerebellar behaviours are thought to offer compact and uniform controlling neuronal populations, yet even here sufficiently long-lasting recordings are rare and have only targeted single neurones. We have performed chronic multi-electrode recordings of Purkinje cell activity during acquisition and extinction of eyeblink conditioning in mice, with an accelerated protocol in which conditioned responses are acquired over two days. These revealed complex dynamics of complex spike activity associated with the conditioned stimulus, the conditioned response and the unconditioned stimulus. In contrast, no systematic modifications of simple spike activity were observed, despite the suitable complex spike activity. The absence of simple spike changes supports neither Marr-Albus-Ito nor Stochastic Gradient Descent theories of cerebellar learning. The perspectives for future experiments testing theories of cerebellar learning are discussed.

FENS Forum 2022
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BOARD NUMBER: S04-443

TWO-PHOTON IMAGING OF CEREBELLAR PURKINJE CELLS DURING FICTIVE LOCOMOTION DISTINGUISHES SENSORY AND MOTOR COMPONENTS OF LOCOMOTION

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Locomotion in mammals is driven by central pattern generators located in the spinal cord, but the cerebellum is required for producing well-coordinated movement (Machado et al 2015). The cerebellum is presumed to achieve coordination by comparing sensory information perceived during locomotion to a model of the expected sensory information self-generated by the locomotor activity and output differences to modulate locomotion. One impediment to testing this hypothesis is isolating the self-generated sensory information from the efferent copy of the CPG in the cerebellum during locomotion. Here we use fictive locomotion in decerebrate mice to separate the sensory and motor components of locomotion and test the hypothesis that the efferent copy of the CPG cycle is highly plastic and forms a negative image in Purkinje cells that cancels expected reafference. To test this hypothesis we compared the rate of simple and complex spikes in Purkinje cells in response to Sural nerve stimulation or to the spontaneous CPG signal. Cell-attached recordings and/or calcium imaging was used to monitor Purkinje cell simple and complex spike activity on the surface of lobule IV/V. Activity was recorded either during fictive locomotion, or after stimulation of the Sural nerve of the right hind-limb, or both. We report that a large proportion of Purkinje cells in the region modulate complex or simple spike rates in response to either the efferent copy, the sensory stimuli, or both and show different patterns of spatial/temporal organization of their responses.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S04-444

IN VIVO TWO-PHOTON IMAGING OF PURKINJE CELL ACTIVITY DURING ADAPTATION TO A NOVEL SENSORIMOTOR MISMATCH PARADIGM

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The ability to learn precise, coordinated motor programs and adapt them to a changing environment is essential for the survival of all animals. A leading theory for how mammalian brains quickly adapt motor programs proposes that the cerebellum uses forward models to compare perceived reafference to expected reafference and makes adjustments to movements based on any mismatches. However, where and how the cerebellum stores expected reafference and how the activity of the cerebellar cortex is affected by differences in perceived and expected reafference is unknown. To probe this, head-fixed mice were trained to use a joystick to control the position of a lick port that delivered water rewards and were forced to adapt to perturbations of the expected position of the lick port. We simultaneously imaged GCaMP7f or GCaMP8f/m expressed in Purkinje cells and observed that complex spike rate remained stable during control conditions but was modulated in specific dendrites during trials in which the mouse adapted to mismatches between expected and perceived position of the lick port. These findings demonstrate that mice adapt to this sensorimotor mismatch paradigm on the same timescale that humans adapt to similar reaching paradigms (5-10 trials; Martin et al., 1996), and supports classical models of supervised learning in which climbing fibers signal mismatches to Purkinje cells via complex spikes.

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BOARD NUMBER: S04-445

A TRASLATIONAL STUDY OF THE CEREBELLAR NEURONAL DOPAMINERGIC SYSTEM AND ITS LINKS TO THE MIDBRAIN DOPAMINERGIC NUCLEI AND ROLE IN DOPAMINE-RELATED BRAIN DISORDERS

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Introduction. Although, a direct involvement of the cerebellum in Parkinson's disease and Schizophrenia were indicated. Data on cerebellar dopaminergic innervation and interconnections to the dopaminergic midbrain nuclei are scantly. In rodents, only extrinsic cerebellar dopaminergic fiber system, originating from the A_{10} dopaminergic area, and few dopaminergic neurons composed by Purkinje neurons of the cerebellar cortex were demonstrated. *Aim.* The goal of this translational study is to explore the human cerebellar neuronal dopaminergic system, by means of Diffusion Magnetic Resonance Tractography (DMRT) brain imaging analysis so as to evaluate the cerebellar interconnections with the dopaminergic nuclei of the midbrain. *Material and methods*. Postmortem fragments of human cerebellum were fixed in an aldehyde picric acid solution, embedded in paraffin, cut into 5 µm sections and subjected to light microscopic immunohistochemistry by using rabbit polyclonal antibodies to dopamine transporter (DAT) or dopamine type 2 receptor (DRD_2). In brain imaging analysis 3T Achieva Philips scanner was used for acquiring T1 weighted 3D TFE sequences. DMRT data using diffusion signal modeling techniques were acquired *Results*. DAT and DRD_2 immunoreactivity were observed in the cerebellar Purkinje neurons in some non-traditional neuron types, and in the dentate nucleus in large and small neuron types. Moreover, we detected direct dentato-nigral and dentato-ventro tegmental interconnections. *Conclusions*. This study demonstrates the occurrence of a human cerebellar dopaminergic system and of direct dentate nucleus interconnections to the midbrain dopaminergic nuclei, which allows us to suggest a cerebellar role in dopaminergic brain disorders.

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BOARD NUMBER: S04-446

FUNCTIONAL SYNAPTIC CONNECTIVITY IN THE CEREBELLAR CORTEX

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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The cerebellum is involved in motor coordination and learning of skilled movements. The cerebellar cortex is divided into repeated anatomo-functional modules that process sensorimotor information from a given body part. This information is conveyed notably by mossy fibres (MF) to granule cells (GC) which send a long axon, the parallel fibres, to Purkinje cells (PC) belonging to several modules. This inter-modular communication may control motor coordination. We have shown that synaptic connectivity at the GC-PC and GC-interneuron connections are stereotyped between individuals (Valera et al, eLife 2016), but individual behavioural features can still be encoded by individual synaptic maps (Spaeth et al., Nat. commun. 2022). Our aims was to determine how inhibitory connectivity maps in the GC-MLI-PC pathway are organized and (2) to investigate these relationships while perturbing behavioural conditions. First, we combined patch clamp recordings of PCs in acute cerebellar slices with systematic photostimulation of RuBi-glutamate using patterned illumination. We showed that inhibitory connectivity maps in the vermal lobule III/IV do not necessarily overlap with excitatory connectivity maps yielding complex intermodular communication rules. Second, we altered the behavioural conditions by allowing mice to run in a wheel for 19 days before establishing the synaptic maps. We found that synaptic maps from trained mice were modified suggesting that both excitatory and inhibitory functional synaptic organization in the cerebellar cortex might be related to locomotor adaptation.

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BOARD NUMBER: S04-447

MULTIDIMENSIONAL CEREBELLAR COMPUTATIONS FOR FLEXIBLE KINEMATIC CONTROL OF MOVEMENTS

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Aims: Maintaining the precision of our movements critically depends on how well our movements adapt to continuously changing environments and states of the body. The cerebellum has been known to be a critical locus for this function, captured by the term sensorimotor adaptation. We addressed the question of circuit-level information processing, which remains poorly understood. Methods: We recorded from mossy fibers (MF, network input) and Purkinje cells (PC, output), from two rhesus monkeys performing a repetitive saccade task. We analyzed the MF firing and PC simple spiking (SS) data to identify a multidimensional, manifold-like structures in both. We also constructed a feed-forward network (FFN) model simulating the MF-to-PC transformation. Results: We found the representations of individual movement parameters in the manifold geometry and dynamics both in MFs and PCs, while they were much more selective in PCs than MFs. Error feedback-driven inputs modulated the PC manifolds in an error-type specific fashion, which predicted the changes in the future movements. Our FFN model revealed that MF activity can accurately predict the PC SS/manifold but only by expanding small variabilities in MF inputs. Conclusion: The cerebellum performs multidimensional adaptive computations by encoding several kinematic parameters concurrently, which enables flexible coordination for sensorimotor learning. A compressed, low dimensional copy of sensorimotor information in the MF inputs transforms to the higher dimensional PC outputs by the cerebellar cortical circuit through variability expansion. This mechanism enables PCs to fine control individual kinematic parameters, which is necessary for the precisions in movements.

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BOARD NUMBER: S04-448

MOTOR PERFORMANCE AND REGIONAL BRAIN AND MUSCLE METABOLISM IN MICE SUBJECTED TO HINDLIMB UNLOADING

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Microgravity induced by space flight has been shown to induce oxidative stress and cause neurochemical and metabolic alterations in specific brain regions. To mimic microgravity, young adult mice were exposed to hindlimb unloading by tail suspension or not (control group) over a 21-day period and, after a 15-day period of recovery, evaluated for motor activity and coordination as well as spatial learning to detect possible long-term behavioral alterations. At the end of the behavioral study, animals were killed and brain as well as forelimb and hindlimb muscles removed for measuring regional cytochrome oxydase activity revealed by histochemistry as a good index of cellular metabolic activity. Both groups of mice increased their body weight in an equivalent manner. The hindlimb unloaded group was impaired on stationary beam and rotorod motor coordination tests, whereas no effect was observed on exploratory activity tests or the Morris water maze. Cytochrome oxidase activity 1) decreased in several precerebellar structures as well as regions involved in the planning and control of movements such as the posterior parietal area and the substantia nigra, and 2) increased in hindlimb muscles, specifically in the small fibers involved in tonic function. The present results confirmed a durable effect of microgravity on vestibulo-cerebellar and motor-related pathways as well as muscle function.

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BOARD NUMBER: S04-449

NEURAL UNDERPINNINGS OF VOCAL PRODUCTION LEARNING AND RHYTHM IN THE SEAL BRAIN

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Seals have two critical prerequisites for studying the evolution of language and music: the ability to flexibly modify their vocalisations (vocal production learning; VPL) and to process rhythm. However, little is known about the neural underpinnings of VPL and rhythm in animal groups other than humans, songbirds, and non-human primates. Here, we investigate the seal's neuroanatomy and connectivity and their possible relationship with VPL and rhythm. Three brains were retrieved post-mortem from weaned seals; one harbour seal—*Phoca vitulina*—and two grey seals—*Halichoerus grypus*) that were either euthanised or had died of natural causes in rehabilitation. Brains were fixed in formalin and scanned with high-resolution MRI to obtain T1, T2, and diffusion weighted images. The anatomical scans were segmented to create an atlas, a labelled template, and a 3D model with volumetric measurements of brain regions. Results show that the seal brain is relatively big and highly convoluted, with a large temporal lobe and cerebellum and similar cortical thickness as found in humans. Planned probabilistic DTI analyses will target the ascending auditory pathway and the pyramidal tracts. We expect to observe strong connections between auditory and motor regions (e.g., Supplementary Motor Area (SMA), dorsal caudate, temporo-parietal area), supporting VPL and rhythmic processing. By bridging the research gap from songbirds to humans and non-human primates, these results will critically inform VPL and rhythm research and may thus elucidate and enrich the ongoing debate on the evolution of language and music.

Pubmed:
34820184: Varola M, Verga L, Sroka MGU, Villanueva S, Charrier I, Ravignani A
Can harbor seals () discriminate familiar conspecific calls after long periods of separation?
The ability to discriminate between familiar and unfamiliar calls may play a key role in pinnipeds' communication and survival, as in the case of mother-pup interactions. Vocal discrimination abilities have been suggested to be more developed in pinniped species with the highest selective pressure such as the otariids; yet, in some group-living phocids, such as harbor seals (), mothers are also able to recognize their pup's voice. Conspecifics' vocal recognition in pups has never been investigated; however, the repeated interaction occurring between pups within the breeding season suggests that long-term vocal discrimination may occur. Here we explored this hypothesis by presenting three rehabilitated seal pups with playbacks of vocalizations from unfamiliar or familiar pups. It is uncommon for seals to come into rehabilitation for a second time in their lifespan, and this study took advantage of these rare cases. A simple visual inspection of the data plots seemed to show more reactions, and of longer duration, in response to familiar as compared to unfamiliar playbacks in two out of three pups. However, statistical analyses revealed no significant difference between the experimental conditions. We also found no significant asymmetry in orientation (left . right) towards familiar and unfamiliar sounds. While statistics do not support the hypothesis of an established ability to discriminate familiar vocalizations from unfamiliar ones in harbor seal pups, further investigations with a larger sample size are needed to confirm or refute this hypothesis.
PeerJ, 2021; 9
34733208: Verga L, Schwartze M, Stapert S, Winkens I, Kotz SA
Dysfunctional Timing in Traumatic Brain Injury Patients: Co-occurrence of Cognitive, Motor, and Perceptual Deficits.
Timing is an essential part of human cognition and of everyday life activities, such as walking or holding a conversation. Previous studies showed that traumatic brain injury (TBI) often affects cognitive functions such as processing speed and

time-sensitive abilities, causing long-term sequelae as well as daily impairments. However, the existing evidence on timing capacities in TBI is mostly limited to perception and the processing of isolated intervals. It is therefore open whether the observed deficits extend to motor timing and to continuous dynamic tasks that more closely match daily life activities. The current study set out to answer these questions by assessing audio motor timing abilities and their relationship with cognitive functioning in a group of TBI patients (= 15) and healthy matched controls. We employed a comprehensive set of tasks aiming at testing timing abilities across perception and production and from single intervals to continuous auditory sequences. In line with previous research, we report functional impairments in TBI patients concerning cognitive processing speed and perceptual timing. Critically, these deficits extended to motor timing: The ability to adjust to tempo changes in an auditory pacing sequence was impaired in TBI patients, and this motor timing deficit covaried with measures of processing speed. These findings confirm previous evidence on perceptual and cognitive timing deficits resulting from TBI and provide first evidence for comparable deficits in motor behavior. This suggests basic co-occurring perceptual and motor timing impairments that may factor into a wide range of daily activities. Our results thus place TBI into the wider range of pathologies with well-documented timing deficits (such as Parkinson's disease) and encourage the search for novel timing-based therapeutic interventions (e.g., employing dynamic and/or musical stimuli) with high transfer potential to everyday life activities.
Front Psychol, 2021; 12
34482729: Hoeksema N, Verga L, Mengede J, van Roessel C, Villanueva S, Salazar-Casals A, Rubio-Garcia A, Ćurčić-Blake B, Vernes SC, Ravignani A
Neuroanatomy of the grey seal brain: bringing pinnipeds into the neurobiological study of vocal learning.
Comparative animal studies of complex behavioural traits, and their neurobiological underpinnings, can increase our understanding of their evolution, including in humans. Vocal learning, a potential precursor to human speech, is one such trait. Mammalian vocal learning is under-studied: most research has either focused on vocal learning in songbirds or its absence in non-human primates. Here, we focus on a highly promising model species for the neurobiology of vocal learning: grey seals (). We provide a neuroanatomical atlas (based on dissected brain slices and magnetic resonance images), a labelled MRI template, a three-dimensional model with volumetric measurements of brain regions, and histological cortical stainings. Four main features of the grey seal brain stand out: (i) it is relatively big and highly convoluted; (ii) it hosts a relatively large temporal lobe and cerebellum; (iii) the cortex is similar to that of humans in thickness and shows the expected six-layered mammalian structure; (iv) there is expression of FoxP2 present in deeper layers of the cortex; is a gene involved in motor learning, vocal learning, and spoken language. Our results could facilitate future studies targeting the neural and genetic underpinnings of mammalian vocal learning, thus bridging the research gap from songbirds to humans and non-human primates. Our findings are relevant not only to vocal learning research but also to the study of mammalian neurobiology and cognition more in general. This article is part of the theme issue 'Vocal learning in animals and humans'.
Philos Trans R Soc Lond B Biol Sci, 2021; 376
33664706: Ferraro S, Nigri A, D'Incerti L, Rosazza C, Sattin D, Rossi Sebastiano D, Visani E, Duran D, Marotta G, Demichelis G, Catricala' E, Kotz S, Verga L, Leonardi M, Cappa S, Bruzzone MG
Corrigendum: Preservation of Language Processing and Auditory Performance in Patients With Disorders of Consciousness: A Multimodal Assessment.
[This corrects the article DOI: 10.3389/fneur.2020.526465.].
Front Neurol, 2021; 12
33408679: Ferraro S, Nigri A, D'Incerti L, Rosazza C, Sattin D, Rossi Sebastiano D, Visani E, Duran D, Marotta G, Demichelis G, Catricala' E, Kotz S, Verga L, Leonardi M, Cappa S, Bruzzone MG
Preservation of Language Processing and Auditory Performance in Patients With Disorders of Consciousness: A Multimodal Assessment.
The impact of language impairment on the clinical assessment of patients suffering from disorders of consciousness (DOC) is unknown or underestimated and may mask the presence of conscious behavior. In a group of DOC patients (= 11; time post-injury range: 5-252 months), we investigated the main neural functional and structural underpinnings of linguistic processing, and their relationship with the behavioral measures of the auditory function using the Coma Recovery Scale-Revised (CRS-R). We assessed the integrity of the brainstem auditory pathways, of the left superior temporal gyrus and arcuate fasciculus, the neural activity elicited by passive listening of an auditory language task, and the mean hemispheric glucose metabolism. Our results support the hypothesis of a relationship between the level of preservation of the investigated structures/functions and the CRS-R auditory subscale scores. Moreover, our findings indicate that patients in minimally conscious state minus (MCS-): (1) when presenting the (at the CRS-R auditory subscale) might be aphasic in the receptive domain, being severely impaired in the core language structures/functions; (2) when presenting the might retain language processing, being almost intact or intact in the core language structures/functions. Despite the small group of investigated patients, our findings provide a grounding of the clinical measures of the CRS-R auditory subscale in the integrity of the underlying auditory

structures/functions. Future studies are needed to confirm our results that might have important consequences for the clinical practice.
Front Neurol, 2020; 11
31515817: Ravignani A, Verga L, Greenfield MD
Interactive rhythms across species: the evolutionary biology of animal chorusing and turn-taking.
The study of human language is progressively moving toward comparative and interactive frameworks, extending the concept of turn-taking to animal communication. While such an endeavor will help us understand the interactive origins of language, any theoretical account for cross-species turn-taking should consider three key points. First, animal turn-taking must incorporate biological studies on animal chorusing, namely how different species coordinate their signals over time. Second, while concepts employed in human communication and turn-taking, such as intentionality, are still debated in animal behavior, lower level mechanisms with clear neurobiological bases can explain much of animal interactive behavior. Third, social behavior, interactivity, and cooperation can be orthogonal, and the alternation of animal signals need not be cooperative. Considering turn-taking a subset of chorusing in the rhythmic dimension may avoid overinterpretation and enhance the comparability of future empirical work.
Ann N Y Acad Sci, 2019; 1453
30946951: Verga L, Kotz SA
Spatial attention underpins social word learning in the right fronto-parietal network.
In a multi- and inter-cultural world, we daily encounter new words. Adult learners often rely on a situational context to learn and understand a new word's meaning. Here, we explored whether interactive learning facilitates word learning by directing the learner's attention to a correct new word referent when a situational context is non-informative. We predicted larger involvement of inferior parietal, frontal, and visual cortices involved in visuo-spatial attention during interactive learning. We scanned participants while they played a visual word learning game with and without a social partner. As hypothesized, interactive learning enhanced activity in the right Supramarginal Gyrus when the situational context provided little information. Activity in the right Inferior Frontal Gyrus during interactive learning correlated with post-scanning behavioral test scores, while these scores correlated with activity in the Fusiform Gyrus in the non-interactive group. These results indicate that attention is involved in interactive learning when the situational context is minimal and suggest that individual learning processes may be largely different from interactive ones. As such, they challenge the ecological validity of what we know about individual learning and advocate the exploration of interactive learning in naturalistic settings.
Neuroimage, 2019; 195
30503716: Galbiati A, Verga L, Giora E, Zucconi M, Ferini-Strambi L
The risk of neurodegeneration in REM sleep behavior disorder: A systematic review and meta-analysis of longitudinal studies.
Several studies report an association between REM Sleep Behavior Disorder (RBD) and neurodegenerative diseases, in particular synucleinopathies. Interestingly, the onset of RBD precedes the development of neurodegeneration by several years. This review and meta-analysis aims to establish the rate of conversion of RBD into neurodegenerative diseases. Longitudinal studies were searched from the PubMed, Web of Science, and SCOPUS databases. Using random-effect modeling, we performed a meta-analysis on the rate of RBD conversions into neurodegeneration. Furthermore, we fitted a Kaplan-Meier analysis and compared the differences between survival curves of different diseases with log-rank tests. The risk for developing neurodegenerative diseases was 33.5% at five years follow-up, 82.4% at 10.5 years and 96.6% at 14 years. The average conversion rate was 31.95% after a mean duration of follow-up of 4.75 ± 2.43 years. The majority of RBD patients converted to Parkinson's Disease (43%), followed by Dementia with Lewy Bodies (25%). The estimated risk for RBD patients to develop a neurodegenerative disease over a long-term follow-up is more than 90%. Future studies should include control group for the evaluation of REM sleep without atonia as marker for neurodegeneration also in non-clinical population and target RBD as precursor of neurodegeneration to develop protective trials.
Sleep Med Rev, 2019; 43
29709607: Bégel V, Verga L, Benoit CE, Kotz SA, Dalla Bella S
Test-retest reliability of the Battery for the Assessment of Auditory Sensorimotor and Timing Abilities (BAASTA).
Perceptual and sensorimotor timing skills can be thoroughly assessed with the Battery for the Assessment of Auditory Sensorimotor and Timing Abilities (BAASTA). The battery has been used for testing rhythmic skills in healthy adults and patient populations (e.g., with Parkinson disease), showing sensitivity to timing and rhythm deficits. Here we assessed the test-retest reliability of the BAASTA in 20 healthy adults. Participants were tested twice with the BAASTA, implemented on a tablet interface, with a 2-week interval. They completed 4 perceptual tasks, namely, duration discrimination, anisochrony detection with tones and music, and the Beat Alignment Test (BAT). Moreover, they completed motor tasks via finger tapping, including unpaced and paced tapping with tones and music, synchronization-continuation, and adaptive tapping to a sequence with a tempo change. Despite high variability among individuals, the results showed good test-retest reliability in most tasks. A slight but significant improvement from test to retest was found in tapping with music, which may reflect a

learning effect. In general, the BAASTA was found a reliable tool for evaluating timing and rhythm skills.
Ann Phys Rehabil Med, 2018; 61
28658646: Verga L, Kotz SA
Help me if I can't: Social interaction effects in adult contextual word learning.
A major challenge in second language acquisition is to build up new vocabulary. How is it possible to identify the meaning of a new word among several possible referents? Adult learners typically use contextual information, which reduces the number of possible referents a new word can have. Alternatively, a social partner may facilitate word learning by directing the learner's attention toward the correct new word meaning. While much is known about the role of this form of 'joint attention' in first language acquisition, little is known about its efficacy in second language acquisition. Consequently, we introduce and validate a novel visual word learning game to evaluate how joint attention affects the contextual learning of new words in a second language. Adult learners either acquired new words in a constant or variable sentence context by playing the game with a knowledgeable partner, or by playing the game alone on a computer. Results clearly show that participants who learned new words in social interaction (i) are faster in identifying a correct new word referent in variable sentence contexts, and (ii) temporally coordinate their behavior with a social partner. Testing the learned words in a post-learning recall or recognition task showed that participants, who learned interactively, better recognized words originally learned in a variable context. While this result may suggest that interactive learning facilitates the allocation of attention to a target referent, the differences in the performance during recognition and recall call for further studies investigating the effect of social interaction on learning performance. In summary, we provide first evidence on the role joint attention in second language learning. Furthermore, the new interactive learning game offers itself to further testing in complex neuroimaging research, where the lack of appropriate experimental set-ups has so far limited the investigation of the neural basis of adult word learning in social interaction.
Cognition, 2017; 168

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BOARD NUMBER: S04-450

PURKINJE CELL MICROZONES MEDIATE DISTINCT KINEMATICS OF A SINGLE MOVEMENT

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

Francois Blot, Joshua White, Amy Van Hattem, Licia Scotti, Vaishnavi Balaji, Chris De Zeeuw, Martijn Schonewille
Erasmus MC, Department Of Neuroscience, Rotterdam, Netherlands

The classification of neuronal subtypes has significantly advanced, yet its relevance for behavior remains unclear. The highly organized flocculus of the cerebellum, known to fine-tune multi-axial eye movements, is an ideal substrate for the study of potential functions of neuronal heterogeneity. Here, we demonstrate that its recently identified subpopulations of 9+ and 9- Purkinje cells exhibit distinct gene expression and electrophysiological profiles, providing evidence for a graded continuum of intrinsic properties among PC subtypes. By identifying and utilizing two Cre-lines that target these floccular domains, we show with high spatial specificity that these subtypes of Purkinje cells participate in separate micromodules with topographically organized connections. Finally, subtype-specific optogenetic excitation results in movements around the same axis in space, yet with distinct kinematic profiles. These results indicate that Purkinje cell microzones can control particular parameters of single movements independent from the spatial reference frame.

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BOARD NUMBER: S04-451

THE PREVALENCE OF DYSTONIC TREMOR AND TREMOR ASSOCIATED WITH DYSTONIA IN PATIENTS WITH CERVICAL DYSTONIA

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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University Hospital Olomouc, Department Of Neurology, Olomouc, Czech Republic

The link between dystonia and tremor has been known for decades, but at present the question arises whether they are two separate illnesses or just different manifestations of one disease with same pathophysiological background. We distinguish two types of tremor in dystonia: dystonic tremor, which appears on body part affected by dystonia, and tremor associated with dystonia (TAWD) in locations where dystonia does not occur, commonly described as coincidental essential tremor. Dystonia has always been considered a basal ganglia disease. However, the theory of neuronal network dysfunction, involving many brain areas, currently prevails. The role of cerebellum seems especially important, which promotes the theory that TAWD might not be just a coincidence of essential tremor and cervical dystonia, but one of the symptoms of cerebellar dysfunction in dystonia. The occurrence of different tremor types in a group of patients with cervical dystonia, was determined by a clinical examination. In total, the study included 123 patients with cervical dystonia, 28 men and 95 women, mean age of patients was 59.8 years. Dystonic tremor was present in 70 patients (56.91%). TAWD was in all 14 cases (11.38%) observed on the upper limbs as a static or intentional tremor. In this study, we point out the presence of TAWD as one of the clinical signs of cervical dystonia, occurring in 11.38% of patients in the studied group. Dystonic tremor occurred in more than half of the patients and appears to be a common part of the clinical picture in patients with cervical dystonia.

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BOARD NUMBER: S04-452

THE NEURONAL DYNAMICS OF CEREBELLAR NUCLEI AND MEDIAL PREFRONTAL CORTEX IN EYEBLINK CONDITIONING ADAPTATION TASK

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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Brain can dynamically control the temporal relationship between sensory inputs and desired movements outputs during daily life. Cerebellum is critically involved in eyeblink conditioning, a classical sensorimotor associative learning paradigm. We sought to examine how cerebellum and other brain regions are involved in regulating the precise timing of conditioned eyeblink response (CR). We trained mice with delay (DEC) or trace eyeblink conditioning (TEC) paradigms. We measured the activity of cerebellar interposed nuclei (IpN) and medial prefrontal cortex (mPFC) during TEC, DEC, and during the conversion between these two paradigms. Pharmacological inhibition of mPFC was also performed in a subgroup of animals. We found that TEC trained mice possess a significantly longer latency between the IpN modulation and CR onset, and IpN neurons could encode for CR onset timing with several different modes. Mice showed quasi-instantaneous adaptation of their CR onset timing while we presented the DEC-trained mice with TEC paradigm, and vice versa. The adaptation of IpN neurons was prominent during DEC-to-TEC conversion, but less during TEC-to-DEC conversion. A group of mPFC neurons rapidly altered their modulation patterns during the paradigm conversion. In addition, silencing mPFC activity selectively blocked the adaptation of CR onsets during both the TEC-to-DEC and TEC-to-DEC conversion, suggesting the functional involvement of mPFC in the rapid adaptation of CR timing. These results illustrate diverse electrophysiology properties of IpN and mPFC neurons that might modulate the timing of associative movements and shed new light on the cerebro-cerebellar mechanisms underlying adaptive temporal control.

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LOCUS COERULEUS MEDIATED STATE SWITCH IN BRAIN STATE DURING MOTOR BEHAVIOR IN THE ZEBRAFISH LARVA.

POSTER SESSION 04 - SECTION: CEREBELLAR COMPUTATION AND MOTOR LEARNING

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[1]Ecole Normale Superieure, L'institut De Biologie, Paris, France, [2]Ecole Normale Superieure, Ibens, Paris, France, [3]Ecole Normale Superierue, Biology, paris, France

The generation of motor behavior has been a crucial question within neuroscience since its emergence as a scientific field. Previously in Zebrafish models, it has been shown that large behavioral activity is associated with a switch in the brain state. Understanding this type of activity is central to our understanding how the brain produces motor behavior. By using zebrafish larva as experimental model in combination with light-sheet microscopy, we monitored whole-brain dynamics with single-neuron resolution while simultaneously recording free tail movement as a behavioral output. We recorded both spontaneous and stimuli-driven activity to be able to compare motor generation between the two. Results show that following both spontaneous and stimuli induced movement there appears to be a Locus Coeruleus (LC) mediated switch in the brain state of the animals. This is characterized by a long-lasting gradual ramping activity of a subpopulation of neurons which are quickly inhibited at the time of activation of the LC. Then, a significant change occurs in terms of the neuronal assembly dynamics across the entire brain. We focus on the ramping subpopulation of neurons to investigate their role in the change of the brain state, their effect of the LC and the behavior of the animal. We hypothesize that this subpopulation is integrating information across the brain to result in motor movement.

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BOARD NUMBER: S04-454

A SPINAL MICROCIRCUIT FOR MUSCLE SEQUENCE ACTIVATION

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Mammals have a remarkable capacity to precisely coordinate body movements to generate complex and functionally relevant motor behaviors. The spinal cord is the final executor of the neuronal code, translating neural signals into detailed and coherent muscle activity along the body. In order to accomplish complex whole-body movements, spinal circuitries should generate interwoven patterns of detailed activity for multiple muscles along the body starting from a common motor command. Here, we examine the sensory-motor components of a spinal microcircuit for multi-joint movements in which a defined population of propriospinal interneurons, the V2a neurons, represents the core element. Combining mouse intersectional genetics with *in vivo* optogenetic and electrophysiology in adult mice, we show that V2a spinal neurons along the spinal cord activate discrete muscles in a topographic fashion. Anatomical tracing and simultaneous recordings of muscles and opto-tagged V2a neurons allowed detailed anatomical characterization and functional dissection of the V2a sensory-motor maps. V2 neurons together with sensory afferents, motor neurons, and muscles form a feedforward descending excitatory system in the spinal cord that simultaneously drives coordinated activity in multiple muscles. Accordingly, prolonged optical activation of V2a neurons in a specific segment appropriately recruit tens of muscles, while their *in vivo* targeted silencing perturbed the sequence of muscle activation in complex motor task as swimming. These data show the anatomical substrate and identify the functional principles of a spinal microcircuit involved in constructing muscle activation sequences, providing the logic on how spinal circuitries sculpt muscle action over time and space for meaningful motor behaviors.

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SPINAL CORD CONTROL OF MANUAL DEXTERITY IN RATS: A FUNCTIONAL MAPPING STUDY

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Manual dexterity, such as object “reaching and grasping” (R&G), is essential to conduct daily tasks. Stereotypic features of these movements suggest that some level of control may be exerted from the spinal cord (SC). In this project, we aimed to unveil the presence of these networks and their rostrocaudal location within the SC. The approach consisted of inflicting excitotoxic injuries to rats at different SC levels covering from C3 to T3 using kainic acid, for exclusively affecting spinal networks while preserving descending commands. Motor deficits were evaluated by comparing the performance, before *vs* after the intervention, in multiple behavioural tests of forelimb muscles’ function that likely require distinct neuronal networks. One-week post-intervention, C3-injured animals showed a significant impairment in R&G, staircase and horizontal ladder tests, but not on other tasks. The performance of rats receiving more caudal injuries remained grossly unaffected. Histological analysis revealed a grey matter loss that correlated with the segments of injection, and which presumably is accompanied by the loss of spinal premotor circuits. To dismiss the possibility of the concomitant loss of motoneurons and/or sensory feedback being the cause of the behavioural deficits observed, a subsequent experiment was conducted in which the C3 dorsal and ventral roots were sectioned, therefore eliminating all its direct afferences and efferences but preserving spinal networks’ integrity. Those animals did not suffer manual dexterity impairments. Our results suggest the presence, at spinal segment C3, of a neuronal network necessary for properly executing forelimb skilled movements.

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SUPRA-PONTINE STRUCTURES MODULATE BRAINSTEM AND SPINAL NETWORKS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

Atiyeh Mohammadshirazi, Monica Colitti, Giuliano Taccola
International School for Advanced Studies, Neuroscience, Trieste, Italy

Supraspinal structures control several spinal motor output and essential rhythmic behaviors. However, current isolated preparations, although of great advantage in deciphering the functional organization of brainstem and spinal networks, are focused on local circuitry. To better characterize the contribution of higher centers to the neuronal pathways involved in respiration and locomotion, we proposed a novel experimental approach. In an in vitro preparation from the isolated CNS of neonatal rodents, a stable respiratory rhythm was simultaneously recorded from cervical and lumbar ventral roots (VRs) with histological morphology preserved for even more than four hours. Selective electrical pulses supplied to pons and medulla evoked distinct responses with staggered onset in rostrocaudal direction, while stimulation of ventrolateral medulla resulted in higher events from homolateral VRs. Moreover, electrical stimulation of a lumbar dorsal root elicited responses even from cervical VRs, albeit smaller and delayed, confirming that ascending pathways were functional. Furthermore, the prototypical fictive locomotion was induced by trains of pulses applied to both the ventrolateral medulla and the dorsal root. When progressively removing higher centers, the frequency of respiration increased after a pontobulbar transection, while burst duration was already reduced after a pre-collicular transection, which also affected the area of lumbar dorsal and ventral root potentials elicited by dorsal root stimulation. In the present study, supra-pontine centers regulate spontaneous respiratory rhythm, as well as electrically-evoked reflex and network responses. Thus, the current approach can clarify the modulatory influence of the brain on caudal micro-circuits.

Pubmed:
31004353: Mohammadshirazi A, Sadrosadat H, Jaberi R, Zareikheirabadi M, Mirsadeghi S, Naghdabadi Z, Ghaneezabadi M, Fardmanesh M, Baharvand H, Kiani S
Combinational therapy of lithium and human neural stem cells in rat spinal cord contusion model.
A large number of treatment approaches have been used for spinal cord injury improvement, a medically incurable disorder, and subsequently stem cell transplantation appears to be a promising strategy. The main objective of this study is to ascertain whether combinational therapy of human neural stem cells (hNSCs) together with lithium chloride improves cell survival, proliferation, and differentiation in a rat spinal contusion model, or not. Contusive spinal cord injury was implemented on Wistar male rats. Experimental groups comprised of: control, hNSCs transplanted, lithium chloride (Li), and hNSCs and lithium chloride (hNSCs + Li). In every experimental group, locomotor activity score and motor evoked potential (MEP) were performed to evaluate motor recovery as well as histological assessments to determine mechanisms of improvement. In accordance with our results, the hNSCs + Li and the Li groups showed significant improvement in locomotor scores and MEP. Also, Histological assessments revealed that transplanted hNSCs are capable of differentiation and migration along the spinal cord. Although NESTIN-positive cells were proliferated significantly in the Lithium group in comparison with control and the hNSCs + Li groups, the quantity of ED1 cells in the hNSCs + Li was significantly larger than the other two groups. Our results demonstrate that combinational therapy of hNSCs with lithium chloride and lithium chloride individually are adequate for ameliorating more than partial functional recovery and endogenous repair in spinal cord-injured rats.
J Cell Physiol, 2019; 234
31982534: Zarei-Kheirabadi M, Sadrosadat H, Mohammadshirazi A, Jaberi R, Sorouri F, Khayyatan F, Kiani S
Human embryonic stem cell-derived neural stem cells encapsulated in hyaluronic acid promotes regeneration in a contusion spinal cord injured rat.
spinal cord injury (SCI) is a traumatic damage that can causes a loss of neurons around the lesion site and resulting in locomotor and sensory deficits. Currently, there is widely attempts in improvement of treatment strategy and cell delivering to the central nervous system (CNS). The usage of hyaluronic acid (HA), the main components of the ECM in CNS tissue and neural stem cells (NSCs) niche, is a good selection that can increase of viability and differentiation of NSCs. Importantly, we demonstrate that encapsulation of human embryonic stem cell derived-neural stem cells (hESC-NS) in HA-based hydrogel can increased differentiation these cells into oligodendrocytes and improved locomotor function.

Int J Biol Macromol, 2020; 148
33826070: Mazzone GL, Mohammadshirazi A, Aquino JB, Nistri A, Taccola G
GABAergic Mechanisms Can Redress the Tilted Balance between Excitation and Inhibition in Damaged Spinal Networks.
Correct operation of neuronal networks depends on the interplay between synaptic excitation and inhibition processes leading to a dynamic state termed balanced network. In the spinal cord, balanced network activity is fundamental for the expression of locomotor patterns necessary for rhythmic activation of limb extensor and flexor muscles. After spinal cord lesion, paralysis ensues often followed by spasticity. These conditions imply that, below the damaged site, the state of balanced networks has been disrupted and that restoration might be attempted by modulating the excitability of sublesional spinal neurons. Because of the widespread expression of inhibitory GABAergic neurons in the spinal cord, their role in the early and late phases of spinal cord injury deserves full attention. Thus, an early surge in extracellular GABA might be involved in the onset of spinal shock while a relative deficit of GABAergic mechanisms may be a contributor to spasticity. We discuss the role of GABA A receptors at synaptic and extrasynaptic level to modulate network excitability and to offer a pharmacological target for symptom control. In particular, it is proposed that activation of GABA A receptors with synthetic GABA agonists may downregulate motoneuron hyperexcitability (due to enhanced persistent ionic currents) and, therefore, diminish spasticity. This approach might constitute a complementary strategy to regulate network excitability after injury so that reconstruction of damaged spinal networks with new materials or cell transplants might proceed more successfully.
Mol Neurobiol, 2021; 58
35173921: Shafiezadeh S, Eshghi M, Dokhaei Z, Mohajeri H, MohammadShirazi A, Mirsadeghi S, Hasani Abharian P
Effect of Transcranial Direct Current Stimulation on Dorsolateral Prefrontal Cortex to Reduce the Symptoms of the Obsessive-Compulsive Disorder.
Obsessive-Compulsive Disorder (OCD) is one of the most common debilitating mental disorders with a prevalence rate of 2% to 3% in the general population. Previous studies have indicated abnormalities in the dorsolateral prefrontal cortex (DLPFC) of OCD patients; thus, we decided to use transcranial Direct Current Stimulation (tDCS) to decline these patients' symptoms.
Basic Clin Neurosci, 2021 Sep-Oct; 12

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BOARD NUMBER: S04-457

PPN-STIMULATION INDUCED FREEZING-RESPONSE AND ITS IMPACT ON THE ACTIVITY OF SPINAL MOTOR CIRCUITS IN FREELY MOVING RATS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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It is well known that stimulation (either electrical or optical) of the midbrain locomotor region (MLR) induces locomotor networks resulting in walking and running gaits (Shik et al 1966). However, it was recently discovered (Carvalho et al 2020) that activating cells in a region within MLR in rats, the pedunculopontine nucleus (PPN), surprisingly induced the opposite effect, which entails a complete arrest of all overt movement including whisking of the vibrissae. To further investigate this paradoxical effect, the PPN region was targeted using the AAV virus with a CamKIIa-dependent expression of an opsin (ChrimsonR) and with an implanted optical fiber (200 µm diameter). After viral expression, approximately 4 weeks later, and confirmation of response, multi-electrode arrays (128-channels, Neuronexus) were implanted in the lumbar spinal cord of adult rats in order to investigate the impact of the PPN stimulation and the freezing response. The lumbar units were recorded before, during and after PPN stimulation. The majority of the spinal units had a reduced neuronal spiking activity during the freezing response. Measurements of motion were performed using accelerometers and video recording. The neuronal subtypes in PPN were investigated using immunohistochemistry, tissue clearing and in-situ hybridization.

Pubmed:

35130533: Sui K, Meneghetti M, Kaur J, Sørensen RJF, Berg RW, Markos C
Adaptive polymer fiber neural device for drug delivery and enlarged illumination angle for neuromodulation.
. Optical fiber devices constitute significant tools for the modulation and interrogation of neuronal circuitry in the mid and deep brain regions. The illuminated brain area during neuromodulation has a direct impact on the spatio-temporal properties of the brain activity and depends solely on the material and geometrical characteristics of the optical fibers. In the present work, we developed two different flexible polymer optical fibers (POFs) with integrated microfluidic channels (MFCs) and an ultra-high numerical aperture (UHNA) for enlarging the illumination angle to achieve efficient neuromodulation.. Three distinct thermoplastic polymers: polysulfone, polycarbonate, and fluorinated ethylene propylene were used to fabricate two step-index UHNA POF neural devices using a scalable thermal drawing process. The POFs were characterized in terms of their illumination map as well as their fluid delivery capability in phantom and adult rat brain slicesA 100-fold reduced bending stiffness of the proposed fiber devices compared to their commercially available counterparts has been found. The integrated MFCs can controllably deliver dye (trypan blue) on-demand over a wide range of injection rates spanning from 10 nl minto 1000 nl min. Compared with commercial silica fibers, the proposed UHNA POFs exhibited an increased illumination area by 17% and 21% under 470 and 650 nm wavelength, respectively. In addition, a fluorescent light recording experiment has been conducted to demonstrate the ability of our UHNA POFs to be used as optical waveguides in fiber photometry.. Our results overcome the current technological limitations of fiber implants that have limited illumination area and we suggest that soft neural fiber devices can be developed using different custom designs for illumination, collection, and photometry applications. We anticipate our work to pave the way towards the development of next-generation functional optical fibers for neuroscience.
J Neural Eng, 2022; 19
26701292: Sámano C, Kaur J, Nistri A
A study of methylprednisolone neuroprotection against acute injury to the rat spinal cord in vitro.
Methylprednisolone sodium succinate (MPSS) has been proposed as a first-line treatment for acute spinal cord injury (SCI). Its clinical use remains, however, controversial because of the modest benefits and numerous side-effects. We investigated if MPSS could protect spinal neurons and glia using an in vitro model of the rat spinal cord that enables recording reflexes, fictive locomotion and morphological analysis of damage. With this model, a differential lesion affecting mainly either neurons or glia can be produced via kainate-evoked excitotoxicity or application of a pathological medium (lacking O2 and glucose), respectively. MPSS (6-10 µM) applied for 24 h after 1-h pathological medium protected astrocytes and oligodendrocytes especially in the ventrolateral white matter. This effect was accompanied by the return of slow, alternating oscillations (elicited

by NMDA and 5-hydroxytryptamine (5-HT)) reminiscent of a sluggish fictive locomotor pattern. MPSS was, however, unable to reverse even a moderate neuronal loss and the concomitant suppression of fictive locomotion evoked by kainate (0.1 mM; 1 h). These results suggest that MPSS could, at least in part, contrast damage to spinal glia induced by a dysmetabolic state (associated to oxygen and glucose deprivation) and facilitate reactivation of spinal networks. Conversely, when even a minority of neurons was damaged by excitotoxicity, MPSS did not protect them nor did it restore network function in the current experimental model.
Neuroscience, 2016; 315
27468970: Kaur J, Flores Gutiérrez J, Nistri A
Neuroprotective effect of propofol against excitotoxic injury to locomotor networks of the rat spinal cord in vitro.
Although neuroprotection to contain the initial damage of spinal cord injury (SCI) is difficult, multicentre studies show that early neurosurgery under general anaesthesia confers positive benefits. An interesting hypothesis is that the general anaesthetic itself might largely contribute to neuroprotection, although in vivo clinical settings hamper studying this possibility directly. To further test neuroprotective effects of a widely used general anaesthetic, we studied if propofol could change the outcome of a rat isolated spinal cord SCI model involving excitotoxicity evoked by 1 h application of kainate with delayed consequences on neurons and locomotor network activity. Propofol (5 μm; 4-8 h) enhanced responses to GABA and depressed those to NMDA together with decrease in polysynaptic reflexes that partly recovered after 1 day washout. Fictive locomotion induced by dorsal root stimuli or NMDA and serotonin was weaker the day after propofol application. Kainate elicited a significant loss of spinal neurons, especially motoneurons, whose number was halved. When propofol was applied for 4-8 h after kainate washout, strong neuroprotection was observed in all spinal areas, including attenuation of motoneuron loss. Although propofol had minimal impact on recovery of electrophysiological characteristics 24 h later, it did not further depress network activity. A significant improvement in disinhibited burst periodicity suggested potential to ameliorate neuronal excitability in analogy to histological data. Functional recovery of locomotor networks perhaps required longer time due to the combined action of excitotoxicity and anaesthetic depression at 24 h. These results suggest propofol could confer good neuroprotection to spinal circuits during experimental SCI.
Eur J Neurosci, 2016; 44
30362615: Petrović A, Kaur J, Tomljanović I, Nistri A, Mladinic M
Pharmacological induction of Heat Shock Protein 70 by celastrol protects motoneurons from excitotoxicity in rat spinal cord in vitro.
The secondary phase of spinal cord injury arising after the primary lesion largely extends the damage severity with delayed negative consequences for sensory-motor pathways. It is, therefore, important to find out if enhancing intrinsic mechanisms of neuroprotection can spare motoneurons that are very vulnerable cells. This issue was investigated with an in vitro model of rat spinal cord excitotoxicity monitored for up to 24 hr after the primary injury evoked by kainate. This study sought to pharmacologically boost the expression of heat shock proteins (HSP) to protect spinal motoneurons using celastrol to investigate if the rat spinal cord can upregulate HSP as neuroprotective mechanism. Despite its narrow range of drug safety in vitro, celastrol was not toxic to the rat spinal cord at 0.75 μM concentration and enhanced the expression of HSP70 by motoneurons. When celastrol was applied either before or after kainate, the number of dead motoneurons was significantly decreased and the nuclear localization of the cell death biomarker AIF strongly inhibited. Nevertheless, electrophysiological recording showed that protection of lumbar motor networks by celastrol was rather limited as reflex activity was impaired and fictive locomotion largely depressed, suggesting that functional deficit persisted, though the networks could express slow rhythmic oscillations. While our data do not exclude further recovery at later times beyond the experimental observations, the present results indicate that the upregulated expression of HSP in the aftermath of acute injury may be an interesting avenue for early protection of spinal motoneurons.
Eur J Neurosci, 2019; 49
29770521: Kaur J, Rauti R, Nistri A
Nicotine-mediated neuroprotection of rat spinal networks against excitotoxicity.
Activation of neuronal nicotinic acetylcholine receptors (nAChRs) by nicotine is reported to protect brain neurons from glutamate excitotoxicity. We inquired whether a similar phenomenon can occur in the rat isolated spinal cord (or spinal slice culture) challenged by a transient (1 hr) application of kainate (a powerful glutamate receptor agonist) to induce excitotoxicity mimicking spinal injury in vitro. We recorded spinal reflexes and fictive locomotion generated by the locomotor central pattern generator before and 24 hr after applying kainate. We also monitored network activity with Ca imaging and counted neurons and glia with immunohistochemical methods. In control conditions, nicotine (1 μM; 4 hr) depressed reflexes and fictive locomotion with slow recovery and no apparent neurotoxicity at 24 hr although synchronous Ca transients appeared in slice cultures. Kainate nearly halved neuron numbers (while sparing glia), decreased reflexes and Ca transients, and suppressed fictive locomotion. When nicotine was applied (4 hr) after washout of kainate, fictive locomotor cycles appeared 24 hr later though with low periodicity, and significant protection of neurons, including motoneurons, was observed. Nicotine applied

together with kainate and maintained for further 4 hr yielded better neuroprotection, improved fictive locomotion expression and reversed the depression of Ca transients. nAChR antagonists did not intensify kainate neurotoxicity and inhibited the neuroprotective effects of nicotine. These data suggest that nicotine was efficacious to limit histological and functional excitotoxic damage probably because it activated and then desensitized nAChRs on excitatory and inhibitory network neurons to prevent triggering intracellular cell death pathways.
Eur J Neurosci, 2018; 47
34502498: Kaur J, Mazzone GL, Aquino JB, Nistri A
Nicotine Neurotoxicity Involves Low Wnt1 Signaling in Spinal Locomotor Networks of the Postnatal Rodent Spinal Cord.
The postnatal rodent spinal cord in-vitro is a useful model to investigate early pathophysiological changes after injury. While low dose nicotine (1 μM) induces neuroprotection, how higher doses affect spinal networks is unknown. Using spinal preparations of postnatal wild-type Wistar rat and Wnt1Cre2:Rosa26Tom double-transgenic mouse, we studied the effect of nicotine (0.5-10 μM) on locomotor networks in-vitro. Nicotine 10 μM induced motoneuron depolarization, suppressed monosynaptic reflexes, and decreased fictive locomotion in rat spinal cord. Delayed fall in neuronal numbers (including motoneurons) of central and ventral regions emerged without loss of dorsal neurons. Conversely, nicotine (0.5-1 μM) preserved neurons throughout the spinal cord and strongly activated the Wnt1 signaling pathway. High-dose nicotine enhanced expression of S100 and GFAP in astrocytes indicating a stress response. Excitotoxicity induced by kainate was contrasted by nicotine (10 μM) in the dorsal area and persisted in central and ventral regions with no change in basal Wnt signaling. When combining nicotine with kainate, the activation of Wnt1 was reduced compared to kainate/sham. The present results suggest that high dose nicotine was neurotoxic to central and ventral spinal neurons as the neuroprotective role of Wnt signaling became attenuated. This also corroborates the risk of cigarette smoking for the foetus/newborn since tobacco contains nicotine.
Int J Mol Sci, 2021; 22
33466339: El Waly B, Escarrat V, Perez-Sanchez J, Kaur J, Pelletier F, Collazos-Castro JE, Debarbieux F
Intravital Assessment of Cells Responses to Conducting Polymer-Coated Carbon Microfibres for Bridging Spinal Cord Injury.
The extension of the lesion following spinal cord injury (SCI) poses a major challenge for regenerating axons, which must grow across several centimetres of damaged tissue in the absence of ordered guidance cues. Biofunctionalized electroconducting microfibres (MFs) that provide biochemical signals, as well as electrical and mechanical cues, offer a promising therapeutic approach to help axons overcome this blind journey. We used poly(3,4-ethylenedioxythiophene)-coated carbon MFs functionalized with cell adhesion molecules and growth factors to bridge the spinal cord after a partial unilateral dorsal quadrant lesion (PUDQL) in mice and followed cellular responses by intravital two-photon (2P) imaging through a spinal glass window. Thy1-CFP//LysM-EGFP//CD11c-EYFP triple transgenic reporter animals allowed real time simultaneous monitoring of axons, myeloid cells and microglial cells in the vicinity of the implanted MFs. MF biocompatibility was confirmed by the absence of inflammatory storm after implantation. We found that the sprouting of sensory axons was significantly accelerated by the implantation of functionalized MFs after PUDQL. Their implantation produced better axon alignment compared to random and misrouted axon regeneration that occurred in the absence of MF, with a most striking effect occurring two months after injury. Importantly, we observed differences in the intensity and composition of the innate immune response in comparison to PUDQL-only animals. A significant decrease of immune cell density was found in MF-implanted mice one month after lesion along with a higher ratio of monocyte-derived dendritic cells whose differentiation was accelerated. Therefore, functionalized carbon MFs promote the beneficial immune responses required for neural tissue repair, providing an encouraging strategy for SCI management.
Cells, 2021; 10
33789065: Kaur J, Mojumdar A
A mechanistic overview of spinal cord injury, oxidative DNA damage repair and neuroprotective therapies.
Despite substantial development in medical treatment strategies scientists are struggling to find a cure against spinal cord injury (SCI) which causes long term disability and paralysis. The prime rationale behind it is the enlargement of primary lesion due to an initial trauma to the spinal cord which spreads to the neighbouring spinal tissues It begins from the time of traumatic event happened and extends to hours and even days. It further causes series of biological and functional alterations such as inflammation, excitotoxicity and ischemia, and promotes secondary lesion to the cord which worsens the life of individuals affected by SCI. Oxidative DNA damage is a stern consequence of oxidative stress linked with secondary injury causes oxidative base alterations and strand breaks, which provokes cell death in neurons. It is implausible to stop primary damage however it is credible to halt the secondary lesion and improve the quality of the patient's life to some extent. Therefore it is crucial to understand the hidden perspectives of cell and molecular biology affecting the pathophysiology of SCI. Thus the focus of the review is to connect the missing links and shed light on the oxidative DNA damages and the functional repair mechanisms, as a consequence of the injury in neurons. The review will also probe the significance of neuroprotective strategies in the present scenario. HIGHLIGHTS• Spinal cord injury, a pernicious condition, causes excitotoxicity and

ischemia, ultimately leading to cell death.• Oxidative DNA damage is a consequence of oxidative stress linked with secondary injury, provoking cell death in neurons.• Base excision repair (BER) is one of the major repair pathways that plays a crucial role in repairing oxidative DNA damages.• Neuroprotective therapies curbing SCI and boosting BER include the usage of pharmacological drugs and other approaches.
Int J Neurosci, 2021;
33521177: Kaur J, Conti E
Dataset on inflammation induced after lumbar puncture.
Neuroinflammation is evident and one of the primary induced responses after central nervous system (CNS) injury, lumbar puncture and CNS surgery. In rare cases, complications could arise after the lumbar puncture or CNS surgery leading to inflammation, bleeding or other problems such as cerebrospinal fluid (CSF) leakage. The present dataset describes the occurrence of such a condition after the dura breakage or postoperative complication leading to the development of neuroinflammation in the adult Wistar rats. Therefore, objective of the study is to report such a rare condition and detect the most reliable glial proteins upregulated 2-3 weeks after the lumbar puncture which may help the neuroscience community to a better understanding their cause of action. In response to neuroinflammation, glial cells leak into the extracellular space, where they can be identified in the CSF or serum and may act as diagnostic biomarkers. Laminectomy was performed at the thoraco-lumbar (T12-L1) region and the dura was punctured. After that, the exposed part was covered with silica gel and adhesive followed by dental cement. The skin was closed using sterile sutures. After, the rats were given buprenorphine (0.05 mg/kg, every 8 h for 3 days) and carprofen (5 mg/kg, once a day for 5 days) as analgesic and anti-inflammatory and baytril (5 mg/kg, once a day for 10 days) as antibiotic drugs. Note, the buprenorphine and lidocaine were also given before starting the procedure. After the laminectomy, the functional outcomes of the rats were tested (starting from the day of surgery to the last day) and the rats which were locomoting normally using all the limbs were included in the study. In case of any decompression, the functional outcomes showing any kind of laming or partial paralysis of hindlimbs were excluded from the study. Unexpected neuroinflammation has occurred 2-3 weeks after performing the given procedure. Data presented in the article implicate active microglia measured by using protein biomarker such as Iba-1 [1, 3] and astrocytes measured by using Glial fibrillary acidic protein (GFAP) [2, 3] and S100 (strongly targets S100B and weakly A1) [7, 8] in the CSF collected two-three weeks after the laminectomy and dura breakage from the adult rats. Later paraformaldehyde (PFA) fixed spinal cord slices were also collected from the animals and immunolabelled with the same biomarkers. Western blots were performed with the collected CSF which showed high expression of glial markers such as GFAP, Iba-1 and S100 which has shown to target S100B with no expression of A1 (or A2, play an important role in neuroprotection) astrocytes which have recently been shown to be produced by microglia at the site of injury [9]. However, S100B [10] and GFAP [2] are known to be adequately discharged by the distinct cells in stress conditions which seems to occur in the present report. In addition, the spinal slices immunolabelled with the same biomarkers also showed increased expression of glia. Cross-talk between microglia-astrocyte in CNS stress is crucial for the neurons to survive and function after the injury. Reactive microglia (shown by high Iba-1 expression) leading to microgliosis comprise the first line of defense to phagocytose the dead cells [11]. Astrocytes act as the second line of defense leading to a process known as astrogliosis and upregulate GFAP and S100B to limit the damage [12]. Further studies are needed to explain the molecular mechanism/s of different astrocytes release such as A2 and their interaction with microglia after the lumbar puncture.
Data Brief, 2021; 34

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BOARD NUMBER: S04-458

ACTIVATION OF DESCENDING BRAINSTEM COMMANDS REVEALS THE DYNAMICS OF MODULAR ORGANIZATION OF THE LOCOMOTOR NETWORKS IN THE SPINAL CORD

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Locomotion empowers animals to move for survival. Locomotor-initiating signals from the brain are funneled through reticulospinal neurons in the brainstem that act on spinal circuits that transform the signal into rhythmic locomotor output. Knowledge about which spinal circuits are targeted by the descending signal and how this signal is transformed into rhythmicity in the cord is scarce. Here, we first show that a brainstem area – encompassing the caudal ventrolateral reticular nucleus and the lateral paragigantocellular nucleus – contains excitatory neurons that directly act to initiate locomotion. Next, we dissected how this glutamatergic descending command interfaces with spinal locomotor circuitries, and deciphered the functional modules of neurons recruited by descending command signals. By calcium imaging, we captured the locomotor initiating command in the spinal cord and reveal an immediate spinal glutamatergic target (iDT) in the intermediate area of the cord. The iDT converts the descending commands into rhythmicity by starting each locomotor cycle, and the signal from there travels to excitatory premotor circuits on the same side of the cord before entering mirroring circuits on the other side of the cord. Inhibitory networks are recruited in strict left-right alternation. Our study visualizes the spatiotemporal dynamics of the locomotor network operation from the brainstem to the spinal cord in real time and reveals a distinct layered composition of spinal locomotor circuits.

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BOARD NUMBER: S04-459

A SENSORY SYSTEM DYNAMICALLY-COUPLED TO THE REISSNER FIBER UNDER TENSION IN THE SPINAL CORD INFORMS STRAIGHTNESS OF THE BODY AXIS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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A decade of work has revealed a sensory system contacting the cerebrospinal fluid (CSF) in the vertebrate spinal cord that detects body curvature and, in turn, modulates posture and locomotor kinematics. This sensory system also informs morphogenesis to straighten the body axis. To detect negative curvature of the spinal cord, CSF-contacting neurons (CSF-cNs) project a ciliated apical extension in close vicinity of the Reissner fiber, a thread-like aggregation of *scospondin* glycoprotein in the CSF that prompts the straightening of the posterior axis. Although recent evidence indicates that the fiber is required for CSF-cN mechanosensory function, the underlying mechanisms and the manner by which it controls morphogenesis are not fully understood. To address these questions, we investigated the dynamic interaction between the Reissner fiber and CSF-cNs in larval zebrafish. We observed that the Reissner fiber is under tension and changes position along the dorsoventral axis. The dynamic mobility of the fiber occurs while nearby CSF-cNs exhibit spontaneous calcium transients, suggesting that physical interaction with the fiber may activate CSF-cNs. To investigate whether the oscillating fiber elicits calcium activity in CSF-cNs, we implemented focal 2-photon-mediated ablation of the fiber and investigated its impact on CSF-cN activity. We further examined the subsequent effect of the Reissner fiber loss on locomotion, posture and morphogenesis. Altogether, our *in vivo* study in larval zebrafish reveals a dynamic interaction between ciliated sensory neurons and the Reissner fiber under tension in the CSF in order to straighten the body axis throughout life.

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BOARD NUMBER: S04-460

LHX9-DERIVED EXCITATORY SPINAL INTERNEURONS CONTROL THE FREQUENCY OF LOCOMOTOR RHYTHM.

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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The ability to move and interact with the surroundings is achieved through a complex motor behaviour called locomotion. Locomotion is controlled by spinal networks that generate rhythm and coordinate left-right and flexor-extensor patterning. Rhythm-generation neurons controlling the frequency of the rhythm are thought to be ipsilaterally projecting and excitatory. Two molecularly-identified glutamatergic neuronal populations, Shox2 non-V2a and Hb9-derived INs, were found to be part of the mouse rhythm-generating networks. However, these molecular classification groups of spinal neurons do not fully account for the entire rhythm-generating population. We have identified new molecular markers using RNA-sequencing of spinal glutamatergic neurons (age P3) that may identify new populations involved in the rhythm-generating circuitries. One of the new populations expresses the transcription factor Lhx9. To uncover the role of this population in the locomotor network, we used tamoxifen-inducible *Lhx9::Cre* mice to genetically manipulate Lhx9::Cre-derived excitatory neurons. We find that this line, throughout the spinal cord, depicts an ipsilaterally-projecting excitatory population in the ventral spinal cord that is non-overlapping with Shox2 neurons. Blocking the synaptic output of Lhx9$^+$ neurons decrease the frequency of locomotor-like activity induced by drug application without affecting the pattern. Optogenetic activation of Lhx9$^+$ neurons at lumbar cord initiates locomotor bursts. It also increases the burst frequency of drug-induced locomotor activities. Together, these data suggest that Lhx9$^+$ neurons are a potential new population involved in the rhythm-generating circuitries of spinal locomotor network.

Pubmed:
34070345: Masini D, Plewnia C, Bertho M, Scalbert N, Caggiano V, Fisone G
A Guide to the Generation of a 6-Hydroxydopamine Mouse Model of Parkinson's Disease for the Study of Non-Motor Symptoms.
In Parkinson's disease (PD), a large number of symptoms affecting the peripheral and central nervous system precede, develop in parallel to, the cardinal motor symptoms of the disease. The study of these conditions, which are often refractory to and may even be exacerbated by standard dopamine replacement therapies, relies on the availability of appropriate animal models. Previous work in rodents showed that injection of the neurotoxin 6-hydroxydopamine (6-OHDA) in discrete brain regions reproduces several non-motor comorbidities commonly associated with PD, including cognitive deficits, depression, anxiety, as well as disruption of olfactory discrimination and circadian rhythm. However, the use of 6-OHDA is frequently associated with significant post-surgical mortality. Here, we describe the generation of a mouse model of PD based on bilateral injection of 6-OHDA in the dorsal striatum. We show that the survival rates of males and females subjected to this lesion differ significantly, with a much higher mortality among males, and provide a protocol of enhanced pre- and post-operative care, which nearly eliminates animal loss. We also briefly discuss the utility of this model for the study of non-motor comorbidities of PD.
Biomedicines, 2021; 9
29686643: Masini D, Bonito-Oliva A, Bertho M, Fisone G
Inhibition of mTORC1 Signaling Reverts Cognitive and Affective Deficits in a Mouse Model of Parkinson's Disease.
Non-motor symptoms, including cognitive deficits and affective disorders, are frequently diagnosed in Parkinson's disease (PD) patients and are only partially alleviated by dopamine replacement therapy. Here, we used a 6-hydroxydopamine (6-OHDA) mouse model of PD to examine the effects exerted on non-motor symptoms by inhibition of the mammalian target of rapamycin complex 1 (mTORC1), which is involved in the control of protein synthesis, cell growth, and metabolism. We show that rapamycin, which acts as an allosteric inhibitor of mTORC1, counteracts the impairment of novel object recognition. A similar effect is produced by PF-4708671, an inhibitor of the downstream target of mTORC1, ribosomal protein S6 kinase (S6K). Rapamycin is also able to reduce depression-like behavior in PD mice, as indicated by decreased immobility in the forced swim test. Moreover, rapamycin exerts anxiolytic effects, thereby reducing thigmotaxis in the open field and increasing

exploration of the open arm in the elevated plus maze. In contrast to rapamycin, administration of PF-4708671 to PD mice does not counteract depression- and anxiety-like behaviors. Altogether, these results identify mTORC1 as a target for the development of drugs that, in combination with standard antiparkinsonian agents, may widen the efficacy of current therapies for the cognitive and affective symptoms of PD.
Front Neurol, 2018; 9

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BOARD NUMBER: S04-461

CHARACTERIZATION OF LONG PROJECTING PROPRIOSPINAL NEURONS IN THE MOUSE SPINAL CORD

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Locomotion is an innate animal behavior that relies on the precise control of limb activity and postural stability. In order to timely coordinate the patterns of trunk and limb muscles contraction, spinal circuits need to share motor programs and sensory information at different segmental levels. Long projecting propriospinal neurons (L-PNs) have been proposed to coordinate the activity of spinal circuits at the intersegmental level. L-PNs reciprocally connect the lumbar, cervical and thoracic spinal levels and recent studies started addressing their roles in motor control. However, a comprehensive molecular, anatomical and functional characterization to precisely define L-PNs diversity and function is still missing. Here, we started to systematically analyze L-PNs at anatomical and molecular levels, in order to pave the way for unraveling their physiological roles. Taking advantage of retrograde viral tracing, we identified six different populations of propriospinal neurons based on their anatomical position and intersegmental connectivity patterns. Moreover, transcriptome analysis revealed the molecular diversity of L-PNs, highlighting the existence of discrete subsets belonging to cardinal spinal progenitor identities. Finally, by taking advantage of viral and intersectional approaches, we started characterizing connectivity and function of a small subset of long ascending propriospinal neurons residing in the dorsal spinal cord.

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RHYTHMIC MOVEMENT BY SPINAL MOTOR NETWORKS IS GOVERNED BY ROTATIONAL POPULATION DYNAMICS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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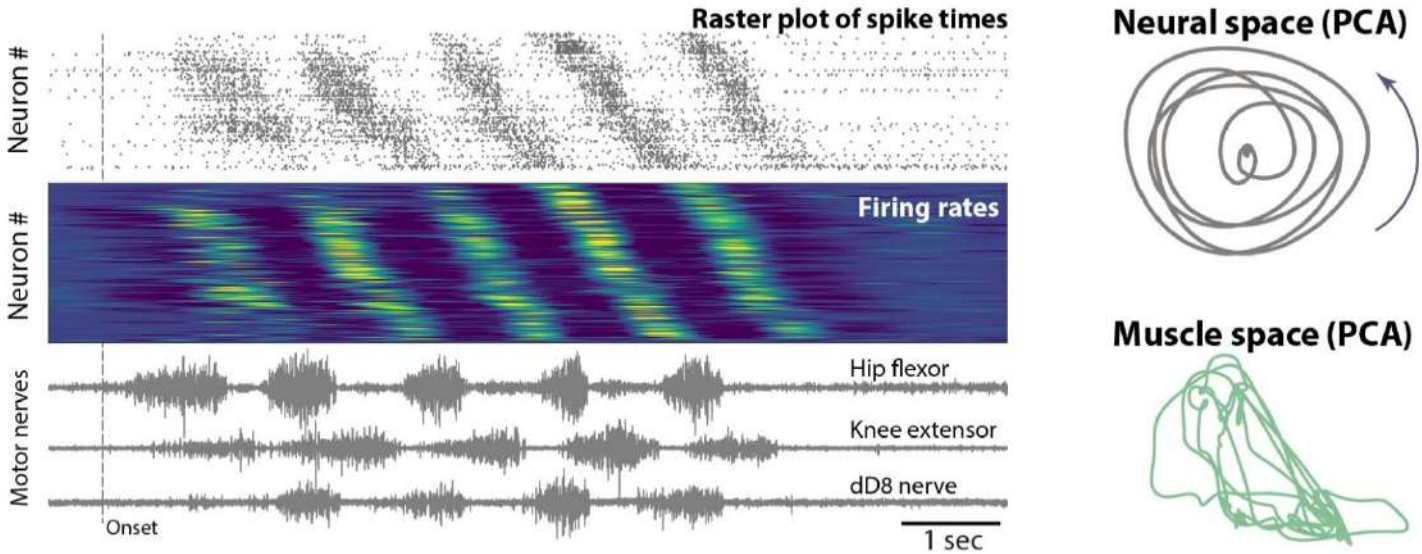
Most of the investigations on spinal rhythm generation are based on motor nerve recordings and single neuron recordings. Since flexor/extensor muscles alternate during movements, it has often been assumed that the generation is accomplished by neuronal modules that alternate in opposition, which single neuron recordings seem to support. However, here we argue that when many neurons are monitored simultaneously a different picture emerges. We recorded hundreds of neurons from the lumbar spinal cord of turtles during rhythmic scratching and found that, rather than alternating, the neuronal population is performing a "rotation", i.e. cycling continuously through all phases (Figure). Rotational dynamics are observed across trials as well as behaviors. Since such rotation is difficult to explain with existing models of alternating neuronal groups, we propose a new theory that accounts for the rotational dynamics. Using a simplified network model, we show that in spinal networks with recurrent excitatory and inhibitory connectivity, there is no need for pacemaker activity or modular structures. Tonic input to the network controls the rhythm and pattern depending on the task. The model reproduces other experimental observations and provides a mechanism for multifunctionality. It also predicts that subsets of neurons, with both inhibitory and excitatory members, have the capacity to either speed up or slow down the movement.



Caption: Raster of phase-sorted neuronal population activity. Middle: Firing rates from top. Bottom: Nerve activity of 3 motor nerves. Right top: neural space exhibit rotational activity (principal components). Bottom: muscle activity has less rotational activity.

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MODULATION OF INTERLIMB CUTANEOUS REFLEXES DURING SPLIT-BELT LOCOMOTION IN THE INTACT CAT

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Abstract: During locomotion, cutaneous reflexes assist in rapidly responding to perturbations, such as when the foot contacts an obstacle to prevent a fall. In quadrupeds and humans, cutaneous reflexes are distributed to all four limbs, participating in a whole-body response, and are modulated by phase to generate functionally appropriate responses. To date, most studies have evaluated cutaneous reflexes during treadmill locomotion at a single speed. Here, we investigated cutaneous reflexes during quadrupedal locomotion in eight intact cats by electrically stimulating the superficial radial and peroneal nerves in all four limbs and by recording fore- and hindlimb muscle activity during split-belt locomotion, which simulates some aspect of walking along a circular path. We collected data during tied-belt (equal left-right speeds at 0.4 and 0.8 m/s) and split-belt (different left-right speeds of 0.4-0.8 and 0.8-0.4 m/s) locomotion. Results showed phase-dependent modulation of long interlimb (homolateral and diagonal) responses during tied- and split-belt locomotion. Descending responses from the forelimbs to hindlimbs were more prominent than ascending responses, suggesting that forelimb cutaneous inputs are stronger or more distributed than those from the hindlimbs. We are currently analyzing whether differences in reflex amplitudes occur between locomotor conditions, which would indicate that signals related to left–right symmetry regulate interlimb cutaneous reflexes between the fore- and hindlimbs. Our results provide a better understanding of the spinal pathways involved in interlimb coordination during locomotion, serving as a basis to assess changes following incomplete spinal cord injury. **Keywords:** cutaneous reflexes, interlimb coordination, locomotion, split-belt

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BOARD NUMBER: S04-464

ELECTROPHYSIOLOGICAL SIGNATURES REVEAL SPINAL INTRINSIC LEARNING MECHANISMS FOR A LASTING SENSORIMOTOR ADAPTATION.

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Neurocircuits within the spinal cord are essential for movement automaticity. However, spinal mechanisms that underlie lasting sensorimotor adaptations remain unclear. Here, we establish a quantitative kinematic framework to characterize a conditioning behavior in which spinal circuits functionally isolated from the brain learn to adapt motor output upon multimodal sensory integration, undergo extinction, and retain learned behavior with repetitive training. *In-vivo* high-density spinal cord recordings from awake behaving mice reveal phase-tuned single unit activities. Using developmental origin as a criterion, optically identified unit recordings uncover that a class of spinal inhibitory interneurons forming recurrent circuits with somatosensory afferents is recruited during learning. We demonstrate that a selective loss-of-function experiment of this dorsally-located population leads to impaired learning. In contrast, population-specific perturbation of any distinct ventral neurons does not disrupt learning. Together, our study reveals neuronal underpinnings that shape lasting motor adaptation, where reliable somatosensory information regulates bottom-up spinal learning.

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COMPUTATIONAL MODELING OF SPINAL LOCOMOTOR CIRCUITS AND NEURAL CONTROL OF LOCOMOTION

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Neural circuits in the spinal cord comprising the locomotor central pattern generator (CPG) generate the locomotor rhythm, control flexor–extensor and left–right coordination, and mediate the descending control of locomotion. Yet, the organization of these neural circuits remains poorly understood. Significant progress has been achieved due to the molecular/genetic identification of several types of spinal interneurons involved in the neural control of locomotion. The specific functions of these interneurons have been suggested based on changes in the locomotor pattern observed in mutant mice lacking particular neuron types or following selective silencing, suppression, or activation of these interneurons. We have developed a computational model of the spinal circuitry based on a suggested connectome of genetically identified interneurons. The model includes the rhythm-generating (RG) circuits, controlling alternating flexor and extensor activity, and several commissural and propriospinal pathways mediating interactions between the RG circuits and providing interlimb coordination and gait control during locomotion. The model reproduces and proposes explanations for multiple experimental data obtained in different studies performed in vivo and in vitro. This includes changes in the locomotor frequency, locomotor pattern, left–right and flexor–extensor coordination, and frequency-dependent expression of locomotor gait in the intact condition as well as following selective ablation, silencing, or activation of particular types of genetically identified interneurons. The model generates a series of testable predictions and provides important insight into the organization and operation of the locomotor CPG, spinal locomotor circuits, and the supra-spinal control of locomotion.

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INTERACTIONS BETWEEN SPINAL CIRCUITS AND AFFERENT FEEDBACK TO CONTROL LOCOMOTION AT DIFFERENT SPEEDS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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To effectively move in complex and changing environments, animals must control locomotor speed and gait, while precisely coordinating and adapting limb movements to the terrain. The underlying neural control involves dynamic interactions between neuronal circuits at different levels of the nervous system, biomechanical properties of the musculoskeletal system, and afferent feedback signals from the periphery. Here, we present a computational neuromechanical model of mouse hindlimb locomotion to study mechanisms of sensorimotor integration and the role of afferent pathways in the stabilization of locomotion at different speeds and under different environmental conditions. The model consists of a neuronal network model of spinal locomotor circuits, coupled with a musculoskeletal model of the mouse hindlimbs. The spinal network includes rhythm-generating and interlimb-coordinating circuits, pattern formation networks forming motor synergies, motoneurons controlling muscle activation and somatosensory afferent feedback connections to all parts of the circuitry. Optimization was used to find connections weights that allow for stable locomotion on different terrains. The proposed connectome of spinal circuits and the organization of afferent feedback allowed the model to closely reproduce characteristics of mouse locomotion at different speeds and gaits; with increasing speed, the model sequentially exhibited walking, running, and hopping gaits. By systematically manipulating feedback gains, we found that feedback pathways serve different functions depending on speed. We suggest that supraspinal control of locomotor speed, besides tonic drive to the rhythm generators and commissural interneurons, includes task-dependent (slow, exploratory, vs. fast, escape-type locomotion) modulation of the gain of sensory afferent pathways to the spinal locomotor circuitry.

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EFFECTS OF STROBOSCOPIC VISUAL TRAINING ON TUG AND 6MWT PERFORMANCES IN SUBJECTS WITH INCOMPLETE SPINAL CORD INJURY EVALUATED USING DEEPLABCUT MARKERLESS POSE ESTIMATION SYSTEM

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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The purpose of this study was to assess the effect of stroboscopic glasses, integrated to a specific motor training, in influencing balance and gait performance in individuals with chronic incomplete spinal cord injury (iSCI). Ten patients with iSCI (AIS D) were enrolled for this study and performed a 6-weeks home-based balance training program, 3 times per week. Individuals were randomly assigned to an experimental group (EG) and placebo control group (PCG; n = 5). EG (n = 5) executed balance training program with active stroboscopic glasses (SVT), while PCG (n = 5) completed the same program of exercise with non-active glasses. At baseline and at the end of the training, gait and balance performances were evaluated using the Timed Up and Go Test (TUG) and the 6 Minutes Walking Test (6MWT). Additionally, 6 normal subjects, as healthy control group, underwent the same assessment once and their data were used as a reference of physiological balance and gait. All performances were recorded with a frontal and a lateral video camera. Videos were analyzed with markerless pose estimation of DeepLabCut (DLC) and a 3D reconstruction of motor performance allowed to investigate spatiotemporal and kinematic features during TUG and 6MWT. Preliminary results suggest that specific balance protocol with SVT modulate balance and gait in chronic iSCI subjects.

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A PARADIGM TO BEHAVIOURALLY DECOUPLE THE TOP-DOWN AND FEEDBACK INFLUENCES ON LOCOMOTOR GAIT

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Locomotion is one of the principal behavioural outputs of the mammalian nervous system, but a lot remains unknown about the neural control principles of its defining features, especially gait. The study of gait at a neural level is complicated by its dependence on factors like leg loading and locomotor speed, as well as by the difficulty to reliably evoke a variety of gaits in genetically amenable species like mice. To overcome these limitations, I propose a head-fixed behavioural paradigm that decouples the speed- and leg loading-related effects on gait using a combination of optogenetic stimulation and body tilt modulation. This paradigm reveals a speed-independent shift in gait preference from left-right alternation to bilateral synchrony at more upward oriented body postures, with this change most prominent in homolateral limb coordination. Upward oriented body tilts, in turn, correlate with an increased relative loading of the hindlimbs and display a consistent relationship with gait during freely moving locomotion. These findings suggest distinct, limb-specific control capacities of the descending and feedback influences on gait, and serve as a principled entry point to a circuit-based study of its implementation.

Pubmed:
33290707: Mitrevica Z, Murray AJ
Orienting Movements: Brainstem Neurons at the Wheel.
The nervous system must constantly adjust its motor output in response to changes in the environment. A new study of the control of orienting movements in mice has identified discrete groups of neurons in the brainstem that connect a sensory integrative area with distinct features of motor behaviour.
Curr Biol, 2020; 30

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OBSTACLE NEGOTIATION DURING LOCOMOTION BEFORE AND AFTER INCOMPLETE SPINAL CORD INJURY

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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During walking, animals avoid and negotiate obstacles. Despite its importance, few studies have investigated obstacle negotiation after incomplete spinal cord injury (SCI). **Aim:** We investigated kinematic and electromyographic adjustments when negotiating obstacles before and after incomplete SCI in adult cats. **Methods: After** training ten cats to negotiate obstacles of different heights (1-9 cm) on a walkway, we implanted electrodes to chronically record fore- and hindlimb muscle activity (EMG, electromyography). After obtaining data in the intact state, we performed a right lateral spinal hemisection at mid-thoracic segments (T6-T7). Data collection resumed 1 week after hemisection for 8 weeks. **Results:** In the intact state, cats stepped over the different obstacles without contact. After hemisection, the right hindpaw (ipsilesional) made frequent contacts (> 50% two and eight weeks after hemisection), and a reflex allowed the limb to step over the obstacle (stumbling corrective response). For 75% of successful crossings, the left hindlimb was the leading limb. In the intact state, right hindlimb flexors, such as sartorius, semitendinosus and tibialis anterior, modulated their activity with obstacle height. However, this modulation was absent after hemisection. Additionally, sartorius EMG amplitude did not increase in unsuccessful crossings, with a delay in activity. Right hindpaw position was also further away from the obstacle in unsuccessful versus successful crossings. **Conclusion:** Incomplete SCI impairs the ability to negotiate obstacles without contact due to improper activation of flexor muscles. A better understanding of the impairments and adjustments after SCI will help develop targeted strategies to restore this voluntary aspect of locomotion.

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NEURAL CONTROL AND BIOMECHANICS OF CAT PAW SHAKE RESPONSE

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Intact and spinal cats produce fast paw oscillations to remove an irritating light object stuck to the paw. There are complex inter-joint coordination and atypical muscle synergies during paw shaking, i.e., co-activation between ankle flexor tibialis anterior and knee extensors vasti. To achieve the proximal-to-distal gradient of segmental angular velocities and accelerations during paw shaking, the nervous system needs to precisely regulate activities of multiple muscles and joints. The goal of this study was to investigate a possible neural and biomechanical organization of paw shaking. We first show experimentally in intact cats that during paw shaking, energy generated by proximal muscle moments is transmitted to distal segments by joint forces. This energy transfer is mostly responsible for the segmental velocity/acceleration proximal-to-distal gradient. To investigate neural mechanisms of paw shaking, we developed a neuromechanical model comprised a half-center CPG, activating hip flexors and extensors, and passive viscoelastic distal muscles that produced length/velocity-depended force. This model reproduced major features of paw shaking found in intact cats: the proximal-to-distal velocity/acceleration gradient, energy transfer by joint forces and energy absorption by distal muscle moments. We also found the atypical co-activation of ankle flexors with knee extensors. Computer simulations revealed important role of the segmental inertia distribution along the hindlimb and muscle viscoelastic properties in the organization of paw shaking. The results of this study suggest that the muscle and joint coordination appears to emerge from interactions between the half-center CPG, inertial properties of hindlimb segments, and muscle length and stretch-velocity feedback.

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HIGH-EFFICIENCY TRANSDIFFERENTIATION OF HUMAN DENTAL PULP STEM CELLS INTO FUNCTIONAL MOTOR NEURON VIA OPTOGENETICS STIMULATION

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Introduction: Optogenetics is known as an advanced biological tool in neuroscience which is able to control the activity of genetically modified cells by light. The goal of this study is to determine optogenetics stimulation in transdifferentiation of Human Dental Pulp Stem Cells (hDPSCs) into functional motor neuron. **Methods:** The hDPSCs impacted third molar were cultured in DMEM/F12 and were infected with lentiviruses carrying CaMKIIa-hChR2 (H134R). To optical stimulation, 5×10^4 Opsin-expressing hDPSCs per well was conducted with blue light (470 nm) pulsing at 15 Hz, 90% Duty Cycle and 10 mW power for 10 s every 90 minutes, 6 times a day for 5 days. Cell viability was evaluated using MTT assay. The expression of motor neuron markers was evaluated by immunocytochemistry (ICC) and motor neuron function was confirmed with co-culture system. **Results:** The reporter gene, mCherry was expressed on hDPSCs 72 hours after lentiviral infection. The highest cell viability was observed in optical stimulated opsin-expressing hDPSCs. The morphological modifications like multiple long dendritic-like cytoplasmic extensions were just seen in Opsin-expressing hDPSCs. Our ICC data revealed islet-1 (motor neuron precursors marker), oligo2 (motor neuron progenitor marker), HB9 (mature motor neuron marker), MAP2 (marker for mature neuron) were just expressed on opsin-expressing hDPSCs and elongated axon-like of differentiated cells had full contact with myotubes. **Conclusion:** Differentiation of hDPSCs using optical stimulation can form functional motor neurons in in vitro situation and might be a suitable option in treatment neurodegenerative disease.

Pubmed:
34147538: Absalan F, Pasandi MS, Ghasemi Hamidabadi H, Saeednia S, Bojnordi MN, Zahiri M, Alizadeh R, Bagher Z
Matrigel enhances differentiation of human adipose tissue-derived stem cells into dopaminergic neuron.
Therapy based stem cells have offered a novel therapeutic approach for the improvement of neurodegenerative diseases, specially Parkinson. Hence, developing a well-established culture model with appropriate stem cells is extremely crucial in regenerative engineering to provide efficient targeted cells. Human adult mesenchymal stem cells derived from adipose tissue (hADSCs) have emerged as a promising source of stem cells due to their unique potentials of self-renewal and differentiation into other stem cells. The purpose of this study was to investigate the differentiation capacity of hADSCs into dopaminergic and neuron-like cells in the 3D culture plate (Matrigel).
Neurosci Lett, 2021; 760
33964606: Darvishi M, Hamidabadi HG, Bojnordi MN, Saeednia S, Zahiri M, Niapour A, Alizadeh R
Differentiation of human dental pulp stem cells into functional motor neuron: In vitro and ex vivo study.
There are several therapeutic options for spinal cord injury (SCI), among these strategies stem cell therapy is a potential treatment. The stem cells based therapies have been investigating in acute phase of clinical trials for promoting spinal repair in humans through replacement of functional neuronal and glial cells. The aim of this study was to evaluate the differentiation of Human Dental Pulp Stem Cells (hDPSCs) into functional motor neuron like cells (MNLCs) and promote neuroregeneration by stimulating local neurogenesis in the adult spinal cord slice culture. The immunocytochemistry analysis demonstrated that hDPSCs were positive for mesenchymal stem cell markers (CD73, CD90 and CD105) and negative for the hematopoietic markers (CD34 and CD45). hDPSCs were induced to neurospheres (via implementing B27, EGF, and bFGF) and then neural stem cells (NSC). The NSC differentiated into MNLCs in two steps: first by Shh and RA and ; then with GDNF and BDNF administration. The NS and the NSC were assessed for Oct4, nestin, Nanog, Sox2 expression while the MNLCs were

evaluated by ISLET1, Olig2, and HB9 genes. Our results showed that hDPSC can be differentiated into motor neuron phenotype with expression of the motor neuron genes. The functionality of MNLCs was demonstrated by FM1-43, intracellular calcium ion shift and co- culture with C2C12. We co-cultivated hDPSCs with adult rat spinal slices in vitro. Immunostaining and hoechst assay showed that hDPSCs were able to migrate, proliferate and integrate in both the anterolateral zone and the edges of the spinal slices.
Tissue Cell, 2021; 72
33636233: Hamidabadi HG, Simorgh S, Kamrava SK, Namjoo Z, Bagher Z, Bojnordi MN, Niapour A, Mojaverrostami S, Saeb MR, Zarrintaj P, Olya A, Alizadeh R
Promoting motor functions in a spinal cord injury model of rats using transplantation of differentiated human olfactory stem cells: A step towards future therapy.
Human olfactory ecto-mesenchymal stem cells (hOE-MSCs) derived from the human olfactory mucosa (OM) can be easily isolated and expanded in cultures while their immense plasticity is maintained. To mitigate ethical concerns, the hOE-MSCs can be also transplanted across allogeneic barriers, making them desirable cells for clinical applications. The main purpose of this study was to evaluate the effects of administering the hOE-MSCs on a spinal cord injury (SCI) model of rats. These cells were accordingly isolated and cultured, and then treated in the neurobasal medium containing serum-free Dulbecco's Modified Essential Medium (DMEM) and Ham's F-12 Medium (DMEM/F12) with 2% B27 for two days. Afterwards, the pre-induced cells were incubated in N2B27 with basic fibroblast growth factor (bFGF), fibroblast growth factor 8b (FGF8b), sonic hedgehog (SHH), and ascorbic acid (vitamin C) for six days. The efficacy of the induced cells was additionally evaluated using immunocytochemistry (ICC) and real-time polymerase chain reaction (RT-PCR). The differentiated cells were similarly transplanted into the SC contusions. Functional recovery was further conducted on a weekly basis for eight consecutive weeks. Moreover, cell integration was assessed via conventional histology and ICC, whose results revealed the expression of choline acetyltransferase (ChAT) marker at the induction stage. According to the RT-PCR findings, the highest expression level of insulin gene-enhancer protein (islet-1), oligodendrocyte transcription factor (Olig2), and homeobox protein HB9 was observed at the induction stage. The number of engraftment cells also rose (approximately by 2.5 % ± 0.1) in the motor neuron-like cells derived from the hOE-MSCs-grafted group compared with the OE-MSCs-grafted one. The functional analysis correspondingly revealed that locomotor and sensory scores considerably improved in the rats in the treatment group. These findings suggested that motor neuron-like cells derived from the hOE-MSCs could be utilized as an alternative cell-based therapeutic strategy for SCI.
Behav Brain Res, 2021; 405
33613883: Soleimani Asl S, Ghasemi Moravej F, Kowsari G, Farhadi MH, Pourhaydar B, Ghasemi Hamidabadi H, Mehdizadeh M
The Effects of 3,4-methylenedioxymethamphetamine on Neurogenesis in the Hippocampus of Male Rats.
The administration of 3,4-methylenedioxymethamphetamine (MDMA) or ecstasy causes memory impairment, whereas neurogenesis improves memory and learning. Hence, this study evaluated the effects of MDMA on neurogenesis in the hippocampus of male rats.
Basic Clin Neurosci, 2020 Jul-Aug; 11
32993279: Goudarzi G, Hamidabadi HG, Bojnordi MN, Hedayatpour A, Niapour A, Zahiri M, Absalan F, Darabi S
Role of cerebrospinal fluid in differentiation of human dental pulp stem cells into neuron-like cells.
Human dental pulp stem cells (hDPSCs) could be differentiated into neuron like-cells under particular microenvironments. It has been reported that a wide range of factors, presented in cerebrospinal fluid (CSF), playing part in neuronal differentiation during embryonic stages, we herein introduce a novel culture media complex to differentiate hDPSCs into neuron-like cells. The hDPSCs were initially isolated and characterized. The CSF was prepared from the Cisterna magna of 19-day-old Wistar rat embryos, embryonic cerebrospinal fluid (E-CSF). The hDPSCs were treated by 5% E-CSF for 2 days, then neurospheres were cultured in DMEM/F12 supplemented with 10 µm retinoic acid (RA), glial-derived neurotrophic factor and brain-derived neurotrophic factor for 6 days. The cells which were cultured in basic culture medium were considered as control group. Morphology of differentiated cells as well as process elongation were examined by an inverted microscope. In addition, the neural differentiation markers (Nestin and MAP2) were studied employing immunocytochemistry. Neuronal-like processes appeared 8 days after treatment. Neural progenitor marker (Nestin) and a mature neural marker (MAP2) were expressed in treated group. Moreover Nissl bodies were found in the cytoplasm of treated group. Taking these together, we have designed a simple protocol for generating neuron-like cells using CSF from the hDPSCs, applicable for cell therapy in several neurodegenerative disorders including Alzheimer's disease.
Anat Cell Biol, 2020; 53
32512152: Niyazi M, Zibaii MI, Chavoshinezhad S, Hamidabadi HG, Dargahi L, Bojnordi MN, Alizadeh R, Heravi M, Karimi H, Hosseini M, Sadeghi Malvajerdi E, Seyednazari M
Neurogenic differentiation of human dental pulp stem cells by optogenetics stimulation.

Human dental pulp stem cells (hDPSCs), a promising source for autologous transplantation in regenerative medicine, have been shown to be able to differentiate into neural precursors. Optogenetics is considered as an advanced biological technique in neuroscience which is able to control the activity of genetically modified stem cells by light. The purpose of this study is to investigate the neurogenic differentiation of hDPSCs following optogenetic stimulation.
J Chem Neuroanat, 2020; 109
32489557: Darvishi M, Ghasemi Hamidabadi H, Sahab Negah S, Moayeri A, Tiraihi T, Mirnajafi-Zadeh J, Jahanbazi Jahan-Abad A, Shojaei A
PuraMatrix hydrogel enhances the expression of motor neuron progenitor marker and improves adhesion and proliferation of motor neuron-like cells.
Cell therapy has provided clinical applications to the treatment of motor neuron diseases. The current obstacle in stem cell therapy is to direct differentiation of stem cells into neurons in the neurodegenerative disorders. Biomaterial scaffolds can improve cell differentiation and are widely used in translational medicine and tissue engineering. The aim of this study was to compare the efficiency of two-dimensional with a three-dimensional culture system in their ability to generate functional motor neuron-like cells from adipose-derived stem cells.
Iran J Basic Med Sci, 2020; 23

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A FORCED PHYSICAL EXERCISE MAINTENANCE PROGRAM AS A MODEL FOR SELECTIVE MANIPULATION OF THE DOPAMINERGIC SYSTEM IN ADOLESCENT RATS.

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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The motor responses during physical activity are the result of a hierarchically arranged central nervous system circuits. Motor programs planned in the prefrontal cortex are refined in different cortical and subcortical circuits. Finally, the motor output arrives to the spinal cord, the first layer in the hierarchy of muscle control. In previous studies we determined that the physical capacity, measured as the maximal response during an incremental test, is dependent of D1 striatal and D2 extra-striatal actions in Sprague-Dawley rats. Here we want to determine the role of dopaminergic antagonists in physical activity maintenance responses. We first defined a model of physical activity maintenance that consisted in performing three incremental tests 72h apart, with active rest in the two sessions between tests to maintain the performance throughout the tests. In the second test, we administered a D1-like receptors antagonist (SCH23390) in a dose of 0.1 mg/kg intraperitoneally or saline (Sodium Chloride 0.9%). Rats administered with SCH23390 decreased the performance in the second test compared to the first and third ones ($p<0.05$). Rats injected with saline were able to maintain the performance throughout the incremental tests. Rats injected with SCH23390 significantly decreased their performance in the second test but recovered performance in the third test. This suggests that a habituation protocol and active rests between tests allow the maintenance of the performance and that the dopaminergic system is involved in the acute maintenance response. Finally, our model reduces the number of experimental animals required for the experimental design.

Pubmed:

32499715: Toval A, Vicente-Conesa F, Martínez-Ortega P, Kutsenko Y, Morales-Delgado N, Garrigos D, Alonso A, Ribeiro Do Couto B, Popović M, Ferran JL
Hypothalamic /, Plasmatic Glucose and Lactate Remain Unchanged During Habituation to Forced Exercise.
It has been demonstrated that physical activity contributes to a healthier life. However, there is a knowledge gap regarding the neural mechanisms producing these effects. One of the keystones to deal with this problem is to use training programs with equal loads of physical activity. However, irregular motor and stress responses have been found in murine exercise models. Habituation to forced exercise facilitates a complete response to a training program in all rodents, reaching the same load of physical activity among animals. Here, it was evaluated if glucose and lactate - which are stress biomarkers - are increased during the habituation to exercise. Sprague-Dawley rats received an 8-days habituation protocol with progressive increments of time and speed of running. Then, experimental and control (non-habituated) rats were subjected to an incremental test. Blood samples were obtained to determine plasmatic glucose and lactate levels before, immediately after and 30 min after each session of training. and mRNA expression was determined by two-step qPCR. Our results revealed that glucose and lactate levels are not increased during the habituation period and tend to decrease toward the end of the protocol. Also, and were not chronically activated by the habituation program. Lactate and glucose, determined after the incremental test, were higher in control rats without previous contact with the wheel, compared with habituated and wheel control rats. These results suggest that the implementation of an adaptive phase prior to forced exercise programs might avoid non-specific stress responses.
Front Physiol, 2020; 11
34040580: Garrigos D, Martínez-Morga M, Toval A, Kutsenko Y, Barreda A, Do Couto BR, Navarro-Mateu F, Ferran JL
A Handful of Details to Ensure the Experimental Reproducibility on the FORCED Running Wheel in Rodents: A Systematic Review.
A well-documented method and experimental design are essential to ensure the reproducibility and reliability in animal research. Experimental studies using exercise programs in animal models have experienced an exponential increase in the

last decades. Complete reporting of forced wheel and treadmill exercise protocols would help to ensure the reproducibility of training programs. However, forced exercise programs are characterized by a poorly detailed methodology. Also, current guidelines do not cover the minimum data that must be included in published works to reproduce training programs. For this reason, we have carried out a systematic review to determine the reproducibility of training programs and experimental designs of published research in rodents using a forced wheel system. Having determined that most of the studies were not detailed enough to be reproducible, we have suggested guidelines for animal research using FORCED exercise wheels, which could also be applicable to any form of forced exercise.
Front Endocrinol (Lausanne), 2021; 12
33394335: Toval A, Garrigos D, Kutsenko Y, Popović M, Do-Couto BR, Morales-Delgado N, Tseng KY, Ferran JL
Dopaminergic Modulation of Forced Running Performance in Adolescent Rats: Role of Striatal D1 and Extra-striatal D2 Dopamine Receptors.
Improving exercise capacity during adolescence impacts positively on cognitive and motor functions. However, the neural mechanisms contributing to enhance physical performance during this sensitive period remain poorly understood. Such knowledge could help to optimize exercise programs and promote a healthy physical and cognitive development in youth athletes. The central dopamine system is of great interest because of its role in regulating motor behavior through the activation of D1 and D2 receptors. Thus, the aim of the present study is to determine whether D1 or D2 receptor signaling contributes to modulate the exercise capacity during adolescence and if this modulation takes place through the striatum. To test this, we used a rodent model of forced running wheel that we implemented recently to assess the exercise capacity. Briefly, rats were exposed to an 8-day period of habituation in the running wheel before assessing their locomotor performance in response to an incremental exercise test, in which the speed was gradually increased until exhaustion. We found that systemic administration of D1-like (SCH23390) and/or D2-like (raclopride) receptor antagonists prior to the incremental test reduced the duration of forced running in a dose-dependent manner. Similarly, locomotor activity in the open field was decreased by the dopamine antagonists. Interestingly, this was not the case following intrastriatal infusion of an effective dose of SCH23390, which decreased motor performance during the incremental test without disrupting the behavioral response in the open field. Surprisingly, intrastriatal delivery of raclopride failed to impact the duration of forced running. Altogether, these results indicate that the level of locomotor response to incremental loads of forced running in adolescent rats is dopamine dependent and mechanistically linked to the activation of striatal D1 and extra-striatal D2 receptors.
Mol Neurobiol, 2021; 58
33980961: Kutsenko Y, Barreda A, Toval A, Garrigos D, Martínez-Morga M, Ribeiro Do Couto B, Ferran JL
Sex-dependent effects of forced exercise in the body composition of adolescent rats.
Determining the body composition during adolescence can predict diseases such as obesity, diabetes, and metabolic syndromes later in life; and physical activity became an effective way to restore changes in body composition. However, current available literature assessing the body composition before, during and after adolescence in female and male rodents by in vivo techniques is scarce. Thus, by using computerized tomography, we aimed to define the baseline of the weight and body composition during the adolescence and young adulthood of female and male Sprague-Dawley rats (on P30, P60 and P90) under standard diet. Then, we determined the effect of 18 days of forced exercise on the body weight and composition during the early adolescence (P27-45). The highest percentual increments in weight, body volume and relative adipose contents occurred during the female and male adolescence. Forced running during the early adolescence decreased weight, body volume and relative adipose delta and increment values in males only. The adolescence of rats is a period of drastic body composition changes, where exercise interventions have sex-dependent effects. These results support a model that could open new research windows in the field of adolescent obesity.
Sci Rep, 2021; 11

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AGE-RELATED DEGENERATION IN THE MOTOR END PLATES AND AXONS OF MICE LEAVES THE MOTONEURON SOMA UNAFFECTED

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Growing evidence from animal models and patients suggests that aging affects motor end plates and thus the innervation of skeletal muscles. The cause and the time course of these degenerative processes are unknown, and they may involve less exercise associated with age and spontaneous degeneration of the end plates, or both. Here we show that there are minor degenerative changes in the motor axons and their terminals while sensory axons remain largely unaffected. Axonal degeneration is already detectable in some of the axons in both the ventral roots and peripheral nerves of 6 months old C57BL/6 mice. These changes were accompanied by an increased number of pathological motor end plates (as compared to 3 months old mice) involving various forms of degenerating end plates in the EDL and TA muscles. At later time points (12, 18 and 24 months) progressive changes are present in both the peripheral nerves and muscles. Number of motoneurons decreases slowly but axonal transport processes appear to be severely affected by aging. Tension recording from these muscles shows a slightly decreasing tetanic force produced by aging animals, while the number of motor units decreases more progressively. Calcium histochemistry displays differential changes in the motor end plates and in the motoneurons. These changes suggest that aging affects mainly the distal parts of the motor unit while the perikaryon remains preserved for long time.

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THE REPRESENTATION OF PROPRIOCEPTIVE SENSORY AND MOTOR ADAPTIVE INFORMATION IN THE CEREBELLUM

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Proprioception is the ability to perceive where one's joints and limbs are located in space. It allows the execution of accurate movements and correction of inaccurate movements. The main target of ascending proprioceptive afferents and a key player in motor control is the cerebellum. The cerebellum has been suggested to use proprioceptive signals to compare the expected to the actual sensory consequences of movement, allowing movements to be adapted and accurately executed. Despite its importance, the neural code for proprioception and how it changes during movement adaptation is poorly understood. To understand how proprioceptive signals are encoded in the cerebellum and how these signals change as movements adapt, awake head-restrained mice were subjected to a novel proprioceptive and a velocity-dependent adaptation task, respectively. In the former, mouse forelimbs were passively displaced with a robotic manipulandum in 8-coplanar directions, with different velocities, amplitudes and starting positions. X-ray assisted video tracking allowed identifying the kinematics of joint angles. In the latter task, mice adapted to a velocity-dependent perturbation while actively pushing the manipulandum. During these tasks, we imaged the climbing fibers (CFs) and Granule cells (GCs), two input streams to the cerebellum, with two-photon microscopy. Our findings, so far, indicate that CFs are tuned to movement velocity, weakly tuned by direction, but not by amplitude, showing no obvious joint angle representation. A further quantification of the neural codes underlying proprioceptive inputs and adaptive motor signals will shed light on the role the two cerebellar input streams play during motor learning.

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TRIDIMENSIONAL ANALYSIS OF NEUROMUSCULAR CIRCUIT DEVELOPMENT IN HUMAN EMBRYOS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Developmental and congenital diseases are a major public health problem but little is known about the ontogeny and morphogenesis of human organs at a cellular scale. Thus, there is a lack of accurate and comprehensive reference human embryology data based on modern technologies. Our lab has recently developed an imaging method combining immunolabelling of human fetal organs or whole embryos, tissue clearing with solvents and 3D imaging with light-sheet microscopy. This has initiated a project aimed at describing the ontogeny of multiple human organs. Here we used this method to analyze the development of the human neuromuscular system during the first trimester of gestation. We are constructing the first accurate map of muscle development (especially of the head) and muscle innervation by simultaneously labelling diverse cell types, such as muscle cells or their precursors, motor and peripheral nerves and progenitors of chondrogenesis and osteogenesis. Datasets were generated from over 20 human embryos and muscles and nerves were segmented individually. This analysis allowed us to follow the development of the orofacial muscles and their motor innervation, which extends and ramifies as development progresses. Furthermore, it enabled us to identify novel muscles at the early stages of development, likely transient as they no longer exist at birth and have never been described before. These data will make possible to build an atlas describing the ontogeneis of muscles and their innervation during the first trimester of development in humans.

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UNRAVELLING THE ROLE OF THE MONOAMINE NEURON SYSTEM IN THE PATHOPHYSIOLOGY OF SMA

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Introduction and Aims: Spinal muscular atrophy(SMA) is a severe neuromuscular disease, affecting infants, caused by deletion or mutations in the survival motor neuron(*SMN*) gene, which mainly results in a progressive degeneration of motor neurons(MN) and muscle atrophy. Recently, three SMN-dependent treatments, with costs and time-window limitations, have been approved. Therefore, novel approaches are urgently needed. Interestingly, analysis of the cerebrospinal fluid(CSF) of SMAI patients revealed that the levels of 5-hydroxyindoleacetic acid(5-HIAA) and homovanillic acid(HVA), the main metabolites of serotonin and catecholamine, respectively, were significantly lower than those in controls, suggesting a role for the monoamine neuron system in the pathophysiology of SMA. Therefore, to identify new potential druggable targets, we investigated possible alterations of catecholamine and serotonin pathways in a SMAD7 mouse model. **Methods**: We investigated by means of RT-PCR, WB and confocal microscopy the expression levels of the enzymes involved in these metabolic pathways and quantified the key metabolites by HPLC, in the brain and in the spinal cord of late symptomatic SMAD7 mice. **Results and Conclusions**: In the brain of late symptomatic SMAD7 mice, mRNA and protein levels of the aromatic L-aminoacid decarboxylase(AADC) and dopamine b-hydroxylase (DbH), the enzymes that convert L-DOPA in dopamine and dopamine in noradrenaline, respectively, were strongly reduced. In particular, AADC showed a reduction in substantia nigra(SN), ventral tegmental area(VTA) and in raphe nuclei(RN). The present study highlights the significant role of these pathways in the pathogenesis of SMA and it might represent an important step to define new pharmacological approach.

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BOARD NUMBER: S04-477

EFFECTS OF EARLY MOVEMENT RESTRICTION ON HINDLIMB MUSCLE IRIS LEVELS IN RAT

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Childhood is a period of construction of the organism, during which interactions with the environment and regular physical activity favor the maturation of the neuronal networks. However, some children exhibit a sensorimotor restriction (SMR) because they are bedridden or they suffer from a neurodevelopmental disorder. In order to better understand the central effects of SMR in these children, we have developed a model of SMR in rats. It consists in casting hindlimbs of the pups from postnatal day (PND) 1 to PND 28. We have previously shown that SMR induces a prominent motor phenotype that includes muscle weakness, locomotor disturbances, spinal hyperreflexia but also reduced somatosensory and motor map areas and disturbance of the excitation/inhibition balance in the sensorimotor cortex. Our objective is to better understand the plasticity processes set up during SMR and our research has focused on irisin. Discovered in 2012, this myokine is secreted by skeletal muscles during physical activity and exerts autocrine, paracrine and also endocrine effects in central nervous system. Irisin is notably known to regulate the expression of brain-derived neurotrophic factor (BDNF), a key regulator of brain plasticity. Thus, we determined irisin level in hindlimb muscles and in plasma. We demonstrated that irisin level varies during development in control rats. In addition, changes are observed in SMR rats according to the age. Whether these changes are involved in cortical changes is in progress.

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BOARD NUMBER: S04-478

SYNAPTIC DRIVE CONTRIBUTING TO RHYTHM GENERATION IN MOTOR NEURONS OF THE INSECT LEG-MUSCLE CONTROL SYSTEM

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Leg stepping is a rhythmic motor behavior based on the interaction between central pattern generators (CPGs) and feedback from leg sensory organs. In the stick insect, individual CPGs provide rhythmic synaptic drive to leg motor neurons, innervating antagonistic muscle pairs, resulting in alternating motor activity. Previous studies revealed that the motor neurons innervating the tibial flexor and extensor muscles receive rhythmic inhibitory synaptic drive from respective premotor CPG networks (Büschges, 1998; Büschges et al. 2004). However, it is not known, whether this is a general mechanism of rhythm generation in motor neurons driving the muscles of all leg joints. Therefore, we studied the synaptic drive from the premotor CPGs to the motor neurons of the other main leg joints, i.e. the Thorax-Coxa and Coxa-Trochanter joints. For this, we recorded intracellularly the activity of motor neurons within the deafferented mesothoracic ganglion during pharmacological activation of the premotor networks that elicits rhythmic activity in motor neurons (Büschges et al. 1995). The synaptic drive to motor neurons was assessed by measuring the input resistance and by stepwise altering the membrane potential during rhythmicity. We show that, similarly to flexor and extensor, rhythmic activity of retractor and protractor motor neurons is generated by phasic inhibitory synaptic inputs. Interestingly and unexpectedly, the synaptic drive contributing to rhythm generation in depressor and levator motor neurons appears to differ. This study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - **233886668/ GRK1960**

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BOARD NUMBER: S04-479

NEURAL CIRCUIT MECHANISMS UNDERLYING SENSORIMOTOR DECISIONS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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From finding nearby food to escaping a predator, animals must respond to sensory cues with appropriate motor actions in order to survive. The neural circuit mechanisms underlying the transformation of sensory information into appropriate motor output remain incompletely understood. To understand these mechanisms better, it would be important to map the entire neural circuitry from the sensory to the motor side in a model organism allowing functional testing of such circuits. Thanks to recent progress in connectomics, allowing the mapping of entire neural circuits in electron microscopy (EM) with synaptic resolution and to the genetic toolbox for cell-specific neuronal manipulations and monitoring, Drosophila larva presents itself as such a model. We will here present our progress on the investigation of the neural circuitry underlying larval responses to an aversive mechanosensory stimulus, the air puff. Drosophila larvae typically respond with either startle or escape behaviours to the air-puff. The selection of one action implies the inhibition of other, mutually exclusive, actions. Using inactivation and optogenetic activation of single neurons in behaving larvae combined with video tracking and automated behavioral detection, we determined the role of interneurons at different sensorimotor stages and found they are differentially involved in startle and escape behaviors. We are now mapping the startle and escape sensorimotor pathways and investigating where and how they could interact. Determining the neural circuit mechanisms underlying competitive interactions in a system where we can trace all connections and manipulate single neurons may bring insights into the neural circuit mechanisms of sensorimotor decisions.

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BOARD NUMBER: S04-480

THE WHOLE-BRAIN IRRADIATION INDUCES SKELETAL MUSCLE DAMAGE IN THE RAT

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Aims: Radiotherapy (RT), a major treatment for brain tumors, can also affect the healthy brain tissue and reduces the quality of life of patients whose feel extreme fatigue during weeks after the brain RT. The etiology of this fatigue remains poorly described. The intimately relationship between brain and muscles could explain the abscopal effects, defined as non-target effects in non-irradiated cells, distant from an irradiated target. The present study aims to assess the impact of brain RT on skeletal muscles and its relationship to fatigue. **Methods:** Adult rats were randomly distributed into control or irradiated groups. Irradiated rats were exposed to a 30 Gy whole-brain irradiation (WBI, 3x10 Gy) with a dedicated irradiator to small animals (X-RAD 225Cx). Complementary approaches: behavioral tests, muscle mass, MRI and histological analyses were employed to examine the effects of cranial irradiation on the skeletal muscles up to 6 months following WBI. **Results:** Irradiated rats displayed fatigue and reduced locomotor activity at short and long terms. The analyzed muscular mass decreased in rats submitted to WBI. Multiparametric MRI imaging of muscles highlighted a dramatic alteration of the fiber organization in irradiated rats as demonstrated by a significant diminution of the mean diffusivity. Moreover, irradiation-induced alteration of fibers was confirmed by histological analyses. These muscular alterations could be attributed to an increased circulating reactive species or pro-inflammatory cytokines. **Conclusions:** This study emphasizes that it is essential to consider skeletal muscle damage resulting from whole-brain irradiation to improve the management of radiotherapy-induced side effects, especially fatigue.

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EFFECTS AND INCREMENTAL TEST RESPONSES BY SHORTENING OF HABITUATION PROTOCOLS DURING ADOLESCENCE OF RATS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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The brain causal mechanisms working on peripheral tissues during a physical activity are poorly understood. In previous studies we developed a model to analyze motor responses based on a habituation program consisting of a progressive increase of the training load. Determine if shortening the habituation program modify responses in exercise capacity, and if this can be related with muscle peripheral adaptations. Sessions of the habituation were developed throughout 2, 4 and 8 days of training. Once the habituation phases were completed, rats were subjected to an incremental exercise test to determine the physical capacity. Samples of gastrocnemius and soleus were used for qPCR and Western blot analysis of markers involved in muscle adaptation. The total time of running during the incremental test was of 37.78 ± 2.65 min for 8 days habituated rats but 15.84 ± 1.22 min for non-habituated rats. In the case of 4 days habituated rats was 36.02 ± 2.67 min with 17.35 ± 2.86 for the control. Finally, the total time of running was of 28.95 ± 3.94 min for 2 days habituated rats, but 19.97 ± 2.57 min for the control. We didn´t observed any differences in selected muscle markers in terms of mRNA molecules or protein concentrations justifying peripheral adaptations. Shortening of habituation period to 2 days habituation period produce a decreased response during the incremental test compared with 4 and 8 day habituated rats. The differences in running can't be justified in terms of muscle peripheral adaptations, strengthening a central nervous system effect.

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MOTOR NEURON AND SPINAL INTERNEURON DIVERSITY SCALE UP DURING FROG METAMORPHOSIS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Frog metamorphosis captures simple swimming and complex, tetrapod limb movement in one organism. Tadpoles first engage in periodic undulatory swimming (NF 37-38); then switch to constant free-swimming (NF 47-50); and finally, to four-limbed movements such as walking, hopping, and scratching (NF 57-adult). We aim to capitalize on this unique metamorphic behavioral switch to determine how spinal neuron diversity differs for swimming and walking. We focus our analysis on motor neurons (MNs) and V1 interneurons (V1s), a class of spinal inhibitory interneurons that modulate motor neuron firing. We find MN and V1 number and transcriptional heterogeneity scale up with the complexity of behavior. The larval tadpole spinal cord contains a largely uniform population of medial motor column MNs and associated V1s. With the emergence of free swimming, MNs and V1s double in number and begin to diversify in their transcriptional profile, acquiring MN populations equivalent to the mouse hypaxial and preganglionic motor columns and V1 subpopulations equivalent to clades. Finally, at metamorphosis, limb motor column neurons are added and V1 number and diversity increase dramatically, with the same transcriptional V1 clade and subclade diversity observed in froglets as in the developing mouse. Our work maps MN and V1 molecular properties onto swim and limb behavior during frog metamorphosis, defining how transcriptional diversity scales up with behavioral complexity. We additionally demonstrate the conservation of MN and V1 molecular organization between the frog, the most ancient tetrapod, and the mouse, a four-limbed mammal.

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BOARD NUMBER: S04-483

ALTERATIONS OF LOCOMOTION IN A MODEL OF INCOMPLETE CERVICAL SPINAL CORD INJURY (SCI) IN PIGS.

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Domestic pig is considered to be a good model for testing neurotherapeutics. A study of the kinematic of the walking cycle was performed in pigs after a cervical SCI (right hemisection with excision of 1 cm of spinal cord). Pigs were filmed while walking on a treadmill at 1, 3 and 5 km/h. We measured knee, ankle, elbow and wrist angles, variation of eye and hip height, relative aft-to-forward movement of the distant part of the legs and step cycle parameters. In uninjured animals, the maximum flexion of every joint during the swing phase increased with speed. Hip and eye height displayed two peaks per stride, coincident with the action of diagonal pairs: right foreleg (RF)/left hindleg (LH) and left foreleg (LF)/right hindleg (RH). After SCI, pigs required 62.3% of body weight support (provided by a harness connected by strain gauges) during the stance of the RF/LH pair. Stride and stance durations for all the limbs (excluding the RF) increased progressively. Ankle and knee extension increased at the end of the stance phase, indicating that the RH was functional and overused. However, the RF was retracted, with negative wrist-shoulder position along the stride. Elbow extension was markedly reduced, and wrist flexion was almost lost. In addition, negative eye height values indicated a tendency of the forepart of the body to fall during the stance of the RF/LH pair. Then, motor impairments and compensations following cervical SCI can be readily characterized by kinematic techniques and force measurements during treadmill locomotion.

Pubmed:
34121450: Cerro PD, Barriga-Martín A, Vara H, Romero-Muñoz LM, Rodríguez-De-Lope Á, Collazos-Castro JE
Neuropathological and Motor Impairments after Incomplete Cervical Spinal Cord Injury in Pigs.
Humans, primates, and rodents with cervical spinal cord injury (SCI) show permanent sensorimotor dysfunction of the upper/forelimb as consequence of axonal damage and local neuronal death. This work aimed at characterizing a model of cervical SCI in domestic pigs in which hemisection with excision of 1 cm of spinal cord was performed to reproduce the loss of neural tissue observed in human neuropathology. Posture and motor control were assessed over 3 months by scales and kinematics of treadmill locomotion. Histological measurements included lesion length, atrophy of the adjacent spinal cord segments, and neuronal death. In some animals, the retrograde neural tracer aminostilbamidine was injected in segments caudal to the lesion to visualize propriospinal projection neurons. Neuronal loss extended for 4-6 mm from the lesion borders and was more severe in the ipsilateral, caudal spinal cord stump. Axonal Wallerian degeneration was observed caudally and rostrally, associated with marked atrophy of the white matter in the spinal cord segments adjacent to the lesion. The pigs showed chronic monoplegia or severe monoparesis of the foreleg ipsilateral to the lesion, whereas the trunk and the other legs had postural and motor impairments that substantially improved during the first month post-lesion. Adaptations of the walking cycle such as those reported for rats and humans ameliorated the negative impact of focal neurological deficits on locomotor performance. These results provide a baseline of behavior and histology in a porcine model of cervical spinal cord hemisection that can be used for translational research in SCI therapeutics.
J Neurotrauma, 2021; 38
30942489: Ordás P, Hernández-Ortego P, Vara H, Fernández-Peña C, Reimúndez A, Morenilla-Palao C, Guadaño-Ferraz A, Gomis A, Hoon M, Viana F, Señarís R
Expression of the cold thermoreceptor TRPM8 in rodent brain thermoregulatory circuits.
The cold- and menthol-activated ion channel transient receptor potential channel subfamily M member 8 (TRPM8) is the principal detector of environmental cold in mammalian sensory nerve endings. Although it is mainly expressed in a subpopulation of peripheral sensory neurons, it has also been identified in non-neuronal tissues. Here, we show, by in situ hybridization (ISH) and by the analysis of transgenic reporter expression in two different reporter mouse strains, that TRPM8 is also expressed in the central nervous system. Although it is present at much lower levels than in peripheral sensory

neurons, we found cells expressing TRPM8 in restricted areas of the brain, especially in the hypothalamus, septum, thalamic reticular nucleus, certain cortices and other limbic structures, as well as in some specific nuclei in the brainstem. Interestingly, positive fibers were also found traveling through the major limbic tracts, suggesting a role of TRPM8-expressing central neurons in multiple aspects of thermal regulation, including autonomic and behavioral thermoregulation. Additional ISH experiments in rat brain demonstrated a conserved pattern of expression of this ion channel between rodent species. We confirmed the functional activity of this channel in the mouse brain using electrophysiological patch-clamp recordings of septal neurons. These results open a new window in TRPM8 physiology, guiding further efforts to understand potential roles of this molecular sensor within the brain.
J Comp Neurol, 2021; 529
30904548: Vara H, Collazos-Castro JE
Enhanced spinal cord microstimulation using conducting polymer-coated carbon microfibers.
Intraspinal microstimulation (ISMS) may help to restore motor functions after spinal cord injury. ISMS caudal to the lesion activates motoneurons and evokes selective movements with graded force in rats and other mammals. We investigated the safety and effectiveness of conducting polymer (CP)-coated carbon microfibers (CMFs) for ISMS. 7-µm-diameter CMFs coated with poly(3,4-ethylenedioxythiophene) doped with poly[(4-styrenesulfonic acid)-co-(maleic acid)] (PEDOT:PSS-co-MA) were used to apply current-controlled biphasic electric pulses at the cervical spinal cord (C7) of anesthetized rats. Electrode performance and motoneuron activation, as readout by voltage transients, cyclic voltammetry, electrochemical impedance spectroscopy, electromyography (EMG) and foreleg kinematics, were investigated as a function of microfiber length (50 µm vs. 250 µm) and presence of polymer coating. The microfibers were very effective in activating specific spinal motoneurons, with the lowest stimulus thresholds varying between -28 µA and -46 µA in the cathodic phase. EMG and kinematic thresholds decreased when the microfiber tip approached the targeted motor nucleus (triceps brachii, t.b.) from the dorsal spinal cord surface. ISMS with polymer-coated CMFs produced higher electrical activity in the t.b. fascicles compared to bare CMFs. PEDOT:PSS-co-MA coating of 250-µm CMFs avoided the generation of unsafe overvoltages for biphasic pulses up to -80/+40 µA in vivo, although the positive effect of the conducting polymer was lost after the application of a few thousands of electric pulses. Thus, CP-coated CMFs may provide an effective and minimally invasive electrode for ISMS; however, polymer optimization is still required to improve its electrical stability and safety for long-term use. Statement of significance Intraspinal microstimulation may restore motor functions after spinal cord injury. In the present study we demonstrate that carbon microfibers (CMFs) coated with the conducting polymer PEDOT:PSS-co-MA can be advantageously used for this purpose. These microfibers allow for both effective and temporarily safe electrical activation of spinal motor circuits with high spatial resolution. The presence of the polymer enhances the effectiveness of the electrical stimuli to recruit spinal motoneurons. Thus, conducting polymer-coated CMFs have potential for the development of advanced neuroprosthetic devices, although further improvements are needed regarding their electrochemical and mechanical stability.
Acta Biomater, 2019; 90
26574911: Vara H, Collazos-Castro JE
Biofunctionalized Conducting Polymer/Carbon Microfiber Electrodes for Ultrasensitive Neural Recordings.
Carbon microfibers (MFs) coated with conducting polymers may provide a solution for long-term recording of activity from individual or small groups of neurons. Attaching cell adhesion molecules to the electro-sensitive surface might further improve electrode-neuron contact, thus enhancing signal stability and fidelity. We fabricated biofunctionalized microelectrodes consisting of 7-µm diameter carbon MFs coated with poly(3,4-ethylenedioxythiophene) doped with poly[(4-styrenesulfonic acid)-co-(maleic acid)] (
ACS Appl Mater Interfaces, 2015; 7
23676497: Marcello E, Saraceno C, Musardo S, Vara H, de la Fuente AG, Pelucchi S, Di Marino D, Borroni B, Tramontano A, Pérez-Otaño I, Padovani A, Giustetto M, Gardoni F, Di Luca M
Endocytosis of synaptic ADAM10 in neuronal plasticity and Alzheimer's disease.
A disintegrin and metalloproteinase 10 (ADAM10), a disintegrin and metalloproteinase that resides in the postsynaptic densities (PSDs) of excitatory synapses, has previously been shown to limit β-amyloid peptide (Aβ) formation in Alzheimer's disease (AD). ADAM10 also plays a critical role in regulating functional membrane proteins at the synapse. Using human hippocampal homogenates, we found that ADAM10 removal from the plasma membrane was mediated by clathrin-dependent endocytosis. Additionally, we identified the clathrin adaptor AP2 as an interacting partner of a previously uncharacterized atypical binding motif in the ADAM10 C-terminal domain. This domain was required for ADAM10 endocytosis and modulation of its plasma membrane levels. We found that the ADAM10/AP2 association was increased in the hippocampi of AD patients compared with healthy controls. Long-term potentiation (LTP) in hippocampal neuronal cultures induced ADAM10 endocytosis through AP2 association and decreased surface ADAM10 levels and activity. Conversely, long-term depression (LTD) promoted ADAM10 synaptic membrane insertion and stimulated its activity. ADAM10 interaction with the synapse-associated protein-97 (SAP97) was necessary for LTD-induced ADAM10 trafficking and required for LTD

maintenance and LTD-induced changes in spine morphogenesis. These data identify and characterize a mechanism controlling ADAM10 localization and activity at excitatory synapses that is relevant to AD pathogenesis.
J Clin Invest, 2013; 123
23300680: de la Peña E, Mälkiä A, Vara H, Caires R, Ballesta JJ, Belmonte C, Viana F
The influence of cold temperature on cellular excitability of hippocampal networks.
The hippocampus plays an important role in short term memory, learning and spatial navigation. A characteristic feature of the hippocampal region is its expression of different electrical population rhythms and activities during different brain states. Physiological fluctuations in brain temperature affect the activity patterns in hippocampus, but the underlying cellular mechanisms are poorly understood. In this work, we investigated the thermal modulation of hippocampal activity at the cellular network level. Primary cell cultures of mouse E17 hippocampus displayed robust network activation upon light cooling of the extracellular solution from baseline physiological temperatures. The activity generated was dependent on action potential firing and excitatory glutamatergic synaptic transmission. Involvement of thermosensitive channels from the transient receptor potential (TRP) family in network activation by temperature changes was ruled out, whereas pharmacological and immunochemical experiments strongly pointed towards the involvement of temperature-sensitive two-pore-domain potassium channels (K(2P)), TREK/TRAAK family. In hippocampal slices we could show an increase in evoked and spontaneous synaptic activity produced by mild cooling in the physiological range that was prevented by chloroform, a K(2P) channel opener. We propose that cold-induced closure of background TREK/TRAAK family channels increases the excitability of some hippocampal neurons, acting as a temperature-sensitive gate of network activation. Our findings in the hippocampus open the possibility that small temperature variations in the brain in vivo, associated with metabolism or blood flow oscillations, act as a switch mechanism of neuronal activity and determination of firing patterns through regulation of thermosensitive background potassium channel activity.
PLoS One, 2012; 7
19487674: Vara H, Onofri F, Benfenati F, Sassoè-Pognetto M, Giustetto M
ERK activation in axonal varicosities modulates presynaptic plasticity in the CA3 region of the hippocampus through synapsin I.
Activity-dependent changes in the strength of synaptic connections in the hippocampus are central for cognitive processes such as learning and memory storage. In this study, we reveal an activity-dependent presynaptic mechanism that is related to the modulation of synaptic plasticity. In acute mouse hippocampal slices, high-frequency stimulation (HFS) of the mossy fiber (MF)-CA3 pathway induced a strong and transient activation of extracellular-regulated kinase (ERK) in MF giant presynaptic terminals. Remarkably, pharmacological blockade of ERK disclosed a negative role of this kinase in the regulation of a presynaptic form of plasticity at MF-CA3 contacts. This ERK-mediated inhibition of post-tetanic enhancement (PTE) of MF-CA3 synapses was both frequency- and pathway-specific and was observed only with HFS at 50 Hz. Importantly, blockade of ERK was virtually ineffective on PTE of MF-CA3 synapses in mice lacking synapsin I, 1 of the major presynaptic ERK substrates, and triple knockout mice lacking all synapsin isoforms displayed PTE kinetics resembling that of wild-type mice under ERK inhibition. These findings reveal a form of short-term synaptic plasticity that depends on ERK and is finely tuned by the firing frequency of presynaptic neurons. Our results also demonstrate that presynaptic activation of the ERK signaling pathway plays part in the activity-dependent modulation of synaptic vesicle mobilization and transmitter release.
Proc Natl Acad Sci U S A, 2009; 106
14620877: Vara H, Muñoz-Cuevas J, Colino A
Age-dependent alterations of long-term synaptic plasticity in thyroid-deficient rats.
Thyroid hormone deficiency during a critical period of development profoundly affects cognitive functions such as attention, learning, and memory, but the synaptic alterations underlying these deficits remain unexplored. The present study examines the effect of congenital hypothyroidism on long-term synaptic plasticity. This plasticity is believed to be essential for learning and memory and for activity-dependent regulation of synapse formation in the developing brain. We found that the neonatal expression of long-term potentiation (LTP), long-term depression (LTD), depotentiation, and de-depression in hippocampal slices from hypothyroid animals was similar to that of controls. To examine the postnatal development of these plasticities, we used slices from neonatal (2-3 weeks) and adult (7-8 weeks) rats. This work demonstrates that the ability to express all these forms of synaptic plasticity is reduced in an age-dependent manner in control rats. LTP and depotentiation are also downregulated in adult hypothyroid rats, but we have found that de-depression is not affected during maturation. In addition, these animals express LTD at ages at which controls fail to induce it. In contrast, input/output experiments have shown greater levels of basal synaptic efficacy in hypothyroid adults, and this effect is probably related to the higher probability of release observed by paired-pulse experiments. Nevertheless, these effects appear to be unrelated to the differences observed in long-term synaptic plasticity, as no correlation was found between basal synaptic efficacy and the degree of LTD and de-depression. Furthermore, the NMDA-receptor antagonist amino-phosphonopentanoic acid (APV) completely blocked LTD, which suggests a postsynaptic locus of this alteration. Because LTD has been associated with novelty acquisition, we

suggest that the greater LTD observed in adult hypothyroid rats might be related to the hyperactivity of these animals. However, other possibilities such as a retarded maturation of synaptic plasticity must be taken into account.
Hippocampus, 2003; 13

11882369: Vara H, Martínez B, Santos A, Colino A
Thyroid hormone regulates neurotransmitter release in neonatal rat hippocampus.
Thyroid hormone is essential for the normal maturation and function of the mammalian CNS. Thyroid hormone deficiency during a critical period of development profoundly affects cognitive functions such as learning and memory. However, the possible electrophysiological alterations that could underlie these learning deficits in hypothyroid animals remain largely unexplored. In this work, we have studied the possible effect of thyroid hormone on short-term synaptic plasticity, which is hypothesized to be a neural substrate of short-term memory. We compared short-term modification of the excitatory postsynaptic potential in hippocampal slices between control and hypothyroid rats. Electrophysiological studies reveal that paired-pulse facilitation is strongly altered in the hypothyroid rats. In addition, hypothyroid rats exhibit an increase in the Ca(2+)-dependent neurotransmitter release. These alterations are basically reversible when thyroid hormone is administered. In order to examine the possible molecular mechanisms underlying these synaptic changes, we compared the expression of synapsin I, synaptotagmin I, syntaxin, and alpha-Ca(2+)/calmodulin kinase II between control and hypothyroid hippocampus. Our results show that the levels of synapsin I and synaptotagmin I are increased in the hypothyroid rats, which suggests that the genes encoding these proteins are implicated in the action of thyroid hormone on neurotransmitter release. Taken together, the results from this study suggest that thyroid hormone may modulate the probability of neurotransmitter release.
Neuroscience, 2002; 110

20805102: Epis R, Marcello E, Gardoni F, Vastagh C, Malinverno M, Balducci C, Colombo A, Borroni B, Vara H, Dell'Agli M, Cattabeni F, Giustetto M, Borsello T, Forloni G, Padovani A, Di Luca M
Blocking ADAM10 synaptic trafficking generates a model of sporadic Alzheimer's disease.
We describe here an innovative, non-transgenic animal model of Alzheimer's disease. This model mimics early stages of sporadic disease, which represents the vast majority of cases. The model was obtained by interfering with the complex between a disintegrin and metalloproteinase domain containing protein 10 (ADAM10), the main α-secretase candidate, and its partner, synapse-associated protein 97, a protein of the postsynaptic density-membrane associated guanylate kinase family. Association of ADAM10 with synapse-associated protein 97 governs enzyme trafficking and activity at synapses. Interfering with the ADAM10/synapse-associated protein 97 complex for 2 weeks by means of a cell-permeable peptide strategy is sufficient to shift the metabolism of the amyloid precursor protein towards amyloidogenesis and allows the reproduction of initial phases of sporadic Alzheimer's disease. After 2 weeks of treatment, we detected progressive Alzheimer's disease-like neuropathology, with an increase of β-amyloid aggregate production and of tau hyperphosphorylation, and a selective alteration of N-methyl-d-aspartic acid receptor subunit composition in the postsynaptic compartment of mouse brain. Behavioural and electrophysiological deficits were also induced by peptide treatment.
Brain, 2010; 133

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BOARD NUMBER: S04-484

THE EMERGENCE OF FIXED POINTS IN INTERLIMB COORDINATION UNDERLIES THE LEARNING OF STABLE GAITS IN MICE

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Complex motor behaviors involve the precise coordination of different body parts. While motor coordination has been extensively studied in expert animals, the behavioral and neural processes involved in its emergence through learning are not well understood. Here, we combined longitudinal behavioral analyses with population recordings from the cerebellum, which is a key region for movement coordination and motor learning. We trained mice to walk on a motorized runged treadmill over multiple days while Ca2+-activity was recorded from cerebellar molecular layer interneurons (MLIs). Motorization and rungs obliged mice to walk slower and with different stride lengths than in their natural gait. High-speed behavioral video recordings allowed us to extract paw trajectories and to assess how mice learned to control stepping patterns in this novel task that required coordination between limbs, and coordination of single limbs with the runged treadmill. Over learning, mice acquired a stable gait pattern (lateral sequence walk). We found that across animals, fixed pairwise swing-stance phase differences between limbs emerge over days. Using a neural population decoder, we show that cerebellar MLIs encode pairwise phase differences of limbs. We finally asked whether fixed pairwise phase relationships emerge through stronger interlimb coupling or rather by an increase in regularity of single-paw stepping patterns. To address these possibilities, we fit paw dynamics to a coupled oscillator model in which intrinsic frequencies change stochastically according to a Hidden Markov Model (HMM). We find that over learning, mice adjust single-limb stepping frequencies so that fixed-point dynamics in interlimb coordination become possible.

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BOARD NUMBER: S04-485

SENSORY ASSOCIATION AND MISMATCH IN THE POSTERIOR PARIETAL CORTEX

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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The posterior parietal cortex (PPC) is a multimodal association area involved in higher cognitive functions such as perceptual decision-making, visual attention, navigation and movement planning. Recently, it has been shown that PPC can report mismatches in sensory sequences, such as the omission of an expected stimulus. Using two-photon calcium imaging coupled with a chronic cranial window in awake head-fixed mice, we show that PPC can report auditory and whisker (tactile) stimuli. Furthermore, PPC can make rapid associations between these stimulus sequences and reliably report mismatches, e.g. in the form of stimulus omissions, unexpected stimuli, and varying stimulus intensities. Our findings reaffirm the role of PPC in comparing the internal model of the external world based on incoming sensory information, as proposed within the framework of predictive processing.

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BOARD NUMBER: S04-486

TOP-DOWN AND BOTTOM-UP INTERACTIONS AT THE POSTERIOR PARIETAL CORTEX

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Sensory perception can be represented as the comparison between feedforward bottom-up sensory inputs and feedback top-down expectations of these sensory stimuli. It was recently shown that a subset of posterior parietal cortex (PPC) neurons can report mismatches in audio tactile sensory sequences. We hypothesize that these mismatch neurons report the balance between the bottom-up (sensory input from S1 and A1) and top-down inputs (feedback from premotor M2). To test our hypothesis, we performed simultaneous dual layer two-photon calcium imaging of M2 axons in layer 1 and of layer 2/3 neurons in the PPC. We show that top-down expectancy of the predicted stimuli is reliably reported by M2 axons, while layer 2/3 PPC neurons report auditory and tactile stimuli, as well as mismatches in the sensory sequence. We demonstrate that the PPC can rapidly learn new associations and subsequently report mismatches, while M2 axons update their expectations to the new association. Our results suggest that M2 potentially provides the top-down prediction, which is then compared to the incoming sensory input at PPC. This leads to the subsequent generation of mismatch responses when top-down and bottom up inputs do not match.

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BOARD NUMBER: S04-487

GRIP DYNAMICS DURING OBJECT MANIPULATION ON THE INTERNATIONAL SPACE STATION

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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AIMS**:** Parabolic-flight studies have shown that the temporal coordination between grip force (GF) and load force (LF) is preserved during object manipulation in extreme gravito-inertial environments such as partial, hyper- or micro- gravity. It was nevertheless observed that participants tended to increase the GF safety margin in microgravity. However, the characteristics of the GF-LF coupling during long-term exposure to microgravity have never been studied. The present experiment aims to fill this gap by studying sensorimotor coordination during object manipulation in astronauts onboard the International Space Station (ISS). METHODS: 10 astronauts performed rhythmic arm movements with an object held in precision grip, both on Earth and during >5-months missions on the ISS. Three different object masses were used, as well as three different oscillation frequencies. RESULTS: A steady GF-LF correlation was rapidly acquired, both on the ground and during spaceflight. Strikingly, the GF/LF ratio at the times of maximum LF was independent of object mass, movement frequency or gravity level. This was achieved by offsetting GF as a function of LF range. Interestingly though, in microgravity the amplitude of GF peaks were dependent on LF direction, despite symmetrical LF peaks. CONCLUSIONS: Our results suggest that tactile and proprioceptive feedback are integrated quickly to update the anticipatory control of grip force and achieve an adequate safety margin in all circumstances. Interestingly, even after several months of exposure to microgravity, up/down asymmetries persisted in the modulation of grip force. Future works should be carried out to pinpoint the cause of this asymmetry.

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BOARD NUMBER: S04-488

COMPARISON OF LATERAL DYNAMIC STABILITY BETWEEN WALKING CATS AND WILD-TYPE MICE

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Among all daily activities, walking accounts for highest proportion of falls in elderly (24%), and falls occur more often in the lateral direction (Robinovitch et al. 2013). The compromised proprioceptive and tactile sensation due to aging or illness contributes to falls (Earhart 2013), although precise mechanisms are not well understood. Genetically-modified mice provided insight into the role of somatosensory feedback in locomotor control (Akay et al., 2014). However, mouse's crouched gait may improve stability and thus reduce comparability of this model with humans. On the other hand, there are remarkable similarities in lateral dynamic stability between walking cats and humans (Burke et al. 2019; Latash et al. 2020). Here we compared lateral dynamic stability between cats and wild-type mice during treadmill walking with a normalized speed (Froud number) of 0.05; 0.4 m/s and 0.1 m/s, respectively. We captured center-of-mass and center-of-pressure on the horizontal plane of wild-type mice walking on a treadmill with transparent belt, and mirror. Cat's kinematics was also computed from full-body motion capture. We determined lateral dynamic stability as the distance between the extrapolated center-of-mass and center-of-pressure positions at onset of ipsilateral double support (Latash et al. 2020) and then scale this distance to the hindlimb step width. The scaled lateral dynamic stability was greater in mice than in cats ($p < 0.05$; 0.508±0.066 vs. 0.158±0.044, respectively). For maximal impact of research with animal models on clinical research, future studies should consider advantages and limitations of these two animal models to study neural mechanisms of locomotor stability.

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BOARD NUMBER: S04-489

DESCENDING NEURON POPULATION ACTIVITY DRIVING LIMB-DEPENDENT BEHAVIORS

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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The brain regulates animal behavior via a population of descending neurons (DNs) projecting to downstream motor circuits in the spinal cord or invertebrate ventral nerve cord (VNC). How DNs as a population efficiently encode the brain's motor intentions remains largely unknown. In *Drosophila melanogaster* the activity of pairs or small sets of `command-like' DNs are sufficient to drive walking, grooming, and escape behaviors. Thus, even a small fraction of ~1300 DNs can elicit complex motor actions. This raises the question of what role the large populations of DNs may play in behaviors. For example, they may modulate core behavioral commands. Alternatively, they may represent parallel pathways eliciting behaviors as a function of sensory context. Here, we address these possibilities using a genetic and optical intersectional approach to record the activity of nearly 100 DNs in the fly during odor-evoked and spontaneous behaviors. We observe that a majority of DNs encode locomotion. Some DNs drive turning and an overlapping set encode walking speed. A small fraction of DNs do not correlate with behavior but appear to encode specific odors. DNs identified from population imaging to encode antennal grooming can be targeted, via their location, functional, and morphological properties, to DNx01 neurons. This highlights the potential for DN population recordings to provide a context for the neural dynamics of genetically-identifiable neurons. These findings reveal that DN populations represent behavior in a largely distributed manner and set the stage for a comprehensive, population-level understanding of how the brain regulates complex limb-dependent motor behaviors

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HEMIFACIAL SPASM IN A 66-YEAR-OLD FILIPINO MALE CAUSED BY VERTEBROBASILAR ARTERY DOLICHOECTASIA: A CASE REPORT

POSTER SESSION 04 - SECTION: CLUSTER 90: MOVEMENT: SPINAL CORD AND MOTOR NEURONS

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Background: Hemifacial spasm (HFS), characterized by unilateral, intermittent contractions of the muscles of facial expression occurs due to vascular compression of the facial nerve at the root exit zone. Its total prevalence is in range of 9.8 to 11 per 100,000 in the total population with very limited knowledge on its prevalence in Asia, most specifically in the Philippines. Direct compression of the facial nerve by Vertebrobasilar dolichoectasia (VBD) is an even rarer cause of HFS, representing 0.7% of cases. **Objective:** This paper presents a case of HFS caused by VBD with multiple cranial nerve compression in a 66-year-old Filipino male presenting with involuntary twitching of the left side of the face. **Case:** A case of a 66-year-old Filipino male who presented with a six-year history of progressive involuntary twitching of the left side of the face, started on Botulinum Toxin periorbital injection treatment. Initial MRI was unremarkable. During subsequent clinic visits, more prominent and sustained contractions of the left side of the face were noted despite more frequent injections with lesser interval. Repeat MRI revealed a tortuous and ectatic vertebrobasilar artery, compressing and dorsally displacing the left Cranial Nerves V, VII and VIII. **Conclusion:** VBD can rarely compress the facial nerve leading to HFS. Although the diagnosis is led by clinical features, MRI is a useful imaging technique for demonstrating the nerve compression. Widely accepted treatment modalities include Botulinum Toxin (BTX) Injections as less invasive procedure, providing symptomatic relief and Microvascular Decompression (MVD) as the more definite treatment.

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BOARD NUMBER: S04-491

FUNCTIONAL CONNECTIVITY MAPPING OF SENSORY PATHWAYS USING FLAVOPROTEIN IMAGING

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Due to the columnar organization of sensory cortical regions the tactile information of individual whiskers, or sensation of low and high frequency sounds separates well in corresponding area, however considerable variance of the fine detailed maps among individual animals was found previously. The aim of the given project is to provide a method for fast mapping of topological organization of thalamocortical circuit. Experiments were carried out on C57BL/6 wild type adult mice, under urethane anesthesia. Electrophysiological recordings were made by silicon probes from somatosensory and auditory cortices, while for flavoprotein imaging (FI) continuous 480nm light illumination was applied for image acquisition. Air puff induced single whisker deflection or 12-25 kHz sinusoid sound waveforms were used for stimulation. Both tactile and auditory stimulation resulted in a topological organization of activation. Silicon probe recordings of high optical activity areas showed immediate and robust response in multi-unit activity, while after clustering, in a small number of neurons elevated firing rate was also found. The injection of retrograde choleratoxin subunit B into optically active barrels proved that the sensory information was originated from ventral posteromedial nucleus of thalamus, and multi- or single unit activity of this thalamic region also showed direct lemniscal input. The FI as method for mapping cortical activity over a large region of brain surface proved to be useful to identify the primary sensory field of applied stimuli. Activation pattern maps provided by FI showed topological organization in both sensory cortices which result was proved by anatomical and electrophysiological experiments.

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BOARD NUMBER: S04-492

ALTERED BODY SCHEMA AFTER VIRTUAL TOOL-USE TRAINING IS ASSOCIATED WITH THE EMERGENCE OF SENSE OF BODY OWNERSHIP AND SENSE OF AGENCY IN HEALTHY AGING

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Aims: We investigated whether training with a virtual tool in augmented reality (AR) has comparable effects on body schema (BS) and sense of ownership and agency in young and older adults, while leveraging AR to partially decouple contributions by visual and vibro-tactile feedback modalities. **Methods:** 34 young and 30 older adults underwent a virtual tool-use training in AR with and without vibro-tactile feedback and learned controlling a virtual gripper to grasp a virtual object. In the visuo-tactile condition compared with visual only, vibro-tactile feedback was applied to palm, thumb and index fingers when the tool touched the object. A tactile distance judgement task (TDJ) was used to assess changes in the BS where participants judged distances between two tactile stimuli applied to their right forearm either in proximodistal or mediolateral orientation. Sense of body ownership and agency after virtual tool-use training were assessed using a questionnaire. **Results** revealed that TDJ estimation errors were reduced after training only for proximodistal orientations, indicating smaller perceived arm length, while effects were stronger for young as compared to older adults. In both age groups sense of agency increased with training, but only in older adults this effect was modulated by changes in BS. Changes in body ownership were depended on BS plasticity in both age groups. We **conclude** that virtual tools are integrated into the existing BS during training, and the emergence of body ownership and sense of agency in healthy aging depends on BS plasticity and is (partly) moderated by type of feedback.

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BOARD NUMBER: S04-493

IDENTIFICATION OF TRIGEMINAL SENSORY NEURONAL TYPES INNERVATING MASSETER MUSCLE

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Understanding masseter muscle (MM) innervation is critical for the study of cell-specific mechanisms of pain induced by temporomandibular disorder (TMDs) or after facial surgery. Here, we identified trigeminal (TG) sensory neuronal subtypes (MM TG neurons) innervating MM fibers, masseteric fascia, tendons, and adjusted tissues. A combination of patch clamp electrophysiology and immunohistochemistry (IHC) on TG neurons back-traced from reporter mouse MM found nine distinct subtypes of MM TG neurons. Of these neurons, 24% belonged to non-peptidergic IB-4^{+}/TRPA1^{-} or IB-4^{+}/TRPA1^{+} groups, while two TRPV1^{+} small-sized neuronal groups were classified as peptidergic/CGRP^{+} One small-sized CGRP^{+} neuronal group had a unique electrophysiological profile and were recorded from Nav1.8^{-} or trkC^{+} neurons. The remaining CGRP^{+} neurons were medium-sized, could be divided into Nav1.8^{-}/trkC^{-} and Nav1.8^{low}/trkC^{+} clusters, and showed large 5HT-induced current. The final two MM TG neuronal groups were trkC^{+} and had no Nav1.8 and CGRP. Among MM TG neurons, TRPV1^{+}/CGRP^{-} (somatostatin^{+}), tyrosine hydroxylase (TH)$^{+}$ (C-LTMR), TRPM8^{+}, MrgprA3^{+}, or trkB^{+} (Aδ-LTMR) subtypes have not been detected. Masseteric muscle fibers, tendons and masseteric fascia in mice and the common marmoset, a new world monkey, were exclusively innervated by either CGRP^{+}/NFH^{+} or CGRP^{-}/NFH^{+} medium-to-large neurons, which we found using a Nav1.8-YFP reporter, and labeling with CGRP, TRPV1, neurofilament heavy chain (NFH) and pgp9.5 antibodies. These nerves were mainly distributed in tendon and at junctions of deep-middle-superficial parts of MM. Overall, the data presented here demonstrates that MM is innervated by a distinct subset of TG neurons, which have unique characteristics and innervation patterns.

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BOARD NUMBER: S04-494

NEUROANATOMICAL TRACING OF ASCENDING PROPRIOCEPTIVE PATHWAYS

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Proprioception is the sensation of where our limbs and body are positioned in space. Muscle spindles, Golgi tendon organs and mechanoreceptors in the joint capsule detect changes in muscle length, tension and changes in joint position, respectively. These organs are innervated by anatomically distinct classes of sensory neurons whose cell bodies lie in the dorsal root ganglia (DRG) and constitute the main proprioceptive afferent system. Beyond controlling basic reflexes, proprioceptive information also ascends to the brain via two major pathways. One terminates in the cerebellum (spino-and cuneo-cerebellar tracks) and allows adapting movements to unexpected perturbations, while the other innervates the cerebral cortex (dorsal column-medial lemniscus pathway) allowing for conscious access to proprioceptive information. Despite its importance, our understanding of how limb proprioception is transmitted to, and represented, in the cortex and cerebellum is limited. Here we use anterograde transsynaptic tracers to identify where, and the extent to which different proprioceptive channels are integrated along ascending pathways in mice. We show that proprioceptive DRG neurons can be labelled after muscle injection of AAV vectors in adult mice. This allows access to mature proprioceptive circuits, without the high toxicity of other virus classes classically used for anterograde tracing.

Pubmed:
34296117: Frezel N, Platonova E, Voigt FF, Mateos JM, Kastli R, Ziegler U, Karayannis T, Helmchen F, Wildner H, Zeilhofer HU
In-Depth Characterization of Layer 5 Output Neurons of the Primary Somatosensory Cortex Innervating the Mouse Dorsal Spinal Cord.
Neuronal circuits of the spinal dorsal horn integrate sensory information from the periphery with inhibitory and facilitating input from higher central nervous system areas. Most previous work focused on projections descending from the hindbrain. Less is known about inputs descending from the cerebral cortex. Here, we identified cholecystokinin (CCK) positive layer 5 pyramidal neurons of the primary somatosensory cortex (CCK S1-corticospinal tract [CST] neurons) as a major source of input to the spinal dorsal horn. We combined intersectional genetics and virus-mediated gene transfer to characterize CCK S1-CST neurons and to define their presynaptic input and postsynaptic target neurons. We found that S1-CST neurons constitute a heterogeneous population that can be subdivided into distinct molecular subgroups. Rabies-based retrograde tracing revealed monosynaptic input from layer 2/3 pyramidal neurons, from parvalbumin positive cortical interneurons, and from thalamic relay neurons in the ventral posterolateral nucleus. Wheat germ agglutinin-based anterograde tracing identified postsynaptic target neurons in dorsal horn laminae III and IV. About 60% of these neurons were inhibitory and about 60% of all spinal target neurons expressed the transcription factor c-Maf. The heterogeneous nature of both S1-CST neurons and their spinal targets suggest complex roles in the fine-tuning of sensory processing.
Cereb Cortex Commun, 2020; 1
31583355: Frezel N, Kratzer G, Verzar P, Bürki J, Weber FA, Zeilhofer HU
Does toe clipping for genotyping interfere with later-in-life nociception in mice?
Genetically modified mice are widely used in studies on human and animal physiology and pharmacology, including pain research. The experimental design usually includes comparisons of genetically modified mice with wild-type littermates, requiring biopsy material for genotyping and methods for unequivocal identification of individual mice. Ethical standards and, in some countries, legislation require that both needs are reached with a single procedure. Clipping of the most distal phalanx of up to two toes per paw (toe clipping) is the favored procedure in most research fields, but it may be problematic in sensory physiology and pain research.
Pain Rep, 2019 May-Jun; 4
31527839: Voigt FF, Kirschenbaum D, Platonova E, Pagès S, Campbell RAA, Kastli R, Schaettin M, Egolf L, van der Bourg A, Bethge P, Haenraets K, Frézel N, Topilko T, Perin P, Hillier D, Hildebrand S, Schueth A, Roebroeck A, Roska B, Stoeckli

ET, Pizzala R, Renier N, Zeilhofer HU, Karayannis T, Ziegler U, Batti L, Holtmaat A, Lüscher C, Aguzzi A, Helmchen F
The mesoSPIM initiative: open-source light-sheet microscopes for imaging cleared tissue.
Light-sheet microscopy is an ideal technique for imaging large cleared samples; however, the community is still lacking instruments capable of producing volumetric images of centimeter-sized cleared samples with near-isotropic resolution within minutes. Here, we introduce the mesoscale selective plane-illumination microscopy initiative, an open-hardware project for building and operating a light-sheet microscope that addresses these challenges and is compatible with any type of cleared or expanded sample (www.mesospim.org).
Nat Methods, 2019; 16
28700081: Haenraets K, Foster E, Johannssen H, Kandra V, Frezel N, Steffen T, Jaramillo V, Paterna JC, Zeilhofer HU, Wildner H
Spinal nociceptive circuit analysis with recombinant adeno-associated viruses: the impact of serotypes and promoters.
Recombinant adeno-associated virus (rAAV) vector-mediated gene transfer into genetically defined neuron subtypes has become a powerful tool to study the neuroanatomy of neuronal circuits in the brain and to unravel their functions. More recently, this methodology has also become popular for the analysis of spinal cord circuits. To date, a variety of naturally occurring AAV serotypes and genetically modified capsid variants are available but transduction efficiency in spinal neurons, target selectivity, and the ability for retrograde tracing are only incompletely characterized. Here, we have compared the transduction efficiency of seven commonly used AAV serotypes after intraspinal injection. We specifically analyzed local transduction of different types of dorsal horn neurons, and retrograde transduction of dorsal root ganglia (DRG) neurons and of neurons in the rostral ventromedial medulla (RVM) and the somatosensory cortex (S1). Our results show that most of the tested rAAV vectors have similar transduction efficiency in spinal neurons. All serotypes analyzed were also able to transduce DRG neurons and descending RVM and S1 neurons via their spinal axon terminals. When comparing the commonly used rAAV serotypes to the recently developed serotype 2 capsid variant rAAV2retro, a > 20-fold increase in transduction efficiency of descending supraspinal neurons was observed. Conversely, transgene expression in retrogradely transduced neurons was strongly reduced when the human synapsin 1 (hSyn1) promoter was used instead of the strong ubiquitous hybrid cytomegalovirus enhancer/chicken β-actin promoter (CAG) or cytomegalovirus (CMV) promoter fragments. We conclude that the use of AAV2retro greatly increases transduction of neurons connected to the spinal cord via their axon terminals, while the hSyn1 promoter can be used to minimize transgene expression in retrogradely connected neurons of the DRG or brainstem. Cover Image for this issue: doi. 10.1111/jnc.13813.
J Neurochem, 2017; 142
27343802: Frezel N, Sohet F, Daneman R, Basbaum AI, Braz JM
Peripheral and central neuronal ATF3 precedes CD4+ T-cell infiltration in EAE.
Experimental allergic encephalomyelitis (EAE), an animal model of multiple sclerosis produced by immunization with myelin oligodendrocyte glycoprotein (MOG) and adjuvants, results from profound T-cell mediated CNS demyelination. EAE is characterized by progressive, ascending motor dysfunction and symptoms of ongoing pain and hypersensitivity, in some cases preceding or concomitant with the motor deficits. In this regard, the EAE model mimics major features of multiple sclerosis, where a central neuropathic pain state is common. Although the latter condition is presumed to arise from a CNS loss of inhibitory controls secondary to the demyelination, dysfunction of sensory neurons may also contribute. Based on our previous studies that demonstrated the utility of monitoring expression of activating transcription factor 3 (ATF3), a sensitive marker of injured sensory neurons, here we followed both ATF3 and CD4+ T cells invasion of sensory ganglia (as well as the CNS) at different stages of the EAE model. We found that ATF3 is induced in peripheral sensory ganglia and brainstem well before the appearance of motor deficits. Unexpectedly, the ATF3 induction always preceded T cell infiltration, typically in adjacent, but non-overlapping regions. Surprisingly, control administration of the pertussis toxin and/or Complete Freund's adjuvants, without MOG, induced ATF3 in sensory neurons. In contrast, T cell infiltration only occurred with MOG. Taken together, our results suggest that the clinical manifestations in the EAE result not only from central demyelination but also from neuronal stress and subsequent pathophysiology of sensory neurons.
Exp Neurol, 2016; 283
23134641: Black JA, Frézel N, Dib-Hajj SD, Waxman SG
Expression of Nav1.7 in DRG neurons extends from peripheral terminals in the skin to central preterminal branches and terminals in the dorsal horn.
Sodium channel Nav1.7 has emerged as a target of considerable interest in pain research, since loss-of-function mutations in SCN9A, the gene that encodes Nav1.7, are associated with a syndrome of congenital insensitivity to pain, gain-of-function mutations are linked to the debiliting chronic pain conditions erythromelalgia and paroxysmal extreme pain disorder, and upregulated expression of Nav1.7 accompanies pain in diabetes and inflammation. Since Nav1.7 has been implicated as playing a critical role in pain pathways, we examined by immunocytochemical methods the expression and distribution of Nav1.7 in rat dorsal root ganglia neurons, from peripheral terminals in the skin to central terminals in the spinal cord dorsal

horn.
Mol Pain, 2012; 8

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BOARD NUMBER: S04-495

INVESTIGATION OF AGE-RELATED CHANGES IN THERMAL Aδ FIBERS

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Studies in animals have shown that Aδ fibers, which underlie cold sensations, have an activation range beyond skin temperature, up to 42°C. Studies investigating changes in temperature perception with age have so far never evaluated the cold sensation of elderly people for temperatures above skin temperature (i.e., between 32°C and 42°C). **Aim:** to evaluate the cold sensations conveyed by Aδ fibers over the temperature range 0-40°C in elderly people to better understand the alteration of thermal perception with age. **Method:** two experiments were performed in young adults and healthy seniors. In experiment 1, participants' cold detection threshold was measured with an adaptive staircase method (baseline temperature 40°C). In experiment 2, 10 stimuli between 36 and 0°C (4°C steps, baseline temperature 40°C) were presented 15 times each. We recorded the rate of cold detection and the associated evoked potentials. **Results:** experiment 1 results' showed that, starting from 40°C, younger subjects have an average cold detection threshold higher than elderly subject (i.e. 35°C vs 34°C). Experiment 2 showed that between 24-36°C, cold discrimination was higher in younger subject than elderly, but no differences were observed between both groups below 24°C. Finally, for each stimulus, the latency of N2P2 component would correspond to the conduction velocity of Aδ fibers. **Conclusion:** all participants, regardless of age, perceive cold above skin temperature and this sensation is supported by Aδ fibers' activity. In contrast, cold discrimination is poorer in older subjects in the 24-36°C range, opening new perspectives for assessing age-related changes in cold-sensitive Aδ fibers.

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BOARD NUMBER: S04-496

SUSTAINED MOTOR ACTIVITY TRIGGERED BY DIRECT MECHANOSENSORY STIMULATION

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Sensory feedback is key for efficient locomotion. Mechanosensory structures exist along each leg to assist in motor coordination by transmitting external cues or proprioceptive information to motor centers in the central nervous system. Nevertheless, how different mechanosensory structures engage these locomotor centers and their underlying circuits remains poorly understood. Here we took advantage of the sophisticated *Drosophila* genetic toolkit to address this question. We tested the role of mechanosensory structures in movement initiation by optogenetically stimulating specific classes of leg sensory structures. We found that stimulation of a wide variety of leg mechanosensory structures is sufficient to initiate forward movement in immobile animals. We also found that while the stimulation of the leg Chordotonal Organ depends on the presence of the central brain to trigger forward movement, stimulation of leg Mechanosensory Bristles (MsB) could trigger cyclic motor activity independently of central brain circuits. Also, although leg MsB-triggered movement lacks inter-leg and intra-leg coordination, antagonistic muscle activity is nevertheless preserved. Moreover, we showed that MsB-induced movement is specific to leg afferents, since stimulation of MsB in other structures lead to different outcomes. This study sheds light on the ability of specific sensory circuits to modulate motor control, including initiation of movement, and how this information can be automatically translated to a motor command by central neuronal pathways.

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BOARD NUMBER: S04-497

COMPARATIVE ANALYSIS OF THE DISTRIBUTION, STRUCTURE AND INNERVATION OF PACINIAN CORPUSCLES ACROSS DIFFERENT MAMMALIAN SPECIES.

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Angie Geraldine Cuenu Velasco[1], Dominica De Thomas Wagner[1], Mario Prsa[2], Christophe Lamy[3], Daniel Huber[4]
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Our ability to sense vibrations depends on diverse mechanoreceptors. Among them, Pacinian corpuscles (PCs) are sensitive to high frequency vibrations. Interestingly, the vibration frequencies with the highest sensitivity seem to change across different mammalian species. Whereas humans show the highest sensitivity around 240Hz, mice are most sensitive around 1000Hz. To understand if this difference in vibrotactile tuning can be related to structural aspects of PCs, we conducted a comparative study of the anatomy, distribution and innervation of PCs in the forelimbs of mice, mouse lemurs and humans. To determine the distribution and innervation in situ we adapted whole-limb tissue-clearing, immuno-staining and light-sheet imaging methods. We demonstrate that all three species have dense distributions of PCs around the bones of their forelimbs, which are innervated by the anterior interosseous nerve. Unlike both primate species, mice seem to lack PCs in the glabrous skin of their hands. We also observed a difference in overall size and organization of the outer core across species. Whereas in humans the size of PCs ranged from 100 to 1850µm, mouse lemurs and mice showed much smaller PCs (15 to 200µm, and 20 to 100µm). Finally, we found differences in the shape and size of the inner core, and are currently confirming this with full PC reconstructions using electron microscopy. Although our analysis revealed substantial differences in size and organization of PCs across different mammalian species, more experiments, including single PC electrophysiology, might be necessary to relate these differences to changes in the best frequency tuning observed.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-498

TOWARDS SENSATION RESTORATION THROUGH ELECTRICAL STIMULATION IN DIABETICS

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Lauren Chee, Noemi Gozzi, Andrea Cimolato, Giacomo Valle, Stanisa Raspopovic
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Aims Diabetic Peripheral Neuropathy (DPN) patients suffer from several comorbidities related to their condition including sensory loss which can lead to reduced balance and mobility. The aim of this study is to provide a preliminary understanding of sensory loss and its restoration through transcutaneous electrical nerve stimulation (TENS) to improve treatment. **Methods** The somatosensory function of healthy and diabetic volunteers was evaluated through quantitative sensory testing (QST) and their nerves were stimulated in the peroneal, tibial, and calcaneal branches that innervate the foot dorsum and sole. The sensitivity of these nerves to electrical stimulation was analyzed through just noticeable difference (JND). The overlap between loss (QST) and restored sensation (TENS) was analyzed in diabetic patients and a comparison in sensitivity between healthy and diabetic patients was drawn. **Results** TENS was shown to partially restore lost sensation in diabetic patients. However, the sensitivity of their nerves was shown to be significantly lower with respect to healthy volunteers. **Conclusions** TENS was able to restore lost sensations in individuals with altered sensation or sensory loss in their feet due to nerve damage. Intuitively, the sensitivity of their damaged nerves is lower than in healthy volunteers, but the successful restoration of some lost sensation area shows promise for TENS as an assistive or therapeutic intervention in DPN patients moving forward.

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BOARD NUMBER: S04-499

DO NOXIOUS STIMULUS NS4 COUD BE A SENSORY ECLIPSE: A SENSORY MODULATION DISORDER?

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Introduction: Plantar irritating stimuli: Noxious stimuli type 4 (Ns4) describes in 1945, witch exacerbating reflex mechanisms, increase or maintain an amplification of related influxes perpetuating or amplifying the nociceptive reflex. Ns4 is defined by: 1) pain revealed by pressure but no expressed, 2) asymmetrical perception of pain on the Ns4 area, 3) loss of spatial discrimination and somesthesia. Pressure/pain plantar thresholds are mediated by C-, Aδ- and Aβ-fiber and could induces sensory modulation dysfunction. Following the beneficial effects of sensorimotor orthoses/insoles, we investigating whether Ns4 could be a “sensory eclipse”. *Method:* 80 subjects with Ns4 (first metatarsal head) and 10 without (control, C) were included. Stimuli consisted of 2-point discriminator delivered: static (at the same time, S) and dynamic: distal to proximal (DP) and proximal to distal (PD) with a randomise distances of 5, 7, 9 mm determined initial situation and after 4 weeks of sensorimotor insoles wearing to Ns4 group. *Result:* significant were observed on S DP and PD between t0 and t4 to Ns4.The reduction of the loos of 7 and 9 mm were higher than 5 mm. The discrimination of 7 and 9 mm were higher in DP > PD than S. These effects did not vary on C. *Discussion:* Results indicates that the spatial configuration of Ns4 noxious stimulation may critically influence sensory modulation of local excitatory/inhibitory activity, and underscores the importance of inhibition during nociceptive processing. After treatment, perception/discrimination of two-point is less than control that we could purpose Ns4 would be a sensory Eclipse.

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BOARD NUMBER: S04-500

TRANSFORMATION OF NEURAL CODING FOR VIBROTACTILE SENSATION ALONG THE ASCENDING SOMATOSENSORY PATHWAY

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Perceiving substrate vibrations is a fundamental component of somatosensation. In mammals, action potentials fired by Pacinian corpuscle afferents are known to reliably time lock to the cycles of a vibration. We recently found that neurons in the somatosensory cortex of mice encode vibration frequency with a rate code tuned to a preferred value. Such surprising feature-selective rate coding raises an important question: how are cyclically entrained action potentials (a temporal code), in afferents of peripheral mechanoreceptors, transformed along the ascending neuraxis into a rate code in the cortex? Here, we traced this transformation with electrophysiological recordings and two-photon calcium imaging along all stages of the ascending somatosensory pathway: primary sensory afferents (mechanoreceptors), dorsal root ganglia, dorsal column nuclei, thalamus and cortex. First, recordings from nerve fibers of primary sensory neurons in lightly anesthetized mice showed that rapidly adapting mechanosensitive units (RAII) display phase-locked spiking for vibrations up to 2000 Hz. This precise temporal code was also found in dorsal column nuclei, but not in thalamus. Second, diverse V-shaped vibrotactile sensitivity curves could be derived from RAII responses and their combination closely resembled perceptual threshold curves obtained with behavioral experiments. Finally, calcium imaging in dorsal column nuclei revealed a topological organization of vibration frequency related to the somatotopic arrangement. By comparing the neural codes at different stages of the somatosensory pathway, we discovered that major neural signal transformations occur between dorsal column nuclei and thalamus. This finding allows modeling the underlying computational principle to reveal novel features of vibrotactile sensation.

Pubmed:
34687665: Sedigh-Sarvestani M, Lee KS, Jaepel J, Satterfield R, Shultz N, Fitzpatrick D
A sinusoidal transformation of the visual field is the basis for periodic maps in area V2.
Retinotopic maps of many visual areas are thought to follow the fundamental principles described for the primary visual cortex (V1), where nearby points on the retina map to nearby points on the surface of V1, and orthogonal axes of the retinal surface are represented along orthogonal axes of the cortical surface. Here we demonstrate a striking departure from this mapping in the secondary visual area (V2) of the tree shrew best described as a sinusoidal transformation of the visual field. This sinusoidal topography is ideal for achieving uniform coverage in an elongated area like V2, as predicted by mathematical models designed for wiring minimization, and provides a novel explanation for periodic banded patterns of intra-cortical connections and functional response properties in V2 of tree shrews as well as several other species. Our findings suggest that cortical circuits flexibly implement solutions to sensory surface representation, with dramatic consequences for large-scale cortical organization.
Neuron, 2021; 109
34504074: Prsa M, Kilicel D, Nourizonoz A, Lee KS, Huber D
A common computational principle for vibrotactile pitch perception in mouse and human.
We live surrounded by vibrations generated by moving objects. These oscillatory stimuli propagate through solid substrates, are sensed by mechanoreceptors in our body and give rise to perceptual attributes such as vibrotactile pitch (i.e. the perception of how high or low a vibration's frequency is). Here, we establish a mechanistic relationship between vibrotactile pitch perception and the physical properties of vibrations using behavioral tasks, in which vibratory stimuli were delivered to the human fingertip or the mouse forelimb. The resulting perceptual reports were analyzed with a model demonstrating that physically different combinations of vibration frequencies and amplitudes can produce equal pitch perception. We found that the perceptually indistinguishable but physically different stimuli follow a common computational principle in mouse and human. It dictates that vibrotactile pitch perception is shifted with increases in amplitude toward the frequency of highest vibrotactile sensitivity. These findings suggest the existence of a fundamental relationship between the seemingly unrelated

concepts of spectral sensitivity and pitch perception.
Nat Commun, 2021; 12
30658859: Lee KS, Vandemark K, Mezey D, Shultz N, Fitzpatrick D
Functional Synaptic Architecture of Callosal Inputs in Mouse Primary Visual Cortex.
Callosal projections are thought to play a critical role in coordinating neural activity between the cerebral hemispheres in placental mammals, but the rules that govern the arrangement of callosal synapses on the dendrites of their target neurons remain poorly understood. Here we describe a high-throughput method to map the functional organization of callosal connectivity by combining in vivo 3D random-access two-photon calcium imaging of the dendritic spines of single V1 neurons with optogenetic stimulation of the presynaptic neural population in the contralateral hemisphere. We find that callosal-recipient spines are more likely to cluster with non-callosal-recipient spines with similar orientation preference. These observations, based on optogenetic stimulation, were confirmed by direct anatomical visualization of callosal synaptic connections using post hoc expansion microscopy. Our results demonstrate, for the first time, that functional synaptic clustering in a short dendritic segment could play a role in integrating distinct neuronal circuits.
Neuron, 2019; 101
27120162: Lee KS, Huang X, Fitzpatrick D
Topology of ON and OFF inputs in visual cortex enables an invariant columnar architecture.
Circuits in the visual cortex integrate the information derived from separate ON (light-responsive) and OFF (dark-responsive) pathways to construct orderly columnar representations of stimulus orientation and visual space. How this transformation is achieved to meet the specific topographic constraints of each representation remains unclear. Here we report several novel features of ON-OFF convergence visualized by mapping the receptive fields of layer 2/3 neurons in the tree shrew (Tupaia belangeri) visual cortex using two-photon imaging of GCaMP6 calcium signals. We show that the spatially separate ON and OFF subfields of simple cells in layer 2/3 exhibit topologically distinct relationships with the maps of visual space and orientation preference. The centres of OFF subfields for neurons in a given region of cortex are confined to a compact region of visual space and display a smooth visuotopic progression. By contrast, the centres of the ON subfields are distributed over a wider region of visual space, display substantial visuotopic scatter, and have an orientation-specific displacement consistent with orientation preference map structure. As a result, cortical columns exhibit an invariant aggregate receptive field structure: an OFF-dominated central region flanked by ON-dominated subfields. This distinct arrangement of ON and OFF inputs enables continuity in the mapping of both orientation and visual space and the generation of a columnar map of absolute spatial phase.
Nature, 2016; 533
22390899: Lee KS, Huang YH, Yen CT
Periaqueductal gray stimulation suppresses spontaneous pain behavior in rats.
Methods for evaluating analgesic effect for spontaneous pain are increasingly important because it is reported by most patients with neuropathic pain. The present study assessed the analgesic effects of periaqueductal gray (PAG) stimulation in the spared nerve injury (SNI) model of neuropathic pain of the rat. Spontaneous rapid paw withdrawal movements were used as the index of spontaneous pain. Deep-brain stimulation in the PAG was performed in rats 3 weeks after SNI. Significant analgesic effects on spontaneous pain behavior were observed at the same stimulation parameter that reversed the reduced mechanical threshold of the von Frey test. Both analgesic effects lasted 30-40min beyond the 3min stimulation period. In summary, PAG stimulation was effective in alleviating spontaneous pain and mechanical allodynia in the SNI rat. The frequency of spontaneous paw lifting, a behavioral index of spontaneous pain used in this study, will be useful for future testing of therapeutic methods.
Neurosci Lett, 2012; 514

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BOARD NUMBER: S04-501

MECHANISM-BASED APPROACH TO CORRECT ATYPICAL SENSORY INFORMATION PROCESSING IN VIVO IN A MOUSE MODEL OF AUTISM

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Atypical sensory experience occurs almost universally in patients with Autism Spectrum Disorders (ASD) and is a strong determinant of other ASD symptoms. The sense of touch, known as somatosensation, is majorly affected in ASD. Fragile X Syndrome is the leading monogenic cause of ASD, and Fmr1-knockout (*Fmr1*$^{-/y}$) mice exhibit atypical processing of tactile sensory stimulation. Previously, we found a link between dysfunctional large-conductance, voltage- and calcium-sensitive potassium channels (BK_{Ca}), somatosensory cortex (S1) hyperexcitability, and sensory hypersensitivity. Aim: This study aimed to correct atypical tactile processing in the L2/3 pyramidal neurons (PN) of the S1-hindpaw (HP) region *in vivo* by pharmacologically targeting BK_{Ca} channels. Methods: We performed *in vivo* whole-cell patch-clamp electrophysiological recordings from S1-HP L2/3 PN in anesthetized *Fmr1*$^{-/y}$ mice, locally applied a specific, blood-brain barrier impermeant BK_{Ca} agonist, BMS191011, and recorded sensory responses to tactile stimuli. Results: Neocortical application of BMS191011 was surprisingly efficient in correcting several alterations in *Fmr1*$^{-/y}$ L2/3 PN. This included correcting the action potential half-width increase, a prominent neuronal hyperexcitability feature of *Fmr1*$^{-/y}$ PN, reducing the exaggerated spontaneous firing rate, and rescuing the stimuli-evoked EPSP parameters. Additionally, BMS191011 significantly diminished the increased variability of features across both the *Fmr1*$^{-/y}$ neuron population and successive stimulation trials. Few parameters, including a cellular source of noise were not corrected. Conclusions: Overall, our data demonstrate that local BMS191011 application can dampen the excitability of the S1 network, and provide strong evidence for the neocortex-level origin of at least certain atypical sensory information processing aspects in *Fmr1*$^{-/y}$ mice.

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BOARD NUMBER: S04-502

ENCODING OF FORELIMB CONSCIOUS PROPRIOCEPTION IN THE MOUSE SOMATOSENSORY CORTEX

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Proprioception is the sensation of where our limbs and body are positioned in space. Despite its importance for accurate movements, a clear understanding of how conscious proprioceptive information is represented in the mammalian cortex remains elusive. To identify where and how proprioceptive stimuli are encoded in the mouse cortex we performed 2-photon calcium imaging during passive forelimb displacement with a robotic manipulandum and cortical optogenetic silencing during a proprioceptive 2AFC discrimination task. Our results show that proprioceptive information is not only encoded in the primary forelimb somatosensory cortex, but also in the adjacent dysgranular/transitional zones and motor areas. These cortical responses have a strong directional tuning, are sensitive to the amplitude of movement but surprisingly not to its velocity. A model based on joints positions of the limb (tracked with X-ray videography) was found to better explain the neuronal activity compared to a model based on endpoint position only. Our results suggest that the mouse homologue of the primate proprioceptive area 3a comprises several overlapping somatosensory/motor cortical areas rather than a single functionally defined zone. A further understanding of the underlying neuronal codes and pathways is the necessary first step towards the development of biomimetic neuro-prosthetics.

Pubmed:
31980584: Alonso I, Sanchez Merlinsky A, Szczupak L
Phase-Specific Motor Efference during a Rhythmic Motor Pattern.
Neuronal circuits that control motor behaviors orchestrate multiple tasks, including the inhibition of self-generated sensory signals. In the hermaphroditic leech, T and P mechanosensory neurons respond to light touch and pressure on the skin, respectively. We show that the low threshold T cells were also sensitive to topological changes of the animal surface, caused by contraction of the muscles that erect the skin annuli. P cells were unresponsive to this movement. Annuli erection is part of the contraction phase of crawling, a leech locomotive behavior. In isolated ganglia, T cells showed phase-dependent IPSPs during dopamine-induced fictive crawling, whereas P cells were unaffected. The timing and magnitude of the T-IPSPs were highly correlated with the activity of the motoneurons excited during the contraction phase. Together, the results suggest that the central network responsible for crawling sends a reafferent signal onto the T cells, concomitant with the signal to the motoneurons. This reafference is specifically targeted at the sensory neurons that are affected by the movements; and it is behaviorally relevant as excitation of T cells affected the rhythmic motor pattern, probably acting upon the rhythmogenic circuit. Corollary discharge is a highly conserved function of motor systems throughout evolution, and we provide clear evidence of the specificity of its targets and timing and of the benefit of counteracting self-generated sensory input. Neuronal circuits that control motor behaviors orchestrate multiple tasks, including inhibition of sensory signals originated by the animal movement, a phenomenon known as corollary discharge. Leeches crawl on solid surfaces through a sequence of elongation and contraction movements. During the contraction, the skin topology changes, affecting a subpopulation of mechanosensory receptors, T (touch) neurons, but not P (pressure) sensory neurons. In the isolated nervous system, T neurons were inhibited during the contraction but not during the elongation phase, whereas P cells were unaffected throughout crawling. Excitation of T cells during the contraction phase temporarily disrupted the rhythmic pattern. Thus, corollary discharge was target (T vs P) and phase (contraction vs elongation) specific, and prevented self-generated signals to perturb motor behaviors.
J Neurosci, 2020; 40

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BOARD NUMBER: S04-503

CORTICAL REORGANIZATION AFTER SPINAL CORD INJURY IS LAYER-SPECIFIC AND TIME-DEPENDENT

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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The cerebral cortex is organized in six layers characterized by cellular composition, input/output connections and network properties. The spontaneous activity and evoked responses to sensory inputs depends on specific layers. A spinal cord injury (SCI) produces a massive sensory deafferentation of the somatosensory cortex (S1) which alters cortical activity. The main aim of this work is to identify the acute and chronic physiological alterations that take place at each cortical layer after SCI. For this purpose, experiments were performed in anesthetized adult rats under control condition (sham lesion), acute SCI and chronic SCI. A vertical array of 32 microelectrodes was lowered into S1 allowing to obtain extracellular recordings of neuronal activity from each cortical layer. First experimental approach was used to study acute effects of complete thoracic SCI. For this purpose, the recordings were obtained continuously under control and immediately after SCI. Second experimental approach was used to study chronic effects of SCI by weekly recordings from injured and sham groups. Results indicate that under acute SCI the infragranular layers significantly increased their response magnitude to peripheral stimulation while supragranular layers showed increased higher frequencies content and also generate more activated states in S1. On the contrary, under chronic SCI conditions supragranular layers returned to control levels of spontaneous activity excitability, while infragranular layers increased them progressively. In conclusion, SCI alters cortical oscillations in individual cortical layers which indicate changes in the network excitability in a time-dependent manner. Cortical reorganization after SCI emerges from additive effects across layers.

Pubmed:
34418097: Zaforas M, Rosa JM, Alonso-Calviño E, Fernández-López E, Miguel-Quesada C, Oliviero A, Aguilar J
Cortical layer-specific modulation of neuronal activity after sensory deprivation due to spinal cord injury.
Cortical areas have the capacity of large-scale reorganization following sensory deafferentation. However, it remains unclear whether this phenomenon is a unique process that homogeneously affects the entire deprived cortical region or whether it is susceptible to changes depending on neuronal networks across distinct cortical layers. Here, we studied how the local circuitry within each layer of the deafferented cortex forms the basis for neuroplastic changes after immediate thoracic spinal cord injury (SCI) in anaesthetized rats. In vivo electrophysiological recordings from deafferented hindlimb somatosensory cortex showed that SCI induces layer-specific changes mediating evoked and spontaneous activity. In supragranular layer 2/3, SCI increased gamma oscillations and the ability of these neurons to initiate up-states during spontaneous activity, suggesting an altered corticocortical network and/or intrinsic properties that may serve to maintain the excitability of the cortical column after deafferentation. On the other hand, SCI enhanced the infragranular layers' ability to integrate evoked sensory inputs leading to increased and faster neuronal responses. Delayed evoked response onsets were also observed in layer 5/6, suggesting alterations in thalamocortical connectivity. Altogether, our data indicate that SCI immediately modifies the local circuitry within the deafferented cortex allowing supragranular layers to better integrate spontaneous corticocortical information, thus modifying column excitability, and infragranular layers to better integrate evoked sensory inputs to preserve subcortical outputs. These layer-specific neuronal changes may guide the long-term alterations in neuronal excitability and plasticity associated with the rearrangements of somatosensory networks and the appearance of central sensory pathologies usually associated with spinal cord injury. KEY POINTS: Sensory stimulation of forelimb produces cortical evoked responses in the somatosensory hindlimb cortex in a layer-dependent manner. Spinal cord injury favours the input statistics of corticocortical connections between intact and deafferented cortices. After spinal cord injury supragranular layers exhibit better integration of spontaneous corticocortical information while infragranular layers exhibit better integration of evoked sensory stimulation. Cortical reorganization is a layer-specific phenomenon.
J Physiol, 2021; 599

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BOARD NUMBER: S04-504

SENSORY INPUT MODULATION RESCUES ALTERATIONS IN PARVALBUMIN CELL CONNECTIVITY AND TEXTURE DISCRIMINATION CAUSED BY TSC1 HAPLOINSUFFICIENCY

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Autism spectrum disorders (ASDs) are characterized by social interaction and communication difficulties as well as repetitive or restricted behaviour. However, a growing number of reports suggest that sensory processing is also affected in a majority of patients with ASDs. In fact, sensory abnormalities are now commonly recognized as diagnostic criteria in ASDs. About 90% of ASD individuals have atypical sensory experiences, described as both hyper- and hypo-reactivity, with abnormal responses to tactile stimulation representing a very frequent finding. However, the neurobiological mechanisms that underlie impaired sensory processing associated with ASDs are poorly understood. Dysregulations of the Mechanistic Target Of Rapamycin Complex 1 (mTORC1) signalling pathway are frequently associated with ASDs, however its implications in sensory processing deficits are less clear. Here, we show that haploinsufficiency of the mTORC1 negative regulator Tsc1 restricted to parvalbumin-expressing GABAergic cells (PV cells) is sufficient to cause increased texture discrimination and whisker-evoked responses in somatosensory cortex. We further find that cortical and thalamocortical glutamatergic inputs to PV cells are selectively reduced in adult mice, whereas these inputs did not appear affected yet in pre-adolescent mice. Finally, we identify a sensitive postnatal window during which limiting sensory inputs by whisker trimming is sufficient to normalize PV cell connectivity in pre-adolescent mice, while rescuing deficits in both PV cell outputs and cortical glutamatergic inputs as well as texture discrimination hyper-sensitivity in adult conditional haploinsufficient mice. These results suggest that manipulating inhibition may help ameliorating sensory processing in disorders associated with dysregulated mTORC1 signalling.

Pubmed:

34135323: Amegandjin CA, Choudhury M, Jadhav V, Carriço JN, Quintal A, Berryer M, Snapyan M, Chattopadhyaya B, Saghatelyan A, Di Cristo G
Sensitive period for rescuing parvalbumin interneurons connectivity and social behavior deficits caused by TSC1 loss.
The Mechanistic Target Of Rapamycin Complex 1 (mTORC1) pathway controls several aspects of neuronal development. Mutations in regulators of mTORC1, such as Tsc1 and Tsc2, lead to neurodevelopmental disorders associated with autism, intellectual disabilities and epilepsy. The correct development of inhibitory interneurons is crucial for functional circuits. In particular, the axonal arborisation and synapse density of parvalbumin (PV)-positive GABAergic interneurons change in the postnatal brain. How and whether mTORC1 signaling affects PV cell development is unknown. Here, we show that Tsc1 haploinsufficiency causes a premature increase in terminal axonal branching and bouton density formed by mutant PV cells, followed by a loss of perisomatic innervation in adult mice. PV cell-restricted Tsc1 haploinsufficient and knockout mice show deficits in social behavior. Finally, we identify a sensitive period during the third postnatal week during which treatment with the mTOR inhibitor Rapamycin rescues deficits in both PV cell innervation and social behavior in adult conditional haploinsufficient mice. Our findings reveal a role of mTORC1 signaling in the regulation of the developmental time course and maintenance of cortical PV cell connectivity and support a mechanistic basis for the targeted rescue of autism-related behaviors in disorders associated with deregulated mTORC1 signaling.
Nat Commun, 2021; 12
34758338: Mirabella F, Desiato G, Mancinelli S, Fossati G, Rasile M, Morini R, Markicevic M, Grimm C, Amegandjin C, Termanini A, Peano C, Kunderfranco P, di Cristo G, Zerbi V, Menna E, Lodato S, Matteoli M, Pozzi D
Prenatal interleukin 6 elevation increases glutamatergic synapse density and disrupts hippocampal connectivity in offspring.
Early prenatal inflammatory conditions are thought to be a risk factor for different neurodevelopmental disorders. Maternal interleukin-6 (IL-6) elevation during pregnancy causes abnormal behavior in offspring, but whether these defects result from altered synaptic developmental trajectories remains unclear. Here we showed that transient IL-6 elevation via injection into pregnant mice or developing embryos enhanced glutamatergic synapses and led to overall brain hyperconnectivity in

offspring into adulthood. IL-6 activated synaptogenesis gene programs in glutamatergic neurons and required the transcription factor STAT3 and expression of the RGS4 gene. The STAT3-RGS4 pathway was also activated in neonatal brains during poly(I:C)-induced maternal immune activation, which mimics viral infection during pregnancy. These findings indicate that IL-6 elevation at early developmental stages is sufficient to exert a long-lasting effect on glutamatergic synaptogenesis and brain connectivity, providing a mechanistic framework for the association between prenatal inflammatory events and brain neurodevelopmental disorders.
Immunity, 2021; 54

30169756: Awad PN, Amegandjin CA, Szczurkowska J, Carriço JN, Fernandes do Nascimento AS, Baho E, Chattopadhyaya B, Cancedda L, Carmant L, Di Cristo G
KCC2 Regulates Dendritic Spine Formation in a Brain-Region Specific and BDNF Dependent Manner.
KCC2 is the major chloride extruder in neurons. The spatiotemporal regulation of KCC2 expression orchestrates the developmental shift towards inhibitory GABAergic drive and the formation of glutamatergic synapses. Whether KCC2's role in synapse formation is similar in different brain regions is unknown. First, we found that KCC2 subcellular localization, but not overall KCC2 expression levels, differed between cortex and hippocampus during the first postnatal week. We performed site-specific in utero electroporation of KCC2 cDNA to target either hippocampal CA1 or somatosensory cortical pyramidal neurons. We found that a premature expression of KCC2 significantly decreased spine density in CA1 neurons, while it had the opposite effect in cortical neurons. These effects were cell autonomous, because single-cell biolistic overexpression of KCC2 in hippocampal and cortical organotypic cultures also induced a reduction and an increase of dendritic spine density, respectively. In addition, we found that the effects of its premature expression on spine density were dependent on BDNF levels. Finally, we showed that the effects of KCC2 on dendritic spine were dependent on its chloride transporter function in the hippocampus, contrary to what was observed in cortex. Altogether, these results demonstrate that KCC2 regulation of dendritic spine development, and its underlying mechanisms, are brain-region specific.
Cereb Cortex, 2018; 28

26780036: Amegandjin CA, Jammow W, Laforest S, Riad M, Baharnoori M, Badeaux F, DesGroseillers L, Murai KK, Pasquale EB, Drolet G, Doucet G
Regional expression and ultrastructural localization of EphA7 in the hippocampus and cerebellum of adult rat.
EphA7 is expressed in the adult central nervous system (CNS), where its roles are yet poorly defined. We mapped its distribution using in situ hybridization (ISH) and immunohistochemistry (IHC) combined with light (LM) and electron microscopy (EM) in adult rat and mouse brain. The strongest ISH signal was in the hippocampal pyramidal and granule cell layers. Moderate levels were detected in habenula, striatum, amygdala, the cingulate, piriform and entorhinal cortex, and in cerebellum, notably the Purkinje cell layer. The IHC signal distribution was consistent with ISH results, with transport of the protein to processes, as exemplified in the hippocampal neuropil layers and weakly stained pyramidal cell layers. In contrast, in the cerebellum, the Purkinje cell bodies were the most strongly immunolabeled elements. EM localized the cell surface-expression of EphA7 essentially in postsynaptic densities (PSDs) of dendritic spines and shafts, and on some astrocytic leaflets, in both hippocampus and cerebellum. Perikaryal and dendritic labeling was mostly intracellular, associated with the synthetic and trafficking machineries. Immunopositive vesicles were also observed in axons and axon terminals. Quantitative analysis in EM showed significant differences in the frequency of labeled elements between regions. Notably, labeled dendrites were ~3-5 times less frequent in cerebellum than in hippocampus, but they were individually endowed with ~10-40 times higher frequencies of PSDs, on their shafts and spines. The cell surface localization of EphA7, being preferentially in PSDs, and in perisynaptic astrocytic leaflets, provides morphologic evidence that EphA7 plays key roles in adult CNS synaptic maintenance, plasticity, or function. J. Comp. Neurol. 524:2462-2478, 2016.
J Comp Neurol, 2016; 524

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BOARD NUMBER: S04-505

DEMYELINATION IMPAIRS LAYER 5 CORTICOTHALAMIC FEEDBACK IN THE SOMATOSENSORY SYSTEM

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Myelination is essential for temporally precise AP propagation. There is emerging evidence that myelin sheath properties can differ widely across brain regions to fine tune temporal delays. Here, we studied the role of L5-to-PoM myelination in timing of corticothalamic feedback *in vitro* and *in vivo*. Using a retrograde AAV targeting approach in combination with a L5-specific Cre-driver mouse line (Rbp4-Cre), we investigated morphological parameters of nodes of Ranvier (noR) and internodes along the axonal projections within the cortex, corpus callosum, striatum and thalamus. After 6 weeks of cuprizone-induced demyelination, we observed, besides internode myelin loss, a diverse pattern of disassembly of nodal compartments, most prominently in the cortex (~20%). To test how demyelination-induced changes in axon architecture affect information transmission we used optogenetic stimulation of L5-to-PoM axons in acute brain slices. The results showed a reduced conduction velocity in demyelinated L5-to-PoM axons and a frequency-dependent impairment of synaptic transmission (20% at 20Hz). Furthermore, to investigate whether corticothalamic feedback to PoM is impaired *in vivo,* we are performing juxtacellular recordings of PoM neurons *in vivo*. In addition, we are using extracellular recordings with Neuropixels probes to record at high-density in S1 and PoM. Strikingly, our preliminary data suggests a global increase of spontaneous activity and an increased delay (~50%) as well as a strong jitter of spike-timing after demyelination. We are currently performing dual whisker- and optogenetic stimulations to test sensory detection and the integration of ascending and descending inputs. Taken together, demyelination disrupts sensory computational processing of the corticothalamic circuit.

Pubmed:
28739523: Jamann N, Jordan M, Engelhardt M
Activity-dependent axonal plasticity in sensory systems.
The rodent whisker-to-barrel cortex pathway is a classic model to study the effects of sensory experience and deprivation on neuronal circuit formation, not only during development but also in the adult. Decades of research have produced a vast body of evidence highlighting the fundamental role of neuronal activity (spontaneous and/or sensory-evoked) for circuit formation and function. In this context, it has become clear that neuronal adaptation and plasticity is not just a function of the neonatal brain, but persists into adulthood, especially after experience-driven modulation of network status. Mechanisms for structural remodeling of the somatodendritic or axonal domain include microscale alterations of neurites or synapses. At the same time, functional alterations at the nanoscale such as expression or activation changes of channels and receptors contribute to the modulation of intrinsic excitability or input-output relationships. However, it remains elusive how these forms of structural and functional plasticity come together to shape neuronal network formation and function. While specifically somatodendritic plasticity has been studied in great detail, the role of axonal plasticity, (e.g. at presynaptic boutons, branches or axonal microdomains), is rather poorly understood. Therefore, this review will only briefly highlight somatodendritic plasticity and instead focus on axonal plasticity. We discuss (i) the role of spontaneous and sensory-evoked plasticity during critical periods, (ii) the assembly of axonal presynaptic sites, (iii) axonal plasticity in the mature brain under baseline and sensory manipulation conditions, and finally (iv) plasticity of electrogenic axonal microdomains, namely the axon initial segment, during development and in the mature CNS.
Neuroscience, 2018; 368
33397944: Jamann N, Dannehl D, Lehmann N, Wagener R, Thielemann C, Schultz C, Staiger J, Kole MHP, Engelhardt M
Sensory input drives rapid homeostatic scaling of the axon initial segment in mouse barrel cortex.
The axon initial segment (AIS) is a critical microdomain for action potential initiation and implicated in the regulation of neuronal excitability during activity-dependent plasticity. While structural AIS plasticity has been suggested to fine-tune neuronal activity when network states change, whether it acts in vivo as a homeostatic regulatory mechanism in behaviorally relevant contexts remains poorly understood. Using the mouse whisker-to-barrel pathway as a model system in combination

with immunofluorescence, confocal analysis and electrophysiological recordings, we observed bidirectional AIS plasticity in cortical pyramidal neurons. Furthermore, we find that structural and functional AIS remodeling occurs in distinct temporal domains: Long-term sensory deprivation elicits an AIS length increase, accompanied with an increase in neuronal excitability, while sensory enrichment results in a rapid AIS shortening, accompanied by a decrease in action potential generation. Our findings highlight a central role of the AIS in the homeostatic regulation of neuronal input-output relations.
Nat Commun, 2021; 12
29305051: Engelhardt M, Hamad MIK, Jack A, Ahmed K, König J, Rennau LM, Jamann N, Räk A, Schönfelder S, Riedel C, Wirth MJ, Patz S, Wahle P
Interneuron synaptopathy in developing rat cortex induced by the pro-inflammatory cytokine LIF.
Exp Neurol, 2018; 302

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BOARD NUMBER: S04-506

CT-FIBERS DENSITY AND NERVE EFFECTS ON CORTICAL TACTILE PROCESSING: A SOMATOSENSORY EVOKED POTENTIALS (SEP) STUDY

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Aims: Somatosensory evoked potentials (SEP) studies essentially characterized short latency components following mainly median nerve stimulations. However, these studies rarely consider 1) the density of C-Tactile fibers, which convey affective information, at the stimulation site, 2) potential difference due to the nerve being stimulated, and 3) middle latency (>30 ms) components. Our aim was to investigate middle latency SEPs following simple mechanical stimulation of two nerves and two skin areas of different CT density. Methods: 18 adults (mean age in years ± sd: 23.2 ± 2.7, 9 males) received 400 vibrotactile stimulations over 4 territories of the right hand (2 nerves: radial/median; 2 CT densities: high (hairy skin)/low (glabrous skin)). A 64-channel ActiveTwo system (Biosemi®, The Netherlands) was used for EEG recording. Results: Four middle latency components were identified: P50, N80, N130 and P200. Significant shorter latency and larger amplitude were found over the contralateral hemisphere. An important fiber effect was found for the N80: high-CT density skin stimulations induced larger amplitude than low-CT density skin stimulations ($F(17) = 15.898$, $p<0.001$). Regarding nerve effects, P50 and N80 amplitudes were more important for median territory than for radial's ($F(17) = 5.946$; $p<0.05$ and $F(17) = 6.336$; $p<0.05$ respectively); N80 latency was longer after median nerve stimulations than radial nerve's ones ($F(17) = 8.266$; $p<0.05$). Conclusions: This study highlighted a CT-fibers density effect, conveying affective information, on the N80. Furthermore, median and radial nerves' stimulations differently affected components before 100 milliseconds, possibly reflecting somatotopy.

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BOARD NUMBER: S04-507

AN ALE META-ANALYSIS OF TEXTURE PERCEPTION

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Aims Humans use active touch to perceive surface characteristics, such as texture, through low-threshold mechanoreceptors in the skin. Previous fMRI studies suggest activations in the primary and secondary somatosensory cortices, primary motor cortex and the premotor cortex for texture perception during active touch. This meta-analysis aimed to collate research articles, using fMRI methods, to identify brain regions associated with texture perception of various stimuli during active touch exploration. Methods A coordinate-based meta-analysis (activation likelihood estimation, ALE) was performed to investigate brain areas implicated in texture discrimination when compared to a non-haptic baseline (e.g. rest or visual control) or to a haptic baseline (e.g. haptic shape or orientation), the latter contrast was employed to highlight areas active due texture processing only. Nine fMRI studies (155 participants; 136 foci) were selected by systematic review. ALE analysis was performed and conjunction and contrast analyses compared processing of texture. Results and conclusions ALE analysis revealed activations in the SI, inferior parietal lobule (IPL), ventral premotor area (PMv) and supramarginal gyrus (SMG) for the texture > non-haptic baseline contrast, as well as the posterior insula (PI) and secondary visual area (VII) for texture > haptic baseline. Activation in the SI and PMv accords with previous investigations of tactile stimulation and voluntary movement. Interestingly, activation identified in the PI may demonstrate sensory processing specific to texture. This meta-analysis reaffirms the role of sensorimotor and posterior parietal regions and uncovered the possible role of the PI in discriminative touch, which has not been elucidated in previous findings.

Pubmed:
31610438: Ogden RS, Henderson J, Slade K, McGlone F, Richter M
The effect of increased parasympathetic activity on perceived duration.
Theories of human temporal perception suggest that changes in physiological arousal distort the perceived duration of events. Behavioural manipulations of sympathetic nervous system (SNS) activity support this suggestion, however the effects of behavioural manipulations of parasympathetic (PSNS) activity on time perception are unclear. The current study examined the effect of a paced respiration exercise known to increase PSNS activity on sub-second duration estimates. Participants estimated the duration of negatively and neutrally valenced images following a period of normal and paced breathing. PSNS and SNS activity were indexed by high-frequency heart-rate variability and pre-ejection period respectively. Paced breathing increased PSNS activity and reduced the perceived duration of the negative and neutrally valenced stimuli relative to normal breathing. The results show that manipulations of PSNS activity can distort time in the absence of a change in SNS activity. They also suggest that activities which increase PSNS activity may be effective in reducing the perceived duration of short events.
Conscious Cogn, 2019; 76
31083698: Ogden RS, Henderson J, McGlone F, Richter M
Time distortion under threat: Sympathetic arousal predicts time distortion only in the context of negative, highly arousing stimuli.
Theoretical models of time perception suggest a simple bottom-up relationship between physiological arousal and perceived duration. Increases in physiological arousal lengthen the perceived duration of events whereas decreases in physiological arousal reduce them. Whilst this relationship has been demonstrated for highly arousing negatively valenced stimuli, it has not been demonstrated for other classes of distorting stimuli (e.g. positively valenced or low arousal stimuli). The current study tested the effect of valence (positive and negative) and arousal level (high and low) on the relationship between physiological arousal and perceived duration. Sympathetic nervous system (SNS) and parasympathetic nervous system (PSNS) activity was measured during a verbal estimation task in which participants judged the duration of high and low

arousal, positive, negative and neutrally valenced IAPS images. SNS and PSNS activity were indexed by measuring Pre-Ejection Period (PEP) and High Frequency Heart-rate Variability (HF-HRV) respectively. SNS reactivity was predicative of perceived duration, but only for high arousal negatively valenced stimuli, with decreases in PEP being associated with longer duration estimates. SNS and PSNS activity was not predictive of perceived duration for the low arousal negative stimuli or the low and high arousal positive stimuli. We therefore propose a new model suggesting that emotional distortions to time result from a combination of bottom-up (physiological arousal) and top-down (threat detection) factors.
PLoS One, 2019; 14

34425248: Mari T, Henderson J, Maden M, Nevitt S, Duarte R, Fallon N
Systematic Review of the Effectiveness of Machine Learning Algorithms for Classifying Pain Intensity, Phenotype or Treatment Outcomes Using Electroencephalogram Data.
Recent attempts to utilize machine learning (ML) to predict pain-related outcomes from Electroencephalogram (EEG) data demonstrate promising results. The primary aim of this review was to evaluate the effectiveness of ML algorithms for predicting pain intensity, phenotypes or treatment response from EEG. Electronic databases MEDLINE, EMBASE, Web of Science, PsycINFO and The Cochrane Library were searched. A total of 44 eligible studies were identified, with 22 presenting attempts to predict pain intensity, 15 investigating the prediction of pain phenotypes and seven assessing the prediction of treatment response. A meta-analysis was not considered appropriate for this review due to heterogeneous methods and reporting. Consequently, data were narratively synthesized. The results demonstrate that the best performing model of the individual studies allows for the prediction of pain intensity, phenotypes and treatment response with accuracies ranging between 62 to 100%, 57 to 99% and 65 to 95.24%, respectively. The results suggest that ML has the potential to effectively predict pain outcomes, which may eventually be used to assist clinical care. However, inadequate reporting and potential bias reduce confidence in the results. Future research should improve reporting standards and externally validate models to decrease bias, which would increase the feasibility of clinical translation. PERSPECTIVE: This systematic review explores the state-of-the-art machine learning methods for predicting pain intensity, phenotype or treatment response from EEG data. Results suggest that machine learning may demonstrate clinical utility, pending further research and development. Areas for improvement, including standardized processing, reporting and the need for better methodological assessment tools, are discussed.
J Pain, 2022; 23

34251684: Byrne A, Hewitt D, Henderson J, Newton-Fenner A, Roberts H, Tyson-Carr J, Fallon N, Giesbrecht T, Stancak A
Investigating the effect of losses and gains on effortful engagement during an incentivized Go/NoGo task through anticipatory cortical oscillatory changes.
Losses usually have greater subjective value (SV) than gains of equal nominal value but often cause a relative deterioration in effortful performance. Since losses and gains induce differing approach/avoidance behavioral tendencies, we explored whether incentive type interacted with approach/avoidance motor-sets. Alpha- and beta-band event-related desynchronization (ERD) was hypothesized to be weakest when participants expected a loss and prepared an inhibitory motor-set, and strongest when participants expected a gain and prepared an active motor-set. It was also hypothesized that effort would modulate reward and motor-set-related cortical activation patterns. Participants completed a cued Go/NoGo task while expecting a reward (+10p), avoiding a loss (-10p), or receiving no incentive (0p); and while expecting a NoGo cue with a probability of either .75 or .25. Pre-movement alpha- and beta-band EEG power was analyzed using the ERD method, and the SV of effort was evaluated using a cognitive effort discounting task. Gains incentivized faster RTs and stronger preparatory alpha band ERD compared to loss and no incentive conditions, while inhibitory motor-sets resulted in significantly weaker alpha-band ERD. However, there was no interaction between incentive and motor-sets. Participants were more willing to expend effort in losses compared to gain trials, although the SV of effort was not associated with ERD patterns or RTs. Results suggest that incentive and approach/avoidance motor tendencies modulate cortical activations prior to a speeded RT movement independently, and are not associated with the economic value of effort. The present results favor attentional explanations of the effect of incentive modality on effort.
Psychophysiology, 2022; 59

34023628: Hewitt D, Byrne A, Henderson J, Newton-Fenner A, Tyson-Carr J, Fallon N, Brown C, Stancak A
Inhibition of cortical somatosensory processing during and after low frequency peripheral nerve stimulation in humans.
Transcutaneous low-frequency stimulation (LFS) elicits long-term depression-like effects on human pain perception. However, the neural mechanisms underlying LFS are poorly understood. We investigated cortical activation changes occurring during LFS and if changes were associated with reduced nociceptive processing and increased amplitude of spontaneous cortical oscillations post-treatment.
Clin Neurophysiol, 2021; 132

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EXPERIENCE-DEPENDENT REPRESENTATION OF SENSORY-, MOTOR- AND DECISION-RELATED ACTIVITY IN PRIMARY SENSORY, MOTOR, AND MEDIAL PREFRONTAL CORTICAL AREAS.

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Goal-directed behavior requires processing of incoming sensory information, making appropriate decisions, and generating relevant motor outputs. Yet, how these diverse aspects of sensorimotor transformation arise within the intricate neuronal networks of the mammalian brain remains to be determined. Here, we investigated sensory, decision, and motor signals in the whisker primary somatosensory cortex (wS1), the medial prefrontal cortex (mPFC), and the tongue-jaw primary motor cortex (tjM1) in mice trained to lick for reward in response to a brief single-whisker stimulus of varying amplitude. Optogenetic inactivation demonstrated the causal involvement of all three areas during task execution. We then performed high-density extracellular recordings of neuronal firing in wS1, mPFC, and tjM1 of mice trained in the whisker-detection task (Trained mice). To understand task-specific neuronal representations, we recorded in another group of mice that were exposed to the same whisker stimuli, but without being associated to reward (Exposed mice). Sensory-evoked activity in the absence of licking (i.e. Miss trials) was almost exclusively found in wS1 and correlated with stimulus amplitude in both Trained and Exposed mice. All three cortical regions were strongly modulated by licking in both Trained and Exposed mice, but only tjM1 neuronal activity was unchanged comparing spontaneous (False alarms) and whisker-evoked (Hit trials) licking. Finally, decision-encoding neurons - with selective activity for Hit trials, but not Miss or False alarm trials - were found mostly in mPFC and wS1. Our results point to distinct yet inter-related roles of cortical regions for goal-directed sensorimotor transformation.

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ULTRASTRUCTURAL COMPARISON OF VPM THALAMOCORTICAL SYNAPSES IN PRIMARY AND SECONDARY SOMATOSENSORY CORTICES

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Thalamocortical synapses are key cellular links in sensory, motor and cognitive information processing. In rodents, ventral posteromedial thalamic nucleus (VPM) axons innervate both layer 4 cells of the vibrissal primary somatosensory cortex (S1) and layer 4 cells of the secondary somatosensory cortex (S2). Despite the “primary” and “secondary” functional characterization of these two areas, it is unknown if differences exist in VPM axon synapse structure and/or their postsynaptic target elements. Here, we set out to 3D measure and compare the ultrastructure of VPM synapses on these two areas and their postsynaptic targets. We microinjected adult male C57B/L6 mice iontophoretically with biotinylated dextran amine (BDA) in VPM to selectively label thalamocortical axon arborizations. Following a 5 day survival, mice were perfused, and their brains sectioned (50µm) into two
parallel series of coronal sections. BDA-labeled VPM axon arborizations were located on a series of sections. Adjacent sections were stained for BDA and included for electron microscopy. Serial image samples of selected areas were obtained with serial sectioning TEM or FIB/SEM electron microscopy. Using EspINA and OpenCAR software labeled boutons were 3D reconstructed. Our preliminary results are consistent with previous evidence that VPM axon synapses are mostly located (>90%) on spiny cell dendrites, and very few on spineless dendrites. We are currently investigating synapse structure differences between thalamocortical boutons in different layers of S1 and adjacent somatic sensory areas. Selective and quantitative data on the subcellular structure of these synapses are important to model interactions of the somatic sensory thalamus and its target areas.

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FUNCTIONAL ADAPTATIONS AND BRAIN-WIDE REMODELING AFTER PERMANENT SENSORY DEPRIVATION IN THE ADULTHOOD.

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Brain networks are remodeled during critical periods of plasticity in the early life following alterations to sensory experience in early life. However, brain-wide remodeling following alterations to neuronal activity during the adulthood are more limited. Therefore a complete picture of this phenomenon has been yet elusive. We studied changes in neuronal activity and connectivity after altering brain networks with permanent unilateral whisker deprivations to the somatosensory system in adult mice. We followed the functional and structural changes evoked by this loss over 6 months. Using whole-brain mapping of Fos and calcium imaging with fiber photometry, we measured dynamic adaptive changes during the first weeks following deprivation, followed by a chronic hypoactivity in the barrel field region of the primary somatosensory cortex. We mapped structural axonal markers at whole-brain scale to generate unbiased maps of changes to axonal densities following adult deprivation. Finally, we used viral tracers to quantify changes to the connectivity of efferent pathways of the barrel field. Deciphering the nature of adult brain plasticity may lead to a breakthrough in our understanding of physiological and pathological alterations to the wiring of mature circuits.

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REPETITIVE SOMATOSENSORY STIMULATION OF A FINGER AFFECTS SOME METRIC ASPECTS OF ITS MENTAL REPRESENTATION

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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[1]Lyon Neuroscience Research Center (CRNL), Impact Team, Inserm U1028, BRON, France, [2]University UCBL Lyon 1, Neuroscience, Villeurbanne, France, [3]Radboud University, Donders Institute, Nijmegen, Netherlands, [4]Hospices Civils de Lyon, Neuro-immersion & Mouvement Et Handicap, Bron, France, [5]Université de Paris, Neurodiderot, Inserm, Paris, France, [6]Université Paris-Saclay, Neurospin-uniact, Cea, Gif-sur-Yvette, France, [7]Birkbeck, University of London, 7. department Of Psychological Sciences, London, United Kingdom, [8]University of Trento, Center For Mind/brain Sciences (cimec), Mattarello, Italy

The ability to represent our body relies on mental body representations (MBR), which are thought to be nourished -and continuously updated- from early stages of tactile input processing in the primary somatosensory cortex (S1). Yet, little is known about MBR susceptibility to changes in S1 plasticity affecting tactile perception. A few studies reported that some interventions, such as local anesthesia, or repetitive transcranial magnetic stimulation, may alter MBR through an induction of plasticity in S1. Repetitive Somatosensory Stimulation (RSS) is a passive mechanical stimulation that, when applied to the index fingertip, changes tactile acuity along with the S1 representation of the stimulated region. Here, we tested whether RSS, beyond affecting tactile perception at relatively low level (spatial discrimination), also alters MBR. To this aim, we ran a double-blind sham-controlled study in thirty-three healthy volunteers performing, before and after 3 hours of RSS on the right index fingertip, three tasks assessing finger size matching, tactile distance judgement and localization abilities on the stimulated finger. Tactile acuity was additionally assessed using the 2-point discrimination task to control for RSS efficacy. The perceived size of the stimulated finger in the template matching task was significantly reduced after RSS only. No differences were observed in the tactile distance judgement nor in the localization abilities. Our results suggest that the perceived finger size is affected by RSS, while other metric aspects appear immune to RSS. The thorough investigation will be presented, deepening our understanding of the building of MBR and enriching its theoretical framework.

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INFORMATION THEORETIC CHARACTERIZATION OF CORTICOTHALAMIC SIGNALING IN THE SOMATOSENSORY SYSTEM OF MICE

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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The thalamus integrates sensory information with descending corticothalamic (CT) signals from cortical layers 5 (L5) and 6 (L6) and converging studies suggest that these pathways are main determinants of information processing in the thalamus. The properties of CT pathways suggest distinct functions: L6 provides facilitating modulatory input to all thalamic nuclei, while L5 pyramidal tract neurons powerfully excite “higher-order” regions of thalamus via large, depressing synapses. Here, we aimed to quantify how these distinct CT pathways functionally interact to control thalamic encoding of sensory information using information theoretic approaches, specifically transfer entropy and the partial information decomposition. To assess interactions between cortical output layers and target thalamic relay neurons, we first validated our approach on simulated data from simple spiking (Izhikevich) and mechanistic (Hodgkin-Huxley) models. We next analyzed 1) simulated data from simple feedforward CT model networks, and 2) silicon probe extracellular spike train data collected from the thalamocortical system (primary somatosensory cortex and ventral posterolateral and posterior medial thalamic nuclei) of anesthetized Ntstr1-cre or Rbp4-cre mice in which L6 or L5 CT neurons, respectively, were “opto-tagged” by activation of Channelrhodopsin-2. We find that both CT information transfer and the synergistic influence of L5 and L6 on thalamic targets depend on cortical output rate and spike timing, and are controlled by the pathway-specific temporal dynamics of CT short-term synaptic plasticity. These findings support the established layer-specific functional roles for CT feedback pathways but also point to a previously unknown role for cooperativity between the two pathways in controlling sensory processing.

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THE STUDY OF TACTILE IMAGERY-INDUCED CHANGES IN SENSORIMOTOR EEG RHYTHMS.

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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In this work, the mu-rhythm of EEG was studied during tactile imagery and tactile stimulation. The goal of the study was to evaluate sensorimotor mu-rhythm event related desynchronization/synchronization (ERD/S) patterns during tactile imagery compared to tactile stimulation. Presented results provide similar EEG patterns of ERD/S during tactile stimulation and imagery. The study involved 16 healthy naive volunteers. The subjects received tactile stimulation, using a vibration motor applied to the inner side of the wrist. After several trials of the tactile stimulation each participant was asked to represent somatosensory feelings mentally. 48 channels EEG were recorded during the experimental session. EEG data was used for spectral analysis with the following calculating ERD/S values in each channel and topographical mapping of EEG activity. Stable ERD patterns were obtained in response to tactile stimulation and tactile imagery, mainly localized contralaterally in the central EEG channels. The intensity of the ERD patterns during imagery conditions was weaker than during the actual stimulation, however there were no statistically significant differences between these conditions. It is important to note that both imagery and real stimulation ERS/S patterns were statistically significantly different from the control condition. During the experimental session we also observed significant gradual increase in ERD values with the trial number during tactile imagery trials. Provided results indicate that not only motor imagery leads to ERD in EEG but also somatosensory imagery. The obtained data complement knowledge about the relationship between the sensorimotor input and mu-rhythm ERD.

Pubmed:
28167121: Vasilyev A, Liburkina S, Yakovlev L, Perepelkina O, Kaplan A
Assessing motor imagery in brain-computer interface training: Psychological and neurophysiological correlates.
Motor imagery (MI) is considered to be a promising cognitive tool for improving motor skills as well as for rehabilitation therapy of movement disorders. It is believed that MI training efficiency could be improved by using the brain-computer interface (BCI) technology providing real-time feedback on person's mental attempts. While BCI is indeed a convenient and motivating tool for practicing MI, it is not clear whether it could be used for predicting or measuring potential positive impact of the training. In this study, we are trying to establish whether the proficiency in BCI control is associated with any of the neurophysiological or psychological correlates of motor imagery, as well as to determine possible interrelations among them. For that purpose, we studied motor imagery in a group of 19 healthy BCI-trained volunteers and performed a correlation analysis across various quantitative assessment metrics. We examined subjects' sensorimotor event-related EEG events, corticospinal excitability changes estimated with single-pulse transcranial magnetic stimulation (TMS), BCI accuracy and self-assessment reports obtained with specially designed questionnaires and interview routine. Our results showed, expectedly, that BCI performance is dependent on the subject's capability to suppress EEG sensorimotor rhythms, which in turn is correlated with the idle state amplitude of those oscillations. Neither BCI accuracy nor the EEG features associated with MI were found to correlate with the level of corticospinal excitability increase during motor imagery, and with assessed imagery vividness. Finally, a significant correlation was found between the level of corticospinal excitability increase and kinesthetic vividness of imagery (KVIQ-20 questionnaire). Our results suggest that two distinct neurophysiological mechanisms might mediate possible effects of motor imagery: the non-specific cortical sensorimotor disinhibition and the focal corticospinal excitability increase. Acquired data suggests that BCI-based approach is unreliable in assessing motor imagery due to its high dependence on subject's innate EEG features (e.g. resting mu-rhythm amplitude). Therefore, employment of additional assessment protocols, such as TMS and psychological testing, is required for more comprehensive evaluation of the subject's motor imagery training efficiency.
Neuropsychologia, 2017; 97

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BOARD NUMBER: S04-514

MAPPING TOUCH ON HANDS AND TOOLS INVOLVES SIMILAR BRAIN DYNAMICS

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Numerous studies have found evidence that tools become incorporated into a plastic neural representation of our body. A prominent hypothesis suggests that our brain re-uses body-based neural processing when we use tools. However, little is known about how this is implemented at the neural level. Here we used the ability to map touch on both tools and body parts as a case study to fill this gap. Neural oscillations in the alpha (8-13 Hz) and beta (15-25 Hz) frequency bands are involved in mapping touch on the body in distinct reference frames. Alpha activity reflects the mapping of touch in external coordinates, whereas beta activity reflects the mapping of touch in skin-centered coordinates. Here, we aimed at pinpointing the role of these oscillations during tool-extended sensing. We recorded participants' oscillatory activity while tactile stimuli were applied to either hands or the tips of hand-held rods. Posture of the hands/tool-tips was uncrossed or crossed at participants' body midline in order for us to disentangle brain responses related to different coordinate systems. Alpha-band activity was modulated similarly across postures when localizing touch on hands and on tools, reflecting the position of touch into external space. Source reconstruction also indicated a similar network of cortical regions involved for tools and hands. The brain seems to use similar oscillatory mechanisms for mapping touch on a rod and on the body, supporting the idea of neural processes being recycled for tool-use. Furthermore, mapping touch on the tool involves mainly the use of external spatial coordinates.

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BOARD NUMBER: S04-515

CHARACTERIZING NEURAL SELECTIVITY IN SENSORY SYSTEMS USING A DATA-DRIVEN INTERPRETABLE FEATURE FINDING METHOD

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Characterizing neural selectivity is a common approach to understand how cortical neurons process information, i.e., the first step to understand the computation mechanism of neurons. Although there have been persistent attempts to investigate neural selectivity, mischaracterization often occurs due to the conventional method using researcher-defined features. Despite the importance of this problem, the reason that causes mischaracterization is not defined and the solution has not been proposed. Here, we suggest possible scenarios of mischaracterization and demonstrate that a data-driven interpretable feature finding (DIFF) method for characterizing neural selectivity can solve this problem. We propose the DIFF method that can find all feasible features given stimuli by fitting neural response vector in multidimensional feature space. It suggests possible features with a score, which reflects the p-value of linear regression analysis. To validate our method, we applied it to simulated data with a wide range of variation and to a real data, i.e., in vivo two-photon Ca2+ imaging data of primary somatosensory cortex neurons using peripheral stimulation, e.g., innocuous brush stroke and noxious forceps pinch. As a result of applying our method to the simulation data, we confirmed that our method works well in a wide range as expected. In the real data, among known-features, brush texture, forcpes texture, and noxiousness features are in high rank, while other features have low probability. Also, this method suggests some unknown features in high ranks. In this study, we identify the DIFF method and demonstrate that our method can be used for finding missing features.

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BOARD NUMBER: S04-516

INDIVIDUAL AND COLLECTIVE AXONAL PROPERTIES UNDERLYING SIGNAL TRANSMISSION MODULARITY

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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In the primate cortex, axonal connections appear both as long-distance fibers and as terminal arborization patches. In the visual cortex, axonal patches represent specific target sites, such as columns of similar orientation preference. Both patch and out-patch axons form axon terminal-like structures suggesting their role in synaptic transmission. However, it is not known whether axons play a similar role in the propagation of activity and dissemination of information within and outside the patches. To answer this question, we reconstructed axons and compared individual axonal properties including thickness, tortuosity and variables related to bouton spacing in addition to collective properties characterizing convergence within and outside the patches for intra-areal, feedforward and feedback connections in horizontal sections of the somatosensory cortex of squirrel monkeys. In patches, axons were thinner and had higher bouton density and stronger convergence than no-patch axons. These findings were consistent across intra- and inter-areal connections. Also, tortuosity was an invariant axonal property. Multivariate analyses indicated bouton density and closeness of unconnected boutons as the major determinants of patch formation. Our results provide evidence that axonal patches are sites of synaptic convergence whereas no-patch axons are more involved in the broadcasting of information in the cerebral cortex. Supported by NIH N

BOARD NUMBER: S093998 and OTKA NN118902. **Corresponding Email: negyessy.laszlo@wigner.hu, yaqubmir@gmail.com**

Pubmed:
32383440: Mir Y, Pálfi E, Roe A, Friedman R, Négyessy L
Corrigendum to "Structural correlates of modular organization of activity propagation in the primate somatosensory cortex" [IBROR 6S (2019) S540].
[This corrects the article DOI: 10.1016/j.ibror.2019.07.1681.].
IBRO Rep, 2019; 7

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BOARD NUMBER: S04-517

SIDE-INVARIANT POSTSYNAPTIC POTENTIALS MEDIATED BY THE CORPUS CALLOSUM IN THE POSTEROLATERAL BARREL CORTEX: MIDLINE FUSION OF THE TWO ROWS A

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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The rodent barrel cortex contains a somatotopic representation of the peripheral whiskers. The decussating trigeminothalamic tract activates the thalamocortical circuit contralateral to the side of a stimulated whisker. Yet, also the stimulation of ipsilateral whiskers can activate the barrel cortex thanks to the callosal innervation. This is scant in most of the barrel cortex and concentrates in its posterolateral aspect where row A is represented, the row lying nearest to the facial midline. Despite the widely known callosal innervation pattern, so far, no study has characterised the ipsilateral receptive fields of highly and scantily callosally innervated areas in relation to whiskers identity. Here, by in vivo whole-cell patch-clamp recordings coupled with sensory stimulations, we show that both inhibitory and excitatory postsynaptic potentials are stronger where callosal innervation is higher. Moreover, with excitatory optogenetics and pharmacological inactivation of the opposite hemisphere, we demonstrate that the strong responses depend on contralateral callosally-projecting neurons. Remarkably, neurons in the posterolateral barrel cortex show a nearly identical response to both right and left row A stimulations. This side-invariant response suggests their participation in the representation of the midline of the whiskers somatosensory space.

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BOARD NUMBER: S04-518

SENSORY CORTICAL DYNAMICS DURING OPTICAL MICROSTIMULATION TRAINING

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Microstimulation of sensory cortex has been used extensively in the study of perception. However, the evolution of neural activity in animals learning to use microstimulation for behavior remains unclear. Here, we employ optical microstimulation of pyramidal neurons in layer (L) 2/3 of mouse primary vibrissal somatosensory cortex (vS1) to study cortical dynamics as mice learn to discriminate the intensity of microstimulation. Tracking activity over weeks using two-photon calcium imaging, we observe a rapid sparsification of the photoresponsive population, with the degree of sparsification predictive of learning speed. Following sparsification, a steady rate of representational drift is attained, with the same number of neurons entering and exiting the photoresponsive population. Moreover, the photoresponsive population comes to increasingly overlap with populations responding to whisker movement and touch. Our results reveal that microstimulation-evoked cortical activity undergoes extensive reorganization during task learning and that the dynamics of this reorganization impact perception.

Pubmed:
32132709: Peron S, Pancholi R, Voelcker B, Wittenbach JD, Ólafsdóttir HF, Freeman J, Svoboda K
Recurrent interactions in local cortical circuits.
Most cortical synapses are local and excitatory. Local recurrent circuits could implement amplification, allowing pattern completion and other computations. Cortical circuits contain subnetworks that consist of neurons with similar receptive fields and increased connectivity relative to the network average. Cortical neurons that encode different types of information are spatially intermingled and distributed over large brain volumes, and this complexity has hindered attempts to probe the function of these subnetworks by perturbing them individually. Here we use computational modelling, optical recordings and manipulations to probe the function of recurrent coupling in layer 2/3 of the mouse vibrissal somatosensory cortex during active tactile discrimination. A neural circuit model of layer 2/3 revealed that recurrent excitation enhances sensory signals by amplification, but only for subnetworks with increased connectivity. Model networks with high amplification were sensitive to damage: loss of a few members of the subnetwork degraded stimulus encoding. We tested this prediction by mapping neuronal selectivity and photoablating neurons with specific selectivity. Ablation of a small proportion of layer 2/3 neurons (10-20, less than 5% of the total) representing touch markedly reduced responses in the spared touch representation, but not in other representations. Ablations most strongly affected neurons with stimulus responses that were similar to those of the ablated population, which is also consistent with network models. Recurrence among cortical neurons with similar selectivity therefore drives input-specific amplification during behaviour.
Nature, 2020; 579

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BOARD NUMBER: S04-519

THE ROLE OF CORTICAL BARRELS IN VIBRISSAL OBJECT TOUCH BEHAVIORS

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Rodent primary vibrissal somatosensory cortex (vS1) is a model system for the study of neural circuits mediating perception due to, among other things, the precise mapping between whiskers and patches of cortex known as ‘barrels’. Perception during single vibrissal behavior may be dependent on individual barrels. However, the specific behaviors for which individual barrels are necessary remains unclear. Lesions in mice performing vibrissal tasks show conflicting effects: both persistent loss of performance several days after vS1 lesion and recovery have been observed. The large spatial extent of most lesion studies further complicates interpretation. We use prolonged exposure to a femtosecond laser beam to induce lesions that typically span 1-2 barrels in mice performing object localization tasks with a single whisker. Mice are pre-implanted with a cranial window over vS1, and lesions are performed without surgery. Lesioning the C2 barrel in mice that must discriminate touch at distinct locations results in persistent, multi-day degradation of task performance. Lesioned mice can nearly immediately learn an object detection task that requires them to simply report the presence of an object. Mice trained on the detection task alone show no deficit upon single barrel lesions in vS1, despite lesions occurring less than an hour prior to the behavioral session. Performance on a more complex detection task featuring multiple response contingencies and a response delay period was also not impaired following single barrel lesion. Thus, individual vS1 barrels are necessary for performing object location discrimination but are not necessary for simple or complex detection behaviors.

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BOARD NUMBER: S04-520

PREDICTIVE PROCESSING OF TACTILE SENSORY INFORMATION IN MICE ENGAGED IN A LOCOMOTION TASK

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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The ability of the mammalian central nervous system to constantly adapt motor commands and optimise behaviour according to the context does not only rely on the efficient analysis of the incoming sensory flow. The cerebral cortex is indeed thought to compute, based on past experience, a dynamic model of our interactions with our environment, allowing to anticipate future sensory inputs. Sensory perception would therefore imply a continuous comparison of the expected sensory inputs with those actually received. The error signals generated in case of deviation between these two types of information would allow the internal model to be updated thereby optimizing future predictions. We aim to study these predictive mechanisms using mice in the context of a whisker-guided locomotion task. To interrogate the possible emergence of top-down error signals in the primary somatosensory cortex at the mesoscopic scale, we record voltage induced fluorescence changes over this area through a bundle of optical fibres in mice trained to avoid an obstacle on their path in complete darkness. By simultaneously recording the animal's movement and whisker motion, this allows us to link cortical dynamics with behaviour at 500 Hz, whilst the animal is navigating and gathering tactile information in an ethologically relevant manner. Studying the dynamics of these components at the millisecond timescale in a familiar context, but also in unexpected situations such as when the obstacle is suddenly removed, thus creating a mismatch between expected and received tactile inputs, we intend to reveal behavioural and neuronal signatures of tactile sensory prediction.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S04-521

MAPPING THE PROJECTION FROM BARREL CORTEX TO THE DIRECT AND INDIRECT PATHWAYS OF DORSAL STRIATUM USING A FUNCTIONAL APPROACH

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Animals take navigational decisions based on sensory cues. Rodents detect objects with their whiskers and navigate accordingly. Neurons of barrel cortex (BC) integrate tactile information and transfer them to the dorsal striatum, the main input region of basal ganglia involved in decision making and motor control. Striatum has two projection-neuron populations that are in distinct pathways, direct (dSPNs) and indirect (iSPNs), which control with opposite manners a given action. Thus, the corticostriatal projection is one important determinant of d/iSPNs activity and basal ganglia outputs. We study the BC/striatum coupling to better understand its function. Our aim is to investigate (1) the topographic organization of the projection, (2) the cortical layers involved (3) the eventual dSPN/iSPN differences. So far, the topography of this projection has mainly been approached with anatomy, from which an organization of connectivity may only be inferred. Here, we studied it with a functional approach in order to map the actual connections. To do so, we patched d/iSPNs in slices and stimulated BC using Laser Scanning Photo-Stimulation. This method detected the soma of neurons in BC that innervated a recorded d/iSPN: presynaptic cortical cells were at sites where the stimulation evoked EPSC in the d/iSPN. The collection of EPSCs evoked by a grid of stimulations was used to compute connectivity maps. Drd1-tomato mice allowed to distinguish dSPNs and iSPNs. Prior to electrophysiology, the mouse's spontaneous behaviour in an open field was recorded in order to link its locomotion to differences in dSPN and iSPN innervations.

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BOARD NUMBER: S04-522

PROCESSING OF TACTILE INPUTS IN THE MOUSE PERIRHINAL CORTEX

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Mammals use their different senses to gather information and interact with our surroundings. Rodents are heavily dependent on their whiskers-mediated touch system to gather information about their surroundings and objects with which they interact with. Tactile information as well as other types of sensory inputs (smell, vision, etc.) converge more prominently in associative cortices, such as the perirhinal cortex (PER), which receives inputs from all sensory areas and other associative regions; however, the topological organization of whiskers-mediated touch system and the circuits processing tactile inputs into the PER remain unclear.
In this work we aim to investigate the neural circuits in the PER involved in the integration of tactile inputs as well as their topological organization. Our retrograde tracing experiments revealed that PER in divided in sensory domains along its rostro-caudal axis. We found that somatosensory areas of the cortex target the rostral part of PER, while more caudal parts receive visual inputs from the postrhinal cortex. We are currently characterizing the circuit architecture integrating tactile inputs in PER with in vitro electrophysiology. Moreover, we aim at unveiling how perirhinal neurons respond to tactile stimulation by performing in vivo electrophysiological recordings with Neuropixel probes in head restrained animals from PER and barrel cortex to characterize population activity in these regions.

Pubmed:
35031630: Cobar LF, Kashef A, Bose K, Tashiro A
Opto-electrical bimodal recording of neural activity in awake head-restrained mice.
Electrical and optical monitoring of neural activity is major approaches for studying brain functions. Each has its own set of advantages and disadvantages, such as the ability to determine cell types and temporal resolution. Although opto-electrical bimodal recording is beneficial by enabling us to exploit the strength of both approaches, it has not been widely used. In this study, we devised three methods of bimodal recording from a deep brain structure in awake head-fixed mice by chronically implanting a gradient-index (GRIN) lens and electrodes. First, we attached four stainless steel electrodes to the side of a GRIN lens and implanted them in a mouse expressing GCaMP6f in astrocytes. We simultaneously recorded local field potential (LFP) and GCaMP6f signal in astrocytes in the hippocampal CA1 area. Second, implanting a silicon probe electrode mounted on a custom-made microdrive within the focal volume of a GRIN lens, we performed bimodal recording in the CA1 area. We monitored LFP and fluorescent changes of GCaMP6s-expressing neurons in the CA1. Third, we designed a 3D-printed scaffold to serve as a microdrive for a silicon probe and a holder for a GRIN lens. This scaffold simplifies the implantation process and makes it easier to place the lens and probe accurately. Using this method, we recorded single unit activity and LFP electrically and GCaMP6f signals of single neurons optically. Thus, we show that these opto-electrical bimodal recording methods using a GRIN lens and electrodes are viable approaches in awake head-fixed mice.
Sci Rep, 2022; 12
34233196: Uemura M, Blankvoort S, Tok SSL, Yuan L, Cobar LF, Lit KK, Tashiro A
A neurogenic microenvironment defined by excitatory-inhibitory neuronal circuits in adult dentate gyrus.
Adult neurogenesis in the dentate gyrus plays a role in adaptive brain functions such as memory formation. Adding new neurons to a specific locus of a neural circuit with functional needs is an efficient way to achieve such an adaptive function. However, it is unknown whether neurogenesis is linked to local functional demands potentially specified by the activity of neuronal circuits. By examining the distribution of neurogenesis and different types of neuronal activity in the dentate gyrus of freely moving adult rats, we find that neurogenesis is positionally associated with active excitatory neurons, some of which show place-cell activity, but is positionally dissociated from a type of interneuron with high-burst tendency. Our finding suggests that the behaviorally relevant activity of excitatory-inhibitory neuronal circuits can define a microenvironment stimulating/inhibiting neurogenesis. Such local regulation of neurogenesis may contribute to strategic recruitment of new neurons to modify functionally relevant neural circuits.
Cell Rep, 2021; 36

27794463: Cobar LF, Yuan L, Tashiro A
Place cells and long-term potentiation in the hippocampus.
Place cells show location-specific firing patterns according to an animal's position in an environment and are thought to contribute to the spatial representation required for self-navigation. Decades of study have extensively characterized the properties of place cells and suggested the involvement of long-term potentiation (LTP), a long-lasting synaptic strengthening, in place cell activity. Here, we review the basic characteristics of place cell activity and the findings that support the idea that LTP contributes to the formation, maintenance, and plasticity of place cell activity.
Neurobiol Learn Mem, 2017; 138

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-523

COEXISTENCE OF STATE, CHOICE AND SENSORY INTEGRATION CODING IN BARREL CORTEX OF MICE PERFORMING NEIGHBORING TACTILE INPUT DISCRIMINATION.

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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How parallel sensory streams are integrated and compared during sensory discrimination remains poorly understood. Taking advantage of the columnar architecture of the mouse barrel field (wS1), we designed a two alternative forced choice task (2AFC) in which mice successfully compared stimulation frequencies applied to two adjacent whiskers. We used two-photon functional imaging and optogenetic interventions to record and manipulate the two corresponding cortical neuronal populations. We found that multi-whisker suppression, a dominant effect in cortical layer II/III allows for comparison of frequencies across the range of tested stimuli. Additionally, we find that behavioral engagement is associated with selective up-modulation of single whisker preferring neurons as compared to neurons responding to combinations of whiskers. However, choice signals prior to the animal's response were surprisingly weak and their neuronal representation correlated little with sensory encoding of frequencies. Importantly, optogenetic silencing of individual barrels using structured light illumination resulted in predicted shifts of psychometric functions. This approach allowed us to relate causally and quantifiably the studied neuronal populations to sensory perception involved in this task. Therefore, our results illustrate the role of wS1 in forming a reliable sensory substrate for neighboring whisker frequency discrimination during engagement in the task. This sensory information is used by downstream areas from which most behavioral variability and choice signals may arise.

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BOARD NUMBER: S04-524

NEURONAL CORRELATE OF MOTIVATIONAL STATES IN THE SECONDARY WHISKER SENSORIMOTOR PATHWAY

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Motivational state can influence performance during perceptual decision-making, yet it is unclear how changes in neuronal representations can alter perception in these states. We adopted a two-whisker discrimination task in head-fixed water-restricted mice to study perceptual decision-making in different states of thirst. Whisker discrimination performance varied with decreasing motivation within individual sessions. Learning dynamics was mainly driven by improvements in the control of motivational state rather than sensorimotor associative learning. Using wide field calcium imaging, optogenetics and anatomical tracing we identified the primary and secondary somatosensory cortex as well as the premotor cortex as part of the cortical network involved in the task. Two-photon calcium imaging across cortical layers in these areas revealed that neural representations of whiskers somatosensation were dependent on the motivational state. In particular, whiskers stimuli could be decoded at best during states of high discrimination performance. These results indicate that the secondary sensorimotor pathway plays a pivotal role during whisker-based goal-directed behaviors.

Pubmed:
30617210: Matteucci G, Bellacosa Marotti R, Riggi M, Rosselli FB, Zoccolan D
Nonlinear Processing of Shape Information in Rat Lateral Extrastriate Cortex.
In rodents, the progression of extrastriate areas located laterally to primary visual cortex (V1) has been assigned to a putative object-processing pathway (homologous to the primate ventral stream), based on anatomical considerations. Recently, we found functional support for such attribution (Tafazoli et al., 2017), by showing that this cortical progression is specialized for coding object identity despite view changes, the hallmark property of a ventral-like pathway. Here, we sought to clarify what computations are at the base of such specialization. To this aim, we performed multielectrode recordings from V1 and laterolateral area LL (at the apex of the putative ventral-like hierarchy) of male adult rats, during the presentation of drifting gratings and noise movies. We found that the extent to which neuronal responses were entrained to the phase of the gratings sharply dropped from V1 to LL, along with the quality of the receptive fields inferred through reverse correlation. Concomitantly, the tendency of neurons to respond to different oriented gratings increased, whereas the sharpness of orientation tuning declined. Critically, these trends are consistent with the nonlinear summation of visual inputs that is expected to take place along the ventral stream, according to the predictions of hierarchical models of ventral computations and a meta-analysis of the monkey literature. This suggests an intriguing homology between the mechanisms responsible for building up shape selectivity and transformation tolerance in the visual cortex of primates and rodents, reasserting the potential of the latter as models to investigate ventral stream functions at the circuitry level. Despite the growing popularity of rodents as models of visual functions, it remains unclear whether their visual cortex contains specialized modules for processing shape information. To addresses this question, we compared how neuronal tuning evolves from rat primary visual cortex (V1) to a downstream visual cortical region (area LL) that previous work has implicated in shape processing. In our experiments, LL neurons displayed a stronger tendency to respond to drifting gratings with different orientations while maintaining a sustained response across the whole duration of the drift cycle. These trends match the increased complexity of pattern selectivity and the augmented tolerance to stimulus translation found in monkey visual temporal cortex, thus revealing a homology between shape processing in rodents and primates.
J Neurosci, 2019; 39
32523998: Matteucci G, Zoccolan D
Unsupervised experience with temporal continuity of the visual environment is causally involved in the development of V1 complex cells.
Unsupervised adaptation to the spatiotemporal statistics of visual experience is a key computational principle that has long been assumed to govern postnatal development of visual cortical tuning, including orientation selectivity of simple cells and position tolerance of complex cells in primary visual cortex (V1). Yet, causal empirical evidence supporting this hypothesis is

scant. Here, we show that degrading the temporal continuity of visual experience during early postnatal life leads to a sizable reduction of the number of complex cells and to an impairment of their functional properties while fully sparing the development of simple cells. This causally implicates adaptation to the temporal structure of the visual input in the development of transformation tolerance but not of shape tuning, thus tightly constraining computational models of unsupervised cortical learning.
Sci Adv, 2020; 6
32490704: Matteucci G, Riggi M, Zoccolan D
A template-matching algorithm for laminar identification of cortical recording sites from evoked response potentials.
In recent years, the advent of the so-called silicon probes has made it possible to homogeneously sample spikes and local field potentials (LFPs) from a regular grid of cortical recording sites. In principle, this allows inferring the laminar location of the sites based on the spatiotemporal pattern of LFPs recorded along the probe, as in the well-known current source-density (CSD) analysis. This approach, however, has several limitations, since it relies on visual identification of landmark features (i.e., current sinks and sources) by human operators - features that can be absent from the CSD pattern if the probe does not span the whole cortical thickness, thus making manual labelling harder. Furthermore, as any manual annotation procedure, the typical CSD-based workflow for laminar identification of recording sites is affected by subjective judgment undermining the consistency and reproducibility of results. To overcome these limitations, we developed an alternative approach, based on finding the optimal match between the LFPs recorded along a probe in a given experiment and a template LFP profile that was computed using 18 recording sessions, in which the depth of the recording sites had been recovered through histology. We show that this method can achieve an accuracy of 79 μm in recovering the cortical depth of recording sites and a 76% accuracy in inferring their laminar location. As such, our approach provides an alternative to CSD that, being fully automated, is less prone to the idiosyncrasies of subjective judgment and works reliably also for recordings spanning a limited cortical stretch.
J Neurophysiol, 2020;
34520476: Matteucci G, Zattera B, Bellacosa Marotti R, Zoccolan D
Rats spontaneously perceive global motion direction of drifting plaids.
Computing global motion direction of extended visual objects is a hallmark of primate high-level vision. Although neurons selective for global motion have also been found in mouse visual cortex, it remains unknown whether rodents can combine multiple motion signals into global, integrated percepts. To address this question, we trained two groups of rats to discriminate either gratings (G group) or plaids (i.e., superpositions of gratings with different orientations; P group) drifting horizontally along opposite directions. After the animals learned the task, we applied a visual priming paradigm, where presentation of the target stimulus was preceded by the brief presentation of either a grating or a plaid. The extent to which rat responses to the targets were biased by such prime stimuli provided a measure of the spontaneous, perceived similarity between primes and targets. We found that gratings and plaids, when used as primes, were equally effective at biasing the perception of plaid direction for the rats of the P group. Conversely, for the G group, only the gratings acted as effective prime stimuli, while the plaids failed to alter the perception of grating direction. To interpret these observations, we simulated a decision neuron reading out the representations of gratings and plaids, as conveyed by populations of either component or pattern cells (i.e., local or global motion detectors). We concluded that the findings for the P group are highly consistent with the existence of a population of pattern cells, playing a functional role similar to that demonstrated in primates. We also explored different scenarios that could explain the failure of the plaid stimuli to elicit a sizable priming magnitude for the G group. These simulations yielded testable predictions about the properties of motion representations in rodent visual cortex at the single-cell and circuitry level, thus paving the way to future neurophysiology experiments.
PLoS Comput Biol, 2021; 17

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BOARD NUMBER: S04-525

LAYER 1 NDNF+ INTERNEURONS CONTROL BILATERAL SENSORY PROCESSING IN A LAYER-DEPENDENT MANNER.

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Bilateral sensory information is indispensable for navigating the world. In most mammals, signals sensed by either side of the midline will ultimately reach the cortex where they will be integrated for perception and appropriate action selection. Even though information transferred across the hemispheres is routed through the corpus callosum, how and which microcircuits are key in integrating it is not well understood. Here we identify an essential role for layer 1 NDNF+ inhibitory cells of mice in integrating bilateral whisker-evoked information in an NMDA receptor-dependent manner. Direct connections from the contralateral cortex and the ipsilateral side activate NDNF+ neurons, which subsequently inhibit the late spiking activity of underlying layer 2/3 neurons, but not layer 5. Our results identify a feed-forward regulatory pathway for bilateral cortical sensory processing of upper layer cortical neurons actuated via layer 1 NDNF+ interneurons.

Pubmed:
26914941: Damilou A, Apostolakis S, Thrapsanioti E, Theleritis C, Smyrnis N
Shared and distinct oculomotor function deficits in schizophrenia and obsessive compulsive disorder.
Detailed analysis of oculomotor function phenotypes in antisaccade, smooth eye pursuit, and active fixation tasks was performed in a sample of 44 patients with schizophrenia, 34 patients with obsessive compulsive disorder (OCD), and 45 matched healthy controls. A common pattern of performance deficits in both schizophrenia and OCD emerged including higher antisaccade error rate, increased latency for corrective antisaccades, as well as higher rates of unwanted saccades in smooth eye pursuit compared to healthy controls. This common pattern could be related to the dysfunction of a network of cognitive control that is present in both disorders, including the dorsolateral prefrontal cortex, the posterior parietal cortex, and the anterior cingulate cortex. In contrast, only patients with schizophrenia showed a specific increase for correct antisaccade mean latency and the intrasubject variability of latency for error prosaccades as well as a decrease in the gain for smooth eye pursuit, suggesting a specific deficit in saccadic motor control and the frontal eye field in schizophrenia that is not present in OCD. A specific deficit in fixation stability (increased frequency of unwanted saccades during active fixation) was observed only for OCD patients pointing to a deficit in the frontostriatal network controlling fixation. This deficit was pronounced for OCD patients receiving additional antipsychotic medication. In conclusion, oculomotor function showed shared and distinct patterns of deviance for schizophrenia and OCD pointing toward shared and specific neurobiological substrates for these psychiatric disorders.
Psychophysiology, 2016; 53

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CEREBELLAR MODULATION OF SENSORY PROCESSING THROUGH A HIGHER ORDER THALAMIC NUCLEUS

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Hind Baba Aissa[1], Anthony Lourdiane[2], Jimena Frontera[1], Marco Diana[1], Patrice Coulon[3], Tom Ruigrok[4], Jean-François Léger[1], Laurent Bourdieu[1], Daniela Popa[1], Clement Lena[1]
[1]Institut de Biologie de l'Ecole Normale Superieure (IBENS), Biologie, Paris, France, [2]ENS, Ibens, PARIS, France, [3]Institut de Neurosciences de la Timone, Biologie, Marseille, France, [4]Erasmus MC, Biology, Rotterdam, Netherlands

Accurate sensory acquisition and perception are key features to survival. Though many parameters underlying the processing of sensory information are known, several aspects are still poorly understood, such as the exact contribution of each cerebral structure. Here, we analyze the cerebellar contribution to sensory processing in the mouse whisker system. Using rabies, a transneuronal retrograde tracer, as well as anterograde tracing from the cerebellar nuclei and retrograde tracer from the primary sensory cortex (S1), we identify an anatomical disynaptic projection from the cerebellar nuclei to S1, involving notably the posterior medial thalamus (POm). Optogenetic stimulations of the dentate nucleus of the cerebellum triggered short-latency responses in S1, and in-vitro recordings reveal the presence of driver-like cerebello-thalamic synapses on the POm. Interestingly, the modulation of this DN-POm pathway induces no strong motor whisker impairments, and its co-activation along with sensory peripheral inputs induces the increased recruitment of POm projections to layer I of sensory cortex. Additionally, pharmacogenetic inhibition of this thalamo-cortical projection tends to disrupt intercortical coherences between sensory and motor cortices in the gamma range during exploratory bouts. Taken together, our results show that the cerebellum targets non-motor cortical areas and can directly modulate sensory-motor processing through a high-order thalamic nucleus, the POm.

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IDENTIFICATION OF A DEVELOPMENTAL SWITCH IN INFORMATION TRANSFER BETWEEN WHISKER S1 AND S2 CORTEX IN MICE

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Linbi Cai
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The whiskers of rodents are a key sensory organ that provides critical tactile information for animal navigation and object exploration throughout life. Previous work has explored the developmental sensory-driven activation of the primary sensory cortex processing whisker information (wS1), also called barrel cortex. This body of work has shown that the barrel cortex is already activated by sensory stimuli during the first post-natal week. However, it is currently unknown when over the course of development these stimuli begin being processed by higher order cortical areas, such as secondary whisker somatosensory area (wS2). Here we investigate the developmental engagement of wS2 by whisker stimuli and the emergence of cortico-cortical communication from wS1 to wS2. Using in vivo wide-field imaging and multi-electrode recordings in control and conditional knock-out mice with thalamocortical innervation defects, we find that wS1 and wS2 are able to process bottom-up information coming from the thalamus from birth. We also identify that it is only at the end of the first postnatal week that wS1 begins to provide functional excitation into wS2, switching to more inhibitory actions after the second postnatal week. Therefore, we have uncovered a developmental window when information transfer between wS1 and wS2 reaches mature function.

Pubmed:
27895587: Su D, Nie Y, Zhu A, Chen Z, Wu P, Zhang L, Luo M, Sun Q, Cai L, Lai Y, Xiao Z, Duan Z, Zheng S, Wu G, Hu R, Tsukamoto H, Lugea A, Liu Z, Pandol SJ, Han YP
Vitamin D Signaling through Induction of Paneth Cell Defensins Maintains Gut Microbiota and Improves Metabolic Disorders and Hepatic Steatosis in Animal Models.
Metabolic syndrome (MetS), characterized as obesity, insulin resistance, and non-alcoholic fatty liver diseases (NAFLD), is associated with vitamin D insufficiency/deficiency in epidemiological studies, while the underlying mechanism is poorly addressed. On the other hand, disorder of gut microbiota, namely dysbiosis, is known to cause MetS and NAFLD. It is also known that systemic inflammation blocks insulin signaling pathways, leading to insulin resistance and glucose intolerance, which are the driving force for hepatic steatosis. Vitamin D receptor (VDR) is highly expressed in the ileum of the small intestine, which prompted us to test a hypothesis that vitamin D signaling may determine the enterotype of gut microbiota through regulating the intestinal interface. Here, we demonstrate that high-fat-diet feeding (HFD) is necessary but not sufficient, while additional vitamin D deficiency (VDD) as a second hit is needed, to induce robust insulin resistance and fatty liver. Under the two hits (HFD+VDD), the Paneth cell-specific alpha-defensins including α-defensin 5 (DEFA5), MMP7 which activates the pro-defensins, as well as tight junction genes, and MUC2 are all suppressed in the ileum, resulting in mucosal collapse, increased gut permeability, dysbiosis, endotoxemia, systemic inflammation which underlie insulin resistance and hepatic steatosis. Moreover, under the vitamin D deficient high fat feeding (HFD+VDD), , a known murine hepatic-pathogen, is substantially amplified in the ileum, while , a beneficial symbiotic, is diminished. Likewise, the VD receptor (VDR) knockout mice exhibit similar phenotypes, showing down regulation of alpha-defensins and MMP7 in the ileum, increased and suppressed . Remarkably, oral administration of DEFA5 restored eubiosys, showing suppression of and increase of in association with resolving metabolic disorders and fatty liver in the HFD+VDD mice. An analysis showed that DEFA5 peptide could directly suppress . Thus, the results of this study reveal critical roles of a vitamin D/VDR axis in optimal expression of defensins and tight junction genes in support of intestinal integrity and eubiosis to suppress NAFLD and metabolic disorders. Front Physiol, 2016; 7

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BOARD NUMBER: S04-528

AN ACTIVE WHISKER-DEPENDENT TASK TO SEEK NEURONAL SIGNATURES OF TACTILE SENSORY PREDICTION IN THE MOUSE SENSORIMOTOR CORTEX

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Fan Yang[1,2], Sophie Hubatz[1,2], Luc Estebanez[1], Thomas Deneux[1], Jean-François Léger[2], Daniel Shulz[1], Laurent Bourdieu[2], Isabelle Ferezou[1]
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Expectancy is an essential function of the nervous system, which allows the organism to react to the external context in an effective way. Cortical circuits are thought to be involved in the computation of an internal model of our interactions with the environment, which generate sensory expectations according to the context, past sensory experience, and current motor actions. According to predictive coding theories, in case of mismatch between such expectations and the actual experience, an error signal is generated, which is key to update the internal model and adjust the motor commands, thus optimising behaviour. To study the neuronal mechanisms underlying predictive coding in active conditions, we designed a new tactile sensorimotor task in which head-fixed mice are trained to contact several times a fixed object with a spared single whisker to obtain a reward. Then, during sessions performed with expert animals, we randomly interleave ‘omission’ trials, in which the object is removed between two whisks, creating a deviance between expected and received tactile inputs. Using high-speed videography of the whisker behaviour, we study how the motor strategy evolves during the learning process, and its rearrangement upon omission trials. Based on this behavioural protocol, which is compatible with chronic and acute electrophysiological recordings, and with wide-field mesoscopic and two-photon optical imaging, we aim to better understand how the primary and secondary somatosensory cortices, in interaction with the primary motor cortex, contribute to the construction of an internal representation of the object, and the emergence of error signals.

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BOARD NUMBER: S04-529

MODULATION OF STRIATAL SENSORY PROCESSING BY BEHAVIOR IN HEALTHY AND DOPAMINE-DEPLETED MICE

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Roberto De La Torre-Martínez[1], Maya Ketzef[1], Gilad Silberberg[2]
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Sensory and motor excitatory cortical afferents and dopaminergic projections from substantia nigra pars compacta converge in the dorsolateral striatum (DLS). In various cortical regions, motor activity was shown to modulate sensory processing, however little is known about sensorimotor interactions in the DLS and the effects of dopamine (DA) depletion. To determine the impact of whisker-related motor activity in the sensory processing in healthy and DA depleted mice, we obtained whole-cell recordings in the DLS of awake mice during the presentation of tactile stimuli. Recorded neurons in DLS responded to both whisker stimulation and spontaneous whisking. Following DA depletion, the representation of spontaneous whisking was reduced in the direct pathway medium spiny neurons (dMSNs) and not in the indirect population (iMSNs). Spontaneous whisking reduced the magnitude of tactile responses in both MSN types, in both healthy and DA depleted mice. Lastly, as previously reported in anesthetized mice, DA depletion reduced the differences between ipsi- and contralateral sensory responses. Our results provide new insights into the sensorimotor integration in the DLS.

Pubmed:

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Phenylalanine-Derived β-Lactam TRPM8 Modulators. Configuration Effect on the Antagonist Activity.
Transient receptor potential cation channel subfamily M member 8 (TRPM8) is a Ca non-selective ion channel implicated in a variety of pathological conditions, including cancer, inflammatory and neuropathic pain. In previous works we identified a family of chiral, highly hydrophobic β-lactam derivatives, and began to intuit a possible effect of the stereogenic centers on the antagonist activity. To investigate the influence of configuration on the TRPM8 antagonist properties, here we prepare and characterize four possible diastereoisomeric derivatives of 4-benzyl-1-[(3'-phenyl-2'-dibenzylamino)prop-1'-yl]-4-benzyloxycarbonyl-3-methyl-2-oxoazetidine. In microfluorography assays, all isomers were able to reduce the menthol-induced cell Ca entry to larger or lesser extent. Potency follows the order 342' > 342' 342' 342' with the most potent diastereoisomer showing a half inhibitory concentration (IC) in the low nanomolar range, confirmed by Patch-Clamp electrophysiology experiments. All four compounds display high receptor selectivity against other members of the TRP family. Furthermore, in primary cultures of rat dorsal root ganglion (DRG) neurons, the most potent diastereoisomers do not produce any alteration in neuronal excitability, indicating their high specificity for TRPM8 channels. Docking studies positioned these β-lactams at different subsites by the pore zone, suggesting a different mechanism than the known -(3-aminopropyl)-2-[(3-methylphenyl)methoxy]--(2-thienylmethyl)-benzamide (AMTB) antagonist.
Int J Mol Sci, 2021; 22
32843690: Bonache MÁ, Martín-Escura C, de la Torre Martínez R, Medina A, González-Rodríguez S, Francesch A, Cuevas C, Roa AM, Fernández-Ballester G, Ferrer-Montiel A, Fernández-Carvajal A, González-Muñiz R
Highly functionalized β-lactams and 2-ketopiperazines as TRPM8 antagonists with antiallodynic activity.
The cool sensor transient receptor potential melastatin channel 8 (TRPM8) is highly expressed in trigeminal and dorsal root ganglia, playing a key role in cold hypersensitivity associated to different peripheral neuropathies. Moreover, these channels are aberrantly expressed in different cancers, and seem to participate in tumor progression, survival and invasion. Accordingly, the search for potent and selective TRPM8 modulators attracted great interest in recent years. We describe new heterocyclic TRPM8 antagonist chemotypes derived from N-cloroalkyl phenylalaninol-Phe conjugates. The cyclization of these conjugates afforded highly substituted β-lactams and/or 2-ketopiperazine (KP) derivatives, with regioselectivity depending on the N-chloroalkyl group and the configuration. These derivatives behave as TRPM8 antagonists in the Ca microfluorometry assay, and confirmed electrophysiologically for the best enantiopure β-lactams 24a and 29a (IC, 1.4 and 0.8 μM). Two putative binding sites by the pore zone, different from those found for typical agonists and antagonists, were

identified by in silico studies for both β-lactams and KPs. β-Lactams 24a and 29a display antitumor activity in different human tumor cell lines (micromolar potencies, A549, HT29, PSN1), but correlation with TRPM8 expression could not be established. Additionally, compound 24a significantly reduced cold allodynia in a mice model of oxaliplatin-induced peripheral neuropathy.
Sci Rep, 2020; 10
31322853: Pérez de Vega MJ, Fernandez-Mendivil C, de la Torre Martínez R, González-Rodríguez S, Mullet J, Sala F, Sala S, Criado M, Moreno-Fernández S, Miguel M, Fernández-Carvajal A, Ferrer-Montiel A, López MG, González-Muñiz R
1-(2',5'-Dihydroxyphenyl)-3-(2-fluoro-4-hydroxyphenyl)-1-propanone (RGM079): A Positive Allosteric Modulator of α7 Nicotinic Receptors with Analgesic and Neuroprotective Activity.
Acetylcholine α7 nicotinic receptors are widely expressed in the brain, where they are involved in the central processing of pain as well as in neuropsychiatric, neurodegenerative, and inflammatory processes. Positive allosteric modulators (PAMs) show the advantage of allowing the selective regulation of different subtypes of acetylcholine receptors without directly interacting with the agonist binding site. Here, we report the preparation and biological activity of a fluoro-containing compound, 1-(2',5'-dihydroxyphenyl)-3-(2-fluoro-4-hydroxyphenyl)-1-propanone (,), that behaves as a potent PAM of the α7 receptors and has a balanced pharmacokinetic profile and antioxidant properties comparable or even higher than well-known natural polyphenols. In addition, compound shows neuroprotective properties in Alzheimer's disease (AD)-toxicity related models. Thus, it causes a concentration-dependent neuroprotective effect against the toxicity induced by okadaic acid (OA) in the human neuroblastoma cell line SH-SY5Y. Similarly, in primary cultures of rat cortical neurons, is able to restore the cellular viability after exposure to OA and amyloid peptide Aβ, with cell death almost completely prevented at 10 and 30 μM, respectively. Finally, compound shows analgesic activity in the complete Freund's adjuvant (CFA)-induced paw inflammation model after intraperitoneal administration.
ACS Chem Neurosci, 2019; 10
29174812: Balsera B, Mulet J, Sala S, Sala F, de la Torre-Martínez R, González-Rodríguez S, Plata A, Naesens L, Fernández-Carvajal A, Ferrer-Montiel A, Criado M, Pérez de Vega MJ, González-Muñiz R
Amino acid and peptide prodrugs of diphenylpropanones positive allosteric modulators of α7 nicotinic receptors with analgesic activity.
α7 Nicotinic acetylcholine receptors (nAChRs) are ion channels implicated in a number of CNS pathological processes, including pain and psychiatric, cognitive and inflammatory diseases. Comparing with orthosteric agonism, positive allosteric modulation of these channels constitutes an interesting approach to achieve selectivity versus other nicotinic receptors. We have recently described new chalcones and 1,3-diphenylpropanones as positive allosteric modulators (PAMs) of α7 nAChRs, which proved to have good analgesic activities but poor pharmacokinetic properties. Here we report the preparation of amino acid and peptide derivatives as prodrugs of these modulators with the aim of improving their in vivo biological activity. While the valine derivative showed very short half life in aqueous solutions to be considered a prodrug, Val-Val and Val-Pro-Val are suitable precursors of the parent 1,3-diphenylpropanones, via chemical and enzymatic transformation, respectively. Compounds 19 (Val-Val) and 21 (Val-Pro-Val), prodrugs of the 2',5',4-trihydroxy-1,3-diphenylpropan-1-one 3, showed significant antinociceptive activity in in vivo assays. The best compound, 21, displayed a better profile in the analgesia test than its parent compound 3, exhibiting about the same potency but long-lasting effects.
Eur J Med Chem, 2018; 143
28883526: de la Torre-Martínez R, Bonache MA, Llabrés-Campaner PJ, Balsera B, Fernández-Carvajal A, Fernández-Ballester G, Ferrer-Montiel A, Pérez de Vega MJ, González-Muñiz R
Synthesis, high-throughput screening and pharmacological characterization of β-lactam derivatives as TRPM8 antagonists.
The mammalian transient receptor potential melastatin channel 8 (TRPM8), highly expressed in trigeminal and dorsal root ganglia, mediates the cooling sensation and plays an important role in the cold hypersensitivity characteristic of some types of neuropathic pain, as well as in cancer. Consequently, the identification of selective and potent ligands for TRPM8 is of great interest. Here, a series of compounds, having a β-lactam central scaffold, were prepared to explore the pharmacophore requirements for TRPM8 modulation. Structure-activity studies indicate that the minimal requirements for potent β-lactam-based TRPM8 blockers are hydrophobic groups (benzyl preferentially or Bu) on R, R, R and R and a short N-alkyl chain (≤3 carbons). The best compounds in the focused library (41 and 45) showed IC values of 46 nM and 83 nM, respectively, in electrophysiology assays. These compounds selectively blocked all modalities of TRPM8 activation, i.e. menthol, voltage, and temperature. Molecular modelling studies using a homology model of TRPM8 identified two putative binding sites, involving networks of hydrophobic interactions, and suggesting a negative allosteric modulation through the stabilization of the closed state. Thus, these β-lactams provide a novel pharmacophore scaffold to evolve TRPM8 allosteric modulators to treat TRPM8 channel dysfunction.
Sci Rep, 2017; 7
28195065: Mathivanan S, de la Torre-Martinez R, Wolf C, Mangano G, Polenzani L, Milanese C, Ferrer-Montiel A
Effect of econazole and benzydamine on sensory neurons in culture.

Econazole is an anti-mycotic agent widely used for the treatment of cutaneous fungal infections, and for the therapy of vaginal candidiasis. Topical application of this azole is generally safe, although some patients have complained of mild burning sensation/cutaneous irritation and itching, especially when administered intravaginally. The underlying mechanisms responsible of these adverse effects are poorly understood, though they suggest excitation of cutaneous nociceptor terminals. We report that exposure of primary cultures of rat nociceptors to econazole augments neuronal excitability. This effect appears mediated by increments in the intracellular Ca by stimulating Ca entry and release from the endoplasmic reticulum. Ca entry was not due to activation of thermo transient receptor potential (TRP) channels, suggesting a different ion channel targeted by the azole. Noteworthy, econazole-evoked responses were potentiated by a pro-inflammatory agent, which resulted in an increase in neuronal excitability. Econazole-elicited action potential firing was significantly abolished by the inflammatory cytokine inhibiting drug benzydamine via blockade of voltage-gated Na (Nav) channels. Collectively, our results indicate that the burning sensation of econazole is due at least in part to modulation of nociceptor excitability, and such sensation is increased in the presence of pro-inflammatory stimuli and blocked by benzydamine. These findings imply that a combination of the azole with benzydamine has the potential to reduce significantly the unpleasant symptoms related to infection and to the adverse effects of topical econazole formulations.
J Physiol Pharmacol, 2016; 67
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1,3-diphenylpropan-1-ones as allosteric modulators of α7 nACh receptors with analgesic and antioxidant properties.
Nicotine acethylcholine receptors (nAChRs) play critical roles in cognitive processes, neuroprotection and inflammation.
Future Med Chem, 2016; 8
26847872: Bertamino A, Ostacolo C, Ambrosino P, Musella S, Di Sarno V, Ciaglia T, Soldovieri MV, Iraci N, Fernandez Carvajal A, de la Torre-Martinez R, Ferrer-Montiel A, Gonzalez Muniz R, Novellino E, Taglialatela M, Campiglia P, Gomez-Monterrey I
Tryptamine-Based Derivatives as Transient Receptor Potential Melastatin Type 8 (TRPM8) Channel Modulators.
Pharmacological modulation of the transient receptor potential melastatin type 8 (TRPM8) is currently under investigation as a new approach for the treatment of pain and other diseases. In this study, a series of N-substituted tryptamines was prepared to explore the structural requirements determining TRPM8 modulation. Using a fluorescence-based screening assay, we identified two compounds acting as an activator (2-(1H-indol-3-yl)-N-(4-phenoxybenzyl)ethanamine, 21) or an inhibitor (N,N-dibenzyl-2-(1H-indol-3-yl)ethanamine, 12) of calcium influx in HEK293 cells. In patch-clamp recordings, compound 21 displayed a significantly higher potency (EC50 = 40 ± 4 μM) and a similar efficacy when compared to menthol; by contrast, compound 12 produced a concentration-dependent inhibition of menthol-induced TRPM8 currents (IC50 = 367 ± 24 nM). Molecular modeling studies using a homology model of a single rat TRPM8 subunit identified a putative binding site located between the VSD and the TRP box, disclosing differences in the binding modes for the agonist and the antagonist.
J Med Chem, 2016; 59
25438056: Fresno N, Pérez-Fernández R, Goicoechea C, Alkorta I, Fernández-Carvajal A, de la Torre-Martínez R, Quirce S, Ferrer-Montiel A, Martín MI, Goya P, Elguero J
Adamantyl analogues of paracetamol as potent analgesic drugs via inhibition of TRPA1.
Paracetamol also known as acetaminophen, is a widely used analgesic and antipyretic agent. We report the synthesis and biological evaluation of adamantyl analogues of paracetamol with important analgesic properties. The mechanism of nociception of compound 6a/b, an analog of paracetamol, is not exerted through direct interaction with cannabinoid receptors, nor by inhibiting COX. It behaves as an interesting selective TRPA1 channel antagonist, which may be responsible for its analgesic properties, whereas it has no effect on the TRPM8 nor TRPV1 channels. The possibility of replacing a phenyl ring by an adamantyl ring opens new avenues in other fields of medicinal chemistry.
PLoS One, 2014; 9
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Chalcones as positive allosteric modulators of α7 nicotinic acetylcholine receptors: a new target for a privileged structure.
The α7 acetylcholine nicotine receptor is a ligand-gated ion channel that is involved in cognition disorders, schizophrenia, pain and inflammation among other diseases. Therefore, the development of new agents that target this receptor has great significance. Positive allosteric modulators might be advantageous, since they facilitate receptor responses without directly interacting with the agonist binding site. Here we report the search for and further design of new positive allosteric modulators having the relatively simple chalcone structure. From the natural product isoliquiritigenin as starting point, chalcones substituted with hydroxyl groups at defined locations were identified as optimal and specific promoters of α7 nicotinic function. The most potent compound (2,4,2',5'-tetrahydroxychalcone, 111) was further characterized showing its potential as

neuroprotective, analgesic and cognitive enhancer, opening the way for future developments around the chalcone structure. Eur J Med Chem, 2014; 86

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BOARD NUMBER: S04-530

MEASURING NEUROLOGICAL DISEASE PROGRESSION IN RODENT MODELS BY QUANTIFYING WHISKER MOVEMENTS

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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[1]Manchester Metropolitan University, Department Of Natural Sciences, Manchester, United Kingdom, [2]University of Göttingen, Institute For Neuroanatomy, Göttingen, Germany, [3]Manchester Metropolitan University, Department Of Life Sciences, Manchester, United Kingdom, [4]Noldus Information Technology BV, Marketing, Wageningen, Netherlands

The current battery of tests used for assessing rodent behaviour consists of either expensive and intrusive methods or requires extensive animal training. They also result in only simple behavioural measures, such as durations or frequencies. We propose that measuring whisker-related exploratory movements offer an alternative way to observe highly quantitative behavioural changes in mice. We have previously demonstrated this by assessing whisker movement differences in mouse models of a wide range of neurological disorders. However, whisker movements have never been studied in tandem, and compared to, other established laboratory tests. Aim: for the first time, we attempt to integrate whisker tracking with a novel texture recognition task (NTR) and to assess NTR effects on whisker exploratory behaviour. Methods: mice were filmed using a high-speed video camera (500 frames per second) in an open arena during a novel object exploration task (NOE). Their whisker and body positions were tracked using freely available software ARTv2. Results: whisker tracking detected differences between mice that had been exposed to the NTR and naïve mice. Specifically, significant differences were found in pre-contact asymmetry and spread as well as during-contact protraction speed of NOE task. We also succeeded in integrating whisker tracking with the NTR task to provide more quantitative sensory and attentional information about the object contacts. Conclusion: whisker movements are a powerful behavioural measurement tool which incorporated within a standard battery of behavioural tests allows detection of additional exploratory-related parameters.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S04-531

CORTICAL CIRCUITS FOR CONTEXT-DEPENDENT SENSORIMOTOR TRANSFORMATION

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Flexible integration of sensory stimuli in a context-dependent manner is a key cognitive process required to generate appropriate behavior. An intriguing question, then, is how the same sensory stimulus can be interpreted differently according to context in order to generate different behavioral responses. We designed a task in which mice were trained to lick for reward in response to a brief single whisker stimulus if it was preceded by a brief Go-Tone presented one second before the whisker stimulus, but not if it was preceded by a NoGo-Tone. Optogenetic inactivation of primary whisker somatosensory cortex (wS1), secondary whisker somatosensory cortex (wS2), secondary whisker motor cortex (wM2) and anterior lateral motor cortex (ALM) during the presentation of the whisker stimulus decreased the probability of licking in the reward window. Inactivation of wM2 and ALM, but not wS1 or wS2, during the delay between the tone and the whisker stimulus reduced licking in the reward window. We investigated the neuronal correlates of context-dependent sensory processing using high-density extracellular recordings combined with high-speed video filming of facial movements. The neuronal response to the whisker stimulus in wS1 and wS2 was higher in Go-context hit trials than in NoGo-context correct rejection trials. wM2 and ALM also showed much stronger responses to whisker stimulus in the Go-context and had prominent persistent activity during the delay period following the Go-tone presentation, even in trials without anticipatory facial movements. These preliminary results point to an important role of frontal areas wM2 and ALM in context-dependent sensorimotor transformation.

Pubmed:

32839617: Yuste R, Hawrylycz M, Aalling N, Aguilar-Valles A, Arendt D, Armañanzas R, Ascoli GA, Bielza C, Bokharaie V, Bergmann TB, Bystron I, Capogna M, Chang Y, Clemens A, de Kock CPJ, DeFelipe J, Dos Santos SE, Dunville K, Feldmeyer D, Fiáth R, Fishell GJ, Foggetti A, Gao X, Ghaderi P, Goriounova NA, Güntürkün O, Hagihara K, Hall VJ, Helmstaedter M, Herculano-Houzel S, Hilscher MM, Hirase H, Hjerling-Leffler J, Hodge R, Huang J, Huda R, Khodosevich K, Kiehn O, Koch H, Kuebler ES, Kühnemund M, Larrañaga P, Lelieveldt B, Louth EL, Lui JH, Mansvelder HD, Marin O, Martinez-Trujillo J, Chameh HM, Mohapatra AN, Munguba H, Nedergaard M, Němec P, Ofer N, Pfisterer UG, Pontes S, Redmond W, Rossier J, Sanes JR, Scheuermann RH, Serrano-Saiz E, Staiger JF, Somogyi P, Tamás G, Tolias AS, Tosches MA, García MT, Wozny C, Wuttke TV, Liu Y, Yuan J, Zeng H, Lein E
A community-based transcriptomics classification and nomenclature of neocortical cell types.
To understand the function of cortical circuits, it is necessary to catalog their cellular diversity. Past attempts to do so using anatomical, physiological or molecular features of cortical cells have not resulted in a unified taxonomy of neuronal or glial cell types, partly due to limited data. Single-cell transcriptomics is enabling, for the first time, systematic high-throughput measurements of cortical cells and generation of datasets that hold the promise of being complete, accurate and permanent. Statistical analyses of these data reveal clusters that often correspond to cell types previously defined by morphological or physiological criteria and that appear conserved across cortical areas and species. To capitalize on these new methods, we propose the adoption of a transcriptome-based taxonomy of cell types for mammalian neocortex. This classification should be hierarchical and use a standardized nomenclature. It should be based on a probabilistic definition of a cell type and incorporate data from different approaches, developmental stages and species. A community-based classification and data aggregation model, such as a knowledge graph, could provide a common foundation for the study of cortical circuits. This community-based classification, nomenclature and data aggregation could serve as an example for cell type atlases in other parts of the body.
Nat Neurosci, 2020; 23
28113298: Ghaderi P, Marateb HR
Muscle Activity Map Reconstruction from High Density Surface EMG Signals With Missing Channels Using Image Inpainting and Surface Reconstruction Methods.
The aim of this study was to reconstruct low-quality High-density surface EMG (HDsEMG) signals, recorded with 2-D

electrode arrays, using image inpainting and surface reconstruction methods.
IEEE Trans Biomed Eng, 2017; 64
30542256: Ghaderi P, Marateb HR, Safari MS
Electrophysiological Profiling of Neocortical Neural Subtypes: A Semi-Supervised Method Applied to Whole-Cell Patch-Clamp Data.
A lot of efforts have been made to understand the structure and function of neocortical circuits. In fact, a promising way to understand the functions of cortical circuits is the classification of the neural types, based on their different properties. Recent studies focused on applying modern computational methods to classify neurons based on molecular, morphological, physiological, or mixed of these criteria. Although there are studies in the literature on /vivo extracellular or intracellular recordings, a study on the classification of neuronal types using whole-cell patch-clamp recordings is still lacking. We thus proposed a novel semi-supervised classification method based on waveform shape of neurons' spikes using whole-cell patch-clamp recordings. We, first, detected spike candidates. Then discriminative features were extracted from the time samples of the spikes using discrete cosine transform. We then extracted the center of clusters using fuzzy c-mean clustering and finally, the neurons were classified using the minimum distance classifier. We distinguished three types of neurons: excitatory pyramidal cells (Pyr) and two types of inhibitory neurons: GABAergic- parvalbumin positive (PV), and somatostatin positive (SST) non-pyramidal cells in layer II/III of the mice primary visual cortex. We used 10-fold cross validation in our study. The classification accuracy for PV, Pyr, and SST was 91.59 ± 1.69, 97.47 ± 0.67, and 89.06 ± 1.99, respectively. Overall, the algorithm correctly classified 92.67 ± 0.54% of the cells, confirming the relative robustness of the discriminant functions. The performance of the method was further assessed on recordings by using a pool of 50 neurons from Allen institute Cell Types Database (5 major subtypes of neurons: Pyr, PV, SST, 5HT3a, and vasoactive intestinal peptide (VIP) cells). Its overall accuracy was 84.13 ± 0.81% on this data set using cross validation framework. The proposed algorithm is thus a promising new tool in recognizing cell's type with high accuracy in laboratories using / whole-cell patch-clamp recording technique. The developed programs and the entire dataset are available online to interested readers.
Front Neurosci, 2018; 12

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BOARD NUMBER: S04-532

CELL TYPE-SPECIFIC ORGANIZATION OF GABAERGIC INTERNEURONS IN A CORTICAL COLUMN

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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[1]Max Planck Institute for Neurobiology of Behavior – caesar, In Silico Brain Sciences, Bonn, Germany, [2]Research Centre Jülich, Institute Of Neuroscience And Medicine, Jülich, Germany, [3]Max Planck Institute for Biological Intelligence, Cortical Column In Silico, Martinsried, Germany

Cortical GABAergic interneurons (INs) have been extensively reported as a rich and diverse class of neurons. At the single cell level, attributes such as morphology and intrinsic physiology exhibit complex patterns of variation, making them difficult to characterize. Here, we systematically assess the degree and character of the variability of these properties across the entire cortical depth of the rat barrel cortex. First, we compute a large set of descriptive features of 306 INs based on (i) their spiking patterns in response to somatic current injections, and (ii) reconstructions of their axon and dendrite morphology, as well as somatic depth location. Then, we group our sample into robust morphological and/or intrinsic physiological types by clustering the extracted features, performing a sensitivity analysis, and evaluating cluster predictability with several classifiers. Finally, we use deep models to provide quantitative insight into the relationships between morphology and intrinsic physiology to group INs, and reveal the degree to which particular features (such as somatic depth location) are predictive of an IN's morphological and/or intrinsic physiological type.

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LOCAL NETWORK EFFECTS OF SINGLE CELL STIMULATION IN THE SOMATOSENSORY CORTEX OF ANESTHETIZED RATS

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Evidence of single cell stimulation affecting cortical activity, animal behavior and presumed percepts keeps accumulating. Nonetheless, the understanding of how these effects are mediated via the local network level remains limited. We aim to complement the current knowledge by investigating the effect of single cell stimulations in rat somatosensory cortex on neurons in the immediate vicinity. To this end, we kept Wistar rats (~300 g) under general anesthesia (Medetomidin, Midazolam, Fentanyl) whilst acutely placing a silicon probe (32 contacts) in the somatosensory cortex and sampling local network activity along the entire cortical depth. At a lateral distance of ~200-300 µm and in any cortical layer, single neurons were stimulated with 220 ms square current pulses (juxtacellular configuration). We stimulated ~100 individual neurons and simultaneously recorded >3000 local network units. On average, the single cells spiked at ~25±15 Hz (maximum instantaneous firing rates of ~100±70 Hz) during stimulation, versus ~3 Hz during baseline. The network recordings contained ~25% single units. While the concerted local network effect of the induced single cell activity was significantly different from baseline, the overall change in firing rate remained below 0.2 Hz. We found that the majority of network units was unaffected by single-cell firing, but a minority of local network units showed significant activity alterations in both directions. In sum, effects of single cell stimulation onto local network units in the somatosensory cortex are small. An in-depth analysis highlights the factors impacting transmission characteristics and helps to understand principles governing single cell effects.

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BOARD NUMBER: S04-534

CONTINUITY WITHIN THE SOMATOSENSORY CORTICAL MAP FACILITATES LEARNING

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Henri Lassagne[1], Dorian Goueytes[2], Daniel Shulz[1], Luc Estebanez[2], Valérie Ego-Stengel[1]
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The topographic organization of sensory cortices is a prominent feature, but its functional role remains controversial. Particularly, it is still unknown how integration of activity within a cortical area depends on its topography during sensory-guided behavior. Here, we trained water-restricted mice expressing channelrhodopsin in cortical excitatory neurons to track a photostimulation bar that rotated continuously over the primary somatosensory cortex (S1). Mice could obtain reward by licking while the photostimulation resided within a specific range of angles. When the photostimulation was aimed at the whisker representation in S1, which contains a continuous representation of the animal whiskers, mice could learn to discriminate angular positions of the bar to obtain rewards. In contrast, they failed to learn the task in three other conditions: 1) when the spatiotemporal continuity of the photostimulation was disrupted, 2) when the photostimulation was aimed at the representation of the trunk and legs in S1, which contains multiple map discontinuities and 3) when the photostimulation was done on the posterior parietal cortex that does not contain a topographic map. Mice demonstrated anticipation of reward availability, specifically when cortical topography enabled to predict future sensory activation. These findings are particularly helpful for designing efficient sensory neuroprostheses that rely on direct cortical stimulation.

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BRADYCARDIA INDUCED BY CARDIAC BIOFEEDBACK TO RAT SOMATOSENSORY CORTEX

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Biofeedback is a therapy that helps humans to recognize and voluntarily control their unconscious bodily functions. The biofeedback-training has been proven effective for clinical application; however, the neural mechanism that enables the biofeedback has not been fully elucidated. Herein, as a pioneer of animals' biofeedback experiments, we established a heartrate (HR)-feedback system using electrocardiograms (ECGs) of rats. We recorded instantaneous HR from ECGs and immediately presented it to rats by electrical stimulation of the right or left barrel cortex depending on whether the current HR was higher or lower than the target HR, respectively. When rats were able to maintain their HRs below the target HR for 10 s, the medial forebrain bundle was stimulated as a neural reward. We demonstrated that rats reduced their HRs by 50% via the cardiac biofeedback. We recorded local field potentials (LFPs) from the anterior cingulate cortex (ACC) and found that the theta oscillation power was enhanced while rats kept their HRs low, consistent with the fact that the theta oscillation power increases in the human ACC during meditation. We investigated the effect of HR-feedback training on anxiety using the elevated plus maze test. The HRs were high when rats walked in the open arms before the feedback training, but the HRs became lower even in the open arms after the training. Our HR-feedback system using model animals will open the door for neuroscientists to investigate the mechanisms and significance of volitional control of cardiac function.

Pubmed:
34078810: Yoshimoto A, Yamashiro K, Ikegaya Y, Matsumoto N
Acute Ramelteon Treatment Maintains the Cardiac Rhythms of Rats during Non-REM Sleep.
Sleep curtailment negatively affects cardiac activities and thus should be ameliorated by pharmacological methods. One of the therapeutic targets is melatonin receptors, which tune circadian rhythms. Ramelteon, a melatonin MT/MT receptor agonist, has recently been developed to modulate sleep-wake rhythms. To date, the sleep-promoting effect of ramelteon has been widely delineated, but whether ramelteon treatment physiologically influences cardiac function is not well understood. To address this question, we recorded electrocardiograms, electromyograms, and electrocorticograms in the frontal cortex and the olfactory bulb of unrestrained rats treated with either ramelteon or vehicle. We detected vigilance states based on physiological measurements and analyzed cardiac and muscular activities. We found that during non-rapid eye movement (non-REM) sleep, heartrate variability was maintained by ramelteon treatment. Analysis of the electromyograms confirmed that neither microarousal during non-REM sleep nor the occupancy of phasic periods during REM sleep was altered by ramelteon. Our results indicate that ramelteon has a remedial effect on cardiac activity by keeping the heartrate variability and may reduce cardiac dysfunction during sleep.
Biol Pharm Bull, 2021; 44
33357785: Yoshimoto A, Yamashiro K, Suzuki T, Ikegaya Y, Matsumoto N
Ramelteon modulates gamma oscillations in the rat primary motor cortex during non-REM sleep.
Sleep disorders adversely affect daily activities and cause physiological and psychiatric problems. The shortcomings of benzodiazepine hypnotics have led to the development of ramelteon, a melatonin MT and MT agonist. Although the sleep-promoting effects of ramelteon have been documented, few studies have precisely investigated the structure of sleep and neural oscillatory activities. In this study, we recorded electrocorticograms in the primary motor cortex, the primary somatosensory cortex and the olfactory bulb as well as electromyograms in unrestrained rats treated with either ramelteon or vehicle. A neural-oscillation-based algorithm was used to classify the behavior of the rats into three vigilance states (e.g., awake, rapid eye movement (REM) sleep, and non-REM (NREM) sleep). Moreover, we investigated the region-, frequency- and state-specific modulation of extracellular oscillations in the ramelteon-treated rats. We demonstrated that in contrast to benzodiazepine treatment, ramelteon treatment promoted NREM sleep and enhanced fast gamma power in the primary motor cortex during NREM sleep, while REM sleep was unaffected. Gamma oscillations locally coordinate neuronal firing, and thus, ramelteon modulates neural oscillations in sleep states in a unique manner and may contribute to off-line

information processing during sleep.
J Pharmacol Sci, 2021; 145
34995981: Shibata Y, Yoshimoto A, Yamashiro K, Ikegaya Y, Matsumoto N
Delayed reinforcement hinders subsequent extinction.
In operant conditioning, animals associate their own behavior with a reinforcer, and the probability of the behavioral responses is increased. This form of learning is called reinforcement. In contrast, when the previously reinforced responses are no longer paired with a reinforcer, these responses are eventually extinguished. The effectiveness of reinforcement depends primarily on time intervals between reinforcers and responses, but it is not fully understood how the intervals affect subsequent extinction. To address this question, we performed electrical stimulation of the rat medial forebrain bundle (MFB), a part of the brain reward system, and an operant task in which the MFB was electrically stimulated 0.1 s (immediate condition) or 1 s (delayed condition) after the rat's nose was poked. During the first half of the task period (a reinforcement period), nose pokes were associated with MFB stimulation. In contrast, during the second half (an extinction period), we did not stimulate the MFB irrespective of nose pokes. We found that rats exhibited increased nose-poke behaviors during the reinforcement period under both conditions, whereas during the extinction period, nose pokes were more persistent in the delayed condition than in the immediate condition. The persistent responses in the extinction period were independent of responses in the reinforcement period. Therefore, reinforcement and extinction are driven by independent neural mechanisms.
Biochem Biophys Res Commun, 2022; 591

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BRAIN ACTIVATION DURING AFFECTIVE TOUCH CHANGES WITH THE “HUNGER HORMONE" GHRELIN

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Daniela M. Pfabigan, Uta Sailer
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The “hunger hormone” ghrelin stimulates appetite and increases food intake. Evidence in both animals and humans suggests that ghrelin also affects the motivation to obtain and consume other types of rewards such as alcohol or drugs. This pre-registered neuroimaging study investigated whether ghrelin’s effects extend to social rewards, namely affective touch, in humans. Sixty-eight volunteers received social-affective touch and control touch on their shins during 3T functional imaging on two test days. On one day, participants stayed fasted, on the other day they received a meal to change ghrelin levels, assessed in plasma at three time points each day. All touch was rated as more pleasant after the meal, but ghrelin levels were not associated with experienced pleasantness. A region-of-interest associated with reward processing, right medial orbitofrontal cortex (mOFC), showed decreased activation during all touch when ghrelin levels were high. During affective touch, larger ghrelin suppression following the meal was associated with higher mOFC activation, which in turn was associated with higher experienced pleasantness. Overall, high ghrelin levels appear to reduce reward-related brain activation, which is in line with suggestions that ghrelin carries a negative valence signal. However, the negative valence did not transfer to subjective experience. The findings suggest that the effects of naturally circulating ghrelin levels might be restricted to conscious perception. The lower touch pleasantness when fasted can more likely be attributed to other factors that come along with energy deprivation, such as altered interoception.

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COEXISTING NEURONAL CODING STRATEGIES IN THE BARREL CORTEX

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Rony Azouz
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During tactile sensation by rodents, whisker movements across surfaces generate complex whisker motions, including discrete, transient stick-slip events, which carry information about surface properties. The characteristics of these events and how the brain encodes this tactile information remain enigmatic. We found that cortical neurons show a mixture of synchronized and non-temporally correlated spikes in their tactile responses. Synchronous spikes convey the magnitude of stick-slip events by numerous aspects of temporal coding. These spikes show preferential selectivity for kinetic and kinematic whisker motion. By contrast, asynchronous spikes in each neuron convey the magnitude of stick-slip events by their discharge rates, response probability, and inter-spike intervals. We further show that the differentiation between these two types of activity is highly dependent on the magnitude of stick-slip events and stimulus and response history. These results suggest that cortical neurons transmit multiple components of tactile information through numerous coding strategies.

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DISENTANGLING A L2/3 VIP CELL TO L4 SST CELL CIRCUIT MOTIF ACROSS PRIMARY SOMATOSENSORY AND VISUAL CORTICES OF MOUSE

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Institute for Neuroanatomy, University Medical Centre Göttingen, Göttingen, Germany

Aims: The vasoactive intestinal polypeptide (VIP) to somatostatin (SST) neuron motif has mostly been studied within cortical L2/3. Connectivity across layers and the role of neuronal subtypes as targets remain unknown. Taking advantage of the recently discovered morphological differences between L4 SSTs of primary somatosensory (S1) and visual (V1) cortices, we studied if morphological differences manifest functionally in the L2/3 VIP to L4 SST motif in S1 and V1 cortices. **Methods:** Fluorescence-expressing transgenic mice were used to target VIPs and SSTs. We performed whole-cell patch-clamp recordings in slices to characterize SST cells and performed paired recordings of L2/3 VIP and L4 SST in S1 and V1. Morphology was systematically recovered. **Results:** 81% of SSTs in S1 and 55% in V1 were classified as non-Martinotti and Martinotti-type respectively, based on observable axonal arborization. L4 SSTs in S1 had lower time constant, lower input resistance and higher rheobase compared to V1. In paired recordings, unitary synaptic properties of VIP onto SST were largely similar. Short-term plasticity (STP) was studied at 1, 10, and 50 Hz. Connections in both cortices either showed no STP at any tested frequency or short-term facilitation during 50Hz stimulation. Strikingly, a distinct population of connections in S1, but not V1, exhibited measurable short-term depression at 50Hz. **Conclusion:** The VIP to SST motif is largely comparable between S1 and V1 in unitary connectivity properties, despite differential morphology of target SSTs. However, the equivalent circuit differs in high-frequency responses, resulting in differential electro-morphological circuit motifs across S1 and V1 cortices.

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CIRCADIAN DYNAMICS OF PAW WITHDRAWAL RESPONSES IN A MOUSE MODEL OF INFLAMMATORY INDUCED MECHANICAL HYPERSENSITIVITY

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

Phong Chau, Somayeh Ezzatpanah, Fred Haugen
National Institute of Occupational Health, Aml, Oslo, Norway

Aims In mammals, the internal molecular circadian systems generate its own oscillation and control the clock genes to keep the daily rhythm of various physiological and behaviour events operated functionally. Understanding of the daily rhythmicity in nociception is important for preventive interventions to minimize occupational pain, as well as standardization of animal studies. Our aim was to investigate daily rhythmicity in nociception and peripheral gene expression. *Methods* To study the phenomenon, we performed the well-established intraplanar Complete Freund's Adjuvant (CFA) model of mechanical allodynia. Mice were housed in a 12:12 hour light-dark cycle and unilaterally injected with CFA in the hind-paw, and saline in the contralateral paw (control). Then, we performed a mechanical stimulation assay of the paw using von-Frey filaments and assayed withdrawal behaviours at specific time points after the onset of light, described as *zeitgeber time* (ZT): 4ZT, 12ZT, 20ZT. Independent behavioural tests were also carried out at different times after CFA injection: 3-, 5- and 7-days post injection. Dorsal root ganglion (DRG) gene expression was studied using mRNA-seq and ddPCR. *Results and conclusions* The mechanical threshold for withdrawal of the CFA-injected paw showed circadian oscillation. It reached the peak during the light phase while it remained lower in the dark phase. The circadian influence on withdrawal behaviour was more evident in the acute phase of inflammation, day 3 after injection and less on day 5, 7. Furthermore, DRG gene expression showed circadian variation. Our data suggest a link between circadian rhythms and mechanical hypersensitivity.

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BARREL CORTEX IS NOT NECESSARY FOR GAP-CROSSING BEHAVIOR IN MICE WITH INTACT WHISKER-PAD

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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The barrel cortex is claimed to be indispensable for complex behavioral tasks. However, it is common that experimenters use partially sensory deprived rodents for the sake of controlling tactile stimulation. For example, rodents can have all but one facial whisker trimmed, a condition called single-whisker experience (SWE), known to cause a functional reorganization of the primary somatosensory maps. During SWE, whisker-dependent behaviors are initially impaired, but recover after cortical whisker associated regions remap. We asked to what extent barrel cortex task-relevant computations in a complex behavioral task rely on a complete whisker-pad. For that, we examined the effects of excitotoxic lesions of the barrel cortex of mice performing the gap-crossing task, a task that requires mice actively use their whiskers to cross over a gap between the two platforms in order to get a reward. Bilateral barrel cortex lesions induced mild behavioral deficits in expert mice with intact whiskers after re-exposure to the task, with mice recovering full task performance by the subsequent session. Similarly, bilateral barrel cortex lesions had no effect on task learning in mice with all whiskers intact. As expected, pre and post task acquisition lesioned groups submitted to SWE showed impaired recovery of previously learned gap-crossing skills. Our results show mice with an intact whisker-pad can reach full recovery of behavioral skills in the gap-crossing task after bilateral barrel cortex lesions, suggesting the functional relevance of primary somatosensory barrel cortex to active detection may rely on its sensory inputs remapping capacities.

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QUANTIFICATION OF AXONAL PROJECTIONS FROM NEURONS LOCATED IN LAYERS 2/3, 5 AND 6 OF MOUSE BARREL CORTEX

POSTER SESSION 04 - SECTION: SOMATOSENSORY PROCESSING

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Ecole Polytechnique Fédérale de Lausanne (EPFL), Brain Mind Institute, Laboratory Of Sensory Processing, Lausanne, Switzerland

Mice are nocturnal animals that rely heavily on their whiskers to sense the outside world. Whisker sensory information arriving from the ventral posterior medial nucleus of the thalamus is processed in the primary whisker somatosensory cortex (also known as the barrel cortex, S1-bfd). Projection neurons in S1-bfd in turn signal to various downstream brain areas including motor cortex, secondary sensory cortex, striatum, superior colliculus, thalamus, pons and brain stem. Different classes of S1-bfd neurons innervate different targets, but the precise organisation and quantification of axonal innervation remains to be determined. Here, we use adenoassociated viral (AAV) vectors to express fluorescent proteins in genetically-defined neuronal classes in S1-bfd. We injected Cre-dependent AAVs into various Cre-driver mice in order to express GFP and/or tdTomato in layer 2/3 (Rasgrf2-dCre), layer 5 (Sim1-Cre, Tlx3-Cre and Rbp4-Cre) and layer 6 (Ntsr1-Cre). After several weeks of expression, the fixed brains were immunostained and cleared through a variant of iDISCO (Renier et al., Cell 2014). Volumetric brain images were acquired by a MesoSPIM light sheet microscope (Voigt et al., Nat. Methods 2019). Voxels containing axons were segmented using TrailMap, a trained 3D convolutional network (Friedmann et al., PNAS 2020). Finally, images were aligned to the Allen mouse brain atlas (Wang et al., Cell 2020), and axonal length was quantified according to the annotated parcellations. Consistent with previous work, we find different projection patterns from each of the Cre-driver lines. Our results provide anatomical bases for functional connectivity contributing to the brain-wide processing of whisker-related sensorimotor information.

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VISUAL PROPERTIES OF CELL-TYPE SPECIFIC CORTICO-COLLICULAR INPUTS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

Katja Reinhard[1,2], Chen Li[1,2], Ben Vermaercke[1,2,3], Alex Calzoni[1,2], Norma Kühn[1,2], Karl Farrow[1,2,4]
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Innate aversive reactions to threat are mediated by circuits through the superior colliculus. In the mouse superior colliculus, a set of genetically identifiable cell types trigger different reactions by connecting relevant visual information from the retina to distinct behaviour-mediating targets. In addition to retinal inputs, these collicular neurons also receive inputs from the visual cortex. However, it is unknown whether the visual features received from cortex and retina are the same. Alternatively, cortical inputs might alter innate reactions by providing information about other aspects of the animal's environment. To address this question, we set out to characterize visual properties of cell-type specific cortico-collicular inputs. The calcium sensor GCaMP6f was expressed in layer 5 cortical cells projecting to $Ntsr^+$ (involved in freezing) or Grp^+ (involved in escape) collicular neurons using transsynaptic rabies tracing in different mouse lines (NTSR-GN209-Cre and GRP-KH288-Cre). We found that unique combinations of visual cortical areas project to different cell types in the colliculus. Further, 2-photon calcium imaging of cortical inputs to $Ntsr^+$ neurons during visual stimulation revealed preferences for looming disks and various combinations of small/big and fast/slow objects. This only partially overlaps with the information that $Ntsr^+$ neurons receive from the retina. Ongoing analysis will reveal whether different cortical areas send distinct distributions of visual features to the colliculus. Finally, by comparing visual preferences of neurons projecting to $Ntsr^+$ and Grp^+ cells, we will determine if cells involved in different behaviours receive the same or different sets of visual information from the cortex.

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FEATURE SELECTIVITY OF COLLICULAR WIDE-FIELD NEURONS IS GENERATED BY STRATIFIED INPUTS AND NONLINEAR DENDRITIC FILTERING

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

Norma Kühn, Chen Li, Natalia Baimacheva, Katja Reinhard, Janne Zimmer, Vincent Bonin, Karl Farrow
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The rules by which neurons combine their inputs to generate the appropriate output are key to brain function. While we know about the computational power of dendrites, how they help to filter sensory information to produce a neuron's output and drive behavior is underexplored due to the inability to trace inputs back to their sensory origin. Here, we address this problem using wide-field (WF) neurons of the mouse superior colliculus; a genetically targetable cell-type that receives direct input from the retina and mediates innate orienting behaviors. To understand how WF neurons combine their inputs, we use a combination of viral tracing and two-photon imaging to measure the visual responses in the dendrites and cell-bodies of WF neurons, as well as from their retinal inputs and local inhibitory inputs. Linear mapping of the retinal and inhibitory signals along the dendrites of WF neurons reveals distinct layers where specific inputs arrive. While a linear model fails to reconstruct the responses of WF cell-bodies, a non-linear model with at least two input layers accounts for the identified input structure and adequately reconstructs the cell-body responses. Our findings suggest that WF neuron dendrites have the capability to nonlinearly filter specific feature combinations from their diverse stratified inputs. To which extent each retinal cell-type contributes to WF signaling, remains to be uncovered by targeted manipulation thereof. Here, we established WF neurons as a unique model system to study the role of dendritic processing on the filtering of sensory information and its impact on behavior.

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DETERMINING THE ROLE OF DEFINED CELL TYPES OF THE MOUSE SUPERIOR COLLICULUS IN VISUAL FIELD-DEPENDENT BEHAVIORS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

Alex Calzoni[1,2,3,4], Chen Li[1,2,3,4], Arnau Sans Dublanc[1,2,3,4], Karl Farrow[1,2,3,4,5]
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Innate behaviors are essential for survival. Visual cues mimicking a predator elicit freezing or escape reactions, whereas those indicative of prey attract an animal's attention and prompt approach responses. Surprisingly, these different outcomes can be triggered by merely altering the position of the stimulus in the visual field. Both aversion and approach are mediated by a set of visuomotor circuits passing through the superior colliculus (SC). We know that certain collicular cell types subserve specific actions via dedicated output pathways. However, how these neurons encode behaviorally- salient visual features and whether their responses and circuits change according to the portion of the visual field they look at remains unknown. This project aims to investigate the role of genetically identified wide-field (WF) and narrow-field (NF) neurons of the mouse SC in driving behaviors evoked by ethologically-relevant stimuli in the upper and lower visual field. To achieve this, we will delineate the brain-wide circuit motifs of WF and NF neurons in different retinotopic regions via viral tracing methods and characterize their response properties to visual stimuli across the visual field with Neuropixels probe recordings. Finally, we will employ optogenetic inactivation of WF and NF cell activity to determine the effect of their retinotopic location on behavior. Together, these results will provide a structural and functional basis to understand how SC topography and cell types underlie visual field-dependent behaviors, and more generally how visual information is encoded and disseminated to trigger the appropriate motor response.

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LARGE-SCALE PAIRED RECORDINGS REVEAL STRONG AND SPECIFIC CONNECTIONS BETWEEN RETINA AND MIDBRAIN.

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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The output of the retina is carried by retinal ganglion cells (RGCs) along parallel functional pathways to multiple areas distributed across the vertebrate brain. Our aim was to better describe the functional organization of the synaptic connections between RGCs and their postsynaptic target neurons. Using tangential insertions of high-density Neuropixels probes in the mouse Superior Colliculus (SC, *Mus musculus*), we discovered that this method allows the measurement of large populations of RGC axons where they form synaptic contacts in SC, *in vivo*. The electrophysiological signature of RGC afferent inputs is made of a triphasic waveform composed of the axonal action potential, the axonal terminal response and, finally, the corresponding responses from the synaptically connected dendrites. Consequently, SC neurons can be recorded simultaneously with their presynaptic RGC inputs at large scales, which we also confirmed is possible in the avian optic tectum (zebra finch, *Taeniopygia guttata*). Taking advantage of the spatial resolution of high-density Neuropixels probes we studied the spatial location of RGC axons in SC and we discovered that the RGC axons isomorphically map the retinal mosaic into the upper visual layers of SC. Functionally, we show that RGC axons connect to their target neurons with limited functional convergence and log-normally distributed connection strength. Overall these results allow us to conclude that the retinotectal connectivity would possibly follow a common organizing principle in mammals and birds that provides a precise and reliable representation of the visual world to neurons in the midbrain.

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THALAMIC PROJECTION GABAERGIC NEURONS: RETINAL INPUT AND FUNCTION IN VIGILANCE STATE TRANSITIONS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Thalamic functions are executed primarily by glutamatergic thalamocortical neurons that extend axons to the cortex and initiate cortico-thalamocortical connectional loops. However, thalamic nucleic can contain GABAergic neurons that project to other subcortical structures but do not directly communicate with the cortex. Recent improvements in the understanding of intergeniculate (IGL) and ventrolateral geniculate (LGv) nuclei development allow us to specifically target and manipulate projecting GABAergic neurons in the mouse thalamus. We mapped the retinal input to thalamus-derived cells in the IGL/LGv complex and discovered that while ipRGC input is dominant, this is not likely to originate from M1-ipRGCs. Using EEG/EMG recordings in freely moving mice kept in standard circadian lighting conditions, we implicate thalamic cells in the IGL/LGv in vigilance state transitions at circadian light changes. Our data suggest that GABA projection neurons play a complementary role to thalamocortical networks in the thalamic control of vigilance states. Keywords: thalamus, intergeniculate leaflet, ventral lateral geniculate nucleus, ipRGCs, sleep, vigilance states, EEG.

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BOARD NUMBER: S04-547

THE VISUAL LAYERS OF THE SUPERIOR COLLICULUS ENCODE SACCADE DIRECTION

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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During active exploration, vision must compensate for visual input generated by retinal displacement: for example, visual responses are thought to be suppressed during voluntary eye movements called saccades. Here we investigated how saccades affect neural activity in the visual layers of the mouse superior colliculus (SC), a crucial visuo-motor structure governing orienting movements. We virally expressed GCaMP6f in the visual layers of SC of GAD-tdTomato mice, and used two-photon imaging to record the activity of excitatory and inhibitory neurons during spontaneous eye movements. Mice were head-fixed on a spherical treadmill in front of a gray screen, while we tracked running speed, eye position and pupil size. We found that, despite the absence of visual stimulation, one third (34±15% st.d., 1262 neurons, 8 mice) of SC neurons responded significantly around saccade onset. Inhibitory neurons (40%) responded almost twice as likely as excitatory neurons (24%). These motor responses were selective for saccade direction: ipsilateral saccades elicited responses in more neurons (53%) than contralateral saccades (27%). 50±15% of responsive neurons were suppressed and 44±11% were activated by saccades, while a minority (7±7%) were activated by one saccade direction and suppressed by the other. Saccade responses did not depend on changes in pupil diameter or locomotion, and were also observed during extracellular spike recordings with Neuropixels probes in complete darkness. In conclusion, we demonstrate that saccades do not merely inhibit visual processing in the SC, rather they are encoded through activation or suppression of neurons that are selective to saccade direction.

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MODULATION BY SEROTONIN OF RETINAL GANGLION CELL BOUTONS IN THE DORSOLATERAL GENICULATE NUCLEUS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Throughout the brain, the processing of sensory information is modulated to guide adaptive behaviors. Here, we investigate how serotonergic axons may specifically gate visual inputs at the level of retinal ganglion cell (RGC) axon terminals, prior to further integration and processing of sensory information in the thalamus. Bulk recordings of RGC axons showed that baseline and visually evoked calcium activity and glutamate release were suppressed by serotonin axon stimulation. Two-photon calcium imaging revealed that retinal axons preferring fullfield changes in luminance were more suppressed than those driven selectively by spatially localized stimuli, even when accounting for differences in each axon's baseline activity. Slice electrophysiology confirmed that serotonergic axon-evoked suppression of retinal glutamate release depends on presynaptic 5-HT1B receptors. Using single-cell sequencing, whole-mount electrophysiology and immunohistochemistry, we demonstrate differences in 5-HT1B receptor gene expression and axonal protein expression between RGCs that prefer fullfield changes in luminance vs. local stimuli. These data suggest a mechanism by which serotonin axons selectively gate specific visual information channels.

Pubmed:
32795442: Liang L, Fratzl A, Reggiani JDS, El Mansour O, Chen C, Andermann ML
Retinal Inputs to the Thalamus Are Selectively Gated by Arousal.
The brain can flexibly filter out sensory information in a manner that depends on behavioral state. In the visual thalamus and cortex, arousal and locomotion are associated with changes in the magnitude of responses to visual stimuli. Here, we asked whether such modulation of visual responses might already occur at an earlier stage in this visual pathway. We measured neural activity of retinal axons using wide-field and two-photon calcium imaging in awake mouse thalamus across arousal states associated with different pupil sizes. Surprisingly, visual responses to drifting gratings in retinal axonal boutons were robustly modulated by arousal level in a manner that varied across stimulus dimensions and across functionally distinct subsets of boutons. At low and intermediate spatial frequencies, the majority of boutons were suppressed by arousal. In contrast, at high spatial frequencies, boutons tuned to regions of visual space ahead of the mouse showed enhancement of responses. Arousal-related modulation also varied with a bouton's preference for luminance changes and direction or axis of motion, with greater response suppression in boutons tuned to luminance decrements versus increments, and in boutons preferring motion along directions or axes of optic flow. Together, our results suggest that differential modulation of distinct visual information channels by arousal state occurs at very early stages of visual processing, before the information is transmitted to neurons in visual thalamus. Such early filtering may provide an efficient means of optimizing central visual processing and perception across behavioral contexts.
Curr Biol, 2020; 30
29632360: Liu J, Reggiani JDS, Laboulaye MA, Pandey S, Chen B, Rubenstein JLR, Krishnaswamy A, Sanes JR
Tbr1 instructs laminar patterning of retinal ganglion cell dendrites.
Visual information is delivered to the brain by >40 types of retinal ganglion cells (RGCs). Diversity in this representation arises within the inner plexiform layer (IPL), where dendrites of each RGC type are restricted to specific sublaminae, limiting the interneuronal types that can innervate them. How such dendritic restriction arises is unclear. We show that the transcription factor Tbr1 is expressed by four mouse RGC types with dendrites in the outer IPL and is required for their laminar specification. Loss of Tbr1 results in elaboration of dendrites within the inner IPL, while misexpression in other cells retargets their neurites to the outer IPL. Two transmembrane molecules, Sorcs3 and Cdh8, act as effectors of the Tbr1-controlled lamination program. However, they are expressed in just one Tbr1 RGC type, supporting a model in which a single transcription factor implements similar laminar choices in distinct cell types by recruiting partially non-overlapping effectors.
Nat Neurosci, 2018; 21


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ANTAGONISTIC VISUOSPATIAL CIRCUITS IN THE SUPERIOR COLLICULUS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Early visual processes are subject to surround modulation thought to be important for perception, yet the circuit mechanisms still remain under debate. We therefore developed a generic optogenetic-based physiological approach that enables the precise determination and selective activation of a visual neuron's center or surround zone in the superficial layer of the superior colliculus (SCs). To study the synaptic interaction between these areas, we used whole-cell recordings from a wide-range of interneuronal subtypes in response to patterned optostimulation of incoming retinal axonal terminals expressing channelrhodopsin. Together with cell-type-specific transsynaptic mapping, in vivo optostimulation, and computational modelling, we demonstrate how local retinal inputs can trigger a strong regime of recurrent interactions amongst glutamatergic and GABAergic SCs interneurons in center and surround zones across the collicular map of visual space. We show that surround modulation, on the other hand, does not result in increased inhibition but it rather modifies the net excitability by weakening the efficacy of local amplification provided by recurrent excitation. This two-step antagonistic computation, which is carried out within the SCs, may provide the flexibility for upstream circuits to intervene and selectively manipulate downstream visual-based behavior.

Pubmed:
29492458: Wei W, Sun P, Li Z, Song K, Su W, Wang B, Liu Y, Zhao J
A surface-display biohybrid approach to light-driven hydrogen production in air.
Solar-to-chemical production by artificial and bioinspired photosynthetic systems is of tremendous interest to help solve current global energy and environmental problems. We developed a bioinorganic hybrid system for photocatalytic hydrogen production under aerobic conditions by combining light-harvesting semiconductors, hydrogenase catalysis, and self-aggregation of whole bacterial cells. We induced hydrogen production via self-photosynthesis in engineered cells, which were originally designed for bioremediation, with in situ biosynthesis of biocompatible cadmium sulfide nanoparticles using a surface-display system. We also introduced a biomimetic silica encapsulation strategy into the engineered cells, enabling this hybrid system to continuously produce hydrogen for 96 hours, even under natural aerobic conditions. This biohybrid catalytic approach may serve as a general strategy for solar-to-chemical production.
Sci Adv, 2018; 4

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BOARD NUMBER: S04-550

RECURRENT CORTICAL CONNECTIVITY IN THE PRIMARY VISUAL CORTEX SUPPORTS ROBUST ENCODING OF NATURAL SENSORY INPUTS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Predictive coding (*PC*) describes the brain as an organ of inference which builds an internal model of its environment to optimize its behaviour. This internal model constantly self-updates by computing the error of its predictions compared to the actual sensory input. Said sensory input comes, in our everyday world, in the form of heterogeneous features distributions, whose variances are highly volatile. Given the mounting evidence for PC to take place in the brain, it is physiologically likely, and mathematically required by PC, that the visual cortex possesses a mechanism that estimates the precision of its sensory inputs. Here, we record the electrophysiological responses of primary visual cortex (*V1*) neurons to naturalistic stimuli of varying precision. Using a model-driven analysis, we report two distinct, layer-specific populations in V1 : infragranular neurons encoding the sensory feature of stimuli, and supragranular neurons co-encoding both the feature and its precision. A machine learning-based neural decoder shows that these precision-specific neural units also implement precision-specific population dynamics. We use a recurrent spiking neural network to causally demonstrate that the presence of precision-selective activity stems from the different strength of recurrent inhibition between cortical neurons. Overall, we provide experimental and computational evidences to pinpoint the neural substrate of precision computations, which is a major milestone to support the theory of predictive coding.

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BOARD NUMBER: S04-551

ASYMMETRIC INFORMATION FLOW IN MOUSE HIGHER VISUAL AREAS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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In visual areas of mammals, there are functionally parallel ventral and dorsal pathways, and visual information is processed hierarchically in each pathway. Mouse higher visual areas (HVAs) can be grouped into ventral streams (LM, LI, P, POR) and dorsal streams (AL, RL, A, AM, PM). Visual hierarchy in mice has been estimated anatomically and physiologically using response latency and others. However, these estimations did not consider the causal relation between lower and higher areas. If visual information flows mostly from lower to higher areas, inhibition of the lower area will suppress the visual response in the higher area, but inhibition of the higher area will not significantly suppress the response in the lower area. We tested this idea with a combination of wide-field calcium imaging and optogenetics. Excitatory opsin was expressed in the inhibitory neurons of mouse visual cortex. While presenting various drifting gratings, we inhibited a specific visual area at a time, then we compared visual responses of each area with and without opto-inhibition of specific visual areas. We found that LM in mice is a gateway of visual information from V1. Inhibiting LM reduced responses of all other HVAs including AL and LI, while inhibiting AL or LI had a minor effect of LM responses. Similar inhibition asymmetry was also observed in the dorsal stream: AL inhibition suppresses RL responses, but not the other way around. Thus, information flows hierarchically between mouse HVAs. Our new method provides a way to determine the cortical hierarchy based on causal relationships.

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A TRANSCRIPTOMIC AXIS PREDICTS STATE MODULATION OF CORTICAL INTERNEURONS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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[Aims] Transcriptomics has revealed that cortical inhibitory neurons exhibit a great diversity of fine molecular subtypes, but it is not known whether these subtypes have correspondingly diverse activity patterns in the living brain. [Methods] We combined in vivo 2-photon calcium imaging of mouse V1 with a novel transcriptomic method to identify mRNAs for 72 selected genes in ex vivo slices. Based on these mRNAs, we classified inhibitory neurons imaged in layers 1-3 into a three-level hierarchy of 5 Families, 11 Types, and 35 Subtypes. [Results] Visual responses differed significantly only across Families: visual stimuli suppressed cells in the Sncg Family while driving cells in the Pvalb, Sst, Vip, and Lamp5 Families. Modulation by brain state differed at all hierarchical levels, but could be largely predicted from a single transcriptomic axis, the first transcriptomic principal component, which also predicted correlations with simultaneously recorded cells. Inhibitory Subtypes that fired more in resting, oscillatory brain states have narrower spikes, lower input resistance, weaker adaptation, and less axon in layer 1 as determined in vitro and express more inhibitory cholinergic receptors, as determined in single-cell RNA sequencing data. Subtypes firing more during arousal have the opposite properties. [Conclusions] We conclude that inhibitory subtypes in primary visual cortex (V1) have diverse correlates with brain state, but that this diversity is organised by a single factor: position along their main axis of transcriptomic variation.

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FUNCTIONAL ANATOMICAL ORGANIZATION OF INTRACORTICAL AND THALAMOCORTICAL PATHWAYS UNDERLYING HIGHER VISUAL REPRESENTATIONS IN THE MOUSE CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Vision in mammals relies on an intricate network of visual areas that integrate and transform retinal inputs into specialized representations, which enable specific visual perception and behavior; however, the underlying circuit organization remains unclear. Here we study the joint functional and anatomical organization of intracortical and thalamocortical visual pathways underlying visual representations in the mouse cortex. Combining in vivo widefield and cellular calcium imaging and genetic labeling, we characterized the cellular populations providing inputs to three specialized higher visual cortical areas (HVAs), anterolateral (AL), posteromedial (PM), and anterior (A) areas, which show specific connectivity and visual tuning. We found a close agreement between long-range connectivity and functional properties as well as diverse degrees of functional target-specificity. Area A and PM avoid connecting to each other and receive nearly non-overlapping inputs from distinct sets of cortical areas. Inputs from common source areas consist of non-overlapping subsets of neurons. The degree of target-specificity varies across intracortical pathways, depending on the diversity and specificity of neurons in the target areas as well as the laminar position of the intracortical projection cells. Lastly, axonal imaging of projections from the lateral posterior thalamic nucleus to HVAs revealed remarkable target-specificity, even greater than those of the cortical pathways. Our findings suggest the coexistence of multiple long-range wiring rules in the visual cortex: while thalamocortical projections play a possible driving role in the emergence of functional specialization in HVAs, intracortical pathways with distinct specificity might modulate the activities of target areas and establish distributed cortical subnetworks.

Pubmed:
32851591: Chen X, Han X, Blanchi B, Guan W, Ge W, Yu YC, Sun YE
Graded and pan-neural disease phenotypes of Rett Syndrome linked with dosage of functional MeCP2.
Rett syndrome (RTT) is a progressive neurodevelopmental disorder, mainly caused by mutations in MeCP2 and currently with no cure. We report here that neurons from R106W MeCP2 RTT human iPSCs as well as human embryonic stem cells after MeCP2 knockdown exhibit consistent and long-lasting impairment in maturation as indicated by impaired action potentials and passive membrane properties as well as reduced soma size and spine density. Moreover, RTT-inherent defects in neuronal maturation could be pan-neuronal and occurred in neurons with both dorsal and ventral forebrain features. Knockdown of MeCP2 led to more severe neuronal deficits as compared to RTT iPSC-derived neurons, which appeared to retain partial function. Strikingly, consistent deficits in nuclear size, dendritic complexity and circuitry-dependent spontaneous postsynaptic currents could only be observed in MeCP2 knockdown neurons but not RTT iPSC-derived neurons. Both neuron-intrinsic and circuitry-dependent deficits of MeCP2-deficient neurons could be fully or partially rescued by re-expression of wild type or T158M MeCP2, strengthening the dosage dependency of MeCP2 on disease phenotypes and also the partial function of the mutant. Our findings thus reveal stable neuronal maturation deficits and unexpectedly, graded sensitivities of neuron-inherent and neural transmission phenotypes towards the extent of MeCP2 deficiency, which is informative for future therapeutic development.
Protein Cell, 2021; 12

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VISION DEPENDENT AND INDEPENDENT PROCESSES SHAPE THE ORGANIZATION OF CORTICO-CORTICAL FEEDBACK IN THE MOUSE VISUAL CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Higher cortical areas influence lower order ones through direct cortico-cortical feedback projections but their precise role in cortical function remains unknown. Feedback connections from lateral-medial visual area (LM) to mouse primary visual cortex (V1) target retinotopically matched locations and their precise organization depends on the tuning properties of individual axons. While the tuning-dependent connectivity of feedback inputs might reflect learned visual statistics, the role of visual experience on the organization of feedback inputs remains uncharacterized. Using simultaneous dual-color 2-photon calcium imaging we recorded from LM axons and V1 neurons in their vicinity and measured the effects of different sensory manipulations on their functional organization. We find that in dark-reared mice, LM inputs remain retinotopically-matched with their targets in V1, but the extent of this alignment depends on the tuning of the feedback axons. Visual experience results in an increase in the proportion of orientation selective distal visual inputs converging into V1 neurons. This increase was mainly from axons representing locations lying along the axis of optic flow expected from forward locomotion. Restricting visual experience to a single specific orientation showed that this experience-dependent increase in feedback innervation is specific for axons tuned to the experienced stimuli. Thus, while the average retinotopic specificity of feedback inputs in V1 is largely independent of visual experience, vision shapes connections between LM and V1 neurons with non-aligned receptive fields to reflect experienced stimulus statistics. Our results suggest that the connectivity of feedback inputs reflects learned spatiotemporal correlations between higher and lower-order neurons.

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TWO DISINHIBITORY CIRCUITS MODULATE VISUAL CORTEX ADAPTATION DURING STIMULUS HABITUATION

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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The visual scene is constantly changing, and the detection of novel stimuli among familiar ones can be crucial for survival. In response to a sustained visual stimulus, pyramidal cells (PCs) in primary visual cortex vary in adaptive properties in a balance that is finely modulated by interneuron activity. PC and interneuron responses also change when the animal habituates to the stimulus, but how their adaptive properties adjust, and the underlying circuit mechanisms are poorly understood. To answer this question, we presented a 10 s visual stimulus repeatedly during 6 sessions while monitoring the activity of different cortical neurons with GCaMP6f. We found that PCs decrease their activity and shift from depression to sensitization upon habituation to the stimulus. Somatostatin interneurons (SSTs) increased their activity undergoing a similar change in adaptive properties to PCs, while parvalbumin (PVs) and vasoactive intestinal polypeptide (VIPs) interneurons dramatically decreased their activities. Optogenetically silencing SSTs we showed that they inhibit PCs more strongly when the stimulus is familiar but that does not affect their adaptive properties overall. Conversely, silencing VIPs revealed that SST adaptive properties are tightly linked to VIP inhibition which is stronger when the stimulus is novel. Our results reveal a concerted change of cortical adaptive properties during habituation towards sensitizing adaptation, and how SST to PV and VIP to SST inhibitory circuits are key to modulate this process disinhibiting PCs.

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VISUAL EXPERIENCE DIFFERENTIALLY AFFECTS THE ORGANIZATION OF CORTICAL FEEDBACK ORIGINATING FROM SUPERFICIAL AND DEEP LAYERS IN THE MOUSE VISUAL CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Perception emerges from the activity of a series of hierarchically organized cortical areas interconnected via ascending feedforward projections and descending feedback projections. Feedback connections from higher visual area lateral-medial area (LM) to primary visual cortex (V1) target retinotopically matched locations and their organization is dependent on the tuning properties of the axons. Neurons in multiple cortical layers send feedback to lower areas. Projections with different laminar origins might play distinct roles in cortical computation. Unveiling the role played by different feedback streams requires comparing their functional organization and how it depends on visual experience. Using laminar-specific driver mouse lines and simultaneous dual-colour 2-photon calcium imaging we measured the functional organization of LM axons arising specifically from Layer 5 (L5) or Layer 2/3 (L2/3) in V1 and how it depends on visual experience. We find that irrespective of visual experience, feedback axons from both L5 and L2/3 are retinotopically matched with their targets in V1. Feedback from L5 relays far more distal information to the V1 inputs compared to feedback from L2/3, predominantly from locations lying along the axis of optic flow from forward locomotion. Visual experience results in increased innervation from horizontally tuned L5, but not L2/3 inputs, relaying information from distal locations lying along the axis of optic flow. Our results suggest that feedback connections originating in the two layers play different roles in cortical computation, with L5 playing a more prominent role in shaping V1 activity depending on learned visual statistics than L2/3.

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CHANDELIER CELLS PROVIDE DIVERSE INPUTS TO CORTICAL NETWORKS IN-VIVO

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Chandelier cells are GABAergic interneurons which receive inputs from diverse brain regions and synapse onto hundreds of nearby excitatory pyramidal cells, precisely targeting their axon initial segments (AIS). They can thus exert a powerful control over the output of a cortical network, and have been suggested to play a key role in organising information processing across brain regions. However, the impact of chandelier cell activity on their local network is poorly understood. Here we used an all-optical approach to both activate and measure the activity of chandelier cells and their postsynaptic partners in the primary visual cortex (V1) and somatosensory cortex (S1) in mice. Network activity was measured using chronic two-photon calcium imaging of neural populations in layer 2/3 while we simultaneously activated chandelier cells using single cell-targeted two-photon optogenetics. Chandelier cells in V1 and S1 were largely unresponsive to visual and whisker stimulation respectively. However, stimulus evoked responses of putative pyramidal cells were modulated by chandelier cell activation. This modulation was heterogeneous across cells and included both facilitation and suppression of responses over distinct timescales. We measured the influence of the same identified chandelier cells longitudinally on the local network in pups over a developmental period (P14-P40) during which the postsynaptic effect of GABA released at the AIS has been postulated to change in polarity. Preliminary results indicate a dynamic and heterogeneous influence of chandelier cells in the developing cortex.

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MODULATION OF THE CORTICAL STATE BY SEROTONERGIC (5-HT) RECEPTORS: A STUDY COMBINING OPTOGENETIC TOOLS AND MULTI-CHANNEL ELECTRODE RECORDINGS IN MOUSE VISUAL CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Serotonin (5-HT) modulates sensory cortical drive by controlling the balance between responses to external inputs and ongoing internal activity. In the visual cortex various 5-HT receptor types that activate different signaling pathways are localized in distinct cell compartments, expressed in non-overlapping populations of interneurons, but widely co-expressed in pyramidal cells. Our previous study, considering 5-HT effects at the level of neuronal populations, showed that activation of the depolarizing 5-HT2A receptor leads to divisive suppression of the gain of visual evoked responses, while the inhibitory 5-HT1A receptor triggers divisive suppression of ongoing activity. However, the interplay of these receptor types and the exact mechanisms by which 5-HT modulates visual cortical circuitries at single cell levels is not fully understood. Here, using optogenetics we stimulated specific 5-HT receptors expressed selectively in either pyramidal or GABAergic cells and recorded single cell activity in the visual cortex of mice using multi-channel silicon probes. Our results indicate that selective activation of the 5-HT2A receptor in pyramidal cells leads to an increase in ongoing activity at the cortical network level. In conjunction with our previous study, these new results hint at polysynaptic mechanisms involved in the overall modulation of activity in the visual cortex. Altogether our findings suggest that 5-HT receptors orchestrate a fine-tuned change of the cortical state that influences the scaling between internal broadcasts and external sensory drive.

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INTERACTION BETWEEN INFORMATION STREAMS IN VISUAL PROCESSING THROUGH INTERAREAL LAYER 1 INHIBITORY CIRCUITS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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In the mouse, the anatomically-separated collicular and geniculate pathways carry distinct spatio-temporal features of a visual scene to the cortex. How such information streams then interact within the cortex and what are the cellular and circuit mechanisms behind this process is still unknown. The Layer 1 circuit of NDNF-positive (neuron-derived neurotrophic factor, L1-NDNF+) interneurons is a particularly good candidate to mediate interactions between information streams. This population is indeed strongly innervated by areas from concurrent pathways (e.g. in the geniculate-recipient primary visual cortex, L1-NDNF+ neurons receive inputs from the collicular streams via latero-posterior thalamic projections) and potently inhibit dendritic integration in the Layer 2/3 network below. In the present study, we investigate multi-pathway integration in the superficial layers of the visual cortex by building a two area spiking network model interconnected by L1-NDNF+ interneuronal circuits. We analyse the conditions, in terms of Layer 1 and Layer 2/3 network connectivity, leading to either a boosting (via disinhibition) or suppressive (via inhibition) effect on stimulus integration following L1-NDNF+ recruitment. We then address this question experimentally using visual stimulation and calcium imaging in the visual cortex of awake behaving mice. Our work suggests that interareal Layer 1 cortical circuits might be a key element of multi-pathway integration in visual processing.

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SPECIALIZED CAUSAL ROLES OF CORTICAL BASAL DENDRITES

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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[Aims] Neurons in layer 2/3 of primary visual cortex (V1) receive most excitatory connections from presynaptic neurons that have similar preferences for stimulus orientation and are located in an elongated region. We asked how pruning the branches of the dendritic tree extending towards different retinotopic territories might affect a neuron's visual tuning. [Methods] We first used two-photon imaging to record the orientation tuning and retinotopy of V1 neurons, reconstructed their dendritic arbors from structural z-stacks, and estimated the visual position of each dendritic segment along the retinotopic map of V1. We then recorded again from the same neurons after 2-photon pruning of the 'parallel' dendrites which extended co-axially with the neuron's preferred orientation, or the 'orthogonal' dendrites, which extended orthogonally. [Results] Parallel pruning drastically reduced responses to all stimuli,significantly decreased orientation selectivity and occasionally shifted preferred orientation. By contrast, orthogonal pruning only caused a mild reduction in response amplitude. In both cases, changes increased progressively with the pruning of a greater dendritic length. [Conclusions] These results demonstrate a specialized role of different basal dendrites in cortex. This specialization may be explained by parallel dendrites extending towards, and receiving more of, the appropriate co-tuned inputs, potentially providing a morphological substrate to orientation selectivity. To test this hypothesis we are currently combining dendritic pruning with synaptic imaging of glutamatergic transmission to reveal the synaptic organization of inputs on these specialized dendrites, and causally test their role.

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LAYER 6 CORTICO-CORTICAL FEEDFORWARD INHIBITION ONTO LAYER 2/3 MEDIATES MULTI-SENSORY INTEGRATION IN PRIMARY VISUAL CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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We constantly update our internal model of the world using information received via our sensory organs. Coherent representations are central to this process that relies on integration of the different types of sensory input. Here we have used a combination of in vivo and in vitro methods in the mouse to explore the possibility that cross-modal connectivity between primary sensory cortices might be mediate this process. Firstly, we find that whisker stimulation suppresses visually driven activity in the primary visual cortex (V1). Using brain-wide retrograde and anterograde trans-synaptic anatomical tracing followed by serial two-photon tomography and deep-learning based 3D detection of labeled cells, we find a direct cortico-cortical (CC) pathway between the barrel field of the primary somatosensory cortex and V1. Secondly, we show that excitatory L6 CC cells are the main source for direct projections to V1 and using opthophysiological experiments reveal fast-spiking interneuron-mediated feedforward inhibition of L2/3 pyramidal cells in V1. Strikingly, the anatomical location of recipient neurons in V1 corresponds to the area in the visual field that overlaps with the external world space where whiskers perform tactile exploration. Hence, our data reveal a functional convergence between somatosensory and visual information streams that overlap in somato-visual space and highlight a role for multisensory integration in primary sensory cortices.

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DIFFERENTIAL SYNAPTIC PROPERTIES OF LONG-RANGE INPUTS CONTACTING L1 INTERNEURONS IN THE PRIMARY VISUAL CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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In the visual system, the processing of visual information is strongly influenced by the behavioral relevance of the stimulus, the animal's internal brain state, as well as the presence of other sensory modalities. These contextual signals are conveyed to primary cortical sensory areas by multiple pathways including thalamic and intra-cortical feedback projections that strongly innervate layer 1 (L1). Thus, by integrating bottom-up sensory input with top-down information, L1 is a key hub structure underlying contextual encoding of sensory information. Yet, the functional architecture of L1 circuits is still poorly defined. Through an optogenetic approach, we dissect the synaptic and connectivity properties of long-range, top-down projections carrying contextual signals to L1 microcircuits in the primary visual cortex. We expressed the light-sensitive opsin channelrhodopsin2 (ChR2) in different associative visual areas (V2L and V2M) and lateral posterior (LP) nucleus of thalamus and recorded from L1 interneurons (L1-INs) while stimulating ChR2-positive axons. At the cellular level, we found that both V2L and V2M inputs preferentially target VIP-negative, NDNF-positive interneurons. Surprisingly, functional synaptic properties were quite heterogeneous across inputs. We found that the NMDA/AMPA ratio was much higher when the stimulated glutamatergic fibers originated from V2M, intermediate for LP and smallest for V2L. Preliminary current clamp recordings suggest that the different proportion of synaptic NMDARs results in pathway-specific integration properties of the multiple top-down inputs contacting L1-INs. In conclusion, we provide anatomical and functional evidence for the existence of L1 microcircuits suggesting differential engagement of L1-INs by long-range inputs in V1.

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EFFECTS OF AROUSAL ON VISUAL SPATIAL SELECTIVITY ACROSS NETWORK, CELLULAR AND SUBTHRESHOLD LEVELS IN MOUSE VISUAL CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Sensory processing depends upon multiple behavioral factors (Vinck et al., 2015; Stringer et al., 2019; Neske et al., 2019). In primary visual cortex (V1) of head-fixed mice, increased arousal during locomotion leads to membrane potential (Vm) depolarization and increased spiking. There remain open questions regarding arousal's effects on vision in the absence of locomotion, and effects on visual spatial selectivity across subthreshold and spiking levels. Here, we performed silicon probe and whole-cell patch-clamp recordings in awake, head-fixed, stationary mice while monitoring spontaneous behavior with high-speed video. We showed brief (0.1s) vertical bars (9° wide) throughout the visual field, and measured effects of pupil size and facial motion energy on V1 spatial responses. In some recordings, we optogenetically identified parvalbumin (PV) and somatostatin (SOM) interneurons. We found that even in stationary mice, changes in pupil size and facial movement correlated with electrophysiological brain state, and both exerted clear effects on visual spatial responses. With increasing arousal, local field potential (LFP) responses were smaller in the center of the receptive field (RF). In contrast, RS neurons (n = 1150) showed increased spiking at the center of the RF, as did PV/FS neurons (n=315), and SOM neurons (n=20). Across cell types, increased arousal generally improved the signal to noise ratio (SNR) of center versus surround visual responses. In excitatory neurons (n=35), subthreshold responses, SNR, spiking, and spatial selectivity generally increased. These findings suggest that even in stationary mice, increased arousal recruits cortical circuits to increase visual SNR without diminishing spatial selectivity.

Pubmed:

34314675: Williams B, Del Rosario J, Muzzu T, Peelman K, Coletta S, Bichler EK, Speed A, Meyer-Baese L, Saleem AB, Haider B
Spatial modulation of dark versus bright stimulus responses in the mouse visual system.
A fundamental task of the visual system is to respond to both increases and decreases of luminance with action potentials (ON and OFF responses). OFF responses are stronger, faster, and more salient than ON responses in primary visual cortex (V1) of both cats and primates, but in ferrets and mice, ON responses can be stronger, weaker, or balanced in comparison to OFF responses. These discrepancies could arise from differences in species, experimental techniques, or stimulus properties, particularly retinotopic location in the visual field, as has been speculated; however, the role of retinotopy for ON/OFF dominance has not been systematically tested across multiple scales of neural activity within species. Here, we measured OFF versus ON responses across large portions of visual space with silicon probe and whole-cell patch-clamp recordings in mouse V1 and lateral geniculate nucleus (LGN). We found that OFF responses dominated in the central visual field, whereas ON and OFF responses were more balanced in the periphery. These findings were consistent across local field potential (LFP), spikes, and subthreshold membrane potential in V1, and were aligned with spatial biases in ON and OFF responses in LGN. Our findings reveal that retinotopy may provide a common organizing principle for spatial modulation of OFF versus ON processing in mammalian visual systems.
Curr Biol, 2021; 31
32554511: Ding L, Chen H, Diamantaki M, Coletta S, Preston-Ferrer P, Burgalossi A
Structural Correlates of CA2 and CA3 Pyramidal Cell Activity in Freely-Moving Mice.
Plasticity within hippocampal circuits is essential for memory functions. The hippocampal CA2/CA3 region is thought to be able to rapidly store incoming information by plastic modifications of synaptic weights within its recurrent network. High-frequency spike-bursts are believed to be essential for this process, by serving as triggers for synaptic plasticity. Given the diversity of CA2/CA3 pyramidal neurons, it is currently unknown whether and how burst activity, assessed during natural behavior, relates to principal cell heterogeneity. To explore this issue, we juxtacellularly recorded the activity of single CA2/CA3 neurons from freely-moving male mice, exploring a familiar environment. In line with previous work, we found that spatial and temporal activity patterns of pyramidal neurons correlated with their topographical position. Morphometric analysis

revealed that neurons with a higher proportion of distal dendritic length displayed a higher tendency to fire spike-bursts. We propose that the dendritic architecture of pyramidal neurons might determine burst-firing by setting the relative amount of distal excitatory inputs from the entorhinal cortex. High-frequency spike-bursts are thought to serve fundamental computational roles within neural circuits. Within hippocampal circuits, spike-bursts are believed to serve as potent instructive signals, which increase the efficiency of information transfer and induce rapid modifications of synaptic efficacies. In the present study, by juxtacellularly recording and labeling single CA2/CA3 neurons in freely-moving mice, we explored whether and how burst propensity relates to pyramidal cell heterogeneity. We provide evidence that, within the CA2/CA3 region, neurons with higher proportion of distal dendritic length display a higher tendency to fire spike-bursts. Thus, the relative amount of entorhinal inputs, arriving onto the distal dendrites, might determine the burst propensity of individual CA2/CA3 neurons during natural behavior.
J Neurosci, 2020; 40
29718804: Coletta S, Zeraati R, Nasr K, Preston-Ferrer P, Burgalossi A
Interspike interval analysis and spikelets in presubicular head-direction cells.
Head-direction (HD) neurons are thought to provide the mammalian brain with an internal sense of direction. These cells, which selectively increase their firing when the animal's head points in a specific direction, use the spike rate to encode HD with a high signal-to-noise ratio. In the present work, we analyzed spike train features of presubicular HD cells recorded juxtacellularly in passively rotated rats. We found that HD neurons could be classified into two groups on the basis of their propensity to fire spikes at short interspike intervals. "Bursty" neurons displayed distinct spike waveforms and were weakly but significantly more modulated by HD compared with "nonbursty" cells. In a subset of HD neurons, we observed the occurrence of spikelets, small-amplitude "spike-like" events, whose HD tuning was highly correlated to that of the co-recorded juxtacellular spikes. Bursty and nonbursty HD cells, as well as spikelets, were also observed in freely moving animals during natural behavior. We speculate that spike bursts and spikelets might contribute to presubicular HD coding by enhancing its accuracy and transmission reliability to downstream targets. NEW & NOTEWORTHY We provide evidence that presubicular head-direction (HD) cells can be classified into two classes (bursty and nonbursty) on the basis of their propensity to fire spikes at short interspike intervals. Bursty cells displayed distinct electrophysiological properties and stronger directional tuning compared with nonbursty neurons. We also provide evidence for the occurrence of spikelets in a subset of HD cells. These electrophysiological features (spike bursts and spikelets) might contribute to the precision and robustness of the presubicular HD code.
J Neurophysiol, 2018; 120
29617670: Diamantaki M, Coletta S, Nasr K, Zeraati R, Laturnus S, Berens P, Preston-Ferrer P, Burgalossi A
Manipulating Hippocampal Place Cell Activity by Single-Cell Stimulation in Freely Moving Mice.
Learning critically depends on the ability to rapidly form and store non-overlapping representations of the external world. In line with their postulated role in episodic memory, hippocampal place cells can undergo a rapid reorganization of their firing fields upon contextual manipulations. To explore the mechanisms underlying such global remapping, we juxtacellularly stimulated 42 hippocampal neurons in freely moving mice during spatial exploration. We found that evoking spike trains in silent neurons was sufficient for creating place fields, while in place cells, juxtacellular stimulation induced a rapid remapping of their place fields to the stimulus location. The occurrence of complex spikes was most predictive of place field plasticity. Our data thus indicate that plasticity-inducing stimuli are able to rapidly bias place cell activity, simultaneously suppressing existing place fields. We propose that such competitive place field dynamics could support the orthogonalization of the hippocampal map during global remapping.
Cell Rep, 2018; 23
29487125: Coletta S, Frey M, Nasr K, Preston-Ferrer P, Burgalossi A
Testing the Efficacy of Single-Cell Stimulation in Biasing Presubicular Head Direction Activity.
To support navigation, the firing of head direction (HD) neurons must be tightly anchored to the external space. Indeed, inputs from external landmarks can rapidly reset the preferred direction of HD cells. Landmark stimuli have often been simulated as excitatory inputs from "visual cells" (encoding landmark information) to the HD attractor network; when excitatory visual inputs are sufficiently strong, preferred directions switch abruptly to the landmark location. In the present work, we tested whether mimicking such inputs via juxtacellular stimulation would be sufficient for shifting the tuning of individual presubicular HD cells recorded in passively rotated male rats. We recorded 81 HD cells in a cue-rich environment, and evoked spikes trains outside of their preferred direction (distance range, 11-178°). We found that HD tuning was remarkably resistant to activity manipulations. Even strong stimulations, which induced seconds-long spike trains, failed to induce a detectable shift in directional tuning. HD tuning curves before and after stimulation remained highly correlated, indicating that postsynaptic activation alone is insufficient for modifying HD output. Our data are thus consistent with the predicted stability of an HD attractor network when anchored to external landmarks. A small spiking bias at the stimulus direction could only be observed in a visually deprived environment in which both average firing rates and directional tuning

were markedly reduced. Based on this evidence, we speculate that, when attractor dynamics become unstable (e.g., under disorientation), the output of HD neurons could be more efficiently controlled by strong biasing stimuli. The activity of head direction (HD) cells is thought to provide the mammalian brain with an internal sense of direction. To support navigation, the firing of HD neurons must be anchored to external landmarks, a process thought to be supported by associative plasticity within the HD system. Here, we investigated these plasticity mechanisms by juxtacellular stimulation of single HD neurons in awake rats. We found that HD coding is strongly resistant to external manipulations of spiking activity. Only in a visually deprived environment was juxtacellular stimulation able to induce a small activity bias in single presubicular neurons. We propose that juxtacellular stimulation can bias HD tuning only when competing anchoring inputs are reduced or not available.
J Neurosci, 2018; 38

27282390: Preston-Ferrer P, Coletta S, Frey M, Burgalossi A
Anatomical organization of presubicular head-direction circuits.
Neurons coding for head-direction are crucial for spatial navigation. Here we explored the cellular basis of head-direction coding in the rat dorsal presubiculum (PreS). We found that layer2 is composed of two principal cell populations (calbindin-positive and calbindin-negative neurons) which targeted the contralateral PreS and retrosplenial cortex, respectively. Layer3 pyramidal neurons projected to the medial entorhinal cortex (MEC). By juxtacellularly recording PreS neurons in awake rats during passive-rotation, we found that head-direction responses were preferentially contributed by layer3 pyramidal cells, whose long-range axons branched within layer3 of the MEC. In contrast, layer2 neurons displayed distinct spike-shapes, were not modulated by head-direction but rhythmically-entrained by theta-oscillations. Fast-spiking interneurons showed only weak directionality and theta-rhythmicity, but were significantly modulated by angular velocity. Our data thus indicate that PreS neurons differentially contribute to head-direction coding, and point to a cell-type- and layer-specific routing of directional and non-directional information to downstream cortical targets.
Elife, 2016; 5

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CONNECTIVITY AND FUNCTION OF CHANDELIER CELLS IN MOUSE PRIMARY VISUAL CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Chandelier cells (ChCs) are a unique subtype of GABAergic cells that exclusively target the axon initial segment of pyramidal neurons (PyNs). Although ChCs were first identified decades ago, their function remains a mystery. Here, we studied the connectivity, plasticity and function of ChCs in mouse primary visual cortex (V1). We performed two-photon calcium imaging in mice performing a behavioral task in a virtual tunnel. We find that in our task, PyNs can be clustered in visual and non-visual cells and that ChC responses align well with those of non-visual cells. We further employed viral tracing, histology and electrophysiology to study their connectivity with PyNs. This revealed that ChCs receive extensive feedback inputs, matching their functional properties. Using opto- and chemogenetics we are currently testing the hypothesis that ChCs preferentially target visual neurons, thus separating feedforward and feedback information streams in the cortical network.

Pubmed:
34570697: Montijn JS, Seignette K, Howlett MH, Cazemier JL, Kamermans M, Levelt CN, Heimel JA
A parameter-free statistical test for neuronal responsiveness.
Neurophysiological studies depend on a reliable quantification of whether and when a neuron responds to stimulation. Simple methods to determine responsiveness require arbitrary parameter choices, such as binning size, while more advanced model-based methods require fitting and hyperparameter tuning. These parameter choices can change the results, which invites bad statistical practice and reduces the replicability. New recording techniques that yield increasingly large numbers of cells would benefit from a test for cell-inclusion that requires no manual curation. Here, we present the parameter-free ZETA-test, which outperforms t-tests, ANOVAs, and renewal-process-based methods by including more cells at a similar false-positive rate. We show that our procedure works across brain regions and recording techniques, including calcium imaging and Neuropixels data. Furthermore, in illustration of the method, we show in mouse visual cortex that (1) visuomotor-mismatch and spatial location are encoded by different neuronal subpopulations and (2) optogenetic stimulation of VIP cells leads to early inhibition and subsequent disinhibition.
Elife, 2021; 10
29920266: Seignette K, Levelt CN
Amblyopia: The Thalamus Is a No-Go Area for Visual Acuity.
When one eye does not function well during development, the visual cortex becomes less responsive to it and visual acuity declines. New research suggests that reduced response strength and deteriorating acuity occur in separate circuits.
Curr Biol, 2018; 28
29184199: Sommeijer JP, Ahmadlou M, Saiepour MH, Seignette K, Min R, Heimel JA, Levelt CN
Thalamic inhibition regulates critical-period plasticity in visual cortex and thalamus.
During critical periods of development, experience shapes cortical circuits, resulting in the acquisition of functions used throughout life. The classic example of critical-period plasticity is ocular dominance (OD) plasticity, which optimizes binocular vision but can reduce the responsiveness of the primary visual cortex (V1) to an eye providing low-grade visual input. The onset of the critical period of OD plasticity involves the maturation of inhibitory synapses within V1, specifically those containing the GABA receptor α1 subunit. Here we show that thalamic relay neurons in mouse dorsolateral geniculate nucleus (dLGN) also undergo OD plasticity. This process depends on thalamic α1-containing synapses and is required for consolidation of the OD shift in V1 during long-term deprivation. Our findings demonstrate that thalamic inhibitory circuits play a central role in the regulation of the critical period. This has far-reaching consequences for the interpretation of studies investigating the molecular and cellular mechanisms regulating critical periods of brain development.
Nat Neurosci, 2017; 20


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FAMILIARITY-EVOKED THETA OSCILLATIONS IN THE MOUSE VISUAL CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Recognizing familiarity and novelty in the environment is critical for animal survival. Surprisingly, very little is known about how animals distinguish familiar from novel stimuli. We have recently discovered persistent stimulus-triggered theta oscillations in the visual cortex of mice, which are specific to the familiarity and spatial frequency features of the stimulus. These oscillations are dependent on the cholinergic muscarinic receptors for both induction and expression. Familiarity-evoked oscillations lead to the decrease in suprathreshold responses to the non-preferred directions of the drifting sinusoidal gratings and to the improved direction selectivity in V1 neurons. We then used optogenetics to directly measure the synaptic strength of the light-triggered EPSCs in V1 neurons in head-fixed mice. We discovered the weakening of the thalamocortical projections coinciding with the strengthening of the intracortical projections in V1 following visual experience and emergence of the familiarity-evoked theta oscillations. Finally, we have discovered theta oscillations in the higher visual areas (HVAs) which are synchronized with the theta oscillations in V1 following visual experience. This synchronization varies between different HVAs and depends on the properties of the visual stimuli.

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FUNCTION-SPECIFIC CORTICOFUGAL CONNECTIVITY PROMOTES THE PLASTICITY OF OPTOKINETIC REFLEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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The visual cortex sends massive corticofugal projection to the brainstem, by which the cortex can modulate the brainstem driven innate behaviors. One example is the optokinetic reflex (OKR), an involuntary eye movement to stabilize retinal images. Recent study showed that the corticofugal projection enabled the visual cortex to potentiate OKR to make up the loss of other ocular behaviors. Despite its importance, the underlying mechanisms remain unclear. We used an interdisciplinary approach to explore the biological substrate and mechanisms of this cortical function. Anatomically, we found that corticofugal neurons innervating the brainstem OKR circuit primarily came from anterior V1 and posterior higher visual areas, and synapsed on one brainstem population. Moreover, behavioral tests showed that above cortical areas and brainstem population were required for the cortical modulation of OKR. Next, with calcium imaging we discovered that those corticofugal neurons shared similar functional properties to their postsynaptic target in the brainstem. Interestingly, following OKR potentiation cortical activity evoked by temporal-nasal motion was boosted only in the neurons preferring temporal-nasal direction. Finally, modelling showed that the direction specific connectivity and activity potentiation of corticofugal neurons provided an efficient way to supply extra cortical innervation in support of OKR potentiation. Altogether, our results suggest that the corticofugal projection and its brainstem partner form a distinct pathway through which visual cortex sends functional relevant information to the brainstem.

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RECAPITULATING THE EVOLUTIONARY TRANSFORMATION OF VISUAL CORTEX ARCHITECTURE IN A TABLETOP EXPERIMENT

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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In the visual cortex of primates and carnivores orientation selective neurons are organized into functional domains that are arranged around so called pinwheels. This structure most likely emerged from a prior rodent-like salt-and-pepper layout. We designed a synthetic biology approach in which we implemented evolutionary transition scenarios by switching between different wiring schemes for thalamic afferents. We used neuronal interface technology to connect a computational model of the retino-thalamic pathway to an in-vitro model of cortical input layer 4 (L4). The latter contained channelrhodopsin expressing principal neurons, either as a primary culture of cortical neurons or an acute brain slice. The two stages were connected via optogenetic holographic stimulation emulating thalamo-cortical synaptic input to L4. We recorded neural activity either with a multielectrode array or by calcium imaging. In the feed-forward model orientation selection in L4 is a result of convergent thalamic input. We implemented such a feed-forward scheme in our system with variable size of orientation domains. We then explored the consequences of scaling the size of orientation domains down to the size of single neurons. Furthermore, we implemented a random wiring scheme. We found that the fraction of orientation selective neurons only weakly decreased with shrinking domain size and even for random thalamo-cortical connections a considerable level of orientation selectivity was maintained. In this case the arrangement of orientation selective cells resembled a sparse salt-and-pepper layout. Our results indicate that evolutionary scaling of orientation domain sizes can induce a self-organized transition to and from a salt-and-pepper layout.

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TO LOOK OR NOT TO LOOK? : A NEURAL NETWORK ROUTING VISUAL DISTRACTIONS INTO BEHAVIOR

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Responding to peripheral visual events during ongoing behavior is critical for survival. Identifying and controlling the neural circuits that route pop-up visual input during planned behavior is important for understanding the versatility of visuospatial behavior. Here, we designed a paradigm in which animals navigate a predicted trajectory and their movement can be interrupted by (1) presenting visual distractors optically projected at random points in space and time or (2) by selective optogenetic stimulation of projection neurons in the intermediate layer of the superior colliculus (SCi) targeting the contralateral gigantocellularis(c.Gi) in the brainstem. We found that both modes are sufficient to interrupt guided navigation with movement patterns reminiscent of natural visual distraction trials. To quantify and assess similarities between the two cases, we employed machine vision techniques to track animal movements, estimated multi-scale postural motifs, and clustered them to achieve unsupervised and systematic categorizations of animal motion. This analysis revealed similar basic motifs, suggesting the involvement of SCi projecting neurons in mediating visual distraction (which we are currently monitoring in vivo). Using transsynaptic circuit mapping, we found that these neurons receive monosynaptic inputs from retinal ganglion cells (RGCs) bypassing the superficial layer by a large extent. Whole-cell recordings showed that there is a weak functional connectivity between SCi projection neurons and channelrhodopsin-expressing RGCs. We propose that the RGC->SCi->c.Gi network is the neural hardware which routes visual distractions into task-dependent behavior.

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COORDINATED ACTIVITY OF TWO CELL-TYPE SPECIFIC CIRCUITS AND THEIR ROLE IN GUIDING BEHAVIORS – INSIGHTS FROM THE MICE SUPERIOR COLLICULUS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Neuronal cell types are arranged in brain wide circuits to guide behavior. Using the mice superior colliculus as a model, it has been demonstrated that optogenetic activation of different genetically targetable collicular cell types leads to various defensive behaviors (passive or active avoidance), engaging distinct but overlapping brain-wide dynamics. However, the optogenetic approach doesn't ideally represent the activation going on in the brain in response to natural stimuli. The aim of this study is to shed light on how cell-type specific processing pathways enable behavioral execution in response to salient, ecologically relevant stimuli. To do that, we focused on two collicular cell types – wide- and narrow-field neurons, and their responses to visual stimuli that mimic predators (e.g., sweeping and looming discs). Freely-moving experiments in combination with chemogenetic inhibition of these neuronal populations resulted in a similar incapability of animals to react to potentially dangerous visual stimuli. This suggests the existence of common processing nodes of those two cell-type specific circuits, where the information about threat encounter converge. We are currently performing experiments to determine whether this is happening locally at the level of the superior colliculus, or globally via recruitment of a set of processing hubs in the brain. We map the local and global activity using high-density Neuropixels probes recordings and functional ultrasound imaging (fUSI), respectively. This work stresses the importance of cell types as functional building blocks in the brain and provides insight into how their coordinated activity can guide behavior.

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BOARD NUMBER: S04-570

CELL-TYPE-SPECIFIC CONTRIBUTIONS TO VISUOMOTOR TRANSFORMATIONS IN THE SUPERIOR COLLICULUS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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How does an animal select which action to take when facing a sudden threat? In mice, most visual inputs are sent directly from the retina to a midbrain structure known as the superior colliculus. In the colliculus, a set of genetically-distinct cell types have been identified that are involved in driving defensive behaviors. However, it remains unclear how these cell types underly the transformation from visual inputs to behavioral actions. In this study, we characterize the organization of visual and behavior representations throughout the superior colliculus and uncover how different cell types contribute to that organization. By presenting threatening and non-threatening visual stimuli while doing electrophysiology recording in the colliculus, we observe a transformation from stimulus-specific encoding to threat encoding along collicular depth. Furthermore, by chemogenetically inhibiting collicular cell types in freely-moving experiments, we find two cell types -- GRP and NTSR1 neurons -- that are both required for visually-evoked defensive behaviors. This suggests that both cell types affect the processing of visual threats to trigger defensive behaviors. Therefore, we are now performing electrophysiology experiments combined with chemogenetic inhibition and optogenetic tagging to find out the role of both cell types in processing visual threats. Taken together, these experiments allow us to uncover the cell-type specific mechanisms underlying the transformation from visual threat to a defensive action in the superior colliculus, thereby hinting towards the importance of cell-type specific populations in the implementation of visuo-motor transformations across the brain.

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BOARD NUMBER: S04-571

A GENETIC SCREEN OF VISUAL PROJECTIONS AND RETINA ORGANIZATION IN ADULT MICE

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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In the mouse, over 30 subtypes of retinal ganglion cells project to at least 40 brain nuclei. The genetic program controlling visual system wiring during development is still largely unknown. To date, a screen for genes affecting the guidance of visual projections has never been performed in mammals. To identify new molecules controlling the development and plasticity of the visual system, we are performing a reverse genetic screen using single-gene knockout mouse lines produced by the JAX Knockout Mouse Phenotyping Program (KOMP2). Visual projections are labeled via intraocular injection of the axonal tracer cholera-toxin subunit B, conjugated to various fluorescent Alexa-dyes. For each line, four mice (two males and two females) are injected, along with age, sex and genetic background matched control animals. Brains are collected and cleared using iDISCO+. To study the topography of visual projections and detect axon targeting defects, cleared brains are imaged in 3D using light-sheet fluorescence microscopy. In parallel, we use the EyeDISCO protocol and TOPRO-3 nuclear staining to study the organization of the retina. We have already been able to image more than 250 lines, corresponding to about 800 brains and 800 eyes. We are developing AI-based analytic pipelines to quantify visual system connectivity in mutant mice. We are also testing various reprocessing strategies to perform secondary screens for axon guidance defects. This project will contribute to our understanding of the neuronal basis of the parallel processing of visual inputs and how circuits involved in image-forming vision or motion detection are established during development.

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BOARD NUMBER: S04-572

EARLY VISUAL DEPRIVATION PROMOTES ASTROCYTE REACTIVITY IN THE VISUAL THALAMUS

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Sensory circuits development is a process that requires peripherally driven sensory signals. The loss of sensory input during the formation of these systems leads to structural and functional changes in stations along the circuit, as the thalamus and cortex. In the visual system, the removal of the eyes at postnatal stages (P3) leads to several changes in the dorsolateral geniculate nucleus (dLGN), the first order visual thalamic nucleus. Among them, postnatal bienucleation causes a decrease in the size of the nucleus which is explained by an increased cell death. Conversely, we observed a specific response in the non-neuronal population in the dLGN. The number of reactive astrocytes (GFAP+) was significantly increased at P6. BrdU labeling showed that reactive astrocytes were not proliferating in the bilateral enucleated mice but that they either become reactive in situ or had migrated from another source. Experiments from single-cell RNA-sequencing will be performed to determine the cell-populations and genes modified in the thalamus following postnatal enucleated condition. We have recently shown that thalamic astrocytes can be converted into specific sensory neurons. Since reactive astrocytes seem to switch to an immature stage and are better predisposed to change their identity into neurons, these experiments in early postnatal deprived mice are setting the scene for astrocyte-to-neuron reprogramming in sensory-deprived models.

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BOARD NUMBER: S04-573

SIGNIFICANCE OF OPTIC FLOW INFORMATION FOR VISUAL COURSE CONTROL IN FREELY FLYING FLIES

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Counteracting involuntary course deviations during flight requires detection of self-motion. In dipteran flies, such as *Drosophila melanogaster*, this information is provided by mechanosensory receptors, located at the base of the halters, as well as by the visual system. In the latter, local motion information is encoded by four sets of directionally-selective T4 (ON-pathway) and T5 cells (OFF-pathway) per column, one for each of the four cardinal direction (right, left, up, down). According to their directional preference, T4 and T5 cells project to four anatomically defined layers of the lobula plate, where they provide synaptic input to large-field course-control neurons. Blocking the synaptic output from T4 and T5 cells renders flies completely blind to visual motion (Bahl et al, 2013; Schilling and Borst, 2015). In our previous study (Leonte et al, 2021), we have shown that in free flight, such motion-blind flies reveal a reduced straightness of inter-saccadic flight segments and are unable to compensate for aerodynamic asymmetries caused by wing damage. The goal of the current study is to investigate free flight performance in flies where visual motion information is not completely blocked but still present for certain directions. To this end, we silence the synaptic output of specific subtypes of T4/T5 cells and then assess the straightness of their inter-saccadic flight segments as recorded in a free flight arena. With this approach, we hope to determine whether flies are still able to correctly infer their self-motion when certain vector components used to compute the optic flow are lacking.

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DYNAMIC AND STATE-DEPENDENT SWITCHING OF BEHAVIOUR IN RESPONSE TO COMPETING VISUAL STIMULI IN DROSOPHILA

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Sensorimotor circuits have classically been studied using defined stimuli that map onto reproducible behavioural responses. However, the numerous features in a complex natural environment would inevitably drive multiple dedicated circuits, generating a combination of competing motor commands. In order to cope with such a scenario, sensorimotor transformations should be selectively modulated depending on the goals and internal state of the animal. Here we developed a novel behavioural paradigm to study how the fruit fly processes competing visual stimuli to generate coherent behaviour. We take advantage of optomotor response and courtship tracking in fruit flies, two distinct visually-guided innate behaviours that can be elicited reliably in our experimental setup. We report that male flies suppress optomotor response to global visual motion while engaged in courtship tracking of female flies. Next, we recapitulate this experiment in virtual reality where the female fly is replaced with a dark dot while the global motion stimulus stays the same. In this setting, males switch between tracking and optomotor response and the probability and dynamics of this switching is governed by the relative strengths of the stimuli determined by their respective saliencies. Our findings show that fruit flies dynamically modulate innate responses to stimuli that are irrelevant to the task that they are engaged in, providing support for attention-like mechanisms in the fly brain. Currently, we are investigating the neuronal mechanism of the switching dynamics by targetedly disrupting octopaminergic and tyraminergic neuromodulatory inputs.

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NEUROMECHANICAL SIMULATION OF ZEBRAFISH VISUOMOTOR COORDINATION

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Abstract: Background and Aim: Zebrafish larvae has gained attention in neuroscience due to its optical accessibility and quantifiable behaviors. While scientists have elucidated the neural mechanisms underlying a variety of visually driven behaviors, such as prey-, escape-, optokinetic-, and optomotor responses (OMR), few studies have explored neuromechanical simulations of both the visuomotor circuits and the body, in order to investigate how sensory-driven behavior depends on neural circuits. Method: Recent advances in the understanding of the neural function and new simulation technologies have made this a feasible goal. We constructed software simulations that reproduce body, water interactions, neural circuits, and the closed-loop visual environments, replicating the experimental settings for live zebrafish behavioral recordings. Results: We implemented a neural network based on real recorded neurons of live zebrafish in a fish-like simulated body that allowed predictions for behavioral responses to different types of stimuli. Our model not only replicates several OMR behaviors reported in the literature, but also predicts novel behaviors to new stimuli that the animal is not confronted within nature (e.g., different stimuli for left and right eyes). Conclusion: Via iterations between simulation, prediction, and experimental validation we incrementally refined the functional elements and connectivity underlying the zebrafish visuomotor
circuits.

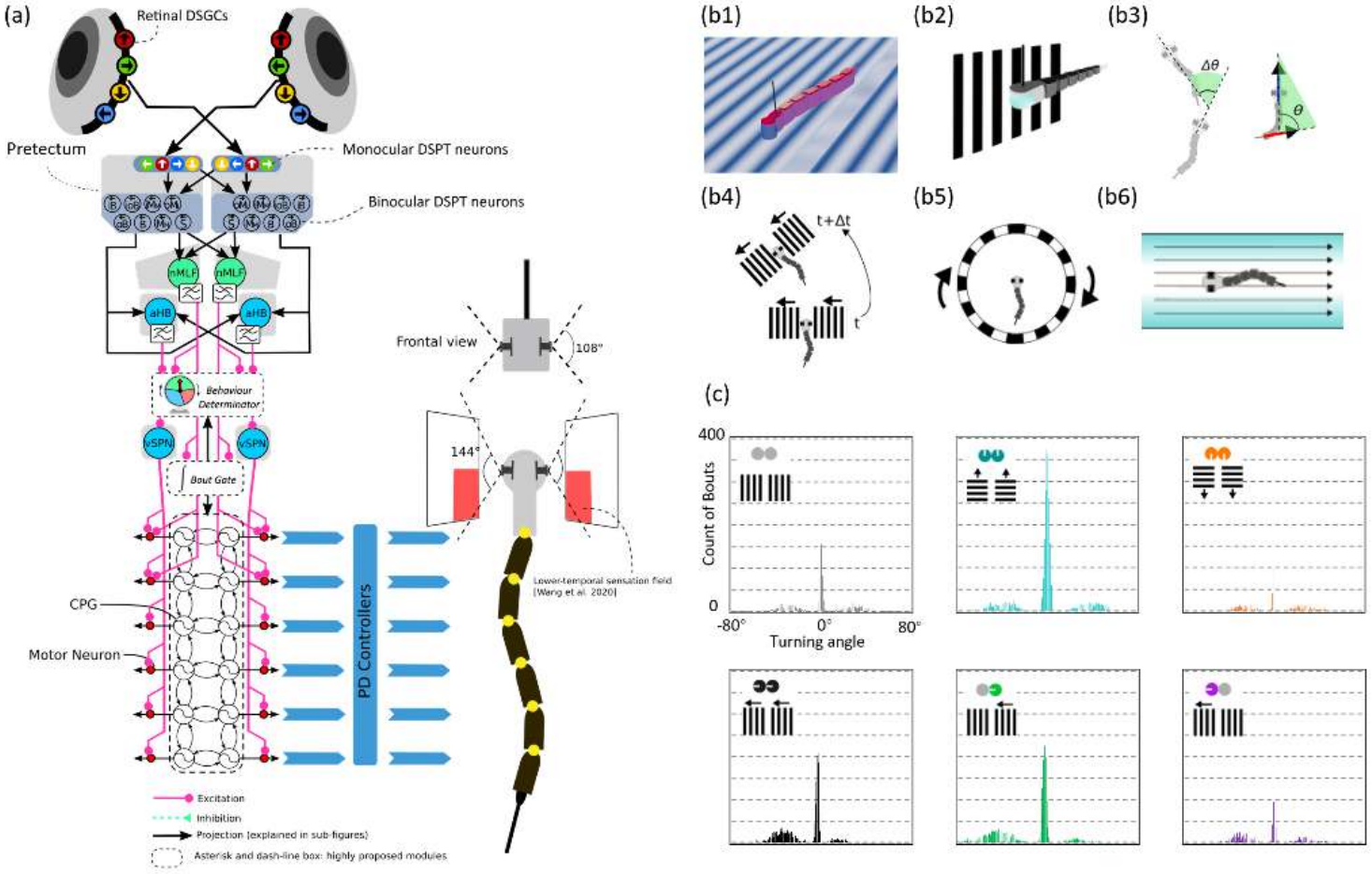

Figure 1: (a) Integrated neural network. (b1) fish body in the simulation. (b2) to (b6) Different visual stimulations that are used to verify the integrated neural mechanism. (c) Example results of free-swimming OMR in response to different visual stimulation (turning angle vs. count of swimming bouts in 20000-secs trials.).

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DEEP LEARNING OF BRAIN SPACETIME TO PREDICT OUTCOME OF VISION RESTORATION THERAPY USING NON-INVASIVE BRAIN STIMULATION

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Background: Vision loss following optic nerve damage does not only impair visual field function in patients, but it also alter Brain Spacetime. As we showed repeatedly, non-invasive transcranial alternating current stimulation (tACS) can enhance residual visual capacity of the brain, however treatment efficacy varies considerably between subjects and the treatment outcomes remains unpredictable. **Methods**: We applied deep neural networks including FFNN, CNN, bidirectional LSTM and Transformer to the task of tACS induced visual recovery outcome. For 20 patients with optic nerve damage, EEG data and visual field information were collected from each subject while performing a visual discrimination task before receiving ACS treatment. EEG data were then processed and decomposed into different frequency bands to build functional brain network per trial in each frequency band on the msec. scale (Brain Spacetime). Node centralities were extracted and then fed into deep neural networks. After receiving tACS treatment, the visual field was evaluated again for each subject to calculate recovery of vision. **Results**: The bidirectional LSTM and FFNN model exhibited similar power of prediction compared to CNN and Transformer. Even so, the bidirectional LSTM model reflected higher accuracy in almost every frequency band compared to FFNN. In contrast, FFNN model had a higher accuracy only in the beta band. The CNN and Transformer were unable to reliably predict outcome. **Conclusions**: AI-based deep neural networks may provide useful information of future therapy outcome to patients before tACS treatment starts which may be of clinical benefit.

Pubmed:
34891420: Xu J, Wu Z, Nurnberger A, Sabel BA
Interhemispheric Cortical Network Connectivity Reorganization Predicts Vision Impairment in Stroke.
Stroke is one of the main causes of disability in human beings, and when the occipital lobe is affected, this leads to partial vision loss (homonymous hemianopia). To understand brain mechanisms of vision loss and recovery, graph theory-based brain functional connectivity network (FCN) analysis was recently introduced. However, few brain network studies exist that have studied if the strength of the damaged FCN can predict the extent of functional impairment. We now characterized the brain FCN using deep neural network analysis to describe multiscale brain networks and explore their corresponding physiological patterns. In a group of 24 patients and 24 controls, Bi-directional long short-term memory (Bi-LSTM) was evaluated to reveal the cortical network pattern learning efficiency compared with other traditional algorithms. Bi-LSTM achieved the best balanced-overall accuracy of 73% with sensitivity of 70% and specificity and 75% in the low alpha band. This demonstrates that bi-directional learning can capture the brain network feature representation of both hemispheres. It shows that brain damage leads to reorganized FCN patterns with a greater number of functional connections of intermediate density in the high alpha band. Future studies should explore how this understanding of brain FCN can be used for clinical diagnostics and rehabilitation.
Annu Int Conf IEEE Eng Med Biol Soc, 2021; 2021
34777199: Xu J, Wu Z, Nürnberger A, Sabel BA
Reorganization of Brain Functional Connectivity Network and Vision Restoration Following Combined tACS-tDCS Treatment After Occipital Stroke.
Non-invasive brain stimulation (NIBS) is already known to improve visual field functions in patients with optic nerve damage and partially restores the organization of brain functional connectivity networks (FCNs). However, because little is known if NIBS is effective also following brain damage, we now studied the correlation between visual field recovery and FCN reorganization in patients with stroke of the central visual pathway. In a controlled, exploratory trial, 24 patients with hemianopia were randomly assigned to one of three brain stimulation groups: transcranial direct current stimulation (tDCS)/transcranial alternating current stimulation (tACS) (ACDC); sham tDCS/tACS (AC); sham tDCS/sham tACS (Sham),

which were compared to age-matched controls (= 24). Resting-state electroencephalogram (EEG) was collected at baseline, after 10 days stimulation and at 2 months follow-up. EEG recordings were analyzed for FCN measures using graph theory parameters, and FCN small worldness of the network and long pairwise coherence parameter alterations were then correlated with visual field performance. ACDC enhanced alpha-band FCN strength in the superior occipital lobe of the lesioned hemisphere at follow-up. A negative correlation (= -0.80) was found between the intact visual field size and characteristic path length (CPL) after ACDC with a trend of decreased alpha-band centrality of the intact middle occipital cortex. ACDC also significantly decreased delta band coherence between the lesion and the intact occipital lobe, and coherence was enhanced between occipital and temporal lobe of the intact hemisphere in the low beta band. Responders showed significantly higher strength in the low alpha band at follow-up in the intact lingual and calcarine cortex and in the superior occipital region of the lesioned hemisphere. While ACDC decreases delta band coherence between intact and damaged occipital brain areas indicating inhibition of low-frequency neural oscillations, ACDC increases FCN connectivity between the occipital and temporal lobe in the intact hemisphere. When taken together with the lower global clustering coefficient in responders, these findings suggest that FCN reorganization (here induced by NIBS) is adaptive in stroke. It leads to greater efficiency of neural processing, where the FCN requires fewer connections for visual processing.
Front Neurol, 2021; 12

34504129: Wu Z, Sabel BA

Spacetime in the brain: rapid brain network reorganization in visual processing and recovery.

Functional connectivity networks (FCN) are the physiological basis of brain synchronization to integrating neural activity. They are not rigid but can reorganize under pathological conditions or during mental or behavioral states. However, because mental acts can be very fast, like the blink of an eye, we now used the visual system as a model to explore rapid FCN reorganization and its functional impact in normal, abnormal and post treatment vision. EEG-recordings were time-locked to visual stimulus presentation; graph analysis of neurophysiological oscillations were used to characterize millisecond FCN dynamics in healthy subjects and in patients with optic nerve damage before and after neuromodulation with alternating currents stimulation and were correlated with visual performance. We showed that rapid and transient FCN synchronization patterns in humans can evolve and dissolve in millisecond speed during visual processing. This rapid FCN reorganization is functionally relevant because disruption and recovery after treatment in optic nerve patients correlated with impaired and recovered visual performance, respectively. Because FCN hub and node interactions can evolve and dissolve in millisecond speed to manage spatial and temporal neural synchronization during visual processing and recovery, we propose "Brain Spacetime" as a fundamental principle of the human mind not only in visual cognition but also in vision restoration.
Sci Rep, 2021; 11

25226999: Cao R, Wu Z, Li H, Xiang J, Chen J

Disturbed connectivity of EEG functional networks in alcoholism: a graph-theoretic analysis.

Generally, an alcoholic's brain shows explicit damage. However, in cognitive tasks, the correlation between the topological structural changes of the brain networks and the brain damage is still unclear. Scalp electrodes and synchronization likelihood (SL) were applied to the constructions of the EGG functional networks of 28 alcoholics and 28 healthy volunteers. The graph-theoretic analysis showed that in cognitive tasks, compared with the healthy control group, the brain networks of alcoholics had smaller clustering coefficients in β1 bands, shorter characteristic path lengths, increased global efficiency, but similar small-world properties. The abnormal topological structure of the alcoholics may be related to the local-function brain damage and the compensation mechanism adopted to complete tasks. This conclusion provides a new perspective for alcohol-related brain damage.
Biomed Mater Eng, 2014; 24

27383393: Wang B, Zhao J, Wu Z, Shang W, Xiang J, Cao R, Li H, Chen J, Zhang H, Yan T

Eccentricity Effects on the Efficiency of Attentional Networks: Evidence From a Modified Attention Network Test.

The effects of eccentricity on the attentional modulation of visual discrimination have been widely studied; however, the substrate of this complex phenomenon is poorly understood. Here, we provided a measure of the effects of eccentricity on three attentional networks: alerting, orienting, and executive attention. Participants (N = 63) were tested with a modified attention network test that included an additional eccentricity variation; this test allowed us to investigate the efficiency of the attentional networks at near and far eccentricities. Compared with targets at the near eccentricity, targets at the far eccentricity generally elicited significantly longer reaction times. We also found the far eccentricity was associated with smaller orienting effect scores and larger executive control scores than the near eccentricity. Interestingly, at the near eccentricity, executive control scores were larger when the spatial information was neutral (no cue, center cue, and double cue), but at the far eccentricity, the scores were larger when the spatial information was valid (spatial cue). We propose that the allocation of attentional resources differed among these cue conditions and influenced the interference caused by conflicting information.
Perception, 2016; 45


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ALPHA ACTIVITY CHANGE DURING IMPLICIT VISUAL STATISTICAL LEARNING

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Introduction Statistical learning (SL) is a cognitive function during which environmental regularities are learned. This phenomenon helps the sensory system to make predictions, thus facilitating stimulus processing. SL has been demonstrated and studied across multiple modalities, but little is known about its neural background. In this study we examined the EEG correlates of implicit, visual SL. Materials and methods Sixty-four channel EEG was recorded on 22 healthy, right-handed volunteers with correct or corrected-to-normal vision (female: 13, mean age: 25.1y). Each participant was presented with a stream of visual stimuli, where eight images formed associative pairs and the remaining eight images were control stimuli. To keep the learning implicit while also maintaining their attention, participants were asked to perform a parallel detection task. Results Based on the literature, we compared the power of low-frequency activity across conditions. Using cluster-based statistics, a significant difference was found in the time-frequency window of 0-400 ms and 8-12 Hz. This difference was predominantly present in the frontal channels and it was mainly caused by a power reduction related to predictable stimuli. Discussion Our results are in line with previous studies, claiming that low-frequency activity is modulated during SL paradigms. We found that the predictability arising from paired stimuli was traceable by decreased alpha-band activity. The early presence of this power change implies dissimilarity during the visual processing of predictable and unpredictable stimuli.

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BOARD NUMBER: S04-578

SONOGENETIC THERAPY FOR VISUAL RESTORATION

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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In recent work, we showed that sonogenetic therapy possesses high spatiotemporal resolution when stimulating the visual cortex (VC) with ultrasounds following the expression of the MscL mechanosensitive ionic channel in cortical neurons. Here we investigated if sonogenetic activation of the VC can generate visual perception using an associative learning test in mice. **Methods:** MscL was expressed by AAV injection in the mouse VC. Mice under water restriction and head-fixed were first taught to associate water delivery with visual light stimulation (LS). Water was delivered 500ms after the LS. Ultrasounds stimulations (US) were then applied using an ultrasound transducer on a cranial window above the VC. **Results:** Injected and non-injected mice increased their licking behavior between the LS and the water delivery from 23% the first day up to 76% the fourth day. Then, LS was replaced by US. The injected mice (n=9) efficacy of US induced response (66%) was close to their LS induced response (65%). Non-injected mice (n=7) efficacy of US induced response (34%) was far from their LS induced (89%). Latency of the first lick was 285.3 ± 12.4 ms (n=15) following LS whereas it decreased to 193.2 ± 12.8 ms (n=9) for US in injected animals. This shorter latency was consistent with direct cortical stimulation. **Conclusion:** We demonstrated that injected mice perceived US in the visual cortex as LS. These results are encouraging for the application of sonogenetics as a novel strategy to restore vision at a cortical level in blind patients with optic nerve atrophy.

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BOARD NUMBER: S04-579

A TWO-WAY LUMINANCE GAIN CONTROL IN THE FLY BRAIN ENSURES LUMINANCE INVARIANCE IN DYNAMIC VISION

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Aim*:* For visual perception to be unaffected by viewing conditions, animals must adapt their sensitivity to changing visual statistics, such as mean illumination. Consistently, photoreceptors in many species control their luminance gain and encode relative changes in luminance, termed contrast. However, this early gain control is insufficient for luminance-invariant contrast estimation in dynamic conditions. Sudden transitions to dim or bright environment would lead to an erroneous, reduced or enhanced perception of contrasts, thus imposing a two-way challenge on contrast estimation. Yet, visual behaviors are luminance invariant, and we aim to understand how the brains achieve such invariance. **Methods:** We combine fly behavior, *in vivo* two-photon calcium imaging and computational modelling approaches. **Results:** In fruit flies, a distinct visual pathway preserves luminance information past photoreceptors and enhances contrast estimation in sudden dim light. Here, we show that the pathway implements a generalized gain correction to tackle the two-way contrast coding deficits. When blocking the output of the luminance-sensitive interneurons, the flies underestimate large contrasts in sudden dim light and overestimate smaller contrasts in sudden bright light. Furthermore, the gain correction improves visibility of very dim stimuli at all contrasts. We formulated an algorithmic model that captures the data with high accuracy and explains how the gain correction is implemented in these widely differing scenarios. **Conclusion:** Our work demonstrates how post-receptor gain correction is key to perceptually relevant vision. Since visual systems of all behaving animals face similar challenges, the corrective gain control might be a universal strategy of visual systems.

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BOARD NUMBER: S04-580

NON-LINEAR CHROMATIC PROCESSING IN THE DROSOPHILA OPTIC LOBE

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Color is an important visual percept that guides various animal behaviors, such as mating and foraging. In order to compute color, the brain compares the relative activation of different wavelength-specific photoreceptors through color opponent signals. This phenomenon has been observed across the animal kingdom, sometimes as early as in photoreceptor terminals themselves. However, how opponent responses are transformed into higher-order chromatic representations such as hue or saturation remains poorly understood. We use the well-defined brain of the fruit fly Drosophila melanogaster to understand how the brain transforms photoreceptor signals into color percepts. The fruit fly retina contains four types of cone-like photoreceptors, which we and others have previously shown to inhibit each other at the axonal level, giving rise to linear color opponent signals. It is unclear how these signals are further transformed in the fly brain. Postsynaptically, color information is relayed to a set of trans-medullary neurons implicated in color-dependent behaviors. However, the responses of these neurons to chromatic visual stimuli has not been reported. We devised a visual stimulus that allows us to map the responses of these neurons across color space and intensities. We find that chromatic information is non-linearly processed by different trans-medullary neural populations to extract specific features of the chromatic space of the animal. We formalize these transformations using simple mathematical models and, we use connectomic data to show how these nonlinearities arise in a biologically constrained model. Here, we define the neural circuit mechanisms that allow for the computation of higher-order chromatic representations.

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BOARD NUMBER: S04-581

VENTRAL STREAM VISUAL AREAS SUPPORT PERCEPTUAL DISCRIMINABILITY OF TEXTURE IMAGES IN THE MOUSE

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Textures are useful visual stimuli for studying the neural underpinnings of object recognition. Textures are sufficiently complex to largely match the statistical properties of natural images, and, unlike natural images, they can be precisely parameterized and synthetized. Here, we studied the cortical processing of textures in mice, asking first whether mice could perceptually discriminate visual textures, and then whether the underlying neural architectures shared similarities with those found in primates. We found that mice could distinguish between types of textures, and between textures and spectrally matched stimuli (scrambles) lacking higher-order statistical features characteristic of textures. Mesoscale calcium imaging revealed that the primary visual cortex (V1) and the secondary ventral visual area (LM), but not other higher visual areas, were differentially activated by textures, relative to scrambles, with a stronger texture selectivity in LM than in V1. Similarly, 2-photon imaging recordings, examined with a regressive model, showed that cell responses in LM were better predicted by the higher-order statistics of textures than in V1. Furthermore, at the population level, textures clustered in activity subspaces that were more separable in LM than in V1, with the distance between clusters significantly correlated with the discrimination performance of the mice. In summary, our results demonstrate texture vision in the mouse with a neural substrate indicating preserved hierarchical coding principles across mammalian species for the processing of naturalistic stimuli.

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BOARD NUMBER: S04-582

REPRESENTATION OF PREY-RELATED VARIABLES IN MOUSE V1 DURING PREY CAPTURE BEHAVIOR

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Neurons in the mouse primary visual cortex (V1) are traditionally described using classical response properties, such as direction/orientation selectivity. These properties are measured by showing moving gratings to a head-fixed animal while recording the ensuing neural responses. This approach is in stark contrast to what mice see and do in nature, where they experience much more complex visual scenes while engaging in behaviors such as foraging and hunting. It is unclear how V1 cells respond during such behaviors. Here, using hunting as a naturalistic behavior, we show that V1 neurons encode a number of hunting-related variables, such as distance and angle to prey. To acquire such data, we combined video tracking of mice hunting live crickets in an open arena and calcium imaging using miniature microscopes. This allowed us to characterize the tuning of hundreds of V1 cells to several behaviorally-relevant variables. We observed that many neurons showed selective tuning to at least one of such variables. At the population level, however, decoding analyses showed that only a subset of these variables can be decoded. These included previously reported quantities, like mouse speed, but also hunting-relevant variables, such as prey distance. Our results therefore show that, despite the “early” positioning of V1 in the visual pathway, its cells can show complex response patterns to scenes that differ greatly from moving gratings. This highlights the importance of recording neuronal responses in freely-moving animals performing ethologically relevant behaviors, as these responses are a step towards understanding how the brain controls complex behaviors.

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BOARD NUMBER: S04-583

SELECTIVE BIDIRECTIONAL MODULATION OF RECIPROCATING NEURONS DURING HIERARCHICAL INTERACTIONS IN THE MOUSE VISUAL CORTEX

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Visual perception arises from a set of hierarchically organised cortical areas, interconnected by ascending and descending projections. While the integration of feedforward inputs is thought to give rise to the increasingly complex visual representations of higher-order neurons, the function of descending inputs is less understood. Recent ex-vivo measurements showed that cortico-cortical projections selectively connect with reciprocating neurons in their target areas, suggesting that they mediate recurrent inter-area computations such as learning and predictive coding. However, whether hierarchical interactions selectively engage with reciprocating neurons in vivo remains unknown. Here we use in vivo 2-photon imaging, optogenetic stimulation and viral retrograde tracers to measure how interactions between the primary visual cortex (V1) and the posteromedial higher visual area (PM) depend on the projection type of the target neurons. Photostimulation of PM-to-V1 feedback axons resulted in a larger reduction of the responses of layer 2/3 neurons to gratings in cells projecting back to PM than in neighbouring ones projecting elsewhere. Similarly, a larger proportion of the PM neurons projecting back to V1 were modulated by V1-to-PM feedforward afferents photostimulation than non-V1 projecting neurons, by showing either an enhancement or a reduction of their visual responses. These results show that both ascending and descending projections in visual cortex selectively modulate reciprocating neurons during visual processing. The bidirectional selective modulation of reciprocating neurons by cortico-cortical projections challenges the classical view of feedforward cortical computation while supporting models of hierarchical computation involving selective recurrent interactions across visual processing stages.

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BOARD NUMBER: S04-584

A BIOPHYSICAL ACCOUNT OF MULTIPLICATION BY A SINGLE NEURON

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Nonlinear, multiplication-like operations carried out by individual nerve cells greatly enhance the computational power of a neural system, but our understanding of their biophysical implementation is scant. Here we pursue this problem in the *Drosophila melanogaster* ON motion vision circuit, in which we record the membrane potentials of direction-selective T4 neurons and of their columnar input elements in response to visual and pharmacological stimuli *in vivo*. Our electrophysiological measurements and conductance-based simulations provide evidence for a passive supralinear interaction between two distinct types of synapse on T4 dendrites. We show that this multiplication-like nonlinearity arises from the coincidence of cholinergic excitation and release from glutamatergic inhibition. The latter depends on the expression of the glutamate-gated chloride channel GluClα in T4 neurons, which sharpens the directional tuning of the cells and shapes the optomotor behaviour of the animals. Interacting pairs of shunting inhibitory and excitatory synapses have long been postulated as an analogue approximation of a multiplication.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S04-585

WHITE MATTER DYNAMICS DEPENDS ON RECOVERY FROM RETINAL LESIONS AND VISUAL STIMULATION IN CATS.

POSTER SESSION 04 - SECTION: VISUAL SYSTEM, FROM RETINA TO CORTEX

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Aims: Temporal assessment of the recovery from binocular central retinal lesions (RL) using simultaneous behavioral and MRI white matter morphology analysis.
Methods: 3 groups of cats participated: *control trained (CT, n=5); retinal lesioned naive (RLN, n=8); retinal lesioned and trained (RLT, n=4).* Behavioral training started 2 weeks after lesion in RLTrained cats, in CT after first scan, and lasted for 3 months. Visual stimulation was based on accelerated discrimination training from a previous study (Burnat et al, 2017). Longitudinal diffusion MRI protocol was applied and 5 scans till 3 months post lesion survival time were acquired (7T Bruker MRI BioSpec). Whole brain and regional analysis was performed (dLGN, Hippocampus, Areas 17/18, 20ab, PMLS, A1, A2, AEV).
Results: RL induced fractional anisotropy (FA) growth up to 5 weeks post-lesion followed by the continuous FA drop of up to 13 weeks post-lesion. In the RLT cats the pattern was similar, showing an increase of FA from 9 weeks post-lesion. CT cats showed continuous growth of FA from 5 weeks post-lesion. Individual FA levels correlated with behavioral performance and or difficulty of the task. Individual Fixel Based Analysis of diffusion data revealed differences in fiber cross section (FC), fiber density (FD) and fiber density and cross-section (FCD), between RLN and CT animals, and in FD and FC between RLN and RLT cats.
Conclusions: Visual stimulation applied shortly after retinal lesions has a longitudinal effect on white matter structure.

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BOARD NUMBER: S04-586

EFFECT OF WHITE MATTER HYPERINTENSITIES OF VASCULAR ORIGIN ON BRAIN POWER SPECTRA PROFILES: A MEG STUDY

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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White matter hyperintensities (WMH) of presumed vascular origin is a common accompaniment of ageing, not only in dementia patients, but also in healthy population. The presence and progression of WMH in the brain have been widely associated to general cognitive impairment and all types of dementia, especially vascular cognitive impairment (VCI). Understand the effect of cerebrovascular damages in brain functioning could help in early diagnosis of different dementia subtypes. The aim of this investigation was to focus the research on the identification of specific electrophysiological signatures related to WMH total volume in the brain. Our data base consists in 350 cognitively healthy participants. All of them underwent a neuropsychological evaluation, MRI assessment (T1 and Flair), and MEG recordings (ten minutes of eyes closed resting state). WMH volume quantification was performed with an automatic detection toolbox, LST (SPM12). We used a completely data-driven approach to evaluate the relationship between power spectrum and the WMH total volume. Our result shows a general slowness pattern related with the severity of the white matter damage, as have been previously reported in the literature. Nevertheless, different patterns were found depending on the volume and etiology of the WMH: age-related or vascular disease. The high incidence and contribution to cognitive impairment of WMH, added to their treatable conditions and the lack of standardized results in the filed provides special importance to the study of robust biomarkers related with cerebrovascular pathologies in old population.

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BOARD NUMBER: S04-587

SPECTRAL PHASOR ANALYSIS FOR QUANTITATION OF AGE- AND DISEASE-RELATED PROTEIN MISFOLDING USING THE AMYLOID DYES BSB AND MCAAD-3

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Aims: This study examines the differences in protein aggregation related to ageing and Alzheimer's disease (AD). The aim of the project is to detect distinct types of amyloid assemblies (particularly ones that are not readily visualized using conventional techniques) and associate the specific types of protein deposits in the human hippocampus with signs of cognitive decline. Methods: Formalin-fixed, paraffin-embedded sections of human hippocampi were stained with conformationally sensitive amyloid dyes BSB and MCAAD-3 and imaged using spectral confocal microscopy. The same sections were then stained with anti-amyloid beta and anti-tau antibodies. Both the greater tissue parenchyma and the obvious protein aggregates were analyzed and quantitated across cases using the spectral phasor approach. Results: Using the varying emission spectra of the two probes, we were able to demonstrate different emission signatures between morphologically similar amyloid plaques across different regions and layers of the hippocampus. Moreover, we found a unique spectral signature of the greater tissue parenchyma across all cases in the AD cohort, where no plaques or tangles were present. Conclusions: Our novel method of staining, imaging and analysis suggested remarkable conformational heterogeneity of both age- and disease-related protein deposits in the hippocampus. The sensitivity of our method outperformed conventional immunohistochemistry with the detected spectral differences between the greater parenchyma of cognitively normal and AD cases indicating a subtle yet widespread proteopathy associated with disease. This subtle spectral change in AD was not present in cognitively unaffected cases exhibiting benign age-related amyloid deposition.

Pubmed:
33705095: Stepanchuk AA, Heyne B, Stys PK
Complex Photophysical Properties of K114 Make for a Versatile Fluorescent Probe for Amyloid Detection.
Protein aggregation is a hallmark of Alzheimer's disease (AD) and many other neurodegenerative disorders. Small organic fluorophores such as Congo Red preferentially bind to cross-β-sheet-rich deposits and have been used to label amyloid plaques and tau tangles in histological samples. However, distinguishing between different conformations of protein aggregates is not trivial. Using silkworm and spider silks (prototypical amyloids) and transgenic AD mouse (5XFAD) and human AD brain samples, we report how spectral confocal microscopy allowed for improved detection and differentiation of protein aggregates based on the unexpected photophysical behavior of the amyloid-specific dye K114. The pH and excitation power had pronounced effects on the emission spectrum and intensity of amyloid-bound K114 fluorescence. When bound to β-sheet-rich assemblies, the emission spectrum of K114 was governed by the local pH of the binding pockets much more than by the pH of the mounting medium, likely due to ionization of titratable phenols. Unexpectedly, exposure to high excitation power caused a permanent increase in fluorescence intensity and a spectral blue-shift. These light-induced fluorescence changes were dependent in a complex manner on laser power, exposure time, pH, and amyloid type examined. The above-mentioned phenomena were observed in silk fibers and Alzheimer brain sections from mouse and human, indicating that this may be a general characteristic of K114 when bound to tightly aggregated macromolecules. Potential mechanisms are discussed, likely involving photoinduced electron transfer. Our findings illustrate how the complex photophysical behavior of amyloid-bound K114 can be exploited for improved detection and differentiation of protein aggregates.
ACS Chem Neurosci, 2021; 12
34958041: Black SAG, Stepanchuk AA, Templeton GW, Hernandez Y, Ota T, Roychoudhury S, Smith EE, Barber PA, Ismail Z, Fischer K, Zwiers A, Poulin MJ, Blennow K, Zetterberg H, Stys PK, Tsutsui S

Diagnosing Alzheimer's Disease from Circulating Blood Leukocytes Using a Fluorescent Amyloid Probe.
Toxic amyloid-β (Aβ) peptides aggregate into higher molecular weight assemblies and accumulate not only in the extracellular space, but also in the walls of blood vessels in the brain, increasing their permeability, and promoting immune cell migration and activation. Given the prominent role of the immune system, phagocytic blood cells may contact pathological brain materials.
J Alzheimers Dis, 2022; 85
34751140: Stepanchuk AA, Barber PA, Lashley T, Joseph JT, Stys PK
Quantitative detection of grey and white matter amyloid pathology using a combination of K114 and CRANAD-3 fluorescence.
Alzheimer's disease (AD) is a neurodegenerative disease that exacts a huge toll on the patient, the healthcare system and society in general. Abundance and morphology of protein aggregates such as amyloid β plaques and tau tangles, along with cortical atrophy and gliosis are used as measures to assess the changes in the brain induced by the disease. Not all of these parameters have a direct correlation with cognitive decline. Studies have shown that only particular protein conformers can be the main drivers of disease progression, and conventional approaches are unable to distinguish different conformations of disease-relevant proteins.
Neurobiol Dis, 2021; 161
34499422: Stepanchuk AA, Joseph JT, Stys PK
Spectral photokinetic conversion of the fluorescent probes BSB and K114 for improved detection of amyloid assemblies.
Cross-β-sheet-rich protein fibrils are infamous for their accumulation in the brains of patients diagnosed with a number of neurodegenerative diseases, including Alzheimer's disease (AD). Disease-relevant fibrils are a result of deviation of the proteins from their native structure to a misfolded state resulting in aggregation and formation of fibrils. In this study, we explored the phenomenon of light-induced fluorescence enhancement of amyloid assemblies stained with two amyloid probes (BSB and K114) using Bombyx mori silk and human AD brain sections. The photoconversion effect, accompanied by an increase in fluorescence intensity and spectral blue-shift, was highly dependent on the chemical structures of the dyes, pH, presence of glycerol and the type of amyloid. The degree of intensity and spectral change over time in response to high laser exposure were quantified and analyzed using custom-written analysis tools. Our findings provide further insight into possible mechanisms of amyloid-mediated photoconversion kinetics of K114 and BSB, and may provide more insight into the molecular nature of various amyloid assemblies.
J Biophotonics, 2021; 14
32799317: Stepanchuk A, Tahir W, Nilsson KPR, Schatzl HM, Stys PK
Early detection of prion protein aggregation with a fluorescent pentameric oligothiophene probe using spectral confocal microscopy.
Misfolding of the prion protein (PrP) and templating of its pathological conformation onto cognate proteins causes a number of lethal disorders of central nervous system in humans and animals, such as Creutzfeldt-Jacob disease, chronic wasting disease and bovine spongiform encephalopathy. Structural rearrangement of PrP into PrP promotes aggregation of misfolded proteins into β-sheet-rich fibrils, which can be visualized by conformationally sensitive fluorescent probes. Early detection of prion misfolding and deposition might provide useful insights into its pathophysiology. Pentameric formyl thiophene acetic acid (pFTAA) is a novel amyloid probe that was shown to sensitively detect various misfolded proteins, including PrP. Here, we compared sensitivity of pFTAA staining and spectral microscopy with conventional methods of prion detection in mouse brains infected with mouse-adapted 22L prions. pFTAA bound to prion deposits in mouse brain sections exhibited a red-shifted fluorescence emission spectrum, which quantitatively increased with disease progression. Small prion deposits were detected as early as 50 days post-inoculation, well before appearance of clinical signs. Moreover, we detected significant spectral shifts in the greater brain parenchyma as early as 25 days post-inoculation, rivaling the most sensitive conventional method (real-time quaking-induced conversion). These results showcase the potential of pFTAA staining combined with spectral imaging for screening of prion-infected tissue. Not only does this method have comparable sensitivity to established techniques, it is faster and technically simpler. Finally, this readout provides valuable information about the spatial distribution of prion aggregates across tissue in the earliest stages of infection, potentially providing valuable pathophysiological insight into prion transmission.
J Neurochem, 2021; 156

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BOARD NUMBER: S04-588

IMAGING THE BRAIN ACROSS THE LIFESPAN: A RAT MODEL OF HEALTHY AGEING

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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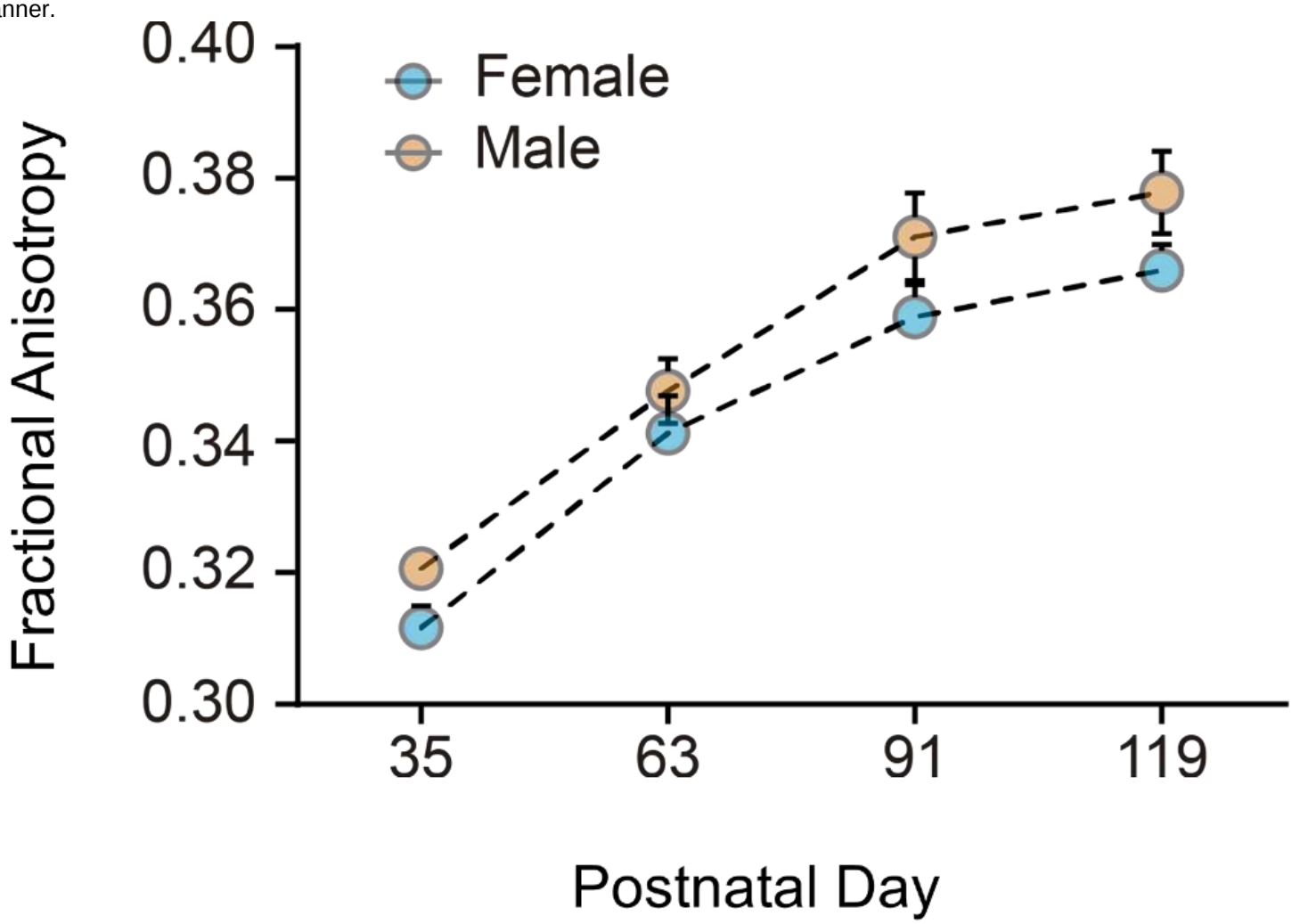
Across the lifespan, the brain is subject to dynamic structural changes which have a deep impact on its healthy and pathological evolution [1]. Recently, magnetic resonance imaging (MRI) in humans uncovered region- and sex-specific trajectories of microstructural integrity decline, suggesting prolonged neoteny in the female brain [2]. Animal models can dissect the neurobiological substrates driving maturation and senescence of the brain and inform on the mechanism underlying sex dimorphism in ageing. **Aims:** 1) To validate a model of healthy ageing in rats, replicating region- and sex-specific trajectories found in humans. 2) To dissect the neurobiological underpinning of the observed trajectories by using advanced MRI biomarkers and immunohistochemistry. **Methods:** We applied a diffusion MRI protocol to a cohort of 28 Wistar rats (14 females) longitudinally from 1 month to 4 months to extract indices of microstructural integrity. 12 rats (6 females) were sacrificed at different time points and processed for immunohistochemistry (GFAP, Iba-1 and myelin basic protein). **Results:** The analysis of MRI biomarkers highlighted region-specific patterns of maturation ($p<0.05$; Time*Region) which follow a quadratic curve for increasing age, with a trend toward difference in the maturation peak in males versus females ($p<0.07$; see figure). Myelin staining intensity varies quadratically with age ($p<0.01$) in a region-specific way. **Conclusions:** Our rat model faithfully reproduces brain-ageing patterns seen in humans, and points to myelin as an important driver of region-specific trajectories of evolution across the lifespan. MRI can provide biomarkers of structural brain development and deterioration in a longitudinal and non-invasive

manner.



Pubmed:
32569785: Eed A, Cerdán Cerdá A, Lerma J, De Santis S
Diffusion-weighted MRI in neurodegenerative and psychiatric animal models: Experimental strategies and main outcomes. Preclinical MRI approaches constitute a key tool to study a wide variety of neurological and psychiatric illnesses, allowing a more direct investigation of the disorder substrate and, at the same time, the possibility of back-translating such findings to human subjects. However, the lack of consensus on the optimal experimental scheme used to acquire the data has led to relatively high heterogeneity in the choice of protocols, which can potentially impact the comparison between results obtained by different groups, even using the same animal model. This is especially true for diffusion-weighted MRI data, where certain experimental choices can impact not only on the accuracy and precision of the extracted biomarkers, but also on their biological meaning. With this in mind, we extensively examined preclinical imaging studies that used diffusion-weighted MRI to investigate neurodegenerative, neurodevelopmental and psychiatric disorders in rodent models. In this review, we discuss the main findings for each preclinical model, with a special focus on the analysis and comparison of the different acquisition strategies used across studies and their impact on the heterogeneity of the findings.
J Neurosci Methods, 2020; 343

FENS Forum 2022
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BOARD NUMBER: S04-589

DECIPHERING MOLECULAR TARGETS OF COMPLETE DIESEL EMISSIONS IN HUMAN OLFACTORY MUCOSA ORIGINATING FROM COGNITIVELY HEALTHY INDIVIDUALS AND PATIENTS WITH ALZHEIMER'S DISEASE.

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Olfaction is governed by olfactory mucosal cells (OM) located in the upper nasal cavity. Impaired olfaction is a common, early sign of neurodegenerative diseases, including Alzheimer's disease (AD). The OM cells are at the forefront of exposure to inhaled air and are thus exposed to a myriad of particles present in ambient air. Recent studies correlate living in highly polluted cities with cognitive impairment and neurodegenerative disease risk, including AD. In this study, we investigated how exposure to air pollution particles affects the OM cell transcriptome. The specific aims were to: 1) Find key molecular mechanisms affected by air pollutant exposure in OM cells; 2) Evaluate differential responses in patient-derived OM cells of cognitively healthy individuals and AD patients. We used a unique, recently developed air-liquid-interface exposure system that mimics real-world traffic-derived air pollutant exposure. Primary OM cells from AD patients and age-matched healthy controls were subjected to exposure to either clean air or to diluted emissions from a heavy-duty diesel engine without aftertreatment for 3 days. After treatment, miRNA and mRNA expression analyses were performed. We found approximately 500 differentially expressed mRNA following exposure, some of which were differentially expressed between healthy and AD cells. Pathway analyses revealed that these genes are involved in the oxidative stress response. This study provides new information on the adverse effects of air pollutant exposure in OM cells and identifies possible biomarkers. It also provides molecular insight into the link between diesel exhaust exposure and AD.

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BOARD NUMBER: S04-590

A MULTI-SITE MAGNETOENCEPHALOGRAPHY (MEG) RESTING-STATE DATASET TO STUDY DEMENTIA: THE BIOFIND DATASET

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Early detection of Alzheimer's Disease (AD) is vital for developing effective treatments. Neuroimaging can detect early brain changes, such as hippocampal atrophy in Mild Cognitive Impairment (MCI), a prodromal state of AD. Machine learning can utilise the many features from high-dimensional neuroimaging data, but many cases are required. While large, public datasets of MCI/AD exist for Magnetic Resonance Imaging (MRI), eg "ADNI", comparable datasets are lacking for Magnetoencephalography (MEG). MEG offers advantages in its millisecond resolution, potentially revealing physiological changes in brain oscillations or connectivity before structural changes are evident with MRI (and unconfounded by vascular changes in functional MRI). Here we describe the "BioFIND" dataset of 324 individuals, approximately half MCI and half controls, who have at least 2 mins of resting-state MEG, plus a T1 structural MRI, from one of two sites (Cambridge and Madrid). To our knowledge, this is the largest publically available MEG dataset for dementia research, available in BIDS format on DPUK platform: https://portal.dementiasplatform.uk/Apply. Initial analyses using Multi-kernel Learning (MKL) of Support Vector Machines (SVM) show that MEG sensor covariance adds complimentary information for MCI classification beyond grey-matter volume from structural MRI. Future possible analyses include source space, measures of functional connectivity (e.g, amplitude or phase), dynamic as well as static connectivity, more advanced classifiers (e.g, deep learning); future plans include adding new participants from ongoing projects, and follow-up diagnoses and other biomarkers where available.

FENS Forum 2022
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BOARD NUMBER: S04-591

ALTERED INFORMATION FLOW FROM FOREBRAIN TO CORTEX IN A RAT MODEL OF EARLY ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Alzheimer's disease (AD), marked by extracellular amyloid-beta (Aβ) plaque accumulation leads to progressive memory and cognitive function loss. Resting-state (RS) functional connectivity (FC), an emerging biomarker of AD, measures only correlative inter-regional relationships. Here, we investigate changes in directed/causal information flow inferred from RS-fMRI measurements in a transgenic rat model (TgF344-AD) of AD at the pre-plaque stage. RS-fMRI data (9.4T Bruker Biospec, 1000 volumes, TR=0.6s) were acquired in anesthetized 4(N=15)- and 6(N=13)-month old TgF344-AD (TG) rats and wild type (WT) littermates (N=11). Preprocessed voxel-level data were parcellated into 126 grey-matter parcels. Normalized directed transfer entropy (NDTE), an information-theoretic approximation of Granger causality, was used to infer directed information flows between region-pairs. Predictive cross-validated accuracy of NDTE and FC to classify genotypes at each age was compared. Salient connections in NDTE's predictive accuracy were identified and their median NDTE was compared between genotypes using Wilcoxon ranksum test. NDTE and FC distinguished WT and TG rats better than chance at 4-months; only NDTE did it at 6-months with 80% accuracy. NDTE accurately classified the two ages only in the TG group implying changes with age predominantly occur in the TG. The most salient connections in NDTE's genotypes classification at both ages were from the forebrain to the cortex. Interestingly, median NDTE for these connections was higher in the TG at 4-months but lower at 6-months. Early impact of AD on basal forebrain, an important modulator of RS networks, could result in altered information flow from it as this study demonstrates.

Pubmed:
34729479: Adhikari MH, Griffis J, Siegel JS, Thiebaut de Schotten M, Deco G, Instabato A, Gilson M, Corbetta M
Effective connectivity extracts clinically relevant prognostic information from resting state activity in stroke.
Recent resting-state functional MRI studies in stroke patients have identified two robust biomarkers of acute brain dysfunction: a reduction of inter-hemispheric functional connectivity between homotopic regions of the same network, and an abnormal increase of ipsi-lesional functional connectivity between task-negative and task-positive resting-state networks. Whole-brain computational modelling studies, at the individual subject level, using undirected effective connectivity derived from empirically measured functional connectivity, have shown a reduction of measures of integration and segregation in stroke as compared to healthy brains. Here we employ a novel method, first, to infer whole-brain directional effective connectivity from zero-lagged and lagged covariance matrices, then, to compare it to empirically measured functional connectivity for predicting stroke versus healthy status, and patient performance (zero, one, multiple deficits) across neuropsychological tests. We also investigated the accuracy of functional connectivity versus model effective connectivity in predicting the long-term outcome from acute measures. Both functional and effective connectivity predicted healthy from stroke individuals significantly better than the chance-level; however, accuracy for the effective connectivity was significantly higher than for functional connectivity at 1- to 2-week, 3-month and 1-year post-stroke. Predictive functional connections mainly included those reported in previous studies (within-network inter-hemispheric and between task-positive and -negative networks intra-hemispherically). Predictive effective connections included additional between-network links. Effective connectivity was a better predictor than functional connectivity of the number of behavioural domains in which patients suffered deficits, both at 2-week and 1-year post-onset of stroke. Interestingly, patient deficits at 1-year time-point were better predicted by effective connectivity values at 2 weeks rather than at 1-year time-point. Our results thus demonstrate that the second-order statistics of functional MRI resting-state activity at an early stage of stroke, derived from a whole-brain effective connectivity, estimated in a model fitted to reproduce the propagation of neuronal activity, has pertinent information for clinical prognosis.
Brain Commun, 2021; 3
33551755: Adhikari MH, Belloy ME, Van der Linden A, Keliris GA, Verhoye M

Resting-State Co-activation Patterns as Promising Candidates for Prediction of Alzheimer's Disease in Aged Mice.
Alzheimer's disease (AD), a neurodegenerative disorder marked by accumulation of extracellular amyloid-β (Aβ) plaques leads to progressive loss of memory and cognitive function. Resting-state fMRI (RS-fMRI) studies have provided links between these two observations in terms of disruption of default mode and task-positive resting-state networks (RSNs). Important insights underlying these disruptions were recently obtained by investigating dynamic fluctuations in RS-fMRI signals in old TG2576 mice (a mouse model of amyloidosis) using a set of quasi-periodic patterns (QPP). QPPs represent repeating spatiotemporal patterns of neural activity of predefined temporal length. In this article, we used an alternative methodology of co-activation patterns (CAPs) that represent instantaneous and transient brain configurations that are likely contributors to the emergence of commonly observed RSNs and QPPs. We followed a recently published approach for obtaining CAPs that divided all time frames, instead of those corresponding to supra-threshold activations of a seed region as done traditionally, to extract CAPs from RS-fMRI recordings in 10 TG2576 female mice and eight wild type littermates at 18 months of age. Subsequently, we matched the CAPs from the two groups using the Hungarian method and compared the temporal (duration, occurrence rate) and the spatial (lateralization of significantly co-activated and co-deactivated voxels) properties of matched CAPs. We found robust differences in the spatial components of matched CAPs. Finally, we used supervised learning to train a classifier using either the temporal or the spatial component of CAPs to distinguish the transgenic mice from the WT. We found that while duration and occurrence rates of all CAPs performed the classification with significantly higher accuracy than the chance-level, blood oxygen level-dependent (BOLD) signals of significantly activated voxels from individual CAPs turned out to be a significantly better predictive feature demonstrating a near-perfect classification accuracy. Our results demonstrate resting-state co-activation patterns are a promising candidate in the development of a diagnostic, and potentially, prognostic RS-fMRI biomarker of AD.
Front Neural Circuits, 2020; 14
29474907: Silva CG, Peyre E, Adhikari MH, Tielens S, Tanco S, Van Damme P, Magno L, Krusy N, Agirman G, Magiera MM, Kessaris N, Malgrange B, Andrieux A, Janke C, Nguyen L
Cell-Intrinsic Control of Interneuron Migration Drives Cortical Morphogenesis.
Interneurons navigate along multiple tangential paths to settle into appropriate cortical layers. They undergo a saltatory migration paced by intermittent nuclear jumps whose regulation relies on interplay between extracellular cues and genetic-encoded information. It remains unclear how cycles of pause and movement are coordinated at the molecular level. Post-translational modification of proteins contributes to cell migration regulation. The present study uncovers that carboxypeptidase 1, which promotes post-translational protein deglutamylation, controls the pausing of migrating cortical interneurons. Moreover, we demonstrate that pausing during migration attenuates movement simultaneity at the population level, thereby controlling the flow of interneurons invading the cortex. Interfering with the regulation of pausing not only affects the size of the cortical interneuron cohort but also impairs the generation of age-matched projection neurons of the upper layers.
Cell, 2018; 172
29112703: Adhikari MH, Deco G, Corbetta M
Reply: Defining a functional network homeostasis after stroke: EEG-based approach is complementary to functional MRI.
Brain, 2017; 140
28334882: Adhikari MH, Hacker CD, Siegel JS, Griffa A, Hagmann P, Deco G, Corbetta M
Decreased integration and information capacity in stroke measured by whole brain models of resting state activity.
While several studies have shown that focal lesions affect the communication between structurally normal regions of the brain, and that these changes may correlate with behavioural deficits, their impact on brain's information processing capacity is currently unknown. Here we test the hypothesis that focal lesions decrease the brain's information processing capacity, of which changes in functional connectivity may be a measurable correlate. To measure processing capacity, we turned to whole brain computational modelling to estimate the integration and segregation of information in brain networks. First, we measured functional connectivity between different brain areas with resting state functional magnetic resonance imaging in healthy subjects (n = 26), and subjects who had suffered a cortical stroke (n = 36). We then used a whole-brain network model that coupled average excitatory activities of local regions via anatomical connectivity. Model parameters were optimized in each healthy or stroke participant to maximize correlation between model and empirical functional connectivity, so that the model's effective connectivity was a veridical representation of healthy or lesioned brain networks. Subsequently, we calculated two model-based measures: 'integration', a graph theoretical measure obtained from functional connectivity, which measures the connectedness of brain networks, and 'information capacity', an information theoretical measure that cannot be obtained empirically, representative of the segregative ability of brain networks to encode distinct stimuli. We found that both measures were decreased in stroke patients, as compared to healthy controls, particularly at the level of resting-state networks. Furthermore, we found that these measures, especially information capacity, correlate with measures of behavioural impairment and the segregation of resting-state networks empirically measured. This study shows that focal

lesions affect the brain's ability to represent stimuli and task states, and that information capacity measured through whole brain models is a theory-driven measure of processing capacity that could be used as a biomarker of injury for outcome prediction or target for rehabilitation intervention.
Brain, 2017; 140
26898464: Kaplan R, Adhikari MH, Hindriks R, Mantini D, Murayama Y, Logothetis NK, Deco G
Hippocampal Sharp-Wave Ripples Influence Selective Activation of the Default Mode Network.
The default mode network (DMN) is a commonly observed resting-state network (RSN) that includes medial temporal, parietal, and prefrontal regions involved in episodic memory [1-3]. The behavioral relevance of endogenous DMN activity remains elusive, despite an emerging literature correlating resting fMRI fluctuations with memory performance [4, 5]-particularly in DMN regions [6-8]. Mechanistic support for the DMN's role in memory consolidation might come from investigation of large deflections (sharp-waves) in the hippocampal local field potential that co-occur with high-frequency (>80 Hz) oscillations called ripples-both during sleep [9, 10] and awake deliberative periods [11-13]. Ripples are ideally suited for memory consolidation [14, 15], since the reactivation of hippocampal place cell ensembles occurs during ripples [16-19]. Moreover, the number of ripples after learning predicts subsequent memory performance in rodents [20-22] and humans [23], whereas electrical stimulation of the hippocampus after learning interferes with memory consolidation [24-26]. A recent study in macaques showed diffuse fMRI neocortical activation and subcortical deactivation specifically after ripples [27]. Yet it is unclear whether ripples and other hippocampal neural events influence endogenous fluctuations in specific RSNs-like the DMN-unitarily. Here, we examine fMRI datasets from anesthetized monkeys with simultaneous hippocampal electrophysiology recordings, where we observe a dramatic increase in the DMN fMRI signal following ripples, but not following other hippocampal electrophysiological events. Crucially, we find increases in ongoing DMN activity after ripples, but not in other RSNs. Our results relate endogenous DMN fluctuations to hippocampal ripples, thereby linking network-level resting fMRI fluctuations with behaviorally relevant circuit-level neural dynamics.
Curr Biol, 2016; 26
26063923: Adhikari MH, Raja Beharelle A, Griffa A, Hagmann P, Solodkin A, McIntosh AR, Small SL, Deco G
Computational modeling of resting-state activity demonstrates markers of normalcy in children with prenatal or perinatal stroke.
Children who sustain a prenatal or perinatal brain injury in the form of a stroke develop remarkably normal cognitive functions in certain areas, with a particular strength in language skills. A dominant explanation for this is that brain regions from the contralesional hemisphere "take over" their functions, whereas the damaged areas and other ipsilesional regions play much less of a role. However, it is difficult to tease apart whether changes in neural activity after early brain injury are due to damage caused by the lesion or by processes related to postinjury reorganization. We sought to differentiate between these two causes by investigating the functional connectivity (FC) of brain areas during the resting state in human children with early brain injury using a computational model. We simulated a large-scale network consisting of realistic models of local brain areas coupled through anatomical connectivity information of healthy and injured participants. We then compared the resulting simulated FC values of healthy and injured participants with the empirical ones. We found that the empirical connectivity values, especially of the damaged areas, correlated better with simulated values of a healthy brain than those of an injured brain. This result indicates that the structural damage caused by an early brain injury is unlikely to have an adverse and sustained impact on the functional connections, albeit during the resting state, of damaged areas. Therefore, these areas could continue to play a role in the development of near-normal function in certain domains such as language in these children.
J Neurosci, 2015; 35
22573672: Adhikari MH, Quilichini PP, Roy D, Jirsa V, Bernard C
Brain state dependent postinhibitory rebound in entorhinal cortex interneurons.
Postinhibitory rebound (PIR) is believed to play an important role in the genesis and maintenance of biological rhythms. While it has been demonstrated during several in vitro studies, in vivo evidence for PIR remains scarce. Here, we report that PIR can be observed in the dorsomedial entorhinal cortex of anesthetized rats, mostly between putatively connected GABAergic interneurons, and that it is more prevalent during the theta (4-6 Hz) oscillation state than the slow (0.5-2 Hz) oscillation state. Functional inhibition was also found to be brain state and postsynaptic cell type dependent but that alone could not explain this brain state dependence of PIR. A theoretical analysis, using two Fitzhugh-Nagumo neurons coupled to an external periodic drive, predicted that the modulation of a faster spiking rate by the slower periodic drive could account for the brain state dependence of PIR. Model predictions were verified experimentally. We conclude that PIR is cell type and brain state dependent and propose that this could impact network synchrony and rhythmogenesis.
J Neurosci, 2012; 32
19616579: Adhikari MH, Heeroma JH, di Bernardo M, Krauskopf B, Richardson MP, Walker MC, Terry JR
Characterisation of cortical activity in response to deep brain stimulation of ventral-lateral nucleus: modelling and experiment.

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Motivated by its success as a therapeutic treatment in other neurological disorders, most notably Parkinson's disease, Deep Brain Stimulation (DBS) is currently being trialled in a number of patients with drug unresponsive epilepsies. However, the mechanisms by which DBS interferes with neuronal activity linked to the disorder are not well understood. Furthermore, there is a need to identify optimized values of parameters (for example in amplitude/frequency space) of the stimulation protocol with which one aims to achieve the desired outcome. In this paper we characterise the system response to stimulation, to gain an understanding of the role different brain regions play in generating the output observed in EEG. We perform a number of experiments in healthy rats, where the ventral-lateral thalamic nucleus is stimulated using a train of square-waves with different frequency and amplitudes. The response to stimulation in the motor cortex is recorded and the drive-response relationship over frequency/amplitude space is considered. Subsequently, we compare the experimental data with simulations of a mean-field model, finding good agreement between the output of the model and the experimental data--both in the time and frequency domains--when considering a transition to oscillatory activity in the cortex as the frequency of stimulation is increased. Overall, our study suggests that mean-field models can appropriately characterise the stimulus-response relationship of DBS in healthy animals. In this way, it constitutes a first step towards the goal of developing a closed-loop feedback control protocol for suppressing epileptic activity, by adaptively adjusting the stimulation protocol in response to EEG activity.
J Neurosci Methods, 2009; 183

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BOARD NUMBER: S04-592

THE APOLIPOPROTEIN E GENOTYPE INFLUENCE ON GLOBAL AMYLOID BETA ACCUMULATION IN NON-DEMENTED ELDERLY

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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The Apolipoprotein E (ApoE) gene is the most important genetic risk factor for Alzheimer's disease (AD) and may act as disease progression modifier. Research has demonstrated the association between the ApoE e4 allele and elevated amyloid beta (Aβ) burden when measured cross-sectionally. However, whether the e4 allele contributes to earlier Aβ accumulation onset or persistent higher accumulation over time is still unclear. Additionally, a genotype effect might be dependent on the accumulation phase. The current project, therefore, investigated ApoE genotype's effect on Aβ accumulation as measured by amyloid positron emission tomography (PET) imaging. Data of 540 non-demented elderly that received PET scans at three or more timepoints were obtained from ADNI and AIBL cohorts. Amyloid values were extracted via centiloid method and analyzed using linear mixed regression considering Aβ baseline status, ApoE genotype, age, gender, and diagnosis. Overall, the sample showed an increase in Aβ over time. ApoE genotype had a direct effect on Aβ load and displayed an indirect relationship with Aβ accumulation via interactions with Aβ baseline status and age. To assess differences in amyloid load trajectories the sample was divided into amyloid baseline positive and negative participants. Within each subgroup, cluster analysis then identified Aβ decrease, stabilization, and increase clusters. Further investigations within these trajectory clusters suggest that the ApoE genotype effect might be group dependent. The three-way interaction of ApoE, amyloid baseline status, and accumulation might explain previous contradictory literature. The trajectories of decreaser, stable, and increaser groups give insights into the complexity of AD's progression.

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BOARD NUMBER: S04-593

ANALYSIS OF SOLUBLE TREM2 LEVELS IN CSF AND PLASMA OF MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE SUBJECTS

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Recent genetic and neuropathological studies have implicated microglia cells to have a causal role in the pathogenesis of Alzheimer's disease (AD). Triggering receptor expressed on myeloid cells 2 (TREM2) is one of the most studied microglial receptors in the context of AD because carriers of its rare variant have an increased risk of disease development. TREM2 is involved in the regulation of myeloid cell number, amyloid β (Aβ) binding, phagocytosis, and inflammatory response. Its ectodomain is processed by a disintegrin and metalloprotease enzymes ADAM10 and ADAM17, and the fragment released in CSF and blood is referred to as soluble TREM2 (sTREM2). We aimed to compare CSF and plasma concentrations of sTREM2 in AD, mild cognitive impairment (MCI), and samples of cognitively normal controls, and to correlate them with the core CSF biomarkers of AD (levels of $A\beta_{1-42}$, total tau [t-tau], and tau phosphorylated at epitope 181 [p-tau181]). Concentrations of sTREM2 were measured in 127 AD, 79 MCI, and 36 control CSF samples and 99 AD, 37 MCI, and 10 control plasma samples using ELISA. The sTREM2 levels in CSF were significantly different among the groups, whereas differences in plasma levels did not reach significance. These results are congruent with previous reports showing elevated sTREM2 levels in CSF of AD subjects. We concluded that the CSF sTREM2 level, but not plasma sTREM2, is a promising biomarker of microglial activation in AD. Funded by the CSF IP-2019-04-3584 and Scientific center of excellence CORE-NEURO GA KK01.1.1.01.0007 funded by the EU Regional Development Fund.

Pubmed:
31636558: Španić E, Langer Horvat L, Hof PR, Šimić G
Role of Microglial Cells in Alzheimer's Disease Tau Propagation.
Uncontrolled immune response in the brain contributes to the progression of all neurodegenerative disease, including Alzheimer's disease (AD). Recent investigations have documented the prion-like features of tau protein and the involvement of microglial changes with tau pathology. While it is still unclear what sequence of events is causal, it is likely that tau seeding potential and microglial contribution to tau propagation act together, and are essential for the development and progression of degenerative changes. Based on available evidence, targeting tau seeds and controlling some signaling pathways in a complex inflammation process could represent a possible new therapeutic approach for treating neurodegenerative diseases. Recent findings propose novel diagnostic assays and markers that may be used together with standard methods to complete and improve the diagnosis and classification of these diseases. In conclusion, a novel perspective on microglia-tau relations reveals new issues to investigate and imposes different approaches for developing therapeutic strategies for AD.
Front Aging Neurosci, 2019; 11
34057084: Babić Leko M, Jurasović J, Nikolac Perković M, Španić E, Sekovanić A, Orct T, Lukinović Škudar V, Bačić Baronica K, Kiđemet-Piskač S, Vogrinc Ž, Pivac N, Borovečki F, Hof PR, Šimić G
The Association of Essential Metals with APOE Genotype in Alzheimer's Disease.
The major confirmed genetic risk factor for late-onset, sporadic Alzheimer's disease (AD) is variant ε4 of apolipoprotein E gene (APOE). It is proposed that ApoE, a protein involved in transport of cholesterol to neurons can cause neurodegeneration in AD through interaction with metals. Previous studies mostly associated copper, iron, zinc, and calcium with ApoE4-mediated toxicity.
J Alzheimers Dis, 2021; 82
31699331: Šimić G, Španić E, Langer Horvat L, Hof PR

Blood-brain barrier and innate immunity in the pathogenesis of Alzheimer's disease.
The pathogenesis of Alzheimer's disease (AD) is only partly understood. This is the probable reason why significant efforts to treat or prevent AD have been unsuccessful. In fact, as of April 2019, there have been 2094 studies registered for AD on the clinicaltrials.gov U.S. National Library of Science web page, of which only a few are still ongoing. In AD, abnormal accumulation of amyloid and tau proteins in the brain are thought to begin 10-20 years before the onset of overt symptoms, suggesting that interventions designed to prevent pathological amyloid and tau accumulation may be more effective than attempting to reverse a pathology once it is established. However, to be successful, such early interventions need to be selectively administered to individuals who will likely develop the disease long before the symptoms occur. Therefore, it is critical to identify early biomarkers that are strongly predictive of AD. Currently, patients are diagnosed on the basis of a variety of clinical scales, neuropsychological tests, imaging and laboratory modalities, but definitive diagnosis can be made only by postmortem assessment of underlying neuropathology. People suffering from AD thus may be misdiagnosed clinically with other primary causes of dementia, and vice versa, thereby also reducing the power of clinical trials. The amyloid cascade hypothesis fits well for the familial cases of AD with known mutations, but is not sufficient to explain sporadic, late-onset AD (LOAD) that accounts for over 95% of all cases. Since the earliest descriptions of AD there have been neuropathological features described other than amyloid plaques (AP) and neurofibrillary tangles (NFT), most notably gliosis and neuroinflammation. However, it is only recently that genetic and experimental studies have implicated microglial dysfunction as a causal factor for AD, as opposed to a merely biological response of its accumulation around AP. Additionally, many studies have suggested the importance of changes in blood-brain barrier (BBB) permeability in the pathogenesis of AD. Here we suggest how these less investigated aspects of the disease that have gained increased attention in recent years may contribute mechanistically to the development of lesions and symptoms of AD.
Prog Mol Biol Transl Sci, 2019; 168
34072960: Šimić G, Tkalčić M, Vukić V, Mulc D, Španić E, Šagud M, Olucha-Bordonau FE, Vukšić M, R Hof P
Understanding Emotions: Origins and Roles of the Amygdala.
Emotions arise from activations of specialized neuronal populations in several parts of the cerebral cortex, notably the anterior cingulate, insula, ventromedial prefrontal, and subcortical structures, such as the amygdala, ventral striatum, putamen, caudate nucleus, and ventral tegmental area. Feelings are conscious, emotional experiences of these activations that contribute to neuronal networks mediating thoughts, language, and behavior, thus enhancing the ability to predict, learn, and reappraise stimuli and situations in the environment based on previous experiences. Contemporary theories of emotion converge around the key role of the amygdala as the central subcortical emotional brain structure that constantly evaluates and integrates a variety of sensory information from the surroundings and assigns them appropriate values of emotional dimensions, such as valence, intensity, and approachability. The amygdala participates in the regulation of autonomic and endocrine functions, decision-making and adaptations of instinctive and motivational behaviors to changes in the environment through implicit associative learning, changes in short- and long-term synaptic plasticity, and activation of the fight-or-flight response via efferent projections from its central nucleus to cortical and subcortical structures.
Biomolecules, 2021; 11

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BOARD NUMBER: S04-594

SEXUALLY DIMORPHIC NEURODEGENERATION AND NEUROINFLAMMATION IN THE HUMAN OLFACTORY BULB IN ALZHEIMER'S DISEASE.

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and mainly diagnosed in females, calling attention to understand sexual dimorphisms. Neurofibrillary tangles of tau and amyloid-β plaques have been described early and preferentially in the olfactory bulb and resonance analysis have reported a reduction in this area. However, whether this diminution is due to loss of neurons or glial cells is unknown. Microglial and astroglial density has been also described in AD, but not in the olfactory bulb. Therefore, the present report aims at describing neural and glial changes in the olfactory bulb using unbiased-stereological methods with specific neural (Neu-N), microglial (Iba-1) and astroglial (GFAP) markers in AD and non-diseased cases (NAD). 58 clinically diagnosed individuals were used (AD= 34 and NAD= 24). These samples from donors were provided by the IDIBAPS, BIOBANC-MUR, BTCIEN and Biobanco Vasco, integrated in the Spanish National Biobanks. Stereological quantification was performed using Stereo Investigator and ImageJ softwares and proteins were quantified by Western-blot (WB). No differences were observed in volume and GFAP. Neurodegeneration was higher in AD males' cases based on stereology, whereas in females by Western-blot. Microgliosis was increased in AD and focused on females. These data constitute the first demonstration of sex differences in neurodegeneration using specific neural markers and confirm the microgliosis in the human olfactory bulb in AD. Sponsored by the UCLM/ERDF (2021-GRIN-31233), Spanish Ministries of Economy and Competitiveness/ERDF (SAF2016-75768-R) and Science and Innovation (PID2019-108659RB-I00) to AMM and Autonomous Government of Castilla- La Mancha/ERDF (SBPLY/17/180501/000430) to AMM and DSS.

Pubmed:
33479244: Flores-Cuadrado A, Saiz-Sanchez D, Mohedano-Moriano A, Lamas-Cenjor E, Leon-Olmo V, Martinez-Marcos A, Ubeda-Bañon I
Astrogliosis and sexually dimorphic neurodegeneration and microgliosis in the olfactory bulb in Parkinson's disease.
Hyposmia is prodromal, and male sex is a risk marker for an enhanced likelihood ratio of Parkinson's disease. The literature regarding olfactory bulb volume reduction is controversial, although the olfactory bulb has been largely reported as an early and preferential site for α-synucleinopathy. These pathological deposits have been correlated with neural loss in Nissl-stained material. However, microgliosis has rarely been studied, and astrogliosis has been virtually neglected. In the present report, α-synucleinopathy (α-synuclein), neurodegeneration (Neu-N), astrogliosis (GFAP), and microgliosis (Iba-1) were quantified, using specific markers and stereological methods. Disease, sex, age, disease duration, and post-mortem interval were considered variables for statistical analysis. No volumetric changes have been identified regarding disease or sex. α-Synucleinopathy was present throughout the OB, mainly concentrated on anterior olfactory nucleus. Neurodegeneration (reduction in Neu-N-positive cells) was statistically significant in the diseased group. Astrogliosis (increased GFAP labeling) and microgliosis (increased Iba-1 labeling) were significantly enhanced in the Parkinson's disease group. When analyzed per sex, neurodegeneration and microgliosis differences are only present in men. These data constitute the demonstration of sex differences in neurodegeneration using specific neural markers, enhanced astrogliosis and increased microgliosis, also linked to male sex, in the human olfactory bulb in Parkinson's disease.
NPJ Parkinsons Dis, 2021; 7

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BOARD NUMBER: S04-595

HIGH-RESOLUTION HISTOLOGICAL MAPPING OF THE HUMAN BRAIN AS A TOOL FOR TRANSLATIONAL PSYCHIATRIC NEUROSCIENCE

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Neuropsychiatric disorders are prevalent diseases whose causes and mechanisms involve cellular, genetic and synaptic alterations that remain elusive. In this context, translating experimental findings to the clinic represents a major challenge. We propose to use high-throughput neurohistological methods to overcome this limitation. We developed a next- generation neurohistopathology pipeline to perform a systematic multiscale analysis of brain pathology in postmortem human brains and identify the cellular, molecular and circuit underpinnings of neurodegenerative and psychiatric disorders. We adapted tissue clearing and labeling techniques, combined with light-sheet microscopy and advanced image-processing tools, to map cell types, gene expression, synaptic connectivity and neuronal circuits in large samples of diseased and non-diseased archival human brains. We combined this approach with whole brain histological mapping of cellular and molecular markers from tissue sections and high-resolution imaging of subcellular structures in pathological hot spots. We demonstrated the validity of this approach in Alzheimer's disease and frontotemporal dementia. Overall, the multimodal mapping of the human brain offers promising avenues for investigating the mechanisms of brain disorders, identifying new biomarkers and devising novel preventive and therapeutic interventions.

Pubmed:
35076178: Jordà-Siquier T, Petrel M, Kouskoff V, Smailovic U, Cordelières F, Frykman S, Müller U, Mulle C, Barthet G
APP accumulates with presynaptic proteins around amyloid plaques: A role for presynaptic mechanisms in Alzheimer's disease?
In Alzheimer's disease (AD), the distribution of the amyloid precursor protein (APP) and its fragments other than amyloid beta, has not been fully characterized. Here, we investigate the distribution of APP and its fragments in human AD brain samples and in mouse models of AD in reference to its proteases, synaptic proteins, and histopathological features characteristic of the AD brain, by combining an extensive set of histological and analytical tools. We report that the prominent somatic distribution of APP observed in control patients remarkably vanishes in human AD patients to the benefit of dense accumulations of extra-somatic APP, which surround dense-core amyloid plaques enriched in APP-Nter. These features are accentuated in patients with familial forms of the disease. Importantly, APP accumulations are enriched in phosphorylated tau and presynaptic proteins whereas they are depleted of post-synaptic proteins suggesting that the extra-somatic accumulations of APP are of presynaptic origin. Ultrastructural analyses unveil that APP concentrates in autophagosomes and in multivesicular bodies together with presynaptic vesicle proteins. Altogether, alteration of APP distribution and its accumulation together with presynaptic proteins around dense-core amyloid plaques is a key histopathological feature in AD, lending support to the notion that presynaptic failure is a strong physiopathological component of AD.
Alzheimers Dement, 2022;
34013204: Haytural H, Jordà-Siquier T, Winblad B, Mulle C, Tjernberg LO, Granholm AC, Frykman S, Barthet G
Distinctive alteration of presynaptic proteins in the outer molecular layer of the dentate gyrus in Alzheimer's disease.
Synaptic degeneration has been reported as one of the best pathological correlates of cognitive deficits in Alzheimer's disease. However, the location of these synaptic alterations within hippocampal sub-regions, the vulnerability of the presynaptic versus postsynaptic compartments, and the biological mechanisms for these impairments remain unknown. Here, we performed immunofluorescence labelling of different synaptic proteins in fixed and paraffin-embedded human hippocampal sections and report reduced levels of several presynaptic proteins of the neurotransmitter release machinery (complexin-1, syntaxin-1A, synaptotagmin-1 and synaptogyrin-1) in Alzheimer's disease cases. The deficit was restricted to the outer molecular layer of the dentate gyrus, whereas other hippocampal sub-fields were preserved. Interestingly, standard markers of postsynaptic densities (SH3 and multiple ankyrin repeat domains protein 2) and dendrites (microtubule-associated

protein 2) were unaltered, as well as the relative number of granule cells in the dentate gyrus, indicating that the deficit is preferentially presynaptic. Notably, staining for the axonal components, myelin basic protein, SMI-312 and Tau, was unaffected, suggesting that the local presynaptic impairment does not result from axonal loss or alterations of structural proteins of axons. There was no correlation between the reduction in presynaptic proteins in the outer molecular layer and the extent of the amyloid load or of the dystrophic neurites expressing phosphorylated forms of Tau. Altogether, this study highlights the distinctive vulnerability of the outer molecular layer of the dentate gyrus and supports the notion of presynaptic failure in Alzheimer's disease.
Brain Commun, 2021; 3
30429473: Barthet G, Jordà-Siquier T, Rumi-Masante J, Bernadou F, Müller U, Mulle C
Presenilin-mediated cleavage of APP regulates synaptotagmin-7 and presynaptic plasticity.
Mutations of the intramembrane protease presenilin (PS) or of its main substrate, the amyloid precursor protein (APP), cause early-onset form of Alzheimer disease. PS and APP interact with proteins of the neurotransmitter release machinery without identified functional consequences. Here we report that genetic deletion of PS markedly decreases the presynaptic levels of the Ca sensor synaptotagmin-7 (Syt7) leading to impaired synaptic facilitation and replenishment of synaptic vesicles. The regulation of Syt7 expression by PS occurs post-transcriptionally and depends on γ-secretase proteolytic activity. It requires the substrate APP as revealed by the combined genetic invalidation of APP and PS1, and in particular the APP-Cterminal fragments which interact with Syt7 and accumulate in synaptic terminals under pharmacological or genetic inhibition of γ-secretase. Thus, we uncover a role of PS in presynaptic mechanisms, through APP cleavage and regulation of Syt7, that highlights aberrant synaptic vesicle processing as a possible new pathway in AD.
Nat Commun, 2018; 9
31649526: Haytural H, Lundgren JL, Köse TB, Jordà-Siquier T, Kalcheva M, Seed Ahmed M, Winblad B, Sundström E, Barthet G, Tjernberg LO, Frykman S
Non-specific Detection of a Major Western Blotting Band in Human Brain Homogenates by a Multitude of Amyloid Precursor Protein Antibodies.
The use of human post-mortem brain material is of great value when investigating which pathological mechanisms occur in human brain, and to avoid translational problems which have for example been evident when translating animal research into Alzheimer disease (AD) clinical trials. The amyloid β (Aβ)-peptide, its amyloid precursor protein (APP) and the intermediate APP-c-terminal fragments (APP-CTFs) are all important players in AD pathogenesis. In order to elucidate which APP CTF that are the most common in brain tissue of different species and developmental stages, and whether there are any differences in these fragments between AD and control brain, we investigated the occurrence of these fragments using different APP c-terminal antibodies. We noticed that whereas the conventional APP-CTFα and CTFβ fragments were most prominent in rat and mouse brain tissue, the major western blotting band detected in human, macaque and guinea pig was of approximately 20 kDa in size, possibly corresponding to the newly discovered APP-CTFη. However, this band was also intensely stained with a total protein stain, as well as by several other antibodies. The staining intensity of the 20 kDa band by the APP antibodies varied considerably between samples and correlated with the staining intensity of this band by the total protein stain. This could potentially be due to non-specific binding of the antibodies to another protein of this size. In-gel digestion and mass spectrometry confirmed that small amounts of APP were present in this band, but many other proteins were identified as well. The major hit of the mass spectrometry analysis was myelin basic protein (MBP) and a myelin removal protocol removed proportionally more of the 20 kDa APP band than the full-length APP and APP-CTFα/β bands. However, the signal could not be immunodepleted with an MBP antibody. In summary, we report on a potentially non-specific western blotting band of approximately 20 kDa and call for precaution when analyzing proteins of this size in human brain tissue.
Front Aging Neurosci, 2019; 11
29696690: Wouterlood FG, Engel A, Daal M, Houwen G, Meinderts A, Jordà Siquier T, Beliën JAM, van Dongen YC, Scheel-Krüger J, Thierry AM, Groenewegen HJ, Deniau JM
Mesencephalic dopamine neurons interfacing the shell of nucleus accumbens and the dorsolateral striatum in the rat.
Parallel corticostriatonigral circuits have been proposed that separately process motor, cognitive, and emotional-motivational information. Functional integration requires that interactions exist between neurons participating in these circuits. This makes it imperative to study the complex anatomical substrate underlying corticostriatonigral circuits. It has previously been proposed that dopaminergic neurons in the ventral mesencephalon may play a role in this circuit interaction. Therefore, we studied in rats convergence of basal ganglia circuits by depositing an anterograde neuroanatomical tracer into the ventral striatum together with a retrograde fluorescent tracer ipsilaterally in the dorsolateral striatum. In the mesencephalon, using confocal microscopy, we looked for possible appositions of anterogradely labeled fibers and retrogradely labeled neurons, "enhancing" the latter via intracellular injection of Lucifer Yellow. Tyrosine hydroxylase (TH) immunofluorescence served to identify dopaminergic neurons. In neurophysiological experiments, we combined orthodromic stimulation in the medial ventral

striatum with recording from ventral mesencephalic neurons characterized by antidromic stimulation from the dorsal striatum. We observed terminal fields of anterogradely labeled fibers that overlap populations of retrogradely labeled nigrostriatal cell bodies in the substantia nigra pars compacta and lateral ventral tegmental area (VTA), with numerous close appositions between boutons of anterogradely labeled fibers and nigrostriatal, TH-immunopositive neurons. Neurophysiological stimulation in the medial ventral striatum caused inhibition of dopaminergic nigrostriatal neurons projecting to the ventrolateral striatal territory. Responding nigrostriatal neurons were located in the medial substantia nigra and adjacent VTA. Our results strongly suggest a functional link between ventromedial, emotional-motivational striatum, and the sensorimotor dorsal striatum via dopaminergic nigrostriatal neurons.
J Neurosci Res, 2018; 96

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MITOCHONDRIAL CA2+ DYNAMICS, OLFACTION AND ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Aim: Alzheimer's disease (AD) is one of the main causes of cognitive decline and the leading cause of dementia in elderly population. AD can be divided in Sporadic (SAD) and Familial (FAD) depending on the absence or presence of autosomal dominant inheritance. Both forms present common clinical features, including neuronal loss and brain amyloid-β (Aβ) deposition. FAD is caused by autosomal mutations in Aβ precursor protein (APP), presenilin-1 (PS1) and presenilin-2 (PS2) genes and is characterized by early onset of symptoms. Based on the assumptions that: i) altered sense of smell is one of the early clinical symptoms of AD, ii) mitochondrial Ca2+ handling is altered in AD and iii) mitochondrial Ca2+ mobilization is involved in the olfactory signalling and in odor memory, we aim to investigate mitochondrial Ca2+ dynamics impairment as a potential mechanism underpinning early olfactory impairment in AD. Methodology: In an established murine model of FAD, we will firstly ascertain at which age Aβ plaque deposition occurs in the olfactory areas. To establish the capability of discriminating odours, animals will perform the habituation/dishabituation test before and after plaque deposition. Additionally, we will investigate cytosolic and mitochondrial Ca2+ dynamics along the olfactory pathway at the early stage of the olfactory defect by 2P-microscope imaging. Results and Conclusions: Preliminary data show that 6-month-old AD mice present olfactory defects respect to controls. Finding the relationship between mitochondrial Ca2+ dynamics and olfactory dysfunctions in AD, possibly identifying a cause-effect relationship, would represent a progression in the comprehension of the AD pathogenesis.

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LINKING PLASMA AMYLOID BETA AND NEUROFILAMENT LIGHT CHAIN TO INTRACORTICAL MYELIN CONTENT IN COGNITIVELY NORMAL OLDER ADULTS

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Evidence suggests that lightly myelinated cortical regions are more vulnerable to aging and Alzheimer's disease (AD). However, it is unknown whether blood AD markers, such as amyloid-b fragment 1-42 ($A\beta_{1-42}$) and neurofilament light chain (NfL), are associated with changes in intracortical myelin content in cognitively normal older adults, and whether these myelin changes may eventually moderate patterns of resting state functional connectivity (rs-FC). One hundred and thirty-three cognitively normal older adults participated in the study. Plasma $A\beta_{1-42}$ and NfL levels were assessed with ultra-sensitive single-molecule array (Simoa) assays, and the ratio T1-weigthed/T2-weighted (T1w/T2w) was employed as a proxy of intracortical myelination. Using cortical surface-based multiple linear regression analyses, we examined: i) whether plasma AD markers were associated with intracortical myelin content at different cortical depths, and ii) whether the relationship between intracortical myelin content and rs-FC was moderated by plasma $A\beta_{1-42}$ and NfL levels. Results showed that decreased plasma $A\beta_{1-42}$ and increased NfL levels were associated with reductions of myelin content in the temporo-parietal-occipital regions and insular cortex, respectively. These associations were most evident in the inner cortical layers. Plasma NfL levels, but not $A\beta_{1-42}$, moderated functional coordination between insula and medial orbitofrontal cortex, presumably due to impaired neural synchronization associated with myelin deficits in the insular cortex. Together, these findings have revealed that plasma levels of $A\beta_{1-42}$ and NfL are able to track intracortical myelin deficits in cognitively normal older adults, which may contribute to identify vulnerability to AD at the earliest stage among the aged population.

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MRI REVEALS REDUCED MICROSTRUCTURAL BRAIN INTEGRITY IN THE APP/SEN1 MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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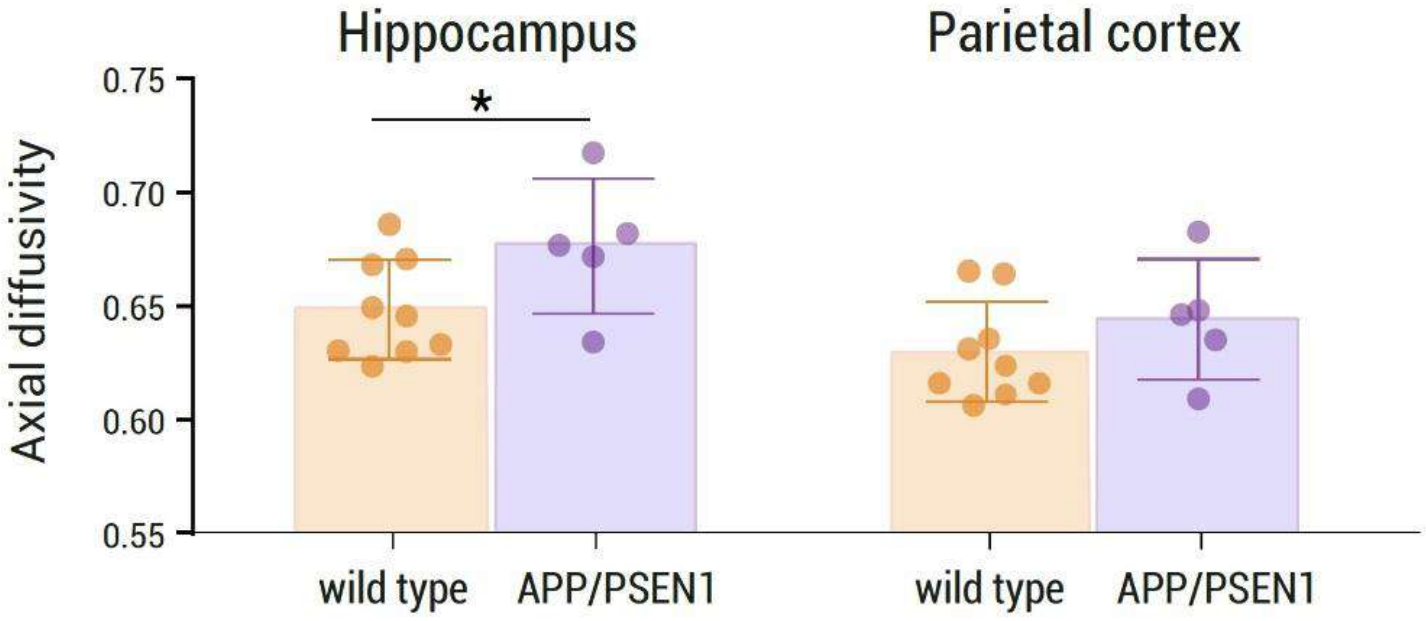
Alzheimer's disease, the most prevalent form of dementia in Europe, is clinically defined by a progressive loss of cognitive functions, but microstructural alterations are known to take place years before pathology is detectable [1]. Early detection of such alterations has therefore high priority. Diffusion-weighted magnetic resonance imaging (dw-MRI) allows characterizing microstructural integrity *in vivo*, revealing increased diffusivity in humans with Alzheimer [3]. Mice models are fundamental to investigate the pathological mechanisms and test interventions. One of the most established models is the APP/PSEN1 mice, known to develop amyloid plaques in parietal cortex and hippocampus from 6 months of age. However, its potential to show similar radiological patterns as found in human disease is unexplored. **Aims:** To use dw-MRI to measure axial diffusivity, an index of microstructural integrity, in the APP/PSEN1 mice, and compare it with controls. **Methods:** We applied a dw-MRI protocol to a cohort of 9 wild type C75BL/6 mice and 5 APP/PSEN1 mice to measure axial diffusivity in parietal cortex and hippocampus. ANOVA with post-hoc t-test was employed to test significant effects of region-of-interest and group. **Results:** ANOVA revealed significant interaction between region-of-interest and group ($F(1,2)=6.280, p=0.028$). Axial diffusivity was significantly increased ($p<0.05$,1-tail; see figure) in the hippocampus in APP/PSEN1 mice compared to control. This biomarker is sensitive to compromised microstructural integrity.
Conclusions: This preliminary study confirms the utility of the model to recapitulate salient radiological features of human Alzheimer's disease. Current work is looking at advanced dw-MRI biomarkers to detect alteration in the glia and neuron morphology.

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DEMYELINATION OF THE FORNIX COMMISSURE AS AN EARLY EVENT IN ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Demyelination is an early event in Alzheimer's disease (AD) previous to neurodegeneration. Demyelination has been identified at the hippocampal formation and the fornix. Specifically, fornix demyelination appears in pre-symptomatic carriers of familial AD mutations, and also in clinically normal older adults with elevated Aβ burden and is associated with accelerated memory decline. The fornix commissure is the connection between both hippocampi and it has been shown that is implicated in recognition memory. We aimed to study the specific demyelination of this connection. In a pilot study, we have assessed demyelination by immunohistochemistry in 10 APP/PS1 mice as a model of AD and 10 wild-type mice as controls from the same colony. We also recruited 10 AD patients, 10 mild cognitive impairment (MCI) patients, and 10 non-demented age-matched controls. We performed 3T nuclear magnetic resonance to analyze by diffusion-tensor imaging the hippocampal commissure of the recruited patients. Also, electroencephalography in the resting state with closed eyes by EEG recordings was performed in all subjects. In AD mice, we observed significant demyelination in the hippocampal commissure compared to controls. In the AD and MCI patients, our preliminary results suggest differences in fractional anisotropy and the apparent diffusion coefficient compared to controls, as well as changes in the interhemispheric phase coherence between the temporal electrodes in the 8-13 Hz frequency band.

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IMAGING COMMON PROTEIN SIGNATURES OF SINGLE CELL-PROTEOPATHY INTERACTIONS ACROSS HUMAN ALZHEIMER'S DISEASE TISSUE USING MULTIPLEXED BEAM IMAGING

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

Bryan Cannon, Kausalia Vijayragavan, Dmitry Tebaykin, Dunja Mrdjen, Jp Oliveria, Noah Greenwald, Alex Baranski, Mike Angelo, Tom Montine, Sean Bendall
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Identifying cell-based and regional phenotypes in human brain tissue is a necessary task to understand neurodegenerative pathologies in Alzheimer's disease. Using MIBI (multiplexed ion-beam imaging) we have simultaneously quantified tissue-level expression of 40 different targets encoding a deep set of neurological phenotypes with nanometer scale spatial resolution. To localize protein expression to specific cells or tissue restricted regions, we use a combination of single cell and single object segmentation methods. Applying this technique to multiple MIBI scans of archival human hippocampus cross-sections, we are able to identify the spatial distribution and expression heterogeneity of glial, neuronal, and endothelial cell types and proteopathies in both cognitively healthy and diseased human brain. Using off-the-shelf and in-house developed computational strategies we are able to isolate unique protein-disease trends in traditional hippocampal neuroanatomy, particularly identifying tau-tangle targeting by microglia in severe Alzheimer's within the CA1 region. Additional work using cross-sample clustering algorithms derived common areas of disease and healthy tissue independent of AD severity. Identification of neurons able to survive in these proteopathy-associated regions may lead to additional work testing the functional significance of the descriptive work outlined here. We believe this will be a straight forward approach to discretize and select features for multiplexed imaging technologies as applied to human brain tissue in both degenerative and non-degenerative contexts.

Pubmed:

32035179: Elliott SE, Kongpachith S, Lingampalli N, Adamska JZ, Cannon BJ, Blum LK, Bloom MS, Henkel M, McGeachy MJ, Moreland LW, Robinson WH
B cells in rheumatoid arthritis synovial tissues encode focused antibody repertoires that include antibodies that stimulate macrophage TNF-α production.
Rheumatoid arthritis (RA) is characterized by the production of anti-citrullinated protein antibodies (ACPAs). To gain insights into the relationship between ACPA-expressing B cells in peripheral blood (PB) and synovial tissue (ST), we sequenced the B cell repertoire in paired PB and ST samples from five individuals with established, ACPA+ RA. Bioinformatics analysis of paired heavy- and light-chain sequences revealed clonally-related family members shared between PB and ST. ST-derived antibody repertoires exhibited reduced diversity and increased normalized clonal family size compared to PB-derived repertoires. Functional characterization showed that seven recombinant antibodies (rAbs) expressed from subject-derived sequences from both compartments bound citrullinated antigens and immune complexes (ICs) formed using one ST-derived rAb stimulated macrophage TNF-α production. Our findings demonstrate B cell trafficking between PB and ST in subjects with RA and ST repertoires include B cells that encode ACPA capable of forming ICs that stimulate cellular responses implicated in RA pathogenesis.
Clin Immunol, 2020; 212
29927104: Elliott SE, Kongpachith S, Lingampalli N, Adamska JZ, Cannon BJ, Mao R, Blum LK, Robinson WH
Affinity Maturation Drives Epitope Spreading and Generation of Proinflammatory Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis.
Rheumatoid arthritis (RA) is characterized by the presence of anti-citrullinated protein antibodies (ACPAs); nevertheless, the origin, specificity, and functional properties of ACPAs remain poorly understood. The aim of this study was to characterize the evolution of ACPAs by sequencing the plasmablast antibody repertoire at serial time points in patients with established RA.
Arthritis Rheumatol, 2018; 70

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ALZHEIMER'S DISEASE GENETIC RISK FACTOR APOE4 IS ASSOCIATED WITH ATTENUATED AUDITORY RESPONSES

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Recent studies have suggested that prodromal stages of Alzheimer's Disease (AD) are accompanied with central auditory system dysfunction, which may be used as early indicators of disease onset and progression. In AD patients of 60 years old and more, atypical patterns of oscillatory entrainment to repetitive sound transients have been reported and suggested as potential neuromarkers of AD. Whether such alterations of auditory functions relate to genetic risk factor of AD (APOE4) at an early age (<30) is unknown. We used EEG recordings to measure auditory responses to repetitive sounds (1 second click trains presented at various frequencies (10-250Hz) in 32 young neurotypical participants). To test whether auditory responsivity is affected by AD risk factor, we compared auditory brain responses from seventeen APOE3 (age mean=21.6, sd=1.8) and fifteen APOE4 carriers (age mean=23.6, sd=4.9). Comparing the magnitude of auditory event related potentials (ERPs) we observed that APOE4 carriers exhibit slightly attenuated P2 and P3 ERP responses as compared to APOE3 carriers. Focusing on Auditory Steady State Response (ASSR) power across frequencies (10-90Hz), we observed that APOE3 carriers exhibit reliably larger neural entrainment than APOE4 carriers (Cohens' d = 0.8, 'large' effect size). This difference was sustained across the peristimulus time course and did vary across stimulus frequencies. Overall, these results suggest that central auditory differences can be detected very early in at-risk populations. Studying these signals could help identify early AD pathology and provide an entry point for therapeutic interventions against neurodegeneration.

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SILENT EPILEPTIC ACTIVITIES: THE MISSING LINK BETWEEN ALZHEIMER'S DISEASE, DISRUPTED SLEEP AND DYSFUNCTIONAL MEMORY CONSOLIDATION?

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Alzheimer's disease (AD) and epileptic activities (EA) were linked through the very first descriptions of AD a century ago and the interest toward this comorbidity exponentially increased in the past decades. These investigations revealed that EA accelerate AD progression, however, given that they are mostly subclinical and occur predominantly during sleep, they may remain undetected and could potentially disrupt sleep-related memory consolidation – although this assumption has not been tested in AD. Moreover, previous clinical studies on this subject focused on non-REM sleep, but preclinical research hints that EA in AD models drastically increase during REM sleep – therefore, consolidation processes could be disrupted both at the systemic and synaptic levels. Here we present the results of a monocentric case-control study exploring the prevalence, the structural and genetic underpinnings and the consequences of subclinical EA in AD during sleep, and particularly REM sleep, in a cohort of mild-to moderate stage AD patients and age-matched controls. These objectives were investigated using neuroimaging methods, genetic testing and a full-night video-EEG, as well as a comprehensive neuropsychological test battery evaluating visual, declarative and episodic memory before the video-EEG and consolidation efficiency directly afterwards. Our study details the distribution of AD-related EA during sleep and is the first to report on the potential memory consolidation deficits they could induce in AD. Finally, these results could have implications from bench to bedside as they pinpoint important divergences between AD models and clinical observations and could potentially lead to improvements in future patient care as well.

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ANTE-MORTEM MAGNETIC RESONANCE IMAGING GREY-WHITE MATTER CONTRAST REGIONAL SIGNATURES OF ALZHEIMER'S DISEASE NEUROPATHOLOGY

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Introduction: Grey matter (GM), white matter (WM) contrast (GWC) in T1-weighted MRI has been shown to decrease with age in healthy populations and cognitive impairment in clinical Alzheimer's Disease (AD). However, the neuropathological determinants of this signal remain unknown. Here, we aimed to study whether GWC is associated with AD neuropathology and longitudinal hippocampal atrophy. **Methods:** 157 patients with neuropathological, clinical data and ante-mortem MRI were selected from the National Alzheimer's Coordinating Center database. Cortical volume and GWC (WM/GM intensity) in T1/MRI were obtained with Freesurfer 6.0. Hippocampal volume was divided into anterior, intermediate and posterior regions using a lab-based algorithm. Correlational analysis was conducted for GWC with age, and GWC residuals after linear regression with age for volume, BRAAK stage (neurofibrillary tangles), CERAD score (neuritic plaque density) and Cognitive Dementia Rating Sum-of-Boxes (CDR-SB). APOE genotypes were compared using one-way-ANOVA. **Results:** GWC was negatively correlated with age in widespread brain areas ($p < 0.05$, FDR), but only the entorhinal cortex showed a positive correlation between volume and GWC. There were no significant correlations between GWC and BRAAK stage, CERAD score, CDR-SB or differences according to APOE genotype. Entorhinal GWC correlated positively only with the posterior hippocampal volume in BRAAK III-IV ($p = 0.031$; $R = 0.34$). **Conclusion:** GWC is highly modulated by age, but not by classical AD neuropathology, APOE genotype or dementia severity in this cohort. Entorhinal GWC correlates with entorhinal and posterior hippocampal volume, suggesting it might reflect an early pathophysiological process distinct from classical AD neuropathology.

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INFLUENCE OF WHITE MATTER HYPERINTENSITIES OF VASCULAR ORIGIN ON BRAIN FUNCTIONAL CONNECTIVITY MEASURED WITH MEG

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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White matter hyperintensities (WMH) of presumed vascular origin is a common accompaniment of ageing, not only in dementia patients, but also in healthy population. In fact, they are present on neuroimaging in practically every individual over 60 years old and its prevalence increases with age. The clinical importance of WMH have been widely reported in the literature. The presence and progression of WMH on the brain have been associated to general cognitive impairment and all types of dementia, especially vascular cognitive impairment (VCI). The aim of this investigation was to focus the research on the identification of specific functional connectivity signatures related to WMH total volume in the brain. 350 cognitively healthy participants were recruited for the present study (aged from 50 to 87). All of them underwent a neuropsychological evaluation, MRI assessment (T1 and Flair), and MEG recordings (ten minutes of eyes closed resting state). WMH volume quantification was performed with an automatic detection toolbox, LST (SPM12). We used a completely data-driven approach to evaluate the relationship between functional connectivity (FC) and the WMH total volume. Interestingly, a dual pattern of FC emerged depending on the WMH volume, probably related to the underlying etiology: aged-related or vascular disease. Early identification and classification of dementia pathogenesis is a crucially important goal for the search for more effective management approaches. These findings could help to understand and try to palliate the contribution of WMH to particular symptoms in mixed dementias, and most subtypes of VCI.

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TEMPORAL AND SPECTRAL ERPS BIOMARKERS IN EARLY STAGES OF DEMENTIA

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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AIM. Improving early dementia diagnosis thanks to EEG using as neural marker Event Related Potentials (ERPs) features associated to a visual recognition task. METHODS. The study involved 93 subjects: 43 SCD (Subjective Cognitive Decline), 38 MCI (Mild Cognitive Impairment) and 12 controls. Patients' condition was assessed with standard clinical procedures. ERPs were decomposed in time-windowed neurocognitive processes associated to visual recognition, i.e, expectation, stimulus encoding, decision and post-decision. For each latent processes and for standard frequency bands, ERPs topographical maps were compared across subjects' groups and response correctness. RESULTS. ERPs topographies varied significantly according to groups during expectation (right occipital, parietal and centro-parietal ROIs), decision (right temporo-parietal, left centro-parieto-frontal ROIs) and post-decision (right/left occipital, right parieto-occipital and left centro-frontal ROIs). The main difference was in the abnormal amplitude and latency of late positive potential as it is associated with cognitive decline. The correctness of the answer affected the ERPs topographies during the encoding period (right central and occipito-parietal ROIs), coherently with the hypothesis that subjects accuracy may depend by initial phases of visual information processing. Spectral topographies displayed a group effect for alpha band (left parietal ROIs), while the correctness was dominant for delta band (left frontal, centro-fronto-parietal ROIs). CONCLUSIONS. These results highlighted ERPs temporal segments and spectral bands that might act as features to differentiate healthy, SCD and MCI condition. A support vector machine based on these signatures was tested and validated in blind, highlighting the possibility to include ERPs biomarkers during the early diagnosis of dementia.

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CAN WE SEE ALZHEIMER THROUGH EYE?

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Introduction One of the most common and prevalent neurodegenerative disorders are Alzheimer's and Dementia which are considered significant causes of mortality and morbidity in today's society particularly among the elderly population.(Erkkinen, Kim et al. 2018)The etiologic and pathophysiologic factors of neuro-degenerative disorders are still complicated and unrecognized. In last decade, the overall mortality rate for deaths cause by dementia and Alzheimer's disease has been generally increasing year-on-year. One of concerning statistics reported by Office for National Statistics , which indicates approximately 52% of increase in amount of people who diagnosed with dementia. Indeed, it is very threatening statistics in deaths of any health condition. From statistical point of view such huge increase on these disorders make and urgent need to understand the mechanisms behind the onset and progression of these heterogeneous diseases (Fig 1). Currently diagnosing Alzheimer's disease is also challenging due to lack of biomarkers. One of the pathological factors of Alzheimer's disease include the accumulation of proteins such as hyper phosphorylated tau and amyloid protein in hippocampal areas of the brain. The detection of these pathological biomarkers are conducted either by performing cerebrospinal fluid analysis, brain imaging or post-mortem brain tissues under microscope. However, these methods are not easily accessible and largely available due to different reasons. These include challenges in collecting certain samples, lack of post-mortem tissues and high-cost of experiment.(Guidoboni, Sacco et al. 2020)

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EEG MICROSTATE TOPOGRAPHIES DISCRIMINATE SUBJECTIVE COGNITIVE DECLINE AND MILD COGNITIVE IMPAIRMENT

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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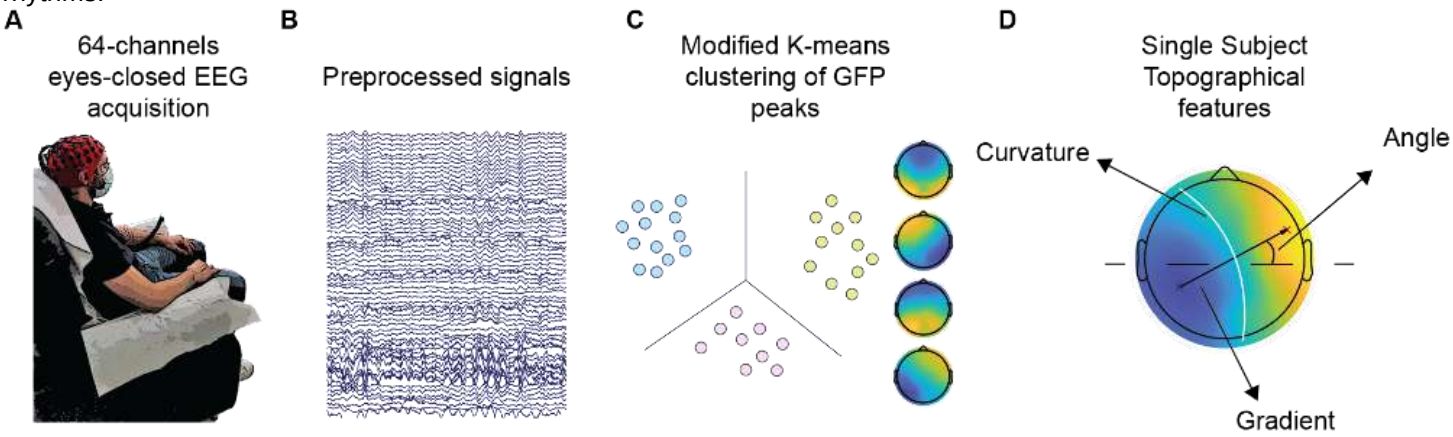
AIMS: Alzheimer’s disease (AD) neurodegeneration may begin up to decades earlier than the appearance of the first symptoms of cognitive decline. In order to prevent irreversible neuronal damage due to dementia, early diagnosis of AD is necessary. Prodromic signs of AD have been usually studied in mild cognitive impairment (MCI), the initial stage of clinical cognitive decline. However, subjective cognitive decline (SCD) is the first pre-clinical sign of possible AD and it has been shown to lead to an increased risk of AD itself. METHODS: To gain insights into the route to AD, we investigated whether it is possible to discriminate between SCD and MCI conditions by recording resting-state electroencephalographic (EEG) signals on a cohort of 81 patients (46 SCD, 35 MCI). For each subject, four microstates that best clustered EEG signal topographies were extracted. We characterized each microstate's topography by introducing a set of features, namely theta angle, gradient, and curvature. RESULTS: A classifier trained on these features reached a cross-validated accuracy of 74.8% (F1-score 74.1%, ROC-AUC 0.88) in discriminating between SCD and MCI and it highlighted differences between the two groups in all microstates topographies. With this work, we show that resting-state EEG signals can possibly be used as a low-cost and non-invasive pre-clinical dementia onset marker. CONCLUSIONS: The proposed topographical features could be employed in future works to gain insights on the specific pathways leading to the pathogenesis of AD, as EEG microstates have been previously related to underlying resting-state networks generating EEG rhythms.



FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-608

DECREASE OF THE EVENT-RELATED THETA POWER IN PATIENTS WITH PARKINSON'S DISEASE DEMENTIA AND LEWY BODY DEMENTIA IN COMPARISON TO ALZHEIMER'S DISEASE DEMENTIA

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Aims: Alzheimer's disease dementia (ADD), Parkinson's disease dementia (PDD), and dementia with Lewy body (DLB) are the most common types of dementia. One of the essential questions in the last years is what electrophysiological differences among these groups of dementia are. EEG Event-related oscillations (EROs) are one of the core methodologies that could show the differences among dementia groups. The present study aimed to investigate the EEG-EROs during the cognitive tasks in patients with ADD, PDD, and DLB. **Methods:** EEG recordings of 20 ADD, 20 PDD, and 17 DLB patients and matched 20 healthy controls (HC) were performed during the auditory and visual oddball paradigms. Event-related power spectrum and phase-locking were analyzed for the theta (4-7 Hz) frequency band. Repeated measures of ANOVA were used for statistical analysis ($p<0.05$). **Results:** There were significant differences among groups for both event-related theta power and phase-locking in the auditory task ($p<0.0001$). All dementia groups had decreased theta power and phase-locking compared to HC. PDD patients had lower theta power than ADD patients ($p<0.005$). Similarly, groups showed significant differences for both event-related theta power and phase-locking in the visual task ($p<0.0001$). All dementia groups had reduced theta phase-locking compared to HC. PDD and DLB patients had lower theta power than HC ($p<0.0001$) and ADD patients ($p<0.005$). **Conclusions:** The present study showed decreased event-related theta responses during auditory and visual cognitive tasks in patients with dementia. The decrease of the event-related theta power is more severe in PDD and DLB patients.

Pubmed:
35169227: Zanin M, Güntekin B, Aktürk T, Yıldırım E, Yener G, Kiyi I, Hünerli-Gündüz D, Sequeira H, Papo D
Telling functional networks apart using ranked network features stability.
Over the past few years, it has become standard to describe brain anatomical and functional organisation in terms of complex networks, wherein single brain regions or modules and their connections are respectively identified with network nodes and the links connecting them. Often, the goal of a given study is not that of modelling brain activity but, more basically, to discriminate between experimental conditions or populations, thus to find a way to compute differences between them. This in turn involves two important aspects: defining discriminative features and quantifying differences between them. Here we show that the ranked dynamical stability of network features, from links or nodes to higher-level network properties, discriminates well between healthy brain activity and various pathological conditions. These easily computable properties, which constitute local but topographically aspecific aspects of brain activity, greatly simplify inter-network comparisons and spare the need for network pruning. Our results are discussed in terms of microstate stability. Some implications for functional brain activity are discussed.
Sci Rep, 2022; 12
34613369: Babiloni C, Noce G, Ferri R, Lizio R, Lopez S, Lorenzo I, Tucci F, Soricelli A, Zurrón M, Díaz F, Nobili F, Arnaldi D, Famà F, Buttinelli C, Giubilei F, Cipollini V, Marizzoni M, Güntekin B, Yıldırım E, Hanoğlu L, Yener G, Gündüz DH, Onorati P, Stocchi F, Vacca L, Maestú F, Frisoni GB, Del Percio C
Resting State Alpha Electroencephalographic Rhythms Are Affected by Sex in Cognitively Unimpaired Seniors and Patients with Alzheimer's Disease and Amnesic Mild Cognitive Impairment: A Retrospective and Exploratory Study.
In the present retrospective and exploratory study, we tested the hypothesis that sex may affect cortical sources of resting

state eyes-closed electroencephalographic (rsEEG) rhythms recorded in normal elderly (Nold) seniors and patients with Alzheimer's disease and mild cognitive impairment (ADMCI). Datasets in 69 ADMCI and 57 Nold individuals were taken from an international archive. The rsEEG rhythms were investigated at individual delta, theta, and alpha frequency bands and fixed beta (14-30 Hz) and gamma (30-40 Hz) bands. Each group was stratified into matched females and males. The sex factor affected the magnitude of rsEEG source activities in the Nold seniors. Compared with the males, the females were characterized by greater alpha source activities in all cortical regions. Similarly, the parietal, temporal, and occipital alpha source activities were greater in the ADMCI-females than the males. Notably, the present sex effects did not depend on core genetic (APOE4), neuropathological (Aβ42/phospho-tau ratio in the cerebrospinal fluid), structural neurodegenerative and cerebrovascular (MRI) variables characterizing sporadic AD-related processes in ADMCI seniors. These results suggest the sex factor may significantly affect neurophysiological brain neural oscillatory synchronization mechanisms underpinning the generation of dominant rsEEG alpha rhythms to regulate cortical arousal during quiet vigilance.
Cereb Cortex, 2022; 32
34460957: Güntekin B, Aktürk T, Arakaki X, Bonanni L, Del Percio C, Edelmayer R, Farina F, Ferri R, Hanoğlu L, Kumar S, Lizio R, Lopez S, Murphy B, Noce G, Randall F, Sack AT, Stocchi F, Yener G, Yıldırım E, Babiloni C
Are there consistent abnormalities in event-related EEG oscillations in patients with Alzheimer's disease compared to other diseases belonging to dementia?
Cerebrospinal and structural-molecular neuroimaging in-vivo biomarkers are recommended for diagnostic purposes in Alzheimer's disease (AD) and other dementias; however, they do not explain the effects of AD neuropathology on neurophysiological mechanisms underpinning cognitive processes. Here, an Expert Panel from the Electrophysiology Professional Interest Area of the Alzheimer's Association reviewed the field literature and reached consensus on the event-related electroencephalographic oscillations (EROs) that show consistent abnormalities in patients with significant cognitive deficits due to Alzheimer's, Parkinson's (PD), Lewy body (LBD), and cerebrovascular diseases. Converging evidence from oddball paradigms showed that, as compared to cognitively unimpaired (CU) older adults, AD patients had lower amplitude in widespread delta (>4 Hz) and theta (4-7 Hz) phase-locked EROs as a function of disease severity. Similar effects were also observed in PD, LBD, and/or cerebrovascular cognitive impairment patients. Non-phase-locked alpha (8-12 Hz) and beta (13-30 Hz) oscillations were abnormally reduced (event-related desynchronization, ERD) in AD patients relative to CU. However, studies on patients with other dementias remain lacking. Delta and theta phase-locked EROs during oddball tasks may be useful neurophysiological biomarkers of cognitive systems at work in heuristic and intervention clinical trials performed in AD patients, but more research is needed regarding their potential role for other dementias.
Psychophysiology, 2022; 59
32339563: Güntekin B, Aktürk T, Yıldırım E, Yılmaz NH, Hanoğlu L, Yener G
Abnormalities in auditory and visual cognitive processes are differentiated with theta responses in patients with Parkinson's disease with and without dementia.
The research on the abnormalities of event-related oscillations in Parkinson's disease (PD) was mostly studied with cognitively normal patients. The present study aims to show the adverse effects of cognitive decline in PD patients via the EEG-Brain Oscillations approach by comparing the electrophysiological responses in two modalities, i.e. auditory, and visual in which PD group show deficit. We conducted a study in which we analyzed event-related theta power and phase-locking during auditory and visual oddball paradigm. Cognitively normal PD (PDCN) patients (N = 15), PD with mild cognitive impairment (PDMCI) patients (N = 22), PD dementia (PDD) patients (N = 11) and healthy controls (HC) (N = 17) were included in the study. Neuropsychological assessments were applied to all participants. There was a gradual decrease in scores of neuropsychological tests (HC, PDCN, PDMCI, PDD, respectively). Most of the neuropsychological test scores of the participants were highly correlated with the theta power and theta phase locking values, especially over frontal-central areas. HC had higher theta phase-locking and power in comparison to PDMCI and PDD. The differentiation between HC and PDCN was specific to frontal-central areas. Theta power and theta phase-locking were decreased overall locations in PDMCI and PDD both during visual and auditory oddball paradigms compared with PDCN. The results indicate that theta responses in PD patients decreased gradually as the cognitive decline increased. We can conclude that complex abnormalities in their neurotransmitter and neuronal signal systems that occur with the progression of the disease could be responsible for these results.
Int J Psychophysiol, 2020; 153
31264726: Güntekin B, Hanoğlu L, Aktürk T, Fide E, Emek-Savaş DD, Ruşen E, Yıldırım E, Yener GG
Impairment in recognition of emotional facial expressions in Alzheimer's disease is represented by EEG theta and alpha responses.
Behavioral studies have shown that the recognition of facial expressions may be impaired in patients with Alzheimer's disease (AD). The identification and recognition of a facial expression might be represented by event-related brain oscillations. The present study aims to analyze EEG event-related oscillations and determine the electrophysiological

indicators of impaired facial expression recognition in AD patients. EEGs of 30 healthy controls and 30 AD patients were recorded during their perception of three different facial expressions (angry, happy, neutral). Event-related power spectrum and phase locking were analyzed in the theta (4-7) and alpha (8-13 Hz) frequency bands with the EEGLAB open toolbox. There was a significant facial Expression × Group interaction ($p < 0.05$) for the theta power spectrum; the healthy control group had higher theta power than the AD group during the perception of angry facial expressions ($p < 0.05$). There was a significant hemisphere difference between the two groups ($p < 0.05$). There was a right hemisphere alpha power dominance in healthy subjects. However, AD patients did not have this alpha power asymmetry. The present study, for the first time in the literature, presents the electrophysiological indicators of impaired recognition of facial expression in AD patients. The current study could be a basis for future studies that will analyze emotional processing in different kinds of dementia patients, and this study may have provided indicators of electrophysiological correlates of behavioral problems observed in clinical practice.
Psychophysiology, 2019; 56

32758480: Güntekin B, Uzunlar H, Çalışoğlu P, Eroğlu-Ada F, Yıldırım E, Aktürk T, Atay E, Ceran Ö
Theta and alpha oscillatory responses differentiate between six-to seven-year-old children and adults during successful visual and auditory memory encoding.
The healthy maturation of the brain is one of the intriguing topics that need to be investigated to understand human brain and child development. The present study aimed to investigate the development of memory processes both for auditory and visual memory using electroencephalography (EEG)-Brain Dynamics methodologies. Sixteen healthy children between the ages of 6 and 7 years and eighteen healthy young adults (age: 21.32 ± 3.28 years) were included in the study. EEG was recorded from 18 channels during the visual and auditory memory paradigms. Two different subtests of the WISC-IV IQ test were applied to all children. Event-related theta (4-7 Hz), alpha (8-13 Hz) power and phase-locking were analyzed. The young adults had higher memory performance than the children for both auditory and visual paradigms. The children had increased theta phase-locking and left alpha power in response to the remembered objects in comparison to the forgotten objects. The young adults had higher theta and alpha phase-locking than the children over the frontal and central locations ($p < 0.05$), and the children had higher parietal-occipital alpha phase-locking than the young adults. There was an increase in alpha power in children, whereas young adults had decreased post-stimulus alpha power in response to memory paradigms. The present study showed that frontocentral theta and alpha phase-locking had an essential role in brain maturation and successful memory performance. Event-related theta and alpha responses could be one of the important indicators of the mature and healthy brain, and these responses could change depending on the maturation state and age.
Brain Res, 2020; 1747

31938578: Yıldırım E, Güntekin B, Hanoğlu L, Algun C
EEG alpha activity increased in response to transcutaneous electrical nervous stimulation in young healthy subjects but not in the healthy elderly.
Transcutaneous Electrical Nerve Stimulation (TENS) is used not only in the treatment of pain but also in the examination of sensory functions. With aging, there is decreased sensitivity to somatosensory stimuli. It is essential to examine the effect of TENS application on the sensory functions in the brain by recording the spontaneous electroencephalogram (EEG) activity and the effect of aging on the sensory functions of the brain during the application. The present study aimed to investigate the effect of the application of TENS on the brain's electrical activity and the effect of aging on the sensory functions of the brain during application of TENS. A total of 15 young (24.2 ± 3.59) and 14 elderly (65.64 ± 4.92) subjects were included in the study. Spontaneous EEG was recorded from 32 channels during TENS application. Power spectrum analysis was performed by Fast Fourier Transform in the alpha frequency band (8-13 Hz) for all subjects. Repeated measures of analysis of variance was used for statistical analysis (< 0.05). Young subjects had increased alpha power during the TENS application and had gradually increased alpha power by increasing the current intensity of TENS (= 0.035). Young subjects had higher alpha power than elderly subjects in the occipital and parietal locations (= 0.073). We can, therefore, conclude that TENS indicated increased alpha activity in young subjects. Young subjects had higher alpha activity than elderly subjects in the occipital and somatosensory areas. To our knowledge, the present study is one of the first studies examining the effect of TENS on spontaneous EEG in healthy subjects. Based on the results of the present study, TENS may be used as an objective method for the examination of sensory impairments, and in the evaluative efficiency of the treatment of pain conditions.
PeerJ, 2020; 8

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BOARD NUMBER: S04-609

THE INTERACTION OF REMINISCENCE THERAPY PLUS WALKING INTERVENTIONS ON COGNITIVE PERFORMANCE AND WELL-BEING OF OLDER ADULTS WITH EARLY STAGE DEMENTIA OF ALZHEIMER TYPE.

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Maynooth University, Psychology Department, Maynooth, Ireland

The population of older adults (60+) is increasing, leading to a pronounced growth of age-related disorders, including Alzheimer's Disease. Therefore, it is a major health challenge to develop treatments and improve quality of life by providing communities with evidence-based programmes. Walking presents cognitive and psychological benefits and can minimise age-related loss in memory-related regions of the brain, notably structural and functional changes to the pre-frontal cortex and hippocampus for older adults and people with Alzheimer's Disease. Reminiscence Therapy (RT) has been shown to produce psychological and cognitive benefits, including improved memory, for older adults with dementia. We used a Public and Patient Involvement (PPI) approach to investigate potential cognitive, and/or well-being benefits when combining these two interventions in a sample of older adults with Alzheimer's Disease. The intervenion group, facilitated by a researcher, were guided through a series of weekly historical walking tours of their local community over a period of four weeks. We compared this group, who walked and were encouraged to recall stories about the locations, with a control group who reminisced using only photos of the same sites. Cognitive measurements (including the MOCA, EAMI Memory Interview and Holden Communication Scale) and well-being measures (CASP-19 questionnaire, Satisfaction With Life Scale SWLS) were also administered. Changes in pre- and post-intervention measures of cognition and well-being will be discussed in the context of potential benefits and future studies. Walking, combined with RT, may represent an inexpensive, accessible, easily implemented and non-invasive therapy for older adults living with Alzheimer's Disease.

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BOARD NUMBER: S04-610

MEDITATION: A POTENTIAL THERAPY TARGETING AMYGDALA FOR THE PREVENTION & REVERSAL OF ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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INTRODUCTION – The most common yet undefeatable, age-associated neurodegenerative disease Alzheimer's Disease (AD) can be prevented with the help of accessible, fruitful, easily learnable & customized practice of meditation whose competence has stood the test of time stanning over 5,000 years. Its role in AD has not been well established indicating a target component or region of the brain. **AIM** – This review aims to highlight the clinical correlation of amygdalar atrophy & activation in AD & the role of meditation in amygdalar deactivation resulting in impedance of cognitive decline. **METHODS** – A review of relevant data from publicly accessible research databases on neurosciences was performed. **RESULTS** – Limbic system component amygdala responsible for processing & memorizing emotional reactions resulting in regulation of social & behavioural responses is found to be influenced in AD. Morphologically, alteration in volume of amygdala results progression of mild cognitive impairment to AD which can be seen as structural shrinkage, distortion, neuronal loss & widespread gliosis. There are numerous researches attesting the positive associations between meditation & regulated amygdalar activity, which plays the vital role in learning & processing of emotional memory. They also acknowledge the ability of meditation to improve neuronal connectivity with prefrontal cortex which is responsible for inducing happiness, reducing fear & anxiety via amygdalar deactivation. **CONCLUSION** – This review presents worthy trappings of meditation in attenuating evolution of AD while impeding cognitive decline & reducing risk factors accompanying AD.

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MOTOR IMAGERY PRESERVATION IN AMYOTROPHIC LATERAL SCLEROSIS: RESULTS FROM A BRAIN-COMPUTER INTERFACE TRIAL

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Aims Patients with amyotrophic lateral sclerosis (ALS) experience progressive motor disability, which causes gradual loss of independence. Motor Imagery (MI)-based Brain-computer interface (BCI) represents a promising approach for controlling exoskeletons, orthosis, and other devices targeted to restore motor function and autonomy. However, the preservation of MI ability in ALS patients is controversial. This study aimed to assess MI in an ALS cohort compared to a group of healthy subjects. Methods We included twenty-six ALS patients (M/F=1.9; disease duration: 28.9±22.6 months; ALSFRS-R: 34.84±7.24) and thirteen healthy controls (M/F=1.6). Age at examination and education were not different between the two groups (Age: ALS, 65.9 + 6.9 years *vs* Controls, 64.4 ± 5.12 years, p=0.5; Education: ALS, 9.7±4.3 years *vs* Controls, 11.3 ± 4.5, p= 0.3). Written informed consent was obtained. The RecoveriX© system (g.tec, Austria) was used for EEG data recording and real-time processing. All subjects participated in an MI-BCI + FES paradigm. The system measured the accuracy in accomplishing the mental task. Correlation between age, education, disease duration, ALSFRS-R, neuropsychological variables and BCI performance has been explored. Results MI-BCI accuracy was not significantly different between ALS patients and healthy controls (p=0.23). This value did not correlate with ALSFRS-R total score (p= 0.9) and upper limbs score (p= 0.9), ECAS total score (p=0.2) and specific scores (p= 0.4), education (p=0.2), and disease' duration (p= 0.8). Conclusions ALS at the intermediate stage does not affect the patients' MI ability. MI BCIs might be adopted for advanced applications to improve autonomy in this devastating disease.

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BOARD NUMBER: S04-612

BIASED AGONISTS OF MUSCARINIC RECEPTORS

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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The disruption of muscarinic signalling is frequently involved in various pathophysiological conditions, including neuropathic pain, neurological and psychiatric disorders, e.g., Alzheimer's disease or schizophrenia. To target these particular conditions, selective modulation of individual muscarinic subtypes to avoid undesired side effects is necessary. High homology of the orthosteric binding site among all muscarinic subtypes makes a finding of orthosteric agonists that bind selectively to individual muscarinic subtypes virtually unattainable. Selective targeting at a particular G-protein mediated signalling pathway by biased agonists, via agonist-specific conformation, can be the way to achieve functional selectivity among individual subtypes of muscarinic receptors. Specifically, the binding of an agonist to one or a subset of functional hot spots within the binding site results in activation of a subset of signalling pathways and thus in ligand-mediated signalling bias. An agonist relatively small in size has a greater chance to bind to a smaller number of functional hot spots than a larger agonist. We demonstrate that newly developed tetrahydropyridin based muscarinic agonists activate only a subset of signalling pathways. They display agonist-specific activation profiles of individual G-protein isoforms, that differ among subtypes. Our results prove that selective activation of individual subtypes of muscarinic receptors by biased agonists can be achieved.

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BOARD NUMBER: S04-613

IDENTIFICATION OF INFECTIOUS AGENTS IN POSTMORTEM BRAIN TISSUE OF PATIENTS WITH ALZHEIMER'S DISEASE

POSTER SESSION 04 - SECTION: ALZHEIMER'S DISEASE AND NEURODEGENERATION

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Background: Currently, there are established genetic and environmental risk factors for Alzheimer's disease (AD). Infectious agents are considered as possible causes of AD since some of them have been detected (ADN or structural components) in the brain tissue of patients suffering from this type of dementia. The aim of this study was to look for and identify infectious agents in *postmortem* brain tissues of patients with AD. **Methods:** Neuropathological changes were studied in fixed brain tissues from patients with a diagnosis of sporadic AD (SAD = 9), familial AD (FAD = 12), and brain samples of healthy individuals (n = 9). In addition, the clinical and demographic characteristics were analyzed. Microarrays, multiplex PCR, panfungal PCR, and immunohistochemistry were used to search for infectious agents in froxen tissue. **Results:** All cases with AD showed high degree of deterioration, according to scales of neuropathological diagnosis. Infectious agents were identified in two brain samples from cases with FAD (Herpes simplex virus type 1or 2, Human herpes virus type 3) and one case from control group was positive to environmental bacteria. **Conclusions:** Although the hypothesis of an infectious origin of AD has been proposed, our results did not confirm direct involvement of infectious microorganism to AD etiology despite the use of different techniques used for the detection of infectious agents. It is possible that infectious agents, in early stages of AD, induce an exacerbated immune response that affects brain tissue, although further studies are needed to confirm this hypothesis.

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BOARD NUMBER: S04-614

BEHAVIORAL CHANGES WITH RELEVANCE TO AFFECTIVE DISORDERS IN DIMETHYLARGININE DIMETHYLAMINOHYDROLASE 1 OVEREXPRESSING MICE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Neuropsychiatric disorders including major depression, bipolar disorder and schizophrenia are a leading cause of disability worldwid
regulation of mood-related behavior by the enzyme dimethylarginine dimethylaminohydrolase 1 (DDAH1). DDAH1 functions to modu
asymmetric dimethylarginine (ADMA). NO itself governs processes including neurotransmission, synaptic plasticity and stress axis a
is highly expressed in the brain and increased levels of ADMA have been observed in patients. However, causality and mechanism
transgenic animals that overexpress a human DDAH1 construct (DDAH1-tg). DDAH1-tg mice show increased levels of expression a
the core phenotypes observed in manic episodes, i.e. increased exploration, hyperactivity, lower habituation in novel environments,
of mood disorders, DDAH1-tg mice also exhibit altered behavioral responses following psychostimulant challenge. In addition, tissue
several relevant brain areas. In conclusion, DDAH1-tg mice display behavioral alterations and changes in neurotransmitter homeost
tool for mechanistic and therapeutic research with relevance to affective disorders.

FENS Forum 2022
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BOARD NUMBER: S04-615

THE VPAC2 RECEPTOR MEDIATES RESILIENCE TO STRESS IN FEMALE, BUT NOT MALE MICE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Aims: The neuropeptide pituitary adenylate cyclase activating peptide (PACAP) has been implicated in the pathophysiology of post-traumatic stress disorder (PTSD). Although PACAP binds to the vasoactive intestinal peptide receptor 2 (VPAC2) it is still unknown whether VPAC2 is involved in the psychopathology of stress-induced psychiatric disease. Here, we hypothesized that administration of the pharmacological agonist of VPAC2, Bay 55-9837, could prevent a variety of stress-induced fear, behavioral despair, and anxiety-like behaviors. Methods: A single injection of saline, (*R,S*)-ketamine, or Bay 55-9837 was administered before or after contextual fear conditioning (CFC) stress in male and female 129S6/SvEv mice. Drug efficacy was assayed using the forced swim test (FST), elevated plus maze (EPM), open field (OF), novelty-suppressed feeding (NSF), contextual fear discrimination (CFD), and Piezo sleep boxes. Brain-wide VPAC2 expression was assayed using immunohistochemistry. Results: Activating VPAC2 prior to stress attenuated learned fear, reduced behavioral despair, suppressed hyponeophagia, and facilitated CFD in female, but not male mice. Prophylactic Bay 55-9837 protected against stress-induced changes in sleep/wake cycles in female mice. Bay 55-9837 administration after stress reduced behavioral despair and hyponeophagia in both sexes. CFD learning upregulated VPAC2 expression in hippocampal CA3 and the agranular insular cortex. Conclusions: Our data indicate that activating the VPAC2 receptor suppresses fear behavior and facilitates CFD learning in female, but not male mice. These results show that VPAC2 critically modulates fear learning and retrieval in a sex-specific manner. Overall, our findings suggest that VPAC2 may be a novel, female-specific target for preventing and treating stress-induced psychiatric disorders.

Pubmed:

29950730: Anacker C, Luna VM, Stevens GS, Millette A, Shores R, Jimenez JC, Chen B, Hen R
Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus.
Adult neurogenesis in the dentate gyrus of the hippocampus is highly regulated by environmental influences, and functionally implicated in behavioural responses to stress and antidepressants. However, how adult-born neurons regulate dentate gyrus information processing to protect from stress-induced anxiety-like behaviour is unknown. Here we show in mice that neurogenesis confers resilience to chronic stress by inhibiting the activity of mature granule cells in the ventral dentate gyrus (vDG), a subregion that is implicated in mood regulation. We found that chemogenetic inhibition of adult-born neurons in the vDG promotes susceptibility to social defeat stress, whereas increasing neurogenesis confers resilience to chronic stress. By using in vivo calcium imaging to record neuronal activity from large cell populations in the vDG, we show that increased neurogenesis results in a decrease in the activity of stress-responsive cells that are active preferentially during attacks or while mice explore anxiogenic environments. These effects on dentate gyrus activity are necessary and sufficient for stress resilience, as direct silencing of the vDG confers resilience whereas excitation promotes susceptibility. Our results suggest that the activity of the vDG may be a key factor in determining individual levels of vulnerability to stress and related psychiatric disorders.
Nature, 2018; 559
31130452: Chen BK, Murawski NJ, Cincotta C, McKissick O, Finkelstein A, Hamidi AB, Merfeld E, Doucette E, Grella SL, Shpokayte M, Zaki Y, Fortin A, Ramirez S
Artificially Enhancing and Suppressing Hippocampus-Mediated Memories.
Emerging evidence indicates that distinct hippocampal domains differentially drive cognition and emotion [1, 2]; dorsal regions encode spatial, temporal, and contextual information [3-5], whereas ventral regions regulate stress responses [6], anxiety-related behaviors [7, 8], and emotional states [8-10]. Although previous studies demonstrate that optically manipulating cells in the dorsal hippocampus can drive the behavioral expression of positive and negative memories, it is

unknown whether changes in cellular activity in the ventral hippocampus can drive such behaviors [11-14]. Investigating the extent to which distinct hippocampal memories across the longitudinal axis modulate behavior could aid in the understanding of stress-related psychiatric disorders known to affect emotion, memory, and cognition [15]. Here, we asked whether tagging and stimulating cells along the dorsoventral axis of the hippocampus could acutely, chronically, and differentially promote context-specific behaviors. Acute reactivation of both dorsal and ventral hippocampus cells that were previously active during memory formation drove freezing behavior, place avoidance, and place preference. Moreover, chronic stimulation of dorsal or ventral hippocampal fear memories produced a context-specific reduction or enhancement of fear responses, respectively, thus demonstrating bi-directional and context-specific modulation of memories along the longitudinal axis of the hippocampus. Fear memory suppression was associated with a reduction in hippocampal cells active during retrieval, while fear memory enhancement was associated with an increase in basolateral amygdala activity. Together, our data demonstrate that discrete sets of cells throughout the hippocampus provide key nodes sufficient to bi-directionally reprogram both the neural and behavioral expression of memory.
Curr Biol, 2019; 29

31600767: Chen BK, Mendez-David I, Luna VM, Faye C, Gardier AM, David DJ, Denny CA
Prophylactic efficacy of 5-HTR agonists against stress.
Enhancing stress resilience could protect against stress-induced psychiatric disorders in at-risk populations. We and others have previously reported that (R,S)-ketamine acts as a prophylactic against stress when administered 1 week before stress. While we have shown that the selective 5-hydroxytryptamine (5-HT) (serotonin) reuptake inhibitor (SSRI) fluoxetine (Flx) is ineffective as a prophylactic, we hypothesized that other serotonergic compounds such as serotonin 4 receptor (5-HTR) agonists could act as prophylactics. We tested if three 5-HTR agonists with varying affinity could protect against stress in two mouse strains by utilizing chronic corticosterone (CORT) administration or contextual fear conditioning (CFC). Mice were administered saline, (R,S)-ketamine, Flx, RS-67,333, prucalopride, or PF-04995274 at varying doses, and then 1 week later were subjected to chronic CORT or CFC. In C57BL/6N mice, chronic Flx administration attenuated CORT-induced weight changes and increased open-arm entries in the elevated plus maze (EPM). Chronic RS-67,333 administration attenuated CORT-mediated weight changes and protected against depressive- and anxiety-like behavior. In 129S6/SvEv mice, RS-67,333 attenuated learned fear in male, but not female mice. RS-67,333 was ineffective against stress-induced depressive-like behavior in the forced swim test (FST), but prevented anxiety-like behavior in both sexes. Prucalopride and PF-04995274 attenuated learned fear and decreased stress-induced depressive-like behavior. Electrophysiological recordings following (R,S)-ketamine or prucalopride administration revealed that both drugs alter AMPA receptor-mediated synaptic transmission in CA3. These data show that in addition to (R,S)-ketamine, 5-HTR agonists are also effective prophylactics against stress, suggesting that the 5-HTR may be a novel target for prophylactic drug development.
Neuropsychopharmacology, 2020; 45

32417852: Chen BK, Luna VM, LaGamma CT, Xu X, Deng SX, Suckow RF, Cooper TB, Shah A, Brachman RA, Mendez-David I, David DJ, Gardier AM, Landry DW, Denny CA
Sex-specific neurobiological actions of prophylactic (R,S)-ketamine, (2R,6R)-hydroxynorketamine, and (2S,6S)-hydroxynorketamine.
Enhancing stress resilience in at-risk populations could significantly reduce the incidence of stress-related psychiatric disorders. We have previously reported that the administration of (R,S)-ketamine prevents stress-induced depressive-like behavior in male mice, perhaps by altering α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-mediated transmission in hippocampal CA3. However, it is still unknown whether metabolites of (R,S)-ketamine can be prophylactic in both sexes. We administered (R,S)-ketamine or its metabolites (2R,6R)-hydroxynorketamine ((2R,6R)-HNK) and (2S,6S)-hydroxynorketamine ((2S,6S)-HNK) at various doses 1 week before one of a number of stressors in male and female 129S6/SvEv mice. Patch clamp electrophysiology was used to determine the effect of prophylactic drug administration on glutamatergic activity in CA3. To examine the interaction between ovarian hormones and stress resilience, female mice also underwent ovariectomy (OVX) surgery and a hormone replacement protocol prior to drug administration. (2S,6S)-HNK and (2R,6R)-HNK protected against distinct stress-induced behaviors in both sexes, with (2S,6S)-HNK attenuating learned fear in male mice, and (2R,6R)-HNK preventing stress-induced depressive-like behavior in both sexes. (R,S)-ketamine and (2R,6R)-HNK, but not (2S,6S)-HNK, attenuated large-amplitude AMPAR-mediated bursts in hippocampal CA3. All three compounds reduced N-methyl-D-aspartate receptor (NMDAR)-mediated currents 1 week after administration. Furthermore, ovarian-derived hormones were necessary for and sufficient to restore (R,S)-ketamine- and (2R,6R)-HNK-mediated prophylaxis in female mice. Our data provide further evidence that resilience-enhancing prophylactics may alter AMPAR-mediated glutamatergic transmission in CA3. Moreover, we show that prophylactics against stress-induced depressive-like behavior can be developed in a sex-specific manner and demonstrate that ovarian hormones are necessary for the prophylactic efficacy of (R,S)-ketamine and (2R,6R)-HNK in female mice.
Neuropsychopharmacology, 2020; 45

32919399: Anacker C, Sydnor E, Chen BK, LaGamma CC, McGowan JC, Mastrodonato A, Hunsberger HC, Shores R, Dixon RS, McEwen BS, Byne W, Meyer-Bahlburg HFL, Bockting W, Ehrhardt AA, Denny CA
Behavioral and neurobiological effects of GnRH agonist treatment in mice-potential implications for puberty suppression in transgender individuals.
In the United States, ~1.4 million individuals identify as transgender. Many transgender adolescents experience gender dysphoria related to incongruence between their gender identity and sex assigned at birth. This dysphoria may worsen as puberty progresses. Puberty suppression by gonadotropin-releasing hormone agonists (GnRHa), such as leuprolide, can help alleviate gender dysphoria and provide additional time before irreversible changes in secondary sex characteristics may be initiated through feminizing or masculinizing hormone therapy congruent with the adolescent's gender experience. However, the effects of GnRH agonists on brain function and mental health are not well understood. Here, we investigated the effects of leuprolide on reproductive function, social and affective behavior, cognition, and brain activity in a rodent model. Six-week-old male and female C57BL/6J mice were injected daily with saline or leuprolide (20 µg) for 6 weeks and tested in several behavioral assays. We found that leuprolide increases hyperlocomotion, changes social preference, and increases neuroendocrine stress responses in male mice, while the same treatment increases hyponeophagia and despair-like behavior in females. Neuronal hyperactivity was found in the dentate gyrus (DG) of leuprolide-treated females, but not males, consistent with the elevation in hyponeophagia and despair-like behavior in females. These data show for the first time that GnRH agonist treatment after puberty onset exerts sex-specific effects on social- and affective behavior, stress regulation, and neural activity. Investigating the behavioral and neurobiological effects of GnRH agonists in mice will be important to better guide the investigation of potential consequences of this treatment for youth experiencing gender dysphoria.
Neuropsychopharmacology, 2021; 46
33766406: Leal Santos S, Stackmann M, Muñoz Zamora A, Mastrodonato A, De Landri AV, Vaughan N, Chen BK, Lanio M, Denny CA
Propranolol Decreases Fear Expression by Modulating Fear Memory Traces.
Posttraumatic stress disorder can develop after a traumatic event and results in heightened, inappropriate fear and anxiety. Although approximately 8% of the U.S. population is affected by posttraumatic stress disorder, only two drugs have been approved by the Food and Drug Administration to treat it, both with limited efficacy. Propranolol, a nonselective β-adrenergic antagonist, has shown efficacy in decreasing exaggerated fear, and there has been renewed interest in using it to treat fear disorders.
Biol Psychiatry, 2021; 89
33631001: Chen BK, Le Pen G, Eckmier A, Rubinstenn G, Jay TM, Denny CA
Fluoroethylnormemantine, A Novel Derivative of Memantine, Facilitates Extinction Learning Without Sensorimotor Deficits.
Memantine, a noncompetitive N-methyl-D-aspartate receptor antagonist, has been approved for use in Alzheimer's disease, but an increasing number of studies have investigated its utility for neuropsychiatric disorders. Here, we characterized a novel compound, fluoroethylnormemtantine (FENM), which was derived from memantine with an extra Fluor in an optimized position for in vivo biomarker labeling. We sought to determine if FENM produced similar behavioral effects as memantine and/or if FENM has beneficial effects against fear, avoidance, and behavioral despair.
Int J Neuropsychopharmacol, 2021; 24
34274107: Chen BK, Luna VM, Shannon ME, Hunsberger HC, Mastrodonato A, Stackmann M, McGowan JC, Rubinstenn G, Denny CA
Fluoroethylnormemantine, a Novel NMDA Receptor Antagonist, for the Prevention and Treatment of Stress-Induced Maladaptive Behavior.
Major depressive disorder is a common, recurrent illness. Recent studies have implicated the NMDA receptor in the pathophysiology of major depressive disorder. (R,S)-ketamine, an NMDA receptor antagonist, is an effective antidepressant but has numerous side effects. Here, we characterized a novel NMDA receptor antagonist, fluoroethylnormemantine (FENM), to determine its effectiveness as a prophylactic and/or antidepressant against stress-induced maladaptive behavior.
Biol Psychiatry, 2021; 90

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BOARD NUMBER: S04-616

A NOVEL ENDOCANNABINOID HYDROLASE FAAH INHIBITOR AS A POTENTIAL ANTIDEPRESSANT INDUCES GENE EXPRESSION CHANGES IN NUCLEUS ACCUMBENS IN A BALB/C MICE ACUTE STRESS MODEL

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Fatty acid amide hydrolase (FAAH) inhibitors have shown antidepressant effects by increasing the bioavailability of anandamide in the central nervous system. We aim to study the new macamide MCH1 (from *Lepidium meyenii*), which shows FAAH inhibitory activity in-vitro. For this purpose, we compared the expression pattern in nucleus accumbens of mice groups receiving 3, 10 and 30 mg/kg i.p. of MCH1 versus vehicle and fluoxetine (FLX) littermates. We used mice exposed to the acute stress model. Real-time polymerase chain reaction (RT-qPCR) analysis shows changes in *Faah, Cnr1, Drn1* and *Drn2* expression levels in MCH1 and FLX groups, compared to the vehicle group. Statistical analysis showed that MCH1 30 mg/kg group had a significant 2.9-fold increase in *Faah* expression compared to vehicle ($p = 0.0352$). Nonetheless, there was no significant difference in expression level in *the Cnr1* gene compared to the group receiving 3 mg/kg MCH1 and the vehicle ($p = 0.8949$). However, we observed that the groups receiving 10 and 30 mg/kg MCH1 and FLX increased the *Cnr1* expression up to 4.3-fold more than the vehicle group ($p = 0.0063$). Moreover, comparing expression levels of *Drd1* and *Drd2* between vehicles and all MCH1 groups shows no statistical difference. Furthermore, we observe a dose-dependent increasing trend of *Drd1* and *Drd2* expression between MCH1 groups. Finally, FLX group shown significant increase in *Drd1* expression compared to 3 mg/kg MCH1 group ($p = 0.0371$) and in *Drd2* expression compared to 3 mg/kg ($p = 0.0027$) and 10 mg/kg ($p = 0.0101$) MCH1 group

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HYPEREXCITABILITY AND WNT/β-CATENIN SIGNALING PATHWAY IN NEURONS DERIVED FROM BIPOLAR DISORDER PATIENTS

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Renata Santos[1], Sara Linker[2], Ana Mendes[2], Maxim Shokhirev[3], Galina Erikson[3], Lynne Randolph-Moore[2], John Kelsoe[4], Martin Alda[5], Fred Gage[2], Maria Marchetto[2]
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Bipolar disorder (BD) is a psychiatric condition characterized by depressive and manic episodes affecting 2% of the world population. Lithium (Li) is the first-line long-term treatment for mood stabilization. Induced pluripotent stem cell (iPSC) modeling of BD using dentate gyrus (DG)-like neurons derived from Li responsive (LR) and Li non-responsive (NR) patients showed neuronal hyperexcitability. Li treatment reversed hyperexcitability on LR neurons indicating that DG neuronal hyperexcitability correlates with patient clinical information and drug response. In this study, we searched for the molecular mechanisms causing hyperexcitability and Li resistance using a combination of cellular techniques, RNA sequencing and electrophysiology. We observed that valproic acid (VPA), a drug used to treat NR patients decreased hyperexcitability in NR neurons. These neurons showed a decrease in Wnt/β-catenin signaling pathway activity and expression of *LEF1* gene that was reversed by VPA treatment. In addition, decreasing *LEF1* expression in control neurons using shRNA caused hyperexcitability. These results indicate that hyperexcitability in NR neurons is related to changes in LEF1 and in Wnt/β-catenin pathway, potentially modulated by VPA treatment. While *LEF1* expression is similar in NR, LR and control neural progenitors, its expression is progressively downregulated over time during neuronal differentiation. The Wnt/β-catenin pathway is essential for hippocampal embryonic development and adult neurogenesis and it was observed that BD patients have consistently smaller volumes of the hippocampus and DG in imaging studies. Our results suggest that Wnt/β-catenin pathway is implicated in hyperexcitability, drug resistance and differentiation of hippocampal DG neurons in an iPSC model of BD.

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EARLY LIFE ADVERSITY PERTURBS MITOSTASIS AND DRIVES INFLAMMAGING IN THE RAT HIPPOCAMPUS

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Early life stressors result in life-long alterations in anxiety and depression-like behaviour, and accelerate neuronal aging. However, the molecular and cellular changes evoked by early adversity that contribute to these phenotype is not delineated. Neurodegenerative disorders are associated with mitochondrial dysfunction and inflammation, and early adversity is also known to enhance neurodegenerative changes in the process of aging. The primary thrust of our project was to gain insights into the contribution of perturbed mitochondrial metabolism and inflammation within limbic neurocircuits in mediating the accelerated aging that has been suggested to be one of the sequelae of early adversity. Using a rodent model of early adversity, maternal separation (MS) which is reported to enhance adult anxiety and depression-like behaviour, accelerate aging, and perturb neuroendocrine stress responses that endure across the life span, we addressed whether this was accompanied by perturbed mitostasis and peripheral/central inflammatory changes. We provide novel information that a history of MS is associated with a robust dysregulation in mitostasis, with a decline noted in mitochondrial biogenesis and enhanced mitophagy, accompanied by an inflammatory state evoked in the hippocampus and prefrontal cortex, which emerges in an age-dependent manner. This is accompanied by impaired mitochondrial function and morphology in middle-aged animals subjected to MS. The changes in the brain are also associated with robust peripheral inflammatory changes, which appear to precede the neuroinflammation. Studies are under way to assess the influence of interventional strategies that may serve to reverse the mitochondrial and inflammatory changes that we observe following early adversity.

Pubmed:
32955432: Pati S, Saba K, Salvi SS, Tiwari P, Chaudhari PR, Verma V, Mukhopadhyay S, Kapri D, Suryavanshi S, Clement JP, Patel AB, Vaidya VA
Chronic postnatal chemogenetic activation of forebrain excitatory neurons evokes persistent changes in mood behavior. Early adversity is a risk factor for the development of adult psychopathology. Common across multiple rodent models of early adversity is increased signaling via forebrain Gq-coupled neurotransmitter receptors. We addressed whether enhanced Gq-mediated signaling in forebrain excitatory neurons during postnatal life can evoke persistent mood-related behavioral changes. Excitatory hM3Dq DREADD-mediated chemogenetic activation of forebrain excitatory neurons during postnatal life (P2-14), but not in juvenile or adult windows, increased anxiety-, despair-, and schizophrenia-like behavior in adulthood. This was accompanied by an enhanced metabolic rate of cortical and hippocampal glutamatergic and GABAergic neurons. Furthermore, we observed reduced activity and plasticity-associated marker expression, and perturbed excitatory/inhibitory currents in the hippocampus. These results indicate that Gq-signaling-mediated activation of forebrain excitatory neurons during the critical postnatal window is sufficient to program altered mood-related behavior, as well as functional changes in forebrain glutamate and GABA systems, recapitulating aspects of the consequences of early adversity.
Elife, 2020; 9
31736725: Salvi SS, Pati S, Chaudhari PR, Tiwari P, Banerjee T, Vaidya VA
Acute Chemogenetic Activation of CamKIIα-Positive Forebrain Excitatory Neurons Regulates Anxiety-Like Behaviour in Mice. Anxiety disorders are amongst the most prevalent mental health disorders. Several lines of evidence have implicated cortical regions such as the medial prefrontal cortex, orbitofrontal cortex, and insular cortex along with the hippocampus in the top-down modulation of anxiety-like behaviour in animal models. Both rodent models of anxiety, as well as treatment with anxiolytic drugs, result in the concomitant activation of multiple forebrain regions. Here, we sought to examine the effects of chemogenetic activation or inhibition of forebrain principal neurons on anxiety and despair-like behaviour. We acutely activated or inhibited Ca/calmodulin-dependent protein kinase II α (CamKIIα)-positive forebrain excitatory neurons using the

hM3Dq or the hM4Di Designer Receptor Exclusively Activated by Designer Drug (DREADD) respectively. Circuit activation was confirmed via an increase in expression of the immediate early gene, c-Fos, within both the hippocampus and the neocortex. We then examined the influence of DREADD-mediated activation of forebrain excitatory neurons on behavioural tests for anxiety and despair-like behaviour. Our results indicate that acute hM3Dq DREADD activation of forebrain excitatory neurons resulted in a significant decline in anxiety-like behaviour on the open field, light-dark avoidance, and the elevated plus maze test. In contrast, hM3Dq DREADD activation of forebrain excitatory neurons did not alter despair-like behaviour on either the tail suspension or forced swim tests. Acute hM4Di DREADD inhibition of CamKIIα-positive forebrain excitatory neurons did not modify either anxiety or despair-like behaviour. Taken together, our results demonstrate that chemogenetic activation of excitatory neurons in the forebrain decreases anxiety-like behaviour in mice.
Front Behav Neurosci, 2019; 13
35115382: Tiwari P, Kapri D, Pradhan A, Balakrishnan A, Chaudhari PR, Vaidya VA
Chronic hM4Di-DREADD-Mediated Chemogenetic Inhibition of Forebrain Excitatory Neurons in Postnatal or Juvenile Life Does Not Alter Adult Mood-Related Behavior.
G-protein-coupled receptors (GPCRs) coupled to G signaling, in particular downstream of monoaminergic neurotransmission, are posited to play a key role during developmental epochs (postnatal and juvenile) in shaping the emergence of adult anxiodepressive behaviors and sensorimotor gating. To address the role of G signaling in these developmental windows, we used a CaMKIIα-tTA::TRE hM4Di bigenic mouse line to express the hM4Di-DREADD (designer receptor exclusively activated by designer drugs) in forebrain excitatory neurons and enhanced G signaling via chronic administration of the DREADD agonist, clozapine--oxide (CNO) in the postnatal window (postnatal days 2-14) or the juvenile window (postnatal days 28-40). We confirmed that the expression of the HA-tagged hM4Di-DREADD was restricted to CaMKIIα-positive neurons in the forebrain, and that the administration of CNO in postnatal or juvenile windows evoked inhibition in forebrain circuits of the hippocampus and cortex, as indicated by a decline in expression of the neuronal activity marker c-Fos. hM4Di-DREADD-mediated inhibition of CaMKIIα-positive forebrain excitatory neurons in postnatal or juvenile life did not impact the weight profile of mouse pups, and also did not influence the normal ontogeny of sensory reflexes. Further, postnatal or juvenile hM4Di-DREADD-mediated inhibition of CaMKIIα-positive forebrain excitatory neurons did not alter anxiety- or despair-like behaviors in adulthood and did not impact sensorimotor gating. Collectively, these results indicate that chemogenetic induction of G signaling in CaMKIIα-positive forebrain excitatory neurons in postnatal and juvenile temporal windows does not appear to impinge on the programming of anxiodepressive behaviors in adulthood.
eNeuro, 2022 Jan-Feb; 9
28867478: Chaudhari PR, Charles SE, D'Souza ZC, Vaidya MM
Hemidesmosomal linker proteins regulate cell motility, invasion and tumorigenicity in oral squamous cell carcinoma derived cells.
BPAG1e and Plectin are hemidesmosomal linker proteins which anchor intermediate filament proteins to the cell surface through β4 integrin. Recent reports indicate that these proteins play a role in various cellular processes apart from their known anchoring function. However, the available literature is inconsistent. Further, the previous study from our laboratory suggested that Keratin8/18 pair promotes cell motility and tumor progression by deregulating β4 integrin signaling in oral squamous cell carcinoma (OSCC) derived cells. Based on these findings, we hypothesized that linker proteins may have a role in neoplastic progression of OSCC. Downregulation of hemidesmosomal linker proteins in OSCC derived cells resulted in reduced cell migration accompanied by alterations in actin organization. Further, decreased MMP9 activity led to reduced cell invasion in linker proteins knockdown cells. Moreover, loss of these proteins resulted in reduced tumorigenic potential. SWATH analysis demonstrated upregulation of N-Myc downstream regulated gene 1 (NDRG1) in linker proteins downregulated cells as compared to vector control cells. Further, the defects in phenotype upon linker proteins ablation were rescued upon loss of NDRG1 in linker proteins knockdown background. These data together indicate that hemidesmosomal linker proteins regulate cell motility, invasion and tumorigenicity possibly through NDRG1 in OSCC derived cells.
Exp Cell Res, 2017; 360
30189187: Dmello C, Sawant S, Chaudhari PR, Dongre H, Ahire C, D'Souza ZC, Charles SE, Rane P, Costea DE, Chaukar D, Kane S, Vaidya M
Aberrant expression of vimentin predisposes oral premalignant lesion derived cells towards transformation.
We have previously reported the aberrant expression of vimentin in human oral premalignant lesions and a 4-Nitroquinoline 1-oxide (4NQO) model of rat lingual carcinogenesis. Hence, we wanted to understand whether the expression of vimentin in early stage contributes to the process of transformation.
Exp Mol Pathol, 2018; 105
22894535: Masurkar SA, Chaudhari PR, Shidore VB, Kamble SP
Effect of biologically synthesised silver nanoparticles on Staphylococcus aureus biofilm quenching and prevention of biofilm formation.

The development of green experimental processes for the synthesis of nanoparticles is a need in the field of nanotechnology. In the present study, the authors reported rapid synthesis of silver nanoparticles using fresh leaves extract of Cymbopogan citratus (lemongrass) with increased stability. The synthesised silver nanoparticles were found to be stable for several months. UV-visible spectrophotometric analysis was carried out to assess the synthesis of silver nanoparticles. The synthesised silver nanoparticles were further characterised by using nanoparticle tracking analyser (NTA), transmission electron microscope (TEM) and energy-dispersive x-ray spectra (EDX). The NTA results showed that the mean size was found to be 32 nm. Silver nanoparticles with controlled size and shape were observed under TEM micrograph. The EDX of the nanoparticles confirmed the presence of elemental silver. These silver nanoparticles showed enhanced quorum quenching activity against Staphylococcus aureus biofilm and prevention of biofilm formation which can be seen under inverted microscope (40X). In the near future, silver nanoparticles synthesised using green methods may be used in the treatment of infections caused by a highly antibiotic resistant biofilm.
IET Nanobiotechnol, 2012; 6

31180046: Dmello C, Srivastava SS, Tiwari R, Chaudhari PR, Sawant S, Vaidya MM
Multifaceted role of keratins in epithelial cell differentiation and transformation.
Keratins, the epithelial-predominant members of the intermediate filament superfamily, are expressed in a pairwise, tissuespecific and differentiation-dependent manner. There are 28 type I and 26 type II keratins, which share a common structure comprising a central coiled coil α-helical rod domain flanked by two nonhelical head and tail domains. These domains harbor sites for major posttranslational modifications like phosphorylation and glycosylation, which govern keratin function and dynamics. Apart from providing structural support, keratins regulate various signaling machinery involved in cell growth, motility, apoptosis etc. However, tissue-specific functions of keratins in relation to cell proliferation and differentiation are still emerging. Altered keratin expression pattern during and after malignant transformation is reported to modulate different signaling pathways involved in tumor progression in a context-dependent fashion. The current review focuses on the literature related to the role of keratins in the regulation of cell proliferation, differentiation and transformation in different types of epithelia.
J Biosci, 2019; 44

25421866: Chaudhari PR, Vaidya MM
Versatile hemidesmosomal linker proteins: structure and function.
Hemidesmosomes are anchoring junctions which connect basal epidermal cells to the extracellular matrix. In complex epithelia like skin, hemidesmosomes are composed of transmembrane proteins like α6β4 integrin, BP180, CD151 and cytoplasmic proteins like BPAG1e and plectin. BPAG1e and plectin are plakin family cytolinker proteins which anchor intermediate filament proteins i.e. keratins to the hemidesmosomal transmembrane proteins. Mutations in BPAG1e and plectin lead to severe skin blistering disorders. Recent reports indicate that these hemidesmosomal linker proteins play a role in various cellular processes like cell motility and cytoskeleton dynamics apart from their known anchoring function. In this review, we will discuss their role in structural and signaling functions.
Histol Histopathol, 2015; 30

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SPHINGOMYELIN SYNTHASES IN DEPRESSION AND ANTIDEPRESSANT TREATMENT

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Major depressive disorder is a severe and common psychiatric disease. Recent studies in mice have revealed that pharmacological inhibition of sphingomyelin synthases (SMS) induces neurogenesis and rapidly improves depression-like behaviour by activating autophagy. However, whether these effects are due to the inhibition of one or both SMS isoforms (SMS1 and SMS2) remains unknown. We aim to elucidate the involvement of SMS1 and SMS2 in depression and to identify their role in the autophagy-dependent antidepressant effect. Therefore, we have compared the activity and expression of SMS under human healthy and pathological conditions. Our first data indicate a sex-independent higher expression of SMS genes in peripheral blood cells from patients with a current major depressive episode (n=129) compared to healthy controls (n=60). In addition, an increase of SMS enzyme activity was observed in female but not male depressed patients compared to controls. To study the specific roles of SMS isoforms on autophagy, we downregulated SMS1 or SMS2 with siRNAs in H4 cells. The *in vitro* studies showed that downregulation of SMS1 (but not SMS2) leads to an accumulation of the autophagic marker LC3B-II in the presence of bafilomycin A1 compared to control cells, indicating an increased autophagic flux. Moreover, the expression of several autophagy related genes (*SQSTM1*/p62, *ULK1*, *PIK3C3*) is increased specifically after SMS1 downregulation. Overall, our results fit with the hypothesis of increased SMS activity in depression and with a specific involvement of SMS1 in autophagy induction, thereby suggesting a potential pharmacological target for the design of fast-acting antidepressants.

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BOARD NUMBER: S04-620

ANTIDEPRESSANT ACTIONS OF KETAMINE ENGAGE CELLULAR MECHANISMS OF ENDOPLASMIC RETICULUM STRESS BY THE EIF2α PATHWAY

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Lluis Miquel Rio[1,2,3], Unai Sarriés Serrano[1,2,3], Verónica Paz[1,2,3], Leticia Campa[1,2,3], Analia Bortolozzi[1,2,3]
[1]Spanish National Research Council (CSIC), Institute Of Biomedical Research Of Barcelona (iibb), Barcelona, Spain, [2]August Pi Sunyer Biomedical Research Institute (IDIBAPS), Systems Neuropharmacology, Barcelona, Spain, [3]Carlos III Institute of Health, Network Centre For Biomedical Research In Mental Health (cibersam), Madrid, Spain

Aims: Depression is a devastating mood disorder that causes profound disability worldwide. Despite the growing number of antidepressant medications available, treatment options for depression are limited. Therefore, it is imperative to understand the etiology and pathophysiology of depression to discover novel therapeutic targets of action. Here, we explore how endoplasmic reticulum (ER) stress might play an important role in the pathophysiology of depression and how the antidepressant ketamine actions involve ER pathways
Methods: We generated a mouse model of ER stress in serotonin (5-HT) neurons using the stressor tunicamycin (200 µg/µl). We examined ER/UPR pathway markers by Western blot, neuroplasticity gene expression (BDNF, TrkB, VEGF, Neuritin, PSD95, and Zif268) by in situ hybridization, 5-HT release by microdialysis, and behavioral depressive-like phenotype. Ketamine (10 mg/kg, i.p.) was used to reverse the ER stress-induced depressive mouse model.
Results: Tunicamycin-induced ER stress in 5-HT neurons left a time-dependent increase in GRP78 and CHOP protein levels. In addition, increased phosphorylation of eIF2α and eEF2 was found, suggesting activation of PERK pathway. Tunicamycin-treated mice exhibited an anxious/depressive phenotype, reduced 5-HT release in the medial prefrontal cortex, and changes in neuroplasticity gene expression in 5-HT projection areas. A single dose of ketamine reversed the depressive phenotype 30 minutes later, which is associated with reduced levels of phosphorylated eIF2α and recovery of BDNF expression.
Conclusions: The results strongly indicate that ER stress and UPR may represent cellular pathogenic mechanisms in the development of mood disorders and that eIF2α pathway is central for the antidepressant activity of ketamine.

Pubmed:
35163729: Pavia-Collado R, Rodríguez-Aller R, Alarcón-Arís D, Miquel-Rio L, Ruiz-Bronchal E, Paz V, Campa L, Galofré M, Sgambato V, Bortolozzi A
Up and Down γ-Synuclein Transcription in Dopamine Neurons Translates into Changes in Dopamine Neurotransmission and Behavioral Performance in Mice.
The synuclein family consists of α-, β-, and γ-Synuclein (α-Syn, β-Syn, and γ-Syn) expressed in the neurons and concentrated in synaptic terminals. While α-Syn is at the center of interest due to its implication in the pathogenesis of Parkinson's disease (PD) and other synucleinopathies, limited information exists on the other members. The current study aimed at investigating the biological role of γ-Syn controlling the midbrain dopamine (DA) function. We generated two different mouse models with: (i) γ-Syn overexpression induced by an adeno-associated viral vector and (ii) γ-Syn knockdown induced by a ligand-conjugated antisense oligonucleotide, in order to modify the endogenous γ-Syn transcription levels in midbrain DA neurons. The progressive overexpression of γ-Syn decreased DA neurotransmission in the nigrostriatal and mesocortical pathways. In parallel, mice evoked motor deficits in the rotarod and impaired cognitive performance as assessed by novel object recognition, passive avoidance, and Morris water maze tests. Conversely, acute γ-Syn knockdown selectively in DA neurons facilitated forebrain DA neurotransmission. Importantly, modifications in γ-Syn expression did not induce the loss of DA neurons or changes in α-Syn expression. Collectively, our data strongly suggest that DA release/re-uptake processes in the nigrostriatal and mesocortical pathways are partially dependent on substantia nigra pars compacta /ventral tegmental area (SNc/VTA) γ-Syn transcription levels, and are linked to modulation of DA transporter function, similar to α-Syn.
Int J Mol Sci, 2022; 23
35022720: Castañé A, Cano M, Ruiz-Avila L, Miquel-Rio L, Celada P, Artigas F, Riga MS
Dual 5-HT3 and 5-HT6 Receptor Antagonist FPPQ Normalizes Phencyclidine-Induced Disruption of Brain Oscillatory Activity

in Rats.
Schizophrenia is a severe mental disorder featuring psychotic, depressive, and cognitive alterations. Current antipsychotic drugs preferentially target dopamine D2-R and/or serotonergic 5-HT2A/1A-R. They partly alleviate psychotic symptoms but fail to treat negative symptoms and cognitive deficits. Here we report on the putative antipsychotic activity of (1-[(3-fluorophenyl)sulfonyl]-4-(piperazin-1-yl)-1H-pyrrolo[3,2-c]quinoline dihydrochloride) (FPPQ), a dual serotonin 5-HT3-R/5-HT6-R antagonist endowed with pro-cognitive properties. FPPQ fully reversed phencyclidine-induced decrease of low-frequency oscillations in the medial prefrontal cortex of anaesthetized rats, a fingerprint of antipsychotic activity. This effect was mimicked by the combined administration of the 5-HT3-R and 5-HT6-R antagonists ondansetron and SB-399 885, respectively, but not by either drug alone. In freely moving rats, FPPQ countered phencyclidine-induced hyperlocomotion and augmentation of gamma and high-frequency oscillations in medial prefrontal cortex, dorsal hippocampus, and nucleus accumbens. Overall, this supports that simultaneous blockade of 5-HT3R and 5-HT6-R-like that induced by FPPQ-can be a new target in antipsychotic drug development.
Int J Neuropsychopharmacol, 2022; 25
34852293: Alarcón-Arís D, Pavia-Collado R, Miquel-Rio L, Coppola-Segovia V, Ferrés-Coy A, Ruiz-Bronchal E, Galofré M, Paz V, Campa L, Revilla R, Montefeltro A, Kordower JH, Vila M, Artigas F, Bortolozzi A
Corrigendum to "Anti-α-synuclein ASO delivered to monoamine neurons prevents α-synuclein accumulation in a Parkinson's disease-like mouse model and in monkeys" [EBioMedicine 2020; 59:102944].
EBioMedicine, 2021; 74
33805843: Pavia-Collado R, Cóppola-Segovia V, Miquel-Rio L, Alarcón-Aris D, Rodríguez-Aller R, Torres-López M, Paz V, Ruiz-Bronchal E, Campa L, Artigas F, Montefeltro A, Revilla R, Bortolozzi A
Intracerebral Administration of a Ligand-ASO Conjugate Selectively Reduces α-Synuclein Accumulation in Monoamine Neurons of Double Mutant Human A30P*A53T*α-Synuclein Transgenic Mice.
α-Synuclein (α-Syn) protein is involved in the pathogenesis of Parkinson's disease (PD). Point mutations and multiplications of the α-Syn, which encodes the gene, are correlated with early-onset PD, therefore the reduction in a-Syn synthesis could be a potential therapy for PD if delivered to the key affected neurons. Several experimental strategies for PD have been developed in recent years using oligonucleotide therapeutics. However, some of them have failed or even caused neuronal toxicity. One limiting step in the success of oligonucleotide-based therapeutics is their delivery to the brain compartment, and once there, to selected neuronal populations. Previously, we developed an indatraline-conjugated antisense oligonucleotide (IND-1233-ASO), that selectively reduces α-Syn synthesis in midbrain monoamine neurons of mice, and nonhuman primates. Here, we extended these observations using a transgenic male mouse strain carrying both A30P and A53T mutant human α-Syn (A30P*A53T*α-Syn). We found that A30P*A53T*α-Syn mice at 4-5 months of age showed 3.5-fold increases in human α-Syn expression in dopamine (DA) and norepinephrine (NE) neurons of the substantia nigra pars compacta (SNc) and locus coeruleus (LC), respectively, compared with mouse α-Syn levels. In parallel, transgenic mice exhibited altered nigrostriatal DA neurotransmission, motor alterations, and an anxiety-like phenotype. Intracerebroventricular IND-1233-ASO administration (100 µg/day, 28 days) prevented the α-Syn synthesis and accumulation in the SNc and LC, and recovered DA neurotransmission, although it did not reverse the behavioral phenotype. Therefore, the present therapeutic strategy based on a conjugated ASO could be used for the selective inhibition of α-Syn expression in PD-vulnerable monoamine neurons, showing the benefit of the optimization of ASO molecules as a disease modifying therapy for PD and related α-synucleinopathies.
Int J Mol Sci, 2021; 22
33303736: Tarrés-Gatius M, Miquel-Rio L, Campa L, Artigas F, Castañé A
Involvement of NMDA receptors containing the GluN2C subunit in the psychotomimetic and antidepressant-like effects of ketamine.
Acute ketamine administration evokes rapid and sustained antidepressant effects in treatment-resistant patients. However, ketamine also produces transient perceptual disturbances similarly to those evoked by other non-competitive NMDA-R antagonists like phencyclidine (PCP). Although the brain networks involved in both ketamine actions are not fully understood, PCP and ketamine activate thalamo-cortical networks after NMDA-R blockade in GABAergic neurons of the reticular thalamic nucleus (RtN). Given the involvement of thalamo-cortical networks in processing sensory information, these networks may underlie psychotomimetic action. Since the GluN2C subunit is densely expressed in the thalamus, including the RtN, we examined the dependence of psychotomimetic and antidepressant-like actions of ketamine on the presence of GluN2C subunits, using wild-type and GluN2C knockout (GluN2CKO) mice. Likewise, since few studies have investigated ketamine's effects in females, we used mice of both sexes. GluN2C deletion dramatically reduced stereotyped (circling) behavior induced by ketamine in male and female mice, while the antidepressant-like effect was fully preserved in both genotypes and sexes. Despite ketamine appeared to induce similar effects in both sexes, some neurobiological differences were observed between male and female mice regarding c-fos expression in thalamic nuclei and cerebellum, and glutamate surge in prefrontal cortex.

In conclusion, the GluN2C subunit may discriminate between antidepressant-like and psychotomimetic actions of ketamine. Further, the abundant presence of GluN2C subunits in the cerebellum and the improved motor coordination of GluN2CKO mice after ketamine treatment suggest the involvement of cerebellar NMDA-Rs in some behavioral actions of ketamine.
Transl Psychiatry, 2020; 10

32810825: Alarcón-Arís D, Pavia-Collado R, Miquel-Rio L, Coppola-Segovia V, Ferrés-Coy A, Ruiz-Bronchal E, Galofré M, Paz V, Campa L, Revilla R, Montefeltro A, Kordower JH, Vila M, Artigas F, Bortolozzi A

Anti-α-synuclein ASO delivered to monoamine neurons prevents α-synuclein accumulation in a Parkinson's disease-like mouse model and in monkeys.

Progressive neuronal death in monoaminergic nuclei and widespread accumulation of α-synuclein are neuropathological hallmarks of Parkinson's disease (PD). Given that α-synuclein may be an early mediator of the pathological cascade that ultimately leads to neurodegeneration, decreased α-synuclein synthesis will abate neurotoxicity if delivered to the key affected neurons.
EBioMedicine, 2020; 59

FENS Forum 2022
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BOARD NUMBER: S04-621

COMBINING PRENATAL AND POSTNATAL STRESS PARADIGMS IN MICE FOR A HIGHLY TRANSLATIONAL MODEL OF DEVELOPMENTAL STRESS EXPOSURE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Sowmya Narayan
Max Planck Institue for Psychiatry, Research Group Neurobiology Of Stress Resilience, Munich, Germany

Early life adversity leads to a higher risk of mood disorders in adulthood, and a growing body of research also implicates the fetal environment's crucial impact on health outcome; however, the biological mechanisms underlying the susceptibility to these disorders due to developmental stress are not yet fully understood. These can be characterized through behavioral assessment and transcriptional profiling in the adult brain following developmental stress exposure. Here we begin this endeavor by assessing the complex social and anxiety-related behavior in adult mice that were exposed to developmental stress. Developmental stress was modeled by a combination of maternal corticosterone (CORT) injections during late pregnancy and a limited nesting and bedding paradigm (ELS) during postnatal days 2-9. Behavioral testing of both sexes in early adulthood (2 months old) revealed higher prevalence of anxious behavior than controls during individual and social assessments, and increased likelihood of taking on a subordinate role in a semi-naturalistic social setting. Furthermore, these phenotypes are present at a more elevated degree in combined treatment CORT+ELS mice than mice that experienced only the prenatal or only the postnatal stressor. As this combination of stress treatments in the mouse is translationally relevant to stress exposure during the human gestational and neurodevelopmental period, we intend to use this model to study the transcriptional response to stress across brain regions moving forward. Through a full characterization of the developmental stress transcription profile in males and females, we hope to unveil more possible targets for early interventions in mood disorders like anxiety and depression.

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BOARD NUMBER: S04-622

ALTERED VENTRAL TEGMENTAL DOPAMINERGIC ACTIVITY IN CHRONIC PAIN INDUCED-DEPRESSION

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Pierre-Alexis Derrien[1], Robin Waegaert[2], Quentin Leboulleux[2], Mélanie Kremer[2], Ipek Yalcin[2], Michel Barrot[1], Jennifer Kaufling[1]
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Neuropathic pain is a chronic pathological condition caused by an injury or disease of the somatosensory system. Almost 30% of patients with chronic pain develop a major depressive disorder (MDD) during their lifespan. The dopaminergic (DA) neurons of the ventral tegmental area (VTA), part of the mesolimbic DA system, are involved in a variety of functions, such as motivation and reward; hence, alterations in this system have been reported in chronic pain and MDD. The lateral habenula (LHb), another key region in mood regulation sends projections to the VTA with a minor direct excitatory and a major indirect inhibitory pathway. Our hypothesis is that VTA DA neurons and more particularly the ones receiving inputs from the LHb can be altered in the comorbidity of chronic pain and MDD. By performing single-cell recording of optogenetically identified VTA DA neurons in anesthetized mice, we studied the basal activity of VTA DA neurons and their response to electrical stimulation of the LHb in a mouse model of neuropathic pain-induced depressive-like behaviors. Our data demonstrated that the tonic activity of VTA DA neurons is increased in animals displaying chronic pain and depressive-like behaviours. Moreover, this increased activity is observed only in VTA DA neurons located contralateral to the paw where the peripheral nerve injury was performed. Finally, the proportion of inhibited neurons by LHb stimulation seems to decrease in neuropathic versus control mice.

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BOARD NUMBER: S04-623

THE ROLE OF MICROGLIA AND THE SPHINGOSINE-1-PHOSPHATE PATHWAY IN NEUROINFLAMMATION. RESULTS OF A PRECLINICAL MODEL OF PERIODONTITIS AND DEPRESSION.

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Javier Robledo-Montaña[1], Marina Muñoz-López[1], María Martinez[2], Leire Virto[2], David Martín-Hernández[1], Juan Carlos Leza[1], Elena Figuero[2], Borja García-Bueno[1]
[1]Universidad Complutense de Madrid UCM, Farmacología Y Toxicología, Madrid, Spain, [2]Universidad Complutense de Madrid UCM, Etep (etiology And Therapy Of Periodontal And Peri-implant Diseases) Research Group (ucm), Madrid, Spain

Background. Depression is a chronic psychiatric disease of multifactorial etiology whose complete pathophysiology understanding remains elusive. Stress and comorbidity with other inflammatory pathologies are risk factors of psychiatric diseases. Epidemiological evidence points out that periodontitis, as a source of low-grade chronic inflammation, could be associated with depression, but the underlying mechanisms have not been fully elucidated yet. **Aims.** We developed a combined model of periodontitis and Chronic Mild Stress (CMS) -previously described in Martinez et al., 2019 - to study the role of the sphingosine-1 phosphate (S1P) pathway in the microglial activation through the S1PR3 receptor during the neuroinflammatory response. **Methods.** Quantitative and morphological studies of microglia in prefrontal cortex slices stained by fluorescence immunohistochemistry against the ionized calcium-binding adapter molecule 1 (IBA1) as well as biochemical analysis via western blotting and RT-qPCR were performed. **Results.** In this study, we observed a significant increase in the microglial population across the prefrontal cortex in the periodontitis, CMS, and the combined model groups compared to control. Moreover, there was a change in their morphology and size that can be related to their reactivity status. S1PR3 receptor expression in the frontal cortex increased after CMS exposure and could modulate the activation of microglia during neuroinflammation and lead to the liberation of pro-inflammatory cytokines. **Conclusion.** Thus, our results indicate that the model causes an increase in the number of microglia cells and a shift in their activation state that could leads to neuroinflammation throught overexpresion of S1PR3.

Pubmed:
31906991: Vargas-Caraveo A, Sayd A, Robledo-Montaña J, Caso JR, Madrigal JLM, García-Bueno B, Leza JC
Toll-like receptor 4 agonist and antagonist lipopolysaccharides modify innate immune response in rat brain circumventricular organs.
The circumventricular organs (CVOs) are blood-brain-barrier missing structures whose activation through lipopolysaccharide (LPS) is a starting point for TLR-driven (Toll-like receptors) neuroinflammation. The aim of this study was to evaluate in the CVO area postrema (AP), subfornical organ (SFO), and median eminence (ME), the inflammatory response to two TLR4 agonists: LPS from Escherichia coli (EC-LPS), the strongest endotoxin molecule described, and LPS from Porphyromonas gingivalis (PG-LPS), a pathogenic bacteria present in the periodontium related to neuroinflammation in neurodegenerative/psychiatric diseases. The response to LPS from the cyanobacteria Rhodobacter sphaeroides (RS-LPS), a TLR4 antagonist with an interesting anti-inflammatory potential, was also assessed.
J Neuroinflammation, 2020; 17
32245674: Sayd A, Vargas-Caraveo A, Perea-Romero I, Robledo-Montaña J, Caso JR, Madrigal JLM, Leza JC, Orio L, Garcia-Bueno B
Depletion of brain perivascular macrophages regulates acute restraint stress-induced neuroinflammation and oxidative/nitrosative stress in rat frontal cortex.
The central nervous system can respond to peripheral immune stimuli through the activation of the neurovascular unit. One of the cellular types implicated are perivascular macrophages (PVMs), hematopoietic-derived brain-resident cells located in the perivascular space. PVMs have been implicated in the immune surveillance and in the regulation of the accumulation/trafficking of macromolecules in brain-blood interfaces. Recent studies suggested that the role of PVMs could vary depending on the nature and duration of the immune challenge applied. Here, we investigate the role of PVMs in stress-induced neuroinflammation and oxidative/nitrosative consequences. The basal phagocytic activity of PVMs was exploited to selectively deplete them by ICV injection of liposomes encapsulating the pro-apoptotic drug clodronate. Acute restraint stress-induced neuroinflammation and oxidative/nitrosative stress in rat brain frontal cortex samples were assessed by western blot

and RT-PCR analyses. The depletion of PVMs: (1) decreased tumor necrosis-α levels (2) prevented the Janus kinase/signal transducers and activators of transcription pathway and increased interleukin-6 receptor protein-expression in stress conditions; (3) prevented the stress-induced Toll-like receptor 4/Myeloid differentiation primary response 88 protein signaling pathway; (4) down-regulated the pro-inflammatory nuclear factor κB/cyclooxygenase-2 pathway; (5) prevented stress-induced lipid peroxidation and the concomitant increase of the endogenous antioxidant mediators nuclear factor (erythroid-derived 2)-like 2, glutathione reductase 1 and Parkinsonism-associated deglycase mRNA expression. Our results point to PVMs as regulators of stress-induced neuroinflammation and oxidative/nitrosative stress. Much more scientific effort is still needed to evaluate whether their selective manipulation is promising as a therapeutic strategy for the treatment of stress-related neuropsychopathologies.
Eur Neuropsychopharmacol, 2020; 34

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BOARD NUMBER: S04-624

THE BLOOD-BRAIN BARRIER PERMEABILITY IN A PRECLINICAL IN VIVO MODEL OF PERIODONTITIS AND DEPRESSION: A STEP FORWARD IN THE UNDERSTANDING OF INFLAMMATORY MECHANISMS

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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[1]Universidad Complutense de Madrid (UCM), Farmacología Y Toxicología, MADRID, Spain, [2]Universidad Complutense de Madrid (UCM), Etep (etiology And Therapy Of Periodontal And Peri-implant Diseases) Research Group (ucm), Universidad Complutense De Madrid Ucm., MADRID, Spain

Background. Depression is a mental illness in which inflammation may play an important role. On the other hand, periodontitis is an inflammatory gum disease caused by an oral multispecies bacterial chronic infection. Despite many underlying aspects remaining unknown, some studies suggest comorbidity between these diseases and point to chronic inflammation as the common denominator. **Aims.** To unravel the potential mechanisms contributing to both pathologies in the frontal cortex of rats. **Methods.** We used a preclinical *in vivo* model of periodontitis and depression (Perio+CMS) (Martínez et al., 2021). The expression of proteins related to the permeability of the blood-brain barrier (BBB) and the sphingosine-1-phosphate (S1P) pathway was analyzed by Western Blot, RT-qPCR, and ELISA techniques. **Results.** The Perio+CMS model downregulated the expression of S1P receptor 1 (S1PR1), which could be involved through RAC1/2/3 signaling in the development and differentiation of endothelial cells to maintain the integrity of the BBB. Moreover, there was a decrease in the expression of some structural proteins (ZO-1, occludin) and an increase in molecules involved in immune trafficking (cell and leukocyte adhesion molecules - ICAM-1, VCAM-1 -, and matrix metalloproteinase 9 - MMP9). **Conclusions.** Thus, the described changes could indicate and increase in the BBB permeability, which may be associated with the induction and promotion of neuroinflammation. These processes could be relevant to the pathophysiology and comorbidity of periodontitis and depression. Moreover, they lay the ground for a better understanding of the interplay between chronic inflammatory diseases and the central nervous system.

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VASCULAR AND BLOOD-BRAIN BARRIER-RELATED CHANGES UNDERLIE STRESS RESPONSES AND RESILIENCE IN FEMALE MICE AND DEPRESSION IN HUMAN TISSUE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Laurence Dion-Albert[1], Alice Cadoret[1], Ellen Doney[1], Fernanda Kaufmann[1], Katarzyna Dudek[1], Béatrice Daigle[1], Lyonna Parise[2], Flurin Cathomas[2], Nalia Samba[3], Nathalie Hudson[4], Manon Lebel[1], Signature Consortium[5], Matthew Campbell[4], Gustavo Turecki[6], Naguib Mechawar[6], Caroline Ménard[1]
[1]CERVO Brain research center, Psychiatry And Neurosciences, Quebec, Canada, [2]Icahn School of Medicine at Mount Sinai, Nash Family Department Of Neuroscience And Friedman Brain Institute, New York, United States of America, [3]Sorbonne Université Paris, Biologie Intégrative Et Physiologie, Paris, France, [4]Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland, [5]Institut universitaire en santé mentale de Montréal, Centre Intégré Universitaire De Santé Et Service Sociaux Est, Montréal, Canada, [6]McGill University, Department Of Psychiatry, Douglas Institute, Montreal, Canada

Aims: Prevalence, symptoms, and treatment of depression suggest that major depressive disorders (MDD) present sex differences. Social stress-induced neurovascular pathology is associated with depressive symptoms in male mice; however, this association is unclear in females. **Methods**: Transcriptional profiling of genes associated with vascular integrity, permeability, angiogenesis, tight junctions, and blood-brain barrier (BBB) formation was performed in the brain of female mice following the 10-day chronic social defeat paradigm. Functional studies using adeno-associated viruses were conducted to confirm the causal role of the BBB in the establishment maladaptive stress behaviors. Endothelium transcriptomic and peripheral blood profiles were established to gain mechanistic insights and uncover novel biomarkers of MDD. **Results**: Chronic social stress promotes BBB alterations in the prefrontal cortex (PFC) of female mice. Targeted disruption of the BBB in this brain region induces anxiety- and depression-like behaviours. By comparing the endothelium cell-specific transcriptomic profiling of the mouse male and female PFC, we identify several pathways and genes involved in maladaptive stress responses and resilience to stress. Furthermore, we confirm that the BBB in the PFC of stressed female mice is leaky. Then, we identify circulating vascular biomarkers of chronic stress, such as soluble E-selectin. Similar changes in circulating soluble E-selectin, BBB gene expression and morphology can be found in blood serum and *postmortem* brain samples from women diagnosed with MDD. **Conclusions**: Altogether, we propose that BBB dysfunction plays an important role in modulating stress responses in female mice and possibly MDD.

Pubmed:
35013188: Dion-Albert L, Cadoret A, Doney E, Kaufmann FN, Dudek KA, Daigle B, Parise LF, Cathomas F, Samba N, Hudson N, Lebel M, , Campbell M, Turecki G, Mechawar N, Menard C
Vascular and blood-brain barrier-related changes underlie stress responses and resilience in female mice and depression in human tissue.
Prevalence, symptoms, and treatment of depression suggest that major depressive disorders (MDD) present sex differences. Social stress-induced neurovascular pathology is associated with depressive symptoms in male mice; however, this association is unclear in females. Here, we report that chronic social and subchronic variable stress promotes blood-brain barrier (BBB) alterations in mood-related brain regions of female mice. Targeted disruption of the BBB in the female prefrontal cortex (PFC) induces anxiety- and depression-like behaviours. By comparing the endothelium cell-specific transcriptomic profiling of the mouse male and female PFC, we identify several pathways and genes involved in maladaptive stress responses and resilience to stress. Furthermore, we confirm that the BBB in the PFC of stressed female mice is leaky. Then, we identify circulating vascular biomarkers of chronic stress, such as soluble E-selectin. Similar changes in circulating soluble E-selectin, BBB gene expression and morphology can be found in blood serum and postmortem brain samples from women diagnosed with MDD. Altogether, we propose that BBB dysfunction plays an important role in modulating stress responses in female mice and possibly MDD.
Nat Commun, 2022; 13
33876886: Doney E, Cadoret A, Dion-Albert L, Lebel M, Menard C
Inflammation-driven brain and gut barrier dysfunction in stress and mood disorders.
Regulation of emotions is generally associated exclusively with the brain. However, there is evidence that peripheral systems

are also involved in mood, stress vulnerability vs. resilience, and emotion-related memory encoding. Prevalence of stress and mood disorders such as major depression, bipolar disorder, and post-traumatic stress disorder is increasing in our modern societies. Unfortunately, 30%-50% of individuals respond poorly to currently available treatments highlighting the need to further investigate emotion-related biology to gain mechanistic insights that could lead to innovative therapies. Here, we provide an overview of inflammation-related mechanisms involved in mood regulation and stress responses discovered using animal models. If clinical studies are available, we discuss translational value of these findings including limitations. Neuroimmune mechanisms of depression and maladaptive stress responses have been receiving increasing attention, and thus, the first part is centered on inflammation and dysregulation of brain and circulating cytokines in stress and mood disorders. Next, recent studies supporting a role for inflammation-driven leakiness of the blood-brain and gut barriers in emotion regulation and mood are highlighted. Stress-induced exacerbated inflammation fragilizes these barriers which become hyperpermeable through loss of integrity and altered biology. At the gut level, this could be associated with dysbiosis, an imbalance in microbial communities, and alteration of the gut-brain axis which is central to production of mood-related neurotransmitter serotonin. Novel therapeutic approaches such as anti-inflammatory drugs, the fast-acting antidepressant ketamine, and probiotics could directly act on the mechanisms described here improving mood disorder-associated symptomatology. Discovery of biomarkers has been a challenging quest in psychiatry, and we end by listing promising targets worth further investigation.
Eur J Neurosci, 2022; 55

31974313: Dudek KA, Dion-Albert L, Lebel M, LeClair K, Labrecque S, Tuck E, Ferrer Perez C, Golden SA, Tamminga C, Turecki G, Mechawar N, Russo SJ, Menard C
Molecular adaptations of the blood-brain barrier promote stress resilience vs. depression.
Preclinical and clinical studies suggest that inflammation and vascular dysfunction contribute to the pathogenesis of major depressive disorder (MDD). Chronic social stress alters blood-brain barrier (BBB) integrity through loss of tight junction protein claudin-5 (cldn5) in male mice, promoting passage of circulating proinflammatory cytokines and depression-like behaviors. This effect is prominent within the nucleus accumbens, a brain region associated with mood regulation; however, the mechanisms involved are unclear. Moreover, compensatory responses leading to proper behavioral strategies and active resilience are unknown. Here we identify active molecular changes within the BBB associated with stress resilience that might serve a protective role for the neurovasculature. We also confirm the relevance of such changes to human depression and antidepressant treatment. We show that permissive epigenetic regulation of expression and low endothelium expression of repressive cldn5-related transcription factor are associated with stress resilience. Region- and endothelial cell-specific whole transcriptomic analyses revealed molecular signatures associated with stress vulnerability vs. resilience. We identified proinflammatory TNFα/NFκB signaling and as mediators of stress susceptibility. Pharmacological inhibition of stress-induced increase in hdac1 activity rescued expression in the NAc and promoted resilience. Importantly, we confirmed changes in expression in the NAc of depressed patients without antidepressant treatment in line with CLDN5 loss. Conversely, many of these deleterious -related molecular changes were reduced in postmortem NAc from antidepressant-treated subjects. These findings reinforce the importance of considering stress-induced neurovascular pathology in depression and provide therapeutic targets to treat this mood disorder and promote resilience.
Proc Natl Acad Sci U S A, 2020; 117

31421056: Dudek KA, Dion-Albert L, Kaufmann FN, Tuck E, Lebel M, Menard C
Neurobiology of resilience in depression: immune and vascular insights from human and animal studies.
Major depressive disorder (MDD) is a chronic and recurrent psychiatric condition characterized by depressed mood, social isolation and anhedonia. It will affect 20% of individuals with considerable economic impacts. Unfortunately, 30-50% of depressed individuals are resistant to current antidepressant treatments. MDD is twice as prevalent in women and associated symptoms are different. Depression's main environmental risk factor is chronic stress, and women report higher levels of stress in daily life. However, not every stressed individual becomes depressed, highlighting the need to identify biological determinants of stress vulnerability but also resilience. Based on a reverse translational approach, rodent models of depression were developed to study the mechanisms underlying susceptibility vs resilience. Indeed, a subpopulation of animals can display coping mechanisms and a set of biological alterations leading to stress resilience. The aetiology of MDD is multifactorial and involves several physiological systems. Exacerbation of endocrine and immune responses from both innate and adaptive systems are observed in depressed individuals and mice exhibiting depression-like behaviours. Increasing attention has been given to neurovascular health since higher prevalence of cardiovascular diseases is found in MDD patients and inflammatory conditions are associated with depression, treatment resistance and relapse. Here, we provide an overview of endocrine, immune and vascular changes associated with stress vulnerability vs. resilience in rodents and when available, in humans. Lack of treatment efficacy suggests that neuron-centric treatments do not address important causal biological factors and better understanding of stress-induced adaptations, including sex differences, could contribute

to develop novel therapeutic strategies including personalized medicine approaches.
Eur J Neurosci, 2021; 53

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BOARD NUMBER: S04-626

INVESTIGATING THE EFFECTS OF AYURVEDIC ANTI-DEPRESSANT DRUG (NARDOSTACHYS JATAMANSI DC.) COMPLEMENTING ALLOPATHIC MEDICATION IN PATIENTS WITH MAJOR DEPRESSIVE DISORDER(MDD) - A DOUBLE-BLIND STUDY

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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BACKGROUND Depression is among the most common mental disorders and Treatment-Resistant Depression (TRD) is also prevalent. *Nardostachys Jatamansi* DC is a promising drug that is present in several Ayurvedic formulations for the complementary treatment of TRD. Investigating the variations in pathophysiological deformities in depression with the administration of the *Ayurvedic* drug by utilizing available neurophysiologic techniques will lead to a better understanding of the disease and its possible management with reduced side effects. **AIM** To evaluate the efficacy of Ayurvedic drug (*Nardostachys Jatamansi* DC.) in treating MDD on clinical improvement assessed by depression scores, cardiac autonomic functions measured by heart rate variability (HRV). **METHODS** In this randomized controlled double-blind study, patients of MDD being treated with Escitalopram were recruited into two groups and given either *Nardostachys Jatamansi* DC. powder or placebo as add-on therapy for 8 weeks. The assessment is based on clinical rating scales and cardiac autonomic functions measured by HRV. **RESULTS** 21 patients have been recruited till now with the majority showing an overall reduction of all rating scores after the completion of treatment. There is an increase in HRV parameters (SDNN, RMSSD, PNN50, Total power & HF nu) and a decrease in LF nu and LF/HF ratio. The unblinding will be done at the end of the study. **CONCLUSION** Cardiac autonomic dysfunction has been observed in depression and our preliminary findings support the same. After unblinding the groups, results may show the possible pathophysiology of the Ayurvedic drug in modulating HRV parameters in the effective management of MDD.

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BOARD NUMBER: S04-627

DOES PHYSICAL EXERCISE OFFER RESILIENCE IN A SOCIAL DEFEAT STRESS MOUSE MODEL?

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Introduction: Several studies support the adoption of physical exercise (PE) as a non-pharmacological approach in depression and anxiety. However, the mechanism underlying the beneficial impact of PE remains poorly studied. **Aims:** To evaluate whether PE provides resilience in a Chronic Social Defeat Stress (CSDS) mice model. **Material & Methods**: Young-adult male C57BL/6 mice (7-8 weeks-old) were used, and four different groups were analyzed: (1) CSDS, (2) PE+CSDS, (3) PE, (4) Control. CSDS mice were subjected to bouts of social defeat by aggressive male CD-1 mice (10 min/day; 10 days). PE+CSDS mice group were subjected to a treadmill PE program prior to CSDS protocol. Mice emotional status (anxiety and depression-like behavior) was analyzed between 24 and 96h after CSDS protocol. Data are presented as mean ± standard deviation (SD). Groups (N=6-28) were compared using ANOVA or Kruskal-Wallis followed by post-hoc multiple comparison tests. Significance was assigned to differences with a $p<0.5$. **Results**: CSDS and PE+CSDS mice group showed social avoidance behavior to CD1. A cluster analysis revealed that a subset of CSDS mice showed a decrease in entries and in time spent in the open arms in the Elevated Plus Maze test. PE did not prevent the observed emotional changes. Other behaviors including exploratory, self-care and anhedonia were not changed by chronic stress and by PE. **Conclusions:** CSDS model induced social avoidance and changes in anxiety-like but not in depression-like behavior. However, PE failed to prevent this CSDS phenotype. **Funded by FCT POCI-01-0145-FEDER-030786**

Pubmed:
33430399: Soares E, Reis J, Rodrigues M, Ribeiro CF, Pereira FC
Circulating Extracellular Vesicles: The Missing Link between Physical Exercise and Depression Management?
Depression is associated with an increased risk of aging-related diseases. It is also seemingly a common psychological reaction to pandemic outbreaks with forced quarantines and lockdowns. Thus, depression represents, now more than ever, a major global health burden with therapeutic management challenges. Clinical data highlights that physical exercise is gaining momentum as a non-pharmacological intervention in depressive disorders. Although it may contribute to the reduction of systemic inflammation associated with depression, the mechanisms underlying the beneficial physical exercise effects in emotional behavior remain to be elucidated. Current investigations indicate that a rapid release of extracellular vesicles into the circulation might be the signaling mediators of systemic adaptations to physical exercise. These biological entities are now well-established intercellular communicators, playing a major role in relevant physiological and pathophysiological functions, including brain cell-cell communication. We also reviewed emerging evidence correlating depression with modified circulating extracellular vesicle surfaces and cargo signatures (e.g., microRNAs and proteins), envisioned as potential biomarkers for diagnosis, efficient disease stratification and appropriate therapeutic management. Accordingly, the clinical data summarized in the present review prompted us to hypothesize that physical exercise-related circulating extracellular vesicles contribute to its antidepressant effects, particularly through the modulation of inflammation. This review sheds light on the triad "physical exercise-extracellular vesicles-depression" and suggests new avenues in this novel emerging field.
Int J Mol Sci, 2021; 22
29589064: Milner J, Cunha A, Gamboa-Cruz C, Reis J, Campos M, António N
Recent major advances in cardiovascular pharmacotherapy.
The field of cardiovascular pharmacotherapy remains extremely active. The aim of this review is to summarize the recent major advances in cardiovascular pharmacotherapy, with a focus on (1) the new approved drug for treatment of heart failure with reduced ejection fraction-sacubitril/valsartan; (2) proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors; (3) the novel reversal agents for non-vitamin K oral anticoagulants (NOACs); and finally, (4) new evidence on pharmacological treatment of coronary artery disease.
Eur J Clin Pharmacol, 2018; 74

33584173: Nicolucci C, Pais ML, Santos AC, Ribeiro FM, Encarnação PMCC, Silva ALM, Castro IF, Correia PMM, Veloso JFCA, Reis J, Lopes MZ, Botelho MF, Pereira FC, Priolli DG
Single Low Dose of Cocaine-Structural Brain Injury Without Metabolic and Behavioral Changes.
Chronic cocaine use has been shown to lead to neurotoxicity in rodents and humans, being associated with high morbidity and mortality rates. However, recreational use, which may lead to addictive behavior, is often neglected. This occurs, in part, due to the belief that exposure to low doses of cocaine comes with no brain damage risk. Cocaine addicts have shown glucose metabolism changes related to dopamine brain activity and reduced volume of striatal gray matter. This work aims to evaluate the morphological brain changes underlying metabolic and locomotor behavioral outcome, in response to a single low dose of cocaine in a pre-clinical study. In this context, a Balb-c mouse model has been chosen, and animals were injected with a single dose of cocaine (0.5 mg/kg). Control animals were injected with saline. A behavioral test, positron emission tomography (PET) imaging, and anatomopathological studies were conducted with this low dose of cocaine, to study functional, metabolic, and morphological brain changes, respectively. Animals exposed to this cocaine dose showed similar open field activity and brain metabolic activity as compared with controls. However, histological analysis showed alterations in the prefrontal cortex and of mice exposed to cocaine. For the first time, it has been demonstrated that a single low dose of cocaine, which can cause no locomotor behavioral and brain metabolic changes, can induce structural damage. These brain changes must always be considered regardless of the dosage used. It is essential to alert the population even against the consumption of low doses of cocaine.
Front Neurosci, 2020; 14

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-628

SLEEP DISTURBANCE AND CHANGES IN OSCILLATORY ACTIVITY IN A MOUSE MODEL OF DEPRESSION: EFFECTS OF SLEEP DEPRIVATION, KETAMINE AND CIRCADIAN CLOCK MODULATION.

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Introduction Sleep disturbances are a critical feature of affective disorders with implications during treatment. The chronic despair mouse (CDM) is a recently established model in which repetitive forced swim stress (FST) produces long-lasting anhedonia- and despair-like phenotypes. In the current pilot study, sleep architecture and oscillatory activity was assessed in the CDM model, compared to non-depressive controls and following anti-depressant treatments (sleep deprivation, ketamine and circardian clockwork modulation via a ROR-α/γ agonist). Materials and methods C57BL/6 mice were surgically implanted with electrocorticography (ECoG) and electromyography (EMG) electrodes in order to assess sleep, plus a deep electrode in the medial pre-frontal cortex (mPFC). 48h recordings were conducted before and after the CDM protocol (five consecutive days 10-minute FST), then before and after 6h sleep deprivation (from light cycle onset), ROR agonist SR1078 (10mg/kg), ketamine (3mg/kg) or vehicle. Sleep assessment and spectral analysis were performed. Results CDM mice exhibited more fragmented sleep, with an increase in slow wave activity (SWA) during slow wave sleep (SWS) and a reduction in theta band oscillations during REM sleep. Sleep deprivation increased REM sleep, reduced SWS fragmentation and increased SWA immediately following treatment. Ketamine produced a sustained increase in mPFC SWA, while all treatments suppressed light cycle gamma-band activity in the mPFC. Conclusions CDM mice showed fragmented sleep, but without REM sleep changes typical of affective disorders. Suppression of gamma activity in the mPFC after all treatments suggests a potential common mechanism or marker of anti-depressant response

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-629

CYTOSKELETON REGULATION AS POSSIBLE CRITICAL HUB OF LITHIUM RESPONSE IN PATIENTS WITH BIPOLAR DISORDER

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Lithium is the first-line treatment for bipolar disorder (BD). However, only 30% of patients respond satisfactorily to this medication. Therefore, the aim of this study is to find biomarkers that help predict clinical response to lithium in BD patients. Olfactory neural progenitors (ONP), cells with a neuronal linage, from patients who respond to lithium (LR), or not (NR) and healthy controls (HC) were cultured with or without lithium. Blood samples (PBMCs) extracted from these patients were used to search for peripheral biomarkers. Proteomic analysis obtained from both ONP and PBMCs showed that PKA signalling pathway was downregulated in cells derived from LR patients when compared to HC, but it was upregulated in LNR. In vitro lithium treatment restored PKA signalling pathway, but exclusively in cells derived from LR patients. PKA signalling pathway regulates cytoskeleton organization and indeed, 45 cytoskeleton-related proteins changed in NR. Moreover, ONP cells from LR and NR patients showed less points of adhesion to the substrate, that it was restored after lithium treatment. ONP cells from NR patients showed an increase in the cell size and morphologic alterations that were not restored by lithium. Changes in cytoskeleton proteins related to PKA signalling pathway and cell size/morphology could serve as differential biomarkers to predict the clinical response to lithium in BD.

Pubmed:
35164940: Suárez-Pereira I, Llorca-Torralba M, Bravo L, Camarena-Delgado C, Soriano-Mas C, Berrocoso E
The Role of the Locus Coeruleus in Pain and Associated Stress-Related Disorders.
The locus coeruleus (LC)-noradrenergic system is the main source of noradrenaline in the central nervous system and is involved intensively in modulating pain and stress-related disorders (e.g., major depressive disorder and anxiety) and in their comorbidity. However, the mechanisms involving the LC that underlie these effects have not been fully elucidated, in part owing to the technical difficulties inherent in exploring such a tiny nucleus. However, novel research tools are now available that have helped redefine the LC system, moving away from the traditional view of LC as a homogeneous structure that exerts a uniform influence on neural activity. Indeed, innovative techniques such as DREADDs (designer receptors exclusively activated by designer drugs) and optogenetics have demonstrated the functional heterogeneity of LC, and novel magnetic resonance imaging applications combined with pupillometry have opened the way to evaluate LC activity in vivo. This review aims to bring together the data available on the efferent activity of the LC-noradrenergic system in relation to pain and its comorbidity with anxiodepressive disorders. Acute pain triggers a robust LC stress response, producing spinal cord-

mediated endogenous analgesia while promoting aversion, vigilance, and threat detection through its ascending efferents. However, this protective biological system fails in chronic pain, and LC activity produces pain facilitation, anxiety, increased aversive memory, and behavioral despair, acting at the medulla, prefrontal cortex, and amygdala levels. Thus, the activation/deactivation of specific LC projections contributes to different behavioral outcomes in the shift from acute to chronic pain.
Biol Psychiatry, 2022; 91
34373893: Llorca-Torralba M, Camarena-Delgado C, Suárez-Pereira I, Bravo L, Mariscal P, Garcia-Partida JA, López-Martín C, Wei H, Pertovaara A, Mico JA, Berrocoso E
Pain and depression comorbidity causes asymmetric plasticity in the locus coeruleus neurons.
There is strong comorbidity between chronic pain and depression, although the neural circuits and mechanisms underlying this association remain unclear. By combining immunohistochemistry, tracing studies and western blotting, with the use of different DREADDS (designer receptor exclusively activated by designer drugs) and behavioural approaches in a rat model of neuropathic pain (chronic constriction injury), we explore how this comorbidity arises. To this end, we evaluated the time-dependent plasticity of noradrenergic locus coeruleus neurons relative to the site of injury: ipsilateral (LCipsi) or contralateral (LCcontra) locus coeruleus at three different time points: short (2 days), mid (7 days) and long term (30-35 days from nerve injury). Nerve injury led to sensorial hypersensitivity from the onset of injury, whereas depressive-like behaviour was only evident following long-term pain. Global chemogenetic blockade of the LCipsi system alone increased short-term pain sensitivity while the blockade of the LCipsi or LCcontra relieved pain-induced depression. The asymmetric contribution of locus coeruleus modules was also evident as neuropathy develops. Hence, chemogenetic blockade of the LCipsi→spinal cord projection, increased pain-related behaviours in the short term. However, this lateralized circuit is not universal as the bilateral chemogenetic inactivation of the locus coeruleus-rostral anterior cingulate cortex pathway or the intra-rostral anterior cingulate cortex antagonism of alpha1- and alpha2-adrenoreceptors reversed long-term pain-induced depression. Furthermore, chemogenetic locus coeruleus to spinal cord activation, mainly through LCipsi, reduced sensorial hypersensitivity irrespective of the time post-injury. Our results indicate that asymmetric activation of specific locus coeruleus modules promotes early restorative analgesia, as well as late depressive-like behaviour in chronic pain and depression comorbidity.
Brain, 2022; 145
35025190: Camarena-Delgado C, Llorca-Torralba M, Suárez-Pereira I, Bravo L, López-Martín C, Garcia-Partida JA, Mico JA, Berrocoso E
Nerve injury induces transient locus coeruleus activation over time: role of the locus coeruleus-dorsal reticular nucleus pathway.
The transition from acute to chronic pain results in maladaptive brain remodeling, as characterized by sensorial hypersensitivity and the ensuing appearance of emotional disorders. Using the chronic constriction injury of the sciatic nerve as a model of neuropathic pain in male Sprague-Dawley rats, we identified time-dependent plasticity of locus coeruleus (LC) neurons related to the site of injury, ipsilateral (LCipsi) or contralateral (LCcontra) to the lesion, hypothesizing that the LC→dorsal reticular nucleus (DRt) pathway is involved in the pathological nociception associated with chronic pain. LCipsi inactivation with lidocaine increased cold allodynia 2 days after nerve injury but not later. However, similar blockade of LCcontra reduced cold allodynia 7 and 30 days after inducing neuropathy but not earlier. Furthermore, lidocaine blockade of the LCipsi or LCcontra reversed pain-induced depression 30 days after neuropathy. Long-term pain enhances phosphorylated cAMP-response element binding protein expression in the DRtcontra but not in the DRtipsi. Moreover, inactivation of the LCcontra→DRtcontra pathway using dual viral-mediated gene transfer of designer receptor exclusively activated by designer drugs produced consistent analgesia in evoked and spontaneous pain 30 days postinjury. This analgesia was similar to that produced by spinal activation of α2-adrenoreceptors. Furthermore, chemogenetic inactivation of the LCcontra→DRtcontra pathway induced depressive-like behaviour in naïve animals, but it did not modify long-term pain-induced depression. Overall, nerve damage activates the LCipsi, which temporally dampens the neuropathic phenotype. However, the ensuing activation of a LCcontra→DRtcontra facilitatory pain projection contributes to chronic pain, whereas global bilateral LC activation contributes to associated depressive-like phenotype.
Pain, 2022; 163
33237429: Delgado-Sequera A, Hidalgo-Figueroa M, Barrera-Conde M, Duran-Ruiz MC, Castro C, Fernández-Avilés C, de la Torre R, Sánchez-Gomar I, Pérez V, Geribaldi-Doldán N, Robledo P, Berrocoso E
Olfactory Neuroepithelium Cells from Cannabis Users Display Alterations to the Cytoskeleton and to Markers of Adhesion, Proliferation and Apoptosis.
Cannabis is the third most commonly used psychoactive substance of abuse, yet it also receives considerable attention as a potential therapeutic drug. Therefore, it is essential to fully understand the actions of cannabis in the human brain. The olfactory neuroepithelium (ON) is a peripheral nervous tissue that represents an interesting surrogate model to study the

effects of drugs in the brain, since it is closely related to the central nervous system, and sensory olfactory neurons are continually regenerated from populations of stem/progenitor cells that undergo neurogenesis throughout life. In this study, we used ON cells from chronic cannabis users and healthy control subjects to assess alterations in relevant cellular processes, and to identify changes in functional proteomic pathways due to cannabis consumption. The ON cells from cannabis users exhibited alterations in the expression of proteins that were related to the cytoskeleton, cell proliferation and cell death, as well as, changes in proteins implicated in cancer, gastrointestinal and neurodevelopmental pathologies. Subsequent studies showed cannabis provoked an increase in cell size and morphological alterations evident through β-Tubulin III staining, as well as, enhanced beta-actin expression and a decrease in the ability of ON cells to undergo cell attachment, suggesting abnormalities of the cytoskeleton and cell adhesion system. Furthermore, these cells proliferated more and underwent less cell death. Our results indicate that cannabis may alter key processes of the developing brain, some of which are similar to those reported in mental disorders like DiGeorge syndrome, schizophrenia and bipolar disorder.
Mol Neurobiol, 2021; 58

34022746: Perez-Caballero L, Soto-Montenegro ML, Desco M, Mico JA, Berrocoso E
Sustained escitalopram administration affects glucose metabolism in the rat brain.
Escitalopram is a selective serotonin reuptake inhibitor (SSRIs) antidepressant, drug that is currently used as first-line agents for the treatment of depression and it is also used in the treatment of other psychiatric disorders. The main goal of this study was to identify which brain areas are affected by escitalopram administration. This study was carried out on male Wistar rats that received escitalopram daily over 14 days and that were studied by 2-deoxy-2[F]fluoro-D-glucose ([F]FDG)-PET on the last day of treatment. Computed tomography (CT) images were acquired immediately before each PET scan and the main effects of drug administration were elucidated by Statistical Parametric Mapping. The results obtained indicated that repeated exposure to escitalopram increased metabolic activity in the retrosplenial and posterior cingulate cortices, while it decreased such activity in the ventral hippocampus, cerebellum, brainstem and midbrain regions, including the raphe nuclei and ventral tegmental area. Therefore, repeated exposure to escitalopram alters the activity of several brain areas closely related to the serotonergic system, and previously identified as key regions in the antidepressant effect induced by SSRIs. Furthermore, some of the changes found, such as the dampened metabolism in the ventral tegmental area, are similar to changes that have been described after treating with other fast-acting antidepressant approaches.
Eur Neuropsychopharmacol, 2021; 51

34501451: Perez-Caballero L, Carceller H, Nacher J, Teruel-Marti V, Pujades E, Casañ-Pastor N, Berrocoso E
Induced Dipoles and Possible Modulation of Wireless Effects in Implanted Electrodes. Effects of Implanting Insulated Electrodes on an Animal Test to Screen Antidepressant Activity.
There is evidence that Deep Brain Stimulation (DBS) produces health benefits in patients even before initiating stimulation. Furthermore, DBS electrode insertion in rat infralimbic cortex (ILC) provokes antidepressant-like effects before stimulation, due to local inflammation and astrogliosis. Consequently, a significant effect of implanting electrodes is suspected. External fields, similar in magnitude to the brain's endogenous fields, induce electric dipoles in conducting materials, in turn influencing neural cell growth through wireless effects. To elucidate if such dipoles influence depressive-like behavior, without external stimulation, the comparative effect of conducting and insulated electrodes along with the glial response is studied in unstressed rats. Naïve and implanted rats with electrically insulated or uninsulated steel electrodes were evaluated in the modified forced swimming test and expression of ILC-glial markers was assessed. An antidepressant-like effect was observed with conducting but not with insulated electrodes. Gliosis was detected in both groups, but astroglial reactivity was larger near uninsulated electrodes. Thus, induced dipoles and antidepressant-like effects were only observed with conducting implants. Such correlation suggests that dipoles induced in electrodes by endogenous fields in turn induce neuron stimulation in a feedback loop between electrodes and neural system. Further research of the effects of unwired conducting implants could open new approaches to regulating neuronal function, and possibly treat neurological disorders.
J Clin Med, 2021; 10

33007320: Alba-Delgado C, Mico JA, Berrocoso E
Neuropathic pain increases spontaneous and noxious-evoked activity of locus coeruleus neurons.
The noradrenergic locus coeruleus nucleus is an important station in both the ascending and descending pain regulatory pathways. These neurons discharge in tonic and phasic modes in response to sensory stimuli. However, few studies have set out to characterize the electrophysiological response of the locus coeruleus to noxious stimuli in conditions of neuropathic pain. Thus, the effects of mechanical nociceptive stimulation of the sciatic nerve area on spontaneous (tonic) and sensory-evoked (phasic) locus coeruleus discharge were studied by extracellular recording in anesthetized rats seven, fourteen and twenty-eight days after chronic constriction injury. Minor significant electrophysiological changes were found seven and fourteen days after nerve injury. However, alterations to the spontaneous activity in both the ipsilateral and contralateral locus coeruleus were found twenty-eight days after nerve constriction, as witnessed by an increase of burst firing incidence and irregular firing patterns. Furthermore, noxious-evoked responses were exacerbated in the contralateral and ipsilateral nucleus

at twenty-eight days after injury, as were the responses evoked when stimulating the uninjured paw. In addition, mechanical stimulation of the hindpaw produced a significant sensitization of neuronal tonic activity after 28 days of neuropathy. In summary, long-term nerve injury led to higher spontaneous activity and exacerbated noxious-evoked responses in the locus coeruleus to stimulation of nerve-injured and even uninjured hindpaws, coinciding temporally with the development of depressive and anxiogenic-like behavior.
Prog Neuropsychopharmacol Biol Psychiatry, 2021; 105
31942167: Bravo L, Llorca-Torralba M, Berrocoso E, Micó JA
Monoamines as Drug Targets in Chronic Pain: Focusing on Neuropathic Pain.
Monoamines are involved in regulating the endogenous pain system and indeed, peripheral and central monoaminergic dysfunction has been demonstrated in certain types of pain, particularly in neuropathic pain. Accordingly, drugs that modulate the monaminergic system and that were originally designed to treat depression are now considered to be first line treatments for certain types of neuropathic pain (e.g., serotonin and noradrenaline (and also dopamine) reuptake inhibitors). The analgesia induced by these drugs seems to be mediated by inhibiting the reuptake of these monoamines, thereby reinforcing the descending inhibitory pain pathways. Hence, it is of particular interest to study the monoaminergic mechanisms involved in the development and maintenance of chronic pain. Other analgesic drugs may also be used in combination with monoamines to facilitate descending pain inhibition (e.g., gabapentinoids and opioids) and such combinations are often also used to alleviate certain types of chronic pain. By contrast, while NSAIDs are thought to influence the monoaminergic system, they just produce consistent analgesia in inflammatory pain. Thus, in this review we will provide preclinical and clinical evidence of the role of monoamines in the modulation of chronic pain, reviewing how this system is implicated in the analgesic mechanism of action of antidepressants, gabapentinoids, atypical opioids, NSAIDs and histaminergic drugs.
Front Neurosci, 2019; 13
30987747: Llorca-Torralba M, Suárez-Pereira I, Bravo L, Camarena-Delgado C, Garcia-Partida JA, Mico JA, Berrocoso E
Chemogenetic Silencing of the Locus Coeruleus-Basolateral Amygdala Pathway Abolishes Pain-Induced Anxiety and Enhanced Aversive Learning in Rats.
Pain affects both sensory and emotional aversive responses, often provoking anxiety-related diseases when chronic. However, the neural mechanisms underlying the interactions between anxiety and chronic pain remain unclear.
Biol Psychiatry, 2019; 85
32437745: Bravo L, Llorca-Torralba M, Suárez-Pereira I, Berrocoso E
Pain in neuropsychiatry: Insights from animal models.
Pain is the most common symptom reported in clinical practice, meaning that it is associated with many pathologies as either the origin or a consequence of other illnesses. Furthermore, pain is a complex emotional and sensorial experience, as the correspondence between pain and body damage varies considerably. While these issues are widely acknowledged in clinical pain research, until recently they have not been extensively considered when exploring animal models, important tools for understanding pain pathophysiology. Interestingly, chronic pain is currently considered a risk factor to suffer psychiatric disorders, mainly stress-related disorders like anxiety and depression. Conversely, pain appears to be altered in many psychiatric disorders, such as depression, anxiety and schizophrenia. Thus, pain and psychiatric disorders have been linked in epidemiological and clinical terms, although the neurobiological mechanisms involved in this pathological bidirectional relationship remain unclear. Here we review the evidence obtained from animal models about the co-morbidity of pain and psychiatric disorders, placing special emphasis on the different dimensions of pain.
Neurosci Biobehav Rev, 2020; 115

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-630

A POTENTIAL ROLE FOR THE STRESS-REGULATED MIR-708 IN AFFECTIVE DISORDERS

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Defects in synaptic plasticity are frequently observed in Major Depressive Disorder (MDD) and Bipolar Disorder (BD). microRNAs (miRNAs) have been implicated in biological pathways regulating brain development and synaptic plasticity, suggesting that they could play a significant role in MDD and BD. miR-708 represents an interesting candidate miRNA, since both miR-708 and its host gene ODZ4/TENM4 are significantly associated with BD based on previous GWAS. Our results show that miR-708 is upregulated in the peripheral blood of healthy human subjects with high genetic predisposition to inherit MDD or BD (Genetic Risk, GR) or that suffered childhood maltreatment (Environmental Risk, ER), and of patients diagnosed with BD or MDD. Furthermore, miR-708 is upregulated in the hippocampus of juvenile rats that underwent social isolation, a model of chronic stress associated with anxiety and depression-like behavior. To investigate the functional impact of elevated miR-708 levels in the hippocampus, we delivered a miR-708-overexpression plasmid via stereotactic injection in adult mice. RNA sequencing of hippocampal RNA revealed differential expression of miR-708 targets involved in the regulation of synaptic transmission. Further bioinformatic analysis showed that the downregulated genes are mostly enriched in inhibitory interneurons, whereas upregulated genes are mostly enriched in glutamatergic neurons of the dentate gyrus and oligodendrocytes. An extensive behavioural characterization of miR-708 overexpressing mice in the context of anxiety, memory, depression, and sociability is currently ongoing. Taken together, our results indicate that stress-induced upregulation of miR-708 alters gene regulatory networks in the hippocampus, with potential implications for cognition and behavior.

Pubmed:
33996819: Gilardi C, Kalebic N
The Ferret as a Model System for Neocortex Development and Evolution.
The neocortex is the largest part of the cerebral cortex and a key structure involved in human behavior and cognition. Comparison of neocortex development across mammals reveals that the proliferative capacity of neural stem and progenitor cells and the length of the neurogenic period are essential for regulating neocortex size and complexity, which in turn are thought to be instrumental for the increased cognitive abilities in humans. The domesticated ferret, , is an important animal model in neurodevelopment for its complex postnatal cortical folding, its long period of forebrain development and its accessibility to genetic manipulation . Here, we discuss the molecular, cellular, and histological features that make this small gyrencephalic carnivore a suitable animal model to study the physiological and pathological mechanisms for the development of an expanded neocortex. We particularly focus on the mechanisms of neural stem cell proliferation, neuronal differentiation, cortical folding, visual system development, and neurodevelopmental pathologies. We further discuss the technological advances that have enabled the genetic manipulation of the ferret . Finally, we compare the features of neocortex development in the ferret with those of other model organisms.
Front Cell Dev Biol, 2021; 9
30905618: Kalebic N, Gilardi C, Stepien B, Wilsch-Bräuninger M, Long KR, Namba T, Florio M, Langen B, Lombardot B, Shevchenko A, Kilimann MW, Kawasaki H, Wimberger P, Huttner WB
Neocortical Expansion Due to Increased Proliferation of Basal Progenitors Is Linked to Changes in Their Morphology.
The evolutionary expansion of the mammalian neocortex (Ncx) is thought to be linked to increased proliferative capacity of basal progenitors (BPs) and their neurogenic capacity. Here, by quantifying BP morphology in the developing Ncx of mouse, ferret, and human, we show that increased BP proliferative capacity is linked to an increase in BP process number. We identify human membrane-bound PALMDELPHIN (PALMD-Caax) as an underlying factor, and we show that it drives BP process growth and proliferation when expressed in developing mouse and ferret Ncx. Conversely, CRISPR/Cas9-mediated disruption of PALMD or its binding partner ADDUCIN-γ in fetal human Ncx reduces BP process numbers and proliferation. We further show that PALMD-induced processes enable BPs to receive pro-proliferative integrin-dependent signals. These findings provide a link between BP morphology and proliferation, suggesting that changes in BP morphology may have

contributed to the evolutionary expansion of the Ncx.
Cell Stem Cell, 2019; 24
30484771: Kalebic N, Gilardi C, Albert M, Namba T, Long KR, Kostic M, Langen B, Huttner WB
Human-specific induces hallmarks of neocortical expansion in developing ferret neocortex.
The evolutionary increase in size and complexity of the primate neocortex is thought to underlie the higher cognitive abilities of humans. is a human-specific gene that, based on its expression pattern in fetal human neocortex and progenitor effects in embryonic mouse neocortex, has been proposed to have a key function in the evolutionary expansion of the neocortex. Here, we study the effects of expression in the developing neocortex of the gyrencephalic ferret. In contrast to its effects in mouse, markedly increases proliferative basal radial glia, a progenitor cell type thought to be instrumental for neocortical expansion, and results in extension of the neurogenic period and an increase in upper-layer neurons. Consequently, the postnatal ferret neocortex exhibits increased neuron density in the upper cortical layers and expands in both the radial and tangential dimensions. Thus, human-specific can elicit hallmarks of neocortical expansion in the developing ferret neocortex.
Elife, 2018; 7

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BOARD NUMBER: S04-631

2-BROMO-LSD: A NON-HALLUCINOGENIC LSD ANALOGUE WITH THERAPEUTIC POTENTIAL FOR MAJOR DEPRESSIVE DISORDER.

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Major depressive disorder (MDD) is the leading cause of disability worldwide. Current pharmacotherapy treatments, such as SSRI's and SNRI's, have long effective latencies, require chronic administration, and show an estimated 30% treatment resistance rate, necessitating the search for more effective, alternate treatments. Interest in psychedelic hallucinogens (e.g., lysergic acid diethylamide [LSD], psilocybin) has seen a resurgence due to their potential for the treatment of neuropsychiatric diseases, including anxiety and depressive disorders. However, psychedelics induce hallucinations which can last for hours, making efficient and cost-effective treatment difficult. Therefore, discovering non-hallucinogenic derivatives with antidepressant properties is of paramount importance. 2-Bromo-LSD (2BLSD, BETR-001) is an LSD derivative with 5-HT2A agonist activity that lacks hallucinogenic effects and has been safely used for cluster headache treatment. Here we examine the anti-depressant and neural plasticity-promoting activity of 2BLSD. Our findings demonstrate that 2BLSD reverses the depression-like behaviors following chronic variable stress in mice and promotes dendritic arbor complexity in cultured primary rat cortical neurons. These results show that 2BLSD may possess a therapeutic potential and represents a promising alternative to psychedelics in the treatment for MDD.

Pubmed:

34391934: Lewis V, Laberge F, Heyland A
Transcriptomic signature of extinction learning in the brain of the fire-bellied toad, Bombina orientalis.
Insight into the molecular and cellular mechanisms of learning and memory from a diverse array of taxa contributes to our understanding of the evolution of these processes. The fire-bellied toad, Bombina orientalis, is a basal anuran amphibian model species who could help us describe shared and divergent characteristics of learning and memory mechanisms between amphibians and other vertebrates, and hence answer questions about the evolution of learning. Utilizing next generation sequencing techniques, we profiled gene expression patterns associated with the extinction of prey-catching conditioning in the brain of the fire-bellied toad. For this purpose, gene expression was at first compared between toads sacrificed after acquisition and extinction of the conditioned response. A second comparison was done between toads submitted to extinction following either short or long acquisition training, which results in toads displaying response extinction or resistance to extinction, respectively. We analyzed brain tissue transcription profiles common to both acquisition and extinction learning, or unique to extinction learning and resistance to extinction, and found significant overlap in gene expression related to molecular pathways involving neuronal plasticity (e.g. structural modification, transcription). However, extinction learning induced a unique GABAergic transcriptomic signal, which may be responsible for suppression of the original response memory. Further, when comparing extinction learning in short- and long-trained groups, short training engaged many pathways related to neuronal plasticity, as expected, but long training engaged molecular pathways related to the suppression of learning through epigenetic mediated transcriptional suppression and inhibitory neurotransmission. Overall, gene expression patterns associated with extinction learning in the fire-bellied toad were similar to those found in mammals submitted to extinction, although some divergent profiles highlighted potential differences in the mechanisms of learning and memory among tetrapods.
Neurobiol Learn Mem, 2021; 184
31992968: Lewis V, Laberge F, Heyland A
Temporal Profile of Brain Gene Expression After Prey Catching Conditioning in an Anuran Amphibian.
A key goal in modern neurobiology is to understand the mechanisms underlying learning and memory. To that end, it is essential to identify the patterns of gene expression and the temporal sequence of molecular events associated with learning and memory processes. It is also important to ascertain if and how these molecular events vary between organisms. In vertebrates, learning and memory processes are characterized by distinct phases of molecular activity involving gene transcription, structural change, and long-term maintenance of such structural change in the nervous system. Utilizing next

generation sequencing techniques, we profiled the temporal expression patterns of genes in the brain of the fire-bellied toad after prey catching conditioning. The fire-bellied toad is a basal tetrapod whose neural architecture and molecular pathways may help us understand the ancestral state of learning and memory mechanisms in tetrapods. Differential gene expression following conditioning revealed activity in molecular pathways related to immediate early genes (IEG), cytoskeletal modification, axon guidance activity, and apoptotic processes. Conditioning induced early IEG activity coinciding with transcriptional activity and neuron structural modification, followed by axon guidance and cell adhesion activity, and late neuronal pruning. While some of these gene expression patterns are similar to those found in mammals submitted to conditioning, some interesting divergent expression profiles were seen, and differential expression of some well-known learning-related mammalian genes is missing altogether. These results highlight the importance of using a comparative approach in the study of the mechanisms of leaning and memory and provide molecular resources for a novel vertebrate model in the relatively poorly studied Amphibia.
Front Neurosci, 2019; 13

21307060: Stephenson R, Lewis V
Behavioural evidence for a sleep-like quiescent state in a pulmonate mollusc, Lymnaea stagnalis (Linnaeus).
The objective of this study was to determine whether the great pond snail, Lymnaea stagnalis, expresses a sleep-like behavioural state. We found that snails spontaneously enter a relatively brief (22±1 min) quiescent state characterized by postural relaxation of the foot, mantle and tentacles, and cessation of radula rasping. Quiescence was reversed ('aroused') by appetitive (sucrose solution) and aversive (tactile) stimuli. Responsiveness to both stimuli was significantly lower in quiescent snails than in active snails. However, tactile stimuli evoked a more sustained defensive response in quiescent snails. Quiescence bouts were consolidated into 'clusters' over an infradian timescale and were only weakly affected by time of day. Clusters contained 7±0.5 bouts, lasted 13±1 h and were separated by long (37±4 h) intervals of almost continuous activity. Analysis of Kaplan-Meier survival curves revealed that the quiescent bout duration was described by an exponential probability distribution (time constant 15±1 min). Active bout duration was described by a bi-exponential probability distribution (time constants 62±4 and 592±48 min). We found no evidence for a 'sleep rebound' mechanism and quiescence expression appeared to be regulated through stochastic processes causing state transitions to resemble a Markovian random walk. We conclude that Lymnaea is a potentially valuable model system for studies of cellular function in sleep.
J Exp Biol, 2011; 214

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BOARD NUMBER: S04-632

EARLY LIFE STRESS TARGETS THE TRANSCRIPTIONAL SIGNATURE AND FUNCTIONAL PROPERTIES OF VOLTAGE GATED-SODIUM (NAV) CHANNELS IN HIPPOCAMPAL NG2+ GLIA

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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The detrimental effects of early life adversity (ELA) on brain development and mental health are well established. While studies have mainly identified neuorns as targets of ELA across species, knowledge about the role of NG2+ cells is still lacking. NG2+ cells are a particularly interesting subpopulation, comprising oligodendrocyte precursor cells, with unique properties, as they form synapses with neurons and respond to stress hormones. Using a mouse model of ELA, we performed molecular profiling in hippocampal NG2+ cells at early postnatal and adult stages. To further dissect the impact of glucocorticoids on ELA-induced transcriptional changes, we integrated our data with Chip-seq data on genomic binding sites of the glucocorticoid receptor (GR). The functional relevance of one candidate, Scn7a, was confirmed by electrophysiological recordings in hippocampal NG2+ cells. First, we have established a link between maternal behavior, activation of the offspring's stress response and heterogeneity in the outcome to ELA. We further showed that ELA targets the hippocampal NG2+ transcriptome with glucocorticoids being an important mediator of the ELA-induced molecular changes. ELA altered the NG2+ transcriptome and these transcriptional effects were linked on stress-induced glucocorticoids. The functional relevance of one ELA candidate gene, Scn7a, could be confirmed by an increase in the density of voltage-gated sodium (Nav) channel activated currents in hippocampal NG2+ cells. Our findings indicate that ELA specifically targets the transcriptional profile and electrophysiological properties of hippocampal NG2+ glia. Considering that Na_v channels are important for NG2+ cell-to-neuron communication, our findings establish novel insights into the pathophysiology of ELA.

Pubmed:
34095364: Treccani G, Yigit H, Lingner T, Schleußner V, Mey F, van der Kooij MA, Wennström M, Herzog DP, Linke M, Fricke M, Schmeisser MJ, Wegener G, Mittmann T, Trotter J, Müller MB
Early life adversity targets the transcriptional signature of hippocampal NG2+ glia and affects voltage gated sodium (Na) channels properties.
The precise mechanisms underlying the detrimental effects of early life adversity (ELA) on adult mental health remain still elusive. To date, most studies have exclusively targeted neuronal populations and not considered neuron-glia crosstalk as a crucially important element for the integrity of stress-related brain function. Here, we have investigated the impact of ELA, in the form of a limited bedding and nesting material (LBN) paradigm, on a glial subpopulation with unique properties in brain homeostasis, the NG2+ cells. First, we have established a link between maternal behavior, activation of the offspring's stress response and heterogeneity in the outcome to LBN manipulation. We further showed that LBN targets the hippocampal NG2+ transcriptome with glucocorticoids being an important mediator of the LBN-induced molecular changes. LBN altered the NG2+ transcriptome and these transcriptional effects were correlated with glucocorticoids levels. The functional relevance of one LBN-induced candidate gene, , could be confirmed by an increase in the density of voltage-gated sodium (Na) channel activated currents in hippocampal NG2+ cells. remained upregulated until adulthood in LBN animals, which displayed impaired cognitive performance. Considering that Na channels are important for NG2+ cell-to-neuron communication, our

findings provide novel insights into the disruption of this process in LBN mice.
Neurobiol Stress, 2021; 15

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BOARD NUMBER: S04-633

MOOD AND COGNITION RELATED ANALYSIS IN DIMETHYLARGININE DIMETHYLAMINOHYDROLASE-1 KNOCKOUT MICE.

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Dimethylarginine dimethylaminohydrolases (DDAH) metabolize asymmetric dimethylarginine (ADMA) the major endogenous inhibitor of nitric oxide synthase (NOS), thereby regulating NO availability. Altered NOS activity, and thus NO levels, are associated with affective disorders. Inline, altered ADMA levels were found in patients with major depression, bipolar disorder, and schizophrenia. Thus, the DDAH/ADMA pathway may play a role in the regulation of NO levels underlying pathology. To investigate the function of the DDAH/ADMA system in neuropathology, we employed a full knockout DDAH1 mice line (DDAH1-ko). We aimed to characterize its impact on mood-related and cognitive behavior and brain morphological and molecular features. DDAH1-ko mice were found to lack DDAH1 mRNA and protein as well as DDAH activity measured by conversion of ADMA to citrulline. DDAH1-ko mice showed a longer active exploration in a novel-object-recognition test and an altered response to an amphetamine challenge paradigm. Based on the decreased response to the psychostimulant, neurotransmitter content was measured and the tissue content of the dopamine metabolite DOPAC was found to be reduced in the caudoputamen and piriform cortex in DDAH1-ko. In contrast to our hypothesis, DDAH1-ko mice showed only minimal behavioral abnormalities compared to littermate wild-type controls. These findings may be explained by the existence of a compensatory mechanism, such as developmental or regulation of NO content through DDAH2 activity. Further research may also focus on the well-established gene-environment interactions in the etiology of neuropsychiatric disorders and thus combine the DDAH1-ko model with external stressors.

Pubmed:

34845253: Kalinina DS, Ptukha MA, Goriainova AV, Merkulyeva NS, Kozlova AA, Murtazina RZ, Shemiakova TS, Kuvarzin SR, Vaganova AN, Volnova AB, Gainetdinov RR, Musienko PE
Role of the trace amine associated receptor 5 (TAAR5) in the sensorimotor functions.
Classical monoamines are well-known modulators of sensorimotor neural networks. However, the role of trace amines and their receptors in sensorimotor function remains unexplored. Using trace amine-associated receptor 5 knockout (TAAR5-KO) mice, that express beta-galactosidase mapping its localization, we observed TAAR5 expression in the Purkinje cells of the cerebellum and the medial vestibular nucleus, suggesting that TAAR5 might be involved in the vestibular and motor control. Accordingly, in various behavioral tests, TAAR5-KO mice demonstrated lower endurance, but better coordination and balance compared to wild-type controls. Furthermore, we found specific changes in striatal local field potentials and motor cortex electrocorticogram, such as a decrease in delta and an increase in theta oscillations of power spectra, respectively. The obtained data indicate that TAAR5 plays a considerable role in regulation postural stability, muscle force, balance, and motor coordination during active movements, likely via modulation of monoaminergic systems at different levels of sensorimotor control involving critical brain areas such as the brainstem, cerebellum, and forebrain.
Sci Rep, 2021; 11
34014421: Kozlova AA, Ragavan VN, Jarzebska N, Lukianova IV, Bikmurzina AE, Rubets E, Suzuki-Yamamoto T, Kimoto M, Mangoni AA, Gainetdinov RR, Weiss N, Bauer M, Markov AG, Rodionov RN, Bernhardt N
Divergent Dimethylarginine Dimethylaminohydrolase Isoenzyme Expression in the Central Nervous System.
The endogenous methylated derivative of L-arginine, N,N-dimethyl-L-arginine (asymmetric dimethylarginine, ADMA), an independent risk factor in many diseases, inhibits the activity of nitric oxide synthases and, consequently, modulates the availability of nitric oxide. While most studies on the biological role of ADMA have focused on endothelial and inducible nitric oxide synthases modulation and its contribution to cardiovascular, metabolic, and renal diseases, a role in regulating neuronal nitric oxide synthases and pathologies of the central nervous system is less understood. The two isoforms of

dimethylarginine dimethylaminohydrolase (DDAH), DDAH1 and DDAH2, are thought to be the main enzymes responsible for ADMA catabolism. A current impediment is limited knowledge on specific tissue and cellular distribution of DDAH enzymes within the brain. In this study, we provide a detailed characterization of the regional and cellular distribution of DDAH1 and DDAH2 proteins in the adult murine and human brain. Immunohistochemical analysis showed a wide distribution of DDAH1, mapping to multiple cell types, while DDAH2 was detected in a limited number of brain regions and exclusively in neurons. Our results provide key information for the investigation of the pathophysiological roles of the ADMA/DDAH system in neuropsychiatric diseases and pave the way for the development of novel selective therapeutic approaches.
Cell Mol Neurobiol, 2021;
33132188: Efimova EV, Kozlova AA, Razenkova V, Katolikova NV, Antonova KA, Sotnikova TD, Merkulyeva NS, Veshchitskii AS, Kalinina DS, Korzhevskii DE, Musienko PE, Kanov EV, Gainetdinov RR
Increased dopamine transmission and adult neurogenesis in trace amine-associated receptor 5 (TAAR5) knockout mice.
Trace amine-associated receptors (TAARs) are a class of sensory G protein-coupled receptors that detect biogenic amines, products of decarboxylation of amino acids. The majority of TAARs (TAAR2-TAAR9) have been described mainly in the olfactory epithelium and considered to be olfactory receptors sensing innate odors. However, there is recent evidence that one of the members of this family, TAAR5, is expressed also in the limbic brain areas receiving projection from the olfactory system and involved in the regulation of emotions. In this study, we further characterized a mouse line lacking TAAR5 (TAAR5 knockout, TAAR5-KO mice) that express beta-galactosidase mapping TAAR5 expression. We found that in TAAR5-KO mice the number of dopamine neurons, the striatal levels of dopamine and its metabolites, as well as striatal levels of GDNF mRNA, are elevated indicating a potential increase in dopamine neuron proliferation. Furthermore, an analysis of TAAR5 beta-galactosidase expression revealed that TAAR5 is present in the major neurogenic areas of the brain such as the subventricular zone (SVZ), the subgranular zone (SGZ) and the less characterized potentially neurogenic zone surrounding the 3rd ventricle. Direct analysis of neurogenesis by using specific markers doublecortin (DCX) and proliferating cell nuclear antigen (PCNA) revealed at least 2-fold increase in the number of proliferating neurons in the SVZ and SGZ of TAAR5-KO mice, but no such markers were detected in mutant or control mice in the areas surrounding the 3rd ventricle. These observations indicate that TAAR5 involved not only in regulation of emotional status but also adult neurogenesis and dopamine transmission. Thus, future TAAR5 antagonists may exert not only antidepressant and/or anxiolytic action but may also provide new treatment opportunity for neurodegenerative disorders such as Parkinson's disease.
Neuropharmacology, 2021; 182
31399838: Zhukov IS, Kubarskaya LG, Tissen IY, Kozlova AA, Dagayev SG, Kashuro VA, Vlasova OL, Sinitca EL, Karpova IV, Gainetdinov RR
Minimal Age-Related Alterations in Behavioral and Hematological Parameters in Trace Amine-Associated Receptor 1 (TAAR1) Knockout Mice.
Since the discovery in 2001, the G protein-coupled trace amine-associated receptor 1 (TAAR1) has become an important focus of research targeted on evaluation of its role in the central nervous system (CNS). Meanwhile, impact of TAAR1 in the peripheral organs is less investigated. Expression of TAAR1 was demonstrated in different peripheral tissues: pancreatic β-cells, stomach, intestines, white blood cells (WBC), and thyroid. However, the role of TAAR1 in regulation of hematological parameters has not been investigated yet. In this study, we performed analysis of anxiety-related behaviors, a complete blood count (CBC), erythrocyte fragility, as well as FT3/FT4 thyroid hormones levels in adult and middle-aged TAAR1 knockout mice. Complete blood count analysis was performed on a Siemens Advia 2120i hematology analyzer and included more than 35 measured and calculated parameters. Erythrocyte fragility test evaluated spherocytosis pathologies of red blood cells (RBC). No significant alterations in essentially all these parameters were found in mice without TAAR1. However, comparative aging analysis has revealed a decreased neutrophils level in the middle-aged TAAR1 knockout mouse group. Minimal alterations in these parameters observed in TAAR1 knockout mice suggest that future TAAR1-based therapies should exert little hematological effect and thus will likely have a good safety profile.
Cell Mol Neurobiol, 2020; 40
32194374: Espinoza S, Sukhanov I, Efimova EV, Kozlova A, Antonova KA, Illiano P, Leo D, Merkulyeva N, Kalinina D, Musienko P, Rocchi A, Mus L, Sotnikova TD, Gainetdinov RR
Trace Amine-Associated Receptor 5 Provides Olfactory Input Into Limbic Brain Areas and Modulates Emotional Behaviors and Serotonin Transmission.
Trace amine-associated receptors (TAARs) are a class of G-protein-coupled receptors found in mammals. While TAAR1 is expressed in several brain regions, all the other TAARs have been described mainly in the olfactory epithelium and the glomerular layer of the olfactory bulb and are believed to serve as a new class of olfactory receptors sensing innate odors. However, there is evidence that TAAR5 could play a role also in the central nervous system. In this study, we characterized a mouse line lacking TAAR5 (TAAR5 knockout, TAAR5-KO) expressing beta-galactosidase mapping TAAR5 expression. We found that TAAR5 is expressed not only in the glomerular layer in the olfactory bulb but also in deeper layers projecting to the

limbic brain olfactory circuitry with prominent expression in numerous limbic brain regions, such as the anterior olfactory nucleus, the olfactory tubercle, the orbitofrontal cortex (OFC), the amygdala, the hippocampus, the piriform cortex, the entorhinal cortex, the nucleus accumbens, and the thalamic and hypothalamic nuclei. TAAR5-KO mice did not show gross developmental abnormalities but demonstrated less anxiety- and depressive-like behavior in several behavioral tests. TAAR5-KO mice also showed significant decreases in the tissue levels of serotonin and its metabolite in several brain areas and were more sensitive to the hypothermic action of serotonin 5-HT1A receptor agonist 8-hydroxy-2-(di--propilamino)tetralin (8-OH-DPAT). These observations indicate that TAAR5 is not just innate odor-sensing olfactory receptor but also serves to provide olfactory input into limbic brain areas to regulate emotional behaviors likely modulation of the serotonin system. Thus, anxiolytic and/or antidepressant action of future TAAR5 antagonists could be predicted. In general, "olfactory" TAAR-mediated brain circuitry may represent a previously unappreciated neurotransmitter system involved in the transmission of innate odors into emotional behavioral responses.
Front Mol Neurosci, 2020; 13

30455454: Deliu E, Arecco N, Morandell J, Dotter CP, Contreras X, Girardot C, Käsper EL, Kozlova A, Kishi K, Chiaradia I, Noh KM, Novarino G

Haploinsufficiency of the intellectual disability gene SETD5 disturbs developmental gene expression and cognition.
SETD5 gene mutations have been identified as a frequent cause of idiopathic intellectual disability. Here we show that Setd5-haploinsufficient mice present developmental defects such as abnormal brain-to-body weight ratios and neural crest defect-associated phenotypes. Furthermore, Setd5-mutant mice show impairments in cognitive tasks, enhanced long-term potentiation, delayed ontogenetic profile of ultrasonic vocalization, and behavioral inflexibility. Behavioral issues are accompanied by abnormal expression of postsynaptic density proteins previously associated with cognition. Our data additionally indicate that Setd5 regulates RNA polymerase II dynamics and gene transcription via its interaction with the Hdac3 and Paf1 complexes, findings potentially explaining the gene expression defects observed in Setd5-haploinsufficient mice. Our results emphasize the decisive role of Setd5 in a biological pathway found to be disrupted in humans with intellectual disability and autism spectrum disorder.
Nat Neurosci, 2018; 21

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BOARD NUMBER: S04-634

OLFACTORY BULBECTOMY INDUCES TIME-DEPENDENT HYPERLOCOMOTION AND GLIOSIS IN THE MALE RAT

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Background. Major depression disorder (MDD) induces a constellation of behavioral deficits including difficulties to adapt to novelty. A working hypothesis of MDD include astrocytic and microglia activation in the central nervous system. The prefrontal cortex (PFC) and hippocampus are two brain regions affected in MDD. Olfactory bulbectomy (OBX) is a well-known preclinical model of depression-related behavior in rodents. **Aims.** In the present study, we tested the hypothesis that OBX induce alterations to adapt to a novel environment as a result of gliosis in the PFC and hippocampus in a time-dependent manner in the rat. **Methods.** To test a possible lack of adaptation to novel places, we measured horizontal and vertical behaviors in the open field test (OFT). To determine whether OBX modulated astrocytic and/or microglial expression, we measured the number and morphology of glial fibrillary acidic protein and Iba1 in the PFC and hippocampus. All these measurements were done in three independent cohorts at three critical times after surgery: one, four and fifteen weeks. **Results.** OBX rats presented hyperlocomotion in the OFT at one and four weeks after surgery, interpreted as a failure of adaptation to novel environment. At four weeks after surgery, OBX rats displayed increased rearing and grooming, suggesting alterations in stress-coping behaviors. The number of astrocytes was increased in the PFC in OBX rats. **Conclusions.** The data from this preclinical animal model supports the immunological theory of MDD. These results add further support to the validity and usefulness of the OBX rat as a model of depression.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-635

ASSESSING POSITIVE AND NEGATIVE VALENCE SYSTEMS TO REFINE ANIMAL MODELS OF BIPOLAR DISORDERS: THE EXAMPLE OF GBR 12909-INDUCED MANIC PHENOTYPE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Mathilde Bigot[1,2], Éléonore Vicq[1], Pierre-Marie Lledo[1], Mariana Alonso[1], Chantal Henry[1,3]
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Bipolar disorders are defined by recurrences of depressive and manic episodes. The pathophysiology is still unknown, and translating clinical symptoms into behaviors explorable in animal models is challenging. Animal models of bipolar disorder do not exist because cyclicity of the disease is impossible to mimic, and it is therefore necessary to study mania and depression models separately. Beyond mood, emotional biases differentiate bipolar states in humans. Mania is associated with positive biases, *e.g.* emotional stimuli become more rewarding and less aversive, and the opposite for depression. We propose to assess behavioral hedonic responses to innately appetitive and aversive olfactory and gustatory cues in mice as proxies for the assigned emotional valence. A mania model is therefore supposed to exhibit positive hedonic bias. Using the GBR 12909 mania model, we observed the classical hyperactivity phenotype, along with low depressive-like but high anxiety-like behaviors. Unexpectedly, GBR 12909-treated mice exhibited strong negative hedonic biases. Consequently, the GBR 12909 model of mania might not be appropriate for studying emotional disturbances associated with mania states. We propose olfactory and gustatory preference tests as crucial assessment for positive and negative valence biases, necessary for precisely characterizing animal models of bipolar disorders.

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BOARD NUMBER: S04-636

THE HYPERACTIVE NATURE OF THE RODENT OLFACTORY BULBECTOMY MODEL: A SYSTEMATIC REVIEW AND META-ANALYSIS

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Major Depressive Disorder (MDD) is a highly impairing and prevalent psychiatric condition. The nature of the disorder makes it difficult to study it in vivo, which supports the use of animal models. One of the most widely used models in MDD research is the rodent model of olfactory bulbectomy (OBX), which should present core depressive-like behaviors like anhedonia or hopelessness. However, many studies also investigate its locomotor activity, which seems to be sometimes exacerbated due to olfactory bulb removal. Regardless of the reason, hyperactivity is seldom a symptom of patients with MDD, which could jeopardize OBX's translatability. To investigate the consistency of these findings, we conducted a systematic review and meta-analysis of the studies that evaluated the locomotor activity of OBX rats or mice in the open field apparat. The protocol for this study was registered on Prospero (CRD42020205536). Duplicates were removed, and records were independently screened by at least two reviewers. A third reviewer resolved the discrepancies. Inclusion criteria were: studies that included OBX rats or mice, a comparator control, and performed the open field test. A total of 305 articles were recruited from PubMed, 569 from Web of Science, and 239 from Scopus. After the duplicates were removed, 630 remained to be selected, and of these, 123 studies were included. After conducting a random-effects meta-analysis, we found an overall positive effect (z = 5,00, p-value < 0.01), indicating that the OBX indeed presents hyperactivity. This raises the question of whether OBX could be regarded as a satisfactory model for MDD.

Pubmed:
34303703: Gasparotto J, Senger MR, Telles de Sá Moreira E, Brum PO, Carazza Kessler FG, Peixoto DO, Panzenhagen AC, Ong LK, Campos Soares M, Reis PA, Schirato GV, Góes Valente WC, Araújo Montoya BO, Silva FP, Fonseca Moreira JC, Dal-Pizzol F, Castro-Faria-Neto HC, Gelain DP
Neurological impairment caused by Schistosoma mansoni systemic infection exhibits early features of idiopathic neurodegenerative disease.
Schistosomiasis, a neglected tropical disease caused by trematodes of the Schistosoma genus, affects over 250 million people around the world. This disease has been associated with learning and memory deficits in children, whereas reduced attention levels, impaired work capacity, and cognitive deficits have been observed in adults. Strongly correlated with poverty and lack of basic sanitary conditions, this chronic endemic infection is common in Africa, South America, and parts of Asia and contributes to inhibition of social development and low quality of life in affected areas. Nonetheless, studies on the mechanisms involved in the neurological impairment caused by schistosomiasis are scarce. Here, we used a murine model of infection with Schistosoma mansoni in which parasites do not invade the central nervous system to evaluate the consequences of systemic infection on neurologic function. We observed that systemic infection with S. mansoni led to astrocyte and microglia activation, expression of oxidative stress-induced transcription factor Nrf2, oxidative damage, Tau phosphorylation, and amyloid-β peptide accumulation in the prefrontal cortex of infected animals. We also found impairment in spatial learning and memory as evaluated by the Morris water maze task. Administration of anthelmintic (praziquantel) and antioxidant (N-acetylcysteine plus deferoxamine) treatments was effective in inhibiting most of these phenotypes, and the combination of both treatments had a synergistic effect to prevent such changes. These data demonstrate new perspectives toward the understanding of the pathology and possible therapeutic approaches to counteract long-term effects of systemic schistosomiasis on brain function.
J Biol Chem, 2021; 297
33681905: Vidor MV, Panzenhagen AC, Martins AR, Cupertino RB, Bandeira CE, Rohde LA, Rovaris DL, Bau CHD, Grevet EH

Attention-deficit/hyperactivity disorder and brain metabolites from proton magnetic resonance spectroscopy: a systematic review and meta-analysis protocol.
Despite major advances in the study of the brain, investigations on neurochemistry in vivo still lack the solid ground of more established methods, such as structural and functional magnetic resonance imaging. Proton magnetic resonance spectroscopy (MRS) is a technique that might potentially fill in this gap. Nevertheless, studies using this approach feature great methodological heterogeneity, such as varying voxel of choice, differences on emphasized metabolites, and absence of a standardized unit. In this study, we present a methodology for creating a systematic review and meta-analysis for this kind of scientific evidence using the prototypical case of attention-deficit/hyperactivity disorder. Systematic review registration: International Prospective Register of Systematic Reviews (PROSPERO), CRD42018112418.
Trends Psychiatry Psychother, 2021 Jan-Mar; 43
33198929: Rosa-Silva HTD, Panzenhagen AC, Espitia-Pérez P, Teixeira AA, Roitman A, Almeida RF, Heimfarth L, Moreira JCF
Effects of foetal and breastfeeding exposure to methylmercury (MeHg) and retinol palmitate (Vitamin A) in rats: Redox parameters and susceptibility to DNA damage in liver.
Methylmercury (MeHg) is known to be a chemical that poses a risk to public health. Exposure to MeHg and vitamin A (VitA) occurs through the ingestion of fish, present in the diet of most pregnant women. The absorption of these elements generates oxidative stress and can generate adaptations for future stressful events. Here, we assessed how exposure to VitA and/or MeHg during the fetal and breastfeeding period modulates the toxicity of MeHg reexposure in adulthood. We focus on redox systems and repairing DNA damage. Male rats (n = 50), were divided into 5 groups. Control received mineral oil; The VitA group received VitA during pregnancy, during breastfeeding and was exposed to MeHg in adulthood; VitA + MeHg received VitA and MeHg during pregnancy and breastfeeding and was exposed to MeHg in adulthood. The single exposure group (SE) was exposed to MeHg only in adulthood; and the MeHg group was pre-exposed to MeHg during pregnancy and breastfeeding and re-exposed to MeHg in adulthood. After treating the animals, we evaluated the redox status and the level of DNA damage in all rats. The results revealed that MeHg significantly decreased the activity of glutathione peroxidase (GPx) and sulfhydryl levels and increased the activity of superoxide dismutase (SOD), glutathione transferase, glutathione and carbonyl in all exposed groups. These results suggest that the second exposure to MeHg directly altered the effects of oxidation and that there were no specific effects associated with exposure during the fetal and breastfeeding periods. In addition, our findings indicate that MDA levels increased in MeHg and SE levels and no differences in MDA levels were observed between the VitA and MeHg + VitA groups. We also observed that animals pretreated exclusively with VitA showed residual damage similar to the control's DNA, while the other groups showed statistically higher levels of damage. In conclusion, low doses of MeHg and VitA during fetal and breastfeeding periods were unable to condition an adaptive response to subsequent exposure to MeHg in adulthood in relation to the observed levels of oxidative damage assessed after exposure.
Mutat Res Genet Toxicol Environ Mutagen, 2020 Oct - Dec; 858-860
32997045: Antonello VS, Panzenhagen AC, Balanzá-Martínez V, Shansis FM
Virtual meetings and social isolation in COVID-19 times: transposable barriers.
Trends Psychiatry Psychother, 2020 Jul-Sep; 42
31809933: Rosa-Silva HTD, Panzenhagen AC, Schmidtt V, Alves Teixeira A, Espitia-Pérez P, de Oliveira Franco Á, Mingori M, Torres-Ávila JF, Schnorr CE, Hermann PRS, Moraes DP, Almeida RF, Moreira JCF
Hepatic and neurobiological effects of foetal and breastfeeding and adulthood exposure to methylmercury in Wistar rats.
Methylmercury (MeHg) is an organic bioaccumulated mercury derivative that strongly affects the environment and represents a public health problem primarily to riparian communities in South America. Our objective was to investigate the hepatic and neurological effects of MeHg exposure during the phases foetal and breast-feeding and adult in Wistar rats. Wistar rats (n = 10) were divided into 3 groups. Control group received mineral oil; The simple exposure (SE) group was exposed only in adulthood (0.5 mg/kg/day); and double exposure (DE) was pre-exposed to MeHg 0.5 mg/kg/day during pregnancy and breastfeeding (±40 days) and re-exposed to MeHg for 45 days from day 100. After, we evaluated possible abnormalities. Behavioral and biochemical parameters in liver and occipital cortex (CO), markers of liver injury, redox and AKT/GSK3β/mTOR signaling pathway. Our results showed that both groups treated with MeHg presented significant alterations, such as decreased locomotion and exploration and impaired visuospatial perception. The rats exposed to MeHg showed severe liver damage and increased hepatic glycogen concentration. The MeHg groups showed significant impairment in redox balance and oxidative damage to liver macromolecules and CO. MeHg upregulated the AKT/GSK3β/mTOR pathway and the phosphorylated form of the Tau protein. In addition, we found a reduction in NeuN and GFAP immunocontent. These results represent the first approach to the hepatotoxic and neural effects of foetal and adult MeHg exposure.
Chemosphere, 2020; 244

30935939: Panzenhagen AC, Bau CHD, Grevet EH, Rovaris DL
An animal model of what? The case of spontaneously hypertensive rats.
Prog Neuropsychopharmacol Biol Psychiatry, 2019; 94
30826386: Leffa DT, Panzenhagen AC, Salvi AA, Bau CHD, Pires GN, Torres ILS, Rohde LA, Rovaris DL, Grevet EH
Systematic review and meta-analysis of the behavioral effects of methylphenidate in the spontaneously hypertensive rat model of attention-deficit/hyperactivity disorder.
The spontaneously hypertensive rats (SHR) are the most widely used model for ADHD. While face and construct validity are consolidated, questions remain about the predictive validity of the SHR model. We aim at summarizing the evidence for the predictive validity of SHR by evaluating its ability to respond to methylphenidate (MPH), the most well documented treatment for ADHD. A systematic review was carried out to identify studies evaluating MPH effects on SHR behavior. Studies (n=36) were grouped into locomotion, attention, impulsivity or memory, and a meta-analysis was performed. Meta-regression, sensitivity, heterogeneity, and publication bias analyses were also conducted. MPH increased attentional and mnemonic performances in the SHR model and decreased impulsivity in a dose-dependent manner. However, MPH did not reduce hyperactivity in low and medium doses, while increased locomotor activity in high doses. Thus, since the paradoxical effect of stimulant in reducing hyperactivity was not observed in the SHR model, our study does not fully support the predictive validity of SHR, questioning their validity as an animal model for ADHD.
Neurosci Biobehav Rev, 2019; 100
28923721: Müller D, Grevet EH, Panzenhagen AC, Cupertino RB, da Silva BS, Kappel DB, Mota NR, Blaya-Rocha P, Teche SP, Vitola ES, Rohde LA, Contini V, Rovaris DL, Schuch JB, Bau CHD
Evidence of sexual dimorphism of HTR1B gene on major adult ADHD comorbidities.
Attention-deficit/hyperactivity disorder (ADHD) is a very common psychiatric disorder across the life cycle and frequently presents comorbidities. Since ADHD is highly heritable, several studies have focused in the underlying genetic factors involved in its etiology. One of the major challenges in this search is the phenotypic heterogeneity, which could be partly attributable to the sexual dimorphism frequently seen in psychiatric disorders. Taking into account the well-known sexual dimorphic effect observed in serotonergic system characteristics, we differentially tested the influence of HTR1B SNPs (rs11568817, rs130058, rs6296 and rs13212041) on ADHD susceptibility and on its major comorbidities according to sex. The sample comprised 564 adults with ADHD diagnosed according to DSM-IV criteria and 635 controls. There was no association of any HTR1B SNPs tested in relation to ADHD susceptibility. As for the comorbidities evaluated, after correction for multiple tests, significant associations were observed for both rs11568817 and rs130058 with substance use disorders (P = 0.009 and P = 0.018, respectively) and for rs11568817 with nicotine dependence (P = 0.025) in men with ADHD. In women with ADHD, the same rs11568817 was associated with generalized anxiety disorder (P = 0.031). The observed effects of rs11568817 G allele presence conferring risk to either substance use disorders or generalized anxiety disorder according to sex, suggest an overall scenario where a higher transcriptional activity of HTR1B, resulting from the presence of this allele, is related to externalizing behaviors in men and internalizing behaviors in women. These results are consistent with and expand previous evidence of sexual dimorphism of the serotoninergic system.
J Psychiatr Res, 2017; 95

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-637

TRANSCRIPTOME SEQUENCING REVEALS KEY GENES AND PATHWAYS IN THE DORSOMEDIAL PREFRONTAL CORTEX OF SUICIDE VICTIMS

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Recent studies revealed that the default mode network (DMN) has an outstanding role in psychiatric disorders. However, expressional alterations related to depression and suicidal behavior have not been reported in the dorsomedial prefrontal cortex (DMPFC), a major component of the dorsal DMN. We used RNA sequencing in DMPFC samples to investigate the molecular changes in suicide victims without any medication for chronic depression as compared to controls. More than 1000 genes differed between the groups using log2FC >±1 and adjusted p-value <0.05 criteria. Particularly the important or intriguing 15 related genes were validated with RT-PCR. Gene set enrichment and protein-protein interaction (PPI) network analysis revealed that the cell surface receptor signaling pathway and synaptic signaling were over-represented in suicide victims suggesting that these processes are involved in suicidal behavior. One of the validated differentially expressed genes was the neuronal Ca(2+) -binding protein 2 (NECAB2). Since NECAB2 may have great importance in modulating glutamatergic functions, and previously had not been fully characterized for its role in mood disorders, we aimed to further characterize its distribution in different layers of the DMPFC by performing in situ hybridization and immunohistochemistry. The distributional data were compared with cell type-specific gene expressional data of the Allen Brain Atlas, based on which we suggest that NECAB2 is located mainly in layer II-IV and VI inhibitory neurons. Our results imply extensive gene expressional alterations in the DMPFC indicating that some of these genes may contribute to the altered mental state and behavior of suicide victims.

Pubmed:

31356449: Luz LL, Fernandes EC, Dora F, Lukoyanov NV, Szucs P, Safronov BV
Trigeminal Aδ- and C-afferent supply of lamina I neurons in the trigeminocervical complex.
Nociceptive trigeminal afferents innervating craniofacial area, eg, facial skin and cranial meninges, project to a broad region in the medullary and upper cervical dorsal horn designated as the trigeminocervical complex. Lamina I neurons in the trigeminocervical complex integrate and relay peripheral inputs, thus playing a key role in both cranial nociception and primary headache syndromes. Because of the technically challenging nature of recording, the long-range trigeminal afferent inputs to the medullary and cervical lamina I neurons were not intensively studied so far. Therefore, we have developed an ex vivo brainstem-cervical cord preparation with attached trigeminal nerve for the visually guided whole-cell recordings from the medullary and cervical lamina I neurons. Two-thirds of recorded neurons generated intrinsic rhythmic discharges. The stimulation of the trigeminal nerve produced a complex effect; it interrupted the rhythmic discharge for hundreds of milliseconds but, if the neuron was silenced by a hyperpolarizing current injection, could elicit a discharge. The monosynaptic inputs from the trigeminal Aδ, high-threshold Aδ, low-threshold C, and C afferents were recorded in the medullary neurons, as well as in the cervical neurons located in the segments C1 to C2 and, to a lesser degree, in C3 to C4. This pattern of supply was consistent with our labelling experiments showing extensive cervical projections of trigeminal afferents. Excitatory inputs were mediated, although not exclusively, through AMPA/kainate and NMDA receptors, whereas inhibitory inputs through both GABA and glycine receptors. In conclusion, the trigeminocervical lamina I neurons receive a complex pattern of long-range monosynaptic and polysynaptic inputs from a variety of the trigeminal nociceptive afferents.
Pain, 2019; 160
31423585: Fazekas EA, Morvai B, Zachar G, Dóra F, Székely T, Pogány Á, Dobolyi A
Neuronal activation in zebra finch parents associated with reintroduction of nestlings.
Recent studies of the brain mechanisms of parental behaviors have mainly focused on rodents. Using other vertebrate taxa, such as birds, can contribute to a more comprehensive, evolutionary view. In the present study, we investigated a passerine

songbird, the zebra finch (Taeniopygia guttata), with a biparental caring system. Parenting-related neuronal activation was induced by first temporarily removing the nestlings, and then, either reuniting the focal male or female parent with the nestlings (parental group) or not (control group). To identify activated neurons, the immediate early gene product, Fos protein, was labeled. Both parents showed an increased level of parental behavior following reunion with the nestlings, and no sexual dimorphism occurred in the neuronal activation pattern. Offspring-induced parental behavior-related neuronal activation was found in the preoptic, ventromedial (VMH), paraventricular hypothalamic nuclei, and in the bed nucleus of the stria terminalis. In addition, the number of Fos-immunoreactive (Fos-ir) neurons in the nucleus accumbens predicted the frequency of the feeding of the nestlings. No difference was found in Fos expression when the effect of isolation or the presence of the mate was examined. Thus, our study identified a number of nuclei involved in parental care in birds and suggests similar regulatory mechanisms in caring females and males. The activated brain regions show similarities to rodents, while a generally lower number of brain regions were activated in the zebra finch. Furthermore, future studies are necessary to establish the role of the apparently avian-specific neuronal activation in the VMH of zebra finch parents.
J Comp Neurol, 2020; 528
32009882: Zachar G, Montagnese C, Fazekas EA, Kemecsei RG, Papp SM, Dóra F, Renner É, Csillag A, Pogány Á, Dobolyi A
Brain Distribution and Sexually Dimorphic Expression of Amylin in Different Reproductive Stages of the Zebra Finch () Suggest Roles of the Neuropeptide in Song Learning and Social Behaviour.
The expression of the recently identified neuropeptide, amylin, is restricted in rodents to the postpartum preoptic area and may play a role in the control of parental behaviours and food intake. These processes are substantially different between bird and rodent parents as birds do not lactate but often show biparental care of the offspring. To establish the presence and role of amylin in the bird brain, in the present study, we investigated the distribution of amylin in brains of adult male and female zebra finches in three different reproductive stages (i.e. paired without young, incubating eggs or provisioning nestlings) and in unpaired control birds living in same sex flocks. Amylin mRNA was identified in the hypothalamus of zebra finch by RT-PCR, which was also used to produce probes for hybridisation. Subsequently, hybridisation histochemistry was performed in brain sections, and the labelling signal was quantified and compared between the groups. Amylin showed a much wider brain distribution than that of rodents. A strong and, in some regions, sexually dimorphic label was found in the striatum and several brain regions of the social behavioural network in both males and females. Many regions responsible for the learning of birdsong also contained amylin-positive neurons, and some regions showed sex differences reflecting the fact that vocalisation is sexually dimorphic in the zebra finch: only males sing. Area X (Ar.X), a striatal song centre present only in males, was labelled in paired but not unpaired male. Ar.X, another song centre, the lateral part of the magnocellular nucleus of the anterior nidopallium (lMAN) also contained amylin and had higher amylin label in paired, as opposed to unpaired birds. The wider distribution of amylin in birds as compared to rodents suggests a more general role of amylin in social or other behaviours in avian species than in mammals. Alternatively, parental care in birds may be a more complex behavioural trait involving a wider set of brain regions. The sex differences in song centres, and the changes with reproductive status suggest a participation of amylin in social behaviours and related changes in the singing of males.
Front Neurosci, 2019; 13
33546359: Lékó AH, Kumari R, Dóra F, Keller D, Udvari EB, Csikós V, Renner É, Dobolyi A
Transcriptome Sequencing in the Preoptic Region of Rat Dams Reveals a Role of Androgen Receptor in the Control of Maternal Behavior.
(1) Background: Preoptic region of hypothalamus is responsible to control maternal behavior, which was hypothesized to be associated with gene expressional changes. (2) Methods: Transcriptome sequencing was first applied in the preoptic region of rat dams in comparison to a control group of mothers whose pups were taken away immediately after parturition and did not exhibit caring behavior 10 days later. (3) Results: Differentially expressed genes were found and validated by quantitative RT-PCR, among them NACHT and WD repeat domain containing 1 (Nwd1) is known to control androgen receptor (AR) protein levels. The distribution of Nwd1 mRNA and AR was similar in the preoptic area. Therefore, we focused on this steroid hormone receptor and found its reduced protein level in rat dams. To establish the function of AR in maternal behavior, its antagonist was administered intracerebroventricularly into mother rats and increased pup-directed behavior of the animals. (4) Conclusions: AR levels are suppressed in the preoptic area of mothers possibly mediated by altered Nwd1 expression in order to allow sustained high-level care for the pups. Thus, our study first implicated the AR in the control of maternal behaviors.
Int J Mol Sci, 2021; 22

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-638

ALTERED CIRCADIAN CLOCK GENE EXPRESSION IN THE MPFC OF MOUSE MODEL OF DEPRESSION AND ITS MODULATION BY RAPID ANTIDEPRESSANT TREATMENTS

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Accumulating evidence correlates circadian dysregulation with major depressive disorder (MDD). Circadian clock oscilation in multiple brain regions are implicated in depression, and their manipulation can affect mood, suggesting potential role in MDD pathophysiology and treatment. To investigate this relationship, we have utilized the chronic despair mouse (CDM) model of depression, induced by 5 days of repeated swim stress, in combination with rapid antidepressant treatments (AD) (6h of sleep deprivation and low-dose ketamine) and circadian clock modulation by RORα/ɣ agonist (SR1078). Tissues from the medial prefrontal cortex (mPFC) and suprachiasmatic nucleus (SCN) of CDM, CDM-treated and naïve (control) mice, prepared at 6 different zeitgeber time points, were analysed via qRT-PCR for the oscillating mRNA expression of the canonical clock genes (CCGs) Bmal1, Per1/2, Cry1/2, Rev-ERBα and RORα. Compared to controls, CDM mice exhibited significant dysregulation of CCGs in the mPFC, including upregulation of positive regulatory loop, downregulation of negative circadian loop and altered acrophase. In contrast, the CCGs expression in the SCN was not significantly altered in CDM mice. AD and SR1078 treatments normalized abnormalities in CCG expression in the mPFC and differentially affected CCGs in the SCN. However, the SCN effects were not correlated with changes in the mPFC. Our results illustrate dysregulation of circadian clockwork in the mPFC, independent of the SCN, in a stress-induced model for depression. Changes of mPFC CCGs expression after treatment suggests for potential role of circadian clock in antidepressant mechanism, providing novel targets to develop circadian-based treatments.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-639

PROGRESSIVE CHANGES IN NEURAL CIRCUITS ACTIVATION, BEHAVIOURAL PHENOTYPES AND MOLECULAR AND MICROBIAL PROFILES DURING DIFFERENT STAGES OF STRESS

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Major depression (MD) is a common, relapsing mental illness that affects millions of people worldwide. One of the most studied risk factors associated with MD is stress. Stress can provoke severe psychiatric disorders such as Major Depression (MD) when becomes insidiously chronic. Here, we want to assess which are the most key changes that transforms a stressful situation from adaptative to pathologic. To do so we performed in mice a multidimensional assessment including a comprehensive behavioral characterization, proteomics, metagenomics and an in-depth assessment of neural circuitry reorganization. We divided our mice into control non-stressed (NS), short-term stress (STS) and long-term stress mice (LTS). STS mice received only two days of stress whereas LTS mice underwent the chronic unpredictable mild stress protocol (CUMS) for 28 days. Results indicate that LTS induces much more severe depressive-like symptoms than STS as demonstrated in different behavioural tasks. Moreover, Mass Spectrometry studies reveal huge differences depending on the duration of the stress. The study of the microbiome in faeces also shows that the changes in the microbiota are mostly observed in LTS mice. Lastly, we identified the activation of different neural ensembles mostly in hippocampal subregions depending on the stress duration. In summary, our work suggests that there is a progression in the molecular, behavioural microbial and functional changes induced by stress. Furthermore, we identified key changes that could explain the transition from an adaptative stressful response to a pathological one. In summary, this work sheds light on the underlying mechanisms of stress-induced major depression.

Pubmed:
30700530: de Pins B, Cifuentes-Díaz C, Farah AT, López-Molina L, Montalban E, Sancho-Balsells A, López A, Ginés S, Delgado-García JM, Alberch J, Gruart A, Girault JA, Giralt A
Conditional BDNF Delivery from Astrocytes Rescues Memory Deficits, Spine Density, and Synaptic Properties in the 5xFAD Mouse Model of Alzheimer Disease.
It has been well documented that neurotrophins, including brain-derived neurotrophic factor (BDNF), are severely affected in Alzheimer's disease (AD), but their administration faces a myriad of technical challenges. Here we took advantage of the early astrogliosis observed in an amyloid mouse model of AD (5xFAD) and used it as an internal sensor to administer BDNF conditionally and locally. We first demonstrate the relevance of BDNF release from astrocytes by evaluating the effects of coculturing WT neurons and BDNF-deficient astrocytes. Next, we crossed 5xFAD mice with pGFAP:BDNF mice (only males were used) to create 5xFAD mice that overexpress BDNF when and where astrogliosis is initiated (5xF:pGB mice). We evaluated the behavioral phenotype of these mice. We first found that BDNF from astrocytes is crucial for dendrite outgrowth and spine number in cultured WT neurons. Double-mutant 5xF:pGB mice displayed improvements in cognitive tasks compared with 5xFAD littermates. In these mice, there was a rescue of BDNF/TrkB downstream signaling activity associated with an improvement of dendritic spine density and morphology. Clusters of synaptic markers, PSD-95 and synaptophysin, were also recovered in 5xF:pGB compared with 5xFAD mice as well as the number of presynaptic vesicles at excitatory synapses. Additionally, experimentally evoked LTP was increased in 5xF:pGB mice. The beneficial effects of conditional BDNF production and local delivery at the location of active neuropathology highlight the potential to use endogenous biomarkers with early onset, such as astrogliosis, as regulators of neurotrophic therapy in AD. Recent evidence places astrocytes as pivotal players during synaptic plasticity and memory processes. In the present work, we first provide evidence that astrocytes are essential for neuronal morphology via BDNF release. We then crossed transgenic mice (5xFAD mice) with the transgenic pGFAP-BDNF mice, which express BDNF under the GFAP promoter. The resultant double-mutant mice 5xF:pGB mice displayed a full rescue of hippocampal BDNF loss and related signaling compared with 5xFAD mice and a

significant and specific improvement in all the evaluated cognitive tasks. These improvements did not correlate with amelioration of β amyloid load or hippocampal adult neurogenesis rate but were accompanied by a dramatic recovery of structural and functional synaptic plasticity.
J Neurosci, 2019; 39
35053183: Pérez-Sisqués L, Solana-Balaguer J, Campoy-Campos G, Martín-Flores N, Sancho-Balsells A, Vives-Isern M, Soler-Palazón F, Garcia-Forn M, Masana M, Alberch J, Pérez-Navarro E, Giralt A, Malagelada C
RTP801/REDD1 Is Involved in Neuroinflammation and Modulates Cognitive Dysfunction in Huntington's Disease.
RTP801/REDD1 is a stress-regulated protein whose levels are increased in several neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's diseases (HD). RTP801 downregulation ameliorates behavioral abnormalities in several mouse models of these disorders. In HD, RTP801 mediates mutant huntingtin (mhtt) toxicity in in vitro models and its levels are increased in human iPSCs, human postmortem putamen samples, and in striatal synaptosomes from mouse models of the disease. Here, we investigated the role of RTP801 in the hippocampal pathophysiology of HD. We found that RTP801 levels are increased in the hippocampus of HD patients in correlation with gliosis markers. Although RTP801 expression is not altered in the hippocampus of the R6/1 mouse model of HD, neuronal RTP801 silencing in the dorsal hippocampus with shRNA containing AAV particles ameliorates cognitive alterations. This recovery is associated with a partial rescue of synaptic markers and with a reduction in inflammatory events, especially microgliosis. Altogether, our results indicate that RTP801 could be a marker of hippocampal neuroinflammation in HD patients and a promising therapeutic target of the disease.
Biomolecules, 2021; 12
34131105: Pérez-Sisqués L, Sancho-Balsells A, Solana-Balaguer J, Campoy-Campos G, Vives-Isern M, Soler-Palazón F, Anglada-Huguet M, López-Toledano MÁ, Mandelkow EM, Alberch J, Giralt A, Malagelada C
RTP801/REDD1 contributes to neuroinflammation severity and memory impairments in Alzheimer's disease.
RTP801/REDD1 is a stress-regulated protein whose upregulation is necessary and sufficient to trigger neuronal death. Its downregulation in Parkinson's and Huntington's disease models ameliorates the pathological phenotypes. In the context of Alzheimer's disease (AD), the coding gene for RTP801, DDIT4, is responsive to Aβ and modulates its cytotoxicity in vitro. Also, RTP801 mRNA levels are increased in AD patients' lymphocytes. However, the involvement of RTP801 in the pathophysiology of AD has not been yet tested. Here, we demonstrate that RTP801 levels are increased in postmortem hippocampal samples from AD patients. Interestingly, RTP801 protein levels correlated with both Braak and Thal stages of the disease and with GFAP expression. RTP801 levels are also upregulated in hippocampal synaptosomal fractions obtained from murine 5xFAD and rTg4510 mice models of the disease. A local RTP801 knockdown in the 5xFAD hippocampal neurons with shRNA-containing AAV particles ameliorates cognitive deficits in 7-month-old animals. Upon RTP801 silencing in the 5xFAD mice, no major changes were detected in hippocampal synaptic markers or spine density. Importantly, we found an unanticipated recovery of several gliosis hallmarks and inflammasome key proteins upon neuronal RTP801 downregulation in the 5xFAD mice. Altogether our results suggest that RTP801 could be a potential future target for theranostic studies since it could be a biomarker of neuroinflammation and neurotoxicity severity of the disease and, at the same time, a promising therapeutic target in the treatment of AD.
Cell Death Dis, 2021; 12
30664624: Montalban E, Al-Massadi O, Sancho-Balsells A, Brito V, de Pins B, Alberch J, Ginés S, Girault JA, Giralt A
Pyk2 in the amygdala modulates chronic stress sequelae via PSD-95-related micro-structural changes.
Major depressive disorder (MDD) is a common disorder with a variety of symptoms including mood alterations, anhedonia, sleep and appetite disorders, and cognitive disturbances. Stressful life events are among the strongest risk factors for developing MDD. At the cellular level, chronic stress results in the modification of dendritic spine morphology and density. Here, we study the role of Pyk2 in the development of depressive-like symptoms induced by a model of chronic unpredictable mild stress (CUMS). Pyk2 is a non-receptor calcium-dependent protein-tyrosine kinase highly expressed in the forebrain principal neurons and involved in spine structure and density regulation. We show that Pyk2 knockout mice are less affected to anxiety-like and anhedonia-like phenotypes induced by the CUMS paradigm. Using region-specific knockout, we demonstrate that this phenotype is fully recapitulated by selective Pyk2 inactivation in the amygdala. We also show that in the absence of Pyk2 the spine alterations, PSD-95 clustering, and NMDA receptors changes induced by the CUMS paradigm are prevented. Our results reveal a possible role for Pyk2 in the response to stress and in synaptic markers expression and spine density regulation in the amygdala. We suggest that Pyk2 contributes to stress-induced responses through micro-structural changes and that its deficit may contribute to the resilience to chronic stress.
Transl Psychiatry, 2019; 9
34758331: Pomeshchik Y, Klementieva O, Gil J, Martinsson I, Hansen MG, de Vries T, Sancho-Balsells A, Russ K, Savchenko E, Collin A, Vaz AR, Bagnoli S, Nacmias B, Rampon C, Sorbi S, Brites D, Marko-Varga G, Kokaia Z, Rezeli M, Gouras GK, Roybon L

Human iPSC-derived hippocampal spheroids: An innovative tool for stratifying Alzheimer disease patient-specific cellular phenotypes and developing therapies.
Stem Cell Reports, 2021; 16
32483154: Fernández-García S, Sancho-Balsells A, Longueville S, Hervé D, Gruart A, Delgado-García JM, Alberch J, Giralt A
Astrocytic BDNF and TrkB regulate severity and neuronal activity in mouse models of temporal lobe epilepsy.
Astrocytes have emerged as crucial regulators of neuronal network activity, synapse formation, and underlying behavioral and cognitive processes. Despite some pathways have been identified, the communication between astrocytes and neurons remains to be completely elucidated. Unraveling this communication is crucial to design potential treatments for neurological disorders like temporal lobe epilepsy (TLE). The BDNF and TrkB molecules have emerged as very promising therapeutic targets. However, their modulation can be accompanied by several off-target effects such as excitotoxicity in case of uncontrolled upregulation or dementia, amnesia, and other memory disorders in case of downregulation. Here, we show that BDNF and TrkB from astrocytes modulate neuronal dysfunction in TLE models. First, conditional overexpression of BDNF from astrocytes worsened the phenotype in the lithium-pilocarpine mouse model. Our evidences pointed out to the astrocytic pro-BDNF isoform as a major player of this altered phenotype. Conversely, specific genetic deletion of BDNF in astrocytes prevented the increase in the number of firing neurons and the global firing rate in an in vitro model of TLE. Regarding to the TrkB, we generated mice with a genetic deletion of TrkB specifically in hippocampal neurons or astrocytes. Interestingly, both lines displayed neuroprotection in the lithium-pilocarpine model but only the mice with genetic deletion of TrkB in astrocytes showed significantly preserved spatial learning skills. These data identify the astrocytic BDNF and TrkB molecules as promising therapeutic targets for the treatment of TLE.
Cell Death Dis, 2020; 11
32589876: Pomeshchik Y, Klementieva O, Gil J, Martinsson I, Hansen MG, de Vries T, Sancho-Balsells A, Russ K, Savchenko E, Collin A, Vaz AR, Bagnoli S, Nacmias B, Rampon C, Sorbi S, Brites D, Marko-Varga G, Kokaia Z, Rezeli M, Gouras GK, Roybon L
Human iPSC-Derived Hippocampal Spheroids: An Innovative Tool for Stratifying Alzheimer Disease Patient-Specific Cellular Phenotypes and Developing Therapies.
The hippocampus is important for memory formation and is severely affected in the brain with Alzheimer disease (AD). Our understanding of early pathogenic processes occurring in hippocampi in AD is limited due to tissue unavailability. Here, we report a chemical approach to rapidly generate free-floating hippocampal spheroids (HSs), from human induced pluripotent stem cells. When used to model AD, both APP and atypical PS1 variant HSs displayed increased Aβ42/Aβ40 peptide ratios and decreased synaptic protein levels, which are common features of AD. However, the two variants differed in tau hyperphosphorylation, protein aggregation, and protein network alterations. NeuroD1-mediated gene therapy in HSs-derived progenitors resulted in modulation of expression of numerous genes, including those involved in synaptic transmission. Thus, HSs can be harnessed to unravel the mechanisms underlying early pathogenic changes in the hippocampi of AD patients, and provide a robust platform for the development of therapeutic strategies targeting early stage AD.
Stem Cell Reports, 2020; 15
32477064: Sancho-Balsells A, Brito V, Fernández B, Pardo M, Straccia M, Ginés S, Alberch J, Hernández I, Arranz B, Canals JM, Giralt A
Lack of Helios During Neural Development Induces Adult Schizophrenia-Like Behaviors Associated With Aberrant Levels of the TRIF-Recruiter Protein WDFY1.
The role of the WDFY1 protein has been studied as a TLR3/4 scaffold/recruiting protein in the immune system and in different oncogenic conditions. However, its function in brain remains poorly understood. We have found that in mice devoid of (He mice), a transcription factor specifically expressed during the development of the immune cells and the central nervous system, there is a permanent and sustained increase of gene expression in the striatum and hippocampus. Interestingly, we observed that WDFY1 protein levels were also increased in the hippocampus and dorsolateral prefrontal cortex of schizophrenic patients, but not in the hippocampus of Alzheimer's disease patients with an associated psychotic disorder. Accordingly, young He mice displayed several schizophrenic-like behaviors related to dysfunctions in the striatum and hippocampus. These changes were associated with an increase in spine density in medium spiny neurons (MSNs) and with a decrease in the number and size of PSD-95-positive clusters in the of the CA1. Moreover, these alterations in structural synaptic plasticity were associated with a strong reduction of neuronal NF-κB in the pyramidal layer of the CA1 in He mice. Altogether, our data indicate that alterations involving the molecular axis Helios-WDFY1 in neurons during the development of core brain regions could be relevant for the pathophysiology of neuropsychiatric disorders such as schizophrenia.
Front Cell Neurosci, 2020; 14

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BOARD NUMBER: S04-640

THE EFFECTS OF SIMVASTATIN ON EMOTIONAL PROCESSING AND INFLAMMATION IN HEALTHY VOLUNTEERS: AN EXPERIMENTAL MEDICINE STUDY

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Shona Waters, Riccardo De Giorgi, Alice Quinton, Philip Cowen, Catherine Harmer
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Background: Simvastatin is commonly prescribed to lower cholesterol. Both simvastatin's anti-inflammatory effects and early clinical evidence suggest that it may be an ideal candidate for repurposing in the treatment of depression. Since emotional processing is an early marker of antidepressant response, understanding simvastatin's influence on emotional processing and inflammation could have significant translational value. *Aims*: To investigate the effects of simvastatin on emotional processing and an inflammatory marker in healthy volunteers. *Methods*: Healthy participants (N=53) were randomised to seven days of simvastatin (N=27) or placebo (N=26) in a double-blind fashion. Questionnaires measuring subjective rates of mood and anxiety, and a battery of tasks assessing emotional processing were then administered. Blood samples for the inflammatory marker C-reactive protein were collected before and after intervention. *Results*: Participants on simvastatin had more positively-valenced intrusions in the emotional recall task ($F_{1,51}$= 4.99, p= 0.03), but also an increase in anxiety scores ($F_{1,51}$= 5.37, p= 0.02) compared to controls. An exploratory analysis of female partcipants (N=27) showed the simvastatin subgroup misclassified fewer facial expressions as sad ($F_{1,25}$= 6.60, p= 0.02) compared to controls. No further statistically significant changes were observed. *Conclusions*: We found limited evidence that seven-day simvastatin use in healthy volunteers may induce a positive emotional bias whilst also being associated with an early increase in subjective anxiety. Similar dissociable effects are seen in acute antidepressant administration. This effect may be more evident in female subjects. Different sample populations and treatment lengths in further experimental medicine and clinical studies may clarify these findings.

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BOARD NUMBER: S04-641

MIR-135 AS A NEW TARGET FOR ANTIDEPRESSANT THERAPY: PRECLINICAL STUDY

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Rubén Pavia-Collado[1,2,3,4], Sharon Manashirov[3,4,5], Esther Ruiz-Bronchal[1,2], Irene Rodríguez-Navarro[1,2], Leticia Campa[1,2,3], Analia Bortolozzi[1,2,3]
[1]Institute of Biomedical Research of Barcelona, Neurosciences And Experimental Therapeutics, Barcelona, Spain, [2]August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Systems Neuropharmacology, Barcelona, Spain, [3]Carlos III Health Institute, Biomedical Research Network Center In Mental Health, Madrid, Spain, [4]miCure Therapeutics, Ltd., Scientific Team, Tel-Aviv, Israel, [5]Max Planck Institute of Psychiatry, Stress Neurobiology And Neurogenetics, Munich, Germany

Aims: Major depressive disorder (MDD) is a major health problem worldwide. Most prescribed antidepressants show limited efficacy and delayed onset of action. Accumulating data support the association between MDD and changes in miRNA pathways. Indeed, low miR-135 levels were found in blood and postmortem brain tissue from depressed patients. *In vitro* and *in vivo* studies showed that miR-135 regulates both serotonin transporter (SERT) and 5-HT_{1A} receptor (5-HT_{1A}R) transcripts. The current study aimed to investigate the antidepressant-like effects of a synthetic miR-135 in a mouse model. **Methods:** We have designed a sertraline conjugated-synthetic miR-135 oligonucleotide (c-miR-135) to be accumulated in serotonin (5-HT) neurons after local infusion into dorsal raphe nucleus (DRN) or administered intranasally in mice. Histological and biochemical approaches and stress-related behavioral tests were performed to examine the potential antidepressant-like effects of c-miR-135. **Results:** Intranasal c-miR-135 administration resulted in its selective accumulation in raphe 5-HT neurons. This elicited a marked antidepressant-like effect in the tail suspension test but did not affect anxiety-like behaviors in the dark-light box. In parallel, c-miR-135 administration significantly decreased SERT and 5-HT_{1A}R protein levels in the DRN, without inducing the loss of TPH-positive neurons, astrogliosis, or microgliosis. Moreover, intranasal c-miR-135 administration modifies the extracellular 5-HT levels in the medial prefrontal cortex. Interestingly, a single dose of c-miR-135 reversed depressive-like behaviors in corticosterone-treated mice. **Conclusion:** These results suggest that miRNAs are useful as molecular targets to develop new therapeutic strategies for MDD. C-miR-135 evokes antidepressant-like responses by selectively targeting neuronal populations, opening a way for translational studies.

Pubmed:
35163729: Pavia-Collado R, Rodríguez-Aller R, Alarcón-Arís D, Miquel-Rio L, Ruiz-Bronchal E, Paz V, Campa L, Galofré M, Sgambato V, Bortolozzi A
Up and Down γ-Synuclein Transcription in Dopamine Neurons Translates into Changes in Dopamine Neurotransmission and Behavioral Performance in Mice.
The synuclein family consists of α-, β-, and γ-Synuclein (α-Syn, β-Syn, and γ-Syn) expressed in the neurons and concentrated in synaptic terminals. While α-Syn is at the center of interest due to its implication in the pathogenesis of Parkinson's disease (PD) and other synucleinopathies, limited information exists on the other members. The current study aimed at investigating the biological role of γ-Syn controlling the midbrain dopamine (DA) function. We generated two different mouse models with: (i) γ-Syn overexpression induced by an adeno-associated viral vector and (ii) γ-Syn knockdown induced by a ligand-conjugated antisense oligonucleotide, in order to modify the endogenous γ-Syn transcription levels in midbrain DA neurons. The progressive overexpression of γ-Syn decreased DA neurotransmission in the nigrostriatal and mesocortical pathways. In parallel, mice evoked motor deficits in the rotarod and impaired cognitive performance as assessed by novel object recognition, passive avoidance, and Morris water maze tests. Conversely, acute γ-Syn knockdown selectively in DA neurons facilitated forebrain DA neurotransmission. Importantly, modifications in γ-Syn expression did not induce the loss of DA neurons or changes in α-Syn expression. Collectively, our data strongly suggest that DA release/re-uptake processes in the nigrostriatal and mesocortical pathways are partially dependent on substantia nigra pars compacta /ventral tegmental area (SNc/VTA) γ-Syn transcription levels, and are linked to modulation of DA transporter function, similar to α-Syn.
Int J Mol Sci, 2022; 23
34852293: Alarcón-Arís D, Pavia-Collado R, Miquel-Rio L, Coppola-Segovia V, Ferrés-Coy A, Ruiz-Bronchal E, Galofré M, Paz V, Campa L, Revilla R, Montefeltro A, Kordower JH, Vila M, Artigas F, Bortolozzi A

Corrigendum to "Anti-α-synuclein ASO delivered to monoamine neurons prevents α-synuclein accumulation in a Parkinson's disease-like mouse model and in monkeys" [EBioMedicine 2020; 59:102944].
EBioMedicine, 2021; 74
33805843: Pavia-Collado R, Cóppola-Segovia V, Miquel-Rio L, Alarcón-Aris D, Rodríguez-Aller R, Torres-López M, Paz V, Ruiz-Bronchal E, Campa L, Artigas F, Montefeltro A, Revilla R, Bortolozzi A
Intracerebral Administration of a Ligand-ASO Conjugate Selectively Reduces α-Synuclein Accumulation in Monoamine Neurons of Double Mutant Human A30P*A53T*α-Synuclein Transgenic Mice.
α-Synuclein (α-Syn) protein is involved in the pathogenesis of Parkinson's disease (PD). Point mutations and multiplications of the α-Syn, which encodes the gene, are correlated with early-onset PD, therefore the reduction in a-Syn synthesis could be a potential therapy for PD if delivered to the key affected neurons. Several experimental strategies for PD have been developed in recent years using oligonucleotide therapeutics. However, some of them have failed or even caused neuronal toxicity. One limiting step in the success of oligonucleotide-based therapeutics is their delivery to the brain compartment, and once there, to selected neuronal populations. Previously, we developed an indatraline-conjugated antisense oligonucleotide (IND-1233-ASO), that selectively reduces α-Syn synthesis in midbrain monoamine neurons of mice, and nonhuman primates. Here, we extended these observations using a transgenic male mouse strain carrying both A30P and A53T mutant human α-Syn (A30P*A53T*α-Syn). We found that A30P*A53T*α-Syn mice at 4-5 months of age showed 3.5-fold increases in human α-Syn expression in dopamine (DA) and norepinephrine (NE) neurons of the substantia nigra pars compacta (SNc) and locus coeruleus (LC), respectively, compared with mouse α-Syn levels. In parallel, transgenic mice exhibited altered nigrostriatal DA neurotransmission, motor alterations, and an anxiety-like phenotype. Intracerebroventricular IND-1233-ASO administration (100 µg/day, 28 days) prevented the α-Syn synthesis and accumulation in the SNc and LC, and recovered DA neurotransmission, although it did not reverse the behavioral phenotype. Therefore, the present therapeutic strategy based on a conjugated ASO could be used for the selective inhibition of α-Syn expression in PD-vulnerable monoamine neurons, showing the benefit of the optimization of ASO molecules as a disease modifying therapy for PD and related α-synucleinopathies.
Int J Mol Sci, 2021; 22
32810825: Alarcón-Arís D, Pavia-Collado R, Miquel-Rio L, Coppola-Segovia V, Ferrés-Coy A, Ruiz-Bronchal E, Galofré M, Paz V, Campa L, Revilla R, Montefeltro A, Kordower JH, Vila M, Artigas F, Bortolozzi A
Anti-α-synuclein ASO delivered to monoamine neurons prevents α-synuclein accumulation in a Parkinson's disease-like mouse model and in monkeys.
Progressive neuronal death in monoaminergic nuclei and widespread accumulation of α-synuclein are neuropathological hallmarks of Parkinson's disease (PD). Given that α-synuclein may be an early mediator of the pathological cascade that ultimately leads to neurodegeneration, decreased α-synuclein synthesis will abate neurotoxicity if delivered to the key affected neurons.
EBioMedicine, 2020; 59
29273501: Alarcón-Arís D, Recasens A, Galofré M, Carballo-Carbajal I, Zacchi N, Ruiz-Bronchal E, Pavia-Collado R, Chica R, Ferrés-Coy A, Santos M, Revilla R, Montefeltro A, Fariñas I, Artigas F, Vila M, Bortolozzi A
Selective α-Synuclein Knockdown in Monoamine Neurons by Intranasal Oligonucleotide Delivery: Potential Therapy for Parkinson's Disease.
Progressive neuronal death in brainstem nuclei and widespread accumulation of α-synuclein are neuropathological hallmarks of Parkinson's disease (PD). Reduction of α-synuclein levels is therefore a potential therapy for PD. However, because α-synuclein is essential for neuronal development and function, α-synuclein elimination would dramatically impact brain function. We previously developed conjugated small interfering RNA (siRNA) sequences that selectively target serotonin (5-HT) or norepinephrine (NE) neurons after intranasal administration. Here, we used this strategy to conjugate inhibitory oligonucleotides, siRNA and antisense oligonucleotide (ASO), with the triple monoamine reuptake inhibitor indatraline (IND), to selectively reduce α-synuclein expression in the brainstem monoamine nuclei of mice after intranasal delivery. Following internalization of the conjugated oligonucleotides in monoamine neurons, reduced levels of endogenous α-synuclein mRNA and protein were found in substantia nigra pars compacta (SNc), ventral tegmental area (VTA), dorsal raphe nucleus (DR), and locus coeruleus (LC). α-Synuclein knockdown by ~20%-40% did not cause monoaminergic neurodegeneration and enhanced forebrain dopamine (DA) and 5-HT release. Conversely, a modest human α-synuclein overexpression in DA neurons markedly reduced striatal DA release. These results indicate that α-synuclein negatively regulates monoamine neurotransmission and set the stage for the testing of non-viral inhibitory oligonucleotides as disease-modifying agents in α-synuclein models of PD.
Mol Ther, 2018; 26

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BOARD NUMBER: S04-642

ROLE OF ADULT HIPPOCAMPAL NEUROGENESIS IN THE ANTIDEPRESSANT EFFECTS OF LACTATE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Previous studies on the antidepressant effects of lactate have shown that chronic administration of lactate improved depressive-like behavior in different animal models (Carrard et al., 2018). The antidepressant effects of lactate are associated with changes in the expression of specific target genes among which Hes5 and p11 are involved in adult hippocampal neurogenesis. These findings led us to investigate the role of adult hippocampal neurogenesis in the antidepressant effects of lactate in the corticosterone model of depression. We found that chronic peripheral injection of lactate counteracted the decreased neural progenitor proliferation and survival induced by corticosterone. In contrast, chronic administration of pyruvate, the oxidized form of lactate, did not produce antidepressant effects and did not prevent the inhibition of neural progenitor proliferation and survival induced by corticosterone. Importantly, depletion of adult hippocampal neurogenesis by the antimitotic drug temozolomide suppressed the antidepressant effects of lactate on behavioral despair and anhedonia in animals chronically treated with corticosterone. *In vitro* studies on hippocampal stem cell cultures revealed that corticosterone decreased cell proliferation and increased ROS production. Consistent with our *in vivo* observations, lactate but not pyruvate suppressed the effect of corticosterone on ROS production and partially counteracted the effect of corticosterone on stem cell proliferation. Similarly to lactate, NADH prevented ROS production elicited by corticosterone and partially reversed the inhibition of stem cell proliferation induced by corticosterone. Together, these data suggest that conversion of lactate to pyruvate with the concomitant production of NADH is necessary for the neurogenic and antidepressant effects of lactate.

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BOARD NUMBER: S04-643

DIFFERENTIAL MODULATION OF ANTERIOR CINGULATE CORTEX SUBREGIONAL CONNECTIVITY BY INTRAVENOUS KETAMINE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

Laith Alexander[1], Peter Hawkins[2], Jennifer Evans[3], Carlos Zarate Jr.[3], Mitul Mehta[2]
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The anterior cingulate cortex (ACC) is thought to be an important locus of ketamine's antidepressant action. Different subregions within the ACC appear to undergo differential changes in connectivity in response to ketamine but have yet to be directly compared. In a double-blind, randomised, placebo-controlled crossover trial, we used functional magnetic resonance imaging (fMRI) to compare changes in resting-state functional connectivity within the perigenual (pg)ACC, rostral subgenual (sg)ACC and caudal sgACC two days after intravenous treatment with ketamine or inactive placebo in a group of patients with treatment-resistant depression (TRD, n=29) *vs.* healthy controls (n=21). Intravenous ketamine (0.5mg/kg over 40mins) induced a robust antidepressant response two days after infusion, and differentially modulated functional connectivity across ACC subregions. The caudal sgACC showed reduced connectivity to key nodes of the default mode network (DMN) in participants with TRD. By contrast, rostral sgACC and pgACC showed increased connectivity to nodes of the DMN in TRD compared to healthy controls. However, these connectivity changes did not correlate with antidepressant responses as measured by the Hamilton Depression Rating Scale. Future work will explore whether functional connectivity changes within the ACC correlate with ketamine's action within specific symptom domains, such as improvements in reward processing or reductions in ruminative thinking.

Pubmed:
33984391: Alexander L, Jelen LA, Mehta MA, Young AH
The anterior cingulate cortex as a key locus of ketamine's antidepressant action.
The subdivisions of the anterior cingulate cortex (ACC) - including subgenual, perigenual and dorsal zones - are implicated in the etiology, pathogenesis and treatment of major depression. We review an emerging body of evidence which suggests that changes in ACC activity are critically important in mediating the antidepressant effects of ketamine, the prototypical member of an emerging class of rapidly acting antidepressants. Infusions of ketamine induce acute (over minutes) and post-acute (over hours to days) modulations in subgenual and perigenual activity, and importantly, these changes can correlate with antidepressant efficacy. The subgenual and dorsal zones of the ACC have been specifically implicated in ketamine's anti-anhedonic effects. We emphasize the synergistic relationship between neuroimaging studies in humans and brain manipulations in animals to understand the causal relationship between changes in brain activity and therapeutic efficacy. We conclude with circuit-based perspectives on ketamine's action: first, related to ACC function in a central network mediating affective pain, and second, related to its role as the anterior node of the default mode network.
Neurosci Biobehav Rev, 2021; 127
33982011: Alexander L, Banai-Tizkar R, Wood CM, Roberts AC
Quantifying anhedonia-like symptoms in marmosets using appetitive Pavlovian conditioning.
Blunted reward responsivity is associated with anhedonia in humans and is a core feature of depression. This protocol describes how to train the common marmoset, , on an appetitive Pavlovian conditioning paradigm to measure behavioral and cardiovascular correlates of anticipatory and consummatory phases of reward processing. We describe how to use intracerebral infusions to manipulate brain regions whose activity is relevant to impaired reward processing in depression and how the paradigm can be used to test antidepressant efficacy. For complete details on the use and execution of this protocol, please refer to Alexander et al. (2019).
STAR Protoc, 2021; 2
33106488: Alexander L, Wood CM, Gaskin PLR, Sawiak SJ, Fryer TD, Hong YT, McIver L, Clarke HF, Roberts AC
Over-activation of primate subgenual cingulate cortex enhances the cardiovascular, behavioral and neural responses to threat.

Stress-related disorders such as depression and anxiety are characterized by enhanced negative emotion and physiological dysfunction. Whilst elevated activity within area 25 of the subgenual anterior cingulate cortex (sgACC/25) has been implicated in these illnesses, it is unknown whether this over-activity is causal. By combining targeted intracerebral microinfusions with cardiovascular and behavioral monitoring in marmosets, we show that over-activation of sgACC/25 reduces vagal tone and heart rate variability, alters cortisol dynamics during stress and heightens reactivity to proximal and distal threat. F-FDG PET imaging shows these changes are accompanied by altered activity within a network of brain regions including the amygdala, hypothalamus and dorsolateral prefrontal cortex. Ketamine, shown to have rapid antidepressant effects, fails to reverse elevated arousal to distal threat contrary to the beneficial effects we have previously demonstrated on over-activation induced reward blunting, illustrating the symptom-specificity of its actions.
Nat Commun, 2020; 11
32958652: Stawicka ZM, Massoudi R, Horst NK, Koda K, Gaskin PLR, Alexander L, Santangelo AM, McIver L, Cockcroft GJ, Wood CM, Roberts AC
Ventromedial prefrontal area 14 provides opposing regulation of threat and reward-elicited responses in the common marmoset.
The ventromedial prefrontal cortex (vmPFC) is a key brain structure implicated in mood and anxiety disorders, based primarily on evidence from correlational neuroimaging studies. Composed of a number of brain regions with distinct architecture and connectivity, dissecting its functional heterogeneity will provide key insights into the symptomatology of these disorders. Focusing on area 14, lying on the medial and orbital surfaces of the gyrus rectus, this study addresses a key question of causality. Do changes in area 14 activity induce changes in threat- and reward-elicited responses within the nonhuman primate, the common marmoset, similar to that seen in mood and anxiety disorders? Area 14 overactivation was found to induce heightened responsivity to uncertain, low-imminence threat while blunting cardiovascular and behavioral anticipatory arousal to high-value food reward. Conversely, inactivation enhanced the arousal to high-value reward cues while dampening the acquisition of cardiovascular and behavioral responses to a Pavlovian threat cue. Basal cardiovascular activity, including heart rate variability and sympathovagal balance, which are dysfunctional in mood and anxiety disorders, are insensitive to alterations in area 14 activity as is the extinction of conditioned threat responses. The distinct pattern of dysregulation compared to neighboring region area 25 highlights the heterogeneity of function within vmPFC and reveals how the effects of area 14 overactivation on positive and negative reactivity mirror symptoms of anhedonia and anxiety that are so often comorbid in mood disorders.
Proc Natl Acad Sci U S A, 2020; 117
31163643: Alexander L, Clarke HF, Roberts AC
A Focus on the Functions of Area 25.
Subcallosal area 25 is one of the least understood regions of the anterior cingulate cortex, but activity in this area is emerging as a crucial correlate of mood and affective disorder symptomatology. The cortical and subcortical connectivity of area 25 suggests it may act as an interface between the bioregulatory and emotional states that are aberrant in disorders such as depression. However, evidence for such a role is limited because of uncertainty over the functional homologue of area 25 in rodents, which hinders cross-species translation. This emphasizes the need for causal manipulations in monkeys in which area 25, and the prefrontal and cingulate regions in which it is embedded, resemble those of humans more than rodents. In this review, we consider physiological and behavioral evidence from non-pathological and pathological studies in humans and from manipulations of area 25 in monkeys and its putative homologue, the infralimbic cortex (IL), in rodents. We highlight the similarities between area 25 function in monkeys and IL function in rodents with respect to the regulation of reward-driven responses, but also the apparent inconsistencies in the regulation of threat responses, not only between the rodent and monkey literatures, but also within the rodent literature. Overall, we provide evidence for a causal role of area 25 in both the enhanced negative affect and decreased positive affect that is characteristic of affective disorders, and the cardiovascular and endocrine perturbations that accompany these mood changes. We end with a brief consideration of how future studies should be tailored to best translate these findings into the clinic.
Brain Sci, 2019; 9
30718320: Zeredo JL, Quah SKL, Wallis CU, Alexander L, Cockcroft GJ, Santangelo AM, Xia J, Shiba Y, Dalley JW, Cardinal RN, Roberts AC, Clarke HF
Glutamate Within the Marmoset Anterior Hippocampus Interacts with Area 25 to Regulate the Behavioral and Cardiovascular Correlates of High-Trait Anxiety.
High-trait anxiety is a risk factor for the development of affective disorders and has been associated with decreased cardiovascular and behavioral responsivity to acute stressors in humans that may increase the risk of developing cardiovascular disease. Although human neuroimaging studies of high-trait anxiety reveals dysregulation in primate cingulate areas 25 and 32 and the anterior hippocampus (aHipp) and rodent studies reveal the importance of aHipp glutamatergic hypofunction, the causal involvement of aHipp glutamate and its interaction with these areas in the primate brain is unknown.

Accordingly, we correlated marmoset trait anxiety scores to their postmortem aHipp glutamate levels and showed that low glutamate in the right aHipp is associated with high-trait anxiety in marmosets. Moreover, pharmacologically increasing aHipp glutamate reduced anxiety levels in highly anxious marmosets in two uncertainty-based tests of anxiety: exposure to a human intruder with uncertain intent and unpredictable loud noise. In the human intruder test, increasing aHipp glutamate decreased anxiety by increasing approach to the intruder. In the unpredictable threat test, animals showed blunted behavioral and cardiovascular responsivity after control infusions, which was normalized by increasing aHipp glutamate. However, this aHipp-mediated anxiolytic effect was blocked by simultaneous pharmacological inactivation of area 25, but not area 32, areas which when inactivated independently reduced and had no effect on anxiety, respectively. These findings provide causal evidence in male and female primates that aHipp glutamatergic hypofunction and its regulation by area 25 contribute to the behavioral and cardiovascular symptoms of endogenous high-trait anxiety. High-trait anxiety predisposes sufferers to the development of anxiety and depression. Although neuroimaging of these disorders and rodent modeling implicate dysregulation in hippocampal glutamate and the subgenual/perigenual cingulate cortices (areas 25/32), the causal involvement of these structures in endogenous high-trait anxiety and their interaction are unknown. Here, we demonstrate that increased trait anxiety in marmoset monkeys correlates with reduced hippocampal glutamate and that increasing hippocampal glutamate release in high-trait-anxious monkeys normalizes the aberrant behavioral and cardiovascular responsivity to potential threats. This normalization was blocked by simultaneous inactivation of area 25, but not area 32. These findings provide casual evidence in primates that hippocampal glutamatergic hypofunction regulates endogenous high-trait anxiety and the hippocampal-area 25 circuit is a potential therapeutic target.
J Neurosci, 2019; 39

30528065: Alexander L, Gaskin PLR, Sawiak SJ, Fryer TD, Hong YT, Cockcroft GJ, Clarke HF, Roberts AC
Fractionating Blunted Reward Processing Characteristic of Anhedonia by Over-Activating Primate Subgenual Anterior Cingulate Cortex.
Anhedonia is a core symptom of depression, but the underlying neurobiological mechanisms are unknown. Correlative neuroimaging studies implicate dysfunction within ventromedial prefrontal cortex, but the causal roles of specific subregions remain unidentified. We addressed these issues by combining intracerebral microinfusions with cardiovascular and behavioral monitoring in marmoset monkeys to show that over-activation of primate subgenual anterior cingulate cortex (sgACC, area 25) blunts appetitive anticipatory, but not consummatory, arousal, whereas manipulations of adjacent perigenual ACC (pgACC, area 32) have no effect. sgACC/25 over-activation also reduces the willingness to work for reward. F-FDG PET imaging reveals over-activation induced metabolic changes in circuits involved in reward processing and interoception. Ketamine treatment ameliorates the blunted anticipatory arousal and reverses associated metabolic changes. These results demonstrate a causal role for primate sgACC/25 over-activity in selective aspects of impaired reward processing translationally relevant to anhedonia, and ketamine's modulation of an affective network to exert its action.
Neuron, 2019; 101

28461477: Wallis CU, Cardinal RN, Alexander L, Roberts AC, Clarke HF
Opposing roles of primate areas 25 and 32 and their putative rodent homologs in the regulation of negative emotion.
Disorders of dysregulated negative emotion such as depression and anxiety also feature increased cardiovascular mortality and decreased heart-rate variability (HRV). These disorders are correlated with dysfunction within areas 25 and 32 of the ventromedial prefrontal cortex (vmPFC), but a causal relationship between dysregulation of these areas and such symptoms has not been demonstrated. Furthermore, cross-species translation is limited by inconsistent findings between rodent fear extinction and human neuroimaging studies of negative emotion. To reconcile these literatures, we applied an investigative approach to the brain-body interactions at the core of negative emotional dysregulation. We show that, in marmoset monkeys (a nonhuman primate that has far greater vmPFC homology to humans than rodents), areas 25 and 32 have causal yet opposing roles in regulating the cardiovascular and behavioral correlates of negative emotion. In novel Pavlovian fear conditioning and extinction paradigms, pharmacological inactivation of area 25 decreased the autonomic and behavioral correlates of negative emotion expectation, whereas inactivation of area 32 increased them via generalization. Area 25 inactivation also increased resting HRV. These findings are inconsistent with current theories of rodent/primate prefrontal functional similarity, and provide insight into the role of these brain regions in affective disorders. They demonstrate that area 32 hypoactivity causes behavioral generalization relevant to anxiety, and that area 25 is a causal node governing the emotional and cardiovascular symptomatology relevant to anxiety and depression.
Proc Natl Acad Sci U S A, 2017; 114

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BOARD NUMBER: S04-644

ALTERATIONS IN MICROGLIA AND THEIR LAG3 CHECKPOINT EXPRESSION IN THE HIPPOCAMPUS OF SUICIDAL BIPOLAR DISORDER PATIENTS

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Bipolar disorder (BD) is a brain disorder characterized by extreme mood fluctuations between mania and depression. Previous studies implicated inflammatory processes, in general, and activation of brain microglia, in particular, in the pathophysiology of BD. Studies in our laboratory demonstrated that modulation of microglia in the hippocampus, mediated by the immune checkpoint molecule lymphocyte-activation gene 3 (LAG3), contributes to the development of depression and its associated brain processes in a mouse model of stress-induced depression. Therefore, in the present study we immunohistochemically stained postmortem hippocampal tissues collected from 15 BD patients and 12 healthy controls (HC) with antibodies to the specific microglia marker P2Y12 as well as to LAG3. Given that BD is associated with high rates of suicide attempts and death, which are more than 10-fold greater than in the general population, and that suicide is also associated with neuroinflammation and microglia activation, the BD group was further divided into a subgroup of suicide completers (BD-S; N=9) and another subgroup of death from other reasons (BD-NS, N=6). Overall, we found increased density of P2Y12-labeled microglia in the BD-S group, as compared with both the HC and BD-NS groups. In HC, 13.3% of all microglia expressed LAG-3 (in contrast with our previous findings in mice, in which most microglia express LAG-3). The percentage of LAG3-expressing microglia was significantly lower in BD-S than in both HC and BD-NS groups. These findings suggest that reduced LAG3 immune checkpoint inhibition contributes to microglia proliferation and suicide in BD.

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A SPECIFIC GPR56/ADGRG1 SPLICING ISOFORM TO MONITOR RESPONSE TO ANTIDEPRESSANT TREATMENT IN PATIENT WITH MAJOR DEPRESSIVE DISORDER: A DIGITAL PCR ASSAY

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Aims: Major depressive disorder (MDD) is a psychiatric disorder, that poses a high burden to society and is still difficult to treat. A recent study showed an association between GPR56/ADGRG1 mRNA, MDD and response to antidepressant treatment in blood and in brain. Among GPR56 transcript isoforms, the S4 isoform has recently been associated with microglial synaptic pruning, while microglia is already known as a central player in MDD. Therefore, we surmise that S4 is the specific isoform associated to MDD and antidepressant response. **Methods**: To test our hypothesis, an *in silico* analysis was first performed to identify the different proteins and transcript isoforms of GPR56. This analysis allowed to design endpoint PCR (PCR) and quantitative digital PCR (dPCR) primers. First, the level of S4 mRNA expression in blood leukocytes was analyzed by reverse transcription (RT) followed by PCR. Second, S4 was assessed by RT-dPCR in leukocytes from a cohort of 46 MDD patients including responders (n=31) and non-responders (n=17) to antidepressant treatment. **Results**: S4 isoform is expressed in leukocytes. The RT-dPCR analysis revealed significant differences between responders and non-responders. Considering that gene expression variations are modest in psychiatric disorders, RT-dPCR allowed a better precision of the measurements and thus offered the possibility to better distinguish small variations in expression levels. **Conclusions**: This study highlighted a link between one of the GPR56 transcript variants and response to antidepressant treatment. Furthermore, we may envisage a role of S4 in the pathophysiological mechanisms of MDD and the response to antidepressants via microglial activity.

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ROLE OF TRPA1 IN LPS-INDUCED ANXIETY-LIKE BEHAVIORS AND COGNITIVE IMPAIRMENTS IN MICE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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The transient receptor potential ankyrin 1 (TRPA1) is a cation channel involved in pain and inflammation. Several studies have reported that TRPA1 receptor modulation could be strategies for the treatment of inflammatory diseases including itch, rheumatoid arthritis, and colitis. However, the role of the TRPA1 receptor in anxiety-like behavior and cognitive impairment caused by LPS has not been studied yet. Thus, we investigated the role of the TRPA1 receptor on LPS-induced anxiety behaviors and cognitive impairments with the TRPA1 knock-out (KO) animal model. Here, open field test (OFT), elevated plus maze (EPM), and social interaction test (SIT) were conducted to observe anxiety-like behaviors. TRPA1 KO mice did not show the anxiety-like behaviors induced by LPS administration. In addition, the memory function was evaluated by using novel object recognition test (NORT) and passive avoidance test (PAT). As a result, LPS-induced memory impairment did not appear in TRPA1 KO mice. Moreover, to investigate the role of TRPA1 in neurotransmitters levels, the concentrations of glutamate and GABA were measured in the prefrontal cortex (PFC) and hippocampus in the mouse brain. Repeated injection of LPS caused the increase in glutamate concentration and the decrease in GABA concentration in PFC. However, this alternation of the neurotransmitter did not appear in TRPA1 KO mice. In conclusion, TRPA1 might play an important role in LPS-induced cognitive and anxiety-like disorders by regulating the levels of glutamate and GABA in the PFC. Therefore, TRPA1 is expected to be a therapeutic target for anxiety and cognitive impairment.

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BOARD NUMBER: S04-647

THYMOSIN-α1 NORMALIZES STRESS-INDUCED ABNORMITIES IN DEPRESSIVE-LIKE BEHAVIORS, THE LYMPHOCYTE SYSTEM, NEUROINFLAMMATION, AND NEUROPLASTICITY THROUGH ERK AND BCL-2 PATHWAYS IN MALE MICE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Aim: Among the therapies for depression, restoration of immune functions is proposed to be beneficial. Therefore, we aimed to explore the antidepressant effects of the immunopotentiator thymosin alpha-1 (Tα1) and the underlying ERK and Bcl-2 pathway. **Methods:** C57 male mice were divided into 8 groups: Control, Tα1 (0.4 mg/kg), PD98059 (0.5 mg/kg), ABT-263 (1.5 mg/kg), stress, stress + Tα1, stress + Tα1 + PD98059, stress + Tα1 + ABT-263. PD98056 and ABT-263 are ERK and Bcl-2 inhibitors, respectively. Mice were exposed to chronic unpredictable mild stress (CUMS) for 10 weeks and treated (i.p.) for the last 3 weeks. Affective behaviors were assessed by sucrose preference and elevated plus test. Brain, meninges, spleen and thymus were collected and markers of lymphocytes, neurotransmitter, neuroplasticity and neuroinflammation were done using flow cytometry, HPLC, Western Blot, IHC and Elisa. **Results:** Main findings showed Tα1 treatment improved CUMS induced anhedonia and anxiety. Tα1 treatment recovered the function of central meningeal lymphocytes and hippocampal neuroinflammation, neurotransmitters as well neuroplasticity after stress, such as the increase in CD4/CD8 ratio, IL-4/INF-γ ratio, 5-HT/5-HIAA, PSD-95, DCX, PERK and Bcl-2/Bax ratio. Tα1 also restored peripheral lymphocytic CD4/CD8 and Th2/Th1 (CD4+IL-4+/CD4+IFN-γ+) ratio in spleen and thymus. Interestingly ERK and Bcl-2 inhibitors partly blocked the effect of Tα1 on the brain, spleen and thymus, suggesting that the actions of this immunopotentiator are via these pathways. **Conclusion:** Tα1 normalize stress-induced abnormities in affective behaviors, lymphocyte system, neuroinflammation, neurotransmitters and neuroplasticity through ERK and Bcl-2 pathway in adult male mice.

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M1 ACETYLCHOLINE RECEPTOR IN SOMATOSTATIN INTERNEURONS MEDIATES CORTICAL EXCITATION/INHIBITION BALANCE AND ANTIDEPRESSANT RESPONSES

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Alterations in glutamatergic and GABAergic function in the medial prefrontal cortex (mPFC) are prevalent in individuals with Major Depressive Disorder (MDD), resulting in impaired excitation/inhibition (E/I) balance that compromises the integrity of signal transfer to limbic regions. Scopolamine, a non-selective muscarinic receptor antagonist, produces rapid antidepressant effects by initially targeting M1-type acetylcholine receptors (M1R) in somatostatin (SST) interneurons. We determined how M1R modulate the balance between GABA and glutamate signaling in mPFC by generating mice with conditional deletion of M1R (*M1$^{f/f}$Sst^{Cre+}*) only in SST interneurons. M1R deletion in SST cells resulted in baseline antidepressant- and anxiolytic-like responses in female mice, accompanied by disinhibition of SST interneurons and increased levels of proteins necessary for glutamatergic and GABAergic function in the mPFC. In contrast, male *M1$^{f/f}$Sst^{Cre+}* mice showed resilience to chronic stress that was positively correlated with levels of proteins relevant to glutamate and GABA signaling in the mPFC. Scopolamine-induced behavioral and molecular effects were absent in male and female mice lacking M1R in SST cells. Finally, chemogenetic inhibition of SST interneurons in the mPFC reversed the baseline decreases in stress-relevant behaviors in female *M1$^{f/f}$Sst^{Cre+}* mice. These findings suggest that restoring cortical plasticity and E/I balance via M1R blockade in SST interneurons could represent a promising strategy for antidepressant development. Support: NIMH (K99MH126098, R01MH105910, R01MH077681), NARSAD (29063).

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BOARD NUMBER: S04-649

EARLY LIFE MATERNAL ATTACHMENT GOVERNS MURINE EPIGENETIC ARCHITECTURE OF THE HIPPOCAMPUS AND MODIFIES ADULTHOOD NEUROCHEMISTRY AND SOCIAL BEHAVIOR

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Maternal care plays a crucial role in conditioning early epigenetic programming during postnatal brain development. Individual variations in predisposition for affective disorders may be exacerbated by alterations in early life maternal care. Stress is recognized as a vital factor that may affect maternal behavior, yet owing to high heterogeneity in stress response, the impact of maternal behavior tends to vary widely among individuals.
Our goal is to understand the connection between inborn stress vulnerability, maternal care, and early epigenetic programming using distinct mouse populations that exhibit opposite poles of the behavioral spectrum (social dominance [Dom] and submissiveness [Sub]).Dom mice are stress resilient; in contrast, Sub mice exhibit stress sensitivity, depressive-like behavior, systemic inflammation, and early aging. We demonstrated that Dom dams exhibit significantly higher maternal attachment towards their pups than Sub dams. Dom pups showed an elevated level of expression of hippocampal DNMT3a at postnatal day (PND) 7 and increased global 5-methylated cytosine (5mC) levels at PND 21. In cross-fostering experiments, Sub pups raised with Dom mothers showed elevated expression of DNMT3a and increased levels of 5mC at PND 21, and further displayed increased sociability and social dominance.Reduced representation bi-sulfite sequencing indicated significant hypermethylation of gene promoters connected with social behavior. Further, cross-fostered Sub mice displayed altered hippocampal monoamine levels, resembling neurochemical profile of Dom mice. Collectively, we speculate that epigenetic patterning sculpted by maternal care during early developmental stages shapes the diversity of gene expression patterns that may dictate lifelong stress sensitivity and social behavior.

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LONGITUDINAL EFFECT OF PSYCHIATRIC MEDICATION ON SUICIDAL IDEATION: A PROSPECTIVE COHORT STUDY.

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Aims We followed-up suicide attempters and ideators with mood disorders. We analyzed the use of psychiatric drugs, and the change in suicidal ideation over time. **Methods** Among 800 suicidal ideators and attempters enrolled in Korean COhort for the Model Predicting A Suicide and Suicide related behavior (K-COMPASS), 639 participants evaluated as having bipolar disorder or major depressive disorder through Korean version of Mini-International Neuropsychiatric Interview were analyzed. Columbia-Suicide Severity Rating Scale and psychiatric drug use was assessed at the baseline, at 1, 3, 6, 9, 12, 18, 24, 30, 36, and 42 months. A linear mixed effect model was used to confirm the fixed effect of psychiatric drug use, type, and time on suicidal ideation intensity. **Results** Suicidal ideations of participants with mood disorders showed a tendency to decrease over time. Suicidal ideations were stronger during periods of psychiatric drug use than during periods of no use, but decreased more rapidly over time. Among psychiatric drugs, mood stabilizers showed a greater decrease in suicidal thoughts over time. On the other hand, in participants with bipolar disorders, there was a negative effect on the amount of change in suicidal ideation during the period of antidepressant use. **Conclusion** Psychiatric medications have a positive effect on the time-dependent reduction of suicidal thoughts in mood disorder patients. The use of antidepressants in patients with bipolar disorder can adversely affect suicidal

Table 1. Comparison of Baseline Characteristics in Mood Disorder Patients

Variables	Major Depressive Disorder (N=543)	Bipolar Disorder (N=96)	t/ x^2	p
Age	42.33±18.35	34.43±13.97	4.019	**<0.001**
Sex			0.481	0.488
Male	296 (54.5%)	56 (58.3%)		
Female	247 (45.5%)	40 (41.7%)		
Marital status			2.600	0.107
Married	181 (33.3%)	72 (75.0%)		
Not married	362 (66.7%)	24 (25.0%)		
Living status			0.026	0.872
Alone	154 (28.4%)	28 (29.2%)		
Not alone	389 (71.6%)	68 (70.8%)		
Employment			0.017	0.897
Employed	405 (74.6%)	71 (74.0%)		
Not employed	138 (25.4%)	25 (26.0%)		
Religion			0.088	0.767
Religious	13 (38.2%)	24 (41.4%)		
Not religious	21 (61.8%)	34 (58.6%)		
Health insurance			3.739	0.053
NIH	409 (75.3%)	81 (84.4%)		
Recipient	134 (24.7%)	15 (15.6%)		

ideation.

Table 2. Effect of Psychiatric Medications on Suicidal Ideation of Mood Disorder Patients over Time

Variables	All Mood Disorders			MDD(N=543)			BPD(N=96)		
	B	SE	P	B	SE	P	B	SE	P
All Psychotropics									
Use	**0.6017**	0.2494	**0.016**	0.6764	0.2576	0.064	-0.0446	0.8848	0.960
Time	**-0.0019**	0.0005	**<0.001**	**-0.0017**	0.0005	**0.002**	**-0.0023**	0.0011	**0.046**
Use x Time	**-0.0021**	0.0010	**0.033**	**-0.0024**	0.0010	**0.018**	**0.0087**	0.0039	**0.028**
Antidepressant									
Use	**0.5273**	0.2125	**0.013**	**0.6651**	0.2307	**0.004**	0.0327	0.5407	0.952
Time	**-0.0012**	0.0006	**0.043**	**-0.0013**	0.0006	**0.047**	-0.0007	0.0016	0.669
Use x Time	0.0003	0.0009	0.760	-0.0009	0.0009	0.358	**0.0054**	0.0021	**0.015**
Mood Stabilizers									
Use	**0.6000**	0.3000	**0.046**	0.6089	0.3789	0.108	0.9027	0.6061	0.138
Time	**-0.0046**	0.0012	**<0.001**	**-0.0039**	0.0019	**0.041**	**-0.0047**	0.0016	**0.004**
Use x Time	**-0.0038**	0.0013	**0.002**	-0.0032	0.0020	0.105	-0.0039	0.0021	0.069
Antipsychotics									
Use	-0.1018	0.2290	0.657	**-0.0912**	0.2530	**0.028**	0.1129	0.6280	0.858
Time	**-0.0020**	0.0007	**0.004**	**-0.0021**	0.0009	**0.015**	-0.0016	0.0013	0.205
Use x Time	-0.0015	0.0009	0.105	-0.0017	0.0010	0.104	0.0021	0.0025	0.414
Hypnotics & Sedatives									
Use	**0.4941**	0.2128	**0.029**	**0.5079**	0.2270	**0.025**	0.5601	0.5862	0.340
Time	-0.0011	0.0006	0.071	-0.0009	0.0007	0.180	-0.0011	0.0014	0.438
Use x Time	-0.0004	0.0009	0.654	-0.0006	0.0009	0.517	0.0035	0.0023	0.139

* Adjusted for control variables: age, sex, and previous suicide attempts.

Table 3. Effect of Psychiatric Medications on Suicidal Ideation of Mood Disorder Patients in 90 Days

Variables	All Mood Disorders			MDD(N=543)			BPD(N=96)		
	B	SE	P	B	SE	P	B	SE	P
All Psychotropics									
Use	0.6897	0.2736	0.012	**0.6973**	0.2827	**0.014**	0.6132	0.9668	0.527
Time	**-0.0103**	0.0048	**0.032**	-0.0018	0.0052	0.726	**-0.0422**	0.0115	**<0.001**
Use x Time	0.0147	0.0121	0.226	0.0189	0.0127	0.137	0.0132	0.0338	0.698
Antidepressant									
Use	-0.3510	0.2343	0.135	**0.5519**	0.2534	**0.030**	-0.3006	0.6055	0.620
Time	-0.0009	0.0051	0.867	0.0005	0.0053	0.931	-0.0132	0.0157	0.401
Use x Time	**0.0224**	0.0087	**0.010**	0.0110	0.0095	0.248	**0.0455**	0.0216	**0.037**
Mood Stabilizers									
Use	**0.7134**	0.3376	**<0.001**	0.5663	0.4129	0.171	1.1627	0.7187	0.108
Time	**-0.0321**	0.0091	**0.035**	-0.0164	0.0133	0.219	**-0.0415**	0.0140	**0.004**
Use x Time	-0.0289	0.0105	0.006	-0.0136	0.0144	0.344	-0.0295	0.0228	0.196
Antispychotics									
Use	-0.0429	0.2564	0.867	-0.1371	0.2816	0.626	0.4099	0.7150	0.567
Time	**-0.0180**	0.0064	**0.005**	-0.0058	0.0075	0.440	**-0.0431**	0.0127	**0.001**
Use x Time	-0.0107	0.0086	0.213	0.0009	0.0095	0.923	-0.0148	0.0220	0.502
Hypnotics & Sedatives									
Use	**0.5158**	0.2398	**0.032**	0.4890	0.2554	0.086	1.0994	0.6727	0.104
Time	-0.0081	0.0053	0.131	-0.0016	0.0057	0.786	**-0.0347**	0.0133	**0.010**
Use x Time	0.0003	0.0090	0.974	0.0058	0.0099	0.553	0.0230	0.0216	0.287

* Adjusted for control variables: age, sex, and previous suicide attempts.

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ACOUSTIC ANALYSIS OF SPEECH FOR SCREENING FOR SUICIDE RISK

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Aims: Assessment of a patient's risk of committing suicide is challenging for health professionals, as it depends largely on voluntary disclosure of the patient. This study aimed to examine the feasibility of using acoustic features as a biomarker for suicidality, independent of speech content. **Methods**: One-hundred patients diagnosed with mood disorder and 88 healthy subjects without psychiatric diagnosis were included in the study. All study participants underwent a semi-structured interview and completed self-report psychiatric questionnaires. Chi-square test and Student's t-test were used to compare baseline characteristics of suicidal (defined as Beck Scale for Suicidal Ideation ≥ 15) and non-suicidal groups. Several machine learning methods were evaluated for detecting suicidality. **Results**: Fifty-seven subjects were classified as being at high risk of suicide among 188 study participants. Suicidal and non-suicidal groups did not differ significantly in distribution of sex, income, and medical comorbidity. Compared to the non-suicidal group, the suicidal group was younger, had greater body mass index, was prescribed with higher dose of antipsychotics, and scored higher in all psychiatric scales such as Patient Health Questionnaire-9, Hamilton's Depression Rating Scale, Beck's Anxiety Inventory, except Barratt Impulsiveness Scale-11. Random Forest Classifier, using 21 acoustic features and two demographic variables, sex and age, correctly identified patients with high suicidality with the mean accuracy of 0.72 and an AUC of 0.69. **Conclusion:** Speech analysis using acoustic features is a promising approach for detection of suicide risk. A machine learning classifier using voice may eventually assist in identifying high-risk patients in clinical setting.

Table 1. Demographic and clinical characteristics of suicidal VS non-suicidal groups

	Suicidal Mean (SD)	Non-suicidal Mean (SD)	*P* value
N	57	131	
Age	29.61 (9.73)	36.61 (11.54)	<0.001
Sex (M/F)	13/44	24/107	0.609
Body mass index	24.72 (4.87)	22.95 (3.52)	0.016
Household income†*	500(50-5000)	500(74-1700)	0.657
Antipsychotics dosage‡*	5(0-504)	0(0-200)	0.071
Medical comorbidity (%)	16.7	9.52	0.638
PHQ	17.42(5.80)	3.40(4.99)	<0.001
HDRS	17.93(4.49)	6.85(5.42)	<0.001
BAI	28.10(16.34)	5.24(9.35)	<0.001
BIS	63.81(7.85)	63.88(8.03)	0.106
BSI	23.37(5.78)	2.71(3.82)	<0.001

†Unit = 10,000 won
‡Antipsychotics dosage converted into the dose equivalent of aripiprazole
*Median (Range)
PHQ: Patient Health Questionnaire-9; HDRS: Hamilton Depression Rating Scale; BAI: Beck Anxiety Inventory; BIS: Barratt Impulsiveness Scale-11; BSI: Beck Scale for Suicidal Ideation

Figure 1. Confusion matrix showing the classification results between suicidal VS non-suicidal groups by acoustic features

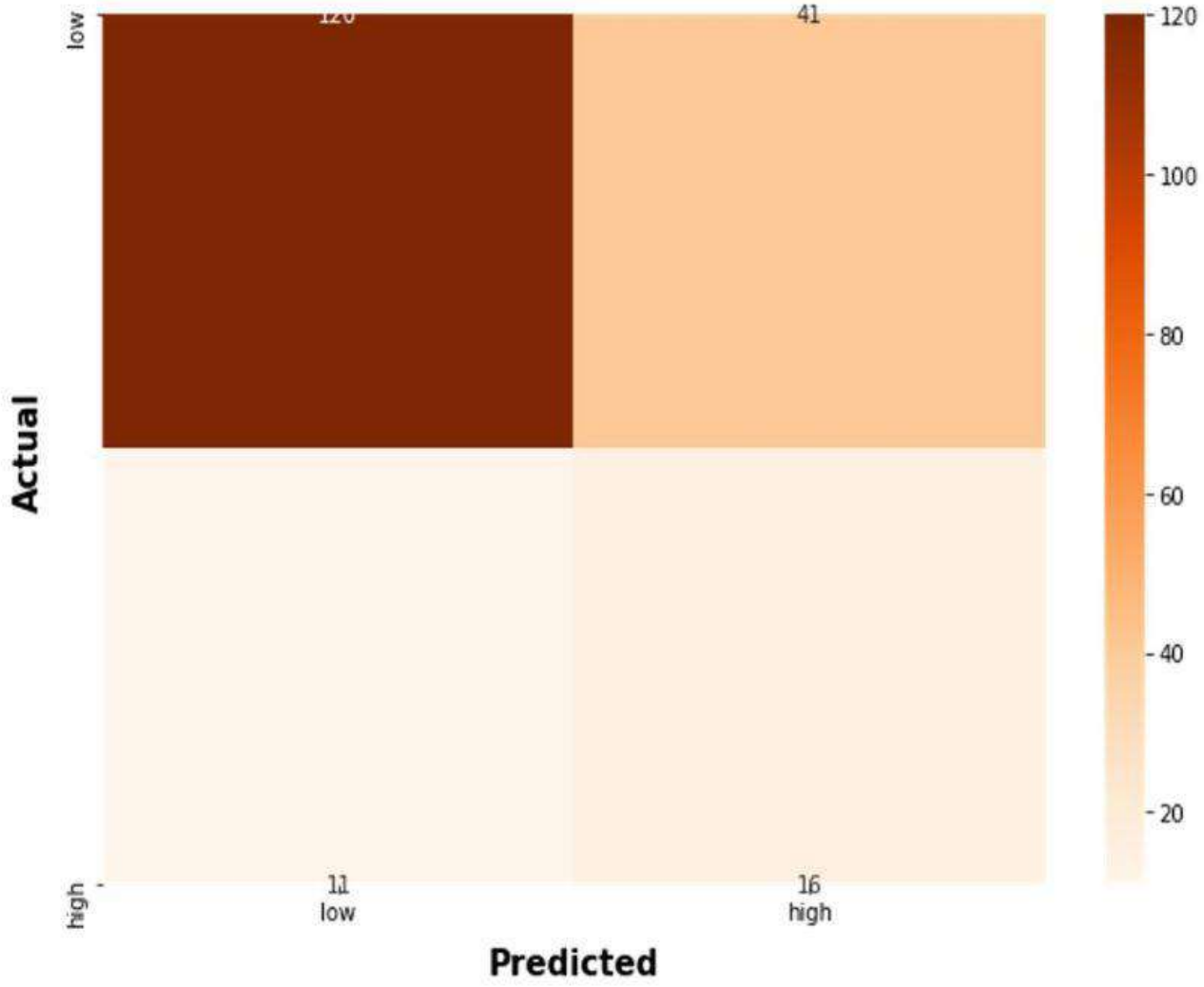
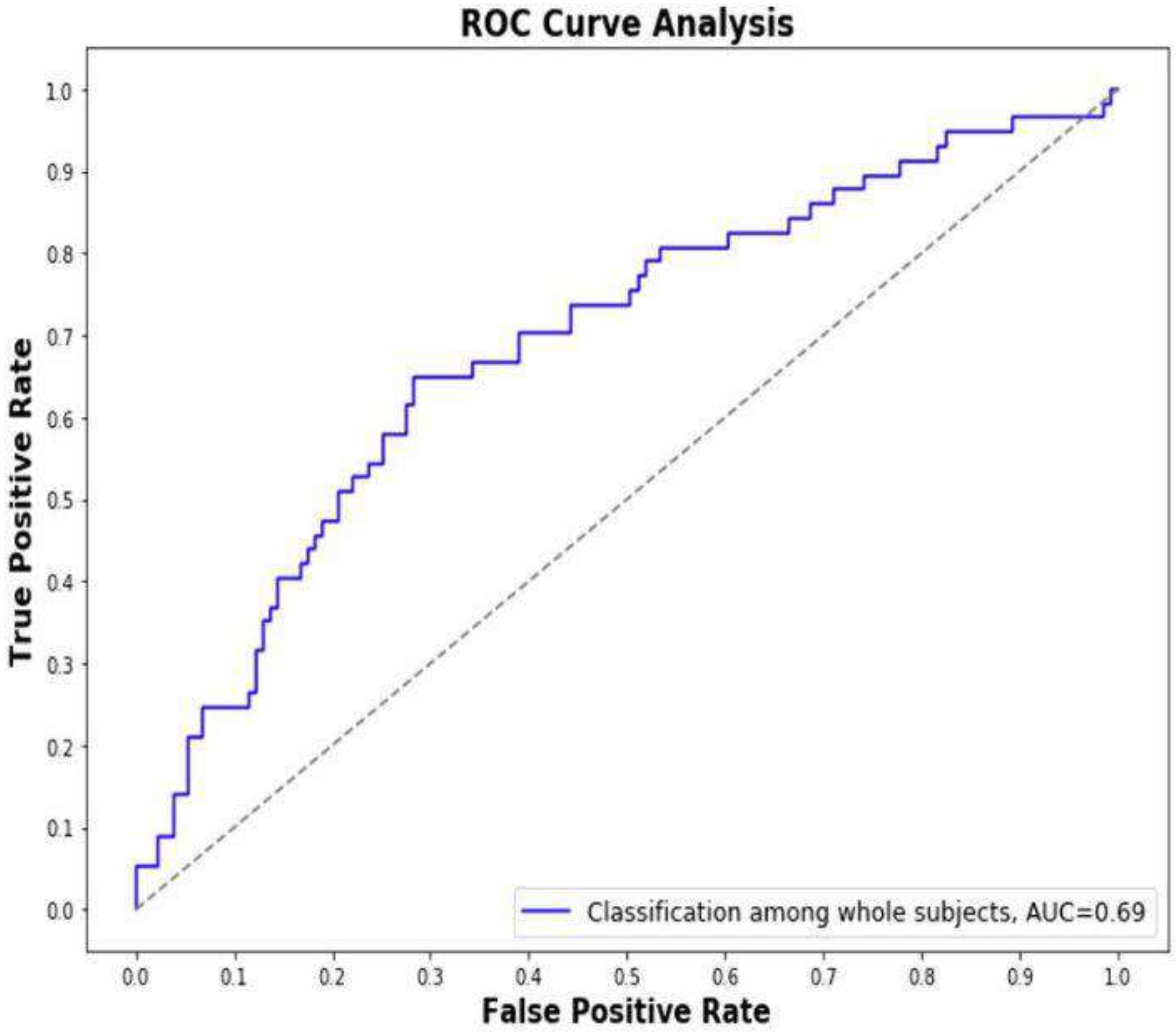


Figure 2. Receiver operating characteristic curve of classification between suicidal VS non-suicidal groups by acoustic features



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NETWORK ANALYSIS BETWEEN SUICIDE-RELATED SYMPTOMS AND SUICIDE ATTEMPT RISK IN PSYCHOTIC DISORDER AND MOOD DISORDER: A PROSPECTIVE COHORT STUDY.

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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We followed-up suicide attempters and ideators with mood disorders over the age of 16 years. We followed up with various psychiatric scales. A network analysis was performed on the relationship between suicide attempts within 6 months and the scale. Among 800 suicidal ideators and attempters enrolled in Korean COhort for the Model Predicting A Suicide and Suicide related behavior (K-COMPASS), 639 participants evaluated as having mood disorder and 56 participants as having psychotic disorders through Korean version of Mini-International Neuropsychiatric Interview were analyzed. Psychiatric scales including the Columbia-Suicide Severity Rating Scale(C-SSRS), Young Mania Rating Scale (YMRS), Alcohol Use Disorders Identification Test (AUDIT), Stress Questionnaire for Korean National Health and Nutrition Examination Survey (KNHANES), Barratt Impulsiveness Scale (BIS), Early Trauma Inventory(ETI), Montgomery Asberg Depressiion Rating Scale(MADRS), Young Mania Rating Scale(YMRS), and Brief Psychiatric Rating Scale(BPRS). Partial correlation network analysis was performed to confirm the effect of psychiatric symptoms on suicidal attempt within 6 months. Participants with mood disorders and psychotic disorders were analyzed separately. In patients with psychotic disorder, various clinical measures such as YMRS, MADRS, PHQ, BAI, AUDIT, and C-SSRS significantly predicted suicide attempts during the follow-up period. On the other hand, in patients with mood disorders, only CSSRS, YMRS, and AUDIT predicted suicide attempts, and the correlation was lower. The correlation stability measured in both models was acceptable as 0.517 and 0.516, respectively. While psychiatric symptoms directly affect suicide attempts in patients with psychotic disorder, factors other than psychiatric symptoms may influence suicide attempts in mood disorder patients.

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THIS ISN'T THE RHYTHM OF THE NIGHT: EFFECTS OF ACUTE DISRUPTION OF THE LIGHT-DARK CYCLE ON DEPRESSIVE SYMPTOMS IN MICE

POSTER SESSION 04 - SECTION: RISK FACTORS AND TREATMENT OF MOOD DISORDERS

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Aims: Circadian rhythms are natural cyclical processes that internally regulate nearly all physiological activities. Since circadian disturbances are a core feature of depression and other mood disorders, we aimed to investigate whether environmental changes in the light-dark (LD) cycle can disrupt the inner clock and subsequently confer susceptibility to develop depressive and anxiety-like behaviors. **Methods:** We used C57BL/6 adult male mice housed under either a 12:12h LD cycle or an altered LD cycle with randomized light hours for 10 days. First, to validate the alteration of circadian machinery, we analyzed several clock-gene expressions at different time-points in brain areas related to mood control (i.e. medial prefrontal cortex (mPFC)) and circadian rhythms (i.e. suprachiasmatic nucleus (SCN)). Additionally, we evaluated the spontaneous locomotor activity considering it provides a measure of circadian fluctuations. Lastly, we performed a battery of tests to assess depression and anxiety-like behaviors. **Results:** Our model of altered LD cycle produces changes in locomotion schedule throughout the day with an increase in activity preceding the start of dark phase. Moreover, it attenuates the rhythmicity of clock-gene expressions in mPFC and subtly impairs their oscillations in SCN. Finally, acute exposure to irregular hours of light did not seem to induce anhedonia, anxiety nor despair-like behavior. **Conclusions:** Our findings suggest that temporary random changes in light-dark cycle may modify behavior's circadian rhythmicity. However, although we show molecular changes in mood-related brain areas, these light shifts are not enough to promote clear mood alterations in adult male mice.

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CORTEX MOLECULAR SIGNATURES ANALYSIS OF A MOUSE MODEL OF KCNQ2-RELATED EPILEPTIC AND DEVELOPMENTAL ENCEPHALOPATHY.

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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KCNQ2-related Epileptic and Developmental Encephalopathies (DEE) are rare and severe epileptic syndromes characterized by seizures in the first 3 months of life, abnormal interictal EEG, and a poor prognosis with severe motor and cognitive consequences. We produced the first knock-in model of the disease. As observed in patients, this model presents a progressive pathology with early epileptic seizures, remission of seizures after several weeks of life, and significant neurological deficits. To better understand the pathophysiology, we compared the proteome and the transcriptome of the prefrontal cortex of the *Kcnq2*-DEE mice at three stages: before seizure onset (P15), during the seizure susceptibility phase (P25-30), and after seizure arrest (P>100) and subsequently performed multi-omics integration. Our results show that SNARE proteins and proteins involved in mitochondrial metabolism are under-expressed in the KI compared to WT at the seizure susceptibility phase (P25-30). Abnormally expressed SNARE or mitochondrial proteins have been described in other types of epilepsy. These results reveal that physiological mechanisms important for neuronal activity are disrupted at early stages in the *KCNQ2*-DEE cortex and that the evolution towards remission of epileptic seizures could be accompanied by specific molecular signatures in the mutated cortex. Similar studies are underway using cortical neurons derived from patients' pluripotent stem cells. Through the integration of human and mouse omics data, we hope to contribute to a better understanding of the neuronal mechanisms at play in *KCNQ2*-DEE and to identify new therapeutic targets.

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ELUCIDATING THE MECHANISMS LEADING TO EPILEPSY IN A DEPDC5 MOUSE MODEL

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Focal cortical dysplasias (FCD) are cortical malformations causing pediatric refractory epilepsies, often requiring surgical resection to control seizures. FCD type II is characterized by a cortical dyslamination and the presence of dysmorphic neurons. Over the past years, both germline and somatic mutations have been found in FCD, in genes of the mTOR (mammalian target of rapamycin) pathway, in particular DEP domain-containing 5 protein (DEPDC5), a repressor of the amino-sensing branch of the mTORC1 (mTOR complex 1) pathway. It has recently been proven that a brain second hit inactivation of this gene causes constitutive activation of mTORC1 and FCDII associated with epilepsy (*Ribierre et al, 2018*). However, little is known on how a loss-of-function of DEPDC5 leads to epilepsy. To get further insights into the underlying pathophysiological mechanisms, we used a *Depdc5* knockout (KO) strain that combines a germline mutation and a neuron-specific deletion of the second allele (*Bacq et al., 2020*). This mouse model exhibits spontaneous epileptic seizures followed by a sudden unexpected death in epilepsy (SUDEP)-like event. We performed multi-electrode arrays (MEA) recordings in cortical brain slices in *Depdc5KO* mice and show hyperexcitability of the network. By performing bulk RNA sequencing on cortical lysates from Depdc5 adult mice, GSE analysis identified multiple dysregulated pathways such as mTOR signaling or mitochondrial functions that may underlie hyperexcitability and seizures, and pinpoint new therapeutic targets to treat children with FCD.

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BOARD NUMBER: S04-656

HEALTHY LIFE-STYLE APPROACHES TO ATTAIN DISEASE MODIFICATIONS IN ACQUIRED EPILEPSIES

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Healthy life-style was reported to improve neurological outcomes after acute brain injuries. In particular, physical activity might improve neurological deficits and reduce seizures in epilepsy. Our study investigates whether aerobic and regular physical exercise reduces the risk of developing epilepsy and disease severity following a focal brain lesion. C57BL66/N adult male mice were given free access to running wheels in their home cage for 3 weeks before being exposed to intra-amygdala kainate to induce a status epilepticus that leads to epilepsy development. Then, injured mice were allowed to run for 6 additional weeks, a time required for chronic epilepsy development. Control mice were similarly treated with kainate but left in their home cage in the absence of running wheels (sedentary mice). Sham mice were prepared as controls for post-mortem histological analysis. Data show that running mice develop status epilepticus of reduced severity (decreased number of spikes, $p<0.05$) vs sedentary mice. Moreover, running mice developed spontaneous seizures of reduced duration ($p<0.05$) vs sedentary mice while epilepsy incidence and seizures frequency were not modified. Nissl staining showed attenuation of hilar interneurons loss in the hippocampus of running mice vs sedentary mice. Data support the beneficial effects of physical activity to improve pathologic outcomes after epileptogenic brain lesions.

Pubmed:
34128226: Di Nunzio M, Di Sapia R, Sorrentino D, Kebede V, Cerovic M, Gullotta GS, Bacigaluppi M, Audinat E, Marchi N, Ravizza T, Vezzani A
Microglia proliferation plays distinct roles in acquired epilepsy depending on disease stages.
Microgliosis occurs in animal models of acquired epilepsy and in patients. It includes cell proliferation that is associated with seizure frequency and decreased neuronal cells in human epilepsy. The role of microglia proliferation in the development of acquired epilepsy is unknown; thus, we examined its contribution to spontaneous seizure, neurodegeneration, and cognitive deficits in different disease phases.
Epilepsia, 2021; 62
34358616: Di Sapia R, Zimmer TS, Kebede V, Balosso S, Ravizza T, Sorrentino D, Castillo MAM, Porcu L, Cattani F, Ruocco A, Aronica E, Allegretti M, Brandolini L, Vezzani A
CXCL1-CXCR1/2 signaling is induced in human temporal lobe epilepsy and contributes to seizures in a murine model of acquired epilepsy.
CXCL1, a functional murine orthologue of the human chemokine CXCL8 (IL-8), and its CXCR1 and CXCR2 receptors were investigated in a murine model of acquired epilepsy developing following status epilepticus (SE) induced by intra-amygdala kainate. CXCL8 and its receptors were also studied in human temporal lobe epilepsy (TLE). The functional involvement of the chemokine in seizure generation and neuronal cell loss was assessed in mice using reparixin (formerly referred to as repertaxin), a non-competitive allosteric inhibitor of CXCR1/2 receptors. We found a significant increase in hippocampal CXCL1 level within 24 h of SE onset that lasted for at least 1 week. No changes were measured in blood. In analogy with human TLE, immunohistochemistry in epileptic mice showed that CXCL1 and its two receptors were increased in hippocampal neuronal cells. Additional expression of these molecules was found in glia in human TLE. Mice were treated with reparixin or vehicle during SE and for additional 6 days thereafter, using subcutaneous osmotic minipumps. Drug-treated mice showed a faster SE decay, a reduced incidence of acute symptomatic seizures during 48 h post-SE, and a delayed time to spontaneous seizures onset compared to vehicle controls. Upon reparixin discontinuation, mice developed spontaneous seizures similar to vehicle mice, as shown by EEG monitoring at 14 days and 2.5 months post-SE. In the same epileptic mice, reparixin reduced neuronal cell loss in the hippocampus vs vehicle-injected mice, as assessed by Nissl staining at completion of EEG monitoring. Reparixin administration for 2 weeks in mice with established chronic seizures, reduced by 2-fold on average seizure number vs pre-treatment baseline, and this effect was reversible upon drug discontinuation. No significant changes in seizure number were measured in vehicle-injected epileptic mice that were EEG monitored in parallel. Data show that CXCL1-IL-8 signaling is activated in experimental and human epilepsy and contributes to acute and chronic seizures in

mice, therefore representing a potential new target to attain anti-ictogenic effects.
Neurobiol Dis, 2021; 158
30639510: David J, Gormley S, McIntosh AL, Kebede V, Thuery G, Varidaki A, Coffey ET, Harkin A
L-alpha-amino adipic acid provokes depression-like behaviour and a stress related increase in dendritic spine density in the pre-limbic cortex and hippocampus in rodents.
Astrocyte dysfunction is implicated in clinical depression. There is a paucity of animal models to assess the role of astrocytes in depression pathogenesis. Refinement of an existing model is described here. Administration of the astrocytic toxin L-alpha aminoadipic acid (L-AAA) to the pre-limbic cortex (PLC) was assessed in rats and mice in tests of anxiety and depression related behaviours. Delivery of L-AAA to the PLC of Wistar rats produced an increase in immobility in the forced swimming test (FST) and reduced exploration in the open field. Delivery to the CA3 subfield of the hippocampus produced a deficit in the novel object relocation task. Delivery of single or two successive doses of L-AAA to the PLC of C57Bl6/J mice was sufficient to induce an increase in immobility in the mouse tail suspension (TST) and FST independently of administration of anaesthetic agent or the surgical procedure. In both mice and rats, L-AAA produced a reduction in immunoreactivity of the astrocytic marker glial fibrillary acidic protein (GFAP) for up to 72 h. L-AAA provoked an increase in the density of apical and basal dendritic spines in mice exposed to the FST when compared to non-FST controls. In summary, L-AAA provokes a region-dependent change in behaviour, a reduction in GFAP immunoreactivity and FST-provoked increased in dendritic spine density in the PLC. This model may be further employed to assess the impact of astroglial integrity on the structural plasticity of neurons and the effect of antidepressant agents on L-AAA-related changes.
Behav Brain Res, 2019; 362

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DEFICITS IN BRAIN ENERGY METABOLISM IN A MOUSE MODEL FOR GLUT1 DEFICIENCY SYNDROME

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Glucose transporter type 1 deficiency syndrome (Glut1DS), also called De Vivo disease, is caused by mutations in *SLC2A1* gene that encodes for glucose transporter type 1 (Glut1). In human, this mutation results in deficient glucose transport to the brain and generates early onset epilepsy, complex movement disorders and cognitive impairment. In order to assess the impact of Glut1 deficiency on brain energy metabolism, we measured glycogen, lactate, and glucose levels in the cerebral cortex and hippocampus of a male and female mouse model for Glut1DS (GLUT1 +/-). A significant decrease in glucose levels was already observed at 2 weeks both in the hippocampus and cerebral cortex of GLUT1 +/- mice compared to wild type (WT) animals (-29% and -42%, respectively) together with a significant reduction in glycogen (-30%) and lactate (-36.9% and -24.7%) levels. In 10-week old mice, similar decreases in glucose, glycogen and lactate hippocampal and cortical levels were observed in GLUT1 +/- compared to WT mice.These data show that Glut1 deficiency has an early and marked impact on brain energy metabolism not limited to glucose levels but extending to other important energy substrates including lactate and glycogen. As astrocytes store glycogen and release lactate, an important energy substrate for neurons and signaling molecule, these data suggest that Glut1DS may affect the metabolic cooperation between astrocytes and neurons. Further elucidation of the mechanisms underlying alterations in the astrocyte-neuron metabolic cooperation in GLUT1+/- mice should help to develop therapeutic targets for the treatment of De Vivo disease.

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BOARD NUMBER: S04-658

TWO CANDIDATE K-CL COTRANSPORTER 2 (KCC2) ENHANCERS PREVENT EPILEPTIFORM ACTIVITY IN VITRO AND IN VIVO

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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In neurons, the cation-chloride cotransporters (CCC) KCC2 and Na-K-Cl cotransporter 1 (NKCC1) control intraneuronal chloride homeostasis and, subsequently, the efficacy and polarity of gamma-aminobutyric acid (GABA) signaling. In many neurological disorders, including epilepsy, reduced KCC2 expression or function may result in depolarizing GABA signaling that may then contribute to pathological activities and seizures. Compensating for the dysregulation of CCC function in the pathology therefore appears as a promising therapeutic strategy. Various compounds derived from library screening were recently identified as candidate KCC2 enhancers. However, their mode of action in cortical neurons and their therapeutic potential in epilepsy remain unknown or even controversial. We first compared the mode of action of these two compounds in rat hippocampal neurons. We showed that both of them enhance KCC2 function and this effect was also accompanied by a direct modulation of GABA signaling. Single particle tracking experiments revealed that both compounds reduce the lateral diffusion of KCC2 in the plasma membrane. This effect was associated with enhanced KCC2 clustering with no change in total or plasmalemmal KCC2 expression. Both compounds effectively suppressed spontaneous, interictal-like discharges recorded in postoperative hippocampal tissue resected from intractable, mesial temporal lobe epilepsy patients. In addition, chronic administration of a Food and Drug Administration (FDA)-approved KCC2 enhancer reduced seizure occurrence by about 67% in a lithium-pilocarpine mouse model of temporal lobe epilepsy. Collectively, our data decipher the mode of action of two candidate KCC2 enhancers in hippocampal neurons and demonstrate for the first time their antiepileptic potential in pharmacoresistant epilepsy.

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THE ABSENCE OF SV2A IN INTERNEURONS LEADS TO EPILEPSY

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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The SV2A protein is a glycoprotein present in the membranes of most synaptic vesicles whose physiological role remains unknown. However, it has been demonstrated that levetiracetam, an effective anti-epileptic drug, binds to SV2A. Moreover, SV2A expression is down-regulated in epileptic foci resected in humans with temporal lobe epilepsy, and *SV2A* full knock-out mice exhibited seizures around post-natal day 7 (P7) and die around P15. AIMS: This project aims to understand how SV2A protein may be involved in epilepsy. First, we wanted to test if the absence of SV2A could lead to seizures appearance after complete brain development. Next, we wished to unravel the sub-population of neurons responsible for seizures onset. METHODS: We induced recombination in Ubiquitin^{ERT2}:SV2A-cKO mice at P70 and observed the obtained phenotype. Next, we compared the phenotype of Dlx5,6:SV2A-cKO (targeting GABAergic interneurons) with Nex:SV2A-cKO (targeting glutamatergic neuron) mice. RESULTS: Ubiquitin^{ERT2}:SV2A-cKO mice recombinated at P70 presented seizures and died one to two months after while WT mice behaved normally. Then, we observed that Dlx5,6:SV2A-cKO mice exhibited lethal spontaneous seizure while Nex:SV2A-cKO had no evident phenotype. Furthermore, Dlx5,6:SV2A-cKO mice present a significant decreased of PV neuron density in the hippocampus. Mass spectrometry analysis revealed that Dlx and Nex:SV2A-cKO synaptosomes present the same modification compared to WT. CONCLUSIONS: Our results show that the absence of SV2A can lead to seizures after the complete brain development. We also observed that GABAergic neurons are sufficient to induce seizures in mice although they seem to bear the same molecular defect as glutamatergic neurons.

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MODELLING MONOGENIC EPILEPSY IN HUMAN BRAIN SLICE CULTURES

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Background Early infantile developmental and epileptic encephalopathies of genetic origin are devastating conditions, but the pathological mechanisms often remain obscure. A major obstacle is the difficulty of studying human cortical brain development, *in utero*. To date, no *in vitro* preparations, not even human cerebral organoids, have been developed that accurately reproduce the complex cellular networks found beyond the first trimester. **Methods** To address this, we established human brain slice cultures prepared from ethically sourced, 14-17 post conception week (pcw) brain tissue (www.hdbr.org). The gross anatomical structures of the marginal zone, cortical plate and subplate are maintained in these cultures for several months, while new synaptic networks form. **Results** We used this model system to examine variants in STXBP1, which encodes a critical presynaptic protein. Specifically, we induced STXBP1 haploinsufficiency, thereby mimicking a common genetic cause of developmental and epileptic encephalopathy. STXBP1 was enriched near spine-like structures and along putative axons of subplate neurons at 16-17 pcw. We achieved a ~50% reduction in STXBP1 expression, using a short hairpin RNA interference introduced by adeno-associated viral vectors, and quantified the effects using confocal microscopy and electrophysiological techniques. The induced STXBP1 haploinsufficiency had divergent effects upon glutamatergic and GABAergic synaptic number and function, without altering subplate neurite length and number. Furthermore, live imaging of synaptic vesicles with reduced STXBP1 levels revealed impaired spontaneous neurotransmitter release with slower release kinetics. **Conclusion** We provide a critical proof-of-principle for how to investigate the aetiology of monogenic epilepsy in prenatal neurodevelopment.

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TBC1D24 INTERACTS WITH V-ATPASE AND REGULATES PH HOMEOSTASIS AND AUTOPHAGY IN NEURONS.

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Background: *Tbc1d24* is a gene mutated in a spectrum of neurological disorders, from mild epilepsy to severe epileptic encephalopathy. TBC1D24 regulates neurite development, synaptic vesicle trafficking and synaptic function; yet the molecular mechanisms mediating these complex roles and their relationship to brain dysfunction are largely unknown. TBC1D24 is unique in containing conserved TBC and TLDc domains; importantly, TLDc proteins have been recently described as interactors of the essential v-ATPase proton pump. **Experimental approach:** First we evaluated the TBC1D24/v-ATPase interaction by immunoprecipitation experiments. Using *Tbc1d24* knockout mouse neurons, we investigated v-ATPase assembly by fractionation experiments, expression of endo-lysosomal and autophagic markers by Western blot and pH regulation by live cell imaging. **Key findings:** TBC1D24 interacts with the v-ATPase cytosolic domain. Loss of TBC1D24 leads to an increase in the cytosolic fraction of ATP6V1A and ATP6V1B2 V1 subunits, an alteration of intracellular organelles acidification and impairment of autophagic flux. This phenotype is accompanied by defects in neurite development, synaptic connectivity and an alteration in synaptic ultrastructure. **Conclusions:** We uncover a novel function for TBC1D24 as regulator of v-ATPase activity and suggest pH and autophagic dysregulation in neurons as key cellular mechanisms that underpin the synaptic defects and pathogenesis in *Tbc1d24* disorders.

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LOSS OF CAPILLARY LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 1 EXPRESSION IN THE HIPPOCAMPUS OF TEMPORAL LOBE EPILEPSY AND ALZHEIMER'S DISEASE PATIENTS

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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[1]Amsterdam UMC, Department Of (neuro)pathology, Amsterdam, Netherlands, [2]University of Amsterdam, Swammerdam Institute For Life Sciences, Center For Neuroscience, Amsterdam, Netherlands, [3]Amsterdam UMC, Department Of Neurosurgery, Amsterdam, Netherlands, [4]Stichting Epilepsie Instellingen Nederland, Sein, Heemstede, Netherlands

Background:
Recently it has been reported that temporal lobe epilepsy (TLE) patients have increased brain amyloid beta (Aβ) expression, a neuropathological hallmark of Alzheimer's Disease (AD). Aβ accumulation might also explain the increased risk of developing epilepsy in AD. Since the low-density lipoprotein receptor-related protein 1 (LRP1) may be involved in Aβ clearance from the brain, we investigated LRP1 expression in the hippocampus of TLE and AD patients. **Methods:** Using immunohistochemistry, we studied the expression and cellular localization of LRP1 on hippocampal sections of autopsy controls (n=20), AD patients (n=12) and resected hippocampal tissue of age-matched TLE patients with hippocampal sclerosis (TLE-HS; n=14). **Results:** We demonstrated strong LRP1 expression at the abluminal side of brain capillaries in control specimens. Optical density analysis showed lower LRP1 expression in capillaries ($p<0.05$) and higher expression in astrocytes ($p<0.05$) in the dentate gyrus, CA1 and CA3 region of the hippocampus from AD and TLE-HS patients as compared to controls. **Conclusions:** Our results suggest that downregulation of LRP1 in brain endothelial cells could be involved in increased perivascular Aβ accumulation. In future studies we will investigate whether restoration of endothelial LRP1 expression can prevent or attenuate the development of epilepsy and/or accumulation of Aβ.

Pubmed:
34276560: de Vrind VAJ, van 't Sant LJ, Rozeboom A, Luijendijk-Berg MCM, Omrani A, Adan RAH
Leptin Receptor Expressing Neurons in the Substantia Nigra Regulate Locomotion, and in The Ventral Tegmental Area Motivation and Feeding.
Leptin is an anorexigenic hormone, important in the regulation of body weight. Leptin plays a role in food reward, feeding, locomotion and anxiety. Leptin receptors (LepR) are expressed in many brain areas, including the midbrain. In most studies that target the midbrain, either all LepR neurons of the midbrain or those of the ventral tegmental area (VTA) were targeted, but the role of substantia nigra (SN) LepR neurons has not been investigated. These studies have reported contradicting results regarding motivational behavior for food reward, feeding and locomotion. Since not all midbrain LepR mediated behaviors can be explained by LepR neurons in the VTA alone, we hypothesized that SN LepR neurons may provide further insight. We first characterized SN LepR and VTA LepR expression, which revealed LepR expression mainly on DA neurons. To further understand the role of midbrain LepR neurons in body weight regulation, we chemogenetically activated VTA LepR or SN LepR neurons in LepR-cre mice and tested for motivational behavior, feeding and locomotion. Activation of VTA LepR neurons in food restricted mice decreased motivation for food reward ($p=0.032$) and food intake ($p=0.020$), but not locomotion. In contrast, activation of SN LepR neurons in food restricted mice decreased locomotion ($p=0.025$), but not motivation for food reward or food intake. Our results provide evidence that VTA LepR and SN LepR neurons serve different functions, i.e. activation of VTA LepR neurons modulated motivation for food reward and feeding, while SN LepR neurons modulated locomotor activity.
Front Endocrinol (Lausanne), 2021; 12
34557909: Asaro A, Sinha R, Bakun M, Kalnytska O, Carlo-Spiewok AS, Rubel T, Rozeboom A, Dadlez M, Kaminska B, Aronica E, Malik AR, Willnow TE
ApoE4 disrupts interaction of sortilin with fatty acid-binding protein 7 essential to promote lipid signaling.
Sortilin is a neuronal receptor for apolipoprotein E (apoE). Sortilin-dependent uptake of lipidated apoE promotes conversion of polyunsaturated fatty acids (PUFA) into neuromodulators that induce anti-inflammatory gene expression in the brain. This neuroprotective pathway works with the apoE3 variant but is lost with the apoE4 variant, the main risk factor for Alzheimer's

disease (AD). Here, we elucidated steps in cellular handling of lipids through sortilin, and why they are disrupted by apoE4. Combining unbiased proteome screens with analyses in mouse models, we uncover interaction of sortilin with fatty acid-binding protein 7 (FABP7), the intracellular carrier for PUFA in the brain. In the presence of apoE3, sortilin promotes functional expression of FABP7 and its ability to elicit lipid-dependent gene transcription. By contrast, apoE4 binding blocks sortilin-mediated sorting, causing catabolism of FABP7 and impairing lipid signaling. Reduced FABP7 levels in the brain of AD patients expressing apoE4 substantiate the relevance of these interactions for neuronal lipid homeostasis. Taken together, we document interaction of sortilin with mediators of extracellular and intracellular lipid transport that provides a mechanistic explanation for loss of a neuroprotective lipid metabolism in AD.
J Cell Sci, 2021; 134

33867112: Omrani A, de Vrind VAJ, Lodder B, Stoltenborg I, Kooij K, Wolterink-Donselaar IG, Luijendijk-Berg MCM, Garner KM, Van't Sant LJ, Rozeboom A, Dickson SL, Meye FJ, Adan RAH
Identification of Novel Neurocircuitry Through Which Leptin Targets Multiple Inputs to the Dopamine System to Reduce Food Reward Seeking.
Leptin reduces the motivation to obtain food by modulating activity of the mesolimbic dopamine (DA) system upon presentation of cues that predict a food reward. Although leptin directly reduces the activity of ventral tegmental area (VTA) DA neurons, the majority of leptin receptor (LepR)-expressing DA neurons do not project to the nucleus accumbens, the projection implicated in driving food reward seeking. Therefore, the precise locus of leptin action to modulate motivation for a food reward is unresolved.
Biol Psychiatry, 2021; 90

31087767: de Vrind VAJ, Rozeboom A, Wolterink-Donselaar IG, Luijendijk-Berg MCM, Adan RAH
Effects of GABA and Leptin Receptor-Expressing Neurons in the Lateral Hypothalamus on Feeding, Locomotion, and Thermogenesis.
The lateral hypothalamus (LH) is known for its role in feeding, and it also regulates other aspects of energy homeostasis. How genetically defined LH neuronal subpopulations mediate LH effects on energy homeostasis remains poorly understood. The behavioral effects of chemogenetically activating LH gamma-aminobutyric acid (GABA) and the more selective population of LH GABA neurons that coexpress the leptin receptor (LepR) were compared.
Obesity (Silver Spring), 2019; 27

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CHANGES OF GOLGI APPARATUS MORPHOLOGY IN EPILEPSY

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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The Golgi apparatus is a highly dynamic organelle, which has many essential functions in cellular mechanisms like lipid metabolism, protein secretion, intracellular signalling and regulation of cell division. The structure of the Golgi apparatus is mainly defined as a stack of disk-like membranes, called the cisternae. The Golgi is highly dynamic and can be fragmented into ministacks under both physiological and pathological conditions. Changes in Golgi structure in neurons were observed in several neurodegenerative disorders including Alzheimer's disease, amyotrophic lateral sclerosis or Parkinson's disease. On the other hand, Golgi apparatus fragmentation was also observed in hippocampal and neocortical neurons during hibernation in Syrian hamster. The role of changes in Golgi apparatus morphology in epilepsy is still poorly understood. In our study, using 3D reconstruction of Golgi apparatus, we have shown for the first time, that Golgi apparatus is fragmented in human brain of epileptic patients. Moreover, this phenomenon was also observed in rat brain upon kainic acid induced seizures. Our results indicate that kainic acid-induced seizures leads to Golgi dispersion in pyramidal and granule neurons. The structural changes of Golgi apparatus in dentate gyrus neurons were reversible and Golgi apparatus morphology returned to normal in 24 hours after KA treatment. These observations suggest that enhanced neuronal activity induces Golgi reorganization, that is not associated with cellular death, but can be rather involved in aberrant neuronal plasticity processes that underlie epilepsy.

Pubmed:
27798233: Roszkowska M, Skupien A, Wójtowicz T, Konopka A, Gorlewicz A, Kisiel M, Bekisz M, Ruszczycki B, Dolezyczek H, Rejmak E, Knapska E, Mozrzymas JW, Wlodarczyk J, Wilczynski GM, Dzwonek J
CD44: a novel synaptic cell adhesion molecule regulating structural and functional plasticity of dendritic spines.
Synaptic cell adhesion molecules regulate signal transduction, synaptic function, and plasticity. However, their role in neuronal interactions with the extracellular matrix (ECM) is not well understood. Here we report that the CD44, a transmembrane receptor for hyaluronan, modulates synaptic plasticity. High-resolution ultrastructural analysis showed that CD44 was localized at mature synapses in the adult brain. The reduced expression of CD44 affected the synaptic excitatory transmission of primary hippocampal neurons, simultaneously modifying dendritic spine shape. The frequency of miniature excitatory postsynaptic currents decreased, accompanied by dendritic spine elongation and thinning. These structural and functional alterations went along with a decrease in the number of presynaptic Bassoon puncta, together with a reduction of PSD-95 levels at dendritic spines, suggesting a reduced number of functional synapses. Lack of CD44 also abrogated spine head enlargement upon neuronal stimulation. Moreover, our results indicate that CD44 contributes to proper dendritic spine shape and function by modulating the activity of actin cytoskeleton regulators, that is, Rho GTPases (RhoA, Rac1, and Cdc42). Thus CD44 appears to be a novel molecular player regulating functional and structural plasticity of dendritic spines.
Mol Biol Cell, 2016; 27
27163367: Konopka A, Zeug A, Skupien A, Kaza B, Mueller F, Chwedorowicz A, Ponimaskin E, Wilczynski GM, Dzwonek J
Cleavage of Hyaluronan and CD44 Adhesion Molecule Regulate Astrocyte Morphology via Rac1 Signalling.
Communication of cells with their extracellular environment is crucial to fulfill their function in physiological and pathophysiological conditions. The literature data provide evidence that such a communication is also important in case of astrocytes. Mechanisms that contribute to the interaction between astrocytes and extracellular matrix (ECM) proteins are still poorly understood. Hyaluronan is the main component of ECM in the brain, where its major receptor protein CD44 is expressed by a subset of astrocytes. Considering the fact that functions of astrocytes are tightly coupled with changes in their morphology (e.g.: glutamate clearance in the synaptic cleft, migration, astrogliosis), we investigated the influence of hyaluronan cleavage by hyaluronidase, knockdown of CD44 by specific shRNA and CD44 overexpression on astrocyte morphology. Our results show that hyaluronidase treatment, as well as knockdown of CD44, in astrocytes result in a

"stellate"-like morphology, whereas overexpression of CD44 causes an increase in cell body size and changes the shape of astrocytes into flattened cells. Moreover, as a dynamic reorganization of the actin cytoskeleton is supposed to be responsible for morphological changes of cells, and this reorganization is controlled by small GTPases of the Rho family, we hypothesized that GTPase Rac1 acts as a downstream effector for hyaluronan and CD44 in astrocytes. We used FRET-based biosensor and a dominant negative mutant of Rac1 to investigate the involvement of Rac1 activity in hyaluronidase- and CD44-dependent morphological changes of astrocytes. Both, hyaluronidase treatment and knockdown of CD44, enhances Rac1 activity while overexpression of CD44 reduces the activity state in astrocytes. Furthermore, morphological changes were blocked by specific inhibition of Rac1 activity. These findings indicate for the first time that regulation of Rac1 activity is responsible for hyaluronidase and CD44-driven morphological changes of astrocytes.
PLoS One, 2016; 11
25300795: Skupien A, Konopka A, Trzaskoma P, Labus J, Gorlewicz A, Swiech L, Babraj M, Dolezyczek H, Figiel I, Ponimaskin E, Wlodarczyk J, Jaworski J, Wilczynski GM, Dzwonek J
CD44 regulates dendrite morphogenesis through Src tyrosine kinase-dependent positioning of the Golgi.
The acquisition of proper dendrite morphology is a crucial aspect of neuronal development towards the formation of a functional network. The role of the extracellular matrix and its cellular receptors in this process has remained enigmatic. We report that the CD44 adhesion molecule, the main hyaluronan receptor, is localized in dendrites and plays a crucial inhibitory role in dendritic tree arborization in vitro and in vivo. This novel function is exerted by the activation of Src tyrosine kinase, leading to the alteration of Golgi morphology. The mechanism operates during normal brain development, but its inhibition might have a protective influence on dendritic trees under toxic conditions, during which the silencing of CD44 expression prevents dendritic shortening induced by glutamate exposure. Overall, our results indicate a novel role for CD44 as an essential regulator of dendritic arbor complexity in both health and disease.
J Cell Sci, 2014; 127
34086671: Skupien-Jaroszek A, Walczak A, Czaban I, Pels KK, Szczepankiewicz AA, Krawczyk K, Ruszczycki B, Wilczynski GM, Dzwonek J, Magalska A
The interplay of seizures-induced axonal sprouting and transcription-dependent Bdnf repositioning in the model of temporal lobe epilepsy.
The Brain-Derived Neurotrophic Factor is one of the most important trophic proteins in the brain. The role of this growth factor in neuronal plasticity, in health and disease, has been extensively studied. However, mechanisms of epigenetic regulation of Bdnf gene expression in epilepsy are still elusive. In our previous work, using a rat model of neuronal activation upon kainate-induced seizures, we observed a repositioning of Bdnf alleles from the nuclear periphery towards the nuclear center. This change of Bdnf intranuclear position was associated with transcriptional gene activity. In the present study, using the same neuronal activation model, we analyzed the relation between the percentage of the Bdnf allele at the nuclear periphery and clinical and morphological traits of epilepsy. We observed that the decrease of the percentage of the Bdnf allele at the nuclear periphery correlates with stronger mossy fiber sprouting-an aberrant form of excitatory circuits formation. Moreover, using in vitro hippocampal cultures we showed that Bdnf repositioning is a consequence of transcriptional activity. Inhibition of RNA polymerase II activity in primary cultured neurons with Actinomycin D completely blocked Bdnf gene transcription and repositioning occurring after neuronal excitation. Interestingly, we observed that histone deacetylases inhibition with Trichostatin A induced a slight increase of Bdnf gene transcription and its repositioning even in the absence of neuronal excitation. Presented results provide novel insight into the role of BDNF in epileptogenesis. Moreover, they strengthen the statement that this particular gene is a good candidate to search for a new generation of antiepileptic therapies.
PLoS One, 2021; 16

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BOARD NUMBER: S04-664

FUNCTIONAL MODULATION OF KV7.2 CHANNELS BY STARGAZIN

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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The M-current is a low-threshold K^+-current that determines neuronal excitability and contributes to dampening repetitive firing. M-channels assemble as tetramers of Kv7.2-7.3 subunits and are targets of neuromodulation. Mutations in the KCNQ2 gene encoding for Kv7.2 subunits are responsible for epilepsy and neurodevelopmental disorders, and a Kv7.2 dominant-negative mouse model displays hyperexcitability, unprovoked seizures and impaired cognition. Even though M-current dysfunction is a common epileptogenic mechanism, little is known about how M-currents are regulated. We have identified stargazin, a well-known regulator of AMPAR trafficking and gating, as a new interactor of Kv7.2 in the cortex. Here, we characterized Kv7.2-stargazin interaction and tested its functional relevance. Co-immunoprecipitation and proximity ligation (PLA) assays revealed that the stargazin-Kv7.2 interaction occurs in neurons, and stargazin co-expression with Kv7.2 potentiated Kv7.2-mediated currents without changing their voltage-dependence, while increasing Kv7.2 cell surface expression. Conversely, stargazin silencing in cultured cortical neurons decreased sensitivity to the M-channel specific blocker XE-991, and reduced the hyperpolarization induced by the M-channel activator retigabine, indicating decreased neuronal M-currents in the absence of stargazin. A variant of stargazin associated with intellectual disability failed to potentiate M-currents when co-expressed with Kv7.2. Additionally, a knock-in mouse model harbouring this stargazin variant showed decreased M-currents and medium after burst-hyperpolarization in pyramidal hippocampal neurons and enhanced susceptibility to pentylenetetrazol-induced seizures. Collectively, our data support a novel function for stargazin in regulating M-channel activity, and highlights that a disrupted stargazing/Kv7.2 interaction may have relevant impact in hyperexcitability disorders.

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BOARD NUMBER: S04-665

FOCAL CORTICAL DYSPLASIA AND SOMATIC MUTATIONS OF THE MTOR PATHWAY: A ROLE FOR NMDARS IN EPILEPTIC ACTIVITY

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

Louison Pineau[1], Emmanuelle Buhler[2], Françoise Watrin[2], Sylvian Bauer[2], Carlos Cardoso[2], Alfonso Represa-Bermejo[2], Nail Burnashev[2], Pierre Szepetowski[2]
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Aims: Abnormal activity of the mTOR signaling pathway leads to severe neurodevelopmental diseases (refractory epilepsy, autism, brain malformations...). These mTORopathies include Tuberous sclerosis complex (TSC) and focal cortical dysplasia (FCD). In FCDs, somatic mutations in *TSC1*, *TSC2* and the eponym *mTOR* gene were identified but the precise mechanisms linking mTOR dysregulation and epilepsy remain to be clarified. We previously reported that aberrant GluN2C-driven NMDA receptor (NMDAR) currents are involved in epileptogenesis caused by defects in the *TSC1* or *TSC2* genes. Whether this mechanism is a peculiarity of TSC genes or would expand to other mTORopathies remains undetermined. Methods: We use our previously characterized rat model of FCD created by *in utero* electroporation in neural progenitors of the dorsal telencephalon of expression vector encoding wild-type or mutant pathogenic mTOR. Activity of neuronal NMDARs was analyzed in acute brain slices in layer IV of somatosensory cortex at around P15 using whole-cell recordings and selective NMDARs inhibitors. Neuronal morphology was analyzed with biocytin labeling and 3D-reconstruction. Ex vivo extracellular recordings were performed to study epileptiform activity. Results: mTOR pathogenic mutation led to changes in neuronal morphology and intrinsic properties. Electroporated mutant but not wild-type neurons displayed increased NMDAR activity mostly sustained by GluN2C-mediated currents. Extracellular recordings revealed GluN2C-dependent spiking activity. Conclusion: Increased synaptic integration caused by slower kinetics of GluN2C-mediated currents might sustain hypersynchronous neuronal networks in FCDs. Broadening Glun2C-related pathogenesis to the mTORopathies spectrum would provide a unified mechanism underlying mTOR-related epileptogenesis and might lead to the design of Glun2C-targeting therapeutic strategies.

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BOARD NUMBER: S04-666

SOMATIC GENETIC MOSAICISM IN NEUROGLIAL PROGENITORS UNDERLIE EPILEPTOGENIC FOCAL BRAIN MALFORMATIONS

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

Sara Baldassari[1], Homa Adle-Biassette[2], Georg Dorfmüller[3], Seyeon Kim[4], Mathilde Chipaux[2], Jeong Ho Lee[5], Stephanie Baulac[1]
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Focal malformations of cortical development (MCD) are important causes of severe pediatric drug-resistant epilepsies subjected to neurosurgery and associated with a spectrum of histological abnormalities. Here, we investigated the role of genetic postzygotic somatic mosaicism in MCD. We performed ultra-deep targeted gene sequencing on matched blood-brain samples of 120 patients with focal cortical dysplasia (FCD) or mild-MCD. We identified mTOR pathway hyperactivating somatic/germline/2-hit mutations in 54% of cases with FCD-II pathology. Somatic variant allele frequencies ranged between 0.4% and 34%, and correlated with the size of the lesion. By laser-capture-microdissection we demonstrated the somatic mutation enrichment in dysmorphic neurons and balloon cells, cytomegalic and mTOR hyperactive cells of neuronal and glial origin, thus suggesting that the mutation occurred in a common neuroglial progenitor. We also identified somatic loss-of-function mutations in the galactose transporter SLC35A2 (important for N-glycosylation) in 33% of mild-MCD cases, with mosaic rates between 12% and 43%. SLC35A2-cases showed white matter anomalies: heterotopic neurons and high-density clusters of oligodendroglial cells (a histological entity called MOGHE). By laser-capture-microdissection, we confirmed the mutation enrichment heterotopic neurons and oligodendrocytes from high-density clusters. Finally, in a cohort of 9 patients with confirmed somatic mutations in the brain tissue, we sequenced cell-free DNA from cerebrospinal fluid collected during neurosurgery and detected the somatic mutations at low mosaicism in 3/9 cases, providing a proof of concept for the presurgical genetic diagnosis in MCD. Our study shows that alterations in mTOR and N-glycosylation pathways are an important cause of a spectrum of focal epileptogenic MCDs.

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BOARD NUMBER: S04-667

CELL-TYPE-SPECIFIC PROFILING OF MICRORNAS DURING EPILEPTOGENESIS: INSIGHTS INTO NEURONS AND MICROGLIA MICRORNA PROFILES IN NORMAL BRAIN FUNCTION AND DISEASE.

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

Leticia Villalba-Benito[1], Omar Mamad[2], Mona Heiland[2], Elizabeth Brindley[2], Elena Langa[2], Aasia Batool[2], Thomas Hill[2], Ngoc Nguyen-Thanh[2], David Henshall[2], Gary Brennan[1]
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Epilepsy is one of the most common neurological conditions, affecting ~50 million people worldwide. Brain insults can trigger pathogenic cellular events including cell death, chronic neuroinflammation, aberrant neurogenesis, and synaptic rewiring, which result in spontaneous seizures. These aberrant functions are usually the result of altered gene networks induced by the precipitating event; however, the full spectrum of such gene dysregulation remains unclear. MicroRNAs, a class of small non-coding RNAs involved in the regulation of gene expression at the post-transcriptional level, have previously been shown to regulate epileptogenic pathways. Nevertheless, how microRNAs shape the specific brain cells response to normal brain function and epilepsy-inciting events remains obscure. To address this, we generated transgenic mice that express a FLAG-tagged-Ago2 in neurons and microglia (using inducible Thy1 and CX3cr1 driven Cre-recombinase), allowing us to elute microRNAs from either neurons or microglia. By using the intra-amygdala kainic-acid technique, epileptogenesis was induced, and FLAG was immunoprecipitated from both lines followed by small RNA-Seq. As a result, the microRNA profiles of neurons and microglia and differential expression of microRNAs during epilepsy development were identified, allowing us to determine the role of specific microRNAs in epilepsy in specific cell types. This will be the most comprehensive *in vivo* map of microRNAs in adult mouse hippocampus, which will illuminate further the complex and nuanced role of microRNAs and individual cell types in epilepsy and in normal brain. Extrapolation of the contribution of specific cell types in epilepsy will facilitate the development of the most efficacious therapeutic targets.

Pubmed:
33750457: Villalba-Benito L, López-López D, Torroglosa A, Casimiro-Soriguer CS, Luzón-Toro B, Fernández RM, Moya-Jiménez MJ, Antiñolo G, Dopazo J, Borrego S
Genome-wide analysis of DNA methylation in Hirschsprung enteric precursor cells: unraveling the epigenetic landscape of enteric nervous system development.
Hirschsprung disease (HSCR, OMIM 142623) is a rare congenital disorder that results from a failure to fully colonize the gut by enteric precursor cells (EPCs) derived from the neural crest. Such incomplete gut colonization is due to alterations in EPCs proliferation, survival, migration and/or differentiation during enteric nervous system (ENS) development. This complex process is regulated by a network of signaling pathways that is orchestrated by genetic and epigenetic factors, and therefore alterations at these levels can lead to the onset of neurocristopathies such as HSCR. The goal of this study is to broaden our knowledge of the role of epigenetic mechanisms in the disease context, specifically in DNA methylation. Therefore, with this aim, a Whole-Genome Bisulfite Sequencing assay has been performed using EPCs from HSCR patients and human controls. Clin Epigenetics, 2021; 13
33407723: Luzón-Toro B, Villalba-Benito L, Fernández RM, Torroglosa A, Antiñolo G, Borrego S
RMRP, RMST, FTX and IPW: novel potential long non-coding RNAs in medullary thyroid cancer.
The relevant role of long non-coding RNAs (lncRNAs) in cancer is currently a matter of increasing interest. Medullary thyroid cancer (MTC) is a rare neuroendocrine tumor (2-5% of all thyroid cancer) derived from the parafollicular C-cells which secrete calcitonin. About 75% of all medullary thyroid cancers are believed to be sporadic medullary thyroid cancer (sMTC), whereas the remaining 25% correspond to inherited cancer syndromes known as Multiple Endocrine Neoplasia type 2 (MEN2). MEN2 syndrome, with autosomal dominant inheritance is caused by germline gain of function mutations in RET proto-oncogene. To date no lncRNA has been associated to MEN2 syndrome and only two articles have been published relating long non-coding RNA (lncRNA) to MTC: the first one linked MALAT1 with sMTC and, in the other, our group determined some new lncRNAs in a small group of sMTC cases in fresh tissue (RMST, FTX, IPW, PRNCR1, ADAMTS9-AS2 and RMRP). The aim of the current study is to validate such novel lncRNAs previously described by our group by using a larger cohort of patients, in

order to discern their potential role in the disease. Here we have tested three up-regulated (RMST, FTX, IPW) and one down-regulated (RMRP) lncRNAs in our samples (formalin fixed paraffin embedded tissues from twenty-one MEN2 and ten sMTC patients) by RT-qPCR analysis. The preliminary results reinforce the potential role of RMST, FTX, IPW and RMRP in the pathogenesis of MTC.
Orphanet J Rare Dis, 2021; 16
33260622: Villalba-Benito L, Torroglosa A, Luzón-Toro B, Fernández RM, Moya-Jiménez MJ, Antiñolo G, Borrego S
ChIP-Seq-Based Approach in Mouse Enteric Precursor Cells Reveals New Potential Genes with a Role in Enteric Nervous System Development and Hirschsprung Disease.
Hirschsprung disease (HSCR) is a neurocristopathy characterized by intestinal aganglionosis which is attributed to a failure in neural crest cell (NCC) development during the embryonic stage. The colonization of the intestine by NCCs is a process finely controlled by a wide and complex gene regulatory system. Several genes have been associated with HSCR, but many aspects still remain poorly understood. The present study is focused on deciphering the PAX6 interaction network during enteric nervous system (ENS) formation. A combined experimental and computational approach was performed to identify PAX6 direct targets, as well as gene networks shared among such targets as potential susceptibility factors for HSCR. As a result, genes related to PAX6 either directly (and) or indirectly (, , and) were identified as putative genes associated with HSCR. Interestingly, is involved in the RET/GDNF/GFRA1 signaling pathway, one of the main pathways implicated in the disease. Our findings represent a new contribution to advance in the knowledge of the genetic basis of HSCR. The investigation of the role of these genes could help to elucidate their implication in HSCR onset.
Int J Mol Sci, 2020; 21
32748823: Torroglosa A, Villalba-Benito L, Fernández RM, Luzón-Toro B, Moya-Jiménez MJ, Antiñolo G, Borrego S
Identification of New Potential LncRNA Biomarkers in Hirschsprung Disease.
Hirschsprung disease (HSCR) is a neurocristopathy defined by intestinal aganglionosis due to alterations during the development of the Enteric Nervous System (ENS). A wide spectrum of molecules involved in different signaling pathways and mechanisms have been described in HSCR onset. Among them, epigenetic mechanisms are gaining increasing relevance. In an effort to better understand the epigenetic basis of HSCR, we have performed an analysis for the identification of long non-coding RNAs (lncRNAs) by qRT-PCR in enteric precursor cells (EPCs) from controls and HSCR patients. We aimed to test the presence of a set lncRNAs among 84 lncRNAs in human EPCs, which were previously related with crucial cellular processes for ENS development, as well as to identify the possible differences between HSCR patients and controls. As a result, we have determined a set of lncRNAs with positive expression in human EPCs that were screened for mutations using the exome data from our cohort of HSCR patients to identify possible variants related to this pathology. Interestingly, we identified three lncRNAs with different levels of their transcripts (, and) between HSCR patients and controls. We propose such lncRNAs as possible regulatory elements implicated in the onset of HSCR as well as potential biomarkers of this pathology.
Int J Mol Sci, 2020; 21
31717449: Luzón-Toro B, Fernández RM, Villalba-Benito L, Torroglosa A, Antiñolo G, Borrego S
Influencers on Thyroid Cancer Onset: Molecular Genetic Basis.
Thyroid cancer, a cancerous tumor or growth located within the thyroid gland, is the most common endocrine cancer. It is one of the few cancers whereby incidence rates have increased in recent years. It occurs in all age groups, from children through to seniors. Most studies are focused on dissecting its genetic basis, since our current knowledge of the genetic background of the different forms of thyroid cancer is far from complete, which poses a challenge for diagnosis and prognosis of the disease. In this review, we describe prevailing advances and update our understanding of the molecular genetics of thyroid cancer, focusing on the main genes related with the pathology, including the different noncoding RNAs associated with the disease.
Genes (Basel), 2019; 10
31355911: Luzón-Toro B, Villalba-Benito L, Torroglosa A, Fernández RM, Antiñolo G, Borrego S
What is new about the genetic background of Hirschsprung disease?
Hirschsprung disease (HSCR) is a rare congenital disorder caused by an incorrect enteric nervous system development due to a failure in migration, proliferation, differentiation and/or survival of enteric neural crest cells. HSCR is a complex genetic disease, where alterations at different molecular levels are required for the manifestation of the disease. In addition, a wide spectrum of mutations affecting many different genes cause HSCR, although the occurrence and severity of HSCR from many cases still remain unexplained. This review summarizes the current knowledge about molecular genetic basis of HSCR.
Clin Genet, 2020; 97
31247956: Torroglosa A, Villalba-Benito L, Luzón-Toro B, Fernández RM, Antiñolo G, Borrego S
Epigenetic Mechanisms in Hirschsprung Disease.
Hirschsprung disease (HSCR, OMIM 142623) is due to a failure of enteric precursor cells derived from neural crest (EPCs) to proliferate, migrate, survive or differentiate during Enteric Nervous System (ENS) formation. This is a complex process which

requires a strict regulation that results in an ENS specific gene expression pattern. Alterations at this level lead to the onset of neurocristopathies such as HSCR. Gene expression is regulated by different mechanisms, such as DNA modifications (at the epigenetic level), transcriptional mechanisms (transcription factors, silencers, enhancers and repressors), postranscriptional mechanisms (3'UTR and ncRNA) and regulation of translation. All these mechanisms are finally implicated in cell signaling to determine the migration, proliferation, differentiation and survival processes for correct ENS development. In this review, we have performed an overview on the role of epigenetic mechanisms at transcriptional and posttranscriptional levels on these cellular events in neural crest cells (NCCs), ENS development, as well as in HSCR.
Int J Mol Sci, 2019; 20
29290961: Torroglosa A, Villalba-Benito L, Fernández RM, Moya-Jiménez MJ, Antiñolo G, Borrego S
knock-down in enteric precursors reveals a possible mechanism by which this methyltransferase is involved in the enteric nervous system development and the onset of Hirschsprung disease.
Hirschsprung disease (HSCR, OMIM 142623) is a pathology that shows a lack of enteric ganglia along of the distal gastrointestinal tract. This aganglionosis is attributed to an abnormal proliferation, migration, differentiation and/or survival of enteric precursor cells (EPCs) derived from neural crest cells (NCCs) during the enteric nervous system (ENS) embryogenesis. DNMT3b methyltransferase is associated with NCCs development and has been shown to be implicated in ENS formation as well as in HSCR. In this study we have aimed to elucidate the specific mechanism underlying the DNMT3b role in such processes. We have performed the knockdown of expression (-KD) in enteric precursor cells (EPCs) to clarify its role on these cells . Moreover, we have analyzed several signaling pathways to determine the mechanisms responsible for the effect caused by -KD in EPCs. Our results seem to support that -KD promotes an increase EPCs proliferation that may be mediated by P53 and P21 activity, since both proteins were observed to be down-regulated in our -KD cultures. Moreover, we observed a down-regulation of and in HSCR patients. These results lead us to propose that DNMT3b could be involved in HSCR through P53 and P21 activity.
Oncotarget, 2017; 8
28740121: Villalba-Benito L, Torroglosa A, Fernández RM, Ruíz-Ferrer M, Moya-Jiménez MJ, Antiñolo G, Borrego S
Overexpression of DNMT3b target genes during Enteric Nervous System development contribute to the onset of Hirschsprung disease.
Hirschsprung disease (HSCR) is attributed to a failure of neural crest cells (NCCs) to migrate, proliferate, differentiate and/or survive in the bowel wall during embryonic Enteric Nervous System (ENS) development. ENS formation is the result from a specific gene expression pattern regulated by epigenetic events, such DNA methylation by the DNA methyltransferases (DNMTs), among other mechanisms. Specifically, DNMT3b de novo methyltransferase is associated with NCCs development and has been shown to be implicated in ENS formation and in HSCR. Aiming to elucidate the specific mechanism underlying the DNMT3b role in such processes, we have performed a chromatin immunoprecipitation coupled with massively parallel sequencing analysis to identify the DNMT3B target genes in enteric precursor cells (EPCs) from mice. Moreover, the expression patterns of those target genes have been analyzed in human EPCs from HSCR patients in comparison with controls. Additionally, we have carried out a search of rare variants in those genes in a HSCR series. Through this approach we found 9 genes showing a significantly different expression level in both groups. Therefore, those genes may have a role in the proper human ENS formation and a failure in their expression pattern might contribute to this pathology.
Sci Rep, 2017; 7

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BOARD NUMBER: S04-668

SUDDEN UNEXPECTED DEATH IN EPILEPSY RELATED TO THE MTOR REPRESSOR DEPDC5

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Aims: Germline loss-of-function mutations in DEPDC5, and in its binding partners (NPRL2/3) of the mammalian target of rapamycin (mTOR) repressor GATOR1 complex, cause focal epilepsies and increase the risk of sudden unexpected death in epilepsy (SUDEP). Here, we asked whether DEPDC5 haploinsufficiency predisposes to primary cardiac defects that could contribute to SUDEP and therefore impact the clinical management of patients at high risk of SUDEP. **Methods:** Clinical cardiac investigations were performed in 16 patients with pathogenic variants in DEPDC5, NPRL2, or NPRL3. Two novel Depdc5 mouse strains, a human HA-tagged Depdc5 strain and a Depdc5 heterozygous knockout with a neuron-specific deletion of the second allele (Depdc5$^{c/-}$), were generated to investigate the role of Depdc5 in SUDEP and cardiac activity during seizures. **Results:** Holter, echocardiographic, and electrocardiographic (ECG) examinations provided no evidence for altered clinical cardiac function in the patient cohort, of whom 3 DEPDC5 patients succumbed to SUDEP and 6 had a family history of SUDEP. There was no cardiac injury at autopsy in a postmortem DEPDC5 SUDEP case. The HA-tagged Depdc5 mouse revealed expression of Depdc5 in the brain, heart, and lungs. Simultaneous electroencephalographic-ECG records on Depdc5$^{c/-}$ mice showed that spontaneous epileptic seizures resulting in a SUDEP-like event are not preceded by cardiac arrhythmia. **Conclusions:** Mouse and human data show neither structural nor functional cardiac damage that might underlie a primary contribution to SUDEP in the spectrum of DEPDC5-related epilepsies.

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BOARD NUMBER: S04-669

HETEROTOPIA SUBTYPE-SPECIFIC MORPHO-ELECTRIC AND CONNECTIVITY PROPERTIES UNDERLIE DISTINCT DYNAMICS OF EPILEPTIFORM ACTIVITY IN MURINE MODELS OF GREY MATTER HETEROTOPIA

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

Jean-Christophe Vermoyal[1], Delphine Hardy[1], Lucas Goirand-Lopez[1], Lucas Silvagnoli[1], Antonin Vinck[1], Aurélien Fortoul[1], Fiona Francis[2,3,4], Silvia Cappello[5], Françoise Watrin[1], Thomas Marissal[1], Jean-Bernard Manent[1]
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Aims: Subcortical band heterotopia (SBH) and periventricular nodular heterotopia (PVNH) are two types of grey matter heterotopia (GMH) causing epilepsy. They present with misplaced neurons, either forming nodules lining the ventricles in PVNH, or forming bands in the white matter in SBH. Here, we compared epileptic circuitries in two murine models of SBH-like and PVNH-like malformations. **Methods**: We performed immunohistochemistry with cortical layer-specific markers to describe neuronal composition in heterotopia. We analyzed intrinsic electrophysiological and morphological properties of heterotopia neurons with whole-cell patch-clamp recordings and post-hoc neuronal reconstructions. We performed principal component analysis and clustering to identify morpho-electric signatures. We studied network excitability and dynamics of epileptiform activity with two-photon calcium imaging, and mapped microcircuits with laser scanning photostimulation of caged glutamate. **Results**: We show that SBH-like and PVNH-like malformations have distinct neuronal composition and histological properties, and are composed of neurons with peculiar morpho-electric properties. Among heterotopia models, we identified 3 morphotypes; two of them being mostly found in the SBH model, the other in the PVNH model. We also identified 2 model-specific electrotypes. In the two models, both the heterotopia and normotopic cortex contributed to epileptiform activity. The heterotopia was however recruited earlier than the normotopic cortex in the SBH model whereas no preference was found in the PVNH model. Last, we observed reciprocal functional connections between the heterotopia and normotopic cortex in the two GMH models. **Conclusions**: Our study will aid in our understanding of altered circuitry in cortical malformations causing epilepsy.

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BOARD NUMBER: S04-670

FCD TYPE-DEPENDENT DYSREGULATION OF MYELINATION IN EXTRATEMPORAL LOBE REGIONS

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Aim: Focal cortical dysplasias (FCDs) are local malformations of the human neocortex and a leading cause of pharmacoresistant epilepsy. FCDs are characterized by local architectural disturbances of the neocortex and often feature a blurred gray-white matter boundary, indicating abnormal white matter myelination. We have recently shown that myelination is also compromised in the gray matter of FCD type IIa in the temporal lobe. In this study, we have analyzed the myelination pattern in FCD type IIa and IIb by focusing on the extratemporal cortical regions. Methods: We characterized the gray matter-associated myelination pathology of FCD IIa, FCD IIb and control specimens. We applied immunohistochemistry to visualize myelinated fibers and ultrastructural analyses of the myelin sheath. Furthermore, *in situ* hybridization was performed to analyze the density of myelinating oligodendrocytes (OLs) and PCR to determine the expression levels of myelin-associated transcripts. Results: We show that the proportion of myelinated gray matter is similar in FCD IIa, IIb and controls with myelinated fibers extending up to layer III. Electron microscopical analysis revealed that the myelin sheaths of layer V axons are significantly thinner in FCD IIa specimens than in controls. In addition, the density of OLs was reduced in FCD IIa, however, contrarily, we observed an increase of cells in FCD IIb. Similarly, the expression levels of myelin-associated transcripts were decreased in FCD IIa and increased in FCD IIb. Conclusion: The results indicate that the myelination pattern is disturbed in extratemporal FCD IIa and IIb cases, depending on the FCD type.

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BOARD NUMBER: S04-671

BRAIN REGION-SPECIFIC EPILEPTOGENESIS IN A CONDITIONAL MOUSE MODEL

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Genetic epilepsies show a large clinical features variability. Even when the same mutation occurs within a family, it often leads to a personal phenotype. This sparked the theory that a mutation induces a regional epileptogenic process which differs among patients causing distinct seizure types. A well-studied epilepsy gene is *SCN1A*, which encodes the voltage-gated sodium channel Na_V 1.1, expressed mainly in GABAergic interneurons throughout the central nervous system (CNS). Pathological variants cause Dravet syndrome (DS), a pharmacoresistant severe developmental and epileptic encephalopathy (DEE) with febrile seizures beginning in early childhood, high seizure frequency and delayed development. The early onset of this disorder suggests that factors in early brain development are crucial for phenotype formation. The most common genetic cause of Dravet syndrome is a loss-of-function (LOF) mutation in the *SCN1A* gene. This LOF leads to epilepsy through hyperexcitability of inhibitory neurons. In this work, a conditional LOF knock-in mouse line expressing a DS-causing mutation in the presence of Cre was used to study developmental and physiological aspects of epileptogenesis. This allowed us to turn on a specific genetic defect locally (in the S1 cortical area or in the hippocampus) and at defined time points (postnatal or adulthood). For this purpose, adeno-associated viruses encoding Cre recombinase were stereotaxically injected into the corresponding regions. We demonstrated that different types of seizures could be induced, which were dependent on both the area injected and the degree of maturation of the brain. Our results thus provide insight into the underlying mechanisms of epileptogenesis.

Pubmed:
30343943: Helbig KL, Lauerer RJ, Bahr JC, Souza IA, Myers CT, Uysal B, Schwarz N, Gandini MA, Huang S, Keren B, Mignot C, Afenjar A, Billette de Villemeur T, Héron D, Nava C, Valence S, Buratti J, Fagerberg CR, Soerensen KP, Kibaek M, Kamsteeg EJ, Koolen DA, Gunning B, Schelhaas HJ, Kruer MC, Fox J, Bakhtiari S, Jarrar R, Padilla-Lopez S, Lindstrom K, Jin SC, Zeng X, Bilguvar K, Papavasileiou A, Xing Q, Zhu C, Boysen K, Vairo F, Lanpher BC, Klee EW, Tillema JM, Payne ET, Cousin MA, Kruisselbrink TM, Wick MJ, Baker J, Haan E, Smith N, Sadeghpour A, Davis EE, Katsanis N, , Corbett MA, MacLennan AH, Gecz J, Biskup S, Goldmann E, Rodan LH, Kichula E, Segal E, Jackson KE, Asamoah A, Dimmock D, McCarrier J, Botto LD, Filloux F, Tvrdik T, Cascino GD, Klingerman S, Neumann C, Wang R, Jacobsen JC, Nolan MA, Snell RG, Lehnert K, Sadleir LG, Anderlid BM, Kvarnung M, Guerrini R, Friez MJ, Lyons MJ, Leonhard J, Kringlen G, Casas K, El Achkar CM, Smith LA, Rotenberg A, Poduri A, Sanchis-Juan A, Carss KJ, Rankin J, Zeman A, Raymond FL, Blyth M, Kerr B, Ruiz K, Urquhart J, Hughes I, Banka S, , Hedrich UBS, Scheffer IE, Helbig I, Zamponi GW, Lerche H, Mefford HC
De Novo Pathogenic Variants in CACNA1E Cause Developmental and Epileptic Encephalopathy with Contractures, Macrocephaly, and Dyskinesias.
Developmental and epileptic encephalopathies (DEEs) are severe neurodevelopmental disorders often beginning in infancy or early childhood that are characterized by intractable seizures, abundant epileptiform activity on EEG, and developmental impairment or regression. CACNA1E is highly expressed in the central nervous system and encodes the α-subunit of the voltage-gated Ca2.3 channel, which conducts high voltage-activated R-type calcium currents that initiate synaptic transmission. Using next-generation sequencing techniques, we identified de novo CACNA1E variants in 30 individuals with DEE, characterized by refractory infantile-onset seizures, severe hypotonia, and profound developmental impairment, often with congenital contractures, macrocephaly, hyperkinetic movement disorders, and early death. Most of the 14, partially recurring, variants cluster within the cytoplasmic ends of all four S6 segments, which form the presumed Ca2.3 channel activation gate. Functional analysis of several S6 variants revealed consistent gain-of-function effects comprising facilitated voltage-dependent activation and slowed inactivation. Another variant located in the domain II S4-S5 linker results in facilitated activation and increased current density. Five participants achieved seizure freedom on the anti-epileptic drug topiramate, which blocks R-type calcium channels. We establish pathogenic variants in CACNA1E as a cause of DEEs and suggest facilitated R-type calcium currents as a disease mechanism for human epilepsy and developmental disorders.

Am J Hum Genet, 2018; 103
31498083: Schwarz N, Uysal B, Welzer M, Bahr JC, Layer N, Löffler H, Stanaitis K, Pa H, Weber YG, Hedrich UB, Honegger JB, Skodras A, Becker AJ, Wuttke TV, Koch H
Long-term adult human brain slice cultures as a model system to study human CNS circuitry and disease.
Most of our knowledge on human CNS circuitry and related disorders originates from model organisms. How well such data translate to the human CNS remains largely to be determined. Human brain slice cultures derived from neurosurgical resections may offer novel avenues to approach this translational gap. We now demonstrate robust preservation of the complex neuronal cytoarchitecture and electrophysiological properties of human pyramidal neurons in long-term brain slice cultures. Further experiments delineate the optimal conditions for efficient viral transduction of cultures, enabling 'high throughput' fluorescence-mediated 3D reconstruction of genetically targeted neurons at comparable quality to state-of-the-art biocytin fillings, and demonstrate feasibility of long term live cell imaging of human cells . This model system has implications toward a broad spectrum of translational studies, regarding the validation of data obtained in non-human model systems, for therapeutic screening and genetic dissection of human CNS circuitry.
Elife, 2019; 8
30849329: Helbig KL, Lauerer RJ, Bahr JC, Souza IA, Myers CT, Uysal B, Schwarz N, Gandini MA, Huang S, Keren B, Mignot C, Afenjar A, Billette de Villemeur T, Héron D, Nava C, Valence S, Buratti J, Fagerberg CR, Soerensen KP, Kibaek M, Kamsteeg EJ, Koolen DA, Gunning B, Schelhaas HJ, Kruer MC, Fox J, Bakhtiari S, Jarrar R, Padilla-Lopez S, Lindstrom K, Jin SC, Zeng X, Bilguvar K, Papavasileiou A, Xing Q, Zhu C, Boysen K, Vairo F, Lanpher BC, Klee EW, Tillema JM, Payne ET, Cousin MA, Kruisselbrink TM, Wick MJ, Baker J, Haan E, Smith N, Sadeghpour A, Davis EE, Katsanis N, , Corbett MA, MacLennan AH, Gecz J, Biskup S, Goldmann E, Rodan LH, Kichula E, Segal E, Jackson KE, Asamoah A, Dimmock D, McCarrier J, Botto LD, Filloux F, Tvrdik T, Cascino GD, Klingerman S, Neumann C, Wang R, Jacobsen JC, Nolan MA, Snell RG, Lehnert K, Sadleir LG, Anderlid BM, Kvarnung M, Guerrini R, Friez MJ, Lyons MJ, Leonhard J, Kringlen G, Casas K, El Achkar CM, Smith LA, Rotenberg A, Poduri A, Sanchis-Juan A, Carss KJ, Rankin J, Zeman A, Raymond FL, Blyth M, Kerr B, Ruiz K, Urquhart J, Hughes I, Banka S, , Hedrich UBS, Scheffer IE, Helbig I, Zamponi GW, Lerche H, Mefford HC
De Novo Pathogenic Variants in CACNA1E Cause Developmental and Epileptic Encephalopathy with Contractures, Macrocephaly, and Dyskinesias.
Am J Hum Genet, 2019; 104

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CELL-TYPE SPECIFIC AND MOLECULAR CHARACTERIZATION OF DEPDC5-MEDIATED EPILEPSY

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Epilepsy affects over 60 million individuals worldwide and is characterized by unprovoked, recurrent seizures and cognitive deficits. Mutations in the DEP-domain-containing protein 5 (*DEPDC5)* gene are a major cause of focal epilepsy. Notably, over 60% of patients with *DEPDC5* mutations are nonresponsive to antiepileptic drugs. DEPDC5 negatively regulates mTORC1, a signaling network that integrates neuronal signals to promote cellular functions. However, the cell-types and molecular mechanism(s) by which DEPDC5-deficiency leads to abnormal, hypersynchronized activity in the brain remain elusive. Given that a) epilepsy is often characterized as a disorder caused by imbalance of excitatory and inhibitory inputs in the brain and b) a full understanding of the role of DEPDC5 in epilepsy requires its dissection at the cellular level, we generated mice lacking *Depdc5* in excitatory (*Depdc5* fb-KO) or inhibitory (*Depdc5 Gad2*-KO) neurons. Interestingly, both models exhibit hyperactive mTORC1 (and unchanged mTORC2 activity), seizures, and cognitive deficits. Importantly, *Depdc5* fb-KO (but not *Depdc5 Gad2*-KO) mice exhibit premature death and, thus, better recapitulate the sudden unexplained death in epilepsy (SUDEP) phenotype observed in patients with *DEPDC5* mutations. More importantly, the SUDEP, seizure and cognitive deficits in *Depdc5* fb-KO mice are fully suppressed by genetic (but not pharmacological) inhibition of mTORC1. Finally, using both phosphoproteomics and genomics, we began to decipher the mechanism by which loss of DEPDC5 leads to epilepsy. Our findings indicate that mTORC1 is the major driver of seizures and SUDEP associated with deficiency in DEPDC5. Moreover, new and selective mTORC1 inhibitors could represent effective therapies for *DEPDC5*-related disorders.

Pubmed:
31636454: Chen CJ, Sgritta M, Mays J, Zhou H, Lucero R, Park J, Wang IC, Park JH, Kaipparettu BA, Stoica L, Jafar-Nejad P, Rigo F, Chin J, Noebels JL, Costa-Mattioli M
Therapeutic inhibition of mTORC2 rescues the behavioral and neurophysiological abnormalities associated with Pten-deficiency.
Dysregulation of the mammalian target of rapamycin (mTOR) signaling, which is mediated by two structurally and functionally distinct complexes, mTORC1 and mTORC2, has been implicated in several neurological disorders. Individuals carrying loss-of-function mutations in the phosphatase and tensin homolog (PTEN) gene, a negative regulator of mTOR signaling, are prone to developing macrocephaly, autism spectrum disorder (ASD), seizures and intellectual disability. It is generally believed that the neurological symptoms associated with loss of PTEN and other mTORopathies (for example, mutations in the tuberous sclerosis genes TSC1 or TSC2) are due to hyperactivation of mTORC1-mediated protein synthesis. Using molecular genetics, we unexpectedly found that genetic deletion of mTORC2 (but not mTORC1) activity prolonged lifespan, suppressed seizures, rescued ASD-like behaviors and long-term memory, and normalized metabolic changes in the brain of mice lacking Pten. In a more therapeutically oriented approach, we found that administration of an antisense oligonucleotide (ASO) targeting mTORC2's defining component Rictor specifically inhibits mTORC2 activity and reverses the behavioral and neurophysiological abnormalities in adolescent Pten-deficient mice. Collectively, our findings indicate that mTORC2 is the major driver underlying the neuropathophysiology associated with Pten-deficiency, and its therapeutic reduction could represent a promising and broadly effective translational therapy for neurological disorders where mTOR signaling is dysregulated.
Nat Med, 2019; 25
29786082: Zhu PJ, Chen CJ, Mays J, Stoica L, Costa-Mattioli M
mTORC2, but not mTORC1, is required for hippocampal mGluR-LTD and associated behaviors.
The mechanistic target of rapamycin complex 1 (mTORC1) has been reported to be necessary for metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD). Here we found that mTORC1-deficient mice exhibit normal hippocampal mGluR-LTD and associated behaviors. Moreover, rapamycin blocks mGluR-LTD in mTORC1-deficient mice.

However, both rapamycin and mGluR activation regulate mTOR complex 2 (mTORC2) activity, and mTORC2-deficient mice show impaired mGluR-LTD and associated behaviors. Thus, mTORC2 is a major regulator of mGluR-LTD.
Nat Neurosci, 2018; 21

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ATP6V1A, A KEY PLAYER FOR LYSOSOMAL FUNCTION AND AUTOPHAGY PROCESS, IS REQUIRED FOR NEURONAL DEVELOPMENT AND SYNAPTIC PLASTICITY

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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ATP6V1A is a member of the vesicular-type adenosine triphosphate (v-ATPase), a ubiquitous multimeric complex that drive the transport of hydrogen ions upon ATP hydrolysis. In neurons, v-ATPase activity is also necessary for neurotransmitter loading into synaptic vesicles. Loss of function mutations in ATP6V1A have been described in patients with epileptic encephalopathy and metabolic disorders; downregulation of ATP6V1A have been recently reported has a hallmark of Alzheimer's disease. In fibroblasts derived from ATP6V1A patients we demonstrated defects in lysosomal homeostasis and autophagy. Yet the role of ATP6V1A in neuronal development and synaptic activity is largely unexplored. We modelled ATP6V1A loss by RNAi-knockdown in rat hippocampal neurons at different developmental stages. We evaluated the neuronal phenotypes by morphological analysis, immunolabelling with lysosomal, autophagy and synaptic markers and patch clamp recordings. Atp6v1a silencing resulted in impairment of neurite arborization and diminished number of glutamatergic synapses accompanied by a significant decrease in the frequency of miniature excitatory postsynaptic currents. These phenotypes were associated with lysosomal alterations and impaired autophagy. By challenging neurons with chemical LTP, we revealed that the autophagy boost followed by the upregulation of glutamatergic synapses were abolished in ATP6V1A silenced neurons compared to control neurons. The data suggest a pivotal role of ATP6V1A in the regulation of neuronal autophagy and in synaptic stabilization and plasticity.

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USING CELLULAR SENESCENCE AS A NOVEL TARGETABLE BIOMARKER IN CHILDHOOD REFRACTORY EPILESPY TO ABOLISH SEIZURES IN A PRECLINICAL MODEL

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Cortical malformations are associated with childhood-onset drug-resistant epilepsy for which surgery is the main therapeutic option to achieve seizure freedom. Focal cortical dysplasia type II (FCDII) is the most frequent cortical malformation among the epilepsy surgery population. FCDII is caused by brain somatic mTOR pathway-activating mutations that occured during neurodevelopment, which produce abnormal cytomegalic cells: dysmorphic neurons and balloon cells. Here we show by *in vitro* recordings of acute cortical slices from resected human FCDII cortex that epileptiform activity correlates with the density of mTOR-hyperactive dysmorphic neurons, supporting their pro-epileptogenic role. We discovered that human surgical FCDII brain tissues express molecular programs of cellular senescence, and further confirmed it at the protein and enzymatic levels, including p53/p16^{INK4A} expression and senescence-associated β-galactosidase activity, specifically in the abnormal cell types. In mTOR-hyperactive mouse models, cellular senescence hallmarks were also present alongside the production of a functional senescence-associated secretory phenotype (SASP). Administration of senolytic molecules that specifically eliminate senescent cells to an Mtor^{S2215F} FCDII mouse model, reduced both the density of dysmorphic-like neurons and the frequency of spontaneous seizures. Our results provide a proof-of-concept that targeting cellular senescence through senotherapy is an efficient approach to control seizures in a preclinical mouse model. These findings pave the way towards the development of ultra-precision medicine with the use of innovative therapeutic strategies selectively targeting mutated cells with a senescent phenotype, and possibly extend it to the spectrum of other 'mTORopathies'.

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VULNERABILITY OF P2Y12-IMMUNOPOSITIVE MICROGLIA IN FOCAL CORTICAL DYSPLASIA TYPE II

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Gliosis is a prominent pathological finding in the epileptic brain. Recently, a growing number of studies suggest that microglia are not only involved in the inflammatory processes, but also monitor different functions of neurons. The somatic junction between microglia and neurons plays an important role in the latter. The morphology of microglia could refer to its function. In our study ramified, hypertrophic, dystrophic, amoeboid, and rod-shaped cells were distinguished. Six surgically removed epileptic cortices with type II focal cortical dysplasia (FCD) were compared to post-mortem control brains from the same cortical regions. Microglia were labeled with P2Y12 immunofluorescence, while neurons were marked with NeuN and their membrane with Kv2.1. The Kv2.1 potassium channel is part of the somatic junction. The densities of all microglia and the proportion of different features of morphologies were measured in layers 3, 5, and the white matter. Furthermore, in layer 3 the coverage of P2Y12-positive microglia were measured on neuronal somata. Our results show that although total density of microglia does not change in FCD compared to control, the proportion of dystrophic cells increases, primarily to the detriment of the ramified ones. In addition, mean microglial coverage is increased in FCD samples. Our results suggest that the functionality of microglia in FCD type II is impaired, causing the increase of dystrophic microglia. The somatic coverage may increase as compensation for the functional impairment. Our plan is the electron microscopic analysis of the microglia-neuron junction in FCD and further investigation of the brain macrophages.

Pubmed:
33355694: Szocsics P, Papp P, Havas L, Watanabe M, Maglóczky Z
Perisomatic innervation and neurochemical features of giant pyramidal neurons in both hemispheres of the human primary motor cortex.
Betz cells-the gigantopyramidal neurons found in high amount in the primary motor cortex-are among of the most characteristic neuronal cells. A part of them contains the calcium-binding protein parvalbumin (PV) in primates. However, less is known about these cells in the human motor cortex despite their important role in different neurological disorders. Therefore, the aim of our study was to investigate the neurochemical features and perisomatic input properties of Betz cells in control human samples with short post-mortem interval. We used different microscopic techniques to investigate the primary motor cortex of both hemispheres. The soma size and density, and expression of PV of the Betz cells were investigated. Furthermore, we used confocal fluorescent and electron microscopy to examine their perisomatic input. The soma size and density showed moderate variability among samples and hemispheres. Post-mortem interval and hemispherical localization did not influence these features. Around 70% of Betz cells expressed PV, but in less intensity than the cortical interneurons. Betz neurons receive dense perisomatic input, which are mostly VIAAT- (vesicular inhibitory amino acid transporter) and PV immunopositive. In the electron microscope, we found PV-immunolabelled terminals with asymmetric-like synaptic structure, too. Terminals with morphologically similar synaptic specialisation were also found among vGluT2- (vesicular glutamate transporter type 2) immunostained terminals contacting Betz cells. Our data suggest that Betz cells' morphological properties showed less variability among subjects and hemispheres than the density of them. Their neurochemical and perisomatic input characteristics support their role in execution of fast and precise movements.
Brain Struct Funct, 2021; 226

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BOARD NUMBER: S04-676

HIPPOCAMPAL ORIGIN OF SPONTANEOUS RECURRENT SEIZURES IN CNTNAP2 KNOCK-OUT MICE

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Focal cortical dysplasia is the most common cause of drug-resistant epilepsy in the pediatric population and the second to third most common etiology in adults, creating a need for further investigation and development of new therapeutic strategies. Several genetic variants underlying malformations of cortical development and epilepsy have been identified, and a familial recessive loss of function in the gene *Cntnap2* was shown to give rise to cortical dysplasia with neurodevelopmental regression after onset of drug-resistant seizures, known as cortical dysplasia-focal epilepsy (CDFE) syndrome. Mice lacking functional *Cntnap2* expression show postnatal features akin to CDFE, such as hyperactivity and deficits in three core ASD behavioral domains, and develop spontaneous seizures around 6 months of age. Using continuous intracranial video-EEG, with bilateral recordings from hippocampus or hippocampus and cortex, after six months of age, we have identified the hippocampus as a potential origin for spontaneous recurrent seizures and an association between an increased number of interictal epileptiform discharges (IED) and seizures. Increased locomotor activity in the open field was still present in *Cntnap2* knock-out (KO) mice after seizure onset while the increase in self-grooming seen at 4-months was decreased after seizure onset. We found no differences in performance of memory tests, either short-term (object location task, after 20 minutes) or long-term (novel object recognition, 24 hours) between WT and *Cntnap2* KO mice at 4 months, while *Cntnap2* KO mice at 7-months, after seizure onset had a significantly decreased discrimination ratio.

Pubmed:
34764308: Gonzalez-Ramos A, Waloschková E, Mikroulis A, Kokaia Z, Bengzon J, Ledri M, Andersson M, Kokaia M
Human stem cell-derived GABAergic neurons functionally integrate into human neuronal networks.
Gamma-aminobutyric acid (GABA)-releasing interneurons modulate neuronal network activity in the brain by inhibiting other neurons. The alteration or absence of these cells disrupts the balance between excitatory and inhibitory processes, leading to neurological disorders such as epilepsy. In this regard, cell-based therapy may be an alternative therapeutic approach. We generated light-sensitive human embryonic stem cell (hESC)-derived GABAergic interneurons (hdIN) and tested their functionality. After 35 days in vitro (DIV), hdINs showed electrophysiological properties and spontaneous synaptic currents comparable to mature neurons. In co-culture with human cortical neurons and after transplantation (AT) into human brain tissue resected from patients with drug-resistant epilepsy, light-activated channelrhodopsin-2 (ChR2) expressing hdINs induced postsynaptic currents in human neurons, strongly suggesting functional efferent synapse formation. These results provide a proof-of-concept that hESC-derived neurons can integrate and modulate the activity of a human host neuronal network. Therefore, this study supports the possibility of precise temporal control of network excitability by transplantation of light-sensitive interneurons.
Sci Rep, 2021; 11
34948040: Waloschková E, Gonzalez-Ramos A, Mikroulis A, Kudláček J, Andersson M, Ledri M, Kokaia M
Human Stem Cell-Derived GABAergic Interneurons Establish Efferent Synapses onto Host Neurons in Rat Epileptic Hippocampus and Inhibit Spontaneous Recurrent Seizures.
Epilepsy is a complex disorder affecting the central nervous system and is characterised by spontaneously recurring seizures (SRSs). Epileptic patients undergo symptomatic pharmacological treatments, however, in 30% of cases, they are ineffective, mostly in patients with temporal lobe epilepsy. Therefore, there is a need for developing novel treatment strategies. Transplantation of cells releasing γ-aminobutyric acid (GABA) could be used to counteract the imbalance between excitation and inhibition within epileptic neuronal networks. We generated GABAergic interneuron precursors from human embryonic stem cells (hESCs) and grafted them in the hippocampi of rats developing chronic SRSs after kainic acid-induced status epilepticus. Using whole-cell patch-clamp recordings, we characterised the maturation of the grafted cells into functional GABAergic interneurons in the host brain, and we confirmed the presence of functional inhibitory synaptic connections from grafted cells onto the host neurons. Moreover, optogenetic stimulation of grafted hESC-derived interneurons reduced the rate

of epileptiform discharges in vitro. We also observed decreased SRS frequency and total time spent in SRSs in these animals in vivo as compared to non-grafted controls. These data represent a proof-of-concept that hESC-derived GABAergic neurons can exert a therapeutic effect on epileptic animals presumably through establishing inhibitory synapses with host neurons.
Int J Mol Sci, 2021; 22
31871869: Vasudevan S, Kajtez J, Bunea AI, Gonzalez-Ramos A, Ramos-Moreno T, Heiskanen A, Kokaia M, Larsen NB, Martínez-Serrano A, Keller SS, Emnéus J
Leaky Optoelectrical Fiber for Optogenetic Stimulation and Electrochemical Detection of Dopamine Exocytosis from Human Dopaminergic Neurons.
In Parkinson's disease, the degeneration of dopaminergic neurons in substantia nigra leads to a decrease in the physiological levels of dopamine in striatum. The existing dopaminergic therapies effectively alleviate the symptoms, albeit they do not revert the disease progression and result in significant adverse effects. Transplanting dopaminergic neurons derived from stem cells could restore dopamine levels without additional motor complications. However, the transplanted cells disperse in vivo and it is not possible to stimulate them on demand to modulate dopamine release to prevent dyskinesia. In order to address these issues, this paper presents a multifunctional leaky optoelectrical fiber for potential neuromodulation and as a cell substrate for application in combined optogenetic stem cell therapy. Pyrolytic carbon coated optical fibers are laser ablated to pattern micro-optical windows to permit light leakage over a large area. The pyrolytic carbon acts as an excellent electrode for the electrochemical detection of dopamine. Human neural stem cells are genetically modified to express the light sensitive opsin channelrhodopsin-2 and are differentiated into dopaminergic neurons on the leaky optoelectrical fiber. Finally, light leaking from the micro-optical windows is used to stimulate the dopaminergic neurons resulting in the release of dopamine that is detected in real-time using chronoamperometry.
Adv Sci (Weinh), 2019; 6
28279192: de la Rosa-Prieto C, Laterza C, Gonzalez-Ramos A, Wattananit S, Ge R, Lindvall O, Tornero D, Kokaia Z
Stroke alters behavior of human skin-derived neural progenitors after transplantation adjacent to neurogenic area in rat brain.
Intracerebral transplantation of human induced pluripotent stem cells (iPSCs) can ameliorate behavioral deficits in animal models of stroke. How the ischemic lesion affects the survival of the transplanted cells, their proliferation, migration, differentiation, and function is only partly understood.
Stem Cell Res Ther, 2017; 8

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BOARD NUMBER: S04-677

MICROGLIA-SPECIFIC KNOCKDOWN OF CORE CLOCK GENE BMAL1 LEADS TO AN ALTERED BEHAVIOURAL PHENOTYPE AND INCREASED SUSCEPTIBILITY TO SEIZURES IN MICE

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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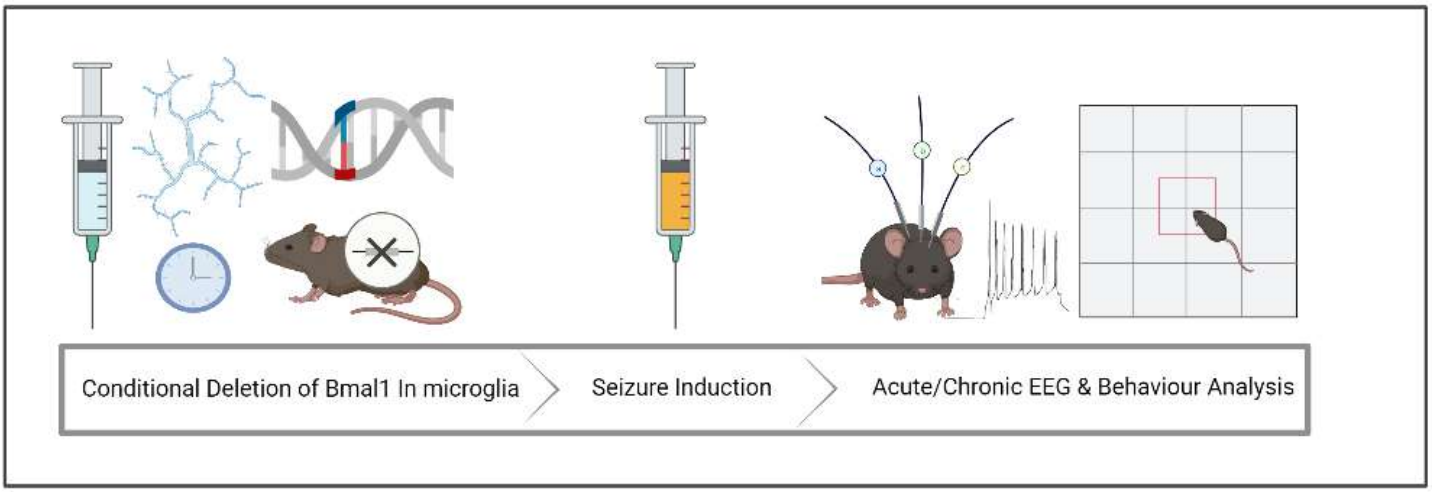
Background: Despite technological advances, epilepsy remains an unsolved clinical issue. Brain inflammation, which is mostly mediated by microglial cells, is a known feature in epilepsy and contributor to the development of spontaneous seizures. Immunity and the inflammatory response is highly regulated by the circadian rhythm; i.e. the 24 hour internal clock governing physiological function via a series of autoregulatory transcriptional-translational feedback loops. Here we explored the impact of the microglial-specific core circadian gene (Bmal1) deletion on behavioural phenotype and seizure susceptibility in mice. **Methods:** Twenty male and female adult Bmal1-Cx3CR1Cre-ER mice bred inhouse were injected with either tamoxifen or vehicle daily (40 mg/kg; IP; 10 days) to induce microglial-specific Bmal1 deletion. Two weeks after recombination, mice underwent a battery of behavioural tests which was analysed via AnyMaze software. A separate cohort was implanted with electrodes for electroencephalographic (EEG) recordings and after recovery underwent the injection of a sub-convulsant dose of kainic acid (KA; IP; 15 mg/kg) to test seizure susceptibility. **Results:** Mice with Bmal1 microglial deletion (KO) displayed a hyperactive behavioural phenotype when compared to their littermate controls. Bmal1-KOs had an increased susceptibility to develop seizures and a significantly increased seizure severity measured by the total EEG power after KA administration at a dose that would not usually trigger seizures in naïve mice. **Conclusions**: Microglial-specific depletion of Bmal1 led to a disrupted behavioural and seizure phenotype in otherwise normal mice. Further studies using long-term EEG will allow to us understand how Bmal1 disruption contributes to the development of epilepsy.



Pubmed:
31612119: Eliwan HO, Watson WRG, Regan I, Philbin B, O'Hare FM, Strickland T, O'Neill A, O'Rourke M, Blanco A, Healy M, Nolan B, Smith O, Molloy EJ
Pediatric Intensive Care: Immunomodulation With Activated Protein C .

Sepsis is major cause of morbidity and mortality in the Pediatric Intensive Care Unit (PICU). PICU patients may develop transient immune deficiency during sepsis. Activated Protein C (APC) has significant anti-inflammatory and cytoprotective effects. Clinical trials of APC in adult sepsis initially showed improved outcome but recent trials showed no benefit in adults or children. We aimed to assess the effects of APC treatment on innate immune responses in children. We compared neutrophil and monocyte responses to lipopolysaccharide (LPS) with and without APC treatment in PICU patients at the time of evaluation for sepsis compared with healthy adults and age-matched pediatric controls. We used flow cytometry to examine cell activation (CD11b expression), function [intracellular reactive oxygen intermediate (ROI) release] and LPS recognition [Toll like Receptor 4 (TLR4) expression]. PICU patients had significantly decreased protein c levels and LPS responses compared with adult and pediatric controls for all parameters. APC reduced LPS-induced neutrophil PICU TLR4 and adult ROI (< 0.05). PICU non-survivors had increased LPS induced neutrophil and monocyte ROI production vs. survivors which was significantly reduced by APC. PICU patients demonstrate significantly reduced endotoxin reactivity which may predispose them to sepsis and alter effective antibacterial responses. APC reduces LPS-induced ROI production in adults and may have a role in treating severely compromised PICU patients especially given that newer APC forms are associated with decreased bleeding risk and enhanced anti-inflammatory effects.
Front Pediatr, 2019; 7
31058120: Aslam S, Strickland T, Molloy EJ
Neonatal Encephalopathy: Need for Recognition of Multiple Etiologies for Optimal Management.
Neonatal encephalopathy (NE) is associated with high mortality and morbidity. Factors predisposing to NE can be antenatal, perinatal, or a combination of both. Antenatal maternal factors, familial factors, genetic predisposition, hypoxic ischemic encephalopathy, infections, placental abnormalities, thrombophilia, coagulation defects, and metabolic disorders all have been implicated in the pathogenesis of NE. At present, therapeutic hypothermia is the only treatment available, regardless of etiology. Recognizing the etiology of NE involved can also guide investigations such as metabolic and sepsis workups to ensure optimal management. Understanding the etiology of NE may allow the development of targeted adjunctive therapies related to the underlying mechanism and develop preventative strategies.
Front Pediatr, 2019; 7
32228505: Zareen Z, Strickland T, Eneaney VM, Kelly LA, McDonald D, Sweetman D, Molloy EJ
Cytokine dysregulation persists in childhood post Neonatal Encephalopathy.
Cytokines are possible mediators of neuroinflammation and associated with adverse outcome in neonatal encephalopathy (NE). Our aim was to explore cytokine response in children with Neonatal Encephalopathy (NE) at school age compared to age-matched controls.
BMC Neurol, 2020; 20
32610169: Dietrick B, Molloy E, Massaro AN, Strickland T, Zhu J, Slevin M, Donoghue V, Sweetman D, Kelly L, O'Dea M, McGowan M, Vezina G, Glass P, Vaidya D, Brooks S, Northington F, Everett AD
Plasma and Cerebrospinal Fluid Candidate Biomarkers of Neonatal Encephalopathy Severity and Neurodevelopmental Outcomes.
To identify candidate biomarkers in both plasma and cerebrospinal fluid (CSF) that are associated with neonatal encephalopathy severity measured by encephalopathy grade, seizures, brain injury by magnetic resonance imaging (MRI), and neurodevelopmental outcomes at 15-30 months.
J Pediatr, 2020; 226
33185287: Zareen Z, Strickland T, Fallah L, McEneaney V, Kelly L, McDonald D, Molloy EJ
Cytokine dysregulation in children with cerebral palsy.
To examine pro- and anti-inflammatory cytokines in children with cerebral palsy (CP) at baseline and in response to endotoxin (lipopolysaccharide), and correlate outcomes compared with age-matched comparisons, to evaluate their ability to mount an immune response.
Dev Med Child Neurol, 2021; 63
33768526: Kelly LA, O'Dea MI, Zareen Z, Melo AM, McKenna E, Strickland T, McEneaney V, Donoghue V, Boylan G, Sweetman D, Butler J, Vavasseur C, Miletin J, El-Khuffash AF, O'Neill LAJ, O'Leary JJ, Molloy EJ
Altered inflammasome activation in neonatal encephalopathy persists in childhood.
Neonatal encephalopathy (NE) is characterized by altered neurological function in term infants and inflammation plays an important pathophysiological role. Inflammatory cytokines interleukin (IL)-1β, IL-1ra and IL-18 are activated by the nucleotide-binding and oligomerization domain (NOD)-, leucine-rich repeat domain (LRR)- and NOD-like receptor protein 3 (NLRP3) inflammasome; furthermore, we aimed to examine the role of the inflammasome multiprotein complex involved in proinflammatory responses from the newborn period to childhood in NE. Cytokine concentrations were measured by multiplex enzyme-linked immunosorbent assay (ELISA) in neonates and children with NE in the absence or presence of lipopolysaccharide (LPS) endotoxin. We then investigated expression of the NLRP3 inflammasome genes, NLRP3, IL-1β and

ASC by polymerase chain reaction (PCR). Serum samples from 40 NE patients at days 1 and 3 of the first week of life and in 37 patients at age 4-7 years were analysed. An increase in serum IL-1ra and IL-18 in neonates with NE on days 1 and 3 was observed compared to neonatal controls. IL-1ra in NE was decreased to normal levels at school age, whereas serum IL-18 in NE was even higher at school age compared to school age controls and NE in the first week of life. Percentage of LPS response was higher in newborns compared to school-age NE. NLRP3 and IL-1β gene expression were up-regulated in the presence of LPS in NE neonates and NLRP3 gene expression remained up-regulated at school age in NE patients compared to controls. Increased inflammasome activation in the first day of life in NE persists in childhood, and may increase the window for therapeutic intervention.
Clin Exp Immunol, 2021; 205

34671050: Siddiqui AM, Islam R, Cuellar CA, Silvernail JL, Knudsen B, Curley DE, Strickland T, Manske E, Suwan PT, Latypov T, Akhmetov N, Zhang S, Summer P, Nesbitt JJ, Chen BK, Grahn PJ, Madigan NN, Yaszemski MJ, Windebank AJ, Lavrov IA
Newly regenerated axons via scaffolds promote sub-lesional reorganization and motor recovery with epidural electrical stimulation.
Here, we report the effect of newly regenerated axons via scaffolds on reorganization of spinal circuitry and restoration of motor functions with epidural electrical stimulation (EES). Motor recovery was evaluated for 7 weeks after spinal transection and following implantation with scaffolds seeded with neurotrophin producing Schwann cell and with rapamycin microspheres. Combined treatment with scaffolds and EES-enabled stepping led to functional improvement compared to groups with scaffold or EES, although, the number of axons across scaffolds was not different between groups. Re-transection through the scaffold at week 6 reduced EES-enabled stepping, still demonstrating better performance compared to the other groups. Greater synaptic reorganization in the presence of regenerated axons was found in group with combined therapy. These findings suggest that newly regenerated axons through cell-containing scaffolds with EES-enabled motor training reorganize the sub-lesional circuitry improving motor recovery, demonstrating that neuroregenerative and neuromodulatory therapies cumulatively enhancing motor function after complete SCI.
NPJ Regen Med, 2021; 6

34621028: Friedes BD, Molloy E, Strickland T, Zhu J, Slevin M, Donoghue V, Sweetman D, Kelly L, O'Dea M, Roux A, Harlan R, Ellis G, Manlhiot C, Graham D, Northington F, Everett AD
Neonatal encephalopathy plasma metabolites are associated with neurodevelopmental outcomes.
To investigate mechanisms of injury and recovery in neonatal encephalopathy (NE), we performed targeted metabolomic analysis of plasma using liquid chromatography with tandem mass spectrometry (LC/MS/MS) from healthy term neonates or neonates with NE.
Pediatr Res, 2021;

34712631: O'Dea MI, Kelly LA, McKenna E, Strickland T, Hurley TP, Butler J, Vavasseur C, El-Khuffash AF, Miletin J, Fallah L, White A, Wyse J, Molloy EJ
Altered Cytokine Endotoxin Responses in Neonatal Encephalopathy Predict MRI Outcomes.
Neonatal encephalopathy (NE) is associated with adverse neurodevelopmental outcome and is linked with systemic inflammation. Pro-inflammatory and anti-inflammatory cytokines are known to play a role in the pathology of NE by activating innate immune cells. Eighty-seven infants were enrolled including 53 infants with NE of whom 52 received therapeutic hypothermia (TH) and 34 term infant healthy controls (TC). Whole blood sampling was performed in the first 4 days of life, and a 14-spot ELISA Multiplex Cytokine Array was carried out on baseline samples or after stimulation with lipopolysaccharide (LPS) as an additional inflammatory stimulus. The cytokine medians were examined for differences between infants with NE and healthy TC; and then short-term outcomes of Sarnat stage, seizures, and MRI brain were examined within the NE group. The potential of LPS stimulation to predict abnormal MRI was explored using receiver operating characteristic (ROC) curves. At baseline, infants with NE had significantly higher levels of erythropoietin (Epo), interleukin (IL)-6, and IL-1ra and significantly lower vascular endothelial growth factor (VEGF) than had controls. All cytokines were increased after LPS stimulation in infants with NE with an excessive Epo and IL-1ra response than in controls. Infants with NE had lower IL-8, IL-2, IL-6, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), VEGF, and interferon (IFN)-γ than controls had following LPS. GM-CSF and IFN-γ, IL-1β, IL-1ra, and VEGF were higher on days 1-2 in NE infants with abnormal neuroimaging. GM-CSF, IFN-γ, and TNF-α levels with LPS stimulation were different upon stimulation between normal and abnormal neuroimaging. TNF-α is the only strong cytokine predictor both pre- and post-LPS stimulation of abnormal brain imaging. Altered cytokine responses are found in infants with NE vs. controls, and more significant differences are unmasked by the additional stimulus of LPS, which potentially improves the predictive power of these cytokines for the detection of abnormal MRIs. Infants with NE undergoing TH demonstrate both trained immunity and tolerance, and understanding these responses will facilitate adjunctive immunomodulatory treatments.
Front Pediatr, 2021; 9

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34528287: Sweetman DU, Strickland T, Isweisi E, Kelly L, Slevin MT, Donoghue V, Meehan J, Boylan G, Murphy JFA, El-Khuffash A, Molloy EJ
Multi-organ dysfunction scoring in neonatal encephalopathy (MODE Score) and neurodevelopmental outcomes.
Neonatal encephalopathy (NE) is associated with an increased risk of multi-organ injury. The lack of standardised definitions for multi-organ dysfunction in NE hinders accurate quantification of these complications.
Acta Paediatr, 2022; 111

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BOARD NUMBER: S04-678

EARLY POSTNATAL TRANSPLANTATION OF HUMAN STEM CELL-DERIVED GABAERGIC INTERNEURONS IN CNTNAP2 KNOCK-OUT MICE

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

Ana Gonzalez-Ramos, Fredrik Berglind, Kerstin Laurin, Diana Hatamian, Mohanad Hayatleh, Anja Vombergar, Marco Ledri, Merab Kokaia, My Andersson
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Focal cortical dysplasia (FCD) is a group of developmental malformations of the cortex manifesting as confined regions of pathology. We have explored a GABAergic cell-based replacement therapy in a mouse model of cortical dysplasia-focal epilepsy syndrome, Cntnap2-/- mice, targeting the hippocampus. Human embryonic stem cell (hESC)-derived interneurons (hdIN) were transplanted unilaterally to the dorsal hippocampus of postnatal day 2 (P2) Cntnap2-/- mice and behavioral tests assessing memory function, cognitive flexibility, anxiety, and locomotor activity were performed at 4- and 7-months of age, followed by 2 weeks of continuous video-EEG monitoring. Histological evaluation confirmed survival of hdINs at all time points studied, up to 9 months post-transplantation (PT), with hdINs migrating along the ipsi- and to the contralateral hippocampus and adjacent structures. However, cell-transplanted animals had increased IEDs and seizures compared to the sham- transplanted group, suggesting that hdIN transplantation does not prevent the development of epilepsy in this model, as hypothesized, but rather enhances it.

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BOARD NUMBER: S04-679

A VERSATILE AND EASY-TO-CUSTOMIZE CONDITIONAL KNOCKOUT MOUSE MODEL TO STUDY THE ROLE OF REST IN EPILEPSY

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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The Repressor Element 1 Silencing Transcription Factor (REST) is an epigenetic master regulator playing a crucial role in the nervous system. REST function was originally described in developmental stages, where it determines neuronal phenotype, however recent studies showed that REST participates in several processes also in the adult brain, including neuronal plasticity and epileptogenesis. In particular, whether REST prevents or promotes epileptogenesis is still debated. We here aim at tackling this issue through an *in vivo* characterization of transgenic murine lines where REST is deleted in specific cellular populations. First, we targeted the excitatory neuron population cloning the calcium/calmodulin-dependent protein kinase II (CaMKII) minimal promoter upstream of either the Cre recombinase or its inactive form. After assessing the specificity of promoter's expression, the transgenes were packaged in an innovative, engineered adeno-associated virus capsid (PHP.eB) able to cross the blood-brain and blood-cerebrospinal fluid barriers, and delivered in the lateral ventricles of 2-month-old $REST^{flox/flox}$ mice. We then characterized the cognitive phenotype (open field, novel object recognition, and social interaction tests) and the seizure propensity. We show that conditional REST knockout (REST-cKO) mice are more anxious, have short-term memory deficits, and are more social than control mice. On the other hand, behavioral evaluation of the susceptibility to epileptic seizures shows that REST-cKO mice are more resistant to chronic, but not acute, pentylenetetrazole (PTZ)-induced seizures, showing a milder phenotype. Overall, these data suggest that the absence of REST is sufficient to induce behavioral alterations and contributes to the progression of the epileptogenic process.

Pubmed:
32988385: Flores Gutiérrez J, De Felice C, Natali G, Leoncini S, Signorini C, Hayek J, Tongiorgi E
Protective role of mirtazapine in adult female Mecp2 mice and patients with Rett syndrome.
Rett syndrome (RTT), an X-linked neurodevelopmental rare disease mainly caused by MECP2-gene mutations, is a prototypic intellectual disability disorder. Reversibility of RTT-like phenotypes in an adult mouse model lacking the Mecp2-gene has given hope of treating the disease at any age. However, adult RTT patients still urge for new treatments. Given the relationship between RTT and monoamine deficiency, we investigated mirtazapine (MTZ), a noradrenergic and specific-serotonergic antidepressant, as a potential treatment.
J Neurodev Disord, 2020; 12

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BOARD NUMBER: S04-680

MTOR COMPLEXES IN EPILEPSY AND SEIZURE DISORDERS

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

James Okoh[1], Jacqunae Mays[1], Alexandre Bacq[2], Hongyi Zhou[1], Khalel Imanbeyev[1], Paymaan Jafar-Nejad[3], Jeffrey Noebels[1], Stephanie Baulac[4], Mauro Costa-Mattioli.[1]
[1]Baylor College of Medicine, Department Of Neuroscience, Houston, United States of America, [2]Institut du Cerveau - Paris Brain Institute - ICM, Sorbonne Université, Inserm, Cnrs, Hôpital De La Pitié Salpêtrière, PARIS, France, [3]Ionis Pharmaceuticals, Research, Carlsbad, United States of America, [4]Institut du Cerveau - Paris Brain Institute - ICM, Sorbonne Université, Inserm, Cnrs, Hôpital De La Pitié Salpêtrière, Paris, France

Epilepsy, a neurological disorder that poses a major threat to public health and presents an enormous economic and social burden, is characterized by spontaneous recurrent seizures. Dysregulation of the mammalian target of rapamycin (mTOR), which functions via two distinct complexes named mTORC1 and mTORC2, has been causally linked to epilepsy. Currently, it is widely believed that hyperactivation of mTORC1, which is sensitive to the drug rapamycin, leads to abnormal network rhythmicity associated with epilepsy. Most of the evidence supporting the role for hyperactivation of mTORC1 in epilepsy relies on its chronic pharmacological inhibition with rapamycin. However, chronic rapamycin treatment, which reduces seizures in several epilepsy models, also inhibits mTORC2. We recently found that genetic inhibition of mTORC2, but not mTORC1, improved behavioral and neurophysiological deficits (including seizures) in mice lacking the mTOR upstream negative regulator, *Pten*. Thus, it remains unclear whether hyperactivation of mTORC1 or mTORC2 leads to abnormal synchronized neuronal firing during epilepsy. To dissect the role of mTOR complexes in epilepsy, we used molecular genetics to selectively silence the activity of either mTORC1 or mTORC2 in forebrain neurons. Starting with the kainic acid (KA) model of epilepsy, we found that selective genetic inhibition of mTORC1 increased acute seizures. Interestingly, we found that inhibition of mTORC2 reduced seizures not only in the KA model, but also in a wide range of epilepsy models. Taken together, these results suggest that mTORC2 is a central driver of seizures and inhibition of mTORC2 presents a novel and broadly applicable therapeutic target.

Pubmed:
33395685: Nolan SO, Hodges SL, Okoh JT, Binder MS, Lugo JN
Prenatal High-Fat Diet Rescues Communication Deficits in Fmr1 Mutant Mice in a Sex-Specific Manner.
Using high-throughput analysis methods, the present study sought to determine the impact of prenatal high-fat dietary manipulations on isolation-induced ultrasonic vocalization production in both male and female Fmr1mutants on postnatal day 9. Prior to breeding, male FVB/129 Fmr1 wildtype and female Fmr1 heterozygous breeding pairs were assigned to 1 of 3 diet conditions: standard lab chow, omega-3 fatty acid-enriched chow, and a diet controlling for the fat increase. Prenatal exposure to omega-3 fatty acids improved reductions in the number of calls produced by Fmr1heterozygotes females. Moreover, diminished spectral purity in the female Fmr1homozygous mouse was rescued by exposure to both high-fat diets, although these effects were not seen in the male Fmr1knockout. Prenatal dietary fat manipulation also influenced several other aspects of vocalization production, such as the number of calls produced and their fundamental frequency, aside from effects due to loss of Fmr1.Specifically, in males, regardless of genotype, prenatal exposure to high omega-3s increased the average fundamental frequency of calls. These data support the need for future preclinical and clinical work elucidating the full potential of prenatal high-fat diets as a novel therapeutic alternative forFragile X syndrome.
Dev Neurosci, 2020; 42
34010641: Asede D, Doddapaneni D, Chavez A, Okoh J, Ali S, Von-Walter C, Bolton MM
Apical intercalated cell cluster: A distinct sensory regulator in the amygdala.
GABAergic neurons regulate different aspects of information processing in the amygdala. Among these are clusters of intercalated cells (ITCs), which have been implicated in fear-related behaviors. Although a few of the ITC clusters have been studied, the functional role of apical ITCs (apITCs) is unknown. Here, we combine monosynaptic rabies tracing with optogenetics and demonstrate that apITCs receive synaptic input from medial geniculate nucleus (MGm), posterior intralaminar nucleus (PIN), and medial dorsal nucleus of the thalamus and from a diverse range of cortical areas including temporal association, entorhinal, insular, piriform, and somatosensory cortex. Upon fear learning, PIN/MGm inputs are

strengthened, indicative of their involvement in fear behaviors. 3-D reconstruction of apITCs reveals local arborization and innervation of the dorsal striatum and lateral amygdala. We further show that apITCs provide sensory feedforward inhibition to LA principal cells, a putative mechanism for controlling plasticity during fear learning.
Cell Rep, 2021; 35
35089938: Nolan SO, Hodges SL, Binder MS, Smith GD, Okoh JT, Jefferson TS, Escobar B, Lugo JN
Dietary rescue of adult behavioral deficits in the Fmr1 knockout mouse.
The current study aimed to further address important questions regarding the therapeutic efficacy of omega-3 fatty acids for various behavioral and neuroimmune aspects of the Fmr1 phenotype. To address these questions, our experimental design utilized two different omega-3 fatty acid administration timepoints, compared to both standard laboratory chow controls ("Standard") and a diet controlling for the increase in fat content ("Control Fat"). In the first paradigm, post-weaning supplementation (after postnatal day 21) with the omega-3 fatty acid diet ("Omega-3") reversed deficits in startle threshold, but not deficits in prepulse inhibition, and the effect on startle threshold was not specific to the Omega-3 diet. However, post-weaning supplementation with both experimental diets also impaired acquisition of a fear response, recall of the fear memory and contextual fear conditioning compared to the Standard diet. The post-weaning Omega-3 diet reduced hippocampal expression of IL-6 and this reduction of IL-6 was significantly associated with diminished performance in the fear conditioning task. In the perinatal experimental paradigm, the Omega-3 diet attenuated hyperactivity and acquisition of a fear response. Additionally, perinatal exposure to the Control Fat diet (similar to a "Western" diet) further diminished nonsocial anxiety in the Fmr1 knockout. This study provides significant evidence that dietary fatty acids throughout the lifespan can significantly impact the behavioral and neuroimmune phenotype of the Fmr1 knockout model.
PLoS One, 2022; 17
34393751: Asede D, Okoh J, Ali S, Doddapaneni D, Bolton MM
Deletion of ErbB4 Disrupts Synaptic Transmission and Long-Term Potentiation of Thalamic Input to Amygdalar Medial Paracapsular Intercalated Cells.
Identification of candidate risk genes and alteration in the expression of proteins involved in regulating inhibitory neuron function in various psychiatric disorders, support the notion that GABAergic neuron dysfunction plays an important role in disease etiology. Genetic variations in neuregulin and its receptor kinase ErbB4, expressed exclusively by GABAergic neurons in the CNS, have been linked with schizophrenia. In the amygdala, ErbB4 is highly expressed in GABAergic intercalated cell clusters (ITCs), which play a critical role in amygdala-dependent behaviors. It is however unknown whether ErbB4 deletion from ITCs affects their synaptic properties and function in amygdala circuitry. Here, we examined the impact of ErbB4 deletion on inhibitory and excitatory circuits recruiting medial paracapsular ITCs (mpITCs) using electrophysiological techniques. Ablation of ErbB4 in mpITCs suppressed NMDA receptor-mediated synaptic transmission at thalamo-mpITC synapses and enhanced thalamic driven GABAergic transmission onto mpITCs. Furthermore, long-term potentiation (LTP) at thalamo-mpITC synapses was compromised in ErbB4 mutant mice, indicating that ErbB4 activity is critical for LTP at these synapses. Together, our findings suggest that ErbB4 deletion from mpITCs disrupts excitation-inhibition balance and learning mechanisms in amygdala circuits.
Front Synaptic Neurosci, 2021; 13
31520894: Hodges SL, Reynolds CD, Nolan SO, Huebschman JL, Okoh JT, Binder MS, Lugo JN
A single early-life seizure results in long-term behavioral changes in the adult Fmr1 knockout mouse.
Fragile X syndrome (FXS) is the leading cause of inherited intellectual disability and a significant genetic contributor to Autism spectrum disorder. In addition to autistic-like phenotypes, individuals with FXS are subject to developing numerous comorbidities, one of the most prevalent being seizures. In the present study, we investigated how a single early-life seizure superimposed on a genetic condition impacts the autistic-like behavioral phenotype of the mouse. We induced status epilepticus (SE) on postnatal day (PD) 10 in Fmr1 wild type (WT) and knockout (KO) mice. We then tested the mice in a battery of behavioral tests during adulthood (PD90) to examine the long-term impact of an early-life seizure. Our findings replicated prior work that reported a single instance of SE results in behavioral deficits, including increases in repetitive behavior, enhanced hippocampal-dependent learning, and reduced sociability and prepulse inhibition ($p < 0.05$). We also observed genotypic differences characteristic of the FXS phenotype in Fmr1 KO mice, such as enhanced prepulse inhibition and repetitive behavior, hyperactivity, and reduced startle responses ($p < 0.05$). Superimposing a seizure on deletion of Fmr1 significantly impacted repetitive behavior in a nosepoke task. Specifically, a single early-life seizure increased consecutive nose poking behavior in the task in WT mice ($p < 0.05$), yet seizures did not exacerbate the elevated stereotypy observed in Fmr1 KO mice ($p > 0.05$). Overall, these findings help to elucidate how seizures in a critical period of development can impact long-term behavioral manifestations caused by underlying gene mutations in Fmr1. Utilizing double-hit models, such as superimposing seizures on the Fmr1 mutation, can help to enhance our understanding of comorbidities in disease models.
Epilepsy Res, 2019; 157
30525120: Holley AJ, Hodges SL, Nolan SO, Binder M, Okoh JT, Ackerman K, Tomac LA, Lugo JN

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A single seizure selectively impairs hippocampal-dependent memory and is associated with alterations in PI3K/Akt/mTOR and FMRP signaling.
A single brief seizure before learning leads to spatial and contextual memory impairment in rodents without chronic epilepsy. These results suggest that memory can be impacted by seizure activity in the absence of epilepsy pathology. In this study, we investigated the types of memory affected by a seizure and the time course of impairment. We also examined alterations to mammalian target of rapamycin (mTOR) and fragile X mental retardation protein (FMRP) signaling, which modulate elements of the synapse and may underlie impairment.
Epilepsia Open, 2018; 3

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BOARD NUMBER: S04-681

NEURAL BASES OF THE BODILY SELF AS REVEALED BY ELECTRICAL BRAIN STIMULATION IN EPILEPTIC PATIENTS

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Introduction: It's been proposed that the bodily self, involving pre-reflective awareness of the body, relies on several bodily experiences, such as self-location, body ownership, agency, first-person perspective, and the perceptual body image. Electrical brain stimulation in patients with pharmacoresistant epilepsy or tumour allows functional brain mapping with a high spatial resolution. The aim of this study is to identify the neural bases of the bodily self from electrical brain stimulation data. **Method:** We conducted a retrospective analysis of 13 984 electrical brain stimulations in 220 patients with pharmaco-resistant epilepsy who underwent stereo-electroencephalography for presurgical evaluation between 2011 and 2020. **Results:** Twenty-nine patients reported a bodily self disturbance during electrical brain stimulation. Electrical brain stimulation mainly disturbed the body image, but also self-location and agency. Changes in the body self were mostly evoked by stimulation of the cingulate cortex, inferior parietal lobule and insula. **Discussion:** The neural bases of the bodily self, as revealed by electrical brain stimulation, are localised in parietal, insular and cingulate regions known to integrate sensory afferents. These regions differ from the cortices involved in reflexive and cognitive levels related to self-representations.

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THE N6-METHYLADENOSINE RNA METHYLATION MACHINERY, A POTENTIAL THERAPEUTIC TARGET FOR TEMPORAL LOBE EPILEPSY

POSTER SESSION 04 - SECTION: EPILEPSY AND EPILEPTOGENESIS

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Temporal Lobe epilepsy is the most severe form of epilepsy in adults. Around 30% of patients are refractory to treatment, hence the need to improve treatments. Epileptogenesis is the period following a brain injury during which the brain becomes epileptic through several molecular and cellular changes potentially driven by gene expression. N6-methyladenosine (m^6A) RNA methylation is the most abundant RNA modification which can drastically alter translational efficiency and mRNA stability. The modification is catalyzed by m^6A writers including METTL3, reversed by m^6A erasers (FTO and ALKBH5) and recognized by m^6A readers like YTHDF2. m^6A is involved in important neurological processes and appears dysregulated in many pathologies like Alzheimer's disease. However, its contribution during epileptogenesis and epilepsy is unknown. Using the intra-amygdala Kainic acid mouse model of epilepsy, we investigate if m^6A methylation processes are disturbed during epileptogenesis and in epilepsy. We assess variation in m^6A machinery protein and RNA levels in hippocampal extracts by western blot and qPCR and then, profile m^6A across the transcriptome using m^6A MeRIP-sequencing. Our data indicates an increase of METTL3 protein levels and m^6A methylation during epileptogenesis. MeRIP-seq revealed extensive differential methylation across the entire transcriptome and an increase of m^6A levels during epileptogenesis. Our results suggest that the m^6A methylation processes are perturbed during epileptogenesis and may contribute to the pathogenic gene dysregulation observed in epilepsy. Further investigation is needed to understand the influence of m^6A modification during epileptogenesis and to assess its inhibition as a new treatment strategy for epilepsy.

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BRAF HYPERACTIVATING MUTATION IN FOREBRAIN GABAERGIC INTERNEURONS INCREASES ANXIETY AND IMPAIRS WORKING MEMORY IN MICE

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Congenital changes leading to aberrant up-regulation of the Ras-mitogen activated protein kinase (Ras/MAPK) signaling cascade can cause a family of developmental disorders collectively called RASopathies. These conditions affect approximately 1 in 1000 individuals and share an overlapping pattern of physical abnormalities (e.g. craniofacial dysmorphology, cardiac malformations) and a variable degree of cognitive impairment. The identification of specific treatments for the RASopathy-associated neurocognitive deficits is still hampered by the lack of understanding of the underlying neuronal mechanisms. Aiming to increase our comprehension of the specific contributions of individual cell types to the neurocognitive alterations found in RASopathies, a conditional mutant mouse was generated with a selected knock-in hyperactivating mutation of the BRAF gene (a central component of the Ras/MAPK pathway) exclusively in forebrain GABAergic interneurons ($BRAF^{Q241R floxed/wt}$-$DLX5/6^{Cre/+}$or DBRAF mice) and its effects on cognitive and emotional behavior were characterized. We observed that DBRAF mice exhibit a strong anxiety phenotype in both the elevated plus maze and the open field tasks, as well as a pronounced deficit in working memory, indicated by reduced spontaneous alternation in the Y-maze task. Additionally, preliminary results suggest that DBRAF mice exhibit a hypoactivation of cells in the granular layer of the dentate gyrus in both dorsal and ventral hippocampus (measured through a cFos immunostaining) 1.5 hours after being tested in the elevated plus maze. These results reveal a potential role of the Ras/MAPK signaling in GABAergic interneurons in the emergence of anxiety and cognitive dysregulations and might contribute to the identification of new potential therapeutic intervention sites. Acknowledgment: German Network of RASopathy Research.

Pubmed:
27992989: Cerón J, Troncoso J
[Facial nerve injuries cause changes in central nervous system microglial cells].
Our research group has described both morphological and electrophysiological changes in motor cortex pyramidal neurons associated with contralateral facial nerve injury in rats. However, little is known about those neural changes, which occur together with changes in surrounding glial cells.
Biomedica, 2016; 36

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BEHAVIORAL AND MOLECULAR CHARACTERIZATION OF A NOVEL MOUSE MODEL OF HERV-W ENVELOPE PROTEIN EXPRESSION

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Human endogenous retroviruses (HERVs) are elements derived from exogenous retroviral infections throughout the evolution of the genome. Evidence implicates elevated expression of HERV type W envelope (ENV) in psychotic disorders, including schizophrenia and bipolar disorder. To gain insights, we generated and characterized a novel mouse model mimicking transgenic expression of this retroviral element in mice. We generated transgenic mice expressing HERV-W ENV under the CAG promoter. Adult transgenic (TG) mice and wild-type (WT) littermates were subjected to behavioral and cognitive tetsung to asses basal locomotor activity, innate anxiety-like behavior, social approach behavior and social recognition memory, sensorimotor gating, and novel object recognition. RNA sequencing of hippocampus was used to identify transcriptional changes in TG mice relative to WT. Through ingenuity pathway analysis (IPA) signaling pathways affected by HERV-W ENV was explored. TG mice displayed behavioral and cognitive anomalies compared to Wt mice; increased locomotor activity in novel environments, impairments in social recognition memory and novel object recognition, and deficits in prepulse inhibition of the acoustic startle reflex. We found 199 genes to be deregulated in the hippocampus of TG mice relative to WT; several genes previously identified as genetic risk variants for schizophrenia and other psychotic disorders. IPA demonstrated that those genes annotated with the functional nodes “neurodevelopmental disorders”, “schizophrenia”, “quantity of dendritic spines” and “synapse formation”. Our data provides causal evidence for HERV-W ENV expression in disrupting behavioral and cognitive functions implicated in schizophrenia. Moreover, we demonstrate that expression of HERV-W ENV has the capacity to change the brain transcriptome and deregulate genes associated with schizophrenia and other psychiatric disorders.

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CEREBELLAR GRADIENT OF VOLUME REDUCTION IN FETAL ALCOHOL SYNDROME: TOWARD A NEUROANATOMICAL MARKER?

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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In Fetal Alcohol Spectrum Disorders (FASD), patients with Fetal Alcohol Syndrome (FAS) or without specific diagnostic features (non-syndromic, NS-FASD) show global brain growth insufficiency. However, some regions were proposed to be more severely reduced, especially the cerebellum. Considering changes in proportions with size (allometric scaling), we sought to specify the cerebellar and intracerebellar damage in paediatric FASD. We applied an original pipeline of cerebellar segmentation (*CERES* combined with *SUIT* to add the vermis) on 3DT1 brain MRI of 52 FAS, 37 NS-FASD and 126 controls (6-20y/o), providing 8 sub-volumes. After covariate (age, sex, site) correction, the scaling relationship ($V_i=bV_t^a$) between these sub-volumes (V_i) and the total brain or cerebellum volume (V_t) enabled us to compared FAS to controls (α=0.05). The cerebellum volume presented negative allometric scaling (a=0.38<<1) in controls, but proportional one (a=0.91»1) in FAS. Five sub-volumes (anterior and posterior hemispheres, whole, anterior, and inferior vermis) showed different intra-cerebellum scaling in FAS than controls. The mean difference between observed sub-volumes in FAS and expected ones according to the scaling law fitted in controls, revealed an anterior-inferior-posterior gradient of severity of volume reduction for the hemispheres (positive-null-negative respectively) and for the vermis (decreasingly negative). For the same five sub-volumes, there were a significant excess of patients with too-small values (<2.5percentile of the scaling distribution of controls) in FAS, but also in NS-FASD. Our study described whole-cerebellum anterior-inferior-posterior gradients of volume reduction in FAS. Furthermore, the recurrent abnormalities identified in FAS were also found in NS-FASD, suggesting a FASD neuroanatomical signature.

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EFFECTS OF NEONATAL HYPOXIA ON THE DEVELOPMENT OF SEROTONERGIC INNERVATION AND COGNITIVE FUNCTIONS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Recent studies suggest that children who experienced mild perinatal hypoxia(MPH), prolonged oxygen deprivation at birth, show long lasting subtle cognitive and behavioral deficits, increasing the risk of learning disabilities, emotional problems or ASD, which becomes more apparent as the child develops. However, not only are the mechanisms underlying MPH not well understood, whether serotonin(5-HT) dysregulations contribute to MPH-induced cognitive problems is also unclear.
We have recently established a MPH mouse model, showing long-term deficits in cognitive flexibility, social interaction, and memory. To investigate whether MPH affects 5-HT system development, we characterized 5-HT expression levels and innervation in the auditory and prefrontal cortex of female and male MPH mice. Immunohistochemistry data suggest 5-HT expression is reduced in adult MPH mice especially in the prefrontal cortex. In addition, HPLC-based quantification revealed altered levels of HIAA or 5-HT in the hippocampus of MPH mice. Assessment of body temperature regulation following pharmacological 5-HT modulation by 5-HT1A receptor agonist also demonstrates deficits in 5-HT auto-receptor function on raphe neurons in MPH mice. Adult MPH mice of both sexes display recognition and spatial memory impairments, social deficits, and dysfunctional cognitive flexibility, but normal anxiety-related behaviour. Chronic treatment with SSRI or 5-HT1A receptor agonist rescues cognitive flexibility and memory impairments observed in MPH mice.
Identifying MPH effects on serotonergic system development will lead to a better understanding of how social, attention, cognitive flexibility and memory dysfunctions associated with MPH occur and may pave a path towards the development of pharmacological strategies for treating children exposed to MPH.

Pubmed:
29325855: Lee KKY, Soutar CN, Dringenberg HC
Gating of long-term potentiation (LTP) in the thalamocortical auditory system of rats by serotonergic (5-HT) receptors.
The neuromodulator serotonin (5-hydroxytryptamine, 5-HT) plays an important role in controlling the induction threshold and maintenance of long-term potentiation (LTP) in the visual cortex and hippocampus of rodents. Serotonergic fibers also innervate the rodent primary auditory cortex (A1), but the regulation of A1 plasticity by 5-HT receptors (5-HTRs) is largely uncharted. Thus, we examined the role of several, predominant 5-HT receptor classes (5-HTRs, 5-HTRs, and 5-HTRs) in gating in vivo LTP induction at A1 synapses of adult, urethane-anesthetized rats. Theta-burst stimulation (TBS) applied to the medial geniculate nucleus resulted in successful LTP induction of field postsynaptic potentials (fPSPs) generated by excitation of thalamocortical and intracortical A1 synapses. Local application (by reverse microdialysis in A1) of the broad-acting 5-HTR antagonist methiothepin suppressed LTP at both thalamocortical and intracortical synapses. In fact, rather than LTP, TBS elicited long-term depression during methiothepin application, an effect that was mimicked by the selective 5-HTR antagonist ketanserin, but not the 5-HTR blocker WAY 100635. Interestingly, antagonism of 5-HTRs by granisetron selectively blocked LTP at thalamocortical, but not intracortical A1 synapses. Further, in the absence of TBS, granisetron application resulted in a pronounced increase in fPSP amplitude, suggesting that 5-HTRs play an important role in regulating baseline (non-potentiated) transmission at A1 synapses. Together, these results indicate that activation of 5-HTRs and 5-HTRs, but not 5-HTRs, exerts a clear, facilitating effect on LTP induction at A1 synapses, allowing 5-HT to act as a powerful regulator of long-term plasticity induction in the fully matured A1 of mammalian species.
Brain Res, 2018; 1683

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UNRAVELLING THE ENDOPHENOTYPIC CHARACTERISTICS OF GENERATED GRIN-RELATED DISORDERS ZEBRAFISH MODELS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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NMDA-type ionotropic glutamate receptors play pivotal roles in synaptic development, plasticity, neural survival, and cognition. Recent advances on Next-Generation Sequencing revealed the association of *de novo* mutations affecting *GRIN* genes (encoding GluN subunits of the NMDAR) with neurodevelopmental disorders, so-called GRIN-related disorders (GRD). GRD is a rare condition with a clinical spectrum dictated by both the affected *GRIN* gene and the functional outcomes of the mutated residue/s, primarily affecting glutamatergic neurotransmission and causing synaptopathies. Accordingly, generation of an *in vivo* library is required to delineate the neurological alterations and ultimately to identify personalized therapeutic approaches for GRDs. In the context of GRD, zebrafish appear as an optimal animal model, since it provides several advantages from biomedical and industrial points of view.
To address this objective, CRISPR-Cas9-based genome editing technology has been applied for the obtention of knockout models of Zebrafish paralogous *GRIN1*, *GRIN2A* and *GRIN2B* genes. Single mutant larvae showed no effect on survival rate, and allowed to define the spatio-temporal expression pattern of *grin* genes in larval stages. Phenotypic assessments have been performed in pharmacological acute GRD models, revealing the presence of both behavioral and motor phenotypes, allowing the optimization of the behavioral paradigms of interest. Currently, the proposed procedure is being used to evaluate the generated GRD models. In the short term, the comprehensive phenotyping of Zebra-GRIN models will allow to define GRD-like alterations and, importantly, to evaluate the therapeutic efficacy of repurposed and EMA-approved putative NMDAR allosteric modulators, to ultimately allow personalized therapies for GRD patients.

Pubmed:
31213567: Soto D, Olivella M, Grau C, Armstrong J, Alcon C, Gasull X, Santos-Gómez A, Locubiche S, Gómez de Salazar M, García-Díaz R, Gratacòs-Batlle E, Ramos-Vicente D, Chu-Van E, Colsch B, Fernández-Dueñas V, Ciruela F, Bayés À, Sindreu C, López-Sala A, García-Cazorla À, Altafaj X
L-Serine dietary supplementation is associated with clinical improvement of loss-of-function -related pediatric encephalopathy.
Autosomal dominant mutations in are associated with severe encephalopathy, but little is known about the pathophysiological outcomes and any potential therapeutic interventions. Genetic studies have described the association between de novo mutations of genes encoding the subunits of the -methyl-d-aspartate receptor (NMDAR) and severe neurological conditions. Here, we evaluated a missense mutation in , causing a proline-to-threonine switch (P553T) in the GluN2B subunit of NMDAR, which was found in a 5-year-old patient with Rett-like syndrome with severe encephalopathy. Structural molecular modeling predicted a reduced pore size of the mutant GluN2B-containing NMDARs. Electrophysiological recordings in a HEK-293T cell line expressing the mutated subunit confirmed this prediction and showed an associated reduced glutamate affinity. Moreover, GluN2B(P553T)-expressing primary murine hippocampal neurons showed decreased spine density, concomitant with reduced NMDA-evoked currents and impaired NMDAR-dependent insertion of the AMPA receptor subunit GluA1 at stimulated synapses. Furthermore, the naturally occurring coagonist d-serine restored function to GluN2B(P553T)-containing NMDARs. l-Serine dietary supplementation of the patient was hence initiated, resulting in the increased abundance of d-serine in the plasma and brain. The patient has shown notable improvements in motor and cognitive performance and communication after 11 and 17 months of l-serine dietary supplementation. Our data suggest that l-serine supplementation might ameliorate -related severe encephalopathy and other neurological conditions caused by glutamatergic signaling deficiency.
Sci Signal, 2019; 12
33043365: Santos-Gómez A, Miguez-Cabello F, García-Recio A, Locubiche-Serra S, García-Díaz R, Soto-Insuga V,

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Guerrero-López R, Juliá-Palacios N, Ciruela F, García-Cazorla À, Soto D, Olivella M, Altafaj X

Disease-associated GRIN protein truncating variants trigger NMDA receptor loss-of-function.

De novo GRIN variants, encoding for the ionotropic glutamate NMDA receptor subunits, have been recently associated with GRIN-related disorders, a group of rare paediatric encephalopathies. Current investigational and clinical efforts are focused to functionally stratify GRIN variants, towards precision therapies of this primary disturbance of glutamatergic transmission that affects neuronal function and brain. In the present study, we aimed to comprehensively delineate the functional outcomes and clinical phenotypes of GRIN protein truncating variants (PTVs)-accounting for ~20% of disease-associated GRIN variants-hypothetically provoking NMDAR hypofunctionality. To tackle this question, we created a comprehensive GRIN PTVs variants database compiling a cohort of nine individuals harbouring GRIN PTVs, together with previously identified variants, to build-up an extensive GRIN PTVs repertoire composed of 293 unique variants. Genotype-phenotype correlation studies were conducted, followed by cell-based assays of selected paradigmatic GRIN PTVs and their functional annotation. Genetic and clinical phenotypes meta-analysis revealed that heterozygous GRIN1, GRIN2C, GRIN2D, GRIN3A and GRIN3B PTVs are non-pathogenic. In contrast, heterozygous GRIN2A and GRIN2B PTVs are associated with specific neurological clinical phenotypes in a subunit- and domain-dependent manner. Mechanistically, cell-based assays showed that paradigmatic pathogenic GRIN2A and GRIN2B PTVs result on a decrease of NMDAR surface expression and NMDAR-mediated currents, ultimately leading to NMDAR functional haploinsufficiency. Overall, these findings contribute to delineate GRIN PTVs genotype-phenotype association and GRIN variants stratification. Functional studies showed that GRIN2A and GRIN2B pathogenic PTVs trigger NMDAR hypofunctionality, and thus accelerate therapeutic decisions for this neurodevelopmental condition.

Hum Mol Genet, 2021; 29

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MEGALENCEPHALIC LEUKOENCEPHALOPATHY WITH SUBCORTICAL CYSTS IS A DEVELOPMENTAL DISORDER OF THE GLIOVASCULAR UNIT

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Absence of the astrocyte-specific membrane protein MLC1 is responsible for megalencephalic leukoencephalopathy with subcortical cysts (MLC), a rare and orphan type of leukodystrophy characterized by early-onset macrocephaly and progressive white matter vacuolation that lead to ataxia, spasticity, and cognitive decline. During postnatal development (from P5 to P15 in the mouse), MLC1 forms a membrane complex with GlialCAM (another astrocytic transmembrane protein) at the junctions between perivascular astrocytic processes. Perivascular astrocytic processes along with blood vessels form the gliovascular unit, where astrocytes participate to several brain vascular functions, including the integrity of the blood-brain barrier, the homeostasis between the brain and the immune system, the transfer of metabolites, and the regulation of cerebral blood flow. Those functions are relying on a specific molecular repertoire that is enriched in perivascular astrocytic processes. Despite MLC1 enrichment in this interface, its role in the physiology and development of the gliovascular unit was not previously known. Using the *Mlc1* knock-out mouse model of MLC, we demonstrated that MLC1 controls the postnatal development and organization of perivascular astrocytic processes, vascular smooth muscle cell contractility, neurovascular coupling, and intraparenchymal interstitial fluid clearance. Our data suggest that MLC is a developmental disorder of the gliovascular unit, and perivascular astrocytic processes and vascular smooth muscle cell maturation defects are primary events in the pathogenesis of MLC and therapeutic targets for this disease.

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UNUSUAL DOUBLE MUTATION IN MECP2 AND CDKL5 GENES IN RETT-LIKE SYNDROME : EFFECT ON GENES EXPRESSION AND GENOTYPE PHENOTYPE CORRELATION

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Rett syndrome (RTT) is a neuro-developmental disorder affecting almost exclusively females and itdivided into classical and atypical forms of the disease. RTT-like syndrome was also described and presents anoverlapping phenotype of RTT. RTT-like syndrome has been associated with several genes including MECP2 andCDKL5 having common biological pathways and regulatory interactions especially during neural maturation andsynaptogenesis. We report patient with Rett-like syndrome for whom clinical features and their progression guided toward the screening of two candidate genes MECP2 and CDKL5 by sequencing. Severity score was evaluated by“Rett Assessment Rating Scale” (R.A.R.S.). Predictions of pahogenicity and functional effects used several bioinformatic tools and qRT-PCR was conducted to evaluate gene expression. Mutational screening revealed two mutations c.1065 C > A (p.S355R) in MECP2 gene and c.616G > A (p.D206N) mutation in CDKL5 gene in the patient with a high R.A.R.S. Bioinformatic investigations predicted a moderate effect of p.S355R in MECP2 gene but a more pathogenic one of p.D206N mutation inCDKL5. Effect of c.616 G > A mutation on structure and stability of CDKL5 mRNA was confirmed by qRT-PCR. Additionally, analysis of gene expression revealed a drastic effect of CDKL5 mutant on its MeCP2 and Dnmt1 substrates and also on its MYCN regulator. In conclusion, the co-existence of the two mutations in CDKL5 and MECP2 genes could explain the severe phenotype in our patient with RTT-Like and is consistent with the data related to the interactions of CDKL5 with MeCP2 and Dnmt1 proteins.

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PHENOTYPE CHARACTERIZATION OF A ZEBRAFISH MODEL FOR THE STUDY OF KCNB1 IN DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHIES

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Developmental and epileptic encephalopathies (DEE) refer to a heterogeneous group of devastating neurodevelopmental and epileptic disorders diagnosed during early childhood. *De novo* variants in *KCNB1,* encoding a voltage-dependent potassium channel, have been reported in DEE patients by displaying loss of function properties. Therefore, we need to better comprehend pathophysiological cascades involving *KCNB1* in DEE pathogenesis. We functionally characterized a transgenic deletion mutant transgenic line targeting and inactivating the zebrafish orthologue of KCNB1 (*kcnb1* gene) to explore neuronal dysregulations in this model. Phenotypic, morphological analyses and electrophysiological recordings were performed during the early stages of development. In parallel, larvae were exposed to the proconvulsant pentylenetetrazol (PTZ) in order to evaluate the epileptogenic threshold of fish. The *kcnb1* knockout model does not reveal any morphological alterations in major organs compared to *kcnb1*+/- and wild-type larvae. However, uncontrolled swimming behavior was recorded at various stages of development (coiling, evoked and spontaneous swimming). PTZ treatment induced an increased and maintained swimming distance in both *kcnb1*+/- and *kcnb1*-/- larvae indicating a greater seizure-susceptibility. These data are in correlation with an increase of c-Fos positive neurons and by greater changes in electrophysiological recordings into the optic tectum of transgenic lines. The *kcnb1* knock-out zebrafish model presents a physiological and behavioral phenotype similar to previous genetic models of epilepsy. Therefore, this novel model will be a useful tool to decipher pathological mechanisms due to *kcnb1* mutations and can pave the way of new therapeutic development for this genetic cause of rare epilepsies and related DEE disorders.

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FUNCTIONAL CHARACTERIZATION OF MONOALLELIC DE NOVO GABAB RECEPTOR VARIANTS IDENTIFIED IN NEUROLOGICAL AND PSYCHIATRIC DISORDERS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Neurons and circuits maintain a constant excitation/inhibition (E/I) balance in response to fluctuations of input activity. $GABA_B$ receptors (GBRs) contribute to the control of the E/I balance by providing prolonged pre- and postsynaptic inhibition in response to ambient and synaptically released GABA. GBRs are obligatory heterodimers composed of GB1 and GB2 subunits. GB2 variants were identified in Rett syndrome ($GB2^{RTT}$/A567T) and epileptic encephalopathy ($GB2^{EE1}$/S695I, $GB2^{EE2}$/I705N) patients (Vuillaume et al., *Ann Neurol, 83*(2), 2018; Yoo et al., *Ann Neurol, 823*(3), 2017). Several GB1 variants (GB1/E368D, GB1/A397V, GB1/A535T and GB1/G673D) were recently identified in patients presenting mild to severe neurodevelopmental delay, epilepsy, intellectual disability, and psychiatric conditions (unpublished). We have functionally analyzed GB1 and GB2 variants in heterologous cells. Consistent with earlier data, we found that $GB2^{RTT}$/A567T and $GB2^{EE2}$/I705N reduce the efficacy of GABA, while $GB2^{EE1}$/S695I is unresponsive to GABA. All GB2 variants exhibit elevated constitutive activity and are partially blocked by the GBR inverse agonist CGP54626. The newly identified GB1/E368D reduces potency and efficacy of GABA, while GB1/E397V and GB1/A535T selectively reduce efficacy of GABA. GB1/G673D receptors are inactive, consistent with a lack of receptor expression at the cell surface. Epileptic seizures in several patients carrying GBR variants suggests that impaired receptor responses to GABA result in an E/I imbalance. Positive allosteric modulators (PAMs) of GBRs could potentially be used to increase potency and efficacy of GABA at the variant receptors. We therefore present a characterization of the activity of the PAM ADX71441 at receptor variants.

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POST-SYNAPTIC SCAFFOLD PROTEIN TANC2 IN PSYCHIATRIC AND SOMATIC DISEASE RISK

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Aims: Understanding the shared genetic aetiology of psychiatric and medical comorbidity in neurodevelopmental disorders (NDDs) could improve patient diagnosis, stratification and treatment options. Rare TANC2 (Tetratricopeptide Repeat, Ankyrin Repeat and Coiled-Coil Containing 2) disrupting variants were pathogenic in NDD patients. This post-synaptic scaffold protein, essential for dendrite formation in synaptic plasticity, plays an unclarified but critical role in development and multi-organ expression suggests pleiotropic functions outside the brain. With focus on *Tanc2*, we aimed to illustrate the potential utility of the International Mouse Phenotyping Consortium (IMPC) preclinical database to identify medical comorbidity risk in NDDs due to pleiotropic gene function. **Methods:** We performed a comprehensive multi-systemic phenotyping analysis of a *Tanc2*$^{-em1/CRISPR/Cas}$ homozygous null mutant mouse model generated for the IMPC using CRISPR/Cas mutagenesis. **Results:** Mutant mice were hyperactive and had impaired sensorimotor gating consistent with NDD patient psychiatric endophenotypes. Yet, a multi-systemic analysis revealed growth failure, hepatocellular damage and aberrant liver function including altered hepatocellular metabolism. Integrative analysis indicates that these disrupted *Tanc2* systemic effects relate to interaction with Hippo developmental signalling pathway proteins and will increase the risk for comorbid somatic disease. **Conclusion:** This highlights how NDD gene pleiotropy can augment medical comorbidity susceptibility underscoring the benefit of holistic NDD patient diagnosis and treatment for which large-scale preclinical functional genomics in the IMPC can provide complementary pleiotropic gene function information.

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NOVEL MPDZ/MUPP1 TRANSGENIC MODELS CONFIRM MPDZ'S ROLE IN SOCIAL-PSYCHOLOGICAL DISORDERS ASSOCIATED WITH HEARING AND VESTIBULAR DYSFUNCTION

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Congenital hydrocephalus is a common heritable anomaly. Multiple PDZ domains (*Mpdz/Mupp1*) gene in implicated in some severe forms of the congenital hydrocephalus in human, associated with a differential diagnosis of hearing loss, often resulting in severe psychiatric, neurological and mental complications. However, the precise role of MPDZ in social-psychological disorders are unknown. Here, we have created two mice line with an absence of the MPDZ/MUPP1 protein only in the forebrain and / or auditory system without hydrocephalus phenotype. We subjected our mice models to a series of behavioural tests in relation to neurodevelopmental disorders such as intellectual disability, autism spectrum disorder as well as in relation to emotional and disruptive Disorders. We also analysed them during development in tasks requiring motor coordination, balance and motor skills. Our data identified the exact nature of emotional, cognitive and social behavioural disorders associated with hearing and vestibular dysfunction specifically linked to the MPDZ gene.

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REVEALING HOW TRNA SPLICING DEFECTS CAUSE PONTOCEREBELLAR HYPOPLASIA USING BRAIN ORGANOIDS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Pontocerebellar hypoplasia type 2 A (PCH2A) is a rare, severe neurodevelopmental disorder that is usually diagnosed shortly after birth. Symptoms include developmental delay, seizures, and deficits in motor control. Anatomically, PCH2A has as hallmarks a cerebellum and pons of reduced size, as well as a progressive small size of the cerebrum. PCH2A is characterized by a single point mutation in TSEN54, which encodes a protein required for tRNA splicing. Despite its unique genetic cause and stereotypical clinical manifestation, the pathological mechanism at the cellular and molecular level is currently unknown and treatment options are limited and symptomatic. We aim to address this gap in knowledge by investigating PCH2A pathology using human brain organoids of patient-derived induced pluripotent stem cells. We characterize the cellular developmental trajectories in cerebellar organoids, since it is the main brain region where hypoplasia is observed. Subsequently, we aim to determine cell type-specific molecular changes in PCH2A through single-cell RNA-sequencing of organoids. We expect that our study will elucidate key disease mechanisms of PCH2A and have implications for other disorders induced by defects in tRNA splicing. Importantly, we will determine at which time point during cerebellar development differences between PCH2A and control organoids emerge. Hence, we will answer the question if PCH2A pathology emerges through the degeneration of existing structures, or whether the formation of the cerebellum is already altered. We will thus reveal a possible interplay between disrupted neurodevelopment and neurodegeneration. Our long-term goal is to define a therapeutic approach for PCH2A.

Pubmed:
34335182: Renner H, Becker KJ, Kagermeier TE, Grabos M, Eliat F, Günther P, Schöler HR, Bruder JM
Cell-Type-Specific High Throughput Toxicity Testing in Human Midbrain Organoids.
Toxicity testing is a crucial step in the development and approval of chemical compounds for human contact and consumption. However, existing model systems often fall short in their prediction of human toxicity because they may not sufficiently recapitulate human physiology. The complexity of three-dimensional (3D) human organ-like cell culture systems ("organoids") can generate potentially more relevant models of human physiology and disease, including toxicity predictions. However, so far, the inherent biological heterogeneity and cumbersome generation and analysis of organoids has rendered efficient, unbiased, high throughput evaluation of toxic effects in these systems challenging. Recent advances in both standardization and quantitative fluorescent imaging enabled us to dissect the toxicities of compound exposure to separate cellular subpopulations within human organoids at the single-cell level in a framework that is compatible with high throughput approaches. Screening a library of 84 compounds in standardized human automated midbrain organoids (AMOs) generated from two independent cell lines correctly recognized known nigrostriatal toxicants. This approach further identified the flame retardant 3,3',5,5'-tetrabromobisphenol A (TBBPA) as a selective toxicant for dopaminergic neurons in the context of human midbrain-like tissues for the first time. Results were verified with high reproducibility in more detailed dose-response experiments. Further, we demonstrate higher sensitivity in 3D AMOs than in 2D cultures to the known neurotoxic effects of the pesticide lindane. Overall, the automated nature of our workflow is freely scalable and demonstrates the feasibility of quantitatively assessing cell-type-specific toxicity in human organoids .
Front Mol Neurosci, 2021; 14
33138918: Renner H, Grabos M, Becker KJ, Kagermeier TE, Wu J, Otto M, Peischard S, Zeuschner D, TsyTsyura Y, Disse

P, Klingauf J, Leidel SA, Seebohm G, Schöler HR, Bruder JM
A fully automated high-throughput workflow for 3D-based chemical screening in human midbrain organoids.
Three-dimensional (3D) culture systems have fueled hopes to bring about the next generation of more physiologically relevant high-throughput screens (HTS). However, current protocols yield either complex but highly heterogeneous aggregates ('organoids') or 3D structures with less physiological relevance ('spheroids'). Here, we present a scalable, HTS-compatible workflow for the automated generation, maintenance, and optical analysis of human midbrain organoids in standard 96-well-plates. The resulting organoids possess a highly homogeneous morphology, size, global gene expression, cellular composition, and structure. They present significant features of the human midbrain and display spontaneous aggregate-wide synchronized neural activity. By automating the entire workflow from generation to analysis, we enhance the intra- and inter-batch reproducibility as demonstrated via RNA sequencing and quantitative whole mount high-content imaging. This allows assessing drug effects at the single-cell level within a complex 3D cell environment in a fully automated HTS workflow.
Elife, 2020; 9

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SIMULTANEOUS PRENATAL ALCOHOL AND CANNABINOID EXPOSURE DURING THE SECOND TRIMESTER AUGMENTS REDUCTIONS IN FETAL CEREBRAL BLOOD FLOW FROM ALCOHOL EXPOSURE ALONE.

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Aims: Investigations of simultaneous prenatal alcohol and cannabinoid (SAC) exposure are currently minimal, although prenatal exposure to either substance can lead to fetal growth deficits. During the second trimester, brain vasculature emerges to support fetal growth and neural development. We therefore investigated whether second-trimester prenatal drug exposure would alter fetal-directed blood flow. **Methods**: We performed high-resolution *in vivo* ultrasound imaging in C57Bl/6J pregnant mice assigned to one of four exposure groups: drug-free control, alcohol or cannabinoid only, or SAC. Drug exposure occurred daily between Gestational Days (G)12-15, via i.p injections of cannabinoid agonist CP-55940 (750µg/kg), while ethanol (95%) was inhaled within vapor chambers for 30min. All dams were imaged on three days of pregnancy: G11 (pre-exposure), G13.5 (peri-exposure) and G16 (post-exposure). **Results:** Compared to controls, both PAE and SAC dams experienced a dip in gestational weight gain while undergoing drug exposure. Furthermore, SAC dams demonstrated higher (+42mg/dL) blood ethanol concentrations than PAE dams. Preliminary ultrasound data suggest that both alcohol and cannabinoid exposures alone produce delayed reductions in fetal blood flow on G16 in the middle cerebral and internal carotid arteries. Notably, SAC fetuses exhibit an augmented reduction in blood supply through the middle cerebral artery (-62%) compared to PAE offspring (-40%). Interestingly, alcohol and cannabinoid exposures lead to acute reductions in umbilical blood flow that recover by G16. **Conclusions**: Our results indicate that prenatal drug exposure may lead to both acute and delayed reductions in fetal-directed blood flow, and SAC may augment deficits specifically in cerebral arterial blood flow.

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CEREBELLAR MALFORMATIONS IN MOUSE MODEL OF WILLIAMS-BEUREN SYNDROME

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder characterized by cardiovascular deficits, hypersociability, and cerebellar ataxia. In order to better understand the cerebellar involvement in causing the neurological symptoms of WBS, we generated a novel mouse model replicating the clinical features of WBS patients. Quantification of the lobulation of the cerebellum, demonstrated that WBS mice showed an enlargement of lobule IV/V in the cerebellar vermis, and by deformation at the onset of the copula pyramidis in the cerebellar hemispheres. In particular in lobule IV/V, we noted a significant decrease of linear density of Purkinje cells. These alterations were specific for the cerebellum, as none of the other relevant brain areas showed significant deformations in WBS mice.
Since the involvement of lobule IV/V in forelimbs movement and in affective states, and copula pyramidis in hindlimb movements, anatomical alterations of these structures in mice may explain social and motor dysregulations seen in humans, suggesting, ulteriorly, how the cerebellum is implicated in motor and non-motor functions and might be involved in WBS behavior.

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PREDICTION OF CHILDREN PSYCHOPHYSIOLOGICAL AGE USING PATTERNS OF AUDITORY EVENT-RELATED POTENTIALS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Although there are various psychological scales for determining whether a child's age corresponds to the population norm, patterns of brain bioelectrical activity could also reflect child development and serve as valuable markers of psychophysiological age. This study aimed to determine the psychophysiological age of children using components of auditory event-related potentials (ERPs). The novelty of the study approach is that age was predicted at an individual level by regression models of machine learning. ERPs were recorded in response to auditory tones presented with different loudness in three groups of children (N=7, age range 4.14-6.42; N=16, 7.08-11.98; N=9, 12.04-17.98). Averaged amplitudes and latencies of ERPs were compared by Kruskal-Wallis Test and served as classification features for Catboost machine learning algorithm. The most informative features are determined by the numbers of times the feature is used in a model. We observed significant group differences in amplitudes of P1, P2, N2 latencies of P1 and P2 components of ERPs ($p<.05$). The classification accuracy between the three age groups was .85, ROC/AUC - .88. The most informative features for correct age recognition were latency of P1 and amplitude of N2. The study findings confirm that auditory ERPs reflect brain developmental changes. The application of the machine learning approach for the classification of ERP data allowed us to determine the psychophysiological age range at the individual level.

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RESCUING COGNITIVE DEFICITS ASSOCIATED TO 22Q11 DELETION SYNDROME: THE IMPORTANCE OF A CORRECT POSTNATAL MITOCHONDRIAL BIOGENESIS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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22q11 deletion syndrome (DS) is a syndrome characterized by structural abnormalities in dendrites maturation, spines formation and by behavioral and cognitive deficits (Karayiorgou et al., 2010). Among the genes deleted in the 22q11, six encode for mitochondrial proteins. Here, we examined mitochondrial genes expression in neuronal and astrocyte cell cultures derived from a mouse model of 22q11DS (Meechan et al., 2009) and found that, among other mitochondrial-related genes, *Bcl-Xl*was specifically downregulated in astrocytes but not in neuronal cells. Similar analysis performed in brain tissues of LgDel mice confirmed that *Bcl-Xl* was significantly downregulated in the prefrontal cortex. We thus checked whether the decreased levels of *Bcl-Xl* could have a role in the neuroanatomical abnormalities associated to 22q11DS. At first, we analysed the mitochondrial distribution and the morphology of astrocytes and neurons *in vivo* by labelling single cells with a plasmid expressing a cytoplasmic fluorescent protein (mTAGBFP2) and a mitochondrial matrix-targeted fluorescent protein (mt-YFP) with IUE. Morphometric analysis of these parameters revealed remarkable differences between the LgDel and control mice. Then, we chronically treated LgDel mice with a biologically active peptide consisting of a domain of BCL-XL fused to the protein transduction domain of the HIV-TAT protein and found a significant rescue of mitochondrial phenotypes and a consistent improvement of astrocytes morphogenesis and of the dendritic and spine abnormalities. Finally, the beneficial effect of TAT peptide was confirmed on cognitive deficits of LgDel mice. *Support: SNFS NCCR "Synapsy" (51NF40-158776) and Swiss National Science Foundation SNSF (310030_185363) and Telethon Italy (GGP20037) (to P.B.)*

Pubmed:
33852851: Zehnder T, Petrelli F, Romanos J, De Oliveira Figueiredo EC, Lewis TL, Déglon N, Polleux F, Santello M, Bezzi P Mitochondrial biogenesis in developing astrocytes regulates astrocyte maturation and synapse formation.
The mechanisms controlling the post-natal maturation of astrocytes play a crucial role in ensuring correct synaptogenesis. We show that mitochondrial biogenesis in developing astrocytes is necessary for coordinating post-natal astrocyte maturation and synaptogenesis. The astrocytic mitochondrial biogenesis depends on the transient upregulation of metabolic regulator peroxisome proliferator-activated receptor gamma (PPARγ) co-activator 1α (PGC-1α), which is controlled by metabotropic glutamate receptor 5 (mGluR5). At tissue level, the loss or downregulation of astrocytic PGC-1α sustains astrocyte proliferation, dampens astrocyte morphogenesis, and impairs the formation and function of neighboring synapses, whereas its genetic re-expression is sufficient to restore the mitochondria compartment and correct astroglial and synaptic defects. Our findings show that the developmental enhancement of mitochondrial biogenesis in astrocytes is a critical mechanism controlling astrocyte maturation and supporting synaptogenesis, thus suggesting that astrocytic mitochondria may be a therapeutic target in the case of neurodevelopmental and psychiatric disorders characterized by impaired synaptogenesis. Cell Rep, 2021; 35

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BEHAVIORAL ANALYSES OF BRAIN-SPECIFIC SNORD116 AND SNORD115 GENES.

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Box C/D small nucleolar RNAs (SNORD) are part of ribonucleoprotein complexes which function mostly in guiding **sequence-specific 2'-O-ribose methylation** of ribosomal RNAs, spliceosomal U6 snRNAs or transfer RNAs. By fine-tuning pre-mRNA splicing and translation, they are now considered as key players in **gene expression regulation**. Intriguingly, some placental mammal-specific SNORD genes are embedded within large imprinted chromosomal domains and show predominant **neuronal expression**. Among these epigenetically-regulated SNORD genes, **SNORD116** and **SNORD115** genes at human Chr15.q11q13 (mouse chr7C) are of particular interest since recent clinical studies suggest that their loss-of-function may contribute to the pathophysiology of the **Prader-Willi syndrome (PWS)**. PWS is a rare human disorder associated with a complex set of metabolic, hormonal and behavioural abnormalities, notably a true obsession with food (hyperphagia) which, in the absence of strict dietary restrictions, leads to morbid obesity. Despite recent undeniable progress, the mode of action of SNORD116 and SNORD115 remain highly elusive. We therefore still need to clarify their biological relevance *in vivo*, not only to unveil novel, unsuspected SNORD-mediated neuronal functions but also to improve our knowledge on the aetiology of PWS. Through the use of novel SNORD-deficient mouse models, we are now probing the involvement of SNORD116 and SNORD115 genes in higher brain functions, with particular attention to **hippocampal and non-hippocampal memories, cognitive flexibility** and **sleep architecture**.

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NEUROINFLAMMATORY MECHANISMS OF PAIN HYPERSENSITIZATION IN A MOUSE MODEL OF ATTENTION DEFICIT/HYPERACTIVITY DISORDER (ADHD).

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Clinical evidence suggest that pain hypersensitivity develops in subjects with attention deficit hyperactivity disorder (ADHD). However, the mechanisms and neural circuits involved in these interactions remain unknown because of the paucity of studies in animal models. We previously validated a mouse model of ADHD obtained by neonatal 6-Hydroxydopamine (6-OHDA) injection. Here, we demonstrated that 6-OHDA mice exhibited a marked sensitization to thermal and mechanical stimuli, suggesting that ADHD conditions increase nociception. In addition, sensitization to pathological inflammatory pain is amplified in 6-OHDA mice as compared to shams. ADHD-related hyperactivity and anxiety, but not inattention and impulsivity, are worsened in persistent inflammatory pain. These results indicate a potential implication of neuroinflammation in the etiology of ADHD. By combining in vivo electrophysiology, optogenetics and behavioral analyses, we demonstrated that the anterior cingulate cortex (ACC) hyperactivity alters the 'ACC – posterior insula' circuit, and triggers changes in spinal networks that underlie pain sensitization. Altogether, our results point to unprecedented mechanisms underlying the comorbidity between ADHD and pain sensitization. They indicate that ADHD and pain sensitization are mutually worsening comorbid disorders. We make the hypothesis that neuroinflammation is a major factor triggering ACC hyperactivity and the associated pain. To test this hypothesis, we aim at identifying potential markers of inflammation and oxidative stress in the ACC, and to study astrocytes and microglia activation. The identification of shared mechanisms, engaging overlapping neuronal circuits and inflammation, and underlying both disorders, is key to better treatments.

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INVESTIGATION OF KCNQ1 FUNCTION IN HUMAN NEURONS WITH A SPECIFIC FOCUS ON INSULIN SIGNALLING

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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KCNQ1 is the pore-forming alpha subunit of a voltage-dependent potassium channel complex. Four KCNQ1 monomers, together with a member of the KCNE family, form the functional potassium channel. The *KCNQ1* gene has been linked to disorders with impaired insulin signalling (so-called insulinopathies) including type 2 diabetes, obsessive compulsive disorder, and Alzheimer's disease. Interestingly, KCNQ1 is known to inhibit insulin secretion in the pancreas, however, its function in the human brain is unknown.
In this project we aim to elucidate the function of KCNQ1 in human neurons with a specific focus on insulin signalling. As a model we use human induced pluripotent stem cells (hiPSCs) which will be differentiated into neuronal stem cells (NSCs) and cortical neurons.
First, we established a neuronal differentiation protocol and analyzed KCNQ1 expression in hiPSCs, NSCs and neurons. All analyzed cell types show KCNQ1 expression. To elucidate KCNQ1 function, we generated KCNQ1 knockout hiPSC lines using CRISPR/Cas9 genome editing. In the next step these hiPSCs will be differentiated into NSCs and further into cortical neurons. The KCNQ1 knockout cells will be compared to isogenic control cells by analyzing different cell properties (e.g. neurite outgrowth, cell type composition, number of synapses), the transcriptome, protein expression and phosphorylation of members of the insulin signalling pathway, and the neuronal function.

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NOVEL MISSENSE MUTATIONS ALTER RELN FUNCTION CAUSING RECESSIVE AND DOMINANT NEURONAL MIGRATION DISORDERS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Reelin (RELN) is a secreted glycoprotein critical for multiple steps of cerebral cortex development in mice. In human *RELN* recessive mutations leading to null alleles were associated with cortical malformations, but were not functionally characterized. Here we identified novel missense *RELN* mutations in both compound and *de novo* heterozygous patients exhibiting an array of neuronal migration disorders (NMDs) as diverse as pachygyria, polymicrogyria and heterotopia. We assessed how different mutations affected RELN function using a set of assays in culture and in the developing cerebral cortex, with the latter being based on the capacity of ectopic expression of RELN *in utero* to drive the formation of caudally positioned neuronal aggregates. Mutations associated with polymicrogyria behave as gain-of-function by forming neuronal aggregates in extracaudal positions, while those with pachygyria behave as loss-of-function by causing defective RELN secretion, neuronal aggregation and migration *in vitro* and *in vivo*. In particular, *de novo* heterozygous RELN mutations act as dominant negative preventing WT RELN secretion, thereby causing dominant NMDs. This work shows that the behavior of the mutant proteins *in vitro* and *in vivo* predicts the severity of cortical malformations, providing valuable insight into the pathogenesis of the associated disorders.

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BOARD NUMBER: S04-703

EARLY BLOCKADE OF SEROTONIN 5-HT6 RECEPTOR-DEPENDENT MTOR ACTIVATION PREVENTS ONSET OF COGNITIVE DEFICITS IN A GENETIC MODEL OF SCHIZOPHRENIA

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Schizophrenia is a devastating mental disorder of neurodevelopmental origin which affects ~1% of the population worldwide. It is characterized by a broad pattern of cognitive symptoms, including for example decreased attention, impaired memory and alterations in social cognition. These symptoms are the earlier observed and are predictive of the risk of transition to schizophrenia. They are poorly controlled by currently available antipsychotics which mainly reduce positive symptoms and, to a lesser extent, negative symptoms. Although improving symptomatic treatments remains an important goal, progress in disease management will likely require shift to novel modes of intervention initiated at an early stage of the disease in high-risk patients. The serotonin 5-HT6 receptor holds special promise, highest receptor densities are found in brain regions involved in mnemonic functions and we previously demonstrated that a sustained non-physiological activation of mechanistic Target Of Rapamycin (mTOR) elicited by 5-HT6 receptors specifically in the prefrontal cortex mediates deficits in social cognition and episodic memory in two developmental models of schizophrenia. We propose a novel disease modifier strategy to prevent emergence of cognitive deficits at the adult stage, in the Disc1-L100P genetic model of schizophrenia. This strategy is based on early blockade of 5-HT6 receptor-operated mTOR signaling during adolescence, a critical phase of prefrontal cortex maturation. Here, we show that early administration of 5-HT6 receptor antagonist or rapamycin prevents the behavioral deficits observed at the adult stage in this model.

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BOARD NUMBER: S04-704

FLNA REGULATES CELL-AUTONOMOUS NEURONAL MATURATION

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Aims: Periventricular Nodular Heterotopia (PNH), the most common type of malformations of cortical development, is characterized by the presence of ectopic neuronal nodules lining the lateral ventricles. Mutations in *Filamin A* (*FLNA*) are the main cause of PNH, which combines epilepsy, intellectual disability, and autistic features. Underlying molecular mechanisms responsible for epileptogenesis remain largely obscure. It has been hypothesized that dysfunctional cortical circuitry, rather than ectopic neurons, may explain the occurrence of seizures. However, the intrinsic role of FLNA in neural circuit formation and function remains poorly understood. Methods: To address this issue, we depleted FLNA from layer II/III pyramidal neurons of mice harboring a Flna conditional allele (*Flna*$^{fl/fl}$) by timed *in utero* electroporation of Cre-recombinase. Results: We found that neuronal-specific loss of *Flna* markedly increase dendritic arborisation by preventing Rac1 activation through its interaction with the Rho-GTPase Activating Protein 24 (Arhgap24). Indeed, we show that *in vivo*, overexpression of Arhgap24 or the dominant-negative form of Rac1 rescue the dendritic overgrowth induced by depletion of Flna. These defects are associated with changes in spine morphology and distribution. Conclusions: These findings suggest that FLNA is crucial for the morpho-functional maturation of L2/3 pyramidal neurons in a cell-autonomous manner and provide strong support for neural circuit dysfunction being a pathological consequence of FLNA mutations.

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BOARD NUMBER: S04-705

TARGETING NEUROINFLAMMATION AS A POTENTIAL THERAPEUTIC INTERVENTION FOR ALTERED PAIN SENSITIVITY IN ATTENTION-DEFICIT HYPERACTIVITY DISORDER MOUSE MODEL

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Attention deficit hyperactivity disorder (ADHD) is a common neurodevelopmental disorder in children and adolescents worldwide. It often coexists with anxiety and aspects of the autism spectrum disorder, among others. Additionally, the prevalence of ADHD symptoms increases dramatically in children with chronic pain. Recent research suggests that increased inflammation during neurodevelopment can trigger ADHD symptoms and disturbances in pain transmission. Our hypothesis is that there are neuroinflammation in specific brain areas could underlie both processes and that targeting inflammation will reduce symptoms and comorbid pain sensitivity. To test the hypothesis, we aim to establish a mice model of ADHD through neonatal lesion with 6-OHDA, which is neurotoxic to dopaminergic neurons. The mice will be treated with Abscisic Acid (ABA) a phytohormone with anti-inflammatory properties as a therapeutic intervention, for a month. The dopamine injury model and ABA effect will be evaluated in various behavioural paradigms (memory, sociability, anxiety) as well as pain (thermal and mechanical sensitivity). In the post-mortem analysis we will analyse the morphology of reactive microglia as an inflammatory biomarker in specific areas involved in the DA projections from the ventral tegmental area and the substantia nigra. Preliminary results indicate that the lesion increases sensitivity thermal stimulation in females and that treatment with ABA counteracts this effect. In addition, there are sex-dependent differences in response to lesion and ABA treatment, which opens interesting perspectives regarding the implication of estrogens in this neurodevelopmental syndrome.

Pubmed:
34942345: Sánchez-Sarasúa S, Meseguer-Beltrán M, García-Díaz C, Beltrán-Bretones MT, ElMlili N, Sánchez-Pérez AM
IRS1 expression in hippocampus is age-dependent and is required for mature spine maintenance and neuritogenesis.
Insulin and insulin-like growth factor type I (IGF-1) play prominent roles in brain activity throughout the lifespan. Insulin/IGF1 signaling starts with the activation of the intracellular insulin receptor substrates (IRS). In this work, we performed a comparative study of IRS1 and IRS2, together with the IGF1 (IGF1R) and insulin (IR) receptor expression in the hippocampus and prefrontal cortex during development. We found that IRS1 and IRS2 expression is prominent during development and declines in the aged hippocampus, contrary to IR, which increases in adulthood and aging. In contrast, IGF1R expression is unaffected by age. Expression patterns are similar in the prefrontal cortex. Neurite development occurs postnatally in the rodent hippocampus and cortex, and it declines in the mature and aged brain and is influenced by trophic factors. In our previous work, we demonstrated that knockdown of IRS1 by shRNA impairs learning and reduces synaptic plasticity in a rat model, as measured by synaptophysin puncta in axons. In this study, we report that shIRS1 alters spine maturation in adult hilar hippocampal neurons. Lastly, to understand the role of IRS1 in neuronal neurite tree, we transfect shIRS1 into primary neuronal cultures and observed that shIRS1 reduced neurite branching and neurite length. Our results demonstrate that IRS1/2 and insulin/IGF1 receptors display different age-dependent expression profiles and that IRS1 is required for spine maturation, demonstrating a novel role for IRS1 in synaptic plasticity.
Mol Cell Neurosci, 2022; 118

FENS Forum 2022
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BOARD NUMBER: S04-706

DIFFERENTIAL PROGENITOR RESPONSES TO MATERNAL INFLAMMATION IN THE DEVELOPING NEOCORTEX

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Infections during pregnancy are associated with an increased risk for disorders such as autism and schizophrenia. Virus infections induce maternal immune system activation (MIA) and the release of several cytokines that cross the placental barrier and severely impact the development of the cerebral cortex. We have previously shown that MIA has a non-uniform impact on cell types in the developing mouse brain. Specifically, MIA displays a developmental time and germinal niche dependent impairment on progenitors that give rise to inhibitory neurons. Progenitor proliferation in the medial (MGE) and caudal ganglionic eminence (CGE) is affected during early- and mid-neurogenesis stages respectively. Furthermore, despite the acute nature of MIA, the impact on neuronal lineages is enduring and continues even after birth leading to physiological dysfunction. Finally, using high-throughput single-nuclei RNA sequencing technology on embryonic tissue, we observed a differential impact on the transcriptome of neuronal cells and their progenitors. We could trace some of the effects of MIA to the expression of cytokine receptors as well as important developmental signaling pathways. Our study hence emphasizes the fundamental differences between neural progenitor cells and how this shapes the development of the neocortex. Understanding the mechanistic basis behind such differential vulnerability is also crucial to resolve the pathogenesis of neurodevelopmental disorders.

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BOARD NUMBER: S04-707

MOSAIC MTORC1 HYPERACTIVITY IN HUMAN CORTICAL SPHEROIDS: A MODEL OF EPILEPSY-RELATED FOCAL CORTICAL DYSPLASIA

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Mutations of the mTOR pathway genes leading to mTORC1 hyperactivity are one of the major causes of genetic focal epilepsies with or without focal cortical dysplasia type II (FCD), a malformation of cortical development. The occurrence of brain-specific somatic mutations leading to mosaic mTORC1 hyperactivity has been reported in a number of FCD II cases. Here we ask whether variable proportions of mutated cells reflect the phenotypic spectrum between patients.
To assess the effect of mTORC1 hyperactivity on human neurodevelopment, we generated mosaic cortical spheroids (CSs) from patient-derived hiPSCs in different proportions to model small FCD or larger FCD such as hemimegalencephaly. First, we demonstrated mTORC1 hyperactivity by assessing the levels of phosphorylated ribosomal protein S6, one of the main downstream mTORC1 targets. Moreover, we found a defect in the formation of neuroectodermal rosettes in high mosaic rate CSs. Strikingly, single-cell transcriptomic profiling and immunohistological assays of these CSs showed a cell fate shift. Lastly, we observed increased neuronal activity in mutated organoids compared to controls using MEA recordings. These findings validate our model as an FCD and an epilepsy model.

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BOARD NUMBER: S04-708

TABLET-BASED MANUAL DEXTERITY ASSESSMENT OF NEUROLOGICAL SOFT SIGNS IN FIRST EPISODE PSYCHOSIS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Neurological symptoms can inform on degree of neurodevelopmental load and may be useful for early detection of psychosis. Aim: to develop tablet-based tasks to provide behavioral markers for detection of first-episode psychosis (FEP). Marker specificity was assessed against groups of: healthy controls, persons with autism spectrum disorder (ASD), and stabilized patients with schizophrenia (SCZ). Tablet markers were compared to scores of conventional neurological soft signs (NSS) and to neurophysiological measures.
Method : 20 persons with FEP, 20 with SCZ, 20 with ASD, and 20 healthy controls were included. Five tablet-tasks assessed different motor and cognitive functions: Finger Recognition (FR) for mental rotation, Rhythm Tapping (RhT) for temporal judgement, Sequence Tapping (ST) for procedural memory, Multi Finger Tapping (MFT) for motor inhibition, and Line Tracking (LT) for visuomotor precision and attentional modulation. NSS and medication data were also collected. Transcranial magnetic stimulation was used to measure resting motor thresholds and short latency intracortical inhibition (SICI).
Results : Compared to controls, FEP patients showed slower reaction times and higher errors in FR, and more variability in rhythmic finger tapping (RhT). ROC curves showed 90% sensitivity and 75% specificity for detection of FEP vs. controls. RhT performance allowed for discrimination between FEP and ASD/SCZ patients. FEP had similar motor threshold but reduced SICI compared to controls and other groups. Some tablet-based measures correlated with NSS in each patient group. In FEP, total NSS correlated with LT duration.
Conclusion : Manual task performance on easy-to-use tablet applications provides promising markers for detection of FEP.

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BOARD NUMBER: S04-709

COGNITIVE IMPAIRMENT IN MICE WITH A GAIN-OF-FUNCTION MUTATION IN RETINOIC ACID RECEPTOR BETA (RARB)

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Retinoic acid (RA) signaling regulates brain development and function. In target cells, RA transduces its signal by binding to heterodimers formed by retinoic acid receptor (RAR) and retinoic X receptor (RXR), which function as transcription factors by binding to RA response elements. Our group has shown that dominant variants in *RARB* cause a disorder characterized by motor and cognitive impairment. We found that these mutations increase the RA-induced transcriptional activity of RARB *in vitro*, suggesting a gain-of-function (GOF) mechanism. We hypothesized that these GOF mutations impair cognition by affecting the development and/or function of the striatum, where RARB is primarily expressed. To investigate this hypothesis, mice carrying the variant p.Arg394Cys homologous to the variant p.Arg387Cys found in 40% of affected individuals were generated. *Rarb*$^{R394C/R394C}$ mice die perinatally whereas *Rarb*$^{R394C/+}$ mice survive and show a motor behavior that is reminiscent of that of the patients. Behavioral assessment of *Rarb*$^{R394C/+}$ mice at P60-65 showed impaired behavior in the contextual fear conditioning paradigm as well as deficits in exploration time and discrimination index between the novel and familiar object in the novel object recognition paradigm. Interestingly, transcriptomic studies of the neonatal and adult Rarb$^{R394C/+}$ striatum showed that downregulated, but not upregulated genes, are significantly enriched for direct targets of RARB, including components of the dopamine signaling pathway. Altogether, these results suggest that p.R394C affects cognition not by acting as a GOF but rather as a Loss-of-function (LOF) allele *in vivo.*

Pubmed:

33420088: Kumar MJV, Shah D, Giridharan M, Yadav N, Manjithaya R, Clement JP
Spatiotemporal analysis of soluble aggregates and autophagy markers in the R6/2 mouse model.
Maintenance of cellular proteostasis is vital for post-mitotic cells like neurons to sustain normal physiological function and homeostasis, defects in which are established hallmarks of several age-related conditions like AD, PD, HD, and ALS. The Spatio-temporal accumulation of aggregated proteins in the form of inclusion bodies/plaques is one of the major characteristics of many neurodegenerative diseases, including Huntington's disease (HD). Toxic accumulation of HUNTINGTIN (HTT) aggregates in neurons bring about the aberrant phenotypes of HD, including severe motor dysfunction, dementia, and cognitive impairment at the organismal level, in an age-dependent manner. In several cellular and animal models, aggrephagy induction has been shown to clear aggregate-prone proteins like HTT and ameliorate disease pathology by conferring neuroprotection. In this study, we used the mouse model of HD, R6/2, to understand the pathogenicity of mHTT aggregates, primarily focusing on autophagy dysfunction. We report that basal autophagy is not altered in R6/2 mice, whilst being functional at a steady-state level in neurons. Moreover, we tested the efficacy of a known autophagy modulator, Nilotinib (Tasigna™), presently in clinical trials for PD, and HD, in curbing mHTT aggregate growth and their potential clearance, which was ineffective in both inducing autophagy and rescuing the pathological phenotypes in R6/2 mice.
Sci Rep, 2021; 11
31143100: Paul A, Nawalpuri B, Shah D, Sateesh S, Muddashetty RS, Clement JP
Differential Regulation of Translation by FMRP Modulates eEF2 Mediated Response on NMDAR Activity.
SYNGAP1, a Synaptic Ras-GTPase activating protein, regulates synapse maturation during a critical developmental window. Heterozygous mutation in ( ) has been shown to cause Intellectual Disability (ID) in children. Recent studies have provided evidence for altered neuronal protein synthesis in a mouse model of . However, the molecular mechanism behind the same is unclear. Here, we report the reduced expression of a known translation regulator, FMRP, during a specific developmental period in mice. Our results demonstrate that FMRP interacts with and regulates the translation of mRNA. We further show reduced translation leads to decreased FMRP level during development in which results in an increase in translation. These developmental changes are reflected in the altered response of eEF2 phosphorylation downstream of NMDA Receptor (NMDAR)-mediated signaling. In this study, we propose a cross-talk between FMRP and SYNGAP1 mediated signaling

which can also explain the compensatory effect of impaired signaling observed in mice.
Front Mol Neurosci, 2019; 12

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-710

PROBIOTIC SUPPLEMENTATION AS A POTENTIAL STRATEGY OF INTERVENTION TO AMELIORATE CLINICAL SYMPTOMS OF THE CDKL5 DEFICIENCY DISORDER

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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In the past few decades, the increasing interest of the scientific community in understanding the interaction between gut microbiota and the brain has brought to novel and important insights into the role of intestinal bacteria in modulating host neural function and development. The involvement of the gut microbiota has been also recently demonstrated for a variety of neuropsychiatric and neurodevelopmental disorders, whose aetiology remains mostly unknown. Cyclin-Dependent Kinase-Like 5 (CDKL5) deficiency disorder (CDD) is a rare X-linked developmental encephalopathy caused by pathogenic variants of the CDKL5 gene. It can manifest in a broad range of clinical symptoms including gastrointestinal dysfunction, a condition that may be related to alterations in the intestinal microbiota composition. Here, we explored whether probiotic supplementation (via Vivomixx®) is able to improve behavioral and neuronal deficits in a CDD murine model. Our results demonstrate a significant amelioration in the nesting performance of Vivomixx-treated knock-out (KO) mice compared to the respective untreated KO, and the same trend was also observed in the Y-maze task. Likewise, the intrinsic optical imaging analysis revealed an amelioration in the visual cortical response of the treated KO mice. The overall picture emerging from this preliminary study indicates an improvement of behavioral and visual deficits in probiotic-treated mice, supporting a potential clinical role for probiotic intervention as a non-invasive way to ameliorate symptoms in CDD patients.

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BOARD NUMBER: S04-711

AUDIOGENIC SEIZURES IN MICE: AN ENTRY POINT TO CENTRAL NERVOUS SYSTEM DISORDERS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Audiogenic seizures, reflex seizures induced by loud sounds, are a common feature of many mouse models for central nervous system disorders such as autism and epilepsy. However, the mechanisms underlying these seizures are still poorly understood. They are thought to reflect an imbalance between neuronal excitation and inhibition in the central auditory pathways. Interestingly both peripheral and central hearing impairments, either acquired or congenital, increase the susceptibility to these seizures. In addition, several areas of the auditory pathway (cochlea, brainstem, inferior colliculus, auditory cortex) are involved in the initiation and propagation of these seizures, but the precise role of each of them remains unclear. Here, we have used a genetic mouse model of deafness highly susceptible to these audiogenic seizures, in order to decipher the mechanisms underlying the generation and propagation of the neuronal hyperactivity characterizing the audiogenic seizures. To do so, we developed a method to record surface activity in several regions simultaneously in awake head-fixed mice before, during and after the seizures. This methodology provides an opportunity to identify the features and origin of audiogenic seizures in several mouse models in order to ascertain and better characterise the neuronal deficits in mouse models of central nervous system disorders.

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BOARD NUMBER: S04-712

DUAL ROLE OF P2X7 RECEPTOR DURING PHYSIOLOGICAL AND PATHOLOGICAL BRAIN DEVELOPMENT AND DENDRITOGENESIS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Aim. Increased concentration of extracellular ATP under pathological conditions activates P2X7 receptor (P2X7R) while its role during early embryonic stages remains unclear, where ATP influences cellular differentiation, proliferation and apoptosis. Our objective is to determine the role of the receptor on the regulation of neuronal outgrowth from primary murine hippocampal neurons. Dendritic branching is studied as a marker for correct brain development and the correlation of abnormal dendritogenesis and human pathologies may offer some understanding of the cognitive deficits related to neurodevelopmental diseases. Methods. Morphological analyses were performed with Sholl analysis from primary hippocampal neurons from P2X7R wild-type (WT) and knockdown mice (KO) obtained from E17.5–E18.5 embryos. To understand the role of P2X7R also in pathogenic conditions, we extended our investigations to primary hippocampal neurons from a maternal immune activation (MIA) model for schizophrenia. A series of tests were performed to study schizophrenia-like behaviours in the young adult offspring. Results. Deficits in dendritic outgrowth were detected in both P2X7R-deficient primary neurons and derived from control embryos exposed to MIA but not KO exposed to MIA. Schizophrenia-like behaviours (deficits in spontaneous alternation in T maze and novel object recognition, PPI) correlated with these changes. Conclusion. P2X7R seems to have different functions regarding the time point in the individual (developmental stages or young adulthood) and being a key element during a pathological inflammatory event (e.g., when it is activated in an immune activation model like MIA during pregnancy, driving schizophrenia-like behaviours)

Pubmed:

30582977: Calovi S, Mut-Arbona P, Sperlágh B
Microglia and the Purinergic Signaling System.
Microglia are the main resident immune-competent cell type of the central nervous system (CNS); these cells are highly sensitive to subtle changes in the chemical environment of the brain. Microglia are activated during diverse conditions, such as apoptosis, trauma, inflammation, and infection. The specific activities of microglia result from the confluence of environmental stimuli and the cellular state. It is likely that several signaling systems with different biological functions operate in competition and/or synergy, thus regulating similar microglial behaviors. The purinergic system is one of the fundamental signaling systems that establish microglial behavior in a wide spectrum of conditions. Adenosine tri-phosphate (ATP) belongs to the purinergic signaling system, which includes P2X, P2Y, and P1 receptors, as well as other proteins participating in ATP secretion and extracellular ATP degradation, and molecules that recognize purines as a ligand. In this review, we focus on the latest pre-clinical and basic purinergic system and microglial research, with particular attention to data collected in vivo and ex vivo. This chapter is divided into sections related to microglial ATP release, ATP degradation, and ATP-related actions mediated by P2X and P2Y receptor activation.
Neuroscience, 2019; 405
7686553: Huang MM, Lipfert L, Cunningham M, Brugge JS, Ginsberg MH, Shattil SJ
Adhesive ligand binding to integrin alpha IIb beta 3 stimulates tyrosine phosphorylation of novel protein substrates before phosphorylation of pp125FAK.
Tyrosine phosphorylation of multiple platelet proteins is stimulated by thrombin and other agonists that cause platelet aggregation and secretion. The phosphorylation of a subset of these proteins, including a protein tyrosine kinase, pp125FAK, is dependent on the platelet aggregation that follows fibrinogen binding to integrin alpha IIb beta 3. In this report, we examined whether fibrinogen binding, per se, triggers a process of tyrosine phosphorylation in the absence of exogenous agonists. Binding of soluble fibrinogen was induced with Fab fragments of an anti-beta 3 antibody (anti-LIBS6) that directly exposes the fibrinogen binding site in alpha IIb beta3. Proteins of 50-68 KD and 140 kD became phosphorylated on tyrosine residues in a fibrinogen-dependent manner. This response did not require prostaglandin synthesis, an increase in cytosolic free calcium, platelet aggregation or granule secretion, nor was it associated with tyrosine phosphorylation of pp125FAK.

Tyrosine phosphorylation of the 50-68-kD and 140-kD proteins was also observed when (a) fibrinogen binding was stimulated by agonists such as epinephrine, ADP, or thrombin instead of by anti-LIBS6; (b) fragment X, a dimeric plasmin-derived fragment of fibrinogen was used instead of fibrinogen; or (c) alpha IIb beta 3 complexes were cross-linked by antibodies, even in the absence of fibrinogen. In contrast, no tyrosine phosphorylation was observed when the ligand consisted of monomeric cell recognition peptides derived from fibrinogen (RGDS or gamma 400-411). Fibrinogen-dependent tyrosine phosphorylation was inhibited by cytochalasin D. These studies demonstrate that fibrinogen binding to alpha IIb beta 3 initiates a process of tyrosine phosphorylation that precedes platelet aggregation and the phosphorylation of pp125FAK. This reaction may depend on the oligomerization of integrin receptors and on the state of actin polymerization, organizational processes that may juxtapose tyrosine kinases with their substrates.
J Cell Biol, 1993; 122

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-713

UNCOVERING NEUROBIOLOGICAL PATHWAYS INVOLVED IN SETBP1 HAPLOINSUFFICIENCY DISORDER DURING EARLY DEVELOPMENT USING HUMAN BRAIN ORGANOIDS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Aims: *SETBP1* haploinsufficiency causes a heterogeneous neurodevelopmental syndrome with a broad range of clinical features including varying intellectual functioning and speech/language impairments. The precise functions of *SETBP1*, encoding the SET-binding protein 1, are yet to be discovered. Therefore, the molecular mechanisms or neuronal pathways by which reduced SETBP1 dosage affects neurodevelopment remain largely unknown. In this study, we aim to dissect these by employing induced pluripotent stem cell (iPSC)-derived brain organoids and transcriptomic approaches. **Methods:** We generated iPSC lines carrying *SETBP1* mutations using CRISPR/Cas9 gene-editing, as well as from *SETBP1* haploinsufficiency patients and their sex-matched parents. We differentiated heterozygous and homozygous knockout iPSCs into brain organoids, analysing their morphology and expression of key marker genes and proteins. Transcriptomic profiles are being investigated via RNA-sequencing at both whole organoid and single-cell levels to identify differences in cell type and lineage. **Results:** *SETBP1* homozygous knockout organoids showed disorganised morphology, with fewer and more elongated ventricles. We detected reduced expression of progenitor markers (*SOX2, PAX6* and *NESTIN),* neuronal marker *TBR1,* and forebrain marker *FOXG1*. Moreover, these *SETBP1*-/- organoids contained significantly fewer neural progenitors and neurons as shown by immunocytochemistry. **Conclusions:** Abnormally low SETBP1 dosage during early organoid development results in fewer neural progenitors and neurons. In the long-term, cell-culture models may provide a platform for testing drug panels with the potential to rescue the aberrant molecular/cellular phenotypes that we observe. Together, this work promises to offer insights into fundamental neuronal mechanisms that go awry in *SETBP1* haploinsufficiency disorder.

Pubmed:
33867525: Jansen NA, Braden RO, Srivastava S, Otness EF, Lesca G, Rossi M, Nizon M, Bernier RA, Quelin C, van Haeringen A, Kleefstra T, Wong MMK, Whalen S, Fisher SE, Morgan AT, van Bon BW
Clinical delineation of SETBP1 haploinsufficiency disorder.
SETBP1 haploinsufficiency disorder (MIM#616078) is caused by haploinsufficiency of SETBP1 on chromosome 18q12.3, but there has not yet been any systematic evaluation of the major features of this monogenic syndrome, assessing penetrance and expressivity. We describe the first comprehensive study to delineate the associated clinical phenotype, with findings from 34 individuals, including 24 novel cases, all of whom have a SETBP1 loss-of-function variant or single (coding) gene deletion, confirmed by molecular diagnostics. The most commonly reported clinical features included mild motor developmental delay, speech impairment, intellectual disability, hypotonia, vision impairment, attention/concentration deficits, and hyperactivity. Although there is a mild overlap in certain facial features, the disorder does not lead to a distinctive recognizable facial gestalt. As well as providing insight into the clinical spectrum of SETBP1 haploinsufficiency disorder, this reports puts forward care recommendations for patient management.
Eur J Hum Genet, 2021; 29
33907317: Morgan A, Braden R, Wong MMK, Colin E, Amor D, Liégeois F, Srivastava S, Vogel A, Bizaoui V, Ranguin K, Fisher SE, van Bon BW
Speech and language deficits are central to SETBP1 haploinsufficiency disorder.
Expressive communication impairment is associated with haploinsufficiency of SETBP1, as reported in small case series. Heterozygous pathogenic loss-of-function (LoF) variants in SETBP1 have also been identified in independent cohorts ascertained for childhood apraxia of speech (CAS), warranting further investigation of the roles of this gene in speech development. Thirty-one participants (12 males, aged 0; 8-23; 2 years, 28 with pathogenic SETBP1 LoF variants, 3 with 18q12.3 deletions) were assessed for speech, language and literacy abilities. Broader development was examined with

standardised motor, social and daily life skills assessments. Gross and fine motor deficits (94%) and intellectual impairments (68%) were common. Protracted and aberrant speech development was consistently seen, regardless of motor or intellectual ability. We expand the linguistic phenotype associated with SETBP1 LoF syndrome (SETBP1 haploinsufficiency disorder), revealing a striking speech presentation that implicates both motor (CAS, dysarthria) and language (phonological errors) systems, with CAS (80%) being the most common diagnosis. In contrast to past reports, the understanding of language was rarely better preserved than language expression (29%). Language was typically low, to moderately impaired, with commensurate expression and comprehension ability. Children were sociable with a strong desire to communicate. Minimally verbal children (32%) augmented speech with sign language, gestures or digital devices. Overall, relative to general development, spoken language and literacy were poorer than social, daily living, motor and adaptive behaviour skills. Our findings show that poor communication is a central feature of SETBP1 haploinsufficiency disorder, confirming this gene as a strong candidate for speech and language disorders.
Eur J Hum Genet, 2021; 29
29397531: Watson LM, Wong MMK, Vowles J, Cowley SA, Becker EBE
A Simplified Method for Generating Purkinje Cells from Human-Induced Pluripotent Stem Cells.
The establishment of a reliable model for the study of Purkinje cells in vitro is of particular importance, given their central role in cerebellar function and pathology. Recent advances in induced pluripotent stem cell (iPSC) technology offer the opportunity to generate multiple neuronal subtypes for study in vitro. However, to date, only a handful of studies have generated Purkinje cells from human pluripotent stem cells, with most of these protocols proving challenging to reproduce. Here, we describe a simplified method for the reproducible generation of Purkinje cells from human iPSCs. After 21 days of treatment with factors selected to mimic the self-inductive properties of the isthmic organiser-insulin, fibroblast growth factor 2 (FGF2), and the transforming growth factor β (TGFβ)-receptor blocker SB431542-hiPSCs could be induced to form En1-positive cerebellar progenitors at efficiencies of up to 90%. By day 35 of differentiation, subpopulations of cells representative of the two cerebellar germinal zones, the rhombic lip (Atoh1-positive) and ventricular zone (Ptf1a-positive), could be identified, with the latter giving rise to cells positive for Purkinje cell progenitor-specific markers, including Lhx5, Kirrel2, Olig2 and Skor2. Further maturation was observed following dissociation and co-culture of these cerebellar progenitors with mouse cerebellar cells, with 10% of human cells staining positive for the Purkinje cell marker calbindin by day 70 of differentiation. This protocol, which incorporates modifications designed to enhance cell survival and maturation and improve the ease of handling, should serve to make existing models more accessible, in order to enable future advances in the field.
Cerebellum, 2018; 17
30249303: Wong MMK, Hoekstra SD, Vowles J, Watson LM, Fuller G, Németh AH, Cowley SA, Ansorge O, Talbot K, Becker EBE
Neurodegeneration in SCA14 is associated with increased PKCγ kinase activity, mislocalization and aggregation.
Spinocerebellar ataxia type 14 (SCA14) is a subtype of the autosomal dominant cerebellar ataxias that is characterized by slowly progressive cerebellar dysfunction and neurodegeneration. SCA14 is caused by mutations in the PRKCG gene, encoding protein kinase C gamma (PKCγ). Despite the identification of 40 distinct disease-causing mutations in PRKCG, the pathological mechanisms underlying SCA14 remain poorly understood. Here we report the molecular neuropathology of SCA14 in post-mortem cerebellum and in human patient-derived induced pluripotent stem cells (iPSCs) carrying two distinct SCA14 mutations in the C1 domain of PKCγ, H36R and H101Q. We show that endogenous expression of these mutations results in the cytoplasmic mislocalization and aggregation of PKCγ in both patient iPSCs and cerebellum. PKCγ aggregates were not efficiently targeted for degradation. Moreover, mutant PKCγ was found to be hyper-activated, resulting in increased substrate phosphorylation. Together, our findings demonstrate that a combination of both, loss-of-function and gain-of-function mechanisms are likely to underlie the pathogenesis of SCA14, caused by mutations in the C1 domain of PKCγ. Importantly, SCA14 patient iPSCs were found to accurately recapitulate pathological features observed in post-mortem SCA14 cerebellum, underscoring their potential as relevant disease models and their promise as future drug discovery tools.
Acta Neuropathol Commun, 2018; 6
28825058: Wong MMK, Watson LM, Becker EBE
Recent advances in modelling of cerebellar ataxia using induced pluripotent stem cells.
The cerebellar ataxias are a group of incurable brain disorders that are caused primarily by the progressive dysfunction and degeneration of cerebellar Purkinje cells. The lack of reliable disease models for the heterogeneous ataxias has hindered the understanding of the underlying pathogenic mechanisms as well as the development of effective therapies for these devastating diseases. Recent advances in the field of induced pluripotent stem cell (iPSC) technology offer new possibilities to better understand and potentially reverse disease pathology. Given the neurodevelopmental phenotypes observed in several types of ataxias, iPSC-based models have the potential to provide significant insights into disease progression, as well as opportunities for the development of early intervention therapies. To date, however, very few studies have successfully used iPSC-derived cells to model cerebellar ataxias. In this review, we focus on recent breakthroughs in

generating human iPSC-derived Purkinje cells. We also highlight the future challenges that will need to be addressed in order to fully exploit these models for the modelling of the molecular mechanisms underlying cerebellar ataxias and the development of effective therapeutics.
J Neurol Neuromedicine, 2017; 2
26136256: Watson LM, Wong MM, Becker EB
Induced pluripotent stem cell technology for modelling and therapy of cerebellar ataxia.
Induced pluripotent stem cell (iPSC) technology has emerged as an important tool in understanding, and potentially reversing, disease pathology. This is particularly true in the case of neurodegenerative diseases, in which the affected cell types are not readily accessible for study. Since the first descriptions of iPSC-based disease modelling, considerable advances have been made in understanding the aetiology and progression of a diverse array of neurodegenerative conditions, including Parkinson's disease and Alzheimer's disease. To date, however, relatively few studies have succeeded in using iPSCs to model the neurodegeneration observed in cerebellar ataxia. Given the distinct neurodevelopmental phenotypes associated with certain types of ataxia, iPSC-based models are likely to provide significant insights, not only into disease progression, but also to the development of early-intervention therapies. In this review, we describe the existing iPSC-based disease models of this heterogeneous group of conditions and explore the challenges associated with generating cerebellar neurons from iPSCs, which have thus far hindered the expansion of this research.
Open Biol, 2015; 5

FENS Forum 2022
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BOARD NUMBER: S04-714

IDENTIFYING THERAPEUTICS TO TREAT RESPIRATORY DEFICITS ASSOCIATED WITH THE CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS)

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

Gabriel Pitollat[1], Laura Cardoit[2], Muriel Thoby-Brisson[3]
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Congenital central hypoventilation syndrome (CCHS) is a rare genetic life-threatening disorder characterized by hypoventilation and lack of central chemosensitivity. CCHS is caused by the mutation of *PHOX2B*, a gene encoding a transcription factor required for the proper development of the autonomic system that includes brainstem circuits controlling breathing. Breathing relies on the preBötzinger complex (preBötC) controlling respiratory rhythmogenesis and the retrotrapezoïd/parafacial respiratory group involved in central chemoception. Mouse models of CCHS harboring the most frequent human *PHOX2B* mutation (+7 alanine; Phox2b$^{27Ala/+}$) recapitulate CCHS breathing anomalies and die at birth from respiratory failures. Using Phox2b$^{27Ala/+}$ embryos we aimed to identify a molecule capable of restoring normal breathing and to decipher the underlying mechanisms. Drug screening suggested that AT

BOARD NUMBER: S002, a derivative of the antibiotic geldanamycin with anti-neuroinflammatory effects, might be a good candidate. To test this we performed electrophysiological recordings of respiratory-related activities on *in vitro* preparations obtained from Phox2b$^{27Ala/+}$ embryos before and after AT

BOARD NUMBER: S002 treatment. We observed that AT

BOARD NUMBER: S002 exposure 1) restored a respiratory frequency comparable to that of wild-type embryos and 2) significantly improved response to acidification (mimicking hypercapnia). These effects resulted from an action on the preBötC network and a modification of the activation state of brainstem microglia, resident macrophages of the nervous system known to play important roles in circuits wiring. Mechanisms involved in chemoception rehabilitation remain to be further investigated. Overall, our work started to establish AT

BOARD NUMBER: S002 as a potential cure against CCHS-associated respiratory deficits. Deciphering its mechanisms of action will help further identifying other candidate molecules.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-715

CEREBRAL BLOOD FLOW ALTERATIONS IN VERY PRETERM-BORN ADULTS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Aim: Preterm birth (i.e., birth before 37 weeks of gestation) is associated with long term alterations in brain structure and function. However, although altered brain perfusion in preterm infants or in animal models of prematurity has previously been reported, it is still unknown whether perfusion changes are still present in preterm born adults. We aimed at investigating this question in a pilot study of 10 very preterm-born adults (born before 32 weeks of gestation) and 12 term-born controls aged 22 to 45 years. **Methods:** Using pseudo-continuous arterial spin labeling MRI with a post-label delay of 1800 ms, and T1-weighted MRI, cerebral blood flow (CBF) maps were derived using the BASIL toolbox from the FMRIB Software Library. Group differences in CBF, controlling for age and gender, were investigated via ANCOVA using the SPSS statistical package. **Results:** We found significantly lower global grey matter CBF in very preterm compared to term-born adults ([F(3,22) = 4.134, p=0.022]). In order to investigate more specifically the location of changes, we used the Harvard oxford atlas to perform a region of interest approach. Significantly lower CBF in widespread cortical regions such as temporal pole ([F(3,22) = 6.333, p=0.007]) and subcortical regions such as the thalamus was observed ([F(3,22) = 7.034, p=0.003]). **Conclusion:** These preliminary results suggest long-lasting reductions in brain perfusion after very preterm birth. Follow up studies with more participants are necessary to confirm this finding and to investigate the impact of neonatal complications such as intraventricular hemorrhage on these CBF differences.

Pubmed:
34126289: Menegaux A, Meng C, Bäuml JG, Berndt MT, Hedderich DM, Schmitz-Koep B, Schneider S, Nuttall R, Zimmermann J, Daamen M, Zimmer C, Boecker H, Bartmann P, Wolke D, Sorg C
Aberrant cortico-thalamic structural connectivity in premature-born adults.
Premature birth is associated with alterations in brain structure, particularly in white matter. Among white matter, alterations in cortico-thalamic connections are present in premature-born infants, and they have been suggested both to last until adulthood and to contribute to impaired cognitive functions. To test these hypotheses, 70 very premature-born adults and 67 full-term controls underwent cognitive testing and diffusion-weighted imaging. Each cortical hemisphere was parcellated into six lobes, from which probabilistic tractography was performed to the thalamus. Connection probability was chosen as metric of structural connectivity. We found increased cortico-thalamic connection probability between left prefrontal cortices and left medio-dorsal thalamus and reduced connection probability between bilateral temporal cortices and bilateral anterior thalami in very premature-born adults. Aberrant prefronto- and temporo-thalamic connection probabilities were correlated with birth weight and days on ventilation, respectively, supporting the suggestion that these connectivity changes relate with the degree of prematurity. Moreover, an increase in left prefronto-thalamic connection probability also correlated with lower verbal comprehension index indicating its relevance for verbal cognition. Together, our results demonstrate that cortico-thalamic structural connectivity is aberrant in premature-born adults, with these changes being linked with impairments in verbal cognitive abilities. Due to corresponding findings in infants, data suggest aberrant development of cortico-thalamic connectivity after premature birth with lasting effects into adulthood.
Cortex, 2021; 141
31841682: Menegaux A, Bäuerlein FJB, Vania A, Napiorkowski N, Neitzel J, Ruiz-Rizzo AL, Müller HJ, Sorg C, Finke K
Linking the impact of aging on visual short-term memory capacity with changes in the structural connectivity of posterior thalamus to occipital cortices.
Aging impacts both visual short-term memory (vSTM) capacity and thalamo-cortical connectivity. According to the Neural Theory of Visual Attention, vSTM depends on the structural connectivity between posterior thalamus and visual occipital cortices (PT-OC). We tested whether aging modifies the association between vSTM capacity and PT-OC structural

connectivity. To do so, 66 individuals aged 20-77 years were assessed by diffusion-weighted imaging used for probabilistic tractography and performed a psychophysical whole-report task of briefly presented letter arrays, from which vSTM capacity estimates were derived. We found reduced vSTM capacity, and aberrant PT-OC connection probability in aging. Critically, age modified the relationship between vSTM capacity and PT-OC connection probability: in younger adults, vSTM capacity was negatively correlated with PT-OC connection probability while in older adults, this association was positive. Furthermore, age modified the microstructure of PT-OC tracts suggesting that the inversion of the association between PT-OC connection probability and vSTM capacity with aging might reflect age-related changes in white-matter properties. Accordingly, our results demonstrate that age-related differences in vSTM capacity links with the microstructure and connectivity of PT-OC tracts.
Neuroimage, 2020; 208
33057208: Menegaux A, Hedderich DM, Bäuml JG, Manoliu A, Daamen M, Berg RC, Preibisch C, Zimmer C, Boecker H, Bartmann P, Wolke D, Sorg C, Stämpfli P
Reduced apparent fiber density in the white matter of premature-born adults.
Premature-born adults exhibit lasting white matter alterations as demonstrated by widespread reduction in fractional anisotropy (FA) based on diffusion-weighted imaging (DWI). FA reduction, however, is non-specific for microscopic underpinnings such as aberrant myelination or fiber density (FD). Using recent advances in DWI, we tested the hypothesis of reduced FD in premature-born adults and investigated its link with the degree of prematurity and cognition. 73 premature- and 89 mature-born adults aged 25-27 years underwent single-shell DWI, from which a FD measure was derived using convex optimization modeling for microstructure informed tractography (COMMIT). Premature-born adults exhibited lower FD in numerous tracts including the corpus callosum and corona radiata compared to mature-born adults. These FD alterations were associated with both the degree of prematurity, as assessed via gestational age and birth weight, as well as with reduced cognition as measured by full-scale IQ. Finally, lower FD overlapped with lower FA, suggesting lower FD underlie unspecific FA reductions. Results provide evidence that premature birth leads to lower FD in adulthood which links with lower full-scale IQ. Data suggest that lower FD partly underpins FA reductions of premature birth but that other processes such as hypomyelination might also take place.
Sci Rep, 2020; 10
30928688: Menegaux A, Napiorkowski N, Neitzel J, Ruiz-Rizzo AL, Petersen A, Müller HJ, Sorg C, Finke K
Theory of visual attention thalamic model for visual short-term memory capacity and top-down control: Evidence from a thalamo-cortical structural connectivity analysis.
In the theory of visual attention (TVA), it is suggested that objects in a visual scene compete for representation in a visual short-term memory (vSTM) store. The race towards the store is assumed to be biased by top-down controlled weighting of the objects according to their task relevance. Only objects that reach the store before its capacity limitation is reached are represented consciously in a given instant. TVA-based computational modeling of participants' performance in whole- and partial-report tasks permits independent parameters of individual efficiency of top-down control α and vSTM storage capacity K to be extracted. The neural interpretation of the TVA proposes recurrent loops between the posterior thalamus and posterior visual cortices to be relevant for generating attentional weights for competing objects and for maintaining selected objects in vSTM. Accordingly, we tested whether structural connectivity between posterior thalamus and occipital cortices (PT-OC) is associated with estimates of top-down control and vSTM capacity. We applied whole- and partial-report tasks and probabilistic tractography in a sample of 37 healthy adults. We found vSTM capacity K to be associated with left PT-OC structural connectivity and a trend-wise relation between top-down control α and right PT-OC structural connectivity. These findings support the assumption of the relevance of thalamic structures and their connections to visual cortex for top-down control and vSTM capacity.
Neuroimage, 2019; 195
28188917: Menegaux A, Meng C, Neitzel J, Bäuml JG, Müller HJ, Bartmann P, Wolke D, Wohlschläger AM, Finke K, Sorg C
Impaired visual short-term memory capacity is distinctively associated with structural connectivity of the posterior thalamic radiation and the splenium of the corpus callosum in preterm-born adults.
Preterm birth is associated with an increased risk for lasting changes in both the cortico-thalamic system and attention; however, the link between cortico-thalamic and attention changes is as yet little understood. In preterm newborns, cortico-cortical and cortico-thalamic structural connectivity are distinctively altered, with increased local clustering for cortico-cortical and decreased integrity for cortico-thalamic connectivity. In preterm-born adults, among the various attention functions, visual short-term memory (vSTM) capacity is selectively impaired. We hypothesized distinct associations between vSTM capacity and the structural integrity of cortico-thalamic and cortico-cortical connections, respectively, in preterm-born adults. A whole-report paradigm of briefly presented letter arrays based on the computationally formalized Theory of Visual Attention (TVA) was used to quantify parameter vSTM capacity in 26 preterm- and 21 full-term-born adults. Fractional anisotropy (FA) of posterior thalamic radiations and the splenium of the corpus callosum obtained by diffusion tensor imaging were analyzed by

tract-based spatial statistics and used as proxies for cortico-thalamic and cortico-cortical structural connectivity. The relationship between vSTM capacity and cortico-thalamic and cortico-cortical connectivity, respectively, was significantly modified by prematurity. In full-term-born adults, the higher FA in the right posterior thalamic radiation the higher vSTM capacity; in preterm-born adults this FA-vSTM-relationship was inversed. In the splenium, higher FA was correlated with higher vSTM capacity in preterm-born adults, whereas no significant relationship was evident in full-term-born adults. These results indicate distinct associations between cortico-thalamic and cortico-cortical integrity and vSTM capacity in preterm-and full-term-born adults. Data suggest compensatory cortico-cortical fiber re-organization for attention deficits after preterm delivery.
Neuroimage, 2017; 150
34171095: Hedderich DM, Menegaux A, Li H, Schmitz-Koep B, Stämpfli P, Bäuml JG, Berndt MT, Bäuerlein FJB, Grothe MJ, Dyrba M, Avram M, Boecker H, Daamen M, Zimmer C, Bartmann P, Wolke D, Sorg C
Aberrant Claustrum Microstructure in Humans after Premature Birth.
Several observations suggest an impact of prematurity on the claustrum. First, the claustrum's development appears to depend on transient subplate neurons of intra-uterine brain development, which are affected by prematurity. Second, the claustrum is the most densely connected region of the mammalian forebrain relative to its volume; due to its effect on pre-oligodendrocytes, prematurity impacts white matter connections and thereby the development of sources and targets of such connections, potentially including the claustrum. Third, due to its high connection degree, the claustrum contributes to general cognitive functioning (e.g., selective attention and task switching/maintaining); general cognitive functioning, however, is at risk in prematurity. Thus, we hypothesized altered claustrum structure after premature birth, with these alterations being associated with impaired general cognitive performance in premature born persons. Using T1-weighted and diffusion-weighted magnetic resonance imaging in 70 very preterm/very low-birth-weight (VP/VLBW) born adults and 87 term-born adults, we found specifically increased mean diffusivity in the claustrum of VP/VLBW adults, associated both with low birth weight and at-trend with reduced IQ. This result demonstrates altered claustrum microstructure after premature birth. Data suggest aberrant claustrum development, which is potentially related with aberrant subplate neuron and forebrain connection development of prematurity.
Cereb Cortex, 2021; 31
34520080: Li H, Menegaux A, Schmitz-Koep B, Neubauer A, Bäuerlein FJB, Shit S, Sorg C, Menze B, Hedderich D
Automated claustrum segmentation in human brain MRI using deep learning.
In the last two decades, neuroscience has produced intriguing evidence for a central role of the claustrum in mammalian forebrain structure and function. However, relatively few in vivo studies of the claustrum exist in humans. A reason for this may be the delicate and sheet-like structure of the claustrum lying between the insular cortex and the putamen, which makes it not amenable to conventional segmentation methods. Recently, Deep Learning (DL) based approaches have been successfully introduced for automated segmentation of complex, subcortical brain structures. In the following, we present a multi-view DL-based approach to segment the claustrum in T1-weighted MRI scans. We trained and evaluated the proposed method in 181 individuals, using bilateral manual claustrum annotations by an expert neuroradiologist as reference standard. Cross-validation experiments yielded median volumetric similarity, robust Hausdorff distance, and Dice score of 93.3%, 1.41 mm, and 71.8%, respectively, representing equal or superior segmentation performance compared to human intra-rater reliability. The leave-one-scanner-out evaluation showed good transferability of the algorithm to images from unseen scanners at slightly inferior performance. Furthermore, we found that DL-based claustrum segmentation benefits from multi-view information and requires a sample size of around 75 MRI scans in the training set. We conclude that the developed algorithm allows for robust automated claustrum segmentation and thus yields considerable potential for facilitating MRI-based research of the human claustrum. The software and models of our method are made publicly available.
Hum Brain Mapp, 2021; 42
31032850: Hedderich DM, Bäuml JG, Berndt MT, Menegaux A, Scheef L, Daamen M, Zimmer C, Bartmann P, Boecker H, Wolke D, Gaser C, Sorg C
Aberrant gyrification contributes to the link between gestational age and adult IQ after premature birth.
Gyrification is a hallmark of human brain development, starting in the second half of gestation in primary cortices, followed by unimodal and then transmodal associative cortices. Alterations in gyrification have been noted in premature-born newborns and children, suggesting abnormal cortical folding to be a permanent feature of prematurity. Furthermore, both gyrification and prematurity are tightly linked with cognitive performance, indicating a link between prematurity, gyrification, and cognitive performance. To investigate this triangular relation, we tested the following two hypotheses: (i) gyrification is aberrant in premature-born adults; and (ii) aberrant gyrification contributes to the impact of prematurity on adult cognitive performance. One hundred and one very premature-born adults (i.e. adults born before 32 weeks of gestation, and/or with birth weight <1500 g) and 111 mature-born adults were assessed by structural MRI and cognitive testing at 27 years of age. Gyrification was measured by local cortical absolute mean curvature (AMC), evaluated through structural MRI. Cognitive performance

was assessed by the Wechsler Adult Intelligence Scale, full-scale IQ test. Two-sample t-tests, regression and mediation analyses were used to assess AMC group differences and the relation between AMC, birth-related variables, and full-scale IQ. Three key findings were identified. First, local AMC was widely increased in fronto-temporo-parietal primary and associative cortices of very premature-born adults. Increase of AMC was inversely associated with gestational age and birth weight and positively associated with medical complications at birth, respectively. Second, increased AMC of temporal associative cortices specifically contributed to the association between prematurity and reduced adult IQ (two-path mediation), indicating that aberrant gyrification of temporal associative cortices is critical for impaired cognitive performance after premature birth. Finally, further investigation of the relationship of gyrification between the early folding postcentral cortices and associative temporal cortices, folding later during neurodevelopment, revealed that the effect of gyrification abnormalities in associative temporal cortices on adult IQ is influenced itself by gyrification abnormalities occurring in the early folding postcentral cortices (three-path mediation). These results indicate that gyrification development across cortical areas in the brain conveys prematurity effects on adult IQ. Overall, these results provide evidence that premature birth leads to permanently aberrant gyrification patterns suggesting an altered neurodevelopmental trajectory. Statistical mediation modelling suggests that both aberrant gyrification itself as well as its propagation across the cortex express aspects of impaired neurodevelopment after premature birth and lead to reduced cognitive performance in adulthood. Thus, markers of gyrification appear as potential candidates for prognosis and treatment of prematurity effects.
Brain, 2019; 142

29712788: Vosberg DE, Zhang Y, Menegaux A, Chalupa A, Manitt C, Zehntner S, Eng C, DeDuck K, Allard D, Durand F, Dagher A, Benkelfat C, Srour M, Joober R, Lepore F, Rouleau G, Théoret H, Bedell BJ, Flores C, Leyton M
Mesocorticolimbic Connectivity and Volumetric Alterations in Mutation Carriers.
The axon guidance cue receptor DCC (deleted in colorectal cancer) plays a critical role in the organization of mesocorticolimbic pathways in rodents. To investigate whether this occurs in humans, we measured (1) anatomical connectivity between the substantia nigra/ventral tegmental area (SN/VTA) and forebrain targets, (2) striatal and cortical volumes, and (3) putatively associated traits and behaviors. To assess translatability, morphometric data were also collected in -haploinsufficient mice. The human volunteers were 20 mutation carriers, 16 relatives, and 20 unrelated healthy volunteers (UHVs; 28 females). The mice were 11 and 16 wild-type C57BL/6J animals assessed during adolescence and adulthood. Compared with both control groups, the human carriers exhibited the following: (1) reduced anatomical connectivity from the SN/VTA to the ventral striatum [: = 0.0005, () = 0.60; UHV: = 0.0029, = 0.48] and ventral medial prefrontal cortex (: = 0.0031, = 0.53; UHV: = 0.034, = 0.35); (2) lower novelty-seeking scores (: = 0.034, = 0.82; UHV: = 0.019, = 0.84); and (3) reduced striatal volume (: = 0.0009, = 1.37; UHV: = 0.0054, = 0.93). Striatal volumetric reductions were also present in mice, and these were seen during adolescence (= 0.0058, = 1.09) and adulthood (= 0.003, = 1.26). Together these findings provide the first evidence in humans that an axon guidance gene is involved in the formation of mesocorticolimbic circuitry and related behavioral traits, providing mechanisms through which mutations might affect susceptibility to diverse neuropsychiatric disorders. Opportunities to study the effects of axon guidance molecules on human brain development have been rare. Here, the identification of a large four-generational family that carries a mutation to the axon guidance molecule receptor gene, , enabled us to demonstrate effects on mesocorticolimbic anatomical connectivity, striatal volumes, and personality traits. Reductions in striatal volumes were replicated in -haploinsufficient mice. Together, these processes might influence mesocorticolimbic function and susceptibility to diverse neuropsychiatric disorders.
J Neurosci, 2018; 38

24742619: Rocchetti J, Isingrini E, Dal Bo G, Sagheby S, Menegaux A, Tronche F, Levesque D, Moquin L, Gratton A, Wong TP, Rubinstein M, Giros B
Presynaptic D2 dopamine receptors control long-term depression expression and memory processes in the temporal hippocampus.
Dysfunctional mesocorticolimbic dopamine signaling has been linked to alterations in motor and reward-based functions associated with psychiatric disorders. Converging evidence from patients with psychiatric disorders and use of antipsychotics suggests that imbalance of dopamine signaling deeply alters hippocampal functions. However, given the lack of full characterization of a functional mesohippocampal pathway, the precise role of dopamine transmission in memory deficits associated with these disorders and their dedicated therapies is unknown. In particular, the positive outcome of antipsychotic treatments, commonly antagonizing D2 dopamine receptors (D2Rs), on cognitive deficits and memory impairments remains questionable.
Biol Psychiatry, 2015; 77

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BOARD NUMBER: S04-716

EARLY AXON GUIDANCE AND SYNAPSE MATURATION DEFECTS IN A ZEBRAFISH MODEL OF MUCOPOLYSACCHARIDOSIS TYPE II

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Mucopolysaccharidosis type II (MPS II) is a rare X-linked lysosomal storage disorder caused by a deficit of the lysosomal enzyme iduronate-2-sulfatase (IDS), a hydrolase involved in the degradation of dermatan and heparan sulfate (HS) glycosaminoglycans. Disease manifestations include progressively severe central nervous system defects resulting in epilepsy, hyperactivity, loss of attention and aggressive behaviors. It is well known that HS plays a critical role in neurodevelopment by shaping the neural extracellular matrix and modulating neuroligand-receptor interactions. The goal of our investigation is to deeply characterize the mechanistic basis of MPS II-related brain defects using an established zebrafish Ids knock out (KO) model. Towards this aim, we preliminarily performed a complementary approach (based on Western Blot, immunofluorescence and other techniques) to investigate axon guidance and synapse maturation defects in KO and age-matched control larvae at early developmental stages. In the former case, we found in KO fish a consistent dysregulation of Netrin1 and Deleted in colorectal cancer (DCC), two well-known molecules involved in axonal chemoattraction. Concerning the synaptic maturation, we detected an overactivation of the canonical Wnt pathway and increased expression of the postsynaptic density protein 95 (PSD-95) in Ids loss of function fish. All abnormalities have been documented before any overt HS accumulation, suggesting that a hierarchy of multiple aberrant cellular and signaling cascades, rather than the simple lysosomal engorgement, underlies the altered MPS II neurophysiology.

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BOARD NUMBER: S04-717

TARGETING BRAIN HTR7 RECEPTOR TO PREVENT HYPOMYELINATION IN A RODENT MODEL OF PERINATAL WHITE MATTER INJURIES

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Children born prematurely are at higher risk to develop brain lesions especially perinatal white matter injury (PWMI). Shreds of evidence in humans and rodents demonstrate that systemic inflammation induces glial reactivity, which is relevant in PWMI physiopathology. A new challenge in perinatal brain injuries is to develop new neuroprotective strategies targeting neuroinflammation to prevent PWMI. Serotonin (5-HT) and its receptors, including HTR7/5-HT7 receptor, might regulate brain inflammation by modulation of glial reactivity. In a perinatal inflammation mouse model of PWMI induced by IL-1β injections, some key lesions can be summarized as (i) systemic inflammation (ii) pro-inflammatory glial reactivity, and (iii) inhibition of oligodendrocyte maturation, leading to PWMI. In this model, *Htr7* mRNA was significantly overexpressed in the anterior cortex, suggesting its key function in PWMI etiology. LP-211, a specific HTR7 agonist with a high affinity that crosses the blood-brain barrier (BBB), was used to modulated HTR7 activity in our model. LP-211 prevented the glial reactivity and the downregulation of myelin basic protein (MBP, a key protein in the myelination process) induced by IL-1β injections. To confirm that the neuroprotective effect of HTR7 activation occurred in the brain and not in the periphery, we compared LP-211 and AS-19 (another HTR7 agonist that does not pass BBB) effects both on systemic inflammation and MBP expression. Both LP-211 and AS-19 did not reduce peripheric inflammation. LP-211, unlike AS-19, was able to rescue MBP optimal expression. Therefore, HTR7 could represent an innovative therapeutic target to protect the developing brain from preterm brain injuries.

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BOARD NUMBER: S04-718

IS THE ADHD BRAIN A SLEEPY BRAIN? ELECTROENCEPHALOGRAPHIC MARKERS OF SLEEP INTRUSIONS IN AWAKE, BEHAVING ADHD ADULTS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Background: Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental condition characterized by above-normal levels of inattentive, hyperactive and/or impulsive behaviors, which seriously challenges everyday-life activities and even reduce the life expectancy of the 5% of individuals affected by this disorder worldwide. However, despite the frequency of this trouble, little is known about its etiology. The frequent observation of sleep disturbances in the ADHD population suggests that attentional disorders may stem from a dysregulation of arousal, depicting the ADHD brain as hypo-vigilant. **Aim:** Test whether ADHD symptoms are at least partially linked to a phenomenon of 'local sleep'. **Methods:** We studied a group of controls and individuals with ADHD performing the CTET attentional task. We recorded their EEG, extracted markers of hypo-vigilance such as theta power and sleep-like slow waves. **Results:** The increase of both theta power and slow waves' amplitude in the ADHD population compared to controls describes sleepier ADHD brains. Moreover, the amplitude of slow waves observed during the task negatively correlated with behavioral performance. **Conclusions:** Consequently, attentional disturbances in ADHD individuals could be partly explained by vigilance troubles. In particular, local sleep intrusions during wakefulness, detected through the presence of sleep-like slow waves, could provide a novel framework to explain attentional lapses, in both the general and ADHD populations, as well as the impulsive manifestations of ADHD. Therefore, these high-amplitude slow waves could be used as a new biomarker, facilitating the diagnosis of ADHD and offering new therapeutic leads. *Keywords : ADHD, hypo-vigilance, local sleep, attentional lapses, impulsivity, electroencephalography*

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BOARD NUMBER: S04-719

HEPARANASE INHIBITION TO PREVENT DEGRADATION OF HEPARAN SULFATE AND ACCUMULATION OF INTERMEDIATE HS DEGRADATION PRODUCTS IN SANFILIPPO SYNDROME

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Mucopolysaccharidosis IIIA (Sanfilippo syndrome) is a hereditary disease caused by mutations in the enzymes responsible for the catabolism of heparan sulfates (HS), leading to lysosomal HS fragments accumulation, multisystemic disease and premature death. Currently, there is no cure and available therapies are scarce. Here, we tested the effects of ip injection of the marine sulphated carbohydrate A5_3 (20µg/g in 40µl 3x/week for 8 weeks) in a mouse model of MPSIIIA from 4 to 12 weeks of age. Both male and female mice were used in this study and were divided into 4 groups (n=14; WT, MPSIIIA, MPSIIIA+A5_3; MPSIIIA+Saline). High field 9.4T ^{1}H-MRSpectroscopy revealed increased levels of glutamate, glutamine, GSH and NAA at 4 weeks in MPSIIIA animals. These alterations indicate dysfunction in the coupling neurons-astrocytes leading to glutamatergic excitotoxicity. Moreover, phosphocreatine and creatine levels were reduced, indicating energetic metabolism failure in the cortex. Treatment with A5_3 partially reversed the alterations in the cortex and hippocampus observed by MRS at 12 weeks. Also, decreased transcription levels of the glutamate transporter Slc1a together with increased IL-1α and TLR4 were observed in MPSIIIA and reversed by treatment. Increased protein expression of LAMP1, GFAP and pro-caspase3 was observed in MPSIIA mice, with no major effects of treatment. Finally, behavioural analysis at 11 weeks revealed decreased exploratory activity and anxiety in MPSIIIA mice, while no difference was observed upon treatment. Despite no evidence of functional protection, A5_3 caused preservation in the cortical metabolism and partial anti-inflammatory effects in MPSIIIA mice.

Pubmed:
34836132: Sanches E, van de Looij Y, Sow S, Toulotte A, da Silva A, Modernell L, Sizonenko S
Dose-Dependent Neuroprotective Effects of Bovine Lactoferrin Following Neonatal Hypoxia-Ischemia in the Immature Rat Brain.
Injuries to the developing brain due to hypoxia-ischemia (HI) are common causes of neurological disabilities in preterm babies. HI, with oxygen deprivation to the brain or reduced cerebral blood perfusion due to birth asphyxia, often leads to severe brain damage and sequelae. Injury mechanisms include glutamate excitotoxicity, oxidative stress, blood-brain barrier dysfunction, and exacerbated inflammation. Nutritional intervention is emerging as a therapeutic alternative to prevent and rescue brain from HI injury. Lactoferrin (Lf) is an iron-binding protein present in saliva, tears, and breast milk, which has been shown to have antioxidant, anti-inflammatory and anti-apoptotic properties when administered to mothers as a dietary supplement during pregnancy and/or lactation in preclinical studies of developmental brain injuries. However, despite Lf's promising neuroprotective effects, there is no established dose. Here, we tested three different doses of dietary maternal Lf supplementation using the postnatal day 3 HI model and evaluated the acute neurochemical damage profile using H Magnetic Resonance Spectroscopy (MRS) and long-term microstructure alterations using advanced diffusion imaging (DTI/NODDI) allied to protein expression and histological analysis. Pregnant Wistar rats were fed either control diet or bovine Lf supplemented chow at 0.1, 1, or 10 g/kg/body weight concentration from the last day of pregnancy (embryonic day 21-E21) to weaning. At postnatal day 3 (P3), pups from both sexes had their right common carotid artery permanently occluded and were exposed to 6% oxygen for 30 min. Sham rats had the incision but neither surgery nor hypoxia episode. At P4, MRS was performed on a 9.4 T scanner to obtain the neurochemical profile in the cortex. At P4 and P25, histological analysis and protein expression were assessed in the cortex and hippocampus. Brain volumes and ex vivo microstructural analysis using DTI/NODDI parameters were performed at P25. Acute metabolic disturbance induced in cortical tissue by HIP3 was reversed with all three doses of Lf. However, data obtained from MRS show that Lf neuroprotective effects were modulated by the dose. Through western blotting analysis, we observed that HI pups supplemented with Lf at 0.1 and 1 g/kg were able to

counteract glutamatergic excitotoxicity and prevent metabolic failure. When 10 g/kg was administered, we observed reduced brain volumes, increased astrogliosis, and hypomyelination, pointing to detrimental effects of high Lf dose. In conclusion, Lf supplementation attenuates, in a dose-dependent manner, the acute and long-term cerebral injury caused by HI. Lf reached its optimal effects at a dose of 1 g/kg, which pinpoints the need to better understand effects of Lf, the pathways involved and possible harmful effects. These new data reinforce our knowledge regarding neuroprotection in developmental brain injury using Lf through lactation and provide new insights into lactoferrin's neuroprotection capacities and limitation for immature brains.
Nutrients, 2021; 13

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BOARD NUMBER: S04-720

NEURODEVELOPMENTAL PATHOGENESIS OF CONGENITAL CYTOMEGALOVIRUS INFECTION: DECIPHERING THE ROLES OF IMMUNE EVENTS IN THE DEVELOPING RAT BRAIN.

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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BOARD NUMBER: S012, Aix-marseille University, Marseille, France, [5]Present Address: INSERM UMR1109, Université De Strasbourg, Strasbourg, France

Aims: Cytomegalovirus (CMV) is a member of the *Herpesviridae* family. Congenital CMV infections represent one leading cause of human neurodevelopmental disorders. Despite their high prevalence (0.7 to 1% of all live births) and possible severity, the pathophysiology remains elusive and no satisfactory prevention or therapy is available. Besides neural-based mechanisms, the possible involvement of immune responses in CMV-related neuropathogenesis has been increasingly questioned. Insights into the early immune events following CMV infection of the developing brain are particularly needed. Methods: We have created a novel model of CMV infection of the rat developing brain by *in utero* injection at E15 of a recombinant rat CMV allowing for GFP expression in the infected cells. Current investigations at various levels non-exhaustively include postnatal phenotyping of rat pups, electrophysiological recordings, microglia- and chemokine-targeting rescue assays, immunophenotyping of brain leukocytes, RNA sequencing of flow-sorted microglia. Results: Rat pups recapitulated several characteristics of the human disease, including altered sensorimotor development and epileptic seizures. Brain immune alterations were detected at the cellular and molecular levels. Our data indicate that fetal microglia on the one hand, and a chemokine encoded by the viral genome on the other hand, might both play pivotal roles in CMV-related neuropathogenesis. Conclusions: Our findings provide novel insights into the pathophysiological mechanisms possibly involved in neuropathogenesis of congenital CMV, and would contribute as proof-of-principle to the future design of innovative therapeutic options.

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BOARD NUMBER: S04-721

BUFFERING OF TRANSCRIPTION RATE BY MRNA HALF DECAY MECHANISMS IS A CONSERVED FEATURE OF RETT SYNDROME MODELS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the transcriptional modulator gene *MECP2*, and patients display global dysregulation of the neuronal transcriptome. However, despite MECP2 being a highly abundant protein, the changes in mRNA levels are surprisingly small in RTT models suggesting that mRNA stability mechanisms significantly shape the mRNA levels in RTT. To uncover the dynamics between transcription and mRNA levels in RTT models, we directly measured transcription rate and mRNA half-life changes in iPSC-derived RTT patient neurons using RATE-seq and RNA-seq from nuclear and whole-cell fractions of RTT mice models. Expectedly, we found thousands of genes with dysregulated transcription rates in both RTT models. Surprisingly, we found that combined frequencies of three dinucleotides were better predictors of transcriptional dysregulation than CA/CG, described DNA-binding sites of MECP2. Moreover, we found a similarly widespread alteration in mRNA stabilities leading to the buffering of transcriptional dysregulation of most genes. Using an unbiased computational approach, we found microRNA and RNA-Binding Protein (RBP) sequence elements enriched in 3'UTRs of buffered genes. We also found a significant number of mRNAs exclusively dysregulated at the stability levels in both RTT models. Our findings identify post-transcriptional mechanisms in humans and mice RTT models that alter mRNA half-life to buffer transcription rate changes uncovering new actionable mechanisms to be explored in translational approaches.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S04-722

UNRAVELLING THE ROLE OF CHROMATIN MODIFIERS IN HUMAN NEURODEVELOPMENT: OBSERVATIONS FROM KABUKI SYNDROME

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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The use of induced pluripotent stem cells (iPSC) as models of development and disease has brought insights into the heterogeneity of cell lineages during early neurodevelopment. This is of relevance to the study of neurodevelopmental disorders (NDD) as their underlying mechanisms remain largely unknown. NDDs are frequently caused by loss-of-function mutations in chromatin-modifying enzymes. **Aim** To understand how these mutations affect neurodevelopmental processes, we studied Kabuki Syndrome (KS), a representative NDD mainly caused by haploinsufficiency of *KMT2D* and *KDM6A*, enzymes involved in histone methylation. **Methods** We created a KS cortical model and differentiated wildtype and KS-patient-derived iPSC lines into neural precursors cells. We profiled the cells using immunocytochemistry, bulk and single-cell transcriptomics, as well as single-cell ATAC and multi-omic sequencing to capture chromatin accessibility and gene expression from the same cells. **Results & Conclusions** Our model highlights an asynchronous delayed differentiation into the cortico-neural lineage. The RNA-seq analysis revealed significant events of differential splicing in *DNMT3*, a DNA methyltransferase and a known NDD gene. Leveraging the chromatin accessibility profile, we identified an enrichment of specific TF binding motifs in KS cells compared to controls. We observed large inter-patient variability, which suggests that different molecular pathways might contribute to the condition. Currently, to control for genetic background effects, we are using CRISPR-Cas9 to create isogenic control iPSC lines and wildtype lines carrying engineered KS disease mutations. We aim to better understand the molecular pathways and developmental stages underlying KS and optimise our model to extend the study to other NDDs.

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BOARD NUMBER: S04-723

A CLOSER LOOK AT CUX1 HETEROZYGOSIS IN THE NEOCORTEX, WHEN ONE COPY IS NOT ENOUGH

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Neurodevelopmental disorders can emerge due to abnormal neuronal function and/or connectivity. Cortical wiring requires a variety of neuronal subtypes whose identity is defined by specific transcription factors (TF). Some of them are dosage-dependent, meaning that a single functional copy of the gene cannot maintain the wild-type phenotype. In the neocortex, upper layer neurons, which participate in the most complex and evolved circuits, are defined by the expression of Cux1 TF. This gene is involved in their dendritogenesis, synaptogenesis and the establishment of interhemispheric projections as shown by knock-down experiments. Moreover, heterozygous patients carrying a mutant Cux1 allele have neurological diseases such as epilepsy, intellectual disability, and autism spectrum disorder. However, the overall cortical development and brain structures seem normal in heterozygous condition. Thus, a deeper characterization of the heterozygous scenario is needed. For this purpose, we characterized the Cux1 heterozygous mouse; studied Cux1 expression in different functional areas of the cortex during development; and analysed their susceptibility to kainate-induced seizures. Our results show a significant reduction in Cux1 expression in the neocortex at postnatal day (P) 10, specially in the barrel field of the somatosensory area. These differences are attenuated in adulthood (P30), suggesting a possible rescue mechanism that leads to a catch-up phenotype. We also demonstrate that Cux1 heterozygosis increases predisposition to develop seizures in mice. Taken together, our findings highlight the relevance of Cux1 levels of expression during cortical development and point to the necessity of investigating the consequences of Cux1 haploinsufficiency at a molecular level.

Pubmed:

31488723: Pappa S, Padilla N, Iacobucci S, Vicioso M, Álvarez de la Campa E, Navarro C, Marcos E, de la Cruz X, Martínez-Balbás MA
PHF2 histone demethylase prevents DNA damage and genome instability by controlling cell cycle progression of neural progenitors.
Histone H3 lysine 9 methylation (H3K9me) is essential for cellular homeostasis; however, its contribution to development is not well established. Here, we demonstrate that the H3K9me2 demethylase PHF2 is essential for neural progenitor proliferation in vitro and for early neurogenesis in the chicken spinal cord. Using genome-wide analyses and biochemical assays we show that PHF2 controls the expression of critical cell cycle progression genes, particularly those related to DNA replication, by keeping low levels of H3K9me3 at promoters. Accordingly, PHF2 depletion induces R-loop accumulation that leads to extensive DNA damage and cell cycle arrest. These data reveal a role of PHF2 as a guarantor of genome stability that allows proper expansion of neural progenitors during development.
Proc Natl Acad Sci U S A, 2019; 116

FENS Forum 2022
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BOARD NUMBER: S04-724

CHARACTERIZATION OF LONG-RANGE MONOAMINERGIC NEUROMODULATORY PROJECTIONS IN FOCAL CORTICAL DYSPLASIA

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Focal Cortical Dysplasia (FCD) represents a large variety of cortical malformations associated with abnormal cortical layering and/or appearance of dysmorphic neuronal elements, often occurring in the frontal cortex. FCD is a common cause of pharmaco-resistant epilepsies and altered local neuronal activity has been reported in human FCD tissue samples. Local cortical microcircuits are extensively modulated by monoaminergic axonal projections arising from the brain stem. Previous analysis of monoaminergic modulatory inputs in human FCD biopsies suggested altered density and distribution of these monoaminergic axons. However, a systematic investigation is still pending. Here we perform a comprehensive analysis of monoaminergic axonal projections, with a focus on dopaminergic (DA) innervation, in human FCD biopsies and in the medial prefrontal cortex (mPFC) of FCD mouse models (mTOR hyperactivation model). Moreover, we analyse the expression of dopamine receptors *Drd1* and *Drd2* via multiplex fluorescent RNA in situ in human specimen and the mPFC of this mouse model. Our preliminary results suggest that the overall DA innervation density is not altered in adult human FCD samples compared to the control area. However, the DA innervation distribution displays a variable lamination pattern between FCD and the control area. In the mTOR hyperactivation mouse model, we observe a transient increase in DA axonal innervation in the deep layers of mPFC during adolescence. *Drd1* and *Drd2* receptors seem differentially expressed in the mTOR-mutated cells compared to normally-developed neurons. Overall, our results suggest alterations in dopaminergic neurotransmission in FCD that may be important for understanding disease mechanisms in FCD.

Pubmed:
34712123: Islam KUS, Meli N, Blaess S
The Development of the Mesoprefrontal Dopaminergic System in Health and Disease.
Midbrain dopaminergic neurons located in the substantia nigra and the ventral tegmental area are the main source of dopamine in the brain. They send out projections to a variety of forebrain structures, including dorsal striatum, nucleus accumbens, and prefrontal cortex (PFC), establishing the nigrostriatal, mesolimbic, and mesoprefrontal pathways, respectively. The dopaminergic input to the PFC is essential for the performance of higher cognitive functions such as working memory, attention, planning, and decision making. The gradual maturation of these cognitive skills during postnatal development correlates with the maturation of PFC local circuits, which undergo a lengthy functional remodeling process during the neonatal and adolescence stage. During this period, the mesoprefrontal dopaminergic innervation also matures: the fibers are rather sparse at prenatal stages and slowly increase in density during postnatal development to finally reach a stable pattern in early adulthood. Despite the prominent role of dopamine in the regulation of PFC function, relatively little is known about how the dopaminergic innervation is established in the PFC, whether and how it influences the maturation of local circuits and how exactly it facilitates cognitive functions in the PFC. In this review, we provide an overview of the development of the mesoprefrontal dopaminergic system in rodents and primates and discuss the role of altered dopaminergic signaling in neuropsychiatric and neurodevelopmental disorders.
Front Neural Circuits, 2021; 15

FENS Forum 2022
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BOARD NUMBER: S04-725

STRUCTURAL PLASTICITY IN THE MONKEY ENTORHINAL AND PERIRHINAL CORTICES FOLLOWING SELECTIVE HIPPOCAMPAL LESION

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Populations of Bcl2-positive immature neurons are present in several regions of the adult mammalian brain, including the amygdala, and the entorhinal and perirhinal cortices. We have previously shown that neonatal and adult hippocampal lesions increase the differentiation of immature neurons in the monkey amygdala. Here, we determined whether similar changes occur in the entorhinal and perirhinal cortices in the same animals. We performed design-based stereological analyses of Nissl-stained and Bcl2-stained sections to estimate the number and soma size of immature and mature neurons in different subdivisions of the entorhinal and perirhinal cortices. We found different lesioned-induced structural changes in the entorhinal and perirhinal cortices following hippocampal lesion. Following neonatal hippocampal lesion, the number of immature neurons was generally higher in the entorhinal and perirhinal cortices, as compared to control and adult-lesioned monkeys. The number of mature neurons was higher in layer III of area Er of the entorhinal cortex, but it did not differ from controls in layer II of area 36 of the perirhinal cortex. Following adult hippocampal lesion, the number of immature neurons was lower in the entorhinal cortex but it did not differ from controls in the perirhinal cortex. The number of small mature neurons was lower in layer II of area 36, but it did not differ from controls in layer III of Er. Consistent with prior findings in the amygdala, hippocampal damage may have influenced neuroblast migration and the differentiation of immature neurons in a subdivision-specific manner in the entorhinal and perirhinal cortices.

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BOARD NUMBER: S04-726

EARLY-LIFE EXPOSURE TO FLUOXETINE INDUCES SPECIFIC PREFRONTAL CORTICAL CIRCUIT ALTERATIONS IN ADULT MICE

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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The prefrontal cortex (PFC) plays a key role in high-level cognitive processes and emotional behaviors. The maturation of PFC circuits is characterized by critical developmental time windows, which shape functional connectivity among neurons. Early life insults can alter PFC function, increasing the risk for brain disorders. Perinatal exposure to fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), affects PFC development and yield significant depressive- and anxiety-like phenotypes in the adult. We found that early postnatal FLX-treatment results in strong hypoactivity and reduced firing rate of putative pyramidal neurons (PNs) of adult mice. *Ex-vivo*, patch-clamp recordings indicate that early-life FLX treatment affects the firing dynamics of a specific subpopulation of PNs, which transiently expresses the serotonin transporter SERT during the PFC critical period (SERT-positivePN). Conversely the excitability of SERT-negative PNs, and parvalbumin-positive interneurons was unaffected by early FLX treatment. Genetic and pharmacological experiments indicate that hypoexcitability of PFC PNs mainly depended on 5HT-7 receptor. Surprisingly, spontaneous glutamatergic and GABAergic neurotransmission onto PFC PNs was unaltered in mice that underwent perinatal FLX treatment. We are currently investigating potential anatomical differences that that could be induced by the early-life insult caused by FLX treatment. Our results suggest potential novel neurobiological mechanisms, underlying detrimental neurodevelopmental consequences of early-life environmental insult.

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BOARD NUMBER: S04-727

MECHANICAL VS THERMAL ANALGESIA INDUCED BY RELAXIN-3/RXFP3 PEPTIDERGIC TRANSMISSION IN ANTERIOR CINGULATE CORTEX AND AMYGDALA

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

Marie Tuifua[1], Thibault Dhellemmes[2], Cynthia Abboub[3], Louison Brochoire[2], Sandra Sánchez-Sarasúa[2], Melina Petrel[4], André Calas[2], Eric Boué-Grabot[2], Akhter Hossain[5], Francisco Olucha-Bordonau[6], Andrew Gundlach[5], Marc Landry[2]
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Affecting around 10% of world population, chronic pain and its related psychiatric comorbidities (depression and anxiety) are major health issues. Pathways and modulation of pain are well documented in the spinal cord, but the implication of neuropeptides in this modulation remains poorly described in the brain. Relaxin-3 neuropeptide displays antidepressant and anxiolytic effects, and our preliminary results indicate an analgesic role in rats and mice. Relaxin-3 is expressed by nucleus incertus neurons that project to different cortical (e.g. anterior cingulate cortex (ACC)) and subcortical (e.g. amygdala) areas of the pain matrix. Because of the prominent expression of the relaxin-3 G protein-coupled receptor (RXFP3) in those areas, we aim at studying the pain modulatory effects of relaxin-3 by using pharmacological, behavioral, and anatomical approaches in a mouse model of persistent inflammatory pain obtained by the injection of Complete Freud's Adjuvant in the hind paw. Intra-amygdalar injection of RXFP3 agonists (A2 or A5) alleviates both mechanical and thermal pain, while intra-ACC injection has an effect only on mechanical sensitization. AAV-mediated chronic release of another RXFP3 agonist (I5/R3) in the ACC prevents mechanical but not thermal sensitization. Multiplex fluorescent in-situ hybridization (RNAscope) shows RXFP3 mRNA expression in somatostatin interneurons both in the ACC and amygdala. We develop quantitative 3D immunolabelling to determine the organisation of relaxin-3/RXFP3 microcircuits. Our data highlight a novel role for this peptide family and suggest their therapeutic potential in persistent pain conditions.

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BOARD NUMBER: S04-728

POSTNATAL IL-4 ADMINISTRATION INDUCES LONG-TERM DYSFUNCTION IN CEREBELLAR-VTA CONNECTIVITY

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Interleukin-4 (IL-4) is a Th2 cytokine that is central to the regulation of allergic inflammation and a critical regulator of microglia. We have recently discovered that postnatal increases in IL-4 induce deficits in cerebellar development, leading to an increase in Purkinje cell excitatory inputs. Moreover, IL-4-treated mice present hyperactivity and increased stereotypies reminiscent of ADHD-like behaviors. Recently, direct projections from the cerebellum to the VTA have been described to modulate activity in this region. Since dopaminergic signaling is highly implicated in the pathophysiology of ADHD, we now aim to probe our animal model for dysfunction in this circuit. To this end, we delivered a viral construct (AAV-ChR2-GFP) to the deep cerebellar nuclei (DCN), allowing us to specifically stimulate DCN-VTA inputs using acute brain slices for whole-cell electrophysiology recordings. Through this, we could corroborate the existence of the recently described DCN-VTA projections, and we also observed that postnatal IL-4 administration was sufficient to induce deficits in short-term plasticity in VTA neurons innerved by DCN afferents. Whole-cell patch clamp was also used to assess whether the presynaptic alterations seen in the previous experiment would alter the number of synapses formed in VTA dopaminergic neurons. These data indicate that IL-4 injected animals present a dysfunctional connection between the cerebellum and the VTA, possibly underlying the hyperactive and impulsive behavioral phenotype previously observed in these mice through a deregulation of the dopaminergic system.

Pubmed:
30926797: Edfawy M, Guedes JR, Pereira MI, Laranjo M, Carvalho MJ, Gao X, Ferreira PA, Caldeira G, Franco LO, Wang D, Cardoso AL, Feng G, Carvalho AL, Peça J
Abnormal mGluR-mediated synaptic plasticity and autism-like behaviours in Gprasp2 mutant mice.
Autism spectrum disorder (ASD) is characterized by dysfunction in social interactions, stereotypical behaviours and high co-morbidity with intellectual disability. A variety of syndromic and non-syndromic neurodevelopmental disorders have been connected to alterations in metabotropic glutamate receptor (mGluR) signalling. These receptors contribute to synaptic plasticity, spine maturation and circuit development. Here, we investigate the physiological role of Gprasp2, a gene linked to neurodevelopmental disabilities and involved in the postendocytic sorting of G-protein-coupled receptors. We show that Gprasp2 deletion leads to ASD-like behaviour in mice and alterations in synaptic communication. Manipulating the levels of Gprasp2 bidirectionally modulates the surface availability of mGluR and produces alterations in dendritic complexity, spine density and synaptic maturation. Loss of Gprasp2 leads to enhanced hippocampal long-term depression, consistent with facilitated mGluR-dependent activation. These findings demonstrate a role for Gprasp2 in glutamatergic synapses and suggest a possible mechanism by which this gene is linked to neurodevelopmental diseases.
Nat Commun, 2019; 10
32492699: Franco LO, Carvalho MJ, Costa J, Ferreira PA, Guedes JR, Sousa R, Edfawy M, Seabra CM, Cardoso AL, Peça J
Social subordination induced by early life adversity rewires inhibitory control of the prefrontal cortex via enhanced Npy1r signaling.
Social hierarchies are present in most mammalian species. In nature, hierarchies offer a tradeoff between reduction of in-group fighting between males, at the expense of an asymmetric sharing of resources. Early life experiences and stress are known to influence the rank an individual attains in adulthood, but the associated cellular and synaptic alterations are poorly understood. Using a maternal separation protocol, we show that care-deprived mice display a long-lasting submissive phenotype, increased social recognition, and enhanced explorative behavior. These alterations are consistent with an adaptation that favors exploration rather than confrontation within a group setting. At the neuronal level, these animals display dendritic atrophy and enhanced inhibitory synaptic inputs in medial prefrontal cortex (mPFC) neurons. To determine what could underlie this synaptic modification, we first assessed global gene expression changes via RNAseq, and next focused

on a smaller subset of putatively altered synaptic receptors that could explain the changes in synaptic inhibition. Using different cohorts of maternally deprived mice, we validated a significant increase in the expression of Npy1r, a receptor known to play a role in maternal care, anxiety, foraging, and regulation of group behavior. Using electrophysiological recordings in adult mice while blocking NPY1R signaling, we determined that this receptor plays a key role in enhancing GABAergic currents in mice that experience maternal deprivation. Taken together, our work highlights the potential of regulating NPY1R in social anxiety disorders and the alterations induced in brain circuitry as a consequence of early life stress and adversity.
Neuropsychopharmacology, 2020; 45
34022277: Costa J, Martins S, Ferreira PA, Cardoso AMS, Guedes JR, Peça J, Cardoso AL
The old guard: Age-related changes in microglia and their consequences.
Among all major organs, the brain is one of the most susceptible to the inexorable effects of aging. Throughout the last decades, several studies in human cohorts and animal models have revealed a plethora of age-related changes in the brain, including reduced neurogenesis, oxidative damage, mitochondrial dysfunction and cell senescence. As the main immune effectors and first responders of the nervous tissue, microglia are at the center of these events. These cells experience irrevocable changes as a result from cumulative exposure to environmental triggers, such as stress, infection and metabolic dysregulation. The age-related immunosenescent phenotype acquired by microglia is characterized by profound modifications in their transcriptomic profile, secretome, morphology and phagocytic activity, which compromise both their housekeeping and defensive functions. As a result, aged microglia are no longer capable of establishing effective immune responses and sustaining normal synaptic activity, directly contributing to age-associated cognitive decline and neurodegeneration. This review discusses how lifestyle and environmental factors drive microglia dysfunction at the molecular and functional level, also highlighting possible interventions to reverse aging-associated damage to the nervous and immune systems.
Mech Ageing Dev, 2021; 197

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-729

ALLERGIES AND IL-4 SHAPE POSTNATAL CEREBELLAR DEVELOPMENT VIA MICROGLIA-MEDIATED NEURONAL PRUNING TO INDUCE ADHD-LIKE BEHAVIORS IN MICE

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Interleukin-4 (IL-4) is a classical Th2 cytokine with pleiotropic functions in allergies, microglia activation and cognitive processes. Epidemiologically, severe allergies such as atopy, asthma and dermatitis share high comorbidity with Attention-Deficit/Hyperactivity Disorder (ADHD). However, the mechanistic link between these conditions is unknown. Here, we find that early postnatal allergies (asthma mouse model of house dust mites) increase the levels of IL-4 in the cerebellum, a brain region that is undergoing profound remodeling during this period. This immune challenge leads to decreased engulfment of granule cells by microglia, deficits in Purkinje cell connectivity and overall hyperactivity in juvenile mice. Interestingly, these dysfunctions are reproduced by peripherally injecting IL-4 alone during this critical period of cerebellar development. We also find that the levels of this cytokine are tightly regulated to allow the physiological phagocytic activity of microglia to prune cerebellar granule cells. We propose that a pathological increase in IL-4 is sufficient to mediate neuronal survival, promote an excessive number of parallel fibers and induce hyperkinetic and impulsive-like behaviors reminiscent of ADHD. Our data provides the first mechanistic link between allergies and hyperactivity, and reinforces the hypothesis that environment-dependent alterations in immune mediators during the postnatal period may impair the maturation of brain regions with protracted development.

Pubmed:
30071357: Cardoso AL, Fernandes A, Aguilar-Pimentel JA, de Angelis MH, Guedes JR, Brito MA, Ortolano S, Pani G, Athanasopoulou S, Gonos ES, Schosserer M, Grillari J, Peterson P, Tuna BG, Dogan S, Meyer A, van Os R, Trendelenburg AU
Towards frailty biomarkers: Candidates from genes and pathways regulated in aging and age-related diseases.
Use of the frailty index to measure an accumulation of deficits has been proven a valuable method for identifying elderly people at risk for increased vulnerability, disease, injury, and mortality. However, complementary molecular frailty biomarkers or ideally biomarker panels have not yet been identified. We conducted a systematic search to identify biomarker candidates for a frailty biomarker panel.
Ageing Res Rev, 2018; 47
34022277: Costa J, Martins S, Ferreira PA, Cardoso AMS, Guedes JR, Peça J, Cardoso AL
The old guard: Age-related changes in microglia and their consequences.
Among all major organs, the brain is one of the most susceptible to the inexorable effects of aging. Throughout the last decades, several studies in human cohorts and animal models have revealed a plethora of age-related changes in the brain, including reduced neurogenesis, oxidative damage, mitochondrial dysfunction and cell senescence. As the main immune effectors and first responders of the nervous tissue, microglia are at the center of these events. These cells experience irrevocable changes as a result from cumulative exposure to environmental triggers, such as stress, infection and metabolic dysregulation. The age-related immunosenescent phenotype acquired by microglia is characterized by profound modifications in their transcriptomic profile, secretome, morphology and phagocytic activity, which compromise both their housekeeping and defensive functions. As a result, aged microglia are no longer capable of establishing effective immune responses and sustaining normal synaptic activity, directly contributing to age-associated cognitive decline and neurodegeneration. This review discusses how lifestyle and environmental factors drive microglia dysfunction at the

molecular and functional level, also highlighting possible interventions to reverse aging-associated damage to the nervous and immune systems.
Mech Ageing Dev, 2021; 197
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Social subordination induced by early life adversity rewires inhibitory control of the prefrontal cortex via enhanced Npy1r signaling.
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Neuropsychopharmacology, 2020; 45
28078993: Viegas ATB, Guedes JR, Oliveira AR, Cardoso AMS, Cardoso ALC
miRNAs: New Biomarkers and Therapeutic Targets in Dementia.
Dementia is a complex pathological state that affects millions of individuals worldwide and is responsible for a huge socioeconomic burden, making it a major health concern of current times. Given the impact of dementia in both patients and caregivers, it is crucial to fully clarify the molecular mechanisms underlying dementia-associated disorders, since without this knowledge our ability to correctly diagnose and treat these diseases is severely hampered.
Curr Pharm Des, 2017; 23
27239545: Guedes JR, Santana I, Cunha C, Duro D, Almeida MR, Cardoso AM, de Lima MC, Cardoso AL
MicroRNA deregulation and chemotaxis and phagocytosis impairment in Alzheimer's disease.
Mononuclear phagocytes play a critical role during Alzheimer's disease (AD) pathogenesis due to their contribution to innate immune responses and amyloid beta (Aβ) clearance mechanisms.
Alzheimers Dement (Amst), 2016; 3
22043967: Cardoso AL, Guedes JR, Pereira de Almeida L, Pedroso de Lima MC
miR-155 modulates microglia-mediated immune response by down-regulating SOCS-1 and promoting cytokine and nitric oxide production.
Innate immunity constitutes the first line of defence against both external and endogenous threats in the brain, and microglia cells are considered key mediators of this process. Recent studies have shown that microRNAs (miRNAs) may play a determinant role in the regulation of gene expression during innate immune responses. The major goal of this work was to investigate the contribution of a specific miRNA - miR-155 - to the modulation of the microglia-mediated immune response. For this purpose, in vitro studies were performed in N9 microglia cells to evaluate changes in the levels of this miRNA following microglia activation. A strong up-regulation of miR-155 expression was observed following microglia exposure to lipopolysaccharide, which was consistent with a decrease in the levels of the suppressor of cytokine signalling 1 (SOCS-1) protein, a key inhibitor of the inflammatory process and a predicted target of miR-155. The miR-155 knockdown by anti-miRNA oligonucleotides up-regulated SOCS-1 mRNA and protein levels and significantly decreased the production of nitric oxide and the expression of inflammatory cytokines and inducible nitric oxide synthase. Finally, treatment of neuronal primary cultures with conditioned medium obtained from microglia cells, in which miR-155 was inhibited before cell activation, decreased inflammatory-mediated neuronal cell death. Overall, our results show that miR-155 has a pro-inflammatory role in microglia and is necessary for the progression of the immune response through the modulation of SOCS-1, suggesting that, in a chronic inflammatory context, miR-155 inhibition can have a neuroprotective effect.
Immunology, 2012; 135
24990149: Guedes JR, Custódia CM, Silva RJ, de Almeida LP, Pedroso de Lima MC, Cardoso AL
Early miR-155 upregulation contributes to neuroinflammation in Alzheimer's disease triple transgenic mouse model.
MicroRNAs (miRNAs) have emerged as a class of small, endogenous, regulatory RNAs that exhibit the ability to epigenetically modulate the translation of mRNAs into proteins. This feature enables them to control cell phenotypes and,

consequently, modify cell function in a disease context. The role of inflammatory miRNAs in Alzheimer's disease (AD) and their ability to modulate glia responses are now beginning to be explored. In this study, we propose to disclose the functional role of miR-155, one of the most well studied immune-related miRNAs in AD-associated neuroinflammatory events, employing the 3xTg AD animal model. A strong upregulation of miR-155 levels was observed in the brain of 12-month-old 3xTg AD animals. This event occurred simultaneously with an increase of microglia and astrocyte activation, and before the appearance of extracellular Aβ aggregates, suggesting that less complex Aβ species, such as Aβ oligomers may contribute to early neuroinflammation. In addition, we investigated the contribution of miR-155 and the c-Jun transcription factor to the molecular mechanisms that underlie Aβ-mediated activation of glial cells. Our results suggest early miR-155 and c-Jun upregulation in the 3xTg AD mice, as well as in Aβ-activated microglia and astrocytes, thus contributing to the production of inflammatory mediators such as IL-6 and IFN-β. This effect is associated with a miR-155-dependent decrease of suppressor of cytokine signaling 1. Furthermore, since c-Jun silencing decreases the levels of miR-155 in Aβ-activated microglia and astrocytes, we propose that miR-155 targeting can constitute an interesting and promising approach to control neuroinflammation in AD.
Hum Mol Genet, 2014; 23
29512082: Cardoso AL, Guedes JR
Quantifying miRNA Deregulation in Alzheimer's Disease.
Analysis of miRNA expression in circulating immune cells, such as monocytes, using qRT-PCR arrays, allows the quantification of a wide range of miRNAs in easily accessible biosamples from Alzheimer's disease patients. This technique enables the identification of differentially expressed miRNAs and provides important clues for the discovery of new miRNA-based biomarkers. Here we describe how to isolate a specific lymphocyte population from human blood samples, CD14 monocytes, and how to extract total RNA, containing short RNAs, from these cells, transcribe the RNA into cDNA and quantify a pre-set of specific miRNAs using customizable PCR plates of 96 or 384 wells.
Methods Mol Biol, 2018; 1750
30926797: Edfawy M, Guedes JR, Pereira MI, Laranjo M, Carvalho MJ, Gao X, Ferreira PA, Caldeira G, Franco LO, Wang D, Cardoso AL, Feng G, Carvalho AL, Peça J
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Nat Commun, 2019; 10
30158892: Guedes JR, Lao T, Cardoso AL, El Khoury J
Roles of Microglial and Monocyte Chemokines and Their Receptors in Regulating Alzheimer's Disease-Associated Amyloid-β and Tau Pathologies.
Chemokines and their receptors have been shown to affect amyloid-β (Aβ) and tau pathologies in mouse models of Alzheimer's disease (AD) by regulating microglia and monocyte-associated neuroinflammation, microglial movement and monocyte recruitment into the brain. These cells in turn can promote and mediate Aβ phagocytosis and degradation and tau phosphorylation. In this review we discuss published work in this field in mouse models of AD and review what is known about the contributions of microglial and monocyte chemokines and their receptors to amyloid and tau pathologies. We focus on the roles of the chemokine/chemokine receptor pairs CCL2/CCR2, CX3CL1/CX3CR1, CCL5/CCR5, CXCL10/CXCR3 and CXCL1/CXCR2, highlighting important knowledge gaps in this field. A full understanding of the functions of chemokines and their receptors in AD may guide the development of novel immunotherapies for this devastating disease.
Front Neurol, 2018; 9

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BOARD NUMBER: S04-730

PHENOTYPIC ALTERATIONS IN THE HYPOGLOSSAL NUCLEUS OF A MOUSE MODEL OF 22Q11.2DS (LGDEL)

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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22q11 DS is caused by a microdeletion of 30-50 genes of chromosome 22. Consistent early perinatal clinical manifestation is dysphagia whose origins remain poorly understood. We have shown that the LgDel mouse mimics the human phenotypic spectrum of 22q11.2 DS, including dysphagia, and alterations of the neuronal excitability in the hypoglossal nucleus (nXII) motor neurons, which control the tongue movement during suckling and swallowing. Here we analyzed whether alteration in neuronal excitability is induced by changes in neurotransmitters content in the nXII. Confocal 3D reconstructions of motor neurons demonstrated dendritic and axonal abnormalities, including degenerating dendrites. Backscatter scanning electron microscopy enabled large-scale analysis of neuronal ultrastructural characteristics in motoneurons affected by the genetic lesion. nXII motoneurons of one-week old LgDel showed dilated mitochondrial cristae, paler mitochondrial matrix, and swollen Golgi and endoplasmic reticulum. At later ages, we found that LgDel nXII motor neurons and interneurons displayed sparse organelles, paler cytoplasm, reduced synaptic frequency and occasional neurodegeneration. We found hypertrophy of perineuronal, perivascular, and perisynaptic astrocytic processes, indicating glial response. To explore alterations in the inhibitory/excitatory afferents, we quantified the number of presynaptic terminals contacting the nXII motor neurons at P7 using SEM. Using postembedding immunogold or confocal imaging, we found a significant decrease in the GABA presynaptic content in nXII of LgDel synapses, supporting the notion of imbalanced neurotransmission levels that could directly affect the functionality of nXII and more generally the feeding and swallowing effector channels. **Support: NICHD: P01HD083157 and P50HD105328.**

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BOARD NUMBER: S04-732

MODULATION OF N-ACYLETHANOLAMINES – PEROXISOME PROLIFERATOR RECEPTOR TYPE GAMMA AXIS COUNTERACTS MEMORY DEFICITS OF PRENATAL AND LACTATION ALCOHOL EXPOSED MICE

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

Alba Garcia[1], Antoni Pastor[2], Rafael De La Torre[1,2], Olga Valverde[1,2]
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Aims: Fetal alcohol spectrum disorder is characterized by physical and behavioral disabilities induced by prenatal and lactation alcohol exposure (PLAE). Since the expanded endocannabinoid system (ECS) is altered by alcohol, we aimed to investigate the role of N-acylethanolamines (NAEs) – peroxisome proliferator activated receptor-gamma (PPARg) axis in PLAE-induced memory deficits. **Methods:** We used pregnant C57BL/6 female mice with time-limited access to either water or a 20% v/v alcohol solution as a model of alcohol binge drinking. First, we analyzed molecules of the expanded ECS in hippocampus and prefrontal cortex at post-partum day (PD) 25 and 70. To investigate the role of NAEs-PPARg axis in the PLAE-induced memory deficits, we performed two pharmacological approaches from PD25 to PD35: i) URB597 (FAAH inhibitor to increase NAE levels) co-administration with GW9662 (PPARg antagonist to block its activation); ii) pioglitatzone administration (PPARg agonist) to explore whether a direct PPARg activation is sufficient. After this, a battery of memory tests was performed at PD60. **Results:** We showed that PLAE induces general reduction of NAEs and PPARg in the hippocampus at PD25. Moreover, URB597 treatment restores memory deficits induced by PLAE through a PPARg-dependent mechanism, as shown when co-administered with GW9662. Finally, PPARg agonist is sufficient to improve the PLAE-induced memory impairments. **Conclusions:** Our findings suggest that either endogenous (by NAEs) or exogenous PPARg activation rescues memory deficits in PLAE mice. Therefore, NAEs-PPARg pathway is involved in said impairments, which indicates this system might be a potential target to counteract cognitive symptoms of PLAE mice.

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BOARD NUMBER: S04-733

CDKL5 SCULPTS FUNCTIONAL CALLOSAL CONNECTIVITY TO PROMOTE COGNITIVE FLEXIBILITY

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Functional connectivity deficits in short and long-range projections have been observed in numerous models of neurodevelopmental disorders. Interhemispheric callosal projection neurons (CPN) represent one of the major long-range projections in the brain and mediate higher-order cognitive function and flexibility. However, little is known about the contribution of CPN in neural network miswiring and behavioral deficits in neurodevelopmental disorders, like the rare CDKL5 Deficiency Disorder (CDD). CDD is caused by de novo mutations in the X-linked gene *CDKL5* and is characterized by early-onset seizures, developmental delays, and cognitive impairment. We explored here whether long-range connectivity of CPN is altered and whether it underlies cognitive deficits in CDD. We found an increase in the number of callosal synaptic inputs but decreased local synaptic connectivity in the cingulate cortex of juvenile CDKL5 KO mice. These deficits were associated with functional hyperconnectivity across higher cognitive areas and homotopic interhemispheric connections, and accompanied by cognitive impairments in adult KO mice. Likewise, selective deletion of CDKL5 in CPNs resulted in increased callosal connectivity, without significantly altering intracortical cingulate network. Notably, these callosal-specific changes were sufficient to recapitulate the cognitive defects observed in CDKL5 KO. Finally, when CDKL5 was selectively expressed only in CPNs, it was sufficient to prevent the cognitive impairments of CDKL5 mutants. Together, these results reveal a novel role of *CDKL5* by demonstrating that it is both necessary and sufficient for proper CPN connectivity and cognitive flexibility and highlights a causal relationship between CPN dysfunction and cognitive impairment in a model of neurodevelopmental disorder.

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BOARD NUMBER: S04-734

SPEECH SIGNAL ANALYSIS TO CLASSIFY BIPOLAR DISORDER SYMPTOMS IN ADHD PATIENTS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Aim Clinical diagnosis in psychiatric is primarily based on behavioral symptoms. A major research issue lies in the identification of biomarkers that could support clinicians' diagnosis. Speech signal processing has been found to be relevant when investigating mood disorders. In this study, we extract speech features from different Verbal Fluency Tests (VFT) conditions to classify comorbid Bipolar Disorder (BD) in adults suffering from Attention Deficit Hyperactivity Disorder (ADHD). **Methods** 57 adults with ADHD and 29 adults with ADHD+BD comorbidity were recruited. Subjects filled out several questionnaires on clinical symptoms before completing five conditions of the VFT. For each condition, speech features were extracted from audio recordings and investigated. Then, a Support Vector Machine – Recursive Features Elimination (SVM-RFE) was performed using a model trained on clinical labels and speech features. The final classification was obtained by combining the marginal classification outcomes from the different conditions using majority voting. Performance scores as accuracy and F1 score were estimated. **Results** Accuracy ranged from 0.72 to 0.89 across the five verbal fluency conditions. After applying the majority voting procedure, accuracy and F1 score increased to 0.94 and 0.95, respectively. Among the ADHD subjects, a correct classification was achieved in 52 out of 57 patients and the 29 subjects with ADHD+BD were correctly classified. The misclassification rates were correlated to the tendency to oscillate from anxiety to depression. **Conclusions** Results highlight that significant information is carried by speech features which could be a promising support for the clinical diagnosis of the ADHD+BD comorbidity.

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BOARD NUMBER: S04-735

THE LATENCY OF AUDITORY EVENT RELATED POTENTIAL P300 PROLONGED IN UNILATERAL HEARING LOSS SCHOOL AGE PUPILS IN MANDARIN LEARNING ENVIRONMENT

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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[1]National Taipei University Of Nursing And Health Sciences, Department Of Speech Language Pathology And Audiology, Taipei, Taiwan, [2]Shin Kong Wu-Ho-Su Memorial Hospital, Department Of Ophthalmology, Taipei, Taiwan, [3]Shin Kong Wu-Ho-Su Memorial Hospital, Department Of Otolaryngology, Taipei, Taiwan, [4]Artise Biomedical Co., Ltd, Artise Biomedical Co., Ltd, Hsinchu, Taiwan, [5]Donghu Elementary School, Donghu Elementary School, Taipei, Taiwan, [6]Taipei Medical University, The Ph.d. Program For Neural Regenerative Medicine, College Of Medical Science And Technology, Taipei, Taiwan

Our study investigated the differences in speech performance and neurophysiological response in groups of school-aged children with unilateral hearing loss (UHL) or typically developed (TD) characteristics. Total 16 primary school-aged children were recruited in our study (UHL=9/TD=7,diagnosed in Shin Kong Wu-Ho-Su Memorial Hospital). Word comprehension is tested by the Peabody Picture Vocabulary Test-Revised (PPVT-R), and the PPVT-R PR value is proportional to the auditory memory score (by The Children's Oral Comprehension Test) in both groups. Later we assessed the latency and amplitude of auditory ERP P300 and found that the latency of auditory ERP P300 in UHL group is prolonged compared with which in TD group. Although UHL pupils have one-side normal hearing, based on our results, long-term one-side hearing deprivation might be the origin of aberrant reorganization of brain areas for auditory or even visual perceptions attributed to speech delay and learning difficulties.
Key words: learning difficulties, unilateral hearing loss, ERP/P300

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-736

TRANSIENT DEVELOPMENTAL INCREASE IN CORTICAL PROJECTIONS TO AMYGDALA GABAERGIC NEURONS CONTRIBUTE TO CIRCUIT DYSFUNCTION FOLLOWING EARLY LIFE STRESS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Early life stress (ELS) results in enduring dysfunction of the cortico-limbic circuitry, underlying emotional and social behavior. However, the neurobiological mechanisms by which ELS affects development of the circuitry remain elusive. Here, we have combined viral tracing and electrophysiological techniques to study the effects of maternal separation (MS) on fronto-limbic connectivity and function in young (P14-21) rats. We report that aberrant prefrontal (mPFC) inputs to basolateral amygdala (BLA) GABAergic interneurons transiently increase the strength of feedforward inhibition in the BLA, which raises LTP induction threshold in MS treated male rats. The enhanced GABAergic activity after MS exposure associates with lower functional synchronization within prefrontal-amygdala networks *in vivo*. Intriguingly, no differences in these parameters were detected in females, which were also resistant to MS dependent changes in anxiety-like behaviors. Impaired plasticity and synchronization during the sensitive period of circuit refinement may contribute to long-lasting functional changes in the prefrontal-amygdaloid circuitry that predispose to neuropsychiatric conditions later on in life.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-737

INVESTIGATION OF THE MECHANISMS FOR NOVEL SHQ1 VARIANTS IN THE PATHOGENESIS OF BRAIN DEVELOPMENTAL DISORDER

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Aims: SHQ1 is an assembly factor of H/ACA ribonucleoproteins (H/ACA RNPs) which are involved in many critical biological pathways, including ribosome biogenesis, mRNA splicing and telomere maintenance. Mutations found in H/ACA RNPs components have been reported to cause dyskeratosis congenita (DKC), a genetic disorder characterized by bone marrow failure and poor telomere maintenance. Here, we identified novel SHQ1 compound heterozygous mutations from a patient with multiple neurological disorders, including developmental delay, movement disorders, epilepsy, and microcephaly. In this study, we aim to investigate the potential role of SHQ1 in brain development. **Methods:** To modulate Shq1 expression in developing brains, we introduced short-hairpin RNA (shRNA) into neural progenitors in the embryonic mouse cortex by utilizing *in utero* electroporation. The distributions and identities of electroporated cells were examined by immunostaining. To elucidate the pathogenic mechanism of SHQ1 mutations, we then performed co-immunoprecipitation to investigate the interaction between SHQ1 and DKC1 which is a core protein of H/ACA RNPs. **Results:** We found that decreased SHQ1 impaired neuronal migration but not neural differentiation during brain development. In addition, we found that most of SHQ1 variants attenuated their binding ability toward DKC1. These results implied SHQ1 mutations may influence brain development through disrupting the assembly and biogenesis of H/ACA RNPs. **Conclusions:** SHQ1 plays an essential role in brain development through regulating the behaviors of neural progenitors and their neuronal progeny. Our study gave a glimpse of the functions of SHQ1 in brain and provided a possible pathogenic mechanism for H/ACA RNPs-related disorders.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-738

MOLECULAR MECHANISM OF NOVEL FOXG1 VARIANTS IN CAUSING CORTICAL MALFORMATIONS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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FOXG1 is an important transcriptional repressor which binds to transcription factors, such as COUP-TF1, to regulate neuronal migration and cell fate during cortical development. Recently, we found four novel FOXG1 variants in patients with FOXG1 syndrome, which has been reported to cause microcephaly and epilepsy. However, the relationship between FOXG1 variants and clinical symptoms remains unclear. In this study, we aimed to elucidate how FOXG1 variants affect transcriptional regulation and lead to cortical malformation. To examine protein expression level of FOXG1 variants, we used western blotting to identify the changes of expression level. Luciferase assay was used to test transcriptional regulatory function of FOXG1 variants, we found that FOXG1 variants fail to repress COUP-TF1 expression through transcriptional abnormality. By using *in utero* electroporation (IUE), we delivered mutant FOXG1 to embryonic cortices to verify the FOXG1 dysfunction effects. We found some FOXG1 loss-of-function variants cause neuronal migration delay 3 days after IUE. In postnatal day 7, cells stayed in layer 4 instead of layer 2/3 and showed spiny stellate neuron morphology. FOXG1 variants also affected the connectivity of callosal projection in corpus callosum. These results were highly correlated with clinical symptoms of FOXG1 syndrome. Our study suggests that disease causing FOXG1 variants not only affect transcriptional regulation but also cause neuronal migration defects and cell fate change during corticogenesis. The correlation between *in vitro* and *in vivo* phenotypes and clinical symptoms may provide a reference for FOXG1 variants in genetic diagnosis.
Keywords: FOXG1, COUP-TF1, FOXG1 Syndrome, Cortical Development, Transcriptional Regulation

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S04-739

A SYSTEMATIC REVIEW ON THE ASSOCIATION OF BIRTH INTERVALS AND RISK OF AUTISM SPECTRUM AND ATTENTION DEFICIT HYPERACTIVITY DISORDERS

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Background This systematic review aimed to assess the relationship of short and long inter-pregnancy intervals (IPIs) with the risk of autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). **Methods** We performed a systematic search on electronic databases including Pubmed, Web of Science, Scopus, and Embase. We included observational studies that evaluated the association between IPIs and the risk of ASD and ADHD. Two reviewers independently screened and then extracted data on study characteristics, IPIs/ birth intervals, and outcome measures. The methodological quality of the included studies was evaluated following the Joanna Briggs Institute (JBI) critical appraisal checklist. **Results** At the final step, 19 out of 161 studies were included in our systematic review. Among them, 16 and 5 studies assessed the association between IPI and the risk of ASD and ADHD, respectively. In 9 studies, findings supported the association between short intervals and an increased risk of ASD. In addition, 7 studies reported significant association between both short and long intervals and an increased risk of ASD. Moreover, 3 studies demonstrated an association between short intervals and ADHD risk, while long birth interval was merely assessed in 2 studies with conflicting results. **Conclusion** This systematic review strongly confirmed the association of short and long birth intervals with ASD and ADHD. Future studies should investigate the mechanisms underlying these associations and the possible modifiers to decrease the risk of such disorders.

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BOARD NUMBER: S04-740

EVALUATING THE ACCURACY OF TABLET-BASED DIGIT-TRACKING FOR POTENTIAL APPLICATION IN NDD DISORDERS IDENTIFICATION

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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Aims: Neurodevelopmental disorders (NDD) such as attention deficit hyperactivity (ADHD), or social communication disorders, are commonly studied in childhood, that have complex clinical phenotypes. Strong overlaps across NDD phenotypes make both group and distinction of each disorder difficult. Recently, digital phenotyping is a new approach aimed at measuring human behavior. Despite promising, this method has been mostly for NDD assessment at lab scale. Digit-tracking, among digital phenotyping, gives a quantitative and qualitative measure of longitudinal and time-dependent behaviour, allowing to gather metrics for curve-matching, acceleration, and continuous dynamic time wrapping (DTW). However, this method has never been applied for NDD classification. In this paper, we explore the feasibility of an ecological processing of digit-tracking data to further give insight into the NDD stratification. Methods: We investigate how digit-tracking combined with clinical phenotypes constitutes a new method for NDD profiling in children aged 7 to 12 years. A total of 50 children performed a task on a touch-screen device. We extracted specific features related to the digit-tracking data and are investigating predicting variables to improve classification of NDD. Results: Our preliminary results strongly suggest that the defined metrics are relevant predictive variables to improve NDD clustering. Conclusions: Digit-tracking is an ecological measure, easily available for digital screening of NDD in large paediatric population. Combined with clinical data, this approach may enhance the capability and sensitivity in early identification and diagnosis of NDD in real-life practice.

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BOARD NUMBER: S04-740a

EPIGENETIC AND TRANSCRIPTOMIC LANDSCAPES SHOW MODIFICATIONS IN POST-MORTEM CEREBRAL CORTEX OF CDKL5 DEFICIENCY DISORDER PATIENTS AND ARE ASSOCIATED WITH BOTH SYNAPTIC AND MYELOARCHITECTURAL DISORGANIZATION

POSTER SESSION 04 - SECTION: NEURODEVELOPMENTAL DISORDERS

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CDKL5 deficiency disorder (CDD) is a rare X-linked neurodevelopmental disease, characterized by drug-resistant epilepsy, autistic-like traits, sensorimotor and visual abnormalities. Currently, very little is known on the neurobiological alterations underlying the disease in humans thus hampering the identification of mechanisms potentially relevant for therapiesCDKL5 is highly expressed in forebrain glutamatergic neurons and can shuttle between the nucleus and the cytoplasm. Data obtained from CDD mouse models revealed that CDKL5 loss primarily leads to morphofunctional synaptic defects. Whether synaptic and/or gene expression impairments are present in CDD patient's brain cells is still unknown. In this study by employing a multidisciplinary approach on post-mortem BA17 cortical samples, we investigated the molecular organization of excitatory synapses in human brains carrying CDKL5 mutations and their role in the regulation of both epigenetic and transcriptional processes. First, we found by using immunofluorescence and western blotting that excitatory postsynaptic compartments in humans share defects with CDKL5 KO mice. By RRBS techniques, we found that CDD brains displayed altered DNA methylation. Intriguingly, RNA-seq analysis revealed that epigenetic alterations were paralleled with changes in genes involved in the formation and/or maintenance of myelin. Finally, we validated by immunohistochemistry the presence of defects in the myeloarchitecture in CDD patients. Our study discloses that both synaptic defects and abnormal myeloarchitecture are a key endophenotype of human CDD.

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Poster Session 05

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-001

ACTIVITY DYNAMICS OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONS DURING VIRTUAL NAVIGATION IN MICE

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The hippocampus plays a critical role in spatial and episodic memory by creating and storing unique representations of visited environments as activity matrices of principal neurons tuned to specific locations. However, the generation, consolidation and use of hippocampal spatial representations during learning and navigating in environments under variable cognitive demands is incompletely understood. To examine the development and flexible reorganization of place coding activity by hippocampal CA1 pyramidal cells (CA1PCs), we implemented a virtual spatial navigation paradigm for head-fixed mice allowing two-photon imaging of Ca2+ activity in CA1PCs. Transgenic Thy1-GCaMP6s mice were implanted with a hippocampal imaging cannula and a metal head plate for head fixation. Water-restricted mice were trained to collect water rewards at specific locations in two visually different virtual environments. We recorded behavioral parameters (speed, licks) and imaged GCaMP6s-mediated Ca2+ signals in hundreds of CA1PCs over consecutive days, including during 1) initial learning, 2) switches between randomly varied or continuous blocks of presented environments, and 3) exposure to a novel environment. Mice were able to efficiently differentiate between the two distinct virtual corridors and learn the location of the reward zone in both, whereas the behavior pattern was disrupted in a novel environment. We observed reliable place cell activity, gradually developing selective representations of familiar environments, global remapping in the new environment, and flexible reward-associated enrichment depending on cognitive demand. Our results indicate refinement of CA1 coding dynamics during learning and in response to subtle and robust changes in the environment and task structure.

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BOARD NUMBER: S05-002

ENTORHINAL GRID-LIKE CODES AND TIME-LOCKED NETWORK DYNAMICS TRACK OTHERS NAVIGATING THROUGH SPACE

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Navigating through crowded, dynamically changing social environments requires the ability to keep track of other individuals. Grid cells in the entorhinal cortex are a central component of self-related navigation but whether they also track others' movement is unclear. Here, we propose that entorhinal grid-like codes make an essential contribution to socio-spatial navigation. Sixty human participants underwent functional magnetic resonance imaging (fMRI) while observing and re-tracing different paths of a demonstrator that navigated a virtual reality environment. Results revealed that grid-like codes in the entorhinal cortex tracked the other individual navigating through space. Further, the activity of grid-like codes was time-locked to increases in co-activation and entorhinal-cortical connectivity that included the striatum, the hippocampus, parahippocampal and right posterior parietal cortices, altogether modulated by accuracy when subsequently re-tracing the paths. This suggests that network dynamics time-locked to entorhinal grid-cell-related activity might serve to distribute information about the 'socio-spatial map' throughout the brain.

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BOARD NUMBER: S05-003

ANIMAL-TO-ANIMAL VARIABILITY IN PARTIAL HIPPOCAMPAL REMAPPING

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Hippocampal place cells form a map of an animal's environment. The hippocampal map changes in response to changes in the environment, a phenomenon known as remapping. Different animals can have different remapping responses to the same environments. This variability across animals in remapping behavior is not well understood. In this work, we analyzed electrophysiological recordings from the CA3 region of the hippocampus performed by Alme et al. (2014), in which five male rats were exposed to 11 different environments, including a variety of repetitions of those environments. To compare the hippocampal maps between two experiences, we computed average rate map correlation coefficients. We found changes in the hippocampal maps between different sessions in the same environment. These changes consisted of partial remapping: a form of remapping in which some place cells maintain their place fields while other place cells remap their place fields. Each animal exhibited partial remapping differently. We discovered that the heterogeneity in animals' hippocampal representational changes is structured: individual animals had consistently different levels of partial remapping across a range of independent comparisons. Our findings highlight that partial hippocampal remapping between repeated environments depends on animal-specific factors.

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BOARD NUMBER: S05-004

EPH-EPHRIN SIGNALING-DEPENDENT ANATOMICAL AND FUNCTIONAL MODULES IN LAYER II OF MEDIAL ENTORHINAL CORTEX

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The layer II of medial entorhinal cortex (MECII) has hexagonally arranged cell clusters of Wolfram syndrome1 (Wfs1)+ pyramidal cells surrounded by Reelin+ stellate cells. Accumulating evidence suggests that cells coding similar spatial information tend to be clustered in MECII, implicating that the Wfs1+ cell clusters may generate the functional modules which contributed to the processing of spatial information. However, it remains unknown the molecular basis for the formation of anatomical cell clusters and whether the anatomical modules affect functional modules. Here, we hypothesized that the cell clusters may be generated by cell-cell-mediated interaction/repulsion. We first comprehensively examined expression patterns of EphBs/ephrin-Bs genes, which are well known as initiators of cell-contact-mediated repulsion signals by fluorescent in situ hybridization. We found that EphB1 was expressed in Reelin+ cells, while ephrin-B2 was expressed in Wfs1+ cells. Loss-of-function studies using knockout (KO) animals revealed that the cell clusters were scattering in the ephrin-B2 hetero knockout (EB2) mice. These results suggest that the EphB1/ephrin-B2 signaling are critical for formation of cell clusters in the MECII. Next, to examine whether anatomical arrangement of cells coding similar spatial information in MECII was impaired in the EB2 mutant, we conducted *in vivo* Ca2+ imaging of MECII in free-moving mouse. The hexagonally arranged periodic firing fields of grid cells was impaired in EB2 mutant, and the grid phase cluster was significantly obscured. These results supported the hypothesis that the anatomical structure in MECII may provide geometrical platform for regulation of grid activities.

Pubmed:

21844201: Tomikawa J, Shimokawa H, Uesaka M, Yamamoto N, Mori Y, Tsukamura H, Maeda K, Imamura T
Single-stranded noncoding RNAs mediate local epigenetic alterations at gene promoters in rat cell lines.
A growing number of noncoding RNAs (ncRNAs) are thought to be involved in sequence-specific alterations of epigenetic processes, mostly causing gene repression. In this study, promoter-associated ncRNAs (pancRNAs >200 nucleotides in size) that were endogenously generated from the sense strand at Map2b, antisense strand at Nefl, and both strands at Vim were investigated regarding their epigenetic potential as positive or negative regulators in rat pheochromocytoma (PC12) and fibroblast (normal rat kidney) cell lines. The respective antisense pancRNAs were associated with several active chromatin marks at the Nefl and Vim promoters. Forced expression of fragments expressing the antisense pancRNAs caused sequence-specific DNA demethylation, whereas a decrease of expression induced methylation of the same sequences. In contrast, perturbing the expression of the two sense pancRNAs did not change the DNA methylation status. These results suggest that a fraction of naturally occurring ncRNAs acts in cis as a single-stranded form and that the transcriptional orientation of pancRNA is important for the establishment of sequence-specific epigenetic modifications consistent with open chromatin structure.
J Biol Chem, 2011; 286
26945044: Yamamoto N, Agata K, Nakashima K, Imamura T
Bidirectional promoters link cAMP signaling with irreversible differentiation through promoter-associated non-coding RNA (pancRNA) expression in PC12 cells.
Bidirectional promoters are the major source of gene activation-associated noncoding RNA (ncRNA). PC12 cells offer an interesting model for understanding the mechanism underlying bidirectional promoter-mediated cell cycle control. Nerve growth factor (NGF)-stimulated PC12 cells elongate neurites, and are in a reversible cell-cycle-arrested state. In contrast, these cells irreversibly differentiate and cannot re-enter the normal cell cycle after NGF plus cAMP treatment. In this study, using directional RNA-seq, we found that bidirectional promoters for protein-coding genes with promoter-associated ncRNA (pancRNA) were enriched for cAMP response element consensus sequences, and were preferred targets for transcriptional regulation by the transcription factors in the cAMP-dependent pathway. A spindle-formation-associated gene, Nusap1 and

pancNusap1 were among the most strictly co-transcribed pancRNA-mRNA pairs. This pancRNA-mRNA pair was specifically repressed in irreversibly differentiated PC12 cells. Knockdown (KD) and overexpression experiments showed that pancNusap1 positively regulated the Nusap1 expression in a sequence-specific manner, which was accompanied by histone acetylation at the Nusap1 promoter. Furthermore, pancNusap1 KD recapitulated the effects of cAMP on cell cycle arrest. Thus, we conclude that pancRNA-mediated histone acetylation contributes to the establishment of the cAMP-induced transcription state of the Nusap1 locus and contributes to the irreversible cell cycle exit for terminal differentiation of PC12 cells.
Nucleic Acids Res, 2016; 44
32277786: Marks WD, Yamamoto N, Kitamura T
Complementary roles of differential medial entorhinal cortex inputs to the hippocampus for the formation and integration of temporal and contextual memory (Systems Neuroscience).
In humans and rodents, the entorhinal cortical (EC)-hippocampal (HPC) circuit is crucial for the formation and recall of memory, preserving both spatial information and temporal information about the occurrence of past events. Both modeling and experimental studies have revealed circuits within this network that play crucial roles in encoding space and context. However, our understanding about the time-related aspects of memory is just beginning to be understood. In this review, we first describe updates regarding recent anatomical discoveries for the EC-HPC network, as several important neural circuits critical for memory formation have been discovered by newly developed neural tracing technologies. Second, we examine the complementary roles of multiple medial entorhinal cortical inputs, including newly discovered circuits, into the hippocampus for the temporal and spatial aspects of memory. Finally, we will discuss how temporal and contextual memory information is integrated in HPC cornu ammonis 1 cells. We provide new insights into the neural circuit mechanisms for anatomical and functional segregation and integration of the temporal and spatial aspects of memory encoding in the EC-HPC networks.
Eur J Neurosci, 2021; 54
33398831: Yamamoto N, Marks WD, Kitamura T
Cell-Type-Specific Optogenetic Techniques Reveal Neural Circuits Crucial for Episodic Memories.
The formation and maintenance of episodic memories are important for our daily life. Accumulating evidence from extensive studies with pharmacological, electrophysiological, and molecular biological approaches has shown that both entorhinal cortex (EC) and hippocampus (HPC) are crucial for the formation and recall of episodic memory. However, to further understand the neural mechanisms of episodic memory processes in the EC-HPC network, cell-type-specific manipulation of neural activity with high temporal resolution during memory process has become necessary. Recently, the technological innovation of optogenetics combined with pharmacological, molecular biological, and electrophysiological approaches has significantly advanced our understanding of the circuit mechanisms for learning and memory. Optogenetic techniques with transgenic mice and/or viral vectors enable us to manipulate the neural activity of specific cell populations as well as specific neural projections with millisecond-scale temporal control during animal behavior. Integrating optogenetics with drug-regulatable activity-dependent gene expression systems has identified memory engram cells, which are a subpopulation of cells that encode a specific episode. Finally, millisecond pulse stimulation of neural activity by optogenetics has further achieved (a) identification of synaptic connectivity between targeted pairs of neural populations, (b) cell-type-specific single-unit electrophysiological recordings, and (c) artificial induction and modification of synaptic plasticity in targeted synapses. In this chapter, we summarize technological and conceptual advancements in the field of neurobiology of learning and memory as revealed by optogenetic approaches in the rodent EC-HPC network for episodic memories.
Adv Exp Med Biol, 2021; 1293
34400533: Yokose J, Marks WD, Yamamoto N, Ogawa SK, Kitamura T
Entorhinal cortical Island cells regulate temporal association learning with long trace period.
Temporal association learning (TAL) allows for the linkage of distinct, nonsynchronous events across a period of time. This function is driven by neural interactions in the entorhinal cortical-hippocampal network, especially the neural input from the pyramidal cells in layer III of medial entorhinal cortex (MECIII) to hippocampal CA1 is crucial for TAL. Successful TAL depends on the strength of event stimuli and the duration of the temporal gap between events. Whereas it has been demonstrated that the neural input from pyramidal cells in layer II of MEC, referred to as Island cells, to inhibitory neurons in dorsal hippocampal CA1 controls TAL when the strength of event stimuli is weak, it remains unknown whether Island cells regulate TAL with long trace periods as well. To understand the role of Island cells in regulating the duration of the learnable trace period in TAL, we used Pavlovian trace fear conditioning (TFC) with a 60-sec long trace period (long trace fear conditioning [L-TFC]) coupled with optogenetic and chemogenetic neural activity manipulations as well as cell type-specific neural ablation. We found that ablation of Island cells in MECII partially increases L-TFC performance. Chemogenetic manipulation of Island cells causes differential effectiveness in Island cell activity and leads to a circuit imbalance that disrupts L-TFC. However, optogenetic terminal inhibition of Island cell input to dorsal hippocampal CA1 during the temporal association period allows for long trace intervals to be learned in TFC. These results demonstrate that Island cells have a

critical role in regulating the duration of time bridgeable between associated events in TAL.
Learn Mem, 2021; 28
33536392: Ideta-Otsuka M, Miyai M, Yamamoto N, Tsuchimoto A, Tamura H, Tanemura K, Shibutani M, Igarashi K
Development of a new in vitro assay system for evaluating the effects of chemicals on DNA methylation.
Epigenetic toxicity, a phenomenon in which chemicals exert epigenetic effects and produce toxicity, has been attracting attention in recent years due to advances in toxicology accompanying the development of life sciences. However, it has been difficult to identify epigenetic toxicants due to the lack of a simple experimental system to evaluate epigenetic toxicity. In this study, we developed a prototype of an in vitro reporter assay system for assessing the effects of chemicals on DNA methylation using two promoters showing different degrees of DNA methylation, Agouti IAP and Daz1 promoters, and a luciferase reporter. The system successfully detected DNA demethylating activity using 5-azacytidine, a chemical having DNA demethylation activity, as a positive control chemical, and demethylation of cytosine of CpG in the promoter was confirmed by pyrosequencing analysis. Next, in order to improve the detection sensitivity of the DNA demethylating activity of this system, we tried to increase the basal level of methylation of the Daz1 promoter by pre-methylase treatment of the reporter vectors. As a result, the detection sensitivity of the system was successfully improved in cells where the basal level of methylation was indeed increased by methylase treatment. Thus, the developed assay system here is effective for the simple evaluation of chemicals that affect DNA methylation.
J Toxicol Sci, 2021; 46
32606367: Kim R, Yamamoto N, Kitamura T
Extra neural ensemble disrupts memory recall.
Nat Neurosci, 2020; 23

FENS Forum 2022
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BOARD NUMBER: S05-005

A PLACE WITH A VIEW: PARIETAL AND HIPPOCAMPAL NEURONAL ACTIVITIES DURING VIRTUAL NAVIGATION IN THE MACAQUE

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Knowing where one is and how to reach a new goal from there requires complex computational processes, which are not yet fully understood. To examine the role of the ventral and lateral intraparietal areas in spatial navigation, we recorded neuronal activity in rhesus monkeys performing an orientation task based on salient visual cues, in a virtual environment. We hypothesize that parietal cortex should play a role, along with the hippocampus, in the processing of the visual cues of the environment to support self-position. Using unsupervised clustering analysis, we showed that position-related activity grouped parietal neurons into clusters representing specific maze locations more strongly than hippocampal neurons. Next, we showed that saccade-related parietal activity was modulated by the maze segment in which these saccades were made more strongly than in the hippocampus. This suggests that task-relevant variables influence the amplitude of saccade-related responses, consistent with a link between oculomotor control and the salience of visual cues. Finally, we showed that parietal cells divided into cells that respond strongly to direct fixation of landmarks, or when landmarks are at the periphery of the visual field. Those two populations displayed different temporal dynamics, taking part respectively in the acquisition of stimuli, and in their anticipation. Overall, the results underline a strong recruitment of parietal cortex during active navigation, upstream the hippocampus, which allows tracking of the animal's position into his environment through a task-based processing of the visual cues, hence shedding light on the neural processes linking place and view.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-006

AN OPEN-SOURCE DEVICE FOR OPEN- AND CLOSED-LOOP VESTIBULAR STIMULATION IN HEAD-FIXED MICE

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Visual virtual reality (VR) is widely used to study cortical processing in awake, behaving mice. It allows for tight control of animal-driven visual stimuli and provides the ability to change the coupling between behaviour and visual stimulus. However, most visual VR approaches render animals motionless in space (i.e., head-fixed), resulting in the vestibular system being taken out of play. Consequently, the head-direction (HD) system, which is primarily driven by vestibular input and plays a pivotal role in navigation, is severely compromised. Here we present a novel experimental apparatus to overcome this limitation. Using an open-source approach, we have built a modular and affordable device allowing the rotation of head-fixed, behaving mice. It can be used in open-loop mode to study vestibular sensory representation and processing. In closed-loop mode, the apparatus allows animals to navigate in rotational space and self-generate vestibular input, providing a better substrate for 2D navigation in virtual environments. We show that our approach is compatible with the electrical recording of brain activity at the cellular level and results in the robust recruitment of HD cells. We further demonstrate its utility by combining the recording of vestibular and visual evoked eye-movements with optogenetic interference of specific neuronal populations.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-007

NOVEL VIRTUAL REALITY-ENABLED PATH INTEGRATION TASK FOR FREELY MOVING RATS.

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Spatial navigation of rodents is supported by a flexible combination of multisensory-based allothetic representation of space and self-referenced path integration. How the two navigational strategies relate to neural representation of space remains poorly understood, in part due to a difficulty in dissociating which strategy is used by the freely-moving behaving animal. In this study we designed a path integration task that relies on a 3D virtual reality beacon to mark an arbitrary homing location in the arena. Rearing behavior serves as a readout of both initiation of outbound foraging and path integration-dependent completion of the inbound trajectory in the darkness. Randomized computer-controlled variation over blocks of trials of the homing location makes the task majorly reliant on path integration. We combine the task with perturbation of the idiothetic sensory inputs via vestibular and optic flow manipulations to understand their respective roles in path integration. We quantify animal performance on the task and effect of perturbation with measures characterizing their trajectories and rearing patterns. In order to understand how spatial representation is related to behavioral strategy of navigation we use wireless silicon probe recordings in the rats performing the task and numerous controls. We quantify the effect of the task and perturbations on spatial coding of the populations of hippocampal place cells.

Pubmed:
31969469: Crego ACG, Štoček F, Marchuk AG, Carmichael JE, van der Meer MAA, Smith KS
Complementary Control over Habits and Behavioral Vigor by Phasic Activity in the Dorsolateral Striatum.
Despite clear evidence linking the basal ganglia to the control of outcome insensitivity (i.e., habit) and behavioral vigor (i.e., its behavioral speed/fluidity), it remains unclear whether or how these functions relate to one another. Here, using male Long-Evans rats in response-based and cue-based maze-running tasks, we demonstrate that phasic dorsolateral striatum (DLS) activity occurring at the onset of a learned behavior regulates how vigorous and habitual it is. In a response-based task, brief optogenetic excitation at the onset of runs decreased run duration and the occurrence of deliberative behaviors, whereas midrun stimulation carried little effect. Outcome devaluation showed these runs to be habitual. DLS inhibition at run start did not produce robust effects on behavior until after outcome devaluation. At that time, when the DLS was plausibly most critically required for performance (i.e., habitual), inhibition reduced performance vigor measures and caused a dramatic loss of habitual responding (i.e., animals quit the task). In a second cue-based "beacon" task requiring behavior initiation at the start of the run and again in the middle of the run, DLS excitation at both time points could improve the vigor of runs. Postdevaluation testing showed behavior on the beacon task to be habitual as well. This pattern of results suggests that one role for phasic DLS activity at behavior initiation is to promote the execution of the behavior in a vigorous and habitual fashion by a diverse set of measures. Our research expands the literature twofold. First, we find that features of a habitual behavior that are typically studied separately (i.e., maze response performance, deliberation movements, running vigor, and outcome insensitivity) are quite closely linked together. Second, efforts have been made to understand "what" the dorsolateral striatum (DLS) does for habitual behavior, and our research provides a key set of results showing "when" it is important (i.e., at behavior initiation). By showing such dramatic control over habits by DLS activity in a phasic time window, plausible real-world applications could involve more informed DLS perturbations to curb intractably problematic habits.
J Neurosci, 2020; 40

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BOARD NUMBER: S05-008

LONG-TERM STABLE SPATIAL REPRESENTATION IN DENTATE GRANULE CELLS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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After long periods, we remain able to recall personal experiences and places associated with long-term memories but the identity of long-term storage sites is controversially discussed. Despite the currently prevailing theory that episodic memories are reallocated to cortical areas after an initial encoding phase within the hippocampus, lesion studies suggest that memories might be partially retained within the hippocampus. Recordings of hippocampal activity, however, ruled out stable long-term storage of spatial information in CA1-3. We therefore tested the hypothesis that sparsely active and spatially tuned DG granule cells (GCs) efficiently store and retrieve episodic memories over extended live spans. We employed two-photon *in vivo* calcium imaging of GCs in mice navigating through virtual environments to investigate the formation, discrimination and recall of spatial memories. We show that formation and stabilization of spatial maps representing novel environments emerge in the DG over the course of 4-5 subsequent days. After map formation, representations remain remarkably stable, with individual GCs even maintaining their place field characteristics for up to 90 days. In marked contrast, CA1 place maps emerge on the first day of exposure but map stability rapidly declines over days. Moreover, artificial increase in GCs excitability using depolarizing DREADDs created novel place cells but their reactivation on subsequent days did not result in the stabilization of their place fields or enlargement of map representations. Thus, GC place cells form long-lasting, highly stable and context-specific ensembles. We propose that this ability is an important requirement for long-term storage of spatial and contextual memories.

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BOARD NUMBER: S05-009

INHIBITORY TUNING IN THE CORTICAL HEAD-DIRECTION SYSTEM REFLECTS THE FOURIER COMPONENTS OF LOCALLY ENCODED FEATURES

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The activity of cortical neurons in sensory and spatial systems results from balanced excitation and inhibition. While excitatory neurons are often fine-tuned to the encoded features, the tuning of inhibitory neurons is not well characterized. We thus aimed to elucidate the principles of cortical inhibitory tuning using a simple neural system encoding a 1-dimensional variable - the head-direction (HD) system. To this end, we recorded neurons in the postsubiculum (PoSub), the primary cortical hub of the HD system. In contrast to canonical, excitatory HD cells, HD tuning curves of fast-spiking interneurons (PoSub-FS cells) were not confined to discrete HD values. However, they were stable across time and were anchored to distal landmarks. Furthermore, we show that when HD tuning curves are decomposed in Fourier space, the average Fourier spectrum of PoSub-FS cell tuning matches that of local PoSub-HD cells. Additionally, while individual HD cells are, by definition, fine-tuned in the HD feature space and thus homogenous in their Fourier spectra, FS cells are narrowly-tuned in Fourier space, resulting in their HD tuning often showing 1-, 2- and even 3-fold radial symmetries. Finally, to determine the origin of PoSub-FS tuning, we optogenetically modulated the gain of the thalamic input to PoSub. This modulation had an exclusively additive effect on PoSub-FS cell tuning, indicating its independence from the thalamic input. These findings suggest that collectively the tuning of PoSub-FS cells is a Fourier transformation of the signal encoded by local excitatory cells. They thus provide new constraints on biologically plausible computational models involving inhibition.

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BOARD NUMBER: S05-010

ROLE OF THE RETROSPLENIAL CORTEX IN VISUALLY-GUIDED NAVIGATION

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Internal representations of self-location in an environment are constantly refined by sensory information. The goal of this study is to dissect the neuronal pathways for updating spatial maps based on visual and tactile cues. We focus on the retrosplenial cortex (RSC), a structure reciprocally connected to both sensory and mnemonic areas throughout the brain. We hypothesize that the RSC-mediated circuits might play different roles in sensory-guided navigation and related learning process. In this study, mice were head-restrained and ran on a linear treadmill with tactile cues. Each recording session consists of three environmental conditions (light, open-loop visual stimulation and darkness, respectively). By combining 2-photon cellular imaging and genetic circuit mapping, we characterized the multimodal representations of RSC neurons and their role in learning. Our results demonstrated that different functional representations of RSC neurons are topographically distributed along the rostral-caudal axis. Cells in the anterior RSC predominantly encoded spatial information, whereas the posterior RSC showed a larger fraction of visually-responsive neurons. Notably, RSC-place cells maintained their firing fields in darkness and were modulated by somatosensory inputs. RSC place cells were initially strongly driven by tactile cues, but became less cue-driven with experiences. Finally, we probed the activity of neurons in teh RSC that project to dorsal subiculum (dS), suggesting that dS receives a mixture of spatial and visual inputs from the RSC. Together, these findings provide insights into diverse representations of RSC, that might serve an integrative role in learning and memory formation.

Pubmed:
33484471: Wei YT, Wu JW, Yeh CW, Shen HC, Wu KP, Vida I, Lien CC
Morpho-physiological properties and connectivity of vasoactive intestinal polypeptide-expressing interneurons in the mouse hippocampal dentate gyrus.
The hippocampus is a key brain structure for cognitive and emotional functions. Among the hippocampal subregions, the dentate gyrus (DG) is the first station that receives multimodal sensory information from the cortex. Local-circuit inhibitory GABAergic interneurons (INs) regulate the excitation-inhibition balance in the DG principal neurons (PNs) and therefore are critical for information processing. Similar to PNs, GABAergic INs also receive distinct inhibitory inputs. Among various classes of INs, vasoactive intestinal polypeptide-expressing (VIP) INs preferentially target other INs in several brain regions and thereby directly modulate the GABAergic system. However, the morpho-physiological characteristics and postsynaptic targets of VIP INs in the DG are poorly understood. Here, we report that VIP INs in the mouse DG are highly heterogeneous based on their morpho-physiological characteristics. In approximately two-thirds of morphologically reconstructed cells, their axons ramify in the hilus. The remaining cells project their axons exclusively to the molecular layer (15%), to both the molecular layer and hilus (10%), or throughout the entire DG layers (8%). Generally, VIP INs display variable intrinsic properties and discharge patterns without clear correlation with their morphologies. Finally, VIP INs are recruited with a long latency in response to theta-band cortical inputs and preferentially innervate GABAergic INs over glutamatergic PNs. In summary, VIP INs in the DG are composed of highly diverse subpopulations and control the DG output via disinhibition.
J Comp Neurol, 2021; 529
29311646: Chang JH, Tsai PH, Wang KY, Wei YT, Chiou SH, Mou CY
Generation of Functional Dopaminergic Neurons from Reprogramming Fibroblasts by Nonviral-based Mesoporous Silica Nanoparticles.
Direct-lineage conversion of the somatic cell by reprogramming, in which mature cells were fully converted into a variety of other cell types bypassing an intermediate pluripotent state, is a promising regenerative medicine approach. Due to the risk of tumorigenesis by viral methods, a non-viral carrier for the delivery of reprogramming factors is very desirable. This study utilized the mesoporous silica nanoparticles (MSNs) as a non-viral delivery system for transduction of the three key factors to

achieve conversion of mouse fibroblasts (MFs) into functional dopaminergic neuron-like cells (denoted as fDA-neurons). At the same time, a neurogenesis inducer, ISX-9, was co-delivered with the MSNs to promote the direct conversion of neuron-like cells. Good transfection efficiency of plasmid@MSN allowed repeated dosing to maintain high exogenous gene expression analyzed by qPCR and the changes in neural function markers were monitored. To further validate the dopaminergic function and the electrophysiological properties of fDA-neurons, the results of ELISA assay showed the high levels of secreted-dopamine in the conditional medium and rich Na/K-channels were observed in the fDA-neurons on Day 22. The results demonstrated that MSN nanocarrier is effective in delivering the reprogramming factors for the conversion of functional dopaminergic neurons from adult somatic cells.
Sci Rep, 2018; 8

27830729: Lee CT, Kao MH, Hou WH, Wei YT, Chen CL, Lien CC

Causal Evidence for the Role of Specific GABAergic Interneuron Types in Entorhinal Recruitment of Dentate Granule Cells. The dentate gyrus (DG) is the primary gate of the hippocampus and controls information flow from the cortex to the hippocampus proper. To maintain normal function, granule cells (GCs), the principal neurons in the DG, receive fine-tuned inhibition from local-circuit GABAergic inhibitory interneurons (INs). Abnormalities of GABAergic circuits in the DG are associated with several brain disorders, including epilepsy, autism, schizophrenia, and Alzheimer disease. Therefore, understanding the network mechanisms of inhibitory control of GCs is of functional and pathophysiological importance. GABAergic inhibitory INs are heterogeneous, but it is unclear how individual subtypes contribute to GC activity. Using cell-type-specific optogenetic perturbation, we investigated whether and how two major IN populations defined by parvalbumin (PV) and somatostatin (SST) expression, regulate GC input transformations. We showed that PV-expressing (PV+) INs, and not SST-expressing (SST+) INs, primarily suppress GC responses to single cortical stimulation. In addition, these two IN classes differentially regulate GC responses to θ and γ frequency inputs from the cortex. Notably, PV+ INs specifically control the onset of the spike series, whereas SST+ INs preferentially regulate the later spikes in the series. Together, PV+ and SST+ GABAergic INs engage differentially in GC input-output transformations in response to various activity patterns.
Sci Rep, 2016; 6

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BOARD NUMBER: S05-011

EVOLUTION OF HIPPOCAMPAL PLACE CELL REPRESENTATIONS IN SLOWLY MORPHING ENVIRONMENTS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The hippocampus forms spatially modulated cell activity patterns that are considered physiological substrates of spatial memories. Like memories, these behave in an attractor-like manner and are reactivated instantaneously in response to corresponding sensory cues. Here, we studied the dynamics of hippocampal network state reactivation during real-time morphing between familiar environments. Rats implanted with tetrodes in CA3 were trained to maintain separate representations for two different light-cue conditions in a square arena. After baseline recording with stable cues, lights for one condition were slowly faded out, while those for the other were faded in over 2 minutes. Data was binned according to local theta and population vectors and specificity indices were calculated based on templates for each stable session. All animals were capable of map switching in an instant teleportation paradigm and showed clear remapping between stable conditions. Morphing between conditions surprisingly resulted in global switching of hippocampal representations only in some rats, but not others. Non-switchers showed predominant activation of the representation matching cue-conditions at the start of morphing and maintained this throughout. A slight increase in cycles showing high correlation with both templates occurred, unrelated to the degree of cue-mixing. Switchers, by contrast, showed a proportional increase resp. decrease in cue-consistent cycles during morphing, resulting in a period with many of repetitive transitions between representations starting at 30-60% of the morphing duration. Our results indicate that CA3 can sustain active spatial representations that contradict environmental cues for a prolonged time, as long as these cues are introduced very slowly.

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BOARD NUMBER: S05-012

NEURONAL REPLAY SEQUENCES IN THE HIPPOCAMPUS OF BATS IN A VERY LARGE ENVIRONMENT (200 METERS)

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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In sleeping or immobile rodents, ensembles of hippocampal place-cells exhibit sequential reactivations of previously-experienced trajectories (termed replay). Such neuronal sequences are thought to be important for memory consolidation. Recently, we found a multifield musltiscale representation in the hippocampus of bats flying in a 200-meter long tunnel, whereby individual place-cells exhibited multiple place-fields with very different sizes (Eliav et al., Science 2021). This surprising finding opened intriguing questions regarding replay in very-large naturalistic environments: Are there ultra-long sequences in bats? Do individual replay-sequences cover the entire environment, or just small parts of it during each sequence? Are the sequences being time-compressed, as in rodents? How is the multifield-multiscale representation manifested in such replay-sequences? To investigate this, we applied a clusterless state-space model to decode sequential reactivations when bats were stationary: during rest-times between flight epochs in the 200-meter long tunnel, and during subsequent sleep. Preliminary analysis revealed time-compressed replay sequences. Surprisingly, these sequences depicted trajectories covering relatively small pieces of the environment (between a few meters and several dozen meters), unlike sequences in rodents in small setups which typically cover the entire environment. These sequences expressed similar time-compression to rodents. These findings provide the first demonstration of hippocampal replay in a non-rodent species. Our findings may have important implications for understanding hippocampal replay in mammals: The piecewise replay of information in this large environment might be akin to using small information-packets for transmitting large messages in artificial communication systems – but here seen for the first time in the biological brain.

Pubmed:

34045327: Eliav T, Maimon SR, Aljadeff J, Tsodyks M, Ginosar G, Las L, Ulanovsky N
Multiscale representation of very large environments in the hippocampus of flying bats.
Hippocampal place cells encode the animal's location. Place cells were traditionally studied in small environments, and nothing is known about large ethologically relevant spatial scales. We wirelessly recorded from hippocampal dorsal CA1 neurons of wild-born bats flying in a long tunnel (200 meters). The size of place fields ranged from 0.6 to 32 meters. Individual place cells exhibited multiple fields and a multiscale representation: Place fields of the same neuron differed up to 20-fold in size. This multiscale coding was observed from the first day of exposure to the environment, and also in laboratory-born bats that never experienced large environments. Theoretical decoding analysis showed that the multiscale code allows representation of very large environments with much higher precision than that of other codes. Together, by increasing the spatial scale, we discovered a neural code that is radically different from classical place codes.
Science, 2021; 372
30318145: Eliav T, Geva-Sagiv M, Yartsev MM, Finkelstein A, Rubin A, Las L, Ulanovsky N
Nonoscillatory Phase Coding and Synchronization in the Bat Hippocampal Formation.
Hippocampal theta oscillations were proposed to be important for multiple functions, including memory and temporal coding of position. However, previous findings from bats have questioned these proposals by reporting absence of theta rhythmicity in bat hippocampal formation. Does this mean that temporal coding is unique to rodent hippocampus and does not generalize to other species? Here, we report that, surprisingly, bat hippocampal neurons do exhibit temporal coding similar to rodents, albeit without any continuous oscillations at the 1-20 Hz range. Bat neurons exhibited very strong locking to the non-rhythmic fluctuations of the field potential, such that neurons were synchronized together despite the absence of oscillations. Further, some neurons exhibited "phase precession" and phase coding of the bat's position-with spike phases shifting earlier as the animal moved through the place field. This demonstrates an unexpected type of neural coding in the mammalian brain-nonoscillatory phase coding-and highlights the importance of synchrony and temporal coding for hippocampal function across species.
Cell, 2018; 175


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BOARD NUMBER: S05-013

A DISTRIBUTED POPULATION CODE FOR OBJECT REPRESENTATION IN CA1 OF THE HIPPOCAMPUS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The hippocampus may, through its place cells that fire in specific locations, construct a cognitive map of the animal's environment. While discrete objects are fundamental parts of the environment, how objects are incorporated into the cognitive map remains incompletely understood. So far, researchers have tried to solve the problem by asking how single cells encode object features and locations. In contrast to the single-cell level of description, we wondered if additional encoding of object features takes place at the neural population level. Using Neuropixels 2.0, we simultaneously record hundreds of cells in CA1 of the hippocampus and, to understand the high-dimensional data, use a combination of decoding algorithms and information theory. We find that population activity reorganises systematically as a function of the animal's distance from the object. Close to the object, the reorganisation of hippocampal activity is large; further away, the reorganisation is small. The reorganisation is distributed across all CA1 cells and does not depend on single-cell properties. The more cells we record, the stronger evidence we find for the reorganisation and its distance-dependence. Our results are suggestive of a novel type of remapping at the population level where objects induce large-scale changes in population activity patterns that are (1) location-dependent; (2) object-relative and (3) elusive at the single-cell level. The computational goal for such a distributed neural code must now be investigated, and our findings underscore the importance of discrete objects for cognitive maps in the hippocampus.

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BOARD NUMBER: S05-014

EXPERIENCE-DEPENDENT REORGANIZATION OF HIPPOCAMPAL CA1 PYRAMIDAL CELL REPRESENTATIONS IN A VIRTUAL CONTEXTUAL GO-NO GO TASK

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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[1]INSTITUTE OF EXPERIMENTAL MEDICINE, Laboratory Of Neuronal Signaling, Budapest, Hungary, [2]INSTITUTE OF EXPERIMENTAL MEDICINE, Laboratory Of Biological Computation, Budapest, Hungary

The hippocampal CA1 region is crucial for contextual memory formation by generating abstract relational maps by pyramidal cells (PCs) selectively tuned to locations or events. While the hippocampal map is thought to support behavioral adaptation, the organization and refinement of representations during this process is poorly understood. Here we aimed to elucidate the dynamic changes in tuning of CA1PCs during the evolution of task-relevant behavioral responses. We trained water-restricted, head-fixed Thy1-GCaMP6s mice to run on a cue-less treadmill in virtual reality environments, and distinguish two visually distinct corridors by selectively licking in a hidden reward zone (RZ) in one of them. We monitored CA1PC activity by two-photon Ca2+ imaging during improvement of task performance, as assessed by measuring running speed and lick rate. We observed enrichment of spatially tuned CA1PC activity at task-relevant locations of the virtual corridors, i.e. at the start and near the RZ. In low performance sessions, tuned cells showed relatively similar activity patterns in rewarded and unrewarded corridors. However, in expert animals, the number of tuned CA1PCs increased selectively in the rewarded corridor and decorrelated representations of the two corridors emerged. Accordingly, the number of corridor-selective CA1PCs increased with learning, especially in the rewarded corridor and predominantly at the RZ. These changes in single neuron tuning during experience were also reflected by an increased difference in the position decoding accuracy between the corridors. Our results suggest that hippocampal CA1PCs may initially generalize between environments but dynamically reorganize their activity according to behavioral relevance of corridor identity.

Pubmed:
31123108: Szőnyi A, Sos KE, Nyilas R, Schlingloff D, Domonkos A, Takács VT, Pósfai B, Hegedüs P, Priestley JB, Gundlach AL, Gulyás AI, Varga V, Losonczy A, Freund TF, Nyiri G
Brainstem nucleus incertus controls contextual memory formation.
Hippocampal pyramidal cells encode memory engrams, which guide adaptive behavior. Selection of engram-forming cells is regulated by somatostatin-positive dendrite-targeting interneurons, which inhibit pyramidal cells that are not required for memory formation. Here, we found that γ-aminobutyric acid (GABA)-releasing neurons of the mouse nucleus incertus (NI) selectively inhibit somatostatin-positive interneurons in the hippocampus, both monosynaptically and indirectly through the inhibition of their subcortical excitatory inputs. We demonstrated that NI GABAergic neurons receive monosynaptic inputs from brain areas processing important environmental information, and their hippocampal projections are strongly activated by salient environmental inputs in vivo. Optogenetic manipulations of NI GABAergic neurons can shift hippocampal network state and bidirectionally modify the strength of contextual fear memory formation. Our results indicate that brainstem NI GABAergic cells are essential for controlling contextual memories.
Science, 2019; 364
29167401: Klinger-Gratz PP, Ralvenius WT, Neumann E, Kato A, Nyilas R, Lele Z, Katona I, Zeilhofer HU
Acetaminophen Relieves Inflammatory Pain through CB Cannabinoid Receptors in the Rostral Ventromedial Medulla.
Acetaminophen (paracetamol) is a widely used analgesic and antipyretic drug with only incompletely understood mechanisms of action. Previous work, using models of acute nociceptive pain, indicated that analgesia by acetaminophen involves an indirect activation of CB receptors by the acetaminophen metabolite and endocannabinoid reuptake inhibitor AM 404. However, the contribution of the cannabinoid system to antihyperalgesia against inflammatory pain, the main indication of acetaminophen, and the precise site of the relevant CB receptors have remained elusive. Here, we analyzed acetaminophen analgesia in mice of either sex with inflammatory pain and found that acetaminophen exerted a dose-dependent antihyperalgesic action, which was mimicked by intrathecally injected AM 404. Both compounds lost their antihyperalgesic activity in mice, confirming the involvement of the cannabinoid system. Consistent with a mechanism downstream of proinflammatory prostaglandin formation, acetaminophen also reversed hyperalgesia induced by intrathecal prostaglandin E

To distinguish between a peripheral/spinal and a supraspinal action, we administered acetaminophen and AM 404 to mice, which lack CB receptors from the peripheral nervous system and the spinal cord. These mice exhibited unchanged antihyperalgesia indicating a supraspinal site of action. Accordingly, local injection of the CB receptor antagonist rimonabant into the rostral ventromedial medulla blocked acetaminophen-induced antihyperalgesia, while local rostral ventromedial medulla injection of AM 404 reduced hyperalgesia in wild-type mice but not in mice. Our results indicate that the cannabinoid system contributes not only to acetaminophen analgesia against acute pain but also against inflammatory pain, and suggest that the relevant CB receptors reside in the rostral ventromedial medulla. Acetaminophen is a widely used analgesic drug with multiple but only incompletely understood mechanisms of action, including a facilitation of endogenous cannabinoid signaling via one of its metabolites. Our present data indicate that enhanced cannabinoid signaling is also responsible for the analgesic effects of acetaminophen against inflammatory pain. Local injections of the acetaminophen metabolite AM 404 and of cannabinoid receptor antagonists as well as data from tissue-specific CB receptor-deficient mice suggest the rostral ventromedial medulla as an important site of the cannabinoid-mediated analgesia by acetaminophen.
J Neurosci, 2018; 38
25485758: Dudok B, Barna L, Ledri M, Szabó SI, Szabadits E, Pintér B, Woodhams SG, Henstridge CM, Balla GY, Nyilas R, Varga C, Lee SH, Matolcsi M, Cervenak J, Kacskovics I, Watanabe M, Sagheddu C, Melis M, Pistis M, Soltesz I, Katona I
Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling.
A major challenge in neuroscience is to determine the nanoscale position and quantity of signaling molecules in a cell type- and subcellular compartment-specific manner. We developed a new approach to this problem by combining cell-specific physiological and anatomical characterization with super-resolution imaging and studied the molecular and structural parameters shaping the physiological properties of synaptic endocannabinoid signaling in the mouse hippocampus. We found that axon terminals of perisomatically projecting GABAergic interneurons possessed increased CB1 receptor number, active-zone complexity and receptor/effector ratio compared with dendritically projecting interneurons, consistent with higher efficiency of cannabinoid signaling at somatic versus dendritic synapses. Furthermore, chronic Δ(9)-tetrahydrocannabinol administration, which reduces cannabinoid efficacy on GABA release, evoked marked CB1 downregulation in a dose-dependent manner. Full receptor recovery required several weeks after the cessation of Δ(9)-tetrahydrocannabinol treatment. These findings indicate that cell type-specific nanoscale analysis of endogenous protein distribution is possible in brain circuits and identify previously unknown molecular properties controlling endocannabinoid signaling and cannabis-induced cognitive dysfunction.
Nat Neurosci, 2015; 18
24607231: Ramikie TS, Nyilas R, Bluett RJ, Gamble-George JC, Hartley ND, Mackie K, Watanabe M, Katona I, Patel S
Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses.
The central amygdala (CeA) is a key structure at the limbic-motor interface regulating stress responses and emotional learning. Endocannabinoid (eCB) signaling is heavily implicated in the regulation of stress-response physiology and emotional learning processes; however, the role of eCBs in the modulation of synaptic efficacy in the CeA is not well understood. Here we describe the subcellular localization of CB1 cannabinoid receptors and eCB synthetic machinery at glutamatergic synapses in the CeA and find that CeA neurons exhibit multiple mechanistically and temporally distinct modes of postsynaptic eCB mobilization. These data identify a prominent role for eCBs in the modulation of excitatory drive to CeA neurons and provide insight into the mechanisms by which eCB signaling and exogenous cannabinoids could regulate stress responses and emotional learning.
Neuron, 2014; 81
22826132: Kato A, Punnakkal P, Pernía-Andrade AJ, von Schoultz C, Sharopov S, Nyilas R, Katona I, Zeilhofer HU
Endocannabinoid-dependent plasticity at spinal nociceptor synapses.
Neuroplastic changes at the spinal synapses between primary nociceptors and second order dorsal horn neurons play key roles in pain and analgesia. NMDA receptor-dependent forms of long-term plasticity have been studied extensively at these synapses, but little is known about possible contributions of the endocannabinoid system. Here, we addressed the role of cannabinoid (CB)1 receptors in activity-dependent plasticity at these synapses. We report that conditional low-frequency stimulation of high-threshold primary sensory nerve fibres paired with depolarisation of the postsynaptic neuron evoked robust long-term depression (LTD)of excitatory synaptic transmission by about 40% in the vast majority (90%) of recordings made in wild-type mice. When recordings were made from global or nociceptor-specific CB(1) receptor-deficient mice (CB(1)(−/−) mice and sns-CB(1)(−/−) mice), the portion of neurons exhibiting LTD was strongly reduced to about 25%. Accordingly, LTD was prevented to a similar extent by the CB1 receptor antagonist AM251 and mimicked by pharmacological activation of CB1 receptors. In a subset of neurons with EPSCs of particularly high stimulation thresholds, we furthermore found that the absence of CB(1) receptors in CB(1)(−/−) and sns-CB(1)(−/−) mice converted the response to the paired conditioning stimulation protocol from LTD to long-term potentiation (LTP). Our results identify CB1 receptor-dependent LTD as a form of synaptic plasticity previously unknown in spinal nociceptors. They furthermore suggest that prevention of LTP may be a

second hither to unknown function of CB1 receptors in primary nociceptors. Both findings may have important implications for our understanding of endogenous pain control mechanisms and of analgesia evoked by cannabinoid receptor agonists.
J Physiol, 2012; 590
22787031: Gregg LC, Jung KM, Spradley JM, Nyilas R, Suplita RL, Zimmer A, Watanabe M, Mackie K, Katona I, Piomelli D, Hohmann AG
Activation of type 5 metabotropic glutamate receptors and diacylglycerol lipase-α initiates 2-arachidonoylglycerol formation and endocannabinoid-mediated analgesia.
Acute stress reduces pain sensitivity by engaging an endocannabinoid signaling circuit in the midbrain. The neural mechanisms governing this process and molecular identity of the endocannabinoid substance(s) involved are unknown. We combined behavior, pharmacology, immunohistochemistry, RNA interference, quantitative RT-PCR, enzyme assays, and lipidomic analyses of endocannabinoid content to uncover the role of the endocannabinoid 2-arachidonoyl-sn-glycerol (2-AG) in controlling pain sensitivity in vivo. Here, we show that footshock stress produces antinociception in rats by activating type 5 metabotropic glutamate receptors (mGlu(5)) in the dorsolateral periaqueductal gray (dlPAG) and mobilizing 2-AG. Stimulation of mGlu(5) in the dlPAG with DHPG [(S)-3,5-dihydroxyphenylglycine] triggered 2-AG formation and enhanced stress-dependent antinociception through a mechanism dependent upon both postsynaptic diacylglycerol lipase (DGL) activity, which releases 2-AG, and presynaptic CB(1) cannabinoid receptors. Pharmacological blockade of DGL activity in the dlPAG with RHC80267 [1,6-bis(cyclohexyloximinocarbonylamino)hexane] and (-)-tetrahydrolipstatin (THL), which inhibit activity of DGL-α and DGL-β isoforms, suppressed stress-induced antinociception. Inhibition of DGL activity in the dlPAG with THL selectively decreased accumulation of 2-AG without altering levels of anandamide. The putative 2-AG-synthesizing enzyme DGL-α colocalized with mGlu(5) at postsynaptic sites of the dlPAG, whereas CB(1) was confined to presynaptic terminals, consistent with a role for 2-AG as a retrograde signaling messenger. Finally, virally mediated silencing of DGL-α, but not DGL-β, transcription in the dlPAG mimicked effects of DGL inhibition in suppressing both endocannabinoid-mediated stress antinociception and 2-AG formation. The results indicate that activation of the postsynaptic mGlu(5)-DGL-α cascade triggers retrograde 2-AG signaling in vivo. This pathway is required for endocannabinoid-mediated stress-induced analgesia.
J Neurosci, 2012; 32
19661434: Pernía-Andrade AJ, Kato A, Witschi R, Nyilas R, Katona I, Freund TF, Watanabe M, Filitz J, Koppert W, Schüttler J, Ji G, Neugebauer V, Marsicano G, Lutz B, Vanegas H, Zeilhofer HU
Spinal endocannabinoids and CB1 receptors mediate C-fiber-induced heterosynaptic pain sensitization.
Diminished synaptic inhibition in the spinal dorsal horn is a major contributor to chronic pain. Pathways that reduce synaptic inhibition in inflammatory and neuropathic pain states have been identified, but central hyperalgesia and diminished dorsal horn synaptic inhibition also occur in the absence of inflammation or neuropathy, solely triggered by intense nociceptive (C-fiber) input to the spinal dorsal horn. We found that endocannabinoids, produced upon strong nociceptive stimulation, activated type 1 cannabinoid (CB1) receptors on inhibitory dorsal horn neurons to reduce the synaptic release of gamma-aminobutyric acid and glycine and thus rendered nociceptive neurons excitable by nonpainful stimuli. Our results suggest that spinal endocannabinoids and CB1 receptors on inhibitory dorsal horn interneurons act as mediators of heterosynaptic pain sensitization and play an unexpected role in dorsal horn pain-controlling circuits.
Science, 2009; 325
19453631: Nyilas R, Gregg LC, Mackie K, Watanabe M, Zimmer A, Hohmann AG, Katona I
Molecular architecture of endocannabinoid signaling at nociceptive synapses mediating analgesia.
Cannabinoid administration suppresses pain by acting at spinal, supraspinal and peripheral levels. Intrinsic analgesic pathways also exploit endocannabinoids; however, the underlying neurobiological substrates of endocannabinoid-mediated analgesia have remained largely unknown. Compelling evidence shows that, upon exposure to a painful environmental stressor, an endocannabinoid molecule called 2-arachidonoylglycerol (2-AG) is mobilized in the lumbar spinal cord in temporal correlation with stress-induced antinociception. We therefore characterized the precise molecular architecture of 2-AG signaling and its involvement in nociception in the rodent spinal cord. Nonradioactive in situ hybridization revealed that dorsal horn neurons widely expressed the mRNA of diacylglycerol lipase-alpha (DGL-alpha), the synthesizing enzyme of 2-AG. Peroxidase-based immunocytochemistry demonstrated high levels of DGL-alpha protein and CB(1) cannabinoid receptor, a receptor for 2-AG, in the superficial dorsal horn, at the first site of modulation of the ascending pain pathway. High-resolution electron microscopy uncovered postsynaptic localization of DGL-alpha at nociceptive synapses formed by primary afferents, and revealed presynaptic positioning of CB(1) on excitatory axon terminals. Furthermore, DGL-alpha in postsynaptic elements receiving nociceptive input was colocalized with metabotropic glutamate receptor 5 (mGluR(5)), whose activation induces 2-AG biosynthesis. Finally, intrathecal activation of mGluR(5) at the lumbar level evoked endocannabinoid-mediated stress-induced analgesia through the DGL-2-AG-CB(1) pathway. Taken together, these findings suggest a key role for 2-AG-mediated retrograde suppression of nociceptive transmission at the spinal level. The striking positioning of the molecular players of 2-AG synthesis and action at nociceptive excitatory synapses suggests that pharmacological

manipulation of spinal 2-AG levels may be an efficacious way to regulate pain sensation.
Eur J Neurosci, 2009; 29
18234884: Nyilas R, Dudok B, Urbán GM, Mackie K, Watanabe M, Cravatt BF, Freund TF, Katona I
Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals.
Endocannabinoids are regarded as retrograde signaling molecules at various types of synapses throughout the CNS. The lipid derivatives anandamide and 2-arachidonoylglycerol (2-AG) are generally thought to be the key molecular players in this process. Previous anatomical and electrophysiological studies provided compelling evidence that the biosynthetic enzyme of 2-AG is indeed localized in the postsynaptic plasma membrane, whereas its target, the CB1 cannabinoid receptor, and the enzyme responsible for its inactivation are both found presynaptically. This molecular architecture of 2-AG signaling is a conserved feature of most synapses and supports the retrograde signaling role of 2-AG. Conversely, the molecular and neuroanatomical organization of synaptic anandamide signaling remains largely unknown. In contrast to its predicted role in retrograde signaling, here we show that N-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD), a biosynthetic enzyme of anandamide and its related bioactive congeners, the N-acylethanolamines (NAEs), is concentrated presynaptically in several types of hippocampal excitatory axon terminals. Furthermore, high-resolution quantitative immunogold labeling demonstrates that this calcium-sensitive enzyme is localized predominantly on the intracellular membrane cisternae of axonal calcium stores. Finally, the highest density of NAPE-PLD is found in mossy terminals of granule cells, which do not express CB1 receptors. Together, these findings suggest that anandamide and related NAEs are also present at glutamatergic synapses, but the sites of their synthesis and action are remarkably different from 2-AG, indicating distinct physiological roles for given endocannabinoids in the regulation of synaptic neurotransmission and plasticity.
J Neurosci, 2008; 28

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BOARD NUMBER: S05-015

PRESENT AND FUTURE: DUAL INFORMATION PROCESSING MODES IN THE HIPPOCAMPAL-MEDIAL ENTORHINAL CIRCUITRY

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Grid cells and place cells are key building blocks of the hippocampal formation's cognitive map. The firing patterns of these cells are usually viewed as encoding an animal's current location; however, previous studies have shown that they can also encode location in the recent past and near future. The relationship between these cells' spatiotemporal coding patterns is unclear. To address this, we analysed the firing patterns of co-recorded medial entorhinal grid cells and CA1 place cells in freely foraging rats. Both cell types mainly encode space prospectively, firing ~150ms before the rat entered a cell's preferred location. Grid cells' time shifts are proportional to their scale; similarly, CA1 place cells' time shifts increase with firing field size. In place cells, firing at different phases of the theta cycle was associated with different time shift lengths. This supports previous hypotheses that the cycle may be used to organise the encoding and retrieval of memories. We speculate that communication between CA1 and mEC may be involved in anticipating future trajectories, while CA1-CA3 communication may be used to compare past and present locations.

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BOARD NUMBER: S05-016

USING A FRAMEWORK OF STATISTICS AND SIMULATIONS TO ADDRESS CLASSIFICATION OF ANGULAR MODULATION IN HIPPOCAMPAL PLACE CELLS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

Jordan Carpenter[1], Jan Sigurd Blackstad[1], David Tingley[2], Edvard Moser[3], May-Britt Moser[3], Benjamin Dunn[4]
[1]NTNU, Kavli Institute For Systems Neuroscience, Trondheim, Norway, [2]Beth Israel Deaconess Medical Center, Division Of Endocrinology, Boston, United States of America, [3]NTNU, Kavli Institute For Systems Neuroscience And Centre For Neural Computation, Trondheim, Norway, [4]NTNU, Department Of Mathematical Sciences, Trondheim, Norway

The current knowledge of spatial maps and spatial coordinate systems in the brain is based to a large extent on neural recordings from animals that move freely in simple open arenas (open fields). In recordings from the hippocampus, neurons have both been shown to represent pure allocentric space (place cells), and to have additional firing rate modulation to angular variables (e.g., place cells with head direction or egocentric bearing modulation). Here, in agreement with previous work, we find indications for modulation of place cell activity by angular variables using previously defined methods for detection in real data from open field environments. However, our findings also raise the possibility that activity in hippocampal neurons can be falsely attributed to angular variables as a result of collinearity and inconsistencies in the analytical methods. By developing a simulation framework composed of functional cell types with various types of spatial tuning and by applying classical statistical approaches, we find higher than expected false positive rates for cells with conjunctive angular or egocentric tuning. False positive rates vary across cell types, behavioral sessions, noise levels, and firing field sizes, pointing to factors that might potentially account for misattribution to angular or egocentric tuning in experimental studies. We propose here that with simple improvements in experimental design and analysis, such issues can be mediated. Further, we suggest that such studies with potentially confounding factors should be investigated routinely and prior to data collection if possible.

Pubmed:
34381214: Tingley D, McClain K, Kaya E, Carpenter J, Buzsáki G
A metabolic function of the hippocampal sharp wave-ripple.
The hippocampus has previously been implicated in both cognitive and endocrine functions. We simultaneously measured electrophysiological activity from the hippocampus and interstitial glucose concentrations in the body of freely behaving rats to identify an activity pattern that may link these disparate functions of the hippocampus. Here we report that clusters of sharp wave-ripples recorded from the hippocampus reliably predicted a decrease in peripheral glucose concentrations within about 10 min. This correlation was not dependent on circadian, ultradian or meal-triggered fluctuations, could be mimicked with optogenetically induced ripples in the hippocampus (but not in the parietal cortex) and was attenuated to chance levels by pharmacogenetically suppressing activity of the lateral septum, which is the major conduit between the hippocampus and the hypothalamus. Our findings demonstrate that a function of the sharp wave-ripple is to modulate peripheral glucose homeostasis, and offer a mechanism for the link between sleep disruption and blood glucose dysregulation in type 2 diabetes.
Nature, 2021; 597

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BOARD NUMBER: S05-017

ERROR CORRECTION IN THE HIPPOCAMPAL-MEDIAL-ENTORHINAL COGNITIVE MAP.

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Hippocampal place cells and medial entorhinal grid cells, border cells and head direction cells are thought to support cognitive map-based navigation. However, they are also known to accumulate error and recent studies have shown that environmental cues have a complex influence on their firing patterns. We investigated the effect of environmental landmarks on error correction in spatially modulated cells during one dimensional navigation in a virtual reality environment, using in vivo chronic multi-tetrode recordings from mouse hippocampus and medial entorhinal cortex. Increasing the number of available visual cues resulted in an increase in the number of firing fields, decrease in the field size and variability, and increase in peak firing rate. A cell-type specific preference for field position relative to cue location was observed. Average drift in field position decreased significantly with increasing number of cues, while precision with which cells corrected their drift significantly increased. This effect was stronger in fields located closer to the visual cues. The presence of a non-visually cued fixed reward location decreased drift, suggesting that error in spatial cell firing can also be modulated by non-visual cues. Increasing the spatial information content of the presented visual cues further increased these stabilizing effects. The above changes resulted in the animal's increased precision in anticipating the reward location. These results suggest that increasing the number and information content of cues in the environment helps correct error in the firing of spatial cells by stabilizing their firing fields and increasing their spatial resolution.

Pubmed:
28663200: Kerekes P, Daret A, Shulz DE, Ego-Stengel V
Bilateral Discrimination of Tactile Patterns without Whisking in Freely Running Rats.
A majority of whisker discrimination tasks in rodents are performed on head-fixed animals to facilitate tracking or control of the sensory inputs. However, head fixation critically restrains the behavior and thus the incoming stimuli compared with those occurring in natural conditions. In this study, we investigated whether freely behaving rats can discriminate fine tactile patterns while running, in particular when stimuli are presented simultaneously on both sides of the snout. We developed a two-alternative forced-choice task in an automated modified T-maze. Stimuli were either a surface with no bars (smooth) or with vertical bars spaced irregularly or regularly. While running at full speed, rats encountered simultaneously the two discriminanda placed on the two sides of the central aisle. Rats learned to recognize regular bars versus a smooth surface in 8 weeks. They solved the task while running at an average speed of 1 m/s, so that the contact with the stimulus lasted <1 typical whisking cycle, precluding the use of active whisking. Whisker-tracking analysis revealed an asymmetry in the position of the whiskers: they oriented toward the rewarded stimulus during successful trials as early as 60 ms after the first possible contact. We showed that the whiskers and activity in the primary somatosensory cortex are involved during the discrimination process. Finally, we identified irregular patterns of bars that the rats can discriminate from the regular one. This novel task shows that freely moving rodents can make simultaneous bilateral tactile discrimination without whisking. The whisker system of rodents is a widely used model to study tactile processing. Rats show remarkable abilities in discriminating surfaces by actively moving their whiskers (whisking) against stimuli, typically sampling them several times. This motor strategy affects considerably the way that tactile information is acquired and thus the way that neuronal networks process the information. However, when rats run at high speed, they protract their whiskers in front of the snout without large movements. Here, we investigated whether rats are able to discriminate regular and irregular patterns of vertical bars while running without whisking. We found that the animals can perform a bilateral simultaneous discrimination without whisking and that this involves both whiskers and barrel cortex activity.
J Neurosci, 2017; 37

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BOARD NUMBER: S05-018

THE RATE OF PLACE CELL REMAPPING DEPENDS ON ACCUMULATED EXPERIENCE WITHIN CONTEXT AND NOT ON PASSAGE OF TIME

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Place cells can represent different spaces and contexts in a process known as *remapping.* Several studies have shown that although place cells are stable throughout a single exposure, they undergo remapping with repeated exposures to the same environment. Why do place cells remap even without any change to the context, and what could modulate this change? To answer this, we recorded place cells in freely moving mice traversing two similar and familiar linear tracks. The mice spent minutes in track A vs. hours in track B in each recording session. We asked whether the accumulated experience in a track (the amount of time spent in it), is the driving force behind the change in representation. We found that the correlations between the spatial representations five hours apart were lower in track B than in track A, in which they spent less time. Our result suggests that spatial representation is a dynamic process that is related to the ongoing experiences within the specific context, such that the more time is spent within the context, the more it is changed. This implies that the remapping of place cells is related to the accumulation of new memories and not to passive forgetting.

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BOARD NUMBER: S05-019

RETROSPLENIAL CORTEX REPRESENTS BOTH LOCAL AND GLOBAL SPACE

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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[1]Laboratoire de Neurosciences Cognitives, Neural Basis Of Spatial Cognition, Marseille, France, [2]Aix-Marseille uUniversity, Centre National De Le Recherche Scientifique (cnrs), Marseille, France

Every day, we navigate between connected rooms to reach goals. This requires a mental map of the environment based on two reference frames: one for each room (local reference frame) and one including all connected rooms (global reference frame). Recent studies in rodents suggest that the retrosplenial cortex (RSC) may simultaneously code for both reference frames. Indeed, single unit recording in rats exploring two symmetrical connected rooms show that RSC contains two distinct functional cell populations: head direction cells (HDC) firing when the animal faces a particular direction thus providing a global directional signal; and bi-directional cells (BDC) displaying distinct directions each specific for one room, suggesting that their activity is anchored to a local space. Here we tested whether these two populations of cells still provide global and local directional signals in environments with two or four visually different connected rooms. We found that HDC firing direction is maintained regardless of the number of rooms. Furthermore, BDC fire in two directions when rodents navigate between two different connected rooms and tend to fire in four directions in four connected rooms. We also observed that non-directional RSC cells show spatial firing patterns that either repeat between rooms (local coding) or remain constant in all rooms (global coding). This indicates that coding of local and global spaces is not limited to HDC and BDC. Altogether, these results confirm that RSC may form global and local reference frames necessary for the construction of a cognitive map which allows navigation in complex environments.

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BOARD NUMBER: S05-020

PERSISTENT HOMOLOGY OF THE UMAP COMPLEX REVEALS TORI IN THE ENTORHINAL CORTEX OF HEAD-FIXED MICE

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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In this work, we uncovered toroidal structure in population activity of medial entorhinal recordings of head-fixed mice using the manifold learning technique, UMAPH. Each neuron had a single receptive field on the hexagonal torus, with the toroidal representation stable across conditions; baseline, dark and gain manipulation (for which the relation between the movement of the running wheel and the linear VR-track was varied). This is consistent with the grid cell tori recently discovered in rats. Furthermore, we decoded the internal dynamics and observed smooth trajectories on the neural manifold. The trajectories evolved in line with the mouse's progression along the VR-track and seemingly made turns which accommodated the continual repetition of the track. Moreover, the gain could be predicted by estimating the length of the internal trajectories. We also observed that even in baseline trials the mapping from the toroidal state space to external space occasionally shifted its representation or drifted over trials. In mice where several MEC subpopulations were recorded, this occurred simultaneously, suggesting a coordinated mapping across toroidal modules at different spatial scales. UMAPH builds on the two most used methods in topological data analysis, UMAP and persistent homology, and we show that the method performs well with synthetic data sets and is able to decode the expected head direction representation of the anterodorsal thalamic nucleus during wake, REM and SWS sleep. We find the UMAPH approach promising, in particular in opening the door to more detailed analysis of grid cell networks in head-fixed animals.

Pubmed:
35022611: Gardner RJ, Hermansen E, Pachitariu M, Burak Y, Baas NA, Dunn BA, Moser MB, Moser EI
Toroidal topology of population activity in grid cells.
The medial entorhinal cortex is part of a neural system for mapping the position of an individual within a physical environment. Grid cells, a key component of this system, fire in a characteristic hexagonal pattern of locations, and are organized in modules that collectively form a population code for the animal's allocentric position. The invariance of the correlation structure of this population code across environments and behavioural states, independent of specific sensory inputs, has pointed to intrinsic, recurrently connected continuous attractor networks (CANs) as a possible substrate of the grid pattern. However, whether grid cell networks show continuous attractor dynamics, and how they interface with inputs from the environment, has remained unclear owing to the small samples of cells obtained so far. Here, using simultaneous recordings from many hundreds of grid cells and subsequent topological data analysis, we show that the joint activity of grid cells from an individual module resides on a toroidal manifold, as expected in a two-dimensional CAN. Positions on the torus correspond to positions of the moving animal in the environment. Individual cells are preferentially active at singular positions on the torus. Their positions are maintained between environments and from wakefulness to sleep, as predicted by CAN models for grid cells but not by alternative feedforward models. This demonstration of network dynamics on a toroidal manifold provides a population-level visualization of CAN dynamics in grid cells.
Nature, 2022; 602

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BOARD NUMBER: S05-021

RAPID WITHIN-SESSION DYNAMICS OF CA1 PLACE CODES IN MICE EXPLORING A NOVEL ENVIRONMENT

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The ability of hippocampal CA1 cells to form and maintain stable spatial codes of an environment across days is well described. Much less is known about the short-term properties of such codes at the first episodes of exploratory behavior in a novel environment. We have imaged CA1 place cells with head-mounted Nvista miniscopes in mice exploring a novel environment in three consequent 15-minute sessions 24h apart. To estimate the within-session stability of place fields, we used a selectivity score calculated for each attendance of a field as a rate of place-specific calcium events. We observed that a significant (25%) part of place fields became stable at the first attendance, while the average latency of place field stabilization equaled 247s at the first session. This latency decreased in the next session in the same environment, however, the average selectivity score demonstrated no cumulation between sessions, starting with similar levels at each session. Importantly, these results were shown non-depending on the retention of place codes on the second session. To verify these results we performed population analysis which revealed latent variables corresponding to trajectories of mice, allowing us to decode the position of animals. Moreover, within-session dynamics of the precision of such decoding was found consistent with the average place field selectivity score dynamics in these animals. Our data thus reveal the fast emergence of place codes in a novel environment and may serve as a basis for further studies. **This study was supported by Russian Ministry of Science and Higher Education Project №075-15-2020-801 and by Russian Science Foundation Project №20-15-00283.**

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BOARD NUMBER: S05-022

DYNAMICS OF DENTATE GYRUS GRANULE CELL ACTIVITY DURING COMBINATORIAL MODIFICATION OF SPACE.

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Theoretical and experimental data support the hypothesis that the Dentate Gyrus (DG) is important for context discrimination. The small number and low activity of granule cells (GCs) engaged in a spatial representation contribute to the sparsification of the rich multimodal input arriving from the entorhinal cortex and the orthogonalization of overlapping information. The DG can efficiently distinguish different spatial contexts, either by the activity of different GCs or by changes in their firing properties. But it is currently unclear which features of a spatial experience induce GC remapping, how newly-formed representations change over time and how they might relate to past experience.
To investigate this, we recorded Ca^{2+} signals from mature GCs in freely moving Thy1-GCaMP mice using head-mounted miniscope during the presentation of three different contextual conditions. First, animals explored two very different novel environments during eight consecutive days. In these conditions, GC activity was sparse and showed high spatial selectivity from the first day of recording. Afterwards, the two contexts were merged into a larger arena by removing a separating wall. Interestingly, this generated an abrupt and progressive increase in the number of recruited GCs during eight consecutive recording days, and an increase in the mean network activity. Intriguingly, the representation of the combined environment included both, GCs that were previously active in one of the separated arenas, and a high number of cells selective for the merged arena. Therefore, a combined environment is represented in the DG as more than the sum of its individual components.

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BOARD NUMBER: S05-023

BEHAVIOUR OF MEDIAL ENTORHINAL CORTEX CELLS DURING A RADIAL EIGHT ARM MAZE TASK

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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How non-grid spatial cells in the medial entorhinal cortex (mEC) behave during navigation is not understood. Also, the role of hippocampus independent replay in the mEC is unknown.The mEC has a myriad of very interesting cells that exhibit spatial properties but are not grid cells per say. The role of these non-grid spatial cells during navigation is still not well understood. The aim of the project is to elucidate the role of this replay and to understand how different cell types in the mEC behave during a spatial task. We performed multichannel, extracellular electrophysiology on rats while they performed a radial eight arm maze task. Rats were implanted with microdrives targeting the dorsal CA1 and layers II/III of mEC while performing an eight arm maze task where they were expected to learn which 3 arms were rewarded. re rewarded. Each trial started with a delay of 1min. Animals performed 30 trials per day.
We establish how the different kinds of spatial cells in mEC behave during this task. We show how grid and non-grid spatial cells behave differently at different phases during the task. We show how at key moments of immobility the trajectory replayed by mEC cells isn't always the same as that replayed by CA1 cells.These results are a demonstration of the influence of non-geometric factors on the activity of the mEC space coding cells. They also demonstrate how independent replay in the mEC could be part of a parallel memory circuit in the brain.

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BOARD NUMBER: S05-024

COORDINATED DYNAMICS OF DIRECTION AND POSITION REPRESENTATIONS IN MEDIAL ENTORHINAL CIRCUITS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Grid cells are believed to provide a universal coordinate system for mapping an animal's ongoing experience. Stereotyped predictive "sweep" trajectories are putatively generated within grid-cell networks, and may serve a specific spatial metric function. To investigate this possibility, we used Neuropixels probes to record from many hundreds of cells in medial entorhinal cortex in rats while they explored 2-D and 1-D environments. First, we identified a novel latent population code, the "internal direction" (ID), which correlated with the animal's head direction but displayed independent theta-paced dynamics, tending to alternately represent directions to the left and right side of the animal's head direction. Surprisingly, when we decoded sweep trajectories from grid cells (or all position-modulated cells), the trajectories aligned to ID much more reliably than to the animal's head direction. Sweeps decoded independently from different grid-cell modules were co-aligned to ID, but with different lengths which were proportional to the module grid spacing. Notably, sweeps never exceeded half the grid spacing – thus, the represented locations were constrained within a local grid "tile". The partially discrepant module sweep trajectories suggest that modules may function as semi-independent, locally predictive maps. Consistent with this idea, module activity levels showed independent behavioural-timescale fluctuations. Finally, we found that decoded sweep trajectories extrapolated the cognitive map into unexplored regions of space: grid-cell maps extended through opaque walls and beyond the edges of elevated running tracks. This supports the notion of sweeps as an automatic and fundamental process underlying the measurement of space, irrespective of its content.

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BOARD NUMBER: S05-025

SPATIAL PERIODIC FIRING IN THE SUBICULUM OF MICE

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Spatial cognition relies on a complex circuitry in which the hippocampal formation seems to be crucial. The subiculum is a region located at the core of this circuit, it receives inputs from grid cells located in the medial entorhinal cortex (MEC) and place cells from the CA1 area of the hippocampus. It integrates input from these two relevant spatial information sources and mediates the output from the hippocampus to cortical and sub-cortical areas also involved in spatial coding. Despite the potential relevance of the subiculum, its role in memory and spatial coding is still poorly understood. Previous work described a very heterogeneous population of spatial neurons in the subiculum, with evidence of its role in coding the geometry of the environment and in spatial navigation in darkness. However, its role in spatial coding remains to be unveiled. With the aim of understanding further the properties of spatial coding in the subiculum, we implanted mice with microdrives mounting tetrodes and multisite electrodes aiming at the CA1 and subicular area, and recorded neuronal activity across different behavioral paradigms. Our results indicate that place cells in CA1 present higher spatial resolution and sharper firing fields than those of subicular neurons. Also, place cells in the CA1 area seem to be differentially modulated by the local field potential. Interestingly, we found pyramidal neurons in the subiculum with periodic firing, the first evidence of this type of regular firing in subicular pyramidal neurons.

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BOARD NUMBER: S05-026

UNIFORMITY OF GRID PHASES IN LARGE ENVIRONMENTS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Grid cells in the medial entorhinal cortex (MEC) show place-modulated activity at specific regions of an environment, generating a symmetrical, hexagonal lattice. The properties of the grid, such as spacing and orientation, vary along the dorso-ventral (D-V) axis of the MEC. Functional clusters of grid cells, called grid modules, share common properties. Within those clusters, each individual cell has a phase, referring to the relative position of their map in the environment. Two different models have been put forward to interpolate the generation of the grid cell network; continuous attractor models (CAN) and feed-forward learning models. One condition for CAN is the random distribution of the grid phases, implying a uniform coverage of space by the grid maps. In opposition, feedforward models can imply a clustered phase distribution. Using neuropixels recordings of rats foraging in an environment of 4x4 meters, we create ideal conditions to study the grid phases of hundreds of neurons. These experiments allow simultaneous recording of multiple D-V levels, providing the unprecedented opportunity to study very large modules. At the same time, small spacing grids show a high number of fields in this megaspace, which allows investigation of the stability of the phase pattern through this large environment. In agreement with the CAN models, our data demonstrate a random, non organised, distribution of phases for most of the grid modules in the MEC.

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BOARD NUMBER: S05-027

HEMISPHERE-SPECIFIC SPATIAL REPRESENTATION BY HIPPOCAMPAL GRANULE CELLS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The dentate gyrus (DG) plays a key role in the emergence of spatial and contextual map representation within the hippocampus during learning. Differences in neuronal activity have been observed across the left and right CA1-3 areas, implying functional lateralization in spatial coding. Whether bilateral differences of DG granule cell (GC) assemblies encoding spatial information exist remains largely unexplored. Here, we employed two-photon calcium imaging in the left or right hemisphere to record the activity of GC populations over five consecutive days in head-fixed mice navigating through familiar and novel virtual environments. Imaging revealed similar mean activity of GCs between both sides. However, spatial tuning, context-selectivity and run-to-run place field reliability was markedly higher for GC place cells in the left than the right hemisphere. Moreover, the proportion of GCs reconfiguring their place fields between contexts was greater in the left DG. Thus, our data suggest that contextual information in GCs is differentially processed between hemispheres, with a high spatial discrimination between environments in the left DG but a bias towards generalization in the right DG. These data provide a potential explanation to recent divergent results about the degree of neuronal discrimination between environments observed in the DG.

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BOARD NUMBER: S05-028

IMPACT OF VISUAL THALAMUS LESION ONTO HIPPOCAMPAL PLACE CELL ACTIVITY.

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Hippocampal place cells are spatially tuned neurons in mammalian brain which are thought to reflect the memory trace of the spatial position of the animal in its environment. Their discovery led to the hypothesis that they are the neural substrate of a ‘cognitive map’ and play a major role in the mapping of the environment. Recently, a very similar spatial activity has been discovered in the dorsal lateral geniculate nucleus (dLGN), a thalamic structure widely known to be involved in early visual information processing. The existence of such activity suggests that a spatial map is formed one synapse after the retina. The aim of this study is to examine the impact of dLGN lesions onto hippocampal function. We performed bilateral excitotoxic lesions (NMDA) in the dLGN and recorded the spatial activity of CA1 hippocampal place cells in freely-moving rats while they explore either a familiar or novel asymmetric environments in total darkness. This allowed us to quantify place cell activity in the absence of visual inputs investigating therefore a putative non-visual role of dLGN in space processing. In both lesioned and SHAM rats, hippocampal place fields appeared to be more linked to specific parts of the environment suggesting a control by the geometric shape of the environment. In addition, hippocampal place fields in lesioned rats were more numerous, larger but less stable than in Sham rats. Overall, the dLGN functionally interacts with hippocampal place cells and may contribute to stabilize and increase the spatial selectivity of hippocampal place cells.

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BOARD NUMBER: S05-029

EFFECTS OF UNILATERAL VESTIBULAR LOSS ON HEAD-DIRECTION CELL ACTIVITY

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Vestibular information processing is essential in various brain' functions including navigation and spatial memory. Unilateral vestibular loss (UVL) induces long-term deficits in various domains of spatial memory associated with altered hippocampal plasticity. The origin of the deficits could be diverse as several pathways relaying vestibular information to the hippocampus have been described, including the head-direction pathway. As head direction cells (HDC) encode specific position of the head in a given environment, they strongly rely on vestibular information which make them a potential target for explaining spatial memory deficits induced by UVL. Particularly, the anterior nucleus (AN) of the thalamus, a key structure for memory and navigation, is known to receive vestibular information and to be the first structure generating proper HDC activity. Here we recorded neuronal activity in the ipsilesional AN before and after UVL in the adult rat. Surprisingly, we observed that HDC activity is still present in the ipsilesional AN after UVL. While other analyses must be conducted to better understand UVL impact on AN's activity, these results suggest that an alteration of the ipsilesional HDC pathway is not a convincing explanation for spatial memory deficits following UVL.

Pubmed:

34419105: El Mahmoudi N, Rastoldo G, Marouane E, Péricat D, Watabe I, Tonetto A, Hautefort C, Chabbert C, Sargolini F, Tighilet B
Breaking a dogma: acute anti-inflammatory treatment alters both post-lesional functional recovery and endogenous adaptive plasticity mechanisms in a rodent model of acute peripheral vestibulopathy.
Due to their anti-inflammatory action, corticosteroids are the reference treatment for brain injuries and many inflammatory diseases. However, the benefits of acute corticotherapy are now being questioned, particularly in the case of acute peripheral vestibulopathies (APV), characterized by a vestibular syndrome composed of sustained spinning vertigo, spontaneous ocular nystagmus and oscillopsia, perceptual-cognitive, posturo-locomotor, and vegetative disorders. We assessed the effectiveness of acute corticotherapy, and the functional role of acute inflammation observed after sudden unilateral vestibular loss.
J Neuroinflammation, 2021; 18
32858093: Rastoldo G, El Mahmoudi N, Marouane E, Pericat D, Watabe I, Toneto A, López-Juárez A, Chabbert C, Tighilet B
Adult and endemic neurogenesis in the vestibular nuclei after unilateral vestibular neurectomy.
We previously revealed adult reactive neurogenesis in deafferented vestibular nuclei following unilateral vestibular neurectomy (UVN) in the feline model. We recently replicated the same surgery in a rodent model and aimed to elucidate the origin and fate of newly generated cells following UVN. We used specific markers of cell proliferation, glial reaction, and cell differentiation in the medial vestibular nucleus (MVN) of adult rats. UVN induced an intense cell proliferation and glial reaction with an increase of GFAP-Immunoreactive (Ir), IBA1-Ir and Olig2-Ir cells 3 days after the lesion in the deafferented MVN. Most of the newly generated cells survived after UVN and differentiated into oligodendrocytes, astrocytes, microglial cells and GABAergic neurons. Interestingly, UVN induced a significant increase in a population of cells colocalizing SOX2 and GFAP 3 days after lesion in the deafferented MVN indicating the probable presence of multipotent cells in the vestibular nuclei. The concomitant increase in BrdU- and SOX2-Ir cells with the presence of SOX2 and GFAP colocalization 3 days after UVN in the deafferented MVN may support local mitotic activity of endemic quiescent neural stem cells in the parenchyma of vestibular nuclei.
Prog Neurobiol, 2021; 196
34943885: Marouane E, El Mahmoudi N, Rastoldo G, Péricat D, Watabe I, Lapôtre A, Tonetto A, Xavier F, Dumas O, Chabbert C, Artzner V, Tighilet B
Sensorimotor Rehabilitation Promotes Vestibular Compensation in a Rodent Model of Acute Peripheral Vestibulopathy by Promoting Microgliogenesis in the Deafferented Vestibular Nuclei.

Acute peripheral vestibulopathy leads to a cascade of symptoms involving balance and gait disorders that are particularly disabling for vestibular patients. Vestibular rehabilitation protocols have proven to be effective in improving vestibular compensation in clinical practice. Yet, the underlying neurobiological correlates remain unknown. The aim of this study was to highlight the behavioural and cellular consequences of a vestibular rehabilitation protocol adapted to a rat model of unilateral vestibular neurectomy. We developed a progressive sensory-motor rehabilitation task, and the behavioural consequences were quantified using a weight-distribution device. This analysis method provides a precise and ecological analysis of posturolocomotor vestibular deficits. At the cellular level, we focused on the analysis of plasticity mechanisms expressed in the vestibular nuclei. The results obtained show that vestibular rehabilitation induces a faster recovery of posturolocomotor deficits during vestibular compensation associated with a decrease in neurogenesis and an increase in microgliogenesis in the deafferented medial vestibular nucleus. This study reveals for the first time a part of the underlying adaptative neuroplasticity mechanisms of vestibular rehabilitation. These original data incite further investigation of the impact of rehabilitation on animal models of vestibulopathy. This new line of research should improve the management of vestibular patients.
Cells, 2021; 10
32547480: Marouane E, Rastoldo G, El Mahmoudi N, Péricat D, Chabbert C, Artzner V, Tighilet B
Identification of New Biomarkers of Posturo-Locomotor Instability in a Rodent Model of Vestibular Pathology.
The vestibular system plays a crucial role in maintaining postural balance. Unilateral vestibular lesions result in a typical syndrome characterized by postural imbalance, altered locomotor patterns and gaze stabilization, as well as cognitive and neurovegetative disorders. One of the main difficulties encountered in the development of new anti-vertigo drugs is the lack of sensitivity in the evaluation of this syndrome. Qualitative assessments of the vestibular syndrome have been developed, but methods of conducting quantitative evaluations are critically lacking. Recently, assessments with a dynamic weight-bearing device (DWB®, Bioseb) revealed postural alterations in rats subjected to unilateral vestibular neurectomy (UVN). Our team is evaluating a new version of this device capable of quantifying additional parameters of postural and locomotor equilibrium. The objective of this study was to use this device to assess these new posturo-locomotor parameters in a rat model of a vestibular pathology. The biomarkers measured by this device are as follows: the barycenter, the support surface and the weight distribution of the rats when they were moving or stationary. Before UVN, the rats showed a symmetric distribution of their weight along the lateral axis. In the acute phase after UVN on the left side, the rats distributed more weight on the right side than on the left side and then distributed more weight on the left side. These results corroborate those presented in our previous study. The support surface of the rats increased between 1 day and 30 days after UVN, and the barycenter distribution reflected the weight distribution. In addition, our results show smaller changes in the weight distributions when the animals are moving compared with when they are stationary in the acute phase after UVN. This study provides new information on the static and dynamic postural balance patterns observed after unilateral vestibular loss in rats. These data are relevant because they objectively quantify the posturo-locomotor component of vestibular syndrome as well as the compensatory strategies used after vestibular loss. These results may guide the development of rehabilitation protocols for vestibular patients and the validation of pharmacological compounds favoring the restoration of equilibrium.
Front Neurol, 2020; 11
32582016: Rastoldo G, Marouane E, El Mahmoudi N, Péricat D, Bourdet A, Timon-David E, Dumas O, Chabbert C, Tighilet B
Quantitative Evaluation of a New Posturo-Locomotor Phenotype in a Rodent Model of Acute Unilateral Vestibulopathy.
Vestibular pathologies are difficult to diagnose. Existing devices make it possible to quantify and follow the evolution of posturo-locomotor symptoms following vestibular loss in static conditions. However, today, there are no diagnostic tools allowing the quantitative and spontaneous analysis of these symptoms in dynamic situations. With this in mind, we used an open-field video tracking test aiming at identifying specific posturo-locomotor markers in a rodent model of vestibular pathology. Using Ethovision XT 14 software (Noldus), we identified and quantified several behavioral parameters typical of unilateral vestibular lesions in a rat model of vestibular pathology. The unilateral vestibular neurectomy (UVN) rat model reproduces the symptoms of acute unilateral peripheral vestibulopathy in humans. Our data show deficits in locomotion velocity, distance traveled and animal mobility in the first day after the injury. We also highlighted alterations in several parameters, such as head and body acceleration, locomotor pattern, and position of the body, as well as "circling" behavior after vestibular loss. Here, we provide an enriched posturo-locomotor phenotype specific to full and irreversible unilateral vestibular loss. This test helps to strengthen the quantitative evaluation of vestibular disorders in unilateral vestibular lesion rat model. It may also be useful for testing pharmacological compounds promoting the restoration of balance. Transfer of these novel evaluation parameters to human pathology may improve the diagnosis of acute unilateral vestibulopathies and could better follow the evolution of the symptoms upon pharmacological and physical rehabilitation.
Front Neurol, 2020; 11

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BOARD NUMBER: S05-030

EXPERIENCE DEPENDENT SPECIFICITY OF PLACE CELL DIRECTIONALITY IN ALZHEIMER'S RAT MODEL

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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On linear track hippocampal place cells fire selectively at specific locations in a direction-dependent manner. Unidirectional cells express firing fields only in one direction while bidirectional cells fire on both. The directionality changes with experience and might be related to memory consolidation as it is higher in familiar environments compared to novel ones, however, its exact nature is not well understood. To understand the development of place cells directionality as possible indicator of memory consolidation in Alzheimer's disease, we compared transgenic 9-12 months old TgF344-AD animals carrying human genes for PS1 and APP with F344 controls. We examined the properties of CA1 place cell activity in familiar and novel linear track environments, namely their directionality and firing field size. Population analysis with respect to forward and reverse directions was also performed. To rule out the possibility of bidirectional cell effect occurred by chance, methods such as spatial selectivity and instantaneous speed were employed. In the familiar environment the measured parameters did not differ significantly between the groups. However, in the novel environment we observed significantly larger firing fields in TgF344-AD animals, as well as a higher proportion of bidirectional place cells compared to controls. The population vector analysis showed a higher similarity in TgF344-AD between neural codes for each direction in the novel environment. These results point towards an impairment in novel information encoding in the Alzheimer model and to its rather functional consolidation in the CA1 hippocampal network.

Pubmed:
34679365: Proskauer Pena SL, Mallouppas K, Oliveira AMG, Zitricky F, Nataraj A, Jezek K
Early Spatial Memory Impairment in a Double Transgenic Model of Alzheimer's Disease TgF-344 AD.
Before the course of Alzheimer's disease fully manifests itself and largely impairs a patient's cognitive abilities, its progression has already lasted for a considerable time without being noticed. In this project, we mapped the development of spatial orientation impairment in an active place avoidance task-a highly sensitive test for mild hippocampal damage. We tested vision, anxiety and spatial orientation performance at four age levels of 4, 6, 9, and 12 months across male and female TgF-344 AD rats carrying human genes for presenilin-1 and amyloid precursor protein. We found a progressive deterioration of spatial navigation in transgenic animals, beginning already at the age of 4 months, that fully developed at 6 months of age across both male and female groups, compared to their age-matched controls. In addition, we described the gradual vision impairment that was accentuated in females at the age of 12 months. These results indicate a rather early onset of cognitive impairment in the TgF-344 AD Alzheimer's disease model, starting earlier than shown to date, and preceding the reported development of amyloid plaques.
Brain Sci, 2021; 11

FENS Forum 2022
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BOARD NUMBER: S05-031

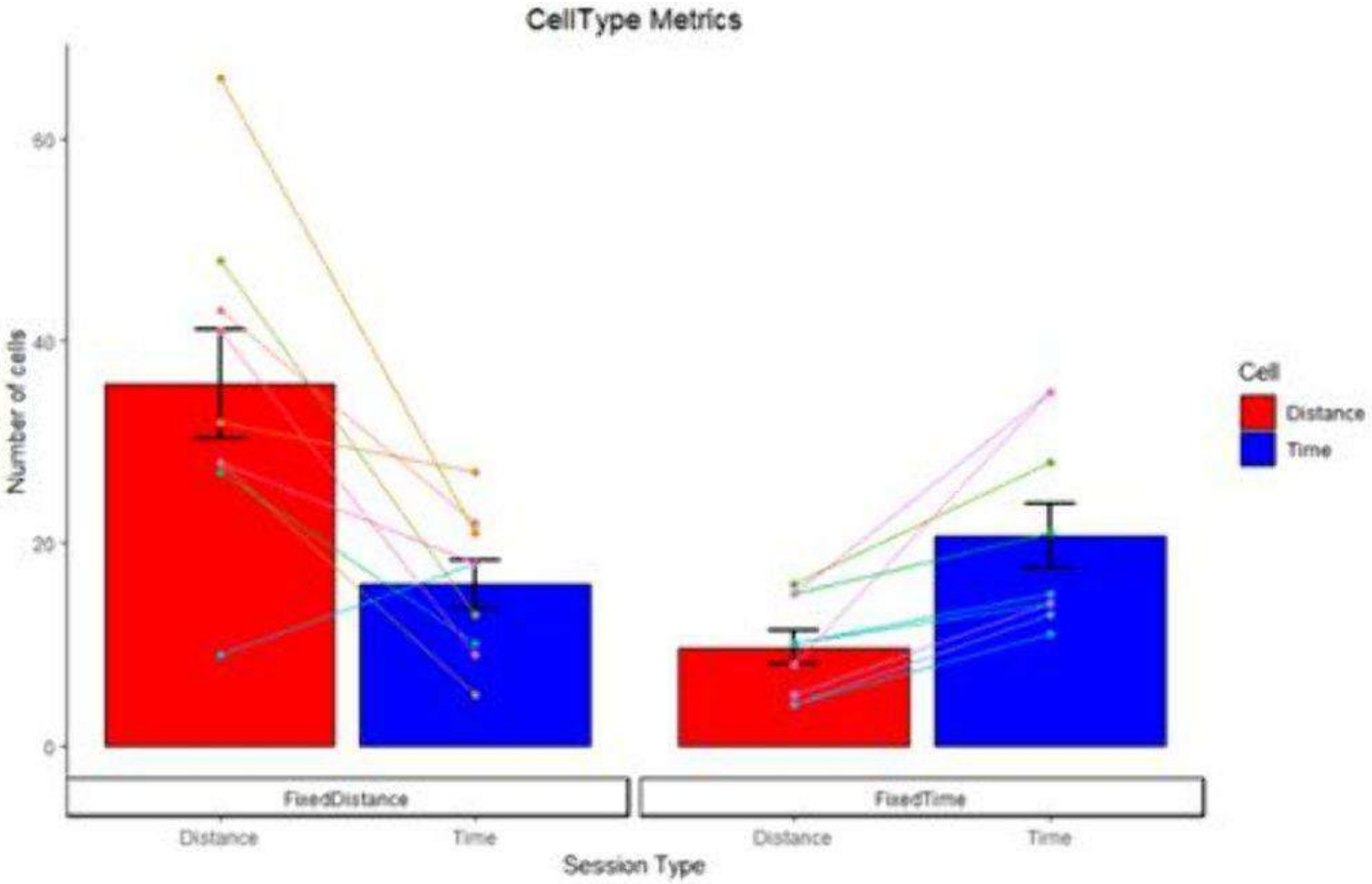
TIME OR DISTANCE: PREDICTIVE CODING OF HIPPOCAMPAL CELLS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

Shai Abramson[1], Benjamin Kraus[2], John White[2], Michael Hasselmo[2], Genela Morris[3], Dori Derdikman[4]
[1]Rappaport Faculty of Medicine and Research Institute, Technion – Israel Institute of Technology, Neuroscience, Haifa, Israel, [2]Boston university, Center For Systems Neuroscience, Boston, United States of America, [3]Tel-Aviv Sourasky Medical Center, Neurosurgery Dept., Tel-Aviv, Israel, [4]Technion, Rappaport Faculty Of Medicine, Haifa, Israel

The discovery of place cells within the hippocampus has pointed to the importance of the hippocampus for navigation. The more recent discovery of hippocampal time cells has broadened the perspective of encoding in the hippocampus. One hypothesis is based on the notion that hippocampal cells deduce location by integrating travelled distance ("path integration"). Accordingly, time cells, which fire at particular times when animals are running on a treadmill without changing location, actually encode accumulated distance on the treadmill. To examine this hypothesis, Kraus et al. [1] performed treadmill experiments in which animals either ran for a fixed-time or a fixed-distance with varying velocities. Two distinct coding modes of hippocampal principal cells were found. Some cells encoded travelled distance and others elapsed time, thus refuting the notion that all hippocampal cells were performing path integration. Using the data from these experiments, we asked whether the two populations depended on the type of task the rats were engaged in. We performed an analysis based on the onset time of each run and defined metrics to evaluate whether the cell is better encoding time or distance. We show (figure 1) that indeed the type of experiment determined the cells' encoding and suggest possible mechanisms of predictive coding which may explain these results. [1] Kraus, B. J., Robinson II, R. J., White, J. A., Eichenbaum, H., & Hasselmo, M. E. (2013). Hippocampal "time cells": time versus path integration. *Neuron*, *78*(6), 1090-

1101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40
20
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FixedDistance
FixedTime
Distance
Time
Distance
Time
Session Type
Cell
Distance
Time

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BOARD NUMBER: S05-032

LOCAL ACTIVATION GENERATES THETA PHASE PRECESSION IN CA1 PYRAMIDAL NEURONS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Multiple studies demonstrated the importance of theta phase precession as a spatial code and as a learning mechanism. However, the components contributing to precession and the locus of generation remain unclear. Here, we tested the hypothesis that a transient position-dependent rate increase of CA1 pyramidal neurons, combined with ongoing theta, suffices for generating precession. We implanted high-density silicon probes with miniature light sources in CA1 expressing ChR2 in pyramidal neurons (five mice). As the mice traversed a linear track, we used closed-loop illumination to generate place- and orientation-dependent spiking. Although illumination was indifferent to theta oscillations, 15% of the pyramidal cells exhibited induced spatial precession. Induced precession was especially prevalent among units with high firing rate gain during ripples, implying that precession is more readily generated in cells tuned to upstream information. During stimulation, rhythmically spiking pyramidal cells shifted to frequencies higher than theta oscillations, indicating induced temporal precession. To determine whether induced precession depends on hippocampal anatomy and inputs, we repeated experiments in parietal neocortex (three mice). Closed-loop illumination led to position-dependent firing of neocortical cells. However, although paced by volume-conducted hippocampal theta, neocortical cells did not exhibit induced precession. Thus, spatial and temporal precession can be generated locally in CA1, at odds with models of inheritance from CA3 or upstream regions. Instead, our results imply that precession is specifically produced by an intra-CA1 generator, which depends on local firing rate increase, theta oscillations, and upstream inputs. Supported by ISF #638/16, CIHR-IDRC-ISF #2558/18, CRCNS BSF#2015577, and ERC #679253

Pubmed:
31705981: Sloin H, Stark E
Response and sample bridging in a primate short-term memory task.
Freely-moving rodents can solve short-term memory (STM) tasks using "response bridging" strategies, relying on motor patterns instead of mnemonic functions. This limits the interpretational power of results yielded by some STM tasks in rodents. To determine whether head-fixed monkeys can employ parallel non-mnemonic strategies, we measured eye position and velocity of two head-fixed monkeys performing a delayed response reaching and grasping task. We found that eye position during the delay period was correlated with reach direction. Moreover, reach direction as well as grasp object could be predicted from eye kinematics during the delay. Both eye velocity and eye position contributed to the prediction of reach direction. These results show that motor signals carry sufficient information to allow monkeys to solve STM tasks without using any mnemonic functions. Thus, the potential of animals to solve STM tasks using motor patterns is more diverse than previously recognized.
Neurobiol Learn Mem, 2019; 166
29940038: Sloin HE, Ruggiero G, Rubinstein A, Smadja Storz S, Foulkes NS, Gothilf Y
Interactions between the circadian clock and TGF-β signaling pathway in zebrafish.
TGF-β signaling is a cellular pathway that functions in most cells and has been shown to play a role in multiple processes, such as the immune response, cell differentiation and proliferation. Recent evidence suggests a possible interaction between TGF-β signaling and the molecular circadian oscillator. The current study aims to characterize this interaction in the zebrafish at the molecular and behavioral levels, taking advantage of the early development of a functional circadian clock and the availability of light-entrainable clock-containing cell lines.
PLoS One, 2018; 13
27354160: Rubinstein A, Bracha N, Rudner L, Zucker N, Sloin HE, Chor B
BioNSi: A Discrete Biological Network Simulator Tool.
Modeling and simulation of biological networks is an effective and widely used research methodology. The Biological Network Simulator (BioNSi) is a tool for modeling biological networks and simulating their discrete-time dynamics, implemented as a

Cytoscape App. BioNSi includes a visual representation of the network that enables researchers to construct, set the parameters, and observe network behavior under various conditions. To construct a network instance in BioNSi, only partial, qualitative biological data suffices. The tool is aimed for use by experimental biologists and requires no prior computational or mathematical expertise. BioNSi is freely available at http://bionsi.wix.com/bionsi , where a complete user guide and a step-by-step manual can also be found.
J Proteome Res, 2016; 15

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BOARD NUMBER: S05-033

CEREBELLAR CONTROL OF A UNITARY SENSE OF DIRECTION

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The head-direction system, a key neural circuit in the animal's navigation system, consists of several anatomical structures with neurons that fire selectively to the animal's head direction. Population activity of these cells exhibits a ubiquitous temporal organization across different regions of the head-direction system, independent of the animal's behavioral state or sensory inputs, creating a single, stable, and persistent head-direction signal. However, the mechanistic processes behind this temporal organization are unknown. Since the cerebellum is involved in processing sensory signals, we examined how suppressing two forms of cerebellar plasticity in Purkinje cells (PKC-dependent synaptic depression and PP2B-dependent potentiation) affects the head-direction system. We first show that head-direction cell pairs from the anterodorsal thalamus and retrosplenial cortex exhibit a robust and stable neuronal coordination in the presence of an external landmark in both controls and cerebellar alteration conditions. However, when the external landmark cue was removed, neuronal coordination between the thalamus and retrosplenial cortex was abolished following the suppression of cerebellar PKC-dependent plasticity. In addition, we identify distinct roles for cerebellar PP2B- and PKC-dependent mechanisms in maintaining a stable thalamocortical head-direction signal during navigation by external landmark and self-motion inputs, respectively. These results identify new roles for the cerebellum in mediating a unitary and stable head-direction signal across the head-direction system.

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BOARD NUMBER: S05-034

THE INTERNAL DIRECTION SIGNAL IN MEDIAL ENTORHINAL CORTEX/PARASUBICULUM IS QUALITATIVELY DIFFERENT FROM THE HEAD DIRECTION SIGNAL IN UPSTREAM BRAIN REGIONS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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A large proportion of cells in the medial entorhinal cortex and parasubiculum (MEC/PaS) encode an internal direction (ID) signal that differs from head direction (HD) with theta-rhythmic, left-right-alternating dynamics (Gardner et al, same session). We were motivated to investigate where and how the ID signal is generated and asked if we could find features of ID in earlier stages of the classical HD circuit. To address this question, we recorded neural activity from hundreds of cells with Neuropixels probes in MEC/PaS and presubiculum (PrS) or anterodorsal thalamus (ADn) in rats during open field foraging, linear track running and sleep. We extracted the directional signals encoded by cells in each region by applying dimensionality reduction methods to population spike trains. The directional signals in ADn/PrS faithfully tracked HD, while the directional signal in MEC/PaS displayed left-right-alternating dynamics. During exploration and REM sleep, we observed occasional ‘decoupling events’ where the directional signals in ADn/PrS and MEC/PaS drifted independently. Except for during ‘decoupling events’, the ID signal in MEC/PaS was centred around the HD signal in ADn/PrS, and the anchoring between the two signals remained unchanged during sleep and after environmental manipulations. In summary, there is a functional split between the directional codes in ADn/PrS, which reliably track HD, and the ID signal in MEC/PaS. The two signals are correlated but differ on the theta-timescale and can transiently decouple. HD and ID are similarly anchored across brain states, suggesting that they might be different directional signals operating within the same cognitive map.

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BOARD NUMBER: S05-035

A FULLY AUTOMATED MAZE APPARATUS TO CHARACTERIZE SPATIAL STRATEGIES IN CLUTTERED ENVIRONMENTS

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Spatial navigation relies on diverse strategies and mechanisms that depend on the nature and familiarity of environments. For instance, animals preferentially use distal landmark/vector-relationship to goals in open environments while they might use the topology of local cues in obstacle-rich environments. How animal trajectories to goal locations develop over time in different environments still remain unclear. For this purpose, we developed a fully motorized maze apparatus, characterized by a central arena enclosure with multiple side doors, a tainted plexiglass cover, and two home boxes that move around the arena perimeter, alternatively serving as start and goal positions for the animal trajectories. We tested two distinct environments: an open arena, with two luminous objects on the periphery acting as orienting cues, and an arena enriched with scattered objects, constraining the animal paths but not the visibility of the two luminous objects on the periphery. On each trial, mice entered the arenas from a randomly chosen start position and had to navigate to the same goal position to receive water rewards. We monitored mouse trajectories across 15 days (10 trials per day). Exit mistake numbers, trajectory lengths and trajectory durations were reduced across days. All mice favored a strategy of circling in counter-clockwise direction in the open arena while they develop preferences for specific trajectory segments in the cluttered arena. These results demonstrate the effectiveness of the apparatus to investigate spatial learning and navigation strategies in cluttered environments.

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BOARD NUMBER: S05-036

ASTROCYTE-NEURON COMMUNICATION IN THE MOUSE HIPPOCAMPUS DURING VIRTUAL NAVIGATION

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Spatial navigation is crucial for animal survival. While previous work identified neuronal place cells in the hippocampus as key cellular correlates of spatial navigation, the involvement of glia in this process is less understood. Recent work demonstrates that intracellular calcium signals in astrocytes, a main type of glial cells, encode information about the animal's position and that information encoded into astrocytes is complementary to that encoded in the activity of nearby hippocampal neurons. Since variations in the intracellular concentration of calcium in astrocytes modulate the activity of surrounding synapses and neurons, we tested whether the perturbation of astrocytic calcium signalling in the hippocampus affects the neuronal representation of space. To this aim, we manipulated astrocytic calcium dynamics using pharmacogenetics, while simultaneously imaging pyramidal neurons in the CA1 hippocampal region of head-fixed mice running in a virtual corridor. We used viral injections to specifically express the Gq-coupled DREADD receptor in astrocytes and GCaMP6f in neurons and we performed two-photon functional imaging before and after DREADD activation with CNO. Preliminary results using information theory show that DREADD activation alters the information content of neuronal place cells. Further analyses of the features of neuronal place cells upon manipulation of astrocytic signalling are currently ongoing. These preliminary findings indicate that perturbation of astrocytic calcium dynamics influences the neuronal representation of space and suggest that the complementary place-dependence of the astrocytic calcium dynamics may facilitate the emergence of dynamic, context-dependent changes in population coding of CA1 neurons.

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BOARD NUMBER: S05-037

MAPPING SPACE WITH INTERNALLY GENERATED THETA SEQUENCES

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Flexible spatial navigation requires the representation of past, present and future locations. In mammals, this is thought to rely on hippocampal place cells. Within each cycle of the theta oscillation, place cells represent a temporally compressed trajectory starting behind the animal and reaching ahead. Assuming the animal ran at its typical running speed through each location, these theta trajectories would start at the position the animal had reached a fixed time interval into the past, and extend to the position the animal would reach a fixed time interval into the future (Parra-Barrero et al., 2021). These behavior-dependent theta sweeps result in longer theta trajectories where animals typically run faster, which is accompanied by bigger place fields with shallower phase precession in those areas. Here we propose a computational model for the neural mechanism underlying these effects. Spatial locations are mapped onto internally generated theta sequences that progress at a fixed pace in a neural space of simulated hippocampal cells by learning the associations between an array of sensory features and the hippocampal cells. As a result, locations mapped onto neighboring cells are further apart the faster the animal runs through an area. Hence, in areas of higher running speed, place fields are larger and less dense, and have shallower phase precession, which in turn leads to longer theta trajectories. Our results highlight the important role of intrinsic network structure and reveal how the hippocampal code can arise from the interplay between predefined internal dynamics, behavior and sensory input.

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BOARD NUMBER: S05-038

FUNCTIONAL INDEPENDENCE OF GRID CELL MODULES DURING HIPPOCAMPAL REMAPPING

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Grid cells in the medial entorhinal cortex (MEC) create a universal metric of space that may be a basis for environment-specific maps and memories in the hippocampus. Grid cells are organized into discrete modules: All cells within a module operate coherently, but it is thought that different modules could, under some circumstances, operate independently of each other. This independence may underlie the generation of unique hippocampal maps for each environment. However, experimental evidence for discordant responses among grid modules is limited, and it has never been shown in conjunction with place cell remapping. To test whether grid modules realign independently during place cell remapping between distinct environments, we chronically implanted Neuropixels probes in MEC and hippocampus in rats to simultaneously record multiple grid modules and place cells. We found that place cells exhibited robust and stable remapping between rooms. There were only slight differences in rotation between grid modules, and this rotation was largely coherent with border and head direction cells. Despite this, each grid module shifted independently between rooms. The difference in the translation of grid modules was large: On average, we estimated that modules were separated by at least 20 cm. Realignment within each module was coherent, consistent with each module functioning as a continuous attractor network. Thus, these findings are the first experimental evidence demonstrating that functionally independent grid cell modules may underlie hippocampal remapping. We are currently investigating how grid modules (and other functional cell types) respond under conditions that elicit partial remapping in place cells.

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BOARD NUMBER: S05-039

GRID CELLS RESCALE TO MATCH GOAL DISTANCE DURING PATH-INTEGRATION

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Spatial navigation rely on both landmarks and path integration information. While grid cells in the medial entorhinal cortex (MEC) display periodic firing fields that are believed to provide an intrinsic metric and to support path-integration, they are also controlled by landmarks as they typically encode stable locations of the environment. It is still unknown whether grid cells can compute reward distances and support goal-direct navigation in contexts that require the abstraction of environmental cues. To test this, we performed silicon probe recordings of MEC cells in mice that were running consecutively in an open field and on a cue-enriched treadmill belt. In the treadmill, the mice needed to use path integration to anticipate a water reward that was delivered after a fixed travel distance (shorter than the belt length). Grid cells identified in the open field showed periodic field activity in the treadmill belt. Interestingly, the spatial period of grid cells varied over time, often matching submultiples of the reward distance or of the belt length. Grid cell ensembles switched synchronously, in a coherent manner, between time windows where grid cell periodicities matched reward distance and time windows where they matched the belt length. During reward distance-matching time windows, the firing fields of grid cells were better aligned to reward locations and mouse anticipatory lickings were more accurate.
These results suggest that grid cells can flexibly rescale to match reward distance and support goal-directed navigation under path integration.

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BOARD NUMBER: S05-040

ASSESSING SPATIAL CODING IN LARGE SCALE ENVIRONMENTS USING VIRTUAL REALITY, ELECTROPHYSIOLOGICAL RECORDINGS AND DEEP LEARNING

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Animals can flexibly navigate their environment by following different paths toward goals. This ability is thought to rely on a cognitive map. Place cells in the hippocampus, a key brain structure for spatial navigation, are pyramidal neurons that fire action potentials whenever an animal is at a specific position within its environment (their place field). Place cell coding and navigation have been studied for decades in small (e.g. 1m in size) homogeneous environments. However, in the wild, animals including rodents can navigate several kilometers away to various food locations through an inhomogeneous landscape. During such large-scale navigation, different parts of the environment could be coded at different spatial resolutions through “nested hierarchies” of coarse and fine grain coding with large and small place fields observed for the same neurons (Eliav et al., 2021; Harland et al., 2021). However, it is unclear how place fields size and number are influenced by specific features of the environment. To address this question, we developed a behavioral task where mice navigate a 30 m long curved path in a 100 m^2 semi-realistic virtual environment with inhomogeneous distribution of visual cues and rewards. In addition, we measure pupil dilation and eye movements to control for visual attention. To better understand how visual information alone could influence the precision of self-location, we further trained an artificial neural network to self-locate using snapshots of the virtual environment. Using this framework, we will investigate the modulation of spatial coding resolution during large-scale navigation in inhomogeneous environments.

FENS Forum 2022
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BOARD NUMBER: S05-041

A TIME-COMPENSATED SUN COMPASS HELPS JUVENILE BALTIC HERRING ORIENT

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The Atlantic herring (*Clupea harengus*), an ecological and economical key species in the Northern Hemisphere, shows pronounced seasonal migratory behaviour from their feeding areas to overwintering grounds to their spawning areas. Goal oriented migration over distances of several hundred kilometres is a difficult task and requires the right cues for orientation and navigation. Fish can use a range of sensory cues for orientation and long-distance navigation, such as magnetic sensing or sun compass. So far, only non-migrating Atlantic herring larvae at the age of 14-28 days post hatching had been tested in different orientation experiments. Results of this study propose the potential use of a sun compass, but could not exclude the use of other orientational cues within the experiment. In our study we tested juveniles shortly before they start their migration in a controlled experiment focusing solely on the sun as a visual cue. The herring showed a significant orientation to the east-southeast (mean vector 108°) which corresponds to a natural migration route. For confirmation of a time-compensated sun compass, we clock-shifted a second experimental group 6 hours backwards. This group showed a significant group orientation towards north-northeast (mean vector 33°). After compensating for the sun azimuth shift during the day, we found no significant difference between the two datasets. For the first time we showed that juvenile Baltic herring use a time-compensated sun compass.

Pubmed:
34257800: Spiecker L, Witte I, Mehlig J, Shah V, Meyerhöfer M, Haehnel PS, Petermann V, Schüler A, More P, Cabezas-Wallscheid N, Horke S, Pautz A, Daiber A, Sasca D, Kindler T, Kleinert H
Deficiency of Antioxidative Paraoxonase 2 (Pon2) Leads to Increased Number of Phenotypic LT-HSCs and Disturbed Erythropoiesis.
Long-term hematopoietic stem cells (LT-HSCs) reside in bone marrow niches with tightly controlled reactive oxygen species (ROS) levels. ROS increase results into LT-HSC differentiation and stem cell exhaustion. Paraoxonase 2 (PON2) has been shown to be important for ROS control.
Oxid Med Cell Longev, 2021; 2021
32503260: Schoepf CL, Zeidler M, Spiecker L, Kern G, Lechner J, Kummer KK, Kress M
Selected Ionotropic Receptors and Voltage-Gated Ion Channels: More Functional Competence for Human Induced Pluripotent Stem Cell (iPSC)-Derived Nociceptors.
Preclinical research using different rodent model systems has largely contributed to the scientific progress in the pain field, however, it suffers from interspecies differences, limited access to human models, and ethical concerns. Human induced pluripotent stem cells (iPSCs) offer major advantages over animal models, i.e., they retain the genome of the donor (patient), and thus allow donor-specific and cell-type specific research. Consequently, human iPSC-derived nociceptors (iDNs) offer intriguingly new possibilities for patient-specific, animal-free research. In the present study, we characterized iDNs based on the expression of well described nociceptive markers and ion channels, and we conducted a side-by-side comparison of iDNs with mouse sensory neurons. Specifically, immunofluorescence (IF) analyses with selected markers including early somatosensory transcription factors (BRN3A/ISL1/RUNX1), the low-affinity nerve growth factor receptor (p75), hyperpolarization-activated cyclic nucleotide-gated channels (HCN), as well as high voltage-gated calcium channels (VGCC) of the Ca2 type, calcium permeable TRPV1 channels, and ionotropic GABA receptors, were used to address the characteristics of the iDN phenotype. We further combined IF analyses with microfluorimetric Ca measurements to address the functionality of these ion channels in iDNs. Thus, we provide a detailed morphological and functional characterization of iDNs, thereby, underpinning their enormous potential as an animal-free alternative for human specific research in the pain field for unveiling pathophysiological mechanisms and for unbiased, disease-specific personalized drug development.
Brain Sci, 2020; 10

31208035: Kalpachidou T, Spiecker L, Kress M, Quarta S
Rho GTPases in the Physiology and Pathophysiology of Peripheral Sensory Neurons.
Numerous experimental studies demonstrate that the Ras homolog family of guanosine triphosphate hydrolases (Rho GTPases) Ras homolog family member A (RhoA), Ras-related C3 botulinum toxin substrate 1 (Rac1) and cell division cycle 42 (Cdc42) are important regulators in somatosensory neurons, where they elicit changes in the cellular cytoskeleton and are involved in diverse biological processes during development, differentiation, survival and regeneration. This review summarizes the status of research regarding the expression and the role of the Rho GTPases in peripheral sensory neurons and how these small proteins are involved in development and outgrowth of sensory neurons, as well as in neuronal regeneration after injury, inflammation and pain perception. In sensory neurons, Rho GTPases are activated by various extracellular signals through membrane receptors and elicit their action through a wide range of downstream effectors, such as Rho-associated protein kinase (ROCK), phosphoinositide 3-kinase (PI3K) or mixed-lineage kinase (MLK). While RhoA is implicated in the assembly of stress fibres and focal adhesions and inhibits neuronal outgrowth through growth cone collapse, Rac1 and Cdc42 promote neuronal development, differentiation and neuroregeneration. The functions of Rho GTPases are critically important in the peripheral somatosensory system; however, their signalling interconnections and partially antagonistic actions are not yet fully understood.
Cells, 2019; 8
29439952: Ebert J, Wilgenbus P, Teiber JF, Jurk K, Schwierczek K, Döhrmann M, Xia N, Li H, Spiecker L, Ruf W, Horke S
Paraoxonase-2 regulates coagulation activation through endothelial tissue factor.
Oxidative stress and inflammation of the vessel wall contribute to prothrombotic states. The antioxidative protein paraoxonase-2 (PON2) shows reduced expression in human atherosclerotic plaques and endothelial cells in particular. Supporting a direct role for PON2 in cardiovascular diseases, deficiency in mice promotes atherogenesis through incompletely understood mechanisms. Here, we show that deregulated redox regulation in deficiency causes vascular inflammation and abnormalities in blood coagulation. In unchallenged mice, we find increased oxidative stress and endothelial dysfunction. Bone marrow transplantation experiments and studies with endothelial cells provide evidence that increased inflammation, indicated by circulating interleukin-6 levels, originates from deficiency in the vasculature. Isolated endothelial cells from mice display increased tissue factor (TF) activity in vitro. Coagulation times were shortened and platelet procoagulant activity increased in mice relative to wild-type controls. Coagulation abnormalities of mice were normalized by anti-TF treatment, demonstrating directly that TF increases coagulation. PON2 reexpression in endothelial cells by conditional reversal of the knockout cassette, restoration in the vessel wall using bone marrow chimeras, or treatment with the antioxidant -acetylcysteine normalized the procoagulant state. These experiments delineate a PON2 redox-dependent mechanism that regulates endothelial cell TF activity and prevents systemic coagulation activation and inflammation.
Blood, 2018; 131
26488403: Kokkinopoulou M, Spiecker L, Messerschmidt C, Barbeck M, Ghanaati S, Landfester K, Markl J
On the Ultrastructure and Function of Rhogocytes from the Pond Snail Lymnaea stagnalis.
Rhogocytes, also termed "pore cells", occur as solitary or clustered cells in the connective tissue of gastropod molluscs. Rhogocytes possess an enveloping lamina of extracellular matrix and enigmatic extracellular lacunae bridged by cytoplasmic bars that form 20 nm diaphragmatic slits likely to act as a molecular sieve. Recent papers highlight the embryogenesis and ultrastructure of these cells, and their role in heavy metal detoxification. Rhogocytes are the site of hemocyanin or hemoglobin biosynthesis in gastropods. Based on electron microscopy, we recently proposed a possible pathway of hemoglobin exocytosis through the slit apparatus, and provided molecular evidence of a common phylogenetic origin of molluscan rhogocytes, insect nephrocytes and vertebrate podocytes. However, the previously proposed secretion mode of the respiratory proteins into the hemolymph is still rather hypothetical, and the possible role of rhogocytes in detoxification requires additional data. Although our previous study on rhogocytes of the red-blooded (hemoglobin-containing) freshwater snail Biomphalaria glabrata provided much new information, a disadvantage was that the hemoglobin molecules were not unequivocally defined in the electron microscope. This made it difficult to trace the exocytosis pathway of this protein. Therefore, we have now performed a similar study on the rhogocytes of the blue-blooded (hemocyanin-containing) freshwater snail Lymnaea stagnalis. The intracellular hemocyanin could be identified in the electron microscope, either as individual molecules or as pseudo-crystalline arrays. Based on 3D-electron microscopy, and supplemented by in situ hybridization, immunocytochemistry and stress response experiments, we provide here additional details on the structure and hemocyanin biosynthesis of rhogocytes, and on their response in animals under cadmium and starvation stress. Moreover, we present an advanced model on the release of synthesized hemocyanin molecules through the slit apparatus into the hemolymph, and the uptake of much smaller particles such as cadmium ions from the hemolymph through the slit apparatus into the cytoplasm.
PLoS One, 2015; 10


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BOARD NUMBER: S05-042

GEOMETRY PRESERVED SCHEMA OF SPACE IN THE ORBITOFRONTAL CORTEX

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Planning efficient navigation strategies requires a geometry preserved internal map of the environment that enables estimating distances between two given locations. Previous research has identified place cells and grid cells in the hippocampus and the parahippocampal cortices that fire specifically when an animal visits a particular location(s), resulting in each location being encoded by different sets of neurons. However, it is unclear how such maps can estimate the distance between two locations that may be represented by entirely separate populations of neurons. Here we report a new kind of spatial map in the orbitofrontal cortex (OFC) that preserves the relative geometry between locations in space. In an environment with multiple equally spaced reward locations, OFC neural ensembles exhibit distinct and ordered firing patterns for each location. Further analysis revealed that the difference in the ensemble firing patterns representing two locations is proportional to the distance between the locations in physical space. Next, to test the generalizability of these maps, we trained animals in two geometrically distinct environments but with identically spaced reward locations. We observed that the OFC formed similar geometry preserved spatial maps of reward locations in both environments which could be further aligned using a linear transformation. Taken together, the OFC forms a geometrically preserved schema of locations that generalizes across environments, making it a potentially crucial brain region for navigational decisions.

Pubmed:
34707289: Basu R, Gebauer R, Herfurth T, Kolb S, Golipour Z, Tchumatchenko T, Ito HT
The orbitofrontal cortex maps future navigational goals.
Accurate navigation to a desired goal requires consecutive estimates of spatial relationships between the current position and future destination throughout the journey. Although neurons in the hippocampal formation can represent the position of an animal as well as its nearby trajectories, their role in determining the destination of the animal has been questioned. It is, thus, unclear whether the brain can possess a precise estimate of target location during active environmental exploration. Here we describe neurons in the rat orbitofrontal cortex (OFC) that form spatial representations persistently pointing to the subsequent goal destination of an animal throughout navigation. This destination coding emerges before the onset of navigation, without direct sensory access to a distal goal, and even predicts the incorrect destination of an animal at the beginning of an error trial. Goal representations in the OFC are maintained by destination-specific neural ensemble dynamics, and their brief perturbation at the onset of a journey led to a navigational error. These findings suggest that the OFC is part of the internal goal map of the brain, enabling animals to navigate precisely to a chosen destination that is beyond the range of sensory perception.
Nature, 2021; 599
28957665: Basu R, Duan X, Taylor MR, Martin EA, Muralidhar S, Wang Y, Gangi-Wellman L, Das SC, Yamagata M, West PJ, Sanes JR, Williams ME
Heterophilic Type II Cadherins Are Required for High-Magnitude Synaptic Potentiation in the Hippocampus.
Hippocampal CA3 neurons form synapses with CA1 neurons in two layers, stratum oriens (SO) and stratum radiatum (SR). Each layer develops unique synaptic properties but molecular mechanisms that mediate these differences are unknown. Here, we show that SO synapses normally have significantly more mushroom spines and higher-magnitude long-term potentiation (LTP) than SR synapses. Further, we discovered that these differences require the Type II classic cadherins, cadherins-6, -9, and -10. Though cadherins typically function via trans-cellular homophilic interactions, our results suggest presynaptic cadherin-9 binds postsynaptic cadherins-6 and -10 to regulate mushroom spine density and high-magnitude LTP in the SO layer. Loss of these cadherins has no effect on the lower-magnitude LTP typically observed in the SR layer, demonstrating that cadherins-6, -9, and -10 are gatekeepers for high-magnitude LTP. Thus, Type II cadherins may uniquely contribute to the specificity and strength of synaptic changes associated with learning and memory.
Neuron, 2017; 96
26575286: Martin EA, Muralidhar S, Wang Z, Cervantes DC, Basu R, Taylor MR, Hunter J, Cutforth T, Wilke SA, Ghosh A,

Williams ME
The intellectual disability gene Kirrel3 regulates target-specific mossy fiber synapse development in the hippocampus.
Synaptic target specificity, whereby neurons make distinct types of synapses with different target cells, is critical for brain function, yet the mechanisms driving it are poorly understood. In this study, we demonstrate Kirrel3 regulates target-specific synapse formation at hippocampal mossy fiber (MF) synapses, which connect dentate granule (DG) neurons to both CA3 and GABAergic neurons. Here, we show Kirrel3 is required for formation of MF filopodia; the structures that give rise to DG-GABA synapses and that regulate feed-forward inhibition of CA3 neurons. Consequently, loss of Kirrel3 robustly increases CA3 neuron activity in developing mice. Alterations in the Kirrel3 gene are repeatedly associated with intellectual disabilities, but the role of Kirrel3 at synapses remained largely unknown. Our findings demonstrate that subtle synaptic changes during development impact circuit function and provide the first insight toward understanding the cellular basis of Kirrel3-dependent neurodevelopmental disorders.
Elife, 2015; 4
25837840: Basu R, Taylor MR, Williams ME
The classic cadherins in synaptic specificity.
During brain development, billions of neurons organize into highly specific circuits. To form specific circuits, neurons must build the appropriate types of synapses with appropriate types of synaptic partners while avoiding incorrect partners in a dense cellular environment. Defining the cellular and molecular rules that govern specific circuit formation has significant scientific and clinical relevance because fine scale connectivity defects are thought to underlie many cognitive and psychiatric disorders. Organizing specific neural circuits is an enormously complicated developmental process that requires the concerted action of many molecules, neural activity, and temporal events. This review focuses on one class of molecules postulated to play an important role in target selection and specific synapse formation: the classic cadherins. Cadherins have a well-established role in epithelial cell adhesion, and although it has long been appreciated that most cadherins are expressed in the brain, their role in synaptic specificity is just beginning to be unraveled. Here, we review past and present studies implicating cadherins as active participants in the formation, function, and dysfunction of specific neural circuits and pose some of the major remaining questions.
Cell Adh Migr, 2015; 9
20430700: Sabarinathan R, Basu R, Sekar K
ProSTRIP: A method to find similar structural repeats in three-dimensional protein structures.
The occurrence of similar structural repeats in a protein structure has evolved through gene duplication. These repeats act as a structural building block and form more than one compact structural and functional unit called a repeat domain. The protein families comprising similar structural repeats are mainly involved in protein-protein interactions as well as binding to other ligand molecules. The identification of internal sequence repeats in the primary structure is not sufficient for the analysis of structural repeats. Thus, a new method called ProSTRIP has been developed using dynamic programming to find the similar structural repeats in a three-dimensional protein structure. The detection of these repeats is made by calculating the protein backbone Calpha angles. An internet computing server is also created by implementing this method and enables graphical visualization of the results. It can be freely accessed at http://cluster.physics.iisc.ernet.in/prostrip/.
Comput Biol Chem, 2010; 34
19646441: Dey T, Basu R, Ghosh SK
Entamoeba invadens: cloning and molecular characterization of chitinases.
Entamoeba histolytica, the causative agent of amebiasis infects through its cyst form and this transmission may be blocked using encystation specific protein as drug target. In this study, we have characterized the enzyme chitinase which express specifically during encystation. The reptilian parasite Entamoeba invadens, used as a model for encystation study contain three chitinases. We report the molecular cloning, over-expression and biochemical characterization of all three E. invadens chitinase. Cloned chitinases were over-expressed in bacterial system and purified by affinity chromatography. Their enzymatic profiles and substrate cleaving patterns were characterized. All of them showed binding affinity towards insoluble chitin though two of them lack the chitin binding domain. All the chitinases cleaved and released dimmers from the insoluble substrate and act as an exochitinase. Homology modeling was also done to understand the substrate binding and cleavage pattern.
Exp Parasitol, 2009; 123

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BOARD NUMBER: S05-043

SIGNATURES OF RAPID PLASTICITY IN HIPPOCAMPAL CA1 REPRESENTATIONS DURING NOVEL EXPERIENCES

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Neurons in the hippocampus exhibit striking selectivity for specific combinations of sensory features, forming representations which are thought to subserve episodic memory. Even during a completely novel experience, ensembles of hippocampal "place cells" are rapidly configured such that the population sparsely encodes visited locations, stabilizing within minutes of the first exposure to a new environment. What cellular mechanisms enable this fast encoding of experience? Here we leverage virtual reality and neural population recordings to dissect the effects of novelty and experience on the dynamics of place field formation. We show that the place fields of many CA1 neurons transiently shift locations and modulate the amplitude of their activity immediately after place field formation, rapid changes in tuning that are predicted by behavioral time scale synaptic plasticity. These correlates were particularly enriched during initial exploration of a novel context and decayed with experience. Our data suggest that novelty modulates the effective learning rate in CA1, favoring rapid mechanisms of field formation to encode new experience.

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BOARD NUMBER: S05-044

MULTIPLEXING OF SPATIAL AND TEMPORAL INFORMATION IN PREFRONTAL CORTEX DURING COMPLEX BEHAVIOR.

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Prefrontal cortex has long been associated with higher order cognitive functions, such as working memory, decision making and rule and category encoding. Neural representations of task-relevant variables have been described in animals performing many behavioral tasks. Single neurons in prefrontal cortex are often selective for multiple variables (mixed selectivity). The principles of how neurons are endowed with functional selectivity are not well understood. We analyzed space and time encoding in rats performing a memory-guided navigation task with two behaviorally important spatial categories, starts and goals. In each trial the animal navigated from one of three start locations to one of three goal locations via one of multiple routes. Using chronically implanted Neuropixels probes, we found that all six locations as well as the category to which it belongs (goal or start) were encoded with high accuracy. While at either start or goal locations, time was encoded by cells' firing ramping down or, more rarely, up, instead of as a sequence of cells' firing. This time code was invariant to the location within a category, even though single neurons could be selective for both time and space. The encoding of spatial locations was roughly orthogonal to time encoding and largely stable over time. The overlap between the population of cells that encoded goal and start locations was higher than expected by chance, pointing towards a functional specialization of cells to encode specific variables relevant to performing behavioral tasks.

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BOARD NUMBER: S05-045

LONG-TERM DYNAMICS OF THE ENTORHINAL GRID CODE

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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The hippocampus and entorhinal cortex (mEC) form an “information loop”: Superficial layers of the mEC provide excitatory input to the hippocampus, and the deep layers of the mEC receive most of their input from the hippocampus. The mEC is crucial for the formation of spatial memory, and its inactivation impairs both recent and remote memory retrieval in navigation tasks- indicating a key role for the entorhinal in long term memory. Recent studies found that hippocampal place codes gradually change over days and weeks when the animal repeatedly visits a fixed familiar environment. However, the long-term dynamics of entorhinal spatial codes have remained unexplored. Here, we developed a novel imaging preparation that enables simultaneous imaging of hundreds of grid cells from up to four different modules within a single field of view in freely behaving mice. By tracking the same neurons over weeks, we longitudinally analyzed the long-term stability of grid cells, and compared it to that of hippocampal CA1 place cells. We found that entorhinal grid cells gradually changed their tuning over days, while maintaining activity rates. Conversely, hippocampal place cells displayed gradual changes in cell activity rates, but their spatial tuning remained relatively stable. Overall, we found a double dissociation between mEC and CA1 neurons with respect to properties of neural code stability, suggesting that different mechanisms may govern representational drift in these circuits. Moreover, our results suggest that stable tuning of entorhinal grid cells is not a prerequisite for stable tuning of hippocampal place cells.

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FUNCTIONALLY DISTINCT HIPPOCAMPAL RHYTHMS AND CIRCUITS PREDICT VALENCE OF THE SUBSEQUENT LOCOMOTION

POSTER SESSION 05 - SECTION: SPATIAL NAVIGATION AND MEMORY

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Theta oscillations are one of the most extensively investigated rhythms of the brain, closely linked to locomotion, diverse types of learning and memory, and controlled by the medial septum circuitry. Previous studies (Whishaw et al, 1973; Bland et al, 2006) have reported that theta frequency predicts different heights in a jump avoidance test. The widely accepted view, in accordance to the “sensorimotor integration model”, is that theta frequency predicts the vigour of the subsequent movement. However, the jump avoidance tests involve a strong fear component, leading to an elusive conclusion whether theta activity predicts solely movement or codes also for the related fear response. To address this conundrum, we designed an experiment in which we performed 2 Photon imaging in combination with oscillations recordings and pharmacological medial septum inhibition, in mice running on a treadmill. To investigate the predictive coding of a motor- and fear- related stimuli, we first introduced a brake, and subsequently an air puff stimulus at two different locations. The analysis of oscillatory activity revealed that theta frequency was largely unaffected by the learned position of the brake, while a strong relationship between the increased frequency and the location of the air puff stimulus was observed. Calcium imaging data revealed that brake- and puff- related cells form largely non-overlapping populations. Finally, we discuss the effect of the medial septum inhibition on both types of coding. These results indicate that distinct hippocampal rhythms and circuits predict the valence of the subsequent locomotor activity rather than movement per se.

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BOARD NUMBER: S05-046

RELATIONSHIPS BETWEEN THE EFFICIENCY OF TEMPORAL INFORMATION PROCESSING ON MILLI- AND SUPRA-SECOND TIME DOMAINS

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Temporal information processing (TIP) constitutes a neural underpinning of many cognitive functions. Although the previous studies indicated several temporal processing levels (or operational processing windows) in TIP, the between-levels relations remain unclear. This study is focussed on relationships between the efficiency on milli- and supra-second time levels and aimed at testing whether the better efficiency on milli-second level is accompanied by the better performance on supra-second one in young subjects. The participants were 118 healthy adults (M_{age} = 23). The measurement included two paradigms: (1) auditory temporal-order judgment task to assess subjects efficiency on millisecond domain, and (2) subjective accentuation task to evaluate their functioning on supra-second one. The former task was indexed by the temporal order threshold defined as the minimum gap between two successive sounds which is necessary to reproduce their order correctly which corresponded to the temporal resolution. During the latter task subjects were presented with various metronome beat frequencies and reported how many sounds they could unite into one perceptual unit indicating the extent of the temporal integration. The results revealed that subjects characterized by more efficient TIP on millisecond level could integrate beats in significantly longer units than those less efficient. Moreover, the former subjects in subjective accentuation relied more on a constant time rather than on the mental counting strategy. These findings provide the new insight into associations between TIP on milli- and supra-second time levels. Supported by National Science Centre, Poland, grant no. 2018/29/B/HS6/02038.

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BOARD NUMBER: S05-047

YOGA ALLEVIATES COGNITIVE IMPAIRMENT AND CARDIAC AUTONOMIC DYSFUNCTION IN BREAST CANCER PATIENTS RECEIVING CHEMOTHERAPY: A RANDOMIZED CONTROLLED STUDY.

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Aim: Evidence suggests that chemotherapy treatment for breast cancer (BC) is associated with adverse effects such as cognitive impairment and cardiac autonomic dysfunction. This study aimed to determine the effect of the Integrated Yoga Therapy Program (IYTP) on cognition, cardiac autonomic function, and quality of life (QOL) in BC patients undergoing chemotherapy. **Methods:** Eighty-two BC patients were randomly assigned to: IYTP intervention (n = 43) or control group (n = 39). All patients underwent assessment at two-time points: baseline (before chemotherapy) and after six cycles of chemotherapy, for the following variables: a) Neuropsychological test battery: assessing various domains of cognition, include learning, memory, recall, focused attention, motor speed, planning and executive functioning; b) Heart rate variability; c) Montreal cognitive assessment scale and d) Perceived stress scale. **Results:** At baseline, both groups showed mild cognitive impairment with moderate stress and normal cardiac autonomic functioning. After six cycles of chemotherapy, the control group showed a significant decline in cognitive abilities in all domains with severe stress and reduced HRV, implying sympathovagal imbalance with sympathetic dominance. Whereas in the yoga group, after six cycles of chemotherapy, the cognitive abilities were preserved, with significant improvements in attention and planning. Furthermore, the yoga group has also shown increased HRV with modulation of cardiac autonomic function towards sympathovagal balance, proposing yoga to be an effective intervention for chemotherapy-induced adverse effects. **Conclusion:** The study suggests yoga as a potential adjuvant intervention for improving various domains of cognition, cardiac autonomic function and QOL in BC patients during chemotherapy.

Pubmed:
31763238: Chellaa R, Soumya MS, Inbaraj G, Nayar R, Saidha PK, Menezes VH, Rajeeva HN
Impact of Hatha Yoga on the Airway Resistances in Healthy Individuals and Allergic Rhinitis Patients.
There have been limited studies on Hatha yoga training as a complementary therapy to manage the symptoms of Allergic Rhinitis. The main Aim of the study was to check the impact of Hatha yogasanas on the Airway resistances in Healthy volunteers, a baseline data can be established and also to study the impact of Hatha yogasanas on the Airway resistances in Allergic Rhinitis patients in Bangalore, India. This is a prospective case series of 51 healthy volunteers (18 Males and 33 Females) Group 1 and 51 Allergic Rhinitis patients (18 Males and 33 Females) Group 2. The Objective analysis of the upper airway resistance was measured using a rhinomanometer and the lower airway resistance was measured using a spirometer. Then the subjects practiced specific Hatha yogasanas for three months. Then the airway resistance tests were again done at 3 months interval. The subjective analysis was done pre yoga and post yoga using the Short form-12 (SF-12) and Sino Nasal Outcome Test (SNOT) Questionnaires to assess the quality of life. The data was analyzed by doing a Paired (2-tailed) Test, using SPSS (Software Package for Social Sciences) version 16. Total Nasal Airway Resistance pre yoga and post yoga in 51 healthy volunteers had significantly reduced at 150 Pa and the Forced Vital Capacity(FVC) pre yoga and post yoga had significantly increased,Forced Expiratory volume (FEV1) & % Residual standard deviation (%RSD) had increased but not significant. The Physical component score (PCS) and Mental component score (MCS) of the SF-12 health survey questionnaire had significantly improved with and the SNOT questionnaire score had significantly reduced. The Total Nasal Airway Resistance in 51 Allergic Rhinitis had significantly reduced at 150 Pa and the FVC pre yoga and post yoga showed increase but change was not significant, FEV1 pre yoga and post yoga had significantly increased, %RSD pre yoga and post yoga had significantly increased. The PCS and MCS of the SF-12 health survey questionnaire had significantly increased and the SNOT questionnaire score had significantly decreased. The scientific documentation of the impact of Hatha Yoga on the

airway resistances can be an eye opener in the management of several other diseases of the airways.
Indian J Otolaryngol Head Neck Surg, 2019; 71

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BOARD NUMBER: S05-048

HOUSEHOLD TOBACCO SMOKING STATUS AND THE TEMPERAMENT DIMENSION OF EFFORTFUL CONTROL AMONG U.S. YOUNG CHILDREN

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Aims: The study objective was to assess the associations between household tobacco smoking status and the temperament dimension of effortful control among U.S. young children. **Methods:** We conducted a secondary analysis of 2019-2020 National Survey of Children's Health data including 11,100 children ages 3-5 years. Household tobacco smoking was defined as the child living with anyone who smoked cigarettes, cigars, or pipe tobacco. We assessed four ordinal response variables that measured effortful control, defined as the ability to manage attention and inhibit or activate behavior as needed, with 5-point scale responses ranging from always to never. Ordinal logistic regression models were built while adjusting for several covariates including child age, sex, race/ethnicity, health status, premature birth; parent education level; and family household structure and federal poverty level. **Results:** About 13% of children lived with a tobacco smoker. Children who lived with a smoker were at decreased odds to always keep working at something until they were finished (AOR=0.56, 95%CI=0.44-0.71) and able to sit still (AOR=0.56, 95%CI=0.44-0.71) compared to children who did not live with a smoker, after covariate adjustment. Children who lived with a smoker were at 1.59 increased odds (95%CI=1.24-2.04) to always be easily distracted than children who did not live with a smoker. No difference was found between household tobacco smoking status and following instructions to complete simple tasks. **Conclusions:** Household smoking is associated with lower effortful control among children ages 3-5 years, which is an important temperament dimension. Findings highlight the need to promote household tobacco cessation.

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BOARD NUMBER: S05-049

DISSOCIATION BETWEEN EXPLICIT AND IMPLICIT PRIORS IN HUMAN VISUAL PERCEPTION

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Perception must operate on noisy inputs that underdetermine sensory likelihood. To overcome this limitation and achieve high accuracy, perception relies on priors. Bayesian inference provides an optimal solution to combine likelihood and priors in perception. Many previous studies investigated the use of priors in normal and pathological perception, but with conflicting results. We propose that it is because priors were explicitly given to subjects or implicit (i.e. learned) in different studies. To test our hypothesis, we designed two experiments to characterize human computations underlying the perception of noisy images, with explicit and implicit priors. We compared choices of 217 subjects to an ideal Bayesian observer (and models capturing deviations from optimality) and quantified the weight of explicit and implicit priors in their inference. Subjects were less optimal when priors were explicit than implicit. In the explicit task, the weight of priors was more heterogeneous (and stronger on average) and the weight of likelihood weaker than in the implicit task. Across individuals, the likelihood weights were highly correlated between the two tasks whereas the prior weights were unrelated, marking a clear dissociation. This conclusion was true when using generative priors and priors adjusted to the subject's learning (using the best model out of 65 variants). Our results demonstrate that for the same visual stimuli, human perception makes a similar use of the likelihood but a different use of explicit and implicit priors. This distinction should be key to study the implementation of Bayesian inference and its alteration in diseases.

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BOARD NUMBER: S05-050

TEMPORAL LOOMING IMPROVES SYNCHRONISATION: ASYMMETRIES IN THE PREDICTION OF ACCELERATING SEQUENCES

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Studies on temporal prediction in sequences often conflate predictability with periodicity. However, recent evidence suggests that the benefits for perception often found in prediction apply just as readily to predictable acceleration. While these sequences are equally predictable, their implications in an ecological setup can be vastly different. An accelerating pattern may suggest, for example, that a critical event is imminent and may therefore require faster reactions. As such, we hypothesized that there would likely be asymmetries in sensorimotor synchronization (SMS) to these sequences, despite their mathematical similarity to deceleration. We asked participants to tap along with accelerating, rhythmic and decelerating sequences, keeping the early portion of the sequence tightly controlled. Participants achieved synchrony more rapidly for the accelerating sequences compared to both rhythm and deceleration. Further, tap variance was significantly lower for acceleration and rhythm relative to deceleration, even when controlling for interval size. These results imply a surprising conclusion: synchronisation is more readily achieved for accelerating sequences relative even to rhythmic stimuli. Further, we manipulated the rules by which intervals changed (i.e., absolute vs proportional). Regardless of the true acceleration rule, participants inherently tapped at the interval predicted by a proportional model, suggesting limited flexibility in our accelerating predictions. The results bare clear links to findings in looming stimuli, whereby growing visual stimuli engender faster reaction times and higher arousal than shrinking. We suggest that models of prediction and SMS take these findings into account by developing dynamical models that account for these nonlinearities in human sequence prediction.

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BOARD NUMBER: S05-051

ACCURACY IN SELF-MONITORING OF TEMPORAL ERRORS IN HUMANS AND RODENTS.

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Humans and rats have the ability to keep track of time with high precision and accuracy in a task where subjects have to press a lever for a certain amount of time to get a reward, suggesting the ability to monitor temporal errors. In a newly developed paradigm, rodents were able to report their temporal errors (Kononowicz, van Wassenhove, & Doyère, 2022). We adapted the procedure to human participants who were required to produce a target time interval and subsequently evaluate their error and compared two types of time production (duration of press vs time between two presses). During the training trials, participants were trained to associate a reward size, which was a function of error magnitude in their time production, with the location of one of two presented cards on the computer screen. The threshold for card/size assignment was set up such that it resulted in a 50% chance of receiving a reward after choosing the illuminated card. In test trials, the reward-card assignments remained, but both cards were illuminated and available for selection, thus, the amount of reward received was driven by the participants' choices. The results suggest that humans perform qualitatively similarly to rats. Participants showed high choice accuracy in test sessions demonstrating that they kept track of the values of the timing variables on which they based their decision. Here we will further focus on the quantitative comparison of human and rat performance in two data sets where both species produced a 2-sec duration.

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BOARD NUMBER: S05-052

VARIABILITY IN THE TIMING OF THE REPETITIVE MOVEMENTS IN PRESCHOOL CHILDREN

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Aims The study aimed to examine the temporal accuracy and precision in repetitive movement task. Methods The subjects were 106 children aged 5 to 6 years. Subjects performed rhythmic tapping with index finger at a frequency of 2 Hz. In the first 20 seconds, the subjects followed the metronome tones (1000 Hz, 50 ms), then the tones ended and the subjects had to continue tapping at the predetermined rate for another 30 seconds. The target inter-response interval (IRI) was 450 ms. The dependent variables were mean IRI, mean SD, and mean absolute error (AE - difference from the target interval). Children completed 5 trials, and for each subject and each trial the mean of IRI, SD and AE were calculated. To check the effect of trials, repeated measures ANOVA was computed. Results IRI mean values for trials dropped from 349.78 to 334.81 ms, from the first to the last trial. Variability expressed in terms of SD was high - mean values ranged from 86.94 to 94.61 ms. Also, children substantially underestimated the target interval in all of the trials. AE ranged from -149.65 up to -161.59 ms from the first to the last trial, which equals 33% to 36% of the target interval. However, none of the three separate within-subjects ANOVAs confirmed the effect of trials on the mean IRI, mean SD and mean AE, respectively. Conclusion Enhanced variability reflects the developmental differences. Follow up study may benefit from partitioning of the variance on the "clock" and motor implementation variance.

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ARE CHILDREN WITH "LIMITED PROSOCIAL EMOTIONS" EMOTIONALLY BLIND? EMOTIONAL PROCESSING AND FACIAL EMOTIONAL EXPRESSIONS IN RESPONSE TO THREE INTERVENTION PROGRAMS

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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The processing of emotions expressed by others have been supported as an important factor in children's moral and social development. Research indicated that the inability of individuals high on Callous-unemotional (CU) traits to recognize emotions might explain their reduced empathic reaction and increased antisocial behavior. This is study applies FaceReader methodology to assess the children's high on CU traits emotional processing and responding to different emotional contexts (i.e., happy, angry). By combining measures of facial emotional expression with socialization practices that enhance parental warmth and cognitive skills (i.e., identification of micro-expressions of different emotions) development, the current study aimed to assess the effectiveness of three different intervention programs in children's ability to respond adaptively. Specifically, we investigated the facial emotional expressions of children high on CU traits (n = 53), prior, immediately after and in a three-months period after the completion of the intervention. We identified an increase of sadness expression in response to distressing cues, and especially in expressions of fear, that can be attributed to an enhancement in children's ability to identify fear and share this emotional expression. The enhanced emotional engagement of children is also supported by an increase in anger and a decreased in surprised emotional expression. Expression of anger serves as an indication of the children response to provocation. Our findings draw research attention in the important influence of the family context and cognitive strategies employed in emotional processing in shaping the emotional functioning of children high on CU traits.

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BOARD NUMBER: S05-054

DOES HALLUCINATION PRONENESS ALTER SENSORY FEEDBACK IN EMOTIONAL SELF-VOICE PERCEPTION?

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Aims: Altered sensory feedback in self-generated speech may lead to auditory verbal hallucinations (AVH) in non-clinical and clinical voice hearers. Unlike non-clinical voice hearers, clinical voice hearers report hearing derogatory voices, accentuating their attentional bias towards negative emotions and salience (threat) misattribution. The current study altered the emotional quality and uncertainty in self-voice perception to elucidate how misattribution of self-generated speech results from neural changes in sensory feedback and/or attentional processes along the psychosis spectrum. **Methods:** Non-voice hearers, non-clinical and clinical voice hearers (age range of 16-40 years) participated in a standard button-press task, combined with EEG to elicit predictable (100% neutral and 100% angry self-voice) and unpredictable (60-40% neutral-angry, 50-50% neutral-angry and 40-60% neutral-angry self-voice) voices. Hallucination proneness (HP) is measured by the Launay Slade Hallucination Scale. Altered sensory feedback was expected to manifest in an event-related amplitude modulation of the N100 component. **Results:** Preliminary results of 15 participants show an N100 suppression effect in response to the fully predictable self-voice conditions, whereas unpredictable voices elicit a reduced N100 suppression effect. Furthermore, our results also show a trend towards an interaction between HP and self-voice quality. **Conclusions:** These findings suggest that sensory feedback processes are sensitive to the changes in the emotional quality of self-voice. Further, HP may alter self-voice processing as self-voice changes from neutral to emotional. These results constitute a critical advancement regarding the nature of AVH.

Pubmed:
31734443: Duggirala SX, Schwartze M, Pinheiro AP, Kotz SA
Interaction of emotion and cognitive control along the psychosis continuum: A critical review.
To better understand how emotion impacts cognitive control is important as both influence adaptive behavior in complex real-life situations. Performance changes in emotion and cognitive control as well as in their interaction are often described in psychotic patients as well as in non-clinical participants who experience psychosis-like symptoms. These changes are linked to low motivation and limited social interaction. However, it is unclear whether these changes are driven by emotion, cognitive control, or an interaction of both. This review provides an overview of neuroimaging evidence on the potential interaction of emotion and cognitive control along the psychosis continuum. The literature confirms that over-sensitivity towards negative and lowered sensitivity towards positive emotional stimuli in tasks exploring emotion-cognitive control interaction are associated with the severity of positive and negative symptoms in psychosis. Changes in the dynamic interplay between emotion and context-sensitive cognitive control, mediated by arousal, motivation, and reward processing may underlie poor interpersonal communication and real-life skills in psychosis. In addition, structural and functional changes in subcortical and cortical associative brain regions (e.g., thalamus, basal ganglia, and angular gyrus) may contribute to alterations in emotion and cognitive control interaction along the psychosis continuum. There is limited evidence on how antipsychotic medication and age at illness-onset affect this interaction.
Int J Psychophysiol, 2020; 147
26774462: Duggirala SX, Saharan S, Raghunathan P, Mandal PK
Stimulus-dependent modulation of working memory for identity monitoring: A functional MRI study.
While the neural correlates of identity monitoring working memory (WM) have been well characterised in literature, the WM subsystems for different types of stimuli have not been established. The aim of our study was to examine the neural network subtending WM for identity monitoring of both verbal and visual stimuli. We used functional magnetic resonance imaging (fMRI) with words, objects, and faces as stimuli in an n-back WM task to delineate the similarities and differences in brain

activation during presentation of verbal and visual stimuli. The results revealed a predominantly left lateralized core fronto-parieto-cerebellar identity WM network comprising bilateral insula, left inferior frontal gyrus, inferior parietal gyrus, and cerebellum that is common to all stimuli. In addition, our results showed stimulus-specific recruitment of brain regions, with exclusive activations in left inferior frontal gyrus and inferior temporal gyrus for identity WM for verbal stimuli, and left middle occipital gyrus and cerebellum for identity WM for visual stimuli. The present study reveals the existence of a central identity WM network for both verbal and visual information, along with activation of distinct verbal and visual representational regions that are sensitive to respective stimuli.
Brain Cogn, 2016; 102

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BOARD NUMBER: S05-055

UNPACKING RESTING STATE DYNAMICS IN HALLUCINATION-PRONE INDIVIDUALS USING A HIDDEN SEMI-MARKOV MODEL

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Aims: Hallucinatory experiences lie on a continuum ranging from healthy to pathological, which can be captured by hallucination proneness (HP). High HP is associated with an increased need for care and aberrant activity in functional networks at rest. However, the temporal dynamics of different networks and their contribution to HP are still elusive. We aim to bridge this gap by assessing changes in brain dynamics linked to HP using a Hidden Semi-Markov Model (HSMM) on resting state EEG (rsEEG) data. **Methods:** HSMM is a generative probabilistic modeling approach that characterizes hidden functional connectivity signatures on a sub-second timescale by allocating neural time-series data to a finite set of discrete hidden brain states. Ten minutes rsEEG data (5 min eyes-open, 5 min eyes-closed) were acquired in a sample of healthy participants varying in HP. The model was applied to cleaned and concatenated (multi-participant) alpha (8-12 Hz) amplitude envelopes. HP was assessed using the Launay-Slade Hallucination Scale. **Results:** Post-hoc statistics such as the state durations and between-state transition probabilities yield insights into the temporal dynamics of underlying functional networks. Preliminary results reveal that high HP relates to altered HSMM state durations. This suggests that the activation time of corresponding functional networks changed, which might indicate dysfunctional network engagement and stability. **Conclusion:** This study provides a new analytic take and perspective on HP in healthy participants. Accurate characterization of altered temporal dynamics linked to HP can inform about predictive disease markers and ultimately favors generating individual risk profiles, differential diagnosis, and targeted intervention.

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BOARD NUMBER: S05-056

THE ROLE OF VIRTUAL REALITY IN THE ASSESSMENT OF TIME PERCEPTION

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Time perception seems to be selectively impaired in right brain damaged (RBD) patients, who underestimate time durations whereas no time deficit has been found following left brain damage (LBD). However, so far time perception has been investigated only using computerized paradigms. Virtual Reality (VR) has recently been used as an effective tool for the assessment and rehabilitation of cognitive deficits. Aim of the present study is to measure the impact of time deficits in everyday life by using VR and computerized tasks. A group of RBD and LBD patients, as well as age matched controls were recruited in the study. Participants were submitted to a time reproduction task using computerized and VR paradigms. In the computerized task, participants were required to reproduce the duration of a previously encoded stimulus. In the VR task, participants were submitted to virtual scenarios where they had to reproduce the duration of 16 actions immediately after having performed them. In the computerized task, RBD patients reproduced *longer* time intervals with respect to controls and LBD. In the VR task, the difference between reproduction and execution revealed that RBD patient were *as accurate as* controls and LBD in reproducing everyday actions. In line with the literature, our study shows the elective role of the right hemisphere in processing time. Moreover, results suggest that patients are more accurate in estimating the duration of a performed action rather than the duration of a visual stimulus, leading to a possible use of VR in the rehabilitation of time deficits.

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BOARD NUMBER: S05-057

INVESTIGATING BRAIN-COGNITION ASSOCIATIONS IN BIPOLAR DISORDER USING CANONICAL CORRELATION ANALYSIS

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Aims: Bipolar Disorder is associated with a range of neuropsychological impairments compared to healthy controls, even in euthymia. Studies have linked cognitive impairment to abnormal brain structure in patient groups, however most studies to date use univariate methods to test brain-cognition associations, which do not account for relationships between cognitive functions. Given that cognitive impairment in mood disorder groups may be hierarchical in nature, we used multivariate methods to investigate associations between neuropsychological performance and brain structure. Methods: N=56 euthymic patients with bipolar disorder and N=26 healthy controls underwent structural MRI scans and completed a neuropsychological test battery. Cognitive scores and cortical thickness for each brain region were standardised based on healthy control data, then Canonical Correlation Analysis was utilised to test associations between cognitive performance and cortical thickness patients and controls separately. Results: In the patient group, the first canonical correlation showed a linear correlation between the first canonical variate pair, with the first canonical variate being associated with cortical thickness in temporal pole, inferior temporal cortex and pericalcarine cortex regions in both hemispheres, as well as insula and frontal pole regions in the right hemisphere, and medial orbitofrontal and parahippocampal regions in the left hemisphere. Performance on tests measuring attention and processing speed were most strongly associated with the first canonical variate in patients. Conclusions: Impairments in attention and processing speed may be particularly associated with cortical abnormalities in patients with Bipolar Disorder.

FENS Forum 2022
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BOARD NUMBER: S05-058

TEMPORAL EXPECTATIONS FACILITATE BEHAVIOUR IN THE ABSENCE OF CONCOMITANT SPATIAL EXPECTATIONS AND IN DYNAMICALLY UNFOLDING ENVIRONMENTS

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Attention can be proactively directed to locations in space, to object features and to points in time. In our environment, spatial, temporal and feature-based expectations interact to shape behaviour. Temporal expectations have been proposed to guide perception when accompanied by congruent spatial expectations. In two complementary studies, we set out to investigate whether cued temporal expectations can guide visual perception, even in the absence of spatial expectations, in a continuously changing environment. On each trial, participants (*online:* N = 49, *in-person*: N = 24) were presented with a stream of bilaterally appearing coloured circles, similar to a dual-stream rapid serial visual presentation task. Each stream was composed of three coloured targets and between 6 and 9 distractors. On each trial one of the targets always appeared at a fixed early time. One appeared at a fixed late time, and one could appear at any time. A coloured cue at the beginning of each trial indicated which of the three target circles was relevant. Specifically, participants were asked to respond to the side (left or right) in which the cued target appeared in any given trial. We found that participants were faster and more accurate in detecting targets that occurred at an expected time (early or late), compared to the randomly timed targets, even though participants had no information regarding the likely location of the target. From these results, we conclude that temporal expectations can improve behaviour in the absence of concomitant spatial expectations and in dynamically unfolding streams of stimuli.

Pubmed:

30021888: Maiarù M, Leese C, Certo M, Echeverria-Altuna I, Mangione AS, Arsenault J, Davletov B, Hunt SP
Selective neuronal silencing using synthetic botulinum molecules alleviates chronic pain in mice.
Chronic pain is a widespread debilitating condition affecting millions of people worldwide. Although several pharmacological treatments for relieving chronic pain have been developed, they require frequent chronic administration and are often associated with severe adverse events, including overdose and addiction. Persistent increased sensitization of neuronal subpopulations of the peripheral and central nervous system has been recognized as a central mechanism mediating chronic pain, suggesting that inhibition of specific neuronal subpopulations might produce antinociceptive effects. We leveraged the neurotoxic properties of the botulinum toxin to specifically silence key pain-processing neurons in the spinal cords of mice. We show that a single intrathecal injection of botulinum toxin conjugates produced long-lasting pain relief in mouse models of inflammatory and neuropathic pain without toxic side effects. Our results suggest that this strategy might be a safe and effective approach for relieving chronic pain while avoiding the adverse events associated with repeated chronic drug administration.
Sci Transl Med, 2018; 10

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BOARD NUMBER: S05-059

IGNORING ASYNCHRONIES AND DELAYS IS NECESSARY FOR THE SENSE OF IMMERSION: EVIDENCE FROM RESULTS IN PATIENTS WITH SCHIZOPHRENIA

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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The sensory consequences of our actions are predicted and adjusted in case of an error. Whether or not small delays in haptic feedback are taken into account depends on the task context. Healthy participants ignore small delays if mingled among large delays. Ignoring small delays may optimize our feeling of control and our sense of being immersed in the environment. Reversely, a deficit at filtering out small asynchronies may decrease the feeling of control. Recent results suggested a difficulty to ignore small asynchronies in patients with schizophrenia and we tested if this difficulty indeed leads to a drop of the feeling of control. 23 patients with schizophrenia, 18 patients with bipolar disorder, and 22 healthy participants performed a pointing task with a haptic device that provided haptic feedback without or with delays, which were processed consciously (65 ms) or unconsciously (15 ms). The trajectory of the hand was measured continuously, to track the deceleration in anticipation of the surface, and its modulation when sensory feedback was stable or unstable. Participants were asked to report their feeling of controlling the device. Following subliminally delayed haptic feedback, the feeling of control decreased, and deceleration durations were adapted, but only in patients with schizophrenia. These effects were correlated with psychotic symptoms. Overreactivity to subliminal delays was observed only when the distribution of delays was volatile. Ignoring small delays may participate to our adaptation and sense of immersion in an ever changing environment. In patients, not ignoring small delays may participate to psychotic symptoms.

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BOARD NUMBER: S05-060

THE SELF PROCESSES IN RUMINATION CONTEXT

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Abstract Flexibly switching between self-and non-self-related processes allows us to accommodate various external and internal demands. This flexibility, however, could be influenced by a particular condition, known as rumination. Rumination is defined as a condition when people continuously think about self-related thoughts. In pathological rumination conditions, people cannot flexibly switch their focus toward external stimuli, causing incoherent thoughts to the relevant condition. This study aims to investigate the relation of rumination and the switching process between self-related and non-self-related focus. We hypothesized that high rumination is associated with reduced flexibility in the self-related switching process. To examine this hypothesis, the rumination level was measured with the Ruminative response scale (RRS). A colour-switching task consisted of preference (self-related) and similarity (non-self-related) conditions that were adopted to examine flexibility. The result showed a unique pattern between rumination and cognitive flexibility with two different context tasks and potential further are suggested to improve the model of rumination measurement in cognitive approach. Keywords: rumination, task switching, self, cognitive flexibility, EEG

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BOARD NUMBER: S05-061

PREDICTION MISMATCH SIGNALLING IN ANTERIOR CINGULATE CORTEX DRIVES TASK-SWITCHING

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Flexibly switching between tasks is a hallmark of cognitive behaviour. During task-switching, animals rapidly update their knowledge of current rules or contexts by constantly evaluating and detecting mismatches between predicted and observed outcomes of events. The anterior cingulate cortex (ACC) is implicated in task-switching, as well as in prediction mismatch signalling. However, the neural circuit mechanisms underlying task-switching are largely unknown. Here we trained mice to perform an attention-switching task in which they alternated between blocks of distinct task rules. Mice typically required a single experience of an expectation violation to accurately adapt their behaviour, differentially responding to the same stimulus under different rules. The behaviour was well-fit to a reinforcement learning (RL) model incorporating context-belief states, but not to a basic RL model. Chronic *in-vivo* two-photon calcium imaging during the task identified prediction-mismatch tuned cells in the ACC but not in V1. These cells maximally fired when a stimulus was expected but not received, and these responses were inhibited when the expected stimulus was received. Crucially, the magnitude of the mismatch responses in the ACC predicted successful behavioural transitions in the subsequent trial. Optogenetic inhibition of the anterior cingulate cortex during the period of mismatch signalling significantly impaired behavioural switching but had no effect once the mismatch signal had ceased. These results suggest an essential role for the ACC in driving rapid behavioural changes in response to changing context using prediction mismatch signals.

Pubmed:
30926781: Jensen TP, Zheng K, Cole N, Marvin JS, Looger LL, Rusakov DA
Multiplex imaging relates quantal glutamate release to presynaptic Ca homeostasis at multiple synapses in situ.
Information processing by brain circuits depends on Ca-dependent, stochastic release of the excitatory neurotransmitter glutamate. Whilst optical glutamate sensors have enabled detection of synaptic discharges, understanding presynaptic machinery requires simultaneous readout of glutamate release and nanomolar presynaptic Ca in situ. Here, we find that the fluorescence lifetime of the red-shifted Ca indicator Cal-590 is Ca-sensitive in the nanomolar range, and employ it in combination with green glutamate sensors to relate quantal neurotransmission to presynaptic Ca kinetics. Multiplexed imaging of individual and multiple synapses in identified axonal circuits reveals that glutamate release efficacy, but not its short-term plasticity, varies with time-dependent fluctuations in presynaptic resting Ca or spike-evoked Ca entry. Within individual presynaptic boutons, we find no nanoscopic co-localisation of evoked presynaptic Ca entry with the prevalent glutamate release site, suggesting loose coupling between the two. The approach enables a better understanding of release machinery at central synapses.
Nat Commun, 2019; 10
32060357: Schill Y, Bijata M, Kopach O, Cherkas V, Abdel-Galil D, Böhm K, Schwab MH, Matsuda M, Compan V, Basu S, Bijata K, Wlodarczyk J, Bard L, Cole N, Dityatev A, Zeug A, Rusakov DA, Ponimaskin E
Serotonin 5-HT receptor boosts functional maturation of dendritic spines via RhoA-dependent control of F-actin.
Activity-dependent remodeling of excitatory connections underpins memory formation in the brain. Serotonin receptors are known to contribute to such remodeling, yet the underlying molecular machinery remains poorly understood. Here, we employ high-resolution time-lapse FRET imaging in neuroblastoma cells and neuronal dendrites to establish that activation of serotonin receptor 5-HT (5-HTR) rapidly triggers spatially-restricted RhoA activity and G13-mediated phosphorylation of cofilin, thus locally boosting the filamentous actin fraction. In neuroblastoma cells, this leads to cell rounding and neurite retraction. In hippocampal neurons in situ, 5-HTR-mediated RhoA activation triggers maturation of dendritic spines. This is paralleled by RhoA-dependent, transient alterations in cell excitability, as reflected by increased spontaneous synaptic activity, apparent shunting of evoked synaptic responses, and enhanced long-term potentiation of excitatory transmission. The 5-HTR/G13/RhoA signaling thus emerges as a previously unrecognized molecular pathway underpinning use-dependent

functional remodeling of excitatory synaptic connections.
Commun Biol, 2020; 3

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BOARD NUMBER: S05-062

EXPLORATORY ATTENTIONAL RESOURCE ALLOCATION IN A PROBABILISTIC FORAGING PARADIGM IN THE MONGOLIAN GERBIL

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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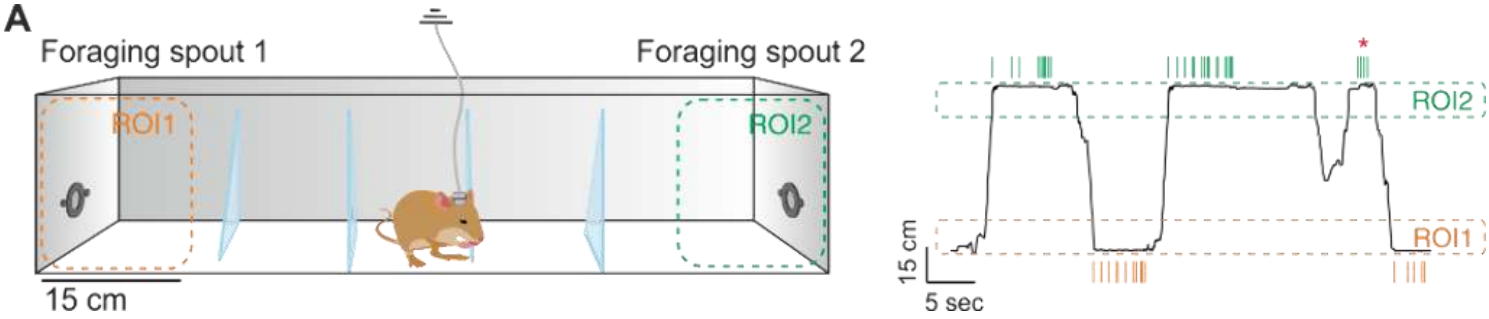
In our constantly changing world, it is necessary to continuously adapt choice options to current needs and if necessary to change our current behavioural strategy and explore the environment for the adaptive reallocation of resources. Eg. Imagine a Mongolian gerbil that forages a desert habitat for distributed food patches. When these patches become exhausted, the gerbil is in an exploitation/exploration dilemma: Should it exploit the current patch further or should it explore an alternative patch, suffering travel costs but enjoying potentially higher food density? The patch leaving decision needs to be made based on probabilistic information and in a potentially changing environment. Here, we present a new behavioural paradigm in the Mongolian gerbil based on a probabilistic foraging paradigm (adapted from Lottem et al., 2018, *Nat Comm*) wherein a random probability schedule, at a given food patch rewards decay exponentially per foraging attempt to zero. In contrast to a deterministic task, rule change cannot be inferred from a single reward omission but is based on accumulating gradual evidence. The stochastic nature of action-outcome associations inevitably leads animals to sample both sides, and hence, animals perform the task at hand without extensive pre-training sessions. Gerbils infer from the statistics of food delivery when to leave a 'depleted site' and explore another site despite the costs of travelling and uncertainty of future reward at another food source. Together, we present here a probabilistic foraging paradigm in the Mongolian gerbil as an ideal model system to investigate exploratory resource allocation.



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BOARD NUMBER: S05-063

MICE REGULATE THEIR ATTENTIONAL INTENSITY AND AROUSAL TO EXPLOIT INCREASES IN TASK UTILITY

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

Jan Willem De Gee, Zakir Mridha, Marisa Hudson, Yanchen Shi, Hannah Ramsaywak, Spencer Smith, Nishad Karediya, Matthew Thompson, Kit Jaspe, Wenhao Zhang, Matthew Mcginley
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Aims: Attention is limited in capacity and costly to utilize. Therefore, organisms are driven by ongoing behavioral incentives to choose *which* stimuli to attend to, and *how much* to attend to them. Global arousal is key to attention, but it is not clear if and how organisms self-regulate their arousal to match attentional intensity to its utility. **Methods:** Here, head-fixed mice licked for sugar-water reward upon detection of temporally unpredictable coherence in a sustained tone-cloud. We manipulated task utility by changing the reward size in blocks of trials, and recorded pupil size and walking speed. **Results:** We observed that detection performance was optimal during intermediate levels of arousal. After increases in task utility, mice stabilized pupil-linked arousal closer to this optimal (intermediate) level and became more sensitive decision-makers, resulting in a higher reward rate. Drift diffusion modeling indicated that evidence accumulation was more efficient and reliable. Periods of low task utility were associated with exploratory rather than resting behaviors. **Conclusions:** In sum, we find evidence for a tri-state model: rest / consolidation during low arousal, optimal task engagement during intermediate arousal, and task disengagement / exploration during high arousal. The self-regulation of arousal partly implements strategic adjustments of attentional intensity. In ongoing work, we are characterizing how reward prediction errors implemented by frontal-sensory interactions and neuromodulatory systems mediate adaptive shifts in attentional state.

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BOARD NUMBER: S05-064

BEHAVIORAL READOUT OF SENSORY-DRIVEN TEMPORAL EXPECTATION IN MICE

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Knowledge regarding the timing of events is critical for all mammals to produce efficient behavioral outcomes. Recent work examining the effect of expectancy based on the presentation of temporally predictable stimulus sequences has revealed that humans are able to extract regularities which in turn improve task performance (Ball et al., 2018). To determine if mice demonstrate a similar capacity, we established an equivalent behavioral paradigm. We trained mice on an audiovisual cue-target paradigm with fixed temporal delay between cue and target events. Mice were required to lick a reward spout at the time of the target event. We then randomly interleaved trials with either a short or long delay between the cue and target stimulus. Mice either encountered more short delay than long delay trials, or vice versa, leading to potential expectation of the temporal structure of target presentations. Our behavioral results indicate that mice can form expectations of the temporal structure of upcoming target events, which we can read-out as distinct patterns of behavior. Assessment of interindividual variability established differences in learning strategies based on the pattern of licking behavior, reaction times and task-related changes in pupil diameter. Ultimately, we aim to understand the underlying neural resources that allow us to continuously adapt to incoming sensory information. We will further investigate the functional activity during this learning paradigm in task-related brain regions.
Ball F., Michels L.E., Thiele C., Noesselt T. (2018). The role of multisensory interplay in enabling temporal expectations. *Cognition* 170:130-146.

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BOARD NUMBER: S05-065

INVESTIGATING THE IMPLICATION OF RAT DORSAL STRIATUM IN ACTION SELECTION USING A CONFLICT TASK

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Action control incurs both the selection of the appropriate response and the inhibition of irrelevant ones. In humans, this is often studied through “conflict tasks” like the Simon task in which stimuli have two dimensions: one, e.g. stimulus intensity, is task-relevant while the other, stimulus position, although task-irrelevant, automatically activates the spatially congruent response leading to interference when the stimulus is contralateral to the response. According to dual route architecture, stimulus intensity is processed in a controlled way, whereas stimulus position is processed automatically and activates the ipsilateral response which needs to be inhibited when stimulus and response side do not match. We developed a T-maze version of the Simon task adapted to rats to further investigate the neural substrates of the dual route architecture. We recorded single unit activity simultaneously from the dorsomedial and dorsolateral striatum, two structures that are generally involved in action-outcome associations or habitual responses, respectively. As in humans, incongruent trials (when the stimulus is contralateral to the required response) lead to longer reaction times and lower accuracy than congruent trials (when the stimulus is ipsilateral to the response). Ongoing analysis of the neural activity will possibly reveal a differential activation of the two striatal subregions. This work will bridge the gap between the studies of action control in rats and humans.

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BOARD NUMBER: S05-066

ATTENTION TRACKING USING A NOVEL DIGITAL INTERFACE IN NON-HUMAN PRIMATES

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Digit-tracking is a novel alternative to eye-tracking for the study of visual attention in human subjects. We presented Gaussian blurred stimuli mimicking the low spatial resolution of the peripheral retina on a touch-sensitive screen. Participants locally unblurred the image by sliding a finger over the display, thus providing a proxy for their eye movements and attention. Due to its simplicity and portability, this technique has a great potential in cognitive science, especially in animal studies. We trained non-human primates (NHPs) subjects to perform a free-viewing task in the laboratory or in their homecage and compared the results to eye-tracking and human digit-tracking results. Attention maps obtained from the two conditions were significantly correlated with eye-tracking and human attention maps. Moreover, we constructed a relevance map for each image by defining regions of interests and calculated the proportion of time spent in these regions. Relevance maps showed that NHPs, like humans, explored relevant features in the social scene like eyes and faces. We also collected eye-tracking and digit-tracking data simultaneously in several sessions and observed high synchrony between them. These results validated the effectiveness of the digit-tracking method in capturing reliable visual exploration behavior in NHPs.

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BOARD NUMBER: S05-067

DREADD-MEDIATED OXYTOCIN NEURONS ACTIVATION IN MACAQUE INCREASED ATTENTION TO EYE AND NOSE AREAS

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Centre National de la Recherche Scientifique, Institut Des Sciences Cognitives - Marc Jeannerod Umr5229, Bron, France

Oxytocin (OT), a neuropeptide mainly produced in the hypothalamus, has been shown to act as a key modulator of social behavior. However, in most human and non-human primates studies, the demonstration of OT effects relies on its external administration by inhalation. Due to the several limitations and pitfalls of OT inhalation, there is an urgent need for more precise tools that allow direct manipulation of oxytocin neurons activity. For that purpose, we induced the expression of an excitatory DREADD (hM3Dq) specifically in OT neurons of a long-tailed macaque. This chemogenetic tool, based on G protein-coupled receptors, have the ability to selectively and reversibly activate DREADD-expressing neurons after a single injection of its ligand. To evaluate the efficiency of the chemogenetic activation of OT neurons, we designed a free viewing task of neutral faces in unconstrained settings by taking advantage of the digit-tracking method. This method consists into presenting on a touch-sensitive display a blurred image simulating the low acuity of peripheral vision. The subject locally unblurred the image by sliding a finger over the display, thus providing a proxy for his eye movements and attention. We found that DREADD-mediated OT neurons activation strongly increased the time spent exploring the eye and nose areas. On the one hand, these results confirm the well-known OT attention enhancement to socially informative facial features. On the other hand, they provide behavioral evidence for the effectiveness of the DREADD-driven endogenous OT release, offering an exciting tool for a better understanding of OT functions in primates.

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BOARD NUMBER: S05-068

THE INFRA-SLOW BRAIN ACTIVITY AFFECTS BEHAVIOR IN CONDITIONS OF UNCERTAINTY

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Objectives: The aim of this study was to investigate the relationship between infra-slow brain activity (ISA) and behaviour in rodents. We developed a method that can measure the strength of phase-amplitude coupling (PAC) between event-related neuronal activity and ISA during a task where mice trained to press a touchscreen during correct stimulus presentation (hits), followed by reward delivery. Specifically, we compared PAC changes in the medial prefrontal cortex (MPFC) during hits versus when touchscreen pressing occurred during distracter stimuli presentation (mistakes) at different levels of certainty. *Methods*: A Ca2+ indicator (GCaMP6f) was expressed in the MPFC of mice that learned the CPT task. Using *in vivo* confocal laser endomicroscopy we recorded, by flexible fiber-optics, neuronal activity synchronized with continuous performance test (CPT) events. The strength of PAC was quantified with Kullback-Leibler Modulation Index (KLMI). To induce uncertainty in behavioral responses, reward contingencies were modified by induction of acute anosmia and introduction of unrewarded hits. *Results:* We found ISA modulated event-related neuronal activity in the MPFC during the CPT. At baseline CPT task, for which mice were well trained, KLMI was higher at the time of mistakes than at the time of hits. Following an altered reward contingency, susceptibility to ISA modulation was larger at the time of hits. This complementarity could be modelled as a change in decisional certainty. *Conclusion:* We found that infra-slow brain activity acts as source of noise at the level of the medial prefrontal cortex, to an extent that can influence behaviour in conditions of uncertainty.

Pubmed:
32283014: Dmytriyeva O, de Diego Ajenjo A, Lundø K, Hertz H, Rasmussen KK, Christiansen AT, Klingelhofer J, Nielsen AL, Hoeber J, Kozlova E, Woldbye DPD, Pankratova S
Neurotrophic Effects of Vascular Endothelial Growth Factor B and Novel Mimetic Peptides on Neurons from the Central Nervous System.
Vascular endothelial growth factor B (VEGFB) is a pleiotropic trophic factor, which in contrast to the closely related VEGFA is known to have a limited effect on angiogenesis. VEGFB improves survival in various tissues including the nervous system, where the effect was observed mainly for peripheral neurons. The neurotrophic effect of VEGFB on central nervous system neurons has been less investigated. Here we demonstrated that VEGFB promotes neurite outgrowth from primary cerebellar granule, hippocampal, and retinal neurons . VEGFB protected hippocampal and retinal neurons from both oxidative stress and glutamate-induced neuronal death. The VEGF receptor 1 (VEGFR1) is required for VEGFB-induced neurotrophic and neuroprotective effects. Using a structure-based approach, we designed short peptides, termed Vefin1-7, mimicking the binding interface of VEGFB to VEGFR1. Vefins were analyzed for their secondary structure and binding to VEGF receptors and compared with previously described peptides derived from VEGFA, another ligand of VEGFR1. We show that Vefins have neurotrophic and neuroprotective effects on primary hippocampal, cerebellar granule, and retinal neurons with potencies comparable to VEGFB. Similar to VEGFB, Vefins were not mitogenic for MCF-7 cancer cells. Furthermore, one of the peptides, Vefin7, even dose-dependently inhibited the proliferation of MCF-7 cells . Unraveling the neurotrophic and neuroprotective potentials of VEGFB, the only nonangiogenic factor of the VEGF family, is promising for the development of neuroprotective peptide-based therapies.
ACS Chem Neurosci, 2020; 11
30529002: Fitzpatrick CM, Runegaard AH, Christiansen SH, Hansen NW, Jørgensen SH, McGirr JC, de Diego Ajenjo A, Sørensen AT, Perrier JF, Petersen A, Gether U, Woldbye DPD, Andreasen JT
Differential effects of chemogenetic inhibition of dopamine and norepinephrine neurons in the mouse 5-choice serial reaction time task.
Attention-deficit/hyperactivity disorder (ADHD) is a psychiatric disorder characterized by inattention, aberrant impulsivity, and hyperactivity. Although the underlying pathophysiology of ADHD remains unclear, dopamine and norepinephrine signaling

originating from the ventral tegmental area (VTA) and locus coeruleus (LC) is thought to be critically involved. In this study, we employ Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) together with the mouse 5-Choice Serial Reaction Time Task (5-CSRTT) to investigate the necessary roles of these catecholamines in ADHD-related behaviors, including attention, impulsivity, and motivation. By selective inhibition of tyrosine hydroxylase (TH)-positive VTA dopamine neurons expressing the Gi-coupled DREADD (hM4Di), we observed a marked impairment of effort-based motivation and subsequently speed and overall vigor of responding. At the highest clozapine N-oxide (CNO) dose tested (i.e. 2 mg/kg) to activate hM4Di, we detected a reduction in locomotor activity. DREADD-mediated inhibition of LC norepinephrine neurons reduced attentional performance in a variable stimulus duration test designed to increase task difficulty, specifically by increasing trials omissions, reducing mean score, and visual processing speed. These findings show that VTA dopamine and LC norepinephrine neurons differentially affect attention, impulsive and motivational control. In addition, this study highlights how molecular genetic probing of selective catecholamine circuits can provide valuable insights into the mechanisms underlying ADHD-relevant behaviors.
Prog Neuropsychopharmacol Biol Psychiatry, 2019; 90

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BOARD NUMBER: S05-069

ASSESSING THE ROLE OF α7 NICOTINIC ACETYLCHOLINE RECEPTORS IN EXECUTIVE FUNCTION USING TOUCHSCREEN TECHNOLOGY IN RAT MODELS

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Dysfunctional α7 nicotinic acetylcholine receptors (α7*nAChRs) and their genetic regulation have been associated with several neuropsychiatric diseases including schizophrenia (SCZ) and Alzheimer's disease. Preclinical studies revealed that α7*nAChR ligands can improve executive function, commonly impaired across many neuropsychiatric disorders. Yet, a translational gap prevents these findings from resulting into clinically effective treatments. Our goal was to refine our understanding of the role of α7*nAChRs in executive function. We generated transgenic rats knockout for the α7 nicotinic subunit gene (α7KO) using the zinc finger nuclease technology. We submitted α7KO and their controls to two tests assessing executive function: the continuous performance task (CPT), a task of sustained attention which involves selection, vigilance and control; and the visual discrimination (VD) task, measuring associative learning and perceptive ability. These tasks were adapted from clinical research in a highly translational manner, thanks to the use of a touchscreen-based experimental setting. Strikingly, α7KO rats showed a response bias towards a global over-responding in the CPT, a strategy that was detrimental to task performance as reflected in a lower discrimination sensitivity - a deficit commonly reported in SCZ patients, and a higher persistence in error making. α7KO and control rats performed equally well and adopted identical strategies in the VD task. As in the CPT, α7KO rats also displayed a global over-responding to the screen in the VD task. Our results suggest that α7nAChRs are crucial to executive functioning for regulating behavioral inhibition, thereby facilitating the performance in tasks demanding high attentional load.

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BOARD NUMBER: S05-070

CEREBELLAR CONTRIBUTIONS TO INTERVAL TIMING

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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The ability to perceive time in the seconds-to-minute range, a mechanism known as interval timing, is important for adaptive behaviours such as foraging and decision-making. Neural substrates underlying interval timing include, amongst others, striatum and medial prefrontal cortex. By comparison, the cerebellum is mainly thought to be important in the control of movements with sub-second timing. However, given the well-established notion that the cerebellum is involved in the generation and updating of internal models through prediction error correction, we hypothesise that the cerebellum may also be involved in learning an internal model of supra-second stimulus time intervals to enable more accurate behaviour. We argue that, while other brain regions keep track of time once the association is acquired, the cerebellum plays a role in establishing temporal predictive associations earlier in learning. In order to test the predictive function of the cerebellum in the supra-second range, we trained rats to associate a sound duration with reward delivery. The effects of temporary chemogenetic inactivation of cerebellar output from the lateral nucleus were investigated at different stages during learning and performance on this task. Our study tests the prediction that cerebellar inactivation will slow learning of a novel stimulus time interval when compared to controls. Consistent with this proposal, preliminary analysis to date indicates that inactivation of cerebellar output does not affect interval timing behaviour after the association has been acquired. Further analysis and experiments to test the role of cerebellar output in learning temporal predictive associations at the supra-second timescale are ongoing.

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BOARD NUMBER: S05-071

THE ROLE OF BETA OSCILLATIONS IN MENTAL TIME TRAVEL

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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The brain is a time machine that tells time and mentally projects the self in the past and in the future. Short temporal intervals are encoded via cortical beta oscillations, whereas the ability to project the self in time, called Mental Time Travel (MTT), requires mental imagery associated with alpha oscillations. To clarify if MTT requires cortical oscillations involved in time processing, i.e., beta, rather than cortical oscillations involved in mental imagery, i.e., alpha, we combined a new task to assess MTT and brain stimulation, i.e., transcranial Alternating Current Stimulation (tACS). Thirty participants see faces of different ages presented one at a time with a phrase describing a life event, happening in middle age and perform a 2-alternative forced choice task: they indicate if it is likely or unlikely that the person has lived the life event 10 years ago (Past Condition) or will live the event in 10 years (Future condition). tACS is administered over the posterior parietal cortex, a key area for MTT and mental imagery. Results show that participants indicate a younger face as more likely to have lived an event 10 years ago during beta-tACS as compared to sham and alpha-tACS. For the future condition there were no significant differences during sham, alpha- and beta-tACS. These findings suggest that self-projection in the past, not in the future, relies on beta oscillations in posterior parietal regions and that beta oscillations are critical for processing also long duration in addition to their well-known role in supporting short durations.

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BOARD NUMBER: S05-072

DO YOU AUTOMATICALLY TRACK TIME?

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Time perception can be assessed with behavior and electroencephalography (EEG). To demonstrate that the brain automatically detects changes in duration, EEG studies have used the Mismatch Negativity (MMN) paradigm in which infrequent duration changes (deviants) in a sequence of frequent durations (standards) elicit a negative wave (the MMN), whether participants paid attention or not to the sequence. However, two shortcomings in the literature were identified: whether the observed MMN results from a violation of the sequence temporal regularity or a change in duration, and whether the temporal scale of durations matter are unsettled questions. Consequently, we conducted an EEG study testing duration changes in rhythmic or non-rhythmic sequences and at two different temporal scales. The sequences consisted of empty intervals, delimited by auditory clicks, separated by fixed or variable (rhythmic or not, respectively) inter-stimulus intervals. Within each sequence, stimuli could be standard or deviant (+/- 30%) durations. We tested two temporal scales, a standard duration of 100 ms or 600 ms (below and above the temporal window of integration at 200 ms). We used control sequences where all durations were equally presented in a randomized order to properly compute differential waves. We hypothesized that because durations below 200 ms are building blocks of perception, they may elicit an MMN, whether embedded in a rhythmic sequence or not. On the contrary, durations above 200ms may elicit an MMN only in rhythmic sequences, as temporal regularities will facilitate the tracking of duration changes.

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BOARD NUMBER: S05-073

OBJETS, SHADOWS AND THE BRAIN

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Immersed in a virtual reality environment, 40 participants (20 female and 20 males) aged 25 years old in average were instructed to perceive and indicate the synchronicity or asynchronicity between a mobile object shadow with regard to a mobile object from the one side, and the synchronicity or asynchronicity between their own body shadow and position in space from the other. Their brain activity was recording using an EEG system and an fNIRS device. Bilateral beta (13.5-30 Hz) oscillations and OxyHb concentrations of frontal and parietal brain areas were analysed as they were considered predictors of perceptual and motor performance. According to the general hypothesis, beta oscillations and OxyHb concentrations associated with the perception of an object shadow would envision the neural activity of the body shadow. It was therefore expected that the fronto-patietal neural activations associated with perception of object shadow would anticipate the neural activations of body shadow. A series of Multiple Regression Analysis (MRA) revealed that body shadow specific modulations in the bilateral frontal areas reflect and infer object shadow relevant sensorimotor perception, and subsequent decision making in 3D virtual environments. The current data suggest the existence of a cortical network in which neural dynamics of object shadows would provide a mechanism for the formation of functional networks during internal re/activation of body relevant cortical representations, that is, a substitution of the physical body. They also suggest that prediction and anticipation along with inference and exploration might be general principles of cortical functioning in real and 3D virtual environments.

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BOARD NUMBER: S05-074

ARE PREDICTIVE PROCESSES ALTERED BY TMS OF THE CEREBELLUM?

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Improving the treatment of Schizophrenia is an important goal in psychiatry. Transcranial magnetic stimulation (TMS) of the Cerebellum was shown to improve symptoms in patients with Schizophrenia. Further, we know that the Cerebellum is related to time perception and prediction on the millisecond level. Interestingly, patients show deficits in time prediction, especially on the milliseconds level. Here, we investigate whether we can alter temporal predictive processes using TMS on Crus I/II (Cerebellum), which are involved in temporal processes. To establish this basic knowledge, we test only healthy controls. In a first MRI session, we obtain anatomical and functional data. Functional data is measured while presenting two different tasks: (1) The variable foreperiod paradigm in order to evoke the hazard function, i.e., the effect of temporal preparation on behavior; (2) A very recently discovered optical illusion, i.e., the collision task, which is interpreted to reflect millisecond predictions of movement and contrast. In the two following sessions, we present those two tasks before and after TMS interventions (treatment or SHAM). The functional MRI will reveal the possible involvement of Crus I/II and its related networks during the variable foreperiod paradigm and during the collision task. Further, we hypothesize that the effect of TMS of Crus I/II will (1) modify the hazard function and (2) alter the illusion perception rate. The study is on-going and I will present preliminary results. If our hypotheses were to be confirmed, we aim to test this approach in patients with Schizophrenia to possibly restore temporal processes.

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THE FUNCTIONAL ROLE OF RESTING-STATE THETA-GAMMA COUPLING AND ITS RELEVANCE TO VISUAL SELECTIVE ATTENTION FOR PATIENTS WITH TIC DISORDER

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Tics are generated as repetitive movements, vocalization, and intrusive thoughts after aberrant sensory gating. Theta phase-gamma power coupling (TGC) has an important role in several different cognitive processes. Although spontaneous brain activity during the resting state is crucial for preparing for cognitive performance, the relationship between the functional role of resting-state TGC and cognitive function of patients with tic disorder (TD) remains unclear. Thirteen patients with TD and 13 healthy children participated in the experiment. Their resting-state TGC was measured while their eyes were closed. The relationship between this value and their clinical outcomes was determined. Resting-state TGC was significantly lower for patients with TD than for healthy children at the global level. While participants' eyes were closed, TGC during the resting state was significantly and negatively correlated with the attention quotient of omission errors according to a visual selective attention test. These findings support the view that TGC reflects information processing and signal interactions at global levels. Additionally, these findings suggest the low efficacy of the dysfunctional resting-state brain network of patients with TD, which is important for attentional processing of visual sensory information. Patients with TD may have difficulty gating irrelevant sensory information while their eyes are closed during the resting state.

Funding: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2019R1A2C4069598). This work was also supported by the Korea Medical Device Development Fund grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Trade, Industry and Energy, the Ministry of Health & Welfare, the Ministry of Food and Drug Safety) (Project Number: 1711138277, KMDF_PR_20200901_0143).

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DISTRACTORS WITH MORE TARGET FEATURES ATTRACT MORE ATTENTION REFLECTED BY N2PC: A CROSS-MODAL EEG STUDY

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Task-irrelevant distractor often captures our attention, and avoiding distraction requires top-down attentional guidance. However, it remains controversial regarding the underlying neural mechanisms of how we handle attentional capture by distractors. Some argue that we proactively suppress task-irrelevant distractors in advance to avoid attentional capture (i.e., signal-suppression hypothesis). Some advocate attentional deployment to the distractor is reduced rather than suppressed (i.e., "no engagement" hypothesis). In contrast, others suggest irrelevant distractors still capture attention but disengaging from it is quicker (i.e., fast disengagement hypothesis). We conducted two cross-modal search tasks with EEG recording to distinguish the above accounts by observing key ERL components: N2pc, Pd, and CCN. To distinguish target- and distractor-related ERL components, we presented distractors 50 to 150 ms before the target. Specifically, the target had a specific visual shape and color, either located in the middle line or the lateral regions, and the salient distractors were varied within-dimension (the same as the target), across-dimension (different color/shape from the target), and across modalities (tactile vibrations). We observed the within-dimension distractor slowed down the reaction time most. The cross-dimension distractors induced minor interference. But the cross-modality distractor did not worsen performance. Distractors containing more target features attracted more attention and caused more attentional engagement reflected by N2pc. The following Pd represented disengagement from the distractor. The tactile distractor captured attention reflected by CCN, but it did not reduce the attentional deployment to the visual target. The current findings suggest the distractor with target-relevant features deteriorated performance because of top-down attentional engagement.

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PSEUDONEGLECT AND HAND PREFERENCE OF SAIMIRI SCIUREUS IN BEHAVIOURAL TASKS

POSTER SESSION 05 - SECTION: TEMPORAL COGNITION, RHYTHMS & ATTENTION

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Converging anatomical, neurological and behavioural evidence demonstrate that manual preference is related to the lateralisation of visuospatial processing. Manual preference has been reported in many primates but remains debated in Saimiri sciureus. Pseudoneglect —an asymmetry in spatial exploration traditionally viewed as an effect of visuospatial brain lateralisation in humans— has never been studied in Saimiri sciureus. In two complementary behavioural tasks, the present study compared manual and visuospatial lateralisation in 15 female Saimiri sciureus. In the line task, ten grapes were aligned and centred in parallel to the animals' eyesight. In the puzzle task, grapes were hidden behind covers in a checkerboard fashion. We then recorded the hand used and the pseudoneglect — i.e. center of gravity of the exploration omissions — during both tasks. Half of the animals showed a strong left (3 animals) or right (4 animals) manual preference for the line task without significant effect in the group (Right vs Left : $p=0.97$). 14 animals showed a hand preference (Left 6 ; Right 8) for the puzzle task (Right vs Left : $p =0.25$). In addition, the difficulty of the task induced a stronger right laterality ($p<0.001$) than left laterality ($p<0.05$). The line task did not reveal any pseudoneglect effect ($p=0.47$) but 7 animals demonstrated an asymmetry in their spatial exploration of the puzzle task (group : $p=0.64$), albeit without any link to manual preference ($r=0.06$, $p=0.82$). These results indicate that Saimiri sciureus, like humans, show a manual preference related to task complexity but not associated with pseudoneglect.

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THE HUMAN DORSAL PREFRONTAL SYSTEM REFLECTS THE SOURCE OF VALUE INFORMATION DURING RISKY DECISIONS

POSTER SESSION 05 - SECTION: DECISION-MAKING

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When making a risky choice, a rational decision-maker integrates outcome magnitude and probability into an expected value (EV). These decision variables could come in two modalities: 1) presented in some descriptive form (e.g., pie-chart) or 2) inferred based on the previous experience. The first modality is mostly used in behavioral (neuro)economics while the latter is adopted in learning paradigms. Growing literature points towards an existence of experience-descriptive "gap". However, it remains unknown whether these two modalities rely on the same neural origins. We designed a two-armed restless bandit task with orthogonally manipulated modality (descriptive vs. experience-based) of magnitude and probability resulting in four decision contexts. While in the MRI-scanner, subjects (N=29) made consecutive choices between two colored shapes each associated with magnitude and probability which either had to be inferred based on the feedback or were displayed on the screen. Analysis of decision accuracy showed similar contributions of magnitude and probability regardless of their modality (p=0.77) with choices better explained by the model that integrated both modalities (Pexp= 0.99). At the neural level we found a dissociation between dorsal (dlPFC/dmPFC) and ventral (vmPFC) prefrontal networks. While the dorsal network differentiated between four decision contexts and showed context-dependent modulations of EV, EV correlates in the vmPFC were modality-nonspecific. Furthermore, the dorsal network showed earlier latency peaks for EV in the experiential relative to the descriptive context in line with the decision reaction times. Together, these results demonstrate a neural discrepancy between descriptive and experiential risky decision-making.

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BOARD NUMBER: S05-079

CONTEXT-DEPENDENCE AND ASYMMETRIC UPDATE IN RISKY DECISION-MAKING

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Economic decisions are influenced by both outcome valence (i.e., whether an outcome entails gain or loss) and risk (the variance of outcomes). It has been shown in previous studies that people have different risk preferences when the prospects' information is described explicitly and when they are learning it by experience. However, the effect of valence on behavioral performance in an experienced-based learning task has been less investigated. To address this question, we used a novel instrumental learning task in 4 behavioral experiments designed to manipulate risk and valence independently while the difference of value between options was kept invariant. Each experiment consists of 2x2 design contexts; one dimension indicates whether the context's valence is loss or gain, and the other dimension is dedicated to whether the correct option is risky or safe. Consistent results in all experiments show a significant effect of prospect's riskiness in behavioral performance. Moreover, we found a significant effect of riskiness, valence and their interactions on subjects' reaction time. Finally, using model-fitting and reinforcement learning simulations, we indicated that a model with a learning rate asymmetry better accounts for behavioral data.

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THE ROLE OF FEEDBACK IN DECISION MAKING UNDER RISK

POSTER SESSION 05 - SECTION: DECISION-MAKING

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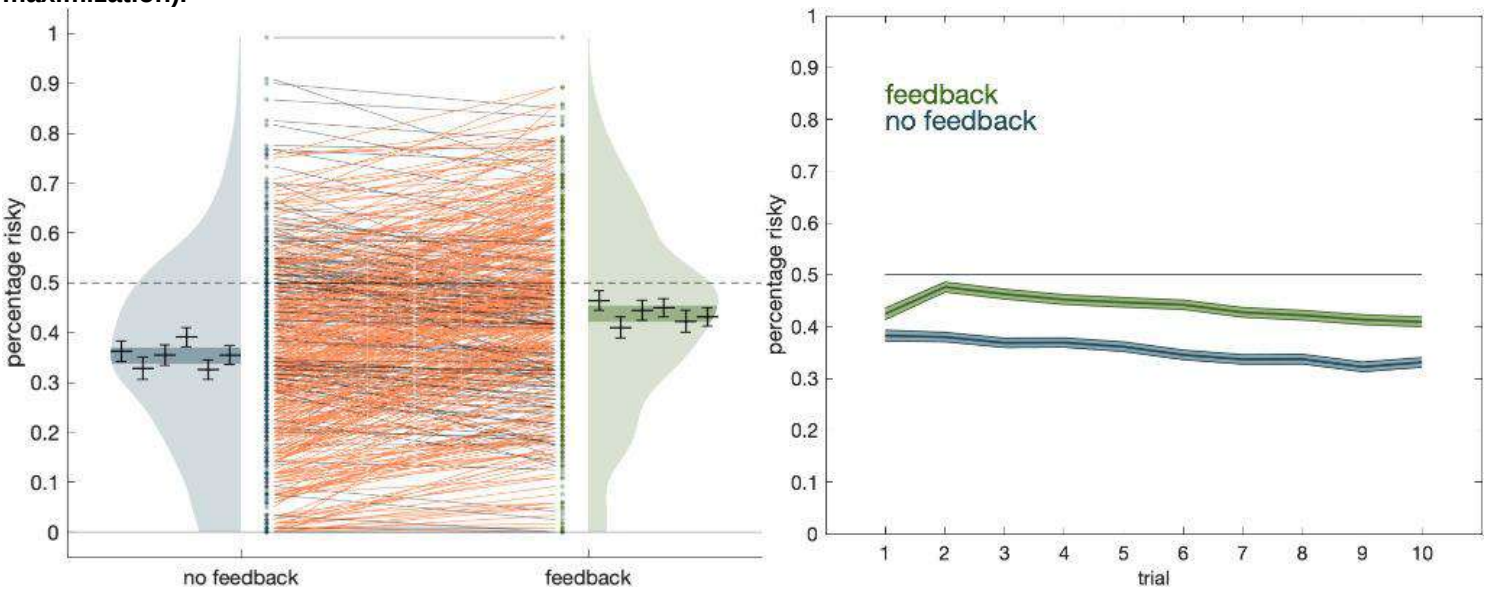
Decision making under risk has been shown to differ depending on whether the outcome of the choice is revealed. We investigated how feedback affects decision making by comparing decisions from description only (outcome not disclosed) with decisions from description and experience (outcome disclosed). We ran a series of six factorial experiments (N=600) using the repeated binary choice task, contrasting a safe versus a risky lottery. We manipulated within-subjects the presence of feedback, which option has a higher expected value(EV), the probability, and the magnitude of the risky option. We also manipulated between-subjects the type of feedback (partial or complete), the presence of instructions at the beginning of each block on the availability of feedback, and whether the safe option was a sure-thing or a low variance lottery. We consistently found that feedback increases risky choice rate. Interestingly, this effect emerges immediately after the subjects realize they will receive feedback, suggesting it is induced by the mere anticipation of feedback, not by learning per se. Consistent with the increase in risky rate, descriptive modeling analysis using a Prospect Theory variant indicated decreased risk aversion and increased weighting for medium and large probabilities when feedback is provided. On the other hand, feedback seems to have no effect on EV-maximization. All in all, we found that feedback makes people more risk-seeking, but not necessarily more accurate (in terms of EV-maximization).



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THE ROLE OF SIBLING AGGRESSION DURING CHILDHOOD IN DECISION-MAKING DURING ADULTHOOD

POSTER SESSION 05 - SECTION: DECISION-MAKING

Stacey Bedwell[1], Natalie Harrison[2], Matthew Brooks[3]
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The role of sibling aggression during childhood in decision-making during adulthood Stacey A. Bedwell[1], Natalie Harrison[2] and Matthew Brooks[3] [1] Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK [2] Department of Psychology, Bath Spa University, Bath, UK [3] Department of Psychology, Manchester Metropolitan University, Manchester, UK Sibling relationships during childhood are known to influence cognitive development, specifically in terms of executive functions associated with the prefrontal cortex. It is understood that the development of prefrontal networks can be influenced by experiences in developmental phases. However, it remains to be understood how experiences of sibling aggression contribute to the development of executive functions, specifically how decision-making is exhibited in adulthood. In the present two studies, we sought to uncover the relationship between experiences of childhood sibling aggression, decision-making style in adulthood and real-time risky decision-making. In study one, reports from 142 adults revealed that using sibling aggression to maintain dominance in childhood was linked to avoidant and spontaneous decision-making in adulthood. In study two, data collected from 75 adults revealed experiences of sibling aggression did not predict risky decision-making. These findings indicate that the types of decisions made may be influenced by childhood sibling aggression, but not the level of risk involved in decisions made.

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BOARD NUMBER: S05-082

CONTEXT INDUCED FALSE MEMORIES OF OUTCOME VALUES

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Here we investigated whether context influences how values are learned and retrieved from memory. Our experiment consisted of a learning task followed by two post-learning memory tasks. These were an explicit valuation task, where participants had to recall the outcome of each symbol, and an implicit choice task, where they had to select the best symbol between a given pair. Crucially, we varied the learning architecture across experimental groups, i.e. which options were presented together during the learning phase. This allowed us to independently manipulate the global rank of an option (i.e. how good the option is compared to all other options in the task) and its local rank (i.e. how good the option is compared to its pairmate). Specifically, we ran three versions of the learning task: in the first two versions (V1&V2), options were presented in fixed pairs during the learning phase (stable local contexts), whereas in the last version (V3), options were presented in all possible combinations (no stable local context). While accuracy during the learning phase was similar across all versions, the learning architecture had a great impact on responses in the post-learning memory tasks. In V3, participants' responses reflected the global rank of the options (i.e. their *absolute* value), whereas in V1&V2, participants' responses reflected a mixture of the global and local ranks of the options (i.e. their *relative* value). We thus demonstrate that irrational preferences and value judgments systematically arise from learning architectures that favour contextual learning.

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EVALUATION OF THE EFFECTIVENESS OF AN EXECUTIVE FUNCTION TRAINING PROGRAM COUPLED WITH TRANSCRANIAL STIMULATION IN BRAIN-INJURED PATIENTS: PRELIMINARY RESULTS OF A SINGLE CASE EXPERIMENTAL DESIGN

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Cognitive remediations (CR) are designed to address the crucial need for symptom improvement in brain-damaged patients with deficit in executive functions. The aim of our study is to investigate the effectiveness of a CR in virtual reality (CoVirtua) coupled with transcranial stimulation (tRNS), in improving performance in ecological situations of patients with dysexecutive syndrome. Five patients were included in a multiple single case study (SCED) that included three phases (A1-B-A2) over 12 weeks. Phases A1 and A2 corresponded to occupational therapy and Phase B corresponded to the CR. The start of phase B was randomized. The intervention lasted four weeks and consisted of four Covirtua sessions per week coupled with a 20-min tRNS session. The effect of the intervention has been assessed by the Goal Attainment Scale (GAS) in the short and long terms. GAS results showed an effect of the intervention in the short term (A1 *vs*. B) and the long term (A1+B *vs.* A2) for 2 objectives for P01 ("Mailbox management" p= 0.01; "Concentration" p = 0.02) and for 1 objective for P03 ("Managing a shopping list" p=0.02). Regarding long-term effects, the intervention showed its effectiveness for 1 objective for P02 (“Following Household chores schedule” p=0.03); and for 2 objectives for P06 (“Following a recipe” p=0.005; “Managing groceries” p=0.02). These first results show of a positive effect of Covirtua coupled with tRNS on executive functioning in daily life, mainly in the long term. Other patients are currently being studied.

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DECISION MAKING AND RISK-TAKING: THE ROLE OF COGNITIVE RESERVE

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Decision-making (DM) competence and risk management are crucial for maintaining independence and autonomy, especially for older adults. The role that Cognitive Reserve (CR) plays in this process is poorly investigated. CR is a pivotal factor, both from a neural and cognitive-functional point of view, in coping with cognitive decline during the later stage of life. According to several authors, it involves efficient and flexible cognitive strategies learned throughout life. Moreover, divergent thinking (DT), was proved to be one of the predictors of CR. The present study aims to investigate the link between CR (assessed with the Cognitive Reserve Test, CoReT), DM styles (investigated with the General Decision Making Style, GDMS) and risk-taking in different domains (assessed with the Domain-Specific Risk-Taking, DOSPERT). Preliminary analyses of data collected in a sample of 32 healthy participants (60-81 years; age: mean= 65.8; SD=5.09; education: mean=14.3; SD=4.87; women F= 46.9%) show a correlation between CR, DM styles and risk-taking. In particular, functional DM styles seem to be related to CR and specifically to the components of DT. On the contrary, non-functional DM styles and risk-taking components are negatively correlated with the creative components of CR. The evidence of a relationship between DM, risk-taking and CR provides the conceptual basis for designing interventions to improve and increase the non-structural component of CR, increasing DT. Such interventions might consequently promote active ageing by supporting DM processes and therefore the efficient management of daily life activities.

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BEHAVIOURAL AND NEURAL MECHANISMS OF COMMITMENT AND ABANDONMENT IN SEQUENTIAL GOAL PURSUIT

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Agents often need to pursue a goal for an extended period of time before any rewards are obtained. In dynamic environments, the relative value of the chosen goal compared to alternatives may change mid-pursuit, meaning agents must evaluate the optimal level of goal commitment. Being too committed to a poorly chosen goal could mean losing out on better options, while being too prone to abandonment risks the agent never completing any goals. We used a sequential goal pursuit task with fMRI to investigate how people move between states of goal selection and goal commitment, how they weigh up the different kinds of pressure to abandon a goal they are committed to (such as frustration with the current goal versus temptation from an alternative), and how this evaluation process varies with sunk costs. Participants' choices in the task were best explained by a sequence sampling model which sampled possible future trajectories of the different goal options. However, beyond capturing choices with this prospective model, participants consistently showed a bias to persevere with the current goal rather than switch to a better option, which worsened with the number of trials invested. The bias to persist was associated with reduced attention allocated to stimuli associated with non-selected goals during an independent spatial working memory task. It was also linked to a decreased representation of alternative goals in the dACC as people progressed through the current goal, suggesting goal commitment is manifested as progressive inhibition of alternative courses of action during goal pursuit.

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SLEEP ONSET IS A CREATIVE SWEET SPOT

POSTER SESSION 05 - SECTION: DECISION-MAKING

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The ability to think creatively is paramount to facing new challenges, but how creativity arises remains mysterious. Here, we show that the brain activity common to the twilight zone between sleep and wakefulness (nonrapid eyemovement sleep stage 1 or N1) ignites creative sparks. Participants (N = 103) were exposed to mathematical problems without knowing that a hidden rule allowed solving them almost instantly. We found that spending at least 15 s in N1 during a resting period tripled the chance to discover the hidden rule (83% versus 30% when participants remained awake), and this effect vanished if subjects reached deeper sleep (N2). We substantiated these results using spectral analyses and discovered an optimal cocktail for creativity (above and beyond sleep stages), consisting of an intermediate level of alpha (a marker of the wake-to-sleep transition) and a low level of delta (which signs sleep depth). Our findings suggest that there is a creative sweet spot within the sleep-onset period, and hitting it requires individuals balancing falling asleep easily against falling asleep too deeply.

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TWO-DIMENSIONAL ADAPTATION OF DECISION VARIABILITY TO REWARD VOLATILITY AND TRAIT COMPULSIVITY

POSTER SESSION 05 - SECTION: DECISION-MAKING

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The variability of reward-guided decisions arises from two distinct sources: imprecise reinforcement learning due to limited resources, and exploratory choices aimed at reducing uncertainty. But whether these two sources of decision variability adapt to specific forms of uncertainty remains unknown. Here we designed a two-armed bandit task in which we compared the effects of reward stochasticity and volatility on decision variability. Across three datasets (total N = 447 participants and 262,560 decisions), we show that humans decrease learning noise and make more ex- ploratory choices in response to volatile but not stochastic rewards. Through theoretical simulations, we demonstrate that these selective effects reflect cost-efficient adaptations to uncertainty. We further show that individual differences in trait compulsivity, measured using a validated transdiagnostic approach, account for variations in exploration but not learning precision. Together, these findings reveal a latent two-dimensional trade-off regulating decision variability under uncertainty.

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LISTEN TO YOURSELF: AN FMRI STUDY OF MOTIVATIONAL INTERVIEWING EFFECTS ON DIETARY DECISION-MAKING IN HEALTHY PARTICIPANTS

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Motivational interviewing (MI) is an evidence-based communicational approach, which aims at resolving a person's ambivalence between changing (CT) and keeping (ST) unhealthy behaviour. However, its neurocognitive underpinnings are unknown. Here, we built on models of economic choices, which propose that decision-making involves a valuation phase during which features, such as for example the healthiness and tastiness of food, become integrated into stimulus value that drives choices. We aimed at testing how MI changes food valuation and food-valuation related brain responses during dietary decision making. Twenty-seven female participants (age=29 ± 2.01 SEM years, BMI = 23.4 ± 1.09 SEM kg/m^2) underwent an MI session from which 5 CT and 5 ST statements were extracted. One week later, during an fMRI session, participants listened to these statements before making a series of dietary choices. The results showed that participants based their food choices more on the healthiness and less on the tastiness of food after listening to CT. This interaction was independent of stimulus value assigned to food, which was similar for CT and ST. On the neural level the vmPFC and dlPFC correlated stronger to the healthiness of food when choices were made after listening to CT statements. The vmPFC correlated more to the tastiness of food when choices were made after listening to ST statements. These findings indicated that CT and ST differentially influenced how healthiness and tastiness features predicted food stimulus value, and how regions within the brain's valuation and cognitive self-regulation systems encoded these features during dietary decision-making.

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BOARD NUMBER: S05-089

PREDICTING A PLANNED EXECUTION OF AGGRESSIVE ACTION: A STUDY FOR PROACTIVE AGGRESSION IN A GREEK-CYPRIOT ADOLESCENT SAMPLE

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Studies regarding narcissism and proactive aggression association have revealed contradicting results. This study aims to investigate whether narcissism, sensation seeking, popularity and media violence exposure predict the expression of a planned, controlled, and purposeful execution of an aggressive act with the aim of achieving a desired goal. The sample consists of 2306 Cypriot adolescents. Narcissism was evaluated with the Narcissistic Personality Inventory (Raskin & Hall, 1979) and Antisocial Process Screening Device-Youth Version (Frick and Hare 2001), proactive aggression with Proactive and Reactive Aggression Questionnaire (Raine et al. 2006), sensation seeking tendencies with Sensation Seeking Scale Form –V (Zuckerman, Eysenck, and Eysenck 1978), popularity with the Peer Pressure Questionnaire (Santor et al., 2000) and self-report for their grade as well as media exposure. A linear regression revealed that narcissism and sensation seeking in combination explain 48.2% of the variance in proactive aggression. When grade is factored in as well as a variable, only an additional 0.2% was explained. Narcissism explained 44% of the variance in proactive aggression, when popularity was factored in to the model 47.6% was explained. Finally, when media violence was added, the variance was explained by 53.7%. The data suggest that narcissism, popularity, and media violence significantly predict proactive aggression in this sample. Results can reveal a great pool of information regarding the type of appropriate interventions for adolescents with narcissistic traits. Hopefully, this study will explain the previous mixed results and also add to the importance of other co-occurring variables.

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LIKING YOUR OWN IDEAS: COMPUTATIONALLY DECIPHERING THE ROLE OF PREFERENCES IN CREATIVITY

POSTER SESSION 05 - SECTION: DECISION-MAKING

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The cognitive and neural mechanisms of creativity are still poorly understood. Formally, creativity is defined as the ability to produce an object/idea that is both original and efficient. Then, creativity should involve an evaluative process of efficiency and originality, interacting with a generative process that generates candidate ideas. Through an original experimental design combining creativity and decision-making tasks, coupled with computational modeling, this study aims to investigate the evaluative component's specific role in creativity mechanisms. The central hypothesis is that valuation involves individual preferences that affect the exploration and/or selection process. Sixty-nine participants completed a free generation of associates task in which they had to provide creative associations in response to cue words. This task was followed by rating tasks in which they had to judge how much they liked their responses and how much associations were appropriate and original. We first found that participants were faster to provide responses they liked more. Then, using random walks applied to semantic networks, combined with decision functions, we found that preferences were more likely to drive the selection phase than the exploration phase of the creative idea generation. We compared the behavior predicted by the model to the behavior of a subgroup of participants excluded for the model development and found strikingly similar behavioral patterns. Finally, we found that preferences parameters and performance in the generation task correlated with creative abilities, measured via classical creativity tests. Altogether, our findings support a new framework for creativity by considering it as a reward-based behavior.

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IMPACT OF DECISION AND ACTION OUTCOMES ON SUBSEQUENT DECISION AND ACTION BEHAVIORS

POSTER SESSION 05 - SECTION: DECISION-MAKING

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While traditionally considered as independent processes, recent studies suggest a close functional relationship between decisions and actions. For instance, humans can trade decision time for movement time to maximize their reward rate between blocks of trials. Besides, it is well established that choice outcomes largely impact subsequent decisions. But the impact of a choice on the next action has never been described, and whether and how a motor outcome influences the next decision is also unknown. We addressed these questions by analyzing trial-to-trial changes of choice and motor behaviors of human subjects instructed to perform successive perceptual decisions expressed with reaching movement whose duration was either bounded or unconstrained in separate tasks. Results indicate that after a bad decision, participants who were not constrained in their action decided more slowly and more accurately. Interestingly, they also shortened their subsequent movement duration by moving faster. Conversely, movement errors not only influenced the speed and the accuracy of the following movement, but those of the decision as well. If the movement had to be slowed down, the decision that precedes that movement was accelerated, and vice versa. These results indicate that decision and action do not only compensate each other over extended time scales, between blocks of trials, but also more locally, from one trial to another. The observed post-outcome adjustments also confirm that in a context allowing reward rate maximization, humans seek in each trial to optimize their global behavior instead of computing their decisions and actions independently of each other.

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THE IMPASSABLE GAP BETWEEN EXPERIENTIAL AND SYMBOLIC VALUES

POSTER SESSION 05 - SECTION: DECISION-MAKING

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To choose between options of different natures, standard decision models presume that a single representational system ultimately indexes their subjective values on a common scale, regardless of how they are constructed. To challenge this assumption, we systematically investigated hybrid decisions between experiential options, whose value is built from past outcomes experience, and symbolic options which describe probabilistic outcomes. We show that participants' choices exhibited a pattern consistent with a systematic neglect of the experiential values. This normatively irrational decision strategy held after accounting for alternative explanations, and persisted when it bore an economic cost. Overall, our results demonstrate that experiential and symbolic values are not symmetrically considered in hybrid decisions, suggesting that they are not commensurable and recruit different representational systems which may be assigned different priority levels in the decision process. These findings challenge the dominant models commonly used in value-based decision-making research.

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BOARD NUMBER: S05-093

INCENTIVE MOTIVATION AFFECTS BELIEF AND CONFIDENCE DIFFERENTLY

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Tracking uncertainty about the environment is crucial for adaptive behavior. However, humans often make errors when estimating probabilities about external states (belief) and their own actions (confidence), particularly being prone to numerous affective and motivational effects. Some theories of motivated cognition propose that a general *desirability bias* affects all probability judgements equally (overestimating the probability of states of the world associated with obtaining rewards or avoiding punishments). Here we challenge this view across multiple perceptual discrimination experiments (total N = 401), where we manipulated choice agency (whether participants were faced with free choices, imposed choices, observed choices, or their own choices from previous trials) and monetary incentives (magnitude and valence of potential outcomes for correct/incorrect choice), measuring their effects on probability judgements about choices being correct. We falsify a generalized desirability bias by demonstrating a) increased probability judgements with net rather than absolute incentive value, and b) higher effects of incentives on probability judgements about self-generated actions. These results show that probability judgements are affected by a *contextual valence effect* rather than a desirability bias, and that judgements about one's own behavior are differentially affected, being more sensitive to this effect.

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USING CITIZEN SCIENCE TO STUDY NEUROCOGNITIVE MECHANISMS AND THEIR RELATION TO MENTAL HEALTH

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Investigating inter-individual differences in cognitive mechanisms is often a challenging endeavor. In-person laboratory testing is slow and sample sizes are small, while online experiments using worker platforms are costly and likely to yield biased samples (e.g. professional participants, restricted age range). Both limitations can affect the generalisability and replicability of findings. Here, we present findings from an alternative approach. We developed a citizen science smartphone app, Brain Explorer (www.brainexplorer.net), that incorporated short versions of two tasks previously used in the laboratory to probe computational mechanisms underlying psychiatric traits such as impulsivity and compulsivity. The first “Treasure Hunt” (TH) game probed mechanisms underlying information search (Hauser et al., 2017) and the second game, “Milky Way” (MW) probed reinforcement learning in changing environments (Skvortsova et al., 2022). Users (N>2500) played both games and additionally answered self-reported surveys targeting compulsivity and impulsivity traits. Here, we investigate how such a citizen science approach affects the psychometric properties for these tasks, revealing moderate test-retest reliability and cross-task associations. Moreover, we show how associations with psychometric properties (here: impulsivity and compulsivity) hold across different task versions and recruitment pathways. Together these results support the usage of short gamified tasks to probe the computational mechanisms underlying psychiatric traits.

Pubmed:

34171289: Wittmann MK, Trudel N, Trier HA, Klein-Flügge MC, Sel A, Verhagen L, Rushworth MFS
Causal manipulation of self-other mergence in the dorsomedial prefrontal cortex.
To navigate social environments, people must simultaneously hold representations about their own and others' abilities. During self-other mergence, people estimate others' abilities not only on the basis of the others' past performance, but the estimates are also influenced by their own performance. For example, if we perform well, we overestimate the abilities of those with whom we are co-operating and underestimate competitors. Self-other mergence is associated with specific activity patterns in the dorsomedial prefrontal cortex (dmPFC). Using a combination of non-invasive brain stimulation, functional magnetic resonance imaging, and computational modeling, we show that dmPFC neurostimulation silences these neural signatures of self-other mergence in relation to estimation of others' abilities. In consequence, self-other mergence behavior increases, and our assessments of our own performance are projected increasingly onto other people. This suggests an inherent tendency to form interdependent social representations and a causal role of the dmPFC in separating self and other representations.
Neuron, 2021; 109
33730554: Miyamoto K, Trudel N, Kamermans K, Lim MC, Lazari A, Verhagen L, Wittmann MK, Rushworth MFS
Identification and disruption of a neural mechanism for accumulating prospective metacognitive information prior to decision-making.
More than one type of probability must be considered when making decisions. It is as necessary to know one's chance of performing choices correctly as it is to know the chances that desired outcomes will follow choices. We refer to these two choice contingencies as internal and external probability. Neural activity across many frontal and parietal areas reflected internal and external probabilities in a similar manner during decision-making. However, neural recording and manipulation approaches suggest that one area, the anterior lateral prefrontal cortex (alPFC), is highly specialized for making prospective, metacognitive judgments on the basis of internal probability; it is essential for knowing which decisions to tackle, given its assessment of how well they will be performed. Its activity predicted prospective metacognitive judgments, and individual variation in activity predicted individual variation in metacognitive judgments. Its disruption altered metacognitive judgments, leading participants to tackle perceptual decisions they were likely to fail.
Neuron, 2021; 109
32868885: Trudel N, Scholl J, Klein-Flügge MC, Fouragnan E, Tankelevitch L, Wittmann MK, Rushworth MFS

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Polarity of uncertainty representation during exploration and exploitation in ventromedial prefrontal cortex.
Environments furnish multiple information sources for making predictions about future events. Here we use behavioural modelling and functional magnetic resonance imaging to describe how humans select predictors that might be most relevant. First, during early encounters with potential predictors, participants' selections were explorative and directed towards subjectively uncertain predictors (positive uncertainty effect). This was particularly the case when many future opportunities remained to exploit knowledge gained. Then, preferences for accurate predictors increased over time, while uncertain predictors were avoided (negative uncertainty effect). The behavioural transition from positive to negative uncertainty-driven selections was accompanied by changes in the representations of belief uncertainty in ventromedial prefrontal cortex (vmPFC). The polarity of uncertainty representations (positive or negative encoding of uncertainty) changed between exploration and exploitation periods. Moreover, the two periods were separated by a third transitional period in which beliefs about predictors' accuracy predominated. The vmPFC signals a multiplicity of decision variables, the strength and polarity of which vary with behavioural context.
Nat Hum Behav, 2021; 5

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BOARD NUMBER: S05-095

EXPLORING TOO MUCH? THE ROLE OF EXPLORATION IN IMPULSIVITY

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Deciding whether to forgo a good choice in favour of exploring a potentially more rewarding alternative is one of the most challenging arbitrations both in human reasoning and in artificial intelligence. Humans show substantial variability in their exploration, and theoretical but only limited empirical work has suggested that excessive exploration is a critical mechanism underlying the psychiatric dimension of impulsivity. We put these theories to test using a large online sample (N=580 healthy adults), dimensional analyses, and computational modelling in a pre-registered study. Capitalising on recent advances in disentangling distinct human exploration strategies, we demonstrate that impulsivity is associated with a specific form of exploration, value-free random exploration (correcting for multiple comparison using Bonferroni correction across 4 parameters x 3 factors, i.e. N=12; r(578)=0.257, p_uncorrected<.001, p_corrected<.001; accounting for age and IQ: r(573)=0.204, p_uncorrected<.001, p_corrected<.001), a computationally light exploration heuristic. Our results not only demonstrate this specific association with impulsivity, but also explore links between exploration and other psychiatric dimensions.

Pubmed:

33393461: Dubois M, Habicht J, Michely J, Moran R, Dolan RJ, Hauser TU
Human complex exploration strategies are enriched by noradrenaline-modulated heuristics.
An exploration-exploitation trade-off, the arbitration between sampling a lesser-known against a known rich option, is thought to be solved using computationally demanding exploration algorithms. Given known limitations in human cognitive resources, we hypothesised the presence of additional cheaper strategies. We examined for such heuristics in choice behaviour where we show this involves a value-free random exploration, that ignores all prior knowledge, and a novelty exploration that targets novel options alone. In a double-blind, placebo-controlled drug study, assessing contributions of dopamine (400 mg amisulpride) and noradrenaline (40 mg propranolol), we show that value-free random exploration is attenuated under the influence of propranolol, but not under amisulpride. Our findings demonstrate that humans deploy distinct computationally cheap exploration strategies and that value-free random exploration is under noradrenergic control.
Elife, 2021; 10
28632961: Kumar P, Waiter GD, Dubois M, Milders M, Reid I, Steele JD
Increased neural response to social rejection in major depression.
Being a part of community is critical for survival and individuals with major depressive disorder (MDD) have a greater sensitivity to interpersonal stress that makes them vulnerable to future episodes. Social rejection is a critical risk factor for depression and it is said to increase interpersonal stress and thereby impairing social functioning. It is therefore critical to understand the neural correlates of social rejection in MDD.
Depress Anxiety, 2017; 34
30252127: Hauser TU, Will GJ, Dubois M, Dolan RJ
Annual Research Review: Developmental computational psychiatry.
Most psychiatric disorders emerge during childhood and adolescence. This is also a period that coincides with the brain undergoing substantial growth and reorganisation. However, it remains unclear how a heightened vulnerability to psychiatric disorder relates to this brain maturation. Here, we propose 'developmental computational psychiatry' as a framework for linking brain maturation to cognitive development. We argue that through modelling some of the brain's fundamental cognitive computations, and relating them to brain development, we can bridge the gap between brain and cognitive development. This in turn can lead to a richer understanding of the ontogeny of psychiatric disorders. We illustrate this perspective with examples from reinforcement learning and dopamine function. Specifically, we show how computational modelling deepens an understanding of how cognitive processes, such as reward learning, effort learning, and social learning might go awry in

psychiatric disorders. Finally, we sketch the promises and limitations of a developmental computational psychiatry.
J Child Psychol Psychiatry, 2019; 60

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BOARD NUMBER: S05-096

TASTE MATTERS: HOW HUNGER RELEVANT FEATURES OF FOOD CONTRIBUTE TO EXPECTANCY-BASED PLACEBO EFFECTS ON HUNGER AND FOOD VALUATION ON THE NEURAL AND BEHAVIORAL LEVEL.

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Aims: This study tested whether cognitive regulation of contextual stimuli, which are relevant to the processing of interoceptive signals mediate expectancy-based placebo effects. **Methods:** The interoceptive outcome measure was hunger reported by 172 healthy female participants, who in the fasted and hungry state were administered a placebo suggested to either curb or enhance appetite. Participants then performed a dietary decision-making task in order to identify with computational modelling and functional magnetic resonance imaging underlying neurocomputational mechanisms of placebo effects on hunger. **Results:** The results replicated an expectancy-based placebo effect on hunger. The curbed-appetite group was less hungry than the enhanced-appetite group. Drift diffusion modelling of reaction times and food choices revealed that this placebo effect on hunger was driven by the enhanced-appetite group, who considered the tastiness of food more and more rapidly during evidence accumulation toward food wanting than the curbed-appetite group. FMRI in a subgroup of 57 participants revealed strong activations of the ventromedial prefrontal cortex, the dorsal anterior cingulate cortex, the precuneus and the posterior insula in response to food value during dietary decision-making. Importantly, these brain responses were stronger in the enhanced-appetite group than in the curbed-appetite group, and this contrast was modulated by how much the tastiness of food predicted food choices. **Conclusions:** These findings indicate that prognostic expectancies shaped the way interoceptive signals such as hunger were experienced through rapid attentional filtering of the tastiness of food, and the modulation of brain regions associated with valuation, attentional control, and secondary somatosensory processing.

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BOARD NUMBER: S05-097

DOES THE STRESS OF THREATENING SOCIAL EVALUATION INCREASE THE PREFERENCE FOR STATUS SIGNALS?

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Previous theoretical and empirical consumer research suggests that social threats, which induce stress and insecurity, lead to increased consumption of status-signaling brands and products as a compensatory behavior. In our current project, we put this hypothesis to a rigorous and pre-registered empirical test, by submitting participant consumers to a social stress protocol, consisting of a mock interview and mental arithmetics in front of an evaluating panel, or a matched control condition. We characterise the response of our participants on a vector of psychological, biological and behavioural measures. Contrasting previous research, our results show that consumers experiencing social-evaluative threat did not show an increased preference for higher status brands, nor increased liking for products advertised with a status-positioning.

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DECISION-MAKING IN DYNAMIC, CONTINUOUSLY EVOLVING ENVIRONMENTS: A NOVEL TASK DESIGN TO RELIABLY QUANTIFY THE FLEXIBILITY OF DECISION FORMATION AND ITS NEURAL SIGNATURES

POSTER SESSION 05 - SECTION: DECISION-MAKING

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How do we choose the best course of action in a dynamic environment? How far back in time do we look for evidence to guide our current choice? To study the sub-components of decision-making, previous research has mostly employed trial-based choice paradigms, where participants choose between two options, the evidence for which remains fixed within (and often across) trials. However, in everyday life we do not make decisions in confined trials. Instead, we have to continuously accumulate information about decision options in the face of sensory noise and temporal uncertainty. Here, we present data from a novel continuous decision task designed to study decision-making in dynamic and temporally extended choice settings. In two EEG studies (N=28, N=23), participants had to continuously monitor a random dot motion display to detect transient changes in the dominant motion. We found that participants adapt the way they accumulate evidence for a decision to the dynamics of their environment: when signal changes were rare (stable environment), they integrated evidence over longer timescales compared to an environment with frequent changes (study 1). Similarly, when the noise in the evidence stream was higher, participants also adopted longer integration time windows as compared to low noise conditions (study 2). These behavioural differences were accompanied by changes in the neural response to changes in the evidence – an EEG signal which resembled the centro-parietal positivity known to track sensory evidence accumulation. Our findings demonstrate the flexibility of decision making in dynamic environments, and the advantages of continuous task designs.

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BOARD NUMBER: S05-099

CONTROLLABILITY REVEALS SPECIFIC SIGNATURES OF INFORMATION SEEKING DURING CHANGES OF MIND UNDER UNCERTAINTY

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Aims. In uncertain environments, seeking information about alternative choice options is essential for adaptive learning and decision-making. However, information seeking is usually confounded with changes-of-mind about the reliability of the preferred option. Here, we exploited the fact that information seeking requires control over which option to sample to isolate its behavioral and neurophysiological signatures. Methods. We employed an adaptive decision-making task that allows comparing the formation and revision of uncertain beliefs between controllable and uncontrollable conditions. We contrasted changes-of-mind occurring with and without control over information sampling. We tested four variants of this cognitive task for ensuring generalisability of our findings (N=84). Results. Behaviorally, we found that changes-of-mind required more evidence against current beliefs in controllable environments. These changes of mind were also associated with reduced confidence, but were nevertheless more likely to be confirmed on the next trial. Computational modelling explained these behavioural effects through more stable beliefs, rather than a reduced sensitivity to incoming evidence in controllable environments. Multimodal neurophysiological recordings showed that changes-of-mind occurring in controllable environments were preceded by a stronger involvement of a dorsal attentional network in magnetoencephalography, and followed by an increased pupil-linked arousal during decision evaluation. Conclusions. By bridging across historically distinct fields of decision research, these findings characterise specific signatures of human information seeking in controllable environments, and indicate that information seeking increases the saliency of evidence perceived as the direct consequence of one's own actions.

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THE EFFECT OF HUNGER ON ATTENTION AND VALUATION IN PREFERENTIAL CHOICE

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Hunger has been shown to affect food choices, such that hungrier participants prefer more hedonic food options. Eye-tracking and neuroimaging studies suggest that hunger induces an attentional bias towards hedonic food options and impairs cognitive control mechanisms as demonstrated by enhanced activation in the dorsolateral prefrontal cortex. Yet, the extent to which this attention-driven impairment applies to other forms of value-based decisions remains elusive. To fill this gap, we asked participants to make three different types of value-based choices (food choice, social preference, and delay discounting; within-subject design) in a hungry and a satiated state while their eye-movements were being recorded. We hypothesize that the effect of hunger on value-based choice is strongest for the food choice task, while we also predict substantial spill-over effects on social preference and delay discounting such that people make more self- and presence-oriented decisions when being hungry. In addition, we predict a modulating role of attention in all three choice tasks. More specifically, participants are expected to attend to hedonic food options as well as to self- and presence-oriented information earlier and longer which modulates the ultimate decision. Behavioral and computational modeling analyses will be conducted to investigate these hypotheses, including the implementation of a dynamic process model of decision making (i.e., a multi-alternative and time-dependent attentional drift diffusion model). We will present the results of our data, which will elucidate the effect of hunger on attentional dynamics and preference formation across various types of value-based decisions.

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BOARD NUMBER: S05-101

EFFICIENT COMPRESSION OF SENSORY INFORMATION DURING CATEGORICAL DECISIONS

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Perceptual decisions rely on a cognitive inference process that extracts the statistics of ambiguous sensory observations through imprecise computations. But theories diverge regarding whether this inference process integrates information in its native sensory space or in a compressed category space defined by current decision alternatives. Here we designed a visual categorization task in which we manipulated the ability of human observers to perform inference in sensory and category spaces. We found that human observers spontaneously integrate sensory information in category space by projecting it on the decision axis, upstream from inference. When observers are forced to integrate sensory information in its native space, they do so with lower precision and larger information loss. Magnetoencephalographic (MEG) brain activity showed compressed neural representations of stimulus and decision signals in conditions where observers perform inference in category space. Together, these findings indicate that humans mitigate the costs of imprecise inference by focusing limited resources on decision-relevant information.

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BOARD NUMBER: S05-102

COGNITIVE PROCESSES AND PERSONALITY TRAITS ASSOCIATED WITH PHENOTYPES OF SUSCEPTIBILITY TO (MIS)INFORMATION.

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Misinformation poses a serious threat to modern societies. Thus, one of the biggest scientific challenges of this decade is to investigate the nature of cognitive mechanisms and personality traits that contribute to the assessment of news' veracity, failures in the discernment of their truthfulness, and the scale of behavioral engagement.
Our research aimed to establish cognitive mechanisms and psychological traits associated with various phenotypes of susceptibility to information.
For this, the participants completed online surveys that consisted of a new scale designed to classify people into one of four phenotypes of susceptibility to true and false information, advanced cognitive tests, and reliable psychological instruments.
The four identified phenotypes: doubters, knowers, duffers, and consumers, showed that believing in misinformation does not imply denying the truth. In contrast, the numerically largest phenotypes encompassed individuals who were either susceptible (consumers) or resistant (doubters), in terms of veracity judgment and behavioral engagement, to any news, regardless of its truthfulness. Significantly less numerous were the phenotypes characterized by excellent and poor discernment of the news' truthfulness (the knowers and the duffers, respectively). The phenotypes significantly differed in sensitivity to positive and negative feedback, cognitive judgment bias, extraversion, conscientiousness, agreeableness, emotional stability, grandiose narcissism, anxiety, and dispositional optimism.
Presented results reveal cognitive and psychological mechanisms underlying susceptibility to (mis)information.
The research leading to these results has received funding from the EEA Financial Mechanism 2014-2021. Project: 2019/35/J/HS6/03498.

FENS Forum 2022
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BOARD NUMBER: S05-103

DECISIONS ARE GUIDED BY LEARNING AND PERCEPTUAL BIASES IN A 2-ALTERNATIVE-FORCED-CHOICE TASK

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Traditionally, improved performance is a key hallmark of learning. However, even if the process of learning may eventually lead to near-perfect performance, the learning trajectory depends on many factors, such as trial order or how the task is represented. In addition, a myriad number of biases affect human and animal performance, for instance serial (Fritsche et al. 2017), choice (Busse et al. 2011, Abrahamyan et al. 2016) or sensory history biases (Akrami et al. 2018, Ashourian et al. 2011) in perceptual decision-making tasks. Here, we find that rats and humans show different learning patterns in a 2-alternative forced-choice (2AFC) task involving the categorization of auditory stimuli based on a perceptual boundary. We show that models based only on feedback-dependent learning, including those incorporating statistical decision confidence, are not sufficient to explain data about the effect of a previous stimulus on the current stimulus. Instead, we identify a stimulus-dependent repulsion effect that contributes to learning in this task. This repulsion effect, in tandem with a feedback-dependent component that may depend on confidence, can recreate patterns in the data with high fidelity. From there, we further isolate the stimulus-dependent component in a separate experiment that limits feedback to only a certain portion of trials and find that purely stimulus-dependent learning can account for the data. We conclude that both stimulus-dependent and feedback-dependent processes are necessary to explain patterns of learning.

FENS Forum 2022
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BOARD NUMBER: S05-104

COMPETING COGNITIVE PRESSURES ON HUMAN EXPLORATION IN THE ABSENCE OF TRADE-OFF WITH EXPLOITATION

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Exploring novel environments through sequential sampling is essential for efficient decision-making under uncertainty. In the laboratory, human exploration has been studied in situations where exploration is traded against reward maximisation. By design, these 'explore-exploit' dilemmas confound the behavioural characteristics of exploration with those of the trade-off itself. Moreover, outside the laboratory, most choices do not yield immediate rewards. Aims: Here we wanted to document the novel patterns of human exploration arising when exploration is released from its trade-off with reward maximisation. Methods: We designed a novel sequential sampling task where exploration can be studied and compared in the presence and absence of a trade-off. Detailed model-based analyses of choice behaviour reveal specific patterns arising only in situations where information seeking is not weighed against reward seeking. Results: As expected, human choices are directed toward the most uncertain option available, but only after an initial sampling phase consisting of choice streaks from each novel option. These findings outline competing cognitive pressures on information seeking: the repeated sampling of the current option (for hypothesis testing), and the directed sampling of the most uncertain option available (for structure mapping). Interestingly, these two forms of information seeking are co-expressed in the same participants. Conclusions: This task overcomes the long-standing confound between the behavioural signatures of exploration and those of explore-exploit dilemmas. Second, our findings provide compelling insights into an undocumented competition between local and global forms of information seeking, that is only expressed when exploration is not traded-off against reward maximisation.

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BOARD NUMBER: S05-105

HISTORY-DEPENDENT PRIORS AS A MODEL FOR ANIMAL BEHAVIOUR IN WORKING MEMORY AND STIMULUS PREDICTION TASKS

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Animals integrate sensory evidence to make decisions. These decisions are by various types of history-dependent biases. Several models have been proposed to account for how humans use past experience to inform decision making. Most normative models assume that humans interpret new sensory evidence by integrating a noisy sensory stimulus with a time-invariant prior. However, subjects rarely have access to the entire sensory evidence at once. Here, we propose that subjects' priors evolve over time following exposure to new sensory evidence. Due to the continuous update of their priors, subjects use an estimated and imperfect prior to making decisions. By simulating a delayed comparison task, we show that our model can account for contraction bias and serial dependency. In addition, our model suggests that individuals can develop a positive feedback loop, due to sequential updates of their priors, and exhibit a form of perceptual confirmation bias. The intensity of this bias depends on internal, individual-dependent parameters and external sensory noise. We test the predictions from our model on human subjects performing a stimulus prediction task. In agreement with our predictions, we observe heterogeneity in participants' performance with some participants showing signatures of perceptual confirmation bias. The complex behaviour shown in this task could not be accounted for by any other model that integrates evidence over time whose prior remains unchanged over time. Instead, our model, based on continuous estimation of priors, provides a simple theoretical framework that accounts for human and animal behaviour in both working memory and stimulus prediction tasks.

Pubmed:
34433026: Kaleb K, Pedrosa V, Clopath C
Network-centered homeostasis through inhibition maintains hippocampal spatial map and cortical circuit function.
Despite ongoing experiential change, neural activity maintains remarkable stability. Although this is thought to be mediated by homeostatic plasticity, what aspect of neural activity is conserved and how the flexibility necessary for learning and memory is maintained is not fully understood. Experimental studies suggest that there exists network-centered, in addition to the well-studied neuron-centered, control. Here we computationally study such a potential mechanism: input-dependent inhibitory plasticity (IDIP). In a hippocampal model, we show that IDIP can explain the emergence of active and silent place cells as well as remapping following silencing of active place cells. Furthermore, we show that IDIP can also stabilize recurrent dynamics while preserving firing rate heterogeneity and stimulus representation, as well as persistent activity after memory encoding. Hence, the establishment of global network balance with IDIP has diverse functional implications and may be able to explain experimental phenomena across different brain areas.
Cell Rep, 2021; 36
32649658: Pedrosa V, Clopath C
The interplay between somatic and dendritic inhibition promotes the emergence and stabilization of place fields.
During the exploration of novel environments, place fields are rapidly formed in hippocampal CA1 neurons. Place cell firing rate increases in early stages of exploration of novel environments but returns to baseline levels in familiar environments. Although similar in amplitude and width, place fields in familiar environments are more stable than in novel environments. We propose a computational model of the hippocampal CA1 network, which describes the formation, dynamics and stabilization of place fields. We show that although somatic disinhibition is sufficient to form place fields, dendritic inhibition along with synaptic plasticity is necessary for place field stabilization. Our model suggests that place cell stability can be attributed to strong excitatory synaptic weights and strong dendritic inhibition. We show that the interplay between somatic and dendritic inhibition balances the increased excitatory weights, such that place cells return to their baseline firing rate after exploration. Our model suggests that different types of interneurons are essential to unravel the mechanisms underlying place field plasticity. Finally, we predict that artificially induced dendritic events can shift place fields even after place field stabilization.

PLoS Comput Biol, 2020; 16
29503184: González-Rueda A, Pedrosa V, Feord RC, Clopath C, Paulsen O
Activity-Dependent Downscaling of Subthreshold Synaptic Inputs during Slow-Wave-Sleep-like Activity In Vivo.
Activity-dependent synaptic plasticity is critical for cortical circuit refinement. The synaptic homeostasis hypothesis suggests that synaptic connections are strengthened during wake and downscaled during sleep; however, it is not obvious how the same plasticity rules could explain both outcomes. Using whole-cell recordings and optogenetic stimulation of presynaptic input in urethane-anesthetized mice, which exhibit slow-wave-sleep (SWS)-like activity, we show that synaptic plasticity rules are gated by cortical dynamics in vivo. While Down states support conventional spike timing-dependent plasticity, Up states are biased toward depression such that presynaptic stimulation alone leads to synaptic depression, while connections contributing to postsynaptic spiking are protected against this synaptic weakening. We find that this novel activity-dependent and input-specific downscaling mechanism has two important computational advantages: (1) improved signal-to-noise ratio, and (2) preservation of previously stored information. Thus, these synaptic plasticity rules provide an attractive mechanism for SWS-related synaptic downscaling and circuit refinement.
Neuron, 2018; 97
32879322: Udakis M, Pedrosa V, Chamberlain SEL, Clopath C, Mellor JR
Interneuron-specific plasticity at parvalbumin and somatostatin inhibitory synapses onto CA1 pyramidal neurons shapes hippocampal output.
The formation and maintenance of spatial representations within hippocampal cell assemblies is strongly dictated by patterns of inhibition from diverse interneuron populations. Although it is known that inhibitory synaptic strength is malleable, induction of long-term plasticity at distinct inhibitory synapses and its regulation of hippocampal network activity is not well understood. Here, we show that inhibitory synapses from parvalbumin and somatostatin expressing interneurons undergo long-term depression and potentiation respectively (PV-iLTD and SST-iLTP) during physiological activity patterns. Both forms of plasticity rely on T-type calcium channel activation to confer synapse specificity but otherwise employ distinct mechanisms. Since parvalbumin and somatostatin interneurons preferentially target perisomatic and distal dendritic regions respectively of CA1 pyramidal cells, PV-iLTD and SST-iLTP coordinate a reprioritisation of excitatory inputs from entorhinal cortex and CA3. Furthermore, circuit-level modelling reveals that PV-iLTD and SST-iLTP cooperate to stabilise place cells while facilitating representation of multiple unique environments within the hippocampal network.
Nat Commun, 2020; 11
28119596: Pedrosa V, Clopath C
The Role of Neuromodulators in Cortical Plasticity. A Computational Perspective.
Neuromodulators play a ubiquitous role across the brain in regulating plasticity. With recent advances in experimental techniques, it is possible to study the effects of diverse neuromodulatory states in specific brain regions. Neuromodulators are thought to impact plasticity predominantly through two mechanisms: the gating of plasticity and the upregulation of neuronal activity. However, the consequences of these mechanisms are poorly understood and there is a need for both experimental and theoretical exploration. Here we illustrate how neuromodulatory state affects cortical plasticity through these two mechanisms. First, we explore the ability of neuromodulators to gate plasticity by reshaping the learning window for spike-timing-dependent plasticity. Using a simple computational model, we implement four different learning rules and demonstrate their effects on receptive field plasticity. We then compare the neuromodulatory effects of upregulating learning rate versus the effects of upregulating neuronal activity. We find that these seemingly similar mechanisms do not yield the same outcome: upregulating neuronal activity can lead to either a broadening or a sharpening of receptive field tuning, whereas upregulating learning rate only intensifies the sharpening of receptive field tuning. This simple model demonstrates the need for further exploration of the rich landscape of neuromodulator-mediated plasticity. Future experiments, coupled with biologically detailed computational models, will elucidate the diversity of mechanisms by which neuromodulatory state regulates cortical plasticity.
Front Synaptic Neurosci, 2016; 8

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BOARD NUMBER: S05-106

PREFERENTIAL DECISIONS BY ASSOCIATION: THE INTERPLAY OF INTERNAL PREFERENCE ON HUMANS' EXTERNAL PERCEPTION

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Every day, everyone makes hundreds of decisions based on different types of information. In perceptual decisions, we aim to make a correct choice based on external information. In preference-based decisions, one's choices are dependent on their internal, subjective evaluations. Although these two types of decisions have been extensively investigated, less is known on direct comparisons between the two decision domains. The current study examined decision performance under the contexts of internal preference and external perception. To this end, sixty human participants performed an internet-based choice paradigm, using identical visual stimuli for both types of decisions. We parametrically morphed geometric shapes to vary between circle-like and diamond-like stimuli. Preference-based decisions, participants remembered mappings between different shapes and food items at different levels of subjective preference, and the participants were then required to decide whether a visual stimulus is associated with food items they prefer. Using the same set of visual stimuli, participants performed perceptual decisions in discriminating whether a given stimulus is more like a circle or a diamond. We showed that assigning internal preference values to geometric shapes led to a lower discriminating sensitivity than perceptual decisions, indexed by a decreased Weber ratio. There was no difference in participants' response bias between the two types of decisions. Preference decisions were associated with longer reaction time than perceptual decisions across stimulus levels. Our results suggested that a common computational process may underly preference-based and perceptual decisions and mapping internal preference onto external perceptual information results in additional noise in the decision-making process.

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BOARD NUMBER: S05-107

RODENTS MONITOR THEIR ERROR IN SELF-GENERATED DURATION ON A SINGLE TRIAL BASIS

POSTER SESSION 05 - SECTION: DECISION-MAKING

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When faced with a deadline, individuals' behavior suggests that they represent the mean and the uncertainty of an internal timer to make near-optimal time-dependent decisions. Whether this ability relies on simple trial-and-error adjustments, or whether it involves richer representations, is unknown. Richer representations enable a possibility of error monitoring, that is, the ability for an individual to assess its internal representation of the world and estimate discrepancy in the absence of external feedback. While rodents show timing behavior, whether they can represent and report temporal errors in their own produced duration on a single trial basis is unknown. We designed a novel paradigm requiring rats to produce a target time interval to receive a reward in a given location depending on the magnitude of their timing errors. During the test-trials, rats had to choose a port corresponding to the error magnitude of their just-produced duration to receive a reward. High choice accuracy demonstrates that rats kept track of the values of the timing variables on which they based their decision. Additionally, they kept a representation of the mapping between those timing values and the target value, as well as the history of the reinforcements. The results show for the first time that rats track their own timing errors, deepening our understanding of error monitoring abilities in rodents and the richness of their representation of elapsed time. This paradigm offers a new way to research the neural architecture underlying the ability of neural systems to monitor their own computations.

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BOARD NUMBER: S05-108

BAYESIAN ANALYSIS OF BEHAVIOURAL STRATEGIES AT TRIAL-RESOLUTION DURING TACTILE REVERSAL LEARNING

POSTER SESSION 05 - SECTION: DECISION-MAKING

Adam Chapman[1], Jasper Teutsch[1,2], Fritjof Helmchen[2], Mark Humphries[3], Silvia Maggi[3], Abhishek Banerjee[1,2]
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The ability to continuously discount evidence based on accumulated sensory and contextual information is an essential component of flexible behaviour. However, how animals modify their behavioural strategies to adapt to environmental changes remains understudied. To study the psychophysics of flexible behaviour, we implemented a Bayesian evidence accumulation model to track the probability of strategy implementation on a trial-by-trial basis. Mice were trained on a 'Go/No-go' tactile reversal learning task. We tested multiple choice-driven, cue-driven and history-dependent strategies, finding that naïve WT mice adopted a choice-driven approach during learning, followed by an increase in cue-driven strategies once expert. Following the reversal, naïve mice adopted choice-driven strategies in a greater proportion, with expert mice showing choice-driven and cue-driven strategies returning to pre-reversal adoption faster than during initial learning. Thus mice evolved their behavioural strategy both during and after learning, with task features guiding choice. Pharmacogenetic silencing of medial and lateral orbitofrontal cortex (mOFC and lOFC, respectively), areas contributing to feature-driven strategies, caused global increases in choice-driven strategy adoption. Silencing mOFC caused more exploratory strategies to emerge in response to reward omission, suggesting decreased bias towards the immediate reward. Lateral OFC silencing showed a sudden shift from purely choice-driven strategies while naive to purely cue-driven strategies at expert, implying a role of the lOFC in leveraging prior knowledge in response to novel stimuli. Our analysis sheds light on rule-based strategies underlying flexible behaviour and critical contributions of mOFC and lOFC in flexibly adapting behavioural strategy implementation through reward feedback and past experience.

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BOARD NUMBER: S05-109

IMPACT OF GAINING OR LOSING ON THE VIGOR OF ARM REACHING MOVEMENTS DURING DECISION-MAKING IN NON-HUMAN PRIMATES

POSTER SESSION 05 - SECTION: DECISION-MAKING

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In essence, decision-making is a higher-order brain function that consists in choosing between options. Basing decisions on expected value (defined as the product of the probability of reward associated to an option and the magnitude of that reward) maximizes future-discounted cumulative reward. Remarkably, people and animals have been shown to respond and move faster to outcomes they value more (i.e., with higher expected values). However, how movement vigor (i.e., latency and velocity of movement) is modulated when actions/choices aim at maximizing gains or minimizing losses is still unclear. To clarify this aspect, we trained two rhesus monkeys in a task where they had to choose by performing arm reaching movements between two lotteries that probabilistically predicted different quantities of gains or losses of the same reward. In doing so, we observed that animals chose the best option less frequently when lotteries involved potential losses rather than potential gains. Additionally, we found that latency (defined by reaction time) and velocity (defined by movement time) were longer when animals tried to minimize their losses rather than maximizing their gains. Finally, slower movements when lotteries involved potential losses was found both for lottery pairs with the same probabilities and different quantities and for lottery pairs with the different probabilities and same quantities. These behavioral results therefore suggest that asymmetry in the processing of gains and losses during decision making in non-human primates is not only observable through choice, but is also reflected in the vigor of arm reaching movements.

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BOARD NUMBER: S05-110

ACCUMULATION OF EVIDENCE DURING PERCEPTUAL DECISION-MAKING IN MICE

POSTER SESSION 05 - SECTION: DECISION-MAKING

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During perceptual decision-making, neural circuits must faithfully represent relevant stimulus features, transform them into decision evidence, accumulate this evidence along the stimulus duration and commit to a choice. However, despite the central role that this computation has in decision-making, the dynamics of evidence accumulation are not yet well understood. Here, we have developed a novel auditory two-alternative forced-choice (2AFC) task, in which head-fixed mice listen to two concomitant, intensity-fluctuating stimuli, each presented through a speaker on either side of the head. Mice have to infer which sound (left or right) was louder on average and lick in the associated spout to receive a water reward. We find that mice can learn the task and show a sigmoid probability of a rightward choice. However, the temporal weighting of the evidence is not generally uniform and can be idiosyncratic as revealed by reverse correlation analysis (i.e. psychophysical kernels). Moreover, we find that mice exhibit a tendency to repeat their previous response and that this trial history bias is best explained as repeating lapses rather than a bias term that alters the decision criteria of the categorization, that is an upward rather than horizontal shift of the psychometric curve (repeating lapse = 0.21 ± 0.04, alternating lapse = 0.05 ± 0.01; p=0.001, n=7 mice, data are mean ± s.e.m., two-tailed paired-samples t-test). Together, our results show that evidence integration is heterogeneous across individuals and that is affected by non-sensory biases that limit subjects' performance.

Pubmed:

34849636: Oude Lohuis MN, Canton AC, Pennartz CMA, Olcese U
Higher Order Visual Areas Enhance Stimulus Responsiveness in Mouse Primary Visual Cortex.
Over the past few years, the various areas that surround the primary visual cortex (V1) in the mouse have been associated with many functions, ranging from higher order visual processing to decision-making. Recently, some studies have shown that higher order visual areas influence the activity of the primary visual cortex, refining its processing capabilities. Here, we studied how in vivo optogenetic inactivation of two higher order visual areas with different functional properties affects responses evoked by moving bars in the primary visual cortex. In contrast with the prevailing view, our results demonstrate that distinct higher order visual areas similarly modulate early visual processing. In particular, these areas enhance stimulus responsiveness in the primary visual cortex, by more strongly amplifying weaker compared with stronger sensory-evoked responses (for instance specifically amplifying responses to stimuli not moving along the direction preferred by individual neurons) and by facilitating responses to stimuli entering the receptive field of single neurons. Such enhancement, however, comes at the expense of orientation and direction selectivity, which increased when the selected higher order visual areas were inactivated. Thus, feedback from higher order visual areas selectively amplifies weak sensory-evoked V1 responses, which may enable more robust processing of visual stimuli.
Cereb Cortex, 2021;

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BOARD NUMBER: S05-111

RULE AWARENESS IN MICE PREDICTS CAPACITY TO GENERALIZE RULES TO NEW STIMULI.

POSTER SESSION 05 - SECTION: DECISION-MAKING

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[1]Radboud University, Donders Institute For Brain, Cognition And Behaviour, Nijmegen, Netherlands, [2]Radboud University Nijmegen, Donders Center For Neuroscience, Nijmegen, Netherlands, [3]Maastricht University, Faculty Of Psychology And Neuroscience, Maastricht, Netherlands, [4]Radboud University Nijmegen, Neuroinformatics, Donders Institute, Nijmegen, Netherlands, [5]Donders Institute for Brain, Cognition and Behaviour, Biophysics, Nijmegen, Netherlands, [6]Ernst Strüngmann Institute for Neuroscience in Cooperation with Max Planck Society, Zero Noise Lab, Frankfurt am Main, Germany

The ability to apply acquired knowledge to new contexts brings clear advantages - when it is confined to appropriately similar situations. Successful behaviour must therefore balance utilizing known rules and acquiring new ones. Here we show that mice apply one of two learning strategies when faced with a visual dicrimination task featuring increasingly varied stimuli: While some mice applied a learned rule to new stimuli without delay (generalizers), others repeatedly acquired a new rule for each new stimulus pair (re-learners). To characterize these different learning strategies, we trained head-fixed mice to approach either horizontal or vertical grey-scale stimuli in a virtual environment, with the range of stimuli increasing gradually. The animals' capacity to generalize their learned orientation preference to new stimuli was predicted by their ability to predict trial outcomes for the original stimuli: Animals that behaved more hesitantly in incorrect than in correct trials, signaling uncertainty about their stimulus choice, were also better able to generalize their behaviour to new stimuli. This suggests that the degree to which different animals adhere to learned rules is a stable cognitive processing style that appears to apply across different contexts. Finally, we connect these two learning styles to neuronal response preferences in primary visual cortex.

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BOARD NUMBER: S05-112

A COMPUTATIONAL AND EXPERIMENTAL FRAMEWORK FOR TESTING THEORIES OF LEARNING

POSTER SESSION 05 - SECTION: DECISION-MAKING

Samuel Liebana Garcia[1], Aeron Laffere[1], Peter Zatka-Haas[1], Rafal Bogacz[2], Armin Lak[1], Andrew Saxe[3]
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Learning to make decisions underlies many aspects of our behavior. As such, understanding the neural mechanisms of learning is a fundamental goal in systems neuroscience. However, despite proliferating theories and large datasets, testing theories of learning remains challenging. Experiments often only approximately control the learning epoch, making the interpretation of learning trajectories difficult; and modern theories of learning (such as deep RL networks) are often complex and hard to fit to data. Here we address this gap with a three-fold approach: first, we introduce a perceptual decision-making paradigm for mice that remains unaltered across the whole learning epoch, such that the learning period is well-controlled (see poster by Laffere et al.). Second, we develop a software toolbox that leverages the automatic differentiation, Just-in-time (JIT) compilation, and GPU acceleration enabled by the JAX software library to enable fast trial-by-trial fitting of diverse and reasonably complex models to behavior. And third, we employ model reduction methods to introduce a class of models that retain key features of more complex theories of learning, such as ‘depth’, while remaining tractable. We describe learning trajectories of tens of mice trained in the task, and show that a `deep’ model with a combined goal-directed (reward-dependent) and habitual (action-dependent) learning rule best fits the data. More broadly, our framework takes a step towards linking modern theories of learning to large-scale data being generated by systems neuroscience, offering a potential route to testing theories of learning in the brain and mind.

Pubmed:

34456673: Mottaghi S, Kohl S, Biemann D, Liebana S, Montaño Crespo RE, Buchholz O, Wilson M, Klaus C, Uchenik M, Münkel C, Schmidt R, Hofmann UG
Bilateral Intracranial Beta Activity During Forced and Spontaneous Movements in a 6-OHDA Hemi-PD Rat Model.
Cortico-basal ganglia beta oscillations (13-30 Hz) are assumed to be involved in motor impairments in Parkinson's Disease (PD), especially in bradykinesia and rigidity. Various studies have utilized the unilateral 6-hydroxydopamine (6-OHDA) rat PD model to further investigate PD and test novel treatments. However, a detailed behavioral and electrophysiological characterization of the model, including analyses of popular PD treatments such as DBS, has not been documented in the literature. We hence challenged the 6-OHDA rat hemi-PD model with a series of experiments (i.e., cylinder test, open field test, and rotarod test) aimed at assessing the motor impairments, analyzing the effects of Deep Brain Stimulation (DBS), and identifying under which conditions excessive beta oscillations occur. We found that 6-OHDA hemi-PD rats presented an impaired performance in all experiments compared to the sham group, and DBS could improve their overall performance. Across all the experiments and behaviors, the power in the high beta band was observed to be an important biomarker for PD as it showed differences between healthy and lesioned hemispheres and between 6-OHDA-lesioned and sham rats. This all shows that the 6-OHDA hemi-PD model accurately represents many of the motor and electrophysiological symptoms of PD and makes it a useful tool for the pre-clinical testing of new treatments when low β (13-21 Hz) and high β (21-30 Hz) frequency bands are considered separately.
Front Neurosci, 2021; 15
32425752: Mottaghi S, Afshari N, Buchholz O, Liebana S, Hofmann UG
Modular Current Stimulation System for Pre-clinical Studies.
Electric stimulators with precise and reliable outputs are an indispensable part of electrophysiological research. From single cells to deep brain or neuromuscular tissue, there are diverse targets for electrical stimulation. Even though commercial systems are available, we state the need for a low-cost, high precision, functional, and modular (hardware, firmware, and software) current stimulation system with the capacity to generate stable and complex waveforms for pre-clinical research. The system presented in this study is a USB controlled 4-channel modular current stimulator that can be expanded and generate biphasic arbitrary waveforms with 16-bit resolution, high temporal precision (µs), and passive charge balancing: the

NES STiM (Neuro Electronic Systems Stimulator). We present a detailed description of the system's structural design, the controlling software, reliability test, and the pre-clinical studies [deep brain stimulation (DBS) in hemi-PD rat model] in which it was utilized. The NES STiM has been tested with MacOS and Windows operating systems. Interfaces to MATLAB source codes are provided. The system is inexpensive, relatively easy to build and can be assembled quickly. We hope that the NES STiM will be used in a wide variety of neurological applications such as Functional Electrical Stimulation (FES), DBS and closed loop neurophysiological research.
Front Neurosci, 2020; 14

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BOARD NUMBER: S05-113

ENGAGEMENT AND STRATEGY: COMPLEMENTARY NEURAL CIRCUITS FOR SELF-DRIVEN SPEED AND ACCURACY CHANGES IN MACAQUES

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Aims Quick decisions are prone to errors, leading to a trade-off between speed and accuracy. In different circumstances animals may chose a faster or more accurate approach; some strategies may be more adaptive depending on the value and difficulty of the current trial, and the richness of the environment. However, subjects may also break this trade-off and increase both speed and accuracy if they are highly motivated. Previous work identified the neural bases of instructed fast response preparation, but little is known about mechanisms influencing self-driven speed and accuracy adjustments. Methods Three macaques performed a binary decision task based on perceptual evidence accumulation with variable reward stakes and intertrial intervals, while in an MRI scanner. Each trial was characterised in a two-dimensional space: along the speed-accuracy trade-off (SAT) axis, trials went from fast-and-inaccurate to slow-and-accurate, and this captured most of the variance. On the perpendicular axis, trials varied from slow and inaccurate (low efficiency) to fast and accurate (high efficiency). We identified the neural correlates of future SAT and efficiency, measured several seconds before each trial. Results Activity in the macaque frontal pole predicted SAT changes, whereas activity in a network including habenula and thalamus predicted future efficiency. Moreover, the same areas mediated the effects of contextual factors such as reward received and reward at stake on self-driven behaviour changes. Conclusions We identified two dissociable neural circuits: a prefrontal one influencing cost-free strategic changes in speed-accuracy trade-off and a subcortical circuit driving costly variations in efficiency depending on animals' motivation.

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BOARD NUMBER: S05-114

ANTICIPATORY CODING OF ANXIETY IN THE VENTRAL HIPPOCAMPUS

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Anxiety is an aversive mood reflecting the expectation of potential threats. The ventral hippocampus (vHip) is a key brain region for the genesis of anxiety responses. Recent studies have shown that anxiety is mediated by the recruitment of pyramidal neurons from the vHip (known as 'anxiety' neurons) targeting various limbic structures. Despite the function of the vHip in triggering emotional responses, whether the vHip also signals the anticipation of an anxiogenic experience remains elusive. We hypothesized that distinct vHip neuronal populations either reflect the direct experience of anxiety or its anticipation. To address this question, we developed a novel trial-based anxiety paradigm, the forced emotional shifting task (FEST), with sequential and separated exposure to an anxiogenic situation (an elevated, bright and open space) or a safe situation (closed and darker space). By performing *in vivo* single-unit recordings from the vHip of freely-moving mice during FEST, we identified both anxiety neurons as well as a distinct neuronal population with ramping activity in the safe configuration of FEST, suggestive of an anticipatory coding for anxiety. The activity of these anticipatory neurons intrinsically depended on the experience of anxiety, indicating that anticipatory neurons are not the mere result of novelty or time processing. Moreover, unpredictable time intervals impaired the formation of ramping/anticipatory neurons which associated with lower anxiety-related activity in vHip, suggestive of a functional interaction between anticipatory and anxiety neurons. Collectively, our results point to distinct neuronal populations within the vHip mediating anxiety or its anticipation.

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BOARD NUMBER: S05-115

RESOLVING DECISION-MAKING DURING EMOTIONAL CONFLICTS BY VENTRAL HIPPOCAMPAL CIRCUITS.

POSTER SESSION 05 - SECTION: DECISION-MAKING

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How the brain computes decisions to support adaptive behaviours under different demands is a long-standing question. Decision-making does not solely rely on cognitive judgements but is likewise influenced by internal states. The ventral hippocampus (vHip) is a higher-order cortical brain region critical for processing emotions such as anxiety. Here, we examined the influence of anxiety levels on decision-making while recording neuronal activity in the vHip and medial prefrontal cortex (mPFC) as mice performed decision-making tasks under emotional conflicts. We observed that the activity of vHip neurons was scaling according to anxiety levels with concomitant remapping of firing fields. This effect was modulated by trajectories with different anxiety levels but was not a mere reflection of novelty. We additionally identified vHip neurons with preferential ‘deliberating’ and ‘anxiety’ features as mice made decisions under emotional conflicts. Using selective optogenetic inhibition of vHip terminals in the mPFC, we showed that mice exhibited biased decision-making selectively during trials with higher emotional conflicts. Collectively, these results suggest that vHip circuits targeting mPFC mediate decision-making under emotional conflicts.

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BOARD NUMBER: S05-116

DIFFERENTIAL CODING OF ABSOLUTE AND RELATIVE VALUE IN THE DROSOPHILA BRAIN.

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Animals use prior experience to assign absolute (good or bad) and also relative (better or worse) value to new experience. These learned values guide appropriate later decision-making. While our understanding of how the dopaminergic system computes absolute value is relatively advanced, the mechanistic underpinnings of relative valuation are unclear. Here we reveal mechanisms of absolute and relative aversive valuation in *Drosophila*. Three types of punishment-sensitive dopaminergic neurons (DANs) drive intensity-scaled plasticity at their respective mushroom body output neuron (MBON) connections to code absolute aversive value. In contrast, by comparing current and previous aversive experiences the MBON-DAN network can code relative aversive value by recruiting a specific subtype of reward-coding DANs which assigns a ‘better than’ value to the lesser of two aversive experiences. This study therefore provides an important functional consequence of having opposing populations of DANs and illustrates how these can operate together as a system within the MB network to code and compare sequential aversive experience to learn relative aversive value.

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BOARD NUMBER: S05-117

MUSHROOM BODY OUTPUT NEURON 02 REGULATES THE TRANSITION FROM GOAL-DIRECTED ACTIONS TO HABITS IN DROSOPHILA

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Goal-directed exploration of the environment allows an animal to learn about the relationships between stimuli and how the environment responds to its actions. In this goal-directed phase, animals can flexibly apply learned relationships to other contexts. However, flexibility usually implies a cost in time, together with higher cognitive and energetic costs. In contrast, the formation of habits ensures fast and efficient behaviors. The learning mechanisms that lead to flexible and efficient behaviors, respectively, interact with each other. During the early, goal-directed phase of such composite operant learning situations, the process that mediates learning about relations in the environment (world-learning) is known to inhibit the process that renders behaviors stereotypic and efficient (self-learning), presumably in order to prevent premature habit-formation. In humans, imbalance between flexible actions and habitual responses can be linked to neuropsychiatric diseases (OCD, drug addiction). We use the fruit fly *Drosophila* to study the interactions between world- and self-learning which mediate the transition mechanisms from goal-directed actions to habitual responses. In *Drosophila* goal-directed behavior inhibits habit formation at the level of the mushroom bodies (MB), such that inhibition of the MBs results in premature habit formation. Here, we present the identification of a single mushroom body output neuron responsible for mediating this highly adaptive interaction between the two learning systems.

Pubmed:
31308381: Lyutova R, Selcho M, Pfeuffer M, Segebarth D, Habenstein J, Rohwedder A, Frantzmann F, Wegener C, Thum AS, Pauls D
Reward signaling in a recurrent circuit of dopaminergic neurons and peptidergic Kenyon cells.
Dopaminergic neurons in the brain of the Drosophila larva play a key role in mediating reward information to the mushroom bodies during appetitive olfactory learning and memory. Using optogenetic activation of Kenyon cells we provide evidence that recurrent signaling exists between Kenyon cells and dopaminergic neurons of the primary protocerebral anterior (pPAM) cluster. Optogenetic activation of Kenyon cells paired with odor stimulation is sufficient to induce appetitive memory. Simultaneous impairment of the dopaminergic pPAM neurons abolishes appetitive memory expression. Thus, we argue that dopaminergic pPAM neurons mediate reward information to the Kenyon cells, and in turn receive feedback from Kenyon cells. We further show that this feedback signaling is dependent on short neuropeptide F, but not on acetylcholine known to be important for odor-shock memories in adult flies. Our data suggest that recurrent signaling routes within the larval mushroom body circuitry may represent a mechanism subserving memory stabilization.
Nat Commun, 2019; 10
28256578: Ferry QR, Lyutova R, Fulga TA
Rational design of inducible CRISPR guide RNAs for de novo assembly of transcriptional programs.
CRISPR-based transcription regulators (CRISPR-TRs) have transformed the current synthetic biology landscape by allowing specific activation or repression of any target gene. Here we report a modular and versatile framework enabling rapid implementation of inducible CRISPR-TRs in mammalian cells. This strategy relies on the design of a spacer-blocking hairpin (SBH) structure at the 5' end of the single guide RNA (sgRNA), which abrogates the function of CRISPR-transcriptional activators. By replacing the SBH loop with ligand-controlled RNA-cleaving units, we demonstrate conditional activation of quiescent sgRNAs programmed to respond to genetically encoded or externally delivered triggers. We use this system to couple multiple synthetic and endogenous target genes with specific inducers, and assemble gene regulatory modules demonstrating parallel and orthogonal transcriptional programs. We anticipate that this 'plug and play' approach will be a valuable addition to the synthetic biology toolkit, facilitating the understanding of natural gene circuits and the design of cell-based therapeutic strategies.
Nat Commun, 2017; 8
25359929: Pauls D, von Essen A, Lyutova R, van Giesen L, Rosner R, Wegener C, Sprecher SG

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Potency of transgenic effectors for neurogenetic manipulation in Drosophila larvae.

Genetic manipulations of neuronal activity are a cornerstone of studies aimed to identify the functional impact of defined neurons for animal behavior. With its small nervous system, rapid life cycle, and genetic amenability, the fruit fly Drosophila melanogaster provides an attractive model system to study neuronal circuit function. In the past two decades, a large repertoire of elegant genetic tools has been developed to manipulate and study neural circuits in the fruit fly. Current techniques allow genetic ablation, constitutive silencing, or hyperactivation of neuronal activity and also include conditional thermogenetic or optogenetic activation or inhibition. As for all genetic techniques, the choice of the proper transgenic tool is essential for behavioral studies. Potency and impact of effectors may vary in distinct neuron types or distinct types of behavior. We here systematically test genetic effectors for their potency to alter the behavior of Drosophila larvae, using two distinct behavioral paradigms: general locomotor activity and directed, visually guided navigation. Our results show largely similar but not equal effects with different effector lines in both assays. Interestingly, differences in the magnitude of induced behavioral alterations between different effector lines remain largely consistent between the two behavioral assays. The observed potencies of the effector lines in aminergic and cholinergic neurons assessed here may help researchers to choose the best-suited genetic tools to dissect neuronal networks underlying the behavior of larval fruit flies.

Genetics, 2015; 199

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BOARD NUMBER: S05-118

PSYCHOMETRIC PROFILE, HETEROGENEITY, AND INTELLECTUAL FUNCTIONING IN FETAL ALCOHOL SPECTRUM DISORDER

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Fetal Alcohol Spectrum Disorders (FASD) are characterized by a variety of cognitive and behavioral disorders, with intellectual, attentional, and executive impairments being the most reported. In clinical practice, the Intelligence Quotient (IQ) is rarely interpreted in this population because of a deemed too strong heterogeneity of the psychometric profile. We propose here an objective characterization of this heterogeneity and a differential analysis between global intellectual functioning and elementary reasoning, in a large retrospective monocentric sample of FASD. Using clinical and psychometric data (WISC 4th or 5th ed.) from 107 children with FASD, with or without fetal alcohol syndrome, we characterized intra-individual heterogeneity (inter-subtest/index variance or excessive difference at 10thp cutoff), searched for profile weaknesses (average at group level, frequency of anomalies at individual level), and specified intellectual functioning in terms of IQ and elementary reasoning (GAI, best reasoning subtest), in comparison to standardization norms and/or a Monte-Carlo simulated population of normalization. Patient performance was poorer than expected on all subtests, with significant weakness in digit memory, letter-number sequencing and coding, and a trend toward better verbal performance. We found no increase in inter-subtest variance or frequency of excessive inter-index differences, but a discordance between the assessment of global efficiency (IQ 32% borderline, 21% deficient) and that of elementary reasoning (12-23% borderline, 2-16% deficient). Our results, which question the notion of cognitive heterogeneity, point to attentional, executive, and procedural cognitive fragility, with strong global repercussions but most often preserving elementary reasoning in at least one modality.

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BOARD NUMBER: S05-119

CENTRAL EEG BETA/ALPHA RATIO PREDICTS THE POPULATION-WIDE CONSUMER BEHAVIOR BETTER THAN THE SURVEY

POSTER SESSION 05 - SECTION: DECISION-MAKING

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Neuroimaging studies have suggested the ability to use the brain activity of a group of individuals to forecast the behavior of a larger independent group at the population level. The current study aimed to forecast the outcome of the digital media campaign using electroencephalography (EEG), eye-tracking, and behavioral measurements. During two studies, 2 groups of participants were exposed to banners, which were used in the real digital marketing campaign of a large food retailer. We calculated EEG, eye-tracking, and behavioral indexes obtained during participants' visual processing of the banners and investigated the relationship between them and the banners' aggregated efficiency calculated based on the decisions of 291,301 Internet users. The central beta/alpha ratio—an EEG-based engagement index—significantly correlated with the aggregated efficiency of banner advertisements. Our multiple linear regression models demonstrated that a combination of EEG and eye-tracking data better explained the market-level efficiency of banner advertisements than behavioral measurements. Overall, our results confirm those of prior studies in neuroforecasting field that the brain activity of a relatively small number of individuals can forecast the aggregate choice of a larger independent group of people.

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FECAL MICROBIOTA TRANSFER REDUCES ALCOHOL PREFERENCE IN STRESSED RATS

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Alcohol use represents a significant health concern, accounting for 4.5% of global disease burden. Only a small proportion of individuals develop persistent alcohol used disorder though. With current pharmacotherapies largely unsatisfying, discovering novel alternatives to prevent alcohol use disorder becomes a priority. Hence, identifying biological markers predicting vulnerability to develop excessive alcohol consumption may lead to real improvement of clinical care. Converging evidence suggests that gut microbiota is capable of influencing immunity, brain and behavior. We thus investigated gut microbiome and signs of peripheral inflammation in stressed rats exhibiting uncontrolled alcohol seeking behaviors defined as: 1)Inability to abstain during a signaled period of reward unavailability, 2)Increased motivation and 3)Persistent alcohol seeking despite aversive foot shocks. Compared to controls, rats exposed to chronic stress during adolescence exhibited impulsive, inattentive and disinhibited behaviors. After 33sessions of daily alcohol (10%weight/volume) self-administration, all rats were screened according to the 3criteria defined above. Majority of the vulnerable group was composed of stressed rats, and most of the resilient group was composed of controls, confirming that stress during adolescence increases the vulnerability to develop AUD-like behavior. All rats were then given access to 2sources of reward: 10%w/v ethanol and saccharine (0.2 %, 0.00625%, 0%), 2consecutive sessions for each concentration, during which stressed rats exhibited a clear-cut preference for alcohol compared to controls. Strikingly, we identify a long-lasting peripheral inflammation in stressed rats (CCL5, IL-4). Not only fecal microbiota transfer lowered stressed rats' preference for alcohol but it restored inflammation modulators levels to those observed in controls.

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BOARD NUMBER: S05-121

SEX AS A FACTOR IN MICE PARADIGMS MODELING ASPECTS OF DEPRESSIVE BEHAVIOR: DIFFERENTIAL RESPONSES TO ANTIDEPRESSANT DRUGS WITH SERT AND DAT BLOCKER PROFILES.

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Depression is a disorder that is twice as common in women than in men, and there are sex differences in the symptomatology and treatment response to this disorder. Mesolimbic dopamine (DA) regulates effort-based decision-making and behavioral activation. Impairments in behavioral activation such as anergia are often seen in people with depression and are highly resistant to treatment. The role of DA in regulating behavioral activation has been extensively studied in male rodents, but little is known about female rodents.**Aims:** We studied the impact of Tetrabenazine (TBZ, a VMAT-2 inhibitor that depletes DA) in male and female CD1 mice on behavioral measures assessing different aspects of depressive-like behavior. Behavioral activation was analyzed using the Forced Swim Test, (FST), social withdrawal was assessed in a Social Interaction (SI) test, anxiety in the Dark and Light box (DL), and sucrose preference and consumption using the two bottle test.**Results:** TBZ increased immobility in the FST both in male and female mice, however it only reduced active behaviors such as climbing in male mice. TBZ did not affect anxiety (as time in the light compartment), SI (as time exploring the conspecific) or sucrose consumption and preference. The DAT blocker bupropion reversed the effect of TBZ in the FST in both sexes. However, the SERT blocker fluoxetine was only effective in female mice. **Conclusions:** These results confirm that DA is involved in behavioral activation. Pro-dopaminergic antidepressants may be able to improve impairments related to anergia, but not other aspects of depression, at least in male mice.

Pubmed:
35151797: Salamone JD, Ecevitoglu A, Carratala-Ros C, Presby RE, Edelstein GA, Fleeher R, Rotolo RA, Meka N, Srinath S, Masthay JC, Correa M
Complexities and paradoxes in understanding the role of dopamine in incentive motivation and instrumental action: Exertion of effort vs. anhedonia.
Instrumental behavior is a very complex and multifaceted process. Behavioral output during instrumental performance is influenced by a variety of factors, including associative conditioning, directional and activational aspects of motivation, affect, action selection and execution, and decision-making functions. Detailed assessments of instrumental behavior can focus on the temporal characteristics of instrumental behavior such as local frequency and response duration, and biophysical measures of response topography such as force output over time. Furthermore, engaging in motivated behavior can require exertion of effort and effort-based decision making. The present review provides an overview of research on the specific deficits in operant behavior induced by dopamine antagonism and depletion. Furthermore, it discusses research on effort-based decision making, and highlights the complexities and seeming paradoxes that are revealed when detailed analyses of operant behavior are conducted, and instrumental behavior is put in the context of factors such as primary or unconditioned food reinforcement, appetite, binge-like eating, and response choice. Although impairments in mesolimbic dopamine are sometimes labeled as being due to "anhedonia", a detailed deconstruction of the findings in this area of research point to a much more complex and nuanced picture of the role that dopamine plays in regulating instrumental behavior. Low doses of DA antagonists and accumbens dopamine depletions blunt the exertion of physical effort as measured by several different challenges in animal studies (e.g., lever pressing, barrier climbing, wheel running), and yet leave fundamental aspects of hedonic reactivity, food motivation, and reinforcement intact. Continued research on the specific features of instrumental behaviors that regulate the sensitivity to impaired dopamine transmission across a number of contexts is important for resolving some of the complexities that are evident in this area of inquiry. These investigations can also provide insights into psychomotor and motivational dysfunctions that are seen in neuropsychiatric conditions such as depression, schizophrenia, and Parkinson's disease.
Brain Res Bull, 2022; 182

34498115: Carratalá-Ros C, Olivares-García R, Martínez-Verdú A, Arias-Sandoval E, Salamone JD, Correa M
Energizing effects of bupropion on effortful behaviors in mice under positive and negative test conditions: modulation of DARPP-32 phosphorylation patterns.
Motivational symptoms such as anergia, fatigue, and reduced exertion of effort are seen in depressed people. To model this, nucleus accumbens (Nacb) dopamine (DA) depletions are used to induce a low-effort bias in rodents tested on effort-based decision-making. We evaluated the effect of the catecholamine uptake blocker bupropion on its own, and after administration of tetrabenazine (TBZ), which blocks vesicular storage, depletes DA, and induces depressive symptoms in humans. Male CD1 mice were tested on a 3-choice-T-maze task that assessed preference between a reinforcer involving voluntary physical activity (running wheel, RW) vs. sedentary activities (sweet food pellet intake or a neutral non-social odor). Mice also were tested on the forced swim test (FST), two anxiety-related measures (dark-light box (DL), and elevated plus maze (EPM)). Expression of phosphorylated DARPP-32 (Thr34 and Thr75) was evaluated by immunohistochemistry as a marker of DA-related signal transduction. Bupropion increased selection of RW activity on the T-maze. TBZ reduced time running, but increased time-consuming sucrose, indicating an induction of a low-effort bias, but not an effect on primary sucrose motivation. In the FST, bupropion reduced immobility, increasing swimming and climbing, and TBZ produced the opposite effects. Bupropion reversed the effects of TBZ on the T-maze and the FST, and also on pDARPP32-Thr34 expression in Nacb core. None of these manipulations affected anxiety-related parameters. Thus, bupropion improved active behaviors, which were negatively motivated in the FST, and active behaviors that were positively motivated in the T-maze task, which has implications for using catecholamine uptake inhibitors for treating anergia and fatigue-like symptoms.
Psychopharmacology (Berl), 2021; 238
34305547: Carratalá-Ros C, López-Cruz L, Martínez-Verdú A, Olivares-García R, Salamone JD, Correa M
Impact of Fluoxetine on Behavioral Invigoration of Appetitive and Aversively Motivated Responses: Interaction With Dopamine Depletion.
Impaired behavioral activation and effort-related motivational dysfunctions like fatigue and anergia are debilitating treatment-resistant symptoms of depression. Depressed people show a bias towards the selection of low effort activities. To determine if the broadly used antidepressant fluoxetine can improve behavioral activation and reverse dopamine (DA) depletion-induced anergia, male CD1 mice were evaluated for vigorous escape behaviors in an aversive context (forced swim test, FST), and also with an exercise preference choice task [running wheel (RW)-T-maze choice task]. In the FST, fluoxetine increased active behaviors (swimming, climbing) while reducing passive ones (immobility). However, fluoxetine was not effective at reducing anergia induced by the DA-depleting agent tetrabenazine, further decreasing vigorous climbing and increasing immobility. In the T-maze, fluoxetine alone produced the same pattern of effects as tetrabenazine. Moreover, fluoxetine did not reverse tetrabenazine-induced suppression of RW time but it reduced sucrose intake duration. This pattern of effects produced by fluoxetine in DA-depleted mice was dissimilar from devaluing food reinforcement by pre-feeding or making the food bitter since in both cases sucrose intake time was reduced but animals compensated by increasing time in the RW. Thus, fluoxetine improved escape in an aversive context but decreased relative preference for active reinforcement. Moreover, fluoxetine did not reverse the anergic effects of DA depletion. These results have implications for the use of fluoxetine for treating motivational symptoms such as anergia in depressed patients.
Front Behav Neurosci, 2021; 15
33493546: Presby RE, Rotolo RA, Hurley EM, Ferrigno SM, Murphy CE, McMullen HP, Desai PA, Zorda EM, Kuperwasser FB, Carratala-Ros C, Correa M, Salamone JD
Sex differences in lever pressing and running wheel tasks of effort-based choice behavior in rats: Suppression of high effort activity by the serotonin transport inhibitor fluoxetine.
Selective serotonin transport (SERT) inhibitors such as fluoxetine are the most commonly prescribed treatments for depression. Although efficacious for many symptoms of depression, motivational impairments such as psychomotor retardation, anergia, fatigue and amotivation are relatively resistant to treatment with SERT inhibitors, and these drugs have been reported to exacerbate motivational deficits in some people. In order to study motivational dysfunctions in animal models, procedures have been developed to measure effort-related decision making, which offer animals a choice between high effort actions leading to highly valued reinforcers, or low effort/low reward options. In the present studies, male and female rats were tested on two different tests of effort-based choice: a fixed ratio 5 (FR5)/chow feeding choice procedure and a running wheel (RW)/chow feeding choice task. The baseline pattern of choice differed across tasks for males and females, with males pressing the lever more than females on the operant task, and females running more than males on the RW task. Administration of the SERT inhibitor and antidepressant fluoxetine suppressed the higher effort activity on each task (lever pressing and wheel running) in both males and females. The serotonin receptor mediating the suppressive effects of fluoxetine is uncertain, because serotonin antagonists with different patterns of receptor selectivity failed to reverse the effects of fluoxetine. Nevertheless, these studies uncovered important sex differences, and demonstrated that the suppressive effects of fluoxetine on high effort activities are not limited to tasks involving food reinforced behavior or appetite

suppressive effects. It is possible that this line of research will contribute to an understanding of the neurochemical factors regulating selection of voluntary physical activity vs. sedentary behaviors, which could be relevant for understanding the role of physical activity in psychiatric disorders.
Pharmacol Biochem Behav, 2021; 202
33471948: Porru S, López-Cruz L, Carratalá-Ros C, Salamone JD, Acquas E, Correa M
Impact of Caffeine on Ethanol-Induced Stimulation and Sensitization: Changes in ERK and DARPP-32 Phosphorylation in Nucleus Accumbens.
Caffeine is frequently consumed with ethanol to reduce the impairing effects induced by ethanol, including psychomotor slowing or incoordination. Both drugs modulate dopamine (DA)-related markers in accumbens (Acb), and Acb DA is involved in voluntary locomotion and locomotor sensitization. The present study determined whether caffeine can affect locomotion induced by acute and repeated ethanol administration in adult male CD-1 mice.
Alcohol Clin Exp Res, 2021; 45
32141919: Correa M, Pardo M, Carratalá-Ros C, Martínez-Verdú A, Salamone JD
Preference for vigorous exercise versus sedentary sucrose drinking: an animal model of anergia induced by dopamine receptor antagonism.
Motivation has activational and directional components. Mesolimbic dopamine is critical for the regulation of behavioral activation and effort-related processes in motivated behaviors. Impairing mesolimbic dopamine function leads to fatigue and anergia, but leaves intact other aspects of reinforce seeking behaviors, such as the consummatory or hedonic component. In male Swiss mice, we characterized the impact of dopamine antagonism on the selection of concurrently presented stimuli that have different vigor requirements. We analyzed running wheel activity versus sucrose solution intake, typically used as a measure of anhedonia. Results are compared with data from nonconcurrent presentation to those stimuli. In the concurrent presentation experiment, control mice preferred to spend time running compared to sucrose intake. Dopamine antagonism shifted relative reinforcer preference, reducing time spent on the running wheel, but actually increasing time-consuming sucrose. Mice increased frequency of bouts for both reinforcers, suggesting that there was fatigue in the running wheel rather than aversion. Moreover, satiation or habituation by preexposing animals to both reinforcers did not shift preferences. In the nonconcurrent experiments, haloperidol reduced running wheel but had no impact on sucrose consumption. Dopamine antagonism did not change preference for sucrose or total volume consumed. Additional correlational analyses indicated that baseline differences in sucrose consumption were independent of baseline running or novelty exploration. Thus, dopamine antagonism seems to have anergic rather than anhedonic effects, and the concurrent presentation in this setting could be useful for assessing preferences based on effort requirements.
Behav Pharmacol, 2020; 31
32082126: Carratalá-Ros C, López-Cruz L, SanMiguel N, Ibáñez-Marín P, Martínez-Verdú A, Salamone JD, Correa M
Preference for Exercise vs. More Sedentary Reinforcers: Validation of an Animal Model of Tetrabenazine-Induced Anergia.
Physical activities can have intrinsic motivational or reinforcing properties. The choice to engage in voluntary physical activity is undertaken in relation to the selection of other alternatives, such as sedentary behaviors, drugs, or food intake. The mesolimbic dopamine (DA) system plays a critical role in behavioral activation or exertion of effort, and DA antagonism or depletion induces anergia in effort-based decision-making tasks. However, little is known about the neural mechanisms underlying the decision-making processes that establish preferences for sedentary vs. activity-based reinforcers. In the present work with male CD1 mice, we evaluated the effect of tetrabenazine (TBZ), a DA-depleting agent, on a three-choice T-maze task developed to assess preference between reinforcers with different behavioral activation requirements and sensory properties [i.e., a running wheel (RW) vs. sweet pellets or a neutral nonsocial odor]. We also studied the effects of TBZ on the forced swim test (FST), which measures climbing and swimming in a stressful setting, and on anxiety tests [dark-light (DL) box and elevated plus maze (EPM)]. In the three-choice task, TBZ reduced time running in the wheel but increased time spent consuming sucrose, thus indicating reduced activation but relatively intact sucrose reinforcement. The effect of TBZ was not mimicked by motivational manipulations that change the value of the reinforcers, such as making the RW aversive or harder to move, food-restricting the animals, inducing a binge-like eating pattern, or introducing social odors. In the FST, TBZ decreased time climbing (most active behavior) and increased immobility but did not affect anxiety in the DL or EPM. These results indicate that the three-choice T-maze task could be useful for assessing DA modulation of preferences for exercise based on activation and effort requirements, differentiating those effects from changes in preference produced by altering physical requirements, food restriction state, and stress during testing.
Front Behav Neurosci, 2019; 13
30237771: Correa M, SanMiguel N, López-Cruz L, Carratalá-Ros C, Olivares-García R, Salamone JD
Caffeine Modulates Food Intake Depending on the Context That Gives Access to Food: Comparison With Dopamine Depletion.
Caffeine is a methylxanthine consumed in different contexts to potentiate alertness and reduce fatigue. However, caffeine

can induce anxiety at high doses. Caffeine is also a minor psychostimulant that seems to act as an appetite suppressant, but there are also reports indicating that it could stimulate appetite. Dopamine also is involved in food motivation and in behavioral activation. In the present series of experiments, we evaluated the effects of acute administration of caffeine on food consumption under different access conditions. CD1 male adult mice had access to highly palatable food (50% sucrose) in a restricted but habitual context, under continuous or intermittent access as well as under anxiogenic, or effortful conditions. Caffeine (2.5-20.0 mg/kg) increased intake at the highest dose under familiar continuous and intermittent access. However, this high dose reduced food intake in the dark-light paradigm. In contrast, a dopamine-depleting agent, tetrabenazine (TBZ; 1.0-8.0 mg/kg) did not affect food intake in any of those experimental conditions. In the T-maze-barrier task that evaluates seeking and taking of food under effortful conditions, caffeine (10.0 mg/kg) decreased latency to reach the food, but did not affect selection of the high-food density arm that required more effort, or the total amount of food consumed. In contrast, TBZ (4.0 mg/kg) reduced selection of the high food density arm with the barrier, thus affecting amount of food consumed. Interestingly, a small dose of caffeine (5.0 mg/kg) was able to reverse the anergia-inducing effects produced by TBZ in the T-maze. These results suggest that caffeine can potentiate or suppress food consumption depending on the context. Moreover, caffeine did not change appetite, and did not impair orientation toward food under effortful conditions, but it rather helped to achieve the goal by improving speed and by reversing performance to normal levels when fatigue was induced by dopamine depletion.
Front Psychiatry, 2018; 9
29655598: SanMiguel N, Pardo M, Carratalá-Ros C, López-Cruz L, Salamone JD, Correa M
Individual differences in the energizing effects of caffeine on effort-based decision-making tests in rats.
Motivated behavior is characterized by activation and high work output. Nucleus accumbens (Nacb) modulates behavioral activation and effort-based decision-making. Caffeine is widely consumed because of its energizing properties. This methylxanthine is a non-selective adenosine A/A receptor antagonist. Adenosine receptors are highly concentrated in Nacb. Adenosine agonists injected into Nacb, shift preference towards low effort alternatives. The present studies characterized effort-related effects of caffeine in a concurrent progressive ratio (PROG)/free reinforcer choice procedure that requires high levels of work output, and generates great variability among different animals. Male Sprague-Dawley rats received an acute dose of caffeine (2.5-20.0 mg/kg, IP) and 30 min later were tested in operant boxes. One group was food-restricted and had to lever pressed for high carbohydrate pellets, another group was non-food-restricted and lever pressed for a high sucrose solution. Caffeine (2.5 and 5.0 mg/kg) increased lever pressing in food-restricted animals that were already high responders. However, in non-restricted animals, caffeine (5.0 and 10.0 mg/kg) increased work output only among low responders. In fact, caffeine (10.0 and 20.0 mg/kg) in non-restricted animals, reduced lever pressing among high responders in the PROG task, and also in a different group of animals lever pressing in an easy task (fixed ratio 7 schedule) that uniformly generates high levels of responding. Caffeine did not modify sucrose preference or consumption under free access conditions. Thus, when animals do not have a homeostatic need, caffeine can help those not very intrinsically motivated to work harder for a more palatable reward. However, caffeine can disrupt performance of animals intrinsically motivated to work hard for a better reward.
Pharmacol Biochem Behav, 2018; 169
29408363: López-Cruz L, San Miguel N, Carratalá-Ros C, Monferrer L, Salamone JD, Correa M
Dopamine depletion shifts behavior from activity based reinforcers to more sedentary ones and adenosine receptor antagonism reverses that shift: Relation to ventral striatum DARPP32 phosphorylation patterns.
The mesolimbic dopamine (DA) system plays a critical role in behavioral activation and effort-based decision-making. DA depletion produces anergia (shifts to low effort options) in animals tested on effort-based decision-making tasks. Caffeine, the most consumed stimulant in the world, acts as an adenosine A/A receptor antagonist, and in striatal areas DA D and D receptors are co-localized with adenosine A and A receptors respectively. In the present work, we evaluated the effect of caffeine on anergia induced by the VMAT-2 inhibitor tetrabenazine (TBZ), which depletes DA. Anergia was evaluated in a three-chamber T-maze task in which animals can chose between running on a wheel (RW) vs. sedentary activities such as consuming sucrose or sniffing a neutral odor. TBZ-caffeine interactions in ventral striatum were evaluated using DARPP-32 phosphorylation patterns as an intracellular marker of DA-adenosine receptor interaction. In the T-maze, control mice spent more time running and much less consuming sucrose or sniffing. TBZ (4.0 mg/kg) reduced ventral striatal DA tissue levels as measured by HPLC, and also shifted preferences in the T-maze, reducing selection of the reinforcer that involved vigorous activity (RW), but increasing consumption of a reinforcer that required little effort (sucrose), at doses that had no effect on independent measures of appetite or locomotion in a RW. Caffeine at doses that had no effect on their own reversed the effects of TBZ on T-maze performance, and also suppressed TBZ-induced pDARPP-32(Thr34) expression as measured by western blot, suggesting a role for D-A interactions. These results support the idea that DA depletion produces anergia, but does not affect the primary motivational effects of sucrose. Caffeine, possibly by acting on A receptors in ventral striatum, reversed the DA depletion effects. It is possible that caffeine, like selective adenosine A2A antagonists, could have some

therapeutic benefit for treating effort-related symptoms.
Neuropharmacology, 2018; 138

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BOARD NUMBER: S05-122

A FORWARD GENETIC SCREEN OF ENU-MUTAGENISED ZEBRAFISH IDENTIFIES LINES SHOWING DEFICITS IN IMPULSE CONTROL

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Impulsivity is defined as acting on the spur of the moment in response to immediate stimuli. Two forms of impulsivity are impulsive action involving deficiency in response inhibition and impulsive choice referring to impairment in delayed gratification. Both forms of impulsivity are associated with many psychiatric disorders including addiction, ADHD and bipolar disorder. While impulse control disorders show moderate to high degrees of heritability, the genetics of impulsivity is not widely studied. We aimed to identify genes and pathways underlying impulsivity using a forward genetic screen of ENU-mutagenised zebrafish. We screened 102 families estimated to cover 7926 dominant and 3554 recessive alleles. We assessed impulsive action using a zebrafish version of 5-choice serial reaction time task. In this task, after the animal learns the association between a stimulus and a reward, a pre-stimulus interval is applied during which a premature response is recorded as a measure of impulsive action. We identified seven candidate families of which one has been further analysed revealing a heritable deficit in impulse control. The exome sequencing of the founder of the family identified 29 candidate mutations of which three are associated with ADHD in GWASs. Our next step is to identify causal mutation and underlying pathways using RNA sequencing. We demonstrated for the first time that a forward genetic screen of zebrafish for impulsivity could identify lines of potential translational relevance to human.

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BOARD NUMBER: S05-123

A RARE VARIANT OF VGLUT3 (P.T8I) IDENTIFIED IN PATIENTS WITH PSYCHIATRIC DISORDERS INDUCES EXCESSIVE HABITS, COCAINE ADDICTION-LIKE AND MALADAPTIVE EATING IN A MOUSE MODEL

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Background. Cholinergic interneurons are major regulators of striatal function and have the particularity to express both the vesicular acetylcholine transporter (VAChT) and the atypical type 3 vesicular glutamate transporter (VGLUT3). VGLUT3 allows cholinergic interneurons to communicate with glutamate as well as to increase acetylcholine vesicular stores by a mechanism named vesicular synergy. We previously reported an increased frequency of allelic variations of *SCL17A8* (encoding VGLUT3) in patients with severe substances use disorders (SUDs); notably of the missense polymorphism p.T8i. **Methods.** The genetic screening of *SCL17A8* mutation was replicated in new samples of patients. Patients with pT8i mutation were extensively phenotyped for cocaine-related disorders. In parallel, a mutant mouse line was generated to understand the molecular, cellular and behavioral impact of the pT8I variant. **Results.** Patients with pT8i allele showed significant increase in clinical severity. At a molecular level, in cellular and animal models, glutamate signaling was not modified by the pT8I allele. In contrast, vesicular synergy and acetylcholine release were severely blunted in VGLUT3$^{T8I/T8I}$ mice. Mutant mice also exhibited an uneven dopaminergic transmission in the dorsal striatum as well as an increased tendency to develop compulsive behaviors for drug or natural reward. Importantly, increasing ACh tone with donepezil in VGLUT3$^{T8I/T8I}$ mice reversed compulsive self-starvation observed in mutant mice. **Conclusions.** The current translational study shows that the VGLUT3-p.T8I mutation can be associated with SUDs and eating disorders. Common mechanisms between addiction and eating disorder could be targeted using cholinergic enhancement with donepezil.

Pubmed:
34491179: El Mestikawy S, Favier M
[Dysregulation of habit formation and vulnerability to eating disorders: Role of striatal cholinergic interneurons].
Med Sci (Paris), 2021 Aug-Sep; 37
33775453: Favier M, Pietrancosta N, El Mestikawy S, Gangarossa G
Leveraging VGLUT3 Functions to Untangle Brain Dysfunctions.
Vesicular glutamate transporters (VGLUTs) were long thought to be specific markers of glutamatergic excitatory transmission. The discovery, two decades ago, of the atypical VGLUT3 has thoroughly modified this oversimplified view. VGLUT3 is strategically expressed in discrete populations of glutamatergic, cholinergic, serotonergic, and even GABAergic neurons. Recent reports show the subtle, but critical, implications of VGLUT3-dependent glutamate co-transmission and its roles in the regulation of diverse brain functions and dysfunctions. Progress in the neuropharmacology of VGLUT3 could lead to decisive

breakthroughs in the treatment of Parkinson's disease (PD), addiction, eating disorders, anxiety, presbycusis, or pain. This review summarizes recent findings on VGLUT3 and its vesicular underpinnings as well as on possible ways to target this atypical transporter for future therapeutic strategies.
Trends Pharmacol Sci, 2021; 42
33164988: Favier M, Janickova H, Justo D, Kljakic O, Runtz L, Natsheh JY, Pascoal TA, Germann J, Gallino D, Kang JI, Meng XQ, Antinora C, Raulic S, Jacobsen JP, Moquin L, Vigneault E, Gratton A, Caron MG, Duriez P, Brandon MP, Neto PR, Chakravarty MM, Herzallah MM, Gorwood P, Prado MA, Prado VF, El Mestikawy S
Cholinergic dysfunction in the dorsal striatum promotes habit formation and maladaptive eating.
Dysregulation of habit formation has been recently proposed as pivotal to eating disorders. Here, we report that a subset of patients suffering from restrictive anorexia nervosa have enhanced habit formation compared with healthy controls. Habit formation is modulated by striatal cholinergic interneurons. These interneurons express vesicular transporters for acetylcholine (VAChT) and glutamate (VGLUT3) and use acetylcholine/glutamate cotransmission to regulate striatal functions. Using mice with genetically silenced VAChT (VAChT conditional KO, VAChTcKO) or VGLUT3 (VGLUT3cKO), we investigated the roles that acetylcholine and glutamate released by cholinergic interneurons play in habit formation and maladaptive eating. Silencing glutamate favored goal-directed behaviors and had no impact on eating behavior. In contrast, VAChTcKO mice were more prone to habits and maladaptive eating. Specific deletion of VAChT in the dorsomedial striatum of adult mice was sufficient to phenocopy maladaptive eating behaviors of VAChTcKO mice. Interestingly, VAChTcKO mice had reduced dopamine release in the dorsomedial striatum but not in the dorsolateral striatum. The dysfunctional eating behavior of VAChTcKO mice was alleviated by donepezil and by l-DOPA, confirming an acetylcholine/dopamine deficit. Our study reveals that loss of acetylcholine leads to a dopamine imbalance in striatal compartments, thereby promoting habits and vulnerability to maladaptive eating in mice.
J Clin Invest, 2020; 130
32559027: Smart K, Nagano-Saito A, Milella MS, Sakae DY, Favier M, Vigneault E, Louie L, Hamilton A, Ferguson SSG, Rosa-Neto P, Narayanan S, El Mestikawy S, Leyton M, Benkelfat C
Metabotropic glutamate type 5 receptor binding availability during dextroamphetamine sensitization in mice and humans.
Glutamate transmission is implicated in drug-induced behavioural sensitization and the associated long-lasting increases in mesolimbic output. Metabotropic glutamate type 5 (mGlu5) receptors might be particularly important, but most details are poorly understood.
J Psychiatry Neurosci, 2021; 46
28994369: Favier M, Carcenac C, Savasta M, Carnicella S
[Motivation and apathy in Parkinson's disease: implication of dopaminergic D receptors].
Med Sci (Paris), 2017; 33
28134302: Favier M, Carcenac C, Drui G, Vachez Y, Boulet S, Savasta M, Carnicella S
Implication of dorsostriatal D3 receptors in motivational processes: a potential target for neuropsychiatric symptoms in Parkinson's disease.
Beyond classical motor symptoms, motivational and affective deficits are frequently observed in Parkinson's disease (PD), dramatically impairing the quality of life of patients. Using bilateral 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra pars compacta (SNc) in rats, we have been able to reproduce these neuropsychiatric/non-motor impairments. The present study describes how bilateral 6-OHDA SNc lesions affect the function of the main striatal dopaminergic (DA) receptor subtypes. Autoradiography was used to measure the levels of striatal DA receptors, and operant sucrose self-administration and neuropharmacological approaches were combined to investigate the causal implication of specific DA receptors subtypes in the motivational deficits induced by a dorsostriatal DA denervation. We found that D3 receptors (DR) exclusively are down-regulated within the dorsal striatum of lesioned rats. We next showed that infusion of a DR antagonist (SB-277011A) in non-lesioned animals specifically disrupts preparatory, but not consummatory behaviors. Our findings reveal an unexpected involvement of dorsostriatal DR in motivational processes. They strongly suggest an implication of dorsostriatal DR in the neuropsychiatric symptoms observed in PD, highlighting this receptor as a potential target for pharmacological treatment.
Sci Rep, 2017; 7
25588931: Carcenac C, Favier M, Vachez Y, Lacombe E, Carnicella S, Savasta M, Boulet S
Subthalamic deep brain stimulation differently alters striatal dopaminergic receptor levels in rats.
High-frequency stimulation (HFS) of the subthalamic nucleus (STN) is recognized as an effective treatment for the motor symptoms of Parkinson's disease (PD), but its mechanisms, particularly as concern dopaminergic transmission, remain unclear. The aim of this study was to evaluate changes in the expression of dopaminergic receptors (D1, D2, and D3 receptors) after prolonged (4 h) unilateral STN-HFS in anesthetized intact rats and rats with total dopaminergic denervation. We used [(3)H]SCH 23390, [(125)I]iodosulpride, and [(125)I]OH-PIPAT to assess the densities of D1R, D2R, and D3R, respectively, within different areas of the striatum-a major input structure of the basal ganglia-including the nucleus

accumbens. We found that STN-HFS increased D1 R levels in almost all of the striatal areas examined, in both intact and denervated rats. By contrast, STN-HFS led to a large decrease in D2 R and D3R levels, limited to the nucleus accumbens and independent of the dopaminergic state of the animals. These data suggest that the influence of STN-HFS on striatal D1 R expression may contribute to its therapeutic effects on motor symptoms, whereas its impact on D2R/D3 R levels in the nucleus accumbens may account for the neuropsychiatric side effects often observed in stimulated PD patients, such as postoperative apathy.
Mov Disord, 2015; 30

24515412: Favier M, Duran T, Carcenac C, Drui G, Savasta M, Carnicella S
Pramipexole reverses Parkinson's disease-related motivational deficits in rats.
Recent evidence suggests that Parkinson's disease affects not only movement, but also cognitive and psychiatric functions. Among these nonmotor complications, apathy, which is defined as a lack of motivation and operationalized as a quantitative reduction in goal-directed behavior, may even precede motor impairments, disappearing with the introduction of dopaminergic (DA) therapies and possibly reappearing with its discontinuation, suggesting a causal role of DA. We recently developed a lesion-based model, with stereotaxic infusion of 6-hydroxydopamine (6-OHDA) into precise areas of the rat SNc or ventral tegmental area and showed, in several operant tasks, that a partial denervation of the nigrostriatal, but not of the mesocorticolimbic, DA system induced profound motivational deficits during instrumental action. We investigated the time course of the effects of nigrostriatal DA denervation on motivation in rats, by assessing the negative effect of SNc bilateral 6-OHDA infusion on preacquired operant behavior, and determining whether the induced deficits were sensitive to the introduction and withdrawal of a clinically relevant PD treatment, the DA D2/D3 receptor agonist, pramipexole (PRA). Partial nigrostriatal DA denervation was accompanied by a significant reduction in operant behavior. This deficit, indicative of a decrease in motivation, was fully reversed by PRA and reappeared after treatment withdrawal. This longitudinal preclinical study provides evidence for the implication of the DA nigrostriatal system in PD-associated apathy. Moreover, by showing a good isomorphy and predictive value, our model highlights the relevance of D2/D3 receptors as potential targets for alleviating apathy in PD.
Mov Disord, 2014; 29

24308494: Favier M, Carcenac C, Drui G, Boulet S, El Mestikawy S, Savasta M
High-frequency stimulation of the subthalamic nucleus modifies the expression of vesicular glutamate transporters in basal ganglia in a rat model of Parkinson's disease.
It has been suggested that glutamatergic system hyperactivity may be related to the pathogenesis of Parkinson's disease (PD). Vesicular glutamate transporters (VGLUT1-3) import glutamate into synaptic vesicles and are key anatomical and functional markers of glutamatergic excitatory transmission. Both VGLUT1 and VGLUT2 have been identified as definitive markers of glutamatergic neurons, but VGLUT 3 is also expressed by non glutamatergic neurons. VGLUT1 and VGLUT2 are thought to be expressed in a complementary manner in the cortex and the thalamus (VL/VM), in glutamatergic neurons involved in different physiological functions. Chronic high-frequency stimulation (HFS) of the subthalamic nucleus (STN) is the neurosurgical therapy of choice for the management of motor deficits in patients with advanced PD. STN-HFS is highly effective, but its mechanisms of action remain unclear. This study examines the effect of STN-HFS on VGLUT1-3 expression in different brain nuclei involved in motor circuits, namely the basal ganglia (BG) network, in normal and 6-hydroxydopamine (6-OHDA) lesioned rats.
BMC Neurosci, 2013; 14

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ACTIVATING NEURONAL ENSEMBLES IN THE NUCLEUS ACCUMBENS ENHANCES COCAINE-CONDITIONED PLACE PREFERENCE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Background: The nucleus accumbens core (NAcore) is a part of the basal ganglia which is involved in motivation, reward-seeking behavior, and reinforcement. Its activation plays a role in substance use disorder and relapse. Neuronal ensembles, i.e, a sparse network of neurons coactivated during a specific behavior, are linked to drug-seeking behavior that leads to relapse. However, whether these ensembles are sufficient for drug and reward seeking is not yet clear. Aims: We seek to establish whether activating the ensemble in the NAcore involved in seeking a reward is sufficient to produce seeking behavior. Methods: To test this, we first performed a cocaine conditioned place preference (CPP) with FosiCre x Ai14 genetically modified mice. CPP models cocaine seeking by exposing an animal to a choice between a drug-paired context and a neutral context. The mice were injected with a Cre- dependent Gq-DREADD (designer receptor exclusively activated by designer drug) virus in the NAcore to allow specific reactivation of the cocaine-seeking ensemble. Results: We found that specific activation of the cocaine-seeking ensemble via administration of clozapine-N-oxide significantly potentiated cocaine seeking. Conclusions: These results show the sufficiency of the cocaine-seeking ensemble in the NAcore to drive drug seeking and advance our understanding of substance use disorder and relapse.

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BOARD NUMBER: S05-125

A SELF-ADJUSTING PROGRESSIVE-SHOCK PROCEDURE TO INVESTIGATE RESISTANCE TO PUNISHMENT: CHARACTERIZATION IN MALE AND FEMALE RATS

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Indifference to harmful consequences is one of the main characteristics of compulsive behaviors and addiction. Animal models of punishment that provide a rapid and effective measure of resistance to punishment could be critical for the investigation of mechanisms underlying this maladaptive behavior. Here, analogous to the progressive ratio (PR) procedure widely used to evaluate appetitive motivation as the response requirement is increased, we developed a self-adjusting progressive shock intensity (PSI) procedure that provides, within a single session, a PSI break point that quantifies the propensity to work for a reward in spite of receiving electric footshock that progressively increases in duration. In both male and female rats, the PSI breaking point was sensitive to 1) the hunger drive; and 2) changes in the qualitative, but not quantitative, incentive value of the reward. In systematic comparisons between PSI and PR procedures in the same rats, we found that both measures are sensitive to manipulations of motivational states, but they are not correlated, suggesting that they measure overlapping but distinct processes. This self-adjusting PSI procedure may represent a useful tool to investigate mechanisms underlying maladaptive behavior that persist in certain individuals despite harmful consequences.

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BOARD NUMBER: S05-126

ROLE OF DOPAMINE NEURONS IN INTER-INDIVIDUAL VARIABILITY DURING SOCIAL LABOR DIVISION TASK IN MICE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Individuals living in group have to adapt to the physical and social constraints of the environment. In that context, access to shared resources leads to the emergence of specific strategies, such as competition or cooperation and division of labor among individuals. To assess individual adaptation and inter-individual variability, we developed a task where mice are tracked continuously across several days in an environment where the food is accessible through a lever press. The lever and the food magazine are far apart and reachable by all the mice at any moment. When living in triads, different individual strategies emerge for food access. Interestingly, when mice are performing the task alone, different behaviors also appear illustrating that a same goal can be achieved in different manners, independently of social pressure. We aim at understanding the neuronal basis of these individual variations. Dopamine (DA) neurons of the ventral tegmental area (VTA) are known to be involved in social behaviors and decision making. We therefore hypothesized that VTA DA neurons play a major role in the different strategies observed in the social labor division task. We address this question using in-vivo electrophysiology in anesthetized mice and fiber photometry technique in freely behaving animals to record VTA DA activity in the different animals' profiles.

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POOR ATTENTIONAL CONTROL AS A BIOMARKER OF VULNERABILITY TO NICOTINE ADDICTION IN MALE BUT NOT IN FEMALE MICE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Tobacco addiction vulnerability is highly variable among smokers and between sexes; thus, the identification of biomarkers for nicotine addiction risk is vital. One property of nicotine is to increase attentional capacities. In fact, a recent study of our laboratory demonstrated that in male rats attentional abilities could predict motivation for nicotine self-administration. Our **aim** was to correlate interindividual and sex differences in attentional control in mice with their vulnerability to nicotine rewarding properties, a crucial step in the initiation of drug taking. **Methods**: Mice underwent the cued-Fixed Consecutive Number task (FCNcue) to examine their attentional control and the Conditioning Place Preference (CPP) for nicotine. **Results**: Mice showed wide heterogeneity in their attentional performances. As a group, males showed better attentional capacities than females. Acute administration of nicotine showed pro-cognitive effects in both sexes. However, while nicotine improved the performance of all males, it only enhanced attention in already good performer females. Principal component analysis using data from the FCNcue and the CPP, showed a link between preference for nicotine and poor attentional control in males but not in females. **Conclusion**: FCNcue allowed us to reveal interindividual variability and sex differences in attentional control in mice. Our multidimensional analysis show a sexual dichotomy for the effects of nicotine. In males, which are more sensitive to nicotine procognitive effects, poor attentional control is a risk factor to develop nicotine addiction. Whereas sensitivity to the procognitive effect of nicotine and poor attentional control are not risk factors to develop nicotine addiction in females

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ANXIETY LEVELS AFTER VICARIOUS SOCIAL DEFEAT STRESS ARE ASSOCIATED WITH VULNERABILITY AND RESILIENCE TO COCAINE-INDUCED CONDITIONED PLACE PREFERENCE IN FEMALE MICE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Exposure to social defeat stress has been associated with enhanced vulnerability of male mice to the rewarding effects of cocaine, although some individuals are resilient to this effect. Our aim was to evaluate whether exposure to vicarious social defeat stress induces anxiety in the elevated plus maze (EPM) and increases the rewarding effects of cocaine in females. On PND 47, 50, 53 and 56, female C57BL/6 mice witnessed an episode of social defeat of a male of the same strain by an aggressive opponent mouse of the OF1 strain. A control group of females spent the same period of time in an empty cage. Both groups of females were tested in the EPM and in the conditioned place preference (CPP) paradigm with 1.5 mg/kg of cocaine (24 hours and 3 weeks after the last episode of repeated social defeat, respectively). Social stress reduced the percentage of time spent in the open arms of the elevated plus maze and increased the CPP score, but these effects were not significant. A subgroup of stressed mice acquired clear CPP and displayed anxiety in the EPM, while another subgroup was resilient to both effects of stress. These results suggest that the absence of short-term anxiety after stress predicts the subsequent resilience of female mice to the potentiating effects of stress on cocaine reward. Funding: Ministry of Science, Innovation and University (Spain), grant PID2020-118945RB-I00.

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EFFECTS OF COHOUSING MICE AND RATS ON STRESS LEVELS AND THE ATTRACTIVENESS OF DYADIC SOCIAL INTERACTION IN C57BL/6J AND CD1 MICE AND SPRAGUE DAWLEY RATS

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Rats may kill mice. Therefore it is standard practice in many research animal housing facilities – despite often very limited space - to separate mice from rats (i.e., the predators) to minimize stress for the mice. We tested the effect of cohousing on the stress levels of mice from either the C57BL/6J (BL6) or the CD1 strain and Sprague Dawley rats by quantifying their fecal corticosterone and metabolites (FCM) concentration and investigated how cohousing impacts a behavioral assay, i.e., conditioned place preference for mouse-mouse or rat-rat social interaction. Mice from the BL6 strain (but not CD1 mice) that were cohoused with rats had significantly increased FCM concentrations, indicative of higher stress levels. In contrast to their elevated stress levels, the attractiveness for contextual cues associated with mouse-mouse social interaction even increased in rat-cohoused mice, albeit nonsignificantly. Thus, cohousing BL6 mice and rats did not impair a behavior of BL6 mice that had proved to be sensitive to social factors, especially handling by humans, in our laboratory. Our findings suggest that the effect of cohousing rats and mice on their stress levels and behavior might be less clearcut than generally assumed and might be overriden by conditions that cannot be controlled, i.e., different deliveries. Our findings can help to use research animal housing resources more efficiently. Publication: Zernig, Ghareh, Berchtold, 2022, Biology (Basel) 11,291. Supported by Austrian Ministry of Science, Research and Economy grant grant BMFWF-80.110/0001-WF/V/3b/2017

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-130

NEUROPHYSIOLOGICAL CORRELATES OF SUB-DIMENSIONS OF ALCOHOL USE DISORDER IN RODENTS

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Aim: Alcohol use disorder (AUD) is the most demanding area of unmet medical needs in psychiatry. Improving the predictive validity of AUD animal models is crucial to bring forward novel and effective medications. **Methods:** Based on the DSM-IV/5 diagnostic criteria, we used a multidimensional operant model to characterize an alcohol addiction prone phenotype in outbred Wistar rats combined with electrophysiological assessments. **Results:** In line with clinical studies rats as humans showed individual variability in the propensity to develop AUD that includes the motivation to seek for alcohol (crit1), increased effort to obtain the substance (crit 2) and continued alcohol drinking despite negative consequences (crit 3). Only a subset of rats met all the aforementioned criteria for AUD (3crit, AUD-prone phenotype), while a larger fraction was considered AUD-resilient (0 crit). In further analysis, by computing a Global Addiction Score (GAS) for each animal, we could establish that GAS correlated with animal distribution within the criteria. Compulsive alcohol seeking was encoded by specific neuronal changes within the basolateral amygdala (BLA), where both presynaptic and postsynaptic activity were depressed in the alcohol addiction-prone phenotype compared to the resilient phenotype. The latter did not differ in neuronal properties compared to water drinking controls. Ex vivo pharmacological manipulation of the BLA further indicated adaptations in mGluR2/3 signalling, specifically in the AUD prone phenotype. **Conclusion:** Our findings suggest that glutamatergic changes within the BLA might contribute to the vulnerability to develop AUD. The mGluR2/3 could thus represent an ideal pharmacological intervention for a target-phenotypic-based treatment in AUD.

Pubmed:

34854073: Domi A, Lunerti V, Petrella M, Domi E, Borruto AM, Ubaldi M, Weiss F, Ciccocioppo R
Genetic deletion or pharmacological blockade of nociceptin/orphanin FQ receptors in the ventral tegmental area attenuates nicotine-motivated behaviour.
The nociceptin/orphanin FQ (N/OFQ)-nociceptin opioid-like peptide (NOP) receptor system is widely distributed in the brain and pharmacological activation of this system revealed therapeutic potential in animal models of substance use disorder. Studies also showed that genetic deletion or pharmacological blockade of NOP receptors confer resistance to the development of alcohol abuse. Here, we have used a genetic and pharmacological approach to evaluate the therapeutic potential of NOP antagonism in smoking cessation.
Br J Pharmacol, 2022; 179
33479741: Domi A, Barbier E, Adermark L, Domi E
Targeting the Opioid Receptors: A Promising Therapeutic Avenue for Treatment in "Heavy Drinking Smokers".
Despite a general decline in tobacco use in the last decades, the prevalence of tobacco smoking in individuals with alcohol use disorder (AUD) remains substantial (45-50%). Importantly, the co-use of both substances potentiates the adverse effects, making it a significant public health problem. Substantial evidence suggests that AUD and Tobacco use disorder (TUD) may share common mechanisms. Targeting these mechanisms may therefore provide more effective therapy. Numerous studies describe a potential role of the endogenous opioid system in both AUD and TUD. Reviewing this literature, we aim to evaluate the efficacy of molecules that target the opioid system as promising therapeutic interventions for treating alcohol and tobacco co-use disorders.
Alcohol Alcohol, 2021; 56
30760988: Domi A, Stopponi S, Domi E, Ciccocioppo R, Cannella N
Sub-dimensions of Alcohol Use Disorder in Alcohol Preferring and Non-preferring Rats, a Comparative Study.
Recent animal models of alcohol use disorder (AUD) are centered in capturing individual vulnerability differences in disease progression. Here, we used genetically selected Marchigian Sardinian alcohol-preferring (msP) and Wistars rats to apply a multidimensional model of AUD adapted from a previously described DSM-IV/DSM-5 multisymptomatic cocaine addiction model. As proof of concept, we hypothesized that msP rats, genetically selected for excessive drinking, would be more prone to develop dependence-like behavior compared to Wistars. Before exposure of animals to alcohol, we monitored basal

anxiety in the elevated plus maze (EPM). Animals were then trained in prolonged operant alcohol self-administration, consisting of 30-min daily sessions for 60 days in total. Each session consisted of two 10-min periods of alcohol reinforcement separated by 10-min interval of non-reinforcement. Following training, we applied three criteria of individual vulnerability for AUD: (1) persistence of lever pressing for alcohol when it was not available; (2) motivation for alcohol in a progressive ratio (PR) schedule of reinforcement; and (3) resistance to punishment when alcohol delivery was anticipated by a foot-shock (0.3 mA). We obtained four groups corresponding to the number of criteria met (0-3 crit). Rats in the 0crit and 1crit groups were characterized as resilient, whereas rats in the 2crit and 3crit groups were characterized as prone to develop a dependent-like phenotype. As predicted, the 2-3crit groups were enriched with msP rats while the 0-1crit groups were enriched in Wistar rats. In further analysis, we calculated the global addiction score (GAS) per subject by the sum of the normalized score (z-score) of each criterion. Results showed GAS was highly correlated with animal distribution within the 3 criteria. Specifically, GAS was negative in the 0-1crit groups, and positive in the 2-3crit groups. A positive correlation between basal anxiety and quantity of alcohol intake was detected in msP rats but not Wistars. In conclusion, we demonstrated that the 0/3criteria model is a suitable approach to study individual differences in AUD and that msP rats, selected for excessive-alcohol drinking, show a higher propensity to develop AUD compared to non-preferring Wistars.
Front Behav Neurosci, 2019; 13

33704789: Domi E, Domi A, Adermark L, Heilig M, Augier E
Neurobiology of alcohol seeking behavior.
Alcohol addiction is a chronic relapsing brain disease characterized by an impaired ability to stop or control alcohol use despite adverse consequences. A main challenge of addiction treatment is to prevent relapse, which occurs in more than >50% of newly abstinent patients with alcohol disorder within 3 months. In people suffering from alcohol addiction, stressful events, drug-associated cues and contexts, or re-exposure to a small amount of alcohol trigger a chain of behaviors that frequently culminates in relapse. In this review, we first present the preclinical models that were developed for the study of alcohol seeking behavior, namely the reinstatement model of alcohol relapse and compulsive alcohol seeking under a chained schedule of reinforcement. We then provide an overview of the neurobiological findings obtained using these animal models, focusing on the role of opioids systems, corticotropin-release hormone and neurokinins, followed by dopaminergic, glutamatergic, and GABAergic neurotransmissions in alcohol seeking behavior.
J Neurochem, 2021; 157

34811469: Adermark L, Lagström O, Loftén A, Licheri V, Havenäng A, Loi EA, Stomberg R, Söderpalm B, Domi A, Ericson M
Astrocytes modulate extracellular neurotransmitter levels and excitatory neurotransmission in dorsolateral striatum via dopamine D2 receptor signaling.
Astrocytes provide structural and metabolic support of neuronal tissue, but may also be involved in shaping synaptic output. To further define the role of striatal astrocytes in modulating neurotransmission we performed in vivo microdialysis and ex vivo slice electrophysiology combined with metabolic, chemogenetic, and pharmacological approaches. Microdialysis recordings revealed that intrastriatal perfusion of the metabolic uncoupler fluorocitrate (FC) produced a robust increase in extracellular glutamate levels, with a parallel and progressive decline in glutamine. In addition, FC significantly increased the microdialysate concentrations of dopamine and taurine, but did not modulate the extracellular levels of glycine or serine. Despite the increase in glutamate levels, ex vivo electrophysiology demonstrated a reduced excitability of striatal neurons in response to FC. The decrease in evoked potentials was accompanied by an increased paired pulse ratio, and a reduced frequency of spontaneous excitatory postsynaptic currents, suggesting that FC depresses striatal output by reducing the probability of transmitter release. The effect by FC was mimicked by chemogenetic inhibition of astrocytes using G-coupled designer receptors exclusively activated by designer drugs (DREADDs) targeting GFAP, and by the glial glutamate transporter inhibitor TFB-TBOA. Both FC- and TFB-TBOA-mediated synaptic depression were inhibited in brain slices pre-treated with the dopamine D2 receptor antagonist sulpiride, but insensitive to agents acting on presynaptic glutamatergic autoreceptors, NMDA receptors, gap junction coupling, cannabinoid 1 receptors, μ-opioid receptors, P2 receptors or GABA receptors. In conclusion, our data collectively support a role for astrocytes in modulating striatal neurotransmission and suggest that reduced transmission after astrocytic inhibition involves dopamine.
Neuropsychopharmacology, 2021;

33160605: Barbier E, Barchiesi R, Domi A, Chanthongdee K, Domi E, Augier G, Augier E, Xu L, Adermark L, Heilig M
Downregulation of Synaptotagmin 1 in the Prelimbic Cortex Drives Alcohol-Associated Behaviors in Rats.
Alcohol addiction is characterized by persistent neuroadaptations in brain structures involved in motivation, emotion, and decision making, including the medial prefrontal cortex, the nucleus accumbens, and the amygdala. We previously reported that induction of alcohol dependence was associated with long-term changes in the expression of genes involved in neurotransmitter release. Specifically, Syt1, which plays a key role in neurotransmitter release and neuronal functions, was downregulated. Here, we therefore examined the role of Syt1 in alcohol-associated behaviors in rats.
Biol Psychiatry, 2021; 89

31685649: Domi E, Caputi FF, Romualdi P, Domi A, Scuppa G, Candeletti S, Atkins A, Heilig M, Demopulos G, Gaitanaris G, Ciccocioppo R, Ubaldi M
Activation of PPARγ Attenuates the Expression of Physical and Affective Nicotine Withdrawal Symptoms through Mechanisms Involving Amygdala and Hippocampus Neurotransmission.
An isoform of peroxisome proliferator-activated receptors (PPARs), PPARγ, is the receptor for the thiazolidinedione class of anti-diabetic medications including pioglitazone. Neuroanatomical data indicate PPARγ localization in brain areas involved in drug addiction. Preclinical and clinical data have shown that pioglitazone reduces alcohol and opioid self-administration, relapse to drug seeking, and plays a role in emotional responses. Here, we investigated the behavioral effect of PPARγ manipulation on nicotine withdrawal in male Wistar rats and in male mice with neuron-specific PPARγ deletion (PPARγ) and their littermate wild-type (PPARγ) controls. Real-time quantitative RT-PCR and RNAscope hybridization assays were used for assessing the levels of expression and cell-type localization of PPARγ during nicotine withdrawal. Brain site-specific microinjections of the PPARγ agonist pioglitazone were performed to explore the role of this system on nicotine withdrawal at a neurocircuitry level. Results showed that activation of PPARγ by pioglitazone abolished the expression of somatic and affective nicotine withdrawal signs in rats and in (PPARγ) mice. This effect was blocked by the PPARγ antagonist GW9662. During early withdrawal and protracted abstinence, the expression of PPARγ increased in GABAergic and glutamatergic cells of the amygdala and hippocampus, respectively. Hippocampal microinjections of pioglitazone reduced the expression of the physical signs of withdrawal, whereas excessive anxiety associated with protracted abstinence was prevented by pioglitazone microinjection into the amygdala. Our results demonstrate the implication of the neuronal PPARγ in nicotine withdrawal and indicates that activation of PPARγ may offer an interesting strategy for smoking cessation. Smoking cessation leads the occurrence of physical and affective withdrawal symptoms representing a major burden to quit tobacco use. Here, we show that activation of PPARγ prevents the expression of both somatic and affective signs of nicotine withdrawal. At molecular levels results show that PPARγ expression increases in GABAergic cells in the hippocampus and in GABA- and glutamate-positive cells in the basolateral amygdala. Hippocampal microinjections of pioglitazone reduce the insurgence of the physical withdrawal signs, whereas anxiety linked to protracted abstinence is attenuated by pioglitazone injected into the amygdala. Our results demonstrate the implication of neuronal PPARγ in nicotine withdrawal and suggest that PPARγ agonism may represent a promising treatment to aid smoking cessation.
J Neurosci, 2019; 39
32676772: Domi E, Domi A, Ubaldi M, Somaini L, Demopulos G, Gaitanaris G, Ciccocioppo R
Further evidence for the involvement of the PPARγ system on alcohol intake and sensitivity in rodents.
Peroxisome Proliferator Activator receptors (PPARs) are intracellular receptors that function as transcription factors, which regulate specific metabolic and inflammatory processes. PPARs are broadly distributed in the body and are also expressed in the central nervous system, especially in areas involved in addiction-related behavioral responses. Recent studies support a role of PPARs in alcoholism and pioglitazone: a PPARγ agonist used for treatment of type 2 diabetes showed efficacy in reducing alcohol drinking, stress-induced relapse, and alcohol withdrawal syndrome in rats.
Psychopharmacology (Berl), 2020; 237
34285372: Borruto AM, Fotio Y, Stopponi S, Petrella M, De Carlo S, Domi A, Ubaldi M, Weiss F, Ciccocioppo R
NOP receptor antagonism attenuates reinstatement of alcohol-seeking through modulation of the mesolimbic circuitry in male and female alcohol-preferring rats.
In patients suffering from alcohol use disorder (AUD), stress and environmental stimuli associated with alcohol availability are important triggers of relapse. Activation of the nociceptin opioid peptide (NOP) receptor by its endogenous ligand Nociceptin/Orphanin FQ (N/OFQ) attenuates alcohol drinking and relapse in rodents, suggesting that NOP agonists may be efficacious in treating AUD. Intriguingly, recent data demonstrated that also blockade of NOP receptor reduced alcohol drinking in rodents. To explore further the potential of NOP antagonism, we investigated its effects on the reinstatement of alcohol-seeking elicited by administration of the α2 antagonist yohimbine (1.25 mg/kg, i.p.) or by environmental conditioning factors in male and female genetically selected alcohol-preferring Marchigian Sardinian (msP) rats. The selective NOP receptor antagonist LY2817412 (0.0, 3.0, 10.0, and 30.0 mg/kg) was first tested following oral (p.o.) administration. We then investigated the effects of LY2817412 (1.0, 3.0, 6.0 µg/µl/rat) microinjected into three candidate mesolimbic brain regions: the ventral tegmental area (VTA), the central nucleus of the amygdala (CeA), and the nucleus accumbens (NAc). We found that relapse to alcohol seeking was generally stronger in female than in male rats and oral administration of LY2817412 reduced yohimbine- and cue-induced reinstatement in both sexes. Following site-specific microinjections, LY2817412 reduced yohimbine-induced reinstatement of alcohol-seeking when administered into the VTA and the CeA, but not in the NAc. Cue-induced reinstatement was suppressed only when LY2817412 was microinjected into the VTA. Infusions of LY2817412 into the VTA and the CeA did not alter saccharin self-administration. These results demonstrate that NOP receptor blockade prevents the reinstatement of alcohol-seeking through modulation of mesolimbic system circuitry, providing further evidence of the therapeutic potential of NOP receptor antagonism in AUD.

Neuropsychopharmacology, 2021; 46
29071769: Stopponi S, Fotio Y, Domi A, Borruto AM, Natividad L, Roberto M, Ciccocioppo R, Cannella N
Inhibition of fatty acid amide hydrolase in the central amygdala alleviates co-morbid expression of innate anxiety and excessive alcohol intake.
Fatty acid amide hydrolase (FAAH) is an enzyme that prominently degrades the major endocannabinoid N-arachidonoylethanolamine (anandamide). Inhibition of this enzyme leads to increased anandamide levels in brain regions that modulate stress and anxiety. Recently, we found that genetically selected Marchigian Sardinian alcohol-preferring (msP) rats display hyperactive FAAH in amygdalar regions that was associated with increased stress sensitivity and a hyper-anxious phenotype. Our previous work has also demonstrated that msPs display an innate preference for and excessive consumption of alcohol, potentially reflecting a form of self-medication to gain relief from hyper-anxious states. Here, we expand on our previous work by microinjecting the selective FAAH inhibitor URB597 (vehicle, 0.03, 0.1 and 1.0 μg per rat) into the central amygdala (CeA) and basolateral amygdala in msP versus non-selected Wistar rats to evaluate the effects of localized FAAH inhibition on operant alcohol self-administration and restraint-induced anxiety using the elevated plus maze. Intra-CeA URB597 significantly reduced alcohol self-administration in msP but not in Wistar rats. Intra-basolateral amygdala URB597 also attenuated alcohol drinking in msPs, although the effect was less pronounced relative to CeA treatment. In contrast, control experiments administering URB597 into the ventral tegmental area produced no genotypic differences in drinking. We also found that URB597 treatment in the CeA significantly reduced the anxiogenic effects of restraint stress in msPs, although no effects were detected in Wistars. Dysregulation of FAAH regulated systems in the major output region of the amygdala may drive the propensity for co-morbid expression of anxiety and excessive alcohol use.
Addict Biol, 2018; 23

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-131

SEX-SPECIFIC ALTERATIONS IN δ OPIOID RECEPTOR EXPRESSION AND FUNCTION AFTER EARLY-LIFE ADVERSITY

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Aims: Early life adversity (ELA) is associated with vulnerabilities to reward-related problems, such as opioid use disorder. To test the hypothesis that ELA perturbs the normal maturation and function of endogenous opioid systems, we employed a naturalistic model of ELA, in which bedding and nesting materials are limited during the first week of life, and examined the impacts of ELA on expression and function of opioid ligands and receptors and on opioid drug seeking behaviors. **Methods:** Adult rats were tested for aspects of opioid addiction-like behaviors including intravenous self-administration, extinction, reinstatement, and economic demand elasticity to measure consumption and motivation for opioids at changing costs following ELA. To gain insight into ELA-induced molecular changes, we employed RT-qPCR for a suite of molecular candidates in reward-related regions. Following on our intriguing findings, we pharmacologically manipulated endogenous opioid signaling during opioid self-administration to test the mechanism of ELA-enhanced opioid seeking. **Results:** ELA led to enhanced motivation for opioid drugs in female rats, in accord with our prior findings. RT-qPCR revealed a sex-specific, selective reduction in δ opioid receptor expression in amygdala and nucleus accumbens of female rats following ELA. Preliminary results from pharmacological manipulation of δ opioid receptors during opioid self-administration suggest a possible mechanism by which ELA may cause vulnerability to addiction. **Conclusions:** ELA causes enduring, sex-specific changes in δ opioid receptor expression, which may underlie the pro-addiction phenotype caused by ELA in female rats.

Pubmed:
35156184: Birnie MT, Levis SC, Mahler SV, Baram TZ
Developmental Trajectories of Anhedonia in Preclinical Models.
This chapter discusses how the complex concept of anhedonia can be operationalized and studied in preclinical models. It provides information about the development of anhedonia in the context of early-life adversity, and the power of preclinical models to tease out the diverse molecular, epigenetic, and network mechanisms that are responsible for anhedonia-like behaviors.Specifically, we first discuss the term anhedonia, reviewing the conceptual components underlying reward-related behaviors and distinguish anhedonia pertaining to deficits in motivational versus consummatory behaviors. We then describe the repertoire of experimental approaches employed to study anhedonia-like behaviors in preclinical models, and the progressive refinement over the past decade of both experimental instruments (e.g., chemogenetics, optogenetics) and conceptual constructs (salience, valence, conflict). We follow with an overview of the state of current knowledge of brain circuits, nodes, and projections that execute distinct aspects of hedonic-like behaviors, as well as neurotransmitters, modulators, and receptors involved in the generation of anhedonia-like behaviors. Finally, we discuss the special case of anhedonia that arises following early-life adversity as an eloquent example enabling the study of causality, mechanisms, and sex dependence of anhedonia.Together, this chapter highlights the power, potential, and limitations of using preclinical models to advance our understanding of the origin and mechanisms of anhedonia and to discover potential targets for its prevention and mitigation.
Curr Top Behav Neurosci, 2022;
33825217: Levis SC, Baram TZ, Mahler SV
Neurodevelopmental origins of substance use disorders: Evidence from animal models of early-life adversity and addiction.
Addiction is a chronic relapsing disorder with devastating personal, societal, and economic consequences. In humans, early-life adversity (ELA) such as trauma, neglect, and resource scarcity are linked with increased risk of later-life addiction, but the brain mechanisms underlying this link are still poorly understood. Here, we focus on data from rodent models of ELA and addiction, in which causal effects of ELA on later-life responses to drugs and the neurodevelopmental mechanisms by which ELA increases vulnerability to addiction can be determined. We first summarize evidence for a link between ELA and

addiction in humans, then describe how ELA is commonly modeled in rodents. Since addiction is a heterogeneous disease with many individually varying behavioral aspects that may be impacted by ELA, we next discuss common rodent assays of addiction-like behaviors. We then summarize the specific addiction-relevant behavioral phenotypes caused by ELA in male and female rodents and discuss some of the underlying changes in brain reward and stress circuits that are likely responsible. By better understanding the behavioral and neural mechanisms by which ELA promotes addiction vulnerability, we hope to facilitate development of new approaches for preventing or treating addiction in those with a history of ELA.
Eur J Neurosci, 2022; 55
33643011: Levis SC, Mahler SV, Baram TZ
The Developmental Origins of Opioid Use Disorder and Its Comorbidities.
Opioid use disorder (OUD) rarely presents as a unitary psychiatric condition, and the comorbid symptoms likely depend upon the diverse risk factors and mechanisms by which OUD can arise. These factors are heterogeneous and include genetic predisposition, exposure to prescription opioids, and environmental risks. Crucially, one key environmental risk factor for OUD is early life adversity (ELA). OUD and other substance use disorders are widely considered to derive in part from abnormal reward circuit function, which is likely also implicated in comorbid mental illnesses such as depression, bipolar disorder, and schizophrenia. ELA may disrupt reward circuit development and function in a manner predisposing to these disorders. Here, we describe new findings addressing the effects of ELA on reward circuitry that lead to OUD and comorbid disorders, potentially shared neural mechanisms. We discuss some of these OUD-related problems in both humans and animals. We also highlight the increasingly apparent, crucial contribution of biological sex in mediating the range of ELA-induced disruptions of reward circuitry which may confer risk for the development of OUD and comorbid neuropsychiatric disorders.
Front Hum Neurosci, 2021; 15
31822817: Levis SC, Bentzley BS, Molet J, Bolton JL, Perrone CR, Baram TZ, Mahler SV
On the early life origins of vulnerability to opioid addiction.
The origins and neural bases of the current opioid addiction epidemic are unclear. Genetics plays a major role in addiction vulnerability, but cannot account for the recent exponential rise in opioid abuse, so environmental factors must contribute. Individuals with history of early life adversity (ELA) are disproportionately prone to opioid addiction, yet whether ELA interacts with factors such as increased access to opioids to directly influence brain development and function, and cause opioid addiction vulnerability, is unknown. We simulated ELA in female rats and this led to a striking opioid addiction-like phenotype. This was characterized by resistance to extinction, increased relapse-like behavior, and, as in addicted humans, major increases in opioid economic demand. By contrast, seeking of a less salient natural reward was unaffected by ELA, whereas demand for highly palatable treats was augmented. These discoveries provide novel insights into the origins and nature of reward circuit malfunction that may set the stage for addiction.
Mol Psychiatry, 2021; 26
30470862: Larson TA, Winkler MC, Stafford J, Levis SC, O'Neill CE, Bachtell RK
Role of dopamine D-like receptors and their modulation by adenosine receptor stimulation in the reinstatement of methamphetamine seeking.
Previous work has demonstrated that dopamine and adenosine receptors are involved in drug-seeking behaviors, yet the pharmacological interactions between these receptors in methamphetamine (MA) seeking are not well characterized. The present studies examined the role of the dopamine D-like receptors in MA seeking and identified the interactive effects of adenosine receptor stimulation.
Psychopharmacology (Berl), 2019; 236
28813640: Brown KT, Levis SC, O'Neill CE, Northcutt AL, Fabisiak TJ, Watkins LR, Bachtell RK
Innate immune signaling in the ventral tegmental area contributes to drug-primed reinstatement of cocaine seeking.
Cocaine addiction is a chronic relapsing disorder characterized by persistent perturbations to an organism's homeostatic processes that result in maladaptive drug seeking. Although considerable attention has been directed at the consequences of neuronal changes following chronic cocaine taking, few studies have examined the role of microglia, the brain's resident immune cells, following chronic cocaine administration. Toll-Like Receptor 4 (TLR4) is a molecular pattern receptor that recognizes pathogens, danger signals, and xenobiotics and induces proinflammatory signaling in the central nervous system. TLR4 is generally considered to be expressed primarily by microglia. Here, we used a rodent model of cocaine addiction to investigate the role of TLR4 in the ventral tegmental area (VTA) in cocaine seeking. Male Sprague-Dawley rats were trained to self-administer cocaine in daily 2-h sessions for 15days. Following self-administration, rats underwent extinction training and were tested in a drug-primed reinstatement paradigm. Pharmacological antagonism of TLR4 in the VTA using lipopolysaccharide from the bacterium Rhodobacter sphaeroides (LPS-RS) significantly reduced cocaine-primed reinstatement of drug seeking but had no effect on sucrose seeking. TLR4 activation within the VTA using the TLR4 activator, lipopolysaccharide, was sufficient to moderately reinstate cocaine seeking. We also assessed changes in proinflammatory cytokine expression in the VTA following cocaine self-administration. Cocaine self-administration increased the expression of

mRNA for the proinflammatory cytokine interleukin-1ß, but not tumor necrosis factor alpha, in the VTA. Pharmacological antagonism of the interleukin-1 receptor in the VTA reduced cocaine-primed drug seeking. These results are consistent with the hypothesis that chronic cocaine produces inflammatory signaling that contributes to cocaine seeking.
Brain Behav Immun, 2018; 67
26874560: O'Neill CE, Newsom RJ, Stafford J, Scott T, Archuleta S, Levis SC, Spencer RL, Campeau S, Bachtell RK
Adolescent caffeine consumption increases adulthood anxiety-related behavior and modifies neuroendocrine signaling.
Caffeine is a commonly used psychoactive substance and consumption by children and adolescents continues to rise. Here, we examine the lasting effects of adolescent caffeine consumption on anxiety-related behaviors and several neuroendocrine measures in adulthood. Adolescent male Sprague-Dawley rats consumed caffeine (0.3g/L) for 28 consecutive days from postnatal day 28 (P28) to P55. Age-matched control rats consumed water. Behavioral testing for anxiety-related behavior began in adulthood (P62) 7 days after removal of caffeine. Adolescent caffeine consumption enhanced anxiety-related behavior in an open field, social interaction test, and elevated plus maze. Similar caffeine consumption in adult rats did not alter anxiety-related behavior after caffeine removal. Characterization of neuroendocrine measures was next assessed to determine whether the changes in anxiety were associated with modifications in the HPA axis. Blood plasma levels of corticosterone (CORT) were assessed throughout the caffeine consumption procedure in adolescent rats. Adolescent caffeine consumption elevated plasma CORT 24h after initiation of caffeine consumption that normalized over the course of the 28-day consumption procedure. CORT levels were also elevated 24h after caffeine removal and remained elevated for 7 days. Despite elevated basal CORT in adult rats that consumed caffeine during adolescence, the adrenocorticotropic hormone (ACTH) and CORT response to placement on an elevated pedestal (a mild stressor) was significantly blunted. Lastly, we assessed changes in basal and stress-induced c-fos and corticotropin-releasing factor (Crf) mRNA expression in brain tissue collected at 7 days withdrawal from adolescent caffeine. Adolescent caffeine consumption increased basal c-fos mRNA in the paraventricular nucleus of the hypothalamus. Adolescent caffeine consumption had no other effects on the basal or stress-induced c-fos mRNA changes. Caffeine consumption during adolescence increased basal Crf mRNA in the central nucleus of the amygdala, but no additional effects of stress or caffeine consumption were observed in other brain regions. Together these findings suggest that adolescent caffeine consumption may increase vulnerability to psychiatric disorders including anxiety-related disorders, and this vulnerability may result from dysregulation of the neuroendocrine stress response system.
Psychoneuroendocrinology, 2016; 67
25328052: O'Neill CE, Levis SC, Schreiner DC, Amat J, Maier SF, Bachtell RK
Effects of adolescent caffeine consumption on cocaine sensitivity.
Caffeine is the most commonly used psychoactive substance, and consumption by adolescents has risen markedly in recent years. We identified the effects of adolescent caffeine consumption on cocaine sensitivity and determined neurobiological changes within the nucleus accumbens (NAc) that may underlie caffeine-induced hypersensitivity to cocaine. Male Sprague-Dawley rats consumed caffeine (0.3 g/l) or water for 28 days during adolescence (postnatal day 28-55; P28-P55) or adulthood (P67-P94). Testing occurred in the absence of caffeine during adulthood (P62-82 or P101-121). Cocaine-induced and quinpirole (D2 receptor agonist)-induced locomotion was enhanced in rats that consumed caffeine during adolescence. Adolescent consumption of caffeine also enhanced the development of a conditioned place preference at a sub-threshold dose of cocaine (7.5 mg/kg, i.p.). These behavioral changes were not observed in adults consuming caffeine for an equivalent period of time. Sucrose preferences were not altered in rats that consumed caffeine during adolescence, suggesting there are no differences in natural reward. Caffeine consumption during adolescence reduced basal dopamine levels and augmented dopamine release in the NAc in response to cocaine (5 mg/kg, i.p.). Caffeine consumption during adolescence also increased the expression of the dopamine D2 receptor, dopamine transporter, and adenosine A1 receptor and decreased adenosine A2A receptor expression in the NAc. Consumption of caffeine during adulthood increased adenosine A1 receptor expression in the NAc, but no other protein expression changes were observed. Together these findings suggest that caffeine consumption during adolescence produced changes in the NAc that are evident in adulthood and may contribute to increases in cocaine-mediated behaviors.
Neuropsychopharmacology, 2015; 40
25301277: Kavanagh KA, Schreiner DC, Levis SC, O'Neill CE, Bachtell RK
Role of adenosine receptor subtypes in methamphetamine reward and reinforcement.
The neurobiology of methamphetamine (MA) remains largely unknown despite its high abuse liability. The present series of studies explored the role of adenosine receptors on MA reward and reinforcement and identified alterations in the expression of adenosine receptors in dopamine terminal areas following MA administration in rats. We tested whether stimulating adenosine A1 or A2A receptor subtypes would influence MA-induced place preference or MA self-administration on fixed and progressive ratio schedules in male Sprague-Dawley rats. Stimulation of either adenosine A1 or A2A receptors significantly reduced the development of MA-induced place preference. Stimulating adenosine A1, but not A2A, receptors reduced MA

self-administration responding. We next tested whether repeated experimenter-delivered MA administration would alter the expression of adenosine receptors in the striatal areas using immunoblotting. We observed no change in the expression of adenosine receptors. Lastly, rats were trained to self-administer MA or saline for 14 days and we detected changes in adenosine A1 and A2A receptor expression using immunoblotting. MA self-administration significantly increased adenosine A1 in the nucleus accumbens shell, caudate-putamen and prefrontal cortex. MA self-administration significantly decreased adenosine A2A receptor expression in the nucleus accumbens shell, but increased A2A receptor expression in the amygdala. These findings demonstrate that MA self-administration produces selective alterations in adenosine receptor expression in the nucleus accumbens shell and that stimulation of adenosine receptors reduces several behavioral indices of MA addiction. Together, these studies shed light onto the neurobiological alterations incurred through chronic MA use that may aid in the development of treatments for MA addiction.
Neuropharmacology, 2015; 89
24562064: O'Neill CE, Hobson BD, Levis SC, Bachtell RK
Persistent reduction of cocaine seeking by pharmacological manipulation of adenosine A1 and A 2A receptors during extinction training in rats.
Adenosine receptor stimulation and blockade have been shown to modulate a variety of cocaine-related behaviors.
Psychopharmacology (Berl), 2014; 231

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-132

INVESTIGATING THE ROLE OF OXYTOCIN IN EARLY ADVERSITY-INDUCED VULNERABILITY TO REWARDING STIMULI

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Early life experiences have the potential role to alter both the brain development and adult behavior. In particular, the formation of the attachment bond is crucial for well-being and mental health, and its disruption has been related to expression of several psychopathologies, such as addiction. Interestingly, oxytocin plays an important role in the formation of the attachment bond and dopaminergic system development, and alterations of oxytocin levels are involved in drug addiction. We have recently showed that an early manipulation (Repeated Cross Fostering, RCF) able to alter the mother-pups bond induces in C57 female mice a behavioral phenotype in adulthood characterized by increased vulnerability to cocaine effects. Given the role of oxytocin in both the formation of attachment bond and the neurobiological mechanisms underlying drug addiction, our aim is to investigate if RCF manipulation affects oxytocin levels that, in turn, are responsible for increased cocaine sensitivity in adulthood. Through ELISA and RT-PCR techniques, we demonstrated that RCF manipulation induces a reduction in both oxytocin and oxytocin receptor levels in the brain of C57 female mice at postnatal day (PND) 5. Moreover, restoring brain oxytocin levels through subcutaneous injection of oxytocin (20ul, 0.2 ng/ul) from PND1 to PND4, adult RCF females show reduced sensitivity for cocaine. These data indicate a critical role for oxytocin in increased sensitivity to cocaine induced by early adversity.

Pubmed:
34660854: Lo Iacono L, Mancini C, Babicola L, Pietrosanto M, Di Segni M, D'Addario SL, Municchi D, Ielpo D, Pascucci T, Cabib S, Ferlazzo F, D'Amato FR, Andolina D, Helmer-Citterich M, Cifani C, Ventura R
Early life adversity affecting the attachment bond alters ventral tegmental area transcriptomic patterning and behavior almost exclusively in female mice.
Early life experiences that affect the attachment bond formation can alter developmental trajectories and result in pathological outcomes in a sex-related manner. However, the molecular basis of sex differences is quite unknown. The dopaminergic system originating from the ventral tegmental area has been proposed to be a key mediator of this process. Here we exploited a murine model of early adversity (Repeated Cross Fostering, RCF) to test how interfering with the attachment bond formation affects the VTA-related functions in a sex-specific manner. Through a comprehensive behavioral screening, within the NiH RDoC framework, and by next-generation RNA-Seq experiments, we analyzed the long-lasting effect of RCF on behavioral and transcriptional profiles related to the VTA, across two different inbred strains of mouse in both sexes. We found that RCF impacted to an extremely greater extent VTA-related behaviors in females than in males and this result mirrored the transcriptional alterations in the VTA that were almost exclusively observed in females. The sexual dimorphism was conserved across two different inbred strains in spite of their divergent long lasting consequences of RCF exposure. Our data suggest that to be female primes a sub-set of genes to respond to early environmental perturbations. This is, to the best of our knowledge, the first evidence of an almost exclusive effect of early life experiences on females, thus mirroring the extremely stronger impact of precocious aversive events reported in clinical studies in women.
Neurobiol Stress, 2021; 15

FENS Forum 2022
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BOARD NUMBER: S05-133

DIFFERENTIAL ROLE OF ROSTROMEDIAL TEGMENTAL NUCLEUS (RMTG) OUTPUTS IN FOOD INTAKE DURING AN OBESOGENIC DIET

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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The rise of obesity prevalence represents a global public health issue. This phenomenon could be explained by a shift in our eating habits towards the consumption of palatable foods, rich in fat and sugar that are associated to a pleasurable sensation. The RMTg is a recently discovered mesencephalic inhibitory brain region projecting to the ventral tegmental area (VTA) and the lateral hypothalamus (LH), two structures involved in hedonic and homeostatic regulation of food intake. Our objective was to investigate the role of the RMTg, as well as the RMTg --> VTA and RMTg --> LH pathways in food intake regulation and associated locomotor behavior. We performed excitotoxic lesions of the RMTg and, in parallel, projection-specific lesions using a combination of viral vectors in rats. Animals were exposed to a 6-week free choice obesogenic diet (fat, 10% sucrose, water, chow). Palatable food was then removed for 4 weeks before a 24h re-access period. Food intake was measured daily, and locomotor activity was recorded at different steps. The RMTg global lesions durably affected sucrose and fat consumption throughout the diet and an increased locomotor activity was observed afterwards., Lesion of the RMTg--> VTA or the RMTg--> LH projections did not modify food intake during the diet. Interestingly, locomotor activity during the removal period and palatable food intake on the re-access day were differentially impacted by the lesions.Our results point towards a role for the RMTg in the control of palatable food intake within a feeding network including both the LH and VTA.

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MATERNAL REWARD FOR DAM RATS: ROLE OF THE TAIL OF THE VENTRAL TEGMENTAL AND IMPACT ON OTHER DOPAMINE-RELATED BRAIN REGIONS

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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The tail of the ventral tegmental area (tVTA) or rostromedial tegmental nucleus, receives lateral habenula inputs and projects to midbrain dopamine neurons, both participating in learning processes predicting the outcomes of actions. Thus, the tVTA is in a critical location into prediction error pathways. Indeed, tVTA GABA neurons display electrophysiological inhibition or activation after reward and aversive stimuli, respectively, and their predictive cues. In the present study, we explored the tVTA contribution in the context of maternal behaviour and its association to dopamine-related brain regions, as lateral habenula, ventral tegmental area, nucleus accumbens and medial prefrontal cortex To this end, pregnant and virgin Sprague-Dawley female rats were housed in pairs. Following 4-5 days after delivery, rats underwent four conditioning sessions. During this period, female rats were separated 1h per day from their pups, which were reintroduced in the homecage in a specific context after separation. On the test day, only half of the females received their pups after separation, to explore the impact of pup-predicting cues on c-Fos expression. Our behavioural results revealed that maternal behaviour during the conditioning sessions was only displayed by lactating females, but not by virgin rats, although they were pup-sensitized. Moreover, we showed that pup-predicting cues induce higher c-Fos in the tVTA of lactating pup-deprived females compared to lactating non-pup deprived and to virgin females, suggesting a role of the tVTA in maternal reward prediction error. Moreover, we explored the possible tVTA impact on the recruitment of dopamine-related brain regions.
Funding: GV/2020/173, UJI-A2019-14, PID2019-107332GB-C21, SENC.

Pubmed:
34461306: Del Rio ML, Nguyen TH, Tesson L, Heslan JM, Gutierrez-Adan A, Fernandez-Gonzalez R, Gutierrez-Arroyo J, Buhler L, Pérez-Simón JA, Anegon I, Rodriguez-Barbosa JI
The impact of CD160 deficiency on alloreactive CD8 T cell responses and allograft rejection.
CD160 is a member of the immunoglobulin superfamily with a pattern of expression mainly restricted to cytotoxic cells. To assess the functional relevance of the HVEM/CD160 signaling pathway in allogeneic cytotoxic responses, exon 2 of the CD160 gene was targeted by CRISPR/Cas9 to generate CD160 deficient mice. Next, we evaluated the impact of CD160 deficiency in the course of an alloreactive response. To that aim, parental donor WT (wild-type) or CD160 KO (knock-out) T cells were adoptively transferred into non-irradiated semiallogeneic F1 recipients, in which donor alloreactive CD160 KO CD4 T cells and CD8 T cells clonally expanded less vigorously than in WT T cell counterparts. This differential proliferative response rate at the early phase of T cell expansion influenced the course of CD8 T cell differentiation and the composition of the effector T cell pool that led to a significant decreased of the memory precursor effector cells (MPECs) / short-lived effector cells (SLECs) ratio in CD160 KO CD8 T cells compared to WT CD8 T cells. Despite these differences in T cell proliferation and differentiation, allogeneic MHC class I mismatched (bm1) skin allograft survival in CD160 KO recipients was comparable to that of WT recipients. However, the administration of CTLA-4.Ig showed an enhanced survival trend of bm1 skin allografts in CD160 KO with respect to WT recipients. Finally, CD160 deficient NK cells were as proficient as CD160 WT NK cells in rejecting allogeneic cellular allografts or MHC class I deficient tumor cells. CD160 may represent a CD28 alternative costimulatory molecule for the modulation of allogeneic CD8 T cell responses either in combination with costimulation blockade or by direct targeting of alloreactive CD8 T cells that upregulate CD160 expression in response to alloantigen stimulation.
Transl Res, 2022; 239
33915805: Pericuesta E, Gutiérrez-Arroyo JL, Sánchez-Calabuig MJ, Gutiérrez-Adán A
Postnatal Catch-Up Growth Programs Telomere Dynamics and Glucose Intolerance in Low Birth Weight Mice.
Low birth weight and rapid postnatal weight gain are independent predictors of obesity and diabetes in adult life, yet the molecular events involved in this process remain unknown. In inbred and outbred mice, this study examines natural

intrauterine growth restriction (IUGR) in relation to body weight, telomere length (TL), glucose tolerance, and growth factor gene (, , , , and) mRNA expression levels in the brain, liver, and muscle at 2- and 10 days of age and then at 3- and 9 months of age. At birth, ~15% of the animals showed IUGR, but by 3 and 9 months, half of these animals had regained the same weight as controls without IUGR (recuperated group). At 10 days, there was no difference in TL between animals undergoing IUGR and controls. However, by 3 and 9 months of age, the recuperated animals had shorter TL than the control and IUGR-non recuperated animals and also showed glucose intolerance. Further, compared to controls, and growth factor mRNA expression was lower in Day 2-IUGR mice, while and mRNA expression was higher in D10-IUGR animals. Moreover, at 3 months of age, only in the recuperated group were brain and liver , , , and expression levels higher than in the control and IUGR-non-recuperated groups. These data indicate that catch-up growth but not IUGR per se affects TL and glucose tolerance, and suggest a role in this latter process of insulin/insulin-like growth signaling pathway gene expression during early development.
Int J Mol Sci, 2021; 22
32842637: Pericuesta E, Laguna-Barraza R, Ramos-Ibeas P, Gutierrez-Arroyo JL, Navarro JA, Vera K, Sanjuan C, Baixeras E, de Fonseca FR, Gutierrez-Adan A
D-Chiro-Inositol Treatment Affects Oocyte and Embryo Quality and Improves Glucose Intolerance in Both Aged Mice and Mouse Models of Polycystic Ovarian Syndrome.
Polycystic ovarian syndrome (PCOS) is the main cause of female infertility. It is a multifactorial disorder with varying clinical manifestations including metabolic/endocrine abnormalities, hyperandrogenism, and ovarian cysts, among other conditions. D--inositol (DCI) is the main treatment available for PCOS in humans. To address some of the mechanisms of this complex disorder and its treatment, this study examines the effect of DCI on reproduction during the development of different PCOS-associated phenotypes in aged females and two mouse models of PCOS. Aged females (8 months old) were treated or not (control) with DCI for 2 months. PCOS models were generated by treatment with dihydrotestosterone (DHT) on Days 16, 17, and 18 of gestation, or by testosterone propionate (TP) treatment on the first day of life. At two months of age, PCOS mice were treated with DCI for 2 months and their reproductive parameters analyzed. No effects of DCI treatment were produced on body weight or ovary/body weight ratio. However, treatment reduced the number of follicles with an atretic cyst-like appearance and improved embryo development in the PCOS models, and also increased implantation rates in both aged and PCOS mice. DCI modified the expression of genes related to oocyte quality, oxidative stress, and luteal sufficiency in cumulus-oocyte complexes (COCs) obtained from the aged and PCOS models. Further, the phosphorylation of AKT, a main metabolic sensor activated by insulin in the liver, was enhanced only in the DHT group, which was the only PCOS model showing glucose intolerance and AKT dephosphorylation. The effect of DCI in the TP model seemed mediated by its influence on oxidative stress and follicle insufficiency. Our results indicate that DCI works in preclinical models of PCOS and offer insight into its mechanism of action when used to treat this infertility-associated syndrome.
Int J Mol Sci, 2020; 21

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BOARD NUMBER: S05-135

THE EFFECTS OF LPS-INDUCED NEUROINFLAMMATION AND AN MGLU2/3 RECEPTOR ANTAGONIST ON INTRACRANIAL SELF-STIMULATION REWARD IN MICE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Our aim was to elucidate how immune system activation induced by bacterial lipopolysaccharide (LPS) administration alters mouse reward-related behaviors. In addition, we analyzed how acute administration of the mGlu2/3 receptor antagonist LY341495 affects intracranial self-stimulation (ICSS). We used an operant ICSS method and adult C57Bl/6J male mice. A bipolar electrode was implanted into the lateral hypothalamus under anesthesia. After recovery, the mice were trained to self-stimulate the brain reward pathway by turning an operant wheel. After reaching a stable baseline stimulation threshold, the mice were treated with LPS (*E.coli* strain 0111:B4, Sigma; 0.5 mg/kg, i.p.) or saline and tested at different time points (4 h to 2 weeks). Several weeks later, the effect of a lower LPS dose (0.05 mg/kg, i.p.) was tested. Also, the effect of LY341495 (0.3-3 mg/kg, i.p.) on ICSS threshold was tested at 1 h and 24 h timepoints. Both doses of LPS acutely increased the stimulation threshold, the lower 0.05-mg/kg dose only at a 4-h time point. The 3-mg/kg dose of LY341495 increased the stimulation threshold, while lower doses had no significant effect on the threshold. In conclusion, C57Bl/6J mice appear to be suitable for ICSS studies by establishing a stable baseline stimulation threshold, showing sensitivity to aversive properties of acute LPS treatment. mGlu2/3 receptor antagonists may have acute aversive properties at high doses. Acknowledgements: Supported by the Finnish Foundation for Alcohol Studies, Orion Research Foundation and Yrjö Jahnsson Foundation.

FENS Forum 2022
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BOARD NUMBER: S05-136

SEX DIFFERENCES IN MOTIVATED BEHAVIOR: RODENT MODELS OF EFFORT-BASED DECISION-MAKING FOR SWEET REINFORCERS.

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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The study of the neural and behavioral components of effort-based decision-making processes in motivated behavior has advanced in the last few decades mainly with studies using male rodent models. However, studies in female animals are still very limited. Aims: We evaluate adult CD1 male and female mice in behavioral activation and effort in tests of motivation. Some of these tests offer choices between several positive reinforcers that require different levels of effort (PROG/choice task and T-maze-RW-pellet-odor task). Sweet reinforcers such as high carbohydrate food or sucrose solutions, and voluntary wheel running were used to evaluate preference for sedentary reinforcers or behaviors that require high levels of activation. Results: Female mice are as active as males in the RW, and they also have a strong preference for the RW compared to more sedentary reinforcers in the T-maze. In the T-maze, females consume higher amounts of sucrose pellets than males under no-choice conditions, but also when they can choose between other reinforcers. In the operant task, males and females do not differ in preference for higher concentrations of sucrose under free concurrent presentation, but females consume higher amounts of the most preferred solution and also work harder to get access to the high concentration of sucrose. This difference between sexes increases when the task becomes more effort-demanding. Conclusions: Females are equally active in a RW and they have a strong preference for this reinforcing activity, but they consume more sucrose reinforcers, and therefore, work harder than males to get access to sucrose.

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BOARD NUMBER: S05-137

COMPARING THE INFLUENCE OF DIFFERENT MONOAMINE TRANSPORTERS ON FLEXIBLE FORAGING

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

Raquel Pinacho[1], Emily Read[1], François Cinotti[1], Dario Sarra[2], Madalena Fonseca[3], Laura Grima[4], Thomas Akam[1], Mark Walton[1]
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Aims: Efficient monoamine signalling plays a key role in the regulation of adaptive decision making. However, previous experiments have tended to utilise tasks where one particular strategy – continued persistence or rapid flexibility, for example – is clearly more beneficial. By contrast, to harvest rewards efficiently in naturalistic environments, animals have to adjust how persistent or flexible to be based on the statistics of their environment. Therefore, we aimed to examine how pharmacological manipulation of monoamine signalling affects how mice respond to a changing foraging environment. **Methods:** Adult DAT-ires-Cre mice (n=18) were trained in an operant foraging task in which both local richness (reward rate within each patch) and global richness (time to travel to a new patch) were manipulated. Once trained, mice were tested after serotonin (SERT), dopamine (DAT) or noradrenaline (NET) transporter blockade (citalopram, 10 mg/kg; GBR12909, 6 mg/kg; or atomoxetine,1 mg/kg respectively), as well as after administration of 5-HT2a (MDL100907, 1 mg/kg) or 2c (SB242084, 0.5 mg/kg) antagonists, following a Latin square, within-subject design. **Results:** Both SERT and DAT, but not NET blockade, increased the time spent foraging within each patch. However, while DAT blockade generally increased patch persistence, the effect of SERT blockade was modulated by local richness, an effect also observed following 5-HT2a blockade. Ongoing work is aimed at understanding these changes by simulating behaviour using foraging models. **Conclusions:** Increasing the availability of synaptic serotonin and dopamine have complementary but distinct effects on foraging patience and persistence.

Pubmed:
32639965: Vidal-Domènech F, Riquelme G, Pinacho R, Rodriguez-Mias R, Vera A, Monje A, Ferrer I, Callado LF, Meana JJ, Villén J, Ramos B
Calcium-binding proteins are altered in the cerebellum in schizophrenia.
Alterations in the cortico-cerebellar-thalamic-cortical circuit might underlie the diversity of symptoms in schizophrenia. However, molecular changes in cerebellar neuronal circuits, part of this network, have not yet been fully determined. Using LC-MS/MS, we screened altered candidates in pooled grey matter of cerebellum from schizophrenia subjects who committed suicide (n = 4) and healthy individuals (n = 4). Further validation by immunoblotting of three selected candidates was performed in two cohorts comprising schizophrenia (n = 20), non-schizophrenia suicide (n = 6) and healthy controls (n = 21). We found 99 significantly altered proteins, 31 of them previously reported in other brain areas by proteomic studies. Transport function was the most enriched category, while cell communication was the most prevalent function. For validation, we selected the vacuolar proton pump subunit 1 (VPP1), from transport, and two EF-hand calcium-binding proteins, calmodulin and parvalbumin, from cell communication. All candidates showed significant changes in schizophrenia (n = 7) compared to controls (n = 7). VPP1 was altered in the non-schizophrenia suicide group and increased levels of parvalbumin were linked to antipsychotics. Further validation in an independent cohort of non-suicidal chronic schizophrenia subjects (n = 13) and non-psychiatric controls (n = 14) showed that parvalbumin was increased, while calmodulin was decreased in schizophrenia. Our findings provide evidence of calcium-binding protein dysregulation in the cerebellum in schizophrenia, suggesting an impact on normal calcium-dependent synaptic functioning of cerebellar circuits. Our study also links VPP1 to suicide behaviours, suggesting a possible impairment in vesicle neurotransmitter refilling and release in these phenotypes.
PLoS One, 2020; 15
28803924: MacDowell KS, Pinacho R, Leza JC, Costa J, Ramos B, García-Bueno B
Differential regulation of the TLR4 signalling pathway in post-mortem prefrontal cortex and cerebellum in chronic schizophrenia: Relationship with SP transcription factors.

Alterations in innate immunity may underlie the pathophysiology of schizophrenia (SZ). Toll-like receptor-4 (TLR4) is a master element of innate immunity. The specificity proteins (SPs), transcription factors recently implicated in SZ, are putative regulatory agents of this. This work was aimed at describing alterations in the TLR4 signalling pathway in postmortem brain prefrontal cortex (PFC) and cerebellum (CB) of 16 chronic SZ patients and 14 controls. The possible association of TLR4 pathway with SP1 and SP4 and SZ negative symptomatology is explored. In PFC, TLR4/myeloid differentiation factor 88 (MyD88)/inhibitory subunit of nuclear factor kappa B alpha (IκBα) protein levels were lower in SZ patients, while nuclear transcription factor-κB (NFκB) activity, cyclooxygenase-2 (COX-2) expression and the lipid peroxidation index malondialdehyde (MDA) appeared increased. The pattern of changes in CB is opposite, except for COX-2 expression that remained augmented and MDA levels unaltered. Network interaction analysis showed that TLR4/MyD88/IκBα/NFκB/COX-2 pathway was coupled in PFC and uncoupled in CB. SP4 co-expressed with TLR4 and NFκB in PFC and both SP1 and SP4 co-expressed with NFκB in CB. In PFC, correlation analysis found an inverse relationship between NFκB and negative symptoms. In summary, we found brain region-specific alterations in the TLR4 signalling pathway in chronic SZ, in which SP transcription factors could participate at different levels. Further studies are required to elucidate the regulatory mechanisms of innate immunity in SZ and its relationship with symptoms.
Prog Neuropsychopharmacol Biol Psychiatry, 2017; 79

27236410: Pinacho R, Villalmanzo N, Meana JJ, Ferrer I, Berengueras A, Haro JM, Villén J, Ramos B
Altered CSNK1E, FABP4 and NEFH protein levels in the dorsolateral prefrontal cortex in schizophrenia.
Schizophrenia constitutes a complex disease. Negative and cognitive symptoms are enduring and debilitating components of the disorder, highly associated to disability and burden. Disrupted neurotransmission circuits in dorsolateral prefrontal cortex (DLPFC) have been related to these symptoms. To identify candidates altered in schizophrenia, we performed a pilot proteomic analysis on postmortem human DLPFC tissue from patients with schizophrenia (n=4) and control (n=4) subjects in a pool design using differential isotope peptide labelling followed by liquid chromatography tandem mass spectrometry (LC-MS/MS). We quantified 1315 proteins with two or more unique peptides, 116 of which showed altered changes. Of these altered proteins, we selected four with potential roles on cell signaling, neuronal development and synapse functioning for further validation: casein kinase I isoform epsilon (CSNK1E), fatty acid-binding protein 4 (FABP4), neurofilament triplet H protein (NEFH), and retinal dehydrogenase 1 (ALDH1A1). Immunoblot validation confirmed our proteomic findings of these proteins being decreased in abundance in the schizophrenia samples. Additionally, we conducted immunoblot validation of these candidates on an independent sample cohort comprising 23 patients with chronic schizophrenia and 23 matched controls. In this second cohort, CSNK1E, FABP4 and NEFH were reduced in the schizophrenia group while ALDH1A1 did not significantly change. This study provides evidence indicating these proteins are decreased in schizophrenia: CSNK1E, involved in circadian molecular clock signaling, FABP4 with possible implication in synapse functioning, and NEFH, important for cytoarchitecture organization. Hence, these findings suggest the possible implication of these proteins in the cognitive and/or negative symptoms in schizophrenia.
Schizophr Res, 2016; 177

27156240: Pinacho R, Vila E, Prades R, Tarragó T, Castro E, Ferrer I, Ramos B
The glial phosphorylase of glycogen isoform is reduced in the dorsolateral prefrontal cortex in chronic schizophrenia.
Reduced glutamatergic activity and energy metabolism in the dorsolateral prefrontal cortex (DLPFC) have been described in schizophrenia. Glycogenolysis in astrocytes is responsible for providing neurons with lactate as a transient energy supply helping to couple glutamatergic neurotransmission and glucose utilization in the brain. This mechanism could be disrupted in schizophrenia. The aim of this study was to explore whether the protein levels of the astrocyte isoform of glycogen phosphorylase (PYGM), key enzyme of glycogenolysis, and the isoform A of Ras-related C3 botulinum toxin substrate 1 (RAC1), a kinase that regulates PYGM activity, are altered in the postmortem DLPFC of chronic schizophrenia patients (n=23) and matched controls (n=23). We also aimed to test NMDAR blockade effect on these proteins in the mouse cortex and cortical astrocytes and antipsychotic treatments in rats. Here we report a reduction in PYGM and RAC1 protein levels in the DLPFC in schizophrenia. We found that treatment with the NMDAR antagonist dizocilpine in mice as a model of psychosis increased PYGM and reduced RAC1 protein levels. The same result was observed in rat cortical astroglial-enriched cultures. 21-day haloperidol treatment increased PYGM levels in rats. These results show that PYGM and RAC1 are altered in the DLPFC in chronic schizophrenia and are controlled by NMDA signalling in the rodent cortex and cortical astrocytes suggesting an altered NMDA-dependent glycogenolysis in astrocytes in schizophrenia. Together, this study provides evidence of a NMDA-dependent transient local energy deficit in neuron-glia crosstalk in schizophrenia, contributing to energy deficits of the disorder.
Schizophr Res, 2016; 177

26049820: Pinacho R, Saia G, Meana JJ, Gill G, Ramos B
Transcription factor SP4 phosphorylation is altered in the postmortem cerebellum of bipolar disorder and schizophrenia subjects.

Transcription factors play important roles in the control of neuronal function in physiological and pathological conditions. We previously reported reduced levels of transcription factor SP4 protein, but not transcript, in the cerebellum in bipolar disorder and associated with more severe negative symptoms in schizophrenia. We have recently reported phosphorylation of Sp4 at S770, which is regulated by membrane depolarization and NMDA receptor activity. The aim of this study was to investigate SP4 S770 phosphorylation in bipolar disorder and its association with negative symptoms in schizophrenia, and to explore the potential relationship between phosphorylation and protein abundance. Here we report a significant increase in SP4 phosphorylation in the cerebellum, but not the prefrontal cortex, of bipolar disorder subjects (n=10) (80% suicide) compared to matched controls (n=10). We found that SP4 phosphorylation inversely correlated with SP4 levels independently of disease status in both areas of the human brain. Moreover, SP4 phosphorylation in the cerebellum positively correlated with negative symptoms in schizophrenia subjects (n=15). Further, we observed that a phospho-mimetic mutation in truncated Sp4 was sufficient to significantly decrease Sp4 steady-state levels, while a non-phosphorylatable mutant showed increased stability in cultured rat cerebellar granule neurons. Our results indicate that SP4 S770 phosphorylation is increased in the cerebellum in bipolar disorder subjects that committed suicide and in severe schizophrenia subjects, and may be part of a degradation signal that controls Sp4 abundance in cerebellar granule neurons. This opens the possibility that modulation of SP4 phosphorylation may contribute to the molecular pathophysiology of psychotic disorders.
Eur Neuropsychopharmacol, 2015; 25

25915526: Pinacho R, Saia G, Fusté M, Meléndez-Pérez I, Villalta-Gil V, Haro JM, Gill G, Ramos B
Phosphorylation of transcription factor specificity protein 4 is increased in peripheral blood mononuclear cells of first-episode psychosis.
Altered expression of transcription factor specificity protein 4 (SP4) has been found in the postmortem brain of patients with psychiatric disorders including schizophrenia and bipolar disorder. Reduced levels of SP4 protein have recently been reported in peripheral blood mononuclear cells in first-episode psychosis. Also, SP4 levels are modulated by lithium treatment in cultured neurons. Phosphorylation of SP4 at S770 is increased in the cerebellum of bipolar disorder subjects and upon inhibition of NMDA receptor signaling in cultured neurons. The aim of this study was to investigate whether SP4 S770 phosphorylation is increased in lymphocytes of first-episode psychosis patients and the effect of lithium treatment on this phosphorylation.
PLoS One, 2015; 10

25175639: Pinacho R, Valdizán EM, Pilar-Cuellar F, Prades R, Tarragó T, Haro JM, Ferrer I, Ramos B
Increased SP4 and SP1 transcription factor expression in the postmortem hippocampus of chronic schizophrenia.
Altered levels of transcription factor specificity protein 4 (SP4) and 1 (SP1) in the cerebellum, prefrontal cortex and/or lymphocytes have been reported in severe psychiatric disorders, including early psychosis, bipolar disorder, and chronic schizophrenia subjects who have undergone long-term antipsychotic treatments. SP4 transgenic mice show altered hippocampal-dependent psychotic-like behaviours and altered development of hippocampal dentate gyrus. Moreover, NMDAR activity regulates SP4 function. The aim of this study was to investigate SP4 and SP1 expression levels in the hippocampus in schizophrenia, and the possible effect of antipsychotics and NMDAR blockade on SP protein levels in rodent hippocampus. We analysed SP4 and SP1 expression levels in the postmortem hippocampus of chronic schizophrenia (n = 14) and control (n = 11) subjects by immunoblot and quantitative RT-PCR. We tested the effect of NMDAR blockade on SP factors in the hippocampus of mouse treated with an acute dose of MK801. We also investigated the effect of subacute treatments with haloperidol and clozapine on SP protein levels in the rat hippocampus. We report that SP4 protein and both SP4 and SP1 mRNA expression levels are significantly increased in the hippocampus in chronic schizophrenia. Likewise, acute treatment with MK801 increased both SP4 and SP1 protein levels in mouse hippocampus. In contrast, subacute treatment with haloperidol and clozapine did not significantly alter SP protein levels in rat hippocampus. These results suggest that SP4 and SP1 upregulation may be part of the mechanisms deregulated downstream of glutamate signalling pathways in schizophrenia and might be contributing to the hippocampal-dependent cognitive deficits of the disorder.
J Psychiatr Res, 2014; 58

25045015: Sun X, Pinacho R, Saia G, Punko D, Meana JJ, Ramos B, Gill G
Transcription factor Sp4 regulates expression of nervous wreck 2 to control NMDAR1 levels and dendrite patterning.
Glutamatergic signaling through N-methyl-d-aspartate receptors (NMDARs) is important for neuronal development and plasticity and is often dysregulated in psychiatric disorders. Mice mutant for the transcription factor Sp4 have reduced levels of NMDAR subunit 1 (NR1) protein, but not mRNA, and exhibit behavioral and memory deficits (Zhou et al., [2010] Human Molecular Genetics 19: 3797-3805). In developing cerebellar granule neurons (CGNs), Sp4 controls dendrite patterning (Ramos et al., [2007] Proc Natl Acad Sci USA 104: 9882-9887). Sp4 target genes that regulate dendrite pruning or NR1 levels are not known. Here we report that Sp4 activates transcription of Nervous Wreck 2 (Nwk2; also known as Fchsd1) and, further, that Nwk2, an F-BAR domain-containing protein, mediates Sp4-dependent regulation of dendrite patterning and cell surface expression of NR1. Knockdown of Nwk2 in CGNs increased primary dendrite number, phenocopying Sp4

knockdown, and exogenous expression of Nwk2 in Sp4-depleted neurons rescued dendrite number. We observed that acute Sp4 depletion reduced levels of surface, but not total, NR1, and this was rescued by Nwk2 expression. Furthermore, expression of Nr1 suppressed the increase in dendrite number in Sp4- or Nwk2- depleted neurons. We previously reported that Sp4 protein levels were reduced in cerebellum of subjects with bipolar disorder (BD) (Pinacho et al., [2011] Bipolar Disorders 13: 474-485). Here we report that Nwk2 mRNA and NR1 protein levels were also reduced in postmortem cerebellum of BD subjects. Our data suggest a role for Sp4-regulated Nwk2 in NMDAR trafficking and identify a Sp4-Nwk2-NMDAR1 pathway that regulates neuronal morphogenesis during development and may be disrupted in bipolar disorder.
Dev Neurobiol, 2015; 75

23540600: Pinacho R, Villalmanzo N, Roca M, Iniesta R, Monje A, Haro JM, Meana JJ, Ferrer I, Gill G, Ramos B
Analysis of Sp transcription factors in the postmortem brain of chronic schizophrenia: a pilot study of relationship to negative symptoms.
Negative symptoms are the most resilient manifestations in schizophrenia. An imbalance in dopamine and glutamate pathways has been proposed for the emergence of these symptoms. SP1, SP3 and SP4 transcription factors regulate genes in these pathways, suggesting a possible involvement in negative symptoms. In this study, we characterized Sp factors in the brains of subjects with schizophrenia and explored a possible association with negative symptoms. We also included analysis of NR1, NR2A and DRD2 as Sp target genes. Postmortem cerebellum and prefrontal cortex from an antemortem clinically well-characterized and controlled collection of elderly subjects with chronic schizophrenia (n = 16) and control individuals (n = 14) were examined. We used the Positive and Negative Syndrome and the Clinical Global Impression Schizophrenia scales, quantitative PCR and immunoblot. SP1 protein and mRNA were reduced in the prefrontal cortex in schizophrenia whereas none of Sp factors were altered in the cerebellum. However, we found that SP1, SP3 and SP4 protein levels inversely correlated with negative symptoms in the cerebellum. Furthermore, NR2A and DRD2 mRNA levels correlated with negative symptoms in the cerebellum. In the prefrontal cortex, SP1 mRNA and NR1 and DRD2 inversely correlated with these symptoms while Sp protein levels did not. This pilot study not only reinforces the involvement of SP1 in schizophrenia, but also suggests that reduced levels or function of SP1, SP4 and SP3 may participate in negative symptoms, in part through the regulation of NMDA receptor subunits and/or Dopamine D2 receptor, providing novel information about the complex negative symptoms in this disorder.
J Psychiatr Res, 2013; 47

22017217: Pinacho R, Villalmanzo N, Lalonde J, Haro JM, Meana JJ, Gill G, Ramos B
The transcription factor SP4 is reduced in postmortem cerebellum of bipolar disorder subjects: control by depolarization and lithium.
Regulation of gene expression is important for the development and function of the nervous system. However, the transcriptional programs altered in psychiatric diseases are not completely characterized. Human gene association studies and analysis of mutant mice suggest that the transcription factor specificity protein 4 (SP4) may be implicated in the pathophysiology of psychiatric diseases. We hypothesized that SP4 levels may be altered in the brain of bipolar disorder (BD) subjects and regulated by neuronal activity and drug treatment.
Bipolar Disord, 2011 Aug-Sep; 13

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BOARD NUMBER: S05-138

GENETICS OF ADDICTION - IDENTIFICATION OF NOVEL GENETIC VARIANTS ASSOCIATED WITH REWARD MECHANISM IN ZEBRAFISH

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

Aleksandra Mech[1], Munise Merteroglu[1], William Havelange[1], Adele Leggieri[1], Saeedeh Husseinian[1], Ian Sealy[2], Elisabeth Busch-Nentwich[2], Caroline Brennan[1]
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Nicotine addiction is one of the major mental health disorders that affects over 29% of the European population and generates enormous cost in medical care and in lost productivity. Previous studies have shown the relevance of genetic factors responsible for a progression to abusive usage, with data from twin studies estimating that approximately 50% of vulnerability to nicotine addiction is due to heritable factors. Here, we developed a novel self-administration assay for juvenile fish to screen ENU-mutagenized zebrafish lines for nicotine seeking. The assay consists of 3 asymmetrically connected chambers with nicotine being administered to one of the chambers by diffusion from a point source thereby setting up a concentration gradient across the 3 chambers. Increased time spent in proximity to the nicotine source is taken as evidence of nicotine seeking behaviour. We screened 54 families covering approximately 3318 dominant and 1037 recessive alleles for nicotine seeking. Wild-type fish showed limited tendency to approach or avoid the nicotine source. However, 10 families of ENU mutagenized fish showed preference for, or aversion to, the nicotine chamber. Two of these families are predicted to carry a dominant mutation affecting the behavior and 8 are predicted to carry recessive mutations. So far, we have shown the heritability of the observed phenotype for five of the mutant lines. Future work will assess the heritability for the remaining families and identify the pathways involved. Increased understanding of the genetics of smoking is necessary to identify novel drug targets and to develop new therapeutic approaches.

Pubmed:
35082702: Mech AM, Merteroglu M, Sealy IM, Teh MT, White RJ, Havelange W, Brennan CH, Busch-Nentwich EM
Behavioral and Gene Regulatory Responses to Developmental Drug Exposures in Zebrafish.
Developmental consequences of prenatal drug exposure have been reported in many human cohorts and animal studies. The long-lasting impact on the offspring-including motor and cognitive impairments, cranial and cardiac anomalies and increased prevalence of ADHD-is a socioeconomic burden worldwide. Identifying the molecular changes leading to developmental consequences could help ameliorate the deficits and limit the impact. In this study, we have used zebrafish, a well-established behavioral and genetic model with conserved drug response and reward pathways, to identify changes in behavior and cellular pathways in response to developmental exposure to amphetamine, nicotine or oxycodone. In the presence of the drug, exposed animals showed altered behavior, consistent with effects seen in mammalian systems, including impaired locomotion and altered habituation to acoustic startle. Differences in responses seen following acute and chronic exposure suggest adaptation to the presence of the drug. Transcriptomic analysis of exposed larvae revealed differential expression of numerous genes and alterations in many pathways, including those related to cell death, immunity and circadian rhythm regulation. Differential expression of circadian rhythm genes did not correlate with behavioral changes in the larvae, however, two of the circadian genes, and , were also differentially expressed at later stages of development, suggesting a long-lasting impact of developmental exposures on circadian gene expression. The immediate-early genes, , and , which are associated with synaptic plasticity, were downregulated by all three drugs and hybridization showed that the expression for all four genes was reduced across all neuroanatomical regions, including brain regions implicated in reward processing, addiction and other psychiatric conditions. We anticipate that these early changes in gene expression in response to drug exposure are likely to contribute to the consequences of prenatal exposure and their discovery might pave the way to therapeutic intervention to ameliorate the long-lasting deficits.
Front Psychiatry, 2021; 12
35124155: Sheardown E, Mech AM, Petrazzini MEM, Leggieri A, Gidziela A, Hosseinian S, Sealy IM, Torres-Perez JV, Busch-Nentwich EM, Malanchini M, Brennan CH
Translational relevance of forward genetic screens in animal models for the study of psychiatric disease.

Psychiatric disorders represent a significant burden in our societies. Despite the convincing evidence pointing at gene and gene-environment interaction contributions, the role of genetics in the etiology of psychiatric disease is still poorly understood. Forward genetic screens in animal models have helped elucidate causal links. Here we discuss the application of mutagenesis-based forward genetic approaches in common animal model species: two invertebrates, nematodes (Caenorhabditis elegans) and fruit flies (Drosophila sp.); and two vertebrates, zebrafish (Danio rerio) and mice (Mus musculus), in relation to psychiatric disease. We also discuss the use of large scale genomic studies in human populations. Despite the advances using data from human populations, animal models coupled with next-generation sequencing strategies are still needed. Although with its own limitations, zebrafish possess characteristics that make them especially well-suited to forward genetic studies exploring the etiology of psychiatric disorders.
Neurosci Biobehav Rev, 2022; 135
32848623: Sleigh JN, Mech AM, Aktar T, Zhang Y, Schiavo G
Altered Sensory Neuron Development in CMT2D Mice Is Site-Specific and Linked to Increased GlyRS Levels.
Dominant, missense mutations in the widely and constitutively expressed gene cause peripheral neuropathy that usually begins in adolescence and principally impacts the upper limbs. Caused by a toxic gain-of-function in the encoded glycyl-tRNA synthetase (GlyRS) enzyme, the neuropathology appears to be independent of the canonical role of GlyRS in aminoacylation. Patients display progressive, life-long weakness and wasting of muscles in hands followed by feet, with frequently associated deficits in sensation. When dysfunction is observed in motor and sensory nerves, there is a diagnosis of Charcot-Marie-Tooth disease type 2D (CMT2D), or distal hereditary motor neuropathy type V if the symptoms are purely motor. The cause of this varied sensory involvement remains unresolved, as are the pathomechanisms underlying the selective neurodegeneration characteristic of the disease. We have previously identified in CMT2D mice that neuropathy-causing mutations perturb sensory neuron fate and permit mutant GlyRS to aberrantly interact with neurotrophin receptors (Trks). Here, we extend this work by interrogating further the anatomy and function of the CMT2D sensory nervous system in mutant mice, obtaining several key results: (1) sensory pathology is restricted to neurons innervating the hindlimbs; (2) perturbation of sensory development is not common to all mouse models of neuromuscular disease; (3) axonal transport of signaling endosomes is not impaired in afferent neurons of all CMT2D mouse models; and (4) expression is selectively elevated in a subset of sensory neurons and linked to sensory developmental defects. These findings highlight the importance of comparative neurological assessment in mouse models of disease and shed light on key proposed neuropathogenic mechanisms in -linked neuropathy.
Front Cell Neurosci, 2020; 14
32703932: Sleigh JN, Mech AM, Schiavo G
Developmental demands contribute to early neuromuscular degeneration in CMT2D mice.
Dominantly inherited, missense mutations in the widely expressed housekeeping gene, GARS1, cause Charcot-Marie-Tooth type 2D (CMT2D), a peripheral neuropathy characterised by muscle weakness and wasting in limb extremities. Mice modelling CMT2D display early and selective neuromuscular junction (NMJ) pathology, epitomised by disturbed maturation and neurotransmission, leading to denervation. Indeed, the NMJ disruption has been reported in several different muscles; however, a systematic comparison of neuromuscular synapses from distinct body locations has yet to be performed. We therefore analysed NMJ development and degeneration across five different wholemount muscles to identify key synaptic features contributing to the distinct pattern of neurodegeneration in CMT2D mice. Denervation was found to occur along a distal-to-proximal gradient, providing a cellular explanation for the greater weakness observed in mutant Gars hindlimbs compared with forelimbs. Nonetheless, muscles from similar locations and innervated by axons of equivalent length showed significant differences in neuropathology, suggestive of additional factors impacting on site-specific neuromuscular degeneration. Defective NMJ development preceded and associated with degeneration, but was not linked to a delay of wild-type NMJ maturation processes. Correlation analyses indicate that muscle fibre type nor synaptic architecture explain the differential denervation of CMT2D NMJs, rather it is the extent of post-natal synaptic growth that predisposes to neurodegeneration. Together, this work improves our understanding of the mechanisms driving synaptic vulnerability in CMT2D and hints at pertinent pathogenic pathways.
Cell Death Dis, 2020; 11
32533580: Mech AM, Brown AL, Schiavo G, Sleigh JN
Morphological variability is greater at developing than mature mouse neuromuscular junctions.
The neuromuscular junction (NMJ) is the highly specialised peripheral synapse formed between lower motor neuron terminals and muscle fibres. Post-synaptic acetylcholine receptors (AChRs), which are found in high density in the muscle membrane, bind to acetylcholine released into the synaptic cleft of the NMJ, thereby enabling the conversion of motor action potentials to muscle contractions. NMJs have been studied for many years as a general model for synapse formation, development and function, and are known to be early sites of pathological changes in many neuromuscular diseases. However, information is limited on the diversity of NMJs in different muscles, how synaptic morphology changes during development, and the

relevance of these parameters to neuropathology. Here, this crucial gap was addressed using a robust and standardised semi-automated workflow called NMJ-morph to quantify features of pre- and post-synaptic NMJ architecture in an unbiased manner. Five wholemount muscles from wild-type mice were dissected and compared at immature (post-natal day, P7) and early adult (P31-32) timepoints. The inter-muscular variability was greater in mature post-synaptic AChR morphology than that of the pre-synaptic motor neuron terminal. Moreover, the developing NMJ showed greater differences across muscles than the mature synapse, perhaps due to the observed distinctions in synaptic growth between muscles. Nevertheless, the amount of nerve to muscle contact was consistent, suggesting that pathological denervation can be reliably compared across different muscles in mouse models of neurodegeneration. Additionally, mature post-synaptic endplate diameters correlated with fibre type, independently of muscle fibre diameter. Altogether, this work provides detailed information on healthy pre- and post-synaptic NMJ morphology from five anatomically and functionally distinct mouse muscles, delivering useful reference data for future comparison with neuromuscular disease models.
J Anat, 2020; 237

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EFFECT OF YOHIMBINE ON VOLUNTARY ETHANOL INTAKE OF ADULT MALE AND FEMALE WISTAR RATS

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

Lourdes De La Torre Vacas, Inmaculada Ruíz García, M. Dolores Escarabajal Arrieta, Ángeles Agüero Zapata
University of Jaen, Psychology, Jaén, Spain

Stress is commonly reported to increase the rewarding effects of alcohol. However, results are not always comparable between laboratories. The numerous variations in experimental methods are contributing to these contradictory results. On the other hand, there is a limited number of studies that have investigated sex differences in stress-alcohol interactions (see Logrip et al., 2018), and the reported results are inconsistent. In this work, we analyze the effect of stress (induced by Yohimbine, 4 mg/kg) on a free choice drinking procedure (the concentration of ethanol was increased gradually from 2% to 10%) in adult Wistar rats. The obtained results show a similar pattern of escalation of alcohol intake as ethanol concentration increases in Yohimbine (YOH) and in vehicle (VEH) injected animals, although YOH-injected animals showed a slightly higher increase of consumption of the 10% ethanol solution. Regarding sex, the results show that female ethanol-intake levels were higher than the exhibited by males. Sex differences in ethanol intake are well documented in rodents with intake in females being higher than that in males (de la Torre et al., 2015). Concerning the possible differential effect of YOH on ethanol intake of male and female rats, the results show absence of significant differences. However, it was found that only in the case of male rats the YOH-injected group ingested a greater amount of 10% ethanol solution than the VEH-injected group. These results may contribute to clarify sex differences in stress-alcohol interactions.

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NEURAL SIGNALING IN THE VTA-ACCUMBENS AXIS CORRELATED WITH CANNABIS EXTRACT EFFECTS ON METHAMPHETAMINE CONDITIONED PLACE PREFERENCE IN MICE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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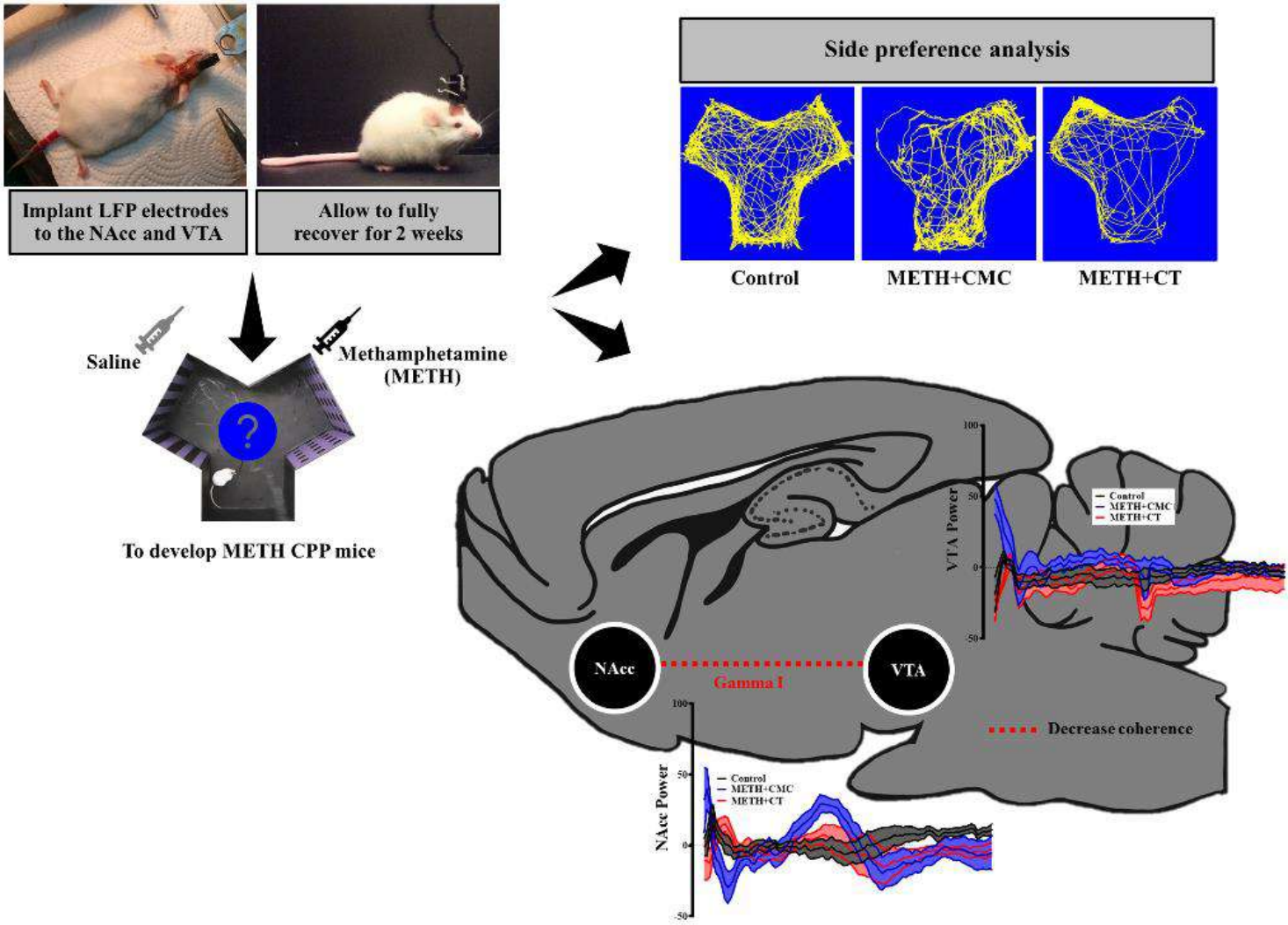
Background: Cannabidiol (CBD) and tetrahydrocannabinol (THC) are natural compounds in *Cannabis* species. A 1:1 ratio of CBD:THC (CT) has been found to improve many psychological illnesses. However, effects of this cannabis mixture on methamphetamine (METH) conditioned place preference (CPP) have not been elucidated. Aims: To evaluate animal behaviors and characterize local filed potential (LFP) oscillations in the nucleus accumbens (NAcc) and ventral tegmental area (VTA) of mice with METH CPP treated with CT Methods: Male albino ICR mice were implanted LFP electrodes and divided into control, METH CPP groups treated with either vehicle (METH+CMC) or 10 mg/kg CT (METH+CT). To induce METH CPP, mice were intraperitoneally injected with 1 mg/kg METH and 0.9% saline in alternative day for 10 sessions and confined to corresponding compartments for 30 min in each session. Control mice were administered with 0.9% saline on all days for both compartments. Animals received treatments 60 min before CPP testing. LFP signals and animal behaviors were recorded simultaneously for 15 min during pre-conditioning and post-conditioning phases. Results: Elevated CPP scores and changes in NAcc thata, gamma I and VTA delta were found in METH+CMC mice. These animals exhibited the enhanced VTA-NAcc gamma I coherence. However, these alterations were significantly attenuated by the cannabis mixture found in METH+CT group. Conclusions: The data confirmed that the cannabis mixture was effective in treatment of METH CPP in mice. The cannabis extract might be useful as an alternative medicine for treatment of methamphetamine

dependence.



Pubmed:

34763040: Nukitram J, Cheaha D, Sengnon N, Wungsintaweekul J, Limsuwanchote S, Kumarnsit E
Ameliorative effects of alkaloid extract from Mitragyna speciosa (Korth.) Havil. Leaves on methamphetamine conditioned place preference in mice.
Mitragyna speciosa (Korth.) Havil., popularly known as Kratom (KT), is a medicinal plant used for pain suppression in Southeast Asia. It has been claimed to assist drug users withdraw from methamphetamine (METH) dependence. However, its use was controversial and not approved yet.
J Ethnopharmacol, 2022; 284

28687506: Cheaha D, Reakkamnuan C, Nukitram J, Chittrakarn S, Phukpattaranont P, Keawpradub N, Kumarnsit E
Effects of alkaloid-rich extract from Mitragyna speciosa (Korth.) Havil. on naloxone-precipitated morphine withdrawal symptoms and local field potential in the nucleus accumbens of mice.
Mitragyna speciosa (Korth.) Havil. (M. speciosa) is among the most well-known plants used in ethnic practice of Southeast Asia. It has gained increasing attention as a plant with potential to substitute morphine in addiction treatment program. However, its action on the central nervous system is controversial.
J Ethnopharmacol, 2017; 208

34098022: Niyomrat K, Cheaha D, Nukitram J, Kumarnsit E
Locomotor activity and resting local field potential oscillatory rhythms of 6-OHDA mouse model of Parkinson's disease in response to acute and repeated treatments with L-dopa.
Phase-amplitude coupling (PAC) of local field potential (LFP) has been recognized as higher-order representation of brain

states. Neuronal loss in the striatum leads to Parkinson's disease (PD) symptoms and modifies LFP oscillation. However, PAC in the striatum of PD mouse model induced by 6-hydroxydopamne (6-OHDA) remained to be investigated. Male Swiss albino ICR mice were implanted with intracranial electrode and injected with 6-OHDA to the left striatum. Levodopa (L-dopa) (10 mg/kg, oral) was used for treatment once a day from day 15-19. Locomotor activity and resting LFP signals were selectively analyzed on day 15 and 19. One-way ANOVA revealed significant decreases in travelled distance induced by 6-OHDA on both days ($p \leq 0.05$). However, the decreased travelled distances were significantly reversed by L-dopa. On day 15, LFP powers of theta, alpha, beta and low gamma waves were significantly increased by 6-OHDA injection and the powers of beta and low gamma were significantly reversed to control level by treatment with L-dopa. On day 19, LFP powers of delta, theta, alpha, beta and low gamma waves were significantly increased by 6-OHDA injection and the powers of low gamma were significantly reversed to control level by treatment with L-dopa. Theta-gamma PAC analyses also confirmed significant increase in modulation index (MI) induced by 6-OHDA on day 19. However, L-dopa failed to significantly reverse the MI to control level. These findings indicated theta-gamma coupling in the striatum of PD mouse model. Taken together, change in striatal theta-gamma PAC might be one of biomarkers in addition to hypokinesia and increased LFP powers that reflect disrupted neural mechanisms in PD mouse model.
Neurosci Lett, 2021; 759

33945805: Nukitram J, Cheaha D, Kumarnsit E

Spectral power and theta-gamma coupling in the basolateral amygdala related with methamphetamine conditioned place preference in mice.

The basolateral amygdala (BLA) plays a crucial role in conditioned place preference (CPP) for addictive drugs. However, neural signaling associated with methamphetamine (METH) craving and seeking remained to be investigated. This study characterized local field potential (LFP) oscillatory patterns in the BLA and conditioned place preference induced by METH-related context. Male Swiss albino ICR mice were deeply anesthetized for LFP intracranial electrode implantation in the BLA. Control and METH groups received sessions to learn to associate saline-paired and METH-paired compartments of the CPP apparatus with saline and METH injections, respectively, for 10 days. LFP signals and exploring behavior were recorded simultaneously during pre- and post-conditioning phases. Time spent in METH-paired compartment was normalized and expressed as CPP scores. Fast Fourier Transform (FFT) algorithm was used to analyze LFP powers of 8 discrete frequency ranges (delta, theta, alpha, beta, gamma I-IV). During post-conditioning phase of METH CPP with METH cues, statistical analysis revealed that METH group significantly increased time spent in METH-paired compartment. Significant suppressions of theta and alpha powers were observed. Phase-amplitude cross frequency coupling analyses confirmed significant increases in maximal modulation index (MI), frequency for phase of slow wave and MI of theta-gamma II coupling. Taken together, LFP oscillation in the BLA was sensitive in association with METH CPP. These research findings might suggest the underlying mechanisms of drug reward learning and adaptive changes in the BLA in acquisition of METH CPP and dependence.
Neurosci Lett, 2021; 756

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A TRANSLATIONAL APPROACH ON REWARD ABNORMALITIES IN ANOREXIA NERVOSA: THE ROLE OF METABOLIC SENSING ON DELAYED GRATIFICATION.

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Reward abnormalities in anorexia nervosa (AN) are characterized by compulsive behaviors but increased sensitivity to delayed gratification. Rodent exposed to food restriction (FR) exhibit strong preference for a running wheel activity in anticipation to food reward, when orexigenic peptides such as ghrelin are elevated in response to fasting. These metabolic sensors drive motivational aspects of feeding modulating the dopaminergic circuit and could acquire reinforcing properties leading to addictive-like behaviors towards undernutrition. We aim to identify metabolic biomarkers associated with sensitivity to delayed gratification in an innovative animal model of chronic undernutrition and in AN patients. We explore the sensitivity to delayed gratification in seven weeks-old female mice before and after a chronic 50% FR with running wheel (FRW) using an innovative operant conditioning paradigm of delay-discounting task (DDT). Clinical data from a cohort of AN will explore the kinetic of reward abnormalities in a human DDT. Data are correlated with metabolic sensors of energy loss (e.g. ghrelin). We validated the DDT in 16 ad-libitum mice, where the preference for a large delayed gratification decreased from 80% to 40% with increasing delay from 0 to 40 sec ($p<0.0001$). We are currently exploring how food restriction modifies response to delayed gratification and perseverative behaviors associated with energy deprivation signals, both in rodent and patients. An innovative step of this project is to identify reliable biomarkers specific for reward abnormalities, possibly involving common pathways in both rodent and humans that might be relevant for diagnosis and prognosis in AN.

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CELLULAR DETERMINANT OF NEGATIVE REINFORCEMENT IN FENTANYL ADDICTION

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Opioids have been used for centuries for their potent effect as painkillers ; however about a third of opioid users will eventually lose control and develop addiction (30%), a number that exceeds the one for a psychostimulant like cocaine (16%) (Anthony et al.,1994). Despite its rewarding property, it is well established that opioids also induce strong dependence, defined by a stereotypical withdrawal syndrome (Comer and Cahill, 2018). This is the case for example, upon cessation of fentanyl consumption, when subjects experience strong dysphoria, tremor, hyperalgesia and sweating. Avoiding this aversive state may contribute to relapse though negative reinforcement (Koob, 2019). While we know much about the circuits of positive reinforcement, much less is known about neural connections of negative reinforcement. To address this question, we use genetic manipulation and in vivo recording in mice precipitate withdrawal with naloxone. We found that withdrawal was associated with an increased activity of central amygdala. Furthermore, we demonstrate that knocking-down MuOR in central amygdala prevent some withdrawal symptoms. We currently explore how and through which projections neurons in the amygdala may contribute to the negative reinforcement.

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TOWARDS DEVELOPING A MOUSE MODEL OF CO-MORBID BINGE-EATING AND -DRINKING.

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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A high rate of co-morbidity exists between binge-eating and binge-drinking disorders, suggesting a common neuropathology. To test this hypothesis, we have worked to develop a mouse model of co-morbid binge-eating and -drinking, using C57BL/6NJ (B6NJ) mice. In our first study, *ad libitum*-fed and -watered female and male B6NJ mice underwent 1-h binge-eating sessions in the morning, every other day. Every afternoon, mice were offered 20 and 40% alcohol for 2 h. As expected, females binge-ate and binge-drank more than males. However, opposite our expectation, mice consuming either SPF or control Chow pellets both exhibited lower alcohol intake on binge-eating days, than on non-eating days, suggesting that mice were regulating their total caloric intake under this concurrent procedure. In a second experiment, mice underwent 10 days of binge-eating (5 days/week), followed by 10 days of binge-drinking. Under this "BE-BD" procedure, females escalated their SPF intake more rapidly than males, and female-SPF mice also consumed the most alcohol on the first day of binge-drinking. Although males did not exhibit behavioral cross-sensitization on day 1 of drinking, both male and female SPF mice consumed more alcohol, overall, than no SPF controls. These data indicate that binge-drinking cross-sensitizes with binge-eating in both male and female B6NJ mice, with females more sensitive to this behavioral phenomenon. Such findings support the notion that common or overlapping mechanisms drive these two forms of excessive behavior, which might account for their high rate of co-morbidity and future work will examine for potential biomolecular correlates within the extended amygdala.

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ROLE OF CYFIP2 ON MEDIAL PREFRONTAL CORTEX TO NUCLEUS ACCUMBENS PATHWAY IN REGULATION OF COCAINE REWARD

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Cytoplasmic FMR1-interacting protein 2 (CYFIP2) consists of the WAVE regulatory complex and is involved in actin polymerization, which contributes to neuronal development and synaptic plasticity through transmitting the Rac1 GTPase signaling to WAVE regulatory complex. It has been reported that S968F point mutation of CYFIP2 is attributed to decreased locomotor response to cocaine administration and facilitates the excessive activation of the WAVE regulatory complex. However, the functional role of which CYFIP2 regulates the rewarding properties of cocaine remains largely unknown. Here, I performed the cocaine reward-related behavioral tests and examined the changes in biochemical and neuronal morphology in nucleus accumbens (NAc) using CYFIP2 S968F knock-in mice to investigate the role of CYFIP2 in regulating cocaine reward. My findings revealed that CYFIP2 S968F attenuated the cocaine behavioral sensitization and conditioned place preference. The CYFIP2 S968F blocked the cocaine-induced neuronal activity of NAc and enhancement of reward-related behaviors by optogenetic activation of NAc or medial prefrontal cortex (mPFC) to NAc pathway. Moreover, CYFIP2 S968F disturbed cocaine-mediated changes in CYFIP2 downstream signaling proteins and dendritic spine plasticity in NAc. These results suggest CYFIP2 plays a role in controlling cocaine-mediated neuronal activity and synaptic plasticity in the mPFC to NAc pathway, and CYFIP2 could serve as a target for regulating cocaine rewards.

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REPEATED ADMINISTRATION OF N-ACETYLCYSTEINE COULD REDUCE EXTINCTION-RESPONDING IN THE MORPHINE CONDITIONED RATS

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Many animal studies and early clinical trials suggested that N-acetylcysteine (NAC) may benefit addiction treatment. The present study tried to evaluate whether chronic administration of systemic NAC during the extinction period could reduce the maintenance of the morphine rewarding properties in the conditioned place preference (CPP) paradigm in the rats. forty-six adult male Wistar rats (190-220 g) were examined with morphine (7 mg/kg; sc) and saline (1 mL/kg; sc) during the 3-day conditioning phase in the CPP paradigm. After the acquisition of morphine CPP, different doses of NAC were daily administered during the extinction period (5, 10, 25, and 50 mg/kg; ip). Conditioning score and locomotor activity were recorded by the video tracking system and Ethovision software after acquisition on the postconditioning day, the extinction period. Daily NAC administration in high doses (25 and 50 mg/kg; ip) reduced extinction-responding compared with the vehicle-control group during the extinction period. These are the first data suggesting that NAC's application during the extinction period could attenuate the morphine reward associated behaviors in the rats which adds to the growing appreciation that the NAC may have potential therapeutic use in combating morphine dependence. It can be consistent with the hypothesis of the involvement of the glutamatergic system in the pathophysiology of addiction.

Pubmed:
34256079: Katebi SN, Torkaman-Boutorabi A, Vousooghi N, Riahi E, Haghparast A
Systemic administration of N-acetylcysteine during the extinction period and on the reinstatement day decreased the maintenance of morphine rewarding properties in the rats.
Many animal studies and early clinical trials suggested that N-acetylcysteine (NAC) may benefit addiction treatment. The present study tried to evaluate whether chronic administration of systemic NAC during the extinction period and acute administration of systemic NAC on the reinstatement day could reduce the maintenance of the morphine rewarding properties in the conditioned place preference (CPP) paradigm in the rats. Ninety-six adult male Wistar rats (190-220 g) were examined with morphine (7 mg/kg; sc) and saline (1 mL/kg; sc) during the 3-day conditioning phase in the CPP paradigm. After the acquisition of morphine CPP, different doses of NAC were daily administered during the extinction period (5, 10, 25, and 50 mg/kg; ip), or 30 min before the CPP test on the reinstatement day (2, 5, 10, 25, and 50 mg/kg; ip). Conditioning score and locomotor activity were recorded by the video tracking system and Ethovision software after acquisition on the post-conditioning day, the extinction period, and reinstatement day. Daily NAC administration in high doses (25 and 50 mg/kg; ip) reduced extinction-responding compared with the vehicle-control group during the extinction period. Although a single injection of NAC in doses 10, 25, 50 mg/kg decreased the reinstatement of morphine-induced CPP, two lower doses (2 and 5 mg/kg) could not significantly reduce the CPP scores. These are the first data suggesting that NAC's application during the extinction period could attenuate the morphine reward-associated behaviors in the rats. Moreover, NAC could inhibit the reinstatement of morphine CPP, which adds to the growing appreciation that the NAC may have potential therapeutic use in combating morphine dependence. It can be consistent with the hypothesis of the involvement of the glutamatergic system in the pathophysiology of addiction.
Behav Brain Res, 2021; 413
30053460: Katebi N, Farahimanesh S, Fatahi Z, Zarrabian S, Haghparast A
Involvement of D1- and D2-like dopamine receptors in the dentate gyrus in the acquisition, expression, and extinction of the morphine-induced conditioned place preference in rats.
In the current study, we investigated the role of intra-dentate gyrus (DG) administration of D1 and/or D2 receptor antagonists on the expression, acquisition, and extinction of morphine-CPP. Cannulae were implanted bilaterally into the DG region in male Wistar rats and CPP was induced by the subcutaneous injection of morphine (5 mg/kg) during a 3-day conditioning phase. Three experimental designs were separately employed in the CPP paradigm during the acquisition, expression and

extinction phases, and different doses (0.25, 1, or 4 µg/0.5 µl saline) of SCH23390, as a selective D1-like receptor antagonist, and sulpiride (0.25, 1, or 4 µg/0.5 µl DMSO), as a selective D2-like receptor antagonist, were bilaterally microinjected into the DG region. Conditioning scores and locomotor activities were recorded during the test. Results showed that the injection of the antagonists into the DG region dose-dependently attenuated the acquisition and expression of the morphine-induced CPP and sulpiride revealed prominent behavioral results compared to SCH23390 in both mentioned phases. Moreover, the blockade of D1- and D2-like receptors shortened the extinction phase of the morphine-induced CPP but had no effect on the locomotor activity. We found that the dopamine receptors within the DG region are involved in the acquisition and expression of morphine-CPP and have a critical role in the association between a morphine-paired context and the rewarding proprieties of morphine.
Behav Brain Res, 2018; 353
24096212: Katebi SN, Razavi Y, Zeighamy Alamdary S, Khodagholi F, Haghparast A
Morphine could increase apoptotic factors in the nucleus accumbens and prefrontal cortex of rat brain's reward circuitry.
The nucleus accumbens (NAc) and prefrontal cortex (PFC) are two parts of neuronal reward circuit involved in motivated and goal-directed behaviors. Some data suggest that morphine is toxic to neurons and induces apoptosis, while other evidence shows that morphine could have beneficial effects against cell death. This study was designed to evaluate the effect of morphine on apoptosis by measuring the expression of apoptotic proteins in two important regions, the NAc and PFC, in the rat brain's reward circuitry. Morphine subchronic administration in different doses (0.5, 5 and 10mg/kg) in conditioned place preference (CPP) paradigm (3 times in 3 days, for each dose in each group of rats) was used to induce its rewarding effect. Then, the expression of four apoptotic factors; Bax, Bcl2, caspase3 and PARP, in the NAc and PFC were assessed using the Western blot technique. All of morphine-treated groups showed increase of apoptotic factors in these regions. In the NAc, morphine significantly increased the Bax/Bcl-2 ratio, caspase3 and PARP in the lowest dose (0.5mg/kg) but in the PFC considerable increase was seen in dose of 5mg/kg. Elevation of apoptotic factors in the NAc and PFC implies that morphine can affect the molecular mechanisms which interfere with apoptosis through different receptors. Our findings suggest that the NAc and PFC may have a different distribution of receptors which become active in different doses of morphine.
Brain Res, 2013; 1540
24281942: Razavi Y, Alamdary SZ, Katebi SN, Khodagholi F, Haghparast A
Morphine-induced apoptosis in the ventral tegmental area and hippocampus after the development but not extinction of reward-related behaviors in rats.
Some data suggest that morphine induces apoptosis in neurons, while other evidences show that morphine could have protective effects against cell death. In this study, we suggested that there is a parallel role of morphine in reward circuitry and apoptosis processing. Therefore, we investigated the effect of morphine on modifications of apoptotic factors in the ventral tegmental area (VTA) and hippocampus (HPC) which are involved in the reward circuitry after the acquisition and extinction periods of conditioned place preference (CPP). In behavioral experiments, different doses of morphine (0.5, 5, and 10 mg/kg) and saline were examined in the CPP paradigm. Conditioning score and locomotor activity were recorded by Ethovision software after acquisition on the post-conditioning day, and days 4 and 8 of extinction periods. In order to investigate the molecular mechanisms in each group, we then dissected the brains and measured the expression of apoptotic factors in the VTA and HPC by western blotting analysis. All of the morphine-treated groups showed an increase of apoptotic factors in these regions during acquisition but not in extinction period. In the HPC, morphine significantly increased the ratio of Bax/Bcl-2, caspases-3, and PARP by the lowest dose (0.5 mg/kg), but, in the VTA, a considerable increase was seen in the dose of 5 mg/kg; promotion of apoptotic factors in the HPC and VTA insinuates that morphine can affect the molecular mechanisms that interfere with apoptosis through different receptors. Our findings suggest that a specific opioid receptor involves in modification of apoptotic factors expression in these areas. It seems that the reduction of cell death in response to high dose of morphine in the VTA and HPC may be due to activation of low affinity opioid receptors which are involved in neuroprotective features of morphine.
Cell Mol Neurobiol, 2014; 34
32925229: Mahmoudi D, Assar N, Mousavi Z, Katebi SN, Azizi P, Haghparast A
The orexin receptors in the ventral tegmental area are involved in the development of sensitization to expression of morphine-induced preference in rats.
Recent studies have shown that orexin neurons in the lateral hypothalamus send a compelling project to the ventral tegmental area (VTA). Besides, orexin-1 (OX1) and orexin-2 (OX2) in the VTA are necessary for the development of morphine-induced place preference. Also, sensitivity to morphine can reinforce the rewarding effects of morphine. The current study aims to determine the role of VTAs orexin receptors in morphine sensitization in rats. In 84 adult male albino Wistar rats, two separate cannulae bilaterally implanted into the VTA. They received intra-VTA infusions of SB334867 (0.1, 1 and 10 nM) and TCS OX2 29 (1, 7 and 20 nM) as OX1 and OX2 receptor antagonists, respectively, 10 min before subcutaneous administration of morphine (5 mg/kg) during 3-day sensitization period. After a 5-day drug-free period, the conditioned place

preference (CPP) paradigm induced by subthreshold doses of morphine (0.5 mg/kg), and CPP scores were measured by EthoVision software. The results revealed that the blockade of both OX1 and OX2 receptors within the VTA reduced the expression of morphine-induced CPP in the sensitized rats. It is plausible that VTAs orexin receptors are involved in the development/acquisition of sensitization to morphine-induced CPP in the rats.
Behav Pharmacol, 2020; 31

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BOARD NUMBER: S05-146

EFFECT OF TESTOSTERONE ON MAINTENANCE OF MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE: ROLE OF ANDROGEN AND MU-OPIOID RECEPTORS EXPRESSION IN PREFRONTAL CORTEX AND NAC OF MALE RATS.

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Rewarding properties of androgens has been suggested. The present study aimed to evaluate the effect of androgen system during the extinction period on morphine induced conditioned place preference (CPP). Androgen and μ-opioid receptor gene expression were also evaluated in PFC and NAC in the rats. CPP was induced by morphine injection (3, 5 and 7 mg/kg, i.p.) for three consecutive days. Testosterone (androgen receptor agonist, 2.5 mg/kg; i.m.) and flutamide (androgen receptor antagonist 10 mg/kg, i.m.) were administered in subsequent extinction period. In 2 castrated groups one group was considered as control and the other one received testosterone during extinction phase. The mRNA expression levels of μ-opioid and androgen receptors in PFC and NAC were evaluated using quantitative Real-time PCR following CPP reinstatement. Testosterone prolonged while flutamide shortened extinction period. Castration facilitated morphine-extinction and testosterone could not reverse this effect. The expression of μ-opioid and androgen receptors were increased in PFC and NAC of castrated animals compared to control group which were revesed by testosterone. In conclusion our results indicated that decreased level of testosterone facilities extinction period in morphine CPP model in rats. This result could be due to the changes in the expression of opioid and androgenic receptors in PFC and NAC. This study confirms the crucial role of androgen system in modulating drug reward. Key words: Androgen, Conditioned place preference, Nucleus accumbens, Morphine, Prefrontal cortex

Pubmed:

35173918: Nikkholgh A, Ahmad Ebrahimi S, Bakhshi E, Zarrindast MR, Asgari Y, Torkaman-Boutorabi A
New Biomarkers Based on Smoking-Related Phenotypes for Smoking Cessation Outcomes of Nicotine Replacement Therapy: A Prospective Study.
Identifying a potent biomarker for smoking cessation can play a key role in predicting prognosis and improving treatment outcomes. This study aimed to evaluate the contribution of new biomarkers based on the levels of Cotinine (Cot) and carbon monoxide (CO) to the short- and long-term quit rates of nicotine replacement therapies (Nicotine Patch [NP] and Nicotine Lozenge [NL]).
Basic Clin Neurosci, 2021 Sep-Oct; 12
35144603: Nourmohammadi S, Yousefi S, Manouchehrabadi M, Farhadi M, Azizi Z, Torkaman-Boutorabi A
Thymol protects against 6-hydroxydopamine-induced neurotoxicity in in vivo and in vitro model of Parkinson's disease via inhibiting oxidative stress.
Parkinson's disease (PD) is a multifactorial movement disorder with the progressive degeneration of the nigrostriatal system that impairs patients' movement ability. Oxidative stress has been found to affect the etiology and pathogenesis of PD. Thymol, a monoterpenic phenol, is one of the most important dietary constituents in thyme species. It has been used in traditional medicine and possesses some properties including antioxidant, free radical scavenging, anti-inflammatory. In this study, in vitro and in vivo experiments were performed with the thymol in order to investigate its potential neuroprotective effects in models of PD.
BMC Complement Med Ther, 2022; 22
34843902: Tarbali S, Zahmatkesh M, Torkaman-Boutorabi A, Khodagholi F
Assessment of lipophilic fluorescence products in β-amyloid-induced cognitive decline: A parallel track in hippocampus, CSF, plasma and erythrocytes.
Oxidative stress implicates in Alzheimer's disease (AD) pathophysiology, and associates with the creation of end products of free radical reactions, are known as lipophilic fluorescent products (LFPs). This study aimed to evaluate the probable parallel alterations in the spectral properties of the LFPs in the hippocampus tissues, cerebrospinal fluid (CSF), plasma, and erythrocytes during AD model induction by intra-cerebroventricular (ICV) amyloid β-protein fragment 25-35 (Aβ) injection.

Exp Gerontol, 2022; 157
34302880: Saberian H, Asgari Taei A, Torkaman-Boutorabi A, Riahi E, Aminyavari S, Naghizadeh A, Farahmandfar M
Effect of histone acetylation on maintenance and reinstatement of morphine-induced conditioned place preference and ΔFosB expression in the nucleus accumbens and prefrontal cortex of male rats.
Recently, epigenetic mechanisms are considered as the new potential targets for addiction treatment. This research was designed to explore the effect of histone acetylation on ΔFosB gene expression in morphine-induced conditioned place preference (CPP) in male rats. CPP was induced via morphine injection (5 mg/kg) for three consecutive days. Animals received low-dose theophylline (LDT) or Suberoylanilide Hydroxamic acid (SAHA), as an histone deacetylase (HDAC) activator or inhibitor, respectively, and a combination of both in subsequent extinction days. Following extinction, a priming dose of morphine (1 mg/kg) was administered to induce reinstatement. H4 acetylation and ΔFosB expression in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) were assessed on the last day of extinction and the following CPP reinstatement. Our results demonstrated that daily administration of SAHA (25 mg/kg; i.p.), facilitated morphine-extinction and decreased CPP score in reinstatement of place preference. Conversely, injections of LDT (20 mg/kg; i.p.) prolonged extinction in animals. Co-administration of LDT and SAHA on extinction days counterbalanced each other, such that maintenance and reinstatement were no different than the control group. The gene expression of ΔFosB was increased by SAHA in NAc and mPFC compared to the control group. Administration of SAHA during extinction days, also altered histone acetylation in the NAc and mPFC on the last day of extinction, but not on reinstatement day. Collectively, administration of SAHA facilitated extinction and reduced reinstatement of morphine-induced CPP in rats. This study confirms the essential role of epigenetic mechanisms, specifically histone acetylation, in regulating drug-induced plasticity and seeking behaviors.
Behav Brain Res, 2021; 414
34256079: Katebi SN, Torkaman-Boutorabi A, Vousooghi N, Riahi E, Haghparast A
Systemic administration of N-acetylcysteine during the extinction period and on the reinstatement day decreased the maintenance of morphine rewarding properties in the rats.
Many animal studies and early clinical trials suggested that N-acetylcysteine (NAC) may benefit addiction treatment. The present study tried to evaluate whether chronic administration of systemic NAC during the extinction period and acute administration of systemic NAC on the reinstatement day could reduce the maintenance of the morphine rewarding properties in the conditioned place preference (CPP) paradigm in the rats. Ninety-six adult male Wistar rats (190-220 g) were examined with morphine (7 mg/kg; sc) and saline (1 mL/kg; sc) during the 3-day conditioning phase in the CPP paradigm. After the acquisition of morphine CPP, different doses of NAC were daily administered during the extinction period (5, 10, 25, and 50 mg/kg; ip), or 30 min before the CPP test on the reinstatement day (2, 5, 10, 25, and 50 mg/kg; ip). Conditioning score and locomotor activity were recorded by the video tracking system and Ethovision software after acquisition on the post-conditioning day, the extinction period, and reinstatement day. Daily NAC administration in high doses (25 and 50 mg/kg; ip) reduced extinction-responding compared with the vehicle-control group during the extinction period. Although a single injection of NAC in doses 10, 25, 50 mg/kg decreased the reinstatement of morphine-induced CPP, two lower doses (2 and 5 mg/kg) could not significantly reduce the CPP scores. These are the first data suggesting that NAC's application during the extinction period could attenuate the morphine reward-associated behaviors in the rats. Moreover, NAC could inhibit the reinstatement of morphine CPP, which adds to the growing appreciation that the NAC may have potential therapeutic use in combating morphine dependence. It can be consistent with the hypothesis of the involvement of the glutamatergic system in the pathophysiology of addiction.
Behav Brain Res, 2021; 413
33955527: Nikkholgh A, Soleimani M, Torkaman-Boutorabi A, Valizadeh B
Evaluation of smoking status: comparison of self-reports with exhaled carbon monoxide analysis in university students in the Islamic Republic of Iran.
Smoking is considered the leading risk factor for many chronic diseases and deaths worldwide. Thus, it is important to determine the number of smokers before implementing tobacco control initiatives. Due to stigma and deterrent measures, it is impossible to access smokers through a self-report questionnaire.
East Mediterr Health J, 2021; 27
33209221: Akbari Z, Reisi P, Torkaman-Boutorabi A, Farahmandfar M
Effect of Pentoxifylline on Apoptotic-Related Gene Expression Profile, Learning and Memory Impairment Induced by Systemic Lipopolysaccharide Administration in the Rat Hippocampus.
Inflammation is one of the effective factors, in the development of functional disorders of the nervous system. Pentoxifylline (PTX) has an inhibitory effect on inflammatory factors. Therefore the aim of this study was to evaluate the effect of PTX on learning, memory and expression of genes, involved in neuronal survival in the rat hippocampus, following systemic lipopolysaccharide (LPS) injection.
Int J Prev Med, 2020; 11

32231769: Torkaman-Boutorabi A, Seifi F, Akbarabadi A, Toolee H, Sadat-Shirazi MS, Vousooghi N, Zarrindast MR
Morphine Exposure and Enhanced Depression-like Behaviour Confronting Chronic Stress in Adult Male Offspring Rat.
Opioid addiction is an important concern in the World. Reports demonstrate that substance use disorder could influence genetic and environmental factors, and children of addicts have a higher rate of psychopathology. In this study, we investigated depression-like behavior among offspring of morphine-exposed rat parents.
Basic Clin Neurosci, 2019 Jul-Aug; 10

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BOARD NUMBER: S05-147

MECP2 CONTROLS DRUG ADDICTION DIFFERENTLY DEPENDING ON CELL TYPE IN THE NUCLEUS ACCUMBENS

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Methyl CpG-binding protein 2 (MeCP2) is a transcriptional regulator that binds to DNA and inhibits or activates the expression of a target gene. Recent studies have reported that MeCP2 plays an important role in addiction regulation in the nucleus accumbens (NAc), which is one of the key reward-related areas. However, little is known about the cell type-specific function of MeCP2 in the mechanisms leading to addiction. Here, we investigated how MeCP2 regulates drug addiction according to the cell type of D1R (dopamine 1 receptor) and D2R (dopamine 2 receptor) neurons, which account for the majority of the NAc. We found that mice repeatedly exposed to cocaine increased their behavioral sensitivity and preference to the drug. Interestingly, repeated exposure to cocaine markedly decreased MeCP2 in D2R neurons, but not in D1R. We also found that when we mimics these changes of MeCP2 by using AAV viruses, MeCP2 knockdown only in D2R neurons of the NAc increased cocaine preference. Additionally, we confirmed whether the alteration in MeCP2 modulates the level of neuronal activity closely related to the regulation of drug response by using MEA (multi-electrode array). We found that a decrease in MeCP2 of the D2R neurons reduces neuronal activity levels of the NAc in response to electrical stimulation. Taken together, MeCP2 in D2R neurons of the NAc is a key mediator in drug addiction. In addition, these results suggest the possibility that MeCP2 may modulate response to the addictive drug by regulating neuronal activity.

Pubmed:
33281509: Bae J, Hong SS, Son LK
Prior failures, laboring in vain, and knowing when to give up: Incremental versus entity theories.
Against intuition, a set of "desirable difficulties" has been touted as a way in which to improve learning and lengthen retention. This includes, for instance, varying the conditions of learning to allow for more active, effortful, or challenging, contexts. In the current paper, we introduce data that show that, on the contrary, learning to know when to take the road may be crucial when it comes to avoiding "laboring in vain." We presented participants with prior problems - either easy or difficult - followed by choices of selecting an easy or a difficult current problem. Our primary goal was to examine the notion that past failures (which are more likely on the difficult prior items) may be a basis for allowing learners to then choose the easy rather than the difficult current problem. In other words, if one has labored in vain already, the easier items may now be more desirable. In addition, we compare the selections that are made between and perspectives, given their fundamentally opposing views on effort. Our results showed that, interestingly, incremental theorists, who generally are proponents of effort, were more likely to select the easy problems, but only when they had experienced failure on prior, and similar, difficult tasks. We interpret these data to suggest that those holding an incremental view may be more in tune with their past efforts, resulting in a Metacognition-by-Experience, or ME strategy, and also hint at its generalizability through cross-cultural comparisons. Metacogn Learn, 2020;

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BOARD NUMBER: S05-148

MICE EXPRESSING ALLELIC VARIANT OR DELETION OF CHRNA5 SHOW INCREASED ALCOHOL CONSUMPTION BUT OPPOSITE MOTIVATIONAL PROFILES: PRECLINICAL SUPPORT FOR CLONINGER ALCOHOLIC SUBTYPES

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Human genetic association studies have linked single nucleotide polymorphisms (SNPs) of the CHRNA5 gene, encoding the α5 nicotinic acetylcholine receptor subunit (α5-nAChR), to an increased risk of alcohol use disorders (AUDs). To understand how α5-nAChR subunit mutations may influence alcohol-drinking behavior and preconsummatory traits relevant to AUDs (anxiety, sensation-seeking, impulsivity), we tested male and female transgenic mice expressing either the most common SNP (D398N_α5KI) or a deletion of the CHRNA5 gene (α5KO) in the elevated-plus maze, novelty-place preference and step-down tasks. Their alcohol consumption was then assessed through an intermittent two-bottle choice self-administration protocol. As this is where α5-nAChR is most densely expressed, we investigated the implication of the α5*nAChR-expressing IPN-GABAergic neurons in these changes using neurospecific reexpression of the subunit in α5KOxGAD-Cre mice. α5KI and α5KO mice both showed alcohol over-consumption for highly concentrated solutions but displayed opposite anxiety-related behaviour (hyper vs hypo-anxious) and behavioural control (impulsive-like vs not impulsive, respectively). These opposite phenotypes strongly evoke characteristics of Cloninger's avoidant (Type-I) and sensation-seeking (Type-II) AUDs. Moreover, viral reexpression of α5-nAChR in IPN-GABAergic neurons decreased alcohol consumption and improved the impulsive-like phenotype observed in α5KO. These results support that mutations of the α5-nAChR subunit resulting in loss of α5*nAChRs function shift the alcohol consumption toward high doses, and in contrast, that α5*nAChR-expressing IPN-GABAergic neurons contribute to its control. Furthermore, we hypothesize that α5-nicotinic mutants may provide a preclinical model of Cloninger's AUD subtypes, which could represent a major step toward the development of personalized and more effective therapeutic strategies.

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BOARD NUMBER: S05-149

SOCIAL EMOTIONAL PROFILES OF TWO STRAINS OF TRANSGENIC MICE EXPRESSING NICOTINIC RECEPTOR MUTATIONS INVOLVED IN ALCOHOL ABUSE.

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Human genetic association studies have linked different single nucleotide polymorphisms (SNPs) of the alpha5-subunit of nicotinic acetylcholine receptors to an increased risk of alcohol use disorders (AUDs). Transgenic mice expressing either a common SNP (D398N, α5KI) or a deletion of the CHRNA5 gene altogether (α5KO) are both prone to alcohol over-consumption but display opposite anxiety-related behaviour (hyper vs hypo-anxious) and behavioural control (impulsive-like vs not impulsive, respectively). These opposite phenotypes strongly evoke characteristics of Cloninger's avoidant (Type-I) and sensation-seeking (Type-II) AUDs. To further explore the possibility that α5-nicotinic mutants may provide a preclinical model of Cloninger's AUD subtypes, we investigated their social-emotional profiles as well as the activity of the amygdalo-hippocampal pathway, involved in social-emotional processes. Emotion recognition abilities were assessed using the Affective State Discrimination Task, and rescuing behaviour using the Restrainer Tube Test. Male α5KO mice were impaired in recognition of a stressed affective state and did not display rescuing behaviour towards trapped peers, or were doing so in an inappropriate manner by assaulting them. In contrast, female α5KI mice exhibited normal emotion recognition and improved rescuing behaviour. Moreover, *in vivo* electrophysiological recordings revealed opposite changes in amygdalo-hippocampal activity in relation with these social-emotional profiles, such as a higher potentiation versus a loss of potentiation of the BLA-vCA1 neurotransmission in α5KI and α5KO respectively. These results further support the parallel between social-emotional profiles of AUD type-I and female α5KI mice, and between AUD type-II and male α5KO mice and the implication of the amygdalo-hippocampal pathway in these differences.

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BOARD NUMBER: S05-150

THE EFFECT OF BLAUTIA WEXLERAE ON FOOD ADDICTIVE-LIKE BEHAVIOR

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Aims: Food addiction is a chronic multifactorial brain disorder resulting from dynamic gene network interactions and environmental factors. A growing body of literature implicates a role of the gut microbiota in various diseases, including eating disorders. Similarities in the microbial composition and functions among healthy individuals suggest that the microbiome is required for proper host health. Previous studies conducted in our laboratory have found differential microbiota signatures in food addiction vulnerable and resilient mice despite identical experimental conditions. We found a significantly higher proportion of *Blautia* spp. in non-addicted mice compared with addicted, thus showing its possible protective role in the development of food addiction. Thus, the present study aimed to investigate the effect of *Blautia* spp. on food addictive-like behavior. **Methods:** We had set up a primary protocol for the anaerobic cultivation of *Blautia wexleare.* As *Blautia wexlerae* is a strict anaerobe, we decided to administer it to mice by gavage to maintain as much viable bacteria as possible. A validated food addiction model was used to analyze the effect of *Blautia wexlerae.* **Results:** Results show that *Blautia* spp. might be beneficial in preventing food addiction. This differential gut microbiota signature was revealed despite similar food intake. **Conclusions:** This novel understanding of the role of gut microbiota in the development of food addiction may open new approaches for the development of biomarkers and innovative therapies for food addiction and related eating disorders. **Acknowledgements:** This work was supported by the Spanish Ministerio de Economía y Competitividad-MINECO' (PID2020-120029GB-I00) and (FPU-2021).

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BOARD NUMBER: S05-151

NORADRENERGIC STIMULATION MODULATES EXTINCTION OF CONDITIONED MEMORIES INDUCED BY COCAINE IN MICE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Addiction is a brain disease in which aberrant learning processes hijack the neural mechanisms necessary to mediate appropriate responses to natural rewards. Maladaptive conditioned memories (context-drug) promote drug-seeking behavior. Extinction involves the formation of new inhibitory memories and prevents drug-directed behaviors. The noradrenergic system (NA) is involved in the consolidation and extinction of drug-associated memories. Our aim is to explore Atomoxetine (ATO: NA reuptake inhibitor) effects on extinction of cocaine-induced conditioned place preference (CPP). C57BL/6 male mice were trained on CPP induced by cocaine (20 mg/kg). Before CPP expression (T1), mice were divided in three groups: SAL-SAL (saline from T1-T6), SAL-ATO (saline on T1, ATO from T2-T6) and ATO-ATO (ATO from T1-T6). ATO was injected 30 min before each test. We performed these experiments at two levels; 1) all mice repeated extinction sessions until the whole group fulfilled the extinction's criteria. 2) We examined individual differences and brains were collected when each mouse reached the extinction's criteria. A two-way ANOVA showed that animals treated with ATO-ATO differed in rate of extinction with mice treated with SAL-SAL ($p=0.0219$). Kolmogorov-Smirnov analysis showed significant differences between SAL-SAL and ATO-ATO on T3 ($p=0.0204$). Additionally, a survival analysis determined that the probabilities to extinguish CPP is higher for the ATO-ATO group than SAL-SAL (T1-T5; $p=0.0377$). These effects were not observed for SAL-ATO group. ATO given before T1 (T1-T6), but not from T2-T6, facilitates extinction of cocaine-induced CPP. These findings indicate that NA modulation of cocaine-CPP extinction might be sensitive to the initial expression of CPP.

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EFFECTS OF THE ACTIVATION OF THE NORADRENERGIC SYSTEM ON RECONSOLIDATION, EXTINCTION, AND SUBSEQUENT REINSTATEMENT OF CONDITIONED MEMORIES ASSOCIATED WITH THE ADMINISTRATION OF COCAINE.

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

Olga Rodríguez-Borillo[1], Lorena Roselló-Jiménez[1], Aitor Sanchez-Hernandez[1], Patricia Ibáñez-Marín[1], Julian Guarque-Chabrera[1,2], Ignasi Melchor Eixea[1], Raúl Pastor[1], Marta Miquel[1,2], Laura Font[1]
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Addiction is a neuroplasticity disorder in which the exposure to environmental stimuli (CS) associated with the drug (US) triggers relapse after abstinence. Memory reconsolidation involves the return to the original CS–US association. Extinction implies a new learning; the CS ceased predicting the US. Following a short CS-alone session, memory reconsolidation is dominant, whereas extended, repeated sessions lead to extinction. β2-adrenergic receptors (β2-ARs) are important for the reconsolidation and extinction of memory. The objective was to evaluate whether the activation of the β2-ARs, would modulate reconsolidation, extinction and reinstatement of cocaine-induced conditioned place preference (CPP). Male C57Bl/6J mice were trained following a cocaine (20 mg/kg)-induced CPP. Following a CPP test, animals were divided into two groups: Exp.1) a short CS-reactivation trial followed by a CPP test (5 series) and Exp.2) 5 consecutive extinction trials followed by a CPP test (2 series). Animals received clenbuterol (β2-ARs agonist; 0, 0.5 and 2 mg/kg) immediately after each trial. Reinstatement was tested 24h, 72h and 30 days after extinction. On Exp.1, activation of β2-ARs had no effect on extinction. However, clenbuterol (0.5 mg/kg; $p = 0.0375$ and 2 mg/kg; $p = 0.0018$) promoted reinstatement 30 days after extinction. On Exp.2, activation of β2-Ars facilitated extinction ($p = 0.0579$) and preliminary data suggested a prevention of reinstatement. Our results indicated that methodological variables (such as the duration of the CS exposure) influence the memory processes activated during retrieval. The effects of β2-Ars activation may be different depending on two dissociable processes; memory reconsolidation and extinction.

Pubmed:
32101989: Morales I, Rodríguez-Borillo O, Font L, Pastor R
Effects of naltrexone on alcohol, sucrose, and saccharin binge-like drinking in C57BL/6J mice: a study with a multiple bottle choice procedure.
Chronic alcohol (ethyl alcohol, EtOH) binging has been associated with long-term neural adaptations that lead to the development of addiction. Many of the neurobiological features of EtOH abuse are shared with other forms of binging, like pathological feeding. The drinking-in-the-dark (DID) paradigm has been used extensively to study the neurobiology of EtOH binge-like drinking due to its ability to promote high intakes relevant to human behavior. DID can also generate high consumption of other tastants, but this procedure has not been fully adapted to study forms of binging behavior that are not alcohol-driven. In the present study, we used a modified version of DID that uses multiple bottle availability to promote even higher levels of EtOH drinking in male C57BL/6J mice and allows a thorough investigation of tastant preferences. We assessed whether administration of systemic naltrexone could reduce binging on EtOH, sucrose, and saccharin separately as well as in combination. Our multiple bottle DID procedure resulted in heightened levels of consumption compared with previously reported data using this task. We found that administration of the opioid receptor antagonist naltrexone reduced intakes of preferred, highly concentrated EtOH, sucrose, and saccharin. We also report that naltrexone was able to reduce overall intakes when animals were allowed to self-administer EtOH, sucrose, or saccharin in combination. Our modified DID procedure provides a novel approach to study binging behavior that extends beyond EtOH to other tastants (i.e. sucrose and artificial sweeteners), and has implications for the study of the neuropharmacology of binge drinking.
Behav Pharmacol, 2020; 31
31713874: Evans O, Rodríguez-Borillo O, Font L, Currie PJ, Pastor R
Alcohol Binge Drinking and Anxiety-Like Behavior in Socialized Versus Isolated C57BL/6J Mice.
Binge alcohol drinking has been characterized as a key feature of alcoholism. The drinking-in-the-dark (DID) preclinical model, a procedure that promotes high levels of ethanol (EtOH) intake in short periods of time, has been extensively used to

investigate neuropharmacological and genetic determinants of binge-like EtOH consumption. Using DID methodology, alcohol-preferring strains of mice such as C57BL/6J (B6) mice consume enough EtOH to achieve blood concentrations (≥1.0 mg/ml) associated with behavioral intoxication (i.e., motor incoordination). DID procedures typically involve the use of socially isolated animals (single-housed prior to and during the experiment). Previous research indicates that stress associated with social isolation can induce anxiety-like behavior and promote increases in EtOH intake. The present study investigates the role of housing conditions in anxiety-like behavior and binge-like EtOH intake using a DID procedure. Alcohol Clin Exp Res, 2020; 44

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BOARD NUMBER: S05-153

THE INTERPEDUNCULAR NUCLEUS ACTS AS A KEY MODULATOR BETWEEN REINFORCEMENT AND AVERSIVE CIRCUITS IN NICOTINE INTAKE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Aims: Nicotine drives reinforcement primarily by activating nicotine acetylcholine receptors (nAChRs) on dopaminergic (DA) neurons in the ventral tegmental area (VTA). But nicotine also acts concurrently on the interpeduncular nucleus (IPN) to induce aversion to the drug. We wish to understand how IPN neurons respond to nicotine *in vivo* and whether this response influences nicotine's reinforcing effect in the VTA. **Methods:** We used *in vivo* electrophysiological and fiber photometry recordings in adult mice, coupled with a novel chemogenetic strategy for manipulating specific nAChR subtypes, to probe nicotinic transmission in the IPN and its influence on VTA DA neurons. **Results:** We show that nicotine activates and inhibits two different populations of IPN neurons starting at low doses that elicit no response in VTA DA neurons. To pharmacologically dissect these effects, we developed two novel mutant mouse models, which allow chemogenetic inhibition of specific nAChR subtypes (β2- and β4-containing receptors). The mutation (E61C) does not alter receptor function, but allows the covalent attachment of an irreversible antagonist (called MPEG4Ch). Infusion of MPEG4Ch in the IPN of these mutant mice, but not of WT mice, leads to potent, pharmacologically-specific and sustained receptor antagonism. We consequently show that β2 and β4 nAChRs differentially contribute to nicotine-induced activation and inhibition. Furthermore, we show that blocking IPN β4 nAChRs significantly reduces the threshold response to nicotine in VTA DA neurons. **Conclusions:** Our results suggest that nicotine's effect on IPN neurons is mediated through specific nAChRs that will ultimately facilitate its action on VTA DA neurons.

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VTA CIRCUITRY SUSTAINS OPPOSITE RESPONSES OF DOPAMINERGIC NEURONS TO DRUGS OF ABUSE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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AIMS: Nicotine, the main psychoactive compound of tobacco, binds on cationic nicotinic receptors (nAChRs) to increase the firing rate of dopaminergic (DA) neurons of the ventral tegmental area (VTA), leading to higher release of DA in target structures and positive reinforcement. Besides rewarding properties, nicotine also promotes negative effects. Recently, we showed heterogeneity in nicotine-induced responses on VTA DA subpopulations targeting nucleus accumbens (NAc) and amygdala (AMg) nuclei. NAc-projecting DA neurons are activated by nicotine and their optogenetic activation is reinforcing. AMg-projecting DA neurons are inhibited by nicotine and their optogenetic silencing is anxiogenic. We address 1) if alcohol, known for its rewarding and anxiolytic/anxiogenic properties, also produces distinct responses on DA subpopulations, and 2) if a circuit-based mechanism could underly drug-induced inhibition. METHODS: Combining *in vivo* juxtacellular or *ex vivo* patch-clamp recordings with injections of retrograde tracers, we investigated extrinsic/intrinsic properties and response profiles to nicotine and ethanol of NAc or AMg-projecting DA neurons. RESULTS: We demonstrated that alcohol induces two opposite responses, such as nicotine, on the same segregated DA subpopulations. NAc-projecting DA neurons are activated by nicotine and ethanol while AMg-projecting DA neurons are inhibited by both drugs. Finally, we start to investigate intrinsic properties, difference in receptor expression and local/distal GABAergic signaling of both populations. CONCLUSION: These results highlight heterogeneity of drug impact on DA subpopulations and raise the question of the role it might play in the balance between the drugs positive and negative effects that regulates their use.

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MOLECULAR CANDIDATES IN THE NUCLEUS ACCUMBENS SHELL INVOLVED IN THE PROTECTIVE EFFECT OF SOCIAL INTERACTION WHEN AVAILABLE AS AN ALTERNATIVE TO COCAINE

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Aims: Social interaction, when available in a distinct context from the one associated with drug consumption, is able to eliminate preference for cocaine and prevents against cocaine relapse. The aim of this study is to investigate how these beneficial effects of social interaction against cocaine are mediated. Methods: Male Sprague Dawley rats underwent stereotaxic surgery for cannula implantation into the brain before being tested in a conditioned place preference (CPP) paradigm. Results: Rats that expressed social CPP had increased nucleus accumbens (NAc) α- calcium/calmodulin dependent protein kinase II (CaMKII) levels. Moreover, rats received an infusion of CaMKII inhibitor in the NAc shell (NAcSh) or core before the test in a concurrent CPP (social *vs* cocaine). Whereas vehicle infusions led to equal preference for both stimuli, inhibition of CaMKII in the NAcSh, but not in the core, shifted rats' preference toward the cocaine-associated context. These results suggest that social interaction reward engages NAcSh CaMKII. In rats expressing cocaine CPP, intracerebroventricular infusion of corticotropin-releasing factor (CRF) led to an increase in cocaine preference, an effect reversed in rats that received the CRF receptor antagonist, α-helical CRF. Importantly, when social interaction was available alternatively to cocaine, the CRF-induced increase in cocaine preference was completely reversed to the level of rats that received α-helical CRF. This reversal was paralleled by a decrease in NAcSh p38 MAPK expression. Conclusion: These findings suggest that social interaction positive effects against cocaine are dichotomized into rewarding effects via CaMKII and anti-stress effects via p38 MAPK, in the NAcSh.

Pubmed:

32650599: El Rawas R, Amaral IM, Hofer A
Is p38 MAPK Associated to Drugs of Abuse-Induced Abnormal Behaviors?
The family members of the mitogen-activated protein kinases (MAPK) mediate a wide variety of cellular behaviors in response to extracellular stimuli. p38 MAPKs are key signaling molecules in cellular responses to external stresses and regulation of pro-inflammatory cytokines. Some studies have suggested that p38 MAPK in the region of the nucleus accumbens is involved in abnormal behavioral responses induced by drugs of abuse. In this review, we discuss the role of the p38 MAPK in the rewarding effects of drugs of abuse. We also summarize the implication of p38 MAPK in stress, anxiety, and depression. We opine that p38 MAPK activation is more closely associated to stress-induced aversive responses rather than drug effects per se, in particular cocaine. p38 MAPK is only involved in cocaine reward, predominantly when promoted by stress. Downstream substrates of p38 that may contribute to the p38 MAPK associated-behavioral responses are proposed. Finally, we suggest p38 MAPK inhibitors as possible therapeutic interventions against stress-related disorders by potentially increasing resilience against stress and addiction relapse induced by adverse experiences.
Int J Mol Sci, 2020; 21
32624295: El Rawas R, Amaral IM, Hofer A
Social interaction reward: A resilience approach to overcome vulnerability to drugs of abuse.
Drug addiction is a multifactorial disorder resulting from the complex interaction between biological, environmental and drug-induced effects. Generally, stress is a well-known risk factor for the development of drug addiction and relapse. While most of the research focuses on risk factors that increase the vulnerability to drugs of abuse, recent studies are focusing on the areas of strength/positive coping approaches that can increase resistance to drugs of abuse. In this review, we concentrate on resilience, seen as a dynamic process, which can allow individuals to positively adapt within the context of a specific risk for psychiatric illness. Here, we discuss the effects of social stress in animal models on drug use, particularly cocaine. In contrast, we suggest social interaction reward when available as an alternative to drug use as an approach contracting negative stress effects and increasing resistance to drug use. Indeed, interventions, which aim at enhancing resilience to stress through the facilitation of social interaction and the enhancement of social support, could be particularly effective in

helping people cope with stress and preventing drug use problems or relapse. Finally, understanding the neurobiological mechanisms underlying protective factors such as social interaction reward should provide the basis for future evidence-based interventions targeting substance abuse and stress-related pathologies.
Eur Neuropsychopharmacol, 2020; 37
34440081: Amaral IM, Hofer A, El Rawas R
Is It Possible to Shift from Down to Top Rank? A Focus on the Mesolimbic Dopaminergic System and Cocaine Abuse.
Impaired social behavior is a common feature of many psychiatric disorders, in particular with substance abuse disorders. Switching the preference of the substance-dependent individual toward social interaction activities remains one of the major challenges in drug dependence therapy. However, social interactions yield to the emergence of social ranking. In this review, we provide an overview of the studies that examined how social status can influence the dopaminergic mesolimbic system and how drug-seeking behavior is affected. Generally, social dominance is associated with an increase in dopamine D receptor binding in the striatum and a reduced behavioral response to drugs of abuse. However, it is not clear whether higher D receptor availability is a result of increased D receptor density and/or reduced dopamine release in the striatum. Here, we discuss the possibility of a potential shift from down to top rank via manipulation of the mesolimbic system. Identifying the neurobiology underlying a potential rank switch to a resilient phenotype is of particular interest in order to promote a positive coping behavior toward long-term abstinence from drugs of abuse and a protection against relapse to drugs. Such a shift may contribute to a more successful therapeutic approach to cocaine addiction.
Biomedicines, 2021; 9
31984611: Lemos C, Salti A, Amaral IM, Fontebasso V, Singewald N, Dechant G, Hofer A, El Rawas R
Social interaction reward in rats has anti-stress effects.
Social interaction in an alternative context can be beneficial against drugs of abuse. Stress is known to be a risk factor that can exacerbate the effects of addictive drugs. In this study, we investigated whether the positive effects of social interaction are mediated through a decrease in stress levels. For that purpose, rats were trained to express cocaine or social interaction conditioned place preference (CPP). Behavioural, hormonal, and molecular stress markers were evaluated. We found that social CPP decreased the percentage of incorrect transitions of grooming and corticosterone to the level of naïve untreated rats. In addition, corticotropin-releasing factor (CRF) was increased in the bed nucleus of stria terminalis after cocaine CPP. In order to study the modulation of social CPP by the CRF system, rats received intracerebroventricular CRF or alpha-helical CRF, a nonselective antagonist of CRF receptors. The subsequent effects on CPP to cocaine or social interaction were observed. CRF injections increased cocaine CPP, whereas alpha-helical CRF injections decreased cocaine CPP. However, alpha-helical CRF injections potentiated social CPP. When social interaction was made available in an alternative context, CRF-induced increase of cocaine preference was reversed completely to the level of rats receiving cocaine paired with alpha-helical CRF. This reversal of cocaine preference was also paralleled by a reversal in CRF-induced increase of p38 MAPK expression in the nucleus accumbens shell. These findings suggest that social interaction could contribute as a valuable component in treatment of substance use disorders by reducing stress levels.
Addict Biol, 2021; 26
35043392: Lopes CR, Amaral IM, Pereira MF, Lopes JP, Madeira D, Canas PM, Cunha RA, Agostinho P
Impact of blunting astrocyte activity on hippocampal synaptic plasticity in a mouse model of early Alzheimer's disease based on amyloid-β peptide exposure.
Amyloid-β peptides (Aβ) accumulate in the brain since early Alzheimer's disease (AD) and dysregulate hippocampal synaptic plasticity, the neurophysiological basis of memory. Although the relationship between long-term potentiation (LTP) and memory processes is well established, there is also evidence that long-term depression (LTD) may be crucial for learning and memory. Alterations in synaptic plasticity, namely in LTP, can be due to communication failures between astrocytes and neurons; however, little is known about astrocytes' ability to control hippocampal LTD, particularly in AD-like conditions. We now aimed to test the involvement of astrocytes in changes of hippocampal LTP and LTD triggered by Aβ , taking advantage of L-α-aminoadipate (L-AA), a gliotoxin that blunts astrocytic function. The effects of Aβ exposure were tested in two different experimental paradigms: ex vivo (hippocampal slices superfusion) and in vivo (intracerebroventricular injection), which were previously validated to impair memory and hippocampal synaptic plasticity, two features of early AD. Blunting astrocytic function with L-AA reduced LTP and LTD amplitude in hippocampal slices from control mice, but the effect on LTD was less evident, suggesting that astrocytes have a greater influence on LTP than on LTD under non-pathological conditions. However, under AD conditions, blunting astrocytes did not consistently alter the reduction of LTP magnitude, but reverted the LTD-to-LTP shift caused by both ex vivo and in vivo Aβ exposure. This shows that astrocytes were responsible for the hippocampal LTD-to-LTP shift observed in early AD conditions, reinforcing the interest of strategies targeting astrocytes to restore memory and synaptic plasticity deficits present in early AD.
J Neurochem, 2022; 160
34944702: Amaral IM, Scheffauer L, Langeder AB, Hofer A, El Rawas R

Rewarding Social Interaction in Rats Increases CaMKII in the Nucleus Accumbens.
Calcium/calmodulin-dependent protein kinase II (CaMKII) is known to be involved in the sensitized locomotor responses and drug-seeking behavior to psychostimulants. However, little is known about the contribution of CaMKII signaling in the nucleus accumbens (NAc) in natural rewards such as social interaction. The present experiments explored the implication of CaMKII signaling in drug versus natural reward. In the NAc of rats expressing cocaine or social interaction conditioned place preference (CPP), αCaMKII activation was induced in those expressing social interaction but not cocaine CPP. In order to investigate the role of NAc CaMKII in the expression of reward-related learning of drug versus non-drug stimuli, we inhibited CaMKII through an infusion of KN-93, a CaMKII inhibitor, directly into the NAc shell or core, before the CPP test in a concurrent paradigm in which social interaction was made available in the compartment alternative to the one associated with cocaine during conditioning. Whereas vehicle infusions led to equal preference to both stimuli, inhibition of CaMKII by a KN-93 infusion before the CPP test in the shell but not the core of the NAc shifted the rats' preference toward the cocaine-associated compartment. Altogether, these results suggest that social interaction reward engages CaMKII in the NAc.
Biomedicines, 2021; 9
34196433: Pereira MF, Amaral IM, Lopes C, Leitão C, Madeira D, Lopes JP, Gonçalves FQ, Canas PM, Cunha RA, Agostinho P
l-α-aminoadipate causes astrocyte pathology with negative impact on mouse hippocampal synaptic plasticity and memory.
Increasing evidence shows that astrocytes, by releasing and uptaking neuroactive molecules, regulate synaptic plasticity, considered the neurophysiological basis of memory. This study investigated the impact of l-α-aminoadipate (l-AA) on astrocytes which sense and respond to stimuli at the synaptic level and modulate hippocampal long-term potentiation (LTP) and memory. l-AA selectivity toward astrocytes was proposed in the early 70's and further tested in different systems. Although it has been used for impairing the astrocytic function, its effects appear to be variable in different brain regions. To test the effects of l-AA in the hippocampus of male C57Bl/6 mice we performed two different treatments (ex vivo and in vivo) and took advantage of other compounds that were reported to affect astrocytes. l-AA superfusion did not affect the basal synaptic transmission but decreased LTP magnitude. Likewise, trifluoroacetate and dihydrokainate decreased LTP magnitude and occluded the effect of l-AA on synaptic plasticity, confirming l-AA selectivity. l-AA superfusion altered astrocyte morphology, increasing the length and complexity of their processes. In vivo, l-AA intracerebroventricular injection not only reduced the astrocytic markers but also LTP magnitude and impaired hippocampal-dependent memory in mice. Interestingly, d-serine administration recovered hippocampal LTP reduction triggered by l-AA (2 h exposure in hippocampal slices), whereas in mice injected with l-AA, the superfusion of d-serine did not fully rescue LTP magnitude. Overall, these data show that both l-AA treatments affect astrocytes differently, astrocytic activation or loss, with similar negative outcomes on hippocampal LTP, implying that opposite astrocytic adaptive alterations are equally detrimental for synaptic plasticity.
FASEB J, 2021; 35
33396297: Amaral IM, Lemos C, Cera I, Dechant G, Hofer A, El Rawas R
Involvement of cAMP-Dependent Protein Kinase in the Nucleus Accumbens in Cocaine Versus Social Interaction Reward.
Evidence suggests that PKA activity in the nucleus accumbens (NAc) plays an essential role in reward-related learning. In this study, we investigated whether PKA is differentially involved in the expression of learning produced by either natural reinforcers or psychostimulants. For that purpose, we inhibited PKA through a bilateral infusion of Rp-cAMPS, a specific PKA inhibitor, directly into the NAc. The effects of PKA inhibition in the NAc on the expression of concurrent conditioned place preference (CPP) for cocaine (drug) and social interaction (natural reward) in rats were evaluated. We found that PKA inhibition increased the expression of cocaine preference. This effect was not due to altered stress levels or decreased social reward. PKA inhibition did not affect the expression of natural reward as intra-NAc Rp-cAMPS infusion did not affect expression of social preference. When rats were trained to express cocaine or social interaction CPP and tested for eventual persisting preference 7 and 14 days after CPP expression, cocaine preference was persistent, but social preference was abolished after the first test. These results suggest that PKA in the NAc is involved in drug reward learning that might lead to addiction and that only drug, but not natural, reward is persistent.
Int J Mol Sci, 2020; 22
30013130: Aguiar AS, Speck AE, Amaral IM, Canas PM, Cunha RA
The exercise sex gap and the impact of the estrous cycle on exercise performance in mice.
Exercise physiology is different in males and females. Females are poorly studied due to the complexity of the estrous cycle and this bias has created an exercise sex gap. Here, we evaluated the impact of sexual dimorphism and of the estrous cycle on muscle strength and running power of C57BL/6 mice. Like men, male mice were stronger and more powerful than females. Exercise-induced increase of O consumption ([Formula: see text]O) and CO production ([Formula: see text]CO) were equal between sexes, indicating that running economy was higher in males. Thermoregulation was also more efficient in males. In females, proestrus increased exercise [Formula: see text]O and [Formula: see text]CO at low running speeds (30-35% female [Formula: see text]O) and estrus worsened thermoregulation. These differences translated into different absolute

and relative workloads on the treadmill, even at equal submaximal [Formula: see text]O and belt speeds. In summary, our results demonstrate the better muscle strength, running power and economy, and exercise-induced thermoregulation of males compared to females. Proestrus and estrus still undermined the running economy and exercise-induced thermoregulation of females, respectively. These results demonstrate an important exercise sex gap in mice.
Sci Rep, 2018; 8

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BOARD NUMBER: S05-156

ATTENUATION OF THE APPETITIVE RESPONSE TO A COCAINE-ASSOCIATED CONTEXT AFTER AN ESCALATING-DOSE DRUG REGIMEN IS ASSOCIATED WITH MALADAPTIVE CHANGES IN THE PREFRONTAL CORTEX

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Aims: Long-term dose-escalating cocaine administration leads to dysregulation of the reward system and initiates processes that ultimately result in weakening of the rewarding effects of cocaine. Here, we studied the influence of an escalating-dose cocaine regimen on rats' drug-associated appetitive behaviour after a withdrawal period, along with changes is HPA axis, opioid and dopaminergic systems' activity in plasma and the prefrontal cortex (PFC). **Method:** We studied the effects of the escalating-dose cocaine regimen in the Conditioned Place Preference (CPP) Test and on 50-kHz ultrasonic vocalisation after withdrawal period. We assessed corticosterone, CART 55-102, β-endorphin, and proopiomelanocortin (POMC) levels in plasma (ELISA), along with mRNA levels for D2 dopaminergic receptor, κ-receptor (KOR), CART, and epigenetic factors: miR-124 and miR-137 in the PFC (PCR). **Results:** Rats subjected to the escalating-dose cocaine regimen spent less time in the cocaine-paired compartment, and presented weaker appetitive vocalisation. These changes were accompanied by a decrease in the corticosterone and CART levels, and an increase in the β-endorphin and POMC levels in plasma, combined with an increase in the mRNA for D2R, but a decrease in the mRNA levels for KOR, and CART in the PFC. We also observed an increase in the miR-124 levels in the PFC. **Conclusions:** The frequent use of high doses of cocaine leads to weakening of its rewarding effects which is possibly related to interplay between HPA axis, dopaminergic and opioid systems in the PFC.
The study was supported by Grant No. 2018/28/C/NZ7/00240 from the National Science Centre, Kraków, in Poland.

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VOLUNTARY ALCOHOL CONSUMPTION ALTERS THE NEUROBIOLOGY UNDERLYING COCAINE-SEEKING

POSTER SESSION 05 - SECTION: REWARD & ADDICTION MECHANISMS

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Aims: While many medications reduce the reinstatement of cocaine-seeking in animals, these agents show little clinical efficacy at preventing relapse in humans. This is possibly due to the fact that an estimated 60-90% of cocaine users also use alcohol, but animal models of polysubstance use are seldom used. Relapse is modeled in animals using the intravenous self-administration (IVSA) extinction-reinstatement paradigm. Here we compare cocaine-seeking, glutamate efflux, and fos activation between rats that undergo cocaine IVSA alone or in combination with oral or IV alcohol. Methods: For sequential self-administration, rats self-administered cocaine for 2 hr/day, followed immediately by 6 hr homecage access to alcohol (20% v/v) and water or water alone. Self-administration continued for 12 days. For simultaneous self-administration, rats underwent IVSA of an alcohol+cocaine solution for 6 hr/day, 2 days/week for 5 weeks. Rats were tested for cued cocaine-seeking. Results: Cocaine self-administration did not alter the amount of alcohol consumed. Post-cocaine alcohol availability did not consistently alter cocaine intake. The robust reinstatement of cocaine-seeking in the sequential cocaine-alcohol group was not accompanied by glutamate efflux in the NAc, as it was in cocaine-only rats. Simultaneous cocaine-alcohol IVSA was robust, with no effect on motivation to seek cocaine or cued reinstatement of cocaine-seeking. Conclusions: While cocaine intake is not consistently increased by alcohol, polysubstance use alters neurobiology underlying drug-seeking, relative to that produced by a single drug. Medications targeting the glutamate transmitter system may not be effective therapies for preventing relapse in cocaine users that also consume alcohol.

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THE PROCOGNITIVE BUT NOT ANTIDEPRESSANT-LIKE EFFECT OF HBK-15 REQUIRES BDNF IN THE UNPREDICTABLE CHRONIC MILD STRESS IN MICE

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

Kinga Sałaciak[1], Klaudia Lustyk[1], Aleksandra Koszałka[1], Monika Głuch-Lutwin[2], Elżbieta Żmudzka[3], Henryk Marona[4], Karolina Pytka[1]
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Aims: Antidepressants are effective in only half of the patients, and their therapeutic effects occur after weeks of treatment. In our previous research, a multimodal compound HBK-15 showed significant antidepressant-like, anxiolytic-like, and memory-enhancing properties in rodents. Given the promising results, we aimed to evaluate the antidepressant-like and procognitive effects of a single administration of HBK-15 in a mouse depression model, the duration of these effects, and their dependence on BDNF. **Methods:** We used a mouse model of the unpredictable chronic mild stress with the sucrose preference and novel object recognition tests as behavioral endpoints to determine the antidepressant-like and procognitive activity, respectively. Next, we determined the BDNF, p-CREB, p-CaMKIV, p-PKA, and p-ERK1/2 levels in the prefrontal cortex and hippocampus using ELISA. To verify whether the pharmacological effects are BDNF-dependent, we used BDNF Val66Met mice. **Results:** We observed that a single administration of HBK-15 reversed reduced sucrose preference and novel object exploration time, and these effects lasted up to 24 h. HBK-15 upregulated the decreased BDNF p-CREB, p-CaMKIV, p-PKA, p-ERK1/2 levels in the prefrontal cortex of the stressed mice. The increase in sucrose preference after HBK-15 injection was still observed in the BDNF Val66Met mice. **Conclusion:** We found that HBK-15 reversed depression-like behaviors and memory impairments and regulated decreased BDNF and p-CREB levels in the prefrontal cortex via all studied pathways. Contrary to the procognitive effect, the antidepressant-like activity of HBK-15 did not depend on BDNF. This study was financed by the National Science Centre, Poland (grant number 2019/34/E/NZ7/00454, 2017/01/X/NZ7/00818).

Pubmed:
33905796: Sałaciak K, Pytka K
Biased agonism in drug discovery: Is there a future for biased 5-HT receptor agonists in the treatment of neuropsychiatric diseases?
Serotonin (5-HT) is one of the fundamental neurotransmitters that contribute to the information essential for an organism's normal, physiological function. Serotonin acts centrally and systemically. The 5-HT receptor is the most widespread serotonin receptor, and participates in many brain-related disorders, including anxiety, depression, and cognitive impairments. The 5-HT receptor can activate several different biochemical pathways and signals through both G protein-dependent and G protein-independent pathways. Preclinical experiments indicate that distinct signaling pathways in specific brain regions may be crucial for antidepressant-like, anxiolytic-like, and procognitive responses. Therefore, the development of new ligands that selectively target a particular signaling pathway(s) could open new possibilities for more effective and safer pharmacotherapy. This review discusses the current state of preclinical studies focusing on the concept of functional selectivity (biased agonism) regarding the 5-HT receptor and its role in antidepressant-like, anxiolytic-like, and procognitive regulation. Such work highlights not only the differential effects of targeted autoreceptors, vs. heteroreceptors, but also the importance of targeting specific downstream intracellular signaling processes, thereby enhancing favorable over unfavorable signaling activation.
Pharmacol Ther, 2021; 227
33919163: Sałaciak K, Koszałka A, Żmudzka E, Pytka K
The Calcium/Calmodulin-Dependent Kinases II and IV as Therapeutic Targets in Neurodegenerative and Neuropsychiatric Disorders.
CaMKII and CaMKIV are calcium/calmodulin-dependent kinases playing a rudimentary role in many regulatory processes in the organism. These kinases attract increasing interest due to their involvement primarily in memory and plasticity and

various cellular functions. Although CaMKII and CaMKIV are mostly recognized as the important cogs in a memory machine, little is known about their effect on mood and role in neuropsychiatric diseases etiology. Here, we aimed to review the structure and functions of CaMKII and CaMKIV, as well as how these kinases modulate the animals' behavior to promote antidepressant-like, anxiolytic-like, and procognitive effects. The review will help in the understanding of the roles of the above kinases in the selected neurodegenerative and neuropsychiatric disorders, and this knowledge can be used in future drug design.
Int J Mol Sci, 2021; 22
34736882: Sałaciak K, Pytka K
Revisiting the sigma-1 receptor as a biological target to treat affective and cognitive disorders.
Depression and cognitive disorders are diseases with complex and not-fully understood etiology. Unfortunately, the COVID-19 pandemic dramatically increased the prevalence of both conditions. Since the current treatments are inadequate in many patients, there is a constant need for discovering new compounds, which will be more effective in ameliorating depressive symptoms and treating cognitive decline. Proteins attracting much attention as potential targets for drugs treating these conditions are sigma-1 receptors. Sigma-1 receptors are multi-functional proteins localized in endoplasmic reticulum membranes, which play a crucial role in cellular signal transduction by interacting with receptors, ion channels, lipids, and kinases. Changes in their functions and expression may lead to various diseases, including depression or memory impairments. Thus, sigma-1 receptor modulation might be useful in treating these central nervous system diseases. Importantly, two sigma-1 receptor ligands entered clinical trials, showing that this compound group possesses therapeutic potential. Therefore, based on preclinical studies, this review discusses whether the sigma-1 receptor could be a promising target for drugs treating affective and cognitive disorders.
Neurosci Biobehav Rev, 2022; 132
30144453: Żmudzka E, Sałaciak K, Sapa J, Pytka K
Serotonin receptors in depression and anxiety: Insights from animal studies.
Serotonin regulates many physiological processes including sleep, appetite, and mood. Thus, serotonergic system is an important target in the treatment of psychiatric disorders, such as major depression and anxiety. This natural neurotransmitter interacts with 7 families of its receptors (5-HT1-7), which cause a variety of pharmacological effects. Using genetically modified animals and selective or preferential agonists and antagonist, numerous studies demonstrated the involvement of almost all serotonin receptor subtypes in antidepressant- or anxiolytic-like effects. In this review, based on animal studies, we discuss the possible involvement of serotonin receptor subtypes in depression and anxiety.
Life Sci, 2018; 210
34451841: Sałaciak K, Malikowska-Racia N, Lustyk K, Siwek A, Głuch-Lutwin M, Kazek G, Popiół J, Sapa J, Marona H, Żelaszczyk D, Pytka K
Synthesis and Evaluation of the Antidepressant-like Properties of HBK-10, a Novel 2-Methoxyphenylpiperazine Derivative Targeting the 5-HT and D Receptors.
The increasing number of patients reporting depressive symptoms requires the design of new antidepressants with higher efficacy and limited side effects. As our previous research showed, 2-methoxyphenylpiperazine derivatives are promising candidates to fulfill these criteria. In this study, we aimed to synthesize a novel 2-methoxyphenylpiperazine derivative, HBK-10, and investigate its in vitro and in vivo pharmacological profile. After assessing the affinity for serotonergic and dopaminergic receptors, and serotonin transporter, we determined intrinsic activity of the compound at the 5-HT and D receptors. Next, we performed behavioral experiments (forced swim test, tail suspension test) to evaluate the antidepressant-like activity of HBK-10 in naïve and corticosterone-treated mice. We also assessed the safety profile of the compound. We showed that HBK-10 bound strongly to 5-HT and D receptors and presented antagonistic properties at these receptors in the functional assays. HBK-10 displayed the antidepressant-like effect not only in naïve animals, but also in the corticosterone-induced mouse depression model, i.e., chronic administration of HBK-10 reversed corticosterone-induced changes in behavior. Moreover, the compound's sedative effect was observed at around 26-fold higher doses than the antidepressant-like ones. Our study showed that HBK-10 displayed a favorable pharmacological profile and may represent an attractive putative treatment candidate for depression.
Pharmaceuticals (Basel), 2021; 14
34832847: Lustyk K, Sałaciak K, Zaręba P, Siwek A, Sapa J, Pytka K
The Antiarrhythmic Activity of Novel Pyrrolidin-2-one Derivative S-75 in Adrenaline-Induced Arrhythmia.
Arrhythmia is a quivering or irregular heartbeat that can often lead to blood clots, stroke, heart failure, and other heart-related complications. The limited efficacy and safety of antiarrhythmic drugs require the design of new compounds. Previous research indicated that pyrrolidin-2-one derivatives possess an affinity for α-adrenergic receptors. The blockade of α-adrenoceptor may play a role in restoring normal sinus rhythm; therefore, we aimed to verify the antiarrhythmic activity of novel pyrrolidin-2-one derivative S-75. In this study, we assessed the influence on sodium, calcium, potassium channels, and

β-adrenergic receptors to investigate the mechanism of action of S-75. Lack of affinity for β-adrenoceptors and weak effects on ion channels decreased the role of these adrenoceptors and channels in the pharmacological activity of S-75. Next, we evaluated the influence of S-75 on normal ECG in rats and isolated rat hearts, and the tested derivative did not prolong the QT interval, which may confirm the lack of the proarrhythmic potential. We tested antiarrhythmic activity in adrenaline-, aconitine- and calcium chloride-induced arrhythmia models in rats. The studied compound showed prophylactic antiarrhythmic activity in the adrenaline-induced arrhythmia, but no significant activity in the model of aconitine- or calcium chloride-induced arrhythmia. In addition, S-75 was not active in the model of post-reperfusion arrhythmias of the isolated rat hearts. Conversely, the compound showed therapeutic antiarrhythmic properties in adrenaline-induced arrhythmia, reducing post-arrhythmogen heart rhythm disorders, and decreasing animal mortality. Thus, we suggest that the blockade of α-adrenoceptor might be beneficial in restoring normal heart rhythm in adrenaline-induced arrhythmia.
Pharmaceuticals (Basel), 2021; 14
33973045: Głuch-Lutwin M, Sałaciak K, Gawalska A, Jamrozik M, Sniecikowska J, Newman-Tancredi A, Kołaczkowski M, Pytka K
The selective 5-HT receptor biased agonists, F15599 and F13714, show antidepressant-like properties after a single administration in the mouse model of unpredictable chronic mild stress.
The prevalence of depression is ever-increasing throughout the population. However, available treatments are ineffective in around one-third of patients and there is a need for more effective and safer drugs.
Psychopharmacology (Berl), 2021; 238
33103555: Sałaciak K, Głuch-Lutwin M, Siwek A, Szafarz M, Kazek G, Bednarski M, Nowiński L, Mitchell E, Jastrzębska-Więsek M, Partyka A, Wesołowska A, Kołaczkowski M, Szkaradek N, Marona H, Sapa J, Pytka K
The antidepressant-like activity of chiral xanthone derivatives may be mediated by 5-HT1A receptor and β-arrestin signalling.
Our previous studies showed that xanthone derivatives with -(2-methoxyphenyl)piperazine fragment have an affinity to the 5-HT1A receptor and show antidepressant-like properties in rodents. In this study, we tested three xanthone derivatives, HBK-1 and its enantiomers, in which we increased the distance between the piperazine and xanthone fragments by using a hydroxypropoxy linker. We hypothesized that this would increase the binding to the 5-HT1A receptor and consequently, pharmacological activity.
J Psychopharmacol, 2020; 34
30410441: Pytka K, Głuch-Lutwin M, Żmudzka E, Sałaciak K, Siwek A, Niemczyk K, Walczak M, Smolik M, Olczyk A, Gałuszka A, Śmieja J, Filipek B, Sapa J, Kołaczkowski M, Pańczyk K, Waszkielewicz A, Marona H
HBK-17, a 5-HT Receptor Ligand With Anxiolytic-Like Activity, Preferentially Activates ß-Arrestin Signaling.
Numerous studies have proven that both stimulation and blockade of 5-HT and the blockade of 5-HT receptors might cause the anxiolytic-like effects. Biased agonists selectively activate specific signaling pathways. Therefore, they might offer novel treatment strategies. In this study, we investigated the anxiolytic-like activity, as well as the possible mechanism of action of 1-[(2,5-dimethylphenoxy)propyl]-4-(2-methoxyphenyl)piperazine hydrochloride (HBK-17). In our previous experiments, HBK-17 showed high affinity for 5-HT and 5-HT receptors and antidepressant-like properties. We performed the four plate test and the elevated plus maze test to determine anxiolytic-like activity. Toward a better understanding of the pharmacological properties of HBK-17 we used various functional assays to determine its intrinsic activity at 5-HT, 5-HT, 5-HT, and D receptors and UHPLC-MS/MS method to evaluate its pharmacokinetic profile. We observed the anxiolytic-like activity of HBK-17 in both behavioral tests and the effect was reversed by the pretreatment with WAY-100635, which proves that 5-HT receptor activation was essential for the anxiolytic-like effect. Moreover, the compound moderately antagonized D, weakly 5-HT and very weakly 5-HT receptors. We demonstrated that HBK-17 preferentially activated ß-arrestin signaling after binding to the 5-HT receptor. HBK-17 was rapidly absorbed after intraperitoneal administration and had a half-life of about 150 min. HBK-17 slightly penetrated the peripheral compartment and showed bioavailability of approximately 45%. The unique pharmacological profile of HBK-17 encourages further experiments to understand its mechanism of action fully.
Front Pharmacol, 2018; 9
32565240: Kotańska M, Mika K, Sałaciak K, Wheeler L, Sapa J, Kieć-Kononowicz K, Pytka K
Pitolisant protects mice chronically treated with corticosterone from some behavioral but not metabolic changes in corticosterone-induced depression model.
Histamine H receptor ligands may have antidepressant and anxiolytic effects. They can also compensate for metabolic disorders, which affect glucose or triglyceride levels. In previous studies, we have shown that pitolisant, a histamine H receptor antagonist/inverse agonist and σ1 receptor agonist, prevented the development of certain metabolic and depressive-like disorders in mice that have been treated chronically with olanzapine.
Pharmacol Biochem Behav, 2020; 196

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BOARD NUMBER: S05-159

THE EFFECT OF SEX AND AGE ON THE ANTIDEPRESSANT- AND ANXIOLYTIC-LIKE ACTIVITY OF HBK-15 IN MICE.

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

Aleksandra Koszałka[1], Kinga Sałaciak[1], Klaudia Lustyk[1], Henryk Marona[2], Karolina Pytka[1]
[1]Faculty of Pharmacy JU MC, Department Of Pharmacodynamics, Kraków, Poland, [2]Faculty of Pharmacy JU MC, Department Of Bioorganic Chemistry, Chair Of Organic Chemistry, Kraków, Poland

Aims: A rapidly burgeoning literature documents copious sex influence on brain anatomy, chemistry, and function. Many regions of the brain responsible for mood and cognitive processes, such as the hippocampus, amygdala, and neocortex, are sexually dimorphic. Therefore, sex matters much more for understanding the effects of the drugs than it has been widely assumed. Taking this into account, we first determined the affinity of HBK-15 for androgen and estrogen receptors. Then we investigated the antidepressant- and anxiolytic-like potential of HBK-15 in female juvenile mice. **Methods:** We used radioligand binding assays to determine the affinity of HBK-15 for androgen and estrogen receptors. We then utilized the forced swim and the tail suspension tests to evaluate the antidepressant-like effect of the compound in female mice. Finally, we used the elevated plus maze test to investigate a possible anxiolytic-like potential. **Results:** HBK-15 did not show any significant affinity for androgen and estrogen receptors. Nor did it decrease the immobility time in the forced swim and tail suspension tests. Similarly, HBK-15 did not increase the open arm entries or the time spent in the open arms of the elevated plus maze. **Conclusion:** We found that HBK-15 did not show affinity towards androgen and estrogen receptors. We also did not observe antidepressant- and anxiolytic-like effects in female juvenile mice. Given the promising results obtained in males, our results indicate the need for further experiments in other age groups to explain the mechanisms of these differences. This study was financed by the National Science Centre, Poland (grant 2019/34/E/NZ7/00454).

Pubmed:
33919163: Sałaciak K, Koszałka A, Żmudzka E, Pytka K
The Calcium/Calmodulin-Dependent Kinases II and IV as Therapeutic Targets in Neurodegenerative and Neuropsychiatric Disorders.
CaMKII and CaMKIV are calcium/calmodulin-dependent kinases playing a rudimentary role in many regulatory processes in the organism. These kinases attract increasing interest due to their involvement primarily in memory and plasticity and various cellular functions. Although CaMKII and CaMKIV are mostly recognized as the important cogs in a memory machine, little is known about their effect on mood and role in neuropsychiatric diseases etiology. Here, we aimed to review the structure and functions of CaMKII and CaMKIV, as well as how these kinases modulate the animals' behavior to promote antidepressant-like, anxiolytic-like, and procognitive effects. The review will help in the understanding of the roles of the above kinases in the selected neurodegenerative and neuropsychiatric disorders, and this knowledge can be used in future drug design.
Int J Mol Sci, 2021; 22

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ELEVATING ANANDAMIDE LEVELS RESTORE DEPRESSION-LIKE PHENOTYPE AND ALTERATIONS IN MICRO-RNAS IN RATS EXPOSED TO EARLY LIFE STRESS

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Introduction Early life stress (ELS) significantly increase predisposition to psychopathologies, including depression. Here, we compared the effects of treatment with the fatty acid amide hydrolase (FAAH) inhibitor, URB597 that increases anandamide levels and the selective serotonin reuptake inhibitor (SSRI), paroxetine, on depressive-like behavior and the expression of microRNAs (miRs) associated with depression and the serotonergic system in the medial prefrontal cortex (mPFC) of rats exposed to ELS. **Methods** Male and female rats were exposed to ELS using the "neglectful mother" paradigm, in which dams had limited access to nesting material, from postnatal day (P)7 to P14. During P45 to P60 (late-adolescence) URB597 (0.4 mg/kg) or paroxetine (5mg/kg), were administered i.p. for 2 weeks. On P90 (adulthood) rats were tested for depressive-like behavior and the expression of miR-16 and miR-135a. **Results** Adult male and female rats demonstrated depressive-like behavior, such as decreased social behavior and increased learned helplessness. Chronic treatment during post-adolescence with URB597, but not paroxetine, reversed these behaviors. In the mPFC, ELS males demonstrated a decrease in miR-16 and ELS females demonstrated a decrease in miR-135a. Importantly, URB597, that reversed depressive-like behavior in both sexes, also normalized mPFC miR-16 and miR-135a expression abnormalities in males and females, respectively. **Conclusions** Our findings show for the first time that enhancing anandamide signaling can prevent ELS-induced decrease in mPFC miRs and associated depression-like phenotype in both sexes. This may advance our knowledge on pathways dysfunctional in depression in cortical areas and suggest a mechanism for the beneficial effects of enhancing endocannabinoid signaling.

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NEW AUGMENTATION STRATEGY IN DEPRESSION: GALANIN (1-15) ENHANCES THE BEHAVIORAL EFFECTS OF FLUOXETINE IN THE OLFACTORY BULBECTOMY RAT.

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

Antonio Flores-Burguess[1], Juan Pedro Pineda Gómez[1], Carmelo Millón[1], Belén Gago[1], Laura García-Durán[2], Noelia Cantero-García[2], Araceli Puigcerver[3], José Ángel Narváez[2], Kjell Fuxe[4], Luis Santín[3], Zaida Díaz-Cabiale[1]
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Major depression is the largest contributor to global disability by years lived with disability. Selective serotonergic reuptake inhibitors, including fluoxetine (FLX), are the most commonly used antidepressant for the treatment of major depression. However, they are effective for remission in only 30% of patients. Recently, we observed that the N-terminal fragment of Galanin [GAL(1-15)] enhanced the antidepressant effects of FLX in naïve animals. In this work, we have analyzed in an animal model of depression, the olfactory bulbectomy (OBX) rats, the effect of GAL(1-15) on FLX-mediated responses in the forced swimming test (FST) and the sucrose preference test (SPT), tests related with despair and anhedonic behaviours. We have also studied the corticosterone levels in OBX rats after the coadministration of GAL(1-15)+FLX. Groups of rats received a subchronic pattern of FLX(10mg/Kg) alone or in combination with GAL(1-15)(1nmol) 15min before the tests. Blood samples for corticosterone assay were collected 1h after the treatments. One-way ANOVA followed by Fisher´s least significant difference test was used. Our results show that GAL(1-15) decreases the immobility time by 50% ($p<0.05$) and increases the swimming time by 30% ($p<0.01$) compared with FLX in the FST, and in the SPT reversed the effects of the OBX procedure increasing the sucrose intake ($p<0.05$) and preference ($p<0.05$). The coadministration of GAL(1-15)(1nmol)+FLX(10mg/kg) also reduced the OBX-increased corticosterone levels by approximately 50% ($p<0.05$). In conclusion, these novelty results suggest using GAL(1-15) in combination with FLX as a novel strategy for treating depression.

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ROLE OF THE ENDOCANNABINOID SYSTEM IN A GENETIC MODEL OF AUTISM BASED ON FMR1 DELETION IN RATS

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Autism spectrum disorder (ASD) has a multifactorial etiology and several studies are investigating ASD etiopathology to develop new therapeutic strategies. Fragile X syndrome (FXS) is the most frequent inherited cause of mental retardation and the leading monogenic cause of autism. In this context, various studies showed the involvement of the endocannabinoid system (ECS) in the pathogenesis of FXS and others focused their attention on the use of cannabinoid compounds in children affected by neurodevelopmental disorders. Beyond anecdotal reports, however, there is limited current evidence supporting such an intervention and the use of cannabinoids remains controversial. In the present work, we studied the role of the endocannabinoid neurotransmission in the autistic-like features displayed by the recently validated Fmr1-$^{\Delta}$exon8 rat model of autism. Wild-type (WT) and Fmr1-$^{\Delta}$exon8 male rats on a Sprague-Dawley background were used. We assessed whether a region-specific alteration in the ECS occurs in Fmr1-$^{\Delta}$exon8 rats by measuring the levels of the main endocannabinoids (AEA and 2-AG). Subsequently, we evaluated in these animals the behavioral effects of drugs targeting the ECS through the novel object recognition, three-chamber and social discrimination tests from adolescence to adulthood. Our results showed alterations in different components of the ECS in a region-specific manner in the brain of Fmr1-$^{\Delta}$exon8 rats. Pharmacological manipulation of endocannabinoid neurotransmission rescued the cognitive and social dysfunctions displayed by Fmr1-$^{\Delta}$exon8 animals. These findings demonstrate that endocannabinoids differentially modulate autistic-like traits in Fmr1-$^{\Delta}$exon8 rats in a brain region-specific manner, suggesting that changes in specific endocannabinoid mechanisms contribute to ASD-related behavioral phenotypes.

Pubmed:

33358985: Carbone E, Manduca A, Cacchione C, Vicari S, Trezza V
Healing autism spectrum disorder with cannabinoids: a neuroinflammatory story.
Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with a multifactorial etiology. Latest researches are raising the hypothesis of a link between the onset of the main behavioral symptoms of ASD and the chronic neuroinflammatory condition of the autistic brain; increasing evidence of this connection is shedding light on new possible players in the pathogenesis of ASD. The endocannabinoid system (ECS) has a key role in neurodevelopment as well as in normal inflammatory responses and it is not surprising that many preclinical and clinical studies account for alterations of the endocannabinoid signaling in ASD. These findings lay the foundation for a better understanding of the neurochemical mechanisms underlying ASD and for new therapeutic attempts aimed at exploiting the renowned anti-inflammatory properties of cannabinoids to treat pathologies encompassed in the autistic spectrum. This review discusses the current preclinical and clinical evidence supporting a key role of the ECS in the neuroinflammatory state that characterizes ASD, providing hints to identify new biomarkers in ASD and promising therapies for the future.
Neurosci Biobehav Rev, 2021; 121
32506112: Schiavi S, Melancia F, Carbone E, Buzzelli V, Manduca A, Peinado PJ, Zwergel C, Mai A, Campolongo P, Vanderschuren LJMJ, Trezza V
Detrimental effects of the 'bath salt' methylenedioxypyrovalerone on social play behavior in male rats.
Methylenedioxypyrovalerone (MDPV) is the most popular synthetic cathinone found in products marketed as 'bath salts', widely abused among teenagers and young adults. Synthetic cathinones have pharmacological effects resembling those of psychostimulants, which are known to disrupt a variety of social behaviors. However, despite the popular use of MDPV by young people in social contexts, information about its effects on social behavior is scarce. To investigate the impact of MDPV on social behavior at young age, and the underlying neurobehavioral mechanisms, we focused on social play behavior. Social play behavior is the most characteristic social behavior displayed by young mammals and it is crucial for neurobehavioral development. Treatment with MDPV reduced social play behavior in both juvenile and young adult male rats,

and its play-suppressant effect was subject to tolerance but not sensitization. As the behavioral effects of MDPV have been ascribed to dopaminergic and noradrenergic neurotransmission, and given the role of these neurotransmitters in social play, we investigated the involvement of dopamine and noradrenaline in the play-suppressant effects of MDPV. The effects of MDPV on social play were blocked by either the α2 adrenoceptor antagonist RX821002 or the dopamine receptor antagonist flupenthixol, given alone or together at sub-effective doses. In sum, MDPV selectively suppresses the most vigorous social behavior of developing rats through both noradrenergic and dopaminergic mechanisms. This study provides important preclinical evidence of the deleterious effects of MDPV on social behavior, and as such increases our understanding of the neurobehavioral effects of this popular cathinone.
Neuropsychopharmacology, 2020; 45

32912100: Schiavi S, Carbone E, Melancia F, Buzzelli V, Manduca A, Campolongo P, Pallottini V, Trezza V
Perinatal supplementation with omega-3 fatty acids corrects the aberrant social and cognitive traits observed in a genetic model of autism based on FMR1 deletion in rats.
Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder for which no treatments exist. Fragile X syndrome (FXS) is the most common form of inherited mental retardation and the most frequent monogenic cause of ASD. Given the lack of pharmacological treatments for ASD, increasing interest is devoted to non-pharmacological approaches, including dietary interventions. Omega-3 polyunsaturated fatty acids (PUFAs) are critical for neurobehavioraldevelopment. This study had two aims: 1. To validatethe recently developed rat model of FXS; 2. To assess the impact of omega-3 PUFAs dietary supplementation during pregnancy and lactation on the altered behavior displayed by rats. Female and wild-type Sprague-Dawley rats were fed with either an omega-3 PUFAs enriched diet or with an isocaloric control diet during pregnancy and lactation. Behavioral experiments were carried out on the infant (Postnatal days (PNDs) 9 and 13), juvenile (PND 35) and adult (PND 90) male offspring. pups showed hypolocomotion, reduced ultrasonic vocalizations (USVs) emission and impaired social discrimination compared to wild-type controls. Juvenile and adult rats showed deficits in the social and cognitive domains, that were counteracted by perinatal omega-3 PUFAs supplementation. Our results support the validity of the rat model to mimic key autistic-like features and support an important role of omega-3 PUFAs during of neurodevelopment. Although the mechanisms underlying the beneficial effects of omega-3 PUFAs supplementation in ASD needs to be clarified, this dietary intervention holds promise to mitigate core and comorbid autistic features.
Nutr Neurosci, 2022; 25

33569830: Manduca A, Carbone E, Schiavi S, Cacchione C, Buzzelli V, Campolongo P, Trezza V
The neurochemistry of social reward during development: What have we learned from rodent models?
Social rewards are fundamental to survival and overall health. Several studies suggest that adequate social stimuli during early life are critical for developing appropriate socioemotional and cognitive skills, whereas adverse social experiences negatively affect the proper development of brain and behavior, by increasing the susceptibility to develop neuropsychiatric conditions. Therefore, a better understanding of the neural mechanisms underlying social interactions, and their rewarding components in particular, is an important challenge of current neuroscience research. In this context, preclinical research has a crucial role: Animal models allow to investigate the neurobiological aspects of social reward in order to shed light on possible neurochemical alterations causing aberrant social reward processing in neuropsychiatric diseases, and they allow to test the validity and safety of innovative therapeutic strategies. Here, we discuss preclinical research that has investigated the rewarding properties of two forms of social interaction that occur in different phases of the lifespan of mammals, that is, mother-infant interaction and social interactions with peers, by focusing on the main neurotransmitter systems mediating their rewarding components. Together, the research performed so far helped to elucidate the mechanisms of social reward and its psychobiological components throughout development, thus increasing our understanding of the neurobiological substrates sustaining social functioning in health conditions and social dysfunction in major psychiatric disorders.
J Neurochem, 2021; 157

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EFFECTS OF ACUTE LYSERGIC ACID DIETHYLAMIDE ON INTERMITTENT ETHANOL AND SUCROSE DRINKING AND INTRACRANIAL SELF-STIMULATION IN C57BL/6 MICE

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Background Psychedelics, like lysergic acid diethylamide (LSD), are again being studied as potential therapies for many neuropsychiatric disorders, including addictions. At the same time, the acute effects of psychedelics on rewarding behaviours have been scarcely studied. *Aims* The current study aimed to clarify if LSD decreases binge-like ethanol drinking in mice, and whether the observed acute effects on ethanol consumption are generalisable to a natural reinforcer, sucrose, and if the effects resulted from aversive or reward-attenuating effects caused by LSD. *Methods* The effects of acute LSD were examined using 2-bottle choice intermittent ethanol (20%) and sucrose drinking (10%), discrete-trial current-intensity threshold method of intracranial self-stimulation, and short-term feeding behaviour assay in C57BL/6 male mice. *Results* The results showed that acute 0.1 mg/kg, but not 0.05 mg/kg, dose (i.p.) of LSD reduced 2-h intermittent ethanol drinking transiently without any prolonged effects. No effects were seen on intermittent 2-h sucrose drinking. The tested LSD doses had no effect on the intracranial self-stimulation current-intensity thresholds, nor did LSD affect the threshold-lowering, or rewarding, effects of simultaneous amphetamine treatment. Further, LSD did have small, acute diminishing effects on 2-h food and water intake. *Conclusions* Based on these results, LSD decreases binge-like ethanol drinking in mice, but only acutely. This effect is not likely to stem from reward-attenuating effects but could be in part due to reduced consummatory behaviour.

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BOARD NUMBER: S05-164

REGULATION OF THE ENDOCANNABINOID SYSTEM BY SEROTONERGIC PSYCHEDELICS: A LIPIDOMIC AND BEHAVIORAL STUDY IN RODENTS

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Serotonergic psychedelics interact with cortical serotonergic $5HT_{2A}$ Gq-coupled receptors and alter consciousness. Studies have shown that serotonergic psychedelics produce distinct transcriptional and translational changes in contrast to the non-hallucinogenic ligands of the $5HT_{2A}$ receptor. Since lipids act as important signaling molecules and are associated both in structure and downstream signaling to the $5HT_{2A}$ receptor, we were interested to study whether there are lipid associated signatures with serotonergic psychedelics and if they regulate the behavioral consequences of psychedelics in rodents. We used LC/MS to profile lipid changes in rat cortical cultures and rat and mouse brain in response to acute treatment with psychedelics. We studied associated behaviors such head-twitch response (HTR) and modified forced swim test (FST). We observe that serotonergic psychedelics like DOI, TCB-2 and 25I-NBOMe produce overlapping lipid profiles distinct from Lisuride, replicating both *in vitro* and *in vivo.* We observe psychedelics mediated upregulation of Diacylglycerol, Monoacylglycerol, Arachidonic acid, prostaglandins and 2-Arachidonoyl glycerol (2-AG) along with others lipids. Since 2-AG is an endocannabinoid agonist of the Gi-coupled CB_1 receptor, we were interested in examining whether serotonergic $5HT_{2A}$ based 2-AG production modulated CB_1 signaling and this had consequences on psychedelic associated behaviors. We observe that AM251 mediated blockade of CB_1 signaling during DOI mediated $5HT_{2A}$ signaling abrogated HTRs in rats but did not impair DOI induced reduction in despair in FST after 24 hours, suggesting synergistic interaction between $5HT_{2A}$ and CB_1 signaling on selective behaviors.

Pubmed:
31736725: Salvi SS, Pati S, Chaudhari PR, Tiwari P, Banerjee T, Vaidya VA
Acute Chemogenetic Activation of CamKIIα-Positive Forebrain Excitatory Neurons Regulates Anxiety-Like Behaviour in Mice. Anxiety disorders are amongst the most prevalent mental health disorders. Several lines of evidence have implicated cortical regions such as the medial prefrontal cortex, orbitofrontal cortex, and insular cortex along with the hippocampus in the top-down modulation of anxiety-like behaviour in animal models. Both rodent models of anxiety, as well as treatment with anxiolytic drugs, result in the concomitant activation of multiple forebrain regions. Here, we sought to examine the effects of chemogenetic activation or inhibition of forebrain principal neurons on anxiety and despair-like behaviour. We acutely activated or inhibited Ca/calmodulin-dependent protein kinase II α (CamKIIα)-positive forebrain excitatory neurons using the hM3Dq or the hM4Di Designer Receptor Exclusively Activated by Designer Drug (DREADD) respectively. Circuit activation was confirmed via an increase in expression of the immediate early gene, c-Fos, within both the hippocampus and the neocortex. We then examined the influence of DREADD-mediated activation of forebrain excitatory neurons on behavioural tests for anxiety and despair-like behaviour. Our results indicate that acute hM3Dq DREADD activation of forebrain excitatory neurons resulted in a significant decline in anxiety-like behaviour on the open field, light-dark avoidance, and the elevated plus maze test. In contrast, hM3Dq DREADD activation of forebrain excitatory neurons did not alter despair-like behaviour on either the tail suspension or forced swim tests. Acute hM4Di DREADD inhibition of CamKIIα-positive forebrain excitatory neurons did not modify either anxiety or despair-like behaviour. Taken together, our results demonstrate that chemogenetic activation of excitatory neurons in the forebrain decreases anxiety-like behaviour in mice.
Front Behav Neurosci, 2019; 13
32714597: Jaggar M, Banerjee T, Weisstaub N, Gingrich JA, Vaidya VA
5-HT receptor loss does not alter acute fluoxetine-induced anxiety and exhibit sex-dependent regulation of cortical immediate

early gene expression.
Acute treatment with the selective serotonin reuptake inhibitor (SSRI), fluoxetine (Flx), induces anxiety-like behavioral effects. The serotonin receptor (5-HT) is implicated in the modulation of anxiety-like behavior, however its contribution to the anxiogenic effects of acute Flx remains unclear. Here, we examined the role of the 5-HT receptor in the effects of acute Flx on anxiety-like behavior, serum corticosterone levels, neural activation and immediate early gene (IEG) expression in stress-responsive brain regions, using 5-HT receptor knockout (5-HT) mice of both sexes. 5-HT and wild-type (WT) male and female mice received a single administration of Flx or vehicle, and were examined for anxiety-like behavior, serum corticosterone levels, FBJ murine osteosarcoma viral oncogene homolog peptide (c-Fos) positive cell numbers in stress-responsive brain regions of the hypothalamus and prefrontal cortex (PFC), and PFC IEG expression. The increased anxiety-like behavior and enhanced corticosterone levels evoked by acute Flx were unaltered in 5-HT mice of both sexes. 5-HT female mice exhibited a diminished neural activation in the hypothalamus in response to acute Flx. Further, 5-HT male, but not female, mice displayed altered baseline expression of several IEGs (brain-derived neurotrophic factor (), , FBJ osteosarcoma gene (), FBJ murine osteosarcoma viral oncogene homolog B (), Fos-like antigen 2 (), Homer scaffolding protein () 1-3 (), Jun proto-oncogene ()) in the PFC. Our results indicate that the increased anxiety and serum corticosterone levels evoked by acute Flx are not influenced by 5-HT receptor deficiency. However, the loss of function of the 5-HT receptor alters the degree of neural activation of the paraventricular nucleus (PVN) of the hypothalamus in response to acute Flx, and baseline expression of several IEGs in the PFC in a sexually dimorphic manner.
Neuronal Signal, 2019; 3

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BOARD NUMBER: S05-165

CANNABIDIOL MODULATES ALTERATIONS IN PFC MICRORNAS IN A RAT MODEL OF DEPRESSION

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Introduction: Accumulating evidence suggests that cannabidiol (CBD) may be an effective and safe anxiolytic agent and potentially also an antidepressant. MicroRNAs (miRNAs; member of small non-coding RNA family) have been recently implicated in development of various neuropsychiatric disorders and in particular depression. Here, we aimed to examine the association between the antidepressant effects of CBD and alterations in brain microRNAs and their serotonergic targets in a rat model for depression, the unpredictable chronic mild stress (UCMS). **Methods:** Male rats were exposed to four weeks of UCMS. During the last 2 weeks they were injected with Vehicle, CBD or the 5HT1a-antagonist WAY100635. Rats were tested for immobility time in the forced swim test, a common phenotype of depression and alterations in miRNAs as well as 5HT1a- and SERT-mRNAs in the prefrontal cortex (mPFC). **Results:** Exposure to UCMS increased immobility time and upregulated the expression of mPFC miR-16 and mirR-135. CBD decreased immobility time in UCMS rats, and this therapeutic-like effect was blocked by WAY100135. Importantly, CBD prevented the UCMS-induced upregulation in miR-16 and mirR-135 and this was not associated with alterations in 5HT1a and SERT mRNA expression. **Conclusions:** CBD has antidepressive-like effects and along with the currently existent knowledge of its safety and high tolerability, it should be examined as an antidepressant in humans as well. We show for the first time that these antidepressive-like effects may be mediated by alterations in the expression of miRNAs.

Pubmed:
34405459: Sabran-Cohen T, Bright U, Mizrachi Zer-Aviv T, Akirav I
Rapamycin prevents the long-term impairing effects of adolescence Δ-9-tetrahydrocannabinol on memory and plasticity in male rats.
Long-lasting cognitive impairment is one of the most central negative consequences related to the exposure to cannabis during adolescence and particularly of Δ-9-tetrahydrocannabinol (THC). The aim of this study was to compare the protracted effects of adolescent versus late-adolescent chronic exposure to THC on short-term memory and plasticity and to examine whether rapamycin, a blocker of the mammalian target of rapamycin (mTOR) pathway, can restore THC-induced deficits in memory and plasticity. Male rats were injected with ascending doses of THC [2.5, 5, 10 mg/kg; intraperitoneally (i.p.)] during adolescence and late-adolescence (post-natal days 30-41 and 45-56, respectively), followed by daily injections of rapamycin (1 mg/kg, i.p.) during the first 10 days of cessation from THC. Thirty days after the last injection, rats were tested for short-term and working memory, anxiety-like behaviour, and plasticity in the pathways projecting from the ventral subiculum (vSub) of the hippocampus to the prefrontal cortex (PFC) and nucleus accumbens (NAc). THC exposure in adolescence, but not late-adolescence, was found to induce long-term deficits in object recognition short-term memory and synaptic plasticity in the hippocampal-accumbens pathway. Importantly, rapamycin rescued these persistent effects of THC administered during adolescence. Our findings show that some forms of memory and plasticity are sensitive to chronic THC administration during adolescence and that rapamycin administered during THC cessation may restore cognitive function and plasticity, thus potentially protecting against the possible long-term harmful effects of THC.
Eur J Neurosci, 2021; 54

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BOARD NUMBER: S05-166

EARLY ADOLESCENCE MK-801-INDUCED BEHAVIORAL AND GENE EXPRESSION ALTERATIONS ARE REVERSED BY ANANDAMIDE HYDROLYSIS INHIBITION: DIFFERENTIAL MODULATION BY CANNABINOID RECEPTOR 1 AND 2

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Aims: NMDA receptor (NMDAr) blockade is commonly used to induce schizophrenia-like behavioral abnormalities, including treatment-resistant negative symptoms and cognitive deficits. Glutamate, GABA and neuroimmune abnormalities in medial-prefrontal cortex (mPFC) are implicated in these symptom clusters. Although endocannabinoid signaling modulates mPFC glutamate and GABA transmission, the mechanisms of its contribution to negative and cognitive symptomatology are unclear. Here, we examined if endocannabinoid activation restores behavioral and molecular deficits induced by early-adolescence NMDAr blockade by cannabinoid receptor 1 (CB1r) or 2 (CB2r)-dependent mechanisms. **Method:** Early-adolescent rats were chronically administered with the NMDAr antagonist MK-801 or Saline. During late adolescence, rats received chronic treatment of either the FAAH inhibitor URB597, URB597+AM215 (CB1r antagonist), URB597+AM630 (CB2r antagonist) or Vehicle. Adult rats were tested for schizophrenia-like behavioral dysfunction, and changes in mPFC mRNA expression of glutamate, GABA, endocannabinoid and neuroinflammatory markers. **Results**: Early-adolescence MK-801 impaired novel object recognition task performance, diminished social interaction, and dysregulated the expression of glutamate, GABA, endocannabinoid and neuroinflammatory markers in the mPFC. While CB1r antagonism prevented URB597's therapeutic influence on social behavior, CB2r antagonism prevented the reversal of recognition memory deficits. This was paralleled by URB597's reversal of CB1r and CB2r expression abnormalities in the basolateral amygdala and prelimbic mPFC, respectively. **Conclusions:** The ability of endocannabinoid enhancement to reverse behavioral and gene expression alterations in the current model impacts our understanding of endocannabinoid-dependent therapeutic mechanisms in schizophrenia models, and may lead to the development of novel anandamide-based treatment venues for schizophrenia treatment resistant symptoms.

Pubmed:
35092675: Bauminger H, Zaidan H, Akirav I, Gaisler-Salomon I
Anandamide Hydrolysis Inhibition Reverses the Long-Term Behavioral and Gene Expression Alterations Induced by MK-801 in Male Rats: Differential CB1 and CB2 Receptor-Mediated Effects.
NMDA receptor blockade in rodents is commonly used to induce schizophrenia-like behavioral abnormalities, including cognitive deficits and social dysfunction. Aberrant glutamate and GABA transmission, particularly in adolescence, is implicated in these behavioral abnormalities. The endocannabinoid system modulates glutamate and GABA transmission, but the impact of endocannabinoid modulation on cognitive and social dysfunction is unclear. Here, we asked whether late-adolescence administration of the anandamide hydrolysis inhibitor URB597 can reverse behavioral deficits induced by early-adolescence administration of the NMDA receptor blocker MK-801. In parallel, we assessed the impact of MK-801 and URB597 on mRNA expression of glutamate and GABA markers. We found that URB597 prevented MK-801-induced novel object recognition deficits and social interaction abnormalities in adult rats, and reversed glutamate and GABA aberrations in the prelimbic PFC. URB597-mediated reversal of MK-801-induced social interaction deficits was mediated by the CB1 receptor, whereas the reversal of cognitive deficits was mediated by the CB2 receptor. This was paralleled by the reversal of CB1 and CB2 receptor expression abnormalities in the basolateral amygdala and prelimbic PFC, respectively. Together, our findings show that interfering with NMDA receptor function in early adolescence has a lasting impact on phenotypes resembling the negative symptoms and cognitive deficits of schizophrenia and on glutamate and GABA marker expression in the PFC. Prevention of behavioral and molecular abnormalities by late-adolescence URB597 via CB1 and CB2 receptors suggests that endocannabinoid stimulation may have therapeutic potential in addressing treatment-resistant symptoms.
Schizophr Bull, 2022;

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BOARD NUMBER: S05-167

IN VITRO AND VIVO PHARMACOLOGICAL CHARACTERIZATION OF THE CLINICALLY VIABLE NOP RECEPTOR ANTAGONIST BTRX-246040

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Aims: the aim of the present study was the in vitro and in vivo pharmacological investigation of BTRX-246040 (also known as LY2940094), a nociceptin/orphanin FQ receptor (NOP) antagonist currently in clinical development as antidepressant. **Methods**: BTRX-246040 has been tested in vitro in the following assays: calcium mobilization in cells expressing NOP and classical opioid receptors and chimeric G proteins, BRET assay measuring NOP interaction with G proteins and β-arrestins, the label free dynamic mass redistribution assay, and the electrically stimulated mouse vas deferens. In vivo, BTRX-246040 antidepressant proprieties have been tested in mice in the forced swimming test (FST) and in the learned helplessness test (LH). Additionally, the ability of BTRX-246040 to modulate adult hippocampal neurogenesis has been investigated. **Results**: in all the in vitro assays BTRX-246040 behaves as a pure, potent and selective NOP antagonist. In vivo, we demonstrated that BTRX-246040 evokes antidepressant effects in mice in the FST (active doses 10 and 30 mg/kg) and in the LH test (30 mg/kg), and, when given before the induction sessions, prevents the development of helplessness. BTRX-246040 did not modulate adult hippocampal neurogenesis, neither in naive nor in stressed mice. **Conclusions**: this study, performed with a clinically viable NOP antagonist corroborates the hypothesis that NOP antagonists can be useful not only as antidepressant drugs, similar to classical antidepressants, but are also as preemptive treatments in patients with severe risk factors for depression.

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ANTIDEPRESSANT-LIKE EFFECTS OF THE IRON CHELATOR DEFERIPRONE IN A MOUSE MODEL OF DEPRESSION

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

Thibault Renoir[1], Volkan Uzungil[1], Connor Aitken[1], Harvey Tran[1], Shanshan Li[1], Jennyfer Payet[2], Matthew Hale[2], Anthony Hannan[1]
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Aims: Since depression severity has been recently reported to correlate with brain iron levels, our study aimed to characterise the potential antidepressant properties of the iron chelator deferiprone. Since depressed individuals who carry the short allele for the serotonin-transporter-linked promotor region are more vulnerable to stress, we will use the serotonin transporter knock-out (5-HTT KO) mouse model. Methods: Using the 5-HTT KO mouse model, we first assessed the behavioural effects of acute deferiprone on the Porsolt swim test (PST) and novelty-suppressed feeding test (NSFT). To determine the relevant brain regions activated by deferiprone, we then measured c-Fos expression and applied network-based analyses to determine the functional connectome and modular characteristics following deferiprone treatment. Results: We found that deferiprone reduced immobility time in the PST in 5-HTT KO mice and reduced latency to feed in the NSFT in both genotypes, suggesting potential antidepressant-like effects. Deferiprone reversed the increase in c-Fos expression induced by swim stress in 5-HTT KO mice in the lateral amygdala. Functional network analyses suggest that hub regions of activity in mice treated with deferiprone include the caudate putamen and prefrontal cortex. The PST-induced increase in network modularity in wild-type mice was not observed in 5-HTT KO mice. Conclusions: Altogether, our data show that the antidepressant-like effects of deferiprone could be underpinned by changes in neuronal activity in the lateral amygdala.

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BOARD NUMBER: S05-169

HBK-15 PREFERENTIALLY ACTIVATES ß-ARRESTIN RECRUITMENT AND GIRK CHANNELS AFTER BINDING TO THE 5-HT1A RECEPTOR AND REVERSES LONG-TERM MEMORY IMPAIRMENTS IN MICE

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Aims: Biased agonists selectively targeting certain signaling pathways started the development of novel pharmacologically active drugs with fewer unwanted effects. Our previous studies proved that a multimodal compound HBK-15 with a high affinity for 5-HT1A receptors possesses rapid antidepressant-like properties. In order to develop the pharmacological profile of HBK-15, we investigated its influence on various signaling pathways connected with the 5-HT1A receptor and potential anti-amnestic properties in rodent models. **Methods:** First, we carried out various cell-based functional assays to determine the intrinsic activity of HBK-15 at the 5-HT1A receptor, i.e., influence on cAMP production, Ca^{2+} mobilization, phosphorylation of ERK1/2, ß-arrestin recruitment, and GIRK activation. Next, to assess the effect of the compound on long-term recognition and spatial memory, we performed the novel object recognition and Morris water maze tests in mice, respectively. We used MK-801 (NMDA receptor antagonist) to induce memory deficits. **Results:** HBK-15 preferentially activated ß-arrestin recruitment and GIRK channels after binding to the 5-HT1A receptor. The efficacy and potency of the compound varied between the signaling pathways. HBK-15 reversed memory deficits induced by MK-801 in both behavioral tests. **Conclusion:** We found that HBK-15 preferentially activated ß-arrestin recruitment and GIRK channels, and showed anti-amnestic properties in mice. Our results indicate that HBK-15 is worth further investigation. This study was financed by the National Science Centre, Poland (grant 2019/34/E/NZ7/00454).

Pubmed:
34832847: Lustyk K, Sałaciak K, Zaręba P, Siwek A, Sapa J, Pytka K
The Antiarrhythmic Activity of Novel Pyrrolidin-2-one Derivative S-75 in Adrenaline-Induced Arrhythmia.
Arrhythmia is a quivering or irregular heartbeat that can often lead to blood clots, stroke, heart failure, and other heart-related complications. The limited efficacy and safety of antiarrhythmic drugs require the design of new compounds. Previous research indicated that pyrrolidin-2-one derivatives possess an affinity for α-adrenergic receptors. The blockade of α-adrenoceptor may play a role in restoring normal sinus rhythm; therefore, we aimed to verify the antiarrhythmic activity of novel pyrrolidin-2-one derivative S-75. In this study, we assessed the influence on sodium, calcium, potassium channels, and β-adrenergic receptors to investigate the mechanism of action of S-75. Lack of affinity for β-adrenoceptors and weak effects on ion channels decreased the role of these adrenoceptors and channels in the pharmacological activity of S-75. Next, we evaluated the influence of S-75 on normal ECG in rats and isolated rat hearts, and the tested derivative did not prolong the QT interval, which may confirm the lack of the proarrhythmic potential. We tested antiarrhythmic activity in adrenaline-, aconitine- and calcium chloride-induced arrhythmia models in rats. The studied compound showed prophylactic antiarrhythmic activity in the adrenaline-induced arrhythmia, but no significant activity in the model of aconitine- or calcium chloride-induced arrhythmia. In addition, S-75 was not active in the model of post-reperfusion arrhythmias of the isolated rat hearts. Conversely, the compound showed therapeutic antiarrhythmic properties in adrenaline-induced arrhythmia, reducing post-arrhythmogen heart rhythm disorders, and decreasing animal mortality. Thus, we suggest that the blockade of α-adrenoceptor might be beneficial in restoring normal heart rhythm in adrenaline-induced arrhythmia.
Pharmaceuticals (Basel), 2021; 14
34451841: Sałaciak K, Malikowska-Racia N, Lustyk K, Siwek A, Głuch-Lutwin M, Kazek G, Popiół J, Sapa J, Marona H, Żelaszczyk D, Pytka K
Synthesis and Evaluation of the Antidepressant-like Properties of HBK-10, a Novel 2-Methoxyphenylpiperazine Derivative Targeting the 5-HT and D Receptors.
The increasing number of patients reporting depressive symptoms requires the design of new antidepressants with higher

efficacy and limited side effects. As our previous research showed, 2-methoxyphenylpiperazine derivatives are promising candidates to fulfill these criteria. In this study, we aimed to synthesize a novel 2-methoxyphenylpiperazine derivative, HBK-10, and investigate its in vitro and in vivo pharmacological profile. After assessing the affinity for serotonergic and dopaminergic receptors, and serotonin transporter, we determined intrinsic activity of the compound at the 5-HT and D receptors. Next, we performed behavioral experiments (forced swim test, tail suspension test) to evaluate the antidepressant-like activity of HBK-10 in naïve and corticosterone-treated mice. We also assessed the safety profile of the compound. We showed that HBK-10 bound strongly to 5-HT and D receptors and presented antagonistic properties at these receptors in the functional assays. HBK-10 displayed the antidepressant-like effect not only in naïve animals, but also in the corticosterone-induced mouse depression model, i.e., chronic administration of HBK-10 reversed corticosterone-induced changes in behavior. Moreover, the compound's sedative effect was observed at around 26-fold higher doses than the antidepressant-like ones. Our study showed that HBK-10 displayed a favorable pharmacological profile and may represent an attractive putative treatment candidate for depression.
Pharmaceuticals (Basel), 2021; 14
29297106: Kotańska M, Lustyk K, Bucki A, Marcinkowska M, Śniecikowska J, Kołaczkowski M
Idalopirdine, a selective 5-HT receptor antagonist, reduces food intake and body weight in a model of excessive eating.
Obesity, from early childhood onwards, is a common societal problem. The overconsumption of sweet, salty and high-fat products are the main factors that cause excessive weight gain. It is therefore necessary to search for new drugs that affect satiety centers and reduce the sense of hunger and caloric intake. It has been suggested that the blockade of 5-HT receptors may reduce food intake, and since idalopirdine is a clinically tested, selective 5HT receptor antagonist, it was chosen to be examined in animal models of obesity. The activity of idalopirdine was measured in the rat model of excessive eating. Animals were on a high caloric diet that consisted of milk chocolate with nuts, cheese, salted peanuts and condensed milk. During a four-week experiment, the rats had constant access to standard feed and water ad libitum. Idalopirdine was administered intraperitoneally at a dose 5 mg/kg b.w./day. To establish whether idalopirdine would effectively suppress the rebound hyperphagia that accompanies refeeding, it was administered after a 20 h food deprivation period. Pica behavior was evaluated after the administration of idalopirdine to confirm that the suppression of food intake was not caused by visceral illness. The effect of the four-week treatment with idalopirdine on the amount of peritoneal adipose tissue, and on lipid and carbohydrate profiles in rats was also examined. The statistical significance was calculated using the one-way ANOVA post-hoc Tukey Multiple Comparison Test or the two-way ANOVA post-hoc Bonferroni Multiple Comparison Test. Idalopirdine significantly reduced caloric intake and prevented the development of obesity in tested animals. Rats, that received idalopirdine, had a smaller amount of adipose tissue in the peritoneum as well as lower glucose, triglyceride and cholesterol levels in comparison to the control group. Moreover, an anorectic action was not caused by abnormalities of the gastrointestinal tract, such as nausea. The obtained results indicate that idalopirdine reduces caloric intake and could be considered for further tests as a potential treatment of obesity.
Metab Brain Dis, 2018; 33
28364694: Pytka K, Młyniec K, Podkowa K, Podkowa A, Jakubczyk M, Żmudzka E, Lustyk K, Sapa J, Filipek B
The role of melatonin, neurokinin, neurotrophic tyrosine kinase and glucocorticoid receptors in antidepressant-like effect.
Over the last few decades, depression has become one of the major public health problems in our society. This problem is connected not only with morbidity, but also with treatment, specifically with the effectiveness of the therapy as well as the concomitant side effects of available antidepressants. Major depressive disorder is a complex clinical entity, including different molecular mechanisms and neurological processes. This complexity is a challenge for scientists seeking to discover an innovatory antidepressant drug with multiple and complementary mechanisms of action. In this review, we discuss the role of melatonin, neurokinin, neurotrophic tyrosine kinase and glucocorticoid receptors in depression and antidepressant-like effects.
Pharmacol Rep, 2017; 69
27298499: Pytka K, Żmudzka E, Lustyk K, Rapacz A, Olczyk A, Gałuszka A, Waszkielewicz A, Marona H, Sapa J, Barbara F
The antidepressant- and anxiolytic-like activities of new xanthone derivative with piperazine moiety in behavioral tests in mice.
Xanthones are flavonoids with numerous activities, including antioxidant, antidepressant., or anxiolytic-like. Therefore, the aim of our study was to determine antidepressant- and anxiolytic-like properties of four xanthone derivatives (3-chloro-5-[(4-methylpiperazin-1-yl)methyl]-9H-xanthen-9-one dihydrochloride [HBK-5], 6-methoxy-2-[(4-methylpiperazin-1-yl) methyl]-9H-xanthen-9-one dihydrochloride, 2-[(4-benzylpiperazin-1-yl) methyl]-6-methoxy-9H-xanthen-9-one dihydrochloride, 2-{[4-(2-methoxyphenyl) piperazin-1-yl] methyl}-9H-xanthen-9-one hydrochloride), as well as the influence on cognitive and motor function of active compounds, using animal models.
Indian J Pharmacol, 2016 May-Jun; 48
26523954: Zaręba P, Dudek M, Lustyk K, Siwek A, Starowicz G, Bednarski M, Nowiński L, Zygmunt M, Sapa J, Malawska B,

Kulig K
Antiarrhythmic and α-Adrenoceptor Antagonistic Properties of Novel Arylpiperazine Derivatives of Pyrrolidin-2-one.
In an effort to develop α-adrenoceptor antagonists with antiarrhythmic activity, we designed a series of pyrrolidin-2-one derivatives. The α1- and α2-adrenorecepor affinities of the new pyrrolidin-2-one derivatives were determined using a radioligand binding assay. The most active compound was then tested in vitro for intrinsic activity toward α(1A)- and α(1B)-adrenoceptors and in vitro for antiarrhythmic activity in epinephrine-induced arrhythmia in rats. The highest affinity for the α1-adrenoceptor (pK(i) = 7.01) was displayed by 1-{4-[4-(2-methoxy-5-chlorophenyl)-piperazin-1-yl]-methyl}-pyrrolidin-2-one (9). 1-[4-(2-Fluorophenyl)-piperazin-1-yl]-methyl-pyrrolidin-2-one (7) showed the highest affinity toward the α2-adrenoceptor (pK(i) = 6.52). Intrinsic activity studies of compound 9 showed that this compound is an antagonist of both α(1A)- (EC50 = 0.5 nM) and α(1B)- (EC50 = 51.0 nM) adrenoceptors. Compound 9 displayed antiarrhythmic activity in rats (ED50 = 5.0 mg/kg (3.13-7.99)). New derivatives of pyrrolidin-2-one with α1 -adrenoceptor affinity were identified. We propose that the antiarrhythmic activity of compound 9 is related to its antagonism of α(1A)- and α(1B)-adrenoceptors.
Arch Pharm (Weinheim), 2015; 348
25813897: Zaręba P, Dudek M, Lustyk K, Siwek A, Starowicz G, Bednarski M, Nowiński L, Raźny K, Sapa J, Malawska B, Kulig K
α-Adrenoceptor antagonistic and hypotensive properties of novel arylpiperazine derivatives of pyrrolidin-2-one.
This study focused on a series of pyrrolidin-2-one derivatives connected via two or four methylene units to arylpiperazine fragment. The compounds obtained for α_1- and α_2-adrenoceptors were assessed. The compound with highest affinity for the α_1-adrenoceptors was 1-{4-[4-(2-chloro-phenyl)-piperazin-1-yl]-butyl}-pyrrolidin-2-one (10 h) with pKi=7.30. Compound with pKi (α_1) $\geqslant$6.44 were evaluated in functional bioassays for intrinsic activity at α_1A- and α_1B-adrenoceptors. All compounds tested were antagonists of the α_1B-adrenoceptors. Additionally, compounds 10e and 10h were α_1A-adrenoceptors antagonist. The dual α_1A-/α_1B-adrenoceptors antagonists, compounds 10e and 10h were also tested in vivo for their hypotensive activity in rats. These compounds, when dosed of 1.0 mg/kg iv in normotensive, anesthetized rats, significantly decreased systolic and diastolic pressure and their hypotensive effects lasted for longer than one hour.
Bioorg Med Chem, 2015; 23

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BOARD NUMBER: S05-170

METABOLISM AS AN ORIGIN OF SEXUAL DIMORPHISM IN MORPHINE-INDUCED ANALGESIA BUT NOT IN THE SETTING OF ANALGESIC TOLERANCE IN MICE

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Aims- In humans and rodents, sex influences morphine analgesia. In the liver and brain, morphine is metabolized into proalgic morphine-3-glucuronide (M3G). We have hypothesized that sexual dimorphism in morphine metabolism and differential metabolic modulation during the setting of analgesic tolerance might contribute to behavioural differences. Thus, we have studied if differences in peripheral and central morphine metabolism exist after acute and chronic morphine treatments in male and female mice. **Methods-** Sexual dimorphism in morphine analgesia and tolerance were studied using the tail-immersion test. Morphine and M3G metabolic kinetics in the blood after acute and chronic morphine treatments were determined using LC-MS/MS. Morphine and M3G were also quantified in several central nervous system (CNS) regions. **Results –** Our results indicate that female mice display weaker morphine analgesia and faster tolerance setting compared to males. In addition, female mice have higher concentrations of proalgic M3G in the blood and several pain-related CNS regions (PAG, amygdala…) than male mice. At the opposite, lower concentrations of morphine were found in these regions. These major differences reflect a major imbalance in the pronociceptive/antinociceptive balance within the CNS. **Conclusion-** Sex differences in morphine analgesic effects were mainly attributable to morphine central metabolism in pain-related CNS regions, consistent with weaker morphine analgesic effects in females. However, the implication of morphine metabolism in analgesic tolerance appeared to be limited.

FENS Forum 2022
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BOARD NUMBER: S05-171

EFFICACY OF A SELECTIVE 5-HT6R ANTAGONIST IN AN INNOVATIVE 2-HIT MOUSE MODEL OF SCHIZOPHRENIA

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Schizophrenic patients are mainly treated with antipsychotic drugs, which efficiently improve positive symptoms (delirium, hallucination…). However, negative symptoms (avolition, anhedonia, social withdrawal…) and cognitive deficits (memory impairment…) remain resistant. The modulation of 5-HT6 receptors appears as a new promising therapeutic strategy since it was shown to improve memory deficits in several preclinical investigations. Our project aims to characterize the efficacy of pharmacological modulation of 5-HT6R in a new animal model of schizophrenia constructed on the combination between two factors (2-hit) including a genetic factor (deletion of serine racemase that synthesizes the NMDA receptor co-agonist D-serine) and an environmental factor (maternal separation for 24h at post-natal day 9). To characterize the new mouse model and assess the efficacy of 5-HT6R modulation, behavioural experiments and electrophysiological recordings in the CA1 region of hippocampus were performed. The 2-hit combination increases spontaneous activity and anxiety-like behaviour, and impairs memory performances (recognition, spatial and working memory). A disruption of synaptic plasticity (long-term potentiation), a mechanism involved in memory processes that requires a strong activation of NMDA receptors, was found in the 2-hit model. Interestingly, the 5-HT6R antagonist SB-271046 facilitates NMDA activation in both control and 2-hit groups. We are now investigating whether this antagonist could restore the expression of synaptic plasticity and counterbalance the memory deficits in the 2-hit model. Thus, our work underlines the interest of targeting 5-HT6R for the treatment of schizophrenia-related negative and cognitive symptoms.

Pubmed:
34239718: Lahogue C, Pinault D
Frontoparietal anodal tDCS reduces ketamine-induced oscillopathies.
During the prodromal phase of schizophrenia with its complex and insidious clinical picture, electroencephalographic recordings detect widespread oscillation disturbances (or oscillopathies) during the wake-sleep cycle. Neural oscillations are electrobiomarkers of the connectivity state within systems. A single-systemic administration of ketamine, a non-competitive NMDA glutamate receptor antagonist, transiently reproduces the oscillopathies with a clinical picture reminiscent of the psychosis prodrome. This acute pharmacological model may help the research and development of innovative treatments against psychotic transition. Transcranial electrical stimulation is recognized as an appropriate non-invasive therapeutic modality since it can increase cognitive performance and modulate neural oscillations with little or no side effects. Therefore, our objective was to set up, in the sedated adult rat, a stimulation method that is able to normalize ketamine-induced increase in gamma-frequency (30-80 Hz) oscillations and decrease in sigma-frequency (10-17 Hz) oscillations. Unilateral and bipolar frontoparietal (FP), transcranial anodal stimulation by direct current (<+1 mA) was applied in ketamine-treated rats. A concomitant bilateral electroencephalographic recording of the parietal cortex measured the stimulation effects on its spontaneously occurring oscillations. A 5 min FP anodal tDCS immediately and quickly reduced, significantly with an intensity-effect relationship, the ketamine-induced gamma hyperactivity, and sigma hypoactivity at least in the bilateral parietal cortex. A duration effect was also recorded. The tDCS also tended to diminish the ketamine-induced delta hypoactivity. These preliminary neurophysiological findings are promising for developing a therapeutic proof-of-concept against neuropsychiatric disorders.
Transl Neurosci, 2021; 12

FENS Forum 2022
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BOARD NUMBER: S05-172

EFFECTS OF L-DOPA, SKF-38393, AND QUINPIROLE ON EXPLORATORY, ANXIETY- AND DEPRESSIVE-LIKE BEHAVIORS IN PUBERTAL FEMALE AND MALE MICE

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Puberty is a period of broad morphological, functional, and chemical reorganization of the brain with significant changes in the emergence of behavior, cognition, and emotions. For example, the dopaminergic system, which is involved in several neuropsychiatric disorders, is overactive during adolescence. However, very little is known regarding the role of dopaminergic receptors in prepubertal behavior. In this study, we investigated the behavioral effects of a single i.p. administration of L-Dopa, SKF-38393 (D1 receptor agonist), and Quinpirole (D2 receptor agonist) in 4 weeks old female and male C57BL/6 mice. We observed that both L-Dopa and Quinpirole decreased exploratory/motor behavior (total distance and number of rearings) and risk assessment/anxiety-like behavior (number of rearings, head dippings, and stretchings) only in females in the open field test (OFT) and elevated plus-maze (EPM) respectively. Males injected with Quinpirole also showed a decrease in risk assessment/anxiety-like behavior (number of rearings, head dippings, and stretchings) in EPM but only L-Dopa injected males showed an increase in behavioral despair/depressive-like behavior in forced swimming test (FST). Our results showed that distinct dopaminergic receptors modulate prepubertal mice behavior in a sex-specific manner, which provides insights into the dynamic of dopaminergic system development in puberty.

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BOARD NUMBER: S05-173

THE BUTYRIC ACID PRECURSOR TRIBUTYRIN MODULATES HIPPOCAMPAL SYNAPTIC PLASTICITY AND PREVENTS SPATIAL MEMORY DEFICITS: ROLE OF PPARγ AND AMPK

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Short chain fatty acids (SCFA), derived from the intestinal fermentation of dietary fiber and contained in dairy products, are gaining interest in relation to their possible beneficial effects on neuropsychological disorders. Specifically, butyric acid (BA), a SCFA, has been shown to reverse certain memory impairments. In order to investigate the impact of this fatty acid on spatial memory process, we have investigated the effect of tributyrin (TB), a prodrug of BA, on i) hippocampus-dependent spatial memory, ii) hippocampal synaptic transmission and plasticity mechanisms, and iii) the expression of genes and proteins relevant to hipocampal glutamatergic transmission. Adolescent male C57BL/6J mice were fed diets with different doses of TB (0.5%, 1% and 3%) acutely (48 h) and chronically (20 weeks). We found that free-feeding intake during 48 h of a diet containing 1% TB prevented, in adolescent but not in adult mice, scopolamine (SCOP)-induced impairment of hippocampus-dependent spatial memory. Moreover, TB treatment up-regulated, within the adolescent hippocampus, gene expression of *Pparg*, leptin and adiponectin receptors, and glutamate receptor subunits (AMPA-2, NMDA-1, NMDA-2A and NMDA-2B). On the other hand, in vitro studies carried out in hippocampal slices, revealed that TB was able to transform early-LTP (e-LTP) into late-LTP (l-LTP) and rescued LTP-inhibition induced by SCOP. TB-induced facilitation of l-LTP was blocked by GW9662 (PPARγ antagonist) and Compound C (AMPK inhibitor). In conclusion, our study suggests that TB has a positive influence on the processes of hippocampal transmission and synaptic plasticity suggesting the involvement of both PPARγ and AMPK on TB-mediated effects.

Pubmed:
35125071: Fernández-Felipe J, Valencia-Avezuela M, Merino B, Somoza B, Cano V, Sanz-Martos AB, Frago LM, Fernández-Alfonso MS, Ruiz-Gayo M, Chowen JA
Effects of saturated versus unsaturated fatty acids on metabolism, gliosis, and hypothalamic leptin sensitivity in male mice.
Development of obesity and its comorbidities is not only the result of excess energy intake, but also of dietary composition. Understanding how hypothalamic metabolic circuits interpret nutritional signals is fundamental to advance towards effective dietary interventions.
Nutr Neurosci, 2022;
34624673: Fernández-Felipe J, Merino B, Sanz-Martos AB, Plaza A, Contreras A, Naranjo V, Morales L, Chowen JA, Cano V, Ruiz-Gayo M, Del Olmo N
Saturated and unsaturated fat diets impair hippocampal glutamatergic transmission in adolescent mice.
Consumption of high-fat diets (HFD) has been associated with neuronal plasticity deficits and cognitive disorders linked to the alteration of glutamatergic disorders in the hippocampus. As young individuals are especially vulnerable to the effects of nutrients and xenobiotics on cognition, we studied the effect of chronic consumption of saturated (SOLF) and unsaturated oil-enriched foods (UOLF) on: i) spatial memory; ii) hippocampal synaptic transmission and plasticity; and iii) gene expression of glutamatergic receptors and hormone receptors in the hippocampus of adolescent and adult mice. Our results show that both SOLF and UOLF impair spatial short-term memory. Accordingly, hippocampal synaptic plasticity mechanisms underlying memory, and gene expression of NMDA receptor subunits are modulated by both diets. On the other hand, PPARγ gene expression is specifically down-regulated in adolescent SOLF individuals and up-regulated in adult UOLF mice.
Psychoneuroendocrinology, 2021; 133
34709416: Piniella D, Martínez-Blanco E, Bartolomé-Martín D, Sanz-Martos AB, Zafra F
Identification by proximity labeling of novel lipidic and proteinaceous potential partners of the dopamine transporter.
Dopamine (DA) transporters (DATs) are regulated by trafficking and modulatory processes that probably rely on stable and transient interactions with neighboring proteins and lipids. Using proximity-dependent biotin identification (BioID), we found

novel potential partners for DAT, including several membrane proteins, such as the transmembrane chaperone 4F2hc, the proteolipid M6a and a potential membrane receptor for progesterone (PGRMC2). We also detected two cytoplasmic proteins: a component of the Cullin1-dependent ubiquitination machinery termed F-box/LRR-repeat protein 2 (FBXL2), and the enzyme inositol 5-phosphatase 2 (SHIP2). Immunoprecipitation (IP) and immunofluorescence studies confirmed either a physical association or a close spatial proximity between these proteins and DAT. M6a, SHIP2 and the Cullin1 system were shown to increase DAT activity in coexpression experiments, suggesting a functional role for their association. Deeper analysis revealed that M6a, which is enriched in neuronal protrusions (filopodia or dendritic spines), colocalized with DAT in these structures. In addition, the product of SHIP2 enzymatic activity (phosphatidylinositol 3,4-bisphosphate [PI(3,4)P]) was tightly associated with DAT, as shown by co-IP and by colocalization of mCherry-DAT with a specific biosensor for this phospholipid. PI(3,4)P strongly stimulated transport activity in electrophysiological recordings, and conversely, inhibition of SHIP2 reduced DA uptake in several experimental systems including striatal synaptosomes and the dopaminergic cell line SH-SY5Y. In summary, here we report several potential new partners for DAT and a novel regulatory lipid, which may represent new pharmacological targets for DAT, a pivotal protein in dopaminergic function of the brain.
Cell Mol Life Sci, 2021; 78

FENS Forum 2022
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BOARD NUMBER: S05-174

GALANIN(1-15) ENHANCED THE ANTIDEPRESSANT-LIKE EFFECTS OF ESCITALOPRAM IN THE OLFACTORY BULBECTOMY RATS IN THE FORCED SWIMMING TEST THROUGH 5-HT1A RECEPTORS.

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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We previously described that Galanin (1-15) [GAL(1-15)] enhances the antidepressant-like effects induced by the SSRI Fluoxetine in the forced swimming test (FST) in naïve rats. In this work, we have analyzed in olfactory bulbectomy rats (OBX) the effect of GAL(1-15)-Escitalopram (ESC) combination in the FST and the involvement of GALR and 5-HT1A receptors in these effects. In the first set of experiments, OBX rats received three injections of ESC (10mg/Kg) (23, 5 and 1 hour) and a single injection of a threshold dose of GAL(1-15) (1nmol) and GALR2 antagonist M871 (3nmol) alone or in combination 15 minutes before the FST. Secondly, we have generated siRNA 5HT1A knockdown rats, and we have evaluated the effects of ESC and GAL(1-15) administration in the FST. One-way ANOVA followed by Fisher´s least significant difference test was used. In the FST, GAL(1-15) (1nmol) enhanced the antidepressant-like effects of ESC, reducing immobility ($p<0.05$) and increasing the swimming time ($p<0.05$). M871 blocked the behavioural effects of GAL(1-15) in the immobility time ($p<0.001$) and in the swimming time ($p<0.05$) in the FST. Moreover, the decrease in 5-HT1AR was sufficient to block GAL(1-15) enhancement of the antidepressant-like effects mediated by ESC. Our results indicate a potent effect of the combination GAL(1-15) with SSRIs in reversed depressive symptoms in the animal model of chronic depression OBX. The results open up the possibility of using GAL(1-15) in combination with SSRIs as a novel strategy for treating depression. Supported by PID2020-114392RB-I00, UMA18-FEDERJA-008 and P20_00026, PI-0083-2019.

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BOARD NUMBER: S05-175

GALANIN (1-15) AND ESCITALOPRAM COMBINATION IN RATS REDUCES ALCOHOL CONSUMPTION IN THE ETHANOL SELF-ADMINISTRATION TEST AND IMPROVES ESCITALOPRAM EFFECTS IN THE FORCED SWIMMING TEST.

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Recently, we described that Galanin(1-15)[GAL(1-15)] enhanced Escitalopram(ESC) effectiveness in depression symptoms. Moreover GAL(1-15) induces a substantial reduction in alcohol consumption. To investigate the effect of GAL(1-15) on ESC-activity in depression-alcoholism comorbidity, we used the ethanol self-administration test and the forced swimming test(FST) in rats, after a chronic alcohol consumption. Also to study if GAL(1-15)+ESC modulate the reward system induced by different reinforcers we have analyzed this combination in the saccharine self-administration test. Groups of rats received three times intraperitoneal injections of ESC (2.5mg/Kg or 7.5mg/Kg) 23, 5 and 1h before the test and one icv injection of GAL(1-15) (0.3nmol or 1nmol) 15 minutes before the test. One-way ANOVA followed by Fisher´s least significant difference test was used. In the saccharin self-administration, the coadministration GAL(1-15)(1nmol) and ESC (2.5mg/Kg) induced a strong reduction in the number of reinforcements of saccharine ($p<0.05$) and in number of active lever presses ($p<0.05$). In the ethanol Self-Administration, GAL(1-15)(0.3nmol) enhanced the reduction of alcohol intake mediated by ESC (2.5mg/Kg). GAL(1-15) decreased the number of alcohol reinforcements ($p<0.01$) and the number of active levers pressed ($p<0.01$) by around 50% induced by ESC. In FST, in rats under a chronic alcohol consumption, GAL(1-15) reversed adverse ESC-mediated effects. Coadministration of GAL(1-15)(1nmol) and ESC (7.5mg/Kg) showed a significant decrease in immobility ($p<0.05$) and an increase in swimming ($p<0.05$) compared with ESC group. The results open up the possibility to use GAL(1-15) in combination with Escitalopram as a novel strategy in AUD comorbidity with depression. Supported by PID2020-114392RB-100, PDC2021-121566-100, P20-00026-R, PI-0083-2019.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S05-176

DELETION OF BETA2 NICOTINIC SUBUNIT IN SPECIFIC TYPES OF CORTICAL GABAERGIC NEURONS CHANGES SOCIAL AND ANXIETY-LIKE BEHAVIOR

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Nicotinic acetylcholine receptors (nAChRs) containing beta2 nicotinic subunit are widely expressed throughout the brain and they have been implicated in the control of various behavioral processes. The widespread expression makes them an important therapeutic target; however, it also poses a requirement for a more precise targeting of nAChRs in specific locations. Until today, tools available for a targeted inhibition or activation of nAChRs remain limited. In the present study, we used a CRISPR/Cas9-based approach to induce a deletion of beta2 nicotinic subunit in the mouse prefrontal cortex in neuropeptide Y- (NPY) and serotonin receptor 5HT3A-expressing neurons. We crossed a mouse line with Cre-inducible expression of Cas9 with respective Cre-drivers and injected the offspring with an AAV vector carrying gRNA targeting CHRNB2 gene. First, we used FISH to characterize beta2 expression in NPY- and 5HT3A-expressing neurons. To analyze the efficiency of the CRISPR/Cas9 indels, we used the fluorescence activated cell sorting (FACS) followed by DNA isolation and sequencing. Then we used a comprehensive behavioral testing to identify domains affected by the beta2 deletion in the targeted neuronal populations. Interestingly, the deletion in either NPY- or 5HT3A-expressing neurons led to an increase in social interactions. In contrast, beta2 deletion in NPY- but not the 5HT3A-expressing neurons decreased anxiety-like behavior during the elevated plus maze. In conclusion, selective deletion of nAChRs in distinct populations of cortical neurons can be achieved by CRISPR/Cas9-based approach and it has different effects on behavior based on the neurons targeted.

FENS Forum 2022
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BOARD NUMBER: S05-177

COMPARING THE EFFICACY OF SELECTIVE NEGATIVE ALLOSTERIC MODULATORS OF α5-CONTAINING GABAA RECEPTORS ON SYNAPTIC INHIBITION AND COGNITIVE DEFICITS IN A MOUSE MODEL OF DOWN SYNDROME

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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We aim to compare α5IA and Basmisanil efficacy on α5-$GABA_AR$-mediated synaptic inhibition, as well as on rescuing cognitive deficits in a Down syndrome (DS) mouse model. They are negative allosteric modulators of α5-$GABA_ARs$ and have been proposed as cognitive-enhancing drugs. Inhibitory postsynaptic currents (IPSCs) evoked on pyramidal neurons (PNs) in L2/L3 by extracellular stimulation on L1 in the somatosensory cortex were recorded by whole-cell patch clamp. In the hippocampus, we recorded IPSCs evoked on CA1 PNs by optogenetic activation of SST axons in the stratum lacunosum moleculare. We also quantified α5IA and Basmisanil concentrations in blood samples from mice following single intraperitoneal administrations of increasing doses of these drugs by LC-MS. Brain samples collected from treated mice were used for ex vivo receptor occupancy measurements. We found a decrease in IPSCs amplitudes recorded from PNs in L2/L3; however, there was a significant variability in drug effect using this approach. Light-evoked IPSCs recorded in hippocampal CA1 PNs were found to be preferentially mediated by α5-$GABA_ARs$, as well as sensitive to α5IA, which corroborates with previous findings. Dose-dependent increase in plasma concentrations was observed for both, although the occupancy at α5-containing $GABA_AR$ was higher for α5IA (~80-100%) than for Basmisanil (~15-30%). Our data suggest that α5IA and Basmisanil have different receptor-binding properties. Next steps will be the study of the effects of these drugs on behavioural phenotypes of a DS mouse model. Optogenetically evoked IPSCs in CA1 PNs seems to be a feasible approach to compare drug efficacy on α5-$GABA_AR$-mediated synaptic inhibition.

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BOARD NUMBER: S05-178

THE INFLUENCE OF G PROTEIN-BIASED AGONISTS OF THE μ-OPIOID RECEPTOR ON ADDICTION-LIKE SYMPTOMS AND BEHAVIOURAL EFFECTS OF MORPHINE IN MICE

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Aims: G protein-biased agonists of the μ-opioid receptor are promising opioid therapeutics, proposed to have diminished side effects and addictive potential. Using animal models, we aimed to assess the addiction-like effects of compounds presenting high G protein bias – PZM21, SR-14968 and SR-17018. We further assessed the impact of these compounds on morphine-induced addictive-like behaviour. **Methods:** Using C57BL/6J mice we determined the effects of these compounds on locomotion, tolerance, physical dependence and reward. Those effects were also tested in mice cotreated with novel agonists and morphine. **Results:** The tested compounds differently affected addictive-like behaviour. PZM21 and SR-17018 did not change animals' locomotion, whereas the treatment with SR-14968 resulted in enhancement of locomotor activity, tolerance of this effect during repeated treatment, and high expression after the incubation period. While PZM21 caused rapid development of antinociceptive tolerance, this effect was delayed in SR-compounds-treated mice. In terms of physical dependence, PZM21 induced low effect, but SR-compounds were highly physically addictive. Both SR-compounds also had rewarding properties, albeit PZM21 was nonrewarding. Finally, the compounds turned out to influence symptoms of morphine addiction, i.e. all the compounds attenuated some symptoms of morphine withdrawal and PZM21 diminished morphine reward. **Conclusions:** Our results suggest that different classes of G protein-biased μ-opioid receptor agonists vary in terms of euphorigenic effects and severity of physical dependence. However, these agonists diminish some symptoms of morphine addiction, thus may be useful in opioid use disorder pharmacotherapy. **Acknowledgments:** The studies were funded by the Polish National Science Centre grants: 2018/31/B/NZ7/03954 and 2019/33/N/NZ7/02378.

Pubmed:
35056950: Kudla L, Bugno R, Podlewska S, Szumiec L, Wiktorowska L, Bojarski AJ, Przewlocki R
Comparison of an Addictive Potential of μ-Opioid Receptor Agonists with G Protein Bias: Behavioral and Molecular Modeling Studies.
Among different approaches to the search for novel-safer and less addictive-opioid analgesics, biased agonism has received the most attention in recent years. Some μ-opioid receptor agonists with G protein bias, including SR compounds, were proposed to induce diminished side effects. However, in many aspects, behavioral effects of those compounds, as well as the mechanisms underlying differences in their action, remain unexplored. Here, we aimed to evaluate the effects of SR-14968 and SR-17018, highly G protein-biased opioid agonists, on antinociception, motor activity and addiction-like behaviors in C57BL/6J mice. The obtained results showed that the compounds induce strong and dose-dependent antinociception. SR-14968 causes high, and SR-17018 much lower, locomotor activity. Both agonists develop reward-associated behavior and physical dependence. The compounds also cause antinociceptive tolerance, however, developing more slowly when compared to morphine. Interestingly, SR compounds, in particular SR-17018, slow down the development of antinociceptive tolerance to morphine and inhibit some symptoms of morphine withdrawal. Therefore, our results indicate that SR agonists possess rewarding and addictive properties, but can positively modulate some symptoms of morphine dependence. Next, we have compared behavioral effects of SR-compounds and PZM21 and searched for a relationship to the substantial differences in molecular interactions that these compounds form with the μ-opioid receptor.
Pharmaceutics, 2021; 14
33835467: Kudla L, Przewlocki R
Influence of G protein-biased agonists of μ-opioid receptor on addiction-related behaviors.
Opioid analgesics remain a gold standard for the treatment of moderate to severe pain. However, their clinical utility is seriously limited by a range of adverse effects. Among them, their high-addictive potential appears as very important, especially in the context of the opioid epidemic. Therefore, the development of safer opioid analgesics with low abuse

potential appears as a challenging problem for opioid research. Among the last few decades, different approaches to the discovery of novel opioid drugs have been assessed. One of the most promising is the development of G protein-biased opioid agonists, which can activate only selected intracellular signaling pathways. To date, discoveries of several biased agonists acting via μ-opioid receptor were reported. According to the experimental data, such ligands may be devoid of at least some of the opioid side effects, such as respiratory depression or constipation. Nevertheless, most data regarding the addictive properties of biased μ-opioid receptor agonists are inconsistent. A global problem connected with opioid abuse also requires the search for effective pharmacotherapy for opioid addiction, which is another potential application of biased compounds. This review discusses the state-of-the-art on addictive properties of G protein-biased μ-opioid receptor agonists as well as we analyze whether these compounds can diminish any symptoms of opioid addiction. Finally, we provide a critical view on recent data connected with biased signaling and its implications to in vivo manifestations of addiction.
Pharmacol Rep, 2021; 73
33821329: Tertil M, Skupio U, Kudla L, Wiktorowska L, Przewlocki R
Astroglial Knockout of Glucocorticoid Receptor Attenuates Morphine Withdrawal Symptoms, but Not Antinociception and Tolerance in Mice.
The development of tolerance and drug dependence limit the clinical application of opioids for the treatment of severe pain. Glucocorticoid receptors (GRs) are among molecular substrates involved in these processes. Most studies focus on the role of neuronal GR, while the involvement of GR on glial cells is not fully understood. To address this issue, we used a transgenic model of conditional GR knockout mice, targeted to connexin 30-expressing astrocytes, treated with repeated doses of morphine. We observed no difference between control mice and astrocytic GR knockouts in the development of antinociceptive tolerance. Nevertheless, when animals were subjected to precipitated withdrawal, knockouts presented some attenuated symptoms, including jumping. Taken together, our data suggest that hippocampal and spinal astrocytic GRs appear to be involved in opioid withdrawal, and drugs targeting the GR may relieve some symptoms of morphine withdrawal without influencing its antinociceptive properties.
Cell Mol Neurobiol, 2021;
33359366: Wiktorowska L, Bilecki W, Tertil M, Kudla L, Szumiec L, Mackowiak M, Przewlocki R
Knockdown of the astrocytic glucocorticoid receptor in the central nucleus of the amygdala diminishes conditioned fear expression and anxiety.
The amygdala is a key structure involved in both physiological and behavioural effects of fearful and stressful stimuli. The central stress response is controlled by the activity of the hypothalamic-pituitary-adrenal (HPA) axis via glucocorticoid hormones, acting mainly through glucocorticoid receptors (GR), widely expressed among different brain regions, including the central nucleus of the amygdala (CeA). Although to date, neuronal GR was postulated to be involved in the mediating stress effects, increasing evidence points to the vital role of glial GR. Here, we aimed to evaluate the role of astrocytic GR in CeA in various aspects of the stress response. We used a lentiviral vector to disrupt an astrocytic GR in the CeA of Aldh1l1-Cre transgenic mice. Astrocytic GR knockdown mice (GR KD) exhibited an attenuated expression of fear-related memory in the fear conditioning paradigm. Interestingly, the consolidation of non-stressful memory in the novel object recognition test remained unchanged. Moreover, GR KD group presented reduced anxiety, measured in the open field test. However, knockdown of astrocytic GR in the CeA did not affect an acute response to stress in the tail suspension test. Taken together, obtained results suggest that astrocytic GR in the CeA promotes aversive memory consolidation and some aspects of anxiety behaviour.
Behav Brain Res, 2021; 402
33053718: Podlewska S, Bugno R, Kudla L, Bojarski AJ, Przewlocki R
Molecular Modeling of μ Opioid Receptor Ligands with Various Functional Properties: PZM21, SR-17018, Morphine, and Fentanyl-Simulated Interaction Patterns Confronted with Experimental Data.
Molecular modeling approaches are an indispensable part of the drug design process. They not only support the process of searching for new ligands of a given receptor, but they also play an important role in explaining particular activity pathways of a compound. In this study, a comprehensive molecular modeling protocol was developed to explain the observed activity profiles of selected μ opioid receptor agents: two G protein-biased μ opioid receptor agonists(PZM21 and SR-17018), unbiased morphine, and the β-arrestin-2-biased agonist,fentanyl. The study involved docking and molecular dynamics simulations carried out for three crystal structures of the target at a microsecond scale, followed by the statistical analysis of ligand-protein contacts. The interaction frequency between the modeled compounds and the subsequent residues of a protein during the simulation was also correlated with the output of in vitro and in vivo tests, resulting in the set of amino acids with the highest Pearson correlation coefficient values. Such indicated positions may serve as a guide for designing new G protein-biased ligands of the μ opioid receptor.
Molecules, 2020; 25
31347704: Kudla L, Bugno R, Skupio U, Wiktorowska L, Solecki W, Wojtas A, Golembiowska K, Zádor F, Benyhe S, Buda S,

Makuch W, Przewlocka B, Bojarski AJ, Przewlocki R
Functional characterization of a novel opioid, PZM21, and its effects on the behavioural responses to morphine.
The concept of opioid ligands biased towards the G protein pathway with minimal recruitment of β-arrestin-2 is a promising approach for the development of novel, efficient, and potentially nonaddictive opioid therapeutics. A recently discovered biased μ-opioid receptor agonist, PZM21, showed analgesic effects with reduced side effects. Here, we aimed to further investigate the behavioural and biochemical properties of PZM21.
Br J Pharmacol, 2019; 176
31254970: Skupio U, Tertil M, Bilecki W, Barut J, Korostynski M, Golda S, Kudla L, Wiktorowska L, Sowa JE, Siwiec M, Bobula B, Pels K, Tokarski K, Hess G, Ruszczycki B, Wilczynski G, Przewlocki R
Astrocytes determine conditioned response to morphine via glucocorticoid receptor-dependent regulation of lactate release.
To date, neurons have been the primary focus of research on the role of glucocorticoids in the regulation of brain function and pathological behaviors, such as addiction. Astrocytes, which are also glucocorticoid-responsive, have been recently implicated in the development of drug abuse, albeit through as yet undefined mechanisms. Here, using a spectrum of tools (whole-transcriptome profiling, viral-mediated RNA interference in vitro and in vivo, behavioral pharmacology and electrophysiology), we demonstrate that astrocytes in the nucleus accumbens (NAc) are an important locus of glucocorticoid receptor (GR)-dependent transcriptional changes that regulate rewarding effects of morphine. Specifically, we show that targeted knockdown of the GR in the NAc astrocytes enhanced conditioned responses to morphine, with a concomitant inhibition of morphine-induced neuronal excitability and plasticity. Interestingly, GR knockdown did not influence sensitivity to cocaine. Further analyses revealed GR-dependent regulation of astroglial metabolism. Notably, GR knockdown inhibited induced by glucocorticoids lactate release in astrocytes. Finally, lactate administration outbalanced conditioned responses to morphine in astroglial GR knockdown mice. These findings demonstrate a role of GR-dependent regulation of astrocytic metabolism in the NAc and a key role of GR-expressing astrocytes in opioid reward processing.
Neuropsychopharmacology, 2020; 45
30487639: Tertil M, Skupio U, Barut J, Dubovyk V, Wawrzczak-Bargiela A, Soltys Z, Golda S, Kudla L, Wiktorowska L, Szklarczyk K, Korostynski M, Przewlocki R, Slezak M
Glucocorticoid receptor signaling in astrocytes is required for aversive memory formation.
Stress elicits the release of glucocorticoids (GCs) that regulate energy metabolism and play a role in emotional memory. Astrocytes express glucocorticoid receptors (GR), but their contribution to cognitive effects of GC's action in the brain is unknown. To address this question, we studied how astrocyte-specific elimination of GR affects animal behavior known to be regulated by stress. Mice with astrocyte-specific ablation of GR presented impaired aversive memory expression in two different paradigms of Pavlovian learning: contextual fear conditioning and conditioned place aversion. These mice also displayed compromised regulation of genes encoding key elements of the glucose metabolism pathway upon GR stimulation. In particular, we identified that the glial, but not the neuronal isoform of a crucial stress-response molecule, Sgk1, undergoes GR-dependent regulation in vivo and demonstrated the involvement of SGK1 in regulation of glucose uptake in astrocytes. Together, our results reveal astrocytes as a central element in GC-dependent formation of aversive memory and suggest their relevance for stress-induced alteration of brain glucose metabolism. Consequently, astrocytes should be considered as a cellular target of therapies of stress-induced brain diseases.
Transl Psychiatry, 2018; 8

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BOARD NUMBER: S05-179

INFLAMMATORY SIGNALLING IN AMPHETAMINE-INDUCED MOUSE MANIA MODEL, THE ROLE OF P2X7 RECEPTOR

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Background: Purinergic dysfunctions play a role in the pathological process of mania and depression, but the essential relationship is not fully understood. Activation of the purinergic P2X7 receptor (P2X7R) plays a central role in inflammation, microglia activation, and IL-1β release, and activates NLRP3 inflammasome. We investigated whether d-amphetamine (AMPH) has an inflammatory profile andwhether P2X7R has an effect through IL-1β in this model. **Methods**: We analysed the modulatory effects of the P2X7R antagonist JNJ 47965567 on behaviour, tissue cytokines, monoamines, brain-derived neurotrophic factor (BDNF), and plasma purine levels in a subchronic d-amphetamine-induced mania model in wild-type and IL-1αβ-double knockout (IL-1αβKO) mice. **Results**: 7-day AMPH treatment caused a significant increase in locomotion of wild-type and IL-1αβKO mice, which was abolished by JNJ 47965567 in both genotype. AMPH mostly did not affect cytokine levels in the prefrontal cortex (PFC), striatum (STR), and hippocampus (HP), and it also increased serotonin content in IL-1αβKO mice only in STR and HP. JNJ 47965567 significantly reduced AMPH-induced dopamine release from STR, while it was not affected by genotype. Changes in purine levels were not affected by AMPH, only by JNJ 47965567. **Conclusion**: Although JNJ 47965567 had an effect on cytokine and purine levels as well as monoamines content, the potencial inflammatory effects of AMPH were contradictory in this model.

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COMPARING THE ANTIDEPRESSANT-LIKE EFFECTS AND NEUROCHEMICAL CORRELATES OF DESIPRAMINE IN MALE AND FEMALE ADULT RATS

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Major depression is one of the most common mental disorders in females, however preclinical studies investigating changes in antidepressant-like efficacy that include this variable are scarce. In this context, the present study compared the antidepressant-like potential and neurochemical correlates of desipramine, a typical tricyclic antidepressant, in female vs. male adult rats. Adult Sprague-Dawley rats (52 females and 89 males) were exposed to the forced-swim test (FST) for 15 min (pre-test) and then treated (i.p.) with 3 pulses of desipramine (5, 10 or 20 mg/kg) or vehicle (0.9% NaCl, 1 ml/kg) administered 23, 5 and 1 h prior to the FST (5-min test). Probable persistent effects were re-evaluated 1 and 3 days post-treatment in the FST. Hippocampal samples were collected 1 h, and 1- and 5-days post-treatment to evaluate cell proliferation (Ki-67+cells) and key neuroplasticity markers (FADD, Cdk-5, p35-p25). Desipramine exerted antidepressant-like effects both in male and female rats by decreasing immobility time in the FST 1 h and 1-day post-treatment. The main molecular results showed that desipramine increased hippocampal cell proliferation 1 h post-treatment both in male and female rats, but regulated some neuroplasticity markers (i.e., decreased FADD, p35/p25 and Cdk-5 protein content) exclusively in male rats. The results demonstrate that although desipramine is capable of inducing a similar antidepressant-like response in male and female rats, there are some sex differences in the mechanisms behind its actions that deserve further studies. PID2020-118582RB-I00 (MCIN/AEI/10.13039/501100011033) and PDR2020-14 (CAIB) to MJG-F; “JUNIOR” program to SL-C (IdISBa).

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BOARD NUMBER: S05-181

EVALUATION OF NEUROTRANSMITTERS (SEROTONIN AND NORADRENALINE) MODULATION IN MICE BRAIN VIA QUERCETIN MEDIATED MONOAMINE OXIDASE INHIBITION IN DEPRESSED MICE

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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The potential of antioxidants defenses against depression were evaluated by this study. The standardized methanolic extract of the vine of C. reflexa was assessed for antidepressant action using various behavioural paradigms such as tail suspension test (TST), forced swim test (FST) and locomotor activity test along with biochemical markers. HPLC fingerprints were obtained. The key constituent of C. reflexa is quercetin which belongs to flavonoids class that are good antioxidants. As per this potential, this natural moiety showed positive activity towards reducing stress mediated depression and showed to have better antidepressant potential. The serotonergic and noradrenergic changes were evaluated using 5-hydroxytryptophan (5-HTP) induced head twitches and yohimbine potentiation tests, respectively. The fluoxetine and phenelzine were used as positive control in the study. The test moeity significantly declined the immobility time in TST (IC50 ~ 50mg/kg) and FST while no significant increase in locomotor count was observed. The extract also significantly increased the 5-HTP induced head twitches and yohimbine induced lethality. The aforesaid results were similar to that caused by standard phenelzine. Furthermore the cortisol and BDNF levels were also determined in the prefrontal cortex as well and the level of certain monoamines and their metabolites was evaluated by using high performance liquid chromatography test. In the mice hippocampus, the C. reflexa extract caused significant elevation of serotonin & noradrenaline compared with the control. Histopathological data also supported the findings. The C. reflexa extract demonstrated antidepressant activity can be attributed to rise in serotonin and noradrenaline levels in the brain via MAO inhibition.

Pubmed:

32450180: Kazmi Z, Zeeshan S, Khan A, Malik S, Shehzad A, Seo EK, Khan S
Anti-epileptic activity of daidzin in PTZ-induced mice model by targeting oxidative stress and BDNF/VEGF signaling.
Epilepsy is a complex and multifactorial neurodegenerative disease described by recurrent seizures. Oxidative stress and dysregulation of brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) are critical factors for the development of epilepsy. Daidzin is well-known for its effective anti-inflammatory and antioxidant potential for centuries. The present study was focused on exploring the anti-epileptic potential of daidzin in the pentylenetetrazole-induced mice model. Daidzin (1, 5, and 10 mg/kg) was administered in the acute study and the dose was optimized. Pretreatment with daidzin remarkably reduced the severity of epileptogenesis in a dose-dependent manner. Moreover, chronic epilepsy was induced in mice by administration of PTZ (35 mg/kg, i.p) every alternative day for 21 days. Results demonstrated that daidzin significantly prevented epileptogenesis and reversed histopathological changes in the hippocampus. It remarkably improved antioxidant (glutathione, glutathione sulfotransferase, superoxide dismutase, and catalase) levels while decreased MDA (malondialdehyde) and nitrite production in the brain. It remarkably improved the expressions of heme oxygenase-1 (HO-1) and BDNF while reduced the expression of VEGF. It remarkably prevented the neuronal apoptosis in the brain tissue. Additionally, spectroscopic analysis such as FTIR (Fourier transform infrared spectroscopy) and DSC (differential scanning calorimetry) revealed that daidzin remarkably prevented PTZ-induced protein damage. HPLC-UV spectrophotometry results demonstrated that there was no peak of aglycone daidzin (metabolite) in the brain sample which specify that the anticonvulsant effect of the compound is due to its direct entry into the brain tissue. Moreover, the molecular docking results showed that daidzin possesses a better binding affinity for ALDH2, estrogen receptor-β, P13k, AKT2, mTORC1, and HIF-1-α proteins. Taken together, the results of the present study showed that daidzin has remarkable neuroprotective and anti-epileptic properties through modulation of oxidative stress, BDNF/VEGF, and apoptotic signaling in the brain tissue of PTZ-kindled mice.
Neurotoxicology, 2020; 79
31079165: Zeeshan S, Naveed M, Khan A, Atiq A, Arif M, Ahmed MN, Kim YS, Khan S
N-Pyrazoloyl and N-thiopheneacetyl hydrazone of isatin exhibited potent anti-inflammatory and anti-nociceptive properties

through suppression of NF-κB, MAPK and oxidative stress signaling in animal models of inflammation.
Hydrazide derivatives constitute an important class of compounds for new drug development as they are reported to possess good anti-inflammatory and analgesic activity. The present study was aimed to investigate the role of newly synthesized hydrazide derivatives N-pyrazoloyl hydrazone of isatin (PHI) and N-thiopheneacetyl hydrazone of isatin (THI) in acute and chronic inflammatory pain models induced by carrageenan and complete Freud's adjuvant (CFA).
Inflamm Res, 2019; 68
30529194: Atiq A, Shal B, Naveed M, Khan A, Ali J, Zeeshan S, Al-Sharari SD, Kim YS, Khan S
Diadzein ameliorates 5-fluorouracil-induced intestinal mucositis by suppressing oxidative stress and inflammatory mediators in rodents.
5-Fluorouracil (5-FU) is one of the most commonly prescribed anti-cancer agent. However, its use is associated with several debilitating adverse effects such as intestinal mucositis (IM) and myelosuppression. Oxidative stress and inflammation are major contributors in the development of mucositis. Diadzein is known for its potent anti-inflammatory and anti-oxidative activities from decades. The present study focused on investigating the effects of diadzein on intestinal mucositis induced by 5-FU by mainly focusing on oxidative stress and inflammatory markers in mice. Mucositis was induced in mice by administration of 5-FU (50 mg/kg, i.p.), once daily for three days and diadzein (1, 5, 10 mg/kg) was administered once daily for seven days. Diadzein pretreatment was found to reduce the severity of mucosal injury in a dose-dependent manner. Diadzein significantly reversed weight loss, relieved diarrhea, and improved histopathological deformities associated with inflammation. Moreover, diadzein remarkably improved the intestinal wall histopathology by reducing inflammatory mediators infiltration and prevented suppression of antioxidants (glutathione, glutathione sulfo-transferase, and catalase) by 5-FU administration. Furthermore, nitrite production in intestinal tissue was reduced by diadzein consistent with the observed modulation of inflammatory markers. Additionally, diadzein also improved the amended microflora profile, by reducing the number of pathogenic bacteria and increasing the abundance of probiotics. Taken together, the behavioral, biochemical and histological outcomes of the present study demonstrates that diadzein has significant anti-mucositis properties in 5-FU induced mucositis model, and the attenuative potential of diadzein might be due to inhibition of oxidative stress and inflammatory mediators.
Eur J Pharmacol, 2019; 843
26134381: Zada W, Zeeshan S, Bhatti HA, Mahmood W, Rauf K, Abbas G
Cinnamomum cassia: an implication of serotonin reuptake inhibition in animal models of depression.
The aim of the study was to explore the traditional use of Cinnamomum cassia against depression. The standardised methanolic extract of the bark of C. cassia was evaluated for antidepressant activity using various behavioural tests, i.e. tail suspension test (TST), forced swim test (FST) and locomotor activity test. The serotonergic and noradrenergic modulation was assessed using 5-hydroxytryptophan (5-HTP)-induced head twitches and yohimbine potentiation tests, respectively. The fluoxetine and phenelzine were used as positive controls in the study. The C. cassia extract significantly decreased the immobility time in TST (maximum effective dose tested was 50 mg/kg) while no effect was observed in FST and locomotor activity test. The extract significantly increased the 5-HTP-induced head twitches while yohimbine-induced lethality remained unaltered. The aforementioned results are similar to that caused by fluoxetine. The standardised methanolic extract of C. cassia demonstrated antidepressant activity that can be attributed to rise in serotonin levels.
Nat Prod Res, 2016; 30
30968231: Naveed M, Khan SZ, Zeeshan S, Khan A, Shal B, Atiq A, Ali H, Ullah R, Zia-Ur-Rehman , Khan S
A new cationic palladium(II) dithiocarbamate exhibits anti-inflammatory, analgesic, and antipyretic activities through inhibition of inflammatory mediators in in vivo models.
Inflammation is being a protective mechanism of the body towards the injury. However, chronic and progressive inflammation may lead to some chronic diseases. Due to the serious unwanted effects associated with available drugs, new and safe anti-inflammatory agents are still required. Therefore, the present study was designed to investigate the anti-inflammatory, analgesics, and antipyretic properties of a new compound (4-benzylpiperidine-1-carbodithioato-κS,S')(1,4-bis-(diphenylphosphino)butane)palladium(II)chloride monohydrate (compound-1) in albino mice models. Compound-1 was characterized by elemental analysis, FT-IR, and multinuclear NMR spectroscopy. Initially, compound-1 was evaluated for cytotoxicity, anti-inflammatory, and analgesic activities by performing MTT assay, carrageenan-, histamine-, serotonin-, and CFA-induced paw edema, mechanical hyperalgesia, thermal hyperalgesia, and mechanical allodynia (0.1, 1, and 10 mg/kg, b.w). Antipyretic activity was evaluated in brewer's yeast-induced model. The pro-inflammatory cytokines were measured by using commercially available ELISA kits. Additionally, nitrite production, antioxidant enzymes, H&E staining, muscle activity and motor coordination, and kidney and liver function tests were also determined. The results demonstrated that compound-1 significantly inhibited inflammation, pain, and febrile responses in all models at a dose of 10 mg/kg without effecting viability of cells in vitro at concentrations up to 100 μM. Similarly, the data clearly demonstrated significant reduction in the pro-inflammatory cytokines and nitrite production while enhancing antioxidant enzymes. Furthermore, pretreatment with

compound-1 did not produce any prominent side effect on kidney, liver, stomach, and muscles. These findings suggest that compound-1 has potent anti-inflammatory-, pain-, and pyrexia-relieving properties. Hence, compound-1 might be a potential candidate for the therapeutic management of chronic inflammation and pain.
Naunyn Schmiedebergs Arch Pharmacol, 2019; 392

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BOARD NUMBER: S05-182

NEUROBEHAVIORAL DEVELOPMENT OF HETEROZYGOUS DAT (DAT-HET) RATS

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Introduction
Among neuropsychiatric disorders in children, attention deficit hyperactivity disorder (ADHD) is one of the most common, difficult to diagnose and treat early. The etiology of this disorder has not yet been established. Rats with a genetically determined dopamine metabolism disorder can probably be used to study the impact of the genetic factor in ADHD pathogenesis. The aim of this work was to study the effect of partial knockout of dopamine transporter (DAT) gene on the neurobehavioral development of rat pups. Methods
Rats were bred following a HET–HET breeding scheme. A total of 18 animals were tested, 4 DAT WT and 12 DAT HET. We analyzed their behavior in 5 tests: surface righting, negative geotaxis, cliff aversion, pendular reflex, grip strength. All experiments were conducted every day starting from 3-rd postnatal day (PND) and performed until the reflex appeared. Genotyping was performed by PCR followed by enzymatic digestion with BtsI MutI NEB.
Results & Discussion
In the surface righting test DAT-HET rats were able to flip onto their feet at 5-th PND on average, which is a day later than DAT-WT (two-tailed Fisher's exact test, p=0.0379). This may be the result of postural imbalances due to deficits in their core. In negative geotaxis, pendular reflex and cliff aversion tests there were no differences between groups. Reflexes appeared in 7th, 8th and 9th PND, respectively.
There were no statistically significant differences between groups in the grip strength test. Research support
The reported study was funded by RSF, project number 22-25-00124.

Pubmed:
33729106: Muruzheva ZM, Traktirov DS, Zubov AS, Pestereva NS, Tikhomirova MS, Karpenko MN
Calpain activity in plasma of patients with essential tremor and Parkinson's disease: a pilot study.
: Essential tremor (ET) and Parkinson's disease (PD) are the two most common movement disorders in adults with similar clinical symptoms, which is hinting towards existence of coincident pathogenesis steps.: The objective of this report is to characterize the relationship between ET and PD severity and the activity of calcium-dependent proteases calpain in plasma.: The study enrolled 12 volunteers for each condition: ET, PD, healthy. We evaluated the stage of PD on the H&Y scale in patients with PD, and the severity of tremor in patients with ET on the FTMS scale. IL-1β, TNFα, IL6, IL10 were determined in plasma using ELISA. Calpain activity was measured using fluorescent substrate and zymography methods.: We demonstrated that the activity of calpains in plasma of patients with PD and ET increased 5.1 and 4.3 times, respectively. The increase of calpain activity in plasma of PD patients correlated with the content of IL-1β, for ET such a connection was not found. At the advanced stages of PD calpain activity in plasma was significantly higher than that of the PD group at the early stage, and this increase was mediated by the increase in m-calpain activity. The increase in the tremor severity in ET did not lead to an increase in the activity of calpains in plasma.: We observed general increase in the activity of calpains in plasma of both PD and ET patients that hints towards presence of the common steps in the pathogenesis of these diseases.
Neurol Res, 2021; 43
33345671: Muruzheva ZM, Ivleva IS, Traktirov DS, Zubov AS, Karpenko MN
The relationship between serum interleukin-1β, interleukin-6, interleukin-8, interleukin-10, tumor necrosis factor-α levels and clinical features in essential tremor.
In recent years, there has been discussion that essential tremor (ET) might be a neurodegenerative disease. Indicators of inflammation are considered as possible biomarkers of neurodegeneration. In this connection, the aim of our study was to identify the relationship between serum inflammation markers and clinical features in ET, including the severity of tremor, cognitive decline, depression.
Int J Neurosci, 2021;


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BOARD NUMBER: S05-183

BEHAVIOURAL MARKERS OF RESILIENCE AND SUSCEPTIBILITY IN A NEURODEVELOPMENTAL TWO-HIT MODEL OF SCHIZOPHRENIA

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Aims: Maternal immune activation (MIA) and postnatal stress are risk factors for neurodevelopmental disorders (NDDs) such as schizophrenia. The current research aimed to characterize the phenotypes resulting from both risk factors in isolation and together. Furthermore, the research aims to identify any putative markers of resilience to either insult. **Method:** A two-hit model for schizophrenia is presented combining MIA via poly(I:C) administration at gestational day 15 and limited bedding and nesting (LBN) from postnatal days 1-10. **Results:** We showed that MIA resulted in cognitive deficits in adolescence and adulthood, with LBN resulting in increased anxiety and cognitive deficits in adolescence. Cognition was used to stratify offspring irrespective of treatment. In adolescence, this resulted in three clusters of typical and deficient cognition as well as one cluster of intermediary performance. This intermediary cluster exhibited recognition memory but at a significantly lower level than typically-performing animals. In adulthood, the procedure resulted in a typical and a deficited group. In adolescence, there was a relationship between MIA and cluster membership in the absence of stress. When offspring were stressed, the relationship was attenuated. In adulthood, MIA offspring were more likely than vehicle offspring to be clustered into the low cognition group. **Conclusions:** Both models result in phenotypes of relevance to NDDs. Unbiased clustering presents the opportunity to identify responders and non-responders for use in subsequent analysis.

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MATERNAL IMMUNE ACTIVATION WITH POLY(I:C) MAY PRODUCE VARIABLE OUTCOMES: COMPARISON OF RESULTS FROM TWO INDEPENDENT EXPERIMENTS AND DIFFERENT CAGING SYSTEMS

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Maternal immune activation has been identified as a significant risk factor for onset of schizophrenia in offspring. We applied 5 mg/kg of poly(I:C) on gestation day 9 to pregnant mouse dams, whose offspring were then stressed during puberty. The experiment was performed twice with two consequential runs of animals, who were housed in different caging systems. The first run was housed in individually ventilated cages (IVC) and we showed impairments in attentional set-shifting in a T-maze and a decreased number of parvalbumin-positive interneurons in the hippocampus as a result of peripubertal stress specifically in females. The second run was housed in open cages, and we found a between-group differences in set-shifting, prepulse inhibition and sensitivity to amphetamine in the open field. However, surprisingly, the poly(I:C) group had less impaired prepulse inhibition and lower sensitivity to amphetamine. There was no between-group difference in these behavioral tasks in the first run of mice housed in IVC. It is therefore possible that caging system has impact on behavioral profile of mice prenatally treated with poly(I:C). This work was supported by Czech Science Foundation (GACR) grants 20-00939S and 21-16667K.

Pubmed:

34838932: Maleninska K, Janikova M, Radostova D, Vojtechova I, Petrasek T, Kirdajova D, Anderova M, Svoboda J, Stuchlik A
Selective deficits in attentional set-shifting in mice induced by maternal immune activation with poly(I:C).
Maternal immune activation has been identified as a significant risk factor for schizophrenia. Using rodent models, past work has demonstrated various behavioral and brain impairments in offspring after immune-activating events. We applied 5 mg/kg of poly(I:C) on gestation day 9 to pregnant mouse dams, whose offspring were then stressed during puberty. We show impairments in attentional set-shifting in a T-maze, and a decreased number of parvalbumin-positive interneurons in the hippocampus as a result of peripubertal stress specifically in females.
Behav Brain Res, 2022; 419
34356631: Janikova M, Mainerova K, Vojtechova I, Petrasek T, Svoboda J, Stuchlik A
Memantine and Riluzole Exacerbate, Rather Than Ameliorate Behavioral Deficits Induced by 8-OH-DPAT Sensitization in a Spatial Task.
Chronic sensitization to serotonin 1A and 7 receptors agonist 8-OH-DPAT induces compulsive checking and perseverative behavior. As such, it has been used to model obsessive-compulsive disorder (OCD)-like behavior in mice and rats. In this study, we tested spatial learning in the 8-OH-DPAT model of OCD and the effect of co-administration of memantine and riluzole-glutamate-modulating agents that have been shown to be effective in several clinical trials. Rats were tested in the active place avoidance task in the Carousel maze, where they learned to avoid the visually imperceptible shock sector. All rats were subcutaneously injected with 8-OH-DPAT (0.25 mg/kg) or saline (control group) during habituation. During acquisition, they were pretreated with riluzole (1 mg/kg), memantine (1 mg/kg), or saline solution 30 min before each session and injected with 8-OH-DPAT ("OH" groups) or saline ("saline" groups) right before the experiment. We found that repeated application of 8-OH-DPAT during both habituation and acquisition significantly increased locomotion, but it impaired the ability to avoid the shock sector. However, the application of 8-OH-DPAT in habituation had no impact on the learning process if discontinued in acquisition. Similarly, memantine and riluzole did not affect the measured parameters in the "saline" groups, but in the "OH" groups, they significantly increased locomotion. In addition, riluzole increased the number of entrances and decreased the maximum time avoided of the shock sector. We conclude that monotherapy with glutamate-modulating agents does not reduce but exacerbates cognitive symptoms in the animal model of OCD.
Biomolecules, 2021; 11
33440912: Brozka H, Alexova D, Radostova D, Janikova M, Krajcovic B, Kubík Š, Svoboda J, Stuchlik A

Plasticity-Related Activity in the Hippocampus, Anterior Cingulate, Orbitofrontal, and Prefrontal Cortex Following a Repeated Treatment with D/D Agonist Quinpirole.
Quinpirole (QNP) sensitization is a well-established model of stereotypical checking relevant to obsessive-compulsive disorder. Previously, we found that QNP-treated rats display deficits in hippocampus-dependent tasks. The present study explores the expression of immediate early genes (IEG) during QNP-induced stereotypical checking in the hippocampus, anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and medial prefrontal cortex (mPFC). Adult male rats were injected with QNP (0.5 mg/mL/kg; = 15) or saline (= 14) daily for 10 days and exposed to an arena enriched with two objects. Visits to the objects and the corners of the arena were recorded. QNP-treated rats developed an idiosyncratic pattern of visits that persisted across experimental days. On day 11, rats were exposed to the arena twice for 5 min and sacrificed. The expression of IEGs and was determined using cellular compartment analysis of temporal activity by fluorescence in situ hybridization. IEG-positive nuclei were counted in the CA1 area of the hippocampus, ACC, OFC, and mPFC. We found significantly fewer IEG-positive nuclei in the CA1 in QNP-treated rats compared to controls. The overlap between IEG expressing neurons was comparable between the groups. We did not observe significant differences in IEG expression between QNP treated and control rats in ACC, OFC, and mPFC. In conclusion, treatment of rats with quinpirole decreases plasticity-related activity in the hippocampus during stereotypical checking.
Biomolecules, 2021; 11
31919978: Ziak J, Weissova R, Jeřábková K, Janikova M, Maimon R, Petrasek T, Pukajova B, Kleisnerova M, Wang M, Brill MS, Kasparek P, Zhou X, Alvarez-Bolado G, Sedlacek R, Misgeld T, Stuchlik A, Perlson E, Balastik M
CRMP2 mediates Sema3F-dependent axon pruning and dendritic spine remodeling.
Regulation of axon guidance and pruning of inappropriate synapses by class 3 semaphorins are key to the development of neural circuits. Collapsin response mediator protein 2 (CRMP2) has been shown to regulate axon guidance by mediating semaphorin 3A (Sema3A) signaling; however, nothing is known about its role in synapse pruning. Here, using newly generated crmp2 mice we demonstrate that CRMP2 has a moderate effect on Sema3A-dependent axon guidance in vivo, and its deficiency leads to a mild defect in axon guidance in peripheral nerves and the corpus callosum. Surprisingly, crmp2 mice display prominent defects in stereotyped axon pruning in hippocampus and visual cortex and altered dendritic spine remodeling, which is consistent with impaired Sema3F signaling and with models of autism spectrum disorder (ASD). We demonstrate that CRMP2 mediates Sema3F signaling in primary neurons and that crmp2 mice display ASD-related social behavior changes in the early postnatal period as well as in adults. Together, we demonstrate that CRMP2 mediates Sema3F-dependent synapse pruning and its dysfunction shares histological and behavioral features of ASD.
EMBO Rep, 2020; 21
30826389: Janikova M, Brozka H, Radostova D, Svoboda J, Stuchlik A
No effect of riluzole and memantine on learning deficit following quinpirole sensitization - An animal model of obsessive-compulsive disorder.
Chronic quinpirole (QNP) sensitization is an established animal model relevant to obsessive-compulsive disorder (OCD) that has been previously shown to induce several OCD-like behavioral patterns, such as compulsive-like checking and increased locomotion.
Physiol Behav, 2019; 204
30210330: Petrasek T, Vojtechova I, Lobellova V, Popelikova A, Janikova M, Brozka H, Houdek P, Sladek M, Sumova A, Kristofikova Z, Vales K, Stuchlík A
The McGill Transgenic Rat Model of Alzheimer's Disease Displays Cognitive and Motor Impairments, Changes in Anxiety and Social Behavior, and Altered Circadian Activity.
The McGill-R-Thy1-APP transgenic rat is an animal model of the familial form of Alzheimer's disease (AD). This model mirrors several neuropathological hallmarks of the disease, including the accumulation of beta-amyloid and the formation of amyloid plaques (in homozygous animals only), neuroinflammation and the gradual deterioration of cognitive functions even prior to plaque formation, although it lacks the tauopathy observed in human victims of AD. The goal of the present study was a thorough characterization of the homozygous model with emphasis on its face validity in several domains of behavior known to be affected in AD patients, including cognitive functions, motor coordination, emotionality, sociability, and circadian activity patterns. On the behavioral level, we found normal locomotor activity in spontaneous exploration, but problems with balance and gait coordination, increased anxiety and severely impaired spatial cognition in 4-7 month old homozygous animals. The profile of social behavior and ultrasonic communication was altered in the McGill rats, without a general social withdrawal. McGill rats also exhibited changes in circadian profile, with a shorter free-running period and increased total activity during the subjective night, without signs of sleep disturbances during the inactive phase. Expression of circadian clock gene was found to be increased in the parietal cortex and cerebellum, while expression was not changed. The clock-controlled gene expression was found to be elevated in the parietal cortex and hippocampus, which might have contributed to the observed changes in circadian phenotype. We conclude that the phenotype in the McGill rat model is not restricted to the

cognitive domain, but also includes gait problems, changes in emotionality, social behavior, and circadian profiles. Our findings show that the model should be useful for the development of new therapeutic approaches targeting not only memory decline but also other symptoms decreasing the quality of life of AD patients.
Front Aging Neurosci, 2018; 10

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EFFECT OF HALOPERIDOL, RISPERIDONE, AND CLOZAPINE ON COGNITIVE SYMPTOMS AND LOCOMOTOR ACTIVITY IN MK-801 MODEL OF SCHIZOPHRENIA IN RATS

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Recent studies suggest that schizophrenia treatment may benefit from a combination of antipsychotic administration with neurosteroids in some cases. As a pilot experiment for a subsequent study of co-application with neurosteroids, we evaluated the efficacy of three widely used antipsychotics (haloperidol, risperidone, and clozapine) in the acute MK-801 model of schizophrenia. Three-month-old males of Wistar rats were trained in the Carousel maze paradigm, a sensitive tool for detecting schizophrenia-like symptoms. This task requires rats to avoid an unmarked section on a slowly rotating arena in four daily sessions (20 minutes each). Prior to each session, rats were administered two i.p. injections. First, they were injected with one of the antipsychotics (haloperidol, risperidone, clozapine) or saline. Second, they received MK-801 (0.2 mg/kg) or saline. In the MK-801 injected animals, none of the antipsychotics reversed the cognitive deficit. In terms of positive symptoms, clozapine attenuated hyperactivity while haloperidol almost blocked the locomotion even in rats without MK-801. These data suggest that clozapine seems to be the most suitable molecule for the follow-up studies aimed at co-administration with neurosteroids. The study was supported by the Czech Health Research Council grant NU20-04-00389.

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BOARD NUMBER: S05-186

ROLE OF THE NEURONAL PRIMARY CILIA-AUTOPHAGY AXIS IN THE REGULATION OF COGNITION DURING AGING

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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During normal brain aging, neurons of the hippocampus, a key structure for learning and memory, present defects in their autophagy machinery which contribute to age-related cognitive decline. Osteocalcin (OCN), a youth-associated hormone, is a direct inducer of autophagy in hippocampal neurons and its administration to aged mice improved memory in an autophagy-dependent manner. Recently, it was shown that the autophagy machinery can be mobilized in response to systemic stimuli by a direct or indirect connection with the primary cilium, an extracellular organelle that acts as a cellular antenna sensing systemic signals, such as hormones. Here we explore the role of the primary cilium as mediator of OCN signaling to modulate autophagy and cognitive fitness. We found that alteration of core ciliary proteins selectively in the hippocampus impaired autophagy machinery, reduced calcium signaling, and led to severe memory deficits. During aging, we found abnormal primary cilia morphology associated with a reduction of major ciliary proteins and restoration of IFT20 ameliorated age-impaired autophagic machinery and memory functions. Lastly, we found that GPR158 (OCN's receptor), is highly present in hippocampal neuronal primary cilia and IFT20 is required to integrate the beneficial effects of OCN on autophagy and memory. These data demonstrate that primary cilia are essential for hippocampal neurons ability to sense the systemic milieu and provide an adaptive response by modulating the autophagic machinery.

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BOARD NUMBER: S05-187

AUTOPHAGY AND ESCRT MACHINERY IMPAIRMENT AND LYSOSOMAL DAMAGE IN FRONTAL CORTEX AND HIPPOCAMPUS OF RATS IN A DEPRESSION-LIKE MODEL

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Introduction: Inflammation seems to be involved in the pathophysiology of depression, while autophagy is essential in the regulation of immunity and inflammation. Autophagy is a lysosome-based degradative pathway whose pathway alterations could lead to other processes hindrance, as membrane dynamics (controlled by the endosomal sorting complex required for transport, ESCRT) or waste products removal by lysosomes. **Aims**: To characterize autophagy markers, ESCRT machinery effectors, and possible lysosomal alterations in frontal cortex (FC) and hippocampus (Hp) of rats exposed to Chronic Mild Stress (CMS). **Methods**: mTOR, ULK1, beclin1, Atgs, SQSTM1, Hrs, STAM2, CHMP6 (among others) were evaluated in FC and Hp by western blot (WB). Fractions of purified lysosomes were tested for lysosomal markers (LAMPs) and proteins (cathepsins, cystatins) by WB. Immunofluorescence approaches were used to study colocalization events between autophagy and lysosomal proteins with neuron and glial cells. **Results**: FC and Hp displayed autophagy and ESCRT imbalances, being FC the most affected by CMS. Several steps of the autophagy pathway were impeded in a region-specific manner. ESCRT proteins were mainly downregulated in FC. CMS increased lysosomal presence in FC and Hp, oppositely modulating proteins in lysosomal fractions of FC and Hp. Astrocytes seemed to exert a chief role in SQSTM1 regulation, while neurons and microglia were the main orchestrators for LAMP2A CMS-derived modifications. **Conclusions**: CMS-induced alterations derived in lysosomal damage in both brain areas, and to the impairment of autophagy and ESCRT components mainly in FC. Autophagy seems to play a concrete role in brain homeostasis according to location.

Pubmed:
26362308: Alcocer-Gómez E, Ulecia-Morón C, Marín-Aguilar F, Rybkina T, Casas-Barquero N, Ruiz-Cabello J, Ryffel B, Apetoh L, Ghiringhelli F, Bullón P, Sánchez-Alcazar JA, Carrión AM, Cordero MD
Stress-Induced Depressive Behaviors Require a Functional NLRP3 Inflammasome.
Depression is a major public health concern in modern society, yet little is known about the molecular link between this condition and neuroinflammation. The inflammasome complex was recently shown to be implicated in depression. The present study shows the implication of NLRP3 inflammasome in animal model of stress-induced depression. Accordingly, we show here that in the absence of a NLRP3 inflammasome, prolonged stress does not provoke depressive behaviors or microglial activation in mice or dampen hippocampal neurogenesis. Indeed, NLRP3 deletion or inhibition of microglial activation impairs the stress-induced alterations associated with depression. According to these findings in animal model, the inflammasome could be a target for new therapeutic interventions to prevent depression in patients.
Mol Neurobiol, 2016; 53
33290967: MacDowell KS, Martín-Hernández D, Ulecia-Morón C, Bris ÁG, Madrigal JLM, García-Bueno B, Caso JR
Paliperidone attenuates chronic stress-induced changes in the expression of inflammasomes-related protein in the frontal cortex of male rats.
Several stress-related neuropsychiatric diseases are related to inflammatory phenomena. Thus, a better understanding of stress-induced immune responses could lead to enhanced treatment alternatives. Little is known about the possible involvement of inflammasomes in the stress-induced proinflammatory response. Antipsychotics have anti-inflammatory effects, but the possible antipsychotic treatment actions on inflammasomes remain unexplored. Our aim was to study whether inflammasomes are involved in the neuroinflammation induced by a paradigmatic model of chronic stress and whether the monoamine receptor antagonist paliperidone can modulate the possible stress-induced inflammasomes activation in the

frontal cortex (FC). Thus, the effects of paliperidone (1 mg/Kg, oral gavage) administered during a chronic restraint stress protocol (6 h/day for 21 days) on the possible stress-related inflammasomes protein induction were evaluated through Western blot in the FC of male Wistar rats. Stress increased protein expression levels of the inflammasome complexes NALP1, NLRP3 and AIM2 and augmented caspase-1 and mature interleukin (IL)-1β protein levels. Paliperidone pre-treatment normalized the protein expression of the inflammasome pathway. In conclusion, our data indicate an induction of inflammasome complexes by chronic restraint stress in the FC of rats. The antipsychotic paliperidone has an inhibitory action on some of the stress-induced inflammasomes stimulation trying to normalize the neuroinflammatory scenario caused by stress. Considering the emerging role of inflammation in neuropsychiatric diseases, the development of new drugs targeting inflammasome pathways is a promising approach for future therapeutic interventions.
Int Immunopharmacol, 2021; 90
31711674: Ferreira DW, Ulecia-Morón C, Alvarado-Vázquez PA, Cunnane K, Moracho-Vilriales C, Grosick RL, Cunha TM, Romero-Sandoval EA
CD163 overexpression using a macrophage-directed gene therapy approach improves wound healing in ex vivo and in vivo human skin models.
Large tissue damage or wounds cause serious comorbidities and represent a major burden for patients, families, and health systems. Due to the pivotal role of immune cells in the proper resolution of inflammation and tissue repair, we focus our current study on the interaction of macrophages with skin cells, and specifically on the effects of CD163 gene induction in macrophages in wound healing. We hypothesize that the over-expression of the scavenger receptor gene CD163 in human macrophages would result in a more efficient wound healing process. Using 3D human wounded skin organotypic tissues, we observed that CD163 overexpression in THP-1 and human primary macrophages induced a more efficient re-epithelization when compared to control cells. Using human primary skin cells and an in vitro scratch assay we observed that CD163 overexpression in THP-1 macrophages promoted a more rapid and efficient wound healing process through a unique interaction with fibroblasts. The addition of CD163-blocking antibody, but not isotype control, blocked the efficient wound healing process induced by CD163 overexpression in macrophages. We found that the co-culture of skin cells and CD163 overexpressing macrophages reduced monocyte chemoattractant protein (MCP)-1 and enhanced tumor growth factor (TGF)-α, without altering interleukin (IL)-6 or TGF-β. Our findings show that CD163 induces a more efficient wound healing and seems to promote a wound milieu with a pro-resolution molecular profile. Our studies set the foundation to study this approach in in vivo clinically relevant settings to test its effects in wound healing processes such as acute major injuries, large surgeries, or chronic ulcers.
Immunobiology, 2020; 225
27615510: Bernal L, Alvarado-Vázquez A, Ferreira DW, Paige CA, Ulecia-Morón C, Hill B, Caesar M, Romero-Sandoval EA
Evaluation of a nanotechnology-based approach to induce gene-expression in human THP-1 macrophages under inflammatory conditions.
Macrophages orchestrate the initiation and resolution of inflammation by producing pro- and anti-inflammatory products. An imbalance in these mediators may originate from a deficient or excessive immune response. Therefore, macrophages are valid therapeutic targets to restore homeostasis under inflammatory conditions. We hypothesize that a specific mannosylated nanoparticle effectively induces gene expression in human macrophages under inflammatory conditions without undesirable immunogenic responses. THP-1 macrophages were challenged with lipopolysaccharide (LPS, 5μg/mL). Polyethylenimine (PEI) nanoparticles grafted with a mannose receptor ligand (Man-PEI) were used as a gene delivery method. Nanoparticle toxicity, Man-PEI cellular uptake rate and gene induction efficiency (GFP, CD14 or CD68) were studied. Potential immunogenic responses were evaluated by measuring the production of tumor necrosis factor-alpha (TNF-α), Interleukin (IL)-6 and IL-10. Man-PEI did not produce cytotoxicity, and it was effectively up-taken by THP-1 macrophages (69%). This approach produced a significant expression of GFP (mRNA and protein), CD14 and CD68 (mRNA), and transiently and mildly reduced IL-6 and IL-10 levels in LPS-challenged macrophages. Our results indicate that Man-PEI is suitable for inducing an efficient gene overexpression in human macrophages under inflammatory conditions with limited immunogenic responses. Our promising results set the foundation to test this technology to induce functional anti-inflammatory genes.
Immunobiology, 2017; 222
28545809: Alvarado-Vazquez PA, Bernal L, Paige CA, Grosick RL, Moracho Vilrriales C, Ferreira DW, Ulecia-Morón C, Romero-Sandoval EA
Macrophage-specific nanotechnology-driven CD163 overexpression in human macrophages results in an M2 phenotype under inflammatory conditions.
M1 macrophages release proinflammatory factors during inflammation. They transit to an M2 phenotype and release anti-inflammatory factors to resolve inflammation. An imbalance in the transition from M1 to M2 phenotype in macrophages contributes to the development of persistent inflammation. CD163, a member of the scavenger receptor cysteine-rich family, is an M2 macrophage marker. The functional role of CD163 during the resolution of inflammation is not completely known.

We postulate that CD163 contributes to the transition from M1 to M2 phenotype in macrophages. We induced CD163 gene in THP-1 and primary human macrophages using polyethylenimine nanoparticles grafted with a mannose ligand (Man-PEI). This nanoparticle specifically targets cells of monocytic origin via mannose receptors. Cells were challenged with a single or a double stimulation of lipopolysaccharide (LPS). A CD163 or empty plasmid was complexed with Man-PEI nanoparticles for cell transfections. Quantitative RT-PCR, immunocytochemistry, and ELISAs were used for molecular assessments. CD163-overexpressing macrophages displayed reduced levels of tumor necrosis factor-alpha (TNF)-α and monocytes chemoattractant protein (MCP)-1 after a single stimulation with LPS. Following a double stimulation paradigm, CD163-overexpressing macrophages showed an increase of interleukin (IL)-10 and IL-1ra and a reduction of MCP-1. This anti-inflammatory phenotype was partially blocked by an anti-CD163 antibody (effects on IL-10 and IL-1ra). A decrease in the release of TNF-α, IL-1β, and IL-6 was observed in CD163-overexpressing human primary macrophages. The release of IL-6 was blocked by an anti-CD163 antibody in the CD163-overexpressing group. Our data show that the induction of the CD163 gene in human macrophages under inflammatory conditions produces changes in cytokine secretion in favor of an anti-inflammatory phenotype. Targeting macrophages to induce CD163 using cell-directed nanotechnology is an attractive and practical approach for inflammatory conditions that could lead to persistent pain, i.e. major surgeries, burns, rheumatoid arthritis, etc.

Immunobiology, 2017; 222

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CANNABIDIOL AS AN ADD-ON THERAPY TO OVERCOME THE SLOW-ONSET AND – POSSIBLY – RESISTANCE TO ANTIDEPRESSANT TREATMENT: INVOLVEMENT OF NAPE-PLD IN THE MEDIAL PREFRONTAL CORTEX

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Antidepressants are the first-line pharmacological treatment for stress-related psychiatric disorders. However, their late-onset of action, frequent side effects and the significant percentage of treatment-resistant patients are important limitations. Cannabidiol (CBD) is a non-psychotomimetic phytocannabinoid and preliminary data showed that CBD (30mg/kg) is faster than the antidepressant escitalopram (ESC; 20mg/kg) to induce an anti-stress effect in mice. We hypothesized that CBD could be beneficial as an add-on therapy to enhance the action of antidepressants. We found that in male mice submitted to either of two different repeated stress protocols, the combination of ESC (10mg/kg) with a low dose of CBD (7.5mg/kg) accelerated the anxiolytic-like effect of the treatment. The anti-stress effect of ESC+CBD, however, was absent in mice in which the expression of the N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD, responsible for synthesizing N-acyl ethanolamines) was deleted in the medial prefrontal cortex (mPFC) using a CRISPR-Cas9-mediated approach. The CRISPR-Cas9-mediated deletion of diacylglycerol lipase, on the other hand, did not prevent the behavioral effect of ESC+CBD in stressed mice. Additionally, in three patients with treatment-resistant depression, after 12 weeks of treatment with CBD as add-on therapy, two patients were considered depression remitted (MADRS Score <10) while one patient successfully responded to CBD adjuvant treatment (>50% improved MADRS score). Our results suggest that CBD might be useful as an add-on therapy for optimizing the action of antidepressants. They also suggest that CBD's beneficial actions depend on the activity of NAPE-PLD in the mPFC. (CEUA: 032/2015-1 / 047/2019). Financial Support: FAPESP, CNPq, L'Oreal-UNESCO.

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ABLATION OF NECROPTOSIS PROTECTS DIABETES ASSOCIATED COGNITIVE DEFICITS & LIPOTOXICITY INDUCED NEURO-GLIA CHANGES

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Aims: Type-2 diabetes (T2DM), and obesity are associated with neuroinflammation and cognitive decline. Necroptosis and programmed necrosis is the major contributing factor to neuroinflammation, neurodegeneration, and metabolic disorder. Necroptosis is best characterized by relative upregulation of phosphorylated-MLKL (mixed-lineage kinase domain-like protein). The present study aims to evaluate the necrostatin (Nec-1) effect on cognitive decline in the experimental T2DM model in C57BL6/J mice and lipotoxicity induced neuro-glia changes in neuro2a and BV2 cells. **Methods:** T2DM was induced in mice by keeping them on a high-fat diet (HFD) for 16 weeks and injecting a single dose of streptozotocin (100 mg/kg,*i.p*) at the 12th week. Nec-1 was administered for 3 weeks at (10 mg/kg, i.p) once every 3-day. Lipotoxicity was induced in neuro2a, and BV2 cells by 200µM palmitate/BSA conjugate, and 50µM of Nec-1 is used to assess their inhibitory effect. neurobehavioral studies, plasma biochemical parameter level was analysed to assess cognitive performances, inflammatory characteristics, and type2 DM. Further, western blot, immunofluorescence, and staining were performed to evaluate the relative protein expression, their cellular localization, amyloid deposit, and neurodegenerative changes. **Results**: Nec-1 improved the cognitive performance of mice and reduced the p-RIPK3-p-MLKL mediated neuroglia changes in the brain tissue, and cells. Nec-1 also reduced the tau and, amyloid-beta load.it has alleviated the p-MLKL mediated mitochondrial and autophagy functionality. **Conclusions:** Necroptosis, a major contributing factor to neuroinflammation and gliosis, is responsible for cognitive decline. The above findings emphasises the central impact of metabolic stress and open the horizon for the need for the development of newer therapies targeting necroptosis.

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EFFECTS OF S-KETAMINE ON COCAINE-SEEKING BEHAVIOR IN RATS

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Cocaine use disorder is a chronic brain disease characterized by compulsive drug-taking and drug-seeking despite harmful consequences. Recent observations suggest glutamatergic transmission and its N-methyl-D-aspartate (NMDA) receptors are implicated in different environmental conditions of cocaine forced abstinence in animals. The aim of this study was to examine the effects of S-ketamine a noncompetitive antagonist of NMDA receptor on cocaine-seeking behavior in rats with a history of cocaine (0.5 mg/kg/infusion) self-administration and different abstinence (condition housing in an enriched or in an isolated environment) and withdrawal with extinction training. S-ketamine was administered acutely (2.5-10 mg/kg, *i.p*.) before exposure to cocaine (10 mg/kg, *i.p*.) or a drug-associated conditional stimulus (a cue) in male male Wistar rats. Our results showed that exposure to different conditions of cocaine abstinence evoked increased active lever presses during cocaine- and cue-induced drug-seeking behavior. S-ketamine (2.5-10 mg/kg, *i.p.*) significantly reduced the effect of the priming dose of cocaine or cue in all conditions of abstinence and withdrawal with extinction training. S-ketamine in investigated doses did not alter locomotor activity in naive rats. Our findings indicate that S-ketamine may have the potential as a novel pharmacotherapies for cocaine addiction to be linked with reduction of relapse. Supported by the statutory funds of the Maj Institute of Pharmacology PAS (Kraków, Poland). We declare no conflict of interest.

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ELEVATION OF ANANDAMIDE BY URB597 MITIGATES COCAINE-SEEKING BEHAVIOUR DURING ABSTINENCE

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Aim: cue-induced cocaine-craving is one of the main factors contributing to relapse in abstinent addicts. The endocannabinoid system represents a potential therapeutic target for the cocaine-related maladaptative alterations that drive drug-seeking behaviour during protracted abstinence. Among them, endocannabinoid mediated long-term depression (eCB-LTD) in the nucleus accumbens is lost after cocaine exposure. However, how this alteration could add to the seeking behaviour in abstinent mice has never been explored. **Methods:** mice underwent an intravenous cocaine (0,75 mg/kg/infusion) self-administration. Afterwards, a group of mice was tested on withdrawal day 1 for cue-induced drug-seeking behaviour. The other groups were treated daily in their home cages with vehicle, the fatty acid amide hydrolase inhibitor URB597 (1 mg/kg ip) or the monoacylglycerol lipase inhibitor MJN110 (5 mg/kg ip) and, on withdrawal day 30, they were tested for cue-induced cocaine-seeking behaviour. Immediately after the test, we dissected or perfused the brains to evaluate the levels of different components of the endocannabinoid system using qPCR and the levels of c-Fos using free-floating immunochemistry in the nucleus accumbens. **Results:** our results show that URB597, but not MJN110, decreased drug-seeking behaviour during abstinence. URB597 also modulated the expression of c-Fos and components of the endocannabinoid system in the nucleus accumbens. **Conclusions:** these results support the therapeutic potential of the cannabinoid-targeted strategies for the treatment of cocaine craving during protracted abstinence from cocaine use. Lastly, the present study suggests that anandamide might constitute the main endocannabinoid involved in the regulation of cue-induced drug-seeking behaviour during withdrawal.

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PROTECTIVE EFFECTS OF PROTAETIA BREVITARSIS LARVAE ON COGNITIVE DECLINE UNDER AMYLOID BETA-INJECTED MICE MODEL

POSTER SESSION 05 - SECTION: ANIMAL MODELS OF PSYCHIATRIC DISORDERS AND PRECLINICAL PHARMACOLOGY

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Amyloid beta (Aβ) accumulation is known to be a major risk factor of Alzheimer's disease. Protaetia brevitarsis larvae, a kind of edible insect, have been used as a traditional medicine. Now, it approved by the Ministry of Food and Drug Safety as food resources in Republic of Korea. In this study, we examined whether P. brevitarsis larvae improved cognitive decline under Aβ-induced mice model. Ethanol extracts of P. brevitarsis larvae is administred to Aβ-induced mice at doses of 100 and 200 mg/kg/day for 14 days. The protective effect of P. brevitarsis larvae from Aβ was determined by behavioral tests using T-maze test, novel object recognition test and water maze test. The Aβ-injected control group showed disorders in both spatial memory and novel object cognitive abilities. However, P. brevitarsis treated groups increased exploration of novel route and novel object, and it also decreased latencies and travelling distances to find the invisible platform in water maze. Therefore, our study demonstrated that administration of P. brevitarsis larvae improved Aβ-induced cognitive decline.

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NEUROINFLAMMATION CONTROL BASED ON AVENANTHRAMIDE-C IS A NEW ALZHEIMER DISEASE TREATMENT STRATEGY

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by cognitive decline and dementia with no effective treatment. As we know, neuroinflammation plays key role during AD life, Here, we investigated a novel compound from oats named avenanthramide-C (Avn-C), on AD-related memory impairment and behavioral deficits in transgenic mouse models. Avn-C, a polyphenol compound found predominantly in oats, has a range of biological properties. Acute hippocampal slices of wild-type or AD transgenic mice were treated with Avn-C in the presence or absence of oligomeric Aβ42. LTP analyses and immunoblotting were performed to assess the effect of Avn-C on Aβ-induced memory impairment. To further investigate the effect of Avn-C on impaired memory and Aβ pathology, two different AD transgenic mice (Tg2576 and 5XFAD) models were orally treated with either Avn-C or vehicle for 2 weeks. They were then assessed for the effect of the treatment on neuropathologies and behavioral impairments. Avn-C reversed impaired LTP in both ex vivo- and in vivo-treated AD mice hippocampus. Oral administration (6 mg/kg per day) for 2 weeks in AD mice leads to improved recognition and spatial memory, reduced caspase-3 cleavage, reversed neuroinflammation, and to accelerated glycogen synthase kinase-3β (pS9GSK-3β) and interleukin (IL-10) levels. Our findings provide evidence, for the first time, that oats' Avn-C reverses the AD-related memory and behavioral impairments, and establish it as a potential candidate for Alzheimer's disease drug development (This work was supported by NRF-2019R1A2C1089108 and the Commercializations Promotion Agency for R&D Outcomes(COMPA) grant funded by the Korea government(MSIT) (2021C100)

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-194

WAT ALTERATIONS IN DIABETIC MICE: ITS CONNECTION AND IMPLICATION IN AD PATHOGENESIS

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease (AD) is a complex disorder and multiple cellular and molecular mechanisms are involved in AD onset and progression. Recent evidences have suggested that metabolic alterations are an important pathological feature in disease progression in AD. Likewise, diabetes and obesity, two mayor metabolic illnesses associated with white adipose tissue expansion, are risk factors for AD. Here, we hypothesize that the white adipose tissue may serve as a key communicator organ between the brain and peripheral metabolic illnesses. We used histological stains, immunohistochemistry and biochemical means to determine changes in the white adipose tissue from WT and db/db mice. Moreover, similar techniques were used in the brain of 3xTg-AD mice that received white fat pads from WT and db/db donors to determine any changes in amyloid and tau pathology. Our study shows that recipient 3xTg-AD mice from db/db fat pads mice develop profound changes in tau pathology due to increased CDK5/p25 expression compared to 3xTg-AD mice that received fad pads from WT mice. This increment in tau level was associated with elevated levels in IL-1β and microglial activation. However, we found that Aβ levels were reduced in recipient 3xTg-AD mice from db/db fat pads compared to 3xTg-AD mice that received fad pads from WT mice. These reduction in Aβ levels were correlated with an increment in microglia phagocytic capacity. Overall, our study demonstrates a novel important crosstalk between AD and diabetes type II through white adipose cells and a differential effect on tau and Aβ pathology.

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BOARD NUMBER: S05-195

CEREBRAL EFFECT OF INTERMITTENT HYPOXIA ON COGNITION AND SENESCENCE FACTORS IN WILD-TYPE AND PS1KI MICE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Aging is the major risk factor for Alzheimer's disease (AD). Hallmarks of cellular senescence have been detected in aging brain and recently in brain from AD patients and AD mice models. In the absence of effective treatment, identifying and treating comorbidities is an interesting treatment strategy. Sleep apnea syndrome (SAS) is common in elderly subjects and is accompanied by intermittent hypoxia (IH). IH may cause stress-induced premature aging and cognitive decline, but the role of cellular senescence in these mechanisms remains largely unknown. To determine whether IH causes premature brain aging and accelerates neurodegeneration, 3-month-old wild-type (WT) and AD transgenic mice (PS1M146Vki; PS1Ki) were exposed to IH or normoxia for 6 weeks (8h/day). We assessed behavioral as well as markers of cellular senescence in the brain. Our results indicate that IH exposure induced a cognitive impairment in WT mice similar to that observed in PS1Ki mice exposed to normoxia. Moreover, we found that IH induced an increase in the expression of markers of cellular senescence in the hippocampus and entorhinal cortex of WT mice. These first data indicate that cellular senescence mediated by SAS could be a potential target for AD prevention.

FENS Forum 2022
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BOARD NUMBER: S05-196

FATE OF HIPPOCAMPAL NEURAL STEM CELLS AT THE SINGLE CELL LEVEL DURING ALZHEIMER'S DISEASE.

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer´s disease (AD) is one of the main neurodegenerative disorders of the 21stcentury involving declining cognitive function characterized by a difficulty in learning new information and memory loss. Currently, AD is considered incurable and barely treatable. This results in an increasing need for patient care, posing enormous social and economic challenges to society and healthcare systems worldwide. Adult neurogenesis, the process by which new neurons arise from neural stem cells (NSCs) in the dentate gyrus of the adult hippocampus, is known to be important for memory formation and learning. Current research of our group suggests that NSCs are affected in their fate decisions by AD. We observed an increased proliferation of NSCs in the pre-plaque phase which is followed by a strong decline in the number of stem cells and adult neurogenesis. The fate of these overactivated cells has not yet been fully elucidated. The aim of the present work was to evaluate the early-onset changes in the neurogenic niche in AD. For this, the fate of NSCs in the pre-plaque phase of AD was determined by *in vivo* fate mapping of individual stem cell clones using the NestinCreER^{T2}/tdTomato mouse model. The fate tracking of the tomato-positive stem cells over a period of 1 month shows increased proliferation of stem- and progenitor cells and the formation of mature astrocytes. Results suggest that increased astrogenesis due to direct differentiation of the NSCs ultimately leads to a decrease in the stem cell pool and consequently reduction of new neurons during AD.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-197

VASCULAR ALTERATIONS IN MIXED MURINE MODELS OF METABOLIC DISORDERS AND ALZHEIMER´S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Aims: While aging remains the main risk factor to suffer Alzheimer´s disease (AD), metabolic disorders, including prediabetes and type 2 diabetes (T2D) also increase the risk to develop dementia. Blood flow and vascular alterations might be one the linking mechanisms underlying the close relationship between AD and T2D. We have analyzed the presence of spontaneous hemorrhages in AD-prediabetes mice and AD-T2D animals. We have also analyzed alterations in brain vascular functionality. **Methods**: AD-prediabetes mice were generated by feeding a high-fat diet to APP/PS1 mice. AD-T2D mice resulted from crossing APP/PS1 and db/db animals. Amyloid-β (Aβ) levels were determined by ELISA. Spontaneous hemorrhages in the brain were quantified by Prussian blue staining. Vessel contractility and red blood cell (RBC) velocity were analyzed *in vivo* and in real time by multiphoton microscopy. **Results**: Soluble Aβ levels were higher in AD-prediabetes and AD-T2D mice. Spontaneous hemorrhages were increased in AD-T2D animals. Vessel contractility was impaired in AD-prediabetes animals and more severely in AD-T2D mice. Moreover, RBC velocity was significantly reduced in AD-T2D animals. **Conclusion**: Vascular complications are observed in AD-prediabetes mice and these effects are more severe in animals harboring AD and T2D, as regularly observed in the clinic, supporting a role for vascular alterations when AD and metabolic disorders coexist. **Funding:** Ministerio de Ciencia e Innovacion (PID2020-115499RB-I0). Subvencion para la Investigación y la Innovación Biomedica y en Ciencias de la Salud I.T.I. Cadiz (PI-0008-2017). Proyectos de I+D+i Universidades. Junta de Andalucia. Union Europea. Andalucia se mueve con Europa P20-00928**.**

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-198

17β-ESTRADIOL AND ESTROGEN-LIKE COMPOUND SHOWS NEUROPROTECTIVE POTENTIAL IN A TRIPLE TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISORDER

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Aims: Based upon our previous experiments we aimed to investigate the neuroprotective effect of 17β-estradiol (E2) in a triple transgenic mouse model of Alzheimer's disorder (3xTg-AD) and to find new estrogen-like compounds (activators of non-genomic estrogen-like signaling – ANGELS) without classical side effects and with neuroprotective potential. **Methods:** The experiment was performed on 6-month-old genetically modified female 3xTg-AD mice. To eliminate the peripheral E2 synthesis ovaries were removed (OVX). After two-week recovery an acute subcutaneous (s.c.) treatment of vehicle, 33ng/g E2 or ANGELS was administered. Y-Maze and Forced Swim Test (FST) were performed 24 hours after treatment. The $A\beta_{1-42}$ plaques, Tau aggregates, and acetylcholinesterase (AChE) fiber loss in the brain were determined with quantitative immunohistochemistry. The uterus was dissected and weighted. **Results:** The uterus weight increased significantly after E2 but not after ANGELS treatment. Behavioural disturbances, both in cognitive (Y-Maze, testing working memory) and depressive-like domain (immobility in FST), were modified by our interventions. The hippocampal region of the 3xTg-AD mice showed the expected pathological hallmarks of AD ($A\beta_{1-42}$ plaques, Tau aggregates). Additionally, significant AChE fiber loss was found in the cortex of 3xTg-AD animals. Our treatments were effective. **Conclusions:** The tested ANGELS proved to lack estrogenic-like side effects *in vivo* and have protective properties in 3xTg-AD mice. We believe that these or similar compounds may provide a novel approach in AD therapy.

Pubmed:
32455953: Gáll Z, Farkas S, Albert Á, Ferencz E, Vancea S, Urkon M, Kolcsár M
Effects of Chronic Cannabidiol Treatment in the Rat Chronic Unpredictable Mild Stress Model of Depression.
Several neuropharmacological actions of cannabidiol (CBD) due to the modulation of the endocannabinoid system as well as direct serotonergic and gamma-aminobutyric acidergic actions have recently been identified. The current study aimed to reveal the effect of a long-term CBD treatment in the chronic unpredictable mild stress (CUMS) model of depression. Adult male Wistar rats (n = 24) were exposed to various stressors on a daily basis in order to induce anhedonia and anxiety-like behaviors. CBD (10 mg/kg body weight) was administered by daily intraperitoneal injections for 28 days (n = 12). The effects of the treatment were assessed on body weight, sucrose preference, and exploratory and anxiety-related behavior in the open field (OF) and elevated plus maze (EPM) tests. Hair corticosterone was also assayed by liquid chromatography-mass spectrometry. At the end of the experiment, CBD-treated rats showed a higher rate of body weight gain (5.94% vs. 0.67%) and sucrose preference compared to controls. A significant increase in vertical exploration and a trend of increase in distance traveled in the OF test were observed in the CBD-treated group compared to the vehicle-treated group. The EPM test did not reveal any differences between the groups. Hair corticosterone levels increased in the CBD-treated group, while they decreased in controls compared to baseline (+36.01% vs. -45.91%). In conclusion, CBD exerted a prohedonic effect in rats subjected to CUMS, demonstrated by the increased sucrose preference after three weeks of treatment. The reversal of the effect of CUMS on hair corticosterone concentrations might also point toward an anxiolytic or antidepressant-like effect of CBD, but this needs further confirmation.
Biomolecules, 2020; 10

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-199

MODELLING SPORADIC AD IN MICE WITH RISK FACTORS APOE4 AND NEUROINFLAMMATION

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Developing effective treatment for Alzheimer's disease (AD) remains a challenge. This can be partially attributed to the fact that the mouse models used in preclinical research largely replicate familial form of AD, while majority of human cases are sporadic; both forms differ widely in the onset and origin of pathology, therefore requiring specific/targeted treatments. In this study, we attempted to model sporadic AD in mice by combining two of the many risk factors that are strongly implicated in AD: ApoE4, a major genetic risk factor, together with neuro-inflammation which plays a pivotal role in AD. We gave ApoE4 knock in (KI) mice, expressing humanized ApoE4, low doses of Lipopolysaccharide (LPS) injections (weekly, for four months) to model chronic neuro-inflammation. We checked these animals for behavioral impairments at 6 months age using Open Field, Y-maze and Barnes Maze Test. LPS induced hypoactivity was observed in the Open Field and Y-maze test in these mice, however spatial learning and memory was intact. We then checked for differences in dendritic spine density, which is a strong correlate of AD, alongside microglia and astrocyte activation. ApoE4 KI mice showed a significant reduction in the number of spines after treatment with LPS, whereas there were no differences in the number of activated microglia and astrocytes. To conclude, although the spine loss observed in this model may indicate an early stage of a sporadic AD phenotype, further studies at later time points are required to see if these mice will also develop a convincing behavioral phenotype.

Pubmed:
33613267: Varinthra P, Ganesan K, Huang SP, Chompoopong S, Eurtivong C, Suresh P, Wen ZH, Liu IY
The 4-(Phenylsulfanyl) butan-2-one Improves Impaired Fear Memory Retrieval and Reduces Excessive Inflammatory Response in Triple Transgenic Alzheimer's Disease Mice.
Alzheimer's disease (AD) is a neurodegenerative disease characterized by an excessive inflammatory response and impaired memory retrieval, including spatial memory, recognition memory, and emotional memory. Acquisition and retrieval of fear memory help one avoid dangers and natural threats. Thus, it is crucial for survival. AD patients with impaired retrieval of fear memory are vulnerable to dangerous conditions. Excessive expression of inflammatory markers is known to impede synaptic transmission and reduce the efficiency of memory retrieval. In wild-type mice, reducing inflammation response can improve fear memory retrieval; however, this effect of this approach is not yet investigated in 3xTg-AD model mice. To date, no satisfactory drug or treatment can attenuate the symptoms of AD despite numerous efforts. In the past few years, the direction of therapeutic drug development for AD has been shifted to natural compounds with anti-inflammatory effect. In the present study, we demonstrate that the compound 4-(phenylsulfanyl) butan-2-one (4-PSB-2) is effective in enhancing fear memory retrieval of wild-type and 3xTg-AD mice by reducing the expression of TNF-α, COX-2, and iNOS. We also found that 4-PSB-2 helps increase dendritic spine density, postsynaptic density protein-95 (PSD-95) expression, and long-term potentiation (LTP) in the hippocampus of 3xTg-AD mice. Our study indicates that 4-PSB-2 may be developed as a promising therapeutic compound for treating fear memory impairment of AD patients.
Front Aging Neurosci, 2021; 13
33230138: Shih YH, Tu LH, Chang TY, Ganesan K, Chang WW, Chang PS, Fang YS, Lin YT, Jin LW, Chen YR
TDP-43 interacts with amyloid-β, inhibits fibrillization, and worsens pathology in a model of Alzheimer's disease.
TDP-43 inclusions are found in many Alzheimer's disease (AD) patients presenting faster disease progression and greater brain atrophy. Previously, we showed full-length TDP-43 forms spherical oligomers and perturbs amyloid-β (Aβ) fibrillization. To elucidate the role of TDP-43 in AD, here, we examined the effect of TDP-43 in Aβ aggregation and the attributed toxicity in mouse models. We found TDP-43 inhibited Aβ fibrillization at initial and oligomeric stages. Aβ fibrillization was delayed specifically in the presence of N-terminal domain containing TDP-43 variants, while C-terminal TDP-43 was not essential for Aβ interaction. TDP-43 significantly enhanced Aβ's ability to impair long-term potentiation and, upon intrahippocampal injection, caused spatial memory deficit. Following injection to AD transgenic mice, TDP-43 induced inflammation, interacted

with Aβ, and exacerbated AD-like pathology. TDP-43 oligomers mostly colocalized with intracellular Aβ in the brain of AD patients. We conclude that TDP-43 inhibits Aβ fibrillization through its interaction with Aβ and exacerbates AD pathology.
Nat Commun, 2020; 11
34794635: Turner KM, Ganesan K, Bradfield LA
Evidence That Compulsive Reward Seeking Has Been Hiding in the Central Dorsal Striatum.
Biol Psychiatry, 2021; 90

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-200

TRANSFER OF A PHARMACOLOGICAL NEUROVASCULAR UNCOUPLING MODEL FROM MICE TO RATS

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Present study's aim was to establish a pharmacologically induced neurovascular uncoupling (NVU) method in rats as a translational animal model of human cognitive decline. Pharmacologically induced NVU with subsequent neurological and cognitive defects was described in mice (Tarantini, 2015), however, no similar procedure has been reported so far in rats. We used 32 male Hannover Wistar rats. NVU was induced by intraperitoneal administration of a pharmacological "cocktail" consisting of MSPPOH (5 mg/kg), L-NAME (10 mg/kg) and indomethacin (1 mg/kg), injected twice daily for 8 consecutive days. Animals were tested in Morris water-maze and fear-conditioning assays. Blood pressure of the animals was also monitored during the treatment. NVU was characterized by the decrease in whisker pad stimulation-induced hyperaemia in the barrel cortex, measured by laser-Doppler probe. Surgery was followed by euthanasia, and brain and small intestine tissue samples were collected for prostaglandin E_2 (PGE2) level measurements. The animals treated with the "cocktail" showed no impairment in their performance in any of the cognitive tasks. However, we observed an overall higher blood pressure in these rats. They also showed a greater than 50 % decrease in CBF. Intestinal bleeding and ulcers were found in some of the treated animals and ELISA assays of the tissue samples revealed significantly decreased levels of PGE2 in both areas. Although we could evoke NVU by the applied mixture of pharmacons, it also induced adverse side effects. Furthermore, the treatment did not cause cognitive impairment. Additional refinements are still required for the development of an applicable model.

Pubmed:
34025423: Gáspár A, Hutka B, Ernyey AJ, Tajti BT, Varga BT, Zádori ZS, Gyertyán I
Intracerebroventricularly Injected Streptozotocin Exerts Subtle Effects on the Cognitive Performance of Long-Evans Rats.
Intracerebroventricularly injected streptozotocin (STZ)-induced learning impairment has been an increasingly used rat model of Alzheimer disease. The evoked pathological changes involve many symptoms of the human disease (cognitive decline, increase in β-amyloid and phospho-tau level, amyloid plaque-like deposits). However, the model has predominantly been used with Wistar rats in the literature. The objective of the current study was to transfer it to Long-Evans rats with the ulterior aim to integrate it in a complex cognitive test battery where we use this strain because of its superior cognitive capabilities. We performed two experiments (EXP1, EXP2) with three months old male animals. At EXP1, rats were treated with 2 × 1.5 mg/kg STZ (based on the literature) or citrate buffer vehicle injected bilaterally into the lateral ventricles on days 1 and 3. At EXP2 animals were treated with 3 × 1.5 mg/kg STZ or citrate buffer vehicle injected in the same way as in EXP1 at days 1, 3, and 5. Learning and memory capabilities of the rats were then tested in the following paradigms: five choice serial reaction time test (daily training, started from week 2 or 8 post surgery in Exp1 or Exp2, respectively, and lasting until the end of the experiment); novel object recognition (NOR) test (at week 8 or 14), passive avoidance (at week 11 or 6) and Morris water-maze (at week 14 or 6). 15 or 14 weeks after the STZ treatment animals were sacrificed and brain phospho-tau/tau protein ratio and β -amyloid level were determined by western blot technique. In EXP1 we could not find any significant difference between the treated and the control groups in any of the assays. In EXP2 we found significant impairment in the NOR test and elevated β-amyloid level in the STZ treated group in addition to slower learning of the five-choice paradigm and a trend for increased phospho-tau/tau ratio. Altogether our findings suggest that the Long-Evans strain may be less sensitive to the STZ treatment than the Wistar rats and higher doses may be needed to trigger pathological changes in these animals. The results also highlight the importance of strain diversity in modelling human diseases.
Front Pharmacol, 2021; 12

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-201

NEURONAL UPTAKE EVALUATION OF NOVEL CARBON NANOFORMS ENCAPSULATED IN POLYMERIC CARRIERS LOADED WITH GALANTAMINE ON PRIMARY NEURONAL CULTURES.

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Aims: The blood-brain barrier restricts penetration of many pharmaceutical agents to the brain leading to low drug accumulation and inadequate targeting of neurons. In this study we tried to investigate the efficacy of a novel carbon nanomolecule in targeting neurons and transferring pharmacologic agents to them. **Methods:** First, we aimed to develop nanoparticles that can successfully absorb and carry galantamine (Gal), an Alzheimer's disease (AD) treating agent, to their final target neurons. For this, hierarchical porous carbon (HPCs) nanoparticles, carrying Gal, were labeled with rhodamine-b and encapsuled in poly-lactic-co-glycolic acid (PLGA) by solid-oil-water modified double emulsification method. Then we elucidated the neuronal uptake of HPC-PLGA-Gal nanoparticles over time and their exact intra-neuronal localization by performing in-vitro experiments on primary neuronal cell cultures, deriving from wild type animals. Neuronal uptake of HPC-PLGA-Gal nanoparticles was also evaluated in vivo, after their intranasal administration to rats. **Results:** Our in-vitro results revealed a rapid, over the first two minutes, neuronal uptake of HPC-PGLA-Gal nanoparticles. Nanoparticles were localized in the cell body and proximal dendrites of neurons on primary neuronal cell cultures. In vivo experimentation confirmed the presence of HPC-PLGA-Gal nanoparticles in neurons of various brain regions within the first hour after a single intranasal administration. **Conclusion:** Conclusively, HPC-PGLA-Gal nanoparticles are capable to efficiently deliver Gal to neurons. Their possible therapeutic potential in the AD brain is under further evaluation on primary neuronal cultures and AD-transgenic rats.

Pubmed:
34941464: Katsenos AP, Davri AS, Simos YV, Nikas IP, Bekiari C, Paschou SA, Peschos D, Konitsiotis S, Vezyraki P, Tsamis KI
New treatment approaches for Alzheimer's disease: preclinical studies and clinical trials centered on antidiabetic drugs.
Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) represent two major chronic diseases that affect a large percentage of the population and share common pathogenetic mechanisms, including oxidative stress and inflammation. Considering their common mechanistic aspects, and given the current lack of effective therapies for AD, accumulating research has focused on the therapeutic potential of antidiabetic drugs in the treatment or prevention of AD.
Expert Opin Investig Drugs, 2022; 31

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-202

CEREBELLAR DYSFUNCTIONS UNDERLIE DEVELOPMENT OF MOTOR SKILLS ALTERATION IN 3XTG-AD MICE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Aims: Alzheimer's disease (AD) is an age-related neurodegenerative disease with progressive memory decline that may be related with the impairment of fine motor skills. The transgenic mouse models of AD are promising tools in understanding the underlying mechanisms. We hypothesized that this early event may be due to an alteration in cerebellar mitochondrial function. **Methods:** Male, 6-8-month-old triple transgenic (3xTg-AD) mice were investigated replicating some pathological hallmarks of the disorder. First, gross motor skills were tested in open field and grip tests. Next, fine motoric ability were checked by food-motivated tests (single pellett retrieval, staircase tests). To confirm that cerebellum was damaged we detected β-Amyloid (Aβ) and tau fibrillary tangels by immunohistochemistry and Western blot (WB). For evaluation of mitochondrial function the cerebellar levels of citochrom C IV subunits mRNA, as well as Bcl2, Bcl-xL and Bax protein levels changes were identified by qPCR and Western blot. **Results:** 3xTg-AD mice has decreased physical activity, muscle strenght and fine motoric. In the cerebellum AD-related deposits were detected. Mitochondrial pathways genes and proteins also showed alterations. **Conclusion:** These results support that the impaired gross and fine motor skills of 3xTg-AD mice are related to cerebellar changes both at the level of AD-related aggregations, as well as at mitochondrial level. We assume that cerebellar changes might be an early sign preceeding cognitive decline in AD.

Pubmed:
35054976: Fazekas CL, Szabó A, Török B, Bánrévi K, Correia P, Chaves T, Daumas S, Zelena D
A New Player in the Hippocampus: A Review on VGLUT3+ Neurons and Their Role in the Regulation of Hippocampal Activity and Behaviour.
Glutamate is the most abundant excitatory amino acid in the central nervous system. Neurons using glutamate as a neurotransmitter can be characterised by vesicular glutamate transporters (VGLUTs). Among the three subtypes, VGLUT3 is unique, co-localising with other "classical" neurotransmitters, such as the inhibitory GABA. Glutamate, manipulated by VGLUT3, can modulate the packaging as well as the release of other neurotransmitters and serve as a retrograde signal through its release from the somata and dendrites. Its contribution to sensory processes (including seeing, hearing, and mechanosensation) is well characterised. However, its involvement in learning and memory can only be assumed based on its prominent hippocampal presence. Although VGLUT3-expressing neurons are detectable in the hippocampus, most of the hippocampal VGLUT3 positivity can be found on nerve terminals, presumably coming from the median raphe. This hippocampal glutamatergic network plays a pivotal role in several important processes (e.g., learning and memory, emotions, epilepsy, cardiovascular regulation). Indirect information from anatomical studies and KO mice strains suggests the contribution of local VGLUT3-positive hippocampal neurons as well as afferentations in these events. However, further studies making use of more specific tools (e.g., Cre-mice, opto- and chemogenetics) are needed to confirm these assumptions.
Int J Mol Sci, 2022; 23
34502322: Török B, Fazekas CL, Szabó A, Zelena D
Epigenetic Modulation of Vasopressin Expression in Health and Disease.
Vasopressin is a ubiquitous molecule playing an important role in a wide range of physiological processes thereby implicated in the pathomechanism of many disorders. Its effect is well characterized through V2 receptors, which regulates the water resorption in kidney, while its vasoconstrictory effect through V1a receptor also received a lot of attention in the maintenance of blood pressure during shock. However, the most striking is its central effect both through the V1b receptors in stress-axis regulation as well as through V1a receptors regulating many aspects of our behavior (e.g., social behavior, learning and memory). Vasopressin has been implicated in the development of depression, due to its connection with chronic stress, as

well as schizophrenia because of its involvement in social interactions and memory processes. Epigenetic changes may also play a role in the development of these disorders. The possible mechanism includes DNA methylation, histone modification and/or micro RNAs, and these possible regulations will be in the focus of our present review.
Int J Mol Sci, 2021; 22
34529674: Szabó A, Schlett K, Szücs A
Conventional measures of intrinsic excitability are poor estimators of neuronal activity under realistic synaptic inputs. Activity-dependent regulation of intrinsic excitability has been shown to greatly contribute to the overall plasticity of neuronal circuits. Such neuroadaptations are commonly investigated in patch clamp experiments using current step stimulation and the resulting input-output functions are analyzed to quantify alterations in intrinsic excitability. However, it is rarely addressed, how such changes translate to the function of neurons when they operate under natural synaptic inputs. Still, it is reasonable to expect that a strong correlation and near proportional relationship exist between static firing responses and those evoked by synaptic drive. We challenge this view by performing a high-yield electrophysiological analysis of cultured mouse hippocampal neurons using both standard protocols and simulated synaptic inputs via dynamic clamp. We find that under these conditions the neurons exhibit vastly different firing responses with surprisingly weak correlation between static and dynamic firing intensities. These contrasting responses are regulated by two intrinsic K-currents mediated by Kv1 and Kir channels, respectively. Pharmacological manipulation of the K-currents produces differential regulation of the firing output of neurons. Static firing responses are greatly increased in stuttering type neurons under blocking their Kv1 channels, while the synaptic responses of the same neurons are less affected. Pharmacological blocking of Kir-channels in delayed firing type neurons, on the other hand, exhibit the opposite effects. Our subsequent computational model simulations confirm the findings in the electrophysiological experiments and also show that adaptive changes in the kinetic properties of such currents can even produce paradoxical regulation of the firing output.
PLoS Comput Biol, 2021; 17
34445795: Chaves T, Fazekas CL, Horváth K, Correia P, Szabó A, Török B, Bánrévi K, Zelena D
Stress Adaptation and the Brainstem with Focus on Corticotropin-Releasing Hormone.
Stress adaptation is of utmost importance for the maintenance of homeostasis and, therefore, of life itself. The prevalence of stress-related disorders is increasing, emphasizing the importance of exploratory research on stress adaptation. Two major regulatory pathways exist: the hypothalamic-pituitary-adrenocortical axis and the sympathetic adrenomedullary axis. They act in unison, ensured by the enormous bidirectional connection between their centers, the paraventricular nucleus of the hypothalamus (PVN), and the brainstem monoaminergic cell groups, respectively. PVN and especially their corticotropin-releasing hormone (CRH) producing neurons are considered to be the centrum of stress regulation. However, the brainstem seems to be equally important. Therefore, we aimed to summarize the present knowledge on the role of classical neurotransmitters of the brainstem (GABA, glutamate as well as serotonin, noradrenaline, adrenaline, and dopamine) in stress adaptation. Neuropeptides, including CRH, might be co-localized in the brainstem nuclei. Here we focused on CRH as its role in stress regulation is well-known and widely accepted and other CRH neurons scattered along the brain may also complement the function of the PVN. Although CRH-positive cells are present on some parts of the brainstem, sometimes even in comparable amounts as in the PVN, not much is known about their contribution to stress adaptation. Based on the role of the Barrington's nucleus in micturition and the inferior olivary complex in the regulation of fine motoric-as the main CRH-containing brainstem areas-we might assume that these areas regulate stress-induced urination and locomotion, respectively. Further studies are necessary for the field.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S05-203

MULTIMODAL ASSESSMENT OF AGING IN THE WILD-TYPE MICE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Aging varies the phenotype of cognitive, physical and organ functions. In order to correctly interpret the mouse model of age-related disorders, it is necessary to clarify the aspects affected by aging in mice. In this study, we performed multimodal experiment combined with behavioral test, magnetic resonance imaging (MRI) and histological study using wild-type mice for comprehensive understanding of aging effect. We conducted behavioral batteries assessing motor, mood, cognitive functions in 3-month-old male C57BL/6J mice and 24-month-old. For the MRI experiment, we used voxel-based morphometry (VBM) to examine the changes of regional brain volumes. For the histological study, we conducted immunohistochemistry in brain decapitated after MRI. 24-month-old mice showed lower muscle strength and open-field activity than 3-month-old mice. Although 24-month-old mice had lower long-term object memory, aversive memory did not impair in both groups. The VBM analysis showed that several cortices, putamen and substantia nigra were larger in 3-month-old mice. On the other hand, 24-month-old mice had bigger anterior commissure, lateral olfactory tract, and corticospinal tract. In the histological results, Iba1-positive cells in white matter was increased in 24-month-old mice. Regression analysis between behavior and regional white matter volume revealed that lateral lemniscus was negatively correlated with open-field activity. Inflammation of lemniscus might affect activity in open-field. According to gray matter analysis, sensory system could be associated with the locomotor behavior. In conclusion, the effects of aging on physical aspects were simple in mice, while cognitive and neurological aspects likely to require consideration of mouse characteristics.

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BOARD NUMBER: S05-204

EFFECT OF BACOPA MONNIERI ON AMYLOID-BETA INDUCED ALZHEIMER'S DISEASE-LIKE PATHOLOGICAL CHANGES

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Background: Alzheimer's disease (AD) is a late-onset neurodegenerative disorder characterized by loss of memory, disordered cognitive function, and caused by the accumulation of amyloid-beta peptide plaque and neurofibrillary tangles of hyperphosphorylated tau. Molecular pathological cause of AD includes oxidative stress, mitochondrial dysfunction, apoptosis, and dysregulation of calcium signaling. Only a few therapeutic drugs are available that intercept the symptoms, besides manifest side effects. *Bacopa monnieri* (BM) is an herb used as neuro-protectant. Several studies have shown its anti-oxidant, anti-cholinesterase, anti-apoptotic, and anti-inflammatory properties. It was also found to prevent neurodegeneration by upregulating BDNF. **Aim:** To explore the effect of BM on Amyloid-beta induced pathological changes in cellular and animal model of AD. **Methodology**: We examined the effect of BM ethanolic extract on cytotoxicity caused by aggregated amyloid-beta using SHSY-5Y cells. In the animal model, anxious behavior was examined in the Open field arena. Furthermore, memory deficits were examined using MWM and NORT tests. Neuronal density was evaluated by cresyl violet stain. **Results**: In this study, the protective effect of BM was found on cell viability. In addition, it has shown the ameliorative effect on the memory deficits and anxious symptoms caused by aggregated Aβ in rats. BM significantly increased neuronal density in hippocampal areas of the rat brain. **Conclusion**: Ethanolic extract of BM has been shown to prevent Amyloid-beta induced cellular toxicity in SHSY-5Y cells. Additionally, BM reverted the memory deficits and also ameliorated neurodegeneration caused by Amyloid-beta in animal models and a further molecular mechanism is needed to explore.

Pubmed:

34963438: Sushma , Mondal AC
Immunotherapeutic Approaches for the Treatment of Neurodegenerative Diseases: Challenges and Outcomes.
Neurodegenerative diseases, being rapidly increasing disorders and the seventh leading cause of death worldwide, have been a great challenge for researchers, affecting cognition, motor activity and other body functioning due to neurodegeneration. Several neurodegenerative diseases are caused by aggregation of proteins which induce the alteration of neuronal function leading to cell death. These proteins are amyloid-β peptide, tau, α-synuclein, and mHTT, which cause Alzheimer's disease, Frontotemporal dementia, Corticobasal degeneration, Progressive supranuclear palsy, Parkinson's disease, Multiple system atrophy, Dementia with Lewy-body and Huntington's disease. Currently available treatments only reduce symptoms and increase life sustainability; however, they possess side effects and are ineffective in curing the diseases.
CNS Neurol Disord Drug Targets, 2021;
31655116: Sushma , Mondal AC
Role of GPCR signaling and calcium dysregulation in Alzheimer's disease.
Alzheimer's disease (AD), a late onset neurodegenerative disorder is characterized by the loss of memory, disordered cognitive function, caused by accumulation of amyloid-β (Aβ) peptide and neurofibrillary tangles (NFTs) in the neocortex and hippocampal brain area. Extensive research has been done on the findings of the disease etiology or pathological causes of aggregation of Aβ and hyperphosphorylation of tau protein without much promising results. Recently, calcium dysregulation has been reported to play an important role in the pathophysiology of AD. Calcium ion acts as one of the major secondary messengers, regulates many signaling pathways involved in cell survival, proliferation, differentiation, transcription and apoptosis. Calcium signaling is one of the major signaling pathways involved in the formation of memory, generation of energy and other physiological functions. It also can modulate function of many proteins upon binding. Dysregulation in calcium homeostasis leads to many physiological changes leading to neurodegenerative diseases including AD. In AD, GPCRs generate secondary messengers which regulate calcium homeostasis inside the cell and is reported to be disturbed in the pathological condition. Calcium channels and receptors present on the plasma membrane and intracellular organelle maintain calcium homeostasis through different signaling mechanisms. In this review, we have summarized the different

calcium channels and receptors involved in calcium dysregulation which in turn play a critical role in the pathogenesis of AD. Understanding the role of calcium channels and GPCRs to maintain calcium homeostasis is an attempt to develop effective AD treatments.

Mol Cell Neurosci, 2019; 101

FENS Forum 2022
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BOARD NUMBER: S05-205

COMBINATION THERAPY OF DONEPEZIL AND ENVIRONMENTAL ENRICHMENT ON MEMORY DEFICITS IN AMYLOID-BETA-INDUCED ALZHEIMER'S DISEASE RATS

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease (AD) is progressive neurodegeneration known as the most common cause of dementia and it is the sixth leading cause of death in elderly people. Given the promising data on the additive effect of combination therapy with donepezil (Aricept), an acetylcholinesterase inhibitor (AChEI), and regarding the similar neuronal mechanisms through them donepezil and environmental enrichment (EE) exert their enhancing effects on cognition, we asked whether simultaneous treatment with two paradigms in amyloid-beta-induced AD rats may lead to greater cognitive improvements than either treatment individually. AD was induced by intrahippocampal injection of amyloid-beta (1-42, 6 µg) and donepezil was orally administrated (4 mg/kg) for 21 days. Environmental enrichment consisted of housing animals in large cages (50× 50× 50 cm) containing a running wheel and differently shaped objects for 21 days. Spatial learning and memory were assessed in the Morris water maze (MWM) and Real-time PCR was performed to assess the expression of brain-derived neurotrophic factor (BDNF) and M1 muscarinic acetylcholine receptor (AchM1R) within the hippocampus. Spatial memory was impaired in AD animals and while neither pretreatment with donepezil nor EE alone could significantly restore spatial memory scores in AD rats, combination therapy was effective. BDNF expression was suppressed in AD rats and pretreatment with donepezil plus EE could increase it to the saline levels. The data suggest that a cholinesterase inhibitor and cognitive stimulation can be used effectively in combination to improve cognitive loss in an AD rat model.

Pubmed:

34820541: Forouzanfar F, Gholami J, Foroughnia M, Payvar B, Nemati S, Khodadadegan MA, Saheb M, Hajali V
The beneficial effects of green tea on sleep deprivation-induced cognitive deficits in rats: the involvement of hippocampal antioxidant defense.
The weight of evidence suggests that sleep is essential for the processes of memory consolidation and sleep deprivation (SD) impairs the retention of long-term memory in both humans and experimental animals, which is associated with oxidative stress damage within the brain. Green tea polyphenols have revealed carcinogenic, antioxidant, anti-, and anti-mutagenic properties. We aimed to investigate the possible protective effect of green tea extract (GTE) and its main active catechin, epigallocatechin-3-gallate (EGCG), on post-training total sleep deprivation (TSD) -induced spatial memory deficits and oxidative stress profile in the hippocampus of the rat.
Heliyon, 2021; 7
34494991: Khodadadegan MA, Negah SS, Saheb M, Gholami J, Arabi MH, Hajali V
Combination effect of exercise and environmental enrichment on cognitive functions and hippocampal neurogenesis markers of rat.
Cognitive decline is one of the most prevalent health problems and is associated with increased healthcare utilization and economic burden. Physical and cognitive training both have positive effects on cognition but have been less applied in combination. We hypothesized that simultaneous cognitive-physical components would yield greater cognitive benefits than single-domain interventions in rats.
Neuroreport, 2021; 32
34232464: Moradi HR, Hajali V, Khaksar Z, Vafaee F, Forouzanfar F, Negah SS
The next step of neurogenesis in the context of Alzheimer's disease.
Among different pathological mechanisms, neuronal loss and neurogenesis impairment in the hippocampus play important roles in cognitive decline in Alzheimer's disease (AD). AD is a progressive and complex neurodegenerative diseases, which is very debilitating. The purpose of this paper is to review recent research into neurogenesis and AD and discuss how pharmacological drugs and herbal active components have impacts on neurogenesis and consequently improve cognitive functions. To date, despite huge research, no effective treatment has been approved for AD. Therefore, an avenue for future research and drug discovery is stimulating adult hippocampal neurogenesis (AHN). Evidence suggests that neurogenesis is

regulated by the pharmacological treatment that may be recommended as a part of prophylaxis and therapeutic options for AD. However, the underlying mechanisms of regulating neurogenesis in AD are not well understood. To this point, we highlight to achieve an efficient treatment in AD by manipulating neurogenesis, it's necessary to target all steps of neurogenesis.
Mol Biol Rep, 2021; 48
31502112: Sahab-Negah S, Hajali V, Moradi HR, Gorji A
The Impact of Estradiol on Neurogenesis and Cognitive Functions in Alzheimer's Disease.
Alzheimer's disease (AD) is described as cognitive and memory impairments with a sex-related epidemiological profile, affecting two times more women than men. There is emerging evidence that alternations in the hippocampal neurogenesis occur at the early stage of AD. Therapies that may effectively slow, stop, or regenerate the dying neurons in AD are being extensively investigated in the last few decades, but none has yet been found to be effective. The regulation of endogenous neurogenesis is one of the main therapeutic targets for AD. Mounting evidence indicates that the neurosteroid estradiol (17β-estradiol) plays a supporting role in neurogenesis, neuronal activity, and synaptic plasticity of AD. This effect may provide preventive and/or therapeutic approaches for AD. In this article, we discuss the molecular mechanism of potential estradiol modulatory action on endogenous neurogenesis, synaptic plasticity, and cognitive function in AD.
Cell Mol Neurobiol, 2020; 40
32405349: Ghadiri T, Gorji A, Vakilzadeh G, Hajali V, Khodagholi F, Sharifzadeh M
Neuronal injury and death following focal mild brain injury: The role of network excitability and seizure.
While traumatic brain injury (TBI) is a predisposing factor for development of post-traumatic epilepsy (PTE), the occurrence of seizures following brain trauma can infuriate adverse consequences of brain injury. However, the effect of seizures in epileptogenesis after mild TBI cannot yet be accurately confirmed. This study was designed to investigate the histopathological and molecular modifications induced by seizures on traumatized brain.
Iran J Basic Med Sci, 2020; 23
31325580: Ghadiri T, Vakilzadeh G, Hajali V, Khodagholi F
Progesterone modulates post-traumatic epileptogenesis through regulation of BDNF-TrkB signaling and cell survival-related pathways in the rat hippocampus.
Female sex hormone, progesterone, in addition to seizure modifying activity is also known as a potential protective agent against various brain injury conditions. Considering the predisposal role of traumatic brain injury (TBI) on developing post-traumatic epilepsy (PTE), the effect of progesterone on post-traumatic epileptogenesis is not investigated yet. Male Wistar rats were given a moderate focal weight drop injury (500 gr) or sham surgery and then progesterone (16 and 32mg/kg) was given daily for two consecutive weeks. On day 15 of injury, seizures were induced by administration of a GABAA receptor antagonist, pentylenetetrazole (PTZ, 30 mg/kg). Seizures were then assessed over a 1-h period using the Racine clinical rating scale. Traumatized animals that received 32 mg/kg progesterone had reduced score, duration of seizures and almost did not show tonic-clonic seizures during 60 min versus the untreated trauma group. In line with behavioral alterations, 32 mg/kg progesterone enhanced the amount of Nrf2 and HO-1 proteins and decreased the level of NF-kB, BDNF, Caspase 3 and ratio of Bax/Bcl-2 in the ipsilateral hippocampus. Additionally, the number of TUNEL-positive apoptotic cells, as well as injured dark neurons in the parietal cortex and hippocampal CA1 of 32 mg/kg-treated animals showed a significant reduction. Administration of 16 mg/kg progesterone elevated production of BDNF, Bax and Caspase 3 and decreased anti-apoptotic Bcl-2 protein. Taken together, an early administration of 32 mg/kg of progesterone after TBI for two weeks post-injury modified seizure activity. Our findings suggest that post-traumatic anti-epileptogenesis property of a high dose of progesterone partly occurs through the manipulation of BDNF-TrkB axis along with control of cell survival pathways.
Neurosci Lett, 2019; 709
34466469: Khalifeh S, Khodamoradi M, Hajali V, Ghazvini H, Eliasy L, Kheradmand A, Farnia V, Akhtari J, Shahveisi K, Ghalehnoei H
Naloxone Ameliorates Spatial Memory Deficits and Hyperthermia Induced by a Neurotoxic Methamphetamine Regimen in Male Rats.
Methamphetamine (METH) as a synthetic psychostimulant is being increasingly recognized as a worldwide problem, which may induce memory impairment. On the other hand, it is well established that naloxone, an opiate antagonist, has some beneficial effects on learning and memory. The present research aimed at evaluating naloxone effects on spatial learning and memory impairment triggered by a neurotoxic regimen of METH in male rats.
Galen Med J, 2019; 8
30597139: Hajali V, Andersen ML, Negah SS, Sheibani V
Sex differences in sleep and sleep loss-induced cognitive deficits: The influence of gonadal hormones.
Males and females can respond differentially to the same environmental stimuli and experimental conditions. Chronic sleep loss is a frequent and growing problem in many modern societies and has a broad variety of negative outcomes for health

and well-being. While much has been done to explore the deleterious effects of sleep deprivation (SD) on cognition in both human and animal studies over the last few decades, very little attention has been paid to the part played by sex differences and gonadal steroids in respect of changes in cognitive functions caused by sleep loss. The effects of gonadal hormones on sleep regulation and cognitive performances are well established. Reduced gonadal function in menopausal women and elderly men is associated with sleep disturbances and cognitive decline as well as dementia, which suggests that sex steroids play a key role in modulating these conditions. Finding out whether there are sex differences in respect of the effect of insufficient sleep on cognition, and how neuroendocrine mediators influence cognitive impairment induced by SD could provide valuable insights into the best therapies for each sex. In this review, we aim to highlight the involvement of sex differences and gonadal hormone status on the severity of cognitive deficits induced by sleep deficiency in both human and animal studies.
Horm Behav, 2019; 108

30472346: Aghaei I, Hajali V, Haghani M, Vaziri Z, Moosazadeh M, Shabani M
Peroxisome proliferator-activated receptor-γ activation attenuates harmaline-induced cognitive impairments in rats.
Cognitive and motor disturbances are serious concerns of the tremors induced by motor disorders. Despite the lack of effective clinical treatment, some potential therapeutic agents have been used to alleviate the cognitive symptoms in the animal models of tremor. Recent studies have shown that PPAR-γ agonists have neuroprotective effects. In the current study, the effects of pioglitazone (PIO), a peroxisome proliferator-activated receptor gamma agonist, on harmaline-induced motor and cognitive impairment were studied. Male Wistar rats were divided into vehicle (normal saline), PIO (20 mg/kg i.p.), harmaline (10 mg/kg, i.p.) and PIO + harmaline (PIO injected 2 h before harmaline) groups. Open field, rotarod, wire grip, foot print and Morris water maze tests were used to evaluate the motor and cognitive performance. The results indicated that administration of PIO attenuated harmaline-induced locomotor, anxiety-like behaviors, and spatial learning and memory impairments, but it partially decreased the tremor score. The neuroprotective and anxiolytic effects of PIO demonstrated in the current study can offer the PPAR-γ receptor agonism as a potential therapeutic agent in the treatment of patients with tremor that manifest mental dysfunction.
J Clin Neurosci, 2019; 59

26704786: Aghaei I, Hajali V, Dehpour A, Haghani M, Sheibani V, Shabani M
Alterations in the intrinsic electrophysiological properties of Purkinje neurons in a rat model of hepatic encephalopathy: Relative preventing effect of PPARγ agonist.
Patients suffering from hepatic cirrhosis (HC) have been shown to have motor and cognitive impairments. The cerebellum, which controls coordinated and rapid movements, is a potential target for the deleterious effects of hyperammonemia induced by bile duct ligation. Therefore, the aim of this study was to determine the mechanisms of motor impairments observed in a rat model of HC and second objective of the current study was to evaluate the possible protective effect of pioglitazone (PIO) on these impairments. Male Wistar rats were used in the current study. Bile duct ligation (BDL) surgery was performed and pioglitazone administration was started two weeks after the surgery for the next four weeks. The effects of pioglitazone on BDL-induced electrophysiological changes of the Purkinje cerebellum neurons were evaluated by Whole-cell patch clamp recordings. Purkinje neurons from the BDL group exhibited significant changes in a number of electrophysiological properties and some alterations partially were counteracted by activation of peroxisome proliferator-activated receptor-γ. Purkinje cells from BDL groups showed a significant increase in the spontaneous firing frequency followed by a decrease in the action potential duration of half-amplitude and spike interval. Chronic administration of pioglitazone could contract this effect of BDL on event frequency and interevent interval, though the difference with the sham group was still significant in the duration of action potential. Results of the current study raise the possibility that BDL may profoundly affect the intrinsic membrane properties of the cerebellar Purkinje neurons and PIO administration can counteract some of these effects.
Brain Res Bull, 2016; 121

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BOARD NUMBER: S05-206

INHIBITORS TARGETING THE INFLAMMASOME AND PYROPTOSIS FOR INTERVENING IN ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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NLRP3 inflammasome is an essential component of innate immunity and its activation leads to the production of interleukin (IL)-1β and IL-18, and promotes inflammatory cell death. Recent studies have indicated a critical role for the dysregulation of NLRP3 inflammasome in the pathogenesis of Alzheimer's disease (AD), where neuroinflammation has been recognized as an essential player. Thus, novel NLRP3 selective inhibitors (NSIs) represent a promising approach to develop AD therapeutics. Our laboratory has recently successfully developed a sulfonamide-containing scaffold into a series of NSIs. Through medicinal chemistry campaign, two generations of compounds have been designed and biologically characterized, and several lead NSIs were identified. Based on the accumulated SAR information, we have designed a new scaffold with improved solubility and drug-like properties to develop NSIs with therapeutic potentials. Through itinerary optimizations, a new lead NSI was identified to bind to the recombinant NLRP3 with a K_D of 10 nM and to inhibit the release of IL-1β from J774A.1 cells with an IC_{50} of 200 nM. Further studies in mice using a PET radiotracer based on this lead structure also demonstrated its brain penetration and selective labeling of the NLRP3 inflammasome in the brain. Our studies also confirmed its in vivo target engagement and selectivity. Collectively, the results strongly encourage developing new analogs based on this novel chemical scaffold as novel NSIs and potential AD therapeutics.

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MEMORY AND VACHT EXPRESSION ARE MODULATED BY STANDARDIZED EXTRACT OF GINKGO BILOBA: A THERAPEUTIC ALTERNATIVE TO ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

Beatriz Muratori[1], Irina Emanuela Da Veiga[1], Gleiciene Medeiros[1], Marco Antônio Prado[2], Vânia Prado[2], Carla Prado[3], Suzete Cerutti[1]
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Alzheimer's disease (AD) is a complex neurodegenerative disorder associated with normal aging that is more prevalent in women. Cholinergic neurotransmission is decreased in patients with AD. It has previously shown that mice with decreased VAChT expression (Knockdown, VAChT KD), a protein necessary for acetylcholine uptake into vesicles, show decreased cholinergic signaling. VAChT KD^{HOM} and VAChT KD^{HET} mice show 65% and 45% decrease in VAChT expression when compared to WT. These mutant mice showed learning and memory impairments, suggesting that they could be used to screen for potential therapeutic drugs to alleviate cognitive deficits in AD. Previous data from our group showed that standardized extract of *Ginkgo biloba* (EGb) improved fear memory retention as well as object recognition memory (ORM) in rats. In this study, we aimed to evaluate whether EGb improves memory in VAChT KD animals. Three-month-old female VAChT KD^{HOM}, VAChT KD^{HET} and WT mice were treated with EGb (250, 500 and 1000 mg/Kg) or vehicle for 30 days and subjected to memory testing and immunohistochemistry for VAChT expression in CA1, CA3 and dentate gyrus of dorsal hippocampal formation. All genotypes treated with EGb showed improved object recognition memory. Further, KD^{HOM} + EGb showed higher expression of VAChT in dentate gyrus. Together, our data suggest that EGb might be used as an alternative for treatment of AD.

Pubmed:
34206011: Muratori BG, Zamberlam CR, Mendes TB, Nozima BHN, Cerutti JM, Cerutti SM
BDNF as a Putative Target for Standardized Extract of -Induced Persistence of Object Recognition Memory.
Despite considerable progress on the study of the effect of standardized extract of (EGb) on memory processes, our understanding of its role in the persistence of long-term memory (LTM) and the molecular mechanism underlying its effect, particularly episodic-like memory, is limited. We here investigated the effects of EGb on the long-term retention of recognition memory and its persistence and BDNF expression levels in the dorsal hippocampal formation (DHF). Adult male Wistar rats (n = 10/group) were handled for 10 min/5 day. On day 6, the animals were treated with vehicle or 0.4 mg/kg diazepam (control groups) or with EGb (250, 500 or 100 mg/kg) 30 min before the training session (TR1), in which the animals were exposed to two sample objects. On day 7, all rats underwent a second training session (TR2) as described in the TR1 but without drug treatment. Object recognition memory (ORM) was evaluated on day 8 (retention test, T1) and day 9 (persistence test, T2). At the end of T1or T2, animals were decapitated, and DHF samples were frozen at -80 °C for analyses of the differential expression of BDNF by Western blotting. EGb-treated groups spent more time exploring the novel object in T2 and showed the highest recognition index (RI) values during the T1 and T2, which was associated with upregulation of BDNF expression in the DHF in a dose-and session-dependent manner. Our data reveal, for the first time, that EGb treatment before acquisition of ORM promotes persistence of LTM by BDNF differential expression.
Molecules, 2021; 26

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BOARD NUMBER: S05-208

THE PROTECTIVE ACTIONS OF DHEA/S AND BDNF AGAINST OXIDATIVE STRESS IN AN IN VITRO MODEL OF VASCULAR DEMENTIA

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

Barbara Vuić[1], Matea Nikolac Perkovic[1], Gordana Nedic Erjavec[1], Lucija Tudor[1], Tina Milos[2], Nela Pivac[2], Marcela Konjevod[2], Dubravka Svob Strac[1]
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Vascular dementia (VaD), the second most common form of dementia, is generally underrecognized and still poorly understood disease. It develops when the brain´s blood supply is blocked or reduced, causing deprivation of oxygen and nutrients and resulting in damage and death of neurons. The brain is highly susceptible to oxidative stress, due to its richness in fatty acids sensitive to peroxidation, as well as due to high oxygen consumption and free radicals accumulation. Oxidative stress in VaD, characterized by the exacerbated production of reactive oxygen species and insufficient antioxidant defense system, increases neuronal cell abnormalities and triggers apoptosis, leading to cognitive dysfunction and dementia. Neurosteroids dehydroepiandrosterone and dehydroepiandrosterone sulfate (DHEA/S) and neurotrophin brain-derived neurotrophic factor (BDNF) have attracted the attention of researchers investigating their involvement in various brain functions such as neural survival, plasticity, cognition and behavior. The aim of this study was to investigate the protective potential of DHEA/S and BDNF in promoting neuronal survival and preventing oxidative stress caused by brain ischemia, a common characteristic of VaD. Oxygen-glucose deprivation (OGD) was performed in primary mouse neurons derived from C57BL/6 mice, and human SH-SY5Y neuroblastoma cells as *in vitro* model of VaD. The cells were cultured in glucose- and serum-free medium in a modular incubator chamber filled with N_2. Before or after OGD, cells were treated with DHEA/S and BDNF and cell viability and oxidative stress parameters were determined. Our results suggested protective effects of DHEA/S and BDNF against oxidative damage induced by the ischemic injury in neuronal cells.

Pubmed:
34093032: Svob Strac D, Konjevod M, Sagud M, Nikolac Perkovic M, Nedic Erjavec G, Vuic B, Simic G, Vukic V, Mimica N, Pivac N
Personalizing the Care and Treatment of Alzheimer's Disease: An Overview.
Alzheimer's disease (AD) is a progressive, complex, and multifactorial neurodegenerative disorder, still without effective and stable therapeutic strategies. Currently, available medications for AD are based on symptomatic therapy, which include acetylcholinesterase (AChE) inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonist. Additionally, medications such as antipsychotic drugs, antidepressants, sedative, and hypnotic agents, and mood stabilizers are used for the management of behavioral and psychological symptoms of dementia (BPSD). Clinical research has been extensively investigated treatments focusing on the hallmark pathology of AD, including the amyloid deposition, tau hyperphosphorylation, neuroinflammation, and vascular changes; however, so far without success, as all new potential drugs failed to show significant clinical benefit. The underlying heterogeneous etiology and diverse symptoms of AD suggest that a precision medicine strategy is required, which would take into account the complex genetic, epigenetic, and environmental landscape of each AD patient. The article provides a comprehensive overview of the literature on AD, the current and potential therapy of both cognitive symptoms as well as BPSD, with a special focus on gut microbiota and epigenetic modifications as new emerging drug targets. Their specific patterns could represent the basis for novel individually tailored approaches aimed to optimize precision medicine strategies for AD prevention and treatment. However, the successful application of precision medicine to AD demands a further extensive research of underlying pathological processes, as well as clinical and biological complexity of this multifactorial neurodegenerative disorder.
Pharmgenomics Pers Med, 2021; 14

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-209

CHRONIC CARBONIC ANHYDRASE INHIBITION IMPROVES COGNITIVE FUNCTION AND REDUCES NEUROPATHOLOGICAL HALLMARKS IN TGCNRD8 MICE, A PRECLINICAL MODEL OF ALZHEIMER'S DISEASE.

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Background: A series of recent observations are shedding light on the involvement of brain carbonic anhydrases (CAs) in neurodegenerative disorders. For instance, a higher expression of the CAII isoform was found in the plasma of Alzheimer's Disease (AD) patients when compared to age-matched control subjects. Moreover, two CA inhibitors (CAIs) prevented Aβ-induced mitochondrial toxicity and cell death in vitro. Despite these encouraging findings, the impact of CAIs in AD animal models were not yet investigated. Aims: To evaluate the efficacy of a pharmacological intervention with acetazolamide (ACTZ), a well-known CAI, against the cognitive impairments and neuropathological alterations observed in TgCRND8 mice. Methods: TgCNRD8 mice (expressing the human amyloid precursor protein carrying the Swedish and Indiana *mutations*) were fed with control or ACTZ-enriched diets (100 and 200 ppm) starting at 6 weeks of age. After 6 or 12 weeks of treatment, animals' cognitive function was evaluated in the social discrimination paradigm. Then, their brains were collected for neurochemical analysis. Results: Chronic ACTZ treatment prevented the cognitive deficits observed in TgCNRD8 mice at both ages. A reduction in the number of β-amyloid plaques (Aβ1-42), in the expression of the β-amyloid pyroglutamic derivative (Aβ N3pE) and in Tau phosphorylation (AT8) were also observed in ACTZ-fed animals compared to the animals fed with control diet. Chronic ACTZ treatment also reduced the levels of caspase-3 in the same areas. Conclusions: Chronic CAIs prevented both behavioral and neuropathological alterations observed in TgCNRD8 animals, suggesting their role as innovative compounds for AD treatment.

Pubmed:

33918940: Rani B, Silva-Marques B, Leurs R, Passani MB, Blandina P, Provensi G
Short- and Long-Term Social Recognition Memory Are Differentially Modulated by Neuronal Histamine.
The ability of recognizing familiar conspecifics is essential for many forms of social interaction including reproduction, establishment of dominance hierarchies, and pair bond formation in monogamous species. Many hormones and neurotransmitters have been suggested to play key roles in social discrimination. Here we demonstrate that disruption or potentiation of histaminergic neurotransmission differentially affects short (STM) and long-term (LTM) social recognition memory. Impairments of LTM, but not STM, were observed in histamine-deprived animals, either chronically (mice lacking the histamine-synthesizing enzyme histidine decarboxylase) or acutely (mice treated with the HDC irreversible inhibitor α-fluoromethylhistidine). On the contrary, restriction of histamine release induced by stimulation of the HR agonist (VUF16839) impaired both STM and LTM. HR agonism-induced amnesic effect was prevented by pre-treatment with donepezil, an acetylcholinesterase inhibitor. The blockade of the HR with ciproxifan, which in turn augmented histamine release, resulted in a procognitive effect. In keeping with this hypothesis, the procognitive effect of ciproxifan was absent in both and αFMH-treated mice. Our results suggest that brain histamine is essential for the consolidation of LTM but not STM in the social recognition test. STM impairments observed after HR stimulation are probably related to their function as heteroreceptors on cholinergic neurons.
Biomolecules, 2021; 11
32571910: Schmidt SD, Costa A, Rani B, Godfried Nachtigall E, Passani MB, Carta F, Nocentini A, de Carvalho Myskiw J, Furini CRG, Supuran CT, Izquierdo I, Blandina P, Provensi G
The role of carbonic anhydrases in extinction of contextual fear memory.
Carbonic anhydrases (CAs; EC 4.2.1.1) are metalloenzymes present in mammals with 16 isoforms that differ in terms of catalytic activity as well as cellular and tissue distribution. CAs catalyze the conversion of CO to bicarbonate and protons and are involved in various physiological processes, including learning and memory. Here we report that the integrity of CA activity in the brain is necessary for the consolidation of fear extinction memory. We found that systemic administration of

acetazolamide, a CA inhibitor, immediately after the extinction session dose-dependently impaired the consolidation of fear extinction memory of rats trained in contextual fear conditioning. d-phenylalanine, a CA activator, displayed an opposite action, whereas C18, a membrane-impermeable CA inhibitor that is unable to reach the brain tissue, had no effect. Simultaneous administration of acetazolamide fully prevented the procognitive effects of d-phenylalanine. Whereas d-phenylalanine potentiated extinction, acetazolamide impaired extinction also when infused locally into the ventromedial prefrontal cortex, basolateral amygdala, or hippocampal CA1 region. No effects were observed when acetazolamide or d-phenylalanine was infused locally into the substantia nigra pars compacta. Moreover, systemic administration of acetazolamide immediately after the extinction training session modulated c-Fos expression on a retention test in the ventromedial prefrontal cortex of rats trained in contextual fear conditioning. These findings reveal that the engagement of CAs in some brain regions is essential for providing the brain with the resilience necessary to ensure the consolidation of extinction of emotionally salient events.
Proc Natl Acad Sci U S A, 2020; 117

31554165: Provensi G, Carta F, Nocentini A, Supuran CT, Casamenti F, Passani MB, Fossati S
A New Kid on the Block? Carbonic Anhydrases as Possible New Targets in Alzheimer's Disease.
The increase in the incidence of neurodegenerative diseases, in particular Alzheimer's Disease (AD), is a consequence of the world's population aging but unfortunately, existing treatments are only effective at delaying some of the symptoms and for a limited time. Despite huge efforts by both academic researchers and pharmaceutical companies, no disease-modifying drugs have been brought to the market in the last decades. Recently, several studies shed light on Carbonic Anhydrases (CAs, EC 4.2.1.1) as possible new targets for AD treatment. In the present review we summarized preclinical and clinical findings regarding the role of CAs and their inhibitors/activators on cognition, aging and neurodegeneration and we discuss future challenges and opportunities in the field.
Int J Mol Sci, 2019; 20

31010921: Provensi G, Schmidt SD, Boehme M, Bastiaanssen TFS, Rani B, Costa A, Busca K, Fouhy F, Strain C, Stanton C, Blandina P, Izquierdo I, Cryan JF, Passani MB
Preventing adolescent stress-induced cognitive and microbiome changes by diet.
Psychological stress during adolescence may cause enduring cognitive deficits and anxiety in both humans and animals, accompanied by rearrangement of numerous brain structures and functions. A healthy diet is essential for proper brain development and maintenance of optimal cognitive functions during adulthood. Furthermore, nutritional components profoundly affect the intestinal community of microbes that may affect gut-brain communication. We adopted a relatively mild stress protocol, social instability stress, which when repeatedly administered to juvenile rats modifies cognitive behaviors and plasticity markers in the brain. We then tested the preventive effect of a prolonged diet enriched with the ω-3 polyunsaturated fatty acids eicosapentaenoic acid, docosahexaenoic acid, and docosapentaenoic acid and vitamin A. Our findings highlight the beneficial effects of this enriched diet on cognitive memory impairment induced by social instability stress, as stressed rats fed the enriched diet exhibited performance undistinguishable from that of nonstressed rats on both emotional and reference memory tests. Furthermore, in stressed rats, the decline in brain-derived neurotrophic factor expression in the hippocampus and shifts in the microbiota composition were normalized by the enriched diet. The detrimental behavioral and neurochemical effects of adolescent stress, as well as the protective effect of the enriched diet, were maintained throughout adulthood, long after the exposure to the stressful environment was terminated. Taken together, our results strongly suggest a beneficial role of nutritional components in ameliorating stress-related behaviors and associated neurochemical and microbiota changes, opening possible new venues in the field of nutritional neuropsychopharmacology.
Proc Natl Acad Sci U S A, 2019; 116

30318340: Misto A, Provensi G, Vozella V, Passani MB, Piomelli D
Mast Cell-Derived Histamine Regulates Liver Ketogenesis via Oleoylethanolamide Signaling.
The conversion of lipolysis-derived fatty acids into ketone bodies (ketogenesis) is a crucial metabolic adaptation to prolonged periods of food scarcity. The process occurs primarily in liver mitochondria and is initiated by fatty-acid-mediated stimulation of the ligand-operated transcription factor, peroxisome proliferator-activated receptor-α (PPAR-α). Here, we present evidence that mast cells contribute to the control of fasting-induced ketogenesis via a paracrine mechanism that involves secretion of histamine into the hepatic portal circulation, stimulation of liver H receptors, and local biosynthesis of the high-affinity PPAR-α agonist, oleoylethanolamide (OEA). Genetic or pharmacological interventions that disable any one of these events, including mast cell elimination, deletion of histamine- or OEA-synthesizing enzymes, and H blockade, blunt ketogenesis without affecting lipolysis. The results reveal an unexpected role for mast cells in the regulation of systemic fatty-acid homeostasis, and suggest that OEA may act in concert with lipolysis-derived fatty acids to activate liver PPAR-α and promote ketogenesis.
Cell Metab, 2019; 29

30110713: Provensi G, Passani MB, Costa A, Izquierdo I, Blandina P
Neuronal histamine and the memory of emotionally salient events.

In this review, we describe the experimental paradigms used in preclinical studies to unravel the histaminergic brain circuits that modulate the formation and retrieval of memories associated with aversive events. Emotionally arousing events, especially bad ones, are remembered more accurately, clearly and for longer periods of time than neutral ones. Maladaptive elaborations of these memories may eventually constitute the basis of psychiatric disorders such as generalized anxiety, obsessive-compulsive disorders and post-traumatic stress disorder. A better understanding of the role of the histaminergic system in learning and memory has not only a theoretical significance but also a translational value. Ligands of histamine receptors are among the most used drugs worldwide; hence, understanding the impact of these compounds on learning and memory may help improve their pharmacological profile and unravel unexplored therapeutic applications. LINKED ARTICLES: This article is part of a themed section on New Uses for 21st Century. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v177.3/issuetoc.
Br J Pharmacol, 2020; 177
29596898: Costa A, Cristiano C, Cassano T, Gallelli CA, Gaetani S, Ghelardini C, Blandina P, Calignano A, Passani MB, Provensi G
Histamine-deficient mice do not respond to the antidepressant-like effects of oleoylethanolamide.
It has been suggested that the bioactive lipid mediator oleoylethanolamide (OEA), a potent agonist of the peroxisome proliferator-activated receptor-alpha (PPAR-α) possesses anti-depressant-like effects in several preclinical models. We recently demonstrated that several of OEA's behavioural actions require the integrity of the brain histaminergic system, and that an intact histaminergic neurotransmission is specifically required for selective serotonin re-uptake inhibitors to exert their anti-depressant-like effect. The purpose of our study was to test if OEA requires the integrity of the histaminergic neurotransmission to exert its antidepressant-like effects. Immobility time in the tail suspension test was measured to assess OEA's potential (10 mg/kg i.p.) as an antidepressant drug in histidine decarboxylase null (HDC) mice and HDC littermates, as well as in PPAR-α and PPAR-α mice. CREB phosphorylation was evaluated using Western blot analysis in hippocampal and cortical homogenates, as pCREB is considered partially responsible for the efficacy of antidepressants. Serotonin release from ventral hippocampi of HDC and HDC mice was measured with in-vivo microdialysis, following OEA administration. OEA decreased immobility time and increased brain pCREB levels in HDC mice, whereas it was ineffective in HDC mice. Comparable results were obtained in PPAR-α and PPAR-α mice. Microdialysis revealed a dysregulation of serotonin release induced by OEA in HDC mice. Our observations corroborate our hypothesis that brain histamine and signals transmitted by OEA interact to elaborate appropriate behaviours and may be the basis for the efficacy of OEA as an antidepressant-like compound.
Neuropharmacology, 2018; 135
28339575: Provensi G, Fabbri R, Munari L, Costa A, Baldi E, Bucherelli C, Blandina P, Passani MB
Histaminergic Neurotransmission as a Gateway for the Cognitive Effect of Oleoylethanolamide in Contextual Fear Conditioning.
The integrity of the brain histaminergic system is necessary for the unfolding of homeostatic and cognitive processes through the recruitment of alternative circuits with distinct temporal patterns. We recently demonstrated that the fat-sensing lipid mediator oleoylethanolamide indirectly activates histaminergic neurons to exerts its hypophagic effects. The present experiments investigated whether histaminergic neurotransmission is necessary also for the modulation of emotional memory induced by oleoylethanolamide in a contextual fear conditioning paradigm.
Int J Neuropsychopharmacol, 2017; 20
27291828: Provensi G, Costa A, Passani MB, Blandina P
Donepezil, an acetylcholine esterase inhibitor, and ABT-239, a histamine H3 receptor antagonist/inverse agonist, require the integrity of brain histamine system to exert biochemical and procognitive effects in the mouse.
Histaminergic H3 receptors (H3R) antagonists enhance cognition in preclinical models and modulate neurotransmission, in particular acetylcholine (ACh) release in the cortex and hippocampus, two brain areas involved in memory processing. The cognitive deficits seen in aging and Alzheimer's disease have been associated with brain cholinergic deficits. Donepezil is one of the acetylcholinesterase (AChE) inhibitor approved for use across the full spectrum of these cognitive disorders. We addressed the question if H3R antagonists and donepezil require an intact histamine neuronal system to exert their procognitive effects. The effect of the H3R antagonist ABT-239 and donepezil were evaluated in the object recognition test (ORT), and on the level of glycogen synthase kinase 3 beta (GSK-3β) phosphorylation in normal and histamine-depleted mice. Systemic administration of ABT-239 or donepezil ameliorated the cognitive performance in the ORT. However, these compounds were ineffective in either genetically (histidine decarboxylase knock-out, HDC-KO) or pharmacologically, by means of intracerebroventricular (i.c.v.) injections of the HDC irreversible inhibitor a-fluoromethylhistidine (a-FMHis), histamine-deficient mice. Western blot analysis revealed that ABT-239 or donepezil systemic treatments increased GSK-3β phosphorylation in cortical and hippocampal homogenates of normal, but not of histamine-depleted mice. Furthermore, administration of the PI3K inhibitor LY294002 that blocks GSK-3β phosphorylation, prevented the procognitive effects of both

drugs in normal mice. Our results indicate that both donepezil and ABT-239 require the integrity of the brain histaminergic system to exert their procognitive effects and strongly suggest that impairments of PI3K/AKT/GSK-3β intracellular pathway activation is responsible for the inefficacy of both drugs in histamine-deficient animals.
Neuropharmacology, 2016; 109
25049422: Provensi G, Coccurello R, Umehara H, Munari L, Giacovazzo G, Galeotti N, Nosi D, Gaetani S, Romano A, Moles A, Blandina P, Passani MB
Satiety factor oleoylethanolamide recruits the brain histaminergic system to inhibit food intake.
Key factors driving eating behavior are hunger and satiety, which are controlled by a complex interplay of central neurotransmitter systems and peripheral stimuli. The lipid-derived messenger oleoylethanolamide (OEA) is released by enterocytes in response to fat intake and indirectly signals satiety to hypothalamic nuclei. Brain histamine is released during the appetitive phase to provide a high level of arousal in anticipation of feeding, and mediates satiety. However, despite the possible functional overlap of satiety signals, it is not known whether histamine participates in OEA-induced hypophagia. Using different experimental settings and diets, we report that the anorexiant effect of OEA is significantly attenuated in mice deficient in the histamine-synthesizing enzyme histidine decarboxylase (HDC-KO) or acutely depleted of histamine via interocerebroventricular infusion of the HDC blocker α-fluoromethylhistidine (α-FMH). α-FMH abolished OEA-induced early occurrence of satiety onset while increasing histamine release in the CNS with an H3 receptor antagonist-increased hypophagia. OEA augmented histamine release in the cortex of fasted mice within a time window compatible to its anorexic effects. OEA also increased c-Fos expression in the oxytocin neurons of the paraventricular nuclei of WT but not HDC-KO mice. The density of c-Fos immunoreactive neurons in other brain regions that receive histaminergic innervation and participate in the expression of feeding behavior was comparable in OEA-treated WT and HDC-KO mice. Our results demonstrate that OEA requires the integrity of the brain histamine system to fully exert its hypophagic effect and that the oxytocin neuron-rich nuclei are the likely hypothalamic area where brain histamine influences the central effects of OEA.
Proc Natl Acad Sci U S A, 2014; 111

FENS Forum 2022
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BOARD NUMBER: S05-210

VOLUNTARY PHYSICAL EXERCISE REGULATES IRON HOMEOSTASIS IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

Irina Belaya[1], Nina Kucháriková[1], Veronika Górová[1], Kai Kysenius[2], Dominic Hare[3,4], Peter Crouch[2], Tarja Malm[1], Mustafa Atalay[5], Anthony White[6], Jeffrey Liddell[2], Katja Kanninen[1]
[1]University of Eastern Finland, A.i. Virtanen Institute For Molecular Sciences, Kuopio, Finland, [2]The University of Melbourne, Department Of Biochemistry And Pharmacology, Melbourne, Australia, [3]The University of Melbourne, School Of Biosciences, Melbourne, Australia, [4]University of Technology Sydney, Atomic Medicine Initiative, Sydney, Australia, [5]University of Eastern Finland, Institute Of Biomedicine, Kuopio, Finland, [6]QIMR Berghofer Medical Research Institute, Mental Health Program, Brisbane, Australia

Iron dyshomeostasis is one of the pathological features of Alzheimer's disease (AD). Excess iron is involved in amyloid beta (Aβ) production and glial cell activation. Moreover, in response to inflammation and iron overload, astrocytes express hepcidin, a main regulator of iron metabolism, and this expression depends on the interleukin-6 (IL-6)/STAT3 pathway. Physical exercise has been demonstrated to have positive effects on cognition, Aβ load, inflammation, and glial activation. Moreover, iron is crucial in oxygen transport and regular exercise may positively affect iron homeostasis, but the underlying mechanisms are still unclear, especially in the context of AD. The aim of this study was to investigate how voluntary physical exercise modulates iron metabolism in the brain and periphery of the 5xFAD mouse model of AD. The 5xFAD male mice and their wild-type (WT) littermates were subjected to voluntary running for 6 months from 1.5 months of age. We measured total iron levels and changes in expression of proteins involved in iron homeostasis in the brain and plasma samples via a variety of biochemical techniques. Our results demonstrate an exercise-induced reduction of Aβ load and an alteration in iron metabolism in the cortex of 5xFAD mice. We show that exercise has an effect on iron metabolism, reducing iron storage protein and hepcidin possibly via an attenuation of the IL-6/STAT3 pathway. Taken together, these findings suggest that voluntary physical exercise modulates iron homeostasis in both WT and AD mice. The molecular pathways involved in this modulation could be possible therapeutic targets against AD.

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BOARD NUMBER: S05-211

PARP MUTATIONS PROTECT FROM MITOCHONDRIAL TOXICITY IN ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease is the most common age-related neurodegenerative disorder. Familial forms of Alzheimer's disease associated with the accumulation of a toxic form of amyloid-β (Aβ) peptides are linked to mitochondrial impairment. The coenzyme nicotinamide adenine dinucleotide (NAD^+) is essential for both mitochondrial bioenergetics and nuclear DNA repair through NAD^+-consuming poly (ADP-ribose) polymerases (PARPs). Here we analysed the metabolomic changes in flies overexpressing Aβ and showed a decrease of metabolites associated with nicotinate and nicotinamide metabolism, which is critical for mitochondrial function in neurons. We show that increasing the bioavailability of NAD^+ protects against Aβ toxicity. Pharmacological supplementation using NAM, a form of vitamin B that acts as a precursor for NAD^+ or a genetic mutation of PARP rescues mitochondrial defects, protects neurons against degeneration and reduces behavioural impairments in a fly model of Alzheimer's disease. Next, we looked at links between PARP polymorphisms and vitamin B intake in patients with Alzheimer's disease. We show that polymorphisms in the human *PARP1* gene or the intake of vitamin B are associated with a decrease in the risk and severity of Alzheimer's disease. We suggest that enhancing the availability of NAD^+ by either vitamin B supplements or the inhibition of NAD^+-dependent enzymes such as PARPs are potential therapies for Alzheimer's disease.

Pubmed:

33120349: Travaglio M, Yu Y, Popovic R, Selley L, Leal NS, Martins LM
Links between air pollution and COVID-19 in England.
In December 2019, a novel disease, coronavirus disease 19 (COVID-19), emerged in Wuhan, People's Republic of China. COVID-19 is caused by a novel coronavirus (SARS-CoV-2) presumed to have jumped species from another mammal to humans. This virus has caused a rapidly spreading global pandemic. To date, over 300,000 cases of COVID-19 have been reported in England and over 40,000 patients have died. While progress has been achieved in managing this disease, the factors in addition to age that affect the severity and mortality of COVID-19 have not been clearly identified. Recent studies of COVID-19 in several countries identified links between air pollution and death rates. Here, we explored potential links between major fossil fuel-related air pollutants and SARS-CoV-2 mortality in England. We compared current SARS-CoV-2 cases and deaths from public databases to both regional and subregional air pollution data monitored at multiple sites across England. After controlling for population density, age and median income, we show positive relationships between air pollutant concentrations, particularly nitrogen oxides, and COVID-19 mortality and infectivity. Using detailed UK Biobank data, we further show that PM was a major contributor to COVID-19 cases in England, as an increase of 1 m in the long-term average of PM was associated with a 12% increase in COVID-19 cases. The relationship between air pollution and COVID-19 withstands variations in the temporal scale of assessments (single-year vs 5-year average) and remains significant after adjusting for socioeconomic, demographic and health-related variables. We conclude that a small increase in air pollution leads to a large increase in the COVID-19 infectivity and mortality rate in England. This study provides a framework to guide both health and emissions policies in countries affected by this pandemic.
Environ Pollut, 2021; 268
33925631: Popovic R, Celardo I, Yu Y, Costa AC, Loh SHY, Martins LM
Combined Transcriptomic and Proteomic Analysis of Perk Toxicity Pathways.
In , endoplasmic reticulum (ER) stress activates the protein kinase R-like endoplasmic reticulum kinase (dPerk). dPerk can also be activated by defective mitochondria in fly models of Parkinson's disease caused by mutations in or . The Perk branch of the unfolded protein response (UPR) has emerged as a major toxic process in neurodegenerative disorders causing a chronic reduction in vital proteins and neuronal death. In this study, we combined microarray analysis and quantitative proteomics analysis in adult flies overexpressing dPerk to investigate the relationship between the transcriptional and translational response to dPerk activation. We identified and as two novel activating transcription factor 4 (dAtf4) regulated

transcripts. Using a combined bioinformatics tool kit, we demonstrated that the activation of dPerk leads to translational repression of mitochondrial proteins associated with glutathione and nucleotide metabolism, calcium signalling and iron-sulphur cluster biosynthesis. Further efforts to enhance these translationally repressed dPerk targets might offer protection against Perk toxicity.
Int J Mol Sci, 2021; 22
34172715: Yu Y, Fedele G, Celardo I, Loh SHY, Martins LM
Parp mutations protect from mitochondrial toxicity in Alzheimer's disease.
Alzheimer's disease is the most common age-related neurodegenerative disorder. Familial forms of Alzheimer's disease associated with the accumulation of a toxic form of amyloid-β (Aβ) peptides are linked to mitochondrial impairment. The coenzyme nicotinamide adenine dinucleotide (NAD) is essential for both mitochondrial bioenergetics and nuclear DNA repair through NAD-consuming poly (ADP-ribose) polymerases (PARPs). Here we analysed the metabolomic changes in flies overexpressing Aβ and showed a decrease of metabolites associated with nicotinate and nicotinamide metabolism, which is critical for mitochondrial function in neurons. We show that increasing the bioavailability of NAD protects against Aβ toxicity. Pharmacological supplementation using NAM, a form of vitamin B that acts as a precursor for NAD or a genetic mutation of PARP rescues mitochondrial defects, protects neurons against degeneration and reduces behavioural impairments in a fly model of Alzheimer's disease. Next, we looked at links between PARP polymorphisms and vitamin B intake in patients with Alzheimer's disease. We show that polymorphisms in the human PARP1 gene or the intake of vitamin B are associated with a decrease in the risk and severity of Alzheimer's disease. We suggest that enhancing the availability of NAD by either vitamin B supplements or the inhibition of NAD-dependent enzymes such as PARPs are potential therapies for Alzheimer's disease.
Cell Death Dis, 2021; 12
33530357: Yu Y, Travaglio M, Popovic R, Leal NS, Martins LM
Alzheimer's and Parkinson's Diseases Predict Different COVID-19 Outcomes: A UK Biobank Study.
In December 2019, a coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), began infecting humans, causing a novel disease, coronavirus disease 19 (COVID-19). This was first described in the Wuhan province of the People's Republic of China. SARS-CoV-2 has spread throughout the world, causing a global pandemic. To date, thousands of cases of COVID-19 have been reported in the United Kingdom, and over 45,000 patients have died. Some progress has been achieved in managing this disease, but the biological determinants of health, in addition to age, that affect SARS-CoV-2 infectivity and mortality are under scrutiny. Recent studies show that several medical conditions, including diabetes and hypertension, increase the risk of COVID-19 and death. The increased vulnerability of elderly individuals and those with comorbidities, together with the prevalence of neurodegenerative diseases with advanced age, led us to investigate the links between neurodegeneration and COVID-19. We analysed the primary health records of 13,338 UK individuals tested for COVID-19 between March and July 2020. We show that a pre-existing diagnosis of Alzheimer's disease predicts the highest risk of COVID-19 and mortality among elderly individuals. In contrast, Parkinson's disease patients were found to have a higher risk of SARS-CoV-2 infection but not mortality from COVID-19. We conclude that there are disease-specific differences in COVID-19 susceptibility among patients affected by neurodegenerative disorders.
Geriatrics (Basel), 2021; 6

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BOARD NUMBER: S05-212

THERAPEUTIC EFFECTS OF BLOOD-BRAIN BARRIER OPENING WITH LOW-INTENSITY PULSED ULTRASOUNDS IN TAU TRANSGENIC P301S MICE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

Maximilien Riche[1,2,3,4], Amandine Geraudie[4], Kevin Beccaria[5], Bertrand Mathon[1,2,3,4], Alexandre Androuin[4,6], Alexandre Carpentier[1,2,3], Benoît Delatour[4]
[1]Sorbonne Universite, Neurosurgery Department, Ap-hp, Pitie Salpetriere Hospital, Paris, France, [2]Sorbonne Universite, Advanced Surgical Research Technology Lab, Paris, France, [3]Sorbonne Universite, Grc 23, Brain Machine Interface, Ap-hp, Pitie Salpetriere Hospital, Paris, France, [4]ICM, Sorbonne Universités, Inserm U1127, Cnrs Umr 7225, Paris, France, [5]Necker Enfants Malades Hospital, Pediatric Neurosurgery Hospital, Paris, France, [6]University of Paris, Institute Of Psychiatry And Neuroscience Of Paris (ipnp), Inserm U1266, Paris, France

Introduction: To date, almost no disease-modifying therapeutics have proven efficacy in Alzheimer's disease. One reason may be the difficulty for drugs to cross the blood-brain barrier (BBB). It has been previously showed that the BBB can be opened safely, transiently and repeatedly, with the application of low-intensity pulsed ultrasound associated to microbubbles (LIPU-MB). Previous studies have also shown that BBB opening by ultrasounds and microbubbles may have beneficial effects on amyloid burden, neurogenesis and neuroinflammation. **Aims**: This study aimed at evaluating the effect of LIPU-MB BBB opening in P301S mice model of tauopathy on neurofibrillary tangles burden. Its secondary aims were to evaluate the effects of LIPU-MB BBB opening on neurogenesis and neuroinflammation. **Methods**: Five sessions (one per week) of LIPU-MB were administered to P301S mice (sonicated group). Sonicated and non-sonicated mice were sacrificed one week after the last session. Immunostaining of neurofibrillary tangles (AT8), neurogenesis (doublecortin) and neuroinflammation (Iba-1) were compared between the sonicated and non-sonicated groups. **Results**: There was a trend for a decrease of cortical neurofibrillary tangles in the sonicated group ($p<.06$). Interestingly, microglial densities were drastically decreased in different brain areas in sonicated mice, when compared to non-sonicated animals ($p<.0001$). Neurogenesis was not significantly different between the two groups in the hippocampus. **Conclusions**: Our results show that LIPU-MB BBB opening decreases microglial cells loads in P301S mice and may impact on tangles pathology. These results could have therapeutical impact for Alzheimer's disease but need to be confirmed in larger cohorts of mice.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-213

NEUROPROTECTION, ANTIOXIDANT, AND ANTI-EXCITOTOXICITY ACTIVITIES OF ACAI BERRY (EUTERPE SP.) POWDER

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease (AD) is characterized by progressive neuronal degeneration and is caused by a variety of pathomechanisms. The existing AD treatments are symptomatic and cause side effects, necessitating the development of more effective medicines. The native South American palm acai berry (*Euterpe sp.*) is a potential source of health-promoting dietary phytochemicals. The dibutyryl cyclic adenosine monophosphate differentiated human cell line TE671 was used to investigate the potential neurotoxicity of acai aqueous extract and neuroprotective ability to reduce the neurotoxicity of L-glutamate (L-Glu). L-Glu, which occurs naturally or as a food additive, can act as an excitotoxin and has been linked to AD development. Cell viability assay using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) showed that acai extract had no toxic effects on neurons at concentrations ranging from 0.001-1000 µg/mL after a 24-hour exposure. Whereas L-Glu concentrations (0.137-100 mM) caused a significant reduction in cell viability after a 24-hour exposure. Acai extract showed significant neuroprotection against L-Glu at 0.137 mM. A 6 hours exposure of L-Glu caused a concentration-dependent increase in reactive oxygen species (ROS) measured via a 2',7'-dichlorofluorescein diacetate (DCFDA) assay. Acai aqueous extract significantly reduced ROS production induced by L-Glu. Whole-cell patch-clamp recordings at a membrane potential of −50 mV showed that acai extract (1000 µg/mL) significantly inhibited L-Glu (300 µM) and glycine (10 µM) activated currents in TE671 cells. In summary, this study demonstrates that acai berry contains nutraceutical components with antioxidant and anti-excitotoxicity activities that may be a beneficial dietary component to limit pathological deficits evidenced in AD.

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BOARD NUMBER: S05-214

TREATMENT WITH NOVEL HIPPO SIGNALING INHIBITOR, XMU-MP-1, AMELIORATES COGNITIVE IMPAIRMENT AND NEURODEGENERATION IN RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease (AD), is a form of dementia and a progressive neurodegenerative disorder often accompanied by aging. Neurodegeneration during the disease progression is majorly driven by cellular apoptosis. Recently, the Hippo signaling pathway has emerged as a key pathway influencing cell survival, proliferation, and apoptotic-related pathophysiological conditions. Several previous studies have demonstrated the contributions of a hyper-activated Hippo signaling pathway in executing cellular death in various neurodegenerative disorders, including AD. However, it remains unknown whether inhibiting Hippo pathway can diminish neurodegeneration and related pathophysiological changes in AD. **Aim:** In this study, our aim was to investigate the beneficial role of Hippo signaling inhibition via a chemical inhibitor, Xmu-mp-1, in the rat model of sporadic AD. **Methods:** Streptozotocin (STZ) was administered intracerebroventricularly via stereotactic surgery in the rat brain to develop AD model. Intraperitoneal administration of Xmu-mp-1 was performed at a dose of 0.5mg/kg body weight for two weeks 7 days post-STZ administration. At 3-week post-surgery, cognitive changes were assessed by behavioural test called Morris Water Maze Test. At the end of behaviour tests, histopathological examination of the rat brain tissue was performed to evaluate changes in the level of Tau phosphorylation and neurodegeneration. **Results:** Xmu-mp-1 treated AD rats exhibit improvement in cognitive deficits. At the tissue level, treatment with Xmu-mp-1 reduced the level of p-Tau and neurodegeneration compared to AD treated with only vehicle. **Conclusion:** These findings indicate a potential therapeutic ability of Hippo pathway inhibition in the management of AD pathophysiology, which needs to be further explored in detail.

Pubmed:
31705587: Sahu MR, Mondal AC
The emerging role of Hippo signaling in neurodegeneration.
Neurodegeneration refers to the complex process of progressive degeneration or neuronal apoptosis leading to a set of incurable and debilitating conditions. Physiologically, apoptosis is important in proper growth and development. However, aberrant and unrestricted apoptosis can lead to a variety of degenerative conditions including neurodegenerative diseases. Although dysregulated apoptosis has been implicated in various neurodegenerative disorders, the triggers and molecular mechanisms underlying such untimely and faulty apoptosis are still unknown. Hippo signaling pathway is one such apoptosis-regulating mechanism that has remained evolutionarily conserved from Drosophila to mammals. This pathway has gained a lot of attention for its tumor-suppressing task, but recent studies have emphasized the soaring role of this pathway in inflaming neurodegeneration. In addition, strategies promoting inactivation of this pathway have aided in the rescue of neurons from anomalous apoptosis. So, a thorough understanding of the relationship between the Hippo pathway and neurodegeneration may serve as a guide for the development of therapy for various degenerative diseases. The current review focuses on the mechanism of the Hippo signaling pathway, its upstream and downstream regulatory molecules, and its role in the genesis of numerous neurodegenerative diseases. The recent efforts employing the Hippo pathway components as targets for checking neurodegeneration have also been highlighted.
J Neurosci Res, 2020; 98
33275833: Sahu MR, Mondal AC
Neuronal Hippo signaling: From development to diseases.
Hippo signaling pathway is a highly conserved and familiar tissue growth regulator, primarily dealing with cell survival, cell proliferation, and apoptosis. The Yes-associated protein (YAP) is the key transcriptional effector molecule, which is under negative regulation of the Hippo pathway. Wealth of studies have identified crucial roles of Hippo/YAP signaling pathway during the process of development, including the development of neuronal system. We provide here, an overview of the contributions of this signaling pathway at multiple stages of neuronal development including, proliferation of neural stem cells (NSCs), migration of NSCs toward their destined niche, maintaining NSCs in the quiescent state, differentiation of NSCs into

neurons, neuritogenesis, synaptogenesis, brain development, and in neuronal apoptosis. Hyperactivation of the neuronal Hippo pathway can also lead to a variety of devastating neurodegenerative diseases. Instances of aberrant Hippo pathway leading to neurodegenerative diseases along with the approaches utilizing this pathway as molecular targets for therapeutics has been highlighted in this review. Recent evidences suggesting neuronal repair and regenerative potential of this pathway has also been pointed out, that will shed light on a novel aspect of Hippo pathway in regenerative medicine. Our review provides a better understanding of the significance of Hippo pathway in the journey of neuronal system from development to diseases as a whole.
Dev Neurobiol, 2021; 81
34165768: Anand SK, Sahu MR, Mondal AC
Induction of oxidative stress and apoptosis in the injured brain: potential relevance to brain regeneration in zebrafish.
Recent findings suggest a significant role of the brain-derived neurotrophic factor (BDNF) as a mediator of brain regeneration following a stab injury in zebrafish. Since BDNF has been implicated in many physiological processes, we hypothesized that these processes are affected by brain injury in zebrafish. Hence, we examined the impact of stab injury on oxidative stress and apoptosis in the adult zebrafish brain. Stab wound injury (SWI) was induced in the right telencephalic hemisphere of the adult zebrafish brain and examined at different time points. The biochemical variables of oxidative stress insult and transcript levels of antioxidant genes were assessed to reflect upon the oxidative stress levels in the brain. Immunohistochemistry was performed to detect the levels of early apoptotic marker protein cleaved caspase-3, and the transcript levels of pro-apoptotic and anti-apoptotic genes were examined to determine the effect of SWI on apoptosis. The activity of antioxidant enzymes, the level of lipid peroxidation (LPO) and reduced glutathione (GSH) were significantly increased in the injured fish brain. SWI also enhanced the expression of cleaved caspase-3 protein and apoptosis-related gene transcripts. Our results indicate induction of oxidative stress and apoptosis in the telencephalon of adult zebrafish brain by SWI. These findings contribute to the overall understanding of the pathophysiology of traumatic brain injury and adult neurogenesis in the zebrafish model and raise new questions about the compensatory physiological mechanisms in response to traumatic brain injury in the adult zebrafish brain.
Mol Biol Rep, 2021; 48
34357519: Anand SK, Sahu MR, Mondal AC
Bacopaside-I Alleviates the Detrimental Effects of Acute Paraquat Intoxication in the Adult Zebrafish Brain.
Paraquat (PQ), an environmental neurotoxicant, causes acute fatal poisoning upon accidental or intentional ingestion (suicidal cases) worldwide. To date, an effective remedy for PQ toxicity is not available. In this study, we have evaluated the therapeutic efficacy of Bacopaside-I (BS-I), an active compound found in the plant extract of Bacopa monnieri (Brahmi), against acute PQ intoxication using zebrafish as a model organism. Adult zebrafish were injected with a dose of either 30 mg/kg or 50 mg/kg PQ. PQ-intoxicated zebrafish showed an increased rate of mortality and oxidative imbalance in their brain. Also, the proliferation of neural cells in the adult zebrafish brain was inhibited. However, when BS-I pretreated zebrafish were intoxicated with PQ, the toxic effects of PQ were ameliorated. PQ treatment also affected the expression of particular genes concerned with the apoptosis and dopamine signaling, which was not altered by BS-I administration. Our results highlight the efficiency of BS-I as a novel therapeutic agent for PQ intoxication. It further compels us to search and evaluate the molecular mechanisms targeted by BS-I to develop a potent therapy for acute PQ intoxication.
Neurochem Res, 2021; 46

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MITOCHONDRIAL PRIMING RESCUES MOLECULAR, PHYSIOLOGICAL AND BEHAVIORAL PATHOLOGICAL OUTCOMES IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

De Veij Mestdagh
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Alzheimer’s disease (AD) is the most prevalent neurodegenerative disease worldwide and remains without effective cure. Increasing evidence is supporting the mitochondrial cascade hypothesis, proposing loss of mitochondrial fitness and subsequent ROS and ATP imbalance, as the foundation of AD pathophysiological outcomes. As such, mitochondria should be seriously considered as pharmacological targets. In this study we tested the effect of SUL-138, a small molecule that preserves mitochondrial network structure and supports mitochondrial bioenergetics via complex I/IV activation, on physiological and behavioural outcomes in APP/PS1 and Wildtype (WT) mice. Subsequently, we investigated the molecular background of SUL-138 treatment using mass-spectrometry and assessed the effect of SUL-138 on A-beta pathology, a key pathological hallmark of AD. 3 month SUL-138 treatment rescued impaired long-term potentiation and hippocampus dependent memory, which was paralleled by a rescue of AD associated and APP/PS1 affected proteins. Consistent with these findings, a decrease in plaque load in SUL-138 treated APP/PS1 mice was observed. In addition, SUL-138 increased synaptic plasticity in WT’s and proteomic analysis showed a genotype unspecific effect of SUL-138 on postsynaptic cytoskeleton proteins and specific proteins involved in metabolism. Taken together, our study points to a shift in metabolic efficiency and postsynaptic organisation as drivers of plasticity enhancement and it demonstrates that targeting mitochondrial bioenergetics by increasing mitochondrial complex I and IV activity is a promising new druggable target for the treatment of Alzheimer's disease.

Pubmed:

34326412: de Veij Mestdagh CF, Timmerman JA, Koopmans F, Paliukhovich I, Miedema SSM, Goris M, van der Loo RJ, Krenning G, Li KW, Mansvelder HD, Smit AB, Henning RH, van Kesteren RE
Torpor enhances synaptic strength and restores memory performance in a mouse model of Alzheimer's disease.
Hibernation induces neurodegeneration-like changes in the brain, which are completely reversed upon arousal. Hibernation-induced plasticity may therefore be of great relevance for the treatment of neurodegenerative diseases, but remains largely unexplored. Here we show that a single torpor and arousal sequence in mice does not induce dendrite retraction and synapse loss as observed in seasonal hibernators. Instead, it increases hippocampal long-term potentiation and contextual fear memory. This is accompanied by increased levels of key postsynaptic proteins and mitochondrial complex I and IV proteins, indicating mitochondrial reactivation and enhanced synaptic plasticity upon arousal. Interestingly, a single torpor and arousal sequence was also sufficient to restore contextual fear memory in an APP/PS1 mouse model of Alzheimer's disease. Our study demonstrates that torpor in mice evokes an exceptional state of hippocampal plasticity and that naturally occurring plasticity mechanisms during torpor provide an opportunity to identify unique druggable targets for the treatment of cognitive impairment.
Sci Rep, 2021; 11
27475000: Talma N, Kok WF, de Veij Mestdagh CF, Shanbhag NC, Bouma HR, Henning RH
Neuroprotective hypothermia - Why keep your head cool during ischemia and reperfusion.
Targeted temperature management (TTM) is the induced cooling of the entire body or specific organs to help prevent ischemia and reperfusion (I/R) injury, as may occur during major surgery, cardiac resuscitation, traumatic brain injury and stroke. Ischemia and reperfusion induce neuronal damage by mitochondrial dysfunction and oxidative injury, ER stress, neuronal excitotoxicity, and a neuroinflammatory response, which may lead to activation of apoptosis pathways.
Biochim Biophys Acta, 2016; 1860

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LOW-DOSE IONIZING RADIATION AS THERAPEUTIC INTERVENTION AGAINST CHRONIC CEREBRAL HYPOPERFUSION-INDUCED COGNITIVE DEFICITS

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Aims: The effects of exposure to low-dose ionizing radiation (LDIR<1Gy) on the brain are not well established. There are increasing evidence to show that LDIR may exert beneficial actions through a radiological hormesis, especially in pathological conditions. Thereby we hypothesized that LDIR could be a therapeutic intervention in some neurological diseases. In this longitudinal study, we characterized the LDIR's effects on brain tissue damage and cognitive decline mediated by chronic cerebral hypoperfusion (HCC) in the rat. **Methods**: HCC was induced on Wistar rats by bilateral and permanent occlusion of the common carotid arteries. The entire brain was irradiated with 0.3 Gy X-rays at one- or seven-days post-occlusion. To assess the effects of LDIR on the hypoperfused brain, behavioral testing (Morris water maze, novel object recognition) and *ex-vivo* analyses evaluating blood brain-barrier permeability (BBB) (Evans blue injection) and neuronal damage (immunohistological studies) were performed. **Results**: LDIR exposure attenuated alterations of spatial learning and spatial reference memory induced by HCC. The working memory deficit driven by brain hypoperfusion was also reduced by irradiation treatment since the recognition index was 0.59±0.08, 0.44±0.24 and 0.56±0.07 for Sham, hypoperfused (HCC) and irradiated hypoperfused (HCC+LDIR) rats respectively. Interestingly, LDIR mitigated BBB permeability since Evans blue concentration in hippocampus was 28±6 µg/L in Sham rats, 51±8 µg/L in HCC rats and 24±2 µg/L in HCC+LDIR rats. **Conclusions**: LDIR modulates the events involved in the development of HCC and improves the cognitive decline in this preclinical model. The LDIR therapy could be promising for cerebrovascular and neurodegenerative diseases.

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EXTRACELLULAR TAU OLIGOMERS IMPAIR SYNAPTIC FUNCTION BY A CONCOMITANT INTRA AND EXTRACELLULAR DETRIMENTAL ACTION ON ASTROCYTES

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

Roberto Piacentini[1], Domenica Donatella Li Puma[1], Giulia Puliatti[1], Cristian Ripoli[1], Francesco Pastore[1], Giacomo Lazzarino[2], Ottavio Arancio[3], Claudio Grassi[1]
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Aims Astrocytes support neuronal function and guarantee neuronal health by handling of glio/neurotransmitters, including ATP and glutamate at the synaptic cleft. Indeed, astrocytic malfunction is associated with synaptic dysregulation and underlies cognitive decline in neurodegenerative diseases, such as tauopathies. Aim of this work was to check whether extracellular tau oligomers (ex-oTau) impair neuronal function specifically targeting astrocytes, reducing gliotransmitter release, and affecting glutamate-dependent synaptic transmission. **Methods** Primary cultures of hippocampal astrocytes, neurons and organotypic brain slices were exposed to ex-oTau (200 nM) for 1 hour. Gliotransmitter handling by astrocytes and synaptic function were studied by Ca^{2+}, Na^{+} and FM1-43 imaging; electrophysiological recordings; high-performance liquid chromatography; Western blot and immunofluorescence. Experimental paradigms avoiding ex-oTau internalization were used to dissect intracellular vs. extracellular effects of oTau. **Results** Ex-oTau uploading in astrocytes led to alteration of intracellular Ca2+ waves and downregulation of glutamate-transporter-1 expression. Uploading-independent Na-K-ATPase mislocalization on plasma membrane was also observed, causing Na^{+} overload in astrocytes. All these actions affected uptake and release of gliotransmitters by astrocytes, including glutamate and ATP, that negatively influenced synaptic function. Notably, we identified glypican 4 as a membrane receptor for oTau internalization and a potential target against tau-induced synaptotoxicity. **Conclusions** We demonstrated that ex-oTau affect synaptic transmission by exerting a complex action on astrocytes, at both intracellular and extracellular levels. The net effect was a dysregulated gliotransmitter signaling that relied on altered intracellular Ca^{2+} signals, reduced expression of glutamate-transporter-1, along with altered function and localization of Na-K-ATPase affecting Na^{+} gradients and Na^{+}-dependent transports.

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ASSOCIATION BETWEEN ADENOSINE A2A RECEPTORS AND CONNEXIN 43 MODULATES HEMICHANNELS ACTIVITY AND ATP RELEASE IN ASTROCYTES EXPOSED TO AMYLOID-β PEPTIDES

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Increasing evidence implicates astrocytes in Alzheimer's disease (AD), a neurodegenerative disorder characterized by memory loss that parallels extracellular accumulation of amyloid-beta peptides (Aβ). Aβ can affect astrocytes function, mainly their ability to uptake and release neuroactive molecules. Astrocytes release ATP, which can be metabolized into adenosine by ecto-5'-nucleotidase, CD73, resulting in adenosine A_{2A} receptors ($A_{2A}R$) activation that in turn bolsters neurodegeneration. AD's brains exhibit increased levels of $A_{2A}R$ and connexins 43 (Cx43), which form astrocytic hemichannels (Cx43-HC) that mediate gliosignals release. **Aims:** investigate a possible association between Cx43-HC and $A_{2A}R$ in astrocytes exposed to Aβ peptides. **Methods:** We used mature primary cultures of cortical astrocytes from Wistar rats exposed to $Aβ_{1-42}$ (1 μM) to mimic pathological conditions of AD. ATP release and HC permeability were evaluated by luciferin-luciferase and ethidium bromide uptake assays, respectively. **Results:** Our results revealed an enhancement in ATP release (180.4±21.5%, $p<0.001$), HC activity (129.7±4.4%, $p<0.001$) and Cx43 total levels (148.9±14.0%, $p<0.05$) and phosphorylation (140.2±5.4%, $p<0.001$) in astrocytes exposed to $Aβ_{1-42}$ relatively to control cells (100%); these effects were mimicked by $A_{2A}R$ activation and counteracted by $A_{2A}R$ antagonism or CD73 blockade. Moreover, proximity ligation assay and co-immunoprecipitation revealed a physical association between $A_{2A}R$ and Cx43. **Conclusions:** Our data identified a feed-forward loop involving $A_{2A}R$ and Cx43-HC in astrocytes, whereby $A_{2A}R$ overfunction increases Cx43-HC activity and ATP release that is converted into adenosine by CD73, to sustain increased $A_{2A}R$ activity in AD-like conditions. **Fundings:** La Caixa Foundation (HP17/00523), Centro2020 (CENTRO-01-0145-FEDER-000008:BrainHealth2020 and CENTRO-01-0246-FEDER-000010) and FCT (FCT,PTDC/NEU-NMC/4154/2014-POCI-01-0145-FEDER-016684 and PTDC/MED-NEU/31274/2017 and UIDB/04539/2020). PhD studentship from FCT of DM (SFRH/BD/139334/2018).

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THE ∆9-TETRAHYDROCANNABINOL AND CANNABIDIOL COMBINATION REDUCES THE EXCESSIVE GLUTAMATERGIC ACTIVITY IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease (AD) is the most common form of dementia and is characterized by a progressive loss of memory and other mental abilities. Current therapies against AD are not totally effective, which highlights the need for new therapeutic strategies. Previous results from our group demonstrated that a combination of non-psychoactive doses of the natural cannabinoids ∆9-tetrahydrocannabinol (THC) and cannabidiol (CBD), the main components of the first cannabis-based medicine approved in many countries, reduces cognitive decline in a mouse model of AD, the APP/PS1 mice. However, the molecular mechanisms underlying this therapeutic effect are not completely understood. Here, we studied the effects of THC and CBD on the glutamate homeostasis and on synaptic plasticity in the hippocampus of APP/PS1 mice because these two processes are known to be altered in AD. Thus, by using in vivo microdialysis and HPLC techniques, we have quantified the glutamate levels in the hippocampus of wild-type and APP/PS1 animals in response to veratridine and the glutamate transporter-1 inhibitor dihydrokainate (DHK) after a chronic treatment with THC and/or CBD. Interestingly, THC+CBD treatment reduced the veratridine-evoked glutamate release in both genotypes and attenuated the enhanced glutamate levels observed in DHK-treated APP/PS1 mice. In contrast, our results by using ballistic labelling demonstrated that THC+CBD chronic treatment does not significantly impact on dendritic spine density and morphology in the hippocampus of APP/PS1 mice. These results suggest that cognitive improvement after THC+CBD treatment could be related with a reduction of the excitotoxicity occurring in our AD model.

Pubmed:
32452760: Miguez-Cabello F, Sánchez-Fernández N, Yefimenko N, Gasull X, Gratacòs-Batlle E, Soto D
AMPAR/TARP stoichiometry differentially modulates channel properties.
AMPARs control fast synaptic communication between neurons and their function relies on auxiliary subunits, which importantly modulate channel properties. Although it has been suggested that AMPARs can bind to TARPs with variable stoichiometry, little is known about the effect that this stoichiometry exerts on certain AMPAR properties. Here we have found that AMPARs show a clear stoichiometry-dependent modulation by the prototypical TARP γ2 although the receptor still needs to be fully saturated with γ2 to show some typical TARP-induced characteristics (i.e. an increase in channel conductance). We also uncovered important differences in the stoichiometric modulation between calcium-permeable and calcium-impermeable AMPARs. Moreover, in heteromeric AMPARs, γ2 positioning in the complex is important to exert certain TARP-dependent features. Finally, by comparing data from recombinant receptors with endogenous AMPAR currents from mouse cerebellar granule cells, we have determined a likely presence of two γ2 molecules at somatic receptors in this cell type. Elife, 2020; 9
30135643: Gratacòs-Batlle E, Olivella M, Sánchez-Fernández N, Yefimenko N, Miguez-Cabello F, Fadó R, Casals N, Gasull X, Ambrosio S, Soto D
Mechanisms of CPT1C-Dependent AMPAR Trafficking Enhancement.
In neurons, AMPA receptor (AMPAR) function depends essentially on their constituent components:the ion channel forming subunits and ion channel associated proteins. On the other hand, AMPAR trafficking is tightly regulated by a vast number of intracellular neuronal proteins that bind to AMPAR subunits. It has been recently shown that the interaction between the GluA1 subunit of AMPARs and carnitine palmitoyltransferase 1C (CPT1C), a novel protein partner of AMPARs, is important in modulating surface expression of these ionotropic glutamate receptors. Indeed, synaptic transmission in CPT1C knockout (KO) mice is diminished supporting a positive trafficking role for that protein. However, the molecular mechanisms of such

modulation remain unknown although a putative role of CPT1C in depalmitoylating GluA1 has been hypothesized. Here, we explore that possibility and show that CPT1C effect on AMPARs is likely due to changes in the palmitoylation state of GluA1. Based on analysis, Ser 252, His 470 and Asp 474 are predicted to be the catalytic triad responsible for CPT1C palmitoyl thioesterase (PTE) activity. When these residues are mutated or when PTE activity is inhibited, the CPT1C effect on AMPAR trafficking is abolished, validating the CPT1C catalytic triad as being responsible for PTE activity on AMPAR. Moreover, the histidine residue (His 470) of CPT1C is crucial for the increase in GluA1 surface expression in neurons and the H470A mutation impairs the depalmitoylating catalytic activity of CPT1C. Finally, we show that CPT1C effect seems to be specific for this CPT1 isoform and it takes place solely at endoplasmic reticulum (ER). This work adds another facet to the impressive degree of molecular mechanisms regulating AMPAR physiology.
Front Mol Neurosci, 2018; 11
33984337: Pérez-Sisqués L, Martín-Flores N, Masana M, Solana-Balaguer J, Llobet A, Romaní-Aumedes J, Canal M, Campoy-Campos G, García-García E, Sánchez-Fernández N, Fernández-García S, Gilbert JP, Rodríguez MJ, Man HY, Feinstein E, Williamson DL, Soto D, Gasull X, Alberch J, Malagelada C
RTP801 regulates motor cortex synaptic transmission and learning.
RTP801/REDD1 is a stress-regulated protein whose upregulation is necessary and sufficient to trigger neuronal death in in vitro and in vivo models of Parkinson's and Huntington's diseases and is up regulated in compromised neurons in human postmortem brains of both neurodegenerative disorders. Indeed, in both Parkinson's and Huntington's disease mouse models, RTP801 knockdown alleviates motor-learning deficits.
Exp Neurol, 2021; 342

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SINOMENINE AND SAFRANAL PROTECT NEURONS AGAINST TWO DIFFERENT MODES OF AMYLOID-BETA-INDUCED TOXICITY.

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease is an age-related neurodegenerative condition characterized by progressive memory loss and other cognitive functions. The pathological hallmarks of this disease are the extracellular deposition of amyloid-β protein and intracellular accumulated hyperphosphorylated protein tau. Gliosis involving the activation of astrocytes and microglia is a crucial outcome of Aβ deposition leading to neuroinflammation. Sinomenine is an alkaloid found in a Chinese medicinal plant, Sinomenium acutum. We found that Sinomenine protects neurons against amyloid-beta-induced indirect toxicity by inhibiting astrocytic activation. It inhibits the release of toxic factors such as reactive oxygen species, nitric oxide, and inflammatory molecules from oligomeric amyloid-beta-treated astrocytes. In addition, Sinomenine protects neurons from toxicity caused by the conditioned medium from oligomeric amyloid-beta-treated astrocytes. Another natural compound, Safranal, isolated from Crocus sativus, has been examined for its efficacy against amyloid-beta-induced toxicity. Safranal prevents amyloid-beta-induced direct toxicity by reducing the levels of toxic factors and caspase-3 activation. Considerable studies have suggested that the enhancement of autophagy could be a therapeutic intervention in AD pathogenesis. The effects of autophagy modulators on amyloid-beta-induced toxic factors, and on proteins specific to different stages of autophagy, is under exploration.

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EVIDENCE FOR PRODROMAL NEUROINFLAMMATION IN A RODENT MODEL OF ALPHA- SYNUCLEINOPATHY

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Toxic aggregation of alpha-synuclein is a key feature of alpha-synucleinopathies, including dementia with Lewy bodies and Parkinson's disease dementia. Early neuroinflammation, mitochondrial dysfunction, and neuronal excitability changes could all be hallmarks of the progression of alpha-synucleinopathy in patients. In addition, transgenic mice expressing human alpha-synuclein exhibit hippocampal and neocortical network hyperexcitability. The current study investigated the early changes caused by abnormal alpha-synuclein in the prefrontal cortex and hippocampus of mice aged 2-4 months expressing human mutant (A30P) alpha-synuclein. We focused on early evidence for neuroinflammation, quantifying changes in astrocytic and microglial activation using immunohistochemistry. Fixed, frozen sections were prepared from the hippocampus and prefrontal cortex of male A30P and age-matched control mice. Immunofluorescence was conducted against GFAP (reactive astrocytes) and Iba-1 (reactive microglia). Sections were imaged using a Nikon N-1 microscope and analysed using the FIJI program. Results showed a significant increase in the percentage area of the CA3 region of hippocampus occupied by reactive astrocyte and microglia cell bodies and their processes (astrocytes; n=3 sections/4 mice, P = <0.033) and Iba-1 (microglia; n=3 sections/4 mice, P = 0.001) in A30P compared to control mice. Morphological changes in glial cells consistent with neuroinflammation were also identified. We aim to establish if these neuroinflammatory changes persist during disease progression in aged A30P mice. We will assess cytokine levels and mitochondrial function in young and old A30P versus control mice. Furthermore, we will determine whether metformin, an anti-hyperglycemic drug with known anti-inflammatory effects, has a neuroprotective role in A30P mice.

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EFFECT OF REPAGLINIDE ON SYMPTOMS ASSOCIATED WITH AGING AND NEURODEGENERATION IN C.ELEGANS AND MUS MUSCULUS

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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The aging process is associated with cognitive decline and an increased risk of suffering neurodegenerative diseases. DREAM is a calcium sensing protein, whose blockade delays the onset of cognitive decline associated with age in mice. One of the drugs capable of blocking DREAM activity is repaglinide (RPG), an antidiabetic drug used clinically. To determine if RPG treatment is capable of delaying the symptoms associated with neurodegeneration, experiments were carried out in two animal models, *C. elegans* and *Mus musculus*. These experiments include thrashing and paralysis assays in worms and behavioral tests, such as open field, object recognition and passive avoidance test in mice. Our results show that chronic treatment with RPG is capable of reducing motor symptoms in models of Parkinson's and Alzheimer's diseases in *C. elegans*, as well as of reversing cognitive deficits in aged mice injected with amyloid oligomers. Taken together, our results suggest that RPG could be an anti-aging drug and a potential treatment for neurodegenerative diseases in humans.

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BOARD NUMBER: S05-223

5XFAD MICE PRESENT MEMORY IMPAIRMENTS AND REDUCED TRKB-FL LEVELS THAT WERE REVERTED AFTER TAT-TRKB ADMINISTRATION

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Introduction: The signalling pathways mediated by the brain-derived neurotrophic factor (BDNF) are strongly impaired in Alzheimer's disease (AD) due to the cleavage of its Tropomyosin-related kinase B-full-length (TrkB-FL) receptor. This cleavage generates an intracellular fragment (TrkB-ICD) with tyrosine kinase activity and accumulates into the nucleus over time, leading to modifications in gene expression and culminating in cognitive impairments. Considering the lack of an effective treatment for AD, our laboratory designed a new peptide, TAT-TrkB, capable of preventing TrkB-FL cleavage and assayed its usefulness for AD-pathology amelioration. Aim: This project aimed to assess the *in vivo* efficacy of TAT-TrkB peptide. Methods: 5xFAD, an AD mouse model and wild-type (wt) mice, with 4 months old, were treated intraperitoneally, with saline solution or TAT-TrkB (25 mg/kg), 5 times a week for 2 months. At 5.5 months old, mice were submitted to several behavioural tests and at 6 months the animals were sacrificed for cellular and molecular analysis. Results: The behavioural tests exposed the capacity of TAT-TrkB to prevent learning and memory impairments observed in vehicle-treated 5xFAD mice (assessed by Morris Water Maze test). Additionality, this compound did not induce anxiety-like behaviours or locomotor deficits in both wt nor 5xFAD mice (assessed by Open Field and Elevated Plus Maze tests). 5xFAD animals showed molecular alterations, including Aβ accumulation and gliosis. The ratio TrkB-ICD/-FL, altered in 5xFAD animals, was re-established by TAT-TrkB administration. Conclusion: The collected results suggest that TAT-TrkB could be a promising therapeutical strategy to prevent/recover the typical cognitive deficits of AD patients.

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EMPAGLIFLOZIN EFFECT ON THE CENTRAL NERVOUS SYSTEM: AN IN VITRO STUDY ON PRIMARY NEURONAL CELL CULTURES

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Aims: Metabolic neuronal pathways and their effect on the central nervous system (CNS) play a key role in neuronal degeneration and death. Considerable number of drugs modulating these pathways have been tested so far. Empagliflozin, a drug initially used for glucose management of type 2 diabetes, comprises a promising novel therapeutic approach tested against neurodegenerative disorders. Methods: Primary neuronal cell cultures were developed from wild type Sprague Dawley rats and neurons were exposed to different concentrations of empagliflozin for 48 hours, during either their early development in the first week, or after two weeks *in vitro*. Neuronal survival was evaluated by MTT assay and development of the dendritic tree was assessed under immunofluorescent microscopy after impregnation of neurons with CM-DiI. A morphometric comparative study was also performed with the ImageJ software and NeuronJ plug-in. Results: Empagliflozin at a concentration between 0,001μM and 8μM didn't significantly alter the survival of the neurons and the development of their dendritic field. However, as expected, higher concentrations resulted in a reduction of total dendritic tree length, branching, and number of spines. Conclusions: Our results show that low and medium concentrations of empagliflozin on neurons do not exert any toxic effect. It is thus appropriate for further *in vitro* and *in vivo* testing as neuroprotective agent against degenerative disorders. However, higher concentrations of empagliflozin in the CNS may cause neurotoxicity, due to glucose deprivation on neurons.

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BIOLOGICAL CHARACTERISATION OF NEW MICRONEUROTROPHIN MIMETICS IN COUNTERING NEURODEGENERATION

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Brain-derived neurotrophic factor (BDNF) is a neurotrophin which regulates neuronal growth and differentiation, along with its receptor tyrosine receptor kinase B (TrkB)[1]. Neurotrophins show promise as therapeutics for neurodegeneration, but they cannot penetrate the blood-brain barrier. Instead, small neurotrophin mimetics could circumvent this obstacle. Here, we present new small BDNF mimetics [steroidal Dehydroepiandrosterone (DHEA) derivatives], with neuroprotective and neurogenic properties. First, we show compounds activate the TrkB pathway, using western blot in primary adult hippocampal neural stem cells (NSCs), showing phosphorylation of TrkB and its downstream targets. What is more, compounds increase proliferation, as assessed via BRDU integration, in absence of EGF/FGF. Strikingly, compounds also reduce cell death after Amyloid-β treatment, assessed through the celltox and MTT assays in hippocampal NSCs. In addition, we show that this effect is specifically carried via TrkB, as treatment with TrkB inhibitor ANA-12 abolishes compound action. Similar to NSCs, compounds also protect hippocampal neurons from Aβ toxicity, tested through tunnel assay and prevent synapse loss after Aβ treatment. To translate this work on a human relevant system, we use neural progenitor cells differentiated from 3 human induced pluripotent stem cell lines and show compounds successfully induce proliferation and prevent Aβ42 induced toxicity. Our work characterises novel BDNF mimetics that show ability to promote neurogenesis and neuroprotection *in vitro* and paves the way for follow up *in vivo* experiments. [1]J. Allen, S., J. Watson, J. & Dawbarn, D. The Neurotrophins and Their Role in Alzheimers Disease. Curr. Neuropharmacol. (2011). doi:10.2174/157015911798376190

Pubmed:
24294081: Radea C, Parmakelis A, Papadogiannis V, Charou D, Triantis KA
The hydrobioid freshwater gastropods (Caenogastropoda, Truncatelloidea) of Greece: new records, taxonomic re-assessments using DNA sequence data and an update of the IUCN Red List Categories.
Hydrobioid freshwater gastropods were collected from mainland and insular Greece. Several threatened taxa, such as Graecoanatolica vegorriticola, Pseudamnicola negropontina, Pseudamnicola pieperi, Pseudobithynia eubooensis and Pseudoislamia balcanica, were recorded from new localities. Trichonia trichonica, which has been considered extinct from its type locality for the last twenty eight years, was re-discovered, whereas the presence of Daphniola exigua, G. vegorriticola, Marstoniopsis graeca, P. pieperi and Pseudobithynia trichonis in their type localities was verified. The taxonomic status of P. negropontina and the newly discovered populations of G. vegorriticola was elucidated using COI sequence data. The new data recorded during this survey indicate that the IUCN status of some Greek endemic hydrobioids needs to be updated. Zookeys, 2013;

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DEPRESSION-INDUCED EARLY ONSET OF ALZHEIMER'S DISEASE IS ASSOCIATED WITH GUT MICROBIOTA IN MICE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease (AD) is the most prevalent form of dementia, yet the current pharmacologic treatments cannot slow or stop the pathology of AD. Interestingly, studies have shown that depression is associated with a greatly increased risk of developing dementia. Moreover, recent studies revealed that a bidirectional communication between the gut microbiota and the brain could play an important in various mental illnesses. In this study, we focused on the effect of depression on the onset of AD and gut microbiota profile in adult mice. 4-months-old APP/PS1 mice and their wild type (WT) littermates were exposed to social defeat-based chronic mild stress to induce depression-like behavior. Interestingly, the "depressed" APP/PS1 mice exhibited earlier onset of cognitive symptoms at 5-6 months, but the age-matched WT mouse model of depression did not develop cognitive impairment until 11 months. Shotgun metagenomic sequencing revealed various taxonomic and metabolic alterations in the gut microbiota of the depressed APP/PS1 mice. Collectively, our data imply that depression in young adulthood is an important risk factor of AD, and that gut microbiota composition and the associated metabolic pathway could be novel therapeutic targets of AD.

Pubmed:

35154497: Lee S, Kim TK, Choi JE, Choi Y, You M, Ryu J, Chun YL, Ham S, Hyeon SJ, Ryu H, Kim HS, Im HI
Dysfunction of striatal MeCP2 is associated with cognitive decline in a mouse model of Alzheimer's disease.
Cerebral Methyl-CpG binding Protein 2 (MeCP2) is involved in several psychiatric disorders that are concomitant with cognitive dysfunction. However, the regulatory function of striatal MeCP2 and its association with Alzheimer's disease (AD) has been largely neglected due to the absence of amyloid plaque accumulation in the striatal region until the later stages of AD progression. Considerable evidence indicates that neuropsychiatric symptoms related to cognitive decline are involved with striatal dysfunction. To this respect, we investigated the epigenetic function of striatal MeCP2 paralleling the pathogenesis of AD. We investigated the brain from amyloid precursor protein (APP)/presenilin1 (PS1) transgenic mice and postmortem brain samples from normal subjects and AD patients. The molecular changes in the brain, particularly in the striatal regions, were analyzed with thioflavin S staining, immunohistochemistry, immunoblotting, and MeCP2 chromatin immunoprecipitation sequencing (ChIP-seq). The cognitive function of APP/PS1 mice was assessed via three behavioral tests: 3-chamber test (3CT), Y-maze test (YMT), and passive avoidance test (PA). A multi-electrode array (MEA) was performed to analyze the neuronal activity of the striatum in APP/PS1 mice. Striatal MeCP2 expression was increased in the younger (6 months) and older (10 months) ages of APP/PS1 mice, and the genome-wide occupancy of MeCP2 in the younger APP/PS1 showed dysregulated binding patterns in the striatum. Additionally, we confirmed that APP/PS1 mice showed behavioral deficits in multiple cognitive behaviors. Notably, defective cognitive phenotypes and abnormal neuronal activity in old APP/PS1 mice were rescued through the knock-down of striatal MeCP2. We found that the MeCP2-mediated dysregulation of the epigenome in the striatum is linked to the defects in cognitive behavior and neuronal activity in the AD animal model, and that this alteration is initiated even in the very early stages of AD pathogenesis. Together, our data indicates that MeCP2 may be a potential target for the diagnosis and treatment of AD at asymptomatic and symptomatic stages.
Theranostics, 2022; 12
34144146: Yang EJ, Kim H, Choi Y, Kim HJ, Kim JH, Yoon J, Seo YS, Kim HS
Modulation of Neuroinflammation by Low-Dose Radiation Therapy in an Animal Model of Alzheimer's Disease.
Recently, several studies have reported that low-dose radiation therapy (RT) suppresses the release of proinflammatory cytokines in inflammatory-degenerative disorders, including Alzheimer disease (AD). AD is the most common cause of dementia, and neuroinflammation is one of the major contributing factors in AD pathogenesis. Therefore, low-dose RT may be used clinically for treating AD. However, the appropriate doses, effects, and underlying mechanisms of RT in AD have not been determined. In this study, we aimed to determine the appropriate RT dose and schedule for AD treatment and to

investigate the therapeutic effects and mechanisms of low-dose RT in AD.
Int J Radiat Oncol Biol Phys, 2021; 111
34437591: Choi Y, Kim B, Ham S, Chung S, Maeng S, Kim HS, Im HI
Subanesthetic ketamine rapidly alters medial prefrontal miRNAs involved in ubiquitin-mediated proteolysis.
Ketamine is a dissociative anesthetic and a non-competitive NMDAR antagonist. At subanesthetic dose, ketamine can relieve pain and work as a fast-acting antidepressant, but the underlying molecular mechanism remains elusive. This study aimed to investigate the mode of action underlying the effects of acute subanesthetic ketamine treatment by bioinformatics analyses of miRNAs in the medial prefrontal cortex of male C57BL/6J mice. Gene Ontology and KEGG pathway analyses of the genes putatively targeted by ketamine-responsive prefrontal miRNAs revealed that acute subanesthetic ketamine modifies ubiquitin-mediated proteolysis. Validation analysis suggested that miR-148a-3p and miR-128-3p are the main players responsible for the subanesthetic ketamine-mediated alteration of ubiquitin-mediated proteolysis through varied regulation of ubiquitin ligases E2 and E3. Collectively, our data imply that the prefrontal miRNA-dependent modulation of ubiquitin-mediated proteolysis is at least partially involved in the mode of action by acute subanesthetic ketamine treatment.
PLoS One, 2021; 16
31795606: Kim B, Choi Y, Kim HS, Im HI
Methyl-CpG Binding Protein 2 in Alzheimer Dementia.
Despite decades of research on Alzheimer disease, understanding the complexity of the genetic and molecular interactions involved in its pathogenesis remains far from our grasp. Methyl-CpG Binding Protein 2 (MeCP2) is an important epigenetic regulator enriched in the brain, and recent findings have implicated MeCP2 as a crucial player in Alzheimer disease. Here, we provide comprehensive insights into the pathophysiological roles of MeCP2 in Alzheimer disease. In particular, we focus on how the alteration of MeCP2 expression can impact Alzheimer disease through risk genes, amyloid-β and tau pathology, cell death and neurodegeneration, and cellular senescence. We suggest that Alzheimer disease can be adversely affected by upregulated MeCP2-dependent repression of risk genes (MEF2C, ADAM10, and PM20D1), increased tau accumulation, and neurodegeneration through neuronal cell death (excitotoxicity and apoptosis). In addition, we propose that the progression of Alzheimer disease could be caused by reduced MeCP2-mediated enhancement of astrocytic and microglial senescence and consequent glial SASP (senescence-associated secretory phenotype)-dependent neuroinflammation. We surmise that any imbalance in MeCP2 function would accelerate or cause Alzheimer disease pathogenesis, implying that MeCP2 may be a potential drug target for the treatment and prevention of Alzheimer disease.
Int Neurourol J, 2019; 23
30718079: Choi Y, Kim H, Choi M, Yang EJ, Takumi T, Kim HS
Fetal neural stem cells from a mouse model of 15q11-13 duplication syndrome exhibit altered differentiation into neurons and astrocytes.
The duplication of human chromosome 15q11-13 is known to be associated with an estimated 1.1% of autism cases. Here, we investigated whether differentiation into neurons and astrocytes is altered in fetal neural stem cells (FNSCs) isolated from the mouse model of 15q11-13 duplication syndrome (patDp/+ mice). In patDp/+ mice-derived FNSCs, multipotency was maintained for a longer period, the population of neurons was downregulated, and that of astrocytes was upregulated significantly after differentiation induction. These results suggest that the dysregulation of FNSCs differentiation could affect cortical development and behavioral deficits in the early postnatal stage shown in the patDp/+ mice.
J Pharmacol Sci, 2019; 139
30118828: Yang EJ, Mahmood U, Kim H, Choi M, Choi Y, Lee JP, Cho JY, Hyun JW, Kim YS, Chang MJ, Kim HS
Phloroglucinol ameliorates cognitive impairments by reducing the amyloid β peptide burden and pro-inflammatory cytokines in the hippocampus of 5XFAD mice.
Among the various causative factors involved in the pathogenesis of Alzheimer's disease (AD), oxidative stress has emerged as an important factor. Phloroglucinol is a polyphenol component of phlorotannin, which is found at sufficient levels in Ecklonia cava (E. cava). Phloroglucinol has been reported to exert antioxidant activities in various tissues. Previously, we reported that the stereotaxic injection of phloroglucinol regulated synaptic plasticity in an AD mouse model. In this study, we aimed to investigate the effects of oral administration of phloroglucinol in AD. The oral administration of phloroglucinol for 2 months attenuated the impairments in cognitive function observed in 6-month-old 5X familial AD (5XFAD) mice, as assessed with the T-maze and Y-maze tests. The administration of phloroglucinol for 2 months in 5XFAD mice caused a reduction in the number of amyloid plaques and in the protein level of BACE1, a major amyloid precursor protein cleavage enzyme, together with γ-secretase. Phloroglucinol also restored the reduction in dendritic spine density and the number of mature spines in the hippocampi of 5XFAD mice. In addition, phloroglucinol-administered 5XFAD mice displayed lower protein levels of GFAP and Iba-1 and mRNA levels of TNF-α and IL-6 compared with vehicle-administered 5XFAD mice. These results demonstrated that phloroglucinol alleviated the neuropathological features and behavioral phenotypes in the 5XFAD mouse model. Taken together, our results suggest that phloroglucinol has therapeutic potential for AD treatment.

Free Radic Biol Med, 2018; 126
28408165: Yang EJ, Mahmood U, Kim H, Choi M, Choi Y, Lee JP, Chang MJ, Kim HS
Alterations in protein phosphorylation in the amygdala of the 5XFamilial Alzheimer's disease animal model.
Alzheimer's disease is the most common disease underlying dementia in humans. Two major neuropathological hallmarks of AD are neuritic plaques primarily composed of amyloid beta peptide and neurofibrillary tangles primarily composed of hyperphosphorylated tau. In addition to impaired memory function, AD patients often display neuropsychiatric symptoms and abnormal emotional states such as confusion, delusion, manic/depressive episodes and altered fear status. Brains from AD patients show atrophy of the amygdala which is involved in fear expression and emotional processing as well as hippocampal atrophy. However, which molecular changes are responsible for the altered emotional states observed in AD remains to be elucidated. Here, we observed that the fear response as assessed by evaluating fear memory via a cued fear conditioning test was impaired in 5XFamilial AD (5XFAD) mice, an animal model of AD. Compared to wild-type mice, 5XFAD mice showed changes in the phosphorylation of twelve proteins in the amygdala. Thus, our study provides twelve potential protein targets in the amygdala that may be responsible for the impairment in fear memory in AD.
J Pharmacol Sci, 2017; 133

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GLYCOGEN SYNTHASE KINASE-3 INHIBITION AFFECTS DOPAMINE METABOLISM BY DECREASING TYROSINE HYDROXYLASE ACTIVITY

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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The activation of presynaptic D2 autoreceptors in the striatum inhibits dopamine synthesis. This effect has been associated to changes in phosphorylation of tyrosine hydroxylase (TH) -the rate-limiting enzyme of brain dopamine biosynthesis-but the signaling mechanisms involved are not yet fully understood. We hypothesize that glycogen synthase kinase-3 (GSK-3) plays a key role in the presynaptic D2 autoreceptor signal transduction pathway, which could be of interest as an alternative target for schizophrenia treatment. We tested the effect of GSK-3 inhibitors CHIR-99021 and SB-216763 on dopamine synthesis and accumulation in rat striatum slices *ex vivo*. We also compared their effects with lithium chloride (LiCl), which is known to have antipsychotic properties and inhibit GSK-3, as well as with UNC9994, a β-arrestin biased D2 receptor agonist. HPLC-EC was used to assess dopamine accumulation, while dopamine synthesis was measured using radioisotopic HPLC-UV method. We have found that CHIR, SB, LiCl and UNC were all able to significantly decrease dopamine accumulation in rat striatum slices ex vivo. Further testing on CHIR revealed that it also significantly decreases dopamine synthesis. We also tested the amount of L-DOPA, given that this metabolite is an indicator of TH activity, and significant decrease in L-DOPA levels was found after CHIR treatment. Our results show that GSK-3 plays an important role in the dopamine synthesis pathway, by affecting the activity of TH. Since GSK-3 is part of the β-arrestin pathway, controlling dopamine hyperactivity through selective targeting could control psychotic symptoms without the adverse effects associated with conventional antipsychotics.

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HIPPOCAMPAL NEUROVASCULAR COUPLING AND SPATIAL WORKING MEMORY IMPAIRMENT IN A RODENT MODEL OF TYPE 2 DIABETES: IMPACT OF DIETARY NITRATE INTERVENTION

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Background: The functional integrity of the brain relies on prompt delivery of metabolic substrates (via local hemodynamic changes) matching the increase in neuronal activity – neurovascular coupling (NVC). Microvascular alterations negatively impact NVC, which contributes to neurodegeneration and cognitive decline, as observed in type 2 Diabetes mellitus (T2DM). **Aim:** We aimed to assess 1) the functionality of NVC in a rodent model of T2DM in connection with cognitive function and 2) if dietary nitrate, a metabolic precursor of the key mediator of NVC – nitric oxide (•NO) – would improve NVC and counteract the cognitive decline in diabetes. **Method:** The functionality of NVC was assessed by laser Doppler flowmetry in the hippocampus of Goto-Kakizaki (GK) rats, an animal model of T2DM. The glycemic profile (glucose tolerance test) and spatial learning and memory (Barnes maze) were also evaluated. The effect of dietary nitrate was assessed via oral supplementation of sodium nitrate for 12 weeks. Age-matched Wistar rats were used as controls. **Results/Conclusions:** Four months old GK rats displayed a significantly impaired spatial learning and memory performance along with a dysregulated glucose tolerance. Additionally, the hemodynamic response to glutamatergic activation (NVC) was lower in GK rats as compared to controls. Dietary nitrate intervention, shown to increase both plasma nitrate and nitrite concentration, abrogated the NVC impairment and improved the spatial memory in GK rats, without changing the glycemic profile. This data supports the potential of dietary nitrate as a therapeutic approach to mitigate cognitive decline in T2DM. **Funding:** COMPETE/FCT-POCI-01-0145-FEDER-000012-HealthyAging2020 and POCI-01-0145-FEDER-029099

Pubmed:
27607739: Rocha BS, Correia MG, Fernandes RC, Gonçalves JS, Laranjinha J
Dietary nitrite induces occludin nitration in the stomach.
The clinical implications of the nitrate-nitrite-nitric oxide pathway have been extensively studied in recent years. However, the physiological impact of bioactive nitrogen oxides produced from dietary nitrate has remained largely elusive. Here, we report a hitherto unrecognized nitrite-dependent nitrating pathway that targets tight junction proteins in the stomach. Inorganic nitrate, nitrite or saliva obtained after the consumption of lettuce were administered by oral gavage to Wistar rats. The enterosalivary circulation of nitrate was allowed to occur for 4 h after which the animals were euthanized and the stomach collected. Nitrated occludin was detected by immunoprecipitation in the gastric epithelium upon inorganic nitrite administration ($p < .05$) but was not observed in the case of inorganic nitrate or human saliva administration. This observation, along with differences in NO production rates from inorganic and salivary nitrite under simulated gastric conditions, suggests that competing reactions at acidic pH determine the production of nitrating agents (NO) or other, more stable, oxides. Accordingly, it is shown in vitro that salivary nitrite yields higher steady state concentrations of NO (0.37 ± 0.01 μM) than sodium nitrite (0.12 ± 0.03 μM). Dietary-dependent reactions involving the production of nitrogen oxides should be further investigated as, in the context of occludin nitration, the consumption of green leafy vegetables (with high nitrate content), if able to modulate gut barrier function, may have important implications in the context of leaky gut disorders.
Free Radic Res, 2016; 50

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NEUROPROTECTIVE EFFECTS OF CUSCUTA CHINENSIS LAM. UNDER THE HYPERGLYCEMIC-ALZHEIMER'S DISEASE IN VIVO MODEL

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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The susceptibility of aged brain to Alzheimer's disease (AD) development is related with several risk factors, including hyperglycemia and amyloid beta (Aβ) accumulation. *Cuscuta chinensis* (*C. chinensis*) Lam. has been reported to display pharmacological effects on the neuronal system and diabetic disorders. Thus, the aim of this study was to investigate whether the *C. chinensis* water extract (CCWE) protects the neuronal damage under the hyperglycemia risk factor model of AD (i.e. the hyperglycemic-Aβ toxic *in vivo* model). Hyperglycemia was induced in male C57BL/6N mice by intraperitoneal (IP) injection of streptozotocin (STZ, 50 mg/kg) for consecutive 3 days and verified by measuring fasting blood glucose levels. And then, a single intracerebroventricular (ICV) injection of $A\beta_{25-35}$ at does of 25 nM was performed. The $A\beta_{25-35}$-injected hyperglycemic mice were administered with 100, 200 mg/kg/day of CCWE for 14 days. The administration of CCWE reduced the levels of lipid peroxidation, reactive oxygen species generation, and nitrite oxide production in brain. Moreover, in association with the modulatory effect on the amyloidogenic pathway, the treatment of CCWE down-regulated the amyloidogenic pathway by declining amyloid precursor protein, presenilin 1, presenilin 2, and beta-secretase. Therefore, the CCWE could not only ameliorate oxidative stress, but also inhibit the amyloidogenic pathway in STZ/$A\beta_{25-35}$-injected mice. Our findings suggest that *C. chinensis* might be a potential candidate for the treatment in hyperglycemic-Alzheimer's disease-like pathological features.

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GUIERA SENEGALENSIS (COMBRETACEAE) LEAVES HYDROETHANOLIC EXTRACT PREVENTS SCOPOLAMINE-INDUCED COGNITIVE DYSFUNCTION BY REGULATING CHOLINERGIC AND ANTIOXIDANT SYSTEMS IN ZEBRAFISH (DANIO RERIO)

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to major cognitive and behavioural dysfunctions. Available drugs do not cure AD but only temporarily improve symptoms. *Guiera senegalensis* (GS) is a plant used in traditional medicine in Africa for the treatment of epilepsy and depression. However, the anti-amnesic property of GS has not yet been evaluated. Here we investigated whether GS leaves hydroethanolic extract prevents scopolamine-induced memory impairment and brain oxidative stress in zebrafish model. Sixty adults zebrafish were divided into six groups: normal control group which received distilled water, negative control group which received scopolamine, positive control group which received galantamine and scopolamine, and test groups which received different doses of GS and scopolamine. Memory was assessed using the Y-maze and novel object recognition (NOR). Acetylcholinesterase (AChE), superoxide dismutase (SOD), Glutathion peroxidase (GPX) and Catalase (CAT) activities, malondialdehyde (MDA), Glutathione (GSH) and protein carbonyl levels were also evaluated. In Y-maze, plant extract resulted in a significant increase of the time spent in the novel arm, total distance travelled, and turn angle. In NOR, preference percentage for the novel object significantly increased in test groups compared to negative control group. Moreover, GS significantly reduced brain AChE activity and significantly suppressed scopolamine-induced oxidative damage by decreasing MDA level, protein carbonyl level and increasing GSH level, SOD, CAT and GPX activities. These findings provide further relevance for the potential use of GS as a natural, alternative treatment against cognitive disorders associated to AD. **Key words:** *Guiera senegalensis*, scopolamine, Alzheimer's disease, memory, acetylcholinesterase.

Pubmed:
32446929: Keugong Wado E, Kubicki M, Ngatanko AHH, Léa Blondelle KD, Jorelle Linda D, Roland RN, Balbine K, Lamshoeft M, Assongalem AE, Foyet HS
Anxiolytic and antidepressant effects of Ziziphus mucronata hydromethanolic extract in male rats exposed to unpredictable chronic mild stress: Possible mechanisms of actions.
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J Ethnopharmacol, 2020; 260

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DIFFERENTIAL ENTRAINMENT OF PREFRONTAL NEURONAL ACTIVITY BY RESPIRATION-RELATED RHYTHMS ACROSS EMOTIONAL STATES

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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At each respiratory cycle, airflow in the nasal cavity induces respiration related oscillations (RR) in the olfactory bulb. These slow oscillations propagate broadly in the neocortex, and have recently been shown to play a role in cognitive function that are independent of olfaction. Among the forebrain areas displaying prominent RR is the medial prefrontal cortex (mPFC), a neocortical region extensively connected with the basal ganglia and the limbic sytsem, and involved in the regulation of emotions. To understand the role that RR might play in emotional control, we recorded single-unit activity in the mPFC of freely behaving mice while simultaneously monitoring respiration by electrophysiological recording from the nasal epithelium. We compared the neuronal activity during spontaneous immobility in the home cage, as a neutral state, to immobility induced by an intense stressor, namely tail suspension. First, we established that intense emotions changed the respiratory pattern and increased the entrainment of the mPFC local field potential by the RR. Secondly, we found that subjecting mice to intense stress shifted the preferred respiratory phase of mPFC single units from inspiration to late expiration with a differential effect on putative interneurons and pyramidal cells. Finally, the proportion of cells modulated by the respiration was changing depending on their laminar location and emotional state of the mice, which might reflect the recruitment of different long-range connections by the RR during stress. Overall, these observations suggest a functional role of the RR in the synchronization of PFC neuronal activity for the processing of emotions.

Pubmed:
35121665: Sauer JF, Folschweiller S, Bartos M
Topographically organized representation of space and context in the medial prefrontal cortex.
Spatial tuning of neocortical pyramidal cells has been observed in diverse cortical regions and is thought to rely primarily on input from the hippocampal formation. Despite the well-studied hippocampal place code, many properties of the neocortical spatial tuning system are still insufficiently understood. In particular, it has remained unclear how the topography of direct anatomical connections from hippocampus to neocortex affects spatial tuning depth, and whether the dynamics of spatial coding in the hippocampal output region CA1, such as remapping in novel environments, is transmitted to the neocortex. Using mice navigating through virtual environments, we addressed these questions in the mouse medial prefrontal cortex, which receives direct input from the hippocampus. We found a rapidly emerging prefrontal representation of space in the absence of task rules, which discriminates familiar from novel environments and is reinstated upon reexposure to the same familiar environment. Topographical analysis revealed a dorsoventral gradient in the representation of the own position, which runs opposite to the innervation density of hippocampal inputs. Jointly, these results reveal a dynamically emerging and topographically organized prefrontal place code during spontaneous locomotion.
Proc Natl Acad Sci U S A, 2022; 119
34790100: Folschweiller S, Sauer JF
Respiration-Driven Brain Oscillations in Emotional Cognition.
Respiration paces brain oscillations and the firing of individual neurons, revealing a profound impact of rhythmic breathing on brain activity. Intriguingly, respiration-driven entrainment of neural activity occurs in a variety of cortical areas, including those involved in higher cognitive functions such as associative neocortical regions and the hippocampus. Here we review recent findings of respiration-entrained brain activity with a particular focus on emotional cognition. We summarize studies from different brain areas involved in emotional behavior such as fear, despair, and motivation, and compile findings of respiration-driven activities across species. Furthermore, we discuss the proposed cellular and network mechanisms by which cortical circuits are entrained by respiration. The emerging synthesis from a large body of literature suggests that the impact of respiration on brain function is widespread across the brain and highly relevant for distinct cognitive functions. These intricate links between respiration and cognitive processes call for mechanistic studies of the role of rhythmic breathing as a timing

signal for brain activity.
Front Neural Circuits, 2021; 15

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COGNITIVE PHENOTYPES AFTER LEFT AND RIGHT HEMISPHERIC STROKE: A LATENT CLASS ANALYSIS

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Aims. Although cognitive impairment after stroke is highly prevalent, most research has not investigated patterns of comorbidity. In this study, we aimed to identify separable cognitive profiles after stroke. **Methods.** We retrospectively analysed data of 2,172 stroke patients (43% left hemisphere, 50% right hemisphere; 81% ischemic; tested on average 19 days post-stroke) across three countries. All patients completed the Oxford Cognitive Screen in their native language, which screens for impairments in language, memory, number, praxis, executive function and attention. To identify cognitive profiles within this cohort, latent class models were estimated. The best model was selected based on three fit indices, the certainty of class assignments and interpretability of classes. **Results.** The best model suggested 13 distinguishable cognitive profiles. The largest group demonstrated no cognitive impairment (30.2%). Five right-lateralized profiles were identified, of which three were characterized by left-sided neglect (neglect without comorbidity, neglect with comorbidity, and neglect with signs of hemianopia). The two other right-lateralized profiles were characterized by non-spatial attention impairments and executive impairment, respectively. Four left-lateralized profiles occurred, of which two were characterized by language impairments (with impaired versus intact praxis and numerical abilities). Four other profiles occurred equally often after left- and right-hemispheric stroke. Age, country, gender and time since stroke did not predict the cognitive profiles. **Conclusions.** A simple distinction of right- and left-lateralized cognitive profiles after stroke does not capture the heterogeneity of post-stroke cognitive comorbidities. Our study presents a big step towards an empirically validated taxonomy of post-stroke cognitive profiles.

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IMPULSIVE BEHAVIOR ASSESSMENT IN A PRECLINICAL MODEL OF STROKE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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Clinical stroke patients show relevant cognitive impairments including inhibitory control deficit; however, preclinical research has focused almost exclusively on motor deficits. In the present work we assess impulsive decision making in a preclinical model of stroke. Male Wistar rats underwent focal ischemia induced in the medial Prefrontal Cortex (mPFC) using bilateral intracerebral injections of endothelin-1, or sham surgery. We evaluated impulsive decision making by the Rodent Gambling task (RGT). The results will be discussed in terms of the effects of stroke on prefrontal cortex damage and the long term alterations of impulsive behaviors such as impulsive risky decision making, perseverative responses and cognitive inflexibility. Preclinical models of focal stroke in the mPFC could help to understand the cognitive impairments and the association with the neuroplastic changes after damage. **Funded by Gobierno de España MCIN/ AEI /10.13039/501100011033/ grant number PGC2018-099117-B-C21, UAL2020-CTSD2068 FEDER I+D+i "Una manera de hacer Europa" and Redes de Investigación Cooperativa Orientadas a Resultados en Salud RICORS-ICTUS RD21/0006/0010.**

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ASSOCIATION OF METABOLITES WITH COGNITIVE DECLINE AND LIPID PATTERN IN ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: ALZHEIMER'S DISEASE: FROM NEUROINFLAMMATION TO NEUROPROTECTION

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The rapid decline in cognition is a major component of many neurodegenerative disorders with the most common type being Alzheimer's disease (AD), as it poses a global challenge both in terms of mortality and exerting pressure on the healthcare system. Delayed disease diagnosis along with the lack of disease modifying treatments mean there is an urgent need to decipher its pathogenesis and underlying biochemistry. It is hypothesised that AD leaves a metabolic signature which can be detectected in the pre-clinical stage. Using an untargeted metabolic profiling approach, metabonomic data acquired from biofluids and tissue samples from AD patients were compared to non-disease controls. In the first study, the relationship between potential metabolites found in human serum and urine and previously recorded cogntive function scores via AD Assessment Scale-Cognition (ADAS-Cog) subset were assessed in training (n = 62; AD patients on donepezil) and validation (n = 75; combination of subgroups e.g., AD) sets. The cohort consisited of controls, stable mild cogntive impairment (MCI), converting MCI and AD subgroups and the feauture exhibiting significant association with cogntition was annotated to be theophylline. Also, to further probe potential mechanisms associated with AD, lipid profiling analysis of tissue samples acquired from AD (n = 102) and control (n = 39) post-mortem human brain expressing triggering recpetor expressed on myeloid cells 2 (*TREM2*; AD risk gene) variants. Preliminary multivariate statistical models (OPLS-DA) for the somatosensory cortex showed lipid features driving separation between sex, group (AD vs. control) and *TREM2* variants.

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PATHOGENIC EFFECTS OF GABAB RECEPTOR ANTIBODIES FROM PATIENTS WITH AUTOIMMUNE ENCEPHALITIS ON SYNAPTIC STRUCTURE AND MEMORY

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Aims: $GABA_B$ receptor ($GABA_BR$) are dimeric G-protein coupled receptors regulating presynaptic neurotransmitter release and postsynaptic excitability. In $GABA_BR$ autoimmune encephalitis IgG antibodies target the B1 subunit of $GABA_BR$ leading to various symptoms, including epilepsy, cognitive disorders and short-term memory loss. Here we aimed to investigate the pathophysiological effect of human serum antibodies on synaptic structure and memory, using staining protocols and behavioral paradigms. Methods: Purified patient-derived immunoglobulin G antibodies (control- and anti-$GABA_BR$-IgG) were used in a mouse model of continuous 14-day cerebroventricular infusion via osmotic pumps. The IgG influence on memory and cognition was examined via novel object recognition test (NOR) and novel object location test (NOL), performed with a retention time of either 3 hours or 24 hours. We preincubated cultivated neurons with control and $GABA_BR$ IgG and co-stained with commercial $GABA_BR$ antibodies and pre- and post-synaptic markers to evaluate preferential IgG binding sites and structural changes in the synaptic area. Results: Evaluation of cognitive function trough behavioral paradigm suggest an influence of the anti-$GABA_BR$-IgG on the consolidation and maintenance of contextually precise memory, but not for the initial encoding of the memory. Super resolution imaging gave further insights on changes in peri-synaptic structure and $GABA_BR$ localization mediated by patients' IgG treatment. Conclusions:
Our results provide insight on differential action of patient IgG on pre- and post-synaptic $GABA_BR$, and elucidated potential mechanism involved in cognitive impairment in this subtype of autoimmune encephalitis. Further experiments will evaluate the influence of patient IgG on $GABA_BR$ B1 subunit localization.

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GENETIC PREDISPOSITION IN AUTOIMMUNE ENCEPHALITIS ASSOCIATED WITH AUTOANTIBODIES AGAINST GLUTAMIC ACID DECARBOXYLASE

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

Marisol Herrera Rivero[1], Christine Strippel[2], Mareike Wendorff[3], Anika Witten[1], Christina Schröter[4], Christopher Nelke[4], Gregor Kuhlenbäumer[5], Frank Leypoldt[6], Andre Franke[3], Sven Meuth[4], Monika Stoll[1], Nico Melzer[4]
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Aims: Autoimmune neurological syndromes (AINS) with autoantibodies against the 65 kDa isoform of the glutamic acid decarboxylase (GAD65) present with limbic encephalitis, cerebellitis and stiff-person-syndrome, or overlap forms. Here, we investigated the genetic predisposition to anti-GAD65 AINS. **Methods:** We performed the first genome-wide association study (GWAS) for anti-GAD65 AINS in a large German cohort of 167 patients and 1,047 population-based controls (N=1,214). GWAS findings were further explored in the cerebrospinal fluid (CSF) proteome of a small and virtually independent cohort of 10 patients and 10 controls. **Results:** Our GWAS identified 16 genome-wide significant ($p<5x10^{-8}$) loci for the susceptibility to anti-GAD65 AINS. The great majority of variants in loci (>90%) mapped to non-coding regions of the genome. Over 40% of the variants are documented regulators of a total of 48 genes in disease relevant cells and tissues, mainly $CD4^+$ T cells and the cerebral cortex. The annotation of epigenomic marks suggested specificity for neurons, monocytes, T cells as well as neural and hematopoietic progenitor cells. A network analysis of the implicated protein-coding genes uncovered a role of protein kinase C beta (*PRKCB* on chr16*)* in the pathophysiology of anti-GAD65 AINS. The larger and stronger locus spanned the human leukocyte antigen (HLA) region (chr6:29488249-31105310, 244 variants, 3 lead variants), implicating mainly non-classical HLA molecules. In the CSF proteome, we found support for this locus through differential levels of HLA-A/B and C4A. **Conclusions:** These findings suggest a strong genetic predisposition with direct functional implications for immunity and neural function in anti-GAD65 AINS.

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THE NEUROCHEMISTRY OF CHRONIC FATIGUE SYNDROME/MYALGIC ENCEPHALOMYELITIS AND LONG-COVID: A MAGNETIC RESONANCE SPECTROSCOPY STUDY AT 7 TESLA

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Background: Chronic fatigue syndrome or myalgic encephalomyelitis (CFS/ME) is often preceded by viral or bacterial infection. Symptoms include pronounced fatigue, post-exertional malaise, autonomic dysregulation, myalgia, and cognitive deficits. Similar symptoms are often present in “long-COVID”, a sequela of infection with the SARS-CoV-2 virus. Available evidence suggests CFS/ME pathology may be linked to neuroinflammation and oxidative stress, resulting in impaired mitochondrial function and energy metabolism. There is a need to understand the pathogenesis of both conditions, particularly given their individual and societal symptomatic burden and the paucity of pharmacological treatment options. **Aims:** Using high-field magnetic resonance spectroscopy (MRS), we aimed to examine central and peripheral symptoms of CFS/ME and long-COVID by measuring biochemical concentrations in the anterior cingulate cortex (ACC) and peripheral muscle. Previous CFS/ME data revealed neurochemical abnormalities in the ACC, which is associated with cognitive, emotional, and pain processing, and autonomic function. Metabolites of interest were markers of energy metabolism (creatine and phosphocreatine); neurotransmitters (glutamate, glutamine and GABA); and glutathione, a marker of oxidative stress. A secondary aim was to determine how neurochemical concentrations relate to symptoms and cognitive performance. **Methods:** 20 CFS/ME (13 tested, recruitment ongoing), 20 long-COVID, and 20 healthy participants underwent MRS scans of the brain and leg at 7 Tesla. Voxels were placed in the pregenual and dorsal ACC, and the right calf muscle. Participants also completed a battery of cognitive tests and questionnaires assessing their symptoms and psychological wellbeing. **Results:** Data analysis is in-progress. Results and conclusions will be presented in the final poster.

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ELECTROPHYSIOLOGICAL CHARACTERISATION OF HIPPOCAMPAL NETWORKS IN ANTI-NMDA RECEPTOR ENCEPHALITIS: FROM SYNAPSE TO CIRCUIT

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Introduction NMDAR-encephalitis is an autoantibody-mediated psychiatric syndrome, presenting with seizure and psychosis. Molecular investigations have delineated a pathway of action of these autoantibodies, whereby NMDARs are translocated out of synaptic compartments and subsequently internalised. This depletion of surface NMDAR expression results in an excitatory hypofunction on hippocampal neurons. **Aims** We aim to characterise a broader-scale functional impact of antibody action, at synaptic-, cellular- and circuit-levels, to finely elucidate the mechanisms by which NMDAR-autoantibodies elicit a hyper-excitable seizure phenotype in clinical settings. **Methods** Using a range of patch-clamp electrophysiological techniques, we examined the synaptic disturbance induced by indirect actions of NMDAR-autoantibodies, encompassing dysfunction at both excitatory and inhibitory inputs to hippocampal cells. **Results** We have identified alterations to the synaptic phenotype of hippocampal neurons, which are compounded by a shift in intrinsic excitability at the cellular- and network-scales. We have further demonstrated a divergence in the pathogenicity of NMDAR-autoantibodies on discrete subpopulations of hippocampal neurons. **Conclusions** Overall, we have characterised an additional disease pathway in NMDAR-encephalitis, whereby alterations in synaptic and cellular functional properties, induced by autoantibodies, are identified at the synaptic and cellular level.

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IMPACT OF AUTOANTIBODIES FROM PATIENTS WITH NMDAR-ENCEPHALITIS ON THE GABAERGIC SYNAPSES

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Autoantibodies targeting the NMDA receptor (NMDAR) have been associated to neuropsychiatric disorders together with memory problems, movement disorders, and epileptic seizures. These autoantibodies cause the internalization of receptors, leading to NMDAR hypofunction. Whether this major dysfunction alter other neurotransmitter systems, such as the GABAergic one, remains an open question. Aim: Here, we aimed to tackle this question and the putative mechanism by which NMDAR autoantibodies could alter the inhibitory transmission. Methods: Monoclonal NMDAR autoantibodies from patients with NMDAR encephalitis or control non-reactive human monoclonal antibody antibodies were used in hippocampal cultured neurons. We performed single nanoparticle tracking and immunofluorescence assays to assess changes in the diffusion and density of the receptors at the GABAergic synapses. Results: We report alteration of the synaptic material at the inhibitory as well as at the excitatory synapse upon exposure to NMDAR autoantibodies. These changes take place at the single receptor level dynamics and at the macroscopic level. Conclusions: Together, our results demonstrate that NMDAR autoantibodies not only affect the target antigen, but also cause alterations in the trafficking and surface distribution of receptors at the inhibitory synapse, providing evidence of the interplay between glutamatergic and GABAergic synapses.

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HUMAN ANTI-GLUN1 AUTOANTIBODIES INDUCE DEFECTIVE SYNAPTIC PLASTICITY DEPENDENT ON CAMKII AND DAPK1 PATHWAYS AS INVESTIGATED BY SUPER-RESOLUTION MICROSCOPY

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Aims: The anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is an autoimmune disorder characterized by specific autoantibodies targeting the GluN1 subunit of postsynaptic NMDARs. We investigated the effect of patient monoclonal anti-GluN1 antibodies on important pathways for synaptic plasticity and function, e.g. synaptic signalling events involving the antagonistic molecules CaMKII and DAPK1. **Methods:** We incubated primary hippocampal cell cultures (div 20) with patient-derived monoclonal anti-GluN1 or control antibodies. Afterwards, we performed additional incubation with the DAPK1 inhibitor TC-DAPK6 and/or LTP induction. We analysed the localization and distribution of the NMDAR GluN1 subunit and the GluA1 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and correlated their expression to a postsynaptic marker with direct stochastic reconstruction microscopy (*d*STORM). Furthermore, we investigated the localisation of CaMKII and DAPK1 compared to a postsynaptic marker using structured illumination microscopy (SIM). **Results:** We established a stable LTP and immunostaining protocol for postsynaptic markers, CaMKII and DAPK1 in primary neuronal cultures for *d*STORM and SIM imaging. We established TC-DAPK6 treatment to inhibit co-localisation of DAPK1 within synapses. Furthermore, we investigated the combined application of LTP, human GluN1 antibodies, and/or TC-DAPK6 on the localisation of GluN1/GluA1 and CaMKII/DAPK1 compared to synaptic markers, thus providing evidence for antibody-induced and CaMKII/DAPK1-dependent synaptic remodelling. **Conclusions:** We observed defective cellular NMDA receptor dependent LTP upon highly specific, patient-derived autoantibodies. Interfering with CaMKII/DAPK1-dependent plasticity pathways by inhibiting DAPK1 function and translocation to extrasynaptic compartments might be beneficial for GluN1 antibody-induced defective LTP.

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PATHOGENIC EFFECTS OF GABAB RECEPTOR ANTIBODIES FROM PATIENTS WITH AUTOIMMUNE ENCEPHALITIS ON NEURONAL SIGNALING AND NETWORK EXCITABILITY

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Aims:
$GABA_B$ receptor ($GABA_BR$) encephalitis is an autoimmune disorder with immunoglobulin G (IgG) antibodies targeting pre- and postsynaptic $GABA_B$Rs. Patients suffer from severe memory dysfunction and epileptic seizures. $GABA_B$Rs are G-protein coupled receptors and mediate complex synaptic signaling in glutamatergic as well as GABAergic neurons by regulating presynaptic neurotransmitter release and postsynaptic excitability. Here we aimed at investigating the pathophysiological effect of human serum antibodies on neuronal synaptic transmission. Methods:
Purified patient-derived IgG antibodies were used in a mouse model of continuous 14-day cerebroventricular infusion via osmotic pumps. To investigate $GABA_BR$ mediated regulation of synaptic transmission, somatic patch-clamp recordings of CA1 pyramidal neurons were performed and special stimulation protocols were applied on afferent fibers. To reveal IgG-effects on $GABA_BR$ function, either the $GABA_BR$ agonist baclofen or the antagonist CGP55845 was applied by bath perfusion. Results:
$GABA_BR$ antibodies affected excitatory synaptic transmission and short-term plasticity of heteroreceptors, as they reduced eEPSC amplitudes and the agonist effect of baclofen onto paired pulse ratios. Furthermore, they block presynaptic autoreceptors on inhibitory synapses, leading to less depression during repetitive IPSC stimulation. In contrast, postsynaptic $GABA_BR$-activated K^+-currents and action potential firing is not influenced by patient's IgGs. Conclusions:
Our results provide evidence that $GABA_BR$ antibodies antagonize the receptor preferably on presynaptic auto- and heteroreceptors, but have less direct pathogenic effect on postsynaptic $GABA_BR$-downstream signaling. These changes may contribute to severe neuronal dysfunction as the basis of memory dysfunction and increased seizure susceptibility.

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ACUTE EFFECTS OF HUMAN MONOCLONAL ANTI-GLUN1 AUTOANTIBODIES ON NMDA-RECEPTOR CHANNEL FUNCTION

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Aim: NMDAR encephalitis is an autoimmune disorder characterized by specific autoantibodies to the aminoterminal domain of the NMDAR GluN1 subunit. This project aims at understanding the acute effects of human monoclonal autoantibodies on NMDA-receptor channel function in detail. **Methods:** To identify changes in glutamate–evoked NMDAR channel responses, a non-host cell line NG108-15 was transfected with NR1/NR2A subunits of NMDARs. We developed a transfection protocol for lower cytotoxicity and optimized membrane integrity. For the fast application of various NMDAR-modulators (including ligands and pathogenic antibodies), a fast solution exchange system was applied, which is able to switch between multiple solutions with a controllable flow rate. To measure the effect of human pathogenic antibodies on acute NMDAR responses, whole-cell patch recordings were applied in both, NR1/NR2A transfected NG108-15 cells and cultured neurons. Moreover, immunostaining followed by imaging using confocal microscopy was performed to evaluate the antibodies' binding kinetics to NMDARs during fast perfusion. **Results:** We established a fast-perfusion antibody-application scheme based on immunostainings. We assessed the influence of monoclonal antibodies on the steady-state and peak amplitude of NMDAR-current in NG108-15 and cultured neurons. At low antibody concentration (10 µg/ml), we found no significant change in the steady-state of NMDAR-current. Furthermore, the influence of these antibodies on the peak-amplitude of NDMAR-currents elucidated the change in the simultaneous opening of NDMARs. **Conclusion:** Human pathogenic antibodies to the NMDAR GluN1 subunit may influence NMDAR channel function. These direct effects may contribute to NMDAR-directed pathology and the development of disease symptoms in patients.

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PATHOPHYSIOLOGICAL RELEVANCE OF NMDAR1-AUTOANTIBODIES DURING GRAY MATTER INFLAMMATION

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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After the discovery of an encephalitis associated with autoantibodies directed against the NMDAR1 subunit of N-methyl-D-aspartate receptors (NMDAR), a vast amount of studies investigated the pathogenicity of these autoantibodies, resulting in some understanding of their function and mode of action. Prior work has shown, that NMDAR are internalized upon binding of NMDAR1-autoantibodies (NMDAR1-AB) resulting in NMDAR-hypofunction. This decrease in surface NMDAR expression potentially explains some psychiatric and neurological symptoms observed in patients with the so-called anti-NMDAR encephalitis. However, the actual pathomechanism(s) that induce encephalitis and drive neuroinflammation are understudied and few studies investigated the pathophysiological relevance of NMDAR1-AB in a neuroinflammatory context. To investigate the effects of NMDAR1-AB during gray matter inflammation, we immunized transgenic (Neurod6$^{tm2.1(cre/ERT2)Kan}$ x Gt(ROSA)26Sor$^{tm1(DTA)Jpmb}$; 'DTA') mice against a cocktail of four NMDAR1-specific peptides and subsequently induced a spatiotemporally defined sterile encephalitis by tamoxifen-dependent ablation of pyramidal neurons. Upon DTA induction, mice developed lasting blood-brain barrier dysfunction, gray matter inflammation and neurodegeneration, particularly in the hippocampus and cortex. Behavioral phenotyping revealed typical hippocampal learning/memory deficits, prefrontal cortical network-dysfunction and hyperlocomotion in DTA mice. Compared to ovalbumin-immunized control mice, mice carrying NMDAR1-AB displayed increased hyperlocomotion, reminiscent of mice injected with the NMDAR antagonist MK-801. Importantly, the presence of NMDAR1-AB did not aggravate the neuroinflammation in DTA mice and immunized non-DTA mice were free of any signs of neuroinflammation. To conclude, while the encephalitic potential of NMDAR1-AB remains to be proven, NMDAR1-AB can modulate the behavioral phenotype of an underlying gray matter inflammation (reference: doi.org/10.1038/s41380-021-01238-3).

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BOARD NUMBER: S05-245

FUNCTIONAL EFFECTS OF HUMAN LGI1 AUTOANTIBODIES ON CA3 PYRAMIDAL NEURONS: A SPECIES-SPECIFIC IN VITRO STUDY IN HUMAN HIPPOCAMPAL SLICE CULTURES

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Leucine-rich glioma-inactivated 1 (LGI1) is a neuronal protein that modulates axonal $K_v1.1$ channels density. $K_v1.1$ channels mediate the dendrotoxin (DTX)-sensitive D-type potassium current, which regulates neuronal action potential firing and excitability. Human autoantibodies against LGI1 are associated with the second most common autoimmune encephalitis characterized by faciobrachial dystonic seizures, memory impairment, and, in some cases, severe uncontrolled seizures. Recently, LGI1 autoantibodies cloned from cerebrospinal fluid of patients with LGI1 encephalitis have been shown to increase neuronal excitability and glutamatergic synaptic transmission of hippocampal CA3 pyramidal neurons in mouse brain slice cultures. The aim of the current study was to investigate the functional effects of human LGI1 autoantibodies in a species-specific manner, namely on human hippocampal tissue derived from epilepsy surgical resections. Upon resection, human hippocampal tissue was sliced and the slices were cultured for 24h in the absence or presence of LGI1 autoantibodies. Next, excitability of CA3 pyramidal neurons was assessed by whole-cell patch clamp experiments together with extracellular recordings of antidromically-evoked population spikes (PS) of CA3 axons. DTX-K was applied as pharmacological control. We found that LGI1 autoantibodies increased human CA3 neuronal excitability and spike latency. In addition, LGI1 increased the amplitude of the antidromic PS, in contrast to the DTX-K effect, which slightly decreased the amplitude, while increasing its delay. We thus confirm that LGI1 autoantibodies increase neuronal excitability underlying seizure generation in human tissue. Further investigation is necessary to understand target specificity of LGI1 autoantibodies at the axonal level.

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BOARD NUMBER: S05-246

CASPR2 AUTOANTIBODIES ELICIT CONCENTRATION-DEPENDENT PERTURBATIONS IN THE REGULATION OF AMPA RECEPTOR TRAFFICKING AND SYNAPTIC PLASTICITY

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Antibodies targeting the cell-adhesion protein CASPR2 have been found in patients with autoimmune synaptic encephalitis, which can present peripheral nerve hyperexcitability, memory loss, cognitive impairments, and seizures. Despite the clear implication of CASPR2 autoantibodies (CASPR2-Abs) in disease, the pathogenic mechanisms ensuing from antibody-mediated perturbations in CASPR2 function are still unclear. Our laboratory recently described a novel role for CASPR2 in the regulation of glutamate AMPA receptors (AMPAR) and cortical excitatory synaptic transmission. Herein, we explored whether patient CASPR2-Abs disrupt excitatory synapse function. To achieve this, we incubated primary cultured neurons with a range of concentrations (0.2, 5, 20 or 50 µg/mL) of purified IgGs from a patient with anti-CASPR2 synaptic encephalitis. We found that CASPR2-Abs alter the synaptic expression of Caspr2 and induce a dose-dependent perturbation in AMPAR trafficking. At lower concentrations, CASPR2-Abs decrease synaptic AMPARs by increasing their internalization from the cell surface. Conversely, endocytosis of AMPARs is hampered at higher CASPR2-Ab concentrations, resulting in a striking synaptic accumulation of surface AMPARs. Moreover, we show that CASPR2-Abs at lower concentrations prevent the synaptic incorporation of AMPARs following chemically-induced long-term potentiation; whereas higher CASPR2-Ab concentrations occlude this effect. Finally, we find that CASPR2-Abs disrupt AMPAR function, since excitatory synaptic transmission in the mouse visual cortex is perturbed following *in vivo* incubation with CASPR2-Abs. Overall, our results suggest that CASPR2-Abs elicit concentration-dependent effects that distinctively perturb the function of CASPR2 in regulating AMPAR trafficking and synaptic plasticity, thus pinpointing the glutamatergic system as a target for pathogenesis in CASPR2 synaptic encephalitis.

Pubmed:
27241695: Fernandes D, Carvalho AL
Mechanisms of homeostatic plasticity in the excitatory synapse.
Brain development, sensory information processing, and learning and memory processes depend on Hebbian forms of synaptic plasticity, and on the remodeling and pruning of synaptic connections. Neurons in networks implicated in these processes carry out their functions while facing constant perturbation; homeostatic responses are therefore required to maintain neuronal activity within functional ranges for proper brain function. Here, we will review in vitro and in vivo studies demonstrating that several mechanisms underlie homeostatic plasticity of excitatory synapses, and identifying participant molecular players. Emerging evidence suggests a link between disrupted homeostatic synaptic plasticity and neuropsychiatric and neurologic disorders. Hebbian forms of synaptic plasticity, such as long-term potentiation (LTP), induce long-lasting changes in synaptic strength, which can be destabilizing and drive activity to saturation. Conversely, homeostatic plasticity operates to compensate for prolonged activity changes, stabilizing neuronal firing within a dynamic physiological range. We review mechanisms underlying homeostatic plasticity, and address how neurons integrate distinct forms of plasticity for proper brain function. This article is part of a mini review series: "Synaptic Function and Dysfunction in Brain Diseases".
J Neurochem, 2016; 139
34818347: Carvalho AL, Fernandes D
It takes two to tango: Concerted protein translation and degradation necessary for synaptic scaling.
Synaptic scaling allows neurons to adjust synaptic strength in response to chronic alterations in neuronal activity. A new study in PLOS Biology identifies a pathway that synergizes protein synthesis and degradation with remodeling of the

microRNA (miRNA)-induced silencing complex (miRISC) to mediate synaptic scaling.
PLoS Biol, 2021; 19
30843029: Fernandes D, Santos SD, Coutinho E, Whitt JL, Beltrão N, Rondão T, Leite MI, Buckley C, Lee HK, Carvalho AL
Disrupted AMPA Receptor Function upon Genetic- or Antibody-Mediated Loss of Autism-Associated CASPR2.
Neuropsychiatric disorders share susceptibility genes, suggesting a common origin. One such gene is CNTNAP2 encoding contactin-associated protein 2 (CASPR2), which harbours mutations associated to autism, schizophrenia, and intellectual disability. Antibodies targeting CASPR2 have also been recently described in patients with several neurological disorders, such as neuromyotonia, Morvan's syndrome, and limbic encephalitis. Despite the clear implication of CNTNAP2 and CASPR2 in neuropsychiatric disorders, the pathogenic mechanisms associated with alterations in CASPR2 function are unknown. Here, we show that Caspr2 is expressed in excitatory synapses in the cortex, and that silencing its expression in vitro or in vivo decreases the synaptic expression of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors and the amplitude of AMPA receptor-mediated currents. Furthermore, Caspr2 loss of function blocks synaptic scaling in vitro and experience-dependent homoeostatic synaptic plasticity in the visual cortex. Patient CASPR2 antibodies decrease the dendritic levels of Caspr2 and synaptic AMPA receptor trafficking, and perturb excitatory transmission in the visual cortex. These results suggest that mutations in CNTNAP2 may contribute to alterations in AMPA receptor function and homoeostatic plasticity, and indicate that antibodies from anti-CASPR2 encephalitis patients affect cortical excitatory transmission.
Cereb Cortex, 2019; 29

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BOARD NUMBER: S05-247

LONG-LASTING DEGRADATION OF HIPPOCAMPAL SPATIAL REPRESENTATION AND MEMORY, IN THE MOUSE MODEL OF ANTI-NMDAR ENCEPHALITIS

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Anti-NMDA receptor encephalitis is the most frequent antibody-mediated encephalitis, in which patients' autoantibodies are directed against subunits of N-methyl-D-aspartate receptors (NMDARs). Patients with this disorder suffer a prolonged recovery process, bearing severe deficits of episodic long-term memory. In an animal model of 14-day continuous cerebroventricular intracranial transfer of antibodies, a decrease of performance in a memory task, along with reduction of synaptic NMDARs, has been reported. Here, we have used the same mouse model to investigate hippocampal neural activity over a period of 80 days. We recorded functional calcium imaging of CA1 neuronal population activity of mice that were freely moving and performing 2 distinct behavioral tasks. The tasks were designed to assess animals' abilities in either a) forming new memories or b) recalling old memories. We found that the CA1 neuronal population's firing activity increased significantly as a result of human NMDAR-antibodies. This high activity state lasted beyond 4 weeks after the infusion stopped. In addition, the decrease of behavioral performance in both memory tasks, as a result of NMDAR-antibodies, was accompanied by the decrease in the amount of spatial information encoded in neural activities and the reduction of place cells' stability over the duration of the experiment.
Our data support a novel methodology to create an amnesic state by using highly specific antibodies for blocking NMDAR function. More importantly, our results can explain the long-term effects of antibody-mediated dysfunction of NMDAR-related circuitry and the slow recovery period in patients with this disease.

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BOARD NUMBER: S05-248

HUMAN CASPR2 ANTIBODIES REVERSIBLY ALTER MEMORY AND THE CASPR2 PROTEIN COMPLEX

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Aims: The encephalitis associated with antibodies against contactin-associated protein-like 2 (CASPR2) is presumably antibody-mediated but the antibody effects and whether they cause behavioral alterations are not well-known. Here, we used a mouse model of patients' IgG transfer and super-resolution microscopy to demonstrate the antibody pathogenicity. **Methods:** IgG from patients with anti-CASPR2 encephalitis or healthy controls were infused into the cerebroventricular system of mice. The levels and colocalization of CASPR2 with transient axonal glycoprotein-1 (TAG1) were determined with Stimulated Emission Depletion (STED) microscopy (40-70nm lateral resolution). Hippocampal clusters of Kv1.1 voltage-gated potassium channels (VGKC) and GluA1-containing AMPA receptors were quantified with confocal microscopy. Behavioral alterations were assessed with standard behavioral paradigms. Cultured neurons were used to determine the levels of intracellular CASPR2 and TAG1 after exposure to patients' IgG. **Results:** Infusion of patients' IgG, but not control IgG, caused memory impairment along with hippocampal reduction of surface CASPR2 clusters and decreased CASPR2/TAG1 colocalization. In cultured neurons, patients' IgG led to an increase of intracellular CASPR2 without affecting TAG1, suggesting selective CASPR2 internalization. Additionally, mice infused with patients' IgG showed decreased levels of Kv1.1 and GluA1 (two CASPR2 regulated proteins). All these alterations and the memory deficit reverted to normal after removing patients' IgG. **Conclusions:** IgG from patients with anti-CASPR2 encephalitis cause reversible memory impairment, inhibit the interaction of CASPR2/TAG1, and decrease the levels of CASPR2 and related proteins (VGKC, AMPAR). These findings demonstrate that patients CASPR2 antibodies are pathogenic and provide support to the use of antibody-removing treatment approaches.

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BOARD NUMBER: S05-249

CHIMERIC AUTOANTIBODY RECEPTOR T CELLS TARGETING AUTOREACTIVE B CELLS IN N-METHYL-D-ASPARTATE (NMDA) RECEPTOR ENCEPHALITIS

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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NMDA receptor (NMDAR) encephalitis is the most common autoimmune encephalitis causing psychosis, epileptic seizures and cognitive impairment. Current treatment options are based on broad immunosuppression or non-selective antibody removal, resulting in often treatment-limiting side effects or insufficient responses. Disease-defining pathogenic autoantibodies bind the NMDAR leading to its internalization and subsequent synaptic alterations. Here, we developed NMDAR-specific Chimeric autoantibody receptor (NMDAR-CAAR) T cells to selectively target the source of disease-causing autoantibodies, anti-NMDAR-specific B cells. NMDAR-CAARs consist of an extracellular NMDAR autoantigen fused to intracellular 4-1BB/CD3ζ domains. After coculture with Nalm6 or K562 cells expressing anti-NMDAR-specific B cell receptors (BCRs), human NMDAR-CAAR T cells released interferon-gamma and granzyme B as markers of T cell effector function and killed target cells in an antigen-specific manner with high specificity. Furthermore, target cell encounter *in vitro* induced proliferation of NMDAR-CAAR T cells. In a NOD/Shi-*scid*/IL-2Rγ^{null} (NOG) mouse model, treatment with NMDAR-CAAR T cells led to depletion of Nalm6 B cells expressing an anti-NMDAR BCR and sustained reduction of autoantibody levels with no notable off-target toxicity. For the first time, this strategy could provide a highly selective treatment of a severe neurological autoimmune disease with reduced side effects, faster remission and better long-term prognosis. These results will the pave the way for clinical trials of CAAR-T cells in patients with antibody-mediated neurological disorders and can be expanded to a broader spectrum of antibody-mediated diseases.

Pubmed:
35076281: Reincke SM, Yuan M, Kornau HC, Corman VM, van Hoof S, Sánchez-Sendin E, Ramberger M, Yu W, Hua Y, Tien H, Schmidt ML, Schwarz T, Jeworowski LM, Brandl SE, Rasmussen HF, Homeyer MA, Stöffler L, Barner M, Kunkel D, Huo S, Horler J, von Wardenburg N, Kroidl I, Eser TM, Wieser A, Geldmacher C, Hoelscher M, Gänzer H, Weiss G, Schmitz D, Drosten C, Prüss H, Wilson IA, Kreye J
SARS-CoV-2 Beta variant infection elicits potent lineage-specific and cross-reactive antibodies.
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Beta variant of concern (VOC) resists neutralization by major classes of antibodies from COVID-19 patients and vaccinated individuals. In this study, serum of Beta-infected patients revealed reduced cross-neutralization of wild-type virus. From these patients, we isolated Beta-specific and cross-reactive receptor-binding domain (RBD) antibodies. The Beta-specificity results from recruitment of VOC-specific clonotypes and accommodation of mutations present in Beta and Omicron into a major antibody class that is normally sensitive to these mutations. The Beta-elicited cross-reactive antibodies share genetic and structural features with wild type-elicited antibodies, including a public VH1-58 clonotype that targets the RBD ridge. These findings advance our understanding of the antibody response to SARS-CoV-2 shaped by antigenic drift, with implications for design of next-generation vaccines and therapeutics. Science, 2022; 375
34546336: Kreye J, Wright SK, van Casteren A, Stöffler L, Machule ML, Reincke SM, Nikolaus M, van Hoof S, Sanchez-Sendin E, Homeyer MA, Cordero Gómez C, Kornau HC, Schmitz D, Kaindl AM, Boehm-Sturm P, Mueller S, Wilson MA, Upadhya MA, Dhangar DR, Greenhill S, Woodhall G, Turko P, Vida I, Garner CC, Wickel J, Geis C, Fukata Y, Fukata M, Prüss H
Encephalitis patient-derived monoclonal GABAA receptor antibodies cause epileptic seizures.
Autoantibodies targeting the GABAA receptor (GABAAR) hallmark an autoimmune encephalitis presenting with frequent seizures and psychomotor abnormalities. Their pathogenic role is still not well-defined, given the common overlap with further autoantibodies and the lack of patient-derived mAbs. Five GABAAR mAbs from cerebrospinal fluid cells bound to various epitopes involving the α1 and γ2 receptor subunits, with variable binding strength and partial competition. mAbs selectively

reduced GABAergic currents in neuronal cultures without causing receptor internalization. Cerebroventricular infusion of GABAAR mAbs and Fab fragments into rodents induced a severe phenotype with seizures and increased mortality, reminiscent of encephalitis patients' symptoms. Our results demonstrate direct pathogenicity of autoantibodies on GABAARs independent of Fc-mediated effector functions and provide an animal model for GABAAR encephalitis. They further provide the scientific rationale for clinical treatments using antibody depletion and can serve as tools for the development of antibody-selective immunotherapies.
J Exp Med, 2021; 218
34016740: Yuan M, Huang D, Lee CD, Wu NC, Jackson AM, Zhu X, Liu H, Peng L, van Gils MJ, Sanders RW, Burton DR, Reincke SM, Prüss H, Kreye J, Nemazee D, Ward AB, Wilson IA
Structural and functional ramifications of antigenic drift in recent SARS-CoV-2 variants.
Neutralizing antibodies (nAbs) elicited against the receptor binding site (RBS) of the spike protein of wild-type severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are generally less effective against recent variants of concern. RBS residues Glu, Lys, and Asn are mutated in variants first described in South Africa (B.1.351) and Brazil (P.1). We analyzed their effects on angiotensin-converting enzyme 2 binding, as well as the effects of two of these mutations (K417N and E484K) on nAbs isolated from COVID-19 patients. Binding and neutralization of the two most frequently elicited antibody families (IGHV3-53/3-66 and IGHV1-2), which can both bind the RBS in alternative binding modes, are abrogated by K417N, E484K, or both. These effects can be structurally explained by their extensive interactions with RBS nAbs. However, nAbs to the more conserved, cross-neutralizing CR3022 and S309 sites were largely unaffected. The results have implications for next-generation vaccines and antibody therapies.
Science, 2021; 373
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High frequency of cerebrospinal fluid autoantibodies in COVID-19 patients with neurological symptoms.
COVID-19 intensive care patients can present with neurological syndromes, usually in the absence of SARS-CoV-2 in cerebrospinal fluid (CSF). The recent finding of some virus-neutralizing antibodies cross-reacting with brain tissue suggests the possible involvement of specific autoimmunity.
Brain Behav Immun, 2021; 93
33058755: Kreye J, Reincke SM, Kornau HC, Sánchez-Sendin E, Corman VM, Liu H, Yuan M, Wu NC, Zhu X, Lee CD, Trimpert J, Höltje M, Dietert K, Stöffler L, von Wardenburg N, van Hoof S, Homeyer MA, Hoffmann J, Abdelgawad A, Gruber AD, Bertzbach LD, Vladimirova D, Li LY, Barthel PC, Skriner K, Hocke AC, Hippenstiel S, Witzenrath M, Suttorp N, Kurth F, Franke C, Endres M, Schmitz D, Jeworowski LM, Richter A, Schmidt ML, Schwarz T, Müller MA, Drosten C, Wendisch D, Sander LE, Osterrieder N, Wilson IA, Prüss H
A Therapeutic Non-self-reactive SARS-CoV-2 Antibody Protects from Lung Pathology in a COVID-19 Hamster Model.
The emergence of SARS-CoV-2 led to pandemic spread of coronavirus disease 2019 (COVID-19), manifesting with respiratory symptoms and multi-organ dysfunction. Detailed characterization of virus-neutralizing antibodies and target epitopes is needed to understand COVID-19 pathophysiology and guide immunization strategies. Among 598 human monoclonal antibodies (mAbs) from 10 COVID-19 patients, we identified 40 strongly neutralizing mAbs. The most potent mAb, CV07-209, neutralized authentic SARS-CoV-2 with an IC value of 3.1 ng/mL. Crystal structures of two mAbs in complex with the SARS-CoV-2 receptor-binding domain at 2.55 and 2.70 Å revealed a direct block of ACE2 attachment. Interestingly, some of the near-germline SARS-CoV-2-neutralizing mAbs reacted with mammalian self-antigens. Prophylactic and therapeutic application of CV07-209 protected hamsters from SARS-CoV-2 infection, weight loss, and lung pathology. Our results show that non-self-reactive virus-neutralizing mAbs elicited during SARS-CoV-2 infection are a promising therapeutic strategy.
Cell, 2020; 183
33032524: Reincke SM, Prüss H, Kreye J
Brain antibody sequence evaluation (BASE): an easy-to-use software for complete data analysis in single cell immunoglobulin cloning.
Repertoire analysis of patient-derived recombinant monoclonal antibodies is an important tool to study the role of B cells in autoimmune diseases of the human brain and beyond. Current protocols for generation of patient-derived recombinant monoclonal antibody libraries are time-consuming and contain repetitive steps, some of which can be assisted with the help of software automation.
BMC Bioinformatics, 2020; 21
33024283: Kreye J, Reincke SM, Prüss H
Do cross-reactive antibodies cause neuropathology in COVID-19?
Nat Rev Immunol, 2020; 20

30843274: Wenke NK, Kreye J, Andrzejak E, van Casteren A, Leubner J, Murgueitio MS, Reincke SM, Secker C, Schmidl L, Geis C, Ackermann F, Nikolaus M, Garner CC, Wardemann H, Wolber G, Prüss H
N-methyl-D-aspartate receptor dysfunction by unmutated human antibodies against the NR1 subunit.
Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is the most common autoimmune encephalitis related to autoantibody-mediated synaptic dysfunction. Cerebrospinal fluid-derived human monoclonal NR1 autoantibodies showed low numbers of somatic hypermutations or were unmutated. These unexpected germline-configured antibodies showed weaker binding to the NMDAR than matured antibodies from the same patient. In primary hippocampal neurons, germline NR1 autoantibodies strongly and specifically reduced total and synaptic NMDAR currents in a dose- and time-dependent manner. The findings suggest that functional NMDAR antibodies are part of the human naïve B cell repertoire. Given their effects on synaptic function, they might contribute to a broad spectrum of neuropsychiatric symptoms. Ann Neurol 2019;85:771-776.
Ann Neurol, 2019; 85
27325695: Ullrich F, Reincke SM, Voss FK, Stauber T, Jentsch TJ
Inactivation and Anion Selectivity of Volume-regulated Anion Channels (VRACs) Depend on C-terminal Residues of the First Extracellular Loop.
Canonical volume-regulated anion channels (VRACs) are crucial for cell volume regulation and have many other important roles, including tumor drug resistance and release of neurotransmitters. Although VRAC-mediated swelling-activated chloride currents (ICl,vol) have been studied for decades, exploration of the structure-function relationship of VRAC has become possible only after the recent discovery that VRACs are formed by differently composed heteromers of LRRC8 proteins. Inactivation of ICl,vol at positive potentials, a typical hallmark of VRACs, strongly varies between native cell types. Exploiting the large differences in inactivation between different LRRC8 heteromers, we now used chimeras assembled from isoforms LRRC8C and LRRC8E to uncover a highly conserved extracellular region preceding the second LRRC8 transmembrane domain as a major determinant of ICl,vol inactivation. Point mutations identified two amino acids (Lys-98 and Asp-100 in LRRC8A and equivalent residues in LRRC8C and -E), which upon charge reversal strongly altered the kinetics and voltage dependence of inactivation. Importantly, charge reversal at the first position also reduced the iodide > chloride permeability of ICl,vol This change in selectivity was stronger when both the obligatory LRRC8A subunit and the other co-expressed isoform (LRR8C or -E) carried such mutations. Hence, the C-terminal part of the first extracellular loop not only determines VRAC inactivation but might also participate in forming its outer pore. Inactivation of VRACs may involve a closure of the extracellular mouth of the permeation pathway.
J Biol Chem, 2016; 291

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BOARD NUMBER: S05-250

AGE-DEPENDENT ROLE OF NMDA RECEPTORS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Aims Ageing affects *N*-methyl-D-aspartate receptors (NMDARs), their expression and function in neuronal and non-neuronal cells. Contribution of NMDARs to pathogenesis of experimental autoimmune encephalomyelitis (EAE) has been investigated but further study is still needed. The aim of this study was to determine whether ageing affects the role of NMDARs in EAE. Methods Memantine, a non-competitive NMDAR antagonist which limits pathological activity of NMDARs while sparing normal synaptic activity, was administered orally from day 7 after immunization to 3- and 24-month-old female Dark Agouti rats. The animals were sacrificed at the peak of the disease. Spinal cord mononuclear cells were analyzed by flow cytometry. Brain tissue was collected for biochemical analysis of redox status and RT-qPCR. Results Semiprophylactic administration of memantine ameliorated clinical disease course, with greater effect in aged rats. Memantine reduced the number, frequency, and reactivation of CD4+ T lymphocytes and increased the relative percentage of CX3CR1-expressing microglia in spinal cord, but to a greater extent in aged rats. Additionally, analysis of brain redox status parameters showed that memantine was more effective in reducing superoxide anion radical, malondialdehyde and advanced oxidation protein products in aged rats than in young ones. In accordance with previous findings, NMDAR inhibition by memantine decreased NADPH oxidase and IL-1β expression and increased the nuclear factor erythroid 2-related factor 2 and heme oxygenase-1 expression, to a greater extent in aged rats. Conclusions The involvement of NMDARs in the pathogenesis of EAE was age-dependent, being more pronounced in aged than in young rats.

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BOARD NUMBER: S05-251

PREVENTIVE EXERCISE COUNTERACTS GLUTAMATERGIC TRANSMISSION DEFECTS IN THE STRIATUM OF MICE WITH EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

Antonietta Gentile[1], Livia Guadalupi[1], Valentina Vanni[1], Diego Fresegna[1], Krizia Sanna[2], Francesca Rizzo[3], Monica Nencini[3], Francesca De Vito[3], Alessandra Musella[1,4], Silvia Caioli[3], Sara Balletta[2], Georgia Mandolesi[1,4], Diego Centonze[2,3]
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Exercise is reported to induce neuroplasticity and to modulate immune response. Both effects may be involved in the benefits of exercise for people with Multiple Sclerosis (MS), a neuroinflammatory disorder characterized by accumulating motor disability. An inflammatory synaptopathy has been shown to contribute to excitotoxicity and neurodegeneration in MS and in mice with experimental autoimmune encephalomyelitis (EAE), animal model of MS. We investigated the effects of preventive voluntary running wheel (exercise) on motor function and EAE-induced alterations of the glutamatergic transmission in the striatum, a subcortical area involved in motor control, by performing behavioral tests, electrophysiology, and biochemical studies. Compared to sedentary-EAE mice, exercise-EAE mice showed reduced clinical deficits and performed better at Rotarod test and grip strength test, suggesting improved motor coordination and neuromuscular function. Exercise prevented myelin loss in spinal cord lysates, consistently with the reduced neurological symptoms. On reverse, in the striatum exercise did not significantly ameliorate demyelination but attenuated both microglia activation and glutamatergic excitotoxicity with a specific effect on the presynaptic compartment. Indeed, the frequency of the spontaneous excitatory postsynaptic events (sEPSCs) was normalized in the EAE-exercise striatum, while the sEPSC kinetics were unaffected. The two main vesicular glutamate transporters (VGLUT-1 and VGLUT-2) that regulate glutamate load into presynaptic vesicles were increased in EAE-striatal protein lysates, but only VGLUT-1 was reduced by exercise. These data suggest that exercise attenuates microglia activation and limits glutamate excitotoxicity in the striatum of EAE mice, by modulating the glutamate release at cortico-striatal synapses, likely contributing to improved motor performance.

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BOARD NUMBER: S05-252

ACTIVATION OF COMPLEMENT C3 IN THE COURSE OF RAT EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

POSTER SESSION 05 - SECTION: NEUROINFLAMMATION AND AUTOIMMUNITY

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Experimental autoimmune encephalomyelitis (EAE) is an animal model for the human demyelinating disease of the central nervous system, such as multiple sclerosis (MS). It is characterized by the infiltration of autoimmune T cells, activation of microglia and astrocytes, and chronic demyelination. It has recently been reported that complement system genes, including complement component C3, are overexpressed in various neuroinflammatory diseases, with complement C3 occasionally being regarded as a marker for toxic astrocytes. The study aims to localize complement C3 in the course of rat EAE immunohistochemically. Complement C3 was found in some glial cells and neurons in control spinal cords. In EAE, increased immunoreactivity of complement C3 was recognized in reactive astrocytes, but not in Iba-1-positive cells in the spinal cord during the peak and recovery stages of EAE. Collectively, this finding suggests that complement C3 from astrocytes activates microglia and macrophages in demyelinating lesions of EAE, resulting in chronic demyelination.

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BOARD NUMBER: S05-253

OLIGODENDROGLIAL NMDA RECEPTORS CONTAINING GLUN3A SUBUNITS: ROLES IN ACTIVITY-DEPENDENT MYELINATION

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Work over the last decade demonstrates that the myelin sheaths enwrapping axons, classically seen as static insulators, are highly dynamic and respond to axonal activity and experience. This phenomenon, called “myelin plasticity”, goes beyond postnatal stages into adulthood and plays key roles in remodeling neuronal circuits to support adaptive behavior or functional recovery upon injury. Here we show that NMDA receptors containing GluN3A subunits (GluN3A-NMDARs) are expressed by oligodendrocyte progenitor cells (OPCs) and required for activity-dependent myelin plasticity. Specifically, we find that genetic removal of GluN3A from young brains accelerates the differentiation of OPCs into oligodendrocytes (OLs) in a variety of brain areas, yielding an upregulation of multiple markers of OL differentiation and myelination that resembles gene expression programs typical of aged OPCs. Functional assays in conditional, OPC-restricted *Grin3a* knockouts further showed that OPCs-lacking GluN3A fail to differentiate in response to chemo-genetic stimulation of callosal axons, and exhibit altered proliferation and differentiation responses to white matter injury. Our study opens an opportunity to develop strategies to re-express GluN3A and boost myelin plasticity that declines during aging and neurodegenerative diseases.

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BOARD NUMBER: S05-254

PRIMARY SENSORY CORTICES OF A MOUSE MODEL OF CDKL5 DEFICIENCY DISORDER SHOW ATYPICAL MYELINATION.

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental condition without a cure caused by mutations in cyclin-dependent kinase-like 5 (*CDKL5*) gene and characterized by early-onset epilepsy, severe cognitive dysfunctions, sensorimotor and intellectual disabilities. CDKL5 is a serine/threonine kinase that is expressed early during postnatal development in neurons where it phosphorylates epigenetic factors, elements of both axonal and dendritic compartment and microtubule associated proteins (MAP1S, EB2) which are crucial in nucleation and assembly of microtubules. Along with neurons, CDKL5 is also expressed in oligodendroglia, underlying myelination process. Although growing evidence indicates that the organization of myelin sheath is severely compromised in autism spectrum disorders and other neurodevelopmental diseases, whether *CDKL5* mutation affects myelin is still unknown. We here evaluated both extent and developmental trajectory of myelination, and the expression of molecules modified by myelin deposition or axonal injury – i.e.: Myelin basic protein (MBP) and neurofilaments (NF)– in both young (PND15) and adult (PND56) *Cdkl5*-KO mice. This analysis showed reduction of both MBP and phospho-NFs expression in both S1 and V1 cortices of *Cdkl5*-KO mice. The g-ratio analysis of myelinated axons investigated by electron microscopy revealed that myelin sheath thickness is decreased in mutant mice. Finally, the density of mature oligodendrocytes was reduced in adult mutant mice whereas oligodendrocyte precursor cells were not affected. In conclusion, our data indicate that primary cortical areas in CDD animals exhibit a global reduction/distortion of myelination process and disclose a novel consequence of CDKL5 loss, likely of pivotal importance for CDD.

FENS Forum 2022
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BOARD NUMBER: S05-255

THE EFFECT OF M1 AND M2 POLARIZED MICROGLIA-DERIVED EXOSOMES ON NEURAL STEM CELL DIFFERENTIATION

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Microglia are corresponding macrophages of Central Nervous System with phagocytic activity; also involved in developmental stages, including neural differentiation. Depending on extracellular signals, microglia polarize into distinctive M1 and M2 phenotypes. The aim of the study is to determine the differential miRNA content of M1 and M2 microglia-derived exosomes, demonstrate exosome uptake and investigate the effects of M1 and M2 microglial exosome on neural differentiation. Assuming that effect of polarized microglia is mediated through miRNAs transported via exosomes; exosomes were isolated from polarized microglia and miRNA contents of exosomes were designated via qPCR panel. Statistically significant upregulation of 6 miRNAs and downregulation 9 miRNAs were determined in M1; while upregulation of 12 miRNAs and downregulation 13 miRNAs were determined in M2 phenotypes. To investigate the effect of exosomes *in vitro*, we used neural stem cells (NSCs) that were differentiated from CGR8 mouse embryonic-stem cells and microglial exosomes. NSCs were cultured with exosomes within 3D-culture that was used to mimic microenvironment within the brain. We visualized exosome uptake by NSCs. The effects of M1 and M2 exosomes on NSCs was investigated via qPCR and immunocytochemistry methods. An increasing trend in neural markers were determined via M2 exosome treatment. Additionally, treatment has been determined to increase neurite outgrowth of NSCs. The observation of altered neural differentiation through microglial exosomes is promising to highlight the pathogenesis of several inflammation-related disorders and will eventually provide a new approach in terms of diagnosis and treatment. This study is supported by TUBITAK (Project no: 218S527)

Pubmed:

34717147: Kiser C, Gonul CP, Olcum M, Genc S
Inhibitory effects of sulforaphane on NLRP3 inflammasome activation.
SFN, a dietary phytochemical, is a significant member of isothiocyanates present in cruciferous vegetables at high levels in broccoli. It is a well-known activator of the Nrf2/ARE antioxidant pathway. Long since, the therapeutic effects of SFN have been widely studied in several different diseases. Other than the antioxidant effect, SFN also exhibits an anti-inflammatory effect through suppression of various mechanisms, including inflammasome activation. Considerably, SFN has been demonstrated to inhibit multiple inflammasomes, including NLRP3 inflammasome. NLRP3 inflammasome induces secretion of pro-inflammatory cytokines and promotes inflammatory cell death. The release of pro-inflammatory cytokines enhances the inflammatory response, in turn leading to tissue damage. These self-propelling inflammatory responses would need modulation with exogenous therapeutic agents to suppress them. SFN is a promising candidate molecule for the mitigation of NLRP3 inflammasome activation, which has been related to the pathogenesis of numerous disorders. In this review, we have provided fundamental knowledge about Sulforaphane, elaborated its characteristics, and evidentially focused on its mechanisms of action with regard to its anti-inflammatory, anti-oxidative, and neuroprotective features. Thereafter, we have summarized both in vitro and in vivo studies regarding SFN effect on NLRP3 inflammasome activation.
Mol Immunol, 2021; 140
31997770: Olcum M, Tastan B, Kiser C, Genc S, Genc K
Microglial NLRP3 inflammasome activation in multiple sclerosis.
Multiple sclerosis (MS) is a chronic, autoimmune and neuroinflammatory disease of the central nervous system (CNS) mediated by autoreactive T cells directed against myelin antigens. Although the crucial role of adaptive immunity is well established in MS, the contribution of innate immunity has only recently been appreciated. Microglia are the main innate immune cells of the CNS. Similar to other myeloid cells, microglia recognize both exogenous and host-derived endogenous

danger signals through pattern recognition receptors (PRRs) localized on their cell surface such as Toll Like receptor 4, or in the cytosol such as NLRP3. The second one is the sensor protein of the multi-molecular NLRP3 inflammasome complex in activated microglia that promotes the maturation and secretion of proinflammatory cytokines, interleukin-1β and interleukin-18. Overactivation of microglia and aberrant activation of the NLRP3 inflammasome have been implicated in the pathogenesis of MS. Indeed, experimental data, together with post-mortem and clinical studies have revealed an increased expression of NLRP3 inflammasome complex elements in microglia and other immune cells. In this review, we focus on microglial NLRP3 inflammasome activation in MS. First, we overview the basic knowledge about MS, microglia and the NLRP3 inflammasome. Then, we summarize studies about microglial NLRP3 inflammasome activation in MS and its animal models. We also highlight experimental therapeutic approaches that target different steps of NLRP inflammasome activation. Finally, we discuss future research avenues and new methods in this rapidly evolving area.
Adv Protein Chem Struct Biol, 2020; 119

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-256

CANNABINOID CB1 RECEPTOR GENE INACTIVATION IN OLIGODENDROCYTE PRECURSORS DISRUPTS OLIGODENDROGENESIS AND MYELINATION IN MICE

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Cannabinoids are known to modulate oligodendrogenesis and developmental CNS myelination. However, the cell-autonomous action of these compounds on oligodendroglial cells *in vivo*, and the molecular mechanisms underlying these effects have not yet been studied. Here, by using oligodendroglial precursor cell (OPC)-targeted genetic mouse models, we show that cannabinoid CB_1 receptors exert an essential role in modulating OPC differentiation at the critical periods of postnatal myelination. We found that selective genetic inactivation of CB_1 receptors in OPCs *in vivo* perturbs oligodendrogenesis and postnatal myelination by altering the RhoA/ROCK signaling pathway, leading to hypomyelination, and motor and cognitive alterations in young adult mice. Conversely, pharmacological CB_1 receptor activation, by inducing E3 ubiquitin ligase-dependent RhoA proteasomal degradation, promotes oligodendrocyte development and CNS myelination in OPCs, an effect that was not evident in OPC-specific CB_1 receptor-deficient mice. Moreover, pharmacological inactivation of ROCK *in vivo* overcame the defects in oligodendrogenesis and CNS myelination, and behavioral alterations found in OPC-specific CB_1 receptor-deficient mice. Overall, this study supports a cell-autonomous role for CB_1 receptors in modulating oligodendrogenesis *in vivo*, which may have a profound impact on the scientific knowledge and therapeutic manipulation of CNS myelination by cannabinoids.

FENS Forum 2022
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BOARD NUMBER: S05-257

TEM AND SBF-SEM ANALYSES OF EPENDYMAL CELLS IN SPINAL CORD OF ADULT RATS REVEALED THEIR SUBSTANTIAL CONTACTS TO VASCULAR BASAL LAMINA AND TO FRACTONE BULBS

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Astrocytes represent major glial population contacting vasculature in CNS, however subpopulations of cells lining ventricular system may be attached to blood vessels too, including ependymal cells (EC) of spinal cord (SC). Immunofluorescent labelling of vimentin revealed that EC in SC of adult rat form basal processes, which are directed toward laminin positive vasculature surrounding the central canal lining. Role of ependymo-vascular connection is not clear yet and only little information is known about extracellular matrix termed fractone bulbs (FB), which develop in SC after birth. In this work we focused on identification of connections between EC, blood vessels and FB in SC using TEM and SBF-SEM. We identified that basal processes of EC form direct contacts to capillary basal lamina or may be attached to narrow extensions resembling to vascular fractones or stems, previously identified in rodent brain. Since no connections between FB and vasculature has been identified using SBF-SEM, we assume that FB are blood vessel independent structures. We observed that complexity of FB containing laminin vary from small to massively branched labyrinths located at interface between EC and neuropil. Electron-dense material was identified on inner membrane of EC contacting FB, with small vesicles present in EC cytoplasm. We also identified vesicles separating from FB indicating potential transport of molecules between these compartments. Our approaches proved that EC of SC contact vasculature, and that FB are organized in SC similarly as was observed in rodent brain. Keywords: fractone bulbs, ependymal cells, vasculature, spinal cord Acknowledgement: Czech-Bioimaging LM2018129, VEGA1/0760/20, APVV-19-0279

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-258

PROTEOMIC AND LIPIDOMIC PROFILING OF DEMYELINATING LESIONS IDENTIFIES FATTY ACIDS AS MODULATORS IN LESION RECOVERY

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

Vini Tiwari[1,2], Horst Penkert[1,2,3], Alix Bertrand[1,2,4], Stephan Breimann[1,5,6], Stephan Müller[1,7], Paul Jordan[8], Mathias Gerl[9,10], Christian Klose[10], Ludovico Castelvetri[1,2], Mar Queralt[1,2], Ilya Levental[11], Stefan Lichtenthaler[1,7,12], Oliver Werz[8], Mikael Simons[1,2,12]
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After demyelinating injury of the central nervous system, resolution of the mounting acute inflammation is crucial for the initiation of a regenerative response. Here, we aim to identify fatty acids and lipid mediators that govern the balance of inflammatory reactions within demyelinating lesions. Using lipidomics, we identify bioactive lipids in the resolution phase of inflammation with markedly elevated levels of n-3 polyunsaturated fatty acids. Using fat-1 transgenic mice, which convert n-6 fatty acids to n-3 fatty acids, we find that reduction of the n-6/n-3 ratio decreases the phagocytic infiltrate. In addition, we observe accelerated decline of microglia/macrophages and enhanced generation of oligodendrocytes in aged mice when n-3 fatty acids are shuttled to the brain. Thus, n-3 fatty acids enhance lesion recovery and may, therefore, provide the basis for pro-regenerative medicines of demyelinating diseases in the central nervous system.

Pubmed:
34706241: Penkert H, Bertrand A, Tiwari V, Breimann S, Müller SA, Jordan PM, Gerl MJ, Klose C, Cantuti-Castelvetri L, Bosch-Queralt M, Levental I, Lichtenthaler SF, Werz O, Simons M
Proteomic and lipidomic profiling of demyelinating lesions identifies fatty acids as modulators in lesion recovery.
After demyelinating injury of the central nervous system, resolution of the mounting acute inflammation is crucial for the initiation of a regenerative response. Here, we aim to identify fatty acids and lipid mediators that govern the balance of inflammatory reactions within demyelinating lesions. Using lipidomics, we identify bioactive lipids in the resolution phase of inflammation with markedly elevated levels of n-3 polyunsaturated fatty acids. Using fat-1 transgenic mice, which convert n-6 fatty acids to n-3 fatty acids, we find that reduction of the n-6/n-3 ratio decreases the phagocytic infiltrate. In addition, we observe accelerated decline of microglia/macrophages and enhanced generation of oligodendrocytes in aged mice when n-3 fatty acids are shuttled to the brain. Thus, n-3 fatty acids enhance lesion recovery and may, therefore, provide the basis for pro-regenerative medicines of demyelinating diseases in the central nervous system.
Cell Rep, 2021; 37
35141565: Bosch-Queralt M, Tiwari V, Damkou A, Vaculčiaková L, Alexopoulos I, Simons M
A fluorescence microscopy-based protocol for volumetric measurement of lysolecithin lesion-associated de- and re-myelination in mouse brain.
Lysolecithin injections into the white matter tracts of the central nervous system are a valuable tool to study remyelination, but evaluating the resulting demyelinating lesion size is challenging. Here, we present a protocol to consistently measure the volume of demyelination and remyelination in mice following brain lysolecithin injections. We describe serial sectioning of the lesion, followed by the evaluation of the demyelinated area in two-dimensional images. We then detail the computation of the volume using our own automated iPython script. For complete details on the use and execution of this profile, please refer to

Bosch-Queralt et al. (2021).
STAR Protoc, 2022; 3

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-259

INJURY-INDUCED UPREGULATION OF FIBRONECTIN AND BMP4 MODULATES THE DIFFERENTIATION OF OLIGODENDROCYTE PROGENITOR CELLS INTO SCHWANN CELLS

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Background: Under homeostatic conditions, axons of the CNS are myelinated by oligodendrocytes. However, in response to demyelinating insults, Schwann cells (SCs), the myelinating glia of the PNS can contribute to CNS remyelination. Although CNS SCs can migrate into the CNS from peripheral sources, lineage tracing studies demonstrated that adult CNS progenitors can also produce SCs *in vivo.* This OPC fate switch is surprising given the early phylogenetic and ontogenetic divergence of the peripheral and central components of the nervous system. **Aims:** We aimed to determine the mechanisms OPCs reprogram towards a SC fate. **Results:** Using single-cell RNA-sequencing we identified a subpopulation of OPCs within experimental demyelinating lesions that co-express SC markers 5 days after injury. Within this SC-primed OPC cluster, differential gene analysis revealed a significant upregulation of genes involved with integrin signalling and bone morphogenetic protein (BMP) activation. We found that OPCs can be differentiated *in vitro* into SCs by exposing OPCs to fibronectin and BMP4, or by directly modulating the expression levels of *OLIG2* and *SOX10*. OPC-derived SCs transcriptionally resemble primary SCs, and when transplanted into the CNS can survive and myelinate host CNS axons with peripheral type myelin. **Conclusions:** These findings show that injury-related changes in the environment can destabilise oligodendroglial transcription factor networks, which enable alternative OPC fate choices. Together, this study reveals insight into the mechanisms underlying OPC cell fate decisions during CNS remyelination.

Pubmed:
33497588: Chen CZ, Neumann B, Förster S, Franklin RJM
Schwann cell remyelination of the central nervous system: why does it happen and what are the benefits?
Myelin sheaths, by supporting axonal integrity and allowing rapid saltatory impulse conduction, are of fundamental importance for neuronal function. In response to demyelinating injuries in the central nervous system (CNS), oligodendrocyte progenitor cells (OPCs) migrate to the lesion area, proliferate and differentiate into new oligodendrocytes that make new myelin sheaths. This process is termed remyelination. Under specific conditions, demyelinated axons in the CNS can also be remyelinated by Schwann cells (SCs), the myelinating cell of the peripheral nervous system. OPCs can be a major source of these CNS-resident SCs-a surprising finding given the distinct embryonic origins, and physiological compartmentalization of the peripheral and central nervous system. Although the mechanisms and cues governing OPC-to-SC differentiation remain largely undiscovered, it might nevertheless be an attractive target for promoting endogenous remyelination. This article will (i) review current knowledge on the origins of SCs in the CNS, with a particular focus on OPC to SC differentiation, (ii) discuss the necessary criteria for SC myelination in the CNS and (iii) highlight the potential of using SCs for myelin regeneration in the CNS.
Open Biol, 2021; 11

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BOARD NUMBER: S05-260

IMMUNOCOMPETENT CEREBRAL SPHEROIDS AS A MODEL SYSTEM TO EVALUATE DRUG-MEDIATED DEMYELINATION AND TO STUDY REMYELINATION

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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In the central nervous system (CNS), oligodendrocytes generate an axon wrapping lipid-rich substance known as myelin. Under certain inflammatory conditions such as Multiple Sclerosis or as a consequence of toxic drug side effects, myelin and/or oligodendrocytes may be destroyed in a process called demyelination.
Potential myelin and/or oligodendrocyte toxicity of new drugs as well as experimental demyelination and myelin repair are mainly evaluated using animal experiments. Due to species differences and the experimental setup these results can often not be translated to humans. The formation of proper myelin sheaths in 2D in vitro culture systems remains a challenge. Moreover, certain aspects such as indirect effects mediated by other CNS resident cell types may not be considered.
Being able to predict side effects of new drugs causing demyelination or neurotoxicity, or to evaluate compounds promoting remyelination in a human 3D in vitro system would not only facilitate safety assessments omitting interspecies differences, but also support efficacy assessment of novel compounds.
Here we show the generation of human iPSC-derived immunocompetent cerebral spheroids consisting of neurons, astrocytes, myelinating oligodendrocytes and microglia. Our data suggest that upon toxin-induced demyelination, the volume of myelin sheaths is decreased, but can efficiently be restored during remyelination and even enhanced by myelination promoting compounds.
These human immunocompetent cerebral spheroids show a high degree of physiological relevance as a tool for safety and efficacy assessment of new drugs. Furthermore, they may serve as a basis for the development of human disease models.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S05-261

IMPACT OF EARLY DISRUPTION OF PARVALBUMIN INTERNEURON-OPC INTERACTIONS ON PREFRONTAL-DEPENDENT COGNITIVE PROCESSES

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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GABAergic inhibitory interneurons have a prominent role in the activity of cortical networks. They act as important regulators of the excitatory output and confer dynamic modulation to neuronal circuits and information processing. Moreover, we previously showed that parvalbumin (PV)-expressing interneurons, the largest proportion of GABAergic neurons of the cerebral cortex, represent the major synaptic input onto oligodendrocyte precursor cells (OPCs) during postnatal development (Orduz et al., 2015 & 2019).
Interestingly, the study of PV interneuron myelination did not attract attention until recently. Using a NG2creERT2;Gcamp3;γ2 (γ2f/f) mice line, we recently demonstrated that disruption of PV interneuron-OPC GABAergic synapses prior to myelination onset resulted in severe PV interneuron myelination defects, impacting inhibitory circuits of the somatosensory cortex as well as whisker-based texture discrimination behavior (Benamer et al., 2020).
Here, we hypothesize that PV interneuron myelination defects impair the function of the medial prefrontal cortex (mPFC), an area implicated in diverse neurodevelopmental disorders. We used a fear conditioning task and found that γ2f/f mice presented extinction retrieval deficits. Since the infralimbic (IL) region has been involved in this process, we evaluated the excitation-inhibition (E-I) balance of its layers 2/3 pyramidal neurons by recording postsynaptic currents in acute brain slices. We found a significant imbalance of the E-I ratio caused by a decreased inhibition. However, no changes in either cell density or distribution of IL PV interneurons were observed.
Taken together, our results show that early postnatal disruption of PV interneuron-OPC synaptic interactions causes deficits in cortical inhibitory circuit function and cognitive processes.

FENS Forum 2022
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BOARD NUMBER: S05-262

ROLE OF ADENOSINE A2B RECEPTORS IN MYELINATION PROCESSES: NEW CHALLENGE IN TREATING MULTIPLE SCLEROSIS

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Differentiation of oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes (OLs) is a key event for axonal myelination in the brain; this process fails during demyelinating pathologies, such as multiple sclerosis (MS). Adenosine is emerging as an important player in oligodendrogliogenesis, by activating its metabotropic receptors and by consequently modulating potassium currents. Moreover, 4-aminopyridine (4-AP), a broad-spectrum potassium channel blocker, can promote axonal conduction and remyelination and it was approved to improve motor skills in MS patients. **Aim:** We studied the functional role of A_{2B}Rs in modulating potassium currents and oligodendrogliogenesis in cultured OPCs and during myelination in dorsal root ganglion (DRG) neurons/OPC co-culture. **Methods:** Patch-clamp recordings coupled to quantitative Real-Time PCR, small-interference RNA techniques, and immunocytochemistry were used. **Results:** BAY60-6583 (1-30 µM), a selective A_{2B}R agonist, inhibited potassium currents in rat OPCs and reduced their differentiation, reported as the decrease in MBP/MAG gene expression. The A_{2B}R silencing or selective A_{2B}Rs antagonists prevented the BAY60-6583 effect on potassium currents and promoted OPC differentiation. Surprisingly, BAY60-6583 increased myelin deposition on DRG axons co-cultured with OPC and enhanced action potential firing in primary DRG culture. **Conclusions:** Our data show that A_{2B}R activation prevents OPC differentiation, by inhibiting potassium currents. However, in OPC-DRG co-cultures A_{2B}Rs increase myelin deposition, probably by enhancing action potential firing in DRG neurons. These results suggest that activation of A_{2B}Rs modulates different functions in oligodendrogliogenesis, depending on their cellular localization, and may represent a valuable target in demyelinating pathologies such as MS.

Pubmed:
34298893: Cherchi F, Bulli I, Venturini M, Pugliese AM, Coppi E
Ion Channels as New Attractive Targets to Improve Re-Myelination Processes in the Brain.
Multiple sclerosis (MS) is the most demyelinating disease of the central nervous system (CNS) characterized by neuroinflammation. Oligodendrocyte progenitor cells (OPCs) are cycling cells in the developing and adult CNS that, under demyelinating conditions, migrate to the site of lesions and differentiate into mature oligodendrocytes to remyelinate damaged axons. However, this process fails during disease chronicization due to impaired OPC differentiation. Moreover, OPCs are crucial players in neuro-glial communication as they receive synaptic inputs from neurons and express ion channels and neurotransmitter/neuromodulator receptors that control their maturation. Ion channels are recognized as attractive therapeutic targets, and indeed ligand-gated and voltage-gated channels can both be found among the top five pharmaceutical target groups of FDA-approved agents. Their modulation ameliorates some of the symptoms of MS and improves the outcome of related animal models. However, the exact mechanism of action of ion-channel targeting compounds is often still unclear due to the wide expression of these channels on neurons, glia, and infiltrating immune cells. The present review summarizes recent findings in the field to get further insights into physio-pathophysiological processes and possible therapeutic mechanisms of drug actions.
Int J Mol Sci, 2021; 22
33510056: Cherchi F, Pugliese AM, Coppi E
Oligodendrocyte precursor cell maturation: role of adenosine receptors.
Oligodendrocyte-formed myelin sheaths allow fast synaptic transmission in the brain and their degeneration leads to demyelinating diseases such as multiple sclerosis. Remyelination requires the differentiation of oligodendrocyte progenitor cells into mature oligodendrocytes but, in chronic neurodegenerative disorders, remyelination fails due to adverse environment. Therefore, a strategy to prompt oligodendrocyte progenitor cell differentiation towards myelinating oligodendrocytes is required. The neuromodulator adenosine, and its receptors (A, A, A and A receptors: AR, AR, AR and

AR), are crucial mediators in remyelination processes. It is known that ARs facilitate oligodendrocyte progenitor cell maturation and migration whereas the ARs initiates apoptosis in oligodendrocyte progenitor cells. Our group of research contributed to the field by demonstrating that AR and AR inhibit oligodendrocyte progenitor cell maturation by reducing voltage-dependent K currents necessary for cell differentiation. The present review summarizes the possible role of adenosine receptor ligands as potential therapeutic targets in demyelinating pathologies such as multiple sclerosis.
Neural Regen Res, 2021; 16
32251679: Coppi E, Cherchi F, Fusco I, Dettori I, Gaviano L, Magni G, Catarzi D, Colotta V, Varano F, Rossi F, Bernacchioni C, Donati C, Bruni P, Pedata F, Cencetti F, Pugliese AM
Adenosine A receptors inhibit K currents and cell differentiation in cultured oligodendrocyte precursor cells and modulate sphingosine-1-phosphate signaling pathway.
Oligodendrocytes are the only myelinating cells in the brain and differentiate from their progenitors (OPCs) throughout adult life. However, this process fails in demyelinating pathologies. Adenosine is emerging as an important player in OPC differentiation and we recently demonstrated that adenosine A receptors inhibit cell maturation by reducing voltage-dependent K currents. No data are available to date about the A receptor (AR) subtype. The bioactive lipid mediator sphingosine-1-phosphate (S1P) and its receptors (S1P) are also crucial modulators of OPC development. An interaction between this pathway and the AR is reported in peripheral cells. We studied the role of ARs in modulating K currents and cell differentiation in OPC cultures and we investigated a possible interplay with S1P signaling. Our data indicate that the AR agonist BAY60-6583 and its new analogue P453 inhibit K currents in cultured OPC and the effect was prevented by the AR antagonist MRS1706, by K channel blockers and was differently modulated by the S1P analogue FTY720-P. An acute (10 min) exposure of OPCs to BAY60-6583 also increased the phosphorylated form of sphingosine kinase 1 (SphK1). A chronic (7 days) treatment with the same agonist decreased OPC differentiation whereas SphK1/2 inhibition exerted the opposite effect. Furthermore, AR was overexpressed during OPC differentiation, an effect prevented by the pan SphK1/2 inhibitor VPC69047. Finally, AR silenced cells showed increased cell maturation, decreased SphK1 expression and enhanced S1P lyase levels. We conclude that ARs inhibit K currents and cell differentiation and positively modulate S1P synthesis in cultured OPCs.
Biochem Pharmacol, 2020; 177
29740323: Fusco I, Ugolini F, Lana D, Coppi E, Dettori I, Gaviano L, Nosi D, Cherchi F, Pedata F, Giovannini MG, Pugliese AM
The Selective Antagonism of Adenosine A Receptors Reduces the Synaptic Failure and Neuronal Death Induced by Oxygen and Glucose Deprivation in Rat CA1 Hippocampus .
Ischemia is a multifactorial pathology characterized by different events evolving in time. Immediately after the ischemic insult, primary brain damage is due to the massive increase of extracellular glutamate. Adenosine in the brain increases dramatically during ischemia in concentrations able to stimulate all its receptors, A, A, A, and A. Although adenosine exerts clear neuroprotective effects through A receptors during ischemia, the use of selective A receptor agonists is hampered by their undesirable peripheral side effects. So far, no evidence is available on the involvement of adenosine A receptors in cerebral ischemia. This study explored the role of adenosine A receptors on synaptic and cellular responses during oxygen and glucose deprivation (OGD) in the CA1 region of rat hippocampus . We conducted extracellular recordings of CA1 field excitatory post-synaptic potentials (fEPSPs); the extent of damage on neurons and glia was assessed by immunohistochemistry. Seven min OGD induced anoxic depolarization (AD) in all hippocampal slices tested and completely abolished fEPSPs that did not recover after return to normoxic condition. Seven minutes OGD was applied in the presence of the selective adenosine A receptor antagonists MRS1754 (500 nM) or PSB603 (50 nM), separately administered 15 min before, during and 5 min after OGD. Both antagonists were able to prevent or delay the appearance of AD and to modify synaptic responses after OGD, allowing significant recovery of neurotransmission. Adenosine A receptor antagonism also counteracted the reduction of neuronal density in CA1 stratum pyramidale, decreased apoptosis at least up to 3 h after the end of OGD, and maintained activated mTOR levels similar to those of controls, thus sparing neurons from the degenerative effects caused by the simil-ischemic conditions. Astrocytes significantly proliferated in CA1 stratum radiatum already 3 h after the end of OGD, possibly due to increased glutamate release. Areceptor antagonism significantly prevented astrocyte modifications. Both A receptor antagonists did not protect CA1 neurons from the neurodegeneration induced by glutamate application, indicating that the antagonistic effect is upstream of glutamate release. The selective antagonists of the adenosine A receptor subtype may thus represent a new class of neuroprotective drugs in ischemia.
Front Pharmacol, 2018; 9
31008816: Coppi E, Cherchi F, Fusco I, Failli P, Vona A, Dettori I, Gaviano L, Lucarini E, Jacobson KA, Tosh DK, Salvemini D, Ghelardini C, Pedata F, Di Cesare Mannelli L, Pugliese AM
Adenosine A3 receptor activation inhibits pronociceptive N-type Ca2+ currents and cell excitability in dorsal root ganglion neurons.

Recently, studies have focused on the antihyperalgesic activity of the A3 adenosine receptor (A3AR) in several chronic pain models, but the cellular and molecular basis of this effect is still unknown. Here, we investigated the expression and functional effects of A3AR on the excitability of small- to medium-sized, capsaicin-sensitive, dorsal root ganglion (DRG) neurons isolated from 3- to 4-week-old rats. Real-time quantitative polymerase chain reaction experiments and immunofluorescence analysis revealed A3AR expression in DRG neurons. Patch-clamp experiments demonstrated that 2 distinct A3AR agonists, Cl-IB-MECA and the highly selective MRS5980, inhibited Ca-activated K (KCa) currents evoked by a voltage-ramp protocol. This effect was dependent on a reduction in Ca influx via N-type voltage-dependent Ca channels, as Cl-IB-MECA-induced inhibition was sensitive to the N-type blocker PD173212 but not to the L-type blocker, lacidipine. The endogenous agonist adenosine also reduced N-type Ca currents, and its effect was inhibited by 56% in the presence of A3AR antagonist MRS1523, demonstrating that the majority of adenosine's effect is mediated by this receptor subtype. Current-clamp recordings demonstrated that neuronal firing of rat DRG neurons was also significantly reduced by A3AR activation in a MRS1523-sensitive but PD173212-insensitive manner. Intracellular Ca measurements confirmed the inhibitory role of A3AR on DRG neuronal firing. We conclude that pain-relieving effects observed on A3AR activation could be mediated through N-type Ca channel block and action potential inhibition as independent mechanisms in isolated rat DRG neurons. These findings support A3AR-based therapy as a viable approach to alleviate pain in different pathologies.
Pain, 2019; 160

33030215: Coppi E, Cherchi F, Sarchielli E, Fusco I, Guarnieri G, Gallina P, Corradetti R, Pedata F, Vannelli GB, Pugliese AM, Morelli A
Acetylcholine modulates K and Na currents in human basal forebrain cholinergic neuroblasts through an autocrine/paracrine mechanism.
The Nucleus Basalis of Meynert (NBM) is the main source of cholinergic neurons in the basal forebrain to be crucially involved in cognitive functions and whose degeneration correlates with cognitive decline in major degenerative pathologies as Alzheimer's and Parkinson's diseases. However, knowledge concerning NBM neurons derived from human brain is very limited to date. We recently characterized a primary culture of proliferating neuroblasts isolated from the human fetal NBM (hfNBM) as immature cholinergic neurons expressing the machinery to synthetize and release acetylcholine. Here we studied in detail electrophysiological features and cholinergic effects in this cell culture by patch-clamp recordings. Our data demonstrate that atropine-blocked muscarinic receptor activation by acetylcholine or carbachol enhanced I and reduced I currents by stimulating G -coupled M2 or phospholipase C-coupled M3 receptors, respectively. Inhibition of acetylcholine esterase activity by neostigmine unveiled a spontaneous acetylcholine release from hfNBM neuroblasts that might account for an autocrine/paracrine signaling during human brain development. Present data provide the first description of cholinergic effects in human NBM neurons and point to a role of acetylcholine as an autocrine/paracrine modulator of voltage-dependent channels. Our research could be of relevance in understanding the mechanisms of cholinergic system development and functions in the human brain, either in health or disease.
J Neurochem, 2021; 157

31132418: Fusco I, Cherchi F, Catarzi D, Colotta V, Varano F, Pedata F, Pugliese AM, Coppi E
Functional characterization of a novel adenosine A receptor agonist on short-term plasticity and synaptic inhibition during oxygen and glucose deprivation in the rat CA1 hippocampus.
Adenosine is an endogenous neuromodulator exerting its biological functions via four receptor subtypes, A, A, A, and A. A receptors (ARs) are expressed at hippocampal level where they are known to inhibit paired pulse facilitation (PPF), whose reduction reflects an increase in presynaptic glutamate release. The effect of ARs on PPF is known to be sensitive not only to AR blockade but also to the AR antagonist DPCPX, indicating that it involves AR activation. In this study we provide the first functional characterization of the newly synthesized non-nucleoside like AR agonist P453, belonging to the amino-3,5-dicyanopyridine series. By extracellular electrophysiological recordings, we demonstrated that P453 mimicked the effect of the prototypical AR agonist BAY60-6583 in decreasing PPF at Schaffer collateral-CA1 synapses in rat acute hippocampal slices. This effect was prevented by two different AR antagonists, PSB603 and MRS1754, and by the AR antagonist DPCPX. We also investigated the functional role of AR during a 2 min of oxygen and glucose deprivation (OGD) insult, known to produce a reversible fEPSP inhibition due to adenosine AR activation. We found that P453 and BAY60-6583 significantly delayed the onset of fEPSP reduction induced by OGD and the effect was blocked by PSB603. We conclude that P453 is a functional AR agonist whose activation decreases PPF by increasing glutamate release at presynaptic terminals and delays AR-mediated fEPSP inhibition during a 2-minute OGD insult.
Brain Res Bull, 2019; 151

33621215: Durante M, Squillace S, Lauro F, Giancotti LA, Coppi E, Cherchi F, Di Cesare Mannelli L, Ghelardini C, Kolar G, Wahlman C, Opejin A, Xiao C, Reitman ML, Tosh DK, Hawiger D, Jacobson KA, Salvemini D
Adenosine A3 agonists reverse neuropathic pain via T cell-mediated production of IL-10.
The A3 adenosine receptor (A3AR) has emerged as a therapeutic target with A3AR agonists to tackle the global challenge of

neuropathic pain, and investigation into its mode of action is essential for ongoing clinical development. Immune cell A3ARs, and their activation during pathology, modulate cytokine release. Thus, the use of immune cells as a cellular substrate for the pharmacological action of A3AR agonists is enticing, but unknown. The present study discovered that Rag-KO mice lacking T and B cells, as compared with WT mice, are insensitive to the anti-allodynic effects of A3AR agonists. Similar findings were observed in interleukin-10 and interleukin-10 receptor knockout mice. Adoptive transfer of CD4+ T cells from WT mice infiltrated the dorsal root ganglion (DRG) and restored A3AR agonist-mediated anti-allodynia in Rag-KO mice. CD4+ T cells from Adora3-KO or Il10-KO mice did not. Transfer of CD4+ T cells from WT mice, but not Il10-KO mice, into Il10-KO mice or Adora3-KO mice fully reinstated the anti-allodynic effects of A3AR activation. Notably, A3AR agonism reduced DRG neuron excitability when cocultured with CD4+ T cells in an IL-10-dependent manner. A3AR action on CD4+ T cells infiltrated in the DRG decreased phosphorylation of GluN2B-containing N-methyl-D-aspartate receptors at Tyr1472, a modification associated with regulating neuronal hypersensitivity. Our findings establish that activation of A3AR on CD4+ T cells to release IL-10 is required and sufficient evidence for the use of A3AR agonists as therapeutics.
J Clin Invest, 2021; 131

30291939: Coppi E, Lana D, Cherchi F, Fusco I, Buonvicino D, Urru M, Ranieri G, Muzzi M, Iovino L, Giovannini MG, Pugliese AM, Chiarugi A

Dexpramipexole enhances hippocampal synaptic plasticity and memory in the rat.

Even though pharmacological approaches able to counteract age-dependent cognitive impairment have been highly investigated, drugs improving cognition and memory are still an unmet need. It has been hypothesized that sustaining energy dynamics within the aged hippocampus can boost memory storage by sustaining synaptic functioning and long term potentiation (LTP). Dexpramipexole (DEX) is the first-in-class compound able to sustain neuronal bioenergetics by interacting with mitochondrial F1Fo-ATP synthase. In the present study, for the first time we evaluated the effects of DEX on synaptic fatigue, LTP induction, learning and memory retention. We report that DEX improved LTP maintenance in CA1 neurons of acute hippocampal slices from aged but not young rats. However, we found no evidence that DEX counteracted two classic parameters of synaptic fatigue such as fEPSP reduction or the train area during the high frequency stimulation adopted to induce LTP. Interestingly, patch-clamp recordings in rat hippocampal neurons revealed that DEX dose-dependently inhibited (IC 814 nM) the I current, a rapidly-inactivating K current that negatively regulates neuronal excitability as well as cognition and memory processes. In keeping with this, DEX counteracted both scopolamine-induced spatial memory loss in rats challenged in Morris Water Maze test and memory retention in rats undergoing Novel Object Recognition. Overall, the present study discloses the ability of DEX to boost hippocampal synaptic plasticity, learning and memory. In light of the good safety profile of DEX in humans, our findings may have a realistic translational potential to treatment of cognitive disorders.
Neuropharmacology, 2018; 143

33353217: Coppi E, Dettori I, Cherchi F, Bulli I, Venturini M, Lana D, Giovannini MG, Pedata F, Pugliese AM

A Adenosine Receptors: When Outsiders May Become an Attractive Target to Treat Brain Ischemia or Demyelination.

Adenosine is a signaling molecule, which, by activating its receptors, acts as an important player after cerebral ischemia. Here, we review data in the literature describing AR-mediated effects in models of cerebral ischemia obtained in vivo by the occlusion of the middle cerebral artery (MCAo) or in vitro by oxygen-glucose deprivation (OGD) in hippocampal slices. Adenosine plays an apparently contradictory role in this receptor subtype depending on whether it is activated on neuro-glial cells or peripheral blood vessels and/or inflammatory cells after ischemia. Indeed, ARs participate in the early glutamate-mediated excitotoxicity responsible for neuronal and synaptic loss in the CA1 hippocampus. On the contrary, later after ischemia, the same receptors have a protective role in tissue damage and functional impairments, reducing inflammatory cell infiltration and neuroinflammation by central and/or peripheral mechanisms. Of note, demyelination following brain ischemia, or autoimmune neuroinflammatory reactions, are also profoundly affected by ARs since they are expressed by oligodendroglia where their activation inhibits cell maturation and expression of myelin-related proteins. In conclusion, data in the literature indicate the ARs as putative therapeutic targets for the still unmet treatment of stroke or demyelinating diseases.
Int J Mol Sci, 2020; 21

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MYELINATION OF PARVALBUMIN INTERNEURONS IS CRITICAL TO MAINTAIN HIGH-FREQUENCY FIRING AND SELF-INHIBITORY NEUROTRANSMISSION

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Erasmus MC, Psychiatry, Rotterdam, Netherlands

Parvalbumin-expressing (PV) interneurons are GABAergic cells that are characterized by their distinct ability to fire at high frequencies, due to a remarkably short action potential (AP) duration and refractory period. These unique firing properties are dependent on a unique complement of sodium and potassium channels with a distinct subcellular localization. Recent studies have highlighted another interesting feature of PV neurons, revealing their extensive myelination throughout the brain. However, the precise function of myelination in PV neurons remains to be elucidated. Here we utilize the cuprizone model of demyelination to investigate how PV interneuron myelination might influence their neuronal physiology. Using whole-cell patch-clamp recordings to examine intrinsic properties of PV neurons, we find that demyelination induces clear alterations in PV neuron firing patterns. Specifically, we find that demyelination causes an increase of AP duration and impairment in the ability of PV interneuron to sustain high frequency firing. Voltage-clamp recordings from PV interneurons confirmed a decrease in Kv-specific current in cuprizone-treated mice compared to control mice. We also found a substantial impairment in PV interneuron self-inhibitory transmission, a feature implicated in temporally coordinating PV neurons during cortical network activity, as well as a significant decrease in the number of PV interneuron autaptic contacts after demyelination. Together, our data provide insight into the importance of PV interneuron myelination, especially in their unique ability to fire at high frequency.

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MYELINATION CLUSTERS MITOCHONDRIA TO PARVALBUMIN INTERNEURON AXONAL DOMAINS

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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[1]Netherlands Institute for Neuroscience, Axonal Signaling Group, Amsterdam, Netherlands, [2]National Kapodistrian University of Athens, Medical School, Athens, Greece, [3]Utrecht University, Cell Biology, Neurobiology And Biophysics, Department Of Biology, Utrecht, Netherlands

Parvalbumin-expressing (PV^+) interneurons are critical for cortical rhythms and a diverse range of important cognitive functions. Recent findings show that PV^+ interneurons require myelination in the proximal axonal arbors to produce fast inhibition and in vivo gamma rhythms. Here, we examined whether PV^+ axon myelination has a role in the metabolic support of axons. To examine such a cell type-specific role of myelination we developed a Cre-dependent AAV methodology to fluorescently label mitochondria specifically in PV^+ interneurons, using the PV-Cre driver line. Detailed anatomical reconstructions of myelinated and unmyelinated PV^+ axonal branches revealed that mitochondria are clustered at higher densities in myelinated internodes compared to unmyelinated branches within the same axon (P = 0.03). Furthermore, after demyelination, using the oligodendrocyte-selective toxin cuprizone, the mitochondrial density in the proximal axonal branches was reduced by ~20% (0.25 vs. 0.19 mitochondria/µm, P = 0.01), and resembled the mitochondrial density in unmyelinated control axons (0.16 mitochondria/µm). Finally, to examine whether myelin affects mitochondrial function of Ca^{2+} buffering (mt-Ca^{2+}) we used two-photon imaging of action-potential evoked mt-Ca^{2+} responses revealing that mitochondria at branch points display robust Ca^{2+} buffering, which was significantly reduced in amplitude upon demyelination (P = 0.01). Together, these findings indicate that in PV^+ interneurons myelination locally increases the mitochondrial content and increases branch point Ca^{2+} buffering, which may serve to orchestrate the local energy demands of the fast-spiking PV^+ axons.

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OPTIMIZATION OF MYELINATION AT MID-AGE: INTERACTION ANALYSIS BETWEEN GREY MATTER AND WHITE MATTER COMPARTMENTS.

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Equal first-authors: PP, VP. **INTRODUCTION:** Brain-networks span grey-matter and white-matter, where myelination enables fast transmission of action-potential. An optimum level of myelination is crucial for constancy of spatiotemporal-perception with aging. Using MR-relaxometry, MRI-DTI, and tissue-histology, we estimated myelination-levels and we probed whether grey-matter exuberance influences white-matter integrity. **METHODS:** We use myelin-stained histology of different ages (5-94 years) to estimate myelin-level using Quantitative-histological analysis. Furthermore, we find myelination-levels using transverse-relaxation rates from MR-relaxometry analysis (252 normal-subjects, age:19-82; relaxametric scanning from Arch Neurol, 60(3):393-398,2003). We formulated optimality equation to account for MR-relaxometry and histology findings. Moreover, we assessed grey-matter-exuberance (volume), vis-a-vis white matter integrity (diffusion-parameters), utilizing MRI-DTI scans of separate 177 normal-subjects (age:20-85). **RESULTS:** We find that maximum myelination occurs at 47.3 and 45.1 years using MR-relaxometry and tissue-histology analysis, respectively. For analyzing white-matter and grey-matter interaction around mid-age(~50years), three age-groups were considered: 50±10 years (mid-age), 20-40 years (young), 60-80 years (old). In Males: we found that grey-matter-volume is stable in earlier-age, and this is followed by white-matter integrity being stable in later-age (diffusivities: $p<10^{-3}$). Contrastingly in Females: grey-matter-volume declines in earlier-age, and this is followed by white-matter integrity being impeded in later-age (diffusivities: $p=0.02$). **CONCLUSION:** For perceptual constancy, neuronal signal-velocity, as reflected by myelination-level, should be high across lifespan. As per the optimality principle, peak myelination at mid-age maintains a basal-level throughout aging. Neural activation transmits from dendrite/soma (cortical grey matter), into myelinated axons (white matter), as an efferent process. MR-relaxometery/MRI-DTI findings attest to this grey-matter effect on white-matter.

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EARLY EXPOSURE TO BISPHENOL A DISTURBS MYELIN LIPID REMODELING IN JUVENILE MICE IN A GENDER DEPENDENT MANNER

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

Vanessa Naffaa[1], Romain Magny[1,2], Anne Regazzetti[1], Juliette Van Steenwinckel[3], Pierre Gressens[3], Olivier Laprévote[1,4], Nicolas Auzeil[1], Anne-Laure Schang[5]
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Neurodevelopmental disorders, including learning disabilities, attention deficits and autism spectrum disorders, are a major public health problem. Endocrine disruptors are incriminated in the growing frequency of these pathologies. Indeed, by mimicking or blocking the effects of hormones, endocrine disruptors can have harmful consequences on brain development. Among the compounds of high concern is bisphenol A (BPA), a ubiquitous environmental pollutant which contaminates the organism mainly through ingestion of contaminated water and food. From conception and then during pregnancy and lactation, fetuses and infants are exposed to BPA from their mother through the placenta and then breast milk. One of the crucial steps of brain development is the establishment of myelin sheaths around axons. Thanks to their high lipid content, myelin sheaths improve the transmission of action potentials and ensure optimal functioning of the brain. We hypothesized that BPA could disrupt myelin sheaths formation in the brain during development. By administering BPA to mice during pregnancy and lactation, we studied the impact of this endocrine disruptor on myelination by analyzing myelin lipid composition in male and female offspring. Two doses of BPA were administered in drinking water, 4 and 40 $\mu g.kg^{-1}$, and lipidomic analysis was performed at three postnatal stages: P15, P30 and P60 by UHPLC-HRMS. Our results suggest that BPA impacts the lipid composition of myelin and that this impact depends on the gender. Ultimately, our study will provide a better understanding of deleterious effects of this pollutant on neurodevelopment.

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CEREBRAL HYPOPERFUSION INDUCED BY CAROTID STENOSIS LEADS TO HYPOXIA IN OLIGODENDROCYTE PRECURSOR CELLS

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Background A recent clinical study demonstrated that white matter lesions (WML) seen in cerebral small vessel disease (cSVD) patients correlated with decreased cerebral blood flow (CBF). We thus hypothesize that cerebral hypoperfusion causes local hypoxia affecting the function of oligodendrocyte precursor cells (OPC), leading to the development of WML. **Methods** Male C57BL6/J male mice (11-week-old) underwent bilateral carotid artery stenosis (BCAS, n=10) or Sham surgery (Sham, n=7). CBF was measured before (at baseline) and after surgery (d0 and d7). At d7, mice were injected with pimonidazole (60mg/kg, IP), a hypoxic probe, prior to sacrifice. Myelin content, and hypoxic cell and OPC density were quantified by immunohistochemistry. Primary mouse OPC were exposed to hypoxic (2% O_2) or normoxic culturing conditions (21% O_2) for 24h. RNA sequencing was performed (Novaseq Illumina), data normalized and differentially expressed genes (DEG) were identified using DEseq2 and enrichment analyses. **Results** CBF was significantly reduced in BCAS at d0 (14,6±5,3%, p=0.02), and at d7 (27,2±3,6%, p<0.0001) compared to baseline. No difference in myelin content was observed. Hypoxic cells and hypoxic OPC in the deep cortical region were increased in BCAS vs Sham (12,9±1,3 vs 7,8±1,1 cells/mm^2, p=0.01, 17,9±2,3% vs 7,9±3,9% cells/mm^2, p=0.04, respectfully). There was no difference in OPC densities. *In vitro*, hypoxia led to 417 DEG in primary OPC, with genes involved in cell migration, differentiation, and angiogenesis. **Conclusion** Cerebral hypoperfusion induced hypoxia in OPC. Signaling pathways involved in myelination and vascularity were regulated in hypoxic OPC in vitro. OPC-vessel signaling pathways are being studied to understand the origin of WML in cSVD.

Pubmed:
32630426: Manukjan N, Ahmed Z, Fulton D, Blankesteijn WM, Foulquier S
A Systematic Review of WNT Signaling in Endothelial Cell Oligodendrocyte Interactions: Potential Relevance to Cerebral Small Vessel Disease.
Key pathological features of cerebral small vessel disease (cSVD) include impairment of the blood brain barrier (BBB) and the progression of white matter lesions (WMLs) amongst other structural lesions, leading to the clinical manifestations of cSVD. The function of endothelial cells (ECs) is of major importance to maintain a proper BBB. ECs interact with several cell types to provide structural and functional support to the brain. Oligodendrocytes (OLs) myelinate axons in the central nervous system and are crucial in sustaining the integrity of white matter. The interplay between ECs and OLs and their precursor cells (OPCs) has received limited attention yet seems of relevance for the study of BBB dysfunction and white matter injury in cSVD. Emerging evidence shows a crosstalk between ECs and OPCs/OLs, mediated by signaling through the Wingless and Int-1 (WNT)/β-catenin pathway. As the latter is involved in EC function (e.g., angiogenesis) and oligodendrogenesis, we reviewed the role of WNT/β-catenin signaling for both cell types and performed a systematic search to identify studies describing a WNT-mediated interplay between ECs and OPCs/OLs. Dysregulation of this interaction may limit remyelination of WMLs and render the BBB leaky, thereby initiating a vicious neuroinflammatory cycle. A better understanding of the role of this signaling pathway in EC-OL crosstalk is essential in understanding cSVD development.
Cells, 2020; 9

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CNS MYELINATION: A ROLE FOR AUTOPHAGIC FUNCTION

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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(Macro)autophagy comprises a major lysosome-dependent degradation mechanism which engulfs, removes, and recycles unwanted cytoplasmic material, including damaged organelles and toxic protein aggregates. Although a few studies implicate autophagy in CNS demyelinating pathologies, its role, particularly in oligodendrocytes and CNS myelin, remains poorly studied. We will present data on the significance of macroautophagy in the central nervous system, focusing on oligodendrocytes and myelin homeostasis. To this end, we have used both *in vitro* and *in vivo* approaches. *In vitro*, both pharmacological and genetic inhibition of autophagy have revealed severe defects in myelin sheet formation, delayed maturation and altered cellular distribution of major myelin protein constituents. *In vivo*, we have utilized a new conditional mutant mouse line that we have generated, in which a core gene of autophagic machinery (*atg5*) is specifically ablated in the myelinating glial cells after tamoxifen administration (*plp-Cre^{ERT2}; atg5$^{fl/fl}$*). Biochemical and ultrastructural analysis of this mouse line has revealed differences in myelin protein levels as well as morphological alterations in conditional mutant animals compared to age-matched controls. In summary, our data support the novel principle that the progression of myelination in the CNS requires the involvement of a fully functional autophagic machinery. We acknowledge funding from the Hellenic Foundation for Research and Innovation (HFRI grant agreement 1676), the National Multiple Sclerosis Society (NMSS, pilot Research Grant), the Hellenic Society for Neuroscience (travel Award to attend FENS Forum Paris 2022), and from The Company of Biologists (Travelling Fellowship).

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FUNCTIONAL ALTERATION OF MYELINATED GABAERGIC NEURONS IN THE HIPPOCAMPUS OF CONTACTIN2- AND 4.1B-DEFICIENT MICE

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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A substantial fraction of myelin, both in mouse and human neocortex and hippocampus, belongs to inhibitory neurons (Micheva et al., 2016; Stedehouder et al., 2017). The role of myelination for axonal conduction is well-established in projection neurons but little is known about its significance in GABAergic interneurons. Myelination is discontinuous along interneuron axons and the mechanisms controlling myelin patterning and segregation of ion channels at the nodes of Ranvier require further investigations. We showed that the Kv1 channels, in complex with the cell adhesion molecules Caspr2, Contactin2/TAG-1, ADAM22, and the scaffolding protein 4.1B, exhibit high expression along the GABAergic axons before myelination and their clustering at juxtaparanodes in cultured hippocampal neurons (Pinatel and Faivre-Sarrailh, 2021; Bonetto et al., 2019). We used Contactin2- and 4.1B-deficient mice expressing tdTomato in parvalbumin and somatostatin Lhx6-expressing cells, two major classes of myelinated GABAergic neurons. Strikingly, the 4.1B-deficient hippocampal inhibitory neurons display a strong alteration of juxtaparanodal Kv1 and nodal Nav channels associated with selective loss of myelin. Using whole-cell patch-clamp recordings on acute slices, we showed that the neuronal excitability of somatostatin interneurons localized in stratum oriens is reduced both in Contactin2- and 4.1B-deficient mice. Finally, we found that the spatial working memory is altered in *4.1B* KO mice using the Y-maze test. In conclusion, we showed that dysmyelination and misdistribution of ion channels at the nodes of Ranvier of Contactin2- and 4.1B-deficient interneurons may induce a network inhibitory/excitatory imbalance.

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PROLACTIN RECEPTOR DEFICIENCY PROMOTES HYPOMYELINATION IN THE CORPUS CALLOSUM OF SUCKLING MICE

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

Ana Ocampo-Ruiz, Dina Vazquez-Carrillo, Marco Dimas-Rufino, Ximena Castillo, Edith Garay, Gonzalo Martinez De La Escalera, Carmen Clapp, Rogelio Arellano, Abraham Cisneros-Mejorado, Yazmin Macotela
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Oligodendrocytes (OLs) are responsible for myelination in the central nervous system. OLs extend their cell membrane surrounding neuronal axons and forming myelin, which accelerates action potential conduction and allow the assembly of complex neural circuits. In this fundamental phenomenon, there is evidence suggesting a regulatory role for the hormone prolactin (PRL). In pregnant mice lacking an allele of the PRL receptor (Prlr), there is a lower rate of oligodendrocyte precursor cell proliferation and decreased myelin production. Moreover, systemic PRL treatment promotes myelin repair in a model of spinal cord demyelination. Here, we explored the effect of Prlr deficiency during the early postnatal stage, a critical window in CNS development, where neonates are exposed to high concentrations of PRL through maternal milk. Using Prlr null mice (Prlr-KO) at postnatal day 12 and their wild type pairs (Prlr-WT), we evaluated myelination by Black Gold II (BGII) staining, immunofluorescence against Myelin Basic Protein (MBP), volumetric evaluation and rt-qPCR. In corpus callosum (CC) of Prlr-KO mice, BGII staining revealed a hypomyelinating phenotype, volumetric analysis showed less volume in this area, and immunofluorescence showed less MBP expression compared to Prlr-WT mice. In agreement, evaluation of the CC mRNA expression of some myelin proteins, showed a significant reduction in the expression of Myelin Proteolipid Protein (PLP) and Myelin Oligodendrocyte Glycoprotein (MOG) in Prlr-KO compared to Prlr-WT mice. Taken together, these data indicates that the lack of Prlr activity leads to hypomyelination in CC and disfavors myelin sheath transcriptional elements.

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TILING THE GLIAL NETWORK: HOW INTERNODAL LENGTH CHANGES DURING DEVELOPMENT.

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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The pattern of myelin internodes plays a crucial role in determining the speed of conduction of electrical signals in the nervous system. Yet, we are still debating whether this process of patterning is stochastic or dynamically responds to extrinsic cues. This project characterizes internodal length during development to assess how it is tuned over time. We precisely quantify internodal length in the corpus callosum and the optic nerve using a novel mouse model of stochastically sparse labeled oligodendrocytes PLP-CreER^{T};STARS. Preliminary results from these experiments reveal that each oligodendrocyte has a wide variety of internodal lengths during early development (postnatal day [P]14): internodes (reported as median [min-max]) are 33 µm long [12 to 89 µm] in the corpus callosum of mice (N=3) and are skewed to shorter internodes (Skewness: 1.22). In the optic nerve, internodes measured at P18 were 77 µm long [51 to 144 µm], while at 7 months old (mo) internodes measured 120 µm long [50 to 192 µm]. We noted a shift in the distribution of internodal length between early (P18) and late (7mo) development: most internodes in the optic nerve at P18 were skewed to shorter internodes (Skewness = 0.74). However, internodes at 7mo adopted a Gaussian distribution (Skewness = 0.07). Further experiments to study the impact of extrinsic cues on internodal length are ongoing. These approaches will not only establish that myelination during development is a dynamic process, but will also provide a model for internodal length determination.

Pubmed:

30418331: Sheehy CK, Beaudry-Richard A, Bensinger E, Theis J, Green AJ
Methods to Assess Ocular Motor Dysfunction in Multiple Sclerosis.
: BACKGROUND:: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system causing the immune-mediated demyelination of the brain, optic nerve, and spinal cord and resulting in ultimate axonal loss and permanent neurological disability. Ocular motor dysfunction is commonly observed in MS but can be frequently overlooked or underappreciated by nonspecialists. Therefore, detailed and quantitative assessment of eye movement function has significant potential for optimization of patient care, especially for clinicians interested in treating visual symptoms or tracking disease progression. METHODS:: A brief history of eye tracking technology followed by a contextualized review of the methods that can be used to assess ocular motor dysfunction in MS-including a discussion of each method's strengths and limitations. We discuss the rationale for interest in this area and describe new tools capable of tracking eye movements as a possible means of monitoring disease. RESULTS/CONCLUSIONS:: This overview should inform clinicians working with patients with MS of how ocular motor deficits can best be assessed and monitored in this population. It also provides a rationale for interest in this field with insights regarding which techniques should be used for studying which classes of eye movements and related dysfunction in the disease.
J Neuroophthalmol, 2018; 38
28847304: Nadeau-Vallee M, Obari D, Beaudry-Richard A, Sierra EM, Beaulac A, Maurice N, Olson DM, Chemtob S
Preterm Birth and Neonatal Injuries: Importance of Interleukin-1 and Potential of Interleukin-1 Receptor Antagonists.
Preterm birth (PTB) is a leading cause of neonatal mortality and morbidity worldwide, and surviving infants are at increased risks of lifelong complications. PTB has been firmly linked to inflammation regardless of infection, specific aetiology or timing of birth. Deleterious inflammation is observed in maternal and fetal tissue, and correlates with the severity of perinatal complications. At present, PTB is treated with tocolytics as though it is exclusively a myometrial contractile disorder. These agents do not address underlying inflammatory processes and are thus vastly ineffective at improving neonatal outcomes. Of all inflammatory mediators, IL-1 is central to the pathophysiology of PTB and most adverse neonatal outcomes. We thus present herein a review of the various effects of IL-1 in utero, with a brief overview of its mechanism of action. We then discuss the potential of different IL-1-targeting agents based on pre-clinical testing in relevant models of PTB and neonatal inflammatory injuries.
Curr Pharm Des, 2017; 23

28830469: Rivera JC, Holm M, Austeng D, Morken TS, Zhou TE, Beaudry-Richard A, Sierra EM, Dammann O, Chemtob S
Retinopathy of prematurity: inflammation, choroidal degeneration, and novel promising therapeutic strategies.
Retinopathy of prematurity (ROP) is an important cause of childhood blindness globally, and the incidence is rising. The disease is characterized by initial arrested retinal vascularization followed by neovascularization and ensuing retinal detachment causing permanent visual loss. Although neovascularization can be effectively treated via retinal laser ablation, it is unknown which children are at risk of entering this vision-threatening phase of the disease. Laser ablation may itself induce visual field deficits, and there is therefore a need to identify targets for novel and less destructive treatments of ROP. Inflammation is considered a key contributor to the pathogenesis of ROP. A large proportion of preterm infants with ROP will have residual visual loss linked to loss of photoreceptor (PR) and the integrity of the retinal pigment epithelium (RPE) in the macular region. Recent studies using animal models of ROP suggest that choroidal degeneration may be associated with a loss of integrity of the outer retina, a phenomenon so far largely undescribed in ROP pathogenesis. In this review, we highlight inflammatory and neuron-derived factors related to ROP progression, as well, potential targets for new treatment strategies. We also introduce choroidal degeneration as a significant cause of residual visual loss following ROP. We propose that ROP should no longer be considered an inner retinal vasculopathy only, but also a disease of choroidal degeneration affecting both retinal pigment epithelium and photoreceptor integrity.
J Neuroinflammation, 2017; 14
32593271: Pietrobon A, Chehadé L, Beaudry-Richard A, Keller BA, Schlossmacher MG
Performance report for a 10-year-old MD/PhD Program: A survey of trainees at the University of Ottawa.
Integrated MD/PhD programs are relatively new in Canada and represent a platform to train the next generation of clinician-scientists. However, MD/PhD programs vary substantially by structure, funding and mentorship opportunities, and there exists a paucity of data on the overall students' successes and challenges. The purpose of this study is to assess objective and subjective metrics of the MD/PhD Program at the University of Ottawa.
Clin Invest Med, 2020; 43
32296110: Beaudry-Richard A, Nadeau-Vallée M, Prairie É, Maurice N, Heckel É, Nezhady M, Pundir S, Madaan A, Boudreault A, Hou X, Quiniou C, Sierra EM, Beaulac A, Lodygensky G, Robertson SA, Keelan J, Adams Waldorf KM, Olson DM, Rivera JC, Lubell WD, Joyal JS, Bouchard JF, Chemtob S
Author Correction: Antenatal IL-1-dependent inflammation persists postnatally and causes retinal and sub-retinal vasculopathy in progeny.
An amendment to this paper has been published and can be accessed via a link at the top of the paper.
Sci Rep, 2020; 10
30089839: Beaudry-Richard A, Nadeau-Vallée M, Prairie É, Maurice N, Heckel É, Nezhady M, Pundir S, Madaan A, Boudreault A, Hou X, Quiniou C, Sierra EM, Beaulac A, Lodygensky G, Robertson SA, Keelan J, Adams Waldorf KM, Olson DM, Rivera JC, Lubell WD, Joyal JS, Bouchard JF, Chemtob S
Antenatal IL-1-dependent inflammation persists postnatally and causes retinal and sub-retinal vasculopathy in progeny.
Antenatal inflammation as seen with chorioamnionitis is harmful to foetal/neonatal organ development including to eyes. Although the major pro-inflammatory cytokine IL-1β participates in retinopathy induced by hyperoxia (a predisposing factor to retinopathy of prematurity), the specific role of antenatal IL-1β associated with preterm birth (PTB) in retinal vasculopathy (independent of hyperoxia) is unknown. Using a murine model of PTB induced with IL-1β injection in utero, we studied consequent retinal and choroidal vascular development; in this process we evaluated the efficacy of IL-1R antagonists. Eyes of foetuses exposed only to IL-1β displayed high levels of pro-inflammatory genes, and a persistent postnatal infiltration of inflammatory cells. This prolonged inflammatory response was associated with: (1) a marked delay in retinal vessel growth; (2) long-lasting thinning of the choroid; and (3) long-term morphological and functional alterations of the retina. Antenatal administration of IL-1R antagonists - 101.10 (a modulator of IL-1R) more so than Kineret (competitive IL-1R antagonist) - prevented all deleterious effects of inflammation. This study unveils a key role for IL-1β, a major mediator of chorioamnionitis, in causing sustained ocular inflammation and perinatal vascular eye injury, and highlights the efficacy of antenatal 101.10 to suppress deleterious inflammation.
Sci Rep, 2018; 8
28148737: Nadeau-Vallée M, Chin PY, Belarbi L, Brien MÈ, Pundir S, Berryer MH, Beaudry-Richard A, Madaan A, Sharkey DJ, Lupien-Meilleur A, Hou X, Quiniou C, Beaulac A, Boufaied I, Boudreault A, Carbonaro A, Doan ND, Joyal JS, Lubell WD, Olson DM, Robertson SA, Girard S, Chemtob S
Antenatal Suppression of IL-1 Protects against Inflammation-Induced Fetal Injury and Improves Neonatal and Developmental Outcomes in Mice.
Preterm birth (PTB) is commonly accompanied by in utero fetal inflammation, and existing tocolytic drugs do not target fetal inflammatory injury. Of the candidate proinflammatory mediators, IL-1 appears central and is sufficient to trigger fetal loss. Therefore, we elucidated the effects of antenatal IL-1 exposure on postnatal development and investigated two IL-1 receptor

antagonists, the competitive inhibitor anakinra (Kineret) and a potent noncompetitive inhibitor 101.10, for efficacy in blocking IL-1 actions. Antenatal exposure to IL-1β induced , , , , and expression in placenta and fetal membranes, and it elevated amniotic fluid IL-1β, IL-6, IL-8, and PGF, resulting in PTB and marked neonatal mortality. Surviving neonates had increased , , , , , , and expression in WBCs, elevated plasma levels of IL-1β, IL-6, and IL-8, increased IL-1β, IL-6, and IL-8 in fetal lung, intestine, and brain, and morphological abnormalities: e.g., disrupted lung alveolarization, atrophy of intestinal villus and colon-resident lymphoid follicle, and degeneration and atrophy of brain microvasculature with visual evoked potential anomalies. Late gestation treatment with 101.10 abolished these adverse outcomes, whereas Kineret exerted only modest effects and no benefit for gestation length, neonatal mortality, or placental inflammation. In a LPS-induced model of infection-associated PTB, 101.10 prevented PTB, neonatal mortality, and fetal brain inflammation. There was no substantive deviation in postnatal growth trajectory or adult body morphometry after antenatal 101.10 treatment. The results implicate IL-1 as an important driver of neonatal morbidity in PTB and identify 101.10 as a safe and effective candidate therapeutic.
J Immunol, 2017; 198

27768863: Zhou TE, Rivera JC, Bhosle VK, Lahaie I, Shao Z, Tahiri H, Zhu T, Polosa A, Dorfman A, Beaudry-Richard A, Costantino S, Lodygensky GA, Lachapelle P, Chemtob S

Choroidal Involution Is Associated with a Progressive Degeneration of the Outer Retinal Function in a Model of Retinopathy of Prematurity: Early Role for IL-1β.

Retinopathy of prematurity (ROP), the most common cause of blindness in premature infants, has long been associated with inner retinal alterations. However, recent studies reveal outer retinal dysfunctions in patients formerly afflicted with ROP. We have recently demonstrated that choroidal involution occurs early in retinopathy. Herein, we investigated the mechanisms underlying the choroidal involution and its long-term impact on retinal function. An oxygen-induced retinopathy (OIR) model was used. In vitro and ex vivo assays were applied to evaluate cytotoxic effects of IL-1β on choroidal endothelium. Electroretinogram was used to evaluate visual function. We found that proinflammatory IL-1β was markedly increased in retinal pigment epithelium (RPE)/choroid and positively correlated with choroidal degeneration in the early stages of retinopathy. IL-1β was found to be cytotoxic to choroid in vitro, ex vivo, and in vivo. Long-term effects on choroidal involution included a hypoxic outer neuroretina, associated with a progressive loss of RPE and photoreceptors, and visual deterioration. Early inhibition of IL-1β receptor preserved choroid, decreased subretinal hypoxia, and prevented RPE/photoreceptor death, resulting in life-long improved visual function in IL-1 receptor antagonist-treated OIR animals. Together, these findings suggest a critical role for IL-1β-induced choroidal degeneration in outer retinal dysfunction. Neonatal therapy using IL-1 receptor antagonist preserves choroid and prevents protracted outer neuroretinal anomalies in OIR, suggesting IL-1β as a potential therapeutic target in ROP.
Am J Pathol, 2016; 186

27512149: Nadeau-Vallée M, Boudreault A, Leimert K, Hou X, Obari D, Madaan A, Rouget R, Zhu T, Belarbi L, Brien MÈ, Beaudry-Richard A, Olson DM, Girard S, Chemtob S

Uterotonic Neuromedin U Receptor 2 and Its Ligands Are Upregulated by Inflammation in Mice and Humans, and Elicit Preterm Birth.

Uterine labor requires the conversion of a quiescent (propregnancy) uterus into an activated (prolabor) uterus, with increased sensitivity to endogenous uterotonic molecules. This activation is induced by stressors, particularly inflammation in term and preterm labor. Neuromedin U (NmU) is a neuropeptide known for its uterocontractile effects in rodents. The objective of the study was to assess the expression and function of neuromedin U receptor 2 (NmU-R2) and its ligands NmU and the more potent neuromedin S (NmS) in gestational tissues, and the possible implication of inflammatory stressors in triggering this system. Our data show that NmU and NmS are uterotonic ex vivo in murine tissue, and they dose-dependently trigger labor by acting specifically via NmU-R2. Expression of NmU-R2, NmU, and NmS is detected in murine and human gestational tissues by immunoblot, and the expression of NmS in placenta and of NmU-R2 in uterus increases considerably with gestation age and labor, which is associated with amplified NmU-induced uterocontractile response in mice. NmU- and NmS-induced contraction is associated with increased NmU-R2-coupled Ca transients, and Akt and Erk activation in murine primary myometrial smooth muscle cells (mSMCs), which are potentiated with gestational age. NmU-R2 is upregulated in vitro in mSMCs and in vivo in uterus in response to proinflammatory interleukin 1beta (IL1beta), which is associated with increased NmU-induced uterocontractile response and Ca transients in murine and human mSMCs; additionally, placental NmS is markedly upregulated in vivo in response to IL1beta. In human placenta at term, immunohistological analysis revealed NmS expression primarily in cytotrophoblasts; furthermore, stimulation with lipopolysaccharide (LPS; Gram-negative endotoxin) markedly upregulates NmS expression in primary human cytotrophoblasts isolated from term placentas. Correspondingly, decidua of women with clinical signs of infection who delivered preterm display significantly higher expression of NmS compared with those without infection. Importantly, in vivo knockdown of NmU-R2 prevents LPS-triggered preterm birth in mice and the associated neonatal mortality. Altogether, our data suggest a critical role for NmU-R2 and its ligands NmU and NmS in preterm labor triggered by infection. We hereby identify NmU-R2 as a relevant target for preterm

birth.
Biol Reprod, 2016; 95

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ANIMAL MODELS FOR DIABETIC PERIPHERAL NEUROPATHIES

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Peripheral neuropathy is one of most common complications in diabetes (1). It occurs in almost half the population with the disease and leads to a poor quality of the patients' life. For this reason, numerous animal models have been developed mimicking the human ailment, with rodents being the main players. Among the murine models of diabetic neuropathy (2) are conventional, streptozotocin-induced, genetically modified and/or high-fat diet-fed mice models, nonobese diabetic (NOD), spontaneously induced Ins2 Akita model, and the leptin-deficient (ob/ob) mice models. Rat models, on the other hand include the streptozotocin-induced diabetic rat models, spontaneously diabetic WBN/Kob rat model, L-fucose-induced neuropathic rat model, the partial sciatic nerve ligated rat model, Otsuka Long-Evans Tokushima Fatty (OLETF) rat model, surgically-induced neuropathic model, and genetically modified Spontaneously Diabetic Torii (SDT) rat model. Chinese hamsters, sand rats are also used for the same purpose. Lastly, non-human primates, play a role into the investigation of the disease. In this review we will present the plethora of animal studies existing today and discuss their efficacy for human study since there are advantages, disadvantages and limitations between the different species and the genetic and nongenetic animal models addressing acute and chronic events leading to diabetic neuropathy. Selected references 1. Pasnoor M, Dimachkie MM, Kluding P, Barohn RJ. Diabetic neuropathy part 1:overview and symmetric phenotypes. Neurol Clin 2013;31(2):425-445. Islam MS. Animal models of diabetic neuropathy: progress since 1960s. J Diabetes Res. 2013;2013:149452. doi: 10.1155/2013/149452. Epub 2013 Jul 29. PMID: 23984428; PMCID: PMC3745837.

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NOVEL C.1799GC>G (P.P600R) SUBSTITUTION IN ATP1A1 CAUSES DEMYELINATING CHARCOT-MARIE-TOOTH IN A CYPRIOT PATIENT

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Charcot-Marie-Tooth (CMT) is a genetically and clinically heterogenous group of inherited neuropathies. CMT affects peripheral nerves and patients typically experience progressive weakness and atrophy of distal muscles. *ATP1A1* is one of the genes that has been recently associated with axonal and intermediate CMT. *ATP1A1* encodes for the α-1 subunit of the Na^+, K^+ ATPase, an integral membrane protein responsible for establishing the electrochemical gradients of Na^+ and K^+ ions across the plasma membrane. Mutations in distinct protein domains affect tissues with high ion transport activity resulting in renal hypomagnesemia, aldosterone-producing adenoma, or neurodevelopmental delay. Using whole exome sequencing, we identified a novel c.1799C>G (p.P600R) missense variant in a Cypriot demyelinating CMT patient with severe sensorineural hearing loss. Functional studies indicated significant reduction of mRNA and protein expression of *ATP1A1* in the patient. In addition, the *ATP1A1*$^{p.P600R}$ transfected HEK cells displayed significant decrease in cell viability, further supporting the pathogenicity of this candidate variant. Thus, we show that *ATP1A1* can cause demyelinating phenotype through loss of function mechanism in addition to previously reported axonal and intermediate phenotypes. Our results further confirm the causative role of *ATP1A1* in peripheral neuropathy and broaden the mutational and phenotypic spectrum of *ATP1A1*-CMT.

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NEURODEVELOPMENTAL ORIGIN OF CORTICAL SATELLITE CELLS

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Cortical satellites cells (CSC) are poorly explored members of the oligodendrocyte lineage that play a role in the regulation of neuronal activity. In the mammalian cerebral cortex, they are found affixed to the soma of pyramidal neurons and to blood vessels, and imbalanced in human pathological brain tissue. As key features of their cellular identity and molecular regulators remain controversial, further characterization of CSC development is essential to gain more insight into their functions during cerebral cortex maturation in physiological and pathological contexts. To probe the developmental origin of CSC and their mode of colonization of the cortical parenchyma, we used the MAGIC Markers multicolor fate mapping strategy and tracked cortical progenitors and all their progeny in the developing mouse brain. Our results show that CSC are generated from cortical progenitor and arrive at their final location after the first postnatal week. The deep cortical layers are colonized first, followed by the upper layers two weeks later. Then, CSC continue to spread in the cortical layers, increasing in numbers until the adult stage. Crossing MAGIC Markers mice with specific inducible Cre transgenic mice allows us to further characterize the progenitors that generate CSC, while tracking over time this poorly studied cell population for which no dedicated marker has been identified so far. Overall, our work contributes to elucidate an enigmatic cell population of the mammalian cerebral cortex by shedding new light on their developmental origin and heterogeneity, which will help us better understand their involvement in neurodevelopmental disorders.

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FUNCTIONAL ROLE OF SATELLITE OLIGODENDROCYTES IN NEOCORTICAL CIRCUITS

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

Maddalena Balia[1], Fernando Sanchez-Roman Teran[1], Vanessa Rouglan[2], Alexandre Favereaux[2], Arne Battefeld[1]
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Oligodendrocytes were considered as a homogeneous population for a long time, however these cells display clear anatomical and transcriptomic heterogeneity. In the somatosensory cortex, oligodendrocytes can be found in direct perisomatic contact with pyramidal neurons. Earlier work established that these satellite oligodendrocytes can detect action potentials and contribute to potassium buffering, therefore modulating the associated neuron's activity. This functional role of satellite oligodendrocytes lead us to hypothesize more complex inter-somatic interactions with their associated neurons, *i.e.* the existence of a direct metabolic coupling. Furthermore, we also hypothesized that satellite oligodendrocytes positioning and function correlates with distinct transcriptional features.
We determined whether (i) satellite oligodendrocytes are specific for distinct cortical neuron populations, (ii) a direct metabolic support of the associated neuron exists, and (iii) a specific transcriptomic profile is associated with this anatomical position of oligodendrocytes.
Using immunohistochemical labelling of murine cortex, we show that satellite oligodendrocytes represent around half of the cortical oligodendrocyte population in layers II to IV. To target different cortical layer V projection neurons, we used a retrograde tracing strategy and found that neurons with different projection targets associate similarly with satellite oligodendrocytes. Electrophysiological recordings of satellite oligodendrocytes and their associated neuron were used to determine the extent of direct metabolic coupling. Targeted whole-cell recordings combined with subsequent single-cell sequencing were used to analyse the transcriptomic profiles of satellite oligodendrocyte - associated neuron pairs. We provide an extended analysis of satellite oligodendrocytes and conclude these cells are an integral part of neocortical circuits.

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GENERATION OF DIFFERENT OLIGODENDROCYTE LINEAGE CELL STATES FOLLOWING DIRECT LINEAGE REPROGRAMMING OF GFAP+ ASTROCYTES

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

Justine Bajohr[1], Hiba Taha[1], Arman Olfat[1], Erica Scott[1,2], Kevin Lee[1], Daniela Lozano-Casasbuenas[1,3], Scott Yuzwa[3], Maryam Faiz[1]
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Oligodendrocyte (OL) loss or dysfunction is a hallmark of many central nervous system (CNS) conditions. Direct lineage reprogramming (DLR) is a new strategy for CNS repair, where a donor cell is converted into a target cell without a pluripotent intermediate. Here we show that astrocytes can be directly reprogrammed to different OL lineage cells *in vitro* using transcription factors (TFs) involved in different stages of OL fate determination. Postnatal cortical astrocytes were purified and transduced with lentiviruses containing three different GFAP-driven TFs. We found that ectopic expression of each of these single TFs produced different types of OL lineage cells. Ectopic expression of a TF important for late OL development resulted in the generation of MBP+ myelinating OLs (mOLs), while expression of an early OL fate determinant generated PDGFRα+ oligodendrocyte progenitor cells (OPCs). Finally, expression of a third TF, expressed throughout OL development, produced cells at various stages of OL lineage development (OPCs, newly forming OLs (nfOLs) and mOLs). Live cell imaging of the reprogramming process confirmed that reprogrammed OLs originate from S100β expressing astrocytes. Finally, single cell RNA sequencing revealed different iOL gene signatures depending on TF delivered. These findings suggest that ectopic expression of TFs in astrocytes results in the generation of OLs at different stages of maturity. These studies lay the groundwork for novel, DLR-based therapeutic strategies for diseases involving OL lineage cell loss with possibilities to tailor iOL production according to the OL type lost in disease.

Pubmed:
30989588: Bajohr J, Faiz M
Direct Lineage Reprogramming in the CNS.
Direct lineage reprogramming is the conversion of one specialized cell type to another without the need for a pluripotent intermediate. To date, a wide variety of cell types have been successfully generated using direct reprogramming, both in vitro and in vivo. These newly converted cells have the potential to replace cells that are lost to disease and/or injury. In this chapter, we will focus on direct reprogramming in the central nervous system. We will review current progress in the field with regards to all the major neural cell types and explore how cellular heterogeneity, both in the starter cell and target cell population, may have implications for direct reprogramming. Finally, we will discuss new technologies that will improve our understanding of the reprogramming process and aid the development of more specific and efficient future CNS-based reprogramming strategies.
Adv Exp Med Biol, 2020; 1212

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A POPULATION OF GREY MATTER OLIGODENDROCYTES ASSOCIATES DIRECTLY WITH THE VASCULATURE

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Oligodendrocyte lineage cells are known to interact with the vasculature in the grey matter. During cortical development oligodendrocyte precursor cells (OPCs) migrate along the vasculature and subsequently detach. Signaling from OPCs influences angiogenesis during early development and release of transforming growth factor ß-1 by OPCs plays a role in maintenance of the blood brain barrier. There is evidence that mature oligodendrocytes continue to interact with the vasculature as they have been occasionally noted being close to blood vessels. In addition, endothelial cell signaling influences activity dependent myelination and early multiple sclerosis lesions can occur around blood vessels. To date, a systematic investigation of oligodendrocyte-vasculature interaction is missing. We studied in an oligodendrocyte reporter mouse line the occurrence and interaction of mature oligodendrocytes with the vasculature in the grey matter using immunohistochemistry, electron microscopy volume imaging coupled with 2-photon branding, and whole-cell electrophysiology. We found that mature oligodendrocytes are regularly closely linked with blood vessels in various brain areas. Correlated light and electron microscopy revealed that parts of the oligodendrocyte cell body is in direct contact with the endothelial basement membrane similar to astrocytic endfeet. Systematic analysis across all neocortical layers showed an increase in vascular associated oligodendrocytes following the overall increase of oligodendrocytes towards deeper cortical layers. We conclude that the association of mature oligodendrocytes with the vasculature is a common feature of all oligodendrocyte lineage cells. Additionally, oligodendrocytes could directly take up metabolites from the vasculature and deterioration of this contact might contribute to the alterations of oligodendrocytes in pathologies.

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OPTICALLY RESOLVED MEMBRANE VOLTAGE CHANGES IN MYELIN

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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A number of ion channels including several potassium and calcium channels have been proposed to be localised in the myelin sheath, but their contribution to myelin membrane voltage changes have not been explored. Investigating spatial dynamics of membrane potential changes could help to understand ion homeostasis of myelin under physiological or pathophysiological conditions. For instance, the potassium inward rectifying channel Kir4.1 has been shown to locate to the inner node and compact myelin where it contributes to potassium buffering during action potentials. Technical limitations of electrode-based voltage measurements do not allow direct recordings of voltage changes at the oligodendrocyte myelin sheath. Therefore, we implemented an imaging strategy to optically measure voltage changes in the myelin membrane in response to neuronal activity with the aim to identify ion channels underlying these responses. We achieved in vivo transfection of oligodendrocytes with an adeno-associated virus expressing the genetically encoded voltage sensor ASAP3 under an oligodendrocyte specific promoter. Five weeks after stereotaxic injection of the virus we prepared acute brain slices of the mouse somatosensory cortex. Using a LED illumination-based epifluorescence live imaging approach, single oligodendrocytes were imaged at 192 Hz during extracellular stimulation of axons at 100 Hz. We recorded voltage responses in control and in the presence of pharmacological blockers at the oligodendrocyte cell body and myelin sheath in wild type and Kir4.1 knock out mice. Our results show that optical measurements of myelin voltage can be a useful tool to study voltage and underlying conductance directly in myelin.

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DEVELOPMENTAL OLIGODENDROGENESIS AND MYELINATION : REVISITING CANONICAL AND NON-CANONICAL SHH SIGNALING

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

Adil El Mesaoudi, Abdelmoumen Kassoussi, Amina Zahaf, Elisabeth Traiffort
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During development, Hedgehog morphogens orchestrate morphogenesis by controlling cell growth, differentiation and migration (Briscoe & Small, 2015). The canonical signaling occurs when Hedgehog proteins bind the transmembrane protein Patched (Ptc), which relieves the repressive activity exerted by Ptc on the key mediator Smoothened (Smo) and leads to a complex downstream signaling cascade involving the transcription factors of the Gli family (Kong et al, 2019). The pathway has been previously involved in the perinatal wave of oligodendrocyte production arising from the germinative zone of the dorsal forebrain (Tong et al, 2015 ; Samanta et al, 2015 ; Winkler et al, 2018) where the canonical pathway has to be first activated and then blocked in order to promote OPC proliferation and differentiation, respectively. However, Shh transcription is maintained at a quite unchanged level during the process of oligodendrogenesis and myelination suggesting that the proteins may still act during myelination by triggering a non canonical pathway. We are currently using specific antibodies in order to characterize the expression of the pathway components in cells other than the neural stem cells where Shh signaling has been identified until now. Our data suggest the expression of the pathway in cells different from the neural stem cells that we are currently characterizing. The maintenance of Shh signals together with the severe downregulation of the main readout of the canonical pathway may likely mask the activity of a non-canonical Shh signaling during developmental myelination.

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BOARD NUMBER: S05-280

CHARACTERIZATION OF DYSFUNCTIONAL OLIGODENDROCYTES AT SINGLE-CELL RESOLUTION

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

Ting Sun[1], Constanze Depp[1], Stefan Berghoff[1], Lena Spieth[1], Andrew Octavian Sasmita[1], Agnes Steixner-Kumar[2], Swati Subramanian[1], Sandra Göbbels[1], Wiebke Möbius[3], Hannelore Ehrenreich[2], Daniel Geschwind[4], Riki Kawaguchi[4], Klaus-Armin Nave[1]
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Oligodendrocytes form myelin and enable the efficient transmission of axon electric impulses in the central nervous system. Dysfunctions of oligodendrocyte and myelin are paralleled by pathological hallmarks such as axonal swellings and reactive gliosis in multiple sclerosis and leukodystrophies. How these pathological side-effects of oligodendrocyte dysfunction arise, however, is incompletely understood. Here, we performed single-nuclei transcriptome sequencing (snRNA-seq) of the cortex and underlying white matter from knockout mouse models of the three most abundant myelin proteins (PLP1, MBP, CNP) that present with subtle oligodendrocyte dysfunctions. We recovered astonishingly distinct oligodendrocyte RNA profiles upon knockout of CNP, PLP1, and MBP. Cell manifold reconstruction, as well as integrative analysis with previously defined cell subpopulations, revealed that mutant oligodendrocytes are mostly trapped at specific intermediate cell states. The imbalanced oligodendrocyte subtype proportions in the mutant brain potentially shift the crosstalk between oligodendrocytes and other cell populations. Our data, therefore, provide a single-cell perspective on the early stages of oligodendrocyte dysfunction with relevance to neurological disorders that present with myelin defects.

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GBA1 INACTIVATION IN OLIGODENDROCYTES AFFECTS MYELINATION AND INDUCES NEURODEGENERATION AND LIPID DYSHOMEOSTASIS IN MICE

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Aims: GBA1 (Glucocerebrosidase 1) mutations are recognized as risk factors for Parkinson's disease (PD). Whilst substantial knowledge is available regarding GBA1 in neurons, the role of glial GBA1 has been mostly overlooked in oligodendrocytes (OL). Nonetheless, white matter alterations have been described in GBA1-linked PD patients. Our aim was to study the relevance of GBA1 in OLs *in vitro* and *in vivo*. Methods: We used *in vitro* models in which GBA1 was inactivated either chemically or genetically to study cell-specific alterations in differentiation and signaling pathways. We generated an OL specific conditional knockout mouse for GBA1. We characterized myelination *in vivo* by quantifying the levels of myelin-related proteins and measuring g-ratio on electron microscopy pictures. Brain lipidic species were quantified by lipidomic analysis. Behavioral tests were also performed. Results: GBA1 inhibition delayed myelination *in vitro* and led to the accumulation of α-synuclein in the Olineu cell line. GBA1 inactivation also altered intracellular cholesterol distribution. Consistently, genetic inactivation of GBA1 in primary cultures altered the maturation of OLs and caused aberrant expression of OL lineage specific markers. *In vivo*, 6-months-old GBA1 deficient mice showed decreased myelin thickness in the striatum and optic nerve, together with axonal degeneration. Quantitative lipidomic analysis of brain extracts showed global anomalies in lipidic species. Gait alterations were detectable at 6 months of age. Conclusions: This is the first demonstration of a contribution from OL dysfunction to GBA1-related neurodegenerative disorders. We showed that GBA1 deficiency leads to defective myelination inducing neuronal degeneration and altered lipid homeostasis.

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EFFECTS OF CRMP2 KNOCKOUT ON MYELINATION IN A SCHIZOPHRENIA MOUSE MODEL

POSTER SESSION 05 - SECTION: GLIAL CELLS AND MYELINATION

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Aims: Myelination is essential to keep signal transduction and communication within the brain at an optimal level. There is evidence for impaired myelination in several neuropsychiatric disorders including Schizophrenia (SZ). This project centers around CNS demyelination as a component of SZ, using a Collapsin Response Mediator Protein 2 (CRMP2) conditional knock out mouse (cKO) model. The aims are to investigate myelin at a functional, structural and ultrastructural levels to elucidate which myelin parameters might be mostly affected. **Methods**: We investigated potential changes in **function** of the largest white matter structure in the brain, corpus callosum (CC), with multielectrode electrophysiology of compound action potentials (CAPs). At the **structural** level, we looked at the volume of CC and number of oligodendrocytes. We examined the **ultrastructure** with 3D axonal and myelin reconstructions of image stacks acquired by Serial Block Face Scanning Electron Microscopy, implementing an image-segmentation pipeline. Several parameters of Nodes of Ranvier (volume, length, and myelin thickness) were manually quantified. **Results:** We saw indication of altered CAP signals and a reduction in CC volume, but not in the number of oligodendrocytes . Preliminary ultrastructural results suggest changes in node of Ranvier structure in CRMP2-cKO animals. **Conclusions:** Together our findings indicate that CRMP2 plays a role in white matter function in a SZ model. The mechanisms by which this happens are still uncertain. Our project has implemented analytic methods to shed light on the role of myelin defects in SZ. The analyses-pipelines can be used in future work on genetic models of neuropsychiatric disorders.

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BOARD NUMBER: S05-283

EXPRESSION OF THE SCHIZOPHRENIA SUSCEPTIBILITY GENE FEZ1 IN THE EARLY FETAL HUMAN FOREBRAIN

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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The protein fasciculation and elongation zeta-1 (FEZ1) has been shown to be involved in axon outgrowth but potentially interacts with various proteins whose roles range from intracellular transport systems to transcription regulation. Genome-wide association studies have identified it as a schizophrenia susceptibility gene. To test whether it may play a role in early brain development, we mapped *FEZ1* expression by region and cell type in the normally developing human forebrain. **Methods:** All tissues were collected with appropriate maternal consent and ethical approval by the Human Developmental Biology Resource (HDBR.org). RNAseq data was obtained from previously published sources. Thin paraffin sections 8-21 post-conceptional weeks (PCW) were used to perform RNAScope in situ hybridization and immunohistochemistry against FEZ1 mRNA and protein and other marker proteins, sometimes in combination. **Results:** Tissue RNAseq data revealed that FEZ1 is highly expressed in the human cerebral cortex between 7 and 17 PCW (top 5% of protein coding genes), and single cell RNAseq at 17-18 PCW confirmed its high expression, predominantly in maturing excitatory neurons. In the cerebral cortex, strong expression of mRNA and protein appeared confined to post-mitotic neurons although low expression was seen in progenitor zones. Protein expression was observed in axon tracts and the subplate at older stages. In the ganglionic eminences and diencephalon, the opposite was observed, with FEZ1 highly expressed in progenitor zones, but largely absent from post-mitotic cells. **Conclusion:** FEZ1 has different expression patterns and potentially diverse functions in discrete forebrain regions during prenatal human development.

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BOARD NUMBER: S05-284

A SIMPLE, RAPID AND EFFICIENT DIFFERENTIATION PROTOCOL FOR THE GENERATION OF INDUCED PLURIPOTENT STEM CELL-DERIVED MOTOR NEURONS FOR AMYOTROPHIC LATERAL SCLEROSIS MODELLING.

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disease characterized by the loss of upper and lower motor neurons (MNs). Most ALS cases are sporadic, with no familial history or known genetic association. Therefore, a large collection of sporadic ALS models is required to identify common underlying mechanisms of pathology or develop new therapeutic interventions. Induced pluripotent stem cells (iPSCs) are a valuable tool for disease modelling, drug screening and cell therapy, especially for sporadic cases as there is no animal model. But the shortages of deriving enriched and functional MNs from iPSCs hinder the utility of the iPSCs model. In combination with small molecules cocktail, we established a rapid, simple, and efficient protocol of spinal MN differentiation. $CHAT^+$ and $MAP2^+$ MNs (90% and 98%) can be derived in 18 days, and they are functionally mature as evidenced by firing strong single action potentials, typical calcium transients in 18 days. And they also showed extensive network firing and responded to the agonists and antagonists of glutamate receptors in 28 days. Using this new protocol, we observed hyperexcitation in MNs derived from sporadic ALS on MEA recording. Above all, a rapid, simple, and efficient spinal MNs differentiation protocol was established and applied to a model of ALS. This protocol enables the generation of large quantities of MN with high purity and maturity, which will provide a basis for modelling ALS and other MN diseases, drug screening and cell therapy.

Pubmed:
32208303: Yang M, Liu M, Ding Y, Vajda A, Ma J, Cui H, O'Brien T, Henshall D, Hardiman O, Shen S
Generation of twelve induced pluripotent stem cell lines from two healthy controls and two patients with sporadic amyotrophic lateral sclerosis.
The majority of amyotrophic lateral sclerosis are sporadic (sALS) with no familial history or known genetic association, therefore a large cohort of disease models are required to identify common mechanisms or to test therapeutic interventions. Here we generated twelve induced pluripotent stem cell (iPSC) lines from human dermal fibroblasts of two healthy individuals and two sALS patients lacking common ALS mutations, using non-integrational Sendai virus expressing reprogramming factors OCT3/4, KLF4, SOX2 and c-MYC. The iPSC lines highly expressed pluripotency markers could be spontaneously differentiated into three embryonic germ layers, with no gross chromosomal aberrations or specific copy number variations.
Stem Cell Res, 2020; 44
31514057: Yang M, Liu M, Vajda A, O'Brien T, Henshall D, Hardiman O, Shen S
Generation of six induced pluripotent stem cell (iPSC) lines from two patients with amyotrophic lateral sclerosis (NUIGi043-A, NUIGi043-B, NUIGi043-C, NUIGi044-A, NUIGi044-B, NUIGi044-C).
In this study, we generated 6 induced pluripotent stem cell (iPSC) lines derived from dermal fibroblasts of patients with sporadic amyotrophic lateral sclerosis (sALS). The fibroblasts were reprogrammed using non-integrating Sendai viruses containing four reprogramming factors OCT3/4, SOX2, KLF4 and C-MYC. The iPSC lines displayed normal molecular karyotype, expressed pluripotency markers and were capable of differentiating into three embryonic germ layers.
Stem Cell Res, 2019; 40
33631419: Ding Y, O'Brien A, Marcó de la Cruz B, Yang M, Lu Y, Qian X, Yang G, McInerney V, Krawczyk J, Lynch SA, Howard L, Allen NM, O'Brien T, Gallagher L, Shen S
Derivation of four iPSC lines from a male ASD patient carrying a deletion in the middle coding region of NRXN1α gene

(NUIGi039-A and NUIGi039-B) and a male sibling control (NUIGi040-A and NUIGi040-B).
NRXN1 deletions are commonly found in autism spectrum disorder (ASD) and other neurodevelopmental/neuropsychiatric disorders. Derivation of induced pluripotent stem cells (iPSCs) from different diseases involving different deletion regions are essential, as NRXN1 may produce thousands of splicing variants. We report here the derivation of iPSCs from a sibling control and an ASD proband carrying de novo heterozygous deletions in the middle region of NRXN1, using a non-integrating Sendai viral kit. The genotype and karyotype of the iPSCs were validated by whole genome SNP array. All iPSC lines highly expressed pluripotency markers and could be differentiated into three germ layers.
Stem Cell Res, 2021; 53
33578364: Ding Y, O'Brien A, de la Cruz BM, Yang M, Fitzgerald J, Yang G, Li W, McInerney V, Krawczyk J, Lynch SA, Howard L, Allen NM, O'Brien T, Gallagher L, Shen S
Derivation of iPSC lines from two patients with autism spectrum disorder carrying NRXN1α deletion (NUIGi041-A, NUIG041-B; NUIGi045-A) and one sibling control (NUIGi042-A, NUIGi042-B).
NRXN1 encodes thousands of splicing variants categorized into long NRXN1α, short NRXN1β and extremely short NRXN1γ, which exert differential roles in neuronal excitation/inhibition. NRXN1α deletions are common in autism spectrum disorder (ASD) and other neurodevelopmental/neuropsychiatric disorders. We derived induced pluripotent stem cells (iPSCs) from one sibling control and two ASD probands carrying NRXN1α, using non-integrating Sendai viral method. All iPSCs highly expressed pluripotency markers and could be differentiated into ectodermal/mesodermal/endodermal cells. The genotype and karyotype of the iPSCs were validated by whole genome SNP array. The availability of the iPSCs offers an opportunity for understanding NRXN1α function in human neurons and in ASD.
Stem Cell Res, 2021; 52
24467897: Shi CC, Feng CC, Yang MM, Li JL, Li XX, Zhao BC, Huang ZJ, Ge RC
Overexpression of the receptor-like protein kinase genes AtRPK1 and OsRPK1 reduces the salt tolerance of Arabidopsis thaliana.
AtRPK1 (AT1G69270) is a leucine-rich repeat receptor-like protein kinase (LRR-RLK) gene in Arabidopsis thaliana. The rice gene O

BOARD NUMBER: S07g0602700 (OsRPK1) is the homolog of AtRPK1. AtRPK1 and OsRPK1 were overexpressed and the expression of AtRPK1 was inhibited by RNAi in A. thaliana. The functional results showed that the degrees of salt tolerance of the 35S:RPK1 A. thaliana plants were significantly lower than that of the control plants. The AtRPK1-RNAi A. thaliana plants exhibited higher salt tolerance than the wild-type plants (Col). The subcellular localisation results showed that the RPK1 proteins were mainly distributed on the cell membrane and that the overexpressed AtRPK1 proteins exhibited a significantly clustered distribution. The physiological analyses revealed that the overexpression of the RPK1 genes increased the membrane permeability in the transgenic A. thaliana plants. In response to salt stress, these plants exhibited an increased Na(+) flux into the cell, which caused greater damage to the cell. The real-time quantitative PCR analysis showed that the expression of the P5CS1 gene was inhibited and the SOS signalling pathway was blocked in the 35S:AtRPK1 A. thaliana plants. These effects at least partially contribute to the salt-sensitive phenotype of the 35S:RPK1 plants.
Plant Sci, 2014; 217-218

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SHORT- AND LONG-DISTANCE EXTRINSIC REGULATION OF INTERNEURON SPECIFICATION AND MIGRATION

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Imbalance between excitatory and inhibitory neurons in the human brain might lead to neurodevelopmental and neuropsychiatric disorders including cortical malformations epilepsy (EP), and autism spectrum disorders (ASD). Here, we propose that the extracellular environment regulates interneuron (IN) differentiation and migration during development, ultimately affecting the excitatory/inhibitory (E/I) balance. Using dorso-ventral cerebral assembloids (dvCAs) and ventral cerebral organoids (vCOs) with mutations in the extracellular matrix gene LGALS3BP, we show that composition of the extracellular medium regulates the migration and molecular differentiation of INs. To investigate how the extracellular environment affects neuronal specification and migration, we characterized the protein content of extracellular vesicles (EVs) secreted from COs carrying a mutation in *LGALS3BP* identified in individual with cortical malformation and neuropsychiatric disorders. These results revealed differences in protein composition. Interestingly, alteration of proteins associated with neuronal migration and ECM composition were altered in mutant EVs contained, suggesting an extrinsic role of LGALS3BP in these processes. Our results indicate that IN migration and molecular differentiation are regulated by short- and long-distance factors released into the extracellular environment.

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CELL-TYPE AND BRAIN-REGION-SPECIFIC EXPRESSION OF PLPPRS AS A MOLECULAR CODE FOR DEVELOPMENTAL NEURON MORPHOGENESIS IN THE CNS

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Phospholipid-phosphatase-related proteins (PLPPRs) are a family of neuron-enriched, developmentally expressed membrane proteins that control glutamatergic synapses, filopodia and branch formation, and growth cone navigation. PLPPRs may form heteromeric complexes suggesting diversified effects on bioactive lipid and small GTPase signaling. Studies have focused on hippocampus and cortex early postnatal development, but PLPPRs expression in subcortical brain tissues during development and adulthood is far from known. Furthermore, there are no studies on co-expression of PLPPRs or their neuron-type expression patterns. Aim: To explore which brain regions and cell types express PLPPRs during development and adulthood. Methods: We used qPCR for quantifying PLPPR mRNA in 5 tissues and 5 developmental stages and developed a custom computational screening tool to mine 4 publicly available mouse brain single-cell RNA-sequencing datasets. Results: Our qPCR analyses suggest ensuing expression of PLPPRs in subcortical brain areas, particularly in structures of the limbic system. Single neuron analysis suggests high PLPPR co-expression in specific adult GABAergic interneuron as well as in cortical and hippocampal glutamatergic subtypes. Conclusions: PLPPRs are expressed at high levels in the adult limbic system, while GABAergic neurons show the highest degree of PLPPR co-expression. This points to a possible regulatory role of PLPPR heteromeric complexes in GABAergic neuron morphogenesis and function. Lastly, our computational screening approach for single cell sequencing datasets provides a tool to collect information about any gene and neuron type of interest. Co-financed by Greece and the European Union-European Regional Development Fund (ERDF); Operational Program “Competitiveness, Entrepreneurship, Innovation” (EPAnEK), NSRF2014-2020(MIS 5047236)

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BOARD NUMBER: S05-287

ASTROCYTE-DERIVED HMGB1 REGULATES GLIOVASCULAR MATURATION IN THE POSTNATAL MOUSE BRAIN

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Moises Freitas-Andrade[1], Cesar Comin[2], Peter Van Dyken[3], Micaël Carrier[4], Jingjing Gong[5], Joanna Raman-Nair[3], Karan Thakur[3], Marie-Ève Tremblay[4], Natalina Salmaso[6], Luciano Da F. Costa[7], Baptiste Lacoste[8]
[1]The Ottawa Hospital Research Institute, Neuroscience Program, Ottawa, Canada, [2]Federal University of São Carlos, Department Of Computer Science, São Carlos, Brazil, [3]Ottawa University, Cellular & Molecular Medicine, Ottawa, Canada, [4]University of Victoria, Division Of Medical Sciences, Victoria, Canada, [5]NanoString Technologies, Inc., Research And Development, Seattle,, United States of America, [6]Carleton University, Department Of Neuroscience, Ottawa, Canada, [7]University of São Paulo, São Carlos Institute Of Physics, Fcm-usp, São Paulo, Brazil, [8]University of Ottawa/ Ottawa Hospital Research Institute/ University of Ottawa Brain and Mind Research Institute, Cellular And Molecular Medicine/ Neuroscience, Ottawa, Canada

Astrocytes are intimately linked with the brain vasculature, through structural and functional relationships that are critical for neuronal health. However, key molecular factors in astrocytes driving these physical and functional associations during development have not yet been identified. Here, we leveraged pan-astrocytic gene *Aldh1l1* as a molecular handle to unmask gliovascular dynamics in the postnatal mouse brain. Using mice with Green Fluorescent Protein expressed under the control of the *Aldh1l1* promoter, we found by immunofluorescence and electron microscopy that astrocytes undergo extensive maturation from postnatal day 5 (P5) and interact with blood vessels more closely from P7 (mostly evident at P14) via their endfeet. Astrocytic endfeet undergo refinement between P7 and P14, after which they exhibit a mature morphology, similar to those found at P21. To identify astrocyte genes critical for gliovascular development during postnatal brain growth, we utilised multiplex spatial RNA sequencing in the cerebral cortex from P0, P5 and P14 mice. We found that high-mobility group box 1 (*Hmgb1*), normally involved in vascular repair in the adult brain, was highly expressed in astrocytes at birth and decreased rapidly. Astrocyte-selective ablation of *Hmgb1* in newborn mice affected astrocytic endfoot placement and disturbed gap junction protein Connexin43 in the long-term. Early removal of astroglial *Hmgb1* also led to transcriptional changes mostly in microvessels and altered endothelial cell ultrastructure. While lack of astroglial *Hmgb1* did not affect vascular permeability or angiogenesis postnatally, it impaired neurovascular coupling in adults. These findings reveal a novel mechanism regulating gliovascular maturation during postnatal development.

Pubmed:

35017640: Bordeleau M, Comin CH, Fernández de Cossío L, Lacabanne C, Freitas-Andrade M, González Ibáñez F, Raman-Nair J, Wakem M, Chakravarty M, Costa LDF, Lacoste B, Tremblay MÈ
Maternal high-fat diet in mice induces cerebrovascular, microglial and long-term behavioural alterations in offspring.
Various environmental exposures during pregnancy, like maternal diet, can compromise, at critical periods of development, the neurovascular maturation of the offspring. Foetal exposure to maternal high-fat diet (mHFD), common to Western societies, has been shown to disturb neurovascular development in neonates and long-term permeability of the neurovasculature. Nevertheless, the effects of mHFD on the offspring's cerebrovascular health remains largely elusive. Here, we sought to address this knowledge gap by using a translational mouse model of mHFD exposure. Three-dimensional and ultrastructure analysis of the neurovascular unit (vasculature and parenchymal cells) in mHFD-exposed offspring revealed major alterations of the neurovascular organization and metabolism. These alterations were accompanied by changes in the expression of genes involved in metabolism and immunity, indicating that neurovascular changes may result from abnormal brain metabolism and immune regulation. In addition, mHFD-exposed offspring showed persisting behavioural alterations reminiscent of neurodevelopmental disorders, specifically an increase in stereotyped and repetitive behaviours into adulthood.
Commun Biol, 2022; 5
35108542: Rurak GM, Simard S, Freitas-Andrade M, Lacoste B, Charih F, Van Geel A, Stead J, Woodside B, Green JR, Coppola G, Salmaso N
Sex differences in developmental patterns of neocortical astroglia: A mouse translatome database.
Astroglial cells are key players in the development and maintenance of neurons and neuronal networks. Astroglia express

steroid hormone receptors and show rapid responses to hormonal manipulations. However, despite important sex differences in the cortex and hippocampus, few studies have examined sex differences in astroglial cells in telencephalic development. To characterize the cortical astroglial translatome in male and female mice across postnatal development, we use translating ribosome affinity purification together with RNA sequencing and immunohistochemistry to phenotype astroglia at six developmental time points. Overall, we find two distinct astroglial phenotypes between early (P1-P7) and late development (P14-adult), independent of sex. We also find sex differences in gene expression patterns across development that peak at P7 and appear to result from males reaching a mature astroglial phenotype earlier than females. These developmental sex differences could have an impact on the construction of neuronal networks and windows of vulnerability to perturbations and disease.
Cell Rep, 2022; 38
32848875: Freitas-Andrade M, Raman-Nair J, Lacoste B
Structural and Functional Remodeling of the Brain Vasculature Following Stroke.
Maintenance of cerebral blood vessel integrity and regulation of cerebral blood flow ensure proper brain function. The adult human brain represents only a small portion of the body mass, yet about a quarter of the cardiac output is dedicated to energy consumption by brain cells at rest. Due to a low capacity to store energy, brain health is heavily reliant on a steady supply of oxygen and nutrients from the bloodstream, and is thus particularly vulnerable to stroke. Stroke is a leading cause of disability and mortality worldwide. By transiently or permanently limiting tissue perfusion, stroke alters vascular integrity and function, compromising brain homeostasis and leading to widespread consequences from early-onset motor deficits to long-term cognitive decline. While numerous lines of investigation have been undertaken to develop new pharmacological therapies for stroke, only few advances have been made and most clinical trials have failed. Overall, our understanding of the acute and chronic vascular responses to stroke is insufficient, yet a better comprehension of cerebrovascular remodeling following stroke is an essential prerequisite for developing novel therapeutic options. In this review, we present a comprehensive update on post-stroke cerebrovascular remodeling, an important and growing field in neuroscience, by discussing cellular and molecular mechanisms involved, sex differences, limitations of preclinical research design and future directions.
Front Physiol, 2020; 11
32661394: Ouellette J, Toussay X, Comin CH, Costa LDF, Ho M, Lacalle-Aurioles M, Freitas-Andrade M, Liu QY, Leclerc S, Pan Y, Liu Z, Thibodeau JF, Yin M, Carrier M, Morse CJ, Dyken PV, Bergin CJ, Baillet S, Kennedy CR, Tremblay MÈ, Benoit YD, Stanford WL, Burger D, Stewart DJ, Lacoste B
Vascular contributions to 16p11.2 deletion autism syndrome modeled in mice.
While the neuronal underpinnings of autism spectrum disorder (ASD) are being unraveled, vascular contributions to ASD remain elusive. Here, we investigated postnatal cerebrovascular development in the 16p11.2 mouse model of 16p11.2 deletion ASD syndrome. We discover that 16p11.2 hemizygosity leads to male-specific, endothelium-dependent structural and functional neurovascular abnormalities. In 16p11.2 mice, endothelial dysfunction results in impaired cerebral angiogenesis at postnatal day 14, and in altered neurovascular coupling and cerebrovascular reactivity at postnatal day 50. Moreover, we show that there is defective angiogenesis in primary 16p11.2 mouse brain endothelial cells and in induced-pluripotent-stem-cell-derived endothelial cells from human carriers of the 16p11.2 deletion. Finally, we find that mice with an endothelium-specific 16p11.2 deletion (16p11.2) partially recapitulate some of the behavioral changes seen in 16p11.2 syndrome, specifically hyperactivity and impaired motor learning. By showing that developmental 16p11.2 haploinsufficiency from endothelial cells results in neurovascular and behavioral changes in adults, our results point to a potential role for endothelial impairment in ASD.
Nat Neurosci, 2020; 23
32110860: Freitas-Andrade M, Bechberger J, Wang J, Yeung KKC, Whitehead SN, Hansen RS, Naus CC
Danegaptide Enhances Astrocyte Gap Junctional Coupling and Reduces Ischemic Reperfusion Brain Injury in Mice.
Ischemic stroke is a complex and devastating event characterized by cell death resulting from a transient or permanent arterial occlusion. Astrocytic connexin43 (Cx43) gap junction (GJ) proteins have been reported to impact neuronal survival in ischemic conditions. Consequently, Cx43 could be a potential target for therapeutic approaches to stroke. We examined the effect of danegaptide (ZP1609), an antiarrhythmic dipeptide that specifically enhances GJ conductance, in two different rodent stroke models. In this study, danegaptide increased astrocytic Cx43 coupling with no significant effects on Cx43 hemichannel activity, in vitro. Using matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI IMS) the presence of danegaptide within brain tissue sections were detected one hour after reperfusion indicating successful transport of the dipeptide across the blood brain barrier. Furthermore, administration of danegaptide in a novel mouse brain ischemia/reperfusion model showed significant decrease in infarct volume. Taken together, this study provides evidence for the therapeutic potential of danegaptide in ischemia/reperfusion stroke.
Biomolecules, 2020; 10

30872361: Freitas-Andrade M, Wang N, Bechberger JF, De Bock M, Lampe PD, Leybaert L, Naus CC
Targeting MAPK phosphorylation of Connexin43 provides neuroprotection in stroke.
Connexin43 (Cx43) function is influenced by kinases that phosphorylate specific serine sites located near its C-terminus. Stroke is a powerful inducer of kinase activity, but its effect on Cx43 is unknown. We investigated the impact of wild-type (WT) and knock-in Cx43 with serine to alanine mutations at the protein kinase C (PKC) site Cx43, the casein kinase 1 (CK1) sites Cx43, and the mitogen-activated protein kinase (MAPK) sites Cx43 (MK4) on a permanent middle cerebral artery occlusion (pMCAO) stroke model. We demonstrate that MK4 transgenic animals exhibit a significant decrease in infarct volume that was associated with improvement in behavioral performance. An increase in astrocyte reactivity with a concomitant decrease in microglial reactivity was observed in MK4 mice. In contrast to WT, MK4 astrocytes displayed reduced Cx43 hemichannel activity. Pharmacological blockade of Cx43 hemichannels with TAT-Gap19 also significantly decreased infarct volume in WT animals. This study provides novel molecular insights and charts new avenues for therapeutic intervention associated with Cx43 function.
J Exp Med, 2019; 216
29575411: Wang JSH, Freitas-Andrade M, Bechberger JF, Naus CC, Yeung KK, Whitehead SN
Matrix-assisted laser desorption/ionization imaging mass spectrometry of intraperitoneally injected danegaptide (ZP1609) for treatment of stroke-reperfusion injury in mice.
This work focuses on direct matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) detection of intraperitoneally (IP)-injected dipeptide ZP1609 in mouse brain tissue. Direct analysis of drug detection in intact tissue sections provides distribution information that can impact drug development. MALDI-IMS capabilities of uncovering drug transport across the blood-brain barrier are demonstrated.
Rapid Commun Mass Spectrom, 2018; 32
29134540: Freitas-Andrade M, She J, Bechberger J, Naus CC, Sin WC
Acute connexin43 temporal and spatial expression in response to ischemic stroke.
Connexin43 (Cx43) gap junctions expressed in astrocytes can significantly impact neuronal survival in stroke. However, little is known regarding Cx43 spatial and temporal expression during the initial stages of brain ischemia. Using immunohistochemistry and Western blot analysis, we examined Cx43 spatial and temporal expression as a function of neuronal injury within the first 24 h after permanent middle cerebral artery occlusion (pMCAO). Western blot analysis showed a significant increase in Cx43 protein expression in the core ischemic area at 2 and 3 h after pMCAO. However, after 6 h of pMCAO Cx43 levels were significantly reduced. This reduction was due to cell death and concomitant Cx43 degradation in the expanding focal ischemic region, while the peri-infarct zone revealed intense Cx43 staining. The neuronal cell-death marker Fluoro-Jade C labeled injured neurons faintly at 1 h post-pMCAO with a time-dependent increase in both intensity and size of punctate staining. In addition, decreased microtubule-associated protein 2 (MAP2) immunoreactivity and thionin staining similarly indicated cell damage beginning at 1 h after pMCAO. Taken together, Cx43 expression is sensitive to neuronal injury and can be detected as early as 2 h post-pMCAO. These findings underscore Cx43 gap junction as a potential early target for therapeutic intervention in ischemic stroke.
J Cell Commun Signal, 2018; 12
28445139: Freitas-Andrade M, Bechberger JF, MacVicar BA, Viau V, Naus CC
Pannexin1 knockout and blockade reduces ischemic stroke injury in female, but not in male mice.
The membrane channel Pannexin 1 (Panx1) mediates apoptotic and inflammatory signaling cascades in injured neurons, responses previously shown to be sexually dimorphic under ischemic conditions. We tested the hypothesis that Panx1 plays an underlying role in mediating sex differences in stroke outcome responses. Middle-aged, 8-9 month old male and female wild type and Panx1 KO mice were subjected to permanent middle cerebral artery (MCA) occlusion, and infarct size and astrocyte and microglia activation were assessed 4 days later. The sexually dimorphic nature of Panx1 deletion was also explored by testing the effect of probenecid a known Panx1 blocker to alter stroke volume. Panx1 KO females displayed significantly smaller infarct volumes (~ 50 % reduction) compared to their wild-type counterparts, whereas no such KO effect occurred in males. This sex-specific effect of Panx1 KO was recapitulated by significant reductions in peri-infarct inflammation and astrocyte reactivity, as well as smaller infarct volumes in probenecid treated females, but not males. Finally, females showed overall, higher Panx1 protein levels than males under ischemic conditions. These findings unmask a deleterious role for Panx1 in response to permanent MCA occlusion, that is unique to females, and provide several new frameworks for understanding sex differences in stroke outcome.
Oncotarget, 2017; 8
28124625: Belousov AB, Fontes JD, Freitas-Andrade M, Naus CC
Gap junctions and hemichannels: communicating cell death in neurodevelopment and disease.
Gap junctions are unique membrane channels that play a significant role in intercellular communication in the developing and mature central nervous system (CNS). These channels are composed of connexin proteins that oligomerize into hexamers to

form connexons or hemichannels. Many different connexins are expressed in the CNS, with some specificity with regard to the cell types in which distinct connexins are found, as well as the timepoints when they are expressed in the developing and mature CNS. Both the main neuronal Cx36 and glial Cx43 play critical roles in neurodevelopment. These connexins also mediate distinct aspects of the CNS response to pathological conditions. An imbalance in the expression, translation, trafficking and turnover of connexins, as well as mutations of connexins, can impact their function in the context of cell death in neurodevelopment and disease. With the ever-increasing understanding of connexins in the brain, therapeutic strategies could be developed to target these membrane channels in various neurological disorders.
BMC Cell Biol, 2017; 18

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BOARD NUMBER: S05-288

REASSESSING THE CONTRIBUTIONS OF CAJAL-RETZIUS CELLS TO CORTICAL DEVELOPMENT

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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In mammals, the cerebral cortex is the region of the brain in charge of motor, sensory and cognitive processing. The correct assembly of cortical architecture during embryogenesis is essential for its normal function and a key player in this process are Cajal-Retzius cells (CR), which are among the first-born pallial neurons. CR migrate tangentially over long distances in the embryonic telencephalon to cover its entire surface and disappear soon after birth. Historically, they have been mostly associated with controlling the radial migration of neocortical glutamatergic neurons and the layering pattern of the cortex. However, more recent work has shown that they also behave as "mobile signalling units, participating in other crucial aspects of cortical development such as patterning and size of functional areas or the establishment of cortical networks. Despite their importance, there is a limited availability of models to study the specific CR contributions to brain development. By using scRNAseq and mouse genetics we have recently developed a novel mouse model largely devoid of CR from early stages of embryogenesis. Our results further point to the critical function that these cells play in correctly shaping the developing brain.

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DECIPHERING HOX TIMER MECHANISMS FOR HUMAN NEURONAL SUBTYPE ENGINEERING.

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Remi Robert[1], Célia Vaslin[1], Vincent Mouilleau[1], Jacques Van Helden[2], Stephane Nedelec[1]
[1]Institut du Fer à Moulin, Neurodevelopment, Paris, France, [2]Institut Francais de Bioinformatique, Bioinformatics, Evry, France

HOX genes encode a large family of transcription factors that orchestrate neural diversification along the hindbrain and spinal cord. Their timely sequential activation during embryogenesis is essential to establish their stereotypical expression profiles and the formation of distinct locomotor circuits. Yet, the timer mechanisms that set the tempo of their activation remain unclear. We recently derived axial progenitors from human pluripotent stem cells in which *HOX* sequential activation is recapitulated. Using this model we demonstrated that the levels of FGF2 and GDF11, two extrinsic factors, are pacing *HOX* temporal induction leading to the efficient specification of distinct motor neuron subtypes found along the human embryonic spinal cord. Transcriptomics and pharmacological analysis indicated that the two cues activate distinct signaling pathways to rapidly and directly induce *HOX* genes together with transcription factors and signal transduction regulators. We are now investigating the role of these targets in the temporal dynamic of *HOX* sequential activation. We should be able to provide a breakthrough in *HOX* timer mechanisms and their links to the specification of neuronal diversity in the human spinal cord. Beside its fundamental biological relevance, considering the pleiotropic role of *HOX* genes decoding these mechanisms will favor *in vitro* human cell and tissue engineering within and beyond the nervous system.

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PROSOMERIC HYPOTHALAMIC DISTRIBUTION OF TYROSINE HYDROXYLASE POSITIVE CELLS IN ADOLESCENT RATS.

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

María Bilbao[1], Daniel Garrigos[2], Marta Martínez-Morga[2], José Ángel Toval[3], Yevheniy Kutsenko[2], Rosario Bautista[2], Alberto Barreda[2], Bruno Ribeiro Do Couto[2], Luis Puelles[2], José Luis Ferrán[2]
[1]Consejo Nacional de Investigaciones Científicas y técnicas (CONICET), Argentina., Facultad De Ciencias Veterinarias. Universidad Nacional De La Pampa, Argentina, Gral Pico, Argentina, [2]University of Murcia, Human Anatomy And Psychobiology, Murcia, Spain, [3]University of Granada, Physical And Sports Education, Granada, Spain

Most of the studies on neurochemical mapping, connectivity and physiology in the hypothalamic region were carried out in rats and under the columnar morphologic paradigm. According to the columnar model, the entire hypothalamic region lies ventrally within the diencephalon, and includes preoptic, anterior, tuberal and mamillary anteroposterior regions. This model is weak in providing little or no experimentally corroborated causal explanation of such subdivisions. In contrast, the modern prosomeric model uses different axial assumptions based on the parallel courses of the brain floor, alar-basal boundary and brain roof (all causally explained), and postulates that the hypothalamus and telencephalon jointly form the secondary prosencephalon. The hypothalamus is divided into two neuromeric (transverse) parts called peduncular and terminal hypothalamus. The classic AP divisions of the columnar hypothalamus are rather seen as dorsoventral subdivisions of the hypothalamic alar and basal plates. We offer here a prosomeric immunohistochemical mapping in the rat of hypothalamic cells expressing tyrosine hydroxylase (TH). TH-positive cells are abundant within the periventricular stratum of the paraventricular and subparaventricular alar domains. In the tuberal region, most labelled cells are found in the acroterminal arcuate nucleus and in the terminal periventricular stratum. The dorsal retrotuberal region contains the A13 cell group of TH-positive cells. In addition, some TH cells appear in the perimamillary and retromamillary regions. The prosomeric model proved useful for determining the precise location of TH-positive cells relative to possible origins of morphogenetic signals, thus aiding potential causal explanation of position-related specification of this hypothalamic cell type.

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NAVIGATING THE SPATIO-TEMPORAL PROGRAMS OF APICAL PROGENITORS ACROSS MOUSE EMBRYONIC DEVELOPMENT

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Along the developing neural tube, progenitor cells of the ventricular zone give rise to neurons populating the brain. The spatial location and temporal progression of these progenitors are critical determinants of the neuronal identities of their progenies, yet little is known about molecular patterning in progenitors. To address this question, we used single-cell RNA sequencing at several embryonic ages together with in silico spatial mapping of neuronal progenitors along the neural tube. We find that progenitors share a program encoding temporal progression regardless of their position and a common spatial program regardless of their age. Together, these results provide an integrative view of dynamic transcriptional programs in central nervous system progenitors.

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BOARD NUMBER: S05-292

PROSOMERIC CHARACTERIZATION OF CHICKEN TH POSITIVE CELLS DURING HYPOTHALAMIC DEVELOPMENT

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Marina Womack Reina[1], Paula Womack[1], Sylvia Bardet[2], Antonia Alonso[1], Ramón Pla[1], Daniel Garrigos[1], Yevheniy Kutsenko[1], Alberto Barreda[1], Bruno Ribeiro Do Couto[1], Luis Puelles[1], José Luis Ferrán[1]
[1]University of Murcia, Human Anatomy And Psychobiology, Murcia, Spain, [2]University of Limoges, Département Génie Biologique - Limoges (iut), Limoges, France

The chicken secondary prosencephalon presents the telencephalon dorsally and the hypothalamus ventrally. The hypothalamic region is identified by two neuromeric parts defined in terms of peduncular and terminal hypothalamus, caudal and rostral respectively. Dorsoventrally, the hypothalamic region is characterized by the paraventricular and subparaventricular alar plate domains, and tuberal/retrotuberal, perimammillary/retroperimammillary and mammillary/retromammillary basal plate domains. Our aim was to determine the hypothalamic distribution of tyrosine hydroxylase (TH) during chicken hypothalamic development following prosomeric references. We performed prosomeric immunohistochemical mapping of TH from chicken developmental stages HH28 to HH46. This mapping was also combined with markers for various hypothalamic nuclei (*Avp*, *Mch*, *Oxt*, *Pomc*, *Sst*, and *Trh*). In stage HH28 TH-positive cells are observed in the mammillary, perimammillary, retromammillary and periretromammillary regions, but also in the caudal part of the retrotuberal domain (precursor of the A13 cell group?). Alar plate TH positive cells are observed as early as stage HH34 in the terminal and peduncular paraventricular alar plate domain. In addition, at this stage, TH-positive cells that are candidates for forming part of the anterobasal nucleus are observed. The prosomeric model provides the clues to pinpoint the location of TH-positive cells during chicken developmental stages, a descriptive framework that became suitable for accurate comparisons with mammals.

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PROSOMERIC PRENATAL HYPOTHALAMIC DISTRIBUTION OF TH-POSITIVE CELLS IN RODENTS

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Paula Womack, Marina Womack Reina, Ramón Pla, Saurabh Gagneja, Daniel Garrigos, Marta Martínez-Morga, Yevheniy Kutsenko, Alberto Barreda, Antonia Alonso, Bruno Ribeiro Do Couto, Luis Puelles, José Luis Ferrán
University of Murcia, Human Anatomy And Psychobiology, Murcia, Spain

According to the prosomeric model, the hypothalamus is located in the ventral alar and basal plate domains of the secondary prosencephalon, rostral to the diencephalon proper. The hypothalamic region is identified by two neuromeric (transverse) parts called peduncular and terminal hypothalamus. Our recent prosomeric mapping during adolescence of rats identifies abundant TH-positive cells within the periventricular stratum of the paraventricular and subparaventricular alar domains. In addition, TH cells were observed in the periventricular stratum of the tuberal region, arcuate nucleus and A13 cell group. In order to identify early prenatal appearance of TH-positive cells, we performed a prosomeric immunohistochemical mapping of tyrosine hydroxylase (TH), from E12.5 to E21.5 developmental stages of the rat. This mapping was also combined with markers for diverse hypothalamic nuclei (*Avp*, *Mch*, *Oxt*, *Pomc*, *Sst*, and *Trh*). At E13.5, most TH positive cells are observed in the acroterminal alar plate domain with increasing numbers in the dorsal tuberal domain, with few of the A13 cell group precursors. At E15.5, *Mch* identifies the peduncular basal plate and the A13 cell group precursors in the same domain. Beginning at E17.5, most TH-positive cells are distributed in the same domains characterized during the adolescence in the rat. The prosomeric model helps to determine the location of prenatal TH-positive cells more precisely than in previous studies. We have an initial framework to begin work on characterizing the origin of each TH neuron in the hypothalamic region.

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BOARD NUMBER: S05-294

A CELL-AUTONOMOUS ROLE FOR PRIMARY CILIA IN LONG-RANGE COMMISSURAL AXON GUIDANCE

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Ciliopathies are characterized by the absence or dysfunction of primary cilia. Patients exhibit a broad spectrum of symptoms, including kidney and liver problems, limb malformations, and very often cognitive impairments. Despite the fact that cognitive impairments are common, how cilia dysfunction affects neuronal development has not been characterized in detail. Here, we show that the primary cilium is required cell-autonomously by neurons during neural circuit formation. In particular, the primary cilium plays a crucial role during axonal pathfinding for the switch in responsiveness of axons at a choice point, or intermediate target. Utilizing animal models and in vivo, ex vivo as well as in vitro experiments, we provide evidences of a critical role played by the primary cilium at the soma level of commissural neurons in transducing long-range guidance signals sensed by axons navigating an intermediate target. In extension of our finding that Shh is required for the rostral turn of post-crossing commissural axons, we show here that the cilium is required for a change in the transcription of axon guidance receptors, which in turn mediate the repulsive response to floorplate-derived Shh shown by post-crossing commissural axons.

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SINGLE-CELL RNA SEQUENCING IN MOUSE REVEALS THAT SCHWANN CELL PRECURSORS REPRESENT A NEURAL CREST-LIKE HUB STATE WITH BIASED MULTIPOTENCY

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Louis Faure[1], Maria Eleni Kastriti[1], François Lallemend[2], Saida Hadjab[2], Igor Adameyko[1]
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For decades, Schwann cell precursors (SCPs) were considered embryonic nerve-associated progenitors generating myelinating and non-myelinating Schwann cells. It recently became evident that SCPs are multipotent like their mother population of the neural crest. SCPs are omnipresent in the body of vertebrate embryos accompanying the peripheral outgrowing innervation. Here we took advantage of single-cell transcriptomics to resolve SCP identity and to answer if SCPs represent the nerve-associated neural crest. To define the transcriptional states of the Schwann cell lineage, we relied on the concept of regulons, integrated transcriptional activation and trajectory analysis. Our results revealed that early SCPs and late migratory crest represent a multipotent “hub” state containing cells biased towards traditional neural crest fates. More advanced SCPs diverge from the crest after being primed towards the terminal Schwann cell fate. Furthermore, we revealed the subtypes of SCPs residing in distinct anatomical locations and identified new paths towards myelination and endoneurial fibroblasts.

Pubmed:
33833454: Kameneva P, Artemov AV, Kastriti ME, Faure L, Olsen TK, Otte J, Erickson A, Semsch B, Andersson ER, Ratz M, Frisén J, Tischler AS, de Krijger RR, Bouderlique T, Akkuratova N, Vorontsova M, Gusev O, Fried K, Sundström E, Mei S, Kogner P, Baryawno N, Kharchenko PV, Adameyko I
Single-cell transcriptomics of human embryos identifies multiple sympathoblast lineages with potential implications for neuroblastoma origin.
Characterization of the progression of cellular states during human embryogenesis can provide insights into the origin of pediatric diseases. We examined the transcriptional states of neural crest- and mesoderm-derived lineages differentiating into adrenal glands, kidneys, endothelium and hematopoietic tissue between post-conception weeks 6 and 14 of human development. Our results reveal transitions connecting the intermediate mesoderm and progenitors of organ primordia, the hematopoietic system and endothelial subtypes. Unexpectedly, by using a combination of single-cell transcriptomics and lineage tracing, we found that intra-adrenal sympathoblasts at that stage are directly derived from nerve-associated Schwann cell precursors, similarly to local chromaffin cells, whereas the majority of extra-adrenal sympathoblasts arise from the migratory neural crest. In humans, this process persists during several weeks of development within the large intra-adrenal ganglia-like structures, which may also serve as reservoirs of originating cells in neuroblastoma.
Nat Genet, 2021; 53
33038290: Zhao J, Faure L, Adameyko I, Sharpe PT
Stem cell contributions to cementoblast differentiation in healthy periodontal ligament and periodontitis.
Loss of tissue attachment as a consequence of bacterial infection and inflammation represents the main therapeutic target for the treatment of periodontitis. Cementoblasts, the cells that produce the mineralized tissue, cementum, that is responsible for connecting the soft periodontal tissue to the tooth, are a key cell type for maintaining/restoring tissue attachment following disease. Here, we identify two distinct stem cell populations that contribute to cementoblast differentiation at different times. During postnatal development, cementoblasts are formed from perivascular-derived cells expressing CD90 and perivascular-associated cells that express Axin2. During adult homeostasis, only Wnt-responsive Axin2+ cells form cementoblasts but following experimental induction of periodontal disease, CD90+ cells become the main source of cementoblasts. We thus show that different populations of resident stem cells are mobilized at different times and during disease to generate precursors for cementoblast differentiation and thus provide an insight into the targeting cells resident cells for novel therapeutic approaches. The differentiation of these stem cells into cementoblasts is however inhibited by bacterial products such as lipopolysaccharides, emphasizing that regeneration of periodontal ligament soft tissue and restoration of attachment will require a multipronged approach.

Stem Cells, 2021; 39
32647059: Klimovich A, Giacomello S, Björklund Å, Faure L, Kaucka M, Giez C, Murillo-Rincon AP, Matt AS, Willoweit-Ohl D, Crupi G, de Anda J, Wong GCL, D'Amato M, Adameyko I, Bosch TCG
Prototypical pacemaker neurons interact with the resident microbiota.
Pacemaker neurons exert control over neuronal circuit function by their intrinsic ability to generate rhythmic bursts of action potential. Recent work has identified rhythmic gut contractions in human, mice, and hydra to be dependent on both neurons and the resident microbiota. However, little is known about the evolutionary origin of these neurons and their interaction with microbes. In this study, we identified and functionally characterized prototypical ANO/SCN/TRPM ion channel-expressing pacemaker cells in the basal metazoan by using a combination of single-cell transcriptomics, immunochemistry, and functional experiments. Unexpectedly, these prototypical pacemaker neurons express a rich set of immune-related genes mediating their interaction with the microbial environment. Furthermore, functional experiments gave a strong support to a model of the evolutionary emergence of pacemaker cells as neurons using components of innate immunity to interact with the microbial environment and ion channels to generate rhythmic contractions.
Proc Natl Acad Sci U S A, 2020; 117
32826903: Faure L, Wang Y, Kastriti ME, Fontanet P, Cheung KKY, Petitpré C, Wu H, Sun LL, Runge K, Croci L, Landy MA, Lai HC, Consalez GG, de Chevigny A, Lallemend F, Adameyko I, Hadjab S
Single cell RNA sequencing identifies early diversity of sensory neurons forming via bi-potential intermediates.
Somatic sensation is defined by the existence of a diversity of primary sensory neurons with unique biological features and response profiles to external and internal stimuli. However, there is no coherent picture about how this diversity of cell states is transcriptionally generated. Here, we use deep single cell analysis to resolve fate splits and molecular biasing processes during sensory neurogenesis in mice. Our results identify a complex series of successive and specific transcriptional changes in post-mitotic neurons that delineate hierarchical regulatory states leading to the generation of the main sensory neuron classes. In addition, our analysis identifies previously undetected early gene modules expressed long before fate determination although being clearly associated with defined sensory subtypes. Overall, the early diversity of sensory neurons is generated through successive bi-potential intermediates in which synchronization of relevant gene modules and concurrent repression of competing fate programs precede cell fate stabilization and final commitment.
Nat Commun, 2020; 11
34612203: Colin A, Micali G, Faure L, Cosentino Lagomarsino M, van Teeffelen S
Two different cell-cycle processes determine the timing of cell division in .
Cells must control the cell cycle to ensure that key processes are brought to completion. In , it is controversial whether cell division is tied to chromosome replication or to a replication-independent inter-division process. A recent model suggests instead that processes may limit cell division with comparable odds in single cells. Here, we tested this possibility experimentally by monitoring single-cell division and replication over multiple generations at slow growth. We then perturbed cell width, causing an increase of the time between replication termination and division. As a consequence, replication became decreasingly limiting for cell division, while correlations between birth and division and between subsequent replication-initiation events were maintained. Our experiments support the hypothesis that both chromosome replication and a replication-independent inter-division process can limit cell division: the two processes have balanced contributions in non-perturbed cells, while our width perturbations increase the odds of the replication-independent process being limiting.
Elife, 2021; 10
33286070: Albergante L, Mirkes E, Bac J, Chen H, Martin A, Faure L, Barillot E, Pinello L, Gorban A, Zinovyev A
Robust and Scalable Learning of Complex Intrinsic Dataset Geometry via ElPiGraph.
Multidimensional datapoint clouds representing large datasets are frequently characterized by non-trivial low-dimensional geometry and topology which can be recovered by unsupervised machine learning approaches, in particular, by principal graphs. Principal graphs approximate the multivariate data by a graph injected into the data space with some constraints imposed on the node mapping. Here we present ElPiGraph, a scalable and robust method for constructing principal graphs. ElPiGraph exploits and further develops the concept of elastic energy, the topological graph grammar approach, and a gradient descent-like optimization of the graph topology. The method is able to withstand high levels of noise and is capable of approximating data point clouds via principal graph ensembles. This strategy can be used to estimate the statistical significance of complex data features and to summarize them into a single consensus principal graph. ElPiGraph deals efficiently with large datasets in various fields such as biology, where it can be used for example with single-cell transcriptomic or epigenomic datasets to infer gene expression dynamics and recover differentiation landscapes.
Entropy (Basel), 2020; 22
29938224: Woo AC, Faure L, Dapa T, Matic I
Heterogeneity of spontaneous DNA replication errors in single isogenic cells.
Despite extensive knowledge of the molecular mechanisms that control mutagenesis, it is not known how spontaneous

mutations are produced in cells with fully operative mutation-prevention systems. By using a mutation assay that allows visualization of DNA replication errors and stress response transcriptional reporters, we examined populations of isogenic cells growing under optimal conditions without exogenous stress. We found that spontaneous DNA replication errors in proliferating cells arose more frequently in subpopulations experiencing endogenous stresses, such as problems with proteostasis, genome maintenance, and reactive oxidative species production. The presence of these subpopulations of phenotypic mutators is not expected to affect the average mutation frequency or to reduce the mean population fitness in a stable environment. However, these subpopulations can contribute to overall population adaptability in fluctuating environments by serving as a reservoir of increased genetic variability.
Sci Adv, 2018; 4

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BOARD NUMBER: S05-296

SPATIO-TEMPORAL DYNAMICS OF STOCHASTIC AXON TARGETING DURING CNS DEVELOPMENT

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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While adult brains present stereotypical wiring diagrams, developmental variation in axonal connectivity contributes to non-heritable behavioral individuality (Linneweber et al. 2020). The question of how and when these individualized wiring patterns emerge and become stable during development remains unexplored. Using long-term intravital live imaging of axonal growth and targeting of Dorsal Clustered Neurons/LC_{14} in the developing central nervous system of intact fruit flies, we show that axon selection is a multi-step process with variable outcomes. First, Notch-mediated lateral inhibition defines a subset of neurons with a potential to innervate distal targets. Live imaging and temporal optogenetic activation of Notch signaling reveal that while $Notch^{ON}$ axons stay near proximal targets, $Notch^{OFF}$ axons alter their growth cone morphology to create single-cell bundles of dynamic parallel fibers extending towards distal targets. Live tracking of microtubule dynamics identifies a second selection step, where a subset of $Notch^{OFF}$ axons randomly stabilize one fiber within their bundle structures. In contrast, $Notch^{OFF}$ axons that do not have microtubule stabilization in their bundles retract before reaching distal targets. We observed a similar axonal splitting/selection process in the developing chick spinal cord suggesting a conserved mechanism. Finally, microtubule developmental pattern predicts the adult wiring pattern of an individual and remain stable during adult life, supporting the idea that a temporal succession of stochastic processes explains the emergence of individual variation in robust wiring patterns.

FENS Forum 2022
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BOARD NUMBER: S05-297

ADHESION MOLECULE AMIGO2 IS INVOLVED IN THE FASCICULATION PROCESS OF THE FASCICULUS RETROFLEXUS.

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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In the habenular complex, the fasciculus retroflexus is the main efference. It is composed by a core, where the medial habenular axons are located, and a shell composed by lateral habenular axons. Both group of fibers share the same initial way and differ in the final segment of the fascicle. The different behavior of the axonal fascicles is regulated by surface molecules. Amigo2, adhesion molecule with immunoglobulin like domain 2, codify for a membrane protein selectively expressed in the medial habenula. We selected this molecule as a candidate to control the medial Habenula axons fasciculation behavior. First, we studied in an Amigo2 lack of function mouse model the development of the habenular efference. The fasciculus retroflexus appeared defasciculated with a variable phenotype. Then, gain of function experiments allowed us to generate not only a more condensed tract but to recover the Amigo2 knock-out phenotype. Amigo2 alterations did not produce any modification in the habenular fibers guidance process. We have demonstrated that Amigo2 plays a role in the fasciculation process of the fasciculus retroflexus. Funding: UMH (VIPROY21/13) to E. Puelles; MICINN/AEI/FEDER (PID2020-118171RB-I00), GVA (PROMETEO/2018/041), ISCIII (“RD16/001/0010”), co-funded by ERDF/ESF, “Investing in your future”, and FTPGB (FTPGB18/SM) to S. Martinez; MECD (FPU16/03853) to V. Company. The Institute of Neurosciences is a “Centre of Excellence Severo Ochoa (SEV-2017-0723)”.

Pubmed:
34722541: Company V, Moreno-Cerdá A, Andreu-Cervera A, Murcia-Ramón R, Almagro-García F, Echevarría D, Martínez S, Puelles E
Role in the Development of the Habenula and the Fasciculus Retroflexus.
is one of the morphogenes that controls the specification and differentiation of neuronal populations in the developing central nervous system. The habenula is a diencephalic neuronal complex located in the most dorsal aspect of the thalamic prosomere. This diencephalic neuronal population is involved in the limbic system and its malfunction is related with several psychiatric disorders. Our aim is to elucidate the role in the habenula and its main efferent tract, the fasciculus retroflexus, development. In order to achieve these objectives, we analyzed these structures development in a lack of function mouse model. The habenula was generated in our model, but it presented an enlarged volume. This alteration was due to an increment in habenular neuroblasts proliferation rate. The fasciculus retroflexus also presented a wider and disorganized distribution and a disturbed final trajectory toward its target. The mid-hindbrain territories that the tract must cross were miss-differentiated in our model. The specification of the habenula is independent. Nevertheless, it controls its precursors proliferation rate. expressed in the isthmic organizer is vital to induce the midbrain and rostral hindbrain territories. The alteration of these areas is responsible for the fasciculus retroflexus axons misroute.
Front Cell Dev Biol, 2021; 9
34169076: Company V, Andreu-Cervera A, Madrigal MP, Andrés B, Almagro-García F, Chédotal A, López-Bendito G, Martinez S, Echevarría D, Moreno-Bravo JA, Puelles E
Netrin 1-Mediated Role of the Substantia Nigra Pars Compacta and Ventral Tegmental Area in the Guidance of the Medial Habenular Axons.
The fasciculus retroflexus is an important fascicle that mediates reward-related behaviors and is associated with different psychiatric diseases. It is the main habenular efference and constitutes a link between forebrain regions, the midbrain, and the rostral hindbrain. The proper functional organization of habenular circuitry requires complex molecular programs to control the wiring of the habenula during development. However, the mechanisms guiding the habenular axons toward their targets remain mostly unknown. Here, we demonstrate the role of the mesodiencephalic dopaminergic neurons (substantia nigra pars compacta and ventral tegmental area) as an intermediate target for the correct medial habenular axons navigation along

the anteroposterior axis. These neuronal populations are distributed along the anteroposterior trajectory of these axons in the mesodiencephalic basal plate. Using and experiments, we determined that this navigation is the result of attraction generated by the mesodiencephalic dopaminergic neurons. This attraction is mediated by the receptor deleted in colorectal cancer (DCC), which is strongly expressed in the medial habenular axons. The increment in our knowledge on the fasciculus retroflexus trajectory guidance mechanisms opens the possibility of analyzing if its alteration in mental health patients could account for some of their symptoms.
Front Cell Dev Biol, 2021; 9
33145610: Murcia-Ramón R, Company V, Juárez-Leal I, Andreu-Cervera A, Almagro-García F, Martínez S, Echevarría D, Puelles E
Neuronal tangential migration from Nkx2.1-positive hypothalamus.
During the development of the central nervous system, the immature neurons suffer different migration processes. It is well known that Nkx2.1-positive ventricular layer give rise to critical tangential migrations into different regions of the developing forebrain. Our aim was to study this phenomenon in the hypothalamic region. With this purpose, we used a transgenic mouse line that expresses the tdTomato reporter driven by the promotor of Nkx2.1. Analysing the Nkx2.1-positive derivatives at E18.5, we found neural contributions to the prethalamic region, mainly in the zona incerta and in the mes-diencephalic tegmental region. We studied the developing hypothalamus along the embryonic period. From E10.5 we detected that the Nkx2.1 expression domain was narrower than the reporter distribution. Therefore, the Nkx2.1 expression fades in a great number of the early-born neurons from the Nkx2.1-positive territory. At the most caudal positive part, we detected a thin stream of positive neurons migrating caudally into the mes-diencephalic tegmental region using time-lapse experiments on open neural tube explants. Late in development, we found a second migratory stream into the prethalamic territory. All these tangentially migrated neurons developed a gabaergic phenotype. In summary, we have described the contribution of interneurons from the Nkx2.1-positive hypothalamic territory into two different rostrocaudal territories: the mes-diencephalic reticular formation through a caudal tangential migration and the prethalamic zona incerta complex through a dorsocaudal tangential migration.
Brain Struct Funct, 2020; 225
32581730: Ádám Á, Kemecsei R, Company V, Murcia-Ramón R, Juarez I, Gerecsei LI, Zachar G, Echevarría D, Puelles E, Martínez S, Csillag A
Gestational Exposure to Sodium Valproate Disrupts Fasciculation of the Mesotelencephalic Dopaminergic Tract, With a Selective Reduction of Dopaminergic Output From the Ventral Tegmental Area.
Gestational exposure to valproic acid (VPA) is known to cause behavioral deficits of sociability, matching similar alterations in human autism spectrum disorder (ASD). Available data are scarce on the neuromorphological changes in VPA-exposed animals. Here, we focused on alterations of the dopaminergic system, which is implicated in motivation and reward, with relevance to social cohesion. Whole brains from 7-day-old mice born to mothers given a single injection of VPA (400 mg/kg b.wt.) on E13.5 were immunostained against tyrosine hydroxylase (TH). They were scanned using the iDISCO method with a laser light-sheet microscope, and the reconstructed images were analyzed in 3D for quantitative morphometry. A marked reduction of mesotelencephalic (MT) axonal fascicles together with a widening of the MT tract were observed in VPA treated mice, while other major brain tracts appeared anatomically intact. We also found a reduction in the abundance of dopaminergic ventral tegmental (VTA) neurons, accompanied by diminished tissue level of DA in ventrobasal telencephalic regions (including the nucleus accumbens (NAc), olfactory tubercle, BST, substantia innominata). Such a reduction of DA was not observed in the non-limbic caudate-putamen. Conversely, the abundance of TH+ cells in the substantia nigra (SN) was increased, presumably due to a compensatory mechanism or to an altered distribution of TH+ neurons occupying the SN and the VTA. The findings suggest that defasciculation of the MT tract and neuronal loss in VTA, followed by diminished dopaminergic input to the ventrobasal telencephalon at a critical time point of embryonic development (E13-E14) may hinder the patterning of certain brain centers underlying decision making and sociability.
Front Neuroanat, 2020; 14
29663710: Company V, Moreno-Bravo JA, Perez-Balaguer A, Puelles E
The Amniote Oculomotor Complex.
The oculomotor (OM) complex is a combination of somatic and parasympatethic neurons. The correct development and wiring of this cranial pair is essential to perform basic functions: eyeball and eyelid movements, pupillary constriction, and lens accommodation. The improper formation or function of this nucleus leads pathologies such as strabismus. We describe the OM organization and function in different vertebrate brains, including chick, mouse, and human. The morphological localization is detailed, as well as the spatial relation with the trochlear nucleus in order to adjust some misleading anatomical topographic descriptions. We detailed the signaling processes needed for the specification of the OM neurons. The transcriptional programs driven the specification and differentiation of these neurons are partially determined. We summarized recent genetic studies that have led to the identification of guidance mechanisms involved in the migration, axon

pathfinding, and targeting of the OM neurons. Finally, we overviewed the pathology associated to genetic malformations in the OM development and related clinical alterations. Anat Rec, 302:446-451, 2019.
Anat Rec (Hoboken), 2019; 302

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INTERPLAY BETWEEN TRANSCRIPTIONAL REGULATION AND NEUROTROPHIC SIGNALING IN RETINAL GANGLION CELL TYPE SPECIFICATION

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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The interplay between transcriptional regulation and neurotrophic signaling in neuronal arbor formation is still poorly understood. In mice the transcription factor Brn3a/Pou4f1 is expressed in most RGCs, and is required for the specification of RGCs with small dendritic arbors. The GDNF receptor Ret is expressed in several RGC types, including some expressing Brn3a, but its role in RGC development is not defined. We used combinatorial genetics involving conditional knock-in reporter alleles at the Brn3a and Ret loci, in combination with retina- or Ret specific Cre drivers, to generate complete or mosaic ablations of either Brn3a or Ret in RGCs and study Brn3a and Ret gene dosage effects on RGC dendritic arbor morphology. We find that mosaic gene dosage manipulation of Brn3a in neurotrophic receptor Ret heterozygote RGCs results in altered cell fate decisions and/or morphological dendritic defects. Specific RGC types are lost if Brn3a is ablated during embryogenesis and only mildly affected by postnatal Brn3a ablation. Sparse but not complete Brn3a heterozygosity combined with complete Ret heterozygosity has striking effects on RGC type distribution, in particular on dendritic arbor morphology. While complete retinal ablation of Ret does not significantly affect Brn3a or Brn3b expression, Brn3a loss of function modestly but significantly affects distribution of Ret co-receptors GFRa1-3, and neurotrophin receptors TrkA and TrkC in RGCs. Based on these observations, we propose that Brn3a and Ret converge onto developmental pathways that control RGC type specification, potentially through a competitive mechanism requiring signaling from the surrounding tissue.

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BOARD NUMBER: S05-299

CELL LINEAGES AND PROGENITOR HETEROGENEITY IN THE CEREBRAL CORTEX

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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The brain contains an extensive catalog of distinct neuronal types. Such diversity emerges through cell specification processes in which progenitors proliferate while navigating a labyrinth of cell fate decisions. Cell lineage plays a pivotal role in neuronal specification, with sibling neurons exhibiting common traits. Decoding the relation between cell lineage and neuronal identity allows for the dissection of the exact progenitor cell and timing in which cell fate decisions occur. In the mouse cerebral cortex, the existence of fate-restricted progenitors remains a long-standing debate. Here, I will present evidence supporting these fate-restricted progenitors exist and may generate different neuronal classes.

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BOARD NUMBER: S05-300

SEMAPHORIN 3A REGULATES AXON GROWTH CONE ELONGATION DURING NEURONAL DIFFERENTIATION

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Introduction: During neuronal development axons navigate to their targets by sensing attractive and repulsive signals through receptors located on their growth cones. Semaphorins (Sema) are the largest family of repellent guidance cues considered essential in guiding axons during development. Class 3 Sema (Sema 3) are the only produced as secreted proteins in mammals, exerting both autocrine and paracrine functions. Interestingly, Sema 3A expression levels are increased in brains of patients with schizophrenia and polymorphisms in Sema 3A or in Sema 3A receptors, Neuropilin1 (Npn1) and Plexin As (PlxnA2), have been associated to neurodevelopmental disorders. **Aim:** We questioned how neural progenitors (NP) grow and differentiate when exposed to increased Sema 3A levels. **Methods:** To this aim, NP were transfected with Sema 3A or exposed to media from human Sema 3A overexpressing microglia. **Results:** Sema 3A overexpression causes axon growth cone retraction in NP. In addition, NP develop an aberrant proximal dendritic branching and die within a few hours after exposure. Axon retraction, disorganized dendritic arborization and neuronal death were prevented by the Sema 3A receptors silencing. Npn-1 or PlxnA2. **Conclusions** Sema3A regulates NP differentiation, as such any insult causing Sema 3A overexpression might affect axonal elongation and dendritic arborization. This will likely influence neuronal organization and connectivity and causes death. Future studies will be enquiring about how an increase in Sema3A signals might lead to neurodevelopmental disorders.

Pubmed:

32580508: Matrone C, Petrillo F, Nasso R, Ferretti G
Fyn Tyrosine Kinase as Harmonizing Factor in Neuronal Functions and Dysfunctions.
Fyn is a non-receptor or cytoplasmatic tyrosine kinase (TK) belonging to the Src family kinases (SFKs) involved in multiple transduction pathways in the central nervous system (CNS) including synaptic transmission, myelination, axon guidance, and oligodendrocyte formation. Almost one hundred years after the original description of Fyn, this protein continues to attract extreme interest because of its multiplicity of actions in the molecular signaling pathways underlying neurodevelopmental as well as neuropathologic events. This review highlights and summarizes the most relevant recent findings pertinent to the role that Fyn exerts in the brain, emphasizing aspects related to neurodevelopment and synaptic plasticity. Fyn is a common factor in healthy and diseased brains that targets different proteins and shapes different transduction signals according to the neurological conditions. We will primarily focus on Fyn-mediated signaling pathways involved in neuronal differentiation and plasticity that have been subjected to considerable attention lately, opening the fascinating scenario to target Fyn TK for the development of potential therapeutic interventions for the treatment of CNS injuries and certain neurodegenerative disorders like Alzheimer's disease.
Int J Mol Sci, 2020; 21
33477654: Brattico E, Bonetti L, Ferretti G, Vuust P, Matrone C
Putting Cells in Motion: Advantages of Endogenous Boosting of BDNF Production.
Motor exercise, such as sport or musical activities, helps with a plethora of diseases by modulating brain functions in neocortical and subcortical regions, resulting in behavioural changes related to mood regulation, well-being, memory, and even cognitive preservation in aging and neurodegenerative diseases. Although evidence is accumulating on the systemic neural mechanisms mediating these brain effects, the specific mechanisms by which exercise acts upon the cellular level are still under investigation. This is particularly the case for music training, a much less studied instance of motor exercise than sport. With regards to sport, consistent neurobiological research has focused on the brain-derived neurotrophic factor (BDNF), an essential player in the central nervous system. BDNF stimulates the growth and differentiation of neurons and synapses. It thrives in the hippocampus, the cortex, and the basal forebrain, which are the areas vital for memory, learning, and higher cognitive functions. Animal models and neurocognitive experiments on human athletes converge in demonstrating that physical exercise reliably boosts BDNF levels. In this review, we highlight comparable early findings obtained with animal

models and elderly humans exposed to musical stimulation, showing how perceptual exposure to music might affect BDNF release, similar to what has been observed for sport. We subsequently propose a novel hypothesis that relates the neuroplastic changes in the human brains after musical training to genetically- and exercise-driven BDNF levels.
Cells, 2021; 10

34453619: Reveglia P, Paolillo C, Ferretti G, De Carlo A, Angiolillo A, Nasso R, Caputo M, Matrone C, Di Costanzo A, Corso G

Challenges in LC-MS-based metabolomics for Alzheimer's disease early detection: targeted approaches versus untargeted approaches.
Alzheimer's disease (AD) is one of the most common causes of dementia in old people. Neuronal deficits such as loss of memory, language and problem-solving are severely compromised in affected patients. The molecular features of AD are Aβ deposits in plaques or in oligomeric structures and neurofibrillary tau tangles in brain. However, the challenge is that Aβ is only one piece of the puzzle, and recent findings continue to support the hypothesis that their presence is not sufficient to predict decline along the AD outcome. In this regard, metabolomic-based techniques are acquiring a growing interest for either the early diagnosis of diseases or the therapy monitoring. Mass spectrometry is one the most common analytical platforms used for detection, quantification, and characterization of metabolic biomarkers. In the past years, both targeted and untargeted strategies have been applied to identify possible interesting compounds.
Metabolomics, 2021; 17

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BOARD NUMBER: S05-301

NON-CELL AUTONOMOUS REGULATION OF NEURONAL CIRCUITS FORMATION AT EARLY STAGES OF DEVELOPMENT IN THE VENTRAL SPINAL CORD

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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So far, there are not so many studies that have explored non-cell autonomous mechanisms involved in the formation of neuronal circuits during the development of the spinal cord. Previous work of the lab demonstrated that the conditional inactivation of Onecut transcription factors in spinal motor neurons cause perturbations in the development of ventral interneuron populations in a non-cell autonomous manner. Moreover, some genes downstream of the Onecut factors that could be implicated in this process were identified by RNA-seq. In this work, we studied the potential role of two of the candidate genes, gfra3 (GDNF Family Receptor Alpha 3) and nt3 (neurotrophin-3), both related to neurotrophic factors, in ventral interneuron differentiation and distribution in the developing spinal cord. Possible cell-autonomous modulation of the motor neurons was also analyzed. For this purpose, their expression was first validated in the spinal cord by In-situ hybridization both in mice and chicken embryos. Then, vectors that allowed us to overexpress the genes of interest specifically in the ventral spinal motor neurons (pHb9-nt3/gfra3-IRES-GFP) were generated and electroporated in the spinal cord of chicken embryos. The possible effects of the induced perturbations on the different neuronal populations were studied at different stages by immunofluorescence techniques. Results showed that the overexpression of neurotrophin-3 in spinal motor neurons alters the differentiation of V2 interneurons in a non-cell autonomous manner. Further experiments in mice will be performed to assess that these genes regulated under the control of Onecut factors contribute to non-cell autonomous regulation of spinal interneuron development.

Pubmed:

29085285: Domínguez-Bajo A, González-Mayorga A, López-Dolado E, Serrano MC
Graphene-Derived Materials Interfacing the Spinal Cord: Outstanding and Findings.
The attractiveness of graphene-derived materials (GDMs) for neural applications has fueled their exploration as components of biomaterial interfaces contacting the brain and the spinal cord. In the last years, an increasing body of work has been published on the ability of these materials to create biocompatible and biofunctional substrates able to promote the growth and activity of neural cells and positively interact with neural tissues when implanted . Encouraging results in the central nervous tissue might impulse the study of GDMs towards preclinical arena. In this mini-review article, we revise the most relevant literature on the interaction of GDMs with the spinal cord. Studies involving the implantation of these materials in the injured spinal cord are first discussed, followed by models with spinal cord slides and a final description of selected results with neural cells . A closing debate of the major conclusions of these results is presented to boost the investigation of GDMs in the field.
Front Syst Neurosci, 2017; 11
34700221: Domínguez-Bajo A, Rosa JM, González-Mayorga A, Rodilla BL, Arché-Núñez A, Benayas E, Ocón P, Pérez L, Camarero J, Miranda R, González MT, Aguilar J, López-Dolado E, Serrano MC
Nanostructured gold electrodes promote neural maturation and network connectivity.
Progress in the clinical application of recording and stimulation devices for neural diseases is still limited, mainly because of suboptimal material engineering and unfavorable interactions with biological entities. Nanotechnology is providing upgraded designs of materials to better mimic the native extracellular environment and attain more intimate contacts with individual neurons, besides allowing for the miniaturization of the electrodes. However, little progress has been done to date on the understanding of the biological impact that such neural interfaces have on neural network maturation and functionality. In this work, we elucidate the effect of a gold (Au) highly ordered nanostructure on the morphological and functional interactions with neural cells and tissues. Alumina-templated Au nanostructured electrodes composed of parallel nanowires of 160 nm in diameter and 1.2 μm in length (Au-NWs), with 320 nm of pitch, are designed and characterized. Equivalent non-structured Au electrodes (Au-Flat) are used for comparison. By using diverse techniques in in vitro cell cultures including live calcium imaging, we found that Au-NWs interfaced with primary neural cortical cells for up to 14 days allow neural networks growth

and increase spontaneous activity and ability of neuronal synchronization, thus indicating that nanostructured features favor neuronal network. The enhancement in the number of glial cells found is hypothesized to be behind these beneficial functional effects. The in vivo effect of the implantation of these nanostructured electrodes and its potential relevance for future clinical applicability has been explored in an experimental model of rat spinal cord injury. Subacute responses to implanted Au-NWs show no overt reactive or toxic biological reactions besides those triggered by the injury itself. These results highlight the translational potential of Au-NWs electrodes for in vivo applications as neural interfaces in contact with central nervous tissues including the injured spinal cord.
Biomaterials, 2021; 279
32761896: Domínguez-Bajo A, Rodilla BL, Calaresu I, Arché-Núñez A, González-Mayorga A, Scaini D, Pérez L, Camarero J, Miranda R, López-Dolado E, González MT, Ballerini L, Serrano MC
Interfacing Neurons with Nanostructured Electrodes Modulates Synaptic Circuit Features.
Understanding neural physiopathology requires advances in nanotechnology-based interfaces, engineered to monitor the functional state of mammalian nervous cells. Such interfaces typically contain nanometer-size features for stimulation and recording as in cell-non-invasive extracellular microelectrode arrays. In such devices, it turns crucial to understand specific interactions of neural cells with physicochemical features of electrodes, which could be designed to optimize performance. Herein, versatile flexible nanostructured electrodes covered by arrays of metallic nanowires are fabricated and used to investigate the role of chemical composition and nanotopography on rat brain cells in vitro. By using Au and Ni as exemplary materials, nanostructure and chemical composition are demonstrated to play major roles in the interaction of neural cells with electrodes. Nanostructured devices are interfaced to rat embryonic cortical cells and postnatal hippocampal neurons forming synaptic circuits. It is shown that Au-based electrodes behave similarly to controls. Contrarily, Ni-based nanostructured electrodes increase cell survival, boost neuronal differentiation, and reduce glial cells with respect to flat counterparts. Nonetheless, Au-based electrodes perform superiorly compared to Ni-based ones. Under electrical stimulation, Au-based nanostructured substrates evoke intracellular calcium dynamics compatible with neural networks activation. These studies highlight the opportunity for these electrodes to excite a silent neural network by direct neuronal membranes depolarization.
Adv Biosyst, 2020; 4
30502723: Domínguez-Bajo A, González-Mayorga A, Guerrero CR, Palomares FJ, García R, López-Dolado E, Serrano MC
Myelinated axons and functional blood vessels populate mechanically compliant rGO foams in chronic cervical hemisected rats.
Neural diseases at the central nervous system including spinal cord injury (SCI) remain therapeutic challenges. Graphene materials are being delineated as alternative tools for neural repair. Herein, the regenerative ability of reduced graphene oxide (rGO) scaffolds to support pivotal features of neural repair at 4 months after SCI is assessed by an interdisciplinary approach. 3D randomly porous foams have been prepared in mechanical compliance with neural cells and tissues (Young's modulus of 1.3 ± 1.0 kPa) as demonstrated by atomic force microscopy techniques applied ex vivo. After implantation, the significant increase in Young's modulus caused by massive cell/protein infiltration does not alter the mechanical performance of the contralateral spinal cord but provides mechanical stability to the lesion. These aerogels appear fully vascularized and populated with neurites, some of them being myelinated excitatory axons. Clinically-inspired magnetic resonance imaging studies demonstrate that the scaffolds significantly reduce perilesional damage with respect to rats without implants and cause no compressive damage in the contralateral hemicord and rostral/caudal regions. The rGO implants do not either alter the rat spontaneous behaviour or induce toxicity in major organs. Finally, preliminary data suggest hints of rGO sheets dissociation and eventual degradation at the injured spinal cord for the first time. In summary, these 3D porous rGO scaffolds are able to induce, without any further biological functionalization, a compilation of positive effects that have been rarely described before, if ever, for any other material implanted in the injured spinal cord.
Biomaterials, 2019; 192
33455347: Domínguez-Bajo A, González-Mayorga A, López-Dolado E, Munuera C, García-Hernández M, Serrano MC
Graphene Oxide Microfibers Promote Regenerative Responses after Chronic Implantation in the Cervical Injured Spinal Cord.
Spinal cord injury (SCI) is characterized by the disruption of neuronal axons and the creation of an inhibitory environment for spinal tissue regeneration. For decades, researchers and clinicians have been devoting a great effort to develop novel therapeutic approaches which include the fabrication of biocompatible implants that could guide neural tissue repair in the lesion site in an attempt to recover the functionality of the nervous tissue. In this context, although fiberlike structures have been hypothesized to serve as a topographical guidance for axonal regrowth, work on the exploration of this type of materials is still limited for SCI. Aiming to develop such guidance platforms, we recently designed and explored reduced graphene oxide materials in the shape of microfibers (rGO-MFs). After preliminary studies to assess the feasibility of their implantation at the injured spinal cord , no evident signs of subacute local toxicity were noticed (10 days of implantation). In this work, we specifically examine for the first time the regenerative potential of these scaffolds, slightly modified in their fabrication for improved reproducibility, when chronically interfaced with a cervical spinal cord injury. After extensive characterization of their

physicochemical properties and experiments with neural progenitor cells, their neural regenerative capacity is investigated in a rat experimental model of SCI after 4 months of implantation (chronic state). Behavioral tests involving the use of forelimbs are performed. Immunofluorescence studies evidence that rGO-MFs scaffolds foster the presence of neuronal structures along with blood vessels both within the epicenter and in the surroundings of the lesion area. Moreover, the inflammatory response does not worsen by the presence of this material. These findings outline the potential of rGO-MF-based scaffolds to promote regenerative features at the injured spinal cord such as axonal and vascular growth. Further studies including biological functionalization might improve their therapeutic potential by a synergistic effect of topographical and chemical cues, thus boosting neural repair after SCI.
ACS Biomater Sci Eng, 2020; 6
32805917: Girão AF, Sousa J, Domínguez-Bajo A, González-Mayorga A, Bdikin I, Pujades-Otero E, Casañ-Pastor N, Hortigüela MJ, Otero-Irurueta G, Completo A, Serrano MC, Marques PAAP
3D Reduced Graphene Oxide Scaffolds with a Combinatorial Fibrous-Porous Architecture for Neural Tissue Engineering.
Graphene oxide (GO) assists a diverse set of promising routes to build bioactive neural microenvironments by easily interacting with other biomaterials to enhance their bulk features or, alternatively, self-assembling toward the construction of biocompatible systems with specific three-dimensional (3D) geometries. Herein, we first modulate both size and available oxygen groups in GO nanosheets to adjust the physicochemical and biological properties of polycaprolactone-gelatin electrospun nanofibrous systems. The results show that the incorporation of customized GO nanosheets modulates the properties of the nanofibers and, subsequently, markedly influences the viability of neural progenitor cell cultures. Interestingly, the partially reduced GO (rGO) nanosheets with larger dimensions trigger the best cell response, while the rGO nanosheets with smaller size provoke an accentuated decrease in the cytocompatibility of the resulting electrospun meshes. Then, the most auspicious nanofibers are synergistically accommodated onto the surface of 3D-rGO heterogeneous porous networks, giving rise to fibrous-porous combinatorial architectures suitable for enhancing adhesion and differentiation of neural cells. By varying the chemical composition of the nanofibers, it is possible to adapt their performance as physical crosslinkers for the rGO sheets, leading to the modulation of both pore size and structural/mechanical integrity of the scaffold. Importantly, the biocompatibility of the resultant fibrous-porous systems is not compromised after 14 days of cell culture, including standard differentiation patterns of neural progenitor cells. Overall, in light of these results, the reported scaffolding approach presents not only an indisputable capacity to support highly viable and interconnected neural circuits but also the potential to unlock novel strategies for neural tissue engineering applications.
ACS Appl Mater Interfaces, 2020; 12
31904927: Fortes Brollo ME, Domínguez-Bajo A, Tabero A, Domínguez-Arca V, Gisbert V, Prieto G, Johansson C, Garcia R, Villanueva A, Serrano MC, Morales MDP
Combined Magnetoliposome Formation and Drug Loading in One Step for Efficient Alternating Current-Magnetic Field Remote-Controlled Drug Release.
We have developed a reproducible and facile one step strategy for the synthesis of doxorubicin loaded magnetoliposomes by using a thin-layer evaporation method. Liposomes of around 200 nm were made of 1,2-dipalmitoyl--glycero-3-phosphocholine (DPPC) and iron oxide nanoparticles (NPs) with negative, positive, and hydrophobic surfaces that were incorporated outside, inside, or between the lipid bilayers, respectively. To characterize how NPs are incorporated in liposomes, advanced cryoTEM and atomic force microscope (AFM) techniques have been used. It was observed that only when the NPs are attached outside the liposomes, the membrane integrity is preserved (lipid melt transition shifts to 38.7 °C with high enthalpy 34.8 J/g) avoiding the leakage of the encapsulated drug while having good colloidal properties and the best heating efficiency under an alternating magnetic field (AMF). These magnetoliposomes were tested with two cancer cell lines, MDA-MB-231 and HeLa cells. First, 100% of cellular uptake was achieved with a high cell survival (above 80%), which is preserved (83%) for doxorubicin-loaded magnetoliposomes. Then, we demonstrate that doxorubicin release can be triggered by remote control, using a noninvasive external AMF for 1 h, leading to a cell survival reduction of 20%. Magnetic field conditions of 202 kHz and 30 mT seem to be enough to produce an effective heating to avoid drug degradation. In conclusion, these drug-loaded magnetoliposomes prepared in one step could be used for drug release on demand at a specific time and place, efficiently using an external AMF to reduce or even eliminate side effects.
ACS Appl Mater Interfaces, 2020; 12

FENS Forum 2022
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BOARD NUMBER: S05-302

ONSET AND TIME COURSE OF EXPRESSION OF ODORANT RECEPTOR GENES DURING MOUSE EMBRYONIC DEVELOPMENT

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Olfactory perception is initiated by the interaction of odorants with odorant receptors (ORs) expressed by olfactory sensory neurons (OSN), which are located within the nasal cavity and are in direct contact with the external environment. A mature OSN is thought to express a single intact OR gene in a monogenic and monoallelic manner. The onset and time course expression of the OR repertoire – the largest gene family in the mouse genome – has not been studied extensively. Here we performed a NanoString transcriptomic study of 1047 OR genes in the whole olfactory mucosa (WOM), from the formation of the olfactory placode at embryonic day (E) 8.5 until postnatal day (PD) 0.5. Expression of some OR genes could be detected by NanoString technology as early as E9.5, and expression by in situ hybridization was detected from E11.5. Coexpression of OR genes occurred in some cells located in the mesenchyme and the prospective olfactory epithelium. Lineage tracing of olfactory progenitor cells was performed at E11.5 by using the UbC-StarTrack clonal method. Altogether, our data reveal transcriptomic and lineage features of how the OR gene family initiates its expression in the putative olfactory epithelium and how it is modulated throughout embryonic development.

Pubmed:
33827819: Herrero-Navarro Á, Puche-Aroca L, Moreno-Juan V, Sempere-Ferràndez A, Espinosa A, Susín R, Torres-Masjoan L, Leyva-Díaz E, Karow M, Figueres-Oñate M, López-Mascaraque L, López-Atalaya JP, Berninger B, López-Bendito G
Astrocytes and neurons share region-specific transcriptional signatures that confer regional identity to neuronal reprogramming.
Neural cell diversity is essential to endow distinct brain regions with specific functions. During development, progenitors within these regions are characterized by specific gene expression programs, contributing to the generation of diversity in postmitotic neurons and astrocytes. While the region-specific molecular diversity of neurons and astrocytes is increasingly understood, whether these cells share region-specific programs remains unknown. Here, we show that in the neocortex and thalamus, neurons and astrocytes express shared region-specific transcriptional and epigenetic signatures. These signatures not only distinguish cells across these two brain regions but are also detected across substructures within regions, such as distinct thalamic nuclei, where clonal analysis reveals the existence of common nucleus-specific progenitors for neurons and astrocytes. Consistent with their shared molecular signature, regional specificity is maintained following astrocyte-to-neuron reprogramming. A detailed understanding of these regional-specific signatures may thus inform strategies for future cell-based brain repair.
Sci Adv, 2021; 7
33151389: Figueres-Oñate M, Sánchez-González R, López-Mascaraque L
Deciphering neural heterogeneity through cell lineage tracing.
Understanding how an adult brain reaches an appropriate size and cell composition from a pool of progenitors that proliferates and differentiates is a key question in Developmental Neurobiology. Not only the control of final size but also, the proper arrangement of cells of different embryonic origins is fundamental in this process. Each neural progenitor has to produce a precise number of sibling cells that establish clones, and all these clones will come together to form the functional adult nervous system. Lineage cell tracing is a complex and challenging process that aims to reconstruct the offspring that arise from a single progenitor cell. This tracing can be achieved through strategies based on genetically modified organisms, using either genetic tracers, transfected viral vectors or DNA constructs, and even single-cell sequencing. Combining different reporter proteins and the use of transgenic mice revolutionized clonal analysis more than a decade ago and now, the availability of novel genome editing tools and single-cell sequencing techniques has vastly improved the capacity of lineage tracing to decipher progenitor potential. This review brings together the strategies used to study cell lineages in the brain and the role they have played in our understanding of the functional clonal relationships among neural cells. In addition, future

perspectives regarding the study of cell heterogeneity and the ontogeny of different cell lineages will also be addressed.
Cell Mol Life Sci, 2021; 78
31543472: Figueres-Oñate M, Sánchez-Villalón M, Sánchez-González R, López-Mascaraque L
Lineage Tracing and Cell Potential of Postnatal Single Progenitor Cells In Vivo.
Understanding the contribution of adult neural progenitor cells (NPCs) and their lineage potential is a great challenge in neuroscience. To reveal progenitor diversity and cell-lineage relationships of postnatal NPCs in the subventricular zone (SVZ), we performed in vivo lineage-tracing genetic analysis using the UbC-StarTrack. We determined the progeny of single SVZ-NPCs, the number of cells per clone, the dispersion of sibling cells, and the cell types within clones. Long-term analysis revealed that both the cell-dispersion pattern and number of cells comprising clones varied depending on the glial/neuronal nature of sibling cells. Sibling-olfactory interneurons were primarily located within the same layer, while sibling-glial cells populated SVZ-adjacent areas. Sibling astrocytes and interneurons did not form big clones, whereas oligodendroglial-lineage clones comprised the largest clones originated in adult brains. These results demonstrate the existence of SVZ postnatal bipotential progenitors that give rise to clones widely dispersed across the olfactory bulb and SVZ-adjacent areas.
Stem Cell Reports, 2019; 13
30970247: Redmond SA, Figueres-Oñate M, Obernier K, Nascimento MA, Parraguez JI, López-Mascaraque L, Fuentealba LC, Alvarez-Buylla A
Development of Ependymal and Postnatal Neural Stem Cells and Their Origin from a Common Embryonic Progenitor.
The adult mouse brain contains an extensive neurogenic niche in the lateral walls of the lateral ventricles. This epithelium, which has a unique pinwheel organization, contains multiciliated ependymal (E1) cells and neural stem cells (B1). This postnatal germinal epithelium develops from the embryonic ventricular zone, but the lineage relationship between E1 and B1 cells remains unknown. Distinct subpopulations of radial glia (RG) cells in late embryonic and early postnatal development either expand their apical domain >11-fold to form E1 cells or retain small apical domains that coalesce into the centers of pinwheels to form B1 cells. Using independent methods of lineage tracing, we show that individual RG cells can give rise to clones containing E1 and B1 cells. This study reveals key developmental steps in the formation of the postnatal germinal niche and the shared cellular origin of E1 and B1 cells.
Cell Rep, 2019; 27
30290178: Tiwari N, Pataskar A, Péron S, Thakurela S, Sahu SK, Figueres-Oñate M, Marichal N, López-Mascaraque L, Tiwari VK, Berninger B
Stage-Specific Transcription Factors Drive Astrogliogenesis by Remodeling Gene Regulatory Landscapes.
A broad molecular framework of how neural stem cells are specified toward astrocyte fate during brain development has proven elusive. Here we perform comprehensive and integrated transcriptomic and epigenomic analyses to delineate gene regulatory programs that drive the developmental trajectory from mouse embryonic stem cells to astrocytes. We report molecularly distinct phases of astrogliogenesis that exhibit stage- and lineage-specific transcriptomic and epigenetic signatures with unique primed and active chromatin regions, thereby revealing regulatory elements and transcriptional programs underlying astrocyte generation and maturation. By searching for transcription factors that function at these elements, we identified NFIA and ATF3 as drivers of astrocyte differentiation from neural precursor cells while RUNX2 promotes astrocyte maturation. These transcription factors facilitate stage-specific gene expression programs by switching the chromatin state of their target regulatory elements from primed to active. Altogether, these findings provide integrated insights into the genetic and epigenetic mechanisms steering the trajectory of astrogliogenesis.
Cell Stem Cell, 2018; 23
30260948: Cerrato V, Parmigiani E, Figueres-Oñate M, Betizeau M, Aprato J, Nanavaty I, Berchialla P, Luzzati F, de'Sperati C, López-Mascaraque L, Buffo A
Multiple origins and modularity in the spatiotemporal emergence of cerebellar astrocyte heterogeneity.
The morphological, molecular, and functional heterogeneity of astrocytes is under intense scrutiny, but how this diversity is ontogenetically achieved remains largely unknown. Here, by quantitative in vivo clonal analyses and proliferation studies, we demonstrate that the major cerebellar astrocyte types emerge according to an unprecedented and remarkably orderly developmental program comprising (i) a time-dependent decline in both clone size and progenitor multipotency, associated with clone allocation first to the hemispheres and then to the vermis(ii) distinctive clonal relationships among astrocyte types, revealing diverse lineage potentials of embryonic and postnatal progenitors; and (iii) stereotyped clone architectures and recurrent modularities that correlate to layer-specific dynamics of postnatal proliferation/differentiation. In silico simulations indicate that the sole presence of a unique multipotent progenitor at the source of the whole astrogliogenic program is unlikely and rather suggest the involvement of additional committed components.
PLoS Biol, 2018; 16
27654510: Figueres-Oñate M, García-Marqués J, López-Mascaraque L
UbC-StarTrack, a clonal method to target the entire progeny of individual progenitors.

Clonal cell analysis defines the potential of single cells and the diversity they can produce. To achieve this, we have developed a novel adaptation of the genetic tracing strategy, UbC-StarTrack, which attributes a specific and unique color-code to single neural precursors, allowing all their progeny to be tracked. We used integrable fluorescent reporters driven by a ubiquitous promoter in PiggyBac-based vectors to achieve inheritable and stable clonal cell labeling. In addition, coupling this to an inducible Cre-LoxP system avoids the expression of non-integrated reporters. To assess the utility of this system, we first analyzed images of combinatorial expression of fluorescent reporters in transfected cells and their progeny. We also validated the efficiency of the UbC-StarTrack to trace cell lineages through in vivo, in vitro and ex vivo strategies. Finally, progenitors located in the lateral ventricles were targeted at embryonic or postnatal stages to determine the diversity of neurons and glia they produce, and their clonal relationships. In this way we demonstrate that UbC-StarTrack can be used to identify all the progeny of a single cell and that it can be employed in a wide range of contexts.
Sci Rep, 2016; 6

27242400: Figueres-Oñate M, López-Mascaraque L
Adult Olfactory Bulb Interneuron Phenotypes Identified by Targeting Embryonic and Postnatal Neural Progenitors.
Neurons are generated during embryonic development and in adulthood, although adult neurogenesis is restricted to two main brain regions, the hippocampus and olfactory bulb. The subventricular zone (SVZ) of the lateral ventricles generates neural stem/progenitor cells that continually provide the olfactory bulb (OB) with new granule or periglomerular neurons, cells that arrive from the SVZ via the rostral migratory stream. The continued neurogenesis and the adequate integration of these newly generated interneurons is essential to maintain homeostasis in the olfactory bulb, where the differentiation of these cells into specific neural cell types is strongly influenced by temporal cues. Therefore, identifying the critical features that control the generation of adult OB interneurons at either pre- or post-natal stages is important to understand the dynamic contribution of neural stem cells. Here, we used in utero and neonatal SVZ electroporation along with a transposase-mediated stable integration plasmid, in order to track interneurons and glial lineages in the OB. These plasmids are valuable tools to study the development of OB interneurons from embryonic and post-natal SVZ progenitors. Accordingly, we examined the location and identity of the adult progeny of embryonic and post-natally transfected progenitors by examining neurochemical markers in the adult OB. These data reveal the different cell types in the olfactory bulb that are generated in function of age and different electroporation conditions.
Front Neurosci, 2016; 10

25852461: Figueres-Oñate M, García-Marqués J, Pedraza M, De Carlos JA, López-Mascaraque L
Spatiotemporal analyses of neural lineages after embryonic and postnatal progenitor targeting combining different reporters.
Genetic lineage tracing with electroporation is one of the most powerful techniques to target neural progenitor cells and their progeny. However, the spatiotemporal relationship between neural progenitors and their final phenotype remain poorly understood. One critical factor to analyze the cell fate of progeny is reporter integration into the genome of transfected cells. To address this issue, we performed postnatal and in utero co-electroporations of different fluorescent reporters to label, in both cerebral cortex and olfactory bulb, the progeny of subventricular zone neural progenitors. By comparing fluorescent reporter expression in the adult cell progeny, we show a differential expression pattern within the same cell lineage, depending on electroporation stage and cell identity. Further, while neuronal lineages arise from many progenitors in proliferative zones after few divisions, glial lineages come from fewer progenitors that accomplish many cell divisions. Together, these data provide a useful guide to select a strategy to track the cell fate of a specific cell population and to address whether a different proliferative origin might be correlated with functional heterogeneity.
Front Neurosci, 2015; 9

25071462: Figueres-Oñate M, Gutiérrez Y, López-Mascaraque L
Unraveling Cajal's view of the olfactory system.
The olfactory system has a highly regular organization of interconnected synaptic circuits from the periphery. It is therefore an excellent model for understanding general principles about how the brain processes information. Cajal revealed the basic cell types and their interconnections at the end of the XIX century. Since his original descriptions, the observation and analysis of the olfactory system and its components represents a major topic in neuroscience studies, providing important insights into the neural mechanisms. In this review, we will highlight the importance of Cajal contributions and his legacy to the actual knowledge of the olfactory system.
Front Neuroanat, 2014; 8

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PERTURBING PUTATIVE NEURONAL FATE DETERMINANTS IN VIVO

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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A number of transcription factors (TFs) are expressed during the neurogenesis and migration of inhibitory neurons from the ganglionic eminences. Diversity is first apparent in the regional expression of a limited number of transcription factors within the ganglionic eminences. However, their functional role in the specification of inhibitory neuron cell state remains unclear. To investigate which transcription factors in the ganglionic eminence facilitate inhibitory neuron fate commitment, we developed a single-cell RNA sequencing compatible CRISPR technique to introduce frameshift mutations to genes in vivo. The CRISPR constructs are first introduced to the embryonic progenitors via in utero electroporation and the perturbed cells are collected postnatally. Differential gene expression analysis allowed us to identify transcription factors that coordinate the gene programs between projection and interneurons. Moreover, we find that these factors coordinate a number of developmental processes such as neuronal differentiation and migration. Furthermore, we developed a novel lineage tracing technique to tag perturbed and non-perturbed progenitors with DNA lineage barcodes. Lineage tracing allowed us to follow clonally related progenitor cells through development and to investigate whether CRISPR perturbations triggered a change in fate.

Pubmed:
34912118: Bandler RC, Vitali I, Delgado RN, Ho MC, Dvoretskova E, Ibarra Molinas JS, Frazel PW, Mohammadkhani M, Machold R, Maedler S, Liddelow SA, Nowakowski TJ, Fishell G, Mayer C
Single-cell delineation of lineage and genetic identity in the mouse brain.
During neurogenesis, mitotic progenitor cells lining the ventricles of the embryonic mouse brain undergo their final rounds of cell division, giving rise to a wide spectrum of postmitotic neurons and glia. The link between developmental lineage and cell-type diversity remains an open question. Here we used massively parallel tagging of progenitors to track clonal relationships and transcriptomic signatures during mouse forebrain development. We quantified clonal divergence and convergence across all major cell classes postnatally, and found diverse types of GABAergic neuron that share a common lineage. Divergence of GABAergic clones occurred during embryogenesis upon cell-cycle exit, suggesting that differentiation into subtypes is initiated as a lineage-dependent process at the progenitor cell level.
Nature, 2022; 601
31371793: Walia R, Ho CC, Lee C, Gilch S, Schatzl HM
Gene-edited murine cell lines for propagation of chronic wasting disease prions.
Prions cause fatal infectious neurodegenerative diseases in humans and animals. Cell culture models are essential for studying the molecular biology of prion propagation. Defining such culture models is mostly a random process, includes extensive subcloning, and for many prion diseases few or no models exist. One example is chronic wasting disease (CWD), a highly contagious prion disease of cervids. To extend the range of cell models propagating CWD prions, we gene-edited mouse cell lines known to efficiently propagate murine prions. Endogenous prion protein (PrP) was ablated in CAD5 and MEF cells, using CRISPR-Cas9 editing. PrP knock-out cells were reconstituted with mouse, bank vole and cervid PrP genes by lentiviral transduction. Reconstituted cells expressing mouse PrP provided proof-of-concept for re-established prion infection. Bank voles are considered universal receptors for prions from a variety of species. Bank vole PrP reconstituted cells propagated mouse prions and cervid prions, even without subcloning for highly susceptible cells. Cells reconstituted with cervid PrP and infected with CWD prions tested positive in prion conversion assay, whereas non-reconstituted cells were negative. This novel cell culture platform which is easily adjustable and allows testing of polymorphic alleles will provide important new insights into the biology of CWD prions.
Sci Rep, 2019; 9

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BOARD NUMBER: S05-304

LRRN2 AND LRRN3A SPECIFY PRECISE RETINO-TECTAL CONNECTIONS IN THE VERTEBRATE VISUAL SYSTEM

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Leucin-rich repeat proteins (LRR) have shown to be key players in several aspects of neuronal circuit development, from axon guidance to synapse formation. Among the LRR proteins, the leucin-rich repeat neuronal (LRRN) family comprises different adhesion molecules, such as Lrrn2 and Lrrn3a (orthologues of Capricious in *Drosophila*), whose expression pattern are conserved across species, from *Drosophila* to human. However, nothing is known about their function in the context of the visual system formation and axonal targeting. Their ortholog Capricious in *Drosophila* governs the synaptic targeting of specific photoreceptors to precise laminae of the fly brain. In vertebrates, we show that *lrrn2 and lrrn3a* are sparsely expressed in retinal ganglion cells (RGCs) and our preliminary data in the zebrafish larva demonstrate that their absence leads to mistargeting defects of a subset of RGCs. Indeed, the axons of this population of RGCs, normally projecting to deep layers of the fish brain retino-recipient optic tectum, are unable to reach these laminae in both *lrrn2* and *3a* mutants. Furthermore, the behavioral analysis of these mutants shows reduced hunting abilities. Together our results indicate that Lrrn2/3a are required for the specification and function of precise retino-tectal circuits, revealing their role for the first time in the vertebrate visual system.

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BOARD NUMBER: S05-305

MOLECULAR AND PHYSICAL FACTORS COORDINATE CAJAL-RETZIUS CELLS MIGRATION IN THE MARGINAL ZONE DURING NEOCORTICAL DEVELOPMENT

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Ana López-Mengual[1,2,3,4], Miriam Segura-Feliu[1,2,3,4], Raimon Sunyer[5,6], Héctor Sanz-Fraile[6], Jorge Otero[6,7], Francina Mesquida-Veny[1,2,3,4], Vanessa Gil[1,2,3,4], Arnau Hervera[1,2,3,4], Isidro Ferrer[4,8], Jordi Soriano[9,10], Xavier Trepat[5,6,11,12], Ramon Farré[6,7,13], Daniel Navajas[6,7,14], José A Del Río[1,2,3,4]
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A key event in brain development is cell migration and distribution, whose are modulated by molecular factors. At early stages of neocortical development, semaphorins, angiogenic factors and chemokines as CXCL12, assume crucial roles in orchestrating neuronal migration and distribution. There is increasing evidence that molecular and mechanical factors are associated to coordinate neuronal migration and axon elongation in brain development. Cajal-Retzius cells are generated in specific proliferative regions in the developing brain and migrate tangentially in the marginal zone covering the neocortical surface. These pioneer neurons play a crucial role in neocortical layered organization by secreting molecules such as Reelin. In this project we explore the intrinsic mechanical properties of the marginal zone of the developing brain in the migratory pathways and brain distribution of Cajal-Retzius cells. We observe a clear relation between mechanical cues and Cajal-Retzius cells migration in the developing brain. These mechanical factors act as a regulatory cue for their migration and distribution between differential stiffness regions on the neocortical surface. Also, Cajal-Retzius cells activity and migration can be modulated by inhibiting mechanotransduction receptors, indicating that can response to mechanical factors. Indeed, Cajal-Retzius cells from the migratory origins display different migratory capacities which may be involved in their differential distribution in the dorsal-lateral axis along the developing marginal zone. In resume, our results indicate that Cajal-Retzius cells migration and distribution are modulated by molecular and mechanical factors, as observed by the by the differential stiffness in their migratory routes and their specific cellular properties of Cajal-Retzius cells.

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BOARD NUMBER: S05-306

TANYCYTE NUCLEOLUS DURING EARLY POSTNATAL DEVELOPMENT AND AGING.

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Tanycytes are highly specialized glial cells lining the infundibular recess. These cells are bipolar and have long basal process penetrating the adjacent nervous tissue of mediabasal hypothalamus. There are 4 types of tanycytes: α1-, α2-, β1- and β2-tanycytes. The nucleolus is one of the important markers reflecting the functional state of a cell. Despite the keen interest of researchers in tanycytes, this important nuclear structure hasn't yet been investigated in these cells. The aim of the research was to study nucleolus organization of tanycytes subpopulations in postnatal development and aging. Using immunohistochemical methods and confocal laser microscopy the brain of young (days 7, 14 and 30 of postnatal development), adult (4-6 months), and old (20 months) male Wistar rats was examined (n=3 for each term). It was established that tanycyte subpopulations are differ in nucleolus organization. Different tanycyte types vary in number, mean diameter and form of nucleolus. These data may indicate a different protein-synthetic activity and functional status of tanycyte subpopulations. For all tanycyte types it was shown that nucleoli diameter increases during postnatal development and aging. The number of nucleoli doesn't change during early postnatal development, but it decreases in aging. These results reflect differentiation processes in tanycytes and increase of synthetic activity in these cells during postnatal development and aging.

Pubmed:

30607910: Fedorova EA, Sufieva DA, Grigorev IP, Korzhevskii DE
[Mast cells of the human pineal gland.]
The purpose of this study was to assess the possibilities of identifying mast cells using different histochemical and immunohistochemical methods and elucidating the features of their localization in the human pineal gland. The undertaken study showed that mast cells are an essential component of the human pineal gland, regardless of age. The data obtained indicate an increase in the number of mast cells in the pineal gland with age. Mast cells are mostly located in the pineal stroma and their preferred location has not been related to concrements, cysts or melanin accumulations. Mast cells in the pineal gland are predominantly non-degranulating, which indicates their inactive state. The detectability of mast cells in the pineal gland depended significantly on the applied method of staining of the preparations. The largest number of mast cells was revealed by tryptase immunohistochemistry, which should be used to accurately determine the population of mast cells of the pineal gland.
Adv Gerontol, 2018; 31
28163253: Agalakova NI, Ivanova TI, Gusev GP, Nazarenkova AV, Sufiyeva DA
Apoptotic death in erythrocytes of lamprey Lampetra fluviatilis induced by ionomycin and tert-butyl hydroperoxide.
The work examined the effects of Ca overload and oxidative damage on erythrocytes of river lamprey Lampetra fluvialtilis. The cells were incubated for 3h with 0.1-5μM Ca ionophore ionomycin in combination with 2.5mM Ca and 10-100μM pro-oxidant agent tert-butyl hydroperoxide (tBHP). The sensitivity of lamprey RBCs to studied compounds was evaluated by the kinetics of their death. Both toxicants induced dose- and time dependent phosphatidylserine (PS) externalization (annexin V-FITC labeling) and loss of membrane integrity (propidium iodide uptake). Highest doses of ionomycin (1-2μM) increased the number of PS-exposed erythrocytes to 7-9% within 3h, while 100μM tBHP produced up to 50% of annexin V-FITC-positive cells. Caspase inhibitor Boc-D-FMK (50μM), calpain inhibitor PD150606 (10μM) and broad protease inhibitor leupeptin (200μM) did not prevent ionomycin-induced PS externalization, whereas tBHP-triggered apoptosis was blunted by Boc-D-FMK. tBHP-dependent death of lamprey erythrocytes was accompanied by the decrease in relative cell size, loss of cell viability, activation of caspases 9 and 3/7, and loss of mitochondrial membrane potential, but all these processes were partially attenuated by Boc-D-FMK. None of examined death-associated events were observed in ionomycin-treated erythrocytes except activation of caspase-9. Incubation with ionomycin did not alter intracellular K and Na content, while exposure to tBHP resulted in 80% loss of K and 2.8-fold accumulation of Na. Thus, lamprey erythrocytes appear to be more susceptible to oxidative damage. Ca overload does not activate the cytosolic death pathways in these cells.
Comp Biochem Physiol C Toxicol Pharmacol, 2017; 194

25051804: Kirik OV, Nazarenkova AV, Sufiyeva DA
[Three-dimensional visualization of the brain ependyma and tanycytes].
The aim of this investigation was to develop an integrated approach to spatial reconstruction of the cells lining the ventricles of the brain using confocal laser microscopy and immunocytochemical reaction to vimentin. The work was performed on paraffin sections of rat brain of different thickness (5 and 10 microm). To visualize the immunocytochemical reaction the fluorescent dyes in the visible range were selected: SYTOX Green selectively staining the nucleus and indocarbocyanin (Cy-3) conjugated with streptavidin. As a result of testing of various processing conditions, the protocol which allows to receive an intensive staining of the structures was developed. The set of fluorochromes proposed in confocal laser microscopy allows to separate easily the channels, to study the structures independently, if needed, and does not require the use of an expensive ultraviolet laser.
Morfologiia, 2014; 145
26390541: Kirik OV, Sufiyeva DA, Nazarenkova AV, Korzhevskiy DE
[STRUCTURAL ORGANIZATION OF THE PROCESSES OF EPENDYMO- CYTES LINING THE LATERAL VENTRICLES OF THE RAT BRAIN].
The aim of this study was to examine the structural organization of processes of ependymocytes lining the lateral ventricles of the rat brain using vimentin immunocytochemistry and confocal laser microscopy. The study was performed on adult male rats (n = 3). It was found that most typical ependymocytes had basal processes, while 1/3 of these cells had none. Some vimentin-immunopositive tanycyte-like cells with long processes appoaching blood vessels, were found inside the ependymal lining In some typical ependymocytes, cytroskeleton wa s formed by intermediate filaments of mixed type containing both vimentin and glial fibrillary acidic protein.
Morfologiia, 2015; 147
27487660: Kirik OV, Sufieyva DA, Nazarenkova AV, Korzhevskiy DE
[CELL CONTACT PROTEIN BETA-CATENIN IN EPENDYMAL AND EPITHELIAL CELLS OF THE CHOROID PLEXUS OF THE CEREBRAL LATERAL VENTRICLES].
The purpose of this study was to examine the distribution pattern of cellular contacts protein beta-catenin in the choroid plexus and ependyma of lateral ventricles of the brain. The study was conducted on frontal sections of the brain of Wistar rats (n = 10) using polyclonal antibodies against beta-catenin. The obtained preparations were analyzed by microscopy in transmitted light and using confocal laser microscopy. To study the distribution of beta-catenin in different projections, three-dimensional reconstruction was performed. The study demonstrated different distribution patterns of this protein in ependyma and choroid plexus. Unlike ependyma, in the cells of the choroid plexus beta-catenin was distributed in the same way as in simple epithelial tissues (on the basal and lateral borders of the cells). This may indicate different tissue attribution of the ependyma and the choroid plexus epithelium, despite their common origin.
Morfologiia, 2016; 149

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DEVELOPMENT AND REGENERATION OF CORNEA INNERVATION

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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The cornea is a transparent and avascular structure in the anterior segment of the eye which allows a good visual acuity and acts as a protective barrier for the ocular surface. It is also the most innervated tissue of the human body and receives sensory inputs from the ophthalmic branch of the trigeminal nerve and autonomic axons from the ciliary and superior cervical ganglia. The features of corneal nerve patterning have been elucidated previously using standard immunostaining and electrophysiology approaches. Recently, the use of genetically modified mouse lines expressing fluorescent proteins or Cre recombinase made possible the direct visualization of corneal nerves. However, the understanding of corneal nerves morphology, distribution and plasticity is still elusive. Here we aimed at describing the development of corneal nerves using the *Phox2b*^{CRE} and *RosaTomato* transgenic lines in which a subset of axons are labelled in the cornea. We perform live imaging of fluorescent axons with confocal spinning disk microscopy and 2-photon. Corneal nerve density increases from early postnatal to adulthood, with axons becoming more centrally organized and with a higher branching complexity. In addition, immune cells (macrophages) have been identified in the corneal surface. We are also analyzing the cross-talk between neuronal and immune cells in the cornea. We follow the dynamics of axon-macrophage interaction over time in *Islet1*^{CRE}Rosa^{Tom}*;Cx3cr1*^{GFP} mice in normal and pathological conditions.

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THE ATYPICAL CILIA OF CHOROID PLEXUS THROUGH DEVELOPMENTAL LENSES

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Kim Hoa Ho[1,2], Valentina Scarpetta[1,3], Chiara Salio[4], Elisa D'Este[5], Marco Sassoè-Pognetto[3], Annarita Patrizi[1]
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Choroid plexus (CP) is located in all brain ventricles and is mainly comprised of epithelial cells. CP produces cerebrospinal fluid (CSF), forms the blood-CSF barrier and has been gaining increasingly significant roles in brain-body communication and neurological diseases. Differentiated CP epithelial cells harbor multiple cilia on the CSF-facing surface, which differ from cilia of the adjacent ependyma. While the latter is well characterized, little is known about CP cilia, hindering efforts to decipher CP-related disorders. **Aim**: Our goal is to shed light on CP ciliogenesis from embryogenesis to senescence, characterizing structural and molecular changes of cilia along the developmental trajectory. **Methods**: Mouse CP cilia from E11.5 to P720 were visualized using multiple microscopic techniques: confocal, STED and TEM. Transcriptomic changes relating to cilia formation and function were investigated by analyzing qPCR and snRNA data of CP in different age groups. **Results:** We discovered a novel mode of multi-ciliation that is spatio-temporally controlled and unique to CP. Our data show that CP cilia are unconventional as they bear characteristics of both primary and motile cilia. Moreover, we demonstrated that CP cilia are formed during early embryogenesis but gradually disappear starting from early postnatal ages, both in mice and humans, accompanied by selective transcriptional changes of related functional genes. **Conclusion:** CP cilia are of a structurally distinct type and represent an important differentiation marker of CP epithelium. Cilia presence and disappearance correlate with CP developmental stages, calling for more investigations on their functions and possible involvement in diseases, such as hydrocephalus.

Pubmed:
33558629: Ho KH, Patrizi A
Assessment of common housekeeping genes as reference for gene expression studies using RT-qPCR in mouse choroid plexus.
Choroid plexus (ChP), a vascularized secretory epithelium located in all brain ventricles, plays critical roles in development, homeostasis and brain repair. Reverse transcription quantitative real-time PCR (RT-qPCR) is a popular and useful technique for measuring gene expression changes and also widely used in ChP studies. However, the reliability of RT-qPCR data is strongly dependent on the choice of reference genes, which are supposed to be stable across all samples. In this study, we validated the expression of 12 well established housekeeping genes in ChP in 2 independent experimental paradigms by using popular stability testing algorithms: BestKeeper, DeltaCq, geNorm and NormFinder. Rer1 and Rpl13a were identified as the most stable genes throughout mouse ChP development, while Hprt1 and Rpl27 were the most stable genes across conditions in a mouse sensory deprivation experiment. In addition, Rpl13a, Rpl27 and Tbp were mutually among the top five most stable genes in both experiments. Normalisation of Ttr and Otx2 expression levels using different housekeeping gene combinations demonstrated the profound effect of reference gene choice on target gene expression. Our study emphasized the importance of validating and selecting stable housekeeping genes under specific experimental conditions.
Sci Rep, 2021; 11

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VISUOMOTOR DEFECTS OF ACHIASMATIC MICE EXPRESSING A TRANSFER-DEFECTIVE VAX1 MUTANT

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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In binocular animals that exhibit stereoscopic visual responses, the axons of retinal ganglion cells (RGCs) connect to brain areas bilaterally by forming a commissure called the optic chiasm (OC). Ventral anterior homeobox 1 (Vax1) contributes to formation of the OC by acting endogenously in cells on RGC axon growth tracks, such as optic stalk and ventral hypothalamus, and exogenously in RGC axons. Here, we generated *Vax1*$^{AA/AA}$ mice expressing the Vax1^{AA} mutant, which is incapable of intercellular transfer. We found that RGC axons cannot take up Vax1^{AA} protein from the axon growth track cells and fail to access the midline. Consequently, RGC axons of *Vax1*$^{AA/AA}$ mice connect exclusively to ipsilateral brain areas, resulting in the losses of visual acuity and visuomotor responses. Together, our study provides physiological evidences for the necessity of intercellular transfer of Vax1 and the importance of the bilateral RGC axon projection in visuomotor responses.

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TCF7L2 - A LINK BETWEEN AUTISM SPECTRUM DISORDER AND ABNORMAL DEVELOPMENT OF THE THALAMUS?

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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[1]University of Warsaw, Centre Of New Technologies, Warsaw, Poland, [2]University of Warsaw, Faculty Of Biology, Warsaw, Poland, [3]Nencki Institute of Experimental Biology PAS, Laboratory Of Electrophysiology, Warsaw, Poland, [4]Nencki Institute of Experimental Biology PAS, Laboratory Of Bioinformatics, Warsaw, Poland, [5]University of Murcia, Department Of Human Anatomy And Psychobiology, Murcia, Spain

The thalamus is a hub for the integration of sensory information and selection of behavioural responses. Anatomical and functional abnormalities in the thalamus and its axonal connections are often identified in neuropsychiatric disorders, such as schizophrenia and autism. Improper development of thalamic nuclei, aberrant growth of thalamocortical axons, or the emergence of atypical electrophysiological properties of thalamic neurons all could potentially contribute to the aetiology of said disorders. Unfortunately, our understanding of the thalamic development and its adult homeostasis was limited, as the molecular mechanisms which govern these processes were poorly characterized. Our studies shown that both depend on the transcription factor TCF7L2, which directly regulates the expression of many genes critical for thalamic development. Our goal was to determine the role of TCF7L2 in the embryonic development and postnatal maturation of the thalamus. To this end we examined mouse embryos with a total knockout of *Tcf7l2* and adolescent/adult mice with thalamus-specific, postnatal knockout of *Tcf7l2*. Anatomy, gene expression patterns, and axon fibres were visualised using Nissl staining, *in situ* hybridization, immunohistochemistry or DiI tracing. RNA-seq and ChIP-seq analyses were performed on thalami of both strains. Postnatal TCF7L2-deficent mice were used for behavioural tests and their brain slices were used for *in vitro* patch-clamp analysis. We show that the development of proper anatomy/cytoarchitecture of the thalamus, molecular identity of its neurons and growth of thalamocortical connections are all regulated by TCF7L2. TCF7L2 is further required for the functional maturation of thalamic neurons, and TCF7L2-deficent mice exhibit abnormal social behaviour.

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ENDOCYTIC RECEPTOR LRP2: A NEW ROLE IN THE NEURAL CREST CELL DYNAMICS

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Morphogenesis during embryonic development involves dynamic changes in the tissue shape and organisation which requires multifaceted molecular events. Specifically, formation of embryonic brain begins when the highly polarized pseudostratified epithelium bends into neural folds which then form a closed neural tube. Endocytic receptor LRP2 (LDL receptor related protein 2) is localised on the apical surface of neuroepithelium and crucial for maintaining the neuroepithelial and neural crest cell (NCC) integrity during embryonic development. NCCs are migrating cells derived from the neuroepithelium through an epithelial-to-mesenchymal transition process and capable of transforming into multiple cell lineages when migrating to distinct locations. Dysregulated neuroepithelial and NCC integrity can lead to congenital anomalies in new-borns, such as neural tube defects and tissue malformation, respectively. We have previously ascertained that the loss of LRP2 is associated with impaired neuroepithelial cell polarity and neural tube closure failure due to disrupted subapical protein interactions harbouring NHERF1 and GIPC1. In addition, we have found that LRP2 is expressed in the NCC population and could contributes to the proper NCC dynamic. Our present study therefore would like to address whether LRP2 indeed plays a role in regulating the early NCC behaviour and understand the associated molecular mechanisms. Phenotypic comparison revealed that *Lrp2*$^{-/-}$ mouse embryos displayed craniofacial abnormalities at E18.5, implying an aberrant NCC dynamic. Spatiotemporal defect in the NCC localisation pattern was observed in *Lrp2*$^{-/-}$ embryos much earlier during embryonic development, starting from E8.5 onwards. Altogether, we conclude that LRP2 function is critical for governing both neuroepithelial and NCC dynamics.

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ROLE OF LAMININ γ1 IN OLFACTORY PLACODE MORPHOGENESIS AND OLFACTORY AXON DEVELOPMENT IN ZEBRAFISH

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Laminins are major components of the extracellular matrix and play a critical role in the formation of basement membranes (BM). Here, we took advantage of the zebrafish *sly* mutation affecting Laminin γ1, to investigate its role in the development of the zebrafish olfactory system, which involves morphogenetic movements shaping the olfactory sensory placode, and the growth of the olfactory axons from the placode to the olfactory bulb in the brain. Using electron microscopy and immmunofluorescence for BM markers, we showed that the integrity of the BM surrounding the olfactory placodes and the brain is strongly affected in the mutant. Using transgenic lines allowing the expression of fluorescent reporters in defined populations of cells at selected stages of development, we performed a quantitative analysis of placode shape, cell movements and dynamic axon behaviours during olfactory system development *in vivo*. We showed that the olfactory placode displays an abnormal morphogenesis in *sly* mutants, characterized by aberrant tissue scattering and elongation. Olfactory axonal projections also display major defects including abnormal exit point from the placode and subsequent perturbed growth and navigation within the brain. Our results point to original roles for Laminins and BMs in neuronal development and tissue morphogenesis, such as tissue encapsulation providing resistance to stretching forces, substrate for the growth of sensory axons and precise definition of their entry point into the brain.

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RCOR2 LOCALIZES IN CENTROSOMES AND INTERACTS WITH TUBULIN

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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REST corepressor 2 (RCOR2) belongs to the RCOR family proteins, which are mainly characterized as repressors of neuronal gene expression in non-neuronal cells. RCOR2 is required for cell proliferation and division of embryonic and neural stem cells, and neural progenitor cells. Although RCOR2 is expressed mainly in neural tissues and has a relevant role in the regulation of cortex development, it is unknown how it is distributed at the cellular level. The present study aimed to characterize the distribution of RCOR2 in neuronal cell lines and tissues. We identified cytoplasmic and nuclear subpopulations of RCOR2 in N2A, PC-12, HT22, and HEK293T cells using high-resolution microscopy. Immune staining for RCOR2 showed large granules within the nucleus and a smaller punctuated pattern in the cytoplasm in neuronal cell lines, whereas kidney cells showed a homogeneous small-punctuated pattern. Like neuronal cell lines, striatum cells showed large granules inside the nucleus. Additionally, we found that RCOR2 interacts with tubulin in interphase and mitotic cells. In metaphase cells, RCOR2 tends to accumulate in centrosomes. In conclusion, RCOR2 shows different distribution patterns according to cell type, and its colocalization with tubulin and centrosomes suggests additional functional roles beyond transcriptional corepression.

Pubmed:

34819154: Rivera C, Verbel-Vergara D, Arancibia D, Lappala A, González M, Guzmán F, Merello G, Lee JT, Andrés ME
Revealing RCOR2 as a regulatory component of nuclear speckles.
Nuclear processes such as transcription and RNA maturation can be impacted by subnuclear compartmentalization in condensates and nuclear bodies. Here, we characterize the nature of nuclear granules formed by REST corepressor 2 (RCOR2), a nuclear protein essential for pluripotency maintenance and central nervous system development.
Epigenetics Chromatin, 2021; 14
32215915: Yarur HE, González MP, Verbel-Vergara D, Andrés ME, Gysling K
Cross-talk between dopamine D1 and corticotropin releasing factor type 2 receptors leads to occlusion of their ERK1/2 signaling.
One manner in which G protein-coupled receptors potentiate, increase, and change their functionality is through the formation of heteromers in a specific cellular context. Previously, we have shown that dopamine D1 receptor (D1R) and the corticotropin releasing factor receptor type-2α (CRF2α) heteromerize in HEK293T cells, enabling D1R to mobilize intracellular calcium in response to D1R agonists. In this study, we further investigated the pharmacological properties of the CRF2α-D1R heteromer and the consequences of the heteromerization in their signaling and subcellular localization when both receptors are co-expressed in HEK293T cells. Using immunoprecipitation assays, we observed that the addition of 10 μM dopamine in the incubation medium significantly decreased the amount of CRF2α on the cell surface of cells expressing both receptors. The presence of agonists of both receptors increased the interaction between CRF2α and D1R as assessed by co-immunoprecipitation. However, the presence of agonists of both receptors resulted in a lesser efficient activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase. Using a synaptosomal preparation of rat prefrontal cortex devoid of post-synaptic elements, we found that CRF2α and D1R co-localize in synaptic terminals of the rat medial prefrontal cortex and that the simultaneous activation of both receptors also occluded phosphorylation of extracellular signal-regulated kinase. These results strengthen the idea that the heteromer CRF2a-D1R is an entity functionally different from each receptor that composes it and suggests that its formation is enhanced by CRF and dopamine co-transmission, as occurs in stress and addiction.
J Neurochem, 2020; 155
29161490: Vergara JG, Verbel-Vergara D, Montesino AM, Pérez-Doria A, Bejarano EE
[Estimation of time detection limit for human cytochrome b in females of Lutzomyia evansi].
Molecular biology techniques have allowed a better knowledge of sources of blood meals in vector insects. However, the usefulness of these techniques depends on both the quantity of ingested blood and the digestion process in the insect.

Biomedica, 2017; 37
27622491: Paternina LE, Verbel-Vergara D, Bejarano EE
Comparison of 16S and COX1 genes mitochondrial regions and their usefulness for genetic analysis of ticks (Acari: Ixodidae).
In recent decades the analysis of mitochondrial genes has been used for population and phylogenetic studies of ticks allowing many advances in their systematics. Mitochondrial ribosomal 16S (16S) subunit is one of the most frequently used among those genes available for tick analysis, whereas cytochrome oxidase gene 1 (COX1) has recently been used and proposed as an alternative to the traditional 16S gene marker.
Biomedica, 2016; 36
26464046: Paternina LE, Verbel-Vergara D, Romero-Ricardo L, Pérez-Doria A, Paternina-Gómez M, Martínez L, Bejarano EE
Evidence for anthropophily in five species of phlebotomine sand flies (Diptera: Psychodidae) from northern Colombia, revealed by molecular identification of bloodmeals.
Identification of the bloodmeal sources of phlebotomine sand flies is fundamental to determining which species are anthropophilic and understanding the transmission of Leishmania parasites in natural epidemiological settings. The objective of this study was to identify sand fly bloodmeals in the mixed leishmaniasis focus of the department of Sucre, northern Colombia. In all 141 engorged female sand flies were analyzed, after being captured in intradomiciliary, peridomiciliary and extradomiciliary habitats with Shannon and CDC traps and by active searching in diurnal resting sites. Bloodmeals were identified by sequencing and analysis of a 358bp fragment of the mitochondrial gene Cytochrome b (CYB) and a 330bp fragment of the nuclear gene prepronociceptin (PNOC). Using both genes 105 vertebrate bloodmeals were identified, with an efficiency of 72% for CYB but only 7% for PNOC. Ten species of vertebrates were identified as providing bloodmeal sources for 8 sand fly species: Homo sapiens (Lutzomyia evansi, Lutzomyia panamensis, Lutzomyia micropyga, Lutzomyia shannoni and Lutzomyia atroclavata), Equus caballus (L. evansi, L. panamensis and Lutzomyia cayennensis cayennensis), Equus asinus (L. evansi and L. panamensis), Bos taurus (L. evansi, L. panamensis and L. c. cayennensis), Tamandua mexicana (L. shannoni and Lutzomyia trinidadensis), Proechimys guyanensis (L. evansi, L. panamensis and L. c. cayennensis), Mabuya sp. (Lutzomyia micropyga), Anolissp. (L. micropyga), Sus scrofa (L. evansi and Lutzomyia gomezi) and Gallus gallus (L. evansi). Cattle, donkeys, humans and pigs were significantly more important than other animals (P=0.0001) as hosts of L. evansi, this being the most abundant sand fly species. The five Lutzomyia species in which blood samples of human origin were detected included L. micropyga and L. atroclavata, constituting the first evidence of anthropophily in both species.
Acta Trop, 2016; 153

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GENERATION OF BRAIN ORGANOIDS WITH IMPROVED NEURAL FATE SPECIFICITY AND BLOCKAGE OF ABERRANT DEVELOPMENT OF MESODERMAL-DERIVED TISSUE

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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AIM Human brain organoids provide a valuable model to interrogate human-specific aspects of neurodevelopmental processes and disease pathology. Despite the advances in generation and manipulation of human induced pluripotent stem cells (hiPSCs), these are known to possess an epigenetic memory, which influences their capacity to differentiate into ectoderm and downstream neural cell types. Here, we explore the potential of using biocompatible and biodegradable 3D-printed polycaprolactone (PCL) scaffolds to constrain hiPSCs into adopting a neural fate, rather than other lineage identities. **METHODS** We use standardized human brain organoid cell culture conditions to compare spherical brain organoids with scaffold-based engineered flat brain organoids (efBOs). Their identity is subsequently analyzed using histological analyses and western blot. **RESULTS** Spherical brain organoids self-organize into regionally distinct parts that correspond to neural tissue, characterized by neural markers (TUJ1, DCX); and cardiac tissue, visualized as a synchronously beating contracting region. The identity of the second is confirmed by the presence of cardiomyocytes (ACTN1) and vessel structures spreading from the heart region (CD31). On the contrary, structural support eliminates the presence of cardiac tissue, as well as it enhances neural differentiation (NES, TUJ1, DCX, MAP2) of efBOs. These develop thick, radially organized neuroectoderm that beings to fold, resembling the gyrification process during human brain development. **CONCLUSIONS** We conclude that structural cues can aid hiPSCs in overriding their intrinsic tendency to preferentially differentiate into one of the three germ layers. By favoring neuronal differentiation, this approach could become a valuable tool for hiPSC-derived brain organoids and modeling neurological disorders.

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UNDERSTANDING ARRHINIA AND ITS MOLECULAR PATHWAYS: WHERE ARE WE NOW?

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Introduction: Congenital arrhinia is a rare medical condition with unknown causes; it has been related to alterations in the eyes, ears, palate, and other midline defects. **Methods:** We conducted a systematic review on MEDLINE, OVID, COCHRANE, and google scholar for clinical and molecular reports of Arhinia. The literature search was restricted to articles in English and Spanish, all the clinical reports were considered and further molecular analysis was done with reports with molecular data analysis of the arhinia spectrum including (Bosman syndrome, isolated arhinia, and holoprosencephaly) were done. Gene assortment was developed through a systematic literature review. Chromosomal aberrations and genetic variants were filtered, classified, and summarized. The arhinia-related genotype was analyzed by developing a protein-protein network using Cytoscape software (String database). The protein-protein network analysis was broken down into two major categories: comprehensive gene co-expression analysis particular to arrhinia and precision gene co-expression analysis suitable to biological features. **Results:** A total of 43 manuscripts were included. A sample of 78 patients in the arhinia spectrum was identified. The most frequent chromosomal aberration was trisomy 9, 13, and 18. Different genes and proteins were found related to the arhinia spectrum such as; PAX6, CHD7, SOX2, OTX2, and SMCHD1. These genes also formed a significant protein-protein interaction with other proteins involved in the biological features, which compromise mainly head and neck development **Discussions:** The present study characterized the clinical and molecular spectrum and suggests the possible pathophysiological pathways, which might be derived from the development of the arhinia spectrum.

Pubmed:

34542925: Pachajoa H, Acosta MA, Alméciga-Díaz CJ, Ariza Y, Diaz-Ordoñez L, Caicedo-Herrera G, Cuartas D, Nastasi-Catanese JA, Ramírez-Montaño D, Silva YK, Moreno L, Satizabal J, Garcia N, Montoya J, Prada C, Porras G, Velasco H, Candelo E
Molecular characterization of mucopolysaccharidosis type IVA patients in the Andean region of Colombia.
Colombia has a high prevalence of mucopolysaccharidosis (MPS) type IVA. Nevertheless, data regarding the mutation spectrum for MPS IVA in this population have not been completely characterized. Forty-seven families and 53 patients from seven different Colombian regions were tested for MPS IVA mutations. We compared the sequences with the N-acetylgalactosamine-6-sulfatase (GALNS) reference sequence NM_000512.4, and gene variants were reported. Bioinformatics analysis was performed using SWISS-MODEL. The mutant proteins were generated by homology from the wild-type GALNS 4FDJ template obtained from the PDB database, and visualization was performed using Swiss-PDBViewer and UCSF Chimera. The predictive analysis was run using different bioinformatic tools, and the deleterious annotation of genetic variants was performed using a neural network. We found that 79% and 21% of the cohort was homozygous and compound heterozygous, respectively. The most frequent mutation observed was p.Gly301Cys (78.3% of alleles), followed by p.Arg386Cys (10.4% of alleles). A novel mutation (p.Phe72Ile) was described and classified in silico as a pathogenic variant. This study reveals the mutation spectrum of MPS IVA in Colombia. The high prevalence of the p.Gly301Cys mutation suggests a founder effect of this variant in the Colombian population that causes diseases in the Andean region (via migration). These data can facilitate genetic counseling, prenatal diagnosis, and the design of therapeutic interventions.
Am J Med Genet C Semin Med Genet, 2021; 187
33815457: Candelo E, Sanz AM, Ramirez-Montaño D, Diaz-Ordoñez L, Granados AM, Rosso F, Nevado J, Lapunzina P, Pachajoa H
A Possible Association Between Zika Virus Infection and CDK5RAP2 Mutation.
Flaviviridae family belongs to the Spondweni serocomplex, which is mainly transmitted by vectors from the genus. Zika virus (ZIKV) is part of this genus. It was initially reported in Brazil in December 2014 as an unknown acute generalized exanthematous disease and was subsequently identified as ZIKV infection. ZIKV became widespread all over Brazil and was linked with potential cases of microcephaly.
Front Genet, 2021; 12


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MONITORING THE COMPLEXIFICATION OF CEREBELLAR AND THALAMIC CELLS DURING EARLY DEVELOPMENT WITH DIFFUSION-WEIGHTED MR SPECTROSCOPY

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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Aims This work aims to assess the potential of diffusion-weighted magnetic resonance spectroscopy (DWMRS) to follow cerebellar and thalamic cell development in the healthy rat brain from P5 to P30. Monitoring non-invasively the morphological development of brain cells in the neonate and infant brain could provide relevant biomarkers for tracking neurodevelopmental disorders. DWMRS is a MR-based method informing about the average morphology (i.e., cyto-architecture) of specific cell types (e.g. neurons, astrocytes) in a large region of interest. It is already implemented in some human scanners, providing the method with a high translational potential. **Methods** 18 Sprague-Dawley rats were scanned at 7T (at P5, P10, P15, P20 and P30), with a DWMRS sequence providing accurate voxel localisation and designed to measure the diffusion properties of metabolites at multiple diffusion times and diffusion-weightings (**FigA**), necessary for cyto-architecture modeling. **Results** Diffusion properties differ between both regions at each age, revealing different microenvironments. Diffusion of total creatine suggests it diffuses in smaller structures at P5/P10 than P20/30, as expected. Diffusion of taurine changes dramatically with age: its cellular compartmentation likely evolves during development. The cyto-architecture modeling relies on the assumption that metabolites primarily diffuse in long fibers. From P15 to P30, cell growth and complexification in both structures is well captured by the data and their modeling, but as expected, different modeling hypotheses may be needed at P5/P10, when the cell body/processes ratio is too high (**FigB/C**).

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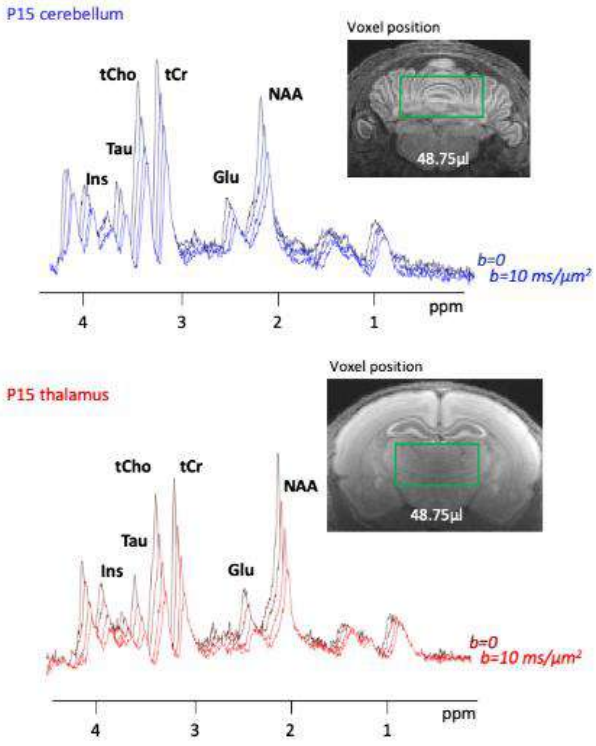
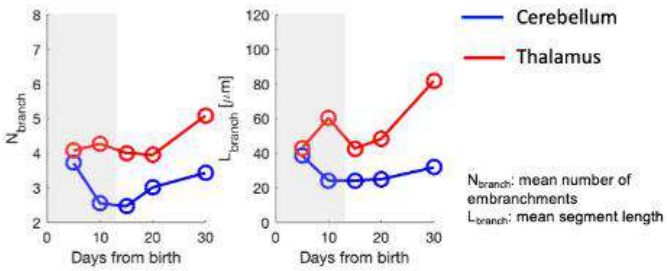
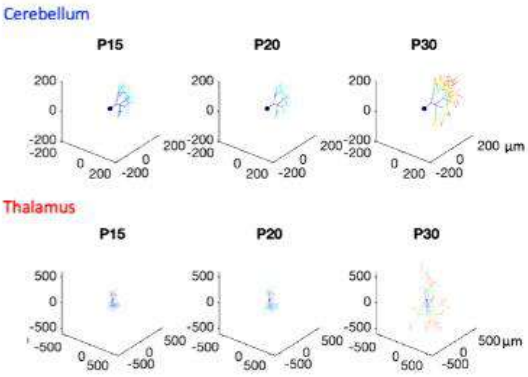
A. Representative high diffusion weightings DWMRS data



B. Cyto-architecture parameters calculated by the modeling of the total creatine long diffusion times data



C. Visualization of the cyto-architecture from P15 to P30 in both regions



Conclusion Overall results show that DWMRS is sensitive to microstructural changes in the developing brain.

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ONTOGENY OF THE CAUDAL NEUROSECRETORY SYSTEM IN ZEBRAFISH REVEALED BY A TRANSGENIC (UTS2A:GFP) LINE.

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Faredin Alejevski[1], Bérénice Bichon[1], Anne-Laure Gaillard[1], Fabrice Girardot[1], Caroline Parmentier[2], Guillaume Pézeron[1], Hervé Tostivint[1]
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The caudal neurosecretory system (CNSS) is a neuroendocrine complex whose existence is restricted to fishes. In teleosts, the neuroendocrine neurons of the CNSS, called Dahlgren cells, are located in the terminal segments of the spinal cord, and project to a neurohaemal organ, the urophysis, from which several neuropeptides, including urotensins, are released. Very little is currently known about the development of the CNSS. The aim of the present study was to study the ontogeny this system by using the zebrafish as a model. For this purpose a Tg(*uts2a : gfp*) fluorescent reporter line was constructed, in which GFP expression is driven by the uts2a gene promoter. The Tg(*uts2a : gfp*) line recapitulates faithfully the endogenous expression profile of the uts2a gene and allows to visualize Dahgren cells. With this line, the first GFP^+ cells could be detected as early as the hatching period in the caudal spinal cord. A few GFP^+ cell processes could be seen to project rostrally towards the brain, but most of them appeared to project towards the urophysis anlage. The morphogenesis of the urophysis could be indirectly followed thanks to the accumulation of GFP^+ fibers within it. In order to visualize the neurovascular interface within the urophysis, a Tg(uts2a : RFP) line was crossed with a vascular endothelial reporter expressing GFP in *kdr* -positive cells. The study of this new line is currently under investigation. In conclusion, the present work provides the basis for further studies on the mechanisms of development of the CNSS in zebrafish.

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THE DEVELOPMENT OF CORNEAL INNERVATION; A 3D ANALYSIS IN MICE AND HUMANS.

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Quentin Rappeneau, Elena Bizzarri, Raphael Blain, Nacim Bouheraoua, Alain Chédotal
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The cornea is the transparent surface of the eye that undergoes a life-long renewal. This tissue is composed of a stratified epithelium supported by a mesenchymal stroma. The cornea is also the most densely innervated tissue in the human body. It primarily receives sensory nerves originating from trigeminal ganglion neurons but also contains axons of the autonomic nervous system. The development of corneal nerves has been relatively well studied in chick embryos and to a lesser extent in mice. However, how the different types of axons invade the embryonic cornea and interact together and with corneal cells remains unknown. Importantly, what we know about the development of corneal innervation during human embryogenesis relies on a unique study performed more than 60 years ago. Our goal is to perform a comprehensive 3D analysis of the development of corneal nerves in mice and humans. We are combining whole-mount immunostaining and 3D imaging of solvent-cleared organs (iDISCO) with Light sheet fluorescence microscopy from embryonic day 12 (E12) to postnatal day 9 (P9) in mice and 6-12 post-conceptional weeks in human. We already validated a large panel of antibodies that allow visualizing embryonic sensory and autonomic nerves and neurons in mice and human embryos. Moreover, we used several transgenic mouse lines in which fluorescent proteins are expressed in all or a subset of corneal nerves. The pathway and developmental time-course of trigeminal, sympathetic and parasympathetic axons and is being analyzed in 3D.

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THE MOLECULAR FOUNDATION OF PROPRIOCEPTOR MUSCLE-TYPE IDENTITY

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Stephan Dietrich[1], Carlos Company[2], Kun Song[3], Elijah Lowenstein[4], Levin Riedel[1], Carmen Birchmeier[4], Gaetano Gargiulo[2], Niccolò Zampieri[1]
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Recent transcriptomic efforts to molecularly profile broad classes of neurons revealed considerable diversity among the major types of somatosensory neurons. In contrast, proprioceptive sensory neurons (PN) seem to represent a more homogenous population and so far, only molecular signatures for the three receptor types - Ia, Ib, II - have been described. In order to precisely adjust motor output according to the biomechanical requirements of different muscles, PN innervate central and peripheral targets with exquisite specificity, implying the existence of molecular programs defining PN muscle-type identities. However, the mechanisms controlling this critical aspect of PN identity are not clear yet. We devised a single cell transcriptomic approach that takes advantage of the topographic organization of the proprioceptive system to reveal features of cardinal proprioceptor muscle-types - epaxial and hypaxial - defined by their connectivity to limb, back, and abdominal muscles. First, we identified and validated molecular signatures for each of these subtypes. Second, we found that molecular programs defining their identities are acquired early in development and maintained until early postnatal stages. Finally, we discovered a role for ephrin-A5, a specific marker for limb-projecting PN, in the control of limb muscles connectivity. Altogether, this work reveals the molecular foundation of proprioceptor muscle-type identity, thus paving the way for studying the development and function of muscle specific sensory feedback circuits.

Pubmed:
25589789: Schmidt A, Dietrich S, Steuer A, Weltmann KD, von Woedtke T, Masur K, Wende K
Non-thermal plasma activates human keratinocytes by stimulation of antioxidant and phase II pathways.
Non-thermal atmospheric pressure plasma provides a novel therapeutic opportunity to control redox-based processes, e.g. wound healing, cancer, and inflammatory diseases. By spatial and time-resolved delivery of reactive oxygen and nitrogen species, it allows stimulation or inhibition of cellular processes in biological systems. Our data show that both gene and protein expression is highly affected by non-thermal plasma. Nuclear factor erythroid-related factor 2 (NRF2) and phase II enzyme pathway components were found to act as key controllers orchestrating the cellular response in keratinocytes. Additionally, glutathione metabolism, which is a marker for NRF2-related signaling events, was affected. Among the most robustly increased genes and proteins, heme oxygenase 1, NADPH-quinone oxidoreductase 1, and growth factors were found. The roles of NRF2 targets, investigated by siRNA silencing, revealed that NRF2 acts as an important switch for sensing oxidative stress events. Moreover, the influence of non-thermal plasma on the NRF2 pathway prepares cells against exogenic noxae and increases their resilience against oxidative species. Via paracrine mechanisms, distant cells benefit from cell-cell communication. The finding that non-thermal plasma triggers hormesis-like processes in keratinocytes facilitates the understanding of plasma-tissue interaction and its clinical application.
J Biol Chem, 2015; 290
29507411: Chatterjee S, Sullivan HA, MacLennan BJ, Xu R, Hou Y, Lavin TK, Lea NE, Michalski JE, Babcock KR, Dietrich S, Matthews GA, Beyeler A, Calhoon GG, Glober G, Whitesell JD, Yao S, Cetin A, Harris JA, Zeng H, Tye KM, Reid RC, Wickersham IR
Nontoxic, double-deletion-mutant rabies viral vectors for retrograde targeting of projection neurons.
Recombinant rabies viral vectors have proven useful for applications including retrograde targeting of projection neurons and monosynaptic tracing, but their cytotoxicity has limited their use to short-term experiments. Here we introduce a new class of double-deletion-mutant rabies viral vectors that left transduced cells alive and healthy indefinitely. Deletion of the viral polymerase gene abolished cytotoxicity and reduced transgene expression to trace levels but left vectors still able to retrogradely infect projection neurons and express recombinases, allowing downstream expression of other transgene

products such as fluorophores and calcium indicators. The morphology of retrogradely targeted cells appeared unperturbed at 1 year postinjection. Whole-cell patch-clamp recordings showed no physiological abnormalities at 8 weeks. Longitudinal two-photon structural and functional imaging in vivo, tracking thousands of individual neurons for up to 4 months, showed that transduced neurons did not die but retained stable visual response properties even at the longest time points imaged.
Nat Neurosci, 2018; 21

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HOW TO MAKE THE MOST FRIENDLY MICE: THE ROLE OF SEROTONIN IN ADRENAL GLAND DEVELOPMENT

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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[1]Karolinska institute, Department Of Phisiology And Pharmacology, Solna, Sweden, [2]Medical University of Vienna, Center for Brain research, Department Of Neuroimmunology, Vienna, Austria, [3]Koltsov Institute of Developmental Biology, Department Of Developmental Neurobiology, Moscow, Russian Federation, [4]Karolinska institute, Department Of Phisiology And Pharmacology, Stockholm, Solna, Sweden, [5]Zakusov institute of pharmacology, Laboratory Of Phycopharmacology, Moscow, Russian Federation, [6]Sechenov First Moscow State Medical University, Department Of Pharmacology, Moscow, Russian Federation, [7]Zakusov institute of pharmacology, Laboratory Of Psychopharmacology, Moscow, Russian Federation, [8]MDC Berlin, Ag Bader, Berlin, Germany, [9]Masaryk University, Department Of Experimental Biology, Brno, Czech Republic, [10]St. Anne's University Hospital, International Clinical Research Center, Brno, Czech Republic, [11]Brno University of Technology, Central European Institute Of Technology, Brno, Czech Republic, [12]University of Pisa, Unit Of Cell And Developmental Biology, Department Of Biology, Pisa, Italy, [13]ICM - Institut du Cerveau | Paris Brain Institute, Sorbonne Université - Cnrs - Inserm, Paris, France, [14]Severtsov Institute of Ecology and Evolution, Department Of Populational Ecology, Moscow, Russian Federation, [15]Karolinska Institute, Department Of Neuroscience, Stockholm, Sweden

Adrenal gland medulla releases catecholamines mediating the bodily and behavioral responses to stress. The chromaffin cells of the adrenal medulla originate from nerve-associated Schwann Cell Precursors (SCP). SCPs lineage progression towards chromaffin cells includes a specific "bridge"-cell-state, with a key marker, the HTR3A gene which encodes a serotonin (5HT) receptor. Therefore, we hypothesized that 5HT plays a role in chromaffin cell development. Moreover, the adrenal gland is the typical location for neuroblastoma and the mechanism controlling differentiation may provide clues for neuroblastoma emergence. We investigated 5HT's role in adrenal gland development by combining single-cell transcriptomics, pharmacological treatments, immunohistochemistry, RNA in situ hybridization, behavioral studies, and evaluation of neuroblastoma cell lines. Following the increase of 5HT in pregnant mice and rats during the "bridge" stage, the number of chromaffin cells in embryos decreased due to a slowdown of "bridge" cells' cell-cycle progression. This mechanism appears to be responsible for preventing the overgrowth of chromaffin cells and may act in an autocrine fashion, as chromaffin cells are 5HT-positive. This results in a decreased number of chromaffin cells in adult life causing a drop in circulating levels of catecholamines and consequently, behavioral changes, rendering animals more cooperative and explorative. Importantly, HTR3Ahigh neuroblastoma cell lines result in tumor formation in contrast to HTR3Alow cells. Moreover, their proliferation is reduced in response to HTR3A receptor stimulation. Taking together, modulation of 5HT signaling during a specific period of chromaffin cell differentiation represents an important mechanism for the control of organ growth, neoplasia development, and animal behavior.

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MULTIPLE REGIONALIZED GENES AND THEIR PUTATIVE NETWORKS IN THE INTERPEDUNCULAR NUCLEUS SUGGEST COMPLEX MECHANISMS OF NEURON DEVELOPMENT AND AXON GUIDANCE

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

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The interpeduncular nucleus (IPN) is a highly conserved limbic structure in the vertebrate brain, located in the isthmus and rhombomere 1. It is formed by various populations that migrate from different sites to the distinct domains within the IPN: the prodromal, rostral interpeduncular, and caudal interpeduncular nuclei. The aim here was to identify genes that are differentially expressed across these domains, characterizing their putative functional roles and interactions. To this end, we screened the 2,038 genes in the Allen Developing Mouse Brain Atlas database expressed at E18.5 and we identified 135 genes expressed within the IPN. The functional analysis of these genes highlighted an overrepresentation of gene families related to neuron development, cell morphogenesis and axon guidance. The interactome analysis within each IPN domain yielded specific networks that mainly involve members of the ephrin/Eph and Cadherin families, transcription factors and molecules related to synaptic neurotransmission. These results bring to light specific mechanisms that might participate in the formation, molecular regionalization, axon guidance and connectivity of the different IPN domains. This genoarchitectonic model of the IPN enables data on gene expression and interactions to be integrated and interpreted, providing a basis for the further study of the connectivity and function of this poorly understood nuclear complex under both normal and pathological conditions.

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MICROFLUIDIC HIGH-THROUGHPUT SCREENING PLATFORM TO SCREEN PRE-CLINICAL STAGE COMPOUND EFFECTS ON NEURITE OUTGROWTH OF HUMAN MOTOR NEURONS POST INJURY

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Jessica Rontard, Aurélie Batut, Delphine Debis, Benoît Maisonneuve, Louise Dubuisson, Mélanie Gleyzes, Margot Libralato, Janaina Vieira, Marion Hochedel, Yannick Calderini, Thibault Honegger
NETRI, Auvergne Rhône Alpes, Lyon, France

There is a significant demand for physiologically relevant models of SCI. The major modeling challenge is to accurately perform the injury only on the neurites without affecting cell viability and to apply the pre-clinical stage compounds on cell soma only. Microfluidic chip designs applied to neuroscience research are based on connection between compartmentalized neuronal populations through guiding neurite outgrowth by using microchannels. The longitude of microchannels acts as a selective barrier for the exclusive passage of axons over dendrites and it promotes the unidirectional growth of the neurites from one compartment to the other. We will present the design of a compartmentalized microfluidic device with three compartments capable of both inducing a localized axotomy and a isolation of soma and axons. Human-induced pluripotent stem cell-derived motor neurons are maintained up to 7 weeks in vitro. We performed a chemically-induced axonal injury on isolated axons in the middle compartment only. We then follow the dynamics of the axonal regeneration using the triangular methodology with and without pharmacological compounds in soma channel. We show that this model can be sucessfully used for quantitative analysis of neurite outgrowth dynamics. To further strengthen the efficacy of those strategies, we will present a new technical feature with addition of a PDMS layer in which the microgrooves are hollowed out. Our data show an increase in the rate of axonal projections into triangular channel and an improvement in axon orientation and elongation.

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BOARD NUMBER: S05-323

EVALUATION OF THE ROLE OF EARLY NEURONAL ACTIVITY ON THE ZEBRAFISH DOPAMINERGIC CELLS DEVELOPMENT, A TRANSCRIPTOMIC STUDY.

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Michael Demarque
NeuroPSI, Neuroinfection And Immunity, saclay, France

Interneuronal communication starts before the establishment of the synapses with alternative forms of neuronal excitability, called here Embryonic Neuronal Excitability (ENE). We recently established the zebrafish as a model to study the developmental contribution of ENE to the differentiation of dopaminergic neurons. Based on previous work in Xenopus, we have validated pharmocological treatments to increase (Veratridine 10 µM) or decrease (TTX 2.5µM, Conotoxin 0.1µM, Nifedipin 0.5µM, Flunarizin 2.5µM) (TCNF) ENE in zebrafish embryos. To identify the genes whose expression was modified in monoaminergic precursors following alterations of ENE, we performed a comparative RNAseq analysis following these validated ENE-increasing or -decreasing treatments. vMAT2, encoded by slc18a2, is the key transporter of monamine neurotransmitters into synaptic vesicles. To restrict the analysis to monoaminergic cells, we FACS-sorted GFP-positive cells from dissociated Tg(VMAT2:GFP) embryos, 24 hours after pharmacological treatment. Bulk RNA-sequencing was conducted on these sorted cells, providing a list of activity-dependent factors in monoaminergic neurons. In this list we identified some transcription factors involved in the specification of the dopaminergic phenotype, including pax6a whose expression is decreased following TCNF exposure and dlx2a whose expression is decreased following veratridine exposure, and interestingly the expression of transcripts related to the innate immune response is also modified. The functional effects of the best candidate genes will be studied by gene inactivation using CRISPR technology. These results provide a convenient framework to study the molecular mechanisms linking ENE to neurotransmitter specification, with relevance to the pathogenesis of neurodevelopmental disorders. plutôt: synaptic networks?

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DEVELOPMENTAL PROCESSES GOVERNING CELL TYPE IDENTITY IN THE CEREBRAL CORTEX

POSTER SESSION 05 - SECTION: EVO-DEVO - EVOLUTIONARY DEVELOPMENTAL NEUROSCIENCE

Katherine Matho[1], Xiaoyin Chen[2], Sweta Srivas[3], Erica Bulzomi[1], Dhananjay Huilgol[3], Z. Josh Huang[1,3]
[1]Cold Spring Harbor Laboratory, Neuroscience, Cold Spring Harbor, United States of America, [2]Allen Brain Institute for Brain Science, Neuroscience, Seattle, United States of America, [3]Duke University, Neuroscience, Durham, United States of America

Major efforts have aimed to classify the excitatory glutamatergic pyramidal neuron (PyN) cell types of cerebral cortex based on molecular, anatomic, and physiological features. Additionally, neurodevelopmental research has reconstructed developmental trajectories for PyNs based on fixed timepoints and pseudotime analyses. However, a significant knowledge gap remains in connecting the embryonic developmental history of PyNs with their ultimate cell fate in the mature, functional brain. PyNs arise from radial glial progenitors (RG) by direct or indirect neurogenesis (dNG or iNG) at specific embryonic timepoints. Advances in mouse genetics, based on decades of prior knowledge of cortical development and candidate marker genes of PyN subsets, have recently provided gene knock-in (KI) mouse lines to access and manipulate PyNs and progenitors. Here, we focus on a population of extratelencephalic PyNs which emerges during embryonic development from both dNG and iNG. We correlate the mode of neurogenesis, whether dNG or iNG, with molecular signatures using spatial transcriptomics in the adult cerebral cortex. This multi-modal approach reveals the effect of a transient developmental event occurring embryonically—dNG versus iNG—on PyN identity as we measure molecular signatures as a cell type readout. Assessing the relationship between developmental origin and cell-types allows us to achieve a finer granularity, required for understanding cortical function.

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HUMAN IPSCS-DERIVED OLIGODENDROCYTES AND ASTROCYTES AS THE FIRST AUTOSOMAL DOMINANT LEUKODYSTROPHY-RELEVANT CELLULAR MODELS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

Martina Lorenzati[1], Marta Ribodino[1], Elena Signorino[1], Ersilia Nicorvo[1], Piercesare Grimaldi[2], Paola Berchialla[3], Luciano Conti[4], Pietro Cortelli[5], Elisa Giorgio[6], Annalisa Buffo[1]
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Autosomal Dominant Leukodystrophy (ADLD) is a rare genetic disease characterized by autonomic dysfunction and movement disorder and associated with white matter loss in the central nervous system. The genetic cause is the presence of three copies of the gene that contains the instructions to produce the lamin B1 (LMNB1) protein, which belongs to a group of structural proteins forming the nuclear membrane of the cell. Pathogenic mechanisms in ADLD have only initially been explored. Moreover, a therapy to treat this disease is not available at the moment. Based on the evidence showing glial pathology in ADLD patients, we generated human glial cells from both ADLD patient- and healthy donor (CTRL)- derived human-induced pluripotent stem cells (hiPSCs). We established a differentiation protocol based on three stages: the commitment to neural progenitors, the production of gliospheres and a further maturation step into authentic oligodendrocytes and astrocytes. Preliminary observations indicate a lower gliogenic potential in the hiPSC ADLD lineages, as revealed by gliosphere production. In ADLD astrocytes an altered expression of LMNB1 is confirmed by RT-qPCR and protein expression, together with morphological alterations affecting the nuclear shape that in turn impacts certain morphological features of the cell body (ramifications, area covered). Functional analyses will be devoted to investigate possible alterations correlated to the LMNB1 higher expression. Thus, the developed model appears as a promising "disease-in-a-dish" platform to further reveal so far unknown dysfunctions of the diseased cells and, prospectively, aid the development of effective therapeutic strategies for this rare genetic disease.

Pubmed:
33790350: Lorenzati M, Boda E, Parolisi R, Bonato M, Borsello T, Herdegen T, Buffo A, Vercelli A
c-Jun N-terminal kinase 1 (JNK1) modulates oligodendrocyte progenitor cell architecture, proliferation and myelination.
During Central Nervous System ontogenesis, myelinating oligodendrocytes (OLs) arise from highly ramified and proliferative precursors called oligodendrocyte progenitor cells (OPCs). OPC architecture, proliferation and oligodendro-/myelino-genesis are finely regulated by the interplay of cell-intrinsic and extrinsic factors. A variety of extrinsic cues converge on the extracellular signal-regulated kinase/mitogen activated protein kinase (ERK/MAPK) pathway. Here we found that the germinal ablation of the MAPK c-Jun N-Terminal Kinase isoform 1 (JNK1) results in a significant reduction of myelin in the cerebral cortex and corpus callosum at both postnatal and adult stages. Myelin alterations are accompanied by higher OPC density and proliferation during the first weeks of life, consistent with a transient alteration of mechanisms regulating OPC self-renewal and differentiation. JNK1 KO OPCs also show smaller occupancy territories and a less complex branching architecture in vivo. Notably, these latter phenotypes are recapitulated in pure cultures of JNK1 KO OPCs and of WT OPCs treated with the JNK inhibitor D-JNKI-1. Moreover, JNK1 KO and WT D-JNKI-1 treated OLs, while not showing overt alterations of differentiation in vitro, display a reduced surface compared to controls. Our results unveil a novel player in the complex regulation of OPC biology, on the one hand showing that JNK1 ablation cell-autonomously determines alterations of OPC proliferation and branching architecture and, on the other hand, suggesting that JNK1 signaling in OLs participates in myelination in vivo.
Sci Rep, 2021; 11
33252173: Giorgio E, Pesce E, Pozzi E, Sondo E, Ferrero M, Morerio C, Borrelli G, Della Sala E, Lorenzati M, Cortelli P, Buffo A, Pedemonte N, Brusco A
A high-content drug screening strategy to identify protein level modulators for genetic diseases: A proof-of-principle in

autosomal dominant leukodystrophy.
In genetic diseases, the most prevalent mechanism of pathogenicity is an altered expression of dosage-sensitive genes. Drugs that restore physiological levels of these genes should be effective in treating the associated conditions. We developed a screening strategy, based on a bicistronic dual-reporter vector, for identifying compounds that modulate protein levels, and used it in a pharmacological screening approach. To provide a proof-of-principle, we chose autosomal dominant leukodystrophy (ADLD), an ultra-rare adult-onset neurodegenerative disorder caused by lamin B1 (LMNB1) overexpression. We used a stable Chinese hamster ovary (CHO) cell line that simultaneously expresses an AcGFP reporter fused to LMNB1 and a Ds-Red normalizer. Using high-content imaging analysis, we screened a library of 717 biologically active compounds and approved drugs, and identified alvespimycin, an HSP90 inhibitor, as a positive hit. We confirmed that alvespimycin can reduce LMNB1 levels by 30%-80% in five different cell lines (fibroblasts, NIH3T3, CHO, COS-7, and rat primary glial cells). In ADLD fibroblasts, alvespimycin reduced cytoplasmic LMNB1 by about 50%. We propose this approach for effectively identifying potential drugs for treating genetic diseases associated with deletions/duplications and paving the way toward Phase II clinical trials.
Hum Mutat, 2021; 42
31143934: Giorgio E, Lorenzati M, Rivetti di Val Cervo P, Brussino A, Cernigoj M, Della Sala E, Bartoletti Stella A, Ferrero M, Caiazzo M, Capellari S, Cortelli P, Conti L, Cattaneo E, Buffo A, Brusco A
Allele-specific silencing as treatment for gene duplication disorders: proof-of-principle in autosomal dominant leukodystrophy.
Allele-specific silencing by RNA interference (ASP-siRNA) holds promise as a therapeutic strategy for downregulating a single mutant allele with minimal suppression of the corresponding wild-type allele. This approach has been effectively used to target autosomal dominant mutations and single nucleotide polymorphisms linked with aberrantly expanded trinucleotide repeats. Here, we propose ASP-siRNA as a preferable choice to target duplicated disease genes, avoiding potentially harmful excessive downregulation. As a proof-of-concept, we studied autosomal dominant adult-onset demyelinating leukodystrophy (ADLD) due to lamin B1 (LMNB1) duplication, a hereditary, progressive and fatal disorder affecting myelin in the CNS. Using a reporter system, we screened the most efficient ASP-siRNAs preferentially targeting one of the alleles at rs1051644 (average minor allele frequency: 0.45) located in the 3' untranslated region of the gene. We identified four siRNAs with a high efficacy and allele-specificity, which were tested in ADLD patient-derived fibroblasts. Three of the small interfering RNAs were highly selective for the target allele and restored both LMNB1 mRNA and protein levels close to control levels. Furthermore, small interfering RNA treatment abrogates the ADLD-specific phenotypes in fibroblasts and in two disease-relevant cellular models: murine oligodendrocytes overexpressing human LMNB1, and neurons directly reprogrammed from patients' fibroblasts. In conclusion, we demonstrated that ASP-silencing by RNA interference is a suitable and promising therapeutic option for ADLD. Moreover, our results have a broad translational value extending to several pathological conditions linked to gene-gain in copy number variations.
Brain, 2019; 142

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INVESTIGATING THE EFFECTS OF KETONE BODY SUPPLEMENTATION ON THE DEVELOPMENT AND DIFFERENTIATION OF CORTICAL NEURAL STEM CELLS.

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims: To understand how a ketogenic diet may influence brain development, we investigated how the primary ketone body Beta-hydroxybutyrate (β-OHB) affected the growth and differentiation of cortical neural stem cells (NSCs) in the presence or absence of potentially confounding culture metabolic substrates. **Methods:** NSCs were first grown in regular glucose (5 mM), low glucose (1 mM), glucose free (0 mM), glucose free without pyruvate, glucose free without glutamine and glucose free without pyruvate and glutamine containing media and supplemented with β-OHB for four days in vitro (DIV). To further investigate cellular development, NSCs were then differentiated using retinoic acid in the same media groups above for five, 10 and 15 DIV. The immunocytochemical markers βIII-tubulin, actin and DAPI were used to assess differentiation, neurite length and number, cell density and structure. MTT assays and microscopic analysis assessed cell viability. Statistical analysis was performed using GraphPad Prism 9. **Results:** Substrate deprivation reduced the growth, viability, and neuronal phenotype of cells. β-OHB induced a growth response in media without glucose and pyruvate at 4 DIV. β-OHB supplementation resulted in a growth response to cell growth, neurite length and neurite number in 1 mM glucose media and media without glucose and glutamine at 10 DIV and 15 DIV. **Conclusion:** These data indicate that β-OHB may rescue NSC growth in response to glucose deprivation. Furthermore, culture medium compositions play a key role in experimental paradigms where multiple fuel sources may offer alternative inputs to glycolytic and oxidative phosphorylation phases of NSC development.

Pubmed:

33389384: Alherz M, Lee D, Alshangiti A, Roddy D, O'Keeffe G, White R, Barry D
The Growth Response to Beta-Hydroxybutyrate in SH-SY5Y Neuroblastoma Cells is Suppressed by Glucose and Pyruvate Supplementation.
Neuroblastoma (NB) is a childhood malignancy of the sympathetic nervous system and is commonly studied using the SH-SY5Y cell line. Its neoplastic and neurodevelopmental manifestations are characterised by a high glucose demand which maintains its high proliferative capacity. This metabolic phenotype may be utilised in dietary therapies such as the ketone diet which alter substrate availability and thus starve NB cells of their preferred biosynthetic requirements. However, the effects of ketone metabolism on cancer growth remain poorly understood due to the involvement of other metabolic substrates in experimental paradigms and complexities underlying the Warburg effect. We investigated how the primary ketone body beta-hydroxybutyrate (βOHB) affects the growth of SH-SY5Y NB cells in the presence or absence of culture metabolic substrates. We demonstrated that while glucose deprivation reduced the growth and viability of SH-SY5Y cells, they proliferated and were initially unaffected by the addition of βOHB. However, a growth response to βOHB was subsequently revealed in media containing low levels of glucose, as well as in glucose and pyruvate deprived conditions. These data shed light on the roles of metabolic substrate availability as key determinants of the responses of SH-SY5Y NB cells to ketone supplementation.
Neurochem Res, 2021; 46
29689338: Barry D, Ellul S, Watters L, Lee D, Haluska R, White R
The ketogenic diet in disease and development.
The ketogenic diet, low in carbohydrates and high in fat, was initially designed to reduce seizure onset in epilepsy. More recent evidence has shown its effectiveness in the treatment of movement and psychological disorders, and in general health maintenance. The cellular significance of ketone body metabolism during development and in the adult central nervous system is being revealed; however, the effects of replacing glucose with ketone bodies as the brain's primary energy source especially in pregnancy are not fully understood. In this mini-review, we highlight key findings related to the functional consequences of ketone body metabolism and monocarboxylic transporter expression throughout development and adulthood. We outline the therapeutic relevance of ketone bodies, and place a spotlight on the known effects of a maternal

ketogenic diet on the developing brain.
Int J Dev Neurosci, 2018; 68

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BOARD NUMBER: S05-327

THE IMPACT OF HUMAN-SPECIFIC DUPLICATED GENES ON HUMAN CORTICAL NEURON DEVELOPMENT AND FUNCTION

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aim: The cerebral cortex is at the core of brain functions that are expanded in the human species. Human cortical pyramidal neurons display distinctive morphological and functional features, as well as prolonged neotenic development. Even tough gene regulatory networks of corticogenesis appear to be largely conserved among all mammals, the emergence of novel duplicated genes in hominid and human genomes (HS genes) may contribute to species specificities of neurodevelopment. Here we investigated the role of such duplicated genes in the maturation of human pyramidal neurons. Methods: Using a model of xenotransplantation of human cortical neurons in the mouse neonatal cortex, we explored the function of the HS gene SRGAP2C, previously implicated in synaptic development by gain of function in the mouse cortex. This model uniquely allows to assess the development and function of human neurons in vivo, from early development to neural circuit integration. We also extended our studies to other HS genes. Results: We found that the loss of function of SRGAP2C leads to a striking acceleration of dendritic spine and synaptic development of human cortical neurons in vivo, both morphologically and functionally. We also identified potential important functions for other HS genes in the control of human-specific neuronal features. Conclusion: Our data demonstrate the key role of SRGAP2C in the control of developmental timing of human neurons in vivo. They constitute the first evidence of the requirement of HS genes in human neurons, pointing to recent gene duplications as a key drive of human cortex evolution.

Pubmed:
34535062: Libé-Philippot B, Vanderhaeghen P
Cellular and Molecular Mechanisms Linking Human Cortical Development and Evolution.
The cerebral cortex is at the core of brain functions that are thought to be particularly developed in the human species. Human cortex specificities stem from divergent features of corticogenesis, leading to increased cortical size and complexity. Underlying cellular mechanisms include prolonged patterns of neuronal generation and maturation, as well as the amplification of specific types of stem/progenitor cells. While the gene regulatory networks of corticogenesis appear to be largely conserved among all mammals including humans, they have evolved in primates, particularly in the human species, through the emergence of rapidly divergent transcriptional regulatory elements, as well as recently duplicated novel genes. These human-specific molecular features together control key cellular milestones of human corticogenesis and are often affected in neurodevelopmental disorders, thus linking human neural development, evolution, and diseases.
Annu Rev Genet, 2021; 55
31761708: Linaro D, Vermaercke B, Iwata R, Ramaswamy A, Libé-Philippot B, Boubakar L, Davis BA, Wierda K, Davie K, Poovathingal S, Penttila PA, Bilheu A, De Bruyne L, Gall D, Conzelmann KK, Bonin V, Vanderhaeghen P
Xenotransplanted Human Cortical Neurons Reveal Species-Specific Development and Functional Integration into Mouse Visual Circuits.
How neural circuits develop in the human brain has remained almost impossible to study at the neuronal level. Here, we investigate human cortical neuron development, plasticity, and function using a mouse/human chimera model in which xenotransplanted human cortical pyramidal neurons integrate as single cells into the mouse cortex. Combined neuronal tracing, electrophysiology, and in vivo structural and functional imaging of the transplanted cells reveal a coordinated developmental roadmap recapitulating key milestones of human cortical neuron development. The human neurons display a prolonged developmental timeline, indicating the neuron-intrinsic retention of juvenile properties as an important component of human brain neoteny. Following maturation, human neurons in the visual cortex display tuned, decorrelated responses to

visual stimuli, like mouse neurons, demonstrating their capacity for physiological synaptic integration in host cortical circuits. These findings provide new insights into human neuronal development and open novel avenues for the study of human neuronal function and disease. VIDEO ABSTRACT.
Neuron, 2019; 104
28705869: Libé-Philippot B, Michel V, Boutet de Monvel J, Le Gal S, Dupont T, Avan P, Métin C, Michalski N, Petit C
Auditory cortex interneuron development requires cadherins operating hair-cell mechanoelectrical transduction.
Many genetic forms of congenital deafness affect the sound reception antenna of cochlear sensory cells, the hair bundle. The resulting sensory deprivation jeopardizes auditory cortex (AC) maturation. Early prosthetic intervention should revive this process. Nevertheless, this view assumes that no intrinsic AC deficits coexist with the cochlear ones, a possibility as yet unexplored. We show here that many GABAergic interneurons, from their generation in the medial ganglionic eminence up to their settlement in the AC, express two cadherin-related (cdhr) proteins, cdhr23 and cdhr15, that form the hair bundle tip links gating the mechanoelectrical transduction channels. Mutant mice lacking either protein showed a major decrease in the number of parvalbumin interneurons specifically in the AC, and displayed audiogenic reflex seizures. - and -expressing interneuron precursors in and mouse embryos, respectively, failed to enter the embryonic cortex and were scattered throughout the subpallium, consistent with the cell polarity abnormalities we observed in vitro. In the absence of adhesion G protein-coupled receptor V1 (adgrv1), another hair bundle link protein, the entry of - and -expressing interneuron precursors into the embryonic cortex was also impaired. Our results demonstrate that a population of newborn interneurons is endowed with specific cdhr proteins necessary for these cells to reach the developing AC. We suggest that an "early adhesion code" targets populations of interneuron precursors to restricted neocortical regions belonging to the same functional area. These findings open up new perspectives for auditory rehabilitation and cortical therapies in patients.
Proc Natl Acad Sci U S A, 2017; 114

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DIFFERENTIATION AND TRANSPLANTATION OF DOPAMINERGIC NEURONS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS IN PARKINSONIAN MARMOSETS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder caused by the death of dopaminergic neurons in the substantia nigra pars compacta (SNc). The loss of dopaminergic neurons in the SNc results in a range of debilitating movement disorders, such as bradykinesia, tremor, rigidity and postural instability in the affected patients. Induced pluripotent stem cells (iPSCs) are specialized cells that can be differentiated into the three germ layers and serve as a valuable source for cell-based replacement therapies. Evidence indicates that stem cell derived dopaminergic neurons are a promising avenue in the search for a viable long-term treatment of PD patients. Here, we report the isolation of self-renewable neural stem cells (NSCs) from human iPSCs derived from PD patients. The NSCs demonstrated the ability to proliferate in response to mitogenic growth factors. The isolated NSCs were further differentiated into neurons of dopaminergic lineage. The dopaminergic neurons co-expressed the tyrosine hydroxylase, β-tubulin and midbrain transcription factors. Quantitative analysis demonstrated that the number of dopaminergic neurons significantly increased on days 1, 2 and 7 in vitro compared to control-treated NSCs. Differentiated neurons were transplanted into the putamen of MPTP-lesioned marmoset model of PD. The animals were monitored for a period of 6 months. The object retrieval task with barrier detour, the PD rating scale (PDRS) and activity analysis were validated for evaluating motor and cognitive functions. The data suggests that grafted iPSC-derived dopaminergic neurons are efficacious in alleviating the behavioral deficits observed in the marmoset model of PD.

Pubmed:
34762921: Oh T, Daadi ES, Kim J, Daadi EW, Chen PJ, Roy-Choudhury G, Bohmann J, Blass BE, Daadi MM
Dopamine D3 receptor ligand suppresses the expression of levodopa-induced dyskinesia in nonhuman primate model of parkinson's disease.
Parkinson's disease (PD) is a complex multisystem, chronic and so far incurable disease with significant unmet medical needs. The incidence of PD increases with aging and the expected burden will continue to escalate with our aging population. Since its discovery in the 1961 levodopa has remained the gold standard pharmacotherapy for PD. However, the progressive nature of the neurodegenerative process in and beyond the nigrostriatal system causes a multitude of side effects, including levodopa-induced dyskinesia within 5 years of therapy. Attenuating dyskinesia has been a significant challenge in the clinical management of PD. We report on a small molecule that eliminates the expression of levodopa-induced dyskinesia and significantly improves PD-like symptoms. The lead compound PD13R we discovered is a dopamine D3 receptor partial agonist with high affinity and selectivity, orally active and with desirable drug-like properties. Future studies are aimed at developing this lead compound for treating PD patients with dyskinesia.
Exp Neurol, 2022; 347
32427127: Lizarraga S, Daadi EW, Roy-Choudhury G, Daadi MM
Age-related cognitive decline in baboons: modeling the prodromal phase of Alzheimer's disease and related dementias.
The aging of brain cells and synaptic loss are the major underlying pathophysiological processes contributing to the progressive decline in cognitive functions and Alzheimer's disease. The difference in cognitive performances observed between adult and aged subjects across species highlights the decline of brain systems with age. The inflection point in age-related cognitive decline is important for our understanding of the pathophysiology of neurodegenerative diseases and for timing therapeutic interventions. Humans and nonhuman primates share many similarities including age-dependent changes in gene expression and decline in neural and immune functions. Given these evolutionary conserved organ systems, complex human-like behavioral and age-dependent changes may be modeled and monitored longitudinally in nonhuman primates. We integrated three clinically relevant outcome measures to investigate the effect of age on cognition, motor function and diurnal activity in aged baboons. We provide evidence of a naturally-occurring age-dependent precipitous decline in movement planning, in learning novel tasks, in simple discrimination and in motivation. These results suggest that baboons aged ~20

years (equivalent to ~60 year old humans) may offer a relevant model for the prodromal phase of Alzheimer's disease and related dementias to investigate mechanisms involved in the precipitous decline in cognitive functions and to develop early therapeutic interventions.
Aging (Albany NY), 2020; 12

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THE MORE, THE BETTER? NEUROPROTECTIVE EFFECTS OF DIFFERENT DRUG- AND STEM CELL-BASED THERAPIES AS NOVEL APPROACHES FOR NEURODEGENERATIVE DISEASES.

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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[1]Universidad de Salamanca, Institute Of Neuroscience Of Castile And Leon, Salamanca, Spain, [2]Universidad de Salamanca, Department Of Statistics, Salamanca, Spain

Current therapeutic approaches based on “one gene, one drug, one disease” philosophy usually offer limited benefits for the treatment of neurodegenerative diseases. However, little is known about the synergy of combined therapies. In this study, the possible synergistic effect of the combination of the endocannabinoid oleoylethanolamide (OEA) and different stem cell (SC) therapies was assessed in a model of aggressive cerebellar degeneration, the PCD mouse. Three different therapies were evaluated: a) OEA (i.p., 10 mg/kg) at post-natal day 12 (P12), b) OEA + bone marrow ablation and $7.5x10^6$ bone marrow SC (BMSC) transplantation at P20, and c) OEA + $2.5x10^5$ mesenchymal SC (MSC) injection at P14-16-18-20-22-24-26-28. Both BMSC and MSC were harvested from healthy GFP-mice and administered through the retro-orbital plexus. *Rotarod* test was performed at P22 and P30, as PCD animals exhibit motor impairments. Finally, at P30, GFP-positive cells from peripheral blood were quantified by flow cytometry, animals were sacrificed, and their brain dissected. Both the neuroprotective effect on Purkinje cells density and morphology, and the analysis of the transplant-derived cells were carried out by immunofluorescence in cerebellar vermis sections. Our preliminary data showed that all the approaches evaluated exerted certain neuroprotection in PCD animals. Contrary to expectations, the most effective treatment was the single OEA treatment, followed by the combined OEA and quasi-continuous MSC transplantation, both at the cellular and behavioral level. Finally, GFP-positive cells were observed in all animals transplanted with BMSC or MSC, although a higher number was found in those treated with BMSC.

Pubmed:

33829414: Pérez-Martín E, Muñoz-Castañeda R, Moutin MJ, Ávila-Zarza CA, Muñoz-Castañeda JM, Del Pilar C, Alonso JR, Andrieux A, Díaz D, Weruaga E
Oleoylethanolamide Delays the Dysfunction and Death of Purkinje Cells and Ameliorates Behavioral Defects in a Mouse Model of Cerebellar Neurodegeneration.
Oleoylethanolamide (OEA) is an endocannabinoid that has been proposed to prevent neuronal damage and neuroinflammation. In this study, we evaluated the effects of OEA on the disruption of both cerebellar structure and physiology and on the behavior of Purkinje cell degeneration (PCD) mutant mice. These mice exhibit cerebellar degeneration, displaying microtubule alterations that trigger the selective loss of Purkinje cells and consequent behavioral impairments. The effects of different doses (1, 5, and 10 mg/kg, i.p.) and administration schedules (chronic and acute) of OEA were assessed at the behavioral, histological, cellular, and molecular levels to determine the most effective OEA treatment regimen. Our in vivo results demonstrated that OEA treatment prior to the onset of the preneurodegenerative phase prevented morphological alterations in Purkinje neurons (the somata and dendritic arbors) and decreased Purkinje cell death. This effect followed an inverted U-shaped time-response curve, with acute administration on postnatal day 12 (10 mg/kg, i.p.) being the most effective treatment regimen tested. Indeed, PCD mice that received this specific OEA treatment regimen showed improvements in motor, cognitive and social functions, which were impaired in these mice. Moreover, these in vivo neuroprotective effects of OEA were mediated by the PPARα receptor, as pretreatment with the PPARα antagonist GW6471 (2.5 mg/kg, i.p.) abolished them. Finally, our in vitro results suggested that the molecular effect of OEA was related to microtubule stability and structure since OEA administration normalized some alterations in microtubule features in PCD-like cells. These findings provide strong evidence supporting the use of OEA as a pharmacological agent to limit severe cerebellar neurodegenerative processes.
Neurotherapeutics, 2021; 18
34916910: Del Pilar C, Lebrón-Galán R, Pérez-Martín E, Pérez-Revuelta L, Ávila-Zarza CA, Alonso JR, Clemente D, Weruaga E, Díaz D

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The Selective Loss of Purkinje Cells Induces Specific Peripheral Immune Alterations.

The progression of neurodegenerative diseases is reciprocally associated with impairments in peripheral immune responses. We investigated different contexts of selective neurodegeneration to identify specific alterations of peripheral immune cells and, at the same time, discover potential biomarkers associated to this pathological condition. Consequently, a model of human cerebellar degeneration and ataxia -the Purkinje Cell Degeneration (PCD) mouse- has been employed, as it allows the study of different processes of selective neuronal death in the same animal, i.e., Purkinje cells in the cerebellum and mitral cells in the olfactory bulb. Infiltrated leukocytes were studied in both brain areas and compared with those from other standardized neuroinflammatory models obtained by administering either gamma radiation or lipopolysaccharide. Moreover, both myeloid and lymphoid splenic populations were analyzed by flow cytometry, focusing on markers of functional maturity and antigen presentation. The severity and type of neural damage and inflammation affected immune cell infiltration. Leukocytes were more numerous in the cerebellum of PCD mice, being located predominantly within those cerebellar layers mostly affected by neurodegeneration, in a completely different manner than the typical models of induced neuroinflammation. Furthermore, the milder degeneration of the olfactory bulb did not foster leukocyte attraction. Concerning the splenic analysis, in PCD mice we found: (1) a decreased percentage of several myeloid cell subsets, and (2) a reduced mean fluorescence intensity in those myeloid markers related to both antigen presentation and functional maturity. In conclusion, the selective degeneration of Purkinje cells triggers a specific effect on peripheral immune cells, fostering both attraction and functional changes. This fact endorses the employment of peripheral immune cell populations as concrete biomarkers for monitoring different neuronal death processes.

Front Cell Neurosci, 2021; 15

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BOARD NUMBER: S05-330

STUDYING NEURONAL PLASTICITY WITH HUMAN CORTICAL NEURONS XENOTRANSPLANTED INTO THE MOUSE VISUAL CORTEX

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

Polona Jager[1], Ben Vermaercke[1], Ryohei Iwata[1], Vincent Bonin[2], Pierre Vanderhaeghen[1]
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Aims Neural plasticity is greatest during development, but decreases into adulthood. Reversing this timeline would have major implications for treating brain diseases. A xenotransplantation method was previously developed in the lab, where human pyramidal neurons are derived from embryonic stem cells and transplanted into the neonatal mouse cortex. Transplanted human neurons retain their species-specific protracted development, displaying juvenile morphology and physiology even in an adult mouse host. Here, we explore whether transplanted human neurons also display prolonged functional plasticity, and investigate if developing human neurons impart their juvenile synaptic properties on the mature mouse circuits, thus increasing their plasticity. Methods We use longitudinal calcium imaging to track sensory responses of human neurons transplanted in the mouse visual cortex, and those of the surrounding adult mouse neurons. Monocular deprivation is performed to study functional plasticity of both neuronal populations. Finally, transsynaptic tracing is applied to selectively study plasticity of mouse neurons presynaptic to human cells. Results Human neurons in the mouse visual cortex respond to visual stimuli, and importantly, adapt their responses according to visual experience of the mouse host, thus displaying plasticity while embedded in the adult mouse circuits. For the adult mouse host neurons, our preliminary data show a plastic change selectively in those synaptically connected to transplanted human cells. Conclusions Xenotransplantation of developing human cortical neurons constitutes an attractive model for studying human neural plasticity *in vivo*, which could be applied to disease modeling, and for future studies on induction of plasticity in an adult brain.

Pubmed:

33522480: Jager P, Moore G, Calpin P, Durmishi X, Salgarella I, Menage L, Kita Y, Wang Y, Kim DW, Blackshaw S, Schultz SR, Brickley S, Shimogori T, Delogu A
Dual midbrain and forebrain origins of thalamic inhibitory interneurons.
The ubiquitous presence of inhibitory interneurons in the thalamus of primates contrasts with the sparsity of interneurons reported in mice. Here, we identify a larger than expected complexity and distribution of interneurons across the mouse thalamus, where all thalamic interneurons can be traced back to two developmental programmes: one specified in the midbrain and the other in the forebrain. Interneurons migrate to functionally distinct thalamocortical nuclei depending on their origin: the abundant, midbrain-derived class populates the first and higher order sensory thalamus while the rarer, forebrain-generated class is restricted to some higher order associative regions. We also observe that markers for the midbrain-born class are abundantly expressed throughout the thalamus of the New World monkey marmoset. These data therefore reveal that, despite the broad variability in interneuron density across mammalian species, the blueprint of the ontogenetic organisation of thalamic interneurons of larger-brained mammals exists and can be studied in mice.
Elife, 2021; 10
27929058: Jager P, Ye Z, Yu X, Zagoraiou L, Prekop HT, Partanen J, Jessell TM, Wisden W, Brickley SG, Delogu A
Tectal-derived interneurons contribute to phasic and tonic inhibition in the visual thalamus.
The release of GABA from local interneurons in the dorsal lateral geniculate nucleus (dLGN-INs) provides inhibitory control during visual processing within the thalamus. It is commonly assumed that this important class of interneurons originates from within the thalamic complex, but we now show that during early postnatal development Sox14/Otx2-expressing precursor cells migrate from the dorsal midbrain to generate dLGN-INs. The unexpected extra-diencephalic origin of dLGN-INs sets them apart from GABAergic neurons of the reticular thalamic nucleus. Using optogenetics we show that at increased firing rates tectal-derived dLGN-INs generate a powerful form of tonic inhibition that regulates the gain of thalamic relay neurons through recruitment of extrasynaptic high-affinity GABA receptors. Therefore, by revising the conventional view of thalamic interneuron ontogeny we demonstrate how a previously unappreciated mesencephalic population controls thalamic relay neuron excitability.

Nat Commun, 2016; 7
25454592: Yu X, Zecharia A, Zhang Z, Yang Q, Yustos R, Jager P, Vyssotski AL, Maywood ES, Chesham JE, Ma Y, Brickley SG, Hastings MH, Franks NP, Wisden W
Circadian factor BMAL1 in histaminergic neurons regulates sleep architecture.
Circadian clocks allow anticipation of daily environmental changes. The suprachiasmatic nucleus (SCN) houses the master clock, but clocks are also widely expressed elsewhere in the body. Although some peripheral clocks have established roles, it is unclear what local brain clocks do. We tested the contribution of one putative local clock in mouse histaminergic neurons in the tuberomamillary nucleus to the regulation of the sleep-wake cycle. Histaminergic neurons are silent during sleep, and start firing after wake onset; the released histamine, made by the enzyme histidine decarboxylase (HDC), enhances wakefulness. We found that hdc gene expression varies with time of day. Selectively deleting the Bmal1 (also known as Arntl or Mop3) clock gene from histaminergic cells removes this variation, producing higher HDC expression and brain histamine levels during the day. The consequences include more fragmented sleep, prolonged wake at night, shallower sleep depth (lower nonrapid eye movement [NREM] δ power), increased NREM-to-REM transitions, hindered recovery sleep after sleep deprivation, and impaired memory. Removing BMAL1 from histaminergic neurons does not, however, affect circadian rhythms. We propose that for mammals with polyphasic/nonwake consolidating sleep, the local BMAL1-dependent clock directs appropriately timed declines and increases in histamine biosynthesis to produce an appropriate balance of wake and sleep within the overall daily cycle of rest and activity specified by the SCN.
Curr Biol, 2014; 24

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ENHANCEMENT OF THE THERAPEUTIC EFFICACY OF PREGABALIN USING MESENCHYMAL STEM CELLS IN AN EXPERIMENTAL MODEL OF PERIPHERAL NEUROPATHY

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Background: Peripheral neuropathy is a common adverse effect observed during use of paclitaxel (PTX) as chemotherapy. Aim: The present investigation was directed to estimate the modulatory effect of bone marrow derived mesenchymal stem cells (BM-MSCs) on pregabalin (PGB) treatment in PTX-induced peripheral neuropathy. Methods: Neuropathic pain was induced in rats by injecting PTX (2 mg/kg, i.p) 4 times every other day. Rats were treated with PGB (30 mg/kg/day, p.o.) for 21 days with or without a single intravenous administration of BM-MSCs. At the end of experiment, behavioral and motor abnormalities were assessed. Animals were then sacrificed for measurement of total antioxidant capacity (TAC), nerve growth factor (NGF), nuclear factor kappa B p65 (NF-κB p65), tumor necrosis factor-alpha (TNF-α), interleukins 6 (IL-6), and active caspase-3 in the sciatic nerve. Moreover, protein expressions of Notch1 receptor, phosphorylated Janus kinase 2 (*p*-JAK2), phosphorylated signal transducer and activator of transcription 3 (*p*-STAT3), and phosphorylated p38 mitogen-activated protein kinase (*p*-p38-MAPK) were estimated. Finally, histological examinations were performed to assess severity of sciatic nerve damage and for estimation of BM-MSCs homing. Results: Combined PGB/BM-MSCs therapy provided an additional improvement toward reducing PTX-induced oxidative stress, neuro-inflammation, and apoptotic markers. Interestingly, BM-MSCs therapy effectively prevented motor impairment observed by PGB treatment. Combined therapy also induced a significant increase in cell homing and prevented PTX-induced sciatic nerve damage in histological examination. Conclusion: The present study highlights a significant role for BM-MSCs in enhancing treatment potential of PGB in peripheral neuropathy and reducing its motor side effects.

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PURKINJE CELL FUSION DYNAMICS IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

Pablo G Téllez De Meneses[1,2,3], Valeria Lorena Cabedo[1,2,3], David Díaz[1,2,3], Eduardo Weruaga[1,2,3], Jorge Valero[1,2,3], José Ramón Alonso[1,2,3]
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Neurodegenerative diseases represent a high economic and health burden for society, and efficient treatments are limited. One of the most promising therapeutic strategies is bone marrow-derived cells (BMDCs) transplantation. Indeed, our group has demonstrated that this strategy improves motor activity in a mouse model of cerebellar ataxia. Furthermore, we and others showed that BMDCs integrate into the central nervous system, either switching from one cell type to another (transdifferentiation) or by cell fusion (forming heterokaryons) as it happens with cerebellar Purkinje cells. Generally, the number of fused Purkinje cells after transplantation is small, which limits their analysis, and makes it difficult to verify the relationship between cell fusion and neuron survival suggested in literature. Nevertheless, few studies have reported a 20-fold increase of cell fusion in mice with experimental autoimmune encephalomyelitis (EAE), a model of induced multiple sclerosis. However, these studies differ in the methodologies used, mainly in experimental timings for transplant, EAE induction and sacrifice. Therefore, we aim to establish an optimal protocol to study cell fusion after BMDCs transplantation in EAE mice. We used different time windows between EAE induction and sacrifice and analysed the number of fused cells. Our data will allow us to estimate fusion rate variations over time, determining the dynamics of cell fusion. This will be crucial for future studies in which we will identify the molecular mechanisms involved in cell fusion by comparing the transcriptome of fused and non-fused Purkinje cells. Support: MICINN/MIU, JCyL, USAL Contact: pabgonses@usal.es, jorgevalero@usal.es, jralonso@usal.es

Pubmed:
33299042: Pérez-Revuelta L, Téllez de Meneses PG, López M, Briñón JG, Weruaga E, Díaz D, Alonso JR
Secretagogin expression in the mouse olfactory bulb under sensory impairments.
The interneurons of the olfactory bulb (OB) are characterized by the expression of different calcium-binding proteins, whose specific functions are not fully understood. This is the case of one of the most recently discovered, the secretagogin (SCGN), which is expressed in interneurons of the glomerular and the granule cell layers, but whose function in the olfactory pathway is still unknown. To address this question, we examined the distribution, generation and activity of SCGN-positive interneurons in the OB of two complementary models of olfactory impairments: Purkinje Cell Degeneration (PCD) and olfactory-deprived mice. Our results showed a significant increase in the density of SCGN-positive cells in the inframitral layers of olfactory-deprived mice as compared to control animals. Moreover, BrdU analyses revealed that these additional SCGN-positive cells are not newly formed. Finally, the neuronal activity, estimated by c-Fos expression, increased in preexisting SCGN-positive interneurons of both deprived and PCD mice -being higher in the later- in comparison with control animals. Altogether, our results suggest that the OB possesses different compensatory mechanisms depending on the type of alteration. Particularly, the SCGN expression is dependent of olfactory stimuli and its function may be related to a compensation against a reduction in sensory inputs.
Sci Rep, 2020; 10

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TRANSPLANTATION OF SPINAL NEURON SUBTYPES GENERATED FROM HUMAN PLURIPOTENT STEM CELLS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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The spinal cord is composed of heterogeneous neuronal populations organised in complex neural circuits that finely control many activities of our body. Traumatic injury or neurodegenerative diseases cause permanent damage and impairment in affected individuals. To date, the possibility to effectively treat these patients remains limited, and new approaches need to be investigated. The capacity of human pluripotent stem cells (hPSCs) to differentiate into a wide range of neuronal cell types makes these cells attractive for neural transplantation therapies aimed at repairing damaged spinal circuitry and/or supporting at risk host neurons. Current challenges for the development of stem cell-based therapies include low efficiency of differentiation protocols, limited capacity to derive specific spinal neural subtypes, and limited knowledge of cell behaviour after intraspinal transplantation. In this study, we developed 2D adherent differentiation protocols to derive progenitors for spinal motoneuron (spMN) and interneuron (spIN) subtypes. Transplantation studies showed the capacity of these progenitors to survive and retain neuronal phenotype *in vivo* after intra-spinal grafting in athymic rats. These results form a valuable platform for investigating the efficacy of cell transplantation for spinal cord injury on a cell type specific basis.

Pubmed:
35180398: Moriarty N, Gantner CW, Hunt CPJ, Ermine CM, Frausin S, Viventi S, Ovchinnikov DA, Kirik D, Parish CL, Thompson LH
A combined cell and gene therapy approach for homotopic reconstruction of midbrain dopamine pathways using human pluripotent stem cells.
Midbrain dopamine (mDA) neurons can be replaced in patients with Parkinson's disease (PD) in order to provide long-term improvement in motor functions. The limited capacity for long-distance axonal growth in the adult brain means that cells are transplanted ectopically, into the striatal target. As a consequence, several mDA pathways are not re-instated, which may underlie the incomplete restoration of motor function in patients. Here, we show that viral delivery of GDNF to the striatum, in conjunction with homotopic transplantation of human pluripotent stem-cell-derived mDA neurons, recapitulates brain-wide mDA target innervation. The grafts provided re-instatement of striatal dopamine levels and correction of motor function and also connectivity with additional mDA target nuclei not well innervated by ectopic grafts. These results demonstrate the remarkable capacity for achieving functional and anatomically precise reconstruction of long-distance circuitry in the adult brain by matching appropriate growth-factor signaling to grafting of specific cell types.
Cell Stem Cell, 2022; 29
34651338: Ermine CM, Nithianantharajah J, O'Brien K, Kauhausen JA, Frausin S, Oman A, Parsons MW, Brait VH, Brodtmann A, Thompson LH
Hemispheric cortical atrophy and chronic microglial activation following mild focal ischemic stroke in adult male rats.
Animal modeling has played an important role in our understanding of the pathobiology of stroke. The vast majority of this research has focused on the acute phase following severe forms of stroke that result in clear behavioral deficits. Human stroke, however, can vary widely in severity and clinical outcome. There is a rapidly building body of work suggesting that milder ischemic insults can precipitate functional impairment, including cognitive decline, that continues through the chronic phase after injury. Here we show that a small infarction localized to the frontal motor cortex of rats following injection of endothelin-1 results in an essentially asymptomatic state based on motor and cognitive testing, and yet produces significant histopathological change including remote atrophy and inflammation that persists up to 1 year. While there is understandably a major focus in stroke research on mitigating the acute consequences of primary infarction, these results point to progressive atrophy and chronic inflammation as additional targets for intervention in the chronic phase after injury. The present rodent model provides an important platform for further work in this area.
J Neurosci Res, 2021; 99
33947043: Kagan BJ, Ermine CM, Frausin S, Parish CL, Nithianantharajah J, Thompson LH

Focal Ischemic Injury to the Early Neonatal Rat Brain Models Cognitive and Motor Deficits with Associated Histopathological Outcomes Relevant to Human Neonatal Brain Injury.
Neonatal arterial ischemic stroke is one of the more severe birth complications. The injury can result in extensive neurological damage and is robustly associated with later diagnoses of cerebral palsy (CP). An important part of efforts to develop new therapies include the on-going refinement and understanding of animal models that capture relevant clinical features of neonatal brain injury leading to CP. The potent vasoconstrictor peptide, Endothelin-1 (ET-1), has previously been utilised in animal models to reduce local blood flow to levels that mimic ischemic stroke. Our previous work in this area has shown that it is an effective and technically simple approach for modelling ischemic injury at very early neonatal ages, resulting in stable deficits in motor function. Here, we aimed to extend this model to also examine the impact on cognitive function. We show that focal delivery of ET-1 to the cortex of Sprague Dawley rats on postnatal day 0 (P0) resulted in impaired learning in a touchscreen-based test of visual discrimination and correlated with important clinical features of CP including damage to large white matter structures.
Int J Mol Sci, 2021; 22
33734599: Viventi S, Frausin S, Howden SE, Lim SY, Finol-Urdaneta RK, McArthur JR, Abu-Bonsrah KD, Ng W, Ivanusic J, Thompson L, Dottori M
In vivo survival and differentiation of Friedreich ataxia iPSC-derived sensory neurons transplanted in the adult dorsal root ganglia.
Friedreich ataxia (FRDA) is an autosomal recessive disease characterized by degeneration of dorsal root ganglia (DRG) sensory neurons, which is due to low levels of the mitochondrial protein Frataxin. To explore cell replacement therapies as a possible approach to treat FRDA, we examined transplantation of sensory neural progenitors derived from human embryonic stem cells (hESC) and FRDA induced pluripotent stem cells (iPSC) into adult rodent DRG regions. Our data showed survival and differentiation of hESC and FRDA iPSC-derived progenitors in the DRG 2 and 8 weeks post-transplantation, respectively. Donor cells expressed neuronal markers, including sensory and glial markers, demonstrating differentiation to these lineages. These results are novel and a highly significant first step in showing the possibility of using stem cells as a cell replacement therapy to treat DRG neurodegeneration in FRDA as well as other peripheral neuropathies.
Stem Cells Transl Med, 2021; 10
29431270: Ermine CM, Wright JL, Frausin S, Kauhausen JA, Parish CL, Stanic D, Thompson LH
Modelling the dopamine and noradrenergic cell loss that occurs in Parkinson's disease and the impact on hippocampal neurogenesis.
Key pathological features of Parkinson's Disease (PD) include the progressive degeneration of midbrain dopaminergic (DA) neurons and hindbrain noradrenergic (NA) neurons. The loss of DA neurons has been extensively studied and is the main cause of motor dysfunction. Importantly, however, there are a range of 'non-movement' related features of PD including cognitive dysfunction, sleep disturbances and mood disorders. The origins for these non-motor symptoms are less clear, but a possible substrate for cognitive decline may be reduced adult-hippocampal neurogenesis, which is reported to be impaired in PD. The mechanisms underlying reduced neurogenesis in PD are not well established. Here we tested the hypothesis that NA and DA depletion, as occurs in PD, impairs hippocampal neurogenesis. We used 6-hydroxydopamine or the immunotoxin dopamine-β-hydroxylase-saporin to selectively lesion DA or NA neurons, respectively, in adult Sprague Dawley rats and assessed hippocampal neurogenesis through phenotyping of cells birth-dated using 5-bromo-2'-deoxyuridine. The results showed no difference in proliferation or differentiation of newborn cells in the subgranular zone of the dentate gyrus after NA or DA lesions. This suggests that impairment of hippocampal neurogenesis in PD likely results from mechanisms independent of, or in addition to degeneration of DA and NA neurons.
Hippocampus, 2018; 28
25747736: Frausin S, Viventi S, Verga Falzacappa L, Quattromani MJ, Leanza G, Tommasini A, Valencic E
Wharton's jelly derived mesenchymal stromal cells: Biological properties, induction of neuronal phenotype and current applications in neurodegeneration research.
Multipotent mesenchymal stromal cells, also known as mesenchymal stem cells (MSC), can be isolated from bone marrow or other tissues, including fat, muscle and umbilical cord. It has been shown that MSC behave in vitro as stem cells: they self-renew and are able to differentiate into mature cells typical of several mesenchymal tissues. Moreover, the differentiation toward non-mesenchymal cell lineages (e.g. neurons) has been reported as well. The clinical relevance of these cells is mainly related to their ability to spontaneously migrate to the site of inflammation/damage, to their safety profile thanks to their low immunogenicity and to their immunomodulation capacities. To date, MSCs isolated from the post-natal bone marrow have represented the most extensively studied population of adult MSCs, in view of their possible use in various therapeutical applications. However, the bone marrow-derived MSCs exhibit a series of limitations, mainly related to their problematic isolation, culturing and use. In recent years, umbilical cord (UC) matrix (i.e. Wharton's jelly, WJ) stromal cells have therefore emerged as a more suitable alternative source of MSCs, thanks to their primitive nature and the easy isolation without

relevant ethical concerns. This review seeks to provide an overview of the main biological properties of WJ-derived MSCs. Moreover, the potential application of these cells for the treatment of some known dysfunctions in the central and peripheral nervous system will also be discussed.
Acta Histochem, 2015 May-Jun; 117

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-334

FUNCTIONAL RECOVERY CAUSED BY HUMAN ADIPOSE TISSUE MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES ADMINISTERED 24H AFTER STROKE IN NORMOTENSIVE RATS AND SOME DIFFERENCES TO HYPERTENSIVE RATS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims: to propose an approach, using extracellular vesicles (EV) secreted from human mesenchymal stem cells isolated from adipose tissue (hAT-MSC), with a prolonged period for therapeutic intervention and a non-invasive route for administering the treatment of stroke. Methods: Wistar rats (90-120 days) were subjected to focal permanent ischemic stroke (IS), 24 hours after, were treated intranasally with EV (200 µg/kg) secreted by hAT-MSC. We analyzed front paws symmetry (Cylinder Task), short- and long-term memory (Open Field and Novel Object Recognition Task) and angiogenesis. Wistar Kyoto Spontaneously hypertensive rats (SHR) (90-120 days) were subjected to IS and we analyzed front paws symmetry (Cylinder Task). Results: In Wistar rats, EV treatment recovered front paws symmetry (Fig 1. A) and short- and long-term memory induced by ischemic stroke. Additionally, we observed stimulation of angiogenesis in the peri-infarction region in animals treated with EVs. For SHR rats, we have preliminary results, where we can observe that the outcome of ischemia induction is very different and with a chronic effect and without spontaneous recovery in the symmetry of the forepaws (Fig 1. B). Conclusions: In line with these findings, our work highlights hAT-MSC-derived EVs as a promising therapeutic strategy for stroke.

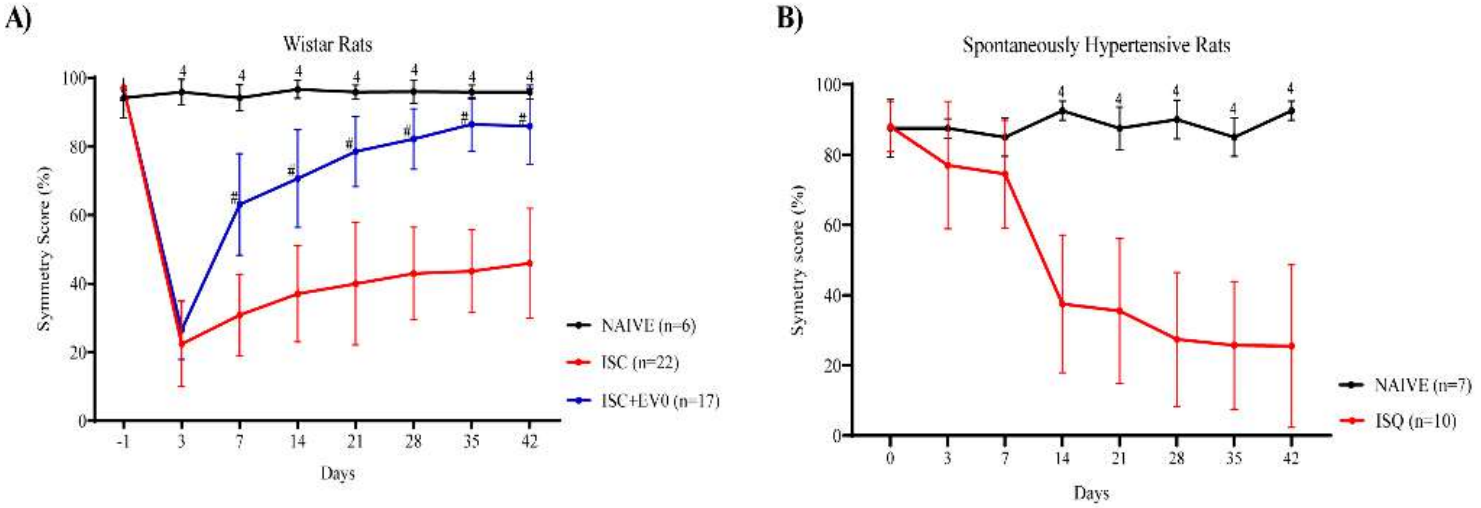

Fig 1. A) Effect of IS and EV treatment on front paws symmetry of Wistar rats. B) Effect of IS on front paws symmetry of SHR rats. Data: means ± SD, two-way ANOVA followed by Tukey's test; [4] $p < 0.0001$ compared ISC to naive; [#] $p < 0.0001$ compared ISC to ISC+EV0.

Pubmed:
33837559: de Moura Alvorcem L, Britto R, Cecatto C, Cristina Roginski A, Rohden F, Nathali Scholl J, Guma FCR, Figueiró F, Umpierrez Amaral A, Zanatta G, Seminotti B, Wajner M, Leipnitz G

Ethylmalonic acid impairs bioenergetics by disturbing succinate and glutamate oxidation and induces mitochondrial permeability transition pore opening in rat cerebellum.
Tissue accumulation and high urinary excretion of ethylmalonic acid (EMA) are found in ethylmalonic encephalopathy (EE), an inherited disorder associated with cerebral and cerebellar atrophy whose pathogenesis is poorly established. The in vitro and in vivo effects of EMA on bioenergetics and redox homeostasis were investigated in rat cerebellum. For the in vitro studies, cerebellum preparations were exposed to EMA, whereas intracerebellar injection of EMA was used for the in vivo evaluation. EMA reduced state 3 and uncoupled respiration in vitro in succinate-, glutamate-, and malate-supported mitochondria, whereas decreased state 4 respiration was observed using glutamate and malate. Furthermore, mitochondria permeabilization and succinate supplementation diminished the decrease in state 3 with succinate. EMA also inhibited the activity of KGDH, an enzyme necessary for glutamate oxidation, in a mixed manner and augmented mitochondrial efflux of α-ketoglutarate. ATP levels were markedly reduced by EMA, reflecting a severe bioenergetic disruption. Docking simulations also indicated interactions between EMA and KGDH and a competition with glutamate and succinate for their mitochondrial transporters. In vitro findings also showed that EMA decreased mitochondrial membrane potential and Ca retention capacity, and induced swelling in the presence of Ca , which were prevented by cyclosporine A and ADP and ruthenium red, indicating mitochondrial permeability transition (MPT). Moreover, EMA, at high concentrations, mildly increased ROS levels and altered antioxidant defenses in vitro and in vivo. Our data indicate that EMA-induced impairment of glutamate and succinate oxidation and MPT may contribute to the pathogenesis of the cerebellum abnormalities in EE.
J Neurochem, 2021; 158
32789760: da Silva JS, Nonose Y, Rohden F, Lukasewicz Ferreira PC, Fontella FU, Rocha A, Brochier AW, Apel RV, de Lima TM, Seminotti B, Amaral AU, Galina A, Souza DO
Guanosine Neuroprotection of Presynaptic Mitochondrial Calcium Homeostasis in a Mouse Study with Amyloid-β Oligomers.
Amyloid-β oligomers (AβOs) toxicity causes mitochondrial dysfunction, leading to synaptic failure in Alzheimer's disease (AD). Considering presynaptic high energy demand and tight Ca regulation, impairment of mitochondrial function can lead to deteriorated neural activity and cell death. In this study, an AD mouse model induced by ICV (intracerebroventricular) injection of AβOs was used to investigate the toxicity of AβOs on presynaptic function. As a therapeutic approach, GUO (guanosine) was given by oral route to evaluate the neuroprotective effects on this AD model. Following 24 h and 48 h from the model induction, behavioral tasks and biochemical analyses were performed, respectively. AβOs impaired object recognition (OR) short-term memory and reduced glutamate uptake and oxidation in the hippocampus. Moreover, AβOs decreased spare respiratory capacity, reduced ATP levels, impaired Ca handling, and caused mitochondrial swelling in hippocampal synaptosomes. Guanosine crossed the BBB, recovered OR short-term memory, reestablished glutamate uptake, recovered mitochondrial Ca homeostasis, and partially prevented mitochondrial swelling. Therefore, this endogenous purine presented a neuroprotective effect on presynaptic mitochondria and should be considered for further studies in AD models.
Mol Neurobiol, 2020; 57
32249669: Scholl JN, de Fraga Dias A, Pizzato PR, Lopes DV, Moritz CEJ, Jandrey EHF, Souto GD, Colombo M, Rohden F, Sévigny J, Pohlmann AR, Guterres SS, Battastini AMO, Figueiró F
Characterization and antiproliferative activity of glioma-derived extracellular vesicles.
To characterize a method to isolate glioma-derived extracellular vesicles (GEVs) and understand their role in immune system modulation and glioma progression. GEVs were isolated by differential centrifugation from C6 cell supernatant and characterized by size and expression of CD9, HSP70, CD39 and CD73. The glioma model was performed by injecting C6 glioma cells into the right striatum of Wistar rats in the following groups: controls (C6 cells alone), coinjection (C6 cells + GEVs) and GEVs by intranasal administration followed by immune cells, tumor size and cells proliferation analyses. GEVs presented uniform size (175 nm), expressed CD9, HSP70, CD39, CD73 and produced adenosine. , we observed a reduction in tumor size, in cell proliferation (Ki-67) and in a regulatory cell marker (FoxP3). GEVs, administered before or at tumor challenge, have antiproliferative properties and reduce regulatory cells in the glioma microenvironment.
Nanomedicine (Lond), 2020; 15
31476364: Figueiró PW, Moreira DS, Dos Santos TM, Prezzi CA, Rohden F, Faccioni-Heuser MC, Manfredini V, Netto CA, Wyse ATS
The neuroprotective role of melatonin in a gestational hypermethioninemia model.
Elevated levels of methionine in blood characterize the hypermethioninemia, which may have genetic or non-genetic origin, as for example from high protein diet. Born rats from hypermethioninemic mothers presented cerebral oxidative stress, inhibition of Na,K-ATPase, memory deficit and ultrastructure cerebral changes. Melatonin is a hormone involved in circadian rhythm and has antioxidant effects. The aim of this study was to verify the possible neuroprotective effects of melatonin administration in hypermethioninemic pregnant rats on damage to biomolecules (Na,K-ATPase, sulfhydryl content and DNA damage index) and behavior (open field, novel object recognition and water maze tasks), as well as its effect on cells

morphology by electron microscopy in offspring. Wistar female rats received methionine (2.68 µmol/g body weight) and/or melatonin (10 mg/kg body weight) by subcutaneous injections during entire pregnancy. Control rats received saline. Biochemical analyzes were performed at 21 and 30 days of life of offspring and behavioral analyzes were performed only at 30 days of age in male pups. Results showed that gestational hypermethioninemia diminished Na,K-ATPase activity and sulfhydryl content and increased DNA damage at 21 and 30 days of life. Melatonin was able to totally prevent Na,K-ATPase activity alteration at 21 days and partially prevent its alteration at 30 days of rats life. Melatonin was unable in to prevent sulfhydryl and DNA damage at two ages. It also improved DNA damage, but not at level of saline animals (controls). Regarding to behavioral tests, data showed that pups exposed to gestational hypermethioninemia decreased reference memory in water maze, spent more time to the center of the open field and did not differentiate the objects in the recognition test. Melatonin was able to prevent the deficit in novel object recognition task. Electron microscopy revealed ultrastructure alterations in neurons of hypermethioninemic at both ages of offspring, whose were prevented by melatonin. These findings suggest that melatonin may be a good neuroprotective to minimize the harmful effects of gestational hypermethioninemia on offspring.
Int J Dev Neurosci, 2019; 78

31257611: Ilha M, Moraes KDS, Rohden F, Martins LAM, Borojevic R, Lenz G, Barbé-Tuana F, Guma FCR
Exogenous expression of caveolin-1 is sufficient for hepatic stellate cell activation.
Caveolin-1 (Cav-1) expression is increased in hepatic stellate cells (HSC) upon liver cirrhosis and it functions as an integral membrane protein of lipid rafts and caveolae that regulates and integrates multiple signals as a platform. This study aimed to evaluate the role of Cav-1 in HSC. Thus, the effects of exogenous expression of Cav-1 in GRX cells, a model of activated HSC, were determined. Here, we demonstrated through evaluating well-known HSC activation markers - such as α-smooth muscle actin, collagen I, and glial fibrillary acidic protein - that up regulation of Cav-1 induced GRX to a more activated phenotype. GRX presented an increased migration, an altered adhesion pattern, a reorganization f-actin cytoskeleton, an arrested cell cycle, a modified cellular ultrastructure, and a raised endocytic flux. Based on this, GRX represents a new cellular model that can be an important tool for understanding of events related to HSC activation. Furthermore, our results reinforce the role of Cav-1 as a molecular marker of HSC activation.
J Cell Biochem, 2019; 120

31194978: Thomé MP, Pereira LC, Onzi GR, Rohden F, Ilha M, Guma FT, Wink MR, Lenz G
Dipyridamole impairs autophagic flux and exerts antiproliferative activity on prostate cancer cells.
Autophagy is a cellular bulk degradation process used as an alternative source of energy and metabolites and implicated in various diseases. Inefficient autophagy in nutrient-deprived cancer cells would be beneficial for cancer therapy making its modulation valuable as a therapeutic strategy for cancer treatment, especially in combination with chemotherapy. Dipyridamole (DIP) is a vasodilator and antithrombotic drug. Its major effects involve the block of nucleoside uptake and phosphodiestesase inhibition, leading to increased levels of intracellular cAMP. Here we report that DIP increases autophagic markers due to autophagic flux blockage, resembling autophagosome maturation and/or closure impairment. Treatment with DIP results in an increased number of autophagosomes and autolysosomes and impairs degradation of SQSTM1/p62. As blockage of autophagic flux decreases the recycling of cellular components, DIP reduced the intracellular ATP levels in cancer cells. Autophagic flux blockage was neither through inhibition of lysosome function nor blockage of nucleoside uptake, but could be prevented by treatment with a PKA inhibitor, suggesting that autophagic flux failure mediated by DIP results from increased intracellular levels of cAMP. Treatment with DIP presented antiproliferative effects in vitro alone and in combination with chemotherapy drugs. Collectively, these data demonstrate that DIP can impair autophagic degradation, by preventing the normal autophagosome maturation, and might be useful in combination anticancer therapy.
Exp Cell Res, 2019; 382

29855847: Teixeira LV, Almeida RF, Rohden F, Martins LAM, Spritzer PM, de Souza DOG
Neuroprotective Effects of Guanosine Administration on In Vivo Cortical Focal Ischemia in Female and Male Wistar Rats.
Guanosine (GUO) has neuroprotective effects in experimental models of brain diseases involving glutamatergic excitotoxicity in male animals; however, its effects in female animals are poorly understood. Thus, we investigated the influence of gender and GUO treatment in adult male and female Wistar rats submitted to focal permanent cerebral ischemia in the motor cortex brain. Female rats were subdivided into non-estrogenic and estrogenic phase groups by estrous cycle verification. Immediately after surgeries, the ischemic animals were treated with GUO or a saline solution. Open field and elevated plus maze tasks were conducted with ischemic and naïve animals. Cylinder task, immunohistochemistry and infarct volume analyses were conducted only with ischemic animals. Female GUO groups achieved a full recovery of the forelimb symmetry at 28-35 days after the insult, while male GUO groups only partially recovered at 42 days, in the final evaluation. The ischemic insult affected long-term memory habituation to novelty only in female groups. Anxiety-like behavior, astrocyte morphology and infarct volume were not affected. Regardless the estrous cycle, the ischemic injury affected differently female and male animals. Thus, this study points that GUO is a potential neuroprotective compound in experimental stroke and that

more studies, considering the estrous cycle, with both genders are recommended in future investigation concerning brain diseases.
Neurochem Res, 2018; 43

29243196: de S Moreira D, Figueiró PW, Siebert C, Prezzi CA, Rohden F, Guma FCR, Manfredini V, Wyse ATS
Chronic Mild Hyperhomocysteinemia Alters Inflammatory and Oxidative/Nitrative Status and Causes Protein/DNA Damage, as well as Ultrastructural Changes in Cerebral Cortex: Is Acetylsalicylic Acid Neuroprotective?
Homocysteine is a sulfur-containing amino acid derived from methionine metabolism. When plasma homocysteine levels exceed 10-15 μM, there is a condition known as hyperhomocysteinemia, which occur as a result of an inborn error of methionine metabolism or by non-genetic causes. Mild hyperhomocysteinemia is considered a risk factor for development of neurodegenerative diseases. The objective of the present study was to evaluate whether acetylsalicylic acid has neuroprotective role on the effect of homocysteine on inflammatory, oxidative/nitrative stress, and morphological parameters in cerebral cortex of rats subjected to chronic mild hyperhomocysteinemia. Wistar male rats received homocysteine (0.03 μmol/g of body weight) by subcutaneous injections twice a day and acetylsalicylic acid (25 mg/Kg of body weight) by intraperitoneal injections once a day from the 30th to the 60th postpartum day. Control rats received vehicle solution in the same volume. Results showed that rats subjected to chronic mild hyperhomocysteinemia significantly increased IL-1β, IL-6, and acetylcholinesterase activity and reduced nitrite levels. Homocysteine decreased catalase activity and immunocontent and superoxide dismutase activity, caused protein and DNA damage, and altered neurons ultrastructure. Acetylsalicylic acid totally prevented the effect of homocysteine on acetylcholinesterase activity and catalase activity and immunocontent, as well as the ultrastructural changes, and partially prevented alterations on IL-1β levels, superoxide dismutase activity, sulfhydryl content, and comet assay. Acetylsalicylic acid per se increased DNA damage index. In summary, our findings showed that chronic chemically induced model of mild hyperhomocysteinemia altered some parameters and acetylsalicylic acid administration seemed to be neuroprotective, at least in part, on neurotoxicity of homocysteine.
Neurotox Res, 2018; 33

29086391: Schweinberger BM, Rodrigues AF, Dos Santos TM, Rohden F, Barbosa S, da Luz Soster PR, Partata WA, Faccioni-Heuser MC, Wyse ATS
Methionine Administration in Pregnant Rats Causes Memory Deficit in the Offspring and Alters Ultrastructure in Brain Tissue.
In the present work, we evaluated the effect of gestational hypermethioninemia on locomotor activity, anxiety, memory, and exploratory behavior of rat offspring through the following behavior tests: open field, object recognition, and inhibitory avoidance. Histological analysis was also done in the brain tissue of pups. Wistar female rats received methionine (2.68 μmol/g body weight) by subcutaneous injections during pregnancy. Control rats received saline. Histological analyses were made in brain tissue from 21 and 30 days of age pups. Another group was left to recover until the 30th day of life to perform behavior tests. Results from open field task showed that pups exposed to methionine during intrauterine development spent more time in the center of the arena. In the object recognition memory task, we observed that methionine administration during pregnancy reduced total exploration time of rat offspring during training session. The test session showed that methionine reduced the recognition index. Regarding to inhibitory avoidance task, the decrease in the step-down latency at 1 and 24 h after training demonstrated that maternal hypermethioninemia impaired short-term and long-term memories of rat offspring. Electron microscopy revealed alterations in the ultrastructure of neurons at 21 and 30 days of age. Our findings suggest that the cell morphological changes caused by maternal hypermethioninemia may be, at least partially, associated to the memory deficit of rat offspring.
Neurotox Res, 2018; 33

24831459: Rohden F, Costa CS, Hammes TO, Margis R, Padoin AV, Mottin CC, Guaragna RM
Obesity associated with type 2 diabetes mellitus is linked to decreased PC1/3 mRNA expression in the Jejunum.
Bariatric surgery is the most effective therapeutic option for obesity and its complications, especially in type 2 diabetes. The aim of this study was to investigate the messenger RNA (mRNA) gene expression of proglucagon, glucose-dependent insulinotropic peptide (GIP), prohormone convertase 1/3 (PC1/3), and dipeptidyl peptidase-IV (DPP-IV) in jejunum cells of the morbidly obese (OB) non type 2 diabetes mellitus (NDM2) and type 2 diabetes mellitus (T2DM), to determine the molecular basis of incretin secretion after bariatric surgery.
Obes Surg, 2014; 24

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ISOLATION AND CHARACTERIZATION OF HPSC-DERIVED STRIATAL PROGENITOR SUBPOPULATIONS FOR CELL THERAPY IN HUNTINGTON'S DISEASE

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims Huntington's disease (HD) is a currently incurable neurodegenerative disease primarily characterized by the loss of striatal medium spiny neurons (MSNs). Cell replacement therapy (CRT) is the only approach focused on structural and functional restoration of atrophied tissue in HD by replenishing the degenerating MSN population, but its clinical translation is limited by the heterogeneity of cell products. The aim of this work is to develop a novel therapeutic strategy to regenerate the brain tissue affected in HD by using cell sorting to generate safe, defined and reproducible cell products. **Methods** Here, we describe the identification of a marker for the selection of human pluripotent stem cell (hPSC)-derived striatal progenitors, and a method for the enrichment of these progenitors from heterogeneous cell populations. Furthermore, we characterize this subpopulation to assess its identity and potential to generate MSNs. Finally, we evaluate the survival of these progenitors following transplantation into the striatum of adult mice. **Results** We demonstrate that this approach reduces the heterogeneity of the final cell product and batch-to-batch variability using both control and HD hPSC lines. Moreover, we show that different neuroblast subtypes with the potential to generate MSNs can be enriched under different conditions. Finally, we provide evidence of the survival and integration into the striatum of the selected progenitors up to one-week post transplantation. **Conclusions** We conclude that the selection of striatal neuroblast populations prior to transplantation has the potential to generate safer and more defined cell products, which can be used to treat HD.

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MATRIX THERAPY COMBINED WITH MESENCHYMAL STEM CELLS-BASED APPROACH IN THE CONTEXT OF BRAIN ISCHEMIA

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Stroke is the leading cause of acquired disability in adults and the second cause of non-degenerative dementia and death. To date, thrombolysis and thrombectomy are the only treatments that can be applied to a limited number of patients (15%) due to a short therapeutic window and there is no therapeutic alternative for the remaining 85% of patients. Stroke provokes a rapid neuronal death and the destruction of the extracellular matrix (ECM), associated to the degradation glycosaminoglycans which are mainly composed of heparan sulfates (HS). In addition, following stroke, numerous preclinical studies showed that mesenchymal stem cells (MSC) are able to protect the brain tissue and enhance recovery, however their actions are limited by the hostile environment in the damaged tissue. The present work showed that combining MSC treatment with the HS mimetic agent, OTR4132, after ischemic stroke conferred a long-lasting neuroprotection and reduced functional deficits in normotensive and hypertensive rats submitted to a 1-hour transient middle cerebral artery occlusion. Beside the beneficial effects of OTR4132 on ECM and as neuroprotectant, the compound favor MSC survival/migration into the ischemic brain. Neuroprotection and functional recovery may be also achieved through secretion of factors and/or extracellular vesicles (EV) by MSC as it has been suggested by many previous studies. Therefore, we also evaluated *in vitro* the effect of OTR4132 on the production of EV from rat MSC and their cargo through different approaches. Altogether, our data underlined that combining matrix and stem cell-based therapies is a promising approach to improve stroke recovery.

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IN VIVO DIRECT REPROGRAMMING OF OPCS INTO GABAERGIC NEURONS IN ADOLESCENT AND ADULT MOUSE BRAIN

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims GABAergic interneurons have been shown to be dysfunctional or lost in many neurological disorders, such as early-onset schizophrenia which affects adolescents, and epilepsy. Therefore, developing strategies for interneuron cell replacement is of great importance. An attractive alternative approach for neural restoration is through direct reprogramming where a somatic cell can be converted into a neuron through ectopic expression of neuronal genes. The technique allows for *in situ* brain repair, i.e., through reprogramming resident cells into neurons. Our group has previously shown that parvalbumin (PV) interneurons can be generated from resident glia (oligodendrocyte progenitor cells; OPCs) via *in vivo* reprogramming in adult mouse striatum. Methods In this study, we have compared *in vivo* reprogramming in adolescent and adult mice using a different combination of transcription factors in tamoxifen-regulated Cre mice. The purpose was to study morphology, functional integration, and diversification of reprogrammed interneurons. Results Preliminary data suggest an improved GABAergic interneuron reprogramming using fewer transcription factors in adolescent mice compared to adult mice. The reprogrammed neurons express some interneuron subtype markers such as PV and can also be seen in other structures apart from the striatum such as the cortex and hippocampus. Functional analysis of the reprogrammed neurons is underway. Conclusions In conclusion, we show that with only two factors we can successfully reprogram OPCs into GABAergic neurons in adolescent and adult mice. This has the potential for cell replacement therapy in schizophrenia and epilepsy.

Pubmed:

34943958: Bruzelius A, Kidnapillai S, Drouin-Ouellet J, Stoker T, Barker RA, Rylander Ottosson D
Reprogramming Human Adult Fibroblasts into GABAergic Interneurons.
Direct reprogramming is an appealing strategy to generate neurons from a somatic cell by forced expression of transcription factors. The generated neurons can be used for both cell replacement strategies and disease modelling. Using this technique, previous studies have shown that γ-aminobutyric acid (GABA) expressing interneurons can be generated from different cell sources, such as glia cells or fetal fibroblasts. Nevertheless, the generation of neurons from adult human fibroblasts, an easily accessible cell source to obtain patient-derived neurons, has proved to be challenging due to the intrinsic blockade of neuronal commitment. In this paper, we used an optimized protocol for adult skin fibroblast reprogramming based on RE1 Silencing Transcription Factor (REST) inhibition together with a combination of GABAergic fate determinants to convert human adult skin fibroblasts into GABAergic neurons. Our results show a successful conversion in 25 days with upregulation of neuronal gene and protein expression levels. Moreover, we identified specific gene combinations that converted fibroblasts into neurons of a GABAergic interneuronal fate. Despite the well-known difficulty in converting adult fibroblasts into functional neurons in vitro, we could detect functional maturation in the induced neurons. GABAergic interneurons have relevance for cognitive impairments and brain disorders, such as Alzheimer's and Parkinson's diseases, epilepsy, schizophrenia and autism spectrum disorders.
Cells, 2021; 10
34324188: Kidnapillai S, Ottosson DR
Functional Assessment of Direct Reprogrammed Neurons In Vitro and In Vivo.
Direct reprogramming is an emerging research field where you can generate neurons from a somatic cell, such as a skin or glial cell by overexpressing neurogenic transcription factors. This technique allows fast generation of subtype-specific and functional neurons from both human and mouse cells. Despite the fact that neurons have been successfully generated both in vitro and in vivo, a more extensive analysis of the induced neurons including phenotypic functional identity or gradual maturity is still lacking. This is an important step for a further development of induced neurons towards cell therapy or disease modeling of neurological diseases. In this protocol, we describe a method for functional assessment of direct reprogrammed neuronal cells both in vitro and in vivo. Using a synapsin-driven reporter, our protocol allows for a direct identification of

the reprogrammed neurons that permits functional assessment using patch-clamp electrophysiology. For in vitro reprogramming we further provide an optimized coating condition that allows a long-term maturation of human induced neurons in vitro.
Methods Mol Biol, 2021; 2352
31535361: Birtele M, Sharma Y, Kidnapillai S, Lau S, Stoker TB, Barker RA, Rylander Ottosson D, Drouin-Ouellet J, Parmar M
Dual modulation of neuron-specific microRNAs and the REST complex promotes functional maturation of human adult induced neurons.
Direct neuronal reprogramming can be achieved using different approaches: by expressing neuronal transcription factors or microRNAs; and by knocking down neuronal repressive elements. However, there still exists a high variability in terms of the quality and maturity of the induced neurons obtained, depending on the reprogramming strategy employed. Here, we evaluate different long-term culture conditions and study the effect of expressing the neuronal-specific microRNAs, miR124 and miR9/9*, while reprogramming with forced expression of the transcription factors Ascl1, Brn2, and knockdown of the neuronal repressor REST. We show that the addition of microRNAs supports neuronal maturation in terms of gene and protein expression, as well as in terms of electrophysiological properties.
FEBS Lett, 2019; 593

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INTRANASAL ADMINISTRATION OF MESENCHYMAL STEM-CELLS-SECRETOME IMPROVES NEUROLOGICAL DEFICITS INDUCED BY PERINATAL ASPHYXIA: A PRECLINICAL STUDY.

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Perinatal asphyxia (PA) is an obstetric complication occurring when the oxygen supply to the newborn is interrupted. This health problem is associated with high morbimortality in preterm and term neonates, and long-term deficits in the surviving neonates. It severely affects the structure and function of brain regions, including hippocampus, neostriatum and white matter. Using an animal model mimicking asphyxia occurring during labour, we investigated on the therapeutic potential of secretome (MSC-S) derived from human adipose mesenchymal stem cells, preconditioned with deferoxamine, intranasally administered. PA was generated by immersing foetus-containing uterine horns in a water bath at 37 °C for 21 min. Thereafter, 16 µL of MSC-S (containing 6 µg of protein derived from 2×10^5 preconditioned-MSC), or vehicle, were intranasally administered 2 h after birth to asphyxia-exposed and control rats, evaluated at postnatal day (P) 1 and P7. Alternatively, pups received a dose of either preconditioned MSC-S or vehicle, at 2 h and P7, evaluated at P14, P30, and P60. The preconditioned MSC-S treatment (i) reversed asphyxia-induced hippocampal oxidative stress; (ii) increased antioxidative Nrf2 translocation and Nrf2 target genes (*ho1, nqo1, gsr, prdx2);* (iii) reduced neuroinflammation; (iv) decreased cleaved-caspase-3 cell-death; (v) prevented hypomyelination, increasing the expression of ASCL and MBP; (vi) improved behavioural deficits, including righting reflex, negative geotaxis, cliff aversion, locomotor activity, motor coordination, anxiety, and recognition memory. Overall, it is proposed that intranasal administration of preconditioned MSC-S is a novel therapeutic strategy to prevent the long-term effects of PA. Acknowledgments: FONDECYT (Grants #1190562; #1200287; #3210771), ANID-Chile 21191287

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MODELLING SYNAPTIC FUNCTION USING HUMAN BRAIN ORGANOIDS WITH MICRO-ELECTRODE ARRAYS AND ELECTRON MICROSCOPY ANALYSIS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims: Human brain organoids derived from induced pluripotent stem cells (iPSCs) are an emerging model in which to better understand neurodevelopmental disorders and test therapeutic efficacy. This project aims to characterise patient-derived brain organoids as an appropriate model for the neurodevelopmental disorder Rett syndrome which is predominantly caused by mutations in the Methyl-CpG binding protein 2 (*MECP2*) gene. **Methods:** MeCP2-deficient and neurotypical control human brain organoids were grown in culture for 3-7 months and characterised morphologically and functionally. Electron microscopy was used to assess the differences in the size and number of synaptic vesicles present at synaptic junctions, as well the number and size of mitochondria. Organoids were also tested on micro-electrode arrays (MEAs) to determine the spike firing rates. Raw data was analysed using GraphPad Prism. **Results:** MeCP2-deficient organoids showed significantly less spike data (indicating lower neural activity) compared to neurotypical control organoids when measured on the MEAs. MeCP2-deficient organoids also showed significantly less vesicles present at synaptic junctions compared to neurotypical control organoids. In addition, despite the number of mitochondria being equal in the patient and control organoids, the average size of the mitochondria were observed to be significantly smaller in the MeCP2-deficient organoids than that of the neurotypical control, validating previously published reports in post-mortem brain and murine studies. **Conclusions:** These observed differences between patient and control brain organoids validates the existing literature as well as provides novel findings and supports the use of brain organoids as a model.

Pubmed:
34713252: Coorey BA, Gold WA
Breaking Boundaries in the Brain-Advances in Editing Tools for Neurogenetic Disorders.
Monogenic neurological disorders are devastating, affecting hundreds of millions of people globally and present a substantial burden to individuals, carers, and healthcare systems. These disorders are predominantly caused by inherited or variants that result in impairments to nervous system development, neurodegeneration, or impaired neuronal function. No cure exists for these disorders with many being refractory to medication. However, since monogenic neurological disorders have a single causal factor, they are also excellent targets for innovative, therapies such as gene therapy. Despite this promise, gene transfer therapies are limited in that they are only suitable for neurogenetic disorders that fit within the technological reach of these therapies. The limitations include the size of the coding region of the gene, the regulatory control of expression (dosage sensitivity), the mode of expression (e.g., dominant negative) and access to target cells. Gene editing therapies are an alternative strategy to gene transfer therapy as they have the potential of overcoming some of these hurdles, enabling the retention of physiological expression of the gene and offers precision medicine-based therapies where individual variants can be repaired. This review focusses on the existing gene editing technologies for neurogenetic disorders and how these propose to overcome the challenges common to neurogenetic disorders with gene transfer therapies as well as their own challenges.
Front Genome Ed, 2021; 3
34512241: Haase FD, Coorey B, Riley L, Cantrill LC, Tam PPL, Gold WA
Pre-clinical Investigation of Rett Syndrome Using Human Stem Cell-Based Disease Models.
Rett syndrome (RTT) is an X-linked neurodevelopmental disorder, mostly caused by mutations in . The disorder mainly affects girls and it is associated with severe cognitive and physical disabilities. Modeling RTT in neural and glial cell cultures and brain organoids derived from patient- or mutation-specific human induced pluripotent stem cells (iPSCs) has advanced our understanding of the pathogenesis of RTT, such as disease-causing mechanisms, disease progression, and cellular and

molecular pathology enabling the identification of actionable therapeutic targets. Brain organoid models that recapitulate much of the tissue architecture and the complexity of cell types in the developing brain, offer further unprecedented opportunity for elucidating human neural development, without resorting to conventional animal models and the limited resource of human neural tissues. This review focuses on the new knowledge of RTT that has been gleaned from the iPSC-based models as well as limitations of the models and strategies to refine organoid technology in the quest for clinically relevant disease models for RTT and the broader spectrum of neurodevelopmental disorders.
Front Neurosci, 2021; 15

31379106: Krishnaraj R, Haase F, Coorey B, Luca EJ, Wong I, Boyling A, Ellaway C, Christodoulou J, Gold WA
Genome-wide transcriptomic and proteomic studies of Rett syndrome mouse models identify common signaling pathways and cellular functions as potential therapeutic targets.
The discovery that Rett syndrome is caused by mutations in the MECP2 gene has provided a major breakthrough in our understanding of the disorder. However, despite this, there is still limited understanding of the underlying pathophysiology of the disorder hampering the development of curative treatments. Over the years, a number of animal models have been developed contributing to our knowledge of the role of MECP2 in development and improving our understanding of how subtle expression levels affect brain morphology and function. Transcriptomic and proteomic studies of animal models are useful in identifying perturbations in functional pathways and providing avenues for novel areas of research into disease. This review focuses on published transcriptomic and proteomic studies of mouse models of Rett syndrome with the aim of providing a summary of all the studies, the reported dysregulated genes and functional pathways that are found to be perturbed. The 36 articles identified highlighted a number of dysfunctional pathways as well as perturbed biological networks and cellular functions including synaptic dysfunction and neuronal transmission, inflammation, and mitochondrial dysfunction. These data reveal biological insights that contribute to the disease process which may be targeted to investigate curative treatments.
Hum Mutat, 2019; 40

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ARTIFICIAL EXTRACELLULAR MATRIX SCAFFOLDS OF MOBILE MOLECULES ENHANCE MATURATION OF HUMAN STEM CELL-DERIVED NEURONS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Human induced pluripotent stem cell (iPSC)-based technologies offer a unique resource for modeling disease and regenerating complex tissues such as the central nervous system (CNS). However, human iPSC models are still fraught with significant technical limitations including inefficient maturation, abnormal aggregation and reduced long-term viability of neurons. These problems are in part due to the absence of synergistic cues derived from the architecture, chemical composition and molecular dynamics of the native extracellular matrix (ECM). We reasoned that establishing a stable and bioactive ECM environment that mimics the adult CNS would facilitate the functional maturation of iPSC-derived neurons. To test this, we utilized peptide amphiphiles (PAs) that have the ability to self-assemble into supramolecular nanofibers capable of morphologically and chemically mimicking the adult CNS ECM. We designed a series of PA nanofibers containing a short bioactive peptide (IKVAV) found in Laminin-alpha-1, which is higher expressed over development in the CNS and plays a major role in neuronal behavior. The newly designed IKVAV-PAs have an almost identical chemical composition, except for a 4 amino acids modification in the non-bioactive domain that makes the IKVAV epitope be displayed in a more or less mobile fashion within the nanofiber. Interestingly, proteomic, biochemical and functional assays reveal that scaffolds with highly mobile molecules of IKVAV lead to enhanced beta-1-integrin pathway activation, reduced aggregation, increased arborization, and mature electrophysiological activity of human iPSC-derived neurons. Our work highlights the importance of designing bioactive ECMs to study the development, function and dysfunction of human neurons *in vitro*.

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INACTIVATION OF HUNTINGTIN ALLELES IN PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS REVEALS WT-HTT ROLES IN STRIATAL DEVELOPMENT AND NEURONAL FUNCTIONS IMPAIRED IN HUNTINGTON DISEASE.

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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The human *HTT* gene encodes a large yet soluble protein named Huntingtin (HTT) ubiquitously expressed in all cells from the fertilized egg onward. This scaffold protein is involved in numerous cellular processes or machinery including intracellular transport, cell division, cell polarity, transcriptional regulation, autophagy, and apoptosis. Although it is best known for its life supporting activity in adult neurons, HTT is involved in several developmental processes such as gastrulation, epithelial morphogenesis, neural tube formation or neuroblast migration. Mutation of HTT cause the devastating neurodegenerative disorder, Huntington's disease (HD). HD is characterized by progressive degeneration in the basal ganglia and cerebral cortex. Neuronal loss is most marked among DARPP32-expressing medium-sized spiny projection neurons in the striatum. Most recent work in human fetuses carrying HD mutations evidenced as well neurodevelopmental defect at early embryonic stages. Here we describe the generation, by CRISPR-Cas9 technology, of HTT-gene edited isogenic clones of a human pluripotent stem cells (h-iPSC) line derived from an HD patient. We isolated and characterized, in particular, homozygous HTT knockout (-/-), wild-type-HTT hemizygote (wt/-) and mutant-HTT hemizygote (-/109Q) iPSC clones. Next, we used this genetic toolkit to explore the specific impact of mut-HTT isoform gain of function, wt-HTT loss of function and HTT gene complete inactivation on hPSC pluripotency, neural and striatal differentiation and neuronal function. Overall, we showed that wt and/or mut-HTT levels modulation do not disrupt h-iPSC pluripotency or neural induction but affect neural cell division, striatal maturation and BDNF axonal transport in neurons.

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THE INTERPLAY BETWEEN CELL SHAPE/SIZE AND FUNCTION IN VITRO: INVESTIGATING THE EFFECT OF AXONAL LENGTH ON HUMAN SPINAL MOTOR NEURONS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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The evolutionary link between cell shape and function is a tightly conserved paradigm, neurons for example present one of the architectural most complex shapes, and moreover neuronal subtypes show a broad variability of their cell sizes. Motorneurons (MNs) are one of the larges neurons and span in a human body up to one meter creating long-range connections between spinal cord and neuromuscular junction. As the axon of a MN spans across the periphery, this cell is confronted with even larger distances in other mammals, highlighting the outstanding variation of size and shape MNs experience. Such extreme cell shapes and sizes create biophysical constraints for the metabolism and homeostasis, which requires functional adaptations utilising basic engineering principles to maintain the integrity of the axon. Using the advantages of in vitro cultures and bioengineering techniques, we developed a platform which allows systematic analysis of axonal length as an isolated variable on biological processes, such as metabolism and homeostasis. This novel bioengineered platform combines iPSC-derived MNs, microfabrication and fluorescently encoded reporters, systematically obtaining arrays of axons up to several centimetres in length, while also allowing live imaging studies. Using the platform, we showed length dependent changes in cytoskeleton composition and changes to growth and dynamic homeostasis of the axoplasm. Moreover, we characterize mitochondrial dynamics and changes in the local protein translation an RNA binding protein concentration at different axonal lengths.

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EXTRINSIC FACTORS ENABLE NEUROGENESIS IN A MODEL OF THE HUMAN DENTATE GYRUS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Modelling development and maturation of the human hippocampus in culture is crucial to elucidate the physiological and pathological mechanisms of its neurogenesis. We established human hippocampal cell cultures by means of controlled neuralization of human induced pluripotent stem cells (hiPSCs). The timely re-activation of WNT signalling in hiPSCs, which were previously driven toward a dorsal telencephalic identity by the combined inhibition of WNT, BMP and TGF-β signalling, produced neural progenitors with a gene expression signature typical of human embryonic dentate gyrus (DG) cells. Key markers of neurogenesis resulted downstream of the NOTCH and WNT pathway and were upregulated in these progenitors. Notably, we found that, in addition to continuous WNT signalling, a specific laminin isoform is crucial to prolonging DG stem state and to extend DG progenitor proliferation for over 200 days in vitro. In fact, transition from laminin 511 to mouse laminin inhibited cell proliferation and promoted the differentiation of DG cells. Interestingly, global gene expression profiles of early and late progenitor cells and neurons suggest that a niche of laminin 511 and WNT signalling is sufficient to maintain a cell population enriched in DG progenitor cells and neurons. Finally, the xenograft of human DG progenitors into the DG of adult immunosuppressed host mice produced efficient integration of neurons, which innervated CA3 layer cells with an area covered by synapses similar to the area covered by endogenous hippocampal neuron synapses. Our observations indicate that neurons produced in the established culture niche resemble bona-fide human DG neurons.

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GENERATION OF A PATIENT SPECIFIC HIPSC-DERIVED NEURONAL MODEL FOR CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS)

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Human-induced pluripotent stem cell (hiPSC) technology enables us to obtain patient-specific cell type models relevant to the disease that could be otherwise unobtainable. This is the case for Congenital Central Hypoventilation Syndrome (CCHS), a rare neonatal disorder of the autonomic nervous system (ANS), characterized by a deficient control of autonomic ventilation and a global autonomic dysfunction. Indeed, the disease-defining gene, PHOX2B encodes a master transcription factor whose role is essential during development of the neural lineages of the ANS. *In vivo* and *in vitro* models of the disease provide a limited representation of human pathophysiology. To clarify the pathogenesis of CCHS, we have generated a hiPSC-derived autonomic neuronal model that fully recapitulates the patient's entire genetic profile. Using a non-integrating Sendai Virus (SeV), we reprogrammed fibroblasts from two CCHS patient's carrying the same genetic mutation but with different clinical manifestation of the disease. Here we show a complete characterization of both patient-derived iPSC lines by karyotyping, morphology study, immunocytochemistry, and qPCR analysis to confirm the presence of gene and protein expression of markers of pluripotency (e.g Nanog, Oct4 and SSEA4). Using a specific autonomic neural differentiation protocol, iPSC lines have been differentiated to neural crest stem cells (NCSCs), which bear the potential to develop into different lineages, among which peripheral autonomic PHOX2B positive neurons that carry the patient's specific mutation. This new personalized disease-in-a-dish model of CCHS opens numerous possibilities to identify molecular and cellular defects induced by the mutations as well as modelling for drug discovery/screening for therapeutic perspectives.

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CULTURED BRAIN ORGANOID SLICES AS A MODEL SYSTEM TO STUDY HUMAN NEURONS AND GLIAL CELLS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Rodents are established as major model systems in brain research. Translation of findings to the human brain, however, is often difficult. Alternative strategies are therefore needed to overcome the resulting setbacks in the development of novel therapeutic approaches to treat human brain disease. A promising new model system are three-dimensional brain organoids derived from human induced pluripotent stem cells (Le et al., 2021). A limitation of this *in vitro* model is that differentiation and maturation of cells require a long-term cultivation. The latter is often accompanied by the development of a necrotic core which arises from insufficient delivery of oxygen and nutrients to deeper regions of the growing organoids. To overcome this drawback, air-liquid interface cerebral organoids were recently introduced (Giandomenico et al., 2019). Adapting this approach, we established slice cultures derived from 11 weeks-old human brain organoids. In brief, organoids were embedded in low-gelling-temperature agarose, cut into 300 µm slices, transferred on membranes and cultured in a humidified incubator. After another 5 weeks in culture, slices showed immunoreactivity for S100ß and MAP2, revealing the presence of (presumed) astrocytes and mature neurons. Ca^{2+} imaging demonstrated that cells exhibit spontaneous intracellular Ca^{2+} signals and respond to glutamate. Taken together, these results suggest that cultured brain organoid slices might serve as a new model system to study the development, cytoarchitecture and physiological properties of human neurons and glial cells under controlled conditions in culture. Supported by the DFG (RU 2795 "Synapses under stress", RO 2327/13-2).

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FUNCTIONAL IMPACTS OF HUNTINGTIN LOWERING ON HUMAN ASTROCYTES DERIVED INDUCED PLURIPOTENT STEM CELLS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims: Huntingtin protein (HTT) is a multifunctional scaffold protein involved in numerous cellular processes including intracellular transport, cell division, cell polarity, ciliogenesis, transcriptional regulation, autophagy or apopotosis. Mutations in HTT are responsible for the devastating neurodegenerative disorder Huntington's disease (HD). Beyond neuronal dysfunctions and loss classically reported in HD, glial cells are as well impaired in the brain of HD patients. Lowering or inactivating the mutant or both, the wild-type and the mutant isoforms of HTT, are most likely the less disputed and direct paths to reduce HD symptoms and may be to cure HD patients. Multiple clinical trials are ongoing or in preparation to test this therapeutic approach despite the fact that the consequence of lowering or losing altogether wt-HTT isoforms in the human brain remains incompletely understood. HTT roles being mostly described in neurons, this issue is felt even more acutely regarding the understanding of HTT roles in astrocytes. Because human astrocytes feature specific morphology, diversity, functions and glia-to-neuron ratios, working on cells from humans is essential to model more precisely human astrocytes functions and interactions with neurons. **Methods**: Here we take advantage of human induced pluripotent stem cells (h-iPSC) technologies to explore the role of HTT protein in h-iPSC derived astrocytes. **Results**: We more specifically study astrocytic wt-HTT and mut-HTT functional alterations by HTT-lowering/editing treatments. **Conclusion**: We address these issues focusing on glutamate homeostasis and the signaling cascade mediated by primary cilia in h-iPSC derived astrocytes cultured alone or co-cultured with synaptically active human cortical neuronal network.

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AUTISM ASSOCIATED CASPR2 AUTO-IMMUNE ANTIBODIES MODIFY THE DEVELOPMENTAL TRAJECTORY AND NETWORK ACTIVITY IN HUMAN BRAIN ORGANOIDS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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The maternal *in utero* environment can contribute to neurodevelopmental disorders in the offspring. There is evidence linking the gestational transfer of brain-reactive antibodies, such as anti-CASPR2, contributing to permanent anomalies in the developing brain. CASPR2 was originally described to be involved in the stabilization of voltage-gated potassium channels (Kv1.1 and Kv1.2) on the myelinated axons, and later to have a role in earlier phases of rodent brain development. Our aim is the development of brain organoids to study this process in a relevant human model during early developmental periods. **Methods:** Here, we evaluate gene expression, synaptic and neurophysiological consequences of exposing brain organoids, for 4 months, to human anti-CASPR2 antibodies, derived from an autoimmune encephalitis patient. **Results:** Our data show a decrease in protein levels of CASPR2, Contactin-2 and Kv1.2 in 6-month-old organoids exposed to anti-CASPR2 antibodies. Neuronal network dynamics was also evaluated and showed increased spontaneous calcium transients in CASPR2-exposed organoids. We also measured spontaneous excitatory postsynaptic currents (sEPSCs) and action potentials (APs) properties. We observed an increase in the frequency and amplitude of sEPSCs, and in the firing rate of APs. Additionally, we observed a decrease in the fast-afterhyperpolarization (fAHP) amplitude. **Conclusions:** These results point to potassium channels dysfunction and consequently to a hyperexcitability phenotype in organoids exposed to anti-CASPR2 antibodies. Moreover, our data highlights the value of using brain organoids exposed to external cues, as robust models to study neurodevelopment.

Pubmed:
33630165: Sequeira DB, Oliveira AR, Seabra CM, Palma PJ, Ramos C, Figueiredo MH, Santos AC, Cardoso AL, Peça J, Santos JM
Regeneration of pulp-dentin complex using human stem cells of the apical papilla: in vivo interaction with two bioactive materials.
To compare the regenerative properties of human stem cells of the apical papilla (SCAPs) embedded in a platelet-rich plasma (PRP) scaffold, when implanted in vivo using an organotypic model composed of human root segments, with or without the presence of the bioactive cements - ProRoot MTA or Biodentine.
Clin Oral Investig, 2021; 25
28078993: Viegas ATB, Guedes JR, Oliveira AR, Cardoso AMS, Cardoso ALC
miRNAs: New Biomarkers and Therapeutic Targets in Dementia.
Dementia is a complex pathological state that affects millions of individuals worldwide and is responsible for a huge socioeconomic burden, making it a major health concern of current times. Given the impact of dementia in both patients and caregivers, it is crucial to fully clarify the molecular mechanisms underlying dementia-associated disorders, since without this knowledge our ability to correctly diagnose and treat these diseases is severely hampered.
Curr Pharm Des, 2017; 23

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IDENTIFICATION OF PRO-ANGIOGENIC FACTORS FOR IN VITRO VASCULARIZATION OF HIPSC-DERIVED BRAIN ORGANOIDS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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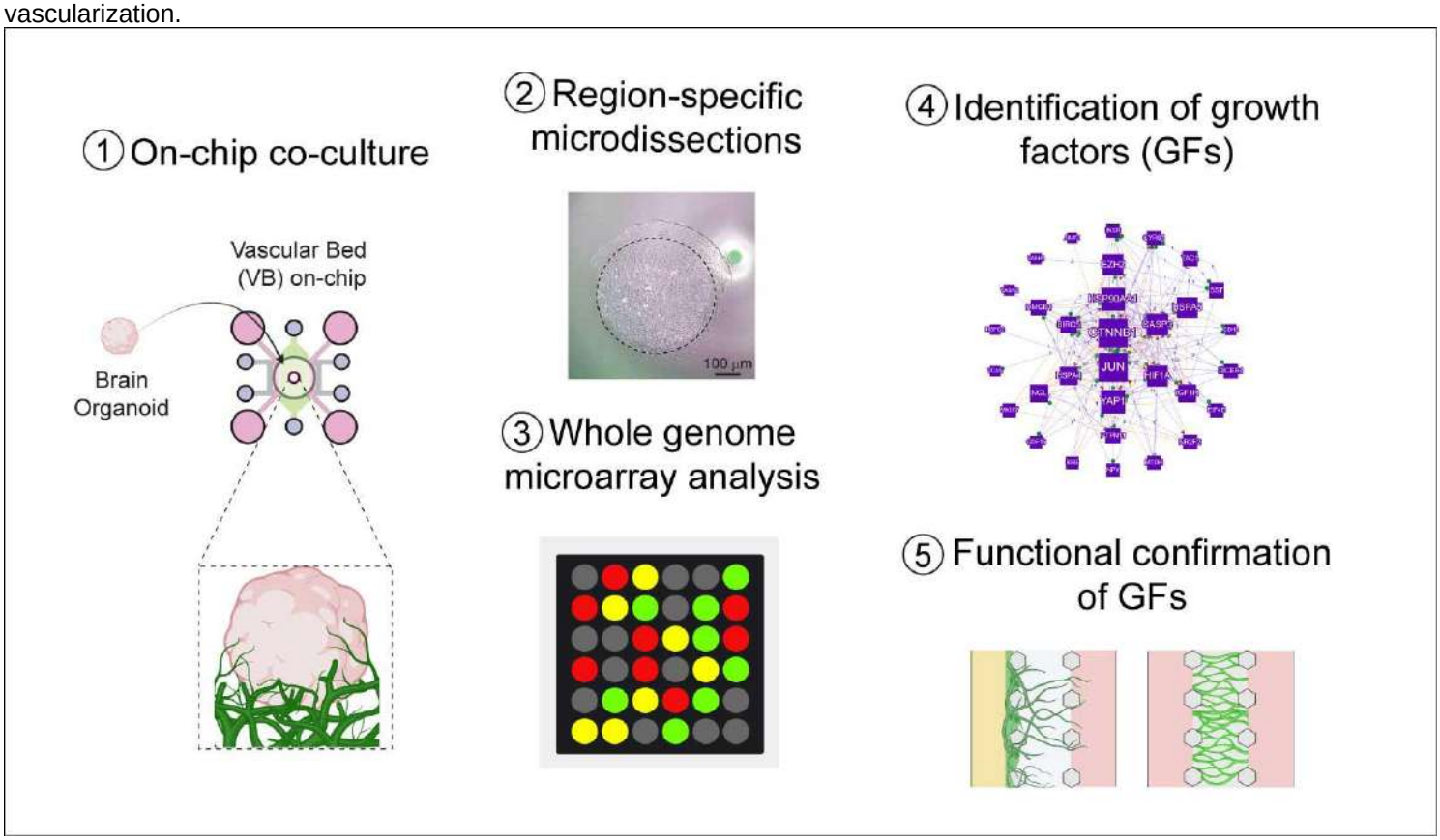
The lack of efficient vascularization protocols is a major limitation for further development and implementation of iPSC-derived cerebral organoids for translational research. Although several attempts have been reported, so far, complete vascularization with a functional capillary network was achieved only via transplantation. In this study, we used a combination of an on-chip culture system and region-specific transcriptome analysis, to identify the molecular program of early angiogenesis in human iPSCs derived brain organoids. Brain organoids were cultured on a preformed 3D vasculature established from HUVEC cells for ten days. Outer cortical layer of cerebral organoids was microdissected using the UnipicK+ microdissection system at several time points (day 28, 31 and 35). Isolated total RNA was then subjected to a full transcriptome analysis using Agilent microarrays. Angiogenic-specific pathways and differentially regulated genes were identified. Several pro-angiogenic factors were tested for organoid vascularization and are discussed in this presentation. This study provides the first whole-genome analysis of cerebral organoid response to in vitro vasculature and highlights putative modifiers for efficient on-chip
vascularization.



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BIOENGINEERED CORTICAL NEURONAL NETWORK (BIOCONNET): A STEM-CELL DERIVED NEURONAL ARRAY WITH DEFINED CIRCUITRY ARCHITECTURE IN VITRO

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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The cortical neuronal network exhibits highly complex and organised network architecture to process functional neuronal communication. Induced pluripotent stem cell (IPSC) technology has provided the possibility to create simplified experimental models for systematic studies to understand the functional properties of the neuronal network. However, IPSC-based models still present the limitation in control of the network formation in a precise architecture, resulting in randomly arranged connections between neurons. This can particularly lead to the high variability of functional maturation because the maturation of neurons is highly dependent on the underlying network connectivity. Therefore, we are developing a controllable cortical network platform using the combination of IPSC-derived cortical neurons, bioengineering techniques that enable us to control the network architecture and its components, including the geometry of the network nodes and directionality of neurites, and applying optogenetic tools to be able to control neuronal activity of the network. Finally, we are using this platform to investigate the impact of different network architectures on cortical neuronal network functionality and maturation.

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GENERATION OF A BLOOD-BRAIN BARRIER MODEL USING CRYOPRESERVED HUMAN IPSC-DERIVED BRAIN MICROVASCULAR ENDOTHELIAL CELLS, PERICYTES, AND ASTROCYTES

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims: Current in vitro models of the Blood-Brain Barrier (BBB) include immortalized or primary brain microvascular endothelial cells (BMEC), pericytes, and astrocytes of human or animal origin. However, these systems do not recreate the physiological barrier function observed in vivo. Cell types derived from human induced pluripotent stem cells (iPSC) offer a promising alternative for BBB model development. **Methods:** Episomally-derived iPSC were differentiated to BMEC, pericytes, and astrocytes using defined protocols and then cryopreserved. Cells were thawed in optimized media and characterized for marker expression and function. Importantly, a Transwell® sandwich model was developed (BMEC on the apical, and astrocytes+pericytes on the basolateral side) to measure barrier function by TEER. **Results:** BMEC expressed high levels (>90%) of CD31, Glut-1, P-glycoprotein, MRP-1, Claudin-5, ZO-1 and transferrin receptor by flow cytometry, distinguishing them from other peripheral endothelial cells. Pericytes expressed high levels of PGDFR-β, NG2, CD13 cell type identity markers and co-expressed Desmin, DLK1, and α-SMA, which is associated with a contractile PC2 subtype for pericytes. Astrocytes not only expressed prototypical glial markers CD44, S100β, and GFAP, but also demonstrated robust glutamate uptake. Most notably, BMEC cultured with pericytes and astrocytes for at least 7 days in the sandwich model yielded TEER values >3000 ohm/cm^2, which is significantly enhanced over other model systems. **Conclusions:** The successful generation of cryopreserved and functional iPSC-derived BMEC, pericytes, and astrocytes has enabled the development of a BBB model that can be used to study barrier function, drug permeability/transport, and neurodegenerative disease.

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DELINEATING THE IMMEDIATE MOLECULAR CONSEQUENCES OF THE GLIOBLASTOMA-ASSOCIATED H3.3K27M MUTATION

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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The histone variant H3.3 is localized at specific regions in the genome, including telomeres as well as promoters of expressed genes and active enhancers. H3.3 has a role in development and its turnover plays a role in neuronal transcription and plasticity. A lysine to methionine substitution in lysine 27 (H3.3K27M) is one of the main causes of Diffuse intrinsic pontine glioma (DIPG) in children, a severe type of lethal cancer occurring in the brain stem. H3.3K27M was shown to have a dominant-negative effect by inhibiting the Polycomb Repressor 2 (PRC2) complex activity and causing a global reduction of H3K27me3. The mechanism by which H3.3K27M causes cancer is yet unknown, however current models refer to its effects on PRC2 activity. In this study, we aimed to identify the immediate, genome-wide, consequences of the H3.3K27M mutation on H3.3 binding, H3 modifications and gene expression dynamics. We managed to distinguish between the dominant-negative effects of H3.3K27M on PRC2 and effects due to the missing lysine and find that mutant cells differentiated to neural progenitors or oligodendrocyte progenitors, retain the expression of genes which are highly expressed in the pluripotent state, suggesting an immature gene expression signature independent on PRC2 activity. H3.3 binding capacity of genes was increased in H3.3K27M presence and was also PRC2 independent. Overall, our findings delineate the immediate consequences of H3.3K27 mutations on global gene expression and demonstrate that K27 is required for both proper H3.3 turnover as well as proper regulation of global gene expression during neural differentiation.

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BOARD NUMBER: S05-352

WHOLE-GENOME CRISPR INTERFERENCE SCREEN IDENTIFIES ARID1A-DEPENDENT GROWTH REGULATORS IN HUMAN INDUCED PLURIPOTENT STEM CELLS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Human development relies on a balance between cellular and environmental clues that regulate fate decision and differentiation. Chromatin remodelers play an essential role in cell fate decisions. Thus, several chromatin remodelers are implicated in developmental diseases and cancer. Specifically, mutations in the ARID1A gene, a member of SWI/SNF complex, are associated with the Coffin-Siris syndrome, a developmental disorder with facial and central nervous system abnormalities. Intriguingly, ARID1A mutations are also observed in 20% of all cancers, which suggests a common mechanism involving cell growth regulation. In this study, we aim to investigate the effect of ARID1A mutation in hiPSC growth and survival. With CRISPR, we introduced 7bp frameshift mutation in the ARID1A gene and produced isogenic ARID1A +/+ and ARID1A+/- hiPSC lines. The mutant line had a lower growth rate compared to the wild type, so we conducted unbiased whole-genome CRISPRi screens to identify genes that modulate growth in an ARID1A-dependent way, and could potentially suppress the mutation effect. We observed that ARID1A$^{+/-}$ line is more sensitive to depletion of proteasome 20S subunit beta 2 (PSMB2), which can be targeted by proteasome inhibitors, thus providing a candidate therapeutic intervention. On the other hand, we showed that EZH2 silencing induces cell differentiation in ARID1A +/+ line but not in ARID1A +/- line, which might illuminate the ARID1A-dependent developmental disease mechanism. Overall, we established an efficient genome-scale method to identify genetic interactions with disease mutations, identified hits that are consistent with the target gene biology, and generated further hypotheses for the disease mechanisms.

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ASSESSING FUNCTIONALITY OF IPSC- BASED NEURONS MODELS OF FAMILIAL DYSAUTONOMIA

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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In recent years, the rise of iPSC technology has created many opportunities for drug discovery and personalized medicine, this ability is most relevant in the research of orphan diseases. Familial Dysautonomia (FD), is an autosomal recessive, rare genetic disorder that affects the development and survival of neurons in the autonomic and sensory nervous system. FD is caused by a mutation in the Elongator like protein-1 (ELP1) gene. To this day available treatments for FD only alleviate the symptoms and there is no therapeutic cure available. In this work we differentiated patient's and paired control's iPSCs into sympathetic neurons, to assess their neuronal function to test efficacy of potential drugs for treatment using our functionality assessment pipeline. Several aspects of neuronal functionality are tested in this pipeline, including electrical activity using a multi electrode array (MEA), vesicular trafficking along neurites using live time-lapse imaging and mitochondrial activity using TMRE mitochondrial membrane potential imaging. The implementation of this pipeline and characterization of FD neuronal functionality will facilitate the process of drug discovery for this devastating disease.

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WNT3A SUPPLEMENTATION INDUCES SPECIFIC HIPPOCAMPAL SIGNATURE IN MURINE BRAIN ORGANOIDS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Brain organoids are self-organized *in vitro* three-dimensional neural structures, which can be exploited in preclinical drug screening tests, neurodevelopmental studies, modeling neuronal diseases and regenerative medicine field. We isolated multipotent neural stem cells from the murine embryonic subgranular zone and developed a highly standardized, reproducible and fast (5 weeks) murine hippocampal brain organoid model. By adding a low concentration of the morphogen Wnt3a, we specifically induced the CA3-hippocampal phenotype. We showed that murine brain organoids progressively differentiate trough early, intermediate, and mature stages, defined by the expression of specific stemness (Vimentin, SOX2), neural progenitors (DCX) and mature neuronal (MAP2) markers. Additionally, functional properties measured by calcium imaging revealed that organoids increased their spontaneous calcium activity during maturation. Subsequently we investigated the specific hippocampal phenotype both via RT-PCR and immunofluorescence. RT-PCR analyses showed a high expression of hippocampal-related genes (e.g. Neurod1, Fzd9, Alk, Grik4) confirming the Wnt3a effectiveness in inducing the hippocampal patterning. We found the expression of the pan-hippocampal (ZBTB20) and CA3 (Ka1) markers in the mature organoids, hippocampal neurons ($ZBTB20^+/Map2^+$ and $Ka1^+/Map2^+$ cells) and a slight expression of the hippocampal CA1 (OCT6) and CA2 (FDZ9) markers. No expression of the DG (PROX1) marker was found. Overall, our results showed the establishment of murine three-dimensional *in vitro* hippocampal brain model that may be a useful tool for high-throughput drug screening and disease modelling. The possibility to generate functional mouse hippocampal organoids could be further exploited as an innovative tool for transplantation and regenerative purposes in pathological conditions, such as epilepsy.

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BIOCOMPATIBLE SCAFFOLDS IMPROVE NEURALIZATION AND REPRODUCIBILITY OF STEM CELL-DERIVED BRAIN ORGANOIDS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aim: Relying on minimal extrinsic interference, whole brain organoids generate multiple brain-like regions with diverse neuronal and glial cell types, as well as non-ectodermal lineage cells. As a result, they notoriously suffer from the “batch effect”, exhibiting highly variable efficiency and reproducibility in differentiation, morphology and size. To improve their homogeneity and anatomical features, hence increase their application in disease modeling and drug discovery, we present a novel highly reproducible protocol. **Methods:** Human induced pluripotent stem cell (hiPSC)-derived standard whole brain organoids (sBOs) and engineered flat brain organoids (efBOs) grown on a biocompatible 3D-printed polycaprolactone (PCL) scaffold are cultured using STEMdiff Cerebral Organoid Kit. Organoids are collected to evaluate the expression of the following makers: PAX6, NES, DCX, TUJ1, MAP2, AQP1, TTR and perform histological study of regional morphology by cresyl violet. **Results:** While sBOs display a loose tissue with large fluid-filled cysts positive for choroid plexus markers AQP1 and TTR, efBOs generate neural tissue with optically translucent neuroectoderm, neural stem cell-containing ventricular zones (PAX6+, NES+) surrounded by newly derived neurons (DCX+, TUJ1+) and mature neurons (MAP2+). **Conclusions:** Combining 3D cell culture with bioengineering, we have developed a protocol with simple tissue culture steps and consistent output with improved tissue architecture, enhanced neuronal differentiation and no fluid-filled cysts. As such, we address the existing shortcomings of human whole brain organoids, strongly increasing their reproducibility.

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MODELLING DRAVET SYNDROME USING HUMAN IPSC-DERIVED NEURAL CIRCUITS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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BACKGROUND/AIMS: Dravet syndrome is a rare form of severe epilepsy with limited treatment options. The disease is primarily caused by mutations in the SCN1A gene, mainly affecting GABAergic inhibitory interneurons. Human induced Pluripotent Stem Cell (hiPSC) technologies have permitted initial studies on neuronal cells derived from Dravet patients. However, an *in vitro* disease model that accounts for the interaction between excitatory and inhibitory components of cortical circuits has yet to be established. With this project we aim to build a comprehensive human iPSC-based disease model for Dravet syndrome suitable for exploring disease phenotypes, performing mechanistic studies, and testing potential therapies. *METHODS*: Our *in vitro* disease model includes a co-culture of cortical excitatory neurons and GABAergic inhibitory interneurons derived from hiPSCs using forward-programming differentiation. Electrophysiological characterisation of individual neuronal populations was coupled with two-colour calcium imaging to evaluate network activity. *RESULTS*: We have generated wildtype and patient-derived hiPSCs suitable for differentiation into inhibitory interneurons and excitatory neurons. The differentiated populations express appropriate markers of mature identity and form functional synapses. Crucially, in mature cultures, single-neuron and network analysis show innate hyperactivity of excitatory neurons in the Dravet model compared to wildtype. *CONCLUSIONS*: Here we present evidence of a previously uncharacterised role for excitatory neurons in Dravet syndrome pathogenesis. This excitatory contribution was previously overlooked in animal and *in vitro* models and could explain the poor efficacy of current treatments. We believe this comprehensive co-culture system will prove an important tool in facilitating the discovery of novel therapeutic approaches.

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IN VITRO MODEL OF ASTROCYTE-NEURON INTERACTIONS AT THE SYNAPSE FOR DRUG DISCOVERY USING HUMAN INDUCED PLURIPOTENT STEM CELLS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims: Astrocytes are the most abundant glial cell type in the human brain. They control synapse formation, maturation, elimination and functions integrating in particular neuronal firing and synaptic transmission. In neurodegenerative or developmental disorders, astrocytes can play multiple roles, either neuroprotective or neurotoxic. Neuro-glial interactions have thus recently become novel targets for therapeutics development. Miniaturized in vitro models of such interactions, allowing high throughput approaches, is key from both fundamental and translational perspectives in drug discovery. Because human astrocytes feature specific morphology, diversity, functions and glia-to-neuron ratios compared to rodents, working on cells from humans is essential for accurate modeling of human neuro-glial interactions. Methods: Here we take advantage of human induced pluripotent stem cells (h-iPSC) technologies and the Neurolead platform, to model, in microplate format, non-cell-autonomous modulations by human astrocytes of the synaptic activity of human cortico-cortical neuronal networks. Results: We first confirmed such h-iPSC derived neuronal network self-organized and synchronized. We evidenced neuronal spontaneous synchronous calcium events measured with a kinetic microplate reader (FDSSµCell). We next showed that this synaptic activity is dependent on network maturation and can be modulated by pharmacological inhibitors of GABA and Glutamatergic synaptic activities. Finally, we explored how astrocyte co-culture changes human cortical neuronal network maturation, connectivity and synchronicity parameters. Conclusion: This in vitro model of “healthy” human neuro-glial interactions in synaptically active neuronal networks can be challenged with different types of neurodegenerative or neurodevelopmental disease determinants and is amenable to high throughput screening for lead discovery and drug optimization for such disorders.

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THERAPEUTICAL APPROACH FOR A NOVEL IN VITRO MODEL OF X-LINKED ADRENOLEUKODYSTROPHY.

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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X-linked adrenoleukodystrophy (X-ALD) is an inborn error of metabolism caused by mutations in ABCD1 gene, which encodes a peroxisomal transmembrane protein called ALDP. This protein is involved in the transport of very-long chain fatty acid (VLCFA) for peroxisomal degradation. X-ALD patients show accumulation of VLCFA in tissue and blood. The outcome observed in patients with cerebral form of X-ALD is a devastating inflammatory demyelination. Unfortunately, there is not an effective therapy for all affected patients. A therapeutical option could be the use of bone marrow derived-mesenchymal stem cells (BMSCs). BMSCs have been proposed as a therapeutical approach in many neurological diseases as they have beneficial effect both in direct (cell-to cell contact) and indirect (paracrine signaling) mechanisms. The development of in vitro models of X-ALD is essential to increase knowledge about this complex disease. Here, we are developing a novel in vitro model to study X-ALD from dental pulp stem cells (DPSCs). We could analyse that DPSCs from a X-ALD patient showed specific phenotypical differences in comparison to healthy DPSCs. Also, X-ALD DPSCs could be differentiated into neural-like cells. Immunocytochemistry experiments revealed that X-ALD neural-like cells showed specific mature neural markers expression. Electrophysiological assays were carried out to further characterize X-ALD neural-like cells. These cells exhibited significant differences in amplitude and kinetics of sodium and potassium currents. Interestingly, when X-ALD neural-like cells were directly co-cultured with BMSCs, they rescued a healthy phenotype. Further investigation of the effect of BMSCs on X-ALD cells may provide relevant insights into X-ALD research.

Pubmed:
32150704: Martinez-Morga M, Medina-Corvalan C, Pérez-García C, Bueno C, Martinez S
[Mechanism of action of cell therapy in hereditary diseases].
Inherited metabolism disorders are serious childhood diseases that lead to significant cognitive impairment and regression of psychomotor development. The pathophysiology of the neural progressive deterioration is usually associated with severe neuroinflammation and demyelination, and as a consequence, neurodegeneration. At the moment they have no adequate treatment and require early and aggressive therapeutic approaches, which entail high mortality rates and, very frequently, low degrees of functional improvement and survival. Bone marrow transplantation and bone marrow mesenchymal cells grafts are therapeutic and experimental therapies that improve the course of these diseases through different mechanisms of action: enzyme replacement, membrane exchange and regulation of the inflammatory process.
Medicina (B Aires), 2020; 80 Suppl 2

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SELF-ORGANIZING MODEL OF HUMAN CAUDAL EMBRYOGENESIS REVEALS SIGNALING PATHWAYS CONTROLLING NEURAL TUBE AND SOMITE MORPHOGENESIS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Understanding human nervous system development and its connectivity with peripheral targets is a major challenge for basic and translational research and requires experimental models of human embryology beyond neural organoids. Locomotor circuits, which control all executive functions of the nervous system, emerge during embryogenesis from the coordinated morphogenesis and patterning of the caudal neural tube and muscle-generating somites. Here, we show that aggregates of human induced pluripotent stem cells can undergo a WNT/TGF-B/BMP-dependent elongation mimicking the caudal extension of the developing embryo. Single-cell and spatial transcriptomics reveal the formation of a typical antero-posterior (AP) axis along which somitic and spinal cord differentiation trajectories self-organize giving rise to a folded functional neural tube surrounded by segmented somites. This organization is established in absence of endoderm, notochord or anterior neural fate. Strikingly, collinear *HOX* transcriptional profiles form along this AP axis and lead to the specification of an organized array of motor neuron subtypes belonging to different locomotor circuits in human embryos. Finally, a proof of principle chemical screen identifies signaling pathways and teratogens impact on human neural tube morphogenesis. Overall, we report the generation of a self-organizing, scalable, tractable model of human caudal embryogenesis amenable to drug screening for basic and translational research.

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NEURODEVELOPMENTAL EFFECTS OF VALPROIC ACID EXPOSURE IN HUMAN BRAIN ORGANOIDS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Valproic acid (VPA) is a widely prescribed anticonvulsant and mood stabilizer. Its antiepileptic properties have been attributed to inhibitory effects on the degradation of gamma-Aminobutyric acid and modulation of ion channel activity. VPA has also been shown to act as a Histone Deacetylase (HDAC) inhibitor, acting indirectly at the level of gene transcription and modulating the expression of transcription factors and growth factors. VPA usage is restricted during pregnancy due to the high risk of the unborn child later developing autism spectrum disorder (ASD) and mental disability. Experiments with VPA using animal models and cell cultures have suggested that the main features observed during development are an imbalance between the excitatory and inhibitory neuronal population, which leads to a hyperactive system. However, to date, little is known on the effects of VPA in the developing human brain, nor how the brain cellular architecture changes due to the effects of this drug. Our aim is to use human brain organoids as an in vitro model to study the effects of chronic exposure to VPA on human brain development and better understand its consequences at the molecular, cellular and circuitry level. Our preliminary data indicate that VPA induces network hyperactivity by lowering the intrinsic excitability of neurons. Even though VPA is now recognized as a major risk factor, our study will help shed light on the aetiology of neurodevelopmental disorders such as ASD and intellectual disability.

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THE ROLE OF CALCIUM SIGNALING DURING NEURAL DEVELOPMENT IN BIPOLAR DISORDER (BPD)

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims: In this project, we propose the use of induced pluripotent stem (IPS) cells from meticulously characterized patients and controls to study neural development in relation to genetic variants of the calcium (Ca^{2+}) channel gene (*CACNA1C*). **Methods:** To provide a study model for BPD, we generated IPS cell lines from human dermal fibroblasts by integration-free all synthetic RNA-based reprogramming system. These IPS lines then differentiated into neural progenitor cells (NPCs) and forebrain glutamatergic neurons by dual SMAD signaling inhibitors, Noggin and SB431542. Starting with NPC and upon neuronal differentiation cells will be used for multiple phenotypic analyses at several time points to determine the role of *CACNA1C* and Ca^{2+} signaling in neural development. **Results:** The study included six BPD patients and six controls genotyped for *CACNA1C* (12 total cell lines). Analyzing the expression of specific cell surface markers and intracellular transcription factors by fluorescence activated cell sorting (FACS) analyzer confirmed the quality of IPS lines which later on were differentiated to NPCs, and then further into forebrain-specific cortical neurons. Immunocytochemical staining using markers of pluripotency (SOX1, TRA-1), neural stem cells (NESTIN) and neurons (TUJ1 and MAP2), confirmed the stage of cells. These cells also express relevant markers on mRNA level and exhibit spontaneous Ca^{2+} activity. **Conclusions:** Cellular reprogramming of adult somatic cells into iPSCs and consequently into neurons which are genetically identical to the donor, enable us to study pathogenesis of mental disorders, including BPD, with disease-permissive genetic contexts.

Pubmed:
34626371: Noborn F, Nikpour M, Persson A, Sihlbom C, Nilsson J, Larson G
A Glycoproteomic Approach to Identify Novel Proteoglycans.
In this chapter, we describe a glycoproteomic approach for the identification of novel chondroitin sulfate proteoglycans (CSPGs) using a combination of biochemical enrichments, enzymatic digestions, and nanoscale liquid chromatography tandem mass spectrometry (nLC-MS/MS) analysis. The identification is achieved by trypsin digestion of CSPG-containing samples, followed by enrichment of chondroitin sulfate (CS) glycopeptides by strong anion exchange chromatography (SAX). The enriched CS glycopeptides are then digested with chondroitinase ABC to depolymerize the CS polysaccharides, generating a residual hexasaccharide structure, composed of the linkage region tetrasaccharide extended with a terminal dehydrated disaccharide, still attached to the peptide. The obtained CS glycopeptides are analyzed by nLC-MS/MS, and the generated data sets are evaluated through proteomic software with adjustment in the settings to allow for glycopeptide identification. This approach has enabled the identification of several novel core proteins in human samples and in Caenorhabditis elegans. Here we specifically describe the procedure for the enrichment and characterization of CS glycopeptides from human cerebrospinal fluid (CSF).
Methods Mol Biol, 2022; 2303
34490248: Noborn F, Nikpour M, Persson A, Nilsson J, Larson G
Expanding the Chondroitin Sulfate Glycoproteome - But How Far?
Chondroitin sulfate proteoglycans (CSPGs) are found at cell surfaces and in connective tissues, where they interact with a multitude of proteins involved in various pathophysiological processes. From a methodological perspective, the identification of CSPGs is challenging, as the identification requires the combined sequencing of specific core proteins, together with the characterization of the CS polysaccharide modification(s). According to the current notion of CSPGs, they are often considered in relation to a functional role in which a given proteoglycan regulates a specific function in cellular physiology. Recent advances in glycoproteomic methods have, however, enabled the identification of numerous novel chondroitin sulfate core proteins, and their glycosaminoglycan attachment sites, in humans and in various animal models. In addition, these methods have revealed unexpected structural complexity even in the linkage regions. These findings indicate that the number and structural complexity of CSPGs are much greater than previously perceived. In light of these findings, the prospect of finding additional CSPGs, using improved methods for structural and functional characterizations, and studying novel sample

matrices in humans and in animal models is discussed. Further, as many of the novel CSPGs are found in low abundance and with not yet assigned functions, these findings may challenge the traditional notion of defining proteoglycans. Therefore, the concept of proteoglycans is considered, discussing whether "a proteoglycan" should be defined mainly on the basis of an assigned function or on the structural evidence of its existence.
Front Cell Dev Biol, 2021; 9
33997891: Nikpour M, Nilsson J, Persson A, Noborn F, Vorontsov E, Larson G
Proteoglycan profiling of human, rat and mouse insulin-secreting cells.
Proteoglycans (PGs) are proteins with glycosaminoglycan (GAG) chains, such as chondroitin sulfate (CS) or heparan sulfate (HS), attached to serine residues. We have earlier shown that prohormones can carry CS, constituting a novel class of PGs. The mapping of GAG modifications of proteins in endocrine cells may thus assist us in delineating possible roles of PGs in endocrine cellular physiology. With this aim, we applied a glycoproteomic approach to identify PGs, their GAG chains and their attachment sites in insulin-secreting cells. Glycopeptides carrying GAG chains were enriched from human pancreatic islets, rat (INS-1 832/13) and mouse (MIN6, NIT-1) insulinoma cell lines by exchange chromatography, depolymerized with GAG lyases, and analyzed by nanoflow liquid chromatography tandem mass spectrometry. We identified CS modifications of chromogranin-A (CgA), islet amyloid polypeptide, secretogranin-1 and secretogranin-2, immunoglobulin superfamily member 10, and protein AMBP. Additionally, we identified two HS-modified prohormones (CgA and secretogranin-1), which was surprising, as prohormones are not typically regarded as HSPGs. For CgA, the glycosylation site carried either CS or HS, making it a so-called hybrid site. Additional HS sites were found on syndecan-1, syndecan-4, nerurexin-2, protein NDNF and testican-1. These results demonstrate that several prohormones, and other constituents of the insulin-secreting cells are PGs. Cell-targeted mapping of the GAG glycoproteome forms an important basis for better understanding of endocrine cellular physiology, and the novel CS and HS sites presented here provide important knowledge for future studies.
Glycobiology, 2021; 31
33757834: Persson A, Nikpour M, Vorontsov E, Nilsson J, Larson G
Domain Mapping of Chondroitin/Dermatan Sulfate Glycosaminoglycans Enables Structural Characterization of Proteoglycans.
Of all posttranslational modifications known, glycosaminoglycans (GAGs) remain one of the most challenging to study, and despite the recent years of advancement in MS technologies and bioinformatics, detailed knowledge about the complete structures of GAGs as part of proteoglycans (PGs) is limited. To address this issue, we have developed a protocol to study PG-derived GAGs. Chondroitin/dermatan sulfate conjugates from the rat insulinoma cell line, INS-1832/13, known to produce primarily the PG chromogranin-A, were enriched by anion-exchange chromatography after pronase digestion. Following benzonase and hyaluronidase digestions, included in the sample preparation due to the apparent interference from oligonucleotides and hyaluronic acid in the analysis, the GAGs were orthogonally depolymerized and analyzed using nano-flow reversed-phase LC-MS/MS in negative mode. To facilitate the data interpretation, we applied an automated LC-MS peak detection and intensity measurement via the Proteome Discoverer software. This approach effectively provided a detailed structural description of the nonreducing end, internal, and linkage region domains of the CS/DS of chromogranin-A. The copolymeric CS/DS GAGs constituted primarily consecutive glucuronic-acid-containing disaccharide units, or CS motifs, of which the N-acetylgalactosamine residues were 4-O-sulfated, interspersed by single iduronic-acid-containing disaccharide units. Our data suggest a certain heterogeneity of the GAGs due to the identification of not only CS/DS GAGs but also of GAGs entirely of CS character. The presented protocol allows for the detailed characterization of PG-derived GAGs, which may greatly increase the knowledge about GAG structures in general and eventually lead to better understanding of how GAG structures are related to biological functions.
Mol Cell Proteomics, 2021; 20
24495449: Nikpour M, Emadi-Baygi M, Fischer U, Niegisch G, Schulz WA, Nikpour P
MTDH/AEG-1 contributes to central features of the neoplastic phenotype in bladder cancer.
Carcinoma of the bladder is the fifth most common cancer whose incidence continues to rise. MTDH/AEG-1 is associated with the initiation and progression of many cancers including breast, hepatocellular, ovarian, and colorectal carcinomas. However, the expression and functional importance of MTDH/AEG-1 in bladder cancer remains unknown. The present study was aimed at exploring the functional role of MTDH/AEG-1 in selected bladder cancer cell lines.
Urol Oncol, 2014; 32
21678030: Palizban A, Nikpour M, Salehi R, Maracy MR
Association of a common variant in TCF7L2 gene with type 2 diabetes mellitus in a Persian population.
Diabetes is one of the most common and challenging health problems. Studies in several nations show that polymorphisms within the transcription factor 7-like 2 genes could be associated with type 2 diabetes (T2D). Therefore, a case-control study was conducted to find the association between SNP rs7903146 and T2D in our population. The study consists of 110 patients referring to clinic and 80 healthy controls randomly selected based on WHO guideline. DNA was extracted from blood and

genotyped by PCR-RFLP with specific primers to amplify a fragment for restriction enzyme (RsaI). A chi-square test was calculated to compare the proportions of genotypes or alleles. Using a logistic regression model, the odds ratio for risk of developing T2D was calculated with and without adjustment for age, sex, and BMI. The frequency of the T allele of rs7903146 (C/T) polymorphism was significantly higher in diabetic subjects (47.3%) compared to that in normal subjects (34.4%). Logistic regression analysis of the rs7903146 polymorphism showed that the odds ratio was 3.71(95% CI: 1.43-9.56; P: 0.008) for the TT genotype and 1.26 (95% CI: 0.67-2.39; P: 0.516) for the CT genotype when compared with the CC genotype. Odds ratio adjusted for age, sex, and BMI have shown similar results. The results show that rs7903146 of TCF7L2 gene is an important susceptibility gene for T2D mellitus in the province of Isfahan, Iran. Our results support the recent findings that rs7903146 of TCF7L2 gene is an important genetic risk factor for the development of T2D in multiple ethnic groups.
Clin Exp Med, 2012; 12

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BOARD NUMBER: S05-362

MIDBRAIN ORGANOIDS WITH MICROGLIA REPRESENT A PROMISING TOOL FOR MODELLING AND STUDYING CELL AUTONOMOUS AND NON-CELL AUTONOMOUS MECHANISMS IN PD AT THE BRAIN TISSUE LEVEL

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Parkinson's disease (PD) is a common neurodegenerative disease affecting over 6 million people globally. Degeneration of the movement-controlling dopaminergic neurons in the midbrain results in the hallmark motor symptoms of PD. There are no curative or disease-modifying treatments for PD. **Aims**: To develop an hiPSC-derived in vitro 3D model system of PD using midbrain organoids (mORGs). The model is complemented with microglia-like cells to facilitate studying the non-cell autonomous mechanisms. Our focus is on optimizing the model for compatibility with functional readouts and for integration with microfluidic devices. **Methods**: Healthy control, patient-derived, and isogenic hiPSCs were seeded onto a silk scaffold to generate mORGs. Microglia progenitors were differentiated separately and co-cultured with the organoids on day 30. Correct patterning was assessed with RT-qPCR for midbrain markers. Immunohistochemical staining for Iba1 and TH were used to visualize microglia and dopaminergic neurons, respectively. Microdialysis was performed to measure extracellular dopamine and dopamine metabolites in the mORGs. **Results**: mORGs express appropriate midbrain-specific genes and contain neurons positive for FoxA2 and TH. Microglia progenitors migrate inside the organoid and give rise to Iba1+ cells with microglia-like morphology, and more ramified morphology after prolonged culture. Dopamine and its metabolites were detectable by HPLC in mORG dialysate. Microdialysis can be performed multiple times on the same mORG without significant damage, allowing for the monitoring of tissue dopamine at multiple time points. **Conclusions**: Our novel model is suitable for studying the effects of microglia on midbrain dopaminergic neurons in PD.

Pubmed:

33919317: Albert K, Niskanen J, Kälvälä S, Lehtonen Š
Utilising Induced Pluripotent Stem Cells in Neurodegenerative Disease Research: Focus on Glia.
Induced pluripotent stem cells (iPSCs) are a self-renewable pool of cells derived from an organism's somatic cells. These can then be programmed to other cell types, including neurons. Use of iPSCs in research has been two-fold as they have been used for human disease modelling as well as for the possibility to generate new therapies. Particularly in complex human diseases, such as neurodegenerative diseases, iPSCs can give advantages over traditional animal models in that they more accurately represent the human genome. Additionally, patient-derived cells can be modified using gene editing technology and further transplanted to the brain. Glial cells have recently become important avenues of research in the field of neurodegenerative diseases, for example, in Alzheimer's disease and Parkinson's disease. This review focuses on using glial cells (astrocytes, microglia, and oligodendrocytes) derived from human iPSCs in order to give a better understanding of how these cells contribute to neurodegenerative disease pathology. Using glia iPSCs in in vitro cell culture, cerebral organoids, and intracranial transplantation may give us future insight into both more accurate models and disease-modifying therapies.
Int J Mol Sci, 2021; 22
32190011: Aaltonen N, Singha PK, Jakupović H, Wirth T, Samaranayake H, Pasonen-Seppänen S, Rilla K, Varjosalo M, Edgington-Mitchell LE, Kasperkiewicz P, Drag M, Kälvälä S, Moisio E, Savinainen JR, Laitinen JT
High-Resolution Confocal Fluorescence Imaging of Serine Hydrolase Activity in Cryosections - Application to Glioma Brain Unveils Activity Hotspots Originating from Tumor-Associated Neutrophils.
Serine hydrolases (SHs) are a functionally diverse family of enzymes playing pivotal roles in health and disease and have emerged as important therapeutic targets in many clinical conditions. Activity-based protein profiling (ABPP) using fluorophosphonate (FP) probes has been a powerful chemoproteomic approach in studies unveiling roles of SHs in various biological systems. ABPP utilizes cell/tissue proteomes and features the FP-warhead, linked to a fluorescent reporter for in-gel fluorescence imaging or a biotin tag for streptavidin enrichment and LC-MS/MS-based target identification. Existing ABPP approaches characterize global SH activity based on mobility in gel or MS-based target identification and cannot reveal the

identity of the cell-type responsible for an individual SH activity originating from complex proteomes.
Biol Proced Online, 2020; 22

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PTK2B REGULATES ELECTRICAL ACTIVITY IN HUMAN NEURONS AND PLAYS A ROLE IN THE Aβ1-42-MEDIATED NEURONAL HYPEREXCITABILITY

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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PTK2B (Protein Tyrosine Kinase 2β) is a Ca^{2+}-activated non-receptor tyrosine kinase, identified as an important late-onset Alzheimer's disease (AD) risk gene and a major player in the mouse hippocampal synaptic plasticity. In this work, we aimed at studying the possible effects of PTK2B in neuronal electrical activity, taking advantage of human-induced pluripotent stem cell (hiPSC)-derived neurons (hiNs). We are using hiPSCs CRRSPR-cas9-edited to produce PTK2B homozygous, and heterozygous clones, spontaneously differentiated or lineage-reprogrammed (Neurog2) into hiNs. We evaluated the protein expression and cell composition using western blot and immunocytochemistry, and the neuronal electrical activity using calcium imaging and multi-electrode array (MEA) electrophysiology. Our data show that PTK2B under-expression leads to an increase in the frequency of calcium spikes, without significant changes in the percentage of active neurons. In KO cells, this is further enhanced, suggesting a dose-dependent effect of PTK2B in the regulation of neuronal activity. Interestingly, acute exposure to $Aβ_{1-42}$ increases the electrical activity in WT and HET but not in KOs, suggesting that PTK2B is necessary for Aβ-mediated neuronal hyperexcitability. MEA reveals changes in the electrical activity of PTK2B KO Neurog2-hiNs, suggesting a role of PTK2B in the regulation of electrical activity in glutamatergic neurons. Our preliminary data suggest that PTK2B regulates important biological processes involved in neuronal electrical activity under normal conditions, as well in an Alzheimer's model. Thus, altered expression of this risk gene in the AD brain could play a role in the neuronal hyperexcitability observed in patients at the early stages of the disease.

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3D HUMAN CORTICAL ORGANOIDS TO INVESTIGATE DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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The human cerebral cortex is characterized by an extraordinary complexity of neuronal and non-neuronal cell types wired together for the execution of high-order cognitive functions. Alterations, during development as well as after birth, in the assembly of cortical circuits can lead to aberrant neuronal activity, shared sign of neurodevelopmental disorders. Developmental and Epileptic Encephalopathy (DEE), a heterogeneous group of epilepsy disorders with a strong genetic component, constitute the most precocious syndromes that can affect infants as early as in the womb. *De novo* mutations in the hyperpolarization-activated cyclic nucleotide gated channels (HCN1,HCN2) are associated with sever and untreatable DEE forms. Both genetic and non-genetic components have been linked to DEE, however the causative mechanisms remain elusive. Here, we aim at decoupling the effect of aberrant activity *per se* from the patient-specific genetic make-up to uncover novel DEE mechanisms. By exploiting a highly reproducible human cortical organoids (hCOs) system, on which acute seizure-like currents are induced, we model infantile/pediatric epilepsy in vitro. In parallel, we generated hCOs from HCN1-DEE patient specific iPSC lines to study HCN1 specific variants. We aim at mapping- at the single-cell level - the epigenetic and transcriptional landscapes of both hCOs models: this will allow to deciphering the epigenetic fingerprints produced by exacerbate activity in distinct cortical neuron types along their trajectory and dissect the specific HCN1 effects on cortical assembly. The integrated analysis will identify molecular fingerprints downstream of aberrant activity *per se* and HCN1-DEE, and provide an invaluable resource for new drug targets for infantile epilepsy.

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N-GLYCOSYLATION OF INDUCED PLURIPOTENT STEM CELLS (IPSCS) AND NEURAL STEM CELLS (NSCS) DERIVED FROM A PERSON WITH DOWN SYNDROME (DS) CAUSED BY TRISOMY 21 (T21)

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aim: To explore the difference in N-glycosylation between euploid disomic (D21) and trisomic (T21) isogenic human induced pluripotent stem cells (iPSCs) and neural stem cells (NSCs) derived from a person with mosaic Down syndrome (DS) trisomy of chromosome 21. Methods: Pellets of isogenic disomic and trisomic iPSCs are derived from a person with mosaic DS. Cells were lysed and (glyco)proteins were extracted. N-glycans were released from cell lysates and then fluorescently labeled. After a clean-up by hydrophilic interaction liquid chromatography (HILIC), labeled glycans were obtained and subsequently separated by HILIC-UHPLC (ultra-high performance liquid chromatography). Data was processed into chromatograms which were separated into 45 glycan peaks. Results: Comparison of iPSCs and NSCs revealed a clear difference in N-glycosylation. There was an increase in the relative abundance of five glycan peaks and a decrease in the abundance of nine glycan peaks in iPSCs when compared to NSCs. Comparison of disomic and trisomic iPSCs revealed no significant difference in the relative abundance of N-glycans. The same was true for disomic and trisomic NSCs. Conclusion: A significant difference was observed in N-glycosylation between isogenic iPSC and NSCs, which falls in line with recently published research that showed NSCs tend to have more N-glycosylation of NrCAM and Plexins compared to iPSCs. The lack of differences in N-glycosylation between disomic and trisomic cells might be explained by the small effect of increased gene expression of chromosome 21 during the pluripotent/stem cell stage, but also due to low levels of complex N-glycosylation in iPSCs (and NSCs).

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VALIDATION OF THE ESSENTIAL ROLE OF THE 16P11.2 ASD CANDIDATE GENE QPRT IN HUMAN STEM CELL-DERIVED INEURONS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Altered neuronal development is discussed as the underlying pathogenic mechanism of autism spectrum disorders (ASD). We previously reported a gene of the ASD associated chromosomal region 16p11.2, *quinolinate phosphoribosyltransferase* (*QPRT*), to be essential for neuronal differentiation but not proliferation of the SH-SY5Y neuroblastoma model. QPRT is part of tryptophan metabolism and catabolizes quinolinic acid (QUIN). QUIN is an NMDA-R agonist and acts neurotoxic for glutamatergic cells. Our findings suggest QPRT to be involved in synaptic development and exlude a QUIN-driven neurotoxicity in the SH-SY5Y model. However, this needs to be replicated in a more mature human neuronal model allowing the investigation of functional glutamatergic and GABAergic neurons. Therefore, we further dissected the role of *QPRT* in human stem cell-derived iNeurons. Specifically, both glutamatergic and GABAergic iNeurons of QPRT-knockout cells were differentiated in co-cultures and compared to isogenic controls. Subsequent cell type-specific analysis was performed for each cell type labeled with a cell type-specific fluorophore. We will present the validity of our model as well as preliminary results of our analysis at morphological (Sholl analysis of e.g., neuritic complexity) and gene expression level of genes involved in tryptophan metabolism and of genes differentially regulated upon knockout of QPRT in the SH-SY5Y model. The analysis will be complemented by transcriptomic and network analysis during the time course of neuronal differentiation. Combining the readouts for morphological and transcriptomic assays will help to further dissect the etiology of ASD in 16p11.2 deletion carriers with the aim to identify new targets for potential pharmacological therapies.

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GENERATION AND CHARACTERIZATION OF HUMAN VENTRAL MIDBRAIN ORGANOIDS DERIVED FROM PARKINSON'S DISEASE-DERIVED AND CONTROL INDUCED PLURIPOTENT STEM CELLS.

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive degeneration of midbrain dopamine neurons. Current treatments mainly rely on pharmacotherapy, which seems to provide beneficial effects only in very early stages of the disease. Having an *in vitro* source of mature midbrain dopamine neurons from patients is paramount to initially reproduce disease phenotypes and subsequently identify disease-modifying drug candidates. In this context, three-dimensional (3D) induced pluripotent stem cell (iPSC)-derived brain organoids play an important role, as they mimic both cell-cell interactions and cytoarchitecture of the *in vivo* environment and also sustain longer term maturation than can be achieved with 2D adherent cultures. The aim of this study is to derive human midbrain organoids (hMOs) from control and PD patient-derived iPSC lines to identify distinctive hallmarks specifically related to the disease pathology. In addition, we perform 3D in vitro co-culture of hMOs with other neuronal and glial cell types to evaluate the impact on neural maturation, network complexity and also cell intrinsic and non-intrinsic disease mechanisms. Our results show successful derivation and long-term survival of hMOs both from control and PD-derived iPSCs and we are currently evaluating disease phenotype. In conclusion, hMOs represent an invaluable tool for developmental studies, disease modelling, and future high-throughput discovery.

Pubmed:
35180398: Moriarty N, Gantner CW, Hunt CPJ, Ermine CM, Frausin S, Viventi S, Ovchinnikov DA, Kirik D, Parish CL, Thompson LH
A combined cell and gene therapy approach for homotopic reconstruction of midbrain dopamine pathways using human pluripotent stem cells.
Midbrain dopamine (mDA) neurons can be replaced in patients with Parkinson's disease (PD) in order to provide long-term improvement in motor functions. The limited capacity for long-distance axonal growth in the adult brain means that cells are transplanted ectopically, into the striatal target. As a consequence, several mDA pathways are not re-instated, which may underlie the incomplete restoration of motor function in patients. Here, we show that viral delivery of GDNF to the striatum, in conjunction with homotopic transplantation of human pluripotent stem-cell-derived mDA neurons, recapitulates brain-wide mDA target innervation. The grafts provided re-instatement of striatal dopamine levels and correction of motor function and also connectivity with additional mDA target nuclei not well innervated by ectopic grafts. These results demonstrate the remarkable capacity for achieving functional and anatomically precise reconstruction of long-distance circuitry in the adult brain by matching appropriate growth-factor signaling to grafting of specific cell types.
Cell Stem Cell, 2022; 29
34022288: Mor ME, Harvey A, Familari M, St Clair-Glover M, Viventi S, de Iongh RU, Cameron FJ, Dottori M
Neural differentiation medium for human pluripotent stem cells to model physiological glucose levels in human brain.
Cortical neurospheres (NSPs) derived from human pluripotent stem cells (hPSC), have proven to be a successful platform to investigate human brain development and neuro-related diseases. Currently, many of the standard hPSC neural differentiation media, use concentrations of glucose (approximately 17.5-25 mM) and insulin (approximately 3.2 μM) that are much greater than the physiological concentrations found in the human brain. These culture conditions make it difficult to analyse perturbations of glucose or insulin on neuronal development and differentiation. We established a new hPSC neural differentiation medium that incorporated physiological brain concentrations of glucose (2.5 mM) and significantly reduced insulin levels (0.86 μM). This medium supported hPSC neural induction and formation of cortical NSPs. The revised hPSC neural differentiation medium, may provide an improved platform to model brain development and to investigate neural differentiation signalling pathways impacted by abnormal glucose and insulin levels.
Brain Res Bull, 2021; 173
33734599: Viventi S, Frausin S, Howden SE, Lim SY, Finol-Urdaneta RK, McArthur JR, Abu-Bonsrah KD, Ng W, Ivanusic J,

Thompson L, Dottori M
In vivo survival and differentiation of Friedreich ataxia iPSC-derived sensory neurons transplanted in the adult dorsal root ganglia.
Friedreich ataxia (FRDA) is an autosomal recessive disease characterized by degeneration of dorsal root ganglia (DRG) sensory neurons, which is due to low levels of the mitochondrial protein Frataxin. To explore cell replacement therapies as a possible approach to treat FRDA, we examined transplantation of sensory neural progenitors derived from human embryonic stem cells (hESC) and FRDA induced pluripotent stem cells (iPSC) into adult rodent DRG regions. Our data showed survival and differentiation of hESC and FRDA iPSC-derived progenitors in the DRG 2 and 8 weeks post-transplantation, respectively. Donor cells expressed neuronal markers, including sensory and glial markers, demonstrating differentiation to these lineages. These results are novel and a highly significant first step in showing the possibility of using stem cells as a cell replacement therapy to treat DRG neurodegeneration in FRDA as well as other peripheral neuropathies.
Stem Cells Transl Med, 2021; 10
32786674: Czuba-Wojnilowicz E, Miellet S, Glab A, Viventi S, Cavalieri F, Cortez-Jugo C, Dottori M, Caruso F
Distribution of Particles in Human Stem Cell-Derived 3D Neuronal Cell Models: Effect of Particle Size, Charge, and Density.
Neurodegenerative diseases are generally characterized by a progressive loss of neuronal subpopulations, with no available cure to date. One of the main reasons for the limited clinical outcomes of new drug formulations is the lack of appropriate in vitro human cell models for research and validation. Stem cell technologies provide an opportunity to address this challenge by using patient-derived cells as a platform to test various drug formulations, including particle-based drug carriers. The therapeutic efficacy of drug delivery systems relies on efficient cellular uptake of the carrier and can be dependent on its size, shape, and surface chemistry. Although considerable efforts have been made to understand the effects of the physiochemical properties of particles on two-dimensional cell culture models, little is known of their effect in three-dimensional (3D) cell models of neurodegenerative diseases. Herein, we investigated the role of particle size (235-1000 nm), charge (cationic and anionic), and density (1.05 and 1.8 g cm) on the interactions of particles with human embryonic stem cell-derived 3D cell cultures of sensory neurons, called sensory neurospheres (sNSP). Templated layer-by-layer particles, with silica or polystyrene cores, and self-assembled glycogen/DNA polyplexes were used. Particles with sizes <280 nm effectively penetrated sNSP. Additionally, effective plasmid DNA delivery was observed up to 6 days post-transfection with glycogen/DNA polyplexes. The findings provide guidance in nanoparticle design for therapies aimed at neurodegenerative diseases, in particular Friedreich's ataxia, whereby sensory neurons are predominantly affected. They also demonstrate the application of 3D models of human sensory neurons in preclinical drug development.
Biomacromolecules, 2020; 21
32270790: Czuba-Wojnilowicz E, Viventi S, Howden SE, Maksour S, Hulme AE, Cortez-Jugo C, Dottori M, Caruso F
Particle-mediated delivery of frataxin plasmid to a human sensory neuronal model of Friedreich's ataxia.
Increasing frataxin protein levels through gene therapy is envisaged to improve therapeutic outcomes for patients with Friedreich's ataxia (FRDA). A non-viral strategy that uses submicrometer-sized multilayered particles to deliver frataxin-encoding plasmid DNA affords up to 27 000-fold increase in frataxin gene expression within 2 days in vitro in a stem cell-derived neuronal model of FRDA.
Biomater Sci, 2020; 8
30977063: Abu-Bonsrah KD, Viventi S, Newgreen DF, Dottori M
Generation of Neural Crest Progenitors from Human Pluripotent Stem Cells.
There are a vast range of diseases and disorders that are neurocristopathic in origin, including Hirschsprung's disease, pheochromocytoma, familial dysautonomia, craniofacial disorders, and melanomas. Having a source of human neural crest cells is highly valuable for investigating potential treatments for such diseases. This chapter describes a robust and well-characterized protocol for deriving neural crest from human pluripotent stem cells (hPSCs), which can then be differentiated to neuronal and non-neuronal lineages. The protocol is adapted to suit hPSC maintenance as a monolayer bulk culture or as manual-passaged colonies, which makes it widely applicable to researchers that may use different systems for hPSC maintenance.
Methods Mol Biol, 2019; 1976
29772357: Viventi S, Dottori M
Modelling the dorsal root ganglia using human pluripotent stem cells: A platform to study peripheral neuropathies.
Sensory neurons of the dorsal root ganglia (DRG) are the primary responders to stimuli inducing feelings of touch, pain, temperature, vibration, pressure and muscle tension. They consist of multiple subpopulations based on their morphology, molecular and functional properties. Our understanding of DRG sensory neurons has been predominantly driven by rodent studies and using transformed cell lines, whereas less is known about human sensory DRG neurons simply because of limited availability of human tissue. Although these previous studies have been fundamental for our understanding of the sensory system, it is imperative to profile human DRG subpopulations as it is becoming evident that human sensory neurons

do not share the identical molecular and functional properties found in other species. Furthermore, there are wide range of diseases and disorders that directly/indirectly cause sensory neuronal degeneration or dysfunctionality. Having an in vitro source of human DRG sensory neurons is paramount for studying their development, unique neuronal properties and for accelerating regenerative therapies to treat sensory neuropathies. Here we review the major studies describing generation of DRG sensory neurons from human pluripotent stem cells and fibroblasts and the gaps that need to be addressed for using in vitro-generated human DRG neurons to model human DRG tissue.
Int J Biochem Cell Biol, 2018; 100
29330377: Alshawaf AJ, Viventi S, Qiu W, D'Abaco G, Nayagam B, Erlichster M, Chana G, Everall I, Ivanusic J, Skafidas E, Dottori M
Phenotypic and Functional Characterization of Peripheral Sensory Neurons derived from Human Embryonic Stem Cells.
The dorsal root ganglia (DRG) consist of a multitude of sensory neuronal subtypes that function to relay sensory stimuli, including temperature, pressure, pain and position to the central nervous system. Our knowledge of DRG sensory neurons have been predominantly driven by animal studies and considerably less is known about the human DRG. Human embryonic stem cells (hESC) are valuable resource to help close this gap. Our previous studies reported an efficient system for deriving neural crest and DRG sensory neurons from hESC. Here we show that this differentiation system gives rise to heterogeneous populations of sensory neuronal subtypes as demonstrated by phenotypic and functional analyses. Furthermore, using microelectrode arrays the maturation rate of the hESC-derived sensory neuronal cultures was monitored over 8 weeks in culture, showing their spontaneous firing activities starting at about 12 days post-differentiation and reaching maximum firing at about 6 weeks. These studies are highly valuable for developing an in vitro platform to study the diversity of sensory neuronal subtypes found within the human DRG.
Sci Rep, 2018; 8
25747736: Frausin S, Viventi S, Verga Falzacappa L, Quattromani MJ, Leanza G, Tommasini A, Valencic E
Wharton's jelly derived mesenchymal stromal cells: Biological properties, induction of neuronal phenotype and current applications in neurodegeneration research.
Multipotent mesenchymal stromal cells, also known as mesenchymal stem cells (MSC), can be isolated from bone marrow or other tissues, including fat, muscle and umbilical cord. It has been shown that MSC behave in vitro as stem cells: they self-renew and are able to differentiate into mature cells typical of several mesenchymal tissues. Moreover, the differentiation toward non-mesenchymal cell lineages (e.g. neurons) has been reported as well. The clinical relevance of these cells is mainly related to their ability to spontaneously migrate to the site of inflammation/damage, to their safety profile thanks to their low immunogenicity and to their immunomodulation capacities. To date, MSCs isolated from the post-natal bone marrow have represented the most extensively studied population of adult MSCs, in view of their possible use in various therapeutical applications. However, the bone marrow-derived MSCs exhibit a series of limitations, mainly related to their problematic isolation, culturing and use. In recent years, umbilical cord (UC) matrix (i.e. Wharton's jelly, WJ) stromal cells have therefore emerged as a more suitable alternative source of MSCs, thanks to their primitive nature and the easy isolation without relevant ethical concerns. This review seeks to provide an overview of the main biological properties of WJ-derived MSCs. Moreover, the potential application of these cells for the treatment of some known dysfunctions in the central and peripheral nervous system will also be discussed.
Acta Histochem, 2015 May-Jun; 117

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BOARD NUMBER: S05-368

NEURONS, ASTROCYTES, AND OLIGODENDROCYTES ARE PRESENT IN SPINAL ORGANOIDS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSC)

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Aims
Human iPSCs can be used to generate 3D organoid cultures that can partially represent neuronal structures. To determine whether spinal organoids can be used to study interactions between distinct cell types, we examined mature spinal organoids for the presence of neuronal and non-neuronal cells. **Methods**
Spinal organoids were grown up to 82 days and fixed at distinct differentiation steps. Following paraformaldehyde fixation, the organoids were cut and immunostained for the presence of neurons (β3-Tubulin, ISL1), astrocytes (GFAP) and oligodendrocytes (MBP, CC1). **Results**
Cells positive for β3-Tubulin and ISL1 were present throughout the spinal organoid structure, marking the presence of distinct neuronal subtypes including motor neurons. Cells expressing GFAP, a marker for reactive astrocytes were present in mature spinal organoids. The presence of oligodendrocytes was determined with antibodies directed to CC1 and MBP. Co-localisation of CC1 in a subset of MBP positive cells indicates the presence of oligodendrocytes at different maturation stages. Apart from oligodendrocyte markers, the cells furthermore resembled the typically expected morphology. **Conclusion**
Here we show that neuronal and non-neuronal cell types are present in spinal organoids, offering a research model for cellular interactions within the spinal cord. Further research should focus on elucidating the functional interactions between the cells in healthy and diseases conditions.

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DISSECTING THE LINK BETWEEN GLIAL CELL SHAPE AND FUNCTION IN VITRO USING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ASTROCYTES

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Astrocytes are glial cells with a dynamic and complex morphology, characterised by their multiple primary branches which then ramify into thousands of peripheral processes. These processes function as contact points between adjacent astrocytes and synapses, allowing astrocytes to perform their key brain functions. Astrocyte shape and process organisation changes based on their subtype, region and in response to their microenvironment, including stress and injury. However, it is not fully understood how an astrocyte's unique and dynamic shape impacts its performance in vital homeostatic functions. To address this, we performed a systematic characterisation on astrocytes differentiated from human induced pluripotent stem cells (iPSC) to investigate how shape is linked to an astrocyte's performance in resting and reactive states. We have optimised the generation of iPSC-derived astrocytes with distinct morphologies using a combination of different defined media with developmentally-relevant cytokines. Quantitative single cell and population level analysis on astrocyte shape uncovered the timepoint of astrocyte shape divergence, plasticity and morphological changes which occur between different astrocytes across varying culture conditions, such as in neuronal co-culture and reactivity. Additionally, we found that the differences in shape between astrocytes translates into distinct functional profiles and characteristics *in vitro*. Our systematic characterisation has revealed a link between dynamic glial shapes and their underlying functional profile, to provide insight on the significance of astrocyte shape changes in both health and injury.

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BOARD NUMBER: S05-370

CANNABIDIVARIN AND NEURAL STEM CELLS, A NEW HOPE FOR RETT SYNDROME?

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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[1]Universidade de Lisboa, Faculdade De Medicina, Lisboa, Portugal, [2]Universidade de Lisboa, Instituto De Farmacologia E Neurociências, Faculdade De Medicina, Lisboa, Portugal, [3]Faculdade de Medicina, Universidade de Lisboa, Instituto De Medicina Molecular João Lobo Antunes, Lisboa, Portugal

Rett Syndrome (RTT) is a rare progressive neurodevelopmental X-linked disorder, caused mostly by mutations in *MECP2* gene. This transcriptional and epigenetic regulator has been proposed to modulate neurogenesis and neuronal maturation, processes known to be affected in both RTT patients and mouse models. Cannabidivarin (CBDV) a non-psychotomimetic cannabinoid, currently undergoing phase 2 clinical trials for medical use in humans, has been reported to bind to TRPV1, with unknown effects on adult neurogenesis. Using the neurosphere assay, *in vitro*, cells were subjected to pharmacological treatments for 2 or 7 days according to the experimental protocol. CBDV-treated cells for 2 days in vitro (DIV2) promoted an increase in cell survival and cell proliferation. While at DIV7, CBDV promoted an increase in neuronal differentiation and inhibited oligodendroglial maturation. Importantly, TRPV1 antagonist 5′-Iodoresiniferatoxin blocked the effects on cell death and cell proliferation mediated by CBDV, further suggesting TRPV1-dependency. *In vivo*, using a female mouse model of RTT, animals were subjected to a chronic treatment with CBDV on the pre-symptomatic stage, followed by a battery of behaviour tests, used to gauge the putative therapeutic effects of CBDV administration. Preliminary data from the Novel Object Recognition test suggested that CBDV ameliorates cognitive impairments in these animals. Additionally, RTT animals show an increased abnormal hippocampal neurogenesis that seems to be attenuated in CBDV treated animals. Taken together, this project explores the novel neurogenic potential of CBDV via TRPV1 which will provide new insights for future research aiming to repurpose CBDV as a viable drug to treat RTT.

Pubmed:
33818505: Soares R, Ribeiro FF, Lourenço DM, Rodrigues RS, Moreira JB, Sebastião AM, Morais VA, Xapelli S
The neurosphere assay: an effective technique to study neural stem cells.
Neural Regen Res, 2021; 16
33022963: Lourenço DM, Ribeiro-Rodrigues L, Sebastião AM, Diógenes MJ, Xapelli S
Neural Stem Cells and Cannabinoids in the Spotlight as Potential Therapy for Epilepsy.
Epilepsy is one of the most common brain diseases worldwide, having a huge burden in society. The main hallmark of epilepsy is the occurrence of spontaneous recurrent seizures, having a tremendous impact on the lives of the patients and of their relatives. Currently, the therapeutic strategies are mostly based on the use of antiepileptic drugs, and because several types of epilepsies are of unknown origin, a high percentage of patients are resistant to the available pharmacotherapy, continuing to experience seizures overtime. Therefore, the search for new drugs and therapeutic targets is highly important. One key aspect to be targeted is the aberrant adult hippocampal neurogenesis (AHN) derived from Neural Stem Cells (NSCs). Indeed, targeting seizure-induced AHN may reduce recurrent seizures and shed some light on the mechanisms of disease. The endocannabinoid system is a known modulator of AHN, and due to the known endogenous antiepileptic properties, it is an interesting candidate for the generation of new antiepileptic drugs. However, further studies and clinical trials are required to investigate the putative mechanisms by which cannabinoids can be used to treat epilepsy. In this manuscript, we will review how cannabinoid-induced modulation of NSCs may promote neural plasticity and whether these drugs can be used as putative antiepileptic treatment.
Int J Mol Sci, 2020; 21
32510488: Soares R, Ribeiro FF, Lourenço DM, Rodrigues RS, Moreira JB, Sebastião AM, Morais VA, Xapelli S
Isolation and Expansion of Neurospheres from Postnatal (P1-3) Mouse Neurogenic Niches.
The neurosphere assay is an extremely useful in vitro technique for studying the inherent properties of neural stem/progenitor cells (NSPCs) including proliferation, self-renewal and multipotency. In the postnatal and adult brain, NSPCs are mainly present in two neurogenic niches: the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone of the

hippocampal dentate gyrus (DG). The isolation of the neurogenic niches from postnatal brain allows obtaining a higher amount of NSPCs in culture with a consequent advantage of higher yields. The close contact between cells within each neurosphere creates a microenvironment that may resemble neurogenic niches. Here, we describe, in detail, how to generate SVZ- and DG-derived neurosphere cultures from 1-3-day-old (P1-3) mice, as well as passaging, for neurosphere expansion. This is an advantageous approach since the neurosphere assay allows a fast generation of NSPC clones (6-12 days) and contributes to a significant reduction in the number of animal usage. By plating neurospheres in differentiative conditions, we can obtain a pseudomonolayer of cells composed of NSPCs and differentiated cells of different neural lineages (neurons, astrocytes and oligodendrocytes) allowing the study of the actions of intrinsic or extrinsic factors on NSPC proliferation, differentiation, cell survival and neuritogenesis.
J Vis Exp, 2020;
30959794: Rodrigues RS, Lourenço DM, Paulo SL, Mateus JM, Ferreira MF, Mouro FM, Moreira JB, Ribeiro FF, Sebastião AM, Xapelli S
Cannabinoid Actions on Neural Stem Cells: Implications for Pathophysiology.
With the increase of life expectancy, neurodegenerative disorders are becoming not only a health but also a social burden worldwide. However, due to the multitude of pathophysiological disease states, current treatments fail to meet the desired outcomes. Therefore, there is a need for new therapeutic strategies focusing on more integrated, personalized and effective approaches. The prospect of using neural stem cells (NSC) as regenerative therapies is very promising, however several issues still need to be addressed. In particular, the potential actions of pharmacological agents used to modulate NSC activity are highly relevant. With the ongoing discussion of cannabinoid usage for medical purposes and reports drawing attention to the effects of cannabinoids on NSC regulation, there is an enormous, and yet, uncovered potential for cannabinoids as treatment options for several neurological disorders, specifically when combined with stem cell therapy. In this manuscript, we review in detail how cannabinoids act as potent regulators of NSC biology and their potential to modulate several neurogenic features in the context of pathophysiology.
Molecules, 2019; 24

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BOARD NUMBER: S05-371

DEVELOPMENT AND CHARACTERIZATION OF AN IN VITRO MODEL OF SSADH DEFICIENCY USING PATIENT IPSC-DERIVED NEURONS TO SUPPORT UNBIASED SCREENING OF NOVEL THERAPEUTIC APPROACHES TO TREATMENT.

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal-recessive neurometabolic disorder caused by bi-allelic mutations in the ALDH5A1 gene. It is the most prevalent inherited disorder of GABA metabolism and is characterized by accumulation of two neuromodulators, GABA and GHB (gamma-hydroxybutyric acid), in the CNS. Previous studies using rodent models have shown that disruption in GABA signaling can lead to dysregulation of mitochondria numbers, turnover, and function. Over the last 30 years, an expanded understanding of pathophysiology based on the corresponding animal model (*Aldh5a1*-/- mice) has emerged but effective pharmacotherapy remains elusive. Alternative models and therapies that address the accumulation of GABA and GHB, and their downstream effects, are needed. In collaboration with the HumanNeuronCore at the Boston Children's Hospital, five clinically-phenotyped patients and unaffected sex-matched parents have been consented and recruited from our SSADH deficiency registry. Fibroblasts have been collected for reprogramming. Three iPSC patient lines and sex-matched parental controls have been generated at the Harvard Stem Cell Institute. We have established the first *in vitro* model of SSADH Deficiency based on iPSC-derived neurons. We successfully generated GABAergic and excitatory neurons based on transcription factor programming (Yang et al., 2017; Zhang et al., 2013) and characterized these models in respect to SSADH deficiency phenotypes such as GABA levels and mitochondria function. Additionally, we performed functional assays to investigate neuronal excitability based on optogenetics and calcium imaging in co-cultures of GABAergic and excitatory neurons to evaluate epileptiform activity in SSADH deficient iPSC-derived neurons and create cell-based models suitable for drug screening.

Pubmed:
34087981: Eberhardt K, Jumo H, D'Amore A, Alecu JE, Ziegler M, Afshar Saber W, Sahin M, Ebrahimi-Fakhari D
Generation and characterization of six human induced pluripotent stem cell lines (iPSC) from three families with AP4M1-associated hereditary spastic paraplegia (SPG50).
Biallelic loss-of-function variants in the subunits of the adaptor protein complex 4 lead to childhood-onset hereditary spastic paraplegia (AP-4-HSP): SPG47 (AP4B1), SPG50 (AP4M1), SPG51 (AP4E1), and SPG52 (AP4S1). Here, we describe the generation of induced pluripotent stem cells (iPSCs) from three AP-4-HSP patients with biallelic, loss-of-function variants in AP4M1 and their sex-matched parents (asymptomatic, heterozygous carriers). Following reprogramming using non-integrating Sendai virus, iPSCs were characterized following standard protocols including karyotyping, embryoid body formation, pluripotency marker expression and STR profiling. These first iPSC lines for SPG50 provide a valuable resource for studying this rare disease and related forms of hereditary spastic paraplegia.
Stem Cell Res, 2021; 53
33861989: Anderson NC, Chen PF, Meganathan K, Afshar Saber W, Petersen AJ, Bhattacharyya A, Kroll KL, Sahin M,
Balancing serendipity and reproducibility: Pluripotent stem cells as experimental systems for intellectual and developmental disorders.
Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) and their differentiation into neural lineages is a revolutionary experimental system for studying neurological disorders, including intellectual and developmental disabilities (IDDs). However, issues related to variability and reproducibility have hindered translating preclinical findings into drug discovery. Here, we identify areas for improvement by conducting a comprehensive review of 58 research articles that utilized iPSC-derived neural cells to investigate genetically defined IDDs. Based upon these findings, we propose recommendations for best practices that can be adopted by research scientists as well as journal editors.

Stem Cell Reports, 2021; 16
32075691: Afshar Saber W, Sahin M
Recent advances in human stem cell-based modeling of Tuberous Sclerosis Complex.
Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by epilepsy, intellectual disability, and benign tumors of the brain, heart, skin, and kidney. Animal models have contributed to our understanding of normal and abnormal human brain development, but the construction of models that accurately recapitulate a human pathology remains challenging. Recent advances in stem cell biology with the derivation of human-induced pluripotent stem cells (hiPSCs) from somatic cells from patients have opened new avenues to the study of TSC. This approach combined with gene-editing tools such as CRISPR/Cas9 offers the advantage of preserving patient-specific genetic background and the ability to generate isogenic controls by correcting a specific mutation. The patient cell line and the isogenic control can be differentiated into the cell type of interest to model various aspects of TSC. In this review, we discuss the remarkable capacity of these cells to be used as a model for TSC in two- and three-dimensional cultures, the potential variability in iPSC models, and highlight differences between findings reported to date.
Mol Autism, 2020; 11
30026684: Afshar Saber W, Gasparoli FM, Dirks MG, Gunn-Moore FJ, Antkowiak M
All-Optical Assay to Study Biological Neural Networks.
We introduce a novel all-optical assay for functional studies of biological neural networks . We created a novel optogenetic construct named OptoCaMP which is a combination of a channelrhodopsin variant (CheRiff) and a red genetically encoded calcium indicator (GECI) (jRCaMP1b). It enables simultaneous optical stimulation and recording from large population of neurons with single-cell readout. Additionally, we have developed a spatio-temporal all-optical assay to simultaneously stimulate a sub-section of a neural network and record evoked calcium activity, in both stimulated and non-stimulated neurons, thus allowing the investigation of the spread of excitation through an interconnected network. Finally, we demonstrate the sensitivity of this assay to the change of neural network connectivity.
Front Neurosci, 2018; 12
30333995: Escobet-Montalbán A, Spesyvtsev R, Chen M, Saber WA, Andrews M, Herrington CS, Mazilu M, Dholakia K
Wide-field multiphoton imaging through scattering media without correction.
Optical approaches to fluorescent, spectroscopic, and morphological imaging have made exceptional advances in the last decade. Super-resolution imaging and wide-field multiphoton imaging are now underpinning major advances across the biomedical sciences. While the advances have been startling, the key unmet challenge to date in all forms of optical imaging is to penetrate deeper. A number of schemes implement aberration correction or the use of complex photonics to address this need. In contrast, we approach this challenge by implementing a scheme that requires no a priori information about the medium nor its properties. Exploiting temporal focusing and single-pixel detection in our innovative scheme, we obtain wide-field two-photon images through various turbid media including a scattering phantom and tissue reaching a depth of up to seven scattering mean free path lengths. Our results show that it competes favorably with standard point-scanning two-photon imaging, with up to a fivefold improvement in signal-to-background ratio while showing significantly lower photobleaching.
Sci Adv, 2018; 4
25420066: Wainger BJ, Buttermore ED, Oliveira JT, Mellin C, Lee S, Saber WA, Wang AJ, Ichida JK, Chiu IM, Barrett L, Huebner EA, Bilgin C, Tsujimoto N, Brenneis C, Kapur K, Rubin LL, Eggan K, Woolf CJ
Modeling pain in vitro using nociceptor neurons reprogrammed from fibroblasts.
Reprogramming somatic cells from one cell fate to another can generate specific neurons suitable for disease modeling. To maximize the utility of patient-derived neurons, they must model not only disease-relevant cell classes, but also the diversity of neuronal subtypes found in vivo and the pathophysiological changes that underlie specific clinical diseases. We identified five transcription factors that reprogram mouse and human fibroblasts into noxious stimulus-detecting (nociceptor) neurons. These recapitulated the expression of quintessential nociceptor-specific functional receptors and channels found in adult mouse nociceptor neurons, as well as native subtype diversity. Moreover, the derived nociceptor neurons exhibited TrpV1 sensitization to the inflammatory mediator prostaglandin E2 and the chemotherapeutic drug oxaliplatin, modeling the inherent mechanisms underlying inflammatory pain hypersensitivity and painful chemotherapy-induced neuropathy. Using fibroblasts from patients with familial dysautonomia (hereditary sensory and autonomic neuropathy type III), we found that the technique was able to reveal previously unknown aspects of human disease phenotypes in vitro.
Nat Neurosci, 2015; 18

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HUMAN DORSAL FOREBRAIN ORGANOIDS HELP TO ELUCIDATE CELL TYPE-SPECIFIC EFFECTS OF MATERNAL IMMUNE ACTIVATION ON FETAL CORTICAL DEVELOPMENT

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Prenatal exposure to maternal immune activation (MIA) during the first trimester of gestation is correlated with long-term deficits in brain development in the offspring, including increased risks for autism spectrum disorder (ASD) in humans. Rodent models show causal links between prenatal exposure to MIA and ASD-like behavioral deficits in pups that are mediated by deficits in protein translation in neurons. While the majority of studies on the effects of MIA focus on neuronal abnormalities, a growing body of evidence suggests that defects in proliferative and neurogenic capacity of neural stem cells may be important contributors to ASD. In order to determine changes in neurogenesis following MIA in human neural stem cells, we treat human iPSC-derived dorsal forebrain organoids with molecular mediators of MIA. We have validated our model by showing that both dorsal forebrain organoids and midgestational human neocortex express the molecular machinery necessary for the response to the mediators of MIA. We have investigated the consequences of signaling pathway activation by mediators of MIA using immunohistochemistry. Single-cell RNA sequencing of more than 10,000 cells showed that dorsal forebrain organoids represent the diversity of cell types in the developing human neocortex. Additionally, we found that mediators of MIA induce differential gene expression in organoids. Currently, we are further characterizing differentially expressed genes in distinct cell types. Taken together, we have established a human in vitro model system of MIA that allows us to untangle cellular and molecular changes at an unprecedented resolution.

Pubmed:

34250020: Sarieva K, Mayer S
The Effects of Environmental Adversities on Human Neocortical Neurogenesis Modeled in Brain Organoids.
Over the past decades, a growing body of evidence has demonstrated the impact of prenatal environmental adversity on the development of the human embryonic and fetal brain. Prenatal environmental adversity includes infectious agents, medication, and substances of use as well as inherently maternal factors, such as diabetes and stress. These adversities may cause long-lasting effects if occurring in sensitive time windows and, therefore, have high clinical relevance. However, our knowledge of their influence on specific cellular and molecular processes of brain development remains scarce. This gap of knowledge can be partially explained by the restricted experimental access to the human embryonic and fetal brain and limited recapitulation of human-specific neurodevelopmental events in model organisms. In the past years, novel 3D human stem cell-based modeling systems, so-called brain organoids, have proven their applicability for modeling early events of human brain development in health and disease. Since their emergence, brain organoids have been successfully employed to study molecular mechanisms of Zika and Herpes simplex virus-associated microcephaly, as well as more subtle events happening upon maternal alcohol and nicotine consumption. These studies converge on pathological mechanisms targeting neural stem cells. In this review, we discuss how brain organoids have recently revealed commonalities and differences in the effects of environmental adversities on human neurogenesis. We highlight both the breakthroughs in understanding the molecular consequences of environmental exposures achieved using organoids as well as the on-going challenges in the field related to variability in protocols and a lack of benchmarking, which make cross-study comparisons difficult.
Front Mol Biosci, 2021; 8
31865524: Vetrovoy O, Sarieva K, Lomert E, Nimiritsky P, Eschenko N, Galkina O, Lyanguzov A, Tyulkova E, Rybnikova E
Pharmacological HIF1 Inhibition Eliminates Downregulation of the Pentose Phosphate Pathway and Prevents Neuronal Apoptosis in Rat Hippocampus Caused by Severe Hypoxia.
The pentose phosphate pathway (PPP) of glucose metabolism in the brain serves as a primary source of NADPH which in turn plays a crucial role in multiple cellular processes, including maintenance of redox homeostasis and antioxidant defense. In our model of protective mild hypobaric hypoxia in rats (3MHH), an inverse correlation between hypoxia-inducible factor-1 (HIF1) activity and mRNA levels of glucose-6-phosphate dehydrogenase (G6PD), the key enzyme of PPP, was observed. In

the present study, it was demonstrated that severe hypobaric hypoxia (SH) induced short-term upregulation of HIF1 alpha-subunit (HIF1α) in the hippocampal CA1 subfield and decreased the activity of G6PD. The levels of NADPH were also reduced, promoting oxidative stress, triggering apoptosis, and neuronal loss. Injection of a HIF1 inhibitor (HIF1i), topotecan hydrochloride (5 mg/kg, i.p.), before SH prevented the upregulation of HIF1α and normalized G6PD activity. In addition, HIF1i injection caused an increase in NADPH levels, normalization of total glutathione levels and of the cellular redox status as well as suppression of free-radical and apoptotic processes. These results demonstrate a new molecular mechanism of post-hypoxic cerebral pathology development which involves HIF1-dependent PPP depletion and support a recently suggested injurious role of HIF1 activation in the acute phase of cerebral hypoxia/ischemia. Application of PPP stimulators in early post-hypoxic/ischemic period might represent a promising neuroprotective strategy. Graphical abstract HIF1-dependent down-regulation of the pentose phosphate pathway contributes to the hypoxia-induced oxidative stress and neuronal apoptosis in the rat hippocampus.
J Mol Neurosci, 2020; 70
28025115: Vetrovoy O, Tulkova E, Sarieva K, Kotryahova E, Zenko M, Rybnikova E
Neuroprotective effect of hypobaric hypoxic postconditioning is accompanied by dna protection and lipid peroxidation changes in rat hippocampus.
The present study was performed to explore the effect of severe hypobaric hypoxia (180Torr, 3h) and severe hypoxia followed by hypoxic postconditioning (360Torr, 2h, 3 episodes) on DNA fragmentation and dynamics of lipid peroxidation products in rat hippocampus. The severe hypoxia induced intense DNA fragmentation in the hippocampus. A persistent decrease of thiobarbituric acid reactive substances in the hippocampus was also detected in response to severe hypoxia while the levels of Schiff bases did not significantly change. The postconditioning prevented severe hypoxia-induced DNA fragmentation, returned the levels of thiobarbituric acid reactive substances to the baseline and decreased the levels of Schiff bases. These findings indicate that the neuroprotective effect of hypoxic postconditioning on hippocampal neurons detected as suppression of hypoxia-induced DNA fragmentation is accompanied by the changes in lipid peroxidation processes.
Neurosci Lett, 2017; 639
30448928: Vetrovoy O, Sarieva K, Galkina O, Eschenko N, Lyanguzov A, Gluschenko T, Tyulkova E, Rybnikova E
Neuroprotective Mechanism of Hypoxic Post-conditioning Involves HIF1-Associated Regulation of the Pentose Phosphate Pathway in Rat Brain.
Post-conditioning is exposure of an injured organism to the same harmful factors but of milder intensity which mobilizes endogenous protective mechanisms. Recently, we have developed a novel noninvasive post-conditioning (PostC) protocol involving three sequential episodes of mild hypobaric hypoxia which exerts pronounced neuroprotective action. In particular, it prevents development of pathological cascades caused by severe hypobaric hypoxia (SH) such as cellular loss, lipid peroxidation, abnormal neuroendocrine responses and behavioural deficit in experimental animals. Development of these post-hypoxic pathological effects has been associated with the delayed reduction of hypoxia-inducible factor 1 (HIF1) regulatory α-subunit levels in rat hippocampus, whereas PostC up-regulated it. The present study has been aimed at experimental examination of the hypothesis that intrinsic mechanisms underlying the neuroprotective and antioxidant effects of PostC involves HIF1-dependent stimulation of the pentose phosphate pathway (PPP). We have observed that SH leads to a decrease of glucose-6-phosphate dehydrogenase (G6PD) activity in the hippocampus and neocortex of rats as well as to a reduction in NADPH and total glutathione levels. This depletion of the antioxidant defense system together with excessive lipid peroxidation during the reoxygenation phase resulted in increased oxidative stress and massive cellular death observed after SH. In contrast, PostC led to normalization of G6PD activity, stabilization of the NADPH and total glutathione levels and thereby resulted in recovery of the cellular redox state and prevention of neuronal death. Our data suggest that stabilization of the antioxidant system via HIF1-associated PPP regulation represents an important neuroprotective mechanism enabled by PostC.
Neurochem Res, 2019; 44

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IDENTIFICATION OF CHANGES IN THE ELECTROPHYSIOLOGICAL ACTIVITIES IN MATURE HUMAN CEREBRAL ORGANOIDS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Human cerebral organoids recapitulate some unique features of human brain development and are increasingly being used as model systems to shed new light on the cellular mechanisms underlying the development of a variety of neurological diseases. However, the presence of functional neural network activities has only recently been demonstrated. Here, we investigate the spontaneous activity frequencies of mature human cerebral organoids using extracellular recordings that reveal the existence of a functional neuronal network. Moreover, we demonstrate an altered functional activity in cerebral organoids derived from individuals with neuronal heterotopia carrying mutations in two different genes, resulting in a higher number of spikes and a higher percentage of high-frequency spikes. We also performed single-cell characterization at functional and morphological levels by patch-clamp recordings and morphological reconstruction of human neurons in 2D, revealing the existence of distinctive and common phenotypes, depending on the specific mutations. Specifically, patients' neurons are hyperexcitable with altered morphology and complexity and an increased number of excitatory pre-synaptic vesicles, that could lead to the observed functional activity. In accordance, transcriptomic and proteomic analyses of mature cerebral organoids suggest alteration at the morphological and synaptic levels. Together, these findings provide an essential base for the use of cerebral organoids to investigate human brain development and disorders at the functional level and illustrate their potential role in therapeutic and personalized treatments.

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TOWARD A BETTER UNDERSTANDING OF ITM2B PATHOGENICITY IN A SPECIFIC RETINAL DYSTROPHY, AND ITS POTENTIAL ROLE IN MITOCHONDRIA

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Our team identified a missense mutation in ITM2B underlying a novel autosomal dominant retinal dystrophy with ganglion cell loss, inner retinal dysfunction and progressive retinal degeneration but the function of ITM2B in the retina and physio-pathological mechanisms remains poorly understood. In a previous work, we obtained patient- and control-derived retinal organoids to model the disease recently showed that ITM2B may interact with mitochondrial proteins, implicated in oxidative stress in human retina. This project aims to investigate ITM2B in mitochondrial function and determined if it is altered in the disease. Two inducted pluripotent cell lines (iPSC) derived from an affected and unaffected sibling were used. ITM2B mitochondrial immunolocalization in 60 days aged organoids was studied using two mitochondrial markers COX5B and ATP-β. Furthermore, IPSC metabolism was analyzed in control and mutant cell lines using a mitostress seahorse assay. ITM2B immuno-localizes with both COX5B and ATB-β mitochondrial markers in retinal organoid sections. No difference was noticed between the mutant and the control organoids suggesting that mutant ITM2B does not modify the localization of the protein at this stage. Furthermore, no difference in oxygen consumption rate was observed in mutant IPSC compared to control cells, but further experiments will be performed in differentiated cells to which to try to highlight a mitochondrial defect in the mutant cell line, as well as a low capacity to respond to stress. Our findings suggest a central role of the mitochondria underlying ITM2B pathogenesis in the retina. Further studies using biochemical assays, respiratory function using sea-horse and

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RAPID, HIGH-EFFICIENCY DIFFERENTIATION OF MOTOR NEURONS FROM HUMAN PLURIPOTENT STEM CELLS

POSTER SESSION 05 - SECTION: PLURIPOTENT STEM CELLS IN DISEASE MODELING

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Human motor neuron (MN) diseases include devastating disorders such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). A reliable human MN model is critical to uncover disease mechanisms. Here we present the STEMdiff™ Motor Neuron Culture System which generates MNs from human pluripotent stem cells (hPSCs) at high efficiency. The hPSCs were aggregated to embryoid bodies (EBs) with STEMdiff™ Motor Neuron Differentiation Kit in an ultra-low attachment or an AggreWell™400 plate. On day 9, EBs were dissociated into single cells and replated for adherent culture. On day 14, the cells were either matured using the STEMdiff™ Motor Neuron Maturation Kit or assessed by immunocytochemistry and qPCR for motor neuron markers BIIITUB, ISL1, and HB9. The MNs were co-cultured with either myotubes generated using MyoCult™ Differentiation Kit (Human) or with microglia generated using STEMdiff™ Microglia Kits for one week, and then analyzed via immunocytochemistry. A highly pure population of MNs was observed at day 14 (BIIITUB: 92.6 ± 3.7%; ISL1: 56.0 ± 13.9%; HB9: 65.2 ± 13.2%; mean ± standard deviation, n = 6) with cervical identity by the expression of HOXA5 through qPCR. After two weeks in the maturation medium, the MNs showed high expression of mature markers including CHAT, MAP2 and SYP. Finally, MNs were successfully co-cultured with hPSC-derived myotubes or microglia for one week. Taken together, STEMdiff™ Motor Neuron Culture System provides a powerful tool to generate hPSC-derived MNs and co-culture systems for in vitro studies of human MN diseases.

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MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION OF SUBICULAR PRINCIPAL CELLS

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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The subiculum is an output region of the hippocampal formation relaying information between the hippocampus proper and entorhinal cortex. While a divergence along its proximo-distal axis has been recognized by early studies, there is increasing evidence for further spatial subdivisions (Ishihara et al., 2020) and a high molecular heterogeneity of its principal cells (Cembrowski et al., 2018). Indeed, a neuroanatomical characterization of the subiculum indicates that the distal part of this area (subiculum-1) diverges in its cytoarchitecture, neuronal density and immunocytochemical staining pattern from the proximal parts (subiculum-2): it has a dense homogenous cell population, with consistent expression of fibronectin-1 (FN1) in contrast to the sparse, layered cytostructure largely lacking FN1 immunoreactivity. In contrast, parvalbumin positive axonal arborization of putative basket cells was found to be high in subiculum-2 but low in the subiculum-1, suggesting a differential organization of the inhibitory system in these two subregions. Therefore, in this study we investigated the electrophysiological and morphological characteristics as well as the inhibitory input of its principal neurons of these two regions in a comparative manner by using whole-cell recordings in combination with intracellular filling in acute mouse slices. To analyze the inhibitory input from PV-positive basket cell, photostimulation was performed in slices from transgenic mice expressing channel rhodopsin-2 under the parvalbumin promoter. Preliminary results indicate that neurons of the subiculum-1 and subiculum-2 diverge in specific morphological characteristics: cell body size, dendritic length and spatial spreading of the apical dendrite, but their intrinsic physiological properties are largely comparable.

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THE ULTRASTRUCTURAL CHARACTERIZATION OF THE CELL POPULATION OF THE MOUSE PARALAMINAR NUCLEUS OF THE AMYGDALA REVEALS THREE STATES OF NEURONAL ACTIVE MATURATION AT POSTNATAL STAGES

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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The paralaminar nucleus (PL) is a region located in the ventral amygdala whose cells show a late maturation profile. Despite its heterogeneous cellular composition, a large population present, at postnatal stages, simple morphology, dense clustering, and expression of doublecortin (DCX) and PSA-NCAM, both markers of immature neurons. Our aim is to characterize the ultrastructure of each cell type in the mouse PL at postnatal stages P7, P14, P21, P28, P60, 7 months and 1 year. Regarding the methodology, we first characterized the PL cell typology by fluorescence immunohistochemistry in paraffin sagittal sections. Subsequently, the ultrastructural characteristics were studied by transmission electron microscopy and *pre-embedding* immuno-gold for a range of molecular markers. Our results indicate that, in the mice PL, there is presence of mature excitatory and inhibitory neurons, immature neurons, oligodendrocytes, OPCs, astrocytes and microglia cells. Focusing on neurons, ultrastructurally, most of DCX^+ neurons presented immature morphology, compacted heterochromatin and reduced cytoplasmatic volume, while DCX^- neurons corresponded to completely mature and developed neurons. However, we found an intermediate neuron population with low expression of DCX but quite developed cytoplasm, suggesting progressive maturation of the PL neurons. Remarkably, we observed some neurons with migratory morphology, directing outside de PL region towards the piriform cortex. In conclusion, the presence of immature DCX^+ cells in the PL of mice which mature at juvenile postnatal stages and may even migrate to other brain areas supports the idea that protracted maturation could provide neuronal plasticity at an important time in the development of the amygdala.

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DEVELOPMENT OF ACD NEURONS IN THE MURINE HIPPOCAMPUS AND PRIMARY SOMATOSENSORY CORTEX

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Cortical principal neurons receive synaptic input at their dendrites and convey them to the soma. After integration, the axon initial segment (AIS) initiates action potentials. Classically, the axon emerges from the soma. However, in axon-carrying dendrite (AcD) neurons, the axon originates from a basal dendrite resulting in potentially distinct functional implications. Although AcD neurons have been described in numerous cortical regions and across species, their developmental profile remains largely unknown. Therefore, this project focuses on the early maturation profile of AcD neurons in comparison to nonAcD neurons in the murine ventral hippocampus (vHC) and primary somatosensory (S1) cortex. Parameters include the occurrance of both subtypes across numerous ages, as well as the maturation of AIS-specific features (e.g. expression of voltage-gated sodium channels and the emergence of GABAergic axo-axonic synapses (AAS)). Data show that in vHC, the number of AcD neurons steadily increases until P28, while in S1, AcD neurons peak at P16. AcD neurons show shorter $Na_V1.6$ channel distribution than nonAcD neurons in both brain regions. Live-cell imaging in cortical slice cultures reveals a striking morphological dynamic in pyramidal neurons, which transform from one type to the other during development. Preliminary data also indicate that the development of AcD neurons has an activity-dependent component. In summary, our data imply that entire cells exhibit morphological plasticity, the regulating mechanisms of which remain to be studied in vivo.

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BIDIRECTIONAL CONTROL OF NEUROVASCULAR COUPLING BY PYRAMIDAL NEURONS

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Neurovascular coupling (NVC), the tight coupling between neural activity and cerebral blood flow, is essential for brain function and integrity. Prostaglandin E2 (PGE2) produced by cyclooxygenase-2 (COX-2) activity of pyramidal cells plays a key role in NVC accounting for about half of the blood perfusion increase induced by sensory stimulation. **Aims:** We sought to determine the regime of pyramidal neurons activity triggering an optimal NVC response. **Methods**: Pyramidal cells were optogenetically stimulated in cortical brain slices of 3-week-old Emx1-Cre::Ai32 mice. Vascular responses were visualized by infrared videomicroscopy and the underlying mechanisms dissected-out by pharmacological approaches. The expression profile of prostaglandin synthesizing enzymes in pyramidal cells was determined by single-cell RT-PCR after patch-clamp. **Results**: Photostimulation of pyramidal cells at low (2 Hz) and high (20 Hz) frequencies elicited, respectively, vasodilation and vasoconstriction requiring COX-2 activity. Molecular profiling revealed that one-third of pyramidal cells expressed COX-2 and terminal synthesizing enzymes for both PGE2 and the vasoconstrictor PGF2a. Optogenetically-induced vasoconstrictions were mediated by the PGE2 receptors EP1 and EP3, but not by the PGF2α receptor. Furthermore, exogenous PGE2 induced vasodilation and vasoconstriction when applied at low (<100nM) and high (>1 µM) concentrations, respectively. Moreover, vasoconstriction was mimicked by an EP1/EP3 agonist. Action potentials and neuropeptide Y, but not constrictive astrocytic pathways, were also required for optogenetically-induced vasoconstrictions. **Conclusions**: These results indicate that pyramidal neurons and PGE2 bidirectionally control NVC. This finding will help to understand the physiopathological adaptations of NVC in neurological disorders.

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ANALYSIS OF THE INTRAAMYGDALAR CONNECTIVITY AND MORPHOLOGICAL CHARACTERIZATION OF PRINCIPAL NEURONS IN THE BASOLATERAL AMYGDALA

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Amygdala refers to a cluster of structurally distinct nuclei in the brain. Its core unit is the basolateral amygdala complex (BLA) and the central amygdala (CeA). The former region includes the lateral (LA), basal (BA) and basomedial (BMA) nuclei. The BLA is involved in numerous cognitive operations, such as fear memory processes. To understand how the amygdala fulfils its various functions, we need to reveal the organization principles of intra-amygdalar connections. Therefore, our aim was to examine the connectivity within the amygdala at single cell and population levels. To this end, we used different tracing techniques corroborated by the *post hoc* reconstruction of 21 principal neurons (PNs) labelled *in vivo* using juxtacellular technique in the mouse brain. The latter provided an opportunity to morphollogically characterize the PNs of the BLA. In accord with previous results, we confirmed the reciprocal connectivity between the LA and BMA and the directionality within the amygdala networks. Considering the anatomical characteristics of the reconstructed cells, we defined groups of BA and LA PNs with different morhological patterns. Additionally, two distinct units were distinguished within the BA: a lateral-posterior one that typically projects to the lateral part of the CeA, and a medial-anterior one that does not. Based on these observations, the lateral part of the BA conveys information towards the CeA, while the medial part forms a separate functional unit with other brain areas, including the prefrontal cortex, suggesting the presence of discrete information flow pathways within the BLA.

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SVCT2 OVEREXPRESSION AND ASCORBIC ACID UPTAKE INCREASE CORTICAL NEURON DIFFERENTIATION, WHICH IS DEPENDENT ON VITAMIN C RECYCLING BETWEEN NEURONS AND ASTROCYTES

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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During brain development, sodium–vitamin C transporter (SVCT2) has been detected primarily in radial glial cells in situ, with low-to-absent expression in cerebral cortex neuroblasts. However, strong SVCT2 expression is observed during the first postnatal days, resulting in increased intracellular concentration of vitamin C. Hippocampal neurons isolated from SVCT2 knockout mice showed shorter neurites and low clustering of glutamate receptors, suggesting that ascorbic acid (AA) and SVCT2 have important roles in postnatal neuronal differentiation and neurite formation. In this study, SVCT2 lentiviral overexpression induced branching and increased synaptic proteins expression in primary cultures of cortical neurons. Analysis in neuroblastoma 2a (Neuro2a) and human subventricular tumor C3 (HSVT-C3) cells showed similar branching results. SVCT2 was mainly observed in the cell membrane and endoplasmic reticulum; however, it was not detected in the mitochondria. Cellular branching in neuronal cells and in a previously standardized neurosphere assay is dependent on the recycling of vitamin C or reduction in dehydroascorbic acid (DHA, produced by neurons) by glial cells. The effect of WZB117, a selective glucose/DHA transporter 1 (GLUT1) inhibitor expressed in glial cells, was also studied. By inhibiting GLUT1 glial cells, a loss of branching is observed in vitro, which is reproduced in the cerebral cortex in situ. We concluded that vitamin C recycling between neurons and astrocyte-like cells is fundamental to maintain neuronal differentiation in vitro and in vivo. The recycling activity begins at the cerebral postnatal cortex when neurons increase SVCT2 expression and concomitantly, GLUT1 is expressed in glial cells.

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BRAIN REGION EVOLUTION BY DUPLICATION-AND-DIVERGENCE

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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How have complex brain regions, circuits, and cell types evolved from simple origins? We are investigating brain region evolution at cell-type resolution in the cerebellar nuclei, the output structures of the cerebellum. In recent work, we applied single-nucleus RNA sequencing in chickens, mice, and humans, STARmap spatial transcriptomic analysis in chicken and mice, and whole-CNS projection mapping in mice. Our work revealed a conserved cell type set containing three classes of region-invariant inhibitory neurons and two classes of region-specific excitatory neurons. This cell type set forms an archetypal cerebellar nucleus that was repeatedly duplicated to create new regions, and thus cerebellar output channels. In excitatory neurons, duplication was accompanied by divergence in gene expression and shifts in projection patterns. By contrast, inhibitory neurons maintained their gene expression signatures. Interestingly, the excitatory cell class that preferentially funnels information to lateral frontal cortices in mice becomes predominant in the massively expanded human Lateral CN. This data provide the first characterization of CN transcriptomic cell types in three species and suggest a model of brain region evolution by duplication and divergence of entire cell type sets. We are now working to extend these findings to tetrapods and to query the evolution of new connectomic cell types using cellular barcoding techniques MAPseq and BARseq in different species.

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TOWARDS TRANSLATING SINGLE-NEURON AXONAL RECONSTRUCTIONS INTO MESO-SCALE STRUCTURAL CONNECTIVITY STATISTICS

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Aims The past decade has seen a tremendous development of connectomics approaches, with single-neuron reconstructions bridging the gap between cellularly resolved micro-circuits and whole-brain bulk axonal projections. However, characterizing brain-wide projection motifs in thousands of neurons is still challenging. We have thus developed a tool for translating ~3000 axonal morphologies into projection statistics across different brain areas, using reconstructed neurons from high throughput repositories and from our recently developed pipeline. **Methods** We first identify neurons with similar axonal morphology and registration coordinates using an in-house neuron as template. Particularly, we apply the Coherent Point Drift method to compute similarity by first finding correspondences between points in target and template neuron followed by determining the mean squared distance between corresponding points. Given the group of most similar neurons, we use the Allen Reference Atlas to measure the distribution of axonal length across cortical layers and subcortical nuclei. **Results** We obtained projection statistics for a group of similar thalamic neurons showing high axonal overlap in layers 2-5 of the lateral visual area and in various thalamic nuclei. In addition, we repeated the procedure with the more densely sampled intra-cortical projection neurons, however results are still preliminary. **Conclusions** Our approach represents a significant extension to existing tools, since it accounts for full axonal trees instead of only regions of interest. Moreover, it is applicable to neurons reconstructed in smaller laboratories and allows for integration with other similarly registered datasets. We further intend to extend our approach to uncover complex projection motifs.

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FOXP1 AND NNOS NEURONAL POPULATIONS IN THE ADULT HUMAN, MOUSE AND RAT SUBTHALAMIC NUCLEUS

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Introduction: The subthalamic nucleus (STN) is a subcortical structure which is an important part of the basal ganglia circuitry. nNOS is an enzyme which produces nitric oxide, a neurotransmitter implicated in learning, memory, and brain plasticity. FOXP1 is a transcription factor whose deficiency causes FOXP1 syndrome – a neurodevelopmental disorder associated with intellectual disability, language deficits and autism. **Aims:** Besides its role in motor control, STN is significantly involved in cognitive and limbic processes in the brain. Because of the importance of NO and FOXP1 in these processes, the aim of our study was to determine the quantity and spatial distribution of FOXP1 and nNOS-IR neurons in the adult human, mouse and rat STN. **Methods:** We performed immunofluorescent double-labelling with antibodies for FOXP1 and nNOS proteins, with HuC/HuD antibody (a pan-neuronal marker) on STN sections of FFPE adult human, mouse, and rat brains. Immunofluorescent slides were imaged using confocal microscopy and used to quantify the colocalization. **Results:** We show that in mouse, the FOXP1 and nNOS populations are clearly separated and almost non-overlapping. FOXP1 neurons group on the antero-ventral portion of STN, while nNOS neurons group on the postero-dorsal portion. In rat, the populations are mostly separated but overlapping and colocalizing in the center. In human, the populations are partially colocalizing and they are not spatially separated but rather intermixed and dispersed evenly throughout the STN. **Conclusions:** This study shows FOXP1 and nNOS expression in the mammalian subthalamic nucleus and may explain the STN involvement in cognitive and limbic processes.

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MOLECULAR ATLAS OF THE ADULT ZEBRAFISH FOREBRAIN AT SINGLE-CELL RESOLUTION REVEALS HOMOLOGIES WITH TERRESTRIAL VERTEBRATES

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Zebrafish is extensively used for investigating fundamental principles underlying the assembly and the function of the vertebrate brain, most commonly for the study of sensory-motor computations in larval zebrafish midbrain and hindbrain. Recent studies have shown that juvenile and adult zebrafish can perform cognitively demanding tasks, which is partly attributed to the maturation of forebrain networks, ancestral to mammalian cortico-limbic structures. Nonetheless, the molecular cytoarchitecture and cell types of adult zebrafish forebrain and its homologies to terrestrial vertebrates are yet to be discovered. In order to identify distinct regions and cell types of the adult zebrafish forebrain, we generated a serial-section transcriptomic atlas for 99 genes at subcellular resolution, and by aligning sections we created a high resolution three-dimensional atlas. For validation and to predict homologies, we integrated our molecular atlas with single-cell RNA sequencing data from zebrafish and several terrestrial vertebrates. Our results revealed multiple excitatory and inhibitory neuron types and non-neuronal cells across the forebrain. We observed that some of these cell types are dispersed widely while others are spatially organized. We also identified novel and anatomically distinct forebrain regions with predicted homologies to mammalian cortical and sub-cortical structures. Finally, we match several of these molecularly identified zebrafish forebrain regions with functional maps based on calcium imaging of ongoing brain activity. Our results highlight the evolutionary conservation and divergence of the anatomy and the cytoarchitecture of zebrafish forebrain with the rest of terrestrial vertebrates.

Pubmed:
33962556: Bredesen BA, Rehmsmeier M
MOCCA: a flexible suite for modelling DNA sequence motif occurrence combinatorics.
Cis-regulatory elements (CREs) are DNA sequence segments that regulate gene expression. Among CREs are promoters, enhancers, Boundary Elements (BEs) and Polycomb Response Elements (PREs), all of which are enriched in specific sequence motifs that form particular occurrence landscapes. We have recently introduced a hierarchical machine learning approach (SVM-MOCCA) in which Support Vector Machines (SVMs) are applied on the level of individual motif occurrences, modelling local sequence composition, and then combined for the prediction of whole regulatory elements. We used SVM-MOCCA to predict PREs in Drosophila and found that it was superior to other methods. However, we did not publish a polished implementation of SVM-MOCCA, which can be useful for other researchers, and we only tested SVM-MOCCA with IUPAC motifs and PREs.
BMC Bioinformatics, 2021; 22
31340029: Bredesen BA, Rehmsmeier M
DNA sequence models of genome-wide Drosophila melanogaster Polycomb binding sites improve generalization to independent Polycomb Response Elements.
Polycomb Response Elements (PREs) are cis-regulatory DNA elements that maintain gene transcription states through DNA replication and mitosis. PREs have little sequence similarity, but are enriched in a number of sequence motifs. Previous methods for modelling Drosophila melanogaster PRE sequences (PREdictor and EpiPredictor) have used a set of 7 motifs and a training set of 12 PREs and 16-23 non-PREs. Advances in experimental methods for mapping chromatin binding factors and modifications has led to the publication of several genome-wide sets of Polycomb targets. In addition to the seven motifs previously used, PREs are enriched in the GTGT motif, recently associated with the sequence-specific DNA binding protein Combgap. We investigated whether models trained on genome-wide Polycomb sites generalize to independent PREs when trained with control sequences generated by naive PRE models and including the GTGT motif. We also developed a

new PRE predictor: SVM-MOCCA. Training PRE predictors with genome-wide experimental data improves generalization to independent data, and SVM-MOCCA predicts the majority of PREs in three independent experimental sets. We present 2908 candidate PREs enriched in sequence and chromatin signatures. 2412 of these are also enriched in H3K4me1, a mark of Trithorax activated chromatin, suggesting that PREs/TREs have a common sequence code.
Nucleic Acids Res, 2019; 47

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A VERSATILE TOOLBOX FOR THE ANALYSIS OF NERVOUS TISSUE ORGANIZATION WITH LIGHT MICROSCOPY

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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The brain is an exceptionally sophisticated organ consisting of billions of cells and trillions of connections that orchestrate our cognition and behavior. To decode its complex connectivity, it is pivotal to disentangle its intricate architecture spanning from cm-sized circuits down to tens of nm-small synapses. To achieve this goal, we have developed CATS - Comprehensive Analysis of nervous Tissue across Scales, a toolbox for obtaining a holistic view of nervous tissue context with fluorescence microscopy. CATS provides rich ultrastructural context by creating contrast between the intra- and extracellular space in a variety of samples types, including slice cultures, perfused brain tissue and clinical samples. It is compatible with common (super-resolution) fluorescence imaging techniques, such as STED and expansion microscopy, as well as labeling of molecular markers. We interface this toolbox with a state-of-a-art machine-learning based analysis pipeline for segmentation and annotation. CATS enables the analysis of key features of nervous tissue connectivity across scales, ranging from whole tissue organization down to synapse architecture. We present the potential of this novel toolbox by reconstructing neuronal in- and output fields in the mouse hippocampus, a feat that so far has only been achieved by electron microscopy. Further, we combine CATS with electrophysiological recordings to investigate structure-function relationships in the brain, apply it to human clinical samples to study tissue context and fully annotate a piece of cerebral organoid, thereby paving the way towards light microscopy-based saturated reconstruction of nervous tissue.

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ROLES OF EARLY MICROGLIA IN THE WIRING OF PERISOMATIC INHIBITION AND CRITICAL PERIODS

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Parvalbumin (PV)-expressing interneurons play important roles at different steps of sensory cortex development and their dysfunction is a hallmark of neurodevelopmental disorders(NDD) as shown by genetic mouse models analyses. PV neurons act as gain modulators via perisomatic inhibition of pyramidal neurons and display, in association with the closure of critical periods(CP), a progressive enwrapping by perineuronal nets(PNNs). We previously found that PV neurons development is altered by embryonic depletion of immune brain resident cells, microglia, as well as by prenatal inflammation mediated by maternal immune activation(MIA). While these results highlighted potential convergence of genetic and immune risks for NND onto PV neuron wiring, they raised questions about both the mechanisms involved as well as the long-term consequences. To address these issues, we are taking advantage of transient embryonic macrophage depletions, MIA and genetic models to explore the development of perisomatic inhibition and how these processes may be perturbed by early alteration in microglia functioning. In parallel, we are investigating the long-term consequences of these early wiring deficits on PV neurons circuit integration and the features of the CP in a well-characterized model of ocular dominance plasticity in the primary visual cortex. To this aims, we are combining morphological studies of PNNs with ocular dominance experiments after monocular deprivation at different developmental stages to assess the kinetics and characteristics of plasticity in these distinct experimental paradigms. Our ongoing results support a novel role for early neuroimmune interactions in the development of PV neurons wiring, with long-lasting impact on cortical circuit properties.

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CORTICAL INTERNEURONS PRESENT MARKED DIFFERENCES IN THEIR PH AND INTRACELLULAR CHLORIDE LEVELS

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Background: Chloride and pH levels inside neurons play an important role in determining neuronal excitability. In particular, the intracellular chloride concentration, $[Cl^-]_i$, dictates the efficacy of fast GABAergic synaptic inhibition; and the activity of most neuronal receptors is highly sensitive to deviations even within the physiological pH range (7.0-7.4). Thus changes in pH and $[Cl^-]_i$ have a very powerful effect on network excitability. However, little is known about how chloride and pH levels differ between cell classes *in vivo*. These measures are now possible thanks to the development of ClopHensor (Arosio et al., 2010), a genetically encoded sensor, and its optimisation for 2-photon microscopy (Sulis Sato et al., 2017). Using ClopHensor, we have demonstrated that diurnal changes in $[Cl^-]_i$ of cortical pyramidal cells have deep effects on the network excitability (Pracucci et al., 2021, biorxiv). Aims: We investigate pH and $[Cl^-]_i$ levels *in vivo* in two major classes of cortical interneurons: Parvalbumin- (PV), and Somatostatin-positive (SST). Methods: We modified ClopHensor by changing the linker sequence, to improve the sensor stability, and we calibrated the new construct. We packaged the sensor into an AAV vector for cell-specific gene delivery (cre/lox system), and used this to target PV and SST interneurons. Results: We present population statistics of pH and $[Cl^-]_i$ in PV and SST interneurons *in vivo*, obtained using 2-photon microscopy. Our measures show marked differences between the two populations in both their pH and $[Cl^-]_i$ values, suggesting the presence of independent regulation mechanisms in these cell classes.

Pubmed:
34166608: Saponaro A, Bauer D, Giese MH, Swuec P, Porro A, Gasparri F, Sharifzadeh AS, Chaves-Sanjuan A, Alberio L, Parisi G, Cerutti G, Clarke OB, Hamacher K, Colecraft HM, Mancia F, Hendrickson WA, Siegelbaum SA, DiFrancesco D, Bolognesi M, Thiel G, Santoro B, Moroni A
Gating movements and ion permeation in HCN4 pacemaker channels.
The HCN1-4 channel family is responsible for the hyperpolarization-activated cation current I/I that controls automaticity in cardiac and neuronal pacemaker cells. We present cryoelectron microscopy (cryo-EM) structures of HCN4 in the presence or absence of bound cAMP, displaying the pore domain in closed and open conformations. Analysis of cAMP-bound and -unbound structures sheds light on how ligand-induced transitions in the channel cytosolic portion mediate the effect of cAMP on channel gating and highlights the regulatory role of a Mg coordination site formed between the C-linker and the S4-S5 linker. Comparison of open/closed pore states shows that the cytosolic gate opens through concerted movements of the S5 and S6 transmembrane helices. Furthermore, in combination with molecular dynamics analyses, the open pore structures provide insights into the mechanisms of K/Na permeation. Our results contribute mechanistic understanding on HCN channel gating, cyclic nucleotide-dependent modulation, and ion permeation.
Mol Cell, 2021; 81
30377377: Alberio L, Locarno A, Saponaro A, Romano E, Bercier V, Albadri S, Simeoni F, Moleri S, Pelucchi S, Porro A, Marcello E, Barsotti N, Kukovetz K, Boender AJ, Contestabile A, Luo S, Moutal A, Ji Y, Romani G, Beltrame M, Del Bene F, Di Luca M, Khanna R, Colecraft HM, Pasqualetti M, Thiel G, Tonini R, Moroni A
A light-gated potassium channel for sustained neuronal inhibition.
Currently available inhibitory optogenetic tools provide short and transient silencing of neurons, but they cannot provide long-lasting inhibition because of the requirement for high light intensities. Here we present an optimized blue-light-sensitive synthetic potassium channel, BLINK2, which showed good expression in neurons in three species. The channel is activated by illumination with low doses of blue light, and in our experiments it remained active over (tens of) minutes in the dark after the illumination was stopped. This activation caused long periods of inhibition of neuronal firing in ex vivo recordings of mouse neurons and impaired motor neuron response in zebrafish in vivo. As a proof-of-concept application, we demonstrated

that in a freely moving rat model of neuropathic pain, the activation of a small number of BLINK2 channels caused a long-lasting (>30 min) reduction in pain sensation.
Nat Methods, 2018; 15
28293893: Cosentino C, Alberio L, Thiel G, Moroni A
Yeast-Based Screening System for the Selection of Functional Light-Driven K Channels.
Ion channels control the electrical properties of cells by opening and closing (gating) in response to a wide palette of environmental and physiological stimuli. Endowing ion channels with the possibility to be gated by remotely applied stimuli, such as light, provides a tool for in vivo control of cellular functions in behaving animals. We have engineered a synthetic light-gated potassium (K) channel by connecting an exogenous plant photoreceptor LOV2 domain to the K channel pore Kcv. Here, we describe the experimental strategy that we have used to evolve the properties of the channel toward full control of light on pore gating. Our method combines rational and random mutagenesis of the channel followed by a yeast-based screening system for light-activated K conductance.
Methods Mol Biol, 2017; 1596
25954011: Cosentino C, Alberio L, Gazzarrini S, Aquila M, Romano E, Cermenati S, Zuccolini P, Petersen J, Beltrame M, Van Etten JL, Christie JM, Thiel G, Moroni A
Optogenetics. Engineering of a light-gated potassium channel.
The present palette of opsin-based optogenetic tools lacks a light-gated potassium (K(+)) channel desirable for silencing of excitable cells. Here, we describe the construction of a blue-light-induced K(+) channel 1 (BLINK1) engineered by fusing the plant LOV2-Jα photosensory module to the small viral K(+) channel Kcv. BLINK1 exhibits biophysical features of Kcv, including K(+) selectivity and high single-channel conductance but reversibly photoactivates in blue light. Opening of BLINK1 channels hyperpolarizes the cell to the K(+) equilibrium potential. Ectopic expression of BLINK1 reversibly inhibits the escape response in light-exposed zebrafish larvae. BLINK1 therefore provides a single-component optogenetic tool that can establish prolonged, physiological hyperpolarization of cells at low light intensities.
Science, 2015; 348

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DOWNREGULATION OF EXTRA-SYNAPTIC DELTA GABAA RECEPTORS IS CORRELATED WITH DISRUPTED TONIC INHIBITION IN AN APP KNOCK-IN MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Aim: We recently reported that Alzheimer's disease (AD) is associated with synaptic hyperexcitability, and is correlated with disrupted tonic inhibition, which is thought to be regulated by extra-synaptic delta-subunit containing gamma-aminobutyric acid receptor (δ-GABAAR). Therefore, this study investigated whether δ-GABAARs altered during AD pathogenesis, and whether pharmacological modulation of this receptor could "normalise" the disrupted tonic inhibition observed in AD. Method: Using the APPNL-F/NL-F knock-in mouse model of AD, age-matched to wild-type mice, we performed immunohistochemistry combined with in vitro electrophysiological whole-cell recordings in the hippocampal CA1 and dentate gyrus (DG) regions. Furthermore, neuroanatomy experiments were performed using post-mortem brain of human AD patients, age-matched to healthy controls. Results: Immunohistochemistry and confocal microscope analysis revealed a downregulation of δ-GABAARs in the presence of the classical hallmarks of AD including amyloid beta plaques and increased astrocytosis in CA1 and DG of 12- 14-months old APPNL-F/NL-F mice compared to age-matched wild-type mice. This observation was consistent in post-mortem brain tissue of AD patients compared to the age-matched controls. Furthermore, there was a cell-type specific co-localisation of the δ-subunit with interneurons containing parvalbumin, and neuropeptide Y, but not in calretinin-expressing cells. Using whole-cell recordings, positive allosteric modulation of the δ-GABAARs using a δ-subunit selective compound 2 "normalised" tonic inhibition and hyperexcitability in the AD model. Conclusions: Our data suggest that δ-GABAARs located in discreet neuronal circuitry of the hippocampus, are downregulated during AD, and modulation of this subunit using a δ-subunit selective agonist, has therapeutic potential.

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FUNCTIONAL AND STRUCTURAL DIVERSITY OF PARVALBUMIN-EXPRESSING INTERNEURONS IN THE MOUSE DENTATE GYRUS

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Fast spiking parvalbumin positive interneurons (PVIs) contribute largely to the unique sparse coding by granule cell (GC) ensembles of the dentate gyrus (DG). Here we provide morphological, neurochemical and electrophysiological evidence for two subtypes of DG PVIs using whole-cell patch clamp recordings of tdTomato-expressing PVIs in acute hippocampal slices. PVIs with soma location in the inner versus outer granule cell layer (PVIis, PVIos, respectively) correlate with distinct structural-functional properties. (1) Reconstructions of the somato-dendritic domain revealed a higher number of basal dendritic arbors and a larger vertical extension of apical dendrites in PVIis than PVIos, pointing to higher dendritic complexity and integration of larger numbers of inputs. (2) PVIis had a more positive resting potential, exhibited a lower spike-threshold and discharged at higher maximal frequency compared to PVIos. (3) Antibody labeling revealed more PVIis expressing mGluR5, a receptor involved in long-term plasticity (LTP) induction at their GC inputs. (4) PVIis show stronger GC-mediated LTP. (5) Paired GC-PVI recordings revealed higher connection probability at PVIis, indicating stronger feedback excitation of PVIis. Perforant path-mediated EPSCs were elicited after shorter latencies, with larger amplitude and faster decay at PVIos, indicating a stronger recruitment by feedforward excitation. Finally, PVI-GC paired recordings revealed similar functional properties of unitary IPSCs, however, failure rate and the CV of individual IPSCs were lower at PVIi-GC synapses with pronounced multiple-pulse depression. In contrast, PVIo-GC synapses lacked dynamic changes. Thus, our data suggest that PVIis provide reliable phasic feedback, whereas PVIos unreliable and fluctuating feedforward inhibition to the DG network.

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SOMATOSTATIN-EXPRESSING NEURONS FROM THE VENTRAL TEGMENTAL AREA INNERVATE DISTANT BRAIN REGIONS.

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Somatostatin-containing (Sst) neurons are a well-known subtype of the inhibitory interneurons in the mammalian brain. At the same time, a major part of known GABAergic long-range projections express Sst neuropeptide and are involved in the synchronization of oscillatory activity of distant brain regions. Such neurons were found in the cortex, amygdala, and hippocampus (Viollet et al., 2008; *Mol Cell Endocrinol*). In our recent work (Nagaeva et al., 2020; *eLife*) we described the Sst population in mouse VTA, showing that these neurons are very heterogeneous: there are three different electrophysiological subtypes, which express GABAergic inhibitory and/or glutamatergic excitatory markers, and some of them even dopaminergic markers. Physiologically, VTA Sst neurons acted as interneurons and inhibited neighboring dopamine cells. Here we report that the mouse VTA Sst neurons also send projections far outside the midbrain. We injected anterograde Cre-dependent viral tracer into the VTA of Sst-CRE mice and found fluorescent Sst axons in the lateral hypothalamus, the medial part of the central amygdala, bilaterally in the paraventricular thalamic nucleus, and in the lateral division of bed nucleus of stria terminalis. Next, we injected a retrograde Cre-dependent viral tracer into each of these target regions and confirmed that VTA Sst axons ended up in the above regions and were not only passing by. Our results demonstrate that Sst neurons in the VTA, as in several other brain regions, can be both interneurons and projection neurons, suggesting that the VTA Sst neurons are a part of the brain's somatostatinergic system.

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NEURENSIN-2: A NOVEL CELL-TYPE-SPECIFIC MEDIATOR OF DEPRESSION

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Major depressive disorder (MDD) is a common psychiatric condition with insufficient available therapies. While the molecular and cellular mechanisms that underlie MDD remain elusive, the hippocampal inhibitory interneurons that dictate local excitation/inhibition balance emerge as key players in MDD and its treatments. Thus, identifying the cellular and molecular pathways that drive functional alterations in these cells may pave the path to the development of improved antidepressants. In this study, we applied the Ribosome Affinity Purification (TRAP) technique in a subtype of hippocampal interneurons, the CCK cells. By this, we were able to analyze the molecular changes in these interneurons in depression. Using this approach, we discovered a new cell-type-specific mediator of depression and anxiety, Neurensin-2. This endosome-associated protein expression is markedly enhanced in the depressed state and is reduced after treatment with traditional antidepressants. Moreover, overexpression and deletion of Neurensin-2 result in depression and stress resilience, respectively. While the molecular mechanism is still unknown, we showed that Neurensin-2 is a robust mediator of the excitatory transmission in the inhibitory neurons. Therefore, we suggest that Neurensin-2 mediates behavior by modifying the function of the inhibitory tone in the hippocampus. The identification of a novel protein that dramatically and adaptively regulates inhibition in the hippocampus provides new insights into how the local E/I balance responds to stress and modulates behavior. Furthermore, these findings implicate Neurensin-2 in the pathophysiology and future treatments of neuropsychiatric disorders, including MDD and anxiety.

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CHARACTERIZATION OF AXO-AXONIC INTERNEURONS TARGETED WITH THE NKX2.1CRE-ER MOUSE LINE.

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Axo-axonic cells (AACs) are GABAergic interneurons targeting with an exquisite specificity the axon initial segment of pyramidal neurons, where axon potentials are generated. This particularity indicates that they may provide an ultimate effect on pyramidal neuron output. Whether this effect is inhibitory or excitatory is still debated, which probably depends on the activity state of pyramidal cells and on their innervation pattern by AACs. Testing these hypothesis has long been challenging due to the absence of genetic tools allowing the selective targeting of AACs. The implementation of the Nkx2.1-CreER mouse line, in which late embryonic tamoxifen induction results in the expression of a chosen reporter in postmitotic AACs, offered a tool to unveil the functions of AACs. However the Nkx2.1Cre-ER model has not been fully characterized, and conflicting data have been reported on the selectivity of ChCs targeting. In this project, we investigated the specificity and efficiency of this novel mouse line for AAC targeting and manipulation in the prefrontal cortex (PFC). To address this question, we used immunohistochemistry and electrophysiological recordings. We found a 70% specificity for AACs in the PFC of Nkx2.1-CreER mice, along with ex-vivo firing properties reminiscent of fast spiking neurons. Our immunohistochemistry study also showed moderate efficiency of this mouse line in targeting AACs. Those results provide a stronger basis for the use of this model for studies using *in vivo* manipulation of AACs activity.

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MORPHOLOGICAL AND PHYSIOLOGICAL FEATURES OF CHANDELIER CELLS IN THE PREFRONTAL CORTEX RECORDED IN TWO TRANSGENIC MOUSE LINES

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Chandelier cells (ChCs) that are present only in cortical structures selectively form synapses on the axon initial segments (AISs) of principal cells (PCs) – the site of action potential generation. Thus, these GABAergic cells are in the position to control the spiking of their postsynaptic partners in the most powerful way. Here, we examined and compared anatomical and electrophysiological features of ChCs sampled in the mouse medial prefrontal cortex (mPFC) of Nkx2.1-Cre and PVeGFP lines. We performed whole-cell recordings in ChCs in acute prefrontal cortical slices that were prepared from the two different transgenic mouse lines. Using paired recordings we found no major differences in the properties of unitary inhibitory postsynaptic currents (uIPSCs) detected in postsynaptic PCs. Recordings were accompanied by intracellular biocytin filling that allowed us *post hoc* morphological analysis of the recorded ChCs. The distribution of axons and dendrites of ChCs sampled in two distinct transgenic mouse lines showed some differences between layers, indicating potentially distinct input and output features. Additionally, we examined the number and the distribution of single ChC boutons along the individual AISs. We observed that the number and the position of boutons, but not the area of innervated segments, were different for ChCs recorded in the two mouse lines. These results show that ChCs in the mPFC are morphologically distinct and show anatomical, but not electrophysiological, differences in their inhibitory connections. The variability of ChCs in prefrontal cortical microcircuits might be an important factor in control of local network operation.

Pubmed:
29954847: Rhomberg T, Rovira-Esteban L, Vikór A, Paradiso E, Kremser C, Nagy-Pál P, Papp OI, Tasan R, Erdélyi F, Szabó G, Ferraguti F, Hájos N
Vasoactive Intestinal Polypeptide-Immunoreactive Interneurons within Circuits of the Mouse Basolateral Amygdala.
In cortical structures, principal cell activity is tightly regulated by different GABAergic interneurons (INs). Among these INs are vasoactive intestinal polypeptide-expressing (VIP+) INs, which innervate preferentially other INs, providing a structural basis for temporal disinhibition of principal cells. However, relatively little is known about VIP+ INs in the amygdaloid basolateral complex (BLA). In this study, we report that VIP+ INs have a variable density in the distinct subdivisions of the mouse BLA. Based on different anatomical, neurochemical, and electrophysiological criteria, VIP+ INs could be identified as IN-selective INs (IS-INs) and basket cells expressing CB1 cannabinoid receptors. Whole-cell recordings of VIP+ IS-INs revealed three different spiking patterns, none of which was associated with the expression of calretinin. Genetic targeting combined with optogenetics and recordings enabled us to identify several types of BLA INs innervated by VIP+ INs, including other IS-INs, basket and neurogliaform cells. Moreover, light stimulation of VIP+ basket cell axon terminals, characterized by CB1 sensitivity, evoked IPSPs in ~20% of principal neurons. Finally, we show that VIP+ INs receive a dense innervation from both GABAergic inputs (although only 10% from other VIP+ INs) and distinct glutamatergic inputs, identified by their expression of different vesicular glutamate transporters.In conclusion, our study provides a wide-range analysis of single-cell properties of VIP+ INs in the mouse BLA and of their intrinsic and extrinsic connectivity. Our results reinforce the evidence that VIP+ INs are structurally and functionally heterogeneous and that this heterogeneity could mediate different roles in amygdala-dependent functions. We provide the first comprehensive analysis of the distribution of vasoactive intestinal polypeptide-expressing (VIP+) interneurons (INs) across the entire mouse amygdaloid basolateral complex (BLA), as well as of their morphological and physiological properties. VIP+ INs in the neocortex preferentially target other INs to form a disinhibitory network that facilitates principal cell firing. Our study is the first to demonstrate the presence of such a disinhibitory circuitry in the BLA. We observed structural and functional heterogeneity of these INs and characterized their input/output connectivity. We also identified several types of BLA INs that, when inhibited, may provide a temporal window for principal cell firing and facilitate associative plasticity, e.g., in fear learning.
J Neurosci, 2018; 38


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MOLECULAR DEVELOPMENTAL TRAJECTORIES UNDERLYING CORTICAL NEUROGLIAFORM CELL DIVERSITY

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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GABAergic interneurons are key inhibitory regulators of neocortical circuit function. Although several dozens of transcriptionally distinct types of interneurons have been identified in the adult mouse neocortex, the developmental molecular mechanisms underlying this diversity remain largely unknown. Here, focusing on neurogliaform cells (NGCs), we combine single-cell transcriptomics, genetic fate-mapping and electrophysiological assessment to show that discrete subtypes of NGCs emerge from a common progenitor domain in the embryonic preoptic area (POA). Using loss-of-function genetic approaches, we confirm that the transcription factor *Tox2* is critical for the proper development of NGCs. Together, these results reveal that NGC diversity emerges from a spatially restricted pool of progenitors and is gradually acquired post-mitotically through diverging molecular programs.

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BOARD NUMBER: S05-396

ROLE OF STRIATAL PTHLH INTERNEURONS IN THE DEVELOPMENT OF NEURODEGENERATIVE DISEASES USING A NEW PTHLH CRE MOUSE MODEL.

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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The Striatum is the main input structure of the basal ganglia system, which is implicated in motor control, emotions, and procedure learning. It is predominantly inhibitory, containing 95% spiny projections neurons and 5% interneurons, most of which are GABAergic. A recent study from Muñoz-Manchado et *al* (Cell Reports, 2018) revealed a striatal population of *Pthlh*-expressing interneurons, containing *Pvalb*-expressing cells in a gradient manner. The level of *Pvalb* expression in this population correlates with electrophysiological and morphological properties (Bengtsson et *al* 2020). It has also been described that *Pvalb*-expressing cells are responsible for strong somatic inhibition onto their target cells, making them crucial for the modulation of basal ganglia circuit output. However, it is not clear how they could influence behaviour and disease. Understanding the *Pthlh* population during development and degenerative processes is crucial to reveal its role and underlying mechanisms in a regular and altered state. With that purpose we designed the Pthlh^{cre}::R26R-tdTomato line, based on a *Pthlh* constitutive knockin mouse crossed with a Rosa26-tdTomato strain, leading to fluorescence labelled *Pthlh* neurons, that will allow us to thoroughly characterize this population. Therefore, we will conduct molecular and histological profiling including connectivity and distribution analysis. Next, we will compare our findings to the situation in *Pthlh* neurons from the 6-hydroxydopamine hemiparkinsonian mouse model, to identify disease-related differences. This study will allow us to have a more complete and deeper understanding of the molecular changes, *Pthlh* cell localization and their contribution in the regulation of the basal ganglia circuits and neurodegenerative disorders.

Pubmed:
33945688: Ezzanad A, Gómez-Oliva R, Escobar-Montaño F, Díez-Salguero M, Geribaldi-Doldan N, Dominguez-Garcia S, Botubol-Ares JM, Reyes CL, Durán-Patrón R, Nunez-Abades P, Macías-Sánchez AJ, Castro C, Hernández-Galán R
Phorbol Diesters and 12-Deoxy-16-hydroxyphorbol 13,16-Diesters Induce TGFα Release and Adult Mouse Neurogenesis.
A small library of phorbol 12,13-diesters bearing low lipophilicity ester chains was prepared as potential neurogenic agents in the adult brain. They were also used in a targeted UHPLC-HRMS screening of the latex of . Two new 12-deoxy-16-hydroxyphorbol 13,16-diesters were isolated, and their structures were deduced using two-dimensional NMR spectroscopy and NOE experiments. The ability of natural and synthetic compounds to stimulate transforming growth factor alpha (TFGα) release, to increase neural progenitor cell proliferation, and to stimulate neurogenesis was evaluated. All compounds that facilitated TGFα release promoted neural progenitor cell proliferation. The presence of two acyloxy moieties on the tigliane skeleton led to higher levels of activity, which decreased when a free hydroxyl group was at C-12. Remarkably, the compound bearing isobutyryloxy groups was the most potent on the TGFα assay and at inducing neural progenitor cell proliferation , also leading to enhanced neurogenesis when administered intranasally to mice.
J Med Chem, 2021; 64
30542270: García-Bernal F, Geribaldi-Doldán N, Domínguez-García S, Carrasco M, Murillo-Carretero M, Delgado-Ariza A, Díez-Salguero M, Verástegui C, Castro C
Protein Kinase C Inhibition Mediates Neuroblast Enrichment in Mechanical Brain Injuries.
Brain injuries of different etiologies lead to irreversible neuronal loss and persisting neuronal deficits. New therapeutic strategies are emerging to compensate neuronal damage upon brain injury. Some of these strategies focus on enhancing endogenous generation of neurons from neural stem cells (NSCs) to substitute the dying neurons. However, the capacity of the injured brain to produce new neurons is limited, especially in cases of extensive injury. This reduced neurogenesis is a consequence of the effect of signaling molecules released in response to inflammation, which act on intracellular pathways, favoring gliogenesis and preventing recruitment of neuroblasts from neurogenic regions. Protein kinase C (PKC) is a family of intracellular kinases involved in several of these gliogenic signaling pathways. The aim of this study was to analyze the role of

PKC isozymes in the generation of neurons from neural progenitor cells (NPCs) and in brain injuries. PKC inhibition , in cultures of NPC isolated from the subventricular zone (SVZ) of postnatal mice, leads differentiation towards a neuronal fate. This effect is not mediated by classical or atypical PKC. On the contrary, this effect is mediated by novel PKCε, which is abundantly expressed in NPC cultures under differentiation conditions. PKCε inhibition by siRNA promotes neuronal differentiation and reduces glial cell differentiation. On the contrary, inhibition of PKCθ exerts a small anti-gliogenic effect and reverts the effect of PKCε inhibition on neuronal differentiation when both siRNAs are used in combination. Interestingly, in cortical brain injuries we have found expression of almost all PKC isozymes found . Inhibition of PKC activity in this type of injuries leads to neuronal production. In conclusion, these findings show an effect of PKCε in the generation of neurons from NPC , and they highlight the role of PKC isozymes as targets to produce neurons in brain lesions.
Front Cell Neurosci, 2018; 12

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BOARD NUMBER: S05-397

MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF DENTATE GYRUS NDNF-EXPRESSING INTERNEURONS

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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The Dentate Gyrus (DG) receives rich multimodal inputs from the entorhinal cortex and translates them into a sparse code for CA3. During the exploration of new environments, groups of coactive DG principal cells (granule cells) emerge, representing the architectural elements of new memories. The activity of granule cells is strongly modulated by a large and heterogeneous inhibitory network. Besides regulating the granule cells assembly dynamics, DG GABAergic interneurons (INs) are also involved in the generation of hippocampal oscillations and pattern separation. However, a clear understanding of how different INs types participate in these mechanisms is still missing. Recently, a novel population of GABAergic INs expressing the neuron-derived neutrophic factor (NDNFIs) has been identified as a key component of cortical network computations. We found that NDNFIs are also present in the DG where they provide dendritic inhibition to granule cells. We investigated their distribution, morphology, electrical properties, and functional connectivity. Based on morphological reconstructions, we distinguished 2 subpopulations of NDNFIs: the neurogliaform-like (NG-L) cells located in the medial and outer molecular layer, and a second class of NDNFIs located between the granular cell layer and the hilus. Despite the morphological differences and the distinct firing pattern properties, they express the same molecular markers, including Reelin and NPY. In accordance with their dendrites distribution, NDNFIs receive synaptic inputs prevalently from the perforant path or the hilus, depending on their soma-location. Combining in-vitro and in-vivo approaches, we provide the first characterization of NDNF-expressing GABAergic interneurons in the DG.

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BOARD NUMBER: S05-398

FUNCTIONAL ANALYSIS OF CHOLINERGIC NEUROMODULATION OF CHANDELIER CELLS FROM SINGLE-CELL TO CIRCUIT

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Chandelier cells (ChCs) are GABAergic cortical interneurons that innervate the axon initial segment of pyramidal neurons, controlling the cell firing output. They are abundant in the association cortical areas, where acetylcholine inputs are essential for normal cognitive performance. Located at the layer1/layer2 boundary, they extend their dendrites towards layer1, suggesting that may receive inputs from other cortical areas and deep nuclei, such as basal forebrain, which projects the strongest cholinergic innervation to layer1, implying a plausible role for ChCs as circuit switches. Our interest lies in studying the role of ChCs in the control of cortical networks, with a special focus on its presumable cholinergic modulation. We have identified cortical ChCs using a mouse model expressing td-Tomato under control of precise Cre- and Flp- dependent promoters. Using immunohistochemical and electrophysiological techniques, we have described the existence of cholinergic neuromodulation of ChCs through specific nicotinic receptors. To clarify its role in the regulation of the prefrontal cortical circuitry, we performed *in vivo* 2-photon imaging experiments in awake animals using GECIs, showing that ChCs present a collective behaviour during arousal. We used DREADDs to modulate their activity to uncover its influence in the control of the excitatory network. Our results demonstrate that prefrontal ChCs are a subpopulation of fast-spiking interneurons modulated by cholinergic inputs activated during arousal states in awake mice, with a prominent role in the control of the pyramidal neurons. We are grateful to C. Cabrera-Romero for excellent technical assistance. Supported by: RyC-2016-19906, PRE2019-087729 (MCI/AEI/FSE,UE), VI PPIT-US and PGC2018-095656-B-I00 (MCI/AEI/FEDER,UE).

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BOARD NUMBER: S05-399

INTRA-INDIVIDUAL PHYSIOMIC ANALYSIS OF EXCITATORY NEURONS AND THEIR SYNAPSES IN THE HUMAN NEOCORTEX

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

Franz Xaver Mittermaier[1], Henrike Planert[1], Sabine Grosser[2], Henrik Alle[1], Pawel Fidzinski[3], Julia Onken[4], Martin Holtkamp[5], Imre Vida[2], Jörg Geiger[1], Yangfan Peng[1]
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Computation within cortical microcircuits is based on functional properties of neurons and their synaptic connections. Heterogeneity of these basic building blocks is suggested to be computationally relevant. While diversity of cortical inhibitory interneurons has been studied extensively, the anatomical, physiological, and molecular differentiation of excitatory neurons is less understood. To address functional heterogeneity of excitatory neurons and their synaptic connections, we focused on human layer 2-3 cortex, which expanded remarkably during evolution. We conducted multi-neuron patch-clamp recordings in brain slices from temporal cortex of 22 patients with pharmacoresistant epilepsy. We recorded up to 80 neurons per patient and characterized the electrophysiological properties of more than 1.000 excitatory neurons. Hierarchical clustering of intrinsic electrophysiological properties yielded functionally distinct excitatory neuron clusters, which were present across individuals. Additionally, we probed more than 9.000 and found over 1.300 monosynaptic connections. We observed that excitatory neurons display non-uniform connectivity patterns and differences in synaptic properties depending on cluster identity. Overall, there is a large diversity in intrinsic electrophysiological and synaptic properties with intra-individual variability being severalfold greater than inter-individual variability. We argue that heterogeneity of excitatory neurons and synapses is a common principle present in all individuals. We conclude that the diversity of cell properties and linked differences in synaptic connectivity point towards excitatory neuron specialization within the temporal cortical microcircuit of the human brain.

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BOARD NUMBER: S05-400

MOLECULAR AND FUNCTIONAL HETEROGENEITY IN DORSAL AND VENTRAL OLIGODENDROCYTE PROGENITOR CELLS OF THE MOUSE FOREBRAIN IN RESPONSE TO DNA DAMAGE

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

Enrica Boda[1], Martina Lorenzati[1], Roberta Parolisi[1], Brian Harding[2], Gianmarco Pallavicini[1], Luca Bonfanti[3], Amanda Moccia[4], Stephanie Bielas[4], Ferdinando Di Cunto[1], Annalisa Buffo[1]
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In the developing mouse forebrain, temporally distinct waves of oligodendrocyte progenitor cells (OPCs) arise from different germinal zones and eventually populate either dorsal or ventral regions, where they present as transcriptionally and functionally equivalent cells. Despite that, developmental heterogeneity influences adult OPC responses upon demyelination. Here we show that accumulation of DNA damage due to ablation of citron-kinase or cisplatin treatment cell-autonomously disrupts OPC fate, resulting in cell death and senescence in the dorsal and ventral subsets, respectively. Such alternative fates are associated with distinct developmental origins of OPCs, and with a different activation of NRF2-mediated anti-oxidant responses. These data indicate that, upon injury, dorsal and ventral OPC subsets show functional and molecular diversity that can make them differentially vulnerable to pathological conditions associated with DNA damage.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S05-401

TRACING GLIA-INTO-NEURON CONVERSION IN THE AGED MOUSE BRAIN USING SINGLE CELL SPATIAL TRANSCRIPTOMICS

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Reports of methods to reprogram glial cells into neurons have open the door to producing new neurons in the adult mammalian brain, including for the replacment of neurons lost in neurodegenerative diseases. Not yet established are the molecular events underlying glia to neuron identity conversion, including identification of the initiating glial cell, the events driving its conversion and subsequent maturation into functional neuron, and the final neuronal subtype produced. We recently demostrated a therapeutically viable approach to generate neurons in the dentate gyrus of aged adult mouse brain by transiently suppressing the RNA binding protein Polypyrimidine Tract Binding Protein-1 using antisense oligonucleotide (ASO) delivered by single injection into the cerebral spinal fluid (CSF). Radial glial-like cells convert into new neurons over a two-month period, aquire mature neuronal character, and functionally integrate into endogenous circuits that modify mouse behavior, thereby opening peospects for production of neurons tto replace those lost during disease or aging. We now utilize a transformative single cell spatial transcriptomics thechnology,
termed Multiplexed Error Robust Fluoresence In Situ Hybridization (MERFISH), to systematically identify the funcionality, localization and specification of the cell origin(s), lineage pathway(s) and newly generated neuron(s) in glia-into-neuron cell conversion. These approaches uncover new mechanistic insights in cell reprograming and might lead to new concept for treating neurodegeneration.

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BOARD NUMBER: S05-402

DLX5/6 LEVELS IN MOUSE GABAERGIC NEURONS AFFECT ADULT PARVALBUMIN-POSITIVE NEURONAL DENSITY AND CONTROL ANXIETY/COMPULSIVE BEHAVIOURS.

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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Introduction: *Dlx5/6* genes encode for two homeobox transcription factors which, during development, are involved in differentiation of Parvalbumin-positive GABAergic inhibitory interneurons (PV). In the adult, these genes continue to be expressed in most forebrain GABAergic neurons where their role is only partially elucidated. PV interneurons are essential for normal brain function and their activity and distribution is altered in psychiatric conditions such as schizophrenia. Brains from autistic and schizophrenic patients and from mouse models of these diseases present regional defects in PV interneurons. Furthermore, large epidemiological studies suggest a link of the *Dlx5/6* locus with schizophrenia, autism spectrum disorder and Rett syndrome. **Results:** Here we evaluate the neuroanatomical and behavioural consequences of targeted *Dlx5/6* gene dosage alterations in adult mouse GABAergic neurons. We compare the effects of homozygous and heterozygous *Dlx5/6* deletions to those of *Dlx5* targeted overexpression. We find a linear correlation between *Dlx5/6* allelic dosage and the density of PV-positive neurons in the adult prelimbic cortex and in the hippocampus.In parallel, we observe that *Dlx5/6* expression levels in GABAergic neurons are also linearly associated with the intensity of anxiety and compulsivity-like behaviours. Higher levels of *Dlx5/6* expression in GABAergic neurons are associated with increased anxiety and compulsivity while deletion of *Dlx5/6* results in reduced anxiety and compulsivity with hyper-vocalization and socialization phenotype. **Conclusion:** Our findings reinforce the notion that regulation of *Dlx5/6* expression is involved in individual cognitive variability and, possibly, in the genesis of certain neuropsychiatric conditions.

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BOARD NUMBER: S05-403

GENETIC ARCHITECTURE OF THE WHITE MATTER CONNECTOME OF THE HUMAN BRAIN

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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White matter tracts form the structural basis of large-scale functional networks in the human brain. We applied brain-wide tractography to diffusion images from 30,810 adult participants (UK Biobank), and found significant heritability for 90 regional connectivity measures and 851 tract-wise connectivity measures. Multivariate genome-wide association analyses identified 355 independently associated lead SNPs across the genome, of which 77% had not been previously associated with human brain metrics. Enrichment analyses implicated neurodevelopmental processes including neurogenesis, neural differentiation, neural migration, neural projection guidance, and axon development, as well as prenatal brain expression especially in stem cells, astrocytes, microglia and neurons. We used the multivariate association profiles of lead SNPs to identify 26 genomic loci implicated in structural connectivity between core regions of the left-hemisphere language network, and also identified 6 loci associated with hemispheric left-right asymmetry of structural connectivity. Polygenic scores for schizophrenia, bipolar disorder, autism spectrum disorder, attention-deficit hyperactivity disorder, left-handedness, Alzheimer's disease, amyotrophic lateral sclerosis, and epilepsy showed significant multivariate associations with structural connectivity, each implicating distinct sets of brain regions with trait-relevant functional profiles. This large-scale mapping study revealed common genetic contributions to the structural connectome of the human brain in the general adult population, highlighting links with polygenic disposition to brain disorders and behavioural traits.

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BOARD NUMBER: S05-404

A NOVEL LAYER4 CORTICOFUGAL CELL TYPE/PROJECTION INVOLVED IN THALAMO-CORTICO-STRIATAL SENSORY PROCESSING

POSTER SESSION 05 - SECTION: CELLULAR ARCHITECTURE OF THE BRAIN

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In sensory cortices, the information flow has been thought to be processed vertically across cortical layers, with layer 4 being the major thalamo-recipient one which relays thalamic signals to layer 2/3, which in turn transmit thalamic information to layer 5 and 6 to then leave the cortex to reach subcortical and cortical long-range structures. Although several exceptions to this model have been described, neurons in layer 4 are still considered to establish only local (i.e., interlaminar and short-range) connections. Here, taking advantage of anatomical, electrophysiological, optogenetic techniques, we describe for the first time a long-range corticostriatal class of pyramidal neurons in layer 4 (CS-L4) of the mouse auditory cortex that receive direct thalamic inputs. The CS-L4 neurons are embedded in a feedforward inhibitory circuit involving local parvalbumin neurons and establish connections in the posterior striatum in yet another feedforward inhibitory **thalamo→cortico(L4)→striatal circuit** to potentially contribute to control the output of striatal spiny projection neurons.Here we propose a new wiring diagram that implemented the old one, in which layer 4 is not only involved in the transfer of thalamic input to the upper layer 2/3 but can exert a direct top-down control, bypassing intracortical processing, of subcortical structure such as the posterior part of the dorsal striatum posing a new conceptual cell element (CS-L4 neurons) for experimental and theoretical work of the cortical function.

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BOARD NUMBER: S05-405

BRIEF SYNAPTIC INHIBITION PERSISTENTLY INTERRUPTS FIRING OF FAST-SPIKING INTERNEURONS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Input-output operations performed by neurons rely on their ability to integrate synaptic activity with their intrinsic electrical properties, a process thought to be constrained by the duration of synaptic events. At variance with this classical view, we find that the sustained firing of CA1 hippocampal fast-spiking (FS) parvalbumin-expressing interneurons (PV-INs) is persistently interrupted for several hundred milliseconds following brief GABAAR-mediated inhibition. A single presynaptic neuron could interrupt PV-INs, occasionally with a single action potential (AP), and reliably with brief bursts of APs. Experiments and a computational model revealed that the persistent interruption of firing maintains neurons in a depolarized, quiescent state through a cell-autonomous mechanism dependent on an inactivating Kv1.1-mediated current and largely inactivated Na+ channels. Strikingly, interrupted PV-INs are highly responsive to Schaffer collateral inputs, with subthreshold stimuli at rest becoming suprathreshold during the interruption. We show that the persistent interruption of firing represents a powerful disinhibitory mechanism that favors spike generation in CA1 pyramidal cells. Overall, our results demonstrate that neuronal silencing can far outlast brief synaptic inhibition owing to a well-tuned interplay between neurotransmitter release and postsynaptic membrane dynamics, thus impacting circuit function.

Pubmed:
32598500: Chamberland S, Timofeeva Y, Evstratova A, Norman CA, Volynski K, Tóth K
Slow-decaying presynaptic calcium dynamics gate long-lasting asynchronous release at the hippocampal mossy fiber to CA3 pyramidal cell synapse.
Action potentials trigger two modes of neurotransmitter release, with a fast synchronous component and a temporally delayed asynchronous release. Asynchronous release contributes to information transfer at synapses, including at the hippocampal mossy fiber (MF) to CA3 pyramidal cell synapse where it controls the timing of postsynaptic CA3 pyramidal neuron firing. Here, we identified and characterized the main determinants of asynchronous release at the MF-CA3 synapse. We found that asynchronous release at MF-CA3 synapses can last on the order of seconds following repetitive MF stimulation. Elevating the stimulation frequency or the external Ca concentration increased the rate of asynchronous release, thus, arguing that presynaptic Ca dynamics is the major determinant of asynchronous release rate. Direct MF bouton Ca imaging revealed slow Ca decay kinetics of action potential (AP) burst-evoked Ca transients. Finally, we observed that asynchronous release was preferentially mediated by Ca influx through P/Q-type voltage-gated Ca channels, while the contribution of N-type VGCCs was limited. Overall, our results uncover the determinants of long-lasting asynchronous release from MF terminals and suggest that asynchronous release could influence CA3 pyramidal cell firing up to seconds following termination of granule cell bursting.
Synapse, 2020; 74
30530093: Chamberland S, Zamora Moratalla A, Topolnik L
Calcium extrusion mechanisms in dendrites of mouse hippocampal CA1 inhibitory interneurons.
Local circuit GABAergic inhibitory interneurons control the integration and transfer of information in many brain regions. Several different forms of plasticity reported at interneuron excitatory synapses are triggered by cell- and synapse-specific postsynaptic calcium (Ca) mechanisms. To support this function, the spatiotemporal dynamics of dendritic Ca elevations must be tightly regulated. While the dynamics of postsynaptic Ca signaling through activation of different Ca sources has been explored, the Ca extrusion mechanisms that operate in interneuron dendrites during different patterns of activity remain largely unknown. Using a combination of whole-cell patch-clamp recordings and two-photon Ca imaging in acute mouse hippocampal slices, we characterized the Ca extrusion mechanisms activated by Ca transients (CaTs) associated with backpropagating action potentials (bAPs) in dendrites of hippocampal CA1 stratum radiatum interneurons. Our data showed that Ca clearance increased as a function of activity, pointing to an activity-dependent recruitment of specific Ca extrusion mechanisms. bAP-CaTs were significantly prolonged in the presence of the plasma membrane Ca ATPase (PMCA) and Na/Ca exchanger (NCX) inhibitors as well as the sarco/endoplasmic reticulum Ca ATPase (SERCA) and the mitochondria Ca

uniporter (MCU) blockers. While PMCA, NCX and SERCA pumps cooperated in the cytosolic Ca removal at a wide range of concentrations, the MCU was only activated at higher Ca loads produced by repetitive interneuron firing. These results identify a division of labor between distinct Ca extrusion mechanisms shaping dendritic Ca dynamics and possibly contributing to activity-dependent regulation of synaptic inputs in interneurons. In addition, the MCU activated by larger Ca levels may be involved in the activity-dependent ATP production or interneuron-selective vulnerability associated with cytosolic Ca overloads under pathological conditions.
Cell Calcium, 2019; 77
29946034: Chamberland S, Timofeeva Y, Evstratova A, Volynski K, Tóth K
Action potential counting at giant mossy fiber terminals gates information transfer in the hippocampus.
Neuronal communication relies on action potential discharge, with the frequency and the temporal precision of action potentials encoding information. Hippocampal mossy fibers have long been recognized as conditional detonators owing to prominent short-term facilitation of glutamate release displayed during granule cell burst firing. However, the spiking patterns required to trigger action potential firing in CA3 pyramidal neurons remain poorly understood. Here, we show that glutamate release from mossy fiber terminals triggers action potential firing of the target CA3 pyramidal neurons independently of the average granule cell burst frequency, a phenomenon we term action potential counting. We find that action potential counting in mossy fibers gates glutamate release over a broad physiological range of frequencies and action potential numbers. Using rapid Ca imaging we also show that the magnitude of evoked Ca influx stays constant during action potential trains and that accumulated residual Ca is gradually extruded on a time scale of several hundred milliseconds. Using experimentally constrained 3D model of presynaptic Ca influx, buffering, and diffusion, and a Monte Carlo model of Ca-activated vesicle fusion, we argue that action potential counting at mossy fiber boutons can be explained by a unique interplay between Ca dynamics and buffering at release sites. This is largely determined by the differential contribution of major endogenous Ca buffers calbindin-D and calmodulin and by the loose coupling between presynaptic voltage-gated Ca channels and release sensors and the relatively slow Ca extrusion rate. Taken together, our results identify a previously unexplored information-coding mechanism in the brain.
Proc Natl Acad Sci U S A, 2018; 115
29308430: Baldy C, Chamberland S, Fournier S, Kinkead R
Sex-Specific Consequences of Neonatal Stress on Cardio-Respiratory Inhibition Following Laryngeal Stimulation in Rat Pups.
The presence of liquid near the larynx of immature mammals triggers prolonged apneas with significant O desaturations and bradycardias. When excessive, this reflex (the laryngeal chemoreflex; LCR) can be fatal. Our understanding of the origins of abnormal LCR are limited; however, perinatal stress and male sex are risk factors for cardio-respiratory failure in infants. Because exposure to stress during early life has deleterious and sex-specific consequences on brain development it is plausible that respiratory reflexes are vulnerable to neuroendocrine dysfunction. To address this issue, we tested the hypothesis that neonatal maternal separation (NMS) is sufficient to exacerbate LCR-induced cardio-respiratory inhibition in anesthetized rat pups. Stressed pups were separated from their mother 3 h/d from postnatal days 3 to 12. At P14-P15, pups were instrumented to monitor breathing, O saturation (So), and heart rate. The LCR was activated by water injections near the larynx (10 µl). LCR-induced apneas were longer in stressed pups than controls; O desaturations and bradycardias were more profound, especially in males. NMS increased the frequency and amplitude of spontaneous EPSCs (sEPSCs) in the dorsal motor nucleus of the vagus (DMNV) of males but not females. The positive relationship between corticosterone and testosterone observed in stressed pups (males only) suggests that disruption of neuroendocrine function by stress is key to sex-based differences in abnormal LCR. Because testosterone application onto medullary slices augments EPSC amplitude only in males, we propose that testosterone-mediated enhancement of synaptic connectivity within the DMNV contributes to the male bias in cardio-respiratory inhibition following LCR activation in stressed pups.
eNeuro, 2017 Nov-Dec; 4
28749338: Chamberland S, Yang HH, Pan MM, Evans SW, Guan S, Chavarha M, Yang Y, Salesse C, Wu H, Wu JC, Clandinin TR, Toth K, Lin MZ, St-Pierre F
Fast two-photon imaging of subcellular voltage dynamics in neuronal tissue with genetically encoded indicators.
Monitoring voltage dynamics in defined neurons deep in the brain is critical for unraveling the function of neuronal circuits but is challenging due to the limited performance of existing tools. In particular, while genetically encoded voltage indicators have shown promise for optical detection of voltage transients, many indicators exhibit low sensitivity when imaged under two-photon illumination. Previous studies thus fell short of visualizing voltage dynamics in individual neurons in single trials. Here, we report ASAP2s, a novel voltage indicator with improved sensitivity. By imaging ASAP2s using random-access multi-photon microscopy, we demonstrate robust single-trial detection of action potentials in organotypic slice cultures. We also show that ASAP2s enables two-photon imaging of graded potentials in organotypic slice cultures and in . These results demonstrate that the combination of ASAP2s and fast two-photon imaging methods enables detection of neural electrical activity with subcellular spatial resolution and millisecond-timescale precision.

Elife, 2017; 6
28411270: Chamberland S, Evstratova A, Tóth K
Short-Term Facilitation at a Detonator Synapse Requires the Distinct Contribution of Multiple Types of Voltage-Gated Calcium Channels.
Neuronal calcium elevations are shaped by several key parameters, including the properties, density, and the spatial location of voltage-gated calcium channels (VGCCs). These features allow presynaptic terminals to translate complex firing frequencies and tune the amount of neurotransmitter released. Although synchronous neurotransmitter release relies on both P/Q- and N-type VGCCs at hippocampal mossy fiber-CA3 synapses, the specific contribution of VGCCs to calcium dynamics, neurotransmitter release, and short-term facilitation remains unknown. Here, we used random-access two-photon calcium imaging together with electrophysiology in acute mouse hippocampal slices to dissect the roles of P/Q- and N-type VGCCs. Our results show that N-type VGCCs control glutamate release at a limited number of release sites through highly localized Ca elevations and support short-term facilitation by enhancing multivesicular release. In contrast, Ca entry via P/Q-type VGCCs promotes the recruitment of additional release sites through spatially homogeneous Ca elevations. Altogether, our results highlight the specialized contribution of P/Q- and N-types VGCCs to neurotransmitter release. In presynaptic terminals, neurotransmitter release is dynamically regulated by the transient opening of different types of voltage-gated calcium channels. Hippocampal giant mossy fiber terminals display extensive short-term facilitation during repetitive activity, with a large several fold postsynaptic response increase. Though, how giant mossy fiber terminals leverage distinct types of voltage-gated calcium channels to mediate short-term facilitation remains unexplored. Here, we find that P/Q- and N-type VGCCs generate different spatial patterns of calcium elevations in giant mossy fiber terminals and support short-term facilitation through specific participation in two mechanisms. Whereas N-type VGCCs contribute only to the synchronization of multivesicular release, P/Q-type VGCCs act through microdomain signaling to recruit additional release sites.
J Neurosci, 2017; 37
26614712: Chamberland S, Tóth K
Functionally heterogeneous synaptic vesicle pools support diverse synaptic signalling.
Synaptic communication between neurons is a highly dynamic process involving specialized structures. At the level of the presynaptic terminal, neurotransmission is ensured by fusion of vesicles to the membrane, which releases neurotransmitter in the synaptic cleft. Depending on the level of activity experienced by the terminal, the spatiotemporal properties of calcium invasion will dictate the timing and the number of vesicles that need to be released. Diverse presynaptic firing patterns are translated to neurotransmitter release with a distinct temporal feature. Complex patterns of neurotransmitter release can be achieved when different vesicles respond to distinct calcium dynamics in the presynaptic terminal. Specific vesicles from different pools are recruited during various modes of release as the particular molecular composition of their membrane proteins define their functional properties. Such diversity endows the presynaptic terminal with the ability to respond to distinct physiological signals via the mobilization of specific subpopulation of vesicles. There are several mechanisms by which a diverse vesicle population could be generated in single presynaptic terminals, including distinct recycling pathways that utilize various adaptor proteins. Several additional factors could potentially contribute to the development of a heterogeneous vesicle pool such as specialized release sites, spatial segregation within the terminal and specialized delivery pathways. Among these factors molecular heterogeneity plays a central role in defining the functional properties of different subpopulations of vesicles.
J Physiol, 2016; 594
25122902: Chamberland S, Evstratova A, Tóth K
Interplay between synchronization of multivesicular release and recruitment of additional release sites support short-term facilitation at hippocampal mossy fiber to CA3 pyramidal cells synapses.
Synaptic short-term plasticity is a key regulator of neuronal communication and is controlled via various mechanisms. A well established property of mossy fiber to CA3 pyramidal cell synapses is the extensive short-term facilitation during high-frequency bursts. We investigated the mechanisms governing facilitation using a combination of whole-cell electrophysiological recordings, electrical minimal stimulation, and random-access two-photon microscopy in acute mouse hippocampal slices. Two distinct presynaptic mechanisms were involved in short-term facilitation, with their relative contribution dependent on extracellular calcium concentration. The synchronization of multivesicular release was observed during trains of facilitating EPSCs recorded in 1.2 mM external Ca(2+) ([Ca(2+)]e). Indeed, covariance analysis revealed a gradual augmentation in quantal size during trains of EPSCs, and application of the low-affinity glutamate receptor antagonist γ-D-glutamylglycine showed an increase in cleft glutamate concentration during paired-pulse stimulation. Whereas synchronization of multivesicular release contributed to the facilitation in 1.2 mM [Ca(2+)]e, variance-mean analysis showed that recruitment of more release sites (N) was likely to account for the larger facilitation observed in 2.5 mM [Ca(2+)]e. Furthermore, this increase in N could be promoted by calcium microdomains of heterogeneous amplitudes observed in single mossy fiber boutons. Our findings suggest that the combination of multivesicular release and the recruitment of additional

release sites act together to increase glutamate release during burst activity. This is supported by the compartmentalized spatial profile of calcium elevations in boutons and helps to expand the dynamic range of mossy fibers information transfer.
J Neurosci, 2014; 34
24671999: Tyan L, Chamberland S, Magnin E, Camiré O, Francavilla R, David LS, Deisseroth K, Topolnik L
Dendritic inhibition provided by interneuron-specific cells controls the firing rate and timing of the hippocampal feedback inhibitory circuitry.
In cortical networks, different types of inhibitory interneurons control the activity of glutamatergic principal cells and GABAergic interneurons. Principal neurons represent the major postsynaptic target of most interneurons; however, a population of interneurons that is dedicated to the selective innervation of GABAergic cells exists in the CA1 area of the hippocampus. The physiological properties of these cells and their functional relevance for network computations remain unknown. Here, we used a combination of dual simultaneous patch-clamp recordings and targeted optogenetic stimulation in acute mouse hippocampal slices to examine how one class of interneuron-specific (IS) cells controls the activity of its GABAergic targets. We found that type 3 IS (IS3) cells that coexpress the vasoactive intestinal polypeptide (VIP) and calretinin contact several distinct types of interneurons within the hippocampal CA1 stratum oriens/alveus (O/A), with preferential innervation of oriens-lacunosum moleculare cells (OLMs) through dendritic synapses. In contrast, VIP-positive basket cells provided perisomatic inhibition to CA1 pyramidal neurons with the asynchronous GABA release and were not connected with O/A interneurons. Furthermore, unitary IPSCs recorded at IS3-OLM synapses had a small amplitude and low release probability but summated efficiently during high-frequency firing of IS3 interneurons. Moreover, the synchronous generation of a single spike in several IS cells that converged onto a single OLM controlled the firing rate and timing of OLM interneurons. Therefore, dendritic inhibition originating from IS cells is needed for the flexible activity-dependent recruitment of OLM interneurons for feedback inhibition.
J Neurosci, 2014; 34
23162426: Chamberland S, Topolnik L
Inhibitory control of hippocampal inhibitory neurons.
Information processing within neuronal networks is determined by a dynamic partnership between principal neurons and local circuit inhibitory interneurons. The population of GABAergic interneurons is extremely heterogeneous and comprises, in many brain regions, cells with divergent morphological and physiological properties, distinct molecular expression profiles, and highly specialized functions. GABAergic interneurons have been studied extensively during the past two decades, especially in the hippocampus, which is a relatively simple cortical structure. Different types of hippocampal inhibitory interneurons control spike initiation [e.g., axo-axonic and basket cells (BCs)] and synaptic integration (e.g., bistratified and oriens-lacunosum moleculare interneurons) within pyramidal neurons and synchronize local network activity, providing a means for functional segregation of neuronal ensembles and proper routing of hippocampal information. Thus, it is thought that, at least in the hippocampus, GABAergic inhibitory interneurons represent critical regulating elements at all stages of information processing, from synaptic integration and spike generation to large-scale network activity. However, this raises an important question: if inhibitory interneurons are fundamental for network computations, what are the mechanisms that control the activity of the interneurons themselves? Given the essential role of synaptic inhibition in the regulation of neuronal activity, it would be logical to expect that specific inhibitory mechanisms have evolved to control the operation of interneurons. Here, we review the mechanisms of synaptic inhibition of interneurons and discuss their role in the operation of hippocampal inhibitory circuits.
Front Neurosci, 2012; 6

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BOARD NUMBER: S05-406

DENDRITIC AXON ORIGIN ENABLES SELECTIVE INFORMATION GATING BY PERISOMATIC INHIBITION IN PYRAMIDAL NEURONS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Information processing in cortical pyramidal neurons involves the specific activation into functional ensembles. In this process, only a minority of neurons is recruited while the majority remains silent. This sparse activation is believed to result from widespread perisomatic inhibition in conjunction with specific synaptic excitation. We have previously shown that in ~50% of hippocampal pyramidal cells the axon emerges from a basal dendrite. Here, we propose that this particular morphology provides a mechanism for selective activation of participating neurons through these morphologically unique axo-dendritic compartments. In awake, head-fixed mice, we found that CA1 pyramidal neurons with a dendritic axon origin displayed a ~4-fold higher firing frequency during network activation compared to neurons with somatic axon origin. This difference was absent outside ripples. Extra- and intracellular recordings in mouse brain slices and computer simulations led us to hypothesize that the axon is forming a functional unit together with the axon-carrying dendrite. We show that excitatory input to axon-carrying dendrites remains efficient even during strong perisomatic inhibition. Other dendrites become uncoupled from this compartment, preventing their input to trigger action potentials. This may likewise apply to all neurons with somatic axon origin. Therefore, cells with axon-carrying dendrites may be privileged members of neuronal ensembles during states of strong perisomatic inhibition, such as fast network oscillations. By this mechanism, activation of inhibitory interneurons and targeted excitation of the respective dendrite may dynamically change the functional network topology, resulting in the activation of defined neuronal ensembles.

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BOARD NUMBER: S05-407

HYPERPOLARIZATION-ACTIVATED CYCLIC NUCLEOTIDE-GATED CHANNELS REGULATE RESPONSE PROBABILITY IN THE TERMINALS OF PARVALBUMIN POSITIVE BASKET CELLS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Columbia University, Department Of Neuroscience, New York, United States of America

Hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels generate the cationic current I_h, which has been found to regulate the excitability of neuronal networks. The function of HCN channels depends, in part, on their subcellular localization. HCN channels are abundantly expressed in the dendrites of pyramidal neurons in hippocampal area CA1 where they act as an inhibitory constraint on dendritic integration and synaptic plasticity. HCN1 is also strongly expressed in presynaptic terminals of parvalbumin-positive inhibitory neuron basket cells (PVBCs). We found that application of HCN channel blockers strongly inhibit IPSCs in CA1 pyramidal neurons evoked by electrical stimulation or PVBC-specific optogenetic stimulation. To investigate the impact of HCN channels in presynaptic terminals, we performed 2-photon imaging in acute brain slices, expressing GCaMP6s specifically in the terminals of CA1 PVBCs. Bath application of the HCN blocker ZD7288 reduced the amplitude of dF/F transients in PVBC terminals elicited by a 5-pulse train of electrical stimulation (30 Hz) delivered to stratum pyramidale. When examining responses to single electrical stimuli, we found that the response of individual terminals to single stimuli was probabilistic; some stimuli elicited a measurable response and some stimuli failed to elicit a response. ZD7288 did not reduce the amplitude of the successful responses to single electrical stimuli, but rather decreased the probability with which single stimuli evoked a successful response. Thus we conclude that HCN channels in the axonal terminals of PVBCs upregulate the probability of terminal activation (and thus vesicle release) rather than the amplitude of the Ca^{2+} response to such activation.

Pubmed:

19151696: Murayama M, Pérez-Garci E, Nevian T, Bock T, Senn W, Larkum ME
Dendritic encoding of sensory stimuli controlled by deep cortical interneurons.
The computational power of single neurons is greatly enhanced by active dendritic conductances that have a large influence on their spike activity. In cortical output neurons such as the large pyramidal cells of layer 5 (L5), activation of apical dendritic calcium channels leads to plateau potentials that increase the gain of the input/output function and switch the cell to burst-firing mode. The apical dendrites are innervated by local excitatory and inhibitory inputs as well as thalamic and corticocortical projections, which makes it a formidable task to predict how these inputs influence active dendritic properties in vivo. Here we investigate activity in populations of L5 pyramidal dendrites of the somatosensory cortex in awake and anaesthetized rats following sensory stimulation using a new fibre-optic method for recording dendritic calcium changes. We show that the strength of sensory stimulation is encoded in the combined dendritic calcium response of a local population of L5 pyramidal cells in a graded manner. The slope of the stimulus-response function was under the control of a particular subset of inhibitory neurons activated by synaptic inputs predominantly in L5. Recordings from single apical tuft dendrites in vitro showed that activity in L5 pyramidal neurons disynaptically coupled via interneurons directly blocks the initiation of dendritic calcium spikes in neighbouring pyramidal neurons. The results constitute a functional description of a cortical microcircuit in awake animals that relies on the active properties of L5 pyramidal dendrites and their very high sensitivity to inhibition. The microcircuit is organized so that local populations of apical dendrites can adaptively encode bottom-up sensory stimuli linearly across their full dynamic range.
Nature, 2009; 457
26936985: Bock T, Stuart GJ
Impact of calcium-activated potassium channels on NMDA spikes in cortical layer 5 pyramidal neurons.
Active electrical events play an important role in shaping signal processing in dendrites. As these events are usually associated with an increase in intracellular calcium, they are likely to be under the control of calcium-activated potassium channels. Here, we investigate the impact of calcium-activated potassium channels onN-methyl-d-aspartate (NMDA) receptor-dependent spikes, or NMDA spikes, evoked by glutamate iontophoresis onto basal dendrites of cortical layer 5 pyramidal neurons. We found that small-conductance calcium-activated potassium channels (SK channels) act to reduce

NMDA spike amplitude but at the same time, also decrease the iontophoretic current required for their generation. This SK-mediated decrease in NMDA spike threshold was dependent on R-type voltage-gated calcium channels and indicates a counterintuitive, excitatory effect of SK channels on NMDA spike generation, whereas the capacity of SK channels to suppress NMDA spike amplitude is in line with the expected inhibitory action of potassium channels on dendritic excitability. Large-conductance calcium-activated potassium channels had no significant impact on NMDA spikes, indicating that these channels are either absent from basal dendrites or not activated by NMDA spikes. These experiments reveal complex and opposing interactions among NMDA receptors, SK channels, and voltage-gated calcium channels in basal dendrites of cortical layer 5 pyramidal neurons during NMDA spike generation, which are likely to play an important role in regulating the way these neurons integrate the thousands of synaptic inputs they receive.
J Neurophysiol, 2016; 115
27630543: Bock T, Stuart GJ
The Impact of BK Channels on Cellular Excitability Depends on their Subcellular Location.
Large conductance calcium-activated potassium channels (or BK channels) fulfil a multitude of roles in the central nervous system. At the soma of many neuronal cell types they control the speed of action potential (AP) repolarization and therefore they can have an impact on neuronal excitability. Due to their presence in nerve terminals they also regulate transmitter release. BK channels have also been shown to be present in the dendrites of some neurons where they can regulate the magnitude and duration of dendritic spikes. Here, we investigate the impact of modulating the activation of BK channels at different locations on the cellular excitability of cortical layer 5 pyramidal neurons. We find that while somatic BK channels help to repolarize APs at the soma and mediate the fast after-hyperpolarization, dendritic BK channels are responsible for repolarization of dendritic calcium spikes and thereby regulate somatic AP burst firing. We found no evidence for a role of dendritic BK channels in the regulation of backpropagating AP amplitude or duration. These experiments highlight the diverse roles of BK channels in regulating neuronal excitability and indicate that their functional impact depends on their subcellular location.
Front Cell Neurosci, 2016; 10
31420457: Bock T, Honnuraiah S, Stuart GJ
Paradoxical Excitatory Impact of SK Channels on Dendritic Excitability.
Dendritic excitability regulates how neurons integrate synaptic inputs and thereby influences neuronal output. As active dendritic events are associated with significant calcium influx they are likely to be modulated by calcium-dependent processes, such as calcium-activated potassium channels. Here we investigate the impact of small conductance calcium-activated potassium channels (SK channels) on dendritic excitability in male and female rat cortical pyramidal neurons and Using local applications of the SK channel antagonist apamin , we show that blocking somatic SK channels enhances action potential output, whereas blocking dendritic SK channels paradoxically reduces the generation of dendritic calcium spikes and associated somatic burst firing. Opposite effects were observed using the SK channel enhancer NS309. The effect of apamin on dendritic SK channels was occluded when R-type calcium channels were blocked, indicating that the inhibitory impact of apamin on dendritic calcium spikes involved R-type calcium channels. Comparable effects were observed Intracellular application of apamin via the somatic whole-cell recording pipette reduced the medium afterhyperpolarization and increased action potential output during UP states. In contrast, extracellular application of apamin to the cortical surface to block dendritic SK channels shifted the distribution of action potentials within UP states from an initial burst to a more distributed firing pattern, while having no impact on overall action potential firing frequency or UP and DOWN states. These data indicate that somatic and dendritic SK channels have opposite effects on neuronal excitability, with dendritic SK channels counter-intuitively promoting rather than suppressing neuronal output. Neurons typically receive input from other neurons onto processes called dendrites, and use electrical events such as action potentials for signaling. As electrical events in neurons are usually associated with calcium influx they can be regulated by calcium-dependent processes. One such process is through the activation of calcium-dependent potassium channels, which usually act to reduce action potential signaling. Although this is the case for calcium-dependent potassium channels found at the cell body, we show here that calcium-dependent potassium channels in dendrites of cortical pyramidal neurons counter-intuitively promote rather than suppress action potential output.
J Neurosci, 2019; 39
33990774: Leroy F, de Solis CA, Boyle LM, Bock T, Lofaro OM, Buss EW, Asok A, Kandel ER, Siegelbaum SA
Enkephalin release from VIP interneurons in the hippocampal CA2/3a region mediates heterosynaptic plasticity and social memory.
The hippocampus contains a diverse array of inhibitory interneurons that gate information flow through local cortico-hippocampal circuits to regulate memory storage. Although most studies of interneurons have focused on their role in fast synaptic inhibition mediated by GABA release, different classes of interneurons express unique sets of neuropeptides, many of which have been shown to exert powerful effects on neuronal function and memory when applied pharmacologically.

However, relatively little is known about whether and how release of endogenous neuropeptides from inhibitory cells contributes to their behavioral role in regulating memory formation. Here we report that vasoactive intestinal peptide (VIP)-expressing interneurons participate in social memory storage by enhancing information transfer from hippocampal CA3 pyramidal neurons to CA2 pyramidal neurons. Notably, this action depends on release of the neuropeptide enkephalin from VIP neurons, causing long-term depression of feedforward inhibition onto CA2 pyramidal cells. Moreover, VIP neuron activity in the CA2 region is increased selectively during exploration of a novel conspecific. Our findings, thus, enhance our appreciation of how GABAergic neurons can regulate synaptic plasticity and mnemonic behavior by demonstrating that such actions can be mediated by release of a specific neuropeptide, rather than through classic fast inhibitory transmission.
Mol Psychiatry, 2022; 27

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BOARD NUMBER: S05-408

PNN-DEPENDENT REGULATION OF THALAMO-CORTICAL INPUTS ONTO PARVALBUMIN INTERNEURONS IN ADULT MOUSE PRIMARY VISUAL CORTEX

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

Lucie Gallet, Alberto Bacci, Laurence Cathala
Sorbonne University, Paris Brain Institute, Paris, France

Sensory, motor and cognitive development of the neocortex requires a critical period (CP) of plasticity, during which neuronal circuits are strongly regulated by experience. Its closure is associated with the accumulation of perineuronal nets (PNNs) around layer 4 parvalbumin (PV)- interneurons. Previous work from the lab has shown that *in vivo* acute PNN removal leads to increased recruitment of PV cells by thalamocortical (TC) inputs, which convey sensory information into the cortex. First, by combining optogenetic and electrical stimulations with patch-clamp recordings in acute V1 slices of adult mice, we have set up protocols for single TC axon activation onto patched layer 4 PV-interneurons. This allowed establishing the properties of thalamic recruitment of PV interneurons in V1 that are poorly known, compared to somatosensory cortex. We found that unitary TC synaptic transmission had high release probability ensuring a reliable transmission. Second, we compared the properties of TC synaptic transmission onto PV cells in control and after acute PNN degradation following *in vivo* ChABC injections, known to reactivate adult cortical plasticity. We found that PNN removal did not affect synaptic strength, short-term plasticity nor the contribution of AMPA and NMDA receptors of unitary TC synaptic transmission. These results suggest that acute disruption of PNNs does not directly affect synaptic transmission and provide compelling evidence that PNNs regulate the structural connectivity between the thalamus and PV interneurons.

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BOARD NUMBER: S05-409

DENDRITIC VOLTAGE SIGNALING IN CEREBELLAR PURKINJE NEURONS DURING ASSOCIATIVE MOTOR LEARNING

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

Lina Koronfel, Christopher Roome, Bernd Kuhn
Okinawa institute of science and technology, Optical Neuroimaging Unit, Okinawa, Japan

The cerebellar cortex is critical for many forms of associative learning, such as the timing of delay eyeblink conditioning. Here, we use two-photon voltage imaging of Purkinje neuron (PN) dendrites to study changes in dendritic activity during associative motor learning in habituated mice. PNs expressing a genetically encoded Ca^{2+} sensor (GCaMP6f) were selected if they reliably responded to an air puff stimulus directed towards the eye. Using a chronic cranial window with access port, the responsive PNs were loaded with the voltage-sensitive dye ANNINE-6plus via electroporation, allowing dendritic voltage imaging *in vivo*. Excitatory and inhibitory postsynaptic voltage signals were recorded with sub-millisecond temporal precision, repeatedly over several weeks, from up to two PNs per mouse. First, we recorded dendritic voltage signals during the presentation of a novel stimulus (blue LED light). Next, we examined dendritic voltage signaling during associative motor learning. In this case, the novel stimulus was associated with an unconditioned stimulus (an aversive air puff directed to the eye). Finally, we examined dendritic voltage signaling while the association between the two stimuli was unlearned. Behavioral recording, at 200Hz, tracked learning and was compared with voltage imaging data on a trial-to-trial basis, to investigate short- and long-term changes in dendritic signaling during the eyeblink conditioning training sessions.

Pubmed:
34362940: Koronfel LM, Kanning KC, Alcos A, Henderson CE, Brownstone RM
Elimination of glutamatergic transmission from Hb9 interneurons does not impact treadmill locomotion.
The spinal cord contains neural circuits that can produce the rhythm and pattern of locomotor activity. It has previously been postulated that a population of glutamatergic neurons, termed Hb9 interneurons, contributes to locomotor rhythmogenesis. These neurons were identified by their expression of the homeobox gene, Hb9, which is also expressed in motor neurons. We developed a mouse line in which Cre recombinase activity is inducible in neurons expressing Hb9. We then used this line to eliminate vesicular glutamate transporter 2 from Hb9 interneurons, and found that there were no deficits in treadmill locomotion. We conclude that glutamatergic neurotransmission by Hb9 interneurons is not required for locomotor behaviour. The role of these neurons in neural circuits remains elusive.
Sci Rep, 2021; 11

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AN ASTROCYTIC SIGNALING LOOP FOR FREQUENCY-DEPENDENT CONTROL OF DENDRITIC INTEGRATION AND SPATIAL LEARNING

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

Kirsten Bohmbach[1], Nicola Masala[2], Eva Schönhense[1], Katharina Hill[1], André Haubrich[2], Andreas Zimmer[3], Thoralf Opitz[2], Heinz Beck[2,4], Christian Henneberger[1,4,5]
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Dendrites of hippocampal CA1 pyramidal cells amplify clustered glutamatergic input by activation of voltage-gated sodium channels and N-methyl-D-aspartate receptors (NMDARs). NMDAR activity depends on the presence of NMDAR co-agonists such as D-serine, but how co-agonists influence dendritic integration is not well understood. Using combinations of whole-cell patch clamp, iontophoretic glutamate application, two-photon excitation fluorescence microscopy and glutamate uncaging we found that exogenous D-serine reduces the threshold of dendritic spikes and increases their amplitude. Triggering an astrocytic mechanism controlling endogenous D-serine supply via endocannabinoid receptors (CBRs) also increased dendritic spiking. Unexpectedly, this pathway was activated by pyramidal cell activity primarily in the theta range, which required HCN channels and astrocytic CB1Rs. Therefore, astrocytes close a positive and frequency-dependent feedback loop between pyramidal cell activity and their integration of dendritic input. Its disruption led to an impairment of spatial memory, which demonstrates its behavioral relevance.

Pubmed:
32966786: Herde MK, Bohmbach K, Domingos C, Vana N, Komorowska-Müller JA, Passlick S, Schwarz I, Jackson CJ, Dietrich D, Schwarz MK, Henneberger C
Local Efficacy of Glutamate Uptake Decreases with Synapse Size.
Synaptically released glutamate is largely cleared by glutamate transporters localized on perisynaptic astrocyte processes. Therefore, the substantial variability of astrocyte coverage of individual hippocampal synapses implies that the efficacy of local glutamate uptake and thus the spatial fidelity of synaptic transmission is synapse dependent. By visualization of sub-diffraction-limit perisynaptic astrocytic processes and adjacent postsynaptic spines, we show that, relative to their size, small spines display a stronger coverage by astroglial transporters than bigger neighboring spines. Similarly, glutamate transients evoked by synaptic stimulation are more sensitive to pharmacological inhibition of glutamate uptake at smaller spines, whose high-affinity N-methyl-D-aspartate receptors (NMDARs) are better shielded from remotely released glutamate. At small spines, glutamate-induced and NMDAR-dependent Ca entry is also more strongly increased by uptake inhibition. These findings indicate that spine size inversely correlates with the efficacy of local glutamate uptake and thereby likely determines the probability of synaptic crosstalk.
Cell Rep, 2020; 32
32160550: King CM, Bohmbach K, Minge D, Delekate A, Zheng K, Reynolds J, Rakers C, Zeug A, Petzold GC, Rusakov DA, Henneberger C
Local Resting Ca Controls the Scale of Astroglial Ca Signals.
Astroglia regulate neurovascular coupling while engaging in signal exchange with neurons. The underlying cellular machinery is thought to rely on astrocytic Ca signals, but what controls their amplitude and waveform is poorly understood. Here, we employ time-resolved two-photon excitation fluorescence imaging in acute hippocampal slices and in cortex in vivo to find that resting [Ca] predicts the scale (amplitude) and the maximum (peak) of astroglial Ca elevations. We bidirectionally manipulate resting [Ca] by uncaging intracellular Ca or Ca buffers and use ratiometric imaging of a genetically encoded Ca indicator to establish that alterations in resting [Ca] change co-directionally the peak level and anti-directionally the amplitude of local Ca transients. This relationship holds for spontaneous and for induced (for instance by locomotion) Ca signals. Our findings uncover a basic generic rule of Ca signal formation in astrocytes, thus also associating the resting Ca level with the physiological "excitability" state of astroglia.

Cell Rep, 2020; 30
29434572: van Campen JS, Hessel EVS, Bohmbach K, Rizzi G, Lucassen PJ, Lakshmi Turimella S, Umeoka EHL, Meerhoff GF, Braun KPJ, de Graan PNE, Joëls M
Stress and Corticosteroids Aggravate Morphological Changes in the Dentate Gyrus after Early-Life Experimental Febrile Seizures in Mice.
Stress is the most frequently self-reported seizure precipitant in patients with epilepsy. Moreover, a relation between ear stress and epilepsy has been suggested. Although ear stress and stress hormones are known to influence seizure threshold in rodents, effects on the development of epilepsy (epileptogenesis) are still unclear. Therefore, we studied the consequences of ear corticosteroid exposure for epileptogenesis, under highly controlled conditions in an animal model. Experimental febrile seizures (eFS) were elicited in 10-day-old mice by warm-air induced hyperthermia, while a control group was exposed to a normothermic condition. In the following 2 weeks, mice received either seven corticosterone or vehicle injections or were left undisturbed. Specific measures indicative for epileptogenesis were examined at 25 days of age and compared with vehicle injected or untreated mice. We examined structural [neurogenesis, dendritic morphology, and mossy fiber sprouting (MFS)] and functional (glutamatergic postsynaptic currents and long-term potentiation) plasticity in the dentate gyrus (DG). We found that differences in DG morphology induced by eFS were aggravated by repetitive (mildly stressful) vehicle injections and corticosterone exposure. In the injected groups, eFS were associated with decreases in neurogenesis, and increases in cell proliferation, dendritic length, and spine density. No group differences were found in MFS. Despite these changes in DG morphology, no effects of eFS were found on functional plasticity. We conclude that corticosterone exposure during early epileptogenesis elicited by eFS aggravates morphological, but not functional, changes in the DG, which partly supports the hypothesis that ear stress stimulates epileptogenesis.
Front Endocrinol (Lausanne), 2018; 9
28122264: Bohmbach K, Schwarz MK, Schoch S, Henneberger C
The structural and functional evidence for vesicular release from astrocytes in situ.
The concept of the tripartite synapse states that bi-directional signalling between perisynaptic astrocyte processes, presynaptic axonal boutons and postsynaptic neuronal structures defines the properties of synaptic information processing. Ca-dependent vesicular release from astrocytes, as one of the mechanisms of astrocyte-neuron communication, has attracted particular attention but has also been the subject of intense debate. In neurons, regulated vesicular release is a strongly coordinated process. It requires a complex release machinery comprised of many individual components ranging from vesicular neurotransmitter transporters and soluble NSF attachment protein receptors (SNARE) proteins to Ca-sensors and the proteins that spatially and temporally control exocytosis of synaptic vesicles. If astrocytes employ similar mechanisms to release neurotransmitters is less well understood. The aim of this review is therefore to discuss recent experimental evidence that sheds light on the central structural components responsible for vesicular release from astrocytes in situ.
Brain Res Bull, 2018; 136

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BOARD NUMBER: S05-411

INTERNEURON-SPECIFIC DENDRITIC COMPUTATIONS IN THE NEOCORTEX

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Neurons receive the majority of their excitatory synaptic inputs on dendrites, which can differ in shape, size, and density of active conductances. This variability can differentially affect dendritic integration and hence neuronal computations. While the role of dendrites in computations performed by excitatory neurons has been substantially investigated, much less is known about the influence of dendritic integration in GABAergic interneurons (INs). Here we compared the dendritic integration properties of soma-targeting parvalbumin (PV^+)-positive and dendritic-targeting somatostatin (SST^+)-positive INs in the primary somatosensory cortex of the mouse. Using two-photon glutamate uncaging we observed that PV^+-INs display mostly sublinear subthreshold input-output relationships, in agreement with local synaptic saturation, as reported for this interneuron subtype in other brain areas. However, SST^+-INs dendrites integrate quasi-synchronous inputs supralinearly in a NMDA-dependent manner, similar to observations from excitatory neurons. While both interneurons have relatively similar dendrite diameters, unitary NMDA/AMPA-EPSCs amplitude ratio was nearly doubled in SST^+-INs. Furthermore, monitoring neurotransmitter release using a glutamate sensor (iGluSnFR) at single unitary contacts in both PV^+-INs and SST^+-INs revealed that short trains of stimuli are associated with multivesicular release with imaging trials detecting zero, one or two quantal release events. Surprisingly, we observed that dendrites of SST^+-INs integrated double-quanta linearly all along the dendritic tree while PV^+-IN dendrites exhibited a progressive somato-dendritic attenuation of the single/double-quanta amplitude ratio as expected for a passive cable. In conclusion, our data demonstrate that IN-specific dendritic integration properties further contribute to the already diverse functional differences between PV^+-INs and SST^+-INs in neocortical microcircuits.

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A MORPHOLOGICAL SUBCLASS OF CA1 PYRAMIDAL CELLS RECEIVES SPECIALISED INTERHEMISPHERIC INPUT ONTO ONE BASAL DENDRITE

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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A large fraction of hippocampal pyramidal cells have axons emerging from a basal or, in rare cases, from the apical dendrite rather than from the soma. We have previously shown that such axon-carrying dendrites (AcD) constitute a privileged pathway for action-potential generation. Here, we studied the distribution of morphological features and the input connectivity of pyramidal cells in the adult mouse hippocampus. We found considerable variability between hippocampal sub-regions ranging from 70% AcD-morphology in the central area CA1 of medial portions of the hippocampus to 5% in dorsal CA1. Comparison of dendritic trees showed that AcD branches are longer than nonAcD branches of both, AcD and non AcD cells, while there is no difference in overall basal dendrite length between the two cell types. To assess the functionalrole of AcD-cells we investigated differences of innervation of AcD and nonAcD cells, using optogenetic stimulation of virus-targeted presynaptic fibres combined with patch-clamp recordings. We found that contralateral inputs from CA3 on the basal dendrite were stronger in AcD-cells compared to nonAcD-neurons. Also, axons from CA3 contra have more putative synapses with AcD-cells focused on the AcD. In contrast to basal dendrites, stimulation of inputs to apical dendrites showed no differences between the two cell types. Similarly, inputs from ipsilateral CA3 and CA2 were not different. These data show a functional asymmetry of synaptic connectivity between cells with and without a privileged dendritic input branch. In summary, our findings may be relevant for understanding cell-specific activation of principal cells in the hippocampal network.

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BOARD NUMBER: S05-413

HETEROGENEOUS DENDRITIC CA2+ SPIKE PROPERTIES IN CA3 PYRAMIDAL NEURONS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Regenerative voltage responses produced by dendrites fundamentally influence input-output transformation of cortical pyramidal neurons. Dendritic Ca^{2+} spikes are generally thought to produce slow afterdepolarization (ADP) following action potentials (APs) that evokes complex spike burst (CSB) at the soma. Pyramidal cells (PCs) of the hippocampal CA3 area - a region essential for associative memory - have highly heterogeneous propensity to produce CSBs, but the underlying putative dendritic Ca^{2+} spikes remained incompletely characterized. We combined patch-clamp recordings with two-photon microscopy in acute rat brain slices to elucidate the biophysical properties of dendritic Ca^{2+} spikes in CA3PCs. Somatic depolarization effectively triggered dendritic Ca^{2+} spikes in the presence of tetrodotoxin. We found large cell-to-cell variability in the kinetic properties of somatically recorded Ca^{2+} spikes, depending on the topographic position of neurons. All Ca^{2+} spike forms were eliminated by an L-type Ca^{2+} channel antagonist, while blockers of other Ca^{2+} channel types (T, R, N, P/Q) had no effect. We assessed the roles of several K^+ channels types (Kv1, Kv2, Kv4, Kv7, BK and SK) applying selective inhibitors. Using dendritic recordings in higher-order apical trunks, we identified distinct types of dendritic Ca^{2+} spikes: 1) ADP-type global Ca^{2+} spikes promoting CSBs, and 2) a novel fast Ca^{2+} spike form generated locally in dendritic subtrees that promotes strictly single APs at the soma. Our results point to unique properties of Ca^{2+} spikes in CA3PCs compared to other PC types and suggest an important role for these spikes in input-output transformation of CA3PCs associative memory functions.

Pubmed:
31015414: Raus Balind S, Magó Á, Ahmadi M, Kis N, Varga-Németh Z, Lőrincz A, Makara JK
Diverse synaptic and dendritic mechanisms of complex spike burst generation in hippocampal CA3 pyramidal cells.
Complex spike bursts (CSBs) represent a characteristic firing pattern of hippocampal pyramidal cells (PCs). In CA1PCs, CSBs are driven by regenerative dendritic plateau potentials, produced by correlated entorhinal cortical and CA3 inputs that simultaneously depolarize distal and proximal dendritic domains. However, in CA3PCs neither the generation mechanisms nor the computational role of CSBs are well elucidated. We show that CSBs are induced by dendritic Ca spikes in CA3PCs. Surprisingly, the ability of CA3PCs to produce CSBs is heterogeneous, with non-uniform synaptic input-output transformation rules triggering CSBs. The heterogeneity is partly related to the topographic position of CA3PCs; we identify two ion channel types, HCN and Kv2 channels, whose proximodistal activity gradients contribute to subregion-specific modulation of CSB propensity. Our results suggest that heterogeneous dendritic integrative properties, along with previously reported synaptic connectivity gradients, define functional subpopulations of CA3PCs that may support CA3 network computations underlying associative memory processes.
Nat Commun, 2019; 10
34817378: Magó Á, Kis N, Lükő B, Makara JK
Distinct dendritic Ca spike forms produce opposing input-output transformations in rat CA3 pyramidal cells.
Proper integration of different inputs targeting the dendritic tree of CA3 pyramidal cells (CA3PCs) is critical for associative learning and recall. Dendritic Ca spikes have been proposed to perform associative computations in other PC types by detecting conjunctive activation of different afferent input pathways, initiating afterdepolarization (ADP), and triggering burst firing. Implementation of such operations fundamentally depends on the actual biophysical properties of dendritic Ca spikes; yet little is known about these properties in dendrites of CA3PCs. Using dendritic patch-clamp recordings and two-photon Ca imaging in acute slices from male rats, we report that, unlike CA1PCs, distal apical trunk dendrites of CA3PCs exhibit distinct forms of dendritic Ca spikes. Besides ADP-type global Ca spikes, a majority of dendrites expresses a novel, fast Ca spike type that is initiated locally without bAPs, can recruit additional Na currents, and is compartmentalized to the activated dendritic subtree. Occurrence of the different Ca spike types correlates with dendritic structure, indicating morpho-functional heterogeneity among CA3PCs. Importantly, ADPs and dendritically initiated spikes produce opposing somatic output: bursts

versus strictly single-action potentials, respectively. The uncovered variability of dendritic Ca spikes may underlie heterogeneous input-output transformation and bursting properties of CA3PCs, and might specifically contribute to key associative and non-associative computations performed by the CA3 network.
Elife, 2021; 10

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BOARD NUMBER: S05-414

DIFFERENT INVOLVEMENT OF AXON-CARRYING DENDRITE VERSUS CANONICAL NEURONS DURING LEARNING PROCESSES

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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The hippocampus is important for the formation of declarative memories. It generates distinct network oscillations, during which functional ensembles are specifically activated. The formation of coherently active ensembles requires integration of multiple synaptic inputs within single neurons. According to current understanding, dendritic excitatory synaptic potentials are integrated at the soma which is directly connected with the axon. Signal flow to the axon can be blocked by perisomatic inhibition which is particularly active during network oscillations. Recently, we have shown that in about 50% of hippocampal CA1 pyramidal neurons the axon emerges from a basal dendrite (AcD, ‘axon-carrying dendrite’). This particular dendrite is largely independent from somatic signal integration and can efficiently convert excitatory inputs into APs, even under conditions of strong perisomatic inhibition. We therefore hypothesize that AcD cells are more active during states of strong perisomatic inhibition. Based on this mechanism, AcD and canonical cells might be differently involved in the formation and consolidation of episodic memory. To test this hypothesis, we trained mice on a spatial memory task (m-maze). Active neurons are expected to express immediate early genes (e.g. cFos), and can be identified by ex vivo staining. Additional, staining of the axon initial segment enabled us to sort task-activated neurons into AcD and canonical cells. Interestingly, nonAcD cells have a two times stronger expression of cFos than AcD after 7 days of training. While the underlying mechanisms are presently unclear it does, however indicate distinct roles of AcD and non-AcD cells during formation and consolidation of memory.

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BOARD NUMBER: S05-415

DENDRITIC PROCESSING IMPLEMENTS SPIKE-TIMING DEPENDENT PLASTICITY (STDP) IN CEREBELLAR GOLGI CELLS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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The existence of a precisely organized neuronal circuitry in the cerebellum is crucial for information processing and induction of long-term synaptic plasticity. This latter determines persistent modifications in neuronal activity and synaptic transmission, providing the basis for timing and adaptation in the cerebellum. At the cerebellar input stage, timing is tightly controlled by Golgi cells (GoCs), inhibitory interneurons that process inputs conveyed by parallel fibers (pfs) and mossy fibers (mfs) on their apical and basal dendrites. Although many works highlighted the complexity of GoCs dendritic organization and synaptic inputs, the mechanisms through which GoCs integrate complex input patterns are still unclear. Based on recent computational model predictions, GoCs dendritic processing might depend on spike time intervals between mfs and pfs inputs, implying spike-timing dependent plasticity (STDP) at the mf-GoC synapse as a key plasticity mechanism of GoCs. To verify the validity of model predictions, GoC STDP was experimentally investigated performing whole-cell patch-clamp recordings in acute coronal slices of mouse cerebellar vermis. STDP showed a peculiar temporal order-dependence, with long-term potentiation and long-term depression elicited when mfs spikes anticipated or followed those on pfs, respectively, within a ms-accurate timing interval. In the presence of NMDA receptor inhibitors, STDP is abolished, corroborating the computational suggestions that NMDA channel unblock at mf-GoC synapses is the coincidence detectors of mfs and pfs activity. Overall, this work shows that dendritic processing is instrumental to STDP and opens new perspectives on the role of GoCs in determining learning and plasticity in the cerebellar circuit.

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BOARD NUMBER: S05-416

HUMAN FAST-SPIKING INHIBITORY NEURONS IN THE NEOCORTEX ACCELERATE THEIR INPUT-OUTPUT FUNCTION WITH SOMATIC HCN CHANNELS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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There is increasing evidence showing species-related differences in neurons of the mammalian brain. Yet, our knowledge of neocortical neuron physiology is heavily based on experiments with rodents and other experimental animals. Human-specific neuronal phenotypes are poorly understood, particularly in the neocortex, where the most complex neuronal operations take place. We studied here a common and abundant type of inhibitory neurons, the fast-spiking basket cell in the neocortex. We found that the inhibitory neurons in human characteristically exhibit hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels in their somatic cell membrane, whereas somatic HCN channels are virtually absent in their rodent neuronal counterparts. With the HCN channels in soma, human fast-spiking inhibitory neurons facilitate their input-output fidelity, accelerate their somatic membrane potential kinetics, and shorten the action potential initiation lag for excitation in soma. With help of computational modeling we demonstrate that somatic HCN channels are well-fitted to facilitate these functions in human fast-spiking neurons. The facilitatory somatic mechanism may have evolved in human inhibitory neurons to accelerate their relatively slow passive membrane potential kinetics which apparently arises from low persistent membrane leakiness compared to their rodent counterparts. With help of somatic HCN channels human fast-spiking cells reach somatic input-output rate similar to their cell type equivalent in rodent.

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PARADOXICAL SOMATO-DENDRITIC DECOUPLING SUPPORTS CORTICAL PLASTICITY DURING REM SLEEP

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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REM sleep is associated with the consolidation of emotional memories encoded by neuronal circuits from the limbic system and prefrontal cortex in mammals. Yet, the underlying neocortical circuits and synaptic mechanisms remain unclear. Here, we found that REM sleep is associated with a somato-dendritic decoupling in pyramidal neurons of the prefrontal cortex, using simultaneous 2-photon calcium imaging and electrophysiological recordings in sleeping mice. This decoupling reflects a shift of inhibitory balance between PV neurons-mediated somatic inhibition and VIP-mediated dendritic disinhibition, mostly driven by neurons from the central medial thalamus. We further showed that REM-specific optogenetic suppression of dendritic activity led to a loss of danger versus safety discrimination during associative learning and a lack of synaptic plasticity, whereas optogenetic release of somatic inhibition resulted in enhanced discrimination and synaptic potentiation. Collectively, our results demonstrated that somato-dendritic decoupling during REM sleep promotes opposite synaptic plasticity mechanisms that optimize emotional response to future behavioral stressors.

Pubmed:
33252331: Aime M, Augusto E, Kouskoff V, Campelo T, Martin C, Humeau Y, Chenouard N, Gambino F
The integration of Gaussian noise by long-range amygdala inputs in frontal circuit promotes fear learning in mice.
Survival depends on the ability of animals to select the appropriate behavior in response to threat and safety sensory cues. However, the synaptic and circuit mechanisms by which the brain learns to encode accurate predictors of threat and safety remain largely unexplored. Here, we show that frontal association cortex (FrA) pyramidal neurons of mice integrate auditory cues and basolateral amygdala (BLA) inputs non-linearly in a NMDAR-dependent manner. We found that the response of FrA pyramidal neurons was more pronounced to Gaussian noise than to pure frequency tones, and that the activation of BLA-to-FrA axons was the strongest in between conditioning pairings. Blocking BLA-to-FrA signaling specifically at the time of presentation of Gaussian noise (but not 8 kHz tone) between conditioning trials impaired the formation of auditory fear memories. Taken together, our data reveal a circuit mechanism that facilitates the formation of fear traces in the FrA, thus providing a new framework for probing discriminative learning and related disorders.
Elife, 2020; 9
29030432: Zhang CL, Aime M, Laheranne E, Houbaert X, El Oussini H, Martin C, Lepleux M, Normand E, Chelly J, Herzog E, Billuart P, Humeau Y
Protein Kinase A Deregulation in the Medial Prefrontal Cortex Impairs Working Memory in Murine Oligophrenin-1 Deficiency.
Classical and systems genetics have identified wide networks of genes associated with cognitive and neurodevelopmental diseases. In parallel to deciphering the role of each of these genes in neuronal or synaptic function, evaluating the response of neuronal and molecular networks to gene loss of function could reveal some pathophysiological mechanisms potentially accessible to nongenetic therapies. Loss of function of the Rho-GAP oligophrenin-1 is associated with cognitive impairments in both human and mouse. Upregulation of both PKA and ROCK has been reported in mice, but it remains unclear whether kinase hyperactivity contributes to the behavioral phenotypes. In this study, we thoroughly characterized a prominent perseveration phenotype displayed by -deficient mice using a Y-maze spatial working memory (SWM) test. We report that deficiency in the mouse generated severe cognitive impairments, characterized by both a high occurrence of perseverative behaviors and a lack of deliberation during the SWM test. and pharmacological experiments suggest that PKA dysregulation in the mPFC underlies cognitive dysfunction in -deficient mice, as assessed using a delayed spatial alternation task results. Functionally, mPFC neuronal networks appeared to be affected in a PKA-dependent manner, whereas hippocampal-PFC projections involved in SWM were not affected in mice. Thus, we propose that discrete gene mutations in intellectual disability might generate "secondary" pathophysiological mechanisms, which are prone to become pharmacological targets for curative strategies in adult patients. Here we report that deficiency generates severe impairments in performance at spatial working

memory tests, characterized by a high occurrence of perseverative behaviors and a lack of decision making. This cognitive deficit is consecutive to PKA deregulation in the mPFC that prevents KO mice to exploit a correctly acquired rule. Functionally, mPFC neuronal networks appear to be affected in a PKA-dependent manner, whereas behaviorally important hippocampal projections were preserved by the mutation. Thus, we propose that discrete gene mutations in intellectual disability can generate "secondary" pathophysiological mechanisms prone to become pharmacological targets for curative strategies in adults.
J Neurosci, 2017; 37

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BOARD NUMBER: S05-418

UNDERSTANDING HOW INTERNEURONS IN THE MEDIAL PREFRONTAL CORTEX MODULATE ASSOCIATIVE RECOGNITION MEMORY

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Associative recognition memory, such as recognising your misplaced house key or finding your car in a carpark, requires the integration of familiarity, spatial location and temporal information that is vital for everyday learning and episodic experience. The medial prefrontal cortex (mPFC) is the central processing unit within the associative recognition memory circuit as it receives long range excitatory inputs from the nucleus reuniens (NRe) and intermediate hippocampus (iHPC), both pathways being critical for learning. It is well known that inhibitory interneurons play essential roles in memory function by balancing excitation and modulating mPFC microcircuits. However, the roles mPFC interneurons have in associative recognition memory and its networks are unknown. Combining AAV1 mediated anterograde tracing, slice electrophysiology and *in vivo* wireless optogenetics, this project investigates; if different interneurons, pavalbumin (PV), somatostatin (SOM), neuron derived neurotrophic factor (NDNF) in mPFC are involved in different phases of recognition memory, the anatomical distribution and possible convergence of afferent excitatory projections from the iHPC and NRe and if these projections differentially engage interneuron subtypes, modulating mPFC network activity. Here we show iHPC and NRe projections have distinct laminar distribution within the prelimbic cortex aswell as layer 1 (L1) targeting of NRe inputs that receive specific iHPC monosynaptic projections. iHPC and NRe inputs differentially recruit PV interneurons and have opposing short term plasticity at L1 NDNF interneurons. SOM electrophysiology and *in vivo* work are ongoing. This work provides an important foundation for understanding the complex role of mPFC interneurons in the circuit function of associative recognition memory.

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BOARD NUMBER: S05-419

INCREASED EXCITABILITY OF PARVALBUMIN-POSITIVE INTERNEURONS IN PREMOTOR CORTICAL AREA IN A MOUSE MODEL OF OBSESSIVE-COMPULSIVE DISORDER

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Obsessive Compulsive Disorder (OCD) is a severe, chronic, and ubiquitous neuropsychiatric disorder that affects 2-3% of worldwide population. Corticostriatal dysfunction is considered a major factor in the pathogenesis of OCD.
In *Sapap3* knockout (KO) mouse, a well-validated model of compulsive behavior, it has been reported that the striatal region receives increased levels of synaptic input from the secondary motor area (M2). M2 is thought to be homologous to the Supplementary Motor Area in humans, an area showing hyperactivity in OCD patients. Cortical disinhibition in OCD patients due to GABAergic deficits has been proposed to be related to its pathogenesis. Therefore, we study, using a combination of *in vitro* and *in vivo* 2-photon experiments, the cortical GABAergic circuitry involving PV^+ interneurons in layer 2/3 of the premotor area (M2). We have developed a *Sapap3* null mouse line that expresses td-Tomato under the control of the PV promoter. The preliminary results show a decrease in the number of PV^+ interneurons in M2. Furthermore, using electrophysiological recording in brain slice preparation, PV+ interneurons show increased input resistance, decreased rheobase and increased firing frequency gain. All together indicate that PV^+ interneurons are hyperexcitable. *In vivo* calcium imaging experiments in awake animals are in progress to confirm the hyperexcitability of PV^+ neurons, and how the excitatory network is affected. We are grateful to C. Cabrera Romero for excellent technical assistance. Supported by: RyC-2016-19906, PRE2019-087729 (MCI/AEI/FSE, UE), VI PPIT-US and US-1264432 (US/JUNTA/FEDER, UE).

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BOARD NUMBER: S05-420

CHARACTERIZATION OF A HABENULA-DRIVEN SEROTONERGIC RECURRENT INHIBITORY NETWORK IN DORSAL RAPHE NUCLEUS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

Michael Lynn[1], Sean Geddes[1], Mohamad Chahrour[1], Sebastien Maillé[1], Emerson Harkin[1], Érik Harvey-Girard[1], Samir Haj-Dahmane[2], Richard Naud[1], Jean-Claude Béïque[1]
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Monoaminergic systems are phylogenetically old hub networks which receive a diverse constellation of long-range synaptic inputs, yet the local processing rules that govern computations over these inputs are poorly understood. Here, we used optogenetic, electrophysiological, computational and behavioral strategies to analyze information transmission from the habenula (LHb) to 5-HT neurons in the dorsal raphe nucleus (DRN). Habenulo-raphe afferents to 5-HT neurons triggered both short-timescale monosynaptic excitation, and inhibition over hundreds of milliseconds which reflected activation of 5-HT1A receptors. Optogenetic and pharmacological manipulations in DRN revealed that 5-HT neurons are organized in a recurrent inhibitory network, refuting the classical model of autocrine activation of 5-HT1ARs. We quantified short-term dynamics at serotonin-serotonin connections with electrical stimulation approaches, revealing dramatic short-term facilitation which was formalized with a linear-nonlinear plasticity model. Next, we outline general principles of information transmission through this circuit with network modeling and targeted slice experiments, including an inversion of response sign at high input intensities, and winner-take-all competition between submodules in DRN. We tested key predictions of our model by combining a classical conditioning paradigm with optogenetic strategies, uncovering a nonlinear transformation whereby stimulating the habenulo-raphe pathway at high frequencies, but not at low frequencies, depressed goal-directed anticipatory licking behavior. We suggest that the computation sustained by this circuit motif in the DRN categorizes synaptic inputs to implement optimal adaption of behavioural policies in dynamic environments.

Pubmed:
33377070: Lynn M, Naud R, Béïque JC
Accurate Silent Synapse Estimation from Simulator-Corrected Electrophysiological Data Using the SilentMLE Python Package.
The proportion of silent (AMPAR-lacking) synapses is thought to be related to the plasticity potential of neural networks. We created a maximum-likelihood estimator of silent synapse fraction based on simulations of the underlying experimental methodology. Here, we provide a set of guidelines for running a Python package on compatible experimental synaptic data. Compared with traditional failure-rate approaches, this synthetic likelihood estimator improves the validity and accuracy of the estimates of the silent synapse fraction. For complete details on the use and execution of this protocol, please refer to Lynn et al. (2020).
STAR Protoc, 2020; 1
32697998: Lynn MB, Lee KFH, Soares C, Naud R, Béïque JC
A Synthetic Likelihood Solution to the Silent Synapse Estimation Problem.
Functional features of synaptic populations are typically inferred from random electrophysiological sampling of small subsets of synapses. Are these samples unbiased? Here, we develop a biophysically constrained statistical framework to address this question and apply it to assess the performance of a widely used method based on a failure-rate analysis to quantify the occurrence of silent (AMPAR-lacking) synapses. We simulate this method in silico and find that it is characterized by strong and systematic biases, poor reliability, and weak statistical power. Key conclusions are validated by whole-cell recordings from hippocampal neurons. To address these shortcomings, we develop a simulator of the experimental protocol and use it to compute a synthetic likelihood. By maximizing the likelihood, we infer silent synapse fraction with no bias, low variance, and superior statistical power over alternatives. Together, this generalizable approach highlights how a simulator of experimental methodologies can substantially improve the estimation of physiological properties.
Cell Rep, 2020; 32
32132221: Maillé S, Lynn M

Reconciling Current Theories of Consciousness.
J Neurosci, 2020; 40
30143180: Vincent-Lamarre P, Lynn M, Béïque JC
The Eloquent Silent Synapse.
The ability of central synapses to undergo long-term potentiation (LTP) still captures the imagination of scientists and has become one of the most fascinating and deeply studied questions in modern neuroscience. By the mid-1990s, however, the field was deeply ensnarled in trying to answer a passionately dichotomous question: is LTP expressed by a pre- or a postsynaptic mechanism? Experimental results that could only be seen by many as being incontrovertibly contradictory presented a perplexing conundrum. However, two papers published in 1995 fundamentally redefined critical assumptions and provided a cunningly simple and elegant solution to an otherwise inextricable impasse.
Trends Neurosci, 2018; 41
28100732: Lynn M, Maillé S
Acute Stress Shapes Synaptic Inhibition within an Amygdala Microcircuit.
J Neurosci, 2017; 37

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-421

ASSESSING FUNCTIONAL PROPERTIES OF PREFRONTAL NETWORKS USING OPTOGENETICS AND PHARMACOLOGY IN LARGE-SCALE MULTI-ELECTRODE ARRAY RECORDINGS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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The ability of brain regions to regulate information processing stems from an intricate and precisely organized network architecture. In the prefrontal cortex (PFC), a meticulously coordinated mosaic of cell types contributes to the regulation of a broad array of executive functions. **Aim:** Here, we detailed how the interaction of excitatory (E) and inhibitory (I) neurons contribute to the activity of broader cortical circuits, allowing for an elaborate repertoire of computations to arise. **Methods:** To examine the contribution of different neuronal subtypes to cortical dynamics, we combined electrophysiology, viral strategies, optogenetics, and pharmacology while recording spiking activity in acute slices of PFC on high-density multi-electrode arrays containing 4096 closely spaced electrodes. **Results:** To parse out the contribution of distinct cell types, we first developed spike sorting techniques that combined spline interpolation and principal component analysis to distinguish regular-spiking excitatory neurons from fast-spiking inhibitory interneurons. We validated this cell-type classification using a targeted combination of viral and optogenetic strategies to selectively activate parvalbumin (PV) interneurons. Next, using a sequential pharmacological approach, we systematically tested the contribution of each connection type to the activity patterns of each cell type. In intact networks, activating parvalbumin (PV) neurons had no effect on their firing rate, indicating complex compensatory mechanisms in the network. However, with GABAergic and glutamatergic transmission blocked, activating PV neurons reliably increased their firing rate. **Conclusion:** Together, these results provide insights on how complex connectivity motifs can affect respective E and I cell population activity.

Pubmed:
27881719: Berberian N, MacPherson A, Giraud E, Richardson L, Thivierge JP
Neuronal pattern separation of motion-relevant input in LIP activity.
In various regions of the brain, neurons discriminate sensory stimuli by decreasing the similarity between ambiguous input patterns. Here, we examine whether this process of pattern separation may drive the rapid discrimination of visual motion stimuli in the lateral intraparietal area (LIP). Starting with a simple mean-rate population model that captures neuronal activity in LIP, we show that overlapping input patterns can be reformatted dynamically to give rise to separated patterns of neuronal activity. The population model predicts that a key ingredient of pattern separation is the presence of heterogeneity in the response of individual units. Furthermore, the model proposes that pattern separation relies on heterogeneity in the temporal dynamics of neural activity and not merely in the mean firing rates of individual neurons over time. We confirm these predictions in recordings of macaque LIP neurons and show that the accuracy of pattern separation is a strong predictor of behavioral performance. Overall, results propose that LIP relies on neuronal pattern separation to facilitate decision-relevant discrimination of sensory stimuli. A new hypothesis is proposed on the role of the lateral intraparietal (LIP) region of cortex during rapid decision making. This hypothesis suggests that LIP alters the representation of ambiguous inputs to reduce their overlap, thus improving sensory discrimination. A combination of computational modeling, theoretical analysis, and electrophysiological data shows that the pattern separation hypothesis links neural activity to behavior and offers novel predictions on the role of LIP during sensory discrimination.
J Neurophysiol, 2017; 117

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-422

INTEGRATION OF MEDIAL AND LATERAL ENTORHINAL CORTEX IN HIPPOCAMPAL AREA CA3

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Distinct brain areas encode different sensory features of the environment and route this information to the hippocampus (HC) where integration can lead to complex representations. However, what are the cellular and circuit mechanisms underlying the association of multisensory information within the brain to permit memory formation and recall remains an open question. To answer this, I am examining how different cortical inputs from medial (MEC) and lateral entorhinal cortex (LEC), carrying spatial and non-spatial sensory information respectively, integrate in single neurons in area CA3 of the hippocampus. CA3 plays a pivotal role in encoding and recall of episodic memory, likely due to its ability to form and reactivate ensembles of neurons. Identifying the circuit interactions driving input-output transformations and network dynamics within CA3 is crucial for understanding the neural computations underlying memory formation and recall. The longstanding dogma in field assumes that the association of information about place and context provided by MEC and LEC is key to forming accurate, complete and long-lasting hippocampal representations. However, this still remains mostly theoretical due to technical limitations. Using slice electrophysiology, dual-color optogenetic stimulation, and pharmacology we have shown that LEC and MEC do converge on to a single CA3 pyramidal neuron. Findings from our study will reveal how this underlying connectivity between these two sensory cortical inputs and their integration of and CA3 PNs drive the input/output transformations and associational plasticity.

Pubmed:
31706697: Hwang EJ, Link TD, Hu YY, Lu S, Wang EH, Lilascharoen V, Aronson S, O'Neil K, Lim BK, Komiyama T
Corticostriatal Flow of Action Selection Bias.
The posterior parietal cortex (PPC) performs many functions, including decision making and movement control. It remains unknown which input and output pathways of PPC support different functions. We addressed this issue in mice, focusing on PPC neurons projecting to the dorsal striatum (PPC-STR) and the posterior secondary motor cortex (PPC-pM2). Projection-specific, retrograde labeling showed that PPC-STR and PPC-pM2 represent largely distinct subpopulations, with PPC-STR receiving stronger inputs from association areas and PPC-pM2 receiving stronger sensorimotor inputs. Two-photon calcium imaging during decision making revealed that the PPC-STR population encodes history-dependent choice bias more strongly than PPC-pM2 or general PPC populations. Furthermore, optogenetic inactivation of PPC-STR neurons or their terminals in STR decreased history-dependent bias, while inactivation of PPC-pM2 neurons altered movement kinematics. Therefore, PPC biases action selection through its STR projection while controlling movements through PPC-pM2 neurons. PPC may support multiple functions through parallel subpopulations, each with distinct input-output connectivity.
Neuron, 2019; 104
29976229: Chute C, Yang X, Meyer K, Yang N, O'Neil K, Kasza I, Eliceiri K, Alexander C, Friedl A
Syndecan-1 induction in lung microenvironment supports the establishment of breast tumor metastases.
Syndecan-1 (Sdc1), a cell surface heparan sulfate proteoglycan normally expressed primarily by epithelia and plasma cells, is aberrantly induced in stromal fibroblasts of breast carcinomas. Stromal fibroblast-derived Sdc1 participates in paracrine growth stimulation of breast carcinoma cells and orchestrates stromal extracellular matrix fiber alignment, thereby creating a migration and invasion-permissive microenvironment. Here, we specifically tested the role of stromal Sdc1 in metastasis.
Breast Cancer Res, 2018; 20
28671694: Peters AJ, Lee J, Hedrick NG, O'Neil K, Komiyama T
Reorganization of corticospinal output during motor learning.
Motor learning is accompanied by widespread changes within the motor cortex, but it is unknown whether these changes are ultimately funneled through a stable corticospinal output channel or whether the corticospinal output itself is plastic. We investigated the consistency of the relationship between corticospinal neuron activity and movement through in vivo two-photon calcium imaging in mice learning a lever-press task. Corticospinal neurons exhibited heterogeneous correlations with movement, with the majority of movement-modulated neurons decreasing activity during movement. Individual cells changed

their activity across days, which led to changed associations between corticospinal activity and movement. Unlike previous observations in layer 2/3, activity accompanying learned movements did not become more consistent with learning; instead, the activity of dissimilar movements became more decorrelated. These results indicate that the relationship between corticospinal activity and movement is dynamic and that the types of activity and plasticity are different from and possibly complementary to those in layer 2/3.
Nat Neurosci, 2017; 20

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BOARD NUMBER: S05-423

PARVALBUMIN-EXPRESSING INHIBITORY NEURONS AT THE MEDIODORSAL NUCLEUS OF THE THALAMUS INHIBIT THE PROLONGED FRONTO-THALAMOCORTICAL ACTIVITY LOOP.

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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The mediodorsal nucleus of the thalamus (MD) forms reciprocal connectivity with the medial prefrontal cortex (mPFC), which has been demonstrated to support the persistent activity in the mPFC observed in short-term memory maintenance. The thalamic reticular nucleus (TRN) neurons have been suggested to be the primary inhibitory input on the MD. Although there are inhibitory interneurons at the MD (MD_{IN}), it hasn't been studied thoroughly. Here, we examined the local inhibition in MD and the possible function of MD_{IN}, with a focus on the regulation of the frontothalamic activity loop using histological, electrophysiological, and behavioral analysis. The MD_{IN}, the majority of which expressed parvalbumin (PV), were found throughout the MD with a slight bias toward the lateral part of the MD. Their dendrites were aspiny and beaded similarly with the dendro-dendritic connections in the inhibitory neurons in sensory thalamic nuclei. MD_{IN} formed inhibitory, ionotropic GABAergic synapses on the local excitatory neurons (MD_{EX}). MD_{IN} received direct excitatory inputs from the mPFC with strong short-term synaptic facilitation. The high-frequency stimulation of mPFC axons, imitating the persistent neural activity during short-term memory, enhanced the chance of action potential evoked in MD_{IN}. In corroboration, inhibitory synaptic inputs were significantly increased by the trains of mPFC activity in MD_{EX}, without TRN inputs. Furthermore, we found that the increased firing of MD_{IN} significantly disturbed the maintenance of short-term memory, suggesting that local inhibition through MD_{IN} may play a regulatory role in the MD-mPFC activity loop by inhibiting prolonged high-frequency frontothalamic excitation transfer during the short-term memory.

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BOARD NUMBER: S05-424

HOMEOSTATIC REGULATION OF CA1 FIRING RATE SET POINTS IN RESPONSE TO CHRONIC HYPERACTIVATION OF INTERNEURONS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Homeostatic mechanisms stabilize activity of neural circuits by keeping firing rates in a given circuitry within a set-point range. Accumulated evidence suggests that mean firing rate (MFR) represents a physiological variable regulated by homeostatic systems. While all the studies *in vivo* have been conducted in primary visual cortex, whether MFRs in the hippocampus are homeostatically regulated and whether this regulation is gated by specific brain states remain unknown. To address this, we applied long-term single-unit recordings in freely behaving mice, along with chronic chemogenetic perturbations of specific neuronal populations – by expressing designer drug (Gq-DREADD) in all inhibitory interneuron types of the CA1 region of the hippocampus. After applying constant dosage of clozapine N-oxide (CNO) via osmotic pump for five days led to an immediate suppression of CA1 pyramidal cells activity that gradually recover after 3 days, returning MFR to its original set point, indicating that MFR and network patterns properties of pyramidal cells in the CA1 area is found under homeostatic regulation. In addition, we analyzed whether the pattern of spikes is also homeostatically regulated and whether homeostatic plasticity is gated by a specific vigilance state. This research may provide new insights on how hippocampal activity set points are regulated in behaving mice and whether homeostatic system is capable to compensate chronic hyperexcitability of interneurons.

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BOARD NUMBER: S05-425

ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION OF TWO TYPES OF VIP-EXPRESSING INTERNEURONS IN THE PRESUBICULUM AND THEIR FACILITATING RESPONSES FOLLOWING OPTOGENETIC STIMULATION OF ANTERIOR THALAMIC FIBERS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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The presubiculum is part of the parahippocampal cortex and plays a fundamental role for orientation in space. Many principal neurons of the presubiculum signal head direction, and show persistent firing when the head of an animal is oriented in a specific preferred direction. GABAergic neurons of the presubiculum seem likely to control the timing, sensitivity and selectivity of head directional signals. Head direction signals reach the presubiculum via the anterior thalamic nuclei (ATN). Their axons directly excite pyramidal cells in layer III, and also Parvalbumin expressing interneurons, which mediate feedforward inhibition. Presubicular Somatostatin interneurons are indirectly excited and mediate a delayed feed-back inhibition. The role of Vasoactive intestinal peptide (VIP) expressing interneurons in the presubicular microcircuit has not yet been addressed. Here, we examined the intrinsic properties of VIP interneurons as well as their input connectivity following photostimulation of ATN axons. Interneurons were recorded from VIPcre::tdTomato brain slices using the patch-clamp technique. Our results show that presubicular VIP interneurons were mostly distributed in superficial layers. Two distinct physiological groups of presubicular VIP interneurons emerged from the unsupervised cluster analysis performed on the electrophysiological parameters. The expression of AAV5-Chronos viral vectors in the ATN of VIP::tdTomato mice let us examine thalamic input connectivity onto VIP interneurons. They were recruited in an activity dependent manner and with facilitating dynamics. Our data provide initial insight into the contribution of VIP interneurons for the integration of thalamic head direction information in the presubiculum.

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BOARD NUMBER: S05-426

PREFRONTAL DISINHIBITION DISRUPTS REVERSAL PERFORMANCE

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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A reduction in prefrontal GABAergic inhibition, so-called neural disinhibition, is a key feature of schizophrenia. Additionally, schizophrenia is characterised by marked reversal learning deficits (Leeson et al.,2009,*BiolPsychiatry*). Reversal learning has mainly been found to require the orbitofrontal, but not prefrontal, cortex (Boulougouris et al.,2007,*BehavBrainRes).* However, we hypothesised that prefrontal disinhibition may impair reversal performance, because such disinhibition causes aberrant prefrontal neuron firing and may, thus, also disrupt processing in projections sites (Bast et al.,2017,*BrJPharmacol*), including the orbitofrontal cortex (Sesack et al.,1989,*JCompNeurol*). We manipulated prefrontal GABAergic activity in rats using local microinfusions of a GABA-A receptor agonist (muscimol), resulting in functional inhibition, and antagonist (picrotoxin), resulting in disinhibition (Pezze et al.,2014,*JNeurosci*). Reversal learning performance was assessed using an operant two-lever repeated reversal paradigm adapted from Brady & Floresco (2015,*JVisExp*). Beyond classical performance measures, including trials-to-criterion and %-correct, we used a Bayesian approach to assess trial-by-trial strategy probabilities to assess how prefrontal GABA manipulations affect the implementation of successful strategies underlying reversal performance. Prefrontal disinhibition, but not inhibition, markedly impaired repeated reversal performance. Although prefrontal disinhibition reduced expression of the old rule, rats with prefrontal disinhibition failed to reduce their adherence to the old rule and to shift to the new rule following reversal. Trial-by-trial strategy analysis suggested that prefrontal disinhibition disrupted the pursuit of strategies supporting reversal performance, including a lose-shift strategy. Overall, although reversal performance does not require prefrontal neural activity (i.e., is unaffected by muscimol inhibition), aberrant prefrontal activity due to disinhibition disrupts reversal performance.

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BOARD NUMBER: S05-427

EXCITATORY ACTION OF GABA/GLYCINE SYNAPTIC ACTIVITY IS FAVORED IN PRENATAL SOD1G93A MOTONEURONS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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We have previously shown that prenatal spinal motoneurons (MNs) of the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis are hyperexcitable and exhibit an altered chloride homeostasis with a more depolarized chloride equilibrium (E_{Cl}). Here, we aimed to verify whether low frequency depolarizing GABAergic/glycinergic postsynaptic potentials (dGPSPs) exert excitation in SOD1^{G93A} MNs before switching to inhibition at high frequency. Such dual effect was evidenced using simulation from SOD-like MNs (Branchereau et al. eLife 2019). E_{Cl} was set below spike threshold and electrical stimulations of the ventro-lateral funiculus were performed at different frequency in order to test the ability of dGPSPs to excite or inhibit the firing activity of E17.5 lumbar spinal MNs from the lateral motor column. Dual effect was more often, but not only, detected in SOD1^{G93A} MNs. WT MNs were classified into two clusters (dual effect or pure inhibition) according to Rm, dual responses being specific to high Rm and pure inhibition to low Rm. This was not the case in SOD1^{G93A} MNs that could express dual, pure inhibition but also pure excitation, whatever Rm value. Simulation showed that pure excitation could be obtained in SOD-like MNs by moving away the inhibitory input from the cell body to dendrites. MNs reconstructions highlighted a distinct morphology between the two WT clusters and VIAAT terminals density / size were smaller in SOD1^{G93A} MNs compared to WT. In conclusion, low density and location of GABA/glycine synaptic inputs, in addition to morphological changes, favor excitatory effects of dGPSP trains in E17.5 SOD1^{G93A} MNs.

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BOARD NUMBER: S05-428

SILENT SYNAPSES IN THE ADULT MOUSE NEOCORTEX

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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Newly generated excitatory synapses in the mammalian cortex lack sufficient AMPA-type glutamate receptors to mediate neurotransmission, resulting in functionally silent synapses that require activity-dependent plasticity to mature. Silent synapses are abundant in early development, where they mediate circuit formation and refinement, but they are thought to be scarce in adulthood. However, adults retain a capacity for neural plasticity and flexible learning that suggests that the formation of new connections is still prevalent. Here, we used super-resolution proteomic imaging to visualize synaptic proteins at 2,234 synapses from layer 5 pyramidal neurons in the primary visual cortex of adult mice. Surprisingly, ~25% of these synapses lack AMPA receptors. These putative silent synapses were located at the tips of thin dendritic protrusions, known as filopodia, which were more abundant by an order of magnitude than previously believed (compromising ~30% of all dendritic protrusions). Physiological experiments revealed that filopodia do indeed lack AMPAR-mediated transmission, but they exhibit NMDAR mediated transmission. We further show that functionally silent synapses on filopodia can be unsilenced via Hebbian plasticity, recruiting new active connections into
a neuron’s input matrix. These results challenge the model that functional connectivity is largely fixed in the adult cortex and demonstrate a new mechanism for flexible control of synaptic wiring that expands the learning capabilities of the mature brain.

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BOARD NUMBER: S05-429

EXCITATORY DRIVE OF CORTICAL FAST-SPIKING GABAERGIC INTERNEURONS IS SET BY D-SERINE ACTING ON NMDA RECEPTORS

POSTER SESSION 05 - SECTION: INTERNEURONS AND DENDRITES

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N-methyl-D-aspartate receptors (NMDARs) populate GABAergic interneurons, where they play a critical role in shaping circuit motifs and memory. However, we are largely ignoring whether and how NMDARs at GABAergic interneurons are gated by signals released in their surrounding microenvironment. Here we explore the dynamics of the co-agonist site occupancy by D-serine and glycine at glutamatergic synapses onto parvalbumin positive GABAergic interneurons in the adolescent prefrontal cortex, an area central to complex cognitive operation. By combining cellular electrophysiology with the use of unique pharmacological interventions and genetic manipulations, we report that the firing activity of layer 5 fast-spiking-PV$^+$ interneurons and their excitatory synaptic coupling with principal neurons is under the control of NMDA receptors which are gated by D-serine but not glycine and that the identity of the co-agonist is not determined by the synaptic regime of the excitatory input. We further show that D-serine-deficient mice, a model of NMDAR hypofunction that exhibits schizophrenia-like phenotypes display attenuated firing pattern of the interneurons and no long-term potentiation. Our study extends the physiological implications of D-serine in brain physiopathology by uncovering its control of inhibitory synaptic networks through NMDARs.

FENS Forum 2022
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BOARD NUMBER: S05-430

REGION SELECTIVE INPUT FROM THE FRONTAL CORTEX MEDIATES INSTANTANEOUS CORRELATION BETWEEN CORTICAL ACTIVITY AND INTRATHALAMIC INHIBITION

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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It has long been known that the neocortex is organized into areas with different cognitive functions. Although emerging data shows that the thalamus is involved in basically all cortical functions, including higher order cognitive processes, we only start to address if and how corticothalamic interactions are regionally specialized. Here we report a qualitative, region-specific difference in the organization of corticothalamic pathways. Using conditional viral tracing and *in vivo* electrophysiological experiments in the layer 5 (L5)-specific Rbp4-Cre and Thy1-Chr2-EYFP mouse lines we show that L5 pyramidal cells of the frontal, but not other cortical regions establish monosynaptic connection with the anterior part of the inhibitory thalamic reticular nucleus (TRN). Optogenetic activation of L5 cells elicited bursts in anterior TRN cells with short latency and high fidelity. Gradually increasing the number of the optogenetically activated L5 cells resulted in gradual change of TRN burst properties including increase in the intraburst frequencies and the number of spikes per burst. Similarly, spontaneous firing pattern of L5-innervated TRN neurons showed tight and gradual correlation with the ongoing frontal cortical activity. While single spike events showed low correlation burst firing was tightly coupled to the frontal population activity. Higher frequency burst with more spikes were correlated with faster, high amplitude cortical LFP events. This correlation disappeared upon optogenetic perturbation of the L5-TRN connection. The data suggest a powerful, region-specific and temporally precise cortical control in frontal cortico-thalamic circuits via the strong coupling of intrathalamic inhibition to the ongoing cortical oscillations.

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BOARD NUMBER: S05-431

DEVELOPMENTAL INCREASE OF INHIBITION DRIVES DECORRELATION OF NEURAL ACTIVITY

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Throughout development, the brain transits from early highly synchronous activity patterns to a mature state with sparse and decorrelated neural activity, yet the mechanisms underlying this process are unknown. The developmental transition has important functional consequences, as the latter state allows for more efficient storage, retrieval and processing of information. Here, we show that, in the mouse medial prefrontal cortex (mPFC), neural activity during the first two postnatal weeks decorrelates following specific spatial patterns. This process is accompanied by a concomitant tilting of excitation/inhibition (E-I) ratio towards inhibition. Using optogenetic manipulations and neural network modeling, we show that the two phenomena are mechanistically linked, and that a relative increase of inhibition drives the decorrelation of neural activity. Accordingly, in two mouse models of neurodevelopmental disorders, subtle alterations in E-I ratio are associated with specific impairments in the correlational structure of spike trains. Finally, capitalizing on EEG data from newborn babies, we show that an analogous developmental transition takes place also in the human brain. Thus, changes in E-I ratio control the (de)correlation of neural activity and, by these means, its developmental imbalance might contribute to the pathogenesis of neurodevelopmental disorders.

Pubmed:
33675685: Bitzenhofer SH, Pöpplau JA, Chini M, Marquardt A, Hanganu-Opatz IL
A transient developmental increase in prefrontal activity alters network maturation and causes cognitive dysfunction in adult mice.
Disturbed neuronal activity in neuropsychiatric pathologies emerges during development and might cause multifold neuronal dysfunction by interfering with apoptosis, dendritic growth, and synapse formation. However, how altered electrical activity early in life affects neuronal function and behavior in adults is unknown. Here, we address this question by transiently increasing the coordinated activity of layer 2/3 pyramidal neurons in the medial prefrontal cortex of neonatal mice and monitoring long-term functional and behavioral consequences. We show that increased activity during early development causes premature maturation of pyramidal neurons and affects interneuronal density. Consequently, altered inhibitory feedback by fast-spiking interneurons and excitation/inhibition imbalance in prefrontal circuits of young adults result in weaker evoked synchronization of gamma frequency. These structural and functional changes ultimately lead to poorer mnemonic and social abilities. Thus, prefrontal activity during early development actively controls the cognitive performance of adults and might be critical for cognitive symptoms in neuropsychiatric diseases.
Neuron, 2021; 109
33793545: Yang W, Chini M, Pöpplau JA, Formozov A, Dieter A, Piechocinski P, Rais C, Morellini F, Sporns O, Hanganu-Opatz IL, Wiegert JS
Anesthetics fragment hippocampal network activity, alter spine dynamics, and affect memory consolidation.
General anesthesia is characterized by reversible loss of consciousness accompanied by transient amnesia. Yet, long-term memory impairment is an undesirable side effect. How different types of general anesthetics (GAs) affect the hippocampus, a brain region central to memory formation and consolidation, is poorly understood. Using extracellular recordings, chronic 2-photon imaging, and behavioral analysis, we monitor the effects of isoflurane (Iso), medetomidine/midazolam/fentanyl (MMF), and ketamine/xylazine (Keta/Xyl) on network activity and structural spine dynamics in the hippocampal CA1 area of adult mice. GAs robustly reduced spiking activity, decorrelated cellular ensembles, albeit with distinct activity signatures, and altered spine dynamics. CA1 network activity under all 3 anesthetics was different to natural sleep. Iso anesthesia most closely resembled unperturbed activity during wakefulness and sleep, and network alterations recovered more readily than with Keta/Xyl and MMF. Correspondingly, memory consolidation was impaired after exposure to Keta/Xyl and MMF, but not Iso. Thus, different anesthetics distinctly alter hippocampal network dynamics, synaptic connectivity, and memory consolidation, with implications for GA strategy appraisal in animal research and clinical settings.

PLoS Biol, 2021; 19
33246578: Chini M, Hanganu-Opatz IL
Prefrontal Cortex Development in Health and Disease: Lessons from Rodents and Humans.
The role of the prefrontal cortex (PFC) takes center stage among unanswered questions in modern neuroscience. The PFC has a Janus-faced nature: it enables sophisticated cognitive and social abilities that reach their maximum expression in humans, yet it underlies some of the devastating symptoms of psychiatric disorders. Accordingly, appropriate prefrontal development is crucial for many high-order cognitive abilities and dysregulation of this process has been linked to various neuropsychiatric diseases. Reviewing recent advances in the field, with a primary focus on rodents and humans, we highlight why, despite differences across species, a cross-species approach is a fruitful strategy for understanding prefrontal development. We briefly review the developmental contribution of molecules and extensively discuss how electrical activity controls the early maturation and wiring of prefrontal areas, as well as the emergence and refinement of input-output circuitry involved in cognitive processing. Finally, we highlight the mechanisms of developmental dysfunction and their relevance for psychiatric disorders.
Trends Neurosci, 2021; 44
31733940: Chini M, Pöpplau JA, Lindemann C, Carol-Perdiguer L, Hnida M, Oberländer V, Xu X, Ahlbeck J, Bitzenhofer SH, Mulert C, Hanganu-Opatz IL
Resolving and Rescuing Developmental Miswiring in a Mouse Model of Cognitive Impairment.
Cognitive deficits, core features of mental illness, largely result from dysfunction of prefrontal networks. This dysfunction emerges during early development, before a detectable behavioral readout, yet the cellular elements controlling the abnormal maturation are still unknown. Here, we address this open question by combining in vivo electrophysiology, optogenetics, neuroanatomy, and behavioral assays during development in mice mimicking the dual genetic-environmental etiology of psychiatric disorders. We report that pyramidal neurons in superficial layers of the prefrontal cortex are key elements causing disorganized oscillatory entrainment of local circuits in beta-gamma frequencies. Their abnormal firing rate and timing relate to sparser dendritic arborization and lower spine density. Administration of minocycline during the first postnatal week, potentially acting via microglial cells, rescues the neuronal deficits and restores pre-juvenile cognitive abilities. Elucidation of the cellular substrate of developmental miswiring causing later cognitive deficits opens new perspectives for identification of neurobiological targets amenable to therapies.
Neuron, 2020; 105
31087437: Oberlander VC, Xu X, Chini M, Hanganu-Opatz IL
Developmental dysfunction of prefrontal-hippocampal networks in mouse models of mental illness.
Despite inherent difficulties to translate human cognitive phenotype into animals, a large number of animal models for psychiatric disorders, such as schizophrenia, have been developed over the last decades. To which extent they reproduce common patterns of dysfunction related to mental illness and abnormal processes of maturation is still largely unknown. While the devastating symptoms of disease are firstly detectable in adulthood, they are considered to reflect profound miswiring of brain circuitry as result of abnormal development. To reveal whether different disease models share common dysfunction early in life, we investigate the prefrontal-hippocampal communication at neonatal age in (a) mice mimicking the abnormal genetic background (22q11.2 microdeletion, DISC1 knockdown), (b) mice mimicking the challenge by environmental stressors (maternal immune activation during pregnancy), (c) mice mimicking the combination of both aetiologies (dual-hit models) and pharmacological mouse models. Simultaneous extracellular recordings in vivo from all layers of prelimbic subdivision (PL) of prefrontal cortex (PFC) and CA1 area of intermediate/ventral hippocampus (i/vHP) show that network oscillations have a more fragmented structure and decreased power mainly in neonatal mice that mimic both genetic and environmental aetiology of disease. These mice also show layer-specific firing deficits in PL. Similar early network dysfunction was present in mice with 22q11.2 microdeletion. The abnormal activity patterns are accompanied by weaker synchrony and directed interactions within prefrontal-hippocampal networks. Thus, only severe genetic defects or combined genetic environmental stressors are disruptive enough for reproducing the early network miswiring in mental disorders.
Eur J Neurosci, 2019; 50
30617212: Xu X, Chini M, Bitzenhofer SH, Hanganu-Opatz IL
Transient Knock-Down of Prefrontal DISC1 in Immune-Challenged Mice Causes Abnormal Long-Range Coupling and Cognitive Dysfunction throughout Development.
Compromised brain development has been hypothesized to account for mental illness. This concept was underpinned by the function of the molecule disrupted-in-schizophrenia 1 (DISC1), which represents an intracellular hub of developmental processes and has been related to cognitive dysfunction in psychiatric disorders. Mice with whole-brain DISC1 knock-down show impaired prefrontal-hippocampal function and cognitive abilities throughout development and at adulthood, especially when combined with early environmental stressors, such as maternal immune activation (MIA). However, the contribution of

abnormal DISC1-driven maturation of either prefrontal cortex (PFC) or hippocampus (HP) to these deficits is still unknown. Here, we use electroporation to restrict the DISC1 knock-down to prefrontal layer II/III pyramidal neurons during perinatal development and expose these mice to MIA as an environmental stressor (dual-hit GE mice, both sexes). Combining electrophysiology and neuroanatomy with behavioral testing, we show that GE mice at neonatal age have abnormal patterns of oscillatory activity and firing in PFC, but not HP. Abnormal firing rates in PFC of GE mice relate to sparser dendritic arborization and lower spine density. Moreover, the long-range coupling within prefrontal-hippocampal networks is decreased at this age. The transient prefrontal DISC1 knock-down was sufficient to permanently perturb the prefrontal-hippocampal communication and caused poorer recognition memory performance at pre-juvenile age. Thus, developmental dysfunction of prefrontal circuitry causes long-lasting disturbances related to mental illness. Hypofrontality is considered a main cause of cognitive deficits in mental disorders, yet the underlying mechanisms are still largely unknown. During development, long before the emergence of disease symptoms, the functional coupling within the prefrontal-hippocampal network, which is the core brain circuit involved in cognitive processing, is reduced. To assess to which extent impaired prefrontal development contributes to the early dysfunction, immune-challenged mice with transient DISC1 knock-down confined to PFC were investigated in their prefrontal-hippocampal communication throughout development by electrophysiology and behavioral testing. We show that perturbing developmental processes of prefrontal layer II/III pyramidal neurons is sufficient to diminish prefrontal-hippocampal coupling and decrease the cognitive performance throughout development.
J Neurosci, 2019; 39

31191258: Chini M, Gretenkord S, Kostka JK, Pöpplau JA, Cornelissen L, Berde CB, Hanganu-Opatz IL, Bitzenhofer SH
Neural Correlates of Anesthesia in Newborn Mice and Humans.
Monitoring the hypnotic component of anesthesia during surgeries is critical to prevent intraoperative awareness and reduce adverse side effects. For this purpose, electroencephalographic (EEG) methods complementing measures of autonomic functions and behavioral responses are in use in clinical practice. However, in human neonates and infants existing methods may be unreliable and the correlation between brain activity and anesthetic depth is still poorly understood. Here, we characterized the effects of different anesthetics on brain activity in neonatal mice and developed machine learning approaches to identify electrophysiological features predicting inspired or end-tidal anesthetic concentration as a proxy for anesthetic depth. We show that similar features from EEG recordings can be applied to predict anesthetic concentration in neonatal mice and humans. These results might support a novel strategy to monitor anesthetic depth in human newborns.
Front Neural Circuits, 2019; 13

29631696: Ahlbeck J, Song L, Chini M, Bitzenhofer SH, Hanganu-Opatz IL
Glutamatergic drive along the septo-temporal axis of hippocampus boosts prelimbic oscillations in the neonatal mouse.
The long-range coupling within prefrontal-hippocampal networks that account for cognitive performance emerges early in life. The discontinuous hippocampal theta bursts have been proposed to drive the generation of neonatal prefrontal oscillations, yet the cellular substrate of these early interactions is still unresolved. Here, we selectively target optogenetic manipulation of glutamatergic projection neurons in the CA1 area of either dorsal or intermediate/ventral hippocampus at neonatal age to elucidate their contribution to the emergence of prefrontal oscillatory entrainment. We show that despite stronger theta and ripples power in dorsal hippocampus, the prefrontal cortex is mainly coupled with intermediate/ventral hippocampus by phase-locking of neuronal firing via dense direct axonal projections. Theta band-confined activation by light of pyramidal neurons in intermediate/ventral but not dorsal CA1 that were transfected by electroporation with high-efficiency channelrhodopsin boosts prefrontal oscillations. Our data causally elucidate the cellular origin of the long-range coupling in the developing brain.
Elife, 2018; 7

28539349: Bellesi M, de Vivo L, Chini M, Gilli F, Tononi G, Cirelli C
Sleep Loss Promotes Astrocytic Phagocytosis and Microglial Activation in Mouse Cerebral Cortex.
We previously found that and its ligand , astrocytic genes involved in phagocytosis, are upregulated after acute sleep deprivation. These results suggested that astrocytes may engage in phagocytic activity during extended wake, but direct evidence was lacking. Studies in humans and rodents also found that sleep loss increases peripheral markers of inflammation, but whether these changes are associated with neuroinflammation and/or activation of microglia, the brain's resident innate immune cells, was unknown. Here we used serial block-face scanning electron microscopy to obtain 3D volume measurements of synapses and surrounding astrocytic processes in mouse frontal cortex after 6-8 h of sleep, spontaneous wake, or sleep deprivation (SD) and after chronic (~5 d) sleep restriction (CSR). Astrocytic phagocytosis, mainly of presynaptic components of large synapses, increased after both acute and chronic sleep loss relative to sleep and wake. MERTK expression and lipid peroxidation in synaptoneurosomes also increased to a similar extent after short and long sleep loss, suggesting that astrocytic phagocytosis may represent the brain's response to the increase in synaptic activity associated with prolonged wake, clearing worn components of heavily used synapses. Using confocal microscopy, we then found that CSR but not SD mice show morphological signs of microglial activation and enhanced microglial phagocytosis of

synaptic elements, without obvious signs of neuroinflammation in the CSF. Because low-level sustained microglia activation can lead to abnormal responses to a secondary insult, these results suggest that chronic sleep loss, through microglia priming, may predispose the brain to further damage. We find that astrocytic phagocytosis of synaptic elements, mostly of presynaptic origin and in large synapses, is upregulated already after a few hours of sleep deprivation and shows a further significant increase after prolonged and severe sleep loss, suggesting that it may promote the housekeeping of heavily used and strong synapses in response to the increased neuronal activity of extended wake. By contrast, chronic sleep restriction but not acute sleep loss activates microglia, promotes their phagocytic activity, and does so in the absence of overt signs of neuroinflammation, suggesting that like many other stressors, extended sleep disruption may lead to a state of sustained microglia activation, perhaps increasing the brain's susceptibility to other forms of damage.
J Neurosci, 2017; 37

27830758: Bellesi M, Bushey D, Chini M, Tononi G, Cirelli C
Contribution of sleep to the repair of neuronal DNA double-strand breaks: evidence from flies and mice.
Exploration of a novel environment leads to neuronal DNA double-strand breaks (DSBs). These DSBs are generated by type 2 topoisomerase to relieve topological constrains that limit transcription of plasticity-related immediate early genes. If not promptly repaired, however, DSBs may lead to cell death. Since the induction of plasticity-related genes is higher in wake than in sleep, we asked whether it is specifically wake associated with synaptic plasticity that leads to DSBs, and whether sleep provides any selective advantage over wake in their repair. In flies and mice, we find that enriched wake, more than simply time spent awake, induces DSBs, and their repair in mice is delayed or prevented by subsequent wake. In both species the repair of irradiation-induced neuronal DSBs is also quicker during sleep, and mouse genes mediating the response to DNA damage are upregulated in sleep. Thus, sleep facilitates the repair of neuronal DSBs.
Sci Rep, 2016; 6

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BOARD NUMBER: S05-432

LOCAL INTEGRATION OF LONG-RANGE INPUTS FROM THE LATERAL ENTORHINAL CORTEX IN HIPPOCAMPAL AREA CA3 DRIVES DENDRITIC NON-LINEARITIES AND SOMATIC OUTPUT

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Functional interactions between the entorhinal cortex and the hippocampus are crucial learning and memory. By integrating context-laden direct inputs from the lateral entorhinal cortex (LEC) with local feedforward dentate gyrus (DG) and feedback CA3 inputs, area CA3 is poised to gate hippocampal information flow and mnemonic function. However, the mechanisms of dendritic integration of LEC inputs in CA3 pyramidal cells and how they contribute to the flexibility of spatial representations displayed by CA3 ensembles remain unknown. Here we use dual color optogenetic circuit mapping *ex vivo* and 2-photon imaging with chemogenetic manipulations *in vivo* to dissect the functional role of LEC excitatory and inhibitory inputs to CA3. We found that LEC direct glutamatergic inputs drove spiking in CA3 interneurons, whereas LEC inhibitory inputs shunted interneuron excitability. In CA3 pyramidal cells, LEC excitatory inputs evoked monosynaptic excitation and disynaptic inhibition which acted to prevent CA3 output. Combined stimulation of LEC excitatory projections with local DG and CA3 recurrent inputs could drive CA3 output, and recruitment of LEC inhibitory projections further boosted CA3 output. Dendritic recordings from CA3 pyramidal cells suggest that this disinhibitory effect may facilitate the generation of dendritic spikes, which ultimately impact somatic excitability. These results demonstrate that LEC inputs can drive CA3 output when coordinated with local inputs, highlighting the role of dendritic integration of LEC signals in CA3 dendrites. To assess the role of this circuit on CA3 spatial coding, we are currently silencing LEC excitatory and inhibitory inputs while imaging CA3 place cell activity *in vivo*.

Pubmed:
24523533: Penzo MA, Robert V, Li B
Fear conditioning potentiates synaptic transmission onto long-range projection neurons in the lateral subdivision of central amygdala.
Recent studies indicate that the lateral subdivision of the central amygdala (CeL) is essential for fear learning. Specifically, fear conditioning induces cell-type-specific synaptic plasticity in CeL neurons that is required for the storage of fear memories. The CeL also controls fear expression by gating the activity of the medial subdivision of the central amygdala (CeM), the canonical amygdala output to areas that mediate defensive responses. In addition to the connection with CeM, the CeL sends long-range projections to innervate extra-amygdala areas. However, the long-range projection CeL neurons have not been well characterized, and their role in fear regulation is unknown. Here we show in mice that a subset of CeL neurons directly project to the midbrain periaqueductal gray (PAG) and the paraventricular nucleus of the thalamus, two brain areas implicated in defensive behavior. These long-range projection CeL neurons are predominantly somatostatin-positive (SOM(+)) neurons, which can directly inhibit PAG neurons, and some of which innervate both the PAG and paraventricular nucleus of the thalamus. Notably, fear conditioning potentiates excitatory synaptic transmission onto these long-range projection CeL neurons. Thus, our study identifies a subpopulation of SOM(+) CeL neurons that may contribute to fear learning and regulate fear expression independent of CeM.
J Neurosci, 2014; 34
25600269: Penzo MA, Robert V, Tucciarone J, De Bundel D, Wang M, Van Aelst L, Darvas M, Parada LF, Palmiter RD, He M, Huang ZJ, Li B
The paraventricular thalamus controls a central amygdala fear circuit.
Appropriate responses to an imminent threat brace us for adversities. The ability to sense and predict threatening or stressful events is essential for such adaptive behaviour. In the mammalian brain, one putative stress sensor is the paraventricular nucleus of the thalamus (PVT), an area that is readily activated by both physical and psychological stressors. However, the role of the PVT in the establishment of adaptive behavioural responses remains unclear. Here we show in mice that the PVT regulates fear processing in the lateral division of the central amygdala (CeL), a structure that orchestrates fear learning and expression. Selective inactivation of CeL-projecting PVT neurons prevented fear conditioning, an effect that can be

accounted for by an impairment in fear-conditioning-induced synaptic potentiation onto somatostatin-expressing (SOM(+)) CeL neurons, which has previously been shown to store fear memory. Consistently, we found that PVT neurons preferentially innervate SOM(+) neurons in the CeL, and stimulation of PVT afferents facilitated SOM(+) neuron activity and promoted intra-CeL inhibition, two processes that are critical for fear learning and expression. Notably, PVT modulation of SOM(+) CeL neurons was mediated by activation of the brain-derived neurotrophic factor (BDNF) receptor tropomysin-related kinase B (TrkB). As a result, selective deletion of either Bdnf in the PVT or Trkb in SOM(+) CeL neurons impaired fear conditioning, while infusion of BDNF into the CeL enhanced fear learning and elicited unconditioned fear responses. Our results demonstrate that the PVT-CeL pathway constitutes a novel circuit essential for both the establishment of fear memory and the expression of fear responses, and uncover mechanisms linking stress detection in PVT with the emergence of adaptive behaviour.
Nature, 2015; 519
28472661: Boehringer R, Polygalov D, Huang AJY, Middleton SJ, Robert V, Wintzer ME, Piskorowski RA, Chevaleyre V, McHugh TJ
Chronic Loss of CA2 Transmission Leads to Hippocampal Hyperexcitability.
Hippocampal CA2 pyramidal cells project into both the neighboring CA1 and CA3 subfields, leaving them well positioned to influence network physiology and information processing for memory and space. While recent work has suggested unique roles for CA2, including encoding position during immobility and generating ripple oscillations, an interventional examination of the integrative functions of these connections has yet to be reported. Here we demonstrate that CA2 recruits feedforward inhibition in CA3 and that chronic genetically engineered shutdown of CA2-pyramidal-cell synaptic transmission consequently results in increased excitability of the recurrent CA3 network. In behaving mice, this led to spatially triggered episodes of network-wide hyperexcitability during exploration accompanied by the emergence of high-frequency discharges during rest. These findings reveal CA2 as a regulator of network processing in hippocampus and suggest that CA2-mediated inhibition in CA3 plays a key role in establishing the dynamic excitatory and inhibitory balance required for proper network function.
Neuron, 2017; 94
29335778: Robert V, Cassim S, Chevaleyre V, Piskorowski RA
Hippocampal area CA2: properties and contribution to hippocampal function.
This review focuses on area CA2 of the hippocampus, as recent results have revealed the unique properties and surprising role of this region in encoding social, temporal and contextual aspects of memory. Originally identified and described by Lorente de No, in 1934, this region of the hippocampus has unique intra-and extra-hippocampal connectivity, sending and receiving input to septal and hypothalamic regions. Recent in vivo studies have indicated that CA2 pyramidal neurons encode spatial information during immobility and play an important role in the generation of sharp-wave ripples. Furthermore, CA2 neurons act to control overall excitability in the hippocampal network and have been found to be consistently altered in psychiatric diseases, indicating that normal function of this region is necessary for normal cognition. With its unique role, area CA2 has a unique molecular profile, interneuron density and composition. Furthermore, this region has an unusual manifestation of synaptic plasticity that does not occur post-synaptically at pyramidal neuron dendrities but through the local network of inhibitory neurons. While much progress has recently been made in understanding the large contribution of area CA2 to social memory formation, much still needs to be learned.
Cell Tissue Res, 2018; 373
30943417: Nasrallah K, Therreau L, Robert V, Huang AJY, McHugh TJ, Piskorowski RA, Chevaleyre V
Routing Hippocampal Information Flow through Parvalbumin Interneuron Plasticity in Area CA2.
The hippocampus is critical for the formation of episodic memory. It is, therefore, important to understand intra-hippocampal circuitry, especially in the often overlooked area CA2. Using specific transgenic mouse lines combined with opto- and chemogenetics, we show that local plasticity of parvalbumin-expressing interneurons in area CA2 allows CA3 input to recruit CA2 pyramidal neurons (PNs), thereby increasing the excitatory drive between CA3 and CA1. CA2 PNs provide both stronger excitation and larger feed-forward inhibition onto deep, compared with superficial, CA1 PNs. This feed-forward inhibition, largely mediated by parvalbumin-expressing interneurons, normalizes the excitatory drive onto deep and superficial CA1 PNs. Finally, we identify a target of CA2 in area CA1, i.e., CA1 PNs, whose soma are located in stratum radiatum. These data provide insight into local hippocampal circuitry and reveal how localized plasticity can potentially control information flow in the larger hippocampal network.
Cell Rep, 2019; 27
32069351: Robert V, Therreau L, Davatolhagh MF, Bernardo-Garcia FJ, Clements KN, Chevaleyre V, Piskorowski RA
The mechanisms shaping CA2 pyramidal neuron action potential bursting induced by muscarinic acetylcholine receptor activation.
Recent studies have revealed that hippocampal area CA2 plays an important role in hippocampal network function. Disruption of this region has been implicated in neuropsychiatric disorders. It is well appreciated that cholinergic input to the

hippocampus plays an important role in learning and memory. While the effect of elevated cholinergic tone has been well studied in areas CA1 and CA3, it remains unclear how changes in cholinergic tone impact synaptic transmission and the intrinsic properties of neurons in area CA2. In this study, we applied the cholinergic agonist carbachol and performed on-cell, whole-cell, and extracellular recordings in area CA2. We observed that under conditions of high cholinergic tone, CA2 pyramidal neurons depolarized and rhythmically fired bursts of action potentials. This depolarization depended on the activation of M1 and M3 cholinergic receptors. Furthermore, we examined how the intrinsic properties and action-potential firing were altered in CA2 pyramidal neurons treated with 10 μM carbachol. While this intrinsic burst firing persisted in the absence of synaptic transmission, bursts were shaped by synaptic inputs in the intact network. We found that both excitatory and inhibitory synaptic transmission were reduced upon carbachol treatment. Finally, we examined the contribution of different channels to the cholinergic-induced changes in neuronal properties. We found that a conductance from Kv7 channels partially contributed to carbachol-induced changes in resting membrane potential and membrane resistance. We also found that D-type potassium currents contributed to controlling several properties of the bursts, including firing rate and burst kinetics. Furthermore, we determined that T-type calcium channels and small conductance calcium-activated potassium channels play a role in regulating bursting activity.
J Gen Physiol, 2020; 152
32999460: Chen S, He L, Huang AJY, Boehringer R, Robert V, Wintzer ME, Polygalov D, Weitemier AZ, Tao Y, Gu M, Middleton SJ, Namiki K, Hama H, Therreau L, Chevaleyre V, Hioki H, Miyawaki A, Piskorowski RA, McHugh TJ
A hypothalamic novelty signal modulates hippocampal memory.
The ability to recognize information that is incongruous with previous experience is critical for survival. Novelty signals have therefore evolved in the mammalian brain to enhance attention, perception and memory. Although the importance of regions such as the ventral tegmental area and locus coeruleus in broadly signalling novelty is well-established, these diffuse monoaminergic transmitters have yet to be shown to convey specific information on the type of stimuli that drive them. Whether distinct types of novelty, such as contextual and social novelty, are differently processed and routed in the brain is unknown. Here we identify the supramammillary nucleus (SuM) as a novelty hub in the hypothalamus. The SuM region is unique in that it not only responds broadly to novel stimuli, but also segregates and selectively routes different types of information to discrete cortical targets-the dentate gyrus and CA2 fields of the hippocampus-for the modulation of mnemonic processing. Using a new transgenic mouse line, SuM-Cre, we found that SuM neurons that project to the dentate gyrus are activated by contextual novelty, whereas the SuM-CA2 circuit is preferentially activated by novel social encounters. Circuit-based manipulation showed that divergent novelty channelling in these projections modifies hippocampal contextual or social memory. This content-specific routing of novelty signals represents a previously unknown mechanism that enables the hypothalamus to flexibly modulate select components of cognition.
Nature, 2020; 586
34003113: Robert V, Therreau L, Chevaleyre V, Lepicard E, Viollet C, Cognet J, Huang AJ, Boehringer R, Polygalov D, McHugh TJ, Piskorowski RA
Local circuit allowing hypothalamic control of hippocampal area CA2 activity and consequences for CA1.
The hippocampus is critical for memory formation. The hypothalamic supramammillary nucleus (SuM) sends long-range projections to hippocampal area CA2. While the SuM-CA2 connection is critical for social memory, how this input acts on the local circuit is unknown. Using transgenic mice, we found that SuM axon stimulation elicited mixed excitatory and inhibitory responses in area CA2 pyramidal neurons (PNs). Parvalbumin-expressing basket cells were largely responsible for the feedforward inhibitory drive of SuM over area CA2. Inhibition recruited by the SuM input onto CA2 PNs increased the precision of action potential firing both in conditions of low and high cholinergic tone. Furthermore, SuM stimulation in area CA2 modulated CA1 activity, indicating that synchronized CA2 output drives a pulsed inhibition in area CA1. Hence, the network revealed here lays basis for understanding how SuM activity directly acts on the local hippocampal circuit to allow social memory encoding.
Elife, 2021; 10
34756987: Moore JJ, Robert V, Rashid SK, Basu J
Assessing Local and Branch-specific Activity in Dendrites.
Dendrites are elaborate neural processes which integrate inputs from various sources in space and time. While decades of work have suggested an independent role for dendrites in driving nonlinear computations for the cell, only recently have technological advances enabled us to capture the variety of activity in dendrites and their coupling dynamics with the soma. Under certain circumstances, activity generated in a given dendritic branch remains isolated, such that the soma or even sister dendrites are not privy to these localized signals. Such branch-specific activity could radically increase the capacity and flexibility of coding for the cell as a whole. Here, we discuss these forms of localized and branch-specific activity, their functional relevance in plasticity and behavior, and their supporting biophysical and circuit-level mechanisms. We conclude by showcasing electrical and optical approaches in hippocampal area CA3, using original experimental data to discuss

experimental and analytical methodology and key considerations to take when investigating the functional relevance of independent dendritic activity.
Neuroscience, 2022; 489

FENS Forum 2022
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BOARD NUMBER: S05-433

SEROTONERGIC MODULATION OF NEURAL ACTIVITY ACROSS THE MOUSE BRAIN

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

Guido Meijer, Joana Catarino, Laura Freitas-Silva, Inês Laranjeira, International Brain Laboratory, Zachary Mainen Champalimaud Foundation, Champalimaud Neuroscience Programme, Lisbon, Portugal

Serotonin (5-HT) is a central neuromodulator which is implicated in the regulation of mood, motivation and cognitive flexibility. 5-HT is released from neurons in the dorsal raphe nucleus (DRN) which project throughout the entire brain. How serotonin release modulates neural dynamics across the brain remains a topic of debate. Previous fMRI and electrophysiological studies in rodents showed that optogenetically stimulating serotonin release resulted in a general suppression of neural activity. Brain-wide fMRI studies, however, lack single neuron resolution and are confounded by the vasoconstrictive effect of serotonin. To date, electrophysiological recording studies were performed under anesthesia or were limited in scope. Therefore, we present an extensive study investigating how serotonin modulates neural spiking activity across the mouse brain. We performed Neuropixel recordings in awake mice while optogenetically stimulating 5-HT neurons in the DRN. Acute Neuropixel recordings were targeted to eight locations, which were recorded in pairs in four recording sessions. We found that stimulating 5-HT release resulted in a substantial suppression of spiking activity in the thalamus, hippocampus and posterior parietal cortex. However, the amygdala, tail of the striatum, orbitofrontal, medial prefrontal, and piriform cortex showed balanced serotonergic enhancement and suppression of neuronal activity. Other target regions, like the superior colliculus, were unaffected. These findings shed light on the functional dynamics of the serotonergic projection system at a single neuron level, and have important implications for the computational role of serotonin in large-scale neural dynamics across the brain.

Pubmed:

34117377: Meijer GT, Arlandis J, Urai AE
There is no mouse: using a virtual mouse to generate training data for video-based pose estimation.
Lab Anim (NY), 2021; 50
32402272: Meijer GT, Marchesi P, Mejias JF, Montijn JS, Lansink CS, Pennartz CMA
Neural Correlates of Multisensory Detection Behavior: Comparison of Primary and Higher-Order Visual Cortex.
We act upon stimuli in our surrounding environment by gathering the multisensory information they convey and by integrating this information to decide on a behavioral action. We hypothesized that the anterolateral secondary visual cortex (area AL) of the mouse brain may serve as a hub for sensorimotor transformation of audiovisual information. We imaged neuronal activity in primary visual cortex (V1) and AL of the mouse during a detection task using visual, auditory, and audiovisual stimuli. We found that AL neurons were more sensitive to weak uni- and multisensory stimuli compared to V1. Depending on contrast, different subsets of AL and V1 neurons showed cross-modal modulation of visual responses. During audiovisual stimulation, AL neurons showed stronger differentiation of behaviorally reported versus unreported stimuli compared to V1, whereas V1 showed this distinction during unisensory visual stimulation. Thus, neural population activity in area AL correlates more closely with multisensory detection behavior than V1.
Cell Rep, 2020; 31
30677428: Meijer GT, Mertens PEC, Pennartz CMA, Olcese U, Lansink CS
The circuit architecture of cortical multisensory processing: Distinct functions jointly operating within a common anatomical network.
Our perceptual systems continuously process sensory inputs from different modalities and organize these streams of information such that our subjective representation of the outside world is a unified experience. By doing so, they also enable further cognitive processing and behavioral action. While cortical multisensory processing has been extensively investigated in terms of psychophysics and mesoscale neural correlates, an in depth understanding of the underlying circuit-level mechanisms is lacking. Previous studies on circuit-level mechanisms of multisensory processing have predominantly focused on cue integration, i.e. the mechanism by which sensory features from different modalities are combined to yield more reliable stimulus estimates than those obtained by using single sensory modalities. In this review, we expand the framework on the circuit-level mechanisms of cortical multisensory processing by highlighting that multisensory processing is a family of

functions - rather than a single operation - which involves not only the integration but also the segregation of modalities. In addition, multisensory processing not only depends on stimulus features, but also on cognitive resources, such as attention and memory, as well as behavioral context, to determine the behavioral outcome. We focus on rodent models as a powerful instrument to study the circuit-level bases of multisensory processes, because they enable combining cell-type-specific recording and interventional techniques with complex behavioral paradigms. We conclude that distinct multisensory processes share overlapping anatomical substrates, are implemented by diverse neuronal micro-circuitries that operate in parallel, and are flexibly recruited based on factors such as stimulus features and behavioral constraints.
Prog Neurobiol, 2019; 174
30337861: Meijer GT, Pie JL, Dolman TL, Pennartz CMA, Lansink CS
Audiovisual Integration Enhances Stimulus Detection Performance in Mice.
The detection of objects in the external world improves when humans and animals integrate object features of multiple sensory modalities. Behavioral and neuronal mechanisms underlying multisensory stimulus detection are poorly understood, mainly because they have not been investigated with suitable behavioral paradigms. Such behavioral paradigms should (i) elicit a robust multisensory gain, (ii) incorporate systematic calibration of stimulus amplitude to the sensory capacities of the individual subject, (iii) yield a high trial count, and (iv) be easily compatible with a large variety of neurophysiological recording techniques. We developed an audiovisual stimulus detection task for head-fixed mice which meets all of these critical behavioral constraints. Behavioral data obtained with this task indicated a robust increase in detection performance of multisensory stimuli compared with unisensory cues, which was maximal when both stimulus constituents were presented at threshold intensity. The multisensory behavioral effect was associated with a change in the perceptual performance which consisted of two components. First, the visual and auditory perceptual systems increased their sensitivity meaning that low intensity stimuli were more often detected. Second, enhanced acuity enabled the systems to better classify whether there was a stimulus or not. Fitting our data to signal detection models revealed that the multisensory gain was more likely to be achieved by integration of sensory signals rather than by stimulus redundancy or competition. This validated behavioral paradigm can be exploited to reliably investigate the neuronal correlates of multisensory stimulus detection at the level of single neurons, microcircuits, and larger perceptual systems.
Front Behav Neurosci, 2018; 12
30222107: Goltstein PM, Meijer GT, Pennartz CM
Conditioning sharpens the spatial representation of rewarded stimuli in mouse primary visual cortex.
Reward is often employed as reinforcement in behavioral paradigms but it is unclear how the visuospatial aspect of a stimulus-reward association affects the cortical representation of visual space. Using a head-fixed paradigm, we conditioned mice to associate the same visual pattern in adjacent retinotopic regions with availability and absence of reward. Time-lapse intrinsic optical signal imaging under anesthesia showed that conditioning increased the spatial separation of mesoscale cortical representations of reward predicting- and non-reward predicting stimuli. Subsequent in vivo two-photon calcium imaging revealed that this improved separation correlated with enhanced population coding for retinotopic location, specifically for the trained orientation and spatially confined to the V1 region where rewarded and non-rewarded stimulus representations bordered. These results are corroborated by conditioning-induced differences in the correlation structure of population activity. Thus, the cortical representation of visual space is sharpened as consequence of associative stimulus-reward learning while the overall retinotopic map remains unaltered.
Elife, 2018; 7
28821672: Meijer GT, Montijn JS, Pennartz CMA, Lansink CS
Audiovisual Modulation in Mouse Primary Visual Cortex Depends on Cross-Modal Stimulus Configuration and Congruency.
The sensory neocortex is a highly connected associative network that integrates information from multiple senses, even at the level of the primary sensory areas. Although a growing body of empirical evidence supports this view, the neural mechanisms of cross-modal integration in primary sensory areas, such as the primary visual cortex (V1), are still largely unknown. Using two-photon calcium imaging in awake mice, we show that the encoding of audiovisual stimuli in V1 neuronal populations is highly dependent on the features of the stimulus constituents. When the visual and auditory stimulus features were modulated at the same rate (i.e., temporally congruent), neurons responded with either an enhancement or suppression compared with unisensory visual stimuli, and their prevalence was balanced. Temporally incongruent tones or white-noise bursts included in audiovisual stimulus pairs resulted in predominant response suppression across the neuronal population. Visual contrast did not influence multisensory processing when the audiovisual stimulus pairs were congruent; however, when white-noise bursts were used, neurons generally showed response suppression when the visual stimulus contrast was high whereas this effect was absent when the visual contrast was low. Furthermore, a small fraction of V1 neurons, predominantly those located near the lateral border of V1, responded to sound alone. These results show that V1 is involved in the encoding of cross-modal interactions in a more versatile way than previously thought. The neural substrate of cross-modal integration is not limited to specialized cortical association areas but extends to primary sensory areas. Using two-

photon imaging of large groups of neurons, we show that multisensory modulation of V1 populations is strongly determined by the individual and shared features of cross-modal stimulus constituents, such as contrast, frequency, congruency, and temporal structure. Congruent audiovisual stimulation resulted in a balanced pattern of response enhancement and suppression compared with unisensory visual stimuli, whereas incongruent or dissimilar stimuli at full contrast gave rise to a population dominated by response-suppressing neurons. Our results indicate that V1 dynamically integrates nonvisual sources of information while still attributing most of its resources to coding visual information.
J Neurosci, 2017; 37
27733611: Lansink CS, Meijer GT, Lankelma JV, Vinck MA, Jackson JC, Pennartz CM
Reward Expectancy Strengthens CA1 Theta and Beta Band Synchronization and Hippocampal-Ventral Striatal Coupling.
The use of information from the hippocampal memory system in motivated behavior depends on its communication with the ventral striatum. When an animal encounters cues that signal subsequent reward, its reward expectancy is raised. It is unknown, however, how this process affects hippocampal dynamics and their influence on target structures, such as ventral striatum. We show that, in rats, reward-predictive cues result in enhanced hippocampal theta and beta band rhythmic activity during subsequent action, compared with uncued goal-directed navigation. The beta band component, also labeled theta's harmonic, involves selective hippocampal CA1 cell groups showing frequency doubling of firing periodicity relative to theta rhythmicity and it partitions the theta cycle into segments showing clear versus poor spike timing organization. We found that theta phase precession occurred over a wider range than previously reported. This was apparent from spikes emitted near the peak of the theta cycle exhibiting large "phase precessing jumps" relative to spikes in foregoing cycles. Neither this phenomenon nor the regular manifestation of theta phase precession was affected by reward expectancy. Ventral striatal neuronal firing phase-locked not only to hippocampal theta, but also to beta band activity. Both hippocampus and ventral striatum showed increased synchronization between neuronal firing and local field potential activity during cued compared with uncued goal approaches. These results suggest that cue-triggered reward expectancy intensifies hippocampal output to target structures, such as the ventral striatum, by which the hippocampus may gain prioritized access to systems modulating motivated behaviors.
J Neurosci, 2016; 36
27545876: Montijn JS, Meijer GT, Lansink CS, Pennartz CM
Population-Level Neural Codes Are Robust to Single-Neuron Variability from a Multidimensional Coding Perspective.
Sensory neurons are often tuned to particular stimulus features, but their responses to repeated presentation of the same stimulus can vary over subsequent trials. This presents a problem for understanding the functioning of the brain, because downstream neuronal populations ought to construct accurate stimulus representations, even upon singular exposure. To study how trial-by-trial fluctuations (i.e., noise) in activity influence cortical representations of sensory input, we performed chronic calcium imaging of GCaMP6-expressing populations in mouse V1. We observed that high-dimensional response correlations, i.e., dependencies in activation strength among multiple neurons, can be used to predict single-trial, single-neuron noise. These multidimensional correlations are structured such that variability in the response of single neurons is relatively harmless to population representations of visual stimuli. We propose that multidimensional coding may represent a canonical principle of cortical circuits, explaining why the apparent noisiness of neuronal responses is compatible with accurate neural representations of stimulus features.
Cell Rep, 2016; 16
34011433: , Aguillon-Rodriguez V, Angelaki D, Bayer H, Bonacchi N, Carandini M, Cazettes F, Chapuis G, Churchland AK, Dan Y, Dewitt E, Faulkner M, Forrest H, Haetzel L, Häusser M, Hofer SB, Hu F, Khanal A, Krasniak C, Laranjeira I, Mainen ZF, Meijer G, Miska NJ, Mrsic-Flogel TD, Murakami M, Noel JP, Pan-Vazquez A, Rossant C, Sanders J, Socha K, Terry R, Urai AE, Vergara H, Wells M, Wilson CJ, Witten IB, Wool LE, Zador AM
Standardized and reproducible measurement of decision-making in mice.
Progress in science requires standardized assays whose results can be readily shared, compared, and reproduced across laboratories. Reproducibility, however, has been a concern in neuroscience, particularly for measurements of mouse behavior. Here, we show that a standardized task to probe decision-making in mice produces reproducible results across multiple laboratories. We adopted a task for head-fixed mice that assays perceptual and value-based decision making, and we standardized training protocol and experimental hardware, software, and procedures. We trained 140 mice across seven laboratories in three countries, and we collected 5 million mouse choices into a publicly available database. Learning speed was variable across mice and laboratories, but once training was complete there were no significant differences in behavior across laboratories. Mice in different laboratories adopted similar reliance on visual stimuli, on past successes and failures, and on estimates of stimulus prior probability to guide their choices. These results reveal that a complex mouse behavior can be reproduced across multiple laboratories. They establish a standard for reproducible rodent behavior, and provide an unprecedented dataset and open-access tools to study decision-making in mice. More generally, they indicate a path toward

achieving reproducibility in neuroscience through collaborative open-science approaches.
Elife, 2021; 10

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LAYER-SPECIFIC STIMULATIONS OF PARVALBUMIN-POSITIVE INTERNEURONS IN MICE ENTRAIN BRAIN RHYTHMS TO DIFFERENT FREQUENCIES

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Neocortical interneurons provide inhibition responsible for organizing neuronal activity into brain oscillations that subserve cognitive functions such as memory, attention or prediction. However, little is known about the interneuron contribution to the entrainment of neocortical oscillations across different layers. Here, using layer-specific optogenetic stimulations with micro-Light-Emitting Diode (µLED) arrays, directed toward parvalbumin-expressing (PV) interneurons in non-anesthetized awake mice, we found that supragranular layer stimulations of PV neurons were most efficient at entraining supragranular local field potential (LFP) oscillations at gamma frequencies (g: 25 - 80 Hz), whereas infragranular layer stimulation of PV neurons better entrained the LFP at delta (d: 2 - 5 Hz) and theta (θ: 6 - 10 Hz) frequencies. At the level of neuronal AP activity, we observed that supragranular regular-spiking (RS) neurons better followed the imposed PV stimulation rhythm than their infragranular counterparts at most frequencies when the stimulation was delivered in their respective layer. Layer-specific intrinsic resonant properties of neurons are hypothesized as one plausible mechanism of the layer-specific rhythm entrainment by the performed brain stimulations.

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ASTROCYTES AS ACTIVE CHANNEL FOR THE MOLECULAR CLOCK SYNCHRONIZATION AMONG SEGREGATED NEURAL POPULATIONS

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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In mammals the suprachiasmatic nucleus of the hypothalamus is considered the master circadian pacemaker which synchronizes all the clocks in the central nervous system and periphery, thus orchestrating rhythms throughout the body. However, little is known about how so many cellular clocks can be effectively synchronized. Here, we investigated the implication of two possible pathways: i) paracrine factors-mediated synchronization and ii) astrocytes-mediated synchronization. We developed a lab-on-a-chip microfluidic device, which allows growing distinct neural populations connected through i) a cell-free channel allowing (or impeding by fluidic compartmentalization) the diffusion of paracrine factors; ii) a network of astrocytes, enabling direct cell-cell transfer of the signals. Results show that both pathways can be involved. Neurons release factors that can diffuse to synchronize a neuronal population. The same factors can also synchronize astrocytes that, in turn, can transmit molecular clocks to more distant neuronal populations. Interestingly, using microfluidic devices featuring different channel lengths, we found that paracrine factors-mediated synchronization occurs only in the case of a short distance between neuronal populations. On the contrary, interconnecting astrocytes define an active channel that can transfer molecular clocks to neural populations also at long distances. By taking advantage of such devices, we also started to investigate if reactive astrocytes affect this signaling. These findings strength the importance of the synergic regulation of clock genes among neurons and astrocytes, and suggest that the network of astrocytes in the brain might have a role in the distribution of regulatory signals for the expression of clock genes.

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BOARD NUMBER: S05-436

FUNCTIONAL INTERACTIONS OF THALAMIC CELLS

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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The thalamic relay cell-reticular cell loop is the origin of diverse oscillations, most notably sleep spindles and absence seizures. Though well characterized in vitro, the direct excitatory and inhibitory connections between the two cell types have not been detected in extracellular population recordings. Here we performed high-density multichannel silicon probe recordings from primary- and higher order somatosensory thalamus of anesthetized and head-restrained mice. Thalamocortical (TC) cells and axon terminals of locally projecting thalamic reticular (nRT) cells were distinguished by their characteristic waveform and autocorrelogram (as reported in Barthó et. al. 2014). TC and nRT units most commonly showed and indirect relationship (nRT cells broadly increasing their firing ~20 ms from TC spikes) likely due to both cells taking part in spindle oscillations. We also found, however, short latency narrow peaks and troughs on several cross-correlograms, indicative of monosynaptic excitation and inhibition, respectively. Both monosynaptic excitation and inhibition occurred between TC and nRT units, not between cells of the same type. In some cases, reciprocal excitation-inhibition was observed between coupled units. On the other hand, TC-TC and nRT-nRT pairs usually showed synchronous activity on a varying time scale. The probability of monosynaptic connections and the degree of synchrony both decreased with physical distance, indicating a strict topography of these connections.

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BOARD NUMBER: S05-437

A COMPENSATORY ACCOUNT OF ALPHA-BAND REORGANIZATION WITH AGE.

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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AIM: Healthy human ageing is marked by several changes in the properties of neuro-electrophysiological recordings. Some markers of ageing reflect underlying structural decline, while others signify secondary compensations that seek to minimize the deleterious consequences of structural loss. For example, white-matter changes are linked to reduced individual peak alpha frequency (IPAF) in ongoing EEG/MEG recordings. However, the causal relationship between white matter and IPAF slowing— whether adverse or compensatory— remains unknown. This work seeks to shed light on the mechanistic basis of frequency slowing with age. **METHOD:** First we analyze a large cohort of adult lifespan MEG data to test our compensatory hypothesis. Next, we employ computational modelling to suggest possible mechanisms of compensation. **RESULTS:** We show that phase-locking at the IPAF remains preserved while the IPAF slows with age. Subsequent in-silico modelling reveals that enhancing inter-areal coupling can cancel the effect of increased axonal transmission delays on network dynamics and preserve brain-wide synchrony at reduced synchronization frequency. **CONCLUSIONS:** Our results indicate that frequency slowing emerges from functional compensation in large-scale brain circuits rather than being a passive fallout of structural decline with age.

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BOARD NUMBER: S05-438

CAUSAL ROLE OF THE CLAUSTRUM IN COORDINATING THE DORSAL ATTENTION NETWORK DURING SUSTAINED ATTENTION

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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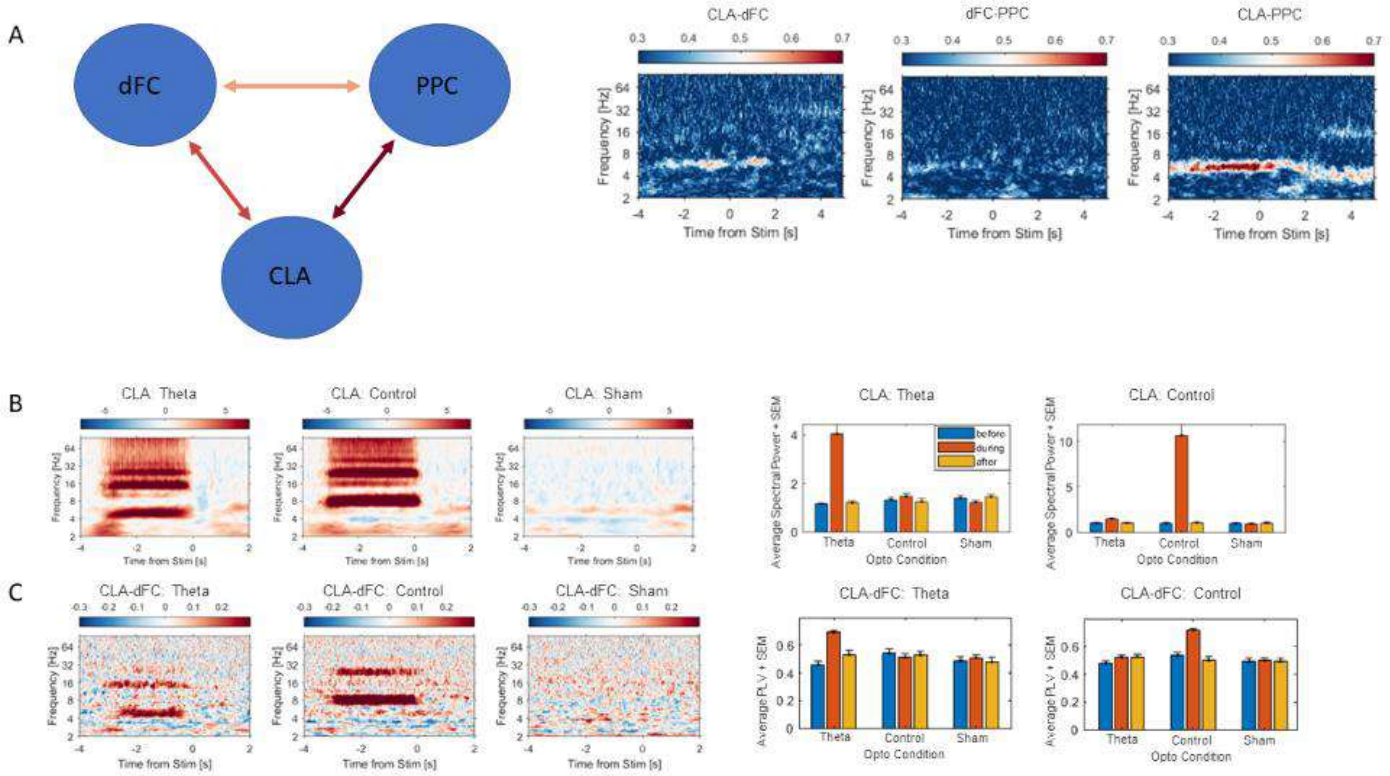
The claustrum is a highly interconnected subcortical structure that has been posited to play a key role in higher order brain functions, such as attention and salience processing. The claustrum has reciprocal anatomical connections with the frontoparietal network which exhibits rhythmic synchronization during the top-down process sustained attention; however, the causal role of oscillations in the claustrum and their role in synchronizing cortical networks remains unknown. We investigated the functional connectivity of the claustrum, dorsal frontal cortex (dFC), and posterior parietal cortex (PPC) in ferrets during the five-choice serial reaction-time task (sustained attention task) by multisite electrophysiological recordings and optogenetic frequency-specific, excitatory stimulation (5 Hz for targeting peak functional connectivity, and 8 Hz as control) in the claustrum. Functional connectivity (CLA-PPC, CLA-dFC) exhibited a task-modulated theta (5 Hz) peak (Panel A). Optogenetic stimulation increased the targeted oscillation in the claustrum for both stimulation frequencies (theta: $F(2,42) = 62.99$, $p < 0.001$; control: $F(2,42) = 272.95$, $p < 0.001$, Panel B) and demonstrated frequency-specific engagement of the CLA-dFC connection (theta: $F(2,42) = 15.76$, $p < 0.001$; control: $F(2,42) = 183.90$, $p < 0.001$, Panel C). In contrast, only the theta stimulation increased behavioral performance by decreasing omission trials by 50% compared to control ($p=0.03$). Our results demonstrate that (1) functional connectivity of the claustrum with the frontoparietal network during a sustained attention task is mediated by theta oscillations and (2) theta oscillations in claustrum may play a causal role in increasing sustained attention by reducing

omissions.



(A) Functional connectivity in CLA-dFC-PPC network. (B) Optogenetic enhancement of oscillations (before, during, and after stimulation) and (C) Functional connectivity (Phase Locking Value, PLV).

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BOARD NUMBER: S05-439

LOCOMOTION INDUCED BY MEDIAL SEPTAL GLUTAMATERGIC NEURONS IS LINKED TO INTRINSICALLY GENERATED PERSISTENT FIRING

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Medial septal glutamatergic neurons are active during theta oscillation and locomotor activity. Prolonged optogenetic activation of medial septal glutamatergic neurons drives theta oscillation and locomotion for extended periods of time outlasting the stimulus duration. However, the cellular and circuit mechanisms supporting the maintenance of both theta oscillation and locomotion remain elusive. Specifically, it remains unclear whether theta-modulated stimulus of glutamatergic neurons is a necessary prerequisite for locomotion, and whether neuronal activity within the medial septum underlies its persistence. In the present study, we show that persistent theta oscillation can be induced in the hippocampus by a brief transient optogenetic activation of medial septal glutamatergic neurons. By blocking synaptic transmission pharmacologically in the medial septum, we observed persistent locomotion upon photoactivation of the glutamatergic neurons while theta oscillation was abolished in the hippocampus. We discovered persistent spiking of medial septal neurons that outlasts the stimulus for several seconds, both in vitro and in vivo. These results led to the conclusion that persistent activity is driven by the intrinsic excitability of medial septal glutamatergic neurons. Finally, using novel Neuropixels electrodes, we recorded a larger number of neurons and discuss how stimulus-evoked changes of a single cell type affects different groups of medial septum neurons, as well as their relation to theta rhythm and locomotion.

Pubmed:
29335356: Neubrandt M, Oláh VJ, Brunner J, Marosi EL, Soltesz I, Szabadics J
Single Bursts of Individual Granule Cells Functionally Rearrange Feedforward Inhibition.
The sparse single-spike activity of dentate gyrus granule cells (DG GCs) is punctuated by occasional brief bursts of 3-7 action potentials. It is well-known that such presynaptic bursts in individual mossy fibers (MFs; axons of granule cells) are often able to discharge postsynaptic CA3 pyramidal cells due to powerful short-term facilitation. However, what happens in the CA3 network after the passage of a brief MF burst, before the arrival of the next burst or solitary spike, is not understood. Because MFs innervate significantly more CA3 interneurons than pyramidal cells, we focused on unitary MF responses in identified interneurons in the seconds-long postburst period, using paired recordings in rat hippocampal slices. Single bursts as short as 5 spikes in <30 ms in individual presynaptic MFs caused a sustained, large increase (tripling) in the amplitude of the unitary MF-EPSCs for several seconds in ivy, axo-axonic/chandelier and basket interneurons. The postburst unitary MF-EPSCs in these feedforward interneurons reached amplitudes that were even larger than the MF-EPSCs during the bursts in the same cells. In contrast, no comparable postburst enhancement of MF-EPSCs could be observed in pyramidal cells or nonfeedforward interneurons. The robust postburst increase in MF-EPSCs in feedforward interneurons was associated with significant shortening of the unitary synaptic delay and large downstream increases in disynaptic IPSCs in pyramidal cells. These results reveal a new cell type-specific plasticity that enables even solitary brief bursts in single GCs to powerfully enhance inhibition at the DG-CA3 interface in the seconds-long time-scales of interburst intervals. The hippocampal formation is a brain region that plays key roles in spatial navigation and learning and memory. The first stage of information processing occurs in the dentate gyrus, where principal cells are remarkably quiet, discharging low-frequency single action potentials interspersed with occasional brief bursts of spikes. Such bursts, in particular, have attracted a lot of attention because they appear to be critical for efficient coding, storage, and recall of information. We show that single bursts of a few spikes in

individual granule cells result in seconds-long potentiation of excitatory inputs to downstream interneurons. Thus, while it has been known that bursts powerfully discharge ("detonate") hippocampal excitatory cells, this study clarifies that they also regulate inhibition during the interburst intervals.
J Neurosci, 2018; 38

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BOARD NUMBER: S05-440

CORTICO-HIPPOCAMPAL BILATERAL COHERENCE IS VARIABLE AND PATHWAY-SPECIFIC: A STUDY WITH FIELD POTENTIAL GENERATORS

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Although most brain structures have one copy in each hemisphere growing evidence indicates that some perform unilaterally beyond the expected coordination of symmetric bodily functions. Particularly, imaging techniques show stronger unilateral activation of high order structures as the cortex and hippocampus. A detailed study will benefit from the unbeaten spatial and temporal resolution of pathway-specific field potentials (FPs). We recorded anesthetized rats with bilateral linear probes spanning the CA1 and Dentate Gyrus (DG). Evoked potentials served to localize the position of recordings. Pathway specific generators were obtained by Independent Component Analysis from depth series of FPs. This avoids strong distortion of FP waveforms from mutually contaminating pathways and uncovers true temporal dynamics. The activity of FP generators in both hemispheres was compared pairwise using cross-correlation and spectral coherence analyses. The cortical input to the DG (perforant pathway) showed intermingled bouts of high and low frequency gamma waves, some of which were bilateral and phase-locked, but others occurred in only one side. Abundant lateralized waves were observed. The FP generator in the stratum lacunosum-moleculare of the CA1 exhibited strong bilateral synchrony, whether it displayed theta or irregular activity. The CA3 input to CA1 showed tight bilateral synchrony during sharp-waves and some short bouts of gamma oscillations, but unilateral waves were frequent. These results indicate a complex bilateral organization of intrinsic and afferent hippocampal pathways that suggest time-varying and pathway-specific coordination of cortical and hippocampal regions. This mode of coordinated but independent operation may require re-defining current theories based on unilateral networks.

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THE COACTIVATION OF NEURONS IN THE PREFRONTAL CORTICO-BASAL GANGLIA NETWORK DISTINGUISHES EFFORT, REWARD AND DECISION IN A NOVEL DECISION-MAKING TASK.

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Levels of effort and reward associated with a given behaviour are encoded by diverse populations of neurons across the forebrain, but it is unclear how this information is integrated across these brain areas for decision-making. In this study, animals were trained to decide whether or not to run to the end of a linear track with varying numbers of obstacles, to receive rewards of different sizes. This task allowed reward and effort to be manipulated independently. Behavioural data (n = 13) confirmed that increasing reward and effort were associated with a higher and lower probability of accepting the reward-effort offer, respectively. Electrophysiological recordings were made simultaneously across the prefrontal cortico-basal ganglia network (medial orbitofrontal cortex, anterior cingulate cortex, ventral pallidum, striatum and subthalamic nucleus) as rats performed this task. A machine learning strategy was used to identify neurons across this network with a statistically significant tendency to coactivate over short timescales (neural ensembles). The expression strengths of some identified coactivity patterns significantly distinguished the reward and effort offered to rats and/or their subsequent decisions on the task. The proportion of neurons in each structure that contributed to these ensembles was used to evaluate the contribution of different brain areas to reward and effort-based decisions. Coordinated activity across diverse populations of forebrain neurons may serve as a mechanism for encoding decision relevant information for effort-based decision making at the network level.

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BOARD NUMBER: S05-442

CHOLINERGIC INDUCTION OF SYNCHRONOUS OSCILLATION IN THE SLUG NEURONAL NETWORK IN VITRO.

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Synchronous oscillatory network is important for cognitive functions of the brain in both vertebrates and invertebrates. In the central nervous system of the terrestrial slugs, spontaneous periodic oscillation (0.5 - 1.0 Hz) is recorded from the surface of the laminar structure of procerebrum (PC), and its frequency changes are suggested to encode the olfactory information and memory. We recently found oscillatory activity is generated spontaneously in dispersed cell culture of PC neurons. Application of acetylcholinesterase inhibitor or nicotine induced synchronous oscillation in *in vitro* network activity. To investigate how such synchronous oscillation is generated, we tested cholinergic activation and compared between synchronous and asynchronous networks. Previous results showed that acetylcholine could be function as a driving force on the synchronous oscillatory activity of the PC neuron network via nicotinic acetylcholine receptors activation *in vivo*. In present study, differences between synchronous and asynchronous network were examined in cultured PC neuron (7-21 days). First, we found a lower excitability and a higher sensibility to cholinergic activation of PC neurons in synchronous networks. Second, muscarinic receptor agonist, pilocarpine, did not induce synchronous oscillation and did not occlude the effects of physostigmine. It is suggested that: (1) *in vitro* synchronous oscillation was induced by activation of cholinergic synaptic transmission via nicotinic ACh receptors; (2) "synchronous *in vitro* networks" were characterized by higher ACh-sensitivity (than "asynchronous networks") together with the lateral inhibition of excessive activity in resting states.

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BOARD NUMBER: S05-443

INCLUDING THE LATERAL HABENULA IN THE STRESS-RELATED FUNCTIONAL NETWORK

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Aims: Stressful experiences induce cellular activation and engage specific plasticity mechanisms and gene expression within the lateral habenula (LHb), providing good insight into stress-related events in this structure. However, little is known about the place of the LHb within the broad network engaged during these types of experiences. **Methods:** Using restraint stress in male Long-Evans rats we performed two studies: one including a single 10-min restraint session and graph theory-based analyses of network activation following c-Fos immunostaining throughout the brain; one including local field potential recordings in the LHb before, during, and following two 3 hours apart 10-min restraint sessions. For the later study we focused our analyses on the different sleep-wake stages. **Results:** Graph theory-based analyses indicated that upon restraint LHb activation correlated with this of the medial prefrontal cortex (mPFC), lateral septum and medial habenula. In addition, the LHb belonged to a community (group of structures sharing more functional connections than with the others) including the mPFC, amygdala, periventricular hypothalamus and midbrain monoaminergic regions. During the electrophysiology experiment, rats could be clustered into two groups based on the intensity of their behavioral response to the first restraint (high and low responders); preliminary analyses revealed in the LHb, following the second restraint, decreased theta (6-9Hz) power during active wake in low responders, and decreased delta (1-3Hz) power during slow-wave sleep in high responders. **Conclusion:** Our results include the LHb in the broad stress-response network and suggest differential implication of LHb oscillations during coping with repeated stressful experience.

Pubmed:
32642914: Durieux L, Mathis V, Herbeaux K, Muller MA, Barbelivien A, Mathis C, Schlichter R, Hugel S, Majchrzak M, Lecourtier L
Involvement of the lateral habenula in fear memory.
Increasing evidence points to the engagement of the lateral habenula (LHb) in the selection of appropriate behavioral responses in aversive situations. However, very few data have been gathered with respect to its role in fear memory formation, especially in learning paradigms in which brain areas involved in cognitive processes like the hippocampus (HPC) and the medial prefrontal cortex (mPFC) are required. A paradigm of this sort is trace fear conditioning, in which an aversive event is preceded by a discrete stimulus, generally a tone, but without the close temporal contiguity allowing for their association based on amygdala-dependent information processing. In a first experiment, we analyzed cellular activations (c-Fos expression) induced by trace fear conditioning in subregions of the habenular complex, HPC, mPFC and amygdala using a factorial analysis to unravel functional networks through correlational analysis of data. This analysis suggested that distinct LHb subregions engaged in different aspects of conditioning, e.g. associative processes and onset of fear responses. In a second experiment, we performed chemogenetic LHb inactivation during the conditioning phase of the trace fear conditioning paradigm and subsequently assessed contextual and tone fear memories. Whereas LHb inactivation did not modify rat's behavior during conditioning, it induced contextual memory deficits and enhanced fear to the tone. These results demonstrate the involvement of the LHb in fear memory. They further suggest that the LHb is engaged in learning about threatening environments through the selection of relevant information predictive of a danger.
Brain Struct Funct, 2020; 225

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BOARD NUMBER: S05-444

ALTERED NEURONAL SPATIOTEMPORAL DYNAMICS AND SENSORY INFORMATION PROCESSING BY ACTIVATION OF CORTICAL ASTROCYTE NETWORK

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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The extraction of behaviouraly relevant sensory information at cortical levels is only possible due to the interaction of parallel neuronal circuitries. However, it is now clear that glial cells, especially astrocytes, form a second network capable to set the basis by which neuronal output is defined in time and space. Here, we used a combination of *in/ex vivo* electrophysiology, behaviour and genetic tools to explored how changes in the spatiotemporal patterns of astrocytic networks alter the integration and processing of sensory information at cellular, network and behavioral level. Our data show that up-regulation of astrocytic network by using Gfap-hMD3q-DREADD increased the duration of spontaneous network synchronization while decreasing the neuronal excitability at cortical level. Such effect was mediated by an enhanced activity of the inhibitory GABAergic transmission onto excitatory pyramidal neurons as shown by whole cell patch clamp recordings. In addition, altered astrocytic network also decreased the strength and propagation of spontaneous activity across layers of the somatosensory cortex, which strongly influenced the processing of sensory information. By using electrical stimulation of hindlimb, we found that Gfap-hM3Dq-DREADD mice exhibited decreased gain of evoked-potentials in response to high-stimulus intensity in L4/5/6 neurons. The altered excitability at cellular and network level was further corroborated at behavior level in which hM3Dq-DREADD mice exhibited increased threshold for temperature response in the hot plate assessment. These results indicate that changes in the spatiotemporal patterns astrocytic network strongly influence the integration and processing of sensory information at cortical levels with consequences to behavior output.

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ROLE OF SST+ INTERNEURONS SCULPING DEVELOPMENTAL DYNAMICS

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Spontaneous coordinated neuronal activity is a hallmark of the developing brain and plays a pivotal role in the formation of neuronal circuits. In contrast, how the interaction between different cell types leads to the complex formation of functional networks remains poorly understood. GABAergic interneurons critically contribute to cortical development starting from neurogenesis and migration, to activity-dependent circuit refinement. Particularly, SST+ interneurons (a subclass of interneurons derived from the medial ganglionic eminence (MGE)), have been suggested to play a prominent role in modulating the functional wiring of early cortical circuits. SST+ cells are among the first type of interneurons to populate and mature within the cortex. However, how these pioneer cells integrate into functional circuits and influence the flow of spontaneous and evoked activity during postnatal development, remains unknown. In this study, we address this issue using a combination of in vivo volumetric and longitudinal calcium imaging in the barrel cortex, to monitor the integration of SST+ cells into functional circuits and their role modulating cortical dynamics at different stages of postnatal development.

Pubmed:
31780329: Duan ZRS, Che A, Chu P, Modol L, Bollmann Y, Babij R, Fetcho RN, Otsuka T, Fuccillo MV, Liston C, Pisapia DJ, Cossart R, De Marco García NV
GABAergic Restriction of Network Dynamics Regulates Interneuron Survival in the Developing Cortex.
During neonatal development, sensory cortices generate spontaneous activity patterns shaped by both sensory experience and intrinsic influences. How these patterns contribute to the assembly of neuronal circuits is not clearly understood. Using longitudinal in vivo calcium imaging in un-anesthetized mouse pups, we show that spatially segregated functional assemblies composed of interneurons and pyramidal cells are prominent in the somatosensory cortex by postnatal day (P) 7. Both reduction of GABA release and synaptic inputs onto pyramidal cells erode the emergence of functional topography, leading to increased network synchrony. This aberrant pattern effectively blocks interneuron apoptosis, causing increased survival of parvalbumin and somatostatin interneurons. Furthermore, the effect of GABA on apoptosis is mediated by inputs from medial ganglionic eminence (MGE)-derived but not caudal ganglionic eminence (CGE)-derived interneurons. These findings indicate that immature MGE interneurons are fundamental for shaping GABA-driven activity patterns that balance the number of interneurons integrating into maturing cortical networks.
Neuron, 2020; 105
31780328: Modol L, Bollmann Y, Tressard T, Baude A, Che A, Duan ZRS, Babij R, De Marco García NV, Cossart R
Assemblies of Perisomatic GABAergic Neurons in the Developing Barrel Cortex.
The developmental journey of cortical interneurons encounters several activity-dependent milestones. During the early postnatal period in developing mice, GABAergic neurons are transient preferential recipients of thalamic inputs and undergo activity-dependent migration arrest, wiring, and programmed cell-death. Despite their importance for the emergence of sensory experience and the role of activity in their integration into cortical networks, the collective dynamics of GABAergic neurons during that neonatal period remain unknown. Here, we study coordinated activity in GABAergic cells of the mouse barrel cortex using in vivo calcium imaging. We uncover a transient structure in GABAergic population dynamics that disappears in a sensory-dependent process. Its building blocks are anatomically clustered GABAergic assemblies mostly composed by prospective parvalbumin-expressing cells. These progressively widen their territories until forming a uniform perisomatic GABAergic network. Such transient patterning of GABAergic activity is a functional scaffold that links the cortex to the external world prior to active exploration. VIDEO ABSTRACT.
Neuron, 2020; 105
28922859: Mòdol L, Sousa VH, Malvache A, Tressard T, Baude A, Cossart R
Spatial Embryonic Origin Delineates GABAergic Hub Neurons Driving Network Dynamics in the Developing Entorhinal Cortex.
Coordinated neuronal activity is essential for the development of cortical circuits. GABAergic hub neurons that function in

orchestrating early neuronal activity through a widespread net of postsynaptic partners are therefore critical players in the establishment of functional networks. Evidence for hub neurons was previously found in the hippocampus, but their presence in other cortical regions remains unknown. We examined this issue in the entorhinal cortex, an initiation site for coordinated activity in the neocortex and for the activity-dependent maturation of the entire entorhinal-hippocampal network. Using an unbiased approach that identifies "driver hub neurons" displaying a high number of functional links in living slices, we show that while almost half of the GABAergic cells single-handedly influence network dynamics, only a subpopulation of cells born in the MGE and composed of somatostatin-expressing neurons located in infragranular layers, spontaneously operate as "driver" hubs. This indicates that despite differences in the origin of interneuron diversity, the hippocampus and entorhinal cortex share similar developmental mechanisms for the establishment of functional circuits.
Cereb Cortex, 2017; 27
25972170: Mòdol L, Santos D, Cobianchi S, González-Pérez F, López-Alvarez V, Navarro X
NKCC1 Activation Is Required for Myelinated Sensory Neurons Regeneration through JNK-Dependent Pathway.
After peripheral nerve injury, axons are able to regenerate, although specific sensory reinnervation and functional recovery are usually worse for large myelinated than for small sensory axons. The mechanisms that mediate the regeneration of different sensory neuron subpopulations are poorly known. The Na(+)-K(+)-Cl(-) cotransporter 1 (NKCC1) is particularly relevant in setting the intracellular chloride concentration. After axotomy, increased NKCC1 phosphorylation has been reported to be important for neurite outgrowth of sensory neurons; however, the mechanisms underlying its effects are still unknown. In the present study we used in vitro and in vivo models to assess the differential effects of blocking NKCC1 activity on the regeneration of different types of dorsal root ganglia (DRGs) neurons after sciatic nerve injury in the rat. We observed that blocking NKCC1 activity by bumetanide administration induces a selective effect on neurite outgrowth and regeneration of myelinated fibers without affecting unmyelinated DRG neurons. To further study the mechanism underlying NKCC1 effects, we also assessed the changes in mitogen-activated protein kinase (MAPK) signaling under NKCC1 modulation. The inhibition of NKCC1 activity in vitro and in vivo modified pJNK1/2/3 expression in DRG neurons. Together, our study identifies a mechanism selectively contributing to myelinated axon regeneration, and point out the role of Cl(-) modulation in DRG neuron regeneration and in the activation of MAPKs, particularly those belonging to the JNK family.
J Neurosci, 2015; 35
24861264: Mòdol L, Casas C, Llidó A, Navarro X, Pallarès M, Darbra S
Neonatal allopregnanolone or finasteride administration modifies hippocampal K(+) Cl(-) co-transporter expression during early development in male rats.
The maintenance of levels of endogenous neurosteroids (NS) across early postnatal development of the brain, particularly to the hippocampus, is crucial for their maturation. Allopregnanolone (Allop) is a NS that exerts its effect mainly through the modulation of the GABAA receptor (GABAAR). During early development, GABA, acting through GABAAR, that predominantly produces depolarization shifts to hyperpolarization in mature neurons, around the second postnatal week in rats. Several factors contribute to this change including the progressive increase of the neuron-specific K(+)/Cl(-) co-transporter 2 (KCC2) (a chloride exporter) levels. Thus, we aimed to analyze whether a different profile of NS levels during development is critical and can alter this natural progression of KCC2 stages. We administrated sustained Allop (20mg/kg) or Finasteride (5α-reductase inhibitor, 50mg/kg) from the 5th postnatal day (PD5) to PD9 and assessed changes in the hippocampal expression of KCC2 at transcript and protein levels as well as its active phosphorylated state in male rats. Taken together data indicated that manipulation of NS levels during early development influence KCC2 levels and point out the importance of neonatal NS levels for the hippocampal development.
J Steroid Biochem Mol Biol, 2014; 143
24813295: Mòdol L, Cobianchi S, Navarro X
Prevention of NKCC1 phosphorylation avoids downregulation of KCC2 in central sensory pathways and reduces neuropathic pain after peripheral nerve injury.
Neuropathic pain after peripheral nerve injury is characterized by loss of inhibition in both peripheral and central pain pathways. In the adult nervous system, the Na(+)-K(+)-2Cl(-) (NKCC1) and neuron-specific K(+)-Cl(-) (KCC2) cotransporters are involved in setting the strength and polarity of GABAergic/glycinergic transmission. After nerve injury, the balance between these cotransporters changes, leading to a decrease in the inhibitory tone. However, the role that NKCC1 and KCC2 play in pain-processing brain areas is unknown. Our goal was to study the effects of peripheral nerve injury on NKCC1 and KCC2 expression in dorsal root ganglia (DRG), spinal cord, ventral posterolateral (VPL) nucleus of the thalamus, and primary somatosensory (S1) cortex. After sciatic nerve section and suture in adult rats, assessment of mechanical and thermal pain thresholds showed evidence of hyperalgesia during the following 2 months. We also found an increase in NKCC1 expression in the DRG and a downregulation of KCC2 in spinal cord after injury, accompanied by later decrease of KCC2 levels in higher projection areas (VPL and S1) from 2 weeks postinjury, correlating with neuropathic pain signs. Administration of bumetanide (30 mg/kg) during 2 weeks following sciatic nerve lesion prevented the previously observed changes in the spinothalamic

tract projecting areas and the appearance of hyperalgesia. In conclusion, the present results indicate that changes in NKCC1 and KCC2 in DRG, spinal cord, and central pain areas may contribute to development of neuropathic pain.
Pain, 2014; 155
24478630: Mòdol L, Mancuso R, Alé A, Francos-Quijorna I, Navarro X
Differential effects on KCC2 expression and spasticity of ALS and traumatic injuries to motoneurons.
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease manifested by progressive muscle atrophy and paralysis due to the loss of upper and lower motoneurons (MN). Spasticity appears in ALS patients leading to further disabling consequences. Loss of the inhibitory tone induced by downregulation of the potassium chloride cotransporter 2 (KCC2) in MN has been proposed to importantly contribute to the spastic behavior after spinal cord injury (SCI). The aim of the present study was to test whether the alterations in the expression of KCC2 are linked to the appearance of spasticity in the SOD(G93A) ALS murine model. We compared SOD(G93A) mice to wild type mice subjected to SCI to mimic the spinal MN disconnection from motor descending pathways, and to sciatic nerve lesion to mimic the loss of MN connectivity to muscle. Electrophysiological results show that loss of motor function is observed at presymptomatic stage (8 weeks) in SOD(G93A) mice but hyperreflexia and spasticity do not appear until a late stage (16 weeks). However, KCC2 was not downregulated despite MN suffered disconnection both from muscles and upper MNs. Further experiments revealed decreased gephyrin expression, as a general marker of inhibitory systems, accompanied by a reduction in the number of Renshaw interneurons. Moreover, 5-HT fibers were increased in the ventral horn of the lumbar spinal cord at late stage of disease progression in SOD1(G93A) mice. Taken together, the present results indicate that spasticity appears late in the ALS model, and may be mediated by a decrease in inhibitory interneurons and an increase of 5-HT transmission, while the absence of down-regulation of KCC2 could rather indicate an inability of MNs to respond to insults.
Front Cell Neurosci, 2014; 8
24011224: Modol L, Casas C, Navarro X, Llidó A, Vallée M, Pallarès M, Darbra S
Neonatal finasteride administration alters hippocampal α4 and δ GABAAR subunits expression and behavioural responses to progesterone in adult rats.
Allopregnanolone is a neurosteroid that has been reported to fluctuate during early developmental stages. Previous experiments reported the importance of neonatal endogenous allopregnanolone levels for the maturation of the central nervous system and particularly for the hippocampus. Changes in neonatal allopregnanolone levels have been related to altered adult behaviour and with psychopathological susceptibility, including anxiety disorders, schizophrenia and drug abuse. However, the mechanism underlying these changes remains to be elucidated. In the present study we assessed changes in hippocampal expression of α4 and δ GABAA receptor (GABAAR) subunits as a consequence of neonatal finasteride (a 5-α reductase inhibitor) administration during early development (PD6 to PD15) in male rats. We observed that the treatment altered the temporal window of the natural peak in the expression of these subunits during development. Additionally, the level of these subunits were higher than in non-handled and control animals in the adult hippocampus. We observed that in adulthood, neonatal finasteride-treated animals presented an anxiogenic-like profile in response to progesterone administration which was absent in the rest of the groups. In conclusion, these results corroborate the relevance of neonatal maintenance of neurosteroid levels for behavioural anxiety responses in the adult, and point to some of the mechanisms involved in this alterations.
Int J Neuropsychopharmacol, 2014; 17
23228522: Mòdol L, Darbra S, Vallèe M, Pallarès M
Alteration of neonatal Allopregnanolone levels affects exploration, anxiety, aversive learning and adult behavioural response to intrahippocampal neurosteroids.
Neurosteroids (NS) are well known to exert modulatory effects on ionotropic receptors. Recent findings indicate that NS could also act as important factors during development. In this sense, neonatal modifications of Allopregnanolone (Allop) levels during critical periods have been demonstrate to alter the morphology of the hippocampus but also other brain structures. The aim of the present work is to screen whether the alterations of Allop levels modify adult CA1 hippocampal response to NS administration. For this purpose, pups were injected with Allop (20 mg/kg s.c.), Finasteride (5α-reductase inhibitor that impedes Allop synthesis) (50 mg/kg s.c.) or Vehicle from postnatal day 5 (P5) to postnatal day 9 (P9). NS levels were tested at P5. To test the behavioural hippocampal response to NS in adulthood, animals were implanted with a bilateral cannula into the CA1 hippocampus at 80 days old and injected with Allop (0.2 µg/0.5 µl), Pregnenolone sulphate (5 ng/0.5 µl) or Vehicle in each hippocampus. After injections animals were tested in the Boisser test to assess exploratory behaviour, the elevated plus maze to assess anxiety and the passive avoidance to test aversive learning. Results indicate that alteration of neonatal Allop or pregnenolone levels (by Allop and Finasteride administration, respectively) suppressed intrahippocampal Allop anxiolytic effect in the EPM. Moreover our results also indicate that manipulation of neonatal Allop levels (Allop and Finast administration) alters exploratory and anxiety-like behaviour and impairs aversive learning in the adulthood. These data point out the role of Allop in the maturation of hippocampal function and behaviour.

Behav Brain Res, 2013; 241
21463656: Mòdol L, Darbra S, Pallarès M
Neurosteroids infusion into the CA1 hippocampal region on exploration, anxiety-like behaviour and aversive learning.
Neurosteroids (NS) are substances synthesised de novo in the brain that have rapid modulatory effects on ionotropic receptors. Specifically, NS can act as positive allosteric modulators of GABAA receptors as pregnanolone or allopregnanolone (Allop), or GABAA negative modulators and NMDA positive modulators as pregnenolone (PREG) or dehydroepiandrosterone (DHEA) and their sulphate esters (PREGS and DHEAS). Given this, their role in anxiety and emotional disturbances has been suggested. In addition, NS such as PREGS or DHEAS have demonstrated a promnesic role in several learning tests. The aim of the present work is to highlight the role that the dorsal (CA1) hippocampus plays in the behavioural profile of NS such as Allop and PREGS in tests assessing exploration, anxiety and aversive learning in rats. For this purpose, animals were administered intrahippocampally with Allop (0.2µg/0.5µl), PREGS (5ng/0.5µl) or vehicle in each hippocampus, and tested in the Boissier and elevated plus maze (EPM) tests. For learning test we have chosen the passive avoidance paradigm. Results indicate that intrahippocampal administration of Allop enhances exploration, reflected in an increase in the total and the inner number of head-dips. Allop-injected animals also showed an increase in the percentage of entries into the open arms of the EPM, suggesting an anxiolytic-like profile. In addition, post-acquisition PREGS administration enhanced passive avoidance retention, while post-acquisition Allop administration had no effects on aversive learning retention. These results point out the important role of the dorsal (CA1) hippocampus in several NS behavioural effects, such as exploration, anxiety, learning and memory.
Behav Brain Res, 2011; 222

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BOARD NUMBER: S05-446

THETA OSCILLATIONS AS A MECHANISM FOR COMMUNICATION BETWEEN CORTICAL SENSORY AREAS, PERIRHINAL CORTEX AND HIPPOCAMPUS DURING SENSORY DETECTION AND MEMORY RECOLLECTION.

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Memory consolidation and recall have been suggested to be driven by coordinated oscillatory activity, such as Hippocampal (HC) theta oscillations, of neuronal ensembles during memory-guided, spatial behaviour. Although multiple studies have demonstrated entrainment of neural assemblies on the hippocampal theta cycle (Vinck et al., 2016) , few investigations have investigated this phenomenon in brain-wide circuits by means of simultaneous multi-area ensemble recordings from distant regions. Additionally, it remains debatable which behavioural parameters most prominently relate to brain-wide coordinated oscillatory entrainment (Mizuseki & Buzsaki, 2014). Here we investigate coherency driven by oscillations between HC and the Visual (V2L), Somatosensory Barrel (S1BF) and Perirhinal (PER) cortices. These areas are interlinked trough anatomical projections and thought to form a cortical hierarchy facilitating the incorporation of sensory information into episodic memory (van Strien et al., 2009). Using a 36-tetrode hyperdrive, we recorded neural activity from four areas (V2, S1BF, PER, HC) simultaneously in freely behaving rats doing a multimodal–two alternative forced choice–object recognition task on a T-maze. The rats were shown one out of two objects under either tactile, visual or multimodal conditions and were rewarded if they responded on the arm of the T-maze associated with the object. Using the pairwise phase consistency we found increased spike-field coherency between HC theta cycles and neurons recorded in V2L (n=310), BR (n=118), PER (n=194) and HC (n=139), during behavioural epochs of sensory detection and memory recollection. Our results highlight the differences in processing between areas S1BF,V2L, PER and HC during the utilization of episodic memory.

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HIGH-DENSITY MULTIELECTRODE ARRAY RECORDINGS REVEAL COMPUTATIONAL COMPLEXITY IN CEREBELLAR CORTICAL PROCESSING

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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The cerebellar cortex has a prominent forward architecture that was interpreted as a predictor of low complexity of spatiotemporal activities compared to the cerebral cortex. However, the complexity of the cerebellar responses has never been assessed experimentally. We quantified the *complexity* of the spatiotemporal responses to mossy-fibers (mfs) stimulation in mouse acute cerebellar slices with the Perturbational Complexity Index (PCI), an index which has been used to summarize the richness of TMS/EEG spatiotemporal patterns in conscious/unconscious human subjects. We computed PCI with an adapted version of the original PCI algorithm, applied to neuronal activity recorded using a high-density multielectrode array (HD–MEA), to study the cerebellar cortical processing at different activity ranges. The *complexity* of granule cells (GrC) layer and PCs responses was quantified for different mfs stimulation frequencies. Our data revealed that PCI increased with the stimulation frequency and it was more pronounced in coronal slices, presumably due to the intense spread of activity along the parallel fibres, with the involvement of molecular layer interneurons (MLI) filtering in shaping PC output. PCI for the GrC and PC populations revealed that complexity in the cerebellar circuit changes dynamically with orientation of the signal flow, revealing that PCs contribution is strikingly different in the sagittal and coronal planes, contrary to GrCs. Finally, the analysis of the temporal and spatial correlation of PCs firing completed the characterization of PCs contribution to shaping the features of the whole network.

Pubmed:
33378395: Masoli S, Ottaviani A, Casali S, D'Angelo E
Cerebellar Golgi cell models predict dendritic processing and mechanisms of synaptic plasticity.
The Golgi cells are the main inhibitory interneurons of the cerebellar granular layer. Although recent works have highlighted the complexity of their dendritic organization and synaptic inputs, the mechanisms through which these neurons integrate complex input patterns remained unknown. Here we have used 8 detailed morphological reconstructions to develop multicompartmental models of Golgi cells, in which Na, Ca, and K channels were distributed along dendrites, soma, axonal initial segment and axon. The models faithfully reproduced a rich pattern of electrophysiological and pharmacological properties and predicted the operating mechanisms of these neurons. Basal dendrites turned out to be more tightly electrically coupled to the axon initial segment than apical dendrites. During synaptic transmission, parallel fibers caused slow Ca-dependent depolarizations in apical dendrites that boosted the axon initial segment encoder and Na-spike backpropagation into basal dendrites, while inhibitory synapses effectively shunted backpropagating currents. This oriented dendritic processing set up a coincidence detector controlling voltage-dependent NMDA receptor unblock in basal dendrites, which, by regulating local calcium influx, may provide the basis for spike-timing dependent plasticity anticipated by theory.
PLoS Comput Biol, 2020; 16

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BOARD NUMBER: S05-448

ATTENTIONAL EFFECTS EMBEDDED IN LARGE-SCALE SYNCHRONIZED NETWORKS

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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We recently demonstrated that rhythmic neuronal oscillations form spatially distinct functional modules that are frequency-dependent but independent of oscillatory power (Vezoli et al., Neuron 2021). Here, we explore this functional heterogeneity with respect to inter-areal anatomical connectivity and in the context of attention. In particular, we explored attentional effects on the strength of rhythmic synchronizations for distinct frequency-specific networks. Two monkeys were trained to perform a visual attention task that required selectively responding to changes to a cued stimulus, while ignoring changes to the uncued stimulus. Recordings were made through high-density micro-electrocorticogram (micro-ECoG) electrode arrays implanted subdurally allowing simultaneous recordings of over 16 interconnected cortical areas distributed across occipital to prefrontal cortices. Attentional effects were characterized by contrasting trials in which the attended stimulus was presented in the hemifield contra- or ipsilateral to the recorded hemisphere (Attend controlateral vs. Attend ipsilateral) across four frequency-bands of interest (theta, beta, high-beta and gamma). This showed that the strength of top-down anatomical projections predicts the strength of attention effects at the top-down targets. We then investigated how rhythm-specific functional interaction networks subserve attention, and show that gamma- and beta-band networks display enhanced interareal influences when mediating behaviorally relevant signals whereas theta- and high-beta-band networks displayed mixed attentional effects. We discuss the implications of these attentional effects on the engagement of both top-down and bottom-up networks in hierarchical processing. JV, AMB and CAB contributed equally

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NEURONAL AVALANCHES DIFFERENTIATE RESTING-STATE AND TASK CONDITIONS IN BRAIN-COMPUTER INTERFACES

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Brain-Computer Interfaces (BCIs) constitute a promising tool, but mastering non-invasive BCI remains a poorly-understood learned skill (1), since the underlying brain processes and their reflection on brain signals are unknown. Brain dynamic is characterized by aperiodic perturbations (i.e. "Neuronal Avalanches") (2) preferentially spreading across the white-matter bundles (3). Different avalanche dynamics might underlie different behaviors. To test our hypothesis we applied the neuronal avalanches approach to MEG data recorded in 19 subjects (aged 28.1 3.6 years, 11 men) during a motor-imagery-based BCI training session (6). We characterized dynamics using avalanche transition matrices (ATM), containing the probability that region j would be active at time t+1 when region i was active at time t. Then, for each subject, we compared the ATMs for the two experimental conditions (Fig. 1). Our results show that all the significantly different edges cluster upon a limited number of brain regions. In particular, the premotor areas appear to differ, bilaterally, between the conditions. The involvement is prominent to the left. Furthermore, the cunei bilaterally are also shown to differ between the two conditions, which might be related to the role these regions play in visual processing. In conclusion, selectively focusing on higher-order dynamics, we pinpointed the regions whose recruitment is affected by the execution of a motor imagery task. Our results suggest that avalanches capture functionally-relevant processes of interest for alternative BCI designing.

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THE TEMPORAL COORDINATION OF LATERAL HYPOTHALAMIC ENSEMBLES DRIVES THE PROGRESSION OF INNATE BEHAVIOURS.

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Innate behaviours are vital for survival, however the neural dynamics that terminate and initiate them promptly are elusive. It is unclear whether the lateral hypothalamus (LH), a key region for the maintenance of innate behaviours, also codes for their progression. LH neurons change their firing rate according to behaviours and the timing of neuronal discharge during network oscillations according to the activity in afferent and efferent regions can be instrumental for engagement in innate behaviours. Here, using electrophysiological recordings in behaving mice, neural decoding, and optogenetics, we studied the dynamics of the neural circuitry entailing LH that underlies engagement in feeding and exploratory behaviours. We investigated the timing of LH neurons during network oscillations, including those in the gamma band (>30 Hz), during food intake and exploration. The timing of neuronal discharge during oscillations was found to be sufficient to reliably decode a relative stage of innate behaviours by the Support Vector Machines. We studied the behavioural necessity of this code using optogenetic re-entrainment of inhibition to LH cells and stimulation of excitatory inputs, and found opposing effects of these manipulations on behavioural progressions. Probabilistic modelling revealed interdependencies between behaviours in their sequential organization. Our results highlight the role of short-time-scale interactions in the organization of hypothalamic information processing and in the regulation of multiple innate behaviours, dysfunctions of which underlie some major neuropsychiatric disorders. We acknowledge support by ERC (772994, FeedHypNet, TK) and DFG (233886668-GRK1960, CC, AC, 431549029-SFB1451, EXC2030 CECAD, TK, SPP1665, 1799/1-2,1799/3-1, AP), ELAN-IZKF FAU, P070, AP.

Pubmed:
34376649: Gao X, Bender F, Soh H, Chen C, Altafi M, Schütze S, Heidenreich M, Gorbati M, Corbu MA, Carus-Cadavieco M, Korotkova T, Tzingounis AV, Jentsch TJ, Ponomarenko A
Place fields of single spikes in hippocampus involve Kcnq3 channel-dependent entrainment of complex spike bursts. Hippocampal pyramidal cells encode an animal's location by single action potentials and complex spike bursts. These elementary signals are believed to play distinct roles in memory consolidation. The timing of single spikes and bursts is determined by intrinsic excitability and theta oscillations (5-10 Hz). Yet contributions of these dynamics to place fields remain elusive due to the lack of methods for specific modification of burst discharge. In mice lacking Kcnq3-containing M-type K channels, we find that pyramidal cell bursts are less coordinated by the theta rhythm than in controls during spatial navigation, but not alert immobility. Less modulated bursts are followed by an intact post-burst pause of single spike firing, resulting in a temporal discoordination of network oscillatory and intrinsic excitability. Place fields of single spikes in one- and two-dimensional environments are smaller in the mutant. Optogenetic manipulations of upstream signals reveal that neither medial septal GABA-ergic nor cholinergic inputs alone, but rather their joint activity, is required for entrainment of bursts. Our results suggest that altered representations by bursts and single spikes may contribute to deficits underlying cognitive disabilities associated with KCNQ3-mutations in humans.
Nat Commun, 2021; 12

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BOARD NUMBER: S05-451

LOCAL DYNAMICS AND DISTANT INTERACTIONS BETWEEN MEDIAL AND LATERAL PREFRONTAL CORTEX DURING PERFORMANCE MONITORING

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Flexible cognition relies on flexible information processing and on dynamical interactions between multiple brain structures. Neural dynamics in and between medial and lateral frontal areas in primates reflect some aspects of flexible information processing, however the detailed mechanisms and dynamics of regional interactions during flexible decision making are still unknown. We acquired and analysed a unique set of simultaneous recordings in the lateral prefrontal cortex and midcingulate cortex in macaques using couples of linear probes. Monkeys performed a 3-arm bandit probabilistic task interacting with a realistic target design (skeuomorphic design) on a touchscreen, a task in which monkeys relied on negative and positive outcomes to make decisions. We analysed the local field potentials from 83 recordings with dual 16 contacts probes, as well as 2827 isolated units recorded across MCC and LPFC regions. We showed that while both LPFC and MCC encoded feedback with sustained coding bridging successive trials, MCC was recruited first and more strongly than LPFC. In addition, Granger causality measures revealed that for a majority of site couples, MCC signals forecasted LPFC, rarely the reverse. These preliminary analyses provide important evidence supporting a model of MCC driving LPFC at the time of adaptation.

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MORPHOLOGICALLY REALISTIC NEURONS ARE ALTERNATIVES TO ARTIFICIAL NEURAL NETWORKS

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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From the perspective of synaptic input and spiking output, a morphologically complex neuron is (almost) equivalent to a single output multilayered artificial neural network. However, it is not known if they can perform equivalent computations. Recently Bicknell and Hausser (2021) showed that a single neuron of complex morphology can be trained to distinguish different synaptic patterns which previously were considered to require multilayer neural networks. Here, using the method of Bicknell and Hausser, we study minimal computational models of a dendritic tree trained on a classification task that requires complex, nonlinear computations. We show which aspects of dendritic morphology and synaptic locations influence feature differentiation and how to select them optimally. Bicknell, B. A., & Häusser, M. (2021). A synaptic learning rule for exploiting nonlinear dendritic computation. *Neuron*, *109*(24), 4001-4017.

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CHARACTERIZATION OF BRAIN NETWORKS USING FUNCTIONAL ULTRASOUND IMAGING

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Aims We often feel bad when witnessing a painful scene, because observing an emotion of a conspecific triggers the same emotion in the observer (Lorenz, 1935; Han et al., 2019). This phenomenon, emotional contagion, has been observed in humans and other species, including rodents (Meyza et al., 2017). Human fMRI studies identified the involved brain circuitry, encompassing several brain regions (Gu et al., 2010). In rodents, invasive techniques can inform on single cell responses and specific connections (Carrillo et al., 2019), but identifying the whole circuit remains difficult. To validate social animal models and gain insight into the evolution of emotional contagion, we need the ability to read-out emotional circuitries in rodents and compare them to those found in humans. By using, as for fMRI, blood flow changes as indirect measure of brain activity, Functional Ultrasound Imaging (fUSI) can bridge this gap, by allowing imaging of big volumes of the brain of awake rodents (Macé et al., 2018). We started with a validation study. **Methods** Using a 128-channels linear ultrasound probe, 2D images of the mouse brain were acquired at Bregma -3 mm during presentation of 25 20 second drifting black-and-white gratings. **Results** Correlation analyses showed increased blood flow with stimulus presentation in the expected regions: the superior colliculi and primary visual cortices. Additionally, the signal was stronger for the contra-lateral hemisphere during stimulation of one eye. **Conclusions** These results show we are able to perform reliable fUSI experiments.

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IS THE WHOLE BRAIN CRITICAL?

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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An increasing body of evidence suggests that healthy brain operates near critical state. Scale-free distributions of neuronal avalanches, the hallmark of criticality, have been described in cortical networks in a variety of species. Yet, the avalanche dynamics in deeper brain structures has remained unelucidated. Here we describe neural avalanches in the amygdala in comparison with the prefrontal cortex in the developing brain using *in vivo* multielectrode recordings in urethane anaesthetized juvenile rats (postnatal days 14-15). Scale-free statistics of neural avalanches in the cortex are expressed in power-law avalanche size distributions with cut-off corresponding to the maximum number of recording sites as also found in our study. However, in the amygdala the cut-off was preceded by a sharp peak at the maximum number of recording sites, meaning that the avalanches spanning over all the electrodes were most abundant in the amygdala. Smaller decay exponents indicated that small avalanches are less prevalent in the amygdala than in the cortex. We further characterized the spread of activity by calculating branching ratio. In critical systems the mean branching ratio is close to one. In line with previous findings in cortical networks, our results show that it is true for the prefrontal cortex whereas avalanches in the amygdala are characterized by higher branching ratio. Taken together, our results suggest that in the amygdala, unlike in the cortex, activity tends to stretch rapidly over the whole region indicating supercritical state.

Pubmed:
28856535: Atanasova T, Kharybina Z, Kaarela T, Huupponen J, Luchkina NV, Taira T, Lauri SE
GluA4 Dependent Plasticity Mechanisms Contribute to Developmental Synchronization of the CA3-CA1 Circuitry in the Hippocampus.
During the course of development, molecular mechanisms underlying activity-dependent synaptic plasticity change considerably. At immature CA3-CA1 synapses in the hippocampus, PKA-driven synaptic insertion of GluA4 AMPA receptors is the predominant mechanism for synaptic strengthening. However, the physiological significance of the developmentally restricted GluA4-dependent plasticity mechanisms is poorly understood. Here we have used microelectrode array (MEA) recordings in GluA4 deficient slice cultures to study the role of GluA4 in early development of the hippocampal circuit function. We find that during the first week in culture (DIV2-6) when GluA4 expression is restricted to pyramidal neurons, loss of GluA4 has no effect on the overall excitability of the immature network, but significantly impairs synchronization of the CA3 and CA1 neuronal populations. In the absence of GluA4, the temporal correlation of the population spiking activity between CA3-CA1 neurons was significantly lower as compared to wild-types at DIV6. Our data show that synapse-level defects in transmission and plasticity mechanisms are efficiently compensated for to normalize population firing rate at the immature hippocampal network. However, lack of the plasticity mechanisms typical for the immature synapses may perturb functional coupling between neuronal sub-populations, a defect frequently implicated in the context of developmentally originating neuropsychiatric disorders.
Neurochem Res, 2019; 44

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-455

SPATIALLY CONFINED OPTOGENETIC PERTURBATIONS IN THE MOUSE MOTOR CORTEX FOR DECOMPOSING LOCAL FIELD POTENTIALS

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Subdural micro electrocorticography (µECoG) arrays capture the superimposed electrical activity of ionic processes in the brain volume beneath. Compared to electroencephalography (EEG) recordings, µECoGs offer a higher spatial resolution and broader frequency range and thus allow a more sensitive readout of the spatio-temporal network dynamics, while remaining minimally invasive compared to intracortical electrodes. Therefore, µECoG recordings possess a great translational potential for the development of brain-computer interfaces (BCIs) and neuroprosthesis. However, a major caveat in interpreting µECoG data is the limited understanding of the physiological origin of the surface local field potential (LFP). The efficiency of future BCIs based on the decoding of ECoG data, requires a profound understanding of the cellular and morphological contribution of different signal sources to the LFP and the advantages of high-density µECoG recordings for the identification of intracortical dynamics. In order to overcome this drawback, we apply joint depth (Neuropixels) and high-resolution µECoG array recordings in the motor cortex of awake and behaving head-fixed mice which where trained to run through a virtual linear track while their 3D paw position was reconstructed using DeepLabCut. This approach allows linking the ongoing cortical dynamics across all layers with the surface LFP. Further, we apply depth-controlled optogenetic modulation of distinct layers of a cortical column using tapered optical fibers and soma-targeted opsins in order to address one isolated laminar source at a time and interrogate its contribution to the depth and surface LFP in a causal manner.

Pubmed:
20865013: Zimmer B, Kuegler PB, Baudis B, Genewsky A, Tanavde V, Koh W, Tan B, Waldmann T, Kadereit S, Leist M
Coordinated waves of gene expression during neuronal differentiation of embryonic stem cells as basis for novel approaches to developmental neurotoxicity testing.
As neuronal differentiation of embryonic stem cells (ESCs) recapitulates embryonic neurogenesis, disturbances of this process may model developmental neurotoxicity (DNT). To identify the relevant steps of in vitro neurodevelopment, we implemented a differentiation protocol yielding neurons with desired electrophysiological properties. Results from focussed transcriptional profiling suggested that detection of non-cytotoxic developmental disturbances triggered by toxicants such as retinoic acid (RA) or cyclopamine was possible. Therefore, a broad transcriptional profile of the 20-day differentiation process was obtained. Cluster analysis of expression kinetics, and bioinformatic identification of overrepresented gene ontologies revealed waves of regulation relevant for DNT testing. We further explored the concept of superimposed waves as descriptor of ordered, but overlapping biological processes. The initial wave of transcripts indicated reorganization of chromatin and epigenetic changes. Then, a transient upregulation of genes involved in the formation and patterning of neuronal precursors followed. Simultaneously, a long wave of ongoing neuronal differentiation started. This was again superseded towards the end of the process by shorter waves of neuronal maturation that yielded information on specification, extracellular matrix formation, disease-associated genes and the generation of glia. Short exposure to lead during the final differentiation phase, disturbed neuronal maturation. Thus, the wave kinetics and the patterns of neuronal specification define the time windows and end points for examination of DNT.
Cell Death Differ, 2011; 18
21434924: Scholz D, Pöltl D, Genewsky A, Weng M, Waldmann T, Schildknecht S, Leist M
Rapid, complete and large-scale generation of post-mitotic neurons from the human LUHMES cell line.
We characterized phenotype and function of a fetal human mesencephalic cell line (LUHMES, Lund human mesencephalic) as neuronal model system. Neurodevelopmental profiling of the proliferation stage (d0, day 0) of these conditionally-immortalized cells revealed neuronal features, expressed simultaneously with some early neuroblast and stem cell markers. An optimized 2-step differentiation procedure, triggered by shut-down of the myc transgene, resulted in uniformly post-mitotic neurons within 5 days (d5). This was associated with down-regulation of some precursor markers and further up-regulation of neuronal genes. Neurite network formation involved the outgrowth of 1-2, often > 500 µm long projections. They showed

dynamic growth cone behavior, as evidenced by time-lapse imaging of stably GFP-over-expressing cells. Voltage-dependent sodium channels and spontaneous electrical activity of LUHMES continuously increased from d0 to d11, while levels of synaptic markers reached their maximum on d5. The developmental expression patterns of most genes and of the dopamine uptake- and release-machinery appeared to be intrinsically predetermined, as the differentiation proceeded similarly when external factors such as dibutyryl-cAMP and glial cell derived neurotrophic factor were omitted. Only tyrosine hydroxylase required the continuous presence of cAMP. In conclusion, LUHMES are a robust neuronal model with adaptable phenotype and high value for neurodevelopmental studies, disease modeling and neuropharmacology.
J Neurochem, 2011; 119
25427445: Genewsky A, Jost I, Busch C, Huber C, Stindl J, Skerka C, Zipfel PF, Rohrer B, Strauß O
Activation of endogenously expressed ion channels by active complement in the retinal pigment epithelium.
Defective regulation of the alternative pathway of the complement system is believed to contribute to damage of retinal pigment epithelial (RPE) cells in age-related macular degeneration. Thus we investigated the effect of complement activation on the RPE cell membrane by analyzing changes in membrane conductance via patch-clamp techniques and Ca(2+) imaging. Exposure of human ARPE-19 cells to complement-sufficient normal human serum (NHS) (25 %) resulted in a biphasic increase in intracellular free Ca(2+) ([Ca(2+)]i); an initial peak followed by sustained Ca(2+) increase. C5- or C7-depleted sera did not fully reproduce the signal generated by NHS. The initial peak of the Ca(2+) response was reduced by sarcoplasmic Ca(2+)-ATPase inhibitor thapsigargin, L-type channel blockers (R)-(+)-BayK8644 and isradipine, transient-receptor-potential (TRP) channel blocker ruthenium-red and ryanodine receptor blocker dantrolene. The sustained phase was carried by CaV1.3 L-type channels via tyrosine-phosphorylation. Changes in [Ca(2+)]I were accompanied by an abrupt hyperpolarization, resulting from a transient increase in membrane conductance, which was absent under extracellular Ca(2+)- or K(+)-free conditions and blocked by (R)-(+)-BayK8644 or paxilline, a maxiK channel inhibitor. Single-channel recordings confirmed the contribution of maxiK channels. Primary porcine RPE cells responded to NHS in a comparable manner. Pre-incubation with NHS reduced H2O2-induced cell death. In summary, in a concerted manner, C3a, C5a and sC5b-9 increased [Ca(2+)]i by ryanodine-receptor-dependent activation of L-type channels in addition to maxi-K channels and TRP channels absent from any insertion of a lytic pore.
Pflugers Arch, 2015; 467
27199662: Dine J, Genewsky A, Hladky F, Wotjak CT, Deussing JM, Zieglgänsberger W, Chen A, Eder M
Local Optogenetic Induction of Fast (20-40 Hz) Pyramidal-Interneuron Network Oscillations in the In Vitro and In Vivo CA1 Hippocampus: Modulation by CRF and Enforcement of Perirhinal Theta Activity.
The neurophysiological processes that can cause theta-to-gamma frequency range (4-80 Hz) network oscillations in the rhinal cortical-hippocampal system and the potential connectivity-based interactions of such forebrain rhythms are a topic of intensive investigation. Here, using selective Channelrhodopsin-2 (ChR2) expression in mouse forebrain glutamatergic cells, we were able to locally, temporally precisely, and reliably induce fast (20-40 Hz) field potential oscillations in hippocampal area CA1 in vitro (at 25°C) and in vivo (i.e., slightly anesthetized NEX-Cre-ChR2 mice). As revealed by pharmacological analyses and patch-clamp recordings from pyramidal cells and GABAergic interneurons in vitro, these light-triggered oscillations can exclusively arise from sustained suprathreshold depolarization (~200 ms or longer) and feedback inhibition of CA1 pyramidal neurons, as being mandatory for prototypic pyramidal-interneuron network (P-I) oscillations. Consistently, the oscillations comprised rhythmically occurring population spikes (generated by pyramidal cells) and their frequency increased with increasing spectral power. We further demonstrate that the optogenetically driven CA1 oscillations, which remain stable over repeated evocations, are impaired by the stress hormone corticotropin-releasing factor (CRF, 125 nM) in vitro and, even more remarkably, found that they are accompanied by concurrent states of enforced theta activity in the memory-associated perirhinal cortex (PrC) in vivo. The latter phenomenon most likely derives from neurotransmission via a known, but poorly studied excitatory CA1→PrC pathway. Collectively, our data provide evidence for the existence of a prototypic (CRF-sensitive) P-I gamma rhythm generator in area CA1 and suggest that CA1 P-I oscillations can rapidly up-regulate theta activity strength in hippocampus-innervated rhinal networks, at least in the PrC.
Front Cell Neurosci, 2016; 10
28890367: Heinz DE, Genewsky A, Wotjak CT
Enhanced anandamide signaling reduces flight behavior elicited by an approaching robo-beetle.
Our current knowledge of the implications of endocannabinoids in fear and anxiety is largely based on fear conditioning paradigms and approach-avoidance conflicts. Here we establish the ethobehavioral beetle mania task (BMT), which confronts mice with an erratically moving robo-beetle. With the help of this task we demonstrate decreased tolerance yet increased avoidance responses to an approaching beetle in high-anxiety behavior (HAB) and BALBc mice compared to C57BL/6N, CD1 and normal-anxiety behavior (NAB) mice. Also DBA/2N mice showed decreased passive and increased active behavior, but followed the robo-beetle more often than HAB and BALBc mice. Treatment with diazepam (1 mg/kg) increased tolerance without affecting avoidance behavior in HAB mice. Treatment with the MAGL inhibitor JZL184 (8 mg/kg) increased flight

behavior, but did not affect tolerance. The FAAH inhibitor URB597 (0.3 mg/kg), however, reduced flight behavior and enhanced tolerance to the robo-beetle. The latter effects were blocked by co-treatment with the CB1 receptor antagonist SR141716A (3 mg/kg), which failed to affect the behavior by itself. Taken together, we validate the BMT as a novel test for studying endocannabinoids beyond traditional paradigms and for assessing active fear responses in mice. Furthermore, we demonstrate panicolytic consequences of pharmacological enhancement of anandamide, but not 2-AG signaling.
Neuropharmacology, 2017; 126
29104536: Genewsky AJ, Wotjak CT
The Endocannabinoid System Differentially Regulates Escape Behavior in Mice.
Among the behaviors, fear or survival responses certainly belong to the most evolutionary conserved ones. However, higher animals possess the ability to adapt to certain environments (e.g., novel foraging grounds), and, therefore, those responses need to be plastic. Previous studies revealed a cell-type specific role of the endocannabinoid system in novelty fear, conditioned fear and active vs. passive avoidance in a shuttle box paradigm. In this study we aim to investigate, whether knocking-out the cannabinoid receptor type-1 (CB1) on cortical glutamatergic (Glu-CB1) or GABAergic (GABA-CB1) neurons differentially affects the level of behavioral inhibition, which could ultimately lead to differences in escape behavior. In this context, we developed a novel behavioral paradigm, the (MWB). Using the MWB task we could show that Glu-CB1 mice have higher levels of behavioral inhibition over the course of repeated testing. GABA-CB1 mice, in contrast, showed significantly lower levels of behavioral inhibition compared to wild-type controls and more escape behavior. These changes in behavioral inhibition and escape behavior cannot be explained by altered levels of arousal, as repeated startle measurements revealed general habituation irrespective of the line and genotype of the animals. Taken together, we could show that CB1 on cortical glutamatergic terminals is important for the acquisition of active avoidance, as the absence of CB1 on these neurons creates a bias toward inhibitory avoidance. This is the case in situations without punishment such as electric footshocks. On the contrary CB1 receptors on GABAergic neurons mediate the acquisition of passive avoidance, as the absence of CB1 on those neurons establishes a strong bias toward escape behavior.
Front Behav Neurosci, 2017; 11
29177007: Genewsky A, Heinz DE, Kaplick PM, Kilonzo K, Wotjak CT
A simplified microwave-based motion detector for home cage activity monitoring in mice.
Locomotor activity of rodents is an important readout to assess well-being and physical health, and is pivotal for behavioral phenotyping. Measuring homecage-activity with standard and cost-effective optical methods in mice has become difficult, as modern housing conditions (e.g. individually ventilated cages, cage enrichment) do not allow constant, unobstructed, visual access. Resolving this issue either makes greater investments necessary, especially if several experiments will be run in parallel, or is at the animals' expense. The purpose of this study is to provide an easy, yet satisfying solution for the behavioral biologist at novice makers level.
J Biol Eng, 2017; 11
29786085: Dedic N, Kühne C, Jakovcevski M, Hartmann J, Genewsky AJ, Gomes KS, Anderzhanova E, Pöhlmann ML, Chang S, Kolarz A, Vogl AM, Dine J, Metzger MW, Schmid B, Almada RC, Ressler KJ, Wotjak CT, Grinevich V, Chen A, Schmidt MV, Wurst W, Refojo D, Deussing JM
Chronic CRH depletion from GABAergic, long-range projection neurons in the extended amygdala reduces dopamine release and increases anxiety.
The interplay between corticotropin-releasing hormone (CRH) and the dopaminergic system has predominantly been studied in addiction and reward, while CRH-dopamine interactions in anxiety are scarcely understood. We describe a new population of CRH-expressing, GABAergic, long-range-projecting neurons in the extended amygdala that innervate the ventral tegmental area and alter anxiety following chronic CRH depletion. These neurons are part of a distinct CRH circuit that acts anxiolytically by positively modulating dopamine release.
Nat Neurosci, 2018; 21
29867370: Almada RC, Genewsky AJ, Heinz DE, Kaplick PM, Coimbra NC, Wotjak CT
Stimulation of the Nigrotectal Pathway at the Level of the Superior Colliculus Reduces Threat Recognition and Causes a Shift From Avoidance to Approach Behavior.
Defensive behavioral responses are essential for survival in threating situations. The superior colliculus (SC) has been implicated in the generation of defensive behaviors elicited by visual, tactile and auditory stimuli. Furthermore, substantia nigra pars reticulata (SNr) neurons are known to exert a modulatory effect on midbrain tectum neural substrates. However, the functional role of this nigrotectal pathway in threating situations is still poorly understood. Using optogenetics in freely behaving mice, we activated SNr projections at the level of the SC, and assessed consequences on behavioral performance in an open field test (OFT) and the beetle mania task (BMT). The latter confronts a mouse with an erratic moving robo-beetle and allows to measure active and passive defensive responses upon frequent encounter of the threatening object. Channelrhodopsin-2 (ChR2)-mediated activation of the inhibitory nigrotectal pathway did not affect anxiety-like and

exploratory behavior in the OFT, but increased the number of contacts between robo-beetle and test mouse in the BMT. Depending on the size of the arena, active avoidance responses were reduced, whereas tolerance and close following of the robo-beetle were significantly increased. We conclude from the data that the nigrotectal pathway plays holds the potential to modulate innate fear by attenuating threat recognition and causing a shift from defensive to approach behavior.
Front Neural Circuits, 2018; 12

33344695: Anderzhanova E, Hafner K, Genewsky AJ, Soliman A, Pöhlmann ML, Schmidt MV, Blum R, Wotjak CT, Gassen NC

The stress susceptibility factor FKBP51 controls S-ketamine-evoked release of mBDNF in the prefrontal cortex of mice.
We report here the involvement of the stress-responsive glucocorticoid receptor co-chaperone FKBP51 in the mechanism of secretion of mature BDNF (mBDNF). We used a novel method combining brain microdialysis with a capillary electrophoresis-based immunoassay, to examine mBDNF secretion in the medial prefrontal cortex (mPFC) in freely moving mice. By combining optogenetic, neurochemical (KCl-evoked depolarization), and transgenic (conditional BDNF knockout mice) means, we have shown that the increase in extracellular mBDNF is determined by neuronal activity. Withal, mBDNF secretion in the mPFC of mice was stimulated by a systemic administration of S-ketamine (10 or 50 mg/kg) or S-hydroxynorketamine (10 mg/kg). KCl- and S-ketamine-evoked mBDNF secretion was strongly dependent on the expression of FKBP51. Moreover, the inability of S-ketamine to evoke a transient secretion in mBDNF in the mPFC in FKBP51- knockout mice matched the lack of antidepressant-like effect of S-ketamine in the tail suspension test. Our data reveal a critical role of FKBP51 in mBDNF secretion and suggest the involvement of mBDNF in the realization of immediate stress-coping behavior induced by acute S-ketamine.
Neurobiol Stress, 2020; 13

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CORTICAL NEURONAL ASSEMBLIES COORDINATE WITH EEG MICROSTATE DYNAMICS DURING RESTING WAKEFULNESS

POSTER SESSION 05 - SECTION: NEURONAL INTERACTIONS AND SYNCHRONIZATION

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Cortical assemblies have been studied in the context of sensory processing, learning, and memory. However, their relation to conscious experience and dynamics across different natural vigilance states remain poorly understood despite their disruption being associated with induced loss of consciousness. To help address this gap in our knowledge, we employed 2-photon calcium imaging of sensorimotor cortex across the full sleep-wake cycle in a head-fixed mouse model. We found no difference in the structural characteristics of assemblies occurring during quiet wakefulness (QW), NREMs, or REMs, despite the latter two vigilance states being associated with significantly reduced levels of consciousness. To determine whether association of local assembly activity with macroscale brain dynamics could help explain this lack of consistent difference between assembly structure in wake versus sleep states, we combined the above imaging techniques with global EEG microstate analysis derived from multi-EEG recordings. When directly compared, evidence of a significant coordination between global EEG microstate dynamics and local cortical assembly activity was found during periods of resting wakefulness, but not sleep. These results suggest that the coordination of cortical assembly activity with global brain dynamics could be a key factor of sustained conscious experience and that a disengagement of local assemblies with global brain states occurs during sleep. More generally, this data suggests that EEG microstates could be an important mechanism for promoting coordination and temporary binding between local activity patterns in physically segregated neural populations in the awake resting brain.

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NEURONAL MECHANISMS OF VISUO-TACTILE INTEGRATION IN MOUSE ASSOCIATIVE CORTICES

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Objects are defined by physical properties that can stimulate different sensory modalities. Multisensory integration (MI) refers to the neural computations that transform signals originating from distinct sensory systems into a unified multimodal representation. However, the cortical circuits involved in producing coherent perceptual experiences of multimodal stimuli remain largely unexplored. In the mouse posterior parietal cortex, the rostrolateral area (RL) of the primary visual cortex (V1) receives direct inputs from both V1 and the primary somatosensory cortex but it is not clear if this is the siege of all visuo-tactile processing. We investigated how visuo-tactile signals are mapped in associative cortical regions and how these representations are used during goal-directed behaviours. Transgenic mice expressing fluorescent calcium indicator GCaMP6f in superficial cortical layers were implanted with a cranial window covering the left posterior hemisphere, providing a large optical access to somatosensory and visual cortical areas. Combining wide-field and two-photon calcium imaging, we recorded at both population and single-cell level in head-fixed mice during passive exposure to combinations of visuo-tactile stimuli. Calcium signals evoked by these stimulations helped to identify non-linear summations of visual and tactile responses in several cortical territories with different functional organizations. We further developed a Go/No-Go visuo-tactile discrimination paradigm where mice were trained to report perceptions of simultaneous visual and tactile stimuli while ignoring unisensory stimuli. We observed that mice exhibited frequent switches between strategies favouring specific sensory modalities during the task. These results illustrate how multiple cortical regions are involved in visuo-tactile MI and potentially support flexible visuo-tactile decision-making.

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BOARD NUMBER: S05-458

POSITION ESTIMATION AT VARYING SENSORY CONFLICTS IN THE HIPPOCAMPUS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

Andrey Sobolev[1], Evgeny Resnik[2], Dustin Fetterhoff[3], Kay Thurley[3], Christian Leibold[4], Anton Sirota[5]
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Estimation of an actual location in space involves simultaneous assessment of information coming from different sensory systems. Multiple behavioral studies demonstrated that independent estimations given by different sensory modalities are combined in an optimal manner. Moreover, while integration of coherent estimations increases response precision, it is reasonable to abandon a less reliable estimation if it is highly conflicting with others. The neural correlates of these mechanisms are still not fully described. We asked if position estimation performed by the hippocampal place cells follows these principles. Using the freely-moving 3D virtual reality for rodents we introduced a translational conflict between the virtual visual and the physical boundary-defined reference frames involved in position estimation and recorded hippocampal neural population in freely-foraging gerbils. Most of the place cells represented position using a weighted combination of sensory estimations, consistent with the concepts of the optimal coding framework. As a population, hippocampal neurons showed mixed representation of both reference frames, balanced between visual and physical environmental geometry depending on the proximity to the boundary and saliency of the visual stimulus. The activity of place fields in small / large conflicts followed the integration / abandonment of position estimations predicted by behavioral studies. Integration of the conflicting sensory information was continuous and led to recalibration of the hippocampal map, producing a new, morphed spatial representation. Finally, patterns of animal behavior parallelled resolution of the conflict predicted by the hippocampal representation in all conflicting conditions connecting altered spatial perception and the corresponding navigation in a mixed environment.

Pubmed:
34525357: Fetterhoff D, Sobolev A, Leibold C
Graded remapping of hippocampal ensembles under sensory conflicts.
Hippocampal place cells are thought to constitute a cognitive map of space derived from multimodal sensory inputs. Alteration of allocentric (visual) cues in a fixed environment is known to induce modulations of place cell activity to varying degrees from rate changes to global remapping. To determine how hippocampal ensembles combine multimodal sensory cues, we examine hippocampal CA1 remapping in Mongolian gerbils in a 1D virtual reality experiment, during which self-motion cues (locomotor, vestibular, and optic flow information) and allocentric visual cues are altered. We observe that self-motion cues are over-represented, but responsiveness to allocentric visual cues, although task-irrelevant, elicits both rate and global remapping in the hippocampal ensemble. We propose that remapping can be reconciled by considering global, partial, and rate remapping on a continuous scale on which the graded change of activity in the entire CA1 population can be interpreted as the expectancy about the animal's spatial environment.
Cell Rep, 2021; 36
24795616: Sobolev A, Stoewer A, Leonhardt A, Rautenberg PL, Kellner CJ, Garbers C, Wachtler T
Integrated platform and API for electrophysiological data.
Recent advancements in technology and methodology have led to growing amounts of increasingly complex neuroscience data recorded from various species, modalities, and levels of study. The rapid data growth has made efficient data access and flexible, machine-readable data annotation a crucial requisite for neuroscientists. Clear and consistent annotation and organization of data is not only an important ingredient for reproducibility of results and re-use of data, but also essential for collaborative research and data sharing. In particular, efficient data management and interoperability requires a unified approach that integrates data and metadata and provides a common way of accessing this information. In this paper we describe GNData, a data management platform for neurophysiological data. GNData provides a storage system based on a data representation that is suitable to organize data and metadata from any electrophysiological experiment, with a

functionality exposed via a common application programming interface (API). Data representation and API structure are compatible with existing approaches for data and metadata representation in neurophysiology. The API implementation is based on the Representational State Transfer (REST) pattern, which enables data access integration in software applications and facilitates the development of tools that communicate with the service. Client libraries that interact with the API provide direct data access from computing environments like Matlab or Python, enabling integration of data management into the scientist's experimental or analysis routines.
Front Neuroinform, 2014; 8

24634654: Sobolev A, Stoewer A, Pereira M, Kellner CJ, Garbers C, Rautenberg PL, Wachtler T
Data management routines for reproducible research using the G-Node Python Client library.
Structured, efficient, and secure storage of experimental data and associated meta-information constitutes one of the most pressing technical challenges in modern neuroscience, and does so particularly in electrophysiology. The German INCF Node aims to provide open-source solutions for this domain that support the scientific data management and analysis workflow, and thus facilitate future data access and reproducible research. G-Node provides a data management system, accessible through an application interface, that is based on a combination of standardized data representation and flexible data annotation to account for the variety of experimental paradigms in electrophysiology. The G-Node Python Library exposes these services to the Python environment, enabling researchers to organize and access their experimental data using their familiar tools while gaining the advantages that a centralized storage entails. The library provides powerful query features, including data slicing and selection by metadata, as well as fine-grained permission control for collaboration and data sharing. Here we demonstrate key actions in working with experimental neuroscience data, such as building a metadata structure, organizing recorded data in datasets, annotating data, or selecting data regions of interest, that can be automated to large degree using the library. Compliant with existing de-facto standards, the G-Node Python Library is compatible with many Python tools in the field of neurophysiology and thus enables seamless integration of data organization into the scientific data workflow.
Front Neuroinform, 2014; 8

33100981: Ferreiro DN, Amaro D, Schmidtke D, Sobolev A, Gundi P, Belliveau L, Sirota A, Grothe B, Pecka M
Sensory Island Task (SIT): A New Behavioral Paradigm to Study Sensory Perception and Neural Processing in Freely Moving Animals.
A central function of sensory systems is the gathering of information about dynamic interactions with the environment during self-motion. To determine whether modulation of a sensory cue was externally caused or a result of self-motion is fundamental to perceptual invariance and requires the continuous update of sensory processing about recent movements. This process is highly context-dependent and crucial for perceptual performances such as decision-making and sensory object formation. Yet despite its fundamental ecological role, voluntary self-motion is rarely incorporated in perceptual or neurophysiological investigations of sensory processing in animals. Here, we present the Sensory Island Task (SIT), a new freely moving search paradigm to study sensory processing and perception. In SIT, animals explore an open-field arena to find a sensory target relying solely on changes in the presented stimulus, which is controlled by closed-loop position tracking in real-time. Within a few sessions, animals are trained via positive reinforcement to search for a particular area in the arena ("target island"), which triggers the presentation of the target stimulus. The location of the target island is randomized across trials, making the modulated stimulus feature the only informative cue for task completion. Animals report detection of the target stimulus by remaining within the island for a defined time ("sit-time"). Multiple "non-target" islands can be incorporated to test psychometric discrimination and identification performance. We exemplify the suitability of SIT for rodents (Mongolian gerbil,) and small primates (mouse lemur,) and for studying various sensory perceptual performances (auditory frequency discrimination, sound source localization, visual orientation discrimination). Furthermore, we show that pairing SIT with chronic electrophysiological recordings allows revealing neuronal signatures of sensory processing under ecologically relevant conditions during goal-oriented behavior. In conclusion, SIT represents a flexible and easily implementable behavioral paradigm for mammals that combines self-motion and natural exploratory behavior to study sensory sensitivity and decision-making and their underlying neuronal processing.
Front Behav Neurosci, 2020; 14

27486397: Zehl L, Jaillet F, Stoewer A, Grewe J, Sobolev A, Wachtler T, Brochier TG, Riehle A, Denker M, Grün S
Handling Metadata in a Neurophysiology Laboratory.
To date, non-reproducibility of neurophysiological research is a matter of intense discussion in the scientific community. A crucial component to enhance reproducibility is to comprehensively collect and store metadata, that is, all information about the experiment, the data, and the applied preprocessing steps on the data, such that they can be accessed and shared in a consistent and simple manner. However, the complexity of experiments, the highly specialized analysis workflows and a lack of knowledge on how to make use of supporting software tools often overburden researchers to perform such a detailed documentation. For this reason, the collected metadata are often incomplete, incomprehensible for outsiders or ambiguous.

Based on our research experience in dealing with diverse datasets, we here provide conceptual and technical guidance to overcome the challenges associated with the collection, organization, and storage of metadata in a neurophysiology laboratory. Through the concrete example of managing the metadata of a complex experiment that yields multi-channel recordings from monkeys performing a behavioral motor task, we practically demonstrate the implementation of these approaches and solutions with the intention that they may be generalized to other projects. Moreover, we detail five use cases that demonstrate the resulting benefits of constructing a well-organized metadata collection when processing or analyzing the recorded data, in particular when these are shared between laboratories in a modern scientific collaboration. Finally, we suggest an adaptable workflow to accumulate, structure and store metadata from different sources using, by way of example, the odML metadata framework.
Front Neuroinform, 2016; 10
24600386: Garcia S, Guarino D, Jaillet F, Jennings T, Pröpper R, Rautenberg PL, Rodgers CC, Sobolev A, Wachtler T, Yger P, Davison AP
Neo: an object model for handling electrophysiology data in multiple formats.
Neuroscientists use many different software tools to acquire, analyze and visualize electrophysiological signals. However, incompatible data models and file formats make it difficult to exchange data between these tools. This reduces scientific productivity, renders potentially useful analysis methods inaccessible and impedes collaboration between labs. A common representation of the core data would improve interoperability and facilitate data-sharing. To that end, we propose here a language-independent object model, named "Neo," suitable for representing data acquired from electroencephalographic, intracellular, or extracellular recordings, or generated from simulations. As a concrete instantiation of this object model we have developed an open source implementation in the Python programming language. In addition to representing electrophysiology data in memory for the purposes of analysis and visualization, the Python implementation provides a set of input/output (IO) modules for reading/writing the data from/to a variety of commonly used file formats. Support is included for formats produced by most of the major manufacturers of electrophysiology recording equipment and also for more generic formats such as MATLAB. Data representation and data analysis are conceptually separate: it is easier to write robust analysis code if it is focused on analysis and relies on an underlying package to handle data representation. For that reason, and also to be as lightweight as possible, the Neo object model and the associated Python package are deliberately limited to representation of data, with no functions for data analysis or visualization. Software for neurophysiology data analysis and visualization built on top of Neo automatically gains the benefits of interoperability, easier data sharing and automatic format conversion; there is already a burgeoning ecosystem of such tools. We intend that Neo should become the standard basis for Python tools in neurophysiology.
Front Neuroinform, 2014; 8

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BOARD NUMBER: S05-459

PROPRIOCEPTIVE DEFICITS AND VISUAL COMPENSATION IN STROKE PATIENTS: A THEORETICAL APPROACH TO REINTERPRET UPPER-LIMB SENSORY ASSESSMENTS.

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Université de Paris, CNRS, Incc, Umr8002, Paris, France

Aim: Recent findings suggest that, when reaching or grasping an object, sensory information is encoded in multiple, parallel reference frames. For example, in some proprioceptive assessments, such as the thumb localization task with eyes closed, proprioceptive signals tend to be re-encoded in visual-space. The process of re-encoding sensory signals, from proprioceptive to visual space, is referred to as “cross-reference transformation”. We propose to apply this concept to the study of proprioceptive deficits post-stroke, distinguishing between proprioceptive inputs available for a given assessment, and the potential cross-reference transformations that ensue during task execution. **Methods and Results:** We reviewed and reinterpreted the outcomes of the most common tests used to assess proprioceptive function in stroke survivors. We identified four main categories of clinical tests, each characterized by a specific processing of proprioceptive signals that may, or may not be re-encoded in different sensory spaces (see

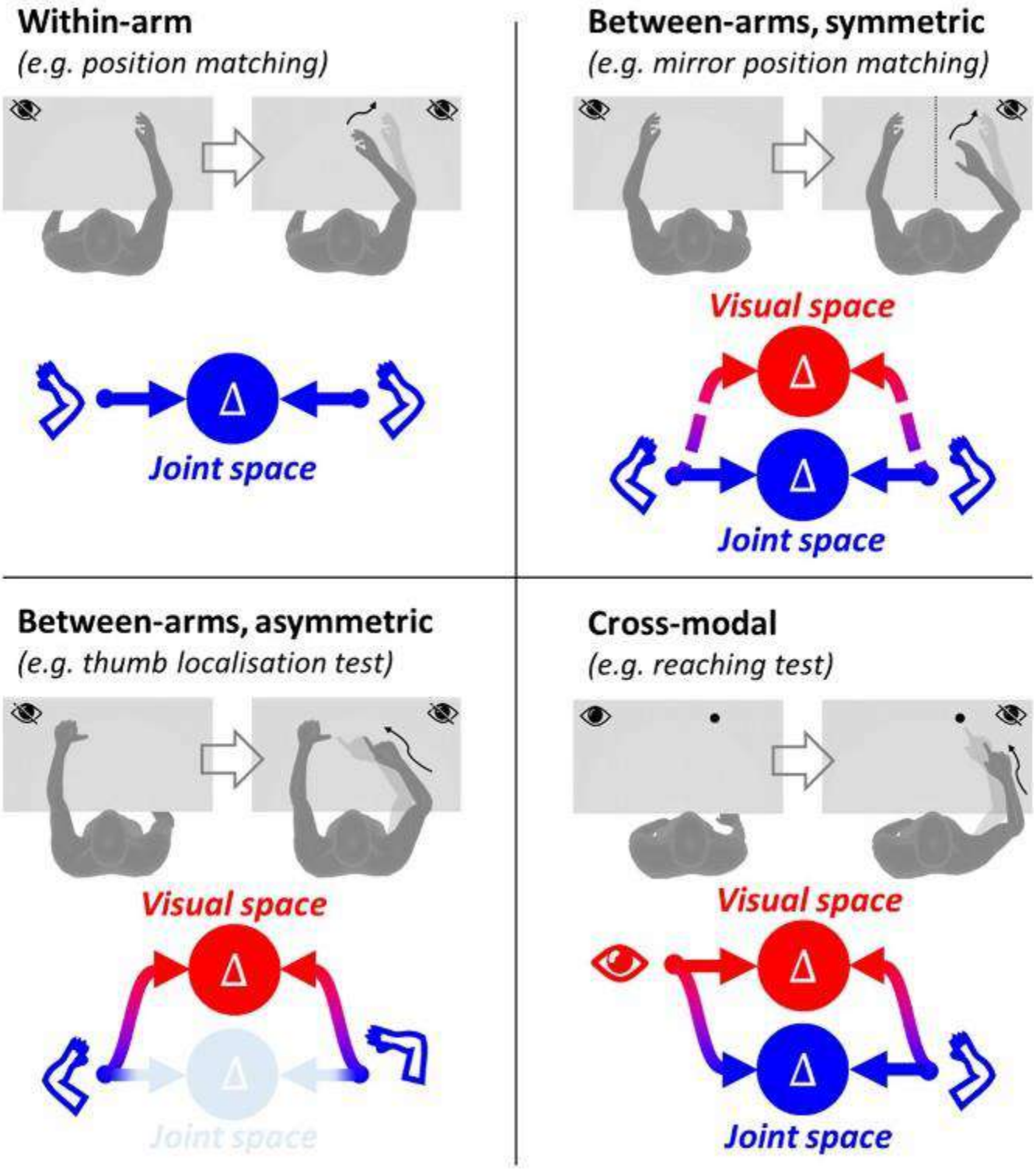

figure). This categorization, together with optimal sensory integration modeling, can reconcile the apparently contradictory results of a large number of clinical studies and can explain functionally why different tests lead, in the same patients, to different outcomes. Similarly, reinterpreting the relation between cerebral structures and proprioceptive functions in the light of our theoretical approach provides a novel rationale for an improved stratification of stroke patients. **Conclusions:** This novel classification of post-stroke sensory deficits may lead to innovative approaches to stroke assessment and rehabilitation.

Targeted training of proprioception and/or cross-reference transformations, depending on the patient's specific of sensory deficit, may be key to improved recovery.

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BOARD NUMBER: S05-460

VISUAL AND TACTILE INTEGRATION OF OBJECT LOCATIONS IN THE MOUSE CORTEX

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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For a stable and coherent perception of the world, the brain combines sensory information from different modalities using distinct reference frames. In the mouse brain, tactile information from the snout's vibrissae passes through the thalamus to the somatotopically organized primary whisker somatosensory cortex (wS1) while the primary visual cortex (V1) contains a retinotopic map of the visual field. For nearby objects, these two sensory streams likely converge in the rostro-lateral (RL) area of the posterior parietal cortex. How such converging multisensory inputs are integrated on a single-cell level, especially for naturalistic stimuli, remains unclear. Here, we investigated how neurons in mouse wS1, V1, and RL integrate visuotactile information about a pole in reach of the whiskers that can be seen and/or felt. Using two-photon calcium imaging, we recorded neurons in L2/3 of head-fixed mice while tracking whisker-pole interactions. Simultaneously, we recorded neural spikes and local field potentials from the primary and secondary thalamus (VPM, PO) by chronically implanting a 64-channel linear array of flexible electrodes. By comparing average activities at different pole locations, we find that subsets of neurons in RL and wS1 show selectivity for specific locations in the near space based on tactile and/or visual input. Using Generalized Linear Models, we find neural activity in RL better predicted by object locations than by whisker kinematics, whereas the opposite holds for wS1. Together, these findings corroborate the notion of RL representing object locations based on visual and tactile information, potentially in a more modality-invariant manner.

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BOARD NUMBER: S05-461

ERP COMPONENTS OF MULTISENSORY SPATIAL REPRESENTATION IN THE VISUAL CORTEX

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Occipital activation has been shown to selectively support spatial information processing independently of the sensory modality involved, suggesting a domain-specific multisensory architecture of the visual cortex. The present study investigates whether this domain specificity is also influenced by the complexity of the spatial representations to be processed. We tested healthy participants in an EEG study involving: i) a spatial bisection task, in which three audio-visual stimuli were reproduced at different spatial locations and participants estimated whether the second stimulus (S2) was spatially farther from the first or the third; ii) a spatial localization task, in which only S2 was presented at specific spatial positions and participants localized it at the left or the right of themselves. To investigate whether the different spatial representations elicited by the audio-visual stimuli in the two tasks modulated the neural activation of visual areas, we measured participants' Event-Related Potentials (ERPs) during their performance. S2 evoked typical visual, auditory and multisensory ERP components in occipital, temporal, and central areas in both tasks. However, occipital ERPs in the initial 200ms after S2 onset were explicitly modulated by the nature of the spatial tasks. A contralateral early occipital component (50-90ms) supported more the representation of complex multisensory spatial information (i.e., the spatial bisection), while a later contralateral occipital response (110-160ms) more strongly sustained the processing of simple spatial representations (i.e., the spatial localization). These results suggest that the neural modulation of visual areas depends on the different spatial factors involved in multisensory processing.

Pubmed:

34551010: Bertonati G, Amadeo MB, Campus C, Gori M
Auditory speed processing in sighted and blind individuals.
Multisensory experience is crucial for developing a coherent perception of the world. In this context, vision and audition are essential tools to scaffold spatial and temporal representations, respectively. Since speed encompasses both space and time, investigating this dimension in blindness allows deepening the relationship between sensory modalities and the two representation domains. In the present study, we hypothesized that visual deprivation influences the use of spatial and temporal cues underlying acoustic speed perception. To this end, ten early blind and ten blindfolded sighted participants performed a speed discrimination task in which spatial, temporal, or both cues were available to infer moving sounds' velocity. The results indicated that both sighted and early blind participants preferentially relied on temporal cues to determine stimuli speed, by following an assumption that identified as faster those sounds with a shorter duration. However, in some cases, this temporal assumption produces a misperception of the stimulus speed that negatively affected participants' performance. Interestingly, early blind participants were more influenced by this misleading temporal assumption than sighted controls, resulting in a stronger impairment in the speed discrimination performance. These findings demonstrate that the absence of visual experience in early life increases the auditory system's preference for the time domain and, consequentially, affects the perception of speed through audition.
PLoS One, 2021; 16
32846109: Battal C, Occelli V, Bertonati G, Falagiarda F, Collignon O
General Enhancement of Spatial Hearing in Congenitally Blind People.
Vision is thought to support the development of spatial abilities in the other senses. If this is true, how does spatial hearing develop in people lacking visual experience? We comprehensively addressed this question by investigating auditory-localization abilities in 17 congenitally blind and 17 sighted individuals using a psychophysical minimum-audible-angle task that lacked sensorimotor confounds. Participants were asked to compare the relative position of two sound sources located in central and peripheral, horizontal and vertical, or frontal and rear spaces. We observed unequivocal enhancement of spatial-hearing abilities in congenitally blind people, irrespective of the field of space that was assessed. Our results conclusively demonstrate that visual experience is not a prerequisite for developing optimal spatial-hearing abilities and that, in striking contrast, the lack of vision leads to a general enhancement of auditory-spatial skills.

Psychol Sci, 2020; 31
30940857: Martel M, Cardinali L, Bertonati G, Jouffrais C, Finos L, Farnè A, Roy AC
Somatosensory-guided tool use modifies arm representation for action.
Tool-use changes both peripersonal space and body representations, with several effects being nowadays termed tool embodiment. Since somatosensation was typically accompanied by vision in most previous tool use studies, whether somatosensation alone is sufficient for tool embodiment remains unknown. Here we address this question via a task assessing arm length representation at an implicit level. Namely, we compared movement's kinematics in blindfolded healthy participants when grasping an object before and after tool-use. Results showed longer latencies and smaller peaks in the arm transport component after tool-use, consistent with an increased length of arm representation. No changes were found in the hand grip component and correlations revealed similar kinematic signatures in naturally long-armed participants. Kinematics changes did not interact with target object position, further corroborating the finding that somatosensory-guided tool use may increase the represented size of the participants' arm. Control experiments ruled out alternative interpretations based upon altered hand position sense. In addition, our findings indicate that tool-use effects are specific for the implicit level of arm representation, as no effect was observed on the explicit estimate of the forearm length. These findings demonstrate for the first time that somatosensation is sufficient for incorporating a tool that has never been seen, nor used before.
Sci Rep, 2019; 9

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BOARD NUMBER: S05-462

LATERAL LINE HAIR CELLS INTEGRATE MECHANICAL AND CHEMICAL CUES TO STEER NAVIGATION

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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The lateral line is a superficial sensory system known to respond to hydrodynamic changes in the environment of aquatic vertebrates, thereby contributing to schooling, predation, and rheotaxis. Whether lateral line hair cells respond to external chemical cues is unknown. Here, we uncover a novel role of the lateral line as a versatile sensory system controlling fish innate locomotion. We find that zebrafish lateral line hair cells express over 45 chemoreceptors capable of detecting diverse chemicals including, among the most enriched ones, ionotropic receptors for serotonin. Serotonin is accumulated in skin neuroepithelial cells, direct neighbors of neuromast hair cells, and we find that this monoamine is released in the water upon injury. We show that lateral line hair cells exhibit large calcium responses to environmental serotonin. The lateral line affects behavior both via its mechanical and chemical sensory functions. First, we reveal that the spontaneous mechanosensory-dependent activity of neuromast hair cells sets the basal rate and speed of bouts during spontaneous swimming. Second, the activation of lateral line hair cells by serotonin shapes the navigation of larval zebrafish by increasing speed and swimming away from the serotonin source. Our results show that lateral line integrates both mechanical and chemical cues to orient navigation. As multiple chemoreceptors are conserved in the inner ear, our study suggests that chemoreception may be a fundamental property of hair cells that could modulate the essentials functions of mechanotransduction or regeneration.

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BOARD NUMBER: S05-463

SYNCHRONIZATION OF MULTISENSORY INFORMATION IN DORSOMEDIAL STRIATUM

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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How the brain operates with different sensory modalities is an essential question in order to understand how sensory feedback engages motor responses. Deficits in multisensory integration have been documented in several neurological problems, such as Parkinson's disease or the attention-deficit and hyperactivity disorder, which involve both the striatum and dopamine transmission. The striatum is the input layer of the basal ganglia and it is associated to sensorimotor functions. The 95% of the striatal neurons are GABAergic projection neurons known as Medium Spiny Neurons (MSNs), which are divided into two subpopulations according to their axonal projections and their different dopamine receptor expression: the direct and indirect pathway. Single MSNs located in the dorsomedial region of the striatum can be activated by different sensory stimuli, such as tactile and visual (Reig & Silberberg, 2014). In this work we studied how dopamine modulates the integration of multisensory inputs. To that end, we obtained in vivo whole-cell recordings in the dorsomedial striatum of anesthetized mice during the presentation of tactile, visual and multisensory stimuli while dopamine is released by optogenetic stimulation. We show that dopamine boosts the synchronization of visual and tactile information specifically in direct pathway MSNs. This synchronization relies on the disinhibition of direct MSNs and is supported by a type-specific corticostriatal pathway. Altogether, our in vivo and in silico results propose a new mechanism underlying the synchronization of sensory information from different modalities mediated by dopamine release that can contribute to the understanding of sensorimotor interactions that occur during fine movements.

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INVESTIGATION OF MULTIMODAL PROCESSING IN THE MOUSE RETROSPLENIAL CORTEX: AN ELECTROPHYSIOLOGY STUDY

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

Shahriar Hosseinjany[1,2], Virginie Oberto[1,3], Yu-Ting Wei[1,4], Asli Ayaz[1,3], Vincent Bonin[1,3,5,6,7]
[1]KU Leuven, Neuro-electronics Research Flanders (nerf), leuven, Belgium, [2]KU Leuven, Faculty Of Science, leuven, Belgium, [3]VIB, Center For Brain & Disease Research, leuven, Belgium, [4]KU LEUVNE (Neuro-Electronics Research Flanders), Psychology, Leuven, Belgium, [5]VIB, KU Leuven, Neuro-electronics Research Flanders (nerf), Leuven, Belgium, [6]imec, VIB, KU Leuven, Neuro-electronics Research Flanders (nerf), Leuven, Belgium, [7]KU Leuven, Department Of Biology & Leuven Brain Institute, Leuven, Belgium

The retrosplenial cortex (RSC) is known to have a key role in a range of cognitive functions, including episodic memory, navigation, imagination, and planning for the future. Based on the RSC connections with sensory regions and hippocampal formation, an integrative processing regime is expected for this area to involve in visuospatial behaviors. However, how the RSC neurons encode multimodal information is poorly understood. We combined acute electrophysiology recording and a head-fixed locomotion assay to characterize the sensory and cognitive signals in different sub-regions of the RSC. Using Neuropixel probes, we recorded single units' activity of the RSC cells from behaving animals during a navigation task in three different conditions including, bright, dark and in the presence of visual stimuli. We used full-field drifting sinusoidal gratings as visual stimuli with different directions, spatial and temporal frequencies. We report the response properties of multiple single units to different stimuli and across behavioral states. In 536 neurons we recorded from different parts of the RSC, we found multiple variables' encoding, including visual selectivity, somatosensory responses, locomotion speed tuning, and behavior-related signals. The number of simultaneously recorded neurons and the high temporal resolution of the recording enabled us to characterize the population and the individual cell's dynamic. We conclude that neuronal activity in the RSC conveys different information about the environment and the animal's behavioral state, as was expected due to previous anatomical and physiological reports. Our study highlights the importance of investigating deep structures of the RSC, which are rarely studied in the literature.

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SEPARATE AUDITORY AND MOVEMENT CONTRIBUTIONS TO SOUND-EVOKED ACTIVITY IN VISUAL CORTEX

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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University of Amsterdam, Swammerdam Institute Of Life Sciences, Amsterdam, Netherlands

To enable appropriate behavioral responses to visual inputs, contextual signals are already integrated with visual signals in primary visual cortex (V1). For example, V1 is also strongly influenced by crossmodal inputs and motor movement. To what extent these two non-visual influences on V1 activity are correlated is nevertheless controversial. Here we show that in awake mice the occurrence of frequency-tuned sound-evoked activity in primary visual cortex is explained to a large extent, but not completely, by instructed and uninstructed orofacial movements. Through task manipulations, pharmacological interventions, multi-area recordings, and optogenetics, we identify two distinct components underlying sound-evoked activity in V1. A sound-related component originates from auditory cortex, has an onset latency of about 27 ms, is transient, and is found predominantly in deep layers of V1. In both naive and trained mice, another motor-related component of V1 activity results from rapid sound-evoked orofacial movements that are both instructed and uninstructed (i.e., licking and whisking). Motor-related activity was found as early as 80-100 ms, mostly in superficial layers of V1, and accounted for most sound-evoked neural activity changes in visual cortex (but, intriguingly, not in auditory cortex). During a multisensory change detection task, these two components profoundly shape visual cortical activity, but in a manner that preserves visual orientation coding. Our study provides a key contribution towards dissociating crossmodal from behavioral signals in V1. This is central to the correct interpretation of multisensory evoked activity and to understanding how distinct sensory modalities interact in visual cortex to support vision.

Pubmed:
30364373: Olcese U, Oude Lohuis MN, Pennartz CMA
Sensory Processing Across Conscious and Nonconscious Brain States: From Single Neurons to Distributed Networks for Inferential Representation.
Neuronal activity is markedly different across brain states: it varies from desynchronized activity during wakefulness to the synchronous alternation between active and silent states characteristic of deep sleep. Surprisingly, limited attention has been paid to investigating how brain states affect sensory processing. While it was long assumed that the brain was mostly disconnected from external stimuli during sleep, an increasing number of studies indicates that sensory stimuli continue to be processed across all brain states-albeit differently. In this review article, we first discuss what constitutes a brain state. We argue that-next to global, behavioral states such as wakefulness and sleep-there is a concomitant need to distinguish bouts of oscillatory dynamics with specific global/local activity patterns and lasting for a few hundreds of milliseconds, as these can lead to the same sensory stimulus being either perceived or not. We define these short-lasting bouts as micro-states. We proceed to characterize how sensory-evoked neural responses vary between conscious and nonconscious states. We focus on two complementary aspects: neuronal ensembles and inter-areal communication. First, we review which features of ensemble activity are conducive to perception, and how these features vary across brain states. Properties such as heterogeneity, sparsity and synchronicity in neuronal ensembles will especially be considered as essential correlates of conscious processing. Second, we discuss how inter-areal communication varies across brain states and how this may affect brain operations and sensory processing. Finally, we discuss predictive coding (PC) and the concept of multi-level representations as a key framework for understanding conscious sensory processing. In this framework the brain implements conscious representations as inferences about world states across multiple representational levels. In this representational hierarchy, low-level inference may be carried out nonconsciously, whereas high levels integrate across different sensory modalities and larger spatial scales, correlating with conscious processing. This inferential framework is used to interpret several cellular and population-level findings in the context of brain states, and we briefly compare its implications to two other theories of consciousness. In conclusion, this review article, provides foundations to guide future studies aiming to uncover the mechanisms of sensory processing and perception across brain states.
Front Syst Neurosci, 2018; 12
32048995: Vertechi P, Lottem E, Sarra D, Godinho B, Treves I, Quendera T, Oude Lohuis MN, Mainen ZF

Inference-Based Decisions in a Hidden State Foraging Task: Differential Contributions of Prefrontal Cortical Areas.
Essential features of the world are often hidden and must be inferred by constructing internal models based on indirect evidence. Here, to study the mechanisms of inference, we establish a foraging task that is naturalistic and easily learned yet can distinguish inference from simpler strategies such as the direct integration of sensory data. We show that both mice and humans learn a strategy consistent with optimal inference of a hidden state. However, humans acquire this strategy more than an order of magnitude faster than mice. Using optogenetics in mice, we show that orbitofrontal and anterior cingulate cortex inactivation impacts task performance, but only orbitofrontal inactivation reverts mice from an inference-based to a stimulus-bound decision strategy. These results establish a cross-species paradigm for studying the problem of inference-based decision making and begins to dissect the network of brain regions crucial for its performance.
Neuron, 2020; 106
34849636: Oude Lohuis MN, Canton AC, Pennartz CMA, Olcese U
Higher Order Visual Areas Enhance Stimulus Responsiveness in Mouse Primary Visual Cortex.
Over the past few years, the various areas that surround the primary visual cortex (V1) in the mouse have been associated with many functions, ranging from higher order visual processing to decision-making. Recently, some studies have shown that higher order visual areas influence the activity of the primary visual cortex, refining its processing capabilities. Here, we studied how in vivo optogenetic inactivation of two higher order visual areas with different functional properties affects responses evoked by moving bars in the primary visual cortex. In contrast with the prevailing view, our results demonstrate that distinct higher order visual areas similarly modulate early visual processing. In particular, these areas enhance stimulus responsiveness in the primary visual cortex, by more strongly amplifying weaker compared with stronger sensory-evoked responses (for instance specifically amplifying responses to stimuli not moving along the direction preferred by individual neurons) and by facilitating responses to stimuli entering the receptive field of single neurons. Such enhancement, however, comes at the expense of orientation and direction selectivity, which increased when the selected higher order visual areas were inactivated. Thus, feedback from higher order visual areas selectively amplifies weak sensory-evoked V1 responses, which may enable more robust processing of visual stimuli.
Cereb Cortex, 2021;
29520000: Lottem E, Banerjee D, Vertechi P, Sarra D, Lohuis MO, Mainen ZF
Activation of serotonin neurons promotes active persistence in a probabilistic foraging task.
The neuromodulator serotonin (5-HT) has been implicated in a variety of functions that involve patience or impulse control. Many of these effects are consistent with a long-standing theory that 5-HT promotes behavioral inhibition, a motivational bias favoring passive over active behaviors. To further test this idea, we studied the impact of 5-HT in a probabilistic foraging task, in which mice must learn the statistics of the environment and infer when to leave a depleted foraging site for the next. Critically, mice were required to actively nose-poke in order to exploit a given site. We show that optogenetic activation of 5-HT neurons in the dorsal raphe nucleus increases the willingness of mice to actively attempt to exploit a reward site before giving up. These results indicate that behavioral inhibition is not an adequate description of 5-HT function and suggest that a unified account must be based on a higher-order function.
Nat Commun, 2018; 9

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CHARACTERIZATION OF DIRECTIONALLY TUNED SIGNALS IN MOUSE PRE- AND POSTSUBICULUM DURING PASSIVE ROTATION USING HIGH-DENSITY PROBES

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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[1]Université Paris Cité, CNRS, Integrative Neuroscience And Cognition Center Umr 8002, Paris, France, [2]Humboldt-Universit t zu Berlin, Bernstein Center for Computational Neuroscience, Institut Für Biologie, Berlin, Germany, [3]Ernst Strüngmann Institute, (esi) For Neuroscience In Cooperation With Max Planck Society, Frankfurt am Main, Germany, [4]Université Paris Cité, SPPIN, Cnrs Umr 8003, Paris, France

The head direction (HD) system functions as the brain's compass system. Vestibular based HD signals combine with visual signals to permit orientation coding with respect to external landmarks. Here we examined the encoding of angularly tuned signals in populations of dorsal postsubicular and ventral presubicular neurons, that can be dominated by HD and/or visual cue position. Head-fixed mice were passively rotated on a motorized stage to cover 360°, in a room, or surrounded by a dome with a white bar. The stage was rotated in a pseudorandom fashion, in clockwise and counterclockwise directions with peak speeds of 140°/sec and accelerations up to 200°/sec^2. We recorded extracellular spiking activity using Neuropixels probes positioned in post- and presubicular cortex. Spikes were sorted using kilosort3 and manually curated with Phy. Angular tuning was quantified by dividing the number of spikes by the occupancy per bin, and the Rayleigh vector (R) was calculated. Bursting index indicated interspike intervals <6ms. We found a total of 380 well-separated units in pre- and postsubiculum (n=5 mice), out of which 61% were directionally tuned (R value >0.3). Typically preferred angular directions of the population covered 360° in the room condition quite uniformly. The presence of a single visual cue could transiently skew the distribution. We examine how the population activity of tuned units allows to decode direction. Most directionally tuned units were non-bursting cells. In conclusion, visual cues can affect angular tuning of pre-and postsubicular cells in head-fixed condition.

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CROSS-DIMENSIONAL INTERFERENCE BETWEEN SPATIAL AND TEMPORAL PROCESSING DURING ROTATIONS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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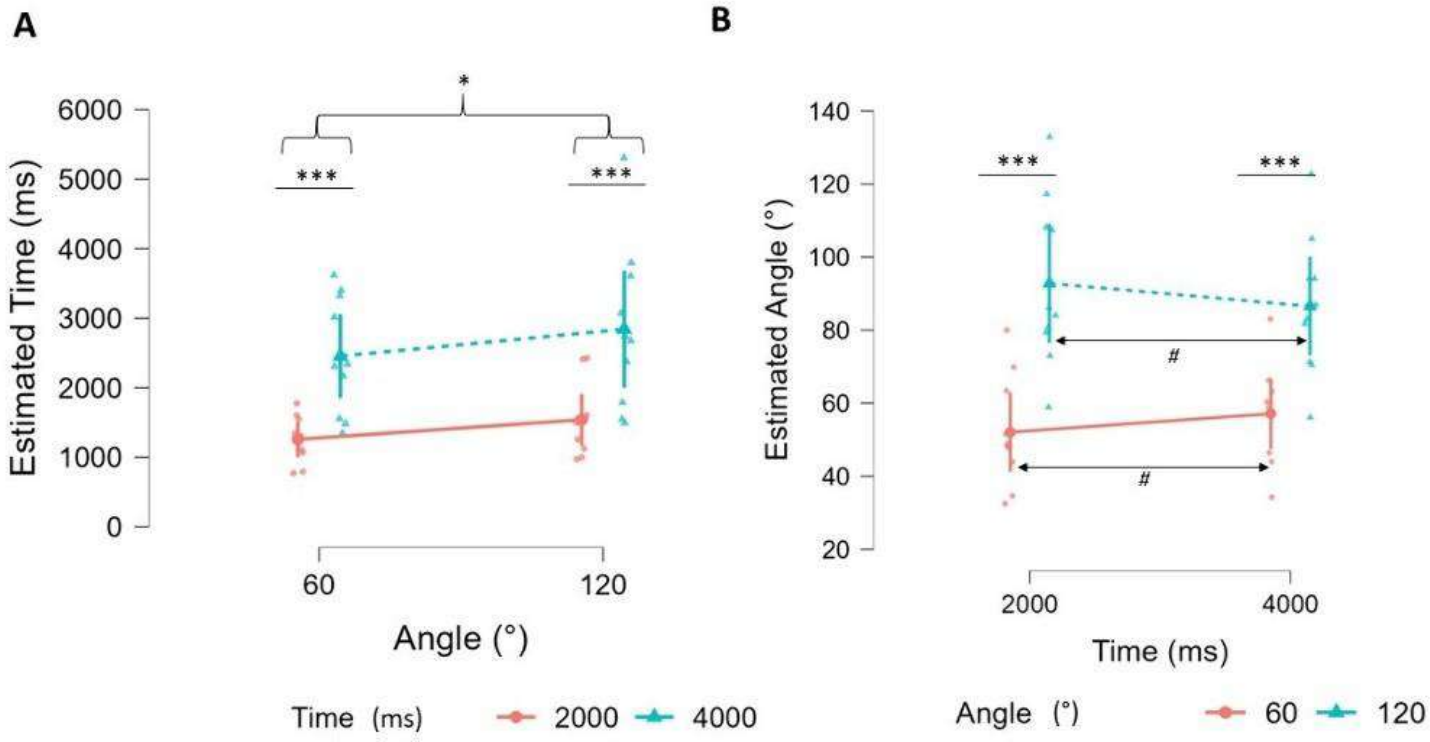
Univ. UNICAEN, INSERM, COMETE, Normandie, Caen, France

Introduction The interaction between time and space dimensions in human brain remains uncertain. Theories suggest an asymmetrical interaction: space perception influences time perception but not the opposite. However, this relationship has never been evaluated in real movement. We aim to study the interaction between time and space perception during rotations. ***Methods*** 25 healthy participants in total darkness performed two tasks after rotation (in random order: 2 or 4 seconds; 60 or 120°; right or left): (1) reproduction of the duration of rotation by pushing a button for the same amount of time or (2) reproduction of the amplitude of the rotation by pushing a button that drove the chair in the same direction. ***Results***



*Preliminary results of Lineal Mixed Model (10 of 25 participants) A) Estimated time based on the angle of rotation (60° or 120°). Rotations of 120° caused an overestimation of time compared to rotations of 60° (p=0.023). B) Estimated angle based on the time of rotation (2 or 4 seconds). Interaction (*p≤0.001*) leaded us to study 60° and 120° separately. For rotations of 60°, amplitude was overestimated comparing 4 to 2 seconds of rotations (p = 0.014). The opposite occurred for rotations of 120°(p=0.018). ***** p≤0.05, ******* p≤0.001, $^{\#}$p≤0.05 (ANOVA repeated measures).* ***Conclusions*** Preliminary results suggest a bidirectional interaction between the two estimates.

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BRAIN-WIDE MAPPING OF PARAHIPPOCAMPAL AND VISUAL NEURAL NETWORKS IN MICE: EVIDENCE FOR FEEDBACK PROJECTIONS FROM THE PERIRHINAL CORTEX TO VISUAL AREAS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Feedback projections from higher-order processing areas to lower-order areas within the visual system have been observed in several species. While much is known about the function of feedforward connections in the visual processing pathway, feedback connections are not as well studied. They are thought to cause behaviors such as (selective) attention or expectations, but the exact mechanism and underlying neural connectivity is still unknown. The perirhinal cortex (PRH) forms the intersection between perceptual and mnemonic areas of the visual processing pathway. Evidence from rats, monkeys and humans suggests that it is involved in both object memory and perceptual tasks, which is reflected in its connectivity. PRH forms numerous feedforward and feedback projections with other areas of the visual processing pathway. However, little is known about the function and organization of feedback projections from PRH, and other parahippocampal regions, to visual areas in mice. Given the widespread availability of genetic and molecular tools, novel opportunities to investigate the function of these feedback projections would be enabled by mapping them in mice. Here we present preliminary data from anterograde and retrograde tract tracing experiments showing feedback projections from PRH, and other parahippocampal regions, to visual areas. We have found first evidence for feedback projections from PRH to lower-order visual areas that were assumed to be exclusive to higher-developed animals such as monkeys or humans. Further, we have identified feedback projections from other parahippocampal regions with specific subregional distribution. Funded from EU Horizon 2020, Specific Grant Agreement No. 945539 (Human Brain Project SGA3).

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BOARD NUMBER: S05-469

BEHAVIORAL ORIGIN OF SOUND-EVOKED ACTIVITY IN MOUSE VISUAL CORTEX

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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[Aims] Many studies suggest that all cortical sensory areas, including primary ones, are multisensory. For instance, mouse primary visual cortex (V1) appears to be influenced by auditory signals. These effects may be due to auditory projections to the visual cortex. However, sounds also change internal state and elicit uninstructed body movements, and both effects have brainwide correlates. It is thus possible that sounds affect visual cortex and other brain regions mainly through changes in state or behavior rather than through direct auditory projections. **[Methods]** We used Neuropixels probes to record the responses of hundreds of neurons in V1 and hippocampus of awake mice to audiovisual stimuli, while filming the mouse. To explore the role of direct projections from auditory to visual cortex, in 3 mice we cut auditory fibers to visual cortex and recorded sound-evoked responses on the cut and uncut sides of the brain. **[Results]** V1 encoded a low-dimensional representation of sounds. The same representation was also present in hippocampus, which barely receives any auditory input. The apparently auditory responses of neurons in V1 and hippocampus were highly correlated with the temporal patterns of movement evoked by the sounds, which were stereotyped across trials and across mice. They were independent of the presence of direct projections from auditory cortex, as they were unaffected by cutting these projections. **[Conclusions]** These results indicate that a large fraction of the multisensory activity that has been widely observed across the brain may have a simpler, behavioral origin.

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BOARD NUMBER: S05-470

A NOVEL EXPERIMENTAL SETUP TO STUDY MULTISENSORY INTEGRATION OF FREELY-SWIMMING ZEBRAFISH DURING RHEOTAXIS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

Orhun Koc[1], Alp Demirel[1], Sevval Izel Solmaz[2], Fatmagül Ibişoğlu[2], Ismail Uyanik[1]
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Separate sensory structures capture information in physically distinct domains. The CNS integrates multimodal information to obtain a unique representation of the environment. Our goal is to identify the dynamics of multisensory integration during free behavior of the animals. We developed a novel experimental setup to study multisensory integration during free rheotaxis behavior of adult *Danio rerio*. The setup is a speed-controlled flow tunnel that allows independent stimulation for visual and mechanosensory systems of zebrafish. Zebrafish orient themselves toward the flow due to their positive-rheotaxis behavior for position holding with minimal energy. Different from classical rheotaxis, our setup involves a transparent cylindrical tube placed within the setup to obscure the flow in its neighborhood. The obstacle forms a low gradient regime, motivating the fish to swim there to reduce its energetic cost during rheotaxis. This tube is attached to a linear actuator that moves horizontally, changing the position of the low gradient regime. Besides, we placed a dark stick inside the cylindrical tube for visual stimulation. Thus, the outer tube and inner stick provide mechanosensory and visual cues, respectively. The key feature of this design is that the movements of the inner stick can be controlled independently from that of the outer tube. We experimented with N=5 adult zebrafish using synchronous and conflicting stimuli. We estimated frequency response functions associated with each sensory structure, assuming a superposition model for the multisensory integration. We are currently examining Bayesian and Kalman filtering models to capture the multisensory behavioral response of the fish.

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BOARD NUMBER: S05-471

DYNAMIC SENSORY REWEIGHTING IN WEAKLY ELECTRIC FISH DURING REFUGE TRACKING IN A FLOW TUNNEL

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Animals are equipped with diverse sensory organs that capture various sensory signals. The CNS integrates such multimodal information to increase reliability and disambiguate complex signals. Our goal is to reveal the filtering mechanisms adopted by CNS to perform multisensory integration under dynamic sensory conditions. We built an experimental setup to identify the dynamics of multisensory integration during refuge tracking behavior of *Apteronotus albifrons*. These fish hide inside refuges and track their movements to remain hidden. To achieve this, *Apteronotus* integrates visual and electrosensory information but (likely) not mechanosensory cues. Our experimental setup examines multisensory behavioral control of *Apteronotus* during refuge tracking considering the effects of these three sensory signals. The setup is a speed-controlled flow tunnel that allows an individual fish to perform refuge tracking under various mechanosensory stimulation levels. We built a transparent refuge attached to a linear actuator. In the dark, this refuge is invisible to the fish, but its longitudinal movements stimulate electrosensory receptors of the fish. We mounted a projector on the linear actuator to project a pattern of vertical stripes onto the refuge, generating visual cues. The refuge is sufficiently translucent so that the fish can see projected stripes from inside the refuge. The key feature is that visual and electrosensory cues can be controlled independently. We conducted experiments with N=5 fish under different sensory conditions and identified the weights associated with each sensory system. We currently investigate models that capture the dynamics of sensory reweighting in relation to the quality of sensory information.

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BOARD NUMBER: S05-472

THE EFFECTS OF SENSORY SALIENCE ON SMOOTH-PURSUIT TRACKING PERFORMANCE OF ZEBRAFISH DURING RHEOTAXIS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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The sensory salience plays a critical role in multisensory behavioral control. Our goal is to investigate the impact of sensory salience on the behavioral performance of *Danio rerio* during a new behavioral assay—target tracking during rheotaxis. We built a speed-controlled swim tunnel for an individual zebrafish to perform rheotaxis. During rheotaxis, zebrafish orient their bodies toward the flow and hold their position to avoid getting dragged. We placed a cylindrical plexiglass tube with a dark inner stick inside the setup to obscure the flow around its neighborhood. The tube is attached to a linear actuator that moves the tube horizontally. When moved, the outer transparent tube generates mechanosensory cues and the dark inner stick generates visual cues. Zebrafish track the movement of the tube to remain around the low gradient regime behind the tube. We varied the sensory salience by changing the diameter of the inner stick (1-5 cm) and the flow speed (10-20 cm/s). We conducted experiments with N=10 adult zebrafish by sinusoidally moving the tubes in 0-2 Hz range. We estimated frequency response functions between the movements of the fish and the tube. Our results show that tracking performance was better for 3cm-tube with a monotonic decrease in both directions. The flow speed did not have a significant effect in tracking. Our results suggest that the tracking behavior might be dominated by vision in light. Thus, we are currently expanding our experiments to dark and testing target objects in different shapes to improve mechanosensory stimulation.

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BOARD NUMBER: S05-473

MULTISENSORY INTEGRATION IN SUPERIOR COLLICULUS AND PRIMARY VISUAL CORTEX OF AWAKE BEHAVING MICE

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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The Superior Colliculus (SC) is a key area for the integration of multisensory stimuli and involved in numerous cognitive functions. Inputs from different modalities reach the SC either directly or via the cortex. Yet, how multisensory integration in SC differs from cortex is not well understood. We therefore performed Neuropixels recordings in visual cortex (V1) and SC of awake mice while presenting visual, auditory and tactile stimuli. A majority of V1 neurons responded to visual stimuli but a large fraction also responded to auditory and tactile stimulation. In SC, responses showed a clear preference for contralateral visual stimuli in the superficial layers, similar to V1, while responses in deeper layers were more evenly distributed across modalities. To quantify multisensory integration, we compared responses to unisensory stimuli and multisensory combinations. Here, multisensory were mostly larger than unisensory responses for all stimulus combinations. Interestingly, multisensory enhancement was more common in V1 while SC neurons showed a larger variety in either enhancement or depression, suggesting a more detailed representation of multisensory stimuli. Lastly, we tested how multisensory integration is affected by behavior. We thus trained mice in a multisensory discrimination task and compared multisensory responses in naive versus trained animals. Here, trained mice showed an increased fraction of tactile neurons and more frequent occurrences of multisensory depression in both V1 and SC. Our results show that multisensory integration happens as early as primary sensory cortex. However, there seemed to be less variability and specificity in the multisensory responses in V1 compared to SC.

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BOARD NUMBER: S05-474

ROLE OF NORADRENERGIC NEURONS IN SHAPING MOTOR PATTERNS DURING AVOIDANCE RESPONSE

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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To ensure their survival, animals need to avoid threats in their environment. Upon encountering aversive cues, animals rapidly integrate sensory information and deploy avoidance responses consisting of freezing and/or semi-automatic motor patterns to flee. The role of noradrenergic modulation in shaping such avoidance responses remains elusive due to the difficulty of accessing these neurons in the brainstem. To tackle this question, we exploit the transparency, genetic accessibility, and high throughput behavioral recordings of larval zebrafish. We first ascertained the recruitment of noradrenergic neurons upon aversive chemosensory and mechanosensory stimulation. Brief unilateral applications of acidic pH and pressure applications of water in the otic vesicle recruited effectively noradrenergic neurons in the medulla oblongata, which triggered a large calcium wave in glial cells descending in the spinal cord and ascending in the brain. We then tested the behavioral relevance of noradrenergic neuron recruitment in free swimming larvae. Pharmacological activation of the adrenergic system drastically reduced the mobility of larval zebrafish, indicating their ability to induce freezing. To dissect the circuit mechanism by which noradrenergic neurons operate, we examined the distribution of alpha-1a adrenergic receptor distribution. We found in the ventromedial medulla punctate clusters of alpha-1A adrenergic receptors onto descending axons projecting to the spinal cord. 2D optogenetic stimulation combined with single-cell electrophysiological recordings will be performed to dissect neuronal versus glial contribution in the induction of freezing. Altogether, this work reveals an essential neuromodulatory pathway to control the arrest of locomotion.

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BOARD NUMBER: S05-475

THE CELLULAR CODING OF TEMPERATURE IN THE MAMMALIAN CORTEX

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Temperature is a fundamental sensory modality separate from touch, with dedicated receptor channels and primary afferent neurons for cool and warm (Blix, 1882; Filingeri, 2016; Vriens et al., 2014). Unlike other modalities, however, the cortical encoding of temperature remains mysterious, with very few cortical neurons reported that respond to non-painful temperature and the presence of a 'thermal cortex' is debated (Bokiniec et al., 2018; Craig et al., 2000; Hellon et al., 1973; Milenkovic et al., 2014; Tsuboi et al., 1993). Using widefield and two-photon calcium imaging in the mouse forepaw system, here we identify cortical neurons that respond to cooling and/or warming with distinct spatial and temporal response properties. Surprisingly, we observed a representation of cool, but not warm, in the primary somatosensory cortex, but cool and warm in the posterior insular cortex (pIC). The representation of thermal information in pIC is robust, somatotopically-arranged and reversible manipulations show a profound impact on thermal perception. Intriguingly, despite being positioned along the same one-dimensional sensory axis, the encoding of cool and warm is distinct, both in highly- and broadly- tuned neurons. Together, our results show that pIC contains the primary cortical representation of skin temperature and may help explain how the thermal system generates sensations of cool and warm.

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BOARD NUMBER: S05-476

INTEGRATION OF SUPRALIMINAL AND SUBLIMINAL MULTISENSORY STIMULI IN VIRTUAL REALITY

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Aims The prevailing theories of consciousness claim that multisensory stimuli cannot be integrated without emerging to consciousness: to empirically test the validity of this assumption, evidence of subliminal visuotactile integration has been checked. **Methods** 6 healthy volunteers were stimulated – unimodally or bimodally, and subliminally or supraliminally – with tactile and/or visual stimuli administered in virtual reality (VR). Tactile stimuli were delivered through Transcutaneous Electrical Nerve Stimulation (TENS) to the right foot dorsum; visual stimuli (semi-transparent circles) appeared on the same location. The multisensory integration of the stimuli was measured through electroencephalography (EEG) and through robust psychophysical models (optimal integration following the MLE model in a 2-Alternative Forced Choice task). **Results** Participants followed the MLE model in the suprathreshold condition. This was not the case in the subliminal condition. Here however, only in the bimodal condition their performance was higher than the chance level. Coherently, EEG analysis showed that bimodal stimuli both in the supra- and subliminal conditions elicited event-related potentials (ERPs) significantly different from those elicited by subliminal unimodal stimuli. **Conclusions** Contrasting popular theories of consciousness, we found evidence of multisensory integration also for subliminal stimuli. The methodological robustness of these results will impact the debate on consciousness and will have relevant implications on the newest frontiers of bionic neurosciences.

Pubmed:
34139247: Baroni M, Frumento S, Cesari V, Gemignani A, Menicucci D, Rutigliano G
Unconscious processing of subliminal stimuli in panic disorder: A systematic review and meta-analysis.
Attentional biases to threat exist in panic disorder (PD), probably related to altered subliminal processing. We systematically reviewed studies investigating subliminal processing in PD. Studies were retrieved from MEDLINE and Scopus®. We meta-analytically compared PD (n = 167) and healthy controls (HC, n = 165) for processing of masked panic-related and neutral words. We also compared subliminal and supraliminal presentations of panic-related words relative to neutral words within PD subjects and HC. We found a significantly enhanced Stroop interference to masked panic-related words in PD vs HC (Hedges' g = 0.60, p = 0.03; Q = 14.83, I = 66.3 %, p = 0.01). While both PD subjects and HC tended to be slower to respond to supraliminal threat words than to neutral words, PD subjects only showed a marginally significant slower response to subliminal panic-related words vs neutral words. Findings remain inconclusive regarding comparison to other mental disorders, neural correlates, and the effect of psychotherapy. Even if possibly flawed by methodological weaknesses, our findings support the existence of a sensitivity to subliminal threat cues in PD, which could be targeted to improve treatment.
Neurosci Biobehav Rev, 2021; 128
34611999: Malloggi E, Menicucci D, Cesari V, Frumento S, Gemignani A, Bertoli A
Lavender aromatherapy: A systematic review from essential oil quality and administration methods to cognitive enhancing effects.
Modern society is reviving the practice of aromatherapy, and lavender is reported being the most worldwide purchased plant for essential oil (EO) extraction. Since recent studies reported cognitive enhancing effects of lavender besides the hypno-inducing effects, a literature review is needed. Considering EO quality and diffusion devices, we conducted a systematic review on the effects of lavender EO inhalation on arousal, attention and memory in healthy subjects. Starting from this new multidisciplinary perspective, cognitive effects were reviewed to link outcomes to effective and reproducible protocols. A systematic search on MEDLINE, ERIC, PsycInfo, Google Scholar, and Scopus databases using Cognitive Atlas and plant-related keywords was conducted. Among the 1,203 articles yielded, 11 met eligibility criteria. Subjects administered with lavender EO displayed arousal decrease and sustained attention increase. Controversial results emerged regarding memory.

Lack of EO quality assessment and protocols heterogeneity did not allow assessing whether different EO composition differentially modulates cognition and whether placebo effect can be discerned from EO effect itself. However, GABAergic pathway modulation exerted by linalool, a major lavender EO constituent, might explain cognitive functions empowerment. We speculate aromatherapy could be a burgeoning cognition enhancing tool, although further investigation is required to reach robust conclusions.
Appl Psychol Health Well Being, 2022; 14
31140116: Hitchcott PK, Menicucci D, Frumento S, Zaccaro A, Gemignani A
The neurophysiological basis of excessive daytime sleepiness: suggestions of an altered state of consciousness.
Excessive daytime sleepiness (EDS) is characterized by difficulty staying awake during daytime, though additional features may be present. EDS is a significant problem for clinical and non-clinical populations, being associated with a range of negative outcomes that also represent a burden for society. Extreme EDS is associated with sleep disorders, most notably the central hypersomnias such as narcolepsy, Kleine-Levin syndrome, and idiopathic hypersomnia (IH). Although investigation of these conditions indicates that EDS results from diminished sleep quality, the underlying cause for this impairment remains uncertain. One possibility could be that previous research has been too narrow in scope with insufficient attention paid to non-sleep-related aspects. Here, we offer a broader perspective in which findings concerning the impact of EDS on cortical functioning are interpreted in relation to current understanding about the neural basis of consciousness. Alterations in the spatial distribution of cortical activity, in particular reduced connectivity of frontal cortex, suggest that EDS is associated with an altered state of consciousness.
Sleep Breath, 2020; 24
34149346: Frumento S, Menicucci D, Hitchcott PK, Zaccaro A, Gemignani A
Systematic Review of Studies on Subliminal Exposure to Phobic Stimuli: Integrating Therapeutic Models for Specific Phobias.
We systematically review 26 papers investigating subjective, behavioral, and psychophysiological correlates of subliminal exposure to phobic stimuli in phobic patients. Stimulations were found to elicit: (1) cardiac defense responses, (2) specific brain activations of both subcortical (e.g., amygdala) and cortical structures, (3) skin conductance reactions, only when stimuli lasted >20 ms and were administered with intertrial interval >20 s. While not inducing the distress caused by current (supraliminal) exposure therapies, exposure to subliminal phobic stimuli still results in successful extinction of both psychophysiological and behavioral correlates: however, it hardly improves subjective fear. We integrate those results with recent bifactorial models of emotional regulation, proposing a new form of exposure therapy whose effectiveness and acceptability should be maximized by a preliminary subliminal stimulation. Systematic Review Registration: identifier [CRD42021129234].
Front Neurosci, 2021; 15
30210407: Costa M, Frumento S, Nese M, Predieri I
Interior Color and Psychological Functioning in a University Residence Hall.
The research exploited a unique architectural setting of a university residence hall composed by six separate buildings that matched for every architectural detail and differed only for the interior color (violet, blue, green, yellow, orange, and red). Four hundred and forty-three students living in the six buildings for an average of 13.33 months participated in a study that assessed color preference (hue and lightness), lightness preference, and the effects of color on studying and mood. The results showed a preference for blue interiors, followed by green, violet, orange, yellow, and red. A preference bias was found for the specific color in which the student lived. Gender differences emerged for the preference of blue and violet. Room-lightness was significantly affected by the interior color. Room ceiling was preferred white. Blue as interior color was considered to facilitate studying activity. The use of differentiated colors in the six buildings was evaluated to significantly facilitate orienting and wayfinding. A significant relation was found between a calm mood and preference for blue.
Front Psychol, 2018; 9

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SIGNATURES OF CORTICAL MULTISENSORY INTEGRATION IN MICE PERFORMING A VISUOTACTILE ACCUMULATION OF EVIDENCE TASK

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Much effort has focused on studying how the brain processes information from individual senses. Yet, the neural mechanisms that allow for multimodal integration aren't well understood. To study how neural circuits integrate visual and tactile information, we developed a multisensory discrimination task for head-fixed mice. Here, sequences of visual, tactile or visuotactile stimuli are presented on both sides of the mouse. After a short delay the mouse has to indicate the side with the higher stimulus rate to obtain a water reward. Mice achieved high accuracy in all conditions, with improved performance in multisensory trials. Using widefield-imaging, we measured cortex-wide activity in transgenic mice, expressing the Ca^{2+}-indicator GCaMP6s in excitatory neurons. Multisensory stimuli evoked higher neuronal activity compared to unisensory stimulation, particularly in the rostrolateral association area RL and the medial frontal cortex (mFC). To better isolate sensory responses we used a linear encoding model. Including a multisensory interaction term explained significantly more information than unisensory regressors alone, especially in parietal and frontal areas. This suggests that cortical multisensory responses are not simply a linear summation of unisensory responses. To causally test the relation between cortical activity and perceptual decisions, we used the inhibitory opsin stGtACR2 to silence excitatory neurons in RL and mFC at different times during the task. Inhibiting mFC during the delay resulted in robust impairments in all conditions, whereas inactivating RL mainly affected visual performance, despite robust multisensory activity. Our results show distributed cortical sensory processing and a convergence of sensory streams in mFC guiding behavior.

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DISTINCT ENCODING OF SELF AND EXTERNAL MOTION IN CORTICAL AND COLLICULAR NETWORKS INVOLVED IN SPATIAL ORIENTATION

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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While self-motion information can be directly derived from the vestibular system, its combination with external visual signals allows the brain to discern the location and motion status of one's self relative to the surrounding world. Recently, vestibular and visual inputs have been shown to converge onto individual neurons in areas involved in spatial navigation, such as visual and retrosplenial cortices in the rodent brain. Yet, the respective contribution of these sensory cues to motion signalling in different brain regions remains unclear. To address this, we performed high-density single-unit recordings from several interconnected areas involved in spatial orientation (the retrosplenial cortex, primary visual cortex, subicular complex, and the superior colliculus (SC)) while head-fixed mice were horizontally rotated and/or presented with a full-field visual motion stimulus. By applying our recently described probabilistic tensor decomposition model to neural spike trains, we extracted motion related variables from the population activity in an unsupervised manner. We observed signals that reflected the temporal dynamics of angular speed and direction of the mouse or the visual scene as well as the animal's acceleration and accumulated angular displacement. The distribution of these signals across different areas was not homogeneous and revealed that motion representation in cortical regions is largely dominated by internally-generated cues. In contrast, the superficial SC mainly encoded external visual motion while deep layers of SC were dominated by self-motion variables. These findings suggest that internally and externally generated motion signals are utilised in different ways by cortical and collicular networks engaged in spatial navigation.

Pubmed:

34788632: Keshavarzi S, Bracey EF, Faville RA, Campagner D, Tyson AL, Lenzi SC, Branco T, Margrie TW
Multisensory coding of angular head velocity in the retrosplenial cortex.
To successfully navigate the environment, animals depend on their ability to continuously track their heading direction and speed. Neurons that encode angular head velocity (AHV) are fundamental to this process, yet the contribution of various motion signals to AHV coding in the cortex remains elusive. By performing chronic single-unit recordings in the retrosplenial cortex (RSP) of the mouse and tracking the activity of individual AHV cells between freely moving and head-restrained conditions, we find that vestibular inputs dominate AHV signaling. Moreover, the addition of visual inputs onto these neurons increases the gain and signal-to-noise ratio of their tuning during active exploration. Psychophysical experiments and neural decoding further reveal that vestibular-visual integration increases the perceptual accuracy of angular self-motion and the fidelity of its representation by RSP ensembles. We conclude that while cortical AHV coding requires vestibular input, where possible, it also uses vision to optimize heading estimation during navigation.
Neuron, 2022; 110
34048426: Tyson AL, Rousseau CV, Niedworok CJ, Keshavarzi S, Tsitoura C, Cossell L, Strom M, Margrie TW
A deep learning algorithm for 3D cell detection in whole mouse brain image datasets.
Understanding the function of the nervous system necessitates mapping the spatial distributions of its constituent cells defined by function, anatomy or gene expression. Recently, developments in tissue preparation and microscopy allow cellular populations to be imaged throughout the entire rodent brain. However, mapping these neurons manually is prone to bias and is often impractically time consuming. Here we present an open-source algorithm for fully automated 3D detection of neuronal somata in mouse whole-brain microscopy images using standard desktop computer hardware. We demonstrate the applicability and power of our approach by mapping the brain-wide locations of large populations of cells labeled with cytoplasmic fluorescent proteins expressed via retrograde trans-synaptic viral infection.
PLoS Comput Biol, 2021; 17
29551490: Vélez-Fort M, Bracey EF, Keshavarzi S, Rousseau CV, Cossell L, Lenzi SC, Strom M, Margrie TW
A Circuit for Integration of Head- and Visual-Motion Signals in Layer 6 of Mouse Primary Visual Cortex.

To interpret visual-motion events, the underlying computation must involve internal reference to the motion status of the observer's head. We show here that layer 6 (L6) principal neurons in mouse primary visual cortex (V1) receive a diffuse, vestibular-mediated synaptic input that signals the angular velocity of horizontal rotation. Behavioral and theoretical experiments indicate that these inputs, distributed over a network of 100 L6 neurons, provide both a reliable estimate and, therefore, physiological separation of head-velocity signals. During head rotation in the presence of visual stimuli, L6 neurons exhibit postsynaptic responses that approximate the arithmetic sum of the vestibular and visual-motion response. Functional input mapping reveals that these internal motion signals arrive into L6 via a direct projection from the retrosplenial cortex. We therefore propose that visual-motion processing in V1 L6 is multisensory and contextually dependent on the motion status of the animal's head.
Neuron, 2018; 98
26400933: Keshavarzi S, Power JM, Albers EH, Sullivan RK, Sah P
Dendritic Organization of Olfactory Inputs to Medial Amygdala Neurons.
The medial amygdala (MeA) is a central hub in the olfactory neural network. It receives vomeronasal information directly from the accessory olfactory bulb (AOB) and main olfactory information largely via odor-processing regions such as the olfactory cortical amygdala (CoA). How these inputs are processed by MeA neurons is poorly understood. Using the GAD67-GFP mouse, we show that MeA principal neurons receive convergent AOB and CoA inputs. Somatically recorded AOB synaptic inputs had slower kinetics than CoA inputs, suggesting that they are electrotonically more distant. Field potential recording, pharmacological manipulation, and Ca(2+) imaging revealed that AOB synapses are confined to distal dendrites and segregated from the proximally located CoA synapses. Moreover, unsynchronized AOB inputs had significantly broader temporal summation that was dependent on the activation of NMDA receptors. These findings show that MeA principal neurons process main and accessory olfactory inputs differentially in distinct dendritic compartments. Significance statement: In most vertebrates, olfactory cues are processed by two largely segregated neural pathways, the main and accessory olfactory systems, which are specialized to detect odors and nonvolatile chemosignals, respectively. Information from these two pathways ultimately converges at higher brain regions, one of the major hubs being the medial amygdala. Little is known about how olfactory inputs are processed by medial amygdala neurons. This study shows that individual principal neurons in this region receive input from both pathways and that these synapses are spatially segregated on their dendritic tree. We provide evidence suggesting that this dendritic segregation leads to distinct input integration and impact on neuronal output; hence, dendritic mechanisms control olfactory processing in the amygdala.
J Neurosci, 2015; 35
24966371: Keshavarzi S, Sullivan RK, Ianno DJ, Sah P
Functional properties and projections of neurons in the medial amygdala.
The medial nucleus of the amygdala (MeA) plays a key role in innate emotional behaviors by relaying olfactory information to hypothalamic nuclei involved in reproduction and defense. However, little is known about the neuronal components of this region or their role in the olfactory-processing circuitry of the amygdala. Here, we have characterized neurons in the posteroventral division of the medial amygdala (MePV) using the GAD67-GFP mouse. Based on their electrophysiological properties and GABA expression, unsupervised cluster analysis divided MePV neurons into three types of GABAergic (Types 1-3) and two non-GABAergic cells (Types I and II). All cell types received olfactory synaptic input from the accessory olfactory bulb and, with the exception of Type 2 GABAergic neurons, sent projections to both reproductive and defensive hypothalamic nuclei. Type 2 GABAergic cells formed a chemically and electrically interconnected network of local circuit inhibitory interneurons that resembled neurogliaform cells of the piriform cortex and provided feedforward inhibition of the olfactory-processing circuitry of the MeA. These findings provide a description of the cellular organization and connectivity of the MePV and further our understanding of amygdala circuits involved in olfactory processing and innate emotions.
J Neurosci, 2014; 34
19505798: Parinejad N, Keshavarzi S, Movahedin M, Raza M
Behavioral and histological assessment of the effect of intermittent feeding in the pilocarpine model of temporal lobe epilepsy.
Temporal lobe epilepsy (TLE) is the most resistant type of epilepsy. Currently available drugs for epilepsy are not antiepileptogenic. A novel treatment for epilepsy would be to block or reverse the process of epileptogenesis. We used intermittent feeding (IF) regimen of the dietary restriction (DR) to study its effect on epileptogenesis and neuroprotection in the pilocarpine model of TLE in rats. The effect of IF regimen on the induction of status epilepticus (SE), the duration of latent period, and the frequency, duration, severity and the time of occurrence of Spontaneous Recurrent Seizures (SRS) were investigated. We also studied the effect of IF regimen on hippocampal neurons against the excitotoxic damage of prolonged SE (about 4h) induced by pilocarpine. The animals (Wistar, male, 200-250g) were divided into four main groups: AL-AL (ad libitum diet throughout), AL-IF (PfS) [IF post-first seizure], AL-IF (PSE) [IF post-SE] and IF-IF (IF diet throughout), and two AL and IF control groups. SE was induced by pilocarpine (350mg/kg, i.p.) and with diazepam (6mg/kg, i.p.) injected after 3h, the behavioral signs of SE terminated at about 4h (AL animals, n=29, 260.43+/-8.74min; IF animals, n=19, 224.32+/-20.73min).

Behavioral monitoring was carried out by 24h video recording for 3 weeks after the first SRS. Rat brains were then prepared for histological study with Nissl stain and cell counting was done in CA1, CA2 and CA3 regions of the hippocampus. The results show that the animals on IF diet had significantly less SE induction and significantly longer duration of latent period (the period of epileptogenesis) was seen in IF-IF group compared to the AL-AL group. The severity of SRS was significantly more in AL-IF (PfS) compared to the AL-IF (PSE) group. These results indicate that IF diet can make rats resistant to the induction of SE and can prolong the process of epileptogenesis. The results of the histological study show that the number of pyramidal neurons was statistically less in CA1, CA2 and CA3 of the hippocampus in the experimental groups compared to the control groups. However, IF regimen could not protect the hippocampal neurons against the excitotoxic injury caused by a prolonged SE. We conclude that IF regimen can significantly influence various behavioral characteristics of pilocarpine model of TLE. Further studies can elaborate the exact mechanisms as well as its possible role in the treatment of human TLE.
Epilepsy Res, 2009; 86

17465369: Keshavarzi S, Nejat F, Kazemi H
Double spinal dysraphism. Report of three cases.
The simultaneous presence of multiple spinal neural tube defects is unusual. There have been only a few of these cases reported in the literature. The authors report on three cases of double spina bifida cystica. One patient had two myelomeningoceles (MMCs) at the cervical and lumbosacral regions, one was noted to have both thoracolumbar and sacral defects, and the third presented with double MMCs at lumbar and lumbosacral levels. All three neonates in these cases underwent surgical treatment and ventriculoperitoneal (VP) shunt insertion for associated hydrocephalus. One child died at the age of 2 months despite a well-functioning VP shunt. The other two patients had no complications. Current models of neural tube closure do not thoroughly explain the mechanisms of multiple spinal dysraphism, but the multisite closure model provides a better understanding of caudal neural tube closure than other closure-site models.
J Neurosurg, 2007; 106

17415183: Nejat F, Keshavarzi S, Monajemzadeh M, Mehdizadeh M, Kalaghchi B
Chronic subdural hematoma associated with subdural rhabdomyosarcoma: case report.
Subdural rhabdomyosarcoma is very rare, and even more unusual is the association between these sarcomas and chronic subdural hematoma. In this report, we present a case of subdural rhabdomyosarcoma that developed in a chronic refractory subdural hematoma.
Neurosurgery, 2007; 60

18723709: Amoli HA, Golozar A, Keshavarzi S, Tavakoli H, Yaghoobi A
Morphine analgesia in patients with acute appendicitis: a randomised double-blind clinical trial.
The administration of analgesics to patients with acute abdominal pain due to acute appendicitis is controversial. A study was undertaken to assess the analgesic effect of morphine in patients with acute appendicitis.
Emerg Med J, 2008; 25

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BOARD NUMBER: S05-479

ASSESSING SENSE OF EMBODIMENT: ITS DIRECT AND INDIRECT EFFECT ON PHYSIOLOGICAL MEASURES

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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We explore the assessment of Sense of Embodiment (SoE), namely the extent to which a surrogate is perceived as one's own. The Rubber Hand Illusion is the pioneer study in this direction. It is hypothesized that physiological measures reflect SoE in a direct and indirect way. If individuals feel strongly embodied, presenting an emotional stimulus like a threat to the surrogate will produce a strong response, as if the stimulus would be presented to their own body. This would lead to a positive correlation between SoE and the measure value during the presentation of emotional stimuli. There may also be an indirect effect. It is postulated that higher degrees of embodiment reduce workload when controlling a surrogate. This indirect effect of embodiment through lower workload would result in a negative correlation between SoE and the measure value, since lower workload results in lower physiological values. We investigated these effects in five experiments, comparing different physiological measures (such as skin conductance response, heart rate, pupil dilation). Our hypothesis was partially accepted, since we did not observe consistent results among the different measures. Pupil dilation, that we propose as novel measure of SoE, resulted in the most sensitive measure. We observed that pupil dilation and SoE are positive and direct correlated in case of emotional stimuli subjected to the surrogate (e.g. a threat), and that the pupil diameter tended to be smaller for participants who experienced a condition designed to provide high SoE compared to one designed to provide low SoE.

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BOARD NUMBER: S05-480

NEURAL ENCODING OF SENSORY "SURPRISE" IN THE MOUSE CORTEX

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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The brain generates predictions of future sensory inputs based on the statistics of recent sensory information. A mismatch between expected and perceived sensory stimuli elicits a "prediction error" or "surprise" signal that is used to update the mental model of the sensory world. Indeed, "surprise" is a major driving force for learning. For example, surprising isolated sensory stimuli triggers a large and widespread electrocortical activity in the mammal brain (the vertex potential) which is coupled with marked changes in behavioral output. However, it is still unclear how "surprise" signals are represented at different hierarchical levels of cortical processing, as well as at different spatiotemporal scales, ranging from individual neurons to large cortical areas. Also, if and how surprise at individual neuron level relates to the widespread surprise-related vertex potential widely studied in humans remains elusive. To answer those questions, we recorded single neuron activity from different hierarchical levels of cortical processing in the mouse brain, i.e. from primary visual or auditory cortices to parietal and prefrontal cortical areas, in response to unexpected visual and auditory sensory stimuli. Our data suggest that neural activity in primary sensory cortices is highly sensitive to abrupt changes in stimulus intensity or pattern, both at the level of single cell and average population activity. These results indicate that detection mechanisms for quick changes in the environment already exist at early stages of sensory processing. Comparing data across different scales and species will lead to a better understanding of how the brain detects and responds to surprising environmental events.

Pubmed:

34977678: Tang Y, Benusiglio D, Lefevre A, Küppers S, Lapies O, Kerspern D, Charlet A, Grinevich V
Viral vectors for opto-electrode recording and photometry-based imaging of oxytocin neurons in anesthetized and socially interacting rats.
Here, we present a step-by-step protocol to target, record, and manipulate the activity of oxytocin neurons in awake rats. The protocol includes a procedure to record the activity of oxytocin neurons from awake and socially interacting rats using opto-electrodes for simultaneous electrophysiological recording and virally based cell-type-specific opto-tagging with Channelrhodopsin 2. Furthermore, we illustrate a procedure for optically guided implantation of optic fiber and imaging of oxytocin neuron population activity expressing calcium indicator GCaMP6s with the fiber photometry technique. For complete details on the use and execution of this protocol, please refer to Tang et al., 2020.
STAR Protoc, 2022; 3
34194303: Lefevre A, Benusiglio D, Tang Y, Krabichler Q, Charlet A, Grinevich V
Oxytocinergic Feedback Circuitries: An Anatomical Basis for Neuromodulation of Social Behaviors.
Oxytocin (OT) is a neuropeptide produced by hypothalamic neurons and is known to modulate social behavior among other functions. Several experiments have shown that OT modulates neuronal activity in many brain areas, including sensory cortices. OT neurons thus project axons to various cortical and subcortical structures and activate neuronal subpopulations to increase the signal-to-noise ratio, and in turn, increases the saliency of social stimuli. Less is known about the origin of inputs to OT neurons, but recent studies show that cells projecting to OT neurons are often located in regions where the OT receptor (OTR) is expressed. Thus, we propose the existence of reciprocal connectivity between OT neurons and extrahypothalamic OTR neurons to tune OT neuron activity depending on the behavioral context. Furthermore, the latest studies have shown that OTR-expressing neurons located in social brain regions also project to other social brain regions containing OTR-expressing neurons. We hypothesize that OTR-expressing neurons across the brain constitute a common network coordinated by OT.
Front Neural Circuits, 2021; 15
33589833: Wahis J, Baudon A, Althammer F, Kerspern D, Goyon S, Hagiwara D, Lefevre A, Barteczko L, Boury-Jamot B, Bellanger B, Abatis M, Da Silva Gouveia M, Benusiglio D, Eliava M, Rozov A, Weinsanto I, Knobloch-Bollmann HS, Kirchner MK, Roy RK, Wang H, Pertin M, Inquimbert P, Pitzer C, Siemens J, Goumon Y, Boutrel B, Lamy CM, Decosterd I, Chatton

JY, Rouach N, Young WS, Stern JE, Poisbeau P, Stoop R, Darbon P, Grinevich V, Charlet A
Astrocytes mediate the effect of oxytocin in the central amygdala on neuronal activity and affective states in rodents.
Oxytocin (OT) orchestrates social and emotional behaviors through modulation of neural circuits. In the central amygdala, the release of OT modulates inhibitory circuits and, thereby, suppresses fear responses and decreases anxiety levels. Using astrocyte-specific gain and loss of function and pharmacological approaches, we demonstrate that a morphologically distinct subpopulation of astrocytes expresses OT receptors and mediates anxiolytic and positive reinforcement effects of OT in the central amygdala of mice and rats. The involvement of astrocytes in OT signaling challenges the long-held dogma that OT acts exclusively on neurons and highlights astrocytes as essential components for modulation of emotional states under normal and chronic pain conditions.
Nat Neurosci, 2021; 24
32719563: Tang Y, Benusiglio D, Lefevre A, Hilfiger L, Althammer F, Bludau A, Hagiwara D, Baudon A, Darbon P, Schimmer J, Kirchner MK, Roy RK, Wang S, Eliava M, Wagner S, Oberhuber M, Conzelmann KK, Schwarz M, Stern JE, Leng G, Neumann ID, Charlet A, Grinevich V
Social touch promotes interfemale communication via activation of parvocellular oxytocin neurons.
Oxytocin (OT) is a great facilitator of social life but, although its effects on socially relevant brain regions have been extensively studied, OT neuron activity during actual social interactions remains unexplored. Most OT neurons are magnocellular neurons, which simultaneously project to the pituitary and forebrain regions involved in social behaviors. In the present study, we show that a much smaller population of OT neurons, parvocellular neurons that do not project to the pituitary but synapse onto magnocellular neurons, is preferentially activated by somatosensory stimuli. This activation is transmitted to the larger population of magnocellular neurons, which consequently show coordinated increases in their activity during social interactions between virgin female rats. Selectively activating these parvocellular neurons promotes social motivation, whereas inhibiting them reduces social interactions. Thus, parvocellular OT neurons receive particular inputs to control social behavior by coordinating the responses of the much larger population of magnocellular OT neurons.
Nat Neurosci, 2020; 23
30954921: Grund T, Tang Y, Benusiglio D, Althammer F, Probst S, Oppenländer L, Neumann ID, Grinevich V
Chemogenetic activation of oxytocin neurons: Temporal dynamics, hormonal release, and behavioral consequences.
Chemogenetics provides cell type-specific remote control of neuronal activity. Here, we describe the application of chemogenetics used to specifically activate oxytocin (OT) neurons as representatives of a unique class of neuroendocrine cells. We injected recombinant adeno-associated vectors, driving the stimulatory subunit hM3Dq of a modified human muscarinic receptor into the rat hypothalamus to achieve cell type-specific expression in OT neurons. As chemogenetic activation of OT neurons has not been reported, we provide systematic analysis of the temporal dynamics of OT neuronal responses in vivo by monitoring calcium fluctuations in OT neurons, and intracerebral as well as peripheral release of OT. We further provide evidence for the efficiency of chemogenetic manipulation at behavioral levels, demonstrating that evoked activation of OT neurons leads to social motivation and anxiolysis. Altogether, our results will be profitable for researchers working on the physiology of neuroendocrine systems, peptidergic modulation of behaviors and translational psychiatry.
Psychoneuroendocrinology, 2019; 106
27242460: Tang Y, Benusiglio D, Grinevich V, Lin L
Distinct Types of Feeding Related Neurons in Mouse Hypothalamus.
The last two decades of research provided evidence for a substantial heterogeneity among feeding-related neurons (FRNs) in the hypothalamus. However, it remains unclear how FRNs differ in their firing patterns during food intake. Here, we investigated the relationship between the activity of neurons in mouse hypothalamus and their feeding behavior. Using tetrode-based in vivo recording technique, we identified various firing patterns of hypothalamic FRNs, which, after the initiation of food intake, can be sorted into four types: sharp increase (type I), slow increase (type II), sharp decrease (type III), and sustained decrease (type IV) of firing rates. The feeding-related firing response of FRNs was rigidly related to the duration of food intake and, to a less extent, associated with the type of food. The majority of these FRNs responded to glucose and leptin and exhibited electrophysiological characteristics of putative GABAergic neurons. In conclusion, our study demonstrated the diversity of neurons in the complex hypothalamic network coordinating food intake.
Front Behav Neurosci, 2016; 10
26478209: Tizzoni M, Sun K, Benusiglio D, Karsai M, Perra N
The Scaling of Human Contacts and Epidemic Processes in Metapopulation Networks.
We study the dynamics of reaction-diffusion processes on heterogeneous metapopulation networks where interaction rates scale with subpopulation sizes. We first present new empirical evidence, based on the analysis of the interactions of 13 million users on Twitter, that supports the scaling of human interactions with population size with an exponent γ ranging between 1.11 and 1.21, as observed in recent studies based on mobile phone data. We then integrate such observations into a reaction- diffusion metapopulation framework. We provide an explicit analytical expression for the global invasion threshold

which sets a critical value of the diffusion rate below which a contagion process is not able to spread to a macroscopic fraction of the system. In particular, we consider the Susceptible-Infectious-Recovered epidemic model. Interestingly, the scaling of human contacts is found to facilitate the spreading dynamics. This behavior is enhanced by increasing heterogeneities in the mobility flows coupling the subpopulations. Our results show that the scaling properties of human interactions can significantly affect dynamical processes mediated by human contacts such as the spread of diseases, ideas and behaviors.
Sci Rep, 2015; 5

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SALIENCE OF MULTISENSORY FEEDBACK MODULATES ACTIVE SENSING MOVEMENTS OF WEAKLY ELECTRIC FISH DURING REFUGE TRACKING IN A FLOW TUNNEL

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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An amazing phenomenon in biological systems is that sensing and motor systems are inherently coupled. Despite its seemingly adverse nature, animals benefit from this coupling by utilizing motor actions to alter the spatiotemporal patterns of sensory information they perceive from the environment—a process known as active sensing. Our goal is to examine the effects of active sensing on sensorimotor control performance during natural behavior of animals. To achieve this, we built an experimental setup to study active sensing in *Apteronotus albifrons* during their refuge tracking behavior. This behavior is a form of image stabilization, where fish swim to remain within a moving refuge. We built a speed-controlled flow tunnel to imitate the natural Amazonian habitats of these fish. We use a cylindrical refuge without flooring to allow video recordings from below. The refuge is attached to a linear actuator that moves longitudinally to provide visual and electrosensory stimulation for the fish. We experimented with N=5 fish using sum-of-sines type stimulus, sweeping 0-2 Hz range. The experiments were repeated with various flow speeds (0-20 cm/s), illumination (0-300 lux), and refuge structures (with and without windows). We recorded the kinematic response of the fish under each sensory condition. We first segregated the tracking response of the fish. Then, we identified the stereotyped active sensing movements. Our results show that active sensing movements increase as the quality of the sensory information decreases. We are currently investigating how these movements help the fish to improve their behavioral performance using a control-theoretic approach.

Pubmed:
34856451: Yüksel S, Aşık MD, Aydin HM, Tönük E, Aydın EY, Bozkurt M
Fabrication of a multi-layered decellularized amniotic membranes as tissue engineering constructs.
As a promising approach in tissue engineering, decellularization has become one of the mostly-studied research areas in tissue engineering thanks to its potential to bring about several advantages over synthetic materials since it can provide a 3-dimensional ECM structure with matching biomechanical properties of the target tissue. Amniotic membranes are the tissues that nurture the embryos during labor. Similarly, these materials have also been proposed for tissue regeneration in several applications. The main drawback in using amniotic membranes is the limited thickness of these materials since most tissues require a 3D matrix for an enhance regeneration. In order to prevent this limitation, here we report a facile fabrication methodology for multilayered amniotic membrane-based tissue constructs. The amniotic membranes of Wistar albino rats were first decellularized with the physical and chemical methods and utilized as scaffolds. Secondly, the prepared decellularized membranes were sutured to form a multilayered 3D structure. Within the study, 7 groups including control (PBS), were prepared based on physical and chemical decellularization methods. UV exposure and freezing techniques were used as a physical decellularization methods while hypertonic medium and SDS (sodium dodecyl sulfate) protocols were used as chemical decellularization methods. The combinations of both protocols were also used. In groups, A was the control and group B was applied just UV. In group C was applied UV and freezing. In addition to UV and freezing, in group D was applied hypertonic solution while group E was applied SDS (0.03 %). In group F was applied UV, freezing, hypertonic solution and SDS (0.03 %). In group G was applied UV, hypertonic solution, SDS (0.03 %) and freezing, respectively. Based on the histological and quantitative analyses, F and G groups were found as the most efficient decellularization protocols in rat amniotic membranes. Then, group F and G decellularized amniotic membranes were used to form scaffolds and thus-formed matrices were further characterized in vitro cell culture studies and mechanical tests. Cytotoxicity analyses performed using MTT showed a good cell viability in F and G groups scaffolds. The percentage viability rate was higher in G group (81.3 %) compared to F (75.33 %) and also cell viability in G group was found more meaningful according to p value which was obtained 0.007. Cellular adhesions after in vitro cell culture and morphology of scaffolds were evaluated by scanning electron

microscopy (SEM). It was observed that the cells cultivated in equal amounts of tissue scaffolds were higher in the F compared to that observed in group G. The mechanical testing with 40 N force revealed 0.77 mm displacement in group F while it was 0.75 mm in group G. Moreover, according to force-controlled test, 2.9 mm displacement of F group and 1.2 mm displacement of G group was measured. As a result, this study shows that the multilayered decellularized amniotic membrane scaffolds support cell survival and adhesion and can form a flexible biomaterial with desired handling properties. Tissue Cell, 2022; 74

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BOARD NUMBER: S05-482

AUDIOVISUAL INTEGRATION IN MOUSE SUPERIOR COLLICULUS IS ADDITIVE AND RARE

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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[Aims] The superior colliculus (SC) receives auditory and visual inputs and is thought to combine them to form a representation of audiovisual space. However, the computational rules behind audiovisual integration are unclear: while electrophysiological studies in SC have emphasized super- & subadditivity, mouse behavior seems consistent with additive integration. We thus asked whether audiovisual integration in the mouse SC might be primarily additive in the majority of neurons. [Methods] We used Neuropixels 1.0 and 2.0 probes to record the responses of ~2,600 SC neurons in awake mice to checkerboard images and pink noise sound bursts presented at varying azimuths, alone or in combination. We then fitted six models to each neuron's response time courses using cross-validation. Models varied in aspects such as spatial selectivity and linearity. Five models were linear and a sixth allowed for nonlinearities. [Results] Neurons responding to both images and sounds were rare (19% of neurons). Most of them had spatial selectivity for images but not for sounds: they responded to sound onsets regardless of position. Only 4% of neurons, mostly in deeper layers, were selective for both auditory and visual position. Practically all audiovisual neurons (98%) were best fitted by an additive model. [Conclusions] We conclude that integration of auditory and visual position in mouse SC is rare, but many neurons additively integrate visual stimuli and sound onsets. We speculate that such sound onset responses may be related to changes in behavioral state.

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BOARD NUMBER: S05-483

MOUSE FRONTAL CORTEX MEDIATES ADDITIVE MULTISENSORY DECISIONS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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[Introduction] To make accurate perceptual decisions, the brain must combine information across sensory modalities. Probability theory suggests that evidence from independent cues should be combined additively, but it is unclear whether mice do this. Here we show that to localize a stimulus, mice combine auditory and visual spatial cues additively, a computation supported by unisensory processing in auditory and visual cortex and additive multisensory integration in frontal cortex. [Methods] We developed an audiovisual localization task where mice turn a wheel to indicate the joint position of an image and a sound. We performed optogenetic inactivation of different spots across dorsal cortex while mice performed this task. We then recorded from >10,000 neurons in the frontal cortex during behaviour. [Results] In this task, mice integrated auditory and visual cues independently. An additive model predicted the mean response of mice to all cue combinations. Optogenetic inactivation of different spots across dorsal cortex demonstrated that auditory and visual areas contribute unisensory information, whereas frontal cortex (secondary motor area, MOs) contributes multisensory information to the mouse's decision. Of the frontal areas recorded, only MOs activity could predict the mouse's upcoming choice. Consistent with the mice's additive behavior, neural activity in MOs reflected an additive combination of visual and auditory signals. Furthermore, an accumulator model applied to the sensory representations of MOs neurons reproduced behaviourally observed choices and reaction times. [Conclusions] Frontal area MOs integrates information from sensory cortices additively, providing a signal that is then transformed into a binary decision by a downstream accumulator.

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BEHAVIOUR-DEPENDENT MODULATION OF INHIBITION IN THE PRIMARY SOMATOSENSORY CORTEX

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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In primary sensory areas stimuli evoked responses are particular sensitive to alterations in behavioural states associated with variations in arousal or locomotion. Recruitment of disinhibitory circuits has been proposed to be important for such behavioural-dependent control of neuronal activity. However, it is unclear if the recruitment of local interneurons during such behaviour transitions is similar across sensory areas and whether it affects responses to sensory stimuli similarly across sensory modalities. In particular, rodents heavily use their whiskers during navigation, however, how locomotion modulates activity in the primary somatosensory cortex (S1) is not well understood. To address this issue, we performed *in vivo* two-photon calcium imaging of somatostatin-positive (SST-INs) and vasoactive intestinal peptide positive (VIP-INs) interneurons, key elements of cortical disinhibitory circuits, in awake head-fixed mice in S1 spontaneously transitioning between rest and running periods. We found in the dark, locomotion strongly recruited SST-INs in the barrel cortex while having no effect in SST-INs in the visual cortex. This difference in SST-INs activity occurred despite the strong recruitment of VIP-INs in the two brain areas. In addition, neither the strength of inhibition provided by VIP-INs nor the sensitivity of SST-INs to cholinergic signalling was different across sensory modalities. We are presently investigating the contribution of thalamic activity to the differential recruitment of SST-INs in V1 and S1 by locomotion. The present results indicate that the recruitment of local inhibitory neurons is different across sensory areas for the same behavioural transition suggesting that impact of arousal in sensory cortices is modality specific.

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THE TEMPORAL DYNAMICS OF AUDIOVISUAL EVIDENCE ACCUMULATION IN HUMANS AND MICE

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Integration of information from multiple sensory modalities over time is necessary for the brain to create a unified percept of the outside world and to perform functions such as decision-making. In natural settings (multi)sensory evidence is not always instantaneously clear, but instead it can be noisy as it can consist of very subtle and sometimes even contradictory stimuli that vary dynamically. A currently unexplored question is how time-varying visual and auditory information is integrated over time during audiovisual decision-making. To study this question we instructed human subjects and trained mice to discriminate between two visual gratings and/or two sound sources based on contrast and loudness that fluctuated in intensity every 50ms. We report that humans and mice based their decisions on early incoming information. Additionally, we demonstrate that audiovisual decision-making in humans was associated with modality dominance switching. The first visual and auditory evidence samples were equally integrated followed by second integration phase of visual dominance after which humans briefly switched to auditory dominance. Mice relied heavily on auditory evidence throughout the stimulus period. This suggests that mice and humans dynamically and differentially use time-varying audiovisual information during decision-making. We further present single-unit activity and local field potentials recorded from the primary visual cortex of task-performing mice. We correlate this neural activity with stimulus fluctuations and behavioral outcome, aiming to identify neural responses during visual processing, cross-modal and multimodal processing reflecting the decision of an animal. Our findings bring us one step closer to revealing the dynamics of multisensory decision-making.

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BOARD NUMBER: S05-486

DO CHANGES ON THE PLANTAR SYSTEM IMPACT THE BINOCULAR FUSION ?

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Background: Multisensory integration is a fundamental brain mechanism, allowing integration and convergences, between a multitude of inputs from the different senses. The purpose of this study is to assess sensory reweighting of the plantar modality on the binocular fusion. Method: Plantar modality contribution was investigated by infection of the foot sole afferent by foams (reduction) and texture (increase). Binocular disruption vision was assessed by the Perceptive Maddox through Vertical Heterophoria (VH) and their Lability (L), because ocular motor compensation is very weak in this plane (only proprioception variations). 40 subjects (28 adults 12 children) with VH were included. VH was randomly scored on foam Crispon Diabet® (CD) and Airgom® (AG) and Black Pyramid (BP, Crispin France). Result: CD and BP induce significant reduction of VH and increase L. These effects did not vary with age. Discussion: Feedback, lack CD AG or upgrade BP of plantar information induce a new sensory situation. Then, somatosensory information is reweighted and impact the binocular fusion with reduction of VH and intensification of L. Only CD inducing reduction depends on the mechanical foam proprieties. We confirm that it is possible to modify visual perception by reducing or increasing, sensory plantar information. Independently of the age, we suggest that the plantar system could affect the visual integration and could influence the visual proprioception correspondingly the binocular fusion. These findings support the hypothesis that plantar sensory variation may be an effective tool for evaluating the relationship between plantar and visual integration.

Pubmed:

32103890: Quercia P, Pozzo T, Marino A, Guillemant AL, Cappe C, Gueugneau N
Children with Dyslexia Have Altered Cross-Modal Processing Linked to Binocular Fusion. A Pilot Study.
The cause of dyslexia, a reading disability characterized by difficulties with accurate and/or fluent word recognition and by poor spelling and decoding abilities, is unknown. A considerable body of evidence shows that dyslexics have phonological disorders. Other studies support a theory of altered cross-modal processing with the existence of a pan-sensory temporal processing deficit associated with dyslexia. Learning to read ultimately relies on the formation of automatic multisensory representations of sounds and their written representation while eyes fix a word or move along a text. We therefore studied the effect of brief sounds on vision with a modification of binocular fusion at the same time (using the Maddox Rod test).
Clin Ophthalmol, 2020; 14
34780497: Lagarrigue Y, Cappe C, Tallet J
Regular rhythmic and audio-visual stimulations enhance procedural learning of a perceptual-motor sequence in healthy adults: A pilot study.
Procedural learning is essential for the effortless execution of many everyday life activities. However, little is known about the conditions influencing the acquisition of procedural skills. The literature suggests that sensory environment may influence the acquisition of perceptual-motor sequences, as tested by a Serial Reaction Time Task. In the current study, we investigated the effects of auditory stimulations on procedural learning of a visuo-motor sequence. Given that the literature shows that regular rhythmic auditory rhythm and multisensory stimulations improve motor speed, we expected to improve procedural learning (reaction times and errors) with repeated practice with auditory stimulations presented either simultaneously with visual stimulations or with a regular tempo, compared to control conditions (e.g., with irregular tempo). Our results suggest that both congruent audio-visual stimulations and regular rhythmic auditory stimulations promote procedural perceptual-motor learning. On the contrary, auditory stimulations with irregular or very quick tempo alter learning. We discuss how regular rhythmic multisensory stimulations may improve procedural learning with respect of a multisensory rhythmic integration process.
PLoS One, 2021; 16
31308621: Quercia P, Pozzo T, Marino A, Guillemant AL, Cappe C, Gueugneau N

Alteration in binocular fusion modifies audiovisual integration in children.
In the field of multisensory integration, vision is generally thought to dominate audiovisual interactions, at least in spatial tasks, but the role of binocular fusion in audiovisual integration has not yet been studied. Using the Maddox test, a classical ophthalmological test used to subjectively detect a latent unilateral eye deviation, we checked whether an alteration in binocular vision in young patients would be able to change audiovisual integration. The study was performed on a group of ten children (five males and five females aged 11.3±1.6 years) with normal binocular vision, and revealed a visual phenomenon consisting of stochastic disappearanceof part of a visual scene caused by auditory stimulation. Indeed, during the Maddox test, brief sounds induced transient visual scotomas (VSs) in the visual field of the eye in front of where the Maddox rod was placed. We found a significant correlation between the modification of binocular vision and VS occurrence. No significant difference was detected in the percentage or location of VS occurrence between the right and left eye using the Maddox rod test orbetween sound frequencies. The results indicate a specific role of the oculomotor system in audiovisual integration in children. This convenient protocol may also have significant interest for clinical investigations of developmental pathologies where relationships between vision and hearing are specifically affected.
Clin Ophthalmol, 2019; 13
19545613: Janin M, Dupui P
The effects of unilateral medial arch support stimulation on plantar pressure and center of pressure adjustment in young gymnasts.
The purpose of this study was to examine the contribution of tactile afferents from the medial arch of the foot on postural control. The center of pressure (CoP) position and right/left plantar pressure distributions of 13 gymnasts, with and without a medial arch support, were recorded by a force platform coupled with a baropedometry analysis. Stimulation of the subject's plantar sole was accomplished using a 3mm thick medial arch insert. Right arch stimulation induced an ipsilateral increase of plantar pressure and a contralateral displacement of the CoP to the left. Left arch support also resulted in an ipsilateral increase in plantar pressure and displacement of the CoP to the right. Stimulation of the plantar arch may induce a perception that the body's center of mass has shifted toward the stimulated foot. To maintain stability, individuals may then shift their CoP in the opposite direction. This response may involve compensatory muscle activation strategies to adjust posture. Clinicians may apply these results in their use of foot orthoses to address postural anomalies in patients.
Neurosci Lett, 2009; 461

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BOARD NUMBER: S05-487

THE CENTRAL COMPLEX OF DROSOPHILA LARVA

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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The Central Complex (CX) is a highly conserved set of neuropils found in insects, that is responsible for integrating sensory inputs (visual, mechanosensory and olfactory) towards spatial navigation decisions (e.g. heading direction, angular velocity and forward velocity), directed locomotion and sleep. In Drosophila, as in other holometabolous insects the central complex is formed during late larval and metamorphic stages. Nevertheless, this brain area has only been characterized in the adult stage of the fruit fly with little attention to its function in earlier stages of development. In this project, we aim at exploring - at synaptic level - whether a simplified version of the central complex exists in Drosophila larvae. Using the mapped electron microscopy volume of the L1 larva, we find that many central complex neuropils extant in the adult are also present in L1, including the noduli (NO), the protocerebral bridge(PB), and a substructure that seems to be the precursor for both the elipsoid body(EB) and the fan-shaped body(FB). Importantly, when describing these structures, we observed that patterns of connecivity characteristic to the adult CX are also present in the larva: (i) strong, direct connections from MBONs to the FB and NO, (ii) high levels of visual input into the PB and EB, but also (iii) strong connnectivity between FB and NO. Interestingly, we find that some lineages contributing to the CX of the larva seem to switch function in the adult, whilst others remain conserved.

Pubmed:
35044677: Luke DP, Lungu L, Friday R, Terhune DB
The chemical induction of synaesthesia.
Preliminary research suggests that experiences resembling synaesthesia are frequently reported under the influence of a diverse range of chemical substances although the incidence, chemical specificity, and characteristics of these effects are poorly understood.
Hum Psychopharmacol, 2022;
34126287: Lungu L, Rothen N, Terhune DB
The time course of synaesthetic colour perception.
Grapheme-colour synaesthesia is a neurodevelopmental condition wherein perception of numbers and letters consistently and involuntarily elicits concurrent experiences of colour photisms. Accumulating evidence suggests that heterogeneity in the visuospatial phenomenology of synaesthesia is attributable to the operation of top-down processes underlying photisms experienced as representations in associator synaesthetes and bottom-up processes subserving photisms experienced as spatially localized in projector synaesthetes. An untested corollary of this hypothesis is that bottom-up mechanisms will actuate earlier photism perception in projector than associator synaesthetes. We tested this prediction in a pre-registered study wherein associators and projectors completed adaptive temporal order judgement tasks for graphemes, colours, and photisms. In corroboration of the hypothesis of differential photism access across subtypes, projectors displayed lower photism colour thresholds than associators whereas the two subtypes did not significantly differ in veridical colour thresholds. Synaesthetes did not differ in grapheme or colour thresholds relative to non-synaesthete controls. These results are consistent with the proposal of differential neural mechanisms underlying photism perception in subtypes of grapheme-colour synaesthesia and warrant renewed attention to heterogeneity in the mechanisms and phenomenology of this condition.
Cortex, 2021; 141
31732472: Lungu L, Stewart R, Luke DP, Terhune DB
Primary visual cortex excitability is not atypical in acquired synaesthesia.
Brain Stimul, 2020 Mar - Apr; 13

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BOARD NUMBER: S05-488

SYNAPTIC PROCESSING IN EURYDENDROID NEURONS IN LARVAL ZEBRAFISH CEREBELLUM DURING ASSOCIATIVE MOTOR LEARNING

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Aims: Our goal was to identify changes in activity in cerebellar output neurons, during the acquisition of a motor behavior. Methods: We recorded Olig2(+) eurydendroid neurons (ENs) in whole-cell and loose-cell attached configurations, in immobilized larvae (dpf 6-8) while monitoring fictive swimming. The unconditioned stimulus, a mild electric tail shock, was repeatedly paired to the offset of the conditioned stimulus, a 2-s blue light. Results: Half fish acquired conditioned swimming responses to the light (CR), results reported below are from learners. Olig2(+) ENs showed robust excitatory and inhibitory synaptic activity, as well as increased firing, during unconditioned and conditioned fictive swimming, with inhibition always far exceeding excitation. Strikingly, at the population level, firing rates of ENs correlated with both EPSC and IPSC amplitude. Most Olig2(+) ENs had a purely motor-associative response and few had a mixed sensory-motor response; we have not seen a topographical distribution of those cells so far. Light-evoked synaptic inputs and an increase in firing preceded conditioned swimming by ~100 ms whereas tactile-related activity was concomitant to unconditioned swimming. Over the course of learning we did not observe modifications in synaptic inputs, only the presence or absence of CRs affected synaptic inputs. Conclusions: Olig2(+) ENs may participate in generating acquired motor responses. However, synaptic inputs from granule cell correlate with motor outcome, suggesting that the synaptic changes at the origin of CRs emergence occur upstream. Our results also suggest that a temporal patterning of EPSCs and IPSCs may allow ENs to fire action potentials despite the overwhelming inhibition.

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BOARD NUMBER: S05-489

INTER- AND INTRA-HEMISPHERIC SOURCES OF VESTIBULAR SIGNALS TO V1

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Sensations often trigger movements, but one's movements also generate sensory experience. Vestibular organs monitor head movements and contribute to the brain's ability to distinguish between self- and externally-generated sensory stimuli. Signals from the vestibular organs are broadcasted throughout the brain, including primary sensory regions such as the primary visual cortex (V1). However, what type of signals relative to head movements reach V1 and what the upstream sources of these signals are, remains poorly understood. Here we show that, in the absence of visual input, V1 neurons encode the direction of head movements as well as the angular velocity and acceleration in a layer specific manner. Moreover, we discovered that the ipsilateral latero-posterior thalamus (LP) and the contralateral visual cortex are key sources of vestibular input to V1. The ipsilateral LP provides the main head movement signal and this signal is biased toward contraversive head movements (i.e. clockwise head movements when recording from left V1). Instead, the contralateral visual cortex provides head-movement signals during ipsiversive head movements. Thus, this study reveals a direction specific recruitment of intra- and inter-hemispheric sources of vestibular signals to V1.

Pubmed:
34507990: Schonewille M, Girasole AE, Rostaing P, Mailhes-Hamon C, Ayon A, Nelson AB, Triller A, Casado M, De Zeeuw CI, Bouvier G
NMDARs in granule cells contribute to parallel fiber-Purkinje cell synaptic plasticity and motor learning.
Long-term synaptic plasticity is believed to be the cellular substrate of learning and memory. Synaptic plasticity rules are defined by the specific complement of receptors at the synapse and the associated downstream signaling mechanisms. In young rodents, at the cerebellar synapse between granule cells (GC) and Purkinje cells (PC), bidirectional plasticity is shaped by the balance between transcellular nitric oxide (NO) driven by presynaptic -methyl-D-aspartate receptor (NMDAR) activation and postsynaptic calcium dynamics. However, the role and the location of NMDAR activation in these pathways is still debated in mature animals. Here, we show in adult rodents that NMDARs are present and functional in presynaptic terminals where their activation triggers NO signaling. In addition, we find that selective genetic deletion of presynaptic, but not postsynaptic, NMDARs prevents synaptic plasticity at parallel fiber-PC (PF-PC) synapses. Consistent with this finding, the selective deletion of GC NMDARs affects adaptation of the vestibulo-ocular reflex. Thus, NMDARs presynaptic to PCs are required for bidirectional synaptic plasticity and cerebellar motor learning.
Proc Natl Acad Sci U S A, 2021; 118
32783882: Bouvier G, Senzai Y, Scanziani M
Head Movements Control the Activity of Primary Visual Cortex in a Luminance-Dependent Manner.
The vestibular system broadcasts head-movement-related signals to sensory areas throughout the brain, including visual cortex. These signals are crucial for the brain's ability to assess whether motion of the visual scene results from the animal's head movements. However, how head movements affect visual cortical circuits remains poorly understood. Here, we discover that ambient luminance profoundly transforms how mouse primary visual cortex (V1) processes head movements. While in darkness, head movements result in overall suppression of neuronal activity; in ambient light, the same head movements trigger excitation across all cortical layers. This light-dependent switch in how V1 processes head movements is controlled by somatostatin-expressing (SOM) inhibitory neurons, which are excited by head movements in dark, but not in light. This study thus reveals a light-dependent switch in the response of V1 to head movements and identifies a circuit in which SOM cells are key integrators of vestibular and luminance signals.
Neuron, 2020; 108
30418871: Bouvier G, Aljadeff J, Clopath C, Bimbard C, Ranft J, Blot A, Nadal JP, Brunel N, Hakim V, Barbour B
Cerebellar learning using perturbations.
The cerebellum aids the learning of fast, coordinated movements. According to current consensus, erroneously active parallel

fibre synapses are depressed by complex spikes signalling movement errors. However, this theory cannot solve the of processing a global movement evaluation into multiple cell-specific error signals. We identify a possible implementation of an algorithm solving this problem, whereby spontaneous complex spikes perturb ongoing movements, create eligibility traces and signal error changes guiding plasticity. Error changes are extracted by adaptively cancelling the average error. This framework, (SGDEGE), predicts synaptic plasticity rules that apparently contradict the current consensus but were supported by plasticity experiments in slices from mice under conditions designed to be physiological, highlighting the sensitivity of plasticity studies to experimental conditions. We analyse the algorithm's convergence and capacity. Finally, we suggest SGDEGE may also operate in the basal ganglia.
Elife, 2018; 7
27052175: Bouvier G, Higgins D, Spolidoro M, Carrel D, Mathieu B, Léna C, Dieudonné S, Barbour B, Brunel N, Casado M
Burst-Dependent Bidirectional Plasticity in the Cerebellum Is Driven by Presynaptic NMDA Receptors.
Numerous studies have shown that cerebellar function is related to the plasticity at the synapses between parallel fibers and Purkinje cells. How specific input patterns determine plasticity outcomes, as well as the biophysics underlying plasticity of these synapses, remain unclear. Here, we characterize the patterns of activity that lead to postsynaptically expressed LTP using both in vivo and in vitro experiments. Similar to the requirements of LTD, we find that high-frequency bursts are necessary to trigger LTP and that this burst-dependent plasticity depends on presynaptic NMDA receptors and nitric oxide (NO) signaling. We provide direct evidence for calcium entry through presynaptic NMDA receptors in a subpopulation of parallel fiber varicosities. Finally, we develop and experimentally verify a mechanistic plasticity model based on NO and calcium signaling. The model reproduces plasticity outcomes from data and predicts the effect of arbitrary patterns of synaptic inputs on Purkinje cells, thereby providing a unified description of plasticity.
Cell Rep, 2016; 15
26627919: Ly R, Bouvier G, Szapiro G, Prosser HM, Randall AD, Kano M, Sakimura K, Isope P, Barbour B, Feltz A
Contribution of postsynaptic T-type calcium channels to parallel fibre-Purkinje cell synaptic responses.
At the parallel fibre-Purkinje cell glutamatergic synapse, little or no Ca(2+) entry takes place through postsynaptic neurotransmitter receptors, although postsynaptic calcium increases are clearly involved in the synaptic plasticity. Postsynaptic voltage-gated Ca(2+) channels therefore constitute the sole rapid postsynaptic Ca(2+) signalling mechanism, making it essential to understand how they contribute to the synaptic signalling. Using a selective T-type calcium channel antagonist, we describe a T-type component of the EPSC that is activated by the AMPA receptor-mediated depolarization of the spine and thus will contribute to the local calcium dynamics. This component can amount up to 20% of the EPSC, and this fraction is maintained even at the high frequencies sometimes encountered in sensory processing. Modelling based on our biophysical characterization of T-type calcium channels in Purkinje cells suggests that the brief spine EPSCs cause the activated T-type channels to deactivate rather than inactivate, enabling repetitive activation.
J Physiol, 2016; 594
25750623: Bidoret C, Bouvier G, Ayon A, Szapiro G, Casado M
Properties and molecular identity of NMDA receptors at synaptic and non-synaptic inputs in cerebellar molecular layer interneurons.
N-methyl-D-aspartate receptors (NMDARs) in cerebellar molecular layer interneurons (MLIs) are expressed and activated in unusual ways: at parallel fibre (PF) synapses they are only recruited by repetitive stimuli, suggesting an extrasynaptic location, whereas their activation by climbing fibre is purely mediated by spillover. NMDARs are thought to play an important role in plasticity at different levels of the cerebellar circuitry. Evaluation of the location, functional properties and physiological roles of NMDARs will be facilitated by knowledge of the NMDAR isoforms recruited. Here we show that MLI-NMDARs activated by both PF and climbing fibre inputs have similar kinetics and contain GluN2B but not GluN2A subunits. On the other hand, no evidence was found of functional NMDARs in the axons of MLIs. At the PF-Purkinje cell (PF-PC) synapse, the activation of GluN2A-containing NMDARs has been shown to be necessary for the induction of long-term depression (LTD). Our results therefore provide a clear distinction between the NMDARs located on MLIs and those involved in plasticity at PF-PC synapses.
Front Synaptic Neurosci, 2015; 7
24277825: Ly R, Bouvier G, Schonewille M, Arabo A, Rondi-Reig L, Léna C, Casado M, De Zeeuw CI, Feltz A
T-type channel blockade impairs long-term potentiation at the parallel fiber-Purkinje cell synapse and cerebellar learning.
CaV3.1 T-type channels are abundant at the cerebellar synapse between parallel fibers and Purkinje cells where they contribute to synaptic depolarization. So far, no specific physiological function has been attributed to these channels neither as charge carriers nor more specifically as Ca(2+) carriers. Here we analyze their incidence on synaptic plasticity, motor behavior, and cerebellar motor learning, comparing WT animals and mice where T-type channel function has been abolished either by gene deletion or by acute pharmacological blockade. At the cellular level, we show that CaV3.1 channels are required for long-term potentiation at parallel fiber-Purkinje cell synapses. Moreover, basal simple spike discharge of the

Purkinje cell in KO mice is modified. Acute or chronic T-type current blockade results in impaired motor performance in particular when a good body balance is required. Because motor behavior integrates reflexes and past memories of learned behavior, this suggests impaired learning. Indeed, subjecting the KO mice to a vestibulo-ocular reflex phase reversal test reveals impaired cerebellum-dependent motor learning. These data identify a role of low-voltage activated calcium channels in synaptic plasticity and establish a role for CaV3.1 channels in cerebellar learning.
Proc Natl Acad Sci U S A, 2013; 110

23922966: Bergerot A, Rigby M, Bouvier G, Marcaggi P
Persistent posttetanic depression at cerebellar parallel fiber to Purkinje cell synapses.
Plasticity at the cerebellar parallel fiber to Purkinje cell synapse may underlie information processing and motor learning. In vivo, parallel fibers appear to fire in short high frequency bursts likely to activate sparsely distributed synapses over the Purkinje cell dendritic tree. Here, we report that short parallel fiber tetanic stimulation evokes a ~7-15% depression which develops over 2 min and lasts for at least 20 min. In contrast to the concomitantly evoked short-term endocannabinoid-mediated depression, this persistent posttetanic depression (PTD) does not exhibit a dependency on the spatial pattern of synapse activation and is not caused by any detectable change in presynaptic calcium signaling. This persistent PTD is however associated with increased paired-pulse facilitation and coefficient of variation of synaptic responses, suggesting that its expression is presynaptic. The chelation of postsynaptic calcium prevents its induction, suggesting that post- to presynaptic (retrograde) signaling is required. We rule out endocannabinoid signaling since the inhibition of type 1 cannabinoid receptors, monoacylglycerol lipase or vanilloid receptor 1, or incubation with anandamide had no detectable effect. The persistent PTD is maximal in pre-adolescent mice, abolished by adrenergic and dopaminergic receptors block, but unaffected by adrenergic and dopaminergic agonists. Our data unveils a novel form of plasticity at parallel fiber synapses: a persistent PTD induced by physiologically relevant input patterns, age-dependent, and strongly modulated by the monoaminergic system. We further provide evidence supporting that the plasticity mechanism involves retrograde signaling and presynaptic diacylglycerol.
PLoS One, 2013; 8

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BOARD NUMBER: S05-490

MULTISENSORY TRAINING INDUCED RECOVERY FROM HEMIANOPIA IN HUMAN PATIENTS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Contralateral blindness (hemianopia) is a common consequence of stroke or trauma that unilaterally damages visual cortex. We previously demonstrated in an animal model that vision is rapidly restored to the blinded hemifield by a noninvasive multisensory training paradigm (see Stein & Rowland, 2020). The paradigm involves repeatedly presenting congruent visual-auditory stimulus pairs in the blinded hemifield. Here we show this paradigm is effective in two human patients whose hemianopia was a consequence of right PCA occlusion. Over the course of several weekly sessions of multisensory training, both regained the ability to detect, verbally report, and accurately point to light flashes in the left hemifield while maintaining fixation. The recovery pattern was like that observed in the animal model: beginning at the sighted field margin and expanding to encompass all locations across the entire hemifield. The recovered vision was robust even when challenged with competitive stimuli in the opposite hemifield and both patients commented on the intensity and color of the stimuli. One could describe the shapes of stationary, flashed, or moving (high contrast) objects everywhere within the formerly blinded field, while the other did so only in more central locations. Both patients reported significant quality of life improvements. Recovery was consistent with a model in which spatiotemporally congruent visual-auditory stimulation engages the brain's mechanisms for multisensory plasticity, through which convergent cross-modal signals enhance the visual sensitivity of multisensory neurons. Supported by NIH R01 EY026916 and a pilot grant from the Wake Forest School of Medicine Clinical Trials and Innovation Center.

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BOARD NUMBER: S05-491

BRAIN MORPHOMETRIC CHANGES IN CONGENITALLY BLIND SUBJECTS - A 7 TESLA MRI STUDY

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Aims: To get a more comprehensive view of cerebral, subcortical and cerebral changes related to congenital blindness using ultra-high field magnetic resonance imaging (MRI). **Methods:** 12 congenitally blind (CB) and 11 matched sighted control (SC) subjects were scanned using 7 Tesla MRI at submillimeter resolution. Data were analyzed using Freesurfer. We assessed changes in cortical grey matter (GM) and white matter (WM) volume, cortical thickness (CT), cortical curvature and subcortical volumes. We segmented subcortical structures into their constituent nuclei. We used “volbrain” to assessed for regional changes in cerebellum. **Results:** CB had overall lower GM and WM volume for whole brain and cerebellum compared to SC. A region-of-interest analysis revealed GM reductions in various visual areas in striate and inferior temporal cortices, (post)central and superior frontal gyri, and right subcallosal gyrus. No significant group differences in CT were found. WM showed a similar pattern of volumetric reductions as those for GM but with additional reductions in precuneus and orbitofrontal cortex. Changes in cortical curvature were mostly situated in occipital cortex. Subcortically, caudate nucleus, putamen, globus pallidum, thalamus and nucleus accumbens were smaller in CB. Within thalamus, there were volumetric reductions in lateral geniculate, ventroposterior, centromedian and ventromedian nuclei. Within hippocampus, left presubiculum and right CA1 were smaller in CB. Reductions in cerebellar GM, WM and CT were also found in CB. **Conclusion:** Our data reveal a multitude of GM and WM reductions in CB, covering occipital, temporal, parietal, prefrontal, subcortical and cerebellar areas.

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A META-ANALYSIS OF STRUCTURAL CHANGES IN CONGENITAL AND LATE-ONSET BLINDNESS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

Samuel Paré[1], Maxime Bleau[2], Ismaël Djerourou[3], Laurence Dricot[4], Maurice Ptito[2], Ron Kupers[5]
[1]Université de Montréal, Ecole D'optométrie, Montréal, Canada, [2]Université de Montréal, Ecole D'optométrie, Montreal, Canada, [3]Université de Montréal, Optometry, Montréal, Canada, [4]Université Catholique de Louvain, Neuroscience, Woluwe-Saint-Lambert, Belgium, [5]University of Copenhagen, Panum Institute, Copenhagen, Denmark

Aim: Numerous reports have described structural brain changes in congenitally blind (CB) and late blind (LB) subjects. However, there is a large variability in reported outcomes. Here, we present results of a meta- analysis of brain morphological changes due to blindness. **Methods:** We performed a complete literature search of five databases (Pubmed, Embase, MEDLINE, Psychinfo, Global Health) in conjunction with an ALE coordinate-based-meta-analysis in early and late-onset blindness in human. Out of 4140 studies screened, 279 were considered eligible. Of these, 65 studies were finally withheld, including 979 CB, 532 LB and 1333 sighted controls (SC). **Results:** Close to half of the studies reported voxel-based or surface-based morphometry data. Diffusion studies accounted for one third. For CB, ALE analysis revealed 3 clusters of reduced volume in cuneus, lentiform and precuneus, and one cluster of increased volume in temporal pole. In addition, CB had decreased cortical thickness (CT) in postcentral gyrus and increased CT in lingual gyrus. CB also had decreased fractional anisotropy (FA) in fusiform, lingual and middle occipital gyri. For LB, ALE revealed two clusters of reduced volume in bilateral cuneus, two of increased volume in the superior temporal and parahippocampal (PHG) gyri and three clusters of reduced FA (sub-gyral, sub-lobar and lingual gyrus). **Conclusion:** Despite the large variability in outcomes across studies, ALE revealed a consistent pattern of changes in CB and LB, consisting of volumetric reductions mostly in visual areas and decreases in FA in visual areas extending into PHG. We found little evidence for “adaptive” changes.

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OPTOGENETIC PROSTHETIC STIMULATION IN THE LATERAL GENICULATE NUCLEUS CAN INDUCE VISUAL-LIKE DETECTION BEHAVIOR IN TREE SHREWS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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The visual thalamic lateral geniculate nucleus (LGN) is a potentially attractive target for visual prosthetics due to its laminar, retinotopic organization. Here we are interested in studying how visual stimulation and optogenetic activation of the LGN impact visually based behavior. We chose the northern tree shrew *T. belangeri*, a small diurnal mammal with a highly developed visual apparatus. In a visual detection paradigm, we used an opaque globe containing a nose-poke at its center to deliver visual stimuli in a retinotopic fashion using a projector in freely moving animals without food or water restriction. Tree shrews had to enter a nose-poke, and remain there until a visual stimulus, a cloud of moving dots resembling a phosphene, was presented at a particular visual field location. They quickly acquired this detection task, achieving over 70% correct performance for stimuli of 2° diameter in size after one week training. Following injection of AAV2-CaMKIIα-ChR2 in LGN, we then studied target detection of brief optogenetic LGN stimulation at various stimulation frequencies by tree shrews, using a battery- and cable-free RF powered light stimulator. Preliminary data suggest that frequencies of 10Hz and 50Hz are more easily detected than other frequencies. Optogenetic target detection was compromised by the addition of a visually presented masking stimulus (moving dot phosphene cloud 60°x75° in size). Our findings suggest that the tree shrew could be a useful animal model system to systematically study how optogenetic interventions in the LGN impact activations across the visual pathways, visual percepts and visually-based behaviors.

Pubmed:
34249778: Wang J, Li Q, Huang Q, Lv M, Li P, Dai J, Zhou M, Xu J, Zhang F, Gao J
Washed Microbiota Transplantation Accelerates the Recovery of Abnormal Changes by Light-Induced Stress in Tree Shrews.
The gut and brain interact constantly in a complex fashion. Its intricacy and intrigue is progressively being revealed in the study of the "gut-brain axis". Among many factors, abnormal light exposure is a potential powerful stressor, which is becoming ever more pervasive in our modern society. However, little is known about how stress, induced by staying up late by light, affects the gut-brain axis. We addressed this question by extending the normal circadian light for four hours at night in fifteen male tree shrews to simulate the pattern of staying up late in humans. The behavior, biochemical tests, microbiota dynamics, and brain structure of tree shrews were evaluated. The simple prolongation of light in the environment resulted in substantial changes of body weight loss, behavioral differences, total sleep time reduction, and an increased level of urine cortisol. These alterations were rescued by the treatment of either ketamine or washed microbiota transplantation (WMT). Importantly, the sustainability of WMT effect was better than that of ketamine. Magnetic Resonance Imaging analysis indicated that ketamine acted on the hippocampus and thalamus, and WMT mainly affected the piriform cortex and lateral geniculate nucleus. In conclusion, long-term light stimulation could change the behaviors, composition of gut microbiota and brain structure in tree shrews. Targeting microbiota thus certainly holds promise as a treatment for neuropsychiatric disorders, including but not limited to stress-related diseases.
Front Cell Infect Microbiol, 2021; 11
34117351: Dimanico MM, Klaassen AL, Wang J, Kaeser M, Harvey M, Rasch B, Rainer G
Aspects of tree shrew consolidated sleep structure resemble human sleep.
Understanding human sleep requires appropriate animal models. Sleep has been extensively studied in rodents, although rodent sleep differs substantially from human sleep. Here we investigate sleep in tree shrews, small diurnal mammals phylogenetically close to primates, and compare it to sleep in rats and humans using electrophysiological recordings from frontal cortex of each species. Tree shrews exhibited consolidated sleep, with a sleep bout duration parameter, τ, uncharacteristically high for a small mammal, and differing substantially from the sleep of rodents that is often punctuated by wakefulness. Two NREM sleep stages were observed in tree shrews: NREM, characterized by high delta waves and spindles, and an intermediate stage (IS-NREM) occurring on NREM to REM transitions and consisting of intermediate delta waves with concomitant theta-alpha activity. While IS-NREM activity was reliable in tree shrews, we could also detect it in

human EEG data, on a subset of transitions. Finally, coupling events between sleep spindles and slow waves clustered near the beginning of the sleep period in tree shrews, paralleling humans, whereas they were more evenly distributed in rats. Our results suggest considerable homology of sleep structure between humans and tree shrews despite the large difference in body mass between these species.
Commun Biol, 2021; 4
31693931: Ni RJ, Wang J, Shu YM, Xu L, Zhou JN
Mapping of c-Fos expression in male tree shrew forebrain.
The tree shrew is susceptible to stimuli. However, mapping of c-Fos expression in male tree shrew forebrain has not been explored. The present results provided the first detailed mapping of c-Fos expression in the forebrain of the tree shrew (Tupaia belangeri chinensis). Acute restraint stress rapidly increased the density of c-Fos-immunoreactive (-ir) neurons in the medial orbital cortex (MO), infralimbic cortex, intermediate part of the lateral septal nucleus (LSi), ventral part of the lateral septal nucleus (LSv), anterior part of the bed nucleus of the stria terminalis, posterior part of the bed nucleus of the stria terminalis (STP), paraventricular nucleus of the hypothalamus, supraoptic nucleus, lateral hypothalamic area, ventromedial hypothalamic nucleus (VMH), and medial amygdaloid nucleus (MeA). Furthermore, a significant increase in c-Fos expression was observed in the MO, LSi, LSv, STP, VMH, arcuate hypothalamic nucleus, anterior amygdaloid area, MeA, and cortical amygdaloid nucleus immediately after acute footshock stress. In addition, the distinct patterns of c-Fos expression in the forebrain were shown in context-, restraint-, or footshock-treated tree shrews. In general, the present study provides the first detailed maps of c-Fos expression in male tree shrew forebrain immediately after various stimuli.
Neurosci Lett, 2020; 714
31113458: Wang J, Xie R, Kou X, Liu Y, Qi C, Liu R, You W, Gao J, Gao X
A protein phosphatase 2A deficit in the hippocampal CA1 area impairs memory extinction.
Protein phosphorylation plays an important role in learning and memory. Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase involved in the regulation of neural synaptic plasticity. Here, to determine if PP2A is necessary for successful learning and memory, we have utilized a Tg (Camk2a-cre) T29-2Stl mice to specific knock down the expression of hippocampal PP2A in mice. By analysing behavioural, we observed that loss of PP2A in the hippocampal CA1 area did not affect the formation of memory but impaired contextual fear memory extinction. We use the electrophysiological recording to find the synaptic mechanisms. The results showed that the basic synapse transmission and synaptic plasticity of PP2A conditional knockout (CKO) mice were impaired. Moreover, PP2A CKO mice exhibited a saturating long-term potentiation inducted by strong theta burst stimulation but no depotentiation after low-frequency stimulation. Taken together, our results provide the evidence that PP2A is involved in synaptic transmission and hippocampus-dependent memory extinction.
Mol Brain, 2019; 12
30590446: Wang J, Kou XL, Chen C, Wang M, Qi C, Wang J, You WY, Hu G, Chen J, Gao J
Hippocampal Wdr1 Deficit Impairs Learning and Memory by Perturbing F-actin Depolymerization in Mice.
WD repeat protein 1 (Wdr1), known as a cofactor of actin-depolymerizing factor (ADF)/cofilin, is conserved among eukaryotes, and it plays a critical role in the dynamic reorganization of the actin cytoskeleton. However, the function of Wdr1 in the central nervous system remains elusive. Using Wdr1 conditional knockout mice, we demonstrated that Wdr1 plays a significant role in regulating synaptic plasticity and memory. The knockout mice exhibited altered reversal spatial learning and fear responses. Moreover, the Wdr1 CKO mice showed significant abnormalities in spine morphology and synaptic function, including enhanced hippocampal long-term potentiation and impaired long-term depression. Furthermore, we observed that Wdr1 deficiency perturbed actin rearrangement through regulation of the ADF/cofilin activity. Taken together, these results indicate that Wdr1 in the hippocampal CA1 area plays a critical role in actin dynamics in associative learning and postsynaptic receptor availability.
Cereb Cortex, 2019; 29
28917660: Huang Q, Nie B, Ma C, Wang J, Zhang T, Duan S, Wu S, Liang S, Li P, Liu H, Sun H, Zhou J, Xu L, Shan B
Stereotaxic F-FDG PET and MRI templates with three-dimensional digital atlas for statistical parametric mapping analysis of tree shrew brain.
Tree shrews are proposed as an alternative animal model to nonhuman primates due to their close affinity to primates. Neuroimaging techniques are widely used to study brain functions and structures of humans and animals. However, tree shrews are rarely applied in neuroimaging field partly due to the lack of available species specific analysis methods.
J Neurosci Methods, 2018; 293
25488282: Wang J, Jing L, Toledo-Salas JC, Xu L
Rapid-onset antidepressant efficacy of glutamatergic system modulators: the neural plasticity hypothesis of depression.
Depression is a devastating psychiatric disorder widely attributed to deficient monoaminergic signaling in the central nervous system. However, most clinical antidepressants enhance monoaminergic neurotransmission with little delay but require 4-8

weeks to reach therapeutic efficacy, a paradox suggesting that the monoaminergic hypothesis of depression is an oversimplification. In contrast to the antidepressants targeting the monoaminergic system, a single dose of the N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine produces rapid (within 2 h) and sustained (over 7 days) antidepressant efficacy in treatment-resistant patients. Glutamatergic transmission mediated by NMDARs is critical for experience-dependent synaptic plasticity and learning, processes that can be modified indirectly by the monoaminergic system. To better understand the mechanisms of action of the new antidepressants like ketamine, we review and compare the monoaminergic and glutamatergic antidepressants, with emphasis on neural plasticity. The pathogenesis of depression may involve maladaptive neural plasticity in glutamatergic circuits that may serve as a new class of targets to produce rapid antidepressant effects.
Neurosci Bull, 2015; 31
24312510: Wang J, Chai A, Zhou Q, Lv L, Wang L, Yang Y, Xu L
Chronic clomipramine treatment reverses core symptom of depression in subordinate tree shrews.
Chronic stress is the major cause of clinical depression. The behavioral signs of depression, including anhedonia, learning and memory deficits, and sleep disruption, result from the damaging effects of stress hormones on specific neural pathways. The Chinese tree shrew (Tupaia belangeri chinensis) is an aggressive non-human primate with a hierarchical social structure that has become a well-established model of the behavioral, endocrine, and neurobiological changes associated with stress-induced depression. The tricyclic antidepressant clomipramine treats many of the core symptoms of depression in humans. To further test the validity of the tree shrew model of depression, we examined the effects of clomipramine on depression-like behaviors and physiological stress responses induced by social defeat in subordinate tree shrews. Social defeat led to weight loss, anhedonia (as measured by sucrose preference), unstable fluctuations in locomotor activity, sustained urinary cortisol elevation, irregular cortisol rhythms, and deficient hippocampal long-term potentiation (LTP). Clomipramine ameliorated anhedonia and irregular locomotor activity, and partially rescued the irregular cortisol rhythm. In contrast, weight loss increased, cortisol levels were even higher, and in vitro LTP was still impaired in the clomipramine treatment group. These results demonstrate the unique advantage of the tree shrew social defeat model of depression.
PLoS One, 2013; 8

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NEURAL REPRESENTATIONS OF DISTANCES IN A TACTILE SPACE IN VISUALLY IMPAIRED AND SIGHTED PERSONS

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

Loes Ottink[1], T. Van Der Geest[2], Christian Doeller[3], Richard Van Wezel[4]
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The human brain can form mental maps of an environment, which can support wayfinding. In such a cognitive map, spatial information such as distances between familiar locations are stored. The hippocampus and entorhinal cortex are essential regions for spatial orientation and navigation. They have been shown to encode distances to goals during navigation, and to store distances in a map-like representation. Most of this research was performed using visual information. In our study, we assessed map formation using tactile information in visually impaired and sighted persons. Furthermore, we explored the role of allocentric and egocentric navigation strategies. We let participants perform an object-location task in a tactile city-like environment, and combined functional magnetic resonance imaging (fMRI) with voxel pattern analysis to assess distance representations in the hippocampus and entorhinal cortex. Behavioural analyses show that both visually impaired and sighted participants have a good mental map of that tactile environment. When testing across all participants in the fMRI results, we found trend level distance representations in the right hippocampus. We found no differential representations between visually impaired and sighted participants. Furthermore, our analyses reveal representations of distances in the right hippocampus and entorhinal cortex in allocentric navigators, but not egocentric navigators. These results suggest map formation in the hippocampus and entorhinal cortex using tactile information. This is in line with ideas of modality-independent coding of space. Furthermore, our findings highlight the importance of implicating navigational strategies in cognitive map research.

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EEG EVOKED ACTIVITY SUGGESTS AMODAL EVIDENCE INTEGRATION IN MULTISENSORY DECISION-MAKING

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Recent works in neuroimaging have revealed neural signatures of evidence integration (O'Connell et al., 2012, Nat Neuro; Philiastides et al., 2014, J Neuro) that reflect the ramping activity of neurons in the parietal cortex. While these experiments focused on unisensory visual and auditory perceptual decision-making, it is unclear to what extent the neural correlates of multisensory evidence integration are shared with their unisensory counterparts. To address this issue, we designed a change detection paradigm in which twenty-one participants monitored a continuous stream of visual random dot motion and auditory tone clouds. The random dot motion was displayed within a circular aperture and consisted of 200 small dots repositioned every 50 ms. The tone clouds consisted of 10 simultaneous 50 ms pure tones drawn from a range of 6 octaves with a resolution of 12 semitones per octave. Participants had to detect unisensory or bimodal changes while continuous EEG was acquired. EEG activity was denoised with spatial filtering techniques to isolate components that capture neural activity most reproducibly evoked by stimulus change onset (de Cheveigné & Simon, 2008, J Neuro Methods). EEG evoked activity could be discriminated between visual and auditory target stimuli highlighting separable encoding of visual and auditory coherence changes. Further analyses revealed a component rising before participants response that echoes evidence accumulation and appeared to be common for both unisensory and redundant audio-visual changes. These results point to a single amodal accumulator that integrates evidence coming from each sensory modality in isolation or a combined bimodal signal.

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ONE-MONTH AUDIO-MOTOR TRAINING IMPROVES SOME SPATIAL ABILITIES OF LATE-BLIND PEOPLE.

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Istituto Italiano di Tecnologia, Unit For Visually Impaired People (u-vip), Genova, Italy

Late blindness represents a unique model to study how spatial abilities develop with visual feedback early in life and subsequently visual deprivation. Research showed that experiencing prolonged visual deprivation in adulthood compromises some complex spatial abilities and underlying neural correlates. We investigated whether training based on audio-motor coupling improved audio-proprioceptive spatial abilities of long-term late blind individuals at behavioral and neurophysiological levels. Six long-term late-blind adults took part in the study. Half participants performed a four-week training with ABBI (Audio Bracelet for Blind Interaction), a wearable device that has been clinically validated as rehabilitative technology to enhance spatial perception in visually impaired people. The other participants were recruited as a control sample performing everyday activities without any training. The training consisted of spatial exercises based on upper-limb movements to be performed in peripersonal and extrapersonal coordinates by relying on the auditory feedback from the bracelet. Before and after one month, a battery of spatial tasks was administrated to all participants consisting of 1) one auditory spatial bisection task with coherent and conflicting spatiotemporal information performed during EEG recording, 2) two auditory localization tasks, and 3) two proprioceptive-motor tasks. Results showed that some auditory and proprioceptive abilities of the late-blind participants who performed the training improved. Moreover, the audio-motor training induced adaptive changes in the early recruitment of visual and auditory areas during the spatial bisection task. To conclude, multimodal rehabilitation seems to help late blind individuals to overcome some of their spatial perception challenges.

Pubmed:
34848173: Amadeo MB, Tonelli A, Campus C, Gori M
Reduced flash lag illusion in early deaf individuals.
When a brief flash is quickly presented aligned with a moving target, the flash typically appears to lag behind the moving stimulus. This effect is widely known in the literature as a flash-lag illusion (FLI). The flash-lag is an example of a motion-induced position shift. Since auditory deprivation leads to both enhanced visual skills and impaired temporal abilities, both crucial for the perception of the flash-lag effect, here we hypothesized that lack of audition could influence the FLI. 13 early deaf and 18 hearing individuals were tested in a visual FLI paradigm to investigate this hypothesis. As expected, results demonstrated a reduction of the flash-lag effect following early deafness, both in the central and peripheral visual fields. Moreover, only for deaf individuals, there is a positive correlation between the flash-lag effect in the peripheral and central visual field, suggesting that the mechanisms underlying the effect in the center of the visual field expand to the periphery following deafness. Overall, these findings reveal that lack of audition early in life profoundly impacts early visual processing underlying the flash-lag effect.
Brain Res, 2022; 1776
34113297: Gori M, Schiatti L, Amadeo MB
Masking Emotions: Face Masks Impair How We Read Emotions.
To date, COVID-19 has spread across the world, changing our way of life and forcing us to wear face masks. This report demonstrates that face masks influence the human ability to infer emotions by observing facial configurations. Specifically, a mask obstructing a face limits the ability of people of all ages to infer emotions expressed by facial features, but the difficulties associated with the mask's use are significantly pronounced in children aged between 3 and 5 years old. These findings are of essential importance, as they suggest that we live in a time that may potentially affect the development of social and emotion reasoning, and young children's future social abilities should be monitored to assess the true impact of the use of masks.
Front Psychol, 2021; 12
32389726: Amadeo MB, Campus C, Gori M
Visual representations of time elicit early responses in human temporal cortex.
Time perception is inherently part of human life. All human sensory modalities are always involved in the complex task of

creating a temporal representation of the external world. However, when representing time, people primarily rely on auditory information. Since the auditory system prevails in many audio-visual temporal tasks, one may expect that the early recruitment of the auditory network is necessary for building a highly resolved and flexible temporal representation in the visual modality. To test this hypothesis, we asked 17 healthy participants to temporally bisect three consecutive flashes while we recorded EEG. We demonstrated that visual stimuli during temporal bisection elicit an early (50-90 ms) response of an extended area of the temporal cortex, likely including auditory cortex too. The same activation did not appear during an easier spatial bisection task. These findings suggest that the brain may use auditory representations to deal with complex temporal representation in the visual system.
Neuroimage, 2020; 217
31899299: Gori M, Amadeo MB, Campus C
Spatial metric in blindness: behavioural and cortical processing.
Visual modality dominates spatial perception and, in lack of vision, space representation might be altered. Here we review our work showing that blind individuals have a strong deficit when performing spatial bisection tasks (Gori et al., 2014). We also describe the neural correlates associated with this deficit, as blind individuals do not show the same ERP response mimicking the visual C1 reported in sighted people during spatial bisection (Campus et al., 2019). Interestingly, the deficit is not always evident in late blind individuals, and it is dependent on blindness duration. We report that the deficit disappears when one presents coherent temporal and spatial cues to blind people. This suggests that they may use time information to infer spatial maps (Gori et al., 2018). Finally, we propose a model to explain why blind individuals are impaired in this task, speculating that a lack of vision drives the construction of a multi-sensory cortical network that codes space based on temporal, rather than spatial, coordinates.
Neurosci Biobehav Rev, 2020; 109
31472192: Amadeo MB, Campus C, Gori M
Time attracts auditory space representation during development.
Vision is the most accurate sense for spatial representation, whereas audition is for temporal representation. However, how different sensory modalities shape the development of spatial and temporal representations is still unclear. Here, 45 children aged 11-13 years were tested to investigate the abilities to evaluate spatial features of auditory stimuli during bisection tasks, while conflicting or non-conflicting spatial and temporal information was delivered. Since audition is fundamental for temporal representation, the hypothesis was that temporal information could influence auditory spatial representation development. Results show a strong interaction between the temporal and the spatial domain. Younger children are not able to build complex spatial representations when the temporal domain is uninformative about space. However, when the spatial information is coherent with the temporal information children of all age are able to decode complex spatial relationships. When spatial and temporal cues are conflicting, younger children are strongly attracted by the temporal instead of spatial information, while older participants result unaffected by the cross-domain conflict. These findings suggest that during development temporal representation of events is used to infer spatial coordinates of the environment, offering important opportunities for new teaching and rehabilitation strategies.
Behav Brain Res, 2019; 376
31415998: Amadeo MB, Campus C, Pavani F, Gori M
Spatial Cues Influence Time Estimations in Deaf Individuals.
Recent studies have reported a strong interaction between spatial and temporal representation when visual experience is missing: blind people use temporal representation of events to represent spatial metrics. Given the superiority of audition on time perception, we hypothesized that when audition is not available complex temporal representations could be impaired, and spatial representation of events could be used to build temporal metrics. To test this hypothesis, deaf and hearing subjects were tested with a visual temporal task where conflicting and not conflicting spatiotemporal information was delivered. As predicted, we observed a strong deficit of deaf participants when only temporal cues were useful and space was uninformative with respect to time. However, the deficit disappeared when coherent spatiotemporal cues were presented and increased for conflicting spatiotemporal stimuli. These results highlight that spatial cues influence time estimations in deaf participants, suggesting that deaf individuals use spatial information to infer temporal environmental coordinates.
iScience, 2019; 19
31406158: Amadeo MB, Störmer VS, Campus C, Gori M
Peripheral sounds elicit stronger activity in contralateral occipital cortex in blind than sighted individuals.
Previous research has shown that peripheral, task-irrelevant sounds elicit activity in contralateral visual cortex of sighted people, as revealed by a sustained positive deflection in the event-related potential (ERP) over the occipital scalp contralateral to the sound's location. This Auditory-evoked Contralateral Occipital Positivity (ACOP) appears between 200-450 ms after sound onset, and is present even when the task is entirely auditory and no visual stimuli are presented at all. Here, we investigate whether this cross-modal activation of contralateral visual cortex is influenced by visual experience. To

this end, ERPs were recorded in 12 sighted and 12 blind subjects during a unimodal auditory task. Participants listened to a stream of sounds and pressed a button every time they heard a central target tone, while ignoring the peripheral noise bursts. It was found that task-irrelevant noise bursts elicited a larger ACOP in blind compared to sighted participants, indicating for the first time that peripheral sounds can enhance neural activity in visual cortex in a spatially lateralized manner even in visually deprived individuals. Overall, these results suggest that the cross-modal activation of contralateral visual cortex triggered by peripheral sounds does not require any visual input to develop, and is rather enhanced by visual deprivation.
Sci Rep, 2019; 9

30760758: Campus C, Sandini G, Amadeo MB, Gori M
Stronger responses in the visual cortex of sighted compared to blind individuals during auditory space representation.
It has been previously shown that the interaction between vision and audition involves early sensory cortices. However, the functional role of these interactions and their modulation due to sensory impairment is not yet understood. To shed light on the impact of vision on auditory spatial processing, we recorded ERPs and collected psychophysical responses during space and time bisection tasks in sighted and blind participants. They listened to three consecutive sounds and judged whether the second sound was either spatially or temporally further from the first or the third sound. We demonstrate that spatial metric representation of sounds elicits an early response of the visual cortex (P70) which is different between sighted and visually deprived individuals. Indeed, only in sighted and not in blind people P70 is strongly selective for the spatial position of sounds, mimicking many aspects of the visual-evoked C1. These results suggest that early auditory processing associated with the construction of spatial maps is mediated by visual experience. The lack of vision might impair the projection of multi-sensory maps on the retinotopic maps used by the visual cortex.
Sci Rep, 2019; 9

30710679: Amadeo MB, Campus C, Gori M
Impact of years of blindness on neural circuits underlying auditory spatial representation.
Early visual deprivation impacts negatively on spatial bisection abilities. Recently, an early (50-90 ms) ERP response, selective for sound position in space, has been observed in the visual cortex of sighted individuals during the spatial but not the temporal bisection task. Here, we clarify the role of vision on spatial bisection abilities and neural correlates by studying late blind individuals. Results highlight that a shorter period of blindness is linked to a stronger contralateral activation in the visual cortex and a better performance during the spatial bisection task. Contrarily, not lateralized visual activation and lower performance are observed in individuals with a longer period of blindness. To conclude, the amount of time spent without vision may gradually impact on neural circuits underlying the construction of spatial representations in late blind participants. These findings suggest a key relationship between visual deprivation and auditory spatial abilities in humans.
Neuroimage, 2019; 191

30240622: Gori M, Amadeo MB, Campus C
Temporal Cues Influence Space Estimations in Visually Impaired Individuals.
Many works have highlighted enhanced auditory processing in blind individuals, suggesting that they compensate for lack of vision with greater sensitivity of the other senses. Few years ago, we demonstrated severely impaired auditory precision in congenitally blind individuals performing an auditory spatial metric task: their thresholds for bisecting three consecutive spatially distributed sounds were seriously compromised, ranging from three times typical thresholds to total randomness. Here, we show that the deficit disappears if blind individuals are presented with coherent temporal and spatial cues. More interestingly, when the audio information is presented in conflict for space and time, sighted individuals are unaffected by the perturbation, whereas blind individuals are strongly attracted by the temporal cue. These results highlight that temporal cues influence space estimations in blind participants, suggesting for the first time that blind individuals use temporal information to infer spatial environmental coordinates.
iScience, 2018; 6

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MULTI-AREA NEURONAL POPULATION DYNAMICS IN MOUSE NEOCORTEX DURING SENSORY DISCRIMINATION

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

Shuting Han[1,2], Fritjof Helmchen[1,2]
[1]University of Zurich, Brain Research Institute, Zurich, Switzerland, [2]University of Zurich / ETH Zurich, Neuroscience Center Zurich, Zurich, Switzerland

Animals adapt to the constantly changing world by learning and predicting the environment and the consequences of their actions. The predictive coding hypothesis states that the brain generates predictions that flow down the hierarchy of neocortical regions, continually comparing them with the actual sensory inputs, and that mismatches are sent up as prediction errors to correct the internal models and generate better predictions. To test this hypothesis, we simultaneously imaged the activity of neuronal populations in mouse somatosensory (S1) cortex and posterior parietal cortex (RL part of PPC) during texture discrimination behavior. Mice were trained to discriminate two textures and then to gradually associate each texture with a distinct auditory cue before texture onset. In expert mice, we randomly introduced trials where the cue and texture are mismatched. We measured GCaMP6f calcium signals in S1 and PPC neurons with a custom-built two-area two-photon microscope. We found that cue-texture mismatch results in erroneous sensory and choice coding of both populations, through disrupted population firing pattern as well as shifted tuning in task-responsive neurons. Furthermore, cross-area interaction is altered when mice shift their predictions to the earlier cue. We will present further results of our ongoing analysis of cross-area interactions.

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ORGANIZATION OF AUDITORY AND AUDIO-VISUAL SPACE IN THE VISUAL CORTEX

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Binding different physical features of objects requires linking multiple sensory stimuli when they are congruent in time and space. The auditory cortex (AC) sends projections to the primary visual cortex (V1), thereby providing a substrate for binding audio-visual stimuli according to their spatiotemporal congruency. While AC neurons show spatial auditory receptive fields, it is not known if information about the spatial location of sounds is relayed to V1, and whether they target neurons with similar receptive fields as themselves. We employed dual-color two-photon calcium imaging and used a 3D array of speakers and LEDs to 1) measure the auditory spatial information that the AC relays to V1 with regard to the visual map of space, and 2) investigate whether multisensory interactions depend on the spatial congruency of auditory and visual stimuli. We found that many AC axons convey information about the location of a sound source to V1. However, unlike visual feedback from the lateral visual cortex, the spatial tuning of AC axons in V1 does not match that of V1 neurons. Consistent with this, audio-visual modulations of both AC axons and V1 neurons did not depend on the spatial congruence of the sound and light stimuli. Nevertheless, we found that audio-visual modulations are projection-specific: visual modulation of auditory responses was only present in AC projections to V1, but not to S1. Our results suggest that inter- and intra-modal cortico-cortical projections follow different organizational rules, and modulation of these inter-modal inputs depends on the modality of the targeted region.

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INTELLIGIBILITY OF AUDIOVISUAL SPEECH DRIVES MULTIVOXEL RESPONSE PATTERNS IN HUMAN SUPERIOR TEMPORAL CORTEX

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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Regions of the human posterior superior temporal gyrus and sulcus (pSTG/S) respond to the visual mouth movements that constitute visual speech and the auditory vocalizations that constitute auditory speech. We examined visual benefit in noisy speech through the lens of multivoxel pattern responses in pSTG/S. fMRI data was collected with from 37 participants. Stimuli consisted of sentences or single words presented in five formats: clear audiovisual (AcV); noisy audiovisual (AnV); clear auditory-only (Ac); noisy auditory-only (An); and visual-only (V). Following the presentation of each item, participants rated intelligibility with a button press. Noisy auditory speech was more intelligible when it was paired with a face video (76% for AnV *vs.* 45% for An). For these conditions, the fMRI data was *post hoc* sorted into intelligible and unintelligible trials. For audiovisual speech, the activity pattern in pSTG/S evoked by intelligible AnV speech was more similar to that evoked by AcV speech (mean r = 0.38) while the activity pattern evoked by unintelligible AnV speech was less similar to AcV speech (mean r = -0.09). A linear mixed-effects model showed this intelligibility difference to be significantly different ($p = 10^{-10}$). Overall, the correlation between patterns was greater for single words (mean r = 0.283) than sentences (mean r = -0.006, $p = 10^{-5}$) without a significant interaction. The successful integration of visual and auditory speech into an intelligible percept produces a characteristic neural signature in pSTG/S for both sentence and word stimuli.

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AGE DIFFERENCES IN VISUO-PROPRIOCEPTIVE ADAPTATION

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

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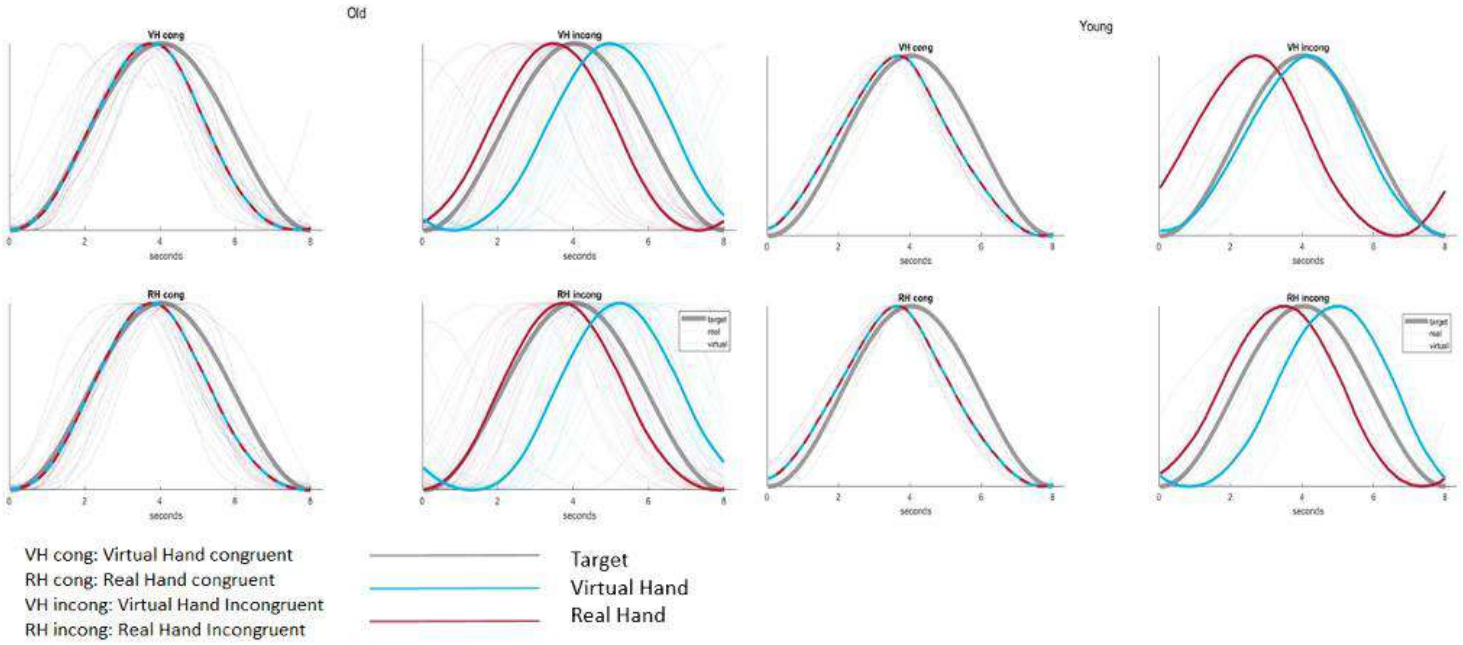
The processing of multisensory signals plays a crucial role in how humans interact with their environment. This key function goes through substantial changes as we age, however, these performance differences also vary depending on the type of stimuli and paradigm. Most virtual reality (VR) environments rely on multisensory integration to appear plausible and realistic. Yet, there is a gap in research regarding the neural correlates of age differences in multisensory integration of visual and proprioceptive stimuli in VR environments. To address this point, we designed an experiment in which groups of older and younger adults control a virtual hand with a data glove during a set of experimental tasks. The first task requires tracking a target with grasping hand movements, and depending on the attentional modality, visual information is either task-relevant or distractor, creating visuo-proprioceptive incongruence. With an additional task we investigate age differences in delay-detection using a version of the same task. During both tasks, we employ functional near-infrared spectroscopy (fNIRS) to measure neural activity in the visual and temporo-parietal areas. Preliminary data of the hand movement trajectories indicate that although there are no age differences in congruent conditions, younger and older adults adapt their movements differently in incongruent conditions. With the assessment of the remaining experimental work, we are aiming to shed light onto the age effects on mechanisms regulating perceptual processing of visuo-proprioceptive stimuli and how this reflects on the development of VR technologies.



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NEURONAL CIRCUIT MECHANISMS OF MULTISENSORY CONTEXT AND INTERNAL STATE INTEGRATION IN DROSOPHILA LARVA

POSTER SESSION 05 - SECTION: MULTISENSORY INTEGRATION

Dylan Manceau[1], Eloïse De Tredern[1], Sandra Autran[1], Tihana Jovanic[2]
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Neuromodulation changes properties of neurons, resulting in a reconfiguration of neural circuits and modulation of behavior. The neural circuit mechanisms responsible for behavioral adaptation to changes in sensory context or internal-state remain unknown. Combining behavior quantification methods and calcium-imaging functional imaging in different feeding-states, we show that short Neuropeptide F, a neuropeptide involved namely in feeding behavior and locomotion, signaling modulates the output of a well-characterized decision circuit in Drosophila larvae according to their feeding state thanks to a differential action on two inhibitory interneurons: Handle-B and Griddle-2. Moreover, we found in the connectome of Drosophila larva a pair of Neuropeptide F releasing neurons that make direct synapses on the Handle-B interneuron. Dense core vesicles can be found in close proximity to these synapses in electron-microscopy images of this neuron, suggesting that neuropeptidergic transmission may be involved in the modulation of Handle-B. Finally, we found numerous 2-hops connexions between peripheric mechanosensory Chordotonal neurons and central feeding-related sensory neurons in the brain, using the connectome data. To study the impact of multisensory integration on decision-making, we developed an assay that allows for the delivery of an appetitive odor on top of an aversive air puff, from the same side of the test arena. First testing of this assay showed that we are able to detect some behavioral disturbances caused by the addition of odor to the air puff.

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CATEGORISING ATTRACTOR DYNAMICS IN NEURAL DATA

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Persistent neural activity is observed in many systems, and is thought to be a neural substrate for holding memories over time delays of a few seconds. When neural activity is not only stimulus-selective but also persistent, outlasting the stimulus that initially drove it, it can be used as a (short-term) memory representation of the stimulus. Such persistent, stimulus-dependent activity is thought to be the neural substrate for memories that last anywhere up to tens of seconds. Attractor networks are models which are used to explain (or at least describe) persistent activity. Attractor dynamics are thought to play a central role in the representation of information and computation in the brain. While many observed phenomena are consistent with their existence, direct proof in the brain remains elusive. In this work, pairwise maximum entropy models are inferred from both real and simulated neural activity. We infer a maximum entropy model from a network of head direction tuned cell recorded from the antero-dorsal thalamic nucleus and the post-subiculum from a freely behaving mouse. The inferred attractor does not however align well with a continuous circle attractor as one would expect for the head direction network. We show that this discrepancy can be explained by the fact that the recorded neurons from this system are sampled from a larger population and we show that the sub-sampling of model neurons leads to similar attractor landscapes.

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BOARD NUMBER: S05-504

EXPLORING THE IMPACT OF EXCITATION-INHIBITION BALANCE THROUGH SYNAPTIC PLACEMENT IN A BIOPHYSICAL MODEL OF CA1 PYRAMIDAL CELL.

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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The hippocampus is widely hypothesized to guide navigation by forming a cognitive map of the surrounding environment. Recent studies show that cells along the CA1 radial axis present differences not only in the molecular and electrophysiological profile but also in the synaptic distributions and the morphology of their dendritic trees. These differences are considered with growing interest because of their potential effect on the computations performed within individual CA1 pyramidal neurons and therefore on the integration of CA3 and EC to produce place fields, building blocks of this cognitive map. To investigate these questions, we developed a computational tool that automatically generates biophysical models of CA1 pyramidal neurons given any reconstructed morphology and allows the user to distribute voltage and calcium gated channels along the somato-dendritic axis. Using an advanced 3D cell reconstruction technique, we experimentally mapped the spatial distribution of all excitatory and inhibitory synapses, obtaining also detailed data about their morphology. Next, we established the neuronal model output baseline of our different morphologies, keeping for all of them the same ionic channel distributions, in line with a previously published model (Poirazi et al., 2003). We simulated grid-like (from EC) and place-like (from CA3) inputs and assessed the differences in dendritic and neuronal responses, including the properties of resulting place cells. In the end, modifying the synaptic distribution and/or the associated weights, we tested how such manipulations could impact the integration of the different input pathways and therefore, the resulting neuronal output.

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BOARD NUMBER: S05-505

IN VITRO ANALYSIS OF HUNTINGTON'S DISEASE IN NEURODEVELOPMENT

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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[1]University of Barcelona; IDIBAPS; CIBERNED, Laboratory Of Stem Cells And Regenerative Medicine, Department Of Biomedical Sciences, Creatio-production And Validation Center Of Advanced Therapies, Faculty Of Medicine And Health Sciences, And Institute Of Neurosciences, Barcelona, Spain, [2]Centro Nacional de Análisis Genómico (CNAG-CRG) - Centre for Genomic Regulation (CRG) - Universitat Pompeu Fabra, Functional Genomics Unit, Barcelona, Spain, [3]Barcelona Supercomputing Center, Life Sciences Department, Barcelona, Spain, [4]University of Barcelona - Universitat Autònoma de Barcelona, Faculty Of Mathematics And Computer Science - Computer Vision Center, Barcelona, Spain

Aims: Huntington's disease (HD) is a monogenic devastating neurodegenerative disorder without available treatment. Evidence indicates that subtle effects during development may lead to impairments in the cellular homeostasis of evolving regional neuronal subpopulations, which in turn may produce adult-onset cell death from normally non-lethal environmental stressors. However, available evidence on the disease onset at cellular level is scarce, especially for the striatum. Thus, our aim is to assess the impact of HD on striatal neurodevelopment and the consequences at a cellular level of HD induced alterations. **Methods:** To investigate human striatal development in both control and HD models, we differentiate three control and three HD human pluripotent stem cell (PSC) lines (two embryonic stem cell lines and four induced PSC lines) in vitro towards a striatal medium spiny neuronal fate. A range of analyses including bulk RNA-seq, single cell RNA-seq (scRNA-seq), and functional assays are performed during differentiation to evaluate the progression of striatal development and how it is altered in HD. **Results:** To integrate and analyze the complete dataset, we apply and implement bioinformatic and machine learning strategies. Concretely, scRNA-seq analysis supports the identification of two main neuroblast populations and differential biomarkers for control and HD cell lines in specific cell clusters. **Conclusions:** Analysis of this diverse range of data sets are integrated to develop a predictive model of striatal development. Using this approach, we anticipate that we will identify genes, signaling pathways and developmental modules to improve our understanding of HD onset and potential therapeutic targets.

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BOARD NUMBER: S05-506

ODOR BACKGROUND INCREASES THE PHEROMONE CODING EFFICIENCY IN MOTH OLFACTORY NEURONS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Insects detect odorants with olfactory receptor neurons (ORNs), located on their antennae. Male moths specifically depend on pheromone-responding neurons (Phe-ORNs) to find females for reproduction purposes, using sex pheromones emitted by female moths. These sex pheromones are a small part of a complex olfactory world and some of the volatile plant compounds (VPCs) found in the environment interfere with the Phe-ORNs. Male moths use VPCs to locate food sources and potential habitats of female moths. Insect olfactory tracking behavior generally results from the integration of multiple information sources, however the effects of VPCs as they naturally appear in the environment have not been studied extensively yet. To this end, we stimulated the ORNs of male *Agrotis ipsilon* with short puffs of pheromone against VPC backgrounds of different concentrations, to mimic the natural environment. We found that the Phe-ORNs have a variable response to different concentrations of the VPC (Z)-3-hexenyl acetate. Of particular interest is a high concentration of the VPC, where we observe an improved coding efficiency per spike of the Phe-ORNs. We confirmed, using regression analysis and other statistical methods, that the accuracy of the stimulus prediction is consistently higher with VPC background. It has been frequently observed that VPC background often suppresses the Phe-ORNs reaction when pheromone is introduced, our research shows that certain VPC concentrations increase the information transmission in Phe-ORNs.

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BOARD NUMBER: S05-507

INTEGRATING MULTIPLE DATASETS TO IDENTIFY CELL TYPE SPECIFIC ASSOCIATIONS WITH ALZHEIMER'S DISEASE PATHOLOGY

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Alzheimer's disease (AD) is a neurodegenerative disorder with complex pathological manifestations and is the leading cause of cognitive decline and dementia in elderly individuals. Given the lack of treatment options, understanding the molecular and cellular changes in AD is essential to identify new therapeutic pathways. In this study, we present a comprehensive investigation of cellular heterogeneity from the temporal cortex region of 40 individuals, comprising healthy donors and individuals with different stages of tau pathology. Using single-nucleus transcriptome analysis of 463,988 nuclei from both gray and white matter of these individuals. We identified cell type-specific subclusters in both neuronal and glial cell types with varying degrees of association with AD pathology. In particular, these associations are present in some layer specific glutamatergic (excitatory) neuronal types, along with other GABAergic (inhibitory) neurons, as well as a few glial cell subtypes. These associations were observed in early as well late pathological progression. To put these findings in broader context, we also integrated our new dataset with other published single nucleus RNA-seq studies that profiled other brain regions such as the entorhinal cortex, prefrontal cortex, and superior frontal gyrus. The integrated analysis of 959,237 total nuclei across 9 different studies/regions identified region-specific subpopulations and associations in these brain regions. Together, our findings start to prioritize specific cell types and pathways for targeted interventions at early and middle stages of pathological spread in AD.

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BOARD NUMBER: S05-508

WWW.HUMOUS.ORG: FRIENDLY AND INTERACTIVE SINGLE-CELL TRANSCRIPTOMIC ONLINE RESOURCE FOR COMPARISON OF GENE EXPRESSION IN DIFFERENT SPECIES AND CONDITIONS ACROSS CORTICOGENESIS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Recent advances in single-cell transcriptomics have led to the exponentially increasing publications of datasets, which, despite being publicly available, require certain expertise in bioinformatics to be used for further studies. Comparisons of distinct datasets also raise the issue of data normalization. Depending on the experimental design, the sequencing depth can vary, as well as the number of genes detected, which can represent a bias for comparative studies. Recent advances in human and human-derived organoid transcriptomics further ask the question of inter-species comparisons: to which extent can we compare human cells with animal or *in vitro* model cells? To which degree are the cell types conserved across species and conditions? And to which extent are the transcriptional landscapes of corresponding cell types comparable between species and conditions? To address these questions, here, we propose an approach to compare the sequential steps of corticogenesis between different species (mouse and human) and different conditions (*in vivo* and *in vitro*) using recently published single-cell transcriptomic datasets.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-509

THE EVENT TIMING-DEPENDENT PLASTICITY RULE CORROBORATES THE KEY ROLE OF DENDRITIC SPIKES FOR LTP INDUCTION AT DISTAL APICAL SYNAPSES IN THE CA1 PYRAMIDAL CELL MODEL

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Aims: It has been shown the Na_v-dSpikes are required for LTP at distal synapses of the CA1 neurons (Kim et al. 2015, doi: 10.7554/eLife.06414). Our goal was to investigate generalization of our synaptic plasticity rule (Jedlicka et al. *2015,* doi: 10.1371/journal.pcbi.1004588) and reduced-morphology computational model of the CA1 cell (Tomko et al. 2021, doi: 10.1038/s41598-021-87002-7) to Kim et al.'s experimental data. *Methods*: Excitatory synapses were distributed on the model CA1 neuron according to experimental data. Synapses changed efficacy according to the Event Timing-Dependent Plasticity (ETDP) rule, in which the presynaptic event is the presynaptic spike, and the postsynaptic event happens when the local PSP exceeds a certain voltage threshold. Four theta-burst stimulation (TBS) protocols were used: (1) TBS alone (2) TBS plus brief somatic current injections (3) TBS plus somatic voltage-clamp (4) modified (weak) TBS. Local application of TTX was simulated by reducing the conductance of dendritic Na_v channels by 50%. *Results:* All four protocols induced LTP of distal apical synapses in our model. The size of LTP was smaller for weak TBS than for the non-modified TBS. After reducing the conductance of Na_v channels so that no dSpikes were generated, we observed no LTP with neither of stimulation protocols. *Conclusions*: Our computational simulations corroborate experimental findings that LTP at the distal apical synapses of the CA1 cells requires Na_v-dSpikes. Thus, we have successfully validated our ETDP rule and the CA1 cell model on these experimental data. *Acknowledgment:* Supported by APVV-19-0435 and BMBF (No. 031L0229) grants.

Pubmed:

33828151: Tomko M, Benuskova L, Jedlicka P
A new reduced-morphology model for CA1 pyramidal cells and its validation and comparison with other models using HippoUnit.
Modeling long-term neuronal dynamics may require running long-lasting simulations. Such simulations are computationally expensive, and therefore it is advantageous to use simplified models that sufficiently reproduce the real neuronal properties. Reducing the complexity of the neuronal dendritic tree is one option. Therefore, we have developed a new reduced-morphology model of the rat CA1 pyramidal cell which retains major dendritic branch classes. To validate our model with experimental data, we used HippoUnit, a recently established standardized test suite for CA1 pyramidal cell models. The HippoUnit allowed us to systematically evaluate the somatic and dendritic properties of the model and compare them to models publicly available in the ModelDB database. Our model reproduced (1) somatic spiking properties, (2) somatic depolarization block, (3) EPSP attenuation, (4) action potential backpropagation, and (5) synaptic integration at oblique dendrites of CA1 neurons. The overall performance of the model in these tests achieved higher biological accuracy compared to other tested models. We conclude that, due to its realistic biophysics and low morphological complexity, our model captures key physiological features of CA1 pyramidal neurons and shortens computational time, respectively. Thus, the validated reduced-morphology model can be used for computationally demanding simulations as a substitute for more complex models.
Sci Rep, 2021; 11
26544038: Jedlicka P, Benuskova L, Abraham WC
A Voltage-Based STDP Rule Combined with Fast BCM-Like Metaplasticity Accounts for LTP and Concurrent "Heterosynaptic" LTD in the Dentate Gyrus In Vivo.
Long-term potentiation (LTP) and long-term depression (LTD) are widely accepted to be synaptic mechanisms involved in learning and memory. It remains uncertain, however, which particular activity rules are utilized by hippocampal neurons to induce LTP and LTD in behaving animals. Recent experiments in the dentate gyrus of freely moving rats revealed an

unexpected pattern of LTP and LTD from high-frequency perforant path stimulation. While 400 Hz theta-burst stimulation (400-TBS) and 400 Hz delta-burst stimulation (400-DBS) elicited substantial LTP of the tetanized medial path input and, concurrently, LTD of the non-tetanized lateral path input, 100 Hz theta-burst stimulation (100-TBS, a normally efficient LTP protocol for in vitro preparations) produced only weak LTP and concurrent LTD. Here we show in a biophysically realistic compartmental granule cell model that this pattern of results can be accounted for by a voltage-based spike-timing-dependent plasticity (STDP) rule combined with a relatively fast Bienenstock-Cooper-Munro (BCM)-like homeostatic metaplasticity rule, all on a background of ongoing spontaneous activity in the input fibers. Our results suggest that, at least for dentate granule cells, the interplay of STDP-BCM plasticity rules and ongoing pre- and postsynaptic background activity determines not only the degree of input-specific LTP elicited by various plasticity-inducing protocols, but also the degree of associated LTD in neighboring non-tetanized inputs, as generated by the ongoing constitutive activity at these synapses.
PLoS Comput Biol, 2015; 11
25698965: Guise M, Knott A, Benuskova L
Enhanced polychronization in a spiking network with metaplasticity.
Computational models of metaplasticity have usually focused on the modeling of single synapses (Shouval et al., 2002). In this paper we study the effect of metaplasticity on network behavior. Our guiding assumption is that the primary purpose of metaplasticity is to regulate synaptic plasticity, by increasing it when input is low and decreasing it when input is high. For our experiments we adopt a model of metaplasticity that demonstrably has this effect for a single synapse; our primary interest is in how metaplasticity thus defined affects network-level phenomena. We focus on a network-level phenomenon called polychronicity, that has a potential role in representation and memory. A network with polychronicity has the ability to produce non-synchronous but precisely timed sequences of neural firing events that can arise from strongly connected groups of neurons called polychronous neural groups (Izhikevich et al., 2004). Polychronous groups (PNGs) develop readily when spiking networks are exposed to repeated spatio-temporal stimuli under the influence of spike-timing-dependent plasticity (STDP), but are sensitive to changes in synaptic weight distribution. We use a technique we have recently developed called Response Fingerprinting to show that PNGs formed in the presence of metaplasticity are significantly larger than those with no metaplasticity. A potential mechanism for this enhancement is proposed that links an inherent property of integrator type neurons called spike latency to an increase in the tolerance of PNG neurons to jitter in their inputs.
Front Comput Neurosci, 2015; 9
22863413: Takac M, Benuskova L, Knott A
Mapping sensorimotor sequences to word sequences: a connectionist model of language acquisition and sentence generation.
In this article we present a neural network model of sentence generation. The network has both technical and conceptual innovations. Its main technical novelty is in its semantic representations: the messages which form the input to the network are structured as sequences, so that message elements are delivered to the network one at a time. Rather than learning to linearise a static semantic representation as a sequence of words, our network rehearses a sequence of semantic signals, and learns to generate words from selected signals. Conceptually, the network's use of rehearsed sequences of semantic signals is motivated by work in embodied cognition, which posits that the structure of semantic representations has its origin in the serial structure of sensorimotor processing. The rich sequential structure of the network's semantic inputs also allows it to incorporate certain Chomskyan ideas about innate syntactic knowledge and parameter-setting, as well as a more empiricist account of the acquisition of idiomatic syntactic constructions.
Cognition, 2012; 125
20510579: Wysoski SG, Benuskova L, Kasabov N
Evolving spiking neural networks for audiovisual information processing.
This paper presents a new modular and integrative sensory information system inspired by the way the brain performs information processing, in particular, pattern recognition. Spiking neural networks are used to model human-like visual and auditory pathways. This bimodal system is trained to perform the specific task of person authentication. The two unimodal systems are individually tuned and trained to recognize faces and speech signals from spoken utterances, respectively. New learning procedures are designed to operate in an online evolvable and adaptive way. Several ways of modelling sensory integration using spiking neural network architectures are suggested and evaluated in computer experiments.
Neural Netw, 2010; 23
17537906: Abraham WC, Logan B, Wolff A, Benuskova L
"Heterosynaptic" LTD in the dentate gyrus of anesthetized rat requires homosynaptic activity.
Heterosynaptic long-term depression (LTD) is conventionally defined as occurring at synapses that are inactive during a time when neighboring synapses are activated by high-frequency stimulation. A new model that combines computational properties of both the Bienenstock, Cooper and Munro model and spike timing-dependent plasticity, however, suggests that such LTD actually may require presynaptic activity in the depressed pathway. We tested experimentally whether presynaptic

activity is in fact necessary for previously described heterosynaptic LTD in lateral perforant path synapses in the dentate gyrus of urethane-anesthetized rats. As predicted by the model, procaine infusion into the lateral path fibers, sufficient to transiently block neural activity in this pathway, prevented the induction of LTD in the lateral path following medial path high-frequency stimulation. These data indicate that the previously described heterosynaptic LTD in the dentate gyrus in vivo is actually a form of homosynaptic LTD, requiring presynaptic activity in the depressed pathway.
J Neurophysiol, 2007; 98

17053995: Benuskova L, Abraham WC
STDP rule endowed with the BCM sliding threshold accounts for hippocampal heterosynaptic plasticity.
We have combined the nearest neighbour additive spike-timing-dependent plasticity (STDP) rule with the Bienenstock, Cooper and Munro (BCM) sliding modification threshold in a computational model of heterosynaptic plasticity in the hippocampal dentate gyrus. As a result we can reproduce (1) homosynaptic long-term potentiation of the tetanized input, and (2) heterosynaptic long-term depression of the untetanized input, as observed in real experiments.
J Comput Neurosci, 2007; 22

11226320: Benusková L, Rema V, Armstrong-James M, Ebner FF
Theory for normal and impaired experience-dependent plasticity in neocortex of adult rats.
We model experience-dependent plasticity in the cortical representation of whiskers (the barrel cortex) in normal adult rats, and in adult rats that were prenatally exposed to alcohol. Prenatal exposure to alcohol (PAE) caused marked deficits in experience-dependent plasticity in a cortical barrel-column. Cortical plasticity was induced by trimming all whiskers on one side of the face except two. This manipulation produces high activity from the intact whiskers that contrasts with low activity from the cut whiskers while avoiding any nerve damage. By a computational model, we show that the evolution of neuronal responses in a single barrel-column after this sensory bias is consistent with the synaptic modifications that follow the rules of the Bienenstock, Cooper, and Munro (BCM) theory. The BCM theory postulates that a neuron possesses a moving synaptic modification threshold, theta(M), that dictates whether the neuron's activity at any given instant will lead to strengthening or weakening of its input synapses. The current value of theta(M) changes proportionally to the square of the neuron's activity averaged over some recent past. In the model of alcohol impaired cortex, the effective theta(M) has been set to a level unattainable by the depressed levels of cortical activity leading to "impaired" synaptic plasticity that is consistent with experimental findings. Based on experimental and computational results, we discuss how elevated theta(M) may be related to (i) reduced levels of neurotransmitters modulating plasticity, (ii) abnormally low expression of N-methyl-d-aspartate receptors (NMDARs), and (iii) the membrane translocation of Ca(2+)/calmodulin-dependent protein kinase II (CaMKII) in adult rat cortex subjected to prenatal alcohol exposure.
Proc Natl Acad Sci U S A, 2001; 98

10695761: Benusková L, Ebner FF, Diamond ME, Armstrong-James M
Computational study of experience-dependent plasticity in adult rat cortical barrel-column.
We model experience-dependent plasticity in the adult rat S1 cortical representation of the whiskers (the barrel cortex) which has been produced by trimming all whiskers on one side of the snout except two. This manipulation alters the pattern of afferent sensory activity while avoiding any direct nerve damage. Our simplified model circuitry represents multiple cortical layers and inhibitory neurons within each layer of a barrel-column. Utilizing a computational model we show that the evolution of the response bias in the barrel-column towards spared whiskers is consistent with synaptic modifications that follow the rules of the Bienenstock, Cooper and Munro (BCM) theory. The BCM theory postulates that a neuron possesses a dynamic synaptic modification threshold, thetaM, which dictates whether the neuron's activity at any given instant will lead to strengthening or weakening of the synapses impinging on it. However, the major prediction of our model is the explanation of the delay in response potentiation in the layer-IV neurons through a masking effect produced by the thresholded monotonically increasing inhibition expressed by either the logarithmic function, h(x) = mu log(1 + x), or by the power function, h(x) = mu x(0.8-0.9), where mu is a constant. Furthermore, simulated removal of the supragranular layers (layers II/III) reduces plasticity of neurons in the remaining layers (IV-VI) and points to the role of noise in synaptic plasticity.
Network, 1999; 10

FENS Forum 2022
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BOARD NUMBER: S05-510

SENSITIVITY OF ELECTRICAL PROPERTIES TO ALTERATIONS OF THE OBLIQUE DENDRITIC ARBOUR IN A DETAILED PYRAMIDAL CELL MODEL

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Morphological properties of a neuron affect its electrical signaling, contributing to the variability of the neuronal responses. Even assigned to the same functional type, neurons can significantly vary in their morphological characteristics. We use detailed electrical models to explore how variability of oblique dendritic tree in layer 5 pyramidal cell can affect its passive and active electrical properties in both soma and apical dendrite. In particular, we assessed the sensitivity of electrical features by applying local alterations of branch oblique diameters. Then, at the soma we measured a set of electrical features and along the apical trunk we recorded calcium transients and voltages generated by back-propagating action potentials. From our preliminary results we observe that the somatic input resistance has a decreased sensitivity to diameters as a function of path distance to soma. With increase in oblique diameters (up to 10%), the firing rate, interspike interval and after hyperpolarization potential increases (up to 20%), while action potential amplitude decreased up to 30%. Moreover, we perform in-silico dendrotomy of oblique branches and show that it can lead to decrease of calcium concentration up to 50% along the trunk during bAP propagation. Surprisingly, contribution to the total calcium concentration in the trunk of each oblique branch can be either positive and negative, and depends on its position within the oblique tree and arborization. These results indicate the impact of oblique tree on the electrical properties of the cell and nonlinear summation of the active dendritic currents along the oblique tree.

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BOARD NUMBER: S05-511

THE IMPACT OF LIGHT SOURCE PROPERTIES, NEURAL MORPHOLOGY AND THE DISTRIBUTION OF LIGHT-GATED ION CHANNELS ON THE EFFECTIVE SPATIAL RESOLUTION OF OPTOGENETIC STIMULATION

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Optogenetic stimulation is a powerful tool for studying the brain with a clinical application in neural prostheses [1]. Crucial for the success of optogenetics-based brain-interfaces is the spatial resolution at which they can control neural activity. Major effort is being invested into improving this aspect through dense arrays of light elements [2] and subcellularly precise expression of light-gated ion channels [3]. However, there is a lack of quantitative understanding on how light source properties, neuron morphology, and the expression of light-gated ion channels constrain the spatial resolution of stimulation. This knowledge is a key for guiding the development of stimulation devices, optogenetic constructs, and for designing and interpreting experiments utilizing optogenetic stimulation. Based on existing computational work [4], we address this question by simulating optogenetic stimulation of channelrhodopsin-2 expressing pyramidal neurons (layer 2 and 5). The simulated scenario resembles illumination with an optical fiber placed on top of the cortex. We find that the spatial stimulation profile strongly depends on the extent of the neuron's morphology unless the expression of channelrhodopsin is somatically confined. Furthermore, the number of expressed channels and the light power affect the spatial stimulation profile similarly. It evolves with increasing magnitude of the two variables from a sparse set of off-center stimulation locations to a centered, laterally decaying stimulation, to a ring-like off-center stimulation. References
1. Sahel et al. (2021). Nat Med.
2. Soltan et al. (2017). IEEE Trans Biomed Circuits Syst.
3. Shemesh et al. (2017). Nat Neurosci.
4. Foutz et al. (2012). J Neurophysiol.

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BOARD NUMBER: S05-512

THE LATERAL ENTORHINAL CORTEX MODULATES DENDRITIC EXCITABILITY IN THE CA1 BY RECRUITING A LOCAL DISINHIBITORY MICROCIRCUIT.

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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The lateral entorhinal cortex (LEC) has been suggested to provide nonspatial contextual information to the hippocampus via direct connections to the distal dendrites of CA1 pyramidal cells. It has been shown that LEC inputs are vital for episodic memory processing, as they encode context-dependent environmental cues in an environment. However, little is known about the circuit interactions between the LEC and the CA1 hippocampal subregion. In this study[1], we combined experimental and computational approaches to reveal the role of LEC inputs in compartment-specific excitation-inhibition dynamics in the CA1 subregion. In particular, we developed a biophysical microcircuit model consisting of a multicompartmental CA1 pyramidal neuron innervated by LEC-driven interneurons, i.e., cholecystokinin (CCK), vasoactive intestinal peptide (VIP), and somatostatin (SST)-expressing cells. All model neurons were heavily validated against experimental evidence through targeted experiments (in the Basu lab). Simulation of LEC-driven input induced local dendritic spikes in the distal dendrites of CA1 pyramidal neurons, as observed in our experiments. To unravel the role of specific interneuron subtypes in dendritic spiking, we simulated their selective deletion. We found that local VIP interneurons in CA1 act as a disinhibitory gate that promotes local dendritic spiking in pyramidal neurons, and this finding was verified experimentally. Furthermore, our model predicts that CCK interneurons act as a potent suppressor of dendritic spikes. These results provide new insight into a LEC-driven GABAergic microcircuit mechanism that gates local, non-linear dendritic computations, which may support dendrite-specific coding of multi-sensory contextual features within the hippocampus. 1. Bilash et al., *bioRxiv*, https://doi.org/10.1101/2022.01.13.476247

Pubmed:
33022227: Geiller T, Vancura B, Terada S, Troullinou E, Chavlis S, Tsagkatakis G, Tsakalides P, Ócsai K, Poirazi P, Rózsa BJ, Losonczy A
Large-Scale 3D Two-Photon Imaging of Molecularly Identified CA1 Interneuron Dynamics in Behaving Mice.
Cortical computations are critically reliant on their local circuit, GABAergic cells. In the hippocampus, a large body of work has identified an unprecedented diversity of GABAergic interneurons with pronounced anatomical, molecular, and physiological differences. Yet little is known about the functional properties and activity dynamics of the major hippocampal interneuron classes in behaving animals. Here we use fast, targeted, three-dimensional (3D) two-photon calcium imaging coupled with immunohistochemistry-based molecular identification to retrospectively map in vivo activity onto multiple classes of interneurons in the mouse hippocampal area CA1 during head-fixed exploration and goal-directed learning. We find examples of preferential subtype recruitment with quantitative differences in response properties and feature selectivity during key behavioral tasks and states. These results provide new insights into the collective organization of local inhibitory circuits supporting navigational and mnemonic functions of the hippocampus.
Neuron, 2020; 108
31907437: Shuman T, Aharoni D, Cai DJ, Lee CR, Chavlis S, Page-Harley L, Vetere LM, Feng Y, Yang CY, Mollinedo-Gajate I, Chen L, Pennington ZT, Taxidis J, Flores SE, Cheng K, Javaherian M, Kaba CC, Rao N, La-Vu M, Pandi I, Shtrahman M, Bakhurin KI, Masmanidis SC, Khakh BS, Poirazi P, Silva AJ, Golshani P
Breakdown of spatial coding and interneuron synchronization in epileptic mice.
Temporal lobe epilepsy causes severe cognitive deficits, but the circuit mechanisms remain unknown. Interneuron death and reorganization during epileptogenesis may disrupt the synchrony of hippocampal inhibition. To test this, we simultaneously recorded from the CA1 and dentate gyrus in pilocarpine-treated epileptic mice with silicon probes during head-fixed virtual navigation. We found desynchronized interneuron firing between the CA1 and dentate gyrus in epileptic mice. Since hippocampal interneurons control information processing, we tested whether CA1 spatial coding was altered in this desynchronized circuit, using a novel wire-free miniscope. We found that CA1 place cells in epileptic mice were unstable and

completely remapped across a week. This spatial instability emerged around 6 weeks after status epilepticus, well after the onset of chronic seizures and interneuron death. Finally, CA1 network modeling showed that desynchronized inputs can impair the precision and stability of CA1 place cells. Together, these results demonstrate that temporally precise intrahippocampal communication is critical for spatial processing.
Nat Neurosci, 2020; 23
34087540: Chavlis S, Poirazi P
Drawing inspiration from biological dendrites to empower artificial neural networks.
This article highlights specific features of biological neurons and their dendritic trees, whose adoption may help advance artificial neural networks used in various machine learning applications. Advancements could take the form of increased computational capabilities and/or reduced power consumption. Proposed features include dendritic anatomy, dendritic nonlinearities, and compartmentalized plasticity rules, all of which shape learning and information processing in biological networks. We discuss the computational benefits provided by these features in biological neurons and suggest ways to adopt them in artificial neurons in order to exploit the respective benefits in machine learning.
Curr Opin Neurobiol, 2021; 70
28316111: Chavlis S, Poirazi P
Pattern separation in the hippocampus through the eyes of computational modeling.
Pattern separation is a mnemonic process that has been extensively studied over the years. It entails the ability -of primarily hippocampal circuits- to distinguish between highly similar inputs, via generating different neuronal activity (output) patterns. The dentate gyrus (DG) in particular has long been hypothesized to implement pattern separation by detecting and storing similar inputs as distinct representations. The ways in which these distinct representations can be generated have been explored in a number of theoretical and computational modeling studies. Here, we review two categories of pattern separation models: those that address the phenomenon in an abstract mathematical fashion and those that delve into the underlying biological mechanisms by taking into account the anatomy and/or physiology of hippocampal circuits. We summarize the strategies, findings and limitations of these modeling approaches in the light of new experimental findings and propose a unifying framework whereby different network, cellular and sub-cellular mechanisms converge to a common goal: controlling sparsity, the key determinant of pattern separation in the DG.
Synapse, 2017; 71
34278605: Primetis E, Chavlis S, Pavlidis P
Evolutionary models of amino acid substitutions based on the tertiary structure of their neighborhoods.
Intra-protein residual vicinities depend on the involved amino acids. Energetically favorable vicinities (or interactions) have been preserved during evolution, while unfavorable vicinities have been eliminated. We describe, statistically, the interactions between amino acids using resolved protein structures. Based on the frequency of amino acid interactions, we have devised an amino acid substitution model that implements the following idea: amino acids that have similar neighbors in the protein tertiary structure can replace each other, while substitution is more difficult between amino acids that prefer different spatial neighbors. Using known tertiary structures for α-helical membrane (HM) proteins, we build evolutionary substitution matrices. We constructed maximum likelihood phylogenies using our amino acid substitution matrices and compared them to widely-used methods. Our results suggest that amino acid substitutions are associated with the spatial neighborhoods of amino acid residuals, providing, therefore, insights into the amino acid substitution process.
Proteins, 2021; 89
30713030: Turi GF, Li WK, Chavlis S, Pandi I, O'Hare J, Priestley JB, Grosmark AD, Liao Z, Ladow M, Zhang JF, Zemelman BV, Poirazi P, Losonczy A
Vasoactive Intestinal Polypeptide-Expressing Interneurons in the Hippocampus Support Goal-Oriented Spatial Learning.
Diverse computations in the neocortex are aided by specialized GABAergic interneurons (INs), which selectively target other INs. However, much less is known about how these canonical disinhibitory circuit motifs contribute to network operations supporting spatial navigation and learning in the hippocampus. Using chronic two-photon calcium imaging in mice performing random foraging or goal-oriented learning tasks, we found that vasoactive intestinal polypeptide-expressing (VIP), disinhibitory INs in hippocampal area CA1 form functional subpopulations defined by their modulation by behavioral states and task demands. Optogenetic manipulations of VIP INs and computational modeling further showed that VIP disinhibition is necessary for goal-directed learning and related reorganization of hippocampal pyramidal cell population dynamics. Our results demonstrate that disinhibitory circuits in the hippocampus play an active role in supporting spatial learning. VIDEO ABSTRACT.
Neuron, 2019; 101
28132825: Danielson NB, Turi GF, Ladow M, Chavlis S, Petrantonakis PC, Poirazi P, Losonczy A
In Vivo Imaging of Dentate Gyrus Mossy Cells in Behaving Mice.
Mossy cells in the hilus of the dentate gyrus constitute a major excitatory principal cell type in the mammalian hippocampus;

however, it remains unknown how these cells behave in vivo. Here, we have used two-photon Ca imaging to monitor the activity of mossy cells in awake, behaving mice. We find that mossy cells are significantly more active than dentate granule cells in vivo, exhibit spatial tuning during head-fixed spatial navigation, and undergo robust remapping of their spatial representations in response to contextual manipulation. Our results provide a functional characterization of mossy cells in the behaving animal and demonstrate their active participation in spatial coding and contextual representation.
Neuron, 2017; 93

27784124: Chavlis S, Petrantonakis PC, Poirazi P

Dendrites of dentate gyrus granule cells contribute to pattern separation by controlling sparsity.

The hippocampus plays a key role in pattern separation, the process of transforming similar incoming information to highly dissimilar, nonverlapping representations. Sparse firing granule cells (GCs) in the dentate gyrus (DG) have been proposed to undertake this computation, but little is known about which of their properties influence pattern separation. Dendritic atrophy has been reported in diseases associated with pattern separation deficits, suggesting a possible role for dendrites in this phenomenon. To investigate whether and how the dendrites of GCs contribute to pattern separation, we build a simplified, biologically relevant, computational model of the DG. Our model suggests that the presence of GC dendrites is associated with high pattern separation efficiency while their atrophy leads to increased excitability and performance impairments. These impairments can be rescued by restoring GC sparsity to control levels through various manipulations. We predict that dendrites contribute to pattern separation as a mechanism for controlling sparsity.
Hippocampus, 2017; 27

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BOARD NUMBER: S05-513

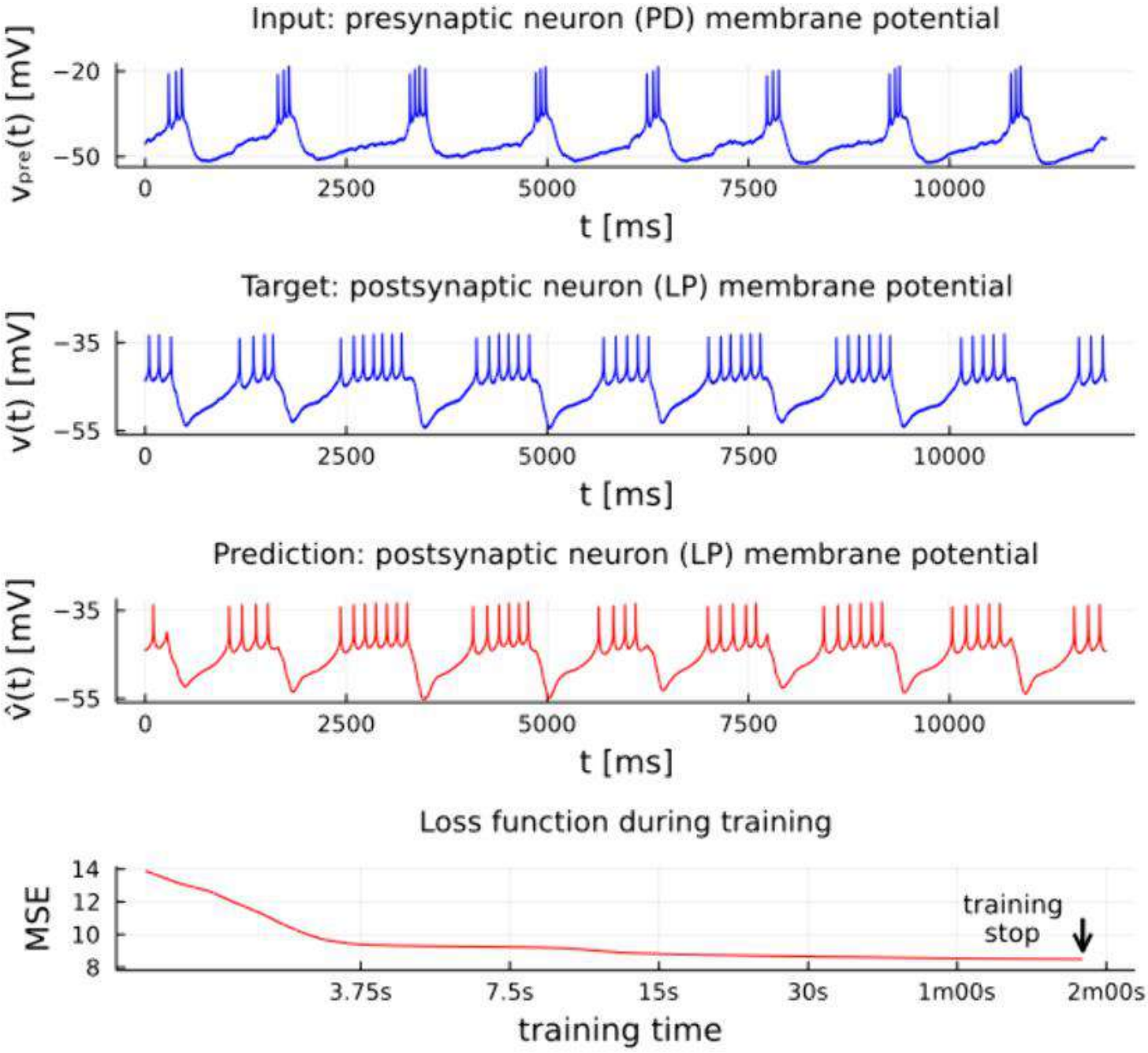
LEARNING A BIOPHYSICAL NEURAL MODEL DURING THE TIME FRAME OF AN EXPERIMENT

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

Thiago Burghi[1], Ekaterina Morozova[2], Sonal Kedia[2], Eve Marder[2], Timothy O'Leary[1]
[1]University of Cambridge, Department Of Engineering, Cambridge, United Kingdom, [2]Brandeis University, Volen Center For Complex Systems, Waltham, United States of America

State-of-the art methods for estimating biophysical neuronal models are typically based on *offline* algorithms that require considerable time and computational resources to run. Consequently, it is currently difficult to estimate models during the relatively short time frame of an electrophysiology experiment. Under moderate uncertainty regarding ion channel kinetics (neglected by some methods), this task is currently unattainable. Solving this challenge is important: rapid or real-time model estimates would open the doors to innovative *closed-loop* experiments incorporating model-based prediction in experiment design. Aims: To develop a data-driven method for rapidly learning a predictive and interpretable model of a single neuron and its synapses, from pre- and post-synaptic voltage measurements, with minimal prior ion channel knowledge. Methods: We exploit novel data-driven neuronal models and adaptive algorithms from control theory. The models respect the neurobiological structure of living neurons (allowing for interpretability) but employ artificial neural networks to capture ion channel dynamics (allowing for rapid estimation). To train our models, we recorded membrane potentials intracellularly from two neurons in the *Cancer borealis* stomatogastric ganglion *in vitro*. In our experimental configuration, we recorded the presynaptic drive in PD neurons simultaneously with postsynaptic cholinergic inhibitory potentials and intervening activity in LP neurons. Results: We consistently obtained accurate biophysical-like models of the LP neuron, from intracellular recordings, in a few minutes using a personal laptop and its embedded GPU. Conclusions: Our results demonstrate that a predictive biophysical-like neuronal model can be rapidly learned during an experiment. The applications to closed-loop experiments involving dynamic clamp are

manifold.


Input: presynaptic neuron (PD) membrane potential
$v_{pre}(t)$ [mV]
−20
−50
0
2500
5000
7500
10000
t [ms]
Target: postsynaptic neuron (LP) membrane potential
v(t) [mV]
−35
−55
0
2500
5000
7500
10000
t [ms]
Prediction: postsynaptic neuron (LP) membrane potential
$\hat{v}(t)$ [mV]
−35
−55
0
2500
5000
7500
10000
t [ms]
Loss function during training
MSE
14
12
10
8
training stop
3.75s
7.5s
15s
30s
1m00s
2m00s
training time

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BOARD NUMBER: S05-514

CALCIUM DYNAMICS SIMULATIONS WITH MORPHOLOGICALLY-DETAILED RECONSTRUCTION OF BERGMANN GLIAL CELL MODEL

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

Laura Keto, Tiina Manninen
Tampere University, Faculty Of Medicine And Health Technology, Tampere, Finland

Calcium dynamics occurring within the highly heterogeneous morphological compartments of Bergmann glial cells play a major role in the functioning of the whole cerebellum. The aim of this research was to build a morphologically-detailed model for a Bergmann glial cell in order to decipher the patterns of calcium signaling in the different morphological compartments. The model consisted of a stem tree, nanoscopic processes attached to the stem tree, and mathematical equations for biophysical properties. The stem tree was built with the NEURON CellBuilder tool based on values found from the literature. For the nanoscopic processes, a 3D reconstruction of a Bergmann glial appendage was recreated based on a video file (Grosche et al. 1999) with AgiSoft Metashape and Blender. A new computational tool ASTRO (Savtchenko et al., 2018) was used to define statistical properties from the reconstructed appendage, assemble the final model, and run the simulations. In this work, for the first time a whole Bergmann glial cell was modeled in the detail of nanoscopic processes that characterize these cells. The spatiotemporal pattern of calcium dynamics across the morphology was influenced by the shape and localization of the lateral processes. Simulations with and without mobile buffers present elucidated the highly variable spatiotemporal dynamics of calcium within the specialized morphological structures of Bergmann glial cells.
Acknowledgements We are very grateful to Prof. Helmut Kettenmann for providing us the video file of Bergmann glia appendage. The work was supported by the Academy of Finland (Nos. 326494, 326495, 345280).

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BOARD NUMBER: S05-515

PERMUTATION TEST FOR COVARIATE SELECTION

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

Fredrik Nevjen, Benjamin Dunn
NTNU, Department Of Mathematical Sciences, Trondheim, Norway

Proper selection of covariates is a critical problem with the ever-growing accessibility to data. The statistical dependencies between the many behavioral covariates and the activity of a neuron make this a non-trivial problem, and there is a need in the field for statistically sound methods. The generalized linear model (GLM) is a common framework to investigate the underlying relationship between the firing of neurons and, in essence, everything else. We address issues that occur when using the GLM framework with neural data. The spike data and our tracked covariates are correlated in time, and there inevitably exist unobserved covariates that affect the activity of the neuron in question. Conditional independence, a core assumption of the GLM, is not fulfilled, and the model is likely misspecified. The problems relate to so called "Nonsense correlations", which are concerns raised initially by Yule (1926) and in modern Neuroscience by Harris (2020). The consequence is that statistical inference and covariate selection methods that build on the GLM assumptions, such as likelihood ratio tests and the Akaike information criterion, are flawed. We utilize a permutation test to construct a forward selection procedure, avoiding the strict dependency on unfulfilled GLM assumptions. Studies on simulated data and electrophysiological recordings with multiple covariates suggest that the method is statistically robust where certain methods prove to be invalid, and also more powerful than methods commonly used in the field.

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BOARD NUMBER: S05-516

BIOPHYSICAL MECHANISMS SUPPORTING MAINTENANCE OF COMPLEX TUNING

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

Andrew Landau, Bernardo Sabatini
Howard Hughes Medical Institute - Harvard Medical School, Department Of Neurobiology, Boston, MA, United States of America

Neural tuning properties are shaped by synaptic plasticity rules that govern the strength of each synaptic input. Hebbian plasticity rules strengthen the connections to correlated inputs that more effectively drive postsynaptic action potentials. While this supports feature detection, it simplifies tuning functions such that neural activity can be described as a mere relay of information already present in correlated inputs. We performed experimental measurements of the calcium signals underlying STDP in cortical L2/3 cells and found that STDP-mediated depression signals are selectively reduced in a subset of dendrites while STDP-mediated potentiation signals are preserved. Using these results as motivation, we constructed STDP models that have a reduction in synaptic depression in a subset of dendrites. We observed that dendrites with less depression can learn and maintain tuning properties that are not observed under conventional Hebbian learning algorithms. We show that this increases the complexity of neural tuning functions by permiting neurons to encode informative features in input with weaker correlations. We conclude by drawing connections between our findings and mutual information.

Pubmed:
31899286: Barrett KC, Barrett FS, Jiradejvong P, Rankin SK, Landau AT, Limb CJ
Classical creativity: A functional magnetic resonance imaging (fMRI) investigation of pianist and improviser Gabriela Montero. Improvisation is sometimes described as instant composition and offers a glimpse into real-time musical creativity. Over the last decade, researchers have built up our understanding of the core neural activity patterns associated with musical improvisation by investigating cohorts of professional musicians. However, since creative behavior calls on the unique individuality of an artist, averaging data across musicians may dilute important aspects of the creative process. By performing case study investigations of world-class artists, we may gain insight into their unique creative abilities and achieve a deeper understanding of the biological basis of musical creativity. In this experiment, functional magnetic resonance imaging and functional connectivity were used to study the neural correlates of improvisation in famed Classical music performer and improviser, Gabriela Montero. GM completed two control tasks of varying musical complexity; for the Scale condition she repeatedly played a chromatic scale and for the Memory condition she performed a given composition by memory. For the experimental improvisation condition, she performed improvisations. Thus, we were able to compare the neural activity that underlies a generative musical task like improvisation to 'rote' musical tasks of playing pre-learned and pre-memorized music. In GM, improvisation was largely associated with activation of auditory, frontal/cognitive, motor, parietal, occipital, and limbic areas, suggesting that improvisation is a multimodal activity for her. Functional connectivity analysis suggests that the visual network, default mode network, and subcortical networks are involved in improvisation as well. While these findings should not be generalized to other samples or populations, results here shed insight into the brain activity that underlies GM's unique abilities to perform Classical-style musical improvisations.
Neuroimage, 2020; 209

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MODELING OF INCREASED EXCITABILITY OF NOCICEPTIVE NEURONS DUE TO DIABETES-INDUCED UPREGULATION OF SOMATIC T-TYPE CA2+ CURRENT

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

Dmytro Duzhyy[1], Sergei Korogod[2], Arsenij Ivasiuk[2], Maxim Matvieienko[2], Nikolay Kononenko[2], Nana Voitenko[3,4], Pavel Belan[2,5]
[1]Bogomoletz Inst. of Physiol. of Natl. Acad. of Sci. of Ukraine, Department Of Sensory Signaling, Kyiv, Ukraine, [2]Bogomoletz Inst. of Physiol. of Natl. Acad. of Sci. of Ukraine, Department Of Molecular Biophysics, Kyiv, Ukraine, [3]Kyiv Academic University, Department Of Molecular Physiology And Biophysics, Kyiv, Ukraine, [4]Dobrobut Academy Medical School, Scientific Centre, Kyiv, Ukraine, [5]Kyiv Academic University, Department Of Biomedicine & Neurosciences, Kyiv, Ukraine

Background: T-type Ca^{2+} channels are known to play an essential role in the development and maintenance of the peripheral diabetic neuropathy (PDN). However, no particular mechanisms relating diabetes-induced upregulation of T-channels in soma of nociceptive DRG neurons to the pathological pain processing in PDN have been suggested. The **aim** of this study is to establish mechanisms that relate diabetes-induced upregulation of T-type channels to hyperexcitability of nociceptive DRG neurons and thus to PDN. **Methods:** Computation experiments were performed on a single-compartment model of isolated DRG neuron soma developed in the NEURON simulation environment. Parameters of simulated voltage-gated Ca^{2+}, Na^{+} and K^{+} currents were fitted by experimental recordings from somas of nociceptive DRG neurons, isolated from control and streptozotocin diabetic rats. **Results**: Computation experiments showed that an increased density of somatic T-type current and a depolarizing shift of its steady-state inactivation, observed in these neurons under diabetic conditions, transforms a single AP in the soma into a burst of multiple APs; thus far, the peripheral nociceptive input can be strongly amplified. This amplification may account for mechanical hyperalgesia observed in diabetic rats. Further increase in the T-type conductivity resulted in sustained AP firing occurred in the absence of external stimulation that might be a mechanism underlying spontaneous pain observed at later stages of diabetes. **Conclusions**: Computation experiments have confirmed causative link between changes in the properties of the T-type current and increased excitability of the DRG neurons under diabetes. Supported by NASU grant 0118U007345 and NRFU grant 2021.01/0435.

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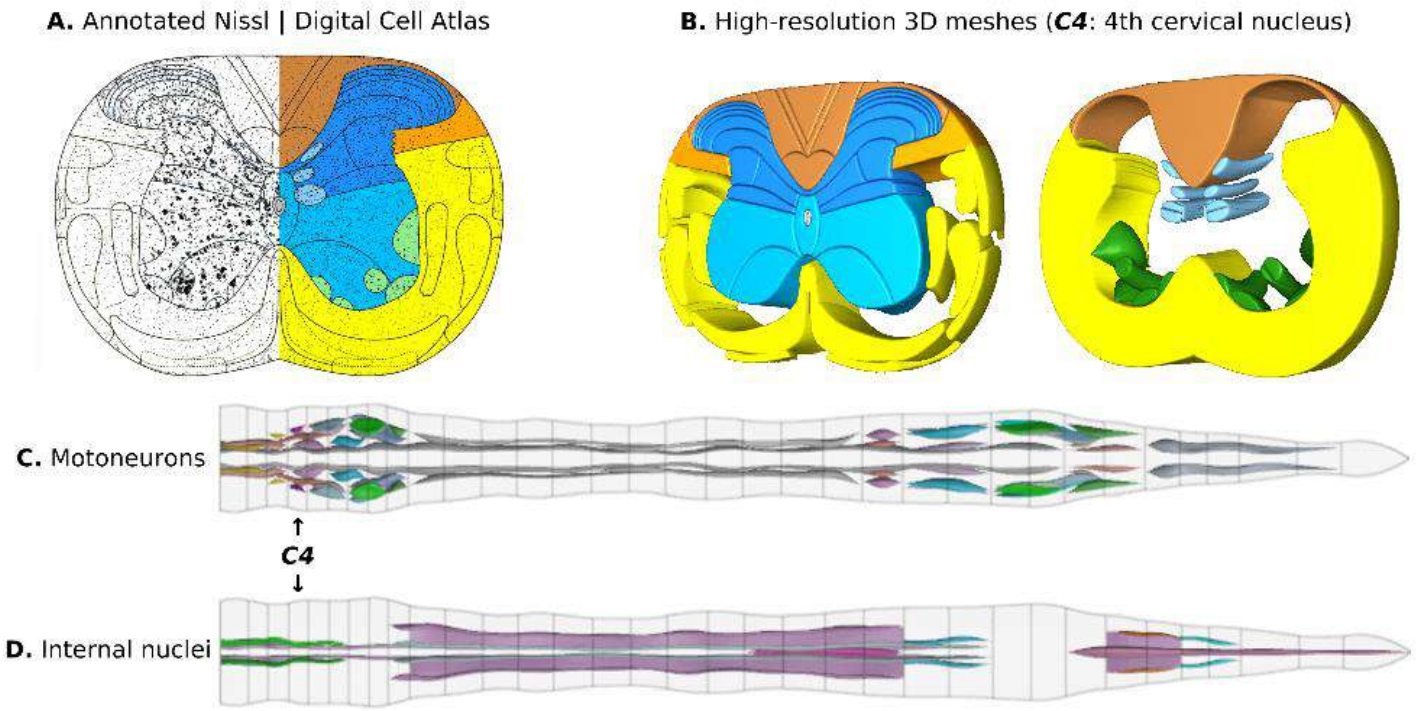
BOARD NUMBER: S05-518

3D CELL ATLAS OF MOUSE SPINAL CORD

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

Ihor Kuras, Henry Markram, Marc-Oliver Gewaltig
EPFL, Blue Brain Project, Geneva, Switzerland



The spinal cord is an elongated nervous structure that together with the brain forms the central nervous system.It relays sensory and motor information between the brain and the body, thus controlling most somatic and autonomic body functions. In recent years, great progress has been made in creating digital atlasses for the mouse brain, convering, regions, cellular composition and connectivity. For the spinal cord, however, such atlasses do not yet exist. Here we present a first step towards a comprehensive digital atlas of the mouse spinal cord, based on annotated Nissl images from the Allen Institute of Brain Science. Our atlas has two parts: the first part is a reference volume, comprising high-resolution 3D meshes for 78 regions across all 34 spinal nuclei. The second part is a cell atlas with the number, densities, and positions of neurons, motoneurons and non-neurons in all regions. We estimate that the mouse spinal cord contains 11,735,398 cells in total with 2,653,186 neurons, 31,280 motoneurons and 9,082,212 glial cells. The neuron-glia ratio in each region depends on the average size of its neurons: in the dorsal horn, where we find the smallest interneurons, the ratio is up to 1:1, while in regions with the largest neurons (motoneurons) the ratio is as low as 1:4. This is consistent with the neuron-glia ratio in the brain. Our atlas will be made available as an online ressource and will be of great value for modelers and anatomists.

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DYNAMIC MODELING OF EYE-HEAD GAZE SHIFTS BY A SPIKING NEURAL NETWORK MODEL OF THE SUPERIOR COLLICULUS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Donders Institute for Brain, Cognition and Behaviour, Radboud University, Department Of Biophysics, Nijmegen, Netherlands

Recent single-unit recordings in head-unrestrained monkeys revealed that saccadic eye-head gaze trajectories and kinematics are encoded by deep-layer midbrain Superior Colliculus (SC) neurons through their cumulative number of spikes and firing rates, respectively.
A previously proposed model for eye-head gaze-control[1] can account for many of the observed properties of eye-head gaze shifts and the SC role. However, in the lumped model, the SC burst profile was described by a simple rectangular pulse that issued a constant desired gaze-velocity signal. We here extended the model by incorporating a spiking neural network of the SC motor map, the output of which is based on the linear summation of individual vectorial spike effects of each recruited neuron. The more realistic spiking activity of the population of recruited SC neurons thus drives eye and head to the target location within a dynamic gaze-velocity feedback loop. In our extended model, we also included a neural mechanism on the cells that explains the small modulatory influence of the initial eye-in-head position on the spiking activity of the neurons, and on the detailed kinematics of gaze shifts. Further, our SC model was made robust to large spatial-temporal variations in its input stimulation by cortical inputs, by tuning its top-down and lateral synaptic connections. Our gaze-control model can thus generate gaze shifts with properties observed in electrophysiological experiments.
[1] B. Kasap and A.J. Van Opstal: Modeling auditory-visual evoked eye-head gaze shifts in dynamic multisteps. J Neurophysiology 119: 1795-1808 (2018).

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NEURONAL AND ASTROCYTIC INTERACTIONS IN SCHIZOPHRENIA: A COMPUTATIONAL MODELLING STUDY

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Astrocytes have been associated with psychiatric disorders such as schizophrenia in several studies. However, there are several different hypotheses regarding the pathological mechanisms of neurons and astrocytes in schizophrenia. In Lenk et al. (2020), we developed a mathematical neuron-astrocyte network model to investigate the influence of astrocytes on neuronal activity. In this study, we used this model to investigate several hypotheses about schizophrenia. In our simulations, reducing the number of astrocytes or neurons leads to decreased glutamate concentration in astrocytes and reduced neuronal network activity. Increased release of ATP (adenosine triphosphate) by astrocytes toward postsynapses also decreased neuronal activity but temporarily increased glutamate concentration. A reduction in the release of the neurotransmitter glutamate and the decreased uptake by astrocytes each resulted in increased network activity. Also, the increase in synaptic weights, i.e., the coupling strength, of excitatory and inhibitory neurons respectively inhibitory neurons led to this result. Our simulations suggest, that the interaction of neurons and astrocytes in schizophrenia should be further should be investigated in more detail.

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BOARD NUMBER: S05-521

A COMPUTATIONAL MODEL OF SLOW WAVE OSCILLATION PROPAGATION ACROSS CORTICAL NETWORKS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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The activity of every neural circuit is limited by anatomical and functional constrains, which will restrict its repertoire of activity patterns. Therefore, the knowledge obtained from the study of the brain spontaneous activity is a valuable resource to understand how those circuits operate during behaviour. We present a computational model of cortical populations under the Slow Wave Oscillation (SWO) regime. This spontaneous brain state is characterized by periods of high spontaneous activity (Up states) intermingled with silent periods (Down states) at the frequency of ~ 1 Hz. It originates in the cortex and from there propagates to other brain regions. While traditional SWO models are focused on single and isolated cortical areas. our study aims to provide a theoretical understating of the contribution of local and long range connections to the properties of the SWO in multiple connected neuronal populations. This includes the study of the neuronal and circuit substrates behind the different Up state attributes across cortical regions and the rostrocaudal preferential directionality of the SWO propagation. With this, we will provide an explanation to the differences in the SWO recorded in slices or brain slabs compared to the intact brain. In conclusion, we have designed a model of SWO propagation along cortical. With this tool, we studied the neuronal and circuit substrates of the propagation of this wave along these networks, unifying the previous knowledge based on *in vivo* and *ex vivo* recordings.

Pubmed:
30557375: Alegre-Cortés J, Soto-Sánchez C, Fernandez E
Multiscale dynamics of interstimulus interval integration in visual cortex.
Although the visual cortex receives information at multiple temporal patterns, much of the research in the field has focused only on intervals shorter than 1 second. Consequently, there is almost no information on what happens at longer temporal intervals. We have tried to address this question recording neuronal populations of the primary visual cortex during visual stimulation with repetitive grating stimuli and intervals ranging from 1 to 7 seconds. Our results showed that firing rate and response stability were dependent of interval duration. In addition, there were collective oscillations with different properties in response to changes in intervals duration. These results suggest that visual cortex could encode visual information at several time scales using oscillations at multiple frequencies.
PLoS One, 2018; 13
27044801: Alegre-Cortés J, Soto-Sánchez C, Pizá ÁG, Albarracín AL, Farfán FD, Felice CJ, Fernández E
Time-frequency analysis of neuronal populations with instantaneous resolution based on noise-assisted multivariate empirical mode decomposition.
Linear analysis has classically provided powerful tools for understanding the behavior of neural populations, but the neuron responses to real-world stimulation are nonlinear under some conditions, and many neuronal components demonstrate strong nonlinear behavior. In spite of this, temporal and frequency dynamics of neural populations to sensory stimulation have been usually analyzed with linear approaches.
J Neurosci Methods, 2016; 267
29375359: Alegre-Cortés J, Soto-Sánchez C, Albarracín AL, Farfán FD, Val-Calvo M, Ferrandez JM, Fernandez E
Toward an Improvement of the Analysis of Neural Coding.
Machine learning and artificial intelligence have strong roots on principles of neural computation. Some examples are the structure of the first perceptron, inspired in the retina, neuroprosthetics based on ganglion cell recordings or Hopfield networks. In addition, machine learning provides a powerful set of tools to analyze neural data, which has already proved its efficacy in so distant fields of research as speech recognition, behavioral states classification, or LFP recordings. However, despite the huge technological advances in neural data reduction of dimensionality, pattern selection, and clustering during the last years, there has not been a proportional development of the analytical tools used for Time-Frequency (T-F) analysis in neuroscience. Bearing this in mind, we introduce the convenience of using non-linear, non-stationary tools, EMD

algorithms in particular, for the transformation of the oscillatory neural data (EEG, EMG, spike oscillations…) into the T-F domain prior to its analysis with machine learning tools. We support that to achieve meaningful conclusions, the transformed data we analyze has to be as faithful as possible to the original recording, so that the transformations forced into the data due to restrictions in the T-F computation are not extended to the results of the machine learning analysis. Moreover, bioinspired computation such as brain-machine interface may be enriched from a more precise definition of neuronal coding where non-linearities of the neuronal dynamics are considered.
Front Neuroinform, 2017; 11
29679647: Sáez M, Ketzef M, Alegre-Cortés J, Reig R, Silberberg G
A New Micro-holder Device for Local Drug Delivery during In Vivo Whole-cell Recordings.
Focal administration of pharmacological agents during in vivo recordings is a useful technique to study the functional properties of neural microcircuits. However, the lack of visual control makes this task difficult and inaccurate, especially when targeting small and deep regions where spillover to neighboring regions is likely to occur. An additional problem with recording stability arises when combining focal drug administration with in vivo intracellular recordings, which are highly sensitive to mechanical vibrations. To address these technical issues, we designed a micro-holder that enables accurate local application of pharmacological agents during in vivo whole-cell recordings. The holder couples the recording and drug delivery pipettes with adjustable distance between the respective tips adapted to the experimental needs. To test the efficacy of the micro-holder we first performed whole-cell recordings in mouse primary somatosensory cortex (S1) with simultaneous extracellular recordings in S1 and motor cortex (M1), before and after local application of bicuculline methiodide (BMI 200 µM). The blockade of synaptic inhibition resulted in increased amplitudes and rising slopes of "Up states", and shortening of their duration. We then checked the usability of the micro-holder in a deeper brain structure, the striatum. We applied tetrodotoxin (TTX 10 µM) during whole-cell recordings in the striatum, while simultaneously obtaining extracellular recordings in S1 and M1. The focal application of TTX in the striatum blocked Up states in the recorded striatal neurons, without affecting the cortical activity. We also describe two different approaches for precisely releasing the drugs without unwanted leakage along the pipette approach trajectory.
Neuroscience, 2018; 381
33599609: Alegre-Cortés J, Sáez M, Montanari R, Reig R
Medium spiny neurons activity reveals the discrete segregation of mouse dorsal striatum.
Behavioral studies differentiate the rodent dorsal striatum (DS) into lateral and medial regions; however, anatomical evidence suggests that it is a unified structure. To understand striatal dynamics and basal ganglia functions, it is essential to clarify the circuitry that supports this behavioral-based segregation. Here, we show that the mouse DS is made of two non-overlapping functional circuits divided by a boundary. Combining in vivo optopatch-clamp and extracellular recordings of spontaneous and evoked sensory activity, we demonstrate different coupling of lateral and medial striatum to the cortex together with an independent integration of the spontaneous activity, due to particular corticostriatal connectivity and local attributes of each region. Additionally, we show differences in slow and fast oscillations and in the electrophysiological properties between striatonigral and striatopallidal neurons. In summary, these results demonstrate that the rodent DS is segregated in two neuronal circuits, in homology with the caudate and putamen nuclei of primates.
Elife, 2021; 10

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BOARD NUMBER: S05-522

INCORPORATION OF INTERNEURONS IN A NETWORK MODEL TO EXPLAIN THE EFFECTS OF SCHIZOPHRENIA-ASSOCIATED GENES ON DELTA OSCILLATIONS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

Jan Fredrik Kismul[1], Torbjørn Ness[2], Gaute Einevoll[2], Marja-Leena Linne[1], Tuomo Mäki-Marttunen[3]
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Recent genome-wide association studies have identified many variants associated with schizophrenia[1]. These genetic variants were suggested to be able to account for the increased power of delta-oscillation, a common biomarker of schizophrenia[2]. In[2] a network of model pyramidal neurons with reduced morphological reconstructions were connected to an inhibitory population of point neurons. In the present study this network model is expanded upon; the interneurons will be replaced with model neurons of simplified morphologies, and a new population of neuroglial cells are added to study the effects of GABA_B receptors. In previous studies GABA_B receptors have been shown to decrease the delta-oscillation power of sleeping rats [3]. In our work, the interneurons are first obtained from the Blue Brain database, and subsequently reduced to 3-compartmental models of the same neurons. They show the same behavior in response to stimulation with a somatic current as the full-morphology level neurons. These additions to the model presented in [2] allow a larger set of model variants of schizophrenia risk genes to be studied in a physiologically more realistic model of cortical circuitry.
Acknowledgements: Academy of Finland(336376) **References**
[1] The Schizophrenia Working Group of the Psychiatric Genomics Consortium
et al. “Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia”. medRxiv (2020)
[2] Tuomo Mäki-Marttunen et al. Cerebral Cortex 29.2 (Nov. 2018), pp. 875–891.
[3] Lucy M. Carracedo et al. Journal of Neuroscience 33.26 (2013), pp. 10750–10761.

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BOARD NUMBER: S05-523

SIMULATING CORTICAL DYNAMICS IN ANATOMICALLY DETAILED NETWORK MODELS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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[1]Johannes Gutenberg University of Mainz, Institute For Physiological Chemistry, Mainz, Germany, [2]Institute of Experimental Epileptology and Cognition Research, University Of Bonn Medical Center, Bonn, Germany, [3]Max Planck Institute for neurobiology of behavior, CAESAR, In Silico Brain Sciences, Bonn, Germany, [4]Zuse-Institut Berlin, Visual And Data-centric Computing, Berlin, Germany

Aims: Rodent barrel cortex receives and processes whisker information. The network dynamics of the barrel cortex is strongly impacted by the cortical structure and cell-type specificity. However, the mechanisms of how structural details and cell-type specificity impact the network dynamics have not yet been fully understood. In the context of the visual cortex, stabilized supralinear network (SSN) models have been suggested as a way to include orientation selectivity and contrast invariance, which makes it a suitable model to study the somatosensory cortex dynamics. Can a connectomically constrained SSN model of the rat barrel cortex be used to interpret the role of cell-type specificity? **Methods:** Here we present an SSN model which incorporates anatomically detailed barrel cortex data which reflects multiple cell types and their neuron-to-neuron connectivity. Our SSN model is consistent with the spiking network activity of approximately 4000 neurons where a single neuron is modelled as a leaky-integrate-and-fire model (LIF). The parameters of LIF neurons are based on biologically plausible data for each specific cell type. The connectivity of LIF neurons is based on an empirically observed statistical connectome model. **Results and Conclusion:** We can now use the model to derive predictions for future experiments and identify cell-type specific activity patterns that contribute to sensory perception.

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BOARD NUMBER: S05-524

CORE NEURONS ARE STRUCTURAL CROSSROADS OF CORTICAL DYNAMICS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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NeuroPSI, Icn, Saclay, France

Two-photon calcium imaging of the behaving mouse cortex shows spiking correlations - called ensembles - thought to underlie perceptual, motor, and higher cognitive functions. The functional specificity and reproducibility of these ensembles would be ensured by distinctive regularly participating cells, called core neurons (here simply ‘cores’). It is assumed that cores' strong interconnections underlie pattern completion and reproducibility. Here we show that this hypothesis is incorrect. Using electron microscopy and 2-photon calcium imaging data of the same cortical tissue, we studied spiking correlations between cell motifs - i.e. significant connectivity patterns. We found that strong interconnection is not the characterizing feature of cores. The majority of cores belong to converging and diverging motifs, and they do not connect preferentially to other cores (low assortativity). On the contrary, cores lie onto many different paths (high betweenness), longer than those passing by other neurons (high eccentricity), making them structural “crossroads”. Based on the strong interconnection assumption, several models have been previously proposed, where strong paths were artificially imposed to obtain ensembles and cores. We present the first model where core neurons are not imposed by ad-hoc wiring. We show instead that topological connectivity - distance-dependent random sampling of connections - is the only necessary and sufficient requirement to reproduce the statistics of spontaneous and evoked ensembles, containing core neurons, recorded across several cortices. Taken together, our analysis of electron microscopy and two-photon data, and our modeling work show that recurrence of cortical dynamics is not a direct consequence of strong structural interconnectivity.

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BOARD NUMBER: S05-525

HUMAN DATA STATE OF THE ART: TOWARDS A HUMAN CORTICAL MICROCIRCUIT

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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The human neocortex is the brain area thought to differentiate us most from other species because it gives us some unique abilities, as speaking. Although the human neocortex has been studied for many years since Ramon y Cajal started last century, little is known about its cell anatomical structure and its cell and circuit functionality. One proven approach to speed up the process of understanding neurons and brain circuits that has been successfully used before, is the use of computational models and simulations. Another approach that aims to make the most out of the little data available for human, is to explicitly compare to and contrast it with better studied cortices from other species, such as rodents. In this study we collected, integrated, and analyzed data on human that were available in the literature and propose various strategies to overcome the missing data, such as generalizing data from other species. We then used it to build a human cortical microcircuit, based on the modelling approach we previously developed to model a rat microcircuit. We found that the literature provided a sufficient amount of data to generate a first draft of a model of microcircuit anatomy, neuronal morphology, connectivity and physiology, when combined with data generalizations. While gaps in data on cell morphologies and electrophysiology had to be filled by adapting corresponding data from other species, in the resulting model still a number of anatomical features emerged that were not present in the rodent model and are thus potentially human-specific.

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BOARD NUMBER: S05-526

MULTI-COMPARTMENTAL RECONSTRUCTION AND SIMULATION OF AN ENTIRE MODULE OF THE MOUSE CEREBELLAR CORTEX

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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[1]University of Pavia, Dep. For Brain And Behavioral Sciences, Pavia, Italy, [2]IRCCS Mondino Foundation, Brain Connectivity Center, Pavia, Italy

Data-driven modelling of the brain requires a neuroinformatic framework implementing a general strategy to accommodate experimental data at different scales. To this end, we have developed the Brain Scaffold Builder (BSB), a tool for structural and functional microcircuit modelling. The BSB provides an organized staged workflow, multiple strategies for cell placement and connectivity, a configuration system capable of including detailed neuronal and synaptic models and the support for multiple simulators with transparent parallel processing. It is provided as an open-source package, applicable for multi-scale modelling of different brain areas. The interfaces with several simulators (NEURON, NEST, Arbor) allow to investigate the same brain region at different levels of resolution, depending on the scientific question. The BSB's effectiveness was tested on the cerebellar network, which has a complex geometry and raises a broad set of modelling challenges. For the first time, an entire module of the mouse cerebellar cortex was reconstructed using morphologically realistic conductance-based multi-compartmental neuron models of granule, Golgi, Purkinje, stellate and basket cells. Different connection rules, such as convergence-divergence, touch detection and voxel intersection, were used to generate the connectome, unifying a collection of scattered experimental data into a coherent construct. Baseline and sensory-burst stimulation were used for functional validation against in vivo recordings, monitoring the impact of subcellular and cellular mechanisms on signal propagation and spatio-temporal processing in the cerebellum. The integration of structural and functional properties through the model provides a new "ground truth" about cerebellar circuit organization capable of predicting neural dynamics in vivo.

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BOARD NUMBER: S05-527

A DETAILED BIOPHYSICAL MODEL OF THE INFERIOR OLIVE

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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The cerebellum is known to play an essential role in the supervised learning of accurately executed and coordinated movements. Cerebellar Purkinje cells (PCs) receive movement-related information at their plastic input synapses, classically thought to be modulated after a movement by error signals. These originate from the Inferior Olive (IO) through the unique climbing fiber to each PC. However, signals coming from the IO during learning have been shown to be more diverse than post-movement errors only, with for instance consistent anticipatory activity being observed in classical conditioning experiments. This plurality of signals may thus contribute to different plasticity mechanisms in the cerebellum. In this context, an accurate description of the IO seems key to understand and model cerebellar learning.
The IO is constituted of a single cell type, assembled in clustered networks highly coupled via electrical gap junctions. Previous work has focused mainly on the experimentally observed synchronous spontaneous oscillations, but individual cell recordings display various complex behaviors contributing to IO excitability that are poorly reproduced by the few models that exist. We thereby aim at developing a more precise biophysical model of networks of IO cells accounting for most of their experimentally measured properties, based on data obtained from the literature and patch-clamp experiments currently in progress. This should deepen our understanding of this key structure and help to understand how it relates to learning in the cerebellum.

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BOARD NUMBER: S05-528

A NOVEL COMPUTATIONAL PLATFORM FOR MIMICKING NEURAL NETWORK ACTIVITY IN ADVANCED IN VITRO NEURAL CONSTRUCTS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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[1]University of Pisa, Department Of Information Engineering, Pisa, Italy, [2]University of Pisa, Centro Di Ricerca “e. Piaggio”, Pisa, Italy

To date, the computational models of biological neural networks do not include the oxygen concentration as a variable influencing the electrical activity of neurons. However, in three-dimensional (3D) vessel-free cell cultures, e.g., brain organoids and assembloids, oxygen may represent a limiting factor for neuron viability and function. We developed a novel model of neuron firing in 3D constructs which accounts for oxygen reaction and diffusion. The model simulates the functional activity of an organoid slice and was developed in Matlab. A single neuron is represented by a Simulink block. The blocks are connected each other to simulate the signal transmission through synapses. Oxygen diffuses through the network according to Fick’s second law and is taken up by neurons fuelling both their metabolic and electrophysiological activities. Specifically, metabolic consumption is modelled with Michaelis-Menten kinetics, while firing activity is simulated with a modified Hodgkin-Huxley model, where the dynamics of sodium and potassium ions, whose activity is oxygen-dependent, are introduced. The outputs of the model (i.e., the spiking activity of the neurons constituting the network) are dependent on the local oxygen concentration within the construct and are consistent with the electrophysiological activity recorded in cerebral organoids. The computational platform can be used to guide experiments, optimising organoid generation and maintenance protocols. It can also help to gain insights into cerebral organoid growth and function. Future developments will include new model configurations, i.e. higher number of neurons, spatial 3D arrangement and different connections, in order to fully resemble the neural network of an organoid.

Pubmed:

33925256: Hasic Telalovic J, Pillozzi S, Fabbri R, Laffi A, Lavacchi D, Rossi V, Dreoni L, Spada F, Fazio N, Amedei A, Iadanza E, Antonuzzo L
A Machine Learning Decision Support System (DSS) for Neuroendocrine Tumor Patients Treated with Somatostatin Analog (SSA) Therapy.
The application of machine learning (ML) techniques could facilitate the identification of predictive biomarkers of somatostatin analog (SSA) efficacy in patients with neuroendocrine tumors (NETs). We collected data from 74 patients with a pancreatic or gastrointestinal NET who received SSA as first-line therapy. We developed three classification models to predict whether the patient would experience a progressive disease (PD) after 12 or 18 months based on clinic-pathological factors at the baseline. The dataset included 70 samples and 15 features. We initially developed three classification models with accuracy ranging from 55% to 70%. We then compared ten different ML algorithms. In all but one case, the performance of the Multinomial Naïve Bayes algorithm (80%) was the highest. The support vector machine classifier (SVC) had a higher performance for the recall metric of the progression-free outcome (97% vs. 94%). Overall, for the first time, we documented that the factors that mainly influenced progression-free survival (PFS) included age, the number of metastatic sites and the primary site. In addition, the following factors were also isolated as important: adverse events G3-G4, sex, Ki67, metastatic site (liver), functioning NET, the primary site and the stage. In patients with advanced NETs, ML provides a predictive model that could potentially be used to differentiate prognostic groups and to identify patients for whom SSA therapy as a single agent may not be sufficient to achieve a long-lasting PFS.
Diagnostics (Basel), 2021; 11

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BOARD NUMBER: S05-529

PROBING THE ROLE OF CUNEATE NUCLEUS AND PRIMARY SOMATOSENSORY CORTEX USING TASK-OPTIMIZED NEURAL NETWORK MODELS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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[1]EPFL, Brain Mind Institute, Center Of Neuroprosthetics, Center Of Intelligent Systems, School Of Life Sciences, Lausanne, Switzerland, [2]EPFL, Brain Mind Institute, Center Of Neuroprosthetics, School Of Life Sciences, Geneva, Switzerland, [3]Northwestern University, Department Of Biomedical Engineering, Chicago, IL, United States of America

Adaptive motor control requires proprioception. However, the principles that govern the processing of proprioception are poorly understood. Here, we employ a task-driven neural network modeling approach to quantitatively test hypotheses about the functional role of proprioceptive neurons in both cuneate nucleus (CN) and somatosensory cortex (area 2). We simulated muscle spindle signals for a large, diverse movement repertoire (following Sandbrink et al., bioRxiv, 2020), and used them to train hundreds of deep neural networks (DNNs) in three behavioral tasks: action recognition, hand localization, and redundancy reduction. We tested these tasks as hypotheses about the function of the ascending proprioceptive pathway by predicting neuronal spiking activity in CN and area 2 from macaques performing a center-out reaching task. To do so, we tracked limb movements using DeepLabCut and inferred the proprioceptive signals via musculoskeletal modeling. We used these as inputs to the task-trained DNNs to linearly regress single-neuron activity. Firstly, we found that network models that perform better on their tasks explain the neural data better, for all tasks and monkeys. This correlation was most pronounced for models trained on action recognition. Secondly, network features of specific tasks explained CN and area 2 neurons differently, while combining representations from different tasks yielded strongly improved predictions, outperforming classical tuning curve and GLMs. Our results suggest that these tasks are sufficient to develop brain-like representations along the proprioceptive pathway. Overall, our work consolidates task-driven modeling as an optimization-based framework to understand sensory systems beyond vision, audition, and touch.

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BOARD NUMBER: S05-530

RETINAL WAVES ALIGN THE CONCENTRIC ORIENTATION MAP IN MOUSE SUPERIOR COLLICULUS TO THE CENTER OF VISION

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Neurons in the mouse superior colliculus (SC) are arranged in an orientation preference map (OPM) that has a concentric organization, which is aligned to the center of vision and the optic flow (Ahmadlou and Heimel, 2015). The developmental mechanisms that underlie this functional map remain unclear. During development, the stage III (S3) retinal waves are known to play important roles in circuit formation. The S3 waves that tend to propagate towards the caudal direction (Gribizis et al., 2019) have impacts on the direction selectivity of the SC neurons (Ge et al., 2021). Besides, the OFF retinal ganglion cells (RGCs) are recruited with a time-delay relative to ON RGCs (Kerschensteiner and Wong, 2008). Here, we propose that these S3 wave properties provide a scaffold to establish the concentric OPM in the mouse SC and its alignment to the optic flow. We test this hypothesis by modeling the orientation-tuned SC neurons that receive ON/OFF retinal inputs. Our results suggest that the OFF delay and the wave direction bias are key factors that regulate the spatial organization of the SC OPM. Specifically, the OFF delay mediates the establishment of orientation-tuned SC neurons, the wave-like activities facilitate the formation of a concentric pattern, and the wave direction biases align the OPM to the center of vision. Taken together, our model suggests that retinal waves may play an instructive role in establishing functional properties of SC neurons and provides a promising mechanism for explaining the correlations between the optic flow and the SC OPM.

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BOARD NUMBER: S05-531

SINGLE-AXON DYNAMICS OF SEROTONERGIC NEURONS IN EX VIVO SYSTEMS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Our group has shown that the self-organization of the brain serotonergic matrix depends in part on the spatiotemporal dynamics of individual serotonergic axons (fibers). The trajectories of these axons are strongly stochastic and can be described by step-wise random walks or fractional Brownian motion (a time-continuous process). The success of these modeling efforts depends on experimental data that can validate their mathematical frameworks and constrain the values of model parameters. However, visualizing this dynamic behavior in vivo is currently extremely difficult. In this study, we developed an ex vivo system of primary mouse brainstem neurons. Using a combination of methods such as confocal microscopy, digital holotomography, STED super-resolution microscopy, and novel 3D hydrogel systems, we investigated serotonergic axons with unprecedented spatiotemporal precision. The dynamics of axon growth cones, branching events, and other key processes were analyzed with respect to the properties of the tunable extracellular environment and to intracellular movements reflected in refractive index changes. These experimental data include the first holotomographic images of serotonergic neurons and axons and provide essential information for predictive modeling of the serotonergic matrix. In addition to the importance of these findings for fundamental neuroscience, they can also support future efforts in the restoration of brain tissue (serotonergic fibers are almost unique in the mammalian brain in their ability to robustly regenerate). The novel approaches developed in this study may be applicable to other "stochastic" axons in the ascending reticular activating system.

Pubmed:
30649856: Janušonis S, Mays KC, Hingorani MT
Serotonergic Axons as 3D-Walks.
Experimental and theoretical research suggests that serotonergic axons (fibers) can be modeled as random walks or stochastic processes. This rigorous approach can support descriptive methods and dynamic control of the ascending reticular activating system, at the level of individual fiber trajectories.
ACS Chem Neurosci, 2019; 10

FENS Forum 2022
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BOARD NUMBER: S05-532

BRIDGING THE GAP BETWEEN ARTIFICIAL MODELS AND CORTICAL CIRCUITS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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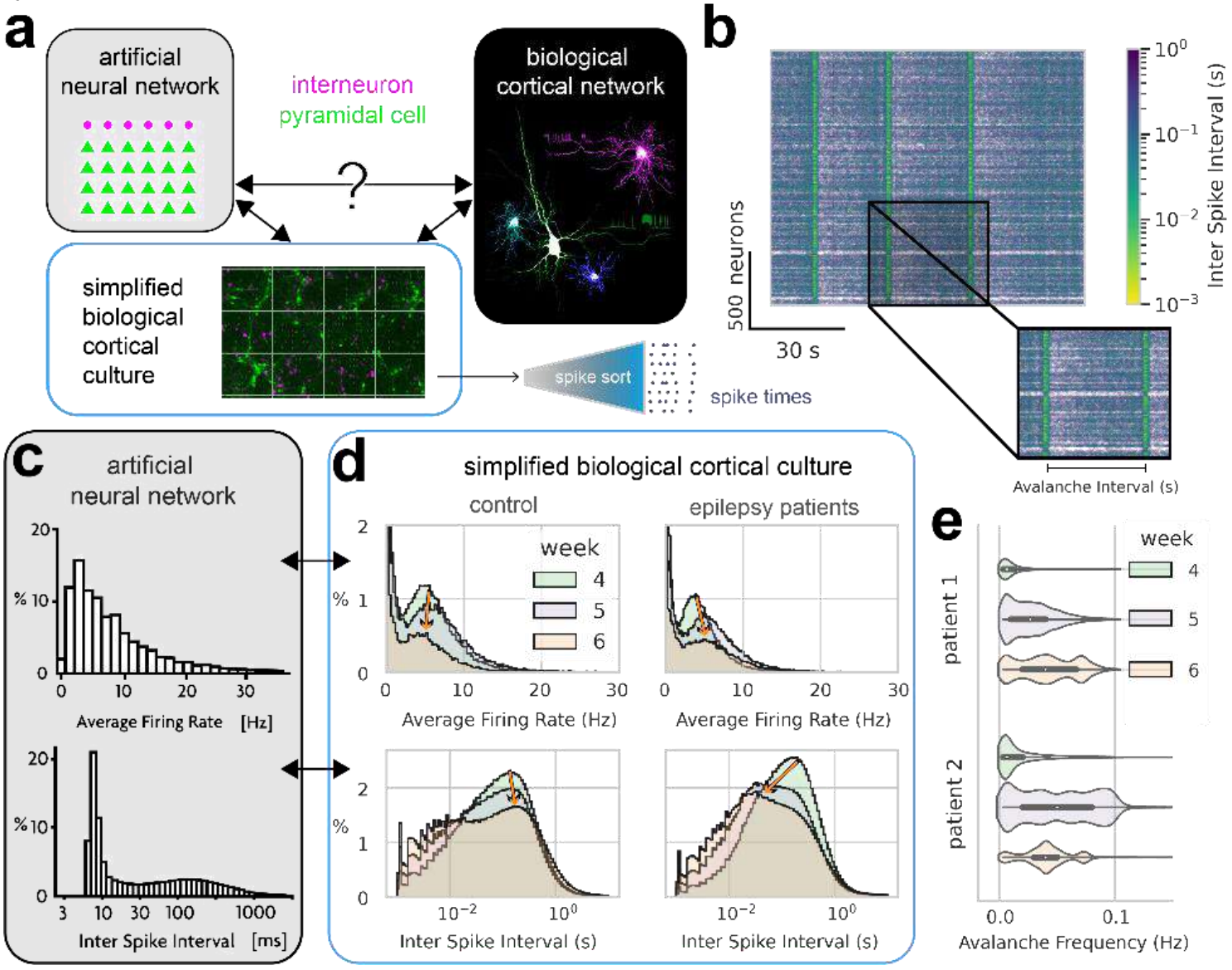
Artificial neural networks simplify complex biological circuits into tractable models for computational exploration and experimentation. However, the simplification of artificial models also undermines their applicability to real brain dynamics. Typical efforts to address this mismatch add complexity to increasingly unwieldy models. Here, we take a different approach; by reducing the complexity of a biological cortical culture, we aim to distil the essential factors of neuronal dynamics and plasticity. We leverage recent advances in growing neurons from human induced pluripotent stem cells (hiPSCs) to analyse *ex vivo* cortical cultures with only two distinct excitatory and inhibitory neuron populations. Over 6 weeks of development, we record from thousands of neurons using high-density microelectrode arrays (HD-MEAs). We compare these dynamics to two-population artificial networks of single-compartment neurons with random sparse connections. Moreover, integrating models and cultures allows us to evaluate the impact of changing architectures over weeks of development, with and without external stimuli. Broadly, the use of simplified cortical cultures enables us to use the repertoire of theoretical neuroscience techniques established over the past decades on artificial network models. Our approach of deriving neural networks from human cells also allows us to directly compare neural dynamics of disease and control. We found that cultures from epilepsy patients have increasingly more synchronous activity over weeks of development. Next, we will test possible interventions, *in silico* and *in vitro*, in a drive for personalised medical care. This work starts bridging an important theoretical-experimental neuroscience gap for advancing our understanding of mammalian neuron

dynamics.



Pubmed:
32453795: Currin CB, Trevelyan AJ, Akerman CJ, Raimondo JV
Chloride dynamics alter the input-output properties of neurons.
Fast synaptic inhibition is a critical determinant of neuronal output, with subcellular targeting of synaptic inhibition able to exert different transformations of the neuronal input-output function. At the receptor level, synaptic inhibition is primarily mediated by chloride-permeable Type A GABA receptors. Consequently, dynamics in the neuronal chloride concentration can alter the functional properties of inhibitory synapses. How differences in the spatial targeting of inhibitory synapses interact with intracellular chloride dynamics to modulate the input-output function of neurons is not well understood. To address this, we developed computational models of multi-compartment neurons that incorporate experimentally parametrised mechanisms to account for neuronal chloride influx, diffusion, and extrusion. We found that synaptic input (either excitatory, inhibitory, or both) can lead to subcellular variations in chloride concentration, despite a uniform distribution of chloride extrusion mechanisms. Accounting for chloride changes resulted in substantial alterations in the neuronal input-output function. This was particularly the case for peripherally targeted dendritic inhibition where dynamic chloride compromised the ability of inhibition to offset neuronal input-output curves. Our simulations revealed that progressive changes in chloride concentration mean that the neuronal input-output function is not static but varies significantly as a

function of the duration of synaptic drive. Finally, we found that the observed effects of dynamic chloride on neuronal output were mediated by changes in the dendritic reversal potential for GABA. Our findings provide a framework for understanding the computational effects of chloride dynamics on dendritically targeted synaptic inhibition.
PLoS Comput Biol, 2020; 16
31295253: Currin CB, Khoza PN, Antrobus AD, Latham PE, Vogels TP, Raimondo JV
Think: Theory for Africa.
PLoS Comput Biol, 2019; 15
30260315: Düsterwald KM, Currin CB, Burman RJ, Akerman CJ, Kay AR, Raimondo JV
Biophysical models reveal the relative importance of transporter proteins and impermeant anions in chloride homeostasis. Fast synaptic inhibition in the nervous system depends on the transmembrane flux of Cl ions based on the neuronal Cl driving force. Established theories regarding the determinants of Cl driving force have recently been questioned. Here, we present biophysical models of Cl homeostasis using the pump-leak model. Using numerical and novel analytic solutions, we demonstrate that the Na/K-ATPase, ion conductances, impermeant anions, electrodiffusion, water fluxes and cation-chloride cotransporters (CCCs) play roles in setting the Cl driving force. Our models, together with experimental validation, show that while impermeant anions can contribute to setting [Cl] in neurons, they have a negligible effect on the driving force for Cl locally and cell-wide. In contrast, we demonstrate that CCCs are well-suited for modulating Cl driving force and hence inhibitory signaling in neurons. Our findings reconcile recent experimental findings and provide a framework for understanding the interplay of different chloride regulatory processes in neurons.
Elife, 2018; 7
27614144: Swart PC, Currin CB, Russell VA, Dimatelis JJ
Early ethanol exposure and vinpocetine treatment alter learning- and memory-related proteins in the rat hippocampus and prefrontal cortex.
This study investigates the effects of early exposure to ethanol on cognitive function and neural plasticity-related proteins in the rat brain. Sprague-Dawley rats were administered 12% ethanol solution (4 g/kg/day i.p.) or saline from P4 to P9. Vinpocetine, a phosphodiesterase type 1 inhibitor, was tested to determine whether it could reverse any changes induced by early ethanol exposure. Hence, from P25 to P31, ethanol-exposed male rats were injected with vinpocetine (20 mg/kg/day i.p.) or vehicle (DMSO) prior to undergoing behavioral testing in the open field and Morris water maze (MWM) tests. Ethanol exposure did not adversely affect spatial memory in the MWM. A key finding in this study was a significant ethanol-induced change in the function of the phosphorylated extracellular signal-related kinase (P-ERK) signaling pathway in the prefrontal cortex (PFC) and dorsal hippocampus (DH) of rats that did not display overt behavioral deficits. The P-ERK/ERK ratio was decreased in the PFC and increased in the DH of ethanol-exposed rats compared with controls. Rats that received vinpocetine in addition to ethanol did not display any behavioral changes but did show alterations in neural plasticity-related proteins. Mitogen-activated protein kinase phosphatase was increased, whereas brain-derived neurotrophic factor was decreased, in the PFC of vinpocetine-treated ethanol-exposed rats, and phosphorylated-glycogen synthase kinase β and synaptophysin were increased in the DH of these rats. This study provides insight into the long-term effects of early ethanol exposure and its interaction with vinpocetine in the rat brain. © 2016 Wiley Periodicals, Inc.
J Neurosci Res, 2017; 95

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BOARD NUMBER: S05-533

NOT IN SHAPE: NEURONAL CELL TYPE CLASSIFICATION USING SPIKE TIMING OR SPATIAL FEATURES

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Brain circuits are composed of different cell types with distinct roles in neuronal network dynamics. Previous work showed that it is possible to distinguish between pyramidal cells and parvalbumin-immunoreactive (PV) interneurons based on features derived from spike waveforms recorded extracellularly. Whether pyramidal and PV cells can be distinguished solely based on features independent of the spike waveform remains unknown. Here, we combined high-density silicon probe recordings from hippocampal region CA1 of freely-moving PV mice with optogenetic tagging. We used the ground truth data to construct cross-validated random-forest models for binary classification, combined with a new chunking approach we created to optimize performance. Features derived from waveforms (morphological features), spike timing (temporal), and the distribution of signals across recording sites (spatial), were used as input to distinct models. Consistent with previous work, the cross-validated morphological model reached near-perfect results with an area-under-the-curve (AUC) of 0.99, considerably above chance level of 0.5. The model based on spike timing alone reached an AUC of 0.95, and the model based on purely spatial features yielded an AUC of 0.89. The results show that both spike timing and the distribution of the signals in space provide unique information about pyramidal and PV cells. The models allow accurate and robust classification of data from animals without ground truth, furthering our understanding of neuronal network dynamics. **Funding: CIHR-IDRC-ISF #2558/18; CRCNS NSF-BSF #2015577; Zimin Institute**

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9-13 July 2022 | Paris, France

BOARD NUMBER: S05-534

NEURAL ASSEMBLIES UNCOVERED BY GENERATIVE MODELING EXPLAIN WHOLE-BRAIN ACTIVITY STATISTICS AND REFLECT STRUCTURAL CONNECTIVITY

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Patterns of endogenous activity in the brain reflect a stochastic exploration of the neuronal state space that is constrained by the underlying assembly organization of neurons. Yet it remains to be shown that this interplay between neurons and their assembly dynamics indeed suffices to generate whole-brain data statistics. Here we recorded the activity from ~ 40, 000 neurons simultaneously in zebrafish larvae, and show that a data-driven network model of neuron-assembly interactions can accurately reproduce the mean activity and pairwise correlation statistics of their spontaneous activity. This model, the compositional Restricted Boltzmann Machine, unveils ~ 200 neural assemblies, which compose neurophysiological circuits and whose various combinations form successive brain states. From this, we mathematically derived an interregional functional connectivity matrix, which is conserved across individual animals and correlates well with structural connectivity. This novel, assembly-based generative model of brain-wide neural dynamics enables physiology-bound perturbation experiments in silico.

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ACTIVITY-DRIVEN DEEP MODELS FOR LEARNING SOUND TRANSFORMATIONS ACROSS THE AUDITORY PATHWAY

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Auditory system neurons present striking nonlinear features in their activity patterns which are difficult to model. To take advantage of model optimization tools now offered by deep networks we recorded a large sample of neurons, using two-photon imaging and electrophysiology, across different stages of the auditory pathway including the cochlea, inferior colliculus, thalamus, and auditory cortex. Based on this dataset, we aimed at capturing stage-to-stage non-linear transformation of auditory information. Given the large number of different stimuli presented and recorded neurons across several trials, we could employ an *activity-driven* framework to map the neuronal activity across stages with multilayer models (multi-layer perceptron) of different complexities, which are trained based on a subset of the dataset and cross-validated on another subset. This allowed us to identify which features of neuronal responses are captured by linear or nonlinear models while preventing overfitting issues. Based on these principles, we derive a scheme to extract nonlinear transformations from data, shedding light on the properties of different stages of the auditory pathway.

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MARKOV CHAIN MONTE CARLO AND DETAILED ELECTRICAL MODELS OF NEURONS: CORRELATIONS AND GENERALISATION

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Detailed electrical models of neurons consist of several ion channels in various compartments yielding models with as many as 35 parameters.
These parameters, such as ion channel conductances, have to be fitted to experimental data via a cost function, but robustness under external perturbations, an important property of biological cells, is rarely studied.
To address this problem, we go beyond methods based on optimisation and use Markov Chain Monte Carlo sampling of the parameters of the electrical models with probability proportional to the cost function.
We show that this approach provides detailed information about the properties of the parameter space, such as the shape of the high dimensional subspace of valid electrical models, or, equivalently, correlations between conductances.
For example, the maximum conductance of sodium in the axon initial segment and soma must not both be small or the threshold current will dramatically increase.
The shape of parameter space allows us to assess stability under perturbations such as morphological changes or current blockades.
We then propose algorithms for selecting a population of robust and low score models.
In addition, we show that by adjusting the size of axon initial segment and soma to match their respective input resistance with one of the dendrites, we further improve the generalisability and consistency of the models. We apply this procedure to various electrical types as means to produce a heterogeneous and robust population of single neuron models, which, in network simulations can provide further insight into how neuronal variability impacts circuit computations.

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SPATIAL AND TEMPORAL CONSTRAINS ON THE EXCITATORY EFFECT OF DEPOLARIZING GABAERGIC INPUTS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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The inhibitory neurotransmitter GABA (γ-amino butyric acid) mediates via ionotropic $GABA_A$ receptors hyperpolarizing or depolarizing membrane responses. While in the healthy adult brain hyperpolarizing responses dominate, depolarizing responses occur during development or after neuropathological insults. Because GABAergic actions depend on membrane potential changes, shunting inhibition and spatiotemporal filtering, the net effect of depolarizing GABAergic responses is hard to predict. To evaluate how the GABA reversal potential (E_{GABA}) influence the excitatory/inhibitory action of GABA, we performed a detailed in-silico study using simple neuronal topologies and distinct spatiotemporal relations between GABAergic and glutamatergic inputs. In these simulations we quantified E_{GABA} required for a net excitatory effect (E_{GABA}^{Thr}). For excitatory current injections E_{GABA}^{Thr} was close to action potential threshold (E_{AP}^{Thr}) for GABAergic synapses located at the soma, while with increasing dendritic distance E_{GABA}^{Thr} shifted to positive values. For excitatory synaptic inputs E_{GABA}^{Thr} was slightly negative to E_{AP}^{Thr} and was shifted to more negative values for dendritic AMPA synapses located proximally to the GABA input. For AMPA synapses located distally to the GABA synapse the dendritic distance had only a minor effect on E_{GABA}^{Thr}. In addition, E_{GABA}^{Thr} depended on the temporal relation between GABA and AMPA inputs, with E_{GABA}^{Thr} approximating resting membrane potential for delayed AMPA inputs. For tonic GABAergic conductances E_{GABA}^{Thr} was negative to E_{AP}^{Thr}. In summary, these results demonstrate that E_{GABA}^{Thr} is negative to E_{AP}^{Thr} for various physiologically relevant situations, suggesting that depolarizing GABAergic responses can mediate excitatory effects even if E_{GABA} does not reach E_{AP}^{Thr}.

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BOARD NUMBER: S05-538

INHIBITORY STABLIZATION IN A CORTICAL NEURAL MASS MODEL

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Strong inhibitory recurrent connections can reduce the tendency for a neural network to become unstable. This is known as inhibitory stabilization and networks that combine unstable excitatory recurrent connections with strong inhibitory feedback are known as Inhibition Stabilized Networks (ISNs). One of the characteristics of ISNs is their “paradoxical response”, which refers to the case where perturbing the inhibitory interneurons by excitatory input results in a decrease in the activity of these neurons after a temporal delay, instead of increasing their activity. Here, we develop a model of populations of neurons across different layers of cortex. Within each layer, there is one population of inhibitory and one population of excitatory neurons. To investigate the presence of ISNs across different layers, we recorded the membrane potentials of neural populations after perturbing inhibitory populations. The perturbation applied was a step increase in the firing rate of the excitatory input. The results shows that layer 2/3 in the model does not operate in the ISN regime as the membrane potential of inhibitory interneurons increases when perturbation is applied to this population. However, layers 4 and 5 operate in the ISN regime as the membrane potential of the inhibitory interneurons decreases after a transition period. These results accord with neurophysiological findings that explored the presence of ISNs across different layers in the cortex. The model may be used to provide insights into how parameter changes may lead to bifurcations resembling epileptic seizures and the relationship of paradoxical response and seizure onset in epileptic brains.

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THE ROLE OF REINFORCEMENT LEARNING IN THE CEREBELLUM BEYOND CLASSICAL THEORIES OF MOTOR CONTROL

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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One of the most influential models of the cerebellar cortex ascribes to its ‘crystalline’ structure the role of an associative learning device in the context of motor control. Albus and Marr, more than 50 years ago, proposed this perceptron-like theory of supervised learning to correct motor outcomes based on sensorimotor predictions. We extend this model, which is characterized by a divergent-convergent architecture and error-gated plasticity, to test new perspectives of its functional repertoire. Our interest revolves around its ability to perform reinforcement learning (RL). This objective is inspired by recent studies that unveiled teaching signals in the cerebellum resembling a reward prediction error used in RL models of basal ganglia, rather than the typical sensory errors used in many computational models. We apply the model to classical cerebellar functions, such as pattern classification or sensorimotor predictions, as well as reinforcement learning tasks. These results demonstrate the ability of the cerebellar architecture to implement a wider range of learning rules than previously appreciated, in particular, in relation to its role beyond motor control and towards cognitive processing.

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BOARD NUMBER: S05-540

NETWORK PATH CONVERGENCE SHAPES LOW-LEVEL PROCESSING IN THE VISUAL CORTEX

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Cognition emerges by counterstream feedforward and feedback interactions in the large-scale hierarchical network of the cerebral cortex. The counterstream, as a topological feature, is captured by the convergence and divergence of paths through directed links. So defined, the convergence degree (CD) reveals hierarchically relevant integrative properties of areas through their inward and outward connections. Although cortical dynamics are rooted in the anatomical network, the relationship of structure and function is far from clear. To understand how topology shapes large-scale cortical functioning, we studied the role of CD in network resilience and Granger causal coupling in a model of hierarchical network dynamics. Our results indicate that topological synchronizability is highly vulnerable to attacking edges based on CD, while global network efficiency depends mostly on edge betweenness. Furthermore, similar to anatomical hierarchy determined by the laminar distribution of connections, CD-based topological hierarchy showed high correlation with causal coupling in feedforward gamma and feedback alpha-beta band synchronizations in a subnetwork including low-level visual cortical areas. In contrast, causal coupling did not correlate with edge betweenness. Considering the entire network, the CD-based hierarchy correlated well with both the anatomical and functional hierarchy for low-level areas that are hierarchically far apart. Conversely, in a large part of the anatomical network where hierarchical distances are small, correlations were not significant. These findings indicate that at lower levels of cortical hierarchy interareal connectivity closely shapes large-scale oscillatory dynamics. However, at higher levels flexibility in hierarchical interactions is maintained to cope with varying demands.

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BOARD NUMBER: S05-541

MODEL-BASED INFERENCE OF CHANGES IN CORTICAL CIRCUIT PARAMETERS USING RECORDINGS OF NEURAL MASS ACTIVITY

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Alterations in cortical circuit parameters impact neural computation and cognition and have been implicated in brain disorders. Existing methods for interrogation of changes in neural circuit configuration are often based on recordings of neural mass signals, such as LFPs, ECoGs, MEGs and EEGs. However, microscopic neural parameters cannot be readily inferred from mass signals. Previous work has suggested that the aperiodic 1/f slope of the power spectrum of mass signals is a robust biomarker of excitation-inhibition (E/I) imbalance. Here we take a step forward from prior work by utilizing a multivariate inference method that accounts for joint changes in several relevant spectral features of mass signals (including aperiodic and periodic spectral components) to accurately predict microscopic neural circuit parameters from mass activity. Using a model of a recurrent cortical microcircuit of interacting excitatory and inhibitory neuronal populations, we systematically varied key neural model parameters to create a large dataset of spectral features that was used to train multivariate regression methods for inferring changes in E/I, mean firing rate of excitatory neurons and strength of the network input. Our model-based inference method was validated in-vivo with a combination of chemogenetic and optogenetic manipulations of E/I and firing rates with LFP and ECoG recordings from mice and ferrets, LFPs in mice at different stages of early development, and a combination of non-invasive pharmacological manipulations of E/I with MEG and EEG recordings in humans. Our results provide a method to estimate circuit parameters from spontaneous and stimulus-evoked activity recorded at different spatial scales.

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BOARD NUMBER: S05-542

EXTRACTING MOTOR COMMANDS FROM NATURAL BEHAVIOR: A CONTROL THEORY APPROACH

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Goal-directed behavior is a key feature of all biological systems. Whether searching for food or running away from a predator, an organism must choose a sequence of actions driven by a series of motor commands to achieve a desired goal. To study the principles shaping action selection, it is necessary to map the continuous space of motor commands forming the organism's behavioral repertoire. Here we record larval zebrafish during natural hunt and extract motor commands accounting for their behavior. Behavior is treated as the response of a dynamic system to an impulse function representing the command. The framework offers predictions of motor commands and properties of their neural representations, including command onset time and duration. Mapping the full space of motor commands will form the first milestone to study sequences of actions in natural behavior settings.

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BOARD NUMBER: S05-543

COMPRESSION-ENABLED INTERPRETABILITY OF VOXEL-WISE ENCODING MODELS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Voxel-wise encoding models based on convolutional neural networks (CNNs) have emerged as state-of-the-art predictive models of brain activity evoked by natural movies. Despite the superior predictive performance of CNN-based models, the huge number of parameters in these models have made them difficult to interpret for domain experts. Here, we investigate the role of model compression in building more interpretable and more stable CNN-based voxel-wise models. We used (1) structural compression techniques to prune less important CNN filters and connections, (2) a receptive field compression method to choose the model receptive fields with optimal center and size, and (3) principal component analysis to reduce the dimensionality of the model. The compressed models reveal increased category-selectivity along the ventral visual pathway with higher stability compared to uncompressed models. Furthermore, we demonstrate that the optimal model receptive fields become larger along the ventral visual pathway, as the receptive fields become more centralized.

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BOARD NUMBER: S05-544

A GRAPH-BASED MODEL OF THE EFFECT OF DEEP BRAIN STIMULATION ON CORTICO-SUBCORTICAL NETWORKS IN THE CONTEXT OF FREEZING OF GAIT IN PARKINSON'S DISEASE

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Currently available treatments of Parkinson's disease (PD) are restricted in their efficiency to alleviate freezing of gait (FoG). One potential therapeutical approach of FoG treatment, which is highlighted in the current literature, represents deep brain stimulation (DBS) of the subthalamic nucleus (STN) and the substantia nigra pars reticulata (SNr), however with clinically heterogeneous results. A computational model explaining the observed STN- or STN+SNr-DBS effects on cortical and brainstem excitability may unravel the complicated brain network interactions underlying the complex phenomenon of DBS. However, large-scale basal ganglia and brainstem models require frequently unavailable biological details or impractical computational capacity. Hence, to date, there is no model that is both computationally effective and accounts for the complex basal ganglia and brainstem circuitry. As an alternative approach, we propose a spontaneous-excitatory-refractory (SER) graph-based network model of the basal ganglia and brainstem. Here, the regions and connections involved in the current gait model and FoG are nodes and edges of the model graph. Every involved region undergoes a simplified, albeit biologically plausible SER dynamical cycle. We assess and compare all combinations of the emerging network dynamics in the healthy, parkinsonian, and DBS states. The network dynamics during the STN+SNr-DBS is markedly different from the standard STN-DBS dynamics. The pattern of the brainstem excitability is closer to the healthy one under the STN+SNr-DBS suggesting its positive effect on FoG. Thus, for the first time, a model accounts for the difference between two stimulation modes and suggests the network mechanism behind them.

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PARAMETER INFERENCE ON BRAIN NETWORK MODELS WITH UNKNOWN NODE DYNAMICS AND SPATIAL HETEROGENEITY

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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Model-based data analysis of whole-brain dynamics links the observed data to model parameters in a network of neural masses. In recent years a special focus was placed on the role of regional variance of model parameters for the emergent activity. Such analyses however depend on the properties of the employed neural mass model, which is often obtained through a series of major simplifications or analogies. Here we propose a data-driven approach where the neural mass model needs not to be specified. Building on the recent progresses in identification of dynamical systems with neural networks, we propose a method to infer from the functional data both the neural mass model representing the regional dynamics as well as the region- and subject-specific parameters, while respecting the known network structure. We demonstrate on two synthetic data sets that our method is able to recover the original model parameters, and that the trained generative model produces dynamics resembling the training data both on the regional level and on the whole-brain level. We further apply the method to resting-state fMRI data from Human Connectome Project. We find that to achieve best fit, the model needs two dimensional state space and three regional parameters, one of which is strongly correlated with the map of genetic expression in human brain. The present approach opens a novel way to the analysis of resting-state fMRI with possible applications in understanding the changes of whole-brain dynamics during aging or in neurodegenerative diseases.

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A MULTIVARIATE MODEL FOR THE ANALYSIS OF NEURAL SPIKE TRAINS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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The current state-of-the-art technology in collecting neurophysiological data allows for simultaneous recording from hundreds of neurons. A proper modelling approach for such data is the point process framework. However, the existing point process models for simultaneous neural spike trains are computationally infeasible. In this work, we introduce a multi-neuron version of our previous work on Skellam process with resetting (SPR). Unlike other models, the multivariate SPR is flexible in capturing the correlation structure among spike trains, it is computationally efficient, and is also biologically justified due to mimicking a neural integration process. Through both simulations and real-data analyses we highlight the strengths and weaknesses of this model.

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LEARNING HETERO-SYNAPTIC DELAYS OF SPIKING NEURONS FOR MOTION DETECTION

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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The response of a biological neuron depends on the precise timing of afferent spikes. This temporal aspect of the neuronal code is essential in understanding information processing in neurobiology and applies particularly well to the output of neuromorphic hardware such as event-based cameras. However, most artificial neuronal models do not take advantage of this minute temporal dimension. Inspired by this neuroscientific observation, we develop a model for the efficient detection of temporal spiking motifs based on a layer of neurons with hetero-synaptic delays. Indeed, the connectivity of the dendritic tree allows to discriminate between different temporal sequences, and we show that this can be formalized as a time-invariant logistic regression that can be trained using labeled data. We apply this model to solve the specific computer vision problem of motion detection and demonstrate its application to synthetic nature videos transformed into event streams similar to the output of event-based cameras. In particular, we quantify how its accuracy can vary with the total computational load. This end-to-end event-driven computational brick could help improve the performance of future spiking neural network (SNN) solutions currently used in neuromorphic chips.

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COMPUTATIONAL STUDY OF GAP JUNCTIONS IN A BIOPHYSICALLY DETAILED MODEL OF THE STRIATAL MICROCIRCUIT

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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We have previously developed a biophysically detailed striatal simulation platform [Hjorth et al, 2020] containing striatal projection neurons of D1 and D2 dopamine subtypes, fast spiking interneurons (FS), cholinergic interneurons and low threshold spiking interneurons, including dopamine modulation [Frost Nylen 2021]. The connectivity of the synapses in the microcircuit is based on touch detection using the software Snudda [Hjorth et al 2021]. Also gap junctions between the FS are placed within the dendritic overlaps. The connection probability and coupling coefficients between gap-junction coupled FS in the model match what has been previously reported in experiments [Koos and Tepper 1999]. Using this in silico platform it becomes possible to investigate both dynamical features and function of the striatal network. For instance, different oscillatory components are seen both in the healthy and diseased basal ganglia, and such oscillatory entrainments may have a causal role in shaping the signal propagation through the basal ganglia [Fischer 2021]. However, the mechanisms behind these oscillations and their functional relevance to the striatum is unclear. We here investigate, in particular, the role of gap junctions between FS neurons for the striatal signal propagation. Our results confirm that when gap junctions are placed using touch detection algorithms the FS subnetwork can detect a change in how synchronous the input is [Hjorth et al 2009]. We furthermore investigate the effect of the embedded gap-junction coupled FS network on the local striatal surrounding microcircuit and activity.

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THE OFT-FORGOTTEN GOLDMAN-HODGKIN-KATZ (GHK) CURRENT EQUATION PREDICTS STABLE SPIKE FIRING IN ULTRATHIN AXONS

POSTER SESSION 05 - SECTION: MODELING THE BRAIN

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In the 1940's and 50's, two constant electric field equations emerged that describe the relationships between voltage and current across a membrane. The more famous GHK *voltage* equation reconciles Nernst potentials of the various ion species with their partial permeabilities. The other, GHK *current* equation, describes the non-linear relationship between current and voltage for different concentration gradients. Hodgkin and Huxley used a simplified, linear model for their formalism, and this well fit the data from squid giant axon. However, when applied to the condition of rapid repetitive firing in ultra-thin axons, the linear HH equations (which have been used in most computational studies) predict that the marked decrease in Na gradient will have dire consequences for the fidelity of spike initiation and propagation. We now show that when the HH formalism is modified to use the GHK *current* equation, spikes are only minimally affected. Thus, whereas the linear model predicts that a 10-fold increase in [Na+]i leads to an 80% decrease in inward current at 0 mV and an equivalent slowing of the rate of rise of an action potential, the non-linear GHK *current* equation predicts only about a 20% change. The effect on propagation velocity is also minimal. These dramatic differences are significant because much neuropil includes axons with diameters smaller than .5 microns. In summary, action potential spans a voltage range that is largely resistant to changes in Na concentration gradient, which ensures stable neuronal interaction in highly active local networks.

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AUTOMATING CONFERENCE ABSTRACT ORGANIZATION AT SCALE WITH NATURAL LANGUAGE PROCESSING

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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HAIR-THIN MULTIMODE FIBER-BASED HOLOGRAPHIC ENDOSCOPE FOR DEEP-BRAIN IN-VIVO STRUCTURAL AND FUNCTIONAL IMAGING OF CELLULAR AND SUBCELLULAR STRUCTURES

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Petra Ondráčková[1], Miroslav Stibůrek[1], Tereza Tučková[1], Sergey Turtaev[2], Martin Šiler[1], Tomáš Pikálek[1], Petr Jákl[1], André Gomes[2], Jana Krejčí[3], Petra Kolbábková[1], Hana Uhlířová[1], Tomáš Čižmár[1,2]
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Exploiting holographic control of light transport through a multimode fiber turns such single hair-thin glass filament into endoscopic instrument performing at submicron resolution. Multimode fiber-based holographic endoscopes (MMFE) thus represent a revolutionary tool for deep brain *in-vivo* imaging. While current state-of-the–art *in-vivo* brain imaging methods struggle from the fundamental trade-off between resolution and penetration depth, MMFE effectively bypasses this technological trap, allowing high resolution imaging in any depth within the brain in the most atraumatic manner. Here we review the performance of a high-speed MMFE system optimised for fluorescent imaging of brain tissue within various living mice models. The instrument is equipped with 110 µm side-view multimode probe, refocusing property, multi-wavelength detection and three-dimensional random access options allowing detection of high speed events. We demonstrate various modes of its application through the observation of fluorescently labelled blood vessels, neurons, neuronal bodies, processes and even subcellular structures such as dendritic spines and lysosomes. Further, exploiting the random access option the instrument allows functional imaging of high speed events as demonstrated by imaging of calcium signaling and red blood cell velocity measurements in single vessels. In conclusion, the present study pushed the technological development forward creating a multimode fiber-based holographic endoscope as a perspective tool for *in-vivo* deep brain imaging.
Acknowledgement: The work was supported from European Regional Development Fund-Project "Holographic endoscopy for in vivo applications" (No. CZ.02.1.01/0.0/0.0/15_003/0000476), European Commission (101016787, CZ.1.05/ 2.1.00/ 01.0017); AS CR (RVO:68081731); ERC (724530); MEYS (CZ.02.1.01/ 0.0/ 0.0/ 15_003/0000476, LO1212).

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BOARD NUMBER: S05-552

PAMAM DENDRIMERS: A VERSATILE DRUG DELIVERY SYSTEM FOR BRAIN DISEASES

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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PAMAM dendrimers are 3-dimensional nanomolecules with multiple applications in biomedical sciences. These dendrimers can carry drugs and DNA/biomolecules to target systems, including those that necessitate the crossing of the blood brain barrier (BBB) to deliver its cargo to the brain. One of the advantages of PAMAM dendrimers, compared to the current viral-based delivery systems, is that these dendrimers can carry and deliver very large plasmids in addition to delivering drugs simultaneously (mixed-cargo). The **aim** of the current study was to analyze the drug delivery applications of our modified-surface PAMAM dendrimers (G4-90/10) in stroke and glioblastoma (GB) models in vitro and in vivo. **Methods:** (1) inject G4-90/10 dendrimers systemically in healthy mice via different routes to analyze their efficiency to cross the BBB; (2) characterize large dendrimer-plasmid complex and its delivery efficiency in healthy rats; (3) complex and deliver a therapeutic gene (hSOX2) in an ischemic stroke rat model; and (4) encapsulate curcumin (which has anti-cancer properties) and deliver this to models of human glioblastoma. **Results:** G4-90/10 dendrimers can: (1) cross the BBB following systemic injections in mice; (2) complex and carry large plasmids that were successfully delivered to the neurons and glial cells in vitro and in vivo in rats; (3) deliver the hSOX2 gene in stroke rats and reduce motor deficits; (4) l specifically target human GB cells for destruction and tended to improve the survivability of GB mice. **Conclusion:** PAMAM dendrimer is a promising versatile cargo delivery vehicle that has multiple applications to treat brain diseases, including GB.

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DEVELOPMENT AND VALIDATION OF ARC NANOBODIES: NEW TOOLS FOR PROBING ARC DYNAMICS AND FUNCTION

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Activity-regulated cytoskeleton-associated (Arc) protein plays key roles in long-term synaptic plasticity, memory, and cognitive flexibility. However, an integral understanding of Arc mechanisms is lacking. Arc is proposed to function as an interaction hub in neuronal dendrites and the nucleus, yet Arc can also form retrovirus-like capsids with proposed roles in intercellular communication. Here, we sought to develop anti-Arc nanobodies (ArcNbs) as new tools for probing Arc dynamics and function. Six ArcNbs representing different clonal lines were selected from immunized alpaca. Western blotting with recombinant ArcNbs fused to a small ALFA-epitope tag demonstrated binding to bacterially-expressed, purified Arc and endogenous Arc from rat cortical tissue. ALFA-ArcNb also provided efficient immunoprecipitation of stimulus-induced Arc after carbachol-treatment of SH-SY5Y neuroblastoma cells and induction of long-term potentiation (LTP) in the rat dentate gyrus in vivo. Epitope mapping showed that all Nbs recognize the Arc C-terminal region containing the retroviral Gag capsid homology domain, comprised of tandem N- and C-lobes. ArcNbs E5 and H11 selectively bound the N-lobe, which harbors a peptide ligand binding pocket specific to mammals. Four additional ArcNbs bound the region containing the C-lobe and terminal tail. For use as genetically-encoded fluorescent intrabodies, we show that ArcNbs fused to mScarlet-I are uniformly expressed, without aggregation, in the cytoplasm and nucleus of HEK293FT cells. Finally, mScarlet-ArcNb H11 expressed as intrabody selectively bound the N-lobe and enabled co-immunoprecipitation of full-length intracellular Arc. Arc nanobodies are versatile tools for live-cell labeling and purification of Arc and analysis of capsid domain specific functions.

FENS Forum 2022
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BOARD NUMBER: S05-554

NEUROANATOMICAL CHARACTERIZATION OF NEUROMEDIN U-EXPRESSING NEURONS IN THE NEWLY DEVELOPED NMU-CRE KNOCK-IN MOUSE MODEL

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Neuromedin U (NMU) is an evolutionary conserved neuropeptide that has been implicated in multiple physiological and pathophysiological processes, such as energy homeostasis, stress response and reward processing. Although central expression of NMU has been addressed previously, the lack of specific and sensitive research tools has prevented a comprehensive anatomical and functional characterization of NMU neurons in the brain. In the present study, we generated a knock-in mouse model constitutively expressing Cre recombinase under the *nmu* endogenous promoter. A multi-level approach using RT-qPCR, *in situ* hybridization, a cross with a reporter mouse line and an adenoviral vector driving Cre-dependent expression of a fluorescent protein successfully validated the mouse model. By using the newly developed Nmu-Cre knock-in mouse, we performed a complete mapping of NMU expression in both male and female adult mouse brain, unveiling a potential new brain circuit of NMU-expressing neurons, comprising areas involved in the modulation of the stress response, drug reward and appetitive motivational states. Taken together, our results strongly suggest that the newly developed Nmu-Cre knock-in mouse model largely and faithfully expresses Cre recombinase in NMU-expressing neurons in the adult mouse brain, without altering endogenous NMU expression. It is presented as a novel, powerful and sensitive tool to explore the role of NMU neurons in mice. Moreover, the neuroanatomical characterization supports the suggested role of NMU in the brain reward system.

Pubmed:

35205652: Garcia-Guasch M, Medrano M, Costa I, Vela E, Grau M, Escrich E, Moral R
Extra-Virgin Olive Oil and Its Minor Compounds Influence Apoptosis in Experimental Mammary Tumors and Human Breast Cancer Cell Lines.
Breast cancer is the most common malignancy among women worldwide. Modifiable factors such as nutrition have a role in its etiology. In experimental tumors, we have observed the differential influence of high-fat diets in metabolic pathways, suggesting a different balance in proliferation/apoptosis. In this work, we analyzed the effects of a diet high in n-6 polyunsaturated fatty acids (PUFA) and a diet high in extra-virgin olive oil (EVOO) on the histopathological features and different cell death pathways in the dimethylbenz(a)anthracene-induced breast cancer model. The diet high in n-6 PUFA had a stimulating effect on the morphological aggressiveness of tumors and their proliferation, while no significant differences were found in groups fed the EVOO-enriched diet in comparison to a low-fat control group. The high-EVOO diet induced modifications in proteins involved in several cell death pathways. In vitro analysis in different human breast cancer cell lines showed an effect of EVOO minor compounds (especially hydroxytyrosol), but not of fatty acids, decreasing viability while increasing apoptosis. The results suggest an effect of dietary lipids on tumor molecular contexts that result in the modulation of different pathways, highlighting the importance of apoptosis in the interplay of survival processes and how dietary habits may have an impact on breast cancer risk.
Cancers (Basel), 2022; 14
33559278: Casanovas M, Jiménez-Rosés M, Cordomí A, Lillo A, Vega-Quiroga I, Izquierdo J, Medrano M, Gysling K, Pardo L, Navarro G, Franco R
Discovery of a macromolecular complex mediating the hunger suppressive actions of cocaine: Structural and functional properties.
Cocaine not only increases brain dopamine levels but also activates the sigma receptor (σ R) that in turn regulates orexigenic receptor function. Identification of interactions involving dopamine D (D R), ghrelin (GHS-R), and σ receptors have been addressed by biophysical techniques and a complementation approach using interfering peptides. The effect of cocaine on

receptor functionality was assayed by measuring second messenger, cAMP and Ca , levels. The effect of acute or chronic cocaine administration on receptor complex expression was assayed by in situ proximity ligation assay. In silico procedures were used for molecular model building. σ R KO mice were used for confirming involvement of this receptor. Upon identification of protomer interaction and receptor functionality, a unique structural model for the macromolecular complex formed by σ R, D R, and GHS-R is proposed. The functionality of the complex, able to couple to both Gs and Gq proteins, is affected by cocaine binding to the σ R, as confirmed using samples from σ R mice. The expression of the macromolecular complex was differentially affected upon acute and chronic cocaine administration to rats. The constructed 3D model is consistent with biochemical, biophysical, and available structural data. The σ R, D R, and GHS-R complex constitutes a functional unit that is altered upon cocaine binding to the σ R. Remarkably, the heteromer can simultaneously couple to two G proteins, thus allowing dopamine to signal via Ca and ghrelin via cAMP. The anorexic action of cocaine is mediated by such complex whose expression is higher after acute than after chronic administration regimens.
Addict Biol, 2021; 26
31899262: De Prins A, Allaoui W, Medrano M, Van Eeckhaut A, Ballet S, Smolders I, De Bundel D
Effects of neuromedin U-8 on stress responsiveness and hypothalamus-pituitary-adrenal axis activity in male C57BL/6J mice.
Neuromedin U (NMU) is a highly conserved neuropeptide that has been implicated in the stress response. To better understand how it influences various aspects of the stress response, we studied the effects of intracerebroventricular NMU-8 administration on stress-related behavior and activity of the hypothalamus-pituitary-adrenal (HPA) axis in male C57BL/6J mice. We investigated these NMU-8 effects when mice remained in their home cage and when they were challenged by exposure to forced swim stress. NMU-8 administration resulted in increased grooming behavior in mice that remained in their home cage and in a significant increase in c-Fos immunoreactivity in the paraventricular hypothalamus (PVH) and arcuate nucleus (ARC). Surprisingly, NMU-8 administration significantly decreased plasma corticosterone concentrations. Furthermore, NMU-8 administration increased immobility in the forced swim test in both naïve mice and mice that were previously exposed to swim stress. The effect of NMU-8 on c-Fos immunoreactivity in the PVH was dependent on previous exposure to swim stress given that we observed no significant changes in mice exposed for the first time to swim stress. In contrast, in the ARC we observed a significant increase in c-Fos immunoreactivity regardless of previous stress exposure. Interestingly, NMU-8 administration also significantly decreased plasma corticosterone concentrations in mice that were exposed to single forced swim stress, while this effect was no longer observed when mice were exposed to forced swim stress for a second time. Taken together, our data indicate that NMU-8 regulates stress responsiveness and suggests that its effects depend on previous stress exposure.
Horm Behav, 2020; 121
30465812: Navarro G, Medrano M, Aguinaga D, Vega-Quiroga I, Lillo A, Jiménez J, Casanovas M, Canela EI, Mallol J, Gysling K, Franco R
Differential effect of amphetamine over the corticotropin-releasing factor CRF receptor, the orexin OX receptor and the CRF-OX heteroreceptor complex.
Stress is one of the factors underlying drug seeking behavior that often goes in parallel with loss of appetite. We here demonstrate that orexin 1 receptors (OXR) may form complexes with the corticotropin releasing factor CRF receptor. Two specific features of the heteromer were a cross-antagonism and a blockade by CRF of OXR signaling. In cells expressing one of the receptors, agonist-mediated signal transduction mechanisms were potentiated by amphetamine. Sigma 1 (σ) and 2 (σ) receptors are targets of drugs of abuse and, despite sharing a similar name, the two receptors are structurally unrelated and their physiological role is not known. We here show that σ receptors interact with CRF receptors and that σ receptors interact with OXR. Moreover, we show that amphetamine effect on CRF receptors was mediated by σR whereas the effect on OX receptors was mediated by σR. Amphetamine did potentiate the negative cross-talk occurring within the CRF-OX receptor heteromer context, likely by a macromolecular complex involving the two sigma receptors and the two GPCRs. Finally, in vivo microdialysis experiments showed that amphetamine potentiated orexin A-induced dopamine and glutamate release in the ventral tegmental area (VTA). Remarkably, the in vivo orexin A effects were blocked by a selective CRFR antagonist. These results show that amphetamine impacts on the OXR-, CRFR- and OXR/CRFR-mediated signaling and that cross-antagonism is instrumental for in vivo detection of GPCR heteromers. This article is part of the Special Issue entitled 'Receptor heteromers and their allosteric receptor-receptor interactions'.
Neuropharmacology, 2019; 152
29876881: Aguinaga D, Medrano M, Cordomí A, Jiménez-Rosés M, Angelats E, Casanovas M, Vega-Quiroga I, Canela EI, Petrovic M, Gysling K, Pardo L, Franco R, Navarro G
Cocaine Blocks Effects of Hunger Hormone, Ghrelin, Via Interaction with Neuronal Sigma-1 Receptors.
Despite ancient knowledge on cocaine appetite-suppressant action, the molecular basis of such fact remains unknown. Addiction/eating disorders (e.g., binge eating, anorexia, bulimia) share a central control involving reward circuits. However, we here show that the sigma-1 receptor (σR) mediates cocaine anorectic effects by interacting in neurons with

growth/hormone/secretagogue (ghrelin) receptors. Cocaine increases colocalization of σR and GHS-R1a at the cell surface. Moreover, in transfected HEK-293T and neuroblastoma SH-SY5Y cells, and in primary neuronal cultures, pretreatment with cocaine or a σR agonist inhibited ghrelin-mediated signaling, in a similar manner as the GHS-R1a antagonist YIL-781. Results were similar in G protein-dependent (cAMP accumulation and calcium release) and in partly dependent or independent (ERK1/2 phosphorylation and label-free) assays. We provide solid evidence for direct interaction between receptors and the functional consequences, as well as a reliable structural model of the macromolecular σR-GHS-R1a complex, which arises as a key piece in the puzzle of the events linking cocaine consumption and appetitive/consummatory behaviors.
Mol Neurobiol, 2019; 56
29483862: Aguinaga D, Medrano M, Vega-Quiroga I, Gysling K, Canela EI, Navarro G, Franco R
Cocaine Effects on Dopaminergic Transmission Depend on a Balance between Sigma-1 and Sigma-2 Receptor Expression.
Sigma σ and σ receptors are targets of cocaine. Despite sharing a similar name, the two receptors are structurally unrelated and their physiological role is unknown. Cocaine increases the level of dopamine, a key neurotransmitter in CNS motor control and reward areas. While the drug also affects dopaminergic signaling by allosteric modulations exerted by σR interacting with dopamine D and D receptors, the potential regulation of dopaminergic transmission by σR is also unknown. We here demonstrate that σR may form heteroreceptor complexes with D but not with D receptors. Remarkably σ, σ, and D receptors may form heterotrimers with particular signaling properties. Determination of cAMP levels, MAP kinase activation and label-free assays demonstrate allosteric interactions within the trimer. Importantly, the presence of σR induces bias in signal transduction as σR ligands increase cAMP signaling whereas reduce MAP kinase activation. These effects, which are opposite to those exerted via σR, suggest that the D receptor-mediated signaling depends on the degree of trimer formation and the differential balance of sigma receptor and heteroreceptor expression in acute versus chronic cocaine consumption. Although the physiological role is unknown, the heteroreceptor complex formed by σ, σ, and D receptors arise as relevant to convey the cocaine actions on motor control and reward circuits and as a key factor in acquisition of the addictive habit.
Front Mol Neurosci, 2018; 11
28717967: Medrano M, Aguinaga D, Reyes-Resina I, Canela EI, Mallol J, Navarro G, Franco R
Orexin A/Hypocretin Modulates Leptin Receptor-Mediated Signaling by Allosteric Modulations Mediated by the Ghrelin GHS-R1A Receptor in Hypothalamic Neurons.
The hypothalamus is a key integrator of nutrient-seeking signals in the form of hormones and metabolites originated in both the central nervous system and the periphery. The main autocrine and paracrine target of orexinergic-related hormones such as leptin, orexin/hypocretin, and ghrelin are neuropeptide Y neurons located in the arcuate nucleus of the hypothalamus. The aim of this study was to investigate the expression and the molecular and functional relationships between leptin, orexin/hypocretin and ghrelin receptors. Biophysical studies in a heterologous system showed physical interactions between them, with potential formation of heterotrimeric complexes. Functional assays showed robust allosteric interactions particularly different when the three receptors are expressed together. Further biochemical and pharmacological assays provided evidence of heterotrimer functional expression in primary cultures of hypothalamic neurons. These findings constitute evidence of close relationships in the action of the three hormones already starting at the receptor level in hypothalamic cells.
Mol Neurobiol, 2018; 55
28102227: Moreno E, Chiarlone A, Medrano M, Puigdellívol M, Bibic L, Howell LA, Resel E, Puente N, Casarejos MJ, Perucho J, Botta J, Suelves N, Ciruela F, Ginés S, Galve-Roperh I, Casadó V, Grandes P, Lutz B, Monory K, Canela EI, Lluís C, McCormick PJ, Guzmán M
Singular Location and Signaling Profile of Adenosine A-Cannabinoid CB Receptor Heteromers in the Dorsal Striatum.
The dorsal striatum is a key node for many neurobiological processes such as motor activity, cognitive functions, and affective processes. The proper functioning of striatal neurons relies critically on metabotropic receptors. Specifically, the main adenosine and endocannabinoid receptors present in the striatum, ie, adenosine A receptor (AR) and cannabinoid CB receptor (CBR), are of pivotal importance in the control of neuronal excitability. Facilitatory and inhibitory functional interactions between striatal AR and CBR have been reported, and evidence supports that this cross-talk may rely, at least in part, on the formation of AR-CBR heteromeric complexes. However, the specific location and properties of these heteromers have remained largely unknown. Here, by using techniques that allowed a precise visualization of the heteromers in situ in combination with sophisticated genetically modified animal models, together with biochemical and pharmacological approaches, we provide a high-resolution expression map and a detailed functional characterization of AR-CBR heteromers in the dorsal striatum. Specifically, our data unveil that the AR-CBR heteromer (i) is essentially absent from corticostriatal projections and striatonigral neurons, and, instead, is largely present in striatopallidal neurons, (ii) displays a striking G protein-coupled signaling profile, where co-stimulation of both receptors leads to strongly reduced downstream signaling, and (iii) undergoes an unprecedented dysfunction in Huntington's disease, an archetypal disease that affects striatal neurons.

Altogether, our findings may open a new conceptual framework to understand the role of coordinated adenosine-endocannabinoid signaling in the indirect striatal pathway, which may be relevant in motor function and neurodegenerative diseases.
Neuropsychopharmacology, 2018; 43
27129257: Navarro G, Aguinaga D, Angelats E, Medrano M, Moreno E, Mallol J, Cortés A, Canela EI, Casadó V, McCormick PJ, Lluís C, Ferré S
A Significant Role of the Truncated Ghrelin Receptor GHS-R1b in Ghrelin-induced Signaling in Neurons.
The truncated non-signaling ghrelin receptor growth hormone secretagogue R1b (GHS-R1b) has been suggested to simply exert a dominant negative role in the trafficking and signaling of the full and functional ghrelin receptor GHS-R1a. Here we reveal a more complex modulatory role of GHS-R1b. Differential co-expression of GHS-R1a and GHS-R1b, both in HEK-293T cells and in striatal and hippocampal neurons in culture, demonstrates that GHS-R1b acts as a dual modulator of GHS-R1a function: low relative GHS-R1b expression potentiates and high relative GHS-R1b expression inhibits GHS-R1a function by facilitating GHS-R1a trafficking to the plasma membrane and by exerting a negative allosteric effect on GHS-R1a signaling, respectively. We found a preferential Gi/o coupling of the GHS-R1a-GHS-R1b complex in HEK-293T cells and, unexpectedly, a preferential Gs/olf coupling in both striatal and hippocampal neurons in culture. A dopamine D1 receptor (D1R) antagonist blocked ghrelin-induced cAMP accumulation in striatal but not hippocampal neurons, indicating the involvement of D1R in the striatal GHS-R1a-Gs/olf coupling. Experiments in HEK-293T cells demonstrated that D1R co-expression promotes a switch in GHS-R1a-G protein coupling from Gi/o to Gs/olf, but only upon co-expression of GHS-R1b. Furthermore, resonance energy transfer experiments showed that D1R interacts with GHS-R1a, but only in the presence of GHS-R1b. Therefore, GHS-R1b not only determines the efficacy of ghrelin-induced GHS-R1a-mediated signaling but also determines the ability of GHS-R1a to form oligomeric complexes with other receptors, promoting profound qualitative changes in ghrelin-induced signaling.
J Biol Chem, 2016; 291
24942731: Moreno E, Andradas C, Medrano M, Caffarel MM, Pérez-Gómez E, Blasco-Benito S, Gómez-Cañas M, Pazos MR, Irving AJ, Lluís C, Canela EI, Fernández-Ruiz J, Guzmán M, McCormick PJ, Sánchez C
Targeting CB2-GPR55 receptor heteromers modulates cancer cell signaling.
The G protein-coupled receptors CB2 (CB2R) and GPR55 are overexpressed in cancer cells and human tumors. Because a modulation of GPR55 activity by cannabinoids has been suggested, we analyzed whether this receptor participates in cannabinoid effects on cancer cells. Here we show that CB2R and GPR55 form heteromers in cancer cells, that these structures possess unique signaling properties, and that modulation of these heteromers can modify the antitumoral activity of cannabinoids in vivo. These findings unveil the existence of previously unknown signaling platforms that help explain the complex behavior of cannabinoids and may constitute new targets for therapeutic intervention in oncology.
J Biol Chem, 2014; 289

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NANOBODIES AS ALLOSTERIC MODULATORS OF MGLU RECEPTOR IN THE TREATMENT OF SCHIZOPHRENIA

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Schizophrenia is a highly debilitating psychiatric disease with a prevalence of roughly 1%. Due to relatively ineffective treatment it currently has a very high societal cost.
In the current understanding of the physiopathology, the disease takes root in a combination of NMDA receptor hypofunction and general glutamatergic hyperstimulation. Current treatment does not address this perturbation and only alleviates symptoms.
Several propositions have been made for treatment that restore NMDA function, but the high side effects of direct glutamatergic stimulation cripple clinical trials and drug development. An alternative approach would be to target mGluR receptors, which are glutamate-responsive GPCR regulating neuronal excitability. These receptors are constituted by a family of 8 proteins that work as dimers. Amongst these, mGlu5 is particularly promising due to its positive action on the NMDA receptor. Unfortunately, the close genetic and structural proximity of mGluRs makes it challenging to develop specific drugs.
Nanobodies, the paratope of a subclass of Camelidae antibodies, are small, potent and very structure-specific peptides that are able to recognize and stabilize specific protein conformations. This property makes them uniquely potent as structural probes or allosteric modulators.
We hypothesized that nanobodies targeting mGluRs might act as modulators of the glutamate system and be viable therapeutic options. We developed nanobodies against different mGluR including mGlu5, and proceeded to improve their function through engineering. Here, we present several engineered nanobodies able to specifically influence mGluR5 activation, and are therefore promising candidates for treatment.

FENS Forum 2022
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BOARD NUMBER: S05-556

A QUICK, EASY AND COST-EFFICIENT METHOD FOR THE HIPPOCAMPAL SUBREGION SPECIFIC DIFFERENTIAL QUANTIFICATION OF 5MC AND 5HMC

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Aims: 5-methyl-cytosine (5mC) and 5-hydroxy-methyl-cytosine (5hmC) are epigenetic modifications that are dynamically regulated throughout neuronal development and function. The discovery of an active demethylation pathway created an increasing interest in these epigenetic marks also in the context of neuroscience. Our goal was to establish an easy, cost-efficient and rapid analysis pipeline allowing for the differential quantification of 5hmC and 5mC in different hippocampal subregions. **Methods:** We combined a hippocampal sub-dissection method using fine syringe needles to selectively isolate regions of interest from slide-mounted hippocampal tissue in a dark field microscope with a liquid-chromatography mass spectrometry (LC-MS) method capable of reliably identifying differential amounts of 5mC and 5hmC. We used mouse brain tissue from wildtype and NEIL3-deficient mice, with NEIL3 being a DNA repair enzyme potentially interacting with methylated DNA bases. We selectively dissected the hippocampal subregions CA1, CA3 and dentate gyrus, isolated nucleic acids (RNA and DNA), verified region specificity via an RNAseq-dataset based on the same samples, and subsequently quantified base modifications of interest via LC-MS. **Results:** Here we show that the differential quantification of DNA base modifications 5mC and 5hmC in subregion specific hippocampal tissue is possible within less than 24h and at reagent/material costs of ca. 5 EUR per sample for the dissection part. **Conclusion:** Our pipeline for the subregion specific quantitative analysis of DNA base modifications can help to add local network-relevance to neuro-epigenetic experiments currently relying on the analysis of e.g. whole-hippocampal tissue. The method appears transferrable to other brain regions or types of tissue.

Pubmed:
33444446: Roliński M, Montaldo NP, Aksu ME, Fordyce Martin SL, Brambilla A, Kunath N, Johansen J, Erlandsen SE, Liabbak NB, Rian K, Bjørås M, Sætrom P, van Loon B
Loss of Mediator complex subunit 13 (MED13) promotes resistance to alkylation through cyclin D1 upregulation.
Alkylating drugs are among the most often used chemotherapeutics. While cancer cells frequently develop resistance to alkylation treatments, detailed understanding of mechanisms that lead to the resistance is limited. Here, by using genome-wide CRISPR-Cas9 based screen, we identify transcriptional Mediator complex subunit 13 (MED13) as a novel modulator of alkylation response. The alkylation exposure causes significant MED13 downregulation, while complete loss of MED13 results in reduced apoptosis and resistance to alkylating agents. Transcriptome analysis identified cyclin D1 (CCND1) as one of the highly overexpressed genes in MED13 knock-out (KO) cells, characterized by shorter G1 phase. MED13 is able to bind to CCND1 regulatory elements thus influencing the expression. The resistance of MED13 KO cells is directly dependent on the cyclin D1 overexpression, and its down-regulation is sufficient to re-sensitize the cells to alkylating agents. We further demonstrate the therapeutic potential of MED13-mediated response, by applying combinatory treatment with CDK8/19 inhibitor Senexin A. Importantly, the treatment with Senexin A stabilizes MED13, and in combination with alkylating agents significantly reduces viability of cancer cells. In summary, our findings identify novel alkylation stress response mechanism dependent on MED13 and cyclin D1 that can serve as basis for development of innovative therapeutic strategies.
Nucleic Acids Res, 2021; 49
34857879: Hildrestrand GA, Rolseth V, Kunath N, Suganthan R, Jensen V, Bugaj AM, Fernandez-Berrocal MS, Sikko SB, Vetlesen S, Kuśnierczyk A, Olsen AK, Gützkow KB, Rowe AD, Wang W, Moldestad O, Syrstad MD, Slupphaug G, Eide L, Klungland A, Sætrom P, Luna L, Ye J, Scheffler K, Bjørås M
NEIL1 and NEIL2 DNA glycosylases modulate anxiety and learning in a cooperative manner in mice.

Oxidative DNA damage in the brain has been implicated in neurodegeneration and cognitive decline. DNA glycosylases initiate base excision repair (BER), the main pathway for oxidative DNA base lesion repair. NEIL1 and NEIL3 DNA glycosylases affect cognition in mice, while the role of NEIL2 remains unclear. Here, we investigate the impact of NEIL2 and its potential overlap with NEIL1 on behavior in knockout mouse models. Neil1Neil2 mice display hyperactivity, reduced anxiety and improved learning. Hippocampal oxidative DNA base lesion levels are comparable between genotypes and no mutator phenotype is found. Thus, impaired canonical repair is not likely to explain the altered behavior. Electrophysiology suggests reduced axonal activation in the hippocampal CA1 region in Neil1Neil2 mice and lack of NEIL1 and NEIL2 causes dysregulation of genes in CA1 relevant for synaptic function. We postulate a cooperative function of NEIL1 and NEIL2 in genome regulation, beyond canonical BER, modulating behavior in mice.
Commun Biol, 2021; 4
34988395: Kunath N, Bugaj AM, Bigonah P, Fernandez-Berrocal MS, Bjørås M, Ye J
DNA repair enzyme NEIL3 enables a stable neural representation of space by shaping transcription in hippocampal neurons.
DNA repair enzymes are essential for the maintenance of the neuronal genome and thereby proper brain functions. Emerging evidence links DNA repair to epigenetic gene regulation; however, its contribution to different transcriptional programs required for neuronal functions remains elusive. In this study, we identified a role of the DNA repair enzyme NEIL3 in modulating the maturation and function of hippocampal CA1 neurons by shaping the CA1 transcriptome during postnatal development and in association with spatial behavior. We observed a delayed maturation in CA1 and identified differentially regulated genes required for hippocampal development. We revealed impaired spatial stability in CA1 place cells and found spatial experience-induced gene expression essential for synaptic plasticity. This is the first study that links molecular underpinnings of DNA repair to the neural basis of spatial cognition beyond animals' behavioral phenotypes, thus shedding light on the molecular determinants enabling a stable neural representation of space.
iScience, 2021; 24
31784530: Montaldo NP, Bordin DL, Brambilla A, Rösinger M, Fordyce Martin SL, Bjørås KØ, Bradamante S, Aas PA, Furrer A, Olsen LC, Kunath N, Otterlei M, Sætrom P, Bjørås M, Samson LD, van Loon B
Alkyladenine DNA glycosylase associates with transcription elongation to coordinate DNA repair with gene expression.
Base excision repair (BER) initiated by alkyladenine DNA glycosylase (AAG) is essential for removal of aberrantly methylated DNA bases. Genome instability and accumulation of aberrant bases accompany multiple diseases, including cancer and neurological disorders. While BER is well studied on naked DNA, it remains unclear how BER efficiently operates on chromatin. Here, we show that AAG binds to chromatin and forms complex with RNA polymerase (pol) II. This occurs through direct interaction with Elongator and results in transcriptional co-regulation. Importantly, at co-regulated genes, aberrantly methylated bases accumulate towards the 3'end in regions enriched for BER enzymes AAG and APE1, Elongator and active RNA pol II. Active transcription and functional Elongator are further crucial to ensure efficient BER, by promoting AAG and APE1 chromatin recruitment. Our findings provide insights into genome stability maintenance in actively transcribing chromatin and reveal roles of aberrantly methylated bases in regulation of gene expression.
Nat Commun, 2019; 10
33159039: Rodriguez-Vargas JM, Martin-Hernandez K, Wang W, Kunath N, Suganthan R, Amé JC, Oliver FJ, Ye J, Bjørås M, Dantzer F
Parp3 promotes astrocytic differentiation through a tight regulation of Nox4-induced ROS and mTorc2 activation.
Parp3 is a member of the Poly(ADP-ribose) polymerase (Parp) family that has been characterized for its functions in strand break repair, chromosomal rearrangements, mitotic segregation and tumor aggressiveness. Yet its physiological implications remain unknown. Here we report a central function of Parp3 in the regulation of redox homeostasis in continuous neurogenesis in mice. We show that the absence of Parp3 provokes Nox4-induced oxidative stress and defective mTorc2 activation leading to inefficient differentiation of post-natal neural stem/progenitor cells to astrocytes. The accumulation of ROS contributes to the decreased activity of mTorc2 as a result of an oxidation-induced and Fbxw7-mediated ubiquitination and degradation of Rictor. In vivo, mTorc2 signaling is compromised in the striatum of naïve post-natal Parp3-deficient mice and 6 h after acute hypoxia-ischemia. These findings reveal a physiological function of Parp3 in the tight regulation of striatal oxidative stress and mTorc2 during astrocytic differentiation and in the acute phase of hypoxia-ischemia.
Cell Death Dis, 2020; 11
26090621: Kunath N, van Groen T, Allison DB, Kumar A, Dozier-Sharpe M, Kadish I
Ghrelin agonist does not foster insulin resistance but improves cognition in an Alzheimer's disease mouse model.
The orexigenic hormone ghrelin, a potential antagonist of the insulin system, ensures sufficient serum glucose in times of fasting. In the race for new therapeutics for diabetes, one focus of study has been antagonizing the ghrelin system in order to improve glucose tolerance. We provide evidence for a differential role of a ghrelin agonist on glucose homeostasis in an Alzheimer's disease mouse model fed a high-glycemic index diet as a constant challenge for glucose homeostasis. The ghrelin agonist impaired glucose tolerance immediately after administration but not in the long term. At the same time, the

ghrelin agonist improved spatial learning in the mice, raised their activity levels, and reduced their body weight and fat mass. Immunoassay results showed a beneficial impact of long-term treatment on insulin signaling pathways in hippocampal tissue. The present results suggest that ghrelin might improve cognition in Alzheimer's disease via a central nervous system mechanism involving insulin signaling.
Sci Rep, 2015; 5

25075439: Anneser J, Kunath N, Krautheim V, Borasio GD
Needs, expectations, and concerns of medical students regarding end-of-life issues before the introduction of a mandatory undergraduate palliative care curriculum.
In the past, implementation of effective palliative care curricula has emerged as a priority in medical education. In order to gain insight into medical students' needs and expectations, we conducted a survey before mandatory palliative care education was introduced in our faculty.
J Palliat Med, 2014; 17

27402596: Kunath N, Müller NCJ, Tonon M, Konrad BN, Pawlowski M, Kopczak A, Elbau I, Uhr M, Kühn S, Repantis D, Ohla K, Müller TD, Fernández G, Tschöp M, Czisch M, Steiger A, Dresler M
Ghrelin modulates encoding-related brain function without enhancing memory formation in humans.
Ghrelin regulates energy homeostasis in various species and enhances memory in rodent models. In humans, the role of ghrelin in cognitive processes has yet to be characterized. Here we show in a double-blind randomized crossover design that acute administration of ghrelin alters encoding-related brain activity, however does not enhance memory formation in humans. Twenty-one healthy young male participants had to memorize food- and non-food-related words presented on a background of a virtual navigational route while undergoing fMRI recordings. After acute ghrelin administration, we observed decreased post-encoding resting state fMRI connectivity between the caudate nucleus and the insula, amygdala, and orbitofrontal cortex. In addition, brain activity related to subsequent memory performance was modulated by ghrelin. On the next day, however, no differences were found in free word recall or cued location-word association recall between conditions; and ghrelin's effects on brain activity or functional connectivity were unrelated to memory performance. Further, ghrelin had no effect on a cognitive test battery comprising tests for working memory, fluid reasoning, creativity, mental speed, and attention. In conclusion, in contrast to studies with animal models, we did not find any evidence for the potential of ghrelin acting as a short-term cognitive enhancer in humans.
Neuroimage, 2016; 142

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BOARD NUMBER: S05-557

BACTRACE A RETROGRADE TOOL FOR MAPPING NEURONAL CONNECTIVITY IN FLIES

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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MRC Laboratory of Molecular Biology, Neurobiology, Cambridge, United Kingdom

The Drosophila brain contains 100,000 neurons with mostly stereotyped connections offering a rich array of well-characterised behaviours to study the basic principles of neural circuits. Extracting those principles relies on the availability of connectivity information. While generating electron microscopy-based connectomes have recently become feasible, they remain prohibitively expensive for many applications, especially those requiring information at the level of many individual flies, genetic backgrounds or live animals. For this reason, an efficient retrograde tracing method to reveal and manipulate inputs to any given neuron remains highly desirable. To address this need, we developed BAcTrace, a fully genetically encoded transsynaptic labelling system based on an engineered form of Clostridium botulinum neurotoxin A (BoNT/A). We re-engineered the toxin in such a way that it can be made in a neuron and transferred to connected partners where it triggers expression of an effector gene such as a fluorescent protein or a neuronal effector gene. We will present the validation of BAcTrace at three synapses along the fly olfactory system: 1. the connection between Olfactory Receptor Neurons and Projection Neurons (PNs), 2. the connection between Kenyon Cells of the mushroom body and PNs and 3. the weaker connections between PNs and Lateral Horn Neurons. We will also present improvements in the genetic implementation and functionality of the system. BAcTrace provides an easy, cheap and fast way to map inputs for any given neuron within living specimens, enabling assessment of changes in connectivity in multiple genetic backgrounds.

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NEW BRAINBOW TRANSGENES FOR SUBCELLULAR MULTICOLOR BARCODING BASED ON QUANTITATIVE MODELLING OF CRE RECOMBINATION IN LOX SITE ARRAYS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Alexandre Marcireau[1], Gabriel Kaddour[1], Mickaël Le[1], Gwenvael Le Dréau[1], Nicolas Auger[1], Samuel Tozer[2], Dragos Niculescu[1], Marion Lerat[1], Simona Esposito[1], Sio-Hoi Ieng[1], Ryad Benosman[1], Jean Livet[1]
[1]Sorbonne Université, Inserm, Institut De La Vision, Paris, France, [2]CNRS, INSERM, PSL Université Paris, Ibens, Ecole Normale Supérieure, Paris, France

Site-specific recombination systems such as Cre/lox are widely used to label cells or control gene expression in model organisms. In Brainbow transgenes, multiple pairs of *lox* sites create mutually exclusive, divergent recombination pathways that trigger the expression of spectrally distinct fluorescent proteins (FPs). These genetic labels are ideal to mark and discriminate neural cells or clones when mapping circuits or studying their development. Transgene configurations that maximize the number and equilibrium of these labels are useful for this purpose, but their design is difficult to optimize. Here, we present a mathematical model based on Markov chains that predicts the recombination outcomes from any given array of lox sites and estimates their respective frequencies. This model accounts for excision and inversion between several types of heterospecific lox sites and distinguishes stable vs. unstable recombination outcomes. We used it to select an improved transgene configuration termed Brainbow-4 that drives stochastic, near-equiprobable and irreversible expression of trichromatic FP markers with only two types of heterospecific lox sites. In addition, all FPs are combined with a shared subcellular tethering sequence, hence solving the difficulty of fusing Brainbow FPs to large proteins. We demonstrate the usefulness of this new configuration to target FPs to a variety of subcellular compartments and express an expanded palette of combinatorial labels from episomal or genome-integrated transgenes. These experiments lay the ground for dense multiplexed single cell and clonal analyses in the developing nervous system. Furthermore, the proposed model may be used to identify other useful genetic barcoding configurations.

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EFFECT OF APOE ε4 ALLELE ON REDOX SIGNATURE IN CIRCULATING EXTRACELLULAR VESICLES FROM COGNITIVELY IMPAIRED WITH NO DEMENTIA PARTICIPANTS CONVERTED TO ALZHEIMER'S DISEASE

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Mohamed Raâfet Ben Khedher[1], Mohamed Haddad[1], Danielle Laurin[2], Charles Ramassamy[1]
[1]Institut National de Recherche Scientifique — centre Armand-Frappier Santé Biotechnologie (INRS-AFSB), Neuropharmacologie Et Toxicologie Du Stress Cellulaire, Laval, Canada, [2]Laval University, Institute Of Nutrition And Functional Foods, Quebec, Canada

Aims: Cumulative clinical evidence supports the reliability of circulating extracellular vesicles (cEVs)-based biomarkers in Alzheimer's disease (AD) diagnosis. The substantial link between apolipoprotein E4 allele (*APOE* ε4) and oxidative stress may underlie enhanced AD risk. Here, we studied the impact of *APOE* ε4 on the level of apolipoproteins with antioxidant activities along with oxidative markers in circulating extracellular vesicles (cEVs) and plasma from cognitively impaired-not demented (CIND) individuals converted to AD (CIND-AD). **Methods:** Apolipoproteins E, J, and D and antioxidant response markers were determined in cEVs and plasma using electrochemical examination, spectrofluorimetry, and chemiluminescence immunoassay. **Results:** For the first time, our study evidences the presence of apoE, apoJ, and apoD in cEVs. The total antioxidant capacity and apoD levels in cEVs, as judged by ROC curves, regression analysis, and cognitive performance correlations, allowed to differentiate CIND *APOE* ε4 carriers from controls and to predict their progression to AD 5 years later. Our data revealed that the reduced antioxidant defense in *APOE* ε4 carriers may contribute to the early pathological cascade of neurodegeneration and that antioxidant supplementation might prevent AD development. **Conclusions:** Our findings support the pathological redox linkage between *APOE* ε4 and AD onset and suggest the use of cEVs oxidative signature in early AD diagnosis.

Pubmed:

34541286: Ben Khedher MR, Haddad M, Laurin D, Ramassamy C
Effect of ε4 allele on levels of apolipoproteins E, J, and D, and redox signature in circulating extracellular vesicles from cognitively impaired with no dementia participants converted to Alzheimer's disease.
The substantial link between apolipoprotein E () ε4 allele and oxidative stress may underlie enhanced Alzheimer's disease (AD) risk. Here, we studied the impact of ε4 on the level of apolipoproteins with antioxidant activities along with oxidative markers in circulating extracellular vesicles (cEVs) and plasma from cognitively impaired-not demented (CIND) individuals converted to AD (CIND-AD).
Alzheimers Dement (Amst), 2021; 13
33537405: Ben Khedher MR, Haddad M, Laurin D, Ramassamy C
Apolipoprotein E4-driven effects on inflammatory and neurotrophic factors in peripheral extracellular vesicles from cognitively impaired, no dementia participants who converted to Alzheimer's disease.
In brain, extracellular vesicles (EVs) play an essential role in the neuron-glia interface and ensure the crosstalk between the brain and the periphery. Some studies now link the pathway dysfunction of the EVs to apolipoprotein E gene variant (ε4) and the risk of progression to Alzheimer's disease (AD). To better understand the role of ε4 in pre-clinical AD, we have determined levels of pathogenic, neurotrophic and inflammatory proteins in peripheral EVs (pEVs) and in plasma from cognitively impaired, no dementia (CIND) participants stratified upon the absence (ε4) or the presence (ε4) of the ε4 allele of .
Alzheimers Dement (N Y), 2021; 7
33637005: Haddad M, Hervé V, Ben Khedher MR, Rabanel JM, Ramassamy C
Glutathione: An Old and Small Molecule with Great Functions and New Applications in the Brain and in Alzheimer's Disease.
Glutathione (GSH) represents the most abundant and the main antioxidant in the body with important functions in the brain related to Alzheimer's disease (AD). Oxidative stress is one of the central mechanisms in AD. We and others have demonstrated the alteration of GSH levels in the AD brain, its important role in the detoxification of advanced glycation end-products and of acrolein, a by-product of lipid peroxidation. Recent studies found a decrease of GSH in several areas of the brain from control, mild cognitive impairment, and AD subjects, which are correlated with cognitive decline. Several strategies

were developed to restore its intracellular level with the l-cysteine prodrugs or the oral administration of γ-glutamylcysteine to prevent alterations observed in AD. To date, no benefit on GSH level or on oxidative biomarkers has been reported in clinical trials. Thus, it remains uncertain if GSH could be considered a potential preventive or therapeutic approach or a biomarker for AD. We address how GSH-coupled nanocarriers represent a promising approach for the functionalization of nanocarriers to overcome the blood/brain barrier (BBB) for the brain delivery of GSH while avoiding cellular toxicity. It is also important to address the presence of GSH in exosomes for its potential intercellular transfer or its shuttle across the BBB under certain conditions. . 35, 270-292.
Antioxid Redox Signal, 2021; 35
34487040: Haddad M, Perrotte M, Ben Khedher MR, Madec E, Lepage A, Fülöp T, Ramassamy C
Levels of Receptor for Advanced Glycation End Products and Glyoxalase-1 in the Total Circulating Extracellular Vesicles from Mild Cognitive Impairment and Different Stages of Alzheimer's Disease Patients.
Growing evidence supports that receptor for advanced glycation end products (RAGE) and glyoxalase-1 (GLO-1) are implicated in the pathophysiology of Alzheimer's disease (AD). Extracellular vesicles (EVs) are nanovesicles secreted by almost all cell types, contribute to cellular communication, and are implicated in AD pathology. Recently, EVs are considered as promising tools to identify reliable biomarkers in AD.
J Alzheimers Dis, 2021; 84
32740712: Ben Khedher MR, Hafsa J, Haddad M, Hammami M
Inhibition of Protein Glycation by Combined Antioxidant and Antiglycation Constituents from a Phenolic Fraction of Sage (Salvia officinalis L.).
Disturbed advanced glycation end products (AGEs)-oxidative stress axis is strongly linked to vascular complications observed in diabetes and other metabolic conditions. Salvia officinalis L. (sage) is a medicinal plant used as an ingredient in foods and beverages and displays a wide range of biological and pharmacological activities including anti-diabetic effects. However, no study has assessed its anti-glycative potential. The aim of this study is to determine the phenolic compounds associated with the anti-glycation and antioxidant potential of sage methanol extract (SME). SME shows similar effects to aminoguanidine on fluorescent AGEs inhibition. It protects albumin damage from glycation (52.9 vs. 50.3%, respectively) by preventing the loss of protein thiol groups (50.0 vs. 44.3%, respectively) and by reducing protein carbonyl accumulation (67.4 vs. 70.5%, respectively). Moreover, linear regression and multivariate analysis support the efficient contribution of SME antioxidant capacity, as judged by DPPH, TBARS and iron chelating tests, in AGEs suppression. Furthermore, HPLC analysis revealed the presence of verbascoside as a novel phenolic constituent identified in sage leaves and suggests that the protective activity is mostly assigned to the presence of rosmarinic acid, resveratrol, quercetin, rutin and luteolin-7-O-glucoside. Likewise, the screening of SME phenolic content supports the contribution of various antioxidant substances to the observed effects. Therefore, a polyphenol enriched sage extract was able to inhibit the formation of AGEs and protein glycation. Our data unveils the promising properties of sage and its bioactive principles in the management of AGEs-mediated vascular complications observed in diabetes and other metabolic disorders.
Plant Foods Hum Nutr, 2020; 75
31623327: Haddad M, Perrotte M, Khedher MRB, Demongin C, Lepage A, Fülöp T, Ramassamy C
Methylglyoxal and Glyoxal as Potential Peripheral Markers for MCI Diagnosis and Their Effects on the Expression of Neurotrophic, Inflammatory and Neurodegenerative Factors in Neurons and in Neuronal Derived-Extracellular Vesicles.
Methylglyoxal (MG) and glyoxal (GO) are suggested to be associated with the development of neurodegenerative pathologies. However, their peripheral levels in relation to cognitive decline and their effects on key factors in neuronal cells are poorly investigated. The aim of this study was to determine their serum levels in MCI (mild cognitive impairment) and Alzheimer's disease (AD) patients, to analyze their effects on the neurotrophic and inflammatory factors, on neurodegenerative markers in neuronal cells and in neuronal derived-extracellular vesicles (nEVs). Our results show that MG and GO levels in serum, determined by HPLC, were higher in MCI. ROC (receiver-operating characteristic curves) analysis showed that the levels of MG in serum have higher sensitivity to differentiate MCI from controls but not from AD. Meanwhile, serum GO levels differentiate MCI from control and AD groups. Cells and nEVs levels of BDNF, PRGN, NSE, APP, MMP-9, ANGPTL-4, LCN2, PTX2, S100B, RAGE, Aβ peptide, pTau T181 and alpha-synuclein were quantified by luminex assay. Treatment of neuronal cells with MG or GO reduced the cellular levels of NSE, PRGN, APP, MMP-9 and ANGPTL-4 and the nEVs levels of BDNF, PRGN and LCN2. Our findings suggest that targeting MG and GO may be a promising therapeutic strategy to prevent or delay the progression of AD.
Int J Mol Sci, 2019; 20
30101547: Ben Khedher MR, Abid M, Jamoussi K, Hammami M
Comprehensive insight into functional interaction between GNB3 C825T and eNOS T-786C, G894T gene polymorphisms and association with susceptibility to diabetic erectile dysfunction.
No study has assessed the possible involvement of endothelial nitric oxide synthase (eNOS) T-786C and G894T and G-

protein β3 subunit (GNB3) C825T polymorphisms with susceptibility to diabetic vasculogenic erectile dysfunction (VED) in North African subjects.
Andrology, 2018; 6
29333341: Ben Khedher MR, Hammami M, Arch JRS, Hislop DC, Eze D, Wargent ET, Kępczyńska MA, Zaibi MS
Preventive effects of leaf extract on insulin resistance and inflammation in a model of high fat diet-induced obesity in mice that responds to rosiglitazone.
(sage) is a native plant to the Mediterranean region and has been used for a long time in traditional medicine for various diseases. We investigated possible anti-diabetic, anti-inflammatory and anti-obesity effects of sage methanol (MetOH) extract in a nutritional mouse model of obesity, inflammation and insulin resistance, as well as its effects on lipolysis and lipogenesis in 3T3-L1 cells.
PeerJ, 2018; 6
29233142: Ben Khedher MR, Bouhajja H, Haj Ahmed S, Abid M, Jamoussi K, Hammami M
Role of disturbed fatty acids metabolism in the pathophysiology of diabetic erectile dysfunction.
Vasculogenic erectile dysfunction (VED) is considered as a common complication among people with type 2 diabetes (T2D). We tested whether changes in fatty acid (FAs) classes measured in erythrocytes are associated with increased risk of diabetic VED along with related risk factors.
Lipids Health Dis, 2017; 16
28507464: Khedher MRB, Khedher SB, Chaieb I, Tounsi S, Hammami M
Chemical composition and biological activities of Salvia officinalis essential oil from Tunisia.
The aim of this study is to evaluate the chemical composition, antioxidant, antimicrobial, insecticidal and allelopathic activities of Tunisia essential oil (SoEO). The SoEO was characterized by the presence of 49 components with camphor (25.14 %), α-thujone (18.83 %), 1,8-cineole (14.14 %), viridiflorol (7.98 %), β-thujone (4.46 %) and β-caryophyllene (3.30 %) as the major components, determined by gas chromatography-mass spectrometry. The level of antioxidant activity, determined by complementary tests, namely 2,2-diphenyl-1-picrylhydrazyl radical-scavenging (IC= 6.7 mg/mL), linoleic acid peroxidation (IC= 9.6 mg/mL) and ferric reducing assays (IC= 28.4 mg/mL), was relatively moderate. The SoEO was also screened for its antimicrobial activity. Good to moderate inhibitions were recorded for most of tested microorganisms. It also exhibited important insecticidal activity against larvae and adults with LC values of 55.99 and 97.43 µl/L air, respectively. The effect of the SoEO on seeds germination and growth showed different activities against radical and hypocotyl elongation of the tested species. These results suggest the potential use of the SoEO as natural antimicrobial preservative in cosmetic, pharmaceutical industry and in pest management.
EXCLI J, 2017; 16

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BOARD NUMBER: S05-560

LIGHT AS AN INVERSE AGONIST INHIBITS CONSTITUTIVE G PROTEIN SIGNALLING OF THE ZEBRAFISH NON-VISUAL OPSIN OPN7B

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Raziye Karapinar
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Optogenetics combines the use of light-sensitive proteins and genetic targeting strategies to allow for precise light-controlled manipulation of cell function and signalling in living tissue. We discovered Opn7b, a non-visual G protein-coupled receptor (GPCR) expressed in zebrafish (*Danio rerio*), as a novel reverse optogenetic tool, which enables the constitutive activation of the $G_{i/o}$ coupled signalling pathway. Our photophysical characterisation shows that Opn7b expressed in HEK cells constitutively activates the $G_{i/o}$ coupled signalling pathway in darkness while illumination with blue/green light inactivates GIRK (G protein-coupled inwardly rectifying K^+) channels. Our patch-clamp studies indicate that light acts as an inverse agonist for Opn7b and can be used as an optogenetic tool to inhibit neuronal networks in the dark and interrupt constitutive inhibition during light stimulation. Consistent with this prediction, recordings in acute slices show that light activation of recombinant expressed Opn7b in cortical pyramidal cells increases neuronal activity. The constitutive activation of $G_{i/o}$ coupled signalling pathways leads to a homeostatic inhibition of neuronal networks, where light stimulation leads to a withdrawal from inhibition inducing generalised epileptiform activity. Indeed, in awake mice, light stimulation of Opn7b expressed in cortical pyramidal cells reliably induces generalised epileptiform activity within a short (<10 s) delay after onset of stimulation. Our study demonstrates a reversed mechanism for G protein-coupled receptor control and Opn7b as a tool for modulating neural circuit properties with the potential to study diseases related to constitutive activity of GPCRs.

Pubmed:

34301944: Karapinar R, Schwitalla JC, Eickelbeck D, Pakusch J, Mücher B, Grömmke M, Surdin T, Knöpfel T, Mark MD, Siveke I, Herlitze S
Reverse optogenetics of G protein signaling by zebrafish non-visual opsin Opn7b for synchronization of neuronal networks. Opn7b is a non-visual G protein-coupled receptor expressed in zebrafish. Here we find that Opn7b expressed in HEK cells constitutively activates the G pathway and illumination with blue/green light inactivates G protein-coupled inwardly rectifying potassium channels. This suggests that light acts as an inverse agonist for Opn7b and can be used as an optogenetic tool to inhibit neuronal networks in the dark and interrupt constitutive inhibition in the light. Consistent with this prediction, illumination of recombinant expressed Opn7b in cortical pyramidal cells results in increased neuronal activity. In awake mice, light stimulation of Opn7b expressed in pyramidal cells of somatosensory cortex reliably induces generalized epileptiform activity within a short (<10 s) delay after onset of stimulation. Our study demonstrates a reversed mechanism for G protein-coupled receptor control and Opn7b as a tool for controlling neural circuit properties.
Nat Commun, 2021; 12
30920724: Tennigkeit SA, Karapinar R, Rudack T, Dreier MA, Althoff P, Eickelbeck D, Surdin T, Grömmke M, Mark MD, Spoida K, Lübben M, Höweler U, Herlitze S, Gerwert K
Design of an Ultrafast G Protein Switch Based on a Mouse Melanopsin Variant.
The primary goal of optogenetics is the light-controlled noninvasive and specific manipulation of various cellular processes. Herein, we present a hybrid strategy for targeted protein engineering combining computational techniques with electrophysiological and UV/visible spectroscopic experiments. We validated our concept for channelrhodopsin-2 and applied it to modify the less-well-studied vertebrate opsin melanopsin. Melanopsin is a promising optogenetic tool that functions as a selective molecular light switch for G protein-coupled receptor pathways. Thus, we constructed a model of the melanopsin G protein complex and predicted an absorption maximum shift of the Y211F variant. This variant displays a narrow blue-shifted action spectrum and twofold faster deactivation kinetics compared to wild-type melanopsin on G protein-coupled inward rectifying K (GIRK) channels in HEK293 cells. Furthermore, we verified the in vivo activity and optogenetic potential for the variant in mice. Thus, we propose that our developed concept will be generally applicable to designing optogenetic tools.
Chembiochem, 2019; 20
31468691: Eickelbeck D, Rudack T, Tennigkeit SA, Surdin T, Karapinar R, Schwitalla JC, Mücher B, Shulman M, Scherlo M,

Althoff P, Mark MD, Gerwert K, Herlitze S
Lamprey Parapinopsin ("UVLamP"): a Bistable UV-Sensitive Optogenetic Switch for Ultrafast Control of GPCR Pathways.
Optogenetics uses light-sensitive proteins, so-called optogenetic tools, for highly precise spatiotemporal control of cellular states and signals. The major limitations of such tools include the overlap of excitation spectra, phototoxicity, and lack of sensitivity. The protein characterized in this study, the Japanese lamprey parapinopsin, which we named UVLamP, is a promising optogenetic tool to overcome these limitations. Using a hybrid strategy combining molecular, cellular, electrophysiological, and computational methods we elucidated a structural model of the dark state and probed the optogenetic potential of UVLamP. Interestingly, it is the first described bistable vertebrate opsin that has a charged amino acid interacting with the Schiff base in the dark state, that has no relevance for its photoreaction. UVLamP is a bistable UV-sensitive opsin that allows for precise and sustained optogenetic control of G protein-coupled receptor (GPCR) pathways and can be switched on, but more importantly also off within milliseconds via lowintensity short light pulses. UVLamP exhibits an extremely narrow excitation spectrum in the UV range allowing for sustained activation of the G pathway with a millisecond UV light pulse. Its sustained pathway activation can be switched off, surprisingly also with a millisecond blue light pulse, minimizing phototoxicity. Thus, UVLamP serves as a minimally invasive, narrow-bandwidth probe for controlling the G pathway, allowing for combinatorial use with multiple optogenetic tools or sensors. Because UVLamP activated G signals are generally inhibitory and decrease cellular activity, it has tremendous potential for health-related applications such as relieving pain, blocking seizures, and delaying neurodegeneration.
Chembiochem, 2020; 21
30793039: Eickelbeck D, Karapinar R, Jack A, Suess ST, Barzan R, Azimi Z, Surdin T, Grömmke M, Mark MD, Gerwert K, Jancke D, Wahle P, Spoida K, Herlitze S
CaMello-XR enables visualization and optogenetic control of G signals and receptor trafficking in GPCR-specific domains.
The signal specificity of G protein-coupled receptors (GPCRs) including serotonin receptors (5-HT-R) depends on the trafficking and localization of the GPCR within its subcellular signaling domain. Visualizing traffic-dependent GPCR signals in neurons is difficult, but important to understand the contribution of GPCRs to synaptic plasticity. We engineered CaMello (Ca-melanopsin-local-sensor) and CaMello-5HT for visualization of traffic-dependent Ca signals in 5-HT-R domains. These constructs consist of the light-activated G coupled melanopsin, mCherry and GCaMP6m for visualization of Ca signals and receptor trafficking, and the 5-HT C-terminus for targeting into 5-HT-R domains. We show that the specific localization of the GPCR to its receptor domain drastically alters the dynamics and localization of the intracellular Ca signals in different neuronal populations in vitro and in vivo. The CaMello method may be extended to every GPCR coupling to the G pathway to help unravel new receptor-specific functions in respect to synaptic plasticity and GPCR localization.
Commun Biol, 2019; 2
27068418: Spoida K, Eickelbeck D, Karapinar R, Eckhardt T, Mark MD, Jancke D, Ehinger BV, König P, Dalkara D, Herlitze S, Masseck OA
Melanopsin Variants as Intrinsic Optogenetic On and Off Switches for Transient versus Sustained Activation of G Protein Pathways.
G-protein-coupled receptors (GPCRs) represent the major protein family for cellular modulation in mammals. Therefore, various strategies have been developed to analyze the function of GPCRs involving pharmaco- and optogenetic approaches [1, 2]. However, a tool that combines precise control of the activation and deactivation of GPCR pathways and/or neuronal firing with limited phototoxicity is still missing. We compared the biophysical properties and optogenetic application of a human and a mouse melanopsin variant (hOpn4L and mOpn4L) on the control of Gi/o and Gq pathways in heterologous expression systems and mouse brain. We found that GPCR pathways can be switched on/off by blue/yellow light. The proteins differ in their kinetics and wavelength dependence to activate and deactivate G protein pathways. Whereas mOpn4L is maximally activated by very short light pulses, leading to sustained G protein activation, G protein responses of hOpn4L need longer light pulses to be activated and decline in amplitude. Based on the different biophysical properties, brief light activation of mOpn4L is sufficient to induce sustained neuronal firing in cerebellar Purkinje cells (PC), whereas brief light activation of hOpn4L induces AP firing, which declines in frequency over time. Most importantly, mOpn4L-induced sustained firing can be switched off by yellow light. Based on the biophysical properties, hOpn4L and mOpn4L represent the first GPCR optogenetic tools, which can be used to switch GPCR pathways/neuronal firing on an off with temporal precision and limited phototoxicity. We suggest to name these tools moMo and huMo for future optogenetic applications.
Curr Biol, 2016; 26

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GOLD AND SILVER NANOPARTICLE-BASED LOCALIZED SURFACE PLASMON RESONANCE SENSOR (LSPR) FOR THE DETECTION OF HISTIDINE AS A POTENTIAL BIOMARKER IN PARKINSON'S DISEASE.

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Objective: Previous literature has proven that histamine has a crucial role in the process of neuroinflammation in Parkinson's Disease (PD) . We can indirectly detect histamine levels in fluid samples by quantifying levels of histidine. Therefore, we developed a gold and silver nanoparticle-based LSPR sensor for the detection of histidine. **Methods:** Au/Ag and Au, Ag nanoparticles were synthesized. Histidine was prepared at different concentrations: 10 mM, 25mM, and 100 mM. UV-Vis spectroscopy was used to determine the absorbance spectrums of the nanoparticles with histidine. Additionally, a spearman analysis was done for the absorbance values measured from 190 nm to 360 nm in all three concentrations in order to evaluate the significance of the study and to observe correlations between the variables. **Results:** In all concentrations a higher absorbance was obtained with the metallic systems. At 10 mM both Au and Ag generated a better signal than histidine alone, at a wavelength of 217 nm with an absorbance of 0.76 at 25 mM, Au emitted the highest signal at a wavelength of 221 with an absorbance of 0.39 and at 100 mM the system Au/Ag generated a better signal at a wavelength of 209.0 at an absorbance of 0.89. Additionally, the conducted spearman analysis indicated $p < 0.001$ in all concentrations. **Conclusions:** We successfully developed an LSPR sensor that improved the detection of histidine. Our findings could help to easily identify abnormal levels of relevant biomarkers in PD and contribute to early detection of this disease.

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A FLEXIBLE AND VERSATILE SYSTEM FOR MULTICOLOR FIBER PHOTOMETRY AND OPTOGENETIC MANIPULATION

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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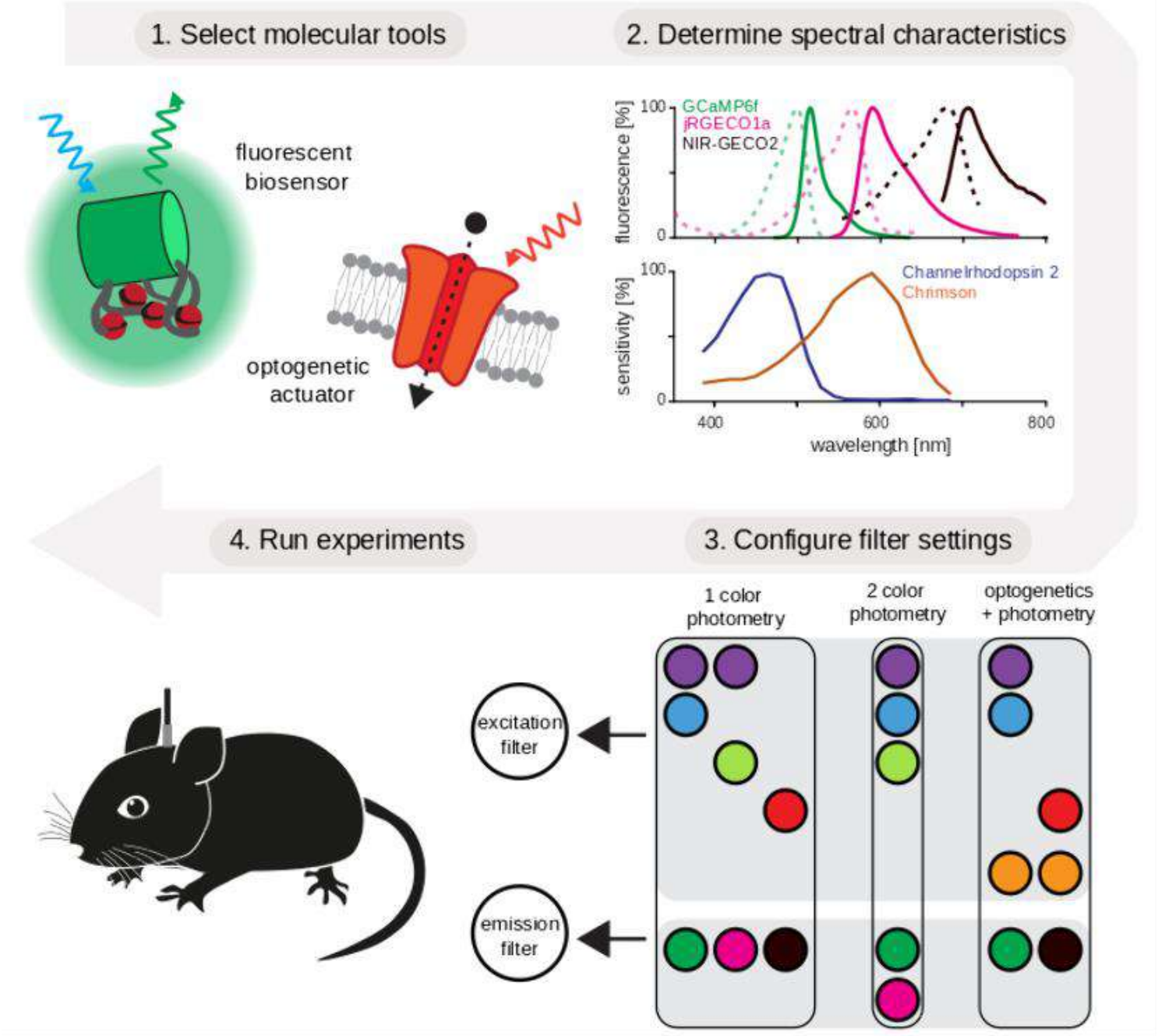
Fiber photometry is a technique of growing popularity in neuroscience research. It is widely used to infer brain activity by recording calcium dynamics in genetically defined populations of neurons. Aside from the wide variety of calcium indicators, other genetically encoded biosensors have recently been engineered to measure membrane potential, neurotransmitter release, pH, or various cellular metabolites, such as ATP or cAMP. Due to the spectral characteristics of these tools, different assemblies of optical hardware are usually needed to reveal the full potential of different biosensors. In addition, combining multiple sensors in one experiment, often requires the investment in more complex equipment, which limits the flexibility of the experimental design. Such constraints often hamper a straightforward implementation of new molecular tools, evaluation of their performance in vivo, and construction of new experimental paradigms – especially if the financial budget is a limiting factor. Here, we propose a novel approach for fiber photometry recordings and optogenetic manipulation. In combination with a multi-color light source and appropriate emission filters, our approach offers remarkable flexibility in experimental design and facilitates the implication of new molecular tools *in vivo* at minimal cost. The ease of assembly, operation, characterization, and customization of this platform holds the potential to foster the development of experimental strategies for multicolor fiber photometry combined with optogenetics far beyond its current

state.



Pubmed:
33793545: Yang W, Chini M, Pöpplau JA, Formozov A, Dieter A, Piechocinski P, Rais C, Morellini F, Sporns O, Hanganu-Opatz IL, Wiegert JS
Anesthetics fragment hippocampal network activity, alter spine dynamics, and affect memory consolidation.
General anesthesia is characterized by reversible loss of consciousness accompanied by transient amnesia. Yet, long-term memory impairment is an undesirable side effect. How different types of general anesthetics (GAs) affect the hippocampus, a brain region central to memory formation and consolidation, is poorly understood. Using extracellular recordings, chronic 2-photon imaging, and behavioral analysis, we monitor the effects of isoflurane (Iso), medetomidine/midazolam/fentanyl (MMF),

and ketamine/xylazine (Keta/Xyl) on network activity and structural spine dynamics in the hippocampal CA1 area of adult mice. GAs robustly reduced spiking activity, decorrelated cellular ensembles, albeit with distinct activity signatures, and altered spine dynamics. CA1 network activity under all 3 anesthetics was different to natural sleep. Iso anesthesia most closely resembled unperturbed activity during wakefulness and sleep, and network alterations recovered more readily than with Keta/Xyl and MMF. Correspondingly, memory consolidation was impaired after exposure to Keta/Xyl and MMF, but not Iso. Thus, different anesthetics distinctly alter hippocampal network dynamics, synaptic connectivity, and memory consolidation, with implications for GA strategy appraisal in animal research and clinical settings.
PLoS Biol, 2021; 19
34798050: Imambocus BN, Zhou F, Formozov A, Wittich A, Tenedini FM, Hu C, Sauter K, Macarenhas Varela E, Herédia F, Casimiro AP, Macedo A, Schlegel P, Yang CH, Miguel-Aliaga I, Wiegert JS, Pankratz MJ, Gontijo AM, Cardona A, Soba P
A neuropeptidergic circuit gates selective escape behavior of Drosophila larvae.
Animals display selective escape behaviors when faced with environmental threats. Selection of the appropriate response by the underlying neuronal network is key to maximizing chances of survival, yet the underlying network mechanisms are so far not fully understood. Using synapse-level reconstruction of the Drosophila larval network paired with physiological and behavioral readouts, we uncovered a circuit that gates selective escape behavior for noxious light through acute and input-specific neuropeptide action. Sensory neurons required for avoidance of noxious light and escape in response to harsh touch, each converge on discrete domains of neuromodulatory hub neurons. We show that acute release of hub neuron-derived insulin-like peptide 7 (Ilp7) and cognate relaxin family receptor (Lgr4) signaling in downstream neurons are required for noxious light avoidance, but not harsh touch responses. Our work highlights a role for compartmentalized circuit organization and neuropeptide release from regulatory hubs, acting as central circuit elements gating escape responses.
Curr Biol, 2022; 32

FENS Forum 2022
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MULTI-MODAL IMAGING OF CORPORA AMYLACEA IN POST MORTEM HUMAN MIDBRAIN

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Aims Corpora amylacea, which contains polysaccharides, accumulates in human brain throughout aging. We aimed to use multi-modal imaging to locate corpora amylacea and investigate their three-dimensional morphological features. Methods Post mortem human brain stem samples (N=4) were scanned using high field MRI (14.1T, T2*weighted 62µm voxels) and X-ray synchrotron-radiation-based phase-contrast microtomography (PhC-µCT, 1µm voxels). Using an automated segmentation pipeline, corpora amylacea were identified on PhC-µCT images and the diameters was calculated. Finally, the samples were sectioned and stained using Klüver-Barrera and Periodic-acid Schiff reaction. Results In both MRI and PhC-µCT, corpora amylacea rich regions showed signal contrast. Using automated segmentation of the PhC-µCT dataset, we detected over 40,000 corpora amylacea located at the intercollicular region that showed hyperintensity. In T2* weighted MRI, corpora amylacea rich regions had low signal due to T2* shortening. The median diameter of corpora amylacea was 12 µm. Sections stained with Periodic-acid Schiff reaction confirmed that these granular bodies are indeed corpora amylacea. Conclusions Corpora amylacea rich region shows noticeable contrast in T2* weighted MRI. In high resolution phase-contrast microtomography, individual corpora amylacea can be
extracted.

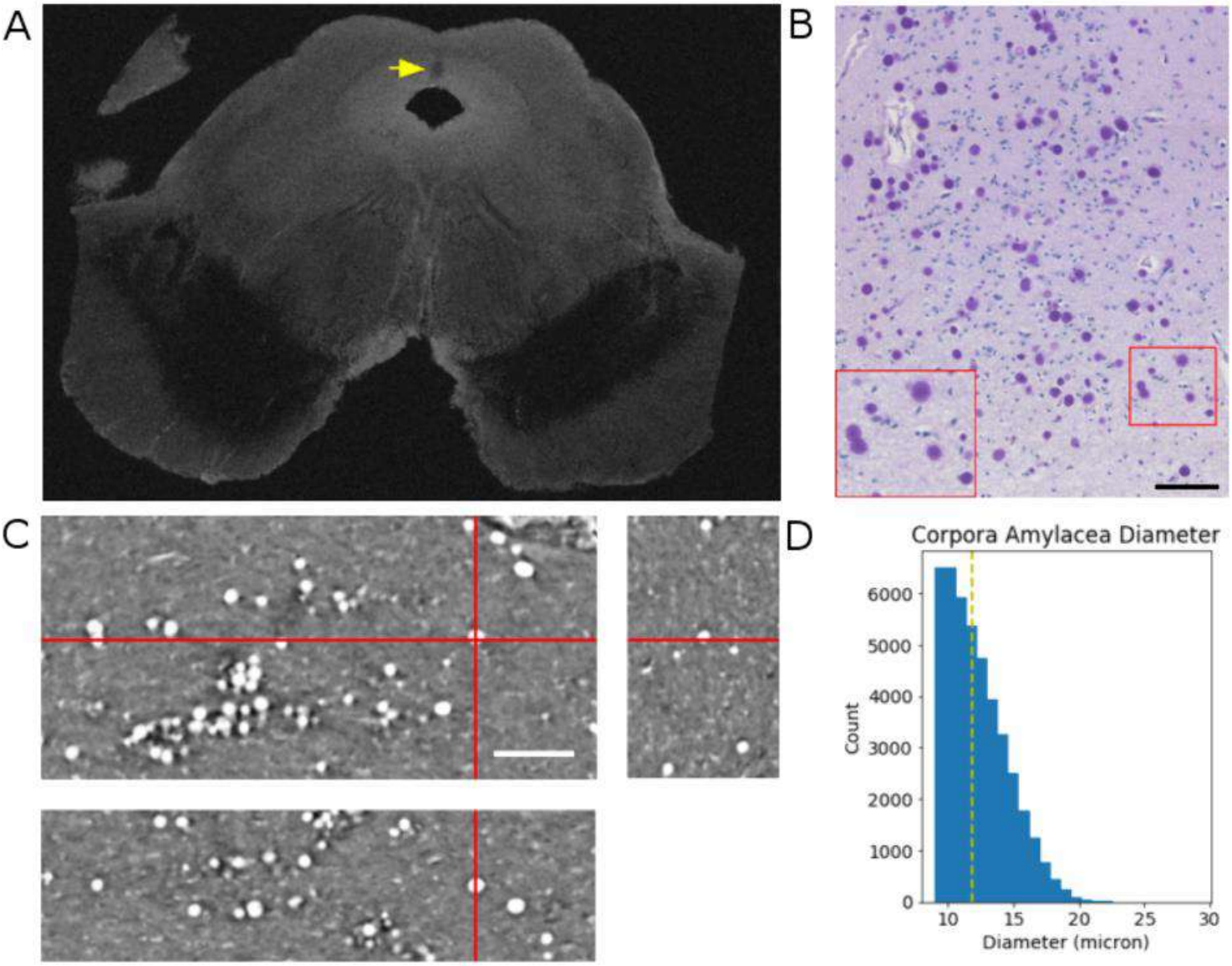

Figure. Multi-modal imaging of corpora amylacea in human midbrain. **A**. T2* weighted MRI image of the axial plane of the midbrain. The arrow points to the corpora amylacea rich region with shortened T2*. **B**. Microscope image of the Periodic-acid Schiff stained section. **C**. Phase-contrast microtomography of corpora amylacea shown with orthogonal planes. **D**. Distribution of corpora amylace diameter. The dashed bar indicates the median value. Scale bars = 100 μm.

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MICROFLUIDIC CHIPS IN A COMPARATIVE STUDY OF MECHANOSENSITIVE PIEZO1 RECEPTORS IN TRIGEMINAL VERSUS DORSAL ROOT GANGLIA NEURONS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Nikita Mikhailov[1], Lidiia Plotnikova[1], Prateek Singh[2], Rashid Giniatullin[1], Riikka Hämäläinen[1]
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Mechanosensitive Piezo1 receptors involved in sensing of pain and pressure were recently suggested as contributors to migraine pain generation. Indeed, migraine is characterized by pulsatile pain and mechanical hyperalgesia associated with activation of trigeminal ganglia (TG) afferents. Interestingly, anti-migraine medicines are ineffective in other types of pain, suggesting some exclusive nociceptive mechanisms underlying migraine generation. To address potential specific role of Piezo1 receptors in migraine pain, we compared activity and expression of Piezo1 receptors in TG neurons vs. neurons of dorsal root ganglia (DRG) which processes innervate the body. We compared results obtained on a conventional imaging setup equipped with multibarrel application system with results obtained on a microfluidic chip-based setup. Surprisingly, despite the higher expression of Piezo1 gene, TG neurons were less responsive to specific stimulation of Piezo1 receptors than DRG neurons suggesting basic low translation or weak traffic to membrane. Additionally, although both conventional and chip-based setups demonstrated similar difference between TG and DRG neurons, this difference was more prominent on the chip-based setup. This demonstrated that some limitations of the conventional approach, e.g. fluid turbulence, may be overcome by microfluidic devices providing laminar solution flow. This research was published in an Open Access International Journal of Molecular Sciences: Mikhailov, N.; Plotnikova, L.; Singh, P.; Giniatullin, R.; Hämäläinen, R.H. Functional Characterization of Mechanosensitive Piezo1 Channels in Trigeminal and Somatic Nerves in a Neuron-on-Chip Model. Int. J. Mol. Sci. 2022, 23, 1370. https://doi.org/10.3390/ijms23031370 NM and RG were supported by H2020 MSCA RISE grant #778405

Pubmed:

35163293: Mikhailov N, Plotnikova L, Singh P, Giniatullin R, Hämäläinen RH
Functional Characterization of Mechanosensitive Piezo1 Channels in Trigeminal and Somatic Nerves in a Neuron-on-Chip Model.
Mechanosensitive ion channels, Piezo1 and 2, are activated by pressure and involved in diverse physiological functions, including senses of touch and pain, proprioception and many more. Understanding their function is important for elucidating the mechanosensitive mechanisms of a range of human diseases. Recently, Piezo channels were suggested to be contributors to migraine pain generation. Migraine is typically characterized by allodynia and mechanical hyperalgesia associated with the activation and sensitization of trigeminal ganglion (TG) nerve fibers. Notably, migraine specific medicines are ineffective for other types of pain, suggesting a distinct underlying mechanism. To address, in a straightforward manner, the specificity of the mechanosensitivity of trigeminal vs. somatic nerves, we compared the activity of Piezo1 channels in mouse TG neurons vs. dorsal root ganglia (DRG) neurons. We assessed the functional expression of Piezo1 receptors using a conventional live calcium imaging setup equipped with a multibarrel application system and utilizing a microfluidic chip-based setup. Surprisingly, the TG neurons, despite higher expression of the gene, were less responsive to Piezo1 agonist Yoda1 than the DRG neurons. This difference was more prominent in the chip-based setup, suggesting that certain limitations of the conventional approach, such as turbulence, can be overcome by utilizing microfluidic devices with laminar solution flow.
Int J Mol Sci, 2022; 23
32487172: Chew S, Lampinen R, Saveleva L, Korhonen P, Mikhailov N, Grubman A, Polo JM, Wilson T, Komppula M, Rönkkö T, Gu C, Mackay-Sim A, Malm T, White AR, Jalava P, Kanninen KM
Urban air particulate matter induces mitochondrial dysfunction in human olfactory mucosal cells.
The adverse effects of air pollutants including particulate matter (PM) on the central nervous system is increasingly reported by epidemiological, animal and post-mortem studies in the last decade. Oxidative stress and inflammation are key consequences of exposure to PM although little is known of the exact mechanism. The association of PM exposure with

deteriorating brain health is speculated to be driven by PM entry via the olfactory system. How air pollutants affect this key entry site remains elusive. In this study, we investigated effects of urban size-segregated PM on a novel cellular model: primary human olfactory mucosal (hOM) cells.
Part Fibre Toxicol, 2020; 17
32508598: Suleimanova A, Talanov M, Gafurov O, Gafarov F, Koroleva K, Virenque A, Noe FM, Mikhailov N, Nistri A, Giniatullin R
Modeling a Nociceptive Neuro-Immune Synapse Activated by ATP and 5-HT in Meninges: Novel Clues on Transduction of Chemical Signals Into Persistent or Rhythmic Neuronal Firing.
Extracellular ATP and serotonin (5-HT) are powerful triggers of nociceptive firing in the meninges, a process supporting headache and whose cellular mechanisms are incompletely understood. The current study aimed to develop, with the neurosimulator NEURON, a novel approach to explore in silico the molecular determinants of the long-lasting, pulsatile nature of migraine attacks. The present model included ATP and 5-HT release, ATP diffusion and hydrolysis, 5-HT uptake, differential activation of ATP P2X or 5-HT3 receptors, and receptor subtype-specific desensitization. The model also tested the role of branched meningeal fibers with multiple release sites. Spike generation and propagation were simulated using variable contribution by potassium and sodium channels in a multi-compartment fiber environment. Multiple factors appeared important to ensure prolonged nociceptive firing potentially relevant to long-lasting pain. Crucial roles were observed in: (i) co-expression of ATP P2X2 and P2X3 receptor subunits; (ii) intrinsic activation/inactivation properties of sodium Nav1.8 channels; and (iii) temporal and spatial distribution of ATP/5-HT release sites along the branches of trigeminal nerve fibers. Based on these factors we could obtain either persistent activation of nociceptive firing or its periodic bursting mimicking the pulsating nature of pain. In summary, our model proposes a novel tool for the exploration of peripheral nociception to test the contribution of clinically relevant factors to headache including migraine pain.
Front Cell Neurosci, 2020; 14
31973098: Della Pietra A, Mikhailov N, Giniatullin R
The Emerging Role of Mechanosensitive Piezo Channels in Migraine Pain.
Recently discovered mechanosensitive Piezo channels emerged as the main molecular detectors of mechanical forces. The functions of Piezo channels range from detection of touch and pain, to control of the plastic changes in different organs. Recent studies suggested the role of Piezo channels in migraine pain, which is supposed to originate from the trigeminovascular nociceptive system in meninges. Interestingly, migraine pain is associated with such phenomenon as mechanical hypersensitivity, suggesting enhanced mechanotransduction. In the current review, we present the data that propose the implication of Piezo channels in migraine pain, which has a distinctive pulsatile character. These data include: (i) distribution of Piezo channels in the key elements of the trigeminovascular nociceptive system; (ii) the prolonged functional activity of Piezo channels in meningeal afferents providing a mechanistical basis for mechanotransduction in nociceptive nerve terminals; (iii) potential activation of Piezo channels by shear stress and pulsating blood flow; and (iv) modulation of these channels by emerging chemical agonists and modulators, including pro-nociceptive compounds. Achievements in this quickly expanding field should open a new road for efficient control of Piezo-related diseases including migraine and chronic pain.
Int J Mol Sci, 2020; 21
30768945: Mikhailov N, Leskinen J, Fagerlund I, Poguzhelskaya E, Giniatullina R, Gafurov O, Malm T, Karjalainen T, Gröhn O, Giniatullin R
Mechanosensitive meningeal nociception via Piezo channels: Implications for pulsatile pain in migraine?
Recent discovery of mechanosensitive Piezo receptors in trigeminal ganglia suggested the novel molecular candidate for generation of migraine pain. However, the contribution of Piezo channels in migraine pathology was not tested yet. Therefore, in this study, we explored a potential involvement of Piezo channels in peripheral trigeminal nociception implicated in generation of migraine pain.
Neuropharmacology, 2019; 149
28496430: Shelukhina I, Mikhailov N, Abushik P, Nurullin L, Nikolsky EE, Giniatullin R
Cholinergic Nociceptive Mechanisms in Rat Meninges and Trigeminal Ganglia: Potential Implications for Migraine Pain.
Parasympathetic innervation of meninges and ability of carbachol, acetylcholine (ACh) receptor (AChR) agonist, to induce headaches suggests contribution of cholinergic mechanisms to primary headaches. However, neurochemical mechanisms of cholinergic regulation of peripheral nociception in meninges, origin place for headache, are almost unknown.
Front Neurol, 2017; 8
28920040: Mikhailov N, V Mamontov O, A Kamshilin A, Giniatullin R
Parasympathetic Cholinergic and Neuropeptide Mechanisms of Migraine.
Migraine mechanisms remain largely uncovered for various reasons including a very high complexity of the neurophysiological mechanisms implicated in this disorder and a plethora of endogenous biologically active compounds

involved in the pathological process. The functional role of parasympathetic innervation of meninges and cholinergic mechanisms of migraine are among little explored issues despite multiple evidence indirectly indicating the role of acetylcholine (ACh) and its analogues in migraine and other types of headache. In the current short review, we discuss morphological, functional, and clinical issues related to the role of ACh and its analogues such as carbachol and nicotine in this most common neurological disorder.
Anesth Pain Med, 2017; 7
30198686: Polyanichko AM, Mikhailov NV, Romanov NM, Baranova YG, Chikhirzhina EV
INTERMOLECULAR INTERACTIONS IN THE SOLUTIONS OF SERUM ALBUMIN.
The mechanisms of intermolecular protein complex formation were studied by the example of monomers, oligomers and aggregates of bovine serum albumin (BSA) depending on the protein concentration, pH and urea concentration. Using dynamic light scattering (DLS), analytical ultracentrifugation (AUC) and PAG electrophoresis, we have shown that there is dynamic equilibrium between monomers and aggregates in BSA solution. Decreasing pH of the solution (4.0—1.0) resulted in increasing sizes of the aggregates. In the solutions with low urea concentrations (below 2 M), the sizes of aggregates decreased, while higher urea concentrations induced formation of larger aggregates due to the unfolding of the protein.
Tsitologiia, 2016; 58

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APPLICATION OF EXTRACELLULAR VESICLES IN A 3D BLOOD-BRAIN BARRIER SPHEROID MODEL

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Viktoriya Sokolova[1], Nina Hagemann[2], Yanis Mouloud[3], Bernd Giebel[3], Dirk Hermann[2], Matthias Epple[1]
[1]Universuty of Duisburg-Essen, Institute For Inorganic Chemistry, Essen, Germany, [2]University Hospital Essen, Department Of Neurology, Essen, Germany, [3]University Hospital Essen, Institute For Transfusion Medicine, Essen, Germany

As a consequence of ischemic stroke, the integrity of the blood-brain barrier (BBB) is affected. Since MSC-EVs improve post ischemic stroke symptoms and can restore the BBB in mice, it was our goal to study the interaction of the BBB with MSC-EVs in more detail. To this end, we have developed a 3D BBB brain model based on a three-dimensional arrangement of three cell types (*i.e.* human astrocytes, pericytes and brain endothelial cells). The uptake of CD63-eGFP labelled mesenchymal stromal cell (MSC)-derived extracellular vesicles (CD63-eGFP$^+$ MSC-EVs) were studied in a two- and three-dimensional co-culture cell model (3D spheroid) by confocal laser scanning microscopy. The spheroid core is composed mainly of astrocytes, covered with pericytes, while brain endothelial cells form the surface layer, establishing the blood-brain barrier that regulates the transport of molecules. In 2D cell co-culture experiments, it was shown that CD63-eGFP$^+$ MSC-EVs readily entered the cells. A similar situation evolved with 3D spheroids, *e.g.* the CD63-eGFP$^+$ MSC-EVs entered the interior of the spheroid. **References:** 1. V. Sokolova, G. Mekky, S.B. van der Meer, M.C. Seeds, A.J. Atala, M. Epple**,** Sci. Rep. **10** (2020) 18033. 2. V. Sokolova, G. Nzou, S.B. van der Meer, T. Ruks, M. Heggen, K. Loza, N. Hagemann, F. Murke, B. Giebel, D.M. Hermann, A.J. Atala, M. Epple, Acta Biomater. 111 (2020) 349-362.

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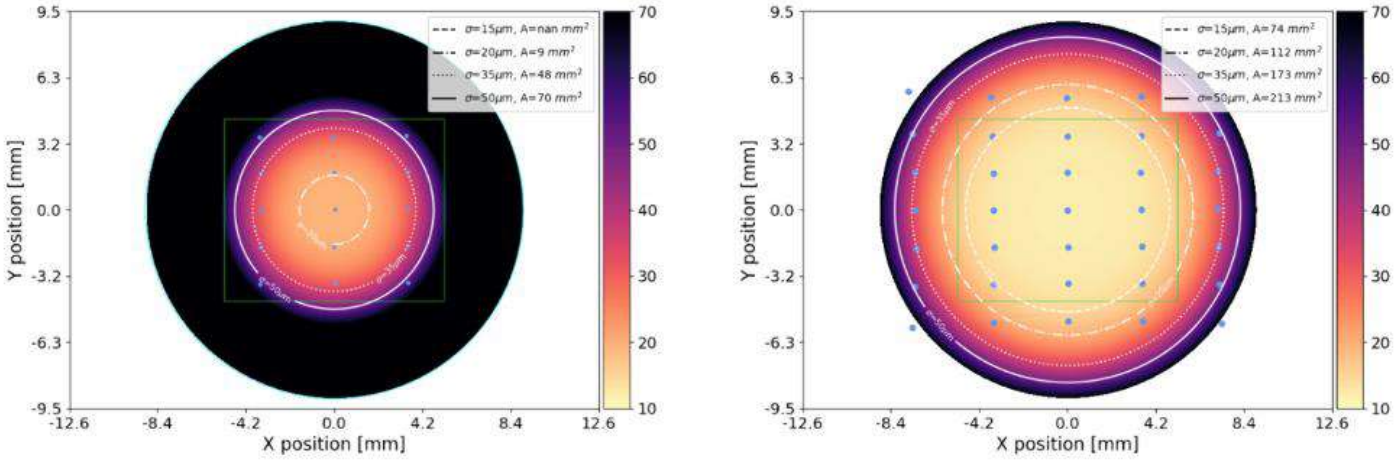
BOARD NUMBER: S05-566

WIDE FIELD OPTICAL IMAGING OF MACAQUE VISUAL CORTEX WITH A CURVED DETECTOR

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Isabelle Racicot[1,2], Frederic Chavane[3], Marc Ferrari[4], Eduard Muslimov[1,4,5], Sandrine Chemla[2], Kevin Blaize[2]
[1]Laboratoire d'Astrophysique de Marseille, Aix-marseille Univ, Cnrs, Cnes, Lam, Marseille, France, [2]Institut de Neurosciences de la Timone, Aix-marseille Universite Cnrs, Marseille, France, [3]Aix-Marseille Université, Institut Des Neurosciences De La Timone, Marseille, France, [4]Laboratoire d'Astrophysique de Marseille, Aix Marseille Universite Cnrs, Marseille, France, [5]Kazan National Research Technical University, Named After A.n. Tupolev Kai, Kazan, Russian Federation

Optical imaging of wide-field cortical areas is limited by the curvature of the brain. To address this issue, we developed a new wide-field optical imaging system adapted to the macaque brain. Our system is composed of a curved detector, an aspherical lens and a ring composed of LEDs providing uniform illumination in four wavelengths relevant for optical imaging, including intrinsic imaging and voltage-sensitive dye (VSD) imaging. The system was fully characterized and compared with the standard optical imaging system currently in use for cortical imaging, and a 3-fold increase of the area with acceptable focus was measured (see figure). Our quantification demonstrated that we could image the visual cortex with a relatively uniform focus over a 16 mm diameter area. This new instrument, by providing good quality imaging over a large field of view, should facilitate the observation of wide-mesoscale phenomena, such as dynamic cortical maps and propagating waves, which are otherwise difficult to observe due to technical limitations of the currently available recording tools.



(a) Original (flat) detector setup (b) Curved detector setup

Figure: Average spot size across the curved field of view at 670 nm. The blue dots represent the positions where the point spread function (PSF) was measured experimentally. The 2D curve is the best power law fit to the data, imposing radial symmetry. White contours indicate the areas where the average spot size reaches 50, 35, 20 and 15 µm. A green rectangle shows the area covered by the flat sensor in the original instrument.

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IMPROVING DATA QUALITY OF 3D RARE ACQUIRED MRI IMAGES AT 9.4 T BY REDUCING ARTIFACTS AND CORRECTING FOR NON-UNIFORM INTENSITY

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Samia Afzal[1], Karl-Heinz Herrmann[2], Martin Krämer[2], Jürgen R. Reichenbach[2], Otto W. Witte[1,3,4,5,6,7], Silvio Schmidt[1,4,6,7]
[1]Jena University Hospital, Hans Berger Department Of Neurology, Jena, Germany, [2]Jena University Hospital, Diagnostic And Interventional Radiology (medical Physics Group), Jena, Germany, [3]Jena University Hospital, Jena Center For Healthy Aging, Jena, Germany, [4]Leibniz Institute on Aging, Aging Research Center Jena, Jena, Germany, [5]Jena University Hospital, Izkf Of The Medical Faculty Jena, Jena, Germany, [6]Jena University Hospital, Department Of Neurology, Biomagnetic Center, Jena, Germany, [7]Jena University Hospital, Brain Imaging Center Jena, Jena, Germany

T2 weighted MR imaging at high, isotropic resolution for morphometric studies is hampered at ultra high field small animal scanners. To achieve good T2 contrast, the required long repetition times (TRs) cause prohibitive long scan times for 3D acquisitions unless high turbo factors can be used in combination with Rapid Acquisition with Relaxation Enhancement (RARE) sequences. However, at 9.4T, severe image artifacts can arise from the faster T2 decay at 9.4T, high signal differences can lead to pronounced Gibbs ringing and the magnetic coil's profile (Transmit-receive-CryoProbe) leads to inhomogeneous image intensities. To reduce these unfavorable image artifacts, we employed filters like T2-decay compensation and 3D-unringing together with N4 bias field correction. Therefore, the filtering effects were analyzed on the signal-to-noise ratio (SNR) along with the evaluation of image contrast and edge effects for images acquired from a T2-weighted RARE sequence performed on a 9.4 T MR scanner. The cumulative application of all three filters delivered an overall higher SNR through the whole image by reducing artifacts. The T2 compensation alone enhanced the image contrast at the selected brain tissues, especially at the larger areas like the brain surface and ventricles. The combined filtering with 3D-unringing provides reliable artifact removal and N4-unbias provides a much more homogenous signal across the T2 weighted whole brain image. The application of a single filter by itself is inadequate for artifact removal. Local tissue contrast and edge sharpness do not suffer from the combined filters, so the filtered data sets might be well suited for morphometric studies.

Pubmed:
33481833: Khaliq A, Ravindran R, Afzal S, Jena PK, Akhtar MW, Ambreen A, Wan YY, Malik KA, Irfan M, Khan IH
Gut microbiome dysbiosis and correlation with blood biomarkers in active-tuberculosis in endemic setting.
Tuberculosis (TB) is the largest infectious disease with 10 million new active-TB patients and1.7 million deaths per year. Active-TB is an inflammatory disease and is increasingly viewed as an imbalance of immune responses to M. tb. infection. The mechanisms of a switch from latent infection to active disease is not well worked out but a shift in the immune responses is thought to be responsible. Increasingly, the role of gut microbiota has been described as a major influencer of the immune system. And because the gut is the largest immune organ, we aimed to analyze the gut microbiome in active-TB patients in a TB-endemic country, Pakistan. The study revealed that Ruminococcacea, Enetrobactericeae, Erysipelotrichaceae, Bifidobacterium, etc. were the major genera associated with active-TB, also associated with chronic inflammatory disease. Plasma antibody profiles against several M. tb. antigens, as specific biomarkers for active-TB, correlated closely with the patient gut microbial profiles. Besides, bcoA gene copy number, indicative of the level of butyrate production by the gut microbiome was five-fold lower in TB patients compared to healthy individuals. These findings suggest that gut health in TB patients is compromised, with implications for disease morbidity (e.g., severe weight loss) as well as immune impairment. PLoS One, 2021; 16

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DEPTH-RESOLVED FIBRE PHOTOMETRY FOR ALZHEIMER'S DISEASE PLAQUE PATHOLOGY IN VIVO

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Nicole Byron[1], Niall Mcalinden[2], Filippo Pisano[3], Jacques Ferreira[1], Marco Pisanello[4], Keith Mathieson[2], Massimo De Vittorio[3], Ferruccio Pisanello[3], Shuzo Sakata[1]
[1]University of Strathclyde, Strathclyde Institute Of Pharmacy And Biomedical Science, Glasgow, United Kingdom, [2]University of Strathclyde, Supa, Institute Of Photonics, Glasgow, United Kingdom, [3]Italian Institute of Technology, Center For Biomolecular Nanotechnology, Arnesano (Lecce), Italy, [4]Optogenix s.r.l, Research And Development, Arnesano, Italy

Development of effective Alzheimer's disease (AD) treatments has been challenging, with limited progression from a lab to clinical setting. Amyloid plaques, a pathological hallmark of AD, have been monitored in humans to determine the success of potential AD treatments. However, only limited technologies are available to monitor amyloid plaques in animal models, in real-time. Here we demonstrate that photometry with a tapered optical fibre allows real-time monitoring of plaque signals with depth resolution, in vivo. We began by performing fibre photometry with a conventional flat fibre, to examine whether plaque signals can be monitored across brain regions of 5xFAD mice, a mouse model of AD. To label plaques, we peripherally administered blood-brain-barrier-permeable plaque marker, Methoxy-x04. We confirmed strong correlation between in vivo fluorescent signals and post-mortem histological plaque signals across depth, indicating that fibre photometry is a feasible approach to assess AD pathology in vivo. To determine if modifications of in vivo fluorescence can be detected, we completed depth-resolved photometry with a tapered fibre to monitor the pharmacokinetic response following Methoxy-x04 administration to 5xFAD mice. We found a steady increase of in vivo fluorescence within 1 hour of Methoxy-x04 injection, indicating that this approach can detect changes in Methoxy-x04 concentration. Our findings advocate this novel approach to be reliable for in vivo, real-time plaque assessment. With changes in Methoxy-x04 concentration being detected, this approach can be used to monitor plaque pathology in response to several pharmaceutical and non-pharmaceutical interventions, helping accelerate prospective treatments from a lab to clinical setting.

Pubmed:
34439940: Byron N, Semenova A, Sakata S
Mutual Interactions between Brain States and Alzheimer's Disease Pathology: A Focus on Gamma and Slow Oscillations. Brain state varies from moment to moment. While brain state can be defined by ongoing neuronal population activity, such as neuronal oscillations, this is tightly coupled with certain behavioural or vigilant states. In recent decades, abnormalities in brain state have been recognised as biomarkers of various brain diseases and disorders. Intriguingly, accumulating evidence also demonstrates mutual interactions between brain states and disease pathologies: while abnormalities in brain state arise during disease progression, manipulations of brain state can modify disease pathology, suggesting a therapeutic potential. In this review, by focusing on Alzheimer's disease (AD), the most common form of dementia, we provide an overview of how brain states change in AD patients and mouse models, and how controlling brain states can modify AD pathology. Specifically, we summarise the relationship between AD and changes in gamma and slow oscillations. As pathological changes in these oscillations correlate with AD pathology, manipulations of either gamma or slow oscillations can modify AD pathology in mouse models. We argue that neuromodulation approaches to target brain states are a promising non-pharmacological intervention for neurodegenerative diseases.
Biology (Basel), 2021; 10

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BOARD NUMBER: S05-569

MRI REVEALS STRUCTURAL CHANGES IN WHITE MATTER OF GD3 SYNTHASE-DEFICIENT MICE

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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We investigated whether *ex vivo* magnetic resonance imaging (MRI) signal is affected by altered membrane (glycosphingo)lipid composition in GD3 synthase deficient (*St8sia1* knock-out, KO) mice. 19 adult (10 *St8sia1* KO and 9 age-matched wild-type, WT) male mice were perfused with 4% paraformaldehyde, their heads rehydrated in PBS with 0.05% sodium azide for 1 to 3 months and scanned using a 9.4T Bruker Biospec® MR scanner to acquire T2-weighted images, diffusion tensor imaging (DTI) data, and T1 relaxation maps. After imaging, selected histological and immunohistochemical markers were analysed in brain tissue sections. The lack of GD3 synthase caused global and widespread changes in MR signal. Interestingly, *St8sia1* KO brains were larger overall, but there were also distinct regional volume differences, with increases in the hippocampus, brain stem, cerebellum and nucleus accumbens, and decreases in cortical and thalamic regions. T1 relaxation time was decreased in *St8sia1* KO mice in many areas but the overall pattern did not match regional volume changes. Analysis of DTI showed an overall increase in mean, axial and radial diffusivity whereas fractional anisotropy (FA) increased in the corpus callosum and decreased in hypothalamus and inferior colliculi. Preliminary histological analysis indicated morphological differences in the white matter of *St8sia1* KO brains, in the same areas demonstrating changed MRI. Observed changes in MRI signal which accompany altered membrane ganglioside pattern, particularly when DTI components are analysed, suggest that MRI is suitable for detection of structural changes in white matter caused by disruption of membrane lipid composition

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BOARD NUMBER: S05-570

CHARACTERIZATION OF NEURONAL CYTO-ARCHITECTURE BY X-RAY PHASE-CONTRAST TOMOGRAPHY

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Mappings of the three-dimensional (3d) cyto-architecture are essential for a quantitative understanding of the human brain. The potential of X-ray phase-contrast computed-tomography (PC-CT) for 3d brain imaging has been recently demonstrated [1,2]. Alzheimer's disease (AD) is a case in point where exact disease mechanisms are still unresolved. Here, we present a granular study of the hippocampus in aging and disease [3], including 3d data, sub-nuclear resolution and advanced analysis tools, completed by a subsequent study on the optimization of experimental settings [4]. To study the human hippocampal cyto-architecture in cohorts of 20 (AD patients and controls), we have implemented a multiscale PC-CT workflow to cover mm^3-sized fields-of-view, and subsequently images of target regions with sub-µm resolution. We used machine learning to segment neurons, followed by optimal transport (OT) analysis to unravel pathological alterations. Opposed to standard statistical tools, OT-analysis allows a more detailed comparison of entire neuron populations. Therefore, OT grants insight into the development of a patient cohort, and hence into the pathology. The workflow allows to conclude a pathway from healthy to pathology without prior hypothesis. We found the prototypical transformation between a structure representing healthy dentate gyrus granule cells and the pathological state: a decrease in the volume of granule cell nuclei, as well as an increase in their electron density and its spatial heterogeneity (reflecting heterochromatin). Our experimental and analysis scheme provides a blueprint for studying the cyto-architecture of the human brain and its alterations associated with neurodegenerative diseases. [1] https://doi.org/10.1038/s41593-020-0704-9 [2] https://doi.org/10.1073/pnas.1801678115 [3] https://doi.org/10.1073/pnas.2113835118 [4] https://doi.org/10.1364/BOE.434885

FENS Forum 2022
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BOARD NUMBER: S05-571

COMPUTATIONAL IMAGE ENHANCEMENT OF MULTIMODE FIBRE BASED HOLOGRAPHIC ENDO-MICROSCOPY: HARNESSING THE MUDDY MODES

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Multi-mode fiber (MMF)-based endoscopes are minimally invasive probes holding big promises for *in vivo* deep-tissue high-resolution imaging [1,2]. By employing wavefront-shaping modulator (DMD) the light propagation through the MMF is controlled resulting in a spot scanned in the focal plane across the fluorescent sample similarly to a scanning microscope. The emitted signal is collected by the same MMF and detected on the photomultiplier tube (PMT). The foci are limited by diffraction as well as the nature of power distribution where a stray optical signal carries typically 20% of input power contaminating the outside of the focal spot. This power is for each position of focal spot distributed in a randomized but reproducible background speckle pattern. We call the focal spots with their speckled background a *muddy modes*. The fluorescence signal generated by excitation with the muddy mode background is incorrectly ascribed to the focal point location and the resulting image suffers from lower contrast and resolution.
Based on the knowledge of the intensity distribution of all muddy modes we present two algorithms for image enhancement. The first reconstruction algorithm of regularized direct pseudo-inversion presumes an intensity independent noise distribution of the detected signal (Gaussian noise), while the approach of regularized iterative algorithm respects the Poisson noise distribution better corresponding to the fluorescent photon signal detection in our experiment. These approaches yield image reconstructions with enhanced contrast and resolution.
1. Ohayon, S., et al., Biomedical Optics Express, 2018.
2. Turtaev S., et al., Light: Science & Applications, 2018.

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BOARD NUMBER: S05-572

DEEP BRAIN STIMULATION OF THE MEDIAL SEPTUM RESTORES BLOOD PERFUSION FOLLOWING PHARMACOLOGIC NMDA ANTAGONISM IN A REGION-DEPENDENT MANNER.

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Reduced function of the *N*-methyl-D-aspartate (NMDA) receptor is a leading model of schizophrenia. In addition to positive and negative symptoms, schizophrenia is characterized by cognitive impairments, such decreased spatial and working memory, for which there are few effective treatments. It has been demonstrated that MK-801, a highly potent and selective NMDA-antagonist, induces spatial memory impairments in rodents (Lee et al., 2013). These spatial memory deficits can be ameliorated by 5 minutes of theta frequency (7.7Hz) deep brain stimulation (DBS) of the medial septal nucleus (MSN) prior to the start of a task. Intriguingly, while MK-801 alters electrophysiological activity, DBS does not appear to have lasting changes on these measures. This suggest that DBS improves memory function through mechanisms other than direct alterations to electrical brain activity. Using functional ultrasound (fUS) we examine blood perfusion across the septo-hippocampal network including the MSN, hippocampus, thalamus, and medial prefrontal cortex following MK-801 administration and MSN theta- and gamma- frequency DBS. We find that intraperitoneal (i.p) injection of 1mg/kg MK-801 reduces blood perfusion in mice across all regions of interest, but at different rates in different brain regions. We further find that 5 minutes of MSN DBS disrupts MK-801- induced reductions to blood perfusion, even after the cessation of stimulation. Together these results (1) indicate that pharmacologically- induced NMDA hypofunction alters blood perfusion in brain regions associated with spatial memory, and (2) that brief DBS of the MSN can attenuate these changes, suggesting that MSN DBS may improve memory function through vascular mechanisms.

Pubmed:

32477237: Crown LM, Bartlett MJ, Wiegand JL, Eby AJ, Monroe EJ, Gies K, Wohlford L, Fell MJ, Falk T, Cowen SL
Sleep Spindles and Fragmented Sleep as Prodromal Markers in a Preclinical Model of LRRK2-G2019S Parkinson's Disease.
Sleep disturbances co-occur with and precede the onset of motor symptoms in Parkinson's disease (PD). We evaluated sleep fragmentation and thalamocortical sleep spindles in mice expressing the p.G2019S mutation of the leucine-rich repeat kinase 2 () gene, one of the most common genetic forms of PD. Thalamocortical sleep spindles are oscillatory events that occur during slow-wave sleep that are involved in memory consolidation. We acquired data from electrocorticography, sleep behavioral measures, and a rotarod-based motor enrichment task in 28 -G2019S knock-in mice and 27 wild-type controls (8-10 month-old males). Sleep was more fragmented in -G2019S mice; sleep bouts were shorter and more numerous, even though total sleep time was similar to controls. -G2019S animals expressed more sleep spindles, and individual spindles were longer in duration than in controls. We then chronically administered the LRRK2-inhibitor MLi-2 in-diet to = 12 -G2019S and = 15 wild-type mice for a within-subject analysis of the effects of kinase inhibition on sleep behavior and physiology. Treatment with MLi-2 did not impact these measures. The data indicate that the -G2019S mutation could lead to reduced sleep quality and altered sleep spindle physiology. This suggests that sleep spindles in -G2019S animals could serve as biomarkers for underlying alterations in sleep networks resulting from the -G2019S mutation, and further evaluation in human -G2019S carriers is therefore warranted.
Front Neurol, 2020; 11
28211999: Parent KL, Hill DF, Crown LM, Wiegand JP, Gies KF, Miller MA, Atcherley CW, Heien ML, Cowen SL
Platform to Enable Combined Measurement of Dopamine and Neural Activity.
Complex behaviors depend on the coordination of the activities of ensembles of neurons and the release of neuromodulators such as dopamine. The mechanisms underlying such coordination are not well-understood due to a lack of instrumentation for combined and real-time monitoring of neuromodulator release and the activities of large ensembles of neurons. Here we describe a measurement platform that allows for the combined monitoring of electrophysiology from a high-density electrode array and dopamine dynamics from a carbon-fiber microelectrode. Integration of these two measurement systems was

achieved through modification of the existing instrumentation. A shared grounded reference electrode was used in both systems to minimize electrical interference. Further, an optional solid-state-relay array positioned between the electrophysiological electrode array and amplifiers was added to provide additional electrical isolation. The capacity of the integrated measurement platform, termed DANA (Dopamine And Neural Activity), to measure action potentials (high frequency) and local-field oscillations (low frequency) was characterized in vitro using an artificial cerebral spinal fluid gelatin. In vivo recordings from the DANA platform in anesthetized rats demonstrated the ability of the system for near-simultaneous measurement of dopamine release and activity from multiple neurons both in distant brain regions (striatum and hippocampus) and within the same brain region (striatum). Furthermore, this system was shown to be sufficiently compact to measure activity in freely moving animals through recording of single-neuron activity, high-frequency local-field oscillations, and dopamine release.
Anal Chem, 2017; 89
24650599: Keuken MC, Bazin PL, Crown L, Hootsmans J, Laufer A, Müller-Axt C, Sier R, van der Putten EJ, Schäfer A, Turner R, Forstmann BU
Quantifying inter-individual anatomical variability in the subcortex using 7 T structural MRI.
Functional magnetic resonance imaging (MRI) data are usually registered into standard anatomical space. However, standard atlases, such as LPBA40, the Harvard-Oxford atlas, FreeSurfer, and the Jülich cytoarchitectonic maps all lack important detailed information about small subcortical structures like the substantia nigra and subthalamic nucleus. Here we introduce a new subcortical probabilistic atlas based on ultra-high resolution in-vivo anatomical imaging from 7 T MRI. The atlas includes six important but elusive subcortical nuclei: the striatum, the globus pallidus internal and external segment (GPi/e), the subthalamic nucleus, the substantia nigra, and the red nucleus. With a sample of 30 young subjects and carefully cross-validated delineation protocols, our atlas is able to capture the anatomical variability within healthy populations for each of the included structures at an unprecedented level of detail. All the generated probabilistic atlases are registered to MNI standard space and are publicly available.
Neuroimage, 2014; 94
32234806: Ehmer I, Crown L, van Leeuwen W, Feenstra M, Willuhn I, Denys D
Evidence for Distinct Forms of Compulsivity in the SAPAP3 Mutant-Mouse Model for Obsessive-Compulsive Disorder.
The specific mechanisms underlying compulsive behavior in obsessive-compulsive disorder (OCD) are unknown. It has been suggested that such compulsivity may have its origin in cognitive dysfunction such as impaired processing of feedback information, received after the completion of goal-directed actions. The signal attenuation (SA) task models such a processing deficit in animals by attenuating the association strength between food reward and audiovisual feedback (signal) presented after performance of an operant response. The compulsive-like responding resulting from SA is well characterized in rats, but was so far not established in mice, a species for which powerful genetic OCD models exist. Thus, first, we demonstrate that the SA task can be implemented in mice and show that attenuation of reward-associated response feedback produces similar behavior in C57BL/6 mice as previously reported in rats. Second, we tested the hypothesis that knock-out mice (SAPAP3), prone to exhibit several OCD-like abnormalities including excessive grooming, show enhanced compulsive-like behavior in the SA task compared with their wild-type (WT) littermates. However, task-related compulsivity measures in SAPAP3 and WT did not yield significant differences, neither following SA nor during "regular" extinction of operant behavior. Thus, compulsive-like instrumental behavior following feedback distortion was not potentiated in compulsively grooming mice, implicating specifically that (1) a general deficit in feedback processing is not related to excessive grooming in SAPAP3 and (2) different manifestations of compulsivity may be driven by independent mechanisms.
eNeuro, 2020 Mar/Apr; 7

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BOARD NUMBER: S05-573

SIMULTANEOUS TWO-PHOTON VOLTAGE OR CALCIUM IMAGING AND MULTI-CHANNEL LOCAL FIELD POTENTIAL RECORDINGS IN THE MOUSE BARREL CORTEX

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Neuronal population activity, both spontaneous and sensory-evoked, is known to generate propagating waves in the cortex. However, high spatiotemporal-resolution mapping of these waves is difficult as calcium imaging, the work horse of current imaging, does not reveal subthreshold activity. Here, we present a new platform combining two-photon voltage or calcium imaging with multi-channel local field potential (LFP) recordings from different layers of the barrel cortex in anesthetized and awake mice. A chronic cranial window with access port allows injecting the voltage-sensitive dye (VSD) ANNINE-6plus or a viral vector expressing GCaMP6f, as well as entering the brain with a multi-channel neural probe. We show both average spontaneous activity and average evoked signals in response to multi-whisker airpuff stimulations. Time domain analysis shows the dependence of the evoked responses on the cortical layer and on the state of the animal, here separated into anesthetized, awake but resting, and running. The simultaneous data acquisition allows to compare the average membrane depolarization measured with ANNINE-6plus with the amplitude and shape of the LFP recordings. The calcium imaging data connects these data sets to the large existing database of this important second messenger. Interestingly, in the calcium imaging data, a few cells showed a decrease in calcium concentration in response to vibrissa stimulation in awake mice. This system offers an innovative multimodal technique to study the spatiotemporal dynamics of neuronal signals through a 3D architecture in vivo, providing novel insights on sensory coding and closing the gap between electrical and optical acquisitions.

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WHAT CAN TRACTOGRAPHY TELL US ABOUT CORTICAL CONNECTIVITY: A WITHIN-ANIMAL AND ACROSS-VALIDATION COMPARISON WITH TRACT TRACING IN MACAQUE

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Aims: Diffusion MRI (dMRI) based tractography is widely used for non-invasive investigations of brain connectivity in human, despite lingering doubts about its validity. Here we evaluate inter-areal weighted connectivity revealed by tractography with that of ground truth tract tracing in the same macaque brain. **Methods**: 42 retrograde tracer injections in 41 brains revealed 3780 pathways that were compared to dMRI tractography in 6 brains, of which 3 brains had tracer injections, thereby allowing within-animal and across-animal comparisons. Post-mortem multi-shell dMRI allowed high-spatial resolution (0.3 mm). One animal had an *in-vivo* dMRI scan (0.75 mm). Whole brain tractographies were computed with MRTrix3 based on the Constrained Spherical Deconvolution to model the local diffusion. Streamlines were seeded from the grey-white matter interface and reconstructed using a probabilistic algorithm associated with the Anatomically Constrained Tractography with multiple cut-off, angle and step size, followed by Spherical-deconvolution Informed Filtering of Tractograms. **Results**: Spearman correlation between tract tracing and tractography using the 42x91 connectivity matrix is 0.54 (averaged correlations: 0.65 for connections < 20 mm, 0.26 for connections of 20-40 mm, -0.09 for connections > 40 mm). Within-animal tract tracing tractography correlation is not higher than across animal, contrasting with within-animal tract tracing correlations. Tract tracing tractography correlations showed marked areal variability. Spatial resolution failed to impact on correlations. **Conclusions:** The present results reveal unsuspected limitations in dMRI tractography to capture the inter-areal connectivity of the cortex.

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BOARD NUMBER: S05-575

ACTIVITY-DEPENDENT STIMULATION TO ASSESS THE EFFECT OF INFRARED-LASER STIMULATION IN SINGLE NEURONS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Near-infrared laser stimulation is becoming popular as a noninvasive stimulation technique and as a promising tool for medical treatments. However, the biophysical basis underlying the neural effect of the laser stimulus is still under study. We have tested the effect of the laser stimulation in neurons of the pond snail *Lymanea stagnalis,* in experimental and in simulation modeling studies. In our experimental protocol, we compared single spike waveforms obtained during control and during laser illumination. The results from that study show that the spike shape is altered during the laser action, with significant changes in the spike duration and the repolarization and depolarization phases. The effect was fully reversible once the laser was off. We used conductance-based models to explain the waveform changes by tuning specific parameters of the equations, effectively replicating the effect. This combination of experimental and modeling study helps us understand with further detail the biophysical effect. In this work we report the results of an activity dependent protocol that selectively turns on and off the laser stimulation during the depolarization or during the hyperpolarization phases of individual action potentials. This protocol allows the detailed characterization of the membrane potential waveform under the laser stimulation in these two phases and the comparison with the biophysical modeling of the neurons. Thus, the effect of the laser on the activation and inactivation of different channels can be assessed. We argue that the protocol can also be generalized for other kinds of activity such as bursts or subthreshold oscillations.

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BOARD NUMBER: S05-576

TOWARD INTEGRATION OF NEURAL PROBES TO CORRELATE RAMAN AND ELECTROPHYSIOLOGICAL SIGNALS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Teresa Jurado-Parras[1], Maria Andriani[2], Elena Cid[1], Antonio Balena[2], Marco Bianco[2], Marco Pisanello[2], Francesco De Angelis[2], Filippo Pisano[2], Massimo De Vittorio[2,3], Ferruccio Pisanello[2], Liset Menendez De La Prida[1]
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AIMS: Understanding the functional organization of the brain is key to map function and dysfunction. Recent advances in neurotechnologies has enabled neuroscientists to work with a set of implantable tools able to capture neural activity from hundreds of neurons simultaneously, to investigate neural circuits with cell-type specificity and to monitor neurotransmitters release in real time. Here, we describe our efforts in combining high-density electrophysiology and vibrational spectroscopy to map the molecular content of specific brain regions. METHODS: Linear array silicon probes and optrodes were used to obtain laminar local field potential (LFP) recordings in head-fixed mice. Raman spectra from laminar regions were measured using micro-structured tapered optical (TF) fiber implants. A TF was coated with aluminum and an optical sensitive region generated by locally milling the thin layer by Focused Ion Beam. RESULTS: Using laminar LFP recordings across the neocortex and hippocampus of awake head-fixed mice, we first established unambiguous criteria to infer recording depth blindly. Then, we mapped depth-resolved Raman signals using micro-structured tapered optical fibers to obtain spectroscopic profiles of the chemical composition of brain parenchyma. We identified specific Raman signatures of fatty acid and triglycerides, as well as lipid and proteins differentially distributed across brain depth, which can be tracked histologically. The sensitive region can extend from 200µm until 1mm, allowing to tailor the spatial resolution of the spectroscopic investigation. CONCLUSION: Integration of electrophysiological and Raman signals may allow for an unprecedented multimodal evaluation of brain structure and function in health and disease.

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BOARD NUMBER: S05-577

EFFECT OF 3D SYNTHETIC MICROSCAFFOLD NICHOID ON THE MORPHOLOGY OF HIPPOCAMPAL NEURONS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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The human brain is the most complex organ in biology. This complexity is due to the intricate connections of brain cells and has so far limited the development of in-vitro models for basic and applied neuroscience. We decided to create a new, reliable, and cost-effective in-vitro system of hippocampal neurons and astrocytes co-culture based on the Nichoid, a 3D microscaffold microfabricated by two-photon laser polymerization technology. After 21 days in culture, we morphologically characterized the 3D spatial organization of astrocytes and neurons within the microscaffold and we compared our observations with the classical 2D co-culture. Using confocal microscopy, we found that cells colonized the entire volume of the 3D devices and had well differentiated. This was further elaborated with Drebrin and PSD95 staining, markers for mature dendritic spines, labelling the majority of neurons both in the 2D and in the 3D co-cultures. Using scanning electron microscopy, we found that neurons in the 3D co-culture displayed a significantly larger amount of dendritic protrusions compared to the 2D co-culture, indicating that neurons growing in a 3D environment may be more prone to connections than those in 2D. Our results show that the Nichoid can act as a 3D device and can be used to investigate structure and morphology of neurons and astrocytes in a 3D volume. In the future, this model can be used as a tool for drug screening and to determine the factors at the basis of different human brain diseases plating cells derived directly from patients.

Pubmed:
34911542: Musi CA, Castaldo AM, Valsecchi AE, Cimini S, Morello N, Pizzo R, Renieri A, Meloni I, Bonati M, Giustetto M, Borsello T
JNK signaling provides a novel therapeutic target for Rett syndrome.
Rett syndrome (RTT) is a monogenic X-linked neurodevelopmental disorder characterized by loss-of-function mutations in the MECP2 gene, which lead to structural and functional changes in synapse communication, and impairments of neural activity at the basis of cognitive deficits that progress from an early age. While the restoration of MECP2 in animal models has been shown to rescue some RTT symptoms, gene therapy intervention presents potential side effects, and with gene- and RNA-editing approaches still far from clinical application, strategies focusing on signaling pathways downstream of MeCP2 may provide alternatives for the development of more effective therapies in vivo. Here, we investigate the role of the c-Jun N-terminal kinase (JNK) stress pathway in the pathogenesis of RTT using different animal and cell models and evaluate JNK inhibition as a potential therapeutic approach.
BMC Biol, 2021; 19
34794851: Little K, Llorián-Salvador M, Scullion S, Hernández C, Simó-Servat O, Del Marco A, Bosma E, Vargas-Soria M, Carranza-Naval MJ, Van Bergen T, Galbiati S, Viganò I, Musi CA, Schlingemann R, Feyen J, Borsello T, Zerbini G, Klaassen I, Garcia-Alloza M, Simó R, Stitt AW,
Common pathways in dementia and diabetic retinopathy: understanding the mechanisms of diabetes-related cognitive decline.
Type 2 diabetes (T2D) is associated with multiple comorbidities, including diabetic retinopathy (DR) and cognitive decline, and T2D patients have a significantly higher risk of developing Alzheimer's disease (AD). Both DR and AD are characterized by a number of pathological mechanisms that coalesce around the neurovascular unit, including neuroinflammation and degeneration, vascular degeneration, and glial activation. Chronic hyperglycemia and insulin resistance also play a significant

role, leading to activation of pathological mechanisms such as increased oxidative stress and the accumulation of advanced glycation end-products (AGEs). Understanding these common pathways and the degree to which they occur simultaneously in the brain and retina during diabetes will provide avenues to identify T2D patients at risk of cognitive decline.
Trends Endocrinol Metab, 2022; 33
34459572: Conz A, Musi CA, Russo L, Borsello T, Colnaghi L
Super-resolution study of PIAS SUMO E3-ligases in hippocampal and cortical neurons.
The SUMOylation machinery is a regulator of neuronal activity and synaptic plasticity. It is composed of SUMO isoforms and specialized enzymes named E1, E2 and E3 SUMO ligases. Recent studies have highlighted how SUMO isoforms and E2 enzymes localize with synaptic markers to support previous functional studies but less information is available on E3 ligases. PIAS proteins - belonging to the protein inhibitor of activated STAT (PIAS) SUMO E3-ligase family - are the best-characterized SUMO E3-ligases and have been linked to the formation of spatial memory in rodents. Whether however they exert their function co-localizing with synaptic markers is still unclear. In this study, we applied for the first time structured illumination microscopy (SIM) to PIAS ligases to investigate the co-localization of PIAS1 and PIAS3 with synaptic markers in hippocampal and cortical murine neurons. The results indicate partial co-localization of PIAS1 and PIAS3 with synaptic markers in hippocampal neurons and much rarer occurrence in cortical neurons. This is in line with previous super-resolution reports describing the co-localization with synaptic markers of other components of the SUMOylation machinery.
Eur J Histochem, 2021; 65
33113832: Colnaghi L, Conz A, Russo L, Musi CA, Fioriti L, Borsello T, Salmona M
Neuronal Localization of SENP Proteins with Super Resolution Microscopy.
SUMOylation of proteins plays a key role in modulating neuronal function. For this reason, the balance between protein SUMOylation and deSUMOylation requires fine regulation to guarantee the homeostasis of neural tissue. While extensive research has been carried out on the localization and function of small ubiquitin-related modifier (SUMO) variants in neurons, less attention has been paid to the SUMO-specific isopeptidases that constitute the human SUMO-specific isopeptidase (SENP)/Ubiquitin-Specific Protease (ULP) cysteine protease family (SENP1-3 and SENP5-7). Here, for the first time, we studied the localization of SENP1, SENP6, and SENP7 in cultured hippocampal primary neurons at a super resolution detail level, with structured illumination microscopy (SIM). We found that the deSUMOylases partially colocalize with pre- and post-synaptic markers such as synaptophysin and drebrin. Thus, further confirming the presence with synaptic markers of the negative regulators of the SUMOylation machinery.
Brain Sci, 2020; 10
32998477: Musi CA, Agrò G, Santarella F, Iervasi E, Borsello T
JNK3 as Therapeutic Target and Biomarker in Neurodegenerative and Neurodevelopmental Brain Diseases.
The c-Jun -terminal kinase 3 (JNK3) is the JNK isoform mainly expressed in the brain. It is the most responsive to many stress stimuli in the central nervous system from ischemia to Aβ oligomers toxicity. JNK3 activity is spatial and temporal organized by its scaffold protein, in particular JIP-1 and β-arrestin-2, which play a crucial role in regulating different cellular functions in different cellular districts. Extensive evidence has highlighted the possibility of exploiting these adaptors to interfere with JNK3 signaling in order to block its action. JNK plays a key role in the first neurodegenerative event, the perturbation of physiological synapse structure and function, known as synaptic dysfunction. Importantly, this is a common mechanism in many different brain pathologies. Synaptic dysfunction and spine loss have been reported to be pharmacologically reversible, opening new therapeutic directions in brain diseases. Being JNK3-detectable at the peripheral level, it could be used as a disease biomarker with the ultimate aim of allowing an early diagnosis of neurodegenerative and neurodevelopment diseases in a still prodromal phase.
Cells, 2020; 9
32087286: Musi CA, Agrò G, Buccarello L, Camuso S, Borsello T
JNK signaling activation in the Ube3a maternal deficient mouse model: its specific inhibition prevents post-synaptic protein-enriched fraction alterations and cognitive deficits in Angelman Syndrome model.
Deficiency of the E3 ubiquitin ligase UBE3A leads to the neurodevelopmental disorder Angelman syndrome (AS), while higher levels are linked to autism spectrum disorder. The mechanisms underlying the downstream effects of UBE3A loss or gain of function in these disorders are still not well understood, and treatments are still lacking. Here, using the Ube3a maternal loss (Ube3a) mouse model, we report an important JNK signaling activation in the hippocampus, cortex and cerebellum correlating with the onset of behavioral defects and biochemical marker alterations in the post-synaptic element, suggesting important spine pathology. JNK activation occurs at 7 and persists up till 23 weeks in Ube3a mice in two different cellular compartments: the nucleus and the post-synaptic protein-enriched fraction. To study JNK's role in Ube3a pathology we treated mice with the specific JNK inhibitor peptide, D-JNKI1, from 7 to 23 weeks of age. Preventing JNK action in vivo restores the post-synaptic protein-enriched fraction defects and the cognitive impairment in these mice. Our results imply a critical role of UBE3A-JNK signaling in the pathogenesis of UBE3A-related disorders. In particular, it was clear that JNK is a

key player in regulating AS synaptic alterations and the correlated cognitive impairments, in fact, its specific inhibition tackles Ube3a pathology. This study sheds new light on the neuronal functions of UBE3A and offers new prospects for understanding the pathogenesis of UBE3A-related disorders.
Neurobiol Dis, 2020; 140
30315879: Buccarello L, Musi CA, Turati A, Borsello T
The Stress c-Jun N-terminal Kinase Signaling Pathway Activation Correlates with Synaptic Pathology and Presents A Sex Bias in P301L Mouse Model of Tauopathy.
Pathological Tau (P-Tau) leads to dementia and neurodegeneration in tauopathies, including Alzheimer's disease. The P301L transgenic mice well mimic human tauopathy features; P-Tau localizes also at the dendritic spine level and this correlates with synaptic markers down-regulation. Importantly, tg females present a more severe pathology compared to male mice. We describe JNK activation in P301L-tg mice, characterizing by P-JNK and P-c-Jun, cleaved-Caspase-3, P-PSD95 and P-Tau (direct JNK-targets) increased levels in tg vs control mice. These data indicate that JNK stress pathway is involved in neuronal degenerative mechanisms of this mouse model. In addition, P-JNK level is higher in females compared to male tg mice, underlying a sexual dimorphism in the JNK pathway activation. The behavioral studies highlight that tg females present major cognitive and locomotor defects, strongly correlated with a more severe synaptic injury, in comparison to tg male. Notably, at the dendritic spine level, JNK is powerfully activated and its level reveals a sexual dimorphism that is coherent with behavioral defects and spine pathology. The P301L's synaptic pathology is characterized by a strong increase of P-PSD95/PSD95 and P-JNK/JNK ratios and by an augmented level of cleaved-Caspase-3 and a decrease of Drebrin level in the post-synaptic elements. These results suggest that JNK plays a key role in synaptopathy of P301L mice. Importantly, until now, there are any efficient treatments against synaptic pathology and JNK could represent an interesting target to tackle P-Tau-induced synaptic pathology. It will be important to test specific JNK inhibitors to verify their potential neuroprotective effect.
Neuroscience, 2018; 393

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BOARD NUMBER: S05-578

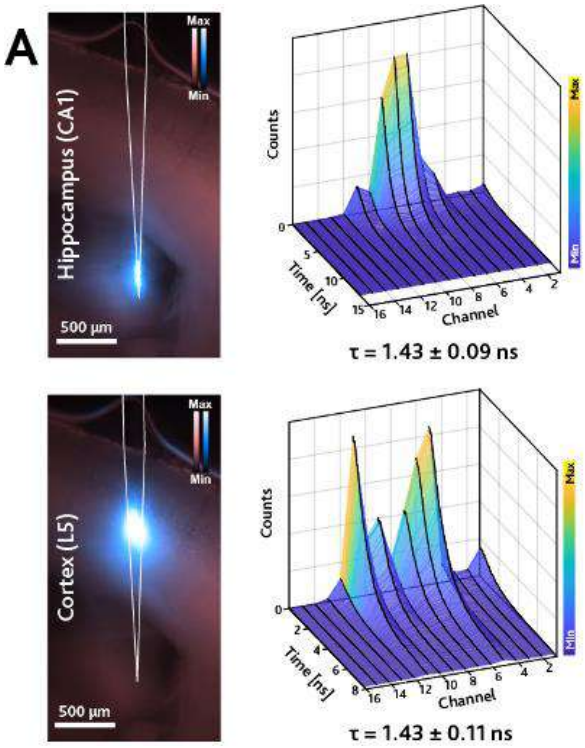
AUGMENTING THE CAPABILITIES OF DEPTH-RESOLVED FIBER PHOTOMETRY IN BRAIN TISSUE WITH TAPERED OPTICAL FIBERS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Marco Bianco[1,2], Marco Pisanello[3], Antonio Balena[4,5], Cinzia Montinaro[2,6], Filippo Pisano[7], Barbara Spagnolo[8], Leonardo Sileo[3], Bernardo Sabatini[9], Massimo De Vittorio[2], Ferruccio Pisanello[2]
[1]Università del Salento, Dipartimento Di Ingegneria Dell'innovazione, Lecce, Italy, [2]Istituto Italiano di Tecnologia, Center For Biomolecular Nanotechnology, Arnesano (Lecce), Italy, [3]Fondazione Istituto Italiano di Tecnologia, Center For Biomolecular Nanotechnologies, Arnesano (LE), Italy, [4]Sorbonne Université, Laboratoire Kastler Brossel, Paris, France, [5]Istituto Italiano di Tecnologia, Center For Biomolecular Nanotechnologies, Arnesano (Lecce), Italy, [6]Università del Salento, Dipartimento Di Scienze E Tecnologie Biologiche E Ambientali, Lecce, Italy, [7]Italian Institute of Technology, Center For Biomolecular Nanotechnology, Arnesano (Lecce), Italy, [8]Fondazione Istituto Italiano di Tecnologia, Center For Biomolecular Technologies, Arnesano (LE), Italy, [9]Howard Hughes Medical Institute - Harvard Medical School, Department Of Neurobiology, Boston, MA, United States of America

Genetically encoded optical actuators and indicators of neural activity allowed interfacing with the mammalian brain by means of light, boosting the development of novel implantable multifunctional neural interfaces able to interrogate neural circuits with cell-type specificity. This generated the need of novel methods to multiplex fluorescence signals collected from the scattering brain tissue with spatial resolution, with optical demultiplexing typically based on space and/or time diversity. We have recently demonstrated that exploiting mode-division multiplexing and demultiplexing in tapered fibers allows detecting optical signal with depth-resolution in the living mouse brain in free-moving animals [*Nat. Methods 16, (2019)*]. In this work we describe how this method can be applied in the context of fluorescence lifetime photometry [*Front. Neurosci. 13, (2019)*], introducing the concept of Depth-Resolved Fluorescence Lifetime Photometry (DR-FLiP)[*Biomed. Opt. Express 12, 993 (2021)*], which exploit mode diversity to augment the capabilities of depth-resolution with time-correlated single photon counting detectors. The approach is also combined with an unconventional far-field detection method based on specific orthogonalization of the Fourier space of the optical fiber output plane[*APL Photonics (2022) doi:10.1063/5.0073594*], paving the way for fully exploiting mode-division in optical neural interfaces based on tapered

fibers.


A
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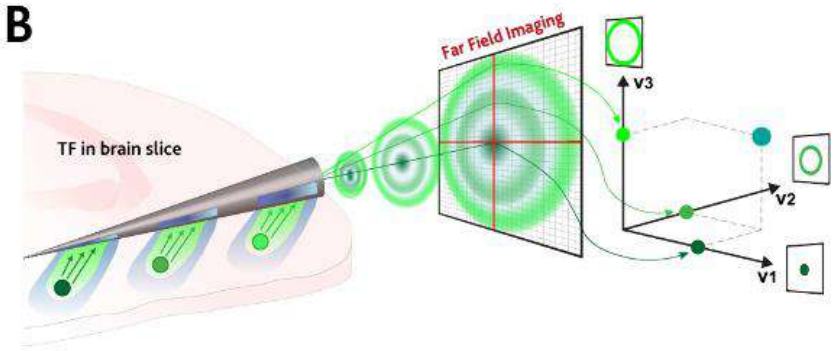
Cortex (L5)
τ = 1.43 ± 0.11 ns


B
Far Field Imaging
TF in brain slice
V1
V2
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BOARD NUMBER: S05-579

CONTINUOUS TRACKING OF METABOLIC CHANGES IN A TRAUMATICALLY INJURED BRAIN USING A MICRODIALYSIS COUPLED TO MID-INFRARED SENSOR

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Chisomo Zimphango[1], Farah Alimagham[1], Monica Killen[1], Agnieszka Zakrzewska[1], Adam Young[1], Núria Marco-García[1], Tanya Hutter[2], Keri Carpenter[1], Peter Hutchinson[1]
[1]University of Cambridge, Clinical Neurosciences, Cambridge, United Kingdom, [2]University of Texas, Walker Department Of Mechanical Engineering, Austin, United States of America

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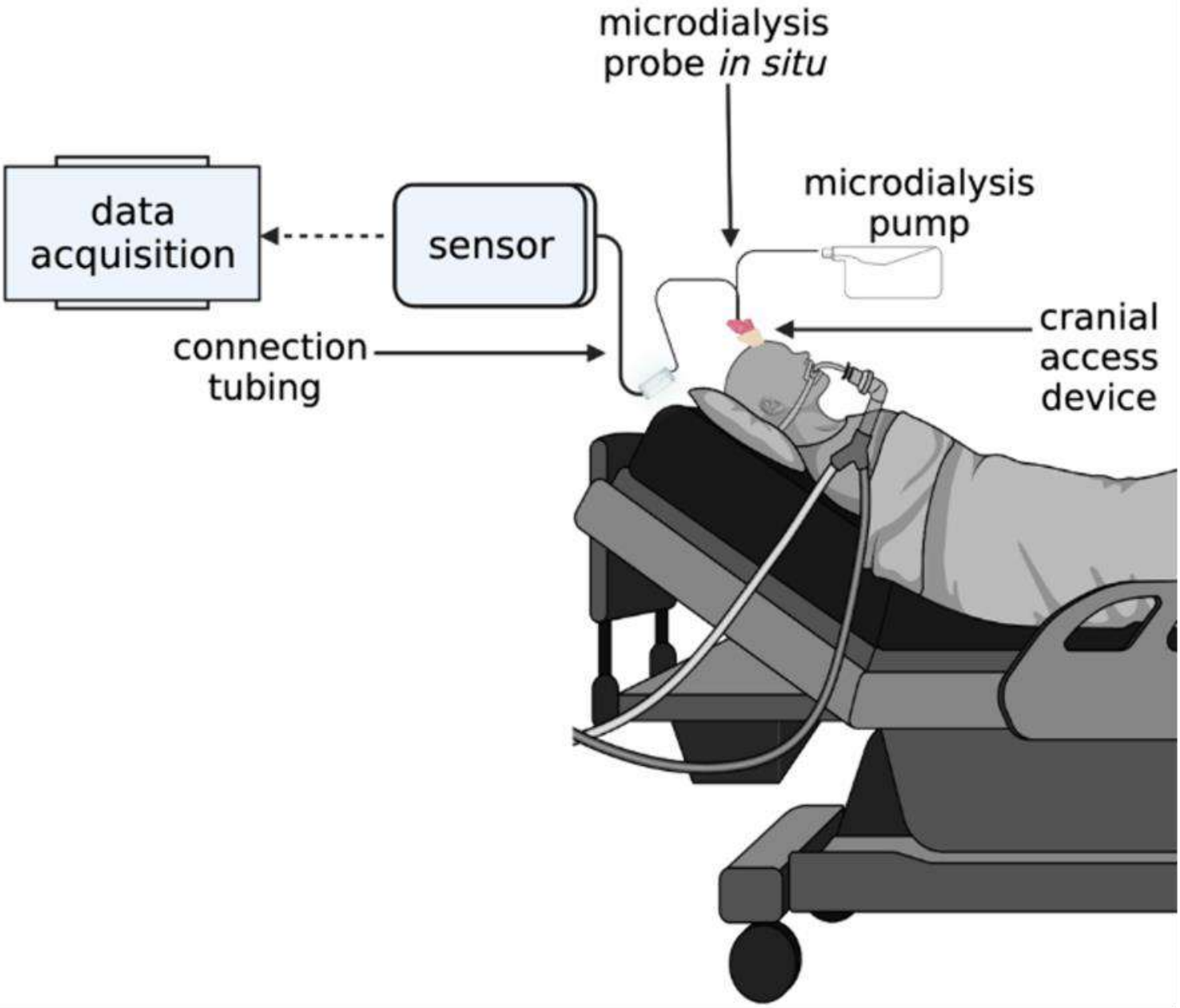

Cerebral metabolic changes in an injured brain can be rapid and dynamic. Therefore, it is imperative that these changes are continuously monitored, thereby enabling timely clinical interventions to treat adverse brain metabolism and consequently improving patients' outcomes. We have integrated cerebral microdialysis (CMD) with a novel mid-infrared (mid-IR) sensor, a system developed by our group, to continuously track dynamic changes in an in-vitro model of brain, and in the brains of TBI patients in the neurocritical care unit. For in vitro studies, CNS perfusion fluid was pumped at 0.3 mL/min, using an M Dialysis 107 pump, through a CMD catheter (M Dialysis 71, 100 kDa cutoff) sited in an external solution composed of CNS perfusion fluid plus human serum albumin (5 mg/mL) and sodium azide (0.05%), to which pure glucose, lactate and pyruvate were added. The microdialysates from this in-vitro brain model were continuously delivered to the mid-IR detector for online analysis. For clinical studies, adult patients with severe TBI (severity evaluated using CT) were assessed for eligibility. Four patients were monitored online using CMD coupled to the mid-IR sensor, for various periods. Following spectral analysis, the findings demonstrate the accuracy and precision of the technology in continuously quantifying cerebral metabolites of a traumatically injured brain. Conclusively, this technology fulfils the clinical need to continuously measure relevant clinical

metabolites albeit more studies are needed to improve its perfomance. Future studies are underway to further refine this technology for enhanced cerebral metabolic change measurements. *Funding:* NIHR HTC, MRC CiC, NIHR i4i

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BOARD NUMBER: S05-580

MOLECULAR CHARACTERIZATION OF ULTRASOUND SENSITIVE PRIMARY SENSORY NEURONS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Elena Brunet[1,2], Sungjae Yoo[2], Eric Debieu[1], Olivier Macherey[1], Emilie Franceschini[1], Aziz Moqrich[2]
[1]Aix-Marseille univ., CNRS, Centrale Marseille, Laboratoire de Mécanique et d'Acoustique, UMR 7031, Turing Center For Living Systems, Marseille, France, [2]Aix-Marseille univ., CNRS, Institut de Biologie du Développement de Marseille, UMR 7288, Turing Center For Living Systems, Marseille, France

Focused UltraSound (US) is a promising noninvasive technology for stimulating neuronal activity. However, the underlying mechanisms are not well understood. At the cellular scale, US-evoked responses have generally been assessed on transfected cells or on brain slices by recording membrane currents or calcium activity. This study aims to explore the direct stimulation of sensory neurons (dorsal root ganglion DRGs and spiral ganglion SGNs) by combining focused US with live-cell calcium imaging. An experimental set-up based on calcium imaging has been developed to monitor changes in calcium signals in individual DRG neurons subjected to US stimuli. The US transducer was positioned with a tilted angle to reduce interference/standing waves between the transducer and the cell culture dish. The US stimulus consisted of a 20 MHz sinusoidal signal with a peak acoustic pressure of 3.5 MPa, and a pulse duration of 1 ms. Calcium images were recorded before, during and after US stimulation. We found that US activated 39 % of DRG neurons (N=94) and 64 % of SGN neurons (N=108). These results demonstrate that focused ultrasound at 20 MHz is capable of activating a small fraction of DRG neurons. This could be explained by the molecular diversity of the two neuronal types considered: DRGs possessing a greater diversity than SGNs. To test this hypothesis, US-positive DRGs and SGNs, and US-negative DRGs were collected and subjected to single cell RNA sequencing to identify the identity of US sensitive neurons and to perhaps unravel candidate genes encoding proteins responsible for US responses.

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BOARD NUMBER: S05-581

ILLUMINATING THE MESOSCALE CONNECTOME: A 100-FIBER INFRARED NEURAL STIMULATION SYSTEM

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Kenneth Schriver[1], Ying Zhang[2], Yipeng Liu[2], Anna Wang Roe[2]
[1]Zhejiang University, Brain Science And Brain Medicine, Hangzhou, China, [2]Zhejiang University, Interdisciplinary Institute Of Neuroscience And Technology, Hangzhou, China

The combination of infrared neural stimulation (INS) with other modalities such as fMRI or optical imaging creates new capabilities for mapping functional mesoscale connectomes in the human and non-human primate brain. To date, most work in this area has been done with one to several optical fibers in contact with cortex or inserting a single fiber into the brain to stimulate regions of interest along the penetration track. To further explore the potential of INS, we have expanded the cortical surface stimulation technique significantly beyond methods using single fibers or linear arrays. We describe here the development and evaluation of a multifiber “bundle” capable of stimulating 100 individual 200 µm diameter sites within a local region of interest (ROI). The fiber bundle is mounted to achieve contact with cortex through a relatively small (5mm diameter) craniotomy. In addition to stimulation of individual sites within the ROI, this multifiber INS system allows exploration of paradigms (such as simultaneous stimulation of multiple sites or programmed sequential stimulation of different sites) to activate different domains in a specific temporal pattern. Preliminary evaluations have been conducted on visual cortex with the fiber bundle mounted in contact with feature-specific functional domains on one hemisphere while monitoring networks of mesoscale BOLD response at brainwide scale. This approach is the first to enable mapping ~100 functionally specified mesoscale networks within a single primate brain.

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MANIPULATION OF NETWORK ACTIVITY IN 3D SPINAL EXPLANTS BY SINGLE CELL LIGHT-ACTIVATION

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Agnes Thalhammer[1], Mario Fontanini[1], Jiuyun Shi[2], Denis Scaini[1], Ljiljana Fruk[3], Bozhi Tian[2], Laura Ballerini[1]
[1]Scuola Internazionale Superiore di Studi Avanzati, Neuroscience Area, Trieste, Italy, [2]University of Chicago, Department Of Chemistry, Chicago, United States of America, [3]University of Cambridge, Dept For Chemical Engineering And Biotechnology, Cambridge, United Kingdom

Selective stimulation of the spinal cord has been in use for decades as treatment for chronic pain. We explored here what implications stimulating single cells might have in network processing by exploiting silicon-based nanoscale photodiodes to optically modulate identified neurons in mammalian spinal cord explants. In order to record activity at a network level, we make use of neuronally expressed genetically encoded Ca^{2+} indicator GCaMP7f. We found that activating single excitatory or inhibitory neurons with near infrared-light stimulation differently affects sensory circuit processing in the dorsal horn: Selective stimulation of a single excitatory neuron can cause potentiation of network activity, reminiscent of 'Central Sensitisation' in chronic pain signaling, the molecular mechanism underlying pain hypersensitivity. Conversely by optically activating single GABAergic interneurons, we could observe a temporary inhibition of resting network activity. Our study introduces nanomaterial-based neural interfaces for optically and selectively stimulating neurons with single cell precision and low invasiveness, a tool with the potential to providing new perspectives in linking brain cell activity to specific behavioral outcome

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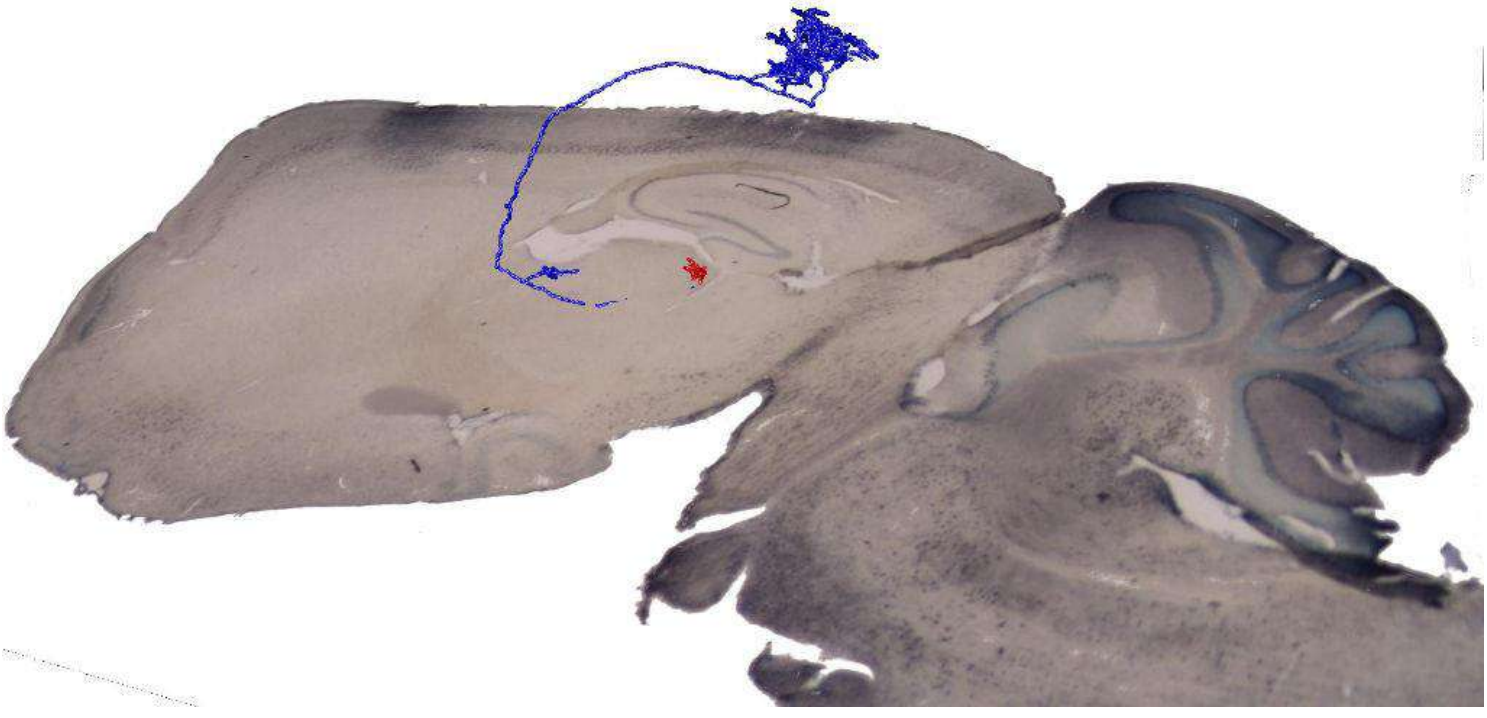
NEW TOOLS FOR THE REGISTRATION OF SERIAL SECTION-TRACED NEURONAL MORPHOLOGIES TO THE ALLEN MOUSE COMMON COORDINATE FRAMEWORK

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Rembrandt Bakker[1,2], Maria Carla Piastra[3], Maria Garcia-Amado[4], Mario Rubio-Teves[4], Nestor Timonidis[1], Francisco Clascá[4], Paul Tiesinga[1]
[1]Radboud University Nijmegen, Neuroinformatics, Donders Institute, Nijmegen, Netherlands, [2]Jülich Research Centre, Institute Of Neuroscience And Medicine (inm-6) And Institute For Advanced Simulation (ias-6) And Jara-institute Brain Structure-function Relationships (inm-10), Jülich, Germany, [3]University of Twente, Institute For Technical Medicine, Enschede, Netherlands, [4]School of Medicine. Autonomous University of Madrid, Department Of Anatomy And Neuroscience, Madrid, Spain

The Allen Common Coordinate Framework version 3 (CCF3) is a standard space for sharing mouse brain data. In recent years, large datasets of 3D long-range projection neurons (LRPN) scattered across the brain and acquired with high throughput techniques were shared in CCF3 [1,2]. Our aim is to augment this with LRPNs from labs that study specific brain circuits. In contrast to the 'industrial' approach that works with 3D-scanned data, these labs typically use Neurolucida to trace neurons from mounted and dried serial brain sections. This results in highly accurate morphologies that often come with functionally relevant metadata (numbers/sizes of terminal boutons, relation to specific tissue microdomains, electrophysiological data or response to experimental manipulations, precise location of cortical layers, etc.) that are lacking in the high-throughput data. Registration to CCF3 involves the warping of a 2D slice-stack into a 3D anatomical volume. If tissue sections are damaged, we use manual initial alignment [3] to find corresponding sections in CCF3, followed by deformable 2D-2D registration. When tissue sections are clean, we automatically register them into a volume [4], followed by deformable 3D-3D registration. In both cases, we apply a final step that aligns known cortical layer borders with the corresponding surfaces in CCF3. The tools are shared via the Jupyterlab extension of the EBRAINS platform [5].



Supported by FLAG-ERA grant NeuronsReunited (NWO 680-91-318, MICINN-AEI PCI2019-111900-2), and by EU H2020 grant agreement 945539 (HBP SGA3). 1. eLife (2016) https://doi.org/10.7554/eLife.10566
2. Nature (2021) https://doi.org/10.1038/s41586-021-03941-1
3. PLOS-One (2019) https://doi.org/10.1371/journal.pone.0216796
4. Neuroinformatics (2016) https://doi.org/10.1007/s12021-015-9286-1
5. https://wiki.ebrains.eu/bin/view/Collabs/morphology-atlas-registration-v2

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BOARD NUMBER: S05-584

PERIPHERAL NERVE REGENERATION: IN VITRO MODEL

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Zuzana Michalová[1], Zuzana Dzurjaskova[2], Ivo Vanický[2]
[1]Pavol Jozef Šafárik University, Department Of Pharmacology, Košice, Slovak Republic, [2]Biomedical Research Center SAS, Institute Of Neurobiology, Košice, Slovak Republic

The study of peripheral nerve repair and regeneration is important in the light of the high clinical incidence of nerve lesions. Although peripheral nerve injuries are capable of some degree of regeneration, the clinical outcome and the functional recovery is rarely complete. Therefore, a number of therapeutic models have been investigated, from cell line-based models to in vivo animal models. However, the need to limit the use of animals in biomedical research as much as possible supports the search for other possibilities. Our study describes in vitro model of peripheral nerve regeneration based on organotypic ex vivo-based models that has much lower impact on animal wellbeing in comparison to in vivo models. Spinal cord sections from P3 rats were used to innervate peripheral sciatic nerve segments. Axon growth across nerve segments can be repeatedly imaged by fluorescence microscopy to define the rate of regeneration, and parental neurons can be labelled in a retrograde manner to identify contributing neurons. The whole mount staining was used for the overall visualization of the axon – spinal cord interactions. Its use allows better observation of regeneration processes as a whole than in conventional staining. This model can be modified using any other peripheral nerves or treated with antibodies and growth factors. The regeneration environment is controlled to an extent that is not possible in vivo, and the use of experimental animals is decreased. The flexibility and control that this technique offers should therefore make it a useful tool for studying the biology of regeneration.

Pubmed:
34979375: Vanický I, Blaško J, Končeková J, Dzurjašková Z, Michalová Z, Székiová E
Formaldehyde-hardened albumin as a non-penetrating embedding matrix for frozen and vibratome sectioning.
In this paper, we describe a protocol for a non-penetrating embedding matrix that can be used for frozen or vibratome sectioning of various formaldehyde-fixed tissue specimens. In our experiments, we wanted to prepare thin frozen sections from miniature specimens for fluorescent staining. As we could not achieve satisfactory results with any of the previously published methods, we have tried to modify the existing protocols, and systematically evaluated the effect of these modifications on the properties of the embedding matrix. The resulting protocol is simple, the matrix gets firmly attached to the tissues, does not cause autofluorescence and enables preparing extremely thin frozen sections. The matrix can be used for 1, embedding miniature specimens from problematic tissues to enable cutting very thin frozen sections, 2, grouping multiple specimens into one large block for simultaneous processing, and 3, dispersing single cells and preparing cell blocks for frozen sectioning.
Acta Histochem, 2022; 124
34959454: Kello M, Kuruc T, Petrova K, Goga M, Michalova Z, Coma M, Rucova D, Mojzis J
Pro-Apoptotic Potential of (L.) Extract and Isolated Physodic Acid in Acute Lymphoblastic Leukemia Model In Vitro.
Acute lymphoblastic leukemia (ALL) is the most frequently diagnosed type of leukemia among children. Although chemotherapy is a common treatment for cancer, it has a wide range of serious side effects, including myelo- and immunosuppression, hepatotoxicity and neurotoxicity. Combination therapies using natural substances are widely recommended to attenuate the adverse effects of chemotherapy. The aim of the present study was to investigate the anti-leukemic potential of extract from the lichen (L.) (PSE) and isolated physodic acid (Phy) in an in vitro ALL model. A screening assay, flow cytometry and Western blotting were used to analyze apoptosis occurrence, oxidative stress, DNA damage and stress/survival/apoptotic pathway modulation induced by the tested substances in Jurkat cells. We demonstrate for the first time that PSE and Phy treatment-induced intrinsic caspase-dependent cell death was associated with increased oxidative stress, DNA damage and cell cycle arrest with the activation of cell cycle checkpoint proteins p53, p21 and p27 and stress/survival kinases p38 MAPK, JNK and PI3K/Akt. Moreover, using peripheral T lymphocytes, we confirmed that PSE and Phy treatment caused minimal cytotoxicity in normal cells, and therefore, these naturally occurring lichen secondary metabolites could be promising substances for ALL therapy.

Pharmaceutics, 2021; 13
31177117: Michalová Z, Čoma M, Kičová M, Gabzdilová J, Dedinská K, Guman T, Hájiková M, Veselinyová D, Giertlova M, Gál P, Šarišský M
Overexpression of Galectin-3 in Chronic Lymphocytic Leukemia Is Associated With 17p Deletion: A Short Report.
Galectins belong to the family of galactose-binding proteins known to play an important role in the processes of cell proliferation, differentiation, migration and neoplastic progression. Herein, we studied the expression of galectin-3 (Gal-3) in chronic lymphocytic leukemia (CLL).
Anticancer Res, 2019; 39
32266820: Kicova M, Michalova Z, Coma M, Gabzdilova J, Dedinska K, Guman T, Bernatova S, Hajikova M, Giertlova M, Veselinyova D, Sarissky M
The expression of CD73 on pathological B-cells is associated with shorter overall survival of patients with CLL.
CD73 is a membrane-bound enzyme that catalyzes the extracellular conversion of adenosine monophosphate to adenosine. Adenosine is thought to play a role in promoting tumor growth and survival together with suppressing the host immune responses, which contribute to the multistep process of tumorigenesis. Here, we studied the expression of this antigen in chronic lymphocytic leukemia (CLL). The expression of CD73 was analyzed by multiparametric flow cytometry on normal and pathological B-cells from peripheral blood and bone marrow samples from 71 patients with CLL. Pathological B-cells expressed significantly lower levels of CD73 than normal B-cells ($p<0.01$). Patients with splenomegaly showed a higher expression of CD73 on pathological B-cells than patients without splenomegaly ($p<0.05$). The expression of CD73 also correlated with beta-2-microglobulin levels ($p<0.05$). Clinically, patients with higher levels of CD73 versus those with lower expression presented with shorter overall survival (median OS of 65 vs. 113 months, $p<0.05$). Our data indicate that CD73 may play a role in CLL pathophysiology, is correlated with poor clinical and biological prognostic factors and may be of potential value as a prognostic marker and therapeutic target.
Neoplasma, 2020; 67

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3D-IMAGING REVEALS CONSERVED CEREBROSPINAL FLUID DRAINAGE VIA MENINGEAL LYMPHATIC VASCULATURE IN MICE AND HUMANS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Laurent Jacob[1,2], Jose De Brito Neto[1], Stephanie Lenck[1], Celine Corcy[3], Farhat Benbelkacem[4], Luiz Henrique Geraldo[2], Yunling Xu[2], Jean-Mickael Thomas[1], Marie-Renee El Kamouh[1], Marie-Claude Potier[1], Stephane Haik[1], Anne Eichmann[2], Jean-Leon Thomas[1]
[1]Paris Brain Institute, Inserm 1127, Paris, France, [2]PARCC Paris research Cardiovacula rcenter, Vascular Development And Disease, Paris, France, [3]Pitie-Salpêtrière Hospital, Sorbonne University, Neuroradiology, Paris, France, [4]Siemens, Healthcare Sas, Saint-Denis, France

Meningeal lymphatic vessels (MLVs) contribute to waste product elimination and immune surveillance in brain tissues. MLVs were identified in the dorsal and caudo-basal regions of the dura mater, where they ensure the clearance of cerebrospinal fluid (CSF). Whether MLVs exist in the complex anterior part of the murine and human skull, and how they connect with the glymphatic system and extracranial lymphatic vasculature remained unclear. Here, we generated three-dimensional (3D) maps of MLV drainage by light-sheet fluorescence microscopy (LSFM) imaging of mouse whole-head preparations following fluorescent OVA-A^{555} tracer injections into the CSF. In humans, we performed real-time magnetic resonance vessel wall imaging (MR-VWI) after systemic gadobutrol injections. We observed a conserved 3D-anatomy of MLVs in mice and humans, and we discovered an extended anterior network around the dural cavernous sinus including multiple capillary beds and exit routes through the foramina of emissary veins. MR-VWI may provide a diagnostic tool for patients with CSF drainage defects and neurological diseases.

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RETRO-TANGO: A RETROGRADE CIRCUIT TRACING METHOD IN DROSOPHILA

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Altar Sorkaç[1], Rareș Moșneanu[1], Anthony Crown[1], Doruk Savaş[1], Mustafa Talay[2], Gilad Barnea[1]
[1]Brown University, Neuroscience, Providence, United States of America, [2]Howard Hughes Medical Institute / Harvard University, Molecular And Cellular Biology, Cambridge, United States of America

Neural circuits are essential for proper animal behavior. Understanding how these circuits are wired is key to deciphering how nervous systems function. Transsynaptic tracing methods, such as *trans*-Tango, are crucial tools in this endeavor. Although a couple of anterograde tracing methods and a targeted retrograde tool have been developed in *Drosophila*, there is still need for a user-friendly retrograde tracing system that is devoid of user bias. Here we describe *retro*-Tango, a modified version of *trans*-Tango that enables retrograde circuit tracing and manipulation in the *Drosophila* nervous system. In this genetically encoded system, a membrane bound ligand, targeted at postsynaptic sites, activates its cognate receptor across the synapse, at the presynaptic site. This, in turn, triggers an intracellular signaling cascade that results in gene expression in the presynaptic neuron. Most importantly, due to the panneuronal expression of its elements, *retro*-Tango does not require the user to make any assumptions regarding connectivity. Therefore, it can be used not only to test hypotheses but also to generate new ones. We validate *retro*-Tango in the olfactory system, in sex-specific circuits, and in the central complex, a brain region responsible for integrating sensory information. For this, we trace connections between the periphery and the central nervous system (CNS) as well as connections within the CNS. Our experiments establish *retro*-Tango as a key method for circuit tracing and manipulation, filling an important gap in *Drosophila* neuroscience research.

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BOARD NUMBER: S05-587

DISYNAPTIC PROJECTIONS FROM THE CEREBELLAR NUCLEI TO THE NEUROMODULATORY SYSTEMS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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AIMS: The influence of the cerebellum on the neuromodulatory systems has received a surge of interest over the past decade. Nevertheless, detailed descriptions of the anatomy underlying the functional connections of the cerebellum with neuromodudatory centers are sparse at best. Well-known monosynaptic projections suggest the existence of disynaptic projections from the cerebellar nuclei (CN) to these modulatory centers, via midbrain areas like superior colliculus (SC) and ventrolateral periaqueductal grey (vlPAG). METHODS: We employed combinations of classical and viral tracing approaches. We injected in the CN, vlPAG and SC to elucidate disynaptic projections from the CN to a variety of modulatory targets. Using confocal scanning and manual quantification combined with stainings for GAD67 and VGLuT2, we investigated the net effect of the projections of interest. RESULTS: We identified disynaptic projections from the cerebellar nuclei via vlPAG and SC to the ventral tegmental area (VTA), Raphe Magnus (RpMg), and Substantia Nigra pars compacta (SNc). Via vlPAG we also found specific projections to locus coeruleus (LC) and basal forebrain (BF). We found the CN to have both excitatory and inhibitory projections to VTA and SNc, while the other targets (RpMg, LC, BF) seem to receive mostly excitatory projections. CONCLUSIONS: Anatomical investigation of disynaptic projections from cerebellum to the neuromodulatory systems support the hypothesis that the cerebellum exerts bidirectional control over such systems.

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THE SYNAPTIC ORGANIZATION OF THE HUMAN ENTORHINAL CORTEX: A 3D ELECTRON MICROSCOPY STUDY ON THE GATEWAY TO HIPPOCAMPUS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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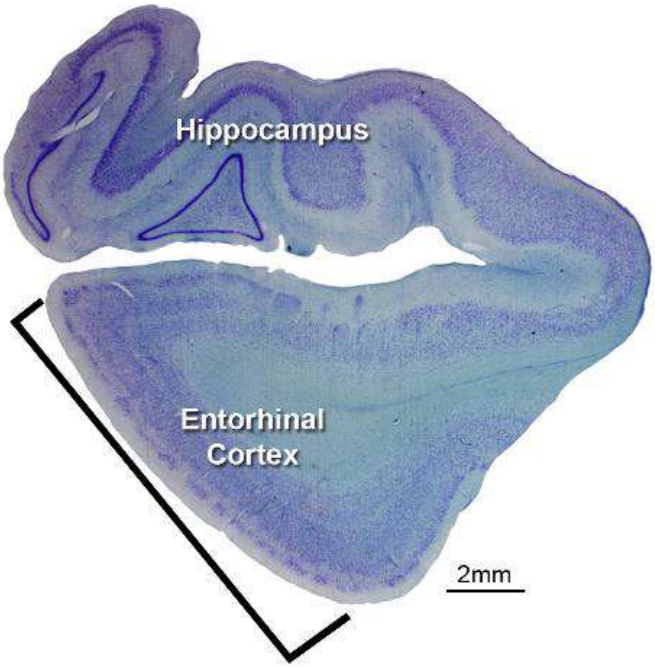
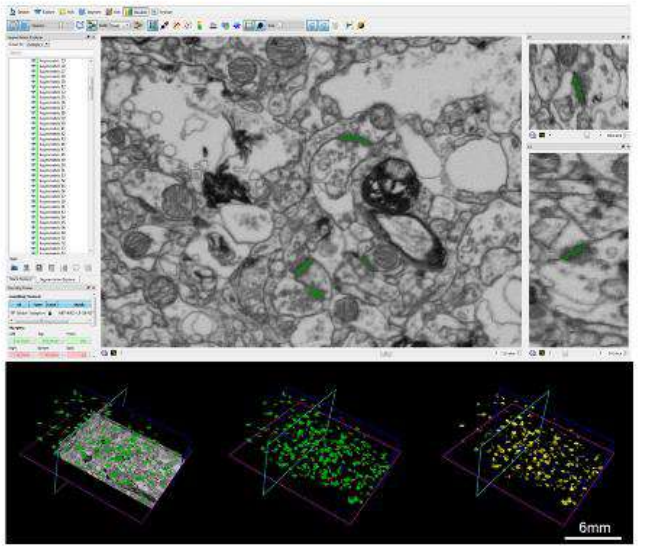
[1]Centro de Tecnología Biomédica, Universidad Politécnica de Madrid, Laboratorio Cajal De Circuitos Corticales, Madrid, Spain, [2]Consejo Superior de Investigaciones Científicas (CSIC), Instituto Cajal, Madrid, Spain, [3]Universidad Autónoma de Madrid, Programa De Doctorado En Neurociencia, Madrid, Spain

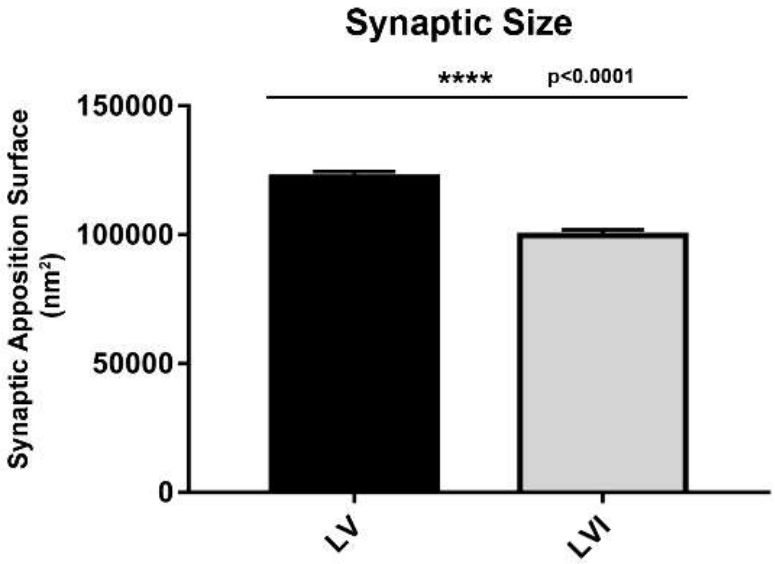
Introduction/Aim: Over the last century, neuroscience has struggled to understand how synaptic organization contributes to the functional organization of the brain. In this work, we have used Focused Ion Bean / Scanning Electron Microscopy (FIB/SEM) to study the synaptology of layer V and VI of the human entorhinal cortex (EC, Figure 1). This area, located in the medial temporal lobe, is essential for memory functions and hippocampal connectivity, and it is one of the first regions affected in Alzheimer´s disease. However, very little is known about the synaptic organization of this region. **Methods:** A total of 2502 synapses from 3 human autopsies were three-dimensionally (3D) reconstructed using FIB/SEM and EspINA software to analyze synaptic density, proportions of asymmetric and symmetric synapses, spatial distribution of synapses, synaptic size, synaptic shape and postsynaptic elements (Figure 1). **Results:** Differences between layers were found in synaptic size, being bigger in layer V (Figure 2). The analysis of postsynaptic elements showed that in layer V most synapses were established on dendritic shafts, whereas in layer VI, synapses were predominantly formed on dendritic spine heads (Figure 2). No significant differences were found regarding synaptic density or other morphological characteristics. **Conclusions:** This study represents the first detailed description of the synaptic organization of layers V and VI from the human EC. Our results emphasize that synaptic organization is layer-specific in this region. Further analyses are being performed to include other layers, in order to generate a detailed synaptic map of the human EC. **Figure 1.**

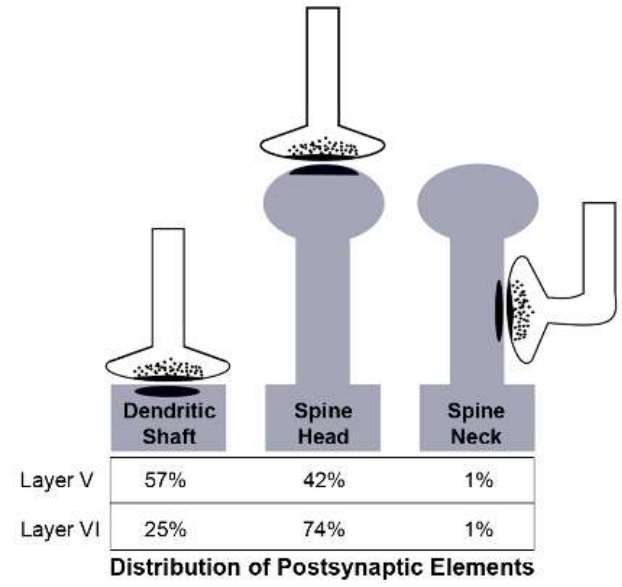




EspINA Software - Synaptic Analysis and 3D Reconstruction

Figure 2.





	Dendritic Shaft	Spine Head	Spine Neck
Layer V	57%	42%	1%
Layer VI	25%	74%	1%

Distribution of Postsynaptic Elements

Pubmed:

34039651: Domínguez-Álvaro M, Montero-Crespo M, Blazquez-Llorca L, Plaza-Alonso S, Cano-Astorga N, DeFelipe J, Alonso-Nanclares L

3D Analysis of the Synaptic Organization in the Entorhinal Cortex in Alzheimer's Disease.

The entorhinal cortex (EC) is especially vulnerable in the early stages of Alzheimer's disease (AD). In particular, cognitive deficits have been linked to alterations in the upper layers of EC. In the present report, we examined Layers II and III from eight human brain autopsies (four subjects with no recorded neurologic alterations and four AD cases). We used stereological methods to assess cortical atrophy of the EC and possible changes in the volume occupied by different cortical elements (neuronal and glial cell bodies; blood vessels; and neuropil). We performed 3D ultrastructural analyses of synapses using focused ion beam/scanning electron microscopy (FIB/SEM) to examine possible alterations related to AD. At the light microscope level, we found a significantly lower volume fraction occupied by neuronal bodies in Layer III and a higher volume fraction occupied by glial cell bodies in Layer II in AD cases. At the ultrastructural level, we observed that (1) there was a significantly lower synaptic density in both layers in AD cases; (2) synapses were larger and more complex in Layer II in AD cases; and (3) there was a greater proportion of small and simple synapses in Layer III in AD cases than in control individuals. These structural differences may play a role in the anatomic basis for the impairment of cognitive functions in AD.

eNeuro, 2021 May-Jun; 8

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BOARD NUMBER: S05-589

SYNAPTIC ORGANIZATION OF THE HUMAN CEREBRAL CORTEX: A 3D-ULTRASTRUCTURAL STUDY OF LAYER III

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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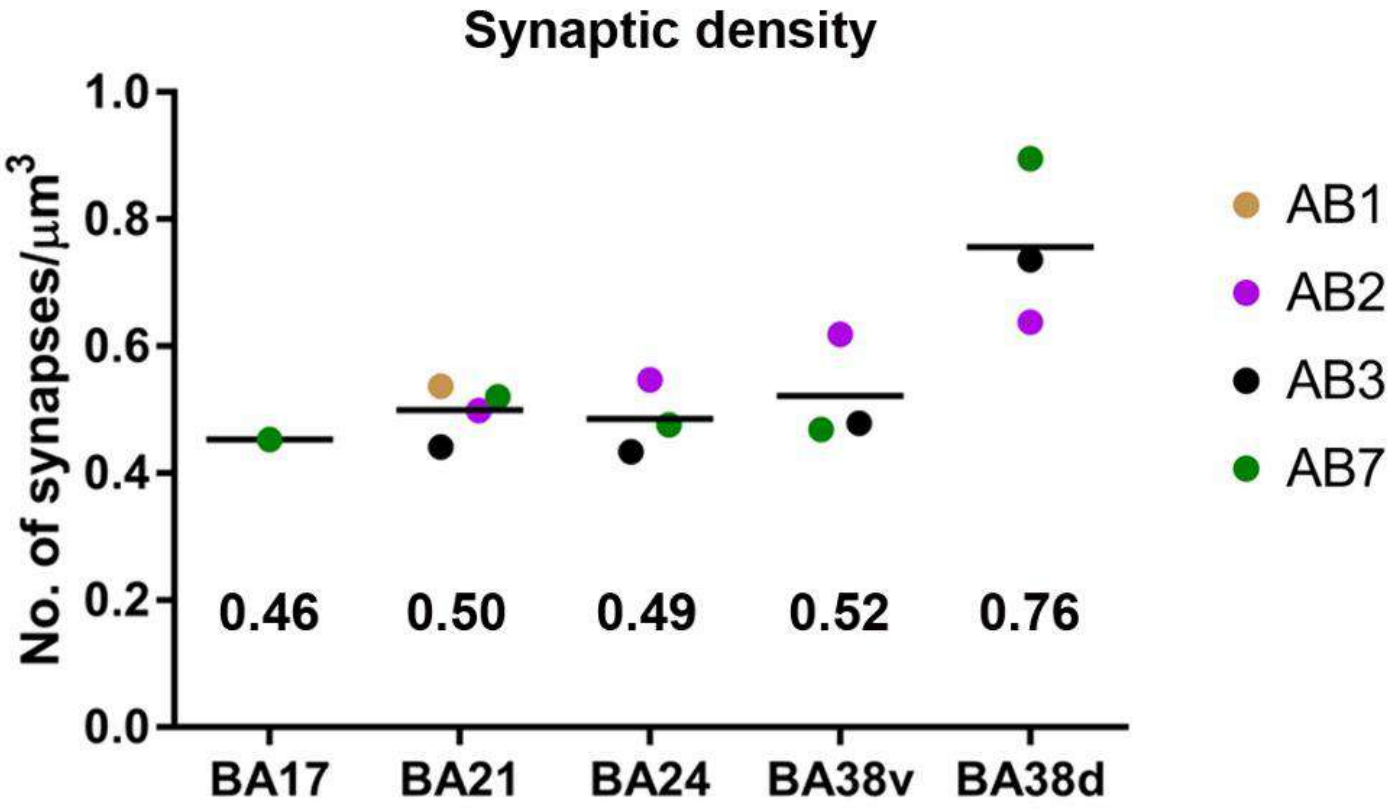
Introduction and aim Layer III pyramidal cell complexity as well as the macroscale connectivity of the human cortex increases from the unimodal primary cortices to the association areas. However, the synaptic organization of these cortical regions are not known. **Methods** We have performed a detailed three-dimensional (3D) ultrastructural analyses in the neuropil of layer III from brain tissue of four human control autopsies, using FIB/SEM technology. Brodmann area (BA) 17, BA21, BA24 and BA38 (ventral and dorsal) were examined. A total of 6877 synapses have been 3D-segmented to analyze the synaptic density, proportions of asymmetric (AS) and symmetric (SS) synapses, spatial distribution of synapses, synaptic size, synaptic shape and postsynaptic elements. **Results** Synaptic densities were similar in all analyzed regions, except in BA38-dorsal which showed a significant higher synaptic density (Figure 1). Besides, in all regions, the ratio of AS:SS synapses was similar (AS 93-95%: SS 7-5%), and the 3D-distribution of synapses fitted to a random distribution. Furthermore, in all regions AS were larger than SS, macular-shaped synapses were smaller than complex-shaped synapses, and they were the most frequent synaptic shape. BA38-dorsal displayed the smallest AS. Most of synapses were found on dendritic spines (range 67-75%), followed by dendritic shafts in all analyzed
regions.



Conclusions The present work suggests that there is not an association between the synaptic characteristics in layer III neuropil and the macroscale connectivity or the complexity of pyramidal cells; and that BA38-dorsal may present a different set of microscale-specific synaptic characteristics.

Pubmed:
33999122: Cano-Astorga N, DeFelipe J, Alonso-Nanclares L
Three-Dimensional Synaptic Organization of Layer III of the Human Temporal Neocortex.
In the present study, we have used focused ion beam/scanning electron microscopy (FIB/SEM) to perform a study of the synaptic organization of layer III of Brodmann's area 21 in human tissue samples obtained from autopsies and biopsies. We analyzed the synaptic density, 3D spatial distribution, and type (asymmetric/symmetric), as well as the size and shape of each synaptic junction of 4945 synapses that were fully reconstructed in 3D. Significant differences in the mean synaptic density between autopsy and biopsy samples were found (0.49 and 0.66 synapses/µm3, respectively). However, in both types of samples (autopsy and biopsy), the asymmetric:symmetric ratio was similar (93:7) and most asymmetric synapses were established on dendritic spines (75%), while most symmetric synapses were established on dendritic shafts (85%). We also compared several electron microscopy methods and analysis tools to estimate the synaptic density in the same brain tissue. We have shown that FIB/SEM is much more reliable and robust than the majority of the other commonly used EM techniques. The present work constitutes a detailed description of the synaptic organization of cortical layer III. Further studies on the rest of the cortical layers are necessary to better understand the functional organization of this temporal cortical region.
Cereb Cortex, 2021; 31
34039651: Domínguez-Álvaro M, Montero-Crespo M, Blazquez-Llorca L, Plaza-Alonso S, Cano-Astorga N, DeFelipe J, Alonso-Nanclares L
3D Analysis of the Synaptic Organization in the Entorhinal Cortex in Alzheimer's Disease.
The entorhinal cortex (EC) is especially vulnerable in the early stages of Alzheimer's disease (AD). In particular, cognitive deficits have been linked to alterations in the upper layers of EC. In the present report, we examined Layers II and III from

eight human brain autopsies (four subjects with no recorded neurologic alterations and four AD cases). We used stereological methods to assess cortical atrophy of the EC and possible changes in the volume occupied by different cortical elements (neuronal and glial cell bodies; blood vessels; and neuropil). We performed 3D ultrastructural analyses of synapses using focused ion beam/scanning electron microscopy (FIB/SEM) to examine possible alterations related to AD. At the light microscope level, we found a significantly lower volume fraction occupied by neuronal bodies in Layer III and a higher volume fraction occupied by glial cell bodies in Layer II in AD cases. At the ultrastructural level, we observed that (1) there was a significantly lower synaptic density in both layers in AD cases; (2) synapses were larger and more complex in Layer II in AD cases; and (3) there was a greater proportion of small and simple synapses in Layer III in AD cases than in control individuals. These structural differences may play a role in the anatomic basis for the impairment of cognitive functions in AD.
eNeuro, 2021 May-Jun; 8

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BOARD NUMBER: S05-590

GLYMPHATIC SYSTEM MODELLED AS PART OF A GUT-BRAIN AXIS ON-A-CHIP PLATFORM TO STUDY BRAIN FLUIDS CLEARANCE IN NEUROINFLAMMATION

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Aim A fluid-clearance pathway in the brain parenchyma, the glymphatic system (GS), was recently re-evaluated in relation to its role in neurodegeneration as controller of the dynamics of neurotoxic proteins. As neuroinflammation is a key feature of neurodegeneration, GS function in this scenario is relevant. We propose a GS on-a-chip-model allowing tunable levels of molecule clearance, to be integrated in a gut-brain axis platform which aims at studying the intestinal microflora impact on brain functionality. Methodology We applied interstitial flows inside our GS model featuring the blood-brain barrier (BBB) and the brain 3D parenchyma and then we coupled the GS to an on-a-chip gut epithelial barrier to simulate the gut-brain connection. The cells used were: intestinal epithelial cells (CaCo2); endothelial cells (bEND.3); astrocytes (C8D1A); neuroglioma cells (H4). We tracked bacteria lipopolysaccharides (LPS) dynamics when administered at gut level and measured downstream H4 inflammatory response under GS clearance. Results The GS model sustained pathophysiological flows and solutes clearance. The on-chip devices allowed long-term culture of all cells. Preliminary results showed inflammatory response inside the 3D brain model, after LPS stimulation, being modulated at different clearance levels suggesting an active role of fluid drainage in controlling neuroinflammatory response. Conclusions Our engineered GS is a suitable tool for modelling *in vitro* pathophysiological drainage of fluids inside the brain parenchyma in the context of microbial neuroinflammation. Acknowledgement Funding received from the European Research Council under the European Union's Horizon 2020 research and innovation program (Grant agreement No. 724734-MINERVA).

Pubmed:
33605282: Perottoni S, Neto NGB, Di Nitto C, Dmitriev RI, Raimondi MT, Monaghan MG
Intracellular label-free detection of mesenchymal stem cell metabolism within a perivascular niche-on-a-chip.
The stem cell niche at the perivascular space in human tissue plays a pivotal role in dictating the overall fate of stem cells within it. Mesenchymal stem cells (MSCs) in particular, experience influential microenvironmental conditions, which induce specific metabolic profiles that affect processes of cell differentiation and dysregulation of the immunomodulatory function. Reports focusing specifically on the metabolic status of MSCs under the effect of pathophysiological stimuli - in terms of flow velocities, shear stresses or oxygen tension - do not model heterogeneous gradients, highlighting the need for more advanced models reproducing the metabolic niche. Organ-on-a-chip technology offers the most advanced tools for stem cell niche modelling thus allowing for controlled dynamic culture conditions while profiling tuneable oxygen tension gradients. However, current systems for live cell detection of metabolic activity inside microfluidic devices require the integration of microsensors. The presence of such microsensors poses the potential to alter microfluidics and their resolution does not enable intracellular measurements but rather a global representation concerning cellular metabolism. Here, we present a metabolic toolbox coupling a miniaturised in vitro system for human-MSCs dynamic culture, which mimics microenvironmental conditions of the perivascular niche, with high-resolution imaging of cell metabolism. Using fluorescence lifetime imaging microscopy (FLIM) we monitor the spatial metabolic machinery and correlate it with experimentally validated intracellular oxygen concentration after designing the oxygen tension decay along the fluidic chamber by in silico models prediction. Our platform allows the metabolic regulation of MSCs, mimicking the physiological niche in space and time, and its real-time monitoring representing a functional tool for modelling perivascular niches, relevant diseases and metabolic-related uptake of pharmaceuticals.
Lab Chip, 2021; 21
33661580: Boeri L, Perottoni S, Izzo L, Giordano C, Albani D
Microbiota-Host Immunity Communication in Neurodegenerative Disorders: Bioengineering Challenges for In Vitro Modeling.

Human microbiota communicates with its host by secreting signaling metabolites, enzymes, or structural components. Its homeostasis strongly influences the modulation of human tissue barriers and immune system. Dysbiosis-induced peripheral immunity response can propagate bacterial and pro-inflammatory signals to the whole body, including the brain. This immune-mediated communication may contribute to several neurodegenerative disorders, as Alzheimer's disease. In fact, neurodegeneration is associated with dysbiosis and neuroinflammation. The interplay between the microbial communities and the brain is complex and bidirectional, and a great deal of interest is emerging to define the exact mechanisms. This review focuses on microbiota-immunity-central nervous system (CNS) communication and shows how gut and oral microbiota populations trigger immune cells, propagating inflammation from the periphery to the cerebral parenchyma, thus contributing to the onset and progression of neurodegeneration. Moreover, an overview of the technological challenges with in vitro modeling of the microbiota-immunity-CNS axis, offering interesting technological hints about the most advanced solutions and current technologies is provided.
Adv Healthc Mater, 2021; 10
33990954: Sardelli L, Perottoni S, Tunesi M, Boeri L, Fusco F, Petrini P, Albani D, Giordano C
Technological tools and strategies for culturing human gut microbiota in engineered in vitro models.
The gut microbiota directly impacts the pathophysiology of different human body districts. Consequently, microbiota investigation is an hot topic of research and its in vitro culture has gained extreme interest in different fields. However, the high sensitivity of microbiota to external stimuli, such as sampling procedure, and the physicochemical complexity of the gut environment make its in vitro culture a challenging task. New engineered microfluidic gut-on-a-chip devices have the potential to model some important features of the intestinal structure, but they are usually unable to sustain culture of microbiota over an extended period of time. The integration of gut-on-a-chip devices with bioreactors for continuous bacterial culture would lead to fast advances in the study of microbiota-host crosstalk. In this review, we summarize the main technologies for the continuous culture of microbiota as upstream systems to be coupled with microfluidic devices to study bacteria-host cells communication. The engineering of integrated microfluidic platforms, capable of sustaining both anaerobic and aerobic cultures, would be the starting point to unveil complex biological phenomena proper of the microbiota-host crosstalks, paving to way to multiple research and technological applications.
Biotechnol Bioeng, 2021; 118

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3D-MICROCHANNALLED SCAFFOLD FOR PERIPHERAL NERVE REGENERATION

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Yuliya Dobropolska[1,2], Sergei Grebenyuk[3], Volodymyr Medvedev[4,5], Taras Petriv[6], Andrii Savytskyi[1], Tetyana Pivneva[2], Adrian Ranga[3], Pavel Belan[7], Nana Voitenko[5]
[1]Kyiv Academic University, Biophysics And Molecular Biology, Kyiv, Ukraine, [2]Bogomoletz Inst. of Physiology of Natl. Acad. of Sci. of Ukraine, Ukraine, Department Of Sensory Signalization, Kyiv, Ukraine, [3]KU Leuven, Department Of Mechanical Engineering, Leuven, Belgium, [4]Bogomolets National Medical University, Neurosurgery, Kyiv, Ukraine, [5]Dobrobut Academy, Scientific Center, Kyiv, Ukraine, [6]The State Institution Romodanov Neurosurgery Institute, Restorative Neurosurgery Department, Kyiv, Ukraine, [7]Bogomoletz Institute of Physiology, Department Of Molecular Biophysics, Kyiv, Ukraine

Peripheral nerve injury (PNI) is a significant medical and social problem and is characterized by long-term limb dysfunction and a high level of disability. Treating PNIs represents a major challenge in reconstructive surgery and regenerative medicine. Here, we investigated the efficacy of motor and sensory functional recovery in a rat model of sciatic nerve injury using a novel polyethyleneglycol (PEG)-based 3Dscaffolds featuring tightly packed oriented microchannels. Addition of pentaerythritol triacrylate (PETA) as a crosslinker improved mechanical and cell adhesion properties of the scaffolds. A unilateral 6-mm sciatic nerve defect was repaired using the 3D micro-channelled scaffold, hollow silicon conduit or neurorrhaphy. Recovery of sensory and motor functions in the injured limb during 6 months after the surgery was assessed by mechanical and thermal sensitivity tests as well as by gait analysis. We found that that the rate and degree of functional recovery were significantly higher in the group with the 3D microchannelled scaffolds than in the ones with other treatment choices. The implantation also provided for successful outgrowth of neuronal and vascular tissues in the scaffold microchannels as shown by confocal microscopy and immunochemistry. Thus, the 3D microchannelled scaffold implanted in the site of sciatic nerve injury, may considerably restore motor and sensory function of the rat's posterior paretic leg.
Supported by NASU grant 0118U007345 and NRFU grant 2021.01/0328

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TEMPORAL TUNING OF PRESYNAPTIC SIGNALS IN CORTICAL AND SPINAL AXONS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Milos Radivojevic[1], Stefan Engblom[2], Yu-Fang Huang[1], Olga Netsyk[3], Anna Rostedt Punga[1]
[1]Uppsala University, Department Of Medical Sciences, Uppsala, Sweden, [2]Uppsala University, Department Of Information Technology, Uppsala, Sweden, [3]Stockholm University, Department Of Biochemistry And Biophysics, Stockholm, Sweden

Intro | Axons are neuronal processes specialized for conduction of action-potentials. Mainly due to technical difficulties to monitor axonal conduction, axonal information processing has been neglected, and axons are classically seen as cables that conduct action-potentials in an all-or-none fashion. Later studies have challenged this view and suggested that axons have much more complex roles than previously thought. Advanced high-density microelectrode arrays (HD-MEAs) bear a potential to reveal complex axonal functionalities. These arrays have not yet been fully exploited, arguably due to lack of methods for complex data analysis and visualization. **Aims** | First part of this project aims at developing methods for automatic tracking and visualisation of signal conduction in mammalian axons. Second part aims at using these methods to investigate conduction dynamics in axons. **Methods** | We cultured rat primary cortical and motor neurons directly on HD-MEAs comprising 26,400 densely-packed microelectrodes. Spike-sorting algorithms were used to reconstruct activities of individual neurons. Kalman filter was used to compute trajectories of axonal conduction. **Results** | We developed a method for tracking axonal conduction in cortical and motor neurons. Spatiotemporal features of tracked signals enabled to reconstruct axonal 'functional morphology'. Tracked trajectories revealed information about waveforms of axonal action-potentials, local conduction velocities and times at which action-potentials arrive at axonal terminals. We found that longer axonal paths provide faster signal conduction compared to shorter paths found in the same neuron. **Conclusions** | Axons tune their local conduction velocities to reduce discrepancies among times at which action-potentials arrive at different axonal terminals.

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IMPROVING NERVE REGENERATION WITH CHITOSAN BLENDED MICRO-GROOVED MEMBRANES

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

Luca Scaccini[1], Roberta Mezzena[1], Antonella Battisti[1], Federica Fregnan[2], Luisa Muratori[2], Stefania Raimondo[2], Giovanna Gambarotta[2], Mariacristina Gagliardi[1], Marco Cecchini[1], Ilaria Tonazzini[1]
[1]NEST, Istituto Nanoscienze-cnr And Scuola Normale Superiore, Pisa, Italy, [2]University of Turin, Department Of Clinical And Biological Sciences, Neuroscience Institute Cavalieri Ottolenghi, Orbassano (TO), Italy

Aims: Chitosan is emerging as a promising FDA-approved, biocompatible biopolymer for tissue engineering. However, its exploitation in regenerative devices can be further improved by blending it with other materials. Also, the critical importance of the nano/micro-structured features of the scaffold surface is overall accepted, impacting the regenerative performance. We have already demonstrated that nano/micro-gratings are capable to direct neuronal and glial cell differentiation, polarization, and migration. Neural cells respond to specific length scales according to the specific cell features. Our study aims to develop microstructured scaffolds for peripheral nerve regeneration, made of biodegradable and soft materials compliant with nerve mechanics. Methods: We develop, by solvent casting, thin, soft films made of chitosan and glycerol, and micro-patterned with directional geometries having different levels of axial symmetry. Their mechanical properties are characterized by scanning probe and light microscopies, and by mechanical and degradation assays. The chitosan-glycerol membranes are tested as in vitro scaffolds with primary cultures of sensitive neurons and Schwann cells, to study cell migration and healing, and as in vivo scaffolds, on a model of Cavernous nerve resection in rats. Results: Overall, we demonstrate that our chitosan-glycerol membranes show optimal physico-mechanical properties, enhancing the neural cell healing response in vitro and also in vivo thanks to their asymmetric directional micropattern. Conclusions: Our results provide information about the production of artificial interfaces for enhancing nerve regeneration, and for the fabrication of novel, bio-compliant neural scaffolds.

Pubmed:
34360664: Scaccini L, Mezzena R, De Masi A, Gagliardi M, Gambarotta G, Cecchini M, Tonazzini I
Chitosan Micro-Grooved Membranes with Increased Asymmetry for the Improvement of the Schwann Cell Response in Nerve Regeneration.
Peripheral nerve injuries are a common condition in which a nerve is damaged, affecting more than one million people every year. There are still no efficient therapeutic treatments for these injuries. Artificial scaffolds can offer new opportunities for nerve regeneration applications; in this framework, chitosan is emerging as a promising biomaterial. Here, we set up a simple and effective method for the production of micro-structured chitosan films by solvent casting, with high fidelity in the micro-pattern reproducibility. Three types of chitosan directional micro-grooved patterns, presenting different levels of symmetricity, were developed for application in nerve regenerative medicine: gratings (GR), isosceles triangles (ISO) and scalene triangles (SCA). The directional patterns were tested with a Schwann cell line. The most asymmetric topography (SCA), although it polarized the cell shaping less efficiently, promoted higher cell proliferation and a faster cell migration, both individually and collectively, with a higher directional persistence of motion. Overall, the use of micro-structured asymmetrical directional topographies may be exploited to enhance the nerve regeneration process mediated by chitosan scaffolds.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S05-594

SEGMENTATION OF THE ANTERIOR HUMAN THALAMUS BASED ON ITS EXCITATORY AFFERENTS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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The connectivity between the anterior part of the human thalamus (ATh) and the frontal cortex is crucial for complex cognitive processes. The ATh is also a major target for deep brain neurosurgery in various neurological disorders such as epilepsy, Parkinson's disease, or essential tremor. Functionally relevant nuclear segmentation is essential for understanding both healthy and pathological processes of the brain, however, a comprehensive division of the human ATh, based on morphological and biological properties, is still lacking. Our aim was to create a reliable nuclear segmentation of the ATh based on its excitatory efferents and cell types. To this end, we used vesicular glutamate transporter types 1 and 2 (vGluT1, vGluT2), calbindin, calretinin, and parvalbumin immunostainings in consecutive sections of post-mortem human thalami to label cortical and subcortical excitatory inputs as well as distinct thalamic cell types. The combination of these markers clearly outlined the major nuclear organisation of the human ATh, however showed major differences compared to the accepted schemes. The size and position of the thalamic motor nuclei as defined by its afferentation were not in accordance with other human thalamus atlases. The composition of excitatory inputs in the mediodorsal nucleus also disagreed with non-human primate and rodent data. The results indicate that the nucleus-specific combinations of afferent systems in the human ATh display both evolutionary conserved and highly derived human features and demonstrate that mapping excitatory afferents can indeed lead to a novel understanding of human thalamic functions.

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BOARD NUMBER: S05-595

QUANTITATIVE, AUTOMATED DETECTION OF EXCITATORY AFFERENTS IN THE ANTERIOR HUMAN THALAMUS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Nuclear parcellation of human thalamus is largely based on subjective, qualitative criteria which may yield segmentation of functionally related areas. Cortical and subcortical afferents carry the most relevant information concerning thalamic function. Thus, in this project we tested whether quantitative, automated analysis of the excitatory inputs in the anterior part of the human thalamus can yield a functionally relevant nuclear segmentation of ATh and whether it can be used to detect alteration in a postmortem COVID patient. We utilized high quality immunolabeling of serial ATh and visualized cortical and subcortical afferents using vesicular glutamate transporter 1 and 2 (vGluT1 and 2 respectively) in postmortem human samples. Automated, high throughput methods provided reliable quantitative data for morphological analysis of the afferentation. vGluT2 immunostaining yielded more informative dataset for nuclear segmentation than vGluT1. Both vGluT2 terminal size distribution as well as vGluT2 terminal density were nucleus specific and dispalyed large intranuclear heterogenity. Despite large interindividual differences among position and size of thalamic nuclei the nucleus specific quantitative features of vGluT2 afferents were remarkably similar between individuals. However, nuclear boundaries based on its excitatory afferents were not in register with accepted thalamus maps (see accompanying poster). In a postmortem COVID patient vGluT2 immunostaining displayed quantitative, nucleus specific alterations compared to control cases, which can, at least partially, explain some of the post COVID symptoms. Our approach allows large scale quantitative characterization of excitatory afferents and its alteration in pathological cases of human ATh and can lead to a rational segmentation of human thalamus.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-596

FROM SKULL TO BRAIN: 3D DENSITY MAPS OF CORTICAL SULCI AS A POWERFUL TOOL FOR PALEONEUROLOGY

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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The timing and emergence of derived cerebral features in the human linage remains a key question in palaeoneurology. In order to reconstruct a timeline of hominin brain evolution using the fossil record, palaeoneurologists have to rely on endocasts which are replicas of the inner table of the bony braincase. The accurate identification of cerebral sulci imprints in endocasts is critical for assessing the topographic extension and structural organisation of cortical regions. This study aims to provide the first documentation of sulcal pattern imprints from the superolateral surface of the cerebrum using a population based atlas technique on extant human endocasts. Human crania from the Pretoria Bone Collection (South Africa) were scanned using micro-CT. Endocasts were virtually extracted, and sulci were automatically detected and manually labelled. A density map method was applied to project all the labels onto an averaged endocast and to visualise the mean distribution of each identified sulcal imprint. This method allowed for the visualisation of inter-individual variation of sulcal imprints, e.g., frontal lobe sulci, correlating with previous brain-MRI studies, and for the first time the extensive overlapping of imprints in historically debated areas on the endocast (e.g., occipital lobe). In providing an innovative, non-invasive, observer-independent method to investigate human endocranial structural organisation, our analytical protocol introduces a promising perspective for discussing critical hypotheses on the evolution of cognitive abilities among hominins. ***Acknowledgements:*** *We thank the South Africa/France (PROTEA) Joint Research Programme (grant number 129923) and the University of Cambridge Harding Distinguished Postgraduate Scholars Programme for financial support.*

Pubmed:

31206664: de Jager EJ, van Schoor AN, Hoffman JW, Oettlé AC, Fonta C, Mescam M, Risser L, Beaudet A
Sulcal pattern variation in extant human endocasts.
Our knowledge of human brain evolution primarily relies on the interpretation of palaeoneurological evidence. In this context, an endocast or replica of the inside of the bony braincase can be used to reconstruct a timeline of cerebral changes that occurred during human evolution, including changes in topographic extension and structural organisation of cortical areas. These changes can be tracked by identifying cerebral imprints, particularly cortical sulci. The description of these crucial landmarks in fossil endocasts is, however, challenging. High-resolution imaging techniques in palaeoneurology offer new opportunities for tracking detailed endocranial neural characteristics. In this study, we use high-resolution imaging techniques to document the variation in extant human endocranial sulcal patterns for subsequent use as a platform for comparison with the fossil record. We selected 20 extant human crania from the Pretoria Bone Collection (University of Pretoria, South Africa), which were detailed using X-ray microtomography at a spatial resolution ranging from 94 to 123 µm (isometric). We used Endex to extract, and Matlab to analyse the cortical imprints on the endocasts. We consistently identified superior, middle and inferior sulci on the frontal lobe; and superior and inferior sulci on the temporal lobe. We were able to label sulci bordering critical functional areas such as Broca's cap. Mapping the sulcal patterns on extant endocasts is a prerequisite for constructing an atlas which can be used for automatic sulci recognition.
J Anat, 2019; 235
29150982: de Jager EJ, Du Plessis AF, Hoffman JW, Oettlé AC, Bosman MC
Visualization Within the Ventricles of the Brain: A Micro-Focus X-Ray Study.
Conceptualization of the ventricular system of the brain by macroscopic studies is complicated by the lack of physical structure of these interconnected cavities. Dissection procedures designed to display the structures in the walls of the ventricles are destructive and not conducive for the appreciation of the ventricular system in its entirety. The application of Micro-focus X-ray tomography affords the possibility to appreciate hidden structures in a nondestructive manner. The aim of

this study was to explore the possibility of using micro-focus X-ray tomography in the three-dimensional (3D) visualization of the ventricular system as well as the various neuroanatomical structures within its walls for educational purposes. Randomly selected embalmed human cadaver brains were scanned at Necsa (South African Nuclear Energy Corporation) housing the MIXRAD laboratory consisting of a Nikon XTH 225 ST micro-focus X-ray tomography facility. A 3D flythrough video of the ventricular system was reconstructed from these scans using software to view the inner surface of the ventricles. Micro-focus X-ray tomography provides feasible means of delivering high-resolution images in a nondestructive way to design a representation of the ventricular system. In addition, structures in the walls of the ventricular system could be appreciated in a novel way. It is envisaged that this 3D-fly-through video of the ventricular system will be valuable when integrated with standard prosections and atlas pictures in the educational setting. Further studies evaluating the use of this integrative visualization of the ventricular system of the brain for its applicability in the educational setting should be performed. Anat Rec, 301:1138-1147, 2018. © 2017 Wiley Periodicals, Inc.
Anat Rec (Hoboken), 2018; 301
30583840: Beaudet A, Clarke RJ, de Jager EJ, Bruxelles L, Carlson KJ, Crompton R, de Beer F, Dhaene J, Heaton JL, Jakata K, Jashashvili T, Kuman K, McClymont J, Pickering TR, Stratford D
The endocast of StW 573 ("Little Foot") and hominin brain evolution.
One of the most crucial debates in human paleoneurology concerns the timing and mode of the emergence of the derived cerebral features in the hominin fossil record. Given its exceptional degree of preservation and geological age (i.e., 3.67 Ma), StW 573 ('Little Foot') has the potential to shed new light on hominin brain evolution. Here we present the first detailed comparative description of the external neuroanatomy of StW 573. The endocast was virtually reconstructed and compared to ten southern African hominin specimens from Makapansgat, Malapa, Sterkfontein and Swartkrans attributed to Australopithecus and Paranthropus. We apply an automatic method for the detection of sulcal and vascular imprints. The endocranial surface of StW 573 is crushed and plastically deformed in a number of locations. The uncorrected and therefore minimum cranial capacity estimate is 408 cm and plots at the lower end of Australopithecus variation. The endocast of StW 573 approximates the rostrocaudally elongated and dorsoventrally flattened endocranial shape seen in Australopithecus and displays a distinct left occipital petalia. StW 573 and the comparative early hominin specimens share a similar sulcal pattern in the inferior region of the frontal lobes that also resembles the pattern observed in extant chimpanzees. The presumed lunate sulcus in StW 573 is located above the sigmoid sinus, as in extant chimpanzees, while it is more caudally positioned in SK 1585 and StW 505. The middle branch of the middle meningeal vessels derives from the anterior branch, as in MH 1, MLD 37/38, StW 578. Overall, the cortical anatomy of StW 573 displays a less derived condition compared to the late Pliocene/early Pleistocene southern African hominins (e.g., StW 505, SK 1585).
J Hum Evol, 2019; 126
32996582: Dumoncel J, Subsol G, Durrleman S, Bertrand A, de Jager E, Oettlé AC, Lockhat Z, Suleman FE, Beaudet A
Are endocasts reliable proxies for brains? A 3D quantitative comparison of the extant human brain and endocast.
Endocasts (i.e., replicas of the inner surface of the bony braincase) constitute a critical proxy for qualifying and quantifying variations in brain shape and organization in extinct taxa. In the absence of brain tissues preserved in the fossil record, endocasts provide the only direct evidence of brain evolution. However, debates on whether or not information inferred from the study of endocasts reflects brain shape and organization have polarized discussions in paleoneurology since the earliest descriptions of cerebral imprints in fossil hominin crania. By means of imaging techniques (i.e., MRIs and CT scans) and 3D modelling methods (i.e., surface-based comparisons), we collected consistent morphological (i.e., shape) and structural (i.e., sulci) information on the variation patterns between the brain and the endocast based on a sample of extant human individuals (N = 5) from the 3D clinical image database of the Steve Biko Academic Hospital in Pretoria (South Africa) and the Hôpitaux Universitaires Pitié Salpêtrière in Paris (France). Surfaces of the brain and endocast of the same individual were segmented from the 3D MRIs and CT images, respectively. Sulcal imprints were automatically detected. We performed a deformation-based shape analysis to compare both the shape and the sulcal pattern of the brain and the endocast. We demonstrated that there is close correspondence in terms of morphology and organization between the brain and the corresponding endocast with the exception of the superior region. By comparatively quantifying the shape and organization of the brain and endocast, this work represents an important reference for paleoneurological studies.
J Anat, 2021; 238
34540572: Herselman R, Lalloo V, Ueckermann V, van Tonder DJ, de Jager E, Spijkerman S, van der Merwe W, du Pisane M, Hattingh F, Stanton D, Hofmeyr R
Adapted full-face snorkel masks as an alternative for COVID-19 personal protection during aerosol generating procedures in South Africa: A multi-centre, non-blinded simulation study.
SARS-CoV-2 has resulted in increased worldwide demand for personal protective equipment (PPE). With pressure from ongoing epidemic and endemic episodes, we assessed an adapted snorkel mask that provides full-face protection for healthcare workers (HCWs), particularly during aerosol-generating procedures. These masks have a custom-made adaptor

which allows the fitment of standard medical respiratory filters. The aim of this study was to evaluate the fit, seal and clinical usability of these masks.
Afr J Emerg Med, 2021; 11

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BOARD NUMBER: S05-597

DETECTION OF CEREBRAL ANEURYSM AND INTRACRANIAL VERTEBRAL DISSECTION USING NON-ENHANCED MAGNETIC RESONANCE IMAGING IN EMERGENCY SETTING: EMPHASIS ON MAGNITUDE IMAGE OF SUSCEPTIBILITY-WEIGHTED IMAGE

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Purpose: To evaluate image features and diagnostic performance of susceptibility-weighted image (SWI) in detection of intracranial vertebral artery dissection (VAD) and unruptured intracranial aneurysm (UIA). **Materials and methods**: From January 2015 to December 2021, symptomatic patients who underwent 3.0 T MR SWI were recruited. For study group, transfemoral cerebral angiography-proven lesions were included, while 1:1 matched control group with MR angiography were included. Image features of SWI were evaluated. Diagnostic performance and interobserver agreements were calculated for detecting VAD with stenosis and UIA greater than 7 mm. **Results:** Total of 110 patients (mean age: 60.92 years, female: 60/110) were included. In the study group (N=55), 21 patients (38.2%) had VAD, while 34 patients (61.8%) had UIA. For SWI-detectable VAD, larger parent artery (PA)-dilatation ratio was observed (1.36 vs. 1.84, p = 0.034). For SWI-detectable UIA, larger PA-dome ratio (1.32 vs. 1.90, p = 0.020) and larger PA-height ratio (1.25 vs. 1.77, p = 0.005) were observed. The diagnostic performance and kappa values for VAD with stenosis were as follow: sensitivity: 91.7 (95% CI: 61.5 – 99.8); specificity: 93.9 (95% CI: 87.2 – 97.7); κ: 0.80. The diagnostic performance for UIA larger than 7 mm were as follow: sensitivity: 87.5 (95% CI: 47.4 – 99.7); specificity: 95.1 (95% CI: 88.9 – 98.4); κ: 0.73. **Conclusion**: SWI-detectable lesions were VAD with larger PA-dilatation ratio, and UIA with larger PA-dome ratio, and PA-height ratio. SWI was able to accurately detect VAD with stenosis and UIA larger than 7 mm with substantial interobserver agreements.

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EVALUATION OF NEURONAL ACTIVATION INDUCED BY ULTRASOUND NEUROSTIMULATION IN MOUSE MOTOR CORTEX

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Aims: Ultrasound neurostimulation (USNS) is a promising modality for rewiring brain pathways and treating non-invasively neuropsychological disorders with high spatiotemporal resolution. However, the optimal USNS parameters for successful brain stimulation warrants further investigation. Here, we investigated the influence of the pair ultrasound frequency/focal spot diameter on motor response and neuronal activity in mice. **Methods:** We transcranially administered USNS to the mouse motor cortex (M1) using eight single-element focused transducers operating at 0.2MHz, 0.4MHz, 0.5MHz and 1MHz. For each frequency, two transducers of different diameters/focal lengths were used. For a given pair of frequency/focal spot, mice were exposed to series of progressively increasing peak negative pressures (PNPs) to evaluate visually the motor responses and then the success rate. Next, stimulations at the highest used PNP were applied to further investigate the corresponding neuronal activity using c-Fos immunostaining. **Results:** Our results showed that the success rate increased significantly with PNP regardless of the frequency and the focal spot diameter. Moreover, significant difference in the success rates was observed when changing the focal spot diameter at a given frequency, wide acoustic beams induce higher success rates. In contrast, the analysis of c-Fos expression revealed that USNS significantly enhanced neuronal activation in the M1 when the focal spot diameter decreased. **Conclusions:** Our data demonstrate that the use of larger focal spots evoked motor responses at the lowest PNPs, while smaller focal spots are required to enhance neuronal activation. Further investigation is required to explore USNS-related changes in excitability and functionalities of the brain circuitries.

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NANOTAG – A NOVEL ANTIBODY-FREE APPROACH FOR EPIGENOMIC PROFILING

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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The expression of genes is regulated by numerous molecular processes inside the nucleus, including posttranslational modifications of histones, the binding of transcription factors to the DNA, and chromatin accessibility. The ability to obtain genome-wide information about these reversible processes provides great insight into the mechanistic regulation of gene expression. Currently, several methods exist to facilitate the profiling of transcription factor binding sites and histone modifications, including chromatin immunoprecipitation followed by sequencing (ChIP-seq) and more recent alternatives, such as cleavage under targets and release using nuclease (CUT&RUN) and cleavage under targets and tagmentation (CUT&Tag), which can be easily applied to low amounts of cells. However, these methods rely on the use of antibodies to capture target sites in the genome, which has disadvantages such as antibody unspecificity and long processing times. Here, we propose a novel method for epigenomic profiling that allows profiling of transcription factors or histone modifications and chromatin accessibility simultaneously in the same cells without antibodies. This approach called NanoTag relies on a protein fusion between the Tn5 transposase and an antibody mimetic, such as the anti-GFP nanobody, to target fusion Tn5 to sites in the genome where the target is localized. This approach is easy, significantly shortens processing times, and reduces background. It provides an additional level of resolution to chromatin accessibility studies applicable to neurosciences.

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SPECIFIC DETECTION AND DELETION OF THE SIGMA-1 RECEPTOR IN NEURONS AND GLIAL CELLS FOR FUNCTIONAL CHARACTERIZATION IN VIVO

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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The chaperon protein sigma-1 receptor (S1R) has been discovered for over forty years. Recent pharmacological studies using S1R exogenous ligands demonstrated a promising therapeutical potential of targeting the S1R for many neurological disorders. Although intensive *in vitro* studies have revealed S1Rs are mainly residing at membranes of the endoplasmic reticulum (ER), the cell-specific in vivo expression pattern of the S1R is still unclear, mainly due to the lack of a reliable detection method which also prevented a comprehensive functional analysis. Here, first, we identified a highly specific antibody using S1R knockout (KO) mice and established an immunohistochemical protocol involving a 1% SDS antigen retrieval step. Second, we characterized the S1R expression in the mouse brain and can demonstrate that the S1R is widely expressed: in principal neurons, interneurons, and all glial cell types. Finally, we generated a novel Cre-dependent S1R conditional KO mouse (S1R flox) to study cell type-specific functions of the S1R. As a proof of concept, we successfully ablated S1R expressions in neurons or microglia employing neuronal and microglial Cre-expressing mice, respectively. In summary, we provide powerful tools to cell-specifically detect, delete and functionally characterize S1Rs *in vivo*.

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IMPROVED NEUROCOGNITIVE OUTCOME IN THE SHORT-TERM FOLLOWING PRIMARY GAMMA KNIFE RADIOSURGERY (GKRS) FOR INTRACRANIAL VASCULAR ANOMALIES

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Objective: To evaluate the effect of GKRS on cognitive functions of patients with vascular anomalies. Methods: A total of 55 patients having vascular anomalies: arteriovenous malformation (AVM) = 37 and cavernous malformation (CM) = 18 without any comorbidities between 2018-2019 were included. Clinical (subjective), radiological (1.5 T MRI), and a multi-domain neuropsychological battery (memory, executive functioning, attention, intelligence) were performed on them pre-GKRS and 6 (n=48) months post-GKRS. Results: Baseline neurocognitive statistical analysis was done on basis of 1. lesion characteristics- hemisphere involved, lesion site, lesion type, lesion volume, and 2: sociodemographic variables- gender, age group, and education group. Before GKRS, it was reported that the CM group performed better than the AVM group in domains of immediate verbal recall (p=0.000*), verbal delayed recall (p=0.012*), and MMSE (p=0.034*). The higher education group performed significantly better in almost all the cognitive domains. No significant difference was seen in the hemisphere involved, lesion site, gender, and age group at pre-GKRS. It was observed that at 6 months, significant improvement was seen in almost all cognitive domains variable except for phonemic fluency, semantic fluency, and psychomotor speed domain variables. At 6 months, 1 patient had subjective complaints of headache and another patient had 2 episodes of seizure. Radiologically the lesion of both patients was static. Rest all patients showed improvement symptomatically. Conclusion: Apart from symptomatic and radiological improvement (resolution of vascular malformations), significant improvement of neurocognitive domains at 6 months ensures a landscape of better neurocognitive outcome and quality of life after GKRS.

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DEVELOPMENT OF A VERSATILE AND COST-EFFICIENT AUTOMATED PLATFORM FOR BRAIN TISSUE MICRODISSECTION, SINGLE CELL ACQUISITION AND ADHESION ANALYSIS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Sophisticated approaches such as laser-based microdissection and cell sorting must be used to acquire specific cells or subanatomical regions from heterogeneous brain tissues. Some of their methodological limitations include tissue preprocessing for laser assisted approaches and conversion of tissue to a suspension of cells for sorting technologies. Moreover, none of the existing instruments provide concurrent single cell acquisition and adhesion force measurement. We have developed an automated and cost-efficient platform for brain tissue microdissection, single cell acquisition, measurements of its adhesion strength and deposition of collected samples into single wells for further molecular analysis or clonal expansion. The platform is based on the capillary based on our vacuum pulse assisted technology with incorporated pressure sensor. Collected volumes are compatible with any downstream single cell analyses such as next generation sequencing. Here, we demonstrate its capabilities for microdissection of fresh and fixed brain tissues, including brain organoid cultures, and isolation of individual cells from various adherent cell lines. Furthermore, cell adhesion force measurements were performed for cells grown on eight different substrates including non-treated, poly-L-lysin, laminin, fibronectin, collagen, gelatin, amin and carboxyl surfaces. This cell adhesion measurement function was incorporated into the main instrument’s software permitting automated analysis and collection. In conclusion, a versatile platform for tissue microdissection, single cell acquisition and adhesion strength measurements was developed that allows for further genomics studies to correlate cell’s location and phenotype with underlying molecular program.

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VALIDATION OF AN INNOVATIVE MILLIFLUIDIC GUT-ON-A-CHIP TO CHALLENGE THE MICROBIOTA-GUT-BRAIN AXIS IN VITRO

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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AIMS The role of gut microbiota in neurodegeneration is becoming an interesting topic and supports the need of novel in vitro tools for its investigation through the dissection of the biochemical pathways involved in the so-called microbiota-gut-brain axis. The ERC-funded project “MINERVA” aims at developing an innovative multiorgan-on-a-chip platform by connecting ad hoc developed organ-on-a-chip devices. Here, we describe the biological validation of the innovative device we developed through the challenging of the gut epithelium of our featured microbiota-gut-brain final platform. METHODS The gut-on-a-chip device was developed by using gut epithelial Caco-2 cells, and characterized by computational simulations jointly with an experimental set of assays including morphology observation, viability tests, trans epithelial electrical resistance (TEER) measure and immunofluorescence assays. RESULTS The device did not alter Caco-2 cell viability, and suitable TEER values have been detected. Morphological analysis confirmed mature cell layer formation with evident villi structures and proper physiologic expression of tight junctions. CONCLUSION Our results confirmed that our innovative device is a suitable tool to model gut epithelium and pave the way to the serially connection of our devices to build up our ERC project microbiota-gut-brain axis platform. ACKNOWLEDGMENT MINERVA project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (Grant Agreement N° 724734).

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GENE EXPRESSION OF THE OXYTOCIN RECEPTOR, C-FOS, AND CGRP IN THE TRIGEMINAL GANGLION IN AN OROFACIAL PAIN MODEL

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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The most devastating pain conditions include orofacial pain disorders. Binding and activating its receptor in primary sensory neurons of the peripheral sensory system, the hypothalamic nonapeptide, oxytocin is supposed to have an antinociceptive role. The purpose of this work was to investigate the gene expression of the oxytocin receptor (OTR), a marker of neuronal activity, c-Fos, and a characteristic neurotransmitter of a substantial portion of the trigeminal afferents, calcitonin gene-related peptide (CGRP) in the trigeminal ganglion (TG) using an animal model of inflammation-induced orofacial pain. Adult Wistar rats (250-300 g) received unilateral injection of carrageenan (100 µl, of 2% w/v) into their vibrissal pads. OTR, c-Fos, and CGRP mRNA expression levels in the TGs were analysed using RT-qPCR. In TGs ipsilateral to the injection site, both the c-Fos and the CGRP mRNAs exhibited higher expression levels, and a moderate increase was observed regarding the OTR mRNA expression one day after the carrageenan administration, compared to controls. The increase in the c-Fos and the CGRP mRNA expression shows that the inflammatory substance injected into the orofacial area stimulates the peptidergic neurons in the TG. Furthermore, we believe that oxytocin can impact the degree of pain transmitted by inflammation-triggered nociceptive neurons by increasing signalling capability due to the increased OTR expression. Since the effects seen in the animal model are similar to those for people, they may contribute to develop new therapies for orofacial pain relief that target the OTRs.

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STANDARDIZATION CRITERIA OF HIPSC DERIVED NEURONS FOR BRAIN-ON-CHIP APPLICATIONS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Brain Organ-on Chips (OoC) is the creation of humanized neural networks culture in relevant microfluidic compartmentalization architecture with the possibility to analyze specific readouts such as functional activity recording. In order to further explore the potential of Brain-on-Chips for preclinical trials and to trigger the adoption by the scientific community, cell culture protocols need to be standardized to achieve a high reproducibility. Here, we present a methodology to control the microfluidic system variability, to optimize culture protocol adapted to specifics of microfluidic technology and to establish analytical methods including validation criteria to quantify axonal outgrowth/ functional activity. We established specific protocols to maintain differentiated neurons derived from hiPSCs (glutamatergic, GABAergic, dopaminergic, motor and sensory neurons) in microfluidic devices. For each cell type, we evaluated the (i) cell morphology and long-term viability, (ii) expression of pluripotency and biological markers by immunofluorescence approach, (iii) growth kinetics and (iv) electrophysiological recordings using multielectrode array (MEA). Reproducibility of cell culture was assessed using semi-automatic image analysis using several cell providers and operators. To conclude, we have applied our methodology to characterize five different neural types, for which Standard Operating Procedures (SOPs) have been developed. We determined and validated specific cell culture renewal media in our devices. We suggest that this validation methodology should be an essential point when using Organ-on-Chip and to facilitate the regulatory acceptance during medicinal product development. Our hope is to open the route to standardize neural cultures for Brain-on-Chip applications.

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HUMAN BRAIN-ORGANOIDS-ON-CHIP: ADVANCED MICROFLUIDIC DEVICE FOR REPRODUCIBLE ORGANOIDS CULTURE

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Alternative methods, such as predictive human-based *in vitro* models are in the spotlight of regulatory bodies. Organs- and Organoids-on-Chips (OoCs) technologies have shown a great potential to reduce the cost and ethical burden of animal studies. Cerebral organoids now appear as a powerful tool to understand complex biological processes with the ability to mimic and recapitulate many features of the human brain. One of the main current challenges is the cell apoptosis described in the center of cerebral organoids leading to a lack of maturation. This is caused by a lack of oxygenation and nutrient supply in the center of this 3D structure. Our objective is to propose an innovative microfluidic system allowing the generation of reproducible cerebral organoids. Here, we develop a microfluidic device with (i) a 3D-Deposition chamber compatible with organoid generation protocol and allowing the developing organoid to expand up to 4 mm in diameter, (ii) a controlled flow to improve exchanges of oxygen, nutrients, and bioactive compounds, and (iii) the compatibility with automation processes to meet the expectations of scientists and pharmaceutical companies. In conclusion, 3D microfluidic *in vitro* models will open the field of multi-OoCs to study toxicology (ADME-tox), as well as the delivery and screening of molecules that target the brain under more physiologically relevant conditions.

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BOARD NUMBER: S05-608

CSF-PRODUCING CHOROID PLEXUS ORGANOIDS MODEL PATHOGEN AND DRUG ENTRY TO THE BRAIN

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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The choroid plexus (ChP) is a highly conserved secretory tissue in the brain. This tissue displays a number of important functions in the brain such as forming a protective epithelial barrier and secreting the cerebrospinal fluid (CSF). The CSF is important for the maintenance of physiological levels of nutrients in the brain, for transport of signalling molecules and growth factors and for its protective role in the regulation of intracranial pressure. To explore the role of the ChP-CSF system, we recently established a protocol to generate ChP organoids using a combination of signalling molecules that are physiologically present during the stages of development of this tissue. These organoids develop ChP and recapitulate fundamental functions of this tissue, namely secretion and formation of a tight epithelial barrier. Combining single-cell RNA-sequencing with immunohistochemical and EM validation, we detected the presence of ChP specific channels and transporters localised on the apical brush border of the ChP epithelium. By testing different compounds, we were able to demonstrate the selective permeability of the ChP barrier in vitro, using NMR. In addition, we noticed the formation of large fluid-filled cysts protruding from the organoids, the contents of which, analysed by mass spectrometry, highly resembles human CSF. Finally, we used this model to test pathogen entry in the brain and we infected the organoids with live SARS-CoV-2. We found that SARS-CoV-2 infects ChP epithelial cells causing damage of this key brain barrier.

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BOARD NUMBER: S05-609

A NOVEL PLATFORM FOR FLUIDIC INTERFACING WITH THE BRAIN

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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The blood-brain barrier (BBB) is a comprehensive set of adaptations in the brain vasculature that ensure compartmentalisation of the neural environment. Any form of systemic intervention is therefore highly hindered by the intrinsic ability of the injected agent to cross the barrier itself. In most applications where efficacy and targeting are paramount, psychoactive agents are generally infused directly into the structure of interest by means of a technique known as convection-enhanced delivery (CED). However, despite its promise of intraparenchymal delivery, CED can still fail to guarantee consistent results due to the reflux of infusate along the insertion track. Within this project, we explored the use of the latest additive manufacturing techniques (i.e. two-photon polymerization) to design an application-specific catheter for pressure-controlled infusions in different animal models. We then proved an increased likelihood of reflux-free infusions both in a brain surrogate and in a mouse model. The presence of the BBB is an equivalent obstacle in the identification of circulating markers of disease. In this context, we exploited the same microfabrication technique to investigate the possibility of sampling circulating cerebrospinal fluid (CSF) in a longitudinal manner. We demonstrated the ability to obtain intraventricular CSF samples that present no blood contamination and at a frequency of extraction that greatly exceeds the alternative technique (i.e. cisterna magna extraction).

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BOARD NUMBER: S05-610

NEUROFILAMANT LIGHT CHAIN (NFL) IMMUNOASSAY FOR THE SMCXPRO™ ASSAY PLATFORM: DEVELOPMENT AND APPLICATIONS

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Objective: The advent of ultrasensitive assay technologies has enabled the measurement of very low-abundance blood-based biomarkers of neurodegenerative conditions, reducing the need for invasive cerebrospinal fluid (CSF) collection. Blood-based neurological biomarkers allow for large scale sample acquisition and screening in research, and allows for the early detection of the transition from healthy to diseased status. We have developed an assay for the detection of neurofilament light chain (NfL) in human serum, plasma and CSF samples in healthy and diseased states. NfL is a valuable neurodegenerative biomarker for accessing progression and treatment of Alzheimer's Disease (AD), Multiple Sclerosis (MS) and Huntington's Disease. Methods: Rigorous assay development procedures were undertaken to produce an NfL immunoassay capable of accurately and reproducibly detecting NfL in human serum, plasma, and CSF samples. Results: A robust kit capable of generating consistent and reliable NfL measurements at sub-pg/mL concentrations has been developed and characterized using Single Molecule Counting (SMC™) ultrasensitive immunoassay technology. The developed assay demonstrates excellent performance characteristics for spike/recovery values and dilutional linearity (90-110% for all three matrices). NfL was detected in all sample types tested. Increased NfL sample levels in AD and MS samples as compared to normal serum, plasma and CSF samples was also observed. Additional data is presented to compare SMC™ NfL results to competitor platform results, as well as multi-site consistency of results utilizing matched samples. Conclusions: Our SMC™ NfL immunoassay kit provides a valuable ultrasensitive tool for NfL research in the areas of neurodegenerative disease and neurological injury.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S05-611

VAS-O-MATIC: A FIJI PLUGIN FOR MICROSCOPY ANALYSIS OF BLOOD-BRAIN BARRIER ORGANIZATION

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Background: In brain, the pericytes enwrapping the microvasculature are major contributors of the blood-brain barrier (BBB) homeostasis. Loss of pericyte coverage is observed in aging and the resulting disruption of the BBB might trigger the onset of neurodegenerative diseases. However, measurement of pericyte coverage is often manual, time-consuming and hence restricted to a few regions of interest. Further, controversy in the field arose due to methodology discrepancy between teams. Here, we provide an automated method for fast and accurate pericyte coverage analysis of entire brain regions. **Methods:** We used FIJI to create Vas-o-Matic, a macro for accurate pericyte coverage measurement in relation to vessel diameter. Vas-o-Matic segments the image into portions which are processed independently to quantify structure metrics along the vascular tree. We compared Vas-o-Matic performance to conventional manual analysis by a human expert on brain slices from young and aged mice. **Results:** Vas-o-Matic dramatically reduced the time spent on measures from the images and allowed diameter-wise analysis of pericyte loss. Power of the analysis was also greatly increased due to thousands of individual measures on whole brain structures. Complementary analyses of the degradation of the vascular basement membrane and of the loss of astrocyte endfeet coverage provided the proof of principle that Vas-o-Matic can be used beyond pericyte coverage and for any component of the BBB. **Conclusions:** Vas-o-Matic is a robust and fast method for the analysis of the fine structure of the BBB.

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BOARD NUMBER: S05-612

DEEP LEARNING AUTOMATIC DETECTION AND QUANTIFICATION OF ADULT NEURAL PROGENITORS REVEALED BY DOUBLE THYMIDINE ANALOG LABELING AND CONFOCAL MICROSCOPY

POSTER SESSION 05 - SECTION: EMERGING TECHNOLOGIES & METHODS

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Neurogenesis, a complex process central to brain development and plasticity, occurs at the neural stem cell niches (NSCN). To characterize NSCN´s cellular composition using double thymidine analogue labelling technique, it is required the detection and quantification of each type of neural progenitors. As these are “rate-determining steps”, we used Cogniflow to automatically identify and quantify neural progenitors cell types revealed by 5 chlorodeoxiuridine (CldU) and 5 Iododeoxiuridine (IdU) double labelling (separated by short and long chases) in adult *Gymnotus omarurom*. The challenge was approached as an Object Detection (OD) task, using a YOLOv5 architecture: models trained to detect CldU+, IdU+ and double labeled (DL) progenitors. We manually labeled confocal images surrounding each nucleus with a class assigned (CldU+, IdU+ or DL) rectangular bounding box. Images were splitted into training (80’%) and validation (20%) sub-datasets. We analyzed the precision of the model as a function of datasets size: 1) 20 confocal images including 264 objects (59 CldU+, 71 IdU and 134 DL) and 2) 30 images (387 objects: 197 for DL, 99 CldU+ and 91 IdU+). To assess the OD task, we used the MeanAveragePrecision@0.5 (mAP@0.5) that considers how precision and recall perform (Intersection over Union threshold= 0.5). The model achieved mAP@0.5 of 69.0% and 72% for 20 and 30 images, respectively. This encouraging result shows the power of the model to perform well even with a small dataset and indicates the importance of increasing the size of data to train future models.

FENS Forum 2022
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BOARD NUMBER: S05-613

PAIN-INDUCED ADAPTATIONS IN THE CLAUSTROCINGULATE PATHWAY

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Pain is a common and complex medical concern that poses a major threat to a person's quality of life. In both humans and other animals, chronic pain is associated with increased activity in the anterior cingulate cortex (ACC), a brain region implicated in the control of attentional and emotional processes. The ACC is nested in a broad cortical and subcortical network whose coordinated activity is thought to give rise to the experience of pain. It is largely unknown, however, how many of these structures adapt to persistent pain states. The present study explores for the first time the role of the claustrum – a thin and functionally elusive structure reciprocally connected to the ACC – in pain processing. Using a combination of *ex vivo* electrophysiology, *in vivo* calcium imaging and chemogenetic approaches, we show that nociceptive stimuli are encoded in the claustrum and that its inhibition can reversibly attenuate mechanical allodynia in a mouse model of complete Freund's adjuvant (CFA)-induced inflammatory pain. Additionally, we reveal differential changes in intrinsic and spontaneous activity of ACC-projecting and non-projecting CLA neurons, along with a weakened synaptic efficacy onto layer 5 ACC pyramidal neurons following prolonged pain. Altogether, our results suggest a role for the claustrocingulate pathway in the modulation of pain behaviors, and open up new avenues of research into the contribution of the CLA in sensory and affective processing.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-614

THE NEURAL CORRELATES OF ACUTE AND CHRONIC PAIN IN SLEEP AND WAKE

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Mechanical pain is processed by the lateral and the medial pain pathways, which code for the sensory-discriminative and the affective-motivational aspects of the experience of pain, respectively. And while in wake the neural correlates of the pain experience are largely revealed, they remain to be elucidated while in sleep. Here we performed multisite electrophysiological recordings in the hind-limb primary somatosensory cortex (S1HL) and the anterior cingulate cortex (ACC) to evaluate the sensory and the emotional processing of pain during the natural sleep cycle of mice in the light phase. In some sessions, animals received mechanical painful stimuli to the planar surface of the hind paws. Our results show for the first time that acute pain reaches both cortices and is processed during natural sleep in mice. In addition, we have observed that high power in low frequencies (1-16 Hz) protect sleep by either suppressing awakening, motor responses or both response types. Interestingly, analysis of the scale-free properties of the S1HL and ACC local field potentials reveals that chronic pain increases the scaling exponents differntly in wake and sleep. This may be indicative of a deficient modulation in the local network that could affect the excitatory-inhibitory balance. Thus, compromising the sensory and emotional processing of pain and, ultimately, impairing behaviour. In summary, these results indicate that the emotional processing of pain remains intact during sleep and that the changes that chronic pain causes in the local network settle rapidly and are long lasting in sleep.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-615

REAL-TIME DECODING OF SPONTANEOUS PAIN FROM TWO-PHOTON MICROSCOPY IMAGES OF BRAIN CELLULAR CALCIUM USING DEEP LEARNING

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Chronic pain remains intractable in millions of patients worldwide. Spontaneous ongoing pain is a major clinical problem of chronic pain and extremely challenging to diagnose and treat compared to stimulus-evoked pain. Although extensive efforts have been made in preclinical studies, there still exists a mismatch in focused pain type between animals and human (i.e. evoked vs. spontaneous), which obstructs the translation of knowledge from preclinical animal models into objective diagnosis and effective new treatments. Here, we developed a deep learning algorithm, named AI-bRNN (Average training, Individual test-bidirectional Recurrent Neural Network), to decode spontaneous pain from brain cellular Ca^{2+}signals recorded by two-photon microscopy imaging in awake, head-fixed mice. AI-bRNN robustly determines the intensity and time point of spontaneous pain even in chronic pain models, and evaluates the efficacy of analgesics in real time. Furthermore, AI-bRNN could be applied to various cell types (neurons and glia), brain areas (cerebral cortex and cerebellum) and somatosensations (itch and pain), proving its versatile performance. These results suggest that our approach offer a clinically relevant, quantitative and real-time preclinical evaluation platform for pain medicine, thereby accelerating the development of new methods for diagnosing and treating human chronic pain patients. **Competing interests** Neurogrin Inc. founded by the authors holds the patent applications related to the contents of this poster abstract (10-2019-0173382 in Korea and PCT/KR2020/006221). **Acknowledgements** This work was supported by grants from the National Research Foundation of Korea funded by the Korean government (NRF-2017M3C7A1025604, NRF-2017M3A9E4057926, and NRF-2019R1A2C2086052 to SKK; NRF-2018R1A5A2025964 and NRF-2017M3C7A1029611 to SJK).

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-616

SENSORY AND CONTEXTUAL MODULATIONS OF PAIN ARE SERVED BY SPATIALLY AND SPECTRALLY DISTINCT BRAIN NETWORK PATTERNS

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Aims: Pain arises from the integration of sensory information about threats to the body and contextual information such as an individual's expectations. To better understand how this integration process is implemented in the human brain, we analyzed an experiment in which brief painful stimuli were applied to 48 healthy human individuals. Methods: In the experiment, sensory and contextual information were independently varied by varying stimulus intensity and expectations, respectively. Based on EEG data recorded during the experiment, the analysis investigated frequency specific connectivity patterns in a network comprising six brain regions which are known to be crucially involved in the cerebral processing of pain. Results: We found that sensory and contextual modulations of pain were associated with spatially and spectrally distinct network patterns. Specifically, we found that expectation alters connectivity at alpha frequencies (8-13 Hz) from prefrontal cortex to somatosensory cortex. Moreover, discrepancies between sensory and contextual information (prediction errors) were associated with connectivity changes at gamma frequencies (60-100 Hz) between prefrontal, anterior cingulate and parietal opercular cortex. These connectivity changes complement previous observations that have indicated that commonly analyzed evoked and oscillatory EEG responses to painful stimuli are sensitive to sensory modulations but not to expectations and prediction errors. Conclusion: The findings indicate how spatially and spectrally distinct network patterns serve sensory and contextual modulations of pain.

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BOARD NUMBER: S05-617

A NEUROIMAGING STUDY OF THE BRAIN CHANGES DUE TO PERIPHERAL NERVE STIMULATION

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Aims: Bionic device connected through peripheral neural implants to the brain of transfemoral amputee could significantly improve quality of life, sensory-motor performance and also reduce phantom limb pain (PLP). However, the effects of the bionic leg on brain re-organization are not explored yet. We aim to present unique neuroimaging data after the neuroprosthesis removal in a patient with transfemoral amputation. Methods: A 37-year-old patient with a left leg amputation underwent a trial with lower limb neuroprosthesis which was removed after 90-days. The brain activations during phantom and healthy leg movements were recorded using task-based fMRI at two-time points (Session-1: 5-days and Session-2: 95-days) after the neuroprosthesis removal. Also, the Neuropathic Pain Symptom Inventory (NPSI) scale was obtained before, during the training period and after removal. Results: Imagined phantom foot movements during Session-2 demonstrated higher activation compared to Session-1 in the right primary somatosensory cortex. During both sessions activation in the motor area restricted to the hemisphere contralateral to the amputation/prosthetic leg was detected. No significant differences were found between Session-1 and Session-2 when the subject performed the task with a healthy leg. No significant differences in the fractional anisotropy of the corticospinal tracts were found between Session-1 and Session-2. PLP was reduced during the trial and partially increased 3 months after neuroprosthesis removal. Conclusions: This study shows the presence of brain neuroplasticity of sensorimotor cortical area induced by 3 months of training and then removal of a bionic leg. Moreover, this neuroplasticity could be also connected to PLP reduction.

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BOARD NUMBER: S05-618

TRIGEMINAL NEUROPATHIC PAIN ALTERS ULTRASONIC VOCALIZATIONS IN RATS RESTORED BY ANALGESIC DRUGS

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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The emission of ultrasonic vocalizations (USVs) has been found to be a reliable measure of the emotional state of rodents. Rats emit 50-kHz calls in appetitive and social situations, whereas 22-kHz calls may index a negative state. It has been suggested that chronic pain causes a reduction in 50-kHz calls, but such a reduction may also indicate anxiety-like behavior. Thus, the aim of the present study is to determine pharmacologically whether the decrease in 50-kHz USVs is due to persistent hyperalgesia or anxiety. For this end, chronic constriction injury of the infraorbital nerve (CCI-ION) or sham surgery was performed in 60 days-old male Wistar rats. On the fifteenth day after surgery animals were treated with local lidocaine, midazolam or carbamazepine to determine their effect on facial mechanical hyperalgesia using Von frey filaments. Independent groups were subjected to the USV recording test for the assessment of social emissions followed to the elevated plus maze (EPM) test for anxiety-like behavior detection. The results showed that CCI-ION induced hyperalgesia, which was attenuated with lidocaine or carbamazepine but not by midazolam. They also developed anxiety-like behavior, which was significantly reduced only with midazolam, and showed a lower number of 50-kHz calls compared to sham rats. Lidocaine and carbamazepine increased the 50-kHz calls emitted by CCI-ION rats, but midazolam did not modify them. These data suggest that pain and anxiety coexist in CCI-ION rats, but can be dissociated by pharmacological interventions. Furthermore, measurement of USV may reflect the emotional component of pain and increase translational capability.

Pubmed:

35042377: Araya EI, Carvalho EC, Andreatini R, Zamponi GW, Chichorro JG
Trigeminal neuropathic pain causes changes in affective processing of pain in rats.
Trigeminal neuropathic pain has been modeled in rodents through the constriction of the infraorbital nerve (CCI-ION). Sensory alterations, including spontaneous pain, and thermal and mechanical hyperalgesia are well characterized, but there is a notable lack of evidence about the affective pain component in this model. Evaluation of the emotional component of pain in rats has been proposed as a way to optimize potential translational value of non-clinical studies. In rats, 22 and 50 kHz ultrasonic vocalizations (USVs) are considered well-established measures of negative and positive emotional states, respectively. Thus, this study tested the hypothesis that trigeminal neuropathic pain would result, in addition to the sensory alterations, in a decrease of 50 kHz USV, which may be related to altered function of brain areas involved in emotional pain processing. CCI-ION surgery was performed on 60-day-old male Wistar rats. 15 days after surgery, von Frey filaments were applied to detect mechanical hyperalgesia, and USV was recorded. At the same timepoint, systemic treatment with d,l-amphetamine (1 mg/kg) allowed investigation of the involvement of the dopaminergic system in USV emission. Finally, brain tissue was collected to assess the change in tyrosine hydroxylase (TH) expression in the nucleus accumbens (NAc) and c-Fos expression in brain areas involved in emotional pain processing, including the prefrontal cortex (PFC), amygdala, and NAc. The results showed that CCI-ION rats presented mechanical hyperalgesia and a significant reduction of environmental-induced 50 kHz USV. Amphetamine caused a marked increase in 50 kHz USV emission in CCI-ION rats. In addition, TH expression was lower in constricted animals and c-Fos analysis revealed an increase in neuronal activation. Taken together, these data indicate that CCI-ION causes a reduction in the emission of environmental-induced appetitive calls concomitantly with facial mechanical hyperalgesia and that both changes may be related to a reduction in the mesolimbic dopaminergic activity.
Mol Pain, 2022 Jan-Dec; 18
34592375: Turnes JM, Araya EI, Barroso AR, Baggio DF, Koren LO, Zanoveli JM, Chichorro JG
Blockade of kappa opioid receptors reduces mechanical hyperalgesia and anxiety-like behavior in a rat model of trigeminal neuropathic pain.
It has been shown that kappa opioid receptor (KOR) antagonists, such as nor-binaltorphimine (nor-BNI), have antinociceptive effects in some pain models that affect the trigeminal system. Also, its anxiolytic-like effect has been extensively

demonstrated in the literature. The present study aimed to investigate the systemic, local, and central effect of nor-BNI on trigeminal neuropathic pain using the infraorbital nerve constriction model (CCI-ION), as well as to evaluate its effect on anxiety-like behavior associated with this model. Animals received nor-BNI systemically; in the trigeminal ganglion (TG); in the subarachnoid space to target the spinal trigeminal nucleus caudalis (Sp5C) or in the central amygdala (CeA) 14 days after CCI-ION surgery. Systemic administration of nor-BNI caused a significant reduction of facial mechanical hyperalgesia and promoted an anxiolytic-like effect, which was detected in the elevated plus-maze and the light-dark transition tests. When administered in the TG or CeA, the KOR antagonist was able to reduce facial mechanical hyperalgesia induced by CCI-ION, but without changing the anxiety-like behavior. Moreover, no change was observed on nociception and anxiety-like behavior after nor-BNI injection into the Sp5C. The present study demonstrated antinociceptive and anxiolytic-like effects of nor-BNI in a model of trigeminal neuropathic pain. The antinociceptive effect seems to be dissociated from the anxiolytic-like effect, at both the sites involved and at the dose need to achieve the effect. In conclusion, the kappa opioid system may represent a promising target to be explored for the control of trigeminal pain and associated anxiety. However, further studies are necessary to better elucidate its functioning and modulatory role in chronic trigeminal pain states.
Behav Brain Res, 2022; 417
32777313: Araya EI, Barroso AR, Turnes JM, Radulski DR, Jaganaught JA, Zampronio AR, Chichorro JG
Toll-like receptor 4 (TLR4) signaling in the trigeminal ganglion mediates facial mechanical and thermal hyperalgesia in rats.
There is increasing evidence that the toll-like receptor 4 (TLR4) signaling pathway contribute to development of hyperalgesia in the trigeminal system. The aim of the present study was to investigate the role of TLR4 in the trigeminal ganglion (TG) in facial hyperalgesia induced by injection of Lipopolysaccharide (LPS) or intraoral mucosal incision, which is an orofacial postoperative pain model, in male Wistar rats. The TLR4 antagonist (LPS-RS, 20 µg/10 µL) was administrated 30 min before LPS injection into the TG (10 µg/10 µL) or oral mucosa (10 µg/50 µL). In the postoperative pain model, rats were treated with LPS-RS (20 µg/10 µL) into the TG for three consecutive days after the incision. Facial heat and mechanical hyperalgesia were assessed hourly after LPS injection or intraoral incision. In addition, expression of NFκB was assessed in the TG on day 3 after intraoral incision. Our results showed that blockade of TLR4 in the TG attenuated facial heat and mechanical hyperalgesia induced by LPS or by mucosal incision, and that both conditions are associated to increase of phosphorylated NFκB in the TG. In conclusion, the present study suggests that activation of TLR4-NFκB signaling pathway in the TG contributes to the development of facial heat and mechanical hyperalgesia and may contribute to pain in inflammatory oral conditions.
Physiol Behav, 2020; 226
32569083: Araya EI, Baggio DF, Koren LO, Andreatini R, Schwarting RKW, Zamponi GW, Chichorro JG
Acute orofacial pain leads to prolonged changes in behavioral and affective pain components.
Acute pain that persists for a few days is associated with a reduction in patients' quality of life. Orofacial persistent pain promotes psychological disorders such as anxiety, impairs daily essential activities such as eating, and results in decreased social interaction. Here, we investigated whether rats subjected to orofacial formalin injection or intraoral incision surgery display persistent facial heat hyperalgesia, ongoing pain, anxiety-like behavior, and changes in ultrasonic vocalization. Orofacial formalin injection or intraoral incision caused facial heat hyperalgesia for 3 days compared with saline-injected and sham animals. In addition, both experimental groups showed a reduction in the number of entries and in the time spent in the open arms in the elevated plus maze test on day 3, suggesting that anxiety-like behavior developed as a consequence of persistent pain. At this time point, both groups also displayed a reduction in the number of 50-kHz calls, specifically in the flat subtype, which suggests a decrease in social communication. Moreover, on day 3 after surgery, systemic morphine produced robust conditioned place preference in rats subjected to intraoral incision compared with sham, and the former group also presented increased spontaneous facial grooming, revealing the presence of ongoing pain. Finally, Western blot and immunohistochemistry analysis showed a reduction in tyrosine hydroxylase expression in the nucleus accumbens, which may reflect a decrease in mesolimbic dopaminergic activity. Altogether, the results demonstrate that acute orofacial pain causes prolonged changes in behavioral and affective pain components, which may be related to dopaminergic changes in the nucleus accumbens.
Pain, 2020; 161
31856582: Araya EI, Turnes JM, Barroso AR, Chichorro JG
Contribution of intraganglionic CGRP to migraine-like responses in male and female rats.
To evaluate whether intraganglionic calcitonin gene-related peptide induced differential migraine-like responses in male and female rats.
Cephalalgia, 2020; 40
31447094: Barroso AR, Araya EI, de Souza CP, Andreatini R, Chichorro JG
Characterization of rat ultrasonic vocalization in the orofacial formalin test: Influence of the social context.
Rats emit ultrasonic vocalizations (USVs) about 22 kHz and 50 kHz sound frequency to communicate the presence of

negative or positive emotional states, respectively. The calling behavior may be influenced by several factors, including environmental factors. Likewise, pain behavior can be modulated according to the social context, and also can be transferred to conspecifics through direct observation and/or social interaction. Herein we investigated if acute pain induction was related to changes in emission of aversive and appetitive calls and how different social contexts affected the nociceptive behavior and USVs. Our results demonstrated that orofacial formalin injection in rats induced aversive calls in addition to the nociceptive behavior, and both are reduced by systemic treatment with morphine (2.5 mg/kg). Exposure of formalin-injected rats to cagemates had no effect on the nociceptive behavior or calls emitted by the demonstrator, but the observer showed emotional contagion of pain. In contrast, exposure of formalin-injected rats to non-cagemates decreased the nociceptive behavior of the demonstrator, without affecting the calls emission. The emotional contagion was not detected in non-cagemates or in cagemates separated by a visual barrier. In conclusion, we suggest that familiarity and the visual contact contributes to emotional contagion of pain. USV analysis may represent an additional measure in the evaluation of the emotional aspect of orofacial pain, and for the study of pain modulation.
Eur Neuropsychopharmacol, 2019; 29
29697717: Claudino R, Nones C, Araya E, Chichorro J
Analgesic Effects of Intranasal Ketamine in Rat Models of Facial Pain.
To assess the analgesic effect of intranasal administration of S-ketamine in different rat models of facial pain.
J Oral Facial Pain Headache, Summer 2018; 32
29678597: Gambeta E, Batista MA, Maschio GP, Turnes JM, Araya EI, Chichorro JG
Anxiety- but not depressive-like behaviors are related to facial hyperalgesia in a model of trigeminal neuropathic pain in rats.
Trigeminal neuralgia (TN) is a painful condition characterized by excruciating facial pain, which has a serious impact on quality of life. Depression and anxiety have been commonly associated with TN, but clinical studies report that these comorbidities are frequently underdiagnosed and undertreated in TN patients. Herein it was investigated if rats submitted to the infraorbital nerve constriction (CION), a model of trigeminal neuropathic pain, would display anxiety- and depressive-like behaviors in addition to the facial sensory changes in different time points after the nerve injury. CION rats developed facial heat hyperalgesia on day 5 after the nerve injury, but at this time point the time spent and the number of entries on open arms in the elevated plus maze (EPM) and the time spent on the lit compartment of light-dark transition test (LDT) was not statistically significant between SHAM and CION groups, suggesting that 5 days after CION animals do not display anxiety-like behavior. On the other hand, around 50% of CION rats developed mechanical allodynia on day 15 postsurgery and the analysis of the time spent and the number of entries on open arms on EPM and the time spent on lit compartment of LDT revealed that only CION-allodynic animals displayed anxiety-like behavior when compared to the SHAM group. The depressive-like behavior was assessed by measuring the time of immobility on the forced swim test (FST) and sucrose preference (SP) in rats previously tested for heat (day 5) and mechanical allodynia (days 15, 30 and 45) induced by CION. The evaluation of immobility time on FST and sucrose preference consumption revealed that both CION rats did not displayed depressive- and anhedonic-like behavior at any time point evaluated. Altogether, these results demonstrate that trigeminal neuropathic pain in rats leads to the development of anxiety-, but not depressive-like behavior, suggesting that the CION model represents a methodology that allows the study of drugs targeting both pain and anxiety.
Physiol Behav, 2018; 191
29570803: Gomes LO, Chichorro JG, Araya EI, de Oliveira J, Rae GA
Facial hyperalgesia due to direct action of endothelin-1 in the trigeminal ganglion of mice.
This study assessed the ability of endothelin-1 (ET-1) to evoke heat hyperalgesia when injected directly into the trigeminal ganglia (TG) of mice and determined the receptors implicated in this effect. The effects of TG ET and ET receptor blockade on alleviation of heat hyperalgesia in a model of trigeminal neuropathic pain induced by infraorbital nerve constriction (CION) were also examined.
J Pharm Pharmacol, 2018; 70
28606782: Araya EI, Nones CFM, Ferreira LEN, Kopruszinski CM, Cunha JMD, Chichorro JG
Role of peripheral and central TRPV1 receptors in facial heat hyperalgesia in streptozotocin-induced diabetic rats.
There is increasing evidence that diabetes may be related to sensory changes in the trigeminal system. Long lasting facial heat hyperalgesia has been described in diabetic rats, but the mechanisms remain to be elucidated. Herein, the contribution of peripheral and central TRPV1 receptors to facial heat hyperalgesia in diabeticrats was investigated. Diabetes was induced in male Wistar rats by streptozotocin (60mg/kg, i.p) and facial heat hyperalgesia was assessed once a week up to four weeks. The role of TRPV1 receptors in the heat hyperalgesia in diabetic rats was evaluated through: 1) the ablation of TRPV1 receptors by resiniferatoxin (RTX) treatment and 2) injection of the TRPV1 antagonist, capsazepine, into the upper lip, trigeminal ganglion or medullary subarachnoid space, at doses that completed prevented the heat hyperalgesia induced by capsaicin in naïve rats. Western blot was used to estimate the changes in TRPV1 expression in diabetic rats. Diabetic rats exhibited facial heat hyperalgesia from the first up to the fourth week after streptozotocin injection, which was prevented by

insulin treatment. Ablation of TRPV1-expressing fibers prevented facial hyperalgesia in diabetic rats. Capsazepine injection in all sites resulted in significant reduction of facial heat hyperalgesia in diabetic rats. Diabetic rats exhibited a significant decrease in TRPV1 expression in the trigeminal nerve, increased expression in the trigeminal ganglion and no changes in subnucleus caudalis when compared to normoglycemic ones. In conclusion, our results suggest that facial heat hyperalgesia in diabetic rats is maintained by peripheral and central TRPV1 receptors activation.
Brain Res, 2017; 1670

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BOARD NUMBER: S05-619

DAILY RECORD WITH PORTABLE EEG IN SCI PATIENTS WITH NEUROPATHIC PAIN

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Neuropathic pain (NP) is a common symptom arising as a direct consequence of a lesion or disease affecting the somatosensory system. The traditional approach to managing NP patients is to initiate treatment with conservative pharmacological therapy before interventional strategies. However, first-line drug treatments have shown modest efficacy with less than 50% of pain relief. Since NP is present in ~70% of patients with spinal cord injury (SCI), people with this pathology represent a reliable population to study NP. Interestingly, previous studies have shown a clear correlation between NP and changes in electroencephalography (EEG), which is a good indicator of the state of the central nervous system. We are employing state-of-the-art digital health technology (the Mobsitudy smartphone app and the BitBrain portable EEG) to collect data from SCI patients daily for one month to better understand NP progression. To date, we have recorded EEG and self-reported symptoms from 6 patients, both at home after one-day training and at the hospital, with the assistance of a technician. Early results show that the recordings suffer from low quality and the strong presence of artifacts. We employed the method Artifact Subspace Reconstruction (ASR). for removing artifacts and analysing the power spectral density of the resulting signals. The initial analysis shows that EEG traces recorded at home are of lower quality than those recorded with a technician at the hospital.

Pubmed:
34226654: Esclarin-Ruz A, Rodríguez-Carrión I, Ceruelo-Abajo S, Palazón-Garcia R, Ayuga-Loro F, Carrasco-Lopez C, Alcobendas-Maestro M, Casado-Lopez RM, Talavera-Diaz F, Soto-León V, Campolo M, Romero-Ganuza FJ, Florensa-Vila J, Garcia-Marco D, Rotondi M, Oliviero A
Phase II/III placebo-controlled randomized trial of safety and efficacy of growth hormone treatment in incomplete chronic traumatic spinal cord injury.
This is a double blind phase II/III placebo-controlled randomized trial of the safety and efficacy of GH treatment in incomplete chronic traumatic spinal cord injury.
Spinal Cord, 2021; 59
29451889: Jimenez S, Mordillo-Mateos L, Dileone M, Campolo M, Carrasco-Lopez C, Moitinho-Ferreira F, Gallego-Izquierdo T, Siebner HR, Valls-Solé J, Aguilar J, Oliviero A
Effects of patterned peripheral nerve stimulation on soleus spinal motor neuron excitability.
Spinal plasticity is thought to contribute to sensorimotor recovery of limb function in several neurological disorders and can be experimentally induced in animals and humans using different stimulation protocols. In healthy individuals, electrical continuous Theta Burst Stimulation (TBS) of the median nerve has been shown to change spinal motoneuron excitability in the cervical spinal cord as indexed by a change in mean H-reflex amplitude in the flexor carpi radialis muscle. It is unknown whether continuous TBS of a peripheral nerve can also shift motoneuron excitability in the lower limb. In 26 healthy subjects, we examined the effects of electrical TBS given to the tibial nerve in the popliteal fossa on the excitability of lumbar spinal motoneurons as measured by H-reflex amplitude of the soleus muscle evoked by tibial nerve stimulation. Continuous TBS was given at 110% of H-reflex threshold intensity and compared to non-patterned regular electrical stimulation at 15 Hz. To disclose any pain-induced effects, we also tested the effects of TBS at individual sensory threshold. Moreover, in a subgroup of subjects we evaluated paired-pulse inhibition of H-reflex. Continuous TBS at 110% of H-reflex threshold intensity induced a short-term reduction of H-reflex amplitude. The other stimulation conditions produced no after effects. Paired-pulse H-reflex inhibition was not modulated by continuous TBS or non-patterned repetitive stimulation at 15 Hz. An effect of pain on the results obtained was discarded, since non-patterned 15 Hz stimulation at 110% HT led to pain scores similar to those induced by EcTBS at 110% HT, but was not able to induce any modulation of the H reflex amplitude. Together, the results provide first time evidence that peripheral continuous TBS induces a short-lasting change in the excitability of spinal motoneurons in lower limb circuitries. Future studies need to investigate how the TBS protocol can be optimized to produce a larger and longer

effect on spinal cord physiology and whether this might be a useful intervention in patients with excessive excitability of the spinal motorneurons.
PLoS One, 2018; 13
28280254: Carrasco-López C, Soto-León V, Céspedes V, Profice P, Strange BA, Foffani G, Oliviero A
Static Magnetic Field Stimulation over Parietal Cortex Enhances Somatosensory Detection in Humans.
The role of neuronal oscillations in human somatosensory perception is currently unclear. To address this, here we use noninvasive brain stimulation to artificially modulate cortical network dynamics in the context of neurophysiological and behavioral recordings. We demonstrate that transcranial static magnetic field stimulation (tSMS) over the somatosensory parietal cortex increases oscillatory power specifically in the alpha range, without significantly affecting bottom-up thalamocortical inputs indexed by the early cortical component of somatosensory evoked potentials. Critically, we next show that parietal tSMS enhances the detection of near-threshold somatosensory stimuli. Interestingly, this behavioral improvement reflects a decrease of habituation to somatosensation. Our data therefore provide causal evidence that somatosensory perception depends on parietal alpha activity. Artificially increasing alpha power by placing a powerful magnetic field over the somatosensory cortex overcomes the natural decline in detection probability of a repeated near-threshold sensory stimulus.
J Neurosci, 2017; 37
28659614: Dileone M, Carrasco-López MC, Segundo-Rodriguez JC, Mordillo-Mateos L, López-Ariztegui N, Alonso-Frech F, Catalan-Alonso MJ, Obeso JA, Oliviero A, Foffani G
Dopamine-dependent changes of cortical excitability induced by transcranial static magnetic field stimulation in Parkinson's disease.
Transcranial static magnetic field stimulation (tSMS) is a recent low-cost non-invasive brain stimulation technique that decreases cortical excitability in healthy subjects. The objective of the present study was to test the ability of tSMS to modulate cortical excitability in patients with Parkinson's disease. We performed a randomized double-blind sham-controlled cross-over study to assess cortical excitability before and immediately after tSMS (or sham) applied for 10 min to the more affected motor cortex of patients with Parkinson's disease. Cortical excitability was quantified by the amplitude of motor evoked potentials (MEPs) elicited by single-pulse transcranial magnetic stimulation (TMS). tSMS significantly decreased MEP amplitudes in patients OFF medication (after overnight withdrawal of dopaminergic drugs), but not ON medication (after an acute dose of levodopa). The between-patients variability of tSMS-induced changes was significantly greater ON medication. The variability ON medication could be partly explained by disease progression, i.e. the more advanced the patient, the more likely it was to observe a switch from inhibitory tSMS plasticity OFF medication to paradoxical facilitatory plasticity ON medication. These results suggest that tSMS induces dopamine-dependent changes of cortical excitability in patients with Parkinson's disease.
Sci Rep, 2017; 7
25595064: Oliviero A, Carrasco-López MC, Campolo M, Perez-Borrego YA, Soto-León V, Gonzalez-Rosa JJ, Higuero AM, Strange BA, Abad-Rodriguez J, Foffani G
Safety Study of Transcranial Static Magnetic Field Stimulation (tSMS) of the Human Cortex.
Transcranial static magnetic field stimulation (tSMS) in humans reduces cortical excitability.
Brain Stimul, 2015 May-Jun; 8
26936413: Carrasco-López C, Jimenez S, Mosqueda-Pozon MC, Pérez-Borrego YA, Alcobendas-Maestro M, Gallego-Izquierdo T, Esclarin-Ruz A, Oliviero A
New Insights from Clinical Assessment of Upper Extremities in Cervical Traumatic Spinal Cord Injury.
Upper extremity function has a strong impact on the quality of life in cervical spinal cord-injured patients. Upper extremity function depends on many factors, such as muscle strength, level of lesion, and extension of the cord damage in its axial axis produced by the injury. These variables can be obtained by the International Standards for Neurological Classification of Spinal Cord Injury, which is the standard for the functional evaluation of traumatic spinal cord injury (SCI) patients. The aim of this study was to describe the relationship between upper limb muscle strength, level of injury, and axial damage with the functionality of upper limb measured using the Jebsen-Taylor Hand Function Test (JTHFT) and the 9 Hole Peg Test (9HPT) in cervical SCI. Twenty-nine patients were included in this study. Our results suggest that both the JTHFT and 9HPT can be similarly used to quantify functional impairment after cervical SCI. Moreover, our data suggest that the upper extremity motor score, JTHFT, and 9HPT strongly correlate with the American Spinal Injury Association (ASIA) impairment scale (graded from A to E), but not with the lesion level. Our findings can be of great importance for the clinician or researchers whose therapeutic interventions have as a main objective to improve upper limb functionality in patients with cervical SCI. We suggest that ASIA impairment scale, ASIA motor score, and functional tests (including JTHFT and/or 9HPT) could be used as outcome measures in cervical SCI clinical trials.
J Neurotrauma, 2016; 33

26085640: Gonzalez-Rosa JJ, Soto-Leon V, Real P, Carrasco-Lopez C, Foffani G, Strange BA, Oliviero A
Static Magnetic Field Stimulation over the Visual Cortex Increases Alpha Oscillations and Slows Visual Search in Humans.
Transcranial static magnetic field stimulation (tSMS) was recently introduced as a promising tool to modulate human cerebral excitability in a noninvasive and portable way. However, a demonstration that static magnetic fields can influence human brain activity and behavior is currently lacking, despite evidence that static magnetic fields interfere with neuronal function in animals. Here we show that transcranial application of a static magnetic field (120-200 mT at 2-3 cm from the magnet surface) over the human occiput produces a focal increase in the power of alpha oscillations in underlying cortex. Critically, this neurophysiological effect of tSMS is paralleled by slowed performance in a visual search task, selectively for the most difficult target detection trials. The typical relationship between prestimulus alpha power over posterior cortical areas and reaction time (RT) to targets during tSMS is altered such that tSMS-dependent increases in alpha power are associated with longer RTs for difficult, but not easy, target detection trials. Our results directly demonstrate that a powerful magnet placed on the scalp modulates normal brain activity and induces behavioral changes in humans.
J Neurosci, 2015; 35

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BOARD NUMBER: S05-620

A MOUSE MODEL FOR THE STUDY OF PAIN, AFFECTIVE DISORDERS AND CARDIOVASCULAR COMORBIDITY

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

Andrea Rodriguez-Lopez[1], Beltrán Álvarez-Pérez[1], Laetitia Degiorgis[2], Xiang Yi Kong[3], Christopher Nielsen[4,5], Bente Halvorsen[3,6], Laura-Adela Harsan[2,7], Rafael Maldonado[1,8]
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Aims: Chronic pain is the leading cause of disability worldwide. It commonly presents associated with affective and cardiovascular disorders, suggesting common molecular mechanisms. The present study aimed to establish a mouse model with central and peripheral mediated co-morbidities allowing to identify extreme populations to evaluate factors to vulnerability. **Methods**: Diversity Outbred male and female adult mice were intravenously injected with an adeno-associated PCSK9-encoding virus and fed with a high fat 0.25% cholesterol-enriched diet to induce the atherosclerotic phenotype. At day 30, a set of mice were subjected to intra-articular administration of monoiodoacetate (MIA) to induce osteoarthritis while another set was injected with saline (SHAM). Thermal hyperalgesia, mechanical allodynia, glucose blood levels and blood pressure were evaluated every two weeks. At the end of the 60-day protocol, a set of motor coordination, behavioural tests were performed. **Results**: MIA-mice showed significant thermal hyperalgesia and mechanical allodynia up to 4 days' post-osteoarthritis induction when compared with SHAM groups. Male mice experienced an increase in glucose blood levels, whereas only female mice experienced a blood pressure transitory increase. MIA-groups developed affective, cognitive, and motor coordination alterations. Different mice subgroups with the core co-morbidities were identified. **Conclusions**: The repeated exposure to these risk factors generates a mouse model of chronic pain, affective disorders and cardiovascular disease. Using this model, different factors of vulnerability to the development of each component of this co-morbid state can be investigated. **Acknowledgements**: This work was supported by *Generalitat de Catalunya* (FI-2021), ICREA (SAF 2021) and European Commission (Grant Agreement: #848099).

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9-13 July 2022 | Paris, France

BOARD NUMBER: S05-621

PARTIAL CRUSH INJURY IN SENSORY AFFERENT PRODUCES LONG-TERM PAIN HYPERSENSITIVITY

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

Hyoung Woo Kim[1], Alexander Daives[2], Sangwook Shim[3], Sena Chung[3], Wheedong Kim[3], Hae Min Han[4], Luuk Wieske[5], Marleen Ja Koel-Simmelink[5], Young Chul Bae[4], Seog Bae Oh[3]
[1]School of Dentistry and Dental Research Institute, Seoul National University, Department Of Neurobiology And Physiology, Seoul, Korea, Republic of, [2]University of Oxford, John Radcliffe Hospital, Nuffield Department Of Clinical Neurosciences, Oxford, United Kingdom, [3]School of Dentistry and Dental Research Institute, Seoul National University, Neurobiology And Physiology, Seoul, Korea, Republic of, [4]Kyungpook National University, Department Of Anatomy And Neurobiology, School Of Dentistry, Daegu, Korea, Republic of, [5]Vrije Universiteit Amsterdam, Department Of Clinical Chemistry, Amsterdam Umc, Amsterdam, Netherlands

Aims Peripheral nerves are capable of regeneration after damage. However, it remains unclear why pain persists even after the apparent functional recovery in many clinical conditions. We aimed to develop a nerve injury model that reflects both chronic pain and axon regeneration, and further identify histological characteristics of the model. **Methods** We compared the classical sciatic nerve crush model (i.e. complete; full crush) and a novel partial (i.e. incomplete) crush injury in adult mice. The partial nerve injury was performed using a custom-modified hemostat. Assays of thermal and mechanically-evoked pain-like behavior were paralleled by transmission electron microscopy and immunohistochemistry at acute (2-7 days) and chronic (>30 days) time-points. **Results** In both crush models, the sciatic nerve function was recovered approximately 15 days after injury. However, only partial crush developed transient thermal and chronic tactile hypersensitivity. The partial crush resulted in a greater number of surviving axons in the distal nerve beyond the window of Wallerian degeneration, and fewer sensory neurons positive for an axotomy marker, ATF3 at day 2 and 7. The axonal morphology at day 30 revealed thinner myelination after partial crush compared to the full crush and control nerves, and it was consistent with a prolonged expression of a sensory regeneration marker, STMN2. **Conclusion** The partial nerve crush, rather than the full crush with greater nerve damage, produces greater sensory hypersensitivity. We also revealed that long-term myelin-thinning correlates with long-term hypersensitivity. Comparing two similar models with distinctive pain phenotypes may yield insights into the pathophysiology of neuropathic pain.

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BOARD NUMBER: S05-622

PRIMING THE BRAIN FOR CHRONIC PAIN: THE IMPACT OF EARLY LIFE FACTORS ON PAIN IN ADOLESCENCE

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Aims To assess the impact of early life factors on adolescent chronic pain outcomes at a behavioural and molecular level. **Methods** Sprague Dawley male and female rats were randomly assigned to either a Standard or High Fat-High Sugar (HFHS) Diet, followed by a No Stress or Maternal Stress (MS) paradigm, and lastly a Sham or Injury condition (n=6M:6F/group). In adolescence, all animals were tested for anxiety-like behaviour and mechanical and thermal nociceptive sensitivity through the elevated plus maze, vonFrey, and hot cold plate tasks respectively. Markers of inflammation and microglia were analyzed through a cytokine panel and RT-qPCR from serum and tissue from the limbic region. Four-way ANOVAs with Sex, Diet, Stress, and Injury as factors were run. **Results** Anxiety-like behaviour increased in males (p <.05). Mechanical nociceptive sensitivity was increased in Female, HFHS Diet, MS, and Injured animals (p's <.05). Thermal nociception was altered by Injury on both the hot and cold plates, while also demonstrating multiple interactions (p's <.05). The cytokine panel revealed Sex and Injury effects in IL18, TNFα, and MIP1α, along with a Diet effect in MIP1α, Sex and Stress effects in IL1β, and numerous interactions (p's <.05). Results from the qPCR demonstrated Sex differences in microglial markers IBA1 and P2RY12, along with Diet effects in TNFα, and interactions in GFAP and IL1β (p's <.05). **Conclusions** Results suggest that early life factors prime the neuroimmune system to overrespond to later stressors and increase susceptibility to chronic pain through persistent inflammation and microglia activation.

Pubmed:

32653883: Salberg S, Sgro M, Brady RD, Noel M, Mychasiuk R
The Development of Adolescent Chronic Pain following Traumatic Brain Injury and Surgery: The Role of Diet and Early Life Stress.
Pain is evolutionarily necessary for survival in that it reduces tissue damage by signaling the body to respond to a harmful stimulus. However, in many circumstances, acute pain becomes chronic, and this is often dysfunctional. Adolescent chronic pain is a growing epidemic with an unknown etiology and limited effective treatment options. Given that the relationship between acute pain and chronic pain is not straightforward, there is a need to better understand the factors that contribute to the chronification of pain. Since early life factors are critical to a variety of outcomes in the developmental and adolescent periods, they pose promise as potential mechanisms that may underlie the transition from acute to chronic pain. This review examines two early life factors: poor diet and adverse childhood experiences (ACEs); they may increase susceptibility to the development of chronic pain following surgical procedures or traumatic brain injury (TBI). Beyond their high prevalence, surgical procedures and TBI are ideal models to prospectively understand mechanisms underlying the transition from acute to chronic pain. Common themes that emerged from the examination of poor diet and ACEs as mechanisms underlying this transition included: prolonged inflammation and microglia activation leading to sensitization of the pain system, and stress-induced alterations to hypothalamic-pituitary-adrenal axis function, where cortisol is likely playing a role in the development of chronic pain. These areas provide promising targets for interventions, the development of diagnostic biomarkers, and suggest that biological treatment strategies should focus on regulating the neuroinflammatory and stress responses in an effort to modulate and prevent the development of chronic pain.
Dev Neurosci, 2020; 42
34296160: Salberg S, Yamakawa GR, Griep Y, Bain J, Beveridge JK, Sun M, McDonald SJ, Shultz SR, Brady RD, Wright DK, Noel M, Mychasiuk R
Pain in the Developing Brain: Early Life Factors Alter Nociception and Neurobiological Function in Adolescent Rats.
Although adverse early experiences prime individuals to be at increased risk for chronic pain, little research has examined the trauma-pain relationship in early life or the underlying mechanisms that drive pathology over time. Given that early experiences can potentiate the nociceptive response, this study aimed to examine the effects of a high-fat, high-sugar (HFHS) diet and early life stress (maternal separation [MS]) on pain outcomes in male and female adolescent rats. Half of the rats also underwent a plantar-incision surgery to investigate how the pain system responded to a mildly painful stimuli in

adolescence. Compared with controls, animals that were on the HFHS diet, experienced MS, or had exposure to both, exhibited increased anxiety-like behavior and altered thermal and mechanical nociception at baseline and following the surgery. Advanced magnetic resonance imaging demonstrated that the HFHS diet and MS altered the maturation of the brain, leading to changes in brain volume and diffusivity within the anterior cingulate, amygdala, corpus callosum, nucleus accumbens, and thalamus, while also modifying the integrity of the corticospinal tracts. The effects of MS and HFHS diet were often cumulative, producing exacerbated pain sensitivity and increased neurobiological change. As early experiences are modifiable, understanding their role in pain may provide targets for early intervention/prevention.
Cereb Cortex Commun, 2021; 2
31583678: Salberg S, Noel M, Burke NN, Vinall J, Mychasiuk R
Utilization of a rodent model to examine the neurological effects of early life adversity on adolescent pain sensitivity.
All children experience pain, and although many recover quickly, some go on to develop chronic pain. Adolescent chronic pain is a growing epidemic. It is unknown why some adolescents recover without incident and others experience persistent pain. Although unexplored, early life adversity may contribute to the development and maintenance of chronic pain. This study investigated the effects and underlying neurobiological mechanisms of an early life stressor on nociceptive (pain) sensitivity and emotional function in male and female Sprague-Dawley rats. Using maternal separation (MS) as an established model of early life stress, we addressed two aims: investigation of the effects of MS on behavior (anxiety and pain sensitivity), and investigation of the effects of MS on mRNA and pathophysiological changes associated with an acutely painful stimulus. Our results indicate that MS increased anxiety-like behavior and altered nociceptive responsivity in adolescent rats, with decreased mechanical withdrawal thresholds indicative of heightened and prolonged pain-related behavior. The MS groups also demonstrated increased expression of genes involved in regulating the stress and fight-or-flight response, mood, and neuroplasticity; as well as increased levels of inflammatory markers. We conclude that nociception, both at the behavioral and molecular level, is altered in response to the MS stressor.
Dev Psychobiol, 2020; 62
31518660: Salberg S, Weerwardhena H, Collins R, Reimer RA, Mychasiuk R
The behavioural and pathophysiological effects of the ketogenic diet on mild traumatic brain injury in adolescent rats.
Mild traumatic brain injury (mTBI), caused by an insult to the head, results in a cascade of molecular imbalance that includes altered glucose metabolism, mitochondrial dysfunction, and increases in reactive oxygen species. Although glucose is the primary energy source for the brain, it becomes an inefficient substrate following injury, and the brain is primed to use alternative substrates (such as ketones). The ketogenic diet (KD), a high-fat, low-carbohydrate diet, forces the brain to utilize ketones over glucose for energy. Given that mTBIs are commonly experienced during adolescence, our study sought to examine the effects of the KD on recovery from mTBI in adolescent rats. This was done via two experiments; the first of which animals were fed the KD prior to a mTBI in order to investigate the neuroprotective potential of the diet, and the second the animals were fed the KD following a mTBI to examine the therapeutic potential. Male and female Sprague Dawley rats were assigned to receive a control standard diet or the KD (either pre-injury or post-injury), then further randomized to receive a sham or mTBI. Animals were tested on 6 behavioural measures designed to examine post-concussive symptomology, and mRNA analysis of the brain and small intestine were performed. Pre-injury exposure to the KD offered some neuroprotection, reducing balance and motor impairments while increasing exploratory behaviour and telomere length. Consumption of the KD following the injury also provided some therapeutic benefit, reducing both anxiety- and depressive-like behaviours. The timing of KD administration also differentially modified expression of prefrontal cortex, hippocampus, and intestinal mRNA for our genes of interest (Fgf2, Iba1, Opa1, Sirt1, Claudin3, OCC, and ZO1) This study demonstrates the neuroprotective and therapeutic potential of the KD for mTBI and warrants further investigation.
Behav Brain Res, 2019; 376
30074871: Salberg S, Christensen J, Yamakawa GR, Lengkeek C, Malik H, Tabor J, Hazari A, Mychasiuk R
A Bump on the Head or Late to Bed: Behavioral and Pathophysiological Effects of Sleep Deprivation after Repetitive Mild Traumatic Brain Injury in Adolescent Rats.
An old wives' tale, and strongly held dogma, maintains that one should be kept awake after a mild traumatic brain injury (mTBI) to prevent a coma. This, however, conflicts with the known benefits of sleep: repair and restoration. We therefore sought to examine the effects of sleep deprivation (SD) in the post-traumatic sleep period on post-concussion symptomology (PCS). Adolescent male and female rats were administered repetitive mTBIs (RmTBI) or sham injuries and were then assigned to 5 h of SD or left undisturbed. All animals were then tested using seven behavioral tasks validated to examine PCS, followed by analysis of serum cytokines, and quantitative real-time PCR for messenger RNA (mRNA) expression. Exposure to 3 SD epochs significantly impaired behavior in 4 of 7 of the measures, while RmTBI also produced dysfunction in 5 of 7 tests, but the effects of SD and RmTBI were not cumulative. SD induced long-lasting changes in serum levels of Tnf-α, IL6, and IL-1ß. mRNA expression in the pre-frontal cortex, hippocampus, hypothalamus, and anterior cingulate cortex was modified in response to SD and RmTBI; but similar to the behavioral measures, the mRNA changes were not cumulative.

Consequently, we report that SD often produced impairments similar or worse than RmTBI, and sleep hygiene should become a priority for adolescent health.
J Neurotrauma, 2018; 35
28988852: Salberg S, Yamakawa G, Christensen J, Kolb B, Mychasiuk R
Assessment of a nutritional supplement containing resveratrol, prebiotic fiber, and omega-3 fatty acids for the prevention and treatment of mild traumatic brain injury in rats.
Children and adolescents have the highest rates of traumatic brain injury (TBI), with mild TBI (mTBI) accounting for most of these injuries. Adolescents are particularly vulnerable and often suffer from post-injury symptomologies that may persist for months. We hypothesized that the combination of resveratrol (RES), prebiotic fiber (PBF), and omega-3 fatty acids (docosahexaenoic acid (DHA)) would be an effective therapeutic supplement for the mitigation of mTBI outcomes in the developing brain. Adolescent male and female Sprague-Dawley rats were randomly assigned to the supplement (3S) or control condition, which was followed by a mTBI or sham insult. A behavioral test battery designed to examine symptomologies commonly associated with mTBI was administered. Following the test battery, tissue was collected from the prefrontal cortex (PFC) and primary auditory cortex for Golgi-Cox analysis of spine density, and for changes in expression of 6 genes (Aqp4, Gfap, Igf1, Nfl, Sirt1, and Tau). 3S treatment altered the behavioral performance of sham animals indicating that dietary manipulations modify premorbid characteristics. 3S treatment prevented injury-related deficits in the longer-term behavior measures, medial prefrontal cortex (mPFC) spine density, and levels of Aqp4, Gfap, Igf1, Nfl, and Sirt1 expression in the PFC. Although not fully protective, treatment with the supplement significantly improved post-mTBI function and warrants further investigation.
Neuroscience, 2017; 365
33001680: Hazari A, Salberg S, Griep Y, Yamakawa GR, Mychasiuk R
Examining changes in rodent temperament following repetitive mild traumatic brain injury in adolescence.
Mild traumatic brain injuries are known to cause a host of symptoms, including headaches, nausea, and depression, that when persistent, are known as postconcussive syndrome. In addition to these overt symptomologies, individuals may experience changes in day-to-day behavior or temperament, which although not meeting criteria for postconcussive diagnosis, does cause distress to the individual. The aim of this study was to determine whether we could measure temperament in a rat and, if so, determine whether temperament is altered in response to repetitive mild traumatic brain injuries (RmTBI). Forty male and female adolescent Sprague-Dawley rats were same-sex pair housed and subjected to RmTBIs or sham injuries. The rats were recorded at 6 different time points throughout the study for the temperament assessment protocol, a measure of the complex behavioral profile of each rat within its dyadic home cage environment. The temperaments were quantified via a novel behavioral scoring algorithm. The rats were also tested on a battery of tests that were designed to measure symptoms of postconcussion syndrome. We determined that rodent temperament is quantifiable, is sex dependent, changes with age, and is modifiable in response to experiential factors such as RmTBI. Rats that received the RmTBIs were significantly less active and showed decreased levels of social interaction compared with their sham-injury counterparts. Moreover, both task switching and recovery patterns for RmTBI rats were dependent on the injury status of their cage mates. Future studies are now required to determine the mechanisms underlying these important changes in temperament. (PsycInfo Database Record (c) 2020 APA, all rights reserved).
Behav Neurosci, 2020; 134
32954298: Bhatt D, Hazari A, Yamakawa GR, Salberg S, Sgro M, Shultz SR, Mychasiuk R
Investigating the cumulative effects of Δ9-tetrahydrocannabinol and repetitive mild traumatic brain injury on adolescent rats.
The prevalence of mild traumatic brain injury is highest amongst the adolescent population and can lead to complications including neuroinflammation and excitotoxicity. Also pervasive in adolescents is recreational cannabis use. Δ9-Tetrahydrocannabinol, the main psychoactive component of cannabis, is known to have anti-inflammatory properties and serves as a neuroprotective agent against excitotoxicity. Thus, we investigated the effects of Δ9-tetrahydrocannabinol on recovery when administered either prior to or following repeated mild brain injuries. Male and female Sprague-Dawley rats were randomly assigned to receive Δ9-tetrahydrocannabinol or vehicle either prior to or following the repeated injuries. Rats were then tested on a behavioural test battery designed to measure post-concussive symptomology. The hippocampus, nucleus accumbens and prefrontal cortex were extracted from all animals to examine mRNA expression changes (, , , , and). We hypothesized that, in both experiments, Δ9-tetrahydrocannabinol administration would provide neuroprotection against mild injury outcomes and confer therapeutic benefit. Δ9-Tetrahydrocannabinol administration following repeated mild traumatic brain injury was beneficial to three of the six behavioural outcomes affected by injury (reducing anxiety and depressive-like behaviours while also mitigating injury-induced deficits in short-term working memory). Δ9-Tetrahydrocannabinol administration following injury also showed beneficial effects on the expression of , and in the hippocampus, nucleus accumbens and prefrontal cortex. There were no notable benefits of Δ9-tetrahydrocannabinol when administered prior to injury, suggesting that Δ9-tetrahydrocannabinol may have potential therapeutic benefit on post-

concussive symptomology when administered post-injury, but not pre-injury.
Brain Commun, 2020; 2
34743137: Beveridge JK, Yeates KO, Madigan S, Stone AL, Wilson AC, Sumpton JE, Salberg S, Mychasiuk R, Noel M
Examining Parent Adverse Childhood Experiences as a Distal Risk Factor in Pediatric Chronic Pain.
Adverse childhood experiences (ACEs; ie, exposure to abuse, neglect, household dysfunction in childhood) are associated with poor mental and physical health outcomes across the lifespan. Emerging research suggests parent ACEs also confer risk for poor child outcomes. The relation between parent ACEs and child pain in youth with chronic pain has not yet been examined. The aim of the current longitudinal study was to examine the associations among parent ACEs, parent health, and child pain, in a clinical sample of youth with chronic pain.
Clin J Pain, 2021; 38
33134755: Beveridge JK, Dobson KS, Madigan S, Yeates KO, Stone AL, Wilson AC, Salberg S, Mychasiuk R, Noel M
Adverse childhood experiences in parents of youth with chronic pain: prevalence and comparison with a community-based sample.
Adverse childhood experiences (ACEs) are common occurrences that are related to poor health outcomes, including chronic pain, in youth and adults. Research suggests that children of parents exposed to ACEs are also at risk of poor outcomes. However, little is known about the risk that ACEs confer for chronic pain across generations. Parent ACEs may play an important role in pediatric chronic pain, given their association with key parent factors (eg, mental and physical health).
Pain Rep, 2020 Nov-Dec; 5

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STUDY OF THE ANTINOCICEPTIVE EFFECT OF MORPHINE EVALUATED BY A NEUROPATHIC PAIN MODEL IN MALE AND FEMALE MICE.

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Morphine is one of the most widely used analgesics in the treatment of moderate and severe pain. However, its clinical use in chronic pain treatment is limited by the addictive potential. The co-administration of morphine with drugs that enhance the analgesic effect and reduce its reinforcing properties, could be an alternative in pain treatment with opioids. In the present study, we propose to determine the lowest effective dose of morphine to ameliorate the nociceptive threshold by using the partial sciatic nerve ligation (PSNL) in male and female Balb/C mice. Von Frey test (VFT) was performed in order to evaluate mechanical allodynia by calculating the nociceptive threshold. Mice were habituated to the environment for four days, baseline responses were measured, and surgery of the right paw was performed in a group of animals with PSNL (PSNL group), and surgery without PSNL was executed in another group (Sham group). Nine days after surgery, morphine (1, 3, 9 mg / kg, i.p.) or saline were administered and VFT was carried out. Our results showed that morphine (3 and 9 mg/kg) was able to reduce neuropathic pain in both sexes ($p < 0.001$). Although some studies have shown that the antinociceptive effect of morphine is more intense in males than in females, it has been suggested that sex differences depend on the strain of mice. These results will allow us to evaluate potential therapeutic targets to enhance the analgesic effect of opiates using the lowest effective dose of morphine (3 mg/kg) in both sexes.

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BOARD NUMBER: S05-624

FUNCTIONAL ALTERATIONS OF INTRINSIC NETWORKS AT VARIOUS STAGES OF NEUROPATHIC PAIN AND COMORBIDITY DEVELOPMENT

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

Silvia Cazzanelli[1,2], Samuel Diebolt[1,2], Adrien Bertolo[1,2], Jérémy Ferrier[2], Mathis Vert[1,2], Thomas Deffieux[1], Bruno Osmanski[2], Mickael Tanter[3], Sophie Pezet[1]
[1]Physics for Medicine, Inserm, Espci Paris, Cnrs, Psl Research University, Paris, France, [2]Iconeus, Iconeus, Paris, France, [3]Physics for Medicine, Inserm, Espci Paris, Cnrs, Psl Research University, Paris, France

Chronic pain is due to abnormal, maladaptive neuronal plasticity in the structures known to be involved in pain perception. Our hypothesis is that the aforementioned maladaptive plasticity in these brain areas could be key mechanisms for the development of comorbidities, such as anxiety and depression. Functional ultrasound (fUS) imaging is a sensitive and versatile neuroimaging modality, able to quantify with a large field of view (indirectly) neuronal activation and measure functional connectivity (FC), an indirect measure of the functionality and strength of brain networks. This study aimed at identifying, using fUS imaging, how the functionality of brain networks is changing in link with neuropathic pain and the comorbidity associated to it. We measured the functional connectivity (FC) at rest in awake, head fixed animals, subjected to neuropathic pain (2W cuffing of the sciatic nerve), or anxiety (8W) or depression (12W). Behavioural tests were carried out to follow the various symptoms. Our results show time-specific alterations of the networks. At the emergence of neuropathic pain, FC within regions involved in the sensory aspect of pain are altered and then gradually mutating overtime (8W-12w) into some changes in areas involved in the emotional aspect of pain, as the Anterior part of the Cingulate cortex and the Insular cortex. In agreement with our hypothesis, these findings suggest that alterations in the regions of the '*pain matrix'* are developing along with the stage of the disease and the associated comorbidities.

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DESCENDING SEROTONERGIC FACILITATION CONTRIBUTION TO DNIC ANALGESIA IN PROLONGED JOINT INFLAMMATORY PAIN

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Background & Aims: The diffuse noxious inhibitory controls (DNIC) are an analgesic paradigm relying on the descending monoaminergic modulation but the underlying mechanisms remain understudied. We sought to study the descending serotonergic system in monoarthritis (MA). *Methods:* Male Wistar rats received tibiotarsal injections of complete Freund's adjuvant or vehicle. Nociceptive hypersensitivity and DNIC were evaluated weekly until day 42. On day 42, the effects of intrathecal ondansetron (5-hydroxytryptamine 3 receptors (5-HT3R) antagonist) were tested on mechanical hyperalgesia and DNIC (before or after the DNIC conditioning stimulus). Spinal 5-HT3R expression and 5-HT levels were quantified via immunofluorescence and HPLC, respectively. The expression of TPH and its co-localization with the phosphorylated extracellular signal-regulated protein kinases 1 and 2 (pERKs1/2; neuronal activation marker) were evaluated at the rostroventral medulla (RVM). *Results:* DNIC was enhanced at earlier timepoints of MA and attenuated on day 42. Ondansetron reversed mechanical hyperalgesia in MA rats. On controls, this effect was observed with 10mg. MA rats showed increased spinal 5-HT3R and 5-HT. At the RVM, TPH and TPH-pERK1/2 double-labeled neurons were increased. Ondansetron restored DNIC in MA rats and at the dose of 10mg it increased DNIC intensity regardless of being administered before or after the conditioning stimulation in MA and controls. *Conclusion:* Our results suggest an enhancement of descending serotonergic facilitation during prolonged MA. The shift towards an enhancement of descending facilitation is likely involved in the attenuation of DNIC. The descending serotonergic system appears to be involved in the maintenance of DNIC rather than its initiation.

Pubmed:

32340137: Pereira-Silva R, Costa-Pereira JT, Alonso R, Serrão P, Martins I, Neto FL
Attenuation of the Diffuse Noxious Inhibitory Controls in Chronic Joint Inflammatory Pain Is Accompanied by Anxiodepressive-Like Behaviors and Impairment of the Descending Noradrenergic Modulation.
The noradrenergic system is paramount for controlling pain and emotions. We aimed at understanding the descending noradrenergic modulatory mechanisms in joint inflammatory pain and its correlation with the diffuse noxious inhibitory controls (DNICs) and with the onset of anxiodepressive behaviours. In the complete Freund's adjuvant rat model of Monoarthritis, nociceptive behaviors, DNICs, and anxiodepressive-like behaviors were evaluated. Spinal alpha2-adrenergic receptors (a2-AR), dopamine beta-hydroxylase (DBH), and noradrenaline were quantified concomitantly with a2-AR pharmacologic studies. The phosphorylated extracellular signal-regulated kinases 1 and 2 (pERK1/2) were quantified in the Locus coeruleus (LC), amygdala, and anterior cingulate cortex (ACC). DNIC was attenuated at 42 days of monoarthritis while present on days 7 and 28. On day 42, in contrast to day 28, noradrenaline was reduced and DBH labelling was increased. Moreover, spinal a2-AR were potentiated and no changes in a2-AR levels were observed. Additionally, at 42 days, the activation of ERKs1/2 was increased in the LC, ACC, and basolateral amygdala. This was accompanied by anxiety- and depressive-like behaviors, while at 28 days, only anxiety-like behaviors were observed. The data suggest DNIC is attenuated in prolonged chronic joint inflammatory pain, and this is accompanied by impairment of the descending noradrenergic modulation and anxiodepressive-like behaviors.
Int J Mol Sci, 2020; 21

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BETAINE AMELIORATES PROVOKED AND ONGOING PAIN IN NERVE-INJURED RATS BY REGULATING KIF17 MEDIATED NR2B ACTIVATION AND NEUROINFLAMMATION

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Aims: The management of neuropathic pain is still a major challenge because of its unresponsiveness to most common treatments and its elusive mechanisms. Betaine has been reported to play an active role in the treatment of various neurological disorders including inflammatory pain. This study aims to investigate the effect of betaine and elucidate its molecular mechanisms in an animal model of neuropathic pain. **Methods:** We assessed multiple pain parameters including thermal sensitivity, static and dynamic mechanical allodynia and ongoing pain. Further molecular analysis was performed using RT-PCR and western blotting to measure the mRNA and protein expressions of NR2B, KIF17 and important markers of nociception such as TRPV1, substance P, CGRP and other inflammatory mediators. **Results:** We found that betaine treatment showed significant and dose-dependent inhibition of both evoked and chronic ongoing pain in rats with nerve injury. Betaine and gabapentin treatment significantly inhibits spontaneous ongoing pain in nerve-injured rats but did not produce any place preference behavior in healthy naïve rats pointing towards their non-addictive analgesic potential. Molecular findings suggested that enhanced expressions of NR2B and KIF-17 along with increased TRPV1, substance P, CGRP, TNF-α, IL-1β, and IL-6 levels in dorsal root ganglion (DRG) and spinal cord of nerve-injured rats which was significantly attenuated on treatment with different doses of betaine in a dose-dependent manner. **Conclusion:** The current findings suggest that inhibition of the expression of KIF-17 mediated NR2B activation and neuroinflammation further leads to significant inhibition of evoked and chronic ongoing pain in nerve-injured rats.

Pubmed:
34856209: Akhilesh , Uniyal A, Gadepalli A, Tiwari V, Allani M, Chouhan D, Ummadisetty O, Verma N, Tiwari V
Unlocking the potential of TRPV1 based siRNA therapeutics for the treatment of chemotherapy-induced neuropathic pain.
Chemotherapy-induced neuropathic pain (CINP) is among the most common clinical complications associated with the use of anti-cancer drugs. CINP occurs in nearly 68.1% of the cancer patients receiving chemotherapeutic drugs. Most of the clinically available analgesics are ineffective in the case of CINP patients as the pathological mechanisms involved with different chemotherapeutic drugs are distinct from each other. CINP triggers the somatosensory nervous system, increases the neuronal firing and activation of nociceptive mediators including transient receptor protein vanilloid 1 (TRPV1). TRPV1 is widely present in the peripheral nociceptive nerve cells and it has been reported that the higher expression of TRPV1 in DRGs serves a critical role in the potentiation of CINP. The therapeutic glory of TRPV1 is well recognized in clinics which gives a promising insight into the treatment of pain. But the adverse effects associated with some of the antagonists directed the scientists towards RNA interference (RNAi), a tool to silence gene expression. Thus, ongoing research is focused on developing small interfering RNA (siRNA)-based therapeutics targeting TRPV1. In this review, we have discussed the involvement of TRPV1 in the nociceptive signaling associated with CINP and targeting this nociceptor, using siRNA will potentially arm us with effective therapeutic interventions for the clinical management of CINP.
Life Sci, 2022; 288
34386880: Uniyal A, Akhilesh , Tiwari V, Gadepalli A, Ummadisetty O, Tiwari V
Epigallocatechin-3-gallate improves chronic alcohol-induced cognitive dysfunction in rats by interfering with neuro-inflammatory, cell death and oxido-nitrosative cascade.
Alcohol consumption for a longer period of time is linked with neuronal damage and an increase in inflammatory signaling resulting in cell death and dementia. Natural compounds are the focus of research due to their high efficacy and good safety profile. Here we have investigated the effect of chronic epigallocatechin-3-gallate (EGCG) administration against the alcohol-induced cognitive deficit rats. Male Wistar rats were exposed to the 12% ethanol (10 g/kg; oral gavage) for ten weeks and treated with EGCG (25, 50, and 100 mg/kg) for the same duration. Ethanol exposure led to the impaired spatial memory and learning in rats assessed using the Morris water maze and elevated plus-maze test. Further, we assessed the role of EGCG in mitigating the oxidative stress, neuroinflammatory and cell death signaling associated markers. Co-administration with

EGCG significantly prevented all the behavioral, biochemical and molecular alterations in the different brain regions of ethanol-treated rats in a dose-dependent manner. EGCG suppressed the acetylcholinesterase activity, increased oxidative-nitrosative stress, cytokines (TNF-alpha and IL-1beta), NF-kappa β and caspase-3 levels in both the cortex and hippocampus of ethanol-treated rats. Our preliminary study demonstrated that EGCG improves the oxido-nitrosative stress, inflammation, and cell death signaling associated with ethanol-induced cognitive dysfunction. This suggests the potential role of EGCG in mitigating the cognitive deficits associated with chronic alcohol consumption.
Metab Brain Dis, 2021; 36
34357554: Uniyal A, Tiwari V, Rani M, Tiwari V
Immune-microbiome interplay and its implications in neurodegenerative disorders.
The neurodegeneration and its related CNS pathologies need an urgent toolbox to minimize the global mental health burden. The neuroimmune system critically regulates the brain maturation and survival of neurons across the nervous system. The chronic manipulated immunological drive can accelerate the neuronal pathology hence promoting the burden of neurodegenerative disorders. The gut is home for trillions of microorganisms having a mutual relationship with the host system. The gut-brain axis is a unique biochemical pathway through which the gut residing microbes connects with the brain cells and regulates various physiological and pathological cascades. The gut microbiota and CNS communicate using a common language that synchronizes the tuning of immune cells. The intestinal gut microbial community has a profound role in the maturation of the immune system as well as the development of the nervous system. We have critically summarised the clinical and preclinical reports from the past a decade emphasising that the significant changes in gut microbiota can enhance the host susceptibility towards neurodegenerative disorders. In this review, we have discussed how the gut microbiota-mediated immune response inclines the host physiology towards neurodegeneration and indicated the gut microbiota as a potential future candidate for the management of neurodegenerative disorders.
Metab Brain Dis, 2022; 37
34324307: Uniyal A, Thakur V, Rani M, Tiwari V, Akhilesh , Gadepalli A, Ummadisetty O, Modi A, Tiwari V
Kinesin Nanomotors Mediated Trafficking of NMDA-Loaded Cargo as A Novel Target in Chronic Pain.
Chronic pain is among the most prevalent burdensome disorders worldwide. The -methyl-d-aspartate (NMDA) receptor system plays a critical role in central sensitization, a primary feature of chronic pain. Despite the proven efficacy of exogenous ligands to this receptor system in preclinical studies, evidence for the clinical efficacy of NMDA antagonists for the treatment of chronic pain is weak. Researchers are studying alternate approaches, rather than direct inhibition of the NMDA receptors in pain processing neurons. This indirect approach utilizes the modulation of molecular switches that regulates the synthesis, maturation, and transport of receptors from cellular organelles to the synaptic membrane. Kinesins are nanomotors that anterogradely transport the cargo using microtubule tracks across the neurons. Various members of the kinesin family, including KIF17, KIF11, KIF5b, and KIF21a, regulate the intracellular transport of NMDA receptors. Pharmacological targeting of these ATP-driven nanomotors could be a useful tool for manipulating the NMDAR functioning. It could provide the potential for the development of a novel strategy for the management of chronic pain.
ACS Chem Neurosci, 2021; 12
33011242: Shaw S, Uniyal A, Gadepalli A, Tiwari V, Belinskaia DA, Shestakova NN, Venugopala KN, Deb PK, Tiwari V
Adenosine receptor signalling: Probing the potential pathways for the ministration of neuropathic pain.
Neuropathic pain is a critical burdensome problem due to the complex interplay of several pathological mechanisms and lack of availability of effective therapeutic interventions. The available therapeutic options are associated with a variety of limitations, including severe side effects, and unmet medical needs, warranting further research to identify and validate potential targets. Adenosine receptors system is a widely studied target, which evidently was successful in alleviation of neuropathic pain in several experimental paradigms, and researchers are putting efforts in building its clinical roadmap. The adenosine receptors act by different mechanisms and targeting adenosine receptors for neuropathic pain includes several important pathways such as p38-mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinases (ERK), brain-derived neurotrophic factor (BDNF) signalling, γ-aminobutyric acid (GABA) as well as the ion channel modulations. Various studies have also shown the relevance of targeting adenosine receptors in chemotherapy-induced neuropathic pain and diabetic neuropathy. Several drugs acting on adenosine receptors have undergone clinical trials for management of neuropathic pain, whereas many other drugs are yet to be studied to find a potential anti-nociceptive agent. In this review, we have discussed the roadmap of adenosine receptors as a potential target for the treatment of neuropathic pain.
Eur J Pharmacol, 2020; 889
31815916: Tiwari V, He SQ, Huang Q, Liang L, Yang F, Chen Z, Tiwari V, Fujita W, Devi LA, Dong X, Guan Y, Raja SN
Activation of μ-δ opioid receptor heteromers inhibits neuropathic pain behavior in rodents.
Several reports support the idea that μ- and δ-opioid receptors (ORs) may exist as heterodimers in brain regions involved in pain signaling. The unique pharmacology of these heteromers may present a novel analgesic target. However, the role of μ-δ heteromers in sensory neurons involved in pain and opioid analgesia remains unclear, particularly during neuropathic pain.

We examined the effects of spinal nerve injury on μ-δ heteromer expression in dorsal root ganglion (DRG) neurons and the effects of a μ-δ heteromer-targeting agonist, CYM51010, on neuropathic pain behavior in rats and mice. An L5 spinal nerve ligation (SNL) in rats significantly decreased μ-δ heteromer expression in L5 DRG but increased heteromer levels in uninjured L4 DRG. Importantly, in SNL rats, subcutaneous injection of CYM51010 inhibited mechanical hypersensitivity in a dose-related manner (EC50: 1.09 mg/kg) and also reversed heat hyperalgesia and attenuated ongoing pain (2 mg/kg, subcutaneously). HEK-293T cell surface-labeled with μ- and δ-ORs internalized both receptors after exposure to CYM51010. By contrast, in cells transfected with μ-OR alone, CYM51010 was significantly less effective at inducing receptor internalization. Electrophysiologic studies showed that CYM51010 inhibited the C-component and windup phenomenon in spinal wide dynamic range neurons of SNL rats. The pain inhibitory effects of CYM51010 persisted in morphine-tolerant rats but was markedly attenuated in μ-OR knockout mice. Our studies show that spinal nerve injury may increase μ-δ heterodimerization in uninjured DRG neurons, and that μ-δ heteromers may be a potential therapeutic target for relieving neuropathic pain, even under conditions of morphine tolerance.
Pain, 2020; 161

29601322: Tiwari V, Anderson M, Yang F, Tiwari V, Zheng Q, He SQ, Zhang T, Shu B, Chen X, Grenald SA, Stephens KE, Chen Z, Dong X, Raja SN, Guan Y
Peripherally Acting μ-Opioid Receptor Agonists Attenuate Ongoing Pain-associated Behavior and Spontaneous Neuronal Activity after Nerve Injury in Rats.
Ongoing neuropathic pain is difficult to treat. The authors examined whether dermorphin [D-Arg2, Lys4] (1-4) amide, a peripherally acting μ-opioid receptor agonist, attenuates ongoing pain-associated manifestations after nerve injury in rats and mice.
Anesthesiology, 2018; 128

28223516: Li Z, Tseng PY, Tiwari V, Xu Q, He SQ, Wang Y, Zheng Q, Han L, Wu Z, Blobaum AL, Cui Y, Tiwari V, Sun S, Cheng Y, Huang-Lionnet JH, Geng Y, Xiao B, Peng J, Hopkins C, Raja SN, Guan Y, Dong X
Targeting human Mas-related G protein-coupled receptor X1 to inhibit persistent pain.
Human Mas-related G protein-coupled receptor X1 (MRGPRX1) is a promising target for pain inhibition, mainly because of its restricted expression in nociceptors within the peripheral nervous system. However, constrained by species differences across , drug candidates that activate MRGPRX1 do not activate rodent receptors, leaving no responsive animal model to test the effect on pain in vivo. Here, we generated a transgenic mouse line in which we replaced mouse with human This humanized mouse allowed us to characterize an agonist [bovine adrenal medulla 8-22 (BAM8-22)] and a positive allosteric modulator (PAM), ML382, of MRGPRX1. Cellular studies suggested that ML382 enhances the ability of BAM8-22 to inhibit high-voltage-activated Ca channels and attenuate spinal nociceptive transmission. Importantly, both BAM8-22 and ML382 effectively attenuated evoked, persistent, and spontaneous pain without causing obvious side effects. Notably, ML382 by itself attenuated both evoked pain hypersensitivity and spontaneous pain in mice after nerve injury without acquiring coadministration of an exogenous agonist. Our findings suggest that humanized mice provide a promising preclinical model and that activating MRGPRX1 is an effective way to treat persistent pain.
Proc Natl Acad Sci U S A, 2017; 114

26900065: Tiwari V, Tiwari V, He S, Zhang T, Raja SN, Dong X, Guan Y
Mas-Related G Protein-Coupled Receptors Offer Potential New Targets for Pain Therapy.
The founding member of the Mas-related G-protein-coupled receptor (Mrgpr) family was discovered in 1986. Since then, many more members of this receptor family have been identified in multiple species, and their physiologic functions have been investigated widely. Because they are expressed exclusively in small-diameter primary sensory neurons, the roles of Mrgpr proteins in pain and itch have been best studied. This review will focus specifically on the current knowledge of their roles in pathological pain and the potential development of new pharmacotherapies targeted at some Mrgprs for the treatment of chronic pain. We will also discuss the limitations and future scope of this receptor family in pain treatment.
Adv Exp Med Biol, 2016; 904

26756519: Tiwari V, Yang F, He SQ, Shechter R, Zhang C, Shu B, Zhang T, Tiwari V, Wang Y, Dong X, Guan Y, Raja SN
Activation of Peripheral μ-opioid Receptors by Dermorphin [D-Arg2, Lys4] (1-4) Amide Leads to Modality-preferred Inhibition of Neuropathic Pain.
Opioids have long been regarded as the most effective drugs for the treatment of severe acute and chronic pain. Unfortunately, their therapeutic efficacy and clinical utility have been limited because of central and peripheral side effects.
Anesthesiology, 2016; 124

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IMPAIRED PAIN TOLERANCE IN AGING: WHAT ROLE FOR LOCAL SKIN BLOOD FLOW?

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Aim: A decrease in pain tolerance is observed in aging, especially when tested with prolonged hot nociceptive stimuli. This type of stimuli activate Aδ and C fibers, the latter also being involved in the control of cutaneous vasomotricity. However, in the elderly, cutaneous vasodilation in response to local skin heating is impaired. This study therefore aims to test whether an alteration in local skin blood flow may participate in the decrease of pain tolerance with age. Methods: 30 young and 40 old participants performed a pain resistance test, in which they placed their hand in an airtight box with the air temperature regulated at 65°C. Participants continuously estimated the pain intensity using a visual analog scale (VAS). The test was 15 minutes long, but participants could end the test earlier if the pain became intolerable. Local blood flow was continuously recorded using laser Doppler flowmetry. Results: The results showed that the elderly have a decreased blood flow compared to the young. We also observed that pain tolerance is impaired in the elderly as 85% of the young subjects resisted pain until the end of the test compared to 50% in the elderly. In the elderly who did not resist until the end of the test, the results showed that blood flow positively correlated with pain rating, whereas no correlation was observed in the other participants. Conclusion: Our results suggest that altered local skin blood flow may influence thermal pain tolerance in aging.

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PRINCIPLES OF NOCICEPTION IN THE ANTERIOR CINGULATE CORTEX

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

Mario Acuna[1], Fernando Kasanetz[2], Paolo De Luna[1], Marta Falkowska[3], Thomas Nevian[3]
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The perception of pain is a multidimensional sensory and emotional/affective experience arising from distributed brain activity. However, the involved brain regions are not specific for pain. Thus, how the cortex distinguishes nociception from other aversive and salient sensory stimuli remains elusive. Using in vivo calcium imaging in mice, we investigated nociceptive in comparison to other sensory coding in the anterior cingulate cortex, a region essential for the emotional processing of pain. The overall population activity, but not single cell responses were required to discriminate nociceptive stimuli and associated behavioral responses. Peripheral nerve injury-induced neuropathic pain led to dysfunctional decoding of sensory events by exacerbation of saliency-detection and impairment of pattern separation and classification, which were reversed by analgesic treatment. These findings provide a novel interpretation for altered nociceptive processing of chronic pain in the brain.

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BOARD NUMBER: S05-629

IMPACT OF LONG-TERM AND SHORT-TERM EXPOSURE OF ENVIRONMENTAL ENRICHMENT ON PAIN-RELATED DEPRESSION IN ADOLESCENT MICE.

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Chronic pain significantly impacts well-being of more than 30% of the world's population. Its comorbidities, such as depression, anxiety, cognitive impairments or sleep disturbances may intensify and exacerbate the overall experience of pain, which consists of physiological as well as psychological aspects. Pharmacological treatments are often effective in reversing these changes, yet carry the risk of side-effects and addiction. Moreover, prescription drugs may be costly, therefore inaccessible to number of individuals. This project aims to characterize the impact of environmental manipulation on animal model of chronic pain, and evaluate if this can prevent the development of anxiodepressive symptoms. In a pilot study, we confirmed the presence of depressive-like behaviors in adult mice after Spared Nerve Injury (SNI) causing long-lasting pain in one hind paw. Subsequently, we randomly assigned 64 mice at 21 days of age into one of eight experimental groups, following two different protocols. Each protocol is applied to four groups: SNI (pain) + standard housing, SNI + enriched environment, sham (control) + standard housing, and sham + enriched environment. Protocols differ in the duration of exposure to enrichment: a) enrichment is interrupted after 6 weeks, simultaneously to conducting SNI/sham surgery; or b) all four groups of animals are housed in the enriched environment until the end of the experiment. Six weeks after surgery, we evaluate mechanical hypersensitivity and depression phenotype, using multiple behavioral tests: von Frey, marble burying, novelty suppressed feeding, splash test, and forced swimming task.

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BOARD NUMBER: S05-630

TOZASERTIB ATTENUATES EVOKED AND ONGOING PAIN IN NERVE INJURED RATS BY INHIBITING KIF MEDIATED INFLAMMATORY SIGNALLING

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Aims: Kinesins (KIF's) are the motor proteins which are recently reported to be involved in the trafficking of nociceptors leading to chronic pain. Aurora kinases are known to be involved in the regulation of KIF proteins which are associated with the activation of NMDA receptors. Here, we investigated the effect of tozasertib, a pan-Aurora kinase inhibitor, on nerve injury-induced evoked and chronic ongoing pain in rats. **Methods:** In this study we evaluated the effect of tozasertib on nerve injury-induced chronic pain. We assessed various pain parameters such as thermal sensitivity, static and dynamic mechanical allodynia and ongoing pain. Further we performed molecular analysis using RT-PCR and western blotting to measure the mRNA and protein expressions of associated markers. **Results:** Tozasertib produced significant and dose-dependent inhibition of both evoked and chronic ongoing pain in nerve injured rats. Tozasertib and gabapentin treatment significantly attenuates spontaneous ongoing pain in nerve injured rats but did not produce any place preference behaviour in healthy naïve rats pointing towards their non-addictive analgesic potential. Western blotting and RT-PCR studies suggested enhanced expressions of NR2B and KIF-17 along with increased NFkβ, TNF-α, IL-1β, and IL-6 levels in DRG and spinal cord of nerve injured rats which was significantly attenuated on treatment with different doses of tozasertib. **Conclusion:** Findings from the current study suggests that inhibition of pan-aurora kinase attenuates KIF-17- NR2B crosstalk mediated activation of inflammatory signaling which further leads to significant inhibition of both evoked and chronic ongoing pain in nerve-injured rats.

Pubmed:

35183034: Uniyal A, Akhilesh , Singh Rathore A, Kumari Keshri P, Pratap Singh S, Singh S, Tiwari V
Inhibition of pan-Aurora kinase attenuates evoked and ongoing pain in nerve injured rats via regulating KIF17-NR2B mediated signaling.
Kinesins (KIF's) are the motor proteins which are recently reported to be involved in the trafficking of nociceptors leading to chronic pain. Aurora kinases are known to be involved in the regulation of KIF proteins which are associated with the activation of N-methyl-D-aspartate (NMDA) receptors. Here, we investigated the effect of tozasertib, a pan-Aurora kinase inhibitor, on nerve injury-induced evoked and chronic ongoing pain in rats and the involvement of kinesin family member 17 (KIF17) and NMDA receptor subtype 2B (NR2B) crosstalk in the same. Rats with chronic constriction injury showed a significantly decreased pain threshold in a battery of pain behavioural assays. We found that tozasertib [10, 20, and 40 mg/kg intraperitoneally (i.p.)] treatment showed a significant and dose-dependent inhibition of both evoked and chronic ongoing pain in rats with nerve injury. Tozasertib (40 mg/kg i.p.) and gabapentin (30 mg/kg i.p.) treatment significantly inhibits spontaneous ongoing pain in nerve injured rats but did not produce any place preference behaviour in healthy naïve rats pointing towards their non-addictive analgesic potential. Moreover, tozasertib (10, 20, and 40 mg/kg i.p.) and gabapentin (30 mg/kg i.p.) treatment did not altered the normal pain threshold in healthy naïve rats and didn't produce central nervous system associated side effects as well. Western blotting and reverse transcription polymerase chain reaction studies suggested enhanced expressions of NR2B and KIF-17 along with increased nuclear factor kappa β (NFkβ), tumour necrosis factor-α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) levels in dorsal root ganglion (DRG) and spinal cord of nerve injured rats which was significantly attenuated on treatment with different does of Tozasertib. Findings from the current study suggests that inhibition of pan-Aurora kinase decreased KIF-17 mediated NR2B activation which further leads to significant inhibition of evoked and chronic ongoing pain in nerve-injured rats.
Int Immunopharmacol, 2022; 106
34856209: Akhilesh , Uniyal A, Gadepalli A, Tiwari V, Allani M, Chouhan D, Ummadisetty O, Verma N, Tiwari V
Unlocking the potential of TRPV1 based siRNA therapeutics for the treatment of chemotherapy-induced neuropathic pain.
Chemotherapy-induced neuropathic pain (CINP) is among the most common clinical complications associated with the use of anti-cancer drugs. CINP occurs in nearly 68.1% of the cancer patients receiving chemotherapeutic drugs. Most of the

clinically available analgesics are ineffective in the case of CINP patients as the pathological mechanisms involved with different chemotherapeutic drugs are distinct from each other. CINP triggers the somatosensory nervous system, increases the neuronal firing and activation of nociceptive mediators including transient receptor protein vanilloid 1 (TRPV1). TRPV1 is widely present in the peripheral nociceptive nerve cells and it has been reported that the higher expression of TRPV1 in DRGs serves a critical role in the potentiation of CINP. The therapeutic glory of TRPV1 is well recognized in clinics which gives a promising insight into the treatment of pain. But the adverse effects associated with some of the antagonists directed the scientists towards RNA interference (RNAi), a tool to silence gene expression. Thus, ongoing research is focused on developing small interfering RNA (siRNA)-based therapeutics targeting TRPV1. In this review, we have discussed the involvement of TRPV1 in the nociceptive signaling associated with CINP and targeting this nociceptor, using siRNA will potentially arm us with effective therapeutic interventions for the clinical management of CINP.
Life Sci, 2022; 288

34723483: Gadepalli A, Akhilesh , Uniyal A, Modi A, Chouhan D, Ummadisetty O, Khanna S, Solanki S, Allani M, Tiwari V
Multifarious Targets and Recent Developments in the Therapeutics for the Management of Bone Cancer Pain.
Bone cancer pain (BCP) is a distinct pain state showing characteristics of both neuropathic and inflammatory pain. On average, almost 46% of cancer patients exhibit BCP with numbers flaring up to as high as 76% for terminally ill patients. Patients suffering from BCP experience a compromised quality of life, and the unavailability of effective therapeutics makes this a more devastating condition. In every individual cancer patient, the pain is driven by different mechanisms at different sites. The mechanisms behind the manifestation of BCP are very complex and poorly understood, which creates a substantial barrier to drug development. Nevertheless, some of the key mechanisms involved have been identified and are being explored further to develop targeted molecules. Developing a multitarget approach might be beneficial in this case as the underlying mechanism is not fixed and usually a number of these pathways are simultaneously dysregulated. In this review, we have discussed the role of recently identified novel modulators and mechanisms involved in the development of BCP. They include ion channels and receptors involved in sensing alteration of temperature and acidic microenvironment, immune system activation, sodium channels, endothelins, protease-activated receptors, neurotrophins, motor proteins mediated trafficking of glutamate receptor, and some bone-specific mechanisms. Apart from this, we have also discussed some of the novel approaches under preclinical and clinical development for the treatment of bone cancer pain.
ACS Chem Neurosci, 2021; 12

34519252: Akhilesh , Baidya ATK, Uniyal A, Das B, Kumar R, Tiwari V
Structure-based virtual screening and molecular dynamics simulation for the identification of sphingosine kinase-2 inhibitors as potential analgesics.
Neuropathic pain is due to an injury or disease of the somatosensory nervous system, which accounts for a significant economical and health burden to society. Due to poor understanding of their underlying mechanisms, the available treatments merely provide symptomatic relief and precipitates a variety of adverse effects. This suggests that there is an unmet medical need that must be addressed with effective strategies for the development of novel therapeutics. Sphingosine kinase 2 (SphK2) is an oncogenic lipid kinase that has emerged as a promising target for chronic pain and other diseases. In the present study, we have explored the structure-based virtual high-throughput screening of the Nuclei of Bioassays, Ecophysiology, and Biosynthesis of Natural Products Database (NuBBE) to identify potent natural products as inhibitors of SphK2. A molecular docking study was performed to calculate binding affinities and specificity to identify potential leads against SphK2. Initially, hits were selected by the implementation of absorption, distribution, metabolism, excretion and toxicity properties, Lipinski rule, and PAINS filters. The top-scoring hits also exhibiting an optimal ADMET profile were subjected to MM/GBSA free binding free energy calculation and molecular dynamics simulation. The results from molecular dynamics simulation revealed a stable ligand -SphK2 complex with protein and ligand RMSD within reasonable limits. Overall, we identified compounds, NuBBE_972 and NuBBE_1107 as potential inhibitors of SphK2 with optimal pharmacokinetic properties which have the potential to be developed as novel therapeutics for the management of chronic pain.Communicated by Ramaswamy H. Sarma.
J Biomol Struct Dyn, 2021;

34386880: Uniyal A, Akhilesh , Tiwari V, Gadepalli A, Ummadisetty O, Tiwari V
Epigallocatechin-3-gallate improves chronic alcohol-induced cognitive dysfunction in rats by interfering with neuro-inflammatory, cell death and oxido-nitrosative cascade.
Alcohol consumption for a longer period of time is linked with neuronal damage and an increase in inflammatory signaling resulting in cell death and dementia. Natural compounds are the focus of research due to their high efficacy and good safety profile. Here we have investigated the effect of chronic epigallocatechin-3-gallate (EGCG) administration against the alcohol-induced cognitive deficit rats. Male Wistar rats were exposed to the 12% ethanol (10 g/kg; oral gavage) for ten weeks and treated with EGCG (25, 50, and 100 mg/kg) for the same duration. Ethanol exposure led to the impaired spatial memory and learning in rats assessed using the Morris water maze and elevated plus-maze test. Further, we assessed the role of EGCG

in mitigating the oxidative stress, neuroinflammatory and cell death signaling associated markers. Co-administration with EGCG significantly prevented all the behavioral, biochemical and molecular alterations in the different brain regions of ethanol-treated rats in a dose-dependent manner. EGCG suppressed the acetylcholinesterase activity, increased oxidative-nitrosative stress, cytokines (TNF-alpha and IL-1beta), NF-kappa β and caspase-3 levels in both the cortex and hippocampus of ethanol-treated rats. Our preliminary study demonstrated that EGCG improves the oxido-nitrosative stress, inflammation, and cell death signaling associated with ethanol-induced cognitive dysfunction. This suggests the potential role of EGCG in mitigating the cognitive deficits associated with chronic alcohol consumption.
Metab Brain Dis, 2021; 36
34357554: Uniyal A, Tiwari V, Rani M, Tiwari V
Immune-microbiome interplay and its implications in neurodegenerative disorders.
The neurodegeneration and its related CNS pathologies need an urgent toolbox to minimize the global mental health burden. The neuroimmune system critically regulates the brain maturation and survival of neurons across the nervous system. The chronic manipulated immunological drive can accelerate the neuronal pathology hence promoting the burden of neurodegenerative disorders. The gut is home for trillions of microorganisms having a mutual relationship with the host system. The gut-brain axis is a unique biochemical pathway through which the gut residing microbes connects with the brain cells and regulates various physiological and pathological cascades. The gut microbiota and CNS communicate using a common language that synchronizes the tuning of immune cells. The intestinal gut microbial community has a profound role in the maturation of the immune system as well as the development of the nervous system. We have critically summarised the clinical and preclinical reports from the past a decade emphasising that the significant changes in gut microbiota can enhance the host susceptibility towards neurodegenerative disorders. In this review, we have discussed how the gut microbiota-mediated immune response inclines the host physiology towards neurodegeneration and indicated the gut microbiota as a potential future candidate for the management of neurodegenerative disorders.
Metab Brain Dis, 2022; 37
34324307: Uniyal A, Thakur V, Rani M, Tiwari V, Akhilesh , Gadepalli A, Ummadisetty O, Modi A, Tiwari V
Kinesin Nanomotors Mediated Trafficking of NMDA-Loaded Cargo as A Novel Target in Chronic Pain.
Chronic pain is among the most prevalent burdensome disorders worldwide. The -methyl-d-aspartate (NMDA) receptor system plays a critical role in central sensitization, a primary feature of chronic pain. Despite the proven efficacy of exogenous ligands to this receptor system in preclinical studies, evidence for the clinical efficacy of NMDA antagonists for the treatment of chronic pain is weak. Researchers are studying alternate approaches, rather than direct inhibition of the NMDA receptors in pain processing neurons. This indirect approach utilizes the modulation of molecular switches that regulates the synthesis, maturation, and transport of receptors from cellular organelles to the synaptic membrane. Kinesins are nanomotors that anterogradely transport the cargo using microtubule tracks across the neurons. Various members of the kinesin family, including KIF17, KIF11, KIF5b, and KIF21a, regulate the intracellular transport of NMDA receptors. Pharmacological targeting of these ATP-driven nanomotors could be a useful tool for manipulating the NMDAR functioning. It could provide the potential for the development of a novel strategy for the management of chronic pain.
ACS Chem Neurosci, 2021; 12
34027667: Uniyal A, Shantanu PA, Vaidya S, Belinskaia DA, Shestakova NN, Kumar R, Singh S, Tiwari V
Tozasertib Attenuates Neuropathic Pain by Interfering with Aurora Kinase and KIF11 Mediated Nociception.
Kinesins are the motor proteins that transport excitatory receptors to the synaptic membrane by forming a complex with receptor cargo leading to central sensitization causing neuropathic pain. Many regulatory proteins govern the transit of receptors by activating kinesin, and Aurora kinases are one of them. In this study, we have performed in silico molecular dynamics simulation to delineate the dynamic interaction of Aurora kinase A with its pharmacological inhibitor, tozasertib. The results from the molecular dynamics study shows that tozasertib-Aurora kinase A complex is stabilized through hydrogen bonding, polar interactions, and water bridges. Findings from the studies suggest that tozasertib treatment significantly attenuates lipopolysaccharide (LPS)-induced increase in oxidonitrosative stress and kif11 overexpression in C6 glial cell lines. Further, we investigated the regulation of kif11 and its modulation by tozasertib in an animal model of neuropathic pain. Two weeks post-CCI surgery we observed a significant increase in pain hypersensitivity and kif11 overexpression in DRG and spinal cord of nerve-injured rats. Tozasertib treatment significantly attenuates enhanced pain hypersensitivity along with the restoration of kif11 expression in DRG and spinal cord and oxidonitrosative stress in the sciatic nerve of injured rats. Our findings demonstrate the potential role of tozasertib for the management of neuropathic pain.
ACS Chem Neurosci, 2021; 12
33964293: Thakur V, Uniyal A, Tiwari V
A comprehensive review on pharmacology of efflux pumps and their inhibitors in antibiotic resistance.
The potential for the build-up of resistance to a particular antibiotic endangers its therapeutic application over time. In recent decades, antibiotic resistance has become one of the most severe threats to public health. It can be attributed to the

relentless and unchecked use of antibiotics in healthcare sectors, cell culture, animal husbandry, and agriculture. Some classic examples of resistance mechanisms employed by bacteria include developing antibiotic degrading enzymes, modifying target sites previously targeted by antibiotics, and developing efflux mechanisms. Studies have shown that while some efflux pumps selectively extrude certain antibiotics, others extrude a structurally diverse class of antibiotics. Such extrusion of a structurally diverse class of antibiotics gives rise to multi-drug resistant (MDR) bacteria. These mechanisms are observed in gram-positive and gram-negative bacteria alike. Therefore, efflux pumps find their place in the list of high-priority targets for the treatment of antibiotic-resistance in bacteria mediated by efflux. Studies showed a significant escalation in bacteria's susceptibility to a particular antibiotic drug when tested with an efflux pump inhibitor (EPI) compared to when it was tested with the antibiotic drug alone. This review discusses the pharmacology, current status, and the future of EPIs in antibiotic resistance.
Eur J Pharmacol, 2021; 903

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-631

BASOLATERAL AMYGDALA INPUT TO ANTERIOR CINGULATE CORTEX MEDIATES PAIN-AVOIDANCE BEHAVIORS

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Pain is caused by dysfunction of the somatosensory system, often due to injury, although it may appear without any apparent cause. When pain becomes chronic, it manifests both as a sensory and affective condition, giving rise to maladaptive behaviors. One typical consequence is the development of pain-related fear, favoring avoidance behaviors when both rewarding and aversive stimuli are present. Dysfunction of the anterior cingulate cortex (ACC) has been associated to negative states occurring in human neuropathy as well as in rodent pain-models. The ACC integrates sensory and affective information and orchestrates appropriate behavioral responses in a changing environment. It receives excitatory projections from the basolateral amygdala (BLA), a brain area extensively studied in the context of fear and exhibiting plasticity in neuropathic pain states. However, whether BLA-ACC circuit is required for pain-related avoidance behaviors remains unknown. Here we are testing the hypothesis that plasticity at BLA-ACC synapses contributes to pain-related avoidance. We use the spared nerve injury (SNI) mouse model of neuropathic pain, which recapitulates behaviors reminiscent of the condition including mechanical allodynia and avoidance in a conflicting approach-avoidance task. Chemogenetic inhibition of BLA-ACC projections normalizes SNI mice's performance in the conflicting task, but not the allodynia behavior. We further show pain-induced increase of pre- and post-synaptic excitatory transmission at BLA inputs onto ACC layer 2/3 pyramidal neurons, but not onto layer 5 or GABA neurons. Altogether our data suggest a projection- and cell type-specific potentiation in the ACC of neuropathic pain mice, potentially contributing to pain-related avoidance behaviors.

Pubmed:

35078926: Hogrefe N, Blom SM, Valentinova K, Ntamati NR, Jonker LJE, Nevian NE, Nevian T
Long-Lasting, Pathway-Specific Impairment of a Novel Form of Spike-Timing-Dependent Long-Term Depression by Neuropathic Pain in the Anterior Cingulate Cortex.
Malfunctioning synaptic plasticity is one of the major mechanisms contributing to the development of chronic pain. We studied spike-timing dependent depression (tLTD) in the anterior cingulate cortex (ACC) of male mice, a brain region involved in processing emotional aspects of pain. tLTD onto layer 5 pyramidal neurons depended on postsynaptic calcium-influx through GluN2B-containing NMDARs and retrograde signaling via nitric oxide to reduce presynaptic release probability. After chronic constriction injury of the sciatic nerve, a model for neuropathic pain, tLTD was rapidly impaired; and this phenotype persisted even beyond the time of recovery from mechanical sensitization. Exclusion of GluN2B-containing NMDARs from the postsynaptic site specifically at projections from the anterior thalamus to the ACC caused the tLTD phenotype, whereas signaling downstream of nitric oxide synthesis remained intact. Thus, transient neuropathic pain can leave a permanent trace manifested in the disturbance of synaptic plasticity in a specific afferent pathway to the cortex. Synaptic plasticity is one of the main mechanisms that contributes to the development of chronic pain. Most studies have focused on potentiation of excitatory synaptic transmission, but very little is known about the reduction in synaptic strength. We have focused on the ACC, a brain region associated with the processing of emotional and affective components of pain. We studied spike-timing dependent LTD, which is a biologically plausible form of synaptic plasticity, that depends on the relative timing of presynaptic and postsynaptic activity. We found a long-lasting and pathway-specific suppression of the induction mechanism for spike-timing dependent LTD from the anterior thalamus to the ACC, suggesting that this pathology might be involved in altered emotional processing in pain.
J Neurosci, 2022; 42
31209376: Valentinova K, Tchenio A, Trusel M, Clerke JA, Lalive AL, Tzanoulinou S, Matera A, Moutkine I, Maroteaux L, Paolicelli RC, Volterra A, Bellone C, Mameli M
Morphine withdrawal recruits lateral habenula cytokine signaling to reduce synaptic excitation and sociability.
The lateral habenula encodes aversive stimuli contributing to negative emotional states during drug withdrawal. Here we report that morphine withdrawal in mice leads to microglia adaptations and diminishes glutamatergic transmission onto raphe-projecting lateral habenula neurons. Chemogenetic inhibition of this circuit promotes morphine withdrawal-like social deficits.

Morphine withdrawal-driven synaptic plasticity and reduced sociability require tumor necrosis factor-α (TNF-α) release and neuronal TNF receptor 1 activation. Hence, habenular cytokines control synaptic and behavioral adaptations during drug withdrawal.
Nat Neurosci, 2019; 22
29074844: Tchenio A, Lecca S, Valentinova K, Mameli M
Limiting habenular hyperactivity ameliorates maternal separation-driven depressive-like symptoms.
Early-life stress, including maternal separation (MS), increases the vulnerability to develop mood disorders later in life, but the underlying mechanisms remain elusive. We report that MS promotes depressive-like symptoms in mice at a mature stage of life. Along with this behavioral phenotype, MS drives reduction of GABA-GIRK signaling and the subsequent lateral habenula (LHb) hyperexcitability-an anatomical substrate devoted to aversive encoding. Attenuating LHb hyperactivity using chemogenetic tools and deep-brain stimulation ameliorates MS depressive-like symptoms. This provides insights on mechanisms and strategies to alleviate stress-dependent affective behaviors.
Nat Commun, 2017; 8
28352112: Valentinova K, Mameli M
Cocaine, cadherins and synaptic plasticity.
Nat Neurosci, 2017; 20
27822183: Tchenio A, Valentinova K, Mameli M
Can the Lateral Habenula Crack the Serotonin Code?
The lateral habenula (LHb) and the serotonergic system both contribute to motivational states by encoding rewarding and aversive signals. Converging evidence suggests that perturbation of these systems is critical for the pathophysiology of mood disorders. Anatomical and functional studies indicate that the serotonergic system and the LHb are interconnected in a forward-feedback loop. However, how serotonin release modifies the synaptic and cellular properties of LHb neurons and whether this has any behavioral repercussions remain poorly investigated. In this review article, we discuss insights gained from rodents and humans regarding the implications of the serotonin system and the LHb in aversion encoding and related disorders. We then describe the type, properties and pharmacology of serotonergic receptors expressed throughout the LHb. Finally, we discuss physiological data reporting how serotonergic signaling modifies synaptic transmission and neuronal activity within the LHb. Altogether, we combine a mechanistic- and circuit-level knowledge to provide an overview on how the LHb integrates serotonergic signals, a process potentially contributing to LHb-dependent encoding of valenced external stimuli.
Front Synaptic Neurosci, 2016; 8
27545888: Valentinova K, Mameli M
mGluR-LTD at Excitatory and Inhibitory Synapses in the Lateral Habenula Tunes Neuronal Output.
Excitatory and inhibitory transmission onto lateral habenula (LHb) neurons is instrumental for the expression of positive and negative motivational states. However, insights into the molecular mechanisms modulating synaptic transmission and the repercussions for neuronal activity within the LHb remain elusive. Here, we report that, in mice, activation of group I metabotropic glutamate receptors triggers long-term depression at excitatory (eLTD) and inhibitory (iLTD) synapses in the LHb. mGluR-eLTD and iLTD rely on mGluR1 and PKC signaling. However, mGluR-dependent adaptations of excitatory and inhibitory synaptic transmission differ in their expression mechanisms. mGluR-eLTD occurs via an endocannabinoid receptor-dependent decrease in glutamate release. Conversely, mGluR-iLTD occurs postsynaptically through PKC-dependent reduction of β2-containing GABAA-R function. Finally, mGluR-dependent plasticity of excitation or inhibition decides the direction of neuronal firing, providing a synaptic mechanism to bidirectionally control LHb output. We propose mGluR-LTD as a cellular substrate that underlies LHb-dependent encoding of opposing motivational states.
Cell Rep, 2016; 16
26628379: Glangetas C, Fois GR, Jalabert M, Lecca S, Valentinova K, Meye FJ, Diana M, Faure P, Mameli M, Caille S, Georges F
Ventral Subiculum Stimulation Promotes Persistent Hyperactivity of Dopamine Neurons and Facilitates Behavioral Effects of Cocaine.
The ventral subiculum (vSUB) plays a key role in addiction, and identifying the neuronal circuits and synaptic mechanisms by which vSUB alters the excitability of dopamine neurons is a necessary step to understand the motor changes induced by cocaine. Here, we report that high-frequency stimulation of the vSUB (HFSvSUB) over-activates ventral tegmental area (VTA) dopamine neurons in vivo and triggers long-lasting modifications of synaptic transmission measured ex vivo. This potentiation is caused by NMDA-dependent plastic changes occurring in the bed nucleus of the stria terminalis (BNST). Finally, we report that the modification of the BNST-VTA neural circuits induced by HFSvSUB potentiates locomotor activity induced by a sub-threshold dose of cocaine. Our findings unravel a neuronal circuit encoding behavioral effects of cocaine in rats and highlight the importance of adaptive modifications in the BNST, a structure that influences motivated behavior as well as maladaptive

behaviors associated with addiction.
Cell Rep, 2015; 13
26059295: Valentinova K, Tchenio A, Meye FJ, Lecca S, Mameli M
[Hell after the pleasure: drug-induced negative symptoms involve lateral habenula].
Med Sci (Paris), 2015; 31
25643299: Meye FJ, Valentinova K, Lecca S, Marion-Poll L, Maroteaux MJ, Musardo S, Moutkine I, Gardoni F, Huganir RL, Georges F, Mameli M
Cocaine-evoked negative symptoms require AMPA receptor trafficking in the lateral habenula.
Addictive substances mediate positive and negative states promoting persistent drug use. However, substrates for aversive effects of drugs remain elusive. We found that, in mouse lateral habenula (LHb) neurons targeting the rostromedial tegmental nucleus, cocaine enhanced glutamatergic transmission, reduced K(+) currents and increased excitability. GluA1 trafficking in LHb was instrumental for these cocaine-evoked modifications and drug-driven aversive behaviors. Altogether, our results suggest that long-lasting adaptations in LHb shape negative symptoms after drug taking.
Nat Neurosci, 2015; 18
24379770: Meye FJ, Lecca S, Valentinova K, Mameli M
Synaptic and cellular profile of neurons in the lateral habenula.
The lateral habenula (LHb) is emerging as a crucial structure capable of conveying rewarding and aversive information. Recent evidence indicates that a rapid increase in the activity of LHb neurons drives negative states and avoidance. Furthermore, the hyperexcitability of neurons in the LHb, especially those projecting to the midbrain, may represent an important cellular correlate for neuropsychiatric disorders like depression and drug addiction. Despite the recent insights regarding the implications of the LHb in the context of reward and aversion, the exact nature of the synaptic and cellular players regulating LHb neuronal functions remains largely unknown. Here we focus on the synaptic and cellular physiology of LHb neurons. First, we discuss the properties of excitatory transmission and the implications of glutamate receptors for long-term synaptic plasticity; second, we review the features of GABAergic transmission onto LHb neurons; and finally, we describe the contribution that neuromodulators such as dopamine (DA) and serotonin may have for LHb neuronal physiology. We relate these findings to the role that the LHb can play in processing aversive and rewarding stimuli, both in health and disease states.
Front Hum Neurosci, 2013; 7

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BOARD NUMBER: S05-632

AAV GENE THERAPY DELIVERING RECOMBINANT DIMERIC PEPTIDES TARGETING PICK1 FULLY RELIEVE CHRONIC NEUROPATHIC PAIN

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Background / Aim Chronic pain arises from central sensitization in the spinal cord, and AMPA receptors (AMPARs) are believed to play a major role. In an earlier study, we showed that a synthetic bivalent peptide targeting the AMPAR-trafficking protein, Protein Interacting with C-Kinase 1 (PICK1), supplied full and temporary pain relief. We hypothesized that the creation and delivery of recombinant dimeric peptides targeting PICK1 could supply similar but long-term effects. Here, we utilized adeno-associated viral (AAV) vectors encoding recombinant peptides, Di-C5, targeting PICK1 under the control of the human synapsin 1-promoter to provide constitutive and pan-neuronal expression in mouse pain models. **Methods** The binding strength of Di-C5 to PICK1 was tested using fluorescence polarization assays. *In vivo*, Di-C5 was given intrathecally to mice exposed to the spared-nerve injury (SNI) model of neuropathic pain. Mechanical paw withdrawal thresholds were assessed using von Frey filaments. **Results** *In vitro*, Di-C5 displayed high binding-affinity towards PICK1(Ki=119nM). In the SNI model, Di-C5 was able to completely prevent mechanical hypersensitivity when given prior to SNI surgery. Di-C5 was also able to reverse an acutely established hypersensitivity when given 2 days following SNI surgery. And finally, Di-C5 was able to gradually attenuate hypersensitivity when administered 35 days after SNI surgery. **Conclusion** We successfully developed a recombinant peptide, Di-C5 supplying complete and persistent reversal of mechanical allodynia in SNI mouse model of neuropathic pain. Together these results suggest that AAV gene therapy aimed at interfering with AMPAR-trafficking may be of clinical relevance in the treatment of chronic pain.

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BOARD NUMBER: S05-633

GREEN LIGHT EXPOSURE ELICITS ANTI-INFLAMMATION, ENDOGENOUS OPIOID RELEASE AND LESSENS SYNAPTIC POTENTIATION TO RELIEVE POST-SURGICAL PAIN IN RATS

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Numerous studies have shown how light exposure could benefits multiple conditions such as seasonal affective disorders, circadian rhythm dysregulations, or even neurodegenerative diseases. However, little is known about its potential benefits in pain management. Postoperative pain severely impacts quality of life and functionality, especially in the elderly. While current pharmacologic methods of pain management are effective in many cases, the associated side effects can limit their use, particularly in elderly population. Non-pharmacological methods would minimize drug reliance, facilitating a reduction of the opioid burden. Green light therapy has been shown to be effective in reducing chronic pain in both humans and rodents; however, the underlying mechanisms of this therapy remain incompletely defined. This study unravels the potential of green light exposure in combination with drugs commonly used in pain management. Through behavioral and pharmacological assays, we have demonstrated that exposure to green light potentiates opioid- and NSAIDS-induced pain relief, minimizing dose requirements to achieve antinociception. Furthermore, these insights gave us directions to delve into the underlying mechanisms of green light in the spinal cord. In a rodent model of postoperative pain, we have shown that green light increased spinal endogenous opioid release, concomitantly to reducing spinal inflammation. Altogether, these results shed light on green exposure mechanisms and provide crucial information about the use of green light in combination with other drug therapies. This evidence is the foundation of future complimentary therapies that will reduce drug prescription and consumption alongside their side effects.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-634

REPETITIVE PREFRONTAL TRANSCRANIAL DIRECT CURRENT STIMULATION ALLEVIATES NEUROPATHIC PAIN VIA NEURAL REMODELLING

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

Zheng Gan, Han Li, Paul Naser, Linette Tan, Manfred Oswald, Rohini Kuner
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Non-invasive transcranial direct current stimulation (tDCS) is gaining momentum as a neuromodulation strategy for chronic pain treatment. So far, there are no studies reporting prefrontal cortex (PFC) tDCS in neuropathic pain states in rodents, and neural circuits mediating PFC tDCS remain to be clarified. Therefore, our study aimed to elucidate the effects of PFC tDCS on neuropathic pain in mice and reveal the underlying neural circuitry. We employed repetitive anodal PFC tDCS in a longitudinal manner in mice with spared nerve injury (SNI). Early-stage application post-nerve injury prevented progressive development of mechanical hypersensitivity and dampened pain-related aversion. Chronic stage application alleviated established mechanical hypersensitivity and suppressed aversion as well as anxiety in neuropathic mice. In vivo tetrode recordings and c-Fos-based mapping were undertaken to address neuronal activity changes. While single PFC tDCS led to major excitation in the neocortex, repetitive PFC tDCS was associated with adaptation and even silencing of activity. PFC GABAergic interneurons and excitatory neurons were recruited differently over distinct temporal phases. Both baseline activity and responsivity to tactile stimuli were altered in several cortical and subcortical areas, including rostral anterior cingulate cortex, insular cortex, ventrolateral periaqueductal grey, and spinal dorsal horn, indicating a drastic remodeling of brain circuits. Our study thus identifies repetitive anodal PFC tDCS as a non-invasive neurostimulation paradigm to achieve long-lasting pain relief in neuropathic pain and yields knowledge on underlying circuits and cellular remodeling in rodents, which can be employed to enhance the efficacy of neurostimulation-based therapies in clinical settings.

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IS IT POSSIBLE TO REDUCE THE DOSE OF CARBAMAZEPINE BY VERAPAMIL COMBINATION IN TRIGEMINAL NEURALGIA PAIN TREATMENTS?

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Trigeminal neuralgia (TN) is a rare facial pain syndrome that immensely affects patients' life quality. Nowadays, Carbamazepine is used clinically in trigeminal neuralgia pain treatment or until operative treatment, but patients do not tolerate the Carbamazepine treatment for long periods due to its side effects. Therefore, we aimed to increase the tolerability or effectiveness of Carbamazepine with combination treatment. The experiment began after randomly dividing the rats into five groups (n=25); Sham, Control, Carbamazepine, Verapamil, and Carbamazepine-Verapamil combination. First, all animals underwent chronic constriction injury of the infraorbital nerve (CCI-IoN) to constitute the animal model of trigeminal neuralgia. Then, the mechanical stimulation test (MST) as a behavioral experiment is carried out by applying a graded series of von Frey filaments (0.02g, 0.16g, 0.4g, 1g, and 2g). The responses of the animals are tested accordingly. When the responses of the Carbamazepine, Verapamil, or control groups to vFF 0,16g and 0,40g filaments were compared statistically, MST scores of Verapamil (1mg) and Carbamazepine (30mg) were found to be significantly different from the control group ($F=6.763$; $p<0.011$; $F=7.860$; $p<0.023$). There was no significant difference between Carbamazepine or Verapamil and sham groups. However, the mean MST scores (1,8) of CBZ 15mg plus Verapamil 1mg (vFF 0.16g) were decreased significantly than the MST scores (2,6 and 2,8) of Verapamil and CBZ by two-way repeated ANOVA ($F=6,615$, $p<0.048$). These results suggest that Verapamil and Carbamazepine combination may be a novel treatment approach to treat neuropathic pain and reduce the side effects of Carbamazepine in TN disease.

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BOARD NUMBER: S05-636

ANTIALLODYNIA AND ANTIHYPERALGESIA EFFECTS OF CERIUM OXIDE NANOPARTICLES IN TREATMENT OF CHRONIC NEUROPATHIC PAIN IN RATS

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Antiallodynia and antihyperalgesia effects of Cerium oxide nanoparticles in treatment of chronic neuropathic pain in rats Fatemeh Forouzanfar[1,2*], Majid Darroudi[3] 1.Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran 2.Department of Neuroscience, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran 3.Nuclear Medicine Research Center, Mashhad University of Medical Sciences, Mashhad, Iran Corresponding author: Fatemeh Forouzanfar, Department of Neuroscience, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran Tel: +98-5138002538, Fax: +98-5138002477, E-mail: forouzanfarff@gmail.com, forouzanfarf@mums.ac.ir **Aims**: Neuropathic pain is often refractory to pain therapies and thus requires exploration of effective drugs. Nanoparticles are reported to have a potential role in the amelioration of neuropathic pain. The aim of this study was to determine the role of cerium oxide nanoparticles in the chronic constriction injury (CCI)-induced neuropathic pain in rats. **Methods**: Cerium oxide nanoparticles was administered intravenously after the neuropathic pain established. Heat hyperalgesia and allodynia were assessed by hot plate, aceton drop, and von frey filament tests, respectively. **Results:** CCI produced significant development in thermal hyperalgesia, mechanical and cold allodynia, in rats. Treatments with cerium oxide nanoparticles decreased thermal hyperalgesia, cold allodynia and mechanical allodynia. **Conclusions**: It is suggested that cerium oxide nanoparticles would be a promising approach for treatment of chronic neuropathic pain.

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BOARD NUMBER: S05-637

ENCODING OF THE UNPLEASANTNESS OF PAIN IN CORTICO-STRIATAL NEURONS OF THE ANTERIOR CINGULATE CORTEX

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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The perception of pain is a multidimensional sensory and emotional/affective experience arising from distributed brain activity. Maladaptive changes in the so called pain matrix are thought to play a role in the chronification of pain. However, how the brain encodes the perception of pain is still elusive. In particular, little is known about the neuronal activity patterns associated to the unpleasantness that characterizes the pain experience.
One of the reasons for this caveat is that in preclinical models, pain was traditionally evaluated by means of reflexive responses. Recently, more attention has been devoted to the affective-motivational consequences of pain, which are complex behaviors that reflect the subject's motivation to make the aversive sensation cease and that require processing of nociceptive information in cortico-limbic circuits.
In order to investigate real-time cortical encoding of the unpleasantness of pain, we first characterized the behavioral repertoire in response to noxious stimuli in female and male mice. We observed that naïve females expressed higher self-attending responses to noxious cold than naïve males, whereas neuropathic pain increased affective/motivational behaviors in both sexes.
Then, using in vivo calcium imaging with a miniature microscope, we monitored neuronal activity in cortico-striatal neurons of the Anterior Cingulate Cortex (CS-ACC), a cell population that may converge nociceptive information to the mesolimbic system. Our preliminary results show that while some neurons responded specifically to sensory stimuli, others encoded preferentially the behavioral manifestation of pain. Thus, our data suggest that CS-ACC neurons may encode a broad variety of information associated to noxious stimuli.

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MULTIMODAL ANALYSIS OF STRUCTURAL PLASTICITY OF CORTICAL GREY MATTER VOLUME IN CHRONIC PAIN

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Grey matter volume (GMV) changes due to chronic pain have been extensively studied in humans, yet the underlying neurobiological mechanisms are poorly understood. The goal of the project is to investigate the cellular underpinnings of GMV alterations in chronic pain and possibly provide a basis for novel strategies for prevention and therapy of this debilitating disease. In a longitudinal study design, a MRI-compatible chronic cranial window was implanted in mice expressing eGFP in all cell nuclei. In vivo imaging of nuclei allows inferences on tissue volume (distances between nuclei) and cell type composition (cell type-specific nuclear features) at different stages of chronic pain. Cell types were identified by a novel algorithm that includes PyRadiomics and deep learning. MRI imaging (voxel-based morphometry, diffusion tensor imaging) was performed in parallel to in vivo two-photon imaging of the anterior cingulate cortex (ACC) at different timepoints up to 12 weeks after the induction of chronic neuropathic pain with the spared nerve injury model (SNI). Additionally, behavioral paradigms were employed during acute pain and progression towards chronic pain. Our preliminary data show depressive and anxious behavior phenotypes in SNI mice. Furthermore, the imaging data revealed nuclei properties and tissue volume changes that significantly correlated with changes in behavior of SNI mice. In summary, the study established a novel multi-modal approach suitable to provide a more comprehensive understanding of the cellular mechanisms underlying changes in GMV caused by chronic pain.

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BOARD NUMBER: S05-639

ACUPUNCTURE EFFECTS ON THE TOLERANCE OF OPIOID ANALGESICS

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Introduction:Opioids are widely used world wide as potent pain relievers. Long-term use of opioids can cause a series of side effects, Such as drowsiness,respiratory depression.What's more,tolerance and physical dependence are the most prominent problems. Tolerance will lead to a decrease in the analgesic effect of opioids,and an increase in patient's demand for drugs. The present study examined if acupuncture could prevent opioid tolerance and a possible neuronal mechanism was also investigated. **Methods**:Male Sprague Dawley rats were anesthetized by sodium pentobarbital(50mg/kg), Their right jugular vein was cannulated for intravenous drug-administration and they were placed in a stereotaxic device, An access hole was made by drill through the skull for the RMTg and placd the guide-cannula at the destination.Then we tested the rat's right foot using a VonFrey filament and recorded changes in the rat's pain perception over 16days. In addition,we also investigated possible involvement of the VTA related GABA pathway using Naloxone,picrotoxin,2-hydroxysaclofen at RMTg. **Results**:The results showed that the pain of the rats increased after 7 days,but remained basically unchanged by acupuncture. In addition,We also demonstrate that acupuncture at SI5 inhibits the effect of morphine through the GABAAreceptors of RMTg. And verified that acupuncture at SI5 can affect the protein level of brain-derived neurotrophic factor(BDNF) in VTA. **Conclusion**:The findings suggest that acupuncture at SI5 can be a useful therapy to prevent the tolerance of opioid analgesics. **Key words:**AcupunctureSI5,Morphine,tolerance,RMTg

This research was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea government (MSIT) (No. 2018R1A5A2025272) and 2020R1A2C1103154.

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BOARD NUMBER: S05-640

CENTRAL NOCICEPTIVE TRANSMISSION MODULATED BY P2X7 IN THE THALAMOCINGULATE CIRCUIT

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Among ATP receptor families, P2X7 receptors differ in many respects from the other subtypes of the P2X1-6 family. The short-term stimulation of P2X7 leads to the activation of cationic currents, upon repeated or prolonged ATP application, and the opening of a large membrane pore can be detected. In view of the great significance of P2X7 receptors involved in pain and inflammation, we set out to investigate the possible role of this receptor in the central modulation of pain signaling in the thalamocingulate path. Our study utilized major *in vivo* and *ex vivo* neuropharmacological methods to record the effects of manipulating P2X7 receptors during neuron transmission with electrical stimulations to mimic nociceptive inputs. Our results show that the P2X7 directly participates in nociceptive transmission, which indicates a clear functional existence of P2X7 in the thalamocingulate path. Our *ex vivo* brain slice data reveal that activation of P2X7 may facilitate the transmission velocity along the thalamocingulate projection and facilitate the neuron firings. Synaptic vesicle release in anterior cingulate cortical neurons could occur by simultaneously increasing not only the known calcium influx but also glutamate and ATP secretion during signal transmission in response to electrical stimulation. The *in vivo* and *ex vivo* observations in this study provide evidence that the ATP receptor P2X7 is present along the major ascending pain path, the thalamocingulate circuit, and plays a neuromodulator role involved in acute pain transmission, which could contribute new insights for short-term anti-nociceptive applications.

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CHARACTERISATION OF THE NEURAL CORRELATES OF CENTRAL SENSITISATION INDUCED BY THE HIGH FREQUENCY STIMULATION (HFS) MODEL IN HEALTHY HUMANS USING FUNCTIONAL MAGNETIC RESONANCE IMAGING (FMRI)

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Aims: Central sensitisation (CS) is characterised by an increase in pain response to noxious stimuli (hyperalgesia). Experimental CS models can elicit hyperalgesia in healthy humans. This study aimed to characterise how CS induced by HFS modulates brain activity as measured by fMRI. ***Methods***: After an initial baseline MRI scan, HFS was applied to the left lower leg of 18 healthy subjects. A second MRI scan was conducted 20 minutes after HFS application. HFS consisted of five 1s trains of 100 Hz electrical pulses separated by 9s intervals. Scans measured blood oxygen level dependent (BOLD) signal changes to 18 punctate mechanical stimuli applied 1cm outside the HFS site (secondary hyperalgesia area). A whole brain, mixed effects analysis with cluster-based correction for multiple comparisons was performed to identify differences in stimulus evoked neural activity post-HFS vs. baseline. ***Results:*** Following HFS, reported mean pain intensity and unpleasentness significantly increased. This was associated with significantly increased activation during the post-HFS scan vs. baseline (mixed effects analysis, $Z > 3.1$, $p < 0.05$) in areas involved in pain perception such as the contralateral posterior insula cortex, mid anterior cingulate cortex, amygdala, hippocampus and nucleus cuneiformis, and bilateral thalamus and secondary somatosensory cortex. ***Conclusions:*** The state of CS induced by HFS consists of increased neural activity in cortical and sub-cortical pain processing areas and key brainstem nuclei such as the nucleus cuneiformis - implicated in both human and animal models of CS. This is consistent with other CS models that have been widely studied using fMRI.

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THE STRUCTURES THAT EXCITE THE CENTRAL AMYGDALA NEURONS AND PAIN NETWORK IN OROFACIAL INFLAMMATORY PAIN

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Pain-associated plastic changes have been documented in various brain regions. Of these modules, the central amygdala (CeA) is one of the core regions of the pain network, playing an essential role in the establishment of "nociplastic pain", a mechanism of pain with an altered nociceptive system. We recently demonstrated that suppression of CeA neurons in an inflammatory pain model of rodents mitigates central sensitization (Arimura et al., 2019, Sugimoto et al., 2021). However, various brain sites other than the CeA show elevated c-Fos expression with diverse time courses after an inflammatory injury. To reveal how these activated brain sites give influence each other, particularly to the CeA, we used a cre-reporter mouse line that allows c-Fos-expression-dependent fluorescence with a combination of a retrograde AAV vector for the Cre-dependent expression of green fluorescent protein injected into the right CeA. After a whisker pad injection of formalin followed by 4-hydroxytamoxifen at 5 hr, we removed the brain and visualized the fluorescence in thin slices. Pain-activated cells with projections to the pain-activated CeA were distributed in the ipsilateral paraventricular thalamus, subthalamic nucleus, interstitial nucleus of the posterior limb of the anterior commissure, basolateral amygdala, lateral parabrachial nucleus and bilateral insular cortex. Neurons in the bed nucleus stria terminalis, ventral paraventricular hypothalamic nucleus and supraoptic nucleus were activated by inflammation but not retrogradely labeled from the CeA. These results suggest that, with exception of a few structures, many brain regions project to the CeA, which might contribute to the activation of the CeA neurons.

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CANNABINOIDS EFFECTS ON NEURAL CORRELATES OF THE PAIN MODULATION SYSTEM IN FIBROMYALGIA SYNDROME

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Abstract Body
Fibromyalgia (FMS) is a syndrome characterized by widespread chronic pain involving all musculoskeletal systems, accompanied by
Despite the advancement in understanding pain mechanisms thanks to various innovations in neuroscience and pain medicine, trea
multidisciplinary therapy, and many patients continue to suffer from chronic pain and associated symptoms.
Although FMS pathophysiology is not fully understood, evidence supports the involvement of several mechanisms, including central
clinical and functional imaging studies.
Consequently, there is great importance in understanding the effect of new treatments on pain mechanisms in FMS. The current res
regulation in FMS in a double-blind, placebo-controlled design. We will test the effect of a single, low dose of THC on psychophysica
(QST), such as temporal summation, conditioned pain modulation (CPM), and offset analgesia. Furthermore, we will characterize th
inhibition using functional MRI. Additionally, the effect of THC on the sympathovagal tone in FMS and its correlation to the patients' s
To our knowledge, this is the first double-blind placebo-controlled trial examining the neural effects of THC in FMS patients.

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PAIN MODULATING TOXIN IDENTIFICATION FROM VENOMS BY HIGH CONTENT SCREENING MICROSCOPY

POSTER SESSION 05 - SECTION: NEUROPATHIC PAIN, ANALGESIA, & THERAPEUTICS

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Background and objective In vertebrates, pain-inducing as well as pain-reducing activities of venoms have been observed. We established a unique approach, monitoring intracellular signalling in nociceptive neurons. This allowed identification of pain-inducing toxins from venoms. However, the capability of this approach in identification of toxins that reduce pain-inducing signalling is not known. Methods Venoms were tested on cultured dissociated primary sensory neurons of rats. For this, dorsal root ganglia were prepared, cells dissociated, and neurons cultured. Then, the selected venoms were added one per wells, cells fixed with PFA, and immunocytochemically stained for activation of PKA-II and/or ERK1/2. Immunocytochemical intensities were acquired by high content screening microscopy on a single cell level and statistically analysed with R. Results We tested over 120 venoms Venoms were considered as potentially sensitizing, if they increased phosphorylation of PKA-II and/or ERK1/2. On the other hand, venoms were considered as potentially desensitizing if they reduced activation of PKA-II and/or ERK1/2 in response to a test stimulus. Thirty four percent of the tested venoms showed pain-initiating signalling whereas 13.8% showed signalling-inhibiting, i.e. potentially analgesic activity. Conclusion Our results indicate that our signalling based high content screening microscopy approach may be a potent screening method to identify potentially analgesic toxins. An advantage against other screening methods is that this is independent of the presence of toxins activating the neurons electrically. This approach therefore may help unmasking of novel analgesic toxins, which now will be purified and characterized from the positive hits of this first round of screening.

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STABILITY OF HYPOTHALAMIC NEURAL POPULATION ACTIVITY DURING SLEEP STATES

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Sleep is a primary and essential biological need for most animals. Many brain regions and neural circuits have been implicated in the regulation of sleep and wakefulness, in particular, the lateral hypothalamic area (LH) is central to the orchestration of sleep–wake states, feeding, energy balance, aversion and goal oriented behaviour. This suggested that some of these cells may share both sleep and metabolic functions. In this study, we longitudinally image the activity of VGat, VGlut2, and Orexin neurons in freely behaving mice over a week using in vivo calcium imaging in freely-moving mice, and compute a categorization of their activity across wake, REM and non-REM states and across days. We found that part (±40%) of the cells remained within the same cluster (wake, NREMS or REMS active), however, a significant portion of these 'recluster' into different categories at different times of the day, and over multiple days. Finally, we described the stability of these clusters upon sleep recovery (sleep deprivation). Our findings shed light on the dynamics and plasticity of several neuronal populations in different sleep-wake states and help to better understand multitasking functions of LH circuits.

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FROM WAKEFULNESS TO SLEEP, FOCUS ON PATIENTS WITH NARCOLEPSY.

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Aims: Narcolepsy type 1 (NT1) is a neurological disorder characterised by excessive daytime sleepiness and sleep attacks, as well as symptoms of supposed dissociations between REM sleep and wakefulness (cataplexy, sleep-related hallucinations, sleep paralysis, lucid dreaming). We aimed at testing this hypothesis of dissociated REM sleep states by studying the entanglement of REM and wake activity through new EEG biomarkers. These new EEG biomarkers of NT1 could in turn help understanding the mechanisms associated with sleep attacks, hallucinations, and other dissociated sleep-wake states during wakefulness. **Methods:** We analysed the nocturnal polysomnography (PSG) recordings of 27 NT1 patients and 24 controls. In the PSG signal, we extracted at different time scales: (1) features based on the hypnogram scored by sleep experts across the entire night, (2) spectral features computed on 30s-long epochs, and (3) features related to sleep microstructure based on the automated detection of slow waves, sleep spindles, and rapid eye movements. **Results:** Our results indicate that NT1 is indeed characterised by a waking state that is more REM-sleep-like and, reciprocally, a REM sleep that is more wake-like than in controls (more alpha power in REM sleep and less in wakefulness for example). **Conclusion:** Characterising the neurophysiology of narcolepsy type 1 as a mixing of wake and REM activity further suggests that NT1 daytime (hallucinations) and nighttime (lucid dreaming) symptoms could be explained by intrusions of REM activity during waking and of wake activity during REM sleep.

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MICROGLIAL TNFα ORCHESTRATES PROTEIN PHOSPHORYLATION DURING THE SLEEP PERIOD AND CONTROLS HOMEOSTATIC SLEEP

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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A sufficient amount of sleep is ensured by a tight homeostatic control. This relies on factors that build-up in the brain with waking and induce sleep proportionally to the length of the previous wake period. Candidate sleep-promoting factors, including adenosine, TNFα and interleukin-1, have been identified but their molecular mechanisms of action remain elusive. Phosphorylation of synaptic proteins has been described as a fundamental process of sleep control. TNFα is a signaling molecule that controls activation of phosphorylation pathways and its expression is restricted to microglia in the brain parenchyma. We therefore hypothesized that microglia-derived TNFα is involved in phosphorylation-based control of homeostatic sleep. In this study, we performed quantitative phosphoproteomic analysis and polygraphic sleep recordings following sleep deprivation in control and microglia-specific TNFα deleted mice. We demonstrate that in the cortex microglial TNFα massively modulates phosphorylation exquisitely during the sleep period. Phospho-substrates of microglial TNFα comprise sleep-promoting kinases, phosphatases and numerous synaptic proteins. These include proteins whose phosphorylation status has been proposed to encode sleep need and control sleep duration. Consistently, lack of microglial TNFα attenuates accumulation of sleep pressure, as measured by slow wave activity, and prevents sleep rebound following forced wake. Together, we establish microglia as a new cellular component of the sleep homeostat. Microglia control homeostatic sleep by releasing TNFα that acts at the neuronal circuitry through phosphorylation.

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ION CONCENTRATIONS IN CEREBROSPINAL FLUID IN WAKEFULNESS, SLEEP AND SLEEP DEPRIVATION IN HEALTHY HUMANS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Sleep is a global state of the brain in which the neurons in the cortex exhibit specific firing patterns. Neuromodulatory transmittors are released into the cortex by projection axons from subcortical nuclei, which presumably regulates and coordinates the transition and stability of sleep and wakefulness across the cortex. It is, however, not known how these neurotransmittors act to change firing patterns in cortical neurons. Pathways including direct modulation of ion channels on neurons as well as modulation via glial cells has been proposed. Recently, a study showed that changes in the extracellular concentration of potassium, as well as of calcium and magnesium, was sufficient to induce transition from sleep to wakefulness in mice. In this study, we investigated if such changes in ion concentrations occur in healthy humans. We collected cerebrospinal fluid from 11 healthy volunteers. Each participant was sampled three times, in randomized order: at 3-5 PM during waking, at 6-7 AM immediately following one night of sleep and at 6-7 AM following one night of sleep deprivation. We could not detect any significant changes in the concentration of calcium or magnesium, but in the concentration of potassium; we saw a small but highly consistent and significant reduction of 0.1 mM (about 3 %) in both sleep and sleep deprivation samples when compared to the samples collected during wakefulness in the afternoon. Our results support a circadian modulation of ion concentrations in the central nervous system which could be involved in sleep regulation in humans.

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BOARD NUMBER: S05-649

SPECTRAL POWER AND TEMPORAL COUPLING OF THE EEG DURING REM SLEEP IN PATIENTS WITH FRONTAL BRAIN TUMOR

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Electrophysiological sleep patterns can be modified because of a brain pathology, such as tumors, which allows us to know in depth what happens when these lesions affect a particular area of the brain, such as the frontal lobes, and which are scant studies about it. Other hand, the cortical brain activity shows electric field oscillations on all temporal and spatial scales. The permanent oscillatory behavior is generated by the intrinsic activity of single neurons and extends to the self-organized motion of large neuronal populations. The aim of this study was to characterize the cerebral electrical activity in patients with frontal brain tumor. Therefore, 10 patients were evaluated by means of polysomnographic recordings. Without comorbid neurological or medical and without surgical treatment, with chemotherapy and radiotherapy. For quantitative EEG (qEEG) analysis, the Fast Fourier Transformation algorithm was used to obtain the spectral power of each participant, channel, and frequency band. Results reveal higher spectral power densities for REM sleep, for all electrodes and for each of the bands; can be observed by patients compared to normative values. Through the analysis of intra and interhemispheric correlation, it is possible to observe the networks that are formed at the level of brain electrical activity. In conclusion, in this study we were able to observe that an alteration in the frontal lobe can generate changes in sleep rhythmogenesis. The presence of this type of pathology, which causes structural and functional changes, allows us to understand the involvement of the frontal lobes during this state.

Pubmed:
27943486: Villarreal-Garza C, Platas A, Martinez-Cannon BA, Bargalló-Rocha E, Aguilar-González CN, Ortega-Leonard V, Ramos-Elías P, Hidalgo-Carrera J, Soto-Perez-de-Celis E
Information Needs and Internet Use of Breast Cancer Survivors in Mexico.
Breast J, 2017; 23

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-650

BRAIN-WIDE RESPONSES TO PROLONGED WAKEFULNESS OUTLINE CRITICAL COMPONENTS OF THE MAMMALIAN SLEEP HOMEOSTAT.

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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According to the two-process model of sleep regulation, the timing and structure of sleep is regulated by the interaction of circadian and homeostatic processes. The first process influences the probability of sleep over the light-dark cycle and is influenced externally by zeitgebers. The second process manifests through a homeostatic requirement for sleep and can be modeled using prior sleep–wake history. More specifically, prolonged wakefulness causes the feeling of tiredness and leads to sleep episodes marked by increased slow wave activity. Currently we lack a circuit level understanding of how prolonged wakefulness influences the brain and engages homeostatic mechanisms that lead to deep recovery sleep. Here we utilize behavioral manipulations, electrophysiology and whole brain imaging of neuronal activity reporters in order to map brain-wide responses to variable levels of sleep pressure across the circadian cycle. This approach reveals discrete regions in the midbrain and diencephalon that are active during varying homeostatic sleep pressure regiments. Using targeted chemogenetic perturbations and inducible cell ablation, we reveal novel roles these regions have in regulating physiological sleep-wake patterns. Our results can be used to constrain current models for sleep regulation and the interactions between sleep homeostasis and circadian rhythms. Additionally, our experimental approach provides a framework to define functionally important regions in behaviors that unfold across long timescales and are difficult to study in isolation.

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BOARD NUMBER: S05-651

REGION-SPECIFIC MODULATION OF ASTROCYTE NETWORK DYNAMICS ACROSS SLEEP-WAKE STATES

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Introduction: An increasing body of literature supports the relevance of astrocytes on sleep-wake regulation and sleep homeostasis. However, most of our understanding remains global and regional specificity is lacking. Here, we sought to investigate changes in astrocytic activity in response to different sleep pressure schedules in two different cortical areas related to sensory and non-sensory thalamic networks. **Methodology:** Mini-endoscopy combined with chronically implanted EEG electrodes were employed to measure GCaMP-Ca2+ astrocytic activity in the anterior cingulate cortex (ACC) and Barrel cortex (BrC) across sleep-wake states at different circadian times and during sleep recovery after extended wake periods (SD). Chemogenetics was used to activate or inhibit astrocytes in a spatial-temporal manner. **Results:** Astrocytic activity in the BrC and ACC differ in wake (~50% vs ~27%) and REM (~0.3 % vs ~6.5%) sleep respectively. Interestingly, stable functional astrocytic networks across days underwent a broad reorganization during the sleep recovery following SD. Remarkably, activation of ACC-astrocytes during the light period - when they were mostly inactive - increased NREM sleep, delta power but not spindles. Whereas inhibition failed to have an effect on sleep, the latency to the first NREM episode was longer and delta power during NREM sleep episodes was decreased in the 1 hrs following CNO injections. **Conclusions**: Collectively, our results indicate a region-specificity of astrocyte network dynamics across sleep-wake states. This specificity is further reflected in the response to sleep need by a long-lasting remodelling of their functional networks after SD, suggesting a spatio-temporal specificity of their sleep functions.

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BOARD NUMBER: S05-652

UNDERSTANDING THE IMPACT OF EARLY MICROEXON MISREGULATION ON ZEBRAFISH SLEEP.

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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The need for sleep or a sleep-like state appears to be universal across the animal kingdom and necessary for survival. Neurodevelopmental alterations found in patients with autism spectrum disorders can disrupt such tightly regulated sleep states. Here, we investigate a highly conserved and neuron-specific splicing program of microexons activated during late neurogenesis and misregulated in autistic individuals. Microexons are believed to fine-tune neurodevelopmental processes such as synaptogenesis and axon guidance, actively contributing to an optimal excitation/inhibition balance in the brain. However, little is known about their impact on behavioral states including sleep/wake patterns. In order to address this, we use a knock-out zebrafish model of the microexon splicing master regulator *srrm3*. Zebrafish show a relatively complex but stereotyped repertoire of movements (bouts) and a diurnal sleep/wake signature. Using high-throughput behavioral tracking of day-night activity and high-resolution recordings of single larvae, we observed a multi-facetted hyperactivity phenotype with sleep loss at night, characterized by a shift in bout type choice as well as increased bout length and frequency. We hypothesize an underlying neuronal activity imbalance caused by early microexon misregulation. To address this hypothesis, we aim to utilize calcium imaging to identify neuronal activity patterns that can explain the sleep disruptions observed as well as neuron-specific bulk RNA sequencing. Our research will not only shed new light into the functional impact of microexons on brain development, but will also contribute to our understanding of brain activity states with a special focus on sleep.

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BOARD NUMBER: S05-653

CHEMOGENETIC ACTIVATION OF VGLUT2-EXPRESSING NEURONS IN THE NODOSE GANGLION OF THE LEFT VAGUS NERVE SUPPRESSES RAPID-EYE-MOVEMENT SLEEP IN MICE

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Aims: When awake, we consciously perceive stimuli from the world that surrounds us. When asleep, our brain instead disconnects from the sensory environment. In contrast to these exteroceptive stimuli, little is known about how interoceptive stimuli are processed by the sleeping brain. The vagus nerve is a mixed sensory-motor nerve that interfaces between the autonomic periphery and the central nervous system. We asked whether stimulating specifically vagal sensory afferents modulates sleep. **Methods:** All experiments are based on viral transfection of vGluT2-expressing neurons in the nodose ganglion of the left vagus nerve, in combination with polysomnographic, local field potential (LFP), fiber photometry and brain temperature recordings in freely moving conditions, or patch-clamp physiology. **Results:** Whole-cell patch-clamp recordings confirmed that optogenetic activation of vagal afferents formed functional glutamatergic synaptic contacts in the brainstem nucleus tractus solitarius. We found that chemogenetic activation of the vagal sensory afferents (1.5-2.5 mg/kg CNO) suppressed rapid-eye-movement sleep (REMS) in a dose-dependent manner, as evident by the lack of its characteristic EEG/EMG correlates. To characterize more comprehensively the physiological correlates of sleep during elevated vagal activity, we are currently analyzing breathing rates and the brain temperature of sleeping mice. The mechanisms underlying these alterations are addressed using fiber photometry techniques in combination with LFP recordings. **Conclusions:** Our findings point to a major role for vagal afferent activity in body-brain physiology that regulates the balanced expression of non-REMS and REMS. Moreover, they indicate that vagus nerve stimulation could offer a non-invasive strategy to improve sleep architecture in pathological conditions.

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BOARD NUMBER: S05-654

UNSUPERVISED CLUSTERING IDENTIFIES LIGHT AND DEEP SLEEP IN DIVERSE MOUSE MODELS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Normal sleep physiology is essential for healthy brain development and aging. Sleep physiology studies rely on the correct classification of brain states within sleep (sleep staging), which is usually constrained by slow scoring methods with fixed rules and definitions of such sleep stages. We hypothesized that unsupervised classification of EEG data would resist the idiosyncrasies of individual recording sessions and unbiasedly identify sleep states within relevant feature spaces. We tested and challenged a workflow for the unsupervised classification of sleep states (WUCSS) that classifies active, REM, and within NREM, deep and light sleep states using head motion and electrophysiological data. We gathered high-quality EEG recordings in mice, combined with accelerometer data in diverse transgenic lines, totaling more than 800 hours. WUCSS works with six feature dimensions, extracted from 4-second epochs and used as inputs for an unsupervised classifier. Features were chosen based on human clinical sleep classification. WUCSS showed high recall, precision, and F1-score compared to manual scoring on active, NREM, and REM sleep states. Within NREM, WUCSS consistently identified two additional clusters that qualify as deep and light sleep states. With just 6 hour long sessions, REM, NREM, deep and light sleep were reliably identified in adult and old mice. The deep and light sleep discrimination allows for a more accurate study of mouse sleep physiology, as evidenced by additional sleep phenotypes identified in CNTNAP2KO mice. We hope that the WUCSS's robustness can be of use to sleep researchers, saving time, resources and ultimately improving sleep staging.

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BOARD NUMBER: S05-655

A COMPREHENSIVE NEURAL SIMULATION OF SLOW-WAVE SLEEP AND HIGHLY RESPONSIVE WAKEFULNESS DYNAMICS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Hallmarks of neural dynamics during different human brain states span spatial scales from neuromodulators acting on microscopic ion channels to macroscopic changes in communication between brain regions. Developing a scale-integrated understanding of neural dynamics has therefore remained challenging. Here, we perform the integration across scales with mean-field modeling of Adaptive Exponential (AdEx) neurons, explicitly incorporating intrinsic properties of excitatory and inhibitory neurons and microscopic parameters, including spike-frequency adaptation, synaptic receptor kinetics, and various membrane conductances. Here, we connect AdEx mean-field neural populations via structural tracts defined by the human connectome, and show that macroscopic, state-dependent dynamics resembling human brain activity emerge that mimic state-dependent signals recorded during both spontaneous and evoked human brain activity. Specifically, modulating microscopic mechanisms enhancing spike-frequency adaptation leads to the emergence of slow-wave dynamics at large scales. Slow waves synchronize across brain areas, shifting the functional connectivity, phase coupling, and power spectral peaks, thus mimicking empirical data describing different brain states. Moreover, evoked activity reproduces observations of more complex spread of stimuli between brain regions during wakefulness compared to slow-wave sleep, analyzed with the perturbational complexity index. The model has been implemented with The Virtual Brain (TVB) simulator, and is open-access in EBRAINS. This approach provides a novel tool to evaluate how changes in microscopic parameters lead to large-scale emergent behavior in the brain, in particular enhanced responsiveness during awake compared to sleeping brain states, and thus offers a more unified, formal understanding of conscious and unconscious states and their associated pathologies.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-656

THE LOCUS COERULEUS IS A GATE FOR NREM-TO-REM SLEEP TRANSITIONS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Aims: The noradrenergic Locus Coeruleus (LC) system is commonly associated to stress and attention during wakefulness and its activity is highly dependent on behavioral states. However, noradrenaline release is high and fluctuates during non-rapid-eye-movement sleep (NREMS) on a close-to-minute infraslow timescale (Osorio-Forero et al., 2021). These fluctuations generate periods free of sleep spindles, during which evoked or spontaneous awakenings are more probable. Here, we asked whether these moments of increased sleep fragility are also involved the regulation of the NREM-to-REMS transitions and the ultradian sleep cycles. *Methods*: We recorded undisturbed sleep-wake behavior in 6 – 10 week-old dopamine-β-hydroxylase-Cre mice expressing optogenetic actuators, together with hippocampal and somatosensory local field potentials and closed-loop optogenetic modulation of LC neurons. Then, we combined these tools with automatic REMS deprivation to explore LC's role in conditions of high REMS pressure. *Results*: Transitions from NREMS-to-REMS were phase-locked to the infraslow cycles of LC fluctuations. Optogenetic activation of LC neurons during NREMS prevented NREMS-to-REMS transitions, even at moments of high REMS pressure. In contrast, inhibition of these cells precipitated REMS. Moreover, optogenetic manipulations at moments of high or low spindle activity refined these effects. *Conclusions*: These observations position the LC as a gate for the regulation of REMS and of the ultradian sleep cycles during unperturbed sleep. Furthermore, the results suggest that the periodic decline of LC activity during NREMS provides windows of opportunity during which NREM to REMS transitions are facilitated. These findings broaden the scope for future mechanistic models in sleep regulation.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-657

SYSTEMIC INFLAMMATION ALTERS SLEEP STATE DYNAMICS AND POWER SPECTRAL CHARACTERISTICS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Sleep disturbances and cognitive impairment are common in sepsis survivors. We used an LPS-mediated rat model of sepsis to study its effects on 1) sleep architecture and spectral characteristics, and 2) network memory processing. Male Long-Evans rats were implanted with tetrodes in hippocampal CA1. Neuronal activity was recorded during exposure to a familiar and novel linear tracks followed by saline (baseline) or LPS (5mg/kg) injection and a 6-hour rest period. Sleep architecture and oscillatory and non-oscillatory spectrum components were analyzed. LPS injection caused extensive sleep fragmentation by frequent wake episodes. While the total amount of NREM and wake were not affected, cumulative time spent in REM was significantly reduced. Spectral power <40 Hz was reduced in NREM and wake, but not in REM. Delta power (1-4 Hz) in NREM decreased and theta power (5-8 Hz) in REM remained stable. NREM spectrum slopes were steeper after LPS injection, but not after saline. LPS also increased the number of sharp-wave-ripples in NREM sleep. In conclusion, LPS caused sleep fragmentation accompanied by steeper NREM slopes possibly indicating changes in excitation/inhibition (E/I) ratio. The increased number of sharp wave ripples might reflect this E/I imbalance as well. As both sleep quality and the off-line memory processing through sharp-wave ripple activity are essential for memory consolidation, their alterations in sepsis might explain some aspects of post-sepsis cognitive impairment. As such, normalizing sleep-related brain activity may be a therapeutic target for sepsis-associated brain dysfunction.

Pubmed:
33727592: Kavkova M, Zikmund T, Kala A, Salplachta J, Proskauer Pena SL, Kaiser J, Jezek K
Contrast enhanced X-ray computed tomography imaging of amyloid plaques in Alzheimer disease rat model on lab based micro CT system.
Amyloid plaques are small (~ 50 μm), highly-dense aggregates of amyloid beta (Aβ) protein in brain tissue, supposed to play a key role in pathogenesis of Alzheimer's disease (AD). Plaques´ in vivo detection, spatial distribution and quantitative characterization could be an essential marker in diagnostics and evaluation of AD progress. However, current imaging methods in clinics possess substantial limits in sensitivity towards Aβ plaques to play a considerable role in AD screening. Contrast enhanced X-ray micro computed tomography (micro CT) is an emerging highly sensitive imaging technique capable of high resolution visualization of rodent brain. In this study we show the absorption based contrast enhanced X-ray micro CT imaging is viable method for detection and 3D analysis of Aβ plaques in transgenic rodent models of Alzheimer's disease. Using iodine contrasted brain tissue isolated from the Tg-F344-AD rat model we show the micro CT imaging is capable of precise imaging of Aβ plaques, making possible to further analyze various aspects of their 3D spatial distribution and other properties.
Sci Rep, 2021; 11

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9-13 July 2022 | Paris, France

BOARD NUMBER: S05-658

THE ROLE OF THE MELANIN CONCENTRATING HORMONE (MCH) SYSTEM IN INCREASED REM SLEEP PROPENSITY AND CATAPLEXY IN NARCOLEPSY.

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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The melanin-concentrating hormone (MCH) neurons play a regulatory role in REM sleep and they dynamically modulate its expression during thermoneutral ambient temperature (Ta) warming (Komagata et al., 2019). Moreover, Ta warming increases REM sleep but decreases cataplexy in narcoleptic hypocretin knock-out(Hcrt-KO) mice. Given the reciprocal inhibition between the Hcrt and MCH systems, we hypothesize that loss of Hcrt may disinhibit MCH activity resulting in the increased REM sleep propensity characteristic of narcolepsy, whereas MCH hypoactivity may exacerbate boundary state instability and favor cataplexy. We investigated the MCH dynamics across the sleep-wake cycle in MCH:cre mice and then evaluated the role of the MCH system in REM sleep and cataplexy in MCH:cre/HcrtKO mice. Fiber photometry approach revealed that MCH-dependent signal increased in anticipation and during REM sleep but dropped during NREM sleep and wake states in both animal models. Exposure to warm Ta pulsing increased REM sleep expression for MCH:cre mice, whereas the same effect was not detected in narcoleptic mice. Surprisingly, MCH activity in MCH:cre/HcrtKO mice also increased in anticipation of cataplexy and decreased at the transition to the wake state. Moreover, optogenetic inhibition of MCH neurons increased cataplexy, but Ta warming appeared to decrease thi effect. In contrast, narcoleptic mice increased REM sleep during the Ta warming condition, but this ability was blunted by optoslencing MCH neurons. Taken together, these results would suggest that the warming effect on cataplexy reduction is independent of the MCH system, that plays a role in driving the REM sleep propensity seen in narcolepsy.

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THE INTEGRATION OF SKIN AND CORE BODY TEMPERATURE IN THE EXPRESSION OF REM SLEEP AND THE ROLE OF THE HYPOTHALAMUS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Rapid eye movement (REM) sleep is preferentially expressed over non-REM sleep during thermoneutral ambient temperature (Ta) warming. The lateral hypothalamus (LH) integrates diverse inputs, including temperature, to modulate sleep-wake expression. Although hypocretin (Hcrt) neurons promote wakefulness, melanin-concentrating hormone (MCH) neurons increase REM sleep during thermoneutral Ta warming. However, how the LH integrates temperature information to modulate REM sleep remains unknown. We hypothesize that skin (TSkin) and core body (TCore) temperature information are integrated in the hypothalamus through the MCH and Hcrt systems. Thermoneutral warm Ta pulsing during the light phase increased wild type (WT) mice's total REM sleep duration and bout number, induced no effect on MCH receptor1 knock-out (MCHR1KO) mice, and increased Hcrt-KO mice's wakefulness while decreasing REM sleep. All groups preserved homeostatic regulation of REM sleep, suggesting that MCH and Hcrt systems are required for the dynamic modulation of Ta-dependent REM sleep, but not for REM sleep homeostasis. We then further investigated brain temperature, TCore and TSkin parameters to investigate whether they may be integrated within the LH to modulate REM sleep. WT and MCHR1KO mice showed clear ultradian TCore cycling and REM sleep nesting at TCore's nadir during the inactive sleep phase, while Hcrt-KO mice showed disrupted ultradian TCore cycling. WT mice dynamically increased REM sleep expression within the TCore cycle as a function of integrated TCore-TSkin, whereas MCHR1KO mice failed to do so. These findings suggest that WT mice integrate TSkin and TCore for dynamic REM sleep expression, where the MCH system plays a critical role.

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BOARD NUMBER: S05-660

SLEEP MACRO- AND MICROSTRUCTURE IN BREAST CANCER SURVIVORS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Complaints of sleep disturbance are prevalent among breast cancer (BC) patients and are predictors of quality of life. Still, electrophysiological measures of sleep are missing in patients, which prevents from understanding the pathophysiological consequences of cancer and its past treatments. Using polysomnography, sleep can be investigated in terms of macro- (e.g. awakenings, sleep stages) and micro- (i.e. cortical activity) structure. We aimed to characterize sleep complaints, and macro- and microstructure in 33 BC survivors untreated by chemotherapy and that had finished radiotherapy since at least 6 months (i.e. out of the acute effects of radiotherapy) compared to 21 healthy controls (HC). Compared to HC, BC patients had a larger number of awakenings (p=0.008); and lower Delta power (p<0.001), related to sleep deepening and homeostasis; greater both Alpha (p=0.002) and Beta power (p<0.001), related to arousal during deep sleep; and lower Theta power (p=0.004), related to emotion regulation during dream sleep. Here we show that patients have increased cortical activity related to arousal and lower activity related to sleep homeostasis compared to controls. These results give additional insights in sleep pathophysiology of BC survivors and suggest sleep homeostasis disruption in non-advanced stages of BC.

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BOARD NUMBER: S05-661

CORTICAL ASTROCYTES AND SLEEP HOMEOSTASIS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Aims: The activity of cortical astrocytes are linked to fluctuations in sleep-wake behaviour. Here we elucidate the role of anterior cingulate cortex astrocytes in sleep homeostasis by assessing how activation of astrocytes contribute to sleep loss and cortical excitability. **Methods:** Using Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetic tools we specifically activated cortical astrocytes in freely behaving female mice. Animals bilaterally injected with GFAP DREADD hM3D(Gq)-mCherry underwent 4h sleep deprivation (SD) starting at ZT0 or left undisturbed. At the end of SD or ZT4, animals were injected with either CNO (0.3 mg/kg, i.p.) or vehicle (saline, i.p.) in a crossover randomised design. In a separate cohort, mice were also stereotaxically injected with AAV1.Camk2a.GCaMP6f and a GRIN lens implanted to observe *in vivo* calcium imaging of cortical neurons. **Results:** We observed a reduction in the fluorescent reporter tag for cortical astrocytes in mice that were sleep deprived. Chemogenetic activation of cingulate/motor cortical astrocytes reduced total sleep time in undisturbed mice for 4hrs post-dose yet had no effect on sleep following SD. **Conclusions:** Our data support a role for cortical astrocytes in sleep-wake states. When sleep-drive is high, such as following sleep loss, supplemental activation of astrocytes are no longer effective in modifying sleep-state.

Pubmed:
29066757: Kovermann P, Hessel M, Kortzak D, Jen JC, Koch J, Fahlke C, Freilinger T
Impaired K binding to glial glutamate transporter EAAT1 in migraine.
SLC1A3 encodes the glial glutamate transporter hEAAT1, which removes glutamate from the synaptic cleft via stoichiometrically coupled Na-K-H-glutamate transport. In a young man with migraine with aura including hemiplegia, we identified a novel SLC1A3 mutation that predicts the substitution of a conserved threonine by proline at position 387 (T387P) in hEAAT1. To evaluate the functional effects of the novel variant, we expressed the wildtype or mutant hEAAT1 in mammalian cells and performed whole-cell patch clamp, fast substrate application, and biochemical analyses. T387P diminishes hEAAT1 glutamate uptake rates and reduces the number of hEAAT1 in the surface membrane. Whereas hEAAT1 anion currents display normal ligand and voltage dependence in cells internally dialyzed with Na-based solution, no anion currents were observed with internal K. Fast substrate application demonstrated that T387P abolishes K-bound retranslocation. Our finding expands the phenotypic spectrum of genetic variation in SLC1A3 and highlights impaired K binding to hEAAT1 as a novel mechanism of glutamate transport dysfunction in human disease.
Sci Rep, 2017; 7
32873441: Hessel M, Pape HC, Seidenbecher T
Stimulation of 5-HT receptors in anterodorsal BNST guides fear to predictable and unpredictable threat.
Through pharmacological manipulation of the serotonergic (5-Hydroxytryptamin, 5-HT) system, combined with behavioral analysis, we tested the hypothesis that fear responses to predictable and unpredictable threat are regulated through stimulation of 5-HT receptors (5-HT-R) in the anterodorsal section of the bed nucleus of the stria terminalis (adBNST). Local adBNST application of 5-HT1A-R antagonist WAY100635 and 5-HT1B-R antagonist NAS-181 before fear retrieval enhanced freezing, 24 h after predictable fear conditioning. In contrast, increased fear responses to unpredictable threat were blocked by 5-HT1A-R agonist Buspirone (given before conditioning or retrieval) and 5-HT1B-R agonist CP-94253 (applied before training). Prolonged fear responses were also blocked by local application of the 5-HT2A-R antagonist R-96544 before fear retrieval, and conversely, local application of the 5-HT2A-R agonist NBOH-2C-CN hydrochloride before fear retrieval enhanced freezing 24 h after predictable conditioning, indicating augmented fear responses. Activation of inhibitory 5-HT1A- or 5-HT1B-Rs and the blockade of the excitatory 5-HT2A-R before unpredictable fear conditioning significantly reduced freezing during retrieval. The results from this study suggest that modulation of inhibitory 5-HT1A/1B-R and/or excitatory 5-HT2A-R activity in the adBNST may represent potential targets for the development of new treatment strategies in anxiety disorders. In addition, this study supports the validity and reliability of the mouse model of modulated fear to predictable and

unpredictable threats to study mechanisms of fear and anxiety in combination with pharmacological manipulations. Eur Neuropsychopharmacol, 2020; 39

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-662

A BENCHMARK FOR SHARP WAVE-RIPPLE DETECTION ALGORITHMS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Sharp Wave Ripples (SWR) are fast oscillatory hippocampal events considered essential for learning, consolidation, and transfer of information. During these events, sequences of neuronal activity underlying previous experiences are replayed in a temporally compressed manner. To better understand the role of SWR, online closed-loop detection and perturbations is needed. To that end, several algorithms for on-line SWR detection have been recently developed. Yet, there is currently no comparison on their performance and no real consensus as to which one to use. Furthermore, algorithms based on Artificial Neural Networks (ANN) are rarely tested outside the controlled conditions they are developed in. Inspired by the seminal role of ImageNet in the development of Deep Learning, we set out to evaluate the performance of two ANN algorithms on a dataset different from the one they were built on, as well as more traditional methods based on ripple power or high synchrony event detection. We observed an overall lower performance with respect to what was stated by the original authors, pointing to a – somehow expected – dependency on the experimental setup and conditions. Specifically, the ANN based algorithms showed the biggest performance drop among the tested methods, suggesting an experimental bias akin to ImageNet's capture bias. This is problematic as it hinders reproducibility and comparison of results between studies. Furthermore, we discuss how specific algorithms may be adapted to different experimental purposes, proposing examples of algorithm tuning to individual data sets, aiming to build a benchmark for adaptive automatic SWR detection.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-663

SLEEP ARCHITECTURE DYNAMICS ACROSS THE LIFESPAN

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Sleep is essential to all animal life. While there are many debates as to why we must sleep, there is no doubt that it is most needed during development. Greater sleep needs in the young are in fact a hallmark of altricial mammals, such as humans and rodents, born in an undeveloped state and whose brain goes through extensive maturation processes postnatally. Rodent models offer a unique opportunity to investigate sleep qualitative and quantitative variations across the lifespan. To that end, we have developed new electrophysiological and computational methods to map sleep architecture in young, adolescent and adult freely-behaving rats. We have designed miniaturised implantable recording probes, that, together with a user-guided sleep scoring program, allow semi-automated classification of sleep stages based on locomotor (camera), EMG (electromyography), ECoG (electrocorticography) and intracerebral (tetrodes) activity. Such methods allowed us to show that rats, like humans, exhibited a steady decrease across the lifespan in sleep duration and marked changes in sleep quality. We specifically observed that while younger animals presented higher amounts of REM (rapid eye movement sleep), older rats showed a significant increase in sleep fragmentation, coinciding with decreased delta power during slow wave sleep. All in all, we present here a novel toolset that allows efficient mapping of sleep architecture across the lifespan while decoding neural dynamics.

Pubmed:
32499648: Romanov RA, Tretiakov EO, Kastriti ME, Zupancic M, Häring M, Korchynska S, Popadin K, Benevento M, Rebernik P, Lallemend F, Nishimori K, Clotman F, Andrews WD, Parnavelas JG, Farlik M, Bock C, Adameyko I, Hökfelt T, Keimpema E, Harkany T
Molecular design of hypothalamus development.
A wealth of specialized neuroendocrine command systems intercalated within the hypothalamus control the most fundamental physiological needs in vertebrates. Nevertheless, we lack a developmental blueprint that integrates the molecular determinants of neuronal and glial diversity along temporal and spatial scales of hypothalamus development. Here we combine single-cell RNA sequencing of 51,199 mouse cells of ectodermal origin, gene regulatory network (GRN) screens in conjunction with genome-wide association study-based disease phenotyping, and genetic lineage reconstruction to show that nine glial and thirty-three neuronal subtypes are generated by mid-gestation under the control of distinct GRNs. Combinatorial molecular codes that arise from neurotransmitters, neuropeptides and transcription factors are minimally required to decode the taxonomical hierarchy of hypothalamic neurons. The differentiation of γ-aminobutyric acid (GABA) and dopamine neurons, but not glutamate neurons, relies on quasi-stable intermediate states, with a pool of GABA progenitors giving rise to dopamine cells. We found an unexpected abundance of chemotropic proliferation and guidance cues that are commonly implicated in dorsal (cortical) patterning in the hypothalamus. In particular, loss of SLIT-ROBO signalling impaired both the production and positioning of periventricular dopamine neurons. Overall, we identify molecular principles that shape the developmental architecture of the hypothalamus and show how neuronal heterogeneity is transformed into a multimodal neural unit to provide virtually infinite adaptive potential throughout life.
Nature, 2020; 582
30830860: Korchynska S, Lutz MI, Borók E, Pammer J, Cinquina V, Fedirko N, Irving AJ, Mackie K, Harkany T, Keimpema E
GPR55 controls functional differentiation of self-renewing epithelial progenitors for salivation.
GPR55, a lipid-sensing receptor, is implicated in cell cycle control, malignant cell mobilization, and tissue invasion in cancer. However, a physiological role for GPR55 is virtually unknown for any tissue type. Here, we localize GPR55 to self-renewing ductal epithelial cells and their terminally differentiated progeny in both human and mouse salivary glands. Moreover, we find GPR55 expression downregulated in salivary gland mucoepidermoid carcinomas and GPR55 reinstatement by antitumor irradiation, suggesting that GPR55 controls renegade proliferation. Indeed, GPR55 antagonism increases cell proliferation and function determination in quasiphysiological systems. In addition, Gpr55-/- mice present ~50% enlarged submandibular glands with many more granulated ducts, as well as disordered endoplasmic reticuli and with glycoprotein content. Next, we

hypothesized that GPR55 could also modulate salivation and glycoprotein content by entraining differentiated excretory progeny. Accordingly, GPR55 activation facilitated glycoprotein release by itself, inducing low-amplitude Ca2+ oscillations, as well as enhancing acetylcholine-induced Ca2+ responses. Topical application of GPR55 agonists, which are ineffective in Gpr55-/- mice, into adult rodent submandibular glands increased salivation and saliva glycoprotein content. Overall, we propose that GPR55 signaling in epithelial cells ensures both the life-long renewal of ductal cells and the continuous availability of saliva and glycoproteins for oral health and food intake.
JCI Insight, 2019; 4
30209240: Alpár A, Zahola P, Hanics J, Hevesi Z, Korchynska S, Benevento M, Pifl C, Zachar G, Perugini J, Severi I, Leitgeb P, Bakker J, Miklosi AG, Tretiakov E, Keimpema E, Arque G, Tasan RO, Sperk G, Malenczyk K, Máté Z, Erdélyi F, Szabó G, Lubec G, Palkovits M, Giordano A, Hökfelt TG, Romanov RA, Horvath TL, Harkany T
Hypothalamic CNTF volume transmission shapes cortical noradrenergic excitability upon acute stress.
Stress-induced cortical alertness is maintained by a heightened excitability of noradrenergic neurons innervating, notably, the prefrontal cortex. However, neither the signaling axis linking hypothalamic activation to delayed and lasting noradrenergic excitability nor the molecular cascade gating noradrenaline synthesis is defined. Here, we show that hypothalamic corticotropin-releasing hormone-releasing neurons innervate ependymal cells of the 3 ventricle to induce ciliary neurotrophic factor (CNTF) release for transport through the brain's aqueductal system. CNTF binding to its cognate receptors on norepinephrinergic neurons in the locus coeruleus then initiates sequential phosphorylation of extracellular signal-regulated kinase 1 and tyrosine hydroxylase with the Ca-sensor secretagogin ensuring activity dependence in both rodent and human brains. Both CNTF and secretagogin ablation occlude stress-induced cortical norepinephrine synthesis, ensuing neuronal excitation and behavioral stereotypes. Cumulatively, we identify a multimodal pathway that is rate-limited by CNTF volume transmission and poised to directly convert hypothalamic activation into long-lasting cortical excitability following acute stress.
EMBO J, 2018; 37
31750562: Korchynska S, Krassnitzer M, Malenczyk K, Prasad RB, Tretiakov EO, Rehman S, Cinquina V, Gernedl V, Farlik M, Petersen J, Hannes S, Schachenhofer J, Reisinger SN, Zambon A, Asplund O, Artner I, Keimpema E, Lubec G, Mulder J, Bock C, Pollak DD, Romanov RA, Pifl C, Groop L, Hökfelt TG, Harkany T
Life-long impairment of glucose homeostasis upon prenatal exposure to psychostimulants.
Maternal drug abuse during pregnancy is a rapidly escalating societal problem. Psychostimulants, including amphetamine, cocaine, and methamphetamine, are amongst the illicit drugs most commonly consumed by pregnant women. Neuropharmacology concepts posit that psychostimulants affect monoamine signaling in the nervous system by their affinities to neurotransmitter reuptake and vesicular transporters to heighten neurotransmitter availability extracellularly. Exacerbated dopamine signaling is particularly considered as a key determinant of psychostimulant action. Much less is known about possible adverse effects of these drugs on peripheral organs, and if in utero exposure induces lifelong pathologies. Here, we addressed this question by combining human RNA-seq data with cellular and mouse models of neuroendocrine development. We show that episodic maternal exposure to psychostimulants during pregnancy coincident with the intrauterine specification of pancreatic β cells permanently impairs their ability of insulin production, leading to glucose intolerance in adult female but not male offspring. We link psychostimulant action specifically to serotonin signaling and implicate the sex-specific epigenetic reprogramming of serotonin-related gene regulatory networks upstream from the transcription factor Pet1/Fev as determinants of reduced insulin production.
EMBO J, 2020; 39
31796600: Fuzik J, Rehman S, Girach F, Miklosi AG, Korchynska S, Arque G, Romanov RA, Hanics J, Wagner L, Meletis K, Yanagawa Y, Kovacs GG, Alpár A, Hökfelt TGM, Harkany T
Brain-wide genetic mapping identifies the indusium griseum as a prenatal target of pharmacologically unrelated psychostimulants.
Psychostimulant use is an ever-increasing socioeconomic burden, including a dramatic rise during pregnancy. Nevertheless, brain-wide effects of psychostimulant exposure are incompletely understood. Here, we performed Fos-CreER-based activity mapping, correlated for pregnant mouse dams and their fetuses with amphetamine, nicotine, and caffeine applied acutely during midgestation. While light-sheet microscopy-assisted intact tissue imaging revealed drug- and age-specific neuronal activation, the indusium griseum (IG) appeared indiscriminately affected. By using GAD67 mice we subdivided the IG into a dorsolateral domain populated by γ-aminobutyric acidergic interneurons and a ventromedial segment containing glutamatergic neurons, many showing drug-induced activation and sequentially expressing Pou3f3/Brn1 and secretagogin (Scgn) during differentiation. We then combined Patch-seq and circuit mapping to show that the ventromedial IG is a quasi-continuum of glutamatergic neurons (IG-) reminiscent of dentate granule cells in both rodents and humans, whose dendrites emanate perpendicularly toward while their axons course parallel with the superior longitudinal fissure. IG- neurons receive VGLUT1 and VGLUT2 excitatory afferents that topologically segregate along their somatodendritic axis. In turn, their efferents terminate in the olfactory bulb, thus being integral to a multisynaptic circuit that could feed information antiparallel to

the olfactory-cortical pathway. In IG- neurons, prenatal psychostimulant exposure delayed the onset of Scgn expression. Genetic ablation of was then found to sensitize adult mice toward methamphetamine-induced epilepsy. Overall, our study identifies brain-wide targets of the most common psychostimulants, among which / neurons of the IG link limbic and olfactory circuits.
Proc Natl Acad Sci U S A, 2019; 116

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-664

A ROLE FOR OREXIN IN REM SLEEP REGULATION

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Orexin (hypocretin) neurons are important for sustaining long periods of wakefulness and their activity has been linked to a variety of behaviors including feeding, motivation, and reward processing. However, the role of orexin in the regulation of REM sleep and its components remains elusive as it was hypothesized that these neurons are silent during sleep. We investigated this paradigm using *in vivo* fiber photometry calcium imaging of orexin neurons of the lateral hypothalamus (LH) combined with EEG/EMG recordings in Hcrt-IRES-Cre mice and we found that the activity of orexin neurons correlates with the phasic components of REM sleep, in particular at enhanced power and faster frequency of theta rhythm. Furthermore, we found increased activity of orexin neurons prior to the termination of REM sleep episodes. Our results suggest a strong neuromodulatory role for the orexin system in the regulation of REM sleep theta oscillations and the termination of the state itself.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-665

SLEEP REGULATION BY THE CORTICAL PROJECTIONS TO THE PREOPTIC NUCLEUS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Sleep is divided into two distinct neurophysiological states : non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. The mechanisms by which these two states alternate remain poorly understood, especially since they can be modulated by learning or stressful situations. To better understand the fine regulation of sleep stages we were interested in the top-down control of high-level cortical structures on sleep-promoting structures. More particularly, we were interested in the projections from the prefrontal cortex (PFC) to the ventrolateral prepotic nucleus (VLPO). This pathway has been anatomically identified but its functionality remains to be determined. In this study, optogenetic tools were used to selectively activate the PFC-VLPO pathway in mouse brain slices as well as in freely moving mice. The sleep scoring was fully performed based on brain signals thanks to electrodes implanted in different structures. Our ex vivo patch-clamp recordings indicate that activation of cortical afferents in the VLPO induces post-synaptic currents in VLPO sleep-promoting neurons, demonstrating the functional nature of this pathway. Our in vivo experiments showed that the PFC-VLPO pathway activation disrupted the theta oscillation during REM sleep but not during wakefulness. Moreover, we found that these optogenetic stimulations induced an increase in ripples density, suggesting that animals went out of REM sleep. Finally, our results indicate that the activation of the PFC-VLPO pathway during REM sleep promotes transitions toward NREM sleep. These results are consistent with the NREM sleep-promoting role of the VLPO.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S05-666

SLEEP CIRCUITS OBSERVED IN DROSOPHILA OVER MULTIPLE DAYS DURING BEHAVIOR

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Neural circuits that control sleep are linked to the function of sleep, but this interaction is only partially understood. In the brain of Drosophila circuits important for sleep control as well as functional circuits important for navigation and memory have been identified in the central complex, an area located in the center of the fly brain. This brain area therefore offers an opportunity to understand the impact of sleep on navigation circuits at the level of small populations of identified cells. Using computational modeling we have started to describe the interaction and dynamics of subsets of these circuits [Flores-Valle et al., Plos. Comp. Biol., 2021]. To experimentally investigate the nature of the interaction between sleep and navigation circuits and to test predictions of our computational model, we have developed an in vivo two-photon imaging method for monitoring behavior and neural activity in head-fixed walking Drosophila for up to one week [Flores-Valle et al., J. Neurosci. Meth., 2021]. We use this method to quantify calcium dynamics in different cell populations important for navigation as well as for sleep over multiple days and nights. We characterize the dynamics of previously described sleep control circuits and identify novel elements of sleep circuits in the fly brain. These experiments provide the first characterization of calcium dynamics of a range of neural populations important for navigation and sleep over extended periods of time during day and night and contribute to an understanding of the interaction of sleep control and function.

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BOARD NUMBER: S05-667

PSYCHEDELIC COMPOUND 5-MEO-DMT INDUCES AN ALTERED WAKE STATE IN MICE

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Introduction: The traditional view that the serotonergic system plays an important role in controlling global sleep-wake states is supported by observations that administration of serotonergic psychedelics has a wake-promoting effect. However, the possibility that potentiating the serotonergic system through psychedelics results in an occurrence of altered states of vigilance has received less attention. Here we tested the hypothesis that serotonergic psychedelics alter fundamental characteristics of sleep and waking, rather than merely affecting the time spent in a specific state of vigilance. *Methods:* We performed chronic EEG and EMG recordings in freely-behaving laboratory mice (n = 7) after injection of a short-lasting psychedelic compound, 5-methoxy-N,N-dimethyltryptamine (5 MeO-DMT, 5 mg/kg) at the beginning of the light period. *Results:* We observed that 5-MeO-DMT administration resulted in an awake state characterised by altered EEG patterns as reflected in reduced theta-frequency (-3.82 % to -73.47 %) and increased spectral power in slow frequency range (9.11 % to 48.60 %). The effects were largely dissipated 1 hour after the injection. *Conclusion:* Our data support the notion that the effects of 5-MeO-DMT on EEG recordings are short-lasting. Importantly, this compound did not merely change the amount and distribution of vigilance states but had an observed effect on state-specific brain activity patterns. Reduced theta-activity and increased slow wave activity during waking after administration of 5-MeO-DMT reflect an occurrence of qualitatively different, “hybrid” or “dissociated” state, having features of both waking and sleep. This project was supported by a BBSRC Scholarship. The Compound was provided by Beckley Psytech.

Pubmed:
35197453: Thomas CW, Blanco-Duque C, Bréant BJ, Goodwin GM, Sharp T, Bannerman DM, Vyazovskiy VV
Psilocin acutely alters sleep-wake architecture and cortical brain activity in laboratory mice.
Serotonergic psychedelic drugs, such as psilocin (4-hydroxy-N,N-dimethyltryptamine), profoundly alter the quality of consciousness through mechanisms which are incompletely understood. Growing evidence suggests that a single psychedelic experience can positively impact long-term psychological well-being, with relevance for the treatment of psychiatric disorders, including depression. A prominent factor associated with psychiatric disorders is disturbed sleep, and the sleep-wake cycle is implicated in the homeostatic regulation of neuronal activity and synaptic plasticity. However, it remains largely unknown to what extent psychedelic agents directly affect sleep, in terms of both acute arousal and homeostatic sleep regulation. Here, chronic electrophysiological recordings were obtained in mice to track sleep-wake architecture and cortical activity after psilocin injection. Administration of psilocin led to delayed REM sleep onset and reduced NREM sleep maintenance for up to approximately 3 h after dosing, and the acute EEG response was associated primarily with an enhanced oscillation around 4 Hz. No long-term changes in sleep-wake quantity were found. When combined with sleep deprivation, psilocin did not alter the dynamics of homeostatic sleep rebound during the subsequent recovery period, as reflected in both sleep amount and EEG slow-wave activity. However, psilocin decreased the recovery rate of sleep slow-wave activity following sleep deprivation in the local field potentials of electrodes targeting the medial prefrontal and surrounding cortex. It is concluded that psilocin affects both global vigilance state control and local sleep homeostasis, an effect which may be relevant for its antidepressant efficacy.
Transl Psychiatry, 2022; 12

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BOARD NUMBER: S05-668

SLEEP STRUCTURE IN JUVENILE AND ADULT ZEBRA FINCHES: BROAD DIFFERENCES IN EEG OSCILLATIONS AND FUNCTIONAL CONNECTIVITY

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Despite their phylogenetic differences and distinct pallial structures, mammals and birds share many features of sleep. However, our current understanding of avian sleep is confined to studies in adult birds. In order to investigate the role of sleep oscillations during brain development, we recorded multi-channel EEG from juvenile and adult zebra finches. We compared the structure of sleep in adults and juveniles by calculating the ratio of slow oscillations to gamma oscillations ("depth of sleep", DOS). We found that the DOS ratio was significantly larger in juveniles compared to adults. Similarly, juveniles underwent state transitions more frequently than adults. A closer look at the multichannel EEG revealed that in addition to global (wide-spread) slow waves, slow oscillations also occurred on a small (local) group of electrode sites. The occurrence of local waves was correlated with the DOS and was higher in frontal sites. Furthermore, we quantified the functional connectivity across adults and juveniles by calculating pair-wise correlations between electrode sites. We observed that the strength of the correlations increased during development and was significantly higher in adults than in juveniles. In addition, we applied graph theory to identify significant spatial networks. The network analysis revealed two main results: 1: The size of highly-correlated subsets of channels was smallest during SWS compared to REM. 2: The highly-correlated networks were smaller in size and more numerous in juveniles compared to adults. Overall, our results suggest that the developing avian brain undergoes significant changes in the oscillatory composition of sleep and connectivity.

FENS Forum 2022
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BOARD NUMBER: S05-669

AN INTERPLAY BETWEEN PLASTICITY AND STABILITY OF CA1 HIPPOCAMPAL CIRCUITS ACROSS AROUSAL STATES IN BEHAVING MICE

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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(Aims) The ability of hippocampal circuits to maintain the balance between stability and flexibility is critical for normal hippocampal functioning. However, to what extent individual neurons and neuronal populations maintain their firing properties across different arousal states remains elusive. ***(Methods)*** To address this question, we combined Ca^{2+} micro-endoscopy in CA1 pyramidal neurons, local field potential and electromyography recordings in behaving and anesthetized mice. ***(Results)*** We found that physiological vigilance states - active wake, quiet wake and non-rapid eye movement sleep (NREMS) - consist of overlapping populations of active cells. At the population level, CA1 displays a unique activity set point, which is highest for active wake and lowest for NREMS and is stable across days. At the single cell level, 32-52% of individual CA1 neurons change their mean rates over short (1 day) and longer (8 days) time periods. Transitions from active wakefulness to NREMS involves a major decrease in activity of most cells, with a stronger decrease in the mean rate of a sub-population of high-firing neurons. Interestingly, drug-induced general anesthesia reveals a unique population of CA1 active cells and only partial overlap with those activated by natural sleep. The firing rates of the common neurons are only weakly correlated between NREMS and anesthesia. ***(Conclusions)*** Altogether, our results suggest that invariant population dynamics during physiological vigilance states emerge despite intrinsically unstable activity of individual neurons, while general anesthesia reveals a unique hippocampal cell population and activity.

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BOARD NUMBER: S05-670

WHOLE BRAIN MAPPING OF ACTIVITY DURING SLEEP AND WAKEFULNESS USING THE TRAP MICE AND IMPROVED DOUBLE FLUORESCENCE METHOD AND AUTOMATIC COUNTING

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Aims Our aim is to identify the populations of neurons activated during paradoxical (REM) sleep and to determine whether introduction of adverse or learning events induce the activation of different populations of neurons than those seen in basal conditions. Methods To this aim, we will use a new genetic TRAP method (2) combining tdtomato expression under Fos promoter with Fos immunohistochemistry. We first examined 3 mice groups: PSR-PSR (2h PS recovery after 48h PS deprivation), PSR-Wake (2h in open field), and Wake-PSR group all receiving 4-OHT 2h after the first condition and perfused 2h after the second condition. We then performed immunofluorescence of cFos and automatic counting of neurons on brain sections of the mice taken every 240um. Results Overall, more cFos and tdtomato labeled neurons were observed in most structures during the W compared to the PSR condition. The dentate gyrus was one of the few structures containing more cFos neurons after PSR than W. As reported recently for the anterior cingulate, retrosplenial and claustrum with DAB staining (Maciel et al., 2021), less double-labeled neurons were observed in the W-PSR and PSR-W conditions than in the PSR-PSR condition in most brain structures examined. Conclusions The development of a new method combining the use of TRAP mice with automatic counting open the path to the study over the whole brain of populations of neurons activated during two different conditions. Further, our results indicate that W and PS are completely different states during which different populations of neurons are activated.

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BOARD NUMBER: S05-671

ELECTROPHYSIOLOGICAL CORRELATES OF DISSOCIATIVE EXPERIENCES IN SLEEP DEPRIVED HEALTHY SUBJECTS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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The fragmented sleep, fragmented mind hypothesis has associated sleep disturbances and dissociative states in subjects with dissociative traits. Resting state EEGs of subjects with dissociative traits show a higher prevalence of slow rhythms albeit with a lower spatial coherence. A labile sleep-wake cycle might support the emergence of dissociative states such derealization, depersonalization, and dissociative amnesia in non-clinical populations even in the absence of background traits. On this basis, we expected that sleep loss could prompt dissociative states, and thus we evaluated dissociative experiences and resting state EEG features (spectral content and phase synchronization) after total sleep deprivation; neuro-phenomenal correlates of dissociative state were identified by correlating changes from baseline to post-deprivation conditions of significant psychometric indices with those of EEG features. We observed a significant increase in perceived altered state of consciousness and in dissociative experiences after sleep deprivation. Correlations between changes in psychometric indices and changes in band spectral contents showed that a higher prefrontal theta was associated with a higher intensity of depersonalization and a lower self-awareness in post sleep deprivation. Correlations between changes in psychometric indices and changes in synchronization showed that a higher intensity of dissociative experiences was paralleled by a higher synchronization increase in alpha, beta, and gamma bands. Acute sleep deprivation might fuel dissociative experiences which in turn might represent a regulatory strategy to face sleep loss, with the establishment of a state of consciousness promoted by a higher synchronization at high frequencies.

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BOARD NUMBER: S05-672

PUPIL SIZE DURING SLEEP INDICATES DISTINCT BRAIN STATES IN HUMANS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Brain states, defined by global activity changes across brain regions, are arguably the most important determinant of behavioral output in mammals. Sleep is the longest state that most animals spend their lives in and comprises distinct sub-states crucial for different physiological and behavioral processes. Classification of different sleep stages is therefore essential for understanding and studying processes taking place throughout sleep in health and disease in both mice and humans. Sleep stage classification has typically been done through polysomnographic recordings, which are precise but complex, expensive and tedious to implement. Other auxiliary measures such as heartbeat, breathing and skin conductance indicate neuromodulator levels that can be used to detect sleep stages; however, these signals are relatively low in time resolution. We have taken inspiration from studies involving eye tracking in wakefulness to monitor brain states and translated them into sleep settings by merging them with novel methods for both mice and humans. We discovered robust correlations between fluctuations in pupil size and brain states as well as the activity of the autonomic nervous system. We furthermore showed that pupils might serve a protective function to preserve the stability of deep sleep. Our research revealed a reliable relationship between eye parameters in sleep and brain states, which has so far been hidden behind closed eyelids. It underlines the importance of developing novel methods for physiological tracking in sleep that can be used for assessments in clinical or research settings.

Pubmed:
29358069: Yüzgeç Ö, Prsa M, Zimmermann R, Huber D
Pupil Size Coupling to Cortical States Protects the Stability of Deep Sleep via Parasympathetic Modulation.
During wakefulness, pupil diameter can reflect changes in attention, vigilance, and cortical states. How pupil size relates to cortical activity during sleep, however, remains unknown. Pupillometry during natural sleep is inherently challenging since the eyelids are usually closed. Here, we present a novel head-fixed sleep paradigm in combination with infrared back-illumination pupillometry (iBip) allowing robust tracking of pupil diameter in sleeping mice. We found that pupil size can be used as a reliable indicator of sleep states and that cortical activity becomes tightly coupled to pupil size fluctuations during non-rapid eye movement (NREM) sleep. Pharmacological blocking experiments indicate that the observed pupil size changes during sleep are mediated via the parasympathetic system. We furthermore found that constrictions of the pupil during NREM episodes might play a protective role for stability of sleep depth. These findings reveal a fundamental relationship between cortical activity and pupil size, which has so far been hidden behind closed eyelids.
Curr Biol, 2018; 28
26180200: Salkoff DB, Zagha E, Yüzgeç Ö, McCormick DA
Synaptic Mechanisms of Tight Spike Synchrony at Gamma Frequency in Cerebral Cortex.
During the generation of higher-frequency (e.g., gamma) oscillations, cortical neurons can exhibit pairwise tight (<10 ms) spike synchrony. To understand how synaptic currents contribute to rhythmic activity and spike synchrony, we performed dual whole-cell recordings in mouse entorhinal cortical slices generating periodic activity (the slow oscillation). This preparation exhibited a significant amount of gamma-coherent spike synchrony during the active phase of the slow oscillation (Up state), particularly among fast-spiking inhibitory interneurons. IPSCs arriving in pairs of either pyramidal or fast-spiking neurons during the Up state were highly synchronized and exhibited significant coherence at frequencies from 10 to 100 Hz, peaking at ~40 Hz, suggesting both synchronous discharge of, and synaptic divergence from, nearby inhibitory neurons. By inferring synaptic currents related to spike generation in simultaneously recorded pyramidal or fast-spiking neurons, we detected a

decay of inhibition ~20 ms before spiking. In fast-spiking interneurons, this was followed by an even larger excitatory input immediately before spike generation. Consistent with an important role for phasic excitation in driving spiking, we found that the correlation of excitatory inputs was highly predictive of spike synchrony in pairs of fast-spiking interneurons. Interestingly, spike synchrony in fast-spiking interneurons was not related to the strength of gap junctional coupling, and was still prevalent in connexin 36 knock-out animals. Our results support the pyramidal-interneuron gamma model of fast rhythmic oscillation in the cerebral cortex and suggest that spike synchrony and phase preference arises from the precise interaction of excitatory-inhibitory postsynaptic currents.
J Neurosci, 2015; 35

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PALLIDIN FUNCTION IN DROSOPHILA SURFACE GLIA REGULATES SLEEP AND IS DEPENDENT ON AMINO ACID AVAILABILITY

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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The Pallidin protein is a component of a multimeric complex named the Biogenesis of Lysosome-related Organelles Complex-1 (BLOC1) that regulates lysosome function. In a gene profiling study we have found *pallidin* mRNA to be strongly upregulated in the cortex of somnolent mice with defective histaminergic transmission, suggesting a link between the cellular and molecular mechanisms controlled by the *pallidin* gene and sleep/wake regulation. To investigate this question, we used a genetic knockdown strategy in *Drosophila* and demonstrated that down-regulation of *pallidin* in the surface glia, the *Drosophila* equivalent of the blood brain barrier, is sufficient to reduce, fragment and delay nighttime sleep at the adult stage and in a circadian clock dependent manner. Other members of the BLOC1 complex appear to be involved in this Pallidin-dependent sleep regulation. In agreement with Pallidin's involvement in amino acid transport, down-regulation of the Large neutral Amino acid Transporter 1 (LAT1)-like transporters JhI-21 and minidiscs, as well as the TOR amino acid signaling, phenocopy the down-regulation of *pallidin*. Supplementing food with essential amino acids normalizes the sleep/wake phenotypes of *pallidin* and *JhI-21* down-regulation. Furthermore, we identify a role for *pallidin* in the subcellular trafficking of JhI-21 in surface glial cells. Finally, we provide evidence that Pallidin function in surface glia is required for GABAergic neurons activation involved in promoting sleep. Taken together, these data identify a novel role for Pallidin that, through LAT1-like transporters subcellular trafficking modulates essential amino acid availability and GABAergic sleep/wake regulation.

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TRPM8 AND CIRCADIAN PHYSIOLOGY

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Most homeostatic functions display circadian oscillations synchronized by light, although ambient and body temperature also regulate the circadian system, especially the peripheral clocks. Because TRPM8 ion channel is a critical cold sensor in mammals and regulates body temperature homeostasis, we investigated its role in circadian regulation. Using TRPM8-deficient mice we studied circadian oscillations of body temperature (Tc) and motor activity in driven and free-running conditions (dark/dark, D/D) and in constant light (L/L). Furthermore, we analyzed the effect of TRPM8 on clockwork in the suprachiasmatic nucleus of the hypothalamus (SCN). Here we show that TRPM8-deficient mice exhibit defective Tc levels and greater oscillations in the absence of external cues, like in constant darkness (D/D). No remarkable changes were observed in the main rhythmic parameters of body temperature. However, in L/L *Trpm8*$^{-/-}$ mice exhibited a Tc with a higher unstable rhythmicity and a significant phase delay in comparison with WT littermates. Interestingly, we found that TRPM8 is expressed in retinal neurons projecting to the SCN, underlining its potential function in the regulation of the master clock. The clock gene Per2 was more expressed in the SCN of *Trpm8*$^{-/-}$ mice, especially during the daytime, accompanied by higher levels of AVP, which regulates many circadian functions, including Tc circadian oscillation. In summary, TRPM8 operates not only as an external thermosensor in skin and mucosae regulating thermal homeostasis, but it might also work as a thermoreceptor in the retina and its projections to the hypothalamus, regulating central circadian clockwork, and consequently influencing Tc circadian oscillations

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ANALYSES OF CIRCRNA EXPRESSION THROUGHOUT CIRCADIAN RHYTHM REVEAL A STRONG LINK BETWEEN CDR1AS AND LIGHT-INDUCED PHASE SHIFTS IN THE SCN

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Cdr1as is a conserved circular RNA (circRNA) enriched in the CNS and important for maintaining brain homeostasis. The loss of Cdr1as results in aberrant synaptic transmission and deregulation of stress response and circadian clock genes. However, it is not known whether the expression of Cdr1as or circRNAs, in general, follows a circadian pattern in different tissues. Here, using newly generated and public RNA-Seq data, we monitor circRNA expression throughout circadian rhythm in various mouse brain regions. We demonstrate that Cdr1as, despite its stable character, has a highly dynamic expression during the circadian cycle in the mouse suprachiasmatic nucleus (SCN). Cdr1as is one of the highest expressed RNAs in a cluster associated with light-induced synaptic transmission and phase shift in the SCN. Further, we identified that another brain enriched circRNA, *mbl*, is also substantially deregulated upon light induction in the fly head. Our study highlights the potential impact of abundant and conserved circRNAs on maintaining a healthy circadian cycle across species.

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CIRCADIAN RHYTHM OF NEURONAL ACTIVITY IN VASOPRESSIN NEURONS OF THE SCN IN MALE AND FEMALE RATS.

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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The hypothalamic suprachiasmatic nucleus (SCN) orchestrates mammalian behavioural and physiological rhythm following the daily light and dark cycle. Assuming that 90 to 95% of the neuronal population are GABAergic, 30% of them co-express the neuropeptide arginine-vasopressin (AVP), also known as anti-diuretic hormone or ADH. AVP neurons contribute to the overall circadian rhythm of SCN neuronal population. They display a pronounced fluctuation of AVP gene as well as cyclic peptide release into the cerebrospinal fluid. These may result of a possible day and night firing activity of these neurons as it was reported a higher frequency of firing during mid-day compared to the mid of the night. A complete activity profile of AVP-expressing neurons has not been shown yet. In this study, we recorded SCN-AVP positive neurons from AVP-eGFP Wistar rats (A kind gift from Dr. Ueta, Japan). Our results show a clear daily oscillation of AVP neuronal firing pattern which is similar in both gender male and female rats. However, females display a lower firing frequency pattern than males which is independent of their oestrus cycle.

Pubmed:
34672042: Thirouin ZS, Bourque CW
Mechanism and function of phasic firing in vasopressin-releasing magnocellular neurosecretory cells.
Magnocellular neurosecretory cells that release vasopressin (MNC) from axon terminals in the neurohypophysis display a unique pattern of action potential firing termed phasic firing. Under basal conditions, only a small proportion of MNC display spontaneous phasic firing. However, acute and chronic conditions that stimulate vasopressin release, such as hemorrhage and dehydration, greatly enhance the number of MNC that fire phasically. Phasic firing optimizes VP neurosecretion at axon terminals by allowing action potential broadening to promote calcium-dependent frequency-facilitation, at the same time as preventing the secretory fatigue caused by spike inactivation that occurs during prolonged continuous stimulation. This review provides an update on our mechanistic understanding of these processes and highlights important gaps in our knowledge that must be addressed in future experiments.
J Neuroendocrinol, 2021; 33
29791836: Ciura S, Prager-Khoutorsky M, Thirouin ZS, Wyrosdic JC, Olson JE, Liedtke W, Bourque CW
Trpv4 Mediates Hypotonic Inhibition of Central Osmosensory Neurons via Taurine Gliotransmission.
The maintenance of hydromineral homeostasis requires bidirectional detection of changes in extracellular fluid osmolality by primary osmosensory neurons (ONs) in the organum vasculosum laminae terminalis (OVLT). Hypertonicity excites ONs in part through the mechanical activation of a variant transient receptor potential vanilloid-1 channel (dn-Trpv1). However, the mechanism by which local hypotonicity inhibits ONs in the OVLT remains unknown. Here, we show that hypotonicity can reduce the basal activity of dn-Trpv1 channels and hyperpolarize acutely isolated ONs. Surprisingly, we found that mice lacking dn-Trpv1 maintain normal inhibitory responses to hypotonicity when tested in situ. In the intact setting, hypotonicity inhibits ONs through a non-cell-autonomous mechanism that involves glial release of the glycine receptor agonist taurine through hypotonicity activated anion channels (HAAC) that are activated subsequent to Ca influx through Trpv4 channels. Our study clarifies how Trpv4 channels contribute to the inhibition of OVLT ONs during hypotonicity in situ.
Cell Rep, 2018; 23
27819299: Ghosh H, Auguadri L, Battaglia S, Simone Thirouin Z, Zemoura K, Messner S, Acuña MA, Wildner H, Yévenes GE, Dieter A, Kawasaki H, O Hottiger M, Zeilhofer HU, Fritschy JM, Tyagarajan SK
Several posttranslational modifications act in concert to regulate gephyrin scaffolding and GABAergic transmission.
GABA receptors (GABARs) mediate the majority of fast inhibitory neurotransmission in the brain via synergistic association with the postsynaptic scaffolding protein gephyrin and its interaction partners. However, unlike their counterparts at glutamatergic synapses, gephyrin and its binding partners lack canonical protein interaction motifs; hence, the molecular

basis for gephyrin scaffolding has remained unclear. In this study, we identify and characterize two new posttranslational modifications of gephyrin, SUMOylation and acetylation. We demonstrate that crosstalk between SUMOylation, acetylation and phosphorylation pathways regulates gephyrin scaffolding. Pharmacological intervention of SUMO pathway or transgenic expression of SUMOylation-deficient gephyrin variants rescued gephyrin clustering in CA1 or neocortical neurons of Gabra2-null mice, which otherwise lack gephyrin clusters, indicating that gephyrin SUMO modification is an essential determinant for scaffolding at GABAergic synapses. Together, our results demonstrate that concerted modifications on a protein scaffold by evolutionarily conserved yet functionally diverse signalling pathways facilitate GABAergic transmission.
Nat Commun, 2016; 7

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WHOLE-BRAIN SIMULATIONS OF WAKEFULNESS, SLOW-WAVE SLEEP, AND ANESTHETIZED STATES IN THE MACAQUE MONKEY

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Recent advances in invasive electrophysiological recordings and non-invasive brain-imaging technology provide a fertile ground to investigate how the anatomical structure of the brain shapes complex brain dynamics in different states of consciousness and in different species. The anatomical connectivity between brain areas is known for macaque monkey with an excellent level of accuracy and is openly available in the COCOMAC database. Here, we have simulated the activity of the whole brain of macaque based on the COCOMAC connectome, using The Virtual Brain simulation platform. We have integrated a biologically-realistic population model based on mean-field models of Adaptive Exponential (AdEx) integrate-and-fire neurons displaying two cell types, Regular Spiking (RS) cells, representing cortical pyramidal neurons, and Fast Spiking (FS) cells, representing inhibitory interneurons. The AdEx mean-field can simulate asynchronous activity states, or Up/Down states and the associated slow-wave activity. We report that from changing the level of adaptation one can switch the entire brain behavior from an asynchronous mode similar to wakefulness to slow-wave activity similar to sleep or anesthesia. We also show that the model can be used to simulate brain signals such as the electrocorticogram or the BOLD signal of functional magnetic resonance imaging. Our TVB-AdEx macaque model replicates functional properties of the macaque brain seen in fMRI in different states of anesthesia. Our bottom-up approach opens the way for mechanistic explanations of data obtained from different sources of experimental data.

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TACKLING THE ELECTROTOPOGRAPHY OF SELF THROUGH THE SPHERE MODEL OF CONSCIOUSNESS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Different models of the Self focused on alpha activity, yet cognitive and contemplative studies emphasized the role of additional frequencies bands. We hypothesized that the hierarchy of Self, within the framework of the Sphere Model of Consciousness (SMC), can be electrophysiologically represented by faster-to-slower frequency bands. SMC posits three types of Self: Narrative Self, involving self-referential processes; Minimal Self, the consciousness of oneself as an immediate and embodied experience in the “here-and-now”; and Overcoming of the Self, in which the sense of Self disappears. Our hypotheses: Gamma (30-100 Hz) and Beta (13-30 Hz) are associated with Narrative Self. Alpha (8-12 Hz) and Theta (4-7 Hz) are associated with Minimal Self. Delta (1-3 Hz) is associated with Overcoming of the Self. We performed a mini-review of the literature regarding states of Self in association with meditation and electrophysiological findings. 257 papers were identified through database search, 222 papers were excluded according to exclusion criteria (Behavioural/fMRI results without EEG; EEG analyses outside of frequency domain). 35 papers were examined. In line with our hypotheses, Narrative Self is related to Gamma and Beta, Minimal Self is associated with Alpha and Theta and Overcoming of the Self is linked to Delta. To our knowledge, this is the first attempt to formulate a topographic map combining Self and electrophysiological results across frequency bands. SMC and proposed electrotopography can contribute to open issues in cognitive and contemplative neuroscience, including the differentiation between higher/altered states of consciousness and their relationship with executive functions.

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FIBER PHOTOMETRY IMAGING OF LOCUS COERULEUS NOREPINEPHRINE ACTIVITIES FOR STUDYING NEUROMODULATORY SPATIOTEMPORAL DYNAMICS ACROSS SLEEP AND WAKEFULNESS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Aims The locus coeruleus (LC) is the main source of norepinephrine (NE) to the forebrain and is involved in multiple functions including regulation of vigilance states. New evidence points to modularity within the LC-NE system, suggesting that distinct efferent pathways may have distinct dynamics. To test this possibility our aim was to validate, in freely sleeping animals, methods to capture dynamics of (i) activities of LC cell bodies and LC subpopulations projecting to forebrain and brainstem regions, and (ii) NE levels in target regions. **Methods** Adult mice were monitored with EEG, EMG, and video during 24-hours of wakefulness and sleep with fiber photometry. Additional experiments combining optogenetics, pupillometry, and noxious stimulation were conducted under moderate isoflurane anesthesia. Fiber photometry was used to either record calcium population activity in LC cell bodies or in LC subpopulations using GCaMP, or to measure levels of free extracellular NE in forebrain regions using $GRAB_{NE}$. **Results** We first validated that under anesthesia LC calcium activity induced by noxious stimulation coincides with pupil dilation. Next, in behaving mice, calcium activities in LC and its subpopulations displayed higher activation in wakefulness compared to sleep, in line with established electrophysiology patterns. In forebrain regions, NE levels are parametrically elevated by LC optogenetic stimulation along with pupil dilation. Finally, NE measurements are possible across 24h recordings, and are modulated by vigilance states. **Conclusions** Fiber photometry allows robust measurements of LC population calcium activities, in LC subpopulations, and extracellular NE levels in forebrain regions, across wakefulness and sleep.

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CYCLICITY OF CEREBRAL GLUTAMATE AND GLUTAMINE LEVELS ACROSS SLEEP-WAKE STATES IN HUMANS

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

Suji Lee, Haejin Hong, Eunji Ha, Rye Young Kim, Hyeonji Lee, Youngeun Shim, Sujung Yoon, In Kyoon Lyoo
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Glutamatergic signaling is critically involved in several brain functions including information processing and neuronal plasticity. However, a sequential pattern of changes in cerebral glutamate and glutamine levels across the sleep-wake cycle has not yet been documented in the living human brain. In this study, we explored whether brain glutamatergic signaling changes during the sleep-wake cycle and how the sleep-wake pattern of glutamate and glutamine levels is perturbed by sleep deprivation. In the current study, healthy participants with a regular sleep-wake cycle schedule were recruited. Using ^{1}H-magnetic resonance spectroscopy, a continuous around-the-clock monitoring approach was implemented to evaluate brain glutamate and glutamine levels during the normal sleep-wake cycle and sleep deprivation period. Continuous monitoring of the metabolites in the prefrontal cortex of the brain indicated that glutamate levels increased and glutamine levels decreased during normal wakefulness. During the night sleep, the opposite direction for metabolite changes was observed such as decreased glutamate levels and increased glutamine levels. Sleep deprivation clearly perturbed the sleep-wake pattern of these cerebral metabolites. Specifically, sustained elevation in glutamate levels was observed during forced wakefulness. Similar to the changes observed in naturalistic wakefulness state, low levels of glutamine persisted throughout forced wakefulness. Our study first documents the distinct sleep-wake pattern of cerebral glutamate and glutamine levels in humans. These findings strongly suggest that sleep plays a central role in brain function through maintaining glutamatergic metabolic homeostasis.

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"CONTROLLING THE GATES" OF REM SLEEP: DUAL MUSCARINIC-NICOTINIC MODULATION OF GABAERGIC TRANSMISSION AT THE REM-S EXECUTIVE AREA

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Intense research in the past decades led to the identification of a relatively well-conserved mesopontine network critically involved in REM sleep (REM-S) and wakefulness (W) control. This network includes a population of 'REM-on' glutamatergic reticular neurons in the *nucleus pontis oralis* (PnO), considered to as the executive area for REM-S onset and maintenance. Based on evidence obtained *in vivo*, a gating mechanism relying on the mutual inhibitory interactions between W-promoting GABAergic and REM-S-promoting cholinergic processes at this area has been proposed as playing a major role in REM-S control. Notwithstanding its probable physiological relevance, this gating mechanism has not been studied in sufficient detail to determine its cellular and synaptic basis. We hypothesised that cholinergic inputs locally inhibit GABAergic synaptic transmission at the PnO. This was evaluated by employing a recently developed *in vitro* model of REM-S motor suppression using electrophysiological and pharmacological approaches. The local micro-application of carbachol elicited either presynaptic depression or facilitation of single evoked GABAergic IPSCs recorded from PnO neurons. Presynaptic depression was replicated by juxtacellular application of muscarine and presynaptic facilitation by nicotine. Using presumed physiological patterns of presynaptic activation (15 Hz stimulation trains) we found that, despite of their opposing effects on short-term plasticity of GABAergic inputs, both muscarine and nicotine reduced the total inhibitory charge transferred to PnO neurons, an effect that was also evoked by carbachol. Our results suggest that a muscarinic-nicotinic cooperation underlies the cholinergic-mediated relief of GABAergic inhibition of PnO neurons responsible for the GABAergic gating mechanism of REM-S.

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IMPAIRED NEURAL FEEDBACK SIGNALING DESPITE ROBUST AUDITORY RESPONSES DURING HUMAN SLEEP

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Sleep in all species is universally defined as a reversible, homeostatically-regulated state of a reduced behavioral responsiveness, with a high arousal threshold in response to external sensory stimulation. However, it remains unclear whether sleep mainly gates motor output or affects responses along sensory pathways, and whether sleep primarily modulates specific aspects of the sensory response such as feedforward vs. feedback signaling. Here, we presented auditory stimuli (e.g. click-trains, words, music) during wakefulness and sleep in epilepsy patients (8 nights / 6 naps from 13 patients), while recording neuronal spiking, microwire LFPs, iEEG, and polysomnography. Auditory stimuli induced robust and selective spiking and high-gamma (80-200 Hz) power responses across the lateral temporal lobe during both NREM and REM sleep. Sleep only moderately attenuated response magnitudes, mainly affecting late responses beyond early auditory cortex and entrainment to rapid click-trains in NREM sleep. By contrast, auditory-induced alpha-beta (10-30Hz) desynchronization (i.e. decreased power) prevalent in wakefulness was strongly reduced in sleep. Thus, extensive auditory responses persist during sleep whereas alpha-beta power decrease, likely reflecting neural feedback processes, is deficient. More broadly, our findings suggest that feedback signalling is key to conscious sensory processing.

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THE ROLE OF SLEEP IN GLYMPHATIC SYSTEM REGULATION

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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The brain parenchyma is devoid of lymphatic vessels. To remove waste products, the brain instead relies on clearance of waste products from the brain to the periphery via the glymphatic system. Strikingly, glymphatic flow of cerebrospinal fluid (CSF) into the brain is limited in wakefulness but triggered during sleep. What drives the enhanced glymphatic activity in sleep is still largely unknown. The aim of this study was to correlate different characteristics of sleep with glymphatic inflow to the brain. A fluorescent tracer was injected into the CSF of mice while electroencephalographic recordings were continuously acquired to monitor the behavioral state. Half of the mice were sleep deprived for 4 hours prior to the experiment, and all mice were either allowed to sleep or were kept awake following the tracer infusion. More tracer was present in the brains of mice that were allowed to sleep after the tracer infusion than mice that were kept awake. The amount of tracer in the brain was positively correlated with the total time spent in sleep, but not delta power. Surprisingly, we found a strong correlation between the amount of tracer in the brain and the number of sleep and wake transitions across experimental groups. These results indicate that entrance of CSF to the brain parenchyma is not merely low in wakefulness and high in sleep, but is also triggered by state transitions and effected by sleep pressure. The exact mechanism behind state transition-induced CSF influx and how it may benefit waste removal requires further investigation.

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MEMORY ENHANCING PROPERTIES OF SLEEP DEPEND ON THE OSCILLATORY AMPLITUDE OF NOREPINEPHRINE

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Sleep microarchitecture is complex and includes short increases in vigilance, known as micro-arousals, as well as restorative processes related to memory consolidation such as sleep spindles. Near-silence of the arousal-promoting nucleus, locus coeruleus (LC), is considered a requirement for sleep, yet LC activity continues during distinct sleep stages suggesting a more complex role. LC releases norepinephrine (NE), but poor temporal resolution of NE measurements has previously limited our insight into the relationship between LC, NE, and sleep structures related to memory performance. Therefore, we set out to study NE during sleep and its connection to micro-arousals and memory-related sleep processes. Combining fiber photometry with EEG and EMG recordings in freely moving mice, we measured LC activity and NE levels across the sleep wake cycle. We found NE levels to oscillate at an infraslow rate during NREM sleep, generating a recurring pattern of micro-arousals alternating with periods of increased sleep spindle activity during NE peak and trough levels, respectively. Optogenetic manipulations of LC revealed that the amplitude of NE oscillations during sleep play a crucial part in memory-related sleep structures: longer NE descents induces spindle-enriched intermediate state and REM sleep, but paradoxically, also increase the likelihood of awakening; shorter NE descents support NREM sleep and micro-arousals. In conclusion NE oscillatory amplitude represents a key target for shaping sleep stages and improving the quality of sleep.

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DOSE-RESPONSE ANALYSIS BETWEEN SMARTPHONE ADDICTION AND SLEEP QUALITY: A SYSTEMATIC REVIEW AND META-ANALYSIS OF OBSERVATIONAL STUDIES

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Study Objective: Several studies have recently been published on the association between smartphone addiction and sleep quality. However, no systematic review or meta-analysis has yet been performed. We aimed to analyse the association between smartphone usage time and sleep quality.
Methods: We searched for articles published up to 13 January 2022 using the Embase and Medline databases. All observational studies were eligible for inclusion. The Newcastle–Ottawa scale was used to evaluate the risk of bias within the studies. We used restricted cubic spline analysis to perform a dose-response analysis.
Results: Seventeen studies with 36,485 participants were included. The pooled odds ratio was 2.28 (confidence interval [CI]: 1.81-2.89, $p<0.00001$), and the heterogeneity was 80%. In the dose-response analysis drawn from five studies, the regression coefficient between daily smartphone usage time (h/day) and poor sleep quality was 1.058 (1.011-1.106), which confirmed a significant association.
Conclusion: This study shows that smartphone addiction is closely associated with sleep quality, such as insomnia, sleep deprivation, and sleep latency. Further studies with a more structured method and high-quality evidence (cohort or case-control) should be conducted.

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BOARD NUMBER: S05-686

PHYSIOLOGICALLY RELEVANT LIGHT STIMULATION LEADS TO LOCAL SIGNATURES OF SLEEP PRESSURE IN THE CONTRALATERAL VISUAL CORTEX IN FREELY MOVING MICE

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Aims: Extended wakefulness leads to a global increase in electroencephalogram (EEG) slow-wave activity (SWA: power density between 0.5-4Hz) during subsequent sleep. SWA is regulated at the local level, which led to the hypothesis that sleep is a use dependent process. There is a scarcity of experimental models that afford the manipulation of sleep locally and physiologically in the neocortex. We present a model for investigating the neurobiological mechanisms of local sleep based on a selective local stimulation during waking. **Methods:** To induce sustained synaptic activity in the mice visual system, we position light-emitting diodes in front of the animal's eyes. EEG and/or 16-channel laminar electrodes were used to record cortical activity. The mice were kept awake for 4 hours starting at light onset under monocular light stimulation (8 Hz, train duration 2 s every 30 s, pulse duration 10 ms) and allowed to sleep undisturbed after the period of visual stimulation. **Results:** During the first hour after sleep deprivation combined with visual stimulation, the occipital EEG spectral power density in part of the SWA frequency range (0.25-2 Hz) was consistently higher in the stimulated visual cortex than the non-stimulated hemisphere [CA1] (mean ± SEM: 50±5.2% vs. 33±5.2%, n=8). A second stimulation generated a bigger difference (63±7% vs. 42±5%, n=8). **Conclusions:** These preliminary results establish a new model for local manipulations of neural activity in freely behaving mice, using peripheral sensory stimulation delivered at physiologically relevant (theta) frequency. This paradigm will be useful for investigating the neurobiological mechanisms of homeostatic sleep need.

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BOARD NUMBER: S05-687

THE EFFECT OF ALTERED NORMOBARIC OXYGEN MANIPULATION ON TMS INDUCED CORTICOSPINAL EXCITABILITY

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Aim: The human brain relies on oxygen for cellular respiration, with a reduction of this supply having profound negative effects on brain function and cognition. Conversely, increased levels of oxygen are proposed to have beneficial and possible therapeutic effects. However, the associated effects of oxygen concentration on cortical excitability (CE) are not well understood. Here we explore how altered levels of oxygen affect CE, using a TMS recruitment curve paradigm. **Methods:** The motor hotspot of the first dorsal interosseous (FDI) was identified using a neuronavigation guided TMS–EMG approach. Resting motor threshold (RMT) was calculated using an adaptive threshold-hunting method based on maximum–likelihood parameter estimation by sequential testing. We applied a repeated measures TMS paradigm, in which participants breathed three different oxygen concentrations (10.5, 21, or 100%), provided in a randomised order, while TMS pulses were delivered between 70–140% of the RMT. **Results**: Motor evoked potentials (MEP's) were calculated by the root mean square of the FDI–EMG response and fitted to a Boltzmann Sigmoid curve. Parameters, including the MEP saturation threshold, curve slope and latency were considered. These results show the changes in CE as a function of oxygen concentration. **Discussion**: These findings have implications for our understanding of the potential importance of cerebral oxygen concentration on neural network activity and related performance. Moreover, it highlights the value of brain stimulation approaches for evaluating the efficacy of hyperbaric oxygen therapy as a treatment for central nervous system related disorders.

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BOARD NUMBER: S05-688

CAPTURING THE ROLE OF OBJECTIVE AND SUBJECTIVE SLEEP MEASURES WITH NEURAL CORRELATES OF COGNITION.

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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Aim: Many neurological disorders present cognitive dysfunction along with high levels of fatigue. However, our understanding of the neurological effects of fatigue on cognition is poor. Generally, research informs of a disconnect between reported measures of objective and subjective sleep, such that individuals are not commonly capable of accurately predicting their own sleep quality and quantities. Here we explore the role of sleep using objective and subjective measures alongside fMRI investigation of cognitive functions. **Method:** Participants (n=25) wore a wrist accelerometer device (GENEActiv) for 7-days to obtain objective measures of sleep and completed the Pittsburgh Sleep Quality Index as a subjective measure of sleep. An MRI protocol consisting of a T1 anatomical scan and task-based fMRI consisting of an n-back and go/no-go task was acquired. A general linear model analysis was used to determine the relationship between fMRI haemodynamic responses, cognitive performance, and sleep measures. **Results:** Here we demonstrate the relationship between objective and subjective measures of sleep quality and quantity, and functional performance in the n-back and go/no-go tasks (accuracy and reaction times). Furthermore, we highlight the corresponding neural network changes associated with perceived and actual sleep quality and subsequent impact on cognitive and behavioural measures. **Conclusion:** Demonstrating the relationship between objective and subjective sleep measures, functional performance and subsequent neural correlates evidences the role of differing sleep measures on cognition in a healthy population. Through this understanding, we can expand further into the role that fatigue plays in cognitive symptoms of neurological disease.

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IS IT POSSIBLE TO DIALOGUE WITH SLEEPWALKERS IN THE MIDST OF AN EPISODE?

POSTER SESSION 05 - SECTION: SLEEP HOMEOSTASIS, REGULATION MECHANISMS & WAKEFULNESS

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We have recently shown that lucid dreamers can behaviorally respond to stimuli while in the midst of a dream during REM sleep. Here, we tested if this ability to communicate with the outside world extends to sleepwalkers (SW), who exhibit complex behaviors (including speech) during slow-wave sleep. Method: 62 SWs filled a questionnaire investigating their memory of their nocturnal episodes, the type and frequency of behaviors exhibited during an episode (e.g., fighting, laughing, opening doors), as well as the relationship between these behaviors, their mental activity, and the environment. Results: 82% of SWs experienced a conversation with a loved one during an episode at least once in their life. While SWs perceived the interlocutor's words accurately in 81.3% of the cases, they often misperceived the speaker as a dream character (47.9%). Similarly, SWs' verbal responses were more frequently (72.9%) related to the dream scenario (e.g., 'there is a monster') than to reality. The dream scenery was mostly distressing (91.1%). SWs could remember having these nightly discussions (66.5%), but their memory was mostly fragmentary (39.5%) or without content (52.6%). Our results suggest that SWs can perceive stimuli from their environment and converse with a real person during an episode. We are planning to confirm this promising finding by sending verbal stimuli during episodes while SWs' sleep/wake states are monitored with video-polysomnography. If validated, our findings would open the possibility to "interview" sleepers in real-time about the content of their dreams, potentially unlocking the mystery of dreams and their functions.

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BOARD NUMBER: S05-689a

EVOKED AND INTRINSIC NEURAL SIGNATURES OF HUMAN RECOVERY-OF-CONSCIOUSNESS

POSTER SESSION 04 - SECTION: MENTAL HEALTH, ADDICTION AND OBSESSIVE-COMPULSIVE DISORDER

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AIMS In this work we aimed to investigate two novel neural markers of Recovery-of-Consciousness (ROC), one based on externally evoked and one based on intrinsic brain processes. First, we examined whether the human brain has different excitability and response to external electrical somatosensory stimulation during anesthesia and after recovery of consciousness. Second, we investigated whether band-limited permutation entropy in various frequency bands of intrinsic brain activity can identify the transition from coma to consciousness. METHODS We recorded brain activity with electrocorticography (ECoG) in 11 patients who underwent awake brain tumor surgery in interleaved blocks of steady state somatosensory electric stimulation and resting state between aesthesia-induced unconsciousness to full wakefulness. RESULTS/CONLUSIONS ERP magnitudes from external stimulation were reduced after ROC. This reduced response is likely attributed to the modulation of bottom-up processing by the more prominent top-down processing in wakefulness. The intrinsic activity showed a sharp increase in peak frequency of ongoing alpha oscillations right at the moment of the patients' first behavioral response independent of eye opening. The presence of this effect in various brain areas, even in ones without a prominent power alpha peak in the spectrum, offers supporting evidence for previously posed hypotheses on the functional role of alpha oscillations as a canonical mechanism for information sampling and sensory integration in the brain, with faster sampling in the awake state. Our findings constitute novel and significant contributions to the understanding of the brain mechanisms that underlie recovery of consciousness.

Poster Session 06

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BOARD NUMBER: S06-001

PRE-TRAUMA BEHAVIOURAL RISK FACTORS OF POSTTRAUMATIC STRESS DISORDER

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Experiencing a traumatic life event results in Posttraumatic Stress Disorder (PTSD) in vulnerable individuals (10-20% of trauma-exposed populations). PTSD can be characterized by extinction-resistant fear memories and generalization of fear to safe contexts. For better treatment options, it is essential to identify risk factors contributing to vulnerability for PTSD. To identify specific predisposing emotional and cognitive domains of fear generalization and impaired fear extinction, we exposed rats to a wide behavioural test battery prior to the trauma. Then subjects were exposed to a traumatic experience utilizing inescapable footshocks. Four weeks later, freezing responses were quantified in an altered/safe context to assess generalization and extinction to differentiate vulnerable and resilient subpopulations (quartiles). Subsequently, we conducted immunohistochemical or gene expression analysis (q-RT PCR of 96 candidate genes) on medial prefrontal cortical (mPFC) samples, respectively. Finally, we virally knocked down CRH gene expression in the mPFC after fear memory consolidation. We found that specific anxiety-like traits and operant learning-like characteristics are predictive factors of PTSD-like symptoms. Furthermore expression of interneuron markers such as CRH, VIP showed major differences with an additional decrease of neuronal activity in the vulnerable population. Immunohistochemical staining confirmed this finding, which is particularly apparent in VIP/CRF-expressing interneurons. Interestingly, CRH knockdown in mPFC decreased fear expression and generalization, thereby recapitulating the resilient phenotype. In conclusion, lower pre-trauma cognitive abilities are vulnerability factors in the development of fear generalization symptoms of PTSD. Moreover, mPFC CRH signaling seems to play a mediatory role in this process.

Pubmed:
30458201: Balogh Z, Szente L, Biro L, Varga ZK, Haller J, Aliczki M
Endocannabinoid interactions in the regulation of acquisition of contextual conditioned fear.
Endocannabinoids (eCBs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG) were shown to be involved in the basis of trauma-induced behavioral changes, particularly contextual conditioned fear, however, their ligand-specific effects and possible interactions are poorly understood. Here we assessed specific eCB effects and interactions on acquisition of contextual conditioned fear employing electric footshocks in a rat model. We selectively increased eCB levels by pharmacological blockade of the degrading enzymes of AEA by URB597 and 2-AG by JZL184 before traumatization either systemically or locally in relevant brain areas, the prelimbic cortex (PrL), ventral hippocampus (vHC) and basolateral amygdala (BLA). Following traumatization, a series of contextual reminders were conducted during which conditioned fear was assessed. While systemic URB597-treatment during traumatization only slightly enhanced the acquisition of contextual conditioned fear, administration of the compound in the PrL and vHC led to the acquisition of stable, lasting conditioned fear, resistant to extinction. These effects of URB597 were blocked by simultaneous administration of JZL184. Similar treatment effects did not occur in the BLA. Treatment effects were not secondary to alterations in locomotor activity or nociception. Our findings suggest that AEA and 2-AG functionally interact in the regulation of acquisition of contextual conditioned fear. AEA signaling in the PrL and vHC is a crucial promoter of fear acquisition while 2-AG potentially modulates this effect. The lack of eCB effects in the BLA suggests functional specificity of eCBs at distinct brain sites.
Prog Neuropsychopharmacol Biol Psychiatry, 2019; 90

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-002

BRAINSTEM SOMATOSTATIN-EXPRESSING CELLS CONTROL THE EMOTIONAL REGULATION OF PAIN BEHAVIOR

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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In mammals, threat-related behavior is typically induced by a noxious physical stressor and is associated with a broad range of behavioral responses such as freezing and avoidance. These behavioral responses are associated with the regulation of pain responses allowing individuals to cope with noxious stimuli. Whereas the structures and mechanisms involved in pain behavior are well documented, little is known about the precise neuronal circuits mediating the emotional regulation of pain behavior. Here we used a combination of behavioral, anatomical, optogenetic, and electrophysiological approaches to show that somatostatin-expressing neurons in the ventrolateral periaqueductal gray matter (vlPAG SST cells) promote antinociceptive responses during the presentation of conditioned stimuli (CS) predicting footshocks. Whereas the optogenetic inhibition of vlPAG SST cells during CS presentation promoted analgesia, their optogenetic activation reduced analgesia by potentiating pain responses in the spinal cord through a relay in the rostral ventromedial medulla (RVM). Together these results identify a brainstem circuit composed of vlPAG SST cells specifically projecting to the RVM and mediating FCA to regulate pain responses during threatful situations.

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BOARD NUMBER: S06-003

UPCONVERSION NANOPARTICLES-MEDIATED OPTOGENETICS : JUGGLING WITH PHOTONS TO CONTROL FEAR

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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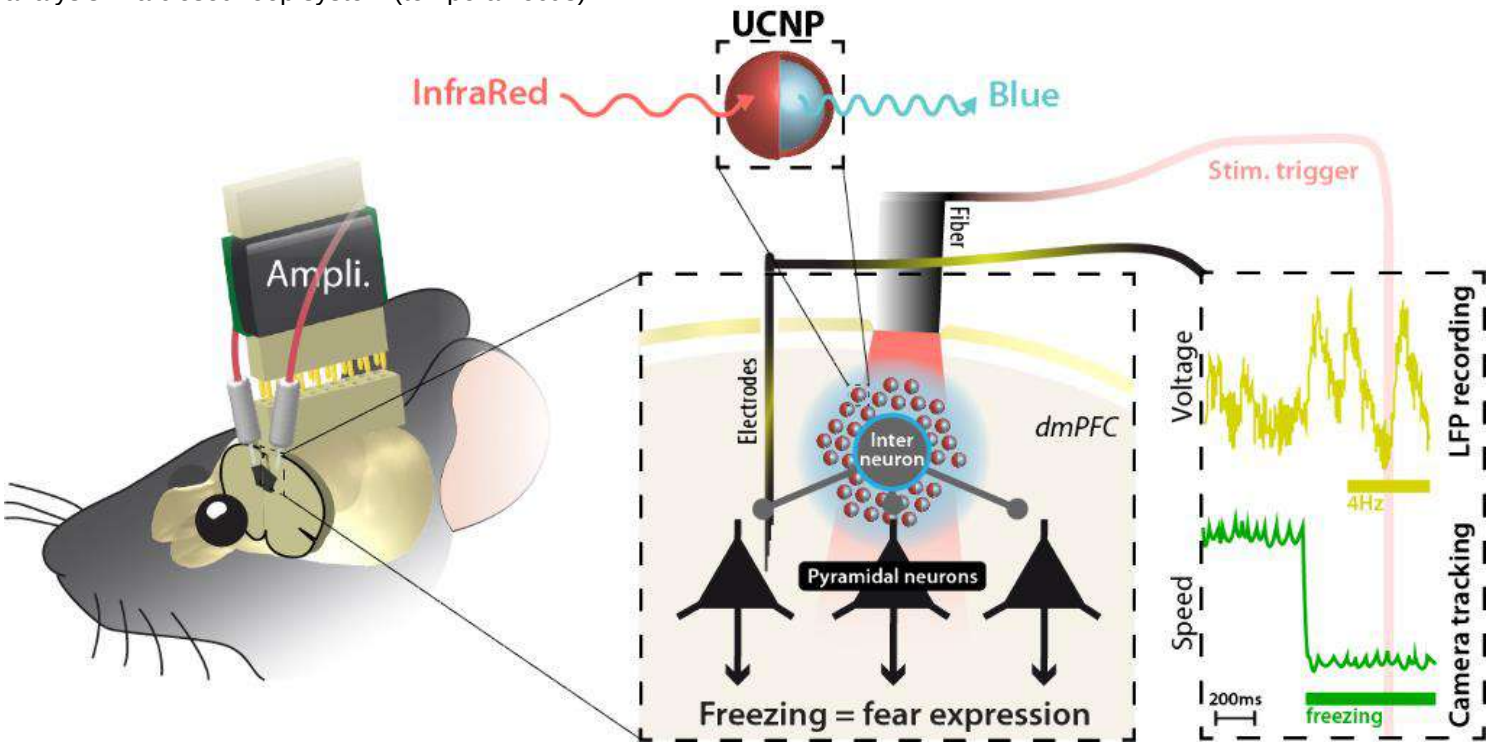
Fear is known to serve essential purposes in protecting individuals from dangerous situations and threats. Dysfunctionnal fear biology is implicated in neuropsychiatric disorders such as pathological anxiety and post-traumatic stress disorder. The understanding of the neuropathophysiology of those disorders is still limited, and therapeutic options (psychotherapy or medication) remain too often ineffective. Works investigating the function of neuronal networks helped highlight the implication of the dorsomedial prefrontal cortex (dmPFC) in fear response. Mice studies, using fear conditioning and dmPFC optogenetic stimulation, have proven that fear behavior can be precisely manipulated. Hence, neuromodulation could be an innovative therapeutic option for fear-linked psychiatric disorders. However, the use of brain stimulation in clinical practice is limited by the unvaforable risk-benefit balance of their use : techniques allowing a precise stimulation are highly invasive, and current non-invasive techniques lack in efficacy and precision. This project aims to develop innovative transcranial optogenetic stimulation techniques, using infrared light that can travel through living tissue, before being converted into visible light at the site of interest by UpConversion NanoParticles (UCNP) (spatial focus). The attached figures rolls out different scales of the project, from the whole animal with its brain implants, to the dmPFC and its neurons, modulated via the light emitted by UCNPs. Moreover this stimulation is conditional, triggered by electrophysiological and behavioral real-time analysis in a closed-loop system (temporal focus).



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BOARD NUMBER: S06-004

PREFRONTAL NEURONAL CIRCUITS OF PASSIVE AND ACTIVE FEAR BEHAVIOURS

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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When facing danger, mammals display a broad range of defensive behaviours including freezing and avoidance behaviors. Several studies emphasized the role of the dorsal medial prefrontal cortex (dmPFC) in encoding the acquisition as well as the expression of freezing behavior. However the role of this structure in processing avoidance behavior and the contribution of distinct prefrontal circuits to both freezing and avoidance responses are largely unknown. To further investigate the role of dmPFC circuits in encoding passive and active fear-coping strategies, we developed in the laboratory a novel behavioural paradigm in which a mouse has the possibility to choose either to passively freeze to an aversive stimulus or to actively avoid it as a function of contextual contingencies. Using this behavioural paradigm where both passive and active behaviors were present, we performed single unit recordings in the dmPFC. We observed large populations of dmPFC neurons encoding either freezing or avoidance behavior. Importantly, avoidance-encoding dmPFC neurons project to the dorsal periaquedecutal gray matter and their optogenetic activation and inhibition, promoted or disrupted avoidance learning, respectively. Likewise, an in-vitro patch-clamp recording analysis after optogenetic activation at dmPFC inputs onto dl/lPAG cells resulted in the development of synaptic potentiation. Our data identify a dmPFC-dPAG circuit involved in the acquisition of avoidance behavior.

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BOARD NUMBER: S06-005

ROLE OF HIPPOCAMPO-PREFRONTAL CIRCUITS IN FEAR MEMORY CONSOLIDATION.

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Fear and anxiety-related disorders are among the most frequent psychiatric conditions with a lifetime prevalence of about 28% in the population worldwide and are therefore a major public health concern. Fear can be modelled in mice with pavlovian fear conditioning (FC) during which mice learn to associate a sound with an electric shock. Concurrent with this learning we observed a subset of prefrontal cortex (PFC) neurons that show synchronous activation during fear expression. We hypothesize that this synchronous activation is the cortical representation of the consolidated associative memory. The ventral part of the hippocampus (vHPC) appears as a likely candidate to orchestrate this consolidation. Hebbian theory posits that memory formation starts in hippocampus before being transferred to cortical regions for long term retention. The ventral region is specifically implicated in emotional memory and send a monosynaptic connection to the PFC. The CA1-field of the vHPC display high frequency oscillations called Sharp-wave-ripples that are crucial for memory consolidation, synchronously activating neurons and strengthening their synaptic weights. To test our hypothesis we used *in vivo* extracellular recordings in the PFC and vHPC during sleep and awake behavior. To investigate the causal role of ripples on fear memory consolidation we used optogenetics to inhibit the pathway from vHPC to PFC during ripples using a closed-loop system. We found that PFC neurons participating in the memory trace display increasing and specific co-activity around SWRs following conditionning, thus highlighting the role of sharp wave ripples in the consolidation of fear neuronal representation and fear memory.

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BOARD NUMBER: S06-006

PHYSIOLOGICAL, NEURONAL AND PHARMACOLOGICAL IDENTIFICATION OF PANIC AND ANXIETY-LIKE STATES DURING FREEZING

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Ethological studies show fear to be a complex, flexible strategy to manage diverse threats. This approach often focuses on behaviour alone despite the important adaptive value of the underexplored neural and somatic states accompanying them. In particular, identifying brain states through their oscillatory markers should shed light on the cognitive strategies implemented to respond to danger. In this context, we designed a U-shaped maze with one arm associated with aversive shocks while the opposite arm was a clear safe zone. Depending on the distance to threat, we observed the full spectrum of fear behaviours. Surprisingly, we observed in both arms behaviourally identical freezing that differed by a host of physiological and neurophysiological characteristics. Shock side freezing is a more aroused state with higher heart rate, breathing, gamma frequency and power and theta oscillation in the hippocampus. On the contrary, safe side freezing is a less aroused state characterized by hippocampal ripples. The difference between these two states is reliably reflected in prefrontal cortex population firing. Moreover during safe-side ripples, these neurons reactivate shock activity suggesting that this state supports offline processing of threat.
We hypothesized that shock side freezing is a panic-like state while safe side freezing anxiety-like and tested this pharmacologically. Panicolytic administration (chronic fluoxetine) specifically modified shock-side freezing whereas anxiolytic (diazepam) had more widespread effects but crucially inhibits ripples, a hallmark of safe side freezing.
These findings allow a clear distinction of panic and anxiety like states based on physiological, neurophysiological, neuronal and pharmacological markers without motor bias.

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BOARD NUMBER: S06-007

SEX-DEPENDENT EFFECTS OF OXR1 BLOCKADE ON ACQUISITION, RETENTION AND EXTINCTION OF ACTIVE AVOIDANCE

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Orexins are a type of neuropeptide which neurons are located in the lateral hypothalamus and project widely across the brain. Orexin A and its receptor orexin-1-receptor (OX1R) are specifically involved in reward, feeding, and emotional memories. Fear is an emotion that helps the arise of adaptative behaviors when a threatening stimulus or context is detected. A maladaptive response during fear learning or its extinction can lead to anxiety or fear-based disorders, such as posttraumatic stress disorder, which are known to affect men and women differently. It has been shown that the selective OX1R blockade enhances the extinction of fear/emotional memories as well as the activation of pro-extinction circuits. The aim of this study is to assess the effects of OX1R blockade on the acquisition and subsequent retention and extinction of an active avoidance task, which involves both classical conditioning of fear and the production of an instrumental response to avoid it. Forty-eight male and female rats were chronically implanted with a canula to icv administer the OX1R blocker (SB334867) or vehicle after each acquisition session. OX1R blockade was found to detriment the acquisition of male rats, while it did not affect female performance. The affectation on acquisition on males did affect further learning sessions, such as the retention, extinction and, the spontaneous recovery. These results suggest that OX1R could be positively involved in the acquisition of conditioning in males but not in females, and support the idea that there might be differences between sexes regarding circuit activity.

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BOARD NUMBER: S06-008

PREFRONTAL CORTEX NEURONAL NETWORK PROCESSES RISK ASSESSMENT BEHAVIORS IN MICE.

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Anxiety and fear are distinct but overlapping emotions that are expressed as different behaviours depending on the risk the situation entails. Here we visualized the activity of medial prefrontal cortex (mPFC) cells with a miniaturized microscope while mice are tested in tasks with increasing anxiety and fear behavioural outcomes. Different ensembles of mPFC cells convey information about the anxiety-like behaviour (identified as mice spend time in the corners) or fear (when mice engage in freezing behaviour) in a dynamic, behaviour- and task-specific, fashion. We also analysed mPFC neural activity at a population level. We show that, at low-risk tasks, anxiety-like behaviour predominates, and its accompanying neural state can fully predict the fear-related neuronal configuration. However, in high-risk tasks, where a potential or a real threat is present, fear behaviour predominates, and its related neuronal representation is able to predict the anxiety configuration. Finally, we show evidence of fear memory retention in the mPFC neuronal network as early as 1 day after contextual fear conditioning training. These data suggest that the mPFC engages partially overlapping neural states during anxiety- or fear-like behaviours, that can be regulated by present or past threatening experiences.

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BOARD NUMBER: S06-009

SEX DIFFERENCES IN A MOUSE SENSORY PRECONDITIONING TASK

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Our daily choices are not always dictated by stimuli that have been directly associated with a potent reinforcer. Indeed, they are often triggered by stimuli that have not been explicitly associated with a reinforcer. These processes are called higher-order conditioning or mediated learning and can be assessed by sensory preconditioning. This task involves repeated simultaneous presentations of two *low-salience* stimuli (e.g. light&tone) and, after, the devaluation of one of the stimuli with an unconditioned stimulus (e.g. foot-shock). These associative processes result in a conditioned response (e.g. fear response) to both the conditioned (direct_learning) and the non-conditioned stimulus (mediated_learning). In our study, male and female mice underwent a protocol of sensory preconditioning divided into 4 different stages: habituation, preconditioning, conditioning, and tests. In habituation, mice freely explore the context where the behavioral paradigm will be performed. In preconditioning, mice were exposed to simultaneous presentation of light and tone. In conditioning, the light was devaluated by pairing a mild foot-shock. Finally, animals performed two tests where we exposed the mice to tone (mediated_cue) and to light (direct_cue) in a different context to avoid fear response elicited by the context where they received the stressor. Our results indicated that females showed a higher sensory-preconditioning performance than males exhibiting a higher percentage of freezing in the presence of mediated cue. No differences were observed in habituation and preconditioning between both sexes. Overall, we implemented a new protocol of light-tone mouse sensory preconditioning and, importantly, we observed sex-dependent behavioral effect in mediated learning.

Pubmed:
27889488: Pinho J, Vale R, Batalha VL, Costenla AR, Dias R, Rombo D, Sebastião AM, de Mendonça A, Diógenes MJ
Enhanced LTP in aged rats: Detrimental or compensatory?
Age-dependent memory deterioration has been well documented and yet an increase in rat hippocampal LTP upon aging has been reported. This poses the question of whether the enhanced LTP is a cause or an attempt to compensate the memory deficits described in aged rats. Hippocampal slices from young, adult and aged Wistar rats were pre-incubated, with an NMDA receptor (NMDAR) antagonist, memantine (1 μM, 4 h), and hippocampal LTP was evaluated. The results show that memantine significantly decreases the larger LTP magnitude recorded in hippocampal slices from aged rats without compromising LTP recorded in slices from young and adult animals. To unveil the impact of in vivo administration of memantine, different doses (1, 5 and 10 mg/kg/day) or saline vehicle solution were intraperitoneally administered, for 15-20 days, to both young and aged animals. Memantine did not significantly affect neither the place learning of young animals, evaluated by Morris Water Maze, nor LTP recorded from hippocampal slices from the same group of animals. However, memantine (5 and 10 mg/kg/day) significantly decreased the large LTP recorded in hippocampal slices from aged animals. Moreover, aged animals treated with memantine (10 mg/kg/day) showed a significantly compromised place learning when compared to aged control animals. Overall, these results suggest that the larger LTP observed in aged animals is a compensatory phenomenon, rather than pathological. The finding that age-dependent blockade of LTP by a NMDAR antagonist leads to learning deficits, implies that the increased LTP observed upon aging may be playing an important role in the learning process.
Neuropharmacology, 2017; 114
32705771: Pinho JS, Castilho M, Sollari JS, Oliveira RF
Innate chemical, but not visual, threat cues have been co-opted as unconditioned stimulus for social fear learning in zebrafish.
Animals can use social information to detect threat in the environment. In particular, social learning allows animals to learn about dangers without incurring in the costs of trial-and-error learning. In zebrafish, both chemical and visual social cues elicit an innate alarm response, which consists of erratic movement followed by freezing behavior. Injured zebrafish release an alarm substance from their skin that elicits the alarm response. Similarly, the sight of conspecifics displaying the alarm response can also elicit the expression of this response in observers. In this study, we investigated if these social cues of

danger can also be used by zebrafish as unconditioned stimulus (US) in learning. We found that only the chemical cue was effective in the social fear conditioning. We suggest that this differential efficacy of social cues results from the fact that the alarm cue is a more reliable indicator of threat, than the sight of an alarmed conspecific. Therefore, although multiple social cues may elicit innate responses not all have been evolutionarily co-opted to act as US in associative learning. Furthermore, the use of the expression of the immediate early genes as markers of neuronal activity showed that chemical social fear conditioning is paralleled by a differential activation of the olfactory bulbs and by a different pattern of functional connectivity across brain regions involved in olfactory processing.
Genes Brain Behav, 2020; 19
32078241: Pinho J, Marcut C, Fonseca R
Actin remodeling, the synaptic tag and the maintenance of synaptic plasticity.
Activity-dependent plasticity of synaptic connections is a hallmark of the mammalian brain and represents a key mechanism for rewiring neural circuits during development, experience-dependent plasticity, and brain disorders. Cellular models of memory, such as long-term potentiation and long-term depression, share common principles to memory consolidation. As for memory, the maintenance of synaptic plasticity is dependent on the synthesis of de novo protein synthesis. The synaptic-tagging and capture hypothesis states that the maintenance of synaptic plasticity is dependent on the interplay between input-specific synaptic tags and the allocation or capture of plasticity-related proteins (PRPs) at activated synapses. The setting of the synaptic tag and the capture of PRPs are independent processes that can occur separated in time and different groups of activated synapses. How are these two processes orchestrated in time and space? Here, we discuss the synaptic-tagging and capture hypothesis in the light of neuronal compartmentalization models and address the role of actin as a putative synaptic tag. If different groups of synapses interact by synaptic-tagging and capture mechanisms, understanding the spatial rules of such interaction is key to define the relevant neuronal compartment. We also discuss how actin modulation can allow an input-specific capture of PRPs and try to conciliate the temporal dynamics of synaptic actin with the maintenance of plasticity. Understanding how multiple synapses interact in time and space is fundamental to predict how neurons integrate information and ultimately how memory is acquired.
IUBMB Life, 2020; 72

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BOARD NUMBER: S06-010

FEAR LEARNING CONDITIONS INFLUENCE HIPPOCAMPAL-DEPENDENT MEMORY AND CONTEXT DISCRIMINATION

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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In contextual fear conditioning, learning occurs when an aversive foot-shock is presented within a context. Hippocampal CA1 neurons integrate sensory and spatial information to form neuronal representations of contexts. Fear learning is expressed in the form of a conditioned response (freezing), which can be restricted to the conditioning context (discrimination) or extended to a similar neutral context (generalization). This capacity to perceive and respond to differences among stimuli has a critical role in learning adapting our behaviour according to past experiences. This work aims to: 1) Identify learning conditions that influence discrimination and 2) elucidate the impact of those conditions on the activity of CA1 neurons using fibre photometry, optogenetics and c-fos expression. Although mice discriminate contexts efficiently before and after conditioning, shock modifies how mice behaviourally express context discrimination. Inter-individual variability suggests that experience-related factors influence discrimination. Correlation analysis showed multiple significant associations of behavioural traits (freezing, locomotion, centre/periphery exploration, shock reactivity) and learning conditions with discrimination. Manipulations of learning conditions (shock reception zone –centre/periphery– and shock intensity) resulted in differences in mice discrimination, in line with the previous correlations. Fibre photometry revealed different levels of activity in CA1 excitatory and inhibitory neurons (Somatostatin and Parvalbumin) depending on mice speed and position (centre/periphery) during exploration. We are now using cell type-specific optogenetics and c-fos tagging of neuronal ensembles to address how CA1 circuitry regulates context discrimination. In conclusion, our results show that discrimination relies on learning conditions that affect the hippocampal circuits involved in memory encoding.

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BOARD NUMBER: S06-011

A FRONTOPARIETAL CIRCUIT REGULATES FEAR RENEWAL IN A NOVEL CONTEXT

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Fear renewal following extinction therapy is a critical issue associated with the treatment of many fear-related disorders. After extinction learning, the contextual effect becomes important to evoke a fear response, and an extinguished fear response can easily return outside of the extinction context (fear renewal). However, brain regions associated with context-dependent retrieval of fear remains poorly understood. Posterior parietal cortex (PPC) receives diverse sensory and cognitive inputs and integrates multisensory signals for making decisions. To study its role in the contextual modulation of fear relapse, pharmacological and optogenetic inhibition were conducted in various contextual situation. Here, we found that the PPC mediates fear renewal in a novel context. Fear relapses were selectively blocked during inhibition of the PPC, only when animals were placed in a novel context, but not in a familiar context. The suppression of the PPC-driven input to the anterior cingulate cortex (ACC) also attenuated fear response. However, fear responses were increased by inhibition of the activity of PPC-ACC neurons innervating to the amygdala. Further study of neural population switching the fear state is needed to understand the fundamental mechanisms underlying relapse of traumatic response even in safe context, and can provide insight into pathologies of traumatic disorder.

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BOARD NUMBER: S06-012

NEURAL CIRCUIT AND MECHANISMS FOR LEARNING RELATIVE AVERSIVE VALUE IN MICE

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Throughout our lifetime, we are constantly approaching or avoiding situations such as places associated with positive (e.g. where food is present) or negative (e.g. where a danger may occur) outcomes, respectively. Surprisingly, very little is known about the neural processes underlying relative aversive value-based learning and choice. Indeed, individual are often confronted to two situations both holding negative outcomes, but each of different intensities, forcing them to choose the less worse option. To understand how such complex decision-making process is selected, it is crucial to identify the brain circuits and mechanisms involved in learning relative (better or worse) aversive value. In a newly-developed conditioned place aversive task, we combined behavioral analysis, c-fos quantification, manipulation and recording of neuronal activity to dissect the neural circuits for learning relative aversive value. We found that while mice can perform both relative and absolute value-based choices, yet they exhibit distinct behavioral strategies. The manipulation and monitoring of identified neurons at the time of the conditioning revealed differential involvement of discrete brain circuits for relative versus absolute value-based learning.

Pubmed:
34922210: Miranda M, Gonzalez Campo C, Birba A, Neely A, Toro-Hernández FD, Faure E, Rojas GM, Ibáñez A, García A
An action-concept processing advantage in a patient with a double motor cortex.
Patients with atrophy in motor brain regions exhibit selective deficits in processing action-related meanings, suggesting a link between movement conceptualization and the amount of regional tissue. Here we examine such a relation in a unique opposite model: a rare patient with a double cortex (due to subcortical band heterotopia) in primary/supplementary motor regions, and no double cortex in multimodal semantic regions. We measured behavioral performance in action- and object-concept processing as well and resting-state functional connectivity. Both dimensions involved comparisons with healthy controls. Results revealed preserved accuracy in action and object categories for the patient. However, unlike controls, the patient exhibited faster performance for action than object concepts, a difference that was uninfluenced by general cognitive abilities. Moreover, this pattern was accompanied by heightened functional connectivity between the bilateral primary motor cortices. This suggests that a functionally active double motor cortex may entail action-processing advantages. Our findings offer new constraints for models of action semantics and motor-region function at large.
Brain Cogn, 2022; 156
34737148: de Landeta AB, Pereyra M, Miranda M, Bekinschtein P, Medina JH, Katche C
Functional connectivity of anterior retrosplenial cortex in object recognition memory.
Recognition memory can rely on three components: "what", "where" and "when". Recently we demonstrated that the anterior retrosplenial cortex (aRSC), like the perirhinal cortex (PRH) and unlike the hippocampus (HP), is required for consolidation of the "what" component. Here, we aimed at studying which brain structures interact with the aRSC to process object recognition (OR) memory in rats. We studied the interaction of six brain structures that are connected to the aRSC during OR memory processing: PRH, medial prefrontal cortex (mPFC), anteromedial thalamic nuclei (AM), medial entorhinal cortex (MEC), anterior cingulate cortex (ACC) and the dorsal HP (dHP). We previously described the role of the PRH and dHP, so we first studied the participation of the mPFC, AM, MEC and ACC in OR memory consolidation by bilateral microinfusions of the GABA receptor agonist muscimol. We observed an impairment in OR long-term memory (LTM) when inactivating the mPFC, the AM and the MEC, but not the ACC. Then, we studied the functional connections by unilateral inactivation of the aRSC and each one of the six structures in the same (ipsilateral) or the opposite (contralateral) hemisphere. Our results showed an amnesic LTM effect in rats with ipsilateral inactivations of aRSC-PRH, aRSC-mPFC, aRSC-AM, or aRSC-MEC. On the other hand, we observed memory impairment when aRSC-ACC were inactivated in opposite hemispheres, and no effect when the aRSC-dHP connection was inactivated. Thus, our ipsilateral inactivation findings reveal that the aRSC and, at

least one brain region required in OR LTM processing are essential to consolidate OR memory. In conclusion, our results show that several cortico-cortical and cortico-thalamic pathways are important for OR memory consolidation.
Neurobiol Learn Mem, 2021; 186
33064924: Miranda M, Morici JF, Gallo F, Piromalli Girado D, Weisstaub NV, Bekinschtein P
Molecular mechanisms within the dentate gyrus and the perirhinal cortex interact during discrimination of similar nonspatial memories.
Differentiating between similar memories is a crucial cognitive function that enables correct episodic memory formation. The ability to separate the components of memories into distinct representations is thought to rely on a computational process known as pattern separation, by which differences are amplified to disambiguate similar events. Although pattern separation has been localized to the dentate gyrus (DG) of the hippocampus and shown to occur in a spatial domain, this cognitive function takes place also during processing of other types of information. In particular, there is some debate on whether the DG participates in pattern separation of nonspatial representations. Considering the classic role of the Prh in the acquisition and storage of object memories in general and tasks with similar features in particular, this cognitive function could rely more heavily on perirhinal regions when object-related information is processed. Here we show that two plasticity-related proteins, BDNF, and Arc, are required in the DG for nonspatial mnemonic differentiation. Moreover, we found that the crucial role of the DG is transient since activity of AMPAR is only required in the Prh but not the DG during differentiated object memory retrieval. Additionally, this memory is not modifiable by postacquisition rhBDNF infusions in the DG that are known to improve memory when given in the Prh. This highlights a differential role of Prh and DG during differentiated object memory consolidation. Additionally, we found that these molecular mechanisms actively interact in the DG and Prh for the formation of distinguishable memories, with infusions of rhBDNF in the Prh being able to rescue mnemonic deficits caused by reduced Arc expression in the DG. These results reveal a complex interaction between plasticity mechanisms in the Prh and DG for nonspatial pattern separation and posit the Prh as the key structure where unique object representations are stored.
Hippocampus, 2021; 31
32587504: Morales C, Morici JF, Miranda M, Gallo FT, Bekinschtein P, Weisstaub NV
Neurophotonics Approaches for the Study of Pattern Separation.
Successful memory involves not only remembering over time but also keeping memories distinct. Computational models suggest that pattern separation appears as a highly efficient process to discriminate between overlapping memories. Furthermore, lesion studies have shown that the dentate gyrus (DG) participates in pattern separation. However, these manipulations did not allow identifying the neuronal mechanism underlying pattern separation. The development of different neurophotonics techniques, together with other genetic tools, has been useful for the study of the microcircuit involved in this process. It has been shown that less-overlapped information would generate distinct neuronal representations within the granule cells (GCs). However, because glutamatergic or GABAergic cells in the DG are not functionally or structurally homogeneous, identifying the specific role of the different subpopulations remains elusive. Then, understanding pattern separation requires the ability to manipulate a temporal and spatially specific subset of cells in the DG and ideally to analyze DG cells activity in individuals performing a pattern separation dependent behavioral task. Thus, neurophotonics and calcium imaging techniques in conjunction with activity-dependent promoters and high-resolution microscopy appear as important tools for this endeavor. In this work, we review how different neurophotonics techniques have been implemented in the elucidation of a neuronal network that supports pattern separation alone or in combination with traditional techniques. We discuss the limitation of these techniques and how other neurophotonic techniques could be used to complement the advances presented up to this date.
Front Neural Circuits, 2020; 14
31376070: Fleitas MFG, Aranda ML, Diéguez HH, Milne G, Langellotti L, Miranda M, Altschuler F, Dorfman D, Rosenstein RE
The "Use It or Lose It" Dogma in the Retina: Visual Stimulation Promotes Protection Against Retinal Ischemia.
Enriched environment (EE) protects the retina from adult rats against ischemia/reperfusion (I/R) injury; however, how the components of EE contribute to the recovery after retinal ischemic damage remains unclear. We analyzed the contribution of social, cognitive, and visual stimulation on functional and histological alterations induced by I/R. Male Wistar rats were submitted to unilateral ischemia by increasing intraocular pressure to 120 mmHg for 40 min. After ischemia, animals were housed in the following conditions: standard environment (SE), enriched environment (EE), novelty environment (NE), standard social environment (SoE), standard visual environment (SVE), or visual environment (VE). In another set of experiments, rats were submitted to bilateral ischemia and housed in SE or EE. At 2 weeks post-ischemia, rats were subjected to electroretinography and histological analysis. EE (but not SoE or NE) afforded functional and histological protection against unilateral ischemia. EE did not induce protection in animals submitted to bilateral ischemia. VE protected retinal function and histology and increased retinal BDNF levels, while a TrkB receptor antagonist prevented the protective effect of VE against I/R damage. In animals submitted to unilateral ischemia, EE and VE induced an increase in c-fos immunoreactivity in the ipsi and contralateral superior colliculus, whereas in animals submitted to bilateral ischemia, no

changes in c-fos-immunoreactivity were observed in either superior colliculus from EE-housed animals. These results support that visual stimulation could be a potent stimulus for driving retinal protection in adult rats through a BDNF/TrkB-dependent mechanism, likely involving the superior colliculus.
Mol Neurobiol, 2020; 57
31440144: Miranda M, Morici JF, Zanoni MB, Bekinschtein P
Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain.
Brain Derived Neurotrophic Factor (BDNF) is a key molecule involved in plastic changes related to learning and memory. The expression of BDNF is highly regulated, and can lead to great variability in BDNF levels in healthy subjects. Changes in BDNF expression are associated with both normal and pathological aging and also psychiatric disease, in particular in structures important for memory processes such as the hippocampus and parahippocampal areas. Some interventions like exercise or antidepressant administration enhance the expression of BDNF in normal and pathological conditions. In this review, we will describe studies from rodents and humans to bring together research on how BDNF expression is regulated, how this expression changes in the pathological brain and also exciting work on how interventions known to enhance this neurotrophin could have clinical relevance. We propose that, although BDNF may not be a valid biomarker for neurodegenerative/neuropsychiatric diseases because of its disregulation common to many pathological conditions, it could be thought of as a marker that specifically relates to the occurrence and/or progression of the mnemonic symptoms that are common to many pathological conditions.
Front Cell Neurosci, 2019; 13
30172952: Miranda M, Kent BA, Morici JF, Gallo F, Saksida LM, Bussey TJ, Weisstaub N, Bekinschtein P
NMDA receptors and BDNF are necessary for discrimination of overlapping spatial and non-spatial memories in perirhinal cortex and hippocampus.
Successful memory involves not only remembering information over time but also keeping memories distinct and less confusable. Discrimination of overlapping representations has been investigated in the dentate gyrus (DG) of the hippocampus and largely in the perirhinal cortex (Prh). In particular, the DG was shown to be important for discrimination of overlapping spatial memories and Prh was shown to be important for discrimination of overlapping object memories. In the present study, we used both a DG-dependent and a Prh-dependent task and manipulated the load of similarity between either spatial or object stimuli during information encoding. We showed that N-methyl-D-aspartate-type glutamate receptors (NMDAr) and BDNF participate of the same cellular network during consolidation of both overlapping object and spatial memories in the Prh and DG, respectively. This argues in favor of conserved cellular mechanisms across regions despite anatomical differences.
Neurobiol Learn Mem, 2018; 155
29717980: Morici JF, Miranda M, Gallo FT, Zanoni B, Bekinschtein P, Weisstaub NV
5-HT2a receptor in mPFC influences context-guided reconsolidation of object memory in perirhinal cortex.
Context-dependent memories may guide adaptive behavior relaying in previous experience while updating stored information through reconsolidation. Retrieval can be triggered by partial and shared cues. When the cue is presented, the most relevant memory should be updated. In a contextual version of the object recognition task, we examined the effect of medial PFC (mPFC) serotonin 2a receptor (5-HT2aR) blockade during retrieval in reconsolidation of competing objects memories. We found that mPFC 5-HT2aR controls retrieval and reconsolidation of object memories in the perirhinal cortex (PRH), but not in the dorsal hippocampus in rats. Also, reconsolidation of objects memories in PRH required a functional interaction between the ventral hippocampus and the mPFC. Our results indicate that in the presence of conflicting information at retrieval, mPFC 5-HT2aR may facilitate top-down context-guided control over PRH to control the behavioral response and object memory reconsolidation.
Elife, 2018; 7
29085903: Miranda M, Kent BA, Morici JF, Gallo F, Weisstaub NV, Saksida LM, Bussey TJ, Bekinschtein P
Molecular Mechanisms in Perirhinal Cortex Selectively Necessary for Discrimination of Overlapping Memories, but Independent of Memory Persistence.
Successful memory involves not only remembering over time but also keeping memories distinct. The ability to separate similar experiences into distinct memories is a main feature of episodic memory. Discrimination of overlapping representations has been investigated in the dentate gyrus of the hippocampus (DG), but little is known about this process in other regions such as the perirhinal cortex (Prh). We found in male rats that perirhinal brain-derived neurotrophic factor (BDNF) is required for separable storage of overlapping, but not distinct, object representations, which is identical to its role in the DG for spatial representations. Also, activity-regulated cytoskeletal-associated protein (Arc) is required for disambiguation of object memories, as measured by infusion of antisense oligonucleotides. This is the first time Arc has been implicated in the discrimination of objects with overlapping features. Although molecular mechanisms for object memory have been shown previously in Prh, these have been dependent on delay, suggesting a role specifically in memory duration. BDNF and Arc

involvement were independent of delay-the same demand for memory persistence was present in all conditions-but only when discrimination of similar objects was required were these mechanisms recruited and necessary. Finally, we show that BDNF and Arc participate in the same pathway during consolidation of overlapping object memories. We provide novel evidence regarding the proteins involved in disambiguation of object memories outside the DG and suggest that, despite the anatomical differences, similar mechanisms underlie this process in the DG and Prh that are engaged depending on the similarity of the stimuli.
eNeuro, 2017 Sep-Oct; 4
28603027: Miranda M, Bekinschtein P
Plasticity Mechanisms of Memory Consolidation and Reconsolidation in the Perirhinal Cortex.
In this review we explore the role of the perirhinal cortex (Prh) in memory, focusing on the cellular and molecular mechanisms that have been described to happen in this structure. The Prh is part of the medial temporal lobe, but the evidences show that it has a different function than that of the hippocampus. In particular, the Prh is known to be important for object recognition memory, although it could have a role in other types of memory. However, despite the fact that object recognition tasks are widely used, information regarding the molecular and cellular mechanisms underlying this type of memory in Prh is lacking. We discuss a series of studies of memory and plasticity in this region and how they might relate. In addition, we propose that Prh could play a role as a "pattern separator" for object memories, similar to the function of the dentate gyrus of the hippocampus in the spatial domain.
Neuroscience, 2018; 370

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BOARD NUMBER: S06-013

SYNCHRONIZATION OF A CEREBELLO-THALAMO-PREFRONTAL PATHWAY REGULATES FEAR EXTINCTION LEARNING

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Failure to suppress aversive memories is a landmark feature of several severe psychiatric conditions, and it is studied using fear extinction paradigms. While it is now well accepted that the cerebellum participates in emotion processing, whether and how the cerebellum affects extinction learning is poorly understood. Here, we establish a cerebello-thalamo-cortical pathway that connects the cerebellar fastigial nucleus (FN) to the lateral subregion of the thalamic mediodorsal nucleus (MD), which sends and receives inputs from the dorsomedial prefrontal cortex (dmPFC). Inhibition or activation of FN input to MD impairs extinction learning. Consistent with this, manipulations of the FN input to the MD increase the bursting of MD neurons, known to prevent extinction learning. Moreover, disrupting FN-MD during extinction of fear memory leads to an enhanced cortico-thalamic coherence of 4Hz oscillations associated with the fear response (freezing). Combined recordings across the FN-MD-dmPFC pathway further indicate that the cerebellum regulates the maintenance of these fear-related dmPFC 4Hz oscillations by providing a destructive interference in the MD. Overall, our results show that, under physiological conditions, the cerebellar activity regulates the fear-related thalamo-cortical patterns of activity and promotes extinction learning.

FENS Forum 2022
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BOARD NUMBER: S06-014

QUANTITATIVE MOLECULAR PROFILING OF BASOLATERAL AMYGDALA NEURONS WITH IDENTIFIED FEAR-RELATED PLASTICITY PHENOTYPES

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Using *in vivo* imaging methods, a previous study from our laboratory showed that during associative learning of tone-shock associations, apparently homogenous principal cells of the mouse basolateral amygdala (BLA) show opposing plasticity of sensory responses. Correlational and decoding analysis revealed that BLA neurons that potentiated or de-potentiated their neural responses for the auditory stimulus paired with a foot shock both contributed to fear memory formation. The molecular mechanisms behind the heterogeneity of the observed plasticity are not understood, therefore we developed a pipeline for quantitative immunostaining of candidate plasticity molecules on functionally characterized BLA cells. First, using sparse, adeno-associated virus-mediated expression of the Ca^{2+}-indicator GCaMP6s and two-photon imaging of neuronal Ca^{2+}-activity in multiple planes through a GRIN lens, we functionally characterized BLA principal neurons before and after auditory fear conditioning. This was followed by perfusion fixation, post-hoc identification of the imaged cells, postprocessing of the tissue and immunostaining. Our pipeline allows for reliably comparing the sensory-evoked Ca^{2+}-responses of individual BLA principal neurons before and after fear learning and for performing multiple rounds of quantitative immunostaining of membrane-associated proteins, postsynaptic receptors, or calcium-binding proteins on the same, functionally identified neurons. These experiments will further broaden our understanding of the molecular mechanisms underlying distinct forms of plasticity during associative learning and fear memory formation.

Pubmed:
31780530: Szőnyi A, Zichó K, Barth AM, Gönczi RT, Schlingloff D, Török B, Sipos E, Major A, Bardóczi Z, Sos KE, Gulyás AI, Varga V, Zelena D, Freund TF, Nyiri G
Median raphe controls acquisition of negative experience in the mouse.
Adverse events need to be quickly evaluated and memorized, yet how these processes are coordinated is poorly understood. We discovered a large population of excitatory neurons in mouse median raphe region (MRR) expressing vesicular glutamate transporter 2 (vGluT2) that received inputs from several negative experience-related brain centers, projected to the main aversion centers, and activated the septohippocampal system pivotal for learning of adverse events. These neurons were selectively activated by aversive but not rewarding stimuli. Their stimulation induced place aversion, aggression, depression-related anhedonia, and suppression of reward-seeking behavior and memory acquisition-promoting hippocampal theta oscillations. By contrast, their suppression impaired both contextual and cued fear memory formation. These results suggest that MRR vGluT2 neurons are crucial for the acquisition of negative experiences and may play a central role in depression-related mood disorders.
Science, 2019; 366
31604297: Szőnyi A
Conducting memory formation.
Science, 2019; 366
31123108: Szőnyi A, Sos KE, Nyilas R, Schlingloff D, Domonkos A, Takács VT, Pósfai B, Hegedüs P, Priestley JB, Gundlach AL, Gulyás AI, Varga V, Losonczy A, Freund TF, Nyiri G
Brainstem nucleus incertus controls contextual memory formation.
Hippocampal pyramidal cells encode memory engrams, which guide adaptive behavior. Selection of engram-forming cells is regulated by somatostatin-positive dendrite-targeting interneurons, which inhibit pyramidal cells that are not required for memory formation. Here, we found that γ-aminobutyric acid (GABA)-releasing neurons of the mouse nucleus incertus (NI) selectively inhibit somatostatin-positive interneurons in the hippocampus, both monosynaptically and indirectly through the inhibition of their subcortical excitatory inputs. We demonstrated that NI GABAergic neurons receive monosynaptic inputs from brain areas processing important environmental information, and their hippocampal projections are strongly activated by

salient environmental inputs in vivo. Optogenetic manipulations of NI GABAergic neurons can shift hippocampal network state and bidirectionally modify the strength of contextual fear memory formation. Our results indicate that brainstem NI GABAergic cells are essential for controlling contextual memories.
Science, 2019; 364
30030438: Takács VT, Cserép C, Schlingloff D, Pósfai B, Szőnyi A, Sos KE, Környei Z, Dénes Á, Gulyás AI, Freund TF, Nyiri G
Co-transmission of acetylcholine and GABA regulates hippocampal states.
The basal forebrain cholinergic system is widely assumed to control cortical functions via non-synaptic transmission of a single neurotransmitter. Yet, we find that mouse hippocampal cholinergic terminals invariably establish GABAergic synapses, and their cholinergic vesicles dock at those synapses only. We demonstrate that these synapses do not co-release but co-transmit GABA and acetylcholine via different vesicles, whose release is triggered by distinct calcium channels. This co-transmission evokes composite postsynaptic potentials, which are mutually cross-regulated by presynaptic autoreceptors. Although postsynaptic cholinergic receptor distribution cannot be investigated, their response latencies suggest a focal, intra- and/or peri-synaptic localisation, while GABA receptors are detected intra-synaptically. The GABAergic component alone effectively suppresses hippocampal sharp wave-ripples and epileptiform activity. Therefore, the differentially regulated GABAergic and cholinergic co-transmission suggests a hitherto unrecognised level of control over cortical states. This novel model of hippocampal cholinergic neurotransmission may lead to alternative pharmacotherapies after cholinergic deinnervation seen in neurodegenerative disorders.
Nat Commun, 2018; 9
27044051: Sos KE, Mayer MI, Cserép C, Takács FS, Szőnyi A, Freund TF, Nyiri G
Cellular architecture and transmitter phenotypes of neurons of the mouse median raphe region.
The median raphe region (MRR, which consist of MR and paramedian raphe regions) plays a crucial role in regulating cortical as well as subcortical network activity and behavior, while its malfunctioning may lead to disorders, such as schizophrenia, major depression, or anxiety. Mouse MRR neurons are classically identified on the basis of their serotonin (5-HT), vesicular glutamate transporter type 3 (VGLUT3), and gamma-aminobutyric acid (GABA) contents; however, the exact cellular composition of MRR regarding transmitter phenotypes is still unknown. Using an unbiased stereological method, we found that in the MR, 8.5 % of the neurons were 5-HT, 26 % were VGLUT3, and 12.8 % were 5-HT and VGLUT3 positive; whereas 37.2 % of the neurons were GABAergic, and 14.4 % were triple negative. In the whole MRR, 2.1 % of the neurons were 5-HT, 7 % were VGLUT3, and 3.6 % were 5-HT and VGLUT3 positive; whereas 61 % of the neurons were GABAergic. Surprisingly, 25.4 % of the neurons were triple negative and were only positive for the neuronal marker NeuN. PET-1/ePET-Cre transgenic mouse lines are widely used to specifically manipulate only 5-HT containing neurons. Interestingly, however, using the ePET-Cre transgenic mice, we found that far more VGLUT3 positive cells expressed ePET than 5-HT positive cells, and about 38 % of the ePET cells contained only VGLUT3, while more than 30 % of 5-HT cells were ePET negative. These data should facilitate the reinterpretation of PET-1/ePET related data in the literature and the identification of the functional role of a putatively new type of triple-negative neuron in the MRR.
Brain Struct Funct, 2017; 222
25381463: Szőnyi A, Mayer MI, Cserép C, Takács VT, Watanabe M, Freund TF, Nyiri G
The ascending median raphe projections are mainly glutamatergic in the mouse forebrain.
The median raphe region (MRR) is thought to be serotonergic and plays an important role in the regulation of many cognitive functions. In the hippocampus (HIPP), the MRR exerts a fast excitatory control, partially through glutamatergic transmission, on a subpopulation of GABAergic interneurons that are key regulators of local network activity. However, not all receptors of this connection in the HIPP and in synapses established by MRR in other brain areas are known. Using combined anterograde tracing and immunogold methods, we show that the GluN2A subunit of the NMDA receptor is present in the synapses established by MRR not only in the HIPP, but also in the medial septum (MS) and in the medial prefrontal cortex (mPFC) of the mouse. We estimated similar amounts of NMDA receptors in these synapses established by the MRR and in local adjacent excitatory synapses. Using retrograde tracing and confocal laser scanning microscopy, we found that the majority of the projecting cells of the mouse MRR contain the vesicular glutamate transporter type 3 (vGluT3). Furthermore, using double retrograde tracing, we found that single cells of the MRR can innervate the HIPP and mPFC or the MS and mPFC simultaneously, and these double-projecting cells are also predominantly vGluT3-positive. Our results indicate that the majority of the output of the MRR is glutamatergic and acts through NMDA receptor-containing synapses. This suggests that key forebrain areas receive precisely targeted excitatory input from the MRR, which is able to synchronously modify activity in those regions via individual MRR cells with dual projections.
Brain Struct Funct, 2016; 221
24407853: Takács VT, Szőnyi A, Freund TF, Nyiri G, Gulyás AI
Quantitative ultrastructural analysis of basket and axo-axonic cell terminals in the mouse hippocampus.

Three functionally different populations of perisomatic interneurons establish GABAergic synapses on hippocampal pyramidal cells: parvalbumin (PV)-containing basket cells, type 1 cannabinoid receptor (CB1)-positive basket cells both of which target somata, and PV-positive axo-axonic cells that innervate axon initial segments. Using electron microscopic reconstructions, we estimated that a pyramidal cell body receives synapses from about 60 and 140 synaptic terminals in the CA1 and CA3 area, respectively. About 60 % of these terminals were PV positive, whereas 35-40 % of them were CB1 positive. Only about 1 % (CA1) and 4 % (CA3) of the somatic boutons were negative for both markers. Using fluorescent labeling, we showed that most of the CB1-positive terminals expressed vesicular glutamate transporter 3. Reconstruction of somatic boutons revealed that although their volumes are similar, CB1-positive boutons are more flat and the total volume of their mitochondria was smaller than that of PV-positive boutons. Both types of boutons contain dense-core vesicles and frequently formed multiple release sites on their targets and innervated an additional soma or dendrite as well. PV-positive boutons possessed small, macular synapses; whereas the total synaptic area of CB1-positive boutons was larger and formed multiple irregular-shaped synapses. Axo-axonic boutons were smaller than somatic boutons, had only one synapse and their ultrastructural parameters were closer to those of PV-positive somatic boutons. Our results represent the first quantitative measurement-using a highly reliable method-of the contribution of different cell types to the perisomatic innervation of pyramidal neurons, and may help to explain functional differences in their output properties.
Brain Struct Funct, 2015; 220

22662211: Cserép C, Szabadits E, Szőnyi A, Watanabe M, Freund TF, Nyiri G
NMDA receptors in GABAergic synapses during postnatal development.
GABA (gamma-aminobutyric-acid), the main inhibitory neurotransmitter in the adult brain, exerts depolarizing (excitatory) actions during development and this GABAergic depolarization cooperates with NMDARs (N-methyl-D-aspartate receptors) to drive spontaneous synchronous activity (SSA) that is fundamentally important for developing neuronal networks. Although GABAergic depolarization is known to assist in the activation of NMDARs during development, the subcellular localization of NMDARs relative to GABAergic synapses is still unknown. Here, we investigated the subcellular distribution of NMDARs in association with GABAergic synapses at the developmental stage when SSA is most prominent in mice. Using multiple immunofluorescent labeling and confocal laser-scanning microscopy in the developing mouse hippocampus, we found that NMDARs were associated with both glutamatergic and GABAergic synapses at postnatal day 6-7 and we observed a direct colocalization of GABA(A)- and NMDA-receptor labeling in GABAergic synapses. Electron microscopy of pre-embedding immunogold-immunoperoxidase reactions confirmed that GluN1, GluN2A and GluN2B NMDAR subunits were all expressed in glutamatergic and GABAergic synapses postsynaptically. Finally, quantitative post-embedding immunogold labeling revealed that the density of NMDARs was 3 times higher in glutamatergic than in GABAergic synapses. Since GABAergic synapses were larger, there was little difference in the total number of NMDA receptors in the two types of synapses. In addition, receptor density in synapses was substantially higher than extrasynaptically. These data can provide the neuroanatomical basis of a new interpretation of previous physiological data regarding the GABA(A)R-NMDAR cooperation during early development. We suggest that during SSA, synaptic GABA(A)R-mediated depolarization assists NMDAR activation right inside GABAergic synapses and this effective spatial cooperation of receptors and local change of membrane potential will reach developing glutamatergic synapses with a higher probability and efficiency even further away on the dendrites. This additional level of cooperation that operates within the depolarizing GABAergic synapse, may also allow its own modification triggered by Ca(2+)-influx through the NMDA receptors.
PLoS One, 2012; 7

21282319: Cserép C, Szonyi A, Veres JM, Németh B, Szabadits E, de Vente J, Hájos N, Freund TF, Nyiri G
Nitric oxide signaling modulates synaptic transmission during early postnatal development.
Early γ-aminobutyric acid mediated (GABAergic) synaptic transmission and correlated neuronal activity are fundamental to network formation; however, their regulation during early postnatal development is poorly understood. Nitric oxide (NO) is an important retrograde messenger at glutamatergic synapses, and it was recently shown to play an important role also at GABAergic synapses in the adult brain. The subcellular localization and network effect of this signaling pathway during early development are so far unexplored, but its disruption at this early age is known to lead to profound morphological and functional alterations. Here, we provide functional evidence--using whole-cell recording--that NO signaling modulates not only glutamatergic but also GABAergic synaptic transmission in the mouse hippocampus during the early postnatal period. We identified the precise subcellular localization of key elements of the underlying molecular cascade using immunohistochemistry at the light--and electron microscopic levels. As predicted by these morpho-functional data, multineuron calcium imaging in acute slices revealed that this NO-signaling machinery is involved also in the control of synchronous network activity patterns. We suggest that the retrograde NO-signaling system is ideally suited to fulfill a general presynaptic regulatory role and may effectively fine-tune network activity during early postnatal development, while GABAergic transmission is still depolarizing.
Cereb Cortex, 2011; 21

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21194001: Zádori D, Nyiri G, Szonyi A, Szatmári I, Fülöp F, Toldi J, Freund TF, Vécsei L, Klivényi P
Neuroprotective effects of a novel kynurenic acid analogue in a transgenic mouse model of Huntington's disease.
Huntington's disease (HD) is a progressive neurodegenerative disorder, the pathomechanism of which is not yet fully understood. Excitotoxicity is known to be involved in the development of HD and antiglutamatergic agents may, therefore, have beneficial neuroprotective effects. One of these agents is the tryptophan metabolite kynurenic acid (KYNA), which is an endogenous NMDA receptor antagonist. However, its pharmacological properties rule out its systemic administration in CNS disorders. We have tested a novel KYNA analogue, N-(2-N,N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide hydrochloride, in the N171-82Q transgenic mouse model of HD. The analogue exhibited several significant effects: it prolonged the survival of the transgenic mice, ameliorated their hypolocomotion, prevented the loss of weight and completely prevented the atrophy of the striatal neurons. The beneficial effects of this KYNA analogue are probably explained by its complex anti-excitotoxic activity. As it did not induce any appreciable side-effect at the protective dose applied in a chronic dosing regime in this mouse model, it appears worthy of further thorough investigations with a view to eventual clinical trials.
J Neural Transm (Vienna), 2011; 118

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BOARD NUMBER: S06-015

TITLE: ROLE OF MICROGLIA IN RECONSOLIDATION-RESISTANT FEAR MEMORIES

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Background: Memory reconsolidation is a transient process which after retrieval stabilizes the labile state of consolidated memory. However, some experimental conditions (boundary conditions) can inhibit reconsolidation to occur that model persistent maladaptive memories observed in post-traumatic stress disorder (PTSD). Studies suggest that alterations in immune status in the central nervous system (CNS) may contribute to PTSD. Together with the emerging role of CNS immune cells, microglia, in regulating synaptic plasticity and memory, this study aims to investigate the role of microglia in the underlying mechanisms of reconsolidation-resistant memories. **Methods:** Mice were trained with auditory fear conditioning with one tone-shock pairing (1P) and ten tone-shock pairings (10P) to develop weak and strong (reconsolidation-resistant) memories respectively and treated for 7 days with PLX5622, a specific antagonist of colony-stimulating factor-1 receptor, for microglia depletion. Then mice were presented with one conditioned stimulus (CS) in a different context to reactivate the fear memory and 24-hours later, reconsolidated memory was tested by presenting one CS. **Results:** We found that 10P group created extinction-resistant memories compared to 1P group and PLX treatment did not alter the freezing behavior of 10P group. Interesting,1P group with PLX treatment showed higher freezing compared to the control and 10P group suggesting microglial association with increased fear memory at recall. **Conclusion:** Our preliminary results demonstrated role of microglia in regulating the maintenance of fear memories. An understanding of the processes determining memory persistence or inhibition could be relevant for the development of new therapeutic interventions for the post-traumatic stress disorder (PTSD).

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BOARD NUMBER: S06-016

A DELAY AND TRACE CONDITIONING PARADIGM FOR HEAD-FIXED LARVAL ZEBRAFISH

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Learning causal relationships is a major step to survive in a dynamic environment. Animals from flies to humans can learn that a conditioned stimulus (CS) predicts an unconditioned stimulus (US) either when the US overlaps with the CS or when a temporal gap (trace period) separates the two stimuli. These two forms of associative learning are known as delay and trace conditioning, respectively. Although in mammals both require brain regions including the cerebellum and amygdala, trace conditioning additionally involves the hippocampus to keep an internal representation of the CS in the trace period. Here we describe a delay and trace conditioning assay for head-fixed larval zebrafish, a small and transparent model organism amenable to whole-brain imaging with cellular resolution. In this conditioning assay, the fish are conditioned to associate a visual stimulus (CS) with an aversive optochemical stimulus (US). A reduction in tail movement during the CS indicated that larvae successfully learned to anticipate the US in both the delay and trace conditions. This reduction was not observed when the CS and US were presented in an unpaired protocol. This is in contrast to previously described delay conditioning tasks in zebrafish larvae using different US, where an increase in swimming was observed. We are now simultaneously recording behavior and neural activity in pan-neural calcium indicator lines, employing a custom light-sheet microscope that allows whole-brain imaging with cellular resolution, with the aim of comparing the neural mechanisms of delay and trace conditioning at the level of the entire brain.

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BOARD NUMBER: S06-017

EFFECTS OF A PSYCHEDELIC 5-HT2A RECEPTOR AGONIST ON ANXIETY-RELATED BEHAVIOR AND FEAR PROCESSING IN MICE

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

Blazej Pedzich[1], Sarah Rubens[1], Mehdi Sekssaoui[2], Anouk Pierre[1], Andries Van Schuerbeek[1], Philippe Marin[2], Joël Bockaert[2], Emmanuel Valjent[3], Carine Bécamel[2], Dimitri De Bundel[1]
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The serotonin 2A (5-HT2A) receptor is a key target underlying the effects of psychedelics on emotional arousal but its role in anxiety and fear processing remains controversial. Using the psychedelic 5-HT2A/5-HT2C receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) and 5-HT2A receptor knockout (KO) mice we investigated the effect of 5-HT2A receptor activation on emotional processing. We show that DOI administration increased exploratory behavior and reduced anxiety-like avoidance in mice through activation of 5-HT2A receptors. Moreover, we found that DOI did not block memory recall but diminished anxiety-like behavior and conditioned freezing in a passive avoidance task. Likewise, DOI administration reduced conditioned freezing in an auditory fear conditioning paradigm, while it did not affect fear extinction learning in wild type (WT) mice. The effect of DOI on conditioned freezing was abolished in 5-HT2A receptor KO mice. Administration of DOI induced a significant increase of c-Fos expression in specific amygdalar nuclei. Moreover, local infusion of the 5-HT2A receptor antagonist M100907 into the amygdala reversed the effect of systemic administration of DOI on conditioned freezing while local administration of DOI into the amygdala was sufficient to suppress conditioned freezing. Our data indicate that activation 5-HT2A receptors in the amygdala suppresses conditioned freezing behavior but we found no no evidence for an effect of DOI on fear extinction.

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BOARD NUMBER: S06-018

AVERSIVE CONTEXTS: HOW TO REMEMBER, DISCRIMINATE AND ADAPT

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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In mammals, survival critically depends on the ability to detect threatening situations and in selecting appropriate defensive behavior. This crucial mechanism is contributed by contextual fear discrimination that largely depends on continuous evaluation of surrounding cues defining specific contexts. Although it is known that contextual discrimination based on specific sensory cues induces efficient defensive strategies, the precise neurobiological substrates governing such mechanism are yet poorly understood. In the laboratory, we previously identified a neuronal projection from the dorsal medial prefrontal cortex (dmPFC) to the periaqueductal gray (PAG), that is selectively activated during contextual fear discrimination. We now aim at the identification of neuronal determinants allowing the building and consolidation of this dmPFC-PAG population, by characterizing how threat-predicting context is associated with specific dmPFC, amygdala (AMG) and hippocampus (HPC) networks to successfully drive adaptive responses. We first developed a novel behavioral paradigm that enables gradual transition of the context, which induced significant changes in the freezing behavior. We next used extracellular recording simultaneously targeting aforementioned brain regions (dmPFC, AMG and HPC) in freely moving and sleeping mice all along the contextual fear paradigm. This allowed us to characterize the physiological determinants of this behavioral adaptation at the cellular and population-level, with the identification of distinct neuronal representation and population coding in discriminative and generalizing mice, both during awake and sleeping periods. These results reveal that, in mice, the cognitive control of emotion related to a context relies on a dynamic encoding of information within the tripartite networks.

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BOARD NUMBER: S06-019

BASOLATERAL AMYGDALA PLASTICITY DURING AUDITORY SECOND-ORDER ASSOCIATIVE LEARNING

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Pavlovian first-order conditioning is an associative learning paradigm characterized by the acquisition of a conditioned response to an initially neutral stimulus which becomes a conditioned stimulus (CS1) by virtue of its association with an unconditioned stimulus (US). Recent data showing that conditioning drives the neuronal population representation of the CS1 in the basal and lateral amygdala (BLA) to align with the US following conditioning supports learning models in which the US representation guides the transformation of the CS1 representation. Although most learning of organisms is arguably higher-order learning, it is currently unknown whether the neuronal mechanism that governs higher-order learning is similar to that of first-order learning (i.e., does the CS1 representation guide the transformation of the CS2-representation during higher-order learning?). We addressed this question by combining second-order conditioning, a form of associative learning wherein the previously conditioned stimulus (CS1) can associate with a second naïve conditioned stimulus (CS2) to elicit a conditioned response, with calcium imaging of principle BLA neurons in freely behaving mice. Longitudinal imaging along the entire paradigm uncovered similarities and differences in the transformation of the CS2 representation during the different stages of the second-order conditioning paradigm (from conditioning through extinction and extinction-retrieval sessions). Optogenetic manipulation confirmed that these CS1-CS2 mediated transformations in the BLA are necessary for second-order learning. These data may provide novel insight into the mechanisms underlying anxiety disorders in humans, which has been proposed to be maintained by higher-order conditioning mechanisms.

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BOARD NUMBER: S06-020

BRAIN-WIDE EPIGENETICS MAPPING OF FEAR MEMORY ENGRAM CELLS

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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A memory engram is thought to be the physical substrate of the memory trace within the brain, which is generally depicted as a neuronal ensemble activated by learning to fire together during encoding and retrieval. Nowadays, emerging evidence supports the postulation that memory engram exhibits a multiscale organization both at a brain network level and at an epigenetic state. However, as engram cells have thus far been visualized in a limited number of brain regions, it remains unclear if engram cell ensembles are broadly distributed across the entire brain to construct the functional connectivity of a memory. Furthermore, as epigenetic mechanisms underlying memory formation and storage have mainly been studied at heterogeneous whole tissue level, engram cell-specific epigenetic modifications have only started to be elucidated. To fill these loopholes, our project aims to generate a composite image of whole brain fear memory engram epigenetics (aka epi-engram) during memory consolidation. By applying iDISCO+ optical clearing, light sheet microscopy and semi-automated 3D colocalization analysis, we overcome the technical bottleneck of whole mount multiple immunostaining to visualize activity-tagged neurons in the TRAP2-tdTomato mouse line, concomitantly with the neuronal activation marker *cfos* and histone modifications. Our data reveal that contextual fear memory formation triggers an increase in recruitment of *cfos*+ *H3S10pK9me3*+ epi-engram cells, which indicates a change from close to open chromatin state for transcriptional activation, in basolateral amygdala, lateral septum, medial septum and anterior cingulate cortex. Ultimately, we hope that such brain-wide epi-engram mapping will illustrate how memory consoliation is orchestrated by chromatin states within engram cells.

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BOARD NUMBER: S06-021

EXPLORING THE ROLE OF CALCINEURIN IN THE EXTINCTION OF AVERSION

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Nowadays, it is widely accepted that memory extinction involves the formation of a new associative rather than unlearning of conditioning. Nevertheless, the cellular and molecular mechanisms that underlie this process are still unclear. In this regard, it has been suggested that kinases and phosphatases modulate the processes of conditioning and extinction, respectively. A body of evidence suggests that protein phosphatase calcineurin (CaN) is involved in the extinction of some behavioral tasks. Indeed, our previous studies showed that conditioned taste aversion (CTA) extinction increases the CaN expression in the insular cortex (IC). CTA is a well-established learning and memory paradigm in which an animal associates a novel taste with nausea. Meanwhile, the IC is a region of the brain that lies in the temporal neocortex and is known for its role in processing aversively motivated learning tasks, like CTA. The present study aimed to evaluate the participation of CaN in the extinction of CTA. To do so, we pharmacologically inhibited calcineurin in the IC of adult male Wistar rats during CTA-extinction. Our results show that CaN inhibition elicits an impairment of CTA extinction, thus revealing that this phosphatase plays an important role in extinction learning. These findings led us to explore the synaptic plasticity associated with the effects of CaN on CTA extinction. In this sense, our preliminary results show that CaN plays an important role in the maintenance of long-term depression in the IC, suggesting that both processes are associated at a molecular level.

Pubmed:
34220454: Reyes-García SE, Escobar ML
Calcineurin Participation in Hebbian and Homeostatic Plasticity Associated With Extinction.
In nature, animals need to adapt to constant changes in their environment. Learning and memory are cognitive capabilities that allow this to happen. Extinction, the reduction of a certain behavior or learning previously established, refers to a very particular and interesting type of learning that has been the basis of a series of therapies to diminish non-adaptive behaviors. In recent years, the exploration of the cellular and molecular mechanisms underlying this type of learning has received increasing attention. Hebbian plasticity (the activity-dependent modification of the strength or efficacy of synaptic transmission), and homeostatic plasticity (the homeostatic regulation of plasticity) constitute processes intimately associated with memory formation and maintenance. Particularly, long-term depression (LTD) has been proposed as the underlying mechanism of extinction, while the protein phosphatase calcineurin (CaN) has been widely related to both the extinction process and LTD. In this review, we focus on the available evidence that sustains CaN modulation of LTD and its association with extinction. Beyond the classic view, we also examine the interconnection among extinction, Hebbian and homeostatic plasticity, as well as emergent evidence of the participation of kinases and long-term potentiation (LTP) on extinction learning, highlighting the importance of the balance between kinases and phosphatases in the expression of extinction. Finally, we also integrate data that shows the association between extinction and less-studied phenomena, such as synaptic silencing and engram formation that open new perspectives in the field.
Front Cell Neurosci, 2021; 15

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BOARD NUMBER: S06-022

MULTISENSORY INTEGRATION AND TONE PLASTICITY IN THE POSTERIOR INSULAR CORTEX DURING AUDITORY FEAR LEARNING

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Aversively-motivated associative learning allows the organism to avoid or reduce harm and thus ensure its survival. Canonically, associative plasticity in the amygdala is thought to underly the process of fear learning (LeDoux, 2000). Interestingly, brain areas upstream to the amygdala like the posterior insular cortex (pInsCx), are known to process auditory, somatosensory and other sensory modalities. Here we used in-vivo optrode recordings in awake behaving mice to study the activity of pInsCx neurons during and following auditory cued fear learning. Significant subpopulations of neurons responded to the footshock and acquired a response to the CS following fear conditioning ("tone learners"). Furthermore, a population that partially overlapped with the tone learners showed a response to the onset of movements following freezing. We next investigated the origin and putative plasticity of the auditory input(s) to the pInsCx. Retrograde tracer experiments showed input neurons to the pInsCx both in the thalamic MGm and in the primary auditory cortex (A1), amongst other areas. Expression of Chronos in either of these presynaptic areas led to robust optogenetically- evoked EPSCs (oEPSCs) in the pInsCx. Interestingly, oEPSCs originating from the A1 showed robust signs of postsynaptic plasticity after fear learning, whereas the ones from the MGm did not. Thus, pInsCx integrates auditory- , footshock- and movement information, and shows plasticity of auditory responses during aversive learning; the latter might be caused by plasticity at a novel A1 to pInsCx connection. These findings suggest an important role for the multisensory pInsCx in fear learning.

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BOARD NUMBER: S06-023

WHOLE-BRAIN NEURONAL ACTIVITY MAPPING DELINEATES ANATOMICAL REGIONS IMPLICATED IN THE TRAUMA VULNERABILITY

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Most individuals experience traumatic events throughout their lifetime, however, only a subpopulation (10-20%) develop a persistent set of biological symptoms referred to as post-traumatic stress disorder (PTSD). Key features of PTSD are the impaired fear extinction and generalisation of fear to safe contexts. Extinction is orchestrated by coordinated operations of several brain regions. Major candidates (i.e. prefrontal cortex, amygdala) have been implicated, however, unbiased large-scale assessments are needed to discover complex networks regulating fear extinction. Pre-trauma differences in behaviour may be a predisposing factor for developing PTSD. To differentiate individuals that are prone or resistant to PTSD, we performed a wide behavioural test battery on adult male Long-Evans rats before a footshock-induced traumatic experience. We discerned subjects based on their freezing response in a safe/altered context four weeks later (i.e. their fear generalisation and extinction). Upper and lower quartiles were identified based on safe context performances as vulnerable and resilient groups and their whole-brain activity patterns were compared and contrasted using complex statistical methods. We compared the two groups based on immunohistochemical labelling with the neuronal activity marker c-Fos. Whole-brain mapping using delineations from the Waxholm Space Atlas identified numerous cortical and subcortical regions distinguishing the investigated groups. The presented model may help understand complex network alterations underlying PTSD and imply new therapeutic targets in the future.

Pubmed:
25485758: Dudok B, Barna L, Ledri M, Szabó SI, Szabadits E, Pintér B, Woodhams SG, Henstridge CM, Balla GY, Nyilas R, Varga C, Lee SH, Matolcsi M, Cervenak J, Kacskovics I, Watanabe M, Sagheddu C, Melis M, Pistis M, Soltesz I, Katona I
Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling.
A major challenge in neuroscience is to determine the nanoscale position and quantity of signaling molecules in a cell type- and subcellular compartment-specific manner. We developed a new approach to this problem by combining cell-specific physiological and anatomical characterization with super-resolution imaging and studied the molecular and structural parameters shaping the physiological properties of synaptic endocannabinoid signaling in the mouse hippocampus. We found that axon terminals of perisomatically projecting GABAergic interneurons possessed increased CB1 receptor number, active-zone complexity and receptor/effector ratio compared with dendritically projecting interneurons, consistent with higher efficiency of cannabinoid signaling at somatic versus dendritic synapses. Furthermore, chronic Δ(9)-tetrahydrocannabinol administration, which reduces cannabinoid efficacy on GABA release, evoked marked CB1 downregulation in a dose-dependent manner. Full receptor recovery required several weeks after the cessation of Δ(9)-tetrahydrocannabinol treatment. These findings indicate that cell type-specific nanoscale analysis of endogenous protein distribution is possible in brain circuits and identify previously unknown molecular properties controlling endocannabinoid signaling and cannabis-induced cognitive dysfunction.
Nat Neurosci, 2015; 18

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-024

SECOND-ORDER FEAR CONDITIONING ENGAGES EPIGENETIC MECHANISMS IN THE AMYGDALA AND PRIMARY SENSORY CORTICES

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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AIMS: A characteristic of post-traumatic stress disorder (PTSD) is fear generalization beyond the initial association between a conditioned stimulus (CS) and an aversive stimulus, which we model using a 2nd order fear conditioning (SOC) paradigm. SOC forms when a 2nd CS (CS2) becomes aversive by association with a CS1 that has been previously associated with an aversive stimulus (1st order conditioning). Here we sought to determine the brain areas and mechanisms underlying SOC. METHODS: Rats underwent SOC using a tone as CS1 and an odor as CS2. We then conducted mapping of cFos and epigenetic marks of histone acetylation (H3Ac, H4ac) and DNA methylation (5MC) expression in multiple brains regions upon encoding and retrieval of SOC using immunohistochemistry. A CS1/CS2 unpaired group was used as a control. RESULTS: Following SOC encoding, cFos expression was elevated in the lateral amygdala (LA). However, upon retrieval of SOC, cFos was elevated in multiple structures including LA, basolateral amygdala (BLA), dorsal and ventral CA1 and CA3, as well as the olfactory piriform cortex (PC) and primary auditory cortex (AuD1). Upon retrieval, higher expressions of 5MC, H3Ac and H4Ac marks were observed in the BLA and AuD1. Moreover, higher expression of H3Ac and H4Ac in the LA and aPC was observed. CONCLUSION: Our preliminary data highlights the role of sensory cortices in SOC memory formation. We suggest a role for epigenetics in the storage of these memories. Epigenetic-mediated plasticity in SOC may have important implications for fear-related disorders such as PTSD.

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BOARD NUMBER: S06-025

NEURAL SIGNATURE OF FEAR RECALL ASSOCIATED WITH STRESS SUSCEPTIBILITY

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Exposure to a severely stressful event can haunt one for life. Flashbacks, spontaneous recollections, and recurrent nightmares of the trauma are amongst the most devastating symptoms of post-traumatic stress disorder (PTSD). Aberrant trauma memory processing seems to lie at the root of these symptoms and is targeted in current PTSD treatment, but the success rate is low as insight into the nature of the memory deviations is lacking. Here we investigated neural activity patterns supporting fear memory recall in a well-established mouse model for PTSD. The model is based on the phenomenon of stress-enhanced fear learning, which builds on the clinical observation that prior stress exposure precipitates PTSD by altering future fear learning. Hence, mice were first exposed to a stressor (intense, unpredictable foot shocks) followed by mild contextual fear conditioning the next day. Since most individuals recover adequately from trauma exposure and stay healthy, we specifically investigated the differences between resilient mice and those susceptible to the long-term behavioral consequences of trauma exposure, which were assessed starting from 1-week post-stress. Recall-induced brain activity was analyzed in response to re-exposure to a) the initial stress context, b) the subsequent conditioned context and c) a similar, yet novel context and compared between resilient and susceptible mice. Since memories are distributed across the entire brain, we assessed whole-brain responses by iDISCO+ (immunolabeling and brain clearing) and subsequent light-sheet microscopy. Moreover, we approximated functional connectivity across memory-related brain regions by calculating within-group inter-subject correlations in activity patterns. Results will be discussed.

FENS Forum 2022
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BOARD NUMBER: S06-026

NEUROTROPHIN-3/TRKC CONTRIBUTION TO FEAR EXTINCTION AND REGULATION OF GLUTAMATERGIC SYNAPSES

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Excessive fear that is resistant to extinction is a prominent feature to many anxiety disorders. Fear extinction, mediated by a brain network comprising the amygdala, hippocampus and medial prefrontal cortex, is a learning process that relies on synaptic plasticity events. The regulation of NMDAR and AMPAR in glutamatergic synapses contributes to this. Neurotrophins regulate synaptic plasticity and have been associated with anxiety disorders and the formation of fear memories. Here, we investigate the role of neurotrophin-3 (NT3)/TrkC receptor signaling in fear extinction and dissect their involvement in the regulation of synaptic NMDAR and AMPAR. We trained C57Bl/6J mice in a contextual fear conditioning and extinction paradigm and studied TrkC activation in the brain fear network, by Western Blot. Moreover, we stimulated cultured hippocampal neurons with NT3 and performed live staining of surface NMDAR and AMPAR subunits, confirming synaptic localization by co-staining with pre- and post-synaptic markers. In our behavioral model, a subset of animals extinguishes fear (>30% reduction in freezing levels) while the remaining fail to do so. Successful extinction was correlated positively with increased TrkC activation in the amygdala at extinction consolidation, and inversely with TrkC activation in the hippocampus at extinction reconsolidation. *In vitro,* NT3 administration increased the synaptic expression of GluN2A, GluN2B and GluA1 but decreased GluA2. Our results suggest that modulation of NT3/TrkC signaling in the brain fear network may contribute to extinction performance. Moreover, NT3 regulates the synaptic expression of glutamate receptors, posing as a putative molecular mechanism underlying NT3/TrkC role in fear extinction.

FENS Forum 2022
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BOARD NUMBER: S06-027

SEX DIFFERENCES IN NEURAL REPRESENTATION OF THREAT IN VENTRAL HIPPOCAMPAL AND PREFRONTAL CORTICAL PROJECTIONS TO NUCLEUS ACCUMBENS

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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BACKGROUND: Although fear is essential for survival, excessive fear characterizes many neuropsychiatric disorders. The nucleus accumbens (NAc) plays a role in encoding and responding to aversive stimuli by integrating input from brain regions including the ventral hippocampus (vHIP) and prefrontal cortex (PFC). Here we investigate how neural activity in these projections is shaped by aversive experiences. METHODS: Using frame-projected independent fiber photometry (FIP) to image *in vivo* calcium activity in male and female mice, we recorded PFC and vHIP NAc-projecting neurons during a Pavlovian fear conditioning paradigm in which mice encounter both threat cues (CS+) predicting shock and neutral cues (CS-) with no outcome. RESULTS: Neural activity in both the vHIP-NAc and PFC-NAc encode aversive experiences. Both pathways show elevated activity to foot-shock with an augmented PFC-NAc response in females compared to males. vHIP-NAc neural activity is significantly elevated at CS+ cue onset, compared to CS-, in males but not females, while the PFC-NAc showed elevated activity at CS+ cue onset in female alone. Furthermore, both pathways exhibited suppression prior to shock. Despite differentiating between cues, this activity did not predict freezing behavior. Consistent with this, pathway-specific chemogenetic inhibition did not impair cue-induced freezing but did lead to attenuation of reward seeking during aversive cues in a conditioned suppression task. CONCLUSIONS: Our findings suggest that both the vHIP and PFC convey information about threat prediction to the NAc medial shell with pathway-specific sex differences that may relate to sex differences in threat processing and stress vulnerability.

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BOARD NUMBER: S06-028

POPULATION LEVEL ENCODING OF THREAT MEMORY IN TEMPORAL NEOCORTEX

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Memory for cues associated with threat is critical for survival, and a leading model for elucidating how sensory information is linked to adaptive behavior by learning. While classical work focused on the contribution of limbic structures, we recently identified the temporal neocortex as an essential site for threat memory to complex, naturalistic auditory stimuli. However, how discriminative threat memory is encoded in this area at the single neuron and population level remains elusive. Here we perform deep brain miniscope calcium imaging in freely moving mice during acquisition and extinction of threat memory to reveal the response dynamics of large populations of neurons in both ventral auditory cortex (AuV) and temporal association cortex (TeA). Our data indicate pronounced dynamics of conditioned stimulus (CS) representation at the single neuron level but stable population responses in both areas before learning. During threat conditioning, mice that form a discriminative memory selectively develop a form of population plasticity that is not observed in generalization and tracks the temporal profile of discrimination learning. Moreover, threat learning increases the signal correlation of CS responses, which remains stable during subsequent extinction learning. At the population level, discriminator and generalizer mice show selective enhancement and reduction of stimulus discrimination during acquisition and extinction, and distinct responses during extinction recall. Moreover, our results reveal that threat learning switches CS encoding from bottom-up sensory responses to a top-down dominated memory representation. These data indicate that temporal neocortex dynamically encodes threat and extinction memory at the level of individual neurons and populations.

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BOARD NUMBER: S06-029

ENCODING OF SPATIAL LONG TERM MEMORIES IN A NEURAL NETWORK: UNDERSTANDING FEAR GENERALIZATION WITH MINISCOPE CALCIUM IMAGING

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Fear memories are highly resistant to forgetting, but environmental details are often lost during consolidation, through a process called fear generalization. I aim to understand the role that the anterodorsal thalamic nucleus (ADn) and the laterodorsal thalamic nucleus (LDn) play during memory consolidation, and how it relates to reduced spatial specificity. Both regions contain a high number of head direction cells (HDc), and they are differently engaged in the recall of recently (1day after) versus remotely (4weeks after) acquired memories. We visualized the real time activity of ADn and LDn neurons at different time points by monitoring calcium activity via a miniaturized microscope implanted in mice brains. Doing so, we identified different population of cells responding to head direction (HDc), as well as shock-responsive cells. We were able to study the overlaps between those populations of cells, as well as their changes in activity over time, from fear conditioning (CFC) to recent and remote recalls. We also identified changes in neuronal activity when mice are in context A (associated to the CFC) and context B (similar, but different to context A) to study ADn and LDn role in memory generalization for recent and remote memory traces. This study aims to directly observe single-cell and population-level mechanics that may explain fear generalization of long-lasting memories.

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BOARD NUMBER: S06-030

DYSREGULATED MIDBRAIN DOPAMINE PREDICTION ERROR SIGNALING MAY UNDERLIE IMPAIRED FEAR EXTINCTION

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Aims: The core mechanism underlying cognitive behavioral therapy, fear extinction, is deficient in many patients suffering from anxiety- and trauma related disorders, resulting in treatment resistance or relapse. This endophenotype is well-modeled in the extinction-impaired 129S1/SvImJ (S1) mouse strain and our groups previously reported that dopamine (DA) precursor L-DOPA facilitated fear extinction in these mice, although the exact mechanism remained unclear. Interestingly, recent studies demonstrate that upon omission of the aversive, unconditioned stimulus during extinction training, DA neurons in the ventral tegmental area (VTA) signal a reward-like prediction error (PE), which is necessary to initiate fear extinction before it dissipates. We hypothesized that PE signaling in S1 mice may be insufficient, while its enhancement would rescue their extinction-deficient phenotype. **Methods & Results:** As opposed to published studies in extinction-competent C57BL/6 mice, optogenetic activation of VTA DA neurons did not facilitate fear extinction in S1 animals. We analyzed VTA DA neuron activity using GCaMP fiber photometry and found that PE signals in S1 mice increased throughout extinction training and retrieval but were reduced in C57BL/6 mice, which was correlated with extinction performance. **Conclusions:** We propose that in S1 mice, VTA PE signals are not properly conveyed to relevant target structures, resulting in deficient extinction acquisition and, due to missing negative feedback, no attenuation of VTA activity. Consequently, dysregulations downstream of the VTA (e.g. insufficient DA release) will have to be investigated as possible neurobiological correlates and novel targets in impaired fear extinction. Supported by FWF (I2433-B26).

Pubmed:
33903668: Fritz EM, Kreuzer M, Altunkaya A, Singewald N, Fenzl T
Altered sleep behavior in a genetic mouse model of impaired fear extinction.
Sleep disturbances are a common complaint of anxiety patients and constitute a hallmark feature of post-traumatic stress disorder (PTSD). Emerging evidence suggests that poor sleep is not only a secondary symptom of anxiety- and trauma-related disorders but represents a risk factor in their development, for example by interfering with emotional memory processing. Fear extinction is a critical mechanism for the attenuation of fearful and traumatic memories and multiple studies suggest that healthy sleep is crucial for the formation of extinction memories. However, fear extinction is often impaired in anxiety- and trauma-related disorders-an endophenotype that is perfectly modelled in the 129S1/SvImJ inbred mouse strain. To investigate whether these mice exhibit altered sleep at baseline that could predispose them towards maladaptive fear processing, we compared their circadian sleep/wake patterns to those of typically extinction-competent C57BL/6 mice. We found significant differences regarding diurnal distribution of sleep and wakefulness, but also sleep architecture, spectral features and sleep spindle events. With regard to sleep disturbances reported by anxiety- and PTSD patients, our findings strengthen the 129S1/SvImJ mouse models' face validity and highlight it as a platform to investigate novel, sleep-focused diagnostic and therapeutic strategies. Whether the identified alterations causally contribute to its pathological anxiety/PTSD-like phenotype will, however, have to be addressed in future studies.
Sci Rep, 2021; 11
33380256: Hofer NT, Pinggera A, Nikonishyna YV, Tuluc P, Fritz EM, Obermair GJ, Striessnig J
Stabilization of negative activation voltages of Cav1.3 L-Type Ca-channels by alternative splicing.
-->Low voltage-activated Cav1.3 L-type Ca-channels are key regulators of neuronal excitability controlling neuronal development and different types of learning and memory. Their physiological functions are enabled by their negative activation voltage-range, which allows Cav1.3 to be active at subthreshold voltages. Alternative splicing in the C-terminus of their pore-forming α1-subunits gives rise to C-terminal long (Cav1.3) and short (Cav1.3) splice variants allowing Cav1.3 to activate at even more negative voltages than Cav1.3. We discovered that inclusion of exons 8b, 11, and 32 in Cav1.3 further shifts activation (-3 to -4 mV) and inactivation (-4 to -6 mV) to more negative voltages as revealed by functional

characterization in tsA-201 cells. We found transcripts of these exons in mouse chromaffin cells, the cochlea, and the brain. Our data further suggest that Cav1.3-containing exons 11 and 32 constitute a significant part of native channels in the brain. We therefore investigated the effect of these splice variants on human disease variants. Splicing did not prevent the gating defects of the previously reported human pathogenic variant S652L, which further shifted the voltage-dependence of activation of exon 11-containing channels by more than -12 mV. In contrast, we found no evidence for gating changes of the missense variant R498L, located in exon 11, which has recently been identified in a patient with an epileptic syndrome. Our data demonstrate that alternative splicing outside the C-terminus involving exons 11 and 32 contributes to channel fine-tuning by stabilizing negative activation and inactivation gating properties of wild-type and mutant Cav1.3 channels.
Channels (Austin), 2021; 15
33192444: Fritz EM, Singewald N, De Bundel D
The Good, the Bad and the Unknown Aspects of Ghrelin in Stress Coping and Stress-Related Psychiatric Disorders.
Ghrelin is a peptide hormone released by specialized X/A cells in the stomach and activated by acylation. Following its secretion, it binds to ghrelin receptors in the periphery to regulate energy balance, but it also acts on the central nervous system where it induces a potent orexigenic effect. Several types of stressors have been shown to stimulate ghrelin release in rodents, including nutritional stressors like food deprivation, but also physical and psychological stressors such as foot shocks, social defeat, forced immobilization or chronic unpredictable mild stress. The mechanism through which these stressors drive ghrelin release from the stomach lining remains unknown and, to date, the resulting consequences of ghrelin release for stress coping remain poorly understood. Indeed, ghrelin has been proposed to act as a stress hormone that reduces fear, anxiety- and depression-like behaviors in rodents but some studies suggest that ghrelin may - in contrast - promote such behaviors. In this review, we aim to provide a comprehensive overview of the literature on the role of the ghrelin system in stress coping. We discuss whether ghrelin release is more than a byproduct of disrupted energy homeostasis following stress exposure. Furthermore, we explore the notion that ghrelin receptor signaling in the brain may have effects independent of circulating ghrelin and in what way this might influence stress coping in rodents. Finally, we examine how the ghrelin system could be utilized as a therapeutic avenue in stress-related psychiatric disorders (with a focus on anxiety- and trauma-related disorders), for example to develop novel biomarkers for a better diagnosis or new interventions to tackle relapse or treatment resistance in patients.
Front Synaptic Neurosci, 2020; 12
31542636: Pierre A, Regin Y, Van Schuerbeek A, Fritz EM, Muylle K, Beckers T, Smolders IJ, Singewald N, De Bundel D
Effects of disrupted ghrelin receptor function on fear processing, anxiety and saccharin preference in mice.
Obesity is a risk factor for stress-related mental disorders such as post-traumatic stress disorder. The underlying mechanism through which obesity affects mental health remains poorly understood but dysregulation of the ghrelin system may be involved. Stress increases plasma ghrelin levels, which stimulates food intake as a potential stress-coping mechanism. However, diet-induced obesity induces ghrelin resistance which in turn may have deleterious effects on stress-coping. In our study, we explored whether disruption of ghrelin receptor function though high-fat diet or genetic ablation affects fear processing, anxiety-like behavior and saccharin preference in mice.
Psychoneuroendocrinology, 2019; 110
28119583: Härtner L, Keil TW, Kreuzer M, Fritz EM, Wenning GK, Stefanova N, Fenzl T
Distinct Parameters in the EEG of the PLP α-SYN Mouse Model for Multiple System Atrophy Reinforce Face Validity.
Multiple system atrophy (MSA) is a neurodegenerative movement disorder characterized by parkinsonian symptoms and cerebellar symptoms. Sleep disturbances also play a crucial role in MSA. One of the most convincing animal models in MSA research is the PLP α-SYN model, but to date no studies on sleep disturbances in this mouse model, frequently found in MSA patients are available. We identified spectral shifts within the EEG of the model, strikingly resembling results of clinical studies. We also characterized muscle activity during REM sleep, which is one of the key symptoms in REM sleep behavioral disorder. Spectral shifts and REM sleep-linked muscle activity were age dependent, supporting Face Validity of the PLP α-SYN model. We also strongly suggest our findings to be critically evaluated for Predictive Validity in future studies. Currently, research on MSA lacks potential compounds attenuating or curing MSA. Future drugs must prove its potential in animal models, for this our study provides potential biomarkers.
Front Behav Neurosci, 2016; 10

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-031

SIMULTANEOUS ENCODING OF FEAR STATE AND THREAT IDENTITY IN PREFRONTAL CORTEX NEURONAL POPULATIONS.

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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In response to specific threats, mammals select a response among a repertoire of different defensive behaviors. The selection and the rapid execution of this response are crucial for animal survival and are determined not only by the nature of the threat but also by the contextual contingencies. Therefore, in order to survive a dangerous situation mammals have to integrate multimodal information regarding the threat, the context and its internal state to rapidly elicit the most adaptive fear response. The neuronal circuits and mechanisms allowing this rapid selection of appropriate defensive fear responses are still largely unknown. To address this question, we used a multi-level approach combining simultaneous electrophysiological recordings and optogenetic manipulations in a novel and unique behavioral paradigm allowing mice to select different defensive behaviors when facing different threats. Using this combination of techniques, we monitored the neuronal activity of dmPFC neurons of mice presented with different threats to demonstrate that at the population level, dmPFC neuronal activity encodes both a general fear state and more specific information about the identity of the threats.

FENS Forum 2022
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BOARD NUMBER: S06-032

CRISPR-BASED EPIGENETIC EDITING OF ENGRAM CELLS IN FEAR MEMORIES

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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The experience of traumatic, life-threatening events gives rise to some of the most enduring forms of fear memories, which can degenerate into a devastating pathological state known as post-traumatic stress disorder (PTSD). Nevertheless, surprisingly little is known about how long-lasting memories are formed and stored. By being at the same time dynamic and stable, epigenetic mechanisms have long been proposed as the molecular substrates for memory, but a cell type, locus-restricted and temporally controlled demonstration thereof has thus far been lacking. Here, we combine *in vivo*epigenetic editing approaches with c-Fos driven engram tagging technologies. First, we show that in transgenic c-Fos::tTA mice, overexpressing the histone acetyl transferase CREB binding protein (CBP) in the dentate gyrus of engram cells increases whole-genome H3K27ac levels and the ability to recall recent fear memories, while overexpressing of HDAC8 has the opposite effects. Then, we go on to show that epigenetic editing of a single site on the genome – using CRISPR-dCas9 coupled to transcriptional activators or repressors – might be sufficient to alter memory performances. Engram-specific, dCas9-VPR mediated upregulation of *Arc*, a master regulator of synaptic plasticity,improves recent fear memory, whilst expression of dCas9-KRAB-MeCP2 in the same cells decreases *Arc* mRNA levelsandimpairs fear memory recall. Overall, these findings show how the modulation of epigenetic marks in a cell type and locus-specific manner can impact neuronal function and memory capacity; at the same time, they deepen our understanding of the molecular mechanisms of traumatic memory storage.

Pubmed:
33343294: Coda DM, Gräff J
Neurogenetic and Neuroepigenetic Mechanisms in Cognitive Health and Disease.
Over the last two decades, the explosion of experimental, computational, and high-throughput technologies has led to critical insights into how the brain functions in health and disease. It has become increasingly clear that the vast majority of brain activities result from the complex entanglement of genetic factors, epigenetic changes, and environmental stimuli, which, when altered, can lead to neurodegenerative and neuropsychiatric disorders. Nevertheless, a complete understanding of the molecular mechanisms underlying neuronal activities and higher-order cognitive processes continues to elude neuroscientists. Here, we provide a concise overview of how the interaction between the environment and genetic as well as epigenetic mechanisms shapes complex neuronal processes such as learning, memory, and synaptic plasticity. We then consider how this interaction contributes to the development of neurodegenerative and psychiatric disorders, and how it can be modeled to predict phenotypic variability and disease risk. Finally, we outline new frontiers in neurogenetic and neuroepigenetic research and highlight the challenges these fields will face in their quest to decipher the molecular mechanisms governing brain functioning.
Front Mol Neurosci, 2020; 13
28191871: Coda DM, Gaarenstroom T, East P, Patel H, Miller DS, Lobley A, Matthews N, Stewart A, Hill CS
Distinct modes of SMAD2 chromatin binding and remodeling shape the transcriptional response to NODAL/Activin signaling.
NODAL/Activin signaling orchestrates key processes during embryonic development via SMAD2. How SMAD2 activates programs of gene expression that are modulated over time however, is not known. Here we delineate the sequence of events that occur from SMAD2 binding to transcriptional activation, and the mechanisms underlying them. NODAL/Activin signaling induces dramatic chromatin landscape changes, and a dynamic transcriptional network regulated by SMAD2, acting via multiple mechanisms. Crucially we have discovered two modes of SMAD2 binding. SMAD2 can bind pre-acetylated nucleosome-depleted sites. However, it also binds to unacetylated, closed chromatin, independently of pioneer factors, where it induces nucleosome displacement and histone acetylation. For a subset of genes, this requires SMARCA4. We find that long term modulation of the transcriptional responses requires continued NODAL/Activin signaling. Thus SMAD2 binding does not linearly equate with transcriptional kinetics, and our data suggest that SMAD2 recruits multiple co-factors during sustained signaling to shape the downstream transcriptional program.

Elife, 2017; 6
25644430: Coda DM, Lingua MF, Morena D, Foglizzo V, Bersani F, Ala U, Ponzetto C, Taulli R
SMYD1 and G6PD modulation are critical events for miR-206-mediated differentiation of rhabdomyosarcoma.
Rhadomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood. RMS cells resemble fetal myoblasts but are unable to complete myogenic differentiation. In previous work we showed that miR-206, which is low in RMS, when induced in RMS cells promotes the resumption of differentiation by modulating more than 700 genes. To better define the pathways involved in the conversion of RMS cells into their differentiated counterpart, we focused on 2 miR-206 effectors emerged from the microarray analysis, SMYD1 and G6PD. SMYD1, one of the most highly upregulated genes, is a H3K4 histone methyltransferase. Here we show that SMYD1 silencing does not interfere with the proliferative block or with the loss anchorage independence imposed by miR-206, but severely impairs differentiation of ERMS, ARMS, and myogenic cells. Thus SMYD1 is essential for the activation of muscle genes. Conversely, among the downregulated genes, we found G6PD, the enzyme catalyzing the rate-limiting step of the pentose phosphate shunt. In this work, we confirmed that G6PD is a direct target of miR-206. Moreover, we showed that G6PD silencing in ERMS cells impairs proliferation and soft agar growth. However, G6PD overexpression does not interfere with the pro-differentiating effect of miR-206, suggesting that G6PD downmodulation contributes to - but is not an absolute requirement for - the tumor suppressive potential of miR-206. Targeting cancer metabolism may enhance differentiation. However, therapeutic inhibition of G6PD is encumbered by side effects. As an alternative, we used DCA in combination with miR-206 to increase the flux of pyruvate into the mitochondrion by reactivating PDH. DCA enhanced the inhibition of RMS cell growth induced by miR-206, and sustained it upon miR-206 de-induction. Altogether these results link miR-206 to epigenetic and metabolic reprogramming, and suggest that it may be worth combining differentiation-inducing with metabolism-directed approaches.
Cell Cycle, 2015; 14
23728344: Taulli R, Foglizzo V, Morena D, Coda DM, Ala U, Bersani F, Maestro N, Ponzetto C
Failure to downregulate the BAF53a subunit of the SWI/SNF chromatin remodeling complex contributes to the differentiation block in rhabdomyosarcoma.
Rhabdomyosarcoma (RMS), the most common soft tissue sarcoma in children and young adults, is characterized by a partially differentiated myogenic phenotype. We have previously shown that the blocking of tumor growth and resumption of differentiation can be achieved by re-expression of miR-206, a muscle-enriched microRNA missing in RMS. In this work, we focused on BAF53a, one of the genes downregulated in miR-206-expressing RMS cells, which codes for a subunit of the SWI/SNF chromatin remodeling complex. Here we show that the BAF53a transcript is significantly higher in primary RMS tumors than in normal muscle, and is a direct target of miR-206. Sustained expression of BAF53a interferes with differentiation in myogenic cells, whereas its silencing in RMS cells increases expression of myogenic markers and inhibits proliferation and anchorage-independent growth. Accordingly, BAF53a silencing also impairs embryonal RMS and alveolar RMS tumor growth, inducing their morphological and biochemical differentiation. These results indicate that failure to downregulate the BAF53a subunit may contribute to the pathogenesis of RMS, and suggest that BAF53a may represent a novel therapeutic target for this tumor.
Oncogene, 2014; 33

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-033

EPIGENETIC PLASTICITY CONTRIBUTES TO NEURONAL COMPETITION DURING MEMORY ALLOCATION

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Long-lasting memories are thought to be stored in a small set of neurons, so-called engram cells. To define a stable engram each region of the brain involved in memory storage recruits between 5 and 20 percent of excitatory neurons. In parallel, recent studies have revealed that the neuronal allocation to participate in the memory engram is not random; rather, post-synaptic neurons with higher excitability than their neighbors are more likely to be selected into the memory trace. However, the precise mechanism that enables neurons to acquire such profile has not yet been investigated. With this project we further explore the process of memory allocation by studying how histone acetylation contributes to neuronal competition during memory formation. To this end, we virally manipulated the histone acetyltransferase (HAT) content of a sparse population of excitatory cells in the lateral amygdala (LA) of wild-type mice. Using patch-clamp recordings, we found that HAT overexpressing neurons are intrinsically more excitable. Furthermore, a higher histone acetylation content increased the probability of individual LA neurons to be selected as part of the engram. Interestingly, mice overexpressing HAT in the LA show a strengthened fear memory response, and, importantly, this fear potentiation is absent when neurons carrying the HAT construct are optogenetically silenced. These findings highlight that epigenetic plasticity is an integral process that co-determined memory allocation.

Pubmed:
29572443: Grosso A, Santoni G, Manassero E, Renna A, Sacchetti B
A neuronal basis for fear discrimination in the lateral amygdala.
In the presence of new stimuli, it is crucial for survival to react with defensive responses in the presence of stimuli that resemble threats but also to not react with defensive behavior in response to new harmless stimuli. Here, we show that in the presence of new uncertain stimuli with sensory features that produce an ambiguous interpretation, discriminative processes engage a subset of excitatory and inhibitory neurons within the lateral amygdala (LA) that are partially different from those engaged by fear processes. Inducing the pharmacogenetic deletion of this neuronal ensemble caused fear generalization but left anxiety-like response, fear memory and extinction processes intact. These data reveal that two opposite neuronal processes account for fear discrimination and generalization within the LA and suggest a potential pathophysiological mechanism for the impaired discrimination that characterizes fear-related disorders.
Nat Commun, 2018; 9
34017129: Silva BA, Astori S, Burns AM, Heiser H, van den Heuvel L, Santoni G, Martinez-Reza MF, Sandi C, Gräff J
A thalamo-amygdalar circuit underlying the extinction of remote fear memories.
Fear and trauma generate some of the longest-lived memories. Despite the corresponding need to understand how such memories can be attenuated, the underlying brain circuits remain unknown. Here, combining viral tracing, neuronal activity mapping, fiber photometry, chemogenetic and closed-loop optogenetic manipulations in mice, we show that the extinction of remote (30-day-old) fear memories depends on thalamic nucleus reuniens (NRe) inputs to the basolateral amygdala (BLA). We found that remote, but not recent (1-day-old), fear extinction activates NRe-to-BLA inputs, which become potentiated upon fear reduction. Furthermore, both monosynaptic NRe-to-BLA and total NRe activity increase shortly before freezing cessation, suggesting that the NRe registers and transmits safety signals to the BLA. Accordingly, pan-NRe and pathway-specific NRe-to-BLA inhibition impairs, whereas their activation facilitates, remote fear extinction. These findings identify the NRe as a crucial BLA regulator for extinction and provide the first functional description of the circuits underlying the attenuation of consolidated fear memories.
Nat Neurosci, 2021; 24

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BOARD NUMBER: S06-034

AMYGDALAR CB2 CANNABINOID RECEPTOR MEDIATES FEAR EXTINCTION DEFICITS INDUCED BY OREXIN-A

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Anxiety disorders are often characterized by an inability to extinguish learned fear responses. Hypocretins/orexins are involved in the regulation of aversive memories, and dysregulation of this system may contribute to the aetiology of fear and anxiety disorders. The aim of this study is to describe the neurobiological mechanisms by which the orexin system modulates fear extinction. Experiments were performed using adult male C57BL/6J and eGFP-CB2R mice. The selective inhibitor of 2-arachidonoylglycerol (2-AG) biosynthesis O7460 abolished the fear extinction deficits induced by orexin-A (OXA). Increased levels of 2-AG were observed in the amygdala of mice treated with OXA which do not extinguish fear. Indeed, a correlation between fear (percentage of freezing) and 2-AG levels was found in this brain region, suggesting that high levels of this endocannabinoid are related to poor extinction. Impairment of fear extinction induced by OXA was associated with increased expression of CB2 cannabinoid receptors (CB2R) in microglial cells of the basolateral amygdala. Consistently, intra-amygdalar administration of the CB2R antagonist AM630 blocked the OXA-induced impairment of fear extinction, whereas the CB1 cannabinoid receptor antagonist rimonabant increased the fear response. Microglial and CB2R expression depletion in the amygdala with PLX5622 chow also prevented these extinction deficits. These results reveal a key role for 2-AG and CB2R probably located in microglial cells of the amygdala in impaired extinction of aversive memories promoted by overactivation of the orexin system.

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BOARD NUMBER: S06-035

RECENT TO REMOTE MEMORY ACTIVITY AND CONNECTIVITY

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Recent memory involves relatively fast processes that take place during the first hours following learning. Remote memories, however, are not yet well-defined. In this research we targeted the transition of a memory from recent to remote recall, to find missing links between these memory stages. For that purpose, we tagged cFos positive cells using genetic manipulation, and using immunohistochemistry within the same mouse, thus allowing comparison of the two populations in each animal. First, we show that neuronal activation throughout different stages in memory consolidation and reconsolidation is consistent in the hippocampus, but not in other memory related brain areas. Secondly, during the transition from recent to remote, several brain structures maintain fixed ensembles, not necessarily similar to the acquisition. In order to define whether the ensembles supporting remote memory are selected based on their connectivity and if they differ from the ensembles supporting recent memory, we marked cells in the CA1 based on their projection target. Recent to remote transition does include recruitment of projection based sub-population; CA1 to ACC projecting cells are more likely to be activated during remote than during recent recall or acquisition. Additionally, hyper activation of hippocampal astrocytes during an acquisition of a memory, increases the activation of the CA1 to ACC projecting cells during recent but not remote recall. Lastly, analyzing projections of active cells from the CA1 during different stages of memory consolidation, using full brain reconstruction, revealed that as time progresses, the CA1 to ACC projections ratio increases, compared to other areas.

Pubmed:
34117643: Refaeli R, Doron A, Benmelech-Chovav A, Groysman M, Kreisel T, Loewenstein Y, Goshen I
Features of hippocampal astrocytic domains and their spatial relation to excitatory and inhibitory neurons.
The mounting evidence for the involvement of astrocytes in neuronal circuits function and behavior stands in stark contrast to the lack of detailed anatomical description of these cells and the neurons in their domains. To fill this void, we imaged >30,000 astrocytes in hippocampi made transparent by CLARITY, and determined the elaborate structure, distribution, and neuronal content of astrocytic domains. First, we characterized the spatial distribution of >19,000 astrocytes across CA1 lamina, and analyzed the morphology of thousands of reconstructed domains. We then determined the excitatory somatic content of CA1 astrocytes, and measured the distance between inhibitory neuronal somata to the nearest astrocyte soma. We find that on average, there are almost 14 pyramidal neurons per domain in the CA1, increasing toward the pyramidal layer midline, compared to only five excitatory neurons per domain in the amygdala. Finally, we discovered that somatostatin neurons are found in close proximity to astrocytes, compared to parvalbumin and VIP inhibitory neurons. This work provides a comprehensive large-scale quantitative foundation for studying neuron-astrocyte interactions.
Glia, 2021; 69
29804835: Adamsky A, Kol A, Kreisel T, Doron A, Ozeri-Engelhard N, Melcer T, Refaeli R, Horn H, Regev L, Groysman M, London M, Goshen I
Astrocytic Activation Generates De Novo Neuronal Potentiation and Memory Enhancement.
Astrocytes respond to neuronal activity and were shown to be necessary for plasticity and memory. To test whether astrocytic activity is also sufficient to generate synaptic potentiation and enhance memory, we expressed the Gq-coupled receptor hM3Dq in CA1 astrocytes, allowing their activation by a designer drug. We discovered that astrocytic activation is not only necessary for synaptic plasticity, but also sufficient to induce NMDA-dependent de novo long-term potentiation in the hippocampus that persisted after astrocytic activation ceased. In vivo, astrocytic activation enhanced memory allocation; i.e., it increased neuronal activity in a task-specific way only when coupled with learning, but not in home-caged mice. Furthermore, astrocytic activation using either a chemogenetic or an optogenetic tool during acquisition resulted in memory recall enhancement on the following day. Conversely, directly increasing neuronal activity resulted in dramatic memory impairment. Our findings that astrocytes induce plasticity and enhance memory may have important clinical implications for cognitive augmentation treatments.
Cell, 2018; 174

30122531: Atlan G, Terem A, Peretz-Rivlin N, Sehrawat K, Gonzales BJ, Pozner G, Tasaka GI, Goll Y, Refaeli R, Zviran O, Lim BK, Groysman M, Goshen I, Mizrahi A, Nelken I, Citri A
The Claustrum Supports Resilience to Distraction.
A barrage of information constantly assaults our senses, of which only a fraction is relevant at any given point in time. However, the neural circuitry supporting the suppression of irrelevant sensory distractors is not completely understood. The claustrum, a circuit hub with vast cortical connectivity, is an intriguing brain structure, whose restrictive anatomy, thin and elongated, has precluded functional investigation. Here, we describe the use of Egr2-CRE mice to access genetically defined claustral neurons. Utilizing conditional viruses for anterograde axonal labeling and retrograde trans-synaptic tracing, we validated this transgenic model for accessing the claustrum and extended the known repertoire of claustral input/output connectivity. Addressing the function of the claustrum, we inactivated CL neurons, chronically as well as acutely, in mice performing an automated two-alternative forced-choice behavioral task. Strikingly, inhibition of CL neurons did not significantly impact task performance under varying delay times and cue durations, but revealed a selective role for the claustrum in supporting performance in the presence of an irrelevant auditory distractor. Further investigation of behavior, in the naturalistic maternal pup-retrieval task, replicated the result of sensitization to an auditory distractor following inhibition of CL neurons. Initiating investigation into the underlying mechanism, we found that activation of CL neurons modulated cortical sensory processing, suppressing tone representation in the auditory cortex. This functional study, utilizing selective genetic access, implicates the claustrum in supporting resilience to distraction, a fundamental aspect of attention.
Curr Biol, 2018; 28
34650207: Rimmerman N, Verdiger H, Goldenberg H, Naggan L, Robinson E, Kozela E, Gelb S, Reshef R, Ryan KM, Ayoun L, Refaeli R, Ashkenazi E, Schottlender N, Ben Hemo-Cohen L, Pienica C, Aharonian M, Dinur E, Lazar K, McLoughlin DM, Zvi AB, Yirmiya R
Microglia and their LAG3 checkpoint underlie the antidepressant and neurogenesis-enhancing effects of electroconvulsive stimulation.
Despite evidence implicating microglia in the etiology and pathophysiology of major depression, there is paucity of information regarding the contribution of microglia-dependent molecular pathways to antidepressant procedures. In this study, we investigated the role of microglia in a mouse model of depression (chronic unpredictable stress-CUS) and its reversal by electroconvulsive stimulation (ECS), by examining the effects of microglia depletion with the colony stimulating factor-1 antagonist PLX5622. Microglia depletion did not change basal behavioral measures or the responsiveness to CUS, but it completely abrogated the therapeutic effects of ECS on depressive-like behavior and neurogenesis impairment. Treatment with the microglia inhibitor minocycline concurrently with ECS also diminished the antidepressant and pro-neurogenesis effects of ECS. Hippocampal RNA-Seq analysis revealed that ECS significantly increased the expression of genes related to neurogenesis and dopamine signaling, while reducing the expression of several immune checkpoint genes, particularly lymphocyte-activating gene-3 (Lag3), which was the only microglial transcript significantly altered by ECS. None of these molecular changes occurred in microglia-depleted mice. Immunohistochemical analyses showed that ECS reversed the CUS-induced changes in microglial morphology and elevation in microglial LAG3 receptor expression. Consistently, either acute or chronic systemic administration of a LAG3 monoclonal antibody, which readily penetrated into the brain parenchyma and was found to serve as a direct checkpoint blocker in BV2 microglia cultures, rapidly rescued the CUS-induced microglial alterations, depressive-like symptoms, and neurogenesis impairment. These findings suggest that brain microglial LAG3 represents a promising target for novel antidepressant therapeutics.
Mol Psychiatry, 2022; 27

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BOARD NUMBER: S06-036

FEAR CIRCUIT-BASED NEUROBEHAVIORAL SIGNATURES AND TRANSCRIPTIONAL NETWORKS PROMOTING RESILIENCE TO CHRONIC SOCIAL STRESS

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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[1]Leibniz Institute for Resilience Research, Molecular And Cellular Mechanisms Of Resilience, Mainz, Germany, [2]Genevention GmbH, Genevention Gmbh, Göttingen, Germany, [3]University of Exeter, Living Systems Institute, Exeter, United Kingdom, [4]Leibniz Institute for Resilience Research, Systemic Mechanisms Of Resilience, Mainz, Germany, [5]Leibniz Institute for Resilience Research, Molecular And Cellular Mechanisms Of Resilience, mainz, Germany

Consistent evidence from human data points to successful threat-safety discrimination and responsiveness to extinction training of aversive memories as key characteristics of resilient individuals. In contrast, susceptible individuals are characterised by generalisation of aversive responses and resistance to extinction. To promote valid cross-species approaches for the identification of resilience mechanisms, we established a translationally informed mouse model enabling the stratification of mice into three phenotypic subgroups following chronic social defeat stress based on their individual ability for threat-safety discrimination and conditioned learning: the *Discriminating-avoiders*, characterised by successful social threat-safety discrimination and successful extinction of social aversive memories; the *Indiscriminate-avoiders*, showing social aversive response generalisation and resistance to extinction of social aversive memories, and the *Non-avoiders* displaying impaired aversive conditioned learning. Employing inter-regional transcriptional analysis revealed distinct functional alternations of the fear circuitry associated with stress resilience and susceptibility. Transcriptional analysis across and between brain regions of the fear circuitry revealed subgroup-specific gene networks and differentially expressed genes, respectively. By modelling translationally relevant phenotypic characteristics, our approach holds the potential to serve as a blueprint for multiple approaches to identifying valid neurobiological mechanisms of resilience in the future.

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BOARD NUMBER: S06-037

THE β-ADRENERGIC RECEPTOR ANTAGONIST PROPRANOLOL ATTENUATES THE ESTABLISHMENT OF CONDITIONED CONTEXT AVERSION IN LABORATORY MICE

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

Çınar Furkan Ilhan[1], Esra Ülke[1], Gonzalo Urcelay[2], Sezen Kışlal[1]
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Introduction: Anticipatory nausea is a type of classical conditioning in which chemotherapy-induced illness is associated with environmental cues in the hospital. Although antiemetic drugs can reduce anticipatory nausea, 30% of patients still suffer from it. We investigated whether β-adrenergic receptor antagonist propranolol impairs the consolidation of conditioned context aversion (CCA), the preclinical model of anticipatory nausea. **Method**: Twelve-week-old CD1 outbred male mice were assigned to the LiCl–Propranolol (n = 11), LiCl–NaCl (n = 9), or NaCl–NaCl (n = 12) group. Five minutes into a 20-minute conditioning trial, the LiCl–Propranolol and LiCl–NaCl groups received an intraperitoneal injection of illness-inducing LiCl (6 mEq/kg); the NaCl–NaCl group received NaCl (0.9%) in a novel context. Immediately after, the LiCl–Propranolol group was injected with propranolol (10mg/kg) and the LiCl–NaCl and NaCl–NaCl groups were injected with NaCl. A retention test (20 minutes) was conducted 72 hours later. Water intake was measured during the conditioning and retention trials. One-way ANOVAs and t-tests were used as statistical tools. **Results:** There were no differences in water intake between groups in the conditioning trial (Figure 1). In the retention test, there was a statistically significant difference between LiCl–NaCl and LiCl–Propranolol ($p = .006$) and LiCl–NaCl and NaCl–NaCl ($p = .01$) but not LiCl–Propranolol and NaCl–NaCl ($p = .83$) (Figure 2). **Conclusion:** Propranolol, an amnestic agent, attenuates the consolidation of CCA, and thereby shows promise to mitigate the stress and discomfort associated with chemotherapy.

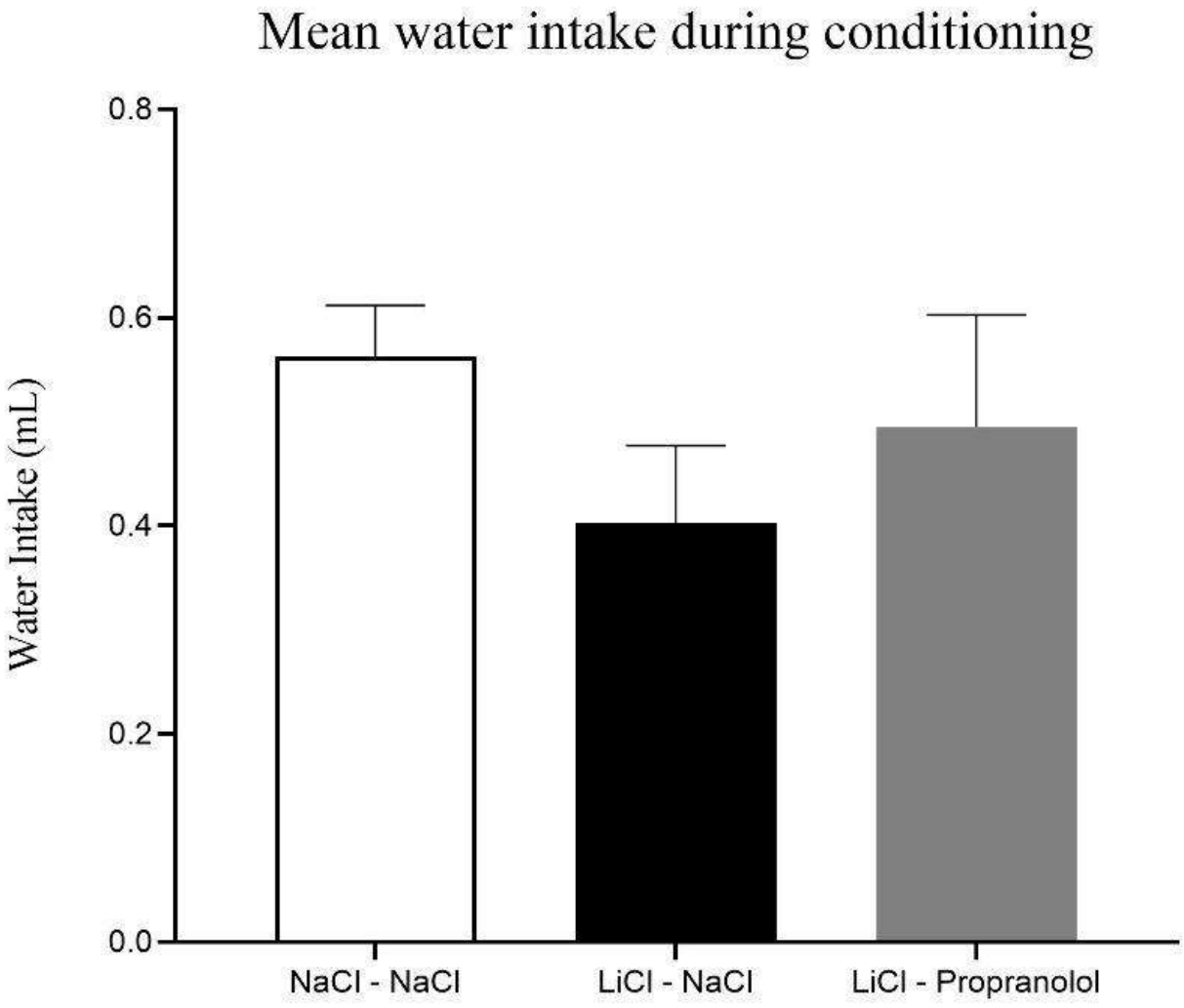
Mean water intake during conditioning
Water Intake (mL)
0.8
0.6
0.4
0.2
0.0
NaCl - NaCl
LiCl - NaCl
LiCl - Propranolol

Figure 1

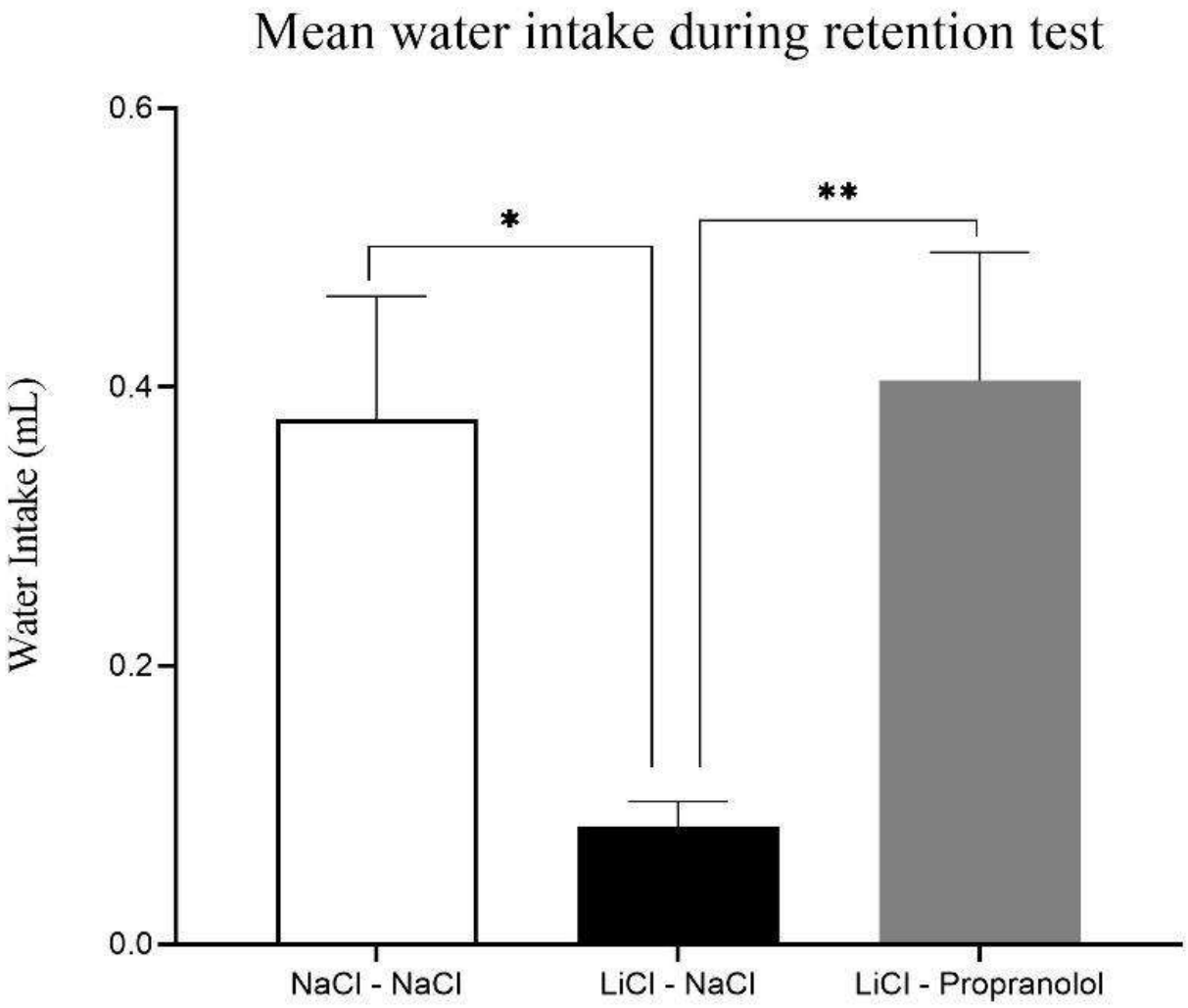
Mean water intake during retention test
Water Intake (mL)
0.6
0.4
0.2
0.0
*
**
NaCl - NaCl
LiCl - NaCl
LiCl - Propranolol

Figure 2

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BOARD NUMBER: S06-038

BLA-MPFC-DMS CIRCUITRY: THE GATEWAY TO THE EFFECT OF FEAR ON ACTION CONTROL

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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When an action is carried out to achieve a desired goal, we operate through the "Goal directed" system. Conversely, behaviors less driven by outcome expectancy are often carried out by the "habitual" system. The same behavior can be executed by either of the systems. The connection and information transfer between them, as well as the ability to switch control when needed, is crucial for adaptive behavior. Dysfunctions in this mechanism are suggested to underlie psychopathologies such as OCD and addiction. Symptoms are known to be affected by stress, possibly through stress-induced changes in action-control neural circuits, involving the cortico-striatal loops. These loops are anatomically similar, yet parallel and functionally different. Habit control involves the sensorimotor cortico-striatal loop going through the dorsolateral striatum (DLS) and sensorimotor cortex. Goal-directed control require cognitive-emotional integration involving the BLA-mPFC pathway and the associative loop going through the dorsomedial striatum (DMS) and the medial prefrontal cortex (mPFC). The latter is an important cortical hub in fear-related behaviors. Here, we use fear conditioning and two distinct behavioral models (Forced alteration delta maze, lever pressing task) allowing mice to produce completely different behaviors theoretically under the same action control. Combined with reward devaluation process, we test the effects of stress on action control in mice. Results from our lab work indicate that behavior can be controlled by both goal-directed and habitual control, and that stressful stimulus promotes habitual control. We use optogenetic manipulations and electrophysiological recordings to examine the effect of fear on the BLA-mPFC-DMS circuit action control.

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BOARD NUMBER: S06-039

EXPLORING THE GUT-MICROBIOTA-BRAIN AXIS IN A SPANISH POPULATION IN THE AFTERMATH OF THE COVID-19 PANDEMIC

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Introduction: The prevalence of anxiety and depression significantly increased in the aftermath of the COVID-19 pandemic. To develop novel treatment strategies, we need to identify all potential role players in these complex disorders. Aim: This study will identify gut microbial features associated with mental health outcomes in individuals with anxiety and depressive symptoms in a Spanish cohort. Methods: Microbial communities from stool samples were profiled in 197 individuals who completed self-reported online questionnaires (March 2021 - Jan 2022). Based on the Centre for Epidemiologic Studies Depression scale, 64 individuals had depressive symptoms, 104 reported state anxiety, and 131 trait anxiety symptoms (based on State-trait anxiety and depression inventory for state anxiety). Furthermore, data about trauma exposure (PTSD Checklist for DSM-5 with Life Events Checklist for DSM-5 and Childhood Trauma Questionnaire-Short Form), quality of life (World Health Organization Quality Of Life questionnaire), health, and lifestyle were also collected. Currently, 16S ribosomal RNA gene V3-4 amplicon sequencing is being performed. Microbial diversity and community structure will be analyzed, together with relative taxonomic abundance at the genus and phylum levels, to identify associations with variables of interest relating to anxiety and depressive symptoms. Results and Conclusion: Gut microbiota data will be analyzed in conjunction with self-report data to identify associations between mental health outcomes and the gut microbiota. Associations with particular taxa will be presented and discussed in the context of novel adjunctive therapies that could alleviate symptoms of anxiety and depression (through the use of prebiotics, probiotics, and synbiotics).

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BOARD NUMBER: S06-040

ALTERED CORTISOL AWAKENING RESPONSE IN RELATIONSHIP WITH TRAUMA IN FUNCTIONAL NEUROLOGICAL DISORDERS

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Psychological stress is a well-known risk factor to develop a functional neurological disorder (FND), a neuropsychiatric condition in which patients experience neurological symptoms in the absence of classical neurological disease. However, only little is known about the biological stress regulation in FND and its role in the aetiology of FND. Investigating the relationship between psychological stressors and a potential dysregulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis in FND could shed a light on the underlying neurobiological mechanisms. We examined potential alterations in the HPA axis in a broad range of cortisol indices, including the cortisol awakening response (CAR), post-awakening cortisol concentration (PACC, AUC_G), and overall hormonal secretion (OHS, AUC_G) of 86 FND patients and 75 healthy controls. Salivary cortisol was collected nine times throughout one day. In parallel presence and severity of traumatic life events were compared in FND patients and controls. Overall, FND patients reported 46% more emotional neglect as compared to healthy controls ($p < 0.001$). Moreover, FND patients had a significantly flatter CAR ($p < 0.001$), and lower AUC_G measures as compared to healthy controls ($p < 0.008$). Using a partial least squares correlation, we found that particularly in FND patients with a history of sexual abuse, trauma might play an important role in the multivariate pattern between trauma history and HPA axis dysfunction. This study supports a stress-diathesis model of FND and showed an association between different attributes of trauma history and potential biological vulnerability in patients with FND.

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BOARD NUMBER: S06-041

TRANSCRANIAL MAGNETIC STIMULATION (TMS) AND SENSE OF AGENCY (SOA) IN FUNCTIONAL NEUROLOGICAL DISORDER (FND); PRELIMINARY DATA

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Patients suffering from functional neurological disorder (FND) present with neurological symptoms not related to an underlying classical neurological disorder. A disrupted sense of agency (SOA) is assumed to be involved in sensorimotor symptom development. The right temporoparietal junction (rTPJ) is a key node of the agency network and has been shown to be a suitable target for neuromodulation of the SOA. The goal of the study is to investigate the effect of a transcranial magnetic stimulation (TMS) over the rTPJ on the SOA in FND patients compared to healthy controls. In a randomized, cross-over, single-blinded trial with active control condition, 23 FND patients and 19 healthy controls were included. In three sessions, every participant underwent excitatory, inhibitory or sham TMS over the rTPJ. Before and after stimulation, participants played a game targeting the SOA during functional magnetic resonance imaging. The game consists of two phases: one with normal SoA (baseline) and one with decreased SoA (turbulence). Preliminary results of whole brain analysis demonstrated that FND patients showed decreased activity in temporoparietal regions compared to healthy controls when contrasting manipulated game-phases versus baseline (p = 0.292, [56 -50 40]). Further, a region-of-interest (ROI) surrounding the subjects' peak activation within a sphere around the rTPJ [62 -34 30] was defined and revealed a lower activation in FND patients compared to controls during turbulence compared to baseline. Further ongoing analyses will determine whether TMS (inhibitory or excitatory) has an effect on these hypoactivation found in patients as well as on behavioural data.

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BOARD NUMBER: S06-042

CORRELATIONS BETWEEN THE WHITE MATTER ANISOTROPY AT THE WHOLE-BRAIN SCALE AND ANXIETY RATINGS

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Diffusion tensor imaging studies of trait anxiety often report that trait anxiety predicts white matter integrity of fiber tracts between frontal regions and the limbic system, e.g. the uncinate fasciculus. However, the whole-brain structural connectivity has been rarely investigated in non-clinical populations despite its particular importance for developing preventive diagnostic markers of pathological anxiety. The aim of the present study was to explore correlations between white matter tracts characteristics and state and trait anxiety inventory (STAI) scores at the whole-brain scale. In addition, we conducted psychological testing twice in order to examine the within-study reproducibility of correlations. The study sample included 25 participants without prior complaints of excess anxiety and clinical history of anxiety disorders. They filled in the STAI twice: one day before and several minutes before the scanning procedure. The 64-direction protocol was applied to obtain diffusion-weighted images. We used the connectometry approach to investigate correlations between the generalized fractional anisotropy (GFA) and STAI scores. Among the reproduced correlations, there were significant associations of both trait and state anxiety self-report ratings with GFA in the corpus callosum and association fibers predominantly in the right hemisphere, including the inferior, superior, and inferior fronto-occipital fasciculus and cingulum bundle. Our findings point to the role of the integrity of these white matter tracts in susceptibility to high trait and state anxiety. The defined local connectome correlations with anxiety ratings could serve as targets for anxiety disorders preventive diagnostics.

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BOARD NUMBER: S06-043

A PRECLINICAL MODEL OF COMORBID ANXIETY AND DEPRESSION

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Approximately half of patients diagnosed with major depressive disorder also suffer from co-occurring anxiety. Comorbid patients have more severe symptoms, are more likely to seek pharmacotherapy, which is less effective in their case. The underlying neurobiological factors behind this phenomenon are not well-known, and currently there are no established animal models to support better understanding. Consequently, we aimed to establish a simple, reliable rodent model of comorbidity with high clinical translational validity. Previously, we developed a behavioral sampling protocol to measure trait-anxiety (rather than commonly measured anxious states) involving repeated testing using the most common anxiety tests. Here, we extended this method to preclinical tests of passive coping, a core symptom of major depression. After examining the correlation of anxiety- and depression-related traits, we observed a subpopulation consistently showing high anxiety and passive coping. By increasing test-repetitions and test-types used, we were able to detect stronger associations between anxiety- and depression-like behavior. Next, the animals underwent the Learned Helplessness (LH) model, which induces a depression-like phenotype in vulnerable subpopulations, while also measuring passive coping. Animals showing high anxiety and passive coping prior to LH were more susceptible to developing learned helplessness in the model. Through a semi-supervised machine learning method we have greatly simplified the testing protocol into two tests that reliably predict an animal's coping style in the LH model. In summary, we developed a simple, reliable model of comorbid anxiety and depression, which will help us understand the underlying neurobiological factors, and improve the efficiency of pharmacotherapy treatment.

Pubmed:
32350040: Varga ZK, Pejtsik D, Biró L, Zsigmond Á, Varga M, Tóth B, Salamon V, Annus T, Mikics É, Aliczki M
Conserved Serotonergic Background of Experience-Dependent Behavioral Responsiveness in Zebrafish ().
Forming effective responses to threatening stimuli requires the adequate and coordinated emergence of stress-related internal states. Such ability depends on early-life experiences and, in connection, the adequate formation of neuromodulatory systems, particularly serotonergic signaling. Here, we assess the serotonergic background of experience-dependent behavioral responsiveness using male and female zebrafish (). For the first time, we have characterized a period during behavioral metamorphosis in which zebrafish are highly reactive to their environment. Absence of social stimuli during this phase established by isolated rearing fundamentally altered the behavioral phenotype of postmetamorphic zebrafish in a challenge-specific manner, partially due to reduced responsiveness and an inability to develop stress-associated arousal state. In line with this, isolation differentially affected whole-brain serotonergic signaling in resting and stress-induced conditions, an effect that was localized in the dorsal pallium and was negatively associated with responsiveness. Administration of the serotonin receptor 1A partial agonist buspirone prevented the isolation-induced serotonin response to novelty in the level of the whole brain and the forebrain as well, without affecting catecholamine levels, and rescued stress-induced arousal along with challenge-induced behaviors, which together indicates functional connection between these changes. In summary, there is a consistent negative association between behavioral responsiveness and serotonergic signaling in zebrafish, which is well recognizable through the modifying effects of developmental perturbation and pharmacological manipulations as well. Our results imply a conserved serotonergic mechanism that context-dependently modulates environmental reactivity and is highly sensitive to experiences acquired during a specific early-life time window, a phenomenon that was previously only suggested in mammals. The ability to respond to challenges is a fundamental factor in survival. We show that zebrafish that lack appropriate social stimuli in a sensitive developmental period show exacerbated alertness in nonstressful conditions while failing to react adequately to stressors. This shift is reflected inversely by central serotonergic signaling, a system that is implicated in numerous mental disorders in humans. Serotonergic changes in brain regions modulating responsivity and behavioral impairment were both prevented by the pharmacological blockade of serotonergic function. These results imply a serotonergic mechanism in zebrafish that transmits early-life experiences to the

later phenotype by shaping stress-dependent behavioral reactivity, a phenomenon that was previously only suggested in mammals. Zebrafish provide new insights into early-life-dependent neuromodulation of behavioral stress-responses.
J Neurosci, 2020; 40
30410116: Varga ZK, Zsigmond Á, Pejtsik D, Varga M, Demeter K, Mikics É, Haller J, Aliczki M
The swimming plus-maze test: a novel high-throughput model for assessment of anxiety-related behaviour in larval and juvenile zebrafish (Danio rerio).
Larval zebrafish (Danio rerio) has the potential to supplement rodent models due to the availability of resource-efficient, high-throughput screening and high-resolution imaging techniques. Although behavioural models are available in larvae, only a few can be employed to assess anxiety. Here we present the swimming plus-maze (SPM) test paradigm, a tool to assess anxiety-related avoidance of shallow water bodies in early developmental stages. The "+" shaped apparatus consists of arms of different depth, representing different levels of aversiveness similarly to the rodent elevated plus-maze. The paradigm was validated (i) in larval and juvenile zebrafish, (ii) after administration of compounds affecting anxiety and (iii) in differentially aversive experimental conditions. Furthermore, we compared the SPM with conventional "anxiety tests" of zebrafish to identify their shared characteristics. We have clarified that the preference of deeper arms is ontogenetically conserved and can be abolished by anxiolytic or enhanced by anxiogenic agents, respectively. The behavioural readout is insensitive to environmental aversiveness and is unrelated to behaviours assessed by conventional tests involving young zebrafish. Taken together, we have developed a sensitive high-throughput test allowing the assessment of anxiety-related responses of zebrafish regardless of developmental stage, granting the opportunity to combine larva-based state-of-the-art methods with detailed behavioral analysis.
Sci Rep, 2018; 8

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INVOLVEMENT OF METHYL-CPG BINDING PROTEIN 2 IN VULNERABILITY TO POST-TRAUMATIC STRESS DISORDER: FROM MICE TO (WO)MEN

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Aims: post-traumatic stress disorder (PTSD) is a mental disorder characterized by symptoms of persistent anxiety arising after trauma exposure in vulnerable individuals. Considering the emerging functions of epigenetics in establishing interindividual differences in PTSD risk, we aimed at translationally addressing the contribution of the X-linked epigenetic regulator Methyl-CpG binding protein 2 (MECP2) in shaping susceptibility to traumatic stress. **Methods:** predisposition to the onset of PTSD-like symptomatology was evaluated in mice carrying hypofunctional MeCP2; the association between peripheral *MECP2* expression and psychometric measures of vulnerability to stress and traumas was assessed in human cohorts. **Results:** after footshock delivery, mutant males displayed lasting cognitive, avoidance and arousal symptoms, mimicking PTSD psychopathology; sex-dependent modulations in stress responsivity were evident, with mutant females releasing lower and males higher glucocorticoids levels compared to controls. In non-traumatized healthy participants *MECP2* underexpression was accompanied by the maladaptive outcomes of childhood adversities, a well-known PTSD risk factor; similarly, in participants exposed to childhood stress and traumatized in adulthood, *MECP2* underexpression paralleled increased PTSD symptom severity. In both cohorts, the association was stronger in women than in men. **Conclusion:** the present results support the relevance of MECP2 hypofunctionality in boosting PTSD risk, suggesting that MECP2-related epigenetic pathways are involved in disease etiology. Gender and exposure to childhood adversities emerge as important factors involved in the association between MECP2 and PTSD susceptibility. Funded by the Italian Ministry of Health #GR-2018-12366210 to BDF and the Deutsche Forschungsgemeinschaft #SFB636/C1 to HF.

Pubmed:

30056123: Vigli D, Cosentino L, Raggi C, Laviola G, Woolley-Roberts M, De Filippis B
Chronic treatment with the phytocannabinoid Cannabidivarin (CBDV) rescues behavioural alterations and brain atrophy in a mouse model of Rett syndrome.
Rett syndrome (RTT) is a rare neurodevelopmental disorder, characterized by severe behavioural and physiological symptoms. RTT is caused by mutations in the MECP2 gene in about 95% of cases and to date no cure is available. The endocannabinoid system modulates several physiological processes and behavioural responses that are impaired in RTT and its deregulation has been associated with neuropsychiatric disorders which have symptoms in common with RTT. The present study evaluated the potential therapeutic efficacy for RTT of cannabidivarin (CBDV), a non-psychotropic phytocannabinoid from Cannabis sativa that presents antagonistic properties on the G protein-coupled receptor 55 (GPR55), the most recently identified cannabinoid receptor. Present results demonstrate that systemic treatment with CBDV (2, 20, 100 mg/Kg ip for 14 days) rescues behavioural and brain alterations in MeCP2-308 male mice, a validated RTT model. The CBDV treatment restored the compromised general health status, the sociability and the brain weight in RTT mice. A partial restoration of motor coordination was also observed. Moreover, increased levels of GPR55 were found in RTT mouse hippocampus, suggesting this G protein-coupled receptor as new potential target for the treatment of this disorder. Present findings highlight for the first time for RTT the translational relevance of CBDV, an innovative therapeutic agent that is under active investigation in the clinical setting.
Neuropharmacology, 2018; 140
30326240: Vigli D, Rusconi L, Valenti D, La Montanara P, Cosentino L, Lacivita E, Leopoldo M, Amendola E, Gross C, Landsberger N, Laviola G, Kilstrup-Nielsen C, Vacca RA, De Filippis B
Rescue of prepulse inhibition deficit and brain mitochondrial dysfunction by pharmacological stimulation of the central serotonin receptor 7 in a mouse model of CDKL5 Deficiency Disorder.
Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5) gene cause CDKL5 Deficiency Disorder (CDD), a rare

neurodevelopmental syndrome characterized by severe behavioural and physiological symptoms. No cure is available for CDD. CDKL5 is a kinase that is abundantly expressed in the brain and plays a critical role in neurodevelopmental processes, such as neuronal morphogenesis and plasticity. This study provides the first characterization of the neurobehavioural phenotype of 1 year old Cdkl5-null mice and demonstrates that stimulation of the serotonin receptor 7 (5-HTR) with the agonist molecule LP-211 (0.25 mg/kg once/day for 7 days) partially rescues the abnormal phenotype and brain molecular alterations in Cdkl5-null male mice. In particular, LP-211 treatment completely normalizes the prepulse inhibition defects observed in Cdkl5-null mice and, at a molecular level, restores the abnormal cortical phosphorylation of rpS6, a downstream target of mTOR and S6 kinase, which plays a direct role in regulating protein synthesis. Moreover, we demonstrate for the first time that mitochondria show prominent functional abnormalities in Cdkl5-null mouse brains that can be restored by pharmacological stimulation of brain 5-HTR.
Neuropharmacology, 2019; 144
31108160: Cosentino L, Vigli D, Franchi F, Laviola G, De Filippis B
Rett syndrome before regression: A time window of overlooked opportunities for diagnosis and intervention.
Rett syndrome (RTT) is a rare neurological disorder primarily affecting females, causing severe cognitive, social, motor and physiological impairments for which no cure currently exists. RTT clinical diagnosis is based on the peculiar progression of the disease, since patients show an apparently normal initial development with a subsequent sudden regression at around 2 years of age. Accumulating evidences are rising doubts regarding the absence of early impairments, hence questioning the concept of regression. We reviewed the published literature addressing the pre-symptomatic stage of the disease in both patients and animal models with a particular focus on behavioral, physiological and brain abnormalities. The emerging picture delineates subtle, but reliable impairments that precede the onset of overt symptoms whose bases are likely set up already during embryogenesis. Some of the outlined alterations appear transient, suggesting compensatory mechanisms to occur in the course of development. There is urgent need for more systematic developmental analyses able to detect early pathological markers to be used as diagnostic tools and precocious targets of time-specific interventions.
Neurosci Biobehav Rev, 2019; 107
31175878: Cosentino L, Vigli D, Medici V, Flor H, Lucarelli M, Fuso A, De Filippis B
Methyl-CpG binding protein 2 functional alterations provide vulnerability to develop behavioral and molecular features of post-traumatic stress disorder in male mice.
Post-traumatic stress disorder (PTSD) is a mental disorder characterized by symptoms of persistent anxiety arising after exposure to traumatic events. Stress susceptibility due to a complex interplay between genetic and environmental factors plays a major role in the disease etiology, although biological underpinnings have not been clarified. We hypothesized that aberrant functionality of the methyl-CpG binding protein 2 (MECP2), a master regulator of experience-dependent epigenetic programming, confers susceptibility to develop PTSD-like symptomatology in the aftermath of traumatic events. Transgenic male mice expressing a truncated form of MeCP2 protein (MeCP2-308) were exposed at adulthood to a trauma in the form of high-intensity footshocks. The presence and duration of PTSD-like symptoms were assessed and compared to those of trauma-exposed wild type littermates and MeCP2-308 mice subjected to a mild stressor. The effects of fluoxetine, a prime pharmacological PTSD treatment, on PTSD-like symptomatology were also explored. Trauma-exposed MeCP2-308 mice showed long-lasting hyperresponsiveness to both correct and incorrect predictors of the trauma and persistent increased avoidance of trauma-related cues. Traumatized MeCP2-308 mice also displayed abnormal post-traumatic plasma levels of the stress hormone corticosterone and altered peripheral gene expression mirroring that of PTSD patients. Fluoxetine improved PTSD-like symptoms in trauma-exposed MeCP2-308 mice. These findings provide evidence that MeCP2 dysfunction results in increased susceptibility to develop PTSD-like symptoms after trauma exposure, and identify trauma-exposed MeCP2-308 mice as a new tool to investigate the underpinnings of PTSD vulnerability.
Neuropharmacology, 2019; 160
33010341: Vigli D, Cosentino L, Pellas M, De Filippis B
Chronic Treatment with Cannabidiolic Acid (CBDA) Reduces Thermal Pain Sensitivity in Male Mice and Rescues the Hyperalgesia in a Mouse Model of Rett Syndrome.
Rett syndrome (RTT) is a rare neurologic disorder, characterized by severe behavioural and physiological symptoms. RTT is caused by mutations in the MECP2 gene in about 95% of cases and to date no cure is available. Recent evidence suggests that non-euphoric phytocannabinoids (pCBs) extracted from Cannabis sativa may represent innovative therapeutic molecules for RTT, with the cannabinoid cannabidivarin having beneficial effects on behavioural and brain molecular alterations in RTT mouse models. The present study evaluated the potential therapeutic efficacy for RTT of cannabidiolic acid (CBDA; 0.2, 2, 20 mg/kg through intraperitoneal injections for 14 days), a pCB that has proved to be effective for the treatment of nausea and anxiety in rodents. This study demonstrates that systemic treatment with the low dose of CBDA has anti-nociceptive effects and reduces the thermal hyperalgesia in 8 month-old MeCP2-308 male mice, a validated RTT mouse model. CBDA did not affect other behavioural or molecular parameters. These results provide support to the antinociceptive effects of CBDA and

stress the need for further studies aimed at clarifying the mechanisms underlying the abnormal pain perception in RTT. Neuroscience, 2021; 453

32492904: Zuliani I, Urbinati C, Valenti D, Quattrini MC, Medici V, Cosentino L, Pietraforte D, Di Domenico F, Perluigi M, Vacca RA, De Filippis B

The Anti-Diabetic Drug Metformin Rescues Aberrant Mitochondrial Activity and Restrains Oxidative Stress in a Female Mouse Model of Rett Syndrome.

Metformin is the first-line therapy for diabetes, even in children, and a promising attractive candidate for drug repurposing. Mitochondria are emerging as crucial targets of metformin action both in the periphery and in the brain. The present study evaluated whether treatment with metformin may rescue brain mitochondrial alterations and contrast the increased oxidative stress in a validated mouse model of Rett syndrome (RTT), a rare neurologic disorder of monogenic origin characterized by severe behavioral and physiological symptoms. No cure for RTT is available. In fully symptomatic RTT mice (12 months old MeCP2-308 heterozygous female mice), systemic treatment with metformin (100 mg/kg ip for 10 days) normalized the reduced mitochondrial ATP production and ATP levels in the whole-brain, reduced brain oxidative damage, and rescued the increased production of reactive oxidizing species in blood. A 10-day long treatment with metformin also boosted pathways related to mitochondrial biogenesis and antioxidant defense in the brain of metformin-treated RTT mice. This treatment regimen did not improve general health status and motor dysfunction in RTT mice at an advanced stage of the disease. Present results provide evidence that systemic treatment with metformin may represent a novel, repurposable therapeutic strategy for RTT.

J Clin Med, 2020; 9

33598674: Napoletani G, Vigli D, Cosentino L, Grieco M, Talamo MC, Lacivita E, Leopoldo M, Laviola G, Fuso A, d'Erme M, De Filippis B

Stimulation of the Serotonin Receptor 7 Restores Brain Histone H3 Acetylation and MeCP2 Corepressor Protein Levels in a Female Mouse Model of Rett Syndrome.

Rett syndrome (RTT) is a rare neurological disorder caused by mutations in the X-linked MECP2 gene, characterized by severe behavioral and physiological impairments for which no cure is available. The stimulation of serotonin receptor 7 (5-HT7R) with its selective agonist LP-211 (0.25 mg/kg/day for 7 days) was proved to rescue neurobehavioral alterations in a mouse model of RTT. In the present study, we aimed at gaining insight into the mechanisms underpinning the efficacy of 5-HT7R pharmacological stimulation by investigating its epigenetic outcomes in the brain of RTT female mice bearing a truncating MeCP2 mutation. Treatment with LP-211 normalized the reduced histone H3 acetylation and HDAC3/NCoR levels, and increased HDAC1/Sin3a expression in RTT mouse cortex. Repeated 5-HT7R stimulation also appeared to strengthen the association between NCoR and MeCP2 in the same brain region. A different profile was found in RTT hippocampus, where LP-211 rescued H3 hyperacetylation and increased HDAC3 levels. Overall, the present data highlight a new scenario on the relationship between histone acetylation and serotoninergic pathways. 5-HT7R is confirmed as a pivotal therapeutic target for the recovery of neuronal function supporting the translational value of this promising pharmacological approach for RTT.

J Neuropathol Exp Neurol, 2021; 80

33655553: Cosentino L, Bellia F, Pavoncello N, Vigli D, D'Addario C, De Filippis B

Methyl-CpG binding protein 2 dysfunction provides stress vulnerability with sex- and zygosity-dependent outcomes.

Stress vulnerability is a critical factor for the development of trauma-related disorders; however, its biological underpinnings are not clear. We demonstrated that dysfunctions in the X-linked epigenetic factor methyl-CpG binding protein 2 (MeCP2) provide trauma vulnerability in male mice. Given the prominent role of sex in stress outcomes, we explored the effects of MeCP2 hypofunctionality in females. Female mice carrying truncated MeCP2 (heterozygous and homozygous) and wild type controls (wt) were tested for fear memory. Stress-induced corticosterone release and brain expression of hypothalamic-pituitary-adrenal (HPA) axis regulatory genes were also evaluated in wt and mutant mice of both sexes. Although heterozygous females displayed a normal stress-related behavioural profile, homozygous mice showed enhanced memory recall for the threatening context compared to wt, thus recapitulating the phenotype previously evidenced in hemizygous males. Interestingly, MeCP2 truncation abolished the sex differences in stress-induced corticosterone release, which was found increased in mutant males, whereas blunted in mutant females in a zygosity-independent manner. Although heterozygous mice did not differ from controls, homozygous females and hemizygous males showed increased hypotalamic Crh and Avp mRNAs and a differentially altered expression of Fkbp5 in cortical areas. Present results demonstrate that in female mice carrying truncated MeCP2, altered stress responsivity is driven by homozygosity, whereas heterozygosity does not lead to maladaptive stress outcomes. MeCP2 dysfunctions thus provide stress vulnerability in mice with sex- and zygosity-dependent outcomes.

Eur J Neurosci, 2022; 55

34201747: Urbinati C, Cosentino L, Germinario EAP, Valenti D, Vigli D, Ricceri L, Laviola G, Fiorentini C, Vacca RA, Fabbri

A, De Filippis B
Treatment with the Bacterial Toxin CNF1 Selectively Rescues Cognitive and Brain Mitochondrial Deficits in a Female Mouse Model of Rett Syndrome Carrying a MeCP2-Null Mutation.
Rett syndrome (RTT) is a rare neurological disorder caused by mutations in the X-linked gene and a major cause of intellectual disability in females. No cure exists for RTT. We previously reported that the behavioural phenotype and brain mitochondria dysfunction are widely rescued by a single intracerebroventricular injection of the bacterial toxin CNF1 in a RTT mouse model carrying a truncating mutation of the gene (MeCP2-308 mice). Given the heterogeneity of mutations in RTT patients, we tested the CNF1 therapeutic efficacy in a mouse model carrying a null mutation (MeCP2-Bird mice). CNF1 selectively rescued cognitive defects, without improving other RTT-related behavioural alterations, and restored brain mitochondrial respiratory chain complex activity in MeCP2-Bird mice. To shed light on the molecular mechanisms underlying the differential CNF1 effects on the behavioural phenotype, we compared treatment effects on relevant signalling cascades in the brain of the two RTT models. CNF1 provided a significant boost of the mTOR activation in MeCP2-308 hippocampus, which was not observed in the MeCP2-Bird model, possibly explaining the differential effects of CNF1. These results demonstrate that CNF1 efficacy depends on the mutation beared by MeCP2-mutated mice, stressing the need of testing potential therapeutic approaches across RTT models.
Int J Mol Sci, 2021; 22
26952805: Romano E, Cosentino L, Laviola G, De Filippis B
Genes and sex hormones interaction in neurodevelopmental disorders.
The prevalence, age of onset and symptomatology of many neurodevelopmental disorders strongly differ between genders. This review examines sex biases in human neurodevelopmental disorders and in validated animal models. A focus is made on disorders of well-established genetic origin, such as Rett syndrome, CDKL5-associated disorders, Fragile X and Down syndrome. Autism is also addressed, given its paradigmatic role as a sex-biased neurodevelopmental disorder. Reviewed literature confirms that a complex interaction between genetic factors and sex hormones may underlie the differential susceptibility of genders and may impact the severity of symptoms in most of the analyzed neurodevelopmental disorders. Even though further studies addressing the advantages and disadvantages conferred by biological sex in this class of disorders are needed to disentangle the underlying mechanisms, present findings suggest that modulation of sex steroid-related pathways may represent an innovative approach for these diseases. Much effort is now expected to unravel the potential therapeutic efficacy of drugs targeting sex hormones-related signaling pathways in neurodevelopmental disorders of well-established genetic origin.
Neurosci Biobehav Rev, 2016; 67

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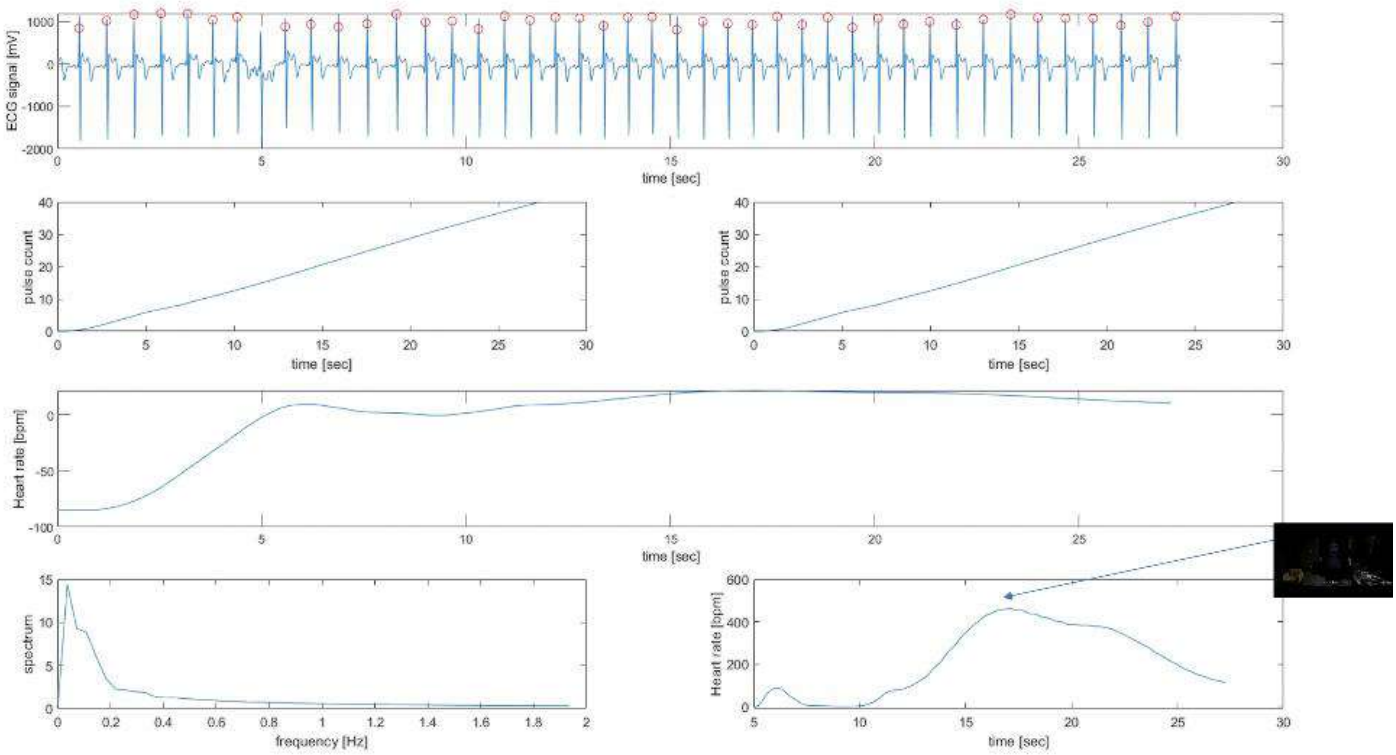
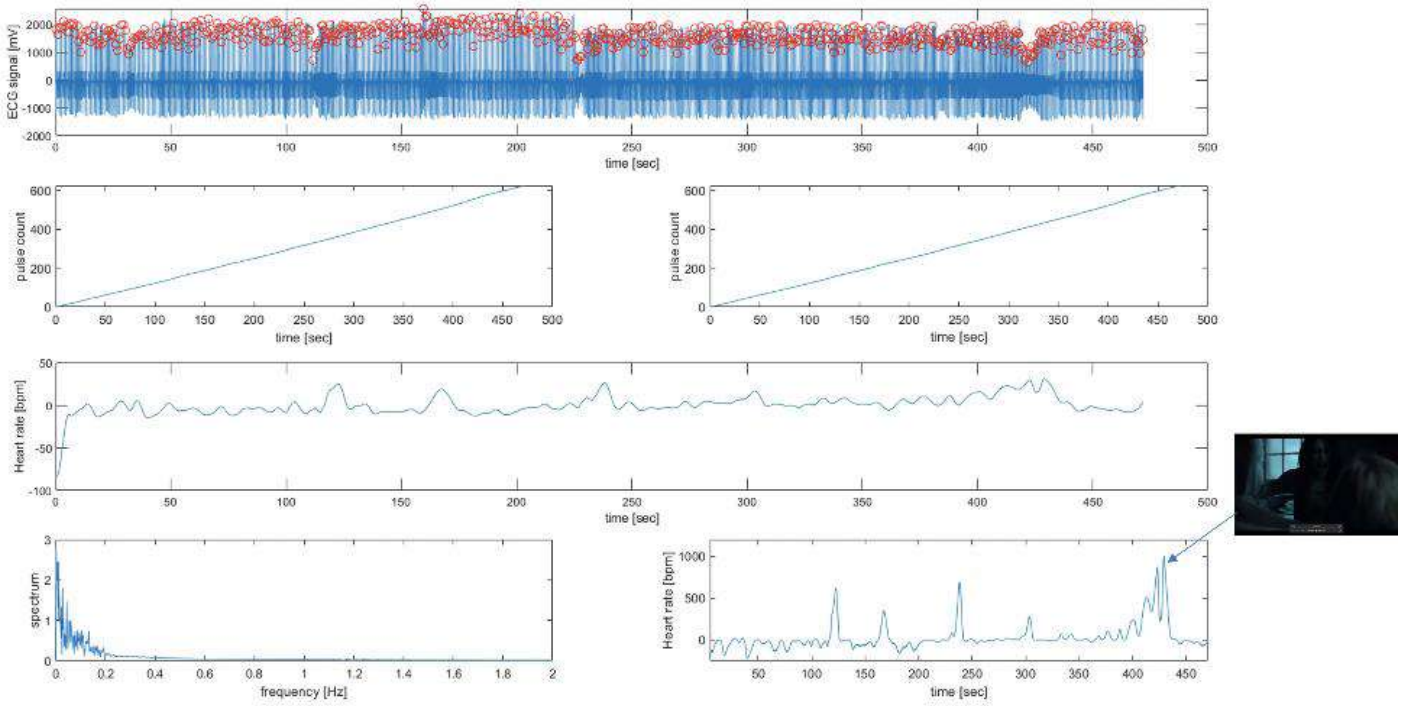
USING THE HEART RATE AS A SENSOR TO DETECT EVOKED RESPONSES OF THE AUTONOMOUS NERVOUS SYSTEM IN BRAIN-HEART COMMUNICATIONS

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

Harish Kambampati
Tor Vergata University of Rome, Department Of Industrial Engineering, Room No:3158, Roma, Italy

Abstract: Observing the instantaneous heart rate variability (HRV) under the sympathetic nervous system while watching scary, thrill, suspense and action videos that influence cardiac muscle activities that trigger automatic physiological reactions. We took males and females aged between 20-60 as subjects, we attached one lead IOT based wireless-enabled ECG to subjects V1 and V2 positions(**V1 is placed to the right of the sternal border, and V2 is placed at the left of the sternal border)** on the chest and recorded the ECG signal data, while they are watching videos which have scary and action scenes at different time intervals and the data were analyzed and observed in MATLAB. Every subject shows a normal heart rate While watching the pleasant and sudden high Heart rate appears at the scary scenes. After detecting the evoked responses at the particular time interval, It matches the scary clip or stressful or action scenes appears, we can understand that Vagus nerve stimulation occurs under the sympathetic nervous system when this subject is in stress or fear conditions. Results are surprisingly reliable and this method can be extended to many other signals whose frequency is changing over time. We used one lead ECG inbuilt with Bluetooth attached to a strap containing two electrodes. “MOVESENSE” IOS Application to record the data from subjects. MATLAB R2021b for analyzing ECG data and Microsoft Excel, IOS Device and Laptop **Keywords:** Heart rate variability (HRV), Autonomous nervous system (ANS) Sympathetic nervous system (SNS), Parasympathetic nervous system (PNS) ECG, Vagus nerve, MATLAB.

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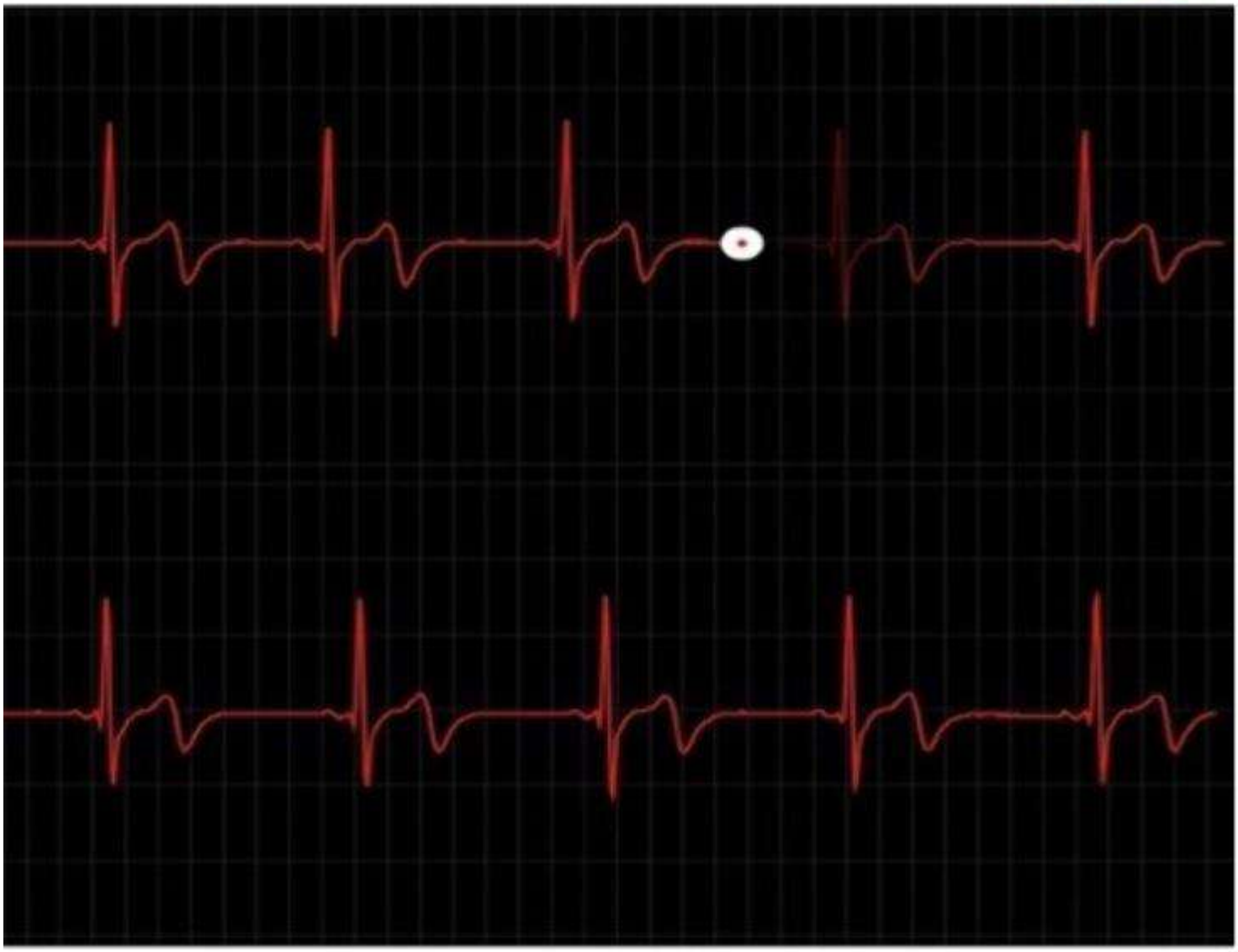
Electrocardiography



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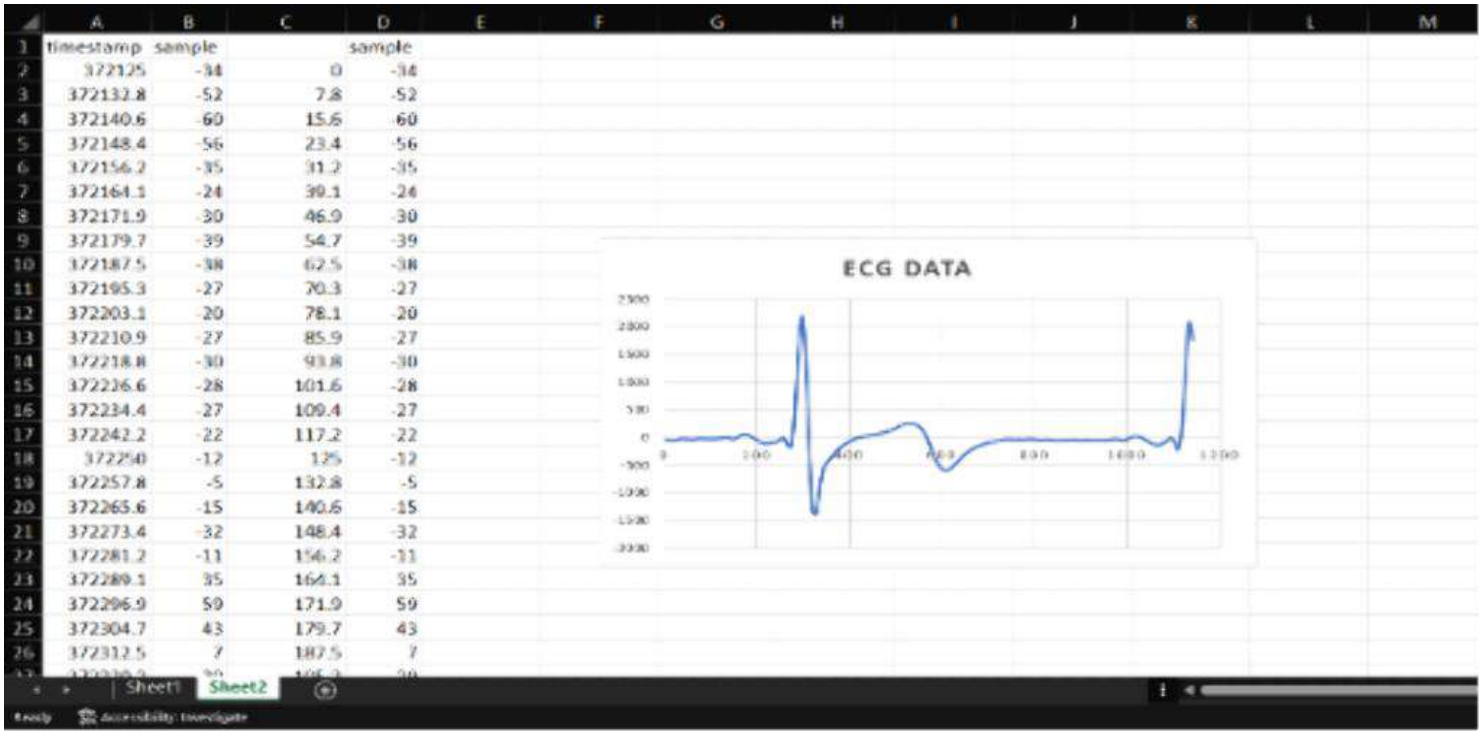

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6	372156.2	-35	31.2	-35
7	372164.1	-24	39.1	-24
8	372171.9	-30	46.9	-30
9	372179.7	-39	54.7	-39
10	372187.5	-38	62.5	-38
11	372195.3	-27	70.3	-27
12	372203.1	-20	78.1	-20
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22	372281.2	-11	156.2	-11
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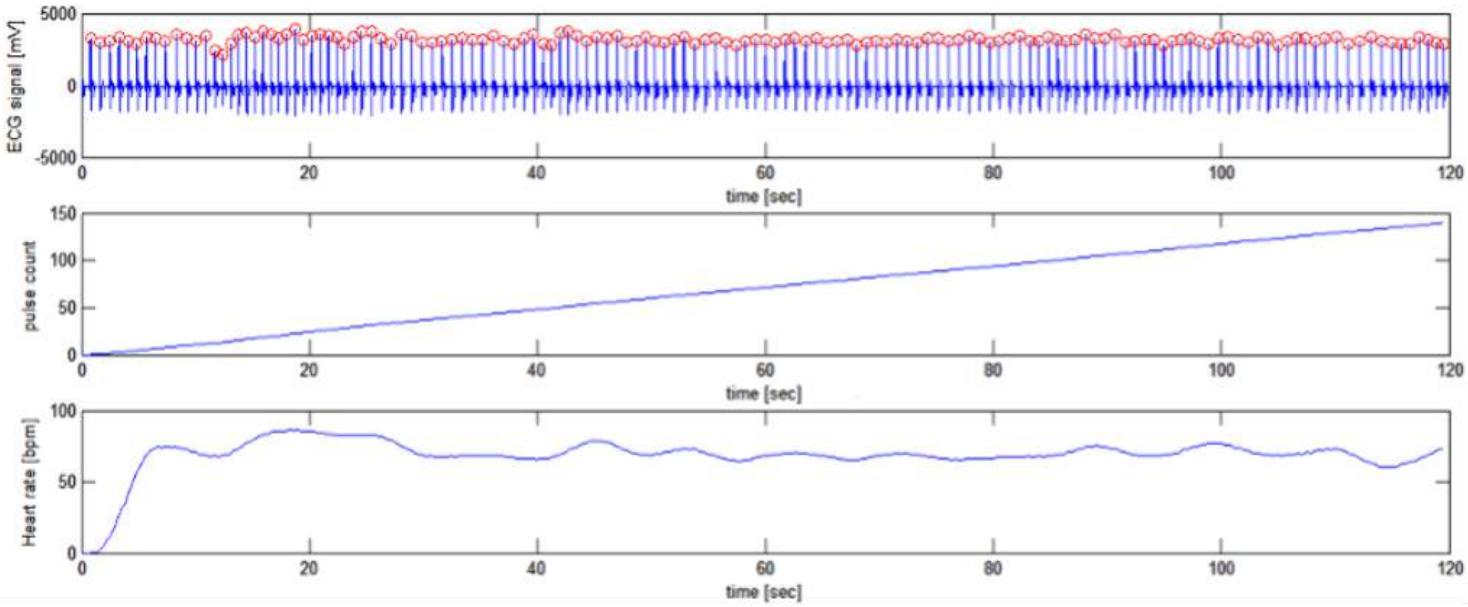

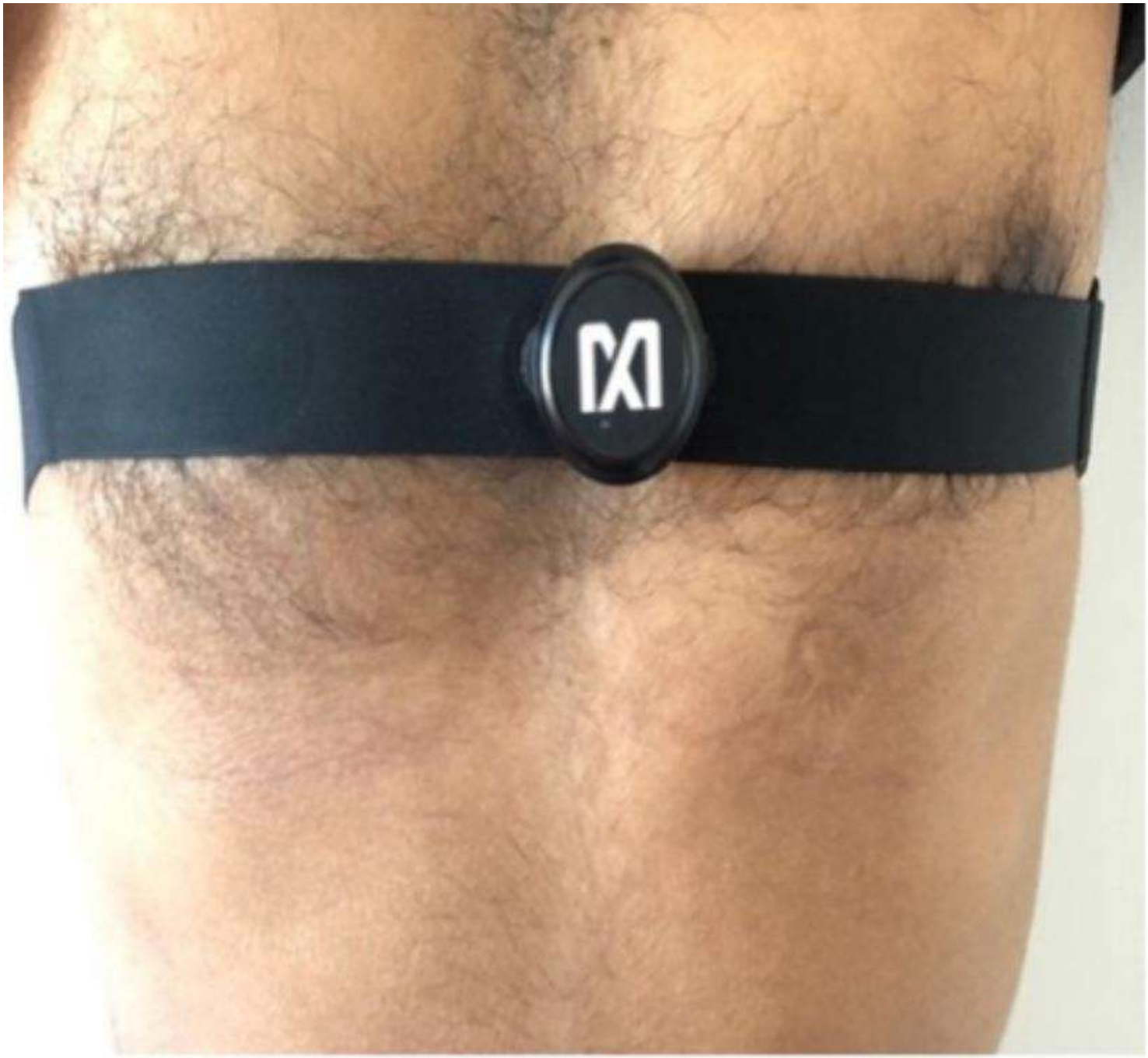

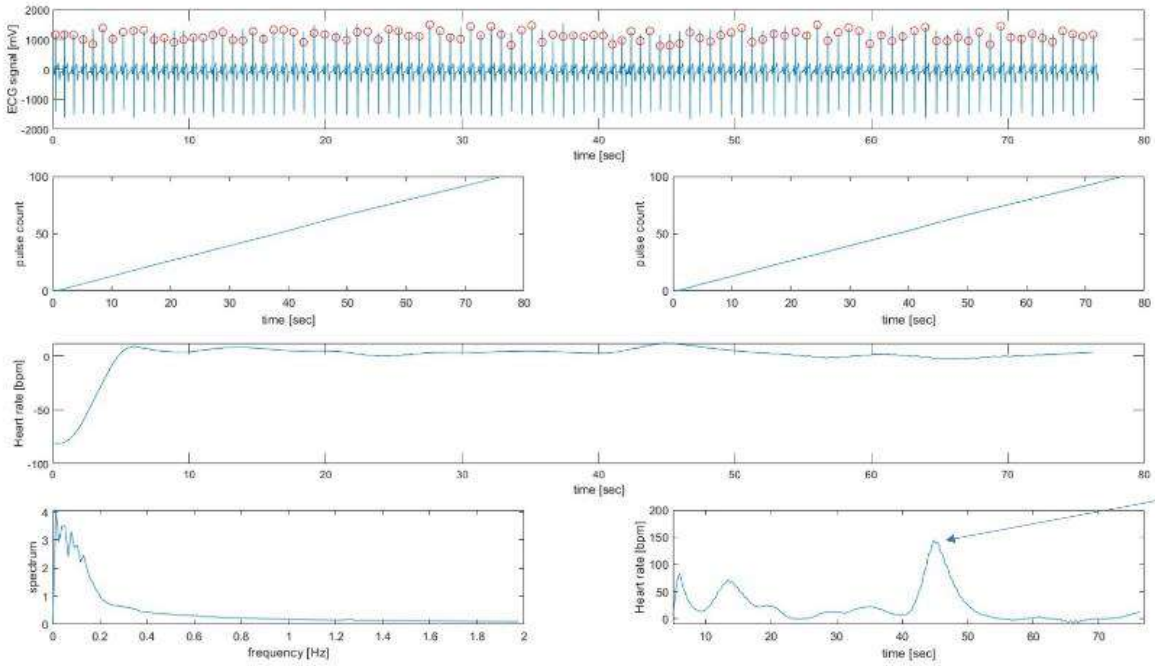

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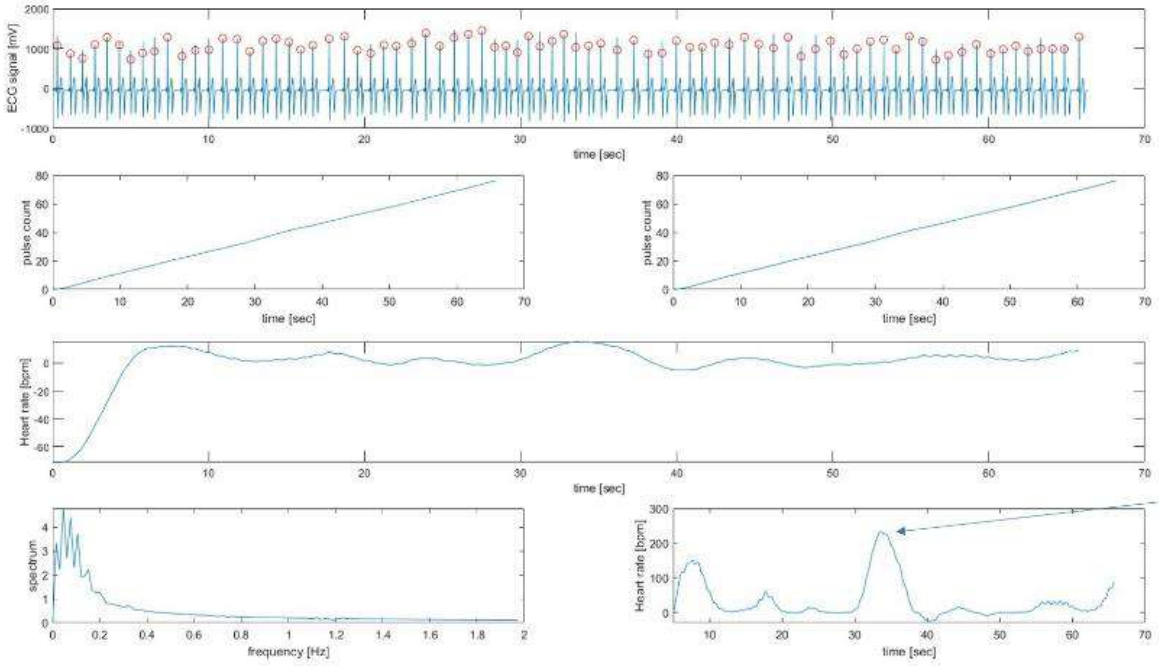

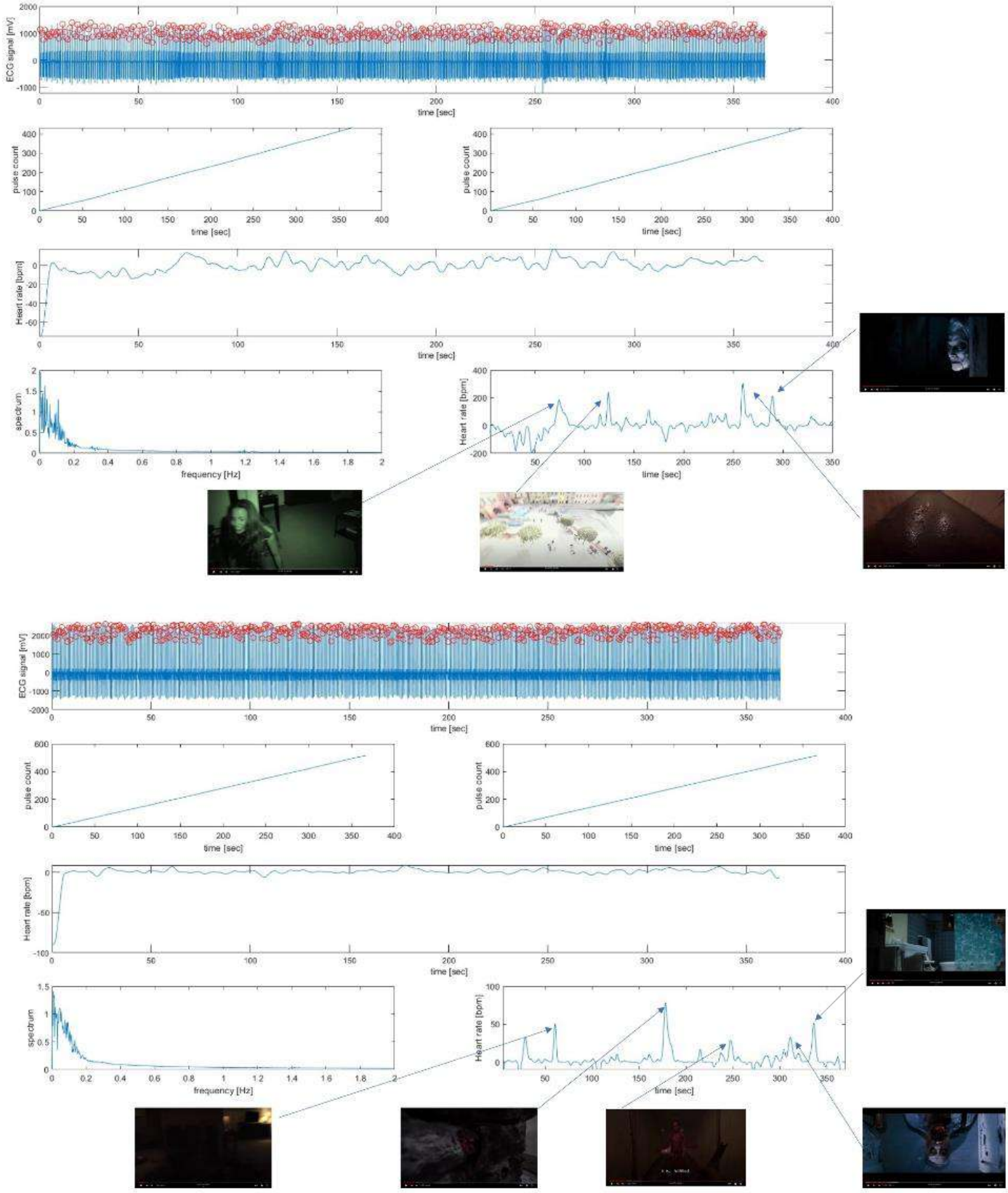
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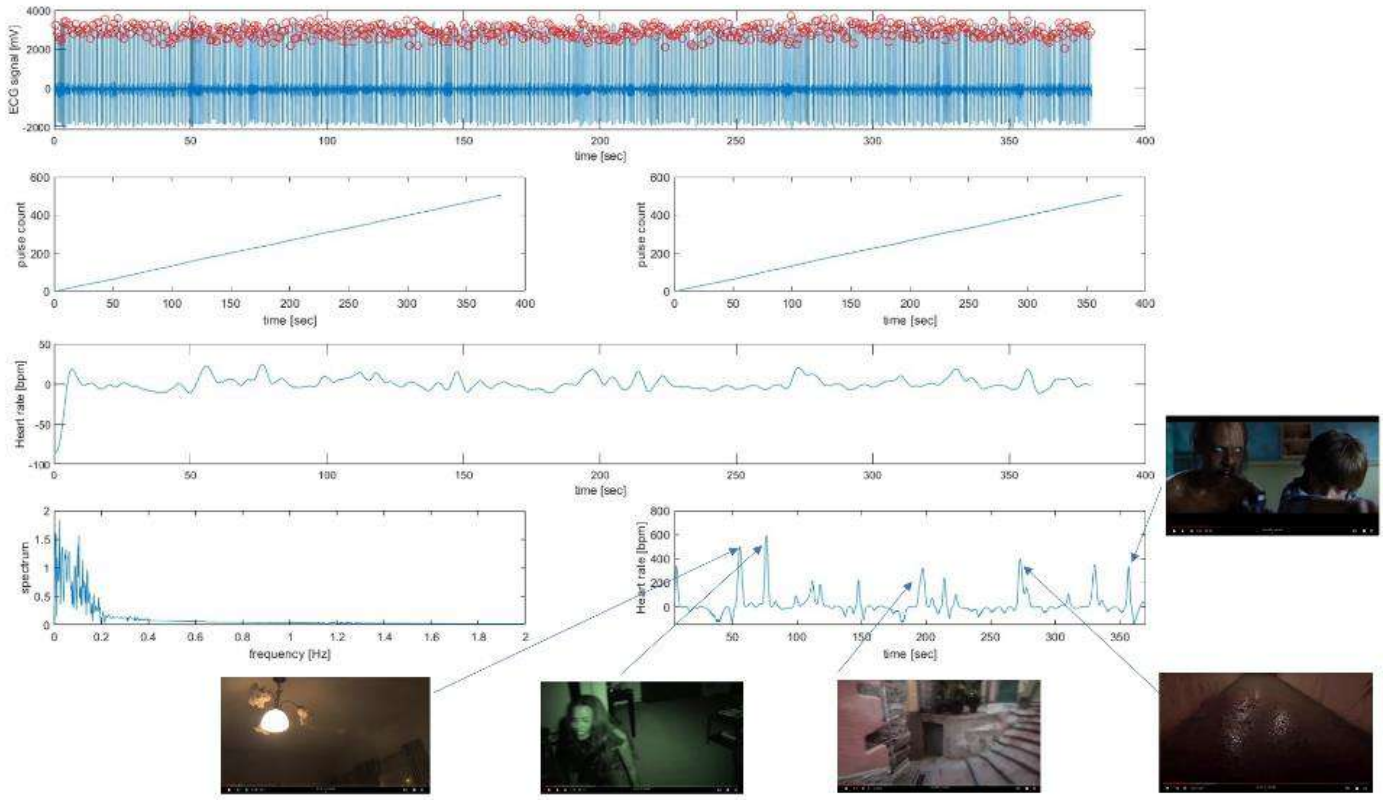
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Heart rate [bpm]
time [sec]

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CONSEQUENCES OF THE LACK OF ONE OR MULTIPLE DYSTROPHIN ISOFORMS ON COGNITION AND BEHAVIOUR IN PREDNISOLONE TREATED MOUSE MODELS OF DUCHENNE MUSCULAR DYSTROPHY

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Duchenne muscular dystrophy (DMD) is a recessive X-linked neuromuscular disorder. Next to severe muscle wasting, patients present with cognitive impairments. Several dystrophin isoforms are expressed in the brain. A correlation between the amount of missing isoforms and cognitive problems has been found in DMD patients, however knowledge about this correlation in mouse models is limited. Standards of care of patients includes corticosteroid treatment, prednisolone (PDN). PDN influences cognition in healthy individuals. The effects of chronic PDN treatment on cognition has not been investigated yet in DMD patients or mouse models. To investigate this, C57BL/6J (WT), *mdx* (lacking Dp427) and *mdx*^{4cv}(lacking Dp427+Dp140) males aged 7 weeks were treated with PDN or placebo and subjected to behavioural tests to analyse anxiety, fear and learning for a duration of 9 weeks. *Mdx*^{4cv} mice showed a more severe anxiety phenotype compared to *mdx* in the open field and dark light box. Both *mdx* and *mdx*^{4cv} males showed an increased fear response in the restraint unconditioned fear test compared to WTs, however no differences were observed between the DMD strains. Chronic PDN treatment did not seem to affect anxiety or fear response in de DMD strains. Results of the memory tasks are still being analysed. These results confirm that also in mice, the amount of missing isoform correlates with the severity of cognitive problems. PDN treatment does not seem to increase anxiety or the fear response. Further analyses will show whether or not the missing isoforms or PDN affect other cognitive processes.

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BOARD NUMBER: S06-047

REACTIVITY TO CONDITIONED THREAT CUES IS DISTINCT FROM EXPLORATORY DRIVE IN THE ELEVATED PLUS-MAZE.

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Commonly used tasks for studying behaviour akin to fear and anxiety in rodent models are pavlovian threat conditioning and the elevated plus maze (EPM) respectively. These two tasks have been used extensively, yet research into whether they interact is scarce. AIMS: We investigated whether recall of an aversive memory, across contextual, odour or auditory modalities, would potentiate anxiety-like behaviour in the elevated plus maze. METHODS: To better characterize potential impacts of prior aversive experience on EPM performance a series of experiments were carried out that systematically varied the time since stimulus presentation and the modality of the conditioning stimulus. Furthermore, we examined whether the EPM measurements taken days before or after threat conditioning measurements held any relationship to conditioned freezing or production of ultrasonic alarm calls. RESULTS: The data did not support that memory recall, even over a series of timepoints, could influence EPM behaviour. Furthermore, there was no correlation between EPM behaviour and conditioned freezing in independent cohorts tested in the EPM before or after auditory threat conditioning. Further analysis found the production of 22kHz ultrasonic vocalisations revealed the strongest responders to a conditioned threat cue. CONCLUSIONS: These results are of particular importance for consideration when using the EPM and threat conditioning to identify individual differences, and the possibility to use the tasks in batteries of tests without cross-task interference. These presented findings call for caution in generalisation of interpretations from the typical range of behaviour in the EPM across other aversively motivated tasks.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-048

THE EFFECT OF THE SOCIAL ISOLATION STRESS ON FEAR EXTINCTION – THE ROLE OF THE DOPAMINERGIC AND ENDOGENOUS OPIOID NEUROTRANSMISSION

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Aims: The aim of the study was to assess the influence of the recurrent social isolation stress on the aversive memory extinction as well as dopaminergic, endogenous opioid neurotransmission in the amygdala. Methods: Male Wistar rats were aversively conditioned and then exposed to three extinction sessions. The control group was only exposed to experimental cage. The conditioned animals were divided into two groups – extinguished group and socially isolated extinguished group. The stressed animals were socially isolated for 48 h before each extinction. After the last exposition to the aversive context, expression of dopamine type 2 (D2) receptors in the subnuclei of the amygdala (immunocytochemistry), KOR expression and D2 (mRNA) and epigenetic factors (miRNA-128 and miRNA-142) in the amygdala were analyzed. Moreover, the levels of beta-endorphin and corticosterone were evaluated in the plasma (ELISA). Results: Social isolation stress decreased the fear extinction rate. After third exposition to aversive context socially isolated animals were characterized by higher expression of D_2 receptors in the basal amygdala compared to controls. Socially isolated extinguished rats were characterized by lower KOR expression in amygdala and plasma beta-endorphins level compared to control group. Moreover, extinguished rats presented lower levels of miRNA-128 and miRNA-142 in the amygdala compared to control and isolated extinguished animals. Conclusions: Observed changes suggest that social isolation stress is an important factor that impairs aversive memory extinction via affecting dopaminergic neurotransmission, endogenous opioid system and epigenetic mechanisms in the amygdala. The study was supported by Grant No 2018/28/C/NZ7/00240, National Science Centre, Krakow, Poland.

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BOARD NUMBER: S06-049

INVESTIGATING THE ROLE OF CEREBELLAR ENDOCANNABINOIDS IN CONDITIONED FEAR EXTINCTION

POSTER SESSION 06 - SECTION: NEUROBIOLOGY OF FEAR AND ANXIETY

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Anxiety disorders can be associated with an individual's inability to extinguish the memory of a conditioned fear response. Endocannabinoids are known to be involved in fear extinction. The cerebellum has also been implicated in fear extinction and its cortex contains the highest density of CB1 receptors. However, the role of endocannabinoids in the cerebellum in relation to fear extinction has not been fully investigated. To assess heterogeneity of behavioural responses during fear extinction, male Sprague Dawley rats (n=12), were tested in an auditory cued fear conditioning paradigm over three days consisting of conditioning, extinction and extinction retention testing. Consistent with previous studies we found that individual animals extinguish their freezing response on different time scales indicating behavioural heterogeneity in fear responses. To investigate the contribution of the endocannabinoid system in the cerebellar cortex the synthesis of the 2 major endocannabinoids, anandamide and 2-AG, were decreased by inhibiting the enzymes NAPE-PLD and DAGL using combined LEI-401 and DO34 respectively, prior to extinction. Animals (n = 16) were implanted with single cannula targeting lobules V/VI of the midline cerebellar vermal cortex to deliver the drugs or vehicle control. Here we present preliminary findings comparing drugs vs vehicle control on the effect of freezing and ultrasonic vocalisations in extinction and extinction retention.

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BOARD NUMBER: S06-050

DIVERSE ROLES OF THE VARIOUS NEURONAL CLASSES IN THE PRELIMBIC CORTEX IN SOCIAL BONDING

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Although many studies indicate a crucial role of the prefrontal cortex (PFC) in social attachment, the involvement of various neuronal classes in that process remains unexplored. Thus, we investigated the effects of the chemogenetic manipulation of the pyramidal neurons (PNs), as well as inhibitory interneurons expressing parvalbumin (PV+), somatostatin (SOM+) and vasoactive intestinal peptide (VIP+) in the prelimbic part (PL) of the PFC, on the spontaneous social interactions with familiar conspecifics. Animals were tested in Eco-HAB, an ecologically relevant, RFID-based system for assessment of spontaneous social behavior in group-housed mice. Using genetically modified mice combined with the PSAM/PSEM-based chemogenetic approach, we performed a time-constrained, cell-specific manipulations in the PL. We tested subjects' social behavior after the systemic administration of the drug (PSEM) activating virally introduced artificial ligand-gated ion channels (PSAM) on the specific cell class. We show that PL-constrained inhibition of the PNs, as well as activation of the PV+ interneurons attenuates sociability by decreasing the time voluntarily spend together. Notably, activating SOM+ and VIP+ cells has no impact on social bonding. Further, alongside the loss of interest in interacting with familiar conspecifics inhibition of the PNs leads to hiper interest in novel social stimuli. Notably, such behavior upon activation of the PV+ and VIP+ interneurons, while the opposite effect, loss of interest in social novelty, is observed when SOM+ cells are activated. Presented data illustrates the diverse roles various neuronal classes play in social bonding and points to the distinct neural underpinnings of processing familiar and novel social stimuli.

Pubmed:
34838526: Puścian A, Bryksa A, Kondrakiewicz L, Kostecki M, Winiarski M, Knapska E
Ability to share emotions of others as a foundation of social learning.
The natural habitats of most species are far from static, forcing animals to adapt to continuously changing conditions. Perhaps the most efficient strategy addressing this challenge consists of obtaining and acting upon pertinent information from others through social learning. We discuss how animals transfer information via social channels and what are the benefits of such exchanges, playing out on different levels, from theperception of socially delivered information to emotional sharing, manifesting themselves across different taxa of increasing biological complexity. We also discuss how social learning is influenced by different factors including pertinence of information for survival, the complexity of the environment, sex, genetic relatedness, and most notably, the relationship between interacting partners. The results appear to form a consistent picture once we shift our focus from emotional contagion as a prerequisite for empathy onto the role of shared emotions in providing vital information about the environment. From this point of view, we can propose approaches that are the most promising for further investigation of complex social phenomena, including learning from others.
Neurosci Biobehav Rev, 2022; 132
32941853: Harda Z, Spyrka J, Jastrzębska K, Szumiec Ł, Bryksa A, Klimczak M, Polaszek M, Gołda S, Zajdel J, Misiołek K, Błasiak A, Rodriguez Parkitna J
Loss of mu and delta opioid receptors on neurons expressing dopamine receptor D1 has no effect on reward sensitivity.
Opioid signaling controls the activity of the brain's reward system. It is involved in signaling the hedonic effects of rewards and has essential roles in reinforcement and motivational processes. Here, we focused on opioid signaling through mu and delta receptors on dopaminoceptive neurons and evaluated the role these receptors play in reward-driven behaviors. We generated a genetically modified mouse with selective double knockdown of mu and delta opioid receptors in neurons expressing dopamine receptor D1. Selective expression of the transgene was confirmed using immunostaining. Knockdown was validated by measuring the effects of selective opioid receptor agonists on neuronal membrane currents using whole-cell

patch clamp recordings. We found that in the nucleus accumbens of control mice, the majority of dopamine receptor D1-expressing neurons were sensitive to a mu or delta opioid agonist. In mutant mice, the response to the delta receptor agonist was blocked, while the effects of the mu agonist were strongly attenuated. Behaviorally, the mice had no obvious impairments. The mutation did not affect the sensitivity to the rewarding effects of morphine injections or social contact and had no effect on preference for sweet taste. Knockdown had a moderate effect on motor activity in some of the tests performed, but this effect did not reach statistical significance. Thus, we found that knocking down mu and delta receptors on dopamine receptor D1-expressing cells does not appreciably affect some of the reward-driven behaviors previously attributed to opioid signaling.
Neuropharmacology, 2020; 180

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BOARD NUMBER: S06-051

AN EXTENDED AMYGDALA-HABENULA CIRCUIT FOR PARENTAL BEHAVIOURS

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Parental behaviours are vital for the optimal development of newborns. Importantly, these behaviours are preserved across species and their correct execution requires the wiring of highly-conserved neuronal circuits. Accordingly, published studies depicted the lateral habenula (LHb) as a region embedded in the parental neuronal network and contributing to parental behaviours, although the functional understanding of its participation in parenting remains unknown. Here, we monitored virgin female mice exposed to foreign pups while employing single-cell calcium imaging, viral-based circuit mapping and genetic approaches. Virgin females display stereotypical parental behaviours including grooming, nest building and retrieval of the pup to a nest. Single-cell microendoscopic calcium imaging shows that a territorially-segregated cluster of LHb cells increased their activity during pup retrieval. We further demonstrate that this population is defined by its excitatory input from the bed nucleus of the stria terminalis (^{BNST}LHb), a central node in the parental circuit. Accordingly, optogenetic activation and inhibition of BNST axons in the LHb promoted and impaired pup retrieval, respectively. Merging imaging of BNST-recipient LHb neurons with decoding analysis highlights specific processing of pup retrieval. Finally, we interrogated the transcriptome of ^{BNST}LHb cells in virgin female mice to reveal i. genetic signatures consistent with a role in parental behaviours and ii. a gene expression similar to mothers but divergent from non-parental virgin male mice. Altogether this study uncovers a neuronal circuit element fundamental for specific parental behaviours.

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BOARD NUMBER: S06-052

BRAIN CODING OF MATERNAL BEHAVIOUR: ROLE OF THE TAIL OF THE VENTRAL TEGMENTAL AREA

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Maternal behaviour entails an increased motivation for pups, which is reflected in a reward-related response occurring in females when pups are present. Motivated behaviours are critically dependent on brain dopamine systems. Thus, understanding its functioning is necessary to elucidate how motivation and reinforcement occur in the brain in response to drugs of abuse or natural rewards, such as social/maternal rewards. The activity of dopaminergic pathways mainly depends on the excitatory and inhibitory balance exerted by glutamatergic and GABAergic afferents to dopamine cells, respectively. The tail of the ventral tegmental area (tVTA) or rostromedial tegmental nucleus (RMTg), is a major inhibitory control center of dopamine midbrain cells, whose integration in reward and aversion brain circuits has been accepted, however there is no evidence of its involvement in social or maternal reward. Thus, in the present study, we explored the role of the activity of the tVTA/RMTg in maternal behaviour in rats. To this end, pregnant Sprague-Dawley female rats underwent stereotaxic implantation of a guide cannula through the tVTA/RMTg (E15-E18). During the first week after delivery, female rats underwent intra-tVTA/RMTg microinjections of vehicle and/or several pharmacological agents (glutamate, DAMGO or muscimol) in a cross-design, in order to explore the impact of the tVTA/RMTg activity (activation and/or inhibition) on maternal behaviour in rats. Our results revealed an involvement of the tVTA/RMTg in maternal reward processing and suggest a possible role of this brain region in the development of maternal behaviour. Funding: GV/2020/173, UJI-A2019-14, PID2019-107332GB-C21, ACIF/2021/330.

Pubmed:

33978813: Ferreira N, Gram H, Sorrentino ZA, Gregersen E, Schmidt SI, Reimer L, Betzer C, Perez-Gozalbo C, Beltoja M, Nagaraj M, Wang J, Nowak JS, Dong M, Willén K, Cholak E, Bjerregaard-Andersen K, Mendez N, Rabadia P, Shahnawaz M, Soto C, Otzen DE, Akbey Ü, Meyer M, Giasson BI, Romero-Ramos M, Jensen PH
Multiple system atrophy-associated oligodendroglial protein p25α stimulates formation of novel α-synuclein strain with enhanced neurodegenerative potential.
Pathology consisting of intracellular aggregates of alpha-Synuclein (α-Syn) spread through the nervous system in a variety of neurodegenerative disorders including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. The discovery of structurally distinct α-Syn polymorphs, so-called strains, supports a hypothesis where strain-specific structures are templated into aggregates formed by native α-Syn. These distinct strains are hypothesised to dictate the spreading of pathology in the tissue and the cellular impact of the aggregates, thereby contributing to the variety of clinical phenotypes. Here, we present evidence of a novel α-Syn strain induced by the multiple system atrophy-associated oligodendroglial protein p25α. Using an array of biophysical, biochemical, cellular, and in vivo analyses, we demonstrate that compared to α-Syn alone, a substoichiometric concentration of p25α redirects α-Syn aggregation into a unique α-Syn/p25α strain with a different structure and enhanced in vivo prodegenerative properties. The α-Syn/p25α strain induced larger inclusions in human dopaminergic neurons. In vivo, intramuscular injection of preformed fibrils (PFF) of the α-Syn/p25α strain compared to α-Syn PFF resulted in a shortened life span and a distinct anatomical distribution of inclusion pathology in the brain of a human A53T transgenic (line M83) mouse. Investigation of α-Syn aggregates in brain stem extracts of end-stage mice demonstrated that the more aggressive phenotype of the α-Syn/p25α strain was associated with an increased load of α-Syn aggregates based on a Förster resonance energy transfer immunoassay and a reduced α-Syn aggregate seeding activity based on a protein misfolding cyclic amplification assay. When injected unilaterally into the striata of wild-type mice, the α-Syn/p25α strain resulted in a more-pronounced motoric phenotype than α-Syn PFF and exhibited a "tropism" for nigro-striatal neurons compared to α-Syn PFF. Overall, our data support a hypothesis whereby oligodendroglial p25α is responsible for generating a highly prodegenerative α-Syn strain in multiple system atrophy.
Acta Neuropathol, 2021; 142

FENS Forum 2022
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BOARD NUMBER: S06-053

INVESTIGATING THE ROLE OF THE DORSAL AND VENTRAL HIPPOCAMPUS IN MICE EMPATHY AND HELPING BEHAVIOUR

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Empathy and helping behaviour are century-old psychological concepts, traditionally attributed solely to humans, but the underlying cognitive and emotional brain mechanisms are poorly understood. Increasing research evidence shows rodents also being capable of displaying empathy and helping each other. While the majority of the rodent studies were performed in rats, it has been more debatable whether mice display helping to a similar extent and which brain regions are governing this type of behaviour. We here show that helping behaviour is prominent in mice too and discuss the effect of stress, familiarity, gender and social hierarchy on the willingness to help. We further sought to understand the role of the dorsal and ventral hippocampal subregions, known to be involved in cognitive and emotional information processing respectively, in innate and learned helping. Using microendoscopic Calcium imaging, we recorded activity in the “helpers” while liberating a “victim”, over multiple days and additional control conditions. We found a subpopulation of cells that was robustly tuned for a heading angle that was facing towards the victim (+/- 10 degrees) throughout the recording, as well as during active attempts to help. We analysed how the cells activity changes during the course of learning, and compared the coding in the dorsal and ventral hippocampal subregions. These results indicate that hippocampal subregions play an important role in innate and learned helping behaviour in mice. Ongoing work investigating the causal relationship between the dorsoventral hippocampal axis and helping behaviour will be discussed.

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BOARD NUMBER: S06-054

MOTIVATION STATES THAT PROMOTE MATERNAL BEHAVIORS IN MICE

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Maternal motivation enables heightened responsivity of mothers towards offspring. While this motivational state is critical to infant survival, little is known about its regulation. Previous work in maternal rodents demonstrates that lesioning ventral tegmental area (VTA) dopamine neurons disrupts maternal motivated behaviors; and these behaviors are restored by manipulating pup sensory salience (i.e., by increasing pup cries) and maternal states (Hansen 1994 Physiol Behav). Additionally, the neurohormone oxytocin is known to convey pup sensory information to VTA (Valtcheva et al., 2021, bioRxiv) and promote VTA-mediated maternal behaviors. We aim to elucidate how infant sensory information and motivation states of the mother are integrated in VTA dopamine neurons to determine the overall levels of maternal motivation that drive context-specific offspring care. We manipulated maternal states by separating mothers (dams) from their litters for several hours. We observed that following separation, dams exhibit a short latency to approach returned pups and prolonged, continuous bouts of crouching over and nursing pups, indicating heightened levels of maternal motivation. Using this behavioral protocol, we observed a significant ($p=0.003$) increase in the density of cFOS+ neurons in the VTA of dams that were separated from their litters ($n=4$) compared to unseparated controls ($n=4$). This is consistent with our electrophysiological recordings in brain slices demonstrating that VTA dopamine neurons of separated dams are differentially modulated by oxytocin compared to unseparated control dams. Our findings suggest that VTA activity is positively associated with maternal motivation and may lower the sensory threshold for maternal behaviors.

Pubmed:
33186550: Blum ID, Keleş MF, Baz ES, Han E, Park K, Luu S, Issa H, Brown M, Ho MCW, Tabuchi M, Liu S, Wu MN
Astroglial Calcium Signaling Encodes Sleep Need in Drosophila.
Sleep is under homeostatic control, whereby increasing wakefulness generates sleep need and triggers sleep drive. However, the molecular and cellular pathways by which sleep need is encoded are poorly understood. In addition, the mechanisms underlying both how and when sleep need is transformed to sleep drive are unknown. Here, using ex vivo and in vivo imaging, we show in Drosophila that astroglial Ca signaling increases with sleep need. We demonstrate that this signaling is dependent on a specific L-type Ca channel and is necessary for homeostatic sleep rebound. Thermogenetically increasing Ca in astrocytes induces persistent sleep behavior, and we exploit this phenotype to conduct a genetic screen for genes required for the homeostatic regulation of sleep. From this large-scale screen, we identify TyrRII, a monoaminergic receptor required in astrocytes for sleep homeostasis. TyrRII levels rise following sleep deprivation in a Ca-dependent manner, promoting further increases in astrocytic Ca and resulting in a positive-feedback loop. Moreover, our findings suggest that astrocytes then transmit this sleep need to a sleep drive circuit by upregulating and releasing the interleukin-1 analog Spätzle, which then acts on Toll receptors on R5 neurons. These findings define astroglial Ca signaling mechanisms encoding sleep need and reveal dynamic properties of the sleep homeostatic control system.
Curr Biol, 2021; 31
30306256: Issa HA, Staes N, Diggs-Galligan S, Stimpson CD, Gendron-Fitzpatrick A, Taglialatela JP, Hof PR, Hopkins WD, Sherwood CC
Comparison of bonobo and chimpanzee brain microstructure reveals differences in socio-emotional circuits.
Despite being closely related, bonobos and chimpanzees exhibit several behavioral differences. For instance, studies indicate that chimpanzees are more aggressive, territorial, and risk-taking, while bonobos exhibit greater social tolerance and higher rates of socio-sexual interactions. To elucidate the potential neuroanatomical variation that accompanies these differences, we examined the microstructure of selected brain areas by quantifying the neuropil fraction, a measure of the relative tissue area occupied by structural elements of connectivity (e.g., dendrites, axons, and synapses) versus cell bodies. In bonobos and chimpanzees, we compared neuropil fractions in the nucleus accumbens (NAc; core and shell), amygdala (whole, accessory basal, basal, central and lateral nuclei), anterior cingulate cortex (ACC; dorsal and subgenual), anterior

insular cortex (AIC), and primary motor cortex (M1). In the dorsal ACC and frontoinsular cortex (FI) we also quantified numbers of von Economo neurons (VENs), a unique subset of neurons thought to be involved in rapid information processing during social interactions. We predicted that the neuropil fraction and number of VENs in brain regions associated with socio-emotional processing would be higher in bonobos. In support of this hypothesis, we found that bonobos had significantly greater neuropil in the central and accessory basal nuclei of the amygdala, as well as layers V-VI of the subgenual ACC. However, we did not find a difference in the numbers of VENs between the two species. These findings support the conclusion that bonobo and chimpanzee brains differ in the anatomical organization of socio-emotional systems that may reflect species-specific variation in behavior.
Brain Struct Funct, 2019; 224

26729507: Ardiccioni C, Clarke OB, Tomasek D, Issa HA, von Alpen DC, Pond HL, Banerjee S, Rajashankar KR, Liu Q, Guan Z, Li C, Kloss B, Bruni R, Kloppmann E, Rost B, Manzini MC, Shapiro L, Mancia F
Structure of the polyisoprenyl-phosphate glycosyltransferase GtrB and insights into the mechanism of catalysis.
The attachment of a sugar to a hydrophobic polyisoprenyl carrier is the first step for all extracellular glycosylation processes. The enzymes that perform these reactions, polyisoprenyl-glycosyltransferases (PI-GTs) include dolichol phosphate mannose synthase (DPMS), which generates the mannose donor for glycosylation in the endoplasmic reticulum. Here we report the 3.0 Å resolution crystal structure of GtrB, a glucose-specific PI-GT from Synechocystis, showing a tetramer in which each protomer contributes two helices to a membrane-spanning bundle. The active site is 15 Å from the membrane, raising the question of how water-soluble and membrane-embedded substrates are brought into apposition for catalysis. A conserved juxtamembrane domain harbours disease mutations, which compromised activity in GtrB in vitro and in human DPM1 tested in zebrafish. We hypothesize a role of this domain in shielding the polyisoprenyl-phosphate for transport to the active site. Our results reveal the basis of PI-GT function, and provide a potential molecular explanation for DPM1-related disease.
Nat Commun, 2016; 7

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BOARD NUMBER: S06-055

EFFECTS OF CHEMOGENETIC MANIPULATIONS OF CRF+ NEURONS IN THE CENTRAL AMYGDALA ON SOCIABILITY IN MICE

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Aims Multiple brain regions are involved in social cognition, including the amygdala, but the mechanisms underlying differences in social approach or avoidance are still poorly understood. Since it has been shown that neurons expressing corticotropin-releasing factor (CRF) regulate sociability in a sex-dependent manner, and that the CeA plays a key role in emotion discrimination, we decided to manipulate CRF-expressing CeA neurons during social interaction using a chemogenetic approach. **Methods** Heterozygous 2-4 month-old CRF-IRES-Cre mice (Jackson Laboratory) were bilaterally injected into the CeA with AAV5-hSyn-DIO-hM4D(Gi)-mCherry, AAV5-hSyn-DIO-hM3D(Gq)-mCherry or AAV5-hSyn-DIO-mCherry vectors. Clozapine N-oxide or vehicle was injected intraperitoneally 30 min before the start of social interaction and appetitive tests. **Results** Manipulation of CRF+ CeA neurons' activity led to a change in social investigation ($F(2,23)=4.887$, $P=0.0170$) but did not change the preference of social novelty or exploration time during social interaction test. At the same time, appetite was reduced as a result of CeA CRF+ neuronal activation ($F(2,35)=4.239$, $P=0.0225$; post hoc tests, $P=0.0030$ in the excitation group). Progressive ratio 4 test showed no changes in motivation of mice. **Conclusions** The role of CRF+ neurons of the CeA in social investigation but not social novelty preference was demonstrated using a chemogenetic approach. Sociability was increased as a result of CeA CRF+ excitation and decreased due to inhibition, while appetite was changed in an opposite manner . These findings together with previous studies indicate that CeA CRF+ neurons modulate not just aversive and appetitive responses, but also play a separate distinct role in social investigation.

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BOARD NUMBER: S06-056

DELINEATING IQ MOTIF AND SEC7 DOMAIN ARFGEF2, IQSEC2 IN RELEVANCE TO SOCIAL DEFICITS: FROM PHYSIOLOGY TO SYNAPSE.

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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A potential candidate associated with the etiology of a wide variety of developmental psychiatric disorders is the PSD-95 binding excitatory post-synaptic molecule, IQ Motif and Sec7 Domain ArfGEF2 (IQSEC2). We seek to elucidate the role of this molecule in physiology at the level of synaptic crosstalk in *Mus musculus*. We used a global and local approach to justify our hypothesis by generating a CRISPR/Cas9-mediated knockout (KO) mouse model and shRNA knockdown of IQSEC2, respectively. Technical details include in-utero electroporation and stereotactic viral injection for selective knockdown and rescue of the target molecule in brain. We have shown the involvement of this molecule in social behavior. Moreover, the excitatory and inhibitory synaptic functions were also found to be altered in Medial Prefrontal Cortex (mPFC) pyramidal neurons in the absence of this molecule. The social deficits and synaptic defects were successfully abolished when we reconstituted the longest isoform of IQSEC2 to the mPFC, a region known to be involved in social decision making, by an adeno-associated viruses (AAV)-mediated gene delivery system. We also studied the early neuron activity marker c-fos, through selected brain regions relevant to social behavior in mice. The mPFC showed an extensive reduction of neuronal activity in our IQSEC2 KO model. Our current findings involve characterization of the type of neurons that are more precisely associated with the physiological deficits observed in the lack of IQSEC2. Disrupted activity pattern as observed from the Excitatory/Inhibitory neuron specific c-fos expression in the mPFC has imparted considerable insight into our preliminary characterization.

Pubmed:

30199849: Devasenapathy S, Midha R, Naskar T, Mehta A, Prajapati B, Ummekulsum M, Sagar R, Singh NC, Sinha S
A pilot Indian family-based association study between dyslexia and Reelin pathway genes, DCDC2 and ROBO1, identifies modest association with a triallelic unit TAT in the gene RELN.
Dyslexia is a neurodevelopmental disorder that manifests as a reading disability despite normal intelligence and adequate educational opportunity. Twin and family studies have indicated a genetic component, while genome-wide studies have implicated a number of susceptibility genes, most of which have direct or indirect roles in neuronal migration. Reelin (RELN) has important biological functions facilitating migration of neurons. Polymorphisms in RELN have been implicated in related disorders like autism and schizophrenia but have not been examined in dyslexia. We hypothesized that not only RELN, but its interactors in the neuronal migration pathway may play roles in the etiology of dyslexia. Twenty two functional variants across six RELN signalling genes (RELN, VLDLR, APOER2, DAB1, LIS1 and NDEL1) and two dyslexia candidate genes (DCDC2 and ROBO1) were analyzed for association in twenty six nuclear and three extended families with individuals affected with dyslexia. Univariate association analysis was suggestive of association (p = 0.01) with rs362746 in RELN which however did not withstand Bonferroni corrections (p = 0.21). Multimarker tests indicated significant association (p = 0.037), based on which we tested for haplotype associations. Although there were no significant haplotypic associations, we found that a three marker unit with rs3808039 and rs2072403 flanking and independently in linkage disequilibrium with rs362746 was significantly overtransmitted (risk allelic combination - TAT) to dyslexia affected individuals in the sample (p = 0.002). Our results suggest preliminary evidence for a new potential risk variant in the RELN locus for dyslexia.
Asian J Psychiatr, 2018; 37
34618621: Singal CMS, Jaiswal P, Mehta A, Saleem K, Seth P
Role of EphrinA3 in HIV-1 Neuropathogenesis.

Glial cells perform important supporting functions for neurons through a dynamic crosstalk. Neuron-glia communication is the major phenomenon to sustain homeostatic functioning of the brain. Several interactive pathways between neurons and astrocytes are critical for the optimal functioning of neurons, and one such pathway is the ephrinA3-ephA4 signaling. The role of this pathway is essential in maintaining the levels of extracellular glutamate by regulating the excitatory amino acid transporters, EAAT1 and EAAT2 on astrocytes. Human immunodeficiency virus-1 (HIV-1) and its proteins cause glutamate excitotoxicity due to excess glutamate levels at sites of high synaptic activity. This study unravels the effects of HIV-1 transactivator of transcription (Tat) from clade B on ephrinA3 and its role in regulating glutamate levels in astrocyte-neuron co-cultures of human origin. It was observed that the expression of ephrinA3 increases in the presence of HIV-1 Tat B, while the expression of EAAT1 and EAAT2 was attenuated. This led to reduced glutamate uptake and therefore high neuronal death due to glutamate excitotoxicity. Knockdown of ephrinA3 using small interfering RNA, in the presence of HIV-1 Tat B reversed the neurotoxic effects of HIV-1 Tat B via increased expression of glutamate transporters that reduced the levels of extracellular glutamate. The in vitro findings were validated in autopsy brain sections from acquired immunodeficiency syndrome patients and we found ephrinA3 to be upregulated in the case of HIV-1-infected patients. This study offers valuable insights into astrocyte-mediated neuronal damage in HIV-1 neuropathogenesis.
ASN Neuro, 2021 Jan-Dec; 13

34685703: Mehta A, Shirai Y, Kouyama-Suzuki E, Zhou M, Yoshizawa T, Yanagawa T, Mori T, Tabuchi K
IQSEC2 Deficiency Results in Abnormal Social Behaviors Relevant to Autism by Affecting Functions of Neural Circuits in the Medial Prefrontal Cortex.
IQSEC2 is a guanine nucleotide exchange factor (GEF) for ADP-ribosylation factor 6 (Arf6), of which protein is exclusively localized to the postsynaptic density of the excitatory synapse. Human genome studies have revealed that the IQSEC2 gene is associated with X-linked neurodevelopmental disorders, such as intellectual disability (ID), epilepsy, and autism. In this study, we examined the behavior and synapse function in IQSEC2 knockout (KO) mice that we generated using CRIPSR/Cas9-mediated genome editing to solve the relevance between IQSEC2 deficiency and the pathophysiology of neurodevelopmental disorders. IQSEC2 KO mice exhibited autistic behaviors, such as overgrooming and social deficits. We identified that up-regulation of c-Fos expression in the medial prefrontal cortex (mPFC) induced by social stimulation was significantly attenuated in IQSEC2 KO mice. Whole cell electrophysiological recording identified that synaptic transmissions mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), N-methyl-D-aspartate receptor (NMDAR), and γ-aminobutyric acid receptor (GABAR) were significantly decreased in pyramidal neurons in layer 5 of the mPFC in IQSEC2 KO mice. Reexpression of IQSEC2 isoform 1 in the mPFC of IQSEC2 KO mice using adeno-associated virus (AAV) rescued both synaptic and social deficits, suggesting that impaired synaptic function in the mPFC is responsible for social deficits in IQSEC2 KO mice.
Cells, 2021; 10

FENS Forum 2022
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BOARD NUMBER: S06-057

CHARACTERIZATION OF CIRCUITRY FUNCTIONS INVOLVING THE PARAVENTRICULAR NUCLEUS OF THE THALAMUS (PVT) IN SOCIAL BEHAVIORS

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Social interaction and connection are crucial factors in shaping mental statements, and positive and negative social experiences would have different impacts on mental status, emotions and behaviors, but the neural circuitry mechanisms of how brain integrates previous social stimuli to mediate behaviors are still unclear. Studies have shown that the paraventricular nucleus of the thalamus (PVT) plays a role in mice's social behaviors, and medial prefrontal cortex (mPFC)-innervated PVT neurons are recruited by social interaction. PVT is also known to be an integrator in processing positive and negative valence, and it modulates mice's motivational behaviors through distinct efferent outputs. It remains elusive whether PVT regulates sociability and motivation through distinct or common neural circuits. Therefore, we set up to investigate the functional connections involving the PVT in social interaction as well as motivational behaviors. To understand the functional roles of PVT-centered connections, we first employed anterograde and retrograde viral tracing and activity-dependent targeting techniques to identify selective afferents and efferents of PVT involved in social interaction in mice. Furthermore, we also found that the anterior and posterior parts of PVT were involved in distinct anatomy connections in processing social stimuli. Causal optogenetic manipulation will be employed to examine the regulatory roles of PVT-involved circuits in sociability and motivational behaviors of the mice.

FENS Forum 2022
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BOARD NUMBER: S06-058

PROSOCIAL AND SELFISH CHOICES DEPEND ON CORTICO-AMYGDALA RECIPROCAL CONNECTIONS

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

Diego Scheggia[1], Filippo La Greca[1], Federica Maltese[2], Giulia Chiacchierini[2], Maria Italia[1], Cinzia Molent[2], Fabrizio Bernardi[2], Nicolò Carrano[1], Elisa Zianni[1], Fabrizio Gardoni[1], Monica Diluca[1], Francesco Papaleo[2]
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Individual choices favoring self-interest or the interest of others depend on context and the relationships between individuals. These social behaviors are conserved in evolution as they greatly influence the survival and everyday life of all mammals. However, the neurobiological bases of choices that benefit others at a personal cost are not understood. *Aims*. To investigate decision-making in a social environment and understand the factors and the underlying neurobiology determining prosocial or selfish decisions. *Methods*. We developed a two-choice social decision-making task in which mice could decide whether to share a positive reinforcement with their conspecifics. We then used fiber photometry recordings and chemogenetic silencing to demonstrate that BLA neurons are involved in the establishment of prosocial decisions. *Results*. Preference for altruistic choices was more evident between familiar males with the highest hierarchical distance, and if the recipient was in direct social contact and in a hungry state. Furthermore, individual variability in the preference for altruistic choices correlated with affective state matching. BLA neurons projecting to the prelimbic region (PL) of the PFC are involved in the development of a preference for altruistic choices. Conversely, PL projections to the BLA modulated self-interest motives on decision-making. *Conclusions*. Here, we reveal the role of the basolateral amygdala (BLA) and its connections with the prefrontal cortex (PFC) in altruistic and selfish choices. This provides a neurobiological comparative model of altruistic and selfish choices with relevance to pathologies associated with dysfunctions in social decision-making

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LESIONS OF NUCLEUS ACCUMBENS SHELL ABOLISH SOCIALLY TRANSMITTED FOOD PREFERENCES

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Aims: The Socially Transmitted Food Preference (STFP) task is a well-established paradigm in rodent research consisting of overwriting the endogenous food preference by the preference of the conspecific. The Nucleus accumbens shell (NAcSh) is an important area in the reward system for adaptative responses, however, its role in this task remained unknown. Therefore, we hypothesized that the integrity of the NAcSh is necessary to show socially transmitted food preferences. Methodology: 36 male Long-Evans rats (observers) received either bilateral NAcSh lesion or sham surgery. In the STFP task, two food options were provided to observer rats and their consumption patterns were measured to determine their individual food preference. Then, they were exposed to a demonstrator rat who was fed with the observer's originally non-preferred food. Afterward, the observer's consumption patterns were assessed again. Two control tasks were performed to rule out potential confounds: anxiety and odor discrimination deficits. Moreover, the histological analysis verified lesions' accuracy. Results: Sham lesioned observer rats changed their food preferences following interaction with the demonstrator, specifically by increasing the intake of their originally non-preferred food. This interaction-related change in preference was not found after NAcSh lesions. The lesion-effects on choice were not the consequence of anxiety or impaired motivation, cognitive flexibility, or sensory or motor function. Conclusion: This experiment suggests that NAcSh lesions result in a deficit in socially transmitted reward reevaluation. These results provide new information about the areas underpinning social learning and contribute to the understanding of social influence on choice and preference.

Pubmed:
34234654: Seidisarouei M, van Gurp S, Pranic NM, Calabus IN, van Wingerden M, Kalenscher T
Distinct Profiles of 50 kHz Vocalizations Differentiate Between Social Versus Non-social Reward Approach and Consumption. Social animals tend to possess an elaborate vocal communication repertoire, and rats are no exception. Rats utilize ultrasonic vocalizations (USVs) to communicate information about a wide range of socially relevant cues, as well as information regarding the valence of the behavior and/or surrounding environment. Both quantitative and qualitative acoustic properties of these USVs are thought to communicate context-specific information to conspecifics. Rat USVs have been broadly categorized into 22 and 50 kHz call categories, which can be further classified into subtypes based on their sonographic features. Recent research indicates that the 50 kHz calls and their various subtype profiles may be related to the processing of social and non-social rewards. However, only a handful of studies have investigated USV elicitation in the context of both social and non-social rewards. Here, we employ a novel behavioral paradigm, the social-sucrose preference test, that allowed us to measure rats' vocal responses to both non-social (i.e., 2, 5, and 10% sucrose) and social reward (interact with a Juvenile rat), presented concurrently. We analyzed adult male Long-Evans rats' vocal responses toward social and non-social rewards, with a specific focus on 50 kHz calls and their 14 subtypes. We demonstrate that rats' preference and their vocal responses toward a social reward were both influenced by the concentration of the non-social reward in the maze. In other words, rats showed a trade-off between time spent with non-social or social stimuli along with increasing concentrations of sucrose, and also, we found a clear difference in the emission of flat and frequency-modulated calls in the social and non-social reward zones. Furthermore, we report that the proportion of individual subtypes of 50 kHz calls, as well as the total USV counts, showed variation across different types of rewards as well. Our findings provide a thorough overview of rat vocal responses toward non-social and social rewards and are a clear depiction of the variability in the rat vocalization repertoire, establishing the role of call subtypes as key players driving context-specific vocal responses of rats.
Front Behav Neurosci, 2021; 15

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-060

CORTICAL ASTROCYTES MODULATE EMOTION DISCRIMINATION THROUGH CANNABINOID SYSTEM

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Within the "*social brain*", the prefrontal cortex (PFC) orchestrate a top-down control of social cognitive processes, including the ability to understand others' emotional states. Accumulating evidence are proving a pivotal role of astrocytes in brain functions and behavior. However, how these glial cells mediate socio-cognitive processes is still unexplored. The endocannabinoid system has been implicated as major modulator of astrocytic activity. Yet, the specific involvement of astrocytic cannabinoid signaling in distinct social behaviors is scarcely addressed. Here, combining *in vivo* chemogenetics and genetics manipulations with a behavioral task designed to study in mice the ability to discriminate conspecifics based on their affective state, we assessed the role of PFC astrocytes in social behavior. Furthermore, we investigated the influence of PFC astrocytic cannabinoid receptor 1 in social behaviors. Our data revealed that activation of cortical astrocytes increase the preference towards emotionally altered mice compared to those in a neutral state. Notably, this ability is abolished following acute and chronic treatment with a cannabinoid agonist (THC). Furthermore, we observed that chemogenetics activation of PFC astrocytes might rescue emotion discrimination deficits induced by systemic THC administration. Lastly, we demonstrated that removing astroglial CB1 within PFC abolish emotion discrimination. Our findings indicate a prominent role of PFC astrocytes and cannabinoid system in emotion recognition abilities.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-061

A BRAINSTEM NEURAL CIRCUIT FOR INSTINCTIVE APPROACH AND AVOIDANCE BEHAVIOR IN MICE

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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To survive animals have to finely regulate their approach and defensive behaviors towards threats. In mice, the brainstem dorsal periaqueductal gray (dPAG) generates innate defensive behaviors towards a multitude of threats, like predators, prey and aggressive conspecifics. Glutamatergic neurons in the anterior cingulate cortex (ACC) project to dPAG and have an inhibitory effect on the structure, promoting approach behaviors. In this study, we used in vivo optogenetic stimulation to study the function and anatomy of the ACC to PAG projection, confirming that stimulation of the projection increases approach behavior towards the mentioned threats, and volume electron microscopy, showing that ACC axons establish axo-dendritic and axo-somatic synapses onto *Vglut2*+ and non-glutamatergic, non-GABAergic cells in dPAG, pointing to the role of a neuromodulator neuron class mediating dPAG inhibition. Moreover, we performed 1 photon calcium imaging recordings in freely moving mice in glutamatergic and GABAergic cells in dPAG, uncovering their activity patterns in correlation with defensive behaviors of interest. Further experiments will analyze the expression profile of dPAG cells, with a focus on ACC synaptic partners, using spatially resolved transcriptomics, and will explore the functional role of dPAG candidate neuromodulatory neurons in defensive behaviors, such as enkephalin expressing neurons. In conclusion, our study characterized the connectivity and function of a brainstem neural circuit that modulates innate defensive behaviors towards aggressive conspecifics, predators and prey in mice. Keywords: dPAG, ACC, defensive behavior, approach and avoidance, optogenetics, volume electron microscopy, 1 photon calcium recordings, enkephalin.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-062

AUTISM SPECTRUM DISORDER-RELATED VOLTAGE-GATED POTASSIUM CHANNEL MUTATION KV7.3 R2C DECREASES DOPAMINE NEURON EXCITABILITY AND SOCIAL INTERACTION IN MICE

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Disruption of ion channel function is increasingly associated with neurodevelopmental disorders (NDDs) such as autism spectrum disorder (ASD). ASD has complex etiology and symptoms, but recent studies suggest that individuals with ASD have disrupted mesostriatal network activity associated with social interaction deficits. Although mutated ion channels play a key role in NDDs, the degree to which they disrupt mesostriatal network activity is not well understood. The Kv7 family of voltage-gated potassium channels encoded by KCNQ1-5 genes are increasingly linked to NDDs including ASD. These channels are broadly expressed, including within the ventral tegmental area (VTA), a nucleus of the mesostriatal pathway that plays an important role in social behavior. Here we assessed the impact of the ASD-associated variant KCNQ3(R2C) on VTA dopamine neuron excitability and social behavior in mice. We used a viral-based strategy to conditionally inactivate endogenous Kv7.3 with CRISPR/SaCas9 and re-express human wildtype (hKv7.3/WT) or mutant (hKv7.3/R2C) KCNQ3. Whole-cell patch clamp electrophysiology in brain slices revealed that hKv7.3/R2C significantly decreased excitability of dopamine neurons. In a social-reward operant task either inactivation of kcnq3 or re-expression of hKv7.3/R2C decreased the number of lever presses performed by experimental mice to receive access to a second mouse. These results suggest that kcnq3 is a critical regulator of dopamine neuron activity, and either kcnq3 inactivation-induced hyperexcitability or hKv7.3/R2C-induced hypoexcitability may both disrupt mesostriatal network activity that results in decreased social interaction.

Pubmed:
34728568: Gamal El-Din TM, Lantin T, Tschumi CW, Juarez B, Quinlan M, Hayano JH, Li J, Zweifel LS, Catterall WA
Autism-associated mutations in K7 channels induce gating pore current.
Autism spectrum disorder (ASD) adversely impacts >1% of children in the United States, causing social interaction deficits, repetitive behaviors, and communication disorders. Genetic analysis of ASD has advanced dramatically through genome sequencing, which has identified >500 genes with mutations in ASD. Mutations that alter arginine gating charges in the voltage sensor of the voltage-gated potassium (K) channel K7 (KCNQ) are among those frequently associated with ASD. We hypothesized that these gating charge mutations would induce gating pore current (also termed ω-current) by causing an ionic leak through the mutant voltage sensor. Unexpectedly, we found that wild-type K7 conducts outward gating pore current through its native voltage sensor at positive membrane potentials, owing to a glutamine in the third gating charge position. In bacterial and human K7 channels, gating charge mutations at the R1 and R2 positions cause inward gating pore current through the resting voltage sensor at negative membrane potentials, whereas mutation at R4 causes outward gating pore current through the activated voltage sensor at positive potentials. Remarkably, expression of the K7.3/R2C ASD-associated mutation in vivo in midbrain dopamine neurons of mice disrupts action potential generation and repetitive firing. Overall, our results reveal native and mutant gating pore current in K7 channels and implicate altered control of action potential generation by gating pore current through mutant K7 channels as a potential pathogenic mechanism in autism.
Proc Natl Acad Sci U S A, 2021; 118
29405480: Tschumi CW, Beckstead MJ
Diverse actions of the modulatory peptide neurotensin on central synaptic transmission.
Neurotensin (NT) is a 13 amino acid neuropeptide that is expressed throughout the central nervous system and is implicated in the etiology of multiple diseases and disorders. Many primary investigations of NT-induced modulation of neuronal excitability at the level of the synapse have been conducted, but they have not been summarized in review form in nearly 30 years. Therefore, the goal of this review is to discuss the many actions of NT on neuronal excitability across brain regions as well as NT circuit architecture. In the basal ganglia as well as other brain nuclei, NT can act through diverse intracellular signaling cascades to enhance or depress neuronal activity by modulating activity of ion channels, ionotropic and

metabotropic neurotransmitter receptors, and presynaptic release of neurotransmitters. Further, NT can produce indirect effects by evoking endocannabinoid release, and recently has itself been identified as a putative retrograde messenger. In the basal ganglia, the diverse actions and circuit architecture of NT signaling allow for input-specific control of reward-related behaviors.
Eur J Neurosci, 2019; 49
30686631: Piccart E, Tschumi CW, Beckstead MJ
Acute and subchronic PCP attenuate D2 autoreceptor signaling in substantia nigra dopamine neurons.
Phencyclidine (PCP) administration is commonly used to model schizophrenia in laboratory animals. While PCP is well-characterized as an antagonist of glutamate-sensitive N-methyl-D-aspartate (NMDA) receptors, its effects on dopamine signaling are not well understood. Here we used whole-cell and cell-attached patch-clamp electrophysiology of substantia nigra dopamine neurons to determine the effects of acute and subchronic PCP exposure on both dopamine D2 autoreceptor-mediated currents and burst firing evoked by glutamate receptor activation. Acute PCP affected D2 autoreceptor-mediated currents through two apparently distinct mechanisms: a low-concentration dopamine transporter (DAT) inhibition and a high-concentration potassium (GIRK) channel inhibition. Subchronic administration of PCP (5 mg/kg, i.p., every 12 h for 7 days) decreased sensitivity to low dopamine concentrations, and also enhanced evoked burst firing of dopamine neurons. These findings suggest the effects of PCP on dopaminergic signaling in the midbrain could enhance burst firing and contribute to the development of schizophreniform behavior.
Eur Neuropsychopharmacol, 2019; 29
30858517: Newman AH, Cao J, Keighron JD, Jordan CJ, Bi GH, Liang Y, Abramyan AM, Avelar AJ, Tschumi CW, Beckstead MJ, Shi L, Tanda G, Xi ZX
Translating the atypical dopamine uptake inhibitor hypothesis toward therapeutics for treatment of psychostimulant use disorders.
Medication-assisted treatments are unavailable to patients with cocaine use disorders. Efforts to develop potential pharmacotherapies have led to the identification of a promising lead molecule, JJC8-091, that demonstrates a novel binding mode at the dopamine transporter (DAT). Here, JJC8-091 and a structural analogue, JJC8-088, were extensively and comparatively assessed to elucidate neurochemical correlates to their divergent behavioral profiles. Despite sharing significant structural similarity, JJC8-088 was more cocaine-like, increasing extracellular DA concentrations in the nucleus accumbens shell (NAS) efficaciously and more potently than JJC8-091. In contrast, JJC8-091 was not self-administered and was effective in blocking cocaine-induced reinstatement to drug seeking. Electrophysiology experiments confirmed that JJC8-091 was more effective than JJC8-088 at inhibiting cocaine-mediated enhancement of DA neurotransmission. Further, when VTA DA neurons in DAT-cre mice were optically stimulated, JJC8-088 produced a significant leftward shift in the stimulation-response curve, similar to cocaine, while JJC8-091 shifted the curve downward, suggesting attenuation of DA-mediated brain reward. Computational models predicted that JJC8-088 binds in an outward facing conformation of DAT, similar to cocaine. Conversely, JJC8-091 steers DAT towards a more occluded conformation. Collectively, these data reveal the underlying molecular mechanism at DAT that may be leveraged to rationally optimize leads for the treatment of cocaine use disorders, with JJC8-091 representing a compelling candidate for development.
Neuropsychopharmacology, 2019; 44
31062485: Tschumi CW, Daszkowski AW, Sharpe AL, Trzeciak M, Beckstead MJ
A history of ethanol drinking increases locomotor stimulation and blunts enhancement of dendritic dopamine transmission by methamphetamine.
Ethanol and psychostimulant use disorders exhibit comorbidity in humans and cross-sensitization in animal models, but the neurobiological underpinnings of this are not well understood. Ethanol acutely increases dopamine neuron excitability, and psychostimulants such as cocaine or methamphetamine increase extracellular dopamine through inhibition of uptake through the dopamine transporter (DAT) and/or vesicular monoamine transporter 2 (VMAT2). Psychostimulants also depress dopamine neuron activity by enhancing dendritic dopamine neurotransmission. Here, we show that mice with a previous history of ethanol drinking are more sensitive to the locomotor-stimulating effects of a high dose (5 mg/kg), but not lower doses (1 and 3 mg/kg) of methamphetamine or any tested dose of cocaine (3, 10, and 18 mg/kg), compared with water-drinking controls. We next investigated the impact of a history of ethanol drinking, in a separate group of mice, on methamphetamine- or cocaine-induced enhancement of dendritic dopamine transmission using whole-cell voltage clamp electrophysiology in mouse brain slices. Methamphetamine, applied at a concentration (10 μM) that affects both DAT and VMAT2, enhanced D2 receptor-mediated inhibitory postsynaptic currents (D2-IPSCs) in both groups, but this effect was blunted in mice with a history of ethanol drinking. As methamphetamine action at VMAT2 disrupts dopamine neurotransmission, these results may suggest enhanced action of methamphetamine at VMAT2. Furthermore, there were no differences in low-dose methamphetamine or cocaine-induced enhancement of D2-IPSCs, suggesting intact DAT function. Disruption of methamphetamine-induced enhancement of dendritic dopamine transmission would result in decreased

inhibition of dopamine neurons, ultimately increasing downstream release and the behavioral effects of methamphetamine.
Addict Biol, 2020; 25
30440089: Lynch WB, Tschumi CW, Sharpe AL, Branch SY, Chen C, Ge G, Li S, Beckstead MJ
Progressively disrupted somatodendritic morphology in dopamine neurons in a mouse Parkinson's model.
Parkinson's disease is characterized by the progressive loss of dopamine neurons in the substantia nigra, leading to severe motor deficits. Although the disease likely begins to develop years before observable motor symptoms, the specific morphological and functional alterations involved are poorly understood.
Mov Disord, 2018; 33
29307543: Tschumi CW, Beckstead MJ
Neurotensin speeds inhibition of dopamine neurons through temporal modulation of GABA and GABA receptor-mediated synaptic input.
Midbrain dopamine neurons play physiological roles in many processes including reward learning and motivated behavior, and are tonically inhibited by γ-aminobutyric acid (GABA)ergic input from multiple brain regions. Neurotensin (NT) is a neuropeptide which acutely modulates midbrain dopamine neuron excitability through multiple mechanisms, one of which is a decrease of GABA-mediated inhibition. However, the mechanisms through which NT depresses GABA signaling are not known. Here we used whole cell patch-clamp electrophysiology of dopamine neurons in mouse brain slices to show that NT acts both presynaptically to increase GABA and postsynaptically to decrease GABA receptor-mediated currents in the substantia nigra. The active peptide fragment NT enhanced GABA signaling presynaptically by causing an increase in the size of the readily releasable pool of GABA via activation of the NT type-1 receptor and protein kinase A. Conversely, NT depressed GABA signaling postsynaptically via the NT type-2 receptor in a process that was modulated by protein kinase C. Both forms of plasticity could be observed simultaneously in single dopamine neurons. Thus, as the kinetics of GABA signaling are significantly faster than those of GABA signaling, NT functionally speeds GABAergic input to midbrain dopamine neurons. This finding contributes to our understanding of how neuropeptide-induced plasticity can simultaneously differentiate and integrate signaling by a single neurotransmitter in a single cell and provides a basis for understanding how neuropeptides use temporal shifts in synaptic strength to encode information.
Neuropharmacology, 2018; 131
26237320: Collins GT, Chen Y, Tschumi C, Rush EL, Mensah A, Koek W, France CP
Effects of consuming a diet high in fat and/or sugar on the locomotor effects of acute and repeated cocaine in male and female C57BL/6J mice.
Drug abuse and obesity are serious public health problems. Dopamine plays a central role in mediating the reinforcing effects of drugs and food. Prolonged use of drugs is known to alter the function and/or sensitivity of many neurotransmitter systems, including dopamine; however, the impact of consuming foods high in fat and/or sugar is less clear. These studies characterized the locomotor effects of acute and repeated cocaine in male and female C57BL/6J mice consuming 1 of 4 diets: (a) standard chow + water; (b) standard chow + 10% sucrose solution; (c) high-fat chow + water; or (d) high-fat chow + 10% sucrose solution. The acute locomotor effects of cocaine (3.2-32.0 mg/kg) were evaluated 4 weeks after initiating dietary conditions; the effects of repeated cocaine administration were evaluated after 5, 6, 7, and 12 weeks. During acute tests, mice consuming a diet high in fat and/or sucrose exhibited greater locomotor responses to cocaine than mice consuming standard chow and water, regardless of sex. Although diet-induced enhancements persisted across repeated cocaine testing, locomotor sensitization developed more rapidly in females drinking sucrose (and consuming either standard or high-fat chow) than in females consuming standard chow and water. In addition to providing evidence that consuming a diet high in fat and/or sugar enhances abuse-related effects of cocaine in ways that might increase vulnerability to abuse cocaine, these studies identified a potentially important sex-related difference in the interaction between nutrition and cocaine effects, with the impacts of sucrose consumption being greater in females than in males.
Exp Clin Psychopharmacol, 2015; 23
30926783: Gomez JA, Perkins JM, Beaudoin GM, Cook NB, Quraishi SA, Szoeke EA, Thangamani K, Tschumi CW, Wanat MJ, Maroof AM, Beckstead MJ, Rosenberg PA, Paladini CA
Ventral tegmental area astrocytes orchestrate avoidance and approach behavior.
The ventral tegmental area (VTA) is a heterogeneous midbrain structure, containing neurons and astrocytes, that coordinates behaviors by integrating activity from numerous afferents. Within neuron-astrocyte networks, astrocytes control signals from distinct afferents in a circuit-specific manner, but whether this capacity scales up to drive motivated behavior has been undetermined. Using genetic and optical dissection strategies we report that VTA astrocytes tune glutamatergic signaling selectively on local inhibitory neurons to drive a functional circuit for learned avoidance. In this circuit, astrocytes facilitate excitation of VTA GABA neurons to increase inhibition of dopamine neurons, eliciting real-time and learned avoidance behavior that is sufficient to impede expression of preference for reward. Loss of one glutamate transporter (GLT-1) from VTA astrocytes selectively blocks these avoidance behaviors and spares preference for reward. Thus, VTA astrocytes selectively

regulate excitation of local GABA neurons to drive a distinct avoidance circuit that opposes approach behavior. Nat Commun, 2019; 10

FENS Forum 2022
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BOARD NUMBER: S06-063

ROLE OF ACETYLCHOLINE RELEASE IN THE PREFRONTAL CORTEX DURING SOCIAL INTERACTION

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Rational Previous studies showed that neuronal nicotinic receptors within the prefrontal cortex are necessary for showing adapted social interactions. However, the precise role of acetylcholine (Ach) in this process has never been studied. Aim: The aim of this study is to understand the role of prefrontal cholinergic input in social interaction. Methods We targeted opsin expression in cholinergic neurons for activating or inhibiting acetylcholine release in the prefrontal cortex of ChAT-IRES-Cre mice while they are interacting socially in dyads of males. We recorded ultrasonic vocalization (USV) that we showed to be a marker of emotional status and to be promoted by social interaction. Result Our results show that inhibition of Ach release increased social approach, together with a decrease of USV frequency. By contrast, Ach release decreased social approach, thus changing social hierarchy within the dyad. Moreover, Ach release increased USV frequencies, suggesting an boost of positive emotions. In addition, Ach modulation always induced escape behaviors from the non stimulated mouse. It is, therefore, possible that increased Ach release in the PFC, that trigger higher USV frequency during social approach, may generate an abnormal emotional signal for the social conspecific, which, in turn escapes more frequently social contacts. Conclusion This study shows that Ach input in the prefrontal cortex modulates social emotional signal, used to establish and maintain social hierarchy.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-064

POSTERIOR INTRALAMINAR THALAMIC CALBINDIN NEURONS CONTRIBUTE TO MATERNAL AND SOCIAL BEHAVIOR IN MICE

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Posterior intralaminar thalamic (PIL) neurons may convey somatosensory information towards hypothalamus and other forebrain areas. Tuberoinfundibular peptide 39 (TIP39) is expressed in PIL neurons. It has been suggested that TIP39 promotes maternal and social behaviors. This neuropeptide is expressed in calbindin positive neurons of the PIL in rats. In contrast, calbindin and TIP39 are located in distinct cell populations in mice. Therefore, in the present study, we focused on the characterization of calbindin-containing PIL neurons in the mice. We determined their projections and examined their function related to maternal, social and anxiety-like behavior using chemogenetics. We injected stimulatory, control and inhibitory (rAAV5-hSyn-DIO-hM4D, Gq,-,Gi/-mCherry) adeno-associated viruses (AAVs) into the PIL of calbindin-Cre mice to induce Cre-dependent cell type specific expression of designer receptors exclusively activated by designer drugs (DREADDs). The validation of the stimulatory DREADDs was verified by appearance of c-Fos following CNO injection.In the virally injected animals, we mapped the projections of PIL calbindin neurons and we performed different behavioral tests. The results demonstrated that stimulation of PIL neurons increased anxiety-related behavior, reduced maternal and social behavior, while the inhibition of the same neurons had opposite effect. Our data suggests that PIL calbindin neurons are involved in the regulation of different behaviors. The authors declare that they have no conflict of interest. Supported by the ÚNKP-21-4 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund. NKFIH-4300-1/2017-NKP_17-0002 and OTKA K134221.

Pubmed:
27300187: Cservenák M, Kis V, Keller D, Dimén D, Menyhárt L, Oláh S, Szabó ÉR, Barna J, Renner É, Usdin TB, Dobolyi A
Maternally involved galanin neurons in the preoptic area of the rat.
Recent selective stimulation and ablation of galanin neurons in the preoptic area of the hypothalamus established their critical role in control of maternal behaviors. Here, we identified a group of galanin neurons in the anterior commissural nucleus (ACN), and a distinct group in the medial preoptic area (MPA). Galanin neurons in ACN but not the MPA co-expressed oxytocin. We used immunodetection of phosphorylated STAT5 (pSTAT5), involved in prolactin receptor signal transduction, to evaluate the effects of suckling-induced prolactin release and found that 76 % of galanin cells in ACN, but only 12 % in MPA were prolactin responsive. Nerve terminals containing tuberoinfundibular peptide 39 (TIP39), a neuropeptide that mediates effects of suckling on maternal motivation, were abundant around galanin neurons in both preoptic regions. In the ACN and MPA, 89 and 82 % of galanin neurons received close somatic appositions, with an average of 2.9 and 2.6 per cell, respectively. We observed perisomatic innervation of galanin neurons using correlated light and electron microscopy. The connection was excitatory based on the glutamate content of TIP39 terminals demonstrated by post-embedding immunogold electron microscopy. Injection of the anterograde tracer biotinylated dextran amine into the TIP39-expressing posterior intralaminar complex of the thalamus (PIL) demonstrated that preoptic TIP39 fibers originate in the PIL, which is activated by suckling. Thus, galanin neurons in the preoptic area of mother rats are innervated by an excitatory neuronal pathway that conveys suckling-related information. In turn, they can be topographically and neurochemically divided into two distinct cell groups, of which only one is affected by prolactin.
Brain Struct Funct, 2017; 222
30872665: Barna J, Dimén D, Puska G, Kovács D, Csikós V, Oláh S, Udvari EB, Pál G, Dobolyi Á
Complement component 1q subcomponent binding protein in the brain of the rat.
Complement component 1q subcomponent binding protein (C1qbp) is a multifunctional protein involved in immune response, energy homeostasis of cells as a plasma membrane receptor, and a nuclear, cytoplasmic or mitochondrial protein. Recent reports suggested its neuronal function, too, possibly in axon maintenance, synaptic function, and neuroplasticity. Therefore,

we addressed to identify C1qbp in the rat brain using in situ hybridization histochemistry and immunolabelling at light and electron microscopic level. C1qbp has a topographical distribution in the brain established by the same pattern of C1qbp mRNA-expressing and protein-containing neurons with the highest abundance in the cerebral cortex, anterodorsal thalamic nucleus, hypothalamic paraventricular (PVN) and arcuate nuclei, spinal trigeminal nucleus. Double labelling of C1qbp with the neuronal marker NeuN, with the astrocyte marker S100, and the microglia marker Iba1 demonstrated the presence of C1qbp in neurons but not in glial cells in the normal brain, while C1qbp appeared in microglia following their activation induced by focal ischemic lesion. Only restricted neurons expressed C1qbp, for example, in the PVN, magnocellular neurons selectively contained C1qbp. Further double labelling by using the mitochondria marker Idh3a antibody suggested the mitochondrial localization of C1qbp in the brain, confirmed by correlated light and electron microscopy at 3 different brain regions. Post-embedding immunoelectron microscopy also suggested uneven C1qbp content of mitochondria in different brain areas but also heterogeneity within single neurons. These data suggest a specific function of C1qbp in the brain related to mitochondria, such as the regulation of local energy supply in neuronal cells.
Sci Rep, 2019; 9

29802523: Oláh S, Cservenák M, Keller D, Fazekas EA, Renner É, Lőw P, Dobolyi A
Prolactin-induced and neuronal activation in the brain of mother mice.
Nursing has important consequences on mothers. To separate the prolactin-mediated and the neuronally-mediated actions of nursing, neurons directly affected by prolactin were visualized using pSTAT5 immunohistochemistry in relation to Fos-expressing neurons in suckled mother mice. In response to pup exposure following 22-h pup deprivation, we found a markedly elevated number of pSTAT5-containing neurons in several brain regions, including the lateral septum, medial amygdaloid nucleus, subparafascicular area, caudal periaqueductal gray, dorsal raphe, lateral parabrachial nucleus, nucleus of the solitary tract, and the periventricular, medial preoptic, paraventricular, arcuate and ventromedial nuclei of the hypothalamus. Pup exposure also induced Fos expression in all of these brain regions except the arcuate and ventromedial hypothalamic nuclei. Bromocriptine treatment known to reduce prolactin levels eliminated pSTAT5 from most brain regions while it did not affect Fos activation following suckling. The degree of colocalization for pSTAT5 and Fos ranged from 8 to 80% in the different brain regions suggesting that most neurons responding to pup exposure in mother mice are driven either by prolactin or direct neuronal input from the pups, while the number of neurons affected by both types of inputs depends on the examined brain area. In addition, both pSTAT5 and Fos were also double-labeled with estrogen receptor alpha (ERα) in mother mice, which revealed a very high degree of colocalization between pSTAT5 and ERα with much less potential interaction between Fos- and ERα-containing neurons suggesting that estrogen-sensitive neurons are more likely to be affected by prolactin than by direct neuronal activation.
Brain Struct Funct, 2018; 223

32612510: Dobolyi A, Oláh S, Keller D, Kumari R, Fazekas EA, Csikós V, Renner É, Cservenák M
Secretion and Function of Pituitary Prolactin in Evolutionary Perspective.
The hypothalamo-pituitary system developed in early vertebrates. Prolactin is an ancient vertebrate hormone released from the pituitary that exerts particularly diverse functions. The purpose of the review is to take a comparative approach in the description of prolactin, its secretion from pituitary lactotrophs, and hormonal functions. Since the reproductive and osmoregulatory roles of prolactin are best established in a variety of species, these functions are the primary subjects of discussion. Different types of prolactin and prolactin receptors developed during vertebrate evolution, which will be described in this review. The signal transduction of prolactin receptors is well conserved among vertebrates enabling us to describe the whole subphylum. Then, the review focuses on the regulation of prolactin release in mammals as we have the most knowledge on this class of vertebrates. Prolactin secretion in response to different reproductive stimuli, such as estrogen-induced release, mating, pregnancy and suckling is detailed. Reproduction in birds is different from that in mammals in several aspects. Prolactin is released during incubation in avian species whose regulation and functional significance are discussed. Little information is available on prolactin in reptiles and amphibians; therefore, they are mentioned only in specific cases to explain certain evolutionary aspects. In turn, the osmoregulatory function of prolactin is well established in fish. The different types of pituitary prolactin in fish play particularly important roles in the adaptation of eutherian species to fresh water environments. To achieve this function, prolactin is released from lactotrophs in hyposmolarity, as they are directly osmosensitive in fish. In turn, the released prolactin acts on branchial epithelia, especially ionocytes of the gill to retain salt and excrete water. This review will highlight the points where comparative data give new ideas or suggest new approaches for investigation in other taxa.
Front Neurosci, 2020; 14

32472169: Csikós V, Varró P, Bódi V, Oláh S, Világi I, Dobolyi A
The mycotoxin deoxynivalenol activates GABAergic neurons in the reward system and inhibits feeding and maternal behaviours.
Deoxynivalenol (DON) or vomitoxin, is a trichothecene mycotoxin produced mainly by Fusarium graminearum and culmorum.

Mycotoxins or secondary metabolic products of mold fungi are micro-pollutants, which may affect human and animal health. The neuronal and behavioural actions of DON were analysed in the present study. To address, which neurons can be affected by DON, the neuronal activation pattern following intraperitoneal injection of DON (1 mg/kg) was investigated in adult male rats and the results were confirmed in mice, too. DON-induced neuronal activation was assessed by c-Fos immunohistochemistry. DON injection resulted in profound c-Fos activation in only the elements of the reward system, such as the accumbens nucleus, the medial prefrontal cortex, and the ventral tegmental area. Further double labelling studies suggested that GABAergic neurons were activated by DON treatment. To study the behavioural relevance of this activation, we examined the effect of DON on feed intake as an example of reward-driven behaviours. Following DON injection, feed consumption was markedly reduced but returned to normal the following day suggesting an inhibitory action of DON on feed intake without forming taste-aversion. To further test how general the effect of DON on goal-directed behaviours is, its actions on maternal behaviour was also examined. Pup retrieval latencies were markedly increased by DON administration, and DON-treated mother rats spent less time with nursing suggesting reduced maternal motivation. In a supplementary control experiment, DON did not induce conditioned place preference arguing against its addictive or aversive actions. The results imply that acute uptake of the mycotoxin DON can influence the reward circuit of the brain and exert inhibitory actions on goal-directed, reward-driven behaviours. In addition, the results also suggest that DON exposure of mothers may have specific implications.
Arch Toxicol, 2020; 94

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BOARD NUMBER: S06-065

A NOVEL BEHAVIOURAL TEST FOR STUDYING SOCIAL AVOIDANCE AND CHASE IN MICE

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Defensive behaviours are a range of strategies an animal employs to cope with threatening stimuli and the exact choice of response depends on the nature and intensity of the perceived danger. Aggression from conspecifics is a common source of threat in social species where aggression is used to ensure access to food, shelter, and mates. Defensive strategies for coping with such risks include avoidance, escape, and submissive displays aimed at de-escalating aggression. We are investigating the neural correlates of social defense in the ventrolateral subdivision of the ventromedial hypothalamus (VMHvl) where we have identified two classes of neurons whose firing encodes a sensory-motor transformation for escape behavior. However, present behavioural tests for social defense are suboptimal because they do not allow for examining the full range of social defense strategies. Here we present the validation and application of a novel circular chase arena that allows us to investigate the neural correlates of the full range of social defensive strategies, including high speed pursuit, passive and active approach, and submissive behaviors. This behavior test has increased ethological validity and for the first time allows for a quantitative behavioral assessment coupled to circuit recording and manipulation for the full repertoire of social defensive behaviors in the laboratory mouse.

Pubmed:
31072928: Fanibunda SE, Deb S, Maniyadath B, Tiwari P, Ghai U, Gupta S, Figueiredo D, Weisstaub N, Gingrich JA, Vaidya ADB, Kolthur-Seetharam U, Vaidya VA
Serotonin regulates mitochondrial biogenesis and function in rodent cortical neurons via the 5-HT receptor and SIRT1-PGC-1α axis.
Mitochondria in neurons, in addition to their primary role in bioenergetics, also contribute to specialized functions, including regulation of synaptic transmission, Ca homeostasis, neuronal excitability, and stress adaptation. However, the factors that influence mitochondrial biogenesis and function in neurons remain poorly elucidated. Here, we identify an important role for serotonin (5-HT) as a regulator of mitochondrial biogenesis and function in rodent cortical neurons, via a 5-HT receptor-mediated recruitment of the SIRT1-PGC-1α axis, which is relevant to the neuroprotective action of 5-HT. We found that 5-HT increased mitochondrial biogenesis, reflected through enhanced mtDNA levels, mitotracker staining, and expression of mitochondrial components. This resulted in higher mitochondrial respiratory capacity, oxidative phosphorylation (OXPHOS) efficiency, and a consequential increase in cellular ATP levels. Mechanistically, the effects of 5-HT were mediated via the 5-HT receptor and master modulators of mitochondrial biogenesis, SIRT1 and PGC-1α. SIRT1 was required to mediate the effects of 5-HT on mitochondrial biogenesis and function in cortical neurons. In vivo studies revealed that 5-HT receptor stimulation increased cortical mtDNA and ATP levels in a SIRT1-dependent manner. Direct infusion of 5-HT into the neocortex and chemogenetic activation of 5-HT neurons also resulted in enhanced mitochondrial biogenesis and function in vivo. In cortical neurons, 5-HT enhanced expression of antioxidant enzymes, decreased cellular reactive oxygen species, and exhibited neuroprotection against excitotoxic and oxidative stress, an effect that required SIRT1. These findings identify 5-HT as an upstream regulator of mitochondrial biogenesis and function in cortical neurons and implicate the mitochondrial effects of 5-HT in its neuroprotective action.
Proc Natl Acad Sci U S A, 2019; 116

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-066

ROLE OF IMMEDIATE EARLY GENES IN NEURAL PLASTICITY DURING SOCIAL DEFEAT

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Defensive behaviors such as social aggression and avoidance are expressed by territorial animals in threatening situations and are crucial for their survival. Activity in the ventrolateral part of the ventromedial hypothalamus (VMHvl) has been shown to mediate both social aggression and avoidance across vertebrates. Interestingly, previous studies have shown that functional reorganization of neural activity that elicits avoidance behavior upon optogenetic stimulation in the VMHvl occurs only after social defeat and is accompanied by the expression of several immediate early genes (IEGs). While IEGs have been used as a proxy for plasticity, the precise nature of plastic changes varies between different brain regions, contexts, and behaviors. Moreover, whether plasticity in the VMHvl is essential for coping with social defeat is still an open question. In this study, we take a transcriptional approach to block induction of plasticity in VMHvl in order to answer this question. We downregulate different IEGs or knockout their master regulators (e.g. serum response factor, SRF) in specific cell types. This approach, for the first time, allows us to manipulate precise molecular pathways and elucidate the molecular mechanisms of plasticity of social defensive behaviors in VMH.

Pubmed:
29991507: Sureka R, Wadhwa R, Thakur SS, Pathak RU, Mishra RK
Comparison of Nuclear Matrix and Mitotic Chromosome Scaffold Proteins in S2 Cells-Transmission of Hallmarks of Nuclear Organization Through Mitosis.
Chromatin condenses several folds to form mitotic chromosomes during cell division and decondenses post-mitotically to reoccupy their nuclear territory and regain their specific transcriptional profile in a precisely lineage specific manner. This necessitates that the features of nuclear architecture and DNA topology persist through mitosis. We compared the proteome of nuclease and high salt resistant fraction of interphase nucleus known as nuclear matrix (NuMat) and an equivalent biochemical fraction in the mitotic chromosome known as mitotic chromosome scaffold (MiCS). Our study elucidates that as much as 67% of the NuMat proteins are retained in the MiCS indicating that the features of nuclear architecture in interphase nucleus are retained on the mitotic chromosomes. Proteins of the NuMat/MiCS have large dynamic range of MS signal and were detected in sub-femtomolar amounts. Chromatin/RNA binding proteins with hydrolase and helicase activity are highly enriched in NuMat as well as MiCS. Although several transcription factors involved in functioning of interphase nucleus are present exclusively in NuMat, protein components responsible for assembly of membrane-less nuclear bodies are uniquely retained in MiCS. Our study clearly indicates that the features of nuclear architecture, in the structural context of NuMat, are retained in MiCS and possibly play an important role in maintenance of cell lineage specific transcriptional status during cell division and thereby, serve as components of cellular memory.
Mol Cell Proteomics, 2018; 17
33289389: Sureka R, Mishra R
Identification of Evolutionarily Conserved Nuclear Matrix Proteins and Their Prokaryotic Origins.
Compared to prokaryotic cells, a typical eukaryotic cell is much more complex along with its endomembrane system and membrane-bound organelles. Although the endosymbiosis theories convincingly explain the evolution of membrane-bound organelles such as mitochondria and chloroplasts, very little is understood about the evolutionary origins of the nucleus, the defining feature of eukaryotes. Most studies on nuclear evolution have not been able to take into consideration the underlying structural framework of the nucleus, attributed to the nuclear matrix (NuMat), a ribonucleoproteinaceous structure. This can largely be attributed to the lack of annotation of its core components. Since NuMat has been shown to provide a structural platform for facilitating a variety of nuclear functions such as replication, transcription, and splicing, it is important to identify its protein components to better understand these processes. In this study, we address this issue using the developing embryos of and and identify 362 core NuMat proteins that are conserved between the two organisms. We further compare our results with publicly available NuMat dataset and cellular localization dataset to define the core homologous NuMat proteins consisting of 252 proteins. We find that of them, 86 protein groups have originated from pre-existing proteins in prokaryotes.

While 36 were conserved across all eukaryotic supergroups, 14 new proteins evolved before the evolution of the last eukaryotic common ancestor and together, these 50 proteins out of the 252 core conserved NuMat proteins are conserved across all eukaryotes, indicating their indispensable nature for nuclear function for over 1.5 billion years of eukaryotic history. Our analysis paves the way to understand the evolution of the complex internal nuclear architecture and its functions.
J Proteome Res, 2021; 20

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BOARD NUMBER: S06-067

REPRESENTATION OF ETHOLOGICAL EVENTS BY BASOLATERAL AMYGDALA NEURONS

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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The accurate interpretation of ethologically-relevant stimuli is crucial for survival. Before deciding whether to approach or avoid a stimulus, an animal processes multisensory cues to develop a new, or activate an existing, internal representation. While the role of BLA neurons in fear-conditioning is well-studied, little is known about how BLA neurons respond during naturalistic events. Cells in the basolateral amygdala were recorded from freely-moving male rats as they interacted with different ethological stimuli: several male and female rats, a moving toy mouse, and sweet rice. 42% of the cells reliably responded to one or more of these stimuli, with over half of these identifying one of the four classes of stimuli to the exclusion of the rest. In addition to strong activation when interacting with their preferred stimulus, these *event-specific* cells signaled micro-behavioral interactions such as social contact with the head or tail of the conspecific. Firing activity persisted in 30% of responsive cells for several minutes after the removal of the eliciting stimulus. Circuit analysis at the sub-millisecond level revealed a directional flow of information from event-specific neurons to less specific *panresponsive* neurons and increases in connection strength after the event. We conclude that individual basolateral amygdala neurons identify specific ethological events and their microstructure, with circuit-wide activity being driven by these event-specific neurons during and after the termination of those events likely facilitating active short-term memory consolidation.

FENS Forum 2022
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BOARD NUMBER: S06-068

THE ROLE OF SOMATOSTATIN INTERNEURONS IN REGULATION OF EMOTIONAL CONTAGION

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Aims The aim of the study was to assess the role of Somatostatin interneurons in amygdala and prefrontal cortex in emotional contagion – the simplest form of empathy in mice. **Methods** To study empathic abilities in mice we employed behavioural paradigm - Remote Transfer of Fear, in which mice are housed in pairs for three weeks, one labelled an Observer, and the other a Demonstrator. In the test session, the Demonstrator is subjected to aversive stimuli (10 foot shocks 0.6mA 1s long), outside of the home cage, while the Observer remains there undisturbed. Then, the Demonstrator returns to the home cage, where it can freely interact with the Observer. First ten minutes of interactions are recorded. Ninety minutes after the onset of the interaction animals were sacrificed for immunochemistry. To check the activity of somatostatin cells we used mice expressing fluorescence marker (dTomato) in somatostatin cells combined with immunochemistry against c-Fos, a standard neuronal novelty marker. **Results** Our results indicate that emotional contagion took place and that somatostatin interneurons in the prefrontal cortex, but not in the amygdala, are involved in the process. We observed increased expression of an immediate early gene (c-Fos) in somatostatin cells in both pre and infralimbic prefrontal cortex. **Conclusions** This study confirms pivotal role of the prefrontal cortex and somatostatin interneurons within this structure in emotional contagion and allows us to study this phenomenon further to better pinpoint its exact neuronal mechanism.

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BOARD NUMBER: S06-069

CONTRIBUTION OF GIRK CHANNELS EXPRESSED IN VTA GABAERGIC NEURONS TO ANXIETY, SOCIAL BEHAVIORS AND HIPPOCAMPAL-DEPENDENT MEMORY

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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G-protein-gated inwardly rectifying potassium (GirK) channels contribute to the neuronal resting membrane potential and cell excitability in many brain regions. In the ventral tegmental area (VTA), GIRK1-containing channels are exclusively expressed in GABAergic cells. Since dopaminergic efferents of the VTA are under the inhibitory control of local GABAergic interneurons, GIRK1-containing channels may play a key role in dopamine release into projection brain areas such as the nucleus accumbens (NAc) -which is involved in anxiety-related and social behaviors- and the hippocampus -crucially implicated in cognition-. We performed intra-VTA injections of Tertiapin-Q, selective blocker of GIRK1-containing channels, to elucidate the effects of the specific modulation of GirK channel activity in VTA GABAergic neurons on anxiety-like behavior, sociability, social competitivenes and hippocampal-dependent contextual memory through a battery of behavioral tests. Subsequently, dopamine content and mitochondrial respiration in the NAc and hippocampus was measured. We found that Tertiapin-Q administration induced a significant decrease in dopamine levels and mitochondrial respiration in the NAc, but not in the hippocampus. However, these changes were not accompanied by behavioral alterations. Our results indicate that GIRK1-containing channels expressed in VTA GABAergic neurons modulate dopaminergic neurotransmission and suggest that higher dose of the drug used in this study might be required to induce significant changes at the behavioral level. *Funding: grants BFU2017-82494-P and PID2020-115823-GB100 funded by MCIN/AEI/10.13039/501100011033 and by the European Union, both to LJ-D and JDN-L, and by intramural funding from EPFL to C.S. GI-L held a predoctoral scholarship from "Plan Propio de Investigación" Programme of UCLM.*

Pubmed:
32698467: Mayordomo-Cava J, Iborra-Lázaro G, Djebari S, Temprano-Carazo S, Sánchez-Rodríguez I, Jeremic D, Gruart A, Delgado-García JM, Jiménez-Díaz L, Navarro-López JD
Impairments of Synaptic Plasticity Induction Threshold and Network Oscillatory Activity in the Hippocampus Underlie Memory Deficits in a Non-Transgenic Mouse Model of Amyloidosis.
In early Alzheimer disease (AD) models synaptic failures and upstreaming aberrant patterns of network synchronous activity result in hippocampal-dependent memory deficits. In such initial stage, soluble forms of Amyloid- (A) peptides have been shown to play a causal role. Among different A species, A has been identified as the biologically active fragment, as induces major neuropathological signs related to early AD stages. Consequently, it has been extensively used to acutely explore the pathophysiological events related with neuronal dysfunction induced by soluble A forms. However, the synaptic mechanisms underlying its toxic effects on hippocampal-dependent memory remain unresolved. Here, in an in vivo model of amyloidosis generated by intracerebroventricular injections of A we studied the synaptic dysfunction mechanisms underlying hippocampal cognitive deficits. At the synaptic level, long-term potentiation (LTP) of synaptic excitation and inhibition was induced in CA1 region by high frequency simulation (HFS) applied to collaterals. A was found to alter metaplastic mechanisms of plasticity, facilitating long-term depression (LTD) of both types of LTP. In addition, aberrant synchronization of hippocampal network activity was found while at the behavioral level, deficits in hippocampal-dependent habituation and recognition memories emerged. Together, our results provide a substrate for synaptic disruption mechanism underlying hippocampal cognitive deficits present in A amyloidosis model.
Biology (Basel), 2020; 9
31875959: Sánchez-Rodríguez I, Djebari S, Temprano-Carazo S, Vega-Avelaira D, Jiménez-Herrera R, Iborra-Lázaro G, Yajeya J, Jiménez-Díaz L, Navarro-López JD
Hippocampal long-term synaptic depression and memory deficits induced in early amyloidopathy are prevented by enhancing

G-protein-gated inwardly rectifying potassium channel activity.
Hippocampal synaptic plasticity disruption by amyloid-β (Aβ) peptides + thought to be responsible for learning and memory impairments in Alzheimer's disease (AD) early stage. Failures in neuronal excitability maintenance seems to be an underlying mechanism. G-protein-gated inwardly rectifying potassium (GirK) channels control neural excitability by hyperpolarization in response to many G-protein-coupled receptors activation. Here, in early in vitro and in vivo amyloidosis mouse models, we study whether GirK channels take part of the hippocampal synaptic plasticity impairments generated by Aβ . In vitro electrophysiological recordings from slices showed that Aβ alters synaptic plasticity by switching high-frequency stimulation (HFS) induced long-term potentiation (LTP) to long-term depression (LTD), which led to in vivo hippocampal-dependent memory deficits. Remarkably, selective pharmacological activation of GirK channels with ML297 rescued both HFS-induced LTP and habituation memory from Aβ action. Moreover, when GirK channels were specifically blocked by Tertiapin-Q, their activation with ML297 failed to rescue LTP from the HFS-dependent LTD induced by Aβ . On the other hand, the molecular analysis of the recorded slices by western blot showed that the expression of GIRK1/2 subunits, which form the prototypical GirK channel in the hippocampus, was not significantly regulated by Aβ . However, immunohistochemical examination of our in vivo amyloidosis model showed Aβ to down-regulate hippocampal GIRK1 subunit expression. Together, our results describe an Aβ-mediated deleterious synaptic mechanism that modifies the induction threshold for hippocampal LTP/LTD and underlies memory alterations observed in amyloidosis models. In this scenario, GirK activation assures memory formation by preventing the transformation of HFS-induced LTP into LTD.
J Neurochem, 2020; 153
34261700: Djebari S, Iborra-Lázaro G, Temprano-Carazo S, Sánchez-Rodríguez I, Nava-Mesa MO, Múnera A, Gruart A, Delgado-García JM, Jiménez-Díaz L, Navarro-López JD
G-Protein-Gated Inwardly Rectifying Potassium (Kir3/GIRK) Channels Govern Synaptic Plasticity That Supports Hippocampal-Dependent Cognitive Functions in Male Mice.
The G-protein-gated inwardly rectifying potassium (Kir3/GIRK) channel is the effector of many G-protein-coupled receptors (GPCRs). Its dysfunction has been linked to the pathophysiology of Down syndrome, Alzheimer's and Parkinson's diseases, psychiatric disorders, epilepsy, drug addiction, or alcoholism. In the hippocampus, GIRK channels decrease excitability of the cells and contribute to resting membrane potential and inhibitory neurotransmission. Here, to elucidate the role of GIRK channels activity in the maintenance of hippocampal-dependent cognitive functions, their involvement in controlling neuronal excitability at different levels of complexity was examined in C57BL/6 male mice. For that purpose, GIRK activity in the dorsal hippocampus CA3-CA1 synapse was pharmacologically modulated by two drugs: ML297, a GIRK channel opener, and Tertiapin-Q (TQ), a GIRK channel blocker. , using dorsal hippocampal slices, we studied the effect of pharmacological GIRK modulation on synaptic plasticity processes induced in CA1 by Schaffer collateral stimulation. , we performed acute intracerebroventricular (i.c.v.) injections of the two GIRK modulators to study their contribution to electrophysiological properties and synaptic plasticity of dorsal hippocampal CA3-CA1 synapse, and to learning and memory capabilities during hippocampal-dependent tasks. We found that pharmacological disruption of GIRK channel activity by i.c.v. injections, causing either function gain or function loss, induced learning and memory deficits by a mechanism involving neural excitability impairments and alterations in the induction and maintenance of long-term synaptic plasticity processes. These results support the contention that an accurate control of GIRK activity must take place in the hippocampus to sustain cognitive functions. Cognitive processes of learning and memory that rely on hippocampal synaptic plasticity processes are critically ruled by a finely tuned neural excitability. G-protein-gated inwardly rectifying K (GIRK) channels play a key role in maintaining resting membrane potential, cell excitability and inhibitory neurotransmission. Here, we demonstrate that modulation of GIRK channels activity, causing either function gain or function loss, transforms high-frequency stimulation (HFS)-induced long-term potentiation (LTP) into long-term depression (LTD), inducing deficits in hippocampal-dependent learning and memory. Together, our data show a crucial GIRK-activity-mediated mechanism that governs synaptic plasticity direction and modulates subsequent hippocampal-dependent cognitive functions.
J Neurosci, 2021; 41
30552869: Lopez-Font I, Iborra-Lazaro G, Sánchez-Valle R, Molinuevo JL, Cuchillo-Ibañez I, Sáez-Valero J
CSF-ApoER2 fragments as a read-out of reelin signaling: Distinct patterns in sporadic and autosomal-dominant Alzheimer disease.
Reelin is a glycoprotein associated with synaptic plasticity and neurotransmission. The malfunctioning of reelin signaling in the brain is likely to contribute to the pathogenesis of Alzheimer's disease (AD). Reelin binding to Apolipoprotein E receptor 2 (ApoER2) activates downstream signaling and induces the proteolytic cleavage of ApoER2, resulting in the generation of soluble fragments. To evaluate the efficiency of reelin signaling in AD, we have quantified the levels of reelin and soluble ectodomain fragments of ApoER2 (ectoApoER2) in the cerebrospinal fluid (CSF). CSF from sporadic AD patients (sAD; n = 14, age 54-83 years) had lower levels of ecto-ApoER2 (~31% reduction; p = .005) compared to those in the age-matched controls (n = 10, age 61-80), and a higher reelin/ecto-ApoER2 ratio. In contrast, autosomal dominant AD patients, carriers of

PSEN1 mutations (ADAD; n = 7, age 31-49 years) had higher ecto-ApoER2 levels (~109% increment; p = .001) and a lower reelin/ecto-ApoER2 ratio than the non-mutation carriers from the same families (n = 7, age 25-47 years). Our data suggest that the levels of ecto-ApoER2 in CSF could be a suitable read-out of an impaired reelin signaling in AD, but also indicate differences between sAD and ADAD.
Clin Chim Acta, 2019; 490

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BOARD NUMBER: S06-070

NEURAL CIRCUITS UNDERLYING SOCIALLY ACQUIRED FEAR MEMORIES IN MICE

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Social learning is one of the most important ways in which animals, especially humans, acquire new information. However, brain mechanisms underlying the processing of fear-related stimuli have been mostly studied in non-human animal models where subjects experience fear directly. Social transmission of fear in non-human animals has comparatively recently emerged. In particular, the study of socially acquired fear and its processing across time from a circuitry perspective that considers the identity of molecules and cells involved is scarce. Several studies support the role of two distinct but interconnected brain regions for observational fear learning and social cognition in rodents, non-human primates and humans: the anterior cingulate cortex (ACC) and the basolateral amygdala (BLA). In this project I will show preliminary results setting up a paradigm to study fear observational learning and memory using mice. By putting particular emphasis in open source tools for stimuli delivery and for behavioral data analysis, we will discuss ongoing and future experiments to further understand the role of both ACC->BLA and BLA->ACC projecting neurons in the consolidation of observational fear memories. We will also consider protocols for studying the reconsolidation of this type of social acquired memories. Studying the mechanisms underlying social fear learning in non-human animals is key to model and better understand vicarious learning in humans and eventually develop therapeutic strategies for disorders of the central nervous system involving deficits of empathy or social learning such as autism.

Pubmed:
32659348: Medina C, de la Fuente V, Tom Dieck S, Nassim-Assir B, Dalmay T, Bartnik I, Lunardi P, de Oliveira Alvares L, Schuman EM, Letzkus JJ, Romano A
LIMK, Cofilin 1 and actin dynamics involvement in fear memory processing.
Long-term memory has been associated with morphological changes in the brain, which in turn tightly correlate with changes in synaptic efficacy. Such plasticity is proposed to rely on dendritic spines as a neuronal canvas on which these changes can occur. Given the key role of actin cytoskeleton dynamics in spine morphology, major regulating factors of this process such as Cofilin 1 (Cfl1) and LIM kinase (LIMK), an inhibitor of Cfl1 activity, are prime molecular targets that may regulate dendritic plasticity. Using a contextual fear conditioning paradigm in mice, we found that pharmacological induction of depolymerization of actin filaments through the inhibition of LIMK causes an impairment in memory reconsolidation, as well as in memory consolidation. On top of that, Cfl1 activity is inhibited and its mRNA is downregulated in CA1 neuropil after re-exposure to the training context. Moreover, by pharmacological disruption of actin cytoskeleton dynamics, the process of memory extinction can either be facilitated or impaired. Our results lead to a better understanding of the role of LIMK, Cfl1 and actin cytoskeleton dynamics in the morphological and functional changes underlying the synaptic plasticity of the memory trace.
Neurobiol Learn Mem, 2020; 173
31434945: de la Fuente V, Medina C, Falasco G, Urrutia L, Kravitz AV, Urbano FJ, Vázquez S, Pedreira ME, Romano A
The lateral neocortex is critical for contextual fear memory reconsolidation.
Memories are a product of the concerted activity of many brain areas. Deregulation of consolidation and reprocessing of mnemonic traces that encode fearful experiences might result in fear-related psychopathologies. Here, we assessed how pre-established memories change with experience, particularly the labilization/reconsolidation of memory, using the whole-brain analysis technique of positron emission tomography in male mice. We found differences in glucose consumption in the lateral neocortex, hippocampus and amygdala in mice that underwent labilization/reconsolidation processes compared to animals that did not reactivate a fear memory. We used chemogenetics to obtain insight into the role of cortical areas in these phases of memory and found that the lateral neocortex is necessary for fear memory reconsolidation. Inhibition of lateral neocortex during reconsolidation altered glucose consumption levels in the amygdala. Using an optogenetic/neuronal recording-based strategy we observed that the lateral neocortex is functionally connected with the amygdala, which, along with retrograde labeling using fluorophore-conjugated cholera toxin subunit B, support a monosynaptic connection between these areas and

poses this connection as a hot-spot in the circuits involved in reactivation of fear memories.
Sci Rep, 2019; 9
29948945: Zalcman G, Federman N, Fiszbein A, de la Fuente V, Ameneiro L, Schor I, Romano A
Sustained CaMKII Delta Gene Expression Is Specifically Required for Long-Lasting Memories in Mice.
Although important information is available on the molecular mechanisms of long-term memory formation, little is known about the processes underlying memory persistence in the brain. Here, we report that persistent gene expression of CaMKIIδ isoform participates in object recognition long-lasting memory storage in mice hippocampus. We found that CaMKIIδ mRNA expression was sustained up to one week after training and paralleled memory retention. Antisense DNA infusion in the hippocampus during consolidation or even after consolidation impairs 7-day- but not 1-day-long memory, supporting a role of CaMKIIδ in memory persistence. CaMKIIδ gene expression was accompanied by long-lasting nucleosome occupancy changes at its promoter. This epigenetic mechanism is described for the first time in a memory process and offers a novel mechanism for persistent gene expression in neurons. CaMKIIδ protein is mainly present in nucleus and presynaptic terminals, suggesting a role in these subcellular compartments for memory persistence. All these results point to a key function of the sustained gene expression of this overlooked CaMKII isoform in long-lasting memories.
Mol Neurobiol, 2019; 56
28084590: Lunardi P, Sachser RM, Sierra RO, Pedraza LK, Medina C, de la Fuente V, Romano A, Quillfeldt JA, de Oliveira Alvares L
Effects of Hippocampal LIMK Inhibition on Memory Acquisition, Consolidation, Retrieval, Reconsolidation, and Extinction.
Long-lasting changes in dendritic spines provide a physical correlate for memory formation and persistence. LIM kinase (LIMK) plays a critical role in orchestrating dendritic actin dynamics during memory processing, since it is the convergent downstream target of both the Rac1/PAK and RhoA/ROCK pathways that in turn induce cofilin phosphorylation and prevent depolymerization of actin filaments. Here, using a potent LIMK inhibitor (BMS-5), we investigated the role of LIMK activity in the dorsal hippocampus during contextual fear memory in rats. We first found that post-training administration of BMS-5 impaired memory consolidation in a dose-dependent manner. Inhibiting LIMK before training also disrupted memory acquisition. We then demonstrated that hippocampal LIMK activity seems to be critical for memory retrieval and reconsolidation, since both processes were impaired by BMS-5 treatment. Contextual fear memory extinction, however, was not sensitive to the same treatment. In conclusion, our findings demonstrate that hippocampal LIMK activity plays an important role in memory acquisition, consolidation, retrieval, and reconsolidation during contextual fear conditioning.
Mol Neurobiol, 2018; 55
26441513: de la Fuente V, Federman N, Zalcman G, Salles A, Freudenthal R, Romano A
NF-κB transcription factor role in consolidation and reconsolidation of persistent memories.
Transcriptional regulation is an important molecular process required for long-term neural plasticity and long-term memory (LTM) formation. Thus, one main interest in molecular neuroscience in the last decades has been the identification of transcription factors that are involved in memory processes. Among them, the nuclear factor κB (NF-κB) family of transcription factors has gained interest due to a significant body of evidence that supports a key role of these proteins in synaptic plasticity and memory. In recent years, the interest was particularly reinforced because NF-κB was characterized as an important regulator of synaptogenesis. This function may be explained by its participation in synapse to nucleus communication, as well as a possible local role at the synapse. This review provides an overview of experimental work obtained in the last years, showing the essential role of this transcription factor in memory processes in different learning tasks in mammals. We focus the review on the consolidation and reconsolidation memory phases as well as on the regulation of immediate-early and late genes by epigenetic mechanisms that determine enduring forms of memories.
Front Mol Neurosci, 2015; 8
25576790: Zalcman G, Federman N, de la Fuente V, Romano A
Nuclear factor kappa B-dependent Zif268 expression in hippocampus is required for recognition memory in mice.
Long-term memory formation requires gene expression after acquisition of new information. The first step in the regulation of gene expression is the participation of transcription factors (TFs) such as nuclear factor kappa B (NF-κB), which are present before the neuronal activity induced by training. It was proposed that the activation of these types of TFs allows a second step in gene regulation by induction of immediate-early genes (IEGs) whose protein products are, in turn, TFs. Between these IEGs, zif268 has been found to play a critical role in long-term memory formation and reprocessing after retrieval. Here we found in mice hippocampus that, on one hand, NF-κB was activated 45 min after training in a novel object recognition (NOR) task and that inhibiting NF-κB immediately after training by intrahippocampal administration of NF-κB Decoy DNA impaired NOR memory consolidation. On the other hand, Zif268 protein expression was induced 45 min after NOR training and the administration of DNA antisense to its mRNA post-training impaired recognition memory. Finally, we found that the inhibition of NF-κB by NF-κB Decoy DNA reduced significantly the training-induced Zif268 increment, indicating that NF-κB is involved in the regulation of Zif268 expression. Thus, the present results support the involvement of NF-κB activity-dependent Zif268

expression in the hippocampus during recognition memory consolidation.
Neurobiol Learn Mem, 2015; 119
25135196: Sol Fustiñana M, de la Fuente V, Federman N, Freudenthal R, Romano A
Protein degradation by ubiquitin-proteasome system in formation and labilization of contextual conditioning memory.
The ubiquitin-proteasome system (UPS) of protein degradation has been evaluated in different forms of neural plasticity and memory. The role of UPS in such processes is controversial. Several results support the idea that the activation of this system in memory consolidation is necessary to overcome negative constrains for plasticity. In this case, the inhibition of the UPS during consolidation impairs memory. Similar results were reported for memory reconsolidation. However, in other cases, the inhibition of UPS had no effect on memory consolidation and reconsolidation but impedes the amnesic action of protein synthesis inhibition after retrieval. The last finding suggests a specific action of the UPS inhibitor on memory labilization. However, another interpretation is possible in terms of the synthesis/degradation balance of positive and negative elements in neural plasticity, as was found in the case of long-term potentiation. To evaluate these alternative interpretations, other reconsolidation-interfering drugs than translation inhibitors should be tested. Here we analyzed initially the UPS inhibitor effect in contextual conditioning in crabs. We found that UPS inhibition during consolidation impaired long-term memory. In contrast, UPS inhibition did not affect memory reconsolidation after contextual retrieval but, in fact, impeded memory labilization, blocking the action of drugs that does not affect directly the protein synthesis. To extend these finding to vertebrates, we performed similar experiments in contextual fear memory in mice. We found that the UPS inhibitor in hippocampus affected memory consolidation and blocked memory labilization after retrieval. These findings exclude alternative interpretations to the requirement of UPS in memory labilization and give evidence of this mechanism in both vertebrates and invertebrates.
Learn Mem, 2014; 21
25043904: de la Fuente V, Federman N, Fustiñana MS, Zalcman G, Romano A
Calcineurin phosphatase as a negative regulator of fear memory in hippocampus: control on nuclear factor-κB signaling in consolidation and reconsolidation.
Protein phosphatases are important regulators of neural plasticity and memory. Some studies support that the Ca(2+) /calmodulin-dependent phosphatase calcineurin (CaN) is, on the one hand, a negative regulator of memory formation and, on the other hand, a positive regulator of memory extinction and reversal learning. However, the signaling mechanisms by which CaN exerts its action in such processes are not well understood. Previous findings support that CaN negatively regulate the nuclear factor kappaB (NF-κB) signaling pathway during extinction. Here, we have studied the role of CaN in contextual fear memory consolidation and reconsolidation in the hippocampus. We investigated the CaN control on the NF-κB signaling pathway, a key mechanism that regulates gene expression in memory processes. We found that post-training intrahippocampal administration of the CaN inhibitor FK506 enhanced memory retention one day but not two weeks after training. Accordingly, the inhibition of CaN by FK506 increased NF-κB activity in dorsal hippocampus. The administration of the NF-κB signaling pathway inhibitor sulfasalazine (SSZ) impeded the enhancing effect of FK506. In line with our findings in consolidation, FK506 administration before memory reactivation enhanced memory reconsolidation when tested one day after re-exposure to the training context. Strikingly, memory was also enhanced two weeks after training, suggesting that reinforcement during reconsolidation is more persistent than during consolidation. The coadministration of SSZ and FK506 blocked the enhancement effect in reconsolidation, suggesting that this facilitation is also dependent on the NF-κB signaling pathway. In summary, our results support a novel mechanism by which memory formation and reprocessing can be controlled by CaN regulation on NF-κB activity.
Hippocampus, 2014; 24
24978317: Federman N, Zalcman G, de la Fuente V, Fustiñana MS, Romano A
Epigenetic mechanisms and memory strength: a comparative study.
Memory consolidation requires de novo mRNA and protein synthesis. Transcriptional activation is controlled by transcription factors, their cofactors and repressors. Cofactors and repressors regulate gene expression by interacting with basal transcription machinery, remodeling chromatin structure and/or chemically modifying histones. Acetylation is the most studied epigenetic mechanism of histones modifications related to gene expression. This process is regulated by histone acetylases (HATs) and histone deacetylases (HDACs). More than 5 years ago, we began a line of research about the role of histone acetylation during memory consolidation. Here we review our work, presenting evidence about the critical role of this epigenetic mechanism during consolidation of context-signal memory in the crab Neohelice granulata, as well as during consolidation of novel object recognition memory in the mouse Mus musculus. Our evidence demonstrates that histone acetylation is a key mechanism in memory consolidation, functioning as a distinctive molecular feature of strong memories. Furthermore, we found that the strength of a memory can be characterized by its persistence or its resistance to extinction. Besides, we found that the role of this epigenetic mechanism regulating gene expression only in the formation of strongest memories is evolutionarily conserved.

J Physiol Paris, 2014 Sep-Dec; 108
24703879: Ogara MF, Belluscio LM, de la Fuente V, Berardino BG, Sonzogni SV, Byk L, Marazita M, Cánepa ET
CDK5-mediated phosphorylation of p19INK4d avoids DNA damage-induced neurodegeneration in mouse hippocampus and prevents loss of cognitive functions.
DNA damage, which perturbs genomic stability, has been linked to cognitive decline in the aging human brain, and mutations in DNA repair genes have neurological implications. Several studies have suggested that DNA damage is also increased in brain disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. However, the precise mechanisms connecting DNA damage with neurodegeneration remain poorly understood. CDK5, a critical enzyme in the development of the central nervous system, phosphorylates a number of synaptic proteins and regulates dendritic spine morphogenesis, synaptic plasticity and learning. In addition to these physiological roles, CDK5 has been involved in the neuronal death initiated by DNA damage. We hypothesized that p19INK4d, a member of the cell cycle inhibitor family INK4, is involved in a neuroprotective mechanism activated in response to DNA damage. We found that in response to genotoxic injury or increased levels of intracellular calcium, p19INK4d is transcriptionally induced and phosphorylated by CDK5 which provides it with greater stability in postmitotic neurons. p19INK4d expression improves DNA repair, decreases apoptosis and increases neuronal survival under conditions of genotoxic stress. Our in vivo experiments showed that decreased levels of p19INK4d rendered hippocampal neurons more sensitive to genotoxic insult resulting in the loss of cognitive abilities that rely on the integrity of this brain structure. We propose a feedback mechanism by which the neurotoxic effects of CDK5-p25 activated by genotoxic stress or abnormal intracellular calcium levels are counteracted by the induction and stabilization of p19INK4d protein reducing the adverse consequences on brain functions.
Biochim Biophys Acta, 2014; 1843

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BOARD NUMBER: S06-071

CHEMOGENETIC EVIDENCE THAT POSTERIOR INTRALAMINAR THALAMIC NEURONS MODULATES AGGRESSIVE BEHAVIOR IN RATS

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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In a previous study, we established the activation of the posterior intralaminar thalamic (PIL) neurons during social interactions between adult female rats. In this study we focused on the role of PIL in intermale aggressive behavior. For manipulation of PIL neurons, adeno-associated virus was stereotaxically injected into the PIL. The virus expressed DREADD fused with mCherry in the infected cells. Excitatory and inhibitory DREADDs were used, activated by clozapine-N-oxide (CNO). Behavioral tests were recorded during the chemogenetic manipulation. After perfusion of the animals, we verified the injection sites and performed histological analysis. We identified the brain areas activated by aggressive behavior using c-Fos method. We found neuronal activation in the infralimbic cortex, medial preoptic area (MPOA) and the lateral septum. To induce aggression, the animals were separated at an early age. The behavioral tests were performed at the age 5 month. On the first day of the experiment, vehicle was injected to the animal. We performed aggressive behavioral test, where an unfamiliar intruder was placed in the subject animal's cage resulting in an aggressive response. On the second day, the same test was repeated starting 1,5 hours after CNO administration. Chemogenetic stimulation significantly decreased aggression and increased the duration of positive valance contact, while inhibiting the PIL resulted in the increase of aggression and decreased the duration of positive valance contact. Based on the results, PIL neurons may participate in the regulation of aggressive behavior conveying sensory inputs from the conspecific to higher brain areas. Grant support: NKFIH-4300-1/2017-NKP_17-00002,OTKA K1342221,EFOP-3.6.3-VEKOP-16-2017-00009

FENS Forum 2022
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BOARD NUMBER: S06-072

PHARMACOLOGICAL INACTIVATION OF THE BED NUCLEUS OF THE STRIA TERMINALIS INCREASES PROSOCIAL BEHAVIOR IN RHESUS MACAQUES

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

Jessica Jacobs, Hannah Waguespack, Rafael Maior, Carolina Campos-Rodriguez, Patrick Forcelli, Ludise Malkova
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The bed nucleus of the stria terminalis (BNST) is a subcortical structure that plays an important role in response to stress, extended-duration fear states, and social behavior. Dysregulation of the BNST has been found in individuals with generalized anxiety and social anxiety disorder and increased activity in the BNST has been found after exposure to aggressive and anxiety-inducing stimuli. Based on these findings, we hypothesized that bilateral inactivation of the BNST in nonhuman primates would result in increased social interaction between conspecifics. To test this hypothesis, we performed intracerebral microinfusions of the GABA-A agonist muscimol directly into the BNST of three adult male rhesus macaques, two additional males served as non-injected partners. Following drug infusion, the experimental animal was placed in an observation cage with a highly familiar non-injected partner and their behavior was recorded for 60 minutes. Behavior was assessed in five dyads. Compared to saline, muscimol infusion into the BNST significantly increased total social contact between animals (paired t-test, $p = 0.03$) but did not notably impact specific social behaviors, such as grooming of the conspecific. An increase in passive social contact between animals approached significance (paired t-test, $p = 0.11$). These results further support a critical role for the BNST in the regulation of social behavior and are consistent with a similar role for the amygdala previously found in our lab. While activation of the BNST is associated with anxiety and aggression, here we show that inactivation of the BNST promotes affiliative social behavior in conspecifics.

Pubmed:
28269776: Heuer E, Jacobs J, Du R, Wang S, Keifer OP, Cintron AF, Dooyema J, Meng Y, Zhang X, Walker LC
Amyloid-Related Imaging Abnormalities in an Aged Squirrel Monkey with Cerebral Amyloid Angiopathy.
Amyloid-related imaging abnormalities (ARIA) in magnetic resonance imaging scans have emerged as indicators of potentially serious side effects in clinical trials of therapeutics for Alzheimer's disease. These anomalies include an edematous type (ARIA-E) that appears as hyperintense (bright) regions by T2-weighted MRI, and a type characterized by the deposition of hemosiderin (ARIA-H) that elicits a hypointense signal, especially in T2* susceptibility weighted images. ARIA in general has been linked to the presence of amyloid-β (Aβ)-type cerebral amyloid angiopathy, an accumulation of misfolded Aβ protein in the vascular wall that impairs the integrity of brain blood vessels. However, the pathobiology of ARIA remains poorly understood, in part due to the absence of an animal model of the disorder that would enable a contemporaneous analysis of tissue integrity in the affected region. Here we describe both ARIA-E and ARIA-H in an aged squirrel monkey (Saimiri sciureus), a nonhuman primate model of naturally occurring cerebral amyloid angiopathy. Histopathologic examination of the anomalous region revealed reactive astrocytosis and microgliosis, infiltration of systemic inflammatory/immune cells, damage to axons and myelin, and hemosiderin deposition. The disruption of axons in particular suggests that ARIA-E could have functional consequences for affected regions. The squirrel monkey model can be useful for studying the pathogenesis and long-term effects of ARIA, and for testing the safety and efficacy of emerging therapies for Alzheimer's disease.
J Alzheimers Dis, 2017; 57
33303679: Napoli JL, Camalier CR, Brown AL, Jacobs J, Mishkin MM, Averbeck BB
Correlates of Auditory Decision-Making in Prefrontal, Auditory, and Basal Lateral Amygdala Cortical Areas.
Spatial selective listening and auditory choice underlie important processes including attending to a speaker at a cocktail party and knowing how (or whether) to respond. To examine task encoding and the relative timing of potential neural substrates underlying these behaviors, we developed a spatial selective detection paradigm for monkeys, and recorded activity in primary auditory cortex (AC), dorsolateral prefrontal cortex (dlPFC), and the basolateral amygdala (BLA). A comparison of neural responses among these three areas showed that, as expected, AC encoded the side of the cue and target characteristics before dlPFC and BLA. Interestingly, AC also encoded the choice of the monkey before dlPFC and around the time of BLA. Generally, BLA showed weak responses to all task features except the choice. Decoding analyses

suggested that errors followed from a failure to encode the target stimulus in both AC and dlPFC, but again, these differences arose earlier in AC. The similarities between AC and dlPFC responses were abolished during passive sensory stimulation with identical trial conditions, suggesting that the robust sensory encoding in dlPFC is contextually gated. Thus, counter to a strictly PFC-driven decision process, in this spatial selective listening task AC neural activity represents the sensory and decision information before dlPFC. Unlike in the visual domain, in this auditory task, the BLA does not appear to be robustly involved in selective spatial processing. We examined neural correlates of an auditory spatial selective listening task by recording single-neuron activity in behaving monkeys from the amygdala, dorsolateral prefrontal cortex, and auditory cortex. We found that auditory cortex coded spatial cues and choice-related activity before dorsolateral prefrontal cortex or the amygdala. Auditory cortex also had robust delay period activity. Therefore, we found that auditory cortex could support the neural computations that underlie the behavioral processes in the task.
J Neurosci, 2021; 41

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CENTRAL AMYGDALA - VENTRAL TEGMENTAL AREA – CORTICAL CIRCUITS MEDIATE INITIATION AND MAINTENANCE OF SOCIAL INTERACTION.

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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[1]Nencki Institute of Experimental Biology Polish Academy of Sciences, Laboratory Of Emotions Neurobiology, Warsaw, Poland, [2]Nencki Institute, Polish Academy of Sciences, Center Of Excellence For Neural Plasticity And Brain Disorders: Braincity, A Nencki-embl Partnership, Warsaw, Poland, [3]Nencki Institute of Experimental Biology, Laboratory Of Emotions Neurobiology, Warsaw, Poland

Social interactions are essential for survival in many species, but the neural mechanisms of initiation and maintaining social interaction remain poorly understood. In this study, we identified a crucial role of the neuronal circuit comprising the central amygdala (CeA), ventral tegmental area (VTA), anterior cingulate cortex (ACC), and orbitofrontal cortex (OFC) in promoting social interaction. Through opsins targeted to behaviorally activated neurons and projection-specific chemogenetic manipulations, we found the projections critical for the initiation and maintenance of social interaction. Moreover, in parallel, we investigated the role of the CeA-VTA-ACC/OFC circuit in food motivation. We found that the CeA-VTA and dopaminergic VTA-ACC and VTA-OFC projections mediate social interaction but not food motivation. Together, these findings establish the role of the CeA-VTA-ACC/OFC circuit in regulating social interaction and provide new insights into the regulation of social and food rewards.

Pubmed:
33848461: Andraka K, Kondrakiewicz K, Rojek-Sito K, Ziegart-Sadowska K, Meyza K, Nikolaev T, Hamed A, Kursa M, Wójcik M, Danielewski K, Wiatrowska M, Kublik E, Bekisz M, Lebitko T, Duque D, Jaworski T, Madej H, Konopka W, Boguszewski PM, Knapska E
Distinct circuits in rat central amygdala for defensive behaviors evoked by socially signaled imminent versus remote danger. Animals display a rich repertoire of defensive responses adequate to the threat proximity. In social species, these reactions can be additionally influenced by the behavior of fearful conspecifics. However, the majority of neuroscientific studies on socially triggered defensive responses focuses on one type of behavior, freezing. To study a broader range of socially triggered reactions and underlying mechanisms, we directly compared two experimental paradigms, mimicking occurrence of the imminent versus remote threat. Observation of a partner currently experiencing aversive stimulation evokes passive defensive responses in the observer rats. Similar interaction with a partner that has just undergone the aversive stimulation prompts animals to increase active exploration. Although the observers display behaviors similar to those of the aversively stimulated demonstrators, their reactions are not synchronized in time, suggesting that observers' responses are caused by the change in their affective state rather than mimicry. Using opsins targeted to behaviorally activated neurons, we tagged central amygdala (CeA) cells implicated in observers' responses to either imminent or remote threat and reactivated them during the exploration of a novel environment. The manipulation revealed that the two populations of CeA cells promote passive or active defensive responses, respectively. Further experiments confirmed that the two populations of cells at least partially differ in expression of molecular markers (protein kinase C-δ [PKC-δ] and corticotropin-releasing factor [CRF]) and connectivity patterns (receiving input from the basolateral amygdala or from the anterior insula). The results are consistent with the literature on single subjects' fear conditioning, suggesting that similar neuronal circuits control defensive responses in social and non-social contexts.
Curr Biol, 2021; 31
28842268: Drozd R, Rojek-Sito K, Rygula R
The trait 'pessimism' does not interact with cognitive flexibility but makes rats more vulnerable to stress-induced motivational deficits: Results from the attentional set-shifting task.
In the present study, we have investigated the effects of the traits 'optimism' and 'pessimism' on cognitive flexibility in an animal model of depression based on chronic restraint stress. For this, first, we trained and tested the rats in a series of ambiguous-cue interpretation (ACI) tests, which allowed us to classify them as 'optimistic' or 'pessimistic'. Subsequently, we re-trained and re-tested the animals in the Attentional Set Shifting Task (ASST), which allowed evaluation of the differences

between 'optimists' and 'pessimists' in terms of cognitive flexibility. Finally, we subjected half of the 'optimistic' and half of the 'pessimistic' rats to chronic (2 weeks) restraint stress and assessed the interaction between cognitive judgement bias and stress in the ASST. Although we did not observe statistically significant effects of the investigated traits and stress on cognitive flexibility, the 'pessimistic' animals subjected to chronic restraint stress showed significantly longer latencies to approach experimental rewards than their 'optimistic' conspecifics. This effect may indicate a stress-induced motivational deficit that is specific to 'pessimistic' animals. The results of the present study, along with our previous reports, indicate that the trait 'pessimism' determines animals' vulnerability to stress.
Behav Brain Res, 2017; 335

24308957: Popik P, Kos T, Pluta H, Nikiforuk A, Rojek K, Ryguła R
Inhibition of the glucocorticoid synthesis reverses stress-induced decrease in rat's 50-kHz ultrasonic vocalizations.
The playful, experimenter-administered manual somatosensory stimulation of rats results in a positive affect that triggers emission of ~50-kHz ultrasonic vocalizations (USVs), which have been proposed to index positive emotions akin to human joy and laughter. Our earlier findings showed that restraint stress decreased rat's tendency to emit 50-kHz USVs. Here we investigated whether the effects of stress on "tickling"-induced vocalizations could be alleviated by the glucocorticoid synthesis inhibitor, metyrapone. After the daily tickling sessions carried out until the USV response to tickling has stabilized, the rats were subjected to either handling, handling and metyrapone treatment, restraint stress lasting one week or the restraint stress and metyrapone treatment. Our results confirmed that animals exposed to restraint stress diminish the number of "tickling"-induced vocalizations as compared to the "tickled" but handled conspecifics. Metyrapone treatment prevented this effect in stressed animals having no effects in handled rats. The off-line analysis revealed that the majority (82-88%) of "tickling"-induced USVs were of the 50-kHz frequency modulated type and that the flat USVs appeared much less frequently (8.5-12%) while the 22-kHz alarm calls appeared sporadically (0.3-8%). Moreover, the acoustic parameters of the 50-kHz frequency modulated and flat USVs resembled the calls described earlier in adult rats. The results of the present study offer a way of identifying anti-stress and perhaps anti-depressant action of novel compounds based on the measurement of a positive affect of animals.
Behav Brain Res, 2014; 260

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BOARD NUMBER: S06-074

INVESTIGATION OF NEURAL ACTIVITY IN VARIOUS BRAIN STRUCTURES IN THE SPATIAL CONTEXT ASSOCIATED WITH SOCIAL INTERACTION.

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Rats are highly social animals, and communication between individuals occurs via means of ultrasonic vocalization. These facts were used to elicit and then evaluate the affective state caused by a 21-day social isolation. To study social-seeking-behavior based on spatial cues, a group of rats were trained in pairs in a specially designed four-corner cage containing light-spatial cues. One individual was placed in a specific corner behind the transparent and perforated plexiglass (conditioned), and the other in the center of the arena (conditioning). This arrangement limited social interactions to those in which the emphasis was on the role of ultrasonic vocalization. After 6 days of training, a single rat tests was performed. To determine the neuronal population activity depicting the early response genes (Arc and Homer1 RNAs) in the nuclei of nerve cells of the various brain structures involved in the emotion and spatial information processing, we used the catFISH fluorescence in situ hybridization methodology. The obtained information became the basis for the mathematical model of the relationship between neuronal activation and behavior and a model of the interplay of brain structures in a social context-seeking task. Interestingly, we demonstrated individual differences in spatial-cued contextual responses in both conditioning and conditioned rats. We observed a different pattern of neuronal activation by colocalizing Arc and Homer1a RNAs in several brain structures and the relationships between them. Our study explores the function of individual brain structures in processing spatial information related to the affective emotional state induced by social interactions. Founding NCN-UMO-2018/29/B/NZ7/02021

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-075

NICOTINIC ACTIVATION OF NPY/AGRP NEURONS OF THE ARCUATE NUCLEUS AND ITS ROLE IN STRESS AND FEEDING

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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In the brain, Neuropeptide Y (NPY) modulates anxiety via amygdalar circuits or regulates energy homeostasis and stress response via hypothalamic nuclei, including the arcuate nucleus (ARC). Within the ARC, the orexogenic NPY/Agouti-related peptide (NPY/AgRP) neurons are responsive to variation of the metabolic state, hormones, GABAergic transmission, and nicotine. Interestingly, nicotine consumption is associated with a reduction of food intake and an increase of the hypothalamo-pituitary-adrenal stress response, whereas NPY release to the hypothalamic paraventricular nucleus (PVN) stimulates feeding behavior. We hypothesize that cholinergic activation of NPY-expressing neurons of the ARC is implicated in the modulation of NPY release. To test this hypothesis, we crossed beta2-flox/flox mice with NPY-IRES-Cre to delete beta2-containing nicotinic acetylcholine receptors (nAChRs) in all the NPY-expressing cells. We used in situ hybridization for NPY and beta2-nAChRs to confirm the presence of nAChRs on NPY/AgRP neurons of the ARC in control animals, and the beta2 deletion in the floxed mutants. Then, we induced stress by restraining and we measured its effect in selected behavioral tasks. Given the sexual dimorphism related to stress and alterations in feeding, we decided to test male and female mice, both during light and dark regimen. In females, the deletion of beta2-nAChRs increases the susceptibility to daily stressors and impairs the preference for social interaction, while in males it influences metabolism and body weight. The phenotype observed suggests a more complex interplay between the cholinergic system and NPY release, with possible recruitment of other types of nAChRs and compensatory mechanisms that need further investigation.

Pubmed:
35165173: Abbondanza A, Ribeiro Bas I, Modrak M, Capek M, Minich J, Tyshkevich A, Naser S, Rangotis R, Houdek P, Sumova A, Dumas S, Bernard V, Janickova H
Nicotinic Acetylcholine Receptors Expressed by Striatal Interneurons Inhibit Striatal Activity and Control Striatal-Dependent Behaviors.
Acetylcholine is an important modulator of striatal activity, and it is vital to controlling striatal-dependent behaviors, including motor and cognitive functions. Despite this significance, the mechanisms determining how acetylcholine impacts striatal signaling are still not fully understood. In particular, little is known about the role of nAChRs expressed by striatal interneurons. In the present study, we used FISH to determine which neuronal types express the most prevalent beta2 nicotinic subunit in the mouse striatum. Our data support a common view that nAChR expression is mostly restricted to striatal interneurons. Surprisingly though, cholinergic interneurons were identified as a population with the highest expression of beta2 nicotinic subunit. To investigate the functional significance of beta2-containing nAChRs in striatal interneurons, we deleted them by injecting the AAV-Cre vector into the striatum of male mice. The deletion led to alterations in several behavioral domains, namely, to an increased anxiety-like behavior, decrease in sociability ratio, deficit in discrimination learning, and increased amphetamine-induced hyperlocomotion and c-Fos expression in mice with beta2 deletion. Further colocalization analysis showed that the increased c-Fos expression was present in both medium spiny neurons and presumed striatal interneurons. The present study concludes that, despite being relatively rare, beta2-containing nAChRs are primarily expressed in striatal neurons by cholinergic interneurons and play a significant role in behavior. A large variety of nAChRs are expressed in the striatum, a brain region that is crucial in the control of behavior. The complexity of receptors with different functions is hindering our understanding of mechanisms through which striatal acetylcholine modulates

behavior. We focused on the role of a small population of beta2-containing nAChRs. We identified neuronal types expressing these receptors and determined their impact in the control of explorative behavior, anxiety-like behavior, learning, and sensitivity to stimulants. Additional experiments showed that these alterations were associated with an overall increased activity of striatal neurons. Thus, the small population of nicotinic receptors represents an interesting target for a modulation of response to stimulant drugs and other striatal-based behavior.
J Neurosci, 2022; 42

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BOARD NUMBER: S06-076

REVERSING ESCALATED COCAINE INTAKE WITH SOCIAL CONTACT AND OPTOGENETIC MODULATION OF THE SUBTHALAMIC NUCLEUS

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Despites decades of neuroscience research on cocaine addiction, only social therapies have proven effective in helping cocaine abusers regain the control of their drug consumption. However, while the immediate presence of a stranger peer, naïve to the drug, was shown to reduce recreational cocaine intake in rats, its effects on later stages of cocaine use disorder has not been investigated. Besides, the subthalamic nucleus (STN) has been identified as a potential surgical target in cocaine addiction, since its manipulation with lesions or deep brain stimulation has been shown to reduce motivation for cocaine, prevent cocaine escalation and re-escalation and reduce compulsive drug seeking. Here, rats first subjected to a cocaine escalation procedure, consisting in extended access (6h) to the drug over 20 days, were then tested for 2h self-administration sessions alone or in presence of an observing peer. We assessed the effects of STN optogenetic inhibition and high-frequency stimulation in these two various conditions. In the alone condition, both optogenetic STN inhibition and stimulation drastically reduced cocaine intake. The presence of a peer reduced the level of cocaine intake in control rats but did not reduce it further in the STN inhibited and stimulated groups. These results confirm the beneficial effects of social contact and STN modulation on cocaine abuse in rats, and suggest that the STN plays a key role in the influence of the peer's presence on drug consumption.

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BOARD NUMBER: S06-077

NEURAL CIRCUIT BASIS UNDERLYING A HUNGER-GATED PARENTAL SWITCH

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Animals have sets of instinctive behaviours such as feeding, drinking, mating, parenting and aggression that are crucial for survival and procreation. Different instinctive behaviours are characterised by stereotypic actions in response to specific cues (e.g. parenting towards infants, aggression towards conspecifics). Despite their stereotypy, these behaviours can exhibit a large degree of flexibility due to experience or changes in internal states. While considerable advances have been made in understanding how individual instinctive behaviours are controlled by dedicated neural circuits, how these circuits interact to balance competing needs is less clear. Specifically, it remains unknown how identical sensory stimuli can result in different, or even opposing, instinctive responses due to organisms' conflicting instinctive drives. Here we address this question by characterising interactions between circuits for food-seeking and those controlling parental behaviour. We find that food deprivation switches female mice from parental behaviour to pup-directed aggression. Remarkably, this 'parental switch' is highly target-specific, rather than a state of general aggressivity. Chemogenetic activation of AgRP neurons in the arcuate nucleus is sufficient to elicit the switch, and we have identified candidate nodes of parenting circuits that are both targeted by AgRP neuron projections and the activity of which is suppressed when animals switch towards pup-directed aggression. We are currently performing projection-specific optogenetic manipulations and *in vivo* recordings from postsynaptic neurons in parenting centres to characterise hunger-induced plasticity mechanisms underlying this drastic behavioural switch.

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BOARD NUMBER: S06-078

THE CIRCUIT BASIS OF OLFACTORY MATE RECOGNITION AND LOCALISATION

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Sex pheromones are key social signals in most animals. In Drosophila a dedicated olfactory channel senses a male pheromone, cis-vaccenyl acetate (cVA) that promotes female courtship while repelling males. Here we show that flies can use cVA information from both antennae for sex-specific orientation. We find that cVA olfactory neurons are exquisitely sensitive to concentration differences in a 5 mm range around a male fly. In line with this, second order projection neurons (PNs) detect bi-antennal differences in cVA concentration, which can reliably encode the angular position of a male. We identify an active circuit mechanism increasing left-right response differences in PNs including an interneuron well placed to perform this function by providing contralateral inhibition. At the third layer of the circuit, one neuron population is selectively tuned to an approaching male with speed dependent responses: an olfactory looming signal. A second population responds tonically to a male fly's presence and controls female mating-decisions. Our results show that the olfactory system generates a range of complex percepts in discrete populations of central neurons. Spatial tuning is generated by integration of bilateral sensory information like the auditory system while separation of what and where pathways is reminiscent of the visual cortex.

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BOARD NUMBER: S06-079

AN EARLY LIFE MATERNAL NEGLECT PARADIGM INDUCES ALTERATIONS ON ANTICIPATORY BEHAVIOUR, PREFRONTAL SIGNALLING AND SOCIAL STATUS STABILITY IN ADULT RATS.

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Adverse early life experiences affect neuronal growth and maturation of dopaminergic circuits that modify behaviour under rewarding conditions. Previous studies demonstrate that rats undergoing denial of expected reward in the form of maternal contact (DER) during early post-natal life develop anhedonia and reduced prefrontal cortex dopaminergic activity. To investigate the potential impact of the DER experience on the manifestation of anticipatory behaviour induced by natural rewards, we evaluated in adult male rats their naive sexual preference and performance as well as their anticipatory behaviour during a 2-phase food anticipation task composed of a context-dependent and a cue-dependent trial. DER rats efficiently spent time in the vicinity of and initiated sexual intercourse with receptive females suggesting an intact sexual reward motivation. Interestingly, during the context-dependent phase of food anticipation DER rats displayed chance level preference for the area where food was expected to be delivered, in contrast to control animals. Moreover, during the subsequent cue-dependent phase DER rats displayed an abnormally rapid loss of preference for the area where food was expected, implying a dysregulated dopaminergic function resulting in accelerated devaluation of the expected reward. This behavioural deficit in DER animals was accompanied by elevated pCREB levels in the ventral prefrontal cortex on the last day of the food anticipation task. In addition, the establishment of food approach priority was significantly disrupted in the groups of DER animals. These findings provide additional highlights to the importance of mother-infant contact on reward associated behavioural strategies, brain activation and establishment of social hierarchy.

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FUNCTIONAL HEMISPHERIC ASYMMETRY OF MEDIAL HABENULA-TO-INTERPEDUNCULAR NUCLEUS SYNAPSES AND ITS IMPLICATIONS IN EMOTION-RELATED BEHAVIORS IN MICE

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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The habenula is a phylogenetically conserved bilateral brain structure known to modulate negative emotions. Hemispheric asymmetry in cortical areas plays a key role in behavior and cognition in human and experimental animals. Although habenular asymmetry is prominent in several vertebrate species, there is no evidence for left-right asymmetry in the mammalian habenula. To investigate the asymmetry in synaptic transmission in the medial habenula (MHb) to the interpeduncular nucleus (IPN) pathway in mice, we performed targeted electrical stimulation of left or right MHb-derived axons in acute slices. We discovered that the probability of neurotransmitter release from left MHb terminals was significantly lower than that of right MHb terminals. Furthermore, activation of presynaptic $GABA_B$ receptors potentiated the release from left MHb terminals significantly stronger than that of right MHb terminals. Finally, we selectively suppressed left or right cholinergic MHb neurons using stereotaxic injection of floxed inhibitory DREADDs-expressing AAV into ChAT-Cre mice and performed cued fear conditioning. Chemogenetic inhibition of left but not right MHb significantly decreased the expression of auditory cue-conditioned fear memory. Our study provides the first evidence for a functional asymmetry of the MHb-IPN pathway in mammals and its potential involvement in emotion-related behavior. Physiologically, the right MHb dominates neurotransmission at rest, whereas activation of $GABA_B$ receptors equalized synaptic strength across hemispheres due to stronger potentiation of left side-derived synapses compared to right side-derived synapses. The side-specific silencing effect on the fear expression may reflect the laterality in the $GABA_B$-mediated regulation of this pathway.

Pubmed:
33913808: Bhandari P, Vandael D, Fernández-Fernández D, Fritzius T, Kleindienst D, Önal C, Montanaro J, Gassmann M, Jonas P, Kulik A, Bettler B, Shigemoto R, Koppensteiner P
GABA receptor auxiliary subunits modulate Cav2.3-mediated release from medial habenula terminals.
The synaptic connection from medial habenula (MHb) to interpeduncular nucleus (IPN) is critical for emotion-related behaviors and uniquely expresses R-type Ca channels (Cav2.3) and auxiliary GABA receptor (GBR) subunits, the K-channel tetramerization domain-containing proteins (KCTDs). Activation of GBRs facilitates or inhibits transmitter release from MHb terminals depending on the IPN subnucleus, but the role of KCTDs is unknown. We therefore examined the localization and function of Cav2.3, GBRs, and KCTDs in this pathway in mice. We show in heterologous cells that KCTD8 and KCTD12b directly bind to Cav2.3 and that KCTD8 potentiates Cav2.3 currents in the absence of GBRs. In the rostral IPN, KCTD8, KCTD12b, and Cav2.3 co-localize at the presynaptic active zone. Genetic deletion indicated a bidirectional modulation of Cav2.3-mediated release by these KCTDs with a compensatory increase of KCTD8 in the active zone in KCTD12b-deficient mice. The interaction of Cav2.3 with KCTDs therefore scales synaptic strength independent of GBR activation.
Elife, 2021; 10

FENS Forum 2022
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BOARD NUMBER: S06-081

SOCIAL ATTENTION AND SOCIAL REINFORCEMENT LEARNING – A NATURALISTIC EYE TRACKING PARADIGM AND COMPUTATIONAL MODELLING OF RESPONSES TO EMOTIONAL POINT-LIGHT-DISPLAYS (PLD'S)

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Attention to socially important regions of the face is a key component of daily social interaction and precedes fundamental social skills such as emotion recognition, nonverbal communication and mentalizing. It is also an essential prerequisite to social learning, i. e. when integrating emotional feedback to one's behavior. Direct eye contact is a salient social signal and, along with the aforementioned social skills, is often found to be atypical in disorders characterized by social impairments such as autism spectrum disorder (ASD). However, studies investigating eye gaze on faces often rely on static picture stimuli, which differ remarkably from real-life social interaction. Here, we developed a novel eye tracking paradigm which uses mobile eye tracking in conjunction with AI face-recognition software, to allow fixations to eyes, mouth and face to be measured during a real-life conversation. Performance in the task was investigated in association to 1) social learning in a reinforcement learning task using point-light-displays (PLD's) of emotional faces and 2) levels of autistic traits in a neurotypical sample measured by the Autism Spectrum Quotient (AQ). Computational modelling of trial-by-trial responses was used to determine more comprehensively which parameters of learning were modulated by attention to socially relevant regions and autistic traits. With this study, we aim to provide a method to investigate social attention in a more naturalistic context, which will pave the way for more precise and valid studies on social attention and ASD.

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BOARD NUMBER: S06-082

COMPUTATIONAL MODEL OF STRATEGIC COORDINATION LEARNING IN BABOONS IS SHAPED BY SOCIAL RANKS

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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The ability to reason about another individual's mental state to predict their behavior is still controversial in non-human primates. This ability would be particularly useful to navigate optimally within a social group, and even more so within a social hierarchy. [E1] Many groups of social primates exhibit hierarchical structures and hierarchy directly influences social interactions and social behavior. Little is known about the computational mechanisms by which hierarchy influences social decision learning. A computational modeling approach might help to elucidate the sophistication of Theory of Mind (ToM) abilities in monkeys and the influence of social hierarchy on their social decision learning. Here we analyzed the behavior of seven baboons (*Papio papio*) who played a Coordination Game (CG) in a unique experimental setup in a social group of eighteen. Mentalizing models captured baboons' behavior when they interacted in a coordination game better than traditional reinforcement learning models or heuristics. Their coordination learning was also influenced by the difference in hierarchical status between individuals coordinating with one another. We provide evidence that coordination learning not only depended on the presence of another individual, but was also modulated by the difference in dominance status between the two individuals. Thus, an influence model that also incorporated the influence of the relative difference of social dominance best explained the learning. These findings provide a computational account of how baboons may actively track social hierarchy social decision making to modulate how they mentalize their influence on the other interacting individual, and to adapt their learning process.

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BOARD NUMBER: S06-083

NEUROCOMPUTATIONAL MECHANISMS ENGAGED IN DETECTING COOPERATIVE AND COMPETITIVE INTENTIONS OF OTHERS

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Humans frequently interact with other agents whose intentions can fluctuate over time between competitive and cooperative strategies. How does the brain decide whether the others' intentions are to cooperate or compete when the nature of the interactions is not explicitly signaled? We used model-based fMRI and a task in which participants thought they were playing with another player. In fact, this agent was an algorithm alternating without signaling between cooperative and competitive strategies. A neurocomputational mechanism (controller) underlying arbitration between competitive and cooperative experts outperforms other learning models in predicting choice behavior. These two mentalizing experts anticipate the effect of there own action on the decision of others either under competitive or cooperative assumption. We found that the ventral striatum and ventromedial prefrontal cortex tracked the reliability of the arbitration process between the two experts at the decision making stage. At the time of receiving the winning/losing outcome, a common network encoded reward Prediction Error (rPE) for trials classified as competitive or cooperative (e.g. Ventral Striatum, rostral Anterior Cingulate Cortex). When the controller attributed competitive intentions, the right Temporoparietal Junction and the right dorsolateral Prefrontal Cortex encoded a rPE more robustly for trials classified as competitive than for trials classified as cooperative. These findings provide a neurocomputational account of how the brain dynamically arbitrates between cooperative and competitive intentions when making adaptive social decisions.

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BOARD NUMBER: S06-085

KNOCKDOWN OF PRIMARY CILIARY COMPONENT GENE IN HYPOTHALAMIC PARAVENTRICULAR NUCLEUS OXYTOCIN NEURONS IMPAIRS SOCIAL BEHAVIOR

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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The neuropeptide oxytocin (Oxt) originating from the paraventricular nucleus of hypothalamus (PVN) broadly modulates social behaviors across diverse species. While the impact of PVN-Oxt system on physiological, social, and cognitive functions is extensively studied, how subcellular organelles maintain the function of Oxt neurons is less explored. Primary cilia are especially known to play an important role in cells among various organelles. The present study investigated how the primary cilia in PVN-Oxt neurons affect the activities of PVN-Oxt neurons and thereby social behaviors. Knockdown of *Ift88*, a ciliogenesis gene, with a specific viral expression of shRNA in PVN-Oxt neurons selectively disrupted social recognition and memory. To determine whether the knockdown of ciliogenesis gene in PVN-Oxt neurons induces change in neuronal response, we performed whole-cell patch-clamp and fiber photometry. It was revealed that the neural activities in *Ift88* knockdown PVN-Oxt neurons display differential patterns in basal activities and social cue-interacting conditions. Overall, we suggest the importance of primary cilia of the PVN-Oxt neurons in the maintenance of baseline activities of PVN-Oxt neurons and the scaling the appropriate sizes of response to different social cues. Our finding also pinpoints the role of Oxt neurons in social cognition, extending recent evidence suggesting a causal role of Oxt neuronal activities in the social investigatory approach. Finally, it will be a first step toward appreciating the clinical implication of ciliary function in PVN-Oxt neurons.

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BOARD NUMBER: S06-086

EXPLORING THE NEURAL SUBSTRATE OF SOCIAL FEAR

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Background: Social anxiety disorder (SAD) or social phobia involves persistent avoidance and fear of a specific social stimulus or all social situations (generalized SAD). It has high lifetime prevalence risks and occurs in significant comorbidity with other neuropsychiatric disorders. Its treatment options are limited and unspecific, mostly because of lack of animal models and proper experimental design to study it. Method: We have developed a novel paradigm that relies on the behavioral response of approach to a neutral social stimulus of the same strain (C57BL/6J) and avoidance learning towards BALB/cJ mice. The paradigm consists of a baseline stage of habituation interleaved with social preference tests with C57BL/6J and BALB/cJ adult mice, followed by habituation in a shock delivery apparatus and social fear conditioning (SFC) session, where the approach of the subject towards BALB/cJ mouse is coupled with mild foot shocks. Early and late recall tests were conducted 20 minutes and 24 hours post-SFC, respectively. The behavioral phenotypes were automatically quantified using custom code in MATLAB (TrackRodent). As subjects, we have used Arc-dVenus transgenic mice, which express a destabilized venus fluorescence protein construct under the immediate-early gene Arc promoter. Results: The quantification of Arc positive cells across the entire forebrain revealed significant activation in the NAc, BLA and mPFC, specifically following the encounter with aversive BALB/cJ mouse, which is consistent with previous reports which show the role of these regions in stress responses and social isolation. A better understanding of social decision-making circuit will help in the emission of social fear with targeted therapy and reduce the risk of comorbid disorders.

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BOARD NUMBER: S06-087

THE INFLUENCE OF CHILDHOOD TRAUMA AND EPIGENETIC VARIATION IN OXTR ON ANXIETY PRONENESS AND STRUCTURAL NEUROIMAGING MEASURES IN A SOUTH AFRICAN ADOLESCENT COHORT

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Aims: Anxiety proneness (AP), an endophenotype of anxiety disorders, is a risk factor for anxiety disorder development. We investigated whether (epi)genetic variation in the oxytocin receptor gene (*OXTR*), implicated in anxiety, interacted with childhood trauma (CT) to influence AP and structural brain differences in adolescents of Xhosa or South African Coloured (SAC) ethnicities. **Methods:** We examined the CT x genetic variant effects on AP (n = 951). We assessed methylation of cytosine-guanine dinucleotide (CpG) units in exon 1 using EpiTYPER MassARRAY technology and examined SNP and methylation contributions to structural MRI data for six bilateral brain regions in a subset of participants grouped according to AP and CT severity (n = 90). **Results:** The rs53576 A allele was associated with increased AP in SAC males and interacted with higher CT to predict increased methylation (chr3:8811004-8811010;chr3:8810970-8810974). Higher CT was associated with increased and decreased AP in SAC male and female rs2254298 GA carriers, respectively. The effect of high CT on methylation (chr3:8811254-8811260) was dependent on AP severity, with increased methylation in the high AP groups. rs2254298 and rs53576 GG genotypes were associated with increased left amygdala volume, with the latter genotype also associated with increased left hippocampal and thalamic volumes. Methylation (chr3:8811027-8811028) was proportionally associated with left thalamus and anterior cingulate cortex (ACC) volumes, with inverse associations between methylation and right amygdala (chr3:8811254-8811260), thalamus (chr3:8811173-8811177) and ACC (chr3:8811004-8811010) volumes prior to multiple testing correction. **Conclusions:** CT and *OXTR* (epi)genetic variation are implicated in AP and brain structure in adolescence.

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BOARD NUMBER: S06-088

A SYSTEMATIC REVIEW OF THE NEURAL BASES OF EMOTIONAL INTELLIGENCE.

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Aims. There has been an undeniable growing interest in Emotional Intelligence (EI) in recent years. Although it has important implications for our social and personal life, little is known about the brain bases that underlie EI. This work aims to review the available literature exploring the neural correlates of EI. Methods. A systematic search following the Cochrane guidelines was conducted in the main databases (Medline, PsycINFO, Scopus, and Web of Science). The inclusion criteria were empirical articles published in a peer-reviewed journal, written in Spanish or English, and that included an objective EI measurement and a neuroimaging technique. Results and Conclusions. Twenty-eight articles were finally identified. According to the results of the selected articles, the main brain regions identified were the dlPFC, OFC, vmPFC, amygdala, and insula. The authors, in general, related these areas to a neural network for social cognition, processes of cognitive and affective integration, and the somatic marker circuitry. Differences between emotional and cognitive intelligence at neural level were also considered. Finally, we identified a number of limitations and propose future lines of research. This research has been funded by the Research Plan of the University of Malaga (project B1-2019_08).

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-089

SOCIAL CUES MODULATE CIRCUIT DYNAMICS TO CONTROL THE CHOICE BETWEEN COMMUNICATION SIGNALS IN DROSOPHILA.

POSTER SESSION 06 - SECTION: CIRCUITS FOR SOCIAL BEHAVIOR

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Communication is multi-modal - when interacting, we speak, gesticulate, and touch. However, the neural computations and circuits that choose these communication signals are unclear. We address this issue in *Drosophila* which thanks to its complex social behavior and genetic toolbox is ideal for dissecting the neural basis of communication. During courtship, male flies produce two types of communication signals to woo the female: air-borne song and substrate-borne vibrations.
Using statistical modeling of pose tracking data alongside signal recordings during courtship, we found that female locomotion controls the choice between song and vibration: When the female moves, the male sings, when she is stationary, he vibrates. Optogenetic control of female locomotion confirms the results.
Combining optogenetics and network models, we next elucidated the circuit mechanisms underlying this choice. Two groups of neurons in the central brain, “P1a“ and “pC2”, drive both signals with complex dynamics: While activation of P1a in solitary males primarily drives persistent vibrations, pC2 induces first song and then vibrations. A circuit model reveals that these complex dynamics emerge from a combination of recurrence and mutual inhibition.
Lastly, we show how sensory cues modify the intrinsic circuit dynamics: P1a is activated by female chemical cues, accordingly we observe higher vibration production in the presence of a dead, immobile female. pC2 is activated when visual motion cues are provided and we find that chasing an alive female drives more song.
Overall, we show how intrinsic circuit dynamics are modified by sensory cues to produce context-dependent signals during social interactions.

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BOARD NUMBER: S06-090

BRAIN STRESS AND NORADRENERGIC SYSTEM MEDIATE THE MECHANISMS UNDERLYING RELAPSE CAUSED BY EXPOSURE TO SOCIAL DEFEAT IN THE NUCLEUS ACCUMBENS IN MORPHINE DEPENDENT MICE

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Aims: Addiction to substances of abuse is a chronic neurological disorder characterized by drug-seeking, compulsive consumption, and frequent relapses after long periods of abstinence. Many studies have proposed that stress has a key role in the development of drug addiction. In this study, we have investigated the influence of *Social Defeat* (SD)in relapse through the model of conditioned-place aversion (CPA). We have also analyzed pERK1/2 expression in nucleus accumbens (NAcc) during this paradigm. **Methods**: 60 C57BL/6J mice were rendered dependent on morphine and injected acutely with naloxone before paired to confinement in a naloxone-associated compartment. After extinction of the aversive memory, animals received CP-154,526 (CRF1 receptor antagonist), propranolol (beta-adrenergic receptor antagonist) or vehicle and were exposed to SD to induce relapse. pERK1/2 expression was analyzed in NAcc by Western Blot. **Results**: Our results show that application of SD after extinction induced relapse. The administration of CP-154,526 and propranolol antagonized this effect. Besides, a significant increase in pERK1/2 expression was observed in NAcc in the group exposed to stress receiving vehicle compared to the non-stress group. **Conclussions:** These results show the important relationship between stress and compulsive drug seeking, as well as a potential therapeutic value of propranolol and CP-154,526 in the treatment of relapses.

Pubmed:

33444599: Navarro-Zaragoza J, Martínez-Laorden E, Teruel-Fernández FJ, Gómez-Murcia V, Cánovas A, Milanés MV, Laorden ML, Almela P
Naloxone-induced conditioned place aversion score and extinction period are higher in C57BL/6J morphine-dependent mice than in Swiss: Role of HPA axis.
Intense associative memories develop between drug-paired contextual cues and the drug withdrawal associated aversive feeling. They have been suggested to contribute to the high rate of relapse. Our study was aimed to elucidate the involvement of hypothalamic-pituitary-adrenocortical (HPA) axis activity in the expression and extinction of aversive memory in Swiss and C57BL/6J (B6) mice. The animals were rendered dependent on morphine by i.p. injection of increasing doses of morphine (10-60 mg/kg). The negative state associated with naloxone (1 mg/kg s.c.) precipitated morphine withdrawal was examined by using conditioned place aversion (CPA) paradigm. B6 mice obtained a higher aversion score and took longer to extinguish the aversive memory than Swiss mice. In addition, corticosterone levels were increased after CPA expression. Moreover, corticosterone levels were decreased during CPA extinction in Swiss mice without changes in B6 mice. Pre-treatment with the selective CRF1 receptor antagonist CP-154,526 before naloxone, impaired morphine-withdrawal aversive memory acquisition and decreased the extinction period. CP-154,526 also antagonized the increased levels of corticosterone observed after CPA expression in Swiss mice, without any changes in B6 mice. These results indicate that HPA axis could be a critical factor governing opioid withdrawal memory storage and retrieval, but in a strain or stock-specific manner. The differences observed between Swiss and B6 mice suggest that the treatment of addictive disorders should consider different individual predisposition to associate the aversive learning with the context.
Pharmacol Biochem Behav, 2021; 201

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BOARD NUMBER: S06-091

STRIATAL MODULATION OF BRAIN CHOLESTEROL METABOLISM DURING ABSTINENCE REDUCES COCAINE SEEKING IN RATS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Cholesterol is one of the major brain lipids and plays an important role in different brain functions. Some studies show that drugs of abuse alter brain cholesterol metabolism, suggesting that cholesterol may participate in the mechanisms of addiction. Here, we investigated whether manipulations of cholesterol metabolism could alter addiction-related behaviors. For this, rats underwent 10 sessions of cocaine self-administration; then at the beginning of abstinence period, using viral approaches we overexpressed CYP46A1 (the main enzyme responsible for cholesterol degradation) or GFP (for control animals) in the dorsal striatum (DSt) or the anterior cingulate cortex (ACC). Six weeks after the last self-administration session, drug seeking was measured in an extinction session. Using a similar protocol, we also investigated the effects of striatal CYP46A1 overexpression on sucrose seeking. Finally, we overexpressed CYP46A1 in the DSt before the beginning of the self-administration of cocaine and measured potential changes in drug intake, motivation and resistance to punishment. We found that overexpression of CYP46A1 in DSt, but not in ACC, reduced the cocaine seeking. CYP46A1 overexpression had no effect on sucrose seeking. No change in levels of cocaine intake, motivation, and resistance to punishment was found when CYP46A1 was overexpressed before self-administration. Our results suggest that manipulating cholesterol metabolism in the DSt during abstinence might reduce risks of relapse to addiction, without changing motivation for natural reward or the direct rewarding effects of the drug. Therefore, we propose that cholesterol metabolism might represent a new therapeutic target to prevent relapse to cocaine addiction.

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BOARD NUMBER: S06-092

UNIQUE GENE EXPRESSION PROFILES IN THE EXTINCTION OF COCAINE AND NICOTINE SELF-ADMINISTRATION.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Epigenetics play a major role in the acquisition and maintenance of drug addiction. While many other drugs have been extensively investigated, nicotine stands out as having been poorly characterized in terms of its epigenetic effects. We used RNA sequencing from the PFC of nicotine (n=15), cocaine (n=8), and saline (n=8) self-administering rats 1 or 6 days after extinction to investigate the persistent changes in gene expression as a result of the drugs administration. Differential expression analyses showed unique expression profiles for cocaine and nicotine both on the first and 6th day of extinction. For example, cocaine self-administration led to an upregulation of a larger number of genes, however, the genes were mostly IEGs associated with acute drug response. Conversely, nicotine extinction led to a regulation of a smaller set of genes involved in the phosphorylation of MAPK pathways. Further network connectivity analyses using WGCNA revealed separate networks associated with general drug administration, nicotine administration, and cocaine administration. Interestingly the module of genes associated with extinction of nicotine self-administration was related to the regulation MAPK pathways. In conclusion, nicotine and cocaine lead to unique changes in RNA expression in the PFC during extinction, that are associated with separate gene networks, thus providing further evidence that it is important to look at these drugs of abuse individually.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-093

THE EFFECTS OF ACUPUNCTURE ON INTRACRANIAL SELF-STIMULATION OF THE MEDIAL FOREBRAIN BUNDLE IN RATS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Aims: The neurobiological substrate for the facilitation of brain stimulation reward and cocaine-seeking behavior is believed to involve the mesolimbic dopamine pathway. We have previously demonstrated that acupuncture at Shenmen (HT7) acupoints suppressed cocaine-induced dopamine release in the nucleus accumbens and cocaine-primed reinstatement through activation of GABA neurons in the ventral tegmental area. The aim of this study was to determine whether acupuncture would alter brain reward thresholds and their responses to cocaine. **Methods:** To assess the effects of HT7 stimulation on brain reward function, rats were used in intracranial self-stimulation paradigm with the frequency-rate curve-shift procedure. Rats were trained to lever-press for electrical stimulation of medial forebrain bundle of the lateral hypothalamus. Brain reward thresholds were monitored following acupuncture stimulation in rats under baseline conditions and exposure to acute cocaine (3.2 mg/kg, i.p.). **Results:** Results showed that acupuncture at HT7 acupoints, but not at Sanyinjiao (SP6) points, triggered a rightward shift of the frequency-rate curve and elevated reward thresholds under baseline conditions in drug-naïve rats. Also, HT7 stimulation tended to attenuate the threshold-lowering effects of cocaine. **Conclusions:** These results provide evidence that acupuncture at HT7 effectively modulates brain reward function.
[Supported by a grant (20182MFDS422 and 20182MFDS425) from Ministry of Food and Drug Safety and National Research Foundation of Korea (NRF) grant funded by the Korea government MSIT (2018R1A5A2025272 and 2019R1I1A1A01052581)]

FENS Forum 2022
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BOARD NUMBER: S06-094

THC EXPOSURE DURING ADOLESCENCE PRODUCES IMPULSIVITY-LIKE BEHAVIOR IN ADULTHOOD IN A WIN 55,212-2 SELF-ADMINISTRATION MICE MODEL

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Rationale Cannabis addiction is a multifactorial chronically relapsing disorder, which lacks effective treatment despite cannabis being the most used illicit drug worldwide. Regular consumption typically begins during adolescence, a critical developmental stage, leading to suggest that adolescent cannabinoid exposure might increase the risk for drug addiction in adulthood. **Aims** This study investigated the development of cannabis addiction in adult male mice after adolescent exposure to the main psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (THC), to unravel the neurobiological correlates of resilience and vulnerability. **Methods** Adolescent mice were exposed to 5 mg/kg of THC from postnatal days 37 to 57. Operant self-administration sessions of WIN 55,212-2 (WIN), a synthetic cannabinoid full agonist (12.5mg/kg/injection), were conducted for 10 days. Mice were tested for three addiction-like criteria (persistence to response, motivation and compulsivity) and two vulnerability phenotypic traits (impulsivity and cue-induced reinstatement of drug-seeking), characterizing addicted and non-addicted mice. qPCRs were performed to detect differentially expressed genes in the mesocorticolimbic pathway of addicted and non-addicted mice. **Results** Adolescent THC-exposed mice displayed an impulsive-like behavior in adulthood during WIN self-administration, that was more pronounced in addicts, while it did not modify the reinforcement of WIN nor the development of cannabis addiction. Moreover, findings showed a downregulated dopamine D_2 receptor (D_2R) expression in the nucleus accumbens (NAc) of THC-exposed mice. Viral-mediated D_2R overexpression was performed to functionally validate these results. **Conclusions** These findings suggest that adolescent THC exposure under these particular conditions produces impulsivity-like behavior in adulthood, possibly mediated by a downregulation in NAc D_2R expression.

Pubmed:
33010795: Valle-León M, Callado LF, Aso E, Cajiao-Manrique MM, Sahlholm K, López-Cano M, Soler C, Altafaj X, Watanabe M, Ferré S, Fernández-Dueñas V, Menchón JM, Ciruela F
Decreased striatal adenosine A-dopamine D receptor heteromerization in schizophrenia.
According to the adenosine hypothesis of schizophrenia, the classically associated hyperdopaminergic state may be secondary to a loss of function of the adenosinergic system. Such a hypoadenosinergic state might either be due to a reduction of the extracellular levels of adenosine or alterations in the density of adenosine A receptors (ARs) or their degree of functional heteromerization with dopamine D receptors (DR). In the present study, we provide preclinical and clinical evidences for this latter mechanism. Two animal models for the study of schizophrenia endophenotypes, namely the phencyclidine (PCP) mouse model and the AR knockout mice, were used to establish correlations between behavioural and molecular studies. In addition, a new AlphaLISA-based method was implemented to detect native AR-DR heteromers in mouse and human brain. First, we observed a reduction of prepulse inhibition in AR knockout mice, similar to that observed in the PCP animal model of sensory gating impairment of schizophrenia, as well as a significant upregulation of striatal DR without changes in AR expression in PCP-treated animals. In addition, PCP-treated animals showed a significant reduction of striatal AR-DR heteromers, as demonstrated by the AlphaLISA-based method. A significant and pronounced reduction of AR-DR heteromers was next demonstrated in postmortem caudate nucleus from schizophrenic subjects, even though both DR and AR were upregulated. Finally, in PCP-treated animals, sub-chronic administration of haloperidol or clozapine counteracted the reduction of striatal AR-DR heteromers. The degree of AR-DR heteromer formation in schizophrenia might constitute a hallmark of the illness, which indeed should be further studied to establish possible correlations with chronic antipsychotic treatments.
Neuropsychopharmacology, 2021; 46

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-095

ROLE OF ACTIVITY-DEPENDENT TRANSMITTER SWITCHING IN DRUG-INDUCED CHANGES IN BEHAVIOR

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Drugs of abuse alter neuronal activity and cause widespread neuroplasticity that results in behavioral alterations. We investigated whether exposure to drugs causes a form of activity-dependent plasticity called neurotransmitter switching. This plasticity occurs when neurons lose the transmitter they have expressed before and gain a different one, and can influence behavior. We treated mice with either phencyclidine or methamphetamine, two drugs that differ in their molecular targets, but both cause hyperactivity of prefrontal cortex glutamatergic neurons. After a 10-day exposure to either drug, we discovered that ~600 prelimbic cortex (PrL) pyramidal neurons have gained GABA and exhibit decreased expression of the vesicular glutamate transporter. Overriding the gain of GABA in PrL glutamatergic neurons, using an RNA-interference strategy, prevented the appearance of drug-induced memory deficits in the novel object recognition test and spontaneous alternation task as well as locomotor sensitization. These results indicate that the switch is causally linked to these behavioral alterations. Because neurotransmitter switching is activity-dependent, we tested whether drug-induced hyperactivity of PrL glutamatergic neurons drives the transmitter switch. To suppress PrL hyperactivity we combined drug-administration with chemogenetic activation of PV+ interneurons that provide perisomatic inhibition of glutamatergic cells. This intervention was sufficient to block the transmitter switch and prevent the changes in behavior. Remarkably, suppression of drug-induced elevation of PrL neuron activity, after the switch has occurred, reversed the change in transmitter identity and rescued the behavioral deficits. The results suggest therapeutic opportunities for mitigating drug-induced cognitive deficits through manipulation of prefrontal cortex electrical activity.

Pubmed:
32434858: Li HQ, Pratelli M, Godavarthi S, Zambetti S, Spitzer NC
Decoding Neurotransmitter Switching: The Road Forward.
Neurotransmitter switching is a form of brain plasticity in which an environmental stimulus causes neurons to replace one neurotransmitter with another, often resulting in changes in behavior. This raises the possibility of applying a specific environmental stimulus to induce a switch that can enhance a desirable behavior or ameliorate symptoms of a specific pathology. For example, a stimulus inducing an increase in the number of neurons expressing dopamine could treat Parkinson's disease, or one affecting the number expressing serotonin could alleviate depression. This may already be producing successful treatment outcomes without our knowing that transmitter switching is involved, with improvement of motor function through physical activity and cure of seasonal depression with phototherapy. This review presents prospects for future investigation of neurotransmitter switching, considering opportunities and challenges for future research and describing how the investigation of transmitter switching is likely to evolve with new tools, thus reshaping our understanding of both normal brain function and mental illness.
J Neurosci, 2020; 40
31243951: Nazzi S, Maddaloni G, Pratelli M, Pasqualetti M
Fluoxetine Induces Morphological Rearrangements of Serotonergic Fibers in the Hippocampus.
Serotonin (5-HT)-releasing fibers show substantial structural plasticity in response to genetically induced changes in 5-HT content. However, whether 5-HT fibers appear malleable also following clinically relevant variations in 5-HT levels that may occur throughout an individual's life has not been investigated. Here, using confocal imaging and 3D modeling analysis in knock-in mice, we show that chronic administration of the antidepressant fluoxetine dramatically affects the morphology of 5-HT fibers innervating the dorsal and ventral hippocampus resulting in a reduced density of fibers. Importantly, GFP fluorescence levels appeared unaffected in the somata of both dorsal and median 5-HT neurons, arguing against potential fluoxetine-mediated down-regulation of the promoter driving GFP expression in the mouse model. In keeping with this notion, mice bearing the pan-serotonergic driver partnered with a Cre-responsive tdTomato allele also showed similar morphological alterations in hippocampal 5-HT circuitry following chronic fluoxetine treatment. Moreover 5-HT fibers innervating the cortex

showed proper density and no overt morphological disorganization, indicating that the reported fluoxetine-induced rearrangements were hippocampus specific. On the whole, these data suggest that 5-HT fibers are shaped in response to subtle changes of 5-HT homeostasis and may provide a structural basis by which antidepressants exert their therapeutic effect.
ACS Chem Neurosci, 2019; 10
30513372: Pratelli M, Pasqualetti M
Serotonergic neurotransmission manipulation for the understanding of brain development and function: Learning from Tph2 genetic models.
Serotonin (5-hydroxytriptamine; 5-HT) is a fascinating neurotransmitter that thanks to an extensive axonal network is released throughout the entire central nervous system (CNS) and exerts its action on the modulation of a countless number of physiological, behavioral and cognitive processes. In addition, cumulating evidences have linked alteration in 5-HT neurotransmission with the onset of psychiatric and neurodevelopmental disorders, such as depression, autisms and schizophrenia. Nevertheless only 5% of the total body content of serotonin exerts its action in the CNS, while the rest is synthetized and stored in peripheral tissues where it acts as an autacoid. In 2003 it became evident that two distinct isoforms of tryptophan hydroxylase (Tph), the rate-limiting enzyme for the synthesis of serotonin, are selectively expressed in peripheral tissues and in the CNS, with Tph2 as the brain specific isoform. In the present review we describe how the discovery of Tph2 has improved our understanding on the role of serotonergic neurotransmission. We mainly focus on the analysis of animal models generated by genetic manipulation of Tph2, in which the synthesis of brain serotonin was either reduced or disrupted. The consequences of an altered serotonergic neurotransmission on brain development, as well as on physiological and behavioral processes will be assessed. Finally, we report on several association studies that have linked single nucleotide polymorphisms (SNPs) in the human TPH2 gene with behavioral disturbances and neuropsychiatric disorders.
Biochimie, 2019; 161
28769763: Maddaloni G, Bertero A, Pratelli M, Barsotti N, Boonstra A, Giorgi A, Migliarini S, Pasqualetti M
Development of Serotonergic Fibers in the Post-Natal Mouse Brain.
Serotonin (5-HT)-synthetizing neurons, which are confined in the nuclei of the rhombencephalon, provide a pervasive innervation of the central nervous system (CNS) and are involved in the modulation of a plethora of functions in both developing and adult brain. Classical studies have described the post-natal development of serotonergic axons as a linear process of terminal field innervation. However, technical limitations have hampered a fine morphological characterization. With the advent of genetic mouse models, the possibility to label specific neuronal populations allowed the rigorous measurement of their axonal morphological features as well as their developmental dynamics. Here, we used the knock-in mouse line, in which GFP expression allows punctual identification of serotonergic neurons and axons, for confocal microscope imaging and we performed 3-dimensional reconstruction in order to morphologically characterize the development of serotonergic fibers in specified brain targets from birth to adulthood. Our analysis highlighted region-specific developmental patterns of serotonergic fiber density ranging from a linear and progressive colonization of the target (Caudate/Putamen, Basolateral Amygdala, Geniculate Nucleus and Substantia Nigra) to a transient increase in fiber density (medial Prefrontal Cortex, Globus Pallidus, Somatosensory Cortex and Hippocampus) occurring with a region-specific timing. Despite a common pattern of early post-natal morphological maturation in which a progressive rearrangement from a dot-shaped to a regular and smooth fiber morphology was observed, starting from post-natal day 28 serotonergic fibers acquire the region specific morphological features present in the adult. In conclusion, we provided novel, target-specific insights on the morphology and temporal dynamics of the developing serotonergic fibers.
Front Cell Neurosci, 2017; 11
28029782: Pacini G, Marino A, Migliarini S, Brilli E, Pelosi B, Maddaloni G, Pratelli M, Pellegrino M, Ferrari A, Pasqualetti M
A Tph2 Reporter Stem Cell Line To Model in Vitro and in Vivo Serotonergic Neuron Development and Function.
Modeling biological systems in vitro has contributed to clarification of complex mechanisms in simplified and controlled experimental conditions. Mouse embryonic stem (mES) cells can be successfully differentiated toward specific neuronal cell fates, thus representing an attractive tool to dissect, in vitro, mechanisms that underlie complex neuronal features. In this study, we generated and characterized a reporter mES cell line, called Tph2, in which the vital reporter GFP replaces the tryptophan hydroxylase 2 (Tph2) gene. Tph2 mES cells selectively express GFP upon in vitro differentiation toward the serotonergic fate, they synthesize serotonin, possess excitable membranes, and show the typical morphological, morphometrical, and molecular features of in vivo serotonergic neurons. Thanks to the vital reporter GFP, we highlighted by time-lapse video microscopy several dynamic processes such as cell migration and axonal outgrowth in living cultures. Finally, we demonstrated that predifferentiated Tph2 cells are able to terminally differentiate, integrate, and innervate the host brain when grafted in vivo. On the whole, the present study introduces the Tph2 mES cell line as a useful tool allowing accurate developmental and dynamic studies and representing a reliable platform for the study of serotonergic neurons in

health and disease.
ACS Chem Neurosci, 2017; 8
28413824: Pratelli M, Migliarini S, Pelosi B, Napolitano F, Usiello A, Pasqualetti M
Perturbation of Serotonin Homeostasis during Adulthood Affects Serotonergic Neuronal Circuitry.
Growing evidence shows that the neurotransmitter serotonin (5-HT) modulates the fine-tuning of neuron development and the establishment of wiring patterns in the brain. However, whether serotonin is involved in the maintenance of neuronal circuitry in the adult brain remains elusive. Here, we use a conditional knockout (cKO) mouse line to assess the impact of serotonin depletion during adulthood on serotonergic system organization. Data show that the density of serotonergic fibers is increased in the hippocampus and decreased in the thalamic paraventricular nucleus (PVN) as a consequence of brain serotonin depletion. Strikingly, these defects are rescued following reestablishment of brain 5-HT signaling via administration of the serotonin precursor 5-hydroxytryptophan (5-HTP). Finally, 3D reconstruction of serotonergic fibers reveals that changes in serotonin homeostasis affect axonal branching complexity. These data demonstrate that maintaining proper serotonin homeostasis in the adult brain is crucial to preserve the correct serotonergic axonal wiring.
eNeuro, 2017 Mar-Apr; 4
26291320: Pelosi B, Pratelli M, Migliarini S, Pacini G, Pasqualetti M
Generation of a Tph2 Conditional Knockout Mouse Line for Time- and Tissue-Specific Depletion of Brain Serotonin.
Serotonin has been gaining increasing attention during the last two decades due to the dual function of this monoamine as key regulator during critical developmental events and as neurotransmitter. Importantly, unbalanced serotonergic levels during critical temporal phases might contribute to the onset of neuropsychiatric disorders, such as schizophrenia and autism. Despite increasing evidences from both animal models and human genetic studies have underpinned the importance of serotonin homeostasis maintenance during central nervous system development and adulthood, the precise role of this molecule in time-specific activities is only beginning to be elucidated. Serotonin synthesis is a 2-step process, the first step of which is mediated by the rate-limiting activity of Tph enzymes, belonging to the family of aromatic amino acid hydroxylases and existing in two isoforms, Tph1 and Tph2, responsible for the production of peripheral and brain serotonin, respectively. In the present study, we generated and validated a conditional knockout mouse line, Tph2flox/flox, in which brain serotonin can be effectively ablated with time specificity. We demonstrated that the Cre-mediated excision of the third exon of Tph2 gene results in the production of a Tph2null allele in which we observed the near-complete loss of brain serotonin, as well as the growth defects and perinatal lethality observed in serotonin conventional knockouts. We also revealed that in mice harbouring the Tph2null allele, but not in wild-types, two distinct Tph2 mRNA isoforms are present, namely Tph2Δ3 and Tph2Δ3Δ4, with the latter showing an in-frame deletion of amino acids 84-178 and coding a protein that could potentially retain non-negligible enzymatic activity. As we could not detect Tph1 expression in the raphe, we made the hypothesis that the Tph2Δ3Δ4 isoform can be at the origin of the residual, sub-threshold amount of serotonin detected in the brain of Tph2null/null mice. Finally, we set up a tamoxifen administration protocol that allows an efficient, time-specific inactivation of brain serotonin synthesis. On the whole, we generated a suitable genetic tool to investigate how serotonin depletion impacts on time-specific events during central nervous system development and adulthood life.
PLoS One, 2015; 10
25098329: Pelosi B, Migliarini S, Pacini G, Pratelli M, Pasqualetti M
Generation of Pet1210-Cre transgenic mouse line reveals non-serotonergic expression domains of Pet1 both in CNS and periphery.
Neurons producing serotonin (5-hydroxytryptamine, 5-HT) constitute one of the most widely distributed neuronal networks in the mammalian central nervous system (CNS) and exhibit a profuse innervation throughout the CNS already at early stages of development. Serotonergic neuron specification is controlled by a combination of secreted molecules and transcription factors such as Shh, Fgf4/8, Nkx2.2, Lmx1b and Pet1. In the mouse, Pet1 mRNA expression appears between 10 and 11 days post coitum (dpc) in serotonergic post-mitotic precursors and persists in serotonergic neurons up to adulthood, where it promotes the expression of genes defining the mature serotonergic phenotype such as tryptophan hydroxylase 2 (Tph2) and serotonin transporter (SERT). Hence, the generation of genetic tools based on Pet1 specific expression represents a valuable approach to study the development and function of the serotonergic system. Here, we report the generation of a Pet1(210)-Cre transgenic mouse line in which the Cre recombinase is expressed under the control of a 210 kb fragment from the Pet1 genetic locus to ensure a reliable and faithful control of somatic recombination in Pet1 cell lineage. Besides Cre-mediated recombination accurately occurred in the serotonergic system as expected and according to previous studies, Pet1(210)-Cre transgenic mouse line allowed us to identify novel, so far uncharacterized, Pet1 expression domains. Indeed, we showed that in the raphe Pet1 is expressed also in a non-serotonergic neuronal population intermingled with Tph2-expressing cells and mostly localized in the B8 and B9 nuclei. Moreover, we detected Cre-mediated recombination also in the developing pancreas and in the ureteric bud derivatives of the kidney, where it reflected a specific Pet1 expression. Thus, Pet1(210)-Cre transgenic mouse line faithfully drives Cre-mediated recombination in all Pet1 expression domains

representing a valuable tool to genetically manipulate serotonergic and non-serotonergic Pet1 cell lineages. PLoS One, 2014; 9

FENS Forum 2022
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BOARD NUMBER: S06-096

A NEURAL CIRCUIT FOR CONTROLLING LONG-TERM VOLUNTARY ALCOHOL CONSUMPTION.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Alcohol-use-disorders (AUDs) are chronic relapsing illnesses, with alcohol often self-medicated to cope with distressing symptoms of various mental health issues. Given that the brain serotonin (5-HT) system is closely involved in stress responses to social isolation, fear, anxiety and depression, we hypothesized that alterations in brain 5-HT transmission contributes to the reinforcement of alcohol drinking behaviour. Long-term alcohol consumption alters the expression and function of 5-HT1A receptors, and raphe 5-HT neurons signaling. We demonstrated that a chronic treatment with partial 5-HT1A receptor agonists reduces ethanol intake, withdrawal-induced anxiety-like behaviour and the deficits in hippocampal neurogenesis after long-term ethanol intake. However, the contribution of 5-HT1A auto- vs heteroreceptors and the role played by raphe 5-HTergic transmission in alcohol consumption is less understood. We used highly selective 5-HT1A agonist (F13640) or biased agonists that preferentially target 5-HT1A auto- (F13714) or heteroreceptors (F5599)to determine which of the 5-HT1A receptor population mediates the effect of 5-HT1A agonists on the reduction of ethanol intake. Brain cannulation and local injections of agonists allowed us to identify which raphe nucleus, dorsal or median, mediates the reduction of ethanol intake following short- vs long-term exposure. We used CRE-dependent DREADDs in *pet1*-5-HT neurons, in combination with local injections of the designer drug CNO to identify 5-HTergic circuits that control long-term ethanol intake. Using this strategy, we discovered that 5-HT neuronal circuits switched after long-term drinking from DR to MR-DG, and suggests that drugs targeting those particular circuits might represent viable therapeutic strategies for the treatment of AUDs.

Pubmed:
35152045: Beecher K, Wang J, Chehrehasa F, Depoortere R, Varney MA, Newman-Tancredi A, Bartlett SE, Belmer A
Dissecting the contribution of 5-HT auto- and heteroreceptors in sucrose overconsumption in mice.
The rise in obesity prevalence has been linked to overconsumption of high-sugar containing food and beverages. Recent evidence suggests that chronic sucrose consumption leads to changes in serotonergic neuroplasticity within the neural circuits involved in feeding control. Although there is a relationship between serotonin signalling in the brain and diet-induced obesity, the specific serotonin (5-HT) receptors or pathways involved remain unknown. The 5-HT receptor subtype plays a role in regulating mood, anxiety, and appetite, and has been associated with reversing addiction to substances of abuse. However, the respective role of 5-HT auto- vs heteroreceptors in sucrose consumption has not been examined. Mice were given controlled access to either 5%, 10% or 25% w/v sucrose, or water as a control, for 12 weeks using the well-established "drinking in the dark" protocol (n = 6-8 mice per group). Ligands selectively targeting 5-HT auto- and/or heteroreceptors (NLX-112, unbiased 5-HT receptor agonist; NLX-101, preferential heteroreceptor agonist; F13714, preferential autoreceptor agonist) were administered i.p. acutely after 6 and 12 weeks of sucrose consumption. The specific involvement of 5-HT receptors in these effects was verified by blockade with the selective 5-HT receptors antagonist WAY-100,635. The specific subpopulation of 5-HT receptors involved in sucrose consumption was dependent on the concentration of sucrose solution and the duration of exposure to sucrose (6 weeks vs 12 weeks). Long-term sucrose consumption leads to accentuated 5-HT autoreceptor function. Thus, targeting 5-HT autoreceptors might represent an effective therapeutic strategy to combat the rise in obesity resulting from the overconsumption of high-sugar diet.
Biomed Pharmacother, 2022; 148
34163325: Beecher K, Alvarez Cooper I, Wang J, Walters SB, Chehrehasa F, Bartlett SE, Belmer A
Long-Term Overconsumption of Sugar Starting at Adolescence Produces Persistent Hyperactivity and Neurocognitive Deficits in Adulthood.
Sugar has become embedded in modern food and beverages. This has led to overconsumption of sugar in children, adolescents, and adults, with more than 60 countries consuming more than four times (>100 g/person/day) the WHO recommendations (25 g/person/day). Recent evidence suggests that obesity and impulsivity from poor dietary habits leads to

further overconsumption of processed food and beverages. The long-term effects on cognitive processes and hyperactivity from sugar overconsumption, beginning at adolescence are not known. Using a well-validated mouse model of sugar consumption, we found that long-term sugar consumption, at a level that significantly augments weight gain, elicits an abnormal hyperlocomotor response to novelty and alters both episodic and spatial memory. Our results are similar to those reported in attention deficit and hyperactivity disorders. The deficits in hippocampal-dependent learning and memory were accompanied by altered hippocampal neurogenesis, with an overall decrease in the proliferation and differentiation of newborn neurons within the dentate gyrus. This suggests that long-term overconsumption of sugar, as that which occurs in the Western Diet might contribute to an increased risk of developing persistent hyperactivity and neurocognitive deficits in adulthood.
Front Neurosci, 2021; 15
31133811: Belmer A, Beecher K, Jacques A, Patkar OL, Sicherre F, Bartlett SE
Axonal Non-segregation of the Vesicular Glutamate Transporter VGLUT3 Within Serotonergic Projections in the Mouse Forebrain.
A subpopulation of raphe 5-HT neurons expresses the vesicular glutamate transporter VGLUT3 with the co-release of glutamate and serotonin proposed to play a pivotal role in encoding reward- and anxiety-related behaviors. Serotonin axons are identifiable by immunolabeling of either serotonin (5-HT) or the plasma membrane 5-HT transporter (SERT), with SERT labeling demonstrated to be only partially overlapping with 5-HT staining. Studies investigating the colocalization or segregation of VGLUT3 within SERT or 5-HT immunolabeled boutons have led to inconsistent results. Therefore, we combined immunohistochemistry, high resolution confocal imaging, and 3D-reconstruction techniques to map and quantify the distribution of VGLUT3 immunoreactive boutons within 5-HT vs. SERT-positive axons in various regions of the mouse forebrain, including the prefrontal cortex, nucleus accumbens core and shell, bed nucleus of the stria terminalis, dorsal striatum, lateral septum, basolateral and central amygdala, and hippocampus. Our results demonstrate that about 90% of 5-HT boutons are colocalized with SERT in almost all the brain regions studied, which therefore reveals that VGLUT3 and SERT do not segregate. However, in the posterior part of the NAC shell, we confirmed the presence of a subtype of 5-HT immunoreactive axons that lack the SERT. Interestingly, about 90% of the 5-HT/VGLUT3 boutons were labeled for the SERT in this region, suggesting that VGLUT3 is preferentially located in SERT immunoreactive 5-HT boutons. This work demonstrates that VGLUT3 and SERT cannot be used as specific markers to classify the different subtypes of 5-HT axons.
Front Cell Neurosci, 2019; 13
30254293: Belmer A, Maroteaux L
Regulation of raphe serotonin neurons by serotonin 1A and 2B receptors.

Neuropsychopharmacology, 2019; 44
29453444: Belmer A, Quentin E, Diaz SL, Guiard BP, Fernandez SP, Doly S, Banas SM, Pitychoutis PM, Moutkine I, Muzerelle A, Tchenio A, Roumier A, Mameli M, Maroteaux L
Positive regulation of raphe serotonin neurons by serotonin 2B receptors.
Serotonin is a neurotransmitter involved in many psychiatric diseases. In humans, a lack of 5-HT receptors is associated with serotonin-dependent phenotypes, including impulsivity and suicidality. A lack of 5-HT receptors in mice eliminates the effects of molecules that directly target serotonergic neurons including amphetamine derivative serotonin releasers, and selective serotonin reuptake inhibitor antidepressants. In this work, we tested the hypothesis that 5-HT receptors directly and positively regulate raphe serotonin neuron activity. By ex vivo electrophysiological recordings, we report that stimulation by the 5-HT receptor agonist, BW723C86, increased the firing frequency of serotonin Pet1-positive neurons. Viral overexpression of 5-HT receptors in these neurons increased their excitability. Furthermore, in vivo 5-HT-receptor stimulation by BW723C86 counteracted 5-HT autoreceptor-dependent reduction in firing rate and hypothermic response in wild-type mice. By a conditional genetic ablation that eliminates 5-HT receptor expression specifically and exclusively from Pet1-positive serotonin neurons (Htr2b mice), we demonstrated that behavioral and sensitizing effects of MDMA (3,4-methylenedioxy-methamphetamine), as well as acute behavioral and chronic neurogenic effects of the antidepressant fluoxetine, require 5-HT receptor expression in serotonergic neurons. In Htr2b mice, dorsal raphe serotonin neurons displayed a lower firing frequency compared to control Htr2b mice as assessed by in vivo extracellular recordings and a stronger hypothermic effect of 5-HT-autoreceptor stimulation was observed. The increase in head-twitch response to DOI (2,5-dimethoxy-4-iodoamphetamine) further confirmed the lower serotonergic tone resulting from the absence of 5-HT receptors in serotonin neurons. Together, these observations indicate that the 5-HT receptor acts as a direct positive modulator of serotonin Pet1-positive neurons in an opposite way as the known 5-HT-negative autoreceptor.
Neuropsychopharmacology, 2018; 43
29391482: Belmer A, Patkar OL, Lanoue V, Bartlett SE
5-HT1A receptor-dependent modulation of emotional and neurogenic deficits elicited by prolonged consumption of alcohol.

Repeated episodes of binge-like alcohol consumption produce anxiety, depression and various deleterious effects including alterations in neurogenesis. While the involvement of the serotonin receptor 1 A (5-HT) in the regulation of anxiety-like behavior and neurogenesis is well documented, its contribution to alcohol withdrawal-induced anxiety and alcohol-induced deficits in neurogenesis is less documented. Using the Drinking-In-the-Dark (DID) paradigm to model chronic long-term (12 weeks) binge-like voluntary alcohol consumption in mice, we show that the selective partial activation of 5-HT receptors by tandospirone (3 mg/kg) prevents alcohol withdrawal-induced anxiety in a battery of behavioral tests (marble burying, elevated-plus-maze, open-field), which is accompanied by a robust decrease in binge-like ethanol intake (1 and 3 mg/kg). Furthermore, using triple immunolabelling of proliferation and neuronal differentiation markers, we show that long-term DID elicits profound deficits in neurogenesis and neuronal fate specification in the dorsal hippocampus that are entirely reversed by a 2-week chronic treatment with the 5-HT partial agonist tandospirone (3 mg/kg/day). Together, our results confirm previous observations that 5-HT receptors play a pivotal role in alcohol drinking behavior and the associated emotional and neurogenic impairments, and suggest that 5-HT partial agonists represent a promising treatment strategy for alcohol abuse.
Sci Rep, 2018; 8

27485750: Belmer A, Klenowski PM, Patkar OL, Bartlett SE
Mapping the connectivity of serotonin transporter immunoreactive axons to excitatory and inhibitory neurochemical synapses in the mouse limbic brain.
Serotonin neurons arise from the brainstem raphe nuclei and send their projections throughout the brain to release 5-HT which acts as a modulator of several neuronal populations. Previous electron microscopy studies in rats have morphologically determined the distribution of 5-HT release sites (boutons) in certain brain regions and have shown that 5-HT containing boutons form synaptic contacts that are either symmetric or asymmetric. In addition, 5-HT boutons can form synaptic triads with the pre- and postsynaptic specializations of either symmetrical or asymmetrical synapses. However, due to the labor intensive processing of serial sections required by electron microscopy, little is known about the neurochemical properties or the quantitative distribution of 5-HT triads within whole brain or discrete subregions. Therefore, we used a semi-automated approach that combines immunohistochemistry and high-resolution confocal microscopy to label serotonin transporter (SERT) immunoreactive axons and reconstruct in 3D their distribution within limbic brain regions. We also used antibodies against key pre- (synaptophysin) and postsynaptic components of excitatory (PSD95) or inhibitory (gephyrin) synapses to (1) identify putative 5-HTergic boutons within SERT immunoreactive axons and, (2) quantify their close apposition to neurochemical excitatory or inhibitory synapses. We provide a 5-HTergic axon density map and have determined the ratio of synaptic triads consisting of a 5-HT bouton in close proximity to either neurochemical excitatory or inhibitory synapses within different limbic brain areas. The ability to model and map changes in 5-HTergic axonal density and the formation of triadic connectivity within whole brain regions using this rapid and quantitative approach offers new possibilities for studying neuroplastic changes in the 5-HTergic pathway.
Brain Struct Funct, 2017; 222

29765841: Belmer A, Patkar OL, Pitman KM, Bartlett SE
Serotonergic Neuroplasticity in Alcohol Addiction.
Alcohol addiction is a debilitating disorder producing maladaptive changes in the brain, leading drinkers to become more sensitive to stress and anxiety. These changes are key factors contributing to alcohol craving and maintaining a persistent vulnerability to relapse. Serotonin (5-Hydroxytryptamine, 5-HT) is a monoamine neurotransmitter widely expressed in the central nervous system where it plays an important role in the regulation of mood. The serotonin system has been extensively implicated in the regulation of stress and anxiety, as well as the reinforcing properties of all of the major classes of drugs of abuse, including alcohol. Dysregulation within the 5-HT system has been postulated to underlie the negative mood states associated with alcohol use disorders. This review will describe the serotonergic (5-HTergic) neuroplastic changes observed in animal models throughout the alcohol addiction cycle, from prenatal to adulthood exposure. The first section will focus on alcohol-induced 5-HTergic neuroadaptations in offspring prenatally exposed to alcohol and the consequences on the regulation of stress/anxiety. The second section will compare alterations in 5-HT signalling induced by acute or chronic alcohol exposure during adulthood and following alcohol withdrawal, highlighting the impact on the regulation of stress/anxiety signalling pathways. The third section will outline 5-HTergic neuroadaptations observed in various genetically-selected ethanol preferring rat lines. Finally, we will discuss the pharmacological manipulation of the 5-HTergic system on ethanol- and anxiety/stress-related behaviours demonstrated by clinical trials, with an emphasis on current and potential treatments.
Brain Plast, 2016; 1

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-097

DISENTANGLING THE MOLECULAR MECHANISMS UNDERLYING THE RETRIEVAL AND EXTINCTION OF MORPHINE WITHDRAWAL-ASSOCIATED MEMORIES IN THE BASOLATERAL AMYGDALA AND DENTATE GYRUS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Opiates prescription remains controversial due to their addictive potential. Relapse in drug use is a significant problem for addiction treatment, being drug-associated memories among its main triggers. Consequently, the extinction of these memories has been proposed as a therapeutic tool. Hence, by using the conditioned place aversion (CPA) paradigm in rats we investigated some of the molecular mechanisms occurring during the retrieval and extinction of morphine withdrawal memories in the basolateral amygdala (BLA) and the hippocampal dentate gyrus (DG), which control emotional and episodic memories, respectively. Retrieval of aversive memories associated with the abstinence syndrome paralleled with decreased mTOR activity and increased Arc and GluN1 expressions in DG. *Arc* mRNA levels in this nucleus very strongly correlated with the CPA score exhibited by the opiate-treated rats. On the other hand, despite the unaltered mTOR phosphorylation Arc augmented in BLA. After the extinction test, Arc and GluN1 expressions raised in both DG and BLA of control and morphine-treated animals. Remarkably, Homer1 expression in both areas correlated almost perfectly with the extinction showed by morphine-dependent animals. Also, Arc expression in DG correlated strongly with the extinction of the CPA manifested by the morphine-treated group. Finally, our results support coordinated activity of some of these neuroplastic proteins for the extinction of morphine activity in a regional-dependent manner. Present data provide evidence of differential expression and activity of synaptic molecules during retrieval and extinction of aversive memories of opiate withdrawal in amygdalar and hippocampal regions that will likely permit the development of therapeutic strategies.

Pubmed:

32648812: Guerrero-Bautista R, Franco-García A, Hidalgo JM, Fernández-Gómez F, Milanés MV, Núñez C
Blockade of D3 receptor prevents changes in DAT and D3R expression in the mesolimbic dopaminergic circuit produced by social stress- and cocaine prime-induced reinstatement of cocaine-CPP.
Cocaine may cause persistent changes in the brain, which are more apparent in DA transporter (DAT) and DA receptor availability within the nucleus accumbens (NAc). On the other hand, the DA D3 receptor (D3R) has emerged as a promising pharmacotherapeutic target for substance use disorders.
J Psychopharmacol, 2020; 34
33803578: Guerrero-Bautista R, Franco-García A, Hidalgo JM, Fernández-Gómez FJ, Ribeiro Do Couto B, Milanés MV, Núñez C
Distinct Regulation of Dopamine D3 Receptor in the Basolateral Amygdala and Dentate Gyrus during the Reinstatement of Cocaine CPP Induced by Drug Priming and Social Stress.
Relapse in the seeking and intake of cocaine is one of the main challenges when treating its addiction. Among the triggering factors for the recurrence of cocaine use are the re-exposure to the drug and stressful events. Cocaine relapse engages the activity of memory-related nuclei, such as the basolateral amygdala (BLA) and the hippocampal dentate gyrus (DG), which are responsible for emotional and episodic memories. Moreover, D3 receptor (D3R) antagonists have recently arisen as a potential treatment for preventing drug relapse. Thus, we have assessed the impact of D3R blockade in the expression of some dopaminergic markers and the activity of the mTOR pathway, which is modulated by D3R, in the BLA and DG during the reinstatement of cocaine-induced conditioned place preference (CPP) evoked by drug priming and social stress. Reinstatement of cocaine CPP paralleled an increasing trend in D3R and dopamine transporter (DAT) levels in the BLA. Social stress, but not drug-induced reactivation of cocaine memories, was prevented by systemic administration of SB-277011-A (a selective D3R antagonist), which was able, however, to impede D3R and DAT up-regulation in the BLA during CPP reinstatement evoked by both stress and cocaine. Concomitant with cocaine CPP reactivation, a diminution in mTOR phosphorylation (activation) in the BLA and DG occurred, which was inhibited by D3R blockade in both nuclei before the social stress episode and only in the BLA when CPP reinstatement was provoked by a cocaine prime. Our data, while supporting a main role for D3R signalling in the BLA in the reactivation of cocaine memories evoked by social stress, indicate

that different neural circuits and signalling mechanisms might mediate in the reinstatement of cocaine-seeking behaviours depending upon the triggering stimuli.
Int J Mol Sci, 2021; 22

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-098

ALLOSTERIC MODULATION OF AHR BY 3,3'-DIINDOLYLMETHANE CAN PREVENT RECOGNITION MEMORY IMPAIRMENT CAUSED BY BINGE-ETHANOL EXPOSURE.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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[1]Universidad Complutense de Madrid, Departamento De Farmacología Y Toxicología, Facultad De Medicina, Madrid, Spain, [2]Instituto de Investigación Sanitaria Hospital 12 de Octubre, Departamento De Farmacología Y Toxicología, Madrid, Spain, [3]Instituto de Salud Carlos III, Red De Trastornos Adictivos, Madrid, Spain, [4]Universidad Complutense de Madrid, Instituto Universitario De Investigación Neuroquímica, Madrid, Spain, [5]University of Massachussets-Chan Medical School, Department Of Neurobiology, Worcester, United States of America

Introduction and Hypothesis Binge drinking, abusive alcohol consumption in a short period of time, leads to serious consequences in memory function. The kynurenine pathway is altered both by ethanol intake and in several pathologies displaying cognitive impairment. Since the main target of kynurenine is the aryl hydrocarbon receptor (AhR), our hypothesis is that allosteric modulation of AhR by 3,3'-diindolylmethane (DIM) prevents binge ethanol-induced memory alterations. **Aims** To assess the effect of treatment with DIM on memory deficits and alterations in glutamate receptor expression and metalloproteinase activity caused by binge ethanol. **Methods** Recognition memory of mice exposed to binge ethanol and treated with DIM was evaluated using the novel object recognition test 20 h after the last dose of ethanol. In addition, metalloproteinase (MMP)-9 and MMP-2 activity and glutamate receptor NMDA expression were measured by zymography and western blot, respectively, at 1 h and 20 h after ethanol. **Results** Binge ethanol induced memory deficits at 20 h which were prevented by DIM treatment. MMP-9 activity was decreased 1 h after the last ethanol dose and increased at 20 h, while DIM prevented the increase in activity at 20 h. NMDAR-2A expression was increased both at 1 h and 20 h after ethanol, and this was prevented by DIM only at 1 h. **Conclusions** The results indicate that binge drinking produced changes in MMP-9 activity and NMDAR-2A expression that were attenuated by DIM, suggesting modulation of AhR may represent a novel target for the prevention of ethanol-induced memory deficits.

Pubmed:
33476641: Morales-Puerto N, Giménez-Gómez P, Pérez-Hernández M, Abuin-Martínez C, Gil de Biedma-Elduayen L, Vidal R, Gutiérrez-López MD, O'Shea E, Colado MI
Addiction and the kynurenine pathway: A new dancing couple?
Drug use poses a serious threat to health systems throughout the world and the number of consumers rises relentlessly every year. The kynurenine pathway, main pathway of tryptophan degradation, has drawn interest in this field due to its relationship with addictive behaviour. Recently it has been confirmed that modulation of kynurenine metabolism at certain stages of the pathway can reduce, prevent or abolish drug seeking-like behaviours in studies with several different drugs. In this review, we present an up-to-date summary of the evidences of a relationship between drug use and the kynurenine pathway, both the alterations of the pathway due to drug use as well as modulation of the pathway as a potential approach to treat drug addiction. The review discusses ethanol, nicotine, cannabis, amphetamines, cocaine and opioids and new prospects in the drug research field are proposed.
Pharmacol Ther, 2021; 223
33607146: Abuin-Martínez C, Vidal R, Gutiérrez-López MD, Pérez-Hernández M, Giménez-Gómez P, Morales-Puerto N, O'Shea E, Colado MI
Increased kynurenine concentration attenuates serotonergic neurotoxicity induced by 3,4-methylenedioxymethamphetamine (MDMA) in rats through activation of aryl hydrocarbon receptor.
3,4-Methylenedioxymethamphetamine (MDMA) is an amphetamine derivative that has been shown to produce serotonergic damage in the brains of primates, including humans, and of rats. Tryptophan, the precursor of serotonin, is primarily degraded through the kynurenine (KYN) pathway, producing among others KYN, the main metabolite of this route. KYN has been reported as an endogenous agonist of the aryl hydrocarbon receptor (AhR), a transcription factor involved in several neurological functions. This study aims to determine the effect of MDMA on the KYN pathway and on AhR activity and to

establish their role in the long-term serotonergic neurotoxicity induced by the drug in rats. Our results show that MDMA induces the activation of the KYN pathway, mediated by hepatic tryptophan 2,3-dioxygenase (TDO). MDMA also activated AhR as evidenced by increased AhR nuclear translocation and CYP1B1 mRNA expression. Autoradiographic quantification of serotonin transporters showed that both the TDO inhibitor 680C91 and the AhR antagonist CH-223191 potentiated the neurotoxicity induced by MDMA, while administration of exogenous l-kynurenine or of the AhR positive modulator 3,3'-diindolylmethane (DIM) partially prevented the serotonergic damage induced by the drug. The results demonstrate for the first time that MDMA increases KYN levels and AhR activity, and these changes appear to play a role in limiting the neurotoxicity induced by the drug. This work provides a better understanding of the physiological mechanisms that attenuate the brain damage induced by MDMA and identify modulation of the KYN pathway and of AhR as potential therapeutic strategies to limit the negative effects of MDMA.
Neuropharmacology, 2021; 187
35189673: Gil de Biedma-Elduayen L, Giménez-Gómez P, Morales-Puerto N, Vidal R, Núñez-de la Calle C, Gutiérrez-López MD, O'Shea E, Colado MI
Influx of kynurenine into the brain is involved in the reduction of ethanol consumption induced by Ro 61-8048 after chronic intermittent ethanol in mice.
The kynurenine pathway has been proposed as a target for modulating drug abuse. We previously demonstrated that inhibition of kynurenine 3-monooxygenase (KMO), using Ro 61-8048, reduces ethanol consumption in a binge drinking model. Here, we investigate the effect of the kynurenine pathway modulation in ethanol-dependent mice.
Br J Pharmacol, 2022;
34389399: Giménez-Gómez P, Ballestín R, Gil de Biedma-Elduayen L, Vidal R, Ferrer-Pérez C, Reguilón MD, O'Shea E, Miñarro J, Colado MI, Rodríguez-Arias M
Decreased kynurenine pathway potentiate resilience to social defeat effect on cocaine reward.
The kynurenine (KYN) pathway of tryptophan (TRP) degradation is activated by stress and inflammatory factors. It is now well established that social stress induces the activation of the immune system, with central inflammation and KYN metabolism being two of the main factors linking stress with depression. The aim of the present study was to evaluate the long-lasting changes in the KYN pathway induced by social defeat (SD) associated with the resilience or susceptibility to an increase in the conditioned rewarding effects of cocaine. Mice were exposed to repeated SD and 3 weeks later, a conditioned place preference (CPP) induced by a subthreshold dose of cocaine (1.5 mg/kg) was developed. KYN levels in plasma, cerebellum, hippocampus, striatum and limbic forebrain were studied at the end of the CPP procedure. Changes in the KYN pathway after exposure to pharmacological (oxytocin and indomethacin) and environmental interventions (environmental enrichment) were also evaluated. Our results showed that defeated susceptible (SD-S) mice had higher conditioning scores than resilient mice (SD-R). In addition, although KYN concentration was elevated in all defeated mice, SD-R mice showed smaller increases in KYN concentration in the cerebellum than SD-S mice. Oxytocin or Indomethacin treatment before SD normalized cocaine-induced CPP, although the increase in the KYN pathway was maintained. However, environmental enrichment before SD normalized cocaine-induced CPP and prevented the increase in the KYN pathway. The present study highlights the role of the KYN pathway and anti-inflammatory drugs acting on TRP metabolism as pharmacological targets to potentiate resilience to social stress effects.
Neuropharmacology, 2021; 197
32360814: Vidal R, García-Marchena N, O'Shea E, Requena-Ocaña N, Flores-López M, Araos P, Serrano A, Suárez J, Rubio G, Rodríguez de Fonseca F, Colado MI, Pavón FJ
Plasma tryptophan and kynurenine pathway metabolites in abstinent patients with alcohol use disorder and high prevalence of psychiatric comorbidity.
Alterations in tryptophan (TRP) metabolism has been linked to drug exposure and mental disorders. However, most of studies have been performed without considering the co-occurrence of both disorders in the context of addiction. This cross-sectional study examines TRP metabolism through the serotonin (5-HT) and kynurenine (KYN) pathways in subjects with alcohol use disorders (AUD) and high prevalence of psychiatric comorbidity.
Prog Neuropsychopharmacol Biol Psychiatry, 2020; 102
29705534: Giménez-Gómez P, Pérez-Hernández M, Gutiérrez-López MD, Vidal R, Abuin-Martínez C, O'Shea E, Colado MI
Increasing kynurenine brain levels reduces ethanol consumption in mice by inhibiting dopamine release in nucleus accumbens.
Recent research suggests that ethanol (EtOH) consumption behaviour can be regulated by modifying the kynurenine (KYN) pathway, although the mechanisms involved have not yet been well elucidated. To further explore the implication of the kynurenine pathway in EtOH consumption we inhibited kynurenine 3-monooxygenase (KMO) activity with Ro 61-8048 (100 mg/kg, i.p.), which shifts the KYN metabolic pathway towards kynurenic acid (KYNA) production. KMO inhibition decreases voluntary binge EtOH consumption and EtOH preference in mice subjected to "drinking in the dark" (DID) and

"two-bottle choice" paradigms, respectively. This effect seems to be a consequence of increased KYN concentration, since systemic KYN administration (100 mg/kg, i.p.) similarly deters binge EtOH consumption in the DID model. Despite KYN and KYNA being well-established ligands of the aryl hydrocarbon receptor (AhR), administration of AhR antagonists (TMF 5 mg/kg and CH-223191 20 mg/kg, i.p.) and of an agonist (TCDD 50 µg/kg, intragastric) demonstrates that signalling through this receptor is not involved in EtOH consumption behaviour. Ro 61-8048 did not alter plasma acetaldehyde concentration, but prevented EtOH-induced dopamine release in the nucleus accumbens shell. These results point to a critical involvement of the reward circuitry in the reduction of EtOH consumption induced by KYN and KYNA increments. PNU-120596 (3 mg/kg, i.p.), a positive allosteric modulator of α7-nicotinic acetylcholine receptors, partially prevented the Ro 61-8048-induced decrease in EtOH consumption. Overall, our results highlight the usefulness of manipulating the KYN pathway as a pharmacological tool for modifying EtOH consumption and point to a possible modulator of alcohol drinking behaviour.
Neuropharmacology, 2018; 135

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-099

ACUTE D3 ANTAGONIST GSK598809 MODULATES NEURAL RESPONSE DURING NEGATIVE EMOTIONAL PROCESSING IN SUBSTANCE DEPENDENCE

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Aim: Negative affective states play a role in the chronic-relapsing nature of addiction and new treatments are needed. We investigated the effects of D3 receptor antagonism on neural response to negative emotional processing in substance dependence. **Methodology:** Functional MRI was used to investigate BOLD response during an evocative image task, following acute administration of GSK598809 (60mg) or placebo in a multi-site, double-blind, pseudo-randomised design. Whole-brain and *a priori* ROI analyses were conducted in abstinent substance dependent individuals (n=19 alcohol-only and n=17 cocaine-alcohol dependent), and matched controls (n=32). The task consisted of aversive (non-drug/alcohol) and neutral images presented in a block design. **Results:** Evocative image processing (aversive>neutral contrast) resulted in increased BOLD response in amygdala, thalamus, and insula, with reductions in medial prefrontal regions including anterior cingulate cortex. No overall differences were found between dependent and controls, but exploratory analysis revealed increased BOLD response in alcohol-only vs alcohol-cocaine groups, suggesting differential task response by substance. GSK598809 was found to enhance BOLD response across groups relative to placebo in the thalamus, caudate, putamen, and pallidum. **Conclusion:** The modulatory effects of GSK598809 on the BOLD response to evocative processing, suggests that D3R antagonism may impact emotional regulation. Enhanced BOLD response to the drug within D3-rich mesolimbic regions is consistent with its pharmacology. However, the non-specific effect across groups, makes it difficult to ascertain whether the response is likely to be restorative in our abstinent population. Further study is needed to determine whether GSK598809 should be recommended as a potential treatment for substance dependence.

FENS Forum 2022
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BOARD NUMBER: S06-100

SEX DIFFERENCES IN EPIGENETIC MECHANISMS OF OPIOIDS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Opioids are the reference treatment for severe pain. Yet their chronic consumption associates with adverse consequences, including addiction, a chronic relapsing disease. One main hypothesis to explain such prolonged effects implicates epigenetic mechanisms. There are well-known clinical differences in opioid addiction across men and women, but whether they are mediated by distinct molecular routes is poorly studied. Here, focusing on the nucleus accumbens, we seek to characterize how epigenetic plasticity, in particular DNA methylation, contributes to sex-differences in opioid effects, combining high-throughput sequencing, optogenetics and behavioral modelling. RNA-Sequencing analyses showed that chronic morphine exposure triggers very distinct gene expression changes across male (n=1546) and female mice (n=1073), with moderate overlap (21.3%). Surprisingly, these effects appear mediated by even more strongly divergent, sex-specific epigenetic processes. Indeed, large collections of genomic sites showing evidence of differential DNA methylation were identified in both males (n=2983) and females (n=3373), with extremely little overlap (<1%). Nevertheless, these sites partly associate with a set of common genes across sexes, suggesting functional convergence. Towards understanding behavioral relevance of these adaptations, we also characterize morphine-induced alterations in neuronal activity (fiber photometry) and reward processing (optogenetics). Preliminary results uncover in vivo kinetics of the decrease in calcium activity of opioid-responsive neurons during morphine exposure. Building on these data, our current studies seek to characterize how experimental manipulation of genes that are epigenetically reprogrammed by morphine may disrupt its impact on neuronal activity and reward function. In the long-term, this may help identify sex-specific therapeutics targets in addiction.

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BOARD NUMBER: S06-101

ERK PHOSPHORYLATION IN DG AND NACC AFTER MORPHINE CPP EXTINCTION. INVOLVEMENT OF MORPHINE ENCAPSULATION IN LIPOSOMES.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Aims: Opioids like morphine have a great ability to induce addiction. It is well established that the faster a drug crosses the CNS, the higher addictive potential produces. Multilamelar (MLV) and pegylated (PEG-L) liposomes ensure prolonged plasmatic drug circulation periods. Because little is known about morphine encapsulation effects in the addictive process, we have investigated the consequences of morphine administration, free or encapsulated, on pERK levels during the conditioned place preference (CPP) paradigm. **Methods:** To evaluate the rewarding effects of free- and encapsulated morphine, mice received an injection of the drug on days 1, 3 and 5, and saline on days 2, 4 and 6. Extinction of CPP was also performed. Body weight gain was calculated. pERK was analyzed by western blot. **Results:** Animals receiving free-morphine, PEG-L and MLV showed a significant lower body weight gain than saline-treated animals. Morphine administration, either free or encapsulated, induced the acquisition of CPP. Animals treated with free-morphine presented preference for the drug-paired compartment for a longer time (43 days), followed by MLV (23 days) and PEG-L (8 days). Besides, a significant decrease in pERK1/2 expression was observed in dentate gyrus in the group treated with free-morphine compared to control group during CPP extinction that was reverted in the MLV group. Similar results were observed in nucleus accumbens. **Conclusions**: This study provides novel data on the potential use of drug delivery systems based on nanotechnology to transform morphine in a drug with less addictive potential, although future studies are necessary to reach solid conclusions.

FENS Forum 2022
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BOARD NUMBER: S06-102

MAGED1 EXPRESSION IN PVT REGULATES ADDICTION THROUGH EPIGENETIC MODIFICATIONS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Our group recently showed that Maged1 inactivation induces insensitivity to cocaine, impairs cocaine-evoked release of dopamine and plasticity in the NAc. However, the molecular mechanisms involving Maged1 in drug addiction was still unknown. Here, we have identified where and how Maged1 regulates addictive behaviours. First, we showed that expression of Maged1 in vGluT2 neurons of the paraventricular thalamus (PVT) is mandatory for a normal cocaine acute effect and cocaine-evoked locomotor sensitization. Second, by transcriptomic, chIP-Seq, and proteomic we discovered an interaction between Maged1, USP7 deubiquitinase -a PRC1 member-, and histone H2A, that is modified by chronic cocaine administration. Third, we demonstrated that chronic cocaine administration increases H2A mono-ubiquitination in prefrontal cortex and the thalamus. Remarkably, this increase was altered in the thalamus after Maged1 inactivation in vGluT2 neurons or by USP7 inhibition, along with impaired cocaine sensitization. These results show a Maged1-UPS7 control of cocaine-induced H2A mono-ubiquitination in the thalamus regulating drug addiction. Finally, to extend our discovery to human, we performed a genomic analysis on polydrug users revealing 9 SNPs mutations on Maged1 conferring a shortened transition to cocaine addiction and one SNP mutation on USP7 associated to reduced aggressivity during cocaine use. Moreover, we confirmed interaction between Maged1 and USP7 in two human cell lines. In conclusion, these findings establish a role for a Maged1-USP7-dependent epigenetic mark in the PVT in chronic cocaine-related behaviours and pave the way for the development of new treatments for cocaine use disorder.

FENS Forum 2022
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BOARD NUMBER: S06-103

ANKK1: A METABOLIC SENSOR OF UNDERNUTRITION IN REWARD CIRCUITS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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AnKK1 gene is encoding for « Ankyrin repeat and kinase domain containing 1 » involved in signal transduction pathways. AnKK1 is adjacent to the DRD2 gene, which regulates reward, food-seeking, and goal-directed behavior. A polymorphism of AnKK1, Taq1A, is associated with a reduction of dopamine signalling and is described to be a risk factor in addictive behaviors. Taq1A is also associated with increased vulnerability to anorexia nervosa (AN), a complex psychiatric disorder that encompasses multiple risk factors and is characterized by an addictive-like compulsive food restriction and physical activity. Aims. We aim to understand how AnKK1 is regulated by chronic undernutrition and its role in AN symptoms using mouse models that mimic the undernutrition state of AN patients. Methods. We measured the expression of AnKK1 in brain structures involved in feeding, goal-directed behavior or reward in young female mice submitted to 50% food restriction with access to a running wheel (FRW). Results. In FRW, we show a reduction of AnKK1 expression in the hypothalamus, dorsal striatum (DS), and nucleus accumbens. We are also exploring a new mouse model in which AnKK1 can be selectively silenced in the DS. This knock-out (KO) doesn't impair learning or spatial memory and we are currently testing its impact on cognitive flexibility, goal-directed and reward-related behaviors that are impaired in AN. Conclusions. These data suggest that Ankk1 is a sensor of undernutrition in brain structures involved in feeding and reward and may have a key role in modulating reward circuits in response to undernutrition.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-104

RO 61-8048 IS AN EFFECTIVE TREATMENT ON THE DIFFERENT PHASES OF FENTANYL INDUCED ADDICTIVE BEHAVIOURS.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Introduction Opioids such as fentanyl are highly effective analgesics widely employed to treat moderate to severe pain. The rise of opioid use outside of their clinical application poses a major public health risk because of the addictive potential and overdose liability of these drugs. Unravelling the molecular mechanisms involved in the addictive behaviour induced by this group of drugs and developing new strategies to lessen their abuse has become a priority for health systems. Mounting evidence points to the kynurenine pathway as a novel target to modulate drug abuse, seeking and reinstatement. **Aims** To assess the effect of the kynurenine monoxygenase inhibitor, Ro 61-8041 on (1) fentanyl-induced addictive behaviours and (2) on kynurenine levels in plasma and hippocampus. **Methods** Male adult Long Evans rats were catheterised in the right yugular vein. Animals underwent operant intravenous self-administration for fentanyl (3,2 µg/kg/infusion). Ro 61-8048 pretreatment (100 mg/kg) was given intraperitoneally 30 minutes before the 2-hour self administration session. **Results** Ro 61-8048 pretreatment lowered fentanyl intake in early acquisition and during maintenance, facilitated extinction and prevented the reinstatement of seeking behavior in the drug priming-induced reinstatement test. Plasma and hippocampal kynurenine levels were increased 2.5 hours after Ro 61-8048 administration. **Conclusions** These findings show for the first time that modulating the kynurenine pathway using Ro 61-8048 positively affects the addictive behaviours induced by fentanyl in rats. Ro 61-8048 is a promising drug that could be useful in the clinical setting in the future.

Pubmed:
35189673: Gil de Biedma-Elduayen L, Giménez-Gómez P, Morales-Puerto N, Vidal R, Núñez-de la Calle C, Gutiérrez-López MD, O'Shea E, Colado MI
Influx of kynurenine into the brain is involved in the reduction of ethanol consumption induced by Ro 61-8048 after chronic intermittent ethanol in mice.
The kynurenine pathway has been proposed as a target for modulating drug abuse. We previously demonstrated that inhibition of kynurenine 3-monooxygenase (KMO), using Ro 61-8048, reduces ethanol consumption in a binge drinking model. Here, we investigate the effect of the kynurenine pathway modulation in ethanol-dependent mice.
Br J Pharmacol, 2022;
33476641: Morales-Puerto N, Giménez-Gómez P, Pérez-Hernández M, Abuin-Martínez C, Gil de Biedma-Elduayen L, Vidal R, Gutiérrez-López MD, O'Shea E, Colado MI
Addiction and the kynurenine pathway: A new dancing couple?
Drug use poses a serious threat to health systems throughout the world and the number of consumers rises relentlessly every year. The kynurenine pathway, main pathway of tryptophan degradation, has drawn interest in this field due to its relationship with addictive behaviour. Recently it has been confirmed that modulation of kynurenine metabolism at certain stages of the pathway can reduce, prevent or abolish drug seeking-like behaviours in studies with several different drugs. In this review, we present an up-to-date summary of the evidences of a relationship between drug use and the kynurenine pathway, both the alterations of the pathway due to drug use as well as modulation of the pathway as a potential approach to treat drug addiction. The review discusses ethanol, nicotine, cannabis, amphetamines, cocaine and opioids and new prospects in the drug research field are proposed.
Pharmacol Ther, 2021; 223
31509716: Giménez-Gómez P, Pérez-Hernández M, O'Shea E, Caso JR, Martín-Hernandez D, Cervera LA, Centelles MLG, Gutiérrez-Lopez MD, Colado MI

Changes in brain kynurenine levels gut microbiota and gut-barrier disruption induced by chronic ethanol exposure in mice. Inflammatory processes have been shown to modify tryptophan (Trp) metabolism. Gut microbiota appears to play a significant role in the induction of peripheral and central inflammation. Ethanol (EtOH) exposure alters gut permeability, but its effects on Trp metabolism and the involvement of gut microbiota have not been studied. We analyzed several parameters of gut-barrier and of peripheral and central Trp metabolism following 2 different EtOH consumption patterns in mice, the binge model, drinking in the dark (DID), and the chronic intermittent (CI) consumption paradigm. Antibiotic treatment was used to evaluate gut microbiota involvement in the CI model. Mice exposed to CI EtOH intake, but not DID, show bacterial translocation and increased plasma LPS immediately after EtOH removal. Gut-barrier permeability to FITC-dextran is increased by CI, and, furthermore, intestinal epithelial tight-junction (TJ) disruption is observed (decreased expression of zonula occludens 1 and occludin) associated with increased matrix metalloproteinase (MMP)-9 activity and iNOS expression. CI EtOH, but not DID, increases kynurenine (Kyn) levels in plasma and limbic forebrain. Intestinal bacterial decontamination prevents the LPS increase but not the permeability to FITC-dextran, TJ disruption, or the increase in MMP-9 activity and iNOS expression. Although plasma Kyn levels are not affected by antibiotic treatment, the elevation of Kyn in brain is prevented, pointing to an involvement of microbiota in CI EtOH-induced changes in brain Trp metabolism. Additionally, CI EtOH produces depressive-like symptoms of anhedonia, which are prevented by the antibiotic treatment thus pointing to an association between anhedonia and the increase in brain Kyn and to the involvement of gut microbiota.-Giménez-Gómez, P., Pérez-Hernández, M., O'Shea, E., Caso, J. R., Martín-Hernández, D., Cervera, L. A., Centelles. M. L. G.-L., Gutiérrez-Lopez, M. D., Colado, M. I. Changes in brain kynurenine levels gut microbiota and gut-barrier disruption induced by chronic ethanol exposure in mice.
FASEB J, 2019; 33
33607146: Abuin-Martínez C, Vidal R, Gutiérrez-López MD, Pérez-Hernández M, Giménez-Gómez P, Morales-Puerto N, O'Shea E, Colado MI
Increased kynurenine concentration attenuates serotonergic neurotoxicity induced by 3,4-methylenedioxymethamphetamine (MDMA) in rats through activation of aryl hydrocarbon receptor.
3,4-Methylenedioxymethamphetamine (MDMA) is an amphetamine derivative that has been shown to produce serotonergic damage in the brains of primates, including humans, and of rats. Tryptophan, the precursor of serotonin, is primarily degraded through the kynurenine (KYN) pathway, producing among others KYN, the main metabolite of this route. KYN has been reported as an endogenous agonist of the aryl hydrocarbon receptor (AhR), a transcription factor involved in several neurological functions. This study aims to determine the effect of MDMA on the KYN pathway and on AhR activity and to establish their role in the long-term serotonergic neurotoxicity induced by the drug in rats. Our results show that MDMA induces the activation of the KYN pathway, mediated by hepatic tryptophan 2,3-dioxygenase (TDO). MDMA also activated AhR as evidenced by increased AhR nuclear translocation and CYP1B1 mRNA expression. Autoradiographic quantification of serotonin transporters showed that both the TDO inhibitor 680C91 and the AhR antagonist CH-223191 potentiated the neurotoxicity induced by MDMA, while administration of exogenous l-kynurenine or of the AhR positive modulator 3,3'-diindolylmethane (DIM) partially prevented the serotonergic damage induced by the drug. The results demonstrate for the first time that MDMA increases KYN levels and AhR activity, and these changes appear to play a role in limiting the neurotoxicity induced by the drug. This work provides a better understanding of the physiological mechanisms that attenuate the brain damage induced by MDMA and identify modulation of the KYN pathway and of AhR as potential therapeutic strategies to limit the negative effects of MDMA.
Neuropharmacology, 2021; 187

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BOARD NUMBER: S06-105

WHOLE-BRAIN CFOS MAPPING OF PSILOCYBIN EFFECTS IN THE MOUSE

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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During the past few years, research on treatments for Psychiatric disorders have been marked by a psychedelic revival. Among the psychedelic substances, psilocybin is being investigated for its therapeutic potential for treating a variety of psychiatric disorders such as obsessive compulsive disorders, depression or substance abuse disorders. Clinical trials are already being conducted but pre-clinical evidence of the impact of psilocybin on the brain are yet to be investigated. Thus the aim of this project was to identify the acute effect of psilocybin injection on the mouse brain. We injected intra-peritoneally wild type C57Bl6 mice with either a psilocybin or a vehicle solution 90 minutes before collecting their brains. The samples were then processed following the iDisco protocol which consist in a clearing protocol coupled with fluorescent immunostaining. Here we labeled the immediate early gene c-Fos considered, in a neuron, as a marker of recent stimulation. After light-sheet microscope imaging and analysis with the Clearmap pipeline we could run a 3D reconstruction of the expression of cFos in whole hemispheres of both groups. With these techniques, we were able to identify brain areas stimulated by the psilocybin and establish a first whole-brain mapping of this psychedelic in the mouse brain.

FENS Forum 2022
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BOARD NUMBER: S06-106

CAPTURING, TRACKING, AND PROFILING COCAINE-RECRUITED NEURONAL ENSEMBLES IN THE NUCLEUS ACCUMBENS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Learned associations between the rewarding effects of drugs and the context in which they are experienced are decisive for precipitated drug-seeking and relapse in addiction. These associative memories are stored in sparse and highly discriminative populations of concomitantly activated neurons defining drug-recruited neuronal ensembles. In this study, we explore the dynamics and molecular mechanisms of both the recruitment of these ensembles upon initial drug exposure and their contribution to the encoding, strengthening and ultimately expression of drug-associated memories. Additionally, we explore the intrinsic and acquired cellular properties favoring the allocation of specific cells to these ensembles and/or predicting their further reactivation. Capitalizing on the activity-dependent labeling in Arc-CreER^{T2} mice (Denny et al., 2014), we captured and permanently tagged (fluorophores, channel-rhodopsin) cocaine-activated cells in the nucleus accumbens for further characterization, optogenetics, and nuclei sorting. We identified subsets of neurons activated at both early and late stages of drug exposure and show that the reactivation of an initial ensemble correlates with behavioral sensitization. Similarly, re-exposure to a cocaine-paired context in a conditioned place preference (CPP) paradigm triggered cocaine ensembles' reactivation. Using optogenetics-mediated artificial reactivation, we found that populations recruited at early versus late stages of drug exposure had opposite roles in CPP expression. Single nucleus RNA Sequencing was then performed on FACS-isolated tagged neurons, and we successfully isolated a cluster of reactivated cells within the initially activated ensemble. Together, this ensemble-specific approach represents a pivotal step in identifying highly specific cellular processes involved in the encoding of pathological memories associated with drug addiction.

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BOARD NUMBER: S06-107

INVOLVEMENT OF NUCLEUS ACCUMBENS PARVALBUMIN INTERNEURONS IN COCAINE SEEKING BEHAVIOR

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Studies demonstrate that the increase in drug-seeking following forced abstinence periods (known as incubation of drug craving) is correlated to adaptations within the neuronal ensembles, which is a small pattern of neurons sparsely distributed in the brain and connected by strong synapses. Nucleus accumbens is a brain region involved in the incubation of drug craving. Parvalbumin interneurons (PVs) from the nucleus accumbens are important neurons involved in drug-behavior. Using restricted (1h/day for 12 days) or extended (6h/day for 12 days) access protocol to cocaine self-administration, we investigated if the transition from occasional to compulsive drug use and the incubation of cocaine craving are related to alterations in the engagement of nucleus acumbens (NAc) parvalbumin interneurons (PVs) in neuronal ensembles activated by drug-related cues. Wistar rats were catheterized for cocaine self-administration, trained and reexposed to drug environment cues after 1 or 30 days of abstinence. Double labeling for Fos and parvalbumin was performed (Ethical Committee number: 4183030918). Both self-administration protocols produced the same NAc PVs recruitment after 1 day of abstinence. The incubation of craving was observed only in rats submitted to extended access to cocaine self-administration and was accompanied by a decrease in the percentage of PVs from NAc core activated by drug cues. Our data suggests that incubation of cocaine craving may be correlated with a shift in the engagement of NAc PVs. Acknowledgements: FAPESP 2018/15505-4 and 2018/14153-7, CAPES

Pubmed:
33346074: Palombo P, Engi SA, Yokoyama TS, Bezerra AG, Curado DF, Anésio A, Leão RM, Santos PCJL, Cruz FC, Galduróz JCF
Effects of biperiden (cholinergic muscarinic m1/m4 receptor antagonist) on ethanol conditioned place preference in mice.
Previous studies suggest that muscarinic cholinergic receptors might act upon the dopamine release in the mesolimbic system and alter drug-reinforcing values related to drug craving.
Neurosci Lett, 2021; 745
33169700: Felipe JM, Palombo P, Bianchi PC, Zaniboni CR, Anésio A, Yokoyama TS, Engi SA, Carneiro-de-Oliveira PE, Planeta CDS, Leão RM, Cruz FC
Dorsal hippocampus plays a causal role in context-induced reinstatement of alcohol-seeking in rats.
Drug addiction is a chronic mental disorder characterized by frequent relapses. Contextual cues associated with drug use to play a critical causal role in drug-seeking behavior. The hippocampus has been implicated in encoding drug associative memories. Here we examine whether the dorsal hippocampus mediates context-induced reinstatement of alcohol-seeking. Male Long-Evans rats were trained to self-administer alcohol in Context A. Alcohol self-administration was extinguished in a distinct context (Context B). On the test day, animals were re-exposed to the alcohol Context A or the extinction Context B. Next, to assess a causal role for the dorsal hippocampus in context-induced alcohol-seeking, on the test day, we injected cobalt chloride (CoCl2; a nonselective synapse inhibitor) or vehicle into the dorsal hippocampus, and 15 min later, rats were tested by re-exposing them to the drug-associated context. The re-exposure to the alcohol-associated Context A reinstated alcohol seeking and increased Fos-positive cells in the dorsal hippocampus neurons (CA1, CA3, and Dentate Gyrus). Pharmacological inactivation with cobalt chloride of the dorsal hippocampus attenuated the reinstatement of alcohol-seeking. Our data suggest that the dorsal hippocampus may be involved in context-induced alcohol-seeking behavior.
Behav Brain Res, 2021; 398
30391542: Anesio A, Barbosa SP, De Luca LA, de Paula PM, Colombari DSA, Colombari E, Andrade CAF, Menani JV
Central muscarinic and LPBN mechanisms on sodium intake.
Central cholinergic activation stimulates water intake, but also NaCl intake when the inhibitory mechanisms are blocked with injections of moxonidine (α adrenergic/imidazoline agonist) into the lateral parabrachial nucleus (LPBN). In the present study, we investigated the involvement of central M and M muscarinic receptors on NaCl intake induced by pilocarpine (non-selective muscarinic agonist) intraperitoneally combined with moxonidine into the LPBN or by muscimol (GABA agonist) into

the LPBN. Male Holtzman rats with stainless steel cannulas implanted bilaterally in the LPBN and in the lateral ventricle were used. Pirenzepine (M muscarinic antagonist, 1 nmol/1 µl) or methoctramine (M muscarinic antagonist, 50 nmol/1 µL) injected intracerebroventricularly (i.c.v.) reduced 0.3 M NaCl and water intake in rats treated with pilocarpine (0.1 mg/100 g of body weight) injected intraperitoneally combined with moxonidine (0.5 nmol/0.2 µL) into the LPBN. In rats treated with muscimol (0.5 nmol/0.2 µL) into the LPBN, methoctramine i.c.v. also reduced 0.3 M NaCl and water intake, however, pirenzepine produced no effect. The results suggest that M and M muscarinic receptors activate central pathways involved in the control of water and sodium intake that are under the influence of the LPBN inhibitory mechanisms.
Brain Res Bull, 2019; 144

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BOARD NUMBER: S06-108

THE EFFECT OF COCAINE AND ALCOHOL POLY-CONSUMPTION ON DRUG SEEKING BEHAVIOR IN YOUNG ADULT RATS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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AIM The poly-consumption of cocaine and alcohol is very prevalent in western countries, moreover, the risk of relapse is very high in recovering addicts. In the light of this, the main objective of this study was to explore the psychobiological mechanism that may be involved in this type of poly-consumption. METHODS Young male and female rats intravenously self-administered during 10 sessions (6h) in Skinner boxes: cocaine and alcohol (C+A; 1mg/kg bodyweight cocaine + 0,133mg/kg bodyweight alcohol); cocaine (Coc; 1mg/kg bodyweight) and 0,9% NaCl (Sal). After a withdrawal period (Wd2 or Wd30), rats were subjected to a relapse test to measure their seeking behaviour. Blood and different brain structures was collected for metabolomic, genetic and protein studies, that are currently in process. RESULTS Self-administration behavior of Coc and C+A groups was significantly higher than Sal group, without differences between sexes. Regarding the relapse test, we observed a higher response rate in Coc and C+A compared to Sal, both on Wd2 and Wd30. However, we only found statistical significance in Coc vs. Sal on Wd30 ($p=0.07$). In the Coc group, but not in C+A, we found a significant higher response in Wd30 vs. Coc Wd2 ($p=0.024$). CONCLUSION This study shows that young adult rats will easily self-administer combined cocaine and alcohol when they have an extended access, without differences between sexes. The drug-seeking behavior test revealed an incubation of craving in the Wd30 Coc group. We believe that this study might contribute to understand the underpinnings of poly-consumption of cocaine and alcohol.

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BOARD NUMBER: S06-109

DOPAMINE D3 RECEPTOR ANTAGONISM BLOCKED THE AKT/MTOR PATHWAY DOWNREGULATION IN THE DENTATE GYRUS AFTER THE REINSTATEMENT OF COCAINE INDUCED CPP EVOKED BY PHYSIOLOGICAL STRESS.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Relapse in cocaine seeking and intake is one of the main challenges when treating its addiction. Among the triggering factors for the recurrence of cocaine use are stressful events. Cocaine relapse engages the activity of memory-related nuclei such as the hippocampal dentate gyrus (DG), which is responsible for episodic memories. Moreover, D3 receptor (D3R) antagonists have recently arisen as a potential treatment for preventing drug relapse. Thus, we have assessed the impact of D3R blockade in the activity of the mammalian (or mechanistic) target of Rapamycin (mTOR) pathway, which is modulated by this receptor, in the DG during the reinstatement of cocaine-induced conditioned place preference (CPP) evoked by tail pinch as a physiological stressor. For that, we systemically administered two doses (24 or 48 mg/kg, i.p.) of SB-277011-A, a selective D3R antagonist, 15 min before the stressful episode. The higher dose of the D3R antagonist blocked the reinstatement of cocaine CPP induced by tail pinch. Additionally, we observed a trend towards diminution of mTOR phosphorylation during the reinstatement of cocaine CPP in the DG, which was inhibited by SB-277011-A (48 mg/kg). We also found a significant increase of phospho-Akt levels in the group treated with the higher dose of the antagonist when compared with animals that received vehicle and, thus, relapsed in cocaine CPP. Our data support a main role for D3R signalling, through the modulation of the Akt/mTOR pathway in the DG, in the reactivation of cocaine memories evoked by physiological stress.

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BOARD NUMBER: S06-110

PREDICTION AND PREVENTION OF COMPULSIVE BEHAVIORS BY CLOSED-LOOP OPTOGENETIC RECRUITMENT OF STRIATAL INTERNEURONS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Dysregulation in the cortico-striatal circuits has been shown to be related to pathologic repetitive behaviors. Striatal positive parvalbumin interneurons (PVIs) receive strong cortical inputs and form a powerful feedforward inhibition network that controls spike timing in medium spiny neurons, thereby are a key element that regulates the striatal output. To prove the role of PV interneurons in reducing aberrant repetitive behaviors we used the Sapap3 mutant mice, which exhibit excessive self-grooming and increased anxiety. Using optogenetics activation of PV interneurons in the dorsomedial striatum, we were able to reduce compulsive behavior to normality. Interestingly, this continuous optogenetic stimulation significantly reduced the number of grooming sequences rather than their duration. Moreover, we found that an increase in power within the 1.5-4 Hz frequency band in the orbitofrontal cortex temporally predicted grooming initialization. Our results from PVI continuous stimulation suggested that the recruitment of PVI was essential at the initiation of the compulsive event. To test this hypothesis, we used a combination of extracellular signal acquisition, optogenetics manipulation, and online data processing in a closed-loop design that allowed us to predict grooming onset and provide on-demand stimulation of striatal PV interneurons. We found that both closed-loop optogenetics and continuous open-loop stimulation robustly decreased excessive grooming. Acute on-demand stimulation had the advantage of requiring significantly less total stimulation delivery. These results highlight how the modulation of PVIs can positively influence the rescuing of pathologic repetitive behaviors and point to their involvement in the dysfunction mechanism that is associated with the cortico-striatal circuits.

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ROLE OF PROPRANOLOL AND CP-154,526 IN RELAPSE CAUSED BY TAIL PINCH ASSOCIATED WITH MORPHINE. EXPRESSION OF PHOSPHORYLATED CREB IN DENTATE GYRUS.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

Maria José Madrid, Ana Fernández-Rodríguez, Alberto Cánovas, Javier Teruel-Fernández, Sofia García-Moreno, Lucia Fernandez-Lopez, M.Luisa Laorden, Javier Navarro-Zaragoza, Pilar Almela
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Aims: Addiction to substances of abuse is a chronic neurological disorder characterized by drug-seeking, compulsive consumption, and frequent relapses after long periods of abstinence. Many studies have proposed that stress has a key role in the development of drug addiction. In this study, we have investigated the influence of *Tail Pinch* (TP) in relapse through the model of conditioned-place aversion (CPA). We have also analyzed pCREB expression in dentate gyrus (DG) during this paradigm. **Methods**: C57BL/6J mice were rendered dependent on morphine and injected acutely with naloxone before paired to confinement in a naloxone-associated compartment. After extinction of the aversive memory, animals received CP-154,526 (CRF1 receptor antagonist), propranolol (beta-adrenergic receptor antagonist) or vehicle and were exposed to TP to induce relapse. pCREB expression was analyzed in DG by Western Blot. **Results**: Our results show that application of TP after extinction induced relapse. The administration of CP-154,526 and propranolol antagonized this effect. Besides, a significant increase in pCREB expression was observed in DG in the group exposed to stress receiving vehicle compared to the non-stress group. **Conclussions**: These results show the important relationship between stress and compulsive drug seeking, as well as a potential therapeutic value of propranolol and CP-154,526 in the treatment of relapses.

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BOARD NUMBER: S06-112

DOPAMINE-GLUTAMATE RECEPTOR HETEROMERIZATION AS A COMMON MOLECULAR SUBSTRATE FOR SUBSTANCE USE DISORDER AND COMORBID DEPRESSION

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Drug addiction is defined as a compulsive pattern of drug-seeking/taking behaviors, with recurrent episodes of abstinence and relapse. Addictive drugs increase dopamine in the nucleus accumbens (NAc), where it persistently shapes excitatory glutamate transmission, thereby hijacking natural reward processing. Herein, we provide evidence, from mice to humans, that an underlying mechanism relies on a drug-evoked heteromerization of glutamate NMDA receptors (NMDAR) with dopamine receptor 1 (D1R) or 2 (D2R) triggered by both psychostimulants and opiates. Using temporally-controlled inhibition of D1R-NMDAR heteromerization, we show their selective implication in early phases of cocaine-evoked, whereas preventing D2R-NMDAR heteromerization blocked the persistence of these adaptations. Notably, interfering with these receptor heteromers spared natural reward processing. Because the high prevalence of comorbidities between addiction and mood disorders suggests that brain dysfunctions underlying drug addiction and other psychiatric disorders may rely on partly shared mechanisms, we asked whether receptor heteromerization in the NAc could constitute a common molecular switch in addiction and depression. Using the chronic defeat stress paradigm as a preclinical model of depression, we indeed found that mice developing depressive-like behavior, but not resilient mice, exhibit an increased dopamine-glutamate receptor heteromerization, which blockades fully prevents the onset and persistence of depressive-like symptoms. These findings contribute to a better understanding of common molecular mechanisms underlying addiction and depression and uncover dopamine-glutamate heteromer as targets with potential therapeutic value for multiple psychiatric diseases associated with alterations in dopamine and glutamate-dependent transmissions.

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BOARD NUMBER: S06-113

AXONAL PATHOLOGY IN ALCOHOL USE DISORDERS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Aim: Europe is the heaviest drinking region in the world, approximately 23 million Europeans are alcohol dependent. A large number of brain alterations has been associated with alcohol use disorders (AUD). A causal link between alcohol and many of these alterations, however, is difficult to establish due to the complexity of AUD and associated comorbidities. In a translational study in AUD patients and the msP rat model, we have shown a causal link between alcohol drinking and a progressing white matter alteration during abstinence. Here, we combine ultra-high field diffusion magnetic resonance imaging (MRI) with immunohistochemistry analysis, with the goal of characterising this alteration. **Methods/results:** We used a total of 28 msP rats, 18 underwent a longitudinal MRI examination with time points at baseline and after one month of alcohol drinking in a two bottles free-choice paradigm. Another 10 age-matched animals (5 controls and 5 exposed to alcohol) were used for histological quantification of myelin basic protein (MBP) and neurofilament to asses myelin content and axonal integrity, respectively, in the corpus callosum and fimbria/fornix. Alcohol consumption reached levels of 4 to 6 g/Kg/day. Multi-shell diffusion imaging combined with multi-compartment modelling unveiled a reduction in the fraction of water undergoing restricted diffusion, a surrogate measure of axonal density, and increase in estimated axonal diameter. The histological analysis showed decreased MBP and increased neurofilament staining intensity in the alcohol group, overall suggesting an early process of axonal degeneration. **Conclusion:** We present convergent evidence of axonal pathology induced by chronic alcohol drinking in rats.

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BOARD NUMBER: S06-114

MODULATING THE EFFECTS OF A LESION IN LVIII OF THE CEREBELLAR VERMIS ON COCAINE-INDUCED CPP THROUGH CHEMOGENETIC INHIBITION OF THE INTERPOSED NUCLEUS ACTIVITY

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Our research group have shown that cocaine-induced conditioned place preference (CPP) increases the activity at LVIII of cerebellar vermis (Carbo-Gas et al. 2014). Furthermore, a lesion of this region increased the probability of cocaine-induced CPP acquisition (Gil-Miravet et al. 2019). Monosynaptic tracing studies indicated that there is a monosynaptic projection from the deep cerebellar nuclei (DCN) to the ventral tegmental area (VTA) that also receives Purkinje axons from LVIII (Gil-Miravet et al. 2020). This projection may control the activity and perineuronal net expression in the medial prefrontal cortex and striatum (Gil-Miravet et al., 2019). The objective of the present investigation was to test whether chemogenetic inhibition of the interposed nucleus (IP) would prevent the effects of a lesion of LVIII on the acquisition of CPP. To that end, we expressed the designer receptors exclusively activated by designer drugs (DREADDs): AAV5-hSyn-hM4D(Gq) or AAV5-CaMKIIa-hM4D(Gq) in quinolinicc acid (QA)-treated rats. The viral vectors and QA were infused by stereotaxic surgery, and DREADDs were activated by intracranial infusion of clozapine N-oxide (CNO) into IP. Our results indicated that the inhibition of the IP prevents the facilitation of cocaine-induced CPP acquisition by QA in lobule VIII and could decrease the activity in other cortico-limbic-striatal regions. Funding: Agencia Estatal de Investigación (PGC2018–095980-B–I00/MCI/AEI/FEDER, UE) Plan Nacional de Drogas 2017 (PND-132400) Programa Estatal de Promoción del Talento y su Empleabilidad en I+D+i (PRE2019-088521)

Pubmed:

34416354: Guarque-Chabrera J, Gil-Miravet I, Olucha-Bordonau F, Melchor-Eixea I, Miquel M
When the front fails, the rear wins. Cerebellar correlates of prefrontal dysfunction in cocaine-induced memory in male rats. Reciprocal pathways connecting the cerebellum to the prefrontal cortex provide a biological and functional substrate to modulate cognitive functions. Dysfunction of both medial prefrontal cortex (mPFC) and cerebellum underlie the phenotypes of several neuropsychiatric disorders that exhibit comorbidity with substance use disorder (SUD). In people with SUD, cue-action-reward associations appears to be particularly strong and salient, acting as powerful motivational triggers for craving and relapse. Studies of cue reactivity in human with SUD have shown cerebellar activations when drug-related cues are presented. Our preclinical research showed that cocaine-induced conditioned preference increases neural activity and upregulates perineuronal nets (PNNs) around Golgi interneurons in the posterior cerebellar cortex. In the present investigation, we aimed at evaluating cerebellar signatures of conditioned preference for cocaine when drug learning is established under mPFC impairment. We used lidocaine to temporarily inactivate in male rats either the Prelimbic (PL) or the Infralimbic (IL) cortices during cocaine-induced conditioning. The inactivation of the IL, but not the PL, encouraged the acquisition of preference for cocaine-related cues, increased posterior cerebellar cortex activity, and upregulated the expression of PNNs around Golgi interneurons. Moreover, IL impairment not only increased vGluT2- and vGAT-related activity around Golgi cells but also regulated PNNs differently on subpopulations of Golgi cells, increasing the number of neurogranin+ PNN-expressing Golgi cells. Our findings suggest that IL dysfunction may facilitate the acquisition of cocaine-induced memory and cerebellar drug-related learning hallmarks. Overall, IL perturbation during cocaine-induced Pavlovian learning increased cerebellar activity and drug effects. Importantly, cerebellum involvement requires a contingent experience with the drug, and it is not the effect of a mere inactivation of IL cortex.
Prog Neuropsychopharmacol Biol Psychiatry, 2022; 112
31808992: Gil-Miravet I, Melchor-Eixea I, Arias-Sandoval E, Vasquez-Celaya L, Guarque-Chabrera J, Olucha-Bordonau F, Miquel M
From back to front: A functional model for the cerebellar modulation in the establishment of conditioned preferences for cocaine-related cues.
It is now increasingly clear that the cerebellum may modulate brain functions altered in drug addiction. We previously

demonstrated that cocaine-induced conditioned preference increased activity at the dorsal posterior cerebellar vermis. Unexpectedly, a neurotoxic lesion at this region increased the probability of cocaine-induced conditioned preference acquisition. The present research aimed at providing an explanatory model for such as facilitative effect of the cerebellar lesion. First, we addressed a tracing study in which we found a direct projection from the lateral (dentate) nucleus to the ventral tegmental area (VTA) that also receives Purkinje axons from lobule VIII in the vermis. This pathway might control the activity and plasticity of the cortico-striatal circuitry. Then we evaluated cFos expression in different regions of the medial prefrontal cortex and striatum after a lesion in lobule VIII before conditioning. Additionally, perineuronal net (PNN) expression was assessed to explore whether the cerebellar lesion might affect synaptic stabilization mechanisms in the medial prefrontal cortex (mPFC). Damage in this region of the vermis induced general disinhibition of the mPFC and striatal subdivisions that receive dopaminergic projections, mainly from the VTA. Moreover, cerebellar impairment induced an upregulation of PNN expression in the mPFC. The major finding of this research was to provide an explanatory model for the function of the posterior cerebellar vermis on drug-related memory. In this model, damage of the posterior vermis would release striatum-cortical networks from the inhibitory tonic control exerted by the cerebellar cortex over VTA, thereby promoting drug effects.
Addict Biol, 2021; 26

FENS Forum 2022
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BOARD NUMBER: S06-115

EFFECT OF COCAINE SELF-ADMINISTRATION ON CEREBELLAR PERINEURONAL NETS COMPONENTS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Addiction could be considered the result of pathological learning in which drug-related memories make the brain inflexible. Perineuronal nets (PNNs) are cartilage-like structures of extracellular matrix which enwrap the cell-body and proximal dendrites of special sets of neurons to stabilize their connections and restrict plasticity. We showed that both drug-induced conditioned memories and protracted abstinence increase expression of PNNs around Golgi inhibitory interneurons in the cerebellar cortex. In cocaine self-administration studies, we found decreased expression of cerebellar PNNs at 24 hours in rats with a limited cocaine experience (ShA). PNN expression around Golgi cells increased after protracted abstinence (28 days) only in rats with extended access to cocaine self-administration (LgA). In the present study, we assessed the relative levels of key components of PNNs on ShA and LgA groups at two times of withdrawal (24h or 28 days). Rats were catheterized and trained to self-administer cocaine (0.75mg/kg/infusion), 1h/day for the ShA group and 6h/day for the LgA group for 12 days. Western-blot analysis showed a reduction in the relative amount of protein (tenascin-R) in the ShA group at 24h when compared to the naïve and LgA groups. At this time point, there was no difference between the LgA group and the naïve group. The results indicate that cocaine self-administration effect on the relative expression of PNN components parallel the dynamic seen with Wisteria Floribunda Agglutinin intensity, suggesting that while a short experience with cocaine can increase plasticity by reducing PNNs, extended access upregulates PNN components and may stabilize drug-induced memory.

Pubmed:
31133834: Miquel M, Nicola SM, Gil-Miravet I, Guarque-Chabrera J, Sanchez-Hernandez A
A Working Hypothesis for the Role of the Cerebellum in Impulsivity and Compulsivity.
Growing evidence associates cerebellar abnormalities with several neuropsychiatric disorders in which compulsive symptomatology and impulsivity are part of the disease pattern. Symptomatology of autism, addiction, obsessive-compulsive (OCD), and attention deficit/hyperactivity (ADHD) disorders transcends the sphere of motor dysfunction and essentially entails integrative processes under control of prefrontal-thalamic-cerebellar loops. Patients with brain lesions affecting the cortico-striatum thalamic circuitry and the cerebellum indeed exhibit compulsive symptoms. Specifically, lesions of the posterior cerebellar vermis cause affective dysregulation and deficits in executive function. These deficits may be due to impairment of one of the main functions of the cerebellum, implementation of forward internal models of the environment. Actions that are independent of internal models may not be guided by predictive relationships or a mental representation of the goal. In this review article, we explain how this deficit might affect executive functions. Additionally, regionalized cerebellar lesions have been demonstrated to impair other brain functions such as the emergence of habits and behavioral inhibition, which are also altered in compulsive disorders. Similar to the infralimbic cortex, clinical studies and research in animal models suggest that the cerebellum is not required for learning goal-directed behaviors, but it is critical for habit formation. Despite this accumulating data, the role of the cerebellum in compulsive symptomatology and impulsivity is still a matter of discussion. Overall, findings point to a modulatory function of the cerebellum in terminating or initiating actions through regulation of the prefrontal cortices. Specifically, the cerebellum may be crucial for restraining ongoing actions when environmental conditions change by adjusting prefrontal activity in response to the new external and internal stimuli, thereby promoting flexible behavioral control. We elaborate on this explanatory framework and propose a working hypothesis for the involvement of the cerebellum in compulsive and impulsive endophenotypes.
Front Behav Neurosci, 2019; 13

33388819: Sanchez-Hernandez A, Nicolas C, Gil-Miravet I, Guarque-Chabrera J, Solinas M, Miquel M
Time-dependent regulation of perineuronal nets in the cerebellar cortex during abstinence of cocaine-self administration.
The probability of structural remodeling in brain circuits may be modulated by molecules of perineuronal nets (PNNs) that restrict neuronal plasticity to stabilize circuits. Animal research demonstrates that addictive drugs can remodel PNNs in different brain regions, including the cerebellum.
Psychopharmacology (Berl), 2021; 238

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-116

STRIATAL CHOLINERGIC INTERNEURONS CONTROL NICOTINE WITHDRAWAL VIA MUSCARINIC SIGNALING

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Striatal cholinergic interneurons (ChIs) provide cholinergic tone to the dorsal striatum and essentially regulate movement control. Nicotine withdrawal leads to perturbations in striatal neurophysiology and motor function, yet the role of striatal ChIs in nicotine withdrawal remains unclear. Here, we demonstrate that RNAi-mediated inhibition of the striatal ChIs leads to effective mitigation of somatic signs of nicotine withdrawal in mice. Bioinformatics analysis of miRNA databases predicted miR-137 as a putative inhibitor of the major sodium channel subtypes expressed in the striatal ChIs. Selective overexpression of miR-137 in the striatal ChIs (ChI^{FIN}, functional inhibition of ChIs) resulted in knockdown of sodium channel subunit $Na_V1.1$ and elevation of action potential threshold, consequently inhibiting action potential generation in the ChIs. In a comprehensive battery of behavioral tests, ChI^{FIN} mice displayed a reduction in somatic signs of nicotine withdrawal. Multi-electrode array recording showed that repeated nicotine exposure enhanced the number of population spikes in the dorsal striatum, which was substantially abolished by ChI^{FIN}. Interestingly, the clinically-approved muscarinic antagonist procyclidine was able to mimic the effect of ChI^{FIN}; procyclidine inhibited the repeated nicotine-evoked increase in striatal population spikes and ameliorated somatic withdrawal signs from nicotine. Collectively, these data indicate that striatal cholinergic interneurons control the motor aspect of nicotine withdrawal, and that drug repurposing of clinically approved muscarinic antagonists is a promising approach for developing new therapeutics against nicotine withdrawal syndrome.

Pubmed:
35138425: Kim B, Tag SH, Kim YS, Cho SN, Im HI
Circulating microRNA miR-137 as a stable biomarker for methamphetamine abstinence.
Stimulant use instigates abstinence syndrome in humans. miRNAs are a critical component for the pathophysiology of stimulant abstinence. Here we sought to identify a miRNA marker of methamphetamine abstinence in the circulating extracellular vesicles (cEVs).
Psychopharmacology (Berl), 2022; 239
34437591: Choi Y, Kim B, Ham S, Chung S, Maeng S, Kim HS, Im HI
Subanesthetic ketamine rapidly alters medial prefrontal miRNAs involved in ubiquitin-mediated proteolysis.
Ketamine is a dissociative anesthetic and a non-competitive NMDAR antagonist. At subanesthetic dose, ketamine can relieve pain and work as a fast-acting antidepressant, but the underlying molecular mechanism remains elusive. This study aimed to investigate the mode of action underlying the effects of acute subanesthetic ketamine treatment by bioinformatics analyses of miRNAs in the medial prefrontal cortex of male C57BL/6J mice. Gene Ontology and KEGG pathway analyses of the genes putatively targeted by ketamine-responsive prefrontal miRNAs revealed that acute subanesthetic ketamine modifies ubiquitin-mediated proteolysis. Validation analysis suggested that miR-148a-3p and miR-128-3p are the main players responsible for the subanesthetic ketamine-mediated alteration of ubiquitin-mediated proteolysis through varied regulation of ubiquitin ligases E2 and E3. Collectively, our data imply that the prefrontal miRNA-dependent modulation of ubiquitin-mediated proteolysis is at least partially involved in the mode of action by acute subanesthetic ketamine treatment.
PLoS One, 2021; 16
32767546: Kim B, Im HI
Chronic nicotine impairs sparse motor learning via striatal fast-spiking parvalbumin interneurons.
Nicotine can diversely affect neural activity and motor learning in animals. However, the impact of chronic nicotine on striatal activity in vivo and motor learning at long-term sparse timescale remains unknown. Here, we demonstrate that chronic nicotine persistently suppresses the activity of striatal fast-spiking parvalbumin interneurons, which mediate nicotine-induced deficit in sparse motor learning. Six weeks of longitudinal in vivo single-unit recording revealed that mice show reduced activity of fast-spiking interneurons in the dorsal striatum during chronic nicotine exposure and withdrawal. The reduced firing

of fast-spiking interneurons was accompanied by spike broadening, diminished striatal delta oscillation power, and reduced sample entropy in local field potential. In addition, chronic nicotine withdrawal impaired motor learning with a weekly sparse training regimen but did not affect general locomotion and anxiety-like behavior. Lastly, the excitatory DREADD hM3Dq-mediated activation of striatal fast-spiking parvalbumin interneurons reversed the chronic nicotine withdrawal-induced deficit in sparse motor learning. Taken together, we identified that chronic nicotine withdrawal impairs sparse motor learning via disruption of activity in striatal fast-spiking parvalbumin interneurons. These findings suggest that sparse motor learning paradigm can reveal the subtle effect of nicotine withdrawal on motor function and that striatal fast-spiking parvalbumin interneurons are a neural substrate of nicotine's effect on motor learning.
Addict Biol, 2021; 26
31795606: Kim B, Choi Y, Kim HS, Im HI
Methyl-CpG Binding Protein 2 in Alzheimer Dementia.
Despite decades of research on Alzheimer disease, understanding the complexity of the genetic and molecular interactions involved in its pathogenesis remains far from our grasp. Methyl-CpG Binding Protein 2 (MeCP2) is an important epigenetic regulator enriched in the brain, and recent findings have implicated MeCP2 as a crucial player in Alzheimer disease. Here, we provide comprehensive insights into the pathophysiological roles of MeCP2 in Alzheimer disease. In particular, we focus on how the alteration of MeCP2 expression can impact Alzheimer disease through risk genes, amyloid-β and tau pathology, cell death and neurodegeneration, and cellular senescence. We suggest that Alzheimer disease can be adversely affected by upregulated MeCP2-dependent repression of risk genes (MEF2C, ADAM10, and PM20D1), increased tau accumulation, and neurodegeneration through neuronal cell death (excitotoxicity and apoptosis). In addition, we propose that the progression of Alzheimer disease could be caused by reduced MeCP2-mediated enhancement of astrocytic and microglial senescence and consequent glial SASP (senescence-associated secretory phenotype)-dependent neuroinflammation. We surmise that any imbalance in MeCP2 function would accelerate or cause Alzheimer disease pathogenesis, implying that MeCP2 may be a potential drug target for the treatment and prevention of Alzheimer disease.
Int Neurourol J, 2019; 23
30277562: Kim B, Im HI
The role of the dorsal striatum in choice impulsivity.
It has long been recognized that the dorsal striatum is an essential brain region for control of action selection based on action-outcome contingency learning, particularly when the available actions are bound to rewarding outcomes. In principle, intertemporal choice in the delay-discounting task-a validated measure of choice impulsivity-involves reward-associated actions that require the recruitment of the dorsal striatum. Here, we conjecture about ways the dorsal striatum is involved in choice impulsivity. Based on a selective body of studies, we begin with a brief history of research on choice impulsivity and the dorsal striatum, and then provide a comprehensive summary of contemporary studies utilizing human neuroimaging and animal models to search for links between choice impulsivity and the dorsal striatum. In particular, we discuss in-depth the converging evidence for the associations of choice impulsivity with the reward valuation coded by the caudate, a ventral-to-dorsal gradient in the dorsal striatum, the origins of striatal afferents, and developmental maturation of frontostriatal connectivity during adolescence.
Ann N Y Acad Sci, 2019; 1451
27230456: Hong H, Kim BS, Im HI
Pathophysiological Role of Neuroinflammation in Neurodegenerative Diseases and Psychiatric Disorders.
Brain diseases and disorders such as Alzheimer disease, Parkinson disease, depression, schizophrenia, autism, and addiction lead to reduced quality of daily life through abnormal thoughts, perceptions, emotional states, and behavior. While the underlying mechanisms remain poorly understood, human and animal studies have supported a role of neuroinflammation in the etiology of these diseases. In the central nervous system, an increased inflammatory response is capable of activating microglial cells, leading to the release of pro-inflammatory cytokines including interleukin (IL)-1β, IL-6, and tumor necrosis factor-α. In turn, the pro-inflammatory cytokines aggravate and propagate neuroinflammation, degenerating healthy neurons and impairing brain functions. Therefore, activated microglia may play a key role in neuroinflammatory processes contributing to the pathogenesis of psychiatric disorders and neurodegeneration.
Int Neurourol J, 2016; 20
26974493: Yeom SY, Son CH, Kim BS, Tag SH, Nam E, Shin H, Kim SH, Gang H, Lee HJ, Choi J, Im HI, Cho IJ, Choi N
Multiplexed Detection of Epigenetic Markers Using Quantum Dot (QD)-Encoded Hydrogel Microparticles.
Epigenetic alterations in gene expression are influenced by experiences and environment, resulting in significant variation of epigenetic markers from individual to individual. Therefore, it is imperative to measure various epigenetic markers simultaneously from samples of individual subjects to accurately analyze the epigenetic markers in biological samples. Moreover, the individualized genome-wide analysis has become a critical technology for recent trends in clinical applications such as early diagnosis and personalized medicine screening of numerous diseases. The array-based detection of modified

histones, conventionally used for multiplexed analysis of epigenetic changes, requires pooling of samples from many subjects to analyze population-wise differences in the expression of histone markers and does not permit individualized analysis. Here, we report multiplexed detection of genome-wide changes in various histone modifications at a single-residue resolution using quantum dot (QD)-encoded polyethylene glycol diacrylate (PEGDA) hydrogel microparticles. To demonstrate the potential of our methodology, we present the simultaneous detection of (1) acetylation of lysine 9 of histone 3 (Ac-H3K9), (2) dimethylation of H3K9 (2Me-H3K9), and (3) trimethylation of H3K9 (3Me-H3K9) from three distinct regions in the brain [nucleus accumbens (NAc), dorsal striatum (DSt), and cerebellum (Cbl)] of cocaine-exposed mice. Our hydrogel-based epigenetic assay enabled relative quantification of the three histone variants from only 10 μL of each brain lysate (protein content = ~ 1 μg/μL) per mouse. We verified that the exposure to cocaine induced a significant increase of acetylation while a notable decrease in methylation in NAc.
Anal Chem, 2016; 88
26759057: Khalid A, Kim BS, Seo BA, Lee ST, Jung KH, Chu K, Lee SK, Jeon D
Gamma oscillation in functional brain networks is involved in the spontaneous remission of depressive behavior induced by chronic restraint stress in mice.
Depression is one of the most prevalent mood disorders, and is known to be associated with abnormal functional connectivity in neural networks of the brain. Interestingly, a significant proportion of patients with depression experience spontaneous remission without any treatment. However, the relationship between electroencephalographic (EEG) functional connectivity and the spontaneous remission in depression remains poorly understood. Here, we investigated regional and network brain activity using EEG signals from a chronic restraint stress (CRS)-induced mouse model of depression. After 1 (CRS1W) or 3 weeks (CRS3W) following the cessation of a 4-week-long CRS, mice were subjected to depression-associated behavioral tasks. EEG signals were obtained from eight cortical regions (frontal, somatosensory, parietal, and visual cortices in each hemisphere).
BMC Neurosci, 2016; 17
25064667: Khalid A, Kim BS, Chung MK, Ye JC, Jeon D
Tracing the evolution of multi-scale functional networks in a mouse model of depression using persistent brain network homology.
Many brain diseases or disorders, such as depression, are known to be associated with abnormal functional connectivity in neural networks in the brain. Some bivariate measures of electroencephalography (EEG) for coupling analysis have been used widely in attempts to explain abnormalities related with depression. However, brain network evolution based on persistent functional connections in EEG signals could not be easily unveiled. For a geometrical exploration of brain network evolution, here, we used persistent brain network homology analysis with EEG signals from a corticosterone (CORT)-induced mouse model of depression. EEG signals were obtained from eight cortical regions (frontal, somatosensory, parietal, and visual cortices in each hemisphere). The persistent homology revealed a significantly different functional connectivity between the control and CORT model, but no differences in common coupling measures, such as cross correlation and coherence, were apparent. The CORT model showed a more localized connectivity and decreased global connectivity than the control. In particular, the somatosensory and parietal cortices were loosely connected in the CORT model. Additionally, the CORT model displayed altered connections among the cortical regions, especially between the frontal and somatosensory cortices, versus the control. This study demonstrates that persistent homology is useful for brain network analysis, and our results indicate that the CORT-induced depression mouse model shows more localized and decreased global connectivity with altered connections, which may facilitate characterization of the abnormal brain network underlying depression.
Neuroimage, 2014; 101
24752658: Kim BS, Lee J, Bang M, Seo BA, Khalid A, Jung MW, Jeon D
Differential regulation of observational fear and neural oscillations by serotonin and dopamine in the mouse anterior cingulate cortex.
The aberrant regulation of serotonin (5-HT) and dopamine (DA) in the brain has been implicated in neuropsychiatric disorders associated with marked impairments in empathy, such as schizophrenia and autism. Many psychiatric drugs bind to both types of receptors, and the anterior cingulate cortex (ACC) is known to be centrally involved with empathy. However, the relationship between the 5-HT/DA system in the ACC and empathic behavior is not yet well known.
Psychopharmacology (Berl), 2014; 231

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THE SAPAP3-KO MOUSE RECONSIDERED AS A COMORBID MODEL EXPRESSING A SPECTRUM OF PATHOLOGICAL REPETITIVE BEHAVIORS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Symptom comorbidity is frequently observed in neuropsychiatric disorders with repetitive behaviours, including obsessive-compulsive disorder (OCD) and Tourette syndrome, and complicates clinical diagnosis and appropriate treatments. Driven by such observations, we reevaluated the behaviour of Sapap3 knockout mice, a current reference mouse model for studying the neurobiology of compulsive-like behaviours. In line with clinical comorbidity observations, we detected previously unreported elements of pathologically repetitive behaviours other than compulsive-like rodent syntactic grooming. Such repetitive behaviours included body and head twitches, aberrant hindpaw scratching, as well as isolated grooming phases. When pharmacologically challenging these phenotypes by a single administration of aripiprazole, a first-line treatment for tic-like symptoms in Tourette Syndrome, we significantly reduced the number of head/body twitches and isolated grooming phases, but not the number of syntactic grooming nor scratching events. We furthermore provide evidence that the flagship-like lesions of Sapap3 knockout mice are due to scratching, not syntactic self-grooming behaviours. Our observations are in line with high comorbidity of tic- and compulsive-like symptoms in Tourette, OCD and trichotillomania patients as well as with the hypothesis of shared neurobiological mechanisms. We suggest to reconsider the Sapap3 knockout mouse model as a comorbid model of pathological repetitive behaviours, including not only compulsive-like but also tic-like elements.

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BOARD NUMBER: S06-118

CHRONIC NICOTINE ALTERS MOTIVATIONAL VALUE OF NATURAL REWARDS THROUGH CIRCUIT-BASED ALTERATIONS IN VTA DOPAMINE NEURONS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Tobacco smoking is a major contributor to the burden of mental health disease worldwide. Nicotine is the primary reinforcing and main addictive component of tobacco. Nicotine addiction is a chronic relapsing disorder associated with multiple psychiatric comorbidities, and few effective interventions currently exist to curb addiction to nicotine. The neurocircuitry underlying nicotine addiction is broad, complex and depends on the stages of the disease process. It is well-acknowledged that nicotine impacts the reward system with prominent alterations of the firing properties of VTA DA neurons consequently biasing the responses to natural and addictive rewards. Yet the underlying mechanisms responsible of VTA DA alterations remain elusive. Here, we exposed mice to chronic nicotine in their drinking water to mimic the prolonged and intermittent nicotine absorption of nicotine in humans. Combining viral tracers and optogenetic approaches, we aim to investigate the effects of long-term nicotine intake on inputs to the reward system, establishing a parallel between circuit-based electrophysiological analyses and behavioral assessments in an operant conditioning task. We will present data showing that chronic nicotine consumption induces cellular alterations within inputs to the reward system that relate to changes in motivational state for natural rewards. These changes may influence the incentive attribution process induced by drugs of abuse in the addiction process.

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ROLE OF THE BLOCKADE OF A2A RECEPTOR IN THE ACQUISITION OF MORPHINE WITHDRAWAL-INDUCED CPA

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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The purinergic system, through the A2A adenosine receptor(A2AR), is involved in addiction induced by different drugs of abuse. It has been previously demonstrated that A2A gene deletion in mice modifies some of the behavioral effects of morphine. To discriminate whether the A2AR participates in the acquisition or in the expression of morphine-induced behaviors and, thus, to design effective pharmacological treatments targeted to A2AR to prevent behaviors responsible for the maintenance and relapse in opiate addiction, we have used the condition place aversion (CPA) paradigm and administered an A2AR antagonist to morphine-dependent and control mice prior the conditioning sessions with naloxone. To induce opiates dependence mice were subcutaneously implanted with morphine (MOR) or placebo (PLA) pellet. An A2RA antagonist (sch58261; 1mg/kg) or its vehicle was administered intraperitoneally 15 min before the conditioning sessions with naloxone (NX; 0.1 mg/kg, s.c.). CPA has been used to study the aversive emotional aspects of naloxone-precipitated morphine. As expected, opiate dependent animals spent significantly less time in the morphine withdrawal-paired chamber during the CPA test comparing to PLA mice. Nonetheless, when sch58261 was injected before naloxone conditioning to morphine dependent mice they spent more time in compartment associated with withdrawal than MOR vehicle mice (&& $p<0.01$ vs MOR vehicle). Our results reveal that the blockade of A2AR in morphine dependent mice prevented the aversion to the withdrawal paired-compartment indicates that this receptor is essential for the acquisition of morphine withdrawal-induced behaviors, pointing out that A2AR antagonists might be effective pharmacological tools to treat addiction to opiates.

Pubmed:

26926421: Gómez-Murcia V, Torrecillas A, de Godos AM, Corbalán-García S, Gómez-Fernández JC
Both idebenone and idebenol are localized near the lipid-water interface of the membrane and increase its fluidity. Idebenone is a synthetic analog of coenzyme Q; both share a quinone moiety but idebenone has a shorter lipophilic tail ending with a hydroxyl group. Differential scanning calorimetry experiments showed that both idebenone and idebenol widened and shifted the phase transition of 1,2-dipalmitoylphosphatidylcholine (DPPC) to a lower temperature and a phase separation with different concentrations of these molecules was observed. Also small angle X-ray diffraction and wide angle X-ray diffraction revealed that both, idebenone and idebenol, induced laterally separated phases in fluid membranes when included in DPPC membranes. Electronic profiles showed that both forms, idebenone and idebenol, reduced the thickness of the fluid membrane. (2)H NMR measurements showed that the order of the membrane decreased at all temperatures in the presence of idebenone or idebenol, the greatest disorder being observed in the segments of the acyl chains close to the lipid-water interface. (1)H NOESY MAS NMR spectra were obtained using 1-palmitoyl-2-oleoyl-phosphatidylcholine membranes and results pointed to a similar location in the membrane for both forms, with the benzoquinone or benzoquinol rings and their terminal hydroxyl group of the hydrophobic chain located near the lipid/water interface of the phospholipid bilayer and the terminal hydroxyl group of the hydrophobic chain of both compounds located at the lipid/water interface. Taken together, all these different locations might explain the different physiological behavior shown by the idebenone/idebenol compared with the ubiquinone-10/ubiquinol-10 pair in which both compounds are differently localized in the membrane.
Biochim Biophys Acta, 2016; 1858
35181375: Gomez-Murcia V, Carvalho K, Thiroux B, Caillierez R, Besegher M, Sergeant N, Buée L, Faivre E, Blum D
Impact of chronic doxycycline treatment in the APP/PS1 mouse model of Alzheimer's disease.
Due to the pathophysiological complexity of Alzheimer's disease, multitarget approaches able to mitigate several pathogenic mechanisms are of interest. Previous studies have pointed to the neuroprotective potential of Doxycycline (Dox), a safe and inexpensive second-generation tetracycline. Dox has been particularly reported to slow down aggregation of misfolded proteins but also to mitigate neuroinflammatory processes. Here, we have evaluated the pre-clinical potential of Dox in the APP/PS1 mouse model of amyloidogenesis. Dox was provided to APP/PS1 mice from the age of 8 months, when animals already exhibit amyloid pathology and memory deficits. Spatial memory was then evaluated from 9 to 10 months of age. Our data demonstrated that Dox moderately improved the spatial memory of APP/PS1 mice without exerting major effect on

amyloid lesions. While Dox did not alleviate overall glial reactivity, we could evidence that it rather enhanced the amyloid-dependent upregulation of several neuroinflammatory markers such as CCL3 and CCL4. Finally, Dox exerted differentially regulated the levels of synaptic proteins in the hippocampus and the cortex of APP/PS1 mice. Overall, these observations support that chronic Dox delivery does not provide major pathophysiological improvements in the APP/PS1 mouse model.
Neuropharmacology, 2022; 209
34469732: Brigas HC, Ribeiro M, Coelho JE, Gomes R, Gomez-Murcia V, Carvalho K, Faivre E, Costa-Pereira S, Darrigues J, de Almeida AA, Buée L, Dunot J, Marie H, Pousinha PA, Blum D, Silva-Santos B, Lopes LV, Ribot JC
IL-17 triggers the onset of cognitive and synaptic deficits in early stages of Alzheimer's disease.
Neuroinflammation in patients with Alzheimer's disease (AD) and related mouse models has been recognized for decades, but the contribution of the recently described meningeal immune population to AD pathogenesis remains to be addressed. Here, using the 3xTg-AD model, we report an accumulation of interleukin-17 (IL-17)-producing cells, mostly γδ T cells, in the brain and the meninges of female, but not male, mice, concomitant with the onset of cognitive decline. Critically, IL-17 neutralization into the ventricles is sufficient to prevent short-term memory and synaptic plasticity deficits at early stages of disease. These effects precede blood-brain barrier disruption and amyloid-beta or tau pathology, implying an early involvement of IL-17 in AD pathology. When IL-17 is neutralized at later stages of disease, the onset of short-memory deficits and amyloidosis-related splenomegaly is delayed. Altogether, our data support the idea that cognition relies on a finely regulated balance of "inflammatory" cytokines derived from the meningeal immune system.
Cell Rep, 2021; 36
33373844: Ausili A, Gómez-Murcia V, Candel AM, Beltrán A, Torrecillas A, He L, Jiang Y, Zhang S, Teruel JA, Gómez-Fernández JC
A comparison of the location in membranes of curcumin and curcumin-derived bivalent compounds with potential neuroprotective capacity for Alzheimer's disease.
Curcumin and two bivalent compounds, namely 17MD and 21MO, both obtained by conjugation of curcumin with a steroid molecule that acts as a membrane anchor, were comparatively studied. When incorporated into 1,2-dipalmitoyl-sn-glycero-3-phosphocholine the compounds showed a very limited solubility in the model membranes. Curcumin and the two bivalent compounds were also incorporated in membranes of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and quenching the fluorescence of pure curcumin or of the curcumin moiety in the bivalent compounds by acrylamide it was seen that curcumin was accessible to this water soluble quencher but the molecule was somehow located in a hydrophobic environment. This was confirmed by quenching with doxyl-phosphatidylcholines, indicating that the curcumin moieties of 17MD and 21MO were in a more polar environment than pure curcumin itself. H NOESY MAS-NMR analysis supports this notion by showing that the orientation of curcumin was parallel to the plane of the membrane surface close to C2 and C3 of the fatty acyl chains, while the curcumin moiety of 17MD and 21MO positioned close to the polar part of the membrane with the steroid moiety in the centre of the membrane. Molecular dynamics studies were in close agreement with the experimental results with respect to the likely proximity of the protons studied by NMR and show that 17MD and 21MO have a clear tendency to aggregate in a fluid membrane. The anchorage of the bivalent compounds to the membrane leaving the curcumin moiety near the polar part may be very important to facilitate the bioactivity of the curcumin moiety when used as anti-Alzheimer drugs.
Colloids Surf B Biointerfaces, 2021; 199
32679397: Gomez-Murcia V, Sandau U, Ferry B, Parrot S, Laurent C, Basquin M, Buée L, Boison D, Blum D
Hyperexcitability and seizures in the THY-Tau22 mouse model of tauopathy.
Epileptic seizures constitute a significant comorbidity of Alzheimer's disease (AD), which are recapitulated in transgenic mouse models of amyloidogenesis. Here, we sought to evaluate the potential role of tau pathology regarding seizure occurrence. To this end, we performed intra-hippocampal electroencephalogram (EEG) recordings and PTZ (pentylenetetrazol) seizure threshold tests in THY-Tau22 transgenic mice of AD-like tau pathology. We demonstrate that despite a lack of spontaneous epileptiform activity in Tau22 mice, the animals display increased PTZ-induced seizure susceptibility and mortality. The increased propensity for induced seizures in THY-Tau22 mutants correlates with astrogliosis and increased expression of adenosine kinase, consistent with increased network excitability. These data support an impact of tau pathology toward AD-associated seizures and suggest that tau pathology may contribute to seizure generation in AD independent of Aβ pathology.
Neurobiol Aging, 2020; 94
31616299: Gómez-Murcia V, Ribeiro Do Couto B, Gómez-Fernández JC, Milanés MV, Laorden ML, Almela P
Liposome-Encapsulated Morphine Affords a Prolonged Analgesia While Facilitating Extinction of Reward and Aversive Memories.
Morphine is thoroughly used for pain control; however, it has a high addictive potential. Opioid liposome formulations produce controlled drug release and have been thoroughly tested for pain treatment although their role in addiction is still unknown. This study investigated the effects of free morphine and morphine encapsulated in unilamellar and multilamellar liposomes on

antinociception and on the expression and extinction of the positive and negative memories associated with environmental cues. The hot plate test was used to measure central pain. The rewarding effects of morphine were analyzed by the conditioned-place preference (CPP) test, and the aversive aspects of naloxone-precipitated morphine withdrawal were evaluated by the conditioned-place aversion (CPA) paradigm. Our results show that encapsulated morphine yields prolonged antinociceptive effects compared with the free form, and that CPP and CPA expression were similar in the free- or encapsulated-morphine groups. However, we demonstrate, for the first time, that morphine encapsulation reduces the duration of reward and aversive memories, suggesting that this technological process could transform morphine into a potentially less addictive drug. Morphine encapsulation in liposomes could represent a pharmacological approach for enhancing extinction, which might lead to effective clinical treatments in drug addiction with fewer side effects.
Front Pharmacol, 2019; 10

31599329: Carvalho K, Faivre E, Pietrowski MJ, Marques X, Gomez-Murcia V, Deleau A, Huin V, Hansen JN, Kozlov S, Danis C, Temido-Ferreira M, Coelho JE, Mériaux C, Eddarkaoui S, Gras SL, Dumoulin M, Cellai L, , Landrieu I, Chern Y, Hamdane M, Buée L, Boutillier AL, Levi S, Halle A, Lopes LV, Blum D
Exacerbation of C1q dysregulation, synaptic loss and memory deficits in tau pathology linked to neuronal adenosine A2A receptor.
Accumulating data support the role of tau pathology in cognitive decline in ageing and Alzheimer's disease, but underlying mechanisms remain ill-defined. Interestingly, ageing and Alzheimer's disease have been associated with an abnormal upregulation of adenosine A2A receptor (A2AR), a fine tuner of synaptic plasticity. However, the link between A2AR signalling and tau pathology has remained largely unexplored. In the present study, we report for the first time a significant upregulation of A2AR in patients suffering from frontotemporal lobar degeneration with the MAPT P301L mutation. To model these alterations, we induced neuronal A2AR upregulation in a tauopathy mouse model (THY-Tau22) using a new conditional strain allowing forebrain overexpression of the receptor. We found that neuronal A2AR upregulation increases tau hyperphosphorylation, potentiating the onset of tau-induced memory deficits. This detrimental effect was linked to a singular microglial signature as revealed by RNA sequencing analysis. In particular, we found that A2AR overexpression in THY-Tau22 mice led to the hippocampal upregulation of C1q complement protein-also observed in patients with frontotemporal lobar degeneration-and correlated with the loss of glutamatergic synapses, likely underlying the observed memory deficits. These data reveal a key impact of overactive neuronal A2AR in the onset of synaptic loss in tauopathies, paving the way for new therapeutic approaches.
Brain, 2019; 142

30123104: Cellai L, Carvalho K, Faivre E, Deleau A, Vieau D, Buée L, Blum D, Mériaux C, Gomez-Murcia V
The Adenosinergic Signaling: A Complex but Promising Therapeutic Target for Alzheimer's Disease.
Alzheimer's disease (AD) is the most common neurodegenerative disorder in elderly people. AD is characterized by a progressive cognitive decline and it is neuropathologically defined by two hallmarks: extracellular deposits of aggregated β-amyloid (Aβ) peptides and intraneuronal fibrillar aggregates of hyper- and abnormally phosphorylated Tau proteins. AD results from multiple genetic and environmental risk factors. Epidemiological studies reported beneficial effects of caffeine, a non-selective adenosine receptors antagonist. In the present review, we discuss the impact of caffeine and of adenosinergic system modulation on AD, in terms of pathology and therapeutics.
Front Neurosci, 2018; 12

29271648: He L, Jiang Y, Liu K, Gomez-Murcia V, Ma X, Torrecillas A, Chen Q, Zhu X, Lesnefsky E, Gomez-Fernandez JC, Xu B, Zhang S
Insights into the Impact of a Membrane-Anchoring Moiety on the Biological Activities of Bivalent Compounds As Potential Neuroprotectants for Alzheimer's Disease.
Bivalent compounds anchoring in different manners to the membrane were designed and biologically characterized to understand the contribution of the anchor moiety to their biological activity as neuroprotectants for Alzheimer's disease. Our results established that the anchor moiety is essential, and we identified a preference for diosgenin, as evidenced by 17MD. Studies in primary neurons and mouse brain mitochondria also identified 17MD as exhibiting activity on neuritic outgrowth and the state 3 oxidative rate of glutamate while preserving the coupling capacity of the mitochondria. Significantly, our studies demonstrated that the integrated bivalent structure is essential to the observed biological activities. Further studies employing bivalent compounds as probes in a model membrane also revealed the influence of the anchor moiety on how they interact with the membrane. Collectively, our results suggest diosgenin to be an optimal anchor moiety, providing bivalent compounds with promising pharmacology that have potential applications for Alzheimer's disease.
J Med Chem, 2018; 61

27908267: Gomez-Murcia V, Montalban MG, Gomez-Fernandez JC, Almela P
Development of Poly(lactide-co-glicolide) Nanoparticles Incorporating Morphine Hydrochloride to Prolong its Circulation in Blood.

Formulations incorporating nanoparticles (NPs) are widely used to prolong drug release. In this regard, poly(lactide-co-glicolide) (PLGA) is often used in their preparation due to its high degree of biocompatibility and biodegradability. In the present study, morphine HCl is incorporated in PLGA-NPs and different preparation alternatives are evaluated for their effects on the properties, stability and capacity of encapsulation.
Curr Pharm Des, 2017; 23

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SUBTHALAMIC NUCLEUS DEEP BRAIN STIMULATION REDUCES ALCOHOL INTAKE IN RATS UNDER INFLUENCE OF PROXIMAL SOCIAL FACTORS.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Drug addiction, like most other addictions, takes place in a social environment that can influence the use of drugs. Alcohol is generally known as a social facilitator due to its disinhibiting properties[1]. Despite these widely known effects, few preclinical studies have so far looked at social factors at the time of consumption (proximal social factors). In rats, we use an alcohol self-administration task to assess the effect of the presence of a peer on alcohol intake. We used operant chambers with two compartments separated by a grid allowing the rats to interact. During sessions, only one rat has access to the alcohol. Our results showed that the presence of a peer increases alcohol intake for consumer rats. Previous studies showed the strong involvement of the subthalamic nucleus (STN) in motivated behavior, particularly in drug seeking[2-3-4]. In this study, we showed that STN deep brain stimulation at 130Hz reduces alcohol seeking when the animal is alone and reduces the influence of the presence of a peer on alcohol consumption.

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TRPA1 CAN MODULATE COCAINE ADDICTION WITHIN GLUTAMATERGIC NEURONS EXTENDING FROM MEDIAL FRONTAL CORTEX TO NUCLEUS ACCUMBENS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Cocaine is a highly addictive stimulant and still cocaine use disorder is a serious problem worldwide. The psychostimulant effect of cocaine is known to be developed through mesocorticolimbic system, yet there is a need to better understand specific mechanism of addiction. Our previous studies have shown that transient receptor potential Ankyrin 1 (TRPA1) channel can be a promising target to treat cocaine addiction. Here, we investigated the role of TRPA1 specifically in the medial prefrontal cortex (mPFC) to nucelus accumbens (NAc) pathway in mice using microinjection and optogenetic techniques. In cocaine psychomotor test, bilateral microinjection of TRPA1 antagonist into the NAc inhibited hyperactivity by acute cocaine treatment and cocaine-primed expression of cocaine sensitization. In the conditioned place preference (CPP) test, intra-NAc infusions of TRPA1 antagonist during conditioning period inhibited cocaine CPP acquisition. In addition, cocaine-primed reinstatement of cocaine CPP was also inhibited by intra-NAc infusions of TRPA1 antagonist on the reinstatement day. Through ELISA-based glutamate assay studies, we found that repeated cocaine injection increased glutamate levels both in the mPFC and NAc which was inhibited by TRPA1 pretreatment. Based on these results, we targeted glutamatergic neurons extending from mPFC to NAc by viral channelrhodopsin expression. When those neurons are specifically activated by optogenetic stimulation, it produced CPP in mice which was attenuated by systemic injection of TRPA1 antagonist. Taken together, present studies suggest that crucial role of TRPA1 in NAc on cocaine addiction and especially glutamatergic neurons extending from mPFC to NAc is considered as major pathway.

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BOARD NUMBER: S06-122

EPIGENETIC PRIMING UNDERLIES LATENT GENE DYSREGULATION IN COCAINE WITHDRAWAL

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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A hallmark of addiction is the ability of drugs of abuse to trigger relapse, even after periods of prolonged abstinence. Growing evidence implicates altered gene regulation in mediating the persistent effects of drugs of abuse in the nucleus accumbens (NAc), a key brain region of reward learning. Changes in the epigenetic landscape are hypothesized to cause the latent dysregulation of gene expression linked to relapse; however, the molecular mechanisms underlying this maladaptive process remains unclear. The NAc is composed of two functionally distinct types of medium spiny neurons (MSNs), the D1 and D2 dopamine receptor-expressing subtypes, thus making the identification of subtype-specific epigenetic changes critical. We surveyed circuit-specific chromatin accessibility in combination with unbiased histone modification profiling by mass spec and ChIP-seq. We discovered that chronic cocaine persistently alters NAc chromatin structure, especially in D1 MSNs, involving dramatic depletion of the histone variant H2A.Z, a recently identified memory suppressor, at key neuronal genes. Curiously, genome accessibility in D1 MSNs is prominently increased at these genes even after prolonged withdrawal, and is linked to aberrant gene expression upon relapse. The histone chaperone ANP32E is promotes the removal of H2A.Z, and we demonstrate that D1 MSN-selective ANP32E knockdown effectively blocks cocaine conditioned place preference (CPP). Together, our studies investigate an emerging view of epigenetic adaptation that may contribute to substance use disorder, providing novel insight into circuit-specific epigenetic priming as an important mechanism whereby drugs of abuse alter gene expression and behavior in lasting ways.

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BOARD NUMBER: S06-123

ANABOLIC ANDROGENIC STEROID USE IN PROFESSIONAL AND AMATEUR ATHLETES: ASSOCIATION WITH OTHER SUBSTANCE USE

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Aim Evidence regarding the association between anabolic androgenic steroid (AAS) and other substance use in athletes is controversial. A low prevalence of polysubstance use in AAS users has been reported by some studies; other studies have hypothesized that AASs use may be a gateway for the consumption of misused substances, especially opioids, possibly due to opioids' analgesic action on muscle pain caused by overtraining or their ability to counteract AAS-induced insomnia/irritability. Yet, the gateway hypothesis remains controversial. Last, AAS-induced alterations of the endogenous opioidergic system may lead to increased sensitivity to opioids. This multisite, cross-sectional study in professional and recreational athletes in Italy, assessed the use of AASs and other substances in biological samples, based on the hypothesis that AAS-using athletes would be more focused on being fit and not take other substances. **Methods** 122 athletes (age=18-45 years; females=38) all completed toxicological examinations, i.e., blood, urine, and hair testing for AASs and misused drugs. **Results** There was slight-to-moderate agreement among the three biological samples used for AAS and other substance testing (Fleiss' κ=0.251-0.474). Contrary to our hypothesis, we found AAS users to consume more misused substances than nonusers (χ^2=0.082, p<0.01), especially cannabinoids (25.8% vs. 18.7%), opioids (16.1% vs. 3.3%), amphetamines (12.9% vs. 4.4%), and cocaine (9.7% vs. 6.6%). **Conclusions** These findings align with some studies in athletes that describe the concurrent use of AASs and other substances, often in a context of polysubstance use. Further research is needed to investigate the association between AAS and other substance use, especially opioids.

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EFFECTS OF THE MONOAMINE STABILIZER OSU6162 ON COMPULSIVE ALCOHOL-RELATED BEHAVIOR IN RATS.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Alcohol addiction is a chronic relapsing disease characterized by the inability to control alcohol consumption, the degradation of affective and emotional state during abstinence and ultimately by the development of compulsive seeking despite knowledge of having negative consequences. Still, the shift between a controlled alcohol consumption and the development of compulsive behavior remain unclear. It has been shown that alcohol-use disorder alters dopaminergic (DA) transmission in both mesolimbic and nigrostriatal pathways, which functionality are fundamental for incentive motivated behaviors in positive and negative reinforcements and motor control. More precisely, it has been shown that acute alcohol consumption increased DA release and phasic DA transmission in the nucleus accumbens (NAcc), while long-term consumption leads to a chronic decrease of DA level, with a tonic hypodopaminergic transmission in the NAcc. We have recently showed that compulsive alcohol-related behavior in rats was rather associated with a decrease of DA release in the anterior dorsolateral striatum (aDLS), and a selective induction of hypodopaminergia in the nigrostriatal pathway triggered compulsive alcohol use in rats. In the current study, we aim to investigate whether restoration of normal dopaminergic transmission by the monoamine stabilizer OSU6162 could restore a normal behavior in compulsive alcohol-related rats.

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PRENATAL HYPOXIA RELATED DISTURBANCES IN GLUCOCORTICOID-DEPENDENT GENE EXPRESSION OF CHRNA7 AND GENES OF GLUTAMATE SYSTEM ARE POSSIBLE MECHANISM OF DEVELOPMENT OF NICOTINE ADDICTION.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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The role of damaging factors in prenatal period as basis of drug addiction in offspring is of great interest. Our investigation aims to study the effects and possible mechanisms of prenatal hypoxia (PSH) on the vulnerability to nicotine addiction in adult 3-months old rats. In PSH group, we have revealed an increased tendency to nicotine consumption in behavioral tests. In an attempt to find out an explanation for the addictive behavior of PSH rats, we previously revealed increased amount of phosphorylated at the 34th threonine residue DARPP-32 protein (known as the relay for dopamine and glutamate signaling) in the nucleus accumbens (NAc). The observed proportion of DARPP-32/phosphoDARPP-32 could not be explained by alterations in the amount of dopamine and its receptors in the NAc. As a protentional, explanation, we consider the decreasing of glutamatergic stimulation of NAc neurons from projections of ventral hippocampus, in which we found decreased glucocorticoid-dependent transcription of genes of glutamate metabolism. Meanwhile, another potential explanation might be increasing expression of the *chrna7* gene, which codes nicotinic acetylcholine receptors of type 7 localized on presynapses of glutamatergic projections to such structures of the mesocortical pathway, as the prefrontal cortex (PFC) and ventral tegmental area (VTA), in which an increase in VGluT2-positive glutamatergic terminals were also found. Thus, the accelerated development of nicotine addiction in experimental rats is probably associated with PSH-related phenotype of acetylcholine and glutamatergic systems, namely with increased glutamatergic stimulation of VTA and PFC neurons and decreased stimulation of NAc from hippocampal glutamatergic projections.

Pubmed:

33389385: Vetrovoy O, Stratilov V, Nimiritsky P, Makarevich P, Tyulkova E
Prenatal Hypoxia Induces Premature Aging Accompanied by Impaired Function of the Glutamatergic System in Rat Hippocampus.
Prenatal hypoxia is among leading causes of progressive brain pathologies in postnatal life. This study aimed to analyze the characteristics of the hippocampal glutamatergic system and behavior of rats in early (2 weeks), adult (3 months) and advanced (18 months) postnatal ontogenesis after exposure to prenatal severe hypoxia (PSH, 180 Torr, 5% O2, 3 h) during the critical period in the formation of the hippocampus (days 14-16 of gestation). We have shown an age-dependent progressive decrease in the hippocampal glutamate levels, a decrease of the neuronal cell number in the CA1 hippocampal region, as well as impairment of spatial long-term memory in the Morris water navigation task. The gradual decrease of glutamate was accompanied by decreased expression of the genes that mediate glutamate metabolism and recycling in the hippocampus. That deficiency apparently correlated with an increase of the metabotropic glutamate receptor type 1 (mGluR1) and synaptophysin expression. Generation of the lipid peroxidation products in the hippocampus of adult rats subjected to prenatal severe hypoxia (PSH rats) was not increased compared to the control animals when tested in a model of glutamate excitotoxicity induced by severe hypoxia. This demonstrates that excessive glutamate sensitivity in PSH rats does not compensate for glutamate deficiency. Our results show a significant contribution of the glutamate system dysfunction to age-associated decrease of this mediator, cognitive decline, and early neuronal loss in PSH rats.
Neurochem Res, 2021; 46
33440383: Vetrovoy O, Tyulkova E, Stratilov V, Baranova K, Nimiritsky P, Makarevich P, Rybnikova E
Long-Term Effects of Prenatal Severe Hypoxia on Central and Peripheral Components of the Glucocorticoid System in Rats.
Prenatal hypoxia is a risk factor for the development of numerous neurological disorders. It is known that the maternal stress response to hypoxia determines the epigenetic impairment of the perinatal expression of glucocorticoid receptors (GR) in the hippocampus of the progeny, but so far no detailed study of how this affects the functional state of the glucocorticoid system

during further ontogenesis has been performed.
Dev Neurosci, 2020; 42

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A NOVEL MOUSE MODEL WITH CONSTITUTIVE ISR ACTIVATION REVEALS NEW INSIGHTS INTO HUMAN DISEASE

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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The integrated stress response (ISR) is a stress sensing pathway that reprograms cellular translation to promote proteostasis in response to nutrient poor conditions, changes in the redox state, infection by double stranded RNA viruses, and accumulation of unfolded proteins in the ER. Aberrant ISR activation has been associated with many neuropathologies of the brain including Vanishing White Matter Disease, Alzheimer Disease, and Downs Syndrome. ISR activation mediates changes in translation via phosphorylation of a translation initiation factor (eIF2), which regulates levels of initiation of protein synthesis. To understand the contribution of the ISR to disease we generated a novel mouse model that expresses a mutant component of the phosphatase that catalyzes removal of the phosphate on eIF2. This mutant phosphatase causes accumulation of phosphorylated eIF2, and thus, sustained activation of the ISR in the brain. Consistent with ISR activation, we saw increased phosphorylated eIF2, and ATF4 target genes in the brain. Previous work had suggested the ISR contributes to impaired cognition in mouse models. We observed impaired memory in contextual fear conditioning experiments, and further studies revealed novel changes associated with the ISR that are relevant to human disease.

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NEUROCOMPUTATIONAL MECHANISMS OF AVOIDANCE BEHAVIOUR IN OBSESSIVE-COMPULSIVE SYMPTOMOLOGY

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Exposure therapy, the first-line treatment for obsessive-compulsive disorder (OCD), relies on aversive conditioning and extinction learning principles. However, little is known about how these principles go awry in unsuccessful exposure therapy. To probe their computational foundations, we designed a novel aversive conditioning task to examine acquisition and extinction learning via explicit contingency reports and avoidance actions. Participants had to learn the aversive contingencies of different stimuli (low (20%), mid (50%), high (80%)). Thereafter, they could perform actions to avoid the probabilistic aversive outcomes, whilst some stimuli were devalued. We collected data from 84 participants as they performed the task online and assessed their OCD symptomatology. We found that individuals scoring high in OCD symptomology overestimated the aversive contingencies of the stimuli initially (r=.27, p=.01) but corrected their biased contingency beliefs after learning (r<014, p>0.19). These individuals also excessively performed avoidance actions for the low contingency stimuli (20%) (r=0.26, p<0.001) and the non-devalued low contingency stimulus after extinction (r=0.37, p<0.001) despite reporting accurate contingency beliefs (r=0.17, p=0.11). This reveals a striking action-belief mismatch in those with high OCD symptoms. Our findings thus suggest that individuals with high OCD scores do not have impairments in learning and reporting accurate contingency beliefs after acquisition and extinction, but yet exhibit excessive avoidance to aversive outcomes of low probability.

Pubmed:
34961624: Seow TXF, Rouault M, Gillan CM, Fleming SM
Reply to: Metacognition, Adaptation, and Mental Health.
Biol Psychiatry, 2022; 91
34334187: Seow TXF, Rouault M, Gillan CM, Fleming SM
How Local and Global Metacognition Shape Mental Health.
Metacognition is the ability to reflect on our own cognition and mental states. It is a critical aspect of human subjective experience and operates across many hierarchical levels of abstraction-encompassing local confidence in isolated decisions and global self-beliefs about our abilities and skills. Alterations in metacognition are considered foundational to neurologic and psychiatric disorders, but research has mostly focused on local metacognitive computations, missing out on the role of global aspects of metacognition. Here, we first review current behavioral and neural metrics of local metacognition that lay the foundation for this research. We then address the neurocognitive underpinnings of global metacognition uncovered by recent studies. Finally, we outline a theoretical framework in which higher hierarchical levels of metacognition may help identify the role of maladaptive metacognitive evaluation in mental health conditions, particularly when combined with transdiagnostic methods.
Biol Psychiatry, 2021; 90
34240338: Seow TXF, Hauser TU
Reliability of web-based affective auditory stimulus presentation.
Web-based experimental testing has seen exponential growth in psychology and cognitive neuroscience. However, paradigms involving affective auditory stimuli have yet to adapt to the online approach due to concerns about the lack of experimental control and other technical challenges. In this study, we assessed whether sounds commonly used to evoke affective responses in-lab can be used online. Using recent developments to increase sound presentation quality, we selected 15 commonly used sound stimuli and assessed their impact on valence and arousal states in a web-based experiment. Our results reveal good inter-rater and test-retest reliabilities, with results comparable to in-lab studies. Additionally, we compared a variety of previously used unpleasant stimuli, allowing us to identify the most aversive among these sounds. Our findings demonstrate that affective sounds can be reliably delivered through web-based platforms, which

help facilitate the development of new auditory paradigms for affective online experiments.
Behav Res Methods, 2022; 54
34131033: Seow TXF, Benoit E, Dempsey C, Jennings M, Maxwell A, O'Connell R, Gillan CM
Model-Based Planning Deficits in Compulsivity Are Linked to Faulty Neural Representations of Task Structure.
Compulsive individuals have deficits in model-based planning, but the mechanisms that drive this have not been established. We examined two candidates-that compulsivity is linked to (1) an impaired model of the task environment and/or (2) an inability to engage cognitive control when making choices. To test this, 192 participants performed a two-step reinforcement learning task with concurrent EEG recordings, and we related the neural and behavioral data to their scores on a self-reported transdiagnostic dimension of compulsivity. To examine subjects' internal model of the task, we used established behavioral and neural responses to unexpected events [reaction time (RT) slowing, P300 wave, and parietal-occipital alpha band power] measured when an unexpected transition occurred. To assess cognitive control, we probed theta power at the time of initial choice. As expected, model-based planning was linked to greater behavioral (RT) and neural (alpha power, but not P300) sensitivity to rare transitions. Critically, the sensitivities of both RT and alpha to task structure were weaker in those high in compulsivity. This RT-compulsivity effect was tested and replicated in an independent pre-existing dataset (= 1413). We also found that mid-frontal theta power at the time of choice was reduced in highly compulsive individuals though its relation to model-based planning was less pronounced. These data suggest that model-based planning deficits in compulsive individuals may arise, at least in part, from having an impaired representation of the environment, specifically how actions lead to future states. Compulsivity is linked to poorer performance on tasks that require model-based planning, but it is unclear what precise mechanisms underlie this deficit. Do compulsive individuals fail to engage cognitive control at the time of choice? Or do they have difficulty in building and maintaining an accurate representation of their environment, the foundation needed to behave in a goal-directed manner? With reaction time and EEG measures in 192 individuals who performed a two-step decision-making task, we found that compulsive individuals are less sensitive to surprising action-state transitions, where they slow down less and show less alpha band suppression following a rare transition. These findings implicate failures in maintaining an accurate model of the world in model-based planning deficits in compulsivity.
J Neurosci, 2021; 41
33080287: Seow TXF, Benoit E, Dempsey C, Jennings M, Maxwell A, McDonough M, Gillan CM
A dimensional investigation of error-related negativity (ERN) and self-reported psychiatric symptoms.
Alterations in error processing are implicated in a range of DSM-defined psychiatric disorders. For instance, obsessive-compulsive disorder (OCD) and generalised anxiety disorder show enhanced electrophysiological responses to errors-i.e. error-related negativity (ERN)-while others like schizophrenia have an attenuated ERN. However, as diagnostic categories in psychiatry are heterogeneous and also highly intercorrelated, the precise mapping of ERN enhancements/impairments is unclear. To address this, we recorded electroencephalograms (EEG) from 196 participants who performed the Flanker task and collected scores on 9 questionnaires assessing psychiatric symptoms to test if a dimensional framework could reveal specific transdiagnostic clinical manifestations of error processing dysfunctions. Contrary to our hypothesis, we found non-significant associations between ERN amplitude and symptom severity of OCD, trait anxiety, depression, social anxiety, impulsivity, eating disorders, alcohol addiction, schizotypy and apathy. A transdiagnostic approach did nothing to improve signal; there were non-significant associations between all three transdiagnostic dimensions (anxious-depression, compulsive behaviour and intrusive thought, and social withdrawal) and ERN magnitude. In these same individuals, we replicated a previously published transdiagnostic association between goal-directed learning and compulsive behaviour and intrusive thought. Possible explanations discussed are (i) that associations between the ERN and psychopathology might be smaller than previously assumed, (ii) that these associations might depend on a greater level of symptom severity than other transdiagnostic cognitive biomarkers, or (iii) that task parameters, such as the ratio of compatible to incompatible trials, might be crucial for ensuring the sensitivity of the ERN to clinical phenomena.
Int J Psychophysiol, 2020; 158
32532686: Gillan CM, Seow TXF
Carving Out New Transdiagnostic Dimensions for Research in Mental Health.
Biol Psychiatry Cogn Neurosci Neuroimaging, 2020; 5
32076008: Seow TXF, Gillan CM
Transdiagnostic Phenotyping Reveals a Host of Metacognitive Deficits Implicated in Compulsivity.
Recent work suggests that obsessive-compulsive disorder (OCD) patients have a breakdown in the relationship between explicit beliefs (i.e. confidence about states) and updates to behaviour. The precise computations underlying this disconnection are unclear because case-control and transdiagnostic studies yield conflicting results. Here, a large online population sample (N = 437) completed a predictive inference task previously studied in the context of OCD. We tested if confidence, and its relationship to action and environmental evidence, were specifically associated with self-reported OCD symptoms or common to an array of psychiatric phenomena. We then investigated if a transdiagnostic approach would reveal

a stronger and more specific match between metacognitive deficits and clinical phenotypes. Consistent with prior case-control work, we found that decreases in action-confidence coupling were associated with OCD symptoms, but also 5/8 of the other clinical phenotypes tested (8/8 with no correction applied). This non-specific pattern was explained by a single transdiagnostic symptom dimension characterized by compulsivity that was linked to inflated confidence and several deficits in utilizing evidence to update confidence. These data highlight the importance of metacognitive deficits for our understanding of compulsivity and underscore how transdiagnostic methods may prove a more powerful alternative over studies examining single disorders.
Sci Rep, 2020; 10
31062300: Seow T, Fleming SM
Perceptual sensitivity is modulated by what others can see.
Previous work has established that social cues such as the direction of others' gaze or their perspective on a scene may influence one's own perceptual judgments. However, up until now it has remained unclear whether such influences are exerted at a perceptual or decisional locus, as most previous studies have used response times as their primary dependent measure. Here, we asked whether perceptual sensitivity is also dependent on social cognition. To test this hypothesis, we asked participants to evaluate whether low-contrast Gabor patterns embedded in noise were visible from either their own or an avatar's perspective. Across three experiments, we found that observers' detection performance was increased if an avatar also shared perception of the stimulus location. By leveraging signal detection modelling, we show that this effect is driven by a change in perceptual sensitivity (d'), independent of decisional or response interference. Furthermore, by "blindfolding" the avatar, we show that the boosting effect of shared perception on detection sensitivity is only obtained when the participant believes the avatar can also see the stimulus, ruling out an influence of low-level directional cues. We interpret these results within a framework in which the avatar's perspective boosts top-down spatial attention by prioritising particular spatial locations at which perception is shared. In summary, we reveal that perceptual sensitivity is modulated by the perspective of others.
Atten Percept Psychophys, 2019; 81
29458997: Rouault M, Seow T, Gillan CM, Fleming SM
Psychiatric Symptom Dimensions Are Associated With Dissociable Shifts in Metacognition but Not Task Performance.
Distortions in metacognition-the ability to reflect on and control other cognitive processes-are thought to be characteristic of poor mental health. However, it remains unknown whether such shifts in self-evaluation are due to specific alterations in metacognition and/or a downstream consequence of changes in decision-making processes.
Biol Psychiatry, 2018; 84
27415631: Koster R, Seow TX, Dolan RJ, Düzel E
Stimulus Novelty Energizes Actions in the Absence of Explicit Reward.
Novelty seeking has been tied to impulsive choice and biased value based choice. It has been postulated that novel stimuli should trigger more vigorous approach and exploration. However, it is unclear whether stimulus novelty can enhance simple motor actions in the absence of explicit reward, a necessary condition for energizing approach and exploration in an entirely unfamiliar situation. In this study human subjects were cued to omit or perform actions in form of button presses by novel or familiar images. We found that subjects' motor actions were faster when cued by a novel compared to a familiar image. This facilitation by novelty was strongest when the delay between cue and action was short, consistent with a link between novelty and impulsive choices. The facilitation of reaction times by novelty was correlated across subjects with trait novelty seeking as measured in the Tridimensional Personality Questionnaire. However, this li between high novelty-seeking and action facilitation was driven by trials with a long delay between cue and action. This prolonged time window of energization following novelty could hint at a mechanistic underpinning of enhanced vigour for approach and exploration frequently postulated for novelty seeking humans. In conclusion, we show that stimulus novelty enhances the speed of a cued motor action. We suggest this is likely to reflect an adaptation to changing environments but may also provide a source of maladaptive choice and impulsive behaviour.
PLoS One, 2016; 11

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SEX DIFFERENCES IN BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF AMPHETAMINE MODULATED BY VASOPRESSIN IN THE LATERAL SEPTUM

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Lateral septum (LS) is a brain structure implicated in addictive behaviors, regulating the activation of dopamine (DA) neurons in the ventral tegmental area (VTA). Extended amygdala vasopressin (AVP) projections to LS are sexually dimorphic and could be responsible for the vulnerability to addiction in a sex-dependent manner. We demonstrated that AVP microinjection in LS reduces amphetamine (AMPH)-induced conditioned place preference (CPP) that it is associated to lower nucleus accumbens (NAc) DA release in male rats. The aim of this work was to examine sex differences in LS AVP system on AMPH-induced behavioral and neurochemical responses in female and male rats. Our results show that LS AVP microinjections avoid the AMPH-induced CPP expression in female rats, which it is reversed by co-microinjection of V_{1A} receptor antagonist. At neurochemical level, intra-LS AVP administration increases LS GABA release, and it decreases VTA DA release only in male rats. Interestingly, our data demonstrate that intra-LS AVP reduces AMPH-induced CPP in rats of both sexes, however at neurochemical level we observed sex differences. This research contributes to the knowledge about sex differences in the role of AVP in LS in regulating the reward circuit and addictive like behaviors. KEYWORDS: amphetamine, lateral septum, dopamine, ventral tegmental area, vasopressin, GABA, CPP This research was funded by ANID-Chile through FONDECYT Grant N°120–0474 to R.S-Z. and G.M.R and DICYT Grant Nº 022101RSSA to G.M.R.

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LOFEXIDINE INHIBITS GASTROINTESTINAL AND SOMATIC, BUT NOT NEGATIVE AFFECTIVE OPIOID WITHDRAWAL SYMPTOMS IN MICE.

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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AIMS The alpha-2 adrenergic agonist lofexidine was repurposed and approved by the FDA for mitigation of opioid withdrawal symptoms. Despite this, there are no published preclinical lofexidine opioid studies. This limits lofexidine's utility as a comparator for drug discovery and hinders the ability to better understand its mechanism of action in mitigating withdrawal. Therefore, we used murine models to assess lofexidine effects on: i) multiple acute opioid withdrawal symptoms; ii) locomotor activity in opioid naïve mice; and iii) acquisition of withdrawal-induced conditioned place aversion (CPA). **METHODS** Male C57BL/6 mice were made physically dependent on oxycodone by repeated administration. Dose-dependent effects of lofexidine on acute gastrointestinal (fecal boli, diarrhea), somatic (tremors), and negative affective (jumping) symptoms were assessed using a naloxone-precipitated oxycodone withdrawal model. Impact on aversive stimulus effects of opioid withdrawal were assessed by examining lofexidine's effects on acquisition of CPA. Locomotor effects of lofexidine were assessed in opioid-naïve mice in an open-field. **RESULTS** Lofexidine reduced paw tremors and gastrointestinal symptoms during withdrawal but had no impact on withdrawal-induced jumping. Lofexidine was sedative and induced thigmotaxis across a broad dose range. Lofexidine did not affect acquisition of withdrawal-induced CPA at either sedating or non-sedating doses. **CONCLUSIONS** Consistent with the clinical literature, lofexidine appears efficacious for managing some, but not all aspects of the withdrawal syndrome. However, effects should be interpreted with caution, as they were not dissociable from pronounced sedation. Notably, lofexidine does not appear to be effective at managing negative affective aspects of the withdrawal syndrome.

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VENTRAL PALLIDAL PERINEURONAL NETS REGULATE OPIOID RELAPSE

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Opioid use disorder remains a major health challenge worldwide. Neuronal activity in the ventral pallidum (VP) is critical for opioid reward, and for driving relapse to opioid seeking, but the precise neuronal mechanisms that mediate these behaviors are incompletely understood. A major population of VP neurons linked to drug relapse is characterized by expression of the calcium binding protein parvalbumin and are ensheathed by specialized extracellular structures known as perineuronal nets (PNNs). However, despite the dense expression of PNNs in the VP, the role of these structures in VP neuronal physiology and opioid seeking remains unknown. To investigate the role of PNNs in the VP on opioid relapse, male and female mice were implanted with intravenous catheters and trained to self administer heroin. Afterwards mice underwent extinction training, followed 24h later by a microinfusion of the PNN-depleting enzyme chondroitinase ABC (ChABC) or vehicle (0.1% BSA) into the VP. The next day, relapse to drug seeking was assessed. VP ChABC microinjection abolished PNNs in the proximity of the microinjector needle and attenuated cue-induced reinstatement of heroin seeking. Ongoing experiments are characterizing the role of PNNs on the physiology of VP neurons. Our results affirm that the VP is a critical regulator for opioid relapse, and expand our knowledge of the opioid addiction circuitry by showing that heroin seeking requires intact PNNs in the VP. Given the importance of PNNs for memory maintenance and behavioral plasticity, therapies aimed at modifying PNN structure might provide a useful novel avenue for treating opioid addiction.

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OXYTOCIN AND OREXIN SYSTEMS BIDIRECTIONALLY REGULATE THE ABILITY OF OPIOID CUES TO BIAS CHOICE DURING RELAPSE

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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As opioid-related fatalities continue to rise, the need for novel opioid use disorder (OUD) treatments could not be more urgent. Two separate hypothalamic neuropeptide systems have shown promise in preclinical OUD models. The oxytocin system, originating in the paraventricular nucleus (PVN), may protect against OUD severity. By contrast, the orexin system, originating in the lateral hypothalamus (LH), may exacerbate OUD severity. Thus, activating the oxytocin system or inhibiting the orexin system are potential therapeutic strategies. The role of these systems with regard to specific OUD outcomes, however, is not fully understood. Here, we probed the therapeutic efficacy of pharmacological interventions targeting the orexin or oxytocin system on two distinct metrics of OUD severity in rats – heroin choice (versus choice for natural reward, i.e., food) and relapse. Using a preclinical model that generates approximately equal choice between heroin and food reward, we examined the impact of exogenously administered oxytocin, an oxytocin receptor antagonist (L-368,899), and a dual orexin receptor antagonist (DORA-12) on opioid choice. Whereas these agents did not alter heroin choice when rewards (heroin and food) were available, both agents significantly reduced heroin relapse in the presence of both types of reward cues when no rewards were available. In addition, we observed that the number of LH orexin neurons and PVN oxytocin neurons correlated with specific behavioral economic variables indicative of heroin versus food motivation. These data identify a novel bidirectional role of the oxytocin and orexin systems in the ability of opioid-related cues to bias choice during relapse.

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EXTINCTION ATTENUATES HYPERALGESIA DURING WITHDRAWAL FROM SELF-ADMINISTERED HEROIN: ROLE OF THE PVT→NAC PATHWAY

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Aim: The paraventricular thalamus (PVT) to the nucleus accumbens (NAc) pathway has been recently identified as a key component in the neural circuitry of opioid use disorder. Here we used chemogenetics, in-vivo optogenetic LTD protocol and fiber photometry to investigate the contribution of the PVT→NAc pathway to heroin withdrawal-mediated hyperalgesia. Using withdrawal-related variables, a machine learning model was trainend to predict individual propensity to relapse. **Methods**: Rats underwent 12d of heroin self-administration. Hyperalgesia was assessed after 14d of withdrawal by using an electronic Von Frey apparatus, after J60 or vehicle pretreatment. A different cohort of rats received an in-vivo optogenetic LTD protocol stimulation (1Hz) after 14d of abstinence, before the cued relapse test. To validate the in-vivo LTD protocol, optogenetic-evoked calcium events were recorded by using fiber photometry before and after LTD stimulation. **Results:** Extinction training significantly attenuated heroin withdrawal-induced hyperalgesia and somatic signs compared to the abstinence group. Chemogenetic inhibition of the PVT→NAc pathway after abstinence reduced hyperalgesia to levels similar to those observed after extinction training. Finally, we found that multiple variables of the addiction model can be used to accurately predict individual relapse rates during cued relapse. **Conclusion:** Here we demonstrated that the PVT→NAc pathway promotes heroin withdrawal-induced hyperalgesia after abstinence but not after extinction training. Moreover, we successfully employed fiber photometry to validate the in-vivo optogenetic LTD protocol in the PVT→NAc pathway. Finally, by using withdrawal-related variables to train a regression machine learning model, we were able to accurately predict the individual propensity to relapse.

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SINGLE-DOSE ETHANOL INTOXICATION CAUSES ACUTE AND LASTING NEURONAL CHANGES IN THE BRAIN

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Alcohol intoxication at early ages is a risk factor for development of addictive behavior. To uncover neuronal molecular correlates of acute ethanol intoxication, we used stable-isotope labeled mice combined with quantitative mass spectrometry to screen over 2000 hippocampal proteins of which 72 changed synaptic abundance up to two-fold after ethanol exposure. Among those were mitochondrial proteins and proteins important for neuronal morphology, including MAP6 and Ankyrin-G. Based on these candidate proteins, we found acute and lasting molecular, cellular, and behavioral changes following a single intoxication in alcohol-naïve mice. Immunofluorescence analysis revealed a shortening of axon initial segments. Longitudinal two-photon *in vivo* imaging showed increased synaptic dynamics and mitochondrial trafficking in axons. Knockdown of mitochondrial trafficking in dopaminergic neurons abolished conditioned alcohol preference in *Drosophila*. This introduces mitochondrial trafficking as a process implicated in reward learning, and highlights the potential of high-resolution proteomics to identify cellular mechanisms relevant for addictive behavior.

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CAFFEINE INTAKE EXERTS DUAL GENOME-WIDE EFFECTS ON HIPPOCAMPAL METABOLISM AND LEARNING-DEPENDENT TRANSCRIPTION

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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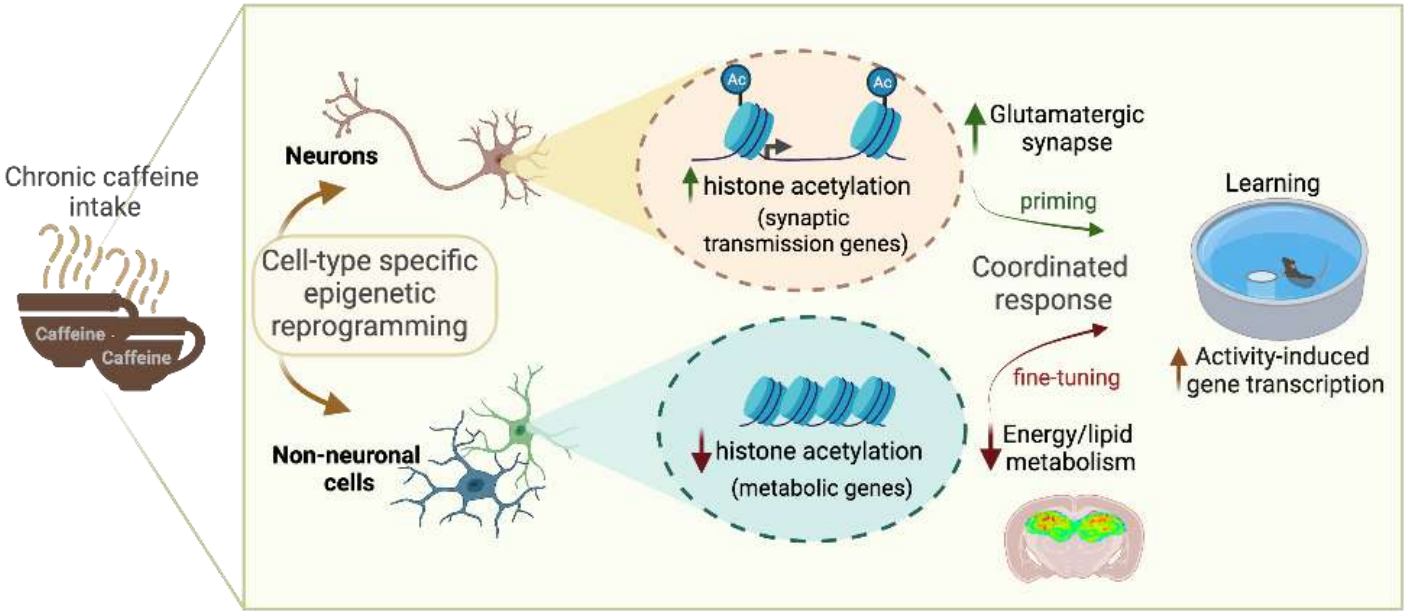
Caffeine is the most consumed psychoactive substance worldwide. Strikingly, molecular pathways engaged by its regular consumption remain unclear. We herein addressed the mechanisms associated with habitual (chronic) caffeine consumption in the mouse hippocampus using untargeted orthogonal-omics techniques. Our results revealed that chronic caffeine exerts concerted pleiotropic effects in the hippocampus, at the epigenomic, proteomic and metabolomic levels. Caffeine lowers metabolic-related processes in the bulk tissue, while it induces neuronal-specific epigenetic changes at synaptic transmission/plasticity-related genes and increased experience-driven transcriptional activity. Altogether, these findings suggest that regular caffeine intake improves the signal-to-noise ratio during information encoding, in part through a fine-tuning of metabolic genes while boosting the salience of information processing during learning in neuronal circuits. Paiva I *et al*, Caffeine intake exerts dual genome-wide effects on hippocampal metabolism and learning-dependent transcription. J Clin Invest. May 10, 2022.

FENS Forum 2022
9-13 July 2022 | Paris, France

https://doi.org/10.1172/JCI149371



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OPIOID WITHDRAWAL ABRUPTLY DISRUPTS AMYGDALA CIRCUIT FUNCTION BY REDUCING PEPTIDE ACTIONS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Withdrawal from opioid drugs has long-lasting effects on neuronal function and synaptic transmission in opioid-sensitive neurons throughout the brain. These persistent changes are thought to drive drug seeking and relapse behaviours, even after protracted abstinence. Despite this, the mechanisms and circuits underlying the altered behaviours following opioid withdrawal are only partially understood, limiting the development of treatments for addiction. Neural circuits within the amygdala mediate drug seeking and relapse behaviours, thus, we aimed to discover whether opioid withdrawal triggered neuroadaptations in opioid-sensitive amygdala cells. Under normal physiological conditions, glutamatergic transmission from principal neurons within the basolateral amygdala to GABAergic intercalated cells within the neighbouring main intercalated cell island is strongly inhibited by endogenous opioid peptides. Using patch-clamp electrophysiology in rat brain slices, we found that opioid withdrawal strongly reduced the ability of met-enkephalin, an opioid peptide, to inhibit this glutamatergic transmission (control inhibition = 25 ± 4 %, n = 12 vs. withdrawal inhibition = 14 ± 3 %, n = 12; unpaired Student's t-test, p = 0.043). Furthermore, we observed that the reduction in opioid inhibition was due to increased degradation of opioid peptides by the enkephalin-degrading peptidase, neprilysin. Our results highlight that opioid peptide activity is decreased during opioid withdrawal; a change that alters synaptic transmission between amygdala nuclei. As amygdala neural circuits are involved in the development of addiction behaviours, restoring endogenous opioid activity within the amygdala during withdrawal may return synaptic transmission to normal, mitigate withdrawal-induced neuroadaptations and rescue the addiction behaviours exhibited following opioid withdrawal.

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BOARD NUMBER: S06-136

ROLE OF A LATERAL HYPOTHALAMUS-LATERAL HABENULA PATHWAY IN COCAINE-INDUCED PSYCHOMOTOR RESPONSES

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

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Administration of cocaine increases synaptic dopamine levels by blocking dopamine reuptake and leads to increased locomotor activity and compulsive drug seeking. It has been suggested that lateral hypothalamus (LH) or lateral habenula (LHb) is associated with cocaine-induced drug seeking behaviors. To explore the role of an LH-LHb pathway in cocaine-induced psychomotor responses, we tested whether activation or inhibition of the LH-LHb pathway affects cocaine-induced locomotion. Cocaine-induced locomotor activity and dopamine release was suppressed by an activation of LH with PEPA, an AMPA receptor agonist. When LH was inhibited by microinjection of GABA mixtures prior to cocaine injection, the cocaine effects were enhanced. Furthermore, optogenetic activation of the LH-LHb pathway attenuated the cocaine-induced locomotion, while optogenetic inhibition of the LH-LHb pathway increased it. These findings suggest that the LH-LHb circuit plays a role in the modulation of cocaine-induced psychomotor responses. Acknowledgement: This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (Nos. 2018R1A5A2025272, 2019R1A2C1002555 and KMDF_PR_20200901_0117

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BOARD NUMBER: S06-137

ALCOHOL USE DISORDER SEVERITY IS LINKED TO ALTERED FUNCTIONAL CONNECTIVITY IN DEFAULT MODE, SALIENCE AND EXECUTIVE CONTROL NETWORKS

POSTER SESSION 06 - SECTION: SUBSTANCE USE AND ABUSE

Elisabeth Jehli[1,2], Matthias Grieder[1], Maria Stein[1], Tobias Bracht[1], Niklaus Denier[1], Franz Moggi[1], Leila Soravia[3]
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Aims Alcohol Use Disorder (AUD) presents an immense burden for patients and society. AUD patients show altered functional connectivity (FC) in default mode (DM), salience and frontoparietal networks [1,2]. This study aims at linking resting state FC in DM, salience and frontoparietal networks of AUD patients with the severity of AUD. **Methods** A total of 43 recently abstinent patients with AUD underwent functional magnetic resonance imaging (fMRI). Functional connectivity in resting-state fMRI data was assessed by using the Conn toolbox [3].The severity of AUD was assessed with the DSM-5 Alcohol Use Disorder Diagnostic Assessment (AUD-S) and Alcohol Use Disorders Identification Test (AUDIT-S) [4,5] scores as well as number of previous detoxifications and duration of problematic drinking. **Results** Higher scores in AUD-S are linked to higher FC in default mode, salience and frontoparietal networks, whereas higher AUDIT-S scores are correlated to higher FC in salience but not DM and frontoparietal networks. Furthermore, the number of previous detoxifications and duration of problematic drinking were linked to higher FC in these networks. **Conclusions** AUD Patients with higher AUD-S and AUDIT-S scores show stronger FC in DM (AUD-S), salience (AUD-S and AUDIT-S) and frontoparietal (AUD-S) networks. Higher number of previous detoxification and longer duration of problematic drinking showed significant positive correlation with FC in all investigated networks. Our results suggest that the neuronal representation of cognitive functioning is increasingly altered with higher AUD severity. Furthermore, the AUD-S seems to be more sensitive in predicting FC alterations in AUD patients when compared to AUDIT-S.

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BOARD NUMBER: S06-138

SOUND PROCESSING IN A MOUSE MODEL OF AUTISM SPECTRUM DISORDER

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in communication and social interaction, restricted interests and repetitive behaviours. Knowledge about ASD neurobiology is still scarce and currently there is no efficient treatment or cure. Although a single cause for ASD is unknown, several candidate genes have emerged from patient studies such as *SHANK3*, a gene that encodes a protein (SHANK3) in the postsynaptic density of excitatory synapses. Previous studies have shown that mutant mice carrying a human ASD-mutation in the *Shank3* gene (InsG3680), exhibit repetitive behaviours, anxiety and social interaction deficits. Social interaction is a complex behaviour that requires the ability to combine sensory information with emotional and cognitive content. Disruptions in sensory processing such as auditory hypersensitivity have also been reported in ASD patients and may relate to their social communication deficits. To test whether *Shank3*-mutant present auditory hypersensitivity, we developed a novel behavioural test where mice can choose between different “soundscapes” and found that *Shank3*-mutant mice tend to prefer quiet environments without sounds. Furthermore, *in vivo* recordings in the primary auditory cortex (A1) of anesthetized *Shank3*-mutant mice are also being performed to test central/higher auditory brain regions. Overall these results seem to be in accordance with the auditory hypersensitivity literature in ASD patients.

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GENETIC HETEROGENEITY SHAPES BRAIN CONNECTIVITY IN PSYCHIATRY

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Polygenicity and genetic heterogeneity pose great challenges for studying mechanisms - such as brain connectivity - underlying neurodevelopmental conditions. Genetic-first approaches have been implemented in neuroimaging studies to address this issue. However, the effects on functional connectivity of genetic risks for psychiatric disorders are largely unknown. Our objectives were to estimate and compare the effect sizes on brain connectivity of neurodevelopmental disorders genomic risk factors with various levels of complexity: oligo-, multi-genic copy number variants (CNVs), and polygenic-scores (PRS) as well as idiopathic psychiatric conditions and traits. Resting-state functional-MRI data were processed using the same pipeline across nine datasets. Twenty-nine connectome-wide association studies were performed to characterize the effects of 15 CNVs (1003 carriers), 7 PRS, 4 idiopathic conditions (1022 individuals with either autism, schizophrenia, bipolar, or ADHD), and 2 traits (31424 non-CNV carriers or unaffected controls). Effect sizes on connectivity were largest for neurodevelopmental psychiatric CNVs (estimates: 0.2 to 0.65 z-score) followed by psychiatric conditions (0.15 to 0.42 z-score), neuroticism and fluid intelligence (0.02 to 0.03 z-score), and PRS (0.01 to 0.02 z-score). Effect sizes of CNVs on connectivity were correlated to their effects on cognition and risk for disease (r=0.9, p=5.93e-06) for each individual brain network. However, effect sizes of CNVs adjusted for the number of genes significantly decreased from small oligogenic to large multigenic CNVs (r=-0.88, p=8.78e-06). PRS had disproportionately low effect sizes on connectivity compared to CNVs conferring similar risk for disease. Heterogeneity and polygenicity impact our ability to detect brain connectivity alterations underlying psychiatric manifestations.

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BOARD NUMBER: S06-140

AGGRESSION AND REWARD PROCESSING IN AUTISM SPECTRUM DISORDER (ASD)

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Aims: Aggression is a common form of challenging behavior among individuals with autism spectrum disorder (ASD). Affecting as many as 2-out-of-3 individuals with ASD, aggression has serious detrimental effects. Aggression often exacerbates core ASD symptoms and is the strongest predictor of parental stress, yet we lack effective treatments. Thus, a neurobiologically informed approach to the understanding and treatment of aggression in ASD is needed. To achieve this, this project is built upon 2 key pieces of evidence: i) aggression was shown to have rewarding properties and ii) the reward system shows aberrant activity following social stimuli in individuals with ASD. We aim to investigate if aggression in ASD has rewarding properties. **Methods:** We used *Neurexin1α* (*Nrxn1α*) knock-out (KO) mice as i) *Nrxn1α* is a category-1 classified ASD-linked gene and ii) because mutations in *Nrxn1α* were associated with aggression in humans and animal models of ASD. Mice were tested for sociability, aggression and conditioned place preference (CPP) for aggression (where 1-out-of-2 contexts is paired with an aggressive encounter). Furthermore, neural activity of the Ventral Tegmental Area (VTA) and Nucleus Accumbens (NAc) were investigated and correlated with behavior. **Results:** Our results show that *Nrxn1α* KO mice exhibit increased aggression compared to their wild-type (WT) littermates. In contrast to WTs, *Nrxn1α* KO mice acquired robust CPP to the aggression paired context, which was corelated with activity levels of the VTA and NAc. **Conclusions:** Our study suggests that aggressive behavior in the *Nrxn1α* KO mice is associated with aberrant recruitment of the reward system.

Pubmed:
34165614: Bourdy R, Hertz A, Filliol D, Andry V, Goumon Y, Mendoza J, Olmstead MC, Befort K
The endocannabinoid system is modulated in reward and homeostatic brain regions following diet-induced obesity in rats: a cluster analysis approach.
Increased availability of high-calorie palatable food in most countries has resulted in overconsumption of these foods, suggesting that excessive eating is driven by pleasure, rather than metabolic need. The behavior contributes to the rise in eating disorders, obesity, and associated pathologies like diabetes, cardiac disease, and cancers. The mesocorticolimbic dopamine and homeostatic circuits are interconnected and play a central role in palatable food intake. The endocannabinoid system is expressed in these circuits and represents a potent regulator of feeding, but the impact of an obesogenic diet on its expression is not fully known.
Eur J Nutr, 2021; 60
31434715: Panasiuk M, Hertz A, Gale-Grant O
Nucleus Accumbens Dopamine Receptor 1 Expressing Neurons Are Instrumental in Appetitive Aggression.
J Neurosci, 2019; 39

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STRIATAL CHOLINERGIC INTERNEURONS DYSFUNCTION AS A SUBSTRATE FOR STEREOTYPIES IN AUTISM SPECTRUM DISORDER

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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TSHZ3 is a risk gene for autism spectrum disorder (ASD). In mice, *Tshz3* deletion causes behavioral abnormalities relevant to the two main domains of ASD (social interaction deficits and restricted/repetitive patterns of behavior) and changes in glutamate synaptic transmission in the cortex and striatum (Caubit et al, 2016; Chabbert et al, 2019). In the adult mouse striatum, the majority (>90%) of cells expressing TSHZ3 are striatal cholinergic interneurons (CINs) and all CINs express TSHZ3, while TSHZ3 expression is absent or partial in the other main cholinergic brain systems. To study the possible role of CINs in the pathophysiology of *Tshz3*-linked ASD, we generated and characterized a conditional knockout (cKO) mouse model with selective *Tshz3* deletion in cholinergic neurons (*Chat-cKO*). *Chat-cKO* mice exhibit repetitive patterns of behavior but no social interaction deficits. While the number of CINs was unchanged, the proportion of CINs classified based on their discharge pattern as "typical" , i.e. showing sustained and regular discharge, and "atypical", with weaker and irregular activity, which is around 2/3 and 1/3 in control mice was inversed in *Chat-cKO*. Whether these populations show different molecular profile and distribution in control mice, how are they affected by *Tshz3* deletion and what are the consequences of the changes in their electrophysiological properties on the function of the corticostriatal network are major issues currently under investigation. Taken together, these results should provide useful insights concerning the cellular and molecular bases linking CIN dysfunction to stereotyped behavior. This study was supported by the ANR-17-CE16-0030 “TSHZ3inASD”.

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ROLE OF GROUP I METABOTROPIC RECEPTORS IN THE SYNAPTIC ALTERATIONS IN THE DORSAL STRIATUM OF THE R451C-NLGN3 MOUSE MODEL OF AUTISM

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism Spectrum Disorders (ASD), comprise heterogeneous neurodevelopmental disorders characterized by early onset of communication and social interaction difficulties, restricted interests, repetitive and stereotyped behaviors. Gene sequencing studies have identified hundreds of genes potentially implicated in ASD, which converge on two main biological pathways: gene expression regulation and neuronal communication. The projection neurons of the striatum, the input nucleus of the basal ganglia, are characterized by a particularly high expression of ASD-
associated genes. Indeed, structural and functional alterations of the striatum were described in ASD patients, and a correlation of the dorso-ventral anatomo-functional subdivisions of the striatum with specific domains of ASD symptoms was proposed. In our previous work, we described the loss of corticostriatal long-term depression (LTD) in the dorsal striatum of the R451C-Nlgn3 knock-in
(KI) mouse model of ASD. LTD was partially rescued by enhancing the endocannabinoid tone or activating CB1 receptors. Here, we aimed at identifying more effective strategies to rescue corticostriatal LTD. Activation of group I metabotropic glutamate receptors (mGlu1 and mGlu5 receptors) activates downstream signaling pathways, involving production of endocannabinoids. We therefore attempted a
pharmacological rescue of LTD in KI mice, by targeting group I mGlu receptors. The mixed mGlu1 and mGlu5 receptor agonist 3,5-DHPG was able to rescue LTD expression. By means of immunoblotting experiments, we found that the amount of mGlu5 receptor protein was significantly
reduced in the synaptosomes from the dorsal striatum of KI mice, suggesting a molecular basis of corticostriatal LTD impairment.

Pubmed:
35034734: Sciamanna G, El Atiallah I, Montanari M, Pisani A
Plasticity, genetics and epigenetics in dystonia: An update.
Dystonia represents a group of movement disorders characterized by involuntary muscle contractions that result in abnormal posture and twisting movements. In the last 20 years several animal models have been generated, greatly improving our knowledge of the neural and molecular mechanism underlying this pathological condition, but the pathophysiology remains still poorly understood. In this review we will discuss recent genetic factors related to dystonia and the current understanding of synaptic plasticity alterations reported by both clinical and experimental research. We will also present recent evidence involving epigenetics mechanisms in dystonia.
Handb Clin Neurol, 2022; 184

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MIMICKING SOCIAL ENVIRONMENT REVEALS OXYTOCIN, VASOPRESSIN AND PLASTICITY VARIATIONS IN MOUSE MODELS OF SOCIAL INTERACTION DEFICITS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Social interaction deficits result from early psychological traumas, genetic and/or environmental factors including autism spectrum disorders which are diagnosed on two behavioral symptoms: deficits in communication and social interaction and restricted and repetitive interests or behaviors. Oxytocin controls all types of social behavior through lifespan and could be involved in the etiology of those heterogenous disorders. First, we compared the effect of two types of social interactions (family or unknown congeners) in three mouse models of social interaction deficits (*Oprm1* KO, *Shank3* KO and isolated mice) and wildtype animals using standardized behavioral apparatus. Our results showed that *Oprm1* and *Shank3* KO mice display unique pattern of social interaction deficits and motor stereotypies whereas isolated mice only present sociability deficits. Then, we assessed if the oxytocin gene family levels are commonly impaired in these models using quantitative PCR. Finally, we studied the kinetic profile of oxytocin and plasticity family gene expression upon stimulation with two types of social stimuli (cage-mate or unknown sex-matched interactor) and a non-social stimulus in *Oprm1* mouse and wildtype brain structures involved in sociability. We identified that all the mouse models have unique and common variations of oxytocin family gene expression. In addition, we found that oxytocin and plasticity family genes respond specifically to cage-mate and/or unknown interactions and is dependent on genotypes, structures and sexual dimorphism. In conclusion, we highlight common and unique features of our three mouse models of social interaction deficits and oxytocin-related mechanisms underlying social interactions.

Pubmed:
32129757: Blanchard A, Gora C, Golinelli-Cohen MP
[The Fe-S protein NfuA, a new key player in the virulence of Pseudomonas aeruginosa].
Med Sci (Paris), 2020; 36

FENS Forum 2022
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SYNAPTIC ALTERATIONS IN THE AUDITORY CORTEX AND HIPPOCAMPUS UNDERLIE SOCIAL DEFICITS IN THE SYNAPSIN II KNOCKOUT MOUSE

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Background: Autism spectrum disorders (ASDs) are heterogeneous neurodevelopmental disorders characterized by two main symptoms: social deficits and repetitive behaviors. Autistic children often exhibit several secondary symptoms including epilepsy. Mutations in the Synapsin2 (SYN2) gene, associated with ASD and epilepsy in humans, are causative for an imbalance between excitatory and inhibitory systems. Synapsins are a family of neuron-specific phosphoproteins which control synaptic vesicle trafficking and modulate neurotransmitter release at the presynaptic terminal. Mice lacking SYN2 (SynII KO) display autistic-like traits with a strong reduction of ultrasonic vocalizations (USV) and social sniffing, repetitive behaviors (self-grooming) and mild cognitive impairments (deficit in social memory and recognition). Furthermore, SynII KO mice display epileptic seizures that appear at 2-3 months of age. Interestingly, we recently showed that the impaired USV phenotype strongly correlates with a reduced functional connectivity in the auditory cortex and hippocampus, two brain regions playing an important role in social behavior. **Aims:** Our goal was to clarify how synaptic alterations in these regions impact on the social brain circuitry generating pathological conditions. **Methods:** We characterized the synaptic alterations in the auditory cortex and hippocampus using western blotting analysis and immunohistochemistry. **Results:** Our results show a significant reduction in presynaptic and postsynaptic markers of the GABAergic system together with alterations in synaptic density in these areas of Syn II KO mice. **Conclusion:** These results indicate that developmental changes in the neural connectivity of specific cortical areas may underlie the epileptic and social phenotype of SynII KO mice.

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HCN CHANNELOPATHY AND AUDITORY HYPERSENSITIVITY IN THE SHANK3 MOUSE MODEL OF ASD

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder affecting 1 in 160 children worldwide. ASD is typically characterized by difficulties in social communication and repetitive behaviors, often associated with hypo- or hypersensitivity to sensory stimuli. It has been hypothesized that ASD impairments in communication might be a consequence of the auditory hypersensitivity often observed in patients, where atypical neuronal activity in auditory cortex could underlie the noxious or unpleasant perception of auditory stimuli. For this reason, patients would avoid auditory stimulation, ultimately affecting their learning, communication, and quality of life. Albeit ASD presents a widely diverse etiology, there is a strong genetic component involved, with the *SHANK3* gene being considered a potential monogenic cause of ASD. Taking advantage from a mouse model of ASD that carries a patient mutation in the *Shank3* gene (*InsG3680-Shank3*), we have been investigating the link between ASD and auditory hypersensitivity. Preliminary data suggests an auditory hypersensitivity behavioral phenotype, together with molecular and functional alterations in the primary auditory cortex (A1) of adult *Shank3* mice. Using LC-MS (liquid chromatography–mass spectrometry) analysis, we found upregulated expression of hyperpolarization-activated cyclic nucleotide gated-channel 1 (HCN1) protein levels, together with increased transcript levels by RT-PCR. Whole-cell patch clamp recordings of intracellular currents mainly mediated by HCN channel (I_h currents), further revealed heightened hyperpolarization in the A1 of adult *Shank3* mice. Together these results suggest that HCN1 channelopathy might contribute to the auditory hypersensitivity behavioral phenotype observed in the *Shank3* mouse model of ASD.

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ATYPICAL WHISKER-DEPENDENT RESPONSES IN CNTNAP2-/- MICE

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Sensory abnormalities are a common feature in autism spectrum disorders (ASDs). Tactile responsiveness is altered in autistic individuals, with hypo-responsiveness being associated with the severity of ASD core symptoms. Similarly, sensory abnormalities have been described in mice lacking ASD-associated genes. Loss-of-function mutations in *CNTNAP2* result in cortical dysplasia-focal epilepsy syndrome (CDFE) and autism. Likewise, *Cntnap2*$^{-/-}$ mice show epilepsy and deficits relevant with core symptoms of human ASDs, and are considered a reliable model to study ASDs. Altered synaptic transmission and synchronicity found in the cerebral cortex of *Cntnap2*$^{-/-}$ mice would suggest a network dysfunction, but their neural substrates have been poorly investigated so far. Here, we investigated functional connectivity within somatosensory areas, whisker-dependent behavior, and whisker-induced c-*fos* mRNA expression in *Cntnap2*$^{+/+}$and *Cntnap2*$^{-/-}$ adult mice. When compared to controls, *Cntnap2*$^{-/-}$ mice showed focal hyper-connectivity within the primary somatosensory cortex (S1), suggesting the presence of impaired somatosensory processing in these mutants. Accordingly, *Cntnap2*$^{-/-}$ mice displayed impaired whisker-dependent discrimination in the textured novel object recognition test (tNORT), increased S1 c-*fos* mRNA induction following whisker stimulation, and upregulation of neocortical excitatory neuron markers compared to controls. S1 functional hyperconnectivity and increased E/I balance might underlie the aberrant whisker-dependent responses observed in *Cntnap2*$^{-/-}$mice, indicating that *Cntnap2* mice are a reliable model to investigate sensory abnormalities that characterize ASDs.

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BOARD NUMBER: S06-147

SHIFTED PARVALBUMIN INTERNEURON STATES ELICIT POST-TRAUMATIC STRESS DISORDER-LIKE MEMORY IN AUTISM

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism Spectrum Disorder (ASD) presents with enhanced sensitivity to stress, yet vulnerability to trauma in this condition remains poorly explored. In this study, we aimed to investigate the predisposition to and impact of Post-Traumatic Stress Disorder (PTSD) in ASD and demonstrated a reciprocal relationship between the two disorders. We revealed that exposure to a mild stressful event induces PTSD-like memory in two mouse models of ASD, unlike in control conditions. Remarkably, PTSD-like memory exacerbated two key core symptoms associated with ASD (social impairments and repetitive movements). We determined that the susceptibility to developing PTSD-like memory in ASD stemmed from prefrontal cortex hyperactivation and changes in fine-tuning of the parvalbumin interneuron firing. These alterations were associated with the dysregulated expression of activity-dependent proteins: the Etv1/Er81 transcription factor, the parvalbumin protein, and the stress-related Neurokinin Receptor 3. Finally, we showed that PTSD-like memory formation in ASD could be prevented by normalizing parvalbumin interneuron activity through the activation of the Neurokinin 3 Receptor. Overall, this study reveals multi-level neurobiological mechanisms that explain the increased vulnerability of developing PTSD in ASD. It provides a framework for examining the impact of stress-induced traumatic memory and interneuron adaptation in autism to ultimately increase the success of therapeutic interventions.

Pubmed:
34722821: Al Abed AS, Sellami A, Ducourneau EG, Bouarab C, Marighetto A, Desmedt A
Protocols to Induce, Prevent, and Treat Post-traumatic Stress Disorder-like Memory in Mice: Optogenetics and Behavioral Approaches.
One of the cardinal features of post-traumatic stress disorder (PTSD) is a paradoxical memory alteration including both for salient trauma-related cues and for the surrounding traumatic context. Interestingly, some clinical studies have suggested that contextual amnesia would causally contribute to the PTSD-related hypermnesia insofar as decontextualized, traumatic memory is prone to be reactivated in contexts that can be very different from the original traumatic context. However, most current animal models of PTSD-related memory focus exclusively on the emotional hypermnesia, , the persistence of a strong fear memory, and do not distinguish normal (adaptive) from pathological (PTSD-like) fear memory, leaving unexplored the hypothetical critical role of contextual amnesia in PTSD-related memory formation, and thus challenging the development of innovative treatments. Having developed the first animal model that precisely recapitulates the two memory components of PTSD in mice (emotional hypermnesia and contextual amnesia), we recently demonstrated that contextual amnesia, induced by optogenetic inhibition of the hippocampus (dorsal CA1), is a causal cognitive process of PTSD-like hypermnesia formation. Moreover, the hippocampus-dependent contextualization of traumatic memory, by optogenetic activation of dCA1 in traumatic condition, prevents PTSD-like hypermnesia formation. Finally, once PTSD-like memory has been formed, the re-contextualization of traumatic memory by its reactivation in the original traumatic context normalizes this pathological fear memory. Revealing the key role of contextual amnesia in PTSD-like memory, this procedure opens a therapeutic perspective based on trauma contextualization and the underlying hippocampal mechanisms.
Bio Protoc, 2021; 11
33867115: Al Abed AS, Reynolds NJ, Dehorter N
A Second Wave for the Neurokinin Tac2 Pathway in Brain Research.
Despite promising advances in basic research of the neurokinin B/Tac2 pathway in both animals and humans, clinical applications are yet to be implemented. This is likely because of our limited understanding of the action of the pathway in the brain. While this system controls neuronal activity in multiple regions, the precise impact of Tac2-induced cellular responses on behavior remains unclear. Recently, elegant studies revealed a key contribution to stress-related behaviors and memory. Here, we discuss the crucial importance of bridging the gap between the Tac2 pathway's involvement in cell physiology and

cognition to comprehend its role in health and disease. We propose that a better understanding of the Tac2 pathway in the brain could provide an essential perspective for basic investigations, which in turn will feed clinical research.
Biol Psychiatry, 2021; 90
33849945: Ahmed NY, Ranjbar-Slamloo Y, Al Abed AS, Gao L, Sontani Y, RCom-H'cheo-Gauthier A, Arabzadeh E, Dehorter N
Er81 Transcription Factor Fine-Tunes Striatal Cholinergic Interneuron Activity and Drives Habit Formation.
The molecular mechanisms tuning cholinergic interneuron (CIN) activity, although crucial for striatal function and behavior, remain largely unexplored. Previous studies report that the Etv1/Er81 transcription factor is vital for regulating neuronal maturation and activity. While Er81 is known to be expressed in the striatum during development, its specific role in defining CIN properties and the resulting consequences on striatal function is unknown. We report here that Er81 is expressed in CINs and its specific ablation leads to prominent changes in their molecular, morphologic, and electrophysiological features. In particular, the lack of Er81 amplifies intrinsic delayed-rectifier and hyperpolarization-activated currents, which subsequently alters the tonic and phasic activity of CINs. We further reveal that Er81 expression is required for normal CIN pause and time-locked responses to sensorimotor inputs in awake mice. Overall, this study uncovers a new cell type-specific control of CIN function in the striatum which drives habit formation in adult male mice. Although previous studies have shown that cholinergic interneurons drive striatal activity and habit formation, the underlying molecular mechanisms controlling their function are unknown. Here we reveal that key cholinergic interneuron physiological properties are controlled by Er81, a transcription factor regulating neuronal activity and development in a cell-specific manner. Moreover, our findings uncover a link between the Er81-dependent molecular control of cholinergic interneuron function and habit formation in mice. These insights will contribute to the future enhancement of our understanding of disorders that involve behavioral inflexibility, such as autism and addiction.
J Neurosci, 2021; 41
33009891: Al Abed AS, Sellami A, Potier M, Ducourneau EG, Gerbeaud-Lassau P, Brayda-Bruno L, Lamothe V, Sans N, Desmedt A, Vanhoutte P, Bennetau-Pelissero C, Trifilieff P, Marighetto A
Age-related impairment of declarative memory: linking memorization of temporal associations to GluN2B redistribution in dorsal CA1.
GluN2B subunits of NMDA receptors have been proposed as a target for treating age-related memory decline. They are indeed considered as crucial for hippocampal synaptic plasticity and hippocampus-dependent memory formation, which are both altered in aging. Because a synaptic enrichment in GluN2B is associated with hippocampal LTP in vitro, a similar mechanism is expected to occur during memory formation. We show instead that a reduction of GluN2B synaptic localization induced by a single-session learning in dorsal CA1 apical dendrites is predictive of efficient memorization of a temporal association. Furthermore, synaptic accumulation of GluN2B, rather than insufficient synaptic localization of these subunits, is causally involved in the age-related impairment of memory. These challenging data identify extra-synaptic redistribution of GluN2B-containing NMDAR induced by learning as a molecular signature of memory formation and indicate that modulating GluN2B synaptic localization might represent a useful therapeutic strategy in cognitive aging.
Aging Cell, 2020; 19
32839437: Al Abed AS, Ducourneau EG, Bouarab C, Sellami A, Marighetto A, Desmedt A
Preventing and treating PTSD-like memory by trauma contextualization.
Post-traumatic stress disorder (PTSD) is characterized by emotional hypermnesia on which preclinical studies focus so far. While this hypermnesia relates to salient traumatic cues, partial amnesia for the traumatic context can also be observed. Here, we show in mice that contextual amnesia is causally involved in PTSD-like memory formation, and that treating the amnesia by re-exposure to all trauma-related cues cures PTSD-like hypermnesia. These findings open a therapeutic perspective based on trauma contextualization and the underlying hippocampal mechanisms.
Nat Commun, 2020; 11
32521268: Robert BJA, Moreau MM, Dos Santos Carvalho S, Barthet G, Racca C, Bhouri M, Quiedeville A, Garret M, Atchama B, Al Abed AS, Guette C, Henderson DJ, Desmedt A, Mulle C, Marighetto A, Montcouquiol M, Sans N
Vangl2 in the Dentate Network Modulates Pattern Separation and Pattern Completion.
The organization of spatial information, including pattern completion and pattern separation processes, relies on the hippocampal circuits, yet the molecular and cellular mechanisms underlying these two processes are elusive. Here, we find that loss of Vangl2, a core PCP gene, results in opposite effects on pattern completion and pattern separation processes. Mechanistically, we show that Vangl2 loss maintains young postmitotic granule cells in an immature state, providing increased cellular input for pattern separation. The genetic ablation of Vangl2 disrupts granule cell morpho-functional maturation and further prevents CaMKII and GluA1 phosphorylation, disrupting the stabilization of AMPA receptors. As a functional consequence, LTP at lateral perforant path-GC synapses is impaired, leading to defects in pattern completion behavior. In conclusion, we show that Vangl2 exerts a bimodal regulation on young and mature GCs, and its disruption leads

to an imbalance in hippocampus-dependent pattern completion and separation processes.
Cell Rep, 2020; 31
28874586: Sellami A, Al Abed AS, Brayda-Bruno L, Etchamendy N, Valério S, Oulé M, Pantaléon L, Lamothe V, Potier M, Bernard K, Jabourian M, Herry C, Mons N, Piazza PV, Eichenbaum H, Marighetto A
Temporal binding function of dorsal CA1 is critical for declarative memory formation.
Temporal binding, the process that enables association between discontiguous stimuli in memory, and relational organization, a process that enables the flexibility of declarative memories, are both hippocampus-dependent and decline in aging. However, how these two processes are related in supporting declarative memory formation and how they are compromised in age-related memory loss remain hypothetical. We here identify a causal link between these two features of declarative memory: Temporal binding is a necessary condition for the relational organization of discontiguous events. We demonstrate that the formation of a relational memory is limited by the capability of temporal binding, which depends on dorsal (d)CA1 activity over time intervals and diminishes in aging. Conversely, relational representation is successful even in aged individuals when the demand on temporal binding is minimized, showing that relational/declarative memory per se is not impaired in aging. Thus, bridging temporal intervals by dCA1 activity is a critical foundation of relational representation, and a deterioration of this mechanism is responsible for the age-associated memory impairment.
Proc Natl Acad Sci U S A, 2017; 114
28730313: Lozan E, Shinkaruk S, Al Abed SA, Lamothe V, Potier M, Marighetto A, Schmitter JM, Bennetau-Pelissero C, Buré C
Derivatization-free LC-MS/MS method for estrogen quantification in mouse brain highlights a local metabolic regulation after oral versus subcutaneous administration.
17β-Estradiol (17β-E) is a steroid with pleiotropic actions. In addition to being a sexual hormone, it is also produced in the brain where it modulates the reproductive axis. It has been shown that 17β-E also acts on synaptic plasticity and plays a role in neurological pathways and in neurodegenerative diseases. Assaying this steroid in the brain is thus interesting to improve our knowledge of 17β-E effects in the brain. However, 17β-E concentration in the central nervous system has been reported to be of a few nanograms per gram wet weight (nanomolar range concentration); therefore, its quantification requires both an efficient extraction process and a sensitive detection method. Herein is presented a derivatization-free procedure based on solid-phase extraction followed by LC-MS/MS analysis, targeted on 17β-E, its isomer17α-E, and its metabolites estrone (E) and estriol (E). This extraction process allowed reaching 96% 17β-E recovery from the mouse brain. Limit of detection (LOD) and limit of quantification (LOQ) values of 0.5 and 2.5 pmol mL, respectively, were reached for both 17α-E and 17β-E. LOD values for E and E were 0.01 and 0.025 pmol mL, respectively. The variation coefficients for intra- and inter-assays were 6 and 14%, respectively, for both estradiol forms. The method was applied to assess estrogen levels in the mouse brain and hippocampus after 17β-E acute (subcutaneous injection) and chronic (drinking water) physiological administration. Total estrogen levels were determined after enzymatic deconjugation and compared to free estrogen levels. While 17α-E was not detected in biological samples, 17β-E and metabolite measurements highlight a local biotransformation of estrogens after physiological administration via drinking water. Graphical abstract Method workflow: After oral or subcutaneous Estradiol administration, mouse brain or hippocampus was removed. Samples were homogenized and prepared according to a liquid-liquid extraction, followed by a solid-phase extraction. Then, LC-MS/MS was optimized to quantify 17ß-E, its isomer17α-E, its metabolites estrone (E) and estriol (E) and their conjugates.
Anal Bioanal Chem, 2017; 409
27038677: Al Abed AS, Sellami A, Brayda-Bruno L, Lamothe V, Noguès X, Potier M, Bennetau-Pelissero C, Marighetto A
Estradiol enhances retention but not organization of hippocampus-dependent memory in intact male mice.
Because estrogens have mostly been studied in gonadectomized females, effects of chronic exposure to environmental estrogens in the general population are underestimated. Estrogens can enhance hippocampus-dependent memory through the modulation of information storage. However, declarative memory, the hippocampus-dependent memory of facts and events, demands more than abilities to retain information. Specifically, memory of repetitive events of everyday life such as "where I parked" requires abilities to organize/update memories to prevent proactive interference from similar memories of previous "parking events". Whether such organizational processes are estrogen-sensitive is unknown. We here studied, in intact young and aged adult mice, drinking-water (1µM) estradiol effects on both retention and organizational components of hippocampus-dependent memory, using a radial-maze task of everyday-like memory. Demand on retention vs organization was manipulated by varying the time-interval separating repetitions of similar events. Estradiol increased performance in young and aged mice under minimized organizational demand, but failed to improve the age-associated memory impairment and diminished performance in young mice under high organizational demand. In fact, estradiol prolonged mnemonic retention of successive events without improving organization abilities, hence resulted in more proactive interference from irrelevant memories. c-Fos imaging of testing-induced brain activations showed that the deterioration of young memory was associated with dentate gyrus dysconnectivity, reminiscent of that seen in aged mice. Our findings support the view that

estradiol is promnesic but also reveal that such property can paradoxically impair memory. These findings have important outcomes regarding health issues relative to the impact of environmental estrogens in the general population.
Psychoneuroendocrinology, 2016; 69
26321020: Potier M, Georges F, Brayda-Bruno L, Ladépêche L, Lamothe V, Al Abed AS, Groc L, Marighetto A
Temporal Memory and Its Enhancement by Estradiol Requires Surface Dynamics of Hippocampal CA1 N-Methyl-D-Aspartate Receptors.
Identifying the underlying cellular mechanisms of episodic memory is an important challenge, since this memory, based on temporal and contextual associations among events, undergoes preferential degradation in aging and various neuropsychiatric disorders. Memory storage of temporal and contextual associations is known to rely on hippocampal N-methyl-D-aspartate receptor (NMDAR)-dependent synaptic plasticity, which depends ex vivo on dynamic organization of surface NMDARs. Whether NMDAR surface trafficking sustains the formation of associative memory, however, remains unknown.
Biol Psychiatry, 2016; 79

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OSCILLATORY ABNORMALITIES AND SENSORY PROCESSING DEFICITS IN NRXN1 RAT AND CNTNAP2 MOUSE MODELS FOR NEURODEVELOPMENTAL DISORDERS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Neurexin 1 (NRXN1) and Contactin-associated protein-like 2 (CNTNAP2) belong to the family of neurexin genes, encoding for presynaptic cell adhesion proteins that are crucial for the proper development of neuronal circuits. While their genetic link to neurodevelopmental disorders is clear, there is limited understanding on how knock-outs of these genes alter complex circuit function. Therefore, we thoroughly investigated the electrophysiological endophenotypes of NRXN1a$^{-/-}$ rats and CNTNAP2$^{-/-}$ mice. Animals were implanted with surface ECoG and intracranial electrodes to study cortico-thalamic-striatal brain circuits. In addition to resting-state oscillations, we assessed auditory responses via chirp-evoked potentials or auditory steady-state responses and mismatch-negativity (MMN) paradigms. For both animal lines we found increased oscillatory power during resting-state conditions, mainly in gamma and higher frequency bands. Conversely, auditory-evoked oscillations appeared mainly altered in NRXN1a$^{-/-}$ rats, showing reduced chirp-evoked power in the theta band confined to cortical brain regions. Responses to simple tones were altered in a genotype-specific manner. Early peaks in the event-related potentials from CNTNAP2$^{-/-}$ showed increased power, but remained normal for NRXN1a$^{-/-}$ rats. Remarkably, in NRXN1a$^{-/-}$ rats the cortical event-related potentials displayed ectopic deflections at 70 ms after tone onset, indicative of abnormal cortical synaptic transmission. Finally, NRXN1a$^{-/-}$ rats showed strong attenuation of the prediction error and impaired adaptation during the MMN paradigm. In conclusion, both NRXN1a$^{-/-}$ rats and CNTNAP2$^{-/-}$ mice showed clear electrophysiological endophenotypes, which help to understand circuit dysfunction and may inform translational biomarkers and support drug development in neurodevelopmental disorders.

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BOARD NUMBER: S06-149

ANALYSIS OF HIPPOCAMPAL PARTICIPATION IN SOCIAL INTERACTIONS IN A GENETIC MODEL OF AUTISTIC SPECTRUM DISORDER

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

Pilar Rodríguez-Martín[1], Almudena Sanz[1], Elisa Cintado[1], Eva Monserrat[1], Inés Colmena[1], Cristina Medina Menéndez[1], Véronique Lefebvre[2], José Luis Trejo[1], Aixa V. Morales[1]
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Many neurodevelopmental disorders associated with deficiencies in social interaction, language difficulties and repetitive behaviours are grouped under the name of autism spectrum disorders (ASD). Although the genetic causes of ASD are complex, one of the genes that have been associated with ASD is *SOX5* (#616803, LAMB-SHAFFER SYNDROME). *Sox5* encodes a transcription factor with important functions in the control of neurogenesis (Li et al., 2022) and in the specification of projection neurons of the cerebral cortex. It has been described that the CA2 region of the hippocampus is fundamental in social behaviour in mice, a region where we have previously shown that Sox5 is expressed (Fernandez-Lamo et al., 2019). Using conditional *Sox5* mutant mice specific for the CA2 region (*Sox5*^{Amigo2}), we have determined that robust lack of Sox5 expression causes PCP4 level decrease in more than half of the pyramidal neurons in CA2. Using several behavioural assays we have determined that Sox5^{Amigo2} mutant mice: i) exhibit normal basic reflexes, weight, locomotion, and anxiety levels; ii) exhibit a good performance in Morris water maze test and iii) present normal social preference. However, males lose social recognition memory while females even show an abnormal preference for a familiar animal over a stranger in social memory tests. Thus, we propose that Sox5^{Amigo2} mice could provide a new model of ASD, based on cellular and functional alterations of the CA2 region of the hippocampus, which serves to understand the hippocampal component in the pathophysiology of ASD and for the testing of new therapeutic strategies.

Pubmed:
35108528: Li L, Medina-Menéndez C, García-Corzo L, Córdoba-Beldad CM, Quiroga AC, Calleja Barca E, Zinchuk V, Muñoz-López S, Rodríguez-Martín P, Ciorraga M, Colmena I, Fernández S, Vicario C, Nicolis SK, Lefebvre V, Mira H, Morales AV
SoxD genes are required for adult neural stem cell activation.
The adult neurogenic niche in the hippocampus is maintained through activation of reversibly quiescent neural stem cells (NSCs) with radial glia-like morphology (RGLs). Here, we show that the expression of SoxD transcription factors Sox5 and Sox6 is enriched in activated RGLs. Using inducible deletion of Sox5 or Sox6 in the adult mouse brain, we show that both genes are required for RGL activation and the generation of new neurons. Conversely, Sox5 overexpression in cultured NSCs interferes with entry in quiescence. Mechanistically, expression of the proneural protein Ascl1 (a key RGL regulator) is severely downregulated in SoxD-deficient RGLs, and Ascl1 transcription relies on conserved Sox motifs. Additionally, loss of Sox5 hinders the RGL activation driven by neurogenic stimuli such as environmental enrichment. Altogether, our data suggest that SoxD genes are key mediators in the transition of adult RGLs from quiescence to an activated mitotic state under physiological situations.
Cell Rep, 2022; 38

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BOARD NUMBER: S06-150

GENERATING A 16P11.2 MOUSE MODEL ON A MIXED GENETIC BACKGROUND STRENGTHENS SOCIAL DEFICITS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorders (ASD) affect more than one percent of the population, impairing social communication and increasing stereotyped behaviours. A micro-deletion of the 16p11.2 chromosomic region has been identified in one percent of ASD patients with intellectual disabilities. In mouse models generated to understand the mechanisms of this deletion, learning and memory difficulties were pervasive, while social communication deficits were scarcer and therefore more difficult to integrate when conducting pre-clinical therapeutic trials. Based on previous study (Arbogast et al. 2016 PLoS genetics), we hypothesized that using a mixed genetic background would increase the face validity of the model, especially in the social domain. We therefore itemized the social deficits displayed by a 16p11.2 mouse model on a hybrid C57BL/6NxC3B background. We recorded the spontaneous behaviours of male and female mice in mixed-genotype groups and in same-genotype female pairs freely moving in a home-cage-like environment. We identified robust social deficits across the different conditions, with mutant mice displaying shorter contacts with other mice, less specific contact types, and less social approaches compared to wild-type mice. In female pairs, these deficits were accompanied by a reduction of the emission of ultrasonic vocalisations, a shortening of their sequences and a modification of their acoustic features compared to wild-type pairs. Altogether, these results showed robust social deficits in a 16p11.2 mouse model on a hybrid background recapitulating more comprehensively the phenotype of patients. This model will therefore be a choice model for further identification of the neuronal circuits involved and pre-clinical targeted therapeutic trials.

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BOARD NUMBER: S06-151

SOMATOSENSORY PROCESSING DEFICITS AND ALTERED CORTICO-HIPPOCAMPAL CONNECTIVITY IN SHANK3B−/− MICE

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Abnormal tactile response is considered an integral feature of Autism Spectrum Disorders (ASDs), and hypo-responsiveness to tactile stimuli is often associated with the severity of ASDs core symptoms. Patients with Phelan-McDermid syndrome (PMS), caused by mutations in the SHANK3 gene, show ASD-like symptoms associated with aberrant tactile responses. However, the neural underpinnings of these somatosensory abnormalities are still poorly understood. Here we investigated, in Shank3b$^{-/-}$ adult mice, the neural substrates of whisker-guided behaviors, a key component of rodents' interaction with the surrounding environment. To this aim, we assessed whisker-dependent behaviors in Shank3b$^{-/-}$ adult mice and age-matched controls, using the textured novel object recognition (tNORT) and whisker nuisance (WN) test. Shank3b$^{-/-}$ mice showed deficits in whisker-dependent texture discrimination in tNORT and behavioral hyporesponsiveness to repetitive whisker stimulation in WN. Notably, sensory hypo-responsiveness was accompanied by a significantly reduced activation of the primary somatosensory cortex (S1) and hippocampus, as measured by c-fos mRNA in situ hybridization, a proxy of neuronal activity following whisker stimulation. Moreover, resting-state fMRI showed a significantly reduced S1- hippocampal connectivity in Shank3b mutant mice. Together, these findings suggest that impaired crosstalk between hippocampus and S1 might underlie Shank3b−/− hypo-reactivity to whisker dependent cues, highlighting a potentially generalizable form of dysfunctional somatosensory processing in ASD.

Pubmed:
34791077: Balasco L, Pagani M, Pangrazzi L, Chelini G, Ciancone Chama AG, Shlosman E, Mattioni L, Galbusera A, Iurilli G, Provenzano G, Gozzi A, Bozzi Y
Abnormal Whisker-Dependent Behaviors and Altered Cortico-Hippocampal Connectivity in Shank3b-/- Mice.
Abnormal tactile response is an integral feature of Autism Spectrum Disorders (ASDs), and hypo-responsiveness to tactile stimuli is often associated with the severity of ASDs core symptoms. Patients with Phelan-McDermid syndrome (PMS), caused by mutations in the SHANK3 gene, show ASD-like symptoms associated with aberrant tactile responses. The neural underpinnings of these abnormalities are still poorly understood. Here we investigated, in Shank3b-/- adult mice, the neural substrates of whisker-guided behaviors, a key component of rodents' interaction with the surrounding environment. We assessed whisker-dependent behaviors in Shank3b-/- adult mice and age-matched controls, using the textured novel object recognition (tNORT) and whisker nuisance (WN) test. Shank3b-/- mice showed deficits in whisker-dependent texture discrimination in tNORT and behavioral hypo-responsiveness to repetitive whisker stimulation in WN. Sensory hypo-responsiveness was accompanied by a significantly reduced activation of the primary somatosensory cortex (S1) and hippocampus, as measured by c-fos mRNA induction, a proxy of neuronal activity following whisker stimulation. Moreover, resting-state fMRI showed a significantly reduced S1-hippocampal connectivity in Shank3b mutants, in the absence of altered connectivity between S1 and other somatosensory areas. Impaired crosstalk between hippocampus and S1 might underlie Shank3b-/- hypo-reactivity to whisker-dependent cues, highlighting a potentially generalizable somatosensory dysfunction in ASD.
Cereb Cortex, 2021;
33256243: Pangrazzi L, Balasco L, Bozzi Y
Natural Antioxidants: A Novel Therapeutic Approach to Autism Spectrum Disorders?
Autism spectrum disorders (ASD) are a group of neurodevelopmental syndromes with both genetic and environmental origins. Several recent studies have shown that inflammation and oxidative stress may play a key role in supporting the pathogenesis and the severity of ASD. Thus, the administration of anti-inflammatory and antioxidant molecules may represent

a promising strategy to counteract pathological behaviors in ASD patients. In the current review, results from recent literature showing how natural antioxidants may be beneficial in the context of ASD will be discussed. Interestingly, many antioxidant molecules available in nature show anti-inflammatory activity. Thus, after introducing ASD and the role of the vitamin E/vitamin C/glutathione network in scavenging intracellular reactive oxygen species (ROS) and the impairments observed with ASD, we discuss the concept of functional food and nutraceutical compounds. Furthermore, the effects of well-known nutraceutical compounds on ASD individuals and animal models of ASD are summarized. Finally, the importance of nutraceutical compounds as support therapy useful in reducing the symptoms in autistic people is discussed.
Antioxidants (Basel), 2020; 9
33228213: Meryk A, Grasse M, Balasco L, Kapferer W, Grubeck-Loebenstein B, Pangrazzi L
Antioxidants N-Acetylcysteine and Vitamin C Improve T Cell Commitment to Memory and Long-Term Maintenance of Immunological Memory in Old Mice.
Aging is characterized by reduced immune responses, a process known as immunosenescence. Shortly after their generation, antigen-experienced adaptive immune cells, such as CD8 and CD4 T cells, migrate into the bone marrow (BM), in which they can be maintained for long periods of time within survival niches. Interestingly, we recently observed how oxidative stress may negatively support the maintenance of immunological memory in the BM in old age. To assess whether the generation and maintenance of immunological memory could be improved by scavenging oxygen radicals, we vaccinated 18-months (old) and 3-weeks (young) mice with alum-OVA, in the presence/absence of antioxidants vitamin C (Vc) and/or N-acetylcysteine (NAC). To monitor the phenotype of the immune cell population, blood was withdrawn at several time-points, and BM and spleen were harvested 91 days after the first alum-OVA dose. Only in old mice, memory T cell commitment was boosted with some antioxidant treatments. In addition, oxidative stress and the expression of pro-inflammatory molecules decreased in old mice. Finally, changes in the phenotype of dendritic cells, important regulators of T cell activation, were additionally observed. Taken together, our data show that the generation and maintenance of memory T cells in old age may be improved by targeting oxidative stress.
Antioxidants (Basel), 2020; 9
32384730: Pangrazzi L, Balasco L, Bozzi Y
Oxidative Stress and Immune System Dysfunction in Autism Spectrum Disorders.
Autism Spectrum Disorders (ASDs) represent a group of neurodevelopmental disorders associated with social and behavioral impairments. Although dysfunctions in several signaling pathways have been associated with ASDs, very few molecules have been identified as potentially effective drug targets in the clinic. Classically, research in the ASD field has focused on the characterization of pathways involved in neural development and synaptic plasticity, which support the pathogenesis of this group of diseases. More recently, immune system dysfunctions have been observed in ASD. In addition, high levels of reactive oxygen species (ROS), which cause oxidative stress, are present in ASD patients. In this review, we will describe the major alterations in the expression of genes coding for enzymes involved in the ROS scavenging system, in both ASD patients and ASD mouse models. In addition, we will discuss, in the context of the most recent literature, the possibility that oxidative stress, inflammation and immune system dysfunction may be connected to, and altogether support, the pathogenesis and/or severity of ASD. Finally, we will discuss the possibility of novel treatments aimed at counteracting the interplay between ROS and inflammation in people with ASD.
Int J Mol Sci, 2020; 21
32047448: Balasco L, Provenzano G, Bozzi Y
Sensory Abnormalities in Autism Spectrum Disorders: A Focus on the Tactile Domain, From Genetic Mouse Models to the Clinic.
Sensory abnormalities are commonly recognized as diagnostic criteria in autism spectrum disorder (ASD), as reported in the last edition of the Diagnostic and Statistical Manual of Mental Disorder (DSM-V). About 90% of ASD individuals have atypical sensory experiences, described as both hyper- and hypo-reactivity, with abnormal responses to tactile stimulation representing a very frequent finding. In this review, we will address the neurobiological bases of sensory processing in ASD, with a specific focus of tactile sensitivity. In the first part, we will review the most relevant sensory abnormalities detected in ASD, and then focus on tactile processing deficits through the discussion of recent clinical and experimental studies. In the search for the neurobiological bases of ASD, several mouse models have been generated with knockout and humanized knockin mutations in many ASD-associated genes. Here, we will therefore give a brief overview of the anatomical structure of the mouse somatosensory system, and describe the somatosensory abnormalities so far reported in different mouse models of ASD. Understanding the neurobiological bases of sensory processing in ASD mouse models may represent an opportunity for a better comprehension of the mechanisms underlying sensory abnormalities, and for the development of novel effective therapeutic strategies.
Front Psychiatry, 2019; 10
33654838: Balasco L, Chelini G, Bozzi Y, Provenzano G

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Whisker Nuisance Test: A Valuable Tool to Assess Tactile Hypersensitivity in Mice.

Abnormal response to tactile stimulation, described as both hyper- and hypo-reactivity, is a common sensory impairment in multiple neuropsychiatric disorders. The neural bases of tactile sensitivity remain so far unknown. In the last years, animal studies have proven to be useful for shedding light on the cellular and molecular mechanism underlying sensory impairments. However, few behavioral tests have been developed in mice for assessing tactile perception abnormalities (, the whisker nuisance [WN] test and the tactile prepulse inhibition assay). Here we provide a modified version of the WN test, which is based on the previously developed method by McNamara (2010). The WN test permits to specifically detect tactile hypo/hyper-sensitivity relative to whisker stimulation in mice. The test starts with a habituation phase in which the mouse familiarizes itself with the experimental cage and the researcher/experimenter. After a sham session, the experimental session begins, consisting of bilateral whisker stimulation with a wooden stick. The advantages of using this protocol are many: it is relatively simple to set with no particular or expensive equipment needed, it is easily reproducible, it allows researchers to assess a variety of behavioral responses to a whisker-specific tactile perception in mice (, fearful behavior, stance, hyperventilation, aggressive behavior and evasiveness) and provides important translational opportunities.
Bio Protoc, 2019; 9

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BOARD NUMBER: S06-152

PREFRONTAL CORTICAL CALRETININ INTERNEURONS INVOLVED IN AUTISM SPECTRUM DISORDER

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder, which is characterized by social deficits and repetitive, stereotypic behavior. A widely accepted hypothesis states that an imbalance of excitatory and inhibitory activity may play an important role in ASD. Our research group previously found the density of calretinin-immunopositive (CR+) neurons decreased in the caudate nucleus in ASD. Thus, we aimed to investigate the distribution of CR+ interneurons across the layers of the dorsolateral prefrontal cortex (DLPFC), Brodmann area 9, an area already proven to be affected in ASD. Formalin-fixed, paraffin-embedded tissue was requested from 13 ASD and 10 control brain samples from the Oxford Brain Bank. After immunohistochemical staining, slides were digitalized with a 3DHistech whole slide scanner. Immunopositive cells were annotated in Aperio ImageScope software after delineating cortical layers based on cytoarchitecture. For the evaluation of the data, linear mixed model and contrasts were used. According to our results the density of CR + interneurons was reduced by 20% in the in layer 2 of DLPFC of the ASD group (p=0,00036), suggesting that these cells may be affected in ASD. Interestingly, Velmeshev et al. (2019) identified VIP (CR+) neurons as one of the clusters with most abundant differentially expressed genes in the same brain region, in a larger cohort of ASD and CTR samples. These results inspire us to explore the cellular background of ASD further, by involving additional cell types and brain areas in forthcoming investigations and applying a multiscale research approach.

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BOARD NUMBER: S06-153

MGLU4 MODULATION OF THALAMO-AMYGDALA SYNAPTIC TRANSMISSION AND RELATION TO AUTISM SPECTRUM DISORDERS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

Julien Genty[1], Francine Acher[2], Cyril Goudet[3], Ingrid Ehrlich[4]
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Autism spectrum disorders (ASD) are neurodevelopmental conditions inducing social behavior impairments and stereotypies. Their neurobiology remains elusive, however activation of metabotropic glutamate receptor 4 (mGlu4) alleviates autistic symptoms in an ASD mouse model. Furthermore, mGlu4 modulates amygdala activity, a hub for processing of emotions and hypothesized to be involved in stereotypic and social behaviors. Here, we investigate connectivity and mGlu4 modulation of different thalamic regions to the basolateral amygdala (BLA) and intercalated cell clusters that may be relevant for ASD. We focused on the MGm/PIN and the paraventricular nucleus of the thalamus (PVT) and elucidated distinct innervation patterns from Calbindin-positive neurons in these regions to the amygdala. Subsequently, we confirmed functional connectivity employing an ex vivo optogenetic approach expressing Channelrhodopsin2 in thalamic regions while monitoring synaptic transmission in the amygdala in the absence and presence of a specific mGlu4 agonist (LSP4-2022). LSP4-2022 decreased glutamatergic transmission and increased paired pulse ratio (PPR), indicating a presynaptic depression. Modulation was found for MGm/PIN inputs to lateral amygdala principal neurons and to dorso-medial and lateral intercalated cell (dmITC and lITCs) clusters. PVT inputs innervated multiple ITCs clusters, including ventro-medial ITCs (vmITCs). Similarly to Mgm/PIN inputs, PVT inputs to dmITCs, lITCs, and vmITCs clusters were also presynaptically modulated by mGlu4. Overall, these thalamic nuclei project onto different amygdala sub-regions, overlapping only at distinct ITC clusters. Thalamic mGlu4-sensitive inputs onto ITCs, could be of great importance considering ITCs´ tightly interconnected network and involvement in emotional regulation.

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IDENTIFYING ENVIRONMENT-DEPENDENT BEHAVIORAL DOMAINS PREDICTIVE OF AUTISM-LIKE PHENOTYPE

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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[1]Erasmus Medical Center, Neuroscience, Rotterdam, Netherlands, [2]University College Dublin, School Of Medicine & Medical Science, Dublin, Ireland, [3]Erasmus MC, Cell Biology & Molecular Genetics, Rotterdam, Netherlands

Genetic and environmental factors alike can contribute to the development of Autism Spectrum Disorder (ASD), making the studies on the underlying neural substrates highly complex. In our study, we aimed to develop a "behavioural fingerprint" for the *Shank2*$^{-/-}$ ASD mouse model raised in standard and enriched environments. To this end we employed a wide range of both discrete, standardized tests such as: elevated-plus-maze, social chamber, marble burying test, Y-maze assay, and open field test; as well as two multi-parametric behavioural assays: the Live Mouse Tracker (described in de Chaumont et al., 2019) and Motion Sequencing (MoSeq) developed by the Datta Lab. Our aim was to integrate our high dimensional data into one single platform, to classify differences in experimental groups along dimensions with maximum consistency and discriminative power. We have found that some behavioral phenotypes of *Shank2*$^{-/-}$ mice, such as impaired burying behavior and behavioral flexibility, as well as hyperactivity and decreased anxiety-like behavior, were consistently altered whereas other features were environment-dependent. Groups raised in enriched housing showed a distinct effect on the behavioral phenotype in both the discrete and multi-parametric assays. *Shank2*$^{-/-}$ mice showed a unique phenotypic profile based on housing conditions using both the Live Mouse tracker and the MoSeq analysis. The described identity domains captured variability in *Shank2*$^{-/-}$ mice reared in different housing conditions, suggesting the feasibility of reducing behavioral test batteries to multi-parametric assays. Together, our results provide a "behavioural fingerprint" of *Shank2*$^{-/-}$ mice, which is shown to be altered by different housing conditions during development.

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REDUCED SOCIAL INTERACTION IN TWO RAT MODELS OF SYNGAP1 HAPLOINSUFFICIENCY

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

Vanesa Salazar-Sanchez, Thomas Watson, Emma Wood, Peter Kind
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SYNGAP1 haploinsufficiency is one of the most common monogenetic causes of intellectual disability (ID) and autism spectrum disorder (ASD). Some major features are seizures, social behaviour, and communication impairments. The SYNGAP protein is a major component of the postsynaptic density (Kim et al.1998). SYNGAP can function as both a scaffolding protein (Walkup et al, 2016) and a regulator of key enzymatic pathways, including ERK and P38MAPKs, via its GTPase activating protein (GAP) domain (Araki et al, 2015). Aim: To understand the role of SYNGAP in regulating social interaction dynamics and to identify the role of the GAP domain in this process. Methods: A conspecific habituation-dishabituation task was developed to assess social interactions. Furthermore, two olfaction tasks were performed to test impairments in detecting non-social and social odours. To distinguish between scaffolding and enzymatic function in social behaviour, two heterozygous rat models were used: one with a GAP-domain deletion (*Syngap*$^{+/\Delta GAP}$) and one with a null mutation (*Syngap*$^{+/-}$). Results: Both *Syngap*$^{+/\Delta GAP}$ and *Syngap*$^{+/-}$ rats demonstrate reduced social exploration with conspecifics compared to wildtype littermates; however, both lines habituate and discriminate. Furthermore, wildtype conspecific, “stimuli” rats show similar social exploration time regardless of the genotype of the paired animal. While both models can detect social and non-social odours, they do display decreased sniffing time in these olfaction tests. Conclusion: These findings suggest that while the enzymatic function of SYNGAP plays a crucial role in general interest in social interaction, SYNGAP does not appear to regulate the ability of animals to recognize novel conspecifics.

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UNDERSTANDING THE CAUSES AND CONSEQUENCES OF INDIVIDUAL VARIATION IN STRIATAL OXTR EXPRESSION IN PRAIRIE VOLES: A MOLECULAR PHENOTYPE RELEVANT TO ASD

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Natural variation in oxytocin receptor (Oxtr) expression increases diversity in social behaviors within and across species. In the socially monogamous prairie vole (Microtus ochrogaster), variation in Oxtr expression in the nucleus accumbens (NAc) is associated with variation in pair bonding, alloparental behavior and resilience to neonatal social neglect. Previously, we found a set of intronic SNPs that largely explain individual variation of Oxtr expression, specifically in the NAc ($r^2>0.7$). We are using ATAC-seq, bulk and single nucleus RNA-seq (scRNA-seq) and nanopore-seq to investigate the mechanistic links between Oxtr SNPs and altered Oxtr gene expression in the NAc, as well as its downstream molecular consequences. RNA-seq revealed differential expression low- and high NAC Oxtr expressing genotypes, in both bulk RNA and single nucleus RNA sequencing data, with Oxtr as a top hit ($q<1x10-7$). The differential expressed gene sets are highly enriched for autism spectrum disorders genes (SFARI 2.0 database, $p<0.0001$), and are largest in astrocytes and oligodendrocytes; cell-types that express no to very little Oxtr. In parallel, we developed an AAV-CRISPR/Cas9 approach that disrupts the Oxtr coding sequence by introducing genetic edits, thereby significantly reducing OXTR binding (>80%). This tools allows for the investigation OXTR signaling-dependent transcriptome changes. By combining CRISPR/Cas9 and sequencing strategies, this project aims to identify and disrupt candidate regulatory regions that contribute to diversity in Oxtr expression and provide mechanistic insight into how individual variation in Oxtr expression shapes diversity in brain function and social behaviors.

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BOARD NUMBER: S06-157

MICE MODELS AND AUTISM SPECTRUM DISORDERS : THE EXAMPLE OF THE SHANK3Δ11/Δ 11 MOUSE.

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorders (ASD) are characterized by atypical social communication and stereotyped behaviors. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are detected in 1-2% of patients with ASD and intellectual disability, but the mechanisms underpinning the symptoms remain largely unknown. To understand the potential mechanisms involved in the behavioral deficits present in ASD with *SHANK3* mutations, we performed a multi-scale characterization of the *Shank3* mutant mice deleted for exon 11 *(Shank3*$^{\Delta 11/\Delta 11}$ mice) that comprises: -A longitudinal study of the behavior of *Shank3*$^{\Delta 11/\Delta 11}$ mice by using the Live Mouse Tracker. -A transcriptomic analysis of gene expression in four brain areas and an analysis of genes/proteins of interest by quantitative RT-PCR and immunostaining. -A study of functional cerebral connectivity with functional ultrasound imaging (fUS). The behavioral analysis revealed decreased locomotor activity, increased stereotyped self-grooming and atypical socio-sexual interaction in *Shank3*$^{\Delta 11/\Delta 11}$ mice compared to wild-type littermates. The transcriptomic analysis, performed on the cortex, hippocampus, striatum and cerebellum, pointed to the striatum as the brain region the most impacted by the *Shank3*$^{\Delta 11}$ deletion. In addition, our results suggest an imbalance between the striosome and matrix compartments of the striatum in *Shank3*$^{\Delta 11/\Delta 11}$ mice. Finally, we did not observe significant alterations in functional connectivity in the brain of *Shank3*$^{\Delta 11/\Delta 11}$ mice at resting state, but we are now investigating it during specific behaviors. Overall, these studies allow a better understanding of the mechanisms involved in ASD with *SHANK3* mutations and may lead to potential new pharmacological treatments for this disorder.

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SEX-DEPENDENT BEHAVIORAL DEFICITS AND NEUROPATHOLOGY IN GENETIC AND ENVIRONMENTAL MOUSE MODELS OF AUTISM SPECTRUM DISORDER

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

Mohamed Jaber, Alexandre Maisterrena, Obelia Haida, Tareq Al Sagheer, Eric Balado, Anais Balbous-Gauthier, Maureen Francheteau, Emmanuel Matas
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Autism spectrum disorders (ASD) is a neurodevelopmental disease, difficult to diagnose and with no curative treatment. A wide range of symptoms have been identified in ASD patients. Among them, motor and gait disorders are often observed but are not fully included within the diagnosis criteria. Determination of cellular disturbances in brain regions underlying motor dysfunction (cerebellum, nigro-striatal pathway and motor cortex) may lead to propose quantitative and earlier diagnosis and potentially help develop new therapeutic approaches. In our study, we aimed at investigating whether various ASD mouse models manifest motor impairments and we strived to determine the neuronal network involved in these deficits. Secondly, we were interested in identifying differences between males and females as the sex ratio in ASD is 3 boys for 1 girl. We used both environmental (valproic acid and Poly IC) and transgenic (Shank3) mouse models to characterize different aspects of their behavior and we determined the neurohistological readouts underlying deficits. Our results demonstrate a decrease of the sociability, fine motor disorders, gait deficits and neuronal loss in ASD mice models. These deficits were present in a sex and model-specific manner. Additionally, a correlation analysis revealed relationships among motor difficulties, low social interactions and decreased number of Purkinje neurons within the cerebellum. This is of importance as it points out that motor disorders in ASD could be used as an early marker of severity of the disease and that the cerebellum could be a targeted for therapeutic strategies.

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STRIATAL DYSFUNCTIONS WITH AGING IN SHANK3 KO MOUSE MODEL OF AUTISM SPECTRUM DISORDERS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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The autism spectrum disorder (ASD) is a non-lethal neurodevelopmental disorder-giving rise to cognitive, communication and affective symptoms, affecting 700 000 patients in France alone. ASD is highly investigated at the neurodevelopmental and juvenile stages and the ageing process in patients is described as an increase in comorbidities leading to a “frail” state over time. In fact, recent studies suggest that adult ASD patients undergo “premature ageing” as they present a significant number of comorbidities very early on in life. The current focus on ASD in development and early life leaves out of sight ASD adults by assuming the persistence of the initial dysfunction documented in children. Seen the time course of appearance of neuronal dysfunction, we hypothesize that the ASD brain undergoes premature ageing in an active state that does not result from developmental phenomena but is an additional ASD symptom focusing on the striatum. Thus, most of animal studies focused on glutamatergic alterations in ASD, leading to lack of knowledge about the GABAergic transmission. The work presented here aims to investigate the evolution of the motor symptoms in Shank3$^{\Delta C/\Delta C}$ mouse model of ASD and of the disturbances of the GABAergic transmission with aging. To assess such disturbances, we used behavioral tests, *ex vivo* electrophysiology, and confocal microscopy. Preliminary results show differences in aging processes regarding striatal MSNs among genotypes.

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ANALYSIS OF COMPLEX SOCIAL BEHAVIOUR DURING AN EXTENDED TIME PERIOD IN A VALPROIC ACID ANIMAL MODEL OF AUTISM SPECTRUM DISORDER

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism Spectrum Disorder (ASD) is a progressive neurodevelopmental disorder characterized mainly by deficits in social communication and stereotyped behaviours and interests. One of the recurrent findings in several ASD animal models are deficits in social interactions. This is generally performed using the three chamber's test paradigm that is simple to implement and use but fails to detect complex social behavior on an extended period of time. Here, we set up a novel procedure entitled the Live Mouse Tracker (LMT) that detects several dozens of complex social behavior during three days in groups of 4 mice. For this we used a well characterised environmental animal model for ASD with valproic acid (VPA) injected to pregnant females at E12.5. Studies were performed on male and females offspring. Comparisons were made within groups of 4 animals with same sex and treatment and within groups of 4 animals with same sex but different treatments (saline versus VPA). We found that VPA mice showed major social deficits and that are different in nature and magnitude in relation with time (from 1 hour to 3 days) and the time period (day versus night). Social deficits were also different when VPA mice were tested together compared to when they are mixed with saline treated mice. This study points out to severe and various impact of VPA treatment on complex social behavior and stresses the need to explore more in depth this behavior in ASD animal models in relation with sex, time period, time window and group composition.

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AUDITORY BRAINSTEM RESPONSES IN THE NRXN1 RAT AND CNTNAP2 MOUSE MODELS FOR NEURODEVELOPMENTAL DISORDERS: A LONGITUDINAL AND PHARMACOLOGICAL STUDY

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Sensory processing abnormalities are core to neurodevelopmental disorders (NDD). In fact, auditory hypersensitivity affects up to 66% of children with autism spectrum disorders (ASDs). Developing efficient therapies require understanding the basic sensory pathways and finding the atypical alterations of the circuit during early development. We used auditory brainstem responses (ABRs) to detect the physiological response to auditory stimuli in two different rodent models of NDD; Nrxn1KO rats and Cntnap2KO mice. ABRs are valuable tools in detecting hearing thresholds and other biomarkers of auditory sensory processing. ABRs are highly translational due to the similarity of signal readout with humans. We performed a longitudinal ABR study, focusing on the different developmental stages of rodents (juvenile, adolescence and adult). Additionally, we tested several pharmacological compounds, which are highly relevant for auditory sensory gating, processing and filtering. We confirmed that ABR's waves increase in amplitude and decrease in latency upon brainstem maturation. In both rodent models, there were significant changes in signal latency and differences with controls. Such differences were detected at multiple ages. The pharmacological tests altered the ABR wave's peaks in a genotype-specific manner. Our results emphasize the role of ABR as a valuable biomarker in ASD and increase our understanding of gene manipulations linked to NDD.

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BOARD NUMBER: S06-162

UNRAVELING THE EFFECT OF ARC DELETION ON VARIOUS BEHAVIORS LINKED TO THE NEUROPSYCHIATRIC DISORDERS IN MICE

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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The neuronal immediate-early gene Arc is a master regulator of different forms of synaptic plasticity in mammals, such as long-term potentiation, depression, and memory consolidation. It is known to be implied in numerous neuropsychiatric disorders, such as schizophrenia, and autism spectrum disorders (ASD). Even though it has been evident that Arc plays an important role in memory, it still has not been investigated whether Arc acts on behaviors impaired in neuropsychiatric disorders. To address this issue, herein we performed broad behavioral phenotyping of *Arc*$^{-/-}$ mice in comparison to wild-type animals in C57BL/6J and mixed C57BL/6J;129S2 backgrounds, to exclude background-specific effects on behavior. We evaluated the implication of *Arc* deletion in different types of behavior impaired in neuropsychiatric disorders, such as social, stereotyped, and anxious-like behaviors, and finally memory tasks. Our results revealed autistic-like behavioral patterns in *Arc*$^{-/-}$ mice associated with other behavioral alterations. In addition to that, we discovered profound effects of the two different genetic backgrounds and sex in these different behavior tests, especially in *Arc*$^{-/-}$ mice. Altogether, we determined the role of Arc in different types of behavior and discovered that its dysregulation may result in severe behavioral impairments, some of which are reported in neuropsychiatric disorders.

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CANNABIDIOL RESCUES AUTISTIC-LIKE SYMPTOMS IN A GENETIC MODEL OF AUTISM BASED ON FMR1 DELETION IN RATS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Among the genetic causes of autism spectrum disorder (ASD), Fragile X syndrome (FXS) is the most common known inherited single-gene disorder with no specific treatment available. Recently, the use of cannabinoid compounds, especially cannabidiol (CBD), an abundant bioactive but non-psychotomimetic constituent of *Cannabis sativa*, has received increasing attention as treatment for the core symptoms and co-morbidities of ASD. Beyond anecdotal reports, however, there is limited current evidence supporting such an intervention and the use of CBD remains controversial. In the present work, we tested the effect of CBD in the autistic-like features displayed by the recently validated *Fmr1-$^{\Delta}$exon 8* rat model of ASD. Wild-type (WT) and *Fmr1-$^{\Delta}$exon 8* male rats on a Sprague-Dawley background were used. We assessed the behavioral effects of systemic administration of CBD in these animals through the novel object recognition, three-chamber and social discrimination tests from adolescence to adulthood and explored the underlying mechanisms. Our results showed that CBD rescued the cognitive and social dysfunctions displayed by *Fmr1-$^{\Delta}$exon 8* animals. Interestingly, intra-hippocampal blockade of the GPR55 receptors prevented the beneficial effect of systemic CBD in the impaired cognitive abilities of *Fmr1-$^{\Delta}$exon 8* rats, thus suggesting that the effects of CBD are mediated, at least in part, by antagonism of the lipid-activated G protein-coupled receptor GPR55 in the hippocampus. These findings demonstrated that CBD reduced autistic-like traits in a genetic model of autism based on FMR1 deletion in rats and provide initial mechanistic insights into its therapeutic actions.

Pubmed:
33358985: Carbone E, Manduca A, Cacchione C, Vicari S, Trezza V
Healing autism spectrum disorder with cannabinoids: a neuroinflammatory story.
Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with a multifactorial etiology. Latest researches are raising the hypothesis of a link between the onset of the main behavioral symptoms of ASD and the chronic neuroinflammatory condition of the autistic brain; increasing evidence of this connection is shedding light on new possible players in the pathogenesis of ASD. The endocannabinoid system (ECS) has a key role in neurodevelopment as well as in normal inflammatory responses and it is not surprising that many preclinical and clinical studies account for alterations of the endocannabinoid signaling in ASD. These findings lay the foundation for a better understanding of the neurochemical mechanisms underlying ASD and for new therapeutic attempts aimed at exploiting the renowned anti-inflammatory properties of cannabinoids to treat pathologies encompassed in the autistic spectrum. This review discusses the current preclinical and clinical evidence supporting a key role of the ECS in the neuroinflammatory state that characterizes ASD, providing hints to identify new biomarkers in ASD and promising therapies for the future.
Neurosci Biobehav Rev, 2021; 121
30201092: Bara A, Manduca A, Bernabeu A, Borsoi M, Serviado M, Lassalle O, Murphy M, Wager-Miller J, Mackie K, Pelissier-Alicot AL, Trezza V, Manzoni OJ
Sex-dependent effects of in utero cannabinoid exposure on cortical function.
Cannabinoids can cross the placenta, thus may interfere with fetal endocannabinoid signaling during neurodevelopment, causing long-lasting deficits. Despite increasing reports of cannabis consumption during pregnancy, the protracted consequences of prenatal cannabinoid exposure (PCE) remain incompletely understood. Here, we report sex-specific differences in behavioral and neuronal deficits in the adult progeny of rat dams exposed to low doses of cannabinoids during gestation. In males, PCE reduced social interaction, ablated endocannabinoid long-term depression (LTD) and heightened excitability of prefrontal cortex pyramidal neurons, while females were spared. Group 1 mGluR and endocannabinoid signaling regulate emotional behavior and synaptic plasticity. Notably, sex-differences following PCE included levels of mGluR1/5 and TRPV1R mRNA. Finally, positive allosteric modulation of mGlu5 and enhancement of anandamide levels

restored LTD and social interaction in PCE adult males. Together, these results highlight marked sexual differences in the effects of PCE and introduce strategies for reversing detrimental effects of PCE.
Elife, 2018; 7
31658362: Manduca A, Servadio M, Melancia F, Schiavi S, Manzoni OJ, Trezza V
Sex-specific behavioural deficits induced at early life by prenatal exposure to the cannabinoid receptor agonist WIN55, 212-2 depend on mGlu5 receptor signalling.
Marijuana is the illicit drug most commonly used among pregnant and breastfeeding women. Different studies reported long-term adverse effects induced by in utero exposure to the main component of marijuana, Δ -tetrahydrocannabinol (THC), both in rodents and in humans. However, little is known about any potential sex-dependent effects of marijuana consumption during pregnancy on newborns at early developmental ages.
Br J Pharmacol, 2020; 177
26860202: Manduca A, Servadio M, Damsteegt R, Campolongo P, Vanderschuren LJ, Trezza V
Dopaminergic Neurotransmission in the Nucleus Accumbens Modulates Social Play Behavior in Rats.
Social play behavior is a highly rewarding form of social interaction displayed by young mammals. Social play is important for neurobehavioral development and it has been found to be impaired in several developmental psychiatric disorders. In line with the rewarding properties of social play, we have previously identified the nucleus accumbens (NAc) as an important site of action for endocannabinoid and opioid modulation of this behavior. NAc dopamine has a well-known role in certain components of reward processes, such as incentive motivation. However, its contribution to the positive emotional aspects of social interactions is less clear. Therefore, we investigated the role of dopaminergic neurotransmission in the NAc in social play behavior in rats. We found that intra-NAc infusion of the dopamine releaser/reuptake inhibitor amphetamine increased social play behavior that was dependent on activation of both D1 and D2 dopamine receptors. This increase in social play behavior was mimicked by intra-NAc infusion of the dopamine receptor agonist apomorphine, but not of the dopamine reuptake inhibitor GBR-12909. Blockade of either D1 or D2 NAc dopamine receptors reduced social play in animals highly motivated to play as a result of longer social isolation before testing. Last, blockade of NAc dopamine receptors prevented the play-enhancing effects of endocannabinoid and opioid receptor stimulation. These findings demonstrate an important modulatory role of NAc dopaminergic neurotransmission in social play. Thus, functional activity in the mesolimbic dopamine pathway plays an important role in adaptive social development, whereas abnormal NAc dopamine function may underlie the social impairments observed in developmental psychiatric disorders such as autism, attention deficit hyperactivity disorder or early-onset schizophrenia.
Neuropsychopharmacology, 2016; 41
23100412: Trezza V, Damsteegt R, Manduca A, Petrosino S, Van Kerkhof LW, Pasterkamp RJ, Zhou Y, Campolongo P, Cuomo V, Di Marzo V, Vanderschuren LJ
Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats.
The brain endocannabinoid system plays a crucial role in emotional processes. We have previously identified an important role for endocannabinoids in social play behavior, a highly rewarding form of social interaction in adolescent rats. Here, we tested the hypothesis that endocannabinoid modulation of social play behavior occurs in brain regions implicated in emotion and motivation. Social play increased levels of the endocannabinoid anandamide in the amygdala and nucleus accumbens (NAc), but not in prefrontal cortex or hippocampus of 4- to 5-week-old male Wistar rats. Furthermore, social play increased phosphorylation of CB1 cannabinoid receptors in the amygdala. Systemic administration of the anandamide hydrolysis inhibitor URB597 increased social play behavior, and augmented the associated elevation in anandamide levels in the amygdala, but not the NAc. Infusion of URB597 into the basolateral amygdala (BLA) increased social play behavior, and blockade of BLA CB1 cannabinoid receptors with the antagonist/inverse agonist SR141716A prevented the play-enhancing effects of systemic administration of URB597. Infusion of URB597 into the NAc also increased social play, but blockade of NAc CB1 cannabinoid receptors did not antagonize the play-enhancing effects of systemic URB597 treatment. Last, SR141716A did not affect social play after infusion into the core and shell subregions of the NAc, while it reduced social play when infused into the BLA. These data show that increased anandamide signaling in the amygdala and NAc augments social play, and identify the BLA as a prominent site of action for endocannabinoids to modulate the rewarding properties of social interactions in adolescent rats.
J Neurosci, 2012; 32
28630250: Manduca A, Bara A, Larrieu T, Lassalle O, Joffre C, Layé S, Manzoni OJ
Amplification of mGlu-Endocannabinoid Signaling Rescues Behavioral and Synaptic Deficits in a Mouse Model of Adolescent and Adult Dietary Polyunsaturated Fatty Acid Imbalance.
Energy-dense, yet nutritionally poor food is a high-risk factor for mental health disorders. This is of particular concern during adolescence, a period often associated with increased consumption of low nutritional content food and higher prevalence of mental health disorders. Indeed, there is an urgent need to understand the mechanisms linking unhealthy diet and mental

disorders. Deficiency in n-3 polyunsaturated fatty acids (PUFAs) is a hallmark of poor nutrition and mood disorders. Here, we developed a mouse model of n-3 PUFA deficiency lasting from adolescence into adulthood. Starting nutritional deficits in dietary n-3 PUFAs during adolescence decreased n-3 PUFAs in both medial prefrontal cortex (mPFC) and nucleus accumbens, increased anxiety-like behavior, and decreased cognitive function in adulthood. Importantly, we discovered that endocannabinoid/mGlu-mediated LTD in the mPFC and accumbens was abolished in adult n-3-deficient mice. Additionally, mPFC NMDAR-dependent LTP was also lacking in the n-3-deficient group. Pharmacological enhancement of the mGlu/eCB signaling complex, by positive allosteric modulation of mGlu or inhibition of endocannabinoid 2-arachidonylglycerol degradation, fully restored synaptic plasticity and normalized emotional and cognitive behaviors in malnourished adult mice. Our data support a model where nutrition is a key environmental factor influencing the working synaptic range into adulthood, long after the end of the perinatal period. These findings have important implications for the identification of nutritional risk factors for disease and design of new treatments for the behavioral deficits associated with nutritional n-3 PUFA deficiency. In a mouse model mimicking n-3 PUFA dietary deficiency during adolescence and adulthood, we found strong increases in anxiety and anhedonia which lead to decreases in specific cognitive functions in adulthood. We found that endocannabinoid/mGlu-mediated LTD and NMDAR-dependent LTP were lacking in adult n-3-deficient mice. Acute positive allosteric modulation of mGlu or inhibition of endocannabinoid degradation normalized behaviors and synaptic functions in n-3 PUFA-deficient adult mice. These findings have important implications for the identification of nutritional risk for disease and the design of new treatments for the behavioral deficits associated with nutritional n-3 PUFAs' imbalance.
J Neurosci, 2017; 37

27899885: Manduca A, Lassalle O, Sepers M, Campolongo P, Cuomo V, Marsicano G, Kieffer B, Vanderschuren LJ, Trezza V, Manzoni OJ

Interacting Cannabinoid and Opioid Receptors in the Nucleus Accumbens Core Control Adolescent Social Play.
Social play behavior is a highly rewarding, developmentally important form of social interaction in young mammals. However, its neurobiological underpinnings remain incompletely understood. Previous work has suggested that opioid and endocannabinoid neurotransmission interact in the modulation of social play. Therefore, we combined behavioral, pharmacological, electrophysiological, and genetic approaches to elucidate the role of the endocannabinoid 2-arachidonoylglycerol (2-AG) in social play, and how cannabinoid and opioid neurotransmission interact to control social behavior in adolescent rodents. Systemic administration of the 2-AG hydrolysis inhibitor JZL184 or the opioid receptor agonist morphine increased social play behavior in adolescent rats. These effects were blocked by systemic pretreatment with either CB1 cannabinoid receptor (CB1R) or mu-opioid receptor (MOR) antagonists. The social play-enhancing effects of systemic morphine or JZL184 treatment were also prevented by direct infusion of the CB1R antagonist SR141716 and the MOR antagonist naloxone into the nucleus accumbens core (NAcC). Searching for synaptic correlates of these effects in adolescent NAcC excitatory synapses, we observed that CB1R antagonism blocked the effect of the MOR agonist DAMGO and, conversely, that naloxone reduced the effect of a cannabinoid agonist. These results were recapitulated in mice, and completely abolished in CB1R and MOR knockout mice, suggesting that the functional interaction between CB1R and MOR in the NAcC in the modulation of social behavior is widespread in rodents. The data shed new light on the mechanism by which endocannabinoid lipids and opioid peptides interact to orchestrate rodent socioemotional behaviors.
Front Behav Neurosci, 2016; 10

24933531: Manduca A, Servadio M, Campolongo P, Palmery M, Trabace L, Vanderschuren LJ, Cuomo V, Trezza V

Strain- and context-dependent effects of the anandamide hydrolysis inhibitor URB597 on social behavior in rats.
Genetic and environmental factors play an important role in the cannabinoid modulation of motivation and emotion. Therefore, the aim of the present study was to test whether anandamide modulation of social behavior is strain- and context-dependent. We tested the effects of the anandamide hydrolysis inhibitor URB597 on social behavior and 50-kHz ultrasonic vocalizations (USVs) in adolescent and adult Wistar and Sprague-Dawley rats tested in different emotionally arousing conditions (familiarity/unfamiliarity to the test cage, low/high light). Under all experimental conditions, adolescent and adult Sprague-Dawley rats displayed higher levels of social behavior and emitted more 50-kHz USVs than Wistar rats. URB597 enhanced social play behavior in adolescent Wistar rats under all experimental conditions. However, URB597 only increased social interaction in adult Wistar rats under unfamiliar/high light conditions. URB597 did not affect adolescent social play behavior and adult social interaction in Sprague-Dawley rats under any experimental condition. Moreover, URB597 increased the USVs emitted during social interaction by adolescent Wistar and adult Sprague-Dawley rats tested under familiar/high light and unfamiliar/high light, respectively. These results show that anandamide has distinct roles in adolescent and adult social behaviors. Anandamide modulation of adolescent social play behavior is strain- but not context-dependent. Conversely, anandamide modulation of adult social behavior and USV emission depends upon both strain and experimental context. Furthermore, these results confirm that profound behavioral differences exist between Wistar and Sprague-Dawley rats, which may explain the sometimes contradictory effects of cannabinoid drugs on emotionality in different strains of rodents.

Eur Neuropsychopharmacol, 2014; 24
24221828: Manduca A, Campolongo P, Palmery M, Vanderschuren LJ, Cuomo V, Trezza V
Social play behavior, ultrasonic vocalizations and their modulation by morphine and amphetamine in Wistar and Sprague-Dawley rats.
Social play behavior is the most characteristic social behavior in young mammals. It is highly rewarding and crucial for proper neurobehavioral development. Despite the importance of genetic factors in normal and pathological social behaviors, little information is available about strain influences on social play.
Psychopharmacology (Berl), 2014; 231
25914159: Manduca A, Morena M, Campolongo P, Servadio M, Palmery M, Trabace L, Hill MN, Vanderschuren LJ, Cuomo V, Trezza V
Distinct roles of the endocannabinoids anandamide and 2-arachidonoylglycerol in social behavior and emotionality at different developmental ages in rats.
To date, our understanding of the relative contribution and potential overlapping roles of the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in the regulation of brain function and behavior is still limited. To address this issue, we investigated the effects of systemic administration of JZL195, that simultaneously increases AEA and 2-AG signaling by inhibiting their hydrolysis, in the regulation of socio-emotional behavior in adolescent and adult rats. JZL195, administered at the dose of 0.01mg/kg, increased social play behavior, that is the most characteristic social activity displayed by adolescent rats, and increased social interaction in adult animals. At both ages, these behavioral effects were antagonized by the CB1 cannabinoid receptor antagonist SR141716A and were associated with increased brain levels of 2-AG, but not AEA. Conversely, at the dose of 1mg/kg, JZL195 decreased general social exploration in adolescent rats without affecting social play behavior, and induced anxiogenic-like effects in the elevated plus-maze test both in adolescent and adult animals. These effects, mediated by activation of CB1 cannabinoid receptors, were paralleled by simultaneous increase in AEA and 2-AG levels in adolescent rats, and by an increase of only 2-AG levels in adult animals. These findings provide the first evidence for a role of 2-AG in social behavior, highlight the different contributions of AEA and 2-AG in the modulation of emotionality at different developmental ages and suggest that pharmacological inhibition of AEA and 2-AG hydrolysis is a useful approach to investigate the role of these endocannabinoids in neurobehavioral processes.
Eur Neuropsychopharmacol, 2015; 25

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BOARD NUMBER: S06-164

BRAIN CIRCUITS IMPLICATED IN AUTISTIC-LIKE BEHAVIORS IN HAPLOINSUFFICIENT NAV1.2 (SCN2A+/-) MICE.

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

Wendy Marcantonio, Massimo Mantegazza, Isabelle Léna
IPMC, Alpes Maritimes, valbonne, France

Autism Spectrum Disorder (ASD) is characterized by deficits in social interactions and communication and restrictive/repetitive behaviors. Excitatory/Inhibitory (E/I) imbalance during brain development has been proposed to contribute to ASD. Mutations in the SCN2A gene, coding for the Nav1.2 voltage-gated sodium channel alpha subunit, has been identified among the most frequent mutations found in patients. Our team previously showed that *Scn2a*$^{+/-}$ mice exhibit core symptoms of ASD, as communication deficits and stereotypic behavior. Our first aim here was to identify the direction of the E/I imbalance using glutamatergic or GABAergic agonists. Glutamatergic agonists injected i.p. in *Scn2a*$^{+/-}$ mice were able to ameliorate social communication and to reduce stereotypic behavior, showing that these mice have global reduced excitatory transmission. Moreover, Western Blot analysis of several brain structures that are often dysfunctional in ASD revealed altered expression of glutamate receptor subunits in *Scn2a*$^{+/-}$ mice. Then, in order to identify brain regions implicated in the behavioral deficits observed in *Scn2a*$^{+/-}$ mice, we locally injected a glutamatergic agonist in different striatal regions. We have observed that reduced excitatory transmission in the Nucleus Accumbens is implicated in social communication deficits in males. We are currently investigating the possible involvement of the dorsal striatum in females. These results will pave the way to experiments in which we will perform optogenetic stimulations of different glutamatergic cortical projections to the striatum in order to rescue the E/I balance and uncover defective neural circuits responsible of behavioral deficits in *Scn2a*$^{+/-}$ mice.

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POGZ DEFICIENCY IN MICE LEADS TO ASD-LIKE BEHAVIORS WITH A MALE-SPECIFIC INCREASE IN SOCIABILITY

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

Reut Suliman-Lavie, Sunny Yanai, Sagiv Shifman
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Genes implicated in autism spectrum disorder (ASD) are enriched for chromatin regulators, but the mechanisms by which they lead to abnormal behavior and cognition remain unclear. POGZ is a key chromatin regulator that, when mutated in humans, leads to "white-Sutton syndrome". The syndrome is characterized by microcephaly, developmental delay, intellectual disability, ASD, and overly friendly behavior. In this study, we generate a mouse model harboring a heterozygous mutation in *Pogz*, mimicking the genetics of the human condition. We characterized this mouse model both anatomically and behaviorally and investigated possible molecular mechanisms that may underlie the observed phenotypes. We characterized *Pogz* heterozygote mutant (*Pogz*$^{+/-}$) male and female mice and found an overall reduction in brain and body weight, more pronounced in males. Adult neurogenesis in these mice was abnormal in proliferation and cell survival. Behavioral assays revealed impairments in motor and spatial learning skills as well as reduced anxiety. Furthermore, we identified an increase in social investigation time of stranger mice and social odors tests in *Pogz*$^{+/-}$ male mice. In agreement, analysis of cFos expression across the brain following exposure to social odors showed increased neuronal activity in regions within the amygdala and hypothalamus only in males *Pogz*$^{+/-}$. Our results establish *Pogz* deficient mouse as a valuable model of "White-Sutton syndrome," showing phenotypes that resemble the human condition, including the overly friendly behavior. Future research is needed to dissect the molecular and neuronal mechanisms for the male-specific changes in social behavior that were accompanied by activation of the amygdala and hypothalamus.

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ROLE OF THE CORTICAL FEEDBACK ON THE NEURONAL REPRESENTATION OF CONTEXTUAL VISUAL INFORMATION IN THE SUPERIOR COLLICULUS OF AN AUTISTIC MOUSE MODEL

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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European Molecular Biology Laboratory, Epigenetics And Neurobiology Unit, Monterotondo, Italy

The brain is a predictive machine. It constantly generates a mental model of the outside world to predict future sensory inputs and adjust behavior accordingly. When the prediction differs from actual sensory inputs, the brain signals prediction errors and uses them to update the mental model to better predict future stimuli. An emerging hypothesis for autism spectrum disorder (ASD) is that symptoms arise from defective interpretation of the environment, due to unbalanced integration of actual sensory evidence and internally generated prior beliefs on the outside world. To test this hypothesis, here we examine neural processing of neurotypical (NT) and Scn2a-haploinsufficient ASD model mice in the context of visual implicit learning in the superior colliculus (SC). The SC is an ideal neural circuit to study an ASD-related imbalance between feedforward and feedback signals in the context of visual learning because it integrates sensory and perceptual information by receiving both direct input from the retina and feedback signals from the primary visual cortex (V1). We recorded the activity of SC neurons and V1 projections via two-photon calcium imaging in head-fixed mice exposed to a visual implicit learning task, where cue and outcome association probabilities change over time, following a Bayesian belief-update scheme. Here we show that 1) the environmental context affects the visual responses of SC to aversive stimuli; 2) ASD and NT mice differ in their neuronal adaptation to the environment; and 3) V1-to-SC feedback is responsible for the context-dependent modulation in both ASD and NT mice.

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DISRUPTED INTERAREAL CORTICAL DYNAMICS AND SENSORIMOTOR LEARNING IN A MOUSE MODEL OF RETT SYNDROME

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

Constantinos Eleftheriou[1,2], Michelle Nicholson Serrano[2], Noha Bahey[2], Victoriana Poon[2], Alexa Schrickel[2], Kirsty Craigie[2], Cristina Martinez Gonzalez[2], Matthew Nolan[1,2], Peter Kind[1,2], Stuart Cobb[1,2], Ian Duguid[1,2]
[1]University of Edinburgh, Simons Initiative For The Developing Brain, Edinburgh, United Kingdom, [2]University of Edinburgh, Centre For Discovery Brain Sciences, Edinburgh, United Kingdom

Rett Syndrome is a devastating neurodevelopmental disorder caused by mutations in the X-linked *MECP2* gene, characterised by a period of normal development prior to the onset of profound cognitive and sensorimotor deficits. While the symptomatic period has been extensively characterised in mouse models of the disorder, much less is known about how loss of MeCP2 impacts cognitive processing prior to the onset of overt motor phenotypes. Given that disrupted MeCP2 expression results in aberrant synaptic connectivity and excitability in individual brain areas, we hypothesised that MeCP2 regulates inter-areal cortical activity dynamics necessary for higher-order brain function. Loss of MeCP2 would result in a breakdown of cortex-wide communication, in turn leading to developmental cognitive deficits prior to phenotypes detectable by conventional symptomatic scoring. We used widefield calcium imaging of the dorsal cortex to assay how loss of MeCP2 affects cortex-wide neural dynamics at rest, during visual stimulation and across visuomotor learning. Female Mecp2$^{+/loxstop}$ mice displayed widespread resting state functional dysconnectivity, suggesting a breakdown in baseline cortical communication. In addition, we found hypoconnectivity during visual stimulation, with sensory information failing to propagate from primary sensory to higher order processing areas. We demonstrate that aberrant functional connectivity and disrupted inter-areal information propagation in Mecp2$^{+/loxstop}$ mice is associated with delayed learning in simple reward association and visual discrimination tasks. Our results suggest that loss of MeCP2 leads to aberrant cortical functional connectivity, which in turn drives sensorimotor processing and learning deficits prior to the onset of overt motor phenotypes.

Pubmed:
34146469: Dacre J, Colligan M, Clarke T, Ammer JJ, Schiemann J, Chamosa-Pino V, Claudi F, Harston JA, Eleftheriou C, Pakan JMP, Huang CC, Hantman AW, Rochefort NL, Duguid I
A cerebellar-thalamocortical pathway drives behavioral context-dependent movement initiation.
Executing learned motor behaviors often requires the transformation of sensory cues into patterns of motor commands that generate appropriately timed actions. The cerebellum and thalamus are two key areas involved in shaping cortical output and movement, but the contribution of a cerebellar-thalamocortical pathway to voluntary movement initiation remains poorly understood. Here, we investigated how an auditory "go cue" transforms thalamocortical activity patterns and how these changes relate to movement initiation. Population responses in dentate/interpositus-recipient regions of motor thalamus reflect a time-locked increase in activity immediately prior to movement initiation that is temporally uncoupled from the go cue, indicative of a fixed-latency feedforward motor timing signal. Blocking cerebellar or motor thalamic output suppresses movement initiation, while stimulation triggers movements in a behavioral context-dependent manner. Our findings show how cerebellar output, via the thalamus, shapes cortical activity patterns necessary for learned context-dependent movement initiation.
Neuron, 2021; 109

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SEX DIFFERENCES IN AUTISM SPECTRUM DISORDER: A PROTEOME ANALYSIS.

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorder (ASD) is with around 1 % of the population the most commonly diagnosed neurodevelopmental disorder. A high difference in the prevalence of cases has been observed in woman and man. The reported male to female ratios varies between 2:1 and 4:1. Moreover, behavioral characteristics differ between the sexes. They tend to be more internalizing in females and externalizing in males. Sex differences have also been found in the behavior of ASD model mice, with females performing similar to control mice and males showing higher rates of restricted repetitive behavior, performing worse in reversal learning tasks and spending less time with a novel mouse in a 3-chamber social approach test. The underlying differences of these sex biases in prevalence and behavior are still unknown. Therefore, our project focusses on analyzing the synaptic proteome of male and female mice of this ASD model, to gain a deeper insight into the mechanisms of ASD and the reasons for the sexual dimorphism. In our analysis we found several proteins to be differentially expressed between female and male ASD model mice. A dimorphism was found in all three investigated brain regions: the hippocampus, the striatum and the cerebellum. Two of the proteins, which were changed in expression, have previously been characterized in ASD and further can be linked to pathways associated with neurodevelopmental disorders. Our efforts should serve as a neurobiological framework to better understand sex differences, in both health and disease.

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EMBRYONIC EXPOSURE TO VALPROIC ACID IMPAIRS VISUAL PREFERENCES FOR FACE-LIKE STIMULI AND ALTERS DOPAMINERGIC DISTRIBUTION AND SIGNALING IN DOMESTIC CHICKS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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In recent years, an involvement of the dopaminergic (DA) population in the regulation of social and affiliative behavior has been largely observed both in human and in animal studies. Moreover, dysfunctions of the same neuromodulatory system have been reported also in children with Autism Spectrum Disorders (ASD) and in different ASD models. To further investigate the role of DA functioning in influencing social behavior, given the importance of early life experiences in shaping social cognitive functions, we chose to study an animal model known for its highly precocial behavioral features: the domestic chick. By embryonically exposing the animals to valproic acid (VPA), a compound largely used to model social disorders, we altered chicks' innate preference for Face-Like stimuli. Given the importance of face processing in ASD, we therefore consider the chick as the ideal model to study the neurobiological bases of such mechanisms. In addition, we examined the anatomical and functional layout of the mesencephalic DA population in VPA-treated chicks by immunolabeling dopaminergic TH^+ neurons and by analyzing the expression levels of several dopaminergic markers in both the mesencephalon and the septum, a DA-innervated brain region associated to social behavior. We observed that VPA exposure affects the rostro-caudal distribution of DA neurons in the mesencephalon and alters the expression of genes involved in DA signaling in the septum. Taken together, our data support the emerging hypothesis of a link between DA dysfunction and social cognitive deficits.

Pubmed:

34858146: Adiletta A, Pedrana S, Rosa-Salva O, Sgadò P
Spontaneous Visual Preference for Face-Like Stimuli Is Impaired in Newly-Hatched Domestic Chicks Exposed to Valproic Acid During Embryogenesis.
Faces convey a great amount of socially relevant information related to emotional and mental states, identity and intention. Processing of face information is a key mechanism for social and cognitive development, such that newborn babies are already tuned to recognize and orient to faces and simple schematic face-like patterns since the first hours of life. Similar to neonates, also non-human primates and domestic chicks have been shown to express orienting responses to faces and schematic face-like patterns. More importantly, existing studies have hypothesized that early disturbances of these mechanisms represent one of the earliest biomarker of social deficits in autism spectrum disorders (ASD). We used VPA exposure to induce neurodevelopmental changes associated with ASD in domestic chicks and tested whether VPA could impact the expression of the animals' approach responses to schematic face-like stimuli. We found that VPA impairs the chicks' preference responses to these social stimuli. Based on the results shown here and on previous studies, we propose the domestic chick as animal model to investigate the biological mechanisms underlying face processing deficits in ASD.
Front Behav Neurosci, 2021; 15
34481832: Spadini S, Racchetti G, Adiletta A, Lamanna J, Moro AS, Ferro M, Zimarino V, Malgaroli A
A novel integrated approach to estimate the mitochondrial content of neuronal cells and brain tissues.
Mitochondria and their dynamics fuel most cellular processes both in physiological and pathological conditions. In the central nervous system, mitochondria sustain synaptic transmission and plasticity via multiple mechanisms which include their redistribution and/or expansion to higher energy demanding sites, sustaining activity changes and promoting morphological circuit adaptations.
J Neurosci Methods, 2021; 363

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RESTING STATE FUNCTIONAL CONNECTIVITY NETWORKS IN TWINS WITH AUTISM: TOWARDS THE TURKISH LONGITUDINAL NEUROIMAGING AUTISM PROJECT (TURK-NAP) USING THE ABİDE DATABASE

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorder -autism- is a lifelong early-onset neurodevelopmental disorder characterized by the 3 main symptom domains (social-communication difficulties, restricted and repetitive behaviors, and sensory abnormalities) (DSM-5; APA, 2013). One of the key characteristics of autism is phenotypical and neurobiological heterogeneity (Geschwind and Levitt, 2007; Minhew and Williams 2007). Big Data sets, such as the Autism Brain Imaging Date Exchange (ABIDE) (Di Martino et al., 2017), reporting both hyperconnectivity and hypoconnectivity, within and between various brain networks. **Aims:** The first goal of the TURK-NAP is to test the hypothesis that autism is associated with both hypoconnectivity and hyperconnectivity, and to characterize relationships between functional connectivity alterations. Second is to examine the clinical scores covering the 3 main symptom domains of the TURK-NAP. Third is to identify biomarkers in autism using Network Neuroscience Tools based on the ABIDE database. **Sample:** The sample of TURK-NAP is planned to consists of the norm study of the Turkish Vineland-II Adaptive Behavior Scales [84 autism (20 girls, 64 boys); 100 healthy developing (50 girls, 50 boys); age range: 8-18] **MRI Data Acquisition:** Resting state fMRI (rs-fMRI) and structural scans acquired at UMRAM (Bilkent University, Ankara) using 3 Tesla MRI (Siemens Magnetom Trio Tim). Resting state scans lasted 7 minutes for each of the children with their eyes closed. **Results:** The correlation between the brain networks and behavioral measurements of the twin sample was calculated by Canonical Correlation Analysis. **Conclusion:** The national and international contributions and limitations of the TURK-NAP Project will be discussed.

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GUT MICROBIOTA FROM AUTISTIC CHILDREN INDUCE CHANGES IN THE CENTRAL NERVOUS SYSTEM OF HEALTHY MICE

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorders (ASD) are a complex group of neurodevelopmental disorders with unclear etiology. ASD manifest in a variety of neuropsychiatric traits, but are most commonly characterized by impaired social interaction and stereotypical repetitive behavior. Current research reports that children with ASD have risk of gastrointestinal disturbances and showcase differences in gut microbiota composition. We hypothesize that transfer of gut microbiota from ASD patients can induce changes in the central nervous system of healthy mice. To test this hypothesis, we used fecal samples of ASD children to perform fecal microbiota transfer (FMT) to adult C57BL/6J mice. We measured the effect of gut microbiota from ASD children on autistic-like behaviors using several behavioral tests. Also, we analyzed gene expression in the hippocampus and medial prefrontal cortex using Real-Time PCR. The transfer of gut microbiota from ASD children to healthy mice affected cognitive functions of healthy animals and influenced changes in ASD associated gene expression. However, the observed effect was minor, possibly due to the gut microbiota variability between samples. These primary results suggest that the gut microbiota might contribute to the cognitive changes observed in ASD patients. Nevertheless, further gut microbiota diversity analysis is required to confirm the success of FMT and to establish the cause of observed changes.

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CHRONIC SODIUM BROMIDE TREATMENT RELIEVES AUTISTIC-LIKE BEHAVIORAL DEFICITS IN THREE MOUSE MODELS OF AUTISM

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism Spectrum Disorders (ASD) are neurodevelopmental disorders whose diagnosis relies on deficient social interaction and communication together with repetitive behavior. To date, no pharmacological treatment has been approved that ameliorates social behavior in patients with ASD. Based on the excitation/inhibition imbalance theory of autism, we hypothesized that bromide ions, long used as an antiepileptic medication, could relieve core symptoms of ASD. We evaluated the effects of chronic sodium bromide (NaBr) administration on autistic-like symptoms in three genetic mouse models of autism: *Oprm1*$^{-/-}$, *Fmr1*$^{-/-}$ and *Shank3*$^{\Delta ex13\text{-}16-/-}$ mice. We showed that chronic NaBr treatment relieved autistic-like behaviors in these three models. In *Oprm1*$^{-/-}$ mice, these beneficial effects were superior to those of chronic bumetanide administration. At transcriptional level, chronic NaBr in *Oprm1* null mice was associated with increased expression of genes coding for chloride ions transporters, $GABA_A$ receptor subunits, oxytocin and mGlu4 receptor. Lastly, we uncovered synergistic alleviating effects of chronic NaBr and a positive allosteric modulator (PAM) of mGlu4 receptor on autistic-like behavior in *Oprm1*$^{-/-}$ mice. We evidenced in heterologous cells that bromide ions behave as PAMs of mGlu4, providing a molecular mechanism for such synergy. Our data reveal the therapeutic potential of bromide ions, alone or in combination with a PAM of mGlu4 receptor, for the treatment of ASDs.

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BOARD NUMBER: S06-173

FACILITATING MGLU4 RECEPTOR ACTIVITY RELIEVES AUTISTIC-LIKE BEHAVIORS IN A MOUSE MODEL OF FRAGILE X SYNDROME

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Fragile X syndrome (FXS) is the most frequent known single gene cause of autism spectrum disorder (ASD). To date, no pharmacological treatment exists that reliably relieves symptoms of autism in FXS. The striatum has been identified from clinical and preclinical studies as a critical brain substrate for autism etiopathology. Here we evidenced altered responses to dopaminergic challenge and modifications of gene expression in the mouse model of FXS, *Fmr1* knockout mice, pointing to striatal dysfunction. Using RNAscope® *in situ* hybridization technique, we revealed a reduction in the activity of D1- and D2-expressing medium spiny neurons (MSNs) in the caudate putamen (CPu) and nucleus accumbens (NAc) following social interaction in *Fmr1*$^{-/-}$ mice. In the NAc, this reduction affected significantly more D1- than D2-MSNs, resulting in an excessive D2-MSN/D1-MSN activity ratio. We had previously shown that administration of a positive allosteric modulator (PAM) of the mGlu4 glutamate receptor, that represses D2-MSN activity, alleviates autistic-like deficits in mouse models of autism or social/reward deficit. In the present study, chronic administration of the mGlu4 PAMs VU0155041 and ADX88178 or the orthosteric mGlu4/mGlu7 agonist LSP4-2022 relieved core autistic symptoms in *Fmr1*$^{-/-}$ mice, whilst chronic administration of the mGlu5 negative allosteric modulator GRN529 failed to rescue social behavior. Finally, administration of the photoswitchable mGlu4 PAM Optogluram in the ventral pallidum was sufficient to restore social interaction in *Fmr1*$^{-/-}$ mice. These results suggest a great therapeutic potential for compounds facilitating mGlu4 activity or, more generally, repressing D2-MSN activity, in FXS and autism.

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BOARD NUMBER: S06-174

LIGHT SHEET IMAGING OF BEHAVIOURALLY ACTIVATED AMYGDALA NEURONS IN THE FRAGILE-X KNOCKOUT RAT MODEL OF AUTISM SPECTRUM DISORDERS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorders (ASD) are characterized by social impairments and restricted behaviours. Activation in key brain regions involved in social and emotional processing, including the amygdala, has been studied using neuroimaging and histology. Nevertheless, changes in neuronal activity in ASD are largely unknown. To evaluated behaviourally activated neurons we used the Fragile-X knockout (Fmr1$^{-/y}$) rat model of ASD combined with whole-brain immunolabeling against cFos. Given the amygdala-dependent emotional processing is altered in Fragile-X syndrome individuals, we focus on behaviourally-induced cFos expression in Fmr1$^{-/y}$ rats compared to wild types (WT). To quantify cFos+ neurons in the amygdala, we created a pipeline combining immunolabeling, a modified iDISCO protocol, light-sheet microscopy, automatic quantification and a custom-made-3D atlas (Basolateral complex: lateral:LA, basal:BA and basomedial:BM; Central nucleus: CeA). We compared WT and Fmr1$^{-/y}$ animals that received a conditioned (CS:light) paired with unconditioned-stimulus (US:foot-shock), to CS-only, and home-caged (naive) groups. Brains were obtained 90 minutes after stimuli, immunolabeled, cleared and imaged. We observed an increase in cell density of cFos+neurons in the BA and BM of shocked WT and Fmr1$^{-/y}$, compared with naive rats. We also observed an increase in WT-CS-only, but not Fmr1$^{-/y}$-CS-only group. No changes were observed in LA or CeA (One-way ANOVA, Tukey post-hoc-test). Our approach identifies differential neuronal activation in the amygdala of Fmr1$^{-/y}$ rats in the CS-only group, but not in the conditioned or naive groups, suggesting that deletion of Fmr1 may cause a reduction in responsiveness of the Basolateral amygdala to sensory events associated with a new environmental context.

Pubmed:

33771986: Bonizzato M, James ND, Pidpruzhnykova G, Pavlova N, Shkorbatova P, Baud L, Martinez-Gonzalez C, Squair JW, DiGiovanna J, Barraud Q, Micera S, Courtine G
Multi-pronged neuromodulation intervention engages the residual motor circuitry to facilitate walking in a rat model of spinal cord injury.
A spinal cord injury usually spares some components of the locomotor circuitry. Deep brain stimulation (DBS) of the midbrain locomotor region and epidural electrical stimulation of the lumbar spinal cord (EES) are being used to tap into this spared circuitry to enable locomotion in humans with spinal cord injury. While appealing, the potential synergy between DBS and EES remains unknown. Here, we report the synergistic facilitation of locomotion when DBS is combined with EES in a rat model of severe contusion spinal cord injury leading to leg paralysis. However, this synergy requires high amplitudes of DBS, which triggers forced locomotion associated with stress responses. To suppress these undesired responses, we link DBS to the intention to walk, decoded from cortical activity using a robust, rapidly calibrated unsupervised learning algorithm. This contingency amplifies the supraspinal descending command while empowering the rats into volitional walking. However, the resulting improvements may not outweigh the complex technological framework necessary to establish viable therapeutic conditions.
Nat Commun, 2021; 12
33769452: Hristova K, Martinez-Gonzalez C, Watson TC, Codadu NK, Hashemi K, Kind PC, Nolan MF, Gonzalez-Sulser A
Medial septal GABAergic neurons reduce seizure duration upon optogenetic closed-loop stimulation.
Seizures can emerge from multiple or large foci in temporal lobe epilepsy, complicating focally targeted strategies such as surgical resection or the modulation of the activity of specific hippocampal neuronal populations through genetic or optogenetic techniques. Here, we evaluate a strategy in which optogenetic activation of medial septal GABAergic neurons, which provide extensive projections throughout the hippocampus, is used to control seizures. We utilized the chronic intrahippocampal kainate mouse model of temporal lobe epilepsy, which results in spontaneous seizures and as is often the case in human patients, presents with hippocampal sclerosis. Medial septal GABAergic neuron populations were

immunohistochemically labelled and were not reduced in epileptic conditions. Genetic labelling with mRuby of medial septal GABAergic neuron synaptic puncta and imaging across the rostral to caudal extent of the hippocampus, also indicated an unchanged number of putative synapses in epilepsy. Furthermore, optogenetic stimulation of medial septal GABAergic neurons consistently modulated oscillations across multiple hippocampal locations in control and epileptic conditions. Finally, wireless optogenetic stimulation of medial septal GABAergic neurons, upon electrographic detection of spontaneous hippocampal seizures, resulted in reduced seizure durations. We propose medial septal GABAergic neurons as a novel target for optogenetic control of seizures in temporal lobe epilepsy.
Brain, 2021; 144

29556028: Asboth L, Friedli L, Beauparlant J, Martinez-Gonzalez C, Anil S, Rey E, Baud L, Pidpruzhnykova G, Anderson MA, Shkorbatova P, Batti L, Pagès S, Kreider J, Schneider BL, Barraud Q, Courtine G
Cortico-reticulo-spinal circuit reorganization enables functional recovery after severe spinal cord contusion.
Severe spinal cord contusions interrupt nearly all brain projections to lumbar circuits producing leg movement. Failure of these projections to reorganize leads to permanent paralysis. Here we modeled these injuries in rodents. A severe contusion abolished all motor cortex projections below injury. However, the motor cortex immediately regained adaptive control over the paralyzed legs during electrochemical neuromodulation of lumbar circuits. Glutamatergic reticulospinal neurons with residual projections below the injury relayed the cortical command downstream. Gravity-assisted rehabilitation enabled by the neuromodulation therapy reinforced these reticulospinal projections, rerouting cortical information through this pathway. This circuit reorganization mediated a motor cortex-dependent recovery of natural walking and swimming without requiring neuromodulation. Cortico-reticulo-spinal circuit reorganization may also improve recovery in humans.
Nat Neurosci, 2018; 21

29386117: Tennant SA, Fischer L, Garden DLF, Gerlei KZ, Martinez-Gonzalez C, McClure C, Wood ER, Nolan MF
Stellate Cells in the Medial Entorhinal Cortex Are Required for Spatial Learning.
Spatial learning requires estimates of location that may be obtained by path integration or from positional cues. Grid and other spatial firing patterns of neurons in the superficial medial entorhinal cortex (MEC) suggest roles in behavioral estimation of location. However, distinguishing the contributions of path integration and cue-based signals to spatial behaviors is challenging, and the roles of identified MEC neurons are unclear. We use virtual reality to dissociate linear path integration from other strategies for behavioral estimation of location. We find that mice learn to path integrate using motor-related self-motion signals, with accuracy that decreases steeply as a function of distance. We show that inactivation of stellate cells in superficial MEC impairs spatial learning in virtual reality and in a real world object location recognition task. Our results quantify contributions of path integration to behavior and corroborate key predictions of models in which stellate cells contribute to location estimation.
Cell Rep, 2018; 22

23708060: Martinez-Gonzalez C, van Andel J, Bolam JP, Mena-Segovia J
Divergent motor projections from the pedunculopontine nucleus are differentially regulated in Parkinsonism.
The pedunculopontine nucleus (PPN) is composed of neurons with different connectivity patterns that express different neurochemical markers, display distinct firing characteristics and are topographically organized in functional domains across its rostro-caudal axis. Previous reports have shown that the caudal region of the PPN is interconnected with motor regions of both the basal ganglia and brainstem/medulla. The co-distribution of ascending and descending motor outputs raises the question as to whether the PPN provides a coordinated or differential modulation of its targets in the basal ganglia and the medulla. To address this, we retrogradely labeled neurons in the two main PPN pathways involved in motor control and determined whether they project to one or both structures, their neurochemical phenotype, and their activity in normal and dopamine depleted rats, as indicated by Egr-1 expression. We show that ascending and descending motor pathways from the PPN arise largely from separate neurons that intermingle in the same region of the PPN, but have a distinct neurochemical composition and are differentially regulated in the Parkinsonian state. Thus, neurons projecting to the subthalamic nucleus consist of cholinergic, calbindin- and calretinin-expressing neurons, and Egr-1 is upregulated following a 6-hydroxydopamine lesion. In contrast, a larger proportion of neurons projecting to the gigantocellular nucleus are cholinergic, none express calbindin and the expression of Egr-1 is not changed by the dopamine lesion. Our results suggest that ascending and descending motor connections of the PPN are largely mediated by different sets of neurons and there are cell type-specific changes in Parkinsonian rats.
Brain Struct Funct, 2014; 219

22356461: Martinez-Gonzalez C, Wang HL, Micklem BR, Bolam JP, Mena-Segovia J
Subpopulations of cholinergic, GABAergic and glutamatergic neurons in the pedunculopontine nucleus contain calcium-binding proteins and are heterogeneously distributed.
Neurons in the pedunculopontine nucleus (PPN) are highly heterogeneous in their discharge properties, their neurochemical markers, their pattern of connectivity and the behavioural processes in which they participate. Three main transmitter

phenotypes have been described, cholinergic, GABAergic and glutamatergic, and yet electrophysiological evidence suggests heterogeneity within these subtypes. To gain further insight into the molecular composition of these three populations in the rat, we investigated the pattern of expression of calcium binding proteins (CBPs) across distinct regions of the PPN and in relation to the presence of other neurochemical markers. Calbindin- and calretinin-positive neurons are as abundant as cholinergic neurons, and their expression follows a rostro-caudal gradient, whereas parvalbumin is expressed by a low number of neurons. We observed a high degree of expression of CBPs by GABAergic and glutamatergic neurons, with a large majority of calbindin- and calretinin-positive neurons expressing GAD or VGluT2 mRNA. Notably, CBP-positive neurons expressing GAD mRNA were more concentrated in the rostral PPN, whereas the caudal PPN was characterized by a higher density of CBP-positive neurons expressing VGluT2 mRNA. In contrast to these two large populations, in cholinergic neurons expression of calretinin is observed only in low numbers and expression of calbindin is virtually non-existent. These findings thus identify novel subtypes of cholinergic, GABAergic and glutamatergic neurons based on their expression of CBPs, and further contribute to the notion of the PPN as a highly heterogeneous structure, an attribute that is likely to underlie its functional complexity.
Eur J Neurosci, 2012; 35

21503154: Martinez-Gonzalez C, Bolam JP, Mena-Segovia J
Topographical organization of the pedunculopontine nucleus.
Neurons in the pedunculopontine nucleus (PPN) exhibit a wide heterogeneity in terms of their neurochemical nature, their discharge properties, and their connectivity. Such characteristics are reflected in their functional properties and the behaviors in which they are involved, ranging from motor to cognitive functions, and the regulation of brain states. A clue to understand this functional versatility arises from the internal organization of the PPN. Thus, two main areas of the PPN have been described, the rostral and the caudal, which display remarkable differences in terms of the distribution of neurons with similar phenotype and the projections that originate from them. Here we review these differences with the premise that in order to understand the function of the PPN it is necessary to understand its intricate connectivity. We support the case that the PPN should not be considered as a homogeneous structure and conclude that the differences between rostral and caudal PPN, along with their intrinsic connectivity, may underlie the basis of its complexity.
Front Neuroanat, 2011; 5

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BOARD NUMBER: S06-175

NEUROPLASTICITY PROFILE AND NEUROGENIC ACTIVITY IN FMR1 KNOCK OUT RATS, A MODEL OF THE FRAGILE X SYNDROME

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Fragile X Syndrome (FXS), is the most common monogenetic leading cause of inherited intellectual disability and autism. Experimental studies have been mainly focused on Fmr1 knock-out (KO) mice while a few studies have investigated behavioral and neurobiological alterations in Fmr1KO rats, given the fact that this is an animal model with improved translational value. In this study we evaluated the biophenotype of Fmr1KO rats with specific behavioral tasks that are related to cognitive functions along with neuroplasticity and neurogenesis biomarkers.

Male 12-16 weeks old rats, LE-Fmr1^{em2Mcwi} referred to as Fmr1 KO (n=20), and WT littermates (n=20), were used. Object recognition and location memory were assayed through the Object Recognition and Object Location Task tests. Neuroplasticity and Neurogenesis indices were evaluated in Prefrontal Cortex, Dorsal and Ventral Hippocampus.

Present findings have shown a specific bio-phenotype of Fmr1KO rats as compared to their WT counterparts. Fmr1KO rats displayed hyperactivity and impaired behavioral indices that are related to specific cognitive functions. In parallel, neuronal plasticity indices, including BDNF/Trkb protein expression levels and neurogenic activity of neural stem cells were found to be genotype dependent.

Based on our findings a differentiated behavioral and plasticity profile along with disrupted neurogenesis was observed between WT and KO Fmr1 rats. Present results contribute to issues related to translational studies focused on cognitive disabilities and autism spectrum disorders.

Co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code:T2EDK-02075).

Pubmed:
34776964: Poulia N, Delis F, Brakatselos C, Ntoulas G, Asprogerakas MZ, Antoniou K
CBD Effects on Motor Profile and Neurobiological Indices Related to Glutamatergic Function Induced by Repeated Ketamine Pre-Administration.
Clinical evidence and experimental studies have shown the psychotomimetic properties induced by ketamine. Moreover, acute or chronic ketamine (KET) administration has been widely used for modeling schizophrenia-like symptomatology and pathophysiology. Several studies have reported the antipsychotic potential of cannabidiol (CBD), while there is limited information on the cannabidiol effect on KET-induced schizophrenia-like impairments. Therefore, the goal of the present study was to evaluate neuroplastic changes induced by repeated KET administration, which is used as an experimental model of schizophrenia-with a behavioral focus on positive-like symptomatology- and to assess the modulatory role of CBD treatment. The present findings have shown a robust increase in motor activity in KET-treated rats, following a 10-day period of chronic administration at the sub-anesthetic dose of 30 mg/kg (i.p), that was reversed to normal by subsequent chronic CBD treatment. Concerning the expression of glutamate receptors, the current findings have shown region-dependent KET-induced constitutional alterations in NMDA and AMPA receptors that were modified by subsequent CBD treatment. Additionally, repeated KET administration increased ERK1/2 phosphorylation state in all regions examined, apart from the ventral hippocampus that was modulated by subsequent CBD treatment. The present results show, for the first time, a

stimulated motor output coupled with a specific glutamatergic-related status and ERK1/2 activation following chronic KET administration that were attenuated by CBD treatment, in a region-dependent manner. These findings provide novel information concerning the antipsychotic potential of CBD using a specific design of chronic KET administration, thus contributing to experimental approaches that mirror the symptomatology and pathophysiology of schizophrenia.
Front Pharmacol, 2021; 12

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BOARD NUMBER: S06-176

FMR1 KO MICE EXHIBIT DEFICITS IN BEHAVIOR, EYE ALIGNMENT, AND CORTICAL ACTIVITY DURING STEREOSCOPIC DEPTH DISCRIMINATION COMPARED TO WILD TYPE MICE

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autistic subjects generally have a higher incidence of strabismus and amblyopia than control subjects. Consequently, autism is associated with an overall deficit in stereoscopic depth perception. Fragile X syndrome (FXS) is the leading genetic cause of autism, driven by a mutation in the FMR1 gene, and leads to a deficit in binocular visual processing. We examined stereo vision in FMR1 knock-out (KO) and wild-type (WT) mice using three different assays: performance on a perceptual task, binocular eye alignment, and disparity selectivity in the visual cortex. Stereoscopic depth perception was significantly impaired using the pole descent visual cliff task in FMR1 KO mice relative to WT mice. While FMR1 KO mice were able to perform the task, they exhibited a larger depth discrimination threshold compared to WT mice. The binocular alignment of FMR1 KO mice had greater variability and offset during both saccades and fixation than WT mice, which reflect characteristics of strabismus. Finally, we measured binocular disparity tuning in the visual cortex of FMR1 KO and WT mice using two-photon microscopy. Disparity tuning in FMR1 KO mice was less selective and disparity-dependent responses were more variable across repeated trials compared to WT mice. Overall, FMR1 KO mice exhibit deficits in binocular vision from the perspective of the visual and motor systems that are analogous to those observed in Fragile X patients and autistic subjects. We propose that the mouse is an effective model for characterizing the consequences of FXS for visual processing.

Pubmed:
33705714: Boone HC, Samonds JM, Crouse EC, Barr C, Priebe NJ, McGee AW
Natural binocular depth discrimination behavior in mice explained by visual cortical activity.
In mice and other mammals, forebrain neurons integrate right and left eye information to generate a three-dimensional representation of the visual environment. Neurons in the visual cortex of mice are sensitive to binocular disparity, yet it is unclear whether that sensitivity is linked to the perception of depth. We developed a natural task based on the classic visual cliff and pole descent tasks to estimate the psychophysical range of mouse depth discrimination. Mice with binocular vision descended to a near (shallow) surface more often when surrounding far (deep) surfaces were progressively more distant. Occlusion of one eye severely impaired their ability to target the near surface. We quantified the distance at which animals make their decisions to estimate the binocular image displacement of the checkerboard pattern on the near and far surfaces. Then, we assayed the disparity sensitivity of large populations of binocular neurons in primary visual cortex (V1) using two-photon microscopy and quantitatively compared this information available in V1 to their behavioral sensitivity. Disparity information in V1 matches the behavioral performance over the range of depths examined and was resistant to changes in binocular alignment. These findings reveal that mice naturally use stereoscopic cues to guide their behavior and indicate a neural basis for this depth discrimination task.
Curr Biol, 2021; 31
31462533: Samonds JM, Choi V, Priebe NJ
Mice Discriminate Stereoscopic Surfaces Without Fixating in Depth.
Stereopsis is a ubiquitous feature of primate mammalian vision, but little is known about if and how rodents such as mice use stereoscopic vision. We used random dot stereograms to test for stereopsis in male and female mice, and they were able to discriminate near from far surfaces over a range of disparities, with diminishing performance for small and large binocular disparities. Based on two-photon measurements of disparity tuning, the range of disparities represented in the visual cortex aligns with the behavior and covers a broad range of disparities. When we examined their binocular eye movements, we found that, unlike primates, mice did not systematically vary relative eye positions or use vergence eye movements when presented with different disparities. Nonetheless, the representation of disparity tuning was wide enough to capture stereoscopic information over a range of potential vergence angles. Although mice share fundamental characteristics of stereoscopic vision with primates and carnivores, their lack of disparity-dependent vergence eye movements and wide

neuronal representation suggests that they may use a distinct strategy for stereopsis. Binocular vision allows us to derive depth information by comparing right and left eye information. We characterized binocular integration in mice because tools exist in these animals to dissect the underlying neural circuitry for binocular vision. Using random dot stereograms, we find that behavior and disparity tuning in the visual cortex share fundamental characteristics with primates, but we did not observe any evidence of disparity-dependent changes in vergence angle. We propose that mice use a distinct strategy of stereopsis compared with primates by using a broad range of disparities to encode depth over a large field of view and to compensate for nonstereoscopic changes in vergence angle that arise during natural behavior.
J Neurosci, 2019; 39

30627645: Samonds JM, Lieberman S, Priebe NJ
Motion Discrimination and the Motion Aftereffect in Mouse Vision.
Prolonged exposure to motion in one direction often leads to the illusion of motion in the opposite direction for stationary objects. This motion aftereffect likely arises across several visual areas from adaptive changes in the balance of activity and competitive interactions. We examined whether or not the mouse was susceptible to this same illusion to determine whether it would be a suitable model for learning about the neural representation of the motion aftereffect. Under a classical conditioning paradigm, mice learned to lick when presented with motion in one direction and not the opposite direction. When the mice were adapted to motion preceding this test, their lick behavior for zero coherence motion was biased for motion in the opposite direction of the adapting stimulus. Overall, lick count versus motion coherence shifted in the opposite direction of the adapting stimulus. This suggests that although the mouse has a simpler visual system compared with primates, it still is subject to the motion aftereffect and may elucidate the underlying circuitry.
eNeuro, 2018 Nov-Dec; 5

30349110: Samonds JM, Geisler WS, Priebe NJ
Natural image and receptive field statistics predict saccade sizes.
Humans and other primates sample the visual environment using saccadic eye movements that shift a high-resolution fovea toward regions of interest to create a clear perception of a scene across fixations. Many mammals, however, like mice, lack a fovea, which raises the question of why they make saccades. Here we describe and test the hypothesis that saccades are matched to natural scene statistics and to the receptive field sizes and adaptive properties of neural populations. Specifically, we determined the minimum amplitude of saccades in natural scenes necessary to provide uncorrelated inputs to model neural populations. This analysis predicts the distributions of observed saccade sizes during passive viewing for nonhuman primates, cats, and mice. Furthermore, disrupting the development of receptive field properties by monocular deprivation changed saccade sizes consistent with this hypothesis. Therefore, natural-scene statistics and the neural representation of natural images appear to be critical factors guiding saccadic eye movements.
Nat Neurosci, 2018; 21

28931608: Samonds JM, Feese BD, Lee TS, Kuhlman SJ
Nonuniform surround suppression of visual responses in mouse V1.
Complex receptive field characteristics, distributed across a population of neurons, are thought to be critical for solving perceptual inference problems that arise during motion and image segmentation. For example, in a class of neurons referred to as "end-stopped," increasing the length of stimuli outside of the bar-responsive region into the surround suppresses responsiveness. It is unknown whether these properties exist for receptive field surrounds in the mouse. We examined surround modulation in layer 2/3 neurons of the primary visual cortex in mice using two-photon calcium imaging. We found that surround suppression was significantly asymmetric in 17% of the visually responsive neurons examined. Furthermore, the magnitude of asymmetry was correlated with orientation selectivity. Our results demonstrate that neurons in mouse primary visual cortex are differentially sensitive to the addition of elements in the surround and that individual neurons can be described as being either uniformly suppressed by the surround, end-stopped, or side-stopped. NEW & NOTEWORTHY Perception of visual scenes requires active integration of both local and global features to successfully segment objects from the background. Although the underlying circuitry and development of perceptual inference is not well understood, converging evidence indicates that asymmetry and diversity in surround modulation are likely fundamental for these computations. We determined that these key features are present in the mouse. Our results support the mouse as a model to explore the neural basis and development of surround modulation as it relates to perceptual inference.
J Neurophysiol, 2017; 118

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BOARD NUMBER: S06-177

AUTISTIC-LIKE BEHAVIORAL EFFECTS OF PRENATAL STRESS IN THE FMR1-KO MOUSE MODEL OF FRAGILE X SYNDROME

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Fragile X Syndrome (FXS) is the most common heritable form of mental retardation and the main monogenic cause of autism spectrum disorder (ASD). FXS is due to a mutation in the X-linked FMR1 gene and is characterized by motor, cognitive and social alterations, mostly overlapping with ASD behavioral phenotypes. The severity of these symptoms and their timing may be exacerbated and/or advanced by environmental adversity interacting with the genetic mutation. We therefore tested the effects of the prenatal exposure to unpredictable chronic stress on the behavioral phenotype of the Fmr1 knock-out (KO) mouse model of FXS, including locomotion, spatial memory, social interaction and communication. Our findings demonstrate the relevance of early environmental stressors in interacting with genetic factors to influence the age-dependent appearance of selected FXS- and ASD-like phenotypes.

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NO INTRINSIC NEUROVISCERAL INTEGRATION IN CHILDREN WITH ASD: AN INVESTIGATION OF AUTONOMIC AROUSAL AND AMYGDALA-FRONTAL CONNECTIVITY

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Aside the social deficits and repetitive behaviors that characterize individuals with an autism spectrum disorder (ASD), extremely high prevalence rates of co-occurring anxiety and (social) stress are often observed. Here, we aim to further investigate the occurrence of autonomic hyperarousal as well as the underlying neural processes in 59 school-aged (8-12 years old) children with ASD compared to 39 age- and IQ-matched typically developing children, thereby focusing on measures indexing heart rate variability (HRV) collected during concurrent neuroimaging during rest (rs-fMRI). In line with the model of Neurovisceral Integration by Thayer et al. (2012), diminished resting HRV was expected to be linked to an overall weaker functional coupling between the amygdala and prefrontal regions in children with ASD, related to the prefrontal inability to inhibit amygdala-centered circuits during states of autonomic hyperarousal. No significant differences in commonly addressed HRV-based estimates of autonomic arousal were noted between ASD and TD children however, indicating no evidence for excessive arousal in ASD children. Additionally, no group differences in functional connectivity between bilateral amygdalae and prefrontal regions were encountered. Significant negative associations between amygdala-frontal coupling and time-domain HRV-based measures of autonomic arousal were noted, but only in TD children. The absence of such central-autonomic associations in children with ASD may suggest a neurovisceral “decoupling” instead of integration, but further research is necessary to address this issue further.

Pubmed:
34880300: Alaerts K, Taillieu A, Daniels N, Soriano JR, Prinsen J
Oxytocin enhances neural approach towards social and non-social stimuli of high personal relevance.
Oxytocin (OT) plays a pivotal role in a variety of complex social behaviors by modulating approach-avoidance motivational tendencies, but recently, its social specificity has been challenged. Here, a randomized, double-blind, placebo-controlled study was conducted with forty young adult men, investigating the effect of a single-dose of OT (24 IU) on behavioral and neural approach-avoidance. Frontal alpha asymmetry, indexing neurophysiological approach-avoidance, was obtained from electroencephalographic recordings while participants were presented with a series of pictures, individually rated in terms of personal relevance (i.e., high versus low positive/negative emotional evocativeness) and categorized as social or non-social. Additionally, participants could prolong (approach) or shorten (avoid) the viewing-time of each picture, providing a measure of behavioral approach-avoidance. Intranasal OT enhanced both behavioral and neural approach (increased viewing-time), particularly towards negatively valenced pictures of both social and non-social nature, thus challenging the notion that OT's effects are specific to social stimuli. Neurally, OT specifically amplified approach-related motivational salience of stimuli that were self-rated to have high personal relevance, but irrespective of their social nature or rated affective valence (positive/negative). Together, these findings provide support to the General Approach-Avoidance Hypothesis of OT, suggesting a role of OT in amplifying the motivational salience of environmental stimuli with high (personal) relevance, but irrespective of their social/non-social nature.Clinical Trial Number: The study design was registered at ClinicalTrials.gov (NCT04443647; 23/06/2020; https://clinicaltrials.gov/ct2/show/NCT04443647).
Sci Rep, 2021; 11
34481326: Alaerts K, Taillieu A, Prinsen J, Daniels N
Tracking transient changes in the intrinsic neural frequency architecture: Oxytocin facilitates non-harmonic relationships between alpha and theta rhythms in the resting brain.
Shifts in the peak frequencies of oscillatory neural rhythms have been put forward as a principal mechanism by which cross-frequency coupling and decoupling is implemented in the brain. This notion is based on the mathematical reality that neural oscillations can only fully synchronize when their peak frequencies form harmonic 2:1 relationships (e.g., f=f/2). Non-

harmonic cross-frequency relationships, on the other hand (based on the irrational golden mean 1.618.:1), provide the highest physiologically possible desynchronized state (reducing the occurrence of spurious, noisy, background coupling), and are therefore anticipated to characterize the resting state of the brain, in which no selective information processing takes place. The present study sought to assess whether the transient occurrence of 1.6:1 non-harmonic and 2:1 harmonic relationships between peak frequencies in the alpha (8-14 Hz) and theta (4-8 Hz) bands - respectively facilitating states of decoupling or coupling between oscillatory rhythms - are impacted by the intranasal administration of a single-dose of oxytocin (OT) or placebo. To do so, continuous resting-state electroencephalography (5 min eyes open, 19 electrodes) was obtained from 96 healthy adult men before and after nasal spray administration. The transient formation of non-harmonic cross-frequency configurations between alpha and theta peak frequencies was significantly increased after OT nasal spray administration, indicating an effect of OT on reducing the intrinsic occurrence of spurious (noisy) background phase synchronizations during resting-state. As a group, the OT group also showed a significant parallel increase in high-frequency and decrease in low-frequency heart rate variability, confirming a homeostatic role of OT in balancing parasympathetic drive. Overall, non-harmonic cross-frequency configurations have been put forward to lay the ground for a healthy neural network allowing the opportunity for an efficient transition from resting state to activity. The observed effects of OT on cross-frequency dynamics are therefore interpreted to reflect a homeostatic role of OT in increasing the signal-to-noise properties of the intrinsic EEG neural frequency architecture, i.e., by precluding the occurrence of 'noisy', unwanted, spurious couplings among neural rhythms in the resting brain.
Psychoneuroendocrinology, 2021; 133
33235329: Prinsen J, Alaerts K
Enhanced mirroring upon mutual gaze: multimodal evidence from TMS-assessed corticospinal excitability and the EEG mu rhythm.
Previous research has demonstrated that eye contact between actor and observer specifically enhances the 'mirroring' of others' actions, as measured by transcranial magnetic stimulation (TMS)-induced motor evoked potentials (MEPs). However, it remains unknown whether other markers of mirror system activation, such as suppression of the EEG mu rhythm (8-13 Hz) over the sensorimotor strip, are also susceptible to perceived eye contact. Here, both TMS-induced MEPs and EEG mu suppression indices were assessed (in separate sessions) while 32 participants (mean age: 24y; 8m) observed a simple hand movement combined with direct or averted gaze from the actor. Both measures were significantly modulated by perceived eye gaze during action observation; showing an increase in MEP amplitude and an attenuation of the mu rhythm during direct vs. averted gaze. Importantly, while absolute MEP and mu suppression scores were not related, a significant association was identified between gaze-related changes in MEPs and mu suppression, indicating that both measures are similarly affected by the modulatory impact of gaze cues. Our results suggest that although the neural substrates underlying TMS-induced MEPs and the EEG mu rhythm may differ, both are sensitive to the social relevance of the observed actions, which might reflect a similar neural gating mechanism.
Sci Rep, 2020; 10
32161366: Alaerts K, Bernaerts S, Prinsen J, Dillen C, Steyaert J, Wenderoth N
Oxytocin induces long-lasting adaptations within amygdala circuitry in autism: a treatment-mechanism study with randomized placebo-controlled design.
Intranasal administration of the neuropeptide oxytocin (IN-OT) is increasingly explored as a potential treatment for targeting the core symptoms of autism spectrum disorder (ASD). To date, however, the impact of multiple-dose IN-OT treatment on human neural circuitry is largely unknown, and also the possibility that long-term IN-OT use may induce long-lasting neural adaptations remains unexplored. Using a double-blind, randomized, placebo-controlled, between-subject design (including 38 adult men with ASD), this treatment-mechanism study showed that 4 weeks of daily oxytocin administration (24 IU/day) significantly altered intrinsic (resting-state fMRI) functional connectivity of the amygdala to core regions of the "social brain" (particularly orbitofrontal cortex and superior temporal sulcus) up to 4 weeks and 1 year post treatment. The neural adaptations in functional coupling of the amygdala to the orbitofrontal cortex were associated with reduced feelings of avoidance toward others and-at the trend level-reduced repetitive behaviors. These observations contribute to a deeper mechanistic understanding of the neural substrates that underlie behavioral effects of multiple-dose IN-OT treatment, and provide initial insights into the long-lasting neural consequences of chronic IN-OT use on amygdala circuitry. Future studies are however warranted to further elucidate the long-term impact of IN-OT treatment on human neural circuitry and its behavioral consequences.
Neuropsychopharmacology, 2020; 45
31541686: Prinsen J, Deschepper A, Maes E, Alaerts K
Attachment styles have a modulatory impact on psychophysiological arousal evoked by reciprocated and unreciprocated gaze.
Gaze processing plays an essential role during social interactions. Here, it was investigated whether variations in attachment

style (secure, anxious and avoidant) were associated with differential expressions of sympathetic autonomic arousal upon live dyadic gaze interactions. To do so, 47 participants were presented with either reciprocated or unreciprocated eye gaze from a live model and skin conductance responses (SCRs) were collected. In line with previous observations, SCRs and subjective ratings of arousal were higher in response to reciprocated, compared to unreciprocated gaze. In terms of the modulation by attachment style, it was shown that participants with low attachment security and high attachment avoidance displayed overall higher sympathetic arousal upon the presentation of the live dyadic gaze cues, irrespective of whether the observed model showed reciprocal or unreciprocated gaze. Together, these observations indicate that attachment styles have a modulatory effect on individuals' psychophysiological responses to dyadic gaze interactions.
Biol Psychol, 2019; 148
31506688: Prinsen J, Alaerts K
Eye contact enhances interpersonal motor resonance: comparing video stimuli to a live two-person action context.
Previous research has shown a link between eye contact and interpersonal motor resonance, indicating that the mirroring of observed movements is enhanced when accompanied with mutual eye contact between actor and observer. Here, we further explored the role of eye contact within a naturalistic two-person action context. Twenty-two participants observed simple hand movements combined with direct or averted gaze presented via a live model in a two-person setting or via video recordings, while transcranial magnetic stimulation was applied over the primary motor cortex (M1) to measure changes in M1 excitability. Skin conductance responses and gaze behavior were also measured to investigate the role of arousal and visual attention herein. Eye contact significantly enhanced excitability of the observer's M1 during movement observation within a two-person setting. Notably, participants with higher social responsiveness (Social Communication subscale of the Social Responsiveness Scale) displayed a more pronounced modulation of M1 excitability by eye gaze. Gaze-related modulations in M1 excitability were, however, not associated with differences in visual attention or autonomic arousal. In summary, the current study highlights the effectiveness and feasibility of adopting paradigms with high ecological validity for studying the modulation of mirror system processes by subtle social cues, such as eye gaze.
Soc Cogn Affect Neurosci, 2019; 14
29885484: Bulthé J, Prinsen J, Vanderauwera J, Duyck S, Daniels N, Gillebert CR, Mantini D, Op de Beeck HP, De Smedt B
Multi-method brain imaging reveals impaired representations of number as well as altered connectivity in adults with dyscalculia.
Two hypotheses have been proposed about the etiology of neurodevelopmental learning disorders, such as dyslexia and dyscalculia: representation impairments and disrupted access to representations. We implemented a multi-method brain imaging approach to directly investigate these representation and access hypotheses in dyscalculia, a highly prevalent but understudied neurodevelopmental disorder in learning to calculate. We combined several magnetic resonance imaging methods and analyses, including univariate and multivariate analyses, functional and structural connectivity. Our sample comprised 24 adults with dyscalculia and 24 carefully matched controls. Results showed a clear deficit in the non-symbolic magnitude representations in parietal, temporal and frontal regions, as well as hyper-connectivity in visual brain regions in adults with dyscalculia. Dyscalculia in adults was thereby related to both impaired number representations and altered connectivity in the brain. We conclude that dyscalculia is related to impaired number representations as well as altered access to these representations.
Neuroimage, 2019; 190
29494953: Prinsen J, Brams S, Alaerts K
To mirror or not to mirror upon mutual gaze, oxytocin can pave the way: A cross-over randomized placebo-controlled trial.
The eyes constitute a highly salient cue to communicate social intent. Previous research showed that direct eye contact between two individuals can readily evoke an increased propensity to 'mirror' other peoples' actions. Considering the implicated role of the prosocial neuropeptide oxytocin (OXT) in enhancing the saliency of social cues and modulating approach/avoidance motivational tendencies, the current study adopted the non-invasive brain stimulation technique transcranial magnetic stimulation (TMS) to explore whether a single dose of intranasal OXT (24 IU) modulated (enhanced) a person's propensity to show heightened mirroring or motor resonance upon salient social cues, such as eye contact. The study involved a double-blind, placebo-controlled, cross-over trial with twenty-seven healthy adult men (19-32 y). By applying single-pulse TMS over the primary motor cortex during movement observation, it was shown that motor resonance was significantly higher when movement observation was accompanied by direct, compared to averted gaze, but that a single dose of OXT did not uniformly enhance this effect. Significant moderations of the treatment effect were noted however, indicating that participants with high self-reports of attachment avoidance displayed a stronger OXT-treatment effect (enhancement of motor resonance upon direct eye contact), compared to participants with low attachment avoidance. Particularly, while participants with high attachment avoidance initially displayed a reduced propensity to increase their motor resonance upon direct eye contact, a single dose of OXT was able to promote an otherwise avoidant individual's propensity to engage in motor resonance upon a salient social cue such as eye contact.

Psychoneuroendocrinology, 2018; 90
28131072: Bernaerts S, Prinsen J, Berra E, Bosmans G, Steyaert J, Alaerts K
Long-term oxytocin administration enhances the experience of attachment.
The neuropeptide 'oxytocin' (OT) is known to play a pivotal role in a variety of complex social behaviors by promoting a prosocial attitude and interpersonal bonding. Previous studies showed that a single-dose of exogenously administered OT can affect trust and feelings of attachment insecurity. With the present study, we explored the effects of two weeks of daily OT administration on measures of state and trait attachment using a double-blind between-subjects randomized placebo-controlled design. In 40 healthy young adult men state and trait attachment were assessed before and after two weeks of daily intranasal OT (24 IU) or placebo using the State Adult Attachment Scale and the Inventory of Parent and Peer Attachment. Mood, social responsiveness and quality of life were additionally assessed as secondary outcome measures. Reductions in attachment avoidance and increases in reports of attachment toward peers were reported after two weeks of OT treatment. Further, treatment-induced changes were most pronounced for participants with less secure attachment towards their peers. indicating that normal variance at baseline modulated treatment response. OT treatment was additionally associated with changes in mood, indicating decreases in feelings of tension and (tentatively) anger in the OT group, not in the placebo group. Further, at the end of the two-week trial, both treatment groups (OT, placebo) reported to experience an increase in social responsiveness and quality of life, but the effects were only specific to the OT-treatment in terms of reports on 'social motivation'. In summary, the observed improvements on state and trait dimensions of attachment after a multiple-dose treatment with OT provide further evidence in support of a pivotal role of OT in promoting the experience of attachment.
Psychoneuroendocrinology, 2017; 78
27939365: Prinsen J, Bernaerts S, Wang Y, de Beukelaar TT, Cuypers K, Swinnen SP, Alaerts K
Direct eye contact enhances mirroring of others' movements: A transcranial magnetic stimulation study.
Direct eye contact is a powerful social cue to regulate interpersonal interactions. Previous behavioral studies showed a link between eye contact and motor mimicry, indicating that the automatic mimicry of observed hand movements is significantly enhanced when direct eye contact exists between the observer and the observed model. In the present study, we aim to investigate the neurophysiological basis of the previously reported behavioral enhancements. Here, transcranial magnetic stimulation (TMS) was applied to assess changes in cortico-motor excitability at the level of the primary motor cortex (M1) to explore whether and how the motor system is facilitated from observing others' hand movements and, in particular, how this process is modulated by eye contact. To do so, motor evoked potentials (MEPs) were collected from two hand muscles while participants received single-pulse TMS and naturally observed video clips of an actor showing hand opening movements or static hands. During the observation, either direct or averted eye gaze was established between the subject and the observed actor. Our findings show a clear effect of eye gaze on observation-induced motor facilitation. This indicates that the mapping or 'mirroring' of others' movements is significantly enhanced when movement observation is accompanied by direct eye gaze compared to averted eye gaze. Our results support the notion that eye contact is a powerful social signal with the ability to direct human non-verbal social behavior. Furthermore, our findings are important for understanding the role of the mirror motor system in the mapping of socially relevant actions.
Neuropsychologia, 2017; 95

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BOARD NUMBER: S06-179

MALE-FEMALE DIFFERENCES IN SOCIAL BEHAVIOUR OF CNTNAP2 MUTANT MICE CORRELATE WITH DISRUPTED SYNAPTIC CONNECTIVITY IN THE ANTERIOR CINGULATE CORTEX AND INCREASED MICROGLIA ACTIVITY

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

Uwe Drescher[1], Matthew Dawson[1], Kevin Gordon-Fleet[1], Vera Tardos[1], Lingxin Yan[1], Zeinab Asgarian[1], Cathy Fernandes[2]
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Autism spectrum disorders (ASD) is a neurodevelopmental disorder encompassing impairments in social interaction, communication, and the exhibition of rigid and repetitive behaviours. Within the large heterogeneity observed for this disorder, there is an apparent higher incidence in boys than in girls, the aetiology of which is poorly understood. We have obtained data from analysis of a mouse line mutant for the ASD risk gene *Cntnap2*, which suggest that these mice are well suited to model the sex bias in ASD. Behavioural analyses uncover defects in social interactions in male, but not female *Cntnap2* mutant mice. This correlates with reduced spine densities of dendrites of pyramidal neurons in layer 1 of the anterior cingulate cortex (ACC) only in male mutant mice. This region represents an integration area of input from multiple brain regions engaged in processing social interaction, which has often been associated with ASD. Microglia, the brain resident immune cells, are known to be sexually dimorphic and to exert crucial functions in brain development by controlling synapse formation and pruning in an activity-dependent manner. Our analysis shows that in L1 of the ACC, microglia only of male mutant mice exert a higher phagocytotic activity, engulfing and removing synapses. Microglia activity appears unchanged comparing female wildtype and mutant mice. The ongoing focus of our lab is to better understand the nature of this sex-biased microglial activation, which leads to disrupted synapse formation in the ACC and reduced social interactions only in male *Cntnap2* mutant mice.

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BOARD NUMBER: S06-180

ENHANCED FEAR MAY LIMIT BEHAVIORAL FLEXIBILITY IN SHANK2 KNOCK-OUT MICE DESPITE OF INTACT LEARNING CAPABILITY

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Impaired behavioral flexibility has been proposed to underlie repetitive and restrictive patterns of behavior associated with autism spectrum disorder (ASD). We investigated whether and how behavioral flexibility is impaired in a mouse model of ASD by testing *Shank2*-knockout (*Shank2*-KO) mice in reversal learning. *Shank2*-KO mice were trained in probabilistic classical conditioning with two odor cues paired with water and air-puff deliveries. Upon the reversal of cue-outcome contingency, male *Shank2*-KO mice were significantly slower than wild-type control mice in reversing their anticipatory licking responses. Male *Shank2*-KO mice also showed stronger anticipatory eye closure responses than wild-type control mice to the air puff. In contrast, female *Shank2*-KO mice were intact in reversal learning and showed normal levels of anticipatory eye closure response. These results raise the possibility that impaired reversal learning in male Shank2-KO mice might be because of enhanced fear. Indeed, male *Shank2*-KO mice showed intact reversal learning when the strong air puff was replaced with a mild air puff. Male *Shank2*-KO mice also showed intact reversal learning between two odor cues predicting reward delivery with different probabilities. These results indicate that enhanced fear may suppress reversal learning despite of intact capability to learn cue-outcome contingency changes in male *Shank2*-KO mice. Our findings suggest that behavioral flexibility may be seriously limited by abnormal emotional responses in ASD.

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BOARD NUMBER: S06-181

SOCIAL DEFICIENCY AND ALTERATIONS OF CHOLINERGIC ACTIVITY IN THE MEDIAL PREFRONTAL CORTEX IN ADULT RAT PRENATALLY EXPOSED TO VALPROIC ACID.

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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The present study was designed to investigate the expression level of the cholinergic (alpha 7 nACh and M1) receptors in the medial prefrontal cortex (mPFC) in the behaviorally characterized rat prenatally exposed to valproic acid (VPA). Experiments were carried out on male offspring prenatally exposed to VPA at the age of 6 month. Sociability and preference for social novelty in VPA treated and control rats were evaluated in a three-chambered social interaction test. At the end of the behavioral experiments half of rats from each group were used in in immunoblotting (n = 6) studies. **Results.** The results of the sociability test showed that both groups spent more time in the compartment with an unfamiliar rat compared to an empty wire cage ($P < 0.001$). The results of the social novelty phase showed that the control rat spent more time in the compartment with an unfamiliar rat compared to familiar rat ($P < 0.001$). The preference for the social novelty in VPA treated rats was not statistically significant ($P = 0.377$). Immunoblotting studies revealed that the mean level of cholinergic (alpha 7 nACh and M1) receptors in the mPFC is significantly higher in VPA treated group as compared to control group ($p < 0.05$). **Summury.** Our results demonstrate that deficit of social behavior in the VPA induced rat model of autism is accompanied by significant changes in cholinergic activity in the mPFC. This work was supported by SRNSFG: Grant # - FR-18-14029

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BOARD NUMBER: S06-182

CHANGES IN SYNAPTIC PROTEINS IN A TRANSGENIC MODEL OF AUTISM

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Changes in the balance of excitatory and inhibitory neurotransmission, especially in the early stages of brain development, are considered to be part of the pathophysiology of autism. Recently, several studies have shown that synaptic abnormalities observed in experimental models of autism are associated with deficits in cytoskeletal and scaffolding proteins and their interactions. The aim of the present studies was to evaluate excitatory and inhibitory neurotransmission markers using transgenic "SH3 and multiple Ankyrin repeat domains 3" (*Shank3*) deficient mice that exhibit behavioral symptomatology of autism spectrum disorders. We demonstrated significant differences in the expression of excitatory glutamatergic markers and postsynaptic scaffolding proteins in pups and adult *Shank3* deficient mice depending on a brain region. We observed a decrease in the expression of inhibitory GABAergic markers and GABA receptor subunits in the brain of *Shank3* deficient mice. Further analyzes indicated lower levels of GABAergic markers in the dopaminergic regions of the brain of adult transgenic mice. We confirmed that the deficit in *Shank3* is accompanied by abnormalities in the number and length of neurites in hippocampal neurons. Transient silencing of *Shank3* gene expression has led to reduced levels of some adhesion synaptic proteins (neurexins, neuroligins). These results suggest that abnormalities in *Shank3* lead to an imbalance in the structural proteins involved in both excitatory and inhibitory synapses. Pathophysiological changes in inhibitory neurotransmission in dopaminergic areas of the brain need to be taken into account when explaining the etiology of autism spectrum disorders. Supported by APVV-20-0114, VEGA 2/0148/21, VEGA 2/0155/20, and SK-FR-19-0015.

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BOARD NUMBER: S06-183

BLOOD RNA SEQUENCING IDENTIFIES DYSREGULATED GENE EXPRESSION IN CHILDREN WITH AUTISM SPECTRUM DISORDER

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Mutations in over 100 genes are known to be implicated in autism spectrum disorder (ASD). Transcriptomics analysis of blood samples may offer clues for pathways dysregulated in ASD. To expand and validate findings of published RNA-sequencing (RNA-seq) studies, we performed RNA-seq of whole blood samples from a discovery cohort of eight male children with ASD compared with nine male age-matched neurotypical control children from the Shaare Zedek Medical Center (SZMC), Jerusalem, Israel. Our RNA-seq found 10 genes with differential expression in the ASD samples compared with controls. Using real-time qPCR, we compared whole blood samples from children with ASD and matched neurotypical children from SZMC for the 10 dysregulated genes detected by RNA-seq. Additionally, we compared our RNA-seq findings with whole blood samples of children with ASD from Phoenix Children's Hospital, Arizona, USA. Our qPCR analysis revealed dysregulated expression levels of several genes in the ASD compared to the control group (to be presented at FENS 2022). The implications of the dysregulated genes for pro-inflammatory phenotypes, immunity, and additional ASD-associated phenotypes will be discussed.

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BOARD NUMBER: S06-184

CDKL5'S ROLE IN MICROTUBULE-BASED TRANSPORT AND COGNITIVE FUNCTION

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Cyclin-dependent kinase like 5 (CDKL5) is a serine-threonine kinase highly enriched in mammalian neurons. *CDKL5* is located on the X-chromosome and its loss of function leads to a severe neurodevelopmental disorder called CDKL5 deficiency disorder (CDD). Our lab showed that CDKL5 phosphorylates the microtubule-associated protein MAP1S and regulates its binding to microtubules. How CDKL5 phosphorylation affects microtubule function is not well understood. To address this question, we generated MAP1S phosphomutant mice, in which the CDKL5 phosphorylation sites S786 and S812 are mutated to Alanine. Using microtubule co-sedimentation assay, we showed that dynein binding to microtubules is severely reduced in the CDKL5 KO and MAP1S phosphomutant brain lysates. Next, we studied dynein motility in CDKL5 KO or MAP1S phosphomutant neurons and their WT littermates using time-lapse imaging. Data showed impaired dynein motility in both CDKL5 KO and MAP1S phosphomutant neurons. It was previously reported that dendrite-specific cargo, AMPA receptor subunit GluA2, is mislocalized to axons when dynein is inhibited. We found that in HA-GluA2 transfected neurons, GluA2 localization in axons is significantly increased, supporting an impairment of dynein function. We also tested if endogenous cargo transport is altered by CDKL5 phosphorylation by using lysosomal protein Lamp1-RFP. Overall, Lamp1 transport was impaired in dendrites but not in axons. Polarized neurons rely on proper cargo transport and delivery to the synapse. This will regulate synapse formation, maintenance and function which is critical for cognitive function. Behavioral phenotyping of MAP1S phosphomutant mice showed increased anxiety, impaired motor performance, social and memory deficits.

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BOARD NUMBER: S06-185

THE NMDA RECEPTOR MODULATOR ZELQUISTINEL DURABLY RELIEVES BEHAVIORAL DEFICITS IN THREE MOUSE MODELS OF AUTISM.

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Aims: Autism spectrum disorders (ASD) are complex neurodevelopmental disorders characterized by deficient social communication and interaction together with restricted, stereotyped behaviors. Currently approved treatments relieve comorbidities rather than core symptoms. Since excitation/inhibition balance and synaptic plasticity are disrupted in ASD, molecules targeting excitatory synaptic transmission appear as highly promising candidates to treat this pathology. **Methods:** Among glutamatergic receptors, NMDA receptor has received particular attention along last decade through the development of novel modulators. Here, we evaluated the effects of positive NMDA receptor modulation by the spirocyclic β-lactam platform chemical zelquistinel (AGN-241751 or GATE-251) on autistic-like symptoms in two genetic and one environmental mouse models of ASD, using two routes of administration. **Results:** A single oral dose of zelquistinel rescued, in a dose-response manner, social deficits and stereotypic behavior in *Shank3*$^{\Delta ex13\text{-}16\text{-}/\text{-}}$ mice. Chronic intraperitoneal administration long-lastingly relieved autistic-like features in these mice. Subchronic mid-dose zelquistinel treatment demonstrated durable effects in *Shank3*$^{\Delta ex13\text{-}16\text{-}/\text{-}}$, *Fmr1*$^{-/-}$ and *in utero* valproate-exposed mice. Carry-over effects were best maintained in the *Fmr1* null mouse model, with social parameters being still fully recovered two weeks after treatment withdrawal. **Conclusions:** Among recently developed NMDA receptor modulators subunits, zelquistinel displays a promising therapeutic potential to relieve core symptoms in ASD patients, with oral bioavailability and long-lasting effects boding well for clinical applications. Efficacy in three mouse models with different etiologies supports high translational value. Further, this compound represents an innovative pharmacological tool to investigate plasticity mechanisms underlying behavioral deficits in animal models of ASD.

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BOARD NUMBER: S06-186

TWO AUTISM-RELATED MOUSE MODELS – DIFFERENCES IN THE HYPOTHALAMIC GENE EXPRESSION OF SYNAPTIC ADHESION MOLECULES AND INHIBITORY NEUROTRANSMITTER MARKERS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Autism spectrum disorder is a neurodevelopmental disease with heterogeneous etiology which is accompanied by defects in social communication and behaviour. Modifications in several genes affecting hypothalamic neuronal cell populations have been suggested to underlie neurodevelopmental abnormalities associated with autistic symptoms. The aim of the present study was to compare gene expression levels of synaptic cell-adhesion molecules and inhibitory neurotransmitter markers in the hypothalamus in two autism-related mouse models at postnatal day 5. *Shank3*- and *Magel2*-deficient mice were used, both known for displaying autism-like behavioral symptoms. We demonstrated significantly higher gene expression levels of cell-adhesion molecules *Neuroligin 2* and *Neurexins 1-2* in the hypothalamus isolated from *Shank3*-deficient mice compared to wild-type animals. Further analysis revealed lower expression levels of GABAergic markers (*Gad65*, *Gad67*) in *Shank3*-deficient mice. In contrast, we found higher gene expression levels of *Neuroligin 2* in *Magel2*-deficient mice, with no such changes observed in the gene expression of *Neurexin 1-2,* when compared to wild-type animals. Reduced expression of some inhibitory GABAergic markers (*Gad65, vGat*) and GABA receptor subunits (*Gabra2*) was found in *Magel2*-deficient mice. Overall, it appears that autism-like conditions are associated with a disrupted balance of cell-adhesion molecules essential for the formation of inhibitory synapses, which is reinforced by observation of altered expression GABAergic markers. In a view of our previous results using these mouse models, alterations in the ratio of excitatory and inhibitory synapses, especially in the early periods of postnatal brain development, are suggested in etiology of autism. Supported by VEGA 2/0148/21, VEGA 2/0155/20 and SK-FR-19-0015.

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BOARD NUMBER: S06-187

FACE PERCEPTION AND AUTISTIC TRAITS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Developmental prosopagnosia, or face blindness, can severely affect higher-order social functions. Prosopagnosia is more common in autism than in the general population and has been suggested to be a potential endophenotype. We hypothesized that poor face recognition predicts socio-communicative autistic traits in a dimensional rather than categorical manner. We recruited 157 adults to an online experiment using the Prolific.co and Gorilla.sc platforms. Subjective face processing deficits were measured with the Prosopagnosia Index 20 (PI-20), and face recognition accuracy was measured with the Oxford Face Matching Task (OFMT). Social and nonsocial (e.g., rigidity) autistic traits were measured with the Comprehensive Autistic Traits Inventory (CATI-Social and CATI-RRB factor scores, respectively). In support of the hypothesis, we found that social autistic traits predicted poor face recognition, whereas the non-social traits did not.

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BOARD NUMBER: S06-188

MODALITY-SPECIFIC ASSOCIATIONS OF SENSORY DEFICITS WITH AUTISTIC TRAITS

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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Atypical sensory processing measured by self- or parent-report co-segregates with quantitative autistic traits (QAT) and has potential endophenotypic properties. It is not known whether this reflects a generalized sensory dysfunction or combinations of modality-specific mechanisms. We combined a Bayesian variable selection method with dominance analysis to obtain a more nuanced understanding of modality-specific associations. We recruited two independent cohorts of adults online to complete the Broad Autism Phenotype Questionnaire and the Glasgow Sensory Questionnaire (GSQ). For each QAT, we performed stochastic search variable selection (SSVS) on GSQ subscales to test which modalities predicted QAT while controlling for uncertainty in the other variables. Dominance analysis was applied to the models identified by SSVS to evaluate the modalities' relative importance in predicting QAT. Only auditory scores reliably predicted all QATs when other modalities were accounted for. The proprioceptive scale, which included motor and interoceptive deficits, specifically predicted communicative QAT. The tactile scale, which included atypical touch/pain/temperature processing, specifically predicted social QAT. Although the findings must be interpreted in light of the limitations of the questionnaires, the study suggests that deficits in the auditory modality may be more likely than those in other senses to be a sensory endophenotype relevant to autism.

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¬FRAGILE X SYNDROME PATIENT-DERIVED NEURONS DEVELOPING IN THE MOUSE BRAIN SHOW FMR1 - DEPENDENT PHENOTYPES

POSTER SESSION 06 - SECTION: AUTISM SPECTRUM DISORDERS

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BACKGROUND Fragile X syndrome (FXS) is characterized by physical abnormalities, anxiety, intellectual disability, hyperactivity, autistic behaviors and seizures. Abnormal neuronal development in Fragile X syndrome (FXS) is poorly understood. Data on FXS patients remain scarce and FXS animal models have failed to yield successful therapies. *In vitro* models do not fully recapitulate the morphology and function of human neurons. METHODS To mimick human neuron development *in vivo*, we co-injected neural precursor cells from FXS patient-derived and corrected isogenic control induced pluripotent stem cells into the brain of neonatal immune-deprived mice. RESULTS The transplanted cells populated the brain and a proportion differentiated into neurons and glial cells. Immunofluorescence and single and bulk RNA sequencing analyses showed accelerated maturation of FXS neurons after an initial delay. Additionally, increased percentages of Arc- and Egr1-positive FXS neurons and wider dendritic protrusions of mature FXS striatal medium spiny neurons suggested an increase in synaptic activity and synaptic strength as compared to control. CONCLUSIONS This transplantation approach provides new insights into the alterations of neuronal development in FXS by facilitating physiological development of cells in a 3D context.

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BOARD NUMBER: S06-190

LOSS OF MCT4 IN MICROGLIA RESULTS IN ALTERED BRAIN DEVELOPMENT AND ANXIETY-LIKE BEHAVIOR

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Aims: Microglia, the tissue-resident macrophages of the central nervous system, actively participate in brain development by supporting neuronal maturation and refining synaptic connections. Accumulating evidence points towards the involvement of cellular metabolism in the regulation of immune cells, including microglia. In particular, lactate, which is key to sustain synaptic activity, also modulates responses of peripheral immune cells. However, its effect on microglia is largely unknown. Here, we aim to shed light on lactate's physiological roles in the modulation of microglial function. **Methods**: We combined confocal microscopy and biochemical assays to investigate how primary microglial cultures respond to lactate exposure. We next generated and characterized a conditional knock out (cKO) mouse model for the monocarboxylate transporter 4 (MCT4), aiming to selectively impair microglial lactate transport. **Results**: We found that lactate exposure leads to the upregulation of MCT4 in microglia. Exogenously given lactate is readily shuttled into primary microglia, and correlates with an increase in lysosomal acidification. Primary cKO microglia exhibit reduced uptake of amyloid beta, a well-known microglial cargo. Two weeks-old cKO mice present alterations in hippocampal microglial density and in CD68+ phagolysosomal structures. This is associated with increased levels of presynaptic markers, indicating that microglial MCT4 depletion is sufficient to affect neuronal circuitries development. In adulthood, cKO mice display an anxiety-like phenotype. **Conclusions**: In summary, this study highlights the importance of microglial MCT4 for brain maturation. Given the established role of microglia in neuropathology, a mechanistic understanding of lactate-dependent microglial modulation may be relevant for targeting microglia in brain diseases.

Pubmed:
34996673: Monsorno K, Buckinx A, Paolicelli RC
Microglial metabolic flexibility: emerging roles for lactate.
Microglia, the resident macrophages of the central nervous system (CNS), play important functions in the healthy and diseased brain. In the emerging field of immunometabolism, progress has been made in understanding how cellular metabolism can orchestrate the key responses of tissue macrophages, such as phagocytosis and inflammation. However, very little is known about the metabolic control of microglia. Lactate, now recognized as a crucial metabolite and a central substrate in metabolic flexibility, is emerging not only as a novel bioenergetic fuel for microglial metabolism but also as a potential modulator of cellular function. Parallels with macrophages will help in understanding how microglial lactate metabolism is implicated in brain physiology and pathology, and how it could be targeted for therapeutic purposes.
Trends Endocrinol Metab, 2022; 33
33992836: Ferlauto L, Vagni P, Fanelli A, Zollinger EG, Monsorno K, Paolicelli RC, Ghezzi D
All-polymeric transient neural probe for prolonged in-vivo electrophysiological recordings.
Transient bioelectronics has grown fast, opening possibilities never thought before. In medicine, transient implantable devices are interesting because they could eliminate the risks related to surgical retrieval and reduce the chronic foreign body reaction. Despite recent progress in this area, the potential of transient bioelectronics is still limited by their short functional lifetime owed to the fast dissolution rate of degradable metals, which is typically a few days or weeks. Here we report that a switch from degradable metals to an entirely polymer-based approach allows for a slower degradation process and a longer lifetime of the transient probe, thus opening new possibilities for transient medical devices. As a proof-of-concept, we fabricated all-polymeric transient neural probes that can monitor brain activity in mice for a few months, rather than a few days or weeks. Also, we extensively evaluated the foreign body reaction around the implant during the probe degradation. This kind of devices might pave the way for several applications in neuroprosthetics.
Biomaterials, 2021; 274

27610074: Provenzano G, Corradi Z, Monsorno K, Fedrizzi T, Ricceri L, Scattoni ML, Bozzi Y
Comparative Gene Expression Analysis of Two Mouse Models of Autism: Transcriptome Profiling of the BTBR and En2 (-/-) Hippocampus.
Autism spectrum disorders (ASD) are characterized by a high degree of genetic heterogeneity. Genomic studies identified common pathological processes underlying the heterogeneous clinical manifestations of ASD, and transcriptome analyses revealed that gene networks involved in synapse development, neuronal activity, and immune function are deregulated in ASD. Mouse models provide unique tools to investigate the neurobiological basis of ASD; however, a comprehensive approach to identify transcriptional abnormalities in different ASD models has never been performed. Here we used two well-recognized ASD mouse models, BTBR T(+) Itpr3 (tf) /J (BTBR) and Engrailed-2 knockout (En2 (-/-)), to identify conserved ASD-related molecular signatures. En2 (-/-) mice bear a mutation within the EN2 transcription factor homeobox, while BTBR is an inbred strain with unknown genetic defects. Hippocampal RNA samples from BTBR, En2 (-/-) and respective control (C57Bl/6J and En2 (+/+)) adult mice were assessed for differential gene expression using microarrays. A total of 153 genes were similarly deregulated in the BTBR and En2 (-/-) hippocampus. Mouse phenotype and gene ontology enrichment analyses were performed on BTBR and En2 (-/-) hippocampal differentially expressed genes (DEGs). Pathways represented in both BTBR and En2 (-/-) hippocampal DEGs included abnormal behavioral response and chemokine/MAP kinase signaling. Genes involved in abnormal function of the immune system and abnormal synaptic transmission/seizures were significantly represented among BTBR and En2 (-/-) DEGs, respectively. Interestingly, both BTBR and En2 (-/-) hippocampal DEGs showed a significant enrichment of ASD and schizophrenia (SCZ)-associated genes. Specific gene sets were enriched in the two models: microglial genes were significantly enriched among BTBR DEGs, whereas GABAergic/glutamatergic postsynaptic genes, FMRP-interacting genes and epilepsy-related genes were significantly enriched among En2 (-/-) DEGs. Weighted correlation network analysis (WGCNA) performed on BTBR and En2 (-/-) hippocampal transcriptomes together identified six modules significantly enriched in ASD-related genes. Each of these modules showed a specific enrichment profile in neuronal and glial genes, as well as in genes associated to ASD comorbidities such as epilepsy and SCZ. Our data reveal significant transcriptional similarities and differences between the BTBR and En2 (-/-) hippocampus, indicating that transcriptome analysis of ASD mouse models may contribute to identify novel molecular targets for pharmacological studies.
Front Neurosci, 2016; 10

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BOARD NUMBER: S06-191

ANALYSIS OF ALZHEIMER'S DISEASE-RELATED SYNAPTIC ALTERATIONS USING MICROFLUIDIC MICROGLIA/NEURON CO-CULTURES

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Aims Microglial cells are resident macrophages of the central nervous system. Genome-wide association studies identified numerous Alzheimer's disease (AD) risk genes highly or exclusively expressed in microglia, supporting their role as major players in AD pathogenesis. We aim to describe the effect of microglia activation on synaptic connectivity and its potential modulation by AD risk genes.
Methods We developed a microfluidic co-culture device where fluidically isolated, mature neuronal synapses are brought in contact with microglia, enabling exclusive genetic and pharmacological interventions to neurons and microglia. We cultured cortical neurons from WT embryonic mice in pre- and postsynaptic chambers for 7 days, after which primary microglia from 10-month-old WT and APP (hAPPJ20, PDGFAPPSw,Ind) mice were added into the synapse chamber. Microglia morphology and synaptic connectivity near microglia were analyzed via immunocytochemistry and confocal microscopy.
Results Exposure to lipopolysaccharide (LPS) activated microglia as evidenced by decreased surface area and increased circularity. Morphological changes were correlated with Interleukin-1 concentration in the medium, indicative of an inflammatory response. We observed a higher sensitivity to LPS in microglia from APP mice compared to WT mice. However, the level of neuronal network disruption near microglia was similar for WT and APP mice.
Conclusions Our microfluidic co-culture device enables specific modulation and high-content analysis of microglial activity and synaptic connectivity in physiological and AD pathological conditions. On-going work is focused on live-cell recordings with fluorescent microglia to characterize their dynamic response to amyloid-β and on harvesting microglia from mice carrying mutations for microglia-related AD risk genes.

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ABSENCE OF FKBP51 IN MURINE MICROGLIA LEADS TO IMPAIRED RESPONSE TO NEUROINFLAMMATORY STIMULUS

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Microglia are one of the major types of glial cells that are essential for normal functioning of the brain. They have a range of functions that extend from protecting the neurons from external threats like infections, to pruning synapses for proper neuronal communications. Microglia can have both anti-inflammatory as well as pro-inflammatory responses, which can lead to neurodegeneration, depending upon the stimulus. Here we studied the role of the co-chaperone FKBP51, a protein closely associated with the stress response and a modulator of glucocorticoid receptor function, in microglia. Mice with microglia specific deletion of FKBP51 were analyzed for behavioral changes using paradigms like Open Field Test, Elevated Plus Maze Test, Social Interaction Test and Home Cage Locomotion Test. They were subsequently exposed to bacterial lipo-polysaccharide (LPS) known to elicit inflammatory response and investigated for behavioral, histopathological and molecular changes. Analysis revealed that the microglia specific deletion of FKBP51 led to reduced anxiety-like behavior along with attenuated response to LPS featured by morphological differences in *Fkbp5*$^{-/-}$ microglia as compared to controls in-vivo, which was concurrent with an in-vitro model. These results not only throw light on the microglia function and homeostasis, but add to our understanding of the role of microglia in stress response on the whole.

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GOLEXANOLONE, A GABAA RECEPTOR MODULATING STEROID ANTAGONIST, REVERSES NEUROINFLAMMATION IN CEREBELLUM AND HIPPOCAMPUS AND RESTORES MOTOR COORDINATION AND COGNITIVE FUNCTION IN HYPERAMMONEMIC RATS

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Background: Neuroinflammation plays a main role in inducing neurological impairment in many pathological situations including hyperammonemia and hepatic encephalopathy. Hyperammonemic rats show increased GABAergic neurotransmission and neuroinflammation in cerebellum and hippocampus which lead to motor incoordination and cognitive impairment. We proposed that reducing GABAergic neurotransmission may reduce neuroinflammation in many pathologies. Golexanolone (also known as GR3027) reduces the potentiation of $GABA_A$ receptors by neurosteroids such as allopregnanolone. **Aims:** to assess if reducing GABAergic neurotransmission in hyperammonemic rats using golexanolone reduces neuroinflammation in hippocampus and cerebellum and restores cognitive function and motor coordination. **Methods:** rats were made hyperammonemic by feeding them an ammonium containing diet. After one week of hyperammonemia, golexanolone was administered daily, using intra-gastric probes. Motor coordination and cognitive function were analyzed after 2-3 weeks of treatment with golexanolone and neuroinflammation at 4 weeks of treatment. **Results:** Hyperammonemic rats show microglia and astrocytes activation in cerebellum associated with impaired motor coordination as assessed in the motorater and CatWalk. They also show microglia and astrocytes activation in hippocampus, associated with impaired spatial memory in the novel object location test and short-term memory in a Y maze. Treatment of hyperammonemic rats with golexanolone reversed microglia and astrocytes activation in cerebellum and hippocampus and restored motor coordination and spatial and short-term memory. **Conclusions:** reducing $GABA_A$ receptors activation by treatment with golexanolone could reduce neuroinflammation and improve cognitive and motor function in patients with hepatic encephalopathy and, likely, in other pathologies associated with neuroinflammation.

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BOARD NUMBER: S06-194

ASTROCYTE ROLE IN MAJOR DEPRESSIVE DISORDER

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Major depressive disorder (MDD) is a severe and debilitating mental illness with a very large socioeconomic impact worldwide. The neurobiology of this disease has been studied for a long time, focused on neuronal alterations; however, the underlying etiology is not yet fully understood. Astrocytes, a glial cell type, have been shown to play relevant roles in synaptic transmission and plasticity, with significant impact on behavioral responses. Evidence collected from human samples and animal models have shown that astrocytes shown particular alterations that might contribute to the pathophysiology of MDD. Here, we aim to investigate the role of astrocytes and their intracellular calcium signaling in this mental disease. Using a corticosterone treatment approach as depressive-like mouse model, we have evaluated the features of astrocyte Ca^{2+} signaling in medial prefrontal cortex (mPFC) from naïve and MDD mice, as well as the impact of astrocytic manipulations for animal behavior. Results: 1- *In vivo* spontaneous and behaviorally-related astrocyte Ca^{2+} signaling was altered in MDD mice. 2- Serotonin-evoked astrocyte Ca^{2+} dynamics were reduced in mPFC slices from MDD mice. 3- Selective chemogenetic activation of astrocytes by Gq-DREADDs in mPFC was able to restore the behavioral deficits shown by MDD mice. Although additional experiments are required, these results reveal the potential impact of astrocyte Ca^{2+} signaling in the pathophysiology of MDD.

Pubmed:
33651263: Gonzalez-Riano C, Tapia-González S, Perea G, González-Arias C, DeFelipe J, Barbas C
Metabolic Changes in Brain Slices over Time: a Multiplatform Metabolomics Approach.
Brain slice preparations are widely used for research in neuroscience. However, a high-quality preparation is essential and there is no consensus regarding stable parameters that can be used to define the status of the brain slice preparation after its collection at different time points. Thus, it is critical to fully characterize the experimental conditions for ex vivo studies using brain slices for electrophysiological recording. In this study, we used a multiplatform (LC-MS and GC-MS) untargeted metabolomics-based approach to shed light on the metabolome and lipidome changes taking place at different time intervals during the brain slice preparation process. We have found significant modifications in the levels of 300 compounds, including several lipid classes and their derivatives, as well as metabolites involved in the GABAergic pathway and the TCA cycle. All these preparation-dependent changes in the brain biochemistry related to the time interval should be taken into consideration for future studies to facilitate non-biased interpretations of the experimental results.
Mol Neurobiol, 2021; 58
32517777: Larramona-Arcas R, González-Arias C, Perea G, Gutiérrez A, Vitorica J, García-Barrera T, Gómez-Ariza JL, Pascua-Maestro R, Ganfornina MD, Kara E, Hudry E, Martinez-Vicente M, Vila M, Galea E, Masgrau R
Sex-dependent calcium hyperactivity due to lysosomal-related dysfunction in astrocytes from APOE4 versus APOE3 gene targeted replacement mice.
The apolipoprotein E (APOE) gene exists in three isoforms in humans: APOE2, APOE3 and APOE4. APOE4 causes structural and functional alterations in normal brains, and is the strongest genetic risk factor of the sporadic form of Alzheimer's disease (LOAD). Research on APOE4 has mainly focused on the neuronal damage caused by defective cholesterol transport and exacerbated amyloid-β and Tau pathology. The impact of APOE4 on non-neuronal cell functions has been overlooked. Astrocytes, the main producers of ApoE in the healthy brain, are building blocks of neural circuits, and Ca signaling is the basis of their excitability. Because APOE4 modifies membrane-lipid composition, and lipids regulate Ca channels, we determined whether APOE4 dysregulates Casignaling in astrocytes.
Mol Neurodegener, 2020; 15
32651909: Mederos S, González-Arias C, Perea G
Melanopsin for Time-Controlling Activation of Astrocyte -Neuron Networks.
Melanopsin, a mammalian G-protein-coupled photopigment, is a novel optical tool which enables studying astrocyte-neuron

networks. Here, we describe the required guidelines to take advantage of this promising optical tool for functional neuron-glia studies. The selective expression of melanopsin in astrocytes allows triggering astrocytic Ca signaling, changes in synaptic transmission, and modifying behavioral responses.
Methods Mol Biol, 2020; 2173
30542276: Mederos S, González-Arias C, Perea G
Astrocyte-Neuron Networks: A Multilane Highway of Signaling for Homeostatic Brain Function.
Research on glial cells over the past 30 years has confirmed the critical role of astrocytes in pathophysiological brain states. However, most of our knowledge about astrocyte physiology and of the interactions between astrocytes and neurons is based on the premises that astrocytes constitute a homogeneous cell type, without considering the particular properties of the circuits or brain nuclei in which the astrocytes are located. Therefore, we argue that more-sophisticated experiments are required to elucidate the specific features of astrocytes in different brain regions, and even within different layers of a particular circuit. Thus, in addition to considering the diverse mechanisms used by astrocytes to communicate with neurons and synaptic partners, it is necessary to take into account the cellular heterogeneity that likely contributes to the outcomes of astrocyte-neuron signaling. In this review article, we briefly summarize the current data regarding the anatomical, molecular and functional properties of astrocyte-neuron communication, as well as the heterogeneity within this communication.
Front Synaptic Neurosci, 2018; 10

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BOARD NUMBER: S06-195

VEGF-A/VEGFR-1: A PAINFUL ASTROCYTE-MEDIATED SIGNALING BLOCKED BY THE ANTI-VEGFR-1 MONOCLONAL ANTIBODY D16F7

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Aim. Chemotherapy-induced neuropathic pain is a clinically relevant adverse effect of several anticancer drugs leading to dose reduction or therapy discontinuation. The lack of knowledge about the mechanisms of neuropathy development and pain chronicization makes chemotherapy-induced neuropathic pain treatment an unmet medical need. In this context, the vascular endothelial growth factor A (VEGF-A) has emerged as a neurotoxicity biomarker in a model of chemotherapy-induced neuropathy, and its decrease has been related to pain relief. Aim was to clarify the VEGF-A-dependent pain signaling in the CNS for individuating new targeted therapeutic approaches. **Methods.** CD-1 male mice were used to investigate the role of VEGF-A/VEGFR-1 in pain signal using pharmacological and genetics tools. Models of neuropathy induced by anticancer drugs were used to study the pain relieving properties of the anti-VEGFR-1 mAb D16F7. **Results.** The intrathecal infusion of VEGF-A induced a dose-dependent noxious hypersensitivity mediated by the VEGF receptor 1 (VEGFR-1). In electrophysiological study, VEGF-A stimulated the spinal nociceptive neurons activity through VEGFR-1. In the spinal cord, VEGF-A increased in astrocytes of animals affected by neuropathy suggesting this cell population as a source of the potent pain mediator. Accordingly, the selective knockdown of astrocytic VEGF-A, by shRNAmir, blocked the development of oxaliplatin-induced neuropathic pain. Besides, D16F7 (previously described as anticancer) effectively relieved neuropathic pain induced by chemotherapeutic agents. **Conclusion.** Astrocyte-released VEGF-A is a new player in the complex neuron-glia network that oversees physiological and pathological pain and D16F7 mAb rises as a potent pain killer strategy.

Pubmed:
35149388: Micheli L, Parisio C, Lucarini E, Carrino D, Ciampi C, Toti A, Ferrara V, Pacini A, Ghelardini C, Di Cesare Mannelli L
Restorative and pain-relieving effects of fibroin in preclinical models of tendinopathy.
The term tendinopathy indicates a wide spectrum of conditions characterized by alterations in tendon tissue homeostatic response and damage to the extracellular matrix. The current pharmacological approach involves the use of nonsteroidal anti-inflammatory drugs and corticosteroids often with unsatisfactory results, making essential the identification of new treatments. In this study, the pro-regenerative and protective effects of an aqueous fibroin solution (0.5-500 μg/mL) against glucose oxidase (GOx)-induced damage in rat tenocytes were investigated. Then, fibroin anti-hyperalgesic and protective actions were evaluated in two models of tendinopathy induced in rats by collagenase or carrageenan injection, respectively. In vitro, 5-10 μg/mL fibroin per se increased cell viability and reverted the morphological alterations caused by GOx (0.1 U/mL). Fibroin 10 μg/mL evoked proliferative signaling upregulating the expression of decorin, scleraxin, tenomodulin ($p < 0.001$), FGF-2, and tenascin-C ($p < 0.01$) genes. Fibroin enhanced the basal FGF-2 and MMP-9 protein concentrations and prevented their GOx-mediated decrease. Furthermore, fibroin positively modulated the production of collagen type I. In vivo, the peri-tendinous injection of fibroin (5 mg) reduced the development of spontaneous pain and hypersensitivity ($p < 0.01$) induced by the intra-tendinous injection of collagenase; the efficacy was comparable to that of triamcinolone. The pain-relieving action of fibroin (peri-tendinous) was confirmed in the model of tendinopathy induced by carrageenan (intra-tendinous) where this fibrous protein was also able to improve tendon matrix organization, normalizing the orientation of collagen fibers. In conclusion, the use of fibroin in tendinopathies is suggested taking advantage of its excellent mechanical properties, pain-relieving effects, and ability to promote tissue regeneration processes.
Biomed Pharmacother, 2022; 148

34680083: Micheli L, Durante M, Lucarini E, Sgambellone S, Lucarini L, Di Cesare Mannelli L, Ghelardini C, Masini E
The Histamine H Receptor Participates in the Anti-Neuropathic Effect of the Adenosine A Receptor Agonist IB-MECA: Role of CD4 T Cells.
A adenosine receptor (AAR) agonists have emerged as potent relievers of neuropathic pain by a T cell-mediated production of IL-10. The H histamine receptor (HR), also implicated in pain modulation, is expressed on T cells playing a preeminent role in its activation and release of IL-10. To improve the therapeutic opportunities, this study aimed to verify the hypothesis of a possible cross-talk between AAR and HR in the resolution of neuropathic pain. In the mouse model of Chronic Constriction Injury (CCI), the acute intraperitoneal co-administration of the AAR agonist IB-MECA (0.5 mg/kg) and the HR agonist VUF 8430 (10 mg/kg), were additive in counteracting mechano-allodynia increasing IL-10 plasma levels. In HR mice, IB-MECA activity was reduced, lower pain relief and lower modulation of plasma IL-1β, TNF-α, IL-6 and IL-10 were shown. The complete anti-allodynia effect of IB-MECA in HR mice was restored after intravenous administration of CD4 T cells obtained from naïve wild type mice. In conclusion, a role of the histaminergic system in the mechanism of AAR-mediated neuropathic pain relief was suggested highlighting the driving force evoked by CD4 T cells throughout IL-10 up-regulation.
Biomolecules, 2021; 11
34649573: Micheli L, Parisio C, Lucarini E, Vona A, Toti A, Pacini A, Mello T, Boccella S, Ricciardi F, Maione S, Graziani G, Lacal PM, Failli P, Ghelardini C, Di Cesare Mannelli L
VEGF-A/VEGFR-1 signalling and chemotherapy-induced neuropathic pain: therapeutic potential of a novel anti-VEGFR-1 monoclonal antibody.
Neuropathic pain is a clinically relevant adverse effect of several anticancer drugs that markedly impairs patients' quality of life and frequently leads to dose reduction or therapy discontinuation. The poor knowledge about the mechanisms involved in neuropathy development and pain chronicization, and the lack of effective therapies, make treatment of chemotherapy-induced neuropathic pain an unmet medical need. In this context, the vascular endothelial growth factor A (VEGF-A) has emerged as a candidate neuropathy hallmark and its decrease has been related to pain relief. In the present study, we have investigated the role of VEGF-A and its receptors, VEGFR-1 and VEGFR-2, in pain signalling and in chemotherapy-induced neuropathy establishment as well as the therapeutic potential of receptor blockade in the management of pain.
J Exp Clin Cancer Res, 2021; 40
34312766: Micheli L, Rajagopalan R, Lucarini E, Toti A, Parisio C, Carrino D, Pacini A, Ghelardini C, Rajagopalan P, Di Cesare Mannelli L
Pain Relieving and Neuroprotective Effects of Non-opioid Compound, DDD-028, in the Rat Model of Paclitaxel-Induced Neuropathy.
Chemotherapy-induced neuropathy (CIN) is a major dose-limiting side effect of anticancer therapy that can compel therapy discontinuation. Inadequate analgesic efficacy of current pharmacological approaches requires the identification of innovative therapeutics and, hence, the purpose of this study is to conduct a preclinical evaluation of the efficacy of DDD-028, a versatile pentacyclic pyridoindole derivative, against paclitaxel-induced neuropathic pain. In two separate experiments, DDD-028 was administered per os acutely (1-25 mg kg) or repeatedly (10 mg kg) in paclitaxel-treated rats. The response to mechanical noxious stimulus (paw pressure) as well as to non-noxious mechanical (von Frey) and thermal (cold plate) stimuli was investigated. Acute administration of DDD-028 induced a dose-dependent anti-neuropathic pain effect in all tests performed. Further, repeated daily treatment for 18 consecutive days (starting the first day of paclitaxel administration) significantly reduced the development of pain over time without the development of tolerance to the anti-hyperalgesic effect. Ex vivo analysis showed that DDD-028 was able to reduce oxidative damage of dorsal root ganglia as evidenced by the increase in the level of carbonylated proteins and the decrease in catalase activity. In the lumbar spinal cord, periaqueductal gray matter, thalamus, and somatosensory cortex 1, DDD-28 significantly prevented the activation of microglia and astrocytes. The pharmacodynamic study revealed that the pain-relieving effects of DDD-028 were fully blocked by both the non-selective nicotinic receptor (nAChR) antagonist mecamylamine and by the selective α7 nAChR antagonist methyllycaconitine. In conclusion, DDD-028 was active in reducing paclitaxel-induced neuropathic pain after single or repeated administrations without tolerance development and displaying a double symptomatic and neuroprotective profile. DDD-028 could represent a valuable candidate for the treatment of CIN.
Neurotherapeutics, 2021; 18
33494253: Micheli L, Vasarri M, Barletta E, Lucarini E, Ghelardini C, Degl'Innocenti D, Di Cesare Mannelli L
Efficacy of Extract against Inflammatory Pain: In Vivo Studies in Mice.
(L.) Delile is traditionally used for its beneficial properties. Recently, promising antioxidant and anti-inflammatory biological properties emerged through studying the in vitro activity of the ethanolic leaves extract (POE). The present study aims to investigate the anti-inflammatory and analgesic role of POE in mice. Inflammatory pain was modeled in CD-1 mice by the intraplantar injection of carrageenan, interleukin IL-1β and formalin. Pain threshold was measured by von Frey and paw pressure tests. Nociceptive pain was studied by the hot-plate test. POE (10-100 mg kg) was administered per os. The paw

soft tissue of carrageenan-treated animals was analyzed to measure anti-inflammatory and antioxidant effects. POE exerted a dose-dependent, acute anti-inflammatory effect able to counteract carrageenan-induced pain and paw oedema. Similar anti-hyperalgesic and anti-allodynic results were obtained when inflammation was induced by IL-1β. In the formalin test, the pre-treatment with POE significantly reduced the nocifensive behavior. Moreover, POE was able to evoke an analgesic effect in naïve animals. Ex vivo, POE reduced the myeloperoxidase activity as well as TNF-α and IL-1β levels; further antioxidant properties were highlighted as a reduction in NO concentration. POE is the candidate for a new valid strategy against inflammation and pain.
Mar Drugs, 2021; 19
32572830: Micheli L, Di Cesare Mannelli L, Del Bello F, Giannella M, Piergentili A, Quaglia W, Carrino D, Pacini A, Ghelardini C
The Use of the Selective Imidazoline I Receptor Agonist Carbophenyline as a Strategy for Neuropathic Pain Relief: Preclinical Evaluation in a Mouse Model of Oxaliplatin-Induced Neurotoxicity.
Anti-cancer therapy based on the repeated administration of oxaliplatin is limited by the development of a disabling neuropathic syndrome with detrimental effects on the patient's quality of life. The lack of effective pharmacological approaches calls for the identification of innovative therapeutic strategies based on new targets. We focused our attention on the imidazoline I receptor (I-R) and in particular on the selective I-R agonist 2-(1-([1,1'-biphenyl]-2-yl)propan-2-yl)-4,5-dihydro-1H-imidazole) (carbophenyline). The purpose of this work was the preclinical evaluation of the efficacy of carbophenyline on oxaliplatin-induced neuropathic pain in mice. Carbophenyline, acutely per os administered (0.1-10 mg kg), induced a dose-dependent anti-hyperalgesic effect that was completely blocked by the pre-treatment with the I-R antagonist 3 or the I/α receptor antagonist efaroxan, confirming the I-R-dependent mechanism. Conversely, pre-treatment with the I-R antagonist BU224 did not block the anti-nociceptive effect evoked by carbophenyline. Repeated oral administrations of carbophenyline (1 mg kg) for 14 days, starting from the first day of oxaliplatin injection, counteracted the development of neuropathic pain in all behavioral tests (cold plate, Von Frey, and paw pressure tests) carried out 24 h after the last carbophenyline treatment on days 7 and 14. In the dorsal horn of the spinal cord, carbophenyline significantly decreased the oxaliplatin-induced astrocyte activation detected by immunofluorescence staining by the specific labelling with GFAP antibody. In conclusion, carbophenyline showed anti-neuropathic properties both after acute and chronic treatment with preventive effect against oxaliplatin-induced astrocyte activation in the spinal cord. Therefore, I-R agonists emerge as a new class of candidates for the management of oxaliplatin-induced neuropathic pain.
Neurotherapeutics, 2020; 17
33071790: Micheli L, Di Cesare Mannelli L, Lucarini E, Parisio C, Toti A, Fiorentino B, Rigamonti MA, Calosi L, Ghelardini C
Intranasal Low-Dose Naltrexone Against Opioid Side Effects: A Preclinical Study.
Opioids are broad spectrum analgesics that are an integral part of the therapeutic armamentarium to combat pain in the clinical practice. Unfortunately, together with analgesia, a number of adverse effects can occur such as nausea, vomiting, constipation, gastrointestinal alterations and cognitive impairments. Naltrexone is a competitive antagonist of opioid receptors commonly used to treat opioid addiction; its oral use against agonists side effects is limited by the decrease of opioids-therapeutic efficacy and own adverse effects. The intranasal delivery of naltrexone could offer a quick and effective achievement of CNS based on extracellular mechanisms including perineural and perivascular transport. The aim of the study was to test the efficacy of intranasal low-dose naltrexone in reducing intraperitoneal morphine and oxycodone side effects in rodents. In mice, 1 μg naltrexone intranasally administered 30 min before opioids reduced cognitive impairments and motor alteration induced by 10 mg kg morphine and 60 mg kg oxycodone in the Passive avoidance and Rota rod tests, respectively. Moreover, naltrexone rebalanced opioid-induced reduction of the intestinal transit and latency of feces expulsion as well as food intake inhibition. Importantly, 1 μg naltrexone instillation did not block analgesia as demonstrated by the Hot plate test. In rats, intranasal naltrexone counteracted the opioid-induced pica phenomenon related to emesis and increased water and palatable food intake. The effects were comparable to that achieved by metoclopramide used as reference drug. Treatments did not influence body weight. Lastly, the safety of the intranasal delivery has been checked by hematoxylin-eosin staining that did not show histological alterations of the nasal cavity. In conclusion, intranasal low-dose naltrexone counteracted morphine and oxycodone induced gastrointestinal and CNS side effects without impairing opioid analgesia. It is a candidate to be a valid clinical strategy deserving deep analysis.
Front Pharmacol, 2020; 11
32570937: Micheli L, Pacini A, Di Cesare Mannelli L, Trallori E, D'Ambrosio R, Bianchini C, Lampertico P, Ghelardini C
Treatment of Non-Alcoholic Steatosis: Preclinical Study of a New Nutraceutical Multitarget Formulation.
Multifactorial pathogenesis of non-alcoholic steatohepatitis (NASH) disease, a wide-spread liver pathology associated with metabolic alterations triggered by hepatic steatosis, should be hit by multitarget therapeutics. We tested a multicomponent food supplement mixture (AP-NHm), whose components have anti-dislipidemic, antioxidant and anti-inflammatory effects, on in vitro and in vivo models of NASH. In vitro, hepatic cells cultures were treated for 24 h with 0.5 mM oleic acid (OA): in the

co-treatment set cells were co-treated with AP-NH mixtures (AP-NHm, 1:3:10 ratio) and in the post-injury set AP-NHm was added for 48 h after OA damage. In vivo, C57BL/6 mice were fed with high-fat diet (HFD) for 12 weeks, inducing NASH at 7th week, and treated with AP-NHm at two dosages (1:3 ratio) in co-treatment or post-injury protocols, while a control group was fed with a standard diet. In in vitro co-treatment protocol, alterations of redox balance, proinflammatory cytokines release and glucose uptake were restored in a dose-dependent manner, at highest dosages also in post-injury regimen. In both regimens, pathologic dyslipidemias were also ameliorated by AP-NHm. In vivo, high-dose-AP-NHm-co-treated-HFD mice dose-dependently gained less body weight, were protected from dyslipidemia, and showed a lower liver weight. Dose-dependently, AP-NHm treatment lowered hepatic LDL, HDL, triglycerides levels and oxidative damage; co-treatment regimen was anti-inflammatory, reducing TNF-α and IL-8 levels. Hepatic lipidic infiltration significantly decreased in co-treated and post-injury-AP-NHm-HFD animals. The multitarget approach with AP-NHm was effective in preventing and reducing NASH-related pathologic features, warranting for the clinical development of this compound.
Nutrients, 2020; 12
32486519: Micheli L, Di Cesare Mannelli L, Mattoli L, Tamimi S, Flamini E, Garetto S, Lucci J, Giovagnoni E, Cinci L, D'Ambrosio M, Luceri C, Ghelardini C
Intra-Articular Route for the System of Molecules 14G1862 from : Pain Relieving and Protective Effects in a Rat Model of Osteoarthritis.
Current pharmacological therapies for the management of chronic articular diseases are far from being satisfactory, so new strategies need to be investigated. We tested the intra-articular pain relieving properties of a system of molecules from a characterized extract (14G1862) in a rat model of osteoarthritis induced by monoiodoacetate (MIA). 14G1862 (0.2-2 mg mL) was intra-articularly (i.a.) injected 7 days after MIA, behavioural and histological evaluations were performed 14, 30 and 60 days after treatments. Moreover, the effect of 14G1862 on nitrate production and iNOS expression in RAW 264.7 macrophages stimulated with LPS was assessed. , 14G1862 treatment attenuated LPS-induced NO production and iNOS expression in a comparable manner to celecoxib. , 14G1862 significantly reduced mechanical allodynia and hyperalgesia, spontaneous pain and motor alterations starting on day 14 up to day 60. The efficacy was higher or comparable to that evoked by triamcinolone acetonide (100 µg i.a.) used as reference drug. Histological evaluation highlighted the improvement of several morphological parameters in MIA + 14G1862-treated animals with particularly benefic effects on joint space and fibrin deposition. In conclusion, i.a. treatment with is a candidate to be a novel effective approach for osteoarthritis therapy.
Nutrients, 2020; 12
31374931: Micheli L, Lucarini E, Trallori E, Avagliano C, De Caro C, Russo R, Calignano A, Ghelardini C, Pacini A, Di Cesare Mannelli L
L. Extract: Alpha-Amylase Inhibition against Metabolic Syndrome in Mice.
To examine the effects of the alpha-amylase inhibitor isoform 1 called phaseolamin, a standardized extract from white kidney beans (L) was tested against the hallmarks of metabolic syndrome. The efficacy of a repeated treatment with extract (500 mg/kg) was compared with metformin (100 mg/kg) and atorvastatin (10 mg/kg) in a model of metabolic syndrome evoked by prolonged high fat diet (HFD; week 1 to week 19) in C57BL/6 mice. Bean extract and compounds administration started after metabolic syndrome establishment (week 11). extract reduced the body weight overtime, as well as effectively lowered glycaemia, triglycerides, and cholesterol. On week 19, bean extract normalized the HFD-evoked tolerance to glucose and insulin. According to the phytochemical characterization, it inhibited the alpha-amylase activity. Animals treated with the extract were rescued from motor impairments and nociceptive threshold alterations induced by HFD. Specific organs analysis revealed that extract decreased hepatic steatosis and lipid peroxidation in liver. It protected the heart from HFD oxidative alterations increasing the expression of the detoxifying enzymes catalase and glutathione reductase, and normalizing NADH dehydrogenase level. The histological analysis of aorta showed a protection about the development of fatty streaks in the muscular layers. In conclusion, a prolonged treatment with the standardized extract of significantly reduced several pathological features related to a metabolic syndrome-like condition; a multifactorial approach that candidates this vegetal product as a possible therapeutic option against metabolic syndrome.
Nutrients, 2019; 11

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BOARD NUMBER: S06-196

EFFECTS OF ADENOSINE A2A RECEPTOR ASTROCYTIC UPREGULATION IN THE MOUSE HIPPOCAMPUS

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

Agathe Launay[1], Kevin Carvalho[1], Thibaut Gauvrit[1], Bryan Thiroux[1], Didier Vieau[1], Emma Augustin[2], Karine Cambon[2], Alexis Bemelmans[3], Gilles Bonvento[2], Stephanie Le Gras[4], Jean-Sébastien Annicotte[5], Luc Buée[1], Emilie Faivre[1], David Blum[1]
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Introduction. Alzheimer's disease (AD) is notably characterized by the intraneuronal aggregation of tau proteins which play important roles in synaptic dysfunctions and memory decline. Studies have reported that chronic caffeine consumption reduces AD risk and cognitive deficits. These protective effects would be ascribed to the blockade of adenosine A_{2A} receptors (A_{2A}Rs) which are pathologically upregulated in the hippocampal astrocytes of AD patients and whose levels have been correlated with the development of cognitive deficits. However, the mechanisms underlying the link between astrocytic A_{2A} upregulation and cognitive deficits remain unknown. **Methods.** To uncover effects of astrocytic A_{2A}R upregulation, we intrahippocampally injected an AAV2/9 virus expressing A_{2A}R (AAV-A2A), or GFP as control (AAV-GFP), under a GFAabc1d astrocyte-specific promoter, in 2m-old C57Bl6/J mice. **Results/expected results.** Our data show that A_{2A}R overexpression in hippocampal astrocytes impairs short-term spatial memory (Y-Maze task) and long-term spatial learning (Barnes Maze task). At the network level, thanks to a DREADD approach, we observed an enhanced neuronal activability in the AAV-A2A hippocampus, compared to the control group, characterized by a higher immediate early gene expression. These changes were associated with altered astrocyte reactivity, morphology and transcriptome. **Conclusions.** Our results demonstrate that A_{2A}R upregulation in hippocampal astrocytes, as seen in the brain of AD patients, is sufficient to alter the astrocytic phenotype, neuronal response and memory. Further experiments in AD mouse models will determine how such A_{2A}R deregulation in astrocytes potentiate the development of AD lesions and their consequences, but also whether astrocytic A_{2A}R downregulation is sufficient to bring benefits.

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DISCOVERING THE BIOLOGICAL BASIS OF NEURONAL-ACTIVITY INDUCED MYELIN REPAIR

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

Dorien Maas[1], Blandine Manot-Saillet[1], Philippe Bun[1], Chloé Habermacher[1], Corinne Poilbout[1], Filippo Rusconi[2], Maria Cecilia Angulo[1,3]
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Enhanced neuronal activity in the healthy brain can induce oligodendrocyte differentiation and *de novo* myelination leading to behavioural changes. Accordingly, clinical studies have identified non-invasive brain stimulation as a therapeutic approach to ameliorate symptomatology in demyelinating diseases such as multiple sclerosis (MS). However, it is unknown whether the benefits of brain stimulation are due to improved myelin repair in this disease (Maas & Angulo, 2021, Front Cell Neurosci). Our lab has recently revealed that in lysolecithin demyelinated lesions in the mouse corpus callosum, optogenetic stimulation of demyelinated axons increases the number of differentiated oligodendrocytes and enhances the number of remyelinated axons (Ortiz et al., 2019, JCI Insight). Nevertheless, the exact manner in which neuronal activity instructs oligodendrocytes to differentiate and remyelinate these active axons remains unknown. To investigate this, we have performed calcium imaging in acute slices of demyelinated lesions in PDGFRαCreER +/- Gcamp6 lox/lox mice and found that at 7, 14 and 21 days post lysolecithin injection there was spontaneous calcium signaling in oligodendroglial cells in the lesion that was significantly enhanced after electrical stimulation of axons. We are presently dissecting the signaling pathways mediating neuron-oligodendroglia communication as well as testing whether these pathways are directly involved in activity-dependent remyelination *in vivo*. Support: L'Oreal for woman in science award, FRM post-doc fellowship, NMSS, ARSEP, EJP Rare Diseases.

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REACTIVE ASTROCYTOSIS AND SYNAPTIC DYSFUNCTION IN POLYAMINE CATABOLISM ACTIVATION

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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In the brain, polyamines play fundamental roles in neuron-astrocyte crosstalk. A transgenic mouse overexpressing the enzyme spermine oxidase (SMOX) in neocortical neurons, Dach-SMOX, was developed as model to study the effect of chronic activation of polyamine catabolism in the brain, generating evidence that these molecules are involved in vulnerability to excitotoxic and oxidative damages. **Aim**: This work aimed to investigate whether and how altered polyamine catabolism may impact synaptic transmission, focusing on neuronal and astrocytic counterparts. **Methods**: The effects of SMOX overexpression were evaluated in purified nerve terminals (synaptosomes) and astrocyte processes (gliosomes) from the neocortex of Dach-SMOX mice by assessing polyamine levels, ezrin and vimentin content, AMPA receptor activation, calcium influx, glutamate release, and catalase activity. **Results**: functional GluA2-lacking AMPA receptors are expressed on synaptosomes and gliosomes from Dach-SMOX, and in synaptosomes of control mice. A lower spermine content was observed in Dach-SMOX gliosomes, whereas in Dach-SMOX synaptosomes polyamine levels were unaffected, suggesting that astrocytes may play a pivotal role in maintaining neuronal polyamine levels. Moreover, the up-regulation of catalase, and the increased expression of vimentin and ezrin, suggest the presence of reactive astrocytosis in Dach-SMOX. This condition is correlated to neuronal suffering, characterized by a down-regulation of catalase in Dach-SMOX synaptosomes and impaired control of AMPA-evoked calcium response. **Conclusion**: Chronic activation of SMOX is responsible for crucial changes in astrocytes, including reduced spermine levels, AMPA-evoked calcium influx, and glutamate release. The consequent reactive astrocytosis leads to neuronal suffering and synaptic dysregulation, confirming polyamine involvement in healthy neuron-astrocyte communication.

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ASTROCYTE ACTIVITY TRIGGERS ADAPTIVE MYELIN PLASTICITY AND INCREASED NEURONAL EXCITABILITY IN THE SOMATOSENSORY CORTEX FOLLOWING SENSORY DEPRIVATION

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Recent data show that horizontal L2/3 axonal projections exhibit increased myelin segments elongation following monocular deprivation. Such adaptive myelin plasticity may improve synaptic efficacy of corticocortical connections with a possible role in cortical maps reorganization after sensory deprivation. Here, we explored the relationship between axonal myelination and cortical reorganization level in distinct layers of the somatosensory cortex following central sensory deprivation. Furthermore, we also investigated the ability of astrocytes, known to provide trophic factors for myelinating glia, to influence the adaptive myelin plasticity. Complete thoracic spinal cord was used to induce sensory deprivation of the cortical areas receiving information from hindlimbs. Fifteen-to-thirty days later, injured and control animals were subjected to electrophysiological recordings using a vertical array lowered into the hindlimb cortex to record evoked potentials in response to contralateral forelimb stimulation. Our experimental design aimed to determine the synaptic connectivity strength between the deprived and intact cortex. Our data showed that sensory deprivation enhanced L2/3 corticocortical connectivity observed as increased response magnitude and slope. Next, we explored whether the increased L2/3 synaptic efficacy was associated to myelin remodelling. While immunostaining against Olig2 showed no changes in deprived nor intact cortices, myelin basic protein staining showed increased intersections and horizontal myelination patterns, indicating increased myelination of corticocortical segments. These changes were not observed in $IP_3R2^{-/-}$ mice exhibiting deficient astrocyte activity, suggesting that astrocytes directly impact myelination. Overall, our data indicate a positive correlation among neuronal excitability and adaptive myelin plasticity that may mediate cortical reorganization through increased L2/3 corticocortical connectivity.

Pubmed:
34418097: Zaforas M, Rosa JM, Alonso-Calviño E, Fernández-López E, Miguel-Quesada C, Oliviero A, Aguilar J
Cortical layer-specific modulation of neuronal activity after sensory deprivation due to spinal cord injury.
Cortical areas have the capacity of large-scale reorganization following sensory deafferentation. However, it remains unclear whether this phenomenon is a unique process that homogeneously affects the entire deprived cortical region or whether it is susceptible to changes depending on neuronal networks across distinct cortical layers. Here, we studied how the local circuitry within each layer of the deafferented cortex forms the basis for neuroplastic changes after immediate thoracic spinal cord injury (SCI) in anaesthetized rats. In vivo electrophysiological recordings from deafferented hindlimb somatosensory cortex showed that SCI induces layer-specific changes mediating evoked and spontaneous activity. In supragranular layer 2/3, SCI increased gamma oscillations and the ability of these neurons to initiate up-states during spontaneous activity, suggesting an altered corticocortical network and/or intrinsic properties that may serve to maintain the excitability of the cortical column after deafferentation. On the other hand, SCI enhanced the infragranular layers' ability to integrate evoked sensory inputs leading to increased and faster neuronal responses. Delayed evoked response onsets were also observed in layer 5/6, suggesting alterations in thalamocortical connectivity. Altogether, our data indicate that SCI immediately modifies the local circuitry within the deafferented cortex allowing supragranular layers to better integrate spontaneous corticocortical information, thus modifying column excitability, and infragranular layers to better integrate evoked sensory inputs to preserve subcortical outputs. These layer-specific neuronal changes may guide the long-term alterations in neuronal excitability and plasticity associated with the rearrangements of somatosensory networks and the appearance of central sensory pathologies usually associated with spinal cord injury. KEY POINTS: Sensory stimulation of forelimb produces cortical evoked responses in the somatosensory hindlimb cortex in a layer-dependent manner. Spinal cord injury favours the input statistics of corticocortical connections between intact and deafferented cortices. After spinal cord injury supragranular layers exhibit better integration of spontaneous corticocortical information while infragranular layers exhibit better integration of evoked

sensory stimulation. Cortical reorganization is a layer-specific phenomenon.
J Physiol, 2021; 599

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BOARD NUMBER: S06-200

EVALUATION OF ASTROCYTES MORPHOLOGICAL CHANGES IN TAUOPATHIES

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Despite tau major expression in neurons in physiological condition, abnormal forms of tau protein are also detected in astrocytes in diverse form of tauopathies. Here, we aimed to investigate the consequences of primary neuronal tau pathology on astrocytes morphology. We injected intravenously Thy-Tau22 transgenic mice, expressing a double mutant form of the human tau protein in neurons, with a PHP.eB AAV to express tdTomato fluorescent protein specifically in astrocytes. We used three-dimensional (3D) reconstruction to perform a detailed analysis of astrocytes complex morphology in the hippocampus combining confocal microscopy and Imaris software analyses. At 9 months of age, tau astrocytes' arborisation is simplified as compared to WT, whereas it is more ramified at 24 months with more branching points. On going 3D sholl analyses show that morphological changes associated to tauopathy are subregion-dependent, with subicular astrocytes being particularly affected. Principal component analysis indicates that the ramification index and number of branches are essential morphological parameters discriminating WT and tau astrocytes. Interestingly, perivascular astrocytic endfeet diameter significantly changed with age in the subiculum. We expect to unravel how tau pathology-induced morphological changes in astrocytes could affect their functions and interactions with neurons.

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DEVELOPMENT OF AN AAV-BASED MODEL OF TAUOPATHY TARGETING RETINAL GANGLION CELLS AND THE MOUSE VISUAL PATHWAY TO STUDY THE ROLE OF MICROGLIA IN TAU SPREADING

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

Pauline Léal[1], Charlotte Duwat[1], Gwennaelle Aurégan[1], Charlène Joséphine[1], Marie-Claude Gaillard[1], Caroline Jan[1], Anne-Sophie Hérard[1], Emmanuel Brouillet[1], Philippe Hantraye[1], Gilles Bonvento[1], Karine Cambon[1], Alexis Bemelmans[2]
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Tauopathy is characterized by neurofibrillary tangles composed of intraneuronal aggregates of hyperphosphorylated Tau protein. In Alzheimer's disease (AD), the spreading of tauopathy being highly correlated with the severity of cognitive symptoms, slowing or stopping it might be a valuable therapeutic strategy. To better understand the underlying mechanisms of Tau spreading, and the role of microglia in this phenomenon, focal models obtained by AAV gene transfer are highly relevant. Several models targeting different areas of the central nervous system already exist. However, due to their physical properties, AAV viral particles tend to efficiently diffuse in the cerebral parenchyma, preventing the discrimination between transduced neurons and secondarily affected ones. Here, we targeted retinal ganglion cells (RGCs) to study Tau spreading in the visual pathway, allowing detection of Tau transgene expression all along the RGCs' axons, up to their target in the dorso-lateral geniculate nucleus (dLGN) and superior colliculus (SC) of the thalamus. Tau was hyperphosphorylated in RGCs, as evidenced by positive staining of pSer422 and AT-8 phospho-specific epitopes. Furthermore, cell-counting showed a progressive degeneration of RGCs. To study the role of microglia in tauopathy, we applied this model to TREM2-deficient mice. RGCs death was reduced in these mice, showing that microglial activation is at least in part responsible for neurodegeneration. Despite tauopathy development in RGCs, we were not able to detect Tau spreading to their target neurons in dLGN or SC. Hence, this model will be useful to study the neuron selective vulnerability to Tau propagation due to their nature or environment.

FENS Forum 2022
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BOARD NUMBER: S06-202

CHRONIC POSTNATAL HM3DQ-DREADD-MEDIATED ACTIVATION OF CAMKIIα-POSITIVE FOREBRAIN EXCITATORY NEURONS MODULATES ADULT GLIAL FUNCTION AND METABOLISM

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

Amartya Pradhan[1], Sthitapranjya Pati[1], Kamal Saba[2,3], Praachi Tiwari[1], Darshana Kapri[1], Anant Patel[2], Vidita Vaidya[1]
[1]Tata Institute of Fundamental Research, Department Of Biological Sciences, Mumbai, India, [2]Centre for Cellular and Molecular Biology, Nmr Microimaging And Spectroscopy, Hyderabad, India, [3]Academy of Scientific and Innovative Research, Council Of Scientific And Industrial Research, Ghaziabad, India

Early adversity increases vulnerability to developing adult psychopathology. Across multiple pre-clinical models of early adversity, glial dysfunction and disruption of amino acid neurotransmission systems have been implicated in contributing to the pathophysiology of mood disorders. Our recent study showed that enhancing Gq signaling in forebrain excitatory neurons during the postnatal window can profoundly impact the emergence of anxio-depressive behaviors, and perturb sensorimotor gating, accompanied by altered neuronal glutamate and GABA metabolism. Given prior evidence that glial dysfunction is associated with perturbed mood behaviors, we hypothesized that postnatally enhancing Gq signaling in the forebrain excitatory neurons may impact glial function in adulthood. To address this, we used a CaMKIIα-tTA::TetO-hM3Dq bigenic mouse line, wherein hM3Dq-DREADD is expressed in CaMKIIα-positive forebrain excitatory neurons, and administered clozapine-N-oxide or vehicle from postnatal day 2 to 14 to investigate the impact on glial metabolism and cytoarchitecture. We show that postnatal hM3Dq-mediated activation of forebrain excitatory neurons increases anxiety-like behavior. We also observe a significant decrease in glutamate and GABA metabolic fluxes in glia within the hippocampus and the neocortex using ^{1}H-[^{13}C]-NMR spectroscopy. Further, we are addressing the impact of enhanced postnatal Gq signaling in CaMKIIα-positive neurons on astrocyte cytoarchitecture and transcription of glia-associated genes. Our findings indicate that chemogenetically driving Gq signaling in the postnatal window in forebrain excitatory neurons results in enhanced anxiety-like behaviors in adulthood, correlated with disrupted glial glutamate and GABA metabolism, a feature that is implicated in pathogenesis of mood disorders.

Pubmed:

35115382: Tiwari P, Kapri D, Pradhan A, Balakrishnan A, Chaudhari PR, Vaidya VA
Chronic hM4Di-DREADD-Mediated Chemogenetic Inhibition of Forebrain Excitatory Neurons in Postnatal or Juvenile Life Does Not Alter Adult Mood-Related Behavior.
G-protein-coupled receptors (GPCRs) coupled to G signaling, in particular downstream of monoaminergic neurotransmission, are posited to play a key role during developmental epochs (postnatal and juvenile) in shaping the emergence of adult anxiodepressive behaviors and sensorimotor gating. To address the role of G signaling in these developmental windows, we used a CaMKIIα-tTA::TRE hM4Di bigenic mouse line to express the hM4Di-DREADD (designer receptor exclusively activated by designer drugs) in forebrain excitatory neurons and enhanced G signaling via chronic administration of the DREADD agonist, clozapine--oxide (CNO) in the postnatal window (postnatal days 2-14) or the juvenile window (postnatal days 28-40). We confirmed that the expression of the HA-tagged hM4Di-DREADD was restricted to CaMKIIα-positive neurons in the forebrain, and that the administration of CNO in postnatal or juvenile windows evoked inhibition in forebrain circuits of the hippocampus and cortex, as indicated by a decline in expression of the neuronal activity marker c-Fos. hM4Di-DREADD-mediated inhibition of CaMKIIα-positive forebrain excitatory neurons in postnatal or juvenile life did not impact the weight profile of mouse pups, and also did not influence the normal ontogeny of sensory reflexes. Further, postnatal or juvenile hM4Di-DREADD-mediated inhibition of CaMKIIα-positive forebrain excitatory neurons did not alter anxiety- or despair-like behaviors in adulthood and did not impact sensorimotor gating. Collectively, these results indicate that chemogenetic induction of G signaling in CaMKIIα-positive forebrain excitatory neurons in postnatal and juvenile temporal windows does not appear to impinge on the programming of anxiodepressive behaviors in adulthood.
eNeuro, 2022 Jan-Feb; 9
35098888: Saini S, Agarwal M, Pradhan A, Pareek S, Singh AK, Dhawan G, Dhawan U, Kumar Y
Exploring the role of framework mutations in enabling breadth of a cross-reactive antibody (CR3022) against the SARS-CoV-2 RBD and its variants of concern.

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Cross-reactive and broadly neutralizing antibodies against surface proteins of diverse strains of rapidly evolving viral pathogens like SARS-CoV-2 can prevent infection and therefore are crucial for the development of effective universal vaccines. While antibodies typically incorporate mutations in their complementarity determining regions during affinity maturation, mutations in the framework regions have been reported as players in determining properties of broadly neutralizing antibodies against HIV and the Influenza virus. We propose an increase in the cross-reactive potential of CR3022 against the emerging SARS- CoV-2 variants of concern through enhanced conformational flexibility. In this study, we use molecular dynamics simulations, mutagenesis, structural modeling, and docking to explore the role of light chain FWR mutations in CR3022, a SARS-CoV anti-spike (S)-protein antibody cross-reactive to the S-protein receptor binding domain of SARS-CoV-2. Our study shows that single substitutions in the light chain framework region of CR3022 with conserved epitopes across SARS-CoV strains allow targeting of diverse antibody epitope footprints that align with the epitopes of recently-categorized neutralizing antibody classes while enabling binding to more than one strain of SARS-CoV-2. Our study has implications for rapid and evolution-based engineering of broadly neutralizing antibodies and reaffirms the role of framework mutations in effective change of antibody orientation and conformation via improved flexibility.Communicated by Ramaswamy H. Sarma.
J Biomol Struct Dyn, 2022;

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-203

CHRONIC LITHIUM ADMINISTRATION IN THE TWITCHER MOUSE

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Krabbe disease (KD; or Globoid cell leukodystrophy) is an autosomal recessive lysosomal storage disorder caused by deficiency of the galactosylceramidase (GALC) enzyme. No cure is currently available for KD. Clinical applied treatments are supportive only. Recently, we demonstrated that two differently acting autophagy inducers (Lithium and Rapamycin) can improve some KD hallmarks in-vitro, laying the foundation for their in-vivo pre-clinical testing. Here, we test Lithium carbonate in-vivo, in the spontaneous mouse model for KD, the Twitcher (TWI) mouse. The drug is administered ad libitum via drinking water (600 mg/L) starting from post natal day 20. We longitudinally monitor the mouse motor performance through the grip strength, the hanging wire and the rotarod tests, and a set of biochemical parameters related to the KD pathogenesis [i.e. GALC enzymatic activity, psychosine (PSY) accumulation and astrogliosis]. Additionally, we investigate the expression of some crucial markers related to the two pathways that could be altered by Lithium: the autophagy and the β-catenin-dependent pathways. Results demonstrate that Lithium has limited rescue effects on the TWI phenotype, mostly related to muscle strength and body weight. We also show that Lithium, with this administration protocol, is unable to stimulate autophagy in the TWI mice central nervous system, whereas it can restore the β-catenin activation status in the TWI sciatic nerve. Overall, these results give for the first time data about the effect of an autophagy inducer on the TWI mouse, providing intriguing inputs to be considered for possible use of Lithium treatment as supportive therapy for KD.

Pubmed:
31799395: Del Grosso A, Galliani M, Angella L, Santi M, Tonazzini I, Parlanti G, Signore G, Cecchini M
Brain-targeted enzyme-loaded nanoparticles: A breach through the blood-brain barrier for enzyme replacement therapy in Krabbe disease.
Lysosomal storage disorders (LSDs) result from an enzyme deficiency within lysosomes. The systemic administration of the missing enzyme, however, is not effective in the case of LSDs with central nervous system (CNS)-involvement. Here, an enzyme delivery system based on the encapsulation of cross-linked enzyme aggregates (CLEAs) into poly-(lactide-co-glycolide) (PLGA) nanoparticles (NPs) functionalized with brain targeting peptides (Ang2, g7 or Tf2) is demonstrated for Krabbe disease, a neurodegenerative LSD caused by galactosylceramidase (GALC) deficiency. We first synthesize and characterize Ang2-, g7- and Tf2-targeted GALC CLEA NPs. We study NP cell trafficking and capability to reinstate enzymatic activity in vitro. Then, we successfully test our formulations in the Twitcher mouse. We report enzymatic activity measurements in the nervous system and in accumulation districts upon intraperitoneal injections, demonstrating activity recovery in the brain up to the unaffected mice level. Together, these results open new therapeutic perspectives for all LSDs with major CNS-involvement.
Sci Adv, 2019; 5
27638607: Del Grosso A, Antonini S, Angella L, Tonazzini I, Signore G, Cecchini M
Lithium improves cell viability in psychosine-treated MO3.13 human oligodendrocyte cell line via autophagy activation.
Globoid cell leukodystrophy (GLD) is a rare, rapidly progressing childhood leukodystrophy triggered by deficit of the lysosomal enzyme galactosylceramidase (GALC) and characterized by the accumulation of galactosylsphingosine (psychosine; PSY) in the nervous system. PSY is a cytotoxic sphingolipid, which leads to widespread degeneration of oligodendrocytes and Schwann cells, causing demyelination. Here we report on autophagy in the human oligodendrocyte cell line MO3.13 treated with PSY and exploitation of Li as an autophagy modulator to rescue cell viability. We demonstrate that PSY causes upregulation of the autophagic flux at the level of autophagosome and autolysosome formation and LC3-II expression. We show that pretreatment with Li, a drug clinically used to treat bipolar disorders, can further stimulate

autophagy, improving cell tolerance to PSY. This Li protective effect is found not to be linked to reduction of PSY-induced oxidative stress and might not stem from a reduction of PSY accumulation. These data provide novel information on the intracellular pathways activated during PSY-induced toxicity and suggest the autophagy pathway as a promising novel therapeutic target for ameliorating the GLD phenotype. © 2016 Wiley Periodicals, Inc.
J Neurosci Res, 2016; 94
29894633: Galliani M, Santi M, Del Grosso A, Cecchettini A, Santorelli FM, Hofmann SL, Lu JY, Angella L, Cecchini M, Signore G
Cross-Linked Enzyme Aggregates as Versatile Tool for Enzyme Delivery: Application to Polymeric Nanoparticles.
Polymeric nanoparticles (NPs) represent one of the most promising tools in nanomedicine and have been extensively studied for the delivery of water-insoluble drugs. However, the efficient loading of therapeutic enzymes and proteins in polymer-based nanostructures remains an open challenge. Here, we report a synthesis method for a new enzyme delivery system based on cross-linked enzyme aggregates (CLEAs) encapsulation into poly(lactide- co-glycolide) (PLGA) NPs. We tested the encapsulation strategy on four enzymes currently investigated for enzyme replacement therapy: palmitoyl protein thioesterase 1 (PPT1; defective in NCL1 disease), galactosylceramidase (GALC; defective in globoid cell leukodystrophy), alpha glucosidase (aGLU; defective in Pompe disease), and beta glucosidase (bGLU; defective in Gaucher's disease). We demonstrated that our system allows encapsulation of enzymes with excellent activity retention (usually around 60%), thus leading to functional and targeted nanostructures suitable for enzyme delivery. We then demonstrated that CLEA NPs efficiently deliver PPT1 in cultured cells, with almost complete enzyme release occurring in 48 h. Finally, we demonstrated that enzymatic activity is fully recovered in primary NCL1 fibroblasts upon treatment with PPT1 CLEA NPs.
Bioconjug Chem, 2018; 29
31108173: Del Grosso A, Angella L, Tonazzini I, Moscardini A, Giordano N, Caleo M, Rocchiccioli S, Cecchini M
Dysregulated autophagy as a new aspect of the molecular pathogenesis of Krabbe disease.
Krabbe disease (KD) is a childhood leukodystrophy with no cure currently available. KD is due to a deficiency of a lysosomal enzyme called galactosyl-ceramidase (GALC) and is characterized by the accumulation in the nervous system of the sphingolipid psychosine (PSY), whose cytotoxic molecular mechanism is not fully known yet. Here, we study the expression of some fundamental autophagy markers (LC3, p62, and Beclin-1) in a KD murine model [the twitcher (TWI) mouse] by immunohistochemistry and Western blot. Moreover, the autophagy molecular process is also shown in primary fibroblasts from TWI and WT mice, with and without PSY treatment. Data demonstrate that large p62 cytoplasmic aggregates are present in the brain of both early and late symptomatic TWI mice. p62 expression is also upregulated in TWI sciatic nerves compared to that measured for WT nerves. In vitro data suggest that this effect might not be fully PSY-driven. Finally, we investigate in vitro the capability of autophagy inducers (Rapamycin, RAP and Resveratrol, RESV) to reinstate the WT phenotype in TWI cells. We show that RAP administration can partially restore the autophagy markers levels, while RESV cannot, indicating a line along which new therapeutic approaches can be developed.
Neurobiol Dis, 2019; 129
33374753: Tonazzini I, Cerri C, Del Grosso A, Antonini S, Allegra M, Caleo M, Cecchini M
Visual System Impairment in a Mouse Model of Krabbe Disease: The Twitcher Mouse.
Krabbe disease (KD, or globoid cell leukodystrophy; OMIM #245200) is an inherited neurodegenerative condition belonging to the class of the lysosomal storage disorders. It is caused by genetic alterations in the gene encoding for the enzyme galactosylceramidase, which is responsible for cleaving the glycosydic linkage of galatosylsphingosine (psychosine or PSY), a highly cytotoxic molecule. Here, we describe morphological and functional alterations in the visual system of the Twitcher (TWI) mouse, the most used animal model of Krabbe disease. We report in vivo electrophysiological recordings showing defective basic functional properties of the TWI primary visual cortex. In particular, we demonstrate a reduced visual acuity and contrast sensitivity, and a delayed visual response. Specific neuropathological alterations are present in the TWI visual cortex, with reduced myelination, increased astrogliosis and microglia activation, and around the whole brain. Finally, we quantify PSY content in the brain and optic nerves by high-pressure liquid chromatography-mass spectrometry methods. An increasing PSY accumulation with time, the characteristic hallmark of KD, is found in both districts. These results represent the first complete characterization of the TWI visual system. Our data set a baseline for an easy testing of potential therapies for this district, which is also dramatically affected in KD patients.
Biomolecules, 2020; 11
30649861: Begarani F, D'Autilia F, Signore G, Del Grosso A, Cecchini M, Gratton E, Beltram F, Cardarelli F
Capturing Metabolism-Dependent Solvent Dynamics in the Lumen of a Trafficking Lysosome.
The eukaryotic cell compartmentalizes into spatially confined, membrane-enclosed, intracellular structures (e. g., organelles, endosomes, and vesicles). Here, peculiar physicochemical properties of the local environment occur and participate in the regulation of ongoing molecular processes. In spite of the huge amount of available environmental probes, experiments on subcellular structures are severely challenged by their three-dimensional (3D) movement. This bottleneck is tackled here by

focusing an excitation light beam in a periodic orbit around the structure of interest. The recorded signal is used as feedback to localize the structure position at high temporal resolution: microseconds along the orbit, milliseconds between orbits. The lysosome is selected as the intracellular target, together with 6-acetyl-2-dimethylaminonaphthalene (ACDAN) as probe of the physicochemical properties of the intralysosomal environment. Generalized polarization (GP) analysis of ACDAN emission is used to get a quantitative view on intralysosomal solvent dipolar relaxation. Thus, raster image correlation spectroscopy (RICS) analysis reveals that the ACDAN GP signal is fluctuating in the micro-to-millisecond time range during natural organelle 3D trafficking. We show that ACDAN GP fluctuations are characteristic of lysosomes in living cells, are selectively abolished by lysosomal basification, and depend on metabolic energy in the form of ATP. We argue that intralysosomal ACDAN GP fluctuates according to the ongoing organelle metabolism. Indeed, we report alterations in amplitude and timing of GP fluctuations in a cellular model of lysosomal storage disorder (LSD). The strategy proposed provides insight into the elusive local environment of a trafficking lysosome and supports similar molecular investigations at the subcellular level.
ACS Nano, 2019; 13
28413720: Parenti N, Del Grosso A, Antoni C, Cecchini M, Corradetti R, Pavone FS, Calamai M
Direct imaging of APP proteolysis in living cells.
Alzheimer's disease is a multifactorial disorder caused by the interaction of genetic, epigenetic and environmental factors. The formation of cytotoxic oligomers consisting of A peptide is widely accepted as being one of the main key events triggering the development of Alzheimer's disease. A peptide production results from the specific proteolytic processing of the amyloid precursor protein (APP). Deciphering the factors governing the activity of the secretases responsible for the cleavage of APP is still a critical issue. Kits available commercially measure the enzymatic activity of the secretases from cells lysates, . By contrast, we have developed a prototypal rapid bioassay that provides visible information on the proteolytic processing of APP directly in living cells. APP was fused to a monomeric variant of the green fluorescent protein and a monomeric variant of the red fluorescent protein at the C-terminal and N-terminal (mChAPPmGFP), respectively. Changes in the proteolytic processing rate in transfected human neuroblastoma and rat neuronal cells were imaged with confocal microscopy as changes in the red/green fluorescence intensity ratio. The significant decrease in the mean red/green ratio observed in cells over-expressing the -secretase BACE1, or the -secretase ADAM10, fused to a monomeric blue fluorescent protein confirms that the proteolytic site is still accessible. Specific siRNA was used to evaluate the contribution of endogenous BACE1. Interestingly, we found that the degree of proteolytic processing of APP is not completely homogeneous within the same single cell, and that there is a high degree of variability between cells of the same type. We were also able to follow with a fluorescence spectrometer the changes in the red emission intensity of the extracellular medium when BACE1 was overexpressed. This represents a complementary approach to fluorescence microscopy for rapidly detecting changes in the proteolytic processing of APP in real time. In order to allow the discrimination between the - and the -secretase activity, we have created a variant of mChAPPmGFP with a mutation that inhibits the -secretase cleavage without perturbing the -secretase processing. Moreover, we obtained a quantitatively robust estimate of the changes in the red/green ratio for the above conditions by using a flow cytometer able to simultaneously excite and measure the red and green fluorescence. Our novel approach lay the foundation for a bioassay suitable to study the effect of drugs or particular conditions, to investigate in an unbiased way the the proteolytic processing of APP in single living cells in order, and to elucidate the causes of the variability and the factors driving the processing of APP.
PeerJ, 2017; 5
35028271: Del Grosso A, Parlanti G, Angella L, Giordano N, Tonazzini I, Ottalagana E, Carpi S, Pellegrino RM, Alabed HBR, Emiliani C, Caleo M, Cecchini M
Chronic lithium administration in a mouse model for Krabbe disease.
Krabbe disease (KD; or globoid cell leukodystrophy) is an autosomal recessive lysosomal storage disorder caused by deficiency of the galactosylceramidase (GALC) enzyme. No cure is currently available for KD. Clinical applied treatments are supportive only. Recently, we demonstrated that two differently acting autophagy inducers (lithium and rapamycin) can improve some KD hallmarks in-vitro, laying the foundation for their in-vivo pre-clinical testing. Here, we test lithium carbonate in-vivo, in the spontaneous mouse model for KD, the Twitcher (TWI) mouse. The drug is administered ad libitum via drinking water (600 mg/L) starting from post natal day 20. We longitudinally monitor the mouse motor performance through the grip strength, the hanging wire and the rotarod tests, and a set of biochemical parameters related to the KD pathogenesis [i.e., GALC enzymatic activity, psychosine (PSY) accumulation and astrogliosis]. Additionally, we investigate the expression of some crucial markers related to the two pathways that could be altered by lithium: the autophagy and the β-catenin-dependent pathways. Results demonstrate that lithium has not a significant rescue effect on the TWI phenotype, although it can slightly and transiently improves muscle strength. We also show that lithium, with this administration protocol, is unable to stimulate autophagy in the TWI mice central nervous system, whereas results suggest that it can restore the β-catenin activation status in the TWI sciatic nerve. Overall, these data provide intriguing inputs for further evaluations of lithium treatment in TWI mice.

JIMD Rep, 2022; 63
30926673: Pellegrini D, Del Grosso A, Angella L, Giordano N, Dilillo M, Tonazzini I, Caleo M, Cecchini M, McDonnell LA
Quantitative Microproteomics Based Characterization of the Central and Peripheral Nervous System of a Mouse Model of Krabbe Disease.
Krabbe disease is a rare, childhood lysosomal storage disorder caused by a deficiency of galactosylceramide beta-galactosidase (GALC). The major effect of GALC deficiency is the accumulation of psychosine in the nervous system and widespread degeneration of oligodendrocytes and Schwann cells, causing rapid demyelination. The molecular mechanisms of Krabbe disease are not yet fully elucidated and a definite cure is still missing. Here we report the first in-depth characterization of the proteome of the Twitcher mouse, a spontaneous mouse model of Krabbe disease, to investigate the proteome changes in the Central and Peripheral Nervous System. We applied a TMT-based workflow to compare the proteomes of the corpus callosum, motor cortex and sciatic nerves of littermate homozygous Twitcher and wild-type mice. More than 400 protein groups exhibited differences in expression and included proteins involved in pathways that can be linked to Krabbe disease, such as inflammatory and defense response, lysosomal proteins accumulation, demyelination, reduced nervous system development and cell adhesion. These findings provide new insights on the molecular mechanisms of Krabbe disease, representing a starting point for future functional experiments to study the molecular pathogenesis of Krabbe disease. Data are available via ProteomeXchange with identifier PXD010594.
Mol Cell Proteomics, 2019; 18
26729821: Lai M, Pifferi M, Bush A, Piras M, Michelucci A, Di Cicco M, del Grosso A, Quaranta P, Cursi C, Tantillo E, Franceschi S, Mazzanti MC, Simi P, Saggese G, Boner A, Pistello M
Gene editing of DNAH11 restores normal cilia motility in primary ciliary dyskinesia.
Primary ciliary dyskinesia (PCD) is a rare autosomal recessive genetic disorder characterised by dysfunction of motile cilia. Ciliary dysmotility causes poor mucociliary clearance and leads to impairment of pulmonary function and severe respiratory infections. PCD has no specific therapy. With the aim to permanently restore gene function and normalise ciliary motility, we used gene editing to replace mutated with wild-type sequence in defective cells.
J Med Genet, 2016; 53

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-204

REACTIVE ASTROCYTES ACQUIRE BENEFICIAL ANTI-AGGREGATION PROPERTIES THROUGH THE JAK2-STAT3 PATHWAY IN HUNTINGTON'S DISEASE

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

María-Ángeles Carrillo-De Sauvage, Laurene Abjean, Miriam Riquelme-Pérez, Lucile Ben Haim, Pauline Gipchtein, Fanny Petit, Anne-Sophie Hérard, Martine Guillermier, Mylène Gaudin, Sueva Bernier, Cameron Héry, Noelle Dufour, Charlène Joséphine, Gilles Bonvento, Alexis Bemelmans, Philippe Hantraye, Julien Flament, Emmanuel Brouillet, Carole Escartin
Laboratoire des maladies neurodégénératives UMR 9199, CNRS, CEA, Université Paris Xi, Fontenay-aux-roses, France

Huntington's disease (HD) is an inherited neurodegenerative disease, characterized by the presence of intracellular aggregates of mutant huntingtin (mHtt). The degeneration of specific neuronal populations in the striatum and cortex is the main cause for clinical symptoms. However, astrocytes may also play a key role in HD. Astrocytes become reactive in response to virtually all pathological situations in the central nervous system. In the brain of HD patients, there is a progressive increase in reactive astrocytes and transcriptomics analysis reveals significant molecular changes in HD astrocytes. The JAK2-STAT3 pathway is a central cascade controlling the reactive response of astrocytes in the brain. We found STAT3 activation in astrocytes in the putamen of HD patients. To pinpoint the role of reactive astrocytes in HD, we generated viral vectors that infect astrocytes in the mouse striatum and activate or inhibit the JAK2-STAT3 pathway. We show that JAK2-STAT3-reactive astrocytes reduce the number and size of mHtt aggregates in striatal neurons in different mouse models of HD, and improved several HD pathological hallmarks. We found that the JAK2-STAT3 pathway controls the expression of proteolytic enzymes in HD astrocytes, enhances proteosomal and lysosomal activities and increase astrocyte capacity to clear mHtt. We also found that JAK2-STAT3-dependent reactive astrocytes produce chaperones, which can be transferred to neurons through exosomes to promote mHtt clearance. We show that astrocytes, through the JAK2-STAT3 pathway, with enhanced proteolysis capacity and anti-aggregation function, can engage in protective reactive responses that could be promoted for HD therapy.

FENS Forum 2022
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BOARD NUMBER: S06-205

CHRONIC GLIAL ACTIVATION CAUSES CHOLINERGIC CELL LOSS IN THE BASAL FOREBRAIN DURING PATHOLOGICAL AGING

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

Rashmi Gamage[1], Ryan Childs[1], Gintara Hornas[1], Ingrid Wagnon[1], Garry Niedermayer[2], Gerald Muench[3], Erika Gyengesi[3]
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Aims: Neuroinflammation leads to gradual neurodegeneration during pathological aging, contributing to disorders like Alzheimer's disease (AD). Basal forebrain cholinergic neuronal (BFCN) loss is associated with AD; however, the underlying mechanism is still unknown. This study investigated the effects of both aging and chronic neuroinflammation on the BFCN, focusing on the medial septum (MS). **Method:** Aged, 12- and 24-months WT and GFAP-IL6 mice (overexpressing IL-6 under the GFAP promoter) were used. Immunohistochemistry combined with unbiased stereology and 3D morphology analysis was performed to estimate the number and morphology of $Iba1^+$ microglia and $ChAT^+$ in the MS, combined with transcriptomic analysis using qPCR. **Results:** Stereological estimation of microglia number displayed a significant increase both at 12 and 24 months in GFAP-IL6 compared to WT. We found a significant, 28% and 38% decrease, in microglial 3D surface area, and volume between WT and GFAP-IL respectively at both 12 and 24 months, indicating increased glial reactivity. Meanwhile, BFCN in the MS displayed a significant, 31% and 30%, decrease in GFAP-IL6 compared to WT, respectively, at both 12 and 24 months. A significant 58% and 67% reduction in the 3D dendritic field surface area, and volume, respectively between WT and GFAP-IL at 12 months; and a significant 41% and 46% decrease between WT at 12 and 24 months respectively, revealing neuronal degeneration. **Conclusion:** We report evidence of the significant impact of both aging and chronic glial activation on the microglial and BFCN numbers and morphology in the MS, resulting in neurodegeneration, as seen in AD.

Pubmed:
33192323: Gamage R, Wagnon I, Rossetti I, Childs R, Niedermayer G, Chesworth R, Gyengesi E
Cholinergic Modulation of Glial Function During Aging and Chronic Neuroinflammation.
Aging is a complex biological process that increases the risk of age-related cognitive degenerative diseases such as dementia, including Alzheimer's disease (AD), Lewy Body Dementia (LBD), and mild cognitive impairment (MCI). Even non-pathological aging of the brain can involve chronic oxidative and inflammatory stress, which disrupts the communication and balance between the brain and the immune system. There has been an increasingly strong connection found between chronic neuroinflammation and impaired memory, especially in AD. While microglia and astrocytes, the resident immune cells of the central nervous system (CNS), exerting beneficial effects during the acute inflammatory phase, during chronic neuroinflammation they can become more detrimental. Central cholinergic circuits are involved in maintaining normal cognitive function and regulating signaling within the entire cerebral cortex. While neuronal-glial cholinergic signaling is anti-inflammatory and anti-oxidative, central cholinergic neuronal degeneration is implicated in impaired learning, memory sleep regulation, and attention. Although there is evidence of cholinergic involvement in memory, fewer studies have linked the cholinergic anti-inflammatory and anti-oxidant pathways to memory processes during development, normal aging, and disease states. This review will summarize the current knowledge of cholinergic effects on microglia and astroglia, and their role in both anti-inflammatory and anti-oxidant mechanisms, concerning normal aging and chronic neuroinflammation. We provided details on how stimulation of α7 nicotinic acetylcholine (α7nACh) receptors can be neuroprotective by increasing amyloid-β phagocytosis, decreasing inflammation and reducing oxidative stress by promoting the nuclear factor erythroid 2-related factor 2 (Nrf2) pathways and decreasing the release of pro-inflammatory cytokines. There is also evidence for astroglial α7nACh receptor stimulation mediating anti-inflammatory and antioxidant effects by inhibiting the nuclear factor-κB (NF-κB) pathway and activating the Nrf2 pathway respectively. We conclude that targeting cholinergic glial interactions between neurons and glial cells α7nACh receptors could regulate neuroinflammation and oxidative stress, relevant to the treatment of several neurodegenerative diseases.
Front Cell Neurosci, 2020; 14
34393713: Chesworth R, Gamage R, Ullah F, Sonego S, Millington C, Fernandez A, Liang H, Karl T, Münch G, Niedermayer G, Gyengesi E

Spatial Memory and Microglia Activation in a Mouse Model of Chronic Neuroinflammation and the Anti-inflammatory Effects of Apigenin.
Chronic neuroinflammation characterized by microglia reactivity is one of the main underlying processes in the initiation and progression of neurodegenerative diseases such as Alzheimer's disease. This project characterized spatial memory during healthy aging and prolonged neuroinflammation in the chronic neuroinflammatory model, glial fibrillary acidic protein-interleukin 6 (GFAP-IL6). We investigated whether chronic treatment with the natural flavonoid, apigenin, could reduce microglia activation in the hippocampus and improve spatial memory. GFAP-IL6 transgenic and wild-type-like mice were fed with apigenin-enriched or control chow from 4 months of age and tested for spatial memory function at 6 and 22 months using the Barnes maze. Brain tissue was collected at 22 months to assess microgliosis and morphology using immunohistochemistry, stereology, and 3D single cell reconstruction. GFAP-IL6 mice showed age-dependent loss of spatial memory recall compared with wild-type-like mice. Chronic apigenin treatment decreased the number of Iba-1 microglia in the hippocampus of GFAP-IL6 mice and changed microglial morphology. Apigenin did not reverse spatial memory recall impairment in GFAP-IL6 mice at 22 months of age. GFAP-IL6 mice may represent a suitable model for age-related neurodegenerative disease. Chronic apigenin supplementation significantly reduced microglia activation, but this did not correspond with spatial memory improvement in the Barnes Maze.
Front Neurosci, 2021; 15
33705934: Childs R, Gamage R, Münch G, Gyengesi E
The effect of aging and chronic microglia activation on the morphology and numbers of the cerebellar Purkinje cells.
Reduced cerebellar volume and motor dysfunction have previously been observed in the GFAP-IL6 murine model of chronic neuroinflammation. This study aims to extend these findings by investigating the effect of microglial activation and ageing on the total number of Purkinje cells and the morphology of their dendritic arborization. Through comparison of transgenic GFAP-IL6 mice and their wild-type counterparts at the ages of 12 and 24-months, we were able to investigate the effects of ageing and chronic microglial activation on Purkinje cells. Unbiased stereology was used to estimate the number of microglia in Iba1 stained tissue and Purkinje cells in calbindin stained tissue. Morphological analyses were made using 3D reconstructions of images acquired from the Golgi-stained cerebellar tissue. We found that the total number of microglia increased by approximately 5 times in the cerebellum of GFAP-IL6 mice compared to their WT littermates. The number of Purkinje cells decreased by as much as 50 % in aged wild type mice and 83 % in aged GFAP-IL6 mice. The remaining Purkinje cells in these cohorts were found to have significant reductions in their total dendritic length and number of branching points, indicating how the complexity of the Purkinje cell dendritic arbor reduces through age and inflammation. GFAP-IL6 mice, when compared to WT mice, had higher levels of microglial activation and more profound neurodegenerative changes in the cerebellum. The presence of constitutive IL6 production, driving chronic neuroinflammation, may account for these neurodegenerative changes in GFAP-IL6 mice.
Neurosci Lett, 2021; 751
34988899: Ullah F, Gamage R, Sen MK, Gyengesi E
The Effects of Modified Curcumin Preparations on Glial Morphology in Aging and Neuroinflammation.
Neuroinflammation is characterized by reactive microglia and astrocytes (collectively called gliosis) in the central nervous system and is considered as one of the main pathological hallmarks in different neurodegenerative diseases such as Alzheimer's disease, age-related dementia, and multiple sclerosis. Upon activation, glia undergoes structural and morphological changes such as the microglial cells swell in size and astrocytes become bushy, which play both beneficial and detrimental roles. Hence, they are unable to perform the normal physiological role in brain immunity. Curcumin, a cytokine suppressive anti-inflammatory drug, has a high proven pre-clinical potency and efficacy to reverse chronic neuroinflammation by attenuating the activation and morphological changes that occur in the microglia and astrocytes. This review will highlight the recent findings on the tree structure changes of microglia and astrocytes in neuroinflammation and the effects of curcumin against the activation and morphology of glial cells.
Neurochem Res, 2022; 47

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BOARD NUMBER: S06-206

ROLE OF ASTROCYTE-MEDIATED PHAGOCYTOSIS IN ANXIETY AND DEPRESSIVE-LIKE DISORDERS

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Abstract: Major Depressive Disorder (MDD) is a complex recurrent and debilitating psychiatric illness, representing one of the leading causes of disability worldwide. Despite the great economic and social burden of MDD, current pharmacotherapeutic strategies have limited efficiency, displaying partial or no response to the treatment. Although extensively studied, the etiopathogenesis of MDD is still not fully understood. Previous research has elucidated the roles of glial cells in brain processes and, among the three glial cell types, astrocytes are the most important determinant of glial pathology in MDD. Astrocytes are integral to synapse functioning and control many aspects of synapse activity, including the proper recognition, engulfment and degradation of redundant synapses through the multiple EGF-like domain protein 10 (MEGF10) pathway. Interestingly, mice astrocytes lacking MEGF10 show a halving of their relative engulfment ability. Moreover, synaptic communication and neuronal connectivity are disrupted in MDD suggesting a putative role for astrocytes in its pathogenesis. We have previously demonstrated that antidepressant drugs promote synaptic elimination in primary astrocyte-neuron co-cultures and in the adult rat prefrontal cortex. This elimination was accompanied by an increase of MEGF10 protein levels in astrocytes. These data point out that astrocytes not only may represent an additional target of antidepressant drugs, but also play an essential role in synaptic network refinement **Objective**: Our aim is to evaluate if a dysfunctional MEGF10 phagocytic pathway might underlie the neurobiology of depression, and whether this represents a potential novel candidate to develop alternative strategies for the treatment of MDD.

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BOARD NUMBER: S06-207

ALTERATION OF THE NEURON-GLIAL GABAERGIC CROSS-TALK IN THE SCIATIC NERVE OF DYSTROPHIC MDX MICE

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Duchenne muscular dystrophy (DMD) is a lethal X-linked muscular disease characterized by the lack of dystrophin, a cytoskeletal protein expressed in muscles and other cell types. Progressive muscle degeneration and impairment of neuromuscular junction stability are the main features of the disease, albeit nothing is known about the growth, integrity, and functional properties of peripheral nerves. These processes might be regulated by bidirectional signals that myelinating and non-myelinating Schwann cells (SCs) establish with both motor and sensory neuron axons. A re-known regulator of SC-neuron cross-talk is the neurotransmitter GABA. Therefore, the aim of the research was to investigate whether DMD would affect motor and sensory neuron physiology and their morpho-functional relationship with SCs. The studies, performed on the sciatic nerve of wild-type and dystrophic *mdx* mice, analyzed the expression and localization of proteins fundamental for myelin sheath integrity and SC-axon signaling. Real time RT-PCR demonstrated a significant reduction in mRNA levels of myelin proteins (e.g. MBP, P0, PMP22, MAG), and inotropic $GABA_A$ (α4, β3, δ) and metabotropic $GABA_B$-1b receptor subunits in *mdx* mice compared to controls. Reduction in protein levels was confirmed by Western immunoblot (e.g MBP, GABAβ3, GABAδ, GABABR1), and corresponded to a decrease in the mean immunofluorescence intensity following confocal microscope analysis. Further preliminary electrophysiological analyses identified a significant reduction in sensory Abeta fiber excitability. Altogether, our findings support the hypothesis of significant alterations of neuron-SC cross-talk in peripheral nerves of *mdx* mice, an intriguing factor that should be considered for the development of new DMD therapeutic strategies.

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BOARD NUMBER: S06-208

SYSTEMIC LIPOPOLYSACCHARIDE EXPOSURE TRIGGERS TLR4-DEPENDENT INFLAMMATORY RESPONSES IN THE MOUSE RETINA IN VIVO

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Aims: Systemic inflammation has been suggested to trigger activation of microglia cells in the retina. The aim of this study is to investigate the effect of systemic lipopolysaccharide (LPS) exposure on the inflammatory status of the mouse retina, *in vivo* and the involvement of Toll-like receptor 4 (Tlr4). Methods: B6(Cg)-Tlr4$^{tm1.2Karp}$ (Tlr4$^{-/-}$) and C57BL/6J mice were intravenously injected with LPS for four consecutive days. Immunohistochemical and flow cytometry analysis, using specific markers for microglia and monocyte-derived macrophages were performed. Electroretinography recordings (ERG) were utilized to investigate potential effects of LPS on retinal function. Transmission Electron Microscopy (TEM) was used to study the effect of LPS on outer plexiform layer organization (OPL). Results: LPS injections led to elevated numbers of microglia/macrophages and accumulation of these cells around retinal blood vessels in C57BL/6J but not in Tlr4$^{-/-}$ mice. Reactive microglia/macrophages were observed at day 4 in the inner and outer retina, while an intermediate activation phenotype was noticed in the outer retina at day 14 post LPS in C57BL/6J animals. In the Tlr4$^{-/-}$ mice reactive microglia was observed in the outer retina at day 14 after the first LPS challenge. ERG recordings, revealed reduced a- and b- wave amplitudes in scotopic conditions in LPS-challenged C57BL/6J mice. A delayed progression of LPS-induced retinal inflammation was observed in Tlr4$^{-/-}$ mice. TEM analysis, showed a disorganization of the OPL ultrastructure upon the LPS challenge, compared to the naïve C57BL/6J mice. Conclusions: Our results suggest that systemic LPS-induced inflammation perturbs retinal homeostasis in a *Tlr4*-dependent manner.

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BOARD NUMBER: S06-209

ASTROCYTE-NEURON INTERPLAY IS CRITICAL FOR ALZHEIMER'S DISEASE PATHOGENESIS AND IS RESCUED BY TRPA1 CHANNEL BLOCKADE

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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The sequence of cellular dysfunctions in preclinical Alzheimer's disease must be understood if we are to plot new therapeutic routes. Hippocampal neuronal hyperactivity is one of the earliest events occurring during the preclinical stages of Alzheimer's disease in both humans and mouse models. The most common hypothesis describes amyloid β accumulation as the triggering factor of the disease but the effects of this accumulation and the cascade of events leading to cognitive decline remain unclear. In mice, we previously showed that amyloid β-dependent TRPA1 channel activation triggers hippocampal astrocyte hyperactivity, subsequently inducing hyperactivity in nearby neurons. In this work, we investigated the potential protection against Alzheimer's disease progression provided by early chronic pharmacological inhibition of TRPA1 channel. To do that, a specific inhibitor of TRPA1 channel (HC030031) was administered intraperitoneally from the onset of amyloid β overproduction in the APP/PS1-21 mouse model of Alzheimer's disease. Short-, medium-, and long-term effects of this chronic pharmacological TRPA1 blockade were characterized at functional (astrocytic and neuronal activity), structural, biochemical, and behavioural levels. Our results revealed that chronic TRPA1 blockade normalizes astrocytic activity, avoids perisynaptic astrocytic process withdrawal, prevents neuronal dysfunction and preserves structural synaptic integrity. These protective effects preserved spatial working-memory in this Alzheimer's disease mouse model. Therefore, the toxic effect of amyloid β on astrocytes triggered by TRPA1 channel activation is pivotal to Alzheimer's disease progression. TRPA1 blockade prevents irreversible neuronal dysfunction, making this channel a potential therapeutic target to promote neuroprotection.

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BOARD NUMBER: S06-210

DEVELOPMENT OF A MOUSE 3D-TRI-CULTURE APPROACH FOR THE ANALYSIS OF NEURON-GLIA INTERACTIONS UNDER PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL CONDITIONS

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Aims: We aim to establish an in vitro primary mouse 3D culture approach that incorporates key constituents of the CNS (neuron, astrocyte, microglia and ECM) for analyzing neuron-glia interactions under physiological and pathophysiological conditions. **Methods:** A collagen-based 3D-culture system was developed and optimized using spectrophotometry, immunofluorescence and western blotting analyses. A 2D murine neuron-astrocyte-microglia tri-culture system was developed and optimized by using different aged donor mice, seeding densities and supplementing with supporting factors (IL-34, TGF-β, cholesterol) for as long as 14 days in vitro. The 3D-tri-culture protocol was evaluated using Western blot and microscopy under inflammation and mechanical stimulation. **Results:** A collagen pre-gelation method was developed and efficiently improved to obtain an homogenous cell distribution without disturbing the dynamic of the gelation process. Slicing and staining protocols and microscopic analyses were established. Under co-culture conditions, the age of donor mice directly correlated with the glial cell density, and inversely with the density of neuronal processes. The highest number of microglia was observed in the high cell density group (1300cells/mm^2). Optimal concentration of supporting factors was determined by evaluating the neuronal network density and reaction to inflammatory stimulation. The collagen-based scaffold is able to sustain tri-culture of neural cells and allows for the development of extensive neuronal networks. **Conclusions:** Murine neurons, astrocytes and microglia can be co-cultured under specific conditions in the presence of selected growth and maintenance factors in both 2D and 3D conditions. The 3D-tri-culture system is suitable of reproducing the neural responses in neuroinflammation paradigms and mechanical injury.

Pubmed:

31105641: Zhang S, Yuan D, Tan G
Neurological Involvement in Primary Systemic Vasculitis.
Primary systemic vasculitis can affect every structure in both the central and peripheral nervous system, causing varied neurological manifestations of neurological dysfunction. Early recognition of the underlying causes of the neurological symptoms can facilitate timely treatment and improve the prognosis. This review highlights the clinical manifestations of primary systemic vasculitis in the nervous system.
Front Neurol, 2019; 10
30165876: Long T, He W, Pan Q, Zhang S, Zhang Y, Liu C, Liu Q, Qin G, Chen L, Zhou J
Microglia P2X4 receptor contributes to central sensitization following recurrent nitroglycerin stimulation.
The mechanism underlying migraine chronification remains unclear. Central sensitization may account for this progression. The microglia P2X4 receptor (P2X4R) plays a pivotal role in the central sensitization of inflammatory and neuropathic pain, but there is no information about P2X4R in migraine. Therefore, the aim of this study was to identify the precise role of microglia P2X4R in chronic migraine (CM).
J Neuroinflammation, 2018; 15
30971286: He W, Long T, Pan Q, Zhang S, Zhang Y, Zhang D, Qin G, Chen L, Zhou J
Microglial NLRP3 inflammasome activation mediates IL-1β release and contributes to central sensitization in a recurrent nitroglycerin-induced migraine model.
Central sensitization is an important mechanism of chronic migraine (CM) and is related to the inflammatory response of microglia. The NOD-like receptor protein 3 (NLRP3) inflammasome may regulate the inflammatory process of microglia in several neurological diseases, but its role in CM is largely unknown. Therefore, the aim of this study was to identify the precise role of microglial NLRP3 in CM.
J Neuroinflammation, 2019; 16
30635915: He W, Zhang Y, Long T, Pan Q, Zhang S, Zhou J
Sphenopalatine Neuralgia: An Independent Neuralgia Entity. Pooled Analysis of a Case Series and Literature Review.

Structural damage or demyelization of the sphenopalatine ganglion may cause sphenopalatine neuralgia (SN). The current International Classification of Headache Disorders, third edition (ICHD-3) regards SN as a phenotype of cluster headache. Whether SN is an independent neuralgia entity has been debated for years.
Headache, 2019; 59
29794430: Pan Q, Zhang Y, Long T, He W, Zhang S, Fan Y, Zhou J
Diagnosis of Vertigo and Dizziness Syndromes in a Neurological Outpatient Clinic.
Dizziness and vertigo are frequent complaints of outpatients in the neurological department. Our objective was to explore the epidemiological category and clinical features of patients with dizziness or vertigo in the neurological outpatient department of a tertiary hospital.
Eur Neurol, 2018; 79

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BOARD NUMBER: S06-211

LRRK2-G2019S MUTATION AT THE INTERFACE OF ASTROCYTE-NEURON CROSS-TALK DURING EARLY POST-NATAL DEVELOPMENT

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Astrocytes play crucial roles in major homeostatic brain mechanisms and are key players in glia-neuron interactions in Parkinson's disease (PD), the second most common neurodegenerative disorder where both genetic and environmental factors contribute to selective degeneration of mesencephalic dopaminergic neurons (mDAns). Among genetic risks, the Leucine-Rich Repeat Kinase 2 (*LRRK2*) G2019S mutation is associated with both sporadic and familial PD. Besides the multiple functions that LRRK2 displays in several cellular processes involved in mDAns homeostasis, mounting evidence implicates LRRK2 in astrocyte's regulatory functions, but whether and how LRRK2 might modulate astrocyte-mDAns interactions is poorly understood. Here, we hypothesize that LRRK2-mediated perturbation of astrocyte-mDAns crosstalk during development might impact on mDAn growth and resistance to toxic insults. Using *in vitro* primary ventral midbrain (VM) astrocyte-mDAn cultures from wild type (WT) LRRK2 and transgenic (TG) G2019S-*LRRK2* mice, we identified G2019S astrocytes as key mediators of mDAn growth impairment and failure to exert neuroprotection against inflammation and oxidative stress-induced mDAn loss. Next, by integrating immunocytochemistry with gene expression and biochemical analyses, we found that G2019S astrocytes displayed exaggerated production of reactive oxygen (ROS) and nitrogen (RNS) species as well a significant up-regulation of proinflammatory cytokines upon low-dose LPS stimulation or moderate MPP^+ exposure. Furthermore, extracellular flux analysis showed significant changes of mitochondrial metabolism of G2019S astrocytes compared to WT-LRRK2 counterparts. Together, these findings provide insight into the role of LRRK2-G2019S in the regulation of astrocyte-mDAn communication as well as astroglia metabolic response to inflammatory stimuli.

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BRAIN REGION SPECIFICITY OF ASTROCYTE-DERIVED EXTRACELLULAR VESICLES: PRESERVATION OF MITOCHONDRIAL FUNCTION IN A CELLULAR MODEL OF PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Aims Astrocytes (AS) are key players in the regulation of dopaminergic neuron homeostasis both in health and disease, such as Parkinson's disease (PD). PD is a neurodegenerative disorder affecting neuronal cell bodies within ventral midbrain (VMB), and their terminals in striatum (STR), with consequent dopamine depletion. When AS are activated by the chemokine CCL3, they exert a robust neuroprotection both in cellular and pre-clinical PD models. We isolated extracellular vesicles (EVs) derived from VMB- and STR-AS, both in basal and CCL3 conditions, to evaluate their possible involvement in the complex cross-talk between AS and neurons. **Methods** EVs were isolated from AS supernatants via ultracentrifugation and characterized by different techniques: nanoparticle tracking analysis, immunogold-transmission electron microscopy and western blotting. The potential of AS-EV was evaluated on differentiated SH-SY5Y cells under neurodegenerative conditions using immunofluorescence and high resolution respirometry. **Results** We found that nigrostriatal AS produce small-EVs (~100 nm), which are positive for classical EV markers. In basal conditions VMB-AS release more EVs than STR-AS, and only VMB-AS respond to CCL3 by producing more EVs. Following internalization by SH-SY5Y cells, we tested AS-EV potential against oxidative stress and mitochondrial toxicity. We observed that both basal but mostly CCL3-AS-EVs fully counteract H_2O_2-induced caspase-3 activation. Furthermore, AS-EVs were able to recover mitochondrial complex I functionality on MPP^+-injured neurons, with only VMB-AS-EVs able to restore ATP production. **Conclusion** These results highlight a novel role for AS-EVs in the propagation of specific intercellular signaling - within the nigrostriatal system - with neuroprotective implications for PD.

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BOARD NUMBER: S06-213

HUMAN IPSC-BASED CELLULAR SYSTEMS TO MODEL AUTOSOMAL DOMINANT LEUKODYSTROPHY

POSTER SESSION 06 - SECTION: GLIA AND DISORDERS OF THE NERVOUS SYSTEM

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Autosomal dominant leukodystrophy (ADLD) is a slowly, progressive, genetic, and fatal neurological disorder. The genetic cause of ADLD is Lamin B1 (LMNB1) overexpression due to coding duplications or noncoding deletions at the LMNB1 locus. Lamin B1 is a component of the inner nuclear membrane of cells and although LMNB1 is ubiquitously expressed, it appears that neurons and glial cells are particularly sensible to LMNB1 dosage. Currently, only symptomatic and palliative treatments are available for this fatal disease. Since its discovery, human induced pluripotent stem cell (hiPSC) technology has open to the generation of novel and pathological-relevant *in vitro* models for Central Nervous System human diseases, for which no appropriate model systems were available. In this work, we describe the reprogramming of peripheral blood mononuclear cell and fibroblast lines derived from ADLD patients carrying different genetic mutations into hiPSCs by Sendai Virus-based method. These hiPSC lines were characterized to assess their pluripotency state by means of qRT-PCR and immunofluorescence assay. Also, embryoid bodies formation assay was used to evaluate their functional pluripotency. In parallel, we set up a procedure for the controlled differentiation of hiPSCs into oligodendrocytes, neurons, and astrocytes. These mature cells were characterized to assess the expression of stage-specific markers by means of qRT-PCR and immunofluorescence assays. In conclusion, patient-derived ADLD hiPSC lines couple to the differentiation protocols that we report represent valuable tools for studies aiming to investigate ADLD-specific alterations at molecular and cellular levels and develop potential target specific drugs.

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BOARD NUMBER: S06-214

ROLE OF SHIP1 AS A MODULATOR OF MICROGLIAL FUNCTION

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Microglia are the innate immune cells of the central nervous system (CNS). They play crucial roles in brain development, repair and plasticity, and are also critically involved in neurodegenerative processes. Genome wide associations studies (GWAS) reveal that hundreds of genetic variants associated with neurodegeneration are found in genes highly expressed in microglia. Nevertheless, the functions of most of these disease risk genes have been so far poorly studied. Here, we investigated how the SH-2 containing inositol 5' polyphosphatase 1 (SHIP1) influences key microglial properties. SHIP1, encoded by Inpp5d, is responsible for the dephosphorylation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to PI(3,4)P2. It is predominantly expressed by microglia in the CNS, and its genetic variants have been associated with late-onset Alzheimer's disease. To investigate SHIP1 loss-of-function *in vitro*, we generated Inpp5d CRISPR/Cas9 single clones of the microglial BV2 cell line, and further validated the findings *ex vivo*, in primary microglia from SHIP1^{floxed} mice. Our results show that SHIP1 depletion in microglia significantly promotes the uptake and degradation of amyloid beta, while affecting synaptosome engulfment to a lesser degree. However, the efficient amyloid degradation is coupled to an impaired lysosomal profile, as indicated by a decrease both in the acidification of the endo/lysosomal compartment and in the levels of mRNAs of lysosomal enzymes. Furthermore, time-lapse imaging by light-sheet microscopy and wound healing assay revealed defective motility in cells lacking SHIP1. In conclusion, this study shows that SHIP1 plays an important role in modulating microglial properties, suggesting possible implications in the pathogenesis of neurodegeneration.

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BOARD NUMBER: S06-215

INVESTIGATING THE ANTI-INFLAMMATORY AND NEUROPROTECTIVE POTENTIAL OF A LESSER-EXPLORED PHYTOCANNABINOID COMPOUND IN ACUTE NEUROINFLAMMATORY MODELS.

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Phytocannabinoids derived from the *Cannabis sativa* plant, have been shown to act as modulators of neuroinflammation, a prominent example being Cannabidiol, which has demonstrated both anti-inflammatory and neuroprotective effects. Several other compounds have been identified from the plant but have been poorly investigated to date. This study examines the properties of a lesser-explored phytocannabinoid “GL4a” (identity of which has been blinded for research purposes) in Toll-like receptor (TLR)-mediated models of acute neuroinflammation. These receptors activate in response to pathogen- and damage-associated signals, which contributes to microglia activation and subsequent neuronal dysfunction. Subtypes TLR2 and 4 are known to play a primary role in the pathogenesis of disorders associated with neuroinflammation, therefore offering an attractive target for therapeutic intervention. We have determined that GL4a can attenuate pro-inflammatory responses in microglial BV2 cells exposed to the TLR2 agonist lipoteichoic acid (LTA). Moreover, we report that these effects extend to mitigation of TLR-mediated inflammatory responses in neuronal cell lines, evidenced by a significant reduction in the production of proinflammatory cytokines TNFα and IL-6 and also nitric oxide. We further assessed potential mechanisms of action by targeting specific signalling pathways, previously-reported to play a role in cannabinoid action. To assess the impact of GL4a on inflammatory-mediated neuronal integrity, we used whole-cell electrophysiology to examine TLR-induced inhibition of a specific potassium current in hippocampal slices as an *in vitro* model of neuronal dysfunction. This on-going, exploratory study reveals anti-inflammatory and neuroprotective effects of a novel, plant-based compound “GL4a”, and supports further interrogation of potential neurotherapeutic properties.

Pubmed:
32485352: Howe AM, Cosgrave A, Ó'Murchú M, Britchfield C, Mulvagh A, Fernandez-Perez I, Dykstra M, Jones AC, Costello DA
Characterising lipoteichoic acid as an in vitro model of acute neuroinflammation.
Toll-like receptor 2 (TLR2) is a primary sensor for pathogens, including those derived from gram-positive bacteria. It can also mediate the effects of endogenous inflammatory signals such as β-amyloid peptide (Aβ), thus promoting the microglial activation and subsequent neuronal dysfunction, characteristic of chronic neuroinflammatory conditions. More recently, a role for TLR2 has been proposed in the pathogenesis of disorders associated with acute inflammation, including anxiety and depression. The current study aims to characterise the acute effects of the TLR2 agonist lipoteichoic acid (LTA) on microglial activation and neuronal integrity, and to evaluate the influence of LTA exposure on sensitivity to the inflammation and neuronal dysfunction associated with Aβ. Using BV2 and N2a cells as an in vitro model, we highlight that acute exposure to LTA robustly promotes inflammatory cytokine and nitric oxide (NO) production in microglia but also in neurons, similar to that reported under longer-term and chronic inflammatory conditions. Moreover, we find that exposure to LTA can enhance sensitivity to subthreshold Aβ, promoting an 'M1'-like phenotype in microglia and provoking dysregulation of neuronal activity in acute hippocampal slices. Anti-inflammatory agents, including mimetics of brain-derived neurotrophic factor (BDNF), have proven effective at alleviating chronic neuroinflammatory complications. We further examined the effects of 7,8,3-trihydroxyflavone (7,8,3-THF), a small-molecule TrkB agonist, on LTA-induced microglial activation. We report that 7,8,3-THF can significantly ameliorate interleukin (IL)-6 and NO production in LTA-stimulated BV2 cells. Taken together, our findings offer support for exploration of TLR2 as a potential target for therapeutic intervention into acute neuroinflammatory conditions. Moreover we propose that exposure to gram-positive bacterial pathogens may promote sensitivity to the inflammatory changes characteristic of the aged brain.
Int Immunopharmacol, 2020; 85

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BOARD NUMBER: S06-216

ROLE OF HSP70 PROTEIN IN SH-SY5Y DIFFERENTIATION AND PROTECTION FROM OXIDATIVE STRESS-DEPENDENT CELL DAMAGE

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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SH-SY5Y cells, a widely used model in *in vitro* neuroscience research, can be differentiated into mature human neurons through different mechanisms including serum deprivation and the use of retinoic acid. Undifferentiated SH-SY5Y cells rapidly proliferate and appear non-polarized, with very few, short processes. When differentiated, these cells extend long, branched processes, stop proliferating and polarize. By using MTT assay, we observed a different sensitivity to oxidative-stress induced cell death between differentiated and undifferentiated cells. Indeed, treatment of undifferentiated cells with hydrogen peroxide (H_2O_2) induces a dose-dependent decrease in cell viability, which is not detectable in differentiated cells. Since Heat shock proteins (HSPs) are key molecules involved in the correct folding of proteins and regulation of redox processes, we speculated their involvement in the modulation of cell survival against H_2O_2-induced cell death. By western blotting analysis, we observed that HSP70 protein was increasingly expressed in differentiated SH-SY5Y cells, in comparison to undifferentiated cells, and that inhibition of HSP70 expression by KNK437 during the process of differentiation induced an increase in differentiated cell sensitivity to H_2O_2-induced cell death and the failure of differentiation process, suggesting that HSP70 expression is necessary to overcome cell stress associated to the process of differentiation. Furthermore, when HSP70 expression was induced by treatment with Oxotremorine, a selective muscarinic acetylcholine receptor agonist, differentiated SH-SY5Y cells became more resistant to oxidative stress-induced cell death. In conclusion our data demonstrate that manipulation of HSP70 signal modulates the process of SH-SY5Y differentiation and survival to oxidative stress-dependent cell damage.

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POWERFUL PROTECTIVE AND ANTIOXIDANT EFFECTS OF LEMON AND GRAPEFRUIT INTEGROPECTIN ON BRAIN CELLS

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Oxidative stress and neuroinflammation are major mechanisms involved in neurological disorders. In the last years, therapeutic benefits of natural products in brain disorders have been intensively explored. Rich in Citrus flavonoids and terpenes and retaining the highly bioactive rhamnogalacturonan RG-I region, IntegroPectin is a new family of pectins recently extracted from citrus processing waste via hydrodynamic cavitation in water only. With the aim of characterizing beneficial effects of IntegroPectin in brain cells, we demonstrated, by MTT assay and morphological measurements, that lemon and grapefruit IntegroPectins rescued cell viability and cell morphology in SH-SY5Y neuronal-like cells treated with H_2O_2. Moreover, IntegroPectin treatment decreased reactive oxygen species (ROS) production (assessed by DCFH-DA assay), preserved mitochondrial membrane potential (evaluated by JC-1 dye) and morphology (analyzed by staining mitochondria with the MitoTracker Deep Red dye) severely impacted by H_2O_2 exposure. Similar results were also observed in microglia HMC3 cells. In addition, pilot experiments suggested that IntegroPectins may modulate inflammatory phenomena. Though preliminary, these results support experimentation of IntegroPectin on preclinical models of complex pathologies marked by extensive phenomena of oxidative stress and inflammation such as neurodegenerative diseases.

Pubmed:
33923111: Nuzzo D, Picone P, Giardina C, Scordino M, Mudò G, Pagliaro M, Scurria A, Meneguzzo F, Ilharco LM, Fidalgo A, Alduina R, Presentato A, Ciriminna R, Di Liberto V
New Neuroprotective Effect of Lemon IntegroPectin on Neuronal Cellular Model.
Lemon IntegroPectin obtained via hydrodynamic cavitation of organic lemon processing waste in water shows significant neuroprotective activity in vitro, as first reported in this study investigating the effects of both lemon IntegroPectin and commercial citrus pectin on cell viability, cell morphology, reactive oxygen species (ROS) production, and mitochondria perturbation induced by treatment of neuronal SH-SY5Y human cells with HO. Mediated by ROS, including HO and its derivatives, oxidative stress alters numerous cellular processes, such as mitochondrial regulation and cell signaling, propagating cellular injury that leads to incurable neurodegenerative diseases. These results, and the absence of toxicity of this new pectic substance rich in adsorbed flavonoids and terpenes, suggest further studies to investigate its activity in preventing, retarding, or even curing neurological diseases.
Antioxidants (Basel), 2021; 10
34502276: Nuzzo D, Scordino M, Scurria A, Giardina C, Giordano F, Meneguzzo F, Mudò G, Pagliaro M, Picone P, Attanzio A, Raimondo S, Ciriminna R, Di Liberto V
Protective, Antioxidant and Antiproliferative Activity of Grapefruit IntegroPectin on SH-SY5Y Cells.
Tested in vitro on SH-SY5Y neuroblastoma cells, grapefruit IntegroPectin is a powerful protective, antioxidant and antiproliferative agent. The strong antioxidant properties of this new citrus pectin, and its ability to preserve mitochondrial membrane potential and morphology, severely impaired in neurodegenerative disorders, make it an attractive therapeutic and preventive agent for the treatment of oxidative stress-associated brain disorders. Similarly, the ability of this pectic polymer rich in RG-I regions, as well as in naringin, linalool, linalool oxide and limonene adsorbed at the outer surface, to inhibit cell proliferation or even kill, at high doses, neoplastic cells may have opened up new therapeutic strategies in cancer research. In order to take full advantage of its vast therapeutic and preventive potential, detailed studies of the molecular mechanism involved in the antiproliferative and neuroprotective of this IntegroPectin are urgently needed.
Int J Mol Sci, 2021; 22


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TLR-MEDIATED ACTIVATION OF MICROGLIA IS ATTENUATED BY THE TERPENE, ERGOLIDE, VIA NF-KB INHIBITION

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Alzheimers Disease(AD) is the leading cause of dementia worldwide. Neuroinflammation is central to the pathology of most neurodegenerative conditions, including AD. Accumulation of the toxic β–Amyloid peptide(Aβ) promotes the chronic activation of microglia; resulting in the progressive loss in neuronal viability, a major characteristic of the disease. Microglial toll-like receptors(TLRs), in particular TLR2 and 4, are among the known receptors for Aβ. Their activation is widely-reported to mediate the inflammatory changes and neuronal dysfunction associated with AD, and has therefore become an interesting avenue of therapeutic investigation. For many years, naturally-occurring compounds have been exploited for their anti-oxidant and anti-inflammatory properties. Previously, ergolide has been reported to attenuate the pro-inflammatory activation of macrophages, following exposure to lipopolysaccharide(LPS) and interferon-ɣ. This study examines whether the anti-inflammatory effects of ergolide extend to the brain, and may offer therapeutic potential for neuroinflammatory disorders. Here we report that the activation of BV2 microglial cells, following exposure to the TLR2 and 4 agonists lipoteichoic acid and LPS respectively, is attenuated by ergolide. Incubation with ergolide significantly reduced the production of the cytokine IL-6, chemokine MCP-1 and nitric oxide from TLR-stimulated microglia. Using a combination of cellular fractionation and luciferase assays, we further demonstrate that ergolide likely exerts its anti-inflammatory effects in microglia via inhibition of NF-κB. Moreover, we evaluate its capacity to mitigate TLR-mediated cell death in a population of vulnerable hippocampal neurons. Taken together, our findings support the further exploration of ergolide as a promising therapeutic agent for neuroinflammatory conditions such as AD.

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BOARD NUMBER: S06-219

NFKB-MEDIATED TOLERANCE IN A CELLULAR MODEL OF NEUROINFLAMMATION: IMPLICATIONS FOR PARKINSON'S DISEASE DOPAMINERGIC NEURODEGENERATION

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons in the substantia nigra *pars compacta* (SNpc). In this region, activation of resident immune cells, such as microglia, take part in the chronic neuroinflammatory scenario that contributes to the process of neuronal death. The main goal of this project is to evaluate the response of microglial cells after repetitive pro-inflammatory stimuli of LPS and IFN-g, in a scenario of PD-like neuroinflammation in vitro. Our hypothesis is that after pro-inflammatory pulses, microglia may change their phenotype and their characteristic reactive features. We performed a set of in vitro experiments applying different strategies of inflammatory treatment to microglia and presented dopaminergic cells. We evaluated features of microglial activation such as cell area, the release of nitrites, the number of cells or the nuclear colocalization of the transcription factor NF-kB, to characterize the phenotype of microglia. Our results show that after repetitive pro-inflammatory pulses, microglial cells seem to reduce the release of nitrites, decrease their cell size, and impede the effective elimination of dopaminergic cells, indicating the establishment of a tolerant state. Repetitive pro-inflammatory stimuli seem to hamper the characteristic translocation of NF-kB into the nucleus, suggesting the mechanistic signalling of the acquired tolerance. These results are critical for a better understanding of the microglial cells behavior after receiving neuroinflammatory stimuli and to know how it may affect the elimination of dopaminergic cells. Further investigation within this research line will be critical to understand the progression of the neurodegenerative process in PD.

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INFLAMMATION ALTERS HUMAN MICROGLIAL NEUROSTEROIDOGENESIS WITH POSSIBLE IMPACT ON NEURAL STEM CELLS DIFFERENTIATION

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Dysregulated microglial activation underlies the insurgence of chronic neuroinflammation and neurodegeneration. The Translocator Protein (TSPO), involved in *de novo* production of neurosteroids, is upregulated in activated microglia and is emerging as an interesting target to control neuroinflammation. **Aims:** Given the anti-inflammatory/neuroprotective properties of neurosteroids, our study aims to evaluate whether inflammation could affect human microglial neurosteroidogenesis. First, the ability of TSPO ligands to restore homeostatic neurosteroid levels was assessed. Furthermore, the effects of microglial conditioned medium (MCM) on human neural stem cell line (ReN cells) differentiation were also evaluated. **Methods:** Microglia were activated by IL-1β administration, and the expression of TSPO and CYP11A1, the enzyme of neurosteroidogenesis rate-limiting step, was evaluated. MCM derived from activated microglia pre-treated with 1μM of XBD-173 and PIGA1138 TSPO ligands or not, was analysed by LC-MS to measure neurosteroid levels. ReN cells were differentiated in presence of MCM for 3 days and immunostained for βIII-Tubulin and GFAP, neuronal and astrocytic markers respectively. **Results:** IL-1β treatment increased TSPO and CYP11A1 expression. LC-MS analysis of IL-1β-stimulated MCM evidenced a reduction in the levels of progesterone, testosterone, and estradiol, while TSPO ligands administration restored their levels. IL-1β-stimulated MCM impaired ReN differentiation into neurons by promoting astrocytogenesis, whereas TSPO ligand/IL-1β MCM rescued neuritic density and reduced GFAP fluorescence intensity. **Conclusions:** Activated microglia reduces the production of anti-inflammatory neurosteroids contributing to the maintenance of a pro-inflammatory microenvironment. TSPO pharmacological stimulation could represent a promising strategy to restore altered neurosteroid levels in order to counteract the negative effects of neuroinflammatory processes.

Pubmed:

33803741: Germelli L, Da Pozzo E, Giacomelli C, Tremolanti C, Marchetti L, Wetzel CH, Barresi E, Taliani S, Da Settimo F, Martini C, Costa B
De novo Neurosteroidogenesis in Human Microglia: Involvement of the 18 kDa Translocator Protein.
Neuroactive steroids are potent modulators of microglial functions and are capable of counteracting their excessive reactivity. This action has mainly been ascribed to neuroactive steroids released from other sources, as microglia have been defined unable to produce neurosteroids de novo. Unexpectedly, immortalized murine microglia recently exhibited this de novo biosynthesis; herein, de novo neurosteroidogenesis was characterized in immortalized human microglia. The results demonstrated that C20 and HMC3 microglial cells constitutively express members of the neurosteroidogenesis multiprotein machinery-in particular, the transduceosome members StAR and TSPO, and the enzyme CYP11A1. Moreover, both cell lines produce pregnenolone and transcriptionally express the enzymes involved in neurosteroidogenesis. The high TSPO expression levels observed in microglia prompted us to assess its role in de novo neurosteroidogenesis. TSPO siRNA and TSPO synthetic ligand treatments were used to reduce and prompt TSPO function, respectively. The TSPO expression downregulation compromised the de novo neurosteroidogenesis and led to an increase in StAR expression, probably as a compensatory mechanism. The pharmacological TSPO stimulation the de novo neurosteroidogenesis improved in turn the neurosteroid-mediated release of Brain-Derived Neurotrophic Factor. In conclusion, these results demonstrated that de novo neurosteroidogenesis occurs in human microglia, unravelling a new mechanism potentially useful for future therapeutic purposes.
Int J Mol Sci, 2021; 22
35018577: Tremolanti C, Cavallini C, Meyer L, Klein C, Da Pozzo E, Costa B, Germelli L, Taliani S, Patte-Mensah C, Mensah-Nyagan AG
Translocator Protein Ligand PIGA1138 Reduces Disease Symptoms and Severity in Experimental Autoimmune Encephalomyelitis Model of Primary Progressive Multiple Sclerosis.
Multiple sclerosis (MS) is an autoimmune and demyelinating disease of the central nervous system (CNS) caused by CNS

infiltration of peripheral immune cells, immune-mediated attack of the myelin sheath, neuroinflammation, and/or axonal/neuronal dysfunctions. Some drugs are available to cope with relapsing-remitting MS (RRMS) but there is no therapy for the primary progressive MS (PPMS). Because growing evidence supports a regulatory role of the translocator protein (TSPO) in neuroinflammatory, demyelinating, and neurodegenerative processes, we investigated the therapeutic potential of phenylindolyilglyoxylamydes (PIGAs) TSPO ligands in myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) mice mimicking the human PPMS. MOG-EAE C57Bl/6-mice were treated by TSPO ligands PIGA839, PIGA1138, or the vehicle. Several methods were combined to evaluate PIGAs-TSPO ligand effects on MOG-EAE symptoms, CNS infiltration by immune cells, demyelination, and axonal damages. PIGA1138 (15 mg/kg) drastically reduced MOG-EAE mice clinical scores, ameliorated motor dysfunctions assessed with the Catwalk device, and counteracted MOG-EAE-induced demyelination by preserving Myelin basic protein (MBP) expression in the CNS. Furthermore, PIGA1138-treatment prevented EAE-evoked decreased neurofilament-200 expression in spinal and cerebellar axons. Moreover, PIGA1138 inhibited peripheral immune-CD45 + cell infiltration in the CNS, suggesting that it may control inflammatory mechanisms involved in PPMS. Concordantly, PIGA1138 enhanced anti-inflammatory interleukin-10 serum level in MOG-EAE mice. PIGA1138-treatment, which increased neurosteroid allopregnanolone production, ameliorated all pathological biomarkers, while PIGA839, unable to activate neurosteroidogenesis in vivo, exerted only moderate/partial effects in MOG-EAE mice. Altogether, our results suggest that PIGA1138-based treatment may represent an interesting possibility to be explored for the innovation of effective therapies against PPMS.
Mol Neurobiol, 2022; 59

30664692: Vitiello M, Evangelista M, Di Lascio N, Kusmic C, Massa A, Orso F, Sarti S, Marranci A, Rodzik K, Germelli L, Chandra D, Salvetti A, Pucci A, Taverna D, Faita F, Gravekamp C, Poliseno L
Antitumoral effects of attenuated Listeria monocytogenes in a genetically engineered mouse model of melanoma.
Attenuated Listeria monocytogenes (Lm-LLO) represents a valuable anticancer vaccine and drug delivery platform. Here we show that in vitro Lm-LLO causes ROS production and, in turn, apoptotic killing of a wide variety of melanoma cells, irrespectively of their stage, mutational status, sensitivity to BRAF inhibitors or degree of stemness. We also show that, when administered in the therapeutic setting to Braf/Pten genetically engineered mice, Lm-LLO causes a strong decrease in the size and volume of primary melanoma tumors, as well as a reduction of the metastatic burden. At the molecular level, we confirm that the anti-melanoma activity exerted in vivo by Lm-LLO depends also on its ability to potentiate the immune response of the organism against the infected tumor. Our data pave the way to the preclinical testing of listeria-based immunotherapeutic strategies against metastatic melanoma, using a genetically engineered mouse rather than xenograft models.
Oncogene, 2019; 38

34340630: Barresi E, Ravichandran R, Germelli L, Angeli A, Baglini E, Salerno S, Marini AM, Costa B, Da Pozzo E, Martini C, Da Settimo F, Supuran C, Cosconati S, Taliani S
Carbonic anhydrase activation profile of indole-based derivatives.
Carbonic Anhydrase Activators (CAAs) could represent a novel approach for the treatment of Alzheimer's disease, ageing, and other conditions that require remedial achievement of spatial learning and memory therapy. Within a research project aimed at developing novel CAAs selective for certain isoforms, three series of indole-based derivatives were investigated. Enzyme activation assay on human CA I, II, VA, and VII isoforms revealed several effective micromolar activators, with promising selectivity profiles towards the brain-associated cytosolic isoform hCA VII. Molecular modelling studies suggested a theoretical model of the complex between hCA VII and the new activators and provide a possible explanation for their modulating as well as selectivity properties. Preliminary biological evaluations demonstrated that one of the most potent CAA is not cytotoxic and is able to increase the release of the brain-derived neurotrophic factor (BDNF) from human microglial cells, highlighting its possible application in the treatment of CNS-related disorders.
J Enzyme Inhib Med Chem, 2021; 36

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CROSSTALK BETWEEN GLIAL CELLS AND C-KIT+ MAST CELLS IN THE ALS DEGENERATING SPINAL CORD

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Background. Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of motor neurons, reactive gliosis and immune cell infiltration into the CNS, resulting in a degenerative cell microenvironment. We have recently reported that mast cells (MC) expressing c-Kit receptor can enter the damaged parenchyma as precursors (Kovacs *et al,* 2021). MCs spatially interact with astrocytes expressing the c-Kit ligand, Stem Cell Factor (SCF), suggesting a mechanism for local MC differentiation and activation. MCs have the potential to degranulate and induce inflammation through the release of mediators such as cytokines, histamine, and proteases. MC-tryptase is a MC-restricted protease that can interact with glial cells through the activation of proteinase activated receptors, representing a major neuroinflammatory mediator. However, the pathogenic significance and interaction between MC-tryptase and glial cells in ALS remain unclear. **Methods.** The expression of MC-tryptase was analyzed in autopsied spinal cords from ALS subjects and controls as well as in the CNS of $SOD1^{G93A}$ mice. Murine tissues were analyzed by flow cytometry and PAGE-western blotting. Crosstalk between MC-tryptase and glial cells was analyzed in primary cultures of glial cells. **Results.** We have identified that MC-tryptase is strongly upregulated in the ALS spinal cord, correlating with the degree of MC infiltration, microgliosis, and defective microvasculature. Downregulation of MC-infiltration with the tyrosine-kinase inhibitor, Masitinib which targets SCF/c-Kit signaling, decreased the MC-tryptase levels. In cell cultures, MC-tryptase was sufficient to modulate the phenotype of microglia and astrocytes. **Conclusions.** Our results suggest a pathogenic mechanism triggered by MC-tryptase that leads to glial activation in ALS, which can be targeted therapeutically.

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STUDY OF THE PHARMACOLOGICAL INHIBITION OF RPTP β/ζ AS A NOVEL STRATEGY TO MODULATE THE LPS-INDUCED LOSS OF NEURONAL PROGENITORS IN THE DENTATE GYRUS

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

María Rodríguez-Zapata, Esther Gramage, Milagros Galán Llario, Marta Vicente-Rodríguez, Teresa Fontán-Baselga, Carmen Pérez-García, Gonzalo Herradón
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Neuroinflammation plays an essential role in the response mechanisms to brain damage. However, prolonged neuroinflammation exert harmful effects on the Central Nervous System. Pleiotrophin (PTN) is a cytokine that modulates neuroinflammation in different contexts by binding to receptor Protein Tyrosine Phosphatase (RPTP) β/ζ. **AIMS:** To test the implication of RPTP β/ζ signalling pathway in lipopolysaccharide (LPS)-induced neuroinflammation and to evaluate the long-term effects of an LPS acute exposure. **METHODS:** Adult male C57BL/6J mice were administered MY10 (60 mg/kg i.g., inhibitor of RPTP β/ζ) or vehicle 1 h before a single injection of LPS (7.5 mg/kg i.p.) or saline 16 h prior to sacrifice. To study the long-term influence of neuroinflammation, another cohort of mice were sacrificed 10 months after a single LPS (5 mg/kg i.p.) or saline administration. In immunohistochemistry assays, we studied changes in ionized calcium-binding adaptor molecule 1 (Iba1, microglial marker) and doublecourtin (DCx, neuronal progenitor marker) expression in dentate gyrus (DG). **RESULTS:** Acute LPS significantly decreases DCx positive cells in the DG. Interestingly, data suggest that treatment with MY10 could prevent this deleterious effect of LPS on hippocampal neurogenesis. However, microglial changes in DG were not modulated by MY10 administration. Regarding long-term LPS effects, no significant differences in Iba1 neither DCx were found. **CONCLUSION:** PRPTP β/ζ appears to be a promising druggable target to modulate the loss of neuronal progenitors induced by LPS acute damage. Further studies are needed to clarify the molecular mechanisms mediating hippoccampal neuroinflammation and the role of the PTN/ RPTP β/ζ signalling pathway.

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VISUALIZATION AND HIGH-THROUGHPUT QUANTIFICATION OF AKT ACTIVITY IN LIVE-CELL NEUROINFLAMMATORY MODELS

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

Jasmine Trigg[1], Gillian Lovell[2], Amber Ward[1], John Rauch[3], Grigory Filonov[3], Susana Alcantara[1], Timothy Dale[1]
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Chronic neuroinflammatory states are associated with the development of several neurodegenerative diseases. The PI3K/Akt signalling pathway has been implicated in these disease processes and holds therapeutic promise as a target to modulate neuroinflammatory responses. Here we demonstrate a robust *in vitro* assay to assess dynamic Akt Kinase activity in real-time. To monitor Akt activity we used astrocytic and immune cell lines stably expressing the Incucyte® Kinase Akt Green/Red Lentivirus Reagent, a genetically-encoded fluorescent kinase translocation reporter whose subcellular localization is phosphorylation dependent. Images were acquired using the Incucyte® Live-Cell Analysis System and Akt activity was quantified using integrated software. To validate inhibition, cells were treated with the allosteric Akt inhibitor MK-2206. Quantification revealed concentration-dependent inhibition of Akt activity across all cell types over 24h, with increased potency for HMC3 microglia, compared to T98G or U87 astrocytes (IC_{50} values of 0.17, 0.73, and 1.29 µM, respectively). Additionally, T98G astrocytes were treated with inhibitors targeting both upstream PI3K and downstream mTOR. Results show concentration-dependent inhibition of Akt activity for all compounds with varying kinetic profiles. Subsequent experiments investigated the effects of immunocompetent cell activation on Akt using inflammatory insults. Lipopolysaccharide (LPS) showed a rapid concentration-dependent increase in Akt activity reaching maximal response by 6h, which was suppressed in the presence of MK-2206, suggestive of the Akt pathway playing an important role in LPS-induced activation. These data exemplify the Incucyte® Kinase Akt Assay as a powerful live-cell approach for assessing Akt activity in neuroinflammatory models and its amenability to screening of therapeutic candidates.

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NEUROINFLAMM-AGING OF THE MICROENVIRONMENT DURING GLIOBLASTOMA PROGRESSION.

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Glioblastoma (GBM) is a lethal brain tumor, supported by a unique microenvironment. By secreting modulating factors, GBM converts innate immune cells activation away from a pro-inflammatory state. Such modulations engage immune cells into a tissue repair mode, promoting tumor growth and invasion. The inflammation status of the microenvironment temporally plays a pivotal role in cancer development. Therefore, studying the inflamm-aging may help to understand the molecular sequence through which GBM affects the brain microenvironment. GL261 glioma cells were injected in the right striatum of immuno-competent C57Bl6J mice and animals were sacrificed after 7, 14, and 21 days (7D, 14D, 21D). The tumor development was assessed through 3D reconstruction of tomographic imaging and brains were processed by immunohistochemistry, immunofluorescence, and western blotting. In the initiation stage, the proliferating tumor (ki67+) triggered astrocytes (GFAP+) reaction and induced the extracellular matrix remodeling (MMP-9, FIB-2). Microglia (TMEM119+; Iba1+) and macrophages (Iba1+) were scarcely represented, and the chemokine-CCL-2- dependent recruitment of inflammatory monocytes appeared to be reduced. The 14D stage showed an evident tumor bulk and the peak of tenascin C (TnC) protein levels, allowing the migration of the tumor cells. At 21D, CD133+ glioma stem cells were out of the primary bulk, which appeared inflamed, necrotic, and infiltrated by macrophages. The contralateral hemisphere was engaged with a secondary tumor mass, showing cyclic pro and anti-tumor features of inflammation. The present study emphasizes the role of inflammation in the microenvironment during the GBM progression, fostering time-dependent therapies in an experimental model similar to human disease.

Pubmed:
33010345: Virtuoso A, De Luca C, Gargano F, Colangelo AM, Papa M
The Spinal Extracellular Matrix Modulates a Multi-level Protein Net and Epigenetic Inducers Following Peripheral Nerve Injury.
The extracellular matrix (ECM) of the central nervous system (CNS) plays a pivotal role in the pathogenesis of several neurodegenerative and neuroinflammatory disorders. Among the major factors, matrix metalloproteinases (MMPs) are actively involved in ECM remodeling and directly affect neuro-glial interactions. Since disease-related functional alterations mostly rely on the proteome, modulation of MMPs activity may be a strategy to correct mechanisms behind neurological disorders. We here investigated modifications of signaling components related to the central pathways in spinal maladaptive plasticity following spared nerve injury (SNI) of the sciatic nerve, and after treatment with the MMPs inhibitor GM6001 for 3 or 8 days. We found that GM6001 reduced the massive astrocytic and microglial activation indicative of reactive gliosis. Functional activity of GM6001 was paralleled by its significant effect on expression levels of the purinergic P2X4 receptor (P2X4R), the transcription factors NFκB and RPBJ, as well as levels of the nerve growth factor (NGF) receptor TrkA. Moreover, we showed that histone deacetylases 1 and 2 (HDAC1, HDAC2) were differentially modulated after SNI and GM6001 treatments for 3 or 8 days. Our data suggest a multi-level network of interactions across ECM and the neuroglial network involving MMPs, the neurotrophin system, intracellular signaling, and epigenetic modifications.
Neuroscience, 2020; 451

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BOARD NUMBER: S06-225

THE LINK BETWEEN ENVIRONMENTAL MYCOTOXIN, OCHRATOXIN A, AND NEURODEGENERATIVE DISEASES

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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UKHSA, Radiation Effects, Didcot, United Kingdom

Aim: Ochratoxin A (OTA) is a common contaminant of crops, foods and drinks. OTA is a known neurotoxin, readily crossing the blood brain barrier yet the role of OTA in neurodegenerative diseases is not understood. This investigation sought to determine whether OTA may act as an environmental hazard linked to neurodegenerative diseases. **Methods**: Undifferentiated SH-SY5Y cells were used as a model to investigate the effect of OTA on MAPT, BAX, P53, BDNF and TPPP gene expression. SH-SY5Y cells were exposed to 1µM or 1nM OTA for 1-2 days or 2pM OTA for 2, 5 or 11 days. Cells were then harvested and pelleted for RNA extraction, followed by cDNA conversion and rt-qPCR to quantify gene expression. **Results**: Exposing cells to 1µM OTA reduced P53, MAPT, and BAX expression at days 1 and 2. The expression of BDNF and TPPP were significantly reduced at day 1 yet significantly increase at day 2 relative to controls. SH-SY5Y cells exposed to a 1nM OTA were observed to have a significant reduction in BAX and P53 only. After 11 days no change in gene expression was observed following a dose of 2pM OTA. **Conclusion**: Gene expression was not altered by 2pM OTA suggesting that low doses of OTA may not trigger neurodegenerative diseases. Downregulation of pro-apoptotic BAX and P53 and upregulation pro-survival BDNF suggest 1µM OTA supresses apoptotic signals and encourages cell survival after just one day. Interestingly, 1µM OTA reduced Alzheimer's disease marker, MAPT expression, while increasing TPPP expression, marker of Parkinson's disease.

Pubmed:
30732655: Hase Y, Ding R, Harrison G, Hawthorne E, King A, Gettings S, Platten C, Stevenson W, Craggs LJL, Kalaria RN
White matter capillaries in vascular and neurodegenerative dementias.
Previous studies suggest white matter (WM) integrity is vulnerable to chronic hypoperfusion during brain ageing. We assessed ~ 0.7 million capillary profiles in the frontal lobe WM across several dementias comprising Alzheimer's disease, dementia with Lewy bodies, Parkinson's disease with dementia, vascular dementia, mixed dementias, post-stroke dementia as well as post-stroke no dementia and similar age ageing and young controls without significant brain pathology. Standard histopathological methods were used to determine microvascular pathology and capillary width and densities in 153 subjects using markers of the basement membrane (collagen IV; COL4) and endothelium (glucose transporter-1; GLUT-1). Variable microvascular pathology including coiled, tortuous, collapsed and degenerated capillaries as well as occasional microaneurysms was present in all dementias. As expected, WM microvascular densities were 20-49% lower than in the overlying cortex. This differential in density between WM and cortex was clearly demonstrated by COL4, which was highly correlated with GLUT-1 densities (Spearman's rho = 0.79, P = 0.000). WM COL4 immunopositive microvascular densities were decreased by ~ 18% across the neurodegenerative dementias. However, we found WM COL4 densities were increased by ~ 57% in post-stroke dementia versus ageing and young controls and other dementias. Using three different methods to measure capillary diameters, we found WM capillaries to be significantly wider by 19-45% compared to those in overlying neocortex apparent with both COL4 and GLUT-1. Remarkably, WM capillary widths were increased by ~ 20% across all dementias compared to ageing and young controls (P < 0.01). We also noted mean WM pathology scores incorporating myelin loss, arteriolosclerosis and perivascular spacing were correlated with COL4 immunopositive capillary widths (Pearson's r = 0.71, P = 0.032). Our key finding indicates that WM capillaries are wider compared to those in the overlying neocortex in controls but they dilate further during dementia pathogenesis. We suggest capillaries undergo restructuring in the deep WM in different dementias. This reflects compensatory changes to retain WM perfusion and integrity during hypoperfusive states in ageing-related dementias.
Acta Neuropathol Commun, 2019; 7
34491346: Gettings SM, Maxeiner S, Tzika M, Cobain MRD, Ruf I, Benseler F, Brose N, Krasteva-Christ G, Vande Velde G, Schönberger M, Althaus M
Two Functional Epithelial Sodium Channel Isoforms Are Present in Rodents despite Pronounced Evolutionary Pseudogenization and Exon Fusion.

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The epithelial sodium channel (ENaC) plays a key role in salt and water homeostasis in tetrapod vertebrates. There are four ENaC subunits (α, β, γ, δ), forming heterotrimeric αβγ- or δβγ-ENaCs. Although the physiology of αβγ-ENaC is well understood, for decades the field has stalled with respect to δβγ-ENaC due to the lack of mammalian model organisms. The SCNN1D gene coding for δ-ENaC was previously believed to be absent in rodents, hindering studies using standard laboratory animals. We analyzed all currently available rodent genomes and discovered that SCNN1D is present in rodents but was independently lost in five rodent lineages, including the Muridae (mice and rats). The independent loss of SCNN1D in rodent lineages may be constrained by phylogeny and taxon-specific adaptation to dry habitats, however habitat aridity does not provide a selection pressure for maintenance of SCNN1D across Rodentia. A fusion of two exons coding for a structurally flexible region in the extracellular domain of δ-ENaC appeared in the Hystricognathi (a group that includes guinea pigs). This conserved pattern evolved at least 41 Ma and represents a new autapomorphic feature for this clade. Exon fusion does not impair functionality of guinea pig (Cavia porcellus) δβγ-ENaC expressed in Xenopus oocytes. Electrophysiological characterization at the whole-cell and single-channel level revealed conserved biophysical features and mechanisms controlling guinea pig αβγ- and δβγ-ENaC function as compared with human orthologs. Guinea pigs therefore represent commercially available mammalian model animals that will help shed light on the physiological function of δ-ENaC.
Mol Biol Evol, 2021; 38

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BOARD NUMBER: S06-226

NECROTIC-LIKE BV-2 MICROGLIAL CELL DEATH INDUCED BY ACUTE EXPOSURE TO METHYLMERCURY

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Methylmercury (MeHg) is an environmental contaminant that especially affects the central nervous system, causing neurological alterations. In response to disturbed microenvironment homeostasis, microglial cells mount an innate immune response contributing to neurodegenerative and neuroinflammatory conditions, a process potentially affected by MeHg exposure. This work aimed to clarify the acute toxic effects of MeHg exposure in microglial cells and associated cell death pathways. BV-2 mouse microglial cells were incubated with MeHg at different concentrations (0.01; 0.1; 1 and 10µM) for 1 hour prior to Lipopolysaccharide (LPS 0.5 µg/mL) exposure for 6 or 24 hours. After cell exposure, the supernatants were harvested and IL-6 and TNF-alpha production and release, ROS production, NO release, iNOS expression, metabolic activity and Propidium Iodide (PI) uptake were assessed. MeHg 10 µM induced a reduction in the production and secretion of pro-inflammatory proteins IL-6, TNF-alpha, iNOS immunoreactivity, release of NO, and ROS formation in BV-2 cells. Furthermore, MeHg 10 µM, with and without LPS stimulation, decreased metabolic activity of BV-2 and increased the number of PI-positive cells (necrotic cell death) when compared to the respective control groups. The short-term effects of a high concentration of MeHg on BV-2 microglial cells lead to impaired production of several pro-inflammatory mediators, as well as a higher microglial cell death via necrosis, compromising their neuroinflammatory response. Clarifying the mechanisms underlying MeHg-induced neurotoxicity and neurodegeneration in brain cells is relevant to better understand acute and long-term chronic neuroinflammatory responses due to MeHg exposure. **Funded by FCT-FUNCAP POCTI-FEDER-02/SAICT/2017/31699**

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BOARD NUMBER: S06-227

GUT-DERIVED METABOLITES AS MODULATORS OF BRAIN INFLAMMATION.

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Gut microbiota has been associated with several neurobehavioural alterations. Some studies highlighted that it can modulates brain inflammation. The aim of the study is to dissect how some gut-derived metabolites can affect brain inflammation and brain functioning. To do so, we explored the effect of these molecules on primary microglia cultures.The first step of the project was to select gut-derived metabolites that are associated with behavioural alterations in patients suffering mood disturbances. Then, we took advantage of an innovative model of primary microglia cells. Quickly, microglia were harvested from adult C57 mice and then a cocktail of molecules was added to the medium in order to make cells acquiring either a quiescent or activated phenotype. Then several gut-derived metabolites were tested in presence or absence of a concomitant LPS stimulation. Several gut-derived metabolites displayed an ability to modulate microglia phenotype in absence of any inflammatory challenge. After LPS stimulation, some metabolites attenuate inflammatory response (i.e cytokines release) and switch in phenotype.Our results revealed that gut microbiota derived metabolites can efficiently modulate microglia phenotype and its response toward inflammatory stimuli *in vitro*.

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BOARD NUMBER: S06-228

ENDOCANNABINOIDS MODULATE AMYLOID-β -INDUCED TRANSGLUTAMINASE 2 EXPRESSION AS A MARKER OF NEUROINFLAMMATION IN MOUSE MODELS

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

Andrea Parente[1], Nicola Gaetano Gatta[1], Rosa Giacca[1], Rosmara Infantino[2], Maria Battipaglia[1], Filomena Capolongo[1], Francesca Guida[2], Sabatino Maione[2,3], Vittorio Gentile[1]
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Amyloid- β 1-42 peptides accumulation and aggregation are the principal hallmarks for Alzheimer Disease (AD) and it triggers to neuroinflammation onset activating Microglia M1 state stimulating pro-inflammatory cytokines. For its properties and enzymatic activites, Transglutaminase 2 (TG2) could be taken in consideration as druggable marker of AD-associated neuroinflammation. Many evidences show a TG2 role during AD-associated neuroinflammation in activated microglia, in association with M1 markers production. To evaluate TG2 inhibition as possible therapeutic approach for AD neuroinflammation, we tested two Endocannabinoids, PEA and its oxazolinic derivative PEA-OXA, with documented anti-inflammatory activity. We analyze TG2 and M1-M2 microglial markers expression to evaluate inflammatory state and define PEA and PEA-OXA neuroprotective activity. Methods : Mouse BV2 microglial cells were treated with fibrillated Aβ1-42 peptides in presence of PEA or PEA-OXA. PEA-OXA effects were studied also in a soluble Amyloid- β mouse model. Real Time PCR and Western Blot analyses were performed to evaluate TG2 expression and M1-M2 microglial markers. Results : TG2 is up-regulated in presence of fibrillated Aβ 1-42 peptide, both at protein and mRNA levels. In presence of PEA and PEA-OXA, TG2 protein and mRNA expression decreases considerably, as M1 markers choosen for the study. Endocannabinoids are able to up-regulate M2 markers Arginase-1 and TREM2 expression. Conclusions : Added to evidences in literature, these data confirm the possibility to consider TG2 as a marker of neuroinflammation. PEA and PEA-OXA have confirmed their anti-inflammatory role suggesting a possible use to block AD patients brain inflammation down-regulating TG2 expression and promoting microglial neuroprotective M2 state.

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GALECTIN-3 ROLE IN THE INTERACTION OF MICROGLIA AND AMYLOID-BETA FIBRILS IN VITRO

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Aims: Microglia are the resident immune cells of the brain. They have been studied in the context of Alzheimer's disease (AD) since are able to take up amyloid-beta (Aβ) fibrils which form plaques, the main hallmark of AD. Nevertheless, microglia play a dual role in this affection: in the beginning they regulate Aβ; but later they become detrimentally activated causing neuroinflammation. Therefore, we sought for a novel way of regulating AD pathogenesis through microglia. Galectin-3, a key protein for microglial activation, has been described to be involved in AD and to interact with Aβ. Herein, we want to characterize the microglial phenotype *in vitro* related to Aβ due to Galectin-3 regulation. **Methods**: We use the BV-2 cell line, a model of murine microglia in combination with recombinant Galectin-3 and Aβ proteins. Different forms of aggregation were characterized through Thioflavin T assay and added to the cells at different timepoints and concentrations. Moreover, we work with Galectin-3 inhibitors and Galectin-3 knockout BV-2 cells. We follow Aβ internalization with live-cell imaging. We also quantify the inflammatory cytokines release through ELISA Mesoscale and identifying the key microglial gene transcripts through quantitative reverse transcription PCR. **Results**: Preliminary results include an increase in the Aβ fibrils internalization and TNFα levels increased in the medium when cells were pretreated with Galectin-3. We expect to identify a phenotype beneficial for the pathogenesis when Galectin-3 is inhibited or knocked out. **Conclusions**: We expect to successfully find a way of maintaining a beneficial activation of microglia throughout AD pathology.

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INVESTIGATION OF THE OLIGODENDROGLIAL CX47 IN THE SPINAL CORD OF THE 5XFAD MOUSE MODEL OF ALZHEIMER DISEASE

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Aims: Alzheimer's disease (AD) is a progressive neurodegenerative disorder with memory loss, cognitive and mobility decline. Central nervous system (CNS) glia, particularly astrocytes and oligodendrocytes are linked to the pathogenesis of Alzheimer's. Glial cells are coupled through gap junctions (GJ) assembled by two hemichannels consisting of six connexin (Cx) proteins on each opposed cell. Recent studies support that brain oligodendrocytic Cx47 might play a pathological role in Alzheimer's progression. The project aim is to investigate oligodendroglial pathology in the spinal cord of the 5xFAD mouse model. Methods: Western blot experiments were performed for the quantification of Cx47 in 5xFAD and non-transgenic mice groups (3 and 12 months). Fluorescence immunohistochemistry (IHC) in 3rd-4th cervical and 2nd-3rd lumbar frozen spinal sections was carried out for Cx47, CC1, Olig2, GFAP, and Iba1 markers. Results: 12-month-old 5xFAD mice showed a time-dependent increased immunoreactivity for GFAP+ astrocytes and Iba1+ microglia in the spinal cord compared to 3-month-old mice. In addition, older 5xFAD mice exhibited increased immunoreactivity for Cx47 in the grey matter of the abovementioned spinal levels and increased protein levels. Lastly, 12M 5xFAD expressed significantly higher levels of both mature and precursor oligodendrocyte populations in the gray matter of the spinal cord in comparison to the younger transgenic mice. Conclusions: We detected severe astrogliosis and increased expression levels of Cx47, which may contribute to Alzheimer's pathogenesis. Future studies will be focused on the investigation of connexins' contribution to the overall glia pathology resulting in motor deficiencies observed in 5xFAD mice.

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MODULATION OF ANANDAMIDE TONE AS AN EFFECTIVE STRATEGY FOR IN VITRO AND IN VIVO STIMULATION OF AUTOPHAGY IN ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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[1]Sapienza University of Rome, Department Of Medico-surgical Sciences And Biotechnologies, Latina, Italy, [2]IRCSS, Santa Lucia Foundation, Roma, Italy, [3]University of L'Aquila, Department Of Biotechnological And Applied Clinical Sciences, L'Aquila, Italy

Endocannabinoids (eCBs) show pleiotropic and pro-homeostatic activities, including changes of inflammatory response within CNS and the ability to drive microglial polarization towards an anti-inflammatory phenotype. The anandamide (AEA) hydrolase fatty-acid-amide-hydrolase (FAAH) was found to be up-regulated in patients with Alzheimer's disease (AD), where the reduced levels of AEA are considered responsible for defective autophagy that leads to increased accumulation of β-amyloid (Aβ). **Aims** By the use of the selective FAAH inhibitor URB597, we have recently shown a shift towards an antiinflammatory phenotype in Aβ-treated BV2 murine microglia cells. Here, we aimed at investigating the possibility that *in vitro* and *in vivo* URB597 treatment can restore autophagy machinery in Aβ-treated microglial cells. **Methods** mRNAs of autophagy marker proteins (ATG-7, Beclin1, LC3, p62), and of the nuclear factor erythroid 2-related factor 2 (Nrf2) were obtained from URB597-treated BV-2 cells with or without Aβ, and were analyzed by real-time PCR. Protein expression was assessed by immunohistochemistry of brain sections (cortex-hippocampus) of URB597-administered transgenic Tg2576 mice, and the results confirmed by Western blot or immunofluorescence. **Results** FAAH inhibition increased the mRNA-expression of ATG-7, Beclin1, LC3, p62 as well as Nrf2 in BV-2 cells. The expression of ATG-7, Beclin1 and LC3 was also increased in BV2 cells and in the brain of URB597-administered transgenic Tg2576 mice, where a decrease of the total number/whole area of Aβ plaques was demonstrated. **Conclusions** The enhancement of eCB tone, and of AEA-mediated signaling, may improve autophagy in ADlike neuropathology.

Pubmed:

34299330: Grieco M, De Caris MG, Maggi E, Armeli F, Coccurello R, Bisogno T, D'Erme M, Maccarrone M, Mancini P, Businaro R
Fatty Acid Amide Hydrolase (FAAH) Inhibition Modulates Amyloid-Beta-Induced Microglia Polarization.
The ability of endocannabinoid (eCB) to change functional microglial phenotype can be explored as a possible target for therapeutic intervention. Since the inhibition of fatty acid amide hydrolase (FAAH), the main catabolic enzyme of anandamide (AEA), may provide beneficial effects in mice model of Alzheimer's disease (AD)-like pathology, we aimed at determining whether the FAAH inhibitor URB597 might target microglia polarization and alter the cytoskeleton reorganization induced by the amyloid-β peptide (Aβ). The morphological evaluation showed that Aβ treatment increased the surface area of BV-2 cells, which acquired a flat and polygonal morphology. URB597 treatment partially rescued the control phenotype of BV-2 cells when co-incubated with Aβ. Moreover, URB597 reduced both the increase of Rho protein activation in Aβ-treated BV-2 cells and the Aβ-induced migration of BV-2 cells, while an increase of Cdc42 protein activation was observed in all samples. URB597 also increased the number of BV-2 cells involved in phagocytosis. URB597 treatment induced the polarization of microglial cells towards an anti-inflammatory phenotype, as demonstrated by the decreased expression of iNOS and pro-inflammatory cytokines along with the parallel increase of Arg-1 and anti-inflammatory cytokines. Taken together, these data suggest that FAAH inhibition promotes cytoskeleton reorganization, regulates phagocytosis and cell migration processes, thus driving microglial polarization towards an anti-inflammatory phenotype.
Int J Mol Sci, 2021; 22
34073983: Armeli F, Bonucci A, Maggi E, Pinto A, Businaro R
Mediterranean Diet and Neurodegenerative Diseases: The Neglected Role of Nutrition in the Modulation of the Endocannabinoid System.
Neurodegenerative disorders are a widespread cause of morbidity and mortality worldwide, characterized by neuroinflammation, oxidative stress and neuronal depletion. The broad-spectrum neuroprotective activity of the

Mediterranean diet is widely documented, but it is not yet known whether its nutritional and caloric balance can induce a modulation of the endocannabinoid system. In recent decades, many studies have shown how endocannabinoid tone enhancement may be a promising new therapeutic strategy to counteract the main hallmarks of neurodegeneration. From a phylogenetic point of view, the human co-evolution between the endocannabinoid system and dietary habits could play a key role in the pro-homeostatic activity of the Mediterranean lifestyle: this adaptive balance among our ancestors has been compromised by the modern Western diet, resulting in a "clinical endocannabinoid deficiency syndrome". This review aims to evaluate the evidence accumulated in the literature on the neuroprotective, immunomodulatory and antioxidant properties of the Mediterranean diet related to the modulation of the endocannabinoid system, suggesting new prospects for research and clinical interventions against neurodegenerative diseases in light of a nutraceutical paradigm.
Biomolecules, 2021; 11
32889506: Fusconi M, Musy I, Valente D, Maggi E, Priori R, Pecorella I, Mastromanno L, Di Cristofano C, Greco A, Armeli F, Candelori F, de Vincentiis M, Gallo A, Businaro R
Immunohistochemical detection of IL-17 and IL-23 improves the identification of patients with a possible diagnosis of Sjogren's syndrome.
The diagnosis of primary Sjogren's syndrome (pSS) continues to be difficult and several patients keep symptomatic for years with different diagnoses before confirmation of pSS. Since the IL-23-IL-17 axis is involved in the etiopathogenesis of pSS we evaluated by immunohistochemistry and morphometric methods the presence of IL-17 as well as IL-23 within minor salivary glands (MSG) obtained from patients with uncertain diagnosis of pSS.
Pathol Res Pract, 2020; 216
32725637: Businaro R, Maggi E, Armeli F, Murray A, Laskin DL
Nutraceuticals as potential therapeutics for vesicant-induced pulmonary fibrosis.
Exposure to vesicants, including sulfur mustard and nitrogen mustard, causes damage to the epithelia of the respiratory tract and the lung. With time, this progresses to chronic disease, most notably, pulmonary fibrosis. The pathogenic process involves persistent inflammation and the release of cytotoxic oxidants, cytokines, chemokines, and profibrotic growth factors, which leads to the collapse of lung architecture, with fibrotic involution of the lung parenchyma. At present, there are no effective treatments available to combat this pathological process. Recently, much interest has focused on nutraceuticals, substances derived from plants, herbs, and fruits, that exert pleiotropic effects on inflammatory cells and parenchymal cells that may be useful in reducing fibrogenesis. Some promising results have been obtained with nutraceuticals in experimental animal models of inflammation-driven fibrosis. This review summarizes the current knowledge on the putative preventive/therapeutic efficacy of nutraceuticals in progressive pulmonary fibrosis, with a focus on their activity against inflammatory reactions and profibrotic cell differentiation.
Ann N Y Acad Sci, 2020; 1480
32575571: De Caris MG, Grieco M, Maggi E, Francioso A, Armeli F, Mosca L, Pinto A, D'Erme M, Mancini P, Businaro R
Blueberry Counteracts BV-2 Microglia Morphological and Functional Switch after LPS Challenge.
Microglia, the innate immune cells of the CNS, respond to brain injury by activating and modifying their morphology. Our study arises from the great interest that has been focused on blueberry (BB) for the antioxidant and pharmacological properties displayed by its components. We analyzed the influence of hydroalcoholic BB extract in resting or lipopolysaccharide (LPS)-stimulated microglia BV-2 cells. BB exerted a protective effect against LPS-induced cytotoxicity, as indicated by cell viability. BB was also able to influence the actin cytoskeleton organization, to recover the control phenotype after LPS insult, and also to reduce LPS-driven migration. We evaluated the activity of Rho and Rac1 GTPases, which regulate both actin cytoskeletal organization and migratory capacity. LPS caused an increase in Rac1 activity, which was counteracted by BB extract. Furthermore, we demonstrated that, in the presence of BB, mRNA expression of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α decreased, as did the immunofluorescence signal of iNOS, whereas that of Arg-1 was increased. Taken together, our results show that, during the inflammatory response, BB extract shifts the M1 polarization towards the M2 phenotype through an actin cytoskeletal rearrangement. Based on that, we might consider BB as a nutraceutical with anti-inflammatory activities.
Nutrients, 2020; 12
32289486: Scarabino D, Peconi M, Broggio E, Gambina G, Maggi E, Armeli F, Mantuano E, Morello M, Corbo RM, Businaro R
Relationship between proinflammatory cytokines (Il-1beta, Il-18) and leukocyte telomere length in mild cognitive impairment and Alzheimer's disease.
Inflammation plays a crucial role in Alzheimer's disease (AD). AD neurodegeneration and concurrent involvement of the peripheral immune system may promote leukocyte division and telomere shortening. We examined genotypes and plasma levels of two proinflammatory cytokines, IL-1beta and IL-18, and leukocyte telomere length (LTL) in patients with mild cognitive impairment (MCI) and AD. We wanted to determine whether changes in plasma IL-1beta and IL-18 levels, together

with LTL shortening, could be diagnostic for disease progression from MCI to AD. Median plasma IL-1beta levels were in the order MCI patients (2.2 pg/ml) < AD patients (4.0 pg/ml), both of which differed significantly from the controls (0.0 pg/ml). In the AD patients, the lowest IL-1beta levels were associated with the presence of the C allele of IL-1beta rs16944 SNP. Median plasma IL-18 levels were in the order MCI patients (116.3 pg/ml) > AD patients (85.8 pg/ml), both of which were significantly higher than in the controls (17.6 pg/ml). Analysis of LTL showed a progressive reduction in the order controls > MCI > AD patients ($p < 0.0001$). Overall LTL reduction was correlated with increased plasma IL-1beta levels, substantiating the hypothesis that inflammatory processes secondary to neuroinflammation may trigger telomere attrition. Changes in plasma IL-1beta and Il-18 levels, and LTL seem to reflect shifts in AD stage; they may have potential use as blood biomarkers to monitor disease onset and progression from MCI to AD.
Exp Gerontol, 2020; 136

FENS Forum 2022
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BOARD NUMBER: S06-232

EXPRESSION OF THE ADENOSINE A2A-A3 RECEPTOR HETEROMER IN DIFFERENT BRAIN REGIONS AND MARKED UPREGULATION IN THE MICROGLIA OF THE TRANSGENIC APPSW,IND ALZHEIMER'S DISEASE MODEL.

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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- Aims: Adenosine binds 4 different mammalian receptors: A_1, A_{2A}, A_{2B} and A_3. These receptors couple two different heterotrimeric G proteins, while A_1 and A_3 couple Gi, A_{2A} and A_{2B} couple Gs/Golf. GPCRs can interact forming heteromeric complexes and acquiring new structural and functional properties. Accordingly, the aim of this study was to look for expression and function of the A_{2A}/A_3 receptor heteromer ($A_{2A}A_3$Het) in HEK-transfected cells, neurons and microglia. **- Methods:** Transfected HEK-293T cells, neuronal and glial primary cultures were characterized by cAMP accumulation and MAPK activation, analyzing A_{2A} and A_3 receptors function. Using in situ proximity ligation assay (PLA) $A_{2A}A_3$Het expression was characterized in primary neurons and microglia of $APP_{Sw,Ind}$ mice model of Alzheimer's disease. **- Results:** The heteromeric context led to a marked decrease of the signaling originating at A_3 receptors. Interestingly from a therapeutic point of view, A_{2A} receptor antagonists overrode the blockade, thus allowing A_3 receptor-mediated signaling. $A_{2A}A_3$Het expression was markedly higher in striatal than in cortical and hippocampal neurons and the expression of the heteromer was markedly enhanced in microglia from the $APP_{Sw,Ind}$ model of Alzheimer's disease. **- Conclusion:** Within this study, we demonstrate that A_{2A}-A_3 can interact forming heteromeric complexes and the receptor heteromer can be an important target for neuroprotection by using A_{2A}R antagonists in order to potentiate the action of A_3R and avoid the blockade by the A_{2A} pro-inflammatory receptor.

Pubmed:
34066933: Franco R, Lillo A, Rivas-Santisteban R, Reyes-Resina I, Navarro G
Microglial Adenosine Receptors: From Preconditioning to Modulating the M1/M2 Balance in Activated Cells.
Neuronal survival depends on the glia, that is, on the astroglial and microglial support. Neurons die and microglia are activated not only in neurodegenerative diseases but also in physiological aging. Activated microglia, once considered harmful, express two main phenotypes: the pro-inflammatory or M1, and the neuroprotective or M2. When neuroinflammation, i.e., microglial activation occurs, it is important to achieve a good M1/M2 balance, i.e., at some point M1 microglia must be skewed into M2 cells to impede chronic inflammation and to afford neuronal survival. G protein-coupled receptors in general and adenosine receptors in particular are potential targets for increasing the number of M2 cells. This article describes the mechanisms underlying microglial activation and analyzes whether these cells exposed to a first damaging event may be ready to be preconditioned to better react to exposure to more damaging events. Adenosine receptors are relevant due to their participation in preconditioning. They can also be overexpressed in activated microglial cells. The potential of adenosine receptors and complexes formed by adenosine receptors and cannabinoids as therapeutic targets to provide microglia-mediated neuroprotection is here discussed.
Cells, 2021; 10
33803075: Casanovas M, Reyes-Resina I, Lillo A, Lillo J, López-Arnau R, Camarasa J, Escubedo E, Navarro G, Franco R
Methamphetamine Blocks Adenosine A Receptor Activation via Sigma 1 and Cannabinoid CB Receptors.
Methamphetamine is, worldwide, one of the most consumed drugs of abuse. One important side effect is neurodegeneration leading to a decrease in life expectancy. The aim of this paper was to check whether the drug affects one of the receptors involved in neurodegeneration/neuroprotection events, namely the adenosine A receptor (AR). First, we noticed that methamphetamine does not affect A functionality if the receptor is expressed in a heterologous system. However, AR becomes sensitive to the drug upon complexes formation with the cannabinoid CB receptor (CBR) and the sigma 1 receptor (σR). Signaling via both adenosine AR and cannabinoid CBR was affected by methamphetamine in cells co-expressing the two receptors. In striatal primary cultures, the AR-CBR heteromer complex was detected and methamphetamine not only altered its expression but completely blocked the AR- and the CBR-mediated activation of the mitogen activated protein kinase (MAPK) pathway. In conclusion, methamphetamine, with the participation of σR, alters the expression and function of

two interacting receptors, AR, which is a therapeutic target for neuroprotection, and CBR, which is the most abundant G protein-coupled receptor (GPCR) in the brain.
Int J Mol Sci, 2021; 22
34758399: Raïch I, Rivas-Santisteban R, Lillo A, Lillo J, Reyes-Resina I, Nadal X, Ferreiro-Vera C, de Medina VS, Majellaro M, Sotelo E, Navarro G, Franco R
Similarities and differences upon binding of naturally occurring Δ-tetrahydrocannabinol-derivatives to cannabinoid CB and CB receptors.
We have here assessed, using Δ-tetrahydrocannabinol (Δ-THC) for comparison, the effect of Δ-tetrahydrocannabinolic acid (Δ-THCA) and of Δ-tetrahydrocannabivarin (Δ9-THCV) that is mediated by human versions of CB, CB, and CB-CB receptor functional units, expressed in a heterologous system. Binding to the CB and CB receptors was addressed in living cells by means of a homogeneous assay. A biphasic competition curve for the binding to the CB receptor, was obtained for Δ-THCV in cells expressing the two receptors. Signaling studies included cAMP level determination, activation of the mitogen-activated protein kinase pathway and ß-arrestin recruitment were performed. The signaling triggered by Δ-THCA and Δ-THCV via individual receptors or receptor heteromers disclosed differential bias, i.e. the bias observed using a given phytocannabinoid depended on the receptor (CB, CB or CB-CB) and on the compound used as reference to calculate the bias factor (Δ-THC, a selective agonist or a non-selective agonist). These results are consistent with different binding modes leading to differential functional selectivity depending on the agonist structure, and the state (monomeric or heteromeric) of the cannabinoid receptor. In addition, on studying Gi-coupling we showed that Δ-THCV and Δ-THCA and Δ-THCV were able to revert the effect of a selective CB receptor agonist, but only Δ9-THCV, and not Δ9-THCA, reverted the effect of arachidonyl-2'-chloroethylamide (ACEA 100 nM) a selective agonist of the CB receptor. Overall, these results indicate that cannabinoids may have a variety of binding modes that results in qualitatively different effects depending on the signaling pathway that is engaged upon cannabinoid receptor activation.
Pharmacol Res, 2021; 174
33559278: Casanovas M, Jiménez-Rosés M, Cordomí A, Lillo A, Vega-Quiroga I, Izquierdo J, Medrano M, Gysling K, Pardo L, Navarro G, Franco R
Discovery of a macromolecular complex mediating the hunger suppressive actions of cocaine: Structural and functional properties.
Cocaine not only increases brain dopamine levels but also activates the sigma receptor (σ R) that in turn regulates orexigenic receptor function. Identification of interactions involving dopamine D (D R), ghrelin (GHS-R), and σ receptors have been addressed by biophysical techniques and a complementation approach using interfering peptides. The effect of cocaine on receptor functionality was assayed by measuring second messenger, cAMP and Ca , levels. The effect of acute or chronic cocaine administration on receptor complex expression was assayed by in situ proximity ligation assay. In silico procedures were used for molecular model building. σ R KO mice were used for confirming involvement of this receptor. Upon identification of protomer interaction and receptor functionality, a unique structural model for the macromolecular complex formed by σ R, D R, and GHS-R is proposed. The functionality of the complex, able to couple to both Gs and Gq proteins, is affected by cocaine binding to the σ R, as confirmed using samples from σ R mice. The expression of the macromolecular complex was differentially affected upon acute and chronic cocaine administration to rats. The constructed 3D model is consistent with biochemical, biophysical, and available structural data. The σ R, D R, and GHS-R complex constitutes a functional unit that is altered upon cocaine binding to the σ R. Remarkably, the heteromer can simultaneously couple to two G proteins, thus allowing dopamine to signal via Ca and ghrelin via cAMP. The anorexic action of cocaine is mediated by such complex whose expression is higher after acute than after chronic administration regimens.
Addict Biol, 2021; 26
31429130: Franco R, Reyes-Resina I, Aguinaga D, Lillo A, Jiménez J, Raïch I, Borroto-Escuela DO, Ferreiro-Vera C, Canela EI, Sánchez de Medina V, Del Ser-Badia A, Fuxe K, Saura CA, Navarro G
Potentiation of cannabinoid signaling in microglia by adenosine A receptor antagonists.
Neuroprotective M2-skewed microglia appear as promising to alter the course of neurodegenerative diseases and G protein-coupled receptors (GPCRs) are potential targets to achieve such microglial polarization. A common feature of adenosine A (A R) and cannabinoid CB (CB R) GPCRs in microglia is that their expression is upregulated in Alzheimer's disease (AD). On the one hand, CB R seems a target for neuroprotection, delaying neurodegenerative processes like those associated to AD or Parkinson's diseases. A R antagonists reduce amyloid burden and improve cognitive performance and memory in AD animal models. We here show a close interrelationship between these two receptors in microglia; they are able to physically interact and affect the signaling of each other, likely due to conformational changes within the A -CB receptor heteromer (A -CB Het). Particularly relevant is the upregulation of A -CB Het expression in samples from the APP , AD transgenic mice model. The most relevant finding, confirmed in both heterologous cells and in primary cultures of microglia, was that blockade of A receptors results in increased CB R-mediated signaling. This heteromer-specific feature suggests that A R antagonists

would potentiate, via microglia, the neuroprotective action of endocannabinoids with implications for AD therapy.
Glia, 2019; 67
34445634: Lillo J, Lillo A, Zafra DA, Miralpeix C, Rivas-Santisteban R, Casals N, Navarro G, Franco R
Identification of the Ghrelin and Cannabinoid CB Receptor Heteromer Functionality and Marked Upregulation in Striatal Neurons from Offspring of Mice under a High-Fat Diet.
Cannabinoids have been reported as orexigenic, i.e., as promoting food intake that, among others, is controlled by the so-called "hunger" hormone, ghrelin. The aim of this paper was to look for functional and/or molecular interactions between ghrelin GHSR1a and cannabinoid CB receptors at the central nervous system (CNS) level. In a heterologous system we identified CB-GHSR1a receptor complexes with a particular heteromer print consisting of impairment of CB receptor/G-mediated signaling. The blockade was due to allosteric interactions within the heteromeric complex as it was reverted by antagonists of the GHSR1a receptor. Cannabinoids acting on the CB receptor did not affect cytosolic increases of calcium ions induced by ghrelin acting on the GHSR1a receptor. In situ proximity ligation imaging assays confirmed the expression of CB-GHSR1a receptor complexes in both heterologous cells and primary striatal neurons. We tested heteromer expression in neurons from offspring of high-fat-diet mouse mothers as they have more risk to be obese. Interestingly, there was a marked upregulation of those complexes in striatal neurons from siblings of pregnant female mice under a high-fat diet.
Int J Mol Sci, 2021; 22
34749800: Rivas-Santisteban R, Lillo A, Lillo J, Rebassa JB, Contestí JS, Saura CA, Franco R, Navarro G
N-Methyl-D-aspartate (NMDA) and cannabinoid CB receptors form functional complexes in cells of the central nervous system: insights into the therapeutic potential of neuronal and microglial NMDA receptors.
The cannabinoid CB receptor (CBR), which is a target to afford neuroprotection, and N-methyl-D-aspartate (NMDA) ionotropic glutamate receptors, which are key in mediating excitatory neurotransmission, are expressed in both neurons and glia. As NMDA receptors are the target of current medication in Alzheimer's disease patients and with the aim of finding neuromodulators of their actions that could provide benefits in dementia, we hypothesized that cannabinoids could modulate NMDA function.
Alzheimers Res Ther, 2021; 13
34955755: Lillo A, Lillo J, Raïch I, Miralpeix C, Dosrius F, Franco R, Navarro G
Ghrelin and Cannabinoid Functional Interactions Mediated by Ghrelin/CB Receptor Heteromers That Are Upregulated in the Striatum From Offspring of Mice Under a High-Fat Diet.
There is evidence of ghrelinergic-cannabinoidergic interactions in the central nervous system (CNS) that may impact on the plasticity of reward circuits. The aim of this article was to look for molecular and/or functional interactions between cannabinoid CB and ghrelin GHS-R1a receptors. In a heterologous system and using the bioluminescence resonance energy transfer technique we show that human versions of cannabinoid CB and ghrelin GHS-R1a receptors may form macromolecular complexes. Such receptor heteromers have particular properties in terms of CB/G-mediated signaling and in terms of GHS-R1a-G-mediated signaling. On the one hand, just co-expression of CBR and GHS-R1a led to impairment of cannabinoid signaling. On the other hand, cannabinoids led to an increase in ghrelin-derived calcium mobilization that was stronger at low concentrations of the CB receptor agonist, arachidonyl-2'-chloroethylamide (ACEA). The expression of CB-GHS-R1a receptor complexes in striatal neurons was confirmed by proximity ligation imaging assays. Upregulation of CB-GHS-R1a- receptor complexes was found in striatal neurons from siblings of pregnant female mice on a high-fat diet. Surprisingly, the expression was upregulated after treatment of neurons with ghrelin (200 nM) or with ACEA (100 nM). These results help to better understand the complexities underlying the functional interactions of neuromodulators in the reward areas of the brain.
Front Cell Neurosci, 2021; 15
32357548: Franco R, Rivas-Santisteban R, Casanovas M, Lillo A, Saura CA, Navarro G
Adenosine A Receptor Antagonists Affects NMDA Glutamate Receptor Function. Potential to Address Neurodegeneration in Alzheimer's Disease.
(1) Background. -methyl d-aspartate (NMDA) ionotropic glutamate receptor (NMDAR), which is one of the main targets to combat Alzheimer's disease (AD), is expressed in both neurons and glial cells. The aim of this paper was to assess whether the adenosine A receptor (AR), which is a target in neurodegeneration, may affect NMDAR functionality. (2) Methods. Immuno-histo/cytochemical, biophysical, biochemical and signaling assays were performed in a heterologous cell expression system and in primary cultures of neurons and microglia (resting and activated) from control and the APP transgenic mice. (3) Results. On the one hand, NMDA and A receptors were able to physically interact forming complexes, mainly in microglia. Furthermore, the amount of complexes was markedly enhanced in activated microglia. On the other hand, the interaction resulted in a novel functional entity that displayed a cross-antagonism, that could be useful to prevent the exacerbation of NMDAR function by using AR antagonists. Interestingly, the amount of complexes was markedly higher in the hippocampal cells from the APP than from the control mice. In neurons, the number of complexes was lesser, probably due to NMDAR not

interacting with the AR. However, the activation of the AR receptors resulted in higher NMDAR functionality in neurons, probably by indirect mechanisms. (4) Conclusions. AR antagonists such as istradefylline, which is already approved for Parkinson's disease (Nouriast in Japan and Nourianz in the US), have potential to afford neuroprotection in AD in a synergistic-like fashion. i.e., via both neurons and microglia.
Cells, 2020; 9

32709103: Lillo A, Martínez-Pinilla E, Reyes-Resina I, Navarro G, Franco R
Adenosine A and A Receptors Are Able to Interact with Each Other. A Further Piece in the Puzzle of Adenosine Receptor-Mediated Signaling.
The aim of this paper was to check the possible interaction of two of the four purinergic P1 receptors, the A and the A. Discovery of the A-A receptor complex was achieved by means of immunocytochemistry and of bioluminescence resonance energy transfer. The functional properties and heteromer print identification were addressed by combining binding and signaling assays. The physiological role of the novel heteromer is to provide a differential signaling depending on the pre-coupling to signal transduction components and/or on the concentration of the endogenous agonist. The main feature was that the heteromeric context led to a marked decrease of the signaling originating at A receptors. Interestingly from a therapeutic point of view, A receptor antagonists overrode the blockade, thus allowing A receptor-mediated signaling. The A-A receptor heteromer print was detected in primary cortical neurons. These and previous results suggest that all four adenosine receptors may interact with each other. Therefore, each adenosine receptor could form heteromers with distinct properties, expanding the signaling outputs derived from the binding of adenosine to its cognate receptors.
Int J Mol Sci, 2020; 21

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BOARD NUMBER: S06-233

HUMAN IPSC-DERIVED TRIDIMENSIONAL-FULL-NETWORKS MODEL TO STUDY MICROGLIA HETEROGENEITY IN ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Alzheimer's disease (AD) is the most common form of dementia. Microglia are the main immune cells of the brain and play key roles in triggering and maintaining neuroinflammation, a fundamental feature of AD. Additionally, Human genome wide association studies have recently demonstrated that most of the AD risk genes were strongly or exclusively expressed by microglia, supporting their critical involvement in AD pathogenesis. However, whether their contribution is detrimental or beneficial is not yet well understood. Several studies suggested the coexistence of different subpopulations of microglia with various phenotypes, which could be responsible for the different and ambivalent responses observed. It is therefore crucial to decipher human microglia heterogeneity. To achieve this goal, we took advantages of recent advances in human induced Pluripotent Stem Cells (hiPSCs) cultures that pave the way for the development of innovative cellular models containing human microglia. To start, we set-up a complex iPSC-derived 3D-full-Networks composed of neural networks supplemented with microglia. We validating that microglial precursors co-cultured with 3D neural networks differentiated into fully functional microglia. We then generated 3D-full-Networks from both healthy donors, AD-patients iPSCs carrying either autosomic dominant or sporadic forms of the disease, and isogenic controls of the AD-lines (generated through CRISPR Cas9 technology). In these different models, we are characterizing the occurrence of several AD-related hallmarks: Ab and Phospho-Tau accumulation, neuroinflammation, neuronal defects, and more specifically microglial alterations. Our final objective will be to characterize the molecular heterogeneity of human microglia through single-cell RNA sequencing and functional genomic analysis.

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BOARD NUMBER: S06-234

EFFECT OF IMPAIRED ENDO-LYSOSOMAL DEGRADATION ON MICROGLIA IN A MOUSE MODEL OF ALZHEIMER´S DISEASE

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Microglia activation and associated neuroinflammation are regarded as key players in Alzheimer´s disease (AD) initiation and progression. Several risk loci for developing sporadic AD were identified in genes involved in microglia phagocytosis and endolysosomal processes. Since old age is still the biggest risk factor for AD, senescent microglia which show reduced degradative capacity are speculated to exaggerate AD pathology. However, the exact role of microglia degradation in AD is still controversial. To answer the question how impaired microglial endolysosomal degradation affects AD pathology, we combined a murine model of familial AD, 5xFAD, with a microglia specific conditional knockout of Rab7 (Rab7ΔMG). Rab7 is involved in the maturation of the late endo-lysosome and it was previously shown that Rab7ΔMG causes reduced lysosomal degradation in aged mice. We confirmed impaired lysosomal function in Rab7ΔMGx5FAD microglia which caused lysosomal accumulation of amyloid beta and reduced amyloid phagocytosis. Since it is well known that subsets of microglia contribute differently to AD pathology we performed single cell sequencing (scSeq) after FACS to investigate the impact of impaired degradation on different microglia clusters. On top we combined this scSeq results with the expression profile of phagocytic active (MX04+) and inactive (MX04-) microglia.

Pubmed:
35042829: Oberländer K, Witte V, Mallien AS, Gass P, Bengtson CP, Bading H
Dysregulation of and expression and an altered excitation-inhibition balance are associated with cognitive deficits in DBA/2 mice.
Differences in the learning associated transcriptional profiles between mouse strains with distinct learning abilities could provide insight into the molecular basis of learning and memory. The inbred mouse strain DBA/2 shows deficits in hippocampus-dependent memory, yet the transcriptional responses to learning and the underlying mechanisms of the impairments are unknown. Comparing DBA/2J mice with the reference standard C57BL/6N mouse strain we verify an enhanced susceptibility to kainic acid induced seizures, confirm impairments in hippocampus-dependent spatial memory tasks and uncover additional behavioral abnormalities including deficits in hippocampus-independent learning. Surprisingly, we found no broad dysfunction of the DBA/2J strain in immediate early gene (IEG) activation but instead report brain region-specific and gene-specific alterations. The learning-associated IEGs , -, and showed no DBA/2J deficits in basal or synaptic activity induced gene expression in hippocampal or cortical primary neuronal cultures or in the CA1, CA3, or retrosplenial cortex following spatial object recognition (SOR) training in vivo. However, the parietal cortex showed reduced and the dentate gyrus showed enhanced SOR-evoked induction of most IEGs. All DBA/2J hippocampal regions exhibited elevated basal expression of inhibin β A () and a learning-associated superinduction of the transcription factor neuronal Per-Arnt-Sim domain protein 4 () known to regulate the synaptic excitation-inhibition balance. In line with this, CA1 pyramidal neurons of DBA/2J mice showed fewer inhibitory and more excitatory miniature postsynaptic currents but no alteration in most other electrophysiological properties or gross dendritic morphology. The dysregulation of and expression and synaptic connectivity may underlie the cognitive deficits and increased susceptibility to seizures of DBA/2J mice.
Learn Mem, 2022; 29
28325690: Yu Y, Oberlaender K, Bengtson CP, Bading H
One nuclear calcium transient induced by a single burst of action potentials represents the minimum signal strength in activity-dependent transcription in hippocampal neurons.
Neurons undergo dramatic changes in their gene expression profiles in response to synaptic stimulation. The coupling of neuronal excitation to gene transcription is well studied and is mediated by signaling pathways activated by cytoplasmic and nuclear calcium transients. Despite this, the minimum synaptic activity required to induce gene expression remains unknown. To address this, we used cultured hippocampal neurons and cellular compartment analysis of temporal activity by

fluorescence in situ hybridization (catFISH) that allows detection of nascent transcripts in the cell nucleus. We found that a single burst of action potentials, consisting of 24.4±5.1 action potentials during a 6.7±1.9s depolarization of 19.5±2.0mV causing a 9.3±0.9s somatic calcium transient, is sufficient to activate transcription of the immediate early gene arc (also known as Arg3.1). The total arc mRNA yield produced after a single burst-induced nuclear calcium transient was very small and, compared to unstimulated control neurons, did not lead to a significant increase in arc mRNA levels measured using quantitative reverse transcriptase PCR (qRT-PCR) of cell lysates. Significantly increased arc mRNA levels became detectable in hippocampal neurons that had undergone 5-8 consecutive burst-induced nuclear calcium transients at 0.05-0.15Hz. These results indicate that a single burst-induced nuclear calcium transient can activate gene expression and that transcription is rapidly shut off after synaptic stimulation has ceased.
Cell Calcium, 2017; 65

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BOARD NUMBER: S06-235

EFFECT OF IMPAIRED MICROGLIAL ENDO-LYSOSOMAL DEGRADATION ON AMYLOID PATHOLOGY IN A MOUSE MODEL OF ALZHEIMER´S DISEASE.

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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To understand the role of microglia degradation in Aß-pathology, neuroinflammation and neurotoxicity, we generated 5xFAD transgenic AD mice with microglia-specific knockout of Rab7 GTPase (Rab7ΔMG x 5xFAD), which is responsible for the fusion of late endosomes and autophagosomes with the lysosome, and thereby promotes lysosomal degradation. We confirmed impaired lysosomal function in Rab7ΔMG x 5xFAD microglia, which also caused lysosomal accumulation of amyloid beta and reduced amyloid phagocytosis. With our single cell sequencing data (presented by Dr. Kristin Oberlander), we obtained detailed information of microglia subclusters. Since previous studies showed that microglia affect Aß-plaque properties, we investigated the effect of Rab7ΔMG x 5xFAD on Aß-plaque number, compaction and morphology. We also examined the effect of the microglial Rab7 depletion on neuronal health status in our mouse model and evaluated neuronal and synaptic markers by immunohistochemistry and western blot.

Pubmed:

23968729: Song Y, Yang X, Li Z, Zhao Y, Fan A
Label-free chemiluminescent ATP aptasensor based on graphene oxide and an instantaneous derivatization of guanine bases.
In this work, a novel label-free chemiluminescent (CL) aptasensor has been developed for rapid and facile detection of adenosine triphosphate (ATP, as model analyte) using graphene oxide (GO) nano-platform. The strategy relies on the preferential binding of GO to single-stranded DNA (ssDNA) over rigid double-stranded DNA (dsDNA) or aptamer-target complexes, and the instantaneous derivative reaction between phenylglyoxal (PGO), a special CL reagent as the signaling molecule, and guanine nucleobases (G) of aptamer strands adsorbed on the surface of GO. In the absence of ATP, the aptamers adsorbed onto the surface of GO leading to a strong background CL signal. Conversely, in the presence of ATP, the aptamers formed the aptamer-ATP complexes which had weak binding ability to GO resulting in a significant CL signal decrease. The CL intensity was adversely related to the ATP concentration in the assay solution. The biosensor's signal decreased linearly with the logarithm of the concentration of ATP from 2 to 80 nmol with a detection limit of 1.4 nmol. The aptasensor also showed high selectivity against cytosine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP). The method presented here holds the advantages of being label-free, cost effective, rapid, sensitive and selective, which would shows great promise for clinical application.
Biosens Bioelectron, 2014; 51
28176668: Yang X, Hutter M, Goh WWB, Bureik M
CYP4Z1 - A Human Cytochrome P450 Enzyme that Might Hold the Key to Curing Breast Cancer.
The human cytochrome P450 (CYP) enzyme CYP4Z1 is a fatty acid hydroxylase which among human CYPs is unique for being much stronger expressed in the mammary gland than in all other tissues. Moreover, it is strongly overexpressed in all subtypes of breast cancer, and some overexpression has also been found in other types of malignancies, such as ovarian, lung, and prostate cancers, respectively. Due to its unique expression pattern it is conceivable that this enzymes' activity might be exploited for a new therapeutic approach. However, the main challenge for a CYP4Z1-based prodrug strategy (CBPS) for the treatment of breast cancer (and possibly other CYP4Z1-positive malignancies) is the identification of candidate prodrugs that can be activated by this enzyme. In this mini-review we summarize the current knowledge about the enzymatic properties of the CYP4Z1 enzyme as well as on the expression pattern of the CYP4Z1 gene in both normal and cancer cells. Moreover, we present the first homology model of this enzyme and give an outlook on its potential use in cancer treatment strategies.
Curr Pharm Des, 2017; 23

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UNVEILING THE ASTROCYTIC-DRIVEN INFLAMMATORY RESPONSE IN ALZHEIMER'S DISEASE – INSIGHTS INTO INFLAMMASOME PATHWAYS

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Alzheimer's disease (AD) is a neurodegenerative disease that has been associated with the activation of multiprotein cytoplasmic complexes, the inflammasomes, which lead to the processing of Caspase-1, culminating in the maturation of Interleukin (IL)-1β and cleavage of Gasdermin D, the effector protein of pyroptosis, an inflammatory type of cell death. Microglia's contribution to the neuroinflammatory environment observed in AD via inflammasome pathways is highly explored. In astrocytes, less is known about inflammasome activation, being believed in the past that these complexes did not assemble in this cell type. However, evidence arose for inflammasome activation in astrocytes, generating controversy regarding this matter. Thus, this project aims to unravel the inflammatory response of astrocytes, under an AD context, focusing on the NLRP3 inflammasome cascade. For this, primary cultures of astrocytes were stimulated with $A\beta_{25-35}$, microglia derived-factors (F), and both stimuli concomitantly for 24h. Expression of NLRP3 components, their assembly in astrocytes, and IL-1β release were analyzed through Western Blot, Immunocytochemistry, and ELISA. Preliminary results showed that F, but not $A\beta_{25-35}$ alone, promoted an increase in NLRP3 domains and in IL-1β release, and this increase was more significant when the two stimuli were applied together. This was coincident with an increase in C3 and PTX3 expression, markers for reactive astrocytes. Altogether, we show that in the presence of F, which mimics microglia presence promoting astrocytic reactivity, $A\beta_{25-35}$ can activate NLRP3 pathway in astrocytes. Future work will explore other inflammasomes, such as NLRP1 and NLRP2, to unveil which pathway contributes most to astrocytic responsiveness.

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MICROGLIAL DIVERSITY IN ALZHEIMER'S DISEASE EARLY STAGES: A KEY TO UNDERSTAND THE DISEASE INITIATION

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Alzheimer's Disease (AD) is the most common form of dementia. It is characterized by behavioral deficits and histological features. Neuroinflammation is another important disease's hallmark, for which microglia are playing key roles. Of note, almost all AD risk genes are highly expressed in microglia, highlighting their crucial role in AD pathogenesis. Whether microglia play beneficial and/or detrimental roles to the disease progression remains heavily debated. In addition, their role in the initiation of the pathology is still poorly understood. The present study was designed to investigate microglial reaction at early stage of AD. The main objectives were to: 1) characterize whether microglial reaction occurs prior to plaques seeding; 2) identify genes and pathways dysregulated at this phase; and 3) determine whether deregulated genes may represent potential early biomarkers. Using the $APP^{swe}/PS1^{dE9}$:$CX3CR1^{+/eGFP}$ AD mice model, we isolated plaque-distant microglia at an early phase of the disease. RNA sequencing revealed significant remodeling of the microglial transcriptome and led to the identification of early markers of the pathology. In particular, we focused on two genes representing potential early biomarkers and investigated their expression in brain tissue from early stage AD mice. Using in-situ hybridization and immunohistochemical approaches, we demonstrate their upregulation in discrete microglial subpopulations. In parallel, we demonstrate that, at this disease's stage, synaptic modifications, microglial phenotypic reactivity and subtle cognitive alterations occur. Our data thus support an early contribution of microglia to AD progression and point to two specific genes that may represent potential early biomarkers and/or therapeutic targets.

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ADENOSINE A2A RECEPTOR ANTAGONISTS BLOCK NMDA RECEPTOR FUNCTION IN APPSW/IND MICE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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N -methyl d -aspartate (NMDA) ionotropic glutamate receptor (NMDAR), which is one of the main targets to combat Alzheimer's disease (AD), is expressed in both neurons and glial cells. The aim of this paper was to assess whether the adenosine A2A receptor (A2A R), which is a target in neurodegeneration, may affect NMDAR functionality. Methods. Immuno-histo/ cytochemical, biophysical, biochemical and signaling assays were performed in a heterologous cell expression system and in primary cultures of neurons and microglia (resting and activated) from control and the APPSw,Ind transgenic mice. Results: On the one hand, NMDA and A2A receptors were able to physically interact forming complexes, mainly in microglia. Furthermore, the amount of complexes was markedly enhanced in activated microglia. On the other hand, the interaction resulted in a novel functional entity that displayed a cross-antagonism, that could be useful to prevent the exacerbation of NMDAR function by using A2A R antagonists. Interestingly, the amount of complexes was markedly higher in the hippocampal cells from the APPSw,Ind than from the control mice. In neurons, the number of complexes was lesser, probably due to NMDAR not interacting with the A2A R. However, the activation of the A2A R receptors resulted in higher NMDAR functionality in neurons, probably by indirect mechanisms. A2A R antagonists such as istradefylline, which is already approved for Parkinson's disease (Nouriast® in Japan and Nourianz® in the US), have potential to afford neuroprotection in AD in a synergistic-like fashion. i.e., via both neurons and microglia.

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METABOLIC REGULATION OF AMYLOID-BETA CLEARANCE VIA MICROGLIA: IMPLICATIONS FOR ALZHEIMER'S DISEASE THERAPEUTICS

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Background: Alzheimer's disease (AD) is the most common cause of dementia and is characterized by pathological deposition of amyloid-β (Aβ) along with neuronal aggregation of hyperphosphorylated tau protein. Abnormal accumulation of Aβ deposits in AD is a critical early event that precedes neurodegeneration and involves an imbalance between the level of Aβ production and clearance. Aβ clearance is primarily a function of microglia, the brain-resident immune cells that are increasingly being implicated in several neuropsychiatric disorders. Microglia are highly sensitive to environmental factors and emerging evidence suggests that microglial functions are controlled by metabolic manipulations. Therefore, the aim of this project is to identify metabolic conditions that could selectively modulate microglial Aβ phagocytosis without substantially harming healthy neurons.
Methods: Human microglia-like (HMC3) cells were incubated with a variety of metabolic nutrients followed by exposure to 488-labelled Aβ (1-42) and fluorescent detection of Aβ uptake by cells. Relative gene expression of proteins involved in uptake and degradation of Aβ was assessed using RT-PCR.
Results: HMC3 cells incubated with lipid-depleted serum significantly upregulated Aβ phagocytosis when compared to lipid-containing serum. Moreover, pre-treatment of cells with HDL and LDL significantly reduced Aβ uptake. RT-PCR indicated that scavenger and lipoprotein receptors, as well as lipid metabolism-related proteins, might be responsible for observed effects. Further molecular underpinnings of the modulation of phagocytosis and its selectivity are the subjects of ongoing investigations.
Conclusions: Preliminary results involving high-throughput metabolic manipulations of HMC3 microglia indicate a role for lipoproteins and lipoprotein receptors in the modulation of microglial phagocytosis.

Pubmed:
34697842: Olajide OA, Iwuanyanwu VU, Lepiarz-Raba I, Al-Hindawi AA, Aderogba MA, Sharp HL, Nash RJ
Garcinia kola and garcinoic acid suppress SARS-CoV-2 spike glycoprotein S1-induced hyper-inflammation in human PBMCs through inhibition of NF-κB activation.
Symptoms and complications associated with severe SARS-CoV-2 infection such as acute respiratory distress syndrome (ARDS) and organ damage have been linked to SARS-CoV-2 spike protein S1-induced increased production of pro-inflammatory cytokines by immune cells. In this study, the effects of an extract of Garcinia kola seeds and garcinoic acid were investigated in SARS-CoV-2 spike protein S1-stimulated human PBMCs. Results of ELISA experiments revealed that Garcinia kola extract (6.25, 12.5, and 25 µg/ml) and garcinoic acid (1.25, 2.5, and 5 µM) significantly reduced SARS-CoV-2 spike protein S1-induced secretion of TNFα, IL-6, IL-1β, and IL-8 in PBMCs. In-cell western assays showed that pre-treatment with Garcinia kola extract and garcinoic acid reduced expressions of both phospho-p65 and phospho-IκBα proteins, as well as NF-κB DNA binding capacity and NF-κB-driven luciferase expression following stimulation of PBMCs with spike protein S1. Furthermore, pre-treatment of PBMCs with Garcinia kola extract prior to stimulation with SARS-CoV-2 spike protein S1 resulted in reduced damage to adjacent A549 lung epithelial cells. These results suggest that the seed of Garcinia kola and garcinoic acid are natural products which may possess pharmacological/therapeutic benefits in reducing cytokine storm in severe SARS-CoV-2 and other coronavirus infections.
Phytother Res, 2021; 35
32418005: Olajide OA, Akande IS, da Silva Maia Bezerra Filho C, Lepiarz-Raba I, de Sousa DP
Methyl 3,4,5-trimethoxycinnamate suppresses inflammation in RAW264.7 macrophages and blocks macrophage-adipocyte interaction.
Methyl 3,4,5-trimethoxycinnamate (MTC) is a bioactive natural phenylpropanoid. We evaluated anti-inflammatory effects of synthetic MTC in RAW264.7 macrophages and RAW264.7-3T3-L1 adipocytes co-culture. Levels of cytokines and

chemokines, as well as NO and PGE in cell supernatants were analysed using ELISAs, Griess assay and enzyme immunoassays, respectively. In-cell cytoblot was used to assess levels of proteins; while DNA binding and reporter gene assays were used to measure transcription factor DNA binding and transcriptional activities, respectively. Glucose uptake in adipocytes was evaluated with 2-deoxy-2-[(7-nitro-2, 1, 3-benzoxadiazol-4-yl) amino]-D-glucose uptake. MTC (5-20 μM) suppressed LPS + IFNγ-induced release of TNFα, IL-6 and IL-1β, as well as NO/iNOS and PGE/COX-2 levels in RAW264.7 cells. Furthermore, there was a reduction in phospho-IκB and phospho-p65 proteins, accompanied by a reduction in total IκB in RAW264.7 cells. Further studies showed that MTC also produced a reduction in NF-κB DNA binding and luciferase activity. Treatment of RAW264.7 cells with MTC (5-20 μM) resulted in enhanced DNA binding of Nrf2 and an increase in ARE-luciferase activity. In a macrophage-adipocyte co-culture, the compound reduced the release of TNFα, IL-6, IL-1β, MCP-1 and RANTES, while enhancing glucose uptake and activation of AMPKα. Our results suggest that MTC produced anti-inflammatory and antioxidant activities in macrophages. MTC also prevented inflammation in macrophage-adipocyte co-culture. The effect of MTC on glucose uptake in adipocytes is proposed to be linked to activation of AMPK.
Inflammopharmacology, 2020; 28
33860869: Olajide OA, Iwuanyanwu VU, Lepiarz-Raba I, Al-Hindawi AA
Induction of Exaggerated Cytokine Production in Human Peripheral Blood Mononuclear Cells by a Recombinant SARS-CoV-2 Spike Glycoprotein S1 and Its Inhibition by Dexamethasone.
An understanding of the pathological inflammatory mechanisms involved in SARS-CoV-2 virus infection is necessary in order to discover new molecular pharmacological targets for SARS-CoV-2 cytokine storm. In this study, the effects of a recombinant SARS-CoV-2 spike glycoprotein S1 was investigated in human peripheral blood mononuclear cells (PBMCs). Stimulation of PBMCs with spike glycoprotein S1 (100 ng/mL) resulted in significant elevation in the production of TNFα, IL-6, IL-1β and IL-8. However, pre-treatment with dexamethasone (100 nM) caused significant reduction in the release of these cytokines. Further experiments revealed that S1 stimulation of PBMCs increased phosphorylation of NF-κB p65 and IκBα, and IκBα degradation. DNA binding of NF-κB p65 was also significantly increased following stimulation with spike glycoprotein S1. Treatment of PBMCs with dexamethasone (100 nM) or BAY11-7082 (1 μM) resulted in inhibition of spike glycoprotein S1-induced NF-κB activation. Activation of p38 MAPK by S1 was blocked in the presence of dexamethasone and SKF 86002. CRID3, but not dexamethasone pre-treatment, produced significant inhibition of S1-induced activation of NLRP3/caspase-1. Further experiments revealed that S1-induced increase in the production of TNFα, IL-6, IL-1β and IL-8 was reduced in the presence of BAY11-7082 and SKF 86002, while CRID3 pre-treatment resulted in the reduction of IL-1β production. These results suggest that SARS-CoV-2 spike glycoprotein S1 stimulated PBMCs to release pro-inflammatory cytokines through mechanisms involving activation of NF-κB, p38 MAPK and NLRP3 inflammasome. It is proposed that the clinical benefits of dexamethasone in COVID-19 are possibly due to its anti-inflammatory activity in reducing SARS-CoV-2 cytokine storm.
Inflammation, 2021; 44
30811877: Velagapudi R, Lepiarz I, El-Bakoush A, Katola FO, Bhatia H, Fiebich BL, Olajide OA
Induction of Autophagy and Activation of SIRT-1 Deacetylation Mechanisms Mediate Neuroprotection by the Pomegranate Metabolite Urolithin A in BV2 Microglia and Differentiated 3D Human Neural Progenitor Cells.
Urolithin A is an anti-inflammatory and neuroprotective gut-derived metabolite from ellagitannins and ellagic acid in pomegranate, berries, and nuts. The roles of SIRT-1 and autophagy in the neuroprotective activity of urolithin A are investigated.
Mol Nutr Food Res, 2019; 63
31634788: Velagapudi R, Jamshaid F, Lepiarz I, Katola FO, Hemming K, Olajide OA
The tiliroside derivative, 3-O-[(E)-(2-oxo-4-(p-tolyl) but-3-en-1-yl] kaempferol produced inhibition of neuroinflammation and activation of AMPK and Nrf2/HO-1 pathways in BV-2 microglia.
Neuroinflammation is now widely accepted as an important pathophysiological mechanism in neurodegenerative disorders, thus providing a critical target for novel compounds. In this study, 3-O-[(E)-(2-oxo-4-(p-tolyl)but-3-en-1-yl] kaempferol (OTBK) prevented the production of pro-inflammatory mediators TNFα, IL-6, PGE and nitrite from BV-2 microglia activated with LPS and IFNγ. These effects were accompanied by reduction in the levels of pro-inflammatory proteins COX-2 and iNOS. Involvement of NF-κB in the anti-inflammatory activity of OTBK was evaluated in experiments showing that the compound prevented phosphorylation, nuclear accumulation and DNA binding of p65 sub-unit induced by stimulation of BV-2 microglia with LPS and IFNγ. Exposure of mouse hippocampal HT22 neurons to conditioned media from LPS + IFNγ-stimulated BV-2 cells resulted in reduced cell viability and generation of cellular reactive oxygen species. Interestingly, conditioned media from LPS/IFNγ-stimulated BV-2 cells which were treated with OTBK did not induce neuronal damage or oxidative stress. OTBK was shown to increase protein levels of phospho-AMPKα, Nrf2 and HO-1 in BV-2 microglia. It was further revealed that OTBK treatment increased Nrf2 DNA binding in BV-2 microglia. The actions of the compound on AMPKα and Nrf2 were shown to contribute to its anti-inflammatory activity as demonstrated by diminished activity in the presence of the AMPK

antagonist dorsomorphin and Nrf2 inhibitor trigonelline. These results suggest that OTBK inhibits neuroinflammation through mechanisms that may involve activation of AMPKα and Nrf2 in BV-2 microglia.
Int Immunopharmacol, 2019; 77

28458100: Velagapudi R, Kumar A, Bhatia HS, El-Bakoush A, Lepiarz I, Fiebich BL, Olajide OA
Inhibition of neuroinflammation by thymoquinone requires activation of Nrf2/ARE signalling.
Thymoquinone is an antioxidant phytochemical that has been shown to inhibit neuroinflammation. However, little is known about the potential roles of intracellular antioxidant signalling pathways in its anti-inflammatory activity. The objective of this study was to elucidate the roles played by activation of the Nrf2/ARE antioxidant mechanisms in the anti-inflammatory activity of this compound. Thymoquinone inhibited lipopolysaccharide (LPS)-induced neuroinflammation through interference with NF-κB signalling in BV2 microglia. Thymoquinone also activated Nrf2/ARE signalling by increasing nuclear localisation, DNA binding and transcriptional activity of Nrf2, as well as increasing protein levels of HO-1 and NQO1. Suppression of Nrf2 activity through siRNA or with the use of trigonelline resulted in the loss of anti-inflammatory activity by thymoquinone. Taken together, our studies show that thymoquinone inhibits NF-κB-dependent neuroinflammation in BV2 microglia, by targeting antioxidant pathway involving activation of both Nrf2/ARE. We propose that activation of Nrf2/ARE signalling pathway by thymoquinone probably results in inhibition of NF-κB-mediated neuroinflammation.
Int Immunopharmacol, 2017; 48

28551846: Velagapudi R, El-Bakoush A, Lepiarz I, Ogunrinade F, Olajide OA
AMPK and SIRT1 activation contribute to inhibition of neuroinflammation by thymoquinone in BV2 microglia.
Thymoquinone is a known inhibitor of neuroinflammation. However, the mechanism(s) involved in its action remain largely unknown. In this study, we investigated the roles of cellular reactive oxygen species (ROS), 5' AMP-activated protein kinase (AMPK) and sirtuin 1 (SIRT1) in the anti-neuroinflammatory activity of thymoquinone. We investigated effects of the compound on ROS generation in LPS-activated microglia using the fluorescent 2',7'-dichlorofluorescin diacetate (DCFDA)-cellular ROS detection. Immunoblotting was used to detect protein levels of p40, gp91, AMPK, LKB1 and SIRT1. Additionally, ELISA and immunofluorescence were used to detect nuclear accumulation of SIRT1. NAD/NADH assay was also performed. The roles of AMPK and SIRT1 in anti-inflammatory activity of thymoquinone were investigated using RNAi and pharmacological inhibition. Our results show that thymoquinone reduced cellular ROS generation, possibly through inhibition of p40 and gp91 protein. Treatment of BV2 microglia with thymoquinone also resulted in elevation in the levels of LKB1 and phospho-AMPK proteins. We further observed that thymoquinone reduced cytoplasmic levels and increased nuclear accumulation of SIRT1 protein and increased levels of NAD. Results also show that the anti-inflammatory activity of thymoquinone was abolished when the expressions of AMPK and SIRT1 were suppressed by RNAi or pharmacological antagonists. Pharmacological antagonism of AMPK reversed thymoquinone-induced increase in SIRT1. Taken together, we propose that thymoquinone inhibits cellular ROS generation in LPS-activated BV2 microglia. It is also suggested that activation of both AMPK and NAD/SIRT1 may contribute to the anti-inflammatory, but not antioxidant activity of the compound in BV2 microglia.
Mol Cell Biochem, 2017; 435

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MICROGLIAL RESPONSE IS A PATHOGENIC DRIVING MECHANISM IN THE NDUFS4 KO MOUSE MODEL OF LEIGH SYNDROME

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Leigh syndrome is a mitochondrial disease that is typically accompanied by a progressive neurodegenerative disorder. Mice lacking the complex I subunit NDUFS4 (*Ndufs4* KO) develop fatal progressive encephalopathy resembling Leigh syndrome. As the disease progresses, neurodegeneration and neuroinflammation increase and are responsible for the neurological symptoms, which include: sever motor decline, respiratory alterations and epilepsy. However, despite the magnitude of the neuroinflammation associated to brain lesions the role of its main cellular components have barely been addressed in the context of *Ndufs4* KO mouse pathology. Therefore, we aim to elucidate the role of microglia, the specialized immune cell of the CNS, on the pathology of the *Ndufs4* KO mice. For that purpose, we have depleted microglia by using PLX3397, an antagonist of the colony-stimulating factor 1 receptor (CSF1R), since CSF-1 is essential for the maintenance of these cells. We daily injected intraperitoneally PLX3397 both control and Ndufs4 KO mice and evaluated the progression of the disease by using the open field and the rotarod test. We also measured the onset of clasping behavior and the respiratory pattern. Moreover, we addressed brain neuroinflammation using IBA-1 and GFAP staining. Our results show that microglial depletion extends survival, delays the onset of the motor phenotype and alleviates brain pathology. We conclude that microglial response contributes to the progression of the pathology, suggesting a causative role of neuroinflammation in the disease in Ndufs4 KO mice and potentially in Leigh patients.

Pubmed:
30636247: Fernández-Gayol O, Sanchis P, Aguilar K, Navarro-Sempere A, Comes G, Molinero A, Giralt M, Hidalgo J
Different Responses to a High-Fat Diet in IL-6 Conditional Knockout Mice Driven by Constitutive GFAP-Cre and Synapsin 1-Cre Expression.
Interleukin-6 (IL-6) is a major cytokine controlling body weight and metabolism, at least in part through actions in the central nervous system (CNS) from local sources.
Neuroendocrinology, 2019; 109
33059703: Sanchis P, Fernández-Gayol O, Comes G, Aguilar K, Escrig A, Giralt M, Palmiter RD, Hidalgo J
A new mouse model to study restoration of interleukin-6 (IL-6) expression in a Cre-dependent manner: microglial IL-6 regulation of experimental autoimmune encephalomyelitis.
Interleukin-6 (IL-6) is a pleiotropic cytokine that controls numerous physiological processes both in basal and neuroinflammatory conditions, including the inflammatory response to experimental autoimmune encephalomyelitis (EAE). IL-6 is produced by multiple peripheral and central cells, and until now, the putative roles of IL-6 from different cell types have been evaluated through conditional cell-specific IL-6 knockout mice. Nevertheless, these mice probably undergo compensatory responses of IL-6 from other cells, which makes it difficult to assess the role of each source of IL-6.
J Neuroinflammation, 2020; 17

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VALIDATION OF NANOPARTICLE-PEPTIDE TARGETING BIOMARKER IN THE BLOOD-BRAIN BARRIER UNDER NEUROINFLAMMATION RELATED TO MULTIPLE SCLEROSIS

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Multiple sclerosis (MS) is a neurodegenerative autoimmune disease characterized by demyelination and manifested by various motor, somatosensory and cognitive symptoms. Demyelinating lesions are accompanied by neuroinflammation and increased blood-brain barrier (BBB) permeability. Advanced imaging techniques as magnetic resonance imaging (MRI) have contributed to detect lesions (and permeability) of the BBB. In addition, the use of magnetic ultra-small particles of iron oxide (USPIO) as contrast agents has been confirmed to improve MRI observations. From a repertoire of peptides detected under neuroinflammation, we previously identified that peptide-88 (P88) has high affinity to laminin-511, an extracellular matrix protein present in the BBB. Laminin has been reported to change its expression and function under neuroinflammation, including MS. This proposal aims to validate the peptide-receptor pair (P88/laminin511) vectorized on USPIO nanoprobes, in order to explore BBB molecular alterations occurring during neuroinflammation as potential tools for use in MRI analysis.We have designed USPIO-P88 nanoprobes and tested cytotoxicity, observing no cell death at concentrations < 4 mM. In addition, we validated the nanoprobes with P88 as a biomarker, under inflammatory conditions, in an *in vitro* model of BBB conformed by brain endothelial cells, astrocytes and pericytes. We have also performed biocompatibility and pharmacokinetic studies *in vivo*, in rats, and additionally tested the marking of the nanoprobe-P88 under induced neuroinflammation. Affinity between P88 and laminin, may provide insight into the role laminin plays in BBB disruption under neuroinflammation, thus laminin (as a target of P88) may be considered a potential biomarker in MS.

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AMYLOID CLEARANCE BY OLIGODENDROCYTE-MEDIATED MICROGLIAL ACTIVATION

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

Soonbong Baek, Seungeun Yeo, Hyun Jin Jung, Jae Myung Jang, Jong-Pil Kim, Youngshik Choe*
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In Alzheimer diseases (AD), microglia, the brain-resident immune cells, protect neural connections against amyloid accumulation and dystrophic neurites. However, the cellular mechanism of microglial activation and recruitment to amyloid plaques is still unclear. In this presentation, we provide evidence that the interaction of amyloid plaque and oligodendrocytes (OLs), a guardian of neural circuits, initiates activation of microglial cells and clearance of the neurotic plaques. Expression of OL-specific immune factor (OSIF) was induced by beta-amyloid, which subsequently promoted the phagocytic activity of microglia. Conditional inhibition of OSIF expression in 5xFAD transgenic background (OSIF-cKO-Tg) increased the spreading of amyloid plaques along with diminished plaque-associated microglia in the cortex. Interestingly, transcriptome analysis showed a Trem2/CD68-positive microglial subtype was reduced in the cortex of OSIF-cKO-Tg mice which is phenotypically similar to microglia-specific *Trem2* knockout in AD model mice. Our presentation illustrates that the OSIF is an important initiator of Trem2-dependent microglial condensation of amyloid plaques to protect neurite damages in AD.

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EFFECT OF PARTHENOLIDE IN LPS-INDUCED MICROGLIAL NEUROINFLAMMATION

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Introduction: Neuroinflammation is one of the most important components related to neurodegenerative processes and can be triggered by various stimuli that lead to the activation of innate immunity receptors and consequently to neurons death. Among the inflammatory pathways studied in neurodegenerative diseases, the pathway mediated by the HMGB1 protein has been shown to be fundamental. HMGB1 released by neurons and microglia into the extracellular space can activate RAGE and TLR4 receptors, which, in turn, activate inflammatory pathways mediated by the gene transcription factor NF-κB, leading to the production of proteins such as IL-6 and TNF-α. Aims: The main objective of this work was to study the effect of Parthenolide on LPS-induced microglial neuroinflammation and its effect on HGMB1 gene expression. Parthenolide is a sesquiterpene lactone of the germacranolide class which occurs naturally in *Tanacetum parthenium* with recognized anti-inflammatory action via NF-κB inhibition. Methods: To evaluate the effects of Parthenolide, murine microglial cells (BV2) were treated with Parthenolide for 30 minutes and then the cells were stimulated with LPS in presence of IFNγ for 24h. Results: Our results shown a decrease of Nitric Oxide and TNF-α production by cells treated with Parthenolide and stimulated with LPS plus IFN-γ when compared with the positive control. Also, preliminary data showed that Parthenolide treatment decreases the *hmgb1*, *tlr4* and *rage* gene expression on cells stimulated with LPS plus IFN-γ. Conclusions: Together, these results contribute to understand the neuroinflammatory scenario and may help the development of new therapeutic strategies related with neuroinflammatory process.

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MOLECULAR LOGIC OF MICROGLIAL ACTIVATION IN ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Genomic-wide association studies (GWAS) and whole exome sequencing (WES) in large cohorts of human subjects have underlined a conspicuous role for microglial cells in the susceptibility to Alzheimer's disease (AD) (Bellenguez et al., 2022). However, the contribution of different microglial states to pathophysiological processes remains elusive. Recent work using single-cell RNA-sequencing (scRNA-seq) or single-nucleus RNA-sequencing (snRNA-seq) has started to unravel the molecular signatures of microglial states in the human AD brain (Mathys et al., 2019; Olah et al., 2020; Srinivasan et al., 2020; Zhou et al., 2020; Morabito et al., 2021). Despite the different classifications used in these studies, they consistently showed that diverse microglial subpopulations can be distinguished in the healthy and diseased adult human brain using molecular profiles revealed by sc/snRNA-seq. However, these studies found remarkably little overlap between microglial gene-expression signatures in human Alzheimer's with those previously identified in mouse models, including disease-associated microglia (DAM, Keren-Shaul et al., 2017). In this work, we took advantage of CellID (Cortal et al., 2021) to identify DAM and homeostatic microglia gene signatures at single cell resolution in three different sc/snRNA-seq datasets generated from healthy and AD brain samples. We also used pseudotime to identify relationships among microglial subsets and SCENIC (Aibar et al., 2018) to study gene regulatory networks and identify master regulatory transcription factors associated with different microglial phenotypes. Our results provide a comprehensive molecular characterization of microglial subtypes in the AD brain and pinpoints potential regulators of microglial states in this pathology.

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DEVELOPMENT OF NOVEL RABBIT MONOCLONAL ANTIBODIES TO CHARACTERIZE MICROGLIAL ACTIVATION STATES IN MURINE MODELS OF ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia worldwide. Neuroinflammation is an important feature of AD pathology, but the precise contribution of neuroinflammation on disease progression is poorly understood. Microglia, the brain's resident macrophages, are likely to play an important role in initiating and maintaining neuroinflammatory responses that contribute directly or indirectly to AD etiology. Several genome-wide association studies in human AD patients identified genes enriched or uniquely expressed in microglia. Moreover, single-cell RNA sequencing (scRNA-seq) studies identified multiple microglia-enriched genes that are upregulated in the context of disease, both in human AD tissue as well as mouse models of AD. Development of tools to these specific genes or gene products can be used to identify disease-associated microglial states and further our understanding of the specific neuroinflammatory responses that contribute to disease. We have developed and validated a cohort of rabbit monoclonal antibodies that can be used to detect these microglial gene products. We used multiplexing techniques to establish microglial enrichment of these targets, including ASC/TMS1, GPNMB, TMEM119, and Galectin-3, in both mouse brain tissue and mouse models of AD. Within this cohort, we highlight Cathepsin D, a lysosomal aspartyl protease involved in protein degradation that is enriched in microglia, particularly in the context of disease. We continue to develop a comprehensive portfolio of monoclonal antibodies to further characterize microglia cellular processes and activation states to understand the role of microglia in neurodegenerative diseases.

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NEUROINFLAMMATORY BOOST OF SYNAPTIC STRIPPING VIA INDUCTION, STRETCHING AND PHAGOCYTOSIS OF SYNAPTIC FILOPODIA

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Despite the accumulating evidence implying activated microglia in the synaptic loss, the sequence of events preceding synaptic phagocytosis or spine retraction remains elusive. Therefore, we performed two-photon time-lapse study of light-directed microglia-synapse interactions in two acute models of neuroinflammation induced in adult mice by bacterial lipopolysaccharide (LPS) administration or by inoculation of Tau/Ab-enriched sarkosyl-insoluble fraction derived from AD patient brain. Both treatments induced activation of microglia, which manifested as characteristic morphological changes and upregulated expression of phagosomal markers, CD68, C3 and C3R, particularly in the model of AD. These changes were associated with decreased surveillance of synapses under baseline condition and establishment of long-lasting contacts between microglia and synapses after single-synapse photoablation. The duration of contacts and expression levels of phagosomal proteins correlated with elevated spine/bouton turnover, generation of synaptic filopodia, and elimination of spines. Several scenarios of spine loss were observed: (i) rapid retraction of spines after their interaction with microglia; (ii) engulfment of spines, followed by endocytosis; and, most surprisingly, (iii) spine engulfment followed by pullout of a spine head filopodia, its stretching during microglia retraction and spine elimination. Our findings highlight multiple common features of synaptic remodeling by microglia in the context of systemic inflammation and AD, and gain a direct insight into the cellular mechanisms of synaptic stripping in these conditions.

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EXTRACELLULAR VESICLES FROM HUMAN IPSC-DERIVED NEURAL STEM CELLS ALLEVIATE MICROGLIAL RESPONSE AND COGNITIVE IMPAIRMENTS IN A CHRONIC NEUROINFLAMMATION MODEL

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Aims: Extracellular vesicles (EVs) released by human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) are enriched with miRNAs and proteins capable of mediating anti-inflammatory activity (Upadhya et al., J. Extracell Vesicles, 9:1809064, 2020). The lack of tumorigenic and immunogenic properties and ability to permeate the entire brain to incorporate into neurons and microglia following intranasal (IN) administrations makes them an attractive biologic for mediating neuroprotection and curtailing chronic neuroinflammation in neurodegenerative disorders. We tested the hypothesis that intranasal administrations of hiPSC-NSC-EVs can alleviate chronic neuroinflammation and cognitive impairments induced by systemic lipopolysaccharide (LPS) challenge. **Methods:** Adult male C57BL/6J mice received intraperitoneal injections of LPS (0.75 mg/Kg) for seven consecutive days. Then, the mice received either vehicle (VEH) or hiPSC-NSC-EVs (~66 x 10^9 EVs/administration, thrice over 7 days). A month later, mice in all groups were investigated for cognitive function with a battery of behavioral tests and euthanized for histological and biochemical studies. **Results:** Mice receiving VEH after LPS displayed impairments in object location memory, spatial recognition memory, and pattern separation function. Such impairments were associated with an increased incidence of activated microglia presenting NLRP3 inflammasomes and an increased concentration of proinflammatory cytokines in the hippocampus. In contrast, in all behavioral tests, mice receiving hiPSC-NSC-EVs after LPS exhibited better cognitive function than mice receiving VEH after LPS. Importantly, these mice exhibited diminished microglial activation with reduced NLRP3 inflammasomes and proinflammatory cytokines. **Conclusion:** Thus, intranasal administrations of hiPSC-NSC-EVs are an efficacious approach to reducing chronic neuroinflammation-associated cognitive impairments.

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IGF-1 EFFECT IN LPS-DERIVED NEUROINFLAMMATION IS MEDIATED BY P110α SUBUNIT OF PI3K AND SHOWS SEXUAL DIFFERENCES.

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Insulin-like growth factor-1 (IGF-1) has been characterized as one of the main neuroprotective molecules. It also actively participates in processes such as synaptic pruning and clearance of harmful aggregates present in most neurodegenerative diseases. However, those functions have been evaluated using male or non-sexed animals.
Systemic bacterial lipopolysaccharide (LPS)-treatment increased in both sexes the expression of GFAP, a reactive astrocyte marker, in the cortex of mice and was blocked by IGF-1 only in males. In primary astrocytes, LPS enhanced the mRNA expression of Toll-like receptors 2 and 4, and proinflammatory factors such as iNOS, IP-10 and cytokines IL-1β, IL-6 and IL-10 in male and female. However, treatment with IGF-1 counteracted cytokines expression in males. Furthermore, reactive astrocyte phagocytosis was modulated by IGF-1 in male astrocytes exclusively. By Western Blot, we observed that IGF-1 was also able to increase AKT-phosphorylation only in male astrocytes, and was reduced using PI3K inhibitors, AG66, TGX-221 and CAL-101, with selectivity towards catalytic p110α, p110β and p110δ isoforms respectively. Although all isoforms interact physically with IGF-1-receptor in both sexes, the expression of p110α is higher in males while the expression of IGF-1-receptor is similar among sexes. AG66 suppressed the IGF-1 effect on cytokine expression and counteracted the IGF-1-produced phagocytosis decrease in male reactive astrocytes.
Taken together, our results suggest that sex differences could be due to a lower expression of the p110α subunit in female and that IGF-1-effects on the inflammatory response and phagocytosis of male reactive astrocytes are mediated by p110α/PI3K subunit.

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BALANCE IS BLISS: EXPLORING THE ROLE OF KYNURENINE 3-MONOOXYGENASE (KMO) IN IMMUNE CHALLENGED MICROGLIA

POSTER SESSION 06 - SECTION: MICROGLIA AND NEUROINFLAMMATION

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Aims: Kynurenine 3-monooxygenase (KMO) is a key enzyme in the kynurenine pathway (KP) of tryptophan degradation, which contains several neuromodulatory metabolites. KMO lies at a critical branch point in the KP and thereby regulates the balance between neuroprotective and neurotoxic kynurenine-derived metabolites associated with the pathogenesis of several neurodegenerative and psychotic disorders. In the central nervous system, KMO is highly expressed in microglial cells, which serve as the primary active immune defence system of the brain. As it has been proposed that KMO plays a central role in regulating neuroinflammation, here we sought to better understand the cellular function of KMO in microglia during inflammation. **Methods:** C20 human microglial cells were challenged for 24 hours with pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ) or bacterial LPS. *Kmo* expression was then measured by RT-qPCR and excreted cytokines quantified using MSD multi-spot assays. **Results:** We found that immune challenged cells dramatically upregulated release of several pro-inflammatory cytokines as compared to untreated cells. Notably, this correlated to a significant increase in *Kmo* expression. Supplementation of microglia with dexamethasone, a synthetic glucocorticoid (GC), was sufficient to dampen the effect of this immune stimulation - yielding a robust decrease in both *Kmo* mRNA and cytokine levels. In contrast, pre-treatment with the KMO inhibitor Ro 61-8048 did not alter cytokine release, suggesting altered *Kmo* expression occurs downstream of immune stimulation. **Conclusions:** Our data suggest that the use of GCs could be a possible therapeutic strategy for diseases associated with neuroinflammation, particularly those with altered KP metabolism.

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A NOVEL KNOCK-IN MODEL RECAPITULATES EARLY EVENTS OF ALZHEIMER'S DISEASE.

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Aims: Alzheimer's disease (AD), primary cause of dementia in the elderly, is characterized at the neuropathological level by extracellular accumulation of amyloid peptide and intraneuronal aggregation of abnormally and hyperphosphorylated Tau proteins. Tau aggregation causes neuronal death and leads to cognitive impairment. Many animal models that try to mimic AD pathology have been obtained by overexpressing both proteins. This study focuses on a new AD mouse model that should develop concomitant pathologies linked to the co-expression of the two humanized proteins, without overexpression. Methods: We have generated this mouse model by crossing a Knock-In humanized mouse model for amyloid peptide (kindly provided by Pr TC Saido) with a Knock-In mouse model for human mutated 1N4R Tau isoform. Double mutant mice (APPxTau) were analyzed at 6 months and compared to single mutants APP or Tau and wild-type littermates. Metabolic, behavior, molecular and biochemical analyses were undertaken in this study. Results: APPxTau mice do not display any particular metabolic and behavior phenotype. Transgenes are expressed at a level similar to endogenous corresponding proteins. An increase in Tau phosphorylation is observed as well as an increasing number of amyloid plaques. Finally, using q-PCR, neuroinflammation markers likely related to amyloid pathology are increased in these APPxTau mice. Conclusion: Although amyloid pathology and Tau hyperphosphorylation are increased in these mice, neuronal degeneration could not be observed. Further investigation is currently done on 12-month-old mice to explore any cognitive impairment as well as neurodegeneration. Therefore, this model likely displays early events of AD.

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BOARD NUMBER: S06-251

CX-DHED EFFICIENTLY REDUCES AMYLOID BETA PRODUCTION AND MEMORY DEFICITS MORE THAN DHED IN A MOUSE MODEL OF ALZHEIMER'S DISEASE BY ADDITIONAL REGULATION ON ALPHA-SECRETASE ACTIVITY

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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We have recently demonstrated that DHED can reduce the generation of amyloid beta peptide by inhibiting beta-secretase (BACE-1) activity. In this study, carboxylated DHED (cx-DHED), a more effective analogue of DHED on APP processing was developed and its mechanism was investigated. Administration of cx-DHED (1mg/kg) for 4 months resulted in more significant lowering of brain amyloid beta peptide than that of DHED in Tg2576 mice. In addition, cx-DHED ameliorates memory impairments and hippocampal cell death in Tg2576 mice. 10mg/kg injection of cx-DHED for 1 month also decreases amyloid beta and cognitive deficits in Tg2576 mice. Treatment of 10 uM cx-DHED impedes amyloid beta peptide production in Tg2576 primary neuronal cells. Furthermore, cx-DHED significantly reducesd beta-secretase activity and increasesd alpha-secretase activity in an enzymatic activity test. These results strongly suggest that cx-DHED may reduce the biosynthesis of amyloid beta peptide by inhibiting BACE-1 and activating alpha-secretase concurrently. These findings arewere confirmed through an induced-fit docking study; both DHED and cx-DHED to beta-secretase showsed similar binding modes, but only cx-DHED to alpha-secretase displayed binding potential. Combined with previous findings of direct inhibition of BACE-1 by DHED, this work indicates that our carboxylating strategy on beta-secretase inhibitors may have the potential to provide new insights into designing novel drugs that target aberrant multiple steps of aberrant APP processing to treat Alzheimer's disease.

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ALZHEIMER'S DISEASE PATHOGENESIS IS INFLUENCED BY SELECTIVE BRAIN IL-6 OVEREXPRESSION

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Increasing evidence suggests that neuroinflammation has a strong contribution in Alzheimer's disease (AD) pathogenesis. Understanding and controlling the mechanisms underlying the interaction between the immune system and the nervous system might be key at the therapeutic level. Interleukin-6 (IL-6), a pleiotropic and multifunctional cytokine, is involved in numerous physiological functions, as it plays a critical role in the homeostasis of the neural tissue and in the pathogenesis of inflammatory disorders including AD. We have examined the role of cerebral IL-6 overexpression in a mouse model of AD, the Tg2576, which courses with progressive loss of brain functions and strong inflammatory processes that accompany the neuropathology of the disease. The effects of IL-6 overexpression on the progression of AD were examined at different levels, including physiology, behavior, and neuropathology. Aged Tg2576 mice showed enhanced locomotor activity and exploration, decreased anxiety-like behavior, and impaired working memory. Overexpression of IL-6 had sex-dependent changes at the behavioral level, affecting locomotion, anxiety-like behavior, and working memory. When assessing the effect of chronic neuroinflammation on motor performance, vertical pole test revealed deteriorated motor function in IL-6-overexpressing mice. Neuropathological analysis revealed extensive plaque-associated chronic glial activation, which has been hypothesized to further promote b-amyloid accumulation and neuroinflammation. IL-6-induced neuroinflammation in AD may promote neurodegeneration, but the evidence suggests that could also play a neuroprotective role by decreasing the amyloid load. Collectively, our results reveal the behavioral and neuropathological impact of brain IL-6 overexpression and suggest that regulating this cytokine may be relevant for the pathogenesis of AD.

Pubmed:

33476547: Klawonn AM, Fritz M, Castany S, Pignatelli M, Canal C, Similä F, Tejeda HA, Levinsson J, Jaarola M, Jakobsson J, Hidalgo J, Heilig M, Bonci A, Engblom D
Microglial activation elicits a negative affective state through prostaglandin-mediated modulation of striatal neurons.
Microglia are activated in many neurological diseases and have been suggested to play an important role in the development of affective disorders including major depression. To investigate how microglial signaling regulates mood, we used bidirectional chemogenetic manipulations of microglial activity in mice. Activation of microglia in the dorsal striatum induced local cytokine expression and a negative affective state characterized by anhedonia and aversion, whereas inactivation of microglia blocked aversion induced by systemic inflammation. Interleukin-6 signaling and cyclooxygenase-1 mediated prostaglandin synthesis in the microglia were critical for the inflammation-induced aversion. Correspondingly, microglial activation led to a prostaglandin-dependent reduction of the excitability of striatal neurons. These findings demonstrate a mechanism by which microglial activation causes negative affect through prostaglandin-dependent modulation of striatal neurons and indicate that interference with this mechanism could milden the depressive symptoms in somatic and psychiatric diseases involving microglial activation.
Immunity, 2021; 54
31799746: Sanchis P, Fernández-Gayol O, Vizueta J, Comes G, Canal C, Escrig A, Molinero A, Giralt M, Hidalgo J
Microglial cell-derived interleukin-6 influences behavior and inflammatory response in the brain following traumatic brain injury.
Traumatic brain injury (TBI) is a major health problem with high rates of mortality and morbidity worldwide. The response of the brain to TBI is orchestrated by a number of cytokines, including interleukin-6 (IL-6). IL-6 is a major cytokine in the central nervous system and it is produced by different cells, such as neurons, glial cells, and endothelial cells. Since glial cells are one of the most important sources and targets of IL-6, we have examined the role of microglia-derived IL-6 in normal conditions and following a model of TBI, cryolesion of the somatosensorial cortex. To this end, tamoxifen-inducible microglial IL-6-deficient (Il6 , using Cx3cr1 model) mice and control (Il6) mice were used. In normal conditions, microglial IL-6

deficiency reduced deambulation and exploratory behavior and decreased anxiety in a sex-dependent manner. The transcriptome profile following cryolesion was dramatically altered 1 day post-lesion in Il6 compared with Il6 mice. However, the phenotype of Il6 mice was less compromised in the following days, suggesting that compensatory mechanisms are at play.
Glia, 2020; 68

31694025: Tribó MJ, Canal C, Baños JE, Robleda G
Pain, Anxiety, Depression, and Quality of Life in Patients with Vulvodynia.
The term vulvodynia refers to vulvar pain of unknown origin lasting at least 3 months. Psychiatric comorbidities are a common feature and, along with pain, may severely affect patients' wellbeing. We aimed to determine the characteristics of pain in vulvodynia, to correlate characteristics with symptoms of anxiety and depression, and to analyse the impact of these factors on patients' quality of life.
Dermatology, 2020; 236

31401302: Escrig A, Canal C, Sanchis P, Fernández-Gayol O, Montilla A, Comes G, Molinero A, Giralt M, Giménez-Llort L, Becker-Pauly C, Rose-John S, Hidalgo J
IL-6 trans-signaling in the brain influences the behavioral and physio-pathological phenotype of the Tg2576 and 3xTgAD mouse models of Alzheimer's disease.
Alzheimer's disease (AD) is the most commonly diagnosed dementia but its underlying pathological mechanisms still unclear. Neuroinflammation and secretion of cytokines such as interleukin-6 (IL-6) accompany the main hallmarks of the disease: amyloid plaques and neurofibrillary tangles. In this study, we analyzed the role of IL-6 trans-signaling in two mouse models of AD, Tg2576 and 3xTg-AD mice. The inhibition of IL-6 trans-signaling partially rescued the AD-induced mortality in females of both models. Before amyloid plaques deposition, it reversed AD-induced changes in exploration and anxiety (but did not affect locomotion) in Tg2576 female mice. However, after plaque deposition the only behavioral trait affected by the inhibition of IL-6 trans-signaling was locomotion. Results in the Morris water maze suggest that cognitive flexibility was reduced by the blocking of the IL-6 trans-signaling in young and old Tg2576 female mice. The inhibition of IL-6 trans-signaling also decreased amyloid plaque burden in cortex and hippocampus, and Aβ and Aβ levels in the cortex, of Tg2576 female mice. The aforementioned changes might be correlated with changes in blood vessels and matrix structure and organization rather than changes in neuroinflammation. 3xTgAD mice showed a very mild phenotype regarding amyloid cascade, but results were in accordance with those of Tg2576 mice. These results strongly suggest that the inhibition of the IL-6 trans-signaling could represent a powerful therapeutic target in AD.
Brain Behav Immun, 2019; 82

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REGULATION OF ALZHEIMER PATHOLOGY BY AMYLOID SEEDS: FROM TOXIC EFFECTS TO THERAPEUTIC OPPORTUNITIES

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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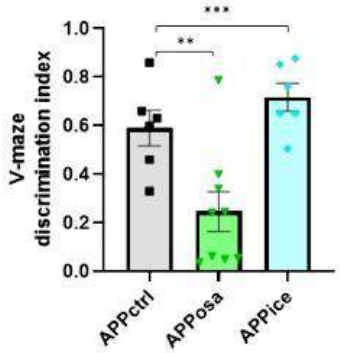
[1]Molecular imaging research center, CEA, Paris-saclay University, Laboratory Of Neurodegenerative Disease, Cnrs, Umr9199, Fontenay-aux-roses, France, [2]INSERM U1216, Grenoble Institut Neurosciences, Grenoble, France, [3]Molecular imaging research center, CEA, Laboratory Of Neurodegenerative Disease, Cnrs, Umr9199, Fontenay-aux-roses, France

Introduction More than 50 mutations have been identified in the amyloid precursor protein (APP) gene that lead to amyloid-β (Aβ) proteins in humans. Mutated Aβ are mostly toxic leading to Alzheimer's disease (AD). Some mutated Aβ can be protective. Our study evaluated the consequences of the transmission of toxic Osaka (E22D, Aβosa) or protective Icelandic (A2T, Aβice) mutations in Aβ plaque-bearing transgenic rodents with high Aβ production. **Methods** Two-month-old $APP_{swe}/PS1_{dE9}$ mice were inoculated in the dentate gyrus (n=10/group) with Aβ variants bearing Osaka or Icelandic mutations. *In vivo* longitudinal follow-up of functional impairments by resting-state functional MRI and behavioral tests (novel object recognition task and water maze test) were performed 4 months post-inoculation (mpi). Then, biochemical (western blot, 6E10) and histological (BAM10) analyses of Aβ were performed in the brain of inoculated mice. **Results** Intracerebral infusion of Aβosa induced memory impairment at 4 mpi (Fig. 1a) and altered functional connectivity in the hippocampus of inoculated animals (Fig. 1b). It increased Aβ plaque load (Fig. 2a) and changed Aβ oligomer patterns (Fig. 2b) at 4 mpi, as compared to mice inoculated with PBS. Interestingly, the protective Aβice reduced significantly cognitive decline and did not alter either brain connectivity (Fig. 1) or Aβ metabolism (Fig. 2). **Conclusion** The inoculation of “good and bad” variants of Aβ to Aβ plaque-bearing mouse models is sufficient to regulate functional impairments, Aβ forms occurrence and Aβ plaque load. The ability to modulate AD pathologies by Aβ species is a promising result for future innovative anti-amyloid therapies.

a.


V-maze discrimination index
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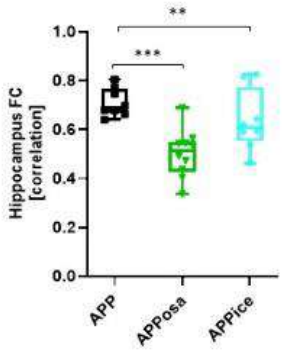
0.8
0.6
0.4
0.2
0.0
***
**
APPctrl
APPosa
APPice

b.


Hippocampus FC [correlation]
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0.8
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0.2
0.0
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APP
APPosa
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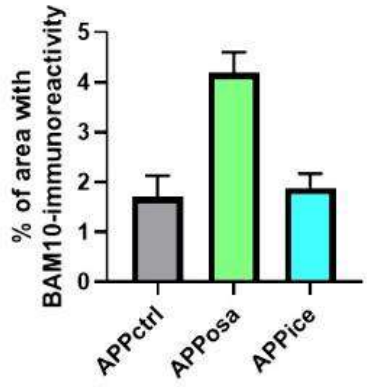
a.


% of area with BAM10-immunoreactivity
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4
3
2
1
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APPctrl
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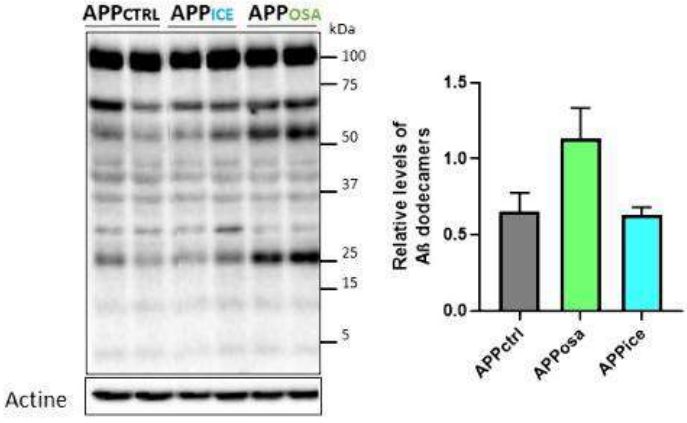
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APPICE
APPOSA
kDa
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75
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37
25
15
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Actine
Relative levels of Aß dodecamers
1.5
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APPctrl
APPosa
APPice


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ALZHEIMER DISEASE: FUNCTIONAL CHARACTERIZATION OF KLVFF ACTIVITY ON NATIVE NICOTINIC RECEPTORS

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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University of Genova, Department Of Pharmacy, Genoa, Italy

Aims: In recent years, the inhibition of beta-amyloid (Aβ) aggregation became a potential strategy for Alzheimer's disease. KLVFF, a small peptide corresponding to the aminoacidic sequence 16-20 of Aβ, reduces dose-dependently Aβ fibrillation. Consequently, the toxic and functional characterization of its brain activity is fundamental for clarifying its potential therapeutic role. Methods: Accordingly, we studied the modulatory role of KLVFF on the cholinergic receptors regulating dopamine and noradrenaline release in rats synaptosomes. Moreover, we evaluated the direct toxicity of KLVFF on synaptic terminals using flow cytometry. Results: In the nucleus accumbens the nicotinic receptors on dopaminergic nerve terminals are inhibited by KLVFF, that closely resembles the full-length Aβ1-40. Moreover, KLVFF entrapped in synaptosomes does not modify the nicotinic receptor's function, suggesting that the external binding to the receptor is required for its activity. Furthermore, the cholinergic agents desformylflustrabromine and galantamine counteracted the KLVFF effect. Remarkably, muscarinic receptors on dopaminergic terminals and nicotinic receptors regulating noradrenaline release in the hippocampus are completely insensitive to KLVFF. Based on our findings, KVLFF mimics Aβ1-40 as a negative modulator of specific nicotinic receptor subtypes affecting dopamine transmission in the rat's brain. In addition, KLVFF does not modify the viability of incubated nor entrapped synaptosomes. The viability was assessed through calcein-AM and annexin-V labelling followed by flow cytometric analysis. Conclusions: The anti-aggregative peptide KLVFF modulates specific nicotinic receptors subtypes without evident toxic effects on the synaptic terminals. Further investigation should be carried out to evaluate the impact of this activity on Alzheimer disease patients.

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ROLE OF AMYLOID PRECURSOR PROTEIN IN ASTROCYTES IN ALZHEIMER´S DISEASE

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Aims: Astrocytes are specialized glial cells that when exposed to inflammatory factors go through a process known as reactive astrogliosis. Reactive astrocytes are a neuropathological feature of Alzheimer's disease (AD). The etiology of AD is often explained by the accumulation of β-amyloid peptides, a proteolytic product of the amyloid precursor protein (APP). However, inflammation also plays a prominent role in the development of AD. How reactive astrocytes participate in the pathogenesis of AD remains poorly understood. Consequently, our goal is to understand how APP affects Astrocytes in AD. **Methods:** To elucidate the role of astrocytes in the pathogenesis of AD we exploited different *in vitro* and *in vivo* models to investigate the role of APP in setting the immune response of astrocytes, measured by biochemical and molecular techniques. **Results:** Our results show that overexpression of APP in human cortical astrocytes induces reactive phenotype, similar to astrocytes found in brains of AD patients. These reactive astrocytes produce significantly increased levels of interferon gamma (INF-γ) in comparison with control astrocytes. Similar positive correlation between APP levels and INF-γ is found also in astrocytes from AD transgenic and traumatic brain injury (TBI) mice models. **Conclusions:** Our data indicate that astrocytes are activated by high levels of APP, which promotes production and secretion of interferons and recapitulates salient features of astrocytes in AD.

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BOARD NUMBER: S06-256

INVISIBLE KILLER: INTRACELLULAR Aβ ACCUMULATION LEADS TO ENDOSOMAL/LYSOSOMAL LEAKAGE IN HIPPOCAMPAL NEURONS

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Karolinska Institutet, Department Of Neurobiology, Care Sciences And Society (nvs), Solna, Sweden

Aims Alzheimer disease (AD) is characterized by amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles. Besides extracellular Aβ plaques, intracellular Aβ was also shown to correlate with AD neuropathology. As an important source of intracellular Aβ, extracellular Aβ can be internalized and accumulate in endocytic vesicles. Previously we treated hippocampal neurons with Aβ42 monomers and detected Aβ42 oligomerization in the late endosomes/lysosomes by Förster resonance energy transfer live cell imaging. However, it is still unclear if those Aβ aggregates can cause vesicle membrane leakage. Here we investigate whether low extracellular concentration of Aβ could be endocytosed and accumulate in late endosomes/lysosomes to high levels and cause endosomal/lysosomal membrane permeabilization in primary neurons. Methods Hippocampal neurons were isolated from the E16-17 C57/BL6 mice embryo brains and cultured for 21 days in vitro. Neurons were treated with Aβ42 with or without fluorescent label at different concentrations for different time periods in an acute or chronic model. Aβ42 concentrations in neuronal vesicles were determined by live cell imaging using confocal microscopy. Immunocytochemistry and Airyscan microscopy were used to detect the loss of endosomal/lysosomal integrity in neurons. Results Treatment with 1 nM Aβ42 for 20 days caused three orders of magnitude higher concentrations in neuronal vesicles than in cell culture medium. Accumulated Aβ42 induced endosomal/lysosomal leakage in hippocampal neurons when Aβ42 concentration reached submillimolar level in those vehicles. Conclusions These data agreed with our model in which extracellular Aβ42 gradually accumulates in neurons and damages the vesicle integrity, and further initiates cellular pathogenesis in AD.

Pubmed:

33926107: Gao Y, Wennmalm S, Winblad B, Schedin-Weiss S, Tjernberg LO
Live Cell FRET Imaging Reveals Amyloid β-Peptide Oligomerization in Hippocampal Neurons.
Amyloid β-peptide (Aβ) oligomerization is believed to contribute to the neuronal dysfunction in Alzheimer disease (AD). Despite decades of research, many details of Aβ oligomerization in neurons still need to be revealed. Förster resonance energy transfer (FRET) is a simple but effective way to study molecular interactions. Here, we used a confocal microscope with a sensitive Airyscan detector for FRET detection. By live cell FRET imaging, we detected Aβ42 oligomerization in primary neurons. The neurons were incubated with fluorescently labeled Aβ42 in the cell culture medium for 24 h. Aβ42 were internalized and oligomerized in the lysosomes/late endosomes in a concentration-dependent manner. Both the cellular uptake and intracellular oligomerization of Aβ42 were significantly higher than for Aβ40. These findings provide a better understanding of Aβ42 oligomerization in neurons.
Int J Mol Sci, 2021; 22
34366358: Yu Y, Gao Y, Winblad B, Tjernberg LO, Schedin-Weiss S
A Super-Resolved View of the Alzheimer's Disease-Related Amyloidogenic Pathway in Hippocampal Neurons.
Processing of the amyloid-β protein precursor (AβPP) is neurophysiologically important due to the resulting fragments that regulate synapse biology, as well as potentially harmful due to generation of the 42 amino acid long amyloid β-peptide (Aβ42), which is a key player in Alzheimer's disease.
J Alzheimers Dis, 2021; 83
30013984: Gao Y, Zhang HL, Xin M, Wang D, Zheng N, Wang S, Xu J, Wang Y, Zhu J, Feng J
Serum Folate Correlates with Severity of Guillain-Barré Syndrome and Predicts Disease Progression.
The aim of this study was to determine the associations between serum folate level and the clinical course and severity of Guillain-Barré syndrome (GBS). We retrospectively enrolled 112 pairs of GBS patients and age- and sex-matched healthy controls with measured serum folate levels. On admission, 21 (18.9%) GBS patients had folate deficiency, of which only two were female patients. Patients with normal folate levels had a shorter disease progression than those with folate deficiency (median progression duration: 6 versus 13 days, < 0.001). Serum folate levels on admission were correlated with progression

duration and Medical Research Council (MRC) sum score in the upper limbs at nadir (r = -0.261, = 0.005; r = -0.208, = 0.03) but not with the duration of hospital stay or GBS disability score (> 0.05). Logistic regression analysis revealed that normal folate levels on admission were an independent predictor of faster GBS progression, along with younger age, intact deep sensation, and a lower MRC sum score on admission. These results show that serum folate levels are correlated with the progression duration and severity of GBS. Further studies are required to confirm the potential of folate level as a biomarker for GBS prognosis.
Biomed Res Int, 2018; 2018

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BOARD NUMBER: S06-257

INTERACTIONS BETWEEN Aβ OLIGOMERS AND PIRB RECEPTOR AT THE SINGLE-MOLECULE LEVEL

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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[1]University of St. Andrews, School Of Biology, St Andrews, United Kingdom, [2]University of Sheffield, Sheffield Institute For Translational Neuroscience (sitran), Sheffield, United Kingdom, [3]University of St. Andrews, School Of Physics And Astronomy, St. Andrews, United Kingdom

Amyloid beta (Aβ) oligomers have been shown to induce a variety of toxic effects in neurons, including pore formation in the plasma membrane and a range of deleterious interactions with cellular receptors. Among these receptors, it has been shown that the Paired-Immunoglobulin-like receptor B (PirB) has a high affinity for Aβ oligomers, and their interaction leads to an impairment in synaptic plasticity in mice models. The surprising divergence of toxicity pathways of Aβ oligomers reported in the literature is likely due to the high structural heterogeneity of these oligomers. Early Aβ oligomers have been shown to preferentially induce membrane damage, while later oligomers of the same aggregation reaction are less prone to create pores while being more inflammatory. Conventional biophysical methods fail to account for these heterogeneous properties of oligomers due to their nanoscale dimensions, low abundance and short lifetime. Using single-molecule and super-resolution imaging techniques, we characterized Aβ populations at the single-oligomer level, allowing us to take structural features into consideration in our biological experiments. We subsequently studied their effect on the trafficking and distribution of individual PirB receptors in primary hippocampal and cortical neurons also by means of single-molecule imaging techniques in living neurons. Our results show that oligomers form at different stages of an aggregation reaction affect the mobility of receptors and their localization in different ways. This approach will help dissecting the conformation-specific toxicity pathway induced by Aβ oligomers, and contribute to assess the relevance of the multiple toxicity pathways associated with Alzheimer’s disease pathogenesis.

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MAIBI: A NEW APPROACH TO DISCOVER POTENTIAL BIOMARKERS IN ALZHEIMER DISEASE USING MALDI IMAGING

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Oxidative stress (OS) is a self-propagating phenomenon caused by an imbalance between oxidant and antioxidant systems, namely an overproduction of reactive oxygen species, which overwhelms the intrinsic antioxidant defenses. In Alzheimer's disease (AD), there is growing evidence supporting the link between ROS-mediated damage and mitochondrial dysfunction, with endoplasmic reticulum stress and proteostasis imbalance. Our previous work demonstrated the protective role of ubiquinol (Ub), the reduced form of coenzyme Q10, in the 3xTg-AD mice model, preventing hypoxia and amyloid-β peptide deposition in hippocampal and neocortical areas at onset (6 months) and advanced (12 months) stages of disease. Aims: to assay the effect of Ub-supplementation on brain proteostasis in the 3xTg-AD model and to discover new biomarkers of disease recovery after Ub treatment. Methodology: 2-month-old wild-type and 3xTg-AD mice were fed during 10 months with standard or Ub-supplemented diet. After performing behavioural tests and euthanasia, brains were processed for MALDI-Imaging. Protein identification was inferred from significantly altered m/z peaks using MaTisse database and clustered using bioinformatics tools (Metaboanalyst 5.0, STRING, Cytoscape and Metascape) and subsequently validated by confocal microscopy. Results: Behavioral tests showed an Ub-dependent rescue in the AD-associated cognitive decline. MALDI-Imaging analysis revealed differential protein signatures in the 3xTg-AD model vs. WT mice in hippocampal and cortex areas. These protein profiles were reset to WT levels upon Ub supplementation. Therefore, several inferred proteins such as CAII and RPL23, closely involved in OS regulation and protein translation respectively, could be potentially used as biomarkers of a mild recovery of the disease.

Pubmed:
32063825: Llanos-González E, Henares-Chavarino ÁA, Pedrero-Prieto CM, García-Carpintero S, Frontiñán-Rubio J, Sancho-Bielsa FJ, Alcain FJ, Peinado JR, Rabanal-Ruíz Y, Durán-Prado M
Interplay Between Mitochondrial Oxidative Disorders and Proteostasis in Alzheimer's Disease.
Although the basis of Alzheimer's disease (AD) etiology remains unknown, oxidative stress (OS) has been recognized as a prodromal factor associated to its progression. OS refers to an imbalance between oxidant and antioxidant systems, which usually consist in an overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which overwhelms the intrinsic antioxidant defenses. Due to this increased production of ROS and RNS, several biological functions such as glucose metabolism or synaptic activity are impaired. In AD, growing evidence links the ROS-mediated damages with molecular targets including mitochondrial dynamics and function, protein quality control system, and autophagic pathways, affecting the proteostasis balance. In this scenario, OS should be considered as not only a major feature in the pathophysiology of AD but also a potential target to combat the progression of the disease. In this review, we will discuss the role of OS in mitochondrial dysfunction, protein quality control systems, and autophagy associated to AD and suggest innovative therapeutic strategies based on a better understanding of the role of OS and proteostasis.
Front Neurosci, 2019; 13

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-259

EARLY IMPAIRMENT OF REFERENCE MEMORY AT THREE MONTHS OF AGE IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE USING THE HELICO MAZE.

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Background: The 5XFAD model of Alzheimer's disease bearing five familial mutations of Alzheimer's disease on human *AbPP* and *PSEN1* transgenes shows deposits of beta amyloid peptide (Ab) as early as 2 months. Given that detecting early dysfunctions in Alzheimer's disease prior to overt pathology is of major interest in the field, we sought to detect memory deficits at earlier stages of the disease in 3-month old 5XFAD male mice. Methods: The Helico Maze a new behavioral task, recently developed and patented, allowing deeper analysis of learning and sub-categories of hippocampal-dependent long-term memory using olfactory cues, was used. Results: Eight 5XFAD and 6 wild-type (WT) male mice at 3 months of age were tested in the Helico Maze. The results demonstrated, for the first time, a starting deficit of pure reference long-term memory. Interestingly, memory impairment was clearly correlated with the amount of Ab deposits in the hippocampus. While we also found significant differences regarding astrogliosis between 5XFAD and WT mice, this was not correlated with memory abilities. Conclusion: These results underlined the efficiency of this new olfactory-dependent behavioral task, easy to use, with a small cohort of mice. Using the Helico Maze may open new avenues to validate efficacy of treatments that target early events related with the amyloid-dependent pathway of the disease and AD progression. M. Migliorati and C. Manrique: Equal contribution

FENS Forum 2022
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BOARD NUMBER: S06-260

THE EFFECT OF DIFFERENT AMYLOID SEEDS AND ANIMAL HOSTS ON AMYLOID PROPAGATION IN ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Abstract text: Alzheimer's Disease is a neurodegenerative proteinopathy in which recent evidence indicates that Aβ can misfold and aggregate into seeds that structurally corrupt native proteins, mimicking a prion-like process of template protein corruption or seeding. In fact, studies show that Aβ deposition can be induced by the intracerebral infusion of seed-containing brain homogenates, and that the characteristics of both the seeding agent and the host, influence the pathologic signature of the Aβ seeds. However, it is still unknown which Aβ-misfolded species are most efficient in triggering the aggregation process and which is the effect of amyloid seeds on different AD models. Methods: Amyloid seeds from AD patients (stage C for amyloid) from the Alzheimer's Disease Research Center (ADRC) at UCI were administered into 7-8-month-old hAβ-KI mice and 3xTg-AD mice. Next, we intracerebrally injected brain homogenates from the human AD patient and 25mo-3xTg-AD mice into the hippocampus of 7-month-old 3xTg-AD mice, which were analyzed at 18 months of age. Results: Our findings demonstrated that amyloid deposition differentially occurs in 3xTg-AD and hAβ-KI mice, and the Aβ aggregates are developed earlier in the familial model. Moreover, the amyloid seeds from the human patient induce more aggressive amyloid pathology compared to seeds from aged 3xTg-AD mice. Conclusion: These results suggest that multiple factors such as the seed, recipient model and time are critical factors that can modulate the amyloid pathology onset and progression. Thus, more profound understanding of these factors will provide key insight on how amyloid pathology progresses in AD.

FENS Forum 2022
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BOARD NUMBER: S06-261

SYNAPTIC ACTIVITY PROMOTES AMYLOIDOGENIC CLEAVAGE OF APP AND SUBSEQUENTLY THE PRODUCTION OF Aβ

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Aim: Accumulation of Aβ peptides is a key player in the development of Alzheimer's disease (AD) physiopathology. Aβ peptides are produced by the sequential cleavage of transmembrane protein APP by proteases BACE-1 and γ-secretase, respectively. Resent findings show increased production of Aβ is driven by synaptic activity. Thus, this study aims at identifying the conditions by which synaptic activity affect Aβ peptides production and APP processing. Methods: For this purpose, we design a plasmid containing human APP695 with and without the Swedish mutation (swe) tagged with mCherry at the c-terminus followed by transfection of this plasmid in murine primary cortical neurons (DIV14). By measuring the fluorescence, we monitored APP processing in live neurons exposed to chemical long-term potentiation (cLTP) protocols (bicuculline [50 mM] and 4-aminopyridine [2.5 mM]) in the presence of BACE inhibitor [5mM]. APP processing was also characterized by Western blotting on murine primary neuronal cultures exposed to cLTP treatment. Results: We showed that exposure to cLTP induced a reduction of APP fluorescence in both APP695. The swe APP displays a 2 folds increase in the reduction of APP fluorescence. Pharmacological inhibition of BACE by B-IV is blocking APP processing driven by synaptic activity in neurons. Conclusions: Our findings suggest that APP processing by amyloidogenic pathway can be monitored in live neurons by measuring the fluorescence of APP-tagged proteins. We showed that synaptic activity promotes BACE-1 cleavage of APP leading to the production of Aβ. These finding highlight the role of synaptic activity in the physiopathology of AD.

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LACK OF ABNORMAL CEREBROVASCULAR FUNCTION IN 5XFAD MODEL OF ALZHEIMER'S DISEASE?

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Neurovascular abnormalities are considered to be the major feature of Alzheimer's disease (AD) and preclinical AD models. Few studies, however, have questioned the traditional view by showing the absence of vascular dysfunction in animal models of AD. Here, we aimed to characterize two major functions of brain vasculature: neurovascular coupling (NVC) and blood-brain barrier (BBB) in 5xFAD mouse model of AD. This model reproduces deposition of amyloid-beta in the brain, but not the neurofibrillary tangles. We used two-photon microscopy in anesthetized 6-9-month-old 5xFAD mice and WT littermate controls. We quantified the NVC by measuring dilation of penetrating arterioles upon whisker-pad stimulation; and the BBB leakage was quantified by measuring the accumulation of small fluorescent tracer the in brain parenchyma. We showed that the baseline diameter of penetrating arterioles (PAs) was similar between WT and 5xFAD mice. We detected no effect of amyloid-beta deposition on the NVC and the BBB: the fraction of dilating PAs, dilation of the PAs, and the BBB leakage were non-significantly different between 5xFAD and WT mice. We concluded that amyloid deposition alone in the 5xFAD model might not be enough to trigger cerebrovascular abnormalities.

Pubmed:
30831173: Zhukov OA, Kazakova TA, Maksimov GV, Brazhe AR
Cost of auditory sharpness: Model-Based estimate of energy use by auditory brainstem "octopus" neurons.
Octopus cells (OCs) of the mammalian auditory brainstem precisely encode timing of fast transient sounds and tone onsets. Sharp temporal fidelity of OCs relies on low resting membrane resistance, which suggests high energy expenditure on maintaining ion gradients across plasma membrane. We provide a model-based estimate of energy consumption in resting and spiking OCs. Our results predict that a resting OC consumes up to 2.6 × 10 ATP molecules (ATPs) per second which remarkably exceeds energy consumption of other CNS neurons. Glucose usage by all OCs in the rat is nevertheless low due to their low number. Major part of the OCs energy use results from the ion mechanisms providing for the low membrane resistance: hyperpolarization-activated mixed cation conductance and low-voltage activated K-conductance. Spatially ordered synapses-a feature of the OCs allowing them to compensate for asynchrony of the synaptic input-brings only a 12% energy saving to OCs excitability cost. Only 13% of total OC energy used for an AP generation (1.5 × 10 ATPs) is associated with the AP generation in the axon initial segment, 64%-with synaptic currents processing and 23%-with keeping resting potential.
J Theor Biol, 2019; 469

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-263

TREM2 MODULATES BINDING, UPTAKE AND DIFFERENTIAL DEPOSITION OF PHOSPHORYLATED Aβ IN ALZHEIMER'S DISEASE BRAINS

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Aim: Rare variants of the Triggering receptor expressed on myeloid cells 2 (TREM2) are associated with increased risk for Alzheimer's disease (AD). The AD-associated TREM2 variants not only affect the binding to Amyloid-β (Aβ) but also alter its deposition in AD brain. Here, we wanted to investigate the binding of TREM2 to aggregation prone Aβ phosphorylated at serine 8 (pSer8-Aβ) and the functional relevance for the deposition in the brains of transgenic mouse models and human AD cases. **Methods:** Interaction with TREM2 was analyzed by BLI, co-immunoprecipitation, and dot blot overlay assays. The deposition characteristics were assessed by immunohistochemistry using generic and phosphorylation-state specific monoclonal antibodies for Aβ with brain sections from APP/PS1 and 5xFAD transgenic TREM2 wildtype and TREM2 knockout mice, and human AD cases with the common and rare variants of TREM2. **Results:** We show the highest affinity interaction of TREM2 to pSer8-Aβ compared to non-phosphorylated Aβ. Also, this modification decreases the TREM2 dependent phagocytosis by primary microglia and in APP transgenic mouse models. Furthermore, we demonstrate characteristic spatio-temporal deposition of pSer8-Aβ in extracellular plaques, vessels and as intraneuronal deposits in brains of transgenic mice and human AD cases with rare TREM2 variants. **Conclusions:** The increased interaction of TREM2 with pSer8-Aβ species is associated with altered deposition in distinct lesions, including extracellular plaques, cerebral amyloid angiopathy, and intraneuronal aggregates. Thus, differential sensing of modified Aβ variants in distinct aggregation states by TREM2 could contribute to the deposition of toxic Aβ species during AD pathogenesis.

Pubmed:
28527954: Karisetty BC, Joshi PC, Kumar A, Chakravarty S
Sex differences in the effect of chronic mild stress on mouse prefrontal cortical BDNF levels: A role of major ovarian hormones.
Depression induced by stress is affected by sex, age and hormonal status of the animal and also by duration and type of the stressors. Moreover, higher prevalence of depression and comorbidities in women than men implies the need to include the sex variable in studies on animal models of depression. The present study was therefore initiated to evaluate the effect of sex and ovarian hormones on depression-like phenotypes in mice exposed to a 21-day Chronic Variable Mild Stress (CVMS) paradigm. Adult male, intact female and, ovariectomized (OVX) female mice exposed to CVMS displayed despair behavior, a depression-like phenotype, in all the groups. However, intact females alone, but not males and OVX females, showed anhedonia, another depression-like phenotype. At the molecular level, the expression of Brain-Derived Neurotrophic Factor (BDNF), a neuropeptide associated with depression, and few other stress-specific genes CRH, NR3C1, CART, and NPY

were measured in the Prefrontal Cortex (PFC) region of the reward circuitry. There was a significant decrease in the BDNF protein expression along with an increase in the mRNA expression of CRH, NR3C1, CART, and NPY in intact females, but not in the other two groups of mice. OVX females resembled males in behavioral and molecular responses to CVMS. 17β-Estradiol (E2) administration, not Progesterone (P4), to OVX female stress mice, mitigated despair and enhanced hedonic capacity with an increased expression of BDNF in PFC. This study strengthens the evidence for the beneficial effects of E2 administration in stress condition.
Neuroscience, 2017; 356
29702163: Wahul AB, Joshi PC, Kumar A, Chakravarty S
Transient global cerebral ischemia differentially affects cortex, striatum and hippocampus in Bilateral Common Carotid Arterial occlusion (BCCAo) mouse model.
Despite the advancements in medical sciences in last few decades, we are yet to develop promising therapeutics for stroke, which is one of the current global non-communicative disease concerns. The occurrence of stroke is likely to increase further due to the transition in our lifestyles. Inadequate knowledge of the sequential region-specific pathological events induced by cerebral ischemia is one of the underlying reasons. Hence to address this, we have used Bilateral Common Carotid Arterial occlusion (BCCAo) model that mimics human cardiac arrest condition. After ischemic insult, motor coordination and cognitive functions were analysed through series of behavioral tasks. Animals were sacrificed and brain regions viz. cortex, striatum and hippocampus, were isolated further for histopathology and molecular investigations. All the molecular studies were performed at 1d and 7d post-ischemia and reperfusion by immunohistochemistry, RT-qPCR and western blot analysis, for hypoxic, inflammatory and apoptotic markers. Our study indicates that unlike cortex and striatum, hippocampus was found to be consistently late ischemic susceptible at a behavioral and molecular level which might be regulated by c-Jun N-terminal kinases (JNK) and AKT signaling mechanism with associative changes in NMDA receptors. This study shows a region-specific temporal molecular response to global ischemia in the brain, which is important to get better insight into the pathophysiology since each region consists of different subsets of neurons that are having different susceptibility and tolerance pattern/level. This insight into a temporally different ischemic response and the underlying molecular basis might help in strategizing the effective therapeutics against stroke.
J Chem Neuroanat, 2018; 92
28473714: Joshi PC, Samineni R, Bhattacharya D, Reddy BR, Veeraval L, Das T, Maitra S, Wahul AB, Karri S, Pabbaraja S, Mehta G, Kumar A, Chakravarty S
A 2-oxa-spiro[5.4]decane scaffold displays neurotrophic, neurogenic and anti-neuroinflammatory activities with high potential for development as a versatile CNS therapeutic.
Following our recent discovery of a new scaffold exhibiting significant neurotrophic and neurogenic activities, a structurally tweaked analogue, embodying a 2-oxa-spiro [5.4]decane framework, has been conceptualised and found to be more potent and versatile. It exhibits enhanced neurotrophic and neurogenic action in in vitro, ex vivo and in vivo models and also shows robust neuroprotection in mouse acute cerebral stroke model. The observed attributes are traceable to the predominant activation of the TrkB-PI3K-AKT-CREB pathway. In addition, it also exhibits remarkable anti-neuroinflammatory activity by concurrently down-regulating pro-inflammatory cytokines IL-1α and IL-6, thereby providing a unique molecule with a trinity of neuroactivities, i.e. neurotrophic, neurogenic and anti-inflammatory. The new chemical entity disclosed here has the potential to be advanced as a versatile therapeutic molecule to treat stroke, depression, and possibly other neuropsychiatric disorders associated with attenuated neurotrophic/ neurogenic activity, together with heightened neuroinflammation.
Sci Rep, 2017; 7
29402744: Reddy CR, Tukaram AG, Mohammed SZ, Dilipkumar U, Babu BN, Chakravarty S, Bhattacharya D, Joshi PC, Grée R
Synthesis and biological evaluation of longanlactone analogues as neurotrophic agents.
Longanlactone analogues were synthesized using a route featuring Friedel-Crafts acylation, Sonogashira coupling and 1,3-dipolar cycloaddition reactions. Structure-activity relationships were investigated for neurotrophic activity. Compound 6 was found to have the most potent neurotrophic activity among all the synthesized analogues in Neuro2a cells as evidenced by a battery of in vitro/cell based assays for assessment of neurogenic and potential neurotrophic activity including neurite outgrowth assay and real time PCR for popular markers of augmented neurotrophic activity. Compound 6 might serve as a template for further development of highly effective neurotrophic molecules.
Bioorg Med Chem Lett, 2018; 28
33519377: Kumar S, Kapadia A, Theil S, Joshi P, Riffel F, Heneka MT, Walter J
Novel Phosphorylation-State Specific Antibodies Reveal Differential Deposition of Ser26 Phosphorylated Aβ Species in a Mouse Model of Alzheimer's Disease.
Aggregation and deposition of amyloid-β (Aβ) peptides in extracellular plaques and in the cerebral vasculature are prominent neuropathological features of Alzheimer's disease (AD) and closely associated with the pathogenesis of AD. Amyloid plaques

in the brains of most AD patients and transgenic mouse models exhibit heterogeneity in the composition of Aβ deposits, due to the occurrence of elongated, truncated, and post-translationally modified Aβ peptides. Importantly, changes in the deposition of these different Aβ variants are associated with the clinical disease progression and considered to mark sequential phases of plaque and cerebral amyloid angiopathy (CAA) maturation at distinct stages of AD. We recently showed that Aβ phosphorylated at serine residue 26 (pSer26Aβ) has peculiar characteristics in aggregation, deposition, and neurotoxicity. In the current study, we developed and thoroughly validated novel monoclonal and polyclonal antibodies that recognize Aβ depending on the phosphorylation-state of Ser26. Our results demonstrate that selected phosphorylation state-specific antibodies were able to recognize Ser26 phosphorylated and non-phosphorylated Aβ with high specificity in enzyme-linked immunosorbent assay (ELISA) and Western Blotting (WB) assays. Furthermore, immunofluorescence analyses with these antibodies demonstrated the occurrence of pSer26Aβ in transgenic mouse brains that show differential deposition as compared to non-phosphorylated Aβ (npAβ) or other modified Aβ species. Notably, pSer26Aβ species were faintly detected in extracellular Aβ plaques but most prominently found intraneuronally and in cerebral blood vessels. In conclusion, we developed new antibodies to specifically differentiate Aβ peptides depending on the phosphorylation state of Ser26, which are applicable in ELISA, WB, and immunofluorescence staining of mouse brain tissues. These site- and phosphorylation state-specific Aβ antibodies represent novel tools to examine phosphorylated Aβ species to further understand and dissect the complexity in the age-related and spatio-temporal deposition of different Aβ variants in transgenic mouse models and human AD brains.
Front Mol Neurosci, 2020; 13

34427354: Joshi P, Riffel F, Satoh K, Enomoto M, Qamar S, Scheiblich H, Villacampa N, Kumar S, Theil S, Parhizkar S, Haass C, Heneka MT, Fraser PE, St George-Hyslop P, Walter J
Differential interaction with TREM2 modulates microglial uptake of modified Aβ species.
Rare coding variants of the microglial triggering receptor expressed on myeloid cells 2 (TREM2) confer an increased risk for Alzheimer's disease (AD) characterized by the progressive accumulation of aggregated forms of amyloid β peptides (Aβ). Aβ peptides are generated by proteolytic processing of the amyloid precursor protein (APP). Heterogeneity in proteolytic cleavages and additional post-translational modifications result in the production of several distinct Aβ variants that could differ in their aggregation behavior and toxic properties. Here, we sought to assess whether post-translational modifications of Aβ affect the interaction with TREM2. Biophysical and biochemical methods revealed that TREM2 preferentially interacts with oligomeric Aβ, and that phosphorylation of Aβ increases this interaction. Phosphorylation of Aβ also affected the TREM2 dependent interaction and phagocytosis by primary microglia and in APP transgenic mouse models. Thus, TREM2 function is important for sensing phosphorylated Aβ variants in distinct aggregation states and reduces the accumulation and deposition of these toxic Aβ species in preclinical models of Alzheimer's disease.
Glia, 2021; 69

34663480: Joshi P, Riffel F, Kumar S, Villacampa N, Theil S, Parhizkar S, Haass C, Colonna M, Heneka MT, Arzberger T, Herms J, Walter J
TREM2 modulates differential deposition of modified and non-modified Aβ species in extracellular plaques and intraneuronal deposits.
Progressive accumulation of Amyloid-β (Aβ) deposits in the brain is a characteristic neuropathological hallmark of Alzheimer's disease (AD). During disease progression, extracellular Aβ plaques undergo specific changes in their composition by the sequential deposition of different modified Aβ species. Microglia are implicated in the restriction of amyloid deposits and play a major role in internalization and degradation of Aβ. Recent studies showed that rare variants of the Triggering Receptor Expressed on Myeloid cells 2 (TREM2) are associated with an increased risk for AD. Post-translational modifications of Aβ could modulate the interaction with TREM2, and the uptake by microglia. Here, we demonstrate that genetic deletion of TREM2 or expression of a disease associated TREM2 variant in mice lead to differential accumulation of modified and non-modified Aβ species in extracellular plaques and intraneuronal deposits. Human brains with rare TREM2 AD risk variants also showed altered deposition of modified Aβ species in the different brain lesions as compared to cases with the common variant of TREM2. These findings indicate that TREM2 plays a critical role in the development and the composition of Aβ deposits, not only in extracellular plaques, but also intraneuronally, that both could contribute to the pathogenesis of AD.
Acta Neuropathol Commun, 2021; 9

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-264

MOLECULAR AND CELLULAR MECHANISMS UNDERLYING THE PATHOGENESIS OF ALZHEIMER'S DISEASE: CHARACTERISATION OF Aβ AND ITS VARIANTS AT THE MONOMER, OLIGOMER AND FIBRIL LEVELS.

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Amyloid beta (Aβ), is a neurotoxic protein associated with Alzheimer's disease that rapidly aggregates into amyloid oligomers, protofibrils and well-defined fibrils. Indeed, some familial early-onset forms of AD are associated with single amino acid Aβ mutations, such as the most toxic Aβ42 mutation Arctic. Based on previous computational predictions of aggregation propensity and a rational mutagenesis study on Aβ peptides, several variants were identified with distinct effects on survival in a *Drosophila* model: E3R Aβ40, E22G Aβ42 (Arctic), I31E/E22G Aβ42 and F19S/L34P Aβ42. Here we have characterized the amyloidogenic cascade of these variants from the monomer, the oligomers and the fibrils using single-molecule force spectroscopy, Thioflavin T binding assay, immunoassay methods and electron microscopy. We found that the Arctic mutation increases the frequency of mechanostable conformers, including hypermechanostable ones, which correlates with an increased aggregation, fibrillogenesis and toxicity associated to SH-SY5Y cell lines. In contrast, I31E/E22G and F19S/L34P mutations in Aβ42 and E3R mutation in Aβ40 caused a decrease in mechanostable conformers and showed lower *in vitro* aggregation as expected, although they exerted similar or even higher cytotoxicity in SH-SY5Y cells. These effects can be attributed to their capacity to form deleterious protofibrillar aggregates instead of well-defined fibrils, which might result in higher toxicity. Deciphering the molecular bases of Aβ pathogenicity, directly linked to its intrinsic properties, should allow us a deeper understand of the complex pathogenic events that are involved in developing Alzheimer's disease in different *in vivo* models (*i.e.*, *Drosophila* constitutive expression vs. human cell lines).

Pubmed:
34852197: Vendrell-Fernández S, Lozano-Picazo P, Cuadros-Sánchez P, Tejero-Ojeda MM, Giraldo R
Conversion of the OmpF Porin into a Device to Gather Amyloids on the Outer Membrane.
Protein amyloids are ubiquitous in natural environments. They typically originate from microbial secretions or spillages from mammals infected by prions, currently raising concerns about their infectivity and toxicity in contexts such as gut microbiota or soils. Exploiting the self-assembly potential of amyloids for their scavenging, here, we report the insertion of an amyloidogenic sequence stretch from a bacterial prion-like protein (RepA-WH1) in one of the extracellular loops (L5) of the abundant outer membrane porin OmpF. The expression of this grafted porin enables bacterial cells to trap on their envelopes the same amyloidogenic sequence when provided as an extracellular free peptide. Conversely, when immobilized on a surface as bait, the full-length prion-like protein including the amyloidogenic peptide can catch bacteria displaying the L5-grafted OmpF. Polyphenolic molecules known to inhibit amyloid assembly interfere with peptide recognition by the engineered OmpF, indicating that this is compatible with the kind of homotypic interactions expected for amyloid assembly. Our study suggests that synthetic porins may provide suitable scaffolds for engineering biosensor and clearance devices to tackle the threat posed by pathogenic amyloids.
ACS Synth Biol, 2022; 11

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-265

TG2 PROMOTES A FAST Aβ PEPTIDE AGGREGATION, DISRUPTS ER-MITOCHONDRIA CONTACT SITES AND NEURONAL FUNCTION IN CELLULAR AND ANIMAL MODELS OF ALZHEIMER DISEASE

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

Jessica Panes, Ana María Marileo, Javiera Gavilan, Oscar Ramirez, Robinson Duran, Patricio Castro, Carlos Felipe Burgos, Gonzalo Yévenes, Gustavo Moraga-Cid, Carola Munoz Montesino, Jorge Fuentealba
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Alzheimer's Disease (AD) is a progressive neurodegenerative disorder. The main factor that has been involved on AD physiopathology is the aberrant aggregation of Amyloid beta peptide (Aβ), that induce Ca^{2+} dyshomeostasis and disruption of mitochondria-associated membranes (MAMs) . Here, we postulate that transglutaminase type 2 (TG2) is a potential target in several steps of the amyloid cascade. Using Hippocampal neuron treated with oligomeric forms of Aβ peptide and aged J20 brain slices, we observed an increase in TG2 transamidating activity and total levels (159 %). Using different molecular techniques we characterize the biochemical features of Aβ aggregates induced by TG2. We observed that TG2-Aβ interaction is energetically favorable with TG2 in open conformation (ΔG $A\beta_{mon}$: -10.9, $A\beta_{dim}$: -9.4, $A\beta_{trim}$: -6.4). We find that TG2 can induce a toxic Aβ assemblies size of ~28 and 55 kDa. Thioflavin T measures showed that TG2 incubation was able to promote Aβ aggregation, increasing lag phase, and maximum peak of fluorescence in stationary phase. We observe an increase in synaptic transmission induced by TG2-Aβ assemblies, that was blockage by inhibitor of TG2. Using PLA technology and Rhod-2 measurements we examine the effect of these aggregates on mitochondrial function in APP/PS1 CAD cells. We observed that after acute exposure to Aβ aggregates in the presence of TG2 a significant two-fold increase in the number of ER-mitochondria contact points and mitochondrial Ca2+ transference (220%). Our results contribute to explaining the key TG2 involvement on sinaptotoxicity and its potentiality as pharmacological target to treat AD.

Pubmed:

34880726: Panes JD, Saavedra P, Pineda B, Escobar K, Cuevas ME, Moraga-Cid G, Fuentealba J, Rivas CI, Rezaei H, Muñoz-Montesino C
PrP as a Transducer of Physiological and Pathological Signals.
After the discovery of prion phenomenon, the physiological role of the cellular prion protein (PrP) remained elusive. In the past decades, molecular and cellular analysis has shed some light regarding interactions and functions of PrP in health and disease. PrP , which is located mainly at the plasma membrane of neuronal cells attached by a glycosylphosphatidylinositol (GPI) anchor, can act as a receptor or transducer from external signaling. Although the precise role of PrP remains elusive, a variety of functions have been proposed for this protein, namely, neuronal excitability and viability. Although many issues must be solved to clearly define the role of PrP , its connection to the central nervous system (CNS) and to several misfolding-associated diseases makes PrP an interesting pharmacological target. In a physiological context, several reports have proposed that PrP modulates synaptic transmission, interacting with various proteins, namely, ion pumps, channels, and metabotropic receptors. PrP has also been implicated in the pathophysiological cell signaling induced by β-amyloid peptide that leads to synaptic dysfunction in the context of Alzheimer's disease (AD), as a mediator of Aβ-induced cell toxicity. Additionally, it has been implicated in other proteinopathies as well. In this review, we aimed to analyze the role of PrP as a transducer of physiological and pathological signaling.
Front Mol Neurosci, 2021; 14
34269182: Panes JD, Wendt A, Ramirez-Molina O, Castro PA, Fuentealba J
Deciphering the role of PGC-1α in neurological disorders: from mitochondrial dysfunction to synaptic failure.
The onset and mechanisms underlying neurodegenerative diseases remain uncertain. The main features of neurodegenerative diseases have been related with cellular and molecular events like neuronal loss, mitochondrial dysfunction and aberrant accumulation of misfolded proteins or peptides in specific areas of the brain. The most prevalent neurodegenerative diseases belonging to age-related pathologies are Alzheimer's disease, Huntington's disease, Parkinson's disease and amyotrophic lateral sclerosis. Interestingly, mitochondrial dysfunction has been observed to occur during the early onset of several neuropathological events associated to neurodegenerative diseases. The master regulator of mitochondrial quality control and energetic metabolism is the transcriptional coactivator peroxisome proliferator-activated

receptor gamma coactivator 1-alpha (PGC-1α). Additionally, it has been observed that PGC-1α appears to be a key factor in maintaining neuronal survival and synaptic transmission. In fact, PGC-1α downregulation in different brain areas (hippocampus, substantia nigra, cortex, striatum and spinal cord) that occurs in function of neurological damage including oxidative stress, neuronal loss, and motor disorders has been seen in several animal and cellular models of neurodegenerative diseases. Current evidence indicates that PGC-1α upregulation may serve as a potent therapeutic approach against development and progression of neuronal damage. Remarkably, increasing evidence shows that PGC-1α deficient mice have neurodegenerative diseases-like features, as well as neurological abnormalities. Finally, we discuss recent studies showing novel specific PGC-1α isoforms in the central nervous system that appear to exert a key role in the age of onset of neurodegenerative diseases and have a neuroprotective function in the central nervous system, thus opening a new molecular strategy for treatment of neurodegenerative diseases. The purpose of this review is to provide an up-to-date overview of the PGC-1α role in the physiopathology of neurodegenerative diseases, as well as establish the importance of PGC-1α function in synaptic transmission and neuronal survival.
Neural Regen Res, 2022; 17
32523530: Panes JD, Godoy PA, Silva-Grecchi T, Celis MT, Ramirez-Molina O, Gavilan J, Muñoz-Montecino C, Castro PA, Moraga-Cid G, Yévenes GE, Guzmán L, Salisbury JL, Trushina E, Fuentealba J
Changes in PGC-1α/SIRT1 Signaling Impact on Mitochondrial Homeostasis in Amyloid-Beta Peptide Toxicity Model.
Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive impairment that increasingly afflicts the elderly population. Soluble oligomers (AβOs) has been implicated in AD pathogenesis: however, the molecular events underlying a role for Aβ are not well understood. We studied the effects of AβOs on mitochondrial function and on key proteins that regulate mitochondrial dynamics and biogenesis in hippocampal neurons and PC-12 cells. We find that AβOs treatment caused a reduction in total Mfn1 after a 2 h exposure (42 ± 11%); while DRP1 increased at 1 and 2 h (205 ± 22% and 198 ± 27%, respectively), correlating to changes in mitochondrial morphology. We also observed that SIRT1 levels were reduced after acute and chronic AβOs treatment (68 ± 7% and 77 ± 6%, respectively); while PGC-1α levels were reduced with the same time treatments (68 ± 8% and 67 ± 7%, respectively). Interestingly, we found that chronic treatment with AβOs increased the levels of pSIRT1 (24 h: 157 ± 18%), and we observed changes in the PGC-1α and p-SIRT1 nucleus/cytosol ratio and SIRT1-PGC-1α interaction pattern after chronic exposure to AβOs. Our data suggest that AβOs induce important changes in the level and localization of mitochondrial proteins related with the loss of mitochondrial function that are mediated by a fast and sustained SIRT1/PGC-1α complex disruption promoting a "non-return point" to an irreversible synaptic failure and neuronal network disconnection.
Front Pharmacol, 2020; 11
31249848: Roa FJ, Peña E, Inostroza E, Sotomayor K, González M, Gutierrez-Castro FA, Maurin M, Sweet K, Labrousse C, Gatica M, Aylwin CF, Mendoza P, Maldonado M, Delgado C, Madariaga J, Panes J, Silva-Grecchi T, Concha II, Moraga-Cid G, Reyes AM, Muñoz-Montesino C, Vera JC, Rivas CI
Data on SVCT2 transporter expression and localization in cancer cell lines and tissues.
The data presented in this article are related to the research paper entitled "Increased expression of mitochondrial sodium-coupled ascorbic acid transporter-2 (mitSVCT2) as a central feature in breast cancer", available in Free Radical Biology and Medicine Journal [1]. In this article, we examined the SVCT2 transporter expression in various breast cancer cell lines using RT-PCR and Western blot assays. In addition, we analyzed the subcellular localization of SVCT2 by immunofluorescence colocalization assays and cellular fractionation experiments. Finally, an analysis of different cancer tissue microarrays immunostained for SVCT2 and imaged by The Human Protein Atlas (https://www.proteinatlas.org) is presented.
Data Brief, 2019; 25
30902760: Peña E, Roa FJ, Inostroza E, Sotomayor K, González M, Gutierrez-Castro FA, Maurin M, Sweet K, Labrousse C, Gatica M, Aylwin CF, Mendoza P, Maldonado M, Delgado C, Madariaga J, Panes J, Silva-Grecchi T, Concha II, Moraga-Cid G, Reyes AM, Muñoz-Montesino C, Vera JC, Rivas CI
Increased expression of mitochondrial sodium-coupled ascorbic acid transporter-2 (mitSVCT2) as a central feature in breast cancer.
The potential role of vitamin C in cancer prevention and treatment remains controversial. While normal human cells obtain vitamin C as ascorbic acid, the prevalent form of vitamin C in vivo, the uptake mechanisms by which cancer cells acquire vitamin C has remained unclear. The aim of this study is to characterize how breast cancer cells acquire vitamin C. For this, we determined the expression of vitamin C transporters in normal and breast cancer tissue samples, and in ZR-75, MCF-7, MDA-231 and MDA-468 breast cancer cell lines. At the same time, reduced (AA) and oxidized (DHA) forms of vitamin C uptake experiments were performed in all cell lines. We show here that human breast cancer tissues differentially express a form of SVCT2 transporter, that is systematically absent in normal breast tissues and it is increased in breast tumors. In fact, estrogen receptor negative breast cancer tissue, exhibit the most elevated SVCT2 expression levels. Despite this, our analysis in breast cancer cell lines showed that these cells are not able to uptake ascorbic acid and depend on glucose

transporter for the acquisition of vitamin C by a bystander effect. This is consistent with our observations that this form of SVCT2 is completely absent from the plasma membrane and is overexpressed in mitochondria of breast cancer cells, where it mediates ascorbic acid transport. This work shows that breast cancer cells acquire vitamin C in its oxidized form and are capable of accumulated high concentrations of the reduced form. Augmented expression of an SVCT2 mitochondrial form appears to be a common hallmark across all human cancers and might have implications in cancer cells survival capacity against pro-oxidant environments.
Free Radic Biol Med, 2019; 135
29079292: Sáez-Orellana F, Fuentes-Fuentes MC, Godoy PA, Silva-Grecchi T, Panes JD, Guzmán L, Yévenes GE, Gavilán J, Egan TM, Aguayo LG, Fuentealba J
P2X receptor overexpression induced by soluble oligomers of amyloid beta peptide potentiates synaptic failure and neuronal dyshomeostasis in cellular models of Alzheimer's disease.
The most common cause of dementia is Alzheimer's disease. The etiology of the disease is unknown, although considerable evidence suggests a critical role for the soluble oligomers of amyloid beta peptide (Aβ). Because Aβ increases the expression of purinergic receptors (P2XRs) in vitro and in vivo, we studied the functional correlation between long-term exposure to Aβ and the ability of P2XRs to modulate network synaptic tone. We used electrophysiological recordings and Ca microfluorimetry to assess the effects of chronic exposure (24 h) to Aβ oligomers (0.5 μM) together with known inhibitors of P2XRs, such as PPADS and apyrase on synaptic function. Changes in the expression of P2XR were quantified using RT-qPCR. We observed changes in the expression of P2X1R, P2X7R and an increase in P2X2R; and also in protein levels in PC12 cells (143%) and hippocampal neurons (120%) with Aβ. In parallel, the reduction on the frequency and amplitude of mEPSCs (72% and 35%, respectively) were prevented by P2XR inhibition using a low PPADS concentration. Additionally, the current amplitude and intracellular Ca signals evoked by extracellular ATP were increased (70% and 75%, respectively), suggesting an over activation of purinergic neurotransmission in cells pre-treated with Aβ. Taken together, our findings suggest that Aβ disrupts the main components of synaptic transmission at both pre- and post-synaptic sites, and induces changes in the expression of key P2XRs, especially P2X2R; changing the neuromodulator function of the purinergic tone that could involve the P2X2R as a key factor for cytotoxic mechanisms. These results identify novel targets for the treatment of dementia and other diseases characterized by increased purinergic transmission.
Neuropharmacology, 2018; 128

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BOARD NUMBER: S06-266

THERAPEUTIC POTENTIAL OF PEPTIDES TARGETING ABETA OLIGOMERS IN ALZHEIMER'S DISEASE.

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Alzheimer's disease (AD) is the most common neurodegenerative disorder characterized by a progressive deficit in memory leading to dementia. Insoluble amyloid beta peptide (Aβ) deposits in the brain are a pathological hallmark of the disease, but soluble Aβ oligomers (Aβo) are considered as the most toxic form for neurons and synapses. Indeed, Aβo induce synapse loss and impair a cellular basis of learning and memory, the long-term potentiation (LTP). In AD patients, Aβ accumulates in the brain decades before disease onset. Therefore, targeting toxic Aβo at early stages appears as a promising strategy to delay disease progression. Thus, we designed a new peptide tool which binds Aβo with high affinity. Using ThT assay, TEM and Western blotting, we showed that this peptide strongly alters Aβ aggregation and reduces Aβo formation. To investigate the potential toxicity of Aβo formed with the peptide, we performed synaptic targeting experiments on neuronal cultures using immunocytochemistry and confocal imaging. Our findings demonstrate that these Aβo are no longer able to bind synapses. Finally, we investigated the functional impact of this peptide on LTP, using electrophysiological experiments on hippocampal slices in the APP/PS1 mouse model of AD. Our results reveal that this blocking peptide rescues LTP in APP/PS1 slices. Altogether, these results indicate that the peptide is a promising candidate for alleviating synaptic defects in AD preclinical models. Further in vivo explorations will help determining the benefits of this blocking strategy on synaptotoxicity, learning and memory alterations in the APP/PS1 mouse model of AD.

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THE ALZHEIMER'S AMYLOID PROTEIN APP AND G(ALPHA)O PHYSICALLY AND FUNCTIONALLY INTERACT TO PROMOTE NEURITOGENESIS

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Aims. Gαo is an alpha subunit of heterotrimeric G proteins, and a highly expressed protein in the nervous system. Long ago, Gαo was reported to interact with the amyloid precursor protein (APP), central to the Alzheimer's pathology. When triggered by an unknown cue, APP prolongs Gαo activation, either by behaving as a guanine nucleotide exchange factor (GEF) or by decreasing Gαo's GTPase-activating protein (GAP) activity. This interaction of unknown function may open new perspectives to the physiological role of APP in the brain. Methods. Gαo interacts with the APP C-terminus at His657-Lys676, in a hydrophobic pocket immediately downstream the APP 653YTSI656 sorting motif. Using SH-SY5Y neuroblastoma cells and rat primary neurons expressing APP S655 phosphomutants, and CB1R-Gαo activating cannabinoids, we have explored APP-Gαo functional interaction in neuritogenesis, and how APP phosphorylation at S655 impacts the physical and functional APP-Gαo interaction. Results. S655 phosphorylation increases/prolongs APP binding to Gαo and Gαo activation. It is also determinant for APP/Gαo-induced neuritogenesis, fine-tuning between the formation and the elongation of cellular pre-neurites in SH-SY5Y cells. In neurons, APP-Gαo co-promote dendritic branching. Two signaling cascades, the Src-STAT3 and ERK1/2 signaling pathways, may be involved, with APP S655 phosphorylation increasing Gαo-induced ERK1/2 activation at 24h. Conclusions. The characterization of the dual neuritogenic effects and signaling activation of the APP-Gαo complex, and the role of APP phosphorylation on its modulation, not only sheds new light on the mechanisms of neuronal differentiation and APP physiology, but might also provide new groundwork to advance the knowledge on AD pathogenesis.

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AMYLOID-B OLIGOMERS DEREGULATE MBP AND MOBP LOCAL PROTEIN SYNTHESIS IN OLIGODENDROCYTES

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

Adhara Gaminde-Blasco[1], Uxue Balantzategi[1], Tania Quintela-López[1], Carlos Matute[1], Elena Alberdi[1], Jimena Baleriola[2]
[1]UPV/EHU, Achucarro Basque Center for Neurosciences, CIBERNED, Neurosciences, LEIOA, Spain, [2]Achucarro Basque Center for Neuroscience, Laboratory Of Local Translation In Neurons And Glia, Leioa, Spain

Myelin degeneration and white matter loss are early events in Alzheimer's disease (AD) that lead to cognitive deficits. One of the hallmarks of AD is the presence of extracellular aggregates of amyloid beta peptide (Aβ), and Aβ oligomers (Abo) have been proposed to induce changes in oligodendrocytes and myelin. However, the underlying molecular mechanisms remain unknown. We found that Aβo alter the translation of MBP and MOBP, one of the major and essential proteins of CNS. Here, we characterized how Aβo control and deregulate the metabolism of *Mbp* and *Mobp* mRNA and its local translation in cultured oligodendrocytes. First, we observed that Aβo increase mRNA and protein levels of MBP and MOBP, as well as of ribonucleoprotein A2 (hnRNPA2), which binds to their 3'UTR. Interestingly, immunofluorescence analysis along with RNA-immunoprecipitation showed an increase in total transport granule number and active granules, together with a higher association between *Mbp* and *Mobp* mRNA and hnRNPA2. Additionally, activation of Fyn with an upregulation in phosphorylation levels of tyrosine residues of hnRNPA2 and direct visualization of newly synthesized proteins by Puro-PLA confirmed that Abo increase the local translation in oligodendrocytes. Consistent with these results, we observed an upregulation of hnRNPA2, MOBP and MBP in the hippocampus of 3xTg-AD mice. Likewise, RNA-seq analysis from oligodendrocytes isolated from 3xTg-AD mice brains revealed changes in genes related to translation and myelination. Taken together, these results suggest that Aβo could be altering the proteostasis, influencing the dynamics of oligodendroglial maturation and myelin, with potential consequences in axonal conduction.

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TARGETED LIPIDOMICS AND METABOLOMICS REVEALS PROGRESSION OF TAU PATHOLOGY IN TRANSGENIC SHR-24 RATS

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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[1]Slovak Academy of Sciences, Institute Of Neuroimmunology, Bratislava, Slovak Republic, [2]Palacký University Olomouc, Faculty Of Medicine And Dentistry, Olomouc, Czech Republic

Tauopathies are a group of neurodegenerative disorders characterized by cerebral atrophy, hyperphosphorylation, abnormal aggregation of tau filaments into intracellular neurofibrillary tangles and chronic neuroinflammation. A role of lipid metabolism dysregulation in aging and neurodegeneration is well established, however still poorly understood. To investigate the impact of tau pathology on lipid metabolism, we have performed a thorough targeted lipidomic and metabolomic analysis of brain tissue, cerebrospinal fluid (CSF) and plasma of transgenic rats expressing human truncated tau. Specific biofluid markers of tau pathology (total-tau and neurofilament-light-chain) and tau hyperphosphorylated and aggregated forms in brain tissue were measured to examine the effect of tau pathology on metabolic profiles. Presence of neurofibrillary pathology resulted in substantially dysregulated lipid metabolism in brain tissue and even more in the CSF. The most profound change was in phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, lysophosphatidylcholine and sphingomyelin subclasses. However, neither of the changes in CSF nor brain lipidome were reflected in the plasma. Further analysis showed that lipid changes correlate with the tau pathology in brain tissue. Moreover, we found that tau pathology induces formation of lipid droplets *in vitro* and *in vivo* . Our results results highlight the importance of lipid metabolism in tau pathology alone, but also main hallmarks of neurodegenerative disesases such as neuroinflammation, glial activation and hyperphoshorylation.

Pubmed:
32079185: Majerova P, Hanes J, Olesova D, Sinsky J, Pilipcinec E, Kovac A
Novel Blood-Brain Barrier Shuttle Peptides Discovered through the Phage Display Method.
Delivery of therapeutic agents into the brain is a major challenge in central nervous system drug development. The blood-brain barrier (BBB) prevents access of biotherapeutics to their targets in the central nervous system and, therefore, prohibits the effective treatment of many neurological disorders. To find blood-brain barrier shuttle peptides that could target therapeutics to the brain, we applied a phage display technology on a primary endothelial rat cellular model. Two identified peptides from a 12 mer phage library, GLHTSATNLYLH and VAARTGEIYVPW, were selected and their permeability was validated using the in vitro BBB model. The permeability of peptides through the BBB was measured by ultra-performance liquid chromatography-tandem mass spectrometry coupled to a triple-quadrupole mass spectrometer (UHPLC-MS/MS). We showed higher permeability for both peptides compared to N-C reversed-sequence peptides through in vitro BBB: for peptide GLHTSATNLYLH 3.3 × 10 cm/s and for peptide VAARTGEIYVPW 1.5 × 10 cm/s. The results indicate that the peptides identified by the in vitro phage display technology could serve as transporters for the administration of biopharmaceuticals into the brain. Our results also demonstrated the importance of proper BBB model for the discovery of shuttle peptides through phage display libraries.
Molecules, 2020; 25
34830461: Olešová D, Majerová P, Hájek R, Piešťanský J, Brumarová R, Michalicová A, Jurkanin B, Friedecký D, Kováč A
GM3 Ganglioside Linked to Neurofibrillary Pathology in a Transgenic Rat Model for Tauopathy.
Glycosphingolipids (GSLs) are amphipathic lipids composed of a sphingoid base and a fatty acyl attached to a saccharide moiety. GSLs play an important role in signal transduction, directing proteins within the membrane, cell recognition, and modulation of cell adhesion. Gangliosides and sulfatides belong to a group of acidic GSLs, and numerous studies report their involvement in neurodevelopment, aging, and neurodegeneration. In this study, we used an approach based on hydrophilic interaction liquid chromatography (HILIC) coupled to high-resolution tandem mass spectrometry (HRMS/MS) to characterize the glycosphingolipid profile in rat brain tissue. Then, we screened characterized lipids aiming to identify changes in glycosphingolipid profiles in the normal aging process and tau pathology. Thorough screening of acidic glycosphingolipids in rat brain tissue revealed 117 ganglioside and 36 sulfatide species. Moreover, we found two ganglioside subclasses that were not previously characterized-GT1b-Ac2 and GQ1b-Ac2. The semi-targeted screening revealed significant changes in the

levels of sulfatides and GM1a gangliosides during the aging process. In the transgenic SHR24 rat model for tauopathies, we found elevated levels of GM3 gangliosides which may indicate a higher rate of apoptotic processes.
Int J Mol Sci, 2021; 22
33147863: Olesova D, Galba J, Piestansky J, Celusakova H, Repiska G, Babinska K, Ostatnikova D, Katina S, Kovac A
A Novel UHPLC-MS Method Targeting Urinary Metabolomic Markers for Autism Spectrum Disorder.
Autism spectrum disorder is a heterogeneous neurodevelopmental disease. Currently, no biomarker of this disease is known. Diagnosis is performed through observation, standardized behavioral scales, and interviews with parents. In practice, diagnosis is often delayed to the average age of four years or even more which adversely affects a child's perspective. A laboratory method allowing to detect the disorder at earlier stages is of a great need, as this could help the patients to start with treatment at a younger age, even prior to the clinical diagnosis. Recent evidence indicates that metabolomic markers should be considered as diagnostic markers, also serving for further differentiation and characterization of different subgroups of the autism spectrum. In this study, we developed an ultra-high performance liquid chromatography-tandem triple quadrupole mass spectrometry method for the simultaneous determination of six metabolites in human urine. These metabolites, namely methylguanidine, N-acetyl arginine, inosine, indole-3-acetic acid, indoxyl sulfate and xanthurenic acid were selected as potential biomarkers according to prior metabolomic studies. The analysis was carried out by means of reversed-phase liquid chromatography with gradient elution. Separation of the metabolites was performed on a Phenomenex Luna Omega Polar C18 (100 × 1.0 mm, 1.6 µm) column at a flow rate of 0.15 mL/min with acetonitrile/water 0.1% formic acid aqueous as the mobile phase. The analysis was performed on a group of children with autism spectrum disorder and age-matched controls. In school children, we have detected disturbances in the levels of oxidative stress markers connected to arginine and purine metabolism, namely methylguanidine and N-acetylargine. Also, products of gut bacteria metabolism, namely indoxyl sulfate and indole-3-acetic acid, were found to be elevated in the patients' group. We can conclude that this newly developed method is fast, sensitive, reliable, and well suited for the quantification of proposed markers.
Metabolites, 2020; 10
31384811: Piestansky J, Galba J, Olesova D, Kovacech B, Kovac A
Determination of immunogenic proteins in biopharmaceuticals by UHPLC-MS amino acid analysis.
Nowadays, there is a growing interest in innovative and more efficient therapeutics-biopharmaceuticals, based on peptides or proteins. There are increased demands on quality control of such therapeutics. One of the methods usually used for characterization and quantification of biopharmaceuticals is amino acid analysis. In this work, a modern advanced analytical method based on precolumn derivatization and reversed-phase ultra high-performance liquid chromatography in combination with single quadrupole mass spectrometer was developed for amino acid analysis in different protein samples-model sample of bovine serum albumin, sample of strong immunogenic protein keyhole limpet hemocyanin, and sample of drug etanercept present in commercially available biopharmaceutical Enbrel. The method used isotopically labeled internal standards and was validated according to the International Council for Harmonisation guideline. The developed method was characterized by favorable performance and validation parameters, such as time of analysis (6 min), specificity, linearity ($r \geq 0.99$), limit of detection (0.009-0.822 µM), limit of quantification (1-2.5 µM), accuracy (recovery in the range 90-102.8%), intra-day (RSD in the range 0.25-11.97%) and inter-day precision (RSD in the range 1.67-11.57%), or stability ($RE \leq 12\%$). According to these findings, the developed amino acid analysis approach is suitable for routine use in areas of peptide/protein quantification, such as quality control laboratories of biopharmaceutical companies.
BMC Chem, 2019; 13
34361026: Piestansky J, Matuskova M, Cizmarova I, Olesova D, Mikus P
Determination of Branched-Chain Amino Acids in Food Supplements and Human Plasma by a CE-MS/MS Method with Enhanced Resolution.
In the presented study, a capillary electrophoresis-mass spectrometry method combining high separation efficiency and sensitive detection has been developed and validated, for the first time, to quantify branched chain amino acids (valine, isoleucine, leucine) in commercial food and sport supplement samples and human plasma samples. The separations were performed in a bare fused silica capillary. The background electrolyte was composed of 500 mM formic acid with pH 2.0. The plasma sample pretreatment was realized by simple protein precipitation with acetonitrile. Injection of a short zone of highly basic electrolyte before the sample injection and application of the negative pressure on the separation were accompanied by enhanced resolution of the isobaric amino acids-isoleucine and leucine. The developed method was characterized by favorable validation parameters, such as linearity ($r > 0.99$), accuracy and precision, the limit of detection, lower limit of quantification, or robustness. These parameters were more than sufficient for the quantification of branched chain amino acids in various samples. The determined concentrations of branched chain amino acids in food and sports supplements were in very good agreement with the content declared by the manufacturer. The investigated concentrations of branched chain amino acids were in the range 294.68-359.24 µM for valine, 91.76-95.67 µM for isoleucine, and 196.78-251.24 µM for leucine. These concentrations fall within the physiological limits. The developed CE-MS/MS method represents a suitable

alternative to traditional approaches used in branched chain amino acid quality control and bioanalysis.
Int J Mol Sci, 2021; 22
33477515: Galba J, Piešťanský J, Kováč A, Olešová D, Cehlár O, Kertys M, Kozlík P, Chaľová P, Tirčová B, Slíž K, Mikuš P
Fast and Sensitive Screening of Oxandrolone and Its Major Metabolite 17-Epi-Oxandrolone in Human Urine by UHPLC-MS/MS with On-Line SPE Sample Pretreatment.
Oxandrolone, a synthetic testosterone analog, is used for the treatment of several diseases associated with weight loss. Unfortunately, oxandrolone is abused by many athletes and bodybuilders due to its strong anabolic effect. We have developed and validated a highly sensitive and rapid on-line SPE-UHPLC-MS/MS method for the determination of oxandrolone and simultaneous identification of its major metabolite 17-epi-oxandrolone in urine matrices. Enrichment of the analytes via an integrated solid-phase extraction was achieved using an Acquity UPLC BEH C18 Column. Subsequently, the chromatographic separation of the on-line preconcentrated sample fraction was achieved using an Acquity HSS T3 C18 Column. For the structural identification of these analytes, a high-resolution mass spectrometer Synapt-G2Si coupled to the Acquity M-class nano-LC system with ionKey source was used. A highly sensitive determination of oxandrolone was achieved using a tandem quadrupole mass spectrometer XEVO TQD. The method was successfully validated in the linear range of oxandrolone from 81.63 pg·mL (limit of quantification, LOQ) to 5000 pg·mL in the human urine matrix. It was applied to the analysis of real urine samples obtained from a healthy volunteer after the oral administration of one dose (10 mg) of oxandrolone. Concentration vs. time dependence was tested in the time interval of 4 h-12 days (after oral administration) to demonstrate the ability of the method to detect the renal elimination of oxandrolone from the human body. Favorable performance parameters along with successful application indicate the usefulness of the proposed method for its routine use in antidoping control labs.
Molecules, 2021; 26
31540027: Piestansky J, Olesova D, Galba J, Marakova K, Parrak V, Secnik P, Secnik P, Kovacech B, Kovac A, Zelinkova Z, Mikus P
Profiling of Amino Acids in Urine Samples of Patients Suffering from Inflammatory Bowel Disease by Capillary Electrophoresis-Mass Spectrometry.
Urine represents a convenient biofluid for metabolomic studies due to its noninvasive collection and richness in metabolites. Here, amino acids are valuable biomarkers for their ability to reflect imbalances of different biochemical pathways. An impact of amino acids on pathology, prognosis and therapy of various diseases, including inflammatory bowel disease (IBD), is therefore the subject of current clinical research. This work is aimed to develop a capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) method for the quantification of the 20 proteinogenic amino acids in human urine samples obtained from patients suffering from IBD and treated with thiopurines. The optimized CE-MS/MS method, with minimum sample preparation (just "dilute and shoot"), exhibited excellent linearity for all the analytes (coefficients of determination were higher than 0.99), with inter-day and intra-day precision yielding relative standard deviations in the range of 0.91-15.12% and with accuracy yielding relative errors in the range of 85.47-112.46%. Total analysis time, an important parameter for the sample throughput demanded in routine practice, was shorter in ca. 17% when compared to established CE-MS methods. Favorable performance of the proposed CE-MS/MS method was also confirmed by the comparison with corresponding ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) method. Consistent data for the investigated amino acid metabolome were obtained using both methods. For the first time, the amino acid profiling by CE-MS approach was applied on the clinical IBD samples. Here, significant differences observed in the concentration levels of some amino acids between IBD patients undergoing thiopurine treatment and healthy volunteers could result from the simultaneous action of the disease and the corresponding therapy. These findings indicate that amino acids analysis could be a valuable tool for the study of mechanism of the IBD treatment by thiopurines.
Molecules, 2019; 24

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BOARD NUMBER: S06-270

IMPACT OF TAUPATHIES ON THE FUNCTIONAL ORGANIZATION OF NEURONAL CULTURES

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Tau protein aggregates are a key element in the development of certain neurodegenerative diseases, defined as tauopathies. Pathological Tau accumulates in the brain and disrupts synaptic communication through different mechanisms, ultimately inducing neurodegeneration. The aim of this project is to understand how tauopathies, and the associated accumulation of pathological tau-protein aggregates, affect the functional connectivity of neuronal networks.
Due to the sheer size of the brain, and the difficulty to monitor tauopathies at a cellular scale, an interesting approach is to use in vitro neuronal circuits. We prepare neuronal cultures derived from mouse cortical tissue and introduce pathological tau protein extracted from different sources. We record the spontaneous activity through fluorescence calcium imaging and apply complex networks analysis to reveal the functional connectivity of the cultures. With this information we can establish aspects such as collective dynamics, the existence of functional modules, and the emergence of synchrony between neurons and their loss upon damage.
Experiments showed a remarkable change in the fluorescence traces between control and tau-treated conditions: cultures treated with pathological tau presented a higher fluorescence intensity as well as a higher number of spikes within bursts. We associate these results to the damage or loss of activity-regulatory mechanisms in the neuronal circuits due to the presence of pathological tau.
The project leading to these results has received funding from “la Caixa” Foundation (ID 100010434) under the agreement LCF/PR/HR19/52160007.

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BOARD NUMBER: S06-271

DYSFUNCTION OF THE GLUTAMATERGIC PHOTORECEPTOR SYNAPSE IN A MOUSE MODEL OF TAUOPATHY

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Alzheimer diseases and tauopathies are mostly characterized by histological features such as retinal ganglion cell loss along amyloid deposits and phosphorylated Tau. We have here investigated the functional alterations of vision in the P301S murine model of tauopathy. Visual impairments were demonstrated by a reduced visual acuity already at 6 months, the pathology onset. Visual signals at the cortical and retinal levels were delayed at 6 and 9 months, respectively. Surprisingly, the retinal output signal was advanced on the OFF pathway but delayed on the ON pathway at 6 months. This antagonistic effect, which relies on a dysfunction of the cone photoreceptor synapse, was associated to changes in the expression of the vesicular glutamate transporter and a microglial reaction. This dysfunction of the retinal glutamatergic synapses provides a novel explanation for visual deficits in tauopathies reinforcing the interest for the retinal model in Alzheimer diseases for diagnostic and therapeutic assessment of treatments.

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BOARD NUMBER: S06-272

REBOXETINE TREATMENT REDUCES NEUROINFLAMMATION AND GLIAL ACTIVATION IN THE P301S MOUSE MODEL OF TAUOPATHY

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Introduction Noradrenaline (NA) is a major modulatory neurotransmitter of the central nervous system (CNS) and besides its actions as a neurotransmitter, it presents a potent anti-inflammatory and neuroprotective effect mainly due to its ability to reduce the activation of glial cells. The main source of NA in the CNS is the Locus Coeruleus (LC), which is the brain region where neurofibrillary tangles of tau first start to accumulate in Alzheimer's disease (AD) patients, being this the earliest detectable AD-like neuropathology. These toxic tau aggregates lead to the death of noradrenergic neurons and a subsequent decrease in NA levels that facilitates the progression of AD. Therefore, treatments to increase NA levels could slow down the progression of AD. Based on this, our main aim was to analyze the effects of a NA elevating drug in a mouse model of tauopathy. **Methods** We treated WT and P301S mice with vehicle or reboxetine, a NA reuptake inhibitor, with mini-osmotic pumps during 28 days. After the treatment we collected brain samples for biochemical analysis. **Results** Our data indicate that reboxetine treatment is able to reduce the neuroinflammation present in the hippocampus of P301S mice through the reduction of astrocytes and microglia activation. **Conclusions** Reboxetine and other NA-elevating drugs could be an interesting therapeutic approach to reduce the neuroinflammation and glial cells over-activation that contribute to the progression of tauopathies such as AD.

Pubmed:

32662924: González-Prieto M, Gutiérrez IL, García-Bueno B, Caso JR, Leza JC, Ortega-Hernández A, Gómez-Garre D, Madrigal JLM
Microglial CX3CR1 production increases in Alzheimer's disease and is regulated by noradrenaline.
The loss of noradrenergic neurons and subsequent reduction of brain noradrenaline (NA) levels are associated with the progression of Alzheimer's disease (AD). This seems to be due mainly to the ability of NA to reduce the activation of microglial cells. We previously observed that NA induces the production of the chemokine Fractalkine/CX3CL1 in neurons. The activation of microglial CX3CR1, sole receptor for CX3CL1, reduces the activation of microglia, which is known to largely contribute to the neuronal damage characteristic of AD. Therefore, alterations of CX3CR1 production in microglia could translate into the enhancement or inhibition of CX3CL1 anti-inflammatory effects. In order to determine if microglial CX3CR1 production is altered in AD and if NA can control it, CX3CR1 expression and synthesis were analyzed in 5xFAD mice and human AD brain samples. In addition, the effects of NA and its reuptake inhibitor reboxetine were analyzed in microglial cultures and mice respectively. Our results indicate that in AD CX3CR1 production is increased in the brain cortex and that reboxetine administration further increases it and enhances microglial reactivity toward amyloid beta plaques. However, direct administration of NA to primary rat microglia or human HMC3 cells inhibits CX3CR1 production, suggesting that microglia responses to NA may be altered in the absence of CX3CL1-producing neurons or other nonmicroglial external factors.
Glia, 2021; 69
31297718: Gutiérrez IL, González-Prieto M, Caso JR, García-Bueno B, Leza JC, Madrigal JLM
Reboxetine Treatment Reduces Neuroinflammation and Neurodegeneration in the 5xFAD Mouse Model of Alzheimer's Disease: Role of CCL2.
The reduction of brain noradrenaline levels is associated to the initiation of Alzheimer's disease and contributes to its progression. This seems to be due mainly to the anti-neuroinflammatory actions of noradrenaline. The analysis of noradrenaline effects on brain cells demonstrates that it also regulates the production of the chemokine CCL2. In the present study, we analyzed the effect of the selective noradrenaline reuptake inhibitor, reboxetine, on the inflammatory and neurodegenerative alterations present in 5xFAD mice, and how the genetic removal of CCL2 affects reboxetine actions. We observed that the removal of CCL2 reduced the memory impairments in 5xFAD mice as well as the neuroinflammatory response, the accumulation of amyloid beta plaques, and the degeneration of neurons in the brain cortex. The administration of reboxetine with osmotic pumps for 28 days also resulted in anti-inflammatory and neuroprotective changes in 5xFAD mice,

even in the absence of CCL2. Yet, 6-month-old CCL2KO mice presented a significant degree of neuroinflammation and neuronal damage. These findings indicate that reboxetine treatment prevents the brain alterations caused by prolonged overproduction of amyloid beta, being these effects independent of CCL2, which is a mediator of the damage caused by amyloid beta in the brain cortex, but necessary for the prevention of the development of neurodegeneration in normal healthy conditions.
Mol Neurobiol, 2019; 56
29950099: Gutiérrez IL, González-Prieto M, García-Bueno B, Caso JR, Leza JC, Madrigal JLM
Alternative Method to Detect Neuronal Degeneration and Amyloid β Accumulation in Free-Floating Brain Sections With Fluoro-Jade.
Fluoro-Jade is a fluorescein-derived fluorochrome which specifically binds to damaged neurons. Due to this characteristic, it is commonly used for the histochemical detection and quantification of neurodegeneration in mounted brain sections. Here, we describe an alternative and simpler histochemistry protocol based on the use of free-floating brain sections. For this purpose, we have used brain slices from wild-type and 5xFAD mice as well as from mice that received an intracerebral injection of oligomeric amyloid beta peptides. We observed that our histochemistry staining procedure allows for a well-defined labeling of degenerating neurons providing a better signal-to-noise ratio staining than the commonly used one. In addition, our modified protocol demonstrates the ability of Fluoro-Jade C to also fluorescently label amyloid beta plaques.
ASN Neuro, 2018 Jan-Dec; 10
29478130: Gutiérrez IL, González-Prieto M, García-Bueno B, Caso JR, Feinstein DL, Madrigal JLM
CCL2 Induces the Production of β2 Adrenergic Receptors and Modifies Astrocytic Responses to Noradrenaline.
The decline in brain noradrenaline levels is associated with the progression of certain neurodegenerative diseases. This seems to be due, at least in part, to the ability of noradrenaline to limit glial activation and to reduce the damage associated with it. Our previous studies of the mechanisms involved in this process indicate that noradrenaline induces the production of the chemokine CCL2 in astrocytes. While CCL2 can protect neurons against certain injuries, its overproduction has also proven to be harmful and to prevent noradrenaline neuroprotective effects. Therefore, in this study, we analyze if the modifications caused to astrocytes by an excessive production of CCL2 may alter their response to noradrenaline. Using primary cultures of rat cortical astrocytes, we observed that CCL2 enhances the production of beta 2 adrenergic receptors in these cells. While this potentiates noradrenaline signaling through cAMP, the activation of the transcription factor CREB is inhibited by CCL2. Furthermore, although CCL2 potentiates noradrenaline induction of glycogenolysis, this does not translate into an augmented release of lactate, one of the processes through which astrocytes help support neurons. Additionally, other neuroprotective actions of noradrenaline, such as the production of brain derived neurotrophic factor and the inhibition of the inducible nitric oxide synthase in astrocytes were modified by CCL2. These data suggest that some of the central nervous system alterations related to CCL2 could be due to its effects on adrenergic receptors and its interference with noradrenaline signaling.
Mol Neurobiol, 2018; 55
27923568: Madrigal JL, Caso JR, García-Bueno B, Gutiérrez IL, Leza JC
Noradrenaline induces CX3CL1 production and release by neurons.
CX3CL1 is a chemokine for which neurons constitute its primary source within the brain. Besides acting as a chemokine, CX3CL1 regulates multiple processes and is known to inhibit microglial activation. Because of this, CX3CL1 is considered as a messenger used by neurons to communicate with microglia. Similarly, the neurotransmitter noradrenaline reduces microglial activation and production of neurotoxic agents. Based on this, the regulation of neuronal CX3CXL1 by noradrenaline was analyzed. In primary cortical neurons, noradrenaline induced the accumulation of CX3CL1 protein and mRNA. Noradrenaline also increased CX3CL1 in its soluble form despite the inhibition of the activity and synthesis of ADAM10 and ADAM17, the main proteases known to cleave CX3CL1 from the neuronal membrane. Noradrenaline-treated neurons displayed a higher degree of dendritic arborization and a characteristic accumulation of CX3CL1 in the dendritic bifurcation zones. The soluble CX3CL1 produced by neurons after noradrenaline treatment, reduced the accumulation of nitrites in microglia. These findings indicate that NA anti-inflammatory actions are mediated by neuronal CX3CL1. In addition, CX3CL1 seems to be involved in the development of neuronal processes stimulated by noradrenaline.
Neuropharmacology, 2017; 114

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POST-TRANSLATIONAL MODIFICATIONS OF TAU PROTEIN IN NEURONAL CELLS EXPOSED TO SATURATED FATTY ACIDS: IMPLICATIONS FOR ALZHEIMER´S DISEASE

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Elevated levels of circulating saturated free fatty acids such as palmitic acid (PA) are associated with obesity and insulin resistance. These metabolic alterations are considered risk factors for developing Alzheimer's disease (AD), as demonstrated by numerous epidemiological studies. Previously, we have found that neuronal exposure to PA induces insulin resistance, increments the intracellular calcium and ROS production, and diminishes the NAD^+/NADH ratio, resulting in decreasing content and activity of Sirtuin1 (Sirt1) deacetylase. However, the precise mechanism triggered after elevated PA on neurons and their association with metabolic disturbances leading to the expression of AD pathological markers, such as post-translational modifications (PTMs) of tau protein have not been elucidated. Therefore, the aim of the present study was to analyze the relationships between the effects of PA metabolism in neuronal cells, differentiated from human neuroblatoma MSN, on protein kinases activation, Sirt1 dysfunction and its consequences in tau phosphorylation and acetylation at AD-related epitopes such as p-S199/202, p-S214, p-S396 and AC-K280. So far, we have observed increased levels of tau phosphorylation and acetylation at specific epitopes such as those mentioned above after 1h or 24 h of PA incubation. These PTMs were mainly associated with activation of GSK3-β and other classical PKCs, and by reduction of Sirt1 activity. Present results provide evidence of how saturated fatty acids metabolism contributes to biochemical alterations in tau protein that resemble some changes found in AD. This work was supported by CONACYT A1-S9559.

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BOARD NUMBER: S06-274

IMPAIRED NEURONAL MATURATION IN A HUMAN IPSC DERIVED CORTICAL ORGANOID MODEL OF TAUOPATHY

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Tauopathies, such as Alzheimer's Disease and Frontotemporal dementia, are characterized by several MAPT mutations which lead to the hyperphosphorylation and accumulation of the microtubule-associated protein TAU, leading to a synaptic and neuronal loss, together with a prominent neuroinflammatory state. Considering the genetically inheritance of these mutations and the late onset of the symptoms, we proposed to investigate the role of the IVS10+16 TAU mutation during neurodevelopment and maturation exploiting patterned cortical organoids generated using both commercial-available hIPSC line that carries that mutation and its isogenic control. We took advantages from different experimental approaches aimed to decipher either gene and protein expression as well as neuronal functionality during both early and late stages of brain development. Gene expression analysis revealed that Tau mutation deeply affects neuronal, astrocytic and synaptic maturation. Moreover, we assessed, through confocal analysis that neurons which carry TAU mutation displayed strong cytoskeleton alterations, characterized by small fragments with lower volume compared to control neurons. Functional analysis of Ca^{2+} oscillations of whole cortical-organoids and 2D neuronal cultures have shown that TAU mutation impairs neuronal activity, in terms of reduced frequency, synchronicity and active neurons. These results suggest that inherited intronic TAU mutation deeply affects neuronal and glial maturation already during neurodevelopment, highlighting the pathophysiological role of TAU in the developing brain and paving the road for the identification of new therapeutical targets.

Pubmed:
34685628: Cordella F, Sanchini C, Rosito M, Ferrucci L, Pediconi N, Cortese B, Guerrieri F, Pascucci GR, Antonangeli F, Peruzzi G, Giubettini M, Basilico B, Pagani F, Grimaldi A, D'Alessandro G, Limatola C, Ragozzino D, Di Angelantonio S
Antibiotics Treatment Modulates Microglia-Synapses Interaction.
'Dysbiosis' of the adult gut microbiota, in response to challenges such as infection, altered diet, stress, and antibiotics treatment has been recently linked to pathological alteration of brain function and behavior. Moreover, gut microbiota composition constantly controls microglia maturation, as revealed by morphological observations and gene expression analysis. However, it is unclear whether microglia functional properties and crosstalk with neurons, known to shape and modulate synaptic development and function, are influenced by the gut microbiota. Here, we investigated how antibiotic-mediated alteration of the gut microbiota influences microglial and neuronal functions in adult mice hippocampus. Hippocampal microglia from adult mice treated with oral antibiotics exhibited increased microglia density, altered basal patrolling activity, and impaired process rearrangement in response to damage. Patch clamp recordings at CA3-CA1 synapses revealed that antibiotics treatment alters neuronal functions, reducing spontaneous postsynaptic glutamatergic currents and decreasing synaptic connectivity, without reducing dendritic spines density. Antibiotics treatment was unable to modulate synaptic function in CX3CR1-deficient mice, pointing to an involvement of microglia-neuron crosstalk through the CX3CL1/CX3CR1 axis in the effect of dysbiosis on neuronal functions. Together, our findings show that antibiotic alteration of gut microbiota impairs synaptic efficacy, suggesting that CX3CL1/CX3CR1 signaling supporting microglia is a major player in in the gut-brain axis, and in particular in the gut microbiota-to-neuron communication pathway.
Cells, 2021; 10
34269204: Cordella F, Brighi C, Soloperto A, Di Angelantonio S
Stem cell-based 3D brain organoids for mimicking, investigating, and challenging Alzheimer's diseases.
Neural Regen Res, 2022; 17
33993189: Brighi C, Salaris F, Soloperto A, Cordella F, Ghirga S, de Turris V, Rosito M, Porceddu PF, D'Antoni C, Reggiani A, Rosa A, Di Angelantonio S
Novel fragile X syndrome 2D and 3D brain models based on human isogenic FMRP-KO iPSCs.
Fragile X syndrome (FXS) is a neurodevelopmental disorder, characterized by intellectual disability and sensory deficits, caused by epigenetic silencing of the FMR1 gene and subsequent loss of its protein product, fragile X mental retardation

protein (FMRP). Delays in synaptic and neuronal development in the cortex have been reported in FXS mouse models; however, the main goal of translating lab research into pharmacological treatments in clinical trials has been so far largely unsuccessful, leaving FXS a still incurable disease. Here, we generated 2D and 3D in vitro human FXS model systems based on isogenic FMR1 knock-out mutant and wild-type human induced pluripotent stem cell (hiPSC) lines. Phenotypical and functional characterization of cortical neurons derived from FMRP-deficient hiPSCs display altered gene expression and impaired differentiation when compared with the healthy counterpart. FXS cortical cultures show an increased number of GFAP positive cells, likely astrocytes, increased spontaneous network activity, and depolarizing GABAergic transmission. Cortical brain organoid models show an increased number of glial cells, and bigger organoid size. Our findings demonstrate that FMRP is required to correctly support neuronal and glial cell proliferation, and to set the correct excitation/inhibition ratio in human brain development.
Cell Death Dis, 2021; 12

33750467: Latina V, Giacovazzo G, Cordella F, Balzamino BO, Micera A, Varano M, Marchetti C, Malerba F, Florio R, Ercole BB, La Regina F, Atlante A, Coccurello R, Di Angelantonio S, Calissano P, Amadoro G
Systemic delivery of a specific antibody targeting the pathological N-terminal truncated tau peptide reduces retinal degeneration in a mouse model of Alzheimer's Disease.
Retina and optic nerve are sites of extra-cerebral manifestations of Alzheimer's Disease (AD). Amyloid-β (Aβ) plaques and neurofibrillary tangles of hyperphosphorylated tau protein are detected in eyes from AD patients and transgenic animals in correlation with inflammation, reduction of synapses, visual deficits, loss of retinal cells and nerve fiber. However, neither the pathological relevance of other post-translational tau modifications-such as truncation with generation of toxic fragments-nor the potential neuroprotective action induced by their in vivo clearance have been investigated in the context of AD retinal degeneration. We have recently developed a monoclonal tau antibody (12A12mAb) which selectively targets the neurotoxic 20-22 kDa NH-derived peptide generated from pathological truncation at the N-terminal domain of tau without cross-reacting with its full-length normal protein. Previous studies have shown that 12A12mAb, when intravenously (i.v.)-injected into 6-month-old Tg2576 animals, markedly improves their AD-like, behavioural and neuropathological syndrome. By taking advantage of this well-established tau-directed immunization regimen, we found that 12A12mAb administration also exerts a beneficial action on biochemical, morphological and metabolic parameters (i.e. APP/Aβ processing, tau hyperphosphorylation, neuroinflammation, synaptic proteins, microtubule stability, mitochondria-based energy production, neuronal death) associated with ocular injury in the AD phenotype. These findings prospect translational implications in the AD field by: (1) showing for the first time that cleavage of tau takes part in several pathological changes occurring in vivo in affected retinas and vitreous bodies and that its deleterious effects are successfully antagonized by administration of the specific 12A12mAb; (2) shedding further insights on the tight connections between neurosensory retina and brain, in particular following tau-based immunotherapy. In our view, the parallel response we detected in this preclinical animal model, both in the eye and in the hippocampus, following i.v. 12A12mAb injection opens novel diagnostic and therapeutic avenues for the clinical management of cerebral and extracerebral AD signs in human beings.
Acta Neuropathol Commun, 2021; 9

32625060: Brighi C, Cordella F, Chiriatti L, Soloperto A, Di Angelantonio S
Retinal and Brain Organoids: Bridging the Gap Between Physiology and Micro-Physiology for the Study of Alzheimer's Diseases.
Recent progress in tissue engineering has led to increasingly complex approaches to investigate human neurodegenerative diseases , such as Alzheimer's disease, aiming to provide more functional and physiological models for the study of their pathogenesis, and possibly the identification of novel diagnostic biomarkers and therapeutic targets. Induced pluripotent stem cell-derived cortical and retinal organoids represent a novel class of three-dimensional models capable to recapitulate with a high similarity the structure and the complexity of the native brain and retinal tissues, thus providing a framework for better mimicking in a dish the patient's disease features. This review aims to discuss progress made over the years in the field of three-dimensional cell culture systems, and the benefits and disadvantages related to a possible application of organoids for the study of neurodegeneration associated with Alzheimer's disease, providing a promising breakthrough toward a personalized medicine approach and the reduction in the use of humanized animal models.
Front Neurosci, 2020; 14

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SPREADING OF P301S AGGREGATED TAU INVESTIGATED IN ORGANOTYPIC MOUSE BRAIN SLICES

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Neurofibrillary tangles composed of hyperphosphorylated tau protein aggregates are a key neuropathological feature of Alzheimer’s disease (AD). Tau pathology is reported to spread throughout the brain in a prion-like fashion through connected brain regions. However, the details of the underlying mechanisms are incompletely understood. The present study aims to examine the spreading of tau proteins using organotypic brain slice cultures. Coronal hippocampal slices (170 µm) were prepared from postnatal (day 8-10) C57BL6 wild-type mice. Collagen hydrogels loaded with different tau proteins (full-length tau, K18 PHF tau, Δ306-311 tau, and P301S aggregated tau) were applied to slices and the spread of tau was assessed by immunohistochemistry after 8 weeks. Collagen hydrogels are an effective protein delivery system subject to natural degradation in 14 days and release tau proteins up to 8 weeks. Only P301S aggregated tau loaded in collagen hydrogels was detectable by Western blotting. Slices with collagen hydrogels loaded with un- and hyperphosphorylated P301S aggregated tau demonstrate significant spreading to the ventral parts of the hippocampal slices compared to empty collagen hydrogels after 8 weeks. Moreover, the spread of P301S aggregated tau occurs in a time-dependent manner. We illustrate that tau spreading can be investigated in organotypic slice cultures using collagen hydrogels to locally and slowly apply tau proteins. P301S aggregated tau significantly spreads to the ventral areas of the slices, suggesting that the disease-relevant aggregated tau form possesses more spreading potential compared to the other tau proteins. Thus, the results offer a novel insight on tau spreading in AD.

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USING VIRAL VECTORS TO STUDY THE SYNERGISTIC DEVELOPMENTAL EFFECTS OF TAU, ALPHA-SYNUCLEIN AND AMYLOID-BETA

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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We want to expand our knowledge on how to study dementia in the mice brain using viral vectors in lieu of transgenic animals. Our research includes tau protein, amyloid-beta and alpha-synuclein and our goal is to understand their synergistic development in dementia pathology. Adeno-associated viruses (AAVs) are modified in-house to carry a gene of interest and are then injected locally. Post mortem analysis shows negligible spread from the injection site. This is important because we wish to study pathology in a limited brain area and have minimal impact on the animal's well-being. In our initial experiments we looked at the tau protein mutation P301S. The goal of the experiment was to evaluate how the mutation impacted transfected cells and the mice in general over a period of 16 weeks. We observed no impact on the mice's general health, locomotion, anxiety or nesting abilities. The AAV-P301S injection causes overexpression of human tau in transfected cells, this was confirmed using immunohistochemistry and western blot. A substantial amount of the tau proteins were hyperphosphorylated (p-tau), this is an early sign of protein malfunction. Tau protein was mainly observed in the neuron cell bodies, while p-tau was also found in dendrites. The mislocation of p-tau to dendritic spines contribute to synaptic loss in Alzheimer's disease. Future experiments will involve other proteins linked to dementia pathology, namely amyloid-beta and alpha-synuclein. Moreover, we will use two-photon imaging to study neuronal activity by imaging Ca2+ transients, and look at the morphology of transfected cells over time.

Pubmed:
29279411: Thompson EH, Lensjø KK, Wigestrand MB, Malthe-Sørenssen A, Hafting T, Fyhn M
Removal of perineuronal nets disrupts recall of a remote fear memory.
Throughout life animals learn to recognize cues that signal danger and instantaneously initiate an adequate threat response. Memories of such associations may last a lifetime and far outlast the intracellular molecules currently found to be important for memory processing. The memory engram may be supported by other more stable molecular components, such as the extracellular matrix structure of perineuronal nets (PNNs). Here, we show that recall of remote, but not recent, visual fear memories in rats depend on intact PNNs in the secondary visual cortex (V2L). Supporting our behavioral findings, increased synchronized theta oscillations between V2L and basolateral amygdala, a physiological correlate of successful recall, was absent in rats with degraded PNNs in V2L. Together, our findings suggest a role for PNNs in remote memory processing by stabilizing the neural network of the engram.
Proc Natl Acad Sci U S A, 2018; 115

FENS Forum 2022
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BOARD NUMBER: S06-277

DOES PRPC HAVE A MAJOR ROLE IN THE PROGRESSION OF TAU PATHOLOGY?

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Tauopathies are a group of neurodegenerative diseases (e.g., Alzheimer's disease (AD)) characterized by the accumulation and deposition of the Tau protein into amyloid structures. The progression of Tau pathology in AD follows a predictable, hierarchical, and stereotyped pattern between anatomically connected areas of the brain. Thus, it is thought that pathological Tau (pTau) protein engages in a self-amplification process known as "seeding" followed by a trans-cellular "spreading" of the disease. However, the precise molecular mechanisms involved remain elusive.
Recently, the cellular prion protein (PrP^c) has been proposed to mediate Aβ-amyloid progression in AD and α-synuclein in Pakinson's disease, yet less is known about its role in the induction and progression of Tau pathology. In this regard, we inoculated in the parietal cortex and corpus callosum of wild-type, Tau knock-out (KO), PrP^c KO (Zürich III), and PrP^c anchorless (Tg44) mice with samples from AD patients. All mice models, except for the Tau KO, revealed similar amounts of pTau aggregates and spreading at 3 and 6 months post-inoculation. Using immunohistochemical techniques, we have determined that such aggregates mainly contain murine endogenous Tau being positives for histopathological pTau markers such as AT8 and PHF-1. Thereby, our preliminary findings suggest that PrP^c does not seem to have a crucial role in the initialization and/or progression of Tau pathology in our tested models, and other mechanisms might run in parallel. Current experiments are directed to determine these alternative processes and whether they are linked to PrP^c.

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SCREENING TYROSINE KINASES FOR THEIR INVOLVEMENT IN SYNAPTOTOXICITY INDUCED BY TAU MICROTUBULE-BINDING REGION FIBRILS

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Aims: Synapse loss is an early event in Alzheimer's disease (AD); yet, mechanisms of synaptotoxicity induced by amyloid-β and tau –hallmarks of AD pathology – remain unclear. We have recently developed a lab-ona-chip device that fluidically isolates synapses of primary neurons from their cell bodies, thereby enabling us to exclusively access synaptic, as well as pre- and postsynaptic regions. Our aim is to identify molecular mechanisms of synaptotoxicity related to AD. **Methods:** Using the said in vitro model, we assessed the impact of exogenously applied monomeric and fibrillar forms of tau microtubule-binding region (tau-MTBR) as well as of full-length tau isoforms on the connectivity of isolated synapses, quantified through distance-based assignment of postsynaptic puncta to presynaptic puncta. After confirming that the fibrillar form of tau-MTBR induces a detrimental effect on mature synapses, we conducted a medium-throughput compound screening, in which we tested small molecule inhibitors of 25 tyrosine kinases for their capacity to block tau-MTBR-induced synaptotoxicity. **Results:** Four compounds that did not impact synapses in the absence of tau-MTBR, but protected them from the detrimental effect were considered potentially protective and subjected to dose-response analysis. Experiments to validate the observed toxic and protective effects using electrophysiological recordings –via microfluidic devices integrated to microelectrode arrays– are under way. **Conclusions:** Deciphering downstream signaling mechanisms in tau-induced synaptotoxicity will help us gain a better understanding of AD pathogenesis and identify potential therapeutic targets.

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BOARD NUMBER: S06-279

LOCAL TAU REDUCTION RESCUES COGNITIVE IMPAIRMENTS AND PATHOLOGICAL PHENOTYPES IN A PRECLINICAL MODEL OF TAUOPATHY

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Aims: Tauopathies are neurodegenerative diseases characterized by intraneuronal accumulation of hyperphosphorilated Tau protein, leading to neuronal dysfunction and neurodegeneration. Recent evidence suggests selective vulnerability of specific brain nuclei to initiate Tau pathology, from where pathological Tau propagates to other areas. Several experimental approaches pointed Tau reduction as a promising therapeutic strategy for tauopathies. Yet, global Tau elimination might affect endogenous physiological functions. Therefore, we aimed to validate a novel strategy for local Tau knock-down into specific brain nuclei in a mouse model of tauopathy. Methods: We analyzed the hTau mouse model of tauopathy, which primarily accumulates hyperphosphorylated Tau in the prefrontal cortex (PFC) and develops cognitive impairments and cortical-pyramidal neuronal firing deficits from 6 months-old. Artificial microRNAs designed to target the *MAPT* transcript were delivered into the PFC of hTau mice by lentiviral vectors, either before (3 months-old) or after (6 months-old) phenotypic onset. Mice were analyzed in behavioral tasks (cognitive and motor) and biochemical, electrophysiological and microPET imaging studies. Tau reduction was determined by RT-qPCR and western blot. Results: microRNAs efficiently reduced endogenous Tau *in vivo* by 50% in the PFC. Tau knock-down from 3 months-old prevented Tau pathology and cognitive impairments. Phenotypic rescue was also observed when microRNAs were administered at mid-stage (6 months-old) of Tau pathology onset. No adverse effects were observed neither in hTau nor wild type mice after microRNAs administration. Conclusions: Our results provide proof of concept for the potential use of microRNAs to locally reduce pathological Tau accumulation as a therapeutic approach for tauopathies.

Pubmed:
34059893: Damianich A, Facal CL, Muñiz JA, Mininni C, Soiza-Reilly M, Ponce De León M, Urrutia L, Falasco G, Ferrario JE, Avale ME
Tau mis-splicing correlates with motor impairments and striatal dysfunction in a model of tauopathy.
Tauopathies are neurodegenerative diseases caused by the abnormal metabolism of the microtubule associated protein tau (MAPT), which is highly expressed in neurons and critically involved in microtubule dynamics. In the adult human brain, the alternative splicing of exon 10 in MAPT pre-mRNA produces equal amounts of protein isoforms with either three (3R) or four (4R) microtubule binding domains. Imbalance in the 3R:4R tau ratio is associated with primary tauopathies that develop atypical parkinsonism, such as progressive supranuclear palsy and corticobasal degeneration. Yet, the development of effective therapies for those pathologies is an unmet goal. Here we report motor coordination impairments in the htau mouse model of tauopathy which harbour abnormal 3R:4R tau isoforms content, and in contrast to TauKO mice, are unresponsive to l-DOPA. Preclinical-PET imaging, array tomography and electrophysiological analyses indicated the dorsal striatum as the candidate structure mediating such phenotypes. Indeed, local modulation of tau isoforms by RNA trans-splicing in the striata of adult htau mice, prevented motor coordination deficits and restored basal neuronal firing. Together, these results suggest that abnormal striatal tau isoform content might lead to parkinsonian-like phenotypes and demonstrate a proof of concept that modulation of tau mis-splicing is a plausible disease-modifying therapy for some primary tauopathies.
Brain, 2021; 144

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SUBSTRATE-SPECIFIC LOSS OF TUBULIN-ALPHA4A POLYGLUTAMYLATION PREVENTS OLIGOMERIZATION OF HYPER-PHOSPHORYLATED TAU AND MICROGLIA ACTIVATION IN BRAIN

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Dissociation of hyper-phosphorylated Tau from neuronal microtubules and its pathological aggregates, are hallmarks in the etiology of tauopathies. The Tau-microtubule interface is subject to polyglutamylation, a reversible posttranslational modification, increasing negative charge at tubulin tails. Here, we asked whether tubulin polyglutamylation may contribute to Tau pathology *in vivo*. Since polyglutamylases modify various proteins other than tubulin, we generated a knock-in mouse carrying gene mutations to abolish Tuba4a polyglutamylation in a substrate-specific manner. We found that Tuba4a lacking polyglutamylation prevents the binding of Tau and MAP2a/b and GSK3 kinase to neuronal microtubules, thereby strongly reducing phospho-Tau levels. Notably, crossbreeding of mutant mice with a tauopathy model, expressing a human Tau transgene, reversed the oligomerization of hyper-phosphorylated Tau and normalized microglia activation in brain. Our data highlight tubulin polyglutamylation as a novel molecular parameter to develop treatment strategies in fighting tauopathies.

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DEFINING SPECIFIC FINGERPRINTS OF NEURODEGENERATIVE DISEASES IN THE RETINA

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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The eye offers a natural window to the brain, as the retina is considered part of the central nervous system. AD, PD and ALS-associated neurodegenerative changes in the brain are accompanied by structural and possible functional changes in the neuroretina and the ocular vasculature. Aim: The present project aimed to define and provide a panel of retinal biomarkers specific to AD and ALS as a tool for monitoring disease progression. Methods: The identification of specific cellular and molecular alterations in human AD and ALS retina were evaluated by immunofluorescence approaches in autoptic human retinal slices from patients versus healthy controls. Images were acquired with a confocal microscope. Results: Our results indicated the presence of TDP43 protein aggregated in the cytoplasm of retinal ganglion bipolar cells of ALS patients, nuclear retinal TDP43 staining was found also in control slices. We could also show an increase of SQSTM1/p62 aggregates in the retina of ALS patients as compared to healthy control. Both TDP43 and SQSTM1/p62 are described as pathological hallmark lesions in the spinal cord and brain of most ALS patients, without clear signs of glial activation. By contrast in AD patients, retinal sections displayed beta-amyloid and tau protein aggregates together with DAM microglia and A1 astrocytes activation, paving the road to the definition of a disease-specific biomarker panel. Conclusions: Retinal biomarkers may be used in vivo as an additional and specific early diagnostic tool, offering the opportunity to longitudinally monitor individuals and therapies over time in a non-invasive and cost-effective way.

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REVISITING TAU INVOLVEMENT IN COMPLEX NEURAL NETWORK REMODELLING THROUGH THE ANALYSIS OF EXTRACELLULAR NEURONAL ACTIVITY EXHIBITED BY ORGANOTYPIC BRAIN SLICE CO-CULTURES

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Background: In regards of tau involvement in tauopathies and the protective effect of tau ablation in epilepsy, understanding tau functions in brain networks is paramount. However, tau functions are difficult to highlight as *tau*^{KO} (*Mapt*$^{-/-}$) neural networks exhibit only elusive phenotypes. **Objectives:** To explore further the physiological role of tau in brain networks, the impact of tau ablation has been investigated during the network remodelling occurring in hippocampal/entorhinal slice co-cultures. **Methods:** We recorded spontaneous extracellular neuronal activity over two weeks from single slice cultures and slice co-cultures established from *wild-type* and *tau*^{KO} animals. We compared bursting activity features and applied concepts and analytical tools intended for the study of network synchrony and connectivity. **Results:** Tau ablation resulted in an anti-synchrony effect on hippocampal/entorhinal slice co-cultures, in line with literature. By focusing on the changes between single culture and co-culture paradigms, we demonstrated that tau ablation led to differential, even opposite, effects at the sub-network scale. Tau ablation induced throughout the duration of the co-culture an anti-synchrony effect within the hippocampal slice likely due to an excitation/inhibition ratio reduction. Conversely, tau ablation led to increased synchrony within the entorhinal slice likely due to homogenization of the connectivity distribution at early stages of the co-culture. **Conclusions:** Our new and powerful methodology to study the role played by a gene/protein in brain networks has confirmed a role of tau in the remodelling of complex neural networks and, for the first time, demonstrate this role is multifaceted and dependent of the sub-network nature.

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LEPTIN PREVENTS AMYLOID-BETA-INDUCED ABERRANT TARGETING OF PHOSPHORYLATED TAU VIA PI 3 KINASE SIGNALLING

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Accumulation of amyloid-β (Aβ) and hyperphosphorylated tau are hallmarks of Alzheimer's disease (AD). Recent studies show that Aβ promotes tau phosphorylation causing migration of tau from axons to dendritic spines and synapses, resulting in synaptic impairment. Aberrant leptin production and signalling has been observed in AD patients. Various studies have identified neuroprotective properties of leptin in models of Aβ-related toxicity. However, the effects of leptin on tau-related synaptic dysfunction are unclear. Here, we show that $A\beta_{1-42}$ treatment (1μM, 1hr) to primary hippocampal neurons causes a significant increase in dendritic p-tau compared to control (167 ± 9%, $p<0.001$, n=36 dendrites), and a significant increase in % colocalisation of p-tau and PSD-95 (synaptic marker) from 43 ± 1.4% to 57 ± 1.4% ($p<0.001$, n=36 dendrites). Treatment with leptin prevents this aberrant targeting of p-tau to dendrites and synapses as dendritic p-tau levels and % colocalisation are not significantly different from control in leptin+$A\beta_{1-42}$ treated neurons ($p>0.05$, n=36 dendrites). PI 3 kinase activation and GSK-3β inhibition were identified as the likely signalling mechanisms underlying the protective effects of leptin. Wortmannin treatment (PI3K inhibitor, 50nM, 1hr) ablated the protective effects of leptin, resulting in a significant increase in dendritic p-tau in wortmannin+$A\beta_{1-42}$+leptin treated neurons (150 ± 6% of control, $p<0.001$, n=36). TCS2002 treatment (GSK-3β inhibitor, 100nM, 1hr) mimicked and occluded the protective effects of leptin, resulting in no significant difference in dendritic p-tau in TCS2002+$A\beta_{1-42}$ and TCS2002+$A\beta_{1-42}$+leptin treated neurons ($p>0.05$, n=36 dendrites). These data further validate the leptin system as a therapeutic target in AD.

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NEDDYLATION-DEPENDENT PROTEIN DEGRADATION IS A NEXUS BETWEEN SYNAPTIC INSULIN RESISTANCE, NEUROINFLAMMATION AND ALZHEIMER'S DISEASE.

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Background: The metabolic syndrome (MetS) is a common pathology in western societies which is characterized by synaptic insulin resistance (IR), high body weight, and high levels of cholesterol. It has been proposed the MetS together with a high amyloid load and low-level neuroinflammation is an important risk factor for Alzheimer's disease (AD). **Aims and methods**: We investigated the biological mechanism behind this interplay establishing an *in vitro* model with the use of primary neurons to induce synaptic insulin resistance. We move then to a mouse model of high-risk aging that includes a high amyloid load, diet induced-obesity, and neuroinflammation. **Results**: We found that neddylation of Cullin 7 induces proteasomal degradation of the Insulin receptor substrate1 (IRS1) inducing synaptic insulin resistance. Moreover, the neddylation activating enzyme inhibitor MLN-4924 restores synaptic IR and cognitive related impairments found in mice with high amyloid load and fed with high-fat diet. **Conclusions:** Our data suggest that neddylation-dependent degradation of IRS1 is the nodal point that might explain the link between synaptic IR, amyloid load and neuroinflammation. Inhibition of neddylation is a molecular entry point to treat cognitive deficits associated with MetS and might be useful to reduce the risk of AD.

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LOWERING SYNAPTOGYRIN-3 LEVELS RESCUES PRESYNAPTIC DEFECTS INDUCED BY PATHOGENIC TAU PATHOLOGY AND OVERCOMES SYNAPSE LOSS AND COGNITIVE DECLINE

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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A group of more than 20 progressive neurodegenerative diseases are pathologically defined by Tau dysfunction; also referred to as Tauopathies, which are characterised by synapse dysfunction and neurodegeneration. The underlying mechanism is unclear. The aim of this study is to elucidate how Tau dysfunction leads to synapse loss. Pathological Tau translocates to the pre-synapse and binds to synaptic vesicles. Binding of Tau to synaptic vesicles is physically mediated by Synaptogyrin-3. In this study, we determined the role of pathogenic Tau at presynaptic terminals by lowering Synaptogyrin-3 levels in mice with Tau pathology. We assessed structural, functional and behavioural effects. We found that heterozygous knockout of Synaptogyrin-3 strongly rescues mutant Tau-induced synaptic defects as well as memory loss. Interestingly, both pre- and postsynapse can be rescued in TauPS19 mice with Synaptogyrin-3 heterozygous knockout. These findings show that reducing Synaptogyrin-3 expression rescues Tau-induced synapse loss and cognitive decline. Together, these findings point to Synaptogyrin-3 as a highly interesting therapeutic candidate to target Tauopathies.

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STUDYING SPORADIC AND FAMILIAL ALZHEIMER´S DISEASE ON IPSC-DERIVED HIPPOCAMPAL AND CORTICAL NEURONS: EFFECT OF APOE AND PRESENILIN1

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Sporadic and familial Alzheimer´s disease (AD) are characterized by a progressive neurodegeneration mainly in memory-related areas such as the entorhinal cortex and hippocampus. The epsilon4 allele of gene encoding apolipoprotein E (APOE) is the strongest genetic risk factor for late-onset AD whereas mutations in PSEN1 gene cause early-onset AD. Increasing evidence shows that APOE4 is associated with diverse aspects of AD pathogenesis, but the impact of APOE alleles on neuronal differentiation, maturation and function remains to be fully elucidated. Furthermore, the effect of G206D-PSEN1 mutation on human neurons has been little explored. To clarify these questions, we obtained induced pluripotent stem cells (iPSCs) from fibroblasts of AD patients carrying the epsilon3 and epsilon4 alleles (in homozygosis) or having G206D-PSEN1 mutation, and from healthy patients. We also used gene-edited iPSC lines homozygous for APOE variants and an APOE knock-out line. Both cortical and hippocampal neurons were generated from human iPSCs by establishing differentiation protocols through the addition of small molecules and growth factors, and their cellular, molecular, functional and neuropathological characterisation was performed. iPSCs-derived neurons expressed cortical and hippocampal markers and showed a functional profile determined by glutamate release, electrical activity and synapse formation visualized by electron microscopy. In addition, release of amyloid-beta 42/40, total Tau and phosphorylated Tau to the culture medium, as well as the presence of amyloid-beta plaques and p-Tau181 aggregates are being analysed. Overall, our results point to specific actions of APOE polymorphism and G206D-PSEN1 mutation affecting neuronal differentiation, dysfunction and neurodegeneration.

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BRAIN MOLECULAR ALTERATIONS ASSOCIATED TO EARLY RECOGNITION DEFICITS IN A NEW MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Alzheimer's disease (AD) is a slow-growing neurodegenerative disease that remains irreversible and incurable today. Early in the disease, both histopathological hallmarks, amyloid plaques and neurofibrillary tangles, affect brain structures involved in memory processes such as the median temporal lobe and the prefrontal cortex. The diagnosis of AD is often late as several years elapse between the beginning of the pathology and the onset of the first cognitive deficits. It is therefore crucial to better understand the earliest stages of AD. We are studying a doubly humanized *App*$^{NL-F}$*/MAPT* double knock-in (dKI) model expressing a humanized App gene with two familiar Alzheimer's disease mutations (*App*$^{NL-F}$) and a humanized tau gene (*MAPT*) (Saito *et al*., 2014 and 2019). This dKI mouse show increased amyloidogenic processing of APP and the six normal human Tau protein isoforms instead of the three murine isoforms. Using a series of sensitive object exploration tasks, we showed that dKI mice displayed a deficit in object-place association memory as soon as 4 months of age, that was associated to molecular alterations related to initial steps of AD. Indeed, western blot studies notably revealed an increase of β-amyloid peptides (pre-plaques stage) in the median temporal lobe whereas an increase of phosphotau proteins was restricted to the perirhinal-entorhinal region and prefrontal cortex, sparing the hippocampus. The similarity between our findings and those reported in the earliest stages of AD suggests that the *App*$^{NL-F}$*/MAPT* dKI model has a high potential to better understand the initial stage of the disease.

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ACTIVATION AND TAU RELATIONS OF HIPPOCAMPAL MICROGLIA IN THE FEMALE 3XTGAD MODEL. A QUESTION OF AGE?

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Alzheimer's disease (AD) involves severe impairment of cognitive and executive functions.Neuropathologically,it is characterized by the deposition of beta-amyloid peptide in extracellular neuritic plaques and the formation of intraneuronal neurofibrillary tangles,which elimination has been the unsuccessful goal of different therapies.However,little is known about the progressing neuroinflammatory process,characterized by an increase in the number and morphological changes of microglial cells at different stages of the disease.The aim of this study is to characterize the morphological differences and tau-relationship of microglial cells in the hippocampus of an aged AD murine model to elucidate whether, associated with age and tau and beta amyloid deposits,there is an active proinflammatory phenotype different from the physiological pattern and how it is related to the formation of tau neurofibrillary tangles. First,we characterized microglial morphology.Twelve female mice (healthy controls and 3xTgAD, n = 6 per group) between 19 and 22 months were used.Ten cells from each animal were randomly analysed using the AnalyzeSkeleton and FracLac extensions of the Image-J program.No significant differences were found between both groups. Secondly,we studied microglial relationship with tau accumulation at different stages (9, 12 and 15 months, n=6).Our results show an increase in both the number of microglia cells and intracellular tau tangles associated with age,as well as a strong positive correlation between both variables. In sum,we must consider the age variable to understand the role of microglial cells in AD neurodegeneration process.Understanding their morphological heterogeneity may be one of the keys of this neurodegenerative disorder. Supported by Universitat Jaume I (PI-UJI2017_17; PPSI 19I358.01/1)

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NEW SMALL MOLECULE LABELLING TOOLS TO INVESTIGATE THE PHYSIOLOGICAL FUNCTIONS OF BETA-SECRETASE BACE1

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Alzheimer´s disease (AD) is the most common form of dementia. A wealth of evidence supports the hypothesis that a major factor in AD is dysregulation of Abeta-homeostasis, which leads to an increased cleavage of amyloid precursor protein by the enzyme beta-site of APP cleaving enzyme 1 (BACE1). Apart from its important role in AD, we recently reported BACE1 as a salient interaction-partner of neuronal and cardiac Kv7-channels independent of proteolysis. We investigated the physiological functions of BACE1 by developing specific, tag- and antibody-free labelling tools. All new compounds are conjugates of BACE1-inhibitor IV linked to different fluorescent dyes. We show here that these fluorescent small molecules bind specifically to the protease with a 1:1 labeling stoichiometry at their catalytic moiety, a crucial property especially for single-molecule and super-resolution microscopy. Using such microscopy, we characterized its biophysical properties and labelling capabilities in over-expressing cell systems, native and fixed neuronal tissue. In addition, the capability to label endogenous and over-expressed BACE1 with high specificity allowed us to perform Acceptor-Photobleaching FRET (Förster resonance energy transfer) assay to evaluate BACE1/Kv7-interactions. Hence, our novel fluorescent inhibitors make it possible to investigate protein-protein interactions and diffusion behavior of BACE1 from the tissue setting down to single molecule level.

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DYRK1A INHIBITION AS A THERAPEUTIC APPROACH FOR ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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There is a critical need for new treatment approaches that can slow or prevent the progression of Alzheimer's disease (AD). Targets that act on multiple relevant pathways could have significant therapeutic potential. Dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1a) phosphorylates both amyloid precursor protein (APP) and tau. Dyrk1a is upregulated in post-mortem brains of AD patients and elevated expression is associated with cognitive deficits. We previously demonstrated that inhibition of Dyrk1 is well-tolerated and reduces amyloid plaques and pathological forms of tau in 3xTg-AD mice if administered after formation of these pathologies. However, while insoluble forms of hyperphosphorylated tau were reduced by Dyrk1 inhibition, overt neurofibrillary tangle (NFT) pathology remained unchanged. Herein, we specifically test the hypothesis that inhibition of Dyrk1 prior to NFT formation will delay the onset of pathology. 3xTg-AD mice were treated chronically, beginning at 6 months of age, prior to NFT pathology. Mice were dosed daily for either 3 or 6 months and amyloid and tau pathology were assessed. We show that chronic Dyrk1 inhibition reduces insoluble forms of amyloid beta peptides (Aβ) and hyper-phosphorylated tau long-term and that these reductions are associated with dramatic delay in the onset of both amyloid plaques and NFTs. In addition, we show that DYR219, a potent and selective small molecule Dyrk1 inhibitor, induces degradation of Dyrk1a protein, likely contributing to its in vivo efficacy. Collectively, these results suggest that therapeutic strategies targeting tau phosphorylation will show the greatest effect if administered very early in the pathogenesis of AD.

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A COMBINED TDP43-TAU CELLULAR MODEL FOR THE UNDERSTANDING OF LATE-NC PROTEINOPATHY

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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TDP-43 protein is known as the major disease protein in amyotrophic lateral sclerosis (ALS) and in the most common variant of frontotemporal lobar degeneration (FTLD). Recently, TDP-43 proteinopathy has been related with a newly recognized type of dementia, limbic-predominant agerelated TDP-43 encephalopathy (LATE-NC). Several studies have shown that almost 50% of patients with LATE-NC also present TDP-43 protein deposits in their brain cells. Although the true role of TDP-43 in LATE-NC and its relationship with pathological forms of Tau is currently unknown, in recent years it has been revealed that they may coexist in exacerbated forms of the disease. The development of a cellular assay aimed to study the behavior of the TDP43-TAR protein in combination with hyperphosphorylated Tau protein may allow us to elucidate new pathways of molecular signaling in LATE disease, as well as to screen compounds that may intercede in the pathological synergy between these two targets. Using this HCS assay in 96 well format, we performed the screening of a small synthetic chemical library of 1,200 compounds. The Z′ factor of the assay was over 0.5 demonstrating the robust performance of the assay. After the screening campaign, the positive compounds were chosen for further testing, based on the strength of the initial response and the lack of cytotoxicity.

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BOARD NUMBER: S06-292

CHARACTERIZATION OF HUMAN TAU PROTEIN IN YEAST CELLS UNDER NORMAL AND STRESS CONDITIONS

POSTER SESSION 06 - SECTION: ALZHEIMER'S AND TAUOPATHIES

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Age-dependent protein aggregation is a conserved phenomenon that is associated with many neurodegenerative diseases, including Alzheimer's disease (AD). AD is characterized by aggregation of Tau, a microtubule-binding protein that is normally soluble and mainly localized to neuronal axons, but can form oligomers and higher-order amyloid-like aggregates that accumulate in soma and dendrites and eventually lead to neuronal death. Although the main risk factor for the onset of AD is aging, the exact causes of Tau protein aggregation are largely unclear. To investigate factors that influence Tau protein aggregation, we expressed human Tau protein fused with fluorescent proteins in yeast *Saccharomyces cerevisiae*. We examined intracellular localization of Tau in young and aged cells, and in cells under different stress conditions, such as glucose starvation, elevated temperature, hyperosmotic and proteotoxic stress caused by toxic amino acid analogue. Furthermore, to study factors affecting Tau oligomerization, we used luminescent reporter NanoBiT in which protein-protein interaction results in the complementation of the luciferase NanoLuc. Results show basal levels of Tau-NanoBiT reporter signal in logarithmically growing wild-type cells, suggesting that Tau oligomerization doesn't occur under normal growth conditions. *Cofinanced by the Research Cooperability Program of the Croatian Science Foundation funded by the EU from the European Social Fund under the Operational Program Efficient Human Resources 2014-2020 (PZS-2019-02-3610); Croatian Science Foundation grants IP-2019-04-3584 and DOK-2018-01-9299, DOK-2021-02-2505; Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience ("Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by the EU through the European Regional Development Fund).*

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BOARD NUMBER: S06-293

CULTURED MOUSE DOPAMINERGIC NEURONS AS A MODEL SYSTEM TO STUDY ALPHA-SYNUCLEIN AGGREGATION AND NEURODEGENERATION IN PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aims: In this study, we aimed to get better insights into mechanisms underlying α-Synuclein (αS) aggregation and neurodegeneration in Parkinson disease (PD). **Methods:** For that, we established primary cultures of mouse dopamine (DA) neurons and chronically exposed them to fibrils 91 (F91) generated from recombinant human αS. We used astrocyte-conditionned medium to enable long-term cultures. **Results:** We established that F91 have an exquisite propensity to seed aggregation of endogenous αS in DA neurons (somas + neurites) through a process that is unrelated to DA metabolism. Until two weeks post-exposure to F91 somal aggregation in DA neurons increased with F91 concentrations and time elapsed since initiation of seeding. There was, however, no evidence of DA cell loss under such treatment conditions. In the same experimental context, neither toxin-induced mitochondrial deficits nor genetically-induced loss of mitochondrial quality control mechanisms promoted F91-mediated αS aggregation or neurodegeneration. Yet, a significant loss of DA neurons (~30%) was detectable three weeks after exposure to F91 (0.5 µM), i.e., at a time point where somal aggregation reaches a plateau phase. This loss was preceded by early deficits in DA uptake, a sensitive marker of DA cell function. Unlike αS aggregation, the loss of DA neurons was prevented by treatment with GDNF suggesting that αS aggregation in DA neurons induces a form of cell death mimicking a state of trophic factor deprivation. **Conclusions:** Overall, we suggest that this model system might be useful to explore PD-related pathomechanisms and test molecules of therapeutic interest for this disorder.

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TRAFFICKING OF THE GLUTAMATE TRANSPORTER IS IMPAIRED IN LRRK2-RELATED PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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The Excitatory Amino Acid Transporter 2 (EAAT2) accounts for the 80% of brain glutamate clearance and it is mainly expressed in astrocytic perisynaptic processes. Noteworthy, deficits in EAAT2 have been associated with neurodegenerative diseases including Parkinson's disease (PD). **AIM**- The aim of this study was to investigate the link between EAAT2 impairment and PD starting from human *post-mortem* samples. **METHODS**- We employed biochemical and imaging techniques to analyze EAAT2 expression in human as well as mouse brains. Purified astrocytic terminals, primary astrocytes and brain organotypic cultures were also applied to study the molecular mechanisms behind EAAT2 dysfunction. **RESULTS**- EAAT2 protein levels are clearly decreased and match with elevated gliosis in PD patients harboring the LRRK2 G2019S mutation, a common cause of familiar PD, and the deficits are less pronounced in the idiopathic PD forms. The decreased expression of the transporter correlates with its reduced functionality in mouse LRRK2 G2019S purified astrocytic terminals. In LRRK2 G2019S knockin mouse brain, the correct surface localization of the endogenous transporter is impaired, resulting in its interaction with a plethora of endo-vesicular proteins. Mechanistically, we report that pathogenic LRRK2 kinase activity delays the recycling of the transporter to the plasma membrane *via* Rabs inactivation, causing its intracellular relocalization and degradation. **CONCLUSIONS**- Our results demonstrate that a molecular and functional connection between EAAT2 and pathogenic LRRK2. Specifically, LRRK2 G2019S interferes with the recycling of EAAT2, pointing to extracellular glutamate overload as a possible contributor to neurodegeneration in PD.

Pubmed:
33629273: Streubel-Gallasch L, Giusti V, Sandre M, Tessari I, Plotegher N, Giusto E, Masato A, Iovino L, Battisti I, Arrigoni G, Shimshek D, Greggio E, Tremblay ME, Bubacco L, Erlandsson A, Civiero L
Parkinson's Disease-Associated LRRK2 Interferes with Astrocyte-Mediated Alpha-Synuclein Clearance.
Parkinson's disease (PD) is a neurodegenerative, progressive disease without a cure. To prevent PD onset or at least limit neurodegeneration, a better understanding of the underlying cellular and molecular disease mechanisms is crucial. Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene represent one of the most common causes of familial PD. In addition, LRRK2 variants are risk factors for sporadic PD, making LRRK2 an attractive therapeutic target. Mutations in LRRK2 have been linked to impaired alpha-synuclein (α-syn) degradation in neurons. However, in which way pathogenic LRRK2 affects α-syn clearance by astrocytes, the major glial cell type of the brain, remains unclear. The impact of astrocytes on PD progression has received more attention and recent data indicate that astrocytes play a key role in α-syn-mediated pathology. In the present study, we aimed to compare the capacity of wild-type astrocytes and astrocytes carrying the PD-linked G2019S mutation in Lrrk2 to ingest and degrade fibrillary α-syn. For this purpose, we used two different astrocyte culture systems that were exposed to sonicated α-syn for 24 h and analyzed directly after the α-syn pulse or 6 days later. To elucidate the impact of LRRK2 on α-syn clearance, we performed various analyses, including complementary imaging, transmission electron microscopy, and proteomic approaches. Our results show that astrocytes carrying the G2019S mutation in Lrrk2 exhibit a

decreased capacity to internalize and degrade fibrillar α-syn via the endo-lysosomal pathway. In addition, we demonstrate that the reduction of α-syn internalization in the Lrrk2 G2019S astrocytes is linked to annexin A2 (AnxA2) loss of function. Together, our findings reveal that astrocytic LRRK2 contributes to the clearance of extracellular α-syn aggregates through an AnxA2-dependent mechanism.
Mol Neurobiol, 2021; 58
34440835: Serpe C, Monaco L, Relucenti M, Iovino L, Familiari P, Scavizzi F, Raspa M, Familiari G, Civiero L, D'Agnano I, Limatola C, Catalano M
Microglia-Derived Small Extracellular Vesicles Reduce Glioma Growth by Modifying Tumor Cell Metabolism and Enhancing Glutamate Clearance through miR-124.
Brain homeostasis needs continuous exchange of intercellular information among neurons, glial cells, and immune cells, namely microglial cells. Extracellular vesicles (EVs) are active players of this process. All the cells of the body, including the brain, release at least two subtypes of EVs, the medium/large EVs (m/lEVs) and small EVs (sEVs). sEVs released by microglia play an important role in brain patrolling in physio-pathological processes. One of the most common and malignant forms of brain cancer is glioblastoma. Altered intercellular communications constitute a base for the onset and the development of the disease. In this work, we used microglia-derived sEVs to assay their effects in vitro on murine glioma cells and in vivo in a glioma model on C57BL6/N mice. Our findings indicated that sEVs carry messages to cancer cells that modify glioma cell metabolism, reducing lactate, nitric oxide (NO), and glutamate (Glu) release. sEVs affect Glu homeostasis, increasing the expression of Glu transporter Glt-1 on astrocytes. We demonstrated that these effects are mediated by miR-124 contained in microglia-released sEVs. The in vivo benefit of microglia-derived sEVs results in a significantly reduced tumor mass and an increased survival of glioma-bearing mice, depending on miR-124.
Cells, 2021; 10
30291939: Coppi E, Lana D, Cherchi F, Fusco I, Buonvicino D, Urru M, Ranieri G, Muzzi M, Iovino L, Giovannini MG, Pugliese AM, Chiarugi A
Dexpramipexole enhances hippocampal synaptic plasticity and memory in the rat.
Even though pharmacological approaches able to counteract age-dependent cognitive impairment have been highly investigated, drugs improving cognition and memory are still an unmet need. It has been hypothesized that sustaining energy dynamics within the aged hippocampus can boost memory storage by sustaining synaptic functioning and long term potentiation (LTP). Dexpramipexole (DEX) is the first-in-class compound able to sustain neuronal bioenergetics by interacting with mitochondrial F1Fo-ATP synthase. In the present study, for the first time we evaluated the effects of DEX on synaptic fatigue, LTP induction, learning and memory retention. We report that DEX improved LTP maintenance in CA1 neurons of acute hippocampal slices from aged but not young rats. However, we found no evidence that DEX counteracted two classic parameters of synaptic fatigue such as fEPSP reduction or the train area during the high frequency stimulation adopted to induce LTP. Interestingly, patch-clamp recordings in rat hippocampal neurons revealed that DEX dose-dependently inhibited (IC 814 nM) the I current, a rapidly-inactivating K current that negatively regulates neuronal excitability as well as cognition and memory processes. In keeping with this, DEX counteracted both scopolamine-induced spatial memory loss in rats challenged in Morris Water Maze test and memory retention in rats undergoing Novel Object Recognition. Overall, the present study discloses the ability of DEX to boost hippocampal synaptic plasticity, learning and memory. In light of the good safety profile of DEX in humans, our findings may have a realistic translational potential to treatment of cognitive disorders.
Neuropharmacology, 2018; 143
30244021: Iovino L, Mutolo D, Cinelli E, Contini M, Pantaleo T, Bongianni F
Breathing stimulation mediated by 5-HT and 5-HT receptors within the preBötzinger complex of the adult rabbit.
Serotonin (5-HT) has been reported to play excitatory effects on respiration by acting on preBötzinger complex (preBötC) neurons in neonatal or juvenile rodents. However, whether its action is circumscribed to the preBötC and present in other animal species, particularly in adult preparations, is unknown. We investigated the respiratory role of 5-HT within the preBötC and neighbouring respiration-related regions. Experiments were performed on α-chloralose-urethane anesthetized, vagotomized, paralyzed and artificially ventilated rabbits making use of bilateral microinjections (30-50 nl). 5-HT caused excitatory effects on respiratory activity only when applied to the preBötC. These effects were mediated by 5-HT and 5-HT receptors as shown by microinjections of specific agonists of the different types of 5-HT receptors. Unexpectedly, the blockade of 5-HT receptors by methysergide or the specific antagonist (S)-WAY 100135 induced excitatory respiratory effects. Microinjections of the 5-HT receptor antagonist ondansetron did not influence respiration, but prevented (S)-WAY 100135-induced responses. The blockade of GABA receptors by bicuculline within the preBötC prevented the effects of the 5-HT receptor agonist 8-OH-DPAT. The involvement of GABAergic inhibition and 5-HT receptor-mediated disinhibition is also corroborated by immunohistochemical data. The results show for the first time in an adult animal preparation that 5-HT plays a pivotal role in the modulation of the preBötC activity probably via both presynaptic and postsynaptic mechanisms and highlight the importance of disinhibition phenomena. Present findings may be relevant to some respiratory disorders in which

an impairment of central 5-HT mechanisms has been reported, such as sleep apnoea and sudden infant death syndrome.
Brain Res, 2019; 1704
32378271: Cinelli E, Mutolo D, Iovino L, Pantaleo T, Bongianni F
Key role of 5-HT receptors in the modulation of the neuronal network underlying the respiratory rhythm generation in lampreys.
In mammals, 5-HTexcitatory respiratory effects imply 5-HT receptor-mediated disinhibition of pre-Bötzinger complex neurons. In the lamprey, 5-HT receptors are involved in the neural control of locomotion, but their role in the respiratory regulation, particularly at the level of the putative respiratory rhythm generator, the paratrigeminal respiratory group (pTRG), is not known. We here investigate the respiratory function of inhibitory 5-HT receptors within the pTRG of the isolated brainstem of the adult lamprey. The 5-HT receptor agonists either bath applied or microinjected into the pTRG did not cause significant effects. However, the selective 5-HT receptor antagonist (S)-WAY 100135 bath applied or microinjected into the pTRG induced depressing respiratory effects or even apnoea, thus revealing that 5-HT exerts a 5-HT receptor-mediated potent tonic influence on respiration and contributes to maintain baseline levels of respiratory activity. Microinjections of strychnine or bicuculline, either alone or in combination, into the pTRG prevented (S)-WAY 100135-induced apnoea. In addition, immunohistochemical studies corroborate the present findings suggesting that 5-HT receptors are widely expressed in close apposition to the soma of glycine-immunoreactive cells located within the pTRG region. The results show that in the lamprey respiratory network, 5-HT exerts a tonic influence on respiration by a potent inhibitory control on both GABAergic and glycinergic mechanisms. The observed disinhibitory effects resemble the excitatory respiratory modulation exerted by 5-HT receptor-mediated inhibition of glycinergic and/or GABAergic neurons present in mammals, supporting the notion that some features of the neuronal network subserving respiratory rhythm generation are highly conserved throughout phylogeny.
Eur J Neurosci, 2020; 52
29369803: Cinelli E, Iovino L, Bongianni F, Pantaleo T, Mutolo D
Inhibitory modulation of the cough reflex by acetylcholine in the caudal nucleus tractus solitarii of the rabbit.
A cholinergic system has been described in the nucleus tractus solitarii (NTS). However, no information is available on the role played by acetylcholine (ACh) in the modulation of the cough reflex within the caudal NTS that has an important function in cough regulation. We addressed this issue making use of bilateral microinjections (30-50 nl) of 10 mM ACh combined with 5 mM physostigmine as well as of 10 mM mecamylamine or 10 mM scopolamine into the caudal NTS of pentobarbital sodium-anesthetized, spontaneously breathing rabbits. Microinjections of ACh/physostigmine caused depressant effects on the cough reflex induced by mechanical and chemical stimulation of the tracheobronchial tree. They also elicited transient increases in respiratory frequency and decreases in abdominal activity. These effects were prevented by scopolamine, but not by mecamylamine. The results show for the first time that ACh exerts an inhibitory modulation of the cough reflex through muscarinic receptors within the caudal NTS. They also may provide hints for novel antitussive approaches.
Respir Physiol Neurobiol, 2018; 257
27466072: Lana D, Iovino L, Nosi D, Wenk GL, Giovannini MG
The neuron-astrocyte-microglia triad involvement in neuroinflammaging mechanisms in the CA3 hippocampus of memory-impaired aged rats.
We examined the effects of inflammaging on memory encoding, and qualitative and quantitative modifications on proinflammatory proteins, apoptosis, neurodegeneration and morphological changes of neuron-astrocyte-microglia triads in CA3 Stratum Pyramidale (SP), Stratum Lucidum (SL) and Stratum Radiatum (SR) of young (3months) and aged rats (20months). Aged rats showed short-term memory impairments in the inhibitory avoidance task, increased expression of iNOS and activation of p38MAPK in SP, increase of apoptotic neurons in SP and of ectopic neurons in SL, and decrease of CA3 pyramidal neurons. The number of astrocytes and their branches length decreased in the three CA3 subregions of aged rats, with morphological signs of clasmatodendrosis. Total and activated microglia increased in the three CA3 subregions of aged rats. In aged rats CA3, astrocytes surrounded ectopic degenerating neurons forming "micro scars" around them. Astrocyte branches infiltrated the neuronal cell body, and, together with activated microglia formed "triads". In the triads, significantly more numerous in CA3 SL and SR of aged rats, astrocytes and microglia cooperated in fragmentation and phagocytosis of ectopic neurons. Inflammaging-induced modifications of astrocytes and microglia in CA3 of aged rats may help clearing neuronal debris derived from low-grade inflammation and apoptosis. These events might be common mechanisms underlying many neurodegenerative processes. The frequency to which they appear might depend upon, or might be the cause of, the burden and severity of neurodegeneration. Targeting the triads may represent a therapeutic strategy which may control inflammatory processes and spread of further cellular damage to neighboring cells.
Exp Gerontol, 2016; 83
27402692: Cinelli E, Iovino L, Bongianni F, Pantaleo T, Mutolo D
GABAA- and glycine-mediated inhibitory modulation of the cough reflex in the caudal nucleus tractus solitarii of the rabbit.
Cough-related sensory inputs from rapidly adapting receptors (RARs) and C fibers are processed by second-order neurons

mainly located in the caudal nucleus tractus solitarii (NTS). Both GABAA and glycine receptors have been proven to be involved in the inhibitory control of second-order cells receiving RAR projections. We investigated the role of these receptors within the caudal NTS in the modulation of the cough reflex induced by either mechanical or chemical stimulation of the tracheobronchial tree in pentobarbital sodium-anesthetized, spontaneously breathing rabbits. Bilateral microinjections (30-50 nl) of the receptor antagonists bicuculline and strychnine as well as of the receptor agonists muscimol and glycine were performed. Bicuculline (0.1 mM) and strychnine (1 mM) caused decreases in peak abdominal activity and marked increases in respiratory frequency due to decreases in both inspiratory time (Ti) and expiratory time (Te), without concomitant changes in arterial blood pressure. Noticeably, these microinjections induced potentiation of the cough reflex consisting of increases in the cough number associated with decreases either in cough-related Ti after bicuculline or in both cough-related Ti and Te after strychnine. The effects caused by muscimol (0.1 mM) and glycine (10 mM) were in the opposite direction to those produced by the corresponding antagonists. The results show that both GABAA and glycine receptors within the caudal NTS mediate a potent inhibitory modulation of the pattern of breathing and cough reflex responses. They strongly suggest that disinhibition is one important mechanism underlying cough regulation and possibly provide new hints for novel effective antitussive strategies.
Am J Physiol Lung Cell Mol Physiol, 2016; 311

32807662: Iovino L, Tremblay ME, Civiero L

Glutamate-induced excitotoxicity in Parkinson's disease: The role of glial cells.
Glutamate is the major excitatory neurotransmitter in the central nervous system. Glutamate transmission efficiency depends on the correct functionality and expression of a plethora of receptors and transporters, located both on neurons and glial cells. Of note, glutamate reuptake by dedicated transporters prevents its accumulation at the synapse as well as non-physiological spillover. Indeed, extracellular glutamate increase causes aberrant synaptic signaling leading to neuronal excitotoxicity and death. Moreover, extrasynaptic glutamate diffusion is strongly associated with glia reaction and neuroinflammation. Glutamate-induced excitotoxicity is mainly linked to an impaired ability of glial cells to reuptake and respond to glutamate, then this is considered a common hallmark in many neurodegenerative diseases, including Parkinson's disease (PD). In this review, we discuss the function of astrocytes and microglia in glutamate homeostasis, focusing on how glial dysfunction causes glutamate-induced excitotoxicity leading to neurodegeneration in PD.
J Pharmacol Sci, 2020; 144

28734063: Cinelli E, Iovino L, Mutolo D

ATP and astrocytes play a prominent role in the control of the respiratory pattern generator in the lamprey.
The paratrigeminal respiratory group (pTRG) is responsible for the respiratory pattern generation in the lamprey. The role of ATP and astrocytes, known to control respiratory activity in mammals, was investigated in the lamprey respiratory network. ATP microinjected into the pTRG induces a biphasic response consisting of marked increases in respiratory frequency mediated by P2X receptors followed by a decrease in the respiratory motor output due to the ATP metabolite adenosine. We provide evidence that astrocytes are involved in the genesis of the normal respiratory pattern, ATP-induced responses and acidification-induced increases of the respiratory activity. The function of astrocytes in rhythmic networks appears to be phylogenetically conserved.
J Physiol, 2017; 595

FENS Forum 2022
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BOARD NUMBER: S06-295

INTERPLAY BETWEEN LEWY PATHOLOGY AND MACROAUTOPHAGY IN MIDBRAIN DOPAMINERGIC NEURONS IN VIVO

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aims. The cardinal motor symptoms of Parkinson's disease (PD) arise from the degeneration of midbrain dopaminergic neurons (DANs) of the substantia nigra pars compacta (SNc). This degenerative process correlates with the presence of Lewy pathology (LP). DANs of the ventral tegmental area (VTA) are less affected in PD. Here, we aim to elucidate the relationship between LP and macroautophagy, a major degradative pathway that should oppose the emergence of LP, in vulnerable and resistant DANs *in vivo*. **Methods.** To characterise the macroautophagic machinery engaged in DANs in response to LP, and investigate the importance of macroautophagy in influencing LP, we compared phenotypes of wildtype and macroautophagy-deficient mice. We induced the development of LP in either SNc or VTA DANs using intracranial injections of Pre-Formed Fibrils (PFFs) of α-synuclein. 1-3 months after PFF injections, we used immunofluorescence, high-resolution microscopy and CellProfiler analyses to localise and quantify LP and macroautophagy-related molecules in DANs. **Results**. Our data in WT mice showed that LAMP1+ puncta cluster at Lewy-like aggregates in DANs, and that clustered puncta are larger than those not associated with aggregates. Both of these phenomena were more pronounced in SNc DANs than VTA DANs. In macroautophagy-deficient mice, LAMP1+ puncta did not cluster at Lewy-like aggregates and were not enlarged. **Conclusions.** Observations in wildtype mice suggest that the induction of LP perturbs macroautophagic machinery in DANs *in vivo*. Comparative observations in macroautophagy-deficient mice suggest that macroautophagy is important for DANs to handle LP and its sequalae.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-296

ROLE OF TGF-BETA SIGNALLING PATHWAY AGAINST ALPHA SYNUCLEIN-INDUCED TOXICITY IN A PARKINSON'S DISEASE CELL MODEL

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is histopathologically defined by the presence of intracellular proteinaceous inclusions known as Lewy bodies (LB). Alpha-synclein (aSyn) has been identified as the main component of LBs. It is generally believed that small intermediates between monomers and large aggregates of aSyn found in LBs are toxic and can lead to the death of dopaminergic neurons in the substantia nigra, resulting in the motor symptoms present in PD. Our group established a cell culture model in which post-mitotic human dopaminergic neurons die in the course of six days upon moderate overexpression of wild-type aSyn. Based on this model, we performed a genome-wide siRNA screening in order to identify genes, which upon their knockdown, can reduce aSyn-induced cytotoxicity. Ultimately, 12 top-hits were identified, whose knockdown protected against aSyn-induced cytotoxicity. 4 of these genes were associated with TGF-beta signalling pathway. Hence, we hypothesized that the knock-down/reduction of the TGF-beta pathway could reduce aSyn-induced cytotoxicity. We aimed at investigating the genes involved in TGF-beta signalling and the mechanism of protectiveness. First, the knockdown efficacy and protectiveness were both further confirmed. Next, we separately knockdown different branches of the pathway to verify if the knockdown/reduction of TGF-beta pathway protected against aSyn-induced cytotoxicity. Our results suggest that the knockdown of TGF-beta pathway can indeed rescue dopaminergic neurons from aSyn-induced toxicity and such effect is aSyn-specific. Altogether, we have identified a potential therapeutic role of TGF-beta signalling pathway against aSyn-induced toxicity. These findings can provide insights that can be used for the development of novel strategies and drugs against synucleinopathies.

Pubmed:

27080132: Chua OW, Wong KK, Ko BC, Chung SK, Chow BK, Lee LT
Role of nuclear factor of activated T-cells 5 in regulating hypertonic-mediated secretin receptor expression in kidney collecting duct cells.
A growing body of evidence suggests that secretin (SCT) is an important element in the osmoregulatory pathway. It is interesting to note that both SCT and its receptor (SCTR) gene are activated upon hyperosmolality in the kidney. However, the precise molecular mechanisms underlying the induction of the SCTR gene expression in response to changes in osmolality have yet to be clarified. Detailed DNA sequence analysis of the promoter regions of the SCTR gene reveals the presence of multiple osmotic response elements (ORE). The ORE is the binding site of a key osmosensitive transactivator, namely, the nuclear factor of activated T-cells 5 (NFAT5). SCTR and NFAT5 are co-expressed in the kidney cortex and medulla collecting duct cells. We therefore hypothesize that NFAT5 is responsible for modulating SCTR expression in hypertonic environments. In this study, we found hypertonicity stimulates the promoter activities and endogenous gene expression of SCTR in mouse kidney cortex collecting duct cells (M1) and inner medulla collecting duct cells (mIMCD3). The overexpression and silencing of NFAT5 further confirmed it to be responsible for the up-regulation of the SCTR gene under hypertonic conditions. A significant increase in the interaction between NFAT5 and the SCTR promoter was also observed following chromatin immunoprecipitation assay. In vivo, osmotic stress up-regulates the SCTR gene in the kidney cortex and medulla of wild-type mice, but does not do so in NFAT5(+/-) animals. Hence, this study provides comprehensive information on how NFAT5 regulates SCTR expression in different osmotic environments.
Biochim Biophys Acta, 2016; 1859
33006159: Han L, Wu KL, Kwan PY, Chua OW, Shum DK, Chan YS
5-HT receptor-mediated attenuation of synaptic transmission in rat medial vestibular nucleus impacts on vestibular-related motor function.
Chemogenetic activation of medial vestibular nucleus-projecting 5-HT neurons resulted in deficits in vestibular-mediated tasks, including negative geotaxis, balance beam and rota-rod tests. The 5-HT receptor mediates the vestibular-related behavioural effects of 5-HT in the vestibular nucleus. 5-HT receptor activation attenuated evoked excitatory postsynaptic

currents and evoked inhibitory postsynaptic currents via a presynaptic mechanism in the vestibular nucleus.
J Physiol, 2021; 599

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BOARD NUMBER: S06-297

NEUROPROTECTIVE EFFECTS OF DHEA(S) AND BDNF IN AN IN VITRO MODEL OF PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Tina Milos[1], Dubravka Svob Strac[2], Matea Nikolac Perkovic[1], Lucija Tudor[2], Gordana Nedic Erjavec[2], Barbara Vuić[2], Marcela Konjevod[2], Nela Pivac[1]
[1]Ruder Boskovic Institute, Division Of Molecular Medicine, Zagreb, Croatia, [2]Ruđer Bošković Institute, Divison Of Molecular Medicine, Zagreb, Croatia

Parkinson's disease (PD) is a neurodegenerative disease characterized by a selective loss of dopaminergic (DA) neurons and accumulation of α-synuclein in the substantia nigra. It is considered that gene mutations, environmental factors and aging are causative factors of PD. Understanding the mechanisms involved in neurodegeneration is the key to identifying potential therapeutic strategies for PD. Neurotoxins, such as rotenone and 6-hydroxidopamine are widely used to induce *in vivo* and *in vitro* model of PD. These neurotoxins induce mitochondrial dysfunction and increase oxidative stress, which leads to neuronal degeneration and cell death by apoptosis. Neurosteroids, dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), are the most abundant steroid hormones and can be synthesized *de novo* in the brain. Levels of DHEA(S) decrease with age and play an important role in brain aging and neurodegeneration. Neurotrophin, brain-derived neurotrophic factor (BDNF) is also involved in neuroprotection and neuroregeneration, while decreased levels of BDNF were observed in the aging process and in neurodegenerative diseases such as PD. In this study we have investigated potential neuroprotective effects of DHEA(S) and BDNF in four different cell models: primary mouse neurons, SH-SY5Y neuroblastoma cells, PC12 and N27 cells. These cells were injured with rotenone or 6-hydroxidopamine to induce an *in vitro* model of PD. In addition, cells were treated with DHEA(S) and BDNF separately and combined to investigate their interactions and neuroprotective potential. The obtained results suggest that DHEA(S) and BDNF may play important role in prevention and treatment of PD.

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INVESTIGATING THE FUNCTION AND THE POTENTIAL NEUROPROTECTIVE ROLE OF NATO3 IN DOPAMINERGIC NEURONS.

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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University of Geneva, Genetics And Evolution, Geneva, Switzerland

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder. PD manifests progressive loss of dopaminergic (DA) neurons in the substantia nigra (SN) pars compacta in the midbrain, leading to a wide array of motor and non-motor disabilities.The high prevalence of the disease and the limited therapeutic options make the discovery of novel targets for PD treatments an urgent need. Accumulating evidence shows that many developmental transcription factors that remain expressed throughout life are implicated in DA neurodegeneration. *Nato3* (*Ferd3l*) encodes a transcription factor required for midbrain DA neuron development and continues to be expressed during adulthood; however, its role in adulthood remains poorly understood. To investigate the post-developmental role of *Nato3* in midbrain DA neurons, we generated a mouse strain that conditionally ablates *Nato3* in differentiated DA neurons (*Nato3* cKO). Employing immunohistochemistry, electron microscopy, and behavioural assays, we found that *Nato3* cKO mice recapitulate two hallmarks of PD: progressive locomotor decline and mitochondrial pathology. These results indicate the critical roles of *Nato3* in the lifelong maintenance of mitochondrial integrity and functioning of midbrain DA neurons. Furthermore, using a combination of RNA-seq analysis and *in vitro* studies in midbrain DA neurons differentiated from induced pluripotent stem cells (iPSCs) derived from PD patients, we are exploring the molecular mechanisms underlying the role of *Nato3* and its neuroprotective potential. Overall, our investigations contribute to identify the transcriptional programmes that maintain DA neurons in the adult brain and will help better understand DA neurodegeneration and how to prevent it.

Pubmed:

33482917: Adhya D, Chennell G, Crowe JA, Valencia-Alarcón EP, Seyforth J, Hosny NA, Yasvoina MV, Forster R, Baron-Cohen S, Vernon AC, Srivastava DP
Application of Airy beam light sheet microscopy to examine early neurodevelopmental structures in 3D hiPSC-derived human cortical spheroids.
The inability to observe relevant biological processes in vivo significantly restricts human neurodevelopmental research. Advances in appropriate in vitro model systems, including patient-specific human brain organoids and human cortical spheroids (hCSs), offer a pragmatic solution to this issue. In particular, hCSs are an accessible method for generating homogenous organoids of dorsal telencephalic fate, which recapitulate key aspects of human corticogenesis, including the formation of neural rosettes-in vitro correlates of the neural tube. These neurogenic niches give rise to neural progenitors that subsequently differentiate into neurons. Studies differentiating induced pluripotent stem cells (hiPSCs) in 2D have linked atypical formation of neural rosettes with neurodevelopmental disorders such as autism spectrum conditions. Thus far, however, conventional methods of tissue preparation in this field limit the ability to image these structures in three-dimensions within intact hCS or other 3D preparations. To overcome this limitation, we have sought to optimise a methodological approach to process hCSs to maximise the utility of a novel Airy-beam light sheet microscope (ALSM) to acquire high resolution volumetric images of internal structures within hCS representative of early developmental time points.
Mol Autism, 2021; 12
32826066: Adhya D, Swarup V, Nagy R, Dutan L, Shum C, Valencia-Alarcón EP, Jozwik KM, Mendez MA, Horder J, Loth E, Nowosiad P, Lee I, Skuse D, Flinter FA, Murphy D, McAlonan G, Geschwind DH, Price J, Carroll J, Srivastava DP, Baron-Cohen S
Atypical Neurogenesis in Induced Pluripotent Stem Cells From Autistic Individuals.
Autism is a heterogeneous collection of disorders with a complex molecular underpinning. Evidence from postmortem brain studies have indicated that early prenatal development may be altered in autism. Induced pluripotent stem cells (iPSCs) generated from individuals with autism with macrocephaly also indicate prenatal development as a critical period for this condition. But little is known about early altered cellular events during prenatal stages in autism.
Biol Psychiatry, 2021; 89


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INSIGHT INTO AN EARLY-ONSET PARKINSON'S DISEASE MUTATION: IMPACT IN ADENOSINE_ A1-A2A RECEPTOR HETEROMERIZATION_

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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[1]University of Barcelona, Department Of Pathology And Experimental Therapeutics, L'Hospitalet de Llobregat, Spain, [2]Institut d'Investigació Biomèdica de Bellvitge (IDIBELL)), Neuropharmacology And Pain Group, L'Hospitalet de Llobregat, Spain, [3]University of Barcelona, Neuroscience Institute, Barcelona, Spain, [4]Universidad Autònoma de Barcelona, Laboratory Of Computational Medicine, Biostatistics Unit, Barcelona, Spain, [5]National Institute of Health, Intramural Research Program, Baltimore, United States of America

Adenosine is an endogenous autacoid purine nucleoside involved in several physiological functions. In the brain, it modulates neurotransmission through inhibitory adenosine A_1 receptors (A_1Rs) and stimulatory A_{2A} receptors (A_{2A}Rs). These G protein-coupled ARs are involved in motor function and related to neurodegenerative diseases such as Parkinson's disease (PD). In line with this, a recent study associated a new autosomal recessive mutation (G279S) within the A_1R gene to the development of early onset PD. Here, we aimed at investigating the impact of this mutation on receptors' structure and function. Our results revealed that the G279S A_1R mutation does not alter receptor's ligand binding, constitutive activity or coupling to transducer proteins (i.e., $G_{\alpha i}$ and $G_{\alpha q}$) in transfected cells. However, G279S mutation reduced A_1R-A_{2A}R heteromer formation and abolished the heteromer-dependent ligand-independent modulation that A_1R exerts over the constitutive and agonist-induced activation of the A_{2A}R. Interestingly, computational studies supported that the G279S A_1R mutation could have a negative effect on the heterodimer interface stability. Overall, our results indicate that G279S mutation does not modify A_1R canonical signalling, whereas it reduces the ability of A_1R to act as a negative allosteric modulator of A_{2A}R function

Pubmed:
30897724: Arana L, Bayón-Cordero L, Sarasola LI, Berasategi M, Ruiz S, Alkorta I
Solid Lipid Nanoparticles Surface Modification Modulates Cell Internalization and Improves Chemotoxic Treatment in an Oral Carcinoma Cell Line.
Solid lipid nanoparticles (SLN) present low toxicity, versatility to incorporate both lipophilic and hydrophilic drugs, controlled drug release and they are easy to scale-up. It is well known that the endocytosis pathway by which SLN are taken up and the subsequent subcellular distribution are crucial for the biological effect of the incorporated drug. In addition, interactions between SLN and cells depend on many factors, such as, the composition of nanoparticle surface. In this work different amounts of phosphatidylethanolamine polyethylene glycol (PE^-PEG) were added to SLN composed of stearic acid, Epikuron 200 and sodium taurodeoxycholate. Characterization of obtained nanoparticle suspensions were performed by the analysis of particle size, polydispersity index, ζ-potential, cell toxicity and cell internalization pathway. We have observed that the presence of PE^-PEG improves active cell internalization of the nanoparticles in an oral adenocarcinoma cell line, reducing non-specific internalization mechanisms. Finally, we have tested the effect of surface coating on the efficiency of incorporated drugs using all-trans retinoic acid as a model drug. We have observed that delivery of this drug into PE^-PEG coated SLN increases its chemotoxic effect compared to non-coated SLN. Therefore, it can be concluded that surface modification with PE^-PEG improves the efficiency and the specificity of the SLN-loaded drug.
Nanomaterials (Basel), 2019; 9

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RABPHILIN-3A AS NOVEL TARGET TO RESCUE ALPHA-SYNUCLEIN INDUCED SYNAPTIC LOSS IN PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Toxic aggregates of the protein alpha-synuclein (αsyn) are considered a key driver of Parkinson's disease (PD) pathology. αsyn mediates synaptic dysfunction at early phases of PD through several mechanisms. αsyn modulates NMDA-type glutamate receptors' (NMDARs) surface levels and activity at the postsynaptic compartment of corticostriatal synapses and our group previously showed that NMDARs retention at postsynaptic sites is strictly correlated with the binding to Rph3A. Moreover, a direct αsyn/Rph3A interaction has been put forward. **Aims**. This work aims at characterizing Rph3A/αsyn interaction and its role in early PD to identify pharmacological approaches able to block αsyn-induced synaptic toxicity. **Methods**. Imaging, biochemical and *in silico* approaches were exploited to investigate Rph3A/αsyn interaction and its role in PD. Experiments were mainly performed using an *in vivo* mouse model of αsyn-induced PD based on striatal injections of αsyn preformed fibrils (PFF) to perform molecular, morphological and motor behavioral evaluation of the early disease phases. **Results**. Firstly, we showed that Rph3A is a novel αsyn interactor at synaptic sites. Then, using the *in vivo* PD model we found that αsyn-PFF in the striatum impaired Rph3A postsynaptic levels, its binding to NMDARs and the overall molecular composition of the postsynaptic compartment. Besides, αsyn-mice showed striatal dendritic spine loss and motor alterations. Interestingly, modulation of Rph3A in the striatum was able to prevent dendritic spine loss and motor defects in αsyn-PFF mice. **Conclusions**. Results indicate that approaches aimed at restoring Rph3A striatal functions represent a strategy to slow-down early detrimental effects of αsyn in PD.

Pubmed:
34297092: Tozzi A, Sciaccaluga M, Loffredo V, Megaro A, Ledonne A, Cardinale A, Federici M, Bellingacci L, Paciotti S, Ferrari E, La Rocca A, Martini A, Mercuri NB, Gardoni F, Picconi B, Ghiglieri V, De Leonibus E, Calabresi P
Dopamine-dependent early synaptic and motor dysfunctions induced by α-synuclein in the nigrostriatal circuit.
Misfolding and aggregation of α-synuclein are specific features of Parkinson's disease and other neurodegenerative diseases defined as synucleinopathies. Parkinson's disease progression has been correlated with the formation and extracellular release of α-synuclein aggregates, as well as with their spread from neuron to neuron. Therapeutic interventions in the initial stages of Parkinson's disease require a clear understanding of the mechanisms by which α-synuclein disrupts the physiological synaptic and plastic activity of the basal ganglia. For this reason, we identified two early time points to clarify how the intrastriatal injection of α-synuclein-preformed fibrils in rodents via retrograde transmission induces time-dependent electrophysiological and behavioural alterations. We found that intrastriatal α-synuclein-preformed fibrils perturb the firing rate of dopaminergic neurons in the substantia nigra pars compacta, while the discharge of putative GABAergic cells of the substantia nigra pars reticulata is unchanged. The α-synuclein-induced dysregulation of nigrostriatal function also impairs, in a time-dependent manner, the two main forms of striatal synaptic plasticity, long-term potentiation and long-term depression. We also observed an increased glutamatergic transmission measured as an augmented frequency of spontaneous excitatory synaptic currents. These changes in neuronal function in the substantia nigra pars compacta and striatum were observed before overt neuronal death occurred. In an additional set of experiments, we were able to rescue α-synuclein-induced alterations of motor function, striatal synaptic plasticity and increased spontaneous excitatory synaptic currents by subchronic treatment with l-DOPA, a precursor of dopamine widely used in the therapy of Parkinson's disease, clearly demonstrating that a dysfunctional dopamine system plays a critical role in the early phases of the disease.
Brain, 2021; 144
32311374: Ferrari E, Cardinale A, Picconi B, Gardoni F
From cell lines to pluripotent stem cells for modelling Parkinson's Disease.
Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by loss of dopaminergic (DAergic) neurons in the substantia nigra (SN) that contributes to the main motor symptoms of the disease. At present, even

if several advancements have been done in the last decades, the molecular and cellular mechanisms involved in the pathogenesis are far to be fully understood. Accordingly, the establishment of reliable in vitro experimental models to investigate the early events of the pathogenesis represents a key issue in the field. However, to mimic and reproduce in vitro the complex neuronal circuitry involved in PD-associated degeneration of DAergic neurons still remains a highly challenging issue. Here we will review the in vitro PD models used in the last 25 years of research, ranging from cell lines, primary rat or mice neuronal cultures to the more recent use of human induced pluripotent stem cells (hiPSCs) and, finally, the development of 3D midbrain organoids.
J Neurosci Methods, 2020; 340
32891865: Price R, Ferrari E, Gardoni F, Mercuri NB, Ledonne A
Protease-activated receptor 1 (PAR1) inhibits synaptic NMDARs in mouse nigral dopaminergic neurons.
Protease-activated receptor 1 (PAR1) is a G protein-coupled receptor (GPCR), whose activation requires a proteolytic cleavage in the extracellular domain exposing a tethered ligand, which binds to the same receptor thus stimulating Gα-, Gα- and Gα proteins. PAR1, activated by serine proteases and matrix metalloproteases, plays multifaceted roles in neuroinflammation and neurodegeneration, in stroke, brain trauma, Alzheimer's diseases, and Parkinson's disease (PD). Substantia nigra pars compacta (SNpc) is among areas with highest PAR1 expression, but current evidence on its roles herein is restricted to mechanisms controlling dopaminergic (DAergic) neurons survival, with controversial data showing PAR1 either fostering or counteracting degeneration in PD models. Since PAR1 functions on SNpc DAergic neurons activity are unknown, we investigated if PAR1 affects glutamatergic transmission in this neuronal population. We analyzed PAR1's effects on NMDARs and AMPARs by patch-clamp recordings from DAergic neurons from mouse midbrain slices. Then, we explored subunit composition of PAR1-sensitive NMDARs, with selective antagonists, and mechanisms underlying PAR1-induced NMDARs modulation, by quantifying NMDARs surface expression. PAR1 activation inhibits synaptic NMDARs in SNpc DAergic neurons, without affecting AMPARs. PAR1-sensitive NMDARs contain GluN2B/GluN2D subunits. Moreover, PAR1-mediated NMDARs hypofunction is reliant on NMDARs internalization, as PAR1 stimulation increases NMDARs intracellular levels and pharmacological limitation of NMDARs endocytosis prevents PAR1-induced NMDARs inhibition. We reveal that PAR1 regulates glutamatergic transmission in midbrain DAergic cells. This might have implications in brain's DA-dependent functions and in neurological/psychiatric diseases linked to DAergic dysfunctions.
Pharmacol Res, 2020; 160
34743951: Italia M, Ferrari E, Di Luca M, Gardoni F
GluA3-containing AMPA receptors: From physiology to synaptic dysfunction in brain disorders.
In the mammalian brain, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (AMPARs) play a fundamental role in the activation of excitatory synaptic transmission and the induction of different forms of synaptic plasticity. The modulation of the AMPAR tetramer subunit composition at synapses defines the functional properties of the receptor. During the last twenty years, several studies have evaluated the roles played by each subunit, from GluA1 to GluA4, in both physiological and pathological conditions. Here, we have focused our attention on GluA3-containing AMPARs, addressing their functional role in synaptic transmission and synaptic plasticity and their involvement in a variety of brain disorders. Although several aspects remain to be fully understood, GluA3 is a widely expressed and functionally relevant subunit in AMPARs involved in several brain circuits, and its pharmacological modulation could represent a novel approach for the rescue of altered glutamatergic synapses associated with neurodegenerative and neurodevelopmental disorders.
Neurobiol Dis, 2021; 161

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NEUROTOXIC EFFECTS OF BETA-SYNUCLEIN AND ITS MUTANTS, P123H AND V70M

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Universitätsmedizin Göttingen, Neurology, Göttingen, Germany

The synuclein protein family is composed of three proteins, alpha-, beta- and gamma-Synuclein (αSyn, βSyn and γSyn). Whereas much work has been put on studying αSyn (due to its apparent toxic role in Synucleinopathies), less attention has been paid to βSyn. In fact, two mutations of this protein, V70M and P123H, have been linked to Dementia with Lewy bodies. Recently, our group showed that these βSyn mutants lead to neurotoxic effects comparable to αSyn's at the level of the mitochondria (V70M) and the synapse (P123H) when overexpressed. In order to understand the mechanisms by which these mutations exert such effects, we study the differential affinity of these proteins for biological membranes. Using techniques such as TEM or DLS, we want to understand how this interaction is regulated and the downstream consequences that these affinities may have on the functioning and trafficking of certain cellular organelles.

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BOARD NUMBER: S06-302

AN ALL IPSC-DERIVED CORTICO-STRIATO-NIGRAL MINICIRCUIT MODELLING PARKINSON'S DISEASE REVEALED ELECTROPHYSIOLOGICAL CHANGES IN MEDIUM SPINY NEURONS COCULTURED WITH DOPAMINERGIC NEURONS CARRYING GBA N370S MUTATION.

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aims: Dysfunction of medium spiny neurons (MSNs) are implicated in motor impairments in Parkinson's Disease (PD). However, most research on MSN pathology in PD has relied on animal models of toxin-induced PD which fail to recapitulate the human nature, genetics and temporal aspects of PD pathogenesis. By developing an all induced pluripotent stem cells (iPSCs)-derived cortico-striato-nigral mini-circuit, we recapitulate the complex connectivity received by MSNs and examine temporal changes of human striatal electrophysiology due to DaNs habouring PD-relevant mutations. Methods: Healthy control iPSC-derived cortical neurons and MSNs, together with dopaminergic neurons (DaNs) derived from either healthy controls or patients carrying GBA N370S mutation were sequentially seeded onto custom 3-chamber microfluidics. Connectivity of different neuronal populations was validated with immunocytochemistry and pairwise chemical stimulation and optical recording. We then performed whole-cell patch clamp with post-hoc labelling to investigate electrophysiological properties of MSNs cocultured with either healthy or GBA N370S DaNs. Results: MSNs cocultured with GBA N370S DaNs exhibited significantly increased intrinsic excitability and reduced current densities of both sodium and potassium conductance. Such changes were observed in 40-45 days *in vitro* (div) MSNs but absent in aged MSNs of up to 75div. Preliminary data suggested alterations in channel-specific kinetics, including recovery time from inactivation and inactivation properties, in aged MSNs. Conclusions: Our results highlight the utility of modelling neurons in a highly physiological manner preserving their endogenous connectivity. They also suggest early electrophysiological dysfunctions in human MSNs induced by surviving GBA N370S DaNs, providing insights into early pathology of MSNs in PD.

Pubmed:

28686207: Song C, Do QB, Antic SD, Knöpfel T
Transgenic Strategies for Sparse but Strong Expression of Genetically Encoded Voltage and Calcium Indicators.
Rapidly progressing development of optogenetic tools, particularly genetically encoded optical indicators, enables monitoring activities of neuronal circuits of identified cell populations in longitudinal in vivo studies. Recently developed advanced transgenic approaches achieve high levels of indicator expression. However, targeting non-sparse cell populations leads to dense expression patterns such that optical signals from neuronal processes cannot be allocated to individual neurons. This issue is particularly pertinent for the use of genetically encoded voltage indicators whose membrane-delimited signals arise largely from the neuropil where dendritic and axonal membranes of many cells intermingle. Here we address this need for sparse but strong expression of genetically encoded optical indicators using a titratable recombination-activated transgene transcription to achieve a Golgi staining-type indicator expression pattern in vivo. Using different transgenic strategies, we also illustrate that co-expression of genetically encoded voltage and calcium indicators can be achieved in vivo for studying neuronal circuit input-output relationships.
Int J Mol Sci, 2017; 18

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BOARD NUMBER: S06-303

EXTRACELLULAR CALCIUM RELEASE MEDIATES POLARIZED MOTILITY AND DISPLACEMENT OF MICROGLIAL CELLS IN A SCENARIO OF PARKINSONIAN NEURODEGENERATION

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Meritxell Roig-Martínez, Paola Casanova, Elena Saavedra, Irina Freitag, Paula Martínez, Roser Masgrau, Carlos Barcia
Institut de Neurociències UAB, Department Of Biochemistry, Barcelona, Spain

Parkinson's disease (PD) is a neurodegenerative disorder in which the loss of dopaminergic neurons, specifically in the Substantia Nigra, causes characteristic motor symptoms and deterioration. The cause of this neuronal loss is still unknown but increasing evidences point to microglial-mediated neuroinflammation as a contributing factor. We propose that dying neurons release Ca^{2+} to the extracellular space that may contribute to the neuroimmune response, particularly provoking changes in microglial cells, which may signal their polarization and motility. Our *in vitro* experiments revealed that microglia internalize and bind extracellular calcium – potentially binding the specific molecule Iba-1 – modifying their shape, and increasing motility. Then, experiments performed *in vivo* showed similar changes. We administered Ca^{2+} to the brain parenchyma to determine the changes in microglial dynamics. We observed slight morphological changes of microglial cells in Ca^{2+}-injected animals. However, when we measured the polarization of microglial cells branches with 3D high-resolution confocal imaging, we found clear differences when compared to saline injections. Knowing that Iba-1 is a Ca^{2+} binding protein specific for microglia and macrophages, we quantified Iba-1 expression after Ca^{2+} administration, and we observed a clear increase that correlates with morphological changes. These results indicate that Iba-1 may be a crucial factor leading these polarizing changes occurred when microglia sense Ca^{2+} changes in their surroundings. These evidences suggest a novel interplay between dying dopaminergic neurons and neighboring microglial cells in which specific Ca^{2+}-dependent responses may contribute to the specific inflammatory response in areas of parkinsonian neurodegeneration.

Pubmed:
31435797: Roig-Martinez M, Saavedra-Lopez E, Casanova PV, Cribaro GP, Barcia C
The MTOC/Golgi Complex at the T-Cell Immunological Synapse.
T cells effectively explore the tissue in search for antigens. When activated, they dedicate a big amount of energy and resources to arrange a complex structure called immunological synapse (IS), containing a particular distribution of molecules defined as supramolecular activation clusters (SMACs), and become polarized toward the target cell in a manner that channels the information specifically. This arrangement is symmetrical and requires the polarization of the MTOC and the Golgi to be operational, especially for the proper delivery of lytic granules and the recycling of molecules three dimensionally segregated at the clustered interface. Alternatively, after the productive encounter, T cells need to rearrange again to newly navigate through the tissue, changing back to a motile state called immunological kinapse (IK). In this IK state, the MTOC and the Golgi apparatus are repositioned and recruited at the back of the T cell to facilitate motility, while the established symmetry of the elements of the SMACs is broken and distributed in a different pattern. Both states, IS and IK, are interchangeable and are mainly orchestrated by the MTOC/Golgi complex, being critical for an effective immune response. Results Probl Cell Differ, 2019; 67
33579378: Cribaro GP, Saavedra-López E, Romarate L, Mitxitorena I, Díaz LR, Casanova PV, Roig-Martínez M, Gallego JM, Perez-Vallés A, Barcia C
Three-dimensional vascular microenvironment landscape in human glioblastoma.
The cellular complexity of glioblastoma microenvironments is still poorly understood. In-depth, cell-resolution tissue analyses of human material are rare but highly necessary to understand the biology of this deadly tumor. Here we present a unique 3D visualization revealing the cellular composition of human GBM in detail and considering its critical association with the neo-vascular niche. Our images show a complex vascular map of human 3D biopsies with increased vascular heterogeneity and altered spatial relationship with astrocytes or glioma-cell counterparts. High-resolution analysis of the structural layers of the blood brain barrier showed a multilayered fenestration of endothelium and basement membrane. Careful examination of T cell position and migration relative to vascular walls revealed increased infiltration corresponding with tumor proliferation. In addition, the analysis of the myeloid landscape not only showed a volumetric increase in glioma-associated microglia and macrophages relative to GBM proliferation but also revealed distinct phenotypes in tumor nest and stroma. Images and data

sets are available on demand as a resource for public access.
Acta Neuropathol Commun, 2021; 9
32954312: Saavedra-López E, Roig-Martínez M, Cribaro GP, Casanova PV, Gallego JM, Pérez-Vallés A, Barcia C
Phagocytic glioblastoma-associated microglia and macrophages populate invading pseudopalisades.
Hypoxic pseudopalisades are a pathological hallmark of human glioblastoma, which is linked to tumour malignancy and aggressiveness. Yet, their function and role in the tumour development have scarcely been explored. It is thought that pseudopalisades are formed by malignant cells escaping from the hypoxic environment, although evidence of the immune component of pseudopalisades has been elusive. In the present work, we analyse the immunological constituent of hypoxic pseudopalisades using high-resolution three-dimensional confocal imaging in tissue blocks from excised tumours of glioblastoma patients and mimic the hypoxic gradient in microfluidic platforms to understand the cellular motility. We visualize that glioblastoma-associated microglia and macrophages abundantly populate pseudopalisades, displaying an elongated kinetic morphology across the pseudopalisades, and are oriented towards the necrotic focus. experiments demonstrate that under hypoxic gradient, microglia show a particular motile behaviour characterized by the increase of cellular persistence in contrast with glioma cells. Importantly, we show that glioblastoma-associated microglia and macrophages utilize fibres of glioma cells as a haptotactic cue to navigate along the anisotropic structure of the pseudopalisades and display a high phagocytic activity at the necrotic border of the pseudopalisades. In this study, we demonstrate that glioblastoma-associated microglia and macrophages are the main immune cells of pseudopalisades in glioblastoma, travelling to necrotic areas to clear the resulting components of the prothrombotic milieu, suggesting that the scavenging features of glioblastoma-associated microglia and macrophages at the pseudopalisades serve as an essential counterpart for glioma cell invasion.
Brain Commun, 2020; 2
34771741: Martínez-Escardó L, Alemany M, Sánchez-Osuna M, Sánchez-Chardi A, Roig-Martínez M, Suárez-García S, Ruiz-Molina D, Vidal N, Plans G, Majós C, Ribas J, Baltrons MA, Bayascas JR, Barcia C, Bruna J, Yuste VJ
Gossypol Treatment Restores Insufficient Apoptotic Function of DFF40/CAD in Human Glioblastoma Cells.
Glioblastoma (GBM) is a highly aggressive brain tumor and almost all patients die because of relapses. GBM-derived cells undergo cell death without nuclear fragmentation upon treatment with different apoptotic agents. Nuclear dismantling determines the point-of-no-return in the apoptotic process. DFF40/CAD is the main endonuclease implicated in apoptotic nuclear disassembly. To be properly activated, DFF40/CAD should reside in the cytosol. However, the endonuclease is poorly expressed in the cytosol and remains cumulated in the nucleus of GBM cells. Here, by employing commercial and non-commercial patient-derived GBM cells, we demonstrate that the natural terpenoid aldehyde gossypol prompts DFF40/CAD-dependent nuclear fragmentation. A comparative analysis between gossypol- and staurosporine-treated cells evidenced that levels of neither caspase activation nor DNA damage were correlated with the ability of each compound to induce nuclear fragmentation. Deconvoluted confocal images revealed that DFF40/CAD was almost completely excluded from the nucleus early after the staurosporine challenge. However, gossypol-treated cells maintained DFF40/CAD in the nucleus for longer times, shaping a ribbon-like structure piercing the nuclear fragments and building a network of bridged masses of compacted chromatin. Therefore, GBM cells can fragment their nuclei if treated with the adequate insult, making the cell death process irreversible.
Cancers (Basel), 2021; 13

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BOARD NUMBER: S06-304

GPR37 N-TERMINAL DOMAIN PROCESSING DEFINES AUTAPTIC RECEPTOR SIGNALLING.

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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GPR37 is an orphan G protein-coupled receptor (GPCR) which has gained attention due to its implication in the pathogenesis of Parkinson's disease (PD). Interestingly, the N-terminus of the receptor (i.e., ecto-GPR37) is subject to metalloproteinase-mediated proteolysis, which leads to several receptor forms at the cell surface. In addition, PD patients have increased amounts of ecto-GPR37 in the cerebrospinal fluid, thus showing an increased expression of GPR37 cleaved isoforms in the brain. Here, we aimed at investigating the impact of ecto-GPR37 on receptor's function and cell viability. To this end, we generated five GPR37 N-terminally truncated constructs ($GPR37^{\Delta 1-54}$, $GPR37^{\Delta 1-171}$, $GPR37^{\Delta 1-199}$, $GPR37^{\Delta 1-219}$, $GPR37^{\Delta 1-247}$) based on the receptor isoforms found in human PD brain samples. Subsequently, we assessed their coupling to different transducers, namely GRK-2, β-Arrestin 2 and G proteins, in HEK293T cells. Thus, GPR37 full length, $GPR37^{\Delta 1-54}$, $GPR37^{\Delta 1-171}$ and $GPR37^{\Delta 1-199}$ showed a robust coupling to GRK-2 and β-Arrestin 2. Also, these truncated forms activate serum response element (SRE)-related signalling pathways which are downstream to β-Arrestin 2. Interestingly, this signalling cascade is attenuated when GPR37 is truncated beyond aminoacid 219. In addition, the impact of GPR37 N-terminally truncated forms expression in SH-SY5Y cells viability was assessed upon 6-hydroxydopamine (6-OHDA) challenge. Thus, the neurotoxic effects of 6-OHDA in SH-SY5Y cells is modulated by the GPR37 N-terminal truncation. Overall, these results provide evidence that ecto-GPR37 may play a key role controlling receptor's constitutive activity and regulating cell viability, which may be relevant to understand its relationship with PD pathogenesis.

Pubmed:
34042202: Mattila SO, Tuhkanen HE, Lackman JJ, Konzack A, Morató X, Argerich J, Saftig P, Ciruela F, Petäjä-Repo UE
GPR37 is processed in the N-terminal ectodomain by ADAM10 and furin.
GPR37 is an orphan G protein-coupled receptor (GPCR) implicated in several neurological diseases and important physiological pathways in the brain. We previously reported that its long N-terminal ectodomain undergoes constitutive metalloprotease-mediated cleavage and shedding, which have been rarely described for class A GPCRs. Here, we demonstrate that the protease that cleaves GPR37 at Glu167↓Gln168 is a disintegrin and metalloprotease 10 (ADAM10). This was achieved by employing selective inhibition, RNAi-mediated downregulation, and genetic depletion of ADAM10 in cultured cells as well as in vitro cleavage of the purified receptor with recombinant ADAM10. In addition, the cleavage was restored in ADAM10 knockout cells by overexpression of the wild type but not the inactive mutant ADAM10. Finally, postnatal conditional depletion of ADAM10 in mouse neuronal cells was found to reduce cleavage of the endogenous receptor in the brain cortex and hippocampus, confirming the physiological relevance of ADAM10 as a GPR37 sheddase. Additionally, we discovered that the receptor is subject to another cleavage step in cultured cells. Using site-directed mutagenesis, the site (Arg54↓Asp55) was localized to a highly conserved region at the distal end of the ectodomain that contains a recognition site for the proprotein convertase furin. The cleavage by furin was confirmed by using furin-deficient human colon carcinoma LoVo cells and proprotein convertase inhibitors. GPR37 is thus the first multispanning membrane protein that has been validated as an ADAM10 substrate and the first GPCR that is processed by both furin and ADAM10. The unconventional N-terminal processing may represent an important regulatory element for GPR37.
FASEB J, 2021; 35
33637132: Morató X, Garcia-Esparcia P, Argerich J, Llorens F, Zerr I, Paslawski W, Borràs E, Sabidó E, Petäjä-Repo UE, Fernández-Dueñas V, Ferrer I, Svenningsson P, Ciruela F
Ecto-GPR37: a potential biomarker for Parkinson's disease.
α-Synuclein has been studied as a potential biomarker for Parkinson's disease (PD) with no concluding results. Accordingly, there is an urgent need to find out reliable specific biomarkers for PD. GPR37 is an orphan G protein-coupled receptor that toxically accumulates in autosomal recessive juvenile parkinsonism. Here, we investigated whether GPR37 is upregulated in

sporadic PD, and thus a suitable potential biomarker for PD.
Transl Neurodegener, 2021; 10
32230915: Fernández-Dueñas V, Qian M, Argerich J, Amaral C, Risseeuw MDP, Van Calenbergh S, Ciruela F
Design, Synthesis and Characterization of a New Series of Fluorescent Metabotropic Glutamate Receptor Type 5 Negative Allosteric Modulators.
In recent years, new drug discovery approaches based on novel pharmacological concepts have emerged. Allosteric modulators, for example, target receptors at sites other than the orthosteric binding sites and can modulate agonist-mediated activation. Interestingly, allosteric regulation may allow a fine-tuned regulation of unbalanced neurotransmitter' systems, thus providing safe and effective treatments for a number of central nervous system diseases. The metabotropic glutamate type 5 receptor (mGluR) has been shown to possess a druggable allosteric binding domain. Accordingly, novel allosteric ligands are being explored in order to finely regulate glutamate neurotransmission, especially in the brain. However, before testing the activity of these new ligands in the clinic or even in animal disease models, it is common to characterize their ability to bind mGluRs in vitro. Here, we have developed a new series of fluorescent ligands that, when used in a new NanoBRET-based binding assay, will facilitate screening for novel mGluR allosteric modulators.
Molecules, 2020; 25
31931958: Escudero-Lara A, Argerich J, Cabañero D, Maldonado R
Disease-modifying effects of natural Δ9-tetrahydrocannabinol in endometriosis-associated pain.
Endometriosis is a chronic painful disease highly prevalent in women that is defined by growth of endometrial tissue outside the uterine cavity and lacks adequate treatment. Medical use of cannabis derivatives is a current hot topic and it is unknown whether phytocannabinoids may modify endometriosis symptoms and development. Here we evaluate the effects of repeated exposure to Δ9-tetrahydrocannabinol (THC) in a mouse model of surgically-induced endometriosis. In this model, female mice develop mechanical hypersensitivity in the caudal abdomen, mild anxiety-like behavior and substantial memory deficits associated with the presence of extrauterine endometrial cysts. Interestingly, daily treatments with THC (2 mg/kg) alleviate mechanical hypersensitivity and pain unpleasantness, modify uterine innervation and restore cognitive function without altering the anxiogenic phenotype. Strikingly, THC also inhibits the development of endometrial cysts. These data highlight the interest of scheduled clinical trials designed to investigate possible benefits of THC for women with endometriosis.
Elife, 2020; 9

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IFNAR1C291* EXPRESSION INDUCES NEURODEGENERATION IN VIVO THROUGH IMPAIRMENTS IN PARKIN-MEDIATED MITOPHAGY

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Parkinson's Disease (PD) is the second most common neurodegenerative disease, with the fastest rising incidence in recent years. While its etiology remains elusive, the role of inflammation in PD is constantly strengthened. We have previously reported a mutation in the interferon receptor gene IFNAR1 (IFNAR1^{C291*}) segregating with disease in a family affected by parkinsonian disorders. Here, we develop a mouse model of PD by overexpressing IFNAR1^{C291*} in neurons of the Substantia Nigra and Prefrontal Cortex. We show behavioral deficits as early as 6 weeks post-injection, and the main hallmarks of PD: dopaminergic neuron loss, mitochondrial dysfunction, protein aggregation and neuroinflammation. Additionally, we mechanistically link Type I Interferons with mitophagy through ISG15, an Interferon-inducible Ubiquitin-like protein capable of enhancing Parkin activation and subsequently clearing damaged mitochondria in neurons.

Pubmed:
33913175: Tresse E, Riera-Ponsati L, Jaberi E, Sew WQG, Ruscher K, Issazadeh-Navikas S
IFN-β rescues neurodegeneration by regulating mitochondrial fission via STAT5, PGAM5, and Drp1.
Mitochondrial homeostasis is essential for providing cellular energy, particularly in resource-demanding neurons, defects in which cause neurodegeneration, but the function of interferons (IFNs) in regulating neuronal mitochondrial homeostasis is unknown. We found that neuronal IFN-β is indispensable for mitochondrial homeostasis and metabolism, sustaining ATP levels and preventing excessive ROS by controlling mitochondrial fission. IFN-β induces events that are required for mitochondrial fission, phosphorylating STAT5 and upregulating PGAM5, which phosphorylates serine 622 of Drp1. IFN-β signaling then recruits Drp1 to mitochondria, oligomerizes it, and engages INF2 to stabilize mitochondria-endoplasmic reticulum (ER) platforms. This process tethers damaged mitochondria to the ER to separate them via fission. Lack of neuronal IFN-β in the Ifnb model of Parkinson disease (PD) disrupts STAT5-PGAM5-Drp1 signaling, impairing fission and causing large multibranched, damaged mitochondria with insufficient ATP production and excessive oxidative stress to accumulate. In other PD models, IFN-β rescues dopaminergic neuronal cell death and pathology, associated with preserved mitochondrial homeostasis. Thus, IFN-β activates mitochondrial fission in neurons through the pSTAT5/PGAM5/ Drp1 pathway to stabilize mitochondria/ER platforms, constituting an essential neuroprotective mechanism.
EMBO J, 2021; 40

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BOARD NUMBER: S06-306

INSULIN-LIKE GROWTH FACTOR II NEUROPROTECTIVE EFFECTS AGAINST MITOCHONDRIAL-OXIDATIVE AND NEURONAL DAMAGE INDUCED BY CORT AND MPP+ IN DOPAMINERGIC NEURONS

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aims: Parkinson's disease (PD) affects 1–3% of the population aged over 65. Stress seems to contribute to PD neuropathology, probably by dysregulation of the hypothalamic–pituitary–adrenal axis. Key factors are oxidative stress, mitochondrial dysfunction and neuronal glucocorticoid-induced toxicity. Insulin-like growth factor II (IGF-II) has shown antioxidant and neuroprotective effects in some neurodegenerative disorders. Therefore, our aim was to study IGF-II protective effects against oxidative damage on a cellular combined model of PD and mild to moderate stress, based on corticosterone (CORT) and the dopaminergic neurotoxin 1-methyl-4-phenylpyridinium (MPP+). **Methods:** The dopaminergic neuronal cell line SN4741 (RRID:CVCL_S466) derived from mouse substantia nigra were exposed to 200 µM MPP+, 0.5 µM CORT or both, with or without 25 ng/mL IGF-II, for 2.5 or 6 h. Cell viability, oxidative stress parameters, mitochondrial and dopamine markers and intracellular signaling pathways were evaluated. **Results:** The administration of MPP+ or CORT individually led to cell damage compared to control situations, whereas the combination of both drugs produced very considerable toxic synergistic effect. IGF-II counteracts the mitochondrial-oxidative damage, protecting dopaminergic neurons from death and neurodegeneration. IGF-II promotes PKC activation and nuclear factor (erythroid-derived 2)-like 2 antioxidant response in a glucocorticoid receptor-dependent pathway, preventing oxidative cell damage and maintaining mitochondrial function. **Conclusions:** IGF-II capacity to protect nigral dopamine neurons against mitochondrial-oxidative damage induced by CORT and MPP+ was demonstrated. Thus, IGF-II is a potential therapeutic tool for prevention and treatment of PD patients suffering mild to moderate emotional stress. **Funding:** UMA18-FEDERJA-004. * These authors contributed equally to this work.

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EXTRACELLULAR CLUSTERIN PREVENTS ALPHA-SYNUCLEIN DISPERSION

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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We hypothesize that clusterin, an extracellular chaperone, reduces propagation of alpha-synuclein (a-syn) from cell to cell, this could result in less protein aggregation inside the cells. Neuron-like SH-SY5Y cells were differentiated during 10 days with DMEM supplemented with 10% foetal calf serum, 10uM retinoic acid and 10ng/ml BDNF. Cells were transfected during 2 days with empty vector (E.V.), a-syn wild-type (WT) or mutants (A30P or A53T) using lipofectamine 3000 and treated with 1ug/ml clusterin diluted in medium. Culture medium, soluble and insoluble protein fractions were collected for Western Blot. Cell viability was evaluated by MTT and Calcein-AM assays. Two-way Anova and $p<0.05$ indicated statistically significant differences. Insoluble a-syn A53T increased threefold compared to E.V., a-syn WT or A30P. Soluble a-syn decreased 30% in cells expressing the mutant A53T, indicating the switch between soluble and insoluble proteins. The increased expression of a-syn promoted an increased release of that protein to culture medium, WT increased 2 fold, A30P increased 2.5 fold and A53T increased 2.8 fold when compared to E.V. Clusterin added to culture medium prevented the increase of insoluble a-synA53T, although no differences were observed in secreted a-syn. Treatment with clusterin mitigated a-syn toxicity demonstrated by MTT where cell metabolism was restored in a-syn A53T-expressing cells. Calcein-AM assay demonstrated decreased number of living cells in all a-syn-expressing cells (5% decrease in a-syn-WT- and in A30P- and 10% in A53T-expressing cells). In conclusion, clusterin traps a-syn in the extracellular medium avoiding its dispersion to surrounding cells and improving cell viability.

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INVESTIGATING TRANSPOSABLE ELEMENTS AS CAUSE OF NEUROINFLAMMATION IN PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Neuroinflammation is known to be a trait in several neurodegenerative diseases. However, its underlying cause is unknown. We hypothesize that aberrant activation of transposable elements (TEs) triggers neuroinflammation in these neurodegenerative disorders, including Parkinson's disease (PD). By performing RNAseq on post-mortem brain tissue samples from PD patients and control individuals, we were able to identify three TE subfamilies that display a significant change in expression: L1PA2, L1HS, and HERV-K. To mechanistically link TEs aberrant activation with an inflammatory response, we are using a CRISPR activation (CRISPRa) system to induce the overexpression of these PD-activated TEs in human induced pluripotent stem cells (iPSCs) and neuroepithelial-like stem cells (NESCs). The iPSCs will then be differentiated into different cell types to obtain an *in vitro* model for TE-based neuroinflammation. In parallel, a CRISPR inhibition (CRISPRi) system will also be used to find whether any transcriptional consequences occur upon dysregulation of our target TEs in the chosen models, given that some TEs were also shown to be downregulated in PD patients. We also want to culture iPSC-derived cerebral organoids to better observe and assess any possible phenotypical effects of the silencing or overexpression of the target TEs. With this project, we hope to uncover a new pathogenic mechanism of PD, which could lead to innovative and clinically relevant research perspectives for this neurodegenerative disorder.

Pubmed:
34624206: Johansson PA, Brattås PL, Douse CH, Hsieh P, Adami A, Pontis J, Grassi D, Garza R, Sozzi E, Cataldo R, Jönsson ME, Atacho DAM, Pircs K, Eren F, Sharma Y, Johansson J, Fiorenzano A, Parmar M, Fex M, Trono D, Eichler EE, Jakobsson J
A cis-acting structural variation at the ZNF558 locus controls a gene regulatory network in human brain development.
The human forebrain has expanded in size and complexity compared to chimpanzees despite limited changes in protein-coding genes, suggesting that gene expression regulation is an important driver of brain evolution. Here, we identify a KRAB-ZFP transcription factor, ZNF558, that is expressed in human but not chimpanzee forebrain neural progenitor cells. ZNF558 evolved as a suppressor of LINE-1 transposons but has been co-opted to regulate a single target, the mitophagy gene SPATA18. ZNF558 plays a role in mitochondrial homeostasis, and loss-of-function experiments in cerebral organoids suggests that ZNF558 influences developmental timing during early human brain development. Expression of ZNF558 is controlled by the size of a variable number tandem repeat that is longer in chimpanzees compared to humans, and variable in the human population. Thus, this work provides mechanistic insight into how a cis-acting structural variation establishes a regulatory network that affects human brain evolution.
Cell Stem Cell, 2022; 29

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THE ROLE OF MICROVASCULAR CHANGES DURING DEVELOPMENT AND PROGRESSION OF PARKINSON'S DISEASE IN A HUMAN ALPHA-SYNUCLEIN OVEREXPRESSION MOUSE MODEL (LINE 61)

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Microvascular alterations have been increasingly recognized in neurodegenerative diseases including Alzheimer's Disease (AD), Parkinson's Disease (PD) and Multiple Sclerosis (MS). Indeed, key cellular processes associated with PD pathogenesis such as accumulation of alpha-synuclein (αSyn) and concomitant oxidative stress and neuroinflammation have been shown to exert detrimental effects on blood-brain barrier (BBB) integrity. In fact, BBB alterations were detected in PD patients but understanding of the underlying mechanism and information on the nature and extent of vascular pathology remain limited. We aimed to study BBB alterations during disease progression in a αSyn PD mouse model and resolve the underlying molecular and cellular mechanisms. Microvascular changes during PD progression were evaluated in the Thy1-αSyn (Masliah line 61) progressive mouse model using two and six month old transgenic human αSyn overexpressing mice and age-matched wildtype control animals. BBB permeability was analyzed by FITC-albumin dye extrusion assay in different brain regions after transcardial perfusion. Additionally expression levels of tight junctions (TJs) that seal the paracellular cleft between endothelial cells, matrix metalloprotease activation that degrade endothelial TJs and basement proteins, as well as endothelial cell activation were analyzed in isolated brain microvessels. Carrier function of LRP1 and Pgp that are expressed by brain microvascular endothelial cells and may influence the αSyn mediated BBB alterations were also evaluated. A better comprehension of BBB alterations during PD progression would bring new insights into the basic understanding of the pathophysiology of PD and may facilitate identification of novel pharmacological targets.

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THE ROLE OF LIPID METABOLISM IN PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a common age-related neurodegenerative disorder with disabling motor symptoms and no available disease modifying treatment. While commonly believed to be a proteinopathy, specifically a synucleinopathy, recent studies have suggested a prominent role for lipids in pathogenesis. Studies have shown that many PD associated genes play a role in lysosomal function in both neuronal and glial cell types which suggest involvement for not only lysosomal clearance of proteins but also lipids in PD. Previously, cell type specific lipid storage changes have been shown in PD patient brains and lipid droplet binding may play a role in the oligomerization of alpha synuclein, the protein present in the disease's hallmark Lewy bodies. Furthermore, fatty acid binding proteins (FABPs), particularly FABP7 and FABP3, have been shown to play a role in lipid droplet formation. In addition to their roles as lipid chaperones, FABPs have been shown to affect metabolic and inflammatory pathways further pointing to their role in lipid metabolism. Investigating changes in lipid storage may provide powerful insights into disease progression and possible disease modifying treatments. By observing differences and FABP7 and FABP3 expression in PD human tissue relative to controls we can understand the role of lipid dysregulation in PD. Additionally, observing differences in lipid droplet formation in neuronal and glial cell lines during lipotoxicity will further elucidate cell type specific changes in lipid metabolism in PD. Preliminary data shows that FABPs may be upregulated in PD.

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BOARD NUMBER: S06-311

THE ROLE OF EEF1A PROTEINS AT SYNAPSES AND IN SYNUCLEINOPATHY

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Synucleinopathies (e.g. Parkinson's disease and dementia with Lewy bodies) are neurodegenerative conditions characterized by an accumulation of **alpha-synuclein** from the beginning of the prodromal phase. Yet, the pathophysiology is far from being completely understood. We have recently identified the synaptic depletion of eukaryotic elongation factor 1A proteins (EEF1A) at early stages of the disease. We now aim at identifying the **function of EEF1A** at synapses and in the pathophysiological process. First, we analyze the **time course** of each EEF1A variant loss in a PDGF-hSyn mouse model overexpressing alpha-synuclein. Furthermore, we develop a **CRISPR/Cas9-mediated protein tagging** as well as **knock-out strategy** for the endogenous EEF1A variants. With these new tools we will access the **precise neuronal localization** of the protein variants and control EEF1A expression in neurons. We also apply fluorescence activated synaptosome sorting to cortical glutamatergic synapses (VGLUT1-Venus x PDGF-hSyn) and explore the impact of early onset synucleinopathy to the proteomics profile. Finally, we aim at exploring the functions of EEF1A variants at synapse, to that end we test local protein translation, synaptic vesicle cycling and spine morphology. Our work will unravel the function of EEF1A at synapses and provides in depth characterization of its implication in the pathophysiology of synucleinopathy.

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IDENTIFYING ALPHA-SYNUCLEIN INTERACTOME MAP IN A MOUSE MODEL USING PROXIMITY-BIOTINILATION.

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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The loss of dopaminergic neurons in the substantia nigra (SN) that project to the striatum causes Parkinson´s disease (PD). The abnormal increase of pre-synaptic alpha-synuclein (aSyn) protein levels or aSyn mutations are one of the main drivers of PD and other synucleinopathies. In spite of the extensive research on this topic, the molecular mechanisms whereby quantitative/qualitative aSyn changes trigger neurodegeneration remain largely unclear. In this study, our working hypothesis is that mutations and/or early increases of aSyn protein levels could facilitate pathological protein-protein interactions (PPIs). The identification and modulation of aSyn PPIs could provide new therapeutic approaches to treat PD and other synucleinopathies.Here, we aimed to identify the early aSyn interactome in a mouse model using a methodology that identifies PPIs through proximity biotinylation. By expressing aSyn fused to a promiscuous biotinilase (BioID2 or TurboID) and adding biotin, proximal stable and transient interacting partners become biotinylated. Biotinylated substrates are affinity purified and identified by mass spectrometry (MS). BioID2 and TurboID-tagged aSyn proteins recapitulate aSyn-dependent neurotoxicity. TurboID-tagged proteins however, displayed a more efficient biotinilase activity. Thus, adeno-associated viral (AAV) vectors encoding TurboID, aSynTurboID and pathological mutant E46KSynTurboID were generated and stereotaxically injected into the SN of mice. AAV9-SynTurboID mice showed aSyn increased levels in the SN and in the striatum, loss of dopaminergic striatal terminals and motor deficits with respect to AAV9-TurboID mice. Pull-down experiments from injected and biotin-treated mice as well as MS analysis are ongoing to identify aSyn PPIs from dopaminergic striatal terminals and SN somas.

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PARKINSON'S DISEASE LRRK2-G2019S DISRUPTS HDAC6-MEDIATED RECRUITMENT OF MISFOLDED PROTEIN AGGREGATES INTO AGGRESOMES

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Mutations in the *LRRK2* gene are the most frequent cause of dominantly inherited Parkinson's disease (PD). LRRK2 PD exhibits Lewy pathology with accumulations of alpha-synuclein and ubiquitin in intracellular aggregates that are indistinguishable from idiopathic PD. LRRK2 is a multi-domain protein with both GTPase and kinase activities which regulates various cellular processes including protein homeostasis. However, how PD-linked LRRK2 mutations may lead to accumulation of ubiquitinated protein aggregates remains unclear. A main cellular pathway to remove aggregated ubiquitinated proteins is aggrephagy. Here, histone deacetylase 6 (HDAC6) tethers ubiquitinated misfolded proteins to the molecular motor cytoplasmic dynein. This promotes the recruitment of protein aggregates into a perinuclear aggresome and their subsequent clearance by macroautophagy. Here we report that LRRK2 regulates HDAC6-dependent aggresome formation, a crucial step in aggrephagy. We found that the LRRK2 Roc-COR domain directly interacts with the HDAC6 deacetylase domains, and that this interaction is regulated by LRRK2 kinase activity. PD mutant LRRK2-G2019S that has increased kinase activity showed reduced interaction with HDAC6, which could be restored using LRRK2 kinase inhibitors. Loss of LRRK2 interaction with HDAC6 prevented recruitment of ubiquitinated proteins into aggresomes. Accordingly, LRRK2-G2019S impaired aggresome formation and this was recapitulated in LRRK2-G2019S patient-derived iAstrocytes. In conclusion our data reveal HDAC6 as a novel interactor of LRRK2 and suggest that deregulation of HDAC6-mediated aggresome formation and aggrephagy could contribute to the pathology of PD.

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BOARD NUMBER: S06-314

NEURONAL HEMOGLOBIN INDUCES LOSS OF DOPAMINERGIC NEURONS IN MOUSE SUBSTANTIA NIGRA, COGNITIVE DEFICITS AND CLEAVAGE OF ENDOGENOUS α-SYNUCLEIN.

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aim Parkinson's disease (PD) presents the selective loss of A9 dopaminergic (DA) neurons of Substantia Nigra *pars compacta* (SNpc) and the presence of intracellular aggregates called Lewy bodies. α-synuclein (α-syn) species truncated at the carboxy-terminal (C-terminal) accumulate in pathological inclusions and promote α-syn aggregation and toxicity. Hemoglobin (Hb) is the major oxygen carrier protein in erythrocytes. In addition, Hb is expressed in A9 DA neurons where it influences mitochondrial activity. Hb overexpression increases cells' vulnerability in a neurochemical model of PD *in vitro* and forms cytoplasmic and nucleolar aggregates upon short-term overexpression in mouse SNpc. The aim of the study was to evaluate the effect of chronic expression of Hb in the SNpc of mice on DA neurons homeostasis. Methods α and β-globin chains were co-expressed in DA cells of SNpc *in vivo* upon stereotaxic injections of an Adeno-Associated Virus isotype 9 (AAV9) and in DA iMN9D cells *in vitro*. Results Long-term Hb over-expression in SNpc induced the loss of about 50% of DA neurons, a mild motor impairment and deficits in recognition and spatial working memory. Hb triggered the formation of endogenous a-synuclein C-terminal truncated species. Similar α-syn fragments were found *in vitro* in DA iMN9D cells over-expressing α and β- globins when treated with pre-formed α-syn fibrils. Conclusion Our study positions Hb as a relevant player in PD pathogenesis for its ability to trigger DA cells' loss *in vivo* and the formation of C-terminal a-synuclein fragments.

Pubmed:

34623427: Espinoza S, Bon C, Valentini P, Pierattini B, Matey AT, Damiani D, Pulcrano S, Sanges R, Persichetti F, Takahashi H, Carninci P, Santoro C, Cotella D, Gustincich S
SINEUPs: a novel toolbox for RNA therapeutics.
RNA molecules have emerged as a new class of promising therapeutics to expand the range of druggable targets in the genome. In addition to 'canonical' protein-coding mRNAs, the emerging richness of sense and antisense long non-coding RNAs (lncRNAs) provides a new reservoir of molecular tools for RNA-based drugs. LncRNAs are composed of modular structural domains with specific activities involving the recruitment of protein cofactors or directly interacting with nucleic acids. A single therapeutic RNA transcript can then be assembled combining domains with defined secondary structures and functions, and antisense sequences specific for the RNA/DNA target of interest. As the first representative molecules of this new pharmacology, we have identified SINEUPs, a new functional class of natural antisense lncRNAs that increase the translation of partially overlapping mRNAs. Their activity is based on the combination of two domains: an embedded mouse inverted SINEB2 element that enhances mRNA translation (effector domain) and an overlapping antisense region that provides specificity for the target sense transcript (binding domain). By genetic engineering, synthetic SINEUPs can potentially target any mRNA of interest increasing translation and therefore the endogenous level of the encoded protein. In this review, we describe the state-of-the-art knowledge of SINEUPs and discuss recent publications showing their potential application in diseases where a physiological increase of endogenous protein expression can be therapeutic.
Essays Biochem, 2021; 65
31584077: Bon C, Luffarelli R, Russo R, Fortuni S, Pierattini B, Santulli C, Fimiani C, Persichetti F, Cotella D, Mallamaci A, Santoro C, Carninci P, Espinoza S, Testi R, Zucchelli S, Condò I, Gustincich S
SINEUP non-coding RNAs rescue defective frataxin expression and activity in a cellular model of Friedreich's Ataxia.
Friedreich's ataxia (FRDA) is an untreatable disorder with neuro- and cardio-degenerative progression. This monogenic disease is caused by the hyper-expansion of naturally occurring GAA repeats in the first intron of the FXN gene, encoding for frataxin, a protein implicated in the biogenesis of iron-sulfur clusters. As the genetic defect interferes with FXN transcription,

FRDA patients express a normal frataxin protein but at insufficient levels. Thus, current therapeutic strategies are mostly aimed to restore physiological FXN expression. We have previously described SINEUPs, natural and synthetic antisense long non-coding RNAs, which promote translation of partially overlapping mRNAs through the activity of an embedded SINEB2 domain. Here, by in vitro screening, we have identified a number of SINEUPs targeting human FXN mRNA and capable to up-regulate frataxin protein to physiological amounts acting at the post-transcriptional level. Furthermore, FXN-specific SINEUPs promote the recovery of disease-associated mitochondrial aconitase defects in FRDA-derived cells. In summary, we provide evidence that SINEUPs may be the first gene-specific therapeutic approach to activate FXN translation in FRDA and, more broadly, a novel scalable platform to develop new RNA-based therapies for haploinsufficient diseases.
Nucleic Acids Res, 2019; 47

31570000: Fasolo F, Patrucco L, Volpe M, Bon C, Peano C, Mignone F, Carninci P, Persichetti F, Santoro C, Zucchelli S, Sblattero D, Sanges R, Cotella D, Gustincich S
The RNA-binding protein ILF3 binds to transposable element sequences in SINEUP lncRNAs.
Transposable elements (TEs) compose about half of the mammalian genome and, as embedded sequences, up to 40% of long noncoding RNA (lncRNA) transcripts. Embedded TEs may represent functional domains within lncRNAs, providing a structured RNA platform for protein interaction. Here we show the interactome profile of the mouse inverted short interspersed nuclear element (SINE) of subfamily B2 (invSINEB2) alone and embedded in antisense (AS) ubiquitin C-terminal hydrolase L1 (Uchl1), an lncRNA that is AS to Uchl1 gene. AS Uchl1 is the representative member of a functional class of AS lncRNAs, named SINEUPs, in which the invSINEB2 acts as effector domain (ED)-enhancing translation of sense protein-coding mRNAs. By using RNA-interacting domainome technology, we identify the IL enhancer-binding factor 3 (ILF3) as a protein partner of AS Uchl1 RNA. We determine that this interaction is mediated by the RNA-binding motif 2 of ILF3 and the invSINEB2. Furthermore, we show that ILF3 is able to bind a free right (Alu) monomer sequence, the embedded TE acting as ED in human SINEUPs. Bioinformatic analysis of Encyclopedia of DNA Elements-enhanced cross-linking immunoprecipitation data reveals that ILF3 binds transcribed human SINE sequences at transcriptome-wide levels. We then demonstrate that the embedded TEs modulate AS Uchl1 RNA nuclear localization to an extent moderately influenced by ILF3. This work unveils the existence of a specific interaction between embedded TEs and an RNA-binding protein, strengthening the model of TEs as functional modules in lncRNAs.-Fasolo, F., Patrucco, L., Volpe, M., Bon, C., Peano, C., Mignone, F., Carninci, P., Persichetti, F., Santoro, C., Zucchelli, S., Sblattero, D., Sanges, R., Cotella, D., Gustincich, S. The RNA-binding protein ILF3 binds to transposable element sequences in SINEUP lncRNAs.
FASEB J, 2019; 33

29453387: Podbevšek P, Fasolo F, Bon C, Cimatti L, Reißer S, Carninci P, Bussi G, Zucchelli S, Plavec J, Gustincich S
Structural determinants of the SINE B2 element embedded in the long non-coding RNA activator of translation AS Uchl1.
Pervasive transcription of mammalian genomes leads to a previously underestimated level of complexity in gene regulatory networks. Recently, we have identified a new functional class of natural and synthetic antisense long non-coding RNAs (lncRNA) that increases translation of partially overlapping sense mRNAs. These molecules were named SINEUPs, as they require an embedded inverted SINE B2 element for their UP-regulation of translation. Mouse AS Uchl1 is the representative member of natural SINEUPs. It was originally discovered for its role in increasing translation of Uchl1 mRNA, a gene associated with neurodegenerative diseases. Here we present the secondary structure of the SINE B2 Transposable Element (TE) embedded in AS Uchl1. We find that specific structural regions, containing a short hairpin, are required for the ability of AS Uchl1 RNA to increase translation of its target mRNA. We also provide a high-resolution structure of the relevant hairpin, based on NMR observables. Our results highlight the importance of structural determinants in embedded TEs for their activity as functional domains in lncRNAs.
Sci Rep, 2018; 8

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BOARD NUMBER: S06-315

PLASMA MEMBRANE CA2+ ATPASE 1 AS A CANDIDATE TO MEDIATE THE DEGENERATION OF DOPAMINERGIC NEURONS BY INFLAMMATION IN PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aims
To identify genes involved in the degeneration of the dopaminergic neurons (DN) of the *substantia nigra* (SN) by inflammation. **Methods**
We performed functional genomic analysis in a rat model that manifests neurodegeneration provoked by inflammation by chronic expression of TNFα in the SN delivered by an adenovector. We validated these findings by qPCR in two other rat models expressing IL-1β and 6OHDA/LPS. To study the functionality of the genes found, we used Drosophila melanogaster (DM) as a model. Behavioural, histological, and physiological analyses were performed. **Results**
We found that the expression of the plasma membrane calcium ATPase 1 (PMCA1) gene was downregulated in the three rat models. To test the relevance of PMCA1 in DN *in vivo*, we specifically decreased its expression in DN using RNA interference in DM. We found that PMCA downregulation in DN induces an increase in intracellular Ca^{2+}, altered Ca^{2+} dynamics, mitochondrial oxidative stress, increments in synaptic vesicles, active synapses and dopamine levels, without cell death, locomotor alterations and lower survival. **Conclusions**
We found PMCA1 is downregulated in three rat models of Parkinson's disease (PD) by inflammation. The reduction of PMCA in DM DN reproduced features of other DM models of PD. Since DN are subjected to Ca^{2+} dependent-pacemaker, the decrease of PMCA1 could provoke a dysregulation in the Ca^{2+} homeostasis, promoting their vulnerability. These results postulate PMCA1 as a participant in the process of vulnerability and degeneration of DN in PD.

Pubmed:
34312939: Erhardt B, Marcora MS, Frenkel L, Bochicchio PA, Bodin DH, Silva BA, Farías MI, Allo MÁ, Höcht C, Ferrari CC, Pitossi FJ, Leal MC
Plasma membrane calcium ATPase downregulation in dopaminergic neurons alters cellular physiology and motor behaviour in Drosophila melanogaster.
The accumulation of Ca and its subsequent increase in oxidative stress is proposed to be involved in selective dysfunctionality of dopaminergic neurons, the main cell type affected in Parkinson's disease. To test the in vivo impact of Ca increment in dopaminergic neurons physiology, we downregulated the plasma membrane Ca ATPase (PMCA), a pump that extrudes cytosolic Ca , by expressing PMCA in Drosophila melanogaster dopaminergic neurons. In these animals, we observed major locomotor alterations paralleled to higher cytosolic Ca and increased levels of oxidative stress in mitochondria. Interestingly, although no overt degeneration of dopaminergic neurons was observed, evidences of neuronal dysfunctionality were detected such as increases in presynaptic vesicles in dopaminergic neurons and in the levels of dopamine in the brain, as well as presence of toxic effects when PMCA was downregulated in the eye. Moreover, reduced PMCA levels were found in a Drosophila model of Parkinson's disease, Parkin knock-out, expanding the functional relevance of PMCA reduction to other Parkinson's disease-related models. In all, we have generated a new model to study motor abnormalities caused by increments in Ca that lead to augmented oxidative stress in a dopaminergic environment, added to a rise in synaptic vesicles and dopamine levels.
Eur J Neurosci, 2021; 54
31669283: Silva BA, Leal MC, Farías MI, Erhardt B, Galeano P, Pitossi FJ, Ferrari CC
Environmental enrichment improves cognitive symptoms and pathological features in a focal model of cortical damage of multiple sclerosis.

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Multiple Sclerosis (MS) is a neuroinflammatory disease affecting white and grey matter, it is characterized by demyelination, axonal degeneration along with loss of motor, sensitive and cognitive functions. MS is a heterogeneous disease that displays different clinical courses: relapsing/remitting MS (RRMS), and MS progressive forms: primary progressive (PPMS) and secondary progressive (SPMS). Cortical damage in the progressive MS forms has considerable clinical relevance due to its association with cognitive impairment and disability progression in patients. One treatment is available for the progressive forms of the disease, but none are specific for cognitive deficits. We developed an animal model that reflects most of the characteristics of the cortical damage, such as cortical neuroinflammation, demyelination, neurodegeneration and meningeal inflammation, which was associated with cognitive impairment. Cognitive rehabilitation, exercise and social support have begun to be evaluated in patients and animal models of neurodegenerative diseases. Environmental enrichment (EE) provides exercise as well as cognitive and social stimulation. EE has been demonstrated to exert positive effects on cognitive domains, such as learning and memory, and improving anxiety-like symptoms. We proposed to study the effect of EE on peripherally stimulated cortical lesion induced by the long term expression of interleukin IL-1β (IL-1β) in adult rats. Here, we demonstrated that EE: 1) reduces the peripheral inflammatory response to the stimulus, 2) ameliorates cognitive deficits and anxiety-like symptoms, 3) modulates neurodegeneration, demyelination and glial activation, 4) regulates neuroinflammation by reducing the expression of pro-inflammatory cytokines and enhancing the expression of anti-inflammatory ones. Our findings correlate with the fact that EE housing could be considered an effective non- pharmacological therapeutic agent that can synergistically aid in the rehabilitation of the disease.
Brain Res, 2020; 1727

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-316

GLUCOCEREBROSIDASE (GCASE) TRANSLOCATION TO LYSOSOMES AND GCASE LEVEL IN PRIMARY MACROPHAGES DERIVED FROM GBA1 MUTATION CARRIERS WITH AND WITHOUT PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Background Mutations in the *GBA1* gene, encoding the lysosomal enzyme glucocerebrosidase (GCase), are the most common genetic risk factor of Parkinson's disease (PD). However, not all *GBA1*-carriers develop PD during their lifetime. It remains unknown if GCase transport is impaired in *GBA1*-carriers and is it related with PD development. **Aim** To assess GCase translocation to lysosomes and its level in primary macrophages derived from *GBA1* mutation carriers with and without PD. **Methods** Mononuclear fraction was isolated from whole blood of patients with *GBA1* mutation with ($GBA1^+/PD^+$)(N=10) or without PD ($GBA1^+/PD^-$)(N=8) and controls (N=11). Primary macrophages were cultured as described earlier (KopytovaA et al., ParkinsonismRelatDisord.2021). The translocation of GCase to lysosomes (marker LAMP2) was assessed in primary macrophages using confocal microscope (×63magnification). Pearson's coefficient was calculated for the degree of colocalization between GCase and LAMP2. Total protein was separated by SDS-PAGE and transferred to PVDF membranes by electroblotting. Primary anti-GBA and anti-GAPDH antibodies were used. **Results** A decreased translocation of GCase to lysosomes, estimated as colocalization of GCase with the lysosomal marker LAMP2, was observed in $GBA1^+/PD^+$ and $GBA1^+/PD^-$ primary macrophages compared to controls (Table 1). Moreover, the degree of translocation of GCase to lysosomes depends on the severity of *GBA1* mutations (Table 1). A decreased relative GCase protein level was observed in $GBA1^+/PD^-$ 0,49(-0,16–1,18) compared to controls 1,82(0,46–3,74)(p=0.012).

Table 1. Pearson's coefficient for GCase and LAMP2 in primary macrophages derived from *GBA1* mutation carriers with and without PD

Patients	Age, years	Sex, male/ female	*GBA1* mutation	Pearson's coefficient, median (min-max)	p-value	Pearson's coefficient depending on the severity of *GBA1* mutation, median (min-max)	p-value
Controls N=11	55,1 ± 6,2	4/7	NA	0,765 (0,615 – 0,992)	-	-	-
$GBA1^+/PD^-$ N=8	53,8 ± 7,4	3/5	"Mild" (N370S, R120W) N=4	0,682 (0,540 – 0,807)	*p<0.0001	0,712 (0,440-0,807)	*p<0.0001
			"Severe" (L444P) N=4			0,658 (0,534-0,717)	*p<0.0001 a p<0.0001
$GBA1^+/PD^+$ N=9	54,5 ± 8,6	2/6	"Mild" (N370S) N=5	0,695 (0,325 – 0,858)	*p<0.0001	0,740 (0,325-0,858)	*p=0.002
			"Severe" (L444P) N=4			0,660 (0,482-0,757)	*p<0.0001 b p<0.0001

** compared to controls*
a compared to "Mild" GBA1 mutation carriers without PD
b compared to "Mild" GBA1 mutation carriers with PD

Conclusions *GBA1* mutation carriers are characterized by impaired GCase transport to lysosomes that depends on mutation severity but is not affected with the disease status. The study was funded by RSF №19-15-00315.

Pubmed:
34680941: Usenko T, Bezrukova A, Basharova K, Panteleeva A, Nikolaev M, Kopytova A, Miliukhina I, Emelyanov A,

Zakharova E, Pchelina S
Comparative Transcriptome Analysis in Monocyte-Derived Macrophages of Asymptomatic Mutation Carriers and Patients with GBA-Associated Parkinson's Disease.
Mutations of the gene, encoding for lysosomal enzyme glucocerebrosidase (GCase), are the greatest genetic risk factor for Parkinson's disease (PD) with frequency between 5% and 20% across the world. N370S and L444P are the two most common mutations in the gene. PD carriers of severe mutation L444P in the gene is characterized by the earlier age at onset compared to N370S. Not every carrier of mutations develop PD during one's lifetime. In the current study we aimed to find common gene expression signatures in PD associated with mutation in the gene (GBA-PD) using RNA-seq. We compared transcriptome of monocyte-derived macrophages of 5 patients with GBA-PD (4 L444P/N, 1 N370S/N) and 4 asymptomatic mutation carriers (GBA-carriers) (3 L444P/N, 1 N370S/N) and 4 controls. We also conducted comparative transcriptome analysis for L444P/N only GBA-PD patients and GBA-carriers. Revealed deregulated genes in GBA-PD independently of mutations (L444P or N370S) were involved in immune response, neuronal function. We found upregulated pathway associated with zinc metabolism in L444P/N GBA-PD patients. The potential important role of in the pathogenesis of GBA-PD was suggested.
Genes (Basel), 2021; 12
34377557: Senkevich KA, Kopytova AE, Usenko TS, Emelyanov AK, Pchelina SN
Parkinson's Disease Associated with GBA Gene Mutations: Molecular Aspects and Potential Treatment Approaches.
Parkinson's disease (PD) is a multifactorial neurodegenerative disease. To date, genome-wide association studies have identified more than 70 loci associated with the risk of PD. Variants in the gene encoding glucocerebrosidase are quite often found in PD patients in all populations across the world, which justifies intensive investigation of this gene. A number of biochemical features have been identified in patients with -associated Parkinson's disease (GBA-PD). In particular, these include decreased activity of glucocerebrosidase and accumulation of the glucosylceramide substrate. These features were the basis for putting forward a hypothesis about treatment of GBA-PD using new strategies aimed at restoring glucocerebrosidase activity and reducing the substrate concentration. This paper discusses the molecular and genetic mechanisms of GBA-PD pathogenesis and potential approaches to the treatment of this form of the disease.
Acta Naturae, 2021 Apr-Jun; 13
33609962: Kopytova AE, Rychkov GN, Nikolaev MA, Baydakova GV, Cheblokov AA, Senkevich KA, Bogdanova DA, Bolshakova OI, Miliukhina IV, Bezrukikh VA, Salogub GN, Sarantseva SV, Usenko TC, Zakharova EY, Emelyanov AK, Pchelina SN
Ambroxol increases glucocerebrosidase (GCase) activity and restores GCase translocation in primary patient-derived macrophages in Gaucher disease and Parkinsonism.
Mutations in the glucocerebrosidase gene (GBA) encoding the lysosomal enzyme glucocerebrosidase (GCase) cause Gaucher disease (GD) and are the most commonly known genetic risk factor for Parkinson disease (PD). Ambroxol is one of the most effective pharmacological chaperones of GCase. Fourteen GD patients, six PD patients with mutations in the GBA gene (GBA-PD), and thirty controls were enrolled. GCase activity and hexosylsphingosine (HexSph) concentration were measured in dried blood and macrophage spots using liquid chromatography coupled with tandem mass spectrometry. The effect of ambroxol on GCase translocation to lysosomes was assessed using confocal microscopy. The results showed that ambroxol treatment significantly increased GCase activity in cultured macrophages derived from patient blood monocytic cell (PBMC) of GD (by 3.3-fold) and GBA-PD patients (by 3.5-fold) compared to untreated cells ($p < 0.0001$ and $p < 0.0001$, respectively) four days after cultivation. Ambroxol treatment significantly reduced HexSph concentration in GD (by 2.1-fold) and GBA-PD patients (by 1.6-fold) ($p < 0.0001$ and $p < 0.0001$, respectively). GD macrophage treatment resulted in increased GCase level and increased enzyme colocalization with the lysosomal marker LAMP2. The possible binding modes of ambroxol to mutant GCase carrying N370S amino acid substitution at pH 4.7 were examined using molecular docking and molecular dynamics simulations. The ambroxol position characterized by minimal binding free energy was observed in close vicinity to the residue, at position 370. Taken together, these data showed that PBMC-derived macrophages could be used for assessing ambroxol therapy response for GD patients and also for GBA-PD patients.
Parkinsonism Relat Disord, 2021; 84
33227372: Usenko TS, Bezrukova AI, Bogdanova DA, Kopytova AE, Senkevich KA, Gracheva EV, Timofeeva AA, Miliukhina IV, Zakharova EY, Emelyanov AK, Pchelina SN
Genetics variants and expression of the SCARB2 gene in the pathogenesis of Parkinson's disease in Russia.
Lysosomal integral membrane protein-2 (LIMP-2), encoded by the SCARB2 gene, is the specific lysosomal receptor for glucocerebrosidase enzyme. Association between rs6812193 and rs68250047 of SCARB2 with PD has been shown in genetic studies, including large genome-wide association studies. The aim of the current study was to determine whether rs6812193 and rs8475 are associated with PD in Russia. rs6812193 and rs8475 were genotyped in a total of 604 PD patients (65 PD patients with positive (fPD) and 539 PD patients with negative family history (sPD)) and 413 controls and also in 17

patients with PD associated with GBA mutations (PD-GBA) and 18 asymptomatic GBA mutation carriers (GBA-Carriers). SCARB2 expression was measured by real-time PCR in CD45+ blood cells in part of individuals in the studied groups. No linkage disequilibrium was shown between rs6812193 and rs8475 in Russian population. Increased PD risk for TT variant of rs8475 (OR = 2.02; p < 0.001) was found in sPD patients but not in fPD. rs6812193 and rs8475 were not associated with age at onset (AAO) of PD. SCARB2 expression level was decreased in GBA-PD patients and GBA-Carriers compared to PD patients (p = 0.02, p = 0.003, respectively) and GBA-Carriers compared to controls (p = 0.013) with no significant difference in PD patients and controls. SCARB2 expression was not modified with rs6812193 and rs8475. In conclusion, rs8475 was associated with PD status. rs6812193 and rs8475 are not genetic modifier of AAO of PD and do not influence on SCARB2 mRNA level in CD45+ blood cells in studied groups. SCARB2 expression could be modified with GBA mutations and is independent of PD status.
Neurosci Lett, 2021; 741
30316070: Emelyanov A, Kulabukhova D, Garaeva L, Senkevich K, Verbitskaya E, Nikolaev M, Andoskin P, Kopytova A, Milyukhina I, Yakimovskii A, Timofeeva A, Prakhova L, Ilves A, Vlasova I, Pchelina S
SNCA variants and alpha-synuclein level in CD45+ blood cells in Parkinson's disease.
Parkinson's disease (PD) is the second most frequent neurodegenerative disorder. Impaired metabolism of alpha-synuclein (SNCA) and its aggregation are implicated in PD pathogenesis. SNCA has been identified as a highly significant genetic risk loci associated with the sporadic form of PD in across populations in GWAS and replicative studies. In this study we conducted a genetic analysis of five SNCA single nucleotide polymorphisms (SNPs) (rs356219, rs2619364, rs11931074, rs2583988, rs356168) in 458 PD patients and 353 from North-West region of Russia. We also assessed an association of studied SNPs with alpha-synuclein levels in homogeneous cell fraction of CD45+ blood cells in PD patients and controls. An association with PD was shown for SNPs rs356219, rs11931074, rs356168. After correction for covariates the significant association with the disease only for rs11931074 and rs356168 was shown. Alpha-synuclein level in peripheral blood CD45+ cells was significantly increased in PD patients compared to control subjects (p = 0.02). The effect of SNCA rs356219 and rs356168 on CD45+ alpha-synuclein level in PD patients and control groups was shown. At the same tame the increase of CD45+ alpha-synuclein level in PD patients was revealed only in risk allele carriers as for rs356219 and rs356168 SNPs. Therefore, our study was the first that demonstrated the increased level of alpha-synuclein in CD45+ blood cells in PD patients and showed that it could be influenced by SNCA rs356168 and rs356219. In conclusion we confirmed the significance of the SNCA locus in the PD development.
J Neurol Sci, 2018; 395
30192031: Pchelina S, Baydakova G, Nikolaev M, Senkevich K, Emelyanov A, Kopytova A, Miliukhina I, Yakimovskii A, Timofeeva A, Berkovich O, Fedotova E, Illarioshkin S, Zakharova E
Blood lysosphingolipids accumulation in patients with parkinson's disease with glucocerebrosidase 1 mutations.
Glucocerebrosidase 1 mutations, the most common genetic contributor to Parkinson's disease (PD), have been associated with decreased glucocerebrosidase enzymatic activity in PD patients with glucocerebrosidase 1 mutations (glucocerebrosidase 1-PD). However, it is unknown whether this decrease in enzymatic activity leads to lysosphingolipid accumulations.
Mov Disord, 2018; 33
30146349: Emelyanov AK, Usenko TS, Tesson C, Senkevich KA, Nikolaev MA, Miliukhina IV, Kopytova AE, Timofeeva AA, Yakimovsky AF, Lesage S, Brice A, Pchelina SN
Mutation analysis of Parkinson's disease genes in a Russian data set.
Common variants and risk factors related to familial and sporadic cases of Parkinson's disease (PD) in diverse populations have been identified at numerous genomic loci. In this study, genetic analysis was performed through a screening of LRRK2 G2019S, GBA mutations (L444P, N370S), and common variants (E326K, T369M) in 762 PD patients and in 400 controls. Next-generation sequencing analysis of 22 PD-related genes in 28 early-onset PD cases from North-Western region of Russia was performed. The frequency of LRRK2 G2019S mutation was 5.8% in familial and 0.5% in sporadic PD cases. The frequency of GBA mutations (L444P, N370S) in PD patients was higher compared to controls (odds ratio [OR] = 6.9, 95% confidence interval [CI], 0.9-53.13, p = 0.031), particularly in patients with early-onset compared to late-onset PD (OR = 3.90 [95% CI, 1.2-13.2], p = 0.009). The frequency of E326K and T369M was twice higher among PD patients than in controls (OR = 2.24, 95% CI 1.05-4.79, p = 0.033). However, the screening of 22 PD-related genes using our novel panel of gene resequencing in our series of 28 early-onset PD failed to identify any mutations. LRRK2 and GBA mutations were found to be common risk factors for PD in North-Western region of Russia.
Neurobiol Aging, 2018; 71
29171494: Senkevich KA, Miliukhina IV, Beletskaia MV, Gracheva EV, Kudrevatykh AV, Nikolaev MA, Emelyanov AK, Kopytova AE, Timofeeva AA, Yakimovskii AF, Pchelina SN
[The clinical features of Parkinson's disease in patients with mutations and polymorphic variants of GBA gene].

Mutations in the glucocerebrosidase gene (GBA) increase the risk of Parkinson's disease (PD) by 6-10 times in all populations and are associated with the early-onset of PD, development of cognitive impairment and presence of psychotic disorders. At the same time, polymorphic variants associated with the twofold increase in the risk of PD were also described in the GBA gene.
Zh Nevrol Psikhiatr Im S S Korsakova, 2017; 117
30188100: Usenko TS, Miroshnikova VV, Bazhenova EA, Nikolaev MA, Brovin DL, Kopytova AE, Panteleeva AA, He J, Semenova IA, Razgildina ND, Neimark AE, Berkovich OA, Belyaeva OD, Baranova EI, Pchelina SN
ITLN1, PPARg AND TNFa GENE EXPRESSION IN VISCERAL ADIPOSE TISSUE.
The adipose tissue is considered today as an endocrine organ in which tissue-specific regulation of gene expression plays a key role in the processes of development of obesity and comorbidities, such as diabetes and cardiovascular disease. The present study is focused on ITLN1, PPARã and TNFá gene expression in intra-abdominal adipose tissue and its effect on the serum levels of omentin 1 and TNFa in individuals with different body mass. It has been shown that serum TNFa level is significantly higher in the subgroup of patients with overweight and obesity (BMI ≥ 25 kg/m2) as compared to individuals with normal body weight (BMI < 25 kg/m2)($p < 0.03$). We have demonstrated that the expression level of the PPARã gene is positively correlated with the ITLN1 gene expression level in the intra-abdominal adipose tissue ($r = 0.516$, $p = 0.020$). Serum level of omentin 1 positively correlates with PPARã mRNA and protein levels in intra-abdominal adipose tissue ($r = 0.550$, $p < 0.05$ and $r = 0.581$, $p < 0.03$, respectively). For the subgroup of patients with overweight and obesity, we have shown negative correlation of the level of TNFá mRNA with PPARã and ITLN1 mRNA levels was shown ($r = –0.549$, $p < 0.05$ and $r = –0.475$, $p < 0.05$, respectively). This study is the first to show a correlation relationship between PPARã gene expression level in the intra-abdominal adipose tissue and the expression and secretion levels of omentin 1.
Tsitologiia, 2017; 59
27780739: Pchelina S, Emelyanov A, Baydakova G, Andoskin P, Senkevich K, Nikolaev M, Miliukhina I, Yakimovskii A, Timofeeva A, Fedotova E, Abramycheva N, Usenko T, Kulabukhova D, Lavrinova A, Kopytova A, Garaeva L, Nuzhnyi E, Illarioshkin S, Zakharova E
Oligomeric α-synuclein and glucocerebrosidase activity levels in GBA-associated Parkinson's disease.
Alpha-synuclein oligomerization plays a key role in the development of Parkinson's disease (PD). Being the most common genetic contributor to PD, glucocerebrosidase 1 (GBA) mutations have been associated with decreased GBA enzymatic activity in PD patients with mutations in the GBA gene (GBA-PD). However, it is unknown whether the activities of other lysosomal hydrolases are being altered in GBA-PD patients and are accompanied by an increase in alpha-synuclein oligomerization. The aim of our study was to estimate GBA enzymatic activity as well as the activities of five other lysosomal hydrolases (galactocerebrosidase, alpha-glucosidase, alpha-galactosidase, sphingomyelinase, alpha-iduronidase) in dried blood spots with assessing plasma oligomeric alpha-synuclein levels in sporadic PD (sPD) patients, in GBA-PD patients and in controls. GBA enzymatic activity and plasma oligomeric alpha-synuclein levels were assessed in sPD patients (N=84), in GBA-PD patients (N=21) and controls (N=62) by LC-MS/MS and ELISA methods accordingly. GBA-PD patients showed lower GBA enzymatic activity compared to controls ($p=0.001$) and to sPD ($p=0.0001$). We also found the reduction of GLA enzymatic activity (but not of other lysosomal hydrolases) in GBA-PD ($p=0.001$). At the same time plasma oligomeric alpha-synuclein levels were increased in GBA-PD group compared to sPD and controls ($p=0.002$ and $p<0.0001$, respectively). Our results suggest that the decrease in enzymatic activity of lysosomal hydrolases in GBA mutation carriers may contribute to PD pathogenesis by increasing the level of neurotoxic oligomeric alpha-synuclein species.
Neurosci Lett, 2017; 636

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SUPER-RESOLVING ALPHA-SYNUCLEIN TRANSMISSION: FROM EXOSOMAL RELEASE TO DOWNREGULATION OF AXONAL RETROGRADE TRANSPORT FLUX IN RECIPIENT NEURONS

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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α-Synuclein (α-syn) is a prion-like protein involved in several neurodegenerative diseases such as Parkinson's disease. Recent studies have demonstrated that α-syn could be released in exosomes, thereby contributing to the propagation of α-syn pathology. However, the function of α-syn-containing exosomes on neuronal function is currently unknown. We recently demonstrated that synaptic activity controls the flux of retrograde carriers emanating from the nerve terminal and carrying survival signalling endosomes[1] and autophagosomes[2,3]. To determine the effect of transmitted α-syn on neuronal trafficking, we used conditioned medium from α-syn transfected hippocampal neurons, that was applied on non-transfected neurons cultured in microfluidic chambers. Following the activity-dependent uptake of cholera toxin-B subunit (CTB), time-lapse imaging of retrograde carriers in recipient neurons was then performed. Conditioned medium from α-syn^{WT}-GFP transfected hippocampal neurons downregulated the frequency of retrograde CTB carriers from non-transfected neurons. This effect was abolished using conditioned medium from α-syn^{A30P}-GFP transfected neurons and upon blockade of exosomal release by mutant Hsp90^{MD7} [4]. Transmitted α-syn^{WT}-mEos could be detected in recipient neurons and exhibited lower mobility compared to mutant α-syn^{A30P}-mEos, suggestive of active interactions. Our data demonstrate that the spreading of α-syn via exosomes controls essential neuronal trafficking flux of survival cues in recipient neurons, an effect lost with the Parkinson's disease α-syn^{A30P} mutant. **References** [1]Wang et al., Nat. Comm. 2016 [2]Wang et al., J Neurosci. 2015 [3]Wang et al., J Cell Biol. 2020 [4]Lauwers et al., Mol Cell. 2018

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BOARD NUMBER: S06-318

LOSS OF GBA ACTIVITY EXACERBATE THE TOXICITY OF ALPHA-SYNUCLEIN OLIGOMERS AND PROTOFIBRILS IN AN IN VITRO MODEL OF PARKINSON'S DISEASE.

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aims : Accumulation of misfolded alpha-synuclein (α-Syn), forming Lewy-bodies, causes mitochondrial stress and impairs autophagy-lysosomal pathway in Parkinson's disease. Mutations on GBA is a strong risk factor for Parkinson's disease. Here, we developed and characterized a novel *in vitro* model of Parkinson's disease,based on GBA-linked lysosomal dysfunctions and α-Syn toxicity **Methods** : Primary cultures of mesencephalic neurons were treated with conduritol B epoxide (CBE, 20 µM), a covalent inhibitor of glucocerebrosidase enzyme (GBA), and were injured with an αSyn solution (250 nM) containing oligomers and protofibrils from 24 hours to 96 hours. Survival of dopaminergic neurons, integrity of the neurite network and lysosomal burden was evaluate at several timepoints after injury with αSyn protofibrils with or without application of CBE. These measurements were evaluated by immunocytochemistry. The effect of ambroxol hydrochloride, a molecular chaperone for GBA, was tested in the model. **Results**: Lysosomal accumulation was the first pathological event observed, 72h after application of αSyn oligomers and protofibrils. Neuronal death and neurite network loss were then evidenced 96h after injury. In presence of CBE, neuronal loss was observed earlier, 72h after αSyn application. These results suggest that toxicity of αSyn protofibrils on dopaminergic neurons was exacerbated by the inhibition of GBA with CBE, demonstrating that lysosomal dysfunction increases αSyn toxicity. Moreover, ambroxol hydrochloride was able to reduce neuronal loss and lysosomal accumulation. **Conclusions**: Altogether, we show here that inhibiting GBA activity exacerbates the toxicity of alpha-synuclein protofibrils on dopaminergic neurons, in *in vitro* models of GBA-linked Parkinson's disease.

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MORPHOLOGICAL AND FUNCTIONAL CHANGES OF NIGRAL DOPAMINE NEURONS IN AN α-SYNUCLEIN OVEREXPRESSING RAT MODEL OF PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Ada Ledonne[1], Mariangela Massaro Cenere[1,2], Emanuela Paldino[3], Vincenza D'Angelo[2], Sebastian Luca D'Addario[1,4], Nicolas Casadei[5], Marcello D'Amelio[1,6], Nicola Berretta[1], Francesca Romana Fusco[3], Rossella Ventura[1,4], Giuseppe Sancesario[2], Ezia Guatteo[1,7], Nicola Biagio Mercuri[1,2]
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The accumulation of α-synuclein (α-syn) fibrils in the intraneuronal inclusions called Lewy bodies and Lewy neurites is the main pathological signature of Parkinson's disease (PD). While several aspects linked to α-syn-dependent pathology (i.e. the ones mainly concerning its spreading, aggregation, and activation of inflammatory and neurodegenerative processes) have been under intense investigation, less attention has been devoted to the factual impact of α-syn overexpression on structure, excitability and functional properties of substantia nigra pars compacta (SNpc) dopamine (DA) neurons, despite this has obvious relevance to comprehending the mechanisms related to PD progression. We aimed at determining the consequences of a prolonged α-synuclein overproduction on somatodendritic morphology and functions of SNpc DA neurons. Thus, we performed immunohistochemistry, stereological DA cell counts, analyses of dendritic arborization, *ex vivo* patch-clamp recordings, and *in vivo* DA microdialysis measurements in a bacterial artificial chromosome (BAC) transgenic PD rat model overexpressing the full-length human α-synuclein (*Snca*$^{+/+}$), and in wildtype (WT) rats of 12-13 months of age. Here, we describe compensatory mechanisms that nigral DA neurons adopt during PD progression to functionally counteract a developing neurodegeneration.

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DEVELOPING 2-D AND 3-D MODELS TO DISENTANGLE THE INTERPLAY BETWEEN MITOCHONDRIAL VULNERABILITY AND INFLAMMATION IN PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Helena Winterberg, Flora Magno, Jana Heneine, Benjamin Galet, Noemi Asfogo, Julie Smeyers, Morwena Latouche, Aurore Tourville, Patrick P. Michel, Jean-Christophe Corvol, Philippe Ravassard, Olga Corti
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Neuroinflammation and mitochondrial dysfunction contribute to the susceptibility and progression of Parkinson's disease (PD), emer
develop 2D and 3D mesencephalic culture models to explore how the hyperactivation of the NLRP3 inflammasome pathway in a Pa
models are established by culturing mouse wildtype (WT) or *Prkn-/-* microglia (P0) on top of WT embryonic mesencephalic neurons
activator 2'(3')-O-(4-benzoylbenzoyl)-adenosine-5'-triphosphate (BzATP). In 3D, we enrich human iPSC-derived midbrain organoids
seeking the optimal conditions for their viable long-term integration in the neuronal environment. Our preliminary results confirm that
/- microglia. As no clear toxicity was observed in the acute treatment paradigm, current modifications involve the chronic exposure to
first indications of increased expression of DA markers in the enriched hMOs. In current work, we investigate the effects of hMDMi o
generated with hMDMi from patients with *PRKN* mutations. Altogether, these approaches should provide novel insight into the comp

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URIC ACID REGULATES NEURON-TO NEURON α-SYNUCLEIN TRANSMISSION IN PARKINSONIAN MICROENVIRONMENT

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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There is ample evidence that α-syn plays a critical role in the pathogenesis of Parkinson's disease (PD) and propagation of α-syn from one brain area to the other areas through neuron-to-neuron transmission could be the primary mechanism for disease progression in parkinsonian patients. The inhibition of α-synuclein transmission throughout brain areas would be the potential disease-modifying strategy to treat PD. UA, a natural antioxidant in the body, has been studied as a potential drug candidate in various neurodegenerative diseases including Parkinson's disease. In the present study, we hypothesized that UA would regulate neuron-to-neuron transmission of α-synuclein in α-synuclein-enriched environment. In a cellular model, high-UA condition reduced the levels of internalized cytosolic α-synuclein not affecting the autophagic pathway and decreased the neuron-to neuron transmission of α-synuclein in α-synuclein-overexpressed BiFC system. In addition, we examined whether elevating serum UA levels in α-synuclein-inoculated parkinsonian model would inhibit extracellular α-synuclein transmission from dorsal striatum to substantia nigra (SN). UA-elevating therapy markedly decreased the neuron-to-neuron transmission of extracellular α-synuclein, which exerted neuroprotective effects on nigral dopaminergic neurons against α-synuclein-enriched conditions. These findings suggest that UA-elevating strategy could be a potential disease-modifying therapy in treatment of parkinson's disease.

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PRIMING MESENCHYMAL STEM CELLS WITH α-SYNUCLEIN ENHANCES NEUROPROTECTIVE PROPERTIES THROUGH INDUCTION OF AUTOPHAGY IN PARKINSONIAN MODELS

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Mesenchymal stem cells (MSCs) are a potential source of cell-based disease-modifying therapy for parkinsonian disorders. As knowledge regarding the therapeutic properties of MSCs accumulates, some obstacles still remain to be overcome, especially, successful clinical translation requires the development of culture systems that mimic the natural MSC niche, while allowing clinical-scale cell expansion without compromising quality and function of the cells. In recent years, priming approaches using bioactive peptide or complement components have been investigated to enhance the therapeutic potential of MSCs. Here, we investigated an innovative priming strategy by conditioning the MSCs with α-synuclein (α-syn). Treatment of naïve MSCs with α-syn upregulated transcriptional factors responsible for regulation of stemness, which was associated with the elevated expression of genes involved in glycolysis and cell re-programming. Primed MSCs with α-syn enhanced the expression of autophagy-regulating miRNA, and exosomes derived from primed MSCs were packed with autophagy-associated miRNA. In a parkinsonian cellular model, primed MSCs with α-syn enhanced neuronal viability relative to naïve MSCs, through the induction of autophagy and lysosome activity. Moreover, several studies using an α-syn over-expressed parkinsonian animal model showed that the pro-survival effect of MSCs on dopaminergic neurons was more prominent in primed MSC-treated mice compared with that in naïve MSC-treated mice. These findings provide a new insight into the role of autophagy in α-syn-induced MSC priming, suggesting that MSC priming with α-syn can be a useful strategy to advance the application of MSCs for the treatment of parkinsonian disorders.

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HIF-1α SIGNALLING AS A THERAPEUTIC STRATEGY IN NEURONAL SURVIVAL

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive neurodegenerative movement disorder characterized by the selective degeneration of a specific subset of dopamine-producing neurons. To date, treatments have focused on compensating dopamine deficiency without addressing the progressive neuronal cell damage. PD related dopaminergic cell death has been associated with environmental factors and genetic predisposition leading to molecular pathway disruption that regulates mitochondrial function, reactive oxygen species and protein degradation. Recent evidence suggests that events causing transient reductions in neuronal oxygen supply (hypoxia) may trigger changes in these key parameters and thereby influence PD development. Indeed, HIF-1α, the master transcriptional regulator of the cellular response to hypoxia, can target these specific disrupted pathways in PD by activating genes involved in mitochondrial fitness, oxidative stress, autophagy and dopamine production, potentially alleviating neuronal damage. Therefore, novel compounds that stabilize HIF-1α may constitute a potential therapeutic strategy for neuronal survival. Our results show how PD-related neuronal stress can block HIF-1α stabilization and influence the molecular processes that lead to neuronal degeneration. Indeed, agents causing mitochondrial damage and increased ROS levels, diminish HIF-1α stabilization in neural cell models. We have subsequently studied a set of novel HIF-1α stabilizing compounds, that reduce PD-related neuronal damage by maintaining mitochondrial integrity, function and dynamics whilst decreasing ROS levels and significantly improving neuronal survival. This constitutes a potential therapeutic strategy to improve neuronal function in PD.

Pubmed:

31781334: Roca-Agujetas V, de Dios C, Lestón L, Marí M, Morales A, Colell A
Recent Insights into the Mitochondrial Role in Autophagy and Its Regulation by Oxidative Stress.
Autophagy is a self-digestive process that degrades intracellular components, including damaged organelles, to maintain energy homeostasis and to cope with cellular stress. Autophagy plays a key role during development and adult tissue homeostasis, and growing evidence indicates that this catalytic process also has a direct role in modulating aging. Although autophagy is essentially protective, depending on the cellular context and stimuli, autophagy outcome can lead to either abnormal cell growth or cell death. The autophagic process requires a tight regulation, with cellular events following distinct stages and governed by a wide molecular machinery. Reactive oxygen species (ROS) have been involved in autophagy regulation through multiple signaling pathways, and mitochondria, the main source of endogenous ROS, have emerged as essential signal transducers that mediate autophagy. In the present review, we aim to summarize the regulatory function of mitochondria in the autophagic process, particularly regarding the mitochondrial role as the coordination node in the autophagy signaling pathway, involving mitochondrial oxidative stress, and their participation as membrane donors in the initial steps of autophagosome assembly.
Oxid Med Cell Longev, 2019; 2019
34439955: Lestón Pinilla L, Ugun-Klusek A, Rutella S, De Girolamo LA
Hypoxia Signaling in Parkinson's Disease: There Is Use in Asking "What HIF?"
Hypoxia is a condition characterized by insufficient tissue oxygenation, which results in impaired oxidative energy production. A reduction in cellular oxygen levels induces the stabilization of hypoxia inducible factor α (HIF-1α), master regulator of the molecular response to hypoxia, involved in maintaining cellular homeostasis and driving hypoxic adaptation through the control of gene expression. Due to its high energy requirement, the brain is particularly vulnerable to oxygen shortage. Thus, hypoxic injury can cause significant metabolic changes in neural cell populations, which are associated with neurodegeneration. Recent evidence suggests that regulating HIF-1α may ameliorate the cellular damage in neurodegenerative diseases. Indeed, the hypoxia/HIF-1α signaling pathway has been associated to several processes linked to Parkinson's disease (PD) including gene mutations, risk factors and molecular pathways such as mitochondrial dysfunction, oxidative stress and protein degradation impairment. This review will explore the impact of hypoxia and HIF-1α signaling on these specific molecular pathways that influence PD development and will evaluate different novel

neuroprotective strategies involving HIF-1α stabilization. Biology (Basel), 2021; 10

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PROTEOMIC ANALYSIS OF THE EFFECTS OF PARKINSON'S DISEASE MIMETICS IN NEURAL CELL POPULATIONS: IDENTIFICATION OF NOVEL BIOMARKERS OF DEGENERATION

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Transglutaminase 2 is a ubiquitous enzyme that belongs to transglutaminase family, which consists of nine multifunctional proteins evolved from the papain family and is expressed in various cells and tissues. It acts by catalyzing the Ca^{2+}-dependent covalent crosslinking between γ-carboxamide group of peptide-bound glutamine and primary amine group of lysine residue bound to a polypeptide or protein, producing a proteolytically-resistant isopeptide bond. TG2 has been shown to be upregulated in various types of neurodegenerative diseases, including Parkinson’s disease (PD). This association between TG2 and neurodegeneration has focused on TG2 structure and mechanism of action, highlighting the importance of TG2 activity and post-translational modifications. Aggregates of α-synuclein have been found in tissues of patients with PD, as well as in other neuronal aging-related diseases, such as Alzheimer's and Huntingtin diseases. Consequently, α-synuclein was found to be a target protein of TG2 in cellular models of PD supported specifically by studies of toxin-induced mitochondrial dysfunction. Our aim was to use neuronal cell culture models that simulate various molecular lesions that occur in a parkinsonian nervous system, such as proteasomal inhibition, mitochondrial complex 1 inhibition and lipid peroxidation, all of which eventually result in protein aggregation in those neurons. The transamidating activity of TG2 was quantified under PD-mimetic conditions in vitro using cell viability and biotin-cadaverine incorporation assays to determine whether the role of TG2 is neuro-protective or neurotoxic under different PD-mimetic conditions and determine its potential as a molecular biomarker of disease and/or a target for therapeutic intervention to slow neuronal aging.

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POTENTIAL ROLE OF THE SECRETOME IN PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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The secretome is comprised of small molecules, proteins and lipids, the importance of which has only started to be unraveled both in health and in pathologies, such as cancer and neurodegenerative diseases. Leucine-riche repeat kinase 2 (LRRK2) is associated to Parkinson disease and was suggested to regulate endolysosomal dynamics, a process which relies particularly on membrane fusion mediated by SNAREs. In addition, we previously found that the interaction between the vesicular SNARE VAMP7 and LRRK1, a molecule closely related to LRRK2, regulated lysosomal secretion (Wang et al, iScience 2018). We recently investigated the biochemical links between LRRK2 and vesicular SNAREs and their potential cellular functions. We found that LRRK2 interacted with the post-Golgi v-SNAREs VAMP4, VAMP7 and VAMP8. Parkinson's disease related R1441C mutant of LRRK2 was the most efficient VAMP4 interactor and lead to impaired VAMP4 subcellular localization. Yeast-2-Hybrid assay identified the interaction of VAMP partner Snapin with the COR domain of LRRK2. LRRK2, Snapin and VAMP4 were found in the same protein complex. Secretomics showed that VAMP4- and VAMP7-KO neuronal cells secreted less pro-VGF, a Parkinson's disease potential biomarker. The R1441C mutant of LRRK2 affected the subcellular localization of VGF and LRRK2 expression inhibited the secretion of pro-VGF. Altogether, these results suggest that LRRK2 might regulate secretion via interaction with VAMP4 and VAMP7 (Filippini et al, SSRN 2021). Here we followed up by investigating the effect of overexpressing WT and pathological LRRK2 mutants in neuronal cells expressing or not VAMP7.

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RARE VARIANTS OF TMEM175 CONCUR WITH PARKINSON'S DISEASE PATHOGENESIS.

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aims: We recently identified 16 novel genes associated with Parkinson's disease (PD). In this study, we focused the attention on the mutations identified in the lysosomal potassium channel TMEM175, necessary for the maintenance of lysosomal acidity. **Methods**: Mutations were identified through the analysis of the our sequence data set (400 cases and 210 controls). The expression profile of *TMEM175* was investigated by qPCR and immunofluorescence (IF). The functional properties of the mutated channel were characterized by patch-clamp technique. The interaction of TMEM175 with AKT regulatory factor was analyzed by co-immunoprecipitation experiments. **Results**: We identified 13 novel deleterious and highly penetrant mutations, affecting important domains of the protein, in 23 PD patients representing the 5.7% of our cohort of patients. None of these variants was identified in healthy subjects, suggesting an important genetic role in PD pathogenesis for this gene. IF experiments in patients-derived fibroblasts showed impaired autophagic flux. Moreover, electrophysiology studies demonstrated the loss of the functional properties of the channel in presence of some mutations identified in patients. Interestingly, these mutations also affected the interaction with the protein kinase AKT, recently identified as a key regulator of TMEM175. Finally, IF analysis on mouse brain showed a higher level of expression of TMEM175 on microglia compared to those observed in neurons, suggesting a possible role of the protein in the inflammatory mechanism underlying neurodegenerative diseases. **Conclusion**: Our data suggest a key role for the mutations identified in *TMEM175* in inflammatory and autophagic processes paving the way for novel therapeutic approaches.

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CONTRIBUTION OF GBA1 MUTATIONS TO AUTOPHAGY-LYSOSOMAL PATHWAY IN PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Mikhail Nikolaev[1,2], Alena Kopytova[1], Artem Izymchenko[1], Margarita Gorchakova[2], Darya Bogdanova[1], Irina Miliukhina[2,3], Yury Kovalchuk[2], Sofya Pchelina[1,2]
[1]Petersburg Nuclear Physics Institute named by B.P.Konstantinov of NRC «Kurchatov Institute», Molecular And Radiation Biophysics Division, Gatchina, Russian Federation, [2]Pavlov First Saint Petersburg State Medical University, Research Center, Saint-Petersburg, Russian Federation, [3]N.P. Bechtereva Institute of the Human Brain, Center For Neurodegenerative Diseases And Botulinum Therapy, Saint-Petersburg, Russian Federation

Backgound GBA1 mutations are the most common known genetic cause of Parkinson's disease (PD). Its biological pathway may be important, since activity of the enzyme encoded by glucocerebrosidase (GCase) is reduced even among individuals with GBA mutations (GBA-carriers). Several studies have also suggested that *GBA1* dysfunction may lead to general lysosomal dysfunction and disruption of autophagy *Aim*
To study the effect of *GBA1* mutations on autophagy in primary macrophages from patients with PD and GBA-carriers. *Materials and Methods* Spontaneous autophagy in primary macrophages of GBA1-PD patients (N=9), GBA1-carriers (N=10) and controls (N=12) was assessed by Autophagy Detection kit (Abcam, UK) using a specific dye autophagosomes marker LC3-II. Primary macrophages were cultured as described earlier (Kopytova A et al., 2021.10;84:112-121) *Results* For the first time we detected an increased concentration of autophagosomes, due to an increased concentration of the LC3-II marker in primary macrophages from GBA1-carriers 14.45 (9.60-21.38) CU and GBA1-PD patients 15.60 (6.70-22.62) CU compared to controls 10.33 (6.21- 12.70) CU ($p=0.003$ and $p=0.027$), respectively. An increased number of autophagosomes in macrophages may indicate a defect in macro-autophagy pathway due to autophagosomes inability for proper fusion with the lysosome. The *GBA1* mutations severity effect on autophagy was not detected. *Conclusion* Our results suggest that GBA1 mutations could lead to autophagy disfunction independent of PD status. The study was funded by RSF №19-15-00315.

Pubmed:
33609962: Kopytova AE, Rychkov GN, Nikolaev MA, Baydakova GV, Cheblokov AA, Senkevich KA, Bogdanova DA, Bolshakova OI, Miliukhina IV, Bezrukikh VA, Salogub GN, Sarantseva SV, Usenko TC, Zakharova EY, Emelyanov AK, Pchelina SN
Ambroxol increases glucocerebrosidase (GCase) activity and restores GCase translocation in primary patient-derived macrophages in Gaucher disease and Parkinsonism.
Mutations in the glucocerebrosidase gene (GBA) encoding the lysosomal enzyme glucocerebrosidase (GCase) cause Gaucher disease (GD) and are the most commonly known genetic risk factor for Parkinson disease (PD). Ambroxol is one of the most effective pharmacological chaperones of GCase. Fourteen GD patients, six PD patients with mutations in the GBA gene (GBA-PD), and thirty controls were enrolled. GCase activity and hexosylsphingosine (HexSph) concentration were measured in dried blood and macrophage spots using liquid chromatography coupled with tandem mass spectrometry. The effect of ambroxol on GCase translocation to lysosomes was assessed using confocal microscopy. The results showed that ambroxol treatment significantly increased GCase activity in cultured macrophages derived from patient blood monocytic cell (PBMC) of GD (by 3.3-fold) and GBA-PD patients (by 3.5-fold) compared to untreated cells ($p < 0.0001$ and $p < 0.0001$, respectively) four days after cultivation. Ambroxol treatment significantly reduced HexSph concentration in GD (by 2.1-fold) and GBA-PD patients (by 1.6-fold) ($p < 0.0001$ and $p < 0.0001$, respectively). GD macrophage treatment resulted in increased GCase level and increased enzyme colocalization with the lysosomal marker LAMP2. The possible binding modes of ambroxol to mutant GCase carrying N370S amino acid substitution at pH 4.7 were examined using molecular docking and molecular dynamics simulations. The ambroxol position characterized by minimal binding free energy was observed in close vicinity to the residue, at position 370. Taken together, these data showed that PBMC-derived macrophages could be used for assessing ambroxol therapy response for GD patients and also for GBA-PD patients.
Parkinsonism Relat Disord, 2021; 84
32336641: Usenko TS, Nikolaev MA, Miliukhina IV, Bezrukova AI, Senkevich KA, Gomzyakova NA, Beltceva YA, Zalutskaya

NM, Gracheva EV, Timofeeva AA, Petrova OA, Semenov AV, Lubimova NE, Totolyan AA, Pchelina SN
Plasma cytokine profile in synucleinophaties with dementia.
Immune response may play a pivotal role in the pathogenesis of the common synucleinopathy as Parkinson's disease (PD) and could be mediated with the accumulation of neurotoxic alpha-synuclein. There is limited evidence for immune response in another synucleinopathy as dementia with Lewy bodies (DLB). Recent data suggest that immune response may contribute to cognitive impairment. We aimed to estimate plasma cytokine profile in patients with synucleinopathies with dementia (PD dementia (PDD), DLB). Plasma cytokine levels (interferon-gamma (IFN-gamma), interleukin (IL)-4 (IL-4), IL-6, IL-10, tumor necrosis factor alpha (TNF-alpha), monocyte chemoattractant protein-1 (MCP-1)). were estimated in 16 patients with DLB, 19 patients with PDD, 28 patients with PD without dementia (PD) and 19 individuals without neurological disorders (controls) using Luminex array system. Cognitive status was assessed with the Mini-Mental State Examination (MMSE). TNF-alpha and IL-6 plasma levels were elevated in patients with synucleinopathies with dementia (DLB, PDD) compared to controls and IL-10 plasma level was increased in PDD compared to controls ($p < 0.05$). IFN-gamma levels were decreased in PD and PDD patients compared to controls ($p < 0.001$, $p = 0.026$, respectively) and in PD patients than in DLB patients ($p = 0.032$). Patients with PD, PDD, and DLB were characterized by increased plasma levels of MCP-1 compared to controls ($p < 0.001$). At the same time, no differences in TNF-alpha, IL-10, IL-6 plasma levels in PD patients compared to controls were found. Our study demonstrated more pronounced immune response in synucleinopathies associated with dementia compared to PD without demetia.
J Clin Neurosci, 2020; 78
30146349: Emelyanov AK, Usenko TS, Tesson C, Senkevich KA, Nikolaev MA, Miliukhina IV, Kopytova AE, Timofeeva AA, Yakimovsky AF, Lesage S, Brice A, Pchelina SN
Mutation analysis of Parkinson's disease genes in a Russian data set.
Common variants and risk factors related to familial and sporadic cases of Parkinson's disease (PD) in diverse populations have been identified at numerous genomic loci. In this study, genetic analysis was performed through a screening of LRRK2 G2019S, GBA mutations (L444P, N370S), and common variants (E326K, T369M) in 762 PD patients and in 400 controls. Next-generation sequencing analysis of 22 PD-related genes in 28 early-onset PD cases from North-Western region of Russia was performed. The frequency of LRRK2 G2019S mutation was 5.8% in familial and 0.5% in sporadic PD cases. The frequency of GBA mutations (L444P, N370S) in PD patients was higher compared to controls (odds ratio [OR] = 6.9, 95% confidence interval [CI], 0.9-53.13, $p = 0.031$), particularly in patients with early-onset compared to late-onset PD (OR = 3.90 [95% CI, 1.2-13.2], $p = 0.009$). The frequency of E326K and T369M was twice higher among PD patients than in controls (OR = 2.24, 95% CI 1.05-4.79, $p = 0.033$). However, the screening of 22 PD-related genes using our novel panel of gene resequencing in our series of 28 early-onset PD failed to identify any mutations. LRRK2 and GBA mutations were found to be common risk factors for PD in North-Western region of Russia.
Neurobiol Aging, 2018; 71
27420620: Miroshnikova VV, Panteleeva AA, Bazhenova EA, Demina EP, Usenko TS, Nikolaev MA, Semenova IA, Neimark AE, He J, Belyaeva OD, Berkovich OA, Baranova EI, Pchelina SN
[Regulation of ABCA1 and ABCG1 gene expression in the intraabdominal adipose tissue].
Tissue specific expression of genes encoding cholesterol transporters ABCA1 and ABCG1 as well as genes encoding the most important transcriptional regulators of adipogenesis - LXRa, LXRb, PPARg and RORa has been investigated in intraabdominal adipose tissue (IAT) samples.A direct correlation between the content of ABCA1 and ABCG1 proteins with RORa protein level ($r=0.480$, $p<0.05$; $r=0.435$, $p<0.05$, respectively) suggests the role of the transcription factor RORa in the regulation of IAT ABCA1 and ABCG1 protein levels. ABCA1 and ABCG1 gene expression positively correlated with obesity indicators such as body mass index (BMI) ($r=0.522$, $p=0.004$; $r=0.594$, $p=0.001$, respectively) and waist circumference ($r=0.403$, $p=0.033$; $r=0.474$, $p=0.013$, respectively). The development of obesity is associated with decreased IAT levels of RORa and LXRb mRNA ($p=0.016$ and $p=0.002$, respectively). These data suggest that the nuclear factor RORa can play a significant role in the regulation of cholesterol metabolism and control IAT expression of ABCA1 and ABCG1, while the level of IAT LXRb gene expression may be an important factor associated with the development of obesity.
Biomed Khim, 2016; 62
27228655: Emelyanov AK, Andoskin PA, Miliukhina IV, Timofeeva AA, Yakimovskii AF, Senkevich KA, Nikolaev MA, Pchelina SN
[SNCA rs356219 AND rs356165 VARIANTS ARE ASSOCIATED WITH PARKINSON'S DISEASE AND INCREASED ALPHA-SYNUCLEIN GENE EXPRESSION IN THE CD45(+)-BLOOD CELLS].
Impaired metabolism of alpha-synuclein (SNCA) and its aggregation are now implicated in the pathogenesis of Parkinson's disease (PD). Previous studies have found association between PD and gene locus, containing the SNCA gene. Meta-analysis have shown high significant association of single nucleotide polymorphisms (SNPs) rs356165 (A/G) and rs356219 (A/G) in the SNCA gene with PD. We genotyped these SNPs in 260 PD patients and 262 controls from north-western region

of Russia. Alleles "G" of rs356165 and rs356219 were associated with increased risk of PD development. Linkage disequilibrium was shown between associated marker alleles. We studied the relationship between rs356165 and rs356219 and levels of mRNA SNCA and alpha-synuclein in CD45+ peripheral blood cells in drug-naive PD patients (n = 43) and controls (n = 39). Alleles "G" of rs356165 and rs356219 were associated with increased levels of SNCA expression (p = 0.046) and high alpha-synuclein levels (p = 0.039) in controls. Our data suggest that rs356165 and rs356219 variants might influence on PD development by upregulating SNCA expression.
Tsitologiia, 2016; 58

25265039: Pchelina SN, Nuzhnyi EP, Emelyanov AK, Boukina TM, Usenko TS, Nikolaev MA, Salogub GN, Yakimovskii AF, Zakharova EY

Increased plasma oligomeric alpha-synuclein in patients with lysosomal storage diseases.
A link between lysosomal storage diseases (LSDs) and neurodegenerative disorders associated with accumulation of presynaptic protein alpha-synuclein has been shown. Particularly, Gaucher disease (GD) patients with a deficiency of the lysosomal enzyme glucocerebrosidase (GBA) and carriers of GBA mutations are at increased risk of Parkinson's disease (PD). It remains unclear whether this link is due to increased alpha-synuclein oligomerization. Here we show that level of oligomeric alpha-synuclein form, associated with PD development, is increased in plasma of GD patients (n=41, median=22.9pg/mL, range1.57-444.58pg/mL; controls (n=40, median=6.02pg/mL, range 1.05-103.14pg/mL, p<0.0001). This difference is absent in GD patients receiving enzyme replacement therapy (ERT) for more than 5 years. Moreover, the levels of alpha-synuclein oligomers in plasma are also higher in patients with other LSDs (Niemann-Pick type C, Krabbe disease, Wolman disease) compared to the median value in controls. Therefore, we suggest that mutations in the GBA gene and at least in several other LSDs genes may be associated with an increase in oligomeric alpha-synuclein in plasma. ERT applied for recovering of GBA functions in GD treatment might decrease formation of plasma oligomeric alpha-synuclein.
Neurosci Lett, 2014; 583

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BOARD NUMBER: S06-328

NOVEL SYNTHETIC 1,5-DIARYL PYRROLE DERIVATIVES PROTECT PC12 CELLS AGAINST 6-OHDA- INDUCED NEUROTOXICITY

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive neurodegenerative disease that affects movement. Its exact etiology is not completely understood. Oxidative stress is considered as one of the factors involved in the pathogenesis of PD. Pyrrole containing compounds are used in a very diverse range of pharmaceuticals products and its derivatives have shown various type of biologic activity such as anti-inflammation and antinociception. we evaluated for the first time, the neuroprotective effect of pyrrole derivative compounds as a potential neuroprotective agent in an *in vitro* model of PD. In the current study we applied three novel synthetic pyrrole derivative compounds 2-(4-chlorophenyl)-5-methyl-1-(4-(trifluoromethoxy)phenyl)-1H-pyrrole, 2-(4-chlorophenyl)-1-(4-methoxyphenyl)-5-methyl-1H-pyrrole and 1-(2-chlorophenyl)-2-(4-chlorophenyl)-5-methyl-1H-pyrrole to investigate their potential neuroprotective effects. PC12 cell line incubated with 6-hydroxydopamine (6-OHDA) to induce cell damage. PC12 cells were pre-treated with pyrrole compounds for 24 hours prior exposed to 6-OHDA. Cell viability assay (MTT), apoptosis (Hoechst staining, Annexin V), oxidative stress (ROS, lipid peroxidation assays), were performed to evaluate their neuroprotective effect. we demonstrated pretreatment with pyrrole derivates compounds for 24 h protected the PC12 cells against 6-OHDA neurotoxicity. This protective role is associated with the increased cell viability. In addition, pyrrole derivates compounds suppressed 6-OHDA mediate cell death by decreasing intracellular ROS and attenuating intracellular lipid peroxidation, which led to apoptosis cell death revealing by Hoechst and Annexin-positive cells.These findings suggest that the synthetic pyrrole derivates exerts neuroprotective effects in this *in vitro* model of PD likely through antioxidation mechanisms. Therefore, they could be considered as the potential neuroprotective strategy to suppress the PD progression.

Pubmed:

31006075: Javid H, Rezayof A, Ghasemzadeh Z, Sardari M
The involvement of ventral hippocampal microglial cells, but not cannabinoid CB1 receptors, in morphine-induced analgesia in rats.
It is well known that glial cells are involved in pain processing. The purpose of the present study was to investigate the possible involvement of the ventral hippocampal (VH) glial cells in morphine-induced analgesia. A tail-flick apparatus was used to measure pain sensitivity in male Wistar rats that were bilaterally cannulated in the VH by stereotaxic surgery. The results showed that intraperitoneal (i.p.) administration of morphine (2.5-7.5 mg/kg) induced analgesia in a time-dependent manner. The blockade of the VH glial cell activation by bilateral microinjection of a glial inhibitor, minocycline (5-15 µg/rat) into the VH with an ineffective dose of morphine (2.5 mg/kg, i.p) significantly increased morphine analgesia. Considering that the endocannabinoid system via CB1 receptors play a crucial role in pain modulation, we also assessed the possible role of the VH cannabinoid CB1 receptors in the functional interaction between minocycline and morphine in acute pain. Our results indicated that intra-VH injection of the cannabinoid CB1 receptor agonist, arachidonylcyclopropylamide (ACPA; 4-12 ng/rat) had no effect on minocycline-induced potentiation of morphine analgesia. It should be considered that intra-VH microinjection of minocycline or ACPA by itself had no effect on tail-flick latency. Our findings suggest that the activation of the VH microglial cells may be involved in mediating pain sensation, because the inhibition of these cells by intra-VH injection of minocycline could potentiate morphine-induced analgesia. Although endocannabinoids have a regulatory role in glia function, the activation of CB1 receptors could not affect the potentiative effect of minocycline on morphine analgesia.
Acta Neurol Belg, 2020; 120

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BOARD NUMBER: S06-329

RESCUE OF DOPAMINE NEURONS FROM IRON-DEPENDENT FERROPTOSIS BY DOXYCYCLINE AND DDMC, A NOVEL DERIVATIVE OF DEMECLOCYCLINE LACKING ANTIBIOTIC ACTIVITY

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Doxycycline (DOX), an old tetracycline (TC) antibiotic primarily used for skin problems, recently demonstrated its efficacy in model systems that relate to Parkinson disease (PD) neurodegeneration. The aim of the present work was two-fold: (i) establish a model system to further characterize DOX-mediated neuroprotection and (ii) take advantage of this system to discover novel TCs having better protective effects than DOX and no antibiotic activity. For that, we established cultures of mouse midbrain dopamine (DA) neurons and implemented culture conditions in which these neurons degenerate progressively by iron-mediated ferroptosis. The non-antibiotic derivative of demeclocycline (DMC), DDMC was synthesized by reductions of the dimethylamino function at position 4 and the hydroxyl group at position 12a of ring A of DMC. We found that DOX and DDMC efficiently protect DA neurons from degeneration, DDMC being, however, far more potent than DOX, regardless of cell culture age. Live imaging of reactive oxygen species (ROS) and mitochondrial membrane potential with DHR-123 and TMRM, respectively, revealed that DOX and DDMC curtailed intracellular ROS production and preserved mitochondrial function. The iron chelator desferrioxamine, the inhibitor of lipid peroxidation Trolox and the ferroptosis inhibitor liproxstatin-1 reproduced all the effects of DOX and DDMC, indicating that both TCs inhibited iron-dependent ferroptosis. Noticeably, non-TC antibiotics such as streptomycin, erythromycin and penicillin-G failed to reproduce DOX-mediated neuroprotection. Overall, our data indicate that DOX and its non-antibiotic derivative DDMC have the potential to interfere efficiently with ferroptosis pathways that could intervene in PD neurodegeneration.

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SAPOSIN C REDUCES LEVELS OF α-SYNUCLEIN AND DISLODGES IT FROM GLUCOSYLCERAMIDE-ENRICHED LIPID MEMBRANES

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aims: This study aims to investigate the molecular mechanisms of the neuroprotective roles of prosaposin (PSAP) and saposin C in α-synuclein pathology in Parkinson's disease (PD). ***Background:*** PSAP is a secreted protein that serves as a neuroprotective factor. PSAP is uptaken by cells and cleaved in the lysosome, yielding four saposins that work as sphingolipid activators. Among four saposins, saposin C is required for the normal function of glucocerebrosidase (GCase). Mutations in the GBA1 gene, which encodes GCase, is one of the most common risk factors of developing PD. Therefore, PSAP and saposin C has been attracting attention as disease modifier of PD. However, the molecular mechanisms underlying the neuroprotective role of PSAP and saposin C remains unclear. ***Methods:*** By genetically modulating the expression levels of PSAP on SH-SY5Y cells, we investigated the effect of PSAP in α-synuclein pathology. Expression levels of α-synuclein in PSAP stable overexpressed cells and PSAP knockdown cells were assessed by qPCR and Western Blot. The binding of α-synuclein to glucosylceramide-enriched lipid membrane under the existence of saposin C was investigated. ***Results:*** Stable overexpression of PSAP significantly decreased both intracellular and extracellular α-synuclein levels. On the contrary, siRNA knockdown of PSAP increased α-synuclein levels. GCase inhibitor treatment demonstrated that the altered levels of α-synuclein were independent of GCase activity. Under the lysosomal pH, saposin C detached α-synuclein from artificial lipid membranes. ***Conclusions:*** PSAP and saposin C can enhance α-synuclein clearance by detaching it from the lysosomal membrane where α-synuclein forms aggregations.

Pubmed:

27439492: Okamura H, Doi M, Goto K, Kojima R
Clock genes and salt-sensitive hypertension: a new type of aldosterone-synthesizing enzyme controlled by the circadian clock and angiotensin II.
With the current societal norm of shiftwork and long working hours, maintaining a stable daily life is becoming very difficult. An irregular lifestyle disrupts circadian rhythms, resulting in the malfunction of body physiology and ultimately leading to lifestyle-related diseases, including hypertension. By analyzing completely arrhythmic Cry1/Cry2 double-knockout (Cry-null) mice, we found salt-sensitive hypertension accompanied by hyperaldosteronism. On the basis of a DNA microarray analysis of the adrenal gland and subsequent biochemical analyses, we discovered that Hsd3b6/HSD3B1, a subtype of 3β-HSD, is markedly overexpressed in aldosterone-producing cells in the Cry-null adrenal cortex. In addition, we found that Hsd3b6/HSD3B1, which converts pregnenolone to progesterone, is a clock-controlled gene and might also be a key enzyme for the regulation of aldosterone biosynthesis, in addition to the previously established CYP11B2, which synthesizes aldosterone from deoxycorticosterone. Importantly, angiotensin II induces HSD3B1 via the transcription factor NGFIB in human adrenocortical H295R cells, similarly to CYP11B2. As HSD3B1 levels are abnormally high in the adrenal aldosterone-producing cells of idiopathic hyperaldosteronism (IHA), the temporal component of this system in the pathophysiology of IHA is a promising area for future research.
Hypertens Res, 2016; 39
29784786: Fustin JM, Kojima R, Itoh K, Chang HY, Ye S, Zhuang B, Oji A, Gibo S, Narasimamurthy R, Virshup D, Kurosawa G, Doi M, Manabe I, Ishihama Y, Ikawa M, Okamura H
Two transcripts regulated by m6A methylation code for two antagonistic kinases in the control of the circadian clock.
The -methylation of internal adenosines (m6A) in mRNA has been quantified and localized throughout the transcriptome. However, the physiological significance of m6A in most highly methylated mRNAs is unknown. It was demonstrated previously that the circadian clock, based on transcription-translation negative feedback loops, is sensitive to the general inhibition of m6A. Here, we show that the Casein Kinase 1 Delta mRNA (), coding for a critical kinase in the control of circadian rhythms, cellular growth, and survival, is negatively regulated by m6A. Inhibition of mRNA methylation leads to increased translation of two alternatively spliced CK1δ isoforms, CK1δ1 and CK1δ2, uncharacterized until now. The

expression ratio between these isoforms is tissue-specific, CK1δ1 and CK1δ2 have different kinase activities, and they cooperate in the phosphorylation of the circadian clock protein PER2. While CK1δ1 accelerates the circadian clock by promoting the decay of PER2 proteins, CK1δ2 slows it down by stabilizing PER2 via increased phosphorylation at a key residue on PER2 protein. These observations challenge the previously established model of PER2 phosphorylation and, given the multiple functions and targets of CK1δ, the existence of two isoforms calls for a re-evaluation of past research when CK1δ1 and CK1δ2 were simply CK1δ.
Proc Natl Acad Sci U S A, 2018; 115

32376902: Fustin JM, Ye S, Rakers C, Kaneko K, Fukumoto K, Yamano M, Versteven M, Grünewald E, Cargill SJ, Tamai TK, Xu Y, Jabbur ML, Kojima R, Lamberti ML, Yoshioka-Kobayashi K, Whitmore D, Tammam S, Howell PL, Kageyama R, Matsuo T, Stanewsky R, Golombek DA, Johnson CH, Kakeya H, van Ooijen G, Okamura H
Methylation deficiency disrupts biological rhythms from bacteria to humans.
The methyl cycle is a universal metabolic pathway providing methyl groups for the methylation of nuclei acids and proteins, regulating all aspects of cellular physiology. We have previously shown that methyl cycle inhibition in mammals strongly affects circadian rhythms. Since the methyl cycle and circadian clocks have evolved early during evolution and operate in organisms across the tree of life, we sought to determine whether the link between the two is also conserved. Here, we show that methyl cycle inhibition affects biological rhythms in species ranging from unicellular algae to humans, separated by more than 1 billion years of evolution. In contrast, the cyanobacterial clock is resistant to methyl cycle inhibition, although we demonstrate that methylations themselves regulate circadian rhythms in this organism. Mammalian cells with a rewired bacteria-like methyl cycle are protected, like cyanobacteria, from methyl cycle inhibition, providing interesting new possibilities for the treatment of methylation deficiencies.
Commun Biol, 2020; 3

32499511: Fustin JM, Ye S, Rakers C, Kaneko K, Fukumoto K, Yamano M, Versteven M, Grünewald E, Cargill SJ, Tamai TK, Xu Y, Jabbur ML, Kojima R, Lamberti ML, Yoshioka-Kobayashi K, Whitmore D, Tammam S, Howell PL, Kageyama R, Matsuo T, Stanewsky R, Golombek DA, Johnson CH, Kakeya H, van Ooijen G, Okamura H
Publisher Correction: Methylation deficiency disrupts biological rhythms from bacteria to humans.
An amendment to this paper has been published and can be accessed via a link at the top of the paper.
Commun Biol, 2020; 3

33328229: Greco CM, Cervantes M, Fustin JM, Ito K, Ceglia N, Samad M, Shi J, Koronowski KB, Forne I, Ranjit S, Gaucher J, Kinouchi K, Kojima R, Gratton E, Li W, Baldi P, Imhof A, Okamura H, Sassone-Corsi P
S-adenosyl-l-homocysteine hydrolase links methionine metabolism to the circadian clock and chromatin remodeling.
Circadian gene expression driven by transcription activators CLOCK and BMAL1 is intimately associated with dynamic chromatin remodeling. However, how cellular metabolism directs circadian chromatin remodeling is virtually unexplored. We report that the S-adenosylhomocysteine (SAH) hydrolyzing enzyme adenosylhomocysteinase (AHCY) cyclically associates to CLOCK-BMAL1 at chromatin sites and promotes circadian transcriptional activity. SAH is a potent feedback inhibitor of S-adenosylmethionine (SAM)-dependent methyltransferases, and timely hydrolysis of SAH by AHCY is critical to sustain methylation reactions. We show that AHCY is essential for cyclic H3K4 trimethylation, genome-wide recruitment of BMAL1 to chromatin, and subsequent circadian transcription. Depletion or targeted pharmacological inhibition of AHCY in mammalian cells markedly decreases the amplitude of circadian gene expression. In mice, pharmacological inhibition of AHCY in the hypothalamus alters circadian locomotor activity and rhythmic transcription within the suprachiasmatic nucleus. These results reveal a previously unappreciated connection between cellular metabolism, chromatin dynamics, and circadian regulation.
Sci Adv, 2020; 6

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BOARD NUMBER: S06-331

LINKS BETWEEN VESICULAR TRAFFICKING DEFECTS, IMPAIRED MITOPHAGY AND NEURODEGENERATION

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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As medical advances have led to increased life expectancy, ageing-related neurodegenerative diseases are posing a major challenge to global health and economy. Mitochondrial dysfunction is one of the key hallmarks of ageing and contributes to a wide range of age-related diseases, including neurodegenerative conditions such as Parkinson's disease (PD). Healthy mitochondrial populations are maintained by the processes of proteolysis and mitophagy. Mitophagy is the main process that regulates the turnover of dysfunctional mitochondria and recent evidence has pointed out the involvement of key vesicular trafficking proteins, the Ras analog in brain (Rab) family in this process. Rabs are GTPases that regulate multiple steps in membrane trafficking and fusion. With our research, we aim to clarify links between Rab proteins, impaired mitophagy and PD. Specifically, we aim to understand how Rab proteins are altered in PD and how their alterations (both in quantity and their localizations) impact mitochondrial disposal in PD. Our recent work on mitochondrial proteome alterations of cells derived from *PARK2*-mutated PD patients further highlighted the importance of Rabs. Our current work utilising SH-SY5Y neuroblastoma cells revealed an upregulation of Rab5 and Rab7 in the whole-cell proteome and Rab9 in the mitochondrial proteome in dopamine-treated cells compared to control cells. The possibility to modulate proper mitochondrial clearance is an unexplored therapeutic possibility in PD treatment. Beyond PD, our data may also be relevant to the understanding of the role of Rab GTPases in the biology of mitochondria in normal physiology and possibly in diseases other than PD.

Pubmed:
34638725: Lualdi M, Shafique A, Pedrini E, Pieroni L, Greco V, Castagnola M, Cucina G, Corrado L, Di Pierro A, De Marchi F, Camillo L, Colombrita C, D'Anca M, Alberio T, D'Alfonso S, Fasano M
Repeat Expansion Affects the Proteome of Primary Skin Fibroblasts in ALS.
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of the corticospinal motor neurons, which ultimately leads to death. The repeat expansion in chromosome 9 open reading frame 72 () represents the most common genetic cause of ALS and it is also involved in the pathogenesis of other neurodegenerative disorders. To offer insights into -mediated pathogenesis, we quantitatively analyzed the proteome of patient-derived primary skin fibroblasts from ALS patients carrying the mutation compared with ALS patients who tested negative for it. Differentially expressed proteins were identified, used to generate a protein-protein interaction network and subjected to a functional enrichment analysis to unveil altered molecular pathways. ALS patients were also compared with patients affected by frontotemporal dementia carrying the repeat expansion. As a result, we demonstrated that the molecular pathways mainly altered in fibroblasts (e.g., protein homeostasis) mirror the alterations observed in -mutated neurons. Moreover, we highlighted novel molecular pathways (nuclear and mitochondrial transports, vesicle trafficking, mitochondrial bioenergetics, glucose metabolism, ER-phagosome crosstalk and Slit/Robo signaling pathway) which might be further investigated as -specific pathogenetic mechanisms. Data are available via ProteomeXchange with the identifier PXD023866.
Int J Mol Sci, 2021; 22
28625547: Lei M, Shafique A, Shang K, Couttas TA, Zhao H, Don AS, Karl T
Contextual fear conditioning is enhanced in mice lacking functional sphingosine kinase 2.
The lipid sphingosine 1-phosphate (S1P) is a potent neuroprotective signalling molecule that signals through its own family of five G-protein coupled receptors. S1P signalling enhances presynaptic glutamate release and is essential for neural development. S1P is synthesized by the enzymes sphingosine kinases 1 and 2 (SPHK1 and SPHK2), of which SPHK2 mRNA and activity is more abundant in the brain. In this study we investigated the consequences of global SphK2 knockout (SphK2) on basic motor capabilities, anxiety, learning, and memory in mice, using a range of tests including the elevated plus maze, the cheeseboard, contextual and cued fear conditioning, and fear extinction. Loss of SphK2 resulted in an 85-90% reduction in brain S1P levels, and was associated with a notably higher freezing response in a novel context. SphK2 knockout mice also exhibited increased contextual fear conditioning but the extinction of contextual fear memory was similar

to control mice. SphK2 mice, contrary to their control littermates, did not respond to cue presentation with increased freezing. Anxiety measures in the elevated plus maze were not different between SphK2 mice and control littermates. Also, knockout mice showed no deficits in neurological reflexes or motor functions, and performed as well as their control littermates in the spatial memory test. Our findings demonstrate that SphK2 is responsible for the vast majority of S1P synthesis in the mouse brain, and plays a role in freezing responses as evaluated in the fear conditioning paradigm.
Behav Brain Res, 2017; 333
27166550: Akhtar H, Hamming OJ, Jan SU, Akhtar S, Terczyn' ska-Dyla E, Siupka P, Shafique A, Hartmann R, Sadia H
Unraveling the molecular mechanism governing the tissue specific expression of IFNλR1.
The functional receptor for type III interferons (IFNs) is a heterodimer of IFNLR1 and IL10R2. IFNLR1 is expressed in a highly tissue specific manner, with epithelial and liver tissue as the prime expressing tissues in humans. However, knowledge about the molecular pathways responsible for regulating the expression of IFNLR1 is yet unknown. In this study, various bioinformatics tools were used to predict the scores of signal peptides of IFNλR1 and IFNαR1, which was considered as an important difference in the expression of both receptors or participation in regulating the IFNLR1 gene. In silico study revealed that the signal peptide of IFNαR1 had more potential than the signal peptide of IFNλR1 but changing the signal peptide of wild type IFNλR1 with the signal peptide of IFNαR1 in wet lab had barely shown any differences. Selective expression of IFNλR1 was considered to be a plus point towards the targeted anti-viral activity of IFNλs but artificial control on its expression will surely make IFNλs a better drug with enhanced activity. The results of this study may help us in contributing some understanding towards the mechanisms involved in the selective expression of IFNLR1 and exceptionalities involved.
Pak J Pharm Sci, 2016; 29

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BOARD NUMBER: S06-332

STRUCTURAL 3D MODELING OF VMAT2 MUTANTS TO IMPROVE DIAGNOSTIC AND THERAPEUTIC TREATMENT OF INFANTILE PARKINSONISM

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Genomic medicine provides considerable potential for novel diagnostic targets and therapeutic solutions. We have previously reported the first mutation in type 2 vesicular monoamine transporter (VMAT2) causing an infantile Parkinsonism phenotype. Since then, many more mutations have been identified in VMAT2, giving rise to a spectrum of symptoms with variable responses to treatment. Here, we analyze the molecular impact of the VMAT2 mutations in silico to better understand the pathophysiology and improve treatment approaches. We based our analysis on the high-confidence three-dimensional structure of the VMAT2 protein produced by the AI-based Alphafold2 algorithm. The model was manually inspected, and mutations were evaluated using the Pymol program. Additionally, residue conservation was assessed with Consurf, and pathogenicity was predicted with Polyphen-2, Mutpred2, and Snpeff. We correlated the VMAT2 structural changes due to mutations with phenotypes. We found that some mutations lead to the removal of important portions of the protein, which impairs the transmembrane transporter function, leaving gene therapy the only effective treatment for patients bearing these mutations. Other mutations cause destabilization of VMAT2 affecting interactions with other proteins and regulatory activities. These alterations are compatible with disease phenotypes explained by reduced serotonin transport, and thus can be treated by modifying serotonin transmission in the brain. Collectively, our structure-phenotype analysis advances our understanding of the disease, and may provide insights for creating personalized management protocols in this disease particularly and in Parkinson’s disease.

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BOARD NUMBER: S06-333

INVESTIGATING THE CELLULAR AND MOLECULAR RESPONSE OF HUMAN DOPAMINERGIC NEURONS TO MITOCHONDRIAL STRESS, WITH A FOCUS ON LONG NON-CODING RNAS

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Jana Heneine[1], Claire Colace-Sauty[1], Justine Guegan[2], Benjamin Galet[1], François-Xavier Lejeune[2], Corinne Pardanaud-Glavieux[1], Olga Corti[1], Philippe Ravassard[1], Hélène Cheval[1]
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Parkinson's Disease (PD) is characterized by the degeneration of the *substantia nigra* dopaminergic (DA) neurons, leading to severe motor symptoms. Several lines of evidence emphasize mitochondrial impairment's role in PD; however, the molecular mechanisms underlying DA neurons' specific vulnerability to mitochondrial stress in PD remain poorly understood. **Objectives:** We aim to unbiasedly characterise the mitochondrial stress response of human DA neurons at the transcriptome level. To discover specific molecular mechanisms underlying their vulnerability to mitochondrial stress, we include analysis of highly cell-specific genomic non-coding elements, such as long non-coding RNAs (lncRNAs) and open regions of the chromatin (ORCs). **Methods:** Using RNA-seq and ATAC-seq, we generated datasets from Lund Human MESencephalic cells-derived DA neurons, in control or mitochondrial stress conditions. We confirmed the activation of several pathways upon stress using RT-QPCR, immunofluorescence and Western Blot. **Results:** We demonstrated, for the first time, concomitant activation of several endoplasmic reticulum Unfolded Protein Response (UPR) pathways in human DA neurons upon mitochondrial stress, leading to apoptotic engagement. Our results also suggested altered DA identity following stress, a potential protective mechanism. Importantly, we identified lncRNAs specifically expressed (19%) or inhibited (33%) following stress. Analysis of these lncRNAs and putative gene targets suggested their contribution to specific steps of DA neurons' stress response, such as translation regulation, mediated by the mTOR pathway, which is altered in PD. **Conclusions:** Altogether this study provides invaluable knowledge to 2) identify critical elements of DA neurons' response to mitochondrial stress 2) understand the specific contribution of lncRNAs to this response.

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SYSTEMS MODELLING OF MITOCHONDRIAL BIOENERGETICS TO EXPLORE MOLECULAR DEFECTS CONTRIBUTING TO PATHOGENESIS IN PARKINSON'S

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Sandeep Chenna[1], Ronald Melki[2], Tina Stummann[3], Orla Watters[1], Marko Kostic[4], Karina Fog[3], Katarina Willen[3], Franziska Walter[1], Heiko Dussmann[1], Jochen Prehn[1], Niamh Connolly[1]
[1]Royal College of Surgeons in Ireland, Physiology & Medical Physics, Dublin, Ireland, [2]Institute Francois Jacob (MIRCen) - CEA, Laboratory Of Neurodegenerative Diseases, Cnrs, Fontenay-aux-Roses Cedex, France, [3]H. Lundbeck A/S, Cell Biology, Valby, Denmark, [4]Teva Pharmaceuticals, Pharmacology, Speciality R&d, Tel Aviv, Israel

Mitochondrial bioenergetic dysfunction is known to play a key role in Parkinson's pathogenesis and other neurodegenerative diseases. As part of the PD-MitoQUANT project (www.pdmitoquant.eu), we combined a systems model of mitochondrial bioenergetics with biochemical studies to pinpoint and explore bioenergetic molecular dysfunction in Parkinson's disease. We first performed sensitivity analysis and unsupervised clustering to computationally predict the impact of mitochondrial respiratory chain defects on key bioenergetic parameters. We also simulated heterogeneous cell populations to explore the effects of single-cell variability on population-level mitochondrial function. We next applied this systems modelling approach to predict the mitochondrial defects underlying experimental phenotypes observed in an alpha-synuclein cellular model of Parkinson's. Model simulations predicted that, while some experimental findings can be explained by isolated mitochondrial defects such as an impaired proton leak or reduced substrate supply, other findings are likely caused by defects outside the mitochondrial respiratory chain. These predictions will be further validated by integrating additional experimental data such as mitochondrial membrane potential (TMRM), ATP and ROS levels. In summary, we here provide an in-depth computational resource detailing the effects of mitochondrial dysfunction on key bioenergetic parameters. Our systems modelling approach informs and complements experimental design to pinpoint critical defects underlying neurodegenerative phenotypes.

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BOARD NUMBER: S06-335

DNA METHYLATION AND EXPRESSION PROFILES OF WHOLE BLOOD IN PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Erika Driver-Dunckley[1], Travis Dunckley[2], Adrienne Henderson[2]
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Parkinson's disease (PD) is the second most common age-related neurodegenerative disease. It is presently only accurately diagnosed at an advanced stage by a series of motor deficits, which are predated by non-motor symptoms manifesting over years. Aberrant epigenetic modifications are non-invasively detectable in blood as potential markers of disease. We performed comparative analyses of the methylome and transcriptome in blood from PD patients and matched controls. Our aim was to characterize DNA methylation and gene expression patterns in whole blood from PD patients as an initial step toward the future goal of identifying molecular markers that could predict, accurately diagnose, or track the progression of PD. We found that differentially expressed genes (DEGs) were involved in the processes of transcription and mitochondrial function and that PD methylation profiles were readily distinguishable from healthy controls. Differentially methylated regions (DMRs) were functionally varied, including near transcription factor nuclear transcription factor Y subunit alpha (*NFYA*), receptor tyrosine kinase *DDR1*, RING finger ubiquitin ligase (*RNF5*), acetyltransferase *AGPAT1*, and vault RNA *VTRNA2-1*. Expression quantitative trait methylation (eQTM) sites were found at long non-coding RNA *PAX8-AS1* and transcription regulator *ZFP57* among others. Functional epigenetic modules were highlighted by *IL18R1*, *PTPRC*, and *ITGB2*. We identified patterns of disease-specific DNA methylation and associated gene expression in whole blood. Our combined analyses extended what we learned from the DEG or DMR results alone. These studies provide a foundation to support the characterization of larger sample cohorts, with the goal of building a thorough, accurate, and non-invasive molecular PD biomarker.

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TRANSCRIPTOMIC CHANGES INDUCED BY PARKINSON'S DISEASE IN THE HUMAN STRIATUM EVALUATED BY SINGLE-CELL RNA SEQUENCING

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Leonardo Garma[1], Lisbeth Harder[1], Juan Barba-Reyes[2], Mónica Diez-Salguero[2], Alberto Serrano-Pozo[3], Patrick Dooley[3], Bradley Hyman[3], Ana Muñoz-Manchado[2]
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Aims Parkinson's disease (PD) is the second most common and the fastest growing neurodegenerative disease. Although some of the underlying cellular and molecular mechanisms of PD have been described we, still lack the insight that allows for the development of efficient therapies beyond dopamine replacement. The goal of our research is to determine the cell type-specific transcriptomic changes and biological pathways that are affected by PD in the human striatum, one of the key brain regions, in order to open new therapeutic approaches. **Methods** We used single-cell RNA sequencing (scRNA-seq) to analyze post-mortem human tissue samples from a broad cohort of PD patients and control subjects. The transcriptomic data was subjected to a computational analysis aimed at discovering the cellular diversity of the striatum and the changes induced by the disease. **Results** Our analysis revealed the cellular composition in the caudate and the putamen, characterizing each cell type by a unique transcriptomic pattern. Among all the cell types, we found that the medium-spiny neurons were the cells most affected by the disease, exhibiting significant changes in the expression of more than 60 genes. Other cell types presented fewer changes that are still under analysis. **Conclusions** PD induces clear cell type-specific alterations in the transcriptome of the human striatum. These alterations span multiple biological pathways, and they include both genes known to be linked to the disease and previously undocumented ones. The dataset generated in this study represents an invaluable source for the identification of disease biomarkers and treatment development.

Pubmed:
32648684: Dipalo M, Caprettini V, Bruno G, Caliendo F, Garma LD, Melle G, Dukhinova M, Siciliano V, Santoro F, De Angelis F
Membrane Poration Mechanisms at the Cell-Nanostructure Interface.
3D vertical nanostructures have become one of the most significant methods for interfacing cells and the nanoscale and for accessing significant intracellular functionalities such as membrane potential. As this intracellular access can be induced by means of diverse cellular membrane poration mechanisms, it is important to investigate in detail the cell condition after membrane rupture for assessing the real effects of the poration techniques on the biological environment. Indeed, differences of the membrane dynamics and reshaping have not been observed yet when the membrane-nanostructure system is locally perturbed by, for instance, diverse membrane breakage events. In this work, new insights are provided into the membrane dynamics in case of two different poration approaches, optoacoustic- and electro-poration, both mediated by the same 3D nanostructures. The experimental results offer a detailed overview on the different poration processes in terms of electrical recordings and membrane conformation.
Adv Biosyst, 2019; 3
31616896: Garma LD, Ferrari LM, Scognamiglio P, Greco F, Santoro F
Inkjet-printed PEDOT:PSS multi-electrode arrays for low-cost in vitro electrophysiology.
Multi-electrode arrays (MEAs) have become a key element in the study of cellular phenomena in vitro. Common modern MEAs are still based on costly microfabrication techniques, making them expensive tools that researchers are pushed to reuse, compromising the reproducibility and the quality of the acquired data. There is a need to develop novel fabrication strategies, able to produce disposable devices that incorporate advanced technologies beyond the standard metal electrodes on rigid substrates. Here we present an innovative fabrication process for the production of polymer-based flexible MEAs. The device fabrication exploited inkjet printing, as this low-cost manufacturing method allows for an easy and reliable patterning of conducting polymers. Poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) was used as the sole conductive element of the MEAs. The physical structure and the electrical properties of the plastic/printed MEAs

(pMEAs) were characterised, showing a low impedance that is maintained also in the long term. The biocompatibility of the devices was demonstrated, and their capability to successfully establish a tight coupling with cells was proved. Furthermore, the pMEAs were used to monitor the extracellular potentials from cardiac cell cultures and to record high quality electrophysiological signals from them. Our results validate the use of pMEAs as in vitro electrophysiology platforms, pushing for the adoption of innovative fabrication techniques and the use of new materials for the production of MEAs.
Lab Chip, 2019; 19

30908502: Garma LD, Matino L, Melle G, Moia F, De Angelis F, Santoro F, Dipalo M
Cost-effective and multifunctional acquisition system for in vitro electrophysiological investigations with multi-electrode arrays.
In vitro multi-electrode array (MEA) technology is nowadays involved in a wide range of applications beyond neuroscience, such as cardiac electrophysiology and bio-interface studies. However, the cost of commercially available acquisition systems severely limits its adoption outside specialized laboratories with high budget capabilities. Thus, the availability of low-cost methods to acquire signals from MEAs is important to allow research labs worldwide to exploit this technology for an ever-expanding pool of experiments independently from their economic possibilities. Here, we provide a comprehensive toolset to assemble a multifunctional in vitro MEA acquisition system with a total cost 80% lower than standard commercial solutions. We demonstrate the capabilities of this acquisition system by employing it to i) characterize commercial MEA devices by means of electrical impedance measurements ii) record activity from cultures of HL-1 cells extracellularly, and iii) electroporate HL-1 cells through nanostructured MEAs and record intracellular signals.
PLoS One, 2019; 14

27580869: Garma LD, Medina M, Juffer AH
Structure-based classification of FAD binding sites: A comparative study of structural alignment tools.
A total of six different structural alignment tools (TM-Align, TriangleMatch, CLICK, ProBis, SiteEngine and GA-SI) were assessed for their ability to perform two particular tasks: (i) discriminating FAD (flavin adenine dinucleotide) from non-FAD binding sites, and (ii) performing an all-to-all comparison on a set of 883 FAD binding sites for the purpose of classifying them. For the first task, the consistency of each alignment method was evaluated, showing that every method is able to distinguish FAD and non-FAD binding sites with a high Matthews correlation coefficient. Additionally, GA-SI was found to provide alignments different from those of the other approaches. The results obtained for the second task revealed more significant differences among alignment methods, as reflected in the poor correlation of their results and highlighted clearly by the independent evaluation of the structural superimpositions generated by each method. The classification itself was performed using the combined results of all methods, using the best result found for each comparison of binding sites. A number of different clustering methods (Single-linkage, UPGMA, Complete-linkage, SPICKER and k-Means clustering) were also used. The groups of similar binding sites (proteins) or clusters generated by the best performing method were further analyzed in terms of local sequence identity, local structural similarity and conservation of analogous contacts with the FAD ligands. Each of the clusters was characterized by a unique set of structural features or patterns, demonstrating that the groups generated truly reflect the structural diversity of FAD binding sites. Proteins 2016; 84:1728-1747. © 2016 Wiley Periodicals, Inc.
Proteins, 2016; 84

26794322: Pietilä I, Prunskaite-Hyyryläinen R, Kaisto S, Tika E, van Eerde AM, Salo AM, Garma L, Miinalainen I, Feitz WF, Bongers EM, Juffer A, Knoers NV, Renkema KY, Myllyharju J, Vainio SJ
Wnt5a Deficiency Leads to Anomalies in Ureteric Tree Development, Tubular Epithelial Cell Organization and Basement Membrane Integrity Pointing to a Role in Kidney Collecting Duct Patterning.
The Wnts can be considered as candidates for the Congenital Anomaly of Kidney and Urinary Tract, CAKUT diseases since they take part in the control of kidney organogenesis. Of them Wnt5a is expressed in ureteric bud (UB) and its deficiency leads to duplex collecting system (13/90) uni- or bilateral kidney agenesis (10/90), hypoplasia with altered pattern of ureteric tree organization (42/90) and lobularization defects with partly fused ureter trunks (25/90) unlike in controls. The UB had also notably less tips due to Wnt5a deficiency being at E15.5 306 and at E16.5 765 corresponding to 428 and 1022 in control ($p<0.02$; $p<0.03$) respectively. These changes due to Wnt5a knock out associated with anomalies in the ultrastructure of the UB daughter epithelial cells. The basement membrane (BM) was malformed so that the BM thickness increased from 46.3 nm to 71.2 nm ($p<0.01$) at E16.5 in the Wnt5a knock out when compared to control. Expression of a panel of BM components such as laminin and of type IV collagen was also reduced due to the Wnt5a knock out. The P4ha1 gene that encodes a catalytic subunit of collagen prolyl 4-hydroxylase I (C-P4H-I) in collagen synthesis expression and the overall C-P4H enzyme activity were elevated by around 26% due to impairment in Wnt5a function from control. The compound Wnt5a+/-;P4ha1+/- embryos demonstrated Wnt5a-/- related defects, for example local hyperplasia in the UB tree. A R260H WNT5A variant was identified from renal human disease cohort. Functional studies of the consequence of the corresponding mouse variant in comparison to normal ligand reduced Wnt5a-signalling in vitro. Together Wnt5a has a novel function in kidney organogenesis by contributing to patterning of UB derived collecting duct development contributing putatively to congenital disease.

PLoS One, 2016; 11
26773655: Garma LD, Juffer AH
Comparison of non-sequential sets of protein residues.
A methodology for performing sequence-free comparison of functional sites in protein structures is introduced. The method is based on a new notion of similarity among superimposed groups of amino acid residues that evaluates both geometry and physico-chemical properties. The method is specifically designed to handle disconnected and sparsely distributed sets of residues. A genetic algorithm is employed to find the superimposition of protein segments that maximizes their similarity. The method was evaluated by performing an all-to-all comparison on two separate sets of ligand-binding sites, comprising 47 protein-FAD (Flavin-Adenine Dinucleotide) and 64 protein-NAD (Nicotinamide-Adenine Dinucleotide) complexes, and comparing the results with those of an existing sequence-based structural alignment tool (TM-Align). The quality of the two methodologies is judged by the methods' capacity to, among other, correctly predict the similarities in the protein-ligand contact patterns of each pair of binding sites. The results show that using a sequence-free method significantly improves over the sequence-based one, resulting in 23 significant binding-site homologies being detected by the new method but ignored by the sequence-based one.
Comput Biol Chem, 2016; 61
22719985: Garma L, Mukherjee S, Mitra P, Zhang Y
How many protein-protein interactions types exist in nature?
"Protein quaternary structure universe" refers to the ensemble of all protein-protein complexes across all organisms in nature. The number of quaternary folds thus corresponds to the number of ways proteins physically interact with other proteins. This study focuses on answering two basic questions: Whether the number of protein-protein interactions is limited and, if yes, how many different quaternary folds exist in nature. By all-to-all sequence and structure comparisons, we grouped the protein complexes in the protein data bank (PDB) into 3,629 families and 1,761 folds. A statistical model was introduced to obtain the quantitative relation between the numbers of quaternary families and quaternary folds in nature. The total number of possible protein-protein interactions was estimated around 4,000, which indicates that the current protein repository contains only 42% of quaternary folds in nature and a full coverage needs approximately a quarter century of experimental effort. The results have important implications to the protein complex structural modeling and the structure genomics of protein-protein interactions.
PLoS One, 2012; 7

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BOARD NUMBER: S06-337

IDENTIFICATION OF POTENTIAL NEW GENES INVOLVED IN AUTOSOMAL RECESSIVE FORMS OF PARKINSON'S DISEASE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Parkinson disease (PD) affects 1% of the population above 65 years. It is characterized by the triad of symptoms: tremor, rigidity, and bradykinesia. To date the identified genes associated with early-onset (EO, >40 years) autosomal recessive (AR) PD only explain 45%, other genes remain to be discovered. The aim of the work is to identify new genes involved in AR EO PD, using consanguineous PD families and applying genotyping on DNA microarrays and NGS technologies. Using a series of 99 families with confirmed consanguinity, we looked for homozygous loss of function or missense mutations predicted deleterious in region of loss of homozygosity. Then we firstfocused on variant shared by at least two families. We identified mutations in *PSMF1* an interactor of *FBXO7*. In one families, it remains only this variant moreover both mutations code for amino acid highly conserved upon evolution. Most of the candidate's genes are private genes highlighting genetic heterogeneity of PD. Therefore, in a second time we hypothesized that some candidate's genes can be involved in a common pathway. Using ClusterProfiler we performed GO term enrichment analyses, then we were able to grouped together some genes and were able to see an statistical enrichment in autophagy pathway. We identified a strong candidate gene for AR-PD: *PSMF1*. Further functional data are needed to strengthen the role of this gene in PD, possibly affecting the proteasome activity and α-synucleine aggregation. We will also continue to investigate pathway analyses in order to identify candidates for PD in our families.

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IS GDNF DOSE ESSENTIAL FOR PARKINSON ’S DISEASE GENE THERAPY SUCCESS?

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Glial cell line-derived neurotrophic factor (GDNF) protects dopaminergic (DA) neurons and reduces motor symptoms, when applied in the striatum, in toxin-induced Parkinson’s disease models. Furthermore, GDNF protects DA neurons against alpha-synuclein propagation in mice model. However, clinical trials based on intraputaminal GDNF delivery have so far failed to demonstrate significant clinical benefits. GDNF interference with dopamine homeostasis via time and dose-dependent neurochemical effects is one of the explanations for poor clinical outcome. Our hypothesis is that these neurochemical effects could be deleterious and mask or reduce GDNF beneficial effect. Methods We have described a doxycycline (dox)-regulated viral vector (AAV-DoxON-GDNF) which allows to finely adjust GDNF dose and period of administration at clinically-acceptable antibiotics doses. We have then injected a high dose of the AAV-DoxON-GDNF vector in order to mimic conditions of GDNF overdosing in a unilateral 6-OHDA rat model. Using Dox regulation, we have administered GDNF at 2 different concentrations: 4-fold (GDNF-LOW) and 20-fold (GDNF-HIGH) higher than the endogenous striatal level. The treatment period was 17 weeks either continuously or transiently for 2 weeks with 2 weeks interruptions. Results At 16 weeks post-injection, the GDNF-LOW group and GDNF-HIGH-intermittent groups showed a significant reduction of the behavioral asymmetry while the GDNF-HIGH-continuous group did not show significant improvements. Immunohistochemistry analysis revealing molecular mechanisms of GDNF pro-survival are supporting the use of low GDNF doses. Conclusions GDNF dose is a parameter to take into account in order to improve the clinical outcome in future trials.

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DETECTION OF NUCLEOTIDE REPEAT EXPANSIONS BY EXOME SEQUENCING OF PARKINSON'S DISEASE PATIENTS

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Short Tandem Repeats represent 10% of the human genome. Beyond a threshold specific to each locus, a progressive disease manifest which very often affects the central nervous system. Repeat expansion in the ATXN2 gene is known to be responsible for spinocerebellar ataxia type 2, but when the expansion is interrupted it can cause Parkinson's Disease. Repeat sequence expansions in PD patients have also been found the ATXN3 gene. High-throughput whole exome sequencing (WES) plays an important role in the identification of monogenic forms of PD but detects mostly point mutations or small insertions. In this context, we analyzed WES data from our cohort of PD patients enriched in familial and early-onset forms, previously excluded from major PD genes, using ExpansionHunter software. This software allows to estimate the size of the repeats in the target genes. The putative expansions were then validated by Repeat Primed PCR. Our cohort consisted of 827 exomes performed with Rochev3 (n=213), MedExome (n=171) or Twist (n=443) kits. Our bioinformatics analysis allowed us to identify 1 patient who had a repeat size within the pathological confidence range (>55 CAG repeats) in ATXN3 and 6 patients with repeat sizes within the pathological range (>32 CAG repeats) in ATXN2, 2 of whom belonged to the same family. Sequence analysis of the ATXN2 repeat is in progress in 5 patients and has revealed the presence of 4 CAA interruptions in another. These results demonstrate the usefulness of bioinformatic detection of expansions using ExpansionHunter in exome sequencing data of PD patients.

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NEUROPROTECTIVE EFFECTS OF A NOVEL DEMECLOCYCLINE DERIVATIVE LACKING ANTIBIOTIC ACTIVITY

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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[1]IMMCA, Tucumán, Capital, San Miguel de Tucumán, Argentina, [2]Paris Brain Institute, Paris, Paris, France, [3]InnoProt, Bizkaia, Derio, Spain, [4]UMR_MD1, Aix-marseille Université, Marseille, France, [5]BioCIS, Universite Paris-saclay, Chatenay Malabry, France

Aims: Recently, the tetracycline antibiotic Demeclocycline (DMC) was reported to prevent neurodegeneration induced by aggregation of the Parkinson's disease (PD)-associated protein a-Synuclein (aSN) (Braun et al., 2021). However, DMC antibiotic activity precludes its potential use for long-term treatments. Our aim was to synthetize a new DMC derivative with no antibiotic activity and better neuroprotective effects. **Methods**: This compound referred as DDMC was synthetized by removal of the dimethylamino function at position 4 and reduction of the hydroxyl group at position 12a on ring A of DMC. Its antibiotic activity was evaluated using Gram (+) and Gram (-) bacterial strains. Inhibition of αSN aggregation assessed *ex-vitro* by ThT and Congo Red assays was complemented by transmission electron microscopy (TEM) analysis and BIS-ANS fluorescence methods. Seeded aggregation induced by αSN preformed fibrils (PFF) was studied on SH-SY5Y-αSN-RFP cells. The pro-inflammatory potential of αSN fibrillary species was tested by measuring the release of TNF-α and glutamate in microglial cells. **Results and conclusions**: DDMC had no antibiotic activity and was more efficient than DMC in preventing αSN amyloid aggregation in *ex-vitro* assays. Using TEM and BIS-ANS methods, we found that fibrillary species formed in the presence of DDMC were morphologically and structurally different. Interestingly, seeding induced in SH-SY5Y cells was significantly reduced when using fibrils formed in the presence of DDMC. Finally, these fibrils were less inflammogenic in microglial cells. Our results suggest that DDMC may be a novel drug candidate for the treatment of PD and other synucleinopathies.

Pubmed:
34722566: Tomas-Grau RH, Ploper D, Ávila CL, Vera Pingitore E, Maldonado Galdeano C, Chaves S, Socias SB, Stagnetto A, Navarro SA, Chahla RE, Aguilar López M, Llapur CJ, Aznar P, Alcorta ME, Costas D, Flores I, Heinze D, Apfelbaum G, Mostoslavsky R, Mostoslavsky G, Cazorla SI, Perdigón GDV, Chehín R
Elevated Humoral Immune Response to SARS-CoV-2 at High Altitudes Revealed by an Anti-RBD "" ELISA.
The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has caused a global pandemic with dramatic health and socioeconomic consequences. The Coronavirus Disease 2019 (COVID-19) challenges health systems to quickly respond by developing new diagnostic strategies that contribute to identify infected individuals, monitor infections, perform contact-tracing, and limit the spread of the virus. In this brief report, we developed a highly sensitive, specific, and precise "" ELISA to correctly discriminate previously SARS-CoV-2-infected and non-infected individuals and study population seroprevalence. Among 758 individuals evaluated for anti-SARS-CoV-2 serology in the province of Tucumán, Argentina, we found a weak correlation between antibodies elicited against the RBD, the receptor-binding domain of the Spike protein, and the nucleocapsid (N) antigens of this virus. Additionally, we detected mild levels of anti-RBD IgG antibodies in 33.6% of individuals diagnosed with COVID-19, while only 19% showed sufficient antibody titers to be considered as plasma donors. No differences in IgG anti-RBD titers were found between women and men, neither in between different age groups ranging from 18 to 60. Surprisingly, individuals from a high altitude village displayed elevated and longer lasting anti-RBD titers compared to those from a lower altitude city. To our knowledge, this is the first report correlating altitude with increased humoral immune response against SARS-CoV-2 infection.
Front Med (Lausanne), 2021; 8
34841388: Chahla RE, Tomas-Grau RH, Cazorla SI, Ploper D, Vera Pingitore E, López MA, Aznar P, Alcorta ME, Vélez EMDM, Stagnetto A, Ávila CL, Maldonado-Galdeano C, Socias SB, Heinze D, Navarro SA, Llapur CJ, Costa D, Flores I, Edelstein A, Kowdle S, Perandones C, Lee B, Apfelbaum G, Mostoslavsky R, Mostoslavsky G, Perdigón G, Chehín RN
Long-term analysis of antibodies elicited by SPUTNIK V: A prospective cohort study in Tucumán, Argentina.

Gam-COVID-Vac (SPUTNIK V) has been granted emergency use authorization in 70 nations and has been administered to millions worldwide. However, there are very few peer-reviewed studies describing its effects. Independent reports regarding safety and effectiveness could accelerate the final approval by the WHO. We aimed to study the long-term humoral immune response in naïve and previously infected volunteers who received SPUTNIK V.
Lancet Reg Health Am, 2022; 6

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BOARD NUMBER: S06-341

IMPACT OF PD IN CAUDATE & PUTAMEN USING SINGLE-CELL TRANSCRIPTOMICS: SPECIAL FOCUS ON OLIGODENDROCYTES AND OPCS

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Juan Manuel Barba Reyes[1], Leonardo Garma[2], Lisbeth Harder[2], Mónica Diez-Salguero[1], Alberto Serrano-Pozo[3], Patrick Dooley[3], Bradley Hyman[3], Ana Muñoz-Manchado[1]
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Parkinson's Disease (PD) is the second most common neurodegenerative disorder. A core feature of this disease is the loss of dopamine signaling in the striatal region of the brain. PD pathology is not fully understood yet which has prevented efficient development of drug treatments of symptoms beyond dopamine replacement that is still fraught with side effects. Oligodendrocytes are the myelinating cells of the central nervous system (CNS), producing multi-layered myelin sheaths around neuronal axons originated from Oligodendrocytes Precursor Cells (OPCs). Oligodendrocytes play a causal role in neurodegenerative disorders such as Multiple Sclerosis, but if these cell types are also involved in the degeneration process of PD is far from understood. Recent investigations have pointed out a potential role of oligodendrocytes in PD: i) Agarwal et al (Nat Commun, 2020) demonstrated in a single-nucleus RNAseq approach from Substantia Nigra and Cortex tissue that Oligodendrocytes differentially expressed genes associated with variants significantly linked with PD in previous Genome Wide Association Studies (GWAS); ii) Bryois et al (Nat Genet, 2020) studied recent GWAS including PD patients, and showed PD to be genetically associated with Oligodendrocytes. Applying single-cell transcriptomics we profiled postmortem nuclei of Putamen and Caudate from PD and control subjects. We provide insights into subtypes of OPCs and Oligodendrocytes in the Putamen and Caudate nucleus and their potential contribution to PD.

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CROSS SPECIES METABOLOMIC STUDY IN PARKINSON'S DISEASE: BRAIN MITOCHONDRIAL REPROGRAMMING AS A BIOMARKER AND THERAPEUTIC TARGET

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

David Mallet[1], Raphaël Goutaudier[1], Thibault Dufourd[1], Sebastien Carnicella[1], Emmanuel Barbier[2], Florence Fauvelle[2], Sabrina Boulet[1]
[1]Grenoble Institute of Neurosciences, Physiopathology Of Motivation, La Tronche, France, [2]Functional neuroimaging and brain perfusion, Grenoble Institute Of Neurosciences, La Tronche, France

Parkinson's disease (PD) remains an incurable neurodegenerative pathology affecting almost 1% of the population beyond the age of 60. The two main factors that account for this therapeutic failure are the late diagnosis, based on the cardinal motor symptoms of the disease appearing when neurodegenerative process has already past rescue, and an incomplete understanding of PD pathophysiology. In this context, using proton nuclear magnetic resonance, we investigated serum and brain metabolic markers in three different animal models of PD, mimicking different stages of the disease assessed by behavioral and histological evaluation, and in 2 cohorts of de novo PD patients. From our translational study, we first highlighted common metabolic dysregulations in serum of the different animal models and PD patients. We propose a promising composite biomarker exhibiting a high level of predictivity for PD diagnosis in its early phase, before motor symptoms appearance. Moreover, mirrored brain and serum dysregulations strongly suggest dysfunctions in the pyruvate metabolism, a central metabolic node to supply cellular energy, as possible therapeutic target. In order to investigate this hypothesis, we therefore pharmacologically blocked mitochondrial pyruvate carrier (MPC) in PD animals.Indeed, as the sole point of entry for pyruvate into the mitochondrial matrix, the MPC plays a crucial role in coordinating glycolytic and mitochondrial activities. MPC blockage improved motor behavior, decreased dopaminergic denervation and reduced mTOR activity and neuroinflammation. This neuroprotective effect could be based on the reorganization of multiple pathways related to energy metabolism.

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LOSS OF 'METABOLIC SPIKES' MAY EXPLAIN DOPAMINE DEPLETION IN PARKINSONS'

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

Chaitanya Chintaluri, Tim Vogels
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In neurons, spontaneous spiking persists in the absence (and even deprivation) of synaptic input. This is perplexing, especially given that spikes are metabolically expensive. Here, we propose a novel theory on the origin of this baseline activity and present its implications using a computational model of substantia nigra dopaminergic neurons (SNcDA) in the context of Parkinsons' disease (PD). Briefly, we hypothesize that neurons keep their ATP levels at a maximum. During synaptic quiescence, neurons are ATP-surplus and ADP-scarce. With ADP availability as the rate-limiting step, ATP production stalls in the mitochondria when energy consumption is low, leading to the formation of toxic Reactive Oxygen Species (ROS)[1,2] which disrupt many cellular processes. We posit that in order to avoid ROS build-up, neurons actively sense their metabolic state and trigger 'metabolic spikes' to restore ATP production. In a metabolic-state-sensing leaky integrate-and-fire neuron we can reproduce the firing patterns reported for the SNcDA neurons[3] and show that the rate of metabolic spiking is primarily dependent on metabolic costs incurred for i) cellular maintenance (i.e., non-spiking costs) and ii) the metabolic cost-per-spike. When we introduce failures to sense, or produce ROS, or when we increase in per-spike costs or non-spiking costs our model neurons cease metabolic spiking, emulating the cellular symptoms of PD[4]. We conclude that metabolic spikes may be an integrated neuronal mechanism that operates in synergy with synaptic integration and forms a basic principle of neural network dynamics. [1]Murphy 2009 [2]Aon et al., 2010 [3]Grace & Bunney 1984 [4]Ehringer & Hornykiewicz 1969

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CHARACTERIZING LRRK2 IN VARYING STATES OF PHOSPHORYLATION

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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LRRK2 harbors several phosphorylation sites clustered into 2 major groups, a heterologous cluster (controlled by upstream kinases and phosphatases) and an autophosphorylation cluster. Work from our team and others suggest that phosphorylation of the heterologous cluster of LRRK2 correlates to healthy phenotypes whereas, dephosphorylation correlates to phenotypes associated to pathological processes corresponding to PD. PP1 and PP2 were identified as the upstream regulators of the heterologous LRRK2 phosphorylation cluster *in vitro*. Therefore, the goal of this study was to examine the phosphoregulating partners of LRRK2 *in vivo* and characterize phenotypes of LRRK2 phosphorylation. First, we confirmed co-immunoprecipitation of LRRK2 with its identified phosphatases in rat brain lysate. Next, as LRRK2 dephosphorylation has been reported to prime LRRK2 for ubiquitination and degradation, purified LRRK2 phosphomutant and wildtype constructs were tested for stability, integrity in solution by nano-DSF. LRRK2 is known to influence retrograde and post-Golgi trafficking pathways based on GTP-binding and Rab29 expression activates LRRK2 kinase activity. We recently showed that Rab29 when co-expressed with LRRK2 results in a hyperactivation of kinase activity of phosphodead LRRK2. Studying membrane recruitment of LRRK2 and its pathogenic mutants by Rab29 to the trans-golgi network has shown interesting data and expanding this assay to determine any biological alterations that the dephosphorylated mutants of LRRK2 could have in terms of recruitment would give use further evidence of the effects the dephosphorylation of LRRK2 has on cells.

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HIPPOCAMPAL DIFFERENTIAL EXPRESSION UNDERLYING THE NEUROPROTECTIVE EFFECT OF Δ9-TETRAHYDROCANNABINOL (THC) MICRODOSE ON OLD MICE

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Delta-9 tetrahydrocannabinol (THC) is the primary psychoactive compound of the Cannabis plant and an exogenous ligand of the endocannabinoid system (ECS). In past studies, we demonstrated that a microdose of THC (0.002 mg/kg, 3–4 orders of magnitude lower than the standard dose for rodents) exerts a long-term neuroprotective effect in model mice, alleviating damage from acute neurological insults and late-onset neurodegenerative pathology. Assays measuring the impact of the THC teratment on gene expression, morphology and biochemistry of the brain identified significant neurogenesis, increased abundance of anti-inflammatory proteins and reversal of gene expression alterations linked to neurodegeneration and aging. In our follow-up research, we use high-throughput transcriptome profiling of various brain region to investigate the potential benefits of the treatment for Parkinsons Disease, including promotion of cell-survival in vunrable dopaminergic neurons, inhibition of chronic microglial inflammation and resistance to oxidative stress. We highlight the modulation of the Transforming growth factor beta (TGF-β) pathway and known Parkinsons Disease therapeutic targets, such as Sgk1. We propose the THC microdose as a novel treatment for Parkinsons disease and discuss its potential disease-modifying properties when used at the pre-clinical stage.

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IMPORTANCE OF MICROENVIRONMENT IN CEREBRAL IN VITRO MODELS FOR PHENOTYPIC SCREENING

POSTER SESSION 06 - SECTION: EMERGING MECHANISMS IN PARKINSON'S DISEASE

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Aim: About 90% of drug-candidates failed in clinical trials, in particular in neurology, due to a lack of efficacy. That highlights a lack of relevance in preclinical models, including *in vitro* models, which do not take into account the microenvironment, composed by glial cells and the Extracellular Matrix (ECM). The objective was to study the influence of the microenvironment in cerebral *in vitro* models, in the frame of Parkinson's Disease (PD). **Methods:** First, we analyzed the influence of astrocytes on Luhmes cell sensitivity, a dopaminergic neuronal cell line, in 2D culture. Then, we developed a hyaluronic acid-based hydroscaffold for 3D cell culture, which mimics the ECM, and study the sensitivity of Luhmes cells in this model. Thirdly, we performed a co-culture of Luhmes cells and astrocytes in this matrix, to form a complex model including both the glial and the matricial microenvironments. **Results:** We observed a protective effect of astrocytes in 2D culture. In the hydroscaffold, Luhmes cells displayed a lower sensitivity compared to 2D culture, that was explained by a partial retention of toxic molecules in the matrix, and differences in neuronal protein expression. In the co-culture, we observed spheroids containing both neurons and astrocytes. **Conclusions:** This work highlighted that the microenvironment of neurons can modify the neuronal response *in vitro*, and should thus be considered carefully in academic research and in drug discovery. This model can be now used to study the microenvironment modifications in pathological conditions, and to develop innovative drugs targeting the microenvironment.

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GENETIC ABLATION OF THE RHO GTPASE RND3 TRIGGERS DEVELOPMENTAL DEFECTS IN INTERNAL CAPSULE AND THE GLOBUS PALLIDUS FORMATION

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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University of Lleida, Irblleida, Lleida, Spain

The forebrain includes the cerebral cortex, the thalamus, and the striatum and globus pallidus (GP) in the subpallium. The formation of these structures and their interconnections by specific axonal tracts take place in a precise and orchestrated time and spatial-dependent manner during development. However, the knowledge of the molecular and cellular mechanisms that are involved is rather limited. Moreover, while many extracellular cues and specific receptors have been shown to play a role in different aspects of nervous system development, including neuron migration and axon guidance, examples of intracellular signaling effectors involved in these processes are sparse. In the present work, we have shown that the atypical RhoGTPase, Rnd3, is expressed very early during brain development and keeps a dynamic expression in several brain regions including the cortex, the thalamus, and the subpallium. By using a gene-trap allele (Rnd3gt) and immunological techniques, we have shown that Rnd3gt/gt embryos display severe defects in striatal and thalamocortical axonal projections (SAs and TCAs, respectively) and defects in GP formation already at early stages. Surprisingly, the corridor, an important intermediate target for TCAs is still present in these mutants. Mechanistically, a conditional genetic deletion approach revealed that Rnd3 is primarily required for the normal development of Medial Ganglionic Eminence-derived structures, such as the GP, and therefore acts non-cell autonomously in SAs and TCAs. In conclusion, we have demonstrated the important role of Rnd3 as an early regulator of subpallium development in vivo and revealed new insights about SAs and TCAs development.

Pubmed:

33576044: Marfull-Oromí P, Fleitas C, Zammou B, Rocandio D, Ballester-Lurbe B, Terrado J, Perez-Roger I, Espinet C, Egea J
Genetic ablation of the Rho GTPase Rnd3 triggers developmental defects in internal capsule and the globus pallidus formation.
The forebrain includes the cerebral cortex, the thalamus, and the striatum and globus pallidus (GP) in the subpallium. The formation of these structures and their interconnections by specific axonal tracts take place in a precise and orchestrated time and spatial-dependent manner during development. However, the knowledge of the molecular and cellular mechanisms that are involved is rather limited. Moreover, while many extracellular cues and specific receptors have been shown to play a role in different aspects of nervous system development, including neuron migration and axon guidance, examples of intracellular signaling effectors involved in these processes are sparse. In the present work, we have shown that the atypical RhoGTPase, Rnd3, is expressed very early during brain development and keeps a dynamic expression in several brain regions including the cortex, the thalamus, and the subpallium. By using a gene-trap allele (Rnd3) and immunological techniques, we have shown that Rnd3 embryos display severe defects in striatal and thalamocortical axonal projections (SAs and TCAs, respectively) and defects in GP formation already at early stages. Surprisingly, the corridor, an important intermediate target for TCAs is still present in these mutants. Mechanistically, a conditional genetic deletion approach revealed that Rnd3 is primarily required for the normal development of Medial Ganglionic Eminence-derived structures, such as the GP, and therefore acts non-cell autonomously in SAs and TCAs. In conclusion, we have demonstrated the important role of Rnd3 as an early regulator of subpallium development in vivo and revealed new insights about SAs and TCAs development.
J Neurochem, 2021; 158
34639085: Olabiyi BF, Fleitas C, Zammou B, Ferrer I, Rampon C, Egea J, Espinet C
proNGF Involvement in the Adult Neurogenesis Dysfunction in Alzheimer's Disease.
In recent decades, neurogenesis in the adult brain has been well demonstrated in a number of animal species, including humans. Interestingly, work with rodents has shown that adult neurogenesis in the dentate gyrus (DG) of the hippocampus is vital for some cognitive aspects, as increasing neurogenesis improves memory, while its disruption triggers the opposite effect. Adult neurogenesis declines with age and has been suggested to play a role in impaired progressive learning and memory loss seen in Alzheimer's disease (AD). Therefore, therapeutic strategies designed to boost adult hippocampal

neurogenesis may be beneficial for the treatment of AD. The precursor forms of neurotrophins, such as pro-NGF, display remarkable increase during AD in the hippocampus and entorhinal cortex. In contrast to mature NGF, pro-NGF exerts adverse functions in survival, proliferation, and differentiation. Hence, we hypothesized that pro-NGF and its p75 neurotrophin receptor (p75NTR) contribute to disrupting adult hippocampal neurogenesis during AD. To test this hypothesis, in this study, we took advantage of the availability of mouse models of AD (APP/PS1), which display memory impairment, and AD human samples to address the role of pro-NGF/p75NTR signaling in different aspects of adult neurogenesis. First, we observed that DG doublecortin (DCX) + progenitors express p75NTR both, in healthy humans and control animals, although the percentage of DCX+ cells are significantly reduced in AD. Interestingly, the expression of p75NTR in these progenitors is significantly decreased in AD conditions compared to controls. In order to assess the contribution of the pro-NGF/p75NTR pathway to the memory deficits of APP/PS1 mice, we injected pro-NGF neutralizing antibodies (anti-proNGF) into the DG of control and APP/PS1 mice and animals are subjected to a Morris water maze test. Intriguingly, we observed that anti-pro-NGF significantly restored memory performance of APP/PS1 animals and significantly increase the percentage of DCX+ progenitors in the DG region of these animals. In summary, our results suggest that pro-NGF is involved in disrupting spatial memory in AD, at least in part by blocking adult neurogenesis. Moreover, we propose that adult neurogenesis alteration should be taken into consideration for better understanding of AD pathology. Additionally, we provide a new molecular entry point (pro-NGF/p75NTR signaling) as a promising therapeutic target in AD.
Int J Mol Sci, 2021; 22

34301831: Fleitas C, Marfull-Oromí P, Chauhan D, Del Toro D, Peguera B, Zammou B, Rocandio D, Klein R, Espinet C, Egea J

FLRT2 and FLRT3 Cooperate in Maintaining the Tangential Migratory Streams of Cortical Interneurons during Development. Neuron migration is a hallmark of nervous system development that allows gathering of neurons from different origins for assembling of functional neuronal circuits. Cortical inhibitory interneurons arise in the ventral telencephalon and migrate tangentially forming three transient migratory streams in the cortex before reaching the final laminar destination. Although migration defects lead to the disruption of inhibitory circuits and are linked to aspects of psychiatric disorders such as autism and schizophrenia, the molecular mechanisms controlling cortical interneuron development and final layer positioning are incompletely understood. Here, we show that mouse embryos with a double deletion of and genes encoding cell adhesion molecules exhibit an abnormal distribution of interneurons within the streams during development, which in turn, affect the layering of somatostatin+ interneurons postnatally. Mechanistically, FLRT2 and FLRT3 proteins act in a noncell-autonomous manner, possibly through a repulsive mechanism. In support of such a conclusion, double knockouts deficient in the repulsive receptors for FLRTs, Unc5B and Unc5D, also display interneuron defects during development, similar to the / mutants. Moreover, FLRT proteins are chemorepellent ligands for developing interneurons , an effect that is in part dependent on FLRT-Unc5 interaction. Together, we propose that FLRTs act through Unc5 receptors to control cortical interneuron distribution in a mechanism that involves cell repulsion. Disruption of inhibitory cortical circuits is responsible for some aspects of psychiatric disorders such as schizophrenia or autism. These defects include interneuron migration during development. A crucial step during this process is the formation of three transient migratory streams within the developing cortex that determine the timing of interneuron final positioning and the formation of functional cortical circuits in the adult. We report that FLRT proteins are required for the proper distribution of interneurons within the cortical migratory streams and for the final laminar allocation in the postnatal cortex. These results expand the multifunctional role of FLRTs during nervous system development in addition to the role of FLRTs in axon guidance and the migration of excitatory cortical neurons.
J Neurosci, 2021; 41

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BOARD NUMBER: S06-348

BALANCING WNT SIGNALING IN EARLY FOREBRAIN DEVELOPMENT

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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During the initial specification of the forebrain, integration of signals from multiple signaling centers is required to establish regional identity of neural progenitor cells (NPCs). WNT signaling pathway is one of the most important signaling pathways in this process. LRPs (LDL receptor related protein) are crucial for WNT signaling and therefore involved in early embryonic forebrain development. LRP5 and LRP6 are well studied as co-receptors for frizzled, the main WNT receptor. However, little is known about the function of LRP4 as a potential modulator of LRP6 in the central nervous system. To shed light on the common and distinct functions of these receptors and to test for interactions among *Lrp* gene family members linked to the WNT pathway, we chose a genetic approach analyzing *Lrp4*, *Lrp6* null mutant mice as well as *Lrp4*$^{-/-}$;*Lrp6*$^{-/-}$ compound mutant mice. Applying mouse genetics, high resolution immunofluorescence imaging, cell culture models and molecular biology approaches I could show the following results: **1)** Developmental disorders of *Lrp6*$^{-/-}$ mutant mice can be partially rescued by loss of LRP4. **2)** *Lrp4* is a genetic modifier of *Lrp6*. **3)** LRP4 is crucial for balancing the proliferative activity versus differentiation of neuronal progenitors in the developing forebrain **4)** LRP4 modulates LRP5 and LRP6 dependent WNT signaling not only during forebrain development but also in a more general context, i.e. in human cell lines.

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ALTERATION OF MOUSE COGNITION AND NEURAL CIRCUITS FORMATION RESULTING FROM MUTATIONS ON THE AUTISM-LINKED GENE NUAK1.

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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INMG - PGNM, Rhone-alpes, LYON, France

The development of functional neural circuits relies on tightly regulated cellular processes controlled by complex cascades of signaling pathways, culminating in the proper development of axon terminals and synaptic connections. A disruption of these molecular mechanisms can lead to life-altering neurodevelopmental disorders such as autism spectrum disorders (ASD), mental retardation, schizophrenia or cognitive defects. Our team identified previously that the autism-associated protein kinase NUAK1 plays a central role in controlling axonal development in the mouse cortex (Courchet et al. Cell 2013). In addition, we recently described that *Nuak1* is haploinsufficient for mouse cortical development (Courchet et al. 2018). Knockout of *Nuak1* leads to alterations of cortical connectivity and a wide-array of behavioral alterations including sociability defects, deficits in learning and memory and abnormal sensory gating. Building on this work, we created a mouse line with a mutation of the *Nuak1* gene mimicking a *de novo* mutation identified in autistic patients. The targeted knock-in mutation (Q434*) was achieved using the Crispr-Cas9 strategy. We will present the first data of characterization of this novel, humanized mouse line. Furthermore we turned to a candidate-approach to identify which signaling pathway and transcription factor could explains the roles of NUAK1 in cued- and contextual fear memory. Overall, the comparison of this model with conditional knockout models will allow to better characterize the cognitive defects associated to *Nuak1* and to identify the alterations in the neural circuits underlying these behavioral alterations. Keywords : autism spectrum disorders, NUAK1, mouse model, memory, CREB.

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BOARD NUMBER: S06-350

CG7101/DTZAP ENCODES A TRANSCRIPTIONAL REGULATOR OF MITOCHONDRIAL BIOLOGY REQUIRED FOR AXONAL OUTGROWTH, CIRCUIT CONNECTIVITY AND BEHAVIOR

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

Iryna Mohylyak[1], Mercedes Bengochea[2], Noemi Asfogo[3], Stein Aerts[4], Olga Corti[3], Bassem Hassan[5]
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Molecular processes that lead to robust circuit wiring during development remain to be fully deciphered. We addressed this question using the Dorsal Cluster Neurons, a higher order visual circuit in *Drosophila*. To understand the molecular underpinnings of circuit formation and identify target genes, we performed analysis of DCNs transcriptome with subsequent RNAi-screen of 75-highly expressed genes to study their role in circuit development, synaptic connectivity and behavior. We identified *CG7101*, a gene encoding a transcription factor homologous to mammalian *TZAP*, known for its role as a negative regulator of telomere length, and proposed to regulate the expression of the *mitochondrial fission process 1* gene. *dTzap* knockdown resulted in a DCN-axons decrease in a target area. Previously we've shown that medulla-targeting DCNs regulate object orientation behavior in flies. Knockdown of *dTzap* impaired this behavior, mimicking the complete silencing of M-DCNs, despite the presence of 65% of axons in the correct target space. Transsynaptic tracing showed that *dTzap* downregulation leads to dramatic loss of postsynaptic connectivity, and decrease was not due to loss of the pre-synaptic proteins, suggesting a different mechanism. *CG7101/dTZAP* ChIPseq data revealed binding to a large number of genes involved in mitochondrial biology such as CG7772/*dMtfp1, pink1*, *drp1*, *ewg* and *atg1*, suggesting that TZAP is a conserved transcriptional regulator of mitochondrial homeostasis. We tested this hypothesis in *Drosophila* and mammalian COS7 cells and confirmed impaired mitochondria morphology and downregulation of key mitochondrial genes. Knockdown of *pink1* phenocopied increased mitochondrial size in axons and reduction of postsynaptic labelling, while its overexpression rescued the phenotypes observed upon *dTzap* knockdown.

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BOARD NUMBER: S06-352

UNRAVELING THE UNEXPECTED FUNCTION OF WILD TYPE AND MUTATED RAD51 PROTEINS IN THE DEVELOPMENT OF THE CORTICOSPINAL TRACT IN MICE

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Aims: RAD51, a protein known for its role in DNA repair, has been linked to congenital mirror movements (CMM). CMM patients perform involuntary movements of one hand that mirror voluntary movements of the other hand. We demonstrated that *RAD51*-mutated CMM patients have abnormal corticospinal tract (CST) midline crossing at the level of the pyramidal decussation. Strikingly, RAD51 is cytoplasmic in the corticospinal neurons in newborn mice, and is present at the pyramidal decussation within the spatio-temporal window corresponding to CST formation. We are investigating the new unexpected role of RAD51 in the development of the CST. **Methods:** We characterized the CST phenotype induced by the deletion or the overexpression of WT or CMM-mutated *Rad51* restricted to the motor cortex using *in utero* electroporation. We analyze the 3D trajectories of corticospinal axons using clearing and an ultramicroscopy method (3DISCO). **Results:** Selective and complete deletion of *Rad51* in the motor cortex does not affect the corticospinal trajectory. In contrast, overexpression of WT *RAD51* or its CMM-mutated variant leads to abnormal tract trajectories in 50% of animals. **Conclusion:** Precise balance of RAD51 levels within corticospinal neurons is mandatory for the correct development of the CST. An altered level of RAD51 could be more detrimental than its absence. This work will help in unraveling the cytoplasmic role of RAD51 in CST development by taking advantage of the CMM paradigm. Our findings will provide new insights into CST development and axon guidance mechanisms.

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THE CONGENITAL MIRROR MOVEMENTS DISORDER REVEALS A NEW HAPLOINSUFFICIENT ROLE OF RAD51 IN THE DEVELOPMENT OF THE CORTICOSPINAL TRACT

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Aims: Mirror movements are involuntary movements of one side of the body that mirror intentional movements on the opposite side. Congenital Mirror Movements (CMM) is a rare genetic disorder with autosomal dominant inheritance, in which mirror movements are the sole or main neurological symptom. CMM is associated with an abnormal decussation of the corticospinal tract, the major motor projection from the cortex to the spinal cord. Surprisingly, *RAD51*, a gene previously known for its key role in homologous recombination in DNA repair, is one of the CMM causative genes. We are investigating this new unexpected role of RAD51 in the development of the nervous system. Methods: We take advantage of our large cohort to investigate the expression of wild-type and mutant RAD51 in CMM patients' lymphoblasts. RAD51 expression is also characterized in the mouse developing nervous system. We further use biochemical approaches to characterize the functions of RAD51 that may be altered in the mutated proteins. Results: RAD51 is present in the cytoplasm of corticospinal neurons and detected at the decussation at the time of midline crossing by their axons. The CMM mutations of *RAD51* are loss-of-function mutations and the non-truncating mutations impact the dimerization of the protein. Conclusions: Through the CMM paradigm, we demonstrate that *RAD51* is haploinsufficient for the corticospinal tract development. Amazingly, our work uncovers a new cytoplasmic role for RAD51, that may require its oligomerization, a key feature of its well-known nuclear recombinase role.

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BOARD NUMBER: S06-354

ROLE OF THE TRANSMEMBRANE TYROSINE KINASE RECEPTOR C-KIT IN THE DEVELOPMENT OF THE OLIVOCEREBELLAR CIRCUIT

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Proper brain development relies on the proliferation, migration, differentiation, recognition and adhesion between different cell populations. Molecular pathways that regulate normal development of other tissues can be involved in brain development as well. One of these pathways is c-KIT/SCF signaling. c-KIT is a transmembrane tyrosine kinase receptor that controls cell survival, proliferation and maintenance of various tissues (e.g. hematopoietic tissue). In the cerebral cortex, c-KIT is involved in the connectivity of callosal fibers to their targets as well as radial migration of neurons. We observed the localization of c-KIT in the cerebellum, whose circuitry and architecture are very well studied. Data from the Allen Brain Atlas show that c-Kit and Scf are expressed in IONs and PCs, respectively, from late embryonic days until adulthood. Using immunolabeling, we showed the localization of c-KIT along the axons of a subset of IONs, and at their glutamatergic synaptic terminals on PCs postnatally, suggesting a potential role of c-KIT/SCF pathway in the establishment of ION-PC connection. To test this hypothesis, we are currently analyzing the consequences of neuron-specific conditional invalidation of c-Kit or Scf in the formation of ION-PC connectivity at the morphological and functional levels. Our results will shed light on the molecular mechanisms regulating the establishment of the olivocerebellar circuit, a network important for motor control and cognition. Furthermore, given that the c-KIT/SCF pathway is an important therapeutic target in cancer, our study will illustrate the potential side effects of those treatments on brain development and function.

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BOARD NUMBER: S06-355

ASYMMETRIC METABOLISM CONTROLS THE ACUTE ACQUISITION OF VERTEBRATE AXON COMPLEXITY

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Asymmetric metabolism controls the acute acquisition of vertebrate axon complexity. Fumi Suomi[1], Masgutova G, Rappe A, Rathod R, Courtoy GE, Adolfs Y, Pasterkamp RJ, Nieminen A, Clotman F and McWilliams TG. **Affiliations** [1]Translational Stem Cell Biology and Metabolism Program, Faculty of Medicine, Biomedicum Helsinki, University of Helsinki, Haartmaninkatu 8, Helsinki 00790-FI **Aims**: Mitochondrial metabolism is critical for developing neurons, yet the full repertoire of metabolic signals that shape developing neuronal architecture remain mysterious. We sought to define the metabolic basis of axon growth in developing mouse post-mitotic neurons. **Methods**: We performed high content morphometric profiling of mouse primary neurons in defined metabolic states, and used temporal fluxomics to define the substrate utilization at distinct morphological stages in concert with transcriptomics and *in vivo* analysis of cleared mutant embryos using iDISCO. **Results**: We reveal that glycolysis promotes the branching and complexity of advancing neurites, with unexpected dynamic changes in glucose utilisation. Our transcriptomics analysis reveals that distinct metabolic states control the expression of growth-inhibitory molecules, and converge on a unique transcription factor family. Genetic ablation of these transcription factors disrupts mouse sensory development organisation in vivo. **Conclusions:** Our findings demonstrate the unexpected developmental importance of glycolytic signalling for the development of vertebrate post-mitotic neurons and link metabolism to a novel family of transcription factors.

Pubmed:
34161705: Collier JJ, Guissart C, Oláhová M, Sasorith S, Piron-Prunier F, Suomi F, Zhang D, Martinez-Lopez N, Leboucq N, Bahr A, Azzarello-Burri S, Reich S, Schöls L, Polvikoski TM, Meyer P, Larrieu L, Schaefer AM, Alsaif HS, Alyamani S, Zuchner S, Barbosa IA, Deshpande C, Pyle A, Rauch A, Synofzik M, Alkuraya FS, Rivier F, Ryten M, McFarland R, Delahodde A, McWilliams TG, Koenig M, Taylor RW
Developmental Consequences of Defective ATG7-Mediated Autophagy in Humans.
Autophagy is the major intracellular degradation route in mammalian cells. Systemic ablation of core autophagy-related () genes in mice leads to embryonic or perinatal lethality, and conditional models show neurodegeneration. Impaired autophagy has been associated with a range of complex human diseases, yet congenital autophagy disorders are rare.
N Engl J Med, 2021; 384
32269837: Suomi F, McWilliams TG
Autophagy in the mammalian nervous system: a primer for neuroscientists.
Autophagy refers to the lysosomal degradation of damaged or superfluous components and is essential for metabolic plasticity and tissue integrity. This evolutionarily conserved process is particularly vital to mammalian post-mitotic cells such as neurons, which face unique logistical challenges and must sustain homoeostasis over decades. Defective autophagy has pathophysiological importance, especially for human neurodegeneration. The present-day definition of autophagy broadly encompasses two distinct yet related phenomena: non-selective and selective autophagy. In this minireview, we focus on established and emerging concepts in the field, paying particular attention to the physiological significance of macroautophagy and the burgeoning world of selective autophagy pathways in the context of the vertebrate nervous system. By highlighting established basics and recent breakthroughs, we aim to provide a useful conceptual framework for neuroscientists interested in autophagy, in addition to autophagy enthusiasts with an eye on the nervous system.
Neuronal Signal, 2019; 3
34725936: Collier JJ, Suomi F, Oláhová M, McWilliams TG, Taylor RW
Emerging roles of ATG7 in human health and disease.
The cardinal stages of macroautophagy are driven by core autophagy-related (ATG) proteins, whose ablation largely abolishes intracellular turnover. Disrupting ATG genes is paradigmatic of studying autophagy deficiency, yet emerging data suggest that ATG proteins have extensive biological importance beyond autophagic elimination. An important example is

ATG7, an essential autophagy effector enzyme that in concert with other ATG proteins, also regulates immunity, cell death and protein secretion, and independently regulates the cell cycle and apoptosis. Recently, a direct association between ATG7 dysfunction and disease was established in patients with biallelic ATG7 variants and childhood-onset neuropathology. Moreover, a prodigious body of evidence supports a role for ATG7 in protecting against complex disease states in model organisms, although how dysfunctional ATG7 contributes to manifestation of these diseases, including cancer, neurodegeneration and infection, in humans remains unclear. Here, we systematically review the biological functions of ATG7, discussing the impact of its impairment on signalling pathways and human pathology. Future studies illuminating the molecular relationship between ATG7 dysfunction and disease will expedite therapies for disorders involving ATG7 deficiency and/or impaired autophagy.
EMBO Mol Med, 2021; 13

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ECTOPIC POSITION OF CSF-CNS IN THE SPINAL CORD OF C57BL/6N MICE IS ASSOCIATED WITH SNPS IN CRB1 AND CYFIP2.

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Cerebrospinal fluid-contacting neurons (CSF-cNs) reside the ependymal lining surrounding the central canal of the spinal cord and brain ventricles. In our previous study, we found that majority of CSF-cNs in the spinal cord of C57Bl/6N mice is in ectopic position, unlike those in the other inspected mice strains and mammalian species. The ectopic position of CSF-cN was observed solely in C57Bl/6N mice, which share common similar genetic profile with mouse strain C57Bl/6J. Therefore, we decided to investigate genetic polymorphisms between 6N and 6J substrains to elucidate possible gene candidate(s) responsible for the ectopic presence of CSF-cNs in C57Bl/6N mouse substrain. First, in the *in silico* analysis, we narrowed down the group of 236 homozygous sequence variants differentiating those two substrains to 5 suspected genes bearing the polymorphisms in 6N substrain. We selected 5 genes (*Crb1*, *Cyfip2*, *Adamts12*, *Plk1* and *Herpud2*) which meet all three criteria; i) mutation of the gene in 6N substrain has deleterious effect on the protein function; ii) the gene is expressed in the spinal cord; and iii) the coding sequence of the gene is conserved among other mice strains. Next, employing the segregation analysis of the F2 progeny derived from parental strains C57Bl/6N and Balb/C, we found that concurrent presence of missense mutations in *Crb1* and *Cyfip2* is closely associated with ectopic distribution of CSF-cNs. Our study adds another piece to the growing body of evidence that the employment of C57Bl/6N mice in particular type of experiments should be carefully reasoned.

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THE ROLE OF HETEROCHROMATIN IN HUMAN BRAIN DEVELOPMENT

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Transposable elements (TEs) are particularly active during human brain development. Aberrant expression and epigenetic dysregulation have been linked to several neurological and psychiatric disorders. During embryonic development, most TEs are silenced via epigenetic mechanisms such as the formation of heterochromatin and DNA methylation since they otherwise can pose a threat to the genomic integrity. In this study, we investigate molecular mechanisms that are in control of TEs activity during human brain development, with a focus on epigenetic mechanisms. Using high throughout CRISPRi screen, we targeted key epigenetic regulators associated with disposition of repressive histone marks as well as DNA methylation. Targets such as TRIM28, SETDB1, HUSH-complex subunits and DNMT1 were knocked down in human neuroepithelial stem cells and induced pluripotent stem cell derived forebrain neural precursors cells and cerebral organoids. Looking at transcriptomic changes after differentiation, we examined the effects of the knock down in human brain development. To get a complete profile on how TEs are regulated and expressed is of great importance to better understand healthy human brain development and various mechanisms underlying brain disorders. Our results will provide further insight on how TEs are epigenetically regulated during human brain development.

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BOARD NUMBER: S06-358

PIEZO2 LINEAGES IN THE DEVELOPING CENTRAL NERVOUS SYSTEM

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Mechanotransduction is the process by which a mechanical stimulus is converted into electrical activity, which is crucial to various physiological phenomenon such as somatic sensation and vascular homeostasis. The mammalian mechanosensitive ion channel PIEZO2 acts as a highly sensitive mechanosensor in diverse cell types, required for mechanotransduction in the proprioceptive and somatosensory systems. Other functions however remain to be clarified, as clinical studies reported that patients who carry PIEZO2 mutations present diverse symptoms including neurodevelopmental disorders, such as Gordon syndrome and Marden-Walker syndrome. Animal models have the potential to yield insights into these open questions. Studies on Piezo2 so far have only focused on adult mice; a thorough analysis of its expression and function over the course of development has yet to be carried out. We used a mouse reporter line to fate-map Piezo2 cell linages which we combined with RNAscope and ISH expression analyses. The results indicate that 1) Piezo2 is widely expressed in various anatomical structures at different developmental stages and 2) Piezo2^{+} lineages give rise to several tissues that have not been hitherto connected to Piezo2 function. Our studies focus on characterizing the identity of these *Piezo2*$^{+}$ descendent cells in the brain by co-expression studies. We found distinct types of *piezo2* lineage cells in the brain, oligodendrocytes, neurons, which can be found in specific brain regions associated with olfaction like the piriform cortex, and endothelial cells. Moreover, we are currently characterizing phenotypic and physiological parameters of Piezo2 gain- and loss-of-function mouse models.

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SPATIOTEMPORAL EXPRESSION OF THE 5-HT6 RECEPTOR IN THE MOUSE BRAIN, FROM EMBRYO TO ADULT

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Pharmacological inhibition of the serotonin 5-HT_6 receptor (5-HT_6R) alleviates the cognitive symptoms observed in neurodevelopmental, neurodegenerative or psychiatric diseases. It is therefore a promising new therapeutic target for these pathologies. However, the molecular mechanisms underlying the beneficial effects of 5-HT_6R blockade remain unknown. This is in part due to the lack of specific antibodies to detect the endogenous receptor and to get a clear picture of where and when it is expressed. In this study, we have used a new mouse model expressing a GFP-tagged 5-HT_6R under the control of its native promoter to characterize the receptor's expression in embryonic and adult brain. We show that the receptor is mainly expressed in projection neurons of the cerebral cortex and the hippocampus as well as in striatal medium size spiny neurons, while it was found in a minor population of interneurons. The receptor was also detected in a large proportion of astrocytes. Finally, we looked at its subcellular distribution in embryonic, post-natal and adult brain. We show that the receptor is located in the primary cilia throughout the life of the mouse, but interestingly, it is relocated to the somato-dendritic compartment at the perinatal stage. This study provides a global view of the 5-HT_6 receptor's expression, which could serve as a basis for further studies aimed at understanding its roles in neurodevelopmental and cognitive processes.

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BOARD NUMBER: S06-360

DMRT2 SPLICING REGULATION IN SEX AND DEVELOPMENT OF THE NERVOUS SYSTEM

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Sexual differences in behaviour emerge from sex-specific molecular and connectivity brain conformations. The genetic factors involved in the sexual differentiation of the nervous system are poorly understood, but they could become vulnerability or protective factors in the etiology of mental disorders. The family of *doublesex*/*mab-3* related transcription factors (DMRT) are conserved as sexual regulators of sexually dimorphic traits across distant species. In flies, sex-specific expression of Doublesex isoforms establishes the morphology and behaviour characteristic of each sex. So far, the presence of a similar sex-specific splicing is unknown. Here, we show a novel alternative transcript of the mouse orthologous *Dmrt2*. This splicing potentially generates two DMRT isoforms with a similar structure to Doublesex, sharing the DM domain but differing in the N-terminal. Both variants are expressed in the nervous system and, remarkably, undergo a switch of predominance at embryonic stage and an interesting upward trend in female brains. We found that *Dmrt2* is widely expressed in the cortex, in corticothalamic projection neurons and GABAergic interneurons. This last population is present in hippocampus and subiculum. It also stands out its expression in several areas associated with sex-specific behaviour such as the lateral preoptic area, septal nucleus, amygdala or periaqueductal grey. Moreover, *Dmrt2* is expressed exclusively in post-mitotic neurons from early embryo until adulthood, suggesting a role in late developmental processes and circuit maintenance. Taken together, our results open the possibility of a novel mechanism to generate brain sexually dimorphic layouts through the sex-specific regulation of Dmrt2.

Pubmed:
32628253: Birkhoff JC, Brouwer RWW, Kolovos P, Korporaal AL, Bermejo-Santos A, Boltsis I, Nowosad K, van den Hout MCGN, Grosveld FG, van IJcken WFJ, Huylebroeck D, Conidi A
Targeted chromatin conformation analysis identifies novel distal neural enhancers of ZEB2 in pluripotent stem cell differentiation.
The transcription factor zinc finger E-box binding protein 2 (ZEB2) controls embryonic and adult cell fate decisions and cellular maturation in many stem/progenitor cell types. Defects in these processes in specific cell types underlie several aspects of Mowat-Wilson syndrome (MOWS), which is caused by ZEB2 haplo-insufficiency. Human ZEB2, like mouse Zeb2, is located on chromosome 2 downstream of a ±3.5 Mb-long gene-desert, lacking any protein-coding gene. Using temporal targeted chromatin capture (T2C), we show major chromatin structural changes based on mapping in-cis proximities between the ZEB2 promoter and this gene desert during neural differentiation of human-induced pluripotent stem cells, including at early neuroprogenitor cell (NPC)/rosette state, where ZEB2 mRNA levels increase significantly. Combining T2C with histone-3 acetylation mapping, we identified three novel candidate enhancers about 500 kb upstream of the ZEB2 transcription start site. Functional luciferase-based assays in heterologous cells and NPCs reveal co-operation between these three enhancers. This study is the first to document in-cis Regulatory Elements located in ZEB2's gene desert. The results further show the usability of T2C for future studies of ZEB2 REs in differentiation and maturation of multiple cell types and the molecular characterization of newly identified MOWS patients that lack mutations in ZEB2 protein-coding exons.
Hum Mol Genet, 2020; 29

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DMRT5 BEYOND THE CORTEX: EARLY ROLE IN THE SEXUAL DIFFERENTIATION OF THE MOUSE LIMBIC SYSTEM

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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The mammalian limbic system is sexually dimorphic and regulates sex-specific innate behaviors such as mating, maternal care and aggression. However, the genetic programs involved in the sexual differentiation of the limbic system are poorly understood. Dmrt transcription factors have emerged as conserved regulators of sex-specific traits across phylogeny. They have been studied mostly in invertebrates, where they play an important role in nervous system sexual differentiation, and in vertebrate cortical development. In fact, mutations in *DMRTA2 (Dmrt5)* lead to a recessive human condition characterized by microcephaly and lissencephaly. In this work we use Dmrt5 null mutant mice, RNA-seq and histological techniques to study Dmrt5 function in the mammalian nervous system beyond the cortex and comparing males and females. In this study we showed that Dmrt5 is broadly expressed in the mouse nervous system, including the vomeronasal organ, olfactory tubercle, or ventral tegmental area (all key nodes in the control of innate behaviors). Furthermore, we showed that Dmrt5 is necessary for the correct specification of the main olfactory epithelium, the olfactory bulb, and several hypothalamic nuclei. Additionally, we found a novel function for Dmrt5 as a suppressor of sex differences in gene expression in the posterior hypothalamic area and midbrain. Taken together, Dmrt5 comes out as a crucial transcription factor for accurately generating limbic regions and integrating sex information. Therefore, by studying the genetic factors involved in sexual differentiation we can find potential factors that either afford protection or generate vulnerability in one sex versus the other for sex-biased mental disorders.

Pubmed:
30953965: Sanzo-Machuca Á, Monje Moreno JM, Casado-Navarro R, Karakuzu O, Guerrero-Gómez D, Fierro-González JC, Swoboda P, Muñoz MJ, Garsin DA, Pedrajas JR, Barrios A, Miranda-Vizuete A
Redox-dependent and redox-independent functions of Caenorhabditis elegans thioredoxin 1.
Thioredoxins (TRX) are traditionally considered as enzymes catalyzing redox reactions. However, redox-independent functions of thioredoxins have been described in different organisms, although the underlying molecular mechanisms are yet unknown. We report here the characterization of the first generated endogenous redox-inactive thioredoxin in an animal model, the TRX-1 in the nematode Caenorhabditis elegans. We find that TRX-1 dually regulates the formation of an endurance larval stage (dauer) by interacting with the insulin pathway in a redox-independent manner and the cGMP pathway in a redox-dependent manner. Moreover, the requirement of TRX-1 for the extended longevity of worms with compromised insulin signalling or under calorie restriction relies on TRX-1 redox activity. In contrast, the nuclear translocation of the SKN-1 transcription factor and increased LIPS-6 protein levels in the intestine upon trx-1 deficiency are strictly redox-independent. Finally, we identify a novel function of C. elegans TRX-1 in male food-leaving behaviour that is redox-dependent. Taken together, our results position C. elegans as an ideal model to gain mechanistic insight into the redox-independent functions of metazoan thioredoxins, overcoming the limitations imposed by the embryonic lethal phenotypes of thioredoxin mutants in higher organisms.
Redox Biol, 2019; 24

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THE ROLE OF PI3K/ AKT/ MTOR PATHWAY IN PROGRAMMED CELL DEATH OF CAJAL-RETZIUS CELLS

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Programmed cell death (PCD) is a critical contributor to nervous system development and its alterations are suggested to participate in the pathophysiology of neurodevelopmental disorders. Cajal-Retzius cells (CRs) play key roles in cortical development and are one of the remarkable examples of PCD in the cerebral cortex. In mice, CRs undergo almost complete PCD in the first two post-natal weeks. The persistence of CRs during postnatal life has been detected in pathological conditions related to epilepsy. It has been shown that the PI3K/ AKT/ mTOR pathway plays an important role in cell survival and its dysregulation is implicated in malformation of cortical development (MCD) and epilepsy. Interestingly we found no evidence of S6 kinase activation in mouse CRs during post-natal stages suggesting that the mTOR pathway is not active after birth. In order to activate it in these cells, we conditionally deleted *Pten* or *Tsc1* genes, which encodes for two inhibitors of the PIK3/AKT/mTOR pathway. Loss of PTEN or TSC1 functions did not affect CRs generation and migration but their morphology at later post-natal stages. In addition, we observed a stronger activation of S6 kinase in the PTEN mutant compared to the TSC1 probably due to parallel activation of PDK1. Finally, activation of the pathway led to CRs persistence after two weeks. Altogether, these results show that the PI3K/ AKT/ mTOR pathway is strongly inhibited in CRs during post-natal stages priming these cells to death.

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THE ATYPICAL KINESIN KIF21B CONTROLS NEURONAL MIGRATION THROUGH REGULATION OF ACTIN CONTRACTION

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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The development of the cerebral cortex progresses through several stages including proliferation, migration and differentiation. Microtubules (MT) and its associated proteins are important regulators of these processes and mutations in genes encoding these components are responsible for cortical malformations, intellectual disabilities and epilepsy. The molecular motors kinesins are key regulators of corticogenesis by regulating cargoes transport and MT cytoskeleton organization. Defective function of kinesins has been related to cortical malformations such as microcephaly, lissencephaly and pachygyria. In this work we studied the physiological roles of Kif21b during corticogenesis, a kinesin that has been shown to be relevant in human cortex development. We showed that Kif21b silencing by *In Utero Electroporation* in mouse embryonic cortices induces morphological changes and locomotion defects in migrating projection neurons. Through rescue experiments with truncated variants of Kif21b we elucidated that its MT motor role is dispensable for migration. Unexpectedly, we identified Kif21b as an actin interacting partner, having a function through which Kif21b regulates actin contraction during the migratory cycle. Altogether, this study unravels unlooked-for physiological functions of Kif21b during neuronal migration.

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ANALYSIS OF APOPTOTIC MARKERS IN RAT SPINAL CORD CELLS DURING POSTNATAL LIFE

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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An important role played by apoptosis and apoptotic-related proteins in many physiological and pathological processes of nervous system makes apoptosis an intriguing study topic for neuroscience. In this work, we focused on the study of apoptosis of cells in the spinal cord of rats in the selected time points during the postnatal life (8, 29, and 90 days) using immunofluorescent detection of active caspase3 (aC3), and protein cleaved PARP-1 (cPARP), which are routinely used as markers of apoptotic cells. Surprisingly, based on the results of stereological quantification, we concluded that the ratio between $aC3^+$ and $cPARP^+$ cells in the spinal cord tissue is 593:1 (P8), 4490:1 (P29), and 487:1 (P90), respectively. To compare the distribution of ac3 and cPARP-1 in the apoptosis, we prepared the primary cell cultures isolated from the spinal cord of 8 days old rats, in which apoptosis was induced using staurosporine treatment and the presence of $aC3^+$ and $cPARP\text{-}1^+$ cells was analyzed in the selected time points afterward. In contrast to the *in vivo* study, the i*n vitro* analysis revealed a similar pattern of distribution of $aC3^+$ and $cPARP^+$ cells in the studied time points after the apoptotic induction. Our data indicate that the aC3 is not an exclusive marker of apoptotic cells in the nervous tissue and may be involved in the non-apoptotic processes during postnatal life. Hence, identification of apoptotic cells by immunodetection of aC3 should be confirmed by other apoptotic markers. Acknowledgment: This study was supported by grants VEGA no.1/0760/20 and APVV-15-0239.

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BOARD NUMBER: S06-365

PRIMARY CILIUM-ELICITED SIGNALLING PATHWAYS AND CORTICAL INTERNEURON MIGRATION

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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During development, cortical interneurons undertake a complex migration in order to reach the developing cortex, in which they will form and integrate functional circuits. Our team and others have shown the importance of a small, dynamic structure called the primary cilium (PC) during this process, to initiate the cortical interneuron migration switch from tangential to radial migratory routes. Although known to be a signalling hub concentrating a wide variety of membrane receptors, the nature of the intracellular pathways elicited at the primary cilium downstream of such receptors, and how they could affect cortical interneuron migration, remain unknown. We here aim at elucidating such pathways by focusing on PC-elicited second messengers such as cAMP and cGMP, which are molecules present in many cellular compartments to transduce a variety of signalling pathways. Thanks to a molecular toolbox developed in collaboration with Xavier Nicol (IDV Paris), we target scavengers or photo-activated constructs specifically to the PC – via a 5HT6 targeting sequence – and respectively buffer or activate cAMP or cGMP levels locally and specifically in the PC. By combining ciliary cAMP/cGMP buffering or photo-activation, our results point at distinct and specific roles for ciliary cAMP and cGMP second messengers in the regulation of cortical interneuron migration. Our data provide new insight into the importance of PC-initiated signalling pathways in interneuron migration. Unravelling these cellular and molecular mechanisms is furthermore crucial to shed new light on our understanding of developmental disorders in which PC formation as well as cortical interneuron migration are defective.

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BOARD NUMBER: S06-366

THE ROLE OF LYSOSOMAL DYSFUNCTION IN CORTICAL DEVELOPMENT IN THE CONTEXT OF HEREDITARY SPASTIC PARAPLEGIA SPG11

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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SPG11 is an autosomal recessive neurodegenerative disorder caused by loss-of-function mutations in the gene *Spg11*, which encodes the protein spatacsin. Typically, symptoms include weakness and rigidity in lower limbs, as well as cognitive impairment, which appear before adolescence and progressively worsen throughout the patient's lifetime. The early onset of this disease has led to the hypothesis that spatacsin loss-of-function may cause impaired neurodevelopment. Accordingly, investigations have shown that SPG11 neural progenitor cells (NPCs) demonstrate impaired proliferation, premature neurogenesis, and widespread transcriptional alterations in pathways implicated in cell cycle regulation and cortical development. Loss of spatacsin is known to impair autophagic lysosome reformation, however there remains a substantial gap in our understanding of the subcellular mechanisms underlying the developmental defects of SPG11. To elucidate the relationship between lysosomal dysfunction and impaired neurodevelopment we are using cortical organoids derived from induced pluripotent stem cells (iPSCs) with truncating mutations in Spg11, along with isogenic controls. Within these 3D structures we are focusing on the radial glial progenitor cells (RGPCs) that are found within rosette-like ventricular formations that mimic early corticogenesis. Thus far we have observed that, within these SPG11 RGPCs, there is apical clustering of lysosomes, as well as a disruption in the expression and/or localization of junctional proteins. We are now aiming to elucidate the subcellular mechanisms that underlie the reduced proliferation and increased differentiation of SPG11 neural progenitor cells, as well as identify potential factors and pathways that can be altered to rescue said function.

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INVESTIGATION OF NEURONAL PHENOTYPES AND MECHANISMS DURING SUBCORTICAL BAND HETEROTOPIA DEVELOPMENT IN EML1 CKO MOUSE

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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The development of the cerebral cortex is a complex and stereotyped process in which perturbations can lead to malformations associated with severe disorders such as epilepsy and intellectual disability. One such malformation, characterized by mislocalized neurons beneath the cortex, is subcortical band heterotopia. Genetic studies in patients and a mutant mouse model of subcortical band heterotopia revealed mutations in the gene coding for the microtubule-associated protein Eml1/EML1. In addition, phenotypic analyses identified abnormal radial glia (RG) progenitor cells contributing to severe forms of this disorder. However, defects in subsequently generated neurons, their mispositioning, and role in heterotopia abnormal function remains to be understood. Using a tissue clearing method iDisco, followed by 3D lightsheet imaging, we characterized neuronal phenotypes in Eml1 conditional knockout (cKO) mouse embryonic cortices, notably identifying abnormal axon bundles. Moreover, comparing single-cell RNA Seq data from wild-type and Eml1 cKO cortices, we identified candidate pathways that might provide causal mechanism for the abnormal cortical phenotypes of the Eml1 cKO mice.

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HYPERACTIVITY OF RAC1 GTPASE AFFECTS THE DIRECTIONAL CONTROL OF MIGRATING INTERNEURONS IN THE EMBRYONIC CORTEX AND RESULTS IN REDUCED INHIBITION AND SUSCEPTIBILITY TO EPILEPSY

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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GTPases of the Rho family are components of signaling pathways linking extracellular signals to the control of cytoskeleton dynamics. Among these, RAC1 plays key roles during brain development, ranging from neuronal migration to neuritogenesis, synaptogenesis, and plasticity. RAC1 activity is positively and negatively controlled by guanine nucleotide exchange factors (GEFs), guanosine nucleotide dissociation inhibitors (GDIs), and GTPase-activating proteins (GAPs), but the specific role of each regulator in vivo is poorly known. ARHGAP15 is a RAC1-specific GAP expressed during development in migrating cortical interneurons (CINs) and in a fraction of most subtypes of adult CINs. During development, loss of ARHGAP15 causes reduced morphological complexity and altered directionality of the leading process of tangentially migrating CINs. Likewise, time-lapse imaging of embryonic CINs reveals a poorly coordinated directional control also during radial migration, possibly due to a hyper-exploratory behavior. In the adult Arhgap15$^{LacZ/LacZ}$ cortex, the observed migration defects lead to subtle layering defects of distinct CIN subtypes, spontaneous subclinical seizures, and increased susceptibility to the pro-epileptic drug pilocarpine. These results indicate that ARHGAP15 imposes a fine negative regulation on RAC1 that is required for normal control of directionality during CIN migration, with consequences on their laminar distribution and inhibitory function.

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GLYCOLYSIS FUELS ACTOMYOSIN CONTRACTION DURING AXONAL GUIDANCE

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Brain wiring relies on stereotyped axonal connections that are established during the development through growth cone navigation in response to environmental guidance cues. Non-muscle myosin II (NMII), acts downstream of repulsive axonal guidance signals. In association with actin filaments, NMII forms the actomyosin complex that is responsible for growth cone retraction. Since NMII function is ATP dependent, we studied the energetic source for actomyosin activity in axonal guidance and then assessed the consequences of metabolic impairment *in vivo*. We first studied, using rat neuronal cultures, which metabolic pathway supplied energy during growth cone retraction by inhibiting mitochondrial or glycolytic ATP synthesis using drugs or siRNA interference. We showed that glycolysis and not mitochondria fuels growth cone retraction in neuronal cultures. Then, by combining confocal, video and STORM microscopy, we found that glycolytic enzymes were enriched at the growth cone during retraction and associated with actomyosin using proximity ligation assay. Furthermore, we confirmed the direct interaction of glycolytic enzymes and actomyosin by co-immunoprecipitation. Finally, we demonstrated that blocking glycolysis *in vivo*, by ketogenic diet (KD) during pregnancy, impaired corticofugal axons wiring. Collectively, our results highlight a tight energy coupling between ATP production and consumption in axonal guidance. We also show evidence that gestational KD is potentially harmful to offspring.

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BOARD NUMBER: S06-370

α-MELANOCYTE STIMULATING HORMONE (α-MSH) AS A TROPHIC FACTOR DURING HYPOTHALAMIC DEVELOPMENT

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Aims: α-MSH is an endogenous neuropeptide involved in energy homeostasis. α-MSH levels increase during the third trimester (3T) in humans and are attenuated in response to high fat diet (HFD) in rodents. 3T is a critical window for synapse formation within the hypothalamus. This period corresponds to the lactation period (LP) in mice, when neurones from the arcuate nucleus of the hypothalamus (ARH) project axons to areas responsible for eating behaviour from P6-16. Undernutrition, overnutrition and inflammation can impair circuit formation, leading to loss of energy homeostasis and excessive energy intake in offspring. Moreover, α-MSH induces axon elongation and increases synaptic proteins *in vitro*. Therefore, we aim to determine how levels of α-MSH fluctuate throughout the LP in mice in a model of malnutrition, HFD, and test whether α-MSH induces axon outgrowth from the ARH. **Methods:** To investigate the fluctuations in α-MSH, we have taken plasma, hypothalamus and pituitary samples from mothers and offspring fed either a control diet or a HFD during LP; we will quantify α-MSH by ELISA. The trophic action of α-MSH on hypothalamic axons will be assessed in an *in-vitro* model of ARH explants. **Hypothesis:** We predict that α-MSH will increase during P6-16, and could be affected by HFD. We anticipate that α-MSH will induce axon elongation *in vitro*. **Conclusions:** α-MSH drives a number of pleiotropic effects throughout development and in maintaining homeostasis. The findings of this study can inform educational outreach for pregnant people and deepen our understanding of brain development.

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FILAMIN A MODULATES DENDRITIC BRANCHING VIA INTEGRIN-AKT AXIS AND ACTIN CYTOSKELETON

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Dendritic architecture is essential during brain development as it lays the foundation of neural signaling. Therefore, control of dendritic branching is crucial for the specificity and capacity of the synaptic input. Integrin receptors on dendrites have been shown to be indispensable for branching mechanisms, however, underlying roles of integrin-accessory proteins are still poorly understood. Here, combining in vitro mouse hippocampal cultures with genetic and pharmacological manipulations, we show that Filamin A (FlnA) plays a crucial role in dendritic branching. First, we demonstrated that altering the FlnA expression levels resulted in dendritic hypertrophy in an extracellular matrix (ECM) dependent manner. Further investigation of these phenotypes revealed that shFlnA and FlnA overexpression mediated dendritic branching are differentially regulated by ECM and ß1-integrin signaling. Moreover, silencing of FlnA resulted to a disturbance in the Akt activity, which might be the downstream mediator of the observed growth effects. Finally, we demonstrated that FlnA overexpression mediated dendritic hypertrophy is critically dependent to actin-crosslinking by FlnA. Together, these results suggest that FlnA is an important regulator of dendritic branching by contributing to integrin downstream signaling and actin dynamics. Supported by the German Research Foundation (STO488/4-1 and CRC779 TPB05).

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BOARD NUMBER: S06-372

THE HIDDEN SIDE OF NCAM FAMILY: NCAM2, A KEY REGULATOR OF NEURONAL MORPHOGENESIS AND AXODENDRITIC ARCHITECTURE

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Neural cell adhesion molecule 2 (NCAM2) is involved in the development and plasticity of the olfactory system. Genetic data have implicated the NCAM2 gene in neurodevelopmental disorders including Down syndrome and autism. NCAM2's functions and partners in cortical areas during development and adulthood have remained largely unknown until not long ago. We studied the role of NCAM2 in cortical areas. We used cell biology and molecular approaches, including loss- and gain-of-function experiments in vitro and in vivo models. We showed that overexpression of NCAM2 in hippocampal neuron cultures leads to minor alterations, but its downregulation severely compromises dendritic architecture, leading to shorter dendritic trees and retraction of dendrites. Furthermore, our data revealed alterations in the axonal tree and deficits in neuronal polarization. In vivo studies confirmed the in vitro phenotype observed and revealed an unexpected role for NCAM2 in cortical migration. Proteomic experiments show that NCAM2 interacts with >100 proteins involved in numerous processes. We described a NCAM2-protein complex with the cytoskeletal-associated proteins MAP2 and 14-3-3γ and ζ. We provide evidence that NCAM2 depletion results in destabilization of the cytoskeleton network and reduced MAP2 signal. Thus, our results demonstrate a role for NCAM2 in dendritic formation, in neural polarization and migration, through interaction of NCAM2 with microtubule-associated proteins and its ability to adapt to the external inputs of the cell and to modify the cytoskeleton accordingly. These functions of NCAM2 may offer promising new therapeutic approaches for the treatment of neurodevelopmental diseases in which NCAM2 is implicated.

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CRMP4-MEDIATED FORNIX DEVELOPMENT INVOLVES SEMAPHORIN-3E SIGNALING PATHWAY

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Neurodevelopmental axonal pathfinding plays a central role in correct brain wiring and subsequent cognitive abilities. Within the growth cone, various intracellular effectors transduce axonal guidance signals by remodeling the cytoskeleton. Semaphorin-3E (Sema3E) is a guidance cue implicated in development of the fornix, a neuronal tract connecting the hippocampus to the hypothalamus. Microtubule-Associated Protein 6 (MAP6) has been shown to be involved in the Sema3E growth-promoting signaling pathway. In this study, we identified the Collapsin Response Mediator Protein 4 (CRMP4) as a MAP6 partner and a crucial effector in Sema3E growth-promoting activity. CRMP4-KO mice displayed abnormal fornix development reminiscent of that observed in Sema3E-KO mice. CRMP4 was shown to interact with the Sema3E tripartite receptor complex within Detergent-*Resistant* Membrane (DRM) domains, and DRM domain integrity was required to transduce Sema3E signaling through the Akt/GSK3 pathway. Finally, we showed that the cytoskeleton-binding domain of CRMP4 is required for Sema3E's growth-promoting activity, suggesting that CRMP4 plays a role at the interface between Sema3E receptors, located in DRM domains, and the cytoskeleton network. As the fornix is affected in many psychiatric diseases, such as schizophrenia, our results provide new insights to better understand the neurodevelopmental components of these diseases.

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BOARD NUMBER: S06-374

FUNCTION OF METEORINS IN COMMISSURAL AXON GUIDANCE

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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In the embryonic nervous system, axons are guided to their specific targets by attractive and repulsive cues in the extracellular environment. Many of these cues are provided by axon guidance molecules, a complex network of receptor/ligand pairs which drive the axons of newly generated neurons towards their correct synaptic partners along well-defined paths. We have identified a family of secreted proteins (the Meteorins) whose members might be novel uncharacterized guidance factors. Via the generation of CRISPR-based mutants for each of the three members of the zebrafish Meteorin family (*mtrn*, *mtrnl1*, *mtrnl2*), we are now using a loss-of-function approach to investigate whether Meteorin proteins are involved in axon guidance, and in particular in the proper development of axonal commissures. Supporting this hypothesis, we observed specific axonal development defects in these mutant fish, including defects in several commissural neuron populations, pointing at a new role of Meteorins in the axon guidance process. We are now interested in the molecular dissection of the cellular pathways in which Meteorin proteins are involved, giving a particular focus on the identification of the so far unknown putative Meteorin receptor(s). In parallel, we are currently developing approaches to validate the identified candidates as *bona fide* Meteorin receptor(s).

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COMPARATIVE STUDY OF CHROMATIN RESPONSES TO NEURONAL STIMULATION REVEALS BDNF-SPECIFIC GENE REGULATORY MECHANISMS THROUGH THE PIONEERING FUNCTION OF TRANSCRIPTION FACTOR FOS

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Neuronal plasticity, the basic of learning and memory, allows the adaptation of the brain to internal and external triggers. Modulations of the genetic regulatory landscape are key to induce long-term structural and functional changes on the cellular level. Since the regulation of gene transcription relies on rearrangements of chromatin structure -i.e. chromatin accessibility and epigenetic modifications- we hypothesize that understanding the chromatin landscape will help discerning the mechanisms linking neuronal stimulation to gene expression changes. To identify features that define stimulation-specific chromatin-to-gene expression programs, we performed temporal profiling of chromatin accessibility and gene transcription in mouse primary cortical neurons upon either Brain-derived neurotrophic factor (BDNF) stimulation or membrane depolarization using KCl. We discovered that enhancer activation is an early event in the regulatory control of BDNF-treated neurons, where the bZIP motif-binding Fos protein pioneers chromatin opening and cooperates with other transcription factors (EGR, Homeobox, CTCF). Depolarization-dependent transcription occurs faster through direct promoter activation, yet with delayed Fos-dependent enhancer activation. Deleting one of such enhancers resulted in decreased Arc expression, a regulator of synaptic plasticity, in a BDNF-dependent manner. Further, we found that chromatin regions that display BDNF-induced accessibility changes are linked to preferential exon usage of neurodevelopmental disorder-related genes and are enriched in SNPs associated with neuropsychiatric diseases by Genome-Wide Association Studies. Thus, we provide a comprehensive view of BDNF-mediated genome regulatory features using comparative genomic approaches. In future research, we aim to understand whether BDNF-specific chromatin rearrangements could condition the neuronal transcriptional response in subsequent encounters to BDNF.

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BOARD NUMBER: S06-376

ALTERED CEREBELLAR BDNF SIGNALING AND SYNAPTIC ANOMALIES IN A MOUSE MODEL OF CHOLESTEROL DYSHOMEOSTASIS

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Niemann-Pick type C1 disease (NPCD) is a lysosomal lipid storage disease caused by mutations in the *Npc1* gene, leading to cerebellar ataxia, neurodegeneration, and progressive dementia. Our previous studies of *Npc1* mutant mice demonstrated reduced proliferation/migration of cerebellar granule cells (GCs) due to the significant downregulation of Shh and BDNF signaling. Using WB and IF analyses, we observed altered expression/localization of BDNF and its receptor TrkB during the first weeks of postnatal life in the mouse *Npc1^{nmf164}*, showing a slow-progressing form of NPCD. GCs from *wt* mice showed a “polarized accumulation” of receptor in the early-endosomes of leading processes necessary for their migration along with cerebellar layers, while *Npc1^{nmf164}* cerebella exhibited a significantly reduced GCs fraction with BDNF-dependent pTrkB polarization. We demonstrated that BDNF/TrkB alterations translate into abnormal GCs differentiation, as displayed by Golgi-staining, which is concomitant with pre- and postsynaptic functional alterations, in the complex neural networks at the glomerulus level. Given that cerebellar anomalies and BDNF downregulation are hallmarks of many neurodevelopmental disorders, such as autism, we characterized early social-communicative behaviors in our model by performing behavioral tests (Ultrasonic Vocalization, Homing, and the Three-Chamber test), commonly used to investigate the social domain. We propose that impaired BDNF signaling is upstream of complex cerebellar circuit deficits that result in dysfunctional synaptic networks, which are common in many neurodevelopmental disorders. Therefore, a better understanding of the additional physiological roles played by BDNF in a lipid dyshomeostasis mouse model is important to elucidate the link between cholesterol metabolism and brain pathology.

Pubmed:
34974125: Camuso S, La Rosa P, Fiorenza MT, Canterini S
Pleiotropic effects of BDNF on the cerebellum and hippocampus: Implications for neurodevelopmental disorders.
Brain-derived neurotrophic factor (BDNF) is one of the most studied neurotrophins in the mammalian brain, essential not only to the development of the central nervous system but also to synaptic plasticity. BDNF is present in various brain areas, but highest levels of expression are seen in the cerebellum and hippocampus. After birth, BDNF acts in the cerebellum as a mitogenic and chemotactic factor, stimulating the cerebellar granule cell precursors to proliferate, migrate and maturate, while in the hippocampus BDNF plays a fundamental role in synaptic transmission and plasticity, representing a key regulator for the long-term potentiation, learning and memory. Furthermore, the expression of BDNF is highly regulated and changes of its expression are associated with both physiological and pathological conditions. The purpose of this review is to provide an overview of the current state of knowledge on the BDNF biology and its neurotrophic role in the proper development and functioning of neurons and synapses in two important brain areas of postnatal neurogenesis, the cerebellum and hippocampus. Dysregulation of BDNF expression and signaling, resulting in alterations in neuronal maturation and plasticity in both systems, is a common hallmark of several neurodevelopmental diseases, such as autism spectrum disorder, suggesting that neuronal malfunction present in these disorders is the result of excessive or reduced of BDNF support. We believe that the more the relevance of the pathophysiological actions of BDNF, and its downstream signals, in early postnatal development will be highlighted, the more likely it is that new neuroprotective therapeutic strategies will be identified in the treatment of various neurodevelopmental disorders.
Neurobiol Dis, 2022; 163
31240667: Ferrari B, Urselli F, Gilodi M, Camuso S, Priori EC, Rangone B, Ravera M, Veneroni P, Zanellato I, Roda E, Osella D, Bottone MG
New Platinum-Based Prodrug Pt(IV)Ac-POA: Antitumour Effects in Rat C6 Glioblastoma Cells.
Gliomas are the most frequent primary tumours of the nervous system, characterised by high degree of malignancy,

widespread invasion and high-rate proliferation. Cisplatin and analogue are currently employed in clinical trials as active chemotherapeutic agents for the systemic treatment of this type of malignancy. Despite therapy benefits, clinical use of these agents is hampered by severe side effects including neurotoxicity. Therefore, the aim of the present study was to analyse the effect of a new compound of platinum(IV) conjugate, named Pt(IV)Ac-POA, which can generate a synergistic antineoplastic action when released along with cisplatin, after a specific reduction reaction within tumour cells. To assess the effects of the novel compound on rat C6 glioma cells, cell cycle and cell death activation analyses were carried out using flow cytometry. Morphological changes and activation of different cell death pathways were evaluated by both transmission electron microscopy and immunofluorescence microscopy. Protein expression was investigated by western blotting analysis. The novel compound Pt(IV)Ac-POA, bearing as axial ligand (2-propynyl)octanoic acid (POA), which is a histone deacetylase inhibitor (HDACi), acts as a prodrug in tumour cells, inducing cell death through different pathways at a concentration lower than those tested for other platinum analogues. The current results showed that Pt(IV)Ac-POA could represent a promising improvement of Pt-based chemotherapy against gliomas, either inducing a chemosensitisation and reducing chemoresistance.
Neurotox Res, 2020; 37

32087286: Musi CA, Agrò G, Buccarello L, Camuso S, Borsello T

JNK signaling activation in the Ube3a maternal deficient mouse model: its specific inhibition prevents post-synaptic protein-enriched fraction alterations and cognitive deficits in Angelman Syndrome model.

Deficiency of the E3 ubiquitin ligase UBE3A leads to the neurodevelopmental disorder Angelman syndrome (AS), while higher levels are linked to autism spectrum disorder. The mechanisms underlying the downstream effects of UBE3A loss or gain of function in these disorders are still not well understood, and treatments are still lacking. Here, using the Ube3a maternal loss (Ube3a) mouse model, we report an important JNK signaling activation in the hippocampus, cortex and cerebellum correlating with the onset of behavioral defects and biochemical marker alterations in the post-synaptic element, suggesting important spine pathology. JNK activation occurs at 7 and persists up till 23 weeks in Ube3a mice in two different cellular compartments: the nucleus and the post-synaptic protein-enriched fraction. To study JNK's role in Ube3a pathology we treated mice with the specific JNK inhibitor peptide, D-JNKI1, from 7 to 23 weeks of age. Preventing JNK action in vivo restores the post-synaptic protein-enriched fraction defects and the cognitive impairment in these mice. Our results imply a critical role of UBE3A-JNK signaling in the pathogenesis of UBE3A-related disorders. In particular, it was clear that JNK is a key player in regulating AS synaptic alterations and the correlated cognitive impairments, in fact, its specific inhibition tackles Ube3a pathology. This study sheds new light on the neuronal functions of UBE3A and offers new prospects for understanding the pathogenesis of UBE3A-related disorders.
Neurobiol Dis, 2020; 140

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BOARD NUMBER: S06-377

EXTRACELLULAR VESICLES UNDERLIE CELL-TYPE-SPECIFIC CROSSTALK DURING HUMAN CORTICAL DEVELOPMENT

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Neuronal differentiation and maturation are guided by both intrinsic and extrinsic factors. Among the extrinsic factors, secreted molecules as well as changes in the extracellular matrix have been shown to regulate these processes (Long & Huttner, 2019, Long et al., 2018). Extracellular vesicles, consisting of secreted vesicles that transfer nucleic acids, lipids, and proteins between cells, have recently been identified as potential modulators in neuronal function (Sharma et al., 2019; Peruzzotti-Jametti et al., 2021). Nevertheless, a comprehensive analysis of the composition and dynamics of EVs in the context of cortical development has not been conducted. Therefore, the aim of this project is to study extracellular vesicle-mediated communication between neural cell types during neurodevelopment. By collecting EVs from both 2D neural cultures and 3D cerebral organoids, we have characterized their protein composition and cell-type specificity in their uptake and release. Our results detected unique protein groups contained in EVs secreted by cerebral organoids at different stages of development and by dorsally or ventrally patterned organoids. Additionally, we observed variances in EV protein content in specific neural cell types (neural progenitors, neurons and astrocytes), and notable differences in the mechanisms of EV uptake. In conclusion, our results highlight the potential role of EVs in cell communication during human cortical development.

Pubmed:
33560471: Jansch C, Ziegler GC, Forero A, Gredy S, Wäldchen S, Vitale MR, Svirin E, Zöller JEM, Waider J, Günther K, Edenhofer F, Sauer M, Wischmeyer E, Lesch KP
Serotonin-specific neurons differentiated from human iPSCs form distinct subtypes with synaptic protein assembly.
Human induced pluripotent stem cells (hiPSCs) have revolutionized the generation of experimental disease models, but the development of protocols for the differentiation of functionally active neuronal subtypes with defined specification is still in its infancy. While dysfunction of the brain serotonin (5-HT) system has been implicated in the etiology of various neuropsychiatric disorders, investigation of functional human 5-HT specific neurons in vitro has been restricted by technical limitations. We describe an efficient generation of functionally active neurons from hiPSCs displaying 5-HT specification by modification of a previously reported protocol. Furthermore, 5-HT specific neurons were characterized using high-end fluorescence imaging including super-resolution microscopy in combination with electrophysiological techniques. Differentiated hiPSCs synthesize 5-HT, express specific markers, such as tryptophan hydroxylase 2 and 5-HT transporter, and exhibit an electrophysiological signature characteristic of serotonergic neurons, with spontaneous rhythmic activities, broad action potentials and large afterhyperpolarization potentials. 5-HT specific neurons form synapses reflected by the expression of pre- and postsynaptic proteins, such as Bassoon and Homer. The distribution pattern of Bassoon, a marker of the active zone along the soma and extensions of neurons, indicates functionality via volume transmission. Among the high percentage of 5-HT specific neurons (~ 42%), a subpopulation of CDH13 + cells presumably designates dorsal raphe neurons. hiPSC-derived 5-HT specific neuronal cell cultures reflect the heterogeneous nature of dorsal and median raphe nuclei and may facilitate examining the association of serotonergic neuron subpopulations with neuropsychiatric disorders.
J Neural Transm (Vienna), 2021; 128
32113967: Forero A, Ku HP, Malpartida AB, Wäldchen S, Alhama-Riba J, Kulka C, Aboagye B, Norton WHJ, Young AMJ, Ding YQ, Blum R, Sauer M, Rivero O, Lesch KP
Serotonin (5-HT) neuron-specific inactivation of Cadherin-13 impacts 5-HT system formation and cognitive function.
Genome-wide screening approaches identified the cell adhesion molecule Cadherin-13 (CDH13) as a risk factor for neurodevelopmental disorders, nevertheless the contribution of CDH13 to the disease mechanism remains obscure. CDH13 is involved in neurite outgrowth and axon guidance during early brain development and we previously provided evidence that constitutive CDH13 deficiency influences the formation of the raphe serotonin (5-HT) system by modifying neuron-radial glia interaction. Here, we dissect the specific impact of CDH13 on 5-HT system development and function using a 5-HT neuron-

specific Cdh13 knockout mouse model (conditional Cdh13 knockout, Cdh13 cKO). Our results show that exclusive inactivation of CDH13 in 5-HT neurons selectively increases 5-HT neuron density in the embryonic dorsal raphe, with persistence into adulthood, and serotonergic innervation of the developing prefrontal cortex. At the behavioral level, adult Cdh13 cKO mice display delayed acquisition of several learning tasks and a subtle impulsive-like phenotype, with decreased latency in a sociability paradigm alongside with deficits in visuospatial memory. Anxiety-related traits were not observed in Cdh13 cKO mice. Our findings further support the critical role of CDH13 in the development of dorsal raphe 5-HT circuitries, a mechanism that may underlie specific clinical features observed in neurodevelopmental disorders.
Neuropharmacology, 2020; 168
29477591: Jansch C, Günther K, Waider J, Ziegler GC, Forero A, Kollert S, Svirin E, Pühringer D, Kwok CK, Ullmann R, Maierhofer A, Flunkert J, Haaf T, Edenhofer F, Lesch KP
Generation of a human induced pluripotent stem cell (iPSC) line from a 51-year-old female with attention-deficit/hyperactivity disorder (ADHD) carrying a duplication of SLC2A3.
Fibroblasts were isolated from a skin biopsy of a clinically diagnosed 51-year-old female attention-deficit/hyperactivity disorder (ADHD) patient carrying a duplication of SLC2A3, a gene encoding neuronal glucose transporter-3 (GLUT3). Patient fibroblasts were infected with Sendai virus, a single-stranded RNA virus, to generate transgene-free human induced pluripotent stem cells (iPSCs). SLC2A3-D2-iPSCs showed expression of pluripotency-associated markers, were able to differentiate into cells of the three germ layers in vitro and had a normal female karyotype. This in vitro cellular model can be used to study the role of risk genes in the pathogenesis of ADHD, in a patient-specific manner.
Stem Cell Res, 2018; 28
29018333: Forero A, Rivero O, Wäldchen S, Ku HP, Kiser DP, Gärtner Y, Pennington LS, Waider J, Gaspar P, Jansch C, Edenhofer F, Resink TJ, Blum R, Sauer M, Lesch KP
Cadherin-13 Deficiency Increases Dorsal Raphe 5-HT Neuron Density and Prefrontal Cortex Innervation in the Mouse Brain.
During early prenatal stages of brain development, serotonin (5-HT)-specific neurons migrate through somal translocation to form the raphe nuclei and subsequently begin to project to their target regions. The rostral cluster of cells, comprising the median and dorsal raphe (DR), innervates anterior regions of the brain, including the prefrontal cortex. Differential analysis of the mouse 5-HT system transcriptome identified enrichment of cell adhesion molecules in 5-HT neurons of the DR. One of these molecules, cadherin-13 (Cdh13) has been shown to play a role in cell migration, axon pathfinding, and synaptogenesis. This study aimed to investigate the contribution of Cdh13 to the development of the murine brain 5-HT system. For detection of Cdh13 and components of the 5-HT system at different embryonic developmental stages of the mouse brain, we employed immunofluorescence protocols and imaging techniques, including epifluorescence, confocal and structured illumination microscopy. The consequence of loss-of-function mutations on brain 5-HT system development was explored in a mouse model of Cdh13 deficiency. Our data show that in murine embryonic brain Cdh13 is strongly expressed on 5-HT specific neurons of the DR and in radial glial cells (RGCs), which are critically involved in regulation of neuronal migration. We observed that 5-HT neurons are intertwined with these RGCs, suggesting that these neurons undergo RGC-guided migration. Cdh13 is present at points of intersection between these two cell types. Compared to wildtype controls, Cdh13-deficient mice display increased cell densities in the DR at embryonic stages E13.5, E17.5, and adulthood, and higher serotonergic innervation of the prefrontal cortex at E17.5. Our findings provide evidence for a role of CDH13 in the development of the serotonergic system in early embryonic stages. Specifically, we indicate that Cdh13 deficiency affects the cell density of the developing DR and the posterior innervation of the prefrontal cortex (PFC), and therefore might be involved in the migration, axonal outgrowth and terminal target finding of DR 5-HT neurons. Dysregulation of CDH13 expression may thus contribute to alterations in this system of neurotransmission, impacting cognitive function, which is frequently impaired in neurodevelopmental disorders including attention-deficit/hyperactivity and autism spectrum disorders.
Front Cell Neurosci, 2017; 11
34728600: Kyrousi C, O'Neill AC, Brazovskaja A, He Z, Kielkowski P, Coquand L, Di Giaimo R, D' Andrea P, Belka A, Forero Echeverry A, Mei D, Lenge M, Cruceanu C, Buchsbaum IY, Khattak S, Fabien G, Binder E, Elmslie F, Guerrini R, Baffet AD, Sieber SA, Treutlein B, Robertson SP, Cappello S
Extracellular LGALS3BP regulates neural progenitor position and relates to human cortical complexity.
Basal progenitors (BPs), including intermediate progenitors and basal radial glia, are generated from apical radial glia and are enriched in gyrencephalic species like humans, contributing to neuronal expansion. Shortly after generation, BPs delaminate towards the subventricular zone, where they further proliferate before differentiation. Gene expression alterations involved in BP delamination and function in humans are poorly understood. Here, we study the role of LGALS3BP, so far known as a cancer biomarker, which is a secreted protein enriched in human neural progenitors (NPCs). We show that individuals with LGALS3BP de novo variants exhibit altered local gyrification, sulcal depth, surface area and thickness in their cortex. Additionally, using cerebral organoids, human fetal tissues and mice, we show that LGALS3BP regulates the position of NPCs. Single-cell RNA-sequencing and proteomics reveal that LGALS3BP-mediated mechanisms involve the extracellular

matrix in NPCs' anchoring and migration within the human brain. We propose that its temporal expression influences NPCs' delamination, corticogenesis and gyrification extrinsically.
Nat Commun, 2021; 12

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BOARD NUMBER: S06-378

INSIGHT INTO CAMK2 SIGNALLING; UNCOVERING SUBSTRATES AND FUNCTIONAL PATHWAYS

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Learning and plasticity depends on functional Ca2+/calmodulin-dependent protein kinase 2 (CAMK2), as established in mutant CAMK2A or CAMK2B mouse models as well as observed in patients suffering from neurodevelopmental disorders with *de novo* mutations in the genes encoding these proteins. Whereas single CAMK2 knockout mice are viable, knockout of both CAMK2A and CAMK2B simultaneously is lethal, suggesting a functional overlap between these two isozymes and critical functions that remain to be uncovered. To gain more understanding of the full function of CAMK2, we generated inducible *Camk2a*$^{f/f}$*;Camk2b*$^{f/f}$*;CAG-Cre*^{ESR} mice and performed mass spectrometry analysis on cortical tissue, focusing on differences in phosphorylation. Our dataset consists of 5622 phosphorylated peptides in 2080 proteins, of which we identify loss of phosphorylation in 130 proteins in our mutant mouse. These proteins are key regulators in the postsynaptic density and glutamatergic signalling. We validate *in vivo* autophosphorylation sites on CAMK2A and CAMK2B and complement the consensus sequence for phosphorylation by CAMK2. In our extensive dataset of potential substrates, we identify novel ones, including proteins that have been implicated in neurodevelopmental disorders. Altogether, our results provide insight into the functional pathways CAMK2 is involved in and could contribute to a more comprehensive understanding of neurodevelopmental disorders.

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BOARD NUMBER: S06-379

ELECTRIC AXON GUIDANCE IN EMBRYONIC CHICK RETINA: MOLECULAR MECHANISM AND IN VITRO OPTIC NERVE FORMATION

POSTER SESSION 06 - SECTION: SIGNALING PATHWAYS IN NEURAL DEVELOPMENT

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Growing axons are directed not only by chemical signals but also by electric fields (EFs) in a process known as galvanotropism. The axons of developing CNS extend towards the cathode *in vitro* or along the voltage gradient generated by neuroepithelial cells *in vivo* (Yamashita, ***BBRC***, 431:280-283, 2013). However, the axon surface molecule that links EF to axon steering remains elusive. Here I show that integrin triggers axon steering by asymmetrically stabilizing microtubules being regulated by directionally EF-moved Ca^{2+}. Retinal strips from chick embryos were embedded in Matrigel, since Matrigel and the optic fiber layer, where retinal ganglion cell (RGC) axons grow expressing integrin α6β1, contain the extracellular matrix ligand. The cathodal growth of RGC axons was enhanced by monoclonal anti-chicken integrin β1 antibodies dose-dependently. Mn^{2+} abolished these EF effects as Mn^{2+} binds to the Ca^{2+}-dependent negative regulatory site in β1 subunit eliminating the Ca^{2+} inhibition. The EF effect was also abolished by the inhibition of intracellular signals for integrin-microtubule stabilization. Since EF moves Ca^{2+} at the axon surface asymmetrically, the less inhibited integrin on the cathodal side would stabilize the more microtubules to steer the axon towards the cathode. The RGC axons converged on the optic nerve head in organotypic cultures. Mn^{2+} abolished this RGC axon convergence. The stem of an optic nerve was reproduced *in vitro* by focusing the EF to mimic the *in vivo* EF. These results strongly support the idea that RGC axons are electrically directed to the future optic disc.

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BOARD NUMBER: S06-380

MUTANT HUNTINGTIN DISRUPTS THE TIMING OF CELLULAR BEHAVIOR OF PROGENITORS IN THE HDHQ7/Q175 HUNTINGTON DISEASE MOUSE MODEL.

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Huntington's disease (HD) is a neurological disease caused by the abnormal expansion of a CAG stretch in the huntingtin gene. Although the HD neuropathology is primarily attributed to cortical and striatal neuronal dysfunction and neurodegeneration in adults, abnormal cortical development occurs in HD gene carriers. In the present study, we used a heterozygous knock-in HD mouse model expressing the full-length mutant HTT ($Hdh^{Q7/Q175}$). This model depicts early onset of characteristic motor defects, abnormal morphology and electrical properties of layer V and II/III cortical neurons. We examined the characteristics of cortical progenitor population in the ventricular zone (VZ) and subventricular zone (SVZ) throughout embryonic corticogenesis and neuronal specification in early post-natal life. Our results revealed that the cell cycle length in HD was increased through the dysregulation of different stage-dependent mechanisms. Early neurogenic divisions gave rise to more transitioning intermediate progenitors that stayed longer in S-phase at the expanse of generating post-mitotic neurons. Proliferation and S-phase rate increased at later stage but not the mitotic index. Finally, we observed defects in radial migration only for late born neurons. These embryonic abnormalities led to the differential post-natal alterations of deep and upper layers. Thus, our study reveals how the HD-causing mutation differentially affects the timing of direct and indirect neurogenesis at different embryonic stages with consequences for post-natal layering.

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BOARD NUMBER: S06-381

NWD1 CONTROLS NSPCS PROLIFERATION THROUGH PURINOSOME FORMATION

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Neural stem/progenitor cells (NSPCs) need a spatiotemporally regulated generation of metabolites to engage in the proper proliferation and differentiation. De novo purine synthesis pathway executed by a sequential reaction mediated by 6 enzymes is tightly controlled depending on the demand of purines. Previous studies demonstrated that these enzymes are assembled as a huge multienzyme complex called purinosome. However, there is no evidence showing the formation or the physiological function of purinosome during brain development. In this study, we identified the novel gene NACHT and WD repeat domain containing protein 1 (Nwd1) expressed strongly in NSPCs and immature neurons in the embryonic mice cerebral cortex (Yamada and Sakakibara, 2018). In addition, we revealed that Nwd1 directly interacts with Paics, one of the purine de novo synthetic enzymes, to regulate the purinosome assembly in NSPCs during cortical development. Nwd1 and Paics were co-localized in purinosome in cultured NPSCs, and altered Nwd1 expression affected purinosome formation. To clarify the physiological function of the purinosome in cortical development, we examined the loss-of-function of purinosome components using in utero electroporation. Nwd1 and Paics knockdown inhibited NSPCs proliferations and induced premature differentiation of NSPCs, resulting in the repression of radial migration. These defects by Nwd1 knockdown frequently caused a periventricular heterotopia, associated with epilepsy and intellectual disability, in the postnatal brain due to the formation of ectopic aggregation of neurons. Taken together, these findings indicate the strict regulation of purinosome assembly is important for proper NSPCs proliferation during cortical development.

Pubmed:

34988397: Yamada S, Sato A, Ishihara N, Akiyama H, Sakakibara SI
Drp1 SUMO/deSUMOylation by Senp5 isoforms influences ER tubulation and mitochondrial dynamics to regulate brain development.
Brain development is a highly orchestrated process requiring spatiotemporally regulated mitochondrial dynamics. Drp1, a key molecule in the mitochondrial fission machinery, undergoes various post-translational modifications including conjugation to the small ubiquitin-like modifier (SUMO). However, the functional significance of SUMOylation/deSUMOylation on Drp1 remains controversial. SUMO-specific protease 5 (Senp5L) catalyzes the deSUMOylation of Drp1. We revealed that a splicing variant of Senp5L, Senp5S, which lacks peptidase activity, prevents deSUMOylation of Drp1 by competing against other Senps. The altered SUMOylation level of Drp1 induced by Senp5L/5S affects mitochondrial morphology probably through controlling Drp1 ubiquitination and tubulation of the endoplasmic reticulum. A dynamic SUMOylation/deSUMOylation balance controls neuronal polarization and migration during the development of the cerebral cortex. These findings suggest a novel role of post-translational modification, in which deSUMOylation enzyme isoforms competitively regulate mitochondrial dynamics via Drp1 SUMOylation levels, in a tightly controlled process of neuronal differentiation and corticogenesis.
iScience, 2021; 24
32344379: Yamada S, Sato A, Sakakibara SI
Nwd1 Regulates Neuronal Differentiation and Migration through Purinosome Formation in the Developing Cerebral Cortex.
Engagement of neural stem/progenitor cells (NSPCs) into proper neuronal differentiation requires the spatiotemporally regulated generation of metabolites. Purines are essential building blocks for many signaling molecules. Enzymes that catalyze de novo purine synthesis are assembled as a huge multienzyme complex called "purinosome." However, there is no evidence of the formation or physiological function of the purinosome in the brain. Here, we showed that a signal transduction ATPases with numerous domains (STAND) protein, NACHT and WD repeat domain-containing 1 (Nwd1), interacted with Paics, a purine-synthesizing enzyme, to regulate purinosome assembly in NSPCs. Altered Nwd1 expression affected purinosome formation and induced the mitotic exit and premature differentiation of NSPCs, repressing neuronal migration and periventricular heterotopia. Overexpression/knockdown of Paics or Fgams, other purinosome enzymes, in the developing brain resulted in a phenocopy of Nwd1 defects. These findings indicate that strict regulation of purinosome

assembly/disassembly is crucial for maintaining NSPCs and corticogenesis.
iScience, 2020; 23
31975032: Akiyama H, Iwasaki Y, Yamada S, Kamiguchi H, Sakakibara SI
Control of cell migration by the novel protein phosphatase-2A interacting protein inka2.
Cell migration is essential for many physiological and pathological processes, including embryonic development, wound healing, immune response and cancer metastasis. Inka2 transcripts are observed in migrating cells during embryonic development, suggesting the involvement of inka2 in cell migration. However, its precise role remains unclear. Here, we found that inka2 controlled focal adhesion dynamics and cell migration, likely by regulating protein phosphatase-2A (PP2A) function. A scratch assay revealed that inka2 shRNA-transfected NIH3T3 cells showed rapid wound closure, indicating an inhibitory effect by inka2 on cell migration. Live-cell imaging of NIH3T3 cells expressing EGFP-paxillin using total internal reflection fluorescence microscopy revealed that inka2 knockdown increased the turnover rate of focal adhesions. Given that PP2A, which consists of catalytic (C), regulatory (B) and scaffolding (A) subunits, is known to regulate focal adhesions, we examined the inka2-PP2A interaction. Immunoprecipitation revealed an association between inka2 and the PP2A C subunit. Binding of Inka2 to the C subunit prevented the association between the A and C subunits, suggesting that inka2 can inhibit PP2A function. Furthermore, both inka2 expression and PP2A inhibition decreased focal adhesion kinase-paxillin interaction, resulting in reduced formation of focal adhesions. We assessed the effect of pharmacological PP2A inhibition on the inka2 knockdown-induced increase in cell migration speed and found that treatment with a PP2A inhibitor negated the accelerated migration of inka2 knockdown cells. These results suggest that inka2 knockdown exerts its effects through PP2A-dependent regulation of focal adhesions. Our findings contribute to a better understanding of the molecular mechanisms underlying cell migration.
Cell Tissue Res, 2020; 380
30004576: Yamada S, Sakakibara SI
Expression profile of the STAND protein Nwd1 in the developing and mature mouse central nervous system.
The orchestrated events required during brain development, as well as the maintenance of adult neuronal plasticity, highly depend on the accurate responses of neuronal cells to various cellular stress or environmental stimuli. Recent studies have defined a previously unrecognized, broad class of multidomain proteins, designated as signal transduction ATPases with numerous domains (STAND), which comprises a large number of proteins, including the apoptotic peptidase activating factor 1 (Apaf1) and nucleotide-binding oligomerization domain-like receptors (NLRs), central players in cell death and innate immune responses, respectively. Although the involvement of STANDs in the central nervous system (CNS) has been postulated in terms of neuronal development and function, it remains largely unclear. Here, we identified Nwd1 (NACHT and WD repeat domain-containing protein 1), as a novel STAND protein, expressed in neural stem/progenitor cells (NSPCs). Structurally, Nwd1 was most analogous to the apoptosis regulator Apaf1, also involved in mitosis and axonal outgrowth regulation in the CNS. Using a specific antibody, we show that, during the embryonic and postnatal period, Nwd1 is expressed in nestin-positive NSPCs in vivo and in vitro, while postnatally it is found in terminally differentiated neurons and blood vessels. At the subcellular level, we demonstrate that Nwd1 is preferentially located in the cytosolic compartment of cultured NSPCs, partially overlapping with cytochrome c. These observations imply that Nwd1 might be involved in the neuronal lineage as a new STAND gene, including having a pro-apoptotic or nonapoptotic role, similar to Apaf1.
J Comp Neurol, 2018; 526

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BOARD NUMBER: S06-382

FATE AND CELL POTENTIAL OF SINGLE PALLIAL PROGENITOR CELLS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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The assemble of the brain from a pool of Neural Progenitor Cells (NPCs) is a complex process. Increasing evidence supports the heterogeneity of NPCs across distinct brain regions. Some studies suggest that progenitor diversity is more restricted to one specific lineage whereas others show a potential cell diversity depending on the spatio-temporal patterning. A transient progenitor named neuroepithelial cells generates Radial Glial Cells (RGC) that produce, in overlapping waves, neurons, astrocytes, NG2-glia and oligodendrocytes. However, NG2-glia is another remarkable cell type that can also act as a progenitor. In adult mouse brains, NG2-glia can generate OLs, Astrocytes or even Neurons. Moreover, our previous works revealed the existence of NG2-progenitors during development, enabling the production of different neural cell lineages. To elucidate the cell potential of single progenitors, our lab developed the UbC-StarTrack, to tag single progenitors with stable and heritable labelling. This strategy, based on the PiggyBac system, consists of the integration of twelve plasmids, using a hyperactive transposase PiggyBac (HyPBase), that codify up to six different fluorescence proteins aim to cytoplasm or nucleus. To target NG2 or GFAP-progenitor cells, we exchanged the CMV-promoter for NG2 or GFAP-promoter in **UbC-(NG2-PB)-StarTrack** or **UbC-(GFAP-PB)-StarTrack**, respectively. After targeting NPCs at different embryonic stages or P0, we performed clonal analyses of the derived-cell progeny at P30. Data showed that GFAP- and NG2-progenitors produce distinct cell types whose differentiation potential changes with time and space. Our results provide new data of the lineage cell potential of NG2 and GFAP-progenitors that strengthen the heterogeneity of NPCs.

Pubmed:
34831460: Ojalvo-Sanz AC, López-Mascaraque L
Gliogenic Potential of Single Pallial Radial Glial Cells in Lower Cortical Layers.
During embryonic development, progenitor cells are progressively restricted in their potential to generate different neural cells. A specific progenitor cell type, the radial glial cells, divides symmetrically and then asymmetrically to produce neurons, astrocytes, oligodendrocytes, and NG2-glia in the cerebral cortex. However, the potential of individual progenitors to form glial lineages remains poorly understood. To further investigate the cell progeny of single pallial GFAP-expressing progenitors, we used the in vivo genetic lineage-tracing method, the . After targeting those progenitors in embryonic mice brains, we tracked their adult glial progeny in lower cortical layers. Clonal analyses revealed the presence of clones containing sibling cells of either a glial cell type (uniform clones) or two different glial cell types (mixed clones). Further, the clonal size and rostro-caudal cell dispersion of sibling cells differed depending on the cell type. We concluded that pallial E14 neural progenitors are a heterogeneous cell population with respect to which glial cell type they produce, as well as the clonal size of their cell progeny.
Cells, 2021; 10
32183100: Sánchez-González R, Figueres-Oñate M, Ojalvo-Sanz AC, López-Mascaraque L
Cell Progeny in the Olfactory Bulb After Targeting Specific Progenitors with Different UbC-StarTrack Approaches.
The large phenotypic variation in the olfactory bulb may be related to heterogeneity in the progenitor cells. Accordingly, the progeny of subventricular zone (SVZ) progenitor cells that are destined for the olfactory bulb is of particular interest, specifically as there are many facets of these progenitors and their molecular profiles remain unknown. Using modified StarTrack genetic tracing strategies, specific SVZ progenitor cells were targeted in E12 mice embryos, and the cell fate of these neural progenitors was determined in the adult olfactory bulb. This study defined the distribution and the phenotypic diversity of olfactory bulb interneurons from specific SVZ-progenitor cells, focusing on their spatial pallial origin, heterogeneity, and genetic profile.
Genes (Basel), 2020; 11
33217041: Melero-Jerez C, Fernández-Gómez B, Lebrón-Galán R, Ortega MC, Sánchez-de Lara I, Ojalvo AC, Clemente D, de Castro F
Myeloid-derived suppressor cells support remyelination in a murine model of multiple sclerosis by promoting oligodendrocyte

precursor cell survival, proliferation, and differentiation.
The most frequent variant of multiple sclerosis (MS) is the relapsing-remitting form, characterized by symptomatic phases followed by periods of total/partial recovery. Hence, it is possible that these patients can benefit from endogenous agents that control the inflammatory process and favor spontaneous remyelination. In this context, there is increasing interest in the role of myeloid-derived suppressor cells (MDSCs) during the clinical course of experimental autoimmune encephalomyelitis (EAE). MDSCs speed up infiltrated T-cell anergy and apoptosis. In different animal models of MS, a milder disease course is related to higher presence/density of MDSCs in the periphery, and smaller demyelinated lesions in the central nervous system (CNS). These observations lead us to wonder whether MDSCs might not only exert an anti-inflammatory effect but might also have direct influence on oligodendrocyte precursor cells (OPCs) and remyelination. In the present work, we reveal for the first time the relationship between OPCs and MDSCs in EAE, relationship that is guided by the distance from the inflammatory core. We describe the effects of MDSCs on survival, proliferation, as well as potent promoters of OPC differentiation toward mature phenotypes. We show for the first time that osteopontin is remarkably present in the analyzed secretome of MDSCs. The ablation of this cue from MDSCs-secretome demonstrates that osteopontin is the main MDSC effector on these oligodendroglial cells. These data highlight a crucial pathogenic interaction between innate immunity and the CNS, opening ways to develop MDSC- and/or osteopontin-based therapies to promote effective myelin preservation and repair in MS patients.
Glia, 2021; 69
31202640: Yanguas-Casás N, Ojalvo-Sanz AC, Martínez-Vázquez A, Goneau MF, Gilbert M, Nieto-Sampedro M, Romero-Ramírez L
Neurostatin and other O-acetylated gangliosides show anti-neuroinflammatory activity involving the NFκB pathway.
In many neuropathologies activated microglia and macrophages cause neurotoxicity and prolong the inflammatory response. We have previously characterized the glycosphingolipid Neurostatin (Nst), which potentially reduces these detrimental mechanisms. Nst, isolated from mammalian brain, is the GD1b ganglioside with O-acetylation of the outer sialic acid residue. Using the enzyme sialate-O-acetyltransferase (SOAT), we obtained several O-acetylated gangliosides and O-propionylated GD1b (PrGD1b). In the present study we investigated the anti-inflammatory effects of these compounds. Nst and other O-acetylated gangliosides reduced nitrite production in microglial cells which were activated with lipopolysaccharide (LPS), but did not affect nitrite production after their stimulation with interferon gamma (IFNγ). Structure-activity relationship analysis showed that Nst was the most active ganglioside as inhibitor of nitrite production. Its ceramide moiety is essential for this, and both, the O-acetylation and the monosaccharide chain are important for the anti-inflammatory activity of the gangliosides. We also found that Nst reduced iNOS, IL-6 and IL-12 transcription in LPS-induced microglia, likely by inhibiting nuclear localization of NFκB. In co-cultures, Nst reduced neuronal cell death caused by LPS-activated microglia. In vivo, Nst diminished microglia activation in a mouse model of acute neuroinflammation. We propose that Nst and other O-acetylated gangliosides are neuroprotective regulators of microglia activity under both physiological and pathological conditions.
Toxicol Appl Pharmacol, 2019; 377

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BOARD NUMBER: S06-383

LANDMARKS OF HUMAN EMBRYONIC DEVELOPMENT INSCRIBED IN SOMATIC MUTATIONS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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The human brain is made of clones that are ultimately traceable to the first postzygotic cell division. Although cell lineage information is fundamental to understanding organismal development, very little direct information is available about humans. We performed high-depth (>250X) whole-genome sequencing (WGS) of multiple tissues from three individuals and identified hundreds of somatic single nucleotide variants (sSNVs). Using variants as “endogenous barcodes” in single cell WGS, we reconstructed early embryonic cell divisions, and estimated their average whole-body mosaic fractions, defined as percentage of cells carrying the sSNV. We revealed asymmetric contributions of early progenitors to extraembryonic tissues and different organs. Identifying first cell generation variants in WGS data from 74 individuals, we found overall asymmetric contributions of the first cell generation clones to the human brain with strong inter-individual variability. Ultra-deep targeted sequencing of clonal sSNVs across 94 biopsies from 17 different organs revealed asymmetries in the clonal contributions to embryonic germ-layers and suggested the onset of gastrulation at ~170 epiblast cells. By analyzing multiple biopsies from several regions of the central nervous system and >1,000 cortical single cells, we estimated ~50-100 founders for the forebrain, and found that early embryonic cell divisions contribute unequally to neuronal and non-neuronal cells. Finally, we performed single nuclei (sn)RNA-seq and snATAC-seq of ~100,000 cortical single cells and identified sSNV lineage markers in a subset of them to couple lineage information with cell type classification. Thus, our study shows that mosaic mutations provide a permanent record of human embryonic development at remarkably high-resolution.

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BOARD NUMBER: S06-384

ROLE OF POST-TRANSLATIONAL MODIFICATION OF NEUROG2 IN HUMAN CORTICOGENESIS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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The neocortex has expanded throughout evolution to give rise to higher-order cognitive abilities. Its enlargement relies on the increased diversity and proliferative capacities of cortical progenitors to increase neuronal production. Therefore, in gyrencephalic species where the neurogenic period is protracted, the regulation of the balance between progenitor maintenance and differentiation is of key importance for advanced intellectual capacities. The control of this balance in the dorsal telencephalon is mediated by feedback regulation between Notch signaling and the proneural transcription factor Neurogenin2 (NEUROG2). As the expression of NEUROG2 alone is sufficient to induce neurogenesis in the neocortex, its regulation at the gene level has been extensively studied. However, recent findings highlight that regulation at the protein level profoundly influences its activity by preventing DNA binding. For example, modulation of the conserved Neurog2 T149 phosphorylation in the developing mouse neocortex results in an altered pool of progenitors and number of neurons in the deep and upper layers. We hypothesize that modulation of the activity of NEUROG2 may regulate the pace of the temporal advance of cortical progenitors down the differentiation landscape. To test this hypothesis in humans, where the protracted neurogenic period is essential, we derived 3D cortical spheroids from CRISPR/Cas9 engineered iPSCs lines to either mimic the phosphorylation or prevent it by replacing the NEUROG2 T149, by an aspartate (T149D) or an alanine (T149A) respectively. We will present the initial results of the analysis on the neurogenic potential of human cortical progenitors in control and NEUROG2 phospho-mutant cortical spheroids.

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SPATIO-TEMPORAL DYNAMICS OF GERMINAL ZONES IN THE MOUSE BRAIN

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Understanding the spatio-temporal identity and dynamics of germinal zone activity during development is essential to understand brain structure and circuit function. Here, we use several parallel birth dating approaches to systematically determine the date of birth of all cells of the embryonic mouse brain. The results show an increased duration of neurogenesis along the caudo-rostral axis of the neural tube, linked to the cycling properties of ventricular zone progenitors. The results are combined in an online atlas providing a relationship between neuronal localization and date of birth.

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BOARD NUMBER: S06-386

LIS1 MUTATION PREVENTS BASAL RADIAL GLIA-LIKE CELL PRODUCTION IN THE MOUSE

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Human cerebral cortical development depends on orchestrated events from progenitor proliferation to neuronal migration and maturation. Progenitor cells include apical radial glia, intermediate progenitors, and basal (or outer) radial glia (bRGs or oRGs). bRGs are abundant in gyrencephalic brains, but are fewer in number in lissencephalic species (e.g. the mouse). The *LIS1* gene, which codes for a dynein regulator, is mutated in human lissencephaly – and has also been associated with microcephaly in some cases. LIS1 was shown to be important during cell division and neuronal migration. Here, we studied the role of Lis1 in progenitors. First, we generated bRG-like cells in the mouse embryonic brain, in order to investigate the role of Lis1 in their formation. This was achieved by *in utero* electroporation of a hominoid-specific gene *TBC1D*3 (coding for a RAB-GAP protein) at mouse embryonic day (E) 14.5. We confirmed that *TBC1D3* expression in wild-type (WT) brain generates numerous Pax6^{+} bRG-like cells which are basally localized. Second, with the same methodology, we assessed the production of these cells in heterozygote *Lis*1 mutant brains. We showed that *Lis*1 depletion in the forebrain from E9.5 prevented subsequent *TBC1D*3-induced bRG-like cell amplification. Third, we observed perturbation of the ventricular zone (VZ) in the mutant. *Lis*1 depletion altered adhesion proteins and mitotic spindle orientations at the ventricular surface and increased the proportion of abventricular mitoses. We conclude that disruption of *Lis*1/LIS1 dosage is likely to be detrimental for appropriate progenitor number and position, contributing to lissencephaly pathogenesis.

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BOARD NUMBER: S06-387

TRANSCRIPTIONAL AND EPIGENETIC CHARACTERIZATION OF PROGENITOR COMPETENCE DURING NEUROGENESIS.

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Embryonic and adult neuronal development is temporally fine-tuned and resilient against errors. Several studies have shed light on how epigenetic mechanisms (histone modifications, chromatin accessibility, and transcription factors) can precisely shape the regulatory landscape. However, little is known about the epigenetic mechanisms regulating the emergence of inhibitory cell types in the ventral forebrain. To better understand how different types of inhibitory neurons emerge and gets integrated into circuits, it is paramount to gain a better understanding of epigenetic regulatory mechanisms and their cross-talk. We profiled single-cell gene expression and chromatin accessibility in progenitor cells of inhibitory neurons to investigate the influence of chromatin accessibility on the regulation of gene expression and fate decisions. Integration of multi-omic data has uncovered cis-regulatory elements that precisely delineate temporal features during the various stages of embryonic development. These domains showed stage-specific chromatin opening and transcription factor motif composition, as well as enhancer-specific chromatin characteristics. We also identified potential transcriptional regulators and performed in vivo perturbation to understand what role they play in inhibitory neuronal development. A growing body of evidence suggest that perturbations in inhibitory neuron development can result in a variety of complex neuropsychiatric disorders, including autism, bipolar disorder, and schizophrenia. As a result, our research sheds light on the epigenetic mechanisms that control cell fate decisions during forebrain development, which is critical for understanding the brain in health and disease.

Pubmed:
35172134: Jansen C, Paraiso KD, Zhou JJ, Blitz IL, Fish MB, Charney RM, Cho JS, Yasuoka Y, Sudou N, Bright AR, Wlizla M, Veenstra GJC, Taira M, Zorn AM, Mortazavi A, Cho KWY
Uncovering the mesendoderm gene regulatory network through multi-omic data integration.
Mesendodermal specification is one of the earliest events in embryogenesis, where cells first acquire distinct identities. Cell differentiation is a highly regulated process that involves the function of numerous transcription factors (TFs) and signaling molecules, which can be described with gene regulatory networks (GRNs). Cell differentiation GRNs are difficult to build because existing mechanistic methods are low throughput, and high-throughput methods tend to be non-mechanistic. Additionally, integrating highly dimensional data composed of more than two data types is challenging. Here, we use linked self-organizing maps to combine chromatin immunoprecipitation sequencing (ChIP-seq)/ATAC-seq with temporal, spatial, and perturbation RNA sequencing (RNA-seq) data from Xenopus tropicalis mesendoderm development to build a high-resolution genome scale mechanistic GRN. We recover both known and previously unsuspected TF-DNA/TF-TF interactions validated through reporter assays. Our analysis provides insights into transcriptional regulation of early cell fate decisions and provides a general approach to building GRNs using highly dimensional multi-omic datasets.
Cell Rep, 2022; 38
33555045: Bright AR, van Genesen S, Li Q, Grasso A, Frölich S, van der Sande M, van Heeringen SJ, Veenstra GJC
Combinatorial transcription factor activities on open chromatin induce embryonic heterogeneity in vertebrates.
During vertebrate gastrulation, mesoderm is induced in pluripotent cells, concomitant with dorsal-ventral patterning and establishing of the dorsal axis. We applied single-cell chromatin accessibility and transcriptome analyses to explore the emergence of cellular heterogeneity during gastrulation in Xenopus tropicalis. Transcriptionally inactive lineage-restricted genes exhibit relatively open chromatin in animal caps, whereas chromatin accessibility in dorsal marginal zone cells more closely reflects transcriptional activity. We characterized single-cell trajectories and identified head and trunk organizer cell clusters in early gastrulae. By integrating chromatin accessibility and transcriptome data, we inferred the activity of transcription factors in single-cell clusters and tested the activity of organizer-expressed transcription factors in animal caps, alone or in combination. The expression profile induced by a combination of Foxb1 and Eomes most closely resembles that observed in the head organizer. Genes induced by Eomes, Otx2, or the Irx3-Otx2 combination are enriched for maternally regulated H3K4me3 modifications, whereas Lhx8-induced genes are marked more frequently by zygotically controlled

H3K4me3. Taken together, our results show that transcription factors cooperate in a combinatorial fashion in generally open chromatin to orchestrate zygotic gene expression.
EMBO J, 2021; 40
31356991: Peñalosa-Ruiz G, Bright AR, Mulder KW, Veenstra GJC
The interplay of chromatin and transcription factors during cell fate transitions in development and reprogramming.
Reprogramming to induced pluripotency through expression of OCT4, SOX2, KLF4, MYC (OSKM) factors is often considered the dedifferentiation of somatic cells. This would suggest that reprogramming represents the reversal of embryonic differentiation. Indeed, molecular events involving the activity of the pluripotency network occur in opposite directions. However, reprogramming and development substantially differ as OSKM bind to accessible regulatory elements in the genome of somatic cells due to their overexpression, including regulatory elements never bound by these factors during normal differentiation. In addition, rewiring the transcriptional network back to pluripotency involves overcoming molecular barriers that protect or stabilize the somatic identity, whereas extrinsic and intrinsic cues will drive differentiation in an energetically favorable landscape in the embryo. This review focuses on how cell fate transitions in reprogramming and development are differentially governed by interactions between transcription factors and chromatin. We also discuss how these interactions shape chromatin architecture and the transcriptional output. Major technological advances have resulted in a better understanding of both differentiation and reprogramming, which is essential to exploit reprogramming regimes for regenerative medicine.
Biochim Biophys Acta Gene Regul Mech, 2019; 1862
30042136: Bright AR, Veenstra GJC
Assay for Transposase-Accessible Chromatin-Sequencing Using Embryos.
The DNA of eukaryotic genomes is packaged into chromatin by nucleosomes. This not only compacts the DNA but also plays a central role in gene regulation and establishment of cellular identity during development. Because of this packaging, the DNA is relatively inaccessible to nucleoplasmic factors; however, regulatory elements such as promoters, enhancers, and insulators are largely kept nucleosome-free. The assay for transposase-accessible chromatin (ATAC-seq) can be used to identify genomic locations of "open" chromatin, footprints of DNA-binding proteins, and positioned nucleosomes. It therefore is a powerful tool for unraveling the dynamic regulatory landscape of chromatin. The method exploits the action of hyperactive prokaryotic Tn-transposase, which preferentially cuts DNA in accessible chromatin and tags the sites with sequencing adaptors. Here we describe an ATAC-seq protocol for use with embryos.
Cold Spring Harb Protoc, 2019; 2019
23921079: Afsal VV, Antony SP, Bright AR, Philip R
Molecular identification and characterization of Type I crustin isoforms from the hemocytes of portunid crabs, Scylla tranquebarica and Portunus pelagicus.
Crustins are cationic antimicrobial peptides of ca. 7-14kDa with a characteristic four-disulphide core containing WAP domain, present in the hemocytes of crustaceans. The present study reports the first crustin sequences from two portunid crabs, viz. the mud crab Scylla tranquebarica (St-Crustin, JQ965930) and the blue swimmer crab Portunus pelagicus (Pp-Crustin, JQ753312). St-Crustin and Pp-Crustin represented the complete cDNA sequence of Type I crustin, with an ORF of 336bp encoding 111aa with a predicted molecular weight of 10kDa and a pI of 8. The signal sequence contained 21aa residues, which was followed by a mature peptide with a WAP domain at the C-terminus. Peptide model of St-Crustin and Pp-Crustin indicated a randomly coiled structure enclosing two β-sheets but no helices. St-Crustin and Pp-Crustin shared significant similarities with crustins of portunid crabs (68-95%) and other crabs (60-73%). Phylogenetic analysis showed that St-Crustin and Pp-Crustin possess the same ancestral origin and have a similar evolutionary status like other crustins, which has subsequently diverged at different phases of evolution. St-Crustin and Pp-Crustin were closely related to crab crustins rather than to the crustins of other crustacean groups. The wide distribution of crustins in crustaceans indicates the importance of these AMPs in the innate immunity. Discovery of novel crustins might pave way to the discovery of promising therapeutic/prophylactic agents in health management and disease control in crustacean aquaculture.
Cell Immunol, 2013 Jul-Aug; 284
23262396: Babu DT, Antony SP, Joseph SP, Bright AR, Philip R
Marine yeast Candida aquaetextoris S527 as a potential immunostimulant in black tiger shrimp Penaeus monodon.
A marine yeast Candida aquaetextoris S527 as a source of immunostimulant in Penaeus monodon was studied. Yeast diet was prepared by incorporating 10% C. aquaetextoris S527 biomass into a standard shrimp diet and administered in P. monodon at different frequencies (daily, once in three days, once in seven days and once in ten days) followed by challenge with white spot syndrome virus (WSSV). Immune parameters such as total protein, total hemocyte count, pro-phenoloxidase, nitroblue tetrazolium reduction, alkaline phosphatase activity and acid phosphatase activity were tested. Expression profile of antimicrobial peptide (AMP) genes viz., anti-lipopolysaccharide factor (ALF), crustin-1, crustin-2, crustin-3, penaeidin-3 and penaeidin-5; immune genes viz., alpha-2-macroglobulin (α-2-M), astakine, peroxinectin, prophenol oxidase (proPO) and

transglutaminase, and WSSV genes viz., DNA polymerase, endonuclease, protein kinase, immediate early gene, latency related gene, ribonucleotide reductase, thymidine kinase and VP28 were analyzed. The study demonstrated that marine yeast diet administered once every seven days conferred better protection to P. monodon against WSSV infection, supported by the hematological and immune gene expression profiles analyzed.
J Invertebr Pathol, 2013; 112

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CALCIUM/CATION-MECHANOSENSING ION CHANNELS ACTIVITY ARE CRUCIAL MEDIATORS FOR MECHANICAL INDUCTION OF RADIAL GLIA

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Aims: During brain development, radial glia is the principal embryonic neural stem cell and form a radial palisade spanning the entire neuroepithelia. We have described that poly (methyl methacrylate) with 2µm linear topographies (ln2PMMA) mimic the structure of radial glia-niche. Ln2PMMA physical signals induce the conversion of cultured astrocytes into functional radial glia cells. However, the molecular mechanisms by which radial glia linage cells sense and respond to ln2PMMA mechanical signals remains poorly understood. **Methods:** We used primary cortical astrocyte cultures from newborn mice, grown for 3DIV in control (glass) and ln2PMMA substrates. We used a custom-developed high-throughput-like image analysis algorithm, allowing simultaneous correlation of lineage markers with nuclei morphology, at the single-cell level. We analyzed expression changes in mechanosensing ion channels by WB and RT-PCR. We validate Ca^{2+}/mechanosensing cation channels implication by pharmacological inhibition of CaMKII (KN93) and non-selective cation channels (GsMTx-4). We finally perform Ca^{2+} imaging and microscopy cross-correlation to identify the activity of radial glia cells (Nestin+/Pax6+). **Results:** Ln2PMMA significantly increase the number of Nestin+/Pax6+ radial glia cells. Cells in ln2PMMA significantly decreased TRPA1 and increased TRPC1 proteins. Moreover, ASIC1 mRNA significantly increased, with a protein increase at the limit of significance in Ln2PMMA. Pharmacological inhibition of CaMKII or non-selective cation channels prevents the increase of Nestin+/Pax6+ radial glia in ln2PMMA, although with different effect on nuclear morphology. **Conclusions:** Calcium/cation-mechanosensing ion channels activity are crucial mediators for mechanical induction of astrocyte dedifferentiation into radial glia. We will discuss changes in Ca^{2+} dynamics in radial glia linage cells.

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EFFECT OF MICROGLIA IN THE VIABILITY OF DOPAMINE NEURONS DEVELOPED IN MESENCEPHALIC ORGANOIDS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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A role of microgial cells on the development and the degeneration of dopamine neurons has been suggested. In this work, we aim to develop a system to study the soluble and cellular interactions of microglia, differentiated from human pluripotent cells, on the development and viability of dopaminergic neurons, growing on mesencephalic organoids. Pluripotent cells were induced for hematopoietic differentiation and after cell sorting with specific surface antigens, microglial cells were allowed to mature. On a separate culture, midbrain organoids were formed after aggregation of pluripotent cells on neural-inducing conditions. We compared two groups: one consisting of a co-culture of microglia-like cells and mesencephalic organoids and another composed of by mesencephalic organoids and conditioned medium from microglia-like cells. We found that the influence of microglia is of great importance for the development of dopaminergic neurons. These interactions occur through cell-cell communication and secretion of diverse molecules. Thus, microglial cells have effects on the viability of dopaminergic neurons in this 3D model, which results in a new tool for the study of neuroimmune interactions. This represents a novel strategy for the study of diverse pathologies, like Parkinson's disease.

Pubmed:
32057806: Santillán-Cigales JJ, Mercado-Gómez OF, Arriaga-Ávila V, Landgrave-Gómez J, Guevara-Guzmán R
Daytime-restricted feeding modulates the expression of inflammatory mediators and diminishes reactive astrogliosis and microgliosis following status epilepticus.
Brain Res, 2020; 1734
27490898: González-Reyes S, Santillán-Cigales JJ, Jiménez-Osorio AS, Pedraza-Chaverri J, Guevara-Guzmán R
Glycyrrhizin ameliorates oxidative stress and inflammation in hippocampus and olfactory bulb in lithium/pilocarpine-induced status epilepticus in rats.
Glycyrrhizin (GL) is a triterpene present in the roots and rhizomes of Glycyrrhiza glabra that has anti-inflammatory, hepatoprotective and neuroprotective effects. Recently, it was demonstrated that GL produced neuroprotective effects on the postischemic brain as well as on the kainic acid injury model in rats. In addition to this, GL also prevented excitotoxic effects on primary cultures. The aims of the present study were to evaluate GL scavenging properties and to investigate GL's effect on oxidative stress and inflammation in the lithium/pilocarpine-induced seizure model in two cerebral regions, hippocampus and olfactory bulb, at acute time intervals (3 or 24h) after status epilepticus (SE). Fluorometric methods showed that GL scavenged three reactive oxygen species: hydrogen peroxide, peroxyl radicals and superoxide anions. In contrast, GL was unable to scavenge peroxynitrite, hydroxyl radicals, singlet oxygen and 2,2-diphenil-1-picrylhydrazyl (DPPH) radicals suggesting that GL is a weak scavenger. Additionally, administration of GL (50mg/kg, i.p.) 30min before pilocarpine administration significantly suppressed oxidative stress. Moreover, malondialdehyde levels were diminished and glutathione levels were maintained at control values in both cerebral regions at 3 and 24 after SE. At 24h after SE, glutathione S-transferase and superoxide dismutase activity increased in the hippocampus, while both glutathione reductase and glutathione peroxidase activity were unchanged in the olfactory bulb at that time. In addition, GL suppressed the induction of the proinflammatory cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) in both cerebral regions evaluated. These results suggest that GL confers protection against pilocarpine damage via antioxidant and anti-inflammatory effects.
Epilepsy Res, 2016; 126

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BOARD NUMBER: S06-390

INVESTIGATING THE EFFECTS OF 16P11.2 DELETION ON CEREBRAL DEVELOPMENT AND INTERNEURON (IN) PRODUCTION USING VENTRAL TELENCEPHALIC ORGANOIDS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Copy number variations of chromosomal region 16p11.2 are genetically linked to 1% of Autism Spectrum Disorder (ASD) cases. The underlying molecular mechanisms linking the deletion to ASD pathophysiology remain largely unknown. **Aims:** Our work investigates whether perturbations in progenitor proliferation and differentiation into interneurons could contribute to the clinical phenotypes of 16p11.2 deletion. **Methods:** We generated ventral organoids from two 16p11.2deletion-CRISPR/Cas9-iPSC lines and three isogenic control lines to mimic ventral telencephalic development. Organoids were analysed using immunohistochemistry and flowcytometry. A combination of flowcytometry and double IdU/BrdU labelling was used to investigate cell cycle kinetics. Linear mixed model analysis was used to assess statistical significance. **Results:** Compared to controls, deletion organoids exhibited a significant increase in the relative neural rosette area, together with a significant increase in COUPTFII expression. No differences were found in the proportions of progenitor and immature neuronal populations. Cell cycle analysis revealed no significant effect of cell line or genotype on the proportion of cells in G1, S or G2M phases, although a trend towards reduced S-phase fractions was noticed in the deletion organoids. Finally, deletion organoids demonstrated a significant increase in total cell cycle duration and durations of G1 and G2M phases. **Conclusions:** Our findings suggest that 16p11.2 deletion impairs the proliferation of ventral progenitors. Prolonged cell cycle and G1 lengths suggest a shift towards asymmetric neurogenic divisions. Whether the interneuron progeny is immature, dysfunctional, or favours a certain interneuron subtype over another is yet to be investigated in detail.

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FATE POTENTIAL OF VENTRAL PROGENITOR CELLS DURING THE COURSE OF NEUROGENESIS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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During neurogenesis, mitotic progenitors located in the ganglionic eminences generate a wide range of inhibitory neurons, that subsequently migrate in distinct telencephalon areas. While the spatial and temporal specification of the individual inhibitory cell types is increasingly understood (Lim et al., 2018; Kelly et al., 2018), little is known about temporal mechanisms driving the generation of the whole pool of inhibitory neurons.
Previous single-cell transcriptomic and epigenomic studies on the excitatory neurons unraveled temporal patterns of genes transmitted from progenitors to their daughter cells and described transcriptional programs and cis-regulatory cascades for corticogenesis (Telley et al., 2019; Di Bella et al., 2021).
We used FlashTag labelling of isochronic cohorts (Govidan et al., 2018), and clonal barcoding methods (Bandler et al. 2021), combined with scRNA-seq, to assess the fate and lineage potential of ganglionic eminence progenitors at different developmental stages of neurogenesis.
Contrarily to their excitatory counterpart, inhibitory neuron progenitors exhibit similar fate potential throughout neurogenesis. However, late-born inhibitory neuron precursors express a transcriptome assortment right after cell-cycle exit, that early-born inhibitory neurons develop only several days after their birthdate. This result indicates a higher degree of transcriptomic maturation of late-born neurons compared to early-born ones, pointing to a possible diversity of lineage potential throughout neurogenesis.

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THE ROLE OF NEUROD2 IN THE DEVELOPMENT OF CEREBELLAR GABAERGIC INTERNEURONS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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In the cerebellar cortex, GABAergic interneurons have an important role in regulating Purkinje cell output. They derive from PAX2 progenitors and specify into diverse mature interneurons in different layers of the cerebellar cortex. Previous research has shown that mice lacking Neurod2, a bHLH transcriptional factor, show significant developmental defects and depletion of a subset of GABAergic interneurons, suggesting a potential role of Neurod2 in the specification of cerebellar GABAergic interneurons. However, how it is involved in their specification trajectory remains unclear. To investigate the role of Neurod2 in determining the specification of GABAergic interneurons in the cerebellar cortex, we combine immunofluorescence approaches to assess the development of the interneuron with single nuclei RNA sequencing technology to investigate the molecular differences in interneurons between wild-type and Neurod2$^{-/-}$ mutant mice. We first observe the absence of basket cells and stellate cells (Parvalbumin+) and significantly increased PAX2+ cell population in the molecular layer in mutant mice from adolescence to adulthood. Interestingly, PAX2+ cells in adult mutant mice express markers of Golgi cells that are normally distributed in the granule cell layer in the wild-type, indicating that these PAX2+ cells are no longer immature but develop in a different trajectory and probably contribute to the maintenance of GABAergic synapses in the molecular layer. Single-nuclei RNA-seq from wild-type and mutant conditions from P0 to P35 confirmed our observation. Together, our findings suggest that Neurod2 is necessary for the development of GABAergic interneurons and plays a role in determining their laminar position of in the cerebellum.

Pubmed:
33973524: Beekhof GC, Osório C, White JJ, van Zoomeren S, van der Stok H, Xiong B, Nettersheim IH, Mak WA, Runge M, Fiocchi FR, Boele HJ, Hoebeek FE, Schonewille M
Differential spatiotemporal development of Purkinje cell populations and cerebellum-dependent sensorimotor behaviors. Distinct populations of Purkinje cells (PCs) with unique molecular and connectivity features are at the core of the modular organization of the cerebellum. Previously, we showed that firing activity of PCs differs between ZebrinII-positive and ZebrinII-negative cerebellar modules (Zhou et al., 2014; Wu et al., 2019). Here, we investigate the timing and extent of PC differentiation during development in mice. We found that several features of PCs, including activity levels, dendritic arborization, axonal shape and climbing fiber input, develop differentially between nodular and anterior PC populations. Although all PCs show a particularly rapid development in the second postnatal week, anterior PCs typically have a prolonged physiological and dendritic maturation. In line herewith, younger mice exhibit attenuated anterior-dependent eyeblink conditioning, but faster nodular-dependent compensatory eye movement adaptation. Our results indicate that specific cerebellar regions have unique developmental timelines which match with their related, specific forms of cerebellum-dependent behaviors.
Elife, 2021; 10

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NORBIN IS REQUIRED FOR THE TRANSITION BETWEEN DIRECT TO INDIRECT NEUROGENESIS IN THE DEVELOPING MOUSE CORTEX

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Background: The cerebral cortex is a highly sophisticated structure organized in neuronal layers dedicated to specific connectivity. Generation of the proper number of neurons in the appropriate layer is essential to cerebral cortex functions. This process depends on a finely regulated balance between the proliferation of neural progenitors-the Radial Glial Cells (RGC)-and their differentiation into neurons through direct or indirect lineages. **Aims:** Many mechanisms involved in this control remain to be discovered. Here we studied the role of Norbin, a cytosolic protein already involved in human epilepsy, schizophrenia and neurodevelopmental delay, in the control of embryonic neurogenesis in the mouse neocortex. **Methods:** Invalidation of Norbin in cortical progenitors was achieved by Nestin Cre-mediated deletion of floxed Norbin alleles at E12.5-E13.5. Analyses of markers of neural progenitors and layer-specific neurons as well as EdU-birthdating experiments were conducted to characterize the effects of Norbin depletion on neurogenesis progression. **Results:** Although initially described as a neuron specific protein, we show that Norbin is also expressed by neural progenitors. The conditional knock-out of Norbin in RGC concomitantly leads to decreased production of intermediate progenitors and alteration of the generation of deep layers neurons, with no effect on RGC number and division rate. **Conclusions:** Our results support the involvement of Norbin in the control of the transition between direct and indirect neurogenesis phases and in the temporal pattern of cortical neuron generation.

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DIFFERENT TYPES OF CORTICAL PROGENITORS CONTRIBUTE TO SPECIFIC ASPECTS OF CORTICAL FOLDING

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Folding of the cerebral cortex is a key phenomenon of mammalian brain evolution and is associated with increases in size and complexity of the brain. The formation of cortex folding relies on the expansion of neuronal progenitors and tangential migration of cortical neurons. In previous work we showed that mice with deletions of FLRT1 and FLRT3 adhesion molecules develop sulci-like cortex folding without alterations of the number of neuronal progenitors, but with changes in radial migration speed. We have used our FLRT 1 and FLR3 double knock out ‘cell migration’ model to ask if the two folding mechanisms synergize and whether the expansion of certain types of progenitors leads to qualitatively different cortical folds. Here, we report that increasing the length of the early cortical stem cell expansion phase by deletion of fibroblast growth factor 10 (FGF10) in the ‘cell migration’ model (*FGF10;Flrt1/3 cTKO*) leads to cortical folding with much increased penetrance and, importantly, with gyrus-like protrusions. Conversely, overproduction of intermediate progenitors by deletion of centrosomal protein 83 (Cep83) in the ‘cell migration’ model (*Cep83;Flrt1/3 cTKO*) leads to cortical folding with much increased sulci-like appearance. These results suggest that expansion of progenitor cells and divergent radial migration of neurons synergize *in vivo* to induce cortical folding. They further indicate that expanding different types of progenitors leads to qualitatively different folding, suggesting that the formation of gyri and sulci requires the timely expansion of distinct progenitors.

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BOARD NUMBER: S06-395

OSCILLATIONS OF MEMBRANE VOLTAGE RECORDED IN HUMAN UNDIFFERENTIATED NEURONS OR RAT OLIGODENDROGLIAL PROGENITOR CELLS REQUIRE THE ACTIVATION OF BIG-CONDUCTANCE K+ (BK) CHANNELS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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The present research stems from an inedited phenomenon that was observed in immature cholinergic neurons isolated from the Nucleus Basalis of Meynert of human foetuses (*hf*NBMs). The Nucleus Basalis of Meynert (NBM) is the main source of cholinergic neurons in the basal forebrain to be crucially involved in cognitive functions and whose degeneration correlates with cognitive decline in major degenerative pathologies as Alzheimer's and Parkinson's diseases. We investigated overall electrophysiological and biochemical properties of *hf*NBM cholinergic neurons by means of whole cell patch clamp recordings or immunofluorescence analysis and we described, for the first time, that these cells display large amplitude, high-frequency, oscillations of membrane voltage when depolarized by a current step injection. This phenomenon, named by us "*voltage waves*", is conceivably due to the activation of BK channels, as confirmed by its sensitivity to iberiotoxin and to low (200 µM) concentration of tetraetylammonium (TEA), considered selective to block BK opening. Immunocytochemical analysis confirmed the expression of BK channels on *hf*NBMs. Of note, a similar phenomenon was also recorded in rat cortical oligodendrocyte progenitor cells (*r*OPCs), which are the only myelinating cells in the brain devoted to the production of myelin either during development or in brain repair/remodelling processes. As for *hf*NBMs, voltage oscillations were blocked by 200 µM TEA. Our data describe "voltage waves" in *hf*NBMs or OPCs by a biophysical point of view and try to dissect the time-window of the BK channel-dependent voltage activity in *hf*NBMs or OPCs at different maturational stages in culture.

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BOARD NUMBER: S06-396

HUMAN-SPECIFIC MODIFIERS OF THE WASH COMPLEX CONTROL CORTICAL NEURON MIGRATION AND FATE SPECIFICATION.

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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The mechanisms underlying human brain evolution constitute one of the most fascinating unresolved questions of biology. We previously identified >30 human-specific gene families highly expressed dynamically during human brain development. Here we focus our study on one such family that contains human-specific paralogs to the ancestral gene WASH1. WASH1 is a well conserved nucleation promoting factor that induces actin polymerization and controls endosome trafficking. We found two human-specific paralogs, WASH1P and WASH3P, to be expressed at high levels during human cortical development. Using in vivo analyses in the mouse and in vitro models, we present evidence that WASH1P can affect cortical neuron migration and fate acquisition, through the inhibition of some of WASH1 ancestral functions. We now investigate the underlying molecular mechanisms and potential relevance to human brain evolution.

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SLOW MATURATION OF DORMANT NEURONAL PRECURSORS IN THE AGED MOUSE BRAIN

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Dormant neuronal precursors (DNP) are intriguing cells capable of maintaining a prolonged state of immaturity in the adult brain, before maturing into functional neurons, as we recently reported. In the adult murine olfactory cortex, we compared neighbouring postnatal-developed neurons (PDN) to maturated DNP and revealed several distinctive features of the latter. We now question whether such differences could result from an extremely slow process of DNP maturation. In the DCX-CreRT2::CAG-fl-EGFP mouse, we permanently labelled DNP at 2 months of age to follow their maturation until 9 months (early) or up to 15 months of age (aged). Age-related differences between early and aged DNP emerged when comparing dendrites, soma, and axon initial segment, implying that DNP maturation is remarkably slow. Furthermore, we questioned the extent of functional maturation of aged DNP with single-cell patch clamp experiments on acute brain slices. Strikingly, functional traits of aged DNP were largely comparable to age-matched PDN. Thus, DNP mature gradually, but slowly, in the aged brain until they become comparable to aged DPN. On one hand, the apparent slowness of maturation is puzzling because the completion of the process may approximate or even exceed the lifespan of feral mice. On the other, the scarce olfactory cues available to caged mice may cause the slow DNP maturation, whereas salient olfactory experiences during adulthood of wild mice may be necessary to stimulate a quicker DNP maturation.

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POTENTIAL ROLE OF FIBROBLAST GROWTH FACTOR RECEPTOR 1 (FGFR1) IN THE DEVELOPMENT OF THE RETROSPLENIAL CORTEX.

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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The retrosplenial cortex (RSP) is located in a medial and caudal region of the brain between the neocortex and the archicortex. This cortical region is part of the limbic circuit and has been implicated in a range of cognitive functions such as emotion, attention, and spatial memory. The RSP has reciprocal connections with the anterior and dorsomedial thalamic nuclei, the hippocampal formation, the amygdaloid complex, and widespread neocortical areas. The RSP has been implicated in the pathophysiology of schizophrenia in both human studies and animal models with hyperactive behavior. Fibroblast growth factors (FGFs) and their receptors (FGFRs) are expressed in the developing and adult central nervous system and they have important roles in the morphogenesis of the mammalian cerebral cortex. Fgfr1gene is expressed in the dorsal VZ, the primordium of the cerebral cortex, also in upper layers of RSP cortex in postnatal stages (Allen Brain Atlas). Previous studies demonstrated that disruption of the Fgfr1 gene causes a decrease in cortical interneurons, decrease of pyramidal neurons in frontal and temporal cortical areas and locomotor hyperactivity. To understand the role of Fgfr1 in RSP development, we conditionally inactivated Fgfr1 in neuroepithelial cells of the CNS (Fgfr1f/f;NesCre+). Our analyses of volume estimation of cortical layers and the quantification of pyramidal neurons and parvalbumin-positive interneurons in the RSP, suggested that the proper formation of the RSP depends upon the function of Fgfr1.

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ALTERATIONS IN THE ANTERIOR CINGULATE AREA AND DENTATE GYRUS IN LIS1 MUTANT MOUSE UNDERLIES A SCHIZOPHRENIA-LIKE PHENOTYPE

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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LIS1 is one of the principal genes related to Type I lissencephaly, a severe human brain malformation characterized by an abnormal neuronal migration in the cortex during embryonic development. This is clinically associated with epilepsy and cerebral palsy in severe cases, as well as a predisposition to developing mental disorders, in cases with a mild phenotype. Although genetic variations in the *LIS1* gene have been associated with the development of schizophrenia, little is known about the underlying neurobiological mechanisms. We have studied how the *Lis1* gene might cause deficits associated with the pathophysiology of schizophrenia using the *Lis1/sLis1* murine model, which involves the deletion of the first coding exon of the *Lis1* gene. Homozygous mice are not viable, but heterozygous animals present abnormal neuronal morphology, cortical dysplasia, and enhanced cortical excitability. We have observed reduced number of cells expressing GABA-synthesizing enzyme glutamic acid decarboxylase 67 (GAD67) in the hippocampus and the anterior cingulate area, as well as fewer parvalbumin-expressing cells in the anterior cingulate cortex in *Lis1/sLis1* mutants compared to control mice. The cFOS protein expression (indicative of neuronal activity) in *Lis1/sLis1* mice was higher in the medial prefrontal (mPFC), perirhinal (PERI), entorhinal (ENT), ectorhinal (ECT) cortices, and hippocampus compared to control mice. Our results suggest that deleting the first coding exon of the *Lis1* gene might cause cortical anomalies associated with the pathophysiology of schizophrenia.

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BOARD NUMBER: S06-400

MOONLIGHTING REGULATION OF NEURONAL CELL FATE DETERMINATION BY ENDOCYTIC ADAPTOR AP-2

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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AP-2 is a heterotetrameric complex that links clathrin and other endocytic proteins to sites of clathrin-mediated endocytosis. Full body knockout of AP-2(μ) in mice causes embryonic lethality before day 3.5 postcoitus (Mitsunari, T. et al., 2005). In contrast, depletion of AP-2(μ) in neurons results in postnatal neurodegeneration and defective synaptic vesicle recycling (Kononenko et al., 2014, Kononenko et al., 2017). However, it does not block plasma membrane retrieval during neuronal activity, questioning the canonical function of AP-2 in neurons and suggesting that AP-2 might perform different functions (known as moonlight functions) in mitotic versus postmitotic cells.
We show that AP-2 controls neuronal progenitor cells (NPCs) proliferation but is not required for neuronal differentiation. In wildtype NPCs, AP-2 can be found at the centrosomes, where it colocalizes with components of the gamma-tubulin complex. Using mass spectrometry analysis, we identified GCP1, GCP2, GCP3, and GCP4 as novel interaction partners of the AP-2 complex. Deletion of AP-2μ in NPCs leads to defects in primary cilia disassembly and centrosome formation, resulting in cell cycle arrest, altered microtubule dynamics, and delayed cell migration. While, in vivo, we observed a tendency to neurogenic division in the SVZ of AP-2(μ) conditional KO mice and accumulation of Dcx+ cells, causing the disorganization of the cortical cytoarchitecture. This phenotype was not reproduced in NPCs treated with clathrin inhibitor PitStop2, suggesting a novel role of AP-2, independent of its function in clathrin-mediated endocytosis, in centrosome formation, trafficking components of the gamma-tubulin complex between the primary cilia basal body and the centrosome

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NEUROGENESIS IN GANGLIONIC EMINENCE IN THE LAST TRIMESTER OF GESTATION

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Background: It is widely accepted that neurogenesis in humans ends around the middle of gestation. However, the subventricular zone of some focal telencephalic domains continues with neuron production throughout the last trimester. **Aims**: The aim of our study was to determine if late neurogenesis might also occur in the major proliferative zones of the telencephalon. **Methods:** We evaluated Nissl, Golgi and immunohistochemically stained human slides from the Zagreb Neuroembryological Collection. **Results**: Between 24-26 postconceptional weeks (pcw) a massive stream of densely packed cells was found leaving the ganglionic eminence (GE), a progenitor domain of the ventral telencephalon that is a major source of cortical GABAergic neurons. Between 32-36 pcw, the GE decreased in size but was clearly distinguishable, and a stream of non-radially oriented MAP-2 positive migratory-like cells still extended towards the dorsal telencephalon. Additionally, volumetric analysis on MRI images was performed to evaluate the developmental pattern of the GE in period from 13 to 34 pcw and found a persistent GE throughout the entire analyzed period. This suggests that the GE maintains its proliferative capabilities up to the end of gestation and that a significant production of cortical GABAergic neurons continues beyond the proliferative period of principal glutamatergic neurons. **Conclusion:** We propose that protracted neurogenesis from the GE contributes to the disproportional increase in the number of calretinin neurons, the most numerous GABAergic neuron population in primates. We further suggest that late fetal production of neurons is not restricted to neurons within the dentate gyrus and rostral migratory stream.

Pubmed:
35124296: Ana K, Iris ŽI, Nina P, Marina R, Tomislav Ć, Snježana S, Andrea B, Milan R, Ivica K
Linking integrity of visual pathways trajectories to visual behavior deficit in very preterm infants.
Low-risk premature infants often develop visual deficits, even in the absence of ophthalmological complications and high-graded brain injury. These complications can be explained by the nature of subtle perinatal lesions and alterations of brain growth due to the prematurity. Subtle brain injuries and vulnerability of axonal pathways can be observed in spatiotemporal context of the white matter segments. The aim of this study was to examine the link between MRI quantitative (brain metrics data) and qualitative features (visibility of 2nd white matter segment - sagittal strata and periventricular crossroads C1-C6) and visual behavior in preterm neonates at term-equivalent age. Seventy-one very preterm infants without high-graded brain injury on MRI and no ocular pathologies were studies. The infants received MRI scans at term-equivalent age. MRI scans were analyzed using (a) simple brain metrics and (b) scoring the visibility of transient structural patterns (sagittal strata and periventricular crossroads). At the median age of 41 PMA weeks infants completed the Neonatal Visual Assessment. Results indicated that visibility of temporal crossroad area C6 and frontal and occipital sagittal strata was positively correlated with visual tracking skills in neonatal period. Furthermore, the visibility of frontal and occipital sagittal strata were strong predictors of total Neonatal Visual Assessment score. The findings confirmed that sagittal strata and periventricular crossroads prominence is a valuable additional marker in perinatal neuroimaging at term-equivalent age. Thus, alteration in MRI appearance of temporal crossroad and sagittal strata may be useful in predicting of visual behavior for very premature born infants.
Infant Behav Dev, 2022; 67
33938657: Banovac I, Katavić V, Blažević A, Bičanić I, Hladnik A, Kovačić N, Petanjek Z
The anatomy lesson of the SARS-CoV-2 pandemic: irreplaceable tradition (cadaver work) and new didactics of digital technology.
To compare the efficacy of different components of online and contact anatomy classes as perceived by medical students.

Croat Med J, 2021; 62
30186667: Maraković J, Marinović T, Jeleč V, Dlaka D, Muller D, Blažević A, Raguž M
Intraspinal calcinosis mimicking intervertebral disc extrusion: A clinical and surgical case report.
Subcutaneous calcinosis is a well-recognized manifestation of systemic sclerosis that usually involves multiple pressure points and may also be found in the paraspinal or intraspinal regions. In this case, intraspinal calcinosis uniquely led to a severe neurological deficit.
Surg Neurol Int, 2018; 9

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BOARD NUMBER: S06-402

A NOVEL FUNCTION FOR MIRK/DYRK1B KINASE IN THE COLUMNAR ORGANIZATION OF MEDIAL LATERAL MOTOR NEURONS IN THE EMBRYONIC CHICK SPINAL CORD

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Aims: Dyrk1B is a dual-specificity kinase involved in growth arrest and differentiation, playing a major role in cancer progression. Our aim is to elucidate the role of Dyrk1B in spinal cord development. **Methods**: *Gain-and-loss-of-function and mechanistic rescue phenotype experiments* were performed in E2 chick neural tube by unilateral *in ovo* electroporation of Dyrk1B/GFP and GFP constructs, administration of AZ191 inhibitor or both of them together respectively. **Results**: Forced expression of Dyrk1B in E2 chick neural tube resulted in increased cell cycle exit and apoptosis at E4, while neuronal differentiation is slightly induced. The observed increased apoptosis in VZ and motor neuron (MN) domain combined to premature cell cycle exit resulted in the reduction of motor neuron progenitors (pMNs), as well as of post-mitotic motor neurons (MNs). This intense ventral phenotype of Dyrk1B function indicates the possible involvement of SHH signaling. In agreement, the real-time qPCR analysis revealed that Dyrk1B overexpression reduces dramatically SHH and Gli3 mRNA levels. At E6, the fewer MNs resulted in the formation of a reduced medial LMC region (LMCm), while no changes were observed at the lateral LMC region (LMCl), or in the medial motor column (MMC). AZ191 administration at E2 showed the opposite phenotype, resulting in increased proliferation at E4, while mechanistic rescue phenotype experiments, applying AZ191 1h after Dyrk1B/GFP electroporation, confirmed the inhibitor specificity. **Conclusions**: We revealed a novel role for Mirk/Dyrk1B kinase in spinal cord development. Dyrk1B overexpression alters the columnar organization of LMCm MNs. The mechanism of Dyrk1B function is under investigation.

Pubmed:
33505600: Kokkorakis N, Gaitanou M
Minibrain-related kinase/dual-specificity tyrosine-regulated kinase 1B implication in stem/cancer stem cells biology.
Dual-specificity tyrosine phosphorylation-regulated kinase 1B (DYRK1B), also known as minibrain-related kinase (MIRK) is one of the best functionally studied members of the DYRK kinase family. DYRKs comprise a family of protein kinases that are emerging modulators of signal transduction pathways, cell proliferation and differentiation, survival, and cell motility. DYRKs were found to participate in several signaling pathways critical for development and cell homeostasis. In this review, we focus on the DYRK1B protein kinase from a functional point of view concerning the signaling pathways through which DYRK1B exerts its cell type-dependent function in a positive or negative manner, in development and human diseases. In particular, we focus on the physiological role of DYRK1B in behavior of stem cells in myogenesis, adipogenesis, spermatogenesis and neurogenesis, as well as in its pathological implication in cancer and metabolic syndrome. Thus, understanding of the molecular mechanisms that regulate signaling pathways is of high importance. Recent studies have identified a close regulatory connection between DYRK1B and the hedgehog (HH) signaling pathway. Here, we aim to bring together what is known about the functional integration and cross-talk between DYRK1B and several signaling pathways, such as HH, RAS and PI3K/mTOR/AKT, as well as how this might affect cellular and molecular processes in development, physiology, and pathology. Thus, this review summarizes the major known functions of DYRK1B kinase, as well as the mechanisms by which DYRK1B exerts its functions in development and human diseases focusing on the homeostasis of stem and cancer stem cells.
World J Stem Cells, 2020; 12

FENS Forum 2022
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BOARD NUMBER: S06-403

MOLECULAR MARKERS OF THE PRENATAL HUMAN SUBTHALAMIC NUCLEUS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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The subthalamic nucleus (STN) is an important node in the basal ganglia circuitry. The developmental origin and molecular markers of STN have been adequately studied in animal models. However, there is a need to elucidate the molecular markers guiding the development of the human STN, as this nucleus is involved in the motor, associative, and limbic functions of the cortico-basal ganglia-thalamocortical circuitry. Moreover, some transcription factors identified in the STN of experimental animals are implicated in several neurodevelopmental disorders, but the structural and molecular changes of STN's neuronal population in these disorders were never thoroughly explored. **Aims** The aim of the study was to identify spatio-temporal expression patterns of transcription factors relevant for the differentiation and specification of STN's neuronal population. **Methods** This study encompassed postmortem, formalin-fixed, paraffin-embedded human fetal brain tissue from the early fetal period (12-15 post-conceptional weeks, PCW) to term. Indirect immunohistochemistry with antibodies for transcription factors FOXA1, FOXP1, FOXP2, BARHL1, and PAX6 was performed on 10µm thin sections. **Results** We show that the developing human STN expresses transcription factors previously shown to be involved in the development of rodent STN. Moreover, the human STN expresses some transcription factors that were not previously described in animal models. **Conclusions** Our preliminary study accentuates the need to study the transcription factors in the developing human STN because the results from animal models cannot fully predict the human STN's molecular markers. Elucidating the STN's transcriptional profile could advance our understanding of its functional properties and its role in neurodevelopmental disorders.

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BOARD NUMBER: S06-404

THE LOGIC OF TEMPORAL PATTERNING IN GENERATING NEURONAL DIVERSITY IN DROSOPHILA TOPC

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Aims: The nervous system is a complex network of distinct cell types organized in a highly stereotypical pattern, each with unique morphology, projection and function. A fundamental goal in neuroscience is deciphering the diversity and heterogeneity of neuronal cells in organization of neural circuits. Neurons are generated through multiple divisions of stem cells. In *Drosophila*, neural progenitors use two axes of information, spatial and temporal, to generate the cellular diversity of the central nervous system. The neuroblasts at the tip of OPC (outer proliferation center) epithelium transit through the series of tTFs as they age and the combinatorial input of spatial and temporal factors allows the generation of different neuronal types. **Methods:** A laser-based flow cytometry, fluorescence-activated cell sorting (FACS Aria III), 10X Genomics single cell technology and NovaSeq 6000 sequencing platform are used to determine the complete gene-expression profile of individual neuroblasts and neurons during development. **Results:** By combining single cell mRNA sequencing with computational methods, we identified the whole series of temporal TFs that specify optic lobe neurons that arise from tOPC region. We also identified complete set of neurons coming from different temporal windows within this region. **Conclusion:** Our results show how the single cell mRNA sequencing can be used to characterize regulation of temporal transcription factors and the way the different set of neurons are generated.

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BOARD NUMBER: S06-405

MOLECULAR INTERPLAY BETWEEN MIR-128 AND ARPP21, A NEURONAL RBP, SHAPES DENDRITIC ARBORS IN THE MOUSE CORTEX

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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miR-128 is a highly abundant, brain-enriched miRNA with important functions in the regulation of migration, dendritic outgrowth and excitability of upper layer (UL) neurons in the mouse cerebral cortex. In vertebrates, miR-128 is located within introns of R3H-domain family RNA binding proteins (RBPs). This arrangement allows miR-128 activity to be leveraged at a second posttranscriptional level: the co-regulation of a subset of miR-128 mRNA targets by the miR-128-2 host gene product, a poly(U)-specific RBP. Surprisingly, ARPP21 target RNAs were specifically enriched for miR-128 binding sites, suggesting that ARPP21 directly antagonizes miR-128 activity on an overlapping set of mRNAs. In contrast to miR-128, we have shown that ARPP21 acts as a positive post-transcriptional regulator, at least in part through interaction with the translation initiation complex. Proximity ligation assays suggest that ARPP21 functionally interacts with multiple 5'-Cap and poly(A) binding complexes. We have shown that miR-128 overexpression inhibits migration to the UL and impairs dendritic arborization. We now show that CRISPR-mediated miR-128 knockdown leads to aberrant apical dendrite orientation and geometry. Persistent expression of miR-128 in radial glia leads to reduced generation and impaired cortical astrogenesis. Manipulation of the two miR-128 host genes, ARPP21 or R3HDM1, oppose the action of miR-128 on UL neuron migration and dendritogenesis by promoting migration and enhancing arbor complexity. We believe these antagonistic functions provide a tunable post-transcriptional switch with relevance for intellectual disability linked to mutations in R3HDM1 and miR-128 target genes.

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BOARD NUMBER: S06-406

THE ALTERATION OF HEME METABOLISM AFFECTS ENERGETIC METABOLISM LEADING TO NEURODEVELOPMENTAL DEFECTS IN MICE

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Heme is an iron-containing porphyrin of vital importance that orchestrates cellular metabolism. Indeed, heme regulates the TCA cycle flux, the activity of the electron transport chain (ETC) complexes, ATP synthesis, and thermogenesis. Proper regulation of energetic metabolism is crucial during neurogenesis and metabolic dysfunctions often result in neurodevelopmental defects. Interestingly, recent data associate defective heme import in the developing brain with microcephaly and hydrocephalus in both humans and mice. Curiously, the heme exporter FLVCR1 is highly expressed in the embryonic nervous tissues and zebrafish lacking FLVCR1 showed hydrocephalus.
To better dissect the role of heme trafficking in the regulation of neurodevelopment, heme levels were genetically manipulated in neuronal progenitors in mice by targeting the plasma membrane heme exporter FLVCR1a.
Mice lacking Flvcr1a in neuronal progenitors (Flvcr1afl/fl; Nes-Cre mice) die perinatally due to severe neurodevelopmental defects: E18,5 Flvcr1afl/fl; Nes-Cre embryos showed microcephaly and hydrocephalus. Analysis of E14,5 Flvcr1afl/fl; NesCre embryos cortex showed increased apoptosis; however, no differences in cell proliferation were detected at this stage. Moreover, a decrease in neural progenitors, as well as a decreased number of differentiating neural cells, were observed in Flvcr1afl/fl; NesCre developing brains compared to controls.
Finally, metabolic analyses showed that the brain of E14,5 Flvcr1afl/fl; NesCre embryos were characterized by decreased activity of enzymes involved in glycolysis, ETC, reduced mitochondrial ATP levels, and increased lipid peroxidation compared to controls.
Together these data reveal an essential role of FLVCR1 during neurodevelopment and suggest that its action is mediated by the fine regulation of energetic metabolism.

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BOARD NUMBER: S06-407

DEFECTIVE INTRACELLULAR CHOLESTEROL MOBILIZATION DERANGES THE PROLIFERATION/DIFFERENTIATION BALANCE OF NEURONAL PRECURSORS IN A MOUSE MODEL OF NIEMANN PICK C DISEASE

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Over recent years, compelling evidence has outlined that developmental trajectories are altered in a genetic rare condition, the Niemann Pick C1 (NPC1) disease. NPC1-deficiency is characterized by late endosomal accumulation of unesterified cholesterol, which leads to impaired cholesterol homeostasis and a broad range of cellular abnormalities, including alterations in mitochondrial electron transport chain and increased oxidative stress (OS).
To investigate the possibility that OS affects the complex sequence of events leading to neuronal maturation, we have established two *in vitro* cell models, using *Npc1* KO mid-gestation embryos as donors: i) neural precursor cells (NSCs), which rapidly grow and form neurospheres *in vitro* when maintained under stemness conditions; and, ii) mouse embryonic fibroblasts (MEFs), which can be easily expanded, obtaining large cell amounts required for biochemical approaches. The NSC self-renewal potential, determined by measuring neurosphere diameter, resulted significantly reduced in neurospheres of *Npc1* KO compared to *wt* ones. Consistently, the expression of Pax6 and Sox2, typical markers of stemness maintenance, was already reduced at DIV1, whereas the expression of differentiation markers, such as beta3-tubulin concomitantly increased. This finding indicates that Npc1-deficiency determines a reduction of NSC proliferation ability, anticipating their switch to differentiation. Of note, differentiating *Npc1* KO neurons, displayed longer neurites and a higher level of neurite sprouting, *i.e*. increased neurite number and branching, when compared to *wt* neurons. Redox signaling mechanisms involved in the regulation of neuronal development were studied in MEFs, observing that the transcriptional activation of antioxidant genes was disturbed in *Npc1* KO MEFs.

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BOARD NUMBER: S06-408

AGO-APP FOR IDENTIFIYING A NEUROGENESIS REGULATING MICRO-RNA NETWORK IN FLY

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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MicroRNAs are small regulatory molecules that negatively control mRNA translation. The microRNA pathway is required for most, if not all, of the developmental processes that have been studied so far. However, many aspects regarding the exact mode of action of microRNAs are still unclear. To decipher the precise role of microRNAs during neurogenesis, we have developed a new in vivo tool called Ago-APP. Ago-APP is based on the controlled expression of a small T6B peptide allowing immuno-precipitation and sequencing of micro-RNAs. We have generated three Drosophila lines where T6B expression can be specifically induced in neuroblasts, neurons or glial cells, respectively. From these lines we have identified sets of micro-RNAs specifically active in one of the 3 cell types. The cell-type specific activity of some of these microRNAs has been verified using reporter fly lines. We have initiated the functional analysis of neuroblast microRNAs. Using overexpression approaches, we confirmed the ability of neuroblast microRNAs to target neurogenic transcription factors and control neurogenesis. However, loss-of-function experiments generally induced no or very few differentiation defects suggesting that microRNAs naturally act synergistically to regulate neurogenesis. To confirm this hypothesis, we generated Drosophila lines harboring sponge constructs designed to simultaneously downregulate more than one microRNA (from 2 to 8). Analysis of these Drosophila lineages, including the ability of neuroblasts to maintain an immature state when sponges are expressed, is ongoing.

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BOARD NUMBER: S06-409

GESTATIONAL EXPOSURE TO FUNGICIDE RESIDUES CORRUPTS NEUROGENESIS AND SYNAPTIC FUNCTIONS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Neurogenesis plays a crucial role during neurodevelopment and its dysfunction can lead to neurodevelopmental disorders. The presence of pesticide residues in the environment represents a threat of which we have recently become aware of. Contaminants could corrupt neurogenesis during gestation, potentially leading to impaired neuronal and synaptic functions. Since the effects of this low-noise contamination have not yet been evaluated on neurodevelopment, we investigated the impact of fungicide residues on WT mice exposed throughout gestation. Mice were exposed to fungicides, cyprodinil, mepanipyrim and pyrimethanil, alone or in cocktail in the drinking water at 0.1 µg/L from gestation to weaning. The dose selected in the drinking water corresponds to the regulatory limit dose allowed by EU. Results showed that gestational exposure to fungicide residues substantially promoted an increase of neural precursor cells at P3. This corrupted neurogenesis was linked to increased levels of β-catenin, through the crosstalk of the PI3K/Akt and Wnt/β-catenin pathways, both involved in cell proliferation. Fungicide exposure also altered protein expression of PSD95 and NMDA receptors in P3 neonates, two targets of the β-catenin signaling pathway. The alteration of the expression of these synaptic proteins leads to synaptic transmission dysfunctions at P18. Exposed-mice are unable to elicit an hippocampal long term depression, which is a key mechanism in memory. Corruption of neurogenesis and synaptic functions by these fungicide residues could be a fertile ground for the development of neurodevelopmental disorders as autism spectrum disorders or other diseases associated with memory deficits.

Pubmed:
34445475: Vignon A, Salvador-Prince L, Lehmann S, Perrier V, Torrent J
Deconstructing Alzheimer's Disease: How to Bridge the Gap between Experimental Models and the Human Pathology? Discovered more than a century ago, Alzheimer's disease (AD) is not only still present in our societies but has also become the most common dementia, with 50 million people worldwide affected by the disease. This number is expected to double in the next generation, and no cure is currently available to slow down or stop the disease progression. Recently, some advances were made due to the approval of the aducanumab treatment by the American Food and Drug Administration. The etiology of this human-specific disease remains poorly understood, and the mechanisms of its development have not been completely clarified. Several hypotheses concerning the molecular mechanisms of AD have been proposed, but the existing studies focus primarily on the two main markers of the disease: the amyloid β peptides, whose aggregation in the brain generates amyloid plaques, and the abnormally phosphorylated tau proteins, which are responsible for neurofibrillary tangles. These protein aggregates induce neuroinflammation and neurodegeneration, which, in turn, lead to cognitive and behavioral deficits. The challenge is, therefore, to create models that best reproduce this pathology. This review aims at gathering the different existing AD models developed in vitro, in cellulo, and in vivo. Many models have already been set up, but it is necessary to identify the most relevant ones for our investigations. The purpose of the review is to help researchers to identify the most pertinent disease models, from the most often used to the most recently generated and from simple to complex, explaining their specificities and giving concrete examples.
Int J Mol Sci, 2021; 22
32014094: Vignon A, Denolly S, Perez-Vargas J, Cosset FL
[The hepatitis delta virus: an easily turncoat pathogen?]
Med Sci (Paris), 2020; 36
29900841: Castino G, Guillemet M, Joly A, Vignon A
[The CRISPR/Cas system: a genome editing tool to develop animal models of viral infections].
Med Sci (Paris), 2018; 34

FENS Forum 2022
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BOARD NUMBER: S06-410

FUNCTIONAL INTEGRATION OF NEWLY GENERATED NEURONS IN MECHANICAL CORTICAL BRAIN INJURIES.

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Introduction: Brain injuries activate neural stem cells in the subventricular zone (SVZ) to produce a higher number of neuroblasts. But neuronal replacement is very limited and does not result in functional recovery. **Aim:** The aim of this work is to see if the treatment with EOF2 is capable of achieving the maturation and integration of these new neuroblasts in the injured region. **Methods:** We have analyzed the effect of the EOF2, that facilitates the release of neuregulin 1 and stimulates neuroblast differentiation, on the generation of new neurons in response to a mechanical cortical brain injury. Using GFP-expressing lentiviral vectors, we have labelled cells of the SVZ before performing mechanical injuries in the primary motor cortex of mice. We have studied the migration towards the injury and have analyzed the functional properties of these cells over the course of 14 to 56 days post injury (dpi). **Results:** Not treated mice show very little or no GFP-labelled cells in the perilesional area. 14 dpi GFP-labelled cells did not show active membrane properties. 28 dpi depolarized resting membrane potentials were observed and 5% of the recorded cells showed action potentials. At this time some recorded cells showed synaptic inputs, which suggest they have integrated into existing circuits. 56 dpi the GFP-labelled neurons showed resting membrane potentials and action potentials values similar to those of mature neurons. **Conclusion:** We conclude that treatment with EOF2 promotes migration of newly generated neuroblasts towards the injury that functionally differentiate into mature neurons.

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BOARD NUMBER: S06-411

DECIPHERING THE DIFFERENTIATION PROGRAM AND FUNCTION OF EPENDYMAL CELLS IN THE ZEBRAFISH BRAIN

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

Percival Paul D'Gama[1], Tao Qiu[2], Mehmet Ilyas Cosacak[3], Dheeraj Rayamajhi[2], Ahsen Konac[4], Jan Hansen[5], Christa Ringers[1], Francisca Acuña-Hinrichsen[4], Subhra Hui[6], Emilie W Olstad[4], Yan Ling Chong[2], Charlton Kang An Lim[2], Astha Gupta[7], Chee Peng Ng[8], Benedikt S . Nilges[7], Nachiket D. Kashikar Kashikar[7], Dagmar Wachten[5], David Lieb[8], Kazu Kikuchi[9], Caghan Kizil[3], Emre Yaksi[4], Sudipto Roy[2], Nathalie Yaksi[1]
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The ependymal cells are specialized cells lining the surface of the brain ventricles and spinal canal. They carry bundles of motile cilia on their apical surface, which beat and contribute to directional CSF flow. They typically originate from radial glia cells and are highly conserved among vertebrates Defects in these cells can lead to a variety of neurological conditions, affecting brain function. Despite this, ependymal cells remain largely understudied. We use zebrafish as a model to characterize ependymal cells and better understand their function in brain development and physiology. In the larval zebrafish brain these cells appear early in development and display a single cilium while multiciliated cells (MCCs) carrying multiple cilia are born later in development. It however remains unclear when and where these MCCs are established and how they contribute to brain function. In our study, we identified two different lineages of motile ciliated cells and showed that these lineages are heterogenous in their expression of *Foxj1* family of ciliary master regulators. Our study also depicts that during development there is a transition from mono- to multiciliated cells (MCCs) driven by *gmnc*. Finally, we revealed the role of these ependymal cells in morphogenesis of the brain by contributing to ventricular development. In conclusion our research depicts that in the zebrafish ependymal cells have a remarkable degree of similarity as well as some differences with mammals.

Pubmed:
34610312: D'Gama PP, Qiu T, Cosacak MI, Rayamajhi D, Konac A, Hansen JN, Ringers C, Acuña-Hinrichsen F, Hui SP, Olstad EW, Chong YL, Lim CKA, Gupta A, Ng CP, Nilges BS, Kashikar ND, Wachten D, Liebl D, Kikuchi K, Kizil C, Yaksi E, Roy S, Jurisch-Yaksi N
Diversity and function of motile ciliated cell types within ependymal lineages of the zebrafish brain.
Motile cilia defects impair cerebrospinal fluid (CSF) flow and can cause brain and spine disorders. The development of ciliated cells, their impact on CSF flow, and their function in brain and axial morphogenesis are not fully understood. We have characterized motile ciliated cells within the zebrafish brain ventricles. We show that the ventricles undergo restructuring through development, involving a transition from mono- to multiciliated cells (MCCs) driven by gmnc. MCCs co-exist with monociliated cells and generate directional flow patterns. These ciliated cells have different developmental origins and are genetically heterogenous with respect to expression of the Foxj1 family of ciliary master regulators. Finally, we show that cilia loss from the tela choroida and choroid plexus or global perturbation of multiciliation does not affect overall brain or spine morphogenesis but results in enlarged ventricles. Our findings establish that motile ciliated cells are generated by complementary and sequential transcriptional programs to support ventricular development.
Cell Rep, 2021; 37

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BOARD NUMBER: S06-412

ROLE IN NEURAL STEM CELL DIFFERENTIATION OF CHD8 AND CHD7, CHROMATIN REMODELERS, IMPLICATED IN AUTISM SPECTRUM DISORDER

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Autism spectrum disorder (ASD) affects approximately 1 in 70 people. Most ASD risk genes are expressed during brain development having roles either in neuronal communication or in regulation of gene expression, with CHD8 chromatin remodeler being the most frequently mutated gene in ASD patients. We previously showed that specific deletion of *Chd8* in oligodendroglia leads to strong defects in oligodendrocyte development and myelination. In addition, mutations in its paralog CHD7 are the cause of CHARGE syndrome, which also presents autistic phenotypes, and we previously showed that *Chd7* specific deletion in oligodendroglia impairs normal oligodendrocyte differentiation and myelination in mouse models and children with *CHD7* mutations. In this study, we aimed to understand the role of these chromatin remodelers in NSC differentiation into different neural subtypes, neurons, astrocytes and oligodendroglia. Using postnatal electroporation to label neural stem cells (NSCs) from neonatal subventricular zone and trace their progeny, we present evidences for an essential role of Chd7 and Chd8 chromatin remodelers in brain NSC differentiation. First, we show that Chd7 and Chd8 bind together to regulatory regions of genes involved in oligodendrogenesis, neurogenesis, and astrogenesis, suggesting they regulate all these biological processes. Second, double knockout NSCs, present a decrease in the total number of cells they generate, with oligodendroglia being the most decreased cell-lineage, followed by a strong reduction in olfactory bulb neurons, and a smaller reduction in astrocytes. We believe that further understanding of Chd7 and Chd8 implication in the NSC differentiation should help to develop therapeutic treatments for ASD patients.

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BOARD NUMBER: S06-413

AQP4 AGGREGATION STATE AFFECTS NEURAL STEM CELL DIFFERENTIATION

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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The glial water channel AQP4 has emerged as an important player regulating neurogenesis in healthy and diseased brain. AQP4 forms ordered structures named Orthogonal Arrays of particles (OAPs) whose size depends on the ratio of its major isoforms, M1 and M23. Consistent with the relevance of controlling cell volume changes during neural development, AQP4 is markedly expressed in Neural Stem Cells (NSCs). AQP4 deletion is known to inhibit proliferation, migration, and neuronal differentiation of NSCs cultured *in vitro* but does not impact glial differentiation. The aim of this study is to investigate the selective role of the OAP-forming M23-AQP4 isoform in regulating NSC differentiation. E14 embryonic NSCs were prepared from WT, OAP-null mice lacking M1-AQP4 isoform and AQP4 knockout (KO) mice. Floating neurospheres were allowed to differentiate for 3, 7, 14 and 21 days and analyzed by immunofluorescence, immunoblotting and functional assays. WT and OAP-null NSCs revealed a progressive decrease in the NSC marker Nestin and time-related increase in GFAP and AQP4, whose level reflected in the upregulated water transport rate. We found that the lack of AQP4 significantly impaired both NSC proliferation and radial migration out of the neurospheres. Interestingly, compared to WT and AQP4 KO, OAP-null NSCs displayed higher viability and faster mobility as well as they failed to migrate in chains. These findings suggest that AQP4 aggregation state may be involved in the mechanobiology of astrocytes during brain development and provide useful information for stem cell technology.

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BOARD NUMBER: S06-414

THE ORGANIZATION OF THE INFERIOR OLIVE IS ALTERED IN TSHZ3 MUTANT MICE

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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In mouse, newborn inferior olivary (IO) neurons derived from the neuroepithelium of the hindbrain rhombic lip. These neuronal precursors migrate tangentially in the submarginal zone from the dorsal to the ventral medulla and stop their migration before reaching the floorplate to form the immature inferior olive nucleus (ION). Near the floor plate, IO neurons first compact into a club-shaped domain, and then they organize in subnuclei that form the characteristic lamellae. The molecular mechanisms controlling the specification and the emergence of precerebral neurons as well as those controlling the migration of IO neurons towards the midline start to be elucidated, however, both the mechanism controlling the final positioning of IO neurons and the compartmentalization of the ION need more investigation. We previously showed that mice deficient for the Teashirt zinc finger homeobox 3 gene (*Tshz3*) die at birth and that *Tshz3* controls the development of diverse components of the circuitry required for breathing. Here, we analyzed the impact of *Tshz3* deletion on the development of the caudal medulla. We report that *Tshz3* inactivation specifically affects the organization of subnuclei as well as the lamellated pattern of the ION. Together our data indicate that *Tshz3* is required for the proper subdivision and final morphology of ION. We previously reported that heterozygous deletion of *TSHZ3* in 19q12 are associated with autism spectrum disorder and these new data might provide insight for understanding the pathophysiological mechanisms of this disorder.

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BOARD NUMBER: S06-415

ROLE OF THE SONIC HEDGEHOG SIGNALING PATHWAY ON THE CORTICAL DEVELOPMENT

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Sonic Hedgehog SHH is a morphogen involved in dorso-ventral patterning of brain during early development, however its role in the development of the cerebral cortex has been poorly investigated. SHH binds to its receptor Patched allowing the translocation of Smoothen in the primary cilium, a cellular compartment acting as a signaling plateform. The canonical SHH dependent pathway control gene expression through activation and repression of Gli transcription factors and depends on intraflagellar trafficking involving specific kinesins, such as KIF7. In the cortex, SHH is involved in the division/migration of principal cortical cells and in the migration of interneurons as they colonize the cortical plate. We analyzed SHH expression in the cortex between E12.5 and E16.5 and investigated the consequence of abnormal canonical SHH-dependent using *Kif7* KO on cortical development. We showed that *Shh* mRNA is expressed at low level in very few cells in the developing cortex whereas SHH protein is present throughout the proliferative and postmitotic cortical layers, suggesting significant extracortical origin for cortical SHH. Deletion of *Kif7* leads to an hyper-activated Gli dependent pathway and to gross morphological defects of the brain. We thus confirmed that the loss of *Kif7* affects cortex neurogenesis, in particular the proliferation and differentiation of principal cortical cells and the migration of cortical interneurons. Afferent and efferent cortical projections were abnormal despite normal dorso-ventral patterning of the forebrain . Our results confirmed a significant contribution of the SHH gli-dependent pathway to the cortex development, with a key role on cortical and GABAergic interneurons migration.

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BOARD NUMBER: S06-416

SEQUENTIAL NEUROGENESIS IN ZEBRAFISH HABENULA GIVE RISE TO DISTINCT FUNCTIONAL MICROCIRCUITS WITH DISTINCT COMPUTATIONAL PROPERTIES

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Habenula is a brain region that relays forebrain activity to monoaminergic brain nuclei and was shown to play an important role in adaptive behaviors. Recently, we revealed that habenula integrates multi-sensory information with the activity of multiple ancestral cortico-limbic forebrain regions in zebrafish. We also showed that as zebrafish develop from larval to juvenile, their performance in cognitively demanding adaptive behaviors increases. These results highlight a developmental maturation and expansion of brain circuits that are required for behavioral flexibility. In line with this maturation, our recent findings showed that sequential neurogenesis in habenula give rise to ensembles of habenular neurons with different spatial locations and correlated ongoing activity. However how sequential neurogenesis in habenula contributes to formation of distinct habenular microcircuits, and how this relates to the integration of the habenula into the cortico-limbic activity and sensory inputs is yet to be discovered. By using a combination of *in vivo* birth-dating with functional brain imagining, we investigated the ongoing and sensory evoked activity of habenular neurons that are born at distinct developmental stages, together with their input regions in dorsal telencephalon. Our results revealed that habenular neurons that are born at distinct developmental time points, integrates information from distinct forebrain ensembles, and encodes sensory information differently. We also showed that distinct microcircuits of habenular neurons marked with molecular markers are populated by different waves of neurogenesis. Our results revealed that sequential neurogenesis in habenula, give rise to habenular ensembles integrating information from distinct sources encoding internal states and environmental cues.

Pubmed:

32917624: Fore S, Acuña-Hinrichsen F, Mutlu KA, Bartoszek EM, Serneels B, Faturos NG, Chau KTP, Cosacak MI, Verdugo CD, Palumbo F, Ringers C, Jurisch-Yaksi N, Kizil C, Yaksi E
Functional properties of habenular neurons are determined by developmental stage and sequential neurogenesis.
The developing brain undergoes drastic alterations. Here, we investigated developmental changes in the habenula, a brain region that mediates behavioral flexibility during learning, social interactions, and aversive experiences. We showed that developing habenular circuits exhibit multiple alterations that lead to an increase in the structural and functional diversity of cell types, inputs, and functional modules. As the habenula develops, it sequentially transforms into a multisensory brain region that can process visual, olfactory, mechanosensory, and aversive stimuli. Moreover, we observed that the habenular neurons display spatiotemporally structured spontaneous activity that shows prominent alterations and refinement with age. These alterations in habenular activity are accompanied by sequential neurogenesis and the integration of distinct neural clusters across development. Last, we revealed that habenular neurons with distinct functional properties are born sequentially at distinct developmental time windows. Our results highlight a strong link between the functional properties of habenular neurons and their precise birthdate.
Sci Adv, 2020; 6

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-417

ALTERED SUBCELLULAR MECHANISMS AND CELL CYCLE IN EML1 MUTANT NEURONAL PROGENITORS : PRIMARY EVENTS LEADING TO A CORTICAL MALFORMATION

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Subcortical band heterotopia (SH) is a cerebral cortical malformation associated with epilepsy and intellectual disability. In this disorder, clusters of neurons are found localized abnormally in the white matter, below the cortex. The microtubule-associated protein Eml1/EML1 was found mutated in SH mice and patients. During corticogenesis in mutant mice, some neuronal progenitors (RG) exhibit an aberrant position, outside their ventricular proliferative zone (VZ), in the region where the heterotopia develops. RG exhibit altered primary cilia suggesting a role of Eml1 associated with this key organelle involved in cell cycle and signalling. Taking advantage of a new murine model that we generated (Eml1 cKO), we are interested in deepening the mechanisms critically leading to the RG phenotype and hence SH formation. We examined the structural and functional abnormalities of centrosomes, as well as protein transport essential for the formation of the cilia, and identified defects in these processes. Also, we characterized the impact of Eml1 on the progress of the cell cycle in RG and showed S-phase and cell cycle lengthening at early corticogenesis before RG detachment. Using multi-OMICS approches we aim to determine key deregulated genes/proteins and interactions to further explain how subcellular abnormalities and cell cycle alterations cause RG detachment upstream of heterotopia. These data will help propose and test an experimental approach for phenotypic rescue, as well as lead to a better understanding of the understudied role of Eml1 in RG behavior, the latter highly regulated during brain evolution.

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BOARD NUMBER: S06-418

INCREASED EXPRESSION OF EHMT1/GLP PROTEIN IN EMBRYONAL NEUROGENESIS, AND SPECIFIC POSTNATAL AND ADULT MOUSE AND RAT BRAIN NEUROGENESIS AREAS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Euchromatin histone methyltransferase 1 (Ehmt1) is a protein which regulates transcription by catalyzing methylation of histones, which then can lead to silencing of gene expression. Haploinsufficiency of the EHMT1 gene, with only 1 functional allele for the Ehmt1/GLP protein, results in humans in a congenital intellectual disability syndrome called Kleefstra Syndrome. Mice with a heterozygous mutation for Ehmt1 (*Ehmt1+/-*) proved to be an excellent animal model to study Kleefstra Syndrome (Balemans et al. 2010, 2013, 2014 and Iacono et al. 2018). Balemans et al. (2013) showed for *Ehmt1+/-* mice that Ehmt1 protein levels in brain cortex, hippocampus, cerebellum and olfactory bulb are 50% lower than levels measured in littermate wildtype mice, using quantitative Western Blot analysis. In this study, we focused on wildtype brain and demonstrated Ehmt1 protein expression in all cells in all parts of mouse and rat brain. Notably, embryonal stage shows high expression, in the postnatal phase the expression decreases, and in adult brain there is a lower, but apparent Ehmt1 protein expression. Interestingly, significantly elevated Ehmt1 protein levels were found in the two known adult rodent neurogenesis areas: the Dentate Gyrus Subgranular layer and the subventricular zone-RMS-Olfactory bulb areas. A correlation between the number of darkly stained Ehmt1-positive cells and the number of DCX-positive cells in the dentate gyrus subgranular layer and in the subventricular zone / RMS indicates a role for Ehmt1 in adult neurogenesis.

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BOARD NUMBER: S06-419

ALDH1L1 DEVELOPMENTAL IMMUNOMORPHOLOGY OF THE FOETAL HUMAN TELENCEPHALON: PRELIMINARY RESULTS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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ALDH1L1 is a specific astrocyte antigen of the mammalian adult brain with a strong pan-astrocyte expression. To better understanding astroglia developmental timeframes in human foetal brain ALDH1L1-immunomorphology analysis was undertaken: foetal human brain autopsies at the prefoetal and early foetal stages (8-20 gestational weeks (gw)) had been studied. ALDH1L1-immunoreactive astroblasts were demonastrated in the basal telencephalon (diagonal band and substantia innominata), ventral striatum (globus pallidus), paleocortical area at the 13-14gw. Only separate immunoreactive fibres were shown in the ventricular zone of ganglionic eminence (GE) at this stage. As gestational weeks increase ALDH1L1-immunoreactive cells were registered through the whole GE, neostriatal and amygdalar areas. Expansion into pallial structures began from the ventrical (vz) and subventrical (svz) zones at 18gw, including paleocortical ventricle. At the beginning of middle foetal period (21-23 gw) numerous ALDH1L1-astrocytes were demonstrated by the neocortical vz-svz and intermediate zone, cortical plate and subcortical zone also demonstrated ALDH1L1-immunoreactivity, but to a lesser extent. Reginal and temporal expansion of ALDH1L1-immunoreactivity in the human foetal telencephalon differs from the GFAP-immunoreactivity patterns (Kharlamova et al., 2018). ALDH1L1-immunoreactive neuroblasts and fibers appeared prior to GFAP-immunoreactivity pattern in the human foetal telencephalon, insular cortex and neocortex became ALDH1L1-immunoreactive also earlier. ALDH1L1-immunomorphological profile evidence for ALDH1L1-astroblasts expansion from the basal (ventral) subpallial structures and paleocortex, into the dorsal extent – dorsal (neo) striatum and cortical areas, whereas GFAP-immunoreactivity firstly appears in the dorsal archaecortical anlag and fimbria, lateral migratory curve and paleocortical marginal zone. This study is supported by the RSF grant # 22-25-00370

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BOARD NUMBER: S06-420

OMEGA-3-DERIVED ENDOCANNABINOIDS PREVENT PRO-INFLAMMATORY CYTOKINE-INDUCED DECREASES IN HUMAN HIPPOCAMPAL NEUROGENESIS AND ASTROGLIOGENESIS, AND INCREASE IN APOPTOSIS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Eicosapentaenoyl ethanolamide (EPEA) and Docosahexaenoyl ethanolamide (DHEA) are omega−3−derived endocannabinoids, synthesised from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively. This study investigated whether EPEA and DHEA treatment prevented the detrimental changes induced by treatment with interleukin−1−beta (IL1-beta) and interleukin−6 (IL6) on neurogenesis, astrogliogenesis, and apoptosis. Human hippocampal progenitors were treated with IL1−beta/IL6 alone or with EPEA/DHEA for 48hrs during differentiation. Doublecortin (DCX)+ neuroblasts, microtubule-associated protein−2 (MAP2)+ neurons, S100 calcium−binding protein−beta (S100−beta)+ astrocytes, and cleaved caspase−3 (CC3)+ apoptotic cells were detected using immunocytochemistry. Treatment with EPEA and DHEA prevented IL1−beta−induced decreases in DCX+ (IL1−beta +EPEA vs IL1−beta: +73.36%, p<0.0001; IL1−beta +DHEA vs IL1−beta: +70.78%, p<0.0001) and MAP2+ (IL1−beta +EPEA vs IL1−beta: +89.32%, p<0.0001; IL1−beta +DHEA vs IL1−beta: +81.38%, p<0.0001), and increase in CC3+ cells(IL1−beta +EPEA vs IL1−beta: −53.05%, p<0.0001; IL1−beta +DHEA vs IL1−beta: −54.67%, p<0.0001). EPEA and DHEA did not prevent IL1−beta−mediated decrease in S100−beta + cells (IL1−beta +EPEA vs IL1−beta: −3.6%, p>0.9999; IL1−beta +DHEA vs IL1−beta: −1.6%, p>0.9999). Treatment with EPEA and DHEA prevented IL6−induced decreases in DCX+ (IL6+EPEA vs IL6: +123.4%, p<0.0001; IL6+DHEA vs IL6: +124.18%, p<0.0001), and S100−beta+ cells(IL6+EPEA vs IL6: +113%, p<0.0001; IL6+DHEA vs IL6: +118.67%, p<0.0001). Only DHEA prevented IL6−induced reduction in MAP2+ (IL6+EPEA vs IL6: +4.8%, p>0.9999; IL6+DHEA vs IL6: +67.12%, p<0.0001), and only EPEA prevented IL6−induced increase in CC3+cells (IL6+EPEA vs IL6: −40.43%, p<0.0001; IL6+DHEA vs IL6: +2.85%, p>0.9999). Our findings demonstrate the anti−inflammatory and neuroprotective roles of EPEA and DHEA against IL1−beta and IL6, and further investigations will discern the underlying mechanisms.

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BOARD NUMBER: S06-421

ROLE OF DIAPH3 IN NEURAL STEM CELL BIOLOGY AND TUMOR DEVELOPMENT.

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Diaphanous(DIAPH)3 belongs to a family of dimeric multidomain proteins called formins that are master regulators of the cytoskeleton in basic cellular processes.In the brain,Diaph3 is specifically expressed in neural stem cells(NSC)and plays an important role during cytokinesis and karyokinesis.Conditional inactivation of Diaph3 in mouse cerebral cortex leads to massive loss of neural progenitors disrupting neurogenesis and causing apoptosis,aneuploiody and microcephaly.We co-inactivated Diaph3 and p53 in dorsal telencephalon by crossing Diaph3/p53 double-floxed mice with Emx1-Cre mice.We report that p53 loss partially restored the disrupted cortical layering and changed the fate of NSCs observed due to Diaph3 loss.We also show that the level of neuronal death observed in Diaph3 single-knockouts was partly rescued by p53 inactivation.However,cells that escape death undergo neoplastic transformation and double mutant mice develop brain tumors with reduced survival.These tumors are identified as Glioblastoma(GBM),the most frequent and aggressive malignant primary brain tumor with a median survival of 15 months in humans.Preliminary studies of animals bearing early olfactory bulb(OB)tumors show that proliferation is increased in the OB and the sub-ventricular zone(SVZ)of the tumoral hemisphere compared to the normal hemisphere.Ongoing studies aim at further analysing the SVZ and the rostral migratory stream(RMS)to detect changes in the fate of NSC and characterize early stages of gliomagenesis as well as generate new markers of GBM as it develops.So far,most of transcriptomic analyses were performed on advanced stages of GBM when the tumor causes detectable behavioral changes.We will combine our mouse models with ultra-high field MRI to compare early stages of GBM and better classify tumors reducing grade-related variability.

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BOARD NUMBER: S06-422

REPROGRAMMING MULLER GLIA TO REGENERATE GANGLION CELLS IN ADULT MOUSE RETINA WITH DEVELOPMENTAL TRANSCRIPTION FACTORS.

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Mammals are unable to regenerate retina. However, vertebrate species such as zebrafish can regenerate a functional retina after injury. In fish, this feat is accomplished by Muller glia (MG). MG in fish respond to injury by reprogramming into neurogenic progenitors that replace lost neurons and eventually restore vision. Our lab has made progress in engineering MG to regenerate neurons in the adult mouse retina in vivo. This was accomplished by designing MG to overexpress Ascl1, a proneural transcription factor required for zebrafish retinal regeneration. We demonstrated that overexpression of Ascl1 can stimulate MG to produce retinal neurons that functionally integrate into circuity. Thus, adding a single proneural gene is sufficient to initiate a “fish-like” regenerative program in mouse. However, in these initial studies, we found that with Ascl1 alone, MG primarily regenerated a single type of neuron called bipolar cells. We hypothesized that the transcription factor used to stimulate reprogramming may bias the neuronal subtype regenerated. In this study, we report a regeneration strategy combining Ascl1 with developmental transcription factors known to specify retinal ganglion cell fate, Pou4f2 and Iselt1. We find the addition of these two transcription factors improves the neurogenic efficacy and shifts the neuronal fate of reprogrammed MG. Using scRNA-seq, scATAC-seq, immunohistochemistry, and electrophysiology we demonstrate that Pou4f2, Islet1, and Ascl1 can stimulate MG to regenerate retinal ganglion-like neurons in vivo in the adult mouse retina. This work provides a proof-of-principle that developmental transcription factors can be redeployed to guide regeneration to specific subtypes of neurons.

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BOARD NUMBER: S06-423

APC/C-CDH1 REGULATES MYELINATION AFTER BIRTH

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Aims The E3 ubiquitin ligase APC/C-Cdh1 plays a key role in the developing brain, where it regulates the onset of neurogenesis during gestation. Whereas neurogenesis is complete at birth, oligodendrogenesis and myelination occur during postnatal brain development. Both processes are temporally regulated by protein turnover via ubiquitination and proteasomal degradation. Then, APC/C-Cdh1 might have a dual function in both pre and postnatal developmental stage. Here, we study the role of APC/C-Cdh1 activity on oligodendroglial lineage and myelination in the postnatal brain. **Methods** We generated Nestin-Cre Cdh1 cKO mice and oligodendrogenesis, myelination, and myelin sheaths were analyzed by electron microscopy, immunohistochemistry, and western blotting at different postnatal days (P0-P21). **Results** Cdh1 deletion resulted in brain morphology alterations, including microcephaly, hydrocephalus, and corpus callosum dysgenesis. Cdh1 loss induced hypomyelination, as revealed by the decreased in myelin (MBP) staining in P14 Cdh1 cKO mice. Myelin ultrastructure analysis at P21 revealed a higher percentage of unmyelinated axons and an increased g-ratio in Cdh1 cKO mice, in comparison to control. Moreover, Cdh1 loss triggered myelin sheath decompaction and disruption, compromising axon integrity and brain function. Finally, the lack of Cdh1 impaired the balance between immature and mature myelinating oligodendrocytes, suggesting a key role of the APC/C-Cdh1 complex in oligodendrogenesis process. **Conclusions** APC/C-Cdh1 activity regulates postnatal myelination, which highlights the impact of Cdh1 in the pathogenesis of neurodevelopmental myelin disorders. Funding by IBSAL; ISCIII (PI21/00727, RD21/0006/0005); FEDER; JCYL (CSI151P20; Escalera de Excelencia CLU-2017-03 Cofinanciado por P.O.FEDER de Castilla y León 14-20)

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BOARD NUMBER: S06-424

DECIPHERING THE MOLECULAR MECHANISM OF PLK1 CONTROL OF ADULT NEURAL STEM CELL ACTIVATION, SELF-RENEWAL AND DIFFERENTIATION

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Adult neural stem cells (NSCs) dwell in specialized microenvironments called niches, being the most studied the subependymal zone along the lateral ventricles, and the subgranular zone of the dentate gyrus. Despite being mainly quiescent, NSCs can be transiently activated by specific signals to proliferate, but they also can re-enter a state of inactivation to avoid exhaustion. The total pool of NSCs and progenitors at a given time point is a consequence of a set of cellular decisions that control quiescence vs. activation, expansion and terminal differentiation. Whether the instructions that guide the making of these decisions are determined by intrinsic properties, by the microenvironment of the niche or a combination of signals is the subject of intense investigation. Deciphering the molecular players that control NSC activation and mode of division is key to understanding adult neurogenesis, and to open a window to develop therapeutic strategies based on the endogenous reparative potential of the neural tissue, especially in the case of brain injury. Polo-like kinase 1 (Plk1) is a serine/threonine kinase with a key role in mitosis regulation. Plk1 has been linked to the regulation of embryonic NSC and progenitor cells mode of division, but to date, whether Plk1 plays a role in mammalian adult NSC division remains unstudied. Our results indicate that Plk1 is a novel intrinsic regulator of NSCs activation and provide evidence of a molecular link between Plk1 and a master regulator of neurogenesis, through which we propose Plk1 controls activation and neuronal differentiation of NSC.

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FOXG1 REGULATES NEURON-GLIA CELL FATE IN THE DEVELOPING NEOCORTEX

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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During neurodevelopment, neurogenesis and gliogenesis are molecularly controlled by transcription factor-mediated interactions. In the mouse cortex, a common pool of progenitors is temporally regulated such that neurogenesis precedes gliogenesis. However, the mechanisms regulating this process during development are not fully understood. Transcription factor Forkhead Box G1 (FOXG1) is known to repress cell cycle exit in the telencephalic progenitors, promote progenitor proliferation and suppress premature differentiation during neurogenesis. We show that Cre-mediated deletion of Foxg1 by *in utero electroporation*, during peak neurogenesis (upper layer neurogenesis), results in premature gliogenesis. In order to lineage trace the progenitors undergoing this cell-fate switch, we use the Mosaic Analysis with Double Marker (MADM) strategy. We identified candidate genes that are possible FOXG1 targets in progenitors, using transcriptomic and transcription factor ChIP-seq analyses from the literature. We find evidence that FOXG1 may act via FGF signaling to control astrogliogenesis, in the neocortical primordium. Our results extend the diverse roles of this transcription factor in neurodevelopment.

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BOARD NUMBER: S06-426

DECODING THE DEVELOPMENT OF THE HUMAN SPINAL CORD BY MULTI-OMICS

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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The human spinal cord has highly diverse cell types, which are generated during development, governed by a series of temporal and spatial events for proper spinal cord assembly and function. However, the molecular regulation of cell fate specification in the human developing spinal cord remains largely unknown. Here, by performing state-of-the-art multi-omics techniques, including single-cell RNA sequencing, spatial transcriptomics and in situ sequencing, we integrate the datasets and provide a comprehensive single-cell and spatial transcriptomic atlas of the developing human spinal cord of the first trimester, from postconceptional week 5 to 12. We observe that all major cell populations in human adult spinal cord have been specified within the first trimester with abundant cell states, and identify the key genes associated with cell fate choices and their spatial expression. Unexpectedly, we find that during early cell fate specification, many human neural progenitor cells have lost their self-renewal capacity. Further, we discover genetic regulation of cell fate specification is different between rodent and human. Finally, we demonstrate how to use our developmental map for molecular cues that direct sequential cellular differentiation and define cells and locations associated to both normal development and spinal cord diseases.

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BOARD NUMBER: S06-427

CROSSTALK BETWEEN PROTOCADHERIN 8 AND TRANSCRIPTION FACTOR DBX1 REGULATE CELL FATE IN THE DEVELOPING CEREBRAL CORTEX

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Normal development of the mammalian central nervous system requires correct tissue patterning, production of the appropriate cell types and establishment of functional neuronal circuits. Transcription factors (TFs) play essential roles in these processes, regulating the expression of target genes responsible for neuronal subtypes specific features. Cell adhesion molecules are key components of neuronal identities that control cell sorting, migration, neurite outgrowth/guidance and synaptogenesis. So far, TFs are known to control neuronal adhesion but not the opposite. Here, using acute gain- and loss-of-function experiments by *in utero* electroporation in the developing mouse telencephalon we demonstrate that ectopic expression of Dbx1, a homeodomain TF acting as a cell fate determinant, leads to increased expression of Protocadherin 8 (*Pcdh8*) and cell aggregation, together with the induction of neuronal fate markers Nurr1 and Pax6. These effects were modulated depending on the region and timing of electroporation. Furthermore, we found that *Pcdh8* expression is required for Dbx1-induced fate specification. Surprisingly, *Pcdh8* overexpression also proved sufficient to induce Dbx1 expression as well as a complete reorganisation of the apico-basal and dorso-ventral axes. Finally, we present evidence that these effects are mediated through regulation of the expression of Notch ligands and promotion of cell cycle exit. Altogether, our work therefore points to cell adhesion molecules as important, yet unexpected, players in the regulation of cell identity and, in particular, Pcdh8 through its crossregulation with the Dbx1 transcription factor.

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BOARD NUMBER: S06-429

EMBRYONIC NUTRITIONAL HYPERGLYCEMIA INHIBITS CELL PROLIFERATION IN THE ZEBRAFISH RETINA

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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Diabetic retinopathy is one of the leading causes of blindness in the world. While there is a major focus on the study of adult diabetic retinopathy, the effects of hyperglycemia during early retinal development are less well studied. Recent work in zebrafish revealed that nutritional hyperglycemia leads to decreased numbers of mature retinal cell types, which was related to an increase in apoptotic cell death and altered cell differentiation (Titialii-Torres and Morris, 2022). However, whether embryonic hyperglycemia impacts cell proliferation in developing zebrafish retinas is unknown. We exposed zebrafish embryos to 50 mM glucose from 10 hours postfertilization to 5 days postfertilization (dpf). We confirmed that hyperglycemia increases apoptotic cell death and decreases rod numbers in the retina at 5 dpf. Interestingly, the increased cell death was mainly observed in the peripheral retina, where most of the progenitor cells lie. To analyze the impact of hyperglycemia in cell proliferation, we quantified mitotic activity using pH3 immunolabeling. We observed a significant decrease in mitotic cells (mainly in the peripheral retina) at 5 dpf. The use of the proliferation marker PCNA also allowed us to detect a decrease in cell proliferation in the outer nuclear and ganglion cell layers of the central retina in hyperglycemic animals. Our results show that nutritional hyperglycemia inhibits cell proliferation in the developing retina, which could be an important contributor to the decrease in mature retinal cell types. Research funded by: Grant ED431C 2021/18 (Xunta de Galicia) to E. Candal. Grant PID2020-115121GB-I00 (MCIN/AEI/10.13039/501100011033) to A. Barreiro-Iglesias.

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BOARD NUMBER: S06-430

MIGRATORY NEURONS OF THE HUMAN CEPHALIC ECTODERM AND THE NEURAL CREST

POSTER SESSION 06 - SECTION: NEURAL STEM CELLS, PROLIFERATION & CELL-FATE

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We report here previously unknown early neuronal populations in humans found outside the brain which have not been described in any other mammalian species.We used a set of neuronal and proliferative markers to reveal the phenotypic characteristic and migratory potential of the earliest neurons of the cephalic ectoderm and the neural crest
We were able to reconstruct cells by high-resolution volume rendering of multichannel 3D data sets from a Zeiss confocal microscope.
Embryos from Carnegie stages (CS) 10-14 (29-33 days post-conception) were obtained from the Human Developmental Biology Resource UK. The preplacodal cephalic epithelium is continuous with the prosencephalic neuroepithelium prior to the fusion of the anterior neuropore. The nasal epiblastic thickening appears at CS11, lateral to the rostral neuropore. By CS12, the major divisions of the brain and the neurogenic placodes are already detectable on the basis of morphological features. The placodal field epithelium is, not mitotically active, with few H3- positive cells restricted to the apical surface of the pseudostratified ectoderm. Notably, we found bipolar TU-20-positive neurons migrating alone several regions of the early cephalic ectoderm, and in mesenchyma adjacent to the oral ectoderm and the neural crest in diencephalon. Some of the TU-20-positive neurons of ectodermal origin migrate into the periocular mesenchyma and extend processes into the eye cup, perhaps providing additional signals to the local stem cell niche. Others invade the cerebral wall of the rostral telencephalon. Thus, the early human cephalic epithelium contains previously unrecognized stem cells generating early migratory neurons that interact with retina and presumptive
cortex. Supported by Knowledge Foundation, The Zvi and Ofra Meitar Family Fund, and Arsenoy Foundation.

FENS Forum 2022
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BOARD NUMBER: S06-431

NEOCORTICAL INHIBITORY SYNAPTIC NETWORKS REFLECT PYRAMIDAL NEURON LINEAGE

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The mammalian neocortex comprises a diverse range of excitatory and inhibitory neuron types. An important question in neuroscience is how the connectivity logic within and between these different cell types is able to generate the processing capabilities of cortical circuits. A growing body of research has investigated how excitatory neurons derived from defined progenitor pools during embryonic development exhibit specific synaptic connectivity patterns in the adult cortex [1-4]. Here we examine excitatory and inhibitory synaptic inputs to cortical L2/3 pyramidal neurons derived from apical intermediate progenitors (aIP-derived) and compare these with neighbouring L2/3 pyramidal neurons derived from other progenitors (OP-derived). By combining in utero labelling methods in mice with paired patch clamp electrophysiological recordings in postnatal brain slices, our experiments reveal that aIP-derived neurons share fewer of their inhibitory synaptic inputs with neighbouring neurons, when compared to either OP-derived or unlabelled neurons. Additionally, the amplitude of shared inhibitory input to aIP-derived neurons was lower than that observed in OP-derived and unlabelled neurons. In contrast, aIP-derived neurons shared similar levels of excitatory synaptic input with other neighbouring neurons. These data suggest that the way in which a cortical pyramidal neuron couples to its local inhibitory synaptic network is reflective of the neuron's progenitor of origin.
References: [1] Ellender et al, 2019: https://doi.org/10.1038/s41467-019-13206-1 [2] Cadwell et al, 2020: https://doi.org/10.7554/eLife.52951 [3] Ohtsuki et al, 2012: https://doi.org/10.1016/j.neuron.2012.05.023 [4] Tyler et al, 2015: https://doi.org/10.1523/JNEUROSCI.0335-15.2015

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BOARD NUMBER: S06-432

SPONTANEOUS ACTIVITY IN THE WHISKER-INNERVATING REGION OF NEONATAL MOUSE TRIGEMINAL GANGLION

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Correlated spontaneous activity occurs in certain systems of the developing mammalian brain during the early postnatal period and is thought to be crucial for the establishment of precise and mature neural circuits following Hebbian principles of plasticity. In the mouse somatosensory system, the barrel cortex shows patchwork-type spontaneous activity during the first postnatal week (Mizuno et al., 2018, Nakazawa et al., 2020). Previous studies suggested that the source of this activity is in the periphery but downstream of the whisker pad (Mizuno et al., 2018), based on which we hypothesized that the trigeminal ganglion (TG) is the source of spontaneous activity in the neonatal barrel cortex. To examine whether the neonatal TG shows spontaneous activity or not, we established a system for imaging activity in the TG *ex vivo*. We identified the whisker-innervating region in the TG by application of DiI to the whisker pads. Using a transgenic mouse line expressing a genetically encoded calcium indicator (GCaMP6s) in the TG neurons and the *ex vivo* calcium imaging system, we found that the neurons in the whisker-innervating region of P4-P6 mouse TG fire spontaneously. This activity originated within the TG itself and was blocked by the chelation of extracellular calcium. Spontaneous activity was also detected in P0-P1 TG but was largely diminished in the adult TG. The spontaneous activity in the TG during the first postnatal week may contribute to thalamocortical circuit refinement as the source of patchwork-type spontaneous activity in the neonatal mouse barrel cortex.

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BOARD NUMBER: S06-433

CHARACTERIZATION OF HIPPOCAMPAL NEURONAL ACTIVITY DURING THE FIRST POSTNATAL WEEK IN-VIVO.

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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During the first post-natal week in-vivo, the immature hippocampus is characterized by spontaneous network activity (SNA), often preceded by myoclonic twitches. While it is believed that these SNAs play an important role in the establishment of neural networks, the underlying mechanisms are poorly understood. We performed electrophysiological recordings of the dorsal CA1 region of the hippocampus in unanesthetized mice from post natal day (P) 4 to 8. At P4, while 48% of hippocampal pyramidal cell layer neurons fired strongly at the SNA onset, 23% fired moderately at the offset and 29% do not change their activity. In contrast, below the pyramidal cell layer (str. radiatum+ str moleculare) 81% of neurons are of the onset type, 5% offset and 14% unresponsive. With age, the proportion of onset neurons decreased in all hippocampal layers, leaving place, at P8 to a majority of unresponsive neurons. We then used optogenetcis to identify and characterise the extracellular activity GABAergic neurons. Preliminary data suggest that GABAergic cells located above and within the pyramidal cell layer are only moderately active during SNAs. The investigation of GABAergic cells below the pyramidal layer is still in progress. The fact that neurons located below the pyramidal cell layer are amongst the most strongly activated during SNAs suggests that they play an important role in the establishment of hippocampal networks. We are now characterising their cellular subtype using optogenetics.

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SPONTANEOUS ACTIVITY OF STRIOSOMAL PROJECTION NEURONS SUPPORTS MATURATION OF STRIATAL INPUTS TO SUBSTANTIA NIGRA DOPAMINERGIC NEURONS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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During neurodevelopment, a critical time window defines the striatum neuronal network maturation, and any impairments in this critical period may lead to neurodevelopmental movement disorders. Due to heterogeneity of striatal spiny projection neurons (SPNs), precise identification of the critical time window for striatum is complicated. We characterized the spatial and temporal profile of neonatal spontaneous activity in the striatum. Using neonatal Drd1a-Cre/tdTomato and Adora2a-Cre/tdTomato mice, we demonstrated a clustered organization of fluorescent direct-pathway SPNs (dSPNs) and indirect-pathway SPNs (iSPNs), which correspond to striosomes. Using *in vitro* electrophysiological recordings, we evaluated domain-specific intrinsic excitability and passive membrane properties of SPNs. Our data show that neuronal activity in developing striatum depends on age and domain. The age-dependent spontaneous activity was more evident in striosomes than in matrix SPNs. In both compartments, iSPNs retained this activity longer than dSPNs. Next, we investigated the role of the SPNs' spontaneous activity in the maturation of the basal ganglia network. We injected at P0 a viral vector to induce expression of the inhibitory DREADDs that specifically respond to clozapine-N-oxide (CNO) in striosomal dorsal striatum SPNs of Oprm1-Cre mice and suppress neuronal activity in neonatal mice. The results show that suppression of spontaneous activity of striosomal SPNs during prospective critical time window causes a significant and persistent decrease in the frequency of the miniature inhibitory postsynaptic currents (mIPSCs) in substantia nigra pars compacta dopaminergic neurons of CNO injected adult mice. These results elucidate the mechanisms possibly underlying neurodevelopmental movement disorders linked to striatal functions.

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A DISTINCTION OF TEMPORAL ODOR PROCESSING IN RATS DURING THE FIRST AND SECOND POSTNATAL WEEK. AN EXPERIMENTAL AND COMPUTATIONAL APPROACH

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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During the first postnatal week of rodents, behaviors such as nipple attachment, orientation and approach to the nest, and mother attachment learning are significantly influenced by olfaction. Our previous study showed that the end of the sensitive period for mother attachment learning in rats at postnatal day (P) 10 could occur due to the maturation of the electric properties of anterior piriform cortex (aPC) pyramidal (pyr) cells. Specifically, the properties of P5 aPC pyr cells and not those of P14 allow for greater cell recruitment during odor conditioning, and GABAergic synaptic transmission profile at P5 contributes to this. Here, we explore how intrinsic maturational properties of pyr cells and GABAergic synaptic transmission in the aPC might contribute to odor processing at P5 and P14. Using a previous olfactory bulb- aPC computational circuit model for P5 and P14 rat pups, updating the latter with experimental EGABA, we simulated a 3s long odor processing. We found that pyr cells of both ages started to fire during the inhalation phase, with P5 pyr cells showing faster recruitment and rising firing rate, twice higher than P14, followed by a quick suppression during the first 50 ms. P14 pyr cells showed slow recruitment and low-rate firing, which was constant throughout the next 50 ms period, in which their firing rate became twice greater than P5 pyr cells. These results suggest that the intrinsic electrophysiological and synaptic maturation properties of aPC provide a neuronal substrate for distinct odor processing at P5 and P14

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DIFFERENTIAL SYNAPTIC INHIBITION PATTERNS ONTO LAYER 5 PYRAMIDAL CELL SUBPOPULATIONS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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In the mammalian neocortex, excitatory pyramidal cells (PC) are assembled in distinct subnetworks, receiving inputs from various sources and projecting outputs to different brain areas. Cortical layer 5 (L5) represents a textbook example of this intricate organisation, where distinct PC subpopulations coexist within the same environment. GABAergic inhibitory interneurons (INs), by providing local inhibition, ensure a proper computation of these multiple information processing streams. While INs were long thought to provide unbiased inhibition onto nearby PC, recent work gathered strong evidence of cell-specific inhibitory connectivity. However, how and when GABAergic INs precisely target and regulate such intermingled excitatory subpopulations remains unclear. Using a combination of viral-based retrograde tracing, transcriptomics and synaptic analysis, we investigated the molecular rules underlying PC subtype-specific inhibition during cortical development. First, we characterised the dynamics of INs synaptic connectivity onto L5 PC subpopulations. Our data demonstrate that neighbouring L5 cortico-cortical and cortico-pontine PC, while residing in the same layer, receive specific combinations of inhibitory inputs. These differential synaptic inhibition patterns emerge early in development and are likely instructed by molecular programs unique to these PC subtypes. Next, we explored how genetic manipulations in vivo could reshape these specific synaptic connectivity motifs onto L5 PC subpopulations. Altogether, our work suggests that PC identity is a crucial determinant of inhibitory circuits assembly.

Pubmed:
34462417: Espana A, Seth H, Jézéquel J, Huang T, Bouchet D, Lepleux M, Gréa H, Bechter K, Schneider M, Hanse E, Groc L
Alteration of NMDA receptor trafficking as a cellular hallmark of psychosis.
A dysfunction of the glutamatergic transmission, especially of the NMDA receptor (NMDAR), constitutes one of the main biological substrate of psychotic disorders, such as schizophrenia. The NMDAR signaling hypofunction, through genetic and/or environmental insults, would cause a neurodevelopmental myriad of molecular, cellular, and network alterations that persist throughout life. Yet, the mechanisms underpinning NMDAR dysfunctions remain elusive. Here, we compared the membrane trafficking of NMDAR in three gold-standard models of schizophrenia, i.e., patient's cerebrospinal fluids, genetic manipulations of susceptibility genes, and prenatal developmental alterations. Using a combination of single nanoparticle tracking, electrophysiological, biochemical, and behavioral approaches in rodents, we identified that the NMDAR trafficking in hippocampal neurons was consistently altered in all these different models. Artificial manipulations of the NMDAR surface dynamics with competing ligands or antibody-induced receptor cross-link in the developing rat brain were sufficient to regulate the adult acoustic startle reflex and compensate for an early pathological challenge. Collectively, we show that the NMDAR trafficking is markedly altered in all clinically relevant models of psychosis, opening new avenues of therapeutical strategies.
Transl Psychiatry, 2021; 11
33536130: Pollak TA, Vincent A, Iyegbe C, Coutinho E, Jacobson L, Rujescu D, Stone J, Jezequel J, Rogemond V, Jamain S, Groc L, David A, Egerton A, Kahn RS, Honnorat J, Dazzan P, Leboyer M, McGuire P
Relationship Between Serum NMDA Receptor Antibodies and Response to Antipsychotic Treatment in First-Episode Psychosis.
When psychosis develops in NMDA receptor (NMDAR) antibody encephalitis, it usually has an acute or subacute onset, and antipsychotic treatment may be ineffective and associated with adverse effects. Serum NMDAR antibodies have been reported in a minority of patients with first-episode psychosis (FEP), but their role in psychosis onset and response to antipsychotic treatment is unclear.

Biol Psychiatry, 2021; 90
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Distinct molecular programs regulate synapse specificity in cortical inhibitory circuits.
How neuronal connections are established and organized into functional networks determines brain function. In the mammalian cerebral cortex, different classes of GABAergic interneurons exhibit specific connectivity patterns that underlie their ability to shape temporal dynamics and information processing. Much progress has been made toward parsing interneuron diversity, yet the molecular mechanisms by which interneuron-specific connectivity motifs emerge remain unclear. In this study, we investigated transcriptional dynamics in different classes of interneurons during the formation of cortical inhibitory circuits in mouse. We found that whether interneurons form synapses on the dendrites, soma, or axon initial segment of pyramidal cells is determined by synaptic molecules that are expressed in a subtype-specific manner. Thus, cell-specific molecular programs that unfold during early postnatal development underlie the connectivity patterns of cortical interneurons.
Science, 2019; 363
29684464: Jézéquel J, Dupuis JP, Maingret F, Groc L
Tracking single membrane targets of human autoantibodies using single nanoparticle imaging.
Over the past decade, an increasing number of neurological and neuropsychiatric diseases have been associated with the expression of autoantibodies directed against neuronal targets, including neurotransmitter receptors. Although cell-based assays are routinely used in clinics to detect the presence of immunoglobulins, such tests often provide heterogeneous outcomes due to their limited sensitivity, especially at low titers. Thus, there is an urging need for new methods allowing the detection of autoantibodies in seropositive patients that cannot always be clinically distinguished from seronegative ones.
J Neurosci Methods, 2018; 304
29606071: Jézéquel J, Lepleux M, Kahn RS, Honnorat J, Leboyer M, Groc L
Molecular Pathogenicity of Anti-NMDA Receptor Autoantibody From Patients With First-Episode Psychosis.
Am J Psychiatry, 2018; 175
29176681: Jézéquel J, Johansson EM, Dupuis JP, Rogemond V, Gréa H, Kellermayer B, Hamdani N, Le Guen E, Rabu C, Lepleux M, Spatola M, Mathias E, Bouchet D, Ramsey AJ, Yolken RH, Tamouza R, Dalmau J, Honnorat J, Leboyer M, Groc L
Dynamic disorganization of synaptic NMDA receptors triggered by autoantibodies from psychotic patients.
The identification of circulating autoantibodies against neuronal receptors in neuropsychiatric disorders has fostered new conceptual and clinical frameworks. However, detection reliability, putative presence in different diseases and in health have raised questions about potential pathogenic mechanism mediated by autoantibodies. Using a combination of single molecule-based imaging approaches, we here ascertain the presence of circulating autoantibodies against glutamate NMDA receptor (NMDAR-Ab) in about 20% of psychotic patients diagnosed with schizophrenia and very few healthy subjects. NMDAR-Ab from patients and healthy subjects do not compete for binding on native receptor. Strikingly, NMDAR-Ab from patients, but not from healthy subjects, specifically alter the surface dynamics and nanoscale organization of synaptic NMDAR and its anchoring partner the EphrinB2 receptor in heterologous cells, cultured neurons and in mouse brain. Functionally, only patients' NMDAR-Ab prevent long-term potentiation at glutamatergic synapses, while leaving NMDAR-mediated calcium influx intact. We unveil that NMDAR-Ab from psychotic patients alter NMDAR synaptic transmission, supporting a pathogenically relevant role.
Nat Commun, 2017; 8
28780967: Jézéquel J, Rogemond V, Pollak T, Lepleux M, Jacobson L, Gréa H, Iyegbe C, Kahn R, McGuire P, Vincent A, Honnorat J, Leboyer M, Groc L
Cell- and Single Molecule-Based Methods to Detect Anti-N-Methyl-D-Aspartate Receptor Autoantibodies in Patients With First-Episode Psychosis From the OPTiMiSE Project.
Circulating autoantibodies against glutamatergic N-methyl-D-aspartate receptor (NMDAR) have been reported in a proportion of patients with psychotic disorders, raising hopes for more appropriate treatment for these antibody-positive patients. However, the prevalence of circulating autoantibodies against glutamatergic NMDAR in psychotic disorders remains controversial, with detection prevalence rates and immunoglobulin classes varying considerably between studies, perhaps because of different detection methods. Here, we compared the results of serum assays for a large cohort of patients with first-episode psychosis using classical cell-based assays in three labs and a single molecule-based imaging method. Most assays and single molecule imaging in live hippocampal neurons revealed the presence of circulating autoantibodies against glutamatergic NMDAR in approximately 5% of patients with first-episode psychosis. However, some heterogeneity between cell-based assays was clearly observed, highlighting the urgent need for new sensitive methods to detect the presence of low-titer autoantibodies against glutamatergic NMDAR in seropositive patients who cannot be clinically identified from

seronegative ones.
Biol Psychiatry, 2017; 82
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Tissue-type plasminogen activator controls neuronal death by raising surface dynamics of extrasynaptic NMDA receptors.
N-methyl-d-aspartate receptors (NMDARs) are ion channels whose synaptic versus extrasynaptic localization critically influences their functions. This distribution of NMDARs is highly dependent on their lateral diffusion at the cell membrane. Each obligatory subunit of NMDARs (GluN1 and GluN2) contains two extracellular clamshell-like domains with an agonist-binding domain and a distal N-terminal domain (NTD). To date, the roles and dynamics of the NTD of the GluN1 subunit in NMDAR allosteric signaling remain poorly understood. Using single nanoparticle tracking in mouse neurons, we demonstrate that the extracellular neuronal protease tissue-type plasminogen activator (tPA), well known to have a role in the synaptic plasticity and neuronal survival, leads to a selective increase of the surface dynamics and subsequent diffusion of extrasynaptic NMDARs. This process explains the previously reported ability of tPA to promote NMDAR-mediated calcium influx. In parallel, we developed a monoclonal antibody capable of specifically blocking the interaction of tPA with the NTD of the GluN1 subunit of NMDAR. Using this original approach, we demonstrate that the tPA binds the NTD of the GluN1 subunit at a lysine in position 178. Accordingly, when applied to mouse neurons, our selected antibody (named Glunomab) leads to a selective reduction of the tPA-mediated surface dynamics of extrasynaptic NMDARs, subsequent signaling and neurotoxicity, both in vitro and in vivo. Altogether, we demonstrate that the tPA is a ligand of the NTD of the obligatory GluN1 subunit of NMDAR acting as a modulator of their dynamic distribution at the neuronal surface and subsequent signaling.
Cell Death Dis, 2016; 7
25225407: Sarabdjitsingh RA, Jezequel J, Pasricha N, Mikasova L, Kerkhofs A, Karst H, Groc L, Joëls M
Ultradian corticosterone pulses balance glutamatergic transmission and synaptic plasticity.
The rodent adrenal hormone corticosterone (CORT) reaches the brain in hourly ultradian pulses, with a steep rise in amplitude before awakening. The impact of a single CORT pulse on glutamatergic transmission is well documented, but it remains poorly understood how consecutive pulses impact on glutamate receptor trafficking and synaptic plasticity. By using high-resolution imaging and electrophysiological approaches, we report that a single pulse of CORT to hippocampal networks causes synaptic enrichment of glutamate receptors and increased responses to spontaneously released glutamatergic vesicles, collectively abrogating the ability to subsequently induce synaptic long-term potentiation. Strikingly, a second pulse of CORT one hour after the first--mimicking ultradian pulses--completely normalizes all aspects of glutamate transmission investigated, restoring the plastic range of the synapse. The effect of the second pulse is precisely timed and depends on a nongenomic glucocorticoid receptor-dependent pathway. This normalizing effect through a sequence of CORT pulses--as seen around awakening--may ensure that hippocampal glutamatergic synapses remain fully responsive and able to encode new stress-related information when daily activities start.
Proc Natl Acad Sci U S A, 2014; 111
29807730: Jézéquel J, Johansson EM, Leboyer M, Groc L
Pathogenicity of Antibodies against NMDA Receptor: Molecular Insights into Autoimmune Psychosis.
Recent years have seen a flourishing literature on detection of circulating autoantibodies against neurotransmitter receptors in patients with neuropsychiatric disorders. These studies have generated hope for a better understanding of the underlying molecular dysfunctions and for appropriate therapeutic strategies. However, the detection of these autoantibodies in healthy subjects, and the lack of mechanistic insights have fostered debate about the pathogenic role of such autoantibodies. Here, we specifically discuss the biological evidence linking autoantibodies directed against the glutamatergic N-methyl-d-aspartate (NMDA) receptor (NMDAR-Abs) and psychosis, emphasising recent single-molecule imaging investigations that unveiled the impaired surface trafficking of NMDAR in the presence of NMDAR-Abs from psychotic patients. Although still in its infancy, the hypothesis that NMDAR-Abs from patients with psychosis play a pathogenic role is thus gaining support, opening avenues of fundamental and translational investigations.
Trends Neurosci, 2018; 41

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BOARD NUMBER: S06-437

EXAMINING DENDRITIC PLASTICITY OF THE BARREL CORTEX LAYER 4 NEURONS VIA IN VIVO HIGH SPATIOTEMPORAL-RESOLUTION IMAGING

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The specific arborization of dendrites determines the inputs the neuron receives, and the accurate dendritic patterns are formed and refined during development in an activity-dependent manner. It is crucial to understand how dendrites elaborate and refine their morphologies during development. Our lab has established two-photon time-lapse in vivo imaging approaches in the neonatal mouse brain to investigate the dynamics of dendritic refinement of cortical neurons (Mizuno et al., Neuron 2014; Nakazawa et al., Nature Commun. 2018). However, the spatiotemporal resolutions of these imaging protocols were not sufficiently high. We here increased the temporal resolution of the imaging system from 8-hour interval to 1-hour interval. We also improved the spatial resolution of dendrite morphology imaging by using a membrane-bound RFP (mRFP) instead of a regular RFP. Neurons labeled with the mRFP visualized the more precise dendritic morphologies comparing to the regular RFP. In-utero electroporation-based Supernova (Mizuno et al., 2014; Luo et al., Sci. Rep. 2016) was used to sparsely label barrel cortex L4 neurons with the mRFP. TCA-GFP mice (Mizuno et al., 2014) were used to enable *in vivo* visualization of the barrel map. Then, we imaged dendrites of the same neurons for 8 hours at postnatal day 4, which is in the middle of the dendritic refinement process. With improved in vivo imaging resolution, we were able to detect precise changes of individual dendrites including those of short-lived dendritic trees and branches. Our imaging also identified several transient refinement features of L4 neuron dendrites. Our recent finding will be discussed.

Pubmed:
29625112: Shen Y, Wang L, Hirose S, Zhou Z, Liu Q
The transcriptional factor Apt regulates neuroblast differentiation through activating CycE expression.
In Drosophila, the thoracic neuroblast 6-4 (NB6-4T) divides asymmetrically into a glial precursor and a neuronal precursor, while the abdominal neuroblast 6-4 (NB6-4A) divides symmetrically to produce two glial cells. The underlying mechanism by which NB6-4T and NB6-4A undergo distinct differentiation is still elusive. Here, we find that the transcription factor Apontic (Apt) exclusively expresses in NB6-4T cells and is involved in regulating NB6-4T differentiation. Loss of Apt results in neuronal precursor loss. Epistasis analysis shows that Apt controls NB6-4T differentiation through activating CycE expression. On the other hand, Gcm suppresses Apt expression in the NB6-4A cell, thus inhibiting CycE expression. Taken together, our findings reveal a Gcm-Apt-CycE axis that regulates neuroblast and glia cell differentiation.
Biochem Biophys Res Commun, 2018; 499

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BOARD NUMBER: S06-438

UNRAVELLING THE NATURE OF NEURAL NETWORKS WITH DIFFERENT RATIOS OF INHIBITORY AND EXCITATORY NEURONS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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INTRODUCTION: To make a working nervous system, only two forces are necessary: excitation and inhibition. The impact of different ratios of GABAergic neurons on the functioning of local cortical networks is so far not completely known. Indeed, through the use of mouse embryonic stem cells (mESCs) it is possible to manipulate neurogenesis and develop neural cultures with glutamatergic and GABAergic neurons. **AIM**: In our work, we aimed to design cortical cell cultures with different ratios of inhibitory and excitatory neurons, with the purpose of observing neuronal differentiation, synaptic formation, and neural activity by the use of intracellular Ca^{2+} live imaging. **METHODS**: The achievement of this goal was validated by qRT-PCR, immunocytodetection and calcium imaging experiments. **RESULTS**: With the use of Cyclopamine and SAG (Smoothened Agonist), we established new protocols to create distinct cultures of excitatory and inhibitory neurons, finding 94% of vGlut2 positive neurons and 80% of GAD65 positive neurons, respectively. We then designed cortical cell populations with different E/I (excitation/inhibition) ratios, developing a physiological (80/20) and an experimental condition (50/50). We performed calcium live imaging experiments with the Fluo3 calcium indicator and with a Lentiviral Vector that expresses GcaMP6s, a genetically encoded calcium indicator, under the control of a synaptic-specific promoter. Our preliminary results suggest that cortical networks with different GABAergic:glutamatergic ratios develop connectivity and spontaneous activity that might be influenced by the different ratio. **CONCLUSIONS**: The setup of an *in vitro* method could be useful to functionally study cortical inhibitory networks and activity and plasticity of cortical networks.

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SUBTHRESHOLD ACTIVITY MAY REGULATE THE MIGRATION OF CORTICAL 5HT3AR-EXPRESSING INTERNEURONS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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During early cortical development, ongoing competition between innate and experience-derived network activity is crucial for the proper functional maturation of the somatosensory system. A prominent feature of this period is the migration of gamma-aminobutyric acid-expressing (GABAergic) interneurons (INs) into the cortical plate. The final steps of IN migration coincide with the rise of K+-Cl- co-transporter (KCC2) expression. Here we studied how migration of 5HT3aR-positive INs in organotypic brain cultures is affected by the manipulation of activity by a sodium channel blocker TTX or KCC2 inhibitor VU0463271 (“VU”). Moreover, we aimed to increase the activity in slices with a lowered concentration of Mg2+ and increased K+ concentration (“MgK”). The impact of the different conditions on network activity was monitored by calcium imaging of Emx1-GCaMP6f mice. Surprisingly, neither MgK nor TTX caused significant alterations in the network activity. Interestingly, in addition to inducing significant reduction of the number of active ROIs, the number of ROIs participating in network events was significantly higher in VU than in control indicating that VU causes synchronization of the subthreshold network activity. Next, we investigated whether the migration of 5HT3aR-expressing INs is affected by this activity. Analysis of saltatory movement indicated reduced motility of 5HT3aR+ INs in organotypic cultures treated with the VU. Migration of INs in slices with TTX or MgK did not differ from the control. These experimental evidence suggests that migration of 5HT3aR+ INs might depend on KCC2 functionality and specific type of network activity. Further studies are required to reveal cell-type specific details.

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BOARD NUMBER: S06-440

VOLTAGE-GATED CALCIUM CHANNELS AUXILIARY SUBUNIT α2δ3-MEDIATED IMPACT ON THE HIPPOCAMPAL NEURONAL NETWORKS.

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Voltage-gated calcium channels (VGGC) are crucial for proper neuronal functioning, and therefore have an implicit impact on the network formation of neurons. The VGGC auxiliary α2δ-subunits have been found to regulate synaptic functioning in a variety of ways, notably synaptic organization, and VGGC recruitment. In this project, we aim to evaluate how α2δ3 expression levels affect neuronal networks in their development. We grow our hippocampal dissociated neuronal culture on microelectrode arrays, allowing for non-invasive extracellular recordings over several weeks. We introduce either overexpression or knock-down of α2δ3 via lentiviral infection and perform extracellular recordings every seven days for a period of 30 days. Thus, observing the entire process of network development. With our analysis pipeline, we extract the functional network structure by statistical dependencies between successive action potentials. Subsequently, different graph theoretical measures are used to compare the network to a matched control (e.g., clustering, connectivity). Preliminary results suggest an aberrant connectivity pattern in both conditions (up- and downregulation of α2δ3) in comparison to control conditions. Specifically, α2δ3 seems to influence GABAergic signaling and interneurons stronger than excitatory neurons. Overall, the knock-down of α2δ3 strengthened short-range over long-range connectivity and induced an excitatory to inhibitory imbalance, leading to a connectivity pattern typical of neuropsychiatric disorders, and particularly prominent in autism spectrum disorder.

Pubmed:
34107849: Heck J, Palmeira Do Amaral AC, Weißbach S, El Khallouqi A, Bikbaev A, Heine M
More than a pore: How voltage-gated calcium channels act on different levels of neuronal communication regulation.
Voltage-gated calcium channels (VGCCs) represent key regulators of the calcium influx through the plasma membrane of excitable cells, like neurons. Activated by the depolarization of the membrane, the opening of VGCCs induces very transient and local changes in the intracellular calcium concentration, known as calcium nanodomains, that in turn trigger calcium-dependent signaling cascades and the release of chemical neurotransmitters. Based on their central importance as concierges of excitation-secretion coupling and therefore neuronal communication, VGCCs have been studied in multiple aspects of neuronal function and malfunction. However, studies on molecular interaction partners and recent progress in omics technologies have extended the actual concept of these molecules. With this review, we want to illustrate some new perspectives of VGCCs reaching beyond their function as calcium-permeable pores in the plasma membrane. Therefore, we will discuss the relevance of VGCCs as voltage sensors in functional complexes with ryanodine receptors, channel-independent actions of auxiliary VGCC subunits, and provide an insight into how VGCCs even directly participate in gene regulation. Furthermore, we will illustrate how structural changes in the intracellular C-terminus of VGCCs generated by alternative splicing events might not only affect the biophysical channel characteristics but rather determine their molecular environment and downstream signaling pathways.
Channels (Austin), 2021; 15
33468057: Weißbach S, Sys S, Hewel C, Todorov H, Schweiger S, Winter J, Pfenninger M, Torkamani A, Evans D, Burger J, Everschor-Sitte K, May-Simera HL, Gerber S
Reliability of genomic variants across different next-generation sequencing platforms and bioinformatic processing pipelines.
Next Generation Sequencing (NGS) is the fundament of various studies, providing insights into questions from biology and medicine. Nevertheless, integrating data from different experimental backgrounds can introduce strong biases. In order to methodically investigate the magnitude of systematic errors in single nucleotide variant calls, we performed a cross-sectional observational study on a genomic cohort of 99 subjects each sequenced via (i) Illumina HiSeq X, (ii) Illumina HiSeq, and (iii) Complete Genomics and processed with the respective bioinformatic pipeline. We also repeated variant calling for the

Illumina cohorts with GATK, which allowed us to investigate the effect of the bioinformatics analysis strategy separately from the sequencing platform's impact.
BMC Genomics, 2021; 22

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-441

STRUCTURAL AND FUNCTIONAL ABNORMALITIES IN THE HIPPOCAMPUS CAUSED BY LIS1 INACTIVATION IN PARVALBUMIN EXPRESSING NEURONS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Mutation of the gene *LIS1* causes lissencephaly, a severe developmental disorder with a deep disorganization of the cerebral cortex. We have studied the consequences of the inactivation of the mouse gene *Lis1* selectively in the neurons expressing parvalbumin (PV). To do that we have breed Lis1KO-flox and PV-cre mouse lines to produce animals (Lis1KO-PV$^+$) in which the *Lis1* gene is inactivated selectively in those neurons expressing PV. In homozygous Lis1KO-PV$^+$ postnatal animals (P14-P18) Nissl staining and NeuN immunohistochemistry showed a disorganized hippocampal CA3 area and the presence of a thin layer of ectopic somas of pyramidal neurons placed in the stratum radiatum / lacunosum of CA3; these ectopic neurons did not express PV. Calbindin staining showed that axons originated in the dentate gyrus reached the area of ectopic neurons. *c-Fos* staining suggested the presence of functional abnormalities since we detected a larger number of *c-Fos* positive neurons in the abnormal CA3 area of mutant animals in comparison with littermate wild type animals (122.0±17.2 and 61.2±11.0 respectively; n=3 for genotype; p=0.007 Student's t test); in contrast, the total number of neurons positive to NeuN in CA3 was similar in mutant and WT animals (335.3±103.7 and 389.4±54.7 respectively; n=3 for genotype; p=0.469 Student's t test). These results show the presence in Lis1KO-PV$^+$ animals of structural and functional abnormalities affecting the hippocampal pyramidal neurons, and suggest the existence of non-cell-autonomous mechanisms that could explain abnormalities in pyramidal neurons after *Lis1* inactivation in PV positive neurons. Funding: MCIN grant PID2020-118171RB-I00 and GVA-Prometeo Grant 2018/041.

Pubmed:
23805080: Martinez S, Andreu A, Mecklenburg N, Echevarria D
Cellular and molecular basis of cerebellar development.
Historically, the molecular and cellular mechanisms of cerebellar development were investigated through structural descriptions and studying spontaneous mutations in animal models and humans. Advances in experimental embryology, genetic engineering, and neuroimaging techniques render today the possibility to approach the analysis of molecular mechanisms underlying histogenesis and morphogenesis of the cerebellum by experimental designs. Several genes and molecules were identified to be involved in the cerebellar plate regionalization, specification, and differentiation of cerebellar neurons, as well as the establishment of cellular migratory routes and the subsequent neuronal connectivity. Indeed, pattern formation of the cerebellum requires the adequate orchestration of both key morphogenetic signals, arising from distinct brain regions, and local expression of specific transcription factors. Thus, the present review wants to revisit and discuss these morphogenetic and molecular mechanisms taking place during cerebellar development in order to understand causal processes regulating cerebellar cytoarchitecture, its highly topographically ordered circuitry and its role in brain function.
Front Neuroanat, 2013; 7
27273333: Bosone C, Andreu A, Echevarria D
GAP junctional communication in brain secondary organizers.
Gap junctions (GJs) are integral membrane proteins that enable the direct cytoplasmic exchange of ions and low molecular weight metabolites between adjacent cells. They are formed by the apposition of two connexons belonging to adjacent cells. Each connexon is formed by six proteins, named connexins (Cxs). Current evidence suggests that gap junctions play an important part in ensuring normal embryo development. Mutations in connexin genes have been linked to a variety of human diseases, although the precise role and the cell biological mechanisms of their action remain almost unknown. Among the big family of Cxs, several are expressed in nervous tissue but just a few are expressed in the anterior neural tube of vertebrates.

Many efforts have been made to elucidate the molecular bases of Cxs cell biology and how they influence the morphogenetic signal activity produced by brain signaling centers. These centers, orchestrated by transcription factors and morphogenes determine the axial patterning of the mammalian brain during its specification and regionalization. The present review revisits the findings of GJ composed by Cx43 and Cx36 in neural tube patterning and discuss Cx43 putative enrollment in the control of Fgf8 signal activity coming from the well known secondary organizer, the isthmic organizer.
Dev Growth Differ, 2016; 58
33383187: Andreu-Cervera A, Catala M, Schneider-Maunoury S
Cilia, ciliopathies and hedgehog-related forebrain developmental disorders.
Development of the forebrain critically depends on the Sonic Hedgehog (Shh) signaling pathway, as illustrated in humans by the frequent perturbation of this pathway in holoprosencephaly, a condition defined as a defect in the formation of midline structures of the forebrain and face. The Shh pathway requires functional primary cilia, microtubule-based organelles present on virtually every cell and acting as cellular antennae to receive and transduce diverse chemical, mechanical or light signals. The dysfunction of cilia in humans leads to inherited diseases called ciliopathies, which often affect many organs and show diverse manifestations including forebrain malformations for the most severe forms. The purpose of this review is to provide the reader with a framework to understand the developmental origin of the forebrain defects observed in severe ciliopathies with respect to perturbations of the Shh pathway. We propose that many of these defects can be interpreted as an imbalance in the ratio of activator to repressor forms of the Gli transcription factors, which are effectors of the Shh pathway. We also discuss the complexity of ciliopathies and their relationships with forebrain disorders such as holoprosencephaly or malformations of cortical development, and emphasize the need for a closer examination of forebrain defects in ciliopathies, not only through the lens of animal models but also taking advantage of the increasing potential of the research on human tissues and organoids.
Neurobiol Dis, 2021; 150
33145610: Murcia-Ramón R, Company V, Juárez-Leal I, Andreu-Cervera A, Almagro-García F, Martínez S, Echevarría D, Puelles E
Neuronal tangential migration from Nkx2.1-positive hypothalamus.
During the development of the central nervous system, the immature neurons suffer different migration processes. It is well known that Nkx2.1-positive ventricular layer give rise to critical tangential migrations into different regions of the developing forebrain. Our aim was to study this phenomenon in the hypothalamic region. With this purpose, we used a transgenic mouse line that expresses the tdTomato reporter driven by the promotor of Nkx2.1. Analysing the Nkx2.1-positive derivatives at E18.5, we found neural contributions to the prethalamic region, mainly in the zona incerta and in the mes-diencephalic tegmental region. We studied the developing hypothalamus along the embryonic period. From E10.5 we detected that the Nkx2.1 expression domain was narrower than the reporter distribution. Therefore, the Nkx2.1 expression fades in a great number of the early-born neurons from the Nkx2.1-positive territory. At the most caudal positive part, we detected a thin stream of positive neurons migrating caudally into the mes-diencephalic tegmental region using time-lapse experiments on open neural tube explants. Late in development, we found a second migratory stream into the prethalamic territory. All these tangentially migrated neurons developed a gabaergic phenotype. In summary, we have described the contribution of interneurons from the Nkx2.1-positive hypothalamic territory into two different rostrocaudal territories: the mes-diencephalic reticular formation through a caudal tangential migration and the prethalamic zona incerta complex through a dorsocaudal tangential migration.
Brain Struct Funct, 2020; 225
34722541: Company V, Moreno-Cerdá A, Andreu-Cervera A, Murcia-Ramón R, Almagro-García F, Echevarría D, Martínez S, Puelles E
Role in the Development of the Habenula and the Fasciculus Retroflexus.
is one of the morphogenes that controls the specification and differentiation of neuronal populations in the developing central nervous system. The habenula is a diencephalic neuronal complex located in the most dorsal aspect of the thalamic prosomere. This diencephalic neuronal population is involved in the limbic system and its malfunction is related with several psychiatric disorders. Our aim is to elucidate the role in the habenula and its main efferent tract, the fasciculus retroflexus, development. In order to achieve these objectives, we analyzed these structures development in a lack of function mouse model. The habenula was generated in our model, but it presented an enlarged volume. This alteration was due to an increment in habenular neuroblasts proliferation rate. The fasciculus retroflexus also presented a wider and disorganized distribution and a disturbed final trajectory toward its target. The mid-hindbrain territories that the tract must cross were miss-differentiated in our model. The specification of the habenula is independent. Nevertheless, it controls its precursors proliferation rate. expressed in the isthmic organizer is vital to induce the midbrain and rostral hindbrain territories. The alteration of these areas is responsible for the fasciculus retroflexus axons misroute.
Front Cell Dev Biol, 2021; 9

30689641: Choi YJ, Laclef C, Yang N, Andreu-Cervera A, Lewis J, Mao X, Li L, Snedecor ER, Takemaru KI, Qin C, Schneider-Maunoury S, Shroyer KR, Hannun YA, Koch PJ, Clark RA, Payne AS, Kowalczyk AP, Chen J
RPGRIP1L is required for stabilizing epidermal keratinocyte adhesion through regulating desmoglein endocytosis.
Cilia-related proteins are believed to be involved in a broad range of cellular processes. Retinitis pigmentosa GTPase regulator interacting protein 1-like (RPGRIP1L) is a ciliary protein required for ciliogenesis in many cell types, including epidermal keratinocytes. Here we report that RPGRIP1L is also involved in the maintenance of desmosomal junctions between keratinocytes. Genetically disrupting the Rpgrip1l gene in mice caused intraepidermal blistering, primarily between basal and suprabasal keratinocytes. This blistering phenotype was associated with aberrant expression patterns of desmosomal proteins, impaired desmosome ultrastructure, and compromised cell-cell adhesion in vivo and in vitro. We found that disrupting the RPGRIP1L gene in HaCaT cells, which do not form primary cilia, resulted in mislocalization of desmosomal proteins to the cytoplasm, suggesting a cilia-independent function of RPGRIP1L. Mechanistically, we found that RPGRIP1L regulates the endocytosis of desmogleins such that RPGRIP1L-knockdown not only induced spontaneous desmoglein endocytosis, as determined by AK23 labeling and biotinylation assays, but also exacerbated EGTA- or pemphigus vulgaris IgG-induced desmoglein endocytosis. Accordingly, inhibiting endocytosis with dynasore or sucrose rescued these desmosomal phenotypes. Biotinylation assays on cell surface proteins not only reinforced the role of RPGRIP1L in desmoglein endocytosis, but also suggested that RPGRIP1L may be more broadly involved in endocytosis. Thus, data obtained from this study advanced our understanding of the biological functions of RPGRIP1L by identifying its role in the cellular endocytic pathway.
PLoS Genet, 2019; 15
34169076: Company V, Andreu-Cervera A, Madrigal MP, Andrés B, Almagro-García F, Chédotal A, López-Bendito G, Martinez S, Echevarría D, Moreno-Bravo JA, Puelles E
Netrin 1-Mediated Role of the Substantia Nigra Pars Compacta and Ventral Tegmental Area in the Guidance of the Medial Habenular Axons.
The fasciculus retroflexus is an important fascicle that mediates reward-related behaviors and is associated with different psychiatric diseases. It is the main habenular efference and constitutes a link between forebrain regions, the midbrain, and the rostral hindbrain. The proper functional organization of habenular circuitry requires complex molecular programs to control the wiring of the habenula during development. However, the mechanisms guiding the habenular axons toward their targets remain mostly unknown. Here, we demonstrate the role of the mesodiencephalic dopaminergic neurons (substantia nigra pars compacta and ventral tegmental area) as an intermediate target for the correct medial habenular axons navigation along the anteroposterior axis. These neuronal populations are distributed along the anteroposterior trajectory of these axons in the mesodiencephalic basal plate. Using and experiments, we determined that this navigation is the result of attraction generated by the mesodiencephalic dopaminergic neurons. This attraction is mediated by the receptor deleted in colorectal cancer (DCC), which is strongly expressed in the medial habenular axons. The increment in our knowledge on the fasciculus retroflexus trajectory guidance mechanisms opens the possibility of analyzing if its alteration in mental health patients could account for some of their symptoms.
Front Cell Dev Biol, 2021; 9
35103859: Domínguez-Sala E, Andreu-Cervera A, Martín-Climent P, Murcia-Ramón R, Martínez S, Geijo-Barrientos E
Properties of the epileptiform activity in the cingulate cortex of a mouse model of LIS1 dysfunction.
Dysfunction of the LIS1 gene causes lissencephaly, a drastic neurological disorder characterized by a deep disruption of the cortical structure. We aim to uncover alterations of the cortical neuronal networks related with the propagation of epileptiform activity in the Lis1/sLis1 mouse, a model lacking the LisH domain in heterozygosis. We did extracellular field-potential and intracellular recordings in brain slices of the anterior cingulate cortex (ACC) or the retrosplenial cortex (RSC) to study epileptiform activity evoked in the presence of bicuculline (10 μM), a blocker of GABA receptors. The sensitivity to bicuculline of the generation of epileptiform discharges was similar in wild type (WT) and Lis1/sLis1 cortex (EC 1.99 and 2.24 μM, respectively). In the Lis1/sLis1 cortex, we observed a decreased frequency of the oscillatory post-discharges of the epileptiform events; also, the propagation of epileptiform events along layer 2/3 was slower in the Lis1/sLis1 cortex (WT 47.69 ± 2.16 mm/s, n = 25; Lis1/sLis1 37.34 ± 2.43 mm/s, n = 15; p = 0.004). The intrinsic electrophysiological properties of layer 2/3 pyramidal neurons were similar in WT and Lis1/sLis1 cortex, but the frequency of the spontaneous EPSCs was lower and their peak amplitude higher in Lis1/sLis1 pyramidal neurons. Finally, the propagation of epileptiform activity was differently affected by AMPA receptor blockers: CNQX had a larger effect in both ACC and RSC while GYKI53655 had a larger effect only in the ACC in the WT and Lis1/sLis1 cortex. All these changes indicate that the dysfunction of the LIS1 gene causes abnormalities in the properties of epileptiform discharges and in their propagation along the layer 2/3 in the anterior cingulate cortex and in the restrosplenial cortex.
Brain Struct Funct, 2022; 227
30692221: Andreu-Cervera A, Anselme I, Karam A, Laclef C, Catala M, Schneider-Maunoury S

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The Ciliopathy Gene Controls Mouse Forebrain Patterning via Region-Specific Modulation of Hedgehog/Gli Signaling. Primary cilia are essential for CNS development. In the mouse, they play a critical role in patterning the spinal cord and telencephalon via the regulation of Hedgehog/Gli signaling. However, despite the frequent disruption of this signaling pathway in human forebrain malformations, the role of primary cilia in forebrain morphogenesis has been little investigated outside the telencephalon. Here we studied development of the diencephalon, hypothalamus and eyes in mutant mice in which the ciliopathy gene is disrupted. At the end of gestation, fetuses displayed anophthalmia, a reduction of the ventral hypothalamus and a disorganization of diencephalic nuclei and axonal tracts. In embryos, we found that the ventral forebrain structures and the rostral thalamus were missing. Optic vesicles formed but lacked the optic cups. In embryos, () expression was virtually lost in the ventral forebrain but maintained in the zona limitans intrathalamica (ZLI), the mid-diencephalic organizer. Gli activity was severely downregulated but not lost in the ventral forebrain and in regions adjacent to the - expressing ZLI. Reintroduction of the repressor form of Gli3 into the background restored optic cup formation. Our data thus uncover a complex role of cilia in development of the diencephalon, hypothalamus and eyes via the region-specific control of the ratio of activator and repressor forms of the Gli transcription factors. They call for a closer examination of forebrain defects in severe ciliopathies and for a search for ciliopathy genes as modifiers in other human conditions with forebrain defects. The Hedgehog (Hh) signaling pathway is essential for proper forebrain development as illustrated by a human condition called holoprosencephaly. The Hh pathway relies on primary cilia, cellular organelles that receive and transduce extracellular signals and whose dysfunctions lead to rare inherited diseases called ciliopathies. To date, the role of cilia in the forebrain has been poorly studied outside the telencephalon. In this paper we study the role of the ciliopathy gene in mouse forebrain development. We uncover complex functions of primary cilia in forebrain morphogenesis through region-specific modulation of the Hh pathway. Our data call for further examination of forebrain defects in ciliopathies and for a search for ciliopathy genes as modifiers in human conditions affecting forebrain development.
J Neurosci, 2019; 39

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BOARD NUMBER: S06-442

DEVELOPMENTAL CHANGES OF NETWORK ACTIVITY IN THE SOMATOSENSORY CORTEX IN THE GLUTAMIC ACID DECARBOXYLASE 67 (GAD67)-GFP MOUSE MODEL.

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Activation of ionotropic and metabotropic GABA-receptors and the subsequent neuronal hyperpolarization plays an important role to stabilize the level of excitation in the developing and adult brain. Here we used a transgenic mouse line expressing the Green Fluorescent Protein (GFP) under the control of the glutamic acid decarboxylase 67 (GAD67) promotor (Tamamaki et al., 2003), which is known to cause impaired GABA synthesis. Our aim was to investigate its impact on cortical network activity during development. We performed electrophysiological multi-electrode array (MEA) and whole-cell patch-clamp recordings in slices of the somatosensory cortex in vitro from GAD67-GFP mice at P8/9, P14/15 and P21/22. Our main results are a decreased spontaneous activity measured by cortical MEA recordings in normal ACSF in P8/9 and P14/15, but not in P21/22 animals as compared to WT-littermate animals. Whole-cell patch clamp recordings from layers II/III pyramidal neurons revealed a decreased frequency, but not amplitude of sIPSCs in GAD67-GFP mice at P14/15. Furthermore, the frequency and amplitude of mIPSCs was decreased in GAD67-GFP mice. Interestingly, the frequency of sEPSCs was also reduced indicating a potentially compensatory downregulation of the glutamatergic transmission. However, amplitude and frequency of evoked eEPSCs was not different compared to WT-mice. These data indicate that the impaired synthesis of GABA in our present GAD67-GFP mouse model does not automatically lead to a hyperexcitable cortical network during development, but it rather triggers homeostatic synaptic processes to keep the excitatory-to-inhibitory ratio balanced. This work was supported by a grant to TM (DFG, CRC 1080, C02).

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-443

RAS-GAPS CONTROL DENDRITIC DEVELOPMENT IN BARREL CORTEX LAYER 4

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The cerebral cortex has a complex yet exquisite network of neuronal circuits which is important for advanced brain functional and cognitive purposes. To explore the molecular mechanisms of neuronal circuit formation, the tactile somatosensory pathway that connects the whiskers and cortex of rodents is useful. The rodent somatosensory barrel cortex layer 4 (L4) comprises a unique 'barrel map' that corresponds to facial whisker patterns. The whisker inputs via the thalamus form the neuronal circuit in the barrel cortex L4 during early postnatal development. Thalamocortical (TC) axon terminals are clustered in the barrels, ring-shaped distributions of neurons in the barrel cortex L4. Dendrites of L4 neurons are oriented toward the barrel centre and receive inputs from the TC axon terminals. These traits make the barrel cortex a useful system to study neuronal circuit development. Our focus is Ras-GTPase activating protein (Ras-GAP) which possibly plays a role in the MAPK and PI-3 kinase pathways. We have created knockdown constructs for Ras-GAPs (NF1 and SynGAP) following which we transfected the constructs to the barrel cortex L4 using in-utero electroporation. We have analysed the effects of Ras-GAPs knockdown in the L4 circuit formation by the histological method. Our results suggest that Ras-GAPs knockdown L4 neurons may show reduced dendritic orientation. We also checked the colocalization of the post-synaptic L4 neuron dendritic spines and pre-synaptic TC axon terminals by confocal imaging. In the meeting, we would like to discuss the possible mechanisms for Ras-GAP-dependent L4 circuit formation.

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BOARD NUMBER: S06-444

THE EMERGENCE OF CORRELATED ACTIVITY IN THE DEVELOPING CORTEX IN VIVO

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Neuronal activity plays a critical role in the developing brain, sculpting cortical circuits and orchestrating somatosensory map formation. During development, neurons undergo successive changes in their activity patterns, which evolve from asynchronous low-frequency single-cell firing in embryonic stages, to correlated activation after birth. In vitro experiments have shown that these early correlated activities, are initially mediated via electrical-synapses and sequentially by chemical-synapses. Interestingly, these major activity changes occur in the rodent brain during a narrow critical time window in the first postnatal week. Thus, our objective is to determine in vivo the cellular and activity-dependent mechanisms underlying network formation during the first postnatal week. In order to investigate when and how different correlated activity patterns emerge and change in space and time we used in vivo two-photon calcium imaging of multineuron dynamics in un-anesthetized mouse pups from birth onwards. We focus on the differential contribution of GABAergic interneurons and glutamatergic cells to such collective early dynamics. We uncover primary forms of correlated activity and show how the developmental timelines of local GABAergic and glutamatergic neurons do not converge until the end of the first postnatal week.

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BOARD NUMBER: S06-445

RULES GOVERNING THE DENDRITIC GROWTH OF MOTION-SENSING NEURONS IN DROSOPHILA

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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During development, neurons grow their dendrites to occupy a specific area to form synapses with their presynaptic partners. This is particularly clear in the case of T4 neurons, the first directionally tuned neurons of the ON pathway in *Drosophila* visual motion detection circuit. T4 neurons are divided into four subtypes, each tuned to motion in one of the four cardinal directions. This functional specificity of T4 neurons is strongly linked to the polarized orientation of their dendrites. Neurons of each subtype have their dendrites oriented primarily opposite to their preferred direction of motion (Takemura et al., 2013). All T4 subtypes connect to the same set of functionally distinct input neurons, but in a subtype-specific spatial order. It is the orientation of their dendrites that determines the correct sampling of inputs in visual space (Shinomiya et al., 2019). Therefore, growing the dendrite in the right orientation is essential for T4 function. We want to understand how T4 neurons manage to grow their dendrites to obtain the proper dendritic orientation corresponding to their subtypes. To understand more about this process and the cues that T4 dendrites read to guide their growth, we use two-photon time-lapse imaging of developing T4 neurons in an ex-vivo brain preparation. The image processing pipeline includes denoising and motion-removal to arrive at a pixel-based description of the area the dendrite covers at each time point during development.

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BOARD NUMBER: S06-446

DEVELOPMENTAL TIMELINE OF THE EMERGENCE OF SOMATIC GABAERGIC INNERVATION IN THE HIPPOCAMPUS OF RODENTS AND PRIMATES

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The adult hippocampus serves multiple cognitive functions that rely on a complex neuronal network able to integrate multiple external inputs onto internally-generated dynamics. One way to better understand the morpho-functional connectome of the adult hippocampus is to analyze how the circuit assembles during development. We have shown that the second postnatal week in the developing mouse marks an abrupt switch in the representation of self-motion in local CA1 dynamics with a transient inhibition in response to motor twitches (Dard et al., 2022). At the same time, we observed the abrupt anatomical and functional emergence of a perisomatic GABAergic coverage in the stratum pyramidale. The beginning of the second postnatal week in the mouse roughly corresponds to the end of the third trimester of pregnancy in humans and of the second trimester in macaques (www.translatingtime.org; Clancy et al., 2007, doi:10.1385/NI:5:1:1). Here we use immunocytochemical labelling directed against different markers of GABAergic afferents such as GAD65, VGAT, synaptotagmin2, parvalbumin, to map the maturation of such perisomatic GABAergic innervation in the hippocampus of humans and non-human primates.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-447

ACTIVITY-DEPENDENT MODULATION OF PGC1α REGULATES THE MATURATION OF CORTICAL PARVALBUMIN INTERNEURONS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Parvalbumin-expressing (PV) interneurons represent the most abundant subclass of cortical interneurons and are essential for the gating of excitatory cells activity. The transcriptional co-activator PGC1α, encoded by the *Ppargc1a* gene (Peroxisome proliferator activated receptor-gamma co-activator 1-alpha), is a master regulator of mitochondrial biogenesis and has been suggested to be important for the proper functioning of PV interneurons. However, the precise pattern of expression of *Ppargc1a* and its role in the maturation of PV interneurons during development are yet to be established. Here, we investigate whether the maturation of cortical PV interneurons is controlled by the activity-dependent modulation of *Ppargc1a* expression levels. We observed that *Ppargc1a* is expressed in selected classes of cortical interneurons during the early postnatal development of the mouse. This includes prospective PV interneurons, in which *Ppargc1a* is enriched before the onset of their terminal differentiation. PGC1α is required for the expression of a PV-specific genetic programme that is altered upon conditional deletion of *Ppargc1a* in interneurons derived from the medial ganglionic eminence. By interfering with the formation of excitatory synapses contacting PV interneurons, we found that *Ppargc1a* expression during development depends on the excitatory inputs received by these cells. Finally, using chemogenetic tools, we also uncover a mechanism by which abnormal network hyperactivation inhibits *Ppargc1a* expression and PV interneuron maturation. Overall, our results suggest that PGC1α functions as a cell-intrinsic molecular hub translating extrinsic cues into transcriptional programmes promoting the maturation of PV interneurons.

Pubmed:
29472441: Mi D, Li Z, Lim L, Li M, Moissidis M, Yang Y, Gao T, Hu TX, Pratt T, Price DJ, Sestan N, Marín O
Early emergence of cortical interneuron diversity in the mouse embryo.
GABAergic interneurons (GABA, γ-aminobutyric acid) regulate neural-circuit activity in the mammalian cerebral cortex. These cortical interneurons are structurally and functionally diverse. Here, we use single-cell transcriptomics to study the origins of this diversity in the mouse. We identify distinct types of progenitor cells and newborn neurons in the ganglionic eminences, the embryonic proliferative regions that give rise to cortical interneurons. These embryonic precursors show temporally and spatially restricted transcriptional patterns that lead to different classes of interneurons in the adult cerebral cortex. Our findings suggest that shortly after the interneurons become postmitotic, their diversity is already patent in their diverse transcriptional programs, which subsequently guide further differentiation in the developing cortex.
Science, 2018; 360

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FUNCTIONAL CONSEQUENCES OF DISRUPTING THE POSTNATAL REGULATION OF CORTICAL CELL NUMBERS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Cortical pyramidal cells and GABAergic interneurons undergo extensive programmed cell death during early postnatal development in the mouse. However, the requirement of programmed cell death for the normal functioning of the cerebral cortex remains to be elucidated. Here we aim to understand how disruption of the normal neuronal composition of the cerebral cortex affects behaviour in mice. Specifically, we studied how changes in the number of pyramidal cells and interneurons in the cerebral cortex may impact cortical function in mice. To this end, we tested mice in which the programmed cell death of pyramidal cells is genetically abolished ($Nex^{Cre/+};Bak^{-/-};Bax^{fl/fl}$) using visual and tactile go/no-go discrimination tasks. We observed no significant differences in the performance of control and mutant mice on neither the visual nor the tactile discrimination task. However, while the training of control mice on the tactile discrimination task improves their ability to subsequently learn the visual discrimination, this transfer of knowledge does not occur in the mutant mice. Our results suggest that artificially increasing the number of pyramidal cells and GABAergic interneurons in the cerebral cortex of mice does not seem to have a significant effect on visual and tactile sensory processing, but it impacts complex cognitive functions like transfer of knowledge. Further experiments are needed to understand how changes in the final number of cortical neurons affect cortical networks and how that might result in the behavioural phenotypes described in this study.

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MATERNAL IMMUNE ACTIVATION DECREASES THE E/I BALANCE AND ACTIVITY OF DOPAMINERGIC NEURONS IN THE VENTRAL TEGMENTAL AREA

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Prenatal infections have been associated with increased risk of developing neuropsychiatric disorders. Animal models are powerful tools for studying how maternal immune activation during pregnancy can alter brain development in the offspring. In particular, rodents whose mother received a pro-inflammatory agent [Poly(I:C)] during gestation show alterations in dopaminergic (DA) circuits reminiscent of schizophrenia-associated phenotypes. How a prenatal immune challenge induces long-lasting alterations of DA signaling is poorly understood. We set out to study the mechanisms by which maternal immune activation causes long-term changes in DA neurons in the ventral tegmental area (VTA). Maternal immune activation was induced by Poly(I:C) administration in pregnant mice at embryonic stage E8.5. Juxta-cellular recordings in the VTA *in vivo* showed decreased neuronal activity of DA neurons in the offspring of Poly(I:C)-treated mice. Using patch-clamp recordings *ex vivo*, we found that VTA DA neurons from Poly(I:C) mice displayed an enhanced voltage sag in response to hyperpolarizing current injections and increased Ih currents, indicating higher expression and/or activity of HCN channels. However, neither the intrinsic firing properties nor the resting membrane potential were altered in Poly(I:C) mice, suggesting that changes in Ih have modest effects on neuronal activity. Next, we studied functional synaptic inputs to DA VTA DA neurons. We observed that the frequency of miniature excitatory, but not inhibitory postsynaptic currents, was strongly diminished in Poly(I:C) mice, indicating impaired excitatory/inhibitory balance in VTA DA neurons. Together, these results suggest that maternal immune activation may reduce VTA DA neuron activity through decreased excitatory synaptic inputs.

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STRIKINGLY DIFFERENT NEUROTRANSMITTER RELEASE STRATEGIES AMONGST INTERNEURON SUBTYPES OF THE OLFACTORY BULB

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Neurons establish morphological polarity by specifying two different compartments: the axon and the somatodendritic domain. This polarity is also functional, because in most neurons dendrites receive the majority of inputs while axons generate output signals via neurotransmitter release. However, many exceptions to this dogma occur in the olfactory bulb (OB), where most GABAergic interneurons are anaxonic and can only release neurotransmitters from their dendrites. OB glutamatergic cells, however, have classic morphological polarity but release neurotransmitters from both axonal and somatodendritic domains. Dendritic release is, therefore, a common feature of OB neurons. A subset of GABAergic interneurons in the OB also release dopamine. These dopaminergic (DA) cells comprise two groups – axonic and anaxonic – depending on the presence or absence of an axon. Here, we provide structural and functional evidence showing that, unlike their anaxonic counterparts, axon-bearing DA neurons rarely if ever release GABA from their dendrites. We injected a Cre-dependent AAV in embryonic VGAT-Cre mice to obtain sparse cell morphology (GFP) plus structural evidence for putative neurotransmitter release sites (synaptophysin-mRuby), finding dendritic mRuby puncta almost exclusively in anaxonic cells. We then obtained electrophysiological recordings in acute slices from DAT-tdT mice, using an auto-evoked inhibition (AEI) protocol to detect dendritic GABA release. All anaxonic neurons displayed an AEI response, while almost all axonic DA cells did not. Our results suggest that axon-bearing DA neurons are the only OB cell type to *not* effect dendritic neurotransmitter release, placing a key spatial constraint on their ability to shape olfactory sensory processing.

Pubmed:
35147186: Tufo C, Poopalasundaram S, Dorrego-Rivas A, Ford MC, Graham A, Grubb MS
Development of the mammalian main olfactory bulb.
The mammalian main olfactory bulb is a crucial processing centre for the sense of smell. The olfactory bulb forms early during development and is functional from birth. However, the olfactory system continues to mature and change throughout life as a target of constitutive adult neurogenesis. Our Review synthesises current knowledge of prenatal, postnatal and adult olfactory bulb development, focusing on the maturation, morphology, functions and interactions of its diverse constituent glutamatergic and GABAergic cell types. We highlight not only the great advances in the understanding of olfactory bulb development made in recent years, but also the gaps in our present knowledge that most urgently require addressing.
Development, 2022; 149

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BOARD NUMBER: S06-451

PERSISTENCE OF CAJAL-RETZIUS CELLS IN THE HIPPOCAMPUS IS REQUIRED FOR THE DEVELOPMENT OF DENDRITIC SPINES OF CA1 PYRAMIDAL CELLS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The hippocampus is a brain structure acknowledged for its role in memory formation, learning and the representation of space. To facilitate this, the hippocampus is comprised of a diverse set of neurons, including Reln$^+$ glutamatergic Cajal-Retzius neurons (CR). Although neocortical CR-cells disappear soon after birth, hippocampal CR-cells persist for an extended period during postnatal development. CR-cells are actively integrated in the hippocampal microcircuit, though their influence on postnatal development is poorly understood. To address this, we injected a Cre-dependent AAV virus expressing diphtheria toxin fragment A in a Pde1c-Cre mouse line at postnatal day 0 (P0) to selectively ablate CR-cells. With this approach, ~90% of hippocampal CR-cells are ablated by P15 and sustained throughout the experimental endpoint at P30. This method enables us to investigate hippocampal development and function when CR-cells are prematurely removed. In vitro electrophysiological experiments show that CR-cell ablation affects the connectivity of CA1 pyramidal cells. Analysis of dendritic spines highlighted significant differences both in density and the type of spines present on CA1 pyramidal cells, in all hippocampal layers, suggesting that removal of CR-cells skews the trajectory of spine development of CA1 pyramidal cells. Thereafter, we performed bulk RNA sequencing from dorsal hippocampus at P15 and P30 to assess underlying genetic mechanisms that CR-cell ablation alters. Synaptic gene ontology analysis show that CR-cell ablation led to negative enrichment of synaptic function and cellular components. Our data suggests that the persistence of CR-cells in the postnatal hippocampus is critical for proper maturation of the hippocampal circuit.

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PRO- AND MATURE-BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) CONTROL NEURONAL CHLORIDE HOMEOSTASIS THROUGH CATION-CHLORIDE CO-TRANSPORTER ACTIVITY KCC2

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The strength of GABAergic inhibitory transmission is dependent on intracellular neuronal chloride concentration ([Cl-]i), mainly regulated by activity of the cation-chloride co-transporter KCC2. Among many molecules controlling its function and affecting neuronal [Cl-]i is brain-derived neurotrophic factor (BDNF). BDNF is synthesized from precursor proteins (proBNDF) that are cleaved to yield to mature BDNF (mBDNF). mBDNF interacts with the TrkB receptor to activate set of TrkB-dependent signaling cascades, whereas proBDNF interacts with the $p75^{NTR}$ to affect distinct cellular responses. Although previous studies showed the implication of BDNF signaling in KCC2 functional activity, the involved cellular mechanisms remain unclear. Here, we investigated the interplay between both forms of BDNF and chloride homeostasis in primary cultures of rat hippocampal neurons using gramicidin perforated-patch recordings, live-cell immunolabeling of KCC2 and NH4(+)-induced pHi shifts. We found that in mBDNF expressing neurons, the value of the reversal potential of GABA responses (EGABA) is more negative in immature neurons, whereas in proBDNF expressing neurons, EGABA is more positive in mature neurons. Analysis of cell surface expression of KCC2 confirms the electrophysiological data showing a KCC2 up-regulation and downregulation in mBDNF and proBDNF expressing neurons respectively. At last, in immature neurons, both forms of BDNF showed a significant increase in KCC2 activity, whereas in mature neurons, pro- and mBDNF modify KCC2 activity in opposite direction. Finally, our findings highlight the significant potential role of BDNF signaling for regulating chloride transport through the control of KCC2 activity and thereby to fine-tune the strength of inhibitory synaptic transmission.

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RAPID PRESYNAPTIC MATURATION IN NATURALLY REGENERATING AXONS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Studying naturally occurring processes of neuronal regeneration has the potential to inform therapeutic attempts at brain repair. Here we examined presynaptic maturation in the regenerating adult mammalian olfactory system, where damaged neurons can naturally re-grow axons to re-establish functional connections with target circuitry. After inducing olfactory sensory neuron (OSN) degeneration with a single dose of the olfactotoxin methimazole (MMZ), we characterised the resultant degeneration and subsequent functional reconnection of OSN axon terminals with their postsynaptic targets in the olfactory bulb (OB). Morphologically, OSN axon terminals degenerated for ~10-15 days post-MMZ injection (DPI), followed by a slower regeneration phase from ~15-45 DPI. Functionally, electrophysiological recordings in acute OB slices revealed a much more rapid increase in OSN-to-OB connectivity that began at 13 DPI and reached control levels just a few days later. Re-connecting axon terminals, whilst capable of robust neurotransmission at these early stages, displayed significantly weaker paired-pulse depression, indicating that their release probability may be lower compared to mature connections. This effect was only present in the most immature synapses, returning rapidly to control levels by 22-30 DPI, and could not be explained by differences in either AMPA receptor desensitisation or presynaptic inhibition. Newly arriving axons also display fully mature levels of presynaptic inhibition in response to dopaminergic D_2 and GABAergic $GABA_B$ agonists. Our data suggest that naturally regenerating axon terminals in the adult mammalian brain are almost fully mature when they reach their postsynaptic targets, but undergo a brief phase of functional maturation after they first re-establish synaptic connectivity.

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INSULIN REGULATES GABAERGIC NEUROTRANSMISSION IN HIPPOCAMPAL NEURONS IN WILD-TYPE MICE AND A MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Aims To investigate the effect of insulin on γ-aminobutyric acid (GABA)-activated currents in hippocampal neurons in Alzheimer's disease (AD) model tg-APPSwe mice in comparison with wild-type mice. **Methods** Whole-cell patch-clamp assay was performed to record GABA-activated currents in hippocampal neurons (dentate gyrus granule cells and CA3 pyramidal cells) of acute brain slices, from wild-type and tg-APPSwe mice at different ages, in the presence or absence of insulin. **Results** In wild-type mice (8-10 weeks old), insulin (1nM) increased the total spontaneous inhibitory postsynaptic current (sIPSC) density in dentate gyrus granule cells from both dorsal and ventral hippocampus, and the extrasynaptic current density in dorsal CA3 pyramidal cells. In addition, insulin increased miniature IPSC (mIPSC) frequency in dentate gyrus granule cells and dorsal CA3 pyramidal cells. In 5-6 months old wild-type and tg-APPSwe mice, insulin modulated neither the total sIPSC density nor extrasynaptic current density. In dentate gyrus granule cells from 5-6 months old tg-APPSwe mice, the total sIPSC density was significantly reduced as compared to wild-type mice and increased by insulin treatment. In contrast, the extrasynaptic current density was increased in tg-APPSwe mice (5-6 and 10-12 months old) as compared with wild-type littermates, but only reduced by insulin in 10-12 months old tg-APPSwe mice. **Conclusions** Insulin differentially modulates GABA-activated neurotransmission in the hippocampus, which depends on neuronal type, subtype of neuronal inhibition, age and disease model. Reference: Hammoud, Netsyk et al. *Acta Physiol (Oxf).* 2021:e13623.

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BOARD NUMBER: S06-455

GABAB RECEPTOR-MEDIATED EFFECTS IN VIP-EXPRESSING INTERNEURONS OF THE DENTATE GYRUS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The dentate gyrus (DG) acts as a gateway to the hippocampus, processing multi-modal information arriving from neocortical areas via the entorhinal cortex. In its netwotk, principal neurons, the granule cells, interact with a morphologically, physiologically, and neurochemically hetergogenous population of inhibitory GABAergic interneurons (INs). Among various IN types, vasoactive intestinal peptide-expressing (VIP) INs are known to selectively target other INs and thereby directly modulate the GABAergic system. GABA acts in addition to ionotropic $GABA_A$ via metabotropic $GABA_B$ receptors. $GABA_B$ receptors mediate diverse effects in cortical networks and are particularly highly expressed in INs, however their action in VIP-INs has not been studied. Therefore. to elucidate the function of $GABA_B$ receptors ($GABA_B$Rs) in the regulation of the electrical activity of DG VIP-INs, the effects of $GABA_B$R agonist and antagonist on VIP-INs were investigated in VIP/tdTomato mouse slices by whole-cell patch-clamp recordings. The result revealed that VIP-INs have small round somata (5-15 µm) located in all subregions of the DG and with distinct axonal projection patterns, and display various input resistances as well as different discharge patterns, as reported recently (Wei et a., 2021). Bath application of the agonist baclofen (10 µM) induced a hyperpolarization and reduced excitability of VIP-INs. This effect was underlied by an outward whole-cell current of 40 ± 10.2 pA in DG VIP-INs which was fully reversed by the application of the antagonist CGP (5 µM). These results suggest that postsynaptic $GABA_B$Rs are highly expressed and modulate the activity of VIP-INs of the DG.

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EXTRACELLULAR RECORDINGS OF MULTIPLE OPTICALLY-TAGGED NEURONS FOR CELL TYPE CLASSIFICATION

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Neuronal networks consist of multiple types of neurons with distinct roles. Extracellular techniques allow monitoring activity at a single spike/single neuron spatiotemporal resolution. Optogenetic stimulation can tag one or two cell types per animal. However, there is no way to determine the type of a recorded neuron in vivo, without optical stimulation or visualization. Here, we generated a ground-truth dataset for classifying four different types of neurons in mouse neocortex and hippocampus. We collected optically-tagged spike data from 19 freely-moving transgenic mice. Every mouse expressed ChR2 under a different promoter: Calcium/calmodulin-dependent kinase (CaMKII), parvalbumin (PV), somatostatin (SOM), or cholecystokinin (CCK). Because some excitatory cells express CCK, we specifically targeted inhibitory CCK cells by viral vector injection into mice transgenic for both CCK and vesicular GABA transporter. From each animal, extracellular data were recorded over several months using high-density silicon probes coupled with optical fibers. Since illumination of excitatory CaMKII cells activates non-CaMKII cells indirectly with millisecond-timescale latency, we identified directly-activated CaMKII cells by white-noise stimulation. For every optically-tagged neuron, we derived a feature set based on spike waveforms, timing, and the propagation of extracellular voltage in space. The four-cell type dataset provides a unique ground-truth basis for training new classifiers. A cross validated classifier trained on this dataset will enable cell type identification in non-optical animals, facilitating more precise interpretation of extracellular recordings and deeper understanding of network dynamics.
Funding: CIHR-IDRC-ISF #2558/18, CRCNS NSF-BSF #2015577, ERC #679253

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ROLE OF THE PRESYNAPTIC SV2A PROTEIN IN THE CONTROL OF EXCITATION/INHIBITION (E/I) BALANCE IN HIPPOCAMPAL CA3 CIRCUITS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The presynaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam, and a widely used tomography synaptic marker. However, the function of SV2A, ubiquitously expressed in the brain, has remained rather elusive. Constitutive SV2A knock-out mice display severe seizures and death after a few weeks, and mutation of the protein in humans can lead to epilepsy. We have examined the electrophysiological consequences of deleting SV2A from either excitatory or inhibitory input onto CA3 pyramidal cells. For this, we have used SV2A-conditional KO mouse in combination with viral gene transfer to allow for expression of the Cre recombinase and an optogenetic tool (ChIEF) in either dentate gyrus granule cells or CA3 interneurons. Removing SV2A from dentate gyrus granule cells led to a decreased E/I ratio during burst activity and this effect was due to an increase in feed-forward inhibition onto the pyramidal cells. Surprisingly, when inhibition was pharmacologically blocked, excitatory mossy fiber to CA3 pyramidal cell responses during high frequency stimulation were also increased. We also show that the absence of SV2A in CA3 interneurons does not affect synaptic transmission onto pyramidal cells. Overall, we show how the absence of SV2A leads to an increased of inhibition of CA3 pyramidal cells, not by directly affecting inhibitory transmission but by increasing excitation of interneurons, intensifying feed-forward inhibition from DG to CA3.

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BOARD NUMBER: S06-458

THE ROLE OF AXOAXONIC CELLS IN CONTROLLING PREFRONTAL FIRING ACTIVITY IN VIVO

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Axoaxonic Cells (AAC) are among the most intriguing GABAergic interneurons of the cerebral cortex. Synapsing with the axon initial segment (AIS) of pyramidal neurons (PN), where action potentials are initiated, they are thought to play a critical role in the modulation of cortical network activities. However, how AAC regulate PN firing activity remains uncertain. *In vitro* studies showed AAC's activation to display both excitatory and inhibitory effects on PN (Szabadics et al., 2006; Glickfeld et al., 2009; Woodruff et al., 2011; Pan-Vazquez et al., 2020). A recent *in vivo* study found an inhibition of 14.9% of PN after AAC optogenetic simulation in the CA1 region of the hippocampus (Dudok et al., 2021). Additionally, in the neocortex, a predominant inhibitory effect of AAC activation was reported (Lu et al., 2017). However, information on the net effect of AAC on neocortical PN is still incomplete and debated. Thus, in this project we leveraged the development of a transgenic mouse line that selectively targets AAC *in vivo,* with inducible Nkx2.1-dependent reporters (He et al., 2016; Taniguchi et al., 2013); and combine large-scale electrophysiological recordings of dorsomedial prefrontal PN with optogenetic activation of AAC *in vivo* in drug-free mice. With this approach, we provide evidence for the net effect of AAC activation on PN's firing in the neocortex. This work provides a better understanding of the regulation of cortical motifs by AAC, which might be altered in neuropsychiatric disorders.

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DENTATE GYRUS INHIBITORY MICROCIRCUIT PROMOTES NETWORK MECHANISMS UNDERLYING MEMORY CONSOLIDATION

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The dentate gyrus (DG) to CA3 pathway plays a key role in encoding experiences that are ultimately consolidated in the anterior cingulate cortex (ACC). Experience-dependent changes among excitatory neurons in the DG-CA3 circuit have been intensely studied. However, DG cells innervate not only CA3 pyramidal neurons but also inhibitory, parvalbumin-positive interneurons to provide strong feed-forward inhibition (FFI) onto CA3 pyramidal neurons. Following learning, FFI onto CA3 is temporarily increased which may be a key element for consolidation and long-term memory storage in hippocampal-cortical networks. The underlying mechanisms through which this inhibitory microcircuit mediates memory consolidation in hippocampal-cortical networks are not well understood. We harnessed a molecular tool to investigate how increased FFI in this microcircuit affects downstream neuronal ensembles and network oscillations during memory consolidation. We performed longitudinal *in vivo* calcium imaging in CA1 and ACC during contextual fear learning in mice with virally enhanced FFI in the DG-CA3 circuit. We found that selectively increasing FFI facilitated formation and maintenance of neuronal representation in both brain regions as it prevented a time-dependent decay of neuronal representations. Furthermore, the specificity of neuronal representation was increased in a time-dependent manner in ACC. Simultaneous recordings of local field potentials (LFPs) in CA1 and ACC revealed that virally enhanced FFI increased coupling of sharp-wave ripples and spindles, a mechanism for hippocampal-cortical communication during memory consolidation. This study links a defined synaptic mechanism in a DG-CA3 inhibitory microcircuit with ensemble dynamics and network oscillations and provides direct evidence for its role in memory consolidation.

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NRG1 HAPLOINSUFFICIENCY ALTERS THE HOMEOSTASIS OF INHIBITORY CORTICAL CIRCUITS

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Neuregulin 1 (Nrg1) is a major schizophrenia (SZ) risk gene that controls the development of neuronal circuits. Nrg1 polymorphisms linked to SZ locate mainly in non-coding regions and they may partially reduce Nrg1 expression. Most previous studies focused on the characterization of mouse models deficient for ErbB4, the receptor for both Nrg1 and Neuregulin 3. Here, we investigated the alterations of cortical circuits in a new mouse model of Nrg1 haploinsufficiency (Nrg1 tm1Lex). To this aim, we used multidisciplinary approaches as Magnetic Resonance Spectroscopy, electrophysiology, quantitative confocal imaging and molecular analysis. We observed changes in the expression of molecules involved in GABAergic neurotransmission, decreased density of Vglut1 excitatory buttons onto Parvalbumin interneurons and decreased frequency of spontaneous inhibitory postsynaptic currents. In the prefrontal cortex, we observed an imbalance of the inhibitory circuits and a decreased number of Parvalbumin interneurons. Conversely, we failed to observe major alterations in excitatory neurons that were previously reported in ErbB4 null mice. Altogether, our work suggests that Nrg1 haploinsufficiency alters the inhibitory circuits in the cortex and provides new insights into molecular synaptic impairment caused by Nrg1 in a preclinical model of SZ.

Pubmed:
34246770: Navarro-Gonzalez C, Carceller H, Benito Vicente M, Serra I, Navarrete M, Domínguez-Canterla Y, Rodríguez-Prieto Á, González-Manteiga A, Fazzari P
Nrg1 haploinsufficiency alters inhibitory cortical circuits.
Neuregulin 1 (NRG1) and its receptor ERBB4 are schizophrenia (SZ) risk genes that control the development of both excitatory and inhibitory cortical circuits. Most studies focused on the characterization ErbB4 deficient mice. However, ErbB4 deletion concurrently perturbs the signaling of Nrg1 and Neuregulin 3 (Nrg3), another ligand expressed in the cortex. In addition, NRG1 polymorphisms linked to SZ locate mainly in non-coding regions and they may partially reduce Nrg1 expression. Here, to study the relevance of Nrg1 partial loss-of-function in cortical circuits we characterized a recently developed haploinsufficient mouse model of Nrg1 (Nrg1). These mice display SZ-like behavioral deficits. The cellular and molecular underpinnings of the behavioral deficits in Nrg1 mice remain to be established. With multiple approaches including Magnetic Resonance Spectroscopy (MRS), electrophysiology, quantitative imaging and molecular analysis we found that Nrg1 haploinsufficiency impairs the inhibitory cortical circuits. We observed changes in the expression of molecules involved in GABAergic neurotransmission, decreased density of Vglut1 excitatory buttons onto Parvalbumin interneurons and decreased frequency of spontaneous inhibitory postsynaptic currents. Moreover, we found a decreased number of Parvalbumin positive interneurons in the cortex and altered expression of Calretinin. Interestingly, we failed to detect other alterations in excitatory neurons that were previously reported in ErbB4 null mice suggesting that the Nrg1 haploinsufficiency does not entirely phenocopies ErbB4 deletions. Altogether, this study suggests that Nrg1 haploinsufficiency primarily affects the cortical inhibitory circuits in the cortex and provides new insights into the structural and molecular synaptic impairment caused by NRG1 hypofunction in a preclinical model of SZ.
Neurobiol Dis, 2021; 157
34369923: Rodríguez-Prieto Á, González-Manteiga A, Domínguez-Canterla Y, Navarro-González C, Fazzari P
A Scalable Method to Study Neuronal Survival in Primary Neuronal Culture with Single-cell and Real-Time Resolution.
Neuronal loss is at the core of many neuropathologies, including stroke, Alzheimer's disease, and Parkinson's disease. Different methods were developed to study the process of neuronal survival upon cytotoxic stress. Most methods are based on biochemical approaches that do not allow single-cell resolution or involve complex and costly methodologies. Presented here is a versatile, inexpensive, and effective experimental paradigm to study neuronal survival. This method takes

advantage of sparse fluorescent labeling of the neurons followed by live imaging and automated quantification. To this aim, the neurons are electroporated to express fluorescent markers and co-cultured with non-electroporated neurons to easily regulate cell density and increase survival. Sparse labeling by electroporation allows a simple and robust automated quantification. In addition, fluorescent labeling can be combined with the co-expression of a gene of interest to study specific molecular pathways. Here, we present a model of stroke as a neurotoxic model, namely, the oxygen-glucose deprivation (OGD) assay, which was performed in an affordable and robust homemade hypoxic chamber. Finally, two different workflows are described using IN Cell Analyzer 2200 or the open-source ImageJ for image analysis for semi-automatic data processing. This workflow can be easily adapted to different experimental models of toxicity and scaled up for high-throughput screening. In conclusion, the described protocol provides an approachable, affordable, and effective in vitro model of neurotoxicity, which can be suitable for testing the roles of specific genes and pathways in live imaging and for high-throughput drug screening.
J Vis Exp, 2021;

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BOARD NUMBER: S06-461

PKA ACTIVITY IN LAYER 2/3 OF SOMATOSENSORY CORTEX IN BEHAVING MICE

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Catecholamines influence animal behavior by controlling the excitability of neurons through G-protein coupled receptors and second messengers including cAMP/PKA signaling. However, little is known about the real-time cellular dynamics of endogenous catecholaminergic transmission in awake animals. Here, we imaged protein kinase A (PKA) activity in dendrites and somata of cortical layer 2/3 of awake mice with two photon microscopy and the single-chromophore PKA sensor GakdYmut with high temporal resolution (6.2Hz). For simultaneous PKA and Ca^{2+} imaging, we combined GakdYmut and the red Ca^{2+} sensor jR-GECO1a. When switching from isoflurane anesthesia (1%) to full wakefulness, the basal PKA activity increased by 22% and 12% in dendrites and somata, respectively (440 dendrites, 190 somata, 5 mice). During anesthesia and wakefulness, the PKA activity was suppressed by the β adrenoceptor agonist propranolol and D1 receptor antagonist sch23390. In contrast, α2 adrenoceptor antagonist yohimbine increased PKA activity. During wakefulness, we detected Ca^{2+} activity increase that synchronized with the locomotion of mice. However voluntary running induced typically biphasic PKA activity: decrease during locomotion followed by an overshooting rebound peaking about 25s after locomotion offset. Individual somata and dendrites showed a continuum of responses from only decrease during running to only increase after locomotion and changed their response type with every locomotion event. PKA activity was not correlated with Ca^{2+} activity in dendrites and somata. Yohimbine increased locomotion induced PKA activity. Our experiments show that α2 and β adrenoceptor set the basal PKA activity and voluntary locomotion causes PKA activity changes mediated by these adrenoceptors.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-462

EFFECTS OF PRENATAL EXPOSURE TO THIACLOPRID, A NEONICOTINOID ON NEUROPLASTICITY IN ZEBRAFISH AND MOUSE

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Aims: Chronic persistence of neonicotinoids raises concerns over its potential adverse effects in vertebrates. We investigated the effects of a thiacloprid on brain neuroplasticity, using zebrafish and mouse models. **Methods:** In the first experiment, we exposed zebrafish eleutheroembryo to 10^{-6} M to 10^{-8} M thiacloprid and collected whole heads 6 days postfertilization. In the second experiment, mice dams were exposed to 0.06, 0.6 and 6mg /kg/day to prenatal and 35-day old male and female offspring euthanized and cingular gyrus, hypothalamus, hippocampus and cerebellum isolated. In mice and zebrafish, markers of neuroplasticity and steroid action were quantified by RT-qPCR. **Results:** Here, thiacloprid did not affect any of the markers investigated in zebrafish eleutheroembryos. In mice amygdala, we observed a significant dose-dependent increase of doublecortin and PCNA and a reduction in aromatase transcription. In the hypothalamus, we found a dose-dependent decrease in ERbeta, nestin, synapsin IIA, synaptophysin neurogenin aromatase BDNF. PCNA was reduced at the lowest dose in the Cerebellum. Finally in the hippocampus, doublecortin, neurogenin, aromatase, nestin and synaptic markers were also reduced in a dose-dependent manner. We did not see any interaction with sex. **Conclusions:** Prenatal exposure to thiacloprid resulted in dose dependent alteration in the neuronal and steroid markers in specific brain areas only in mice, not in zebrafish. This could be due to the dose used or receptor specificity indicating the need for further investigations on the effects of neonicotinoids in the developing vertebrate brain.

Pubmed:
26007202: Kunikullaya KU, Goturu J, Muradi V, Hukkeri PA, Kunnavil R, Doreswamy V, Prakash VS, Murthy NS
Combination of music with lifestyle modification versus lifestyle modification alone on blood pressure reduction - A randomized controlled trial.
To evaluate the change in blood pressure (BP) after 3 months of music intervention combined with lifestyle modifications, in comparison with conventional lifestyle modifications.
Complement Ther Clin Pract, 2016; 23
26365454: Kunikullaya KU, Goturu J, Muradi V, Hukkeri PA, Kunnavil R, Doreswamy V, Prakash VS, Murthy NS
Music versus lifestyle on the autonomic nervous system of prehypertensives and hypertensives--a randomized control trial.
Ragas of Indian music are said to be beneficial in normalizing blood pressure (BP). The objective of this study was to evaluate the effect of passive listening to relaxing raga on the autonomic functions of hypertensives and prehypertensives and provide scientific evidence.
Complement Ther Med, 2015; 23
26486461: Kunikullaya KU, Purushottam N, Prakash V, Mohan S, Chinnaswamy R
Correlation of serum uric acid with heart rate variability in hypertension.
Autonomic dysfunction with dominant sympathetic tone is a common finding among hypertensives and prehypertensives. Uric acid is one of the independent predictors of hypertension. There are very few studies which have shown a relationship between the autonomic tone and uric acid generation pathway among prehypertensives and hypertensives. Aim of the study was to estimate and correlate serum uric acid levels with autonomic function as measured by heart rate variability (HRV) among prehypertensives and hypertensives.
Hipertens Riesgo Vasc, 2015 Oct-Dec; 32

21151382: Kunikullaya KU, Kirthi SK, Venkatesh D, Goturu J
Heart rate variability changes in business process outsourcing employees working in shifts.
Irregular and poor quality sleep is common in business process outsourcing (BPO) employees due to continuous shift working. The influence of this on the cardiac autonomic activity was investigated by the spectral analysis of heart rate variability (HRV).
Indian Pacing Electrophysiol J, 2010; 10
28076315: Kunikullaya KU, Vijayaraghava A, Asha P, Kunnavil R, MuraliMohan BV
Meteorological parameters and pollutants on asthma exacerbation in Bangalore, India - an ecological retrospective time-series study.
Literature has shown a significant association between asthma exacerbations and pollutant levels during that time. There is very limited evidence in India, especially Bangalore, for impacts of meteorological changes and pollution on asthma hospital admissions in adults. The objective was to study the impact of air pollution and meteorological parameters on asthma exacerbation in Bangalore.
J Basic Clin Physiol Pharmacol, 2017; 28
35071064: Srinivasa R, Furtado SV, Kunikullaya KU, Biradar S, Jayakumar D, Basu E
Surgical Management of Spinal Tuberculosis - A Retrospective Observational Study from a Tertiary Care Center in Karnataka.
Tuberculosis (TB) is a common infectious disorder in developing countries. A significant load of patients with extrapulmonary TB are diagnosed in our institute, mostly involving the spine.
Asian J Neurosurg, 2021 Oct-Dec; 16
22133471: Kunikullaya U K, Ananthakrishnan V, Goturu J
Robert Tigerstedt and the discovery of renin - a revisit.
Int J Cardiol, 2012; 158
24701471: K P, Kunikullaya U K, Goturu J
Glycosylated Haemoglobin (HbA1c) - A Marker of Circulating Lipids in Type 2 Diabetic Patients.
Diabetic patients with concomitant dyslipidemia are often soft targets for cardiovascular disease and deaths. An early intervention to normalize circulating lipids has been shown to reduce cardiovascular morbidity and mortality. Glycosylated hemoglobin (HbA1c) is routinely used as a marker to indicate long-term glycemic control.
J Clin Diagn Res, 2014; 8
28847142: Kunikullaya U. K, Kumar M. A, Ananthakrishnan V, Jaisri G
Stress as a Cause of Recurrent Aphthous Stomatitis and Its Correlation with Salivary Stress Markers.
Stress causes an increase in cortisol and amylase. Recurrent aphthous stomatitis (RAS) results due to a multitude of causes, amongst which stress is one of the most important. Aim of the study was to estimate the level of stress, serum cortisol, salivary cortisol, amylase and electrolytes in subjects with RAS. 34 subjects with RAS (cases) were compared with 34 controls. Stress was measured using state trait anxiety inventory (STAI). Serum cortisol (Radioimmunoassay), Salivary cortisol, amylase (ELISA) and electrolytes (Flame photometry) were measured. Statistical analysis was done using SPSS 18.0 version software. The mean STAI scores were 48.71 ± 4.6 in cases and 46.74 ± 6.4 in controls (P = 0.13). The mean salivary cortisol concentration was 3.35 ± 1.8 ng/dl in cases and 3.65 ± 2.5 ng/dl in controls (P = 0.78). The mean salivary amylase was 155.09 ± 116.1 U/ml in cases and 128.74 ± 86.3 U/ml in controls (P = 0.49). The salivary sodium (0.24 ± 0.4 in both groups) and potassium (0.65 ± 0.5 in cases and 0.82 ± 0.4 in controls; P = 0.07) was not different in the two groups (electrolytes in mEq/dl). No correlation was seen between the salivary stress markers and STAI scores. Though stress was higher in RAS group none of the measured parameters were different from the control group. Stress may cause RAS but, in this study, there was no change in the salivary homeostasis.
Chin J Physiol, 2017; 60
33482336: Kunikullaya U K, Kunnavil R, Vijayadas , Goturu J, Prakash VS, Murthy NS
Normative data and gender differences in heart rate variability in the healthy young individuals aged 18-30 years, a South Indian cross-sectional study.
Indian Pacing Electrophysiol J, 2021 Mar-Apr; 21

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BOARD NUMBER: S06-463

EXPOSURE TO BLUE LIGHT AT NIGHT DURING ADOLESCENCE INDUCES NEUROTRANSMITTER PLASTICITY IN THE AMYGDALA AFFECTING EMOTIONAL RESPONSES IN MICE

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Anxiety and mood disorders are the most common mental health problem in adolescents. Human studies showed that prolonged blue light exposure at night was associated with anxiety, lower self-control, and less social interactions in adolescents. **Aim:** The aim of this study is to test whether blue light affects amygdala neuroplasticity during adolescence regulating emotional responses to reveal whether prolonged blue light exposure represents an early risk factor for developing psychiatric disorders. **Methods:** We developed a new light paradigm to mimic human adolescent light exposure in mice: a prolonged light phase (19hrs/day) with light exposure during the night phase. Adolescent mice were exposed to either such light protocol or to control conditions (12hrs/day) for 4 weeks, then tested for anxiety-like behavior. Immunofluorescence, chemogenetic approach and fiber photometry were used to establish the effect of blue light on neurotransmitter plasticity in the amygdala and emotional responses. **Results:** Exposure to blue light protocol increased anxiety-like responses and induced a significance difference in the number of GABAergic and somatostatin neurons in the amygdala in adolescent mice. *In vivo* Ca^{2+} recording revealed increased somatostatin activity in the amygdala associated with impaired social behavior in adolescent mice exposed to blue light protocol. Chemogenetic inhibition of amygdala GABAergic neurons interfered with light-mediated neurotransmitter plasticity and reduced anxiety states in adolescent mice. **Conclusions:** Our data indicate that prolonged blue light exposure induces a form of neuroplasticity in the amygdalamediating anxiety and risky choice behaviors during adolescence, revealing new strategies to advance mental health in human adolescents.

Pubmed:

34850507: McCarthy MJ, Gottlieb JF, Gonzalez R, McClung CA, Alloy LB, Cain S, Dulcis D, Etain B, Frey BN, Garbazza C, Ketchesin KD, Landgraf D, Lee HJ, Marie-Claire C, Nusslock R, Porcu A, Porter R, Ritter P, Scott J, Smith D, Swartz HA, Murray G
Neurobiological and behavioral mechanisms of circadian rhythm disruption in bipolar disorder: A critical multi-disciplinary literature review and agenda for future research from the ISBD task force on chronobiology.
Symptoms of bipolar disorder (BD) include changes in mood, activity, energy, sleep, and appetite. Since many of these processes are regulated by circadian function, circadian rhythm disturbance has been examined as a biological feature underlying BD. The International Society for Bipolar Disorders Chronobiology Task Force (CTF) was commissioned to review evidence for neurobiological and behavioral mechanisms pertinent to BD.
Bipolar Disord, 2022; 24
33798546: Porcu A, Mostallino R, Serra V, Melis M, Sogos V, Beggiato S, Ferraro L, Manetti F, Gianibbi B, Bettler B, Corelli F, Mugnaini C, Castelli MP
COR758, a negative allosteric modulator of GABA receptors.
Allosteric modulators of G protein coupled receptors (GPCRs), including GABARs (GABARs), are promising therapeutic candidates. While several positive allosteric modulators (PAM) of GABARs have been characterized, only recently the first negative allosteric modulator (NAM) has been described. In the present study, we report the characterization of COR758, which acts as GABAR NAM in rat cortical membranes and CHO cells stably expressing GABARs (CHO-GABA). COR758 failed to displace the antagonist [H]CGP54626 from the orthosteric binding site of GABARs showing that it acts through an allosteric binding site. Docking studies revealed a possible new allosteric binding site for COR758 in the intrahelical pocket of the GABA monomer. COR758 inhibited basal and GABAR-stimulated O-(3-[Sthio)-triphosphate ([S]GTPγS) binding in brain membranes and blocked the enhancement of GABAR-stimulated [S]GTPγS binding by the PAM GS39783. Bioluminescent resonance energy transfer (BRET) measurements in CHO-GABA cells showed that COR758 inhibited G protein activation by GABA and altered GABAR subunit rearrangements. Additionally, the compound altered GABAR-mediated signaling such as baclofen-induced inhibition of cAMP production in transfected HEK293 cells, agonist-induced Ca mobilization as well as baclofen and the ago-PAM CGP7930 induced phosphorylation of extracellular signal-regulated kinases (ERK1/2) in CHO-

GABA cells. COR758 also prevented baclofen-induced outward currents recorded from rat dopamine neurons, substantiating its property as a NAM for GABARs. Altogether, these data indicate that COR758 inhibits G protein signaling by GABARs, likely by interacting with an allosteric binding-site. Therefore, COR758 might serve as a scaffold to develop additional NAMs for therapeutic intervention.
Neuropharmacology, 2021; 189
32487727: Porcu A, Vaughan M, Nilsson A, Arimoto N, Lamia K, Welsh DK
Vulnerability to helpless behavior is regulated by the circadian clock component CRYPTOCHROME in the mouse nucleus accumbens.
The nucleus accumbens (NAc), a central component of the midbrain dopamine reward circuit, exhibits disturbed circadian rhythms in the postmortem brains of depressed patients. We hypothesized that normal mood regulation requires proper circadian timing in the NAc, and that mood disorders are associated with dysfunctions of the NAc cellular circadian clock. In mice exhibiting stress-induced depression-like behavior (helplessness), we found altered circadian clock function and high nighttime expression of the core circadian clock component CRYPTOCHROME (CRY) in the NAc. In the NAc of helpless mice, we found that higher expression of CRY is associated with decreased activation of dopamine 1 receptor-expressing medium spiny neurons (D1R-MSNs). Furthermore, D1R-MSN-specific CRY-knockdown in the NAc reduced susceptibility to stress-induced helplessness and increased NAc neuronal activation at night. Finally, we show that CRY inhibits D1R-induced G protein activation, likely by interacting with the Gs protein. Altered circadian rhythms and CRY expression were also observed in human fibroblasts from major depressive disorder patients. Our data reveal a causal role for CRY in regulating the midbrain dopamine reward system, and provide a mechanistic link between the NAc circadian clock and vulnerability to depression.
Proc Natl Acad Sci U S A, 2020; 117
31625128: Porcu A, Gonzalez R, McCarthy MJ
Pharmacological Manipulation of the Circadian Clock: A Possible Approach to the Management of Bipolar Disorder.
Bipolar disorder (BD) is a mood disorder with genetic and neurobiological underpinnings, characterized primarily by recurrent episodes of mania and depression, with notable disruptions in rhythmic behaviors such as sleep, energy, appetite and attention. The chronobiological links to BD are further supported by the effectiveness of various treatment modalities such as bright light, circadian phase advance, and mood-stabilizing drugs such as lithium that have effects on the circadian clock. Over the past 30 years, the neurobiology of the circadian clock has been exquisitely described and there now exists a detailed knowledge of key signaling pathways, neurotransmitters and signaling mechanisms that regulate various dimensions of circadian clock function. With this new wealth of information, it is becoming increasingly plausible that new drugs for BD could be made by targeting molecular elements of the circadian clock. However, circadian rhythms are multidimensional and complex, involving unique, time-dependent factors that are not typically considered in drug development. We review the organization of the circadian clock in the central nervous system and briefly summarize data implicating the circadian clock in BD. We then consider some of the unique aspects of the circadian clock as a drug target in BD, discuss key methodological considerations and evaluate some of the candidate clock pathways and systems that could serve as potential targets for novel mood stabilizers. We expect this work will serve as a roadmap to facilitate the development of compounds acting on the circadian clock for the treatment of BD.
CNS Drugs, 2019; 33
29861175: Walton ZE, Patel CH, Brooks RC, Yu Y, Ibrahim-Hashim A, Riddle M, Porcu A, Jiang T, Ecker BL, Tameire F, Koumenis C, Weeraratna AT, Welsh DK, Gillies R, Alwine JC, Zhang L, Powell JD, Dang CV
Acid Suspends the Circadian Clock in Hypoxia through Inhibition of mTOR.
Recent reports indicate that hypoxia influences the circadian clock through the transcriptional activities of hypoxia-inducible factors (HIFs) at clock genes. Unexpectedly, we uncover a profound disruption of the circadian clock and diurnal transcriptome when hypoxic cells are permitted to acidify to recapitulate the tumor microenvironment. Buffering against acidification or inhibiting lactic acid production fully rescues circadian oscillation. Acidification of several human and murine cell lines, as well as primary murine T cells, suppresses mechanistic target of rapamycin complex 1 (mTORC1) signaling, a key regulator of translation in response to metabolic status. We find that acid drives peripheral redistribution of normally perinuclear lysosomes away from perinuclear RHEB, thereby inhibiting the activity of lysosome-bound mTOR. Restoring mTORC1 signaling and the translation it governs rescues clock oscillation. Our findings thus reveal a model in which acid produced during the cellular metabolic response to hypoxia suppresses the circadian clock through diminished translation of clock constituents.
Cell, 2018; 174
29681926: Porcu A, Riddle M, Dulcis D, Welsh DK
Photoperiod-Induced Neuroplasticity in the Circadian System.
Seasonal changes in light exposure have profound effects on behavioral and physiological functions in many species,

including effects on mood and cognitive function in humans. The mammalian brain's master circadian clock, the suprachiasmatic nucleus (SCN), transmits information about external light conditions to other brain regions, including some implicated in mood and cognition. Although the detailed mechanisms are not yet known, the SCN undergoes highly plastic changes at the cellular and network levels under different light conditions. We therefore propose that the SCN may be an essential mediator of the effects of seasonal changes of day length on mental health. In this review, we explore various forms of neuroplasticity that occur in the SCN and other brain regions to facilitate seasonal adaptation, particularly altered phase distribution of cellular circadian oscillators in the SCN and changes in hypothalamic neurotransmitter expression.
Neural Plast, 2018; 2018
29407764: Porcu A, Melis M, Turecek R, Ullrich C, Mocci I, Bettler B, Gessa GL, Castelli MP
Rimonabant, a potent CB1 cannabinoid receptor antagonist, is a Gα protein inhibitor.
Rimonabant is a potent and selective cannabinoid CB1 receptor antagonist widely used in animal and clinical studies. Besides its antagonistic properties, numerous studies have shown that, at micromolar concentrations rimonabant behaves as an inverse agonist at CB1 receptors. The mechanism underpinning this activity is unclear. Here we show that micromolar concentrations of rimonabant inhibited Gα-type G proteins, resulting in a receptor-independent block of G protein signaling. Accordingly, rimonabant decreased basal and agonist stimulated [S]GTPγS binding to cortical membranes of CB1- and GABA-receptor KO mice and Chinese Hamster Ovary (CHO) cell membranes stably transfected with GABA or D2 dopamine receptors. The structural analog of rimonabant, AM251, decreased basal and baclofen-stimulated GTPγS binding to rat cortical and CHO cell membranes expressing GABA receptors. Rimonabant prevented G protein-mediated GABA and D2 dopamine receptor signaling to adenylyl cyclase in Human Embryonic Kidney 293 cells and to G protein-coupled inwardly rectifying K channels (GIRK) in midbrain dopamine neurons of CB1 KO mice. Rimonabant suppressed GIRK gating induced by GTPγS in CHO cells transfected with GIRK, consistent with a receptor-independent action. Bioluminescent resonance energy transfer (BRET) measurements in living CHO cells showed that, in presence or absence of co-expressed GABA receptors, rimonabant stabilized the heterotrimeric Gαi/o-protein complex and prevented conformational rearrangements induced by GABA receptor activation. Rimonabant failed to inhibit Gαs-mediated signaling, supporting its specificity for Gα-type G proteins. The inhibition of Gα protein provides a new site of rimonabant action that may help to understand its pharmacological and toxicological effects occurring at high concentrations.
Neuropharmacology, 2018; 133
27578262: Porcu A, Lobina C, Giunta D, Solinas M, Mugnaini C, Castelli MP
In vitro and in vivo pharmacological characterization of SSD114, a novel GABAB positive allosteric modulator.
Positive allosteric modulators (PAMs) of the GABA receptor have emerged as a novel approach to the pharmacological manipulation of the GABA receptor, enhancing the effects of receptor agonists with few side effects. Here, we identified N-cyclohexyl-4-methoxy-6-(4-(trifluoromethyl)phenyl)pyrimidin-2-amine (SSD114) as a new compound with activity as a GABA PAM in in vitro and in vivo assays. SSD114 potentiated GABA-stimulated [S]GTPγS binding to native GABA receptors, whereas it had no effect when used alone. Its effect on GTPγS stimulation was suppressed when GABA-induced activation was blocked with CGP54626, a competitive antagonist of the GABA receptor. SSD114 failed to potentiate WIN55,212,2-, morphine- and quinpirole-induced [S]GTPγS binding to cortical and striatal membranes, respectively, indicating that it is a selective GABA PAM. Increasing SSD114 fixed concentrations induced a leftward shift of the GABA concentration-response curve, enhancing the potency of GABA rather than its efficacy. SSD114 concentration-response curves in the presence of fixed concentrations of GABA (1, 10, and 20μM) revealed a potentiating effect on GABA-stimulated binding of [S]GTPγS to rat cortical membranes, with EC values in the low micromolar range. Bioluminescence resonance energy transfer (BRET) experiments in Chinese Hamster Ovary (CHO)-cells expressing GABA receptors showed that SSD114 potentiates the GABA inhibition of adenylyl-cyclase mediated by GABA receptors. Our compound is also effective in vivo potentiating baclofen-induced sedation/hypnosis in mice, with no effect when tested alone. These findings indicate that SSD114, a molecule with a different chemical structure compared to known GABA PAMs, is a novel GABA PAM with potential usefulness in the GABA-receptor research field.
Eur J Pharmacol, 2016; 791
26686391: De Luca MA, Castelli MP, Loi B, Porcu A, Martorelli M, Miliano C, Kellett K, Davidson C, Stair JL, Schifano F, Di Chiara G
Native CB1 receptor affinity, intrinsic activity and accumbens shell dopamine stimulant properties of third generation SPICE/K2 cannabinoids: BB-22, 5F-PB-22, 5F-AKB-48 and STS-135.
In order to investigate the in vivo dopamine (DA) stimulant properties of selected 3rd generation Spice/K2 cannabinoids, BB-22, 5F-PB-22, 5F-AKB-48 and STS-135, their in vitro affinity and agonist potency at native rat and mice CB1 receptors was studied. The compounds bind with high affinity to CB1 receptors in rat cerebral cortex homogenates and stimulate CB1-induced [(35)S]GTPγS binding with high potency and efficacy. BB-22 and 5F-PB-22 showed the lowest Ki of binding to CB1 receptors (0.11 and 0.13 nM), i.e., 30 and 26 times lower respectively than that of JWH-018 (3.38 nM), and a potency (EC50,

2.9 and 3.7 nM, respectively) and efficacy (Emax, 217% and 203%, respectively) as CB1 agonists higher than JWH-018 (EC50, 20.2 nM; Emax, 163%). 5F-AKB-48 and STS-135 had higher Ki for CB1 binding, higher EC50 and lower Emax as CB1 agonists than BB-22 and 5F-PB-22 but still comparatively more favourable than JWH-018. The agonist properties of all the compounds were abolished or drastically reduced by the CB1 antagonist/inverse agonist AM251 (0.1 μM). No activation of G-protein was observed in CB1-KO mice. BB-22 (0.003-0.01 mg/kg i.v.) increased dialysate DA in the accumbens shell but not in the core or in the medial prefrontal cortex, with a bell shaped dose-response curve and an effect at 0.01 mg/kg and a biphasic time-course. Systemic AM251 (1.0 mg/kg i.p.) completely prevented the stimulant effect of BB-22 on dialysate DA in the NAc shell. All the other compounds increased dialysate DA in the NAc shell at doses consistent with their in vitro affinity for CB1 receptors (5F-PB-22, 0.01 mg/kg; 5F-AKB-48, 0.1 mg/kg; STS-135, 0.15 mg/kg i.v.). 3rd generation cannabinoids can be even more potent and super-high CB1 receptor agonists compared to JWH-018. Future research will try to establish if these properties can explain the high toxicity and lethality associated with these compounds.
Neuropharmacology, 2016; 105

23544432: Mugnaini C, Pedani V, Casu A, Lobina C, Casti A, Maccioni P, Porcu A, Giunta D, Lamponi S, Solinas M, Dragoni S, Valoti M, Colombo G, Castelli MP, Gessa GL, Corelli F
Synthesis and pharmacological characterization of 2-(acylamino)thiophene derivatives as metabolically stable, orally effective, positive allosteric modulators of the GABAB receptor.
Two recently reported hit compounds, COR627 and COR628, underpinned the development of a series of 2-(acylamino)thiophene derivatives. Some of these compounds displayed significant activity in vitro as positive allosteric modulators of the GABAB receptor by potentiating GTPγS stimulation induced by GABA at 2.5 and 25 μM while failing to exhibit intrinsic agonist activity. Compounds were also found to be effective in vivo, potentiating baclofen-induced sedation/hypnosis in DBA mice when administered either intraperitoneally or intragastrically. Although displaying a lower potency in vitro than the reference compound GS39783, the new compounds 6, 10, and 11 exhibited a higher efficacy in vivo: combination of these compounds with a per se nonsedative dose of baclofen resulted in shorter onset and longer duration of the loss of righting reflex in mice. Test compounds showed cytotoxic effects at concentrations comparable to or higher than those of GS39783 or BHF177.
J Med Chem, 2013; 56

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BOARD NUMBER: S06-464

INFERRING LIGAND-RECEPTOR INTERACTIONS BETWEEN GABAERGIC AND GLUTAMATERGIC CELLS DURING SOMATOSENSORY CORTEX DEVELOPMENT

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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The cerebral cortex contains two main neuronal types: excitatory glutamatergic neurons (GlutNs) & inhibitory GABAergic interneurons (INs) that each comprise different subtypes differentially distributing in six layers in a stereotyped fashion. GlutNs settle in the cortex before INs during cortex development, and several studies suggest that the preferred laminar allocation of IN subtypes to specific cortical layers is extrinsically regulated by GlutN subtypes. However, the cellular and molecular mechanisms by which different IN subtypes are allocated to specific cortical layers remain elusive. We hypothesize that a ligand/receptor (LR) molecular code orchestrates stereotypical laminar allocation and synaptic connectivity between INs and GlutNs. In order to identify this molecular code, we performed single-cell RNA sequencing from somatosensory cortex of mice at P0, P2, P5, P8, P16 and P30, which span the neonatal critical window of cortex invasion and synaptic connectivity by INs. By integrating to this collection published datasets corresponding to earlier and later time points using advanced bioinformatic methods, we could identify every main neuronal cell type and its transcriptomic evolution along 16 time points ranging from E11 to P56. To decrypt a putative cell-cell interaction molecular code by which IN subtypes would be recruited by and connect specific domains of GlutN subtypes during the neonatal critical window, we constructed a ligand-receptor atlas between these cell types. This bioinformatic ligand-receptor atlas unmasks shared and specific interactions that might instruct the stereotypic lamination and/or connectivity of neuronal subtypes.

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BOARD NUMBER: S06-465

ACTIVATION OF THE GABA-A RECEPTOR IN THE NEONATE MOUSE RESULTED IN SEX-DEPENDENT CORTICAL ALTERATIONS DURING DEVELOPMENT AND BEHAVIOURAL DEFICITS IN ADULTHOOD

POSTER SESSION 06 - SECTION: E/I BALANCE AND PLASTICITY IN DEVELOPING CIRCUITS

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Introduction: Appropriate excitatory/inhibitory balance is crucial for correct establishment of the neural circuit underlying normal brain development. Disruptions to this balance have been proposed in the aetiology of several neurodevelopmental disorders (NDDs), in which GABAergic signalling is impaired and neuronal morphology is changed. There are sex differences in incidence of NDDs and therefore it is possible that the neurobiological effect of altered GABA signalling is also sex-dependent. Here, we aimed to investigate whether pharmacological alteration of GABA signalling in early development results in sex-dependent changes in neuronal development and behaviour. Methods: Cortical neuronal cultures derived from postnatal day (P) 0 male and female mice were treated on Day-In-Vitro (DIV) 3 with $GABA_A$ receptor agonist muscimol at 0 and 5μM doses and assessed on DIV7. In vivo experiments involved performing intraperitoneal injections of 0.5mg/kg muscimol or saline in postnatal male and female mice P3, 4 and 5. Brains were collected for cortical structural analysis at P6 and 20. Behavioural studies were conducted at P60. Results: In the P0 cultures, treatment with 5μM of Muscimol reduced neural branching complexity of neurons cultured from male mice and reduced neurite length of females. In vivo experiments showed muscimol treatment increased cortical thickness and was statistically significant only within the male population. These changes were accompanied by behavioural sex-dependent impairments in adulthood. Conclusion: These data demonstrated that excitatory/inhibitory imbalance, impairs neural development by altering neuronal morpho-functional maturation in a sex-dependent manner, likely explaining the varied pathophysiology and neurodevelopmental diagnosis that result from the excitatory/inhibitory imbalance.

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BOARD NUMBER: S06-466

DIFFERENTIAL CONTROL OF PYRAMIDAL NEURON ACTIVITY IN TWO VISUAL CORTICAL AREAS BY AN ELUSIVE INHIBITORY INTERNEURON SUBTYPE

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Cortical inhibitory GABAergic neurons are a highly heterogeneous cell population, whose specific connectivity patterns contribute to a division of labor within cortical circuits. Perisomatic inhibition is provided by parvalbumin (PV)- and cannabinoid receptor type 1 (CB1)-expressing basket cells (BCs). Despite the large body of evidence indicating the crucial role of PV cells in orchestrating cortical networks, the connectivity and function of CB1 BCs is still unknown. In order to identify the circuit properties of CB1 interneurons, we combined mouse genetics, *in vitro* whole-cell patch clamp recordings and *in vivo* two-photon calcium imaging in awake head-fixed mice during resting and running periods. We found that the intra- and infra-laminar connectivity pattern of CB1 BCs strongly differs between primary (V1) and medial secondary visual cortex (V2M). Tonic CB1 signaling suppresses GABA release from CB1 BCs onto layer 2/3 pyramidal neurons (PNs) in V2M, but not V1. In vivo, visual area-specific tonic CB1 signaling is responsible for a much higher but less coordinated spontaneous activity of PNs in V2M, as compared to V1. Numerical simulations confirmed that area-specific morpho-functional properties of CB1 interneurons are essential for the different cortical dynamics observed in V1 and V2M. We are currently investigating how CB1-mediated plasticity of inhibition affects locomotion-induced modulation of PN activity in the two areas. Our findings indicate that CB1 BCs operate a visual area-dependent dynamic inhibitory control of PNs that will likely play a major role in visual perception.

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BOARD NUMBER: S06-467

DORSAL RAPHE MODULATES THE ONGOING ACTIVITY, FUNCTIONAL CONNECTIVITY AND SENSORY RESPONSES OF ZEBRAFISH FOREBRAIN CIRCUITS

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Our emotions have significant impact on the way we perceive the world surrounding us. In fact, making correct decisions by integrating the environmental stimuli with our internal state is crucial for survival. However, neural mechanisms underlying this integration are unknown. The dorsal raphe nucleus (DRN) is implicated in regulation of the internal state through its serotonergic projections. Here, we investigated the modulation of the ongoing forebrain activity, functional connectivity, sensory processing, and behavior by the serotonergic projections of the DRN. To do this, we chemogenetically ablated the serotonergic neurons in the DRN of the zebrafish, and compared the neural, and behavioral activity between the control animals and DRN-ablated animals. Using two-photon calcium imaging, we recorded the forebrain activity of 3 weeks-old juvenile zebrafish at cellular resolution, together with animal's locomotory behavior in virtual reality. We observed the DRN ablation led to prominent alterations in functional connectivity and sensory responses to aversive mechanical vibrations, across multiple forebrain regions associated with ancestral limbic circuitry. We also revealed that DRN ablation resulted in an increase in animals' behavioral response to novel environments and aversive mechanical vibrations. We are currently investigating how the activity of DRN relates to the activity of distinct forebrain circuitry. Taken together, our data point to broad regulation of forebrain circuits by DRN.

Pubmed:

34416179: Bartoszek EM, Ostenrath AM, Jetti SK, Serneels B, Mutlu AK, Chau KTP, Yaksi E
Ongoing habenular activity is driven by forebrain networks and modulated by olfactory stimuli.
Ongoing neural activity, which represents internal brain states, is constantly modulated by the sensory information that is generated by the environment. In this study, we show that the habenular circuits act as a major brain hub integrating the structured ongoing activity of the limbic forebrain circuitry and the olfactory information. We demonstrate that ancestral homologs of amygdala and hippocampus in zebrafish forebrain are the major drivers of ongoing habenular activity. We also reveal that odor stimuli can modulate the activity of specific habenular neurons that are driven by this forebrain circuitry. Our results highlight a major role for the olfactory system in regulating the ongoing activity of the habenula and the forebrain, thereby altering brain's internal states.
Curr Biol, 2021; 31
32917624: Fore S, Acuña-Hinrichsen F, Mutlu KA, Bartoszek EM, Serneels B, Faturos NG, Chau KTP, Cosacak MI, Verdugo CD, Palumbo F, Ringers C, Jurisch-Yaksi N, Kizil C, Yaksi E
Functional properties of habenular neurons are determined by developmental stage and sequential neurogenesis.
The developing brain undergoes drastic alterations. Here, we investigated developmental changes in the habenula, a brain region that mediates behavioral flexibility during learning, social interactions, and aversive experiences. We showed that developing habenular circuits exhibit multiple alterations that lead to an increase in the structural and functional diversity of cell types, inputs, and functional modules. As the habenula develops, it sequentially transforms into a multisensory brain region that can process visual, olfactory, mechanosensory, and aversive stimuli. Moreover, we observed that the habenular neurons display spatiotemporally structured spontaneous activity that shows prominent alterations and refinement with age. These alterations in habenular activity are accompanied by sequential neurogenesis and the integration of distinct neural clusters across development. Last, we revealed that habenular neurons with distinct functional properties are born sequentially at distinct developmental time windows. Our results highlight a strong link between the functional properties of habenular neurons and their precise birthdate.
Sci Adv, 2020; 6
29141024: Padula MC, Schaer M, Scariati E, Mutlu AK, Zöller D, Schneider M, Eliez S
Quantifying indices of short- and long-range white matter connectivity at each cortical vertex.

Several neurodevelopmental diseases are characterized by impairments in cortical morphology along with altered white matter connectivity. However, the relationship between these two measures is not yet clear. In this study, we propose a novel methodology to compute and display metrics of white matter connectivity at each cortical point. After co-registering the extremities of the tractography streamlines with the cortical surface, we computed two measures of connectivity at each cortical vertex: the mean tracts' length, and the proportion of short- and long-range connections. The proposed measures were tested in a clinical sample of 62 patients with 22q11.2 deletion syndrome (22q11DS) and 57 typically developing individuals. Using these novel measures, we achieved a fine-grained visualization of the white matter connectivity patterns at each vertex of the cortical surface. We observed an intriguing pattern of both increased and decreased short- and long-range connectivity in 22q11DS, that provides novel information about the nature and topology of white matter alterations in the syndrome. We argue that the method presented in this study opens avenues for additional analyses of the relationship between cortical properties and patterns of underlying structural connectivity, which will help clarifying the intrinsic mechanisms that lead to altered brain structure in neurodevelopmental disorders.
PLoS One, 2017; 12
28502658: Esquivelzeta Rabell J, Mutlu K, Noutel J, Martin Del Olmo P, Haesler S
Spontaneous Rapid Odor Source Localization Behavior Requires Interhemispheric Communication.
Navigation, finding food sources, and avoiding danger critically depend on the identification and spatial localization of airborne chemicals. When monitoring the olfactory environment, rodents spontaneously engage in active olfactory sampling behavior, also referred to as exploratory sniffing [1]. Exploratory sniffing is characterized by stereotypical high-frequency respiration, which is also reliably evoked by novel odorant stimuli [2, 3]. To study novelty-induced exploratory sniffing, we developed a novel, non-contact method for measuring respiration by infrared (IR) thermography in a behavioral paradigm in which novel and familiar stimuli are presented to head-restrained mice. We validated the method by simultaneously performing nasal pressure measurements, a commonly used invasive approach [2, 4], and confirmed highly reliable detection of inhalation onsets. We further discovered that mice actively orient their nostrils toward novel, previously unexperienced, smells. In line with the remarkable speed of olfactory processing reported previously [3, 5, 6], we find that mice initiate their response already within the first sniff after odor onset. Moreover, transecting the anterior commissure (AC) disrupted orienting, indicating that the orienting response requires interhemispheric transfer of information. This suggests that mice compare odorant information obtained from the two bilaterally symmetric nostrils to locate the source of the novel odorant. We further demonstrate that asymmetric activation of the anterior olfactory nucleus (AON) is both necessary and sufficient for eliciting orienting responses. These findings support the view that the AON plays an important role in the internostril difference comparison underlying rapid odor source localization.
Curr Biol, 2017; 27
24845162: Fountain DM, Schaer M, Mutlu AK, Schneider M, Debbané M, Eliez S
Congenital heart disease is associated with reduced cortical and hippocampal volume in patients with 22q11.2 deletion syndrome.
There is increasing evidence that congenital heart disease (CHD) affects brain structure, but little is known about the long-term trajectory of brain maturation and its impact on the cognitive development of patients with CHD. We proposed to address this question in a longitudinally-followed cohort of individuals with 22q11.2 deletion syndrome (22q11DS), the most common microdeletion syndrome in humans.
Cortex, 2014; 57
23999732: Schneider M, Schaer M, Mutlu AK, Menghetti S, Glaser B, Debbané M, Eliez S
Clinical and cognitive risk factors for psychotic symptoms in 22q11.2 deletion syndrome: a transversal and longitudinal approach.
22q11.2 deletion syndrome (22q11DS) is associated with increased risk for schizophrenia. Better identifying risk factors for the emergence of psychotic symptoms in this population is needed to improve clinical assessment and early interventions. Schizophrenia spectrum disorders, hallucinations and delusions were characterized in an original sample of 104 individuals with 22q11DS. Further analysis of positive and negative symptoms was performed in a subsample of 59 individuals. Finally, longitudinal data available in 56 patients were used to explore the developmental trajectories of psychotic symptoms as well as the associations between psychotic symptoms and cognitive functioning. Schizophrenia spectrum disorders and psychotic symptoms were frequent in adolescent and adults with 22q11DS. The severity of hallucinations and non-persecutory delusional ideas discriminated patients at ultra-high risk for conversion to psychosis. Whereas approximately one-third of patients experienced an emergence of psychotic symptoms during a 4-year interval, 20 % displayed transient symptoms. Individuals with psychotic symptoms were characterized by a lower cognitive functioning in the context of the 22q11DS. The present study adds important data on the characteristics and developmental trajectory of psychotic symptoms in this population. This information may ultimately help clinicians dealing with these patients to reduce the duration of untreated psychosis and improve outcome.

Eur Child Adolesc Psychiatry, 2014; 23
23721724: Mutlu AK, Schneider M, Debbané M, Badoud D, Eliez S, Schaer M
Sex differences in thickness, and folding developments throughout the cortex.
While significant differences in male and female brain structures have commonly been reported, only a few studies have focused on the sex differences in the way the cortex matures over time. Here, we investigated cortical thickness maturation between the age of 6 to 30 years, using 209 longitudinally-acquired brain MRI scans. Significant sex differences in the trajectories of cortical thickness change with age were evidenced using non-linear mixed effects models. Similar statistical analyses were computed to quantify the differences between cortical gyrification changes with age in males and females. During adolescence, we observed a statistically significant higher rate of cortical thinning in females compared to males in the right temporal regions, the left temporoparietal junction and the left orbitofrontal cortex. This finding is interpreted as a faster maturation of the social brain areas in females. Concomitantly, statistically significant sex differences in cortical folding changes with age were observed only in one cluster of the right prefrontal regions, suggesting that the mechanisms underlying cortical thickness and gyrification changes with age are quite distinct. Sexual dimorphism in the developmental course of the cortical maturation may be associated with the different age of onset and clinical presentation of many psychiatric disorders between males and females.
Neuroimage, 2013; 82
23282849: Bartussek J, Mutlu AK, Zapotocky M, Fry SN
Limit-cycle-based control of the myogenic wingbeat rhythm in the fruit fly Drosophila.
In many animals, rhythmic motor activity is governed by neural limit cycle oscillations under the control of sensory feedback. In the fruit fly Drosophila melanogaster, the wingbeat rhythm is generated myogenically by stretch-activated muscles and hence independently from direct neural input. In this study, we explored if generation and cycle-by-cycle control of Drosophila's wingbeat are functionally separated, or if the steering muscles instead couple into the myogenic rhythm as a weak forcing of a limit cycle oscillator. We behaviourally tested tethered flying flies for characteristic properties of limit cycle oscillators. To this end, we mechanically stimulated the fly's 'gyroscopic' organs, the halteres, and determined the phase relationship between the wing motion and stimulus. The flies synchronized with the stimulus for specific ranges of stimulus amplitude and frequency, revealing the characteristic Arnol'd tongues of a forced limit cycle oscillator. Rapid periodic modulation of the wingbeat frequency prior to locking demonstrates the involvement of the fast steering muscles in the observed control of the wingbeat frequency. We propose that the mechanical forcing of a myogenic limit cycle oscillator permits flies to avoid the comparatively slow control based on a neural central pattern generator.
J R Soc Interface, 2013; 10
29501561: Mutlu K, Rabell JE, Martin Del Olmo P, Haesler S
IR thermography-based monitoring of respiration phase without image segmentation.
Respiratory rate is an essential parameter in biomedical research and clinical applications. Most respiration measurement techniques in preclinical animal models require surgical implantation of sensors. Current clinical measurement modalities typically involve attachment of sensors to the patient, causing discomfort. We have previously developed a non-contact approach to measuring respiration phase in head-restrained rodents using infrared (IR) thermography. While the non-invasive nature of IR thermography offers many advantages, it also bears the complexity of extracting respiration signals from videos. Previously reported algorithms involve image segmentation to identify the nose in IR videos and extract breathing-relevant pixels which is particularly challenging if the videos have low contrast or suffer from suboptimal focusing.
J Neurosci Methods, 2018; 301

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-468

ASYMMETRICAL INFLUENCE OF THE SUPERIOR COLLICULUS ON THE MIDBRAIN DOPAMINERGIC SYSTEM VIA IPSILATERAL DIRECT EXCITATION AND CONTRALATERAL INDIRECT INHIBITION RELIED BY THE ROSTROMEDIAL TEGMENTAL NUCLEUS

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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[1]Institute of Zoology and Biomedical Research, Jagiellonian University, Department Of Neurophysiology And Chronobiology, Krakow, Poland, [2]Institute of Applied Psychology, Jagiellonian University, Department Of Neurobiology And Neuropsychology, Krakow, Poland

Dopaminergic neurons of the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) control orienting and approach toward important stimuli; this information is provided primarily by the superior colliculus (SC) which processes contralateral sensory data. Since interhemispheric imbalance of striatal dopamine release impacts the direction of animal's movement, we aimed to describe lateralization of neuronal pathway descending form SC to the dopaminergic system and its inhibitory input – the rostromedial tegmental nucleus (RMTg). The anatomy of the circuit was studied using anterograde, retrograde and transsynaptic tract tracing techniques. Multichannel electrophysiological recordings *in vivo* and *ex vivo*, combined with optogenetics were performed to study the circuit's physiology. We demonstrated that SC innervates predominantly ipsilateral SNc/VTA. Simultaneously, SC innervates contralateral RMTg; these recipient neurons send dense ipsilateral projection to SNc/VTA. Unilateral SC activation excited primarily contralateral RMTg neurons. SC stimulation *in vivo* was only slightly more prevalent in exciting ipsilateral dopaminergic neurons, however, activating SC-originating axon terminals *ex vivo* excited exclusively ipsilateral dopaminergic neurons. By manipulating SC-contralateral RMTg circuit we demonstrated SC's ability to inhibit contralateral dopaminergic neurons. Overall, SC may excite ipsilateral dopaminergic neurons directly while inhibiting contralateral ones indirectly via RMTg. This may cause an interhemispheric imbalance of striatal dopamine release, which in turn may affect animal's direction of movement and orientation. Since SC processes sensory information from the contralateral side of the body, the described brain circuit would promote orienting and approach in the direction of incoming sensory stimuli. Funding: National Science Centre, 2017/27/N/NZ4/00785 and 2020/36/T/NZ4/00341.

Pubmed:
28606743: Solecki WB, Szklarczyk K, Klasa A, Pradel K, Dobrzański G, Przewłocki R
Alpha-adrenergic receptor blockade in the VTA modulates fear memories and stress responses.
Activity of the ventral tegmental area (VTA) and its terminals has been implicated in the Pavlovian associative learning of both stressful and rewarding stimuli. However, the role of the VTA noradrenergic signaling in fear responses remains unclear. We aimed to examine how alpha-adrenergic receptor (α-AR) signaling in the VTA affects conditioned fear. The role of α-AR was assessed using the micro-infusions into the VTA of the selective antagonists (0.1-1μg/0.5μl prazosin and 1μg/0.5μl terazosin) in acquisition and expression of fear memory. In addition, we performed control experiments with α-AR blockade in the mammillary bodies (MB) - a brain region with α-AR expression adjacent to the VTA. Intra-VTA but not intra-MB α-AR blockade prevented formation and retrieval of fear memories. Importantly, local administration of α-AR antagonists did not influence footshock sensitivity, locomotion or anxiety-like behaviors. Similarly, α-AR blockade in the VTA had no effects on negative affect measured as number of 22kHz ultrasonic vocalizations during fear conditioning training. We propose that noradrenergic signaling in the VTA via α-AR regulates formation and retrieval of fear memories but not other behavioral responses to stressful environmental stimuli. It enhances the encoding of environmental stimuli by the VTA to form and retrieve conditioned fear memories and to predict future behavioral outcomes. Our results provide novel insight into the role of the VTA α-AR signaling in the regulation of stress responsiveness and fear memory.
Eur Neuropsychopharmacol, 2017; 27
28635140: Solecki WB, Szklarczyk K, Pradel K, Kwiatkowska K, Dobrzański G, Przewłocki R
Noradrenergic signaling in the VTA modulates cocaine craving.
Exposure to drug-associated cues evokes drug-seeking behavior and is regarded as a major cause of relapse. Conditional stimulus upregulates noradrenaline (NA) system activity, but the drug-seeking behavior depends particularly on phasic

dopamine signaling downstream from the ventral tegmental area (VTA). The VTA dopamine-ergic activity is regulated via the signaling of alpha -adrenergic and alpha -adrenergic receptors (α -ARs and α -ARs); thus, the impact of the conditional stimulus on drug-seeking behavior might involve NAergic signaling in the VTA. To date, the role of VTA ARs in regulating cocaine seeking was not studied. We found that cocaine seeking under extinction conditions in male Sprague-Dawley rats was attenuated by intra-VTA prazosin or terazosin-two selective α -AR antagonists. In contrast, cocaine seeking was facilitated by intra-VTA administration of the selective α -AR agonist phenylephrine as well as α -AR antagonist RX 821002, whereas the selective β-AR antagonist propranolol had no effects. In addition, blockade of α -AR in the VTA prevented α -AR antagonist-induced enhancement of cocaine seeking. Importantly, the potential non-specific effects of the VTA AR blockade on cocaine seeking could be excluded, because none of the AR antagonists influenced sucrose seeking under extinction conditions or locomotor activity in the open field test. These results demonstrate that NAergic signaling potently and selectively regulates cocaine seeking during early cocaine withdrawal via VTA α -AR and α -AR but not β-AR. Our findings provide new insight into the NAergic mechanisms that underlie cocaine craving.
Addict Biol, 2018; 23

29138105: Pradel K, Blasiak T, Solecki WB
Adrenergic Receptor Agonists' Modulation of Dopaminergic and Non-dopaminergic Neurons in the Ventral Tegmental Area.
The ventral tegmental area (VTA) neuronal population consists of dopaminergic (DAergic) and non-DAergic neurons (mainly GABAergic), the activity of which is intertwined with VTA behavioral functions. Both DAergic and GABAergic neurons in the VTA have been shown to express adrenergic receptors (ARs) and respond to AR stimulation. The aim of the present study was to demonstrate the effects of selective AR agonists on DAergic and non-DAergic neuronal activity in the central and lateral parts of the VTA using in vivo electrophysiological recording combined with microiontophoretic drug application in anaesthetized rats. Administration of phenylephrine, a selective α-AR agonist, while having an inhibitory effect on putative DAergic neurons (11% decrease in firing rate), induced a clear excitatory effect (59% increase in firing rate) on putative non-DAergic neurons. In contrast, application of clonidine, a selective α-AR agonist, or isoprenaline, a selective β-adrenergic receptor agonist, did not change the firing rate of either DAergic or non-DAergic neurons but influenced the firing pattern of non-DAergic cells only. Our results suggest that noradrenaline modulates activity of VTA neurons in vivo primarily via α, but also via β- and α-AR to a lesser extent. Furthermore, we show that α-AR activation has contrasting effects on putative DAergic and non-DAergic neurons. We hypothesize that the phenylephrine-induced inhibition of putative DAergic neurons results from activation of GABAergic terminals present at the site of drug application. Such a mechanism is further supported by the observed α-AR-induced excitation of putative GABAergic VTA neurons.
Neuroscience, 2018; 375

31478293: Solecki W, Wilczkowski M, Pradel K, Karwowska K, Kielbinski M, Drwięga G, Zajda K, Blasiak T, Soltys Z, Rajfur Z, Szklarczyk K, Przewłocki R
Effects of brief inhibition of the ventral tegmental area dopamine neurons on the cocaine seeking during abstinence.
Preclinical studies strongly suggest that cocaine seeking depends on the neuronal activity of the ventral tegmental area (VTA) and phasic dopaminergic (DA) signaling. Notably, VTA pharmacological inactivation or dopamine receptor blockade in the forebrain may induce behavioral inhibition in general and acute aversive states in particular, thus reducing cocaine seeking indirectly. Such artifacts hinder successful translation of these findings in clinical studies and practice. Here, we aimed to evaluate if dynamic VTA manipulations effectively reduce cocaine seeking. We used male tyrosine hydroxylase (TH) IRES-Cre rats along with optogenetic tools to inhibit directly and briefly VTA DA neurons during conditioned stimulus (CS)-induced cocaine seeking under extinction conditions. The behavioral effects of optogenetic inhibition were also assessed in the real-time dynamic place aversion, conditioned place aversion, and CS-induced food-seeking tests. We found that brief and nondysphoric/nonsedative pulses of VTA photo-inhibition (1 s every 9 s, ie, for 10% of time) attenuated CS-induced cocaine seeking under extinction conditions in rats expressing archaerhodopsin selectively on the TH neurons. Furthermore, direct inhibition of the VTA DA activity reduced CS-induced cocaine seeking 24 hours after photo-modulation. Importantly, such effect appears to be selective for cocaine seeking as similar inhibition of the VTA DA activity had no effect on CS-induced food seeking. Thus, briefly inhibiting VTA DA activity during CS-induced cocaine seeking drastically and selectively reduces seeking without behavioral artifacts such as sedation or dysphoria. Our results point to the therapeutic possibilities of coupling nonpharmacologic treatments with extinction training in reducing cocaine addiction.
Addict Biol, 2020; 25

32997815: Chrobok L, Jeczmien-Lazur JS, Pradel K, Klich JD, Bubka M, Wojcik M, Kepczynski M, Lewandowski MH
Circadian actions of orexins on the retinorecipient lateral geniculate complex in rat.
Rhythmic processes in living organisms are controlled by biological clocks. The orexinergic system of the lateral hypothalamus carries circadian information to provide arousal for the brain during the active phase. Here, we show that orexins exert an excitatory action in three parts of the lateral geniculate nucleus (LGN), in particular upon directly retinorecipient neurons in the non-image forming visual structures. We provide evidence for the high nocturnal levels of

orexins with stable circadian expression of predominant orexin receptor 2 in the LGN. Our data additionally establish the convergence of orexinergic and pituitary adenylate cyclase (PAC)-activating peptide/PAC1 receptor systems (used by melanopsin-expressing retinal ganglion cells), which directly regulates responses to the retinal input. These results help us better understand circadian orexinergic control over the non-image forming subcortical visual system, forming the animal's preparedness for the behaviourally active night.
J Physiol, 2021; 599
33741724: Pradel K, Drwięga G, Błasiak T
Superior Colliculus Controls the Activity of the Rostromedial Tegmental Nuclei in an Asymmetrical Manner.
Dopaminergic (DA) neurons of the midbrain are involved in controlling orienting and approach of animals toward relevant external stimuli. The firing of DA neurons is regulated by many brain structures; however, the sensory input is provided predominantly by the ipsilateral superior colliculus (SC). It is suggested that SC also innervates the contralateral rostromedial tegmental nucleus (RMTg)-the main inhibitory input to DA neurons. Therefore, this study aimed to describe the physiology and anatomy of the SC-RMTg pathway. To investigate the anatomic connections within the circuit of interest, anterograde, retrograde, and transsynaptic tract-tracing studies were performed on male Sprague Dawley rats. We have observed that RMTg is monosynaptically innervated predominantly by the lateral parts of the intermediate layer of the contralateral SC. To study the physiology of this neuronal pathway, we conducted electrophysiological experiments combined with optogenetics; the activity of RMTg neurons was recorded using silicon probes, while either contralateral or ipsilateral SC was optogenetically stimulated. Obtained results revealed that activation of the contralateral SC excites the majority of RMTg neurons, while stimulation of the ipsilateral SC evokes similar proportions of excitatory or inhibitory responses. Consequently, single-unit recordings showed that the activation of RMTg neurons innervated by the contralateral SC, or stimulation of contralateral SC-originating axon terminals within the RMTg, inhibits midbrain DA neurons. Together, the anatomy and physiology of the discovered brain circuit suggest its involvement in the orienting and motivation-driven locomotion of animals based on the direction of external sensory stimuli. Dopaminergic neurons are the target of predominantly ipsilateral, excitatory innervation originating from the superior colliculus. However, we demonstrate in our study that SC inhibits the activity of dopaminergic neurons on the contralateral side of the brain via the rostromedial tegmental nucleus. In this way, sensory information received by the animal from one hemifield could induce opposite effects on both sides of the dopaminergic system. It was shown that the side to which an animal directs its behavior is a manifestation of asymmetry in dopamine release between left and right striatum. Animals tend to move oppositely to the hemisphere with higher striatal dopamine concentration. This explains how the above-described circuit might guide the behavior of animals according to the direction of incoming sensory stimuli.
J Neurosci, 2021; 41
34053067: Chrobok L, Klich JD, Jeczmien-Lazur JS, Pradel K, Palus-Chramiec K, Sanetra AM, Piggins HD, Lewandowski MH
Daily changes in neuronal activities of the dorsal motor nucleus of the vagus under standard and high-fat diet.
Recently, we found that the dorsal vagal complex displays autonomous circadian timekeeping properties The dorsal motor nucleus of the vagus (DMV) is an executory part of this complex - a source of parasympathetic innervation of the gastrointestinal tract Here, we reveal daily changes in the neuronal activities of the rat DMV, including firing rate, intrinsic excitability and synaptic input - all of these peaking in the late day Additionally, we establish that short term high-fat diet disrupts these daily rhythms, boosting the variability in the firing rate, but blunting the DMV responsiveness to ingestive cues These results help us better understand daily control over parasympathetic outflow and provide evidence on its dependence on the high-fat diet ABSTRACT: The suprachiasmatic nuclei (SCN) of the hypothalamus function as the brain's primary circadian clock, but circadian clock genes are also rhythmically expressed in several extra-SCN brain sites where they can exert local temporal control over physiology and behaviour. Recently, we found that the hindbrain dorsal vagal complex possesses strong daily timekeeping capabilities, with the area postrema and nucleus of the solitary tract exhibiting the most robust clock properties. The possibility that the executory part of this complex - the dorsal motor nucleus of the vagus (DMV) - also exhibits daily changes has not been extensively studied. The DMV is the source of vagal efferent motoneurons that regulate gastric motility and emptying and consequently influence meal size and energy homeostasis. We used a combination of multi-channel electrophysiology and patch clamp recordings to gain insight into effects of time of day and diet on these DMV cells. We found that DMV neurons increase their spontaneous activity, excitability and responsiveness to metabolic neuromodulators at late day and this was paralleled with an enhanced synaptic input to these neurons. A high-fat diet typically damps circadian rhythms, but we found that consumption of a high-fat diet paradoxically amplified daily variation of DMV neuronal activity, while blunting the neurons responsiveness to metabolic neuromodulators. In summary, we show for the first time that DMV neural activity changes with time of day, with this temporal variation modulated by diet. These findings have clear implications for our understanding of the daily control of vagal efferents and parasympathetic outflow.

J Physiol, 2022; 600
34490628: Chrobok L, Klich JD, Sanetra AM, Jeczmien-Lazur JS, Pradel K, Palus-Chramiec K, Kepczynski M, Piggins HD, Lewandowski MH
Rhythmic neuronal activities of the rat nucleus of the solitary tract are impaired by high-fat diet - implications for daily control of satiety.
Temporal partitioning of daily food intake is crucial for survival and involves the integration of internal circadian states and external influences such as the light-dark cycle and dietary composition. These intrinsic and extrinsic factors are interdependent with misalignment of circadian rhythms promoting body weight gain, while consumption of a calorie-dense diet elevates the risk of obesity and blunts circadian rhythms. Recently, we defined the circadian properties of the dorsal vagal complex of the brainstem, a structure implicated in the control of food intake and autonomic tone, but whether and how 24 h rhythms in this area are influenced by diet remains unresolved. Here we focused on a key structure of this complex, the nucleus of the solitary tract (NTS). We used a combination of immunohistochemical and electrophysiological approaches together with daily monitoring of body weight and food intake to interrogate how the neuronal rhythms of the NTS are affected by a high-fat diet. We report that short-term consumption of a high-fat diet increases food intake during the day and blunts NTS daily rhythms in neuronal discharge. Additionally, we found that a high-fat diet dampens NTS responsiveness to metabolic neuropeptides, and decreases orexin immunoreactive fibres in this structure. These alterations occur without prominent body weight gain, suggesting that a high-fat diet acts initially to reduce activity in the NTS to disinhibit mechanisms that suppress daytime feeding. KEY POINTS: The dorsal vagal complex of the rodent hindbrain possesses intrinsic circadian timekeeping mechanisms In particular, the nucleus of the solitary tract (NTS) is a robust circadian oscillator, independent of the master suprachiasmatic clock Here, we reveal that rat NTS neurons display timed daily rhythms in their neuronal activity and responsiveness to ingestive cues These daily rhythms are blunted or eliminated by a short-term high-fat diet, together with increased consumption of calories during the behaviourally quiescent day Our results help us better understand the circadian control of satiety by the brainstem and its malfunctioning under a high-fat diet.
J Physiol, 2022; 600
34533886: Chrobok L, Jeczmien-Lazur JS, Bubka M, Pradel K, Klekocinska A, Klich JD, Ridla Rahim A, Myung J, Kepczynski M, Lewandowski MH
Daily coordination of orexinergic gating in the rat superior colliculus-Implications for intrinsic clock activities in the visual system.
The orexinergic system delivers excitation for multiple brain centers to facilitate behavioral arousal, with its malfunction resulting in narcolepsy, somnolence, and notably, visual hallucinations. Since the circadian clock underlies the daily arousal, a timed coordination is expected between the orexin system and its target subcortical visual system, including the superior colliculus (SC). Here, we use a combination of electrophysiological, immunohistochemical, and molecular approaches across 24 h, together with the neuronal tract-tracing methods to investigate the daily coordination between the orexin system and the rodent SC. Higher orexinergic input was found to occur nocturnally in the superficial layers of the SC, in time for nocturnal silencing of spontaneous firing in this visual brain area. We identify autonomous daily and circadian expression of clock genes in the SC, which may underlie these day-night changes. Additionally, we establish the lateral hypothalamic origin of the orexin innervation to the SC and that the SC neurons robustly respond to orexin A via OX receptor in both excitatory and GABA receptor-dependent inhibitory manners. Together, our evidence elucidates the combination of intrinsic and extrinsic clock mechanisms that shape the daily function of the visual layers of the SC.
FASEB J, 2021; 35
35078925: Trenk A, Walczak M, Szlaga A, Pradel K, Blasiak A, Blasiak T
Bidirectional Communication between the Pontine Nucleus Incertus and the Medial Septum Is Carried Out by Electrophysiologically-Distinct Neuronal Populations.
Theta oscillations are key brain rhythm involved in memory formation, sensorimotor integration, and control of locomotion and behavioral states. Generation and spatiotemporal synchronization of theta oscillations rely on interactions between brain nuclei forming a large neural network, which includes pontine nucleus incertus (NI). Here we identified distinct populations of NI neurons, based on the relationship of their firing to hippocampal waves, with a special focus on theta oscillations, and the direction and type of interaction with the medial septum (MS) in male, urethane-anesthetized rats. By recording NI neuronal firing and hippocampal LFP, we described NI neurons that fire action potentials in a theta phase-independent or theta phase-locked and delta wave-independent or delta wave-locked manner. Among hippocampal activity-independent NI neurons, irregular, slow-firing, and regular, fast-firing cells were observed, while hippocampal oscillation-/wave-locked NI neurons were of a bursting or nonbursting type. By projection-specific optotagging, we revealed that only fast-firing theta phase-independent NI neurons innervate the MS, rarely receiving feedback information. In contrast, the majority of theta-bursting NI neurons were inhibited by MS stimulation, and this effect was mediated by direct GABAergic input. Described NI neuronal populations differ in reciprocal connections with the septohippocampal system, plausibly forming separate neuronal loops.

Our results suggest that theta phase-independent NI neurons participate in theta rhythm generation through direct innervation of the MS, while theta-bursting NI neurons further transmit the rhythmic signal received from the MS to stabilize and/or strengthen rhythmic activity in other structures. The generation and spatiotemporal synchronization of theta oscillations rely on interactions between nuclei forming a large neural network, part of which is the pontine nucleus incertus (NI). Here we describe that within NI there are populations of neurons that can be distinguished based on the relationship of their firing to hippocampal theta oscillations and delta waves. We show that these neuronal populations largely do not have reciprocal connections with the septohippocampal system, but form separate neuronal loops. Our results suggest that medial septum (MS)-projecting, fast-firing, theta phase-independent NI neurons may participate in theta rhythm generation through direct innervation of the MS, while theta-bursting NI neurons may further transmit the rhythmic signal received from the MS to other structures.
J Neurosci, 2022; 42

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BOARD NUMBER: S06-469

WILL WORK FOR EFFECT: HOW RESPONSE-CONTINGENT PERCEPTUAL EFFECTS REINFORCE BEHAVIOR

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Successfully manipulating one's environment has previously been argued to be inherently rewarding, assumingly affecting performance by influencing patterns of response selection. Studies in humans have shown that information about the effectiveness of a response, even when it merely indicates that the response led to a perceptual change (e.g., a brief 'flash'), reinforces the response that is credited with this, value-neutral, effect (i.e., effectiveness-feedback). On the one hand, the motivating effect of being effective could be a higher order phenomena relying on a higher cognitive mechanisms, yet on the other, this could be a basic mechanism perfecting and shaping even the simplest responses. In order to test this question, in this study, we tested the effectiveness-feedback in mice using a newly designed behavioral task. Based on previous work in humans, which established that only immediate response-effects were reinforcing we predicted that effectiveness-feedback, in the form of light appearing immediately after a lever press, will reinforce the mice's behavior to press the relevant lever more than a delayed appearance of light (delayed effectiveness-feedback). The behavioral experiment provides substantial support that this is indeed the case, implying that the (motivating) effects on behavior are driven by environmental information about one's own effectiveness. The presence of such an effect in mice indicates for the first time, that as in humans, other animals are also intrinsically motivated by an effectiveness-feedback with striking resemblance to the parameters that determine 'effects' in humans. This suggests that effectiveness is a basic mechanism in shaping behavior.

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INPUT AND OUTPUT CONNECTIVITY MAPPING REVEALS INTEGRATING PROPERTIES OF CORTICOCLAUSTRAL AND INTRACLAUSTRAL CIRCUITS

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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University of Oxford, Department Of Physiology, Anatomy, And Genetics, Oxford, United Kingdom

The claustrum is highly interconnected with many structures in the brain, but our knowledge of the organizing principles governing its vast connectivity remains incomplete. The present study investigates the architecture of circuits between the claustrum and neocortex and within the claustrum itself. Immunohistochemistry, retrograde tracing, *in vitro* whole-cell patch-clamp electrophysiology, dual-color optogenetic circuit mapping, and *in vivo* calcium imaging of neural activity were used to assess whether claustrum neurons combine inputs from multiple cortical areas and what impact claustrum neurons have on the cortex. We determined that individual claustrum neurons frequently integrate inputs from more than one cortical site, most commonly between regions of the frontal cortex. Additionally, we found that neurons in the claustrum receive inputs from an array of sensory and associative cortical areas, albeit to a lesser extent. Retrograde labeling further indicated that input integration from frontal cortical regions depends on the output target of claustrum neurons. Optogenetic mapping revealed that intraclaustral connectivity was far more frequent than previously reported in coronal slices, particularly among neurons that did not share the same output target. Finally, individual claustrum axons recorded *in vivo* displayed clear responses to multiple sensory modalities. Our findings shed light on the organizing principles of claustrum connectivity, demonstrating a clear relationship between cortical projections, local claustral connectivity, and claustral outputs.

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POSITIVE AND SHARP BIPHASIC EXTRACELLULAR WAVEFORMS CORRESPOND TO DENDRITIC AND AXONAL SPIKES

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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In extracellular models, spike waveforms differ between neuronal compartments: negative peaks near the soma, and positive or biphasic waveforms next to other compartments. Experimental reports suggest that both positive and biphasic waveforms correspond to axonal potentials. However, the prior work employed relatively small datasets or anesthetized preparations, and did not monitor simultaneously multiple waveforms of the same unit. Here, we analyzed 10,199 units recorded by high-density silicon probes from neocortex and hippocampus of 17 freely-moving mice. We divided units into three categories based on the waveform recorded at the highest amplitude channel: negative units ("Nunits"), sharp biphasic units, and positive units ("Punits"). We found 869 biphasic units, 648 Punits, and 889 "multipolar" Nunits with simultaneously-monitored biphasic or positive spikes. Punits exhibited firing rates and refractory periods similar to Nunits, but sharper waveforms which were compactly distributed in space. In multipolar units, positive spikes preceded negative spikes by under 0.1 ms, with jitter under 0.2 ms. This suggest that positive spikes represent the extracellular signature of dendritic return currents resulting from spikes generated at the axon initial segment. In CA1 recordings, the fraction of biphasic units was highest in stratum radiatum. In some cases, spikes of physiologically-verified inhibitory cells were followed by spiking of biphasic units with sub-millisecond delay and jitter. These observations suggest that biphasic spikes correspond to axonal potentials. Together, the results suggest that positive and sharp biphasic extracellular spikes represent dendritic and axonal potentials, respectively.
Funding: CRCNS NSF-BSF #2015577, ERC #679253, ISF #638/16, ISF #1871/17, and CIHR-IDRC-ISF #2558/18

Pubmed:
33760740: Tarnavsky Eitan A, Someck S, Zajac M, Socher E, Stark E
Outan: An On-Head System for Driving µLED Arrays Implanted in Freely Moving Mice.
In the intact brain, neural activity can be recorded using sensing electrodes and manipulated using light stimulation. Silicon probes with integrated electrodes and µLEDs enable the detection and control of neural activity using a single implanted device. Miniaturized solutions for recordings from small freely moving animals are commercially available, but stimulation is driven by large, stationary current sources. We designed and fabricated a current source chip and integrated it into a headstage PCB that weighs 1.37 g. The proposed system provides 10-bit resolution current control for 32 channels, driving µLEDs with up to 4.6 V and sourcing up to 0.9 mA at a refresh rate of 5 kHz per channel. When calibrated against a µLED probe, the system allows linear control of light output power, up to 10 µW per µLED. To demonstrate the capabilities of the system, synthetic sequences of neural spiking activity were produced by driving multiple µLEDs implanted in the hippocampal CA1 area of a freely moving mouse. The high spatial, temporal, and amplitude resolution of the system provides a rich variety of stimulation patterns. Combined with commercially available sampling headstages, the system provides an easy to use back-end, fully utilizing the bi-directional potential of integrated opto-electronic arrays.
IEEE Trans Biomed Circuits Syst, 2021; 15
32746022: Noked O, Levi A, Someck S, Amber-Vitos O, Stark E
Bidirectional Optogenetic Control of Inhibitory Neurons in Freely-Moving Mice.
Optogenetic manipulations of excitable cells enable activating or silencing specific types of neurons. By expressing two types of exogenous proteins, a single neuron can be depolarized using light of one wavelength and hyperpolarized with another. However, routing two distinct wavelengths into the same brain locality typically requires bulky optics that cannot be implanted on the head of a freely-moving animal.
IEEE Trans Biomed Eng, 2021; 68

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BOARD NUMBER: S06-472

THE NEURAL CIRCUIT OF ENHANCED POST-TRAUMATIC THREAT DETECTION

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Anxiety disorders, and PTSD particularly are associated with heightened levels of arousal. This study aimed to investigate neural networks, which regulate threat detection, and long term changes after traumatic experiences. We were interested in neural activity in the cortico-basal ganglia related circuits which may facilitate threat detection by inhibiting the nigro-tectal pathway including the substantia-nigra(SC) and its connection to the superior colliculus(SC). The striatum, the main input region of the basal-ganglia receives projections from the medial-prefrontal-cortex(mPFC), potentially allowing the introduction of affective information into the basal-ganglia to modulate threat detection and behavioral responses thereto. We combined in-vivo electrophysiology and optogenetics, while behaviorally assessing threat detection mice, post an inescapable foot-shock. A new paradigm was developed, involving a randomly moving robotic bug(robo-beetle), creating incidental interactions with the mouse in the arena. We found significant differences in fear related behaviors, such as altered timing and distance of avoidance and escaping onset, higher fleeing velocities following anticipated contacts with the robo-beetle and overall, more instances of fear related actions following shock. A direct mPFC–SC pathway, as well as a yet to be described connection, branching out of the mPFC onto both, the DMS and SC was found. Most recorded SC neurons precede fleeing onsets by few milliseconds and correlate with the anxiety induced shifts in flight initiation timing, as well as defensive distance. Results suggest a direct contribution of SC units in flight behavior, as well as plastic changes in the top down connectivity from the mPFC onto the SC, involved in enhanced threat detection.

Pubmed:
25783195: Zubedat S, Aga-Mizrachi S, Cymerblit-Sabba A, Ritter A, Nachmani M, Avital A
Methylphenidate and environmental enrichment ameliorate the deleterious effects of prenatal stress on attention functioning. Either pre- or post-natal environmental factors seem to play a key role in brain and behavioral development and to exert long-term effects. Increasing evidence suggests that exposure to prenatal stress (PS) leads to motor and learning deficits and elevated anxiety, while enriched environment (EE) shows protective effects. The dopaminergic system is also sensitive to environmental life circumstances and affects attention functioning, which serves as the preliminary gate to cognitive processes. However, the effects of methylphenidate (MPH) on the dopaminergic system and attentional functioning, in the context of these life experiences, remain unclear. Therefore, we aimed to examine the effects of EE or PS on distinct types of attention, along with possible effects of MPH exposure. We found that PS impaired selective attention as well as partial sustained attention, while EE had beneficial effects. Both EE and MPH ameliorated the deleterious effects of PS on attention functioning. Considering the possible psychostimulant effect of MPH, we examined both anxiety-like behavior as well as motor learning. We found that PS had a clear anxiogenic effect, whereas EE had an anxiolytic effect. Nevertheless, the treatment with both MPH and/or EE recovered the deleterious effects of PS. In the motor-learning task, the PS group showed superior performance while MPH led to impaired motor learning. Performance decrements were prevented in both the PS + MPH and EE + MPH groups. This study provides evidence that peripubertal exposure to EE (by providing enhanced sensory, motor, and social opportunities) or MPH treatments might be an optional therapeutic intervention in preventing the PS long-term adverse consequences.
Stress, 2015; 18

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BOARD NUMBER: S06-473

UNDERSTANDING THE INTERPLAY BETWEEN FEAR ANXIETY AND THE STEREOTYPIC BEHAVIOR OF GROOMING IN DAT-CRE MICE

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Repetitive behavior is an integral part of several neurological/mental disorders. In disorders such as Obsessive Compulsive Disorder (OCD) or syndromes like Tourette, patients are unable to cease some repetitive activities, despite a great inconvenience, impaired functioning, injuries and social problems. Excessive repetitive behavior has been explained as both impairment in reward processing and an imbalance between Stimuli-Response (S-R) and Action-Outcome (A-O) systems. Striatal activity, and in particular Dopamine (DA) projections to the Dorsal Striatum (DS) and ventral (VS) striatum has been tied with reward processing and S-R/A-O behaviors. In addition, evidence show that DA projections to the striatum are involved in psychopathology such as OCD and Tourette. Anxiety is known to exacerbate compulsive behaviors. It has been suggested that fear modulate S-R/A-O behaviors by affecting striatal activity. In the current study we are focused on the role of striatal Afferent DA projections in the interaction between anxiety and grooming behavior – induced and spontaneous. We bilaterally injected 80 DAT-Cre mice with retrograde AAV containing hM4D to either the DS or the VS. All animals went through a behavioral paradigm involving fear conditioning, which allowed us to test the augmentation effects of fear and anxiety on grooming behavior and the role played in this interaction by DA neurons belonging to distinct pathways. We tested different aspects of grooming behavior (duration, timing, bout lengths) and found different effects for both DA pathways in different types of grooming and the interaction between with the effects of anxiety.

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CONTROL OF NEOCORTICAL TOP-DOWN INFORMATION BY NDNF INTERNEURONS IN LAYER 1

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Mounting evidence identifies neocortical layer 1 (L1) as a central hub for processing of internally-generated top-down information encoding previous experiences and current aims. These signals are thought to be controlled and shaped by L1 interneurons, the only resident neurons in this lamina. Here we leverage our recent identification of the first selective marker for a subset of L1 interneurons (neuron derived neurotrophic factor, NDNF) to dissect the connectivity, potential subtypes and circuit function of these neurons. *In vitro* slice recordings in combination with optogenetics indicate that, in addition to their established output to pyramidal neuron dendrites, NDNF interneurons also supply inhibition to interneurons in deeper layers expressing the markers parvalbumin (PV) and vasoactive intestinal peptide (VIP). Large-scale *in vivo* recordings together with optogenetics reveal how this widespread connectivity combines to shape the circuit function of NDNF cells. Since recent research implies the existence of NDNF interneuron subpopulations distinguished by the expression of neuropeptide Y (NDNF/NPY cells), we furthermore validate and employ intersectional viral targeting. Our data indicate that both NDNF and NDNF/NPY interneurons comprise a large proportion of late-spiking cells, and provide inhibitory inputs to L2/3 pyramidal neurons. Intriguingly, NDNF and NDNF/NPY interneurons engage in persistent, autonomous firing after repeated stimulation, a feature we observe only rarely in PV cells and not in non-fast spiking interneurons and pyramidal cells. In conclusion, NDNF and likely NDNF/NPY interneurons control large parts of the circuit, enabling top-down input to these cells to function as a master regulator in neocortex.

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BOARD NUMBER: S06-475

OPTOGENETIC DECONSTRUCTION OF CORTICAL OLFACTORY CODES

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Many neural circuits exhibit reproducible sequences of activity that correlate with perception, action, or distinct internal states. Neural sequences lasting on the order of tens to hundreds of milliseconds are proposed to mediate essential cognitive processes including navigation, memory encoding and retrieval, and sensory discrimination. However, the extent to which the temporal structure of these sequences impacts the activity of downstream reader circuits remains relatively unexplored. In the rodent olfactory system, odors activate stereotyped spatiotemporal sequences of olfactory bulb glomeruli that project to downstream piriform cortex (PCx). Using targeted patterned optogenetic stimulation of glomeruli in mice while recording from large populations of PCx neurons, we have revealed that neural activity in PCx is highly sensitive to the specific timing of sequences of glomerular inputs with millisecond resolution. We hypothesize that the temporal specificity of these responses is implemented by the interplay of intra-cortical excitation and inhibition which, together, form a delay-line architecture that governs the temporal selectivity of PCx neurons. We are currently testing this hypothesis by stimulating sequences of glomeruli while optogenetically suppressing either PCx inhibitory interneurons or subsets of excitatory PCx cells. These findings will provide novel insights into the computational principles and mechanisms that underlie the encoding and decoding of precise neural sequences in cortical circuits.

Pubmed:
33080204: Blazing RM, Franks KM
Neuroscience: Illuminating Principles of Odor Coding.
Neuroscientists still are not sure what makes any two odors smell alike. A new study uses light to manipulate the sensory cells in our nose that respond to odors and reveals that both the timing and identity of activated cells influence odor perception.
Curr Biol, 2020; 30
32422571: Blazing RM, Franks KM
Odor coding in piriform cortex: mechanistic insights into distributed coding.
Olfaction facilitates a large variety of animal behaviors such as feeding, mating, and communication. Recent work has begun to reveal the logic of odor transformations that occur throughout the olfactory system to form the odor percept. In this review, we describe the coding principles and mechanisms by which the piriform cortex and other olfactory areas encode three key odor features: odor identity, intensity, and valence. We argue that the piriform cortex produces a multiplexed odor code that allows non-interfering representations of distinct features of the odor stimulus to facilitate odor recognition and learning, which ultimately drives behavior.
Curr Opin Neurobiol, 2020; 64

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NEURONAL AND HEMODYNAMIC RESTING STATE ACTIVITY DURING ACUTE HYPOXIA USING CALCIUM AND INTRINSIC OPTICAL IMAGING IN MICE

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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The brain uses the most oxygen of all organs in the human body. A hindered oxygen supply to the brain can cause a lower cognition. How this decrease in cognition happens during hypoxic episodes is still obscure, but might be related with altered functional connectivity of the brain. Even in the absence of stimuli, the brain has a certain activation pattern known as the resting state network (RSN). These can be observed both through neuronal and hemodynamic activity using calcium and intrinsic optical imaging respectively. In this study, we aimed to use these tools to better understand how the RSN are modified under different levels of hypoxia in awake mice. To do so, we exposed 8 mice to different levels of oxygen (12%, 10% and 8%) in an enclosure with controlled environment. At the same time, we monitored changes in calcium activity (using GCaMP6), HbO and HbR on the surface of the cortex with a multiwavelength intrinsic optical imaging. We compared these findings with spontaneous activity during normoxia. We found decreases in the hemodynamic network activity, that were consistent over mice. The changes became more apparent with lower levels of oxygen. This suggests that oxygen levels alter RSN dynamics, which could be linked to decreased cognition. By investigating the functional connectivity changes during lowered levels of atmospheric oxygen, we shed light on the mechanisms in play during global hypoxia. This opens the door to further works into the study of neurovascular coupling and the neural substrate of consciousness.

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BOARD NUMBER: S06-477

RETROSPLENIAL CORTEX ACTIVITY: A HUB IN A PARADOXICAL SLEEP NETWORK?

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Rapid eye movement (REM, also called paradoxical) sleep correlates with enhanced cellular activity of region-specific thalamo-cortical circuit and subcortical structures including the hippocampus, midbrain or hypothalamus. This REM sleep specific neuronal activity is hypothesized to promote structural plasticity and provide a window for the consolidation of contextual and emotional memories previously acquired during wakefulness, yet the underlying mechanism remains unclear. Amongst the cortical structures, the activity of neurons located in the retrosplenial cortex (RSC) is increased during REM sleep. Yet, cortical single cell-to-whole brain circuit connections and its role in REM sleep function remains unknown. Here we characterized the activity of RSC microcircuit across the sleep-wake cycle using simultaneous 2-photon calcium imaging and electrophysiological recordings in spontaneously head-restrained sleeping mice. We observed a REM sleep-specific reduction of pyramidal cell somatic activity concomitant to the activation of the interneurons expressing either parvalbumin, somatostatin or vasoactive intestinal peptide. Collectively, these observations suggested a region-specific regulation of excitatory/inhibitory balance in RSC during REM sleep that may contribute to information integration, memory consolidation and ultimately behavioural optimization.

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BOARD NUMBER: S06-478

MULTIDIRECTIONAL PROPAGATION OF SPW-R COMPLEXES IN THE RAT HIPPOCAMPUS, IN VITRO

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Sharp wave-ripple (SPW-R) complexes are observed in the mammalian hippocampus on EEG during slow-wave sleep and immobility. These synchronous population discharges are considered to play an essential role in memory consolidation and the replay of wakefulness-acquired memory. An in vitro SPW-R model was investigated on rat hippocampal slices using a 24-channel linear electrode. Instead of assuming a classical trisynaptic circle of information flow from the DG to the CA3 (Type I), our studies showed that SPW-Rs could also be propagated in the opposite direction (Type II), or generated simultaneously in both areas (Type III). Based on these results, it can be stated that bidirectional information flow occurs between the DG and the CA3 region of the rat hippocampus. At the initiation site of the SPW-R complexes, more cells were activated than at other regions. Pyramidal cells of the CA3 fired more and showed denser connectivity than granule cells of the DG. A metabotropic glutamate receptor agonist, DCG-IV was used to investigate the role of the mossy fibres in the generation of SPW-Rs. In DCG-IV bath, the recurrence frequency of Type I SPW-Rs increased, while that of Type II SPW-Rs showed a slight drop. The propagation of SPW-Rs decelerated, while the LFPg deflections and the superimposed multiunit activities were reduced. The role of reverse information flow might be to intensify the information packages transmitted by the SPW-R complexes by adding another network to the trisynaptic circle for more efficient memory consolidation.

Pubmed:
35008628: Tóth EZ, Szabó FG, Kandrács Á, Molnár NO, Nagy G, Bagó AG, Erőss L, Fabó D, Hajnal B, Rácz B, Wittner L, Ulbert I, Tóth K
Perisomatic Inhibition and Its Relation to Epilepsy and to Synchrony Generation in the Human Neocortex.
Inhibitory neurons innervating the perisomatic region of cortical excitatory principal cells are known to control the emergence of several physiological and pathological synchronous events, including epileptic interictal spikes. In humans, little is known about their role in synchrony generation, although their changes in epilepsy have been thoroughly investigated. This paper demonstraits how parvalbumin (PV)- and type 1 cannabinoid receptor (CB1R)-positive perisomatic interneurons innervate pyramidal cell bodies, and their role in synchronous population events spontaneously emerging in the human epileptic and non-epileptic neocortex, in vitro. Quantitative electron microscopy showed that the overall, PV+ and CB1R+ somatic inhibitory inputs remained unchanged in focal cortical epilepsy. On the contrary, the size of PV-stained synapses increased, and their number decreased in epileptic samples, in synchrony generating regions. Pharmacology demonstrated-in conjunction with the electron microscopy-that although both perisomatic cell types participate, PV+ cells have stronger influence on the generation of population activity in epileptic samples. The somatic inhibitory input of neocortical pyramidal cells remained almost intact in epilepsy, but the larger and consequently more efficient somatic synapses might account for a higher synchrony in this neuron population. This, together with epileptic hyperexcitability, might make a cortical region predisposed to generate or participate in hypersynchronous events.
Int J Mol Sci, 2021; 23
32409039: Márton G, Tóth EZ, Wittner L, Fiáth R, Pinke D, Orbán G, Meszéna D, Pál I, Győri EL, Bereczki Z, Kandrács Á, Hofer KT, Pongrácz A, Ulbert I, Tóth K
The neural tissue around SU-8 implants: A quantitative in vivo biocompatibility study.
The use of SU-8 material in the production of neural sensors has grown recently. Despite its widespread application, a detailed systematic quantitative analysis concerning its biocompatibility in the central nervous system is lacking. In this immunohistochemical study, we quantified the neuronal preservation and the severity of astrogliosis around SU-8 devices implanted in the neocortex of rats, after a 2 months survival. We found that the density of neurons significantly decreased up

to a distance of 20 µm from the implant, with an averaged density decrease to 24 ± 28% of the control. At 20 to 40 µm distance from the implant, the majority of the neurons was preserved (74 ± 39% of the control) and starting from 40 µm distance from the implant, the neuron density was control-like. The density of synaptic contacts - examined at the electron microscopic level - decreased in the close vicinity of the implant, but it recovered to the control level as close as 24 µm from the implant track. The intensity of the astroglial staining significantly increased compared to the control region, up to 560 µm and 480 µm distance from the track in the superficial and deep layers of the neocortex, respectively. Electron microscopic examination revealed that the thickness of the glial scar was around 5-10 µm thin, and the ratio of glial processes in the neuropil was not more than 16% up to a distance of 12 µm from the implant. Our data suggest that neuronal survival is affected only in a very small area around the implant. The glial scar surrounding the implant is thin, and the presence of glial elements is low in the neuropil, although the signs of astrogliosis could be observed up to about 500 µm from the track. Subsequently, the biocompatibility of the SU-8 material is high. Due to its low cost fabrication and more flexible nature, SU-8 based devices may offer a promising approach to experimental and clinical applications in the future.
Mater Sci Eng C Mater Biol Appl, 2020; 112
31523807: Kandrács Á, Hofer KT, Tóth K, Tóth EZ, Entz L, Bagó AG, Erőss L, Jordán Z, Nagy G, Fabó D, Ulbert I, Wittner L
Presence of synchrony-generating hubs in the human epileptic neocortex.
•Initiation of pathological synchronous events such as epileptic spikes and seizures is linked to the hyperexcitability of the neuronal network in both humans and animals. •In the present study, we show that epileptiform interictal-like spikes and seizures emerged in human neocortical slices by blocking GABA receptors, following the disappearance of the spontaneously occurring synchronous population activity. •Large variability of temporally and spatially simple and complex spikes was generated by tissue from epileptic patients, whereas only simple events appeared in samples from non-epileptic patients. •Physiological population activity was associated with a moderate level of principal cell and interneuron firing, with a slight dominance of excitatory neuronal activity, whereas epileptiform events were mainly initiated by the synchronous and intense discharge of inhibitory cells. •These results help us to understand the role of excitatory and inhibitory neurons in synchrony-generating mechanisms, in both epileptic and non-epileptic conditions.
J Physiol, 2019; 597
29178354: Tóth K, Hofer KT, Kandrács Á, Entz L, Bagó A, Erőss L, Jordán Z, Nagy G, Sólyom A, Fabó D, Ulbert I, Wittner L
Hyperexcitability of the network contributes to synchronization processes in the human epileptic neocortex.
Hyperexcitability and hypersynchrony of neuronal networks are thought to be linked to the generation of epileptic activity in both humans and animal models. Here we show that human epileptic postoperative neocortical tissue is able to generate two different types of synchronies in vitro. Epileptiform bursts occurred only in slices derived from epileptic patients and were hypersynchronous events characterized by high levels of excitability. Spontaneous population activity emerged in both epileptic and non-epileptic tissue, with a significantly lower degree of excitability and synchrony, and could not be linked to epilepsy. These results help us to understand better the role of excitatory and inhibitory neuronal circuits in the generation of population events, and to define the subtle border between physiological and pathological synchronies.
J Physiol, 2018; 596
25209976: Hofer KT, Kandrács Á, Ulbert I, Pál I, Szabó C, Héja L, Wittner L
The hippocampal CA3 region can generate two distinct types of sharp wave-ripple complexes, in vitro.
Hippocampal sharp wave-ripples (SPW-Rs) occur during slow wave sleep and behavioral immobility and are thought to play an important role in memory formation. We investigated the cellular and network properties of SPW-Rs with simultaneous laminar multielectrode and intracellular recordings in a rat hippocampal slice model, using physiological bathing medium. Spontaneous SPW-Rs were generated in the dentate gyrus (DG), CA3, and CA1 regions. These events were characterized by a local field potential gradient (LFPg) transient, increased fast oscillatory activity and increased multiple unit activity (MUA). Two types of SPW-Rs were distinguished in the CA3 region based on their different LFPg and current source density (CSD) pattern. Type 1 (T1) displayed negative LFPg transient in the pyramidal cell layer, and the associated CSD sink was confined to the proximal dendrites. Type 2 (T2) SPW-Rs were characterized by positive LFPg transient in the cell layer, and showed CSD sinks involving both the apical and basal dendrites. In both types, consistent with the somatic CSD source, only a small subset of CA3 pyramidal cells fired, most pyramidal cells were hyperpolarized, while most interneurons increased firing rate before the LFPg peak. Different neuronal populations, with different proportions of pyramidal cells and distinct subsets of interneurons were activated during T1 and T2 SPW-Rs. Activation of specific inhibitory cell subsets-with the possible leading role of perisomatic interneurons-seems to be crucial to synchronize distinct ensembles of CA3 pyramidal cells finally resulting in the expression of different SPW-R activities. This suggests that the hippocampus can generate dynamic changes in its activity stemming from the same excitatory and inhibitory circuits, and so, might provide the cellular and network basis for an input-specific and activity-dependent information transmission.
Hippocampus, 2015; 25


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BOARD NUMBER: S06-479

CELL-SPECIFIC SYNAPTIC WIRING WITHIN THE HIPPOCAMPAL CA3 NETWORK

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Aims: The hippocampus is crucial for learning and memory, and the interconnected pyramidal neurons of its CA3 area appear uniquely configured for memory storage. However, while the overall information flow through the hippocampus has been long understood, little is known about individual cell wiring, which will dictate the rules of information processing. We aim to establish the microcircuit architecture of CA3, to understand its capabilities for memory formation. **Methods:** Using simultaneous multi-cellular patch-clamp recordings from up to 8 neurons in acute slices of mouse hippocampus, and post-hoc imaging of recorded cells, we determined the connectivity, synaptic properties, and arrangement of the CA3 network. **Results:** We recorded 49 synaptic connections between morphologically identified CA3 pyramidal neurons (Connection probability: 3.2 %), which show a non-random circuit architecture, containing sub-networks of highly interconnected cells. Unique subtypes of excitatory pyramidal neurons can be identified by functional and anatomical properties. We demonstrate that these subtypes have specific wiring rules, adding a further layer of complexity to the CA3 network. Connectivity between pyramidal neurons and local fast-spiking interneurons was more abundant than principal neuron interconnectivity (19 % connectivity, 48/250 confirmed connections), with unique synaptic properties that will differentially process excitatory activity in a frequency and spatially dependent manner. **Conclusions:** Our results shed new light on the microcircuit arrangement of the CA3 network. Non-random principal neuron connectivity and cellular heterogeneity may increase the processing capacity of the network, and suggest that distinct pyramidal subtypes could have specific roles in encoding life experiences within neuronal ensembles.

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BOARD NUMBER: S06-480

AWAKE PERCEPTION IS ASSOCIATED WITH DEDICATED NEURONAL ASSEMBLIES IN CEREBRAL CORTEX

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Neural activity in sensory cortex combines stimulus responses and ongoing activity, but it remains unclear whether they reflect the same underlying dynamics or separate processes. Here, using two-photon imaging of neuronal populations, we show that during wakefulness, the neuronal assemblies evoked by sounds in the auditory cortex and thalamus are specific to the stimulus and distinct from the assemblies observed in ongoing activity. In contrast, during isoflurane and ketamine-medetomidine anesthesia, evoked assemblies are indistinguishable from ongoing assemblies in the cortex. Using a visualization of thalamocortical fibers, we show that in all cases, the thalamocortical input remains distinct between spontaneous and sensory input, suggesting that the change of dynamics is essential of cortical origin. We also find that a strong remapping of sensory responses accompanies this dynamical state change produced by anesthesia. Together, these results show that the awake cortex engages dedicated neuronal assemblies in response to sensory inputs, which we suggest is a network correlate of sensory perception. ---

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BOARD NUMBER: S06-481

SELECTIVE INHIBITION OF EXCITATORY NEURONS ALTERS THE EMERGENT BURSTING DYNAMICS OF IN VITRO NEURAL NETWORKS

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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[1]Norwegian University of Science and Technology (NTNU), Department Of Neuromedicine And Movement Science (inb), Trondheim, Norway, [2]Umeå University Hospital, Department Of Clinical Science, Division Of Neuroscience, Umeå, Sweden, [3]Umeå University Hospital, Department Of Community Medicine And Rehabilitation, Division Of Rehabilitation Medicine, Umeå, Sweden

Introduction At maturity, in vitro neural network activity is dominated by network bursts which play an important role in Hebbian plasticity for structural and functional connectivity. Network bursts are vital for neural information processing and are strong indicators of network computation. While bursting is the consequence of balanced excitatory-inhibitory interactions, the underlying functional mechanisms that facilitate this are still poorly understood. This is partly because we have limited knowledge of and access to the connections themselves. Hence, in our research we targeted specific synapses to investigate how plasticity rules may facilitate or hinder adaptive and maladaptive functional responses to network perturbation. *Materials and methods* In vitro neural networks of rat cortical neurons were transduced with adeno-associated viral vector encoding the inhibitory designer receptors exclusively activated by designer drugs (DREADDs) hM4Di protein, specifically targeted to excitatory neurons. The DREADDs were acutely activated by the novel synthetic ligand deschloroclozapine (DCZ), and network wide activity was recorded using microelectrode arrays (MEAs) before, during and after DCZ perturbation. *Preliminary results and analysis in progress* DCZ application to neural networks expressing DREADDs suppressed network bursting for up to 2hrs. Post washout, high frequency bursts reappeared by 2hrs, and were maintained for up to 24hrs. There was also evidence of increased synaptic strengths at several electrodes post washout, which were also maintained 24hrs later. *Conclusion* Global inhibition of excitatory neurons triggers homeostatic adaptive mechanisms by inducing rapid short-term facilitation. This study presents a novel method to investigate emergence in biological networks through manipulation of underlying network components.

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BOARD NUMBER: S06-482

HOW SINGLE GABAERGIC NEURONS SHAPE LOCAL CIRCUIT DYNAMICS: AN ALL-OPTICAL APPROACH

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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GABAergic neurons shape the spatial and temporal organization of collective neuronal dynamics, thus playing a major role in both the developing and adult brain. However, it remains unclear how single GABAergic neurons causally impact the coordination of local neuronal assemblies *in vivo*. To address this question, we have developed a custom optical setup allowing to combine 2-photon calcium imaging and holographic optogenetic stimulation at single-cell resolution. We used soma-targeted ChroME and GtaCR1 opsins to excite and inhibit specific GABAergic cells with high spatiotemporal precision. We have applied this all-optical approach to the neonatal developing barrel cortex and the adult CA1 hippocampus in mice to shed light on the functional organization of developing and adult cortical networks.

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BOARD NUMBER: S06-483

DIFFERENT MODULATORY EFFECTS OF SEROTONIN AND 5-HT2A RECEPTOR SUBTYPE ACTIVATION ON SENSORIMOTOR AND MEDIAL PREFRONTAL BASAL GANGLIA CIRCUITS

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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The cortical information is transferred to the *substantia nigra pars reticulata* (SNr) through the sensorimotor (SM) and medial prefrontal (mPF) basal ganglia (BG) circuits that are implicated in motor and cognitive/motivational behaviours, respectively. All components of the BG circuits receive serotonergic (5-HT) innervation and express low/moderate densities of 5-HT2A receptors. This study investigated the effect of 5-HT on SM and mPF BG circuits and the specific contribution of 5-HT_{2A} receptors on their function. For that purpose, in vivo single-unit extracellular recordings of the lateral (included in the SM circuit) and medial (in the mPF circuit) SNr neurons along with simultaneous electrical stimulation of the motor and mPF cortex were performed in vehicle-, pCPA- and acute fluoxetine-treated anaesthetized rats, before, and after the administration of highly selective 5-HT_{2A} receptor agonist TCB-2. Results revealed a territory-dependent dissociation in 5-HT modulation over the SNr, as only the lateral SNr neuron activity was under the tonic inhibitory control of 5-HT. After fluoxetine, the cortico-SNr transmission through both SM and mPF circuits was altered. TCB-2 (50-200 µg/kg, i.v.) increased the medial SNr neuronal firing rate and had preferential action on the mPF circuit, inducing an imbalance between the functionality of trans-striatal pathways and favouring cortical information transfer via the direct pathway. Overall, these data contribute to a better understanding of the role of 5-HT in cortico-BG information processing and clarify the potential role that 5-HT_{2A} receptor-targeting drugs may have as a therapeutic in BG related disorders. Funding: GIU19/092, SAF2016-77758-R. L.G. is supported by UPV/EHU fellowship.

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BOARD NUMBER: S06-484

TOPOLOGY OF STRIATAL MOTIFS IN HEALTH AND DISEASE

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Simulating large-scale networks of neurons is an important approach when interpreting and synthesising different types of experimental data from healthy as well as from diseased brains. Here we focus on the striatum, the main input stage and largest nucleus of the basal ganglia. Basal ganglia are involved in motor learning, action-selection and reinforcement learning. Dysfunction in these areas lead to a variety of brain disorders like Parkinson's disease (PD), a neurodegenerative disorder that affects nerve cells. Understanding diseases related changes in the topology of striatal microcircuit can provide insights into striatal dynamics in both healthy and disease states. To characterise the topology of striatal network we used a nearly full-scale digital reconstruction of the mouse striatal microcircuitry (Hjorth, Kozlov et al., 2020). Neurodegeneration was modelled as progressive loss of the most distal fragments of the dendritic arbours. We are applying algebraic topology, in particular directed cliques (simplices), to investigate the local structural connectivity in striatum, and how structural features shape network dynamics. We show that progressive dendritic degeneration not only alters the global connection probabilities but also dramatically affects statistics of simplices particularly at the later PD stage. We found that interneurons despite being in minority can have a surprisingly large effect on the distribution of simplices. These results suggest that interneurons play a crucial role in shaping the striatal network structure as well as the dynamics.

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BOARD NUMBER: S06-485

UNDERSTANDING THE ROLE OF VIP+ INHIBITORY INTERNEURONS IN THE RETINA

POSTER SESSION 06 - SECTION: MICROCIRCUITS AND NETWORK DYNAMICS

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Understanding the role of inhibitory interneurons in shaping sensory computations is a major purpose in neuroscience. The inhibitory neurons expressing the vasoactive intestinal peptide (VIP+ cells) have been extensively studied in the cortex, but their exact role in the retina remains unclear. Anatomical studies suggest that these VIP+ amacrines cells have both inhibitory GABAergic synapses and gap-junctions [1-3]. However, the role of VIP+ amacrines cells in visual information processing in the retina is still unclear. In particular, we don't know how these two types of synapses can impact ganglion cells activity. Here we used optogenetic tools to stimulate VIP+ cells in two different ways: full-field stimulation and 2-photon holographic stimulation to target individual VIP+ cells with high resolution, while we recorded the impact of these stimulations on ganglion cells. We show that VIP+ amacrines cells can (i) activate ON ganglion cells, and (ii) suppress the activity of OFF ganglion cells, even over very long distances (up to 400µm). The activation of ON ganglion cells did not depend on GABAergic transmission, suggesting it results from gap junctions. Our results thus demonstrate that VIP+ cells differentially modulate different types of ganglion cells through different types of synapses. This suggests that these VIP+ cells could both (i) synchronize the response of ON ganglion cells populations, and (ii) participate in lateral inhibition to ganglion cells. Our results show that VIP+ differentially modulate different ganglion cell types and suggest that they play an important role in information processing in the retina.

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EVOLUTION OF CROSS-FREQUENCY COUPLING BETWEEN ENDOGENOUS OSCILLATIONS OVER THE TEMPORAL CORTEX IN VERY PREMATURE NEONATES

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Temporal theta activity in coalescence with slow-wave (TTA-SW) is one of the first neurobiomarkers of the neurodevelopment of perisylvian networks in the electroencephalography (EEG) of premature neonates. Dynamic changes in the microstructure and activity within neural networks are reflected in the EEG. Slow oscillation slope can reflect synaptic strength, and cross-frequency coupling (CFC), associated with several putative functions in adults, can reflect neural communication. Here, we investigated the evolution of CFC, in terms of SW theta phase-amplitude coupling (PAC), during the course of very early development between 25 and 32 wGA in 23 premature neonates. We used high-resolution EEG and dipole models as spatial filters to extract the source waveforms corresponding to TTA-SW. We also carried out nonlinear phase dependent correlation measurements to examine whether the characteristics of the SW slopes are associated with TTA-SW coupling. We show that neurodevelopment leads to temporal accumulation of the SW theta PAC towards the trough of SW. Steepness of the negative going slope of SW determined the degree of this coupling. Systematic modulation of SW-TTA CFC during development is a signature of the complex development of local cortico-cortical perisylvian networks and distant thalamo-cortical neural circuits driving this nested activity over the perisylvian networks.

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BOARD NUMBER: S06-487

SENSORIMOTOR BRAIN OSCILLATIONS IN HUMAN TODDLERS

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Evidence, in the last decades, revealed a rhythmic modulation that involved sensorimotor processing. Natural behaviour relies on a dynamic interaction between multimodal sensorimotor loops.
To study these rhythms in the early years of life, we consider the non-nutritive sucking (NNS) behaviour in 17 typical infants/toddlers from 6 months to 3 years. While the pattern generating circuit of rhythmic oromotor activity is located in the brainstem, the quality of sucking behaviour is probably modulated by more cranial structures (e.g., cortico-spinal). So, we investigated how sensorimotor cortical rhythms (i.e. μ, β) are involved in this behaviour. Considering that a relationship between these EEG rhythms and movement speed has been previously reported, we hypothesized a proportional relationship between sucking frequency and sensorimotor oscillations during baseline, sucking or pre-sucking periods.
First, we performed a linear regression analysis between sucking frequency and spectral activity for μ1 [8-10Hz), μ2 [10-13Hz), β1 [14-21) and β2 [21-32) bands, measured as spectral power during baseline and sucking periods and their ratio. We found a correlation between sucking frequency with μ1 and β activity during baseline and sucking period, respectively. Then, we performed a time-frequency analysis which revealed the role of β1 activity in the pre-movement phase.
These results suggest the existence of different sensorimotor communication channels from birth and support the role of increased β rhythm in sensorimotor coordination. Acknowledgments: This research was partially supported by the European Research Council (PI Monica Gori; Grant agreement No. 948349).

Pubmed:
34051686: Campus C, Signorini S, Vitali H, De Giorgis V, Papalia G, Morelli F, Gori M
Sensitive period for the plasticity of alpha activity in humans.
Visual experience is crucial for the development of neural processing. For example, alpha activity development is a vision-dependent mechanism. Indeed, studies report no alpha activity is present in blind adults. Nevertheless, studies have not investigated the developmental trajectory of this activity in infants and children with blindness. Here, we hypothesize that the difference in neural activity of blind compared to sighted subjects is: absent at birth, progressive with age, specifically occipital and linked to a gradual motor impairment. Therefore, we consider spectral power of resting-state EEG and its association with motor impairment indices, in blind subjects and in sighted controls between 0 and 11 years of age. Blind subjects show posterior alpha activity during the first three years of life, although weaker and slower maturing compared to sighted subjects. The first great differentiation between blind and sighted subjects occurs between 3 and 6 years of age. Starting in this period, reduced alpha activity increases the probability of motor impairment in blind subjects, likely because of impaired perception/interaction. These results show that visual experience mediates the neural mechanisms generating alpha oscillations during the first years of life, suggesting that it is a sensitive period for the plasticity of this process.
Dev Cogn Neurosci, 2021; 49

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BOARD NUMBER: S06-488

ASSESSING FUNCTIONAL NETWORKS DYNAMIC THROUGH HIGH-DENSITY LONGITUDINAL EEG RECORDINGS IN A NEW MOUSE MODEL OF EARLY ALZHEIMER'S DISEASE

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Alzheimer's disease (AD) is a neurodegenerative pathology characterized by a progressive and irreversible deterioration of cognitive functions, especially memory. As first neuropathological hallmarks arise decades before first memory deficits, a critical challenge in current research on AD is to find a reliable and early marker of the disease. Recently, our team showed that a new AD mouse model, the humanized *App*$^{NL-F}$*xMAPT* double knock-in (dKI) model, could generate key information on the initial disease stage. Indeed, 4-month-old dKI mice present early recognition deficit in an object-place association task and a disorganization of medial temporal lobe internal communication. To better characterize network alteration in the early stage of the disease, we performed long-term high-density EEG recording starting at 2-month of age in dKI and control littermate mice. Using functional brain network dynamic analyses, our preliminary results tend to show differences between genotypes, even at this early disease stage. This prompted us to test the effect of a non-invasive visual stimulation on network functional connectivity. Indeed, recent studies have shown that 40 Hz gamma entrainment via visual stimulation protocol (vGENUS) can reduce AD impact by improving cognitive performance, while mechanisms underlying these effects remain unclear. We first showed that vGENUS effectively rescued memory deficits in young dKI mice in the object-place association task. Strikingly, the main effects on dynamic were not at the gamma entrainment frequency but on other brain rhythms. Our results will provide new insight into early network dynamic alterations and the beneficial effect of a non-invasive gamma visual stimulation in AD.

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BOARD NUMBER: S06-489

REVISITING NEURAL BASIS OF BELIEF UPDATING: P300, PUPIL DILATION AND BETA RHYTHM AS RELIABLE INDICES OF A UNITARY PROCESS

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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AIMS A crucial step in making decisions is the updating of *a priori* beliefs based on current evidence. Belief updating is accompanied by three psychophysiological phenomena: P300, pupil dilation and electroencephalogram (EEG) power decrease, which some have linked to activity in norepinephrine-Locus Coeruleus system. However, these indices – albeit co-occurring – have been found uncorrelated across stimulus repetitions and participants, contradicting their alleged common origin. We aimed at re-examining this relationship employing a simple paradigm (3-stimulus oddball), thus avoiding confounding factors such as uncertainty and complexity; moreover, we aimed at overcoming these difficulties by separating out the impact of different factors (mainly, motor- vs. stimulus-related) on pupil and EEG responses. **METHODS** Event-related potentials (ERP) were derived from group-level (N=19) independent component analysis. Event-related spectral perturbations were computed *via* Short-Time Fourier Transform of those components. General Linear Model (GLM) was applied to identify EEG and pupil responses' predictors. **RESULTS** Besides confirming the co-occurrence of P300-like ERP, beta-band desynchronization and pupil dilation following target-oddball stimulus, we demonstrated a positive linear correlation among them (Pearson's correlation across participants, $r(19) = 0.60$, $p < 0.01$), which only emerged for stimulus-related component of pupil and EEG responses. **CONCLUSIONS** Our results highlight the complexity of pupil responses, which incorporate diverse factors that may confound the association of pupillometric and electrophysiological indices. However, once this complexity is appropriately modelled, phasic pupil dilation becomes predictive of P300-like ERP and beta-band suppression, supporting their inter-dependent nature, and proposing pupillometry as a useful tool for monitoring these physiological events.

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BOARD NUMBER: S06-490

INDIVIDUAL DIFFERENCES IN DOMINANT THETA-BAND FREQUENCY DURING ASSOCIATIVE MEMORY ENCODING

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Individual differences in brain electrophysiologic activity have been recognized a long time ago. Electroencephalographic (EEG) indices such as individual alpha (IAF) and gamma (IGF) frequency have been extensively investigated as biomarkers of individual differences in baseline neurophysiological activity and in response to different stimuli. In contrast, individual differences in dominant theta frequency have received considerably less attention. Given that theta-band EEG activity underlies memory processes, better knowledge of its individual difference, besides improving understanding of the physiology of memory processes, is of foremost importance for personalization of future non-invasive neuromodulation treatment attempts for memory impairments (such as in dementia and other neurodegenerative disorders). The aim of this study was to explore individual differences in individual theta-band frequency (ITF) from a scalp EEG recorded during associative memory (AM) encoding. In a sample of 42 healthy young adults (age 20 – 35; 50% female) we extracted the frequencies (2-10 Hz, in 0.5 Hz steps) with the highest event-related spectral perturbation from 19 overlapping time windows and six centroparietal electrodes from the EEG recorded during successful encoding in the AM task. The ITF was defined as modal frequency (in the 4-8Hz range) in the given time x electrode matrix. The method showed 93% success rate and the range of variability of the extracted ITFs. We present and discuss individual differences in theta-band activity during AM encoding, together with ITF-extraction challenges.

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BOARD NUMBER: S06-491

40-HZ STIMULATION DURING NREM SLEEP INDUCES A SPECIFIC AUDITORY STEADY-STATE RESPONSE AND AN INCREASE IN THE SLEEP-SPINDLE POWER ASSOCIATED WITH UP AND DOWN CORTICAL STATES

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Auditory Steady-State Response (ASSR) consists in a rhythmic brain activity resulting from sound stimuli presented periodically. This brain response is close to the stimulation frequency with a highest magnitude at 40 Hz. The ASSR has been evaluated as an assessment tool in different physiological conditions. For instance, it has been shown that ASSR is modulated by anesthesia and different vigilance states. In particular, it has been reported that ASSR decreases in NREM sleep compared to wake. Nevertheless, at a finer scale, it is not clear how this type of stimulation can induce brain changes when applied during up or down phases of slow oscillations (SO) characterizing NREM sleep. To assess this, we performed auditory stimulation during nights of 11 healthy subjects recorded with a scalp EEG system in a 10-20 configuration. Stimuli consisted of 40-Hz trains of white noise (400 ms). We classified stimulation events that fell around SO positive or negative peaks and for them we computed the 40-Hz ASSR through the Iter-Trial Coherence (ITC). We report a marked ITC with higher levels in frontal and central electrodes for both up and down states with no statistical difference between them. Furthermore, when compared to sham events, stimulation during both positive and negative peaks induced a strong significant enhancement ($p < 0.05$) of sleep spindle power for the majority of electrodes around 1 sec after the stimulus onset. Taken together, our results suggest that 40-Hz auditory stimulation can be an efficient way for inducing specif brain responses during sleep.

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BOARD NUMBER: S06-492

DATA-DRIVEN EEG THETA AND ALPHA COMPONENTS ARE ASSOCIATED WITH SUBJECTIVE EXPERIENCE DURING RESTING STATE

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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The resting-state paradigm is frequently applied in EEG research; however, it is associated with the inability to control participant's thoughts. To quantify subjects' subjective experiences at rest, the Amsterdam Resting-State Questionnaire (ARSQ) was introduced covering ten dimensions of mind wandering. We aimed to estimate associations between subjective experiences and data-driven EEG components extracted using frequency Principal Components Analysis (f-PCA). 5 minutes of resting-state EEG data of 226 subjects were used to evaluate data-driven EEG components extracted with f-PCA. The Bayesian correlation approach was implemented to assess associations between ARSQ domains assessed after resting and f-PCA outcomes. Three alpha components, one beta, one theta, and one delta component were identified corresponding to previous reports on the resting state EEG. Only frontal midline theta component peaking at 5.5 Hz and alpha component peaking at 9 Hz were positively associated with individual ratings of Sleepiness ($r=0.198$, $BF10 =7.115$) and Comfort ($r=0.200$, $BF10 =7.676$). No other correlations emerged. The inverse model of sLORETA for the theta component showed the main activation of the voxels at limbic lobe and anterior cingulate. The correlations observed between theta component and Sleepiness and alpha component and Comfort are in line with previously reported associations on EEG and fMRI signals, pointing to the relevance of assessments of spontaneous thoughts occurring during the resting state.

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BOARD NUMBER: S06-493

ABNORMAL PATTERNS OF SLEEP AND WAKING BEHAVIORS ARE ACCOMPANIED BY INCREASED SLOW GAMMA POWER IN AN ANK3 MOUSE MODEL OF EPILEPSY-MOOD DISORDER COMORBIDITY

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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The ANK3 gene is a leading bipolar disorder candidate gene in humans. Previous studies showed that a bipolar disorder (BD)-associated variant of ANK3 (ANK3-1b) leads to increased firing threshold and diminished action potential dynamic range of parvalbumin (PV) interneurons and absence epilepsy in mice, thus providing a potential mechanistic link between epilepsy and BD. To better understand the impact of PV interneurons on network activity and behavior in these mice, we examined spectral correlates of behaviors seen in *Ank3-1b* knockout (KO) mice during overnight home-cage activity using paired video-EEG recordings. PV interneuron dysfunction and aberrant gamma rhythms have been implicated in BD. Also, the fast-spiking properties of PV interneurons make them important for generating and modulating high frequency gamma oscillations. Thus, we anticipated changes in the power of EEG signals in the gamma frequency range (> 25 Hz) during behaviors related to BD symptoms seen in human patients such as changes in sleep and activity levels. *Ank3-1b* KO mice exhibited an overall increase in slow gamma (~25-45 Hz) power compared to controls, and slow gamma power correlated with seizure phenotype severity across behaviors. Notably, during sleep, increased slow gamma power co-occurred with decreases in time spent in the rapid eye movement (REM) phase. We also identified a repetitive behaviors phenotype in *Ank3-1b* KO mice that co-occurred with increased slow gamma power. These results further support the association of *Ank3-1b* with BD and suggest modulation of gamma oscillations as a potential therapeutic target.

Pubmed:

23414504: Orson FM, Rossen RD, Shen X, Lopez AY, Wu Y, Kosten TR
Spontaneous development of IgM anti-cocaine antibodies in habitual cocaine users: effect on IgG antibody responses to a cocaine cholera toxin B conjugate vaccine.
In cocaine vaccine studies, only a minority of subjects made strong antibody responses. To investigate this issue, IgG and IgM antibody responses to cocaine and to cholera toxin B (CTB-the carrier protein used to enhance immune responses to cocaine) were measured in sera from the 55 actively vaccinated subjects in a Phase IIb randomized double-blind placebo-controlled trial (TA-CD 109).
Am J Addict, 2013 Mar-Apr; 22
23010423: Singh RA, Kosten TA, Kinsey BM, Shen X, Lopez AY, Kosten TR, Orson FM
Dose-dependent changes in the locomotor responses to methamphetamine in BALB/c mice: low doses induce hypolocomotion.
The overall goal of the present study was to determine the effects of different doses of (+)-methamphetamine (meth) on locomotor activity of Balb/C mice. Four experiments were designed to test a wide range of meth doses in BALB/c female mice. In Experiment 1, we examined locomotor activity induced by an acute administration of low doses of meth (0.01 and 0.03mg/kg) in a 90-min session. Experiment 2 was conducted to test higher meth doses (0.3-10mg/kg). In Experiment 3, separate sets of mice were pre-treated with various meth doses once or twice (one injection/week) prior to a locomotor challenge with a low meth dose. Finally, in Experiment 4, we tested whether locomotor activation would be affected by pretreatment with a low or moderate dose of meth one month prior to the low meth dose challenge. Results show that low doses of meth induce hypolocomotion whereas moderate to high doses induce hyperlocomotion. Prior exposure to either one moderate or high dose of meth or to two, low doses of meth attenuated the hypolocomotor effect of a low meth dose one week later. This effect was also attenuated in mice tested one month after administration of a moderate meth dose. These results show that low and high doses of meth can have opposing effects on locomotor activity. Further, prior exposure to the drug leads to tolerance, rather than sensitization, of the hypolocomotor response to low meth doses.
Pharmacol Biochem Behav, 2012; 103
23022610: Shen XY, Kosten TA, Lopez AY, Kinsey BM, Kosten TR, Orson FM
A vaccine against methamphetamine attenuates its behavioral effects in mice.

Vaccines have treatment potential for methamphetamine (MA) addiction. We tested whether a conjugate vaccine against MA (succinyl-methamphetamine-keyhole limpet hemocyanin carrier protein; SMA-KLH) would generate MA antibodies and alter MA-induced behaviors.
Drug Alcohol Depend, 2013; 129
27956739: Lopez AY, Wang X, Xu M, Maheshwari A, Curry D, Lam S, Adesina AM, Noebels JL, Sun QQ, Cooper EC
Ankyrin-G isoform imbalance and interneuronopathy link epilepsy and bipolar disorder.
ANK3, encoding the adaptor protein Ankyrin-G (AnkG), has been implicated in bipolar disorder by genome-wide association studies. ANK3 has multiple alternative first exons, and a bipolar disorder-associated ANK3 variant has been shown to reduce the expression of exon 1b. Here we identify mechanisms through which reduced ANK3 exon 1b isoform expression disrupts neuronal excitation-inhibition balance. We find that parvalbumin (PV) interneurons and principal cells differentially express ANK3 first exon subtypes. PV interneurons express only isoforms containing exon 1b, whereas excitatory principal cells express exon 1e alone or both 1e and 1b. In transgenic mice deficient for exon 1b, PV interneurons lack voltage-gated sodium channels at their axonal initial segments and have increased firing thresholds and diminished action potential dynamic range. These mice exhibit an Ank3 gene dosage-dependent phenotype including behavior changes modeling bipolar disorder, epilepsy and sudden death. Thus ANK3's important association with human bipolar susceptibility may arise from imbalance between AnkG function in interneurons and principal cells and resultant excessive circuit sensitivity and output. AnkG isoform imbalance is a novel molecular endophenotype and potential therapeutic target.
Mol Psychiatry, 2017; 22

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STUDYING GAMMA OSCILLATIONS AND CONSCIOUSNESS DURING SLEEP USING SSVEPS

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Consciousness has been the focus of many scientific works over the past decades, but the debate about how to best study or define it is nowhere near settled. Brain oscillations in Gamma range (> 30 Hz) were highlighted in the literature devoted to Neural Correlates of Consciousness as a potentially relevant biomarker, usually assessed through EEG. Here, we focus on Gamma-range oscillations during wakefulness and sleep, where REM and NREM stages represent different states of (un)consciousness. Steady-State Visually Evoked Potentials (SSVEPs) have proven to be an effective way to study brain oscillations, even during sleep (Sharon & Nir, 2018). In this study, we demonstrate the feasibility of recording SSVEPs in the Gamma range during different sleep stages. A fully mobile setup is employed, including a custom-made sleep mask with LEDs to induce periodic visual flicker, along with the mobile EEG system Mentalab Explore. This novel method allows for a more naturalistic and effective study of oscillations during sleep, while ensuring a greatly improved Signal-To-Noise Ratio of the EEG data. Further studies based on this technique will be applied to larger samples for validation, as well as clinical populations with Disorders of Consciousness.

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MATHEMATICAL SIMULATION ENLIGHTENMENT AND EXPERIMENTAL IMPROVEMENT OF TDCS IN A MODEL OF PSYCHOTIC TRANSITION: A TRANSLATIONAL STUDY

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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The transcranial Direct Current Stimulation (tDCS) is a non-invasive promising neuromodulation approach to treat psychotic disorders, like schizophrenia. During the psychotic transition, symptoms are associated with disturbance of neuronal oscillations (oscillopathies), notably in the Cortico-Thalamo-Cortical (CTC) system. A state that can be mimicked in mammals by injection of a psychotomimetic dose of the NMDA receptor antagonist ketamine. It was demonstrated in rats that a unilateral fronto-parietal anodal tDCS can reduced ketamine-induced oscillopathies, with a differential efficiency on hemispheres. Our pre-clinical translational study proposes to bilaterally normalize ketamine-induced oscillopathies and to provide insights about CTC response to tDCS. To this aim, stimulation parameters are experimentally optimized and a mathematical model of the CTC neuronal populations was designed. In sedated rats, while recording fronto-parietal electroencephalogram, an acute ketamine administration was performed (2.5 mg/kg), followed by anodal or cathodal inter-hemispheric tDCS (from ± 0.5 to ± 2 mA). Parallelly, reproducing experimental conditions in numerical simulations brought information on subcortical states. Experimental results reveal that anodic and cathodic inter-hemispheric stimulation has a coherent spatio-temporal effect on both hemispheres, and are able to damped the peak effect of ketamine. Also, the +1 mA stimulation normalize oscillopathies 40 minutes after ketamine injection (versus 80-90 minutes in the sham condition). Moreover, numerical simulations highlighted the importance of delta rhythm synchronicity in psychosis and showed that anodal tDCS globally increase functional connectivity in the CTC circuit. These results draw the contours of an innovative preventive treatment by neuromodulation in psychiatry and lighten tDCS influence on networks.

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EFFECTIVE CONNECTIVITY ANALYSIS BASED ON COUPLED NEURAL MASS MODEL FOR TMS-EEG DATA

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Functional or effective connectivity analysis of the whole brain activity based on non-invasive recording has been gaining importance for understanding the mechanisms of interactions between brain regions during cognitive functions. While most of the analysis methods proposed so far are data-driven (model-free) ones, the methods utilizing the dynamical characteristics of neural activity might arrow us to understand the mechanism of interaction between brain regions more deeply. To this end, we employed coupled neural mass model to EEG data as the interregional brain dynamics to mathematically describe the measured neural activity. We derived parameters of the model such that it could reproduce characteristics of the measured data such as in the frequency- and temporal domains by minimizing difference in power spectrum density distribution and permutation patterns of time-series distribution between measured and simulated activity. In the present study, the proposed method was applied to EEG data with Transcranial Magnetic Stimulation (TMS) to see if it could detect TMS effects as a change in the parameter of inter-regional connection. As a result, (i) it was implicated that the proposed method successfully estimated model parameters that could reproduced the characteristics of EEG activity, (ii) the proposed method could detect TMS effect particularly in the regions near the TMS target, (iii) the parameter estimation was robust between different sessions conducted for identical subjects.

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TITLE : INFLUENCE OF BEHAVIORAL ACTIVITY LEVEL ON THE OCCURRENCE OF ABSENCE EPILEPTIC SEIZURES IN GAERS RATS

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Aim : Absence epilepsy is a generalized epilepsy characterized by sudden and brief loss of consciousness and affecting mostly children and teenagers, due to a large amout of remittance after puberty. Predicting the probability of the occurrence of an absence seizure may not only help to prevent seizures, but also to better understand the mechanism that triggers them. In this study, we investigated the interraction between two behavioral states (active and resting) and the occurrence of seizures. **Method :** Electroencephalographic recordings were performed continuously over a long period (5-7 days) to determine the occurrence of seizures in the Genetic Absence Epileptic Rat of Strasbourg (GAERS) model. In addition, the electrooculography, neck electromyography and head acceleration signals allowed the identification of active and resting behavioral states. **Result :** We found that seizures occur more often around the transition between periods of activity and rest, especially when the animal moves from an active to a resting state. Furthermore, in the epileptic rats, the active phase lasted longer on average than in controls. **Conclusions :** These results suggests that transition states, by acting on the level of excitability of the neuronal networks, could be a determining factor in the seizure triggering process. Furthermore, longer active phase in epileptic rats could participate in the development of commorbidities like increased anxiety and learning deficits.

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THE SPECTRAL AND FRACTAL NEURODYNAMICAL FEATURES AS A SIGNATURE OF CORTICAL AREAS: AN INSIGHT FROM MONTREAL NEUROLOGICAL INSTITUTE INTRACRANIAL SEEG

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Measuring power spectral density (PSD) and Higuchi fractal dimension (HFD) of intracranial sEEG, from Montreal Neurological Institute, we investigate the neurodynamics of 21 subjects in primary motor (M1), somatosensory (S1), and auditory (A1) cortices across wakefulness, N2, N3, and REM sleep stages. We aim to assess if the spectral and fractal features of the local ongoing electrical activity allow distinguishing cortical areas across different states and to evaluate whether a measure of fractal properties, typical of complex systems, catch physiological features possibly missed by linear spectral analyses. We observed specific spectral properties in wakefulness, where the prevailing frequencies above beta band were in M1, the alpha band waves were prevalent in S1, and the delta in A1. We also detected a transition from higher to lower frequencies depending on the sleep depth in the three regions of interest. On the contrary, HFD showed the ability to describe the sEEG complexity by being constantly higher in M1 than in S1, which in turn is higher than A1, steadily through all states of wakefulness and sleep. The core result of this study is to have highlighted the existence of typical features in distinct cortical parcels and that these properties maintain even during sleep. Moreover, this study confirmed our hypothesis that the measures of complexity (such as HDF) can detect the specificities of the ongoing electrical activity better than the linear ones. A distinction of neurodynamical properties of specific primary cortices supports the efforts in cortical parcelling based on their local electrical activity.

Pubmed:
34727186: Armonaite K, Bertoli M, Paulon L, Gianni E, Balsi M, Conti L, Tecchio F
Neuronal Electrical Ongoing Activity as Cortical Areas Signature: An Insight from MNI Intracerebral Recording Atlas.
The time course of the neuronal activity in the brain network, the neurodynamics, reflects the structure and functionality of the generating neuronal pools. Here, using the intracranial stereo-electroencephalographic (sEEG) recordings of the public Montreal Neurological Institute (MNI) atlas, we investigated the neurodynamics of primary motor (M1), somatosensory (S1) and auditory (A1) cortices measuring power spectral densities (PSD) and Higuchi fractal dimension (HFD) in the same subject (M1 vs. S1 in 16 subjects, M1 vs. A1 in 9, S1 vs. A1 in 6). We observed specific spectral features in M1, which prevailed above beta band, S1 in the alpha band, and A1 in the delta band. M1 HFD was higher than S1, both higher than A1. A clear distinction of neurodynamics properties of specific primary cortices supports the efforts in cortical parceling based on this expression of the local cytoarchitecture and connectivity. In this perspective, we selected within the MNI intracortical database a first set of primary motor, somatosensory and auditory cortices' representatives to query in recognizing ongoing patterns of neuronal communication. Potential clinical impact stands primarily in exploiting such exchange patterns to enhance the efficacy of neuromodulation intervention to cure symptoms secondary to neuronal activity unbalances.
Cereb Cortex, 2021;

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INTRINSIC ORGANIZATION OF HIPPOCAMPAL OSCILLATIONS

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Neuronal oscillations, a hallmark of mammalian brain, well preserved during evolution, enable synchronization of neural activity within and across brain regions, thus promoting the precise temporal coordination of neural processes underlying high cognitive functions. Oscillatory activity, occurring simultaneously in a given area at any given time, is thought to represent a physiological unit of a given brain state. Here, an analytical method has been developed to investigate how simultaneously occurring network oscillations, occurring at different frequencies, contribute to build up the network state space in the CA1 region of the hippocampus. To this aim, mice were chronically implanted with tetrodes/silicon probes during sleep and exploration of an arena during wakefulness. Power signals in distinct frequency bands ranging from 0.5 to 200 Hz, were extracted from local field potentials in *stratum pyramidale, radiatum and lacunosum moleculare* and were used to construct the state space of oscillations. The UMAP algorithm was employed to project the multi-dimensional (D) state space into 2D projection. With this approach, significant differences in the organization of oscillatory activity were detected among wake, REM and non-REM sleep. In addition, this method allowed unveiling state dependent coupling among different oscillations as well as alterations in state transitions after exploration of a novel arena suggesting the existence of a plasticity process at the network level. Finally, this approach allowed to study the firing activity of different neurons in the context of different brain states and their modulation by distinct network oscillations and *vice versa.*

Pubmed:
31379628: Modi B, Pimpinella D, Pazienti A, Zacchi P, Cherubini E, Griguoli M
Possible Implication of the CA2 Hippocampal Circuit in Social Cognition Deficits Observed in the Neuroligin 3 Knock-Out Mouse, a Non-Syndromic Animal Model of Autism.
Autism spectrum disorders (ASDs) comprise a heterogeneous group of neuro-developmental abnormalities with a strong genetic component, characterized by deficits in verbal and non-verbal communication, impaired social interactions, and stereotyped behaviors. In a small percentage of cases, ASDs are associated with alterations of genes involved in synaptic function. Among these, relatively frequent are mutations/deletions of genes encoding for neuroligins (NLGs). NLGs are postsynaptic adhesion molecules that, interacting with their presynaptic partners neurexins, ensure the cross talk between pre- and postsynaptic specializations and synaptic stabilization, a condition needed for maintaining a proper excitatory/inhibitory balance within local neuronal circuits. We have focused on mice lacking NLG3 (NLG3 knock-out mice), animal models of a non-syndromic form of autism, which exhibit deficits in social behavior reminiscent of those found in ASDs. Among different brain areas involved in social cognition, the CA2 region of the hippocampus has recently emerged as a central structure for social memory processing. Here, recordings from anesthetized animals and recordings from hippocampal slices have been used to assess the dynamics of neuronal signaling in the CA2 hippocampal area. experiments from NLG3-deficient mice revealed a selective impairment of spike-related slow wave activity in the CA2 area and a significant reduction in oscillatory activity in the theta and gamma frequencies range in both CA2 and CA3 regions of the hippocampus. These network effects were associated with an increased neuronal excitability in the CA2 hippocampal area. recordings from CA2 principal cells in slices obtained from NLG3 knock-out animals unveiled a strong excitatory/inhibitory imbalance in this region accompanied by a strong reduction of perisomatic inhibition mediated by CCK-containing GABAergic interneurons. These data clearly suggest that the selective alterations in network dynamics and GABAergic signaling observed in the CA2 hippocampal region of NLG3 knock-out mice may account for deficits in social memory reminiscent of those observed in autistic patients.
Front Psychiatry, 2019; 10
34661306: Basilico B, Ferrucci L, Ratano P, Golia MT, Grimaldi A, Rosito M, Ferretti V, Reverte I, Sanchini C, Marrone MC, Giubettini M, De Turris V, Salerno D, Garofalo S, St-Pierre MK, Carrier M, Renzi M, Pagani F, Modi B, Raspa M, Scavizzi F, Gross CT, Marinelli S, Tremblay MÈ, Caprioli D, Maggi L, Limatola C, Di Angelantonio S, Ragozzino D

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Microglia control glutamatergic synapses in the adult mouse hippocampus.

Microglia cells are active players in regulating synaptic development and plasticity in the brain. However, how they influence the normal functioning of synapses is largely unknown. In this study, we characterized the effects of pharmacological microglia depletion, achieved by administration of PLX5622, on hippocampal CA3-CA1 synapses of adult wild type mice. Following microglial depletion, we observed a reduction of spontaneous and evoked glutamatergic activity associated with a decrease of dendritic spine density. We also observed the appearance of immature synaptic features and higher levels of plasticity. Microglia depleted mice showed a deficit in the acquisition of the Novel Object Recognition task. These events were accompanied by hippocampal astrogliosis, although in the absence ofneuroinflammatory condition. PLX-induced synaptic changes were absent in Cx3cr1 mice, highlighting the role of CX3CL1/CX3CR1 axis in microglia control of synaptic functioning. Remarkably, microglia repopulation after PLX5622 withdrawal was associated with the recovery of hippocampal synapses and learning functions. Altogether, these data demonstrate that microglia contribute to normal synaptic functioning in the adult brain and that their removal induces reversible changes in organization and activity of glutamatergic synapses.
Glia, 2022; 70

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BOARD NUMBER: S06-500

DO SPONTANEOUS ALPHA OSCILLATIONS PREDICT SUBJECTIVE TIME DURING RESTING-STATE?

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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In time perception research, alpha rhythms were seminally proposed to embody the internal clock, but most studies attempting to link time estimates with spontaneous alpha dynamics have failed to provide conclusive evidence. Here, we revisit this hypothesis with a new approach. We recorded 63 human participants for a few minutes of resting-state with magnetoencephalography (MEG). Before the MEG recording, participants were not instructed to attend to the passage of time; after the MEG recording, they were asked to estimate how long the recording lasted. Participants retrospectively underestimated the duration they spent in resting-state. We found that the power of alpha rhythms (7-14 Hz) during resting-state linearly predicted participants' retrospective time estimates and specifically, the duration of alpha bursts (more than their amplitude) was a predictor of retrospective duration. As a control condition, a subgroup of 25 participants from the same pool of participants performed a prospective duration estimation task in which they knew ahead of the MEG recording they would have to attend to the passage of time. In this case no relation between the alpha rhythm and prospective time estimates was found. Moreover, at the time scale of minutes, we found no evidence that alpha peak frequency would be linked to retrospective or prospective timing. In the absence of attention to time and task demands, alpha bursts may embody discrete states of awareness like timestamps in our episodic landscape, from which accurate duration estimates can be recollected retrospectively. **Keywords** : episodic timing, retrospective, prospective, time perception, alpha bursts, M/EEG

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BOARD NUMBER: S06-501

PATTERNS OF PREFRONTAL-HIPPOCAMPAL CIRCUIT DEVELOPMENT IN JUVENILE MICE IN HEALTH AND A GENETIC RISK MODEL FOR SCHIZOPHRENIA

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Higher cognitive abilities, such as the capacity to hold information temporarily, defined as working-memory (WM), critically depend on prefrontal-hippocampal coupling. On the flip side, poor cognitive performance in neuropsychiatric disorders results from prefrontal-hippocampal miswiring. We previously showed that the communication between prefrontal cortex (PFC) and hippocampus (HP) emerges early in life and that neonatal dysfunction of both areas is present long before the cognitive deficits. However, it is still unknown, how developing prefrontal-hippocampal circuits underlie the maturation of WM at juvenile age and whether network dysfunction towards late development predicts poorer WM performance in disease. To fill this knowledge gap, we monitor the patterns of neuronal and network activity in the PFC and HP throughout adolescent development (postnatal day (P) 16-60) in control and Df16(A)$^{+/-}$ mice that model human 22q11.2 microdeletions of neuropsychiatric disorders. Both groups are investigated during resting state (i.e. no task) and WM testing. During resting state, prefrontal gamma oscillations and firing in control mice progressively augment in their frequency and amplitude peaking around P30, and decrease afterwards. These developmental gamma features are impaired in the Df16(A)$^{+/-}$ model. After a transient decrease at around P16-20, theta band coupling between PFC and HP stabilizes at a high level along the juvenile development, being accompanied by an increase in WM performance. The prefrontal-hippocampal coupling is lower in Df16(A)$^{+/-}$ mice and disrupted during WM task. Thus, juvenile age represents a developmental time window of critical relevance for prefrontal-hippocampal function and cognitive maturation in health and disease.

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BOARD NUMBER: S06-502

CORTICAL STATES DYNAMICS ACROSS THE MOUSE SLEEP WAKE CYCLE

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Cortical states dynamics across the mouse sleep wake cycle The close resemblance between the electroencephalogram (EEG) observed during paradoxical sleep (or Rapid Eye Movement sleep, REM) and the EEG patterns observed during the fundamentally different cognitive state of wakefulness led Llinas and Paré (1991) to propose that REM and wakefulness are equivalent functional states, although the weight given to sensory inputs differs. The aim of this project is to investigate how the mouse whiskers sensory inputs are encoded in the cortex throughout the sleep-wake cycle. We performed local field potential (LFP) recordings in the primary somatosensory (S1) and motor (M1) cortices, combined with EEG and electromyogram recordings and the video-tracking of whisker movements. Our results reveal an acceleration of EEG Theta oscillations in REM concomitant with whisker movements. At the level of S1-LFP, we observe during REM the presence of spindle oscillations, typically associated with slow-wave-sleep, but exclusively outside whisking periods. The spectral analysis of S1-LFP also exhibits a significant decrease of the 2-5Hz power range during whisker movements, in both wakefulness and REM. This modulation in 2-5Hz power associated with whisker movements is also observed in M1-LFP and on the EEG but only in wakefulness. Therefore, our data show a modulation of the cortical activity associated with whisker movements that is restricted to S1 in REM, and which might underlie a more local sensory processing as well as a decrease in cortico-cortical integration during this state.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-503

PROPAGATING SPIKING ACTIVITY IN THE THALAMUS OF ANESTHETIZED RODENTS

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Propagating waves are involved in many aspects of brain functions such as neural computations in sensory and motor systems, or regulation of synaptic events; both during wakefulness and sleep. The subject of our research was the thalamus, which plays an essential role in generating both slow oscillations and sleep spindles. Previous research on the propagation of thalamic oscillations is mostly based on in vitro studies. However, the application of multi-shank and high-density silicon-based probes now allows us to investigate the firing patterns of neuronal populations both at high spatial and temporal resolution in vivo. Using these techniques, we investigated whether propagating waves can be recorded in the thalamus of anesthetized mice and rats. We used NeuroNexus multi-shank probes (having 64 or 128 recording sites) and high-density Neuropixels probes with 384 channels (selectable from 960 sites) to record spontaneous activity patterns from several nuclei of the thalamus simultaneously. Our preliminary results indicate that, for both species, propagating firing activity appears principally in higher-order thalamic nuclei (e.g., Po or LDVL). Furthermore, we found that spatiotemporal patterns of these traveling neuronal waves are highly dependent on the actual brain state. Propagating patterns of sleep spindles were mainly observed in first-order nuclei (e.g., in the VPM). Our findings also suggest that the appearance and properties of propagating neuronal activity observed in the thalamus in vivo might be significantly influenced by a number of factors including the depth of anesthesia, the type of anesthetic used, or the degree of synchronization of the thalamocortical network.

Pubmed:
34267214: Horváth C, Tóth LF, Ulbert I, Fiáth R
Dataset of cortical activity recorded with high spatial resolution from anesthetized rats.
Publicly available neural recordings obtained with high spatial resolution are scarce. Here, we present an electrophysiological dataset recorded from the neocortex of twenty rats anesthetized with ketamine/xylazine. The wideband, spontaneous recordings were acquired with a single-shank silicon-based probe having 128 densely-packed recording sites arranged in a 32 × 4 array. The dataset contains the activity of a total of 7126 sorted single units extracted from all layers of the cortex. Here, we share raw neural recordings, as well as spike times, extracellular spike waveforms and several properties of units packaged in a standardized electrophysiological data format. For technical validation of our dataset, we provide the distributions of derived single unit properties along with various spike sorting quality metrics. This large collection of in vivo data enables the investigation of the high-resolution electrical footprint of cortical neurons which in turn may aid their electrophysiology-based classification. Furthermore, the dataset might be used to study the laminar-specific neuronal activity during slow oscillation, a brain rhythm strongly involved in neural mechanisms underlying memory consolidation and sleep.
Sci Data, 2021; 8

FENS Forum 2022
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BOARD NUMBER: S06-504

WHAT IS A BURST? TRANSIENT BETA BAND PHENOMENA IN SINGLE-TRIAL FRONTAL OSCILLATORY DYNAMICS HAVE DIFFERENT PROPERTIES DEPENDING ON THE BURST DETECTION METHOD USED.

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Motor and cognitive processes are reliably accompanied by trial-averaged differences in the beta band (12-35 Hz) localized to sensorimotor and frontal areas. Yet, this act of averaging appears to do a disservice to the true underlying dynamic. It is increasingly clear that non-sustained transient 'burst-like' rhythms are the predominant feature in the beta band. Whilst the analysis of the properties of bursts of oscillation in cortex is an increasing feature of the field, the methods used to detect and identify those bursts shows significant variation, and as such the definition of what constitutes a burst must vary as well. Here we systematically compare published burst detection methods – power threshold and cycle-by-cycle approaches - on simulated and real neural data. First, we simulate realistic neural data with bursts that have different distributions of burst characteristics and evaluate how each method classifies bursts. Second, we use each method to detail the characteristics of the bursts recovered in real data from mouse and macaque recordings (local field potential and ECoG); and detail the differences of burst characteristics recovered by each method. Our analyses show that phenomena considered to be "beta bursts" by different detection methods have strikingly different oscillatory and temporal properties. They may have very different functional implications and should not all be grouped together. These results have methodological implications for the field, but more fundamentally they reveal multiple phenomena in neural data that could be considered "bursts", with different properties.

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BOARD NUMBER: S06-505

CLOSED-LOOP PHASE-DEPENDENT OPTOGENETIC MODULATION OF MOTOR CORTICAL THETA OSCILLATIONS

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Neuronal oscillations are a prominent feature of motor cortical local field potentials (LFPs) and abnormalities in oscillatory activity have been linked to several disorders. Theta-modulated gamma frequency pulses of alternating current stimulation modulate human motor learning, however, it is unclear how these frequencies modulate motor cortical activity at the microcircuit level. Here, we aimed to develop a method of bidirectionally modulating theta and gamma coupled oscillations in the motor cortex using closed-loop optogenetic stimulation of parvalbumin-expressing (PV) interneurons. Motor cortical LFPs and single units were recorded in PV-Cre mice in which PV-interneurons were transfected with Channelrhodopsin-2 allowing reliable modulation of ongoing spiking activity with blue light. Using our recently developed phase-tracking system, pulses of blue-light were delivered at four target phases of the ongoing theta oscillation in the motor cortical LFP. Light was delivered over a quarter of the theta cycle, either as a continuous pulse or a burst of three pulses at gamma frequency. Both the continuous pulse and gamma frequency stimulation modulated theta power in a phase-dependent manner, with stimulation targeted to the ascending and descending phases suppressing and amplifying theta power respectively. This study demonstrates that theta power can be modulated using phase-dependent closed-loop optogenetic stimulation of PV-expressing interneurons and that this can be achieved using gamma frequency stimulation. These methods can be used to uncover the mechanism through which oscillatory manipulations modulate cortical activity and aid their future development.

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BOARD NUMBER: S06-506

MICROGLIA-MEDIATED REORGANIZATION OF ADOLESCENT PREFRONTAL CIRCUITRY UNDERLIES ADULT COGNITIVE ABILITIES

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Brain development is not a linear process. The relevance of critical time windows of development has been extensively documented for sensory systems, yet it is still a matter of debate, whether similar processes occur in brain areas accounting for cognitive behavior, such as the prefrontal cortex. Our recent data demonstrate that during neonatal development, perturbation of prefrontal activity dramatically disrupts adult network function and cognition. However, the protracted timeline of the maturation of higher-order cognitive abilities, leads to the hypothesis that critical windows for adult prefrontal functioning extend to the adolescent period. To prove this hypothesis, we combine chronic extracellular recordings and optogenetic manipulations with in-depth morphological and behavioral assessments of mice from early pre-juvenile age until adulthood. We show that neuronal firing complexity, oscillatory phase-amplitude synchrony, and excitation/inhibition ratio of prefrontal network activity increase almost linearly with age. In contrast, oscillatory gamma power and firing rates of regular spiking neurons deviate from a linear transition and peak during early adolescence. The timeline of these functional changes relates to a temporal augmentation of dendritic complexity of pyramidal neurons and increased pruning of excitatory postsynaptic compartments of these neurons by microglia cells. Pharmacological inactivation of microglia cells during adolescence but not adult age leads to a lasting increase in broadband gamma power and shifts the excitation/inhibition ratio towards excitation, causing ultimately poorer working memory abilities. These results support the presence of a critical time windows during adolescent prefrontal development and uncover mechanisms by which neuronal-microglial interactions shape circuitry and cognitive behavior.

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BOARD NUMBER: S06-507

FOURIER-BASED WAVEFORM ANALYSIS DISSOCIATES HUMAN CORTICAL ALPHA RHYTHMS

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Human cortical alpha oscillations are non-sinusoidal, and the wave shape of neuronal oscillations is an emerging indicator for neurological and neuropsychiatric disease. However, wave-shape analysis has been largely limited to particularly dominant rhythms and has been unable to cover the full space of possible waveform features. Here, we contribute a novel frequency-domain based waveform-analysis method that allows for a parsimonious and comprehensive analysis of non-sinusoidal wave shapes. We applied this novel approach to human cortical alpha oscillations recorded during resting-state using magnetoencephalography (MEG). Our approach allowed us to dissociate up to six different alpha-frequency waveforms, highlighting the potential of our wave-shape analysis approach for the dissociation of neural rhythms in clinical and basic neuroscience.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-508

CHARACTERIZATION OF SLOW OSCILLATIONS AND SPINDLES DURING SLEEP FROM THE JUVENILE TO THE PERI-ADOLESCENT DEVELOPMENTAL STAGE IN RATS

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Sleep is known to be an important mediator of memory consolidation through the precise temporal coordination of cortical slow oscillations (SOs) and thalamic spindles during slow-wave sleep. These oscillatory hallmarks have been well investigated in adulthood, however, a description of their temporal coordination across early development is missing. We therefore recorded the electroencephalogram (EEG) from skull electrodes over the frontal and parietal cortex in juvenile and peri-adolescent rats at postnatal day (PD)25 and PD31 roughly corresponding to human early childhood and late childhood. We found an increase in the amplitude of frontal and parietal SOs from PD25 to PD31. Additionally, we identified an increase in the number and density of frontal spindles from PD25 to PD31, while no such increase was observed for parietal spindles. Spindle power increased in both frontal and parietal regions in parallel with the increase observed in SO amplitude. As the temporal synchrony between SOs and spindles, we found a similar percentage of spindles coupled to a SOs at both ages, in frontal and parietal recordings. However, phase-locking spindles to the upstate of an SO showed a different topographical trajectory: at frontal recording sites consistent SO-spindle phase-locking was still absent at PD25 and emerged only at PD31 whereas at parietal recording sites, SO-spindle phase-locking was present already at PD25 without further changes at PD31. These results shed light on a gradual emergence of the SO-spindle coupling during development with different trajectories in frontal and posterior cortex regions.

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BOARD NUMBER: S06-509

SPECTRAL FINGERPRINTS OF CORTICAL NEUROMODULATION

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Pupil size has been established as a versatile marker of noradrenergic and cholinergic neuromodulation, which has profound effects on neuronal processing, cognition, and behavior. However, little is known about the cortical control and effects of pupil-linked neuromodulation. Here, we show that pupil dynamics are tightly coupled to temporally, spectrally, and spatially specific modulations of local and large-scale cortical population activity in the human brain. We quantified the dynamics of band-limited cortical population activity in resting human subjects using magnetoencephalography and investigated how neural dynamics were linked to simultaneously recorded pupil dynamics. Our results show that pupil-linked neuromodulation does not merely affect cortical population activity in a stereotypical fashion. Instead, we identified three frontal, precentral, and occipitoparietal networks, in which local population activity with distinct spectral profiles in the theta, beta, and alpha bands temporally preceded and followed changes in pupil size. Furthermore, we found that amplitude coupling at ~16 Hz in a large-scale frontoparietal network predicted pupil dynamics. Our results unravel network-specific spectral fingerprints of cortical neuromodulation in the human brain that likely reflect both the causes and effects of neuromodulation.

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BOARD NUMBER: S06-510

EFFECT OF CLEAR AND DEGRADED NATURAL SPEECH STIMULATION ON PUPIL RESPONSE FROM EYE-TRACKING DATA: A PILOT STUDY

POSTER SESSION 06 - SECTION: OSCILLATIONS AND NEURAL DYNAMICS

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Several studies showed that an increasing the degradation level of speech will lead to an increase in the pupil dilation response (Zekveld, 2018). However, those studies were designed to test the hypothesis with audio-visual stimuli. This study aimed to evaluate the influence of degraded speech on the pupil dilation response during only listening. In the experiment, participants just listened to the soundtrack of the movie “Forrest Gump” for 2 hours, and eye movements were recorded during the three different conditions: Clear Stimuli (CS), Degraded Stimuli (S2), and Highly Degraded Stimuli (N4). No visual stimuli were presented during the experiment. Participants were told explicitly to focus on the fixation cross on the screen. We hypothesized that an increased response in pupil dilation response is observed when the degradation level of sound increases as well. Due to the small sample size, we conducted micro statistical analysis by applying Kruskal-Wallis Test for each subject separately. Despite the results did not provide a comprehensive conclusion, they might imply that pupil dilation response does not increase in the event of degraded sound during the absence of visual stimuli. Hereupon one possible speculation might be that pupil dilation response is not caused by degraded sound directly. The reason for increased pupil dilation is that when one sensory system is not able to provide information, the other sensory system increases its effort and/or performance to derive information only if the corresponding stimulus is present.

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BOARD NUMBER: S06-511

ENHANCING SCALP SLEEP SLOW OSCILLATIONS AND SLEEP SPINDLES THROUGH TARGETED CLOSED-LOOP AUDITORY STIMULATION BASED ON IN-EAR EEG ELECTRODES

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Targeted closed-loop auditory stimulation during sleep has proven to be an efficient way to enhance endogenous brain activities. Recently, different studies have shown that this stimulation technique can also improve memory recalling processes, making it a promise tool for boosting cognitive functions during sleep. Nevertheless, classical experimental setups require the connection, by a trained staff, of scalp EEG electrodes which could be poorly tolerated by subjects. For this reason, in this work we evaluated the possibility to implement an auditory stimulation system based on in-ear EEG sensors. First, we designed especial in-ear electrodes connected in a bipolar configuration. Then, we implemented a previously validated algorithm [1] for the online detection of positive peaks from the in-ear-recorded slow oscillations (SO) of NREM sleep. Upon the detection of a valid peak, we delivered two sound clicks through especial headband speakers. We connected standard C4 and F4 scalp EEG contacts for comparison purposes. We validated the system through naps of 11 healthy subjects. We report that a precise targeted SO phase detection was possible on the in-ear channel, obtaining a preferred stimulation phase in the 70.62% of the cases for the first stimulus. In addition, we observed a strong SO amplitude and spindle power (11-16 Hz) enhancement in the scalp channels after in-ear stimulation compared with a sham condition where the actual sound was not delivered ($p < 0.05$). We believe that the presented in-ear setup represents an important technological advancement that could lead new functional and clinical applications in the future.

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BOARD NUMBER: S06-512

DEEP LEARNING-BASED SPIKE SORTING ON EDGE DEVICES

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Today's multi-channel silicon-based probes can generate large amounts of data, providing finer resolution and enabling better separation of the spike clusters. To cope with the increased complexity, several algorithms (so-called spike sorting algorithms) were developed to provide a faster and more accurate data analysis tool. The aim of our project was to assess the performance of deep learning methods and their efficiency regarding the evaluation of high complexity extracellular neural data on edge devices like a tensor processing unit (TPU). A supervised deep learning model was trained to identify and classify extracellular signals. The architecture of the model was selected from a list of well-known, optimized architectures used in the field of deep learning computer vision. The training data consisted of samples of 128x128 dimension semi-synthetic data in order to provide the most accurate ground truth to the model during training. After quantization, the model was transferred and tested on a Coral Development Board Mini, containing a Coral Edge TPU. During the initial supervised training of the model, a mean average precision of 63.04 was achieved on the test dataset. The inference speed of the initial model, with single-precision floating-point weights, could reach 3,17 msec/sample on a high-performance PC, while the quantized model on the development board reached an average speed of 90 msec/sample. Considering the promising results, a conclusion can be made, that by further optimizing the model in the future, a highly efficient, real-time method can be developed for spike sorting, enabling an efficient and near-state-of-art performance on edge devices.

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BOARD NUMBER: S06-513

COCHLEAR SPIRAL GANGLION-ON-A-CHIP

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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AIMS A key limitation of cochlear implant (CI) performance is the electrical interface with spiral ganglion neurons (SGNs). Therefore, we aim to develop a Spiral Ganglion-on-a-Chip,which combines 3D replication of the anatomy and core structures of the cochlea, along with embedded SGNs and multielectrode arrays (MEAs) to record neuronal responses expected in humans in a controlled and measurable in vitro system. **METHODS** The model consists of 3 main elements: 1)Rat SGNs and human induced pluripotent stem cell (hiPSC)-derived SGNs (in separate models) 2)Custom-made MEAs to measure cellular electrical activity and 3)Custom-designed 3D printed microfluidic chips to replicate the structure of the human cochlea. We focused on developing both the cellular and device aspects of the Spiral Ganglion-on-a-Chip.Our microfluidic device design has been iteratively optimised in order to ensure: accurate anatomical and electrical conductivity representation of the scala tympani(where CIs sit in the cochlea) and reproducible 3D printing and subsequent casting of the precise features of the PDMS casted model. **RESULTS** A casted PDMS device demonstrated good casting of all required microfeatures.Rat SGNs and glial cells were seeded in initial Cochlea-on-a-Chip prototypes and survived for over a month.SGNs extended neurites through the microchannels.We also developed human auditory neuron-like cells from human induced pluripotent stem cells (hiPSCs).These hiPSC-derived SGNs displayed both a similar morphology to rat SGNs and express a neuronal marker TUJ1. **CONCLUSIONS** Spiral Ganglion-on-a-chip that we have been developing and optimizing will enable us to optimize current and future CI stimulation strategies and chemical and biological therapeutics for hearing loss.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-514

LOW-DISTORTION AND LOW-NOISE CMOS AMPLIFIER FOR HIGH-CHANNEL-COUNT NEUROELECTRONIC INTERFACES

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Aims We present electrical and electrophysiological measurements of a new CMOS neural signals preamplifier. The design addresses the common problem of neural amplifiers with tunable lower cutoff frequency: high nonlinear distortion (>10% of Total Harmonic Distortion) of slow (~ 1 Hz), large-amplitude (a few mV) signals. Methods The main goal of the presented design was to reduce significantly the nonlinear distortion introduced by the standard AC coupling circuit while preserving low noise, low power and small silicon area of the amplifier. Results The measured THD values were below 0.8% for the frequency range 1-10000 Hz and amplitudes up to 10 mV p-p. The noise measured with Neuronexus probe (177 μm^2 iridium sites) was 6.5 µV and 7.2 µV in 1-300 Hz, 300–10000 Hz range respectively. The chip recorded wide-band electrophysiological signals with high-amplitude (up to 5 mV) field potentials i.e. slow sleep waves and responses evoked in rat barrel cortex by whisker stimulation. Spikes (up to about 1 mV) were also clearly visible. Conclusions Our preamplifier enables recording of the full spectrum of electrophysiological signals with minimal distortion, low noise and adjustable lower cutoff frequency (0.1-10 Hz). The design with its small silicon area (0.0046 mm^2) and low power consumption (7 µW) can become the base for future high-channel-count neuroelectronic interfaces. The complete amplifier with a second gain stage and analog/digital converter is currently under development. Funding This work was supported by Polish National Science Centre grant DEC-2013/10/M/NZ4/00268 (PH). B.T.-J, P.J. have been partially supported by the EU Project POWR.03.02.00-00-I004/16.

Pubmed:
33946209: Trzpil-Jurgielewicz B, Dąbrowski W, Hottowy P
Analysis and Reduction of Nonlinear Distortion in AC-Coupled CMOS Neural Amplifiers with Tunable Cutoff Frequencies. Integrated CMOS neural amplifiers are key elements of modern large-scale neuroelectronic interfaces. The neural amplifiers are routinely AC-coupled to electrodes to remove the DC voltage. The large resistances required for the AC coupling circuit are usually realized using MOSFETs that are nonlinear. Specifically, designs with tunable cutoff frequency of the input high-pass filter may suffer from excessive nonlinearity, since the gate-source voltages of the transistors forming the pseudoresistors vary following the signal being amplified. Consequently, the nonlinear distortion in such circuits may be high for signal frequencies close to the cutoff frequency of the input filter. Here we propose a simple modification of the architecture of a tunable AC-coupled amplifier, in which the bias voltages of the transistors forming the pseudoresistor are kept constant independently of the signal levels, what results in significantly improved linearity. Based on numerical simulations of the proposed circuit designed in 180 nm technology we analyze the Total Harmonic Distortion levels as a function of signal frequency and amplitude. We also investigate the impact of basic amplifier parameters-gain, cutoff frequency of the AC coupling circuit, and silicon area-on the distortion and noise performance. The post-layout simulations of the complete test ASIC show that the distortion is very significantly reduced at frequencies near the cutoff frequency, when compared to the commonly used circuits. The THD values are below 1.17% for signal frequencies 1 Hz-10 kHz and signal amplitudes up to 10 mV peak-to-peak. The preamplifier area is only 0.0046 mm and the noise is 8.3 µV in the 1 Hz-10 kHz range. To our knowledge this is the first report on a CMOS neural amplifier with systematic characterization of THD across complete range of frequencies and amplitudes of neuronal signals recorded by extracellular electrodes.
Sensors (Basel), 2021; 21

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-515

ENGINEERING A STRETCHABLE NERVE-ON-CHIP PLATFORM TO STUDY THE CHANGES IN NERVE CONDUCTION UNDER TENSION IN VITRO

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

Blandine Clément, Tobias Ruff, Léo Sifringer, Sophie Girardin, Sean Weaver, Simon Steffens, Margherita Razzoli, János Vörös
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Peripheral Neuropathy (PN) is a disease of nerves that can be induced by mechanical stress. Neuropathies are still an irreversible condition as available therapies are limited to symptomatic treatment. Development of restorative treatment requires efficient tools. Current *in vitro* methods mostly rely on random cell cultures lacking the biological complexity necessary to generate translatable results. Thus, nerve-on-a-chip models have recently gained interest as they can emulate better the *in vivo* architecture. However, their development on rigid glass multielectrode arrays (MEA) does not allow to study how mechanical stretch induces neuropathy or can be a regenerative strategy for nerve damage[1]. To address this, we aim to establish a stretchable *in vitro* nerve model platform.The platform consists of a polydimethylsiloxane (PDMS) microstructure[2] with seeding wells connected to 10-8 µm wide directional axon guidance channels that all converge into a mm size nerve-forming output channel. The PDMS microstructure aims to be mounted onto a customized stretchable MEA substrate. When seeding rat DRGs spheroids, we could grow several mm long DRGs axons within our PDMS microstructure on glass. Within 30 days, cells had already formed an axon fascicle in the *in vitro* nerve channel and were viable over 100 days. Moreover, co-cultured Schwann cells elicited myelination around some axonal segments. Overall, our initial feasibility experiments demonstrate the potential of the proposed methodology to facilitate the understanding of the role of tensile stress in neuropathy. *References* Shah et al. (2014), Neural Regeneration Research, 9(16): 1498-1501 Forró, C. et al. (2018), Biosensors and Bioelectronics, 122, 75-87.

FENS Forum 2022
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BOARD NUMBER: S06-516

MULTISCALE PATTERNING OF NEURONAL CIRCUITS

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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A key feature of the brain is organization on multiple scales whether it be nanodomains within the synapse, cortical columns, or the hippocampus. It is likely that such stereotyped organization is required for meaningful neural dynamics. However, experimental limitations make studying the dynamics of neural circuits across several spatiotemporal orders of magnitude *in vivo* difficult in the best of cases. While previous patterned *in vitro* systems provided simple analogs for studying neuronal circuits,[1,2] the connectivity was guided between small groups of ~20-50 neurons while the individual connections between neurons were not influenced. To better approximate the degree of organization *in vivo*, we have developed micro/nanofluidic structures able to guide connectivity from synapses to circuits. By using the difference in diameter of main axonal branches, ~450nm for rat hippocampal neurons, versus dendritic spine diameters, ~100nm, we patterned regions with nanochannels where synapses were able to form between pre and postsynaptic neurons. To validate if spines crossing nanochannels created functional synapses we monitored the calcium flux within the afferent axon and the spine head, neck, and dendrite of the postsynaptic neuron[3]. Though our aim is to use this patterning method to investigate fundamentals of neural computation *in vitro* with a specific focus on Hebbian learning, broader interest may be in using it to investigate the dynamics of individual synapses. [1] Forró, Csaba, et al. *Biosensors and Bioelectronics* 122 (2018) [2] Ihle, Stephan J., et al. *Biosensors and Bioelectronics* 201 (2022). [3] Mateus and Weaver et al. *Bioarxiv* (2022)

FENS Forum 2022
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BOARD NUMBER: S06-517

THE EFFECT OF NANOSTRUCTURATION OF SEMI-CONDUCTOR OR POLYMER MATERIALS IN NEURAL CELL CULTURES: IMPLICATIONS FOR NEURAL IMPLANT DESIGN

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Nanowires can be used in a broad range of bio-applications among which are neural implants for brain computer interface or neuroprostheses. We have shown that neurons from the Central Nervous System thrive when cultured on vertical arrays of semi-conductor nanowires (NWs), whereas the growth of glial cells on such arrays is limited compared to when cultured on flat substrates. However, semi-conductor nanowires present challenges in terms of integration in neural implants, such as their integration in a flexible substrate and their resistance to corrosion. We have analyzed the interaction of neuronal cells with NWs made from insulator polymers that are usually used for neural implants. For this purpose, we performed retinal and cortical cell cultures on SU8 and parylene-C polymer NWs. Four µm long SU-8 NWs positively influenced cell adhesion and neurite network formation compared to 1 µm long SU-8 NWs and flat SU-8 substrates. However, flat parylene-C was found to be the best polymer. Although we anticipate that parylene-C NWs might improve cell behavior, it has not yet been possible to obtain parylene-C NWs longer than 2µm. Taken together, these results suggest that arrays of nanowires are promising nanomaterials for designing neural interfaces and that the type of material and shape/dimensions of such nanomaterials play an important role.

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BOARD NUMBER: S06-518

IN SEARCH OF NEUROPHYSIOLOGICAL BIOMARKERS OF BINGE DRINKING AND EMOTIONAL EATING AMONG YOUTH

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Youth is a critical period of development in which heavy alcohol consumption has been related to significant physical, cognitive and emotional alterations. Along with this, it is increasingly common to find adolescents who, besides binge drinking, also present emotional eating, i.e., they eat excessively and unhealthily in the face of stressful events with the aim of reducing their anxiety state. Several studies have found a high degree of comorbidity between eating disorders and alcohol consumption. Despite the relevance of the topic and the consequences that binge eating behaviors have for young people, scarce studies have been focused on this topic and it is necessary to develop more preventive research.
Given that binge eating behaviors usually appear after stressful events in young people, we study in a sample of 18 to 25 year olds whether high levels of anxiety are related to intensive alcohol consumption and emotional eating. For this purpose, we propose an innovative virtual reality methodology that allows us to recreate a homogeneous and controlled stressful situation for all participants. Once exposed to this situation, we analyze whether there are psychological and/or neurophysiological differences between young people who binge eat and those who do not in this type of anxious context. Among these neurophysiological variables, we conduct a study of brain electrical activity by electroencephalogram (EEG). Thus, our main goal is to find possible biomarkers that allow to detect young people at risk of developing possible impulse control disorders, such as substance abuse or eating behavior disorders throughout life.

Pubmed:
33829973: Sampedro-Piquero P, Moreno-Fernández RD
Building Resilience with Aerobic Exercise: Role of FKBP5.
Both preclinical and clinical studies have pointed that aerobic exercise, at moderate doses, is beneficial at all stages of life by promoting a range of physiological and neuroplastic adaptations that reduce the anxiety response. Previous research about this topic has repeatedly described how the regular practice of aerobic exercise induces a positive regulation of neuroplasticity and neurogenesis-related genes, as well as a better control of the HPA axis function. However, limited progress has been carried out in the integration of neuroendocrine and neuroplastic changes, as well as in introducing new factors to understand how aerobic exercise can promote resilience to future stressful conditions. Resilience is defined as the ability to adapt to stress while maintaining healthy mental and physical performance. Consistent findings point to an important role of FKBP5, the gene expressing FK506-binding protein 51 (FKBP51), as a strong inhibitor of the glucocorticoid receptor (GR), and thus, an important regulator of the stress response. We propose that aerobic exercise could contribute to modulate FKBP5 activity acting as a potential therapeutic approach for mood disorders. In this sense, aerobic exercise is well known for increasing the growth factor BDNF, which by downstream pathways could affect the FKBP5 activity. Therefore, our manuscript has the aim of analyzing how FKBP5 could constitute a promising target of aerobic exercise promoting resilient-related phenotypes.
Curr Neuropharmacol, 2021; 19
31811875: Moreno-Fernández RD, Rosell-Valle C, Bacq A, Zanoletti O, Cifuentes M, Pérez-Martín M, Gavito AL, García-Fernández MI, Estivill-Torrús G, Rodríguez de Fonseca F, Santín LJ, Sandi C, Pedraza C
LPA receptor and chronic stress: Effects on behaviour and the genes involved in the hippocampal excitatory/inhibitory balance.
The LPA receptor, one of the six characterized G protein-coupled receptors (LPA) through which lysophosphatidic acid acts, is likely involved in promoting normal emotional behaviours. Current data suggest that the LPA-LPAreceptor pathway may be involved in mediating the negative consequences of stress on hippocampal function. However, to date, there is no available information regarding the mechanisms whereby the LPA receptor mediates this adaptation. To gain further insight into how the LPA-LPA pathway may prevent the negative consequences of chronic stress, we assessed the effects of the continuous

delivery of LPA on depressive-like behaviours induced by a chronic restraint stress protocol. Because a proper excitatory/inhibitory balance seems to be key for controlling the stress response system, the gene expression of molecular markers of excitatory and inhibitory neurotransmission was also determined. In addition, the hippocampal expression of mineralocorticoid receptor genes and glucocorticoid receptor genes and proteins as well as plasma corticosterone levels were determined. Contrary to our expectations, the continuous delivery of LPA in chronically stressed animals potentiated rather than inhibited some (e.g., anhedonia, reduced latency to the first immobility period), though not all, behavioural effects of stress. Furthermore, this treatment led to an alteration in the genes coding for proteins involved in the excitatory/inhibitory balance in the ventral hippocampus and to changes in corticosterone levels. In conclusion, the results of this study reinforce the assumption that LPA is involved in emotional regulation, mainly through the LPA receptor, and regulates the effects of stress on hippocampal gene expression and hippocampus-dependent behaviour.
Neuropharmacology, 2020; 164
31244601: Tabbai S, Moreno-Fernández RD, Zambrana-Infantes E, Nieto-Quero A, Chun J, García-Fernández M, Estivill-Torrús G, Rodríguez de Fonseca F, Santín LJ, Oliveira TG, Pérez-Martín M, Pedraza C
Effects of the LPA Receptor Deficiency and Stress on the Hippocampal LPA Species in Mice.
Lysophosphatidic acid (LPA) is an important bioactive lipid species that functions in intracellular signaling through six characterized G protein-coupled receptors (LPA). Among these receptors, LPA is a strong candidate to mediate the central effects of LPA on emotion and may be involved in promoting normal emotional behaviors. Alterations in this receptor may induce vulnerability to stress and predispose an individual to a psychopathological disease. In fact, mice lacking the LPA receptor exhibit emotional dysregulation and cognitive alterations in hippocampus-dependent tasks. Moreover, the loss of this receptor results in a phenotype of low resilience with dysfunctional coping in response to stress and induces anxiety and several behavioral and neurobiological changes that are strongly correlated with mood disorders. In fact, our group proposes that maLPA1-null mice represent an animal model of anxious depression. However, despite the key role of the LPA-LPA-pathway in emotion and stress coping behaviors, the available information describing the mechanisms by which the LPA-LPA-pathway regulates emotion is currently insufficient. Because activation of LPA requires LPA, here, we used a Matrix-Assisted Laser Desorption/ Ionization mass spectrometry-based approach to evaluate the effects of an LPA receptor deficiency on the hippocampal levels of LPA species. Additionally, the impact of stress on the LPA profile was also examined in both wild-type (WT) and the Malaga variant of LPA1-null mice (maLPA-null mice). Mice lacking LPA did not exhibit gross perturbations in the hippocampal LPA species, but the LPA profile was modified, showing an altered relative abundance of 18:0 LPA. Regardless of the genotype, restraint stress produced profound changes in all LPA species examined, revealing that hippocampal LPA species are a key target of stress. Finally, the relationship between the hippocampal levels of LPA species and performance in the elevated plus maze was established. To our knowledge, this study is the first to detect, identify and profile LPA species in the hippocampus of both LPA-receptor null mice and WT mice at baseline and after acute stress, as well as to link these LPA species with anxiety-like behaviors. In conclusion, the hippocampal LPA species are a key target of stress and may be involved in psychopathological conditions.
Front Mol Neurosci, 2019; 12
30990134: Sampedro-Piquero P, Moreno-Fernandez RD
The Forgotten Cells: Role of Astrocytes in Mood Disorders During the Aging.

Curr Neuropharmacol, 2019; 17
30061118: Moreno-Fernández RD, Nieto-Quero A, Gómez-Salas FJ, Chun J, Estivill-Torrús G, Rodríguez de Fonseca F, Santín LJ, Pérez-Martín M, Pedraza C
Effects of genetic deletion versus pharmacological blockade of the LPA receptor on depression-like behaviour and related brain functional activity.
Animal models of psychopathology are particularly useful for studying the neurobiology of depression and characterising the subtypes. Recently, our group was the first to identify a possible relationship between the LPA receptor and a mixed anxiety-depression phenotype. Specifically, maLPA-null mice exhibited a phenotype characterised by depressive and anxious features. However, the constitutive lack of the gene encoding the LPA receptor () can induce compensatory mechanisms that might have resulted in the observed deficits. Therefore, in the present study, we have compared the impact of permanent loss and acute pharmacological inhibition of the LPA receptor on despair-like behaviours and on the functional brain map associated with these behaviours, as well as on the degree of functional connectivity among structures. Although the antagonist (intracerebroventricularly administered Ki16425) mimicked some, but not all, effects of genetic deletion of the LPA receptor on the results of behavioural tests and engaged different brain circuits, both treatments induced depression-like behaviours with an agitation component that was linked to functional changes in key brain regions involved in the stress response and emotional regulation. In addition, both Ki16425 treatment and LPA receptor deletion modified the functional brain maps in a way similar to the changes observed in depressed patients. In summary, the pharmacological and genetic

approaches could ultimately assist in dissecting the function of the LPA receptor in emotional regulation and brain responses, and a combination of those approaches might provide researchers with an opportunity to develop useful drugs that target the LPA receptor as treatments for depression, mainly the anxious subtype.This article has an associated First Person interview with the first author of the paper.
Dis Model Mech, 2018; 11
29608953: Sampedro-Piquero P, Moreno-Fernández RD, Mañas-Padilla MC, Gil-Rodríguez S, Gavito AL, Pavón FJ, Pedraza C, García-Fernández M, Ladrón de Guevara-Miranda D, Santín LJ, Castilla-Ortega E
Training memory without aversion: Appetitive hole-board spatial learning increases adult hippocampal neurogenesis.
Learning experiences are potent modulators of adult hippocampal neurogenesis (AHN). However, the vast majority of findings on the learning-induced regulation of AHN derive from aversively-motivated tasks, mainly the water maze paradigm, in which stress is a confounding factor that affects the AHN outcome. Currently, little is known regarding the effect of appetitively-motivated training on AHN. Hence we studied how spatial learning to find food rewards in a hole-board maze modulates AHN (cell proliferation and immature neurons) and AHN-related hippocampal neuroplasticity markers (BDNF, IGF-II and CREB phosphorylation) in mice. The 'Trained' mice were tested for both spatial reference and working memory and compared to 'Pseudotrained' mice (exposed to different baited holes in each session, thus avoiding the reference memory component of the task) and 'Control' mice (exposed to the maze without rewards). In contrast to Pseudotrained and Control mice, the number of proliferating hippocampal cells were reduced in Trained mice, but they notably increased their population of immature neurons assessed by immunohistochemistry. This evidence shows that hole-board spatial reference learning diminishes cell proliferation in favor of enhancing young neurons' survival. Interestingly, the enhanced AHN in the Trained mice (specifically in the suprapyramidal blade) positively correlated with their reference memory performance, but not with their working memory. Furthermore, the Trained animals increased the hippocampal protein expression of all the neuroplasticity markers analyzed by western blot. Results show that the appetitively-motivated hole-board task is a useful paradigm to potentiate and/or investigate AHN and hippocampal plasticity minimizing aversive variables such as fear or stress.
Neurobiol Learn Mem, 2018; 151
29512441: Sampedro-Piquero P, Moreno-Fernandez RD
Editorial: Brain Basis of Resilience and its Effect on Mood Disorders in the Aging.
Curr Neuropharmacol, 2018; 16
29480526: Ladrón de Guevara-Miranda D, Moreno-Fernández RD, Gil-Rodríguez S, Rosell-Valle C, Estivill-Torrús G, Serrano A, Pavón FJ, Rodríguez de Fonseca F, Santín LJ, Castilla-Ortega E
Lysophosphatidic acid-induced increase in adult hippocampal neurogenesis facilitates the forgetting of cocaine-contextual memory.
Erasing memories of cocaine-stimuli associations might have important clinical implications for addiction therapy. Stimulating hippocampal plasticity by enhancing adult hippocampal neurogenesis (AHN) is a promising strategy because the addition of new neurons may not only facilitate new learning but also modify previous connections and weaken retrograde memories. To investigate whether increasing AHN prompted the forgetting of previous contextual cocaine associations, mice trained in a cocaine-induced conditioned place preference (CPP) paradigm were administered chronic intracerebroventricular infusions of lysophosphatidic acid (LPA, an endogenous lysophospholipid with pro-neurogenic actions), ki16425 (an LPA receptor antagonist) or a vehicle solution, and they were tested 23 days later for CPP retention and extinction. The results of immunohistochemical experiments showed that the LPA-treated mice exhibited reduced long-term CPP retention and an approximately twofold increase in the number of adult-born hippocampal cells that differentiated into mature neurons. Importantly, mediation analyses confirmed a causal role of AHN in reducing CPP maintenance. In contrast, the ki16425-treated mice displayed aberrant responses, with initially decreased CPP retention that progressively increased across the extinction sessions, leading to no effect on AHN. The pharmacological treatments did not affect locomotion or general exploratory or anxiety-like responses. In a second experiment, normal and LPA -receptor-deficient mice were acutely infused with LPA, which revealed that LPA -mediated signaling was required for LPA-induced proliferative actions. These results suggest that the LPA/LPA pathway acts as a potent in vivo modulator of AHN and highlight the potential usefulness of pro-AHN strategies to treat aberrant cognition in those addicted to cocaine.
Addict Biol, 2019; 24
28699486: Moreno-Fernandez RD, Tabbai S, Castilla-Ortega E, Perez-Martin M, Estivill-Torrus G, Rodriguez de Fonseca F, Santin LJ, Pedraza C
Stress, Depression, Resilience and Ageing: A Role for the LPA-LPA1 Pathway.
Chronic stress affects health and the quality of life, with its effects being particularly relevant in ageing due to the psychobiological characteristics of this population. However, while some people develop psychiatric disorders, especially depression, others seem very capable of dealing with adversity. There is no doubt that along with the identification of

neurobiological mechanisms involved in developing depression, discovering which factors are involved in positive adaptation under circumstances of extreme difficulty will be crucial for promoting resilience.
Curr Neuropharmacol, 2018; 16

28375206: Moreno-Fernández RD, Pérez-Martín M, Castilla-Ortega E, Rosell Del Valle C, García-Fernández MI, Chun J, Estivill-Torrús G, Rodríguez de Fonseca F, Santín LJ, Pedraza C

maLPA1-null mice as an endophenotype of anxious depression.

Anxious depression is a prevalent disease with devastating consequences and a poor prognosis. Nevertheless, the neurobiological mechanisms underlying this mood disorder remain poorly characterized. The LPA1 receptor is one of the six characterized G protein-coupled receptors (LPA1-6) through which lysophosphatidic acid acts as an intracellular signalling molecule. The loss of this receptor induces anxiety and several behavioural and neurobiological changes that have been strongly associated with depression. In this study, we sought to investigate the involvement of the LPA1 receptor in mood. We first examined hedonic and despair-like behaviours in wild-type and maLPA1 receptor null mice. Owing to the behavioural response exhibited by the maLPA1-null mice, the panic-like reaction was assessed. In addition, c-Fos expression was evaluated as a measure of the functional activity, followed by interregional correlation matrices to establish the brain map of functional activation. maLPA1-null mice exhibited anhedonia, agitation and increased stress reactivity, behaviours that are strongly associated with the psychopathological endophenotype of depression with anxiety features. Furthermore, the functional brain maps differed between the genotypes. The maLPA1-null mice showed increased limbic-system activation, similar to that observed in depressive patients. Antidepressant treatment induced behavioural improvements and functional brain normalisation. Finally, based on validity criteria, maLPA1-null mice are proposed as an animal model of anxious depression. Here, for we believe the first time, we have identified a possible relationship between the LPA1 receptor and anxious depression, shedding light on the unknown neurobiological basis of this subtype of depression and providing an opportunity to explore new therapeutic targets for the treatment of mood disorders, especially for the anxious subtype of depression.
Transl Psychiatry, 2017; 7

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FROM DATA TO KNOWLEDGE: AN OPEN, FULLY-AUTOMATED ELECTROENCEPHALOGRAPHY PIPELINE FOR BIOMARKER DISCOVERY

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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[1]Technical University of Munich, Neurology, School Of Medicine, Munich, Germany, [2]Technical University of Munich, Tum-neuroimaging Center, School Of Medicine, Munich, Germany, [3]Technical University of Munich, Center For Interdisciplinary Pain Medicine,school Of Medicine, Munich, Germany

Aims Biomarker discovery through non-invasive brain imaging techniques such as electroencephalography (EEG) requires reproducible and transparent methods applied to large-scale datasets. We developed a comprehensive and standardized EEG pipeline ranging from data acquisition to analysis and interpretation of findings. It allows an easy and large-scale application of EEG to study changes of brain function in various neuropsychiatric disorders. *Methods* We followed open science practices and the FAIR principles of scientific data management in the design of the pipeline. We designed it specifically –but not exclusively– for mobile EEG devices with dry electrodes. These allow much faster acquisition of data than traditional EEG setups with wet electrodes. Standardized EEG BIDS format was used for reading and storing the data. For preprocessing and analysis, we used elements from two open-source and widely used EEG toolboxes (EEGlab, fieldtrip). Finally, to facilitate interpretation of findings and the application to different neuropsychiatric conditions, we accompany our EEG pipeline with standardized patient reported outcomes that characterize overlapping symptoms across neuropsychiatric disorders (e.g., pain, fatigue, anxiety). *Results* We developed a comprehensive EEG pipeline for fast acquisition, preprocessing and analysis of resting state EEG data. It automatically preprocesses the data and extracts physiologically meaningful EEG features (power, connectivity, network characterization). It facilitates the creation of large-scale datasets and fosters sharing and reusability of the data. It is easy-to-use and applicable to a wide range of neuropsychiatric disorders. *Conclusions* Our pipeline promotes open and reproducible research on brain dysfunction in neuropsychiatric diseases, an indispensable step for robust biomarker discovery.

Pubmed:
35050961: Heitmann H, Gil Ávila C, Nickel MM, Ta Dinh S, May ES, Tiemann L, Hohn VD, Tölle TR, Ploner M
Longitudinal resting-state electroencephalography in patients with chronic pain undergoing interdisciplinary multimodal pain therapy.
Chronic pain is a major healthcare issue posing a large burden on individuals and society. Converging lines of evidence indicate that chronic pain is associated with substantial changes of brain structure and function. However, it remains unclear which neuronal measures relate to changes of clinical parameters over time and could thus monitor chronic pain and treatment responses. We therefore performed a longitudinal study in which we assessed clinical characteristics and resting-state electroencephalography data of 41 patients with chronic pain before and 6 months after interdisciplinary multimodal pain therapy. We specifically assessed electroencephalography measures that have previously been shown to differ between patients with chronic pain and healthy people. These included the dominant peak frequency; the amplitudes of neuronal oscillations at theta, alpha, beta, and gamma frequencies; as well as graph theory-based measures of brain network organization. The results show that pain intensity, pain-related disability, and depression were significantly improved after interdisciplinary multimodal pain therapy. Bayesian hypothesis testing indicated that these clinical changes were not related to changes of the dominant peak frequency or amplitudes of oscillations at any frequency band. Clinical changes were, however, associated with an increase in global network efficiency at theta frequencies. Thus, changes in chronic pain might be reflected by global network changes in the theta band. These longitudinal insights further the understanding of the brain mechanisms of chronic pain. Beyond, they might help to identify biomarkers for the monitoring of chronic pain.
Pain, 2021;
34983852: Nickel MM, Tiemann L, Hohn VD, May ES, Gil Ávila C, Eippert F, Ploner M
Temporal-spectral signaling of sensory information and expectations in the cerebral processing of pain.
The perception of pain is shaped by somatosensory information about threat. However, pain is also influenced by an individual's expectations. Such expectations can result in clinically relevant modulations and abnormalities of pain. In the

brain, sensory information, expectations (predictions), and discrepancies thereof (prediction errors) are signaled by an extended network of brain areas which generate evoked potentials and oscillatory responses at different latencies and frequencies. However, a comprehensive picture of how evoked and oscillatory brain responses signal sensory information, predictions, and prediction errors in the processing of pain is lacking so far. Here, we therefore applied brief painful stimuli to 48 healthy human participants and independently modulated sensory information (stimulus intensity) and expectations of pain intensity while measuring brain activity using electroencephalography (EEG). Pain ratings confirmed that pain intensity was shaped by both sensory information and expectations. In contrast, Bayesian analyses revealed that stimulus-induced EEG responses at different latencies (the N1, N2, and P2 components) and frequencies (alpha, beta, and gamma oscillations) were shaped by sensory information but not by expectations. Expectations, however, shaped alpha and beta oscillations before the painful stimuli. These findings indicate that commonly analyzed EEG responses to painful stimuli are more involved in signaling sensory information than in signaling expectations or mismatches of sensory information and expectations. Moreover, they indicate that the effects of expectations on pain are served by brain mechanisms which differ from those conveying effects of sensory information on pain.
Proc Natl Acad Sci U S A, 2022; 119

33863863: May ES, Gil Ávila C, Ta Dinh S, Heitmann H, Hohn VD, Nickel MM, Tiemann L, Tölle TR, Ploner M
Dynamics of brain function in patients with chronic pain assessed by microstate analysis of resting-state electroencephalography.
Chronic pain is a highly prevalent and severely disabling disease that is associated with substantial changes of brain function. Such changes have mostly been observed when analyzing static measures of resting-state brain activity. However, brain activity varies over time, and it is increasingly recognized that the temporal dynamics of brain activity provide behaviorally relevant information in different neuropsychiatric disorders. Here, we therefore investigated whether the temporal dynamics of brain function are altered in chronic pain. To this end, we applied microstate analysis to eyes-open and eyes-closed resting-state electroencephalography data of 101 patients suffering from chronic pain and 88 age- and sex-matched healthy controls. Microstate analysis describes electroencephalography activity as a sequence of a limited number of topographies termed microstates that remain stable for tens of milliseconds. Our results revealed that sequences of 5 microstates, labelled with the letters A to E, consistently described resting-state brain activity in both groups in the eyes-closed condition. Bayesian analysis of the temporal characteristics of microstates revealed that microstate D has a less predominant role in patients than in controls. As microstate D has previously been related to attentional networks and functions, these abnormalities might relate to dysfunctional attentional processes in chronic pain. Subgroup analyses replicated microstate D changes in patients with chronic back pain, while patients with chronic widespread pain did not show microstates alterations. Together, these findings add to the understanding of the pathophysiology of chronic pain and point to changes of brain dynamics specific to certain types of chronic pain.
Pain, 2021; 162

33845173: May ES, Hohn VD, Nickel MM, Tiemann L, Gil Ávila C, Heitmann H, Sauseng P, Ploner M
Modulating Brain Rhythms of Pain Using Transcranial Alternating Current Stimulation (tACS) - A Sham-Controlled Study in Healthy Human Participants.
Chronic pain is a major health care problem. A better mechanistic understanding and new treatment approaches are urgently needed. In the brain, pain has been associated with neural oscillations at alpha and gamma frequencies, which can be targeted using transcranial alternating current stimulation (tACS). Thus, we investigated the potential of tACS to modulate pain and pain-related autonomic activity in an experimental model of chronic pain in 29 healthy participants. In 6 recording sessions, participants completed a tonic heat pain paradigm and simultaneously received tACS over prefrontal or somatosensory cortices at alpha or gamma frequencies or sham tACS. Concurrently, pain ratings and autonomic responses were collected. Using the present setup, tACS did not modulate pain or autonomic responses. Bayesian statistics confirmed a lack of tACS effects in most conditions. The only exception was alpha tACS over somatosensory cortex where evidence was inconclusive. Taken together, we did not find significant tACS effects on tonic experimental pain in healthy humans. Based on our present and previous findings, further studies might apply refined stimulation protocols targeting somatosensory alpha oscillations. TRIAL REGISTRATION: The study protocol was pre-registered at ClinicalTrials.gov (NCT03805854). PERSPECTIVE: Modulating brain oscillations is a promising approach for the treatment of pain. We therefore applied transcranial alternating current stimulation (tACS) to modulate experimental pain in healthy participants. However, tACS did not modulate pain, autonomic responses, or EEG oscillations. These findings help to shape future tACS studies for the treatment of pain.
J Pain, 2021; 22

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A NEW REAL-TIME EEG SOURCE LOCALIZATION METHOD

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Aims: Electroencephalography (EEG) provides insights for monitoring brain activity. Unfortunately, although scalp EEG can provide clinically useful information, its spatial resolution is low, making it difficult to determine the specific brain locations involved. However, noninvasive EEG-based source localization methods computationally estimate the locations, orientations, and strengths of the electric currents in the brain generating scalp potentials. Using anatomically faithful 3D head models, these approaches enable the accurate localization of epileptogenic foci, improve surgical efficacy, contribute to the improvement of critical care monitoring, and provide guidance for patient-tailored treatment. Our goal is to determine whether our method of source localization, called "Renormalization", is helpful for obtaining real-time feedback. Methods: Our approach is based on the lead field theory. Unlike other methods, ours computes the so-called “visibility field” of the recording system through a “3D Renormalisation” process. Then, to determine the spatial location and the strength of sources producing the potential scalp maps, this field is used to calculate a unique solution to the neuroelectric inverse problem. Results: We used the “Renormalisation” method with an “ANT Neuro eego sports” system and an anatomically realistic 3D head model. The visibility field characterizes the regions in which the recording system concentrates its source identification ability. We used it to provide an accurate real-time spatial mapping of electrical brain activity and characterization (localization, strength, orientation) of localized and distributed sources. Conclusions: By its specificities, we expect that our method will contribute to a better understanding of brain functions and improve the care of brain-injured patients.

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ASSESSING THE HYPEREXCITABILITY OF THE EPILEPTIC BRAIN BY BURST-SUPPRESSION EEG REACTIVITY

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Aim: The burst-suppression (BS) patterns emerging on the electroencephalogram (EEG) in deep anesthetic coma are reactive to external stimulation. We aimed to investigate whether this BS reactivity is increased in the epileptic brain.
Methods: Acute experiments were carried out in adult Wistar Albino Glaxo Rijswijk (WAG/Rij) rats, a commonly used genetic model of absence epilepsy which presents characteristic episodes of spike-and-wave discharges (SWD) lasting about 6 seconds on the wake EEG. Age-matched Wistar rats served as control. Cortical EEG recordings were carried out during isoflurane anesthesia. The BS patterns were quantified by the suppression ratio (SR), measuring the fraction of time spent in suppression, over 1-minute intervals. Investigations were carried out at a baseline SR of 40% - 80%. Intermittent photic stimulation (IPS) was delivered to one eye at 0.5 Hz in 1-minute epochs. Given the predominantly crossed visual pathways, the SR was assessed ipsilaterally to minimize the confounding effect of the visual evoked potentials. A BS reactivity index (BSRI) was defined as the reduction in SR that occurred during IPS, relative to the baseline SR recorded just prior to IPS.
Results: The IPS under deep anesthesia did not trigger SWDs. The mean BSRI was 0.2 in controls. In WAG/Rij the mean BSRI was increased by 55%. The difference was reduced after ethosuximide and paradoxically increased after carbamazepine at doses that had no effect on controls.
Conclusion: Our data suggest that measures of burst-suppression EEG reactivity could be useful to assess the hyperexcitability of the epileptic brain.

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BOARD NUMBER: S06-522

ASSESSMENT OF THE RAT ISCHEMIC BRAIN BY BURST-SUPPRESSION EEG REACTIVITY

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Aim: The electroencephalographic (EEG) assessment of the diffuse ischemic brain injury remains methodologically challenging. The aim of this study was to investigate the impairment in EEG reactivity to intermittent photic stimulation (IPS) following experimental global cerebral ischemia. Rather than focusing on continuous EEG measures, we tested the reactivity of the discontinuous burst-suppression (BS) patterns induced in deep anesthetic coma. Methods: Male Wistar rats were surgically exposed to a mild global cerebral ischemia by electrocauterization of the vertebral arteries and the subsequent clamping of both common carotid arteries for 5 minutes under laser doppler control. A group of rats exposed to sham surgery served as controls. Cortical EEG recordings were carried out at 48 hours after surgery when all rats appeared clinically recovered. The BS patterns, induced by an overdose of Chloral hydrate, were quantified by the suppression ratio (SR), measuring the fraction of time spent in suppression, over 1-minute intervals. The IPS was delivered to one eye at 0.5 Hz in 1-minute epochs. The BS reactivity index (BSRI) was defined as the reduction in the ipsilateral SR that occurred during IPS, relative to the baseline SR recorded just prior to IPS. Results: At a baseline SR of 40%-80%, the mean BSRI was 0.27 in controls. In contrast, the mean BSRI was about 3-fold smaller in the rats exposed to GCI. The amplitude of the visual evoked potentials was similar between the groups. Conclusion: Our data suggest that measures of burst-suppression EEG reactivity are sensitive to detect the post-ischemic brain injury.

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NON-INVASIVE MASTOID STIMULATION INFLUENCES VISUAL EMOTIONAL INFORMATION PERCEPTION

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Vagus nerve stimulation is a modern effective method of neuromodulation. Due to reducing anxiety and burnout auricular VNS improves the cognitive function. The aim of the current study was to evaluate the effects of the non-invasive vagus nerve stimulation (VNS) and sham stimulation (SHAM) on visual emotional information perception. 6 right-handed male volunteers aged 18-22 years (Mage= 18.00) participated in VNS study. 5 right-handed male volunteers aged 18-22 years (Mage= 18.00) participated in SHAM study. We used the combination of pleasant meditative classical music and a slow bi-polar wave (0.1-0.2 Hz) of electrical non-invasive transcutaneous auricular vagus nerve stimulation for 5 minutes. The set of 4 VNS was performed at intervals of 3 days. The participants were presented a set of alarming images, taken from the NAPS database (The Nencki Affective Picture System). After 1th session of VHS event-related EEG activity analysis detected activation of cortical structures involved in the stimulus processing (verbal memory (Fz) and cognitive processes (P3)) 600-800 ms after visual stimuli exposition. 4th VHS session leaded to changes of the temporal pattern of processing visual emotional information: we observed the activation of processes associated with emotional understanding (750 ms after the stimuli presentation), processes associated with attention, judgments formation and verbal memory (1200 ms). We may conclude that vagus nerve stimulation has enhanced the cognitive processes involved in the processing of stimuli and changed the temporal pattern of processing visual emotional information.

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PERFORMANCE OF NOVEL EEG-BASED ARTEFACT DETECTION METHODS IN SIMULTANEOUS NEUROPHYSIOLOGICAL AND HEMODYNAMIC RECORDINGS

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Aims: Simultaneous EEG-fMRI holds promise as a method offering novel insights into spatiotemporal circuit-level mechanisms in humans. Artefacts are induced in the EEG by the MR environment, caused by magnetic field gradient switches and electrode movement from scalp pulsations related to cardiac activity. Canonical methods rely upon unmet assumptions and the presence of additional recordings for artefact detection: gradient switch (GS) onsets and ECG. Without these, artefacts cannot be corrected, and the dataset deemed unusable. We have developed methods that enable the detection of GS/cardioballistic (CB) artefacts in the absence of additional/useable recordings. Objective: Validate the performance of our alternative methods against the canonical standard. **Methods:** 64-channel EEG-fMRI data from 26 healthy subjects during eyes-closed rest was analysed. GS onsets from the MR scanner and ECG were recorded. Sensitivity/specificity values were compared for pre-recorded (canonical) GS onsets versus those estimated in our automated method. Similarly, sensitivity/specificity values were compared for CB onsets as estimated from the ECG (canonical, Christov, 2004) versus our EEG-based semi-automated approach. **Results:** GS onset detection: Pre-recorded onsets yielded 100% sensitivity/100% specificity; Our method yielded 100% sensitivity/100% specificity. CB onset detection: Canonical standard yielded onsets with a mean sensitivity of 98.06% (std=1.74%) and specificity of 99.13% (std=1.77%). Our method yielded a mean sensitivity of 99.14% (std=1.42%) and specificity of 98.82% (std=1.05%). **Conclusions:** Our alternative methods (applicable to single-channel data) allow for artefact estimation from clean EEG data to a similar standard as canonical methods in the absence/useability of additional signals with clear advantages to the canonical approaches.

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A DEEP LEARNING APPROACH TO MOTOR UNIT NUMBER ESTIMATION

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Transcutaneous stimulation of a peripheral nerve with currents of increasing intensity evokes over the innervated muscle a compound motor action potential (CMAP) of increasing amplitude until all motor units are activated. Motor unit number estimation (MUNE) from such a muscle scan (MScan) remains a challenging problem, as it relies on fitting complex mathematical models. We propose a novel approach to MUNE, by harnessing the advances in deep neuronal networks. MScanFit (Bostock H., Muscle Nerve 2016: 889) introduced a simplified MScan model characterizing each motor unit by 3 parameters: amplitude, threshold and threshold variability. In addition, the model included a baseline noise and a cumulated noise of the of the amplitude variability. By varying all the MScanFit model parameters, we generated about 100000 simulated MScans, in the form of 256 x 256 pixels grayscale density images. This simulated dataset with known number of motor units was used for training a deep convolutional network, using a Visual Geometry Group type architecture terminating with a regression layer, referred to as EXMUNE.
We compared MScanFit MUNE and EXMUNE on real MScan recordings collected by stimulating the median nerve at wrist and recording the CMAP over the abductor pollicis brevis muscle. We found that in healthy volunteers, the MUNE was similar for the 2 methods. Nevertheless, in chronic inflammatory demyelinating polyneuropathy and in amyotrophic lateral sclerosis, the loss in motor units detected by EXMUNE was more concordant with conventional needle electromyography.
Our data suggest that deep learning can improve MUNE from MScan in pathology.

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A DYNAMIC CLAMP PROTOCOL TO ARTIFICIALLY MODIFY CELL CAPACITANCE

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Dynamics of excitable cells and networks depend on the membrane time constant, set by membrane resistance and capacitance. Whereas pharmacological and genetic manipulations of ionic conductances of excitable membranes are routine in electrophysiology, experimental control over capacitance remains a challenge. Here, we present capacitance clamp (CapClamp), an approach that allows to mimic a modified capacitance in biological neurons via an unconventional application of the dynamic clamp technique. We first demonstrate the feasibility to quantitatively modulate capacitance in a mathematical neuron model and then confirm the functionality of capacitance clamp in in vitro experiments in granule cells of rodent dentate gyrus with up to threefold virtual capacitance changes. In addition, we show proof-of-concept applications of the CapClamp to study temporal integration, energy consumption and bifurcations of neuronal dynamics. The feedback scheme underlying the CapClamp is easy to adopt in existing dynamic clamp setups and we provide corresponding code for open source dynamic clamp software (RELACS and RTXI). Clamping capacitance provides a flexible tool to investigate the consequences of actual changes of capacitance such as in neuronal growth. As the CapClamp can alter the membrane time constant via capacitance changes while leaving ionic conductances unaffected, it further lends itself to general investigations of neural dynamics, e.g. improving fitting of conductance-based neuron models. Altogether, the presented CapClamp constitutes a novel technique to decipher mechanisms of neuronal signaling in ways that were so far inaccessible to experimental electrophysiology.

Pubmed:
32031523: Pfeiffer P, Egorov AV, Lorenz F, Schleimer JH, Draguhn A, Schreiber S
Clusters of cooperative ion channels enable a membrane-potential-based mechanism for short-term memory.
Across biological systems, cooperativity between proteins enables fast actions, supra-linear responses, and long-lasting molecular switches. In the nervous system, however, the function of cooperative interactions between voltage-dependent ionic channels remains largely unknown. Based on mathematical modeling, we here demonstrate that clusters of strongly cooperative ion channels can plausibly form bistable conductances. Consequently, clusters are permanently switched on by neuronal spiking, switched off by strong hyperpolarization, and remain in their state for seconds after stimulation. The resulting short-term memory of the membrane potential allows to generate persistent firing when clusters of cooperative channels are present together with non-cooperative spike-generating conductances. Dynamic clamp experiments in rodent cortical neurons confirm that channel cooperativity can robustly induce graded persistent activity - a single-cell based, multistable mnemonic firing mode experimentally observed in several brain regions. We therefore propose that ion channel cooperativity constitutes an efficient cell-intrinsic implementation for short-term memories at the voltage level.
Elife, 2020; 9
34134979: Peng Y, Barreda Tomas FJ, Pfeiffer P, Drangmeister M, Schreiber S, Vida I, Geiger JRP
Spatially structured inhibition defined by polarized parvalbumin interneuron axons promotes head direction tuning.
In cortical microcircuits, it is generally assumed that fast-spiking parvalbumin interneurons mediate dense and nonselective inhibition. Some reports indicate sparse and structured inhibitory connectivity, but the computational relevance and the underlying spatial organization remain unresolved. In the rat superficial presubiculum, we find that inhibition by fast-spiking interneurons is organized in the form of a dominant super-reciprocal microcircuit motif where multiple pyramidal cells recurrently inhibit each other via a single interneuron. Multineuron recordings and subsequent 3D reconstructions and analysis further show that this nonrandom connectivity arises from an asymmetric, polarized morphology of fast-spiking interneuron axons, which individually cover different directions in the same volume. Network simulations assuming topographically organized input demonstrate that such polarized inhibition can improve head direction tuning of pyramidal cells in comparison to a "blanket of inhibition." We propose that structured inhibition based on asymmetrical axons is an overarching spatial connectivity principle for tailored computation across brain regions.

Sci Adv, 2021; 7
31038241: Manrique-Castano D, van Casteren A, Bouazza-Arostegui B, MacDonald DI, Pfeiffer P
ENCODS: A novel initiative to inspire young neuroscientists.
Eur J Neurosci, 2019; 49

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BOARD NUMBER: S06-527

ULTRASENSITIVE DOPAMINE DETECTION WITH GRAPHENE MULTITRANSISTOR ARRAYS

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Dopamine is a monoamine neurotransmitter with critical roles in the human brain and body. Abnormal alterations in the levels of dopamine can have severe consequences and underly brain disorders such as Parkinson's Disease (PD), Alzheimer's Disease, and Attention Deficit and Hyperactive Disorder. Detecting physiologically relevant dopamine concentrations with high sensitivity in the brain or brain-derived biological samples could bring new early diagnostic and therapeutic approaches for these disorders. However, conventional methodologies to detect dopamine typically have reduced selectivity or sensitivity or long detection cycles. In this work, we developed a novel miniaturized biosensor for dopamine detection based on graphene field-effect transistor (gFET) arrays functionalized with a selective dopamine-specific DNA aptamer. The integration of micron-sized gFETs in an array format permitted label-free ultrasensitive dopamine detection, yielding stable measurements by high-throughput sample measurement replication. With our novel biosensor we reliably detected ultra-low dopamine concentrations in various media, from physiological ionic-strength buffers, such as undiluted phosphate-buffered saline (PBS) and artificial cerebral spinal fluid (aCSF), to complex biological samples. We report record limits-of-detection (LODs) for dopamine, as low as 1 aM (10^{-18}), and wide sensing ranges up to 100 µM (10^{-8}). Furthermore, we show that the multitransistor arrays can detect minimal changes in dopamine concentrations in small working volume biological CSF and striatal brain homogenate samples from a mouse model of PD. These promising results can pave the way for novel point-of-care devices and academic and pharmaceutical research tools that require stable high-throughput detection of physiologically and clinically relevant dopamine concentrations.

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MEMBRANE-TARGETED LIGHT-SENSITIVE COMPOUND FOR OPTO-PORATION

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Light-driven modulation of neuronal activity at high Spatio-temporal resolution is becoming of high interest in neuroscience. Existing approaches, such as optogenetics, have shown promising results and, as an alternative, we envision using light-sensitive compounds that act without genetic manipulation. Here we engineered and characterized a novel polyconjugated compound (BV-1) that spontaneously and efficiently partitions into the lipid bilayer of primary hippocampal neurons after loading it in the external medium. Neuronal activity is not affected in the dark, whereas millisecond pulses of cyan light induce a robust and persistent depolarization. Our data, based on molecular dynamics simulation, high-speed atomic force microscopy imaging, electrophysiological recordings, and live imaging experiments on lipid bilayers and primary neuronal cultures, suggest the occurrence of two independent light-driven mechanisms: i) formation of pore-like structures that increase membrane permeability to Na^+ and ii) impairment of voltage-gated Na^+ channels activity by putative inhibition of their inactivation-gate. Within seven days after initial incubation, BV-1 is partially internalized, and light-driven membrane depolarization is consequently reduced but still possible to trigger. These properties open up to diverse applications, such as the possibility to employ this compound to perform patch-clamp experiments in a light-controlled perforated configuration. The main advantage of BV-1 is the possibility of modulating membrane perforation using light as a trigger and with significantly faster kinetics compared to conventional antibiotics used for the perforated-patch electrophysiological technique.

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AUTOMATED, TWO-PHOTON-TARGETED, MULTI PATCH-CLAMP ROBOT

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Whole-cell patch-clamp allows for the study of ion channel biophysics, membrane properties, and synaptic responses in intact brains. Multi-patching promises to elucidate the functional and connectivity properties of neuronal microcircuits, but the high skill and labor required (and low throughput) hinders its widespread dissemination. Automated multi-patching may solve this problem, but current 'blind' autopatchers are impractical for targeting secondary cell types. We have developed an automated two-photon-targeted quad-channel patch-clamping technology platform for ex vivo and in vivo electrophysiology, extending the system of Annecchino et al (Neuron 2017,95:1048-55). The platform comprises a two-photon microscope, four micromanipulators fitted with mechanical stability clamps, custom-developed electronically-controlled pressure regulator, patch-clamp amplifier and DAQs. Control is via a custom-developed LabView program which acquires frames directly from the microscope. In ex vivo mode (brain slices), a 3D-stack of the target cells is acquired, and an algorithm detects their center of mass using CFS (contrast focus score). All pipettes then simultaneously step towards their target cells, leaving them in contact. Then the sealing protocol is activated. For in vivo, a closed-loop algorithm is used, where pipettes approach their targets in a step-by-step mode, while the microscope repeatedly acquires images around the targets. After each trial, pipettes are automatically cleaned and returned to their previous positions, allowing for their reuse. Our targeted quad patch-clamp system allows scalable and reproducible electrophysiology studies to be conducted across a variety of laboratory settings, offering for the first time robotically automated recording of subthreshold signals from multiple genetically and optically targeted cells simultaneously.

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HUNTING THE MAGNETIC SIGNATURE OF ACTION POTENTIALS

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Magnetic signals are reference-free, pass through tissue undisturbed and include directional information. Intracranially recorded, they would be a great addition to electrophysiology, potentially providing intracellular information by means of extracellular recording. We have previously demonstrated that magnetic signatures of visually evoked potentials can be recorded with ~30x30µm sensors based on giant magnetoresistance (GMR). Here, we investigate whether it is also possible to record the magnetic signature of single action potentials (APs) with GMRs. We combined GMR and electrophysiological recordings in anesthetized rats (n=8). We limited the voltage across the sensor to avoid tissue warming. When averaging the magnetic field triggered on the thresholded electric spikes, we identified an artifact induced by common electro-magnetic noise on the two sensor types. This was avoided when using spikes from putative single units, identified by spike sorting. When focusing on spikes from 73 clean single-unit clusters with a high number of events (>7000 spikes) we could not identify any apparent signature in the magnetic signal on the sensor close to the putative single units. However, when looking at data from a second magnetic sensor, at 250µm distance, we found a delayed response in the magnetic signal. We hypothesize that this is not the magnetic signature of the AP, but a network effect following the event. We are still convinced that it will be possible to record magnetic signatures of single APs, but the current GMRs are not sensitive enough since the expected signal is very small.

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A NON-INVASIVE TECHNIQUE FOR RECORDING THE ELECTRICAL ACTIVITY OF THE HUMAN SPINAL CORD

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Aims: This work aims to develop a non-invasive technique to record the electrical activity of the spinal cord by means of active surface electrodes. **Methods:** 64 active electrodes are placed on the skin of the back in 6 columns parallel to the sagittal midline, covering from the cervical (C2) to the thoracic (T8) spinal segments. Interelectrode distance is ~3 cm across both rows and columns. Healthy volunteers participated in 3 different experiments: (1) a passive, fixed rate transcutaneous electrical stimulation of the median nerve; (2) two discrimination tasks, entailing either (a) passive texture stimulation or (b) active object manipulation; (3) finger tapping of different motor sequences at different levels of complexity. A number of post-processing and artifact correction techniques (referencing, ICA, adaptive filtering, and Canonical Correlation Analysis) were employed.
Results: Preliminary results confirmed the possibility of obtaining artifact-free evoked electrical potentials reflecting the electrical activity of the cord following the different types of tasks employed. Importantly, these results are obtained without aggressive preprocessing and extensive computational resources.
Conclusions: A specific electrode montage combined with the preprocessing and analysis pipeline developed for this project might contribute to the development of a non-invasive technology that allows recording the electrical activity of the spinal cord. Such a technique would have important implications in basic and clinical investigations, as well as in the development of novel BCI applications.

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BOARD NUMBER: S06-532

TRANSIENT MOTIFS IN NEURONAL DENDRITES REVEALED BY NANOPIPETTE ELECTROPHYSIOLOGY

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Although dendrites act as carriers of synaptic inputs, the nanoscale dimension of their microdomains precludes establishing direct and stable recordings for a sustained period of time, leaving their voltage dynamics poorly understood. To overcome this challenge of electrophysiological access, we developed quartz nanopipettes. Using these nanopipettes we were able to achieve recordings from neuronal dendrites lasting tens of minutes. Identification and characterisation of behavioural dynamics within the spontaneous activity recorded from primary rat and mice neuronal cultures with these nanopipettes revealed a diversity of voltage oscillation patterns. These complex voltage dynamics are a previously unexplored contribution to dendritic computations and may represent intrinsic activity or possibly reflect neuronal network activity.

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ARTEFACTS-FREE OPTOGENETICS IN DEEP BRAIN REGIONS WITH MULTIFUNCTIONAL TAPERED OPTICAL FIBERS

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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AIMS: The advent of optogenetics has generated the need of novel implantable tools able to optically control and electrically monitor neural activity. This poses an important technology challenge: the stimulation and readout volumes should overlap as much as possible, requiring light emitters and recording sites very close one to each other. However, this generates photoelectric artifacts due to direct electrode illumination during the stimulation train. In this work we propose a method to exploit mode division in implantable tapered optical fibers to direct stimulation light away from the recording pad. **METHODS:** Tapered optical fibers were coated with a stack of electric and insulating layers patterned with non-planar microfabrication techniques to realize optical emitting points (hereafter referred to as windows) and extracellular recording pads as close as 10µm. The optical behavior of the device was simulated by using Monte-Carlo simulation methods. The obtained multifunctional probes were tested in vivo in Thy1-ChR2 mice. **RESULTS:** We realized multiple devices with different combinations of window/electrode sizes, that were then characterized in terms of light emission patterns and electrochemical impedance. The devices were fist characterized in vitro to confirm the absence of photoelectric noise at power densities ranging from 2 to 10 mW/mm^2, at both high and low frequencies. This allowed for a good superposition of the stimulated and recorded volumes, enabling artefact-free optogenetics in deep brain regions of the living mouse brain.

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AN ELECTROPHYSIOLOGICAL INVESTIGATION OF THE EFFECTS OF DIRECT CURRENT STIMULATION OF THE TRIGEMINAL NERVE ON THE RAT HIPPOCAMPUS

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Background: Transcranial direct current stimulation (tDCS) is a neuromodulation method where low amplitude direct current is passed through scalp electrodes to create a weak electric field in the brain. tDCS was shown to affect many different functions, including memory. Previously it was assumed that the weak field in the brain could explain all the observed neuromodulatory effects. More recent evidence suggests the DC field in the skin may stimulate cranial nerves, such as the trigeminal nerve. These nerves then indirectly cause the observed neuromodulatory effects. However, this indirect mechanism is not fully understood yet. **Methods:** In 4 male Sprague Dawley rats we applied DC stimulation to the trigeminal nerve (0.25 – 3 mA, 3 minutes) while making single unit electrophysiological recordings from the hippocampus (CA1, CA3 and dentate gyrus) using a 32 channel silicon probes. **Results:** The results showed that trigeminal nerve DC stimulation using amplitudes similar to those of conventional tDCS in humans caused increasing spike rates across multiple locations in the hippocampus. This increase lasted up to an hour. **Conclusions:** We showed that tDCS applied to the trigeminal nerve caused a neuromodulatory effect at the level of the hippocampus. These results support the hypothesis that tDCS may work via an indirect peripheral route. The observed effects on the hippocampus may be driven by trigeminal nerve stimulation causing noradrenergic release. More studies could further help to fully understand this tDCS indirect mechanism.

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AN ELECTROPHYSIOLOGICAL INVESTIGATION OF THE EFFECTS OF DIRECT CURRENT STIMULATION OF THE TRIGEMINAL NERVE IN THE RAT BRAINSTEM

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Transcranial direct current stimulation (tDCS) is a popular non-invasive brain stimulation method used to modulate many different behavioral and cognitive functions. When low amplitude DC current is passed through the tDCS electrodes on the scalp it is generally assumed that the weak electric field in the brain causes the observed neuromodulation effects. Interestingly, very recent evidence suggests that the stronger electric field in the scalp may stimulate cranial nerves, such as the trigeminal nerve, which indirectly causes the neuromodulation effect. However, the electrophysiological effects of DC stimulation on the trigeminal nerve have, to the best of our knowledge, never before been studied. In 7 male Sprague Dawley rats, we applied direct current (DC) stimulation to the trigeminal nerve (0.25 – 3 mA, 1 minute) while making single-unit electrophysiological recordings from the trigeminal nuclei in the brainstem (primary sensory nucleus of the trigeminal nerve (NVsnpr), and the mesencephalic nucleus, MeV) using a 32 channel silicon probes. The results for 98 single units showed that trigeminal nerve DC stimulation using amplitudes similar to those of conventional tDCS in humans caused an increase in spiking in the trigeminal nuclei. Spike rates decreased after DC stimulation was stopped. The trigeminal nuclei are a key step in transmitting information from the periphery to the cortex. Results show for the first time that DC stimulation of the trigeminal nerve causes an increase in the trigeminal nuclei activity. These results support the idea that some of the observed tDCS effects could be caused by cranial nerve stimulation.

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THE DEFAULT EEG MACROSTATE REACTIVITY TO THE SUBJECT'S OWN NAME DEPENDS ON AWARENESS

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Aim: We recently proposed that the brain electrical activity is comprised of a sequence of alternating oscillatory states with repeating topographic spectral distributions on scalp electroencephalography (EEG), referred to as oscillatory macrostates. The macrostate showing the largest decrease in probability of occurrence measured in percent (reactivity) during sensory stimulation was labelled as the default EEG macrostate (DEM). The aim of this study was to assess whether awareness influences the DEM reactivity (DER). Methods: We included 11 middle cerebral artery ischemic stroke patients with impaired awareness having a median Glasgow Coma Scale (GCS) of 6//15 and a group of 11 matched healthy controls. EEG recordings were carried out during auditory 1-minute stimulation epochs repeating either the subject's own name (SON) or the name in reverse (rSON) which had no discernable meaning at the same acoustic level. The stimuli were generated using a voice synthesizer. The DEM was identified across 3 SON epochs alternating with 3 rSON epochs at 2-minute intervals. The DER was measured from the 1-minute quiet baseline preceding each stimulation epoch (EP3646784B1). The difference in mean DER between SON and rSON epochs, was measured by the salient EEG reactivity (SER). Results: The DER to rSON was about 27% in both controls and patients. The SER was 12.4 ± 2.7 % (Mean ± SEM) in controls and only1.3 ± 1.9 % in the patients (Mann Whitney $p<0.01$). The patient SER increased with the GCS (Pearson $r=0.71$). Conclusion: Our data suggest that the default EEG macrostate reactivity depends on awareness.

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REPEAT AT-HOME SAMPLING OF GAMIFIED BEHAVIOURAL TASKS AND WEARABLE DRY-EEG CAN MATCH THE QUALITY OF LAB-BASED SYSTEMS

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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Task-driven electroencephalography (EEG) is typically conducted with high-density wet sensor systems, under controlled laboratory conditions. This reduces the size and breadth of cohorts, and limits the technology's potential as an objective marker of functional neurophysiology for patient care and new therapies development. Recent advances have enabled the creation of wireless, 'dry' EEG systems, and easy-to-use engaging tasks emulating standard laboratory paradigms, that can be operated repeatedly by naïve users at-home without expert supervision. We report data from a 4-week long study (n=30 healthy males, mean age=25.6years). Participants were asked to complete gamified tasks unsupervised at home using a Cumulus Neuroscience 16-sensor headset, with tablet for task presentation. We evaluated adherence to the study protocol; quantified data quality by computing the Standard Measurement Error over repeated sessions; and compared results with a reference wet EEG dataset collected from a similar population (Luck et al., 2021). In total, 684 sessions were collected. Participants successfully completed 96% of sessions and reported a mean usability score of 88.3 on the SUS scale. Characteristic event-related potential (ERP) components – the P300 and error-related negativity – were observed in the Oddball and Flanker tasks, respectively. Aggregation of ERPs across sessions (2-4, depending on the task) resulted in grand average signal quality comparable to that of single-session data collected by experts in a laboratory setting using wet EEG. These results indicate that easy-to-use task-driven EEG is a suitable tool for cognitive neuroscience investigations, and has the potential to provide objective, frequent tracking of biomarkers of functional neurophysiology.

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A USER-FRIENDLY HOME-BASED VEP TASK, WITH SELF-ADMINISTERED DRY WIRELESS EEG, IS A FEASIBLE REAL-WORLD SURROGATE MARKER OF LTP.

POSTER SESSION 06 - SECTION: NEURO-ELECTRONICS & NEURO-ENGINEERING

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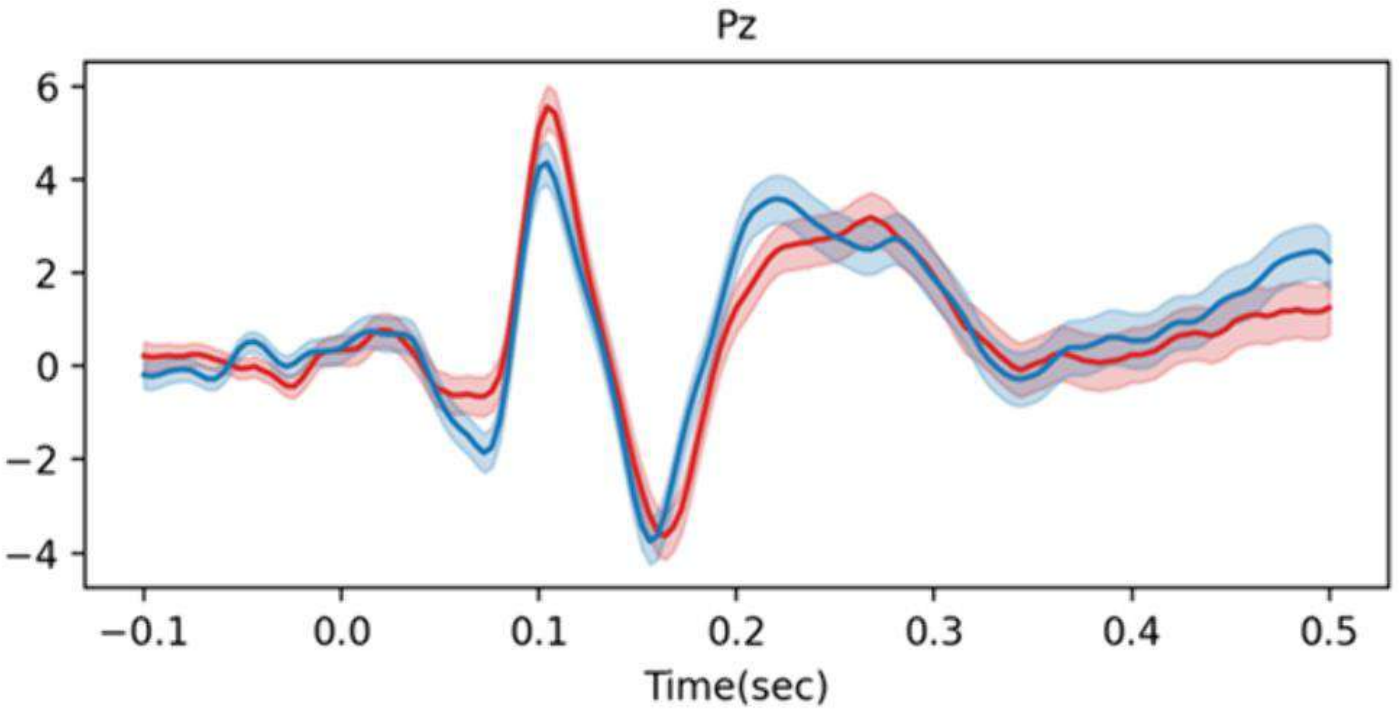
Background: Neuroplasticity is often altered in neurodegenerative and psychiatric disorders, and so has relevance as a marker of disease and response to therapy. In preclinical science measures are obtained invasively. In humans measures are high-burden (eg TMS; lab-based EEG) or difficult to link to intervention (eg BNDF).
Aims: The visual evoked potential (VEP) modulation task is based on a paradigm that induces LTP in animals. We evaluated the feasibility of home-based self administration with an easy-to-use dry EEG platform.
Methods: Twelve adults aged 22-48 (7 male, mean age 35.8 [SD 8.8]) received a tablet and Cumulus Neuroscience headset to home. Instructions were given by email and brief video-call. Participants viewed full field checkerboard reversals while counting centrally presented coloured circles. VEPs before and after 5 minutes of 2Hz potentiation were compared. Participants completed 1 session lasting ~11 minutes.
Results: Peak amplitudes of VEP components C1, P1, N1 and P1-N1 peak-to-peak difference were compared at electrode sites O1, O2 and Pz using Fpz as reference (Oz not included in headset configuration). At electrode site Pz there was a significant mean increase in P1 peak amplitude following potentiation (1.52μV [SD 2.76]; t(11)= 3.17; p= .004 uncorrected). **Conclusions:** A VEP modulation paradigm eliciting LTP-like correlates of cortical plasticity can be successfully self-administered in a low-burden session lasting less than 15 minutes. *Figure 1. Group mean pre- (blue) and post- (red)*

potentiation VEPs (n = 12). Y-axis is amplitude in μV. Shading shows 95% confidence intervals.



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CHRONIC FUNCTIONAL ULTRASOUND IMAGING COMBINED WITH BEHAVIOR TRACKING ON FREELY MOVING RATS PERFORMING SPATIAL EXPLORATION

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Brain-wide functional images of freely moving animals are key to understanding how cognitive behaviors may emerge from dynamic activation across different areas of the underlying neural circuitry. Functional Ultrasound (fUS) imaging has recently been demonstrated to robustly record brain-wide cerebral blood volume (CBV) dynamics as an indirect measure of neural activity over several weeks or months in freely moving rodents. Hardware and software interface to couple unrestrained cognitive experiments and fUS data is essential and can provide reliable use of this technique in a wide variety of behavioral paradigms. Here we propose studying freely moving rats in naturalistic behavior exploring an open arena using fUS. Spatial information is extracted using DeepLabCut library and custom pipeline. fUS imaging technology is optimized using a very light 15MHz piezo composite probe and highly flexible cable driven by an Ultrafast Ultrasound scanner (Iconeus One, Iconeus, France). With minimal preprocessing methods on the fUS data, we show encouraging results correlating changes in the CBV of specific brain regions, such as the dorsal hippocampus, with the speed of the animal during its exploration of the environment. In depth analysis of the signal suggests a sequence of activation within these brain regions during animal locomotion. Functional Ultrasound emerges as an interesting tool for unconstrained deep brain imaging during spatial navigation in
rodents.

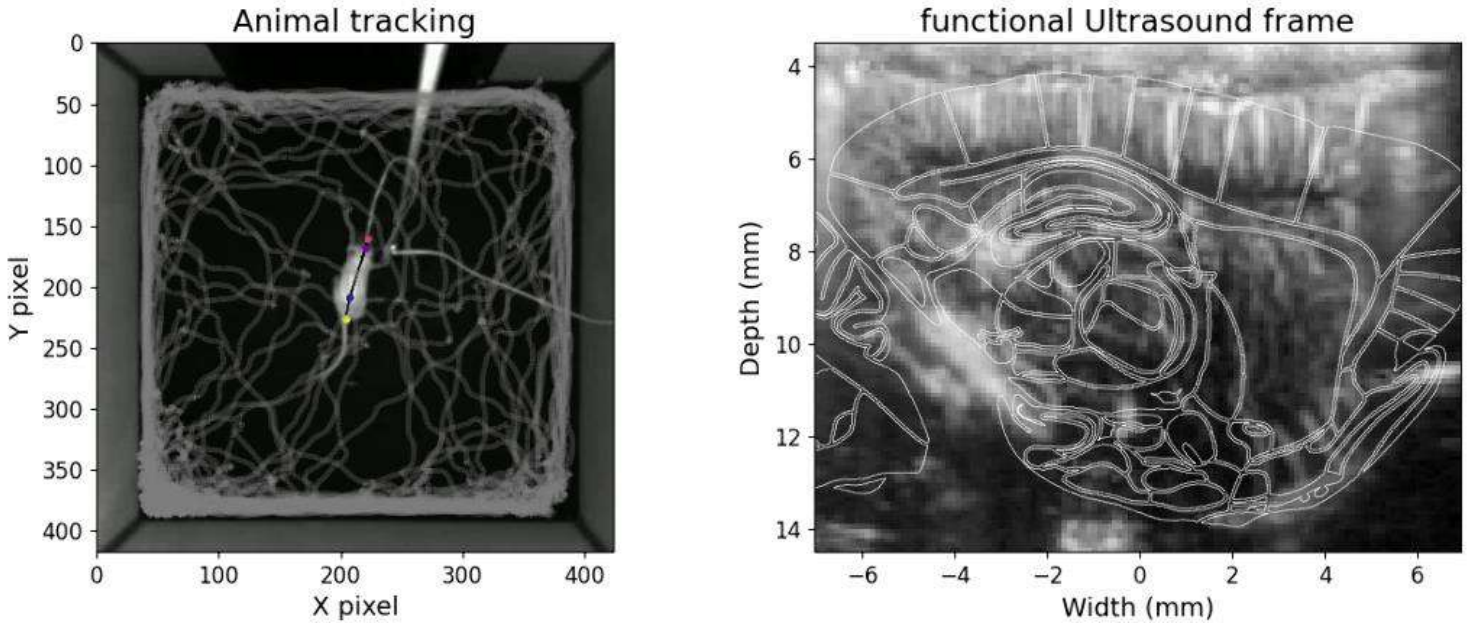

Figure 1: Simultaneous spatial navigation tracking and freely moving fUS imaging in sagittal view.

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MRI ATLAS OF THE PITUITARY GLAND AND AUTOMATIC ATLAS SEGMENTATION.

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Introduction. The hypophysis is an endocrine gland located at the base of the brain, weighs less than 1 g and has an average volume of 8 mm^3, it is divided into 2 lobes: an anterior and a posterior. It is established that the disturbance in the secretion of its hormones would be involved in the genesis of eating disorders (ED). **Aims.** The purpose of this study is to create an ATLAS of the hypophysis in order to allow an automatic segmentation, and thus facilitate its morphological and physiological exploration in ED. **Methods.** The anterior and posterior parts of the pituitary glands of 26 healthy subjects were manually delineated on high-resolution MRI T1 images, and automatic atlas-based segmentation of the hypohysis was tested against manual delineation in 32 patients with anorexia nervosa, and 18 athletes. **Results.** With manual delineation, an average total volume of 730 mm^3, 634 mm^3 for the anterior lobe and 96 mm3 for the posterior division. Using the atlas, we get successivly 622 mm^3, 559,7 mm^3 and 62,57 mm^3. The superposition of the voxels with a dice index of 71,1% for the anterior lobe, 46,6% for the posterior lobe, and 71,7% for the global volume. We further describe a morphological modification of the hypophysis in patients with ED and athletes. **Conclusion.** Automatic segmentation atlas-based of hypophysis can subtitute to a manual delineation. The hypophysis atlas could serve for the extraction of binding potentiels (BP), and provide informations on the pathophysiology of this gland in ED and other pathologies.

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BOARD NUMBER: S06-541

EVIDENCE FOR FUNCTIONAL CONNECTIVITY PATTERN CHANGES IN FRONTOTEMPORAL DEMENTIA PATIENTS USING CONNECTOME GRADIENT MAPPING

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Frontotemporal dementia (FTD) is a neurodegenerative syndrome with a broad range of clinical manifestations making its early detection and progression monitoring difficult. It is believed that functional connectivity underlies cognitive function which makes it a potential meaningful biomarker. Functional connectivity studies in FTD have provided findings suggesting changes in resting-state networks. However, many of the analysis methods used are either far from the biological processes or restricted to a priori defined networks. Recent work has revealed the emergence of a brain network hierarchy during neurodevelopment, separating immediate environment processing from transmodal cortices; this is assumed to support higher-order cognition. We included 36 behavioural-FTD patients (bvFTD) and 50 controls. We applied a novel whole-brain analysis, connectome-gradient-mapping, to resting-state fMRI data. This approach extracts gradients representing measures of functional similarity, enabling the extent of segregation between networks to be assessed. bvFTD patients showed disruption in macroscale hierarchy affecting integration and segregation of unimodal and transmodal networks. In bvFTD patients, we observed a significant shift of cognitive/behavioural networks (Limbic and Default-mode), which are less segregated from the primary networks (visual and auditory). We found the Default-mode and Limbic networks seem particularly vulnerable in FTD, loosing their evolutionarily-derived characteristics of segregation. These networks underlie complex phenotypes observed in FTD. Next, we will evaluate whether this model could explain the main symptoms of patients, such as disinhibition (Stroop/Hayling tests), alterations in social cognition (miniSEA) and worse general cognition (MMSE). Overall, this method applied to early and/or presymptomatic stages will be of interest in future therapeutic studies for neurodegeneration.

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CHEMOGENETIC STIMULATION OF OXYTOCINERGIC NEURONS DYNAMICALLY MODULATES FMRI CONNECTIVITY

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Oxytocin (OXT) is a key modulator of complex socio-affective behavior. However, the brain-wide networks endogenously modulated by OXT remain undetermined. Here, we combine chemogenetics and fMRI[1] to map the topography and dynamics of brain networks engaged by endogenously-released OXT. To this aim, we crossed mice harboring a double-floxed DREADD activator hM3Dq[2] with OXT-specific *Cre*-recombinase mice[3]. We show that the resulting OXT-hM3Dq mice allow physiologically- and behaviorally-relevant release of OXT. Chemo-fMRI mapping of OXT-evoked release revealed prominent functional activation of parietal cortical areas, hypothalamus and dorsal hippocampus. Subsequent resting-state fMRI upon DREADD-based OXT stimulation revealed robustly increased functional connectivity between limbic components of the social brain, and reduced prefrontal-hippocampal cross-talk. Notably, these effects were accompanied by dynamic changes in connectivity characterized by altered coactivation pattern (CAP)[1] topography and distinctive, state-specific changes in fMRI state occurrence. Our work reveals for the first time the network targeted of endogenously released OXT in the mammalian brain, and suggest that endogenous OXT can rapidly and robustly alter functional cross-talk between key components of the social brain via temporal re-organization of network dynamics. 1. Gutierrez-Barragan, D., Basson, M. A., Panzeri, S. & Gozzi, A. Infraslow State Fluctuations Govern Spontaneous fMRI Network Dynamics. *Curr. Biol.* 29, 2295-2306.e5 (2019). 2. Giorgi, A. *et al.* Brain-wide Mapping of Endogenous Serotonergic Transmission via Chemogenetic fMRI. *Cell Rep.* 21, 910–918 (2017). 3. Wu, Z. *et al.* An Obligate Role of Oxytocin Neurons in Diet Induced Energy Expenditure. *PLoS One* 7, (2012).

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-543

OPTIMIZED AWAKE RAT FMRI STRATEGY FOR UNBIASED CONNECTOMIC INVESTIGATIONS

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Aims: In recent years, fMRI has been established in awake rodents, but one confound facing researchers is that acute stress caused by head fixation and loud acoustic imaging noise is likely to bias the datasets: stress not only impacts the neurobiological function of the brain, but also increases the likelihood of head motion during imaging. Effective habituation may serve to improve the experimental outcome. **Methods:** We assessed faecal corticosterone metabolite levels in rats during gradual habituation to awake fMRI, by conducting an ELISA on the faecal boli. Each sample was analyzed in triplicate. Moreover, we determined the robustness of our procedure exposing the awake rats to an auditory task. **Results:** Although corticosterone levels became elevated immediately after gradual daily habituation began, prolonged daily habituation that involved incremental exposure to the various scanner conditions (physical restraint, head fixation, noise) resulted in a return of corticosterone levels to pre-handling levels. The auditory task revealed the activation of the expected auditory structures, including the auditory cortex which is a very sensitive region – and therefore challenging to detect. **Conclusions:** Our data show how effective acclimatization to the awake fMRI procedures can be achieved by gradual exposure to the experimental conditions over a period of three weeks of training. In the absence of prolonged habituation, experimental data are likely to be confounded by the influence of stress-related elevations in corticosterone. **Acknowledgments:** This work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) (SFB 874/B3, B5, project number: 122679504; SFB 1280/A01, A04: project number: 316803389).

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INVESTIGATING THE EFFECTS OF FLOTATION RESTRICTED ENVIRONMENT STIMULATION THERAPY ON NEURAL NETWORKS IN CHRONIC PAIN PATIENTS VIA FUNCTIONAL MAGNETIC RESONANCE IMAGING

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Millions of Americans suffer from chronic lower back pain (CLBP). Current clinical approaches focus primarily on analgesia through high-strength opioids or invasive surgical interventions. However, these management strategies fail to consider a holistic perspective such as the biopsychosocial approach, which accounts for the long-term effects of both physical and emotional stress caused by CLBP. These factors have a profound effect on key neural networks like the somatosensory and default mode networks. A promising intervention to reduce stress and has altered key brain networks of interest in healthy participants, is Flotation Restricted Environment Stimulation Therapy (REST). REST flotation tanks are designed to reduce auditory, visual, thermal, and tactile stimulation thus allowing the brain to be at rest in a sensory deprived environment. Numerous studies have shown this to be an effective stress management tool with physiological, subjective, and functional effects. This study was designed to evaluate the effects of flotation REST on the neural networks affected by CLBP. Participants underwent a functional magnetic resonance imaging (fMRI) session before and after engaging in six flotation REST session. These scans were contrasted against control participants in a zero-gravity chair. Significant differences was found in resting state connectivity in the somatosensory and default mode networks across conditions. Also, there were significant differences in the prefrontal cortex across over time in the experimental group but not in the control group. These neural changes coupled with subjective alterations in pain show promise for flotation REST as a potential supplemental treatment for CLBP.

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FRACTIONAL AMPLITUDE OF LOW-FREQUENCY FLUCTUATION AND REGIONAL HOMOGENEITY IN DOWN SYNDROME. A RELATION WITH COGNITIVE OUTCOME

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Introduction: Although clinical and genetic characteristics in the Down Syndrome (DS) population have been described, few neuroimaging research has deepened in this population. Aims: study the whole brain resting state using fractional amplitude of low-frequency fluctuation (fALFF) and regional homogeneity (ReHo) strategies to find differences in spontaneous brain activity among young people with DS and controls. Moreover, this study aims also to find if differences may be related to the neuropsychological evaluation. Methods: 18 persons with DS (M=28,67, SD=4,18) and 18 controls (M=28,56, SD=4,26) matched by chronological age participated in this study. A resting-state registration was done for all the participants, as well as a neuropsychological evaluation, including KBIT-II and phonological and semantic fluency. Results: Regarding fALLF and ReHo analysis, results show significant differences in these frequencies in the whole brain between both compared groups. In this sense, increased and reduced fALLF in DS can reveal abnormal spontaneous activity in some regions. In the same line as in fALFF, results in ReHo show considerable differences between both groups. Regarding correlation analysis with cognitive outcomes, there is a big significant relationship. Moreover, the regressions predicting the signal extraction also have good results. Conclusions: Results are congruent with structural and functional studies done with the same population. Both data-driven methods have shown high sensitivity to find differences between the two populations, and the relationship with neuropsychological evaluation is clear. To our knowledge, it is the first time to explore regional spontaneous brain activity in a population with DS compared to controls.

Pubmed:
33801471: Figueroa-Jimenez MD, Carbó-Carreté M, Cañete-Massé C, Zarabozo-Hurtado D, Peró-Cebollero M, Salazar-Estrada JG, Guàrdia-Olmos J
Complexity Analysis of the Default Mode Network Using Resting-State fMRI in Down Syndrome: Relationships Highlighted by a Neuropsychological Assessment.
Studies on complexity indicators in the field of functional connectivity derived from resting-state fMRI (rs-fMRI) in Down syndrome (DS) samples and their possible relationship with cognitive functioning variables are rare. We analyze how some complexity indicators estimated in the subareas that constitute the default mode network (DMN) might be predictors of the neuropsychological outcomes evaluating Intelligence Quotient (IQ) and cognitive performance in persons with DS.
Brain Sci, 2021; 11
33757302: Cañete-Massé C, Carbó-Carreté M, Peró-Cebollero M, Guàrdia-Olmos J
Task-Related Brain Connectivity Activation Functional Magnetic Resonance Imaging in Intellectual Disability Population: A Meta-Analytic Study.
Neuroimaging studies of intellectual disability (ID) have been published over the last three decades, but the findings are often inconsistent, and therefore, the neural correlates of ID remain elusive. This article aims to study the different publications in task-functional magnetic resonance imaging (fMRI) and different ID populations to make a qualitative and quantitative analysis on this field. After duplicates were removed, only 10 studies matching our inclusion criteria were incorporated. Moreover, a quality assessment of the included studies was done. Qualitative results of the different articles were analyzed, separated by type of task and type of ID. Seed-based d mapping (SDM) software was used. The right temporal gyrus was more activated in control subjects than in ID. Concretely, the right temporal gyrus is implicated in many cognitive domains as semantic memory processing and language. Moreover, it can be highly influenced by the type of task used in every study. Heterogeneity was not detected. A jackknife sensitivity analysis was also estimated to improve the analysis reliability, and

both results were confirmed. More task-fMRI studies on ID must be published to add larger samples to address the pathophysiological questions more directly. Impact statement In this article, the state-of-the-art in the field of functional magnetic resonance imaging (fMRI) and intellectual disability (ID) is reviewed. Moreover, we perform a meta-analysis of every article's results to summarize the principal outcomes in the field. It is very relevant because it has become the first meta-analytic study to overcome all the principal studies published in fMRI and ID to find the principal neurological substrates while the subjects are performing a task.
Brain Connect, 2021; 11
33636235: Figueroa-Jiménez MD, Cañete-Massé C, Carbó-Carreté M, Zarabozo-Hurtado D, Guàrdia-Olmos J
Structural equation models to estimate dynamic effective connectivity networks in resting fMRI. A comparison between individuals with Down syndrome and controls.
Emerging evidence suggests that an effective or functional connectivity network does not use a static process over time but incorporates dynamic connectivity that shows changes in neuronal activity patterns. Using structural equation models (SEMs), we estimated a dynamic component of the effective network through the effects (recursive and nonrecursive) between regions of interest (ROIs), taking into account the lag 1 effect. The aim of the paper was to find the best structural equation model (SEM) to represent dynamic effective connectivity in people with Down syndrome (DS) in comparison with healthy controls. Twenty-two people with DS were registered in a functional magnetic resonance imaging (fMRI) resting-state paradigm for a period of six minutes. In addition, 22 controls, matched by age and sex, were analyzed with the same statistical approach. In both groups, we found the best global model, which included 6 ROIs within the default mode network (DMN). Connectivity patterns appeared to be different in both groups, and networks in people with DS showed more complexity and had more significant effects than networks in control participants. However, both groups had synchronous and dynamic effects associated with ROIs 3 and 4 related to the upper parietal areas in both brain hemispheres as axes of association and functional integration. It is evident that the correct classification of these groups, especially in cognitive competence, is a good initial step to propose a biomarker in network complexity studies.
Behav Brain Res, 2021; 405
33179859: Figueroa-Jimenez MD, Cañete-Massé C, Carbó-Carreté M, Zarabozo-Hurtado D, Peró-Cebollero M, Salazar-Estrada JG, Guàrdia-Olmos J
Resting-state default mode network connectivity in young individuals with Down syndrome.
Down syndrome (DS) is a chromosomal disorder that causes intellectual disability. Few studies have been conducted on functional connectivity using resting-state fMRI (functional magnetic resonance imaging) signals or more specifically, on the relevant structure and density of the default mode network (DMN). Although data on this issue have been reported in adult DS individuals (age: >45 years), the DMN properties in young DS individuals have not been studied. The aim of this study was to describe the density and structure of the DMN network from fMRI signals in young DS (age: <36 years).
Brain Behav, 2021; 11
33003398: Carbó-Carreté M, Cañete-Massé C, Figueroa-Jiménez MD, Peró-Cebollero M, Guàrdia-Olmos J
Relationship between Quality of Life and the Complexity of Default Mode Network in Resting State Functional Magnetic Resonance Image in Down Syndrome.
The study of the Default Mode Network (DMN) has been shown to be sensitive for the recognition of connectivity patterns between the brain areas involved in this network. It has been hypothesized that the connectivity patterns in this network are related to different cognitive states.
Int J Environ Res Public Health, 2020; 17
32711678: Carbó-Carreté M, Cañete-Massé C, Peró-Cebollero M, Guàrdia-Olmos J
Eliminate the effect of severity of the Personal Outcomes Scale: Linear regression in persons with intellectual disability.
The Personal Outcomes Scale (POS) is used to assess quality of life (QoL) in people with intellectual disability (ID) but the results are influenced by the severity of the disability. To address this issue, we present the standardization of the Spanish adaptation of the POS. One of the limitations of the Classical Test Theory is the differential effect in some items due to the effect of an external variable. For this reason, we propose the use of multiple linear regressions.
Psicothema, 2020; 32
32395104: Carbó-Carreté M, Cañete-Massé C, Peró-Cebollero M, Guàrdia-Olmos J
Using fMRI to Assess Brain Activity in People With Down Syndrome: A Systematic Review.
In the last few years, many investigations have focused on brain activity in general and in populations with different pathologies using non-invasive techniques such as electroencefalography (EEG), positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and magnetic resonance imaging (MRI). However, the use of non-invasive techniques to detect brain signals to evaluate the cognitive activity of people with Down syndrome (DS) has not been sufficiently addressed. The objective of this study is to describe the state-of-the-art in fMRI techniques for recording brain signals in people with DS. A systematic review was performed based on PRISMA recommendations; only nine papers on this

topic have been published. Three independent researchers selected all relevant information from each paper. Analyses of information concordance showed a high value of agreement between researchers. Although few relevant works have been published, the use of fMRI in people with DS is becoming an appropriate option to study brain function in this population. Of the nine identified papers, five used task designs, and four used resting-state paradigms. Thus, we emphasize the need to incorporate rigorous cognitive activity procedures in evaluations of the DS population. We suggest several factors (such as head correction movements and paired sample techniques) that must be considered when designing an fMRI study with a task or a resting-state paradigm in a DS population.
Front Hum Neurosci, 2020; 14
30127761: Cañete-Massé C, Peró-Cebollero M, Gudayol-Ferré E, Guàrdia-Olmos J
Longitudinal Estimation of the Clinically Significant Change in the Treatment of Major Depression Disorder.
Although major depressive disorder is usually treated with antidepressants, only 50-70% of the patients respond to this treatment. This study applied Jacobson and Truax's (1991) methodology (reliable change index, RCI) to a sample of depressive patients being treated with one of two antidepressants to evaluate their functioning and the effect of certain variables such as severity and age. Seventy-three depressive patients medicated with Escitalopram (= 37) or Duloxetine (= 36) were assessed using the Hamilton depression rating scale over a 24-week period. They indicate that the RCI stabilizes in an absolute way starting in week 16, and it is not until week 24 that all of the patients become part of the functional population. We found limited statistical significance with respect to the RCI and the external variables. Our study suggests the need to accompany the traditional statistical methodology with some other clinical estimation systems capable of going beyond a simple subtraction between pre and posttreatment values. Hence, it is concluded that RCI estimations could be stronger and more stable than the classical statistical techniques.
Front Psychol, 2018; 9

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BOARD NUMBER: S06-546

VOXELBOXPLUS - A NOVEL TOOL FOR DYNAMIC NETWORK LOCALIZATION OF BRAIN FUNCTIONAL ACTIVITY IN DEMENTIA USING RESTING STATE-FMRI: IMPLICATIONS ON DETECTION, TREATMENT, AND MANAGEMENT

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

Rimjhim Agrawal[1], Akshay Kumaar M[1], Vatsala Nema[2], Dilip Rajeswari[3], Ruchi Sharma[1], Sahana Hegde[1], Laina Emmanuel[1], Ranganayaki Sathyanarayanan[1]
[1]BrainSight Technology Private Limited, Science And Technology, Bangalore, India, [2]Indian Institute of Science Education and Research Bhopal, Electrical Engineering And Computer Sciences, Bhopal, India, [3]Columbia University Irving Medical Center, Pathology And Cell Biology, New York, India

The etiology of dementia has a multifactorial contribution impacted by clinical, cognitive, and cultural factors. The complexity associated with the disease poses a major challenge in terms of its diagnosis and treatment. The goal is to develop neuroimaging indicators that can better treat dementia by understanding brain activity and network relationships using network localization and intrinsic connectivity. This research proposes a novel method named ‘VoxelBox+’ for dynamic network localization of several functional networks of the brain using a sophisticated functional MRI preprocessing pipeline called the ‘VoxelBox’ and Dynamic Canonical Independent Component Analysis to cluster brain activations and map 9 different networks based on Human Connectome Project’s atlas with adaptive template-matching, thresholding, and post-processing. We used 100 subjects in Healthy Controls and Mild Alzheimer’s disease data from the Oasis-3 brain dataset for the network analysis with VoxelBox+. A two-sample t-test was performed for the group-level analysis to find the networks that highly corresponded to Alzheimer's disease. The proposed study found significant differences in the activation of DMN, CEN, DAN, Sensory Motor, and Salience networks between HC and AD subjects with a localization score of ~90%. VoxelBox+’s robust network localization and analysis can prove to be more beneficial for understanding neuropsychiatric symptoms and cognitive-behaviours in patients with dementia at ease using resting state functional MRI.

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BOARD NUMBER: S06-547

USE OF PCASL MRI SEQUENCE TO STUDY BRAIN PERFUSION AND VASCULAR PERMEABILITY AFTER BLOOD BRAIN BARRIER OPENING: LIMITATIONS AND PERSPECTIVES.

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Objective: Contrast enhancement after Gadolinium injection is the gold-standard method for visualizing the opening of the blood-brain barrier. However, it does not allow the assessment of permeability for a smaller molecule such as water. In this study, we evaluated multi-PLD pCASL as a method to assess the change in permeability. **Methods:** BBBO is performed on one hemisphere (Rats, n=5) by US emission at 0.3MPa following the injection of 200µL of microbubbles (SONOVUE-8µL/mL). Control acquisitions (7T/Bruker) are immediately performed. Then, pCASL acquisitions were performed using a LT of 2sec and different PLDs from 10ms to 1100ms. For BBBO visualization, the injection of 300µL of Gadolinium (DOTAREM-0,5mmol/ml) has been followed by T1map acquisition. **Results:** A decrease in ASL signal was observed in the exposed hemisphere (-45.5%) with a recovery within 60min. The volume with ASL signal change is larger than the volume with post-Gd-DOTA contrast enhancement. **Discussion:** The decrease in ASL signal observed in US-impacted areas has two possible causes: a decrease in perfusion related to vasoconstriction *[1]* or, an increase in membrane permeability *[2]*. Despite the multi-PLD method failed to determine their respective contribution, dynamic and spatial evaluation of the ASL signal suggests that the first hypothesis is the most likely. BBBO is generally intended to facilitate drugs permeation to the parenchyma, thus, it would be important to take into account the decrease in brain perfusion resulting in a reduction of the effective amount of drug that reaches the targeted area. *[1] Parkes LM. MRM 2002 [2] Eunice Cho. JCBFM 2011.*

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ASSESSING NEURAL ACTIVITY OF MUSICIANS WITH MUSIC PERFORMANCE ANXIETY: CREATION AND VALIDATION OF THE FMRI SOCIAL-EVALUATIVE MUSIC PERFORMANCE TASK

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

Kayla Boileau[1], Nicole Stanson[2], Kheana Barbeau-Julien[3], Umara Hansen[1], Gilles Comeau[2], Lydia Fang[1], Andra Smith[1]
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Aims: Music performance anxiety (MPA) is prevalent amongst musicians and can affect musicians' cognition, physiological arousal, and behaviors. However, few studies have focused on the neurophysiological aspects of MPA. This study aimed to create and validate an fMRI task for assessing neural activity associated with MPA. Methods: Twenty musicians with MPA performed a piece of music, individually, in front of judges and rated their subjective anxiety levels. The performance was recorded and was used to create an anxiety-provoking fMRI task that was administered during an MRI scanning session. During the task, participants viewed blocks of videos of themselves performing, videos of another musician's performance, and videos of judges. Results: When examining neural activity during the participant video and panel judging minus neural activity during the control video and panel judging while covarying anxiety scores, there was increased activity in the postcentral gyrus (bilaterally), the superior frontal gyrus, and the middle frontal gyrus. When examining the same contrast without covarying anxiety scores, there was still increased activity in the postcentral gyrus bilaterally and in the superior frontal gyrus; however, the middle frontal gyrus cluster was no longer significant. Conclusions: A new social-evaluative music performance task was successfully implemented in the MRI environment. Results showed brain activity that indirectly represented MPA in areas related to self-reference. Activation related to anxiety scores included regions of the default mode network (DMN), which has been found in previous studies. However, this task was able to tap into this relationship specifically to assess music performance anxiety.

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COST FUNCTION MASKING ARTIFICIALLY INFLATES GROUP LEVEL DIFFERENCES IN PROCESSING OF MAGNETIC RESONANCE IMAGING DATA FOR PATHOLOGICAL PATIENT POPULATIONS.

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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[1]Berlin Institute of Health at Charité Universitätsmedizin Berlin, Brain Simulation Section, Berlin, Germany, [2]Charité Universitätsmedizin Berlin, Department Of Neurology With Experimental Neurology, Berlin, Germany, [3]Bernstein Center for Computational Neuroscience Berlin, Bccn, Berlin, Germany, [4]Charité – Universitätsmedizin Berlin, Department Of Neurology And Experimental Neurology, Berlin, Germany

Aims: Cost function masking (CFM) is routinely used to register magnetic resonance imaging (MRI) volumes across modalities or to common templates (Radwan et al. 2021, Andersen et al. 2010, Brett et al. 2001) in the presence of pathological artifacts, such as lesions and tumors. While the impact of pathological abnormalities on downstream analyses is well documented (Andersen et al. 2010, King et al. 2020), the effects of CFM have not yet been thoroughly investigated. Here we aim to quantify the impact on common downstream processing steps. Specifically, we illustrate that applying CFM to a treatment group exhibiting pathological abnormalities in brain structure but not to the control group(s) can affect volumetrics in a statistically biased way, which can in turn influence end-goal hypothesis tests. **Methods** We registered 33 brain extracted T1w images from the ADNI healthy controls dataset to MNI152 template space with and without CFM, creating an artificial grouping based on the same brain volumes. The registered images were segmented using *FSL_fast* (Zhang et al. 2001), and cortical ribbon and white matter masks were compared between groups using dice coefficients and volume ratios (Taha et al. 2015). **Results** We found a strong impact of CFM on tissue segmentation.

Segmentation	Metric	Mean (FSL / ANTS)	Standard deviation (FSL / ANTS)
Cortical ribbon	Dice coefficient	0.8607 / 0.8895	0.0612 / 0.0188
White matter	Dice coefficient	0.8916 / 0.9106	0.0504 / 0.0144
Cortical ribbon	Volume difference	2.885% / 2.369%	1.463% / 0.966%
White matter	Volume difference	3.047% / 0.908 %	1.488% / 0.551%

Table 1 Agreement metrics for CFM versus no CFM segmentation results. With linear registrations using FSL (left) and ANTS (right) software.

Conclusions These findings suggest that statistical group comparisons can be inflated when CFM is applied asymmetrically. Therefore, methods must be developed to account for the bias induced by CFM and related correction methods, particularly when group differences are small. Crucially, the effect on volume estimates impacts downstream analyses, such as connectomics.

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DOSING THE TRIP: DOSE-RESPONSE ANTAGONIST-CONTROLLED STUDY OF PERSISTENT PSILOCYBIN EFFECTS ON AFFECTIVE BEHAVIOR AND FUNCTIONAL CONNECTIVITY

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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[1]Faculdade de Medicina, Universidade de Lisboa, Instituto De Farmacologia E Neurociências, Lisboa, Portugal, [2]Universidade de Lisboa, Faculdade de Medicina, Instituto De Medicina Molecular João Lobo Antunes, Lisboa, Portugal, [3]University of Paris, Institute Of Psychiatry And Neuroscience Of Paris, Paris, France

Interest in the development of 5-HT2AR agonists, such as psilocybin, for the treatment of multiple neuropsychiatric disorders, has greatly increased. Indeed, in both humans and rodents, psilocybin has been shown to induce persistent mood improvements and alterations in resting-state functional connectivity (rsFC) patterns. However, it remains unknown whether such effects are dose- and/or 5-HT2AR-dependent. Here we aimed at filling these gaps using three cohorts of 10-week-old male C57BL6/J mice. At the behavioral level, alterations in anxiety- and depression-like behaviors (elevated plus maze, open field, and forced swim tests) were assessed 7-days after pretreatment with the selective 5-HT2AR antagonist MDL100907 (vehicle or 0.5mg/kg; i.p.) and subsequent psilocybin administration (vehicle, 1, 2.5 or 5mg/kg; i.p.). Corresponding rsFC alterations are being assessed by functional ultrasound (fUS) recordings in awake mice, immediately after psilocybin (vehicle, 1, 5 or 10mg/kg; i.p.) administration, as well as 7-days after. Furthermore, the role of 5-HT2AR activation will be assessed in a further cohort, by MDL100907 pretreatment (vehicle or 0.5mg/kg; i.p.) previous to psilocybin injection. Behavioral results evidenced a significant dose-dependent anxiolytic-like, but not antidepressant-like, effect of psilocybin, which was abolished by MDL100907 pretreatment, suggesting that 5-HT2AR activation is necessary for these actions. These findings will hold relevance for further mechanistic pre-clinical study of these compounds, in addition to serving as a first step in dissecting the relationship between psilocybin-induced changes in functional connectivity and behavior. Funding: H2020-WIDESPREAD-05-2017-Twinning (EpiEpinet;No.952455), FCT (PTDC/MED-FAR/4834/2021[AMS], SFRH/BD/147505/2019[MF-F]).

Pubmed:
32077067: Ventura P, Fernandes T, Pereira A, Guerreiro JC, Farinha-Fernandes A, Delgado J, Ferreira MF, Faustino B, Raposo I, Wong AC
Holistic word processing is correlated with efficiency in visual word recognition.
Holistic processing of visual words (i.e., obligatory encoding of/attending to all letters of a word) could be a marker of expert word recognition. In the present study, we thus examined for the first time whether there is a direct relation between the word-composite effect (i.e., all parts of a visual word are fully processed when observers perform a task on a word part) and fast access to the orthographic lexicon by visual word experts (i.e., fluent adult readers). We adopted an individual differences approach and used the word-frequency effect (i.e., faster recognition of high- than low-frequency words) in an independent lexical decision task as a proxy of fast access to lexical orthographic representations. Fluent readers with larger word-composite effect showed smaller word-frequency effect. This correlation was mainly driven by an association between a larger composite effect and faster lexical decision on low-frequency words, probably because these lexical representations are less stable and integrated/unitized, hence allowing differentiating among fluent readers. We thus showed that holistic processing of visual words is indeed related to higher efficiency in visual word recognition by skilled readers.
Atten Percept Psychophys, 2020; 82
29756509: Ventura P, Delgado J, Ferreira M, Farinha-Fernandes A, Guerreiro JC, Faustino B, Leite I, Wong AC
Hemispheric asymmetry in holistic processing of words.
Holistic processing has been regarded as a hallmark of face perception, indicating the automatic and obligatory tendency of the visual system to process all face parts as a perceptual unit rather than in isolation. Studies involving lateralized stimulus presentation suggest that the right hemisphere dominates holistic face processing. Holistic processing can also be shown with other categories such as words and thus it is not specific to faces or face-like expertize. Here, we used divided visual field presentation to investigate the possibly different contributions of the two hemispheres for holistic word processing. Observers performed same/different judgment on the cued parts of two sequentially presented words in the complete

composite paradigm. Our data indicate a right hemisphere specialization for holistic word processing. Thus, these markers of expert object recognition are domain general.
Laterality, 2019; 24
30430939: Castanheira L, Ferreira MF, Sebastião AM, Telles-Correia D
Anxiety Assessment in Pre-clinical Tests and in Clinical Trials: A Critical Review.
The identification of anxious symptoms is crucial to diagnose anxiety disorders, as well as to monitor their treatment in clinical practice and research. The aim of this review is to discuss the different ways of assessing anxiety in clinical research, including clinical trials, and the different kinds of animal behavioral tests used to study anxiety and test the efficacy of anxiolytics in pre-clinical studies. In clinical practice, a categorical classification (such as the Diagnostic and Statistical Manual of Mental Disorders and the International Statistical Classification of Diseases and Related Health Problems) distinguishes the cases of the disease versus non-disease. Some structured and semi-structured interviews can be used to arrive at these diagnoses. On the other hand, anxiety can also be assessed using a dimensional approach, through self-report or hetero-evaluation questionnaires. Regarding the assessment of anxiety in animals, several behavioral tests are described and evaluated, namely the Social Interaction Test, Elevated Plus Maze and Open Field Test. Under a critical view, these two approaches are presented and discussed, in order to improve the outcome of research in this field.
Curr Top Med Chem, 2018; 18
30430942: Ferreira MF, Castanheira L, Sebastião AM, Telles-Correia D
Depression Assessment in Clinical Trials and Pre-clinical Tests: A Critical Review.
Depression is deeply rooted in human behavior. The development of new antidepressants demands the creation of animal models to investigate new drugs, which potentially could work as antidepressants. The aim of this review is to discuss the different ways of assessing depression in clinical research, including clinical trials, and the different animal behavioral tests used to study depression and test the efficacy of antidepressants in pre-clinical studies. In clinical practice, a categorical classification, such as the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Statistical Classification of Diseases and Related Health Problems (ICD) can be used for diagnosis, through the use of structured and semi-structured interviews. On the other hand, depression can also be assessed using a dimensional approach, through self- or clinicianrated questionnaires. Regarding the assessment of the efficacy of antidepressants in animal models, several tests are routinely used, namely the Forced Swim Test, the Modified Forced Swim Test, the Tail Suspension Test and the Sucrose Preference Test. These tests are informative, providing that the following rules are taken into account: 1) more than one test is used, with coherent results; 2) secondary drug effects, the most frequent being putative changes in motor activity, are taken into account and properly controlled with specific tests run concomitantly; 3) each test and specific protocol is validated with data from at least a gold standard antidepressant drug. We herein briefly discuss the potential and limitations of each of those tests.
Curr Top Med Chem, 2018; 18
32311420: Rei N, Rombo DM, Ferreira MF, Baqi Y, Müller CE, Ribeiro JA, Sebastião AM, Vaz SH
Hippocampal synaptic dysfunction in the SOD1 mouse model of Amyotrophic Lateral Sclerosis: Reversal by adenosine AR blockade.
Amyotrophic Lateral Sclerosis (ALS) mostly affects motor neurons, but non-motor neural and cognitive alterations have been reported in ALS mouse models and patients. Here, we evaluated if time-dependent biphasic changes in synaptic transmission and plasticity occur in hippocampal synapses of ALS SOD1 mice. Recordings were performed in hippocampal slices of SOD1 and age-matched WT mice, in the pre-symptomatic and symptomatic stages. We found an enhancement of pre-synaptic function and increased adenosine A receptor levels in the hippocampus of pre-symptomatic mice. In contrast, in symptomatic mice, there was an impairment of long-term potentiation (LTP) and a decrease in NMDA receptor-mediated synaptic currents, with AR levels also being increased. Chronic treatment with the AR antagonist KW-6002, rescued LTP and AR values. Altogether, these findings suggest an increase in synaptic function during the pre-symptomatic stage, followed by a decrease in synaptic plasticity in the symptomatic stage, which involves over-activation of AR from early disease stages.
Neuropharmacology, 2020; 171
30959794: Rodrigues RS, Lourenço DM, Paulo SL, Mateus JM, Ferreira MF, Mouro FM, Moreira JB, Ribeiro FF, Sebastião AM, Xapelli S
Cannabinoid Actions on Neural Stem Cells: Implications for Pathophysiology.
With the increase of life expectancy, neurodegenerative disorders are becoming not only a health but also a social burden worldwide. However, due to the multitude of pathophysiological disease states, current treatments fail to meet the desired outcomes. Therefore, there is a need for new therapeutic strategies focusing on more integrated, personalized and effective approaches. The prospect of using neural stem cells (NSC) as regenerative therapies is very promising, however several issues still need to be addressed. In particular, the potential actions of pharmacological agents used to modulate NSC activity are highly relevant. With the ongoing discussion of cannabinoid usage for medical purposes and reports drawing attention to

the effects of cannabinoids on NSC regulation, there is an enormous, and yet, uncovered potential for cannabinoids as treatment options for several neurological disorders, specifically when combined with stem cell therapy. In this manuscript, we review in detail how cannabinoids act as potent regulators of NSC biology and their potential to modulate several neurogenic features in the context of pathophysiology.
Molecules, 2019; 24
33285234: Gomes JI, Farinha-Ferreira M, Rei N, Gonçalves-Ribeiro J, Ribeiro JA, Sebastião AM, Vaz SH
Of adenosine and the blues: The adenosinergic system in the pathophysiology and treatment of major depressive disorder. Major depressive disorder (MDD) is the foremost cause of global disability, being responsible for enormous personal, societal, and economical costs. Importantly, existing pharmacological treatments for MDD are partially or totally ineffective in a large segment of patients. As such, the search for novel antidepressant drug targets, anchored on a clear understanding of the etiological and pathophysiological mechanisms underpinning MDD, becomes of the utmost importance. The adenosinergic system, a highly conserved neuromodulatory system, appears as a promising novel target, given both its regulatory actions over many MDD-affected systems and processes. With this goal in mind, we herein review the evidence concerning the role of adenosine as a potential player in pathophysiology and treatment of MDD, combining data from both human and animal studies. Altogether, evidence supports the assertions that the adenosinergic system is altered in both MDD patients and animal models, and that drugs targeting this system have considerable potential as putative antidepressants. Furthermore, evidence also suggests that modifications in adenosine signaling may have a key role in the effects of several pharmacological and non-pharmacological antidepressant treatments with demonstrated efficacy, such as electroconvulsive shock, sleep deprivation, and deep brain stimulation. Lastly, it becomes clear from the available literature that there is yet much to study regarding the role of the adenosinergic system in the pathophysiology and treatment of MDD, and we suggest several avenues of research that are likely to prove fruitful.
Pharmacol Res, 2021; 163

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BOARD NUMBER: S06-551

A CUSTOM CRANIAL WINDOW IMPLANT FOR LONG-TERM WHOLE-BRAIN FUNCTIONAL ULTRASOUND IMAGING IN BEHAVING MICE

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Behavior emerges from the interplay of multiple circuits across the brain, which emphasizes the importance of observing brain processes at the global scale. Functional ultrasound imaging (fUS) is a unique tool capable of recording rich whole-brain activity in behaving mice. However, high-quality fUS requires direct access to the brain through a cranial window and volumetric imaging requires a large field-of-view, the combination of which makes the associated surgical intervention technically challenging. To simplify and standardize this procedure, we developed a 3D printable resin-based cranial window implant for mice that is compatible with long-term whole-brain imaging during behavioral tasks. We provide a guide for the assembly and installation of the implant as well as a head fixation design compatible with a variety of behavioral setups, including a running wheel and holding tube. We evaluated the robustness and durability of this design by presenting head-fixed mice with a visual stimulation paradigm at over a month after implantation while recording whole-brain activity using fUS. In addition to obtaining brain-wide activation maps in response to visual stimulation, we further demonstrate how the low-profile design of the implant and head fixation facilitates the unobstructed extraction of various behavioral readouts from videographic recordings. This window is acoustically and optically transparent and in practice can be combined with electrode or optic fiber implantations. Therefore, this custom implant can complement a variety of functional neuroimaging modalities, including but not limited to fUS, that benefit from chronic access to a large area of the brain during behavioral mouse experiments.

Pubmed:

31656937: Edelman BJ, Meng J, Suma D, Zurn C, Nagarajan E, Baxter BS, Cline CC, He B
Noninvasive neuroimaging enhances continuous neural tracking for robotic device control.
Brain-computer interfaces (BCIs) utilizing signals acquired with intracortical implants have achieved successful high-dimensional robotic device control useful for completing daily tasks. However, the substantial amount of medical and surgical expertise required to correctly implant and operate these systems significantly limits their use beyond a few clinical cases. A noninvasive counterpart requiring less intervention that can provide high-quality control would profoundly impact the integration of BCIs into the clinical and home setting. Here, we present and validate a noninvasive framework utilizing electroencephalography (EEG) to achieve the neural control of a robotic device for continuous random target tracking. This framework addresses and improves upon both the "brain" and "computer" components by respectively increasing user engagement through a continuous pursuit task and associated training paradigm, and the spatial resolution of noninvasive neural data through EEG source imaging. In all, our unique framework enhanced BCI learning by nearly 60% for traditional center-out tasks and by over 500% in the more realistic continuous pursuit task. We further demonstrated an additional enhancement in BCI control of almost 10% by using online noninvasive neuroimaging. Finally, this framework was deployed in a physical task, demonstrating a near seamless transition from the control of an unconstrained virtual cursor to the real-time control of a robotic arm. Such combined advances in the quality of neural decoding and the practical utility of noninvasive robotic arm control will have major implications on the eventual development and implementation of neurorobotics by means of noninvasive BCI.
Sci Robot, 2019; 4
28914232: Johnson NN, Carey J, Edelman BJ, Doud A, Grande A, Lakshminarayan K, He B
Combined rTMS and virtual reality brain-computer interface training for motor recovery after stroke.
Combining repetitive transcranial magnetic stimulation (rTMS) with brain-computer interface (BCI) training can address motor impairment after stroke by down-regulating exaggerated inhibition from the contralesional hemisphere and encouraging ipsilesional activation. The objective was to evaluate the efficacy of combined rTMS + BCI, compared to sham rTMS + BCI, on motor recovery after stroke in subjects with lasting motor paresis.

J Neural Eng, 2018; 15
26276986: Edelman BJ, Baxter B, He B
EEG Source Imaging Enhances the Decoding of Complex Right-Hand Motor Imagery Tasks.
Sensorimotor-based brain-computer interfaces (BCIs) have achieved successful control of real and virtual devices in up to three dimensions; however, the traditional sensor-based paradigm limits the intuitive use of these systems. Many control signals for state-of-the-art BCIs involve imagining the movement of body parts that have little to do with the output command, revealing a cognitive disconnection between the user's intent and the action of the end effector. Therefore, there is a need to develop techniques that can identify with high spatial resolution the self-modulated neural activity reflective of the actions of a helpful output device.
IEEE Trans Biomed Eng, 2016; 63
34706219: Choy M, Dadgar-Kiani E, Cron GO, Duffy BA, Schmid F, Edelman BJ, Asaad M, Chan RW, Vahdat S, Lee JH
Repeated hippocampal seizures lead to brain-wide reorganization of circuits and seizure propagation pathways.
Repeated seizure activity can lead to long-term changes in seizure dynamics and behavior. However, resulting changes in brain-wide dynamics remain poorly understood. This is due partly to technical challenges in precise seizure control and in vivo whole-brain mapping of circuit dynamics. Here, we developed an optogenetic kindling model through repeated stimulation of ventral hippocampal CaMKII neurons in adult rats. We then combined fMRI with electrophysiology to track brain-wide circuit dynamics resulting from non-afterdischarge (AD)-generating stimulations and individual convulsive seizures. Kindling induced widespread increases in non-AD-generating stimulation response and ipsilateral functional connectivity and elevated anxiety. Individual seizures in kindled animals showed more significant increases in brain-wide activity and bilateral functional connectivity. Onset time quantification provided evidence for kindled seizure propagation from the ipsilateral to the contralateral hemisphere. Furthermore, a core of slow-migrating hippocampal activity was identified in both non-kindled and kindled seizures, revealing a novel mechanism of seizure sustainment and propagation.
Neuron, 2022; 110
34333106: Edelman BJ, Ielacqua GD, Chan RW, Asaad M, Choy M, Lee JH
High-sensitivity detection of optogenetically-induced neural activity with functional ultrasound imaging.
Whole-brain imaging approaches and optogenetic manipulations are powerful tools to map brain-wide neural circuits in vivo. To date, functional magnetic resonance imaging (fMRI) provides the most comprehensive evaluation of such large-scale circuitry. However, functional ultrasound imaging (fUSI) has recently emerged as a complementary imaging modality that can extend such measurements towards the context of diverse behavioral states and tasks. Nevertheless, in order to properly interpret the fUSI signal during these complicated scenarios, it must first be carefully validated against well-established technologies, such as fMRI, in highly controlled experimental settings. Here, to address this need, we compared subsequent fMRI and fUSI recordings in response to direct neuronal activation via optogenetics in the same animals under an identical anesthetic protocol. Specifically, we applied various intensities of light stimulation to the primary motor cortex (M1) of mice and compared the spatiotemporal dynamics of the elicited fMRI and fUSI signals. Overall, our general linear model analysis (t-scores) and time series analysis (z-scores) revealed that fUSI was more sensitive than fMRI for detecting optogenetically-induced neuronal activation. Local field potential recordings in the bilateral M1 and striatum also better co-localized with fUSI activation patterns than those of fMRI. Finally, the fUSI response contained distinct arterial and venous components that provide vascular readouts of neuronal activity with vessel-type specificity.
Neuroimage, 2021; 242
33049729: Suma D, Meng J, Edelman BJ, He B
Spatial-temporal aspects of continuous EEG-based neurorobotic control.
The goal of this work is to identify the spatio-temporal facets of state-of-the-art electroencephalography (EEG)-based continuous neurorobotics that need to be addressed, prior to deployment in practical applications at home and in the clinic.Nine healthy human subjects participated in five sessions of one-dimensional (1D) horizontal (LR), 1D vertical (UD) and two-dimensional (2D) neural tracking from EEG. Users controlled a robotic arm and virtual cursor to continuously track a Gaussian random motion target using EEG sensorimotor rhythm modulation via motor imagery (MI) commands. Continuous control quality was analyzed in the temporal and spatial domains separately.Axis-specific errors during 2D tasks were significantly larger than during 1D counterparts. Fatigue rates were larger for control tasks with higher cognitive demand (LR, left- and right-hand MI) compared to those with lower cognitive demand (UD, both hands MI and rest). Additionally robotic arm and virtual cursor control exhibited equal tracking error during all tasks. However, further spatial error analysis of 2D control revealed a significant reduction in tracking quality that was dependent on the visual interference of the physical device. In fact, robotic arm performance was significantly greater than that of virtual cursor control when the users' sightlines were not obstructed.This work emphasizes the need for practical interfaces to be designed around real-world tasks of increased complexity. Here, the dependence of control quality on cognitive task demand emphasizes the need for decoders that facilitate the translation of 1D task mastery to 2D control. When device footprint was accounted for, the introduction of a

physical robotic arm improved control quality, likely due to increased user engagement. In general, this work demonstrates the need to consider both the physical footprint of devices, the complexity of training tasks, and the synergy of control strategies during the development of neurorobotic control.
J Neural Eng, 2020; 17
29752228: Edelman BJ, Meng J, Gulachek N, Cline CC, He B
Exploring Cognitive Flexibility With a Noninvasive BCI Using Simultaneous Steady-State Visual Evoked Potentials and Sensorimotor Rhythms.
EEG-based brain-computer interface (BCI) technology creates non-biological pathways for conveying a user's mental intent solely through noninvasively measured neural signals. While optimizing the performance of a single task has long been the focus of BCI research, in order to translate this technology into everyday life, realistic situations, in which multiple tasks are performed simultaneously, must be investigated. In this paper, we explore the concept of cognitive flexibility, or multitasking, within the BCI framework by utilizing a 2-D cursor control task, using sensorimotor rhythms (SMRs), and a four-target visual attention task, using steady-state visual evoked potentials (SSVEPs), both individually and simultaneously. We found no significant difference between the accuracy of the tasks when executing them alone (SMR-57.9% ± 15.4% and SSVEP-59.0% ± 14.2%) and simultaneously (SMR-54.9% ± 17.2% and SSVEP-57.5% ± 15.4%). These modest decreases in performance were supported by similar, non-significant changes in the electrophysiology of the SSVEP and SMR signals. In this sense, we report that multiple BCI tasks can be performed simultaneously without a significant deterioration in performance; this finding will help drive these systems toward realistic daily use in which a user's cognition will need to be involved in multiple tasks at once.
IEEE Trans Neural Syst Rehabil Eng, 2018; 26
34336364: Edelman BJ, Johnson N, Sohrabpour A, Tong S, Thakor N, He B
Systems Neuroengineering: Understanding and Interacting with the Brain.
In this paper, we review the current state-of-the-art techniques used for understanding the inner workings of the brain at a systems level. The neural activity that governs our everyday lives involves an intricate coordination of many processes that can be attributed to a variety of brain regions. On the surface, many of these functions can appear to be controlled by specific anatomical structures; however, in reality, numerous dynamic networks within the brain contribute to its function through an interconnected web of neuronal and synaptic pathways. The brain, in its healthy or pathological state, can therefore be best understood by taking a systems-level approach. While numerous neuroengineering technologies exist, we focus here on three major thrusts in the field of systems neuroengineering: neuroimaging, neural interfacing, and neuromodulation. Neuroimaging enables us to delineate the structural and functional organization of the brain, which is key in understanding how the neural system functions in both normal and disease states. Based on such knowledge, devices can be used either to communicate with the neural system, as in neural interface systems, or to modulate brain activity, as in neuromodulation systems. The consideration of these three fields is key to the development and application of neuro-devices. Feedback-based neuro-devices require the ability to sense neural activity (via a neuroimaging modality) through a neural interface (invasive or noninvasive) and ultimately to select a set of stimulation parameters in order to alter neural function via a neuromodulation modality. Systems neuroengineering refers to the use of engineering tools and technologies to image, decode, and modulate the brain in order to comprehend its functions and to repair its dysfunction. Interactions between these fields will help to shape the future of systems neuroengineering-to develop neurotechniques for enhancing the understanding of whole-brain function and dysfunction, and the management of neurological and mental disorders.
Engineering (Beijing), 2015; 1
34334804: He B, Baxter B, Edelman BJ, Cline CC, Ye W
Noninvasive Brain-Computer Interfaces Based on Sensorimotor Rhythms.
Brain-computer interfaces (BCIs) have been explored in the field of neuroengineering to investigate how the brain can use these systems to control external devices. We review the principles and approaches we have taken to develop a sensorimotor rhythm EEG based brain-computer interface (BCI). The methods include developing BCI systems incorporating the control of physical devices to increase user engagement, improving BCI systems by inversely mapping scalp-recorded EEG signals to the cortical source domain, integrating BCI with noninvasive neuromodulation strategies to improve learning, and incorporating mind-body awareness training to enhance BCI learning and performance. The challenges and merits of these strategies are discussed, together with recent findings. Our work indicates that the sensorimotor-rhythm-based noninvasive BCI has the potential to provide communication and control capabilities as an alternative to physiological motor pathways.
Proc IEEE Inst Electr Electron Eng, 2015; 103

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BOARD NUMBER: S06-552

IDENTIFICATION OF FUNCTIONAL BIOMARKERS OF DEMYELINATION IN TWO ANIMAL MODELS OF MULTIPLE SCLEROSIS WITH FUNCTIONAL ULTRASOUND IMAGING

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Multiple Sclerosis (MS) is a chronically evolving inflammatory disease of the central nervous system caused by focal demyelination. To develop new treatments, new imaging technologies are needed at the preclinical stage to foster therapeutical development. Functional ultrasound imaging (fUSI) has recently been demonstrated to robustly measure brain cerebral blood volume (CBV) dynamics as an indirect measure of neural activity. **Aim:** We postulated that fUSI could allow the definition of biomarkers of demyelinisation / remyelinisation in animal models of MS. The aim of this study was to improve the reproducibility of the experimental conditions and signal processing, in order to use it in preclinical studies involving model systems and pharmacology. **Methods:** We studied the changes of CBV induced by whiskers stimulation in naïve C57Bl6 mice, Cuprizone or Lysolecithin injected mice (N=8 animals/ group), imaged at 5-10 Weeks and 7-14-21 days, respectively. **Results:** First we developed a protocol to trigger the whiskers stimulations and reduce user-dependent variability. The parameters of the stimulation (frequency, duration of stimulation) were optimized, along with the plane of imaging through a fully-automatic ultrasound-based neuro-navigation approach. Finally, this protocol was used in two animal models of MS and showed contrasting results (60% increase CBV in Cuprizone, 50% decrease CBV in Lysolecithin-induced models). **Conclusion**: The measure of evoked hemodynamic response is not a good biomarker of demyelinisation per se, but uncovers interesting mechanisms on demyelination-induced vascular alterations in these models.

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BOARD NUMBER: S06-553

STIMULUS AND SINGLE NEURONS INDUCED CEREBRAL BLOOD FLOW SIGNALS IN BEHAVING NON-HUMAN PRIMATES

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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The ability to simultaneously record brain activity at multiple temporal and spatial scales is a highly complex quest . In non-human primates (NHP), a combination of fMRI and electrophysiological recordings has been undertaken to elucidate the contributions of spiking activity to the neurovascular response, a question largely debated for several years (Logothetis et al., 2001; Mukamel et al., 2005). Functional ultrasound imaging (fUSi) has recently become a valid but indirect measure to explore brain function in many species, including NHP (Dizeux et al., 2018; Blaize et al., 2020). Even with limited motion artifacts and well-controlled anesthetic states, comparisons between fUSi and extracellular neuronal responses remain an extreme challenge to understand neuronal processing in primates. Here, in reproducible experimental sessions, we present a new set-up achieving simultaneous recordings of cerebral blood flow (CBV) and nearby single-unit activities (SUA) in two behaving macaques' primary visual and fronto-medial cortices. We computed task-induced and SUA-induced CBV activation maps using Generalized Linear Models (GLM) commonly used in fMRI studies. Thereby, using the high spatial resolution of fUSi (~100*100*400 µm^3), our results demonstrate that SUA provides a significant estimate of the neurovascular response. These results have important implications for interpreting functional imaging findings as fMRI or fUSi and physiological inferences guided by explorations of direct neurophysiological activities in awake primates and humans.

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FUNCTIONAL STUDIES OF NEURON-ASTROCYTE INTERACTIONS IN VIVO

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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NeuroLead is a drug discovery platform, coordinated by Theranexus, Collège de France and CEA, dedicated to neuron-astrocyte communication in physiological and pathological conditions. We present here two complementary *in vivo* approaches to evaluate neuroglial interactions based on calcium signaling and neurovascular coupling. Mice expressing GCaMP6f under the GFAP promoter were lens implanted in the nucleus accumbens to record astroglial calcium fluorescence variations with a head-mountable epifluorescent miniature microscope. Animals were habituated to the system by holding a dummy miniscope for 7 days before to perform a three-chamber test. The imaging system was set up to detect TTL pulses generated by the behavior system to synchronize imaging frames and mouse behavioral activities. We recorded calcium activity of accumbal astrocytes in freely behaving mice performing simultaneously behavioral tests. We found that calcium transients were triggered when mice were interacting in a three-chamber test. In addition to this approach, a functional neuroimaging technique based on neurovascular coupling was designed to locally monitor cerebral blood dynamics in rodents. Functional ultrasound imaging allows, with high spatio-temporal resolution and sensitivity, to record cerebral blood volume dynamics and evaluate functional connectome patterns. Moreover, fUS can be performed in anaesthetized or awake state, during a pharmacological challenge and/or using different stimuli. Through this imaging platform, we present here our ability to monitor both astroglial calcium- and cerebral blood- dynamics using *in vivo* miniscope and functional ultrasound imaging approach respectively. Thus, this *in vivo* platform may constitute a new step to move forward in drug development for neurological disorders.

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COMBINING HIGH-RESOLUTION FUNCTIONAL ULTRASOUND (FUS)- AND FMRI-IMAGING IN THE SAME HUMAN SUBJECT

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

Sadaf Soloukey[1,2], Ellen Collée[2], Luuk Verhoef[1], Djaina Satoer[2], Clemens Dirven[2], Eelke Bos[2], Joost Schouten[2], Bastian Generowicz[2], Frits Mastik[1], Chris De Zeeuw[1,3], Sebastiaan Koekkoek[1], Arnaud Vincent[2], Marion Smits[4], Pieter Kruizinga[1]
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Aims In contrast to many conventional functional brain imaging modalities, functional Ultrasound (fUS) combines high sensitivity with submillimeter-subsecond spatiotemporal resolution, high-depth penetration and large fields of view. Since its inception over a decade ago there has been no study comparing fUS-acquired functional maps and its gold standard counterpart of BOLD-fMRI imaging. Here we present our first results of combined fUS-fMRI imaging in the same human subject. **Methods** fUS stools on high-sensitivity ultrafast µDoppler ultrasound imaging of microvascular hemodynamics, including changes in cerebral blood flow (CBF) and cerebral blood volume (CBV). Through the principle of neurovascular coupling, these hemodynamics serve as an indirect measure of neuronal activity. In a number of patients undergoing awake craniotomy surgery for tumor removal, we performed paired functional testing with pre-operative fMRI and intra-operative fUS, respectively. Building on clinically available neuro-navigation software, we co-registered both modalities to study spatiotemporal overlap. **Results** In a range of simple motor, visual and language-related functional tasks we were able to confirm fUS-based functional regions using the pre-operative fMRI-map. Our fUS functional maps presented with unprecedented micrometer-millisecond precision, even at depths of over > 5 cm. What is more, fUS was able to concomitantly visualize the in-vivo microvascular morphology underlying these functional hemodynamics. **Conclusions** We present the first in-human confirmation of spatial overlap in functional regions between fUS and fMRI, serving as an important milestone towards further clinical and neuroscientific maturity of fUS as a new cutting-edge functional brain imaging modality.

Pubmed:
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Diagnosing embodiment should become part of our repertoire.
Disabil Rehabil, 2021; 43
34580478: Kathe C, Michoud F, Schönle P, Rowald A, Brun N, Ravier J, Furfaro I, Paggi V, Kim K, Soloukey S, Asboth L, Hutson TH, Jelescu I, Philippides A, Alwahab N, Gandar J, Huber D, De Zeeuw CI, Barraud Q, Huang Q, Lacour SP, Courtine G
Wireless closed-loop optogenetics across the entire dorsoventral spinal cord in mice.
Optoelectronic systems can exert precise control over targeted neurons and pathways throughout the brain in untethered animals, but similar technologies for the spinal cord are not well established. In the present study, we describe a system for ultrafast, wireless, closed-loop manipulation of targeted neurons and pathways across the entire dorsoventral spinal cord in untethered mice. We developed a soft stretchable carrier, integrating microscale light-emitting diodes (micro-LEDs), that conforms to the dura mater of the spinal cord. A coating of silicone-phosphor matrix over the micro-LEDs provides mechanical protection and light conversion for compatibility with a large library of opsins. A lightweight, head-mounted, wireless platform powers the micro-LEDs and performs low-latency, on-chip processing of sensed physiological signals to control photostimulation in a closed loop. We use the device to reveal the role of various neuronal subtypes, sensory pathways and supraspinal projections in the control of locomotion in healthy and spinal-cord injured mice.
Nat Biotechnol, 2022; 40
33038598: Soloukey S, de Rooij JD, Drenthen J, De Zeeuw CI, Huygen FJPM, Harhangi BS
Unilateral L2-Level DRG-stimulation evokes bilateral CPG-Like motor response in a patient with chronic pain.
Brain Stimul, 2020 Nov - Dec; 13
33749941: Soloukey S, Drenthen J, Osterthun R, de Vos CC, De Zeeuw CI, Huygen FJPM, Harhangi BS
How to Identify Responders and Nonresponders to Dorsal Root Ganglion-Stimulation Aimed at Eliciting Motor Responses in

Chronic Spinal Cord Injury: Post Hoc Clinical and Neurophysiological Tests in a Case Series of Five Patients.
While integrity of spinal pathways below injury is generally thought to be an important factor in the success-rate of neuromodulation strategies for spinal cord injury (SCI), it is still unclear how the integrity of these pathways conveying the effects of stimulation should be assessed. In one of our institutional case series of five patients receiving dorsal root ganglion (DRG)-stimulation for elicitation of immediate motor response in motor complete SCI, only two out of five patients presented as responders, showing immediate muscle activation upon DRG-stimulation. The current study focuses on post hoc clinical-neurophysiological tests performed within this patient series to illustrate their use for prediction of spinal pathway integrity, and presumably, responder-status.
Neuromodulation, 2021; 24
32289689: Soloukey S, Drenthen J, Osterthun R, de Rooij JD, De Zeeuw CI, Huygen FJPM, Harhangi BS
Bilateral L2 dorsal root ganglion-stimulation suppresses lower limb spasticity following chronic motor complete Spinal Cord Injury: A case report.
Brain Stimul, 2020 May - Jun; 13
32706445: Soloukey S, de Rooij JD, Osterthun R, Drenthen J, De Zeeuw CI, Huygen FJPM, Harhangi BS
The Dorsal Root Ganglion as a Novel Neuromodulatory Target to Evoke Strong and Reproducible Motor Responses in Chronic Motor Complete Spinal Cord Injury: A Case Series of Five Patients.
Current strategies for motor recovery after spinal cord injury (SCI) aim to facilitate motor performance through modulation of afferent input to the spinal cord using epidural electrical stimulation (EES). The dorsal root ganglion (DRG) itself, the first relay station of these afferent inputs, has not yet been targeted for this purpose. The current study aimed to determine whether DRG stimulation can facilitate clinically relevant motor response in motor complete SCI.
Neuromodulation, 2021; 24
31998060: Soloukey S, Vincent AJPE, Satoer DD, Mastik F, Smits M, Dirven CMF, Strydis C, Bosch JG, van der Steen AFW, De Zeeuw CI, Koekkoek SKE, Kruizinga P
Functional Ultrasound (fUS) During Awake Brain Surgery: The Clinical Potential of Intra-Operative Functional and Vascular Brain Mapping.
Oncological neurosurgery relies heavily on making continuous, intra-operative tumor-brain delineations based on image-guidance. Limitations of currently available imaging techniques call for the development of real-time image-guided resection tools, which allow for reliable functional and anatomical information in an intra-operative setting. Functional ultrasound (fUS), is a new mobile neuro-imaging tool with unprecedented spatiotemporal resolution, which allows for the detection of small changes in blood dynamics that reflect changes in metabolic activity of activated neurons through neurovascular coupling. We have applied fUS during conventional awake brain surgery to determine its clinical potential for both intra-operative functional and vascular brain mapping, with the ultimate aim of achieving maximum safe tumor resection.
Front Neurosci, 2019; 13

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BOARD NUMBER: S06-556

BENCHMARKING OF INDIVIDUAL-LEVEL PREPROCESSING STRATEGIES FOR PHARMACO-FUS

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Functional ultrasound (fUS) imaging, a novel and highly sensitive neuroimaging modality with previously unequaled spatiotemporal resolution (1kHz, 100µm), is becoming an increasingly important tool for the study of functional connectivity in awake animals. However, fUS data preprocessing presents significant challenges, in particular considering the confounding effects of artefacts caused by animal motion. While steps which have been previously selected to mitigate these artefacts have often been inspired by fMRI preprocessing pipelines, including global signal regression (GSR), scrubbing and bandpass filtering, their implementation and the order in which they are performed is often controversial and can be critical. Here, we report benchmarking results of the performance of several preprocessing pipelines previously used in functional connectivity studies using fUS imaging, along with new methods inspired by recent advances in fMRI preprocessing. We tested the algorithms on two fUS datasets from longitudinal studies of functional connectivity alterations following pharmacological treatments in mice. This study illustrates the effects that different preprocessing pipelines can have on subsequent statistical analyses and shows that special care must be taken when preprocessing fUS data. The best preprocessing pipeline, selected according to previously established metrics of noise removal, has been implemented in a fUS analysis package, as well as a user-friendly web-application that enables end-to-end analysis of functional ultrasound data acquired in longitudinal pharmacological studies.

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FUNCTIONAL FINGERPRINTING OF DRUGS ON BRAIN ACTIVATION AND CONNECTIVITY PATTERNS IN THE AWAKE MOUSE BRAIN

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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[1]Université de Paris, INSERM U1266, Institute Of Psychiatry And Neuroscience Of Paris, Paris, France, [2]Physics for Medicine, Inserm, Espci Paris, Cnrs, Psl Research university, Paris, France, [3]Physics for Medicine, Inserm, Espci Paris, Cnrs, Psl Research University, Paris, France

Functional imaging of hemodynamic signals as a proxy for neuronal activations allowed the emergence of Functional Connectivity (FC) studies non invasively in humans. However, translation back to pre-clinics remains challenging as traditional scanners require almost complete immobility of animals, usually achieved through anaesthesia which interfere with FC. We show here that functional ultrasound brain imaging (fUS) is a good alternative for FC measurement to characterise the pharmacological fingerprints of different drugs in mobile rodents. We describe a standardised platform optimising the different steps of FC measures in awake behaving animals : preparation surgery, habituation to head fixed apparatus, imaging sessions for longitudinal follow up of treatment, data management, fUS oriented analysis pipeline. We compared the effects of opioids and cannabinoids on FC by using specific agonists of the μ-opioid (MOR) and CB1 receptors (CB1R). We report reproducible changes in cortico-cortical, thalamo-cortical and hippocampal connectivity. Additionally, we observe a significant increase in cerebral blood volume (CBV) preceding the shift in FC. Finally, the trigeminal ganglia appears as a main source of signal at baseline with a massive down-regulation mediated by both CB1R and MOR activation. All the observed effects were dose-dependent and reversed after antagonist pre-treatment. The opioid effect was absent in MOR knock-out mice. We conclude that a fast increase of CBV precedes the establishment of a FC fingerprint associated with a change of brain state correlating with known behaviour features. The study of these FC patterns should allow to accelerate screening of drug effects and identify new potential therapies.

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NON-INVASIVE TRANSCRANIAL WHOLE BRAIN ANGIOGRAPHY AND HEMODYNAMIC QUANTIFICATION AT THE MICROSCOPIC SCALE IN RODENTS

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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In the last decade, Ultrafast ultrasound imaging at thousands of frames per second combined with the intravenous injection of 1-3 µm gas microbubbles led to the concept of Ultrasound Localization Microscopy (ULM). This new modality breaks the fundamental trade-off between spatial resolution and penetration depth of Ultrasound imaging and enables the deep and non-invasive mapping of cerebral vasculature at the microscopic scale both in preclinical and clinical configurations. Additionally, ULM provides quantitative information about hemodynamics (local microbubble speed in the 1-30 mm/s range and microbubble flow). It exhibits several distinct compartments of the vascular tree (arterioles, branching capillaries, venules) and can be spatially linked to the functional and anatomic information through a process of registration to an atlas. For now, ULM has mainly been restricted to single-plane imaging. For 3D imaging, matrix probes still lack sensitivity and impose a great electronic complexity. Here, we introduce a multilinear stacked probe combining four high sensitivity linear ultrasound probes (4x64 elements, 15MHz) to image the whole brain at once. Our protocol is implemented on the Iconeus One ultrasound scanner (256 channels) and consists in translating the probe to four positions to obtain sixteen super-resolution planes in 15 minutes. We achieve a whole-brain quantification of the mouse vascular properties and characterize the spatial heterogeneities of the hemodynamic response under the influence of a vasodilator drug (isoflurane inhalation) in different brain regions.
Whole-brain transcranial angiography at microscopic scale based on ULM imaging gives new insights on the spatial heterogeneities of the vascular system and its associated hemodynamics.

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BOARD NUMBER: S06-559

EVALUATION OF THE RELAXIVITY AND CONTRAST ENHANCEMENT OF A NEW MRI MOLECULAR PROBE TARGETING TAU LESIONS IN MOUSE BRAINS : A PILOT STUDY

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Aims : Hyperphosphorylated Tau deposits are a hallmark of Alzheimer's disease. Contrary to amyloid plaques, which have endogenous MRI contrast[1], their detection requires targeted molecular MRI contrast agents (CA)[2]. This study evaluates an anti-tau-gadolinium (Gd) CA prototype based on camelid single-domain antibody fragments in P301S transgenic mouse model of tauopathy[3]. **Methods** : CA relaxivity parameters were measured *in vitro* at 11.7T. To bypass the blood-brain barrier, 6 µL of CA (0.5mM [Gd]), Gd (0.45mM [Gd]) or Phosphate Buffer Saline (PBS) were stereotactically injected in 7 month-old P301S mice (right hemisphere). Three to 5h after injection, *in vivo* T1-weighted (T_1w) and T2*-weighted (T_2*w) images were acquired with a Bruker cryoprobe™. Contrast-to-noise ratios (CNR) between ipsilateral and contralateral hemispheres were measured. **Results** : r1 and r2 values were measured to 8.6 and 15.3 $mM^{-1}.s^{-1}$ respectively, with r1 of the same order of magnitude as Dotarem – Gd-based MRI CA reference. A diffuse contrast enhancement was observed on T_1w images in the ipsilateral hemisphere compared to the contralateral one following both CA and Gd injections (Fig1). The CNR enhancement induced by the CA was 30% higher than that induced by Gd alone. No effect was observed following PBS injection. Except for the needle tract, T_2*w images presented no contrast
anomaly.

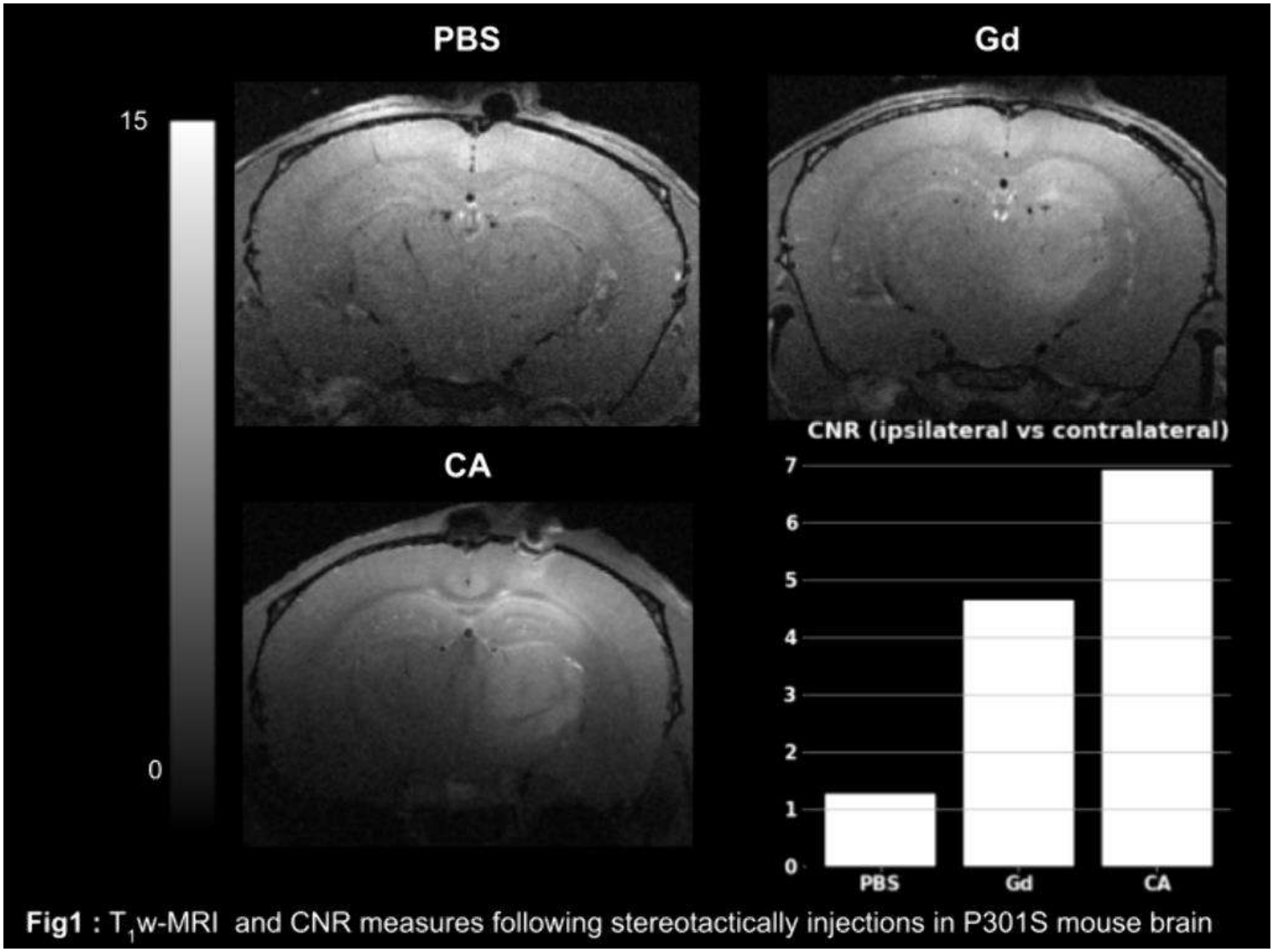

Fig1 : T_1w-MRI and CNR measures following stereotactically injections in P301S mouse brain

Conclusions : The good relaxivity of our CA allowed enhanced contrast compared to Gd alone. Those preliminary results should help detecting Tau deposits and the specificity will be validated with histology. **References :** [1]Petiet, Neurobiol. Aging, 2012 [2]Li, JCR, 2016 [3]Yoshiyama, Neuron, 2007

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BOARD NUMBER: S06-560

WHOLE-BRAIN 3D TRANSCRANIAL FUNCTIONAL CONNECTIVITY OF THE MOUSE BRAIN WITH MULTIARRAY FUNCTIONAL ULTRASOUND IMAGING

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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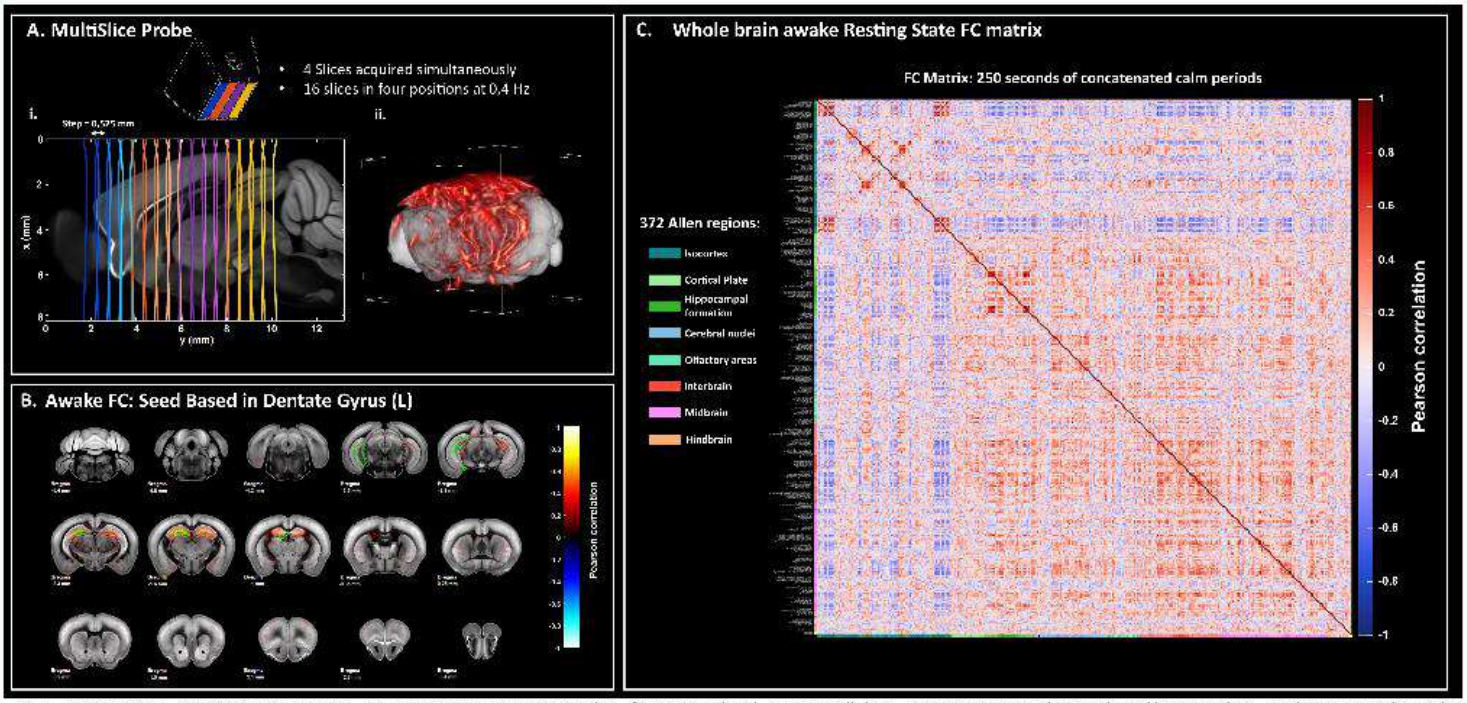
Functional Ultrasound (fUS) recently allowed 2D or 3D transcranial functional imaging in awake mice thanks to motorized linear or matrix arrays [1]. However, the tradeoff between the field of view and temporal resolution introduced by motorized scanning prevents acquiring whole-brain resting state Functional Connectivity (rsFC) whereas the limited sensitivity of matrix arrays prevents transcranial imaging in mice. Here we propose a new hybrid approach dedicated to 3D FC in the whole brain. A 15 MHz MultiArray probe was developed based on four high-sensitive compact linear arrays (4x64 transducers, pitch 110 µm, Iconeus, Paris, France), mounted on a 4-axis motor stage for automatic positioning and scanning both driven by the Iconeus One scanner. 3D rsFC was characterized in both anesthetized and head-fixed awake mice experiments. During rsFC acquisitions (20 minutes) the power Doppler was acquired in 4 slices simultaneously during 0.4s (200 compounded frames at 500 Hz) for four positions, resulting in 16 contiguous slices (525 µm step) acquired in 2.4s. After automatic atlas-based registration and preprocessing (temporal filtering, global mean regression), correlation matrices were computed with 372 Allen regions. Strong cortical and hippocampal bilateral connectivity patterns were found, and seed maps analysis allowed the quantification of long-ranged connectivity in the entire mouse brain. The MultiArray approach is a suitable solution for 3D functional imaging in awake and anesthetized mice without craniotomy. The wide field of view combined with the high sensitivity opens new opportunities to perform unbiased 3D functional connectivity measurements in the whole mouse

brain.



A.i. Principle of IcoPrime 4D MultiArray Probe: the four stacked arrays (blue, orange, purple and yellow color code) scanning the whole brain in four positions (represented with the color gradient) at a volume rate of 0.4 Hz. **ii. 3D Power Doppler angiography** registered on Allen Atlas. **B. Seed Based map (left Dentate Gyrus)** overlayed with the Allen two-photon template after Resting State FC experiment in awake mice, showing long ranged connectivity patterns. **C. Whole brain Resting State FC matrix in awake mice** computed on 250 s acquisituion and after automatic signal extraction in 372 volumic ROIs.

[1] A. Bertolo *et al.*, « Whole-Brain 3D Activation and Functional Connectivity Mapping in Mice using Transcranial Functional Ultrasound Imaging », *J. Vis. Exp.*, nº 168, p. 62267, févr. 2021, doi: 10.3791/62267

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BOARD NUMBER: S06-561

ACTIVITY ALTERATIONS OF VARIOUS BRAIN REGIONS IN ALCOHOL INTOXICATED DRIVERS: A SYSTEMATIC REVIEW AND META ANALYSIS OF FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDIES

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Aim: Drunk driving is one of the main causes of traffic accidents. Years of functional magnetic resonance imaging (fMRI) research have revealed various effects of alcohol intoxication on drivers' brains, but there is no comprehensive summary of the exactly involved brain regions. Our aim was to conduct a meta-analysis to develop more effective intervention efforts for alcohol use disorder. **Methods:** Medline and other available databases were systematically searched to retrieve fMRI studies in the field of alcohol intoxication in drivers in comparison to non-intoxicated drivers. Articles were screened based on eligibility criteria. Meta-analysis was performed with the ALE method in GingerALE2.3.6 on MNI and Talairach coordinates from included studies. ALE maps were set at a threshold of $P<0.05$ and viewed with Mango software. **Results**: 4 articles were included in the meta-analysis. In comparison to non-inebriated drivers, ALE analysis revealed that alcohol-intoxicated drivers have significantly lower activation of the medial region of the right superior frontal gyrus, left thalamus, and left anterior cingulate gyrus. They also had higher activation in the right parietal operculum, right planum polare, right precentral gyrus, medial segment of right superior frontal gyrus, right superior frontal gyrus, left angular gyrus, left superior frontal gyrus, left superior motor cortex, and left superior frontal gyrus. **Conclusion**: This study is the first meta-analysis to offer key hyper- and hypo-activation regions of the brain while drunk driving. The obtained regional abnormalities in this study might serve as biomarkers to effectively understand the underlying brain mechanisms of impaired driving performance while intoxicated.

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BOARD NUMBER: S06-562

BRAIN TISSUE PULSATIONS (BTPS) IN ACUTE STROKE USING TRANSCRANIAL TISSUE DOPPLER TECHNIQUE (TCTD)

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

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Aims: To investigate if brain tissue pulsations (BTPs) could be affected by the presence of acute stroke, and to explore the clinical potential of measuring BTPs using ultrasound in patients with ischaemic, haemorrhagic (ICH), and transient ischaemic attacks (TIA) compared to non-stroke control group. **Methods:** Recordings were obtained using a novel Transcranial Tissue Doppler (TCTD) technique. TCTD involves using a pair of 2 MHz ultrasound probes placed on the patient forehead. MATLAB software was used 'in house' for TCTD signal analysis, including bulk BTP amplitude, time to peak (TtP), and other physiological measurements. **Results:** BTPs were measured in 13 control subjects, 20 acute ischaemic stroke, 5 ICH patients and 12 TIA patients. A paired Wilcoxon signed rank test found no significant difference in bulk BTP amplitude and TtP between affected and unaffected hemispheres in stroke patients or between right and left sides for control subjects. A Kruskal-Wallis test found no significant differences in forehead combined bulk BTP amplitude and TtP between the control group and other groups (p = 0.06 and p = 0.43, respectively). Spearman's rank-order correlation test found a significant correlation between bulk BTP and MAP for ICH patients (r=1, p=0.01]. No association was found between bulk BTP amplitude and TtP with PP and MAP for other stroke patients. **Conclusion:** This study found no obvious disruptions to BTPs in patients with mild-moderate stroke. Overall, BTP amplitude and TtP seem unable to provide a good diagnostic indicator for stroke.

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BOARD NUMBER: S06-563

THREE-DIMENSIONAL VISUALIZATION OF CEREBRAL BLOOD VESSELS AND NEURAL CHANGES IN THICK ISCHEMIC RAT BRAIN SLICES USING TISSUE CLEARING

POSTER SESSION 06 - SECTION: FUNCTIONAL IMAGING OF THE BRAIN

Jongsoon Won
BINAREE, Laboratory, Daegu, Korea, Republic of

Blood vessels are structurally three-dimensional (3D) and precisely connected. Conventional histological methods are unsuitable for their analysis due to the destruction of the functionally important topological 3D vascular structures. Tissue optical clearing techniques allow extensive volume imaging and the analysis of vast information without destruction. This study applied a tissue clearing technique to acquire high-resolution 3D images of rat brain vasculature by light-sheet and confocal microscopies. The rat underwent middle cerebral artery occlusion for 45 min followed by 24 h reperfusion with lectin injected directly into the heart for vascular staining afterward. For the 3D images of vascular, 3-mm thick brain slices were reconstructed using tissue clearing and light-sheet microscopy. Subsequently, after 3D rendering, the fitting of blood vessels to a filament model was used for analysis. Therefore, this resulted in the vessel diameter and density in the ischemic region decreasing significantly compared to those with contralesional non-ischemic regions. Immunostaining of 0.5-mm thick brain slices showed considerable neuronal loss and increased fluorescence intensity of astrocytes in the ipsilateral region. Therefore, these methods can provide more accurate data by broadening the scope of the analyzed “Region of Interests” to study the 3D cerebrovascular system and observation of neuronal changes observed in various brain disorders.

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BOARD NUMBER: S06-564

CENTRIFUGATION-INDUCED HYPERGRAVITY, A NEW APPROACH TO MODULATE THE BLOOD-BRAIN BARRIER PERMEABILITY IN MICE?

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Modifications of gravity levels induce generalized adaptation of mammalian physiology including vascular, brain, muscle, bone and immunity functions. It is known that centrifugation, increasing gravity level, affects the vestibular system and may be one of the sources of these physiological changes. As a crucial interface between the vascular system and the brain, the blood-brain barrier (BBB) acts as a filter to protect neurons from pathogens and inflammation. Our previous results suggested that centrifugation (24h, 2g) induce a BBB leakage in hippocampus measured by IgG extravasation (Dubayle et al., 2020). In order to characterize the opening size of BBB, we have performed i.v. injection of FITC-dextrans with different sizes (40, 70, 150 kDa). Their presences have been revealed directly in brain slices analyzed by microscopy (Nanozoomer). Currently, antisense oligonucleotides (ASON) are used to target specifically protein isoforms and reduce their expression. To be usable in neurosciences as therapeutical approaches ASON must pass throught the BBB (Boursereau et al., 2015). In the second part of this study, we have i.v. injected FAM-ASON (asANGPT2, 7kDa) and also analyzed by microscopy. We revealed fluorescent molecules in hippocampus, thalamus and cortex and also in liver as positive control organ. In conclusion, the crossing of BBB, from the blood to the brain parenchyma, depends on the size but also the nature of the molecules. Our experiences should be consolidated to validate centrifugation as non-invasive approach (i.v. administration) to increase delivery of pharmacological drug into the brain. **Keywords:** blood brain barrier, permeability, hypergravity, antisens oligonucleotide, dextran

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BOARD NUMBER: S06-565

DORSOLATERAL PERIAQUEDUCTAL GREY MATTER STIMULATION MODIFIES LARYNGEAL ACTIVITY AND SUBGLOTTIC PRESSURE IN SPONTANEOUSLY BREATHING ANAESTHETIZED RATS

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

Marta Gonzalez-Garcia[1,2,3], Manuel Víctor López-González[1,2,3], Laura Carrillo-Franco[1,3], Claudia Casermeiro-Garcia[1], Amelia Diaz-Casares[1,2,3], Marc Stefan Dawid-Milner[1,2,3]
[1]University of Málaga, Department Of Human Physiology, Faculty Of Medicine, Málaga, Spain, [2]University of Malaga, Unit Of Neurophysiology Of The Autonomic Nervous System (cimes), Malaga, Spain, [3]University of Malaga, Biomedical Research Institute Of Malaga (ibima), Malaga, Spain

Aims The aim of this study was to characterize the relations between mesencephalic regions (dlPAG) involved in cardiorespiratory control and their possible role in modulating laryngeal activity and their effects on vocalization. **Methods** Experimental studies were carried out with non-inbred male rats (n=14), SPF, Sprague-Dawley (250-300 g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2 mg/ kg, i.v., as necessary). A double up and down tracheal cannulation was performed to measure subglottic pressure and airflow. Subglottic pressure was recorded with an aneroid transducer (Hugo Sachs Elektronik D-7801, ± 0,1 psi) by passing a stream of humidified warm medical air upwards with a thermal mass digital air flow meter controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V). Bilateral parietostomy allowed access to dlPAG. Microinjections of PBS-Evans Blue (250 nl, pH 7.4 ± 0.1, 5-s duration) or glutamate (0,25M, 250 nl) were performed. Respiratory flow, pleural pressure, blood pressure, heart rate and ECG activity were also recorded. **Results** dlPAG PBS-Evans Blue microinjections did not produce any significant changes in any of the cardiorespiratory variables recorded. However, glutamate microinjections within the dlPAG evoked a decrease of laryngeal resistance (subglottal pressure) ($p<0,001$) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p<0,001$), together with a pressor ($p<0,05$) and tachycardic response ($p<0,001$). **Conclusions** The results of our study contribute with new data on the role of the mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

Pubmed:
32812210: López-González MV, González-García M, Peinado-Aragonés CA, Barbancho MÁ, Díaz-Casares A, Dawid-Milner MS
Pontine A5 region modulation of the cardiorespiratory response evoked from the midbrain dorsolateral periaqueductal grey. Connections between the midbrain dorsolateral periaqueductal grey (dlPAG) and the pontine A5 region have been shown. The stimulation of both regions evokes similar cardiovascular responses: tachycardia and hypertension. Accordingly, we have studied the interactions between dlPAG and A5 region in spontaneously breathing anesthetized rats. dlPAG was electrically stimulated (20-30 µA 1-ms pulses were given for 5 s at 100 Hz). Changes in the evoked cardiorespiratoy response were analysed before and after ipsilateral microinjections of muscimol (GABAergic agonist, 50 nl, 0.25 nmol, 5 s) within the A5 region. Electrical stimulation of the dlPAG produces, in the rat, a response characterized by tachypnoea ($p < 0.001$), hypertension ($p < 0.001$) and tachycardia ($p < 0.001$). The increase in respiratory rate was due to a decrease in expiratory time ($p < 0.01$). Pharmacological inhibition of the A5 region with muscimol produced a marked reduction of the tachycardia ($p < 0.001$) and the tachypnoea ($p < 0.01$) evoked from the dlPAG. Finally, to assess functional interactions between A5 and dlPAG, extracellular activity of putative A5 neurones were recorded during dlPAG electrical stimulation. Forty A5 cells were recorded, 16 of which were affected by dlPAG stimulation (40%). 4 cells showed activation, 5 cells excitation and 7 cells decreased spontaneous activity to dlPAG stimulation ($p < 0.001$). These results confirm a link between the A5 region and dlPAG. The potential role of these connections in the modulation of dlPAG evoked cardiorespiratory responses and their possible clinical implications are discussed.
J Physiol Biochem, 2020; 76

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-566

CUNEIFORM NUCLEUS STIMULATION MODIFIES LARYNGEAL ACTIVITY AND SUBGLOTTIC PRESSURE IN SPONTANEOUSLY BREATHING ANAESTHETIZED RATS

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

Laura Carrillo-Franco[1,2], Manuel Víctor López-González[2,3,4], Marta Gonzalez-Garcia[2,4,5], Claudia Casermeiro-Garcia[3], Amelia Diaz-Casares[2,3,4], Marc Stefan Dawid-Milner[2,3,4]
[1]Universidad de Malaga, Fisiología Humana, Histología Humana, Anatomía Patológica Y Educación Física Deportiva, Malaga, Spain, [2]University of Malaga, Biomedical Research Institute Of Malaga (ibima), Malaga, Spain, [3]University of Málaga, Department Of Human Physiology, Faculty Of Medicine, Málaga, Spain, [4]University of Malaga, Unit Of Neurophysiology Of The Autonomic Nervous System (cimes), Malaga, Spain, [5]Universidad de Malaga, Fisiologia Humana, Malaga, Spain

Aims The aim of this study was to characterize the electrophysiological relationships between the CnF and those pontine-medullary neuronal circuits involved in cardiorespiratory control and in changes of laryngeal caliber, to understand their role in laryngeal control and its effect on vocalization. **Methods** Experimental studies were carried out with non-inbred male rats (n=14), SPF, Sprague-Dawley (250-300 g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2 mg/ kg, i.v., as necessary). A double up and down tracheal cannulation was performed to measure subglottic pressure and airflow. Subglottic pressure was recorded with an aneroid transducer (Hugo Sachs Elektronik D-7801, ± 0,1 psi) by passing a stream of humidified warm medical air upwards with a thermal mass digital air flow meter controller (Bronkhorst-Hi-Tec F-201CV-AGD-22-V). Bilateral parietostomy allowed access to CnF. Microinjections of PBS-Evans Blue (250 nl, pH 7.4 ± 0.1, 5-s duration) or glutamate (0,25M, 250 nl) were performed. Respiratory flow, pleural pressure, blood pressure, heart rate and ECG activity were also recorded. **Results** CnF PBS-Evans Blue microinjections did not produce any significant changes in any of the cardiorespiratory variables recorded. However, glutamate microinjections within the CnF evoked a decrease of laryngeal resistance (subglottal pressure) ($p<0,001$) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p<0,001$), together with a pressor ($p<0,05$) and tachycardic response ($p<0,001$). **Conclusions** These results contribute with new data on the role of the mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

FENS Forum 2022
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BOARD NUMBER: S06-567

SMALL EXTRACELLULAR VESICLES FROM PERIPHERAL BLOOD OF AGED MICE PASS THE BLOOD-BRAIN BARRIER AND INDUCE GLIAL CELL ACTIVATION

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Introduction: Extracellular vesicles (EVs), including small EVs (sEVs), are involved in neuroinflammation and neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Yet, increased neuroinflammation can also be detected in the aging brain, and it is associated with increased glial activation. Changes in EV concentration are reported in aging tissues and senescence cells, suggesting a role of EVs in the process of aging. **Aims:** To investigate the effect of peripheral extracellular vesicles (EVs) from aged animals on neuroinflammation, specifically on glial activation. **Methods:** Extracellular vesicles (EVs) were isolated from the peripheral blood of young (3 months) and aged (24 months) C57BL/6J wildtype mice and injected into the peripheral blood from young animals via vein tail injections. The localization of EVs and the expression of selected genes involved in glial cell activation, including *Gfap*, *Tgf-β*, *Cd68*, and *Iba1*, were assessed in brain tissue 30 minutes, 4 hours, and 24 hours after injection. **Results:** We found that small EVs from peripheral blood of aged mice but not from young mice altered gene expression in the brains of young animals. In particular, the expression of the specific astrocyte marker, *Gfap*, was significantly increased, indicating a strong response of this glial cell type. **Conclusions:** Our study shows that small EVs from aged mice can pass the blood-brain barrier (BBB) and induce glial cell activation.

Pubmed:
34923482: Barth E, Srivastava A, Wengerodt D, Stojiljkovic M, Axer H, Witte OW, Kretz A, Marz M
Age-dependent expression changes of circadian system-related genes reveal a potentially conserved link to aging.
The circadian clock system influences the biology of life by establishing circadian rhythms in organisms, tissues, and cells, thus regulating essential biological processes based on the day/night cycle. Circadian rhythms change over a lifetime due to maturation and aging, and disturbances in the control of the circadian system are associated with several age-related pathologies. However, the impact of chronobiology and the circadian system on healthy organ and tissue aging remains largely unknown. Whether aging-related changes of the circadian system's regulation follow a conserved pattern across different species and tissues, hence representing a common driving force of aging, is unclear. Based on a cross-sectional transcriptome analysis covering 329 RNA-Seq libraries, we provide indications that the circadian system is subjected to aging-related gene alterations shared between evolutionarily distinct species, such as , , , and . We discovered differentially expressed genes by comparing tissue-specific transcriptional profiles of mature, aged, and old-age individuals and report on six genes (, , , , , and) of the circadian system, which show conserved aging-related expression patterns in four organs of the species examined. Our results illustrate how the circadian system and aging might influence each other in various tissues over a long lifespan and conceptually complement previous studies tracking short-term diurnal and nocturnal gene expression oscillations.
Aging (Albany NY), 2021; 13
34054686: Gaur N, Huss E, Prell T, Steinbach R, Guerra J, Srivastava A, Witte OW, Grosskreutz J
Monocyte-Derived Macrophages Contribute to Chitinase Dysregulation in Amyotrophic Lateral Sclerosis: A Pilot Study.
Neuroinflammation significantly contributes to Amyotrophic Lateral Sclerosis (ALS) pathology. In lieu of this, reports of elevated chitinase levels in ALS are interesting, as they are established surrogate markers of a chronic inflammatory response. While post-mortem studies have indicated glial expression, the cellular sources for these moieties remain to be fully understood. Therefore, the objective of this pilot study was to examine whether the peripheral immune system also contributes to chitinase dysregulation in ALS. The temporal expression of CHIT1, CHI3L1, and CHI3L2 in non-polarized monocyte-derived macrophages (MoMas) from ALS patients and healthy controls (HCs) was examined. We demonstrate that while CHIT1 and CHI3L1 display similar temporal expression dynamics in both groups, profound between-group differences were noted for these targets at later time-points i.e., when cells were fully differentiated. CHIT1 and CHI3L1 expression were

significantly higher in MoMas from ALS patients at both the transcriptomic and protein level, with CHI3L1 levels also being influenced by age. Conversely, CHI3L2 expression was not influenced by disease state, culture duration, or age. Here, we demonstrate for the first time, that in ALS, circulating immune cells have an intrinsically augmented potential for chitinase production that may propagate chronic neuroinflammation, and how the ageing immune system itself contributes to neurodegeneration.
Front Neurol, 2021; 12
33309863: Srivastava A, Barth E, Ermolaeva MA, Guenther M, Frahm C, Marz M, Witte OW
Tissue-specific Gene Expression Changes Are Associated with Aging in Mice.
Aging is a complex process that can be characterized by functional and cognitive decline in an individual. Aging can be assessed based on the functional capacity of vital organs and their intricate interactions with one another. Thus, the nature of aging can be described by focusing on a specific organ and an individual itself. However, to fully understand the complexity of aging, one must investigate not only a single tissue or biological process but also its complex interplay and interdependencies with other biological processes. Here, using RNA-seq, we monitored changes in the transcriptome during aging in four tissues (including brain, blood, skin and liver) in mice at 9 months, 15 months, and 24 months, with a final evaluation at the very old age of 30 months. We identified several genes and processes that were differentially regulated during aging in both tissue-dependent and tissue-independent manners. Most importantly, we found that the electron transport chain (ETC) of mitochondria was similarly affected at the transcriptome level in the four tissues during the aging process. We also identified the liver as the tissue showing the largest variety of differentially expressed genes (DEGs) over time. Lcn2 (Lipocalin-2) was found to be similarly regulated among all tissues, and its effect on longevity and survival was validated using its orthologue in Caenorhabditis elegans. Our study demonstrated that the molecular processes of aging are relatively subtle in their progress, and the aging process of every tissue depends on the tissue's specialized function and environment. Hence, individual gene or process alone cannot be described as the key of aging in the whole organism.
Genomics Proteomics Bioinformatics, 2020; 18
31606727: Barth E, Srivastava A, Stojiljkovic M, Frahm C, Axer H, Witte OW, Marz M
Conserved aging-related signatures of senescence and inflammation in different tissues and species.
Increasing evidence indicates that chronic inflammation and senescence are the cause of many severe age-related diseases, with both biological processes highly upregulated during aging. However, until now, it has remained unknown whether specific inflammation- or senescence-related genes exist that are common between different species or tissues. These potential markers of aging could help to identify possible targets for therapeutic interventions of aging-associated afflictions and might also deepen our understanding of the principal mechanisms of aging. With the objective of identifying such signatures of aging and tissue-specific aging markers, we analyzed a multitude of cross-sectional RNA-Seq data from four evolutionarily distinct species (human, mouse and two fish) and four different tissues (blood, brain, liver and skin). In at least three different species and three different tissues, we identified several genes that displayed similar expression patterns that might serve as potential aging markers. Additionally, we show that genes involved in aging-related processes tend to be tighter controlled in long-lived than in average-lived individuals. These observations hint at a general genetic level that affect an individual's life span. Altogether, this descriptive study contributes to a better understanding of common aging signatures as well as tissue-specific aging patterns and supplies the basis for further investigative age-related studies.
Aging (Albany NY), 2019; 11
28110102: Frahm C, Srivastava A, Schmidt S, Mueller J, Groth M, Guenther M, Ji Y, Priebe S, Platzer M, Witte OW
Transcriptional profiling reveals protective mechanisms in brains of long-lived mice.
The brain plays a central role in organismal aging but is itself most sensitive to aging-related functional impairments and pathologies. Insights into processes underlying brain aging are the basis to positively impact brain health. Using high-throughput RNA sequencing and quantitative polymerase chain reaction (PCR), we monitored cerebral gene expression in mice throughout their whole lifespan (2, 9, 15, 24, and 30 months). Differentially expressed genes were clustered in 6 characteristic temporal expression profiles, 3 of which revealed a distinct change between 24 and 30 months, the period when most mice die. Functional annotation of these genes indicated a participation in protection against cancer and oxidative stress. Specifically, the most enriched pathways for the differentially expressed genes with higher expression at 30 versus 24 months were found to be glutathione metabolism and chemokine signaling pathway, whereas those lower expressed were enriched in focal adhesion and pathways in cancer. We therefore conclude that brains of very old mice are protected from certain aspects of aging, in particular cancer, which might have an impact on organismal health and lifespan.
Neurobiol Aging, 2017; 52
21354960: Mishra J, Kumar A, Sinha A, Das S,
Ingenuity in pattern recognition: a novel bioinformatics approach towards lung cancer identification.
Lung cancer is the significant contributor of increased cancer deaths. Earlier research indicates that the environmental factors have a major role in causing lung cancer. It is evident that the pathway of lung cancer includes both RAS-mediated and non-

RAS-mediated mechanisms. Clinical findings point to a wide variety of other cancers contributing to lung cancer incidence. Such a scenario makes identification of lung cancer difficult and thus identifying its mechanisms can contribute to the society. Our study involves the RAS subfamily that includes a set of proteins, which cause an over expression of cancer-causing genes like M-ras and initiate tumour formation in lungs.
Int J Bioinform Res Appl, 2010; 6

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-568

MIMICKING BLOOD BRAIN BARRIER IN MICROFLUIDIC MODELS

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Aim: Blood brain barrier (BBB) is an extremely important structure for maintaining brain homeostasis. We thus aimed to reproduce appropriate physiological conditions by carrying out experiments in microfluidic lab-on-a-chip models that could mimic both architecture and functionality of BBB. One objective was to demonstrate biocompatibility of various transparent materials used for manufacturing the microfluidic devices with brain microvascular endothelial cells test them (bEnd.3 cell line) for mimicking BBB on a chip. **Methods:** For the fabrication of microfluidic networks in glass or polymeric substrates, we applied laser assisting etching methods to tailor material shape and physical characteristics. We then tested biocompatibility of the modified material surfaces by evaluation of actin fibres integrity and cytoskeleton with bEnd.3 cell line. Preliminary tests were also performed on microfluidic devices using human microvascular endothelial cells. For checking the BBB monolayer integrity, the ZO-1 tight junction proteins were immunostained and analysed by confocal fluorescence microscopy. **Results:** Our results have shown that most modified surfaces are biocompatible. The integrity of the BBB was demonstrated using optimized protocols for high-resolution microscopy visualization of the ZO-1 immunostained junction proteins. **Conclusions:** Our analyses have shown that various transparent materials modified by laser processing methods are suitable for BBB-on-a-chip studies. These materials were found appropriate to develop an adequate BBB model that will be further proposed as an *in vitro* tool for evaluating pathological conditions such as Alzheimer's disease and Parkinson's disease.

Pubmed:

34205550: Staicu CE, Jipa F, Axente E, Radu M, Radu BM, Sima F
Lab-on-a-Chip Platforms as Tools for Drug Screening in Neuropathologies Associated with Blood-Brain Barrier Alterations.
Lab-on-a-chip (LOC) and organ-on-a-chip (OOC) devices are highly versatile platforms that enable miniaturization and advanced controlled laboratory functions (i.e., microfluidics, advanced optical or electrical recordings, high-throughput screening). The manufacturing advancements of LOCs/OOCs for biomedical applications and their current limitations are briefly discussed. Multiple studies have exploited the advantages of mimicking organs or tissues on a chip. Among these, we focused our attention on the brain-on-a-chip, blood-brain barrier (BBB)-on-a-chip, and neurovascular unit (NVU)-on-a-chip applications. Mainly, we review the latest developments of brain-on-a-chip, BBB-on-a-chip, and NVU-on-a-chip devices and their use as testing platforms for high-throughput pharmacological screening. In particular, we analyze the most important contributions of these studies in the field of neurodegenerative diseases and their relevance in translational personalized medicine.
Biomolecules, 2021; 11
34253298: Stoica R, Rusu CM, Staicu CE, Burlacu AE, Radu M, Radu BM
Ca homeostasis in brain microvascular endothelial cells.
Blood brain barrier (BBB) is formed by the brain microvascular endothelial cells (BMVECs) lining the wall of brain capillaries. Its integrity is regulated by multiple mechanisms, including up/downregulation of tight junction proteins or adhesion molecules, altered Ca homeostasis, remodeling of cytoskeleton, that are confined at the level of BMVECs. Beside the contribution of BMVECs to BBB permeability changes, other cells, such as pericytes, astrocytes, microglia, leukocytes or neurons, etc. are also exerting direct or indirect modulatory effects on BBB. Alterations in BBB integrity play a key role in multiple brain pathologies, including neurological (e.g. epilepsy) and neurodegenerative disorders (e.g. Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis etc.). In this review, the principal Ca signaling pathways in brain microvascular endothelial cells are discussed and their contribution to BBB integrity is emphasized. Improving the knowledge of Ca homeostasis alterations in BMVECa is fundamental to identify new possible drug targets that diminish/prevent BBB permeabilization in neurological and neurodegenerative disorders.

Int Rev Cell Mol Biol, 2021; 362
32785177: Caravia L, Staicu CE, Radu BM, Condrat CE, Crețoiu D, Bacalbașa N, Suciu N, Crețoiu SM, Voinea SC
Altered Organelle Calcium Transport in Ovarian Physiology and Cancer.
Calcium levels have a huge impact on the physiology of the female reproductive system, in particular, of the ovaries. Cytosolic calcium levels are influenced by regulatory proteins (i.e., ion channels and pumps) localized in the plasmalemma and/or in the endomembranes of membrane-bound organelles. Imbalances between plasma membrane and organelle-based mechanisms for calcium regulation in different ovarian cell subtypes are contributing to ovarian pathologies, including ovarian cancer. In this review, we focused our attention on altered calcium transport and its role as a contributor to tumor progression in ovarian cancer. The most important proteins described as contributing to ovarian cancer progression are inositol trisphosphate receptors, ryanodine receptors, transient receptor potential channels, calcium ATPases, hormone receptors, G-protein-coupled receptors, and/or mitochondrial calcium uniporters. The involvement of mitochondrial and/or endoplasmic reticulum calcium imbalance in the development of resistance to chemotherapeutic drugs in ovarian cancer is also discussed, since Ca channels and/or pumps are nowadays regarded as potential therapeutic targets and are even correlated with prognosis.
Cancers (Basel), 2020; 12
31936634: Staicu CE, Predescu DV, Rusu CM, Radu BM, Cretoiu D, Suciu N, Crețoiu SM, Voinea SC
Role of microRNAs as Clinical Cancer Biomarkers for Ovarian Cancer: A Short Overview.
Ovarian cancer has the highest mortality rate among gynecological cancers. Early clinical signs are missing and there is an urgent need to establish early diagnosis biomarkers. MicroRNAs are promising biomarkers in this respect. In this paper, we review the most recent advances regarding the alterations of microRNAs in ovarian cancer. We have briefly described the contribution of miRNAs in the mechanisms of ovarian cancer invasion, metastasis, and chemotherapy sensitivity. We have also summarized the alterations underwent by microRNAs in solid ovarian tumors, in animal models for ovarian cancer, and in various ovarian cancer cell lines as compared to previous reviews that were only focused the circulating microRNAs as biomarkers. In this context, we consider that the biomarker screening should not be limited to circulating microRNAs per se, but rather to the simultaneous detection of the same microRNA alteration in solid tumors, in order to understand the differences between the detection of nucleic acids in early vs. late stages of cancer. Moreover, in vitro and in vivo models should also validate these microRNAs, which could be very helpful as preclinical testing platforms for pharmacological and/or molecular genetic approaches targeting microRNAs. The enormous quantity of data produced by preclinical and clinical studies regarding the role of microRNAs that act synergistically in tumorigenesis mechanisms that are associated with ovarian cancer subtypes, should be gathered, integrated, and compared by adequate methods, including molecular clustering. In this respect, molecular clustering analysis should contribute to the discovery of best biomarkers-based microRNAs assays that will enable rapid, efficient, and cost-effective detection of ovarian cancer in early stages. In conclusion, identifying the appropriate microRNAs as clinical biomarkers in ovarian cancer might improve the life quality of patients.
Cells, 2020; 9

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BOARD NUMBER: S06-569

INVESTIGATING THE EXPRESSION AND FUNCTIONAL ROLE OF KV7 CHANNELS IN BRAIN ENDOTHELIAL CELLS

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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The blood-brain barrier (BBB) protects brain parenchyma from neurotoxic agents and regulates the composition of the brain interstitial fluid that is crucial for the proper functioning of neurons. Such properties are determined by the presence of tight junctions (TJs) between brain endothelial cells (ECs) that limit the paracellular flow of ions and solutes, and by specific transporters and ion channels expressed in EC. Whilst solute transporters in ECs have been thoroughly studied, the role of ion channels have been poorly investigated. In this study we evaluated the expression and the function of the five members of the voltage-gated Kv7 potassium channels family in *in vitro* models of BBB. Quantitative PCR (Q-PCR) and immunofluorescence experiments showed the expression of Kv7.1, Kv7.4 and Kv7.5 in clonal ECs (BEND cells), brain microvessels (BMVs), and primary ECs, with Kv7.5 being localized at cell-cell junction sites; instead, the expression of Kv7.2 and Kv7.3 was negligible. In BMVs, Kv7.4 was also expressed in pericytes that, along with astrocytes, form the neurovascular unit. In BEND cells cultured in Transwell inserts, pharmacological activation of Kv7 channels with the specific opener retigabine (10 μM) reduced paracellular flux of fluorescent dextran; this effect was prevented by the selective Kv7 blocker XE-991 (10 μM). Immunofluorescence experiments using a TJ-specific marker (Zonula Occludens-1) revealed that retigabine also reduced the number of breaks in the continuity of TJs between ECs. Overall, these results show that Kv7 channels are expressed in brain ECs, and suggest that their pharmacological modulation regulates BBB permeability.

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BOARD NUMBER: S06-570

THE HIGHLY REACTIVE DICARBONYL COMPOUND, METHYLGLYOXAL, REGULATES THE PURINERGIC SIGNALING PATHWAYS IN BRAIN ENDOTHELIUM

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Aim: Inducing oxidative stress in the endothelium of brain vasculature can lead to DNA damage, increase the intracellular calcium concentration, cell death. Considering the major role of blood-brain barrier (BBB) to ensure cerebral homeostasis, the goal of this study is to understand the murine cerebral microvasculature endothelial cells behavior after 24 hours' exposure to methylglyoxal (MGO), known for its ability to generate reactive oxygen species (ROS). Methods: Murine brain microvasculature endothelial cells, bEnd.3 cell line, were exposed to MGO (1-1000 µM) and tested for the cellular viability (MTT assay), cellular response to ATP (calcium imaging assay) and ROS production. Specific inhibitors for the purinergic signaling pathway: phospholipase C inhibitor (U73122), inositol-triphosphate receptor inhibitor (2-APB) and P2Y2 receptor inhibitor (ARC 118925XX) were used to modulate the MGO effect. Results: High concentrations of methylglyoxal (over 250 µM) significantly enhanced ROS production (5-10 times) and diminished cellular viability (down to 40% for 1000 µM MGO). The ATP response was tested in the range of 0-250 µM MGO where no significant change in viability and ROS production was observed. Exposure to MGO induced a stimulatory effect on the amplitude (~50%) and area (~50%) of the calcium transient accompanied by a reduction of its latency (~50%). The inhibitors used in these experiments modulated the effects induced by MGO treatment. Conclusions: We demonstrated that MGO modulates the purinergic signaling pathway in brain microvascular endothelium. These results are relevant in understanding the BBB behavior in glycation and oxidative stress conditions associated to various neuropathologies.

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BOARD NUMBER: S06-571

ACTIVATION OF NOTCH1 SIGNALING PREVENTS THE BBB DISRUPTION IN THE INTRACEREBRAL HEMORRHAGE MODEL VIA ENHANCEMENT OF MITOCHONDRIAL FUNCTION

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Endothelial cells (ECs) in cerebral vessels are a primary target for acute brain injury and the dysfunction of ECs leads to secondary inflammatory injury through blood-brain barrier (BBB) disruption. Although quality control of BBB is important to prevent acute brain injury, modulatory signaling of it still needs further investigation. According to our previous report, the defect of mitochondrial respiration in ECs leads to profound BBB disruption but the downstream signaling pathway was not revealed. To identify the link between a mitochondrial defect in ECs and BBB disruption, we isolated cerebral vessels from TEKCRIF1 mouse and performed RNA sequencing. 908 (p-value 0.05) differentially expressed genes were identified. Among the candidate signaling from transcriptome analysis, we focused on the Notch1 signaling which closely correlated to BBB maintenance. With the intracerebral hemorrhage (ICH) mouse model, we observed the decrease of Notch1 signaling and mitochondrial OxPhos complex expression that results in BBB disruption. To assess the function of Notch1 signaling in BBB disruption, we injected an agonist of Notch1 receptor into the ICH mouse model. With the treatment, we found the prevention of BBB disruption and decrease of injury area of ICH model accompanying alteration of mitochondrial OxPhos complex. Taken together, we suggest that activation of Notch1 signaling could be prevention of acute brain injury through quality control of BBB, and one of the modulatory signaling between mitochondrial defect and BBB disruption.

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BOARD NUMBER: S06-572

MICROGLIA SHAPE A GENETIC CEREBRAL SMALL VESSEL DISEASE

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Cerebral small vessel diseases are a common source of disability. While most cases are sporadic, genetic forms provide insight into the cellular and molecular mechanisms underlying the vessel pathology. Mutations in the *NEMO* gene are the cause of incontinentia pigmenti (IP) that is associated with a cerebral small vessel disease. To model IP, we deleted *Nemo* in the brain endothelial cells of mice ($Nemo^{beKO}$). In addition to the known loss of brain capillaries, we found an increase in the number of microglia in the brains of $Nemo^{beKO}$ mice. To explore their function, we ablated microglia by feeding mice a PLX-5622 containing diet. Ablation of microglia improved the anxiety-like phenotype of mice in the open field and lowered the seizure threshold as determined by maximal electroshocks. In parallel, the number of remnant dead capillaries, so-called string vessels, and of activated astrocytes slightly increased. In summary, microglia aggravate the functional consequences of a small vessel disease. Interfering with microglia activation may represent a strategy to treat cerebral small vessel diseases.

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BOARD NUMBER: S06-573

BRAIN ENDOTHELIAL GαQ/11 KNOCKOUT INDUCES SYSTEMIC METABOLIC CHANGES

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

Dorothea Ziemens, Marius Richter, Nina Feller, Helge Müller-Fielitz, Markus Schwaninger, Jan Wenzel
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Cerebral endothelial dysfunction refers to the loss of physiological properties and functionality of the brain endothelium. The impaired reactivity of the vasculature leads to an insufficient supply of energy substrates like glucose and insufficient removal of molecules such as carbon dioxide (CO_2) from the brain tissue. CO_2 is a well-known metabolic end product of cellular metabolism and has effects on brain areas that control essential functions such as respiration. Recently, CO_2-sensitive neurons have also been described in brain areas relevant to energy homeostasis. The extent to which endothelial dysfunction and its consequences like impaired CO_2 removal contribute to the development of metabolic changes remains unknown. To this purpose, we developed a mouse model mimicking cerebral endothelial dysfunction that is induced by a knockout of the $G\alpha_{q/11}$ signalling pathway specifically in brain endothelial cells ($G\alpha_{q/11}^{beKO}$ mice). These mice show a loss of vascular reactivity to stimuli such as CO_2. Mice were examined for energy metabolism in a basal and diet-induced obese state. We could show that under basal conditions, $G\alpha_{q/11}^{beKO}$ mice show differences in body composition compared to control mice, and along with that, we found an impaired glucose metabolism by using different measurements. Under the influence of a high-fat diet, $G\alpha_{q/11}^{beKO}$ mice showed a decrease and dampened circadian rhythmicity. With our experiments, we obtained strong evidence for the influence of an impaired vascular reactivity in the brain on the peripheral metabolism. Our findings suggest a link between cerebral endothelial dysfunction and the outcome and progression of diet-induced obesity.

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BOARD NUMBER: S06-574

TWO-PHOTON IMAGING REVEALS VESSEL TYPE-SPECIFIC LOSS OF THE GLYCOCALYX AT THE BLOOD-BRAIN BARRIER DURING APOM/S1PR1 SIGNALING IMPAIRMENT IN VIVO.

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Background: Aging, stroke, and chronic brain disorders are accompanied by neurotoxic increases in adsorptive-mediated transcytosis (AMT), a non-specific transport of proteins across the blood-brain barrier (BBB). We have shown that impairment of sphingosine-1-phosphate receptor 1 (S1PR1) signaling at the BBB leads to detrimental increases in AMT. Two features stood out: only arterioles were affected, and the BBB could be rapidly rescued by selective S1PR1 agonism. However, the mechanisms remained unknown. **Hypothesis:** Glycocalyx is a meshwork of glycoproteins and proteoglycans, constituting a non-cellular component of the BBB. Negatively-charged glycocalyx lines the luminal side of the endothelial cells and can putatively repel plasma proteins from entering AMT. We hypothesized that S1PR1 signaling impairment causes glycocalyx loss, which might explain AMT increase. **Methods:** Glycocalyx is seldom preserved *ex vivo*. Therefore; a) we used two-photon microscopy (2PM) to image BBB in mice with impaired S1PR1 signaling (*Apom*$^{-/-}$mice); and b) we developed a 2PM photobleaching approach to quantify the extent of glycocalyx changes at different categories of cerebral microvessels, i.e., arterioles, capillaries, and venules *in vivo.* **We show that:** (i) S1PR1 signaling impairment led to glycocalyx loss; and exclusively in arterioles, which mirrored the vascular zonation of AMT increase; (ii) S1PR1 stimulation that was sufficient to rapidly rescue AMT (<150 min) did not reinstate the glycocalyx within the same time frame; (iii) Glycocalyx recovery occurred much later, over days, and upon recurrent agonism of S1PR1. **Conclusions:** Glycocalyx loss may contribute to AMT increase, but the reconstitution of the glycocalyx is not critical to normalizing AMT.

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BOARD NUMBER: S06-575

APPROACHING IN-VIVO BBB PERMEABILITIES WITH ENGINEERED BLOOD-BRAIN BARRIER-ON-CHIP

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Accurate modeling of the Blood-Brain Barrier (BBB) is crucial in maintaining brain homeostasis and is the key barrier to understanding drug pharmacokinetics.

Our microfluidic BBB-on-chip platform design includes static conditions of the brain-side of the BBB, as well as the dynamic conditions of the brain microvasculature within the same platform, mimicking in-vivo conditions. Our BBB-on-chip is established using a co-culture of endothelial cells and astrocytes, cultured on opposing sides of a semipermeable membrane. The confluence of cell layers is achieved approximately after 7 days of culture when the barrier properties are validated.

In-vitro BBB generated with either mono or cultured brain endothelial cells and astrocytes showed marked improvement in Papp (apparent permeabilities) when cultured under the flow conditions. Mouse BBB-on-chip showed a Papp for 70 kDa Dextran to be 1.7E-7 cm/s, which is astonishingly close to in-vivo observed Papp of 1.5E-7 cm/s.

We have demonstrated that flow shear stress and dynamic flow conditions are crucial for improving in-vitro BBB permeabilities. Our microfluidic BBB model accurately reflects the in vivo conditions of the BBB by modeling both the static conditions of the brain-side of the BBB, as well as the dynamic conditions of the brain microvasculature within the same platform. As the highest-throughput platform currently in the field, our BBB model enables the development and screening of new therapeutics for neurological disorders still lacking effective treatments, such as Alzheimer's and Parkinson's disease.

Pubmed:
30424394: Nguyen HT, Thach H, Roy E, Huynh K, Perrault CM
Low-Cost, Accessible Fabrication Methods for Microfluidics Research in Low-Resource Settings.
Microfluidics are expected to revolutionize the healthcare industry especially in developing countries since it would bring portable, easy-to-use, self-contained diagnostic devices to places with limited access to healthcare. To date, however, microfluidics has not yet been able to live up to these expectations. One non-negligible factor can be attributed to inaccessible prototyping methods for researchers in low-resource settings who are unable to afford expensive equipment and/or obtain critical reagents and, therefore, unable to engage and contribute to microfluidics research. In this paper, we present methods to create microfluidic devices that reduce initial costs from hundreds of thousands of dollars to about $6000 by using readily accessible consumables and inexpensive equipment. By including the scientific community most embedded and aware of the requirements of healthcare in developing countries, microfluidics will be able to increase its reach in the research community and be better informed to provide relevant solutions to global healthcare challenges.
Micromachines (Basel), 2018; 9

FENS Forum 2022
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BOARD NUMBER: S06-576

EFFECT OF AGEING ON THE CEREBRAL HEMODYNAMICS IN THE MARMOSET MONKEY

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Aim Cerebrovascular function declines during normal ageing and appears to be closely linked to brain function alteration in the elderly. We adapted dedicated MRI sequences to analyze blood flow dynamics in arterial and venous intracranial vessels, using a non-human primate model of significant interest for preclinical research, the marmoset monkey *Callithrix jacchus*. **Methods** Imaging sequences were acquired on a 7T MRI (Bruker Biospec, voxel size: 0,15x0,15X1mm3) on two groups of male and female marmosets: 10 young (4 years old) and 9 aged (9 years old). Internal carotid arteries, basilar artery, superior and inferior sagittal sinuses were first identified by Time Of Flight (TOF) sequences and flow velocity in these different vessels was recorded by Phase Contrast (PC) MRI synchronized with ECG signal. **Results** The main significant results show that during the cardiac cycle: -The blood flow peaks earlier in the basilary trunk than in the carotids. -The arterial flow peak occurs earlier in the aged than in the young marmoset brains. -The time interval between the arterial- and the venous flow peaks is shorter in old than in young marmosets. **Conclusions** These results reflect vessel rigidity and tissue compliance changes occurring during ageing in the vascular network. They have not been shown in human studies, in which blood flow dynamics are usually synchronized with peripheral pulse measured on the fingertip. They suggest that the time courses of the different vascular flows could contribute as early markers of brain ageing and brain pathologies. ***Supported by ANR Hanuman 18-CE45-0014-01***

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BOARD NUMBER: S06-577

CEREBROVASCULAR REACTIVITY TO HYPERCAPNIA AND NEUROVASCULAR COUPLING EXPLORE DIFFERENT VASCULAR BEDS

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Background: Cerebrovascular reactivity (CVR) to hypercapnia is the gold standard to evaluate vascular diseases with BOLD fMRI. In contrast to neurovascular coupling, the spatial and temporal dynamics of cellular and vascular responses to CO_2 along the vascular arbor remain unclear. **Aim:** Our goal was to investigate and compare the dynamics of CVR and neurovascular coupling along the vascular arbor in the mouse barrel cortex. **Methods:** Using two-photon imaging in sedated mice, implanted with a chronic cranial window, we measured vascular and cellular responses (pyramidal cells, smooth muscle cells, pericytes, endothelial cells and astrocytes expressing GCaMP6) to CO_2 (20%, 10 s) and whisker stimulations from pial arterioles to veins. **Results:** As previously reported, whisker stimulation triggered fast activation of neurons and smooth muscle cells, followed within ~1 s by dilation of penetrating arterioles and systematic increases of blood velocity in downstream capillaries and veins. In contrast, CO_2 stimulation generated a different sequence of events: in pial and penetrating arterioles, CO_2 triggered an initial velocity decrease, a 4 s delayed Ca^{2+} drop in smooth muscle cells, a further delayed dilation and blood flow increase (7 and 13 s, respectively). At the level of capillaries, the effect of CO_2 was sparse and small. In opposition to other cells of the neurovascular unit, pyramidal cell activity was not affected by CO_2. **Conclusions:** Overall, our data suggest that CVR to hypercapnia reports mostly the function of large arteriolar vessels whereas neurovascular coupling investigates the function of the entire neurovascular unit.

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BOARD NUMBER: S06-578

MEASUREMENT OF BLOOD FLOW VELOCITY WITH LASER SCANNING MICROSCOPY: MODELLING AND COMPARISON OF LINE-SCAN IMAGE-PROCESSING ALGORITHMS.

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Aim: Blood flow is commonly monitored to follow brain activity. At the cellular level, laser scanning microscopy is the technique of choice to measure blood velocity with line-scans and compare data sets across animal models and experimental conditions. Although velocity differences as low as a few percent have been reported, the extent to which measurements depend on the choice of experimental parameters and the image analysis is too often ignored. We have previously shown how to suppress the errors resulting from scanning by providing an unbiased algorithm to calculate velocities. Here, we investigated the errors resulting from the interplay of acquisition parameters and image-analysis algorithms. Methods: Analysis of blood flow velocity from line-scans requires specific image-processing algorithms, such as angle measurements, Line-Scanning Particle Image Velocimetry or Fourier transformation of line-scan images. We developed mathematical models for each of them. We also provide a software generating artificial line-scan images to test the models. Results: Our models predict the experimental conditions under which each image-processing algorithm gives velocity measurements with a given accuracy. We apply our approach to different vessel types and give the parameter space available for each image-processing algorithm. We validate our models with artificial line-scans. Conclusion: Our study provides efficient tools to the community of vascular physiologists and imaging neuroscientists that monitor blood flow changes to assess normal and pathological brain function.

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BOARD NUMBER: S06-579

MODELING THE RELATION BETWEEN NEURONAL ACTIVITY AND THE BOLD SIGNAL VIA ASTROCYTIC CALCIUM DYNAMICS

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Functional magnetic resonance imaging (fMRI) relies on the coupling between neuronal and vascular activity, but the mechanisms behind this coupling are still under discussion. Recent experimental evidence suggests that calcium signaling may play a significant role in neurovascular coupling. However, it is still controversial where this calcium signal is located (in neurons or elsewhere), how it operates and how relevant is its role. In this work we introduce a biologically plausible model of the neurovascular coupling and we show that calcium signaling in astrocytes can explain the main aspects of the dynamics of the coupling. We find that calcium signaling can explain so-far unrelated features such as the linear and non-linear regimes, the negative vascular response (undershoot) and the emergence of a (calcium-driven) Hemodynamic Response Function. We analyze how information is coded and transmitted from the neuronal to the vascular system and we find that frequency modulation of astrocytic calcium dynamics plays a key role in this process. Our work provides a framework to link neuronal activity to the BOLD signal, and vice-versa, where neuronal activity can be inferred from the BOLD signal. This opens new ways to link known alterations of astrocytic calcium signaling in neurodegenerative diseases (e.g. Alzheimer's and Parkinson's diseases) with detectable changes in neurovascular coupling. Finally, in our modeling framework the neuronal-astrocyte pathway is built upon a mean-field description of neuronal activity, which makes it suitable for its implementation in large-scale simulations. We show an example of such an implementation for whole-brain simulations.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S06-580

IN MICE AND HUMANS, BRAIN VASCULAR BARRIER HOMEOSTASIS AND CONTRACTILITY ARE ACQUIRED POSTNATALLY

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Although great efforts to characterize the embryonic phase of brain vascular system development have been made, its postnatal maturation has barely been described. Here, we compared the molecular and functional properties of brain vascular cells on postnatal day (P)5 vs. P15, via a transcriptomic analysis of purified mouse cortical microvessels (MVs) and the identification of vascular-cell-type-specific or -preferentially expressed transcripts. We found that endothelial cells (EC), vascular smooth muscle cells (VSMC) and fibroblasts (FB) follow specific molecular and functional maturation programs over this time period. In particular, ECs acquire P-glycoprotein (P-gP)-mediated efflux capacities. The arterial VSMC network expands and becomes contractile resulting in a greater cerebral blood flow (CBF). Samples of human brain cortex showed the same postnatal maturation process. Thus, the early postnatal phase is a critical period during which essential properties of cerebral blood vessels, i.e. the endothelial efflux of xenobiotics and other molecules, and the VSMC contractility required for vessel tone and brain perfusion, are acquired and mature.

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BOARD NUMBER: S06-581

CHARACTERIZING THE OXYGEN INITIAL DIP IN THE BRAIN OF ANESTHETIZED AND AWAKE MICE

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Background: An ongoing controversy in brain metabolism and imaging based on deoxyhemoglobin detection is whether increases in neural activity cause a local and rapid decrease in oxygen concentration (the "Po_2 initial dip") preceding functional hyperemia. Here, we reinvestigated the issue by targeting a distinct neuronal network in the olfactory bulb, the glomerulus most sensitive to ethyl tiglate (GMS_{ET}) and a site of strong activation and energy consumption upon ET stimulation. **Aim:** Our goal was to determine the experiment conditions under which a Po_2 initial dip is detected near activated synapses, in the neuropil and in capillaries. **Method:** Using two-photon fluorescence and phosphorescence lifetime imaging in Thy1-GCaMP6 mice implanted with a cranial window for more than 2 weeks, we measured vascular and tissue Po_2 with the oxygen sensor Oxyphor 2P, simultaneously with neuronal calcium signals in the GMS_{ET}. Measurements were performed in mice anesthetized (Ketamine/Domitor) and awake. **Results/Conclusions:** In anesthetized mice, a transient dip in vascular Po_2 was detected in the GMS_{ET} when functional hyperemia was slightly delayed, but its amplitude was minute. The vascular Po_2 dip was not observed in glomeruli responding non-specifically to ET and was poorly influenced by resting Po_2. In awake mice, this dip in Po_2 was absent in capillaries as well as, surprisingly, in the neuropil. This demonstrates that in awake mice recovered from brain surgery, neurovascular coupling is too fast and efficient to reveal any dip in Po_2.

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BOARD NUMBER: S06-582

ASTROCYTIC CA2+ SIGNALS PARTAKE IN INHIBITORY NEUROVASCULAR COUPLING IN A BRAIN STATE-DEPENDENT MANNER.

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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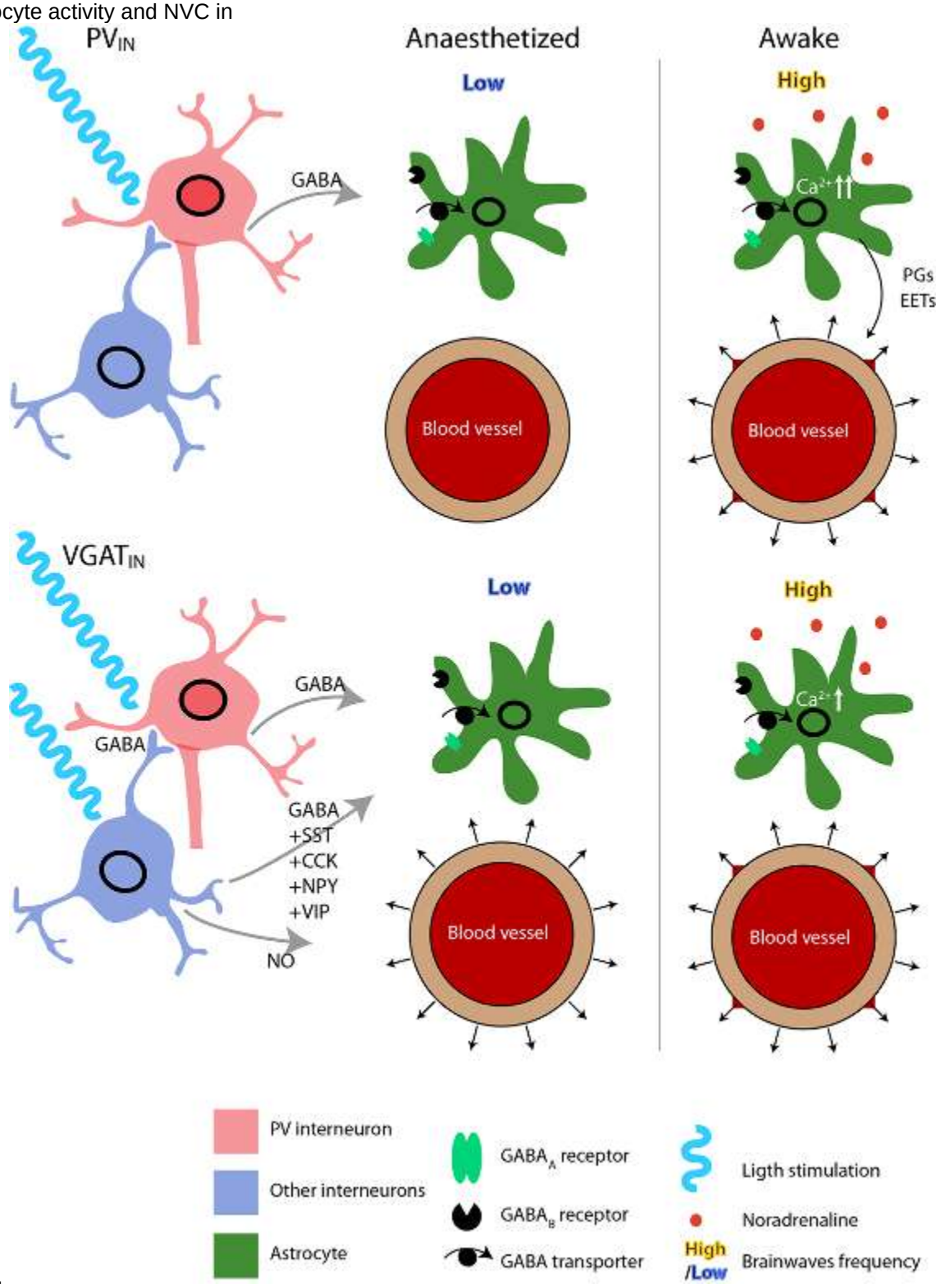
Neurovascular coupling (NVC) modulates cerebral blood flow to match increased metabolic demand during neuronal excitation. Activation of inhibitory interneurons also increase blood flow, but the basis for this inhibitory NVC is unclear. We performed two-photon microscopy in awake mice to examine the correlation between astrocytic Ca^{2+} and NVC, evoked by activity in either all ($VGAT_{IN}$) or parvalbumin-positive GABAergic interneurons (PV_{IN}). Optogenetic stimulation of $VGAT_{IN}$ and PV_{IN} in the somatosensory cortex triggered astrocytic Ca^{2+} increases that were abolished by anaesthesia. PV_{IN} evoked astrocytic Ca^{2+} responses with a short latency that preceded NVC, whereas $VGAT_{IN}$ evoked Ca^{2+} increases that were delayed relative to the NVC response. The early onset in PV_{IN} evoked Ca^{2+} increases dependent on noradrenaline release from locus coeruleus, which also affected inhibitory NVC. Therefore, inhibitory NVC mechanisms should be studied in awake mice and, though the relationship between interneuron activity and astrocytic Ca^{2+} is complex, there is a correlation between

astrocyte activity and NVC in



PV$_{IN}$.

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BOARD NUMBER: S06-583

AN ATLAS OF THE DEVELOPING POST-NATAL CEREBRAL VASCULATURE

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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The brain is densely perfused by the vascular network, which provides nutrients and oxygen to support neuronal function. The architecture of the cerebral vasculature addresses specific constrains of the neural tissues, including the near absence of energy storage and very high metabolic demand. Despite the clear observation that both the vascular density and the metabolic demands are heterogenous across the brain, whether and how neuronal activity controls the vascular topology is still debated for several reasons: first, there is no correlation between the densities of neurons and blood vessels. Second, the organization of the vascular and neuronal networks don’t match closely. Third, there is disagreement in the literature on whether modulating neuronal activity levels can lead to a remodeling of the vasculature or not. To better understand the relationship between the metabolic need of the different neural cell types and the topology of the adult vascular network, we built a 3D developmental atlas of the brain vasculature. For this, we generated the annotation maps and templates for the developing mouse brain to align vascular datasets onto. We next optimized a series of computational tools to measure and classify the organization of the different brain regions. We used these tools to generate developmental trajectories for the various brain regions and tested how a lack of neuronal activity affects their developmental. This work also revealed how the vascular network can cater differently to the metabolic needs of both the developing and adult brain.

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BOARD NUMBER: S06-584

IMAGERY EVALUATION OF CEREBROVASCULAR INFLAMMATION AND FUNCTIONAL CONSEQUENCES INDUCED BY SUBLETHAL DOSES OF SARIN SURROGATE: NON-INVASIVE IMAGING METHODS TO PREDICT LONG-TERM DEFICITS.

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Aims. Organophosphate (OP) compounds are highly toxic chemicals widely used as pesticides, but also as chemical warfare nerve agent (NA). The incidence of accidental poisoning with OP pesticides is associated with nearly 100,000 annual deaths worldwide for 2 million reported cases of intoxication. More worryingly, the use of neurotoxic weapons demonstrates the ever-persistent threat. NA exposure leads to the irreversible inhibition of cholinesterases, affecting both the peripheral and the central nervous system (CNS). In the CNS, if the cholinergic hyperactivity is not quickly stopped, it will lead to seizure and epileptic activity that will induce brain damages, neuronal degeneration, BBB dysfunction, cerebral edema and significant neuroinflammation. However, the effects of NA on the neurovascular system remain poorly understood. This work aimed to explore anatomical and functional neurovascular consequences of an exposure of a sarin surrogate (NIMP). **Methods**. For that purpose, MRI molecular imaging of the adhesion molecules (VCAM-1) was used to uncover neurovascular inflammation *in vivo* after 2 different sublethal doses of a sarin analog exposition (0.5LD50 and 0.9LD50). Functional consequences were measured on the somatosensory cortex using speckle contrast imaging. **Results**. We demonstrate that cerebrovascular inflammation was already perceptible 6 hours after exposure up to 7 days for the highest dose. Neurovascular coupling was altered up to 1 month after NIMP exposure whatever the dose as shown by speckle imaging. **Conclusions.** This method allows to detect neurovascular inflammation after mild OP exposure which would improve the diagnosis and management of patients to avoid long-term cerebral impact.

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BOARD NUMBER: S06-585

HUMAN IPSC-BASED MILLIFLUIDIC MODEL OF THE BBB/BRAIN AS PART OF THE MICROBIOTA-GUT-BRAIN AXIS MINERVA PLATFORM

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Aims The human brain is a complex machinery whose pathological perturbation leads to several disorders, including Alzheimer's disease (AD). Engineering brain structure and neural networks is challenging, also because we need to simulate fluid movements inside the parenchyma. In the frame of the MINERVA project that wants to reproduce in vitro the microbiota-gut-brain axis [http://www.minerva.polimi.it/] we developed a novel "brain-on-a-chip" using a fluidic model featuring the blood brain barrier (BBB) and main neural cell types. **Methods** We developed a millifluidic, optically accessible "on-a-chip" device that can host 2D or 3D hydrogel-embedded cells. To reproduce the BBB we cultured at opposite sides of a Transwell membrane human induced pluripotent stem cells (IPSCs) differentiated to astrocytes or endothelial cells. We tested the barrier integrity by analyzing transepithelial electric resistance (TEER) and FITC-dextrane permeability. IPSCs-derived neurons, microglia and astrocytes were also used to recapitulate brain parenchyma in a second on-a-chip device. **Results** Our millifluidic models of BBB and neural cells had comparable viability respect to static condition. The BBB device featured barrier properties and the whole system confirmed its suitability for the MINERVA aims. **Conclusions** IPSCs are a promising in vitro tool for disease modeling. Our millifluidic model can host iPSCs maintaining cell viability and BBB integrity. IPSCs derived from AD patients or genetically modified could be easily integrated to create a fluidic AD pathological model. **Acknowledgement** MINERVA project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement N° 724734).

Pubmed:
35017031: Boeri L, Donnaloja F, Campanile M, Sardelli L, Tunesi M, Fusco F, Giordano C, Albani D
Using integrated meta-omics to appreciate the role of the gut microbiota in epilepsy.
The way the human microbiota may modulate neurological pathologies is a fascinating matter of research. Epilepsy is a common neurological disorder, which has been largely investigated in correlation with microbiota health and function. However, the mechanisms that regulate this apparent connection are scarcely defined, and extensive effort has been conducted to understand the role of microbiota in preventing and reducing epileptic seizures. Intestinal bacteria seem to modulate the seizure frequency mainly by releasing neurotransmitters and inflammatory mediators. In order to elucidate the complex microbial contribution to epilepsy pathophysiology, integrated meta-omics could be pivotal. In fact, the combination of two or more meta-omics approaches allows a multifactorial study of microbial activity within the frame of disease or drug treatments. In this review, we provide information depicting and supporting the use of multi-omics to study the microbiota-epilepsy connection. We described different meta-omics analyses (metagenomics, metatranscriptomics, metaproteomics and metabolomics), focusing on current technical challenges in stool collection procedures, sample extraction methods and data processing. We further discussed the current advantages and limitations of using the integrative approach of multi-omics in epilepsy investigations.
Neurobiol Dis, 2022; 164
33990954: Sardelli L, Perottoni S, Tunesi M, Boeri L, Fusco F, Petrini P, Albani D, Giordano C
Technological tools and strategies for culturing human gut microbiota in engineered in vitro models.
The gut microbiota directly impacts the pathophysiology of different human body districts. Consequently, microbiota investigation is an hot topic of research and its in vitro culture has gained extreme interest in different fields. However, the high sensitivity of microbiota to external stimuli, such as sampling procedure, and the physicochemical complexity of the gut environment make its in vitro culture a challenging task. New engineered microfluidic gut-on-a-chip devices have the potential to model some important features of the intestinal structure, but they are usually unable to sustain culture of microbiota over an extended period of time. The integration of gut-on-a-chip devices with bioreactors for continuous bacterial culture would lead to fast advances in the study of microbiota-host crosstalk. In this review, we summarize the main technologies for the

continuous culture of microbiota as upstream systems to be coupled with microfluidic devices to study bacteria-host cells communication. The engineering of integrated microfluidic platforms, capable of sustaining both anaerobic and aerobic cultures, would be the starting point to unveil complex biological phenomena proper of the microbiota-host crosstalks, paving to way to multiple research and technological applications.
Biotechnol Bioeng, 2021; 118
33661580: Boeri L, Perottoni S, Izzo L, Giordano C, Albani D
Microbiota-Host Immunity Communication in Neurodegenerative Disorders: Bioengineering Challenges for In Vitro Modeling.
Human microbiota communicates with its host by secreting signaling metabolites, enzymes, or structural components. Its homeostasis strongly influences the modulation of human tissue barriers and immune system. Dysbiosis-induced peripheral immunity response can propagate bacterial and pro-inflammatory signals to the whole body, including the brain. This immune-mediated communication may contribute to several neurodegenerative disorders, as Alzheimer's disease. In fact, neurodegeneration is associated with dysbiosis and neuroinflammation. The interplay between the microbial communities and the brain is complex and bidirectional, and a great deal of interest is emerging to define the exact mechanisms. This review focuses on microbiota-immunity-central nervous system (CNS) communication and shows how gut and oral microbiota populations trigger immune cells, propagating inflammation from the periphery to the cerebral parenchyma, thus contributing to the onset and progression of neurodegeneration. Moreover, an overview of the technological challenges with in vitro modeling of the microbiota-immunity-CNS axis, offering interesting technological hints about the most advanced solutions and current technologies is provided.
Adv Healthc Mater, 2021; 10
33542304: Jacchetti E, Nasehi R, Boeri L, Parodi V, Negro A, Albani D, Osellame R, Cerullo G, Matas JFR, Raimondi MT
The nuclear import of the transcription factor MyoD is reduced in mesenchymal stem cells grown in a 3D micro-engineered niche.
Smart biomaterials are increasingly being used to control stem cell fate in vitro by the recapitulation of the native niche microenvironment. By integrating experimental measurements with numerical models, we show that in mesenchymal stem cells grown inside a 3D synthetic niche both nuclear transport of a myogenic factor and the passive nuclear diffusion of a smaller inert protein are reduced. Our results also suggest that cell morphology modulates nuclear proteins import through a partition of the nuclear envelope surface, which is a thin but extremely permeable annular portion in cells cultured on 2D substrates. Therefore, our results support the hypothesis that in stem cell differentiation, the nuclear import of gene-regulating transcription factors is controlled by a strain-dependent nuclear envelope permeability, probably related to the reorganization of stretch-activated nuclear pore complexes.
Sci Rep, 2021; 11
32222473: Tripathy D, Migazzi A, Costa F, Roncador A, Gatto P, Fusco F, Boeri L, Albani D, Juárez-Hernández JL, Musio C, Colombo L, Salmona M, Wilhelmus MMM, Drukarch B, Pennuto M, Basso M
Increased transcription of transglutaminase 1 mediates neuronal death in in vitro models of neuronal stress and Aβ1-42-mediated toxicity.
Alzheimer's disease (AD) is the most common cause of dementia. At the pre-symptomatic phase of the disease, the processing of the amyloid precursor protein (APP) produces toxic peptides, called amyloid-β 1-42 (Aβ 1-42). The downstream effects of Aβ 1-42 production are not completely uncovered. Here, we report the involvement of transglutaminase 1 (TG1) in in vitro AD models of neuronal toxicity. TG1 was increased at late stages of the disease in the hippocampus of a mouse model of AD and in primary cortical neurons undergoing stress. Silencing of TGM1 gene was sufficient to prevent Aβ-mediated neuronal death. Conversely, its overexpression enhanced cell death. TGM1 upregulation was mediated at the transcriptional level by an activator protein 1 (AP1) binding site that when mutated halted TGM1 promoter activation. These results indicate that TG1 acts downstream of Aβ-toxicity, and that its stress-dependent increase makes it suitable for pharmacological intervention.
Neurobiol Dis, 2020; 140
32092706: Boeri L, Jacchetti E, Soncini M, Negro A, Albani D, Raimondi MT
Advantages and limitations of a supernegative GFP in facilitating MyoD intracellular tracking.
Despite intracellular molecular dynamics being fundamental to understand pathological, biomechanical or biochemical events, several processes are still not clear because of the difficulty of monitoring and measuring these phenomena. To engineer an effective fluorescent tool useful to improve protein intracellular tracking studies, we fused a supernegative green fluorescent protein, (-30)GFP, to a myogenic transcription factor, MyoD. The (-30)GFP-MyoD was able to pass the plasma membrane when complexed with cationic lipids. Fluorescence confocal microscopy showed the protein delivery in just 3 hours with high levels of protein transduction efficiency. Confocal acquisitions also confirmed the maintenance of the MyoD nuclear localization. To examine how the supernegative GFP influenced MyoD activity, we did gene expression analyses, which showed an inhibitory effect of (-30)GFP on transcription factor function. This negative effect was possibly due to a

charge-driven interference mechanism, as suggested by further investigations by molecular dynamics simulations. Summarizing these results, despite the functional limitations related to the charge structural characteristics that specifically affected MyoD function, we found (-30)GFP is a suitable fluorescent label for improving protein intracellular tracking studies, such as nucleocytoplasmic transport in mechanotransduction.
Methods Appl Fluoresc, 2020; 8
31628607: Boeri L, Albani D, Raimondi MT, Jacchetti E
Mechanical regulation of nucleocytoplasmic translocation in mesenchymal stem cells: characterization and methods for investigation.
Mesenchymal stem cells (MSCs) have immune-modulatory and tissue-regenerative properties that make them a suitable and promising tool for cell-based therapy application. Since the bio-chemo-mechanical environment influences MSC fate and behavior, the understanding of the mechanosensors involved in the transduction of mechanical inputs into chemical signals could be pivotal. In this context, the nuclear pore complex is a molecular machinery that is believed to have a key role in force transmission and in nucleocytoplasmic shuttling regulation. To fully understand the nuclear pore complex role and the nucleocytoplasmic transport dynamics, recent advancements in fluorescence microscopy provided the possibility to study passive and facilitated nuclear transports also in mechanically stimulated cell culture conditions. Here, we review the current available methods for the investigation of nucleocytoplasmic shuttling, including photo-perturbation-based approaches, fluorescence correlation spectroscopy, and single-particle tracking techniques. For each method, we analyze the advantages, disadvantages, and technical limitations. Finally, we summarize the recent knowledge on mechanical regulation of nucleocytoplasmic translocation in MSC, the relevant progresses made so far, and the future perspectives in the field.
Biophys Rev, 2019; 11
31569428: Boeri L, Izzo L, Sardelli L, Tunesi M, Albani D, Giordano C
Advanced Organ-on-a-Chip Devices to Investigate Liver Multi-Organ Communication: Focus on Gut, Microbiota and Brain.
The liver is a key organ that can communicate with many other districts of the human body. In the last few decades, much interest has focused on the interaction between the liver and the gut microbiota, with their reciprocal influence on biosynthesis pathways and the integrity the intestinal epithelial barrier. Dysbiosis or liver disorders lead to0 epithelial barrier dysfunction, altering membrane permeability to toxins. Clinical and experimental evidence shows that the permeability hence the delivery of neurotoxins such as LPS, ammonia and salsolinol contribute to neurological disorders. These findings suggested multi-organ communication between the gut microbiota, the liver and the brain. With a view to in vitro modeling this liver-based multi-organ communication, we describe the latest advanced liver-on-a-chip devices and discuss the need for new organ-on-a-chip platforms for in vitro modeling the in vivo multi-organ connection pathways in physiological and pathological situations.
Bioengineering (Basel), 2019; 6
30868253: Izzo L, Tunesi M, Boeri L, Laganà M, Giordano C, Raimondi MT
Influence of the static magnetic field on cell response in a miniaturized optically accessible bioreactor for 3D cell culture.
Hydraulic sealing is a crucial condition for the maintenance of sterility during long term operation of microfluidic bioreactors. We developed a miniaturized optically accessible bioreactor (MOAB) allowing perfused culture of 3D cellularised constructs. In the MOAB, the culture chambers are sealed by magnets that generate a weak static magnetic field (SMF). Here, we predicted computationally the exact level of SMF to which cells are subjected during culture in the MOAB and we assessed its influence on the viability, metabolic activity and gene expression of neuroblastoma-derived cells cultured up to seven days. The predicted SMF ranged from 0.32 to 0.57 T using an axial-symmetric model of a single chamber, whereas it ranged from 0.35 to 0.62 T using a 3D model of the complete device. Cell function was evaluated in SH-SY5Y neuroblastoma cells at 2 and 7 days of culture in the MOAB, compared to 2D monolayer, 3D non-perfused constructs, and 3D perfused constructs cultured in a modified MOAB with magnet-free sealing. We measured the cell metabolic activity normalized by the DNA content and the expression levels of heat-shock protein 70 (Hsp-70), Bcl-2 and Bax. We found that the level of SMF applied to cells in the MOAB did not influence their metabolic activity and exerted a stressful effect in 2D monolayer, not confirmed in 3D conditions, neither static not perfused. Instead, the magnets provided a significantly greater hydraulic sealing in long-term culture, thus the MOAB might be potentially exploitable for the development of reliable in vitro models of neurodegeneration.
Biomed Microdevices, 2019; 21
30309378: Paracchini L, Beltrame L, Boeri L, Fusco F, Caffarra P, Marchini S, Albani D, Forloni G
Exome sequencing in an Italian family with Alzheimer's disease points to a role for seizure-related gene 6 (SEZ6) rare variant R615H.
The typical familial form of Alzheimer's disease (FAD) accounts for about 5% of total Alzheimer's disease (AD) cases. Presenilins (PSEN1 and PSEN2) and amyloid-β (A4) precursor protein (APP) genes carry all reported FAD-linked mutations. However, other genetic loci may be involved in AD. For instance, seizure-related gene 6 (SEZ6) has been reported in brain

development and psychiatric disorders and is differentially expressed in the cerebrospinal fluid of AD cases. Alzheimers Res Ther, 2018; 10

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BOARD NUMBER: S06-586

INVOLVEMENT OF MENINGEAL SENSORY NERVES IN THE PATHOMECHANISM OF SUBARACHNOID HEMORRHAGE

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Background Intracranial sensory nerves, mainly arise from trigeminal ganglion (TG) have a functional role on cerebrovascular tone due to the release of the vasodilator mediator calcitonin-gene related peptide (CGRP). An imbalance of vasoactive mechanisms may result in prolonged spasm. Cerebral vasospasm is a serious consequence of s*ubarachnoid hemorrhage* (SAH)- the bleeding between brain covering layers, the meninges. Diminished CGRP-content in cerebral nerves following SAH had been described. Our study aims to investigate the involvement of CGRP-containing meningeal afferents in the pathomechanism of SAH. *Methods* SAH was evoked by intracisternal injection of autologous blood in rats. 72 hours after the injection, the outermost meningeal layer, dura mater encephali and TG were removed. The density of CGRP-containing nerves was determined by immunohistochemical analysis in dural preparations. Retrograde tracer was used to identify trigeminal region innervating the dura and CGRP-immunopositive cells were counted. ATF3, as an injury marker was used to determine the damage of neurons. Furthermore, expression level of CGRP in TG was measured by RT-PCR 72 hours following SAH. *Results* Our results showed a decreased density of CGRP-positive meningeal afferents following SAH. However, the number of CGRP-immunopositive trigeminal neurons was retained with moderately increased expression of CGRP in TG. The number of ATF3-immunopositive neurons was not increased comparing to control. *Conclusion* Decreased CGRP-content of meningeal afferents supports that vasoactive sensory functions diminish after SAH. However, retained CGRP synthesis shows that not damage of primary sensory neurons is the reason of decreased amount of CGRP in sensory nerves intracranially.

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BOARD NUMBER: S06-587

HETEROGENEITY AND DEVELOPMENTAL DYNAMICS OF LYVE-1 PERIVASCULAR MACROPHAGES DISTRIBUTION IN THE MOUSE BRAIN

POSTER SESSION 06 - SECTION: CEREBROVASCULAR PHYSIOLOGY

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Brain perivascular macrophages (PVMs) are border-associated macrophages situated along blood vessels in the Virchow-Robin space and are thus found at a unique anatomical position between the endothelium and the parenchyma. Owing to their location and phagocytic capabilities, PVMs are regarded as important components that regulate various aspects of brain physiology in health and pathophysiological states. Here, we used LYVE-1 to identify PVMs in the mouse brain using used brain-tissue sections and cleared whole-brains to learn about how they are distributed within the brain and across different developmental postnatal stages. We find that LYVE-1$^+$PVMs associate with the vasculature in different patterns and proportions depending on vessel diameter or arterio-venous differentiation. LYVE-1$^+$PVMs relate to blood vessels in a brain-region-dependent manner. We show that their postnatal distribution is developmentally dynamic and peaks at P10-P20 depending on the brain region. PVMs express CD206 (another non-lymphatic marker of PVMs) and LYVE-1 at different ratio throughout the brain suggesting that PVMs possess a unique molecular signature. We further demonstrate that their density is reduced in the APP/PS1 mouse model of Alzheimer's Disease inversely proportional to beta-amyloid deposits. Interestingly, we found that PVMs conserve CD206 but not LYVE-1 expression in APP/PS1 compared to WT, thus losing their lymphatic and possibly drainage abilities. In conclusion, our results reveal unexpected heterogeneity and dynamics of LYVE-1$^+$PVMs, with selective coverage in brain vasculature, compatible with potential unexplored roles for this population of PVMs in postnatal development, and in regulating brain functions in steady-state and disease conditions.

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BOARD NUMBER: S06-588

T-TYPE CALCIUM CURRENT IN PKCγ NEURONS GATING CHRONIC PAIN IN THE DORSAL SPINAL CORD IN MICE

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Pain management with current analgesic drugs is often a failure: 20% of the population suffers from chronic pain. In the quest for alternative targets, our team has shown that low-threshold calcium channels (T-type channels), and in particular the Cav3.2 isoform, are targets of interest in the search for new pain medications (Candelas et al. 2019; François et al. 2015). The roles of these channels, in the somatosensory system may be involved in the development and maintenance of chronic pain. Genetic ablation of Cav3.2 in the dorsal horn of the spinal cord is sufficient to induce an anti-allodynic effect in mice. This putative therapeutic target, Cav3.2, is homogeneously expressed in excitatory interneurons expressing protein kinase C-γ (PKCγ) in the spinal dorsal horn. These PKCγ neurons play a key role in neuropathic pain, during which they transmit non-nociceptive excitation to nociceptive projection neurons. By combining electrophysiology, immunohistology, and clarification techniques, we were able to examine the effect of pharmacological and/or genetic inhibition of T-type channels selectively in PKCγ neurons or in the entire spinal network. Our results suggest multiple roles for Cav3.2 in PKCγ-expressing neurons. In a mouse model of neuropathic pain (spared nerve injury: SNI), we found time-dependent remodelling of synaptic currents in PKCγ neurons and genetic inactivation of Cav3.2 in PKCγ-expressing neurons does not always oppose its effect to that of SNI. Follow-up experiments will illustrate the role of other T-type channel isoforms (Cav3.1 and Cav3.3), using a viral/sh-RNA strategy targeted to PKCγ expressing neurons in sham and SNI mice.

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SPINAL CAV3.2 T-TYPE CHANNELS IMPACT ON NEUROPATHIC PAIN: TOWARD TRANSLATING RODENT FINDINGS TO HUMAN PATHOPHYSIOLOGY

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Cav3.2 T-type calcium channels are present throughout nociceptive pathways, from free endings of primary afferent neurons up to structures of the pain matrix. Previous studies focalized on primary afferent, have shown its important pronociceptive role in acute and pathological rodent pain models. At the first pit stop of somatosensory information processing in the dorsal horn of the spinal cord, Cav3.2 is abundant however; its role remains to be resolved. Thanks to local viral delivery of Cre recombinase in spinal cord of Cav3.2$^{GFP\text{-}flox}$ KI mice; we achieved a robust local Cav3.2 dorsal horn-specific KO. This spinal KO prophylactically and to a lesser extent curatively alleviates all the cardinal signs of neuropathic pain. Nonetheless, in a clinical perspective, we believe it is imperative to extend this approach to study human spinal cord biology. This will guide the priority and relevance of our future studies in mouse and serve as a foundation for understanding molecular changes that accompany human chronic pain. In that respect we developed, a unique research axis with the Montpellier hospital on human spinal and dorsal roots tissues collected from human donors during organ transplant procedures. The presentation will highlight these approaches including recent molecular and functional data obtained from human tissues.

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BOARD NUMBER: S06-590

THE HETEROGENEITY OF SYNAPTIC NMDA RECEPTOR RESPONSES WITHIN INDIVIDUAL LAMINA I PAIN PROCESSING NEURONS IS CONSERVED ACROSS SEX AND SPECIES

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Excitatory glutamatergic NMDA receptors (NMDARs) are key regulators of spinal pain processing, and yet the biophysical properties of NMDARs in dorsal horn nociceptive neurons remain poorly understood. Despite the clear clinical importance, it is unknown whether the molecular and functional properties of NMDAR synaptic responses are conserved between males and females as well as from rodents to humans. To address these translational gaps, we systematically compared individual and averaged excitatory synaptic responses from lamina I pain processing neurons of adult Sprague Dawley rats and human organ donors, including both sexes. By combining patch-clamp recordings of outward miniature excitatory postsynaptic currents with non-biased data analysis approaches, we uncovered a wide range in decay constants of excitatory synaptic events within individual lamina I neurons. Decay constants of quantal synaptic responses were distributed in a continuum from 1-20 ms to greater than 1000 ms, suggesting that individual lamina I neurons may contain AMPA receptor (AMPAR)-only as well as GluN2A-, GluN2B-, and GluN2D-dominated synaptic events. This intraneuronal heterogeneity in AMPA receptor- and NMDAR-mediated decay kinetics was conserved across both sex and species. The amplitude of synaptic responses was also conserved between event types and across sex for both rats and humans. The vast heterogeneity in decay rates of excitatory synaptic events within individual lamina I pain processing neurons may enable synapse-specific forms of plasticity and sensory integration within dorsal horn nociceptive networks.

Pubmed:
33871884: Harding EK, Dedek A, Bonin RP, Salter MW, Snutch TP, Hildebrand ME
The T-type calcium channel antagonist, Z944, reduces spinal excitability and pain hypersensitivity.
T-type voltage-gated calcium channels are an emerging therapeutic target for neurological disorders including epilepsy and pain. Inhibition of T-type channels reduces the excitability of peripheral nociceptive sensory neurons and reverses pain hypersensitivity in male rodent pain models. However, administration of peripherally restricted T-type antagonists failed to show efficacy in multiple clinical and preclinical pain trials, suggesting that inhibition of peripheral T-type channels alone may be insufficient for pain relief.
Br J Pharmacol, 2021; 178
31135041: Dedek A, Xu J, Kandegedara CM, Lorenzo LÉ, Godin AG, De Koninck Y, Lombroso PJ, Tsai EC, Hildebrand ME
Loss of STEP61 couples disinhibition to N-methyl-d-aspartate receptor potentiation in rodent and human spinal pain processing.
Dysregulated excitability within the spinal dorsal horn is a critical mediator of chronic pain. In the rodent nerve injury model of neuropathic pain, BDNF-mediated loss of inhibition (disinhibition) gates the potentiation of excitatory GluN2B N-methyl-d-aspartate receptor (NMDAR) responses at lamina I dorsal horn synapses. However, the centrality of this mechanism across pain states and species, as well as the molecular linker involved, remain unknown. Here, we show that KCC2-dependent disinhibition is coupled to increased GluN2B-mediated synaptic NMDAR responses in a rodent model of inflammatory pain, with an associated downregulation of the tyrosine phosphatase STEP61. The decreased activity of STEP61 is both necessary and sufficient to prime subsequent phosphorylation and potentiation of GluN2B NMDAR by BDNF at lamina I synapses. Blocking disinhibition reversed the downregulation of STEP61 as well as inflammation-mediated behavioural hypersensitivity. For the first time, we characterize GluN2B-mediated NMDAR responses at human lamina I synapses and show that a human ex vivo BDNF model of pathological pain processing downregulates KCC2 and STEP61 and upregulates phosphorylated GluN2B at dorsal horn synapses. Our results demonstrate that STEP61 is the molecular brake that is lost following KCC2-dependent disinhibition and that the decrease in STEP61 activity drives the potentiation of excitatory GluN2B NMDAR responses in rodent and human models of pathological pain. The ex vivo human BDNF model may thus form a

translational bridge between rodents and humans for identification and validation of novel molecular pain targets.
Brain, 2019; 142
27926876: Hildebrand ME, Xu J, Dedek A, Li Y, Sengar AS, Beggs S, Lombroso PJ, Salter MW
Potentiation of Synaptic GluN2B NMDAR Currents by Fyn Kinase Is Gated through BDNF-Mediated Disinhibition in Spinal Pain Processing.
In chronic pain states, the neurotrophin brain-derived neurotrophic factor (BDNF) transforms the output of lamina I spinal neurons by decreasing synaptic inhibition. Pain hypersensitivity also depends on N-methyl-D-aspartate receptors (NMDARs) and Src-family kinases, but the locus of NMDAR dysregulation remains unknown. Here, we show that NMDAR-mediated currents at lamina I synapses are potentiated in a peripheral nerve injury model of neuropathic pain. We find that BDNF mediates NMDAR potentiation through activation of TrkB and phosphorylation of the GluN2B subunit by the Src-family kinase Fyn. Surprisingly, we find that Cl-dependent disinhibition is necessary and sufficient to prime potentiation of synaptic NMDARs by BDNF. Thus, we propose that spinal pain amplification is mediated by a feedforward mechanism whereby loss of inhibition gates the increase in synaptic excitation within individual lamina I neurons. Given that neither disinhibition alone nor BDNF-TrkB signaling is sufficient to potentiate NMDARs, we have discovered a form of molecular coincidence detection in lamina I neurons.
Cell Rep, 2016; 17

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BOARD NUMBER: S06-591

INFLAMMATION TRIGGERS HOMEOSTATIC PROCESSES IN THE TRIGEMINAL PAIN PATHWAY

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Trigeminal pain pathway involves the detection of noxious stimulus by trigeminal ganglion (TG) nociceptors, which innervate orofacial areas and terminate in the trigeminal nucleus caudalis (TNc). TNc projection neurons (PNs) integrate nociceptive input from the periphery with local interneurons activity, and transmit the information to higher brain regions. Although the anatomy of the trigeminal pathway is well characterized, its physiological function is less understood. Here we ask how nociceptive input is encoded by TNc PNs, and how inflammation affects their properties and function and hence their output. We generated a mouse model, which enables the identification of TNc PNs as well as activation of TG nociceptors using optogenetics. Behaviorally, we show that optogenetic activation of TG nociceptive fibers evokes nocifensive response. Physiologically, we demonstrate that TNc PNs differ in their responses to optogenetic stimulation of the nociceptive axons, with two prominent responses: (1) excitation and (2) excitation followed by inhibition. We further characterized TNc PNs during inflammation using UV-keratitis model which results with corneal inflammation. TNc PNs in the inflammatory condition displayed similar distribution of responses to activation of the nociceptive axons. Surprisingly, in these conditions, PNs displayed reduced excitability, as manifested by a reduction in their input resistance and reduced firing response to current injections. Corneal inflammation in the UV-keratitis model is known to result with increased excitability of TG nociceptive fibers, implying increased activation of TNc PNs. We suggest that TNc PNs reduce their excitability as a regulatory mechanism to reduce their activity and maintain homeostasis during inflammation.

Pubmed:
33203851: Suliman-Lavie R, Title B, Cohen Y, Hamada N, Tal M, Tal N, Monderer-Rothkoff G, Gudmundsdottir B, Gudmundsson KO, Keller JR, Huang GJ, Nagata KI, Yarom Y, Shifman S
Pogz deficiency leads to transcription dysregulation and impaired cerebellar activity underlying autism-like behavior in mice. Several genes implicated in autism spectrum disorder (ASD) are chromatin regulators, including POGZ. The cellular and molecular mechanisms leading to ASD impaired social and cognitive behavior are unclear. Animal models are crucial for studying the effects of mutations on brain function and behavior as well as unveiling the underlying mechanisms. Here, we generate a brain specific conditional knockout mouse model deficient for Pogz, an ASD risk gene. We demonstrate that Pogz deficient mice show microcephaly, growth impairment, increased sociability, learning and motor deficits, mimicking several of the human symptoms. At the molecular level, luciferase reporter assay indicates that POGZ is a negative regulator of transcription. In accordance, in Pogz deficient mice we find a significant upregulation of gene expression, most notably in the cerebellum. Gene set enrichment analysis revealed that the transcriptional changes encompass genes and pathways disrupted in ASD, including neurogenesis and synaptic processes, underlying the observed behavioral phenotype in mice. Physiologically, Pogz deficiency is associated with a reduction in the firing frequency of simple and complex spikes and an increase in amplitude of the inhibitory synaptic input in cerebellar Purkinje cells. Our findings support a mechanism linking heterochromatin dysregulation to cerebellar circuit dysfunction and behavioral abnormalities in ASD.
Nat Commun, 2020; 11
26445872: Libster AM, Title B, Yarom Y
Corticotropin-releasing factor increases Purkinje neuron excitability by modulating sodium, potassium, and Ih currents. Corticotropin-releasing factor (CRF) is a neuromodulator closely associated with stress responses. It is synthesized and released in the central nervous system by various neurons, including neurons of the inferior olive. The targets of inferior olivary neurons, the cerebellar Purkinje neurons (PNs), are endowed with CRF receptors. CRF increases the excitability of PNs in vivo, but the biophysical mechanism is not clear. Here we examine the effect of CRF on the firing properties of PNs using acute rat cerebellar slices. CRF increased the PN firing rate, regardless of whether they were firing tonically or switching between firing and quiescent periods. Current- and voltage-clamp experiments showed that the increase in firing rate was associated with a voltage shift of the activation curve of the persistent sodium current and hyperpolarizing-activated current, as well as activation of voltage-dependent potassium current. The multiple effects on various ionic currents, which

are in agreement with the possibility that activation of CRF receptors triggers several intracellular pathways, are manifested as an increase excitability of PN.
J Neurophysiol, 2015; 114

30933959: Barkai O, Puig S, Lev S, Title B, Katz B, Eli-Berchoer L, Gutstein HB, Binshtok AM
Platelet-derived growth factor activates nociceptive neurons by inhibiting M-current and contributes to inflammatory pain.
Endogenous inflammatory mediators contribute to the pathogenesis of pain by acting on nociceptors, specialized sensory neurons that detect noxious stimuli. Here, we describe a new factor mediating inflammatory pain. We show that platelet-derived growth factor (PDGF)-BB applied in vitro causes repetitive firing of dissociated nociceptor-like rat dorsal root ganglion neurons and decreased their threshold for action potential generation. Injection of PDGF-BB into the paw produced nocifensive behavior in rats and led to thermal and mechanical pain hypersensitivity. We further detailed the biophysical mechanisms of these PDGF-BB effects and show that PDGF receptor-induced inhibition of nociceptive M-current underlies PDGF-BB-mediated nociceptive hyperexcitability. Moreover, in vivo sequestration of PDGF or inhibition of the PDGF receptor attenuates acute formalin-induced inflammatory pain. Our discovery of a new pain-facilitating proinflammatory mediator, which by inhibiting M-current activates nociceptive neurons and thus contributes to inflammatory pain, improves our understanding of inflammatory pain pathophysiology and may have important clinical implications for pain treatment.
Pain, 2019; 160

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BOARD NUMBER: S06-592

CIRCADIAN REGULATION OF TRIGEMINAL PAIN CIRCUITS

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Circadian characteristics of pain have been described in humans and animal models, but the underlying mechanisms remain unclear. Preliminary data from our group suggest that trigeminal nociceptor activity and orofacial pain behaviour vary depending on the time of day. Patch clamp recordings from acute trigeminal nucleus caudalis (TNC) slices revealed differences in excitatory drive between two time points assessed, with increased spontaneous input to layer I TNC neurons at the time point corresponding to decreased pain behaviour. At this time point, activation of primary nociceptors using the TRPV1 agonist capsaicin did not affect the frequency of sEPSCs in presence of the $GABA_A$ blocker picrotoxin. Surprisingly, in absence of synaptic blockers, a third of neurons at this time point showed reduced sEPSC rates when capsaicin was applied. Furthermore, sIPSC rate was modulated by capsaicin depending on the time of day. Global knockout of the clock genes *Cry1* and *Cry2*, and sensory neuron-specific ablation of *Bmal1* abolished the variation of synaptic currents between time points. Collectively, the data supports the role of circadian mechanisms in intraspinal modulation of trigeminal nociceptive traffic.

Pubmed:
31166606: Brickley S, Zirpel F
Developing more effective seizure therapies requires more selective drugs.
J Physiol, 2019; 597
35088415: Bampali K, Koniuszewski F, Silva LL, Rehman S, Vogel FD, Seidel T, Scholze P, Zirpel F, Garon A, Langer T, Willeit M, Ernst M
Tricyclic antipsychotics and antidepressants can inhibit α5-containing GABA receptors by two distinct mechanisms.
Many psychotherapeutic drugs, including clozapine, display polypharmacology and act on GABA receptors. Patients with schizophrenia show alterations in function, structure and molecular composition of the hippocampus, and a recent study demonstrated aberrant levels of hippocampal α5 subunit-containing GABA receptors. The purpose of this study is to investigate the effects of tricyclic compounds on α5 subunit-containing receptor subtypes.
Br J Pharmacol, 2022;

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ULTRASOUND LOCALIZATION MICROSCOPY AND FUNCTIONAL ULTRASOUND IMAGING REVEAL ATYPICAL FEATURES OF THE TRIGEMINAL GANGLION VASCULATURE

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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The trigeminal ganglion (TG) contains clusters of cell bodies of *highly specialized sensory neurons* innervating the ophthalmic, maxillary and the mandibular orofacial regions. The functional imaging within the TG is highly challenging due to anatomical features: small size and deep localization. Aims: This study aimed at imaging of both TG blood vasculature at microscopic resolution and hemodynamic responses evoked by orofacial stimulations in anesthetized rats using functional ultrasound imaging (fUSI). Methods: fUSI of TG was done in anesthetized rats. Peripheral stimulations included mechanical (soft brush) and chemical (capsaisin) corneal stimulations and mechanical stimulations of the ophthalmic and maxillary territories using von Frey filaments. Results: Despite the small number of sensory neurons within the TG, fUSI was able to quantify highly localized hemodynamic response in the ipsilateral TG, constituting the first demonstration of functional somatotopy**.** The *in vivo* quantitative imaging of the TG's vasculature using Ultrafast Localisation Microscopy revealed particular features of the vascularization (strong density and high speed of blood flow) of the area containing the sensory neurons, that are likely the origin of this strong vaso-trigeminal response. Conclusions: This innovative imaging approach opens the path for future studies on the mechanisms underlying changes in trigeminal local blood flow and evoked hemodynamic responses, key mechanisms and readouts for the understanding and treatment of debilitating trigeminal pain conditions.

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ROLE OF HYPERPOLARIZATION ACTIVATED CURRENTS IN THE RESONANCE BEHAVIOR OF SUPERFICIAL DORSAL HORN NEURONS

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Rhythmic activity is essential for nervous system function. The ability of individual neurons to integrate and encode information from inputs in defined frequency bands depends on their intrinsic resonant properties. The aims of this work were to examine the subthreshold resonance in superficial dorsal horn neurons (sDHN) and its relation to hyperpolarization activated currents (Ih). Whole-cell recordings were obtained from sDHN using in vitro horizontal spinal cord slices from adult mice. Sinusoidal current injection was applied to analyze resonance behavior. Voltage-clamp recordings were obtained from the same neurons to study the presence of Ih and their characteristics. Computer-modelled Ih was injected in real neurons using dynamic-clamp to further assess the influence of this current in resonance. Fifteen percent of the recorded neurons presented a clear resonance peak at a frequency between 1-3 Hz, most of these neurons showed Ih. In each individual neuron, the magnitude of the resonance peak was related to the density of Ih. H currents with different kinetic properties were recorded in sDHN, and therefore slow and fast Ih currents were modelled. In dynamic clamp experiments, injection of both types of currents reproduced the resonance behavior observed in neurons that expressed real Ih. Our results demonstrate that the presence of Ih produced neuronal resonance in sDHN. This behavior may be implicated in somatosensory processing at the spinal cord level, and the modulation of these currents may serve as a regulatory mechanism for neuronal integration. Financial support was obtained from the University of Alcala, grant no. PIUAH21/CCS-039

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DUAL PI3Kδ/γ INHIBITOR DUVELISIB PREVENTS DEVELOPMENT OF CHEMOTHERAPY INDUCED NEUROPATHIC PAIN

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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The development of painful paclitaxel-induced peripheral neuropathy (PIPN) represents a major dose-limiting side effect of paclitaxel chemotherapy. Our experiments show a promising effect of duvelisib (Copiktra™), a novel FDA approved PI3Kδ/γ isoform-specific inhibitor, in preventing increased mechanical sensitivity and pronociceptive signalling in dorsal root ganglia (DRG) and spinal cord dorsal horn (SCDH) in rat and mouse model of PIPN. Duvelisib blocked the development of mechanical hyperalgesia in both males and females. Duvelisib also prevented paclitaxel-induced sensitization of TRPV1 receptors, increased PI3K/Akt-signalling in small-diameter DRG neurons and an increase of $CD68^{+}$ cells within DRGs. Specific optogenetic stimulation of inhibitory neurons combined with patch-clamp recording revealed that duvelisib prevented paclitaxel-induced reduction of inhibitory currents in SCDH excitatory neurons. Enhanced excitatory and reduced inhibitory neurotransmission in the SCDH following PIPN was also alleviated by duvelisib application. In summary, duvelisib showed a promising ability to prevent neuropathic pain in PIPN. The potential use of our findings in human medicine may be augmented by the fact that duvelisib is an FDA approved drug with known side effects. This work was supported by the Grant Agency of the Czech Republic GACR 20-19136S.

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BOARD NUMBER: S06-596

TAFA4 RELIEVES INJURY-INDUCED MECHANICAL ALLODYNIA THROUGH LRP1 AND MODULATION OF SPINAL A-TYPE POTASSIUM CURRENTS

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Pain, whether acute or persistent, is a serious medical problem worldwide. However, its management remains unsatisfactory, and new analgesic molecules are therefore required. We show here that TAFA4 reverses inflammatory, postoperative and spared nerve injury (SNI)-induced mechanical hypersensitivity in male and female mice. TAFA4-mediated analgesia requires functional LRP1, as LRP1 inhibition *in vivo* abolished its analgesic effect. SNI induces a selective decrease in I_A in outer lamina II excitatory interneurons (L-IIo-ExIN), accompanied by a concomitant increase in I_A and decrease in I_h in inner lamina II inhibitory interneurons (L-IIi-InhIN). Remarkably, TAFA4 rescued these SNI-induced current alterations in an LRP1-dependent manner in both interneuron subtypes. Finally, SNI decreased the density of TAFA4$^+$ terminals in the spinal region, as shown by high levels of microglial activation. Our findings provide mechanistic insight into the mechanism by which TAFA4 reverses injury-induced mechanical hypersensitivity, by restoring normal spinal neuron activity, and highlight the considerable potential of TAFA4 as a treatment for pain.

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SEX DIFFERENCES IN CHLORIDE HOMEOSTASIS OF C-FIBER PRIMARY AFFERENTS IN THE SPINAL CORD DORSAL HORN.

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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[1]Université Laval, Faculty Of Medicine, Québec, Canada, [2]CERVO Brain Research Centre, Department Of Psychiatry And Neuroscience, Quebec City, Canada

Introduction: Intracellular Cl^- concentration ($[Cl^-]i$) is high in primary sensory neurons due to the activity of the Na^+-K^+-Cl^- cotransporter 1 (NKCC1), causing greater Cl^- accumulation than typically seen in CNS neurons. Consequently, central terminals of primary afferents in the spinal dorsal horn experience depolarization upon activation of $GABA_A$ receptors ($GABA_AR$). Thus, regulation of $[Cl^-]_i$ in these terminals may significantly affect transmitter release. Determining the exact $[Cl^-]_i$ in C-fiber terminals is pivotal to understand sensory processing. Methods: To image $[Cl^-]_i$ we used the genetically-encoded ratiometric Cl^- sensor, superclomeleon, which was virally transduced selectively in C-fibers in NaV1.8-cre male and female mice. We did 2-photon microscopy in acute spinal cord slices and on the acutely delaminated spinal cord in anesthetized mice. The $GABA_AR$ agonist and antagonist muscimol and bicuculline, as well as the NKCC1 antagonist bumetanide, were used to modulate $[Cl^-]_i$ in afferent terminals in the dorsal horn. NKCC1 protein and mRNA levels in the dorsal root ganglia were evaluated with western blot and RNAScope. Results: We found that $[Cl^-]_i$ in C-fibers was significantly higher in males than females. Bumetanide significantly decreased $[Cl^-]_i$ in males but not in females. Bicuculline increased significantly $[Cl^-]_i$ in females but not males C-fibers indicating a more important contribution of tonic $GABA_A$ signaling to $[Cl^-]_i$ in females. NKCC1 protein and mRNA were also significantly lower in females than males, consistent with the functional data. Conclusion: Presynaptic inhibition appears to be under distinct control by GABAergic inhibition between sexes, which should be taken into consideration in future studies.

Pubmed:

30009812: Khalilzadeh E, Azarpey F, Hazrati R, Vafaei Saiah G
Evaluation of different classes of histamine H and H receptor antagonist effects on neuropathic nociceptive behavior following tibial nerve transection in rats.
It seems that histamine release in the site of neuronal injury could contribute to the neuropathic pain mechanism. In the present study, we investigated the anti-allodynic effects of chronic administration of different classes of histamine H and H receptor antagonists on neuropathic nociceptive behavior following tibial nerve transection (TNT) in rats. Peripheral neuropathy was induced by TNT surgery. We performed acetone tests (AT) to record cold allodynia, Von Frey tests (VFT) to measure mechanical allodynia, double plate test (DPT) to evaluate thermal place preference/avoidance and open field test (OFT) for evaluation of animal activity. TNT rats showed a significant mechanical and cold allodynia compared to the sham group. Chlorpheniramine (5 and 15 mg/kg, i.p) significantly attenuated cold allodynia and prevented cold plate avoidance behavior and at the dose of 15 mg/kg remarkably decreased mechanical allodynia. Fexofenadine (10 and 30 mg/kg, p.o) significantly attenuated the mechanical allodynia and prevented cold plate avoidance. Ranitidine (5 and 15 mg/kg, i.p) significantly prevented cold plate avoidance behavior and at the dose of 15 mg/kg notably improved mechanical and cold allodynia. Famotidine (1 and 3 mg/kg, p.o) was ineffective on all nociceptive tests. Gabapantin (100 mg/kg, p.o) significantly improved all types of nociceptive behaviors. These results indicate that both blood brain barrier penetrating (chlorpheniramine) and poorly penetrating (fexofenadine) histamine H receptor antagonists could improve the neuropathic pain sign, but only the blood brain barrier penetrating histamine H receptor antagonist (ranitidine) could produce anti-allodynic effects in the TNT model of neuropathic pain in rats.
Eur J Pharmacol, 2018; 834
27651809: Khalilzadeh E, Hazrati R, Saiah GV
Effects of topical and systemic administration of Eugenia caryophyllata buds essential oil on corneal anesthesia and analgesia.
Clinical studies suggest that essential oil of Eugenia caryophyllata (Clove) buds (EOEC) is efficacious in the treatment of dental pain. In the present study, we investigated the analgesic and local anesthetic effects of EOEC and its possible

mechanisms of action in acute corneal pain in rats. EOEC was extracted by hydro-distillation in a Clevenger type apparatus from clove buds. The acute corneal pain was induced by applying a drop (40 µl) of 5 M NaCl solution on the corneal surface, and the numbers of eye wipes were counted during the first 30 s. The mechanical sensation of the cornea was evaluated by calibrated Von Frey filaments. Systemic administration of EOEC (100 and 200 mg/kg, SC) and morphine (2.5 and 5 mg/kg, IP) produced a significant antinociceptive effect in acute corneal pain. Pretreatment with naloxone or atropine prevented the EOEC-induced analgesia. However, L-arginine and methylene blue did not change the suppressive effect of EOEC on corneal pain response. Topical application of EOEC, eugenol and lidocaine significantly decreased corneal sensitivity. Combination treatments of eugenol (25 µg) with lidocaine (0.5%) and EOEC (50 µg) with lidocaine (0.5%) also significantly suppressed corneal sensitivity. Systemic administration of EOEC produced analgesia in the acute corneal pain through mechanisms that involved both opioidergic and cholinergic systems. In addition, topical instillation of EOEC, eugenol, and lidocaine produced local anesthesia in the rat cornea. Sub-anesthetic doses of EOEC or eugenol produced a significant local anesthetic effect when concurrently used with the sub-anesthetic dose of lidocaine.
Res Pharm Sci, 2016; 11

FENS Forum 2022
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BOARD NUMBER: S06-598

ANALYSIS OF EFFECTIVE CONNECTIVITY BETWEEN DORSAL HORN NUERONS AND PRIMARY AFFERENTS FROM ADULT MICE

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Aim. Excitability of central terminals from primary afferents is modulated by spinal interneurons located in the superficial dorsal horn. Our aim was to identify and characterise these neurons. **Methods**. We used horizontal slices of the lumbar spinal cord from adult mice maintained *in vitro*. Slices were 500 µm thick and kept superficial laminae and dorsal roots. Electrode matrixes were used to record spontaneous activity from 5-20 dorsal horn neurons simultaneously. Recordings from primary afferents were obtained with micro-suction electrodes from teased dorsal rootlets. Effective connectivity analysis was performed with an artificial intelligence tool built in house adapting the C5.0 algorithm. The Mathews Correlation Coefficient (MCC) was used as a single metric to estimate effective connectivity. **Results**. Successful recordings were obtained from 10 primary afferents and 50 dorsal horn neurons. High MCC values were obtained for 7 neurons which were considered putative presynaptic neurons. All of them exhibited irregular patterns of spontaneous activity. Five neurons produced characteristic short- high frequency bursts of activity at irregular intervals. In one case, two neurons with high MCC were related to the same afferent. In another experiment, one single neuron had high MCC values related to the 4 afferents recorded. For 2 afferents it was not possible to find neurons with relevant MCC values. **Conclusions**. In the superficial dorsal horn, putative presynaptic neurons to primary afferents have a characteristic pattern of spontaneous activity consisting of brisk bursts of activity at irregular intervals. Financial support from the University of Alcala grant no. PIUAH21/CCS-039

Pubmed:

29950700: Lucas-Romero J, Rivera-Arconada I, Roza C, Lopez-Garcia JA
Origin and classification of spontaneous discharges in mouse superficial dorsal horn neurons.
Superficial laminae of the spinal cord possess a considerable number of neurons with spontaneous activity as reported in vivo and in vitro preparations of several species. Such neurons may play a role in the development of the nociceptive system and/or in the spinal coding of somatosensory signals. We have used electrophysiological techniques in a horizontal spinal cord slice preparation from adult mice to investigate how this activity is generated and what are the main patterns of activity that can be found. The results show the existence of neurons that fire regularly and irregularly. Within each of these main types, it was possible to distinguish patterns of spontaneous activity formed by single action potentials and different types of bursts according to intra-burst firing frequency. Activity in neurons with irregular patterns was blocked by a mixture of antagonists of the main neurotransmitter receptors present in the cord. Approximately 82% of neurons with a regular firing pattern were insensitive to synaptic antagonists but their activity was inhibited by specific ion channel blockers. It is suggested that these neurons generate endogenous activity due to the functional expression of hyperpolarisation-activated and persistent sodium currents driving the activity of irregular neurons.
Sci Rep, 2018; 8
33796015: Pozo-Jimenez P, Lucas-Romero J, Lopez-Garcia JA
Discovering Effective Connectivity in Neural Circuits: Analysis Based on Machine Learning Methodology.
As multielectrode array technology increases in popularity, accessible analytical tools become necessary. Simultaneous recordings from multiple neurons may produce huge amounts of information. Traditional tools based on classical statistics are either insufficient to analyze multiple spike trains or sophisticated and expensive in computing terms. In this communication, we put to the test the idea that AI algorithms may be useful to gather information about the effective connectivity of neurons in local nuclei at a relatively low computing cost. To this end, we decided to explore the capacity of the algorithm C5.0 to retrieve information from a large series of spike trains obtained from a simulated neuronal circuit with a known structure. Combinatory, iterative and recursive processes using C5.0 were built to examine possibilities of increasing the performance of a direct application of the algorithm. Furthermore, we tested the applicability of these processes to a reduced dataset obtained from original biological recordings with unknown connectivity. This was obtained in house from a mouse preparation of the spinal cord. Results show that this algorithm can retrieve neurons monosynaptically connected to the target in

simulated datasets within a single run. Iterative and recursive processes can identify monosynaptic neurons and disynaptic neurons under favorable conditions. Application of these processes to the biological dataset gives clues to identify neurons monosynaptically connected to the target. We conclude that the work presented provides substantial proof of concept for the potential use of AI algorithms to the study of effective connectivity.
Front Neuroinform, 2021; 15

FENS Forum 2022
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BOARD NUMBER: S06-599

ROLE OF GABA LEVELS IN MODULATION OF INJURY-INDUCED PAIN

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Increasing evidence suggests that loss of spinal inhibition (disinhibition) is believed to underlie several forms of chronic pain (Braz et al., 2014; Sandkuhler, 2009; Yaksh, 1989; Zeilhofer et al., 2012). One of the most contested hypotheses proposes that disinhibition is due to loss of a subset of inhibitory interneurons and/or a reduction in γ-aminobutyric acid (GABA) synthesis and release (Castro-Lopes et al., 1993; Hughes and Todd, 2020; Moore et al., 2002; Petitjean et al., 2015; Polgar et al., 2005; Polgar and Todd, 2008). Here, we provide new evidence supporting the importance of spinal GABA contents in modulation of injury-induced mechanical pain. We showed that *Gad1* haplodeficiency, the main GABA synthetizing enzyme, causes a significant decrease of synaptic GABA in both sexes, and that this decrease was more pronounced in female mice. In line with this, GAD haplodeficiency caused an exacerbated formalin-evoked pain selectively in female mice, and both sexes exhibited a prolonged injury-induced mechanical hypersensitivity in both sexes in three different pain models: the Zymosan A as a model of inflammatory pain, the Brennan test as a model of postoperative pain, and the CCI model of neuropathic pain. Our data provide new elements supporting the importance of GABA levels in modulation of injury-induced pain.

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BOARD NUMBER: S06-600

DOWNREGULATION OF PARVALBUMIN PROTEIN IN DORSAL HORN INTERNEURONS ELICITS MECHANICAL PAIN HYPERSENSITIVITY.

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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The nervous system processes sensory information through the precise coordination of neuronal networks and their synaptic firing patterns. In the spinal cord, the segregation of touch and pain inputs is mediated in part by the output of a subset of tonic firing inhibitory neurons (PVIN) that express the parvalbumin protein. The parvalbumin protein (PVp) is a calcium (Ca2+)-binding protein that buffers the accumulation of calcium following a train of action potential to allow for tonic firing. Here, using Western blot, qPCR, and immunohistochemistry, we find that nerve injury causes a decrease in PVp expression in PVINs and decreased their inhibitory output, ultimately resulting in mechanical allodynia, where innocuous inputs produce a painful response. We also examined the causality of this observation by reducing PVp in otherwise healthy animals and observe that PVIN lose their ability to fire tonically and the mice develop mechanical allodynia. To directly relate the firing activity of PVIN to mechanical allodynia, we produced a virus consisting of an inhibitory pharmacogenetic tool (hM4D) and an excitatory opsin (oChIEF) inserted in a Cre/FlpO-dependent manner and injected it in in *PV::cre;FLP-tdTomato* mice. Mice were implanted with an optic fiber to deliver pulses of light on the lumbar segment of the spinal cord. The injection of CNO to silence PVIN resulted in mechanical allodynia. However, simultaneous photo-activation of PVIN caused an alleviation of mechanical hypersensitivity. Our results indicate an essential role for PVp-mediated calcium buffering and precise PVIN firing frequency modulation in the development of mechanical allodynia after nerve injury.

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BOARD NUMBER: S06-601

SPINAL CODING OF THERMONOCICPETION IN ADULT WISTAR RAT

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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In the nervous system, specialized sensory neurons (i.e nociceptors) are able to detect internal and external stimuli of various nature and intensity, such as mecanical deformation, heat, cold or irritation caused by chemicals. Peripheral nociceptors make synapses with secondary nuyerons or interneurons of the dorsal horn of the spinal cord where nociceptives information are first integrated then send to the suprapsinal centers centers of pain. Nociceptive informations are mainfly integrated in the superficial layers of the dorsal horn while deeper layers tend to receive a convergence of both nociceptive and non nociceptive informations. It is generally considered that hot nociceptive informations are carried by unmyalinated C fibers while cold informations are perceived by thinly myelinated Aδ fibers. However, how these informations are integrated whithin the spinal cord is still unclear. This study aims to further caracterize the spinal integration of thermal nociceptive inforamtion, specifically by the wide dynamic range neurons of the deep layers that received convergent informations from the periphery. Using a novel peltier thermode allowing a wide range of stimulation in various temprature and duration, we caracterized the response of spinal wide dynamic range neurons after applying various innocuous and noxious thermal stimuli.

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BOARD NUMBER: S06-602

MIF INHIBITOR (ISO-1) REDUCES PAIN HYPERSENSITIVITY IN A MODEL OF PERIPHERAL NEUROPATHY

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Fyziologický ústav AV ČR, Laboratory Of Pain Research, Prague, Czech Republic

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine, and its genetic deletion prevents the development of hypersensitivity induced by peripheral nerve injury or inflammation. MIF exerts its biological functions mainly through binding to the putative membrane receptor, CD74. MIF tautomerase inhibitor (S,R)-3(4-hydroxyphenyl)- 4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) binds to the MIF active site and blocks the MIF-CD74 binding. Our aim was to examine whether systemic treatment with ISO-1 alleviates the hypersensitivity induced by peripheral neuropathy and modulates synaptic transmission at the spinal cord level. Peripheral neuropathy was induced by chronic constriction injury (CCI) of the sciatic nerve. Electronic Von Frey test to assess mechanical sensitivity and patch-clamp recordings of excitatory and inhibitory postsynaptic currents (EPSCs, IPSCs) from superficial dorsal horn neurons in mouse spinal cord slices were used. Inhibitory currents were evoked by optical stimulation of channelrhodopsin expressing inhibitory neurons. Mechanical allodynia induced by CCI was prevented with the ISO-1 treatment. CCI also significantly increased the spontaneous EPSCs frequency and decreased the amplitude of the light-evoked IPSCs in excitatory neurons. The systemic ISO-1 treatment largely diminished these pathological effects on nociceptive synaptic transmission induced by the CCI. Our results suggest that ISO-1 treatment reduces the development of hypersensitivity after nerve injury. One of the underlying mechanisms of the ISO-1 effect could be balancing the CCI-induced changes of excitatory and inhibitory synaptic transmission in the spinal cord dorsal horn.

Pubmed:
35042769: Adamek P, Heles M, Bhattacharyya A, Pontearso M, Slepicka J, Palecek J
Dual PI3Kδ/γ Inhibitor Duvelisib Prevents Development of Neuropathic Pain in Model of Paclitaxel-Induced Peripheral Neuropathy.
The development of painful paclitaxel-induced peripheral neuropathy (PIPN) represents a major dose-limiting side effect of paclitaxel chemotherapy. Here we report a promising effect of duvelisib (Copiktra), a novel FDA-approved PI3Kδ/γ isoform-specific inhibitor, in preventing paclitaxel-induced pain-like behavior and pronociceptive signaling in DRGs and spinal cord dorsal horn (SCDH) in rat and mouse model of PIPN. Duvelisib blocked the development of mechanical hyperalgesia in both males and females. Moreover, duvelisib prevented paclitaxel-induced sensitization of TRPV1 receptors, and increased PI3K/Akt signaling in small-diameter DRG neurons and an increase of CD68 cells within DRGs. Specific optogenetic stimulation of inhibitory neurons combined with patch-clamp recording revealed that duvelisib inhibited paclitaxel-induced weakening of inhibitory, mainly glycinergic control on SCDH excitatory neurons. Enhanced excitatory and reduced inhibitory neurotransmission in the SCDH following PIPN was also alleviated by duvelisib application. In summary, duvelisib showed a promising ability to prevent neuropathic pain in PIPN. The potential use of our findings in human medicine may be augmented by the fact that duvelisib is an FDA-approved drug with known side effects. We show that duvelisib, a novel FDA-approved PI3Kδ/γ isoform-specific inhibitor, prevents the development of paclitaxel-induced pain-like behavior in males and females and prevents pronociceptive signaling in DRGs and spinal cord dorsal horn in rat and mouse model of paclitaxel-induced peripheral neuropathy.
J Neurosci, 2022; 42

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BOARD NUMBER: S06-603

PRESYNAPTIC GABAERGIC INHIBITION CONTRIBUTES TO PUNCTATE ALLODYNIA CIRCUITS ACTIVATION IN INFLAMMATORY PAIN

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Aims: Mechanical allodynia occurs in distinct forms in inflammatory pain, including dynamic and punctate mechanical allodynia. Our previous study indicates presynaptic GABAergic inhibition switches to excitation in nociceptors under inflammatory pain conditions, but contribution of presynaptic inhibition to these distinct mechanical allodynia forms is not clear. Methods: To investigate the role of presynaptic inhibition in these circuits, we generated mice conditionally lacking GABAA receptors in DRG nociceptor (SNS-GABAAβ3-/-). We used an IEG-based dual epoch method to simultaneously visualize the population correlates and the circuitry involved in these two contrasting types of mechanical allodynia in the spinal cord dorsal horn at a single-cell resolution. Results: In conditions of inflammatory pain induced by paw inflammation, SNS-GABAAβ3-/- mice did not develop punctate mechanical allodynia, while dynamic mechanical allodynia was still preserved. We observed that punctate and dynamic mechanical stimuli shape distinct activation patterns of intermingled neurons in the spinal cord dorsal horn under inflammatory pain conditions. Only the neuronal population activated by punctate mechanical stimuli was significantly reduced in SNS-GABAAβ3-/- mice as compared to control littermates. Conclusions: Collectively with our previous studies, these results support the hypothesis that presynaptic GABAergic inhibition switches to excitation in nociceptors under inflammatory pain conditions, and particularly modulates punctate mechanical allodynia. Moreover, this study is amongst the first to visualize the circuit population of these two distinct forms of mechanical allodynia and demonstrates the distinct, but intermingled, patterns shared between them.

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BOARD NUMBER: S06-604

INTEGRATIVE ANALYSIS OF DESCENDING PAIN MODULATION PATHWAYS

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Persistent mechanical pain caused by inflammation or nerve injury is a debilitating clinical problem. The spinal cord-projecting neurons in the rostral ventromedial medulla (RVM^{SC}neurons) play active roles in pain facilitation. However, the underlying circuitry and molecular mechanisms remain largely unknown. Here we show that acute activation of $OPRM1^{+}RVM^{SC}$neurons does not facilitate pain in normal mice, but activity of these neurons is required for both initiation and maintenance of mechanical hypersensitivity in mouse models of inflammatory and neuropathic pain. Surprisingly, we find that the lateral superior colliculus rather than the traditionally assumed periaqueductal gray, provides the excitatory input onto the $OPRM1^{+}RVM^{SC}$ neurons that drives mechanical hypersensitivity. Combining Ribotag RNA profiling and pathway manipulation, we establish that collicular inputs are essential for upregulating pseudokinase CaMKv in the $OPRM1^{+}RVM^{SC}$ neurons after nerve injury, and demonstrate that up- or down- regulation of CaMKv is sufficient to drive or reverse mechanical hypersensitivity. Together, our results reveal a collicular-medulla-spinal cord pathway that drives persistent pain and substantiate CaMKv as a key molecular determinant of mechanical hypersensitivity.

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BOARD NUMBER: S06-605

CENTRALLY EXPRESSED CAV3.2 T-TYPE CALCIUM CHANNEL IS CRITICAL FOR THE INITIATION AND MAINTENANCE OF NEUROPATHIC PAIN

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Cav3.2 T-type calcium channel is a major molecular actor of neuropathic pain in peripheral sensory neurons, but its involvement at the supra-spinal level is almost unknown. Using the spared nerve injury neuropathic pain model, we combined in vitro/in vivo electrophysiological approaches and behavioral tests in a murine model that allows the identification of the Cav3.2 channels and their conditional deletion in specific areas. We show that in the Anterior Pretectum (APT), a hub of connectivity of the somatosensory system involved in pain perception, Cav3.2 channels are expressed in a sub-population of GABAergic neurons co-expressing parvalbumin (PV). In these PV-expressing neurons, Cav3.2 channels contribute to a high frequency bursting activity, which is increased in mice that undergo the spared nerve injury. Specific deletion of Cav3.2 channels in APT neurons reduced both the initiation and maintenance of mechanical and cold allodynia. These data are a direct demonstration that centrally expressed Cav3.2 channels also play a fundamental role in pain pathophysiology.

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BOARD NUMBER: S06-606

INVOLVEMENT OF PARVALBUMIN-POSITIVE GABAERGIC NEURONS IN BASAL FOREBRAIN MODULATION IN A MOUSE MODEL OF NEUROPATHIC PAIN

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

Marie-Luise Edenhofer, Michaela Kress, Kai Kummer
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Chronic neuropathic pain is a public health issue that affects ~5 % of the European population and is caused by a lesion or disease of the somatosensory system. The central mechanisms underlying the chronification of pain are not yet fully understood. The medial prefrontal cortex receives input from basal forebrain (BF) cholinergic neurons that is altered after nerve injury. Parvalbumin (PV)-positive GABAergic neurons are of special importance for this regulation, as those neurons produce widespread inhibition. To elucidate the role of PV neurons in the pain-associated changes of BF cholinergic neurons, we investigated the electrophysiological properties of BF-PV neurons. PV neurons were selectively labelled and acute brain slices were prepared one week after spared nerve injury (SNI). After SNI, BF-PV neurons demonstrated decreased firing rates in response to depolarizing current injections compared to sham controls. Consistently, the area under the curve (AUC) of the action potential frequency against injected currents was reduced in SNI mice. As postsynaptic transmission can influence the observed decrease in BF-PV neuron excitability, we recorded spontaneous inhibitory (sIPSC) and excitatory (sEPSC) postsynaptic currents. Neurons recorded from brain slices obtained from SNI treated mice demonstrated a reduced frequency of sEPSCs, suggesting a reduction of excitatory inputs. Our findings suggest that alterations in cholinergic synaptic transmission in neuropathic pain may be caused by reduced inhibitory inputs mediated by local BF-PV neurons. In order to elucidate the direct nature of this PV-to-ChAT signaling, we will perform patch-clamp recordings from ChAT neurons in response to optogenetic activation of BF-PV neurons.

Pubmed:

35003840: May MA, Kummer KK, Edenhofer ML, Choconta JL, Kress M, Ritsch-Marte M, Jesacher A
Simultaneous scattering compensation at multiple points in multi-photon microscopy.
The two-photon fluorescence imaging depth has been significantly improved in recent years by compensating for tissue scattering with wavefront correction. However, in most approaches the wavefront corrections are valid only over a small sample region on the order of 1 to 10 µm. In samples where most scattering structures are confined to a single plane, sample conjugate correction geometries can increase the observable field to a few tens of µm. Here, we apply a recently introduced fast converging scheme for sensor-less scattering correction termed "Dynamic Adaptive Scattering compensation Holography" (DASH) in a sample conjugate configuration with a high pixel count nematic liquid crystal spatial light modulator (LC-SLM). Using a large SLM allows us to simultaneously correct for scattering at multiple field points, which can be distributed over the entire field of view provided by the objective lens. Despite the comparably slow refresh time of LC-SLMs, we achieve correction times on the order of 10 s per field point, which we show is sufficiently fast to counteract scattering at multiple sites in living mouse hippocampal tissue slices.
Biomed Opt Express, 2021; 12
34450173: Oemer G, Edenhofer ML, Wohlfarter Y, Lackner K, Leman G, Koch J, Cardoso LHD, Lindner HH, Gnaiger E, Dubrac S, Zschocke J, Keller MA
Fatty acyl availability modulates cardiolipin composition and alters mitochondrial function in HeLa cells.
The molecular assembly of cells depends not only on the balance between anabolism and catabolism but to a large degree on the building blocks available in the environment. For cultured mammalian cells, this is largely determined by the composition of the applied growth medium. Here, we study the impact of lipids in the medium on mitochondrial membrane architecture and function by combining LC-MS/MS lipidomics and functional tests with lipid supplementation experiments in an otherwise serum-free and lipid-free cell culture model. We demonstrate that the composition of mitochondrial cardiolipins strongly depends on the lipid environment in cultured cells and favors the incorporation of essential linoleic acid over other fatty acids. Simultaneously, the mitochondrial respiratory complex I activity was altered, whereas the matrix-localized enzyme citrate synthase was unaffected. This raises the question on a link between membrane composition and respiratory control. In summary, we found a strong dependency of central mitochondrial features on the type of lipids contained in the growth

medium. This underlines the importance of considering these factors when using and establishing cell culture models in biomedical research. In summary, we found a strong dependency of central mitochondrial features on the type of lipids contained in the growth medium.
J Lipid Res, 2021; 62

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9-13 July 2022 | Paris, France

BOARD NUMBER: S06-607

EXCITABILITY OF INJURED AND NON-INJURED NEURONS AFTER SPARED NERVE INJURY

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Aims. Various models of neuropathic pain demonstrate that nerve injury leads to hyperexcitability of primary sensory neurons. The spared nerve injury (SNI) model produces a close arrangement of injured and non-injured primary sensory neurons within the same dorsal root ganglion (DRG). We aim to distinguish the excitability of injured and non-injured of primary sensory neurons after SNI. Methods. We used transgenic B6-Tg(ATF3::GFP)Wlf mice that express GFP under the promoter of ATF3, highly upregulated by axonal injury and used as marker of injured neurons. Non-injured neurons from the spared sural nerve were labelled using retrograde tracer FastBlue (FB). ATF3+ and FB+ neurons were sorted by FACS. Extracellular electrophysiological recordings were perfomed using multi-electrode arrays (MEA) at 2, 4, 7 and 14 days after SNI. Whole-cell patch clamp recordings of DRG neurons were performed to reveal changes in current-evoked excitability. Results. The MEA recordings indicated an increased spontaneous activity in the ATF3+ injured neurons at the different timepoints after SNI. The FB+ non-injured neurons showed an increase only 2 days after SNI. In the patch clamp recordings, the ipsilateral FB+ neurons showed a higher firing frequency rate than the contralateral FB+ neurons. ATF3+ neurons only showed a different firing pattern, as illustrated by the change in different AP features. Conclusions. Hyperexcitability is present with different patterns in injured and adjacent non-injured DRG neurons after SNI. This study suggests that injured neurons are more prone to fire spontaneously, while non-injured neurons are hypersensitive to stimulation.

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CONTRIBUTION OF PERIPHERAL NEURONAL ACTIVITY TO SPINAL MICROGLIAL REACTIVITY IN CHRONIC PAIN

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Chronic neuropathic pain is a complex pathology with a strong neuroimmune interplay. Abnormal peripheral activity arriving from primary sensory neurons of the dorsal root ganglia (DRG) contributes to spinal microglia reactivity after nerve injury and previous studies in rats demonstrated that electrical stimulation of the peripheral nerve induces spinal microglial activation. Our aim is to further investigate the contribution of nociceptive and non-nociceptive inputs to spinal microglial reactivity in the context of chronic pain. Method: we first performed electrical stimulation on the sciatic nerve in CX3CR1-eGFP mice. 2 days after stimulation, we quantified GFP signal density and cell proliferation (Ki-67) using immunohistochemistry on L3-L5 dorsal horn. Then, we used optogenetic tools and blue light was applied onto sciatic nerve to selectively activate all fiber types with Advilin-Cre/ChR2-TdTomato mice or nociceptive fibers only in SNS-Cre/ChR2-TdTomato mice. Results: we observe microglial reactivity in the spinal cord after electrical and optogenetic stimulation when compared to the controls ($p=0.0199$). Similarly, light activation of Advilin-ChR2 nociceptive and non-nociceptive neurons induces microglial reactivity in the spinal cord. Activation of SNS-ChR2 nociceptive neurons only induces also microglial reactivity. Conclusion: intense activity from nociceptive neurons is sufficient to induce spinal microglia reactivity. Same experiments performed in non-nociceptive ChR2 transgenic lines will allow us to determine whether the activation of nociceptive fibers is not only sufficient but also necessary for the development of neuroinflammation and neuropathic pain.

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THE PARABRACHIAL NUCLEUS TO THE CENTRAL AMYGDALA SYSTEM REGULATES LONG-LASTING WIDESPREAD SENSITIZATION IN THE RODENT MODEL OF NOCIPLASTIC PAIN

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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Widespread sensitization is one of the hallmark symptoms of nociplastic pain, characterized by augmented nociception at various body sites not necessarily associated with injury/inflammation. We have demonstrated that inflammation in a trigeminally innervated region results in robust expression of c-fos protein in the bilateral parabrachial nucleus (PBN) and the right central amygdala (CeA), and this excitation was accompanied by bilateral hindpaw sensitization in a manner critically dependent on CeA neuronal activity (Sugimoto et al, 2021; Miyazawa et al, 2018). Here we asked whether the PBN-CeA network plays specific roles in this formalin-induced long-lasting sensitization. Single-injection of formalin into the left upper lip decreased the bilateral paw withdrawal threshold for >10 days, and this sensitization was counteracted by intraperitoneal administration of 30 mg/kg pregabalin. The PBN neurons activated in this model were visualized and selectively stimulated using FosTRAP mice and AAV-mediated transfection of ChR2 into the PBN and CeA. The activated CeA neurons showed a higher incidence of postsynaptic responses to light-evoked stimulation of the fibers arising from the activated PBN neurons than those from the general population of PBN neurons. These observations indicate that PBN neurons and CeA neurons form specific robust connections that regulate CeA neuronal activity and thus modulate the expression of widespread sensitization.

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BOARD NUMBER: S06-610

ROLE OF VOLTAGE-DEPENDENT POTASSIUM CURRENTS ON TRIGEMINAL NEUROPATHIC PAIN

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

Ana Abreu, Beatriz Szwarc, André Bastos, Pedro Lima
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Neuropathic pain affecting the orofacial area is extremely debilitating and remains a therapeutic challenge. Conditions caused by trigeminal nerve injury are characterised by a state of hyperexcitability throughout all levels of the trigeminal axis. This work aimed to characterise the electrophysiological properties of the most peripheral element of this axis, by studying the potassium currents recorded from small-diameter neurones isolated from the trigeminal ganglion of animals with chronic constriction of the infraorbital nerve (CCI-ION) and comparing them to those recorded from naïve animals. The CCI-ION model was induced on female Wistar rats by ligation of a distal segment of the infraorbital nerve, exposed through a small facial incision. The mechanical sensitivity threshold of the ipsilateral whisker pad was reduced for up to 28 days. After this period, the trigeminal ganglia were harvested and dissociated. When compared with neurones from naïve rats, the activation of the potassium channels became more voltage-dependent, and the slower current component became more easily activated, with a hyperpolarized profile. The voltage dependence of inactivation was also altered with surgery: a current component with slower inactivation became more prominent and more voltage-sensitive in the neurons isolated from CCI-ION rats. Combined, these changes lead to increased activity of the potassium voltage-dependent channels, which might represent a mechanism necessary to sustain the higher activity rate typical of the chronic pain state. Such results agree with previous findings on a model of chronic inflammatory orofacial pain and the CCI model of the sciatic nerve.

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A NOVEL ANALGESIC PATHWAY FROM PARVOCELLULAR OXYTOCIN NEURONS TO THE PERIAQUEDUCTAL GRAY

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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The hypothalamic neuropeptide, oxytocin (OT), exerts prominent analgesic effects via central and peripheral action. Here we discovered a novel subset of OT neurons whose projections preferentially terminate on OT receptor (OTR)-expressing neurons in the ventrolateral periaqueductal gray (vlPAG). Using a newly generated line of transgenic rats (OTR-IRES-Cre), we determined that most of the vlPAG OTR expressing cells being targeted by OT projections are GABAergic in nature. Both optogenetically-evoked axonal OT release in the vlPAG as well as chemogenetic activation of OTR vlPAG neurons results in a long-lasting overall increase of vlPAG neuronal activity. This then leads to an indirect suppression of sensory neuron activity in the spinal cord and strong analgesia. Finally, we describe a novel OT→vlPAG→spinal cord circuit that seems critical for analgesia in the context of both inflammatory and neuropathic pain.

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BOARD NUMBER: S06-612

REPROGRAMMING THE TOPOLOGY OF THE NOCICEPTIVE CIRCUIT IN C. ELEGANS RESHAPES SEXUAL BEHAVIOR

POSTER SESSION 06 - SECTION: PHYSIOLOGY OF PAIN CIRCUITS

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In sexually reproducing species, males and females respond to environmental sensory cues and transform the input into sexually dimorphic traits. Yet, how sexually dimorphic behavior is encoded in the nervous system is poorly understood. We characterize the sexually dimorphic nociceptive behavior in *C. elegans* – hermaphrodites present a lower pain threshold than males in response to aversive stimuli, and study the underlying neuronal circuits, which are composed of the same neurons that are wired differently. By imaging receptor expression, calcium responses and glutamate secretion, we show that sensory transduction is similar in the two sexes, and therefore explore how downstream network topology shapes dimorphic behavior. We generated a computational model that replicates the observed dimorphic behavior, and used this model to predict simple network rewirings that would switch the behavior between the sexes. We then showed experimentally, using genetic manipulations, artificial gap junctions, automated tracking and optogenetics, that these subtle changes to male connectivity result in hermaphrodite-like aversive behavior *in-vivo*, while hermaphrodite behavior was more robust to perturbations. Strikingly, when presented with aversive cues, rewired males were compromised in finding mating partners, suggesting that the network topology that enables efficient avoidance of noxious cues would have a reproductive "cost". To summarize, we present a deconstruction of a neural circuit design that controls sexual behavior, and how to reprogram it. More broadly, our results are an example of how common neuronal circuits changed their function during evolution by subtle topological rewirings to account for different environmental and sexual needs.

Pubmed:
32857970: Salzberg Y, Pechuk V, Gat A, Setty H, Sela S, Oren-Suissa M
Synaptic Protein Degradation Controls Sexually Dimorphic Circuits through Regulation of DCC/UNC-40.
Sexually dimorphic circuits underlie behavioral differences between the sexes, yet the molecular mechanisms involved in their formation are poorly understood. We show here that sexually dimorphic connectivity patterns arise in C. elegans through local ubiquitin-mediated protein degradation in selected synapses of one sex but not the other. Specifically, synaptic degradation occurs via binding of the evolutionary conserved E3 ligase SEL-10/FBW7 to a phosphodegron binding site of the netrin receptor UNC-40/DCC (Deleted in Colorectal Cancer), resulting in degradation of UNC-40. In animals carrying an undegradable unc-40 gain-of-function allele, synapses were retained in both sexes, compromising the activity of the circuit without affecting neurite guidance. Thus, by decoupling the synaptic and guidance functions of the netrin pathway, we reveal a critical role for dimorphic protein degradation in controlling neuronal connectivity and activity. Additionally, the interaction between SEL-10 and UNC-40 is necessary not only for sex-specific synapse pruning, but also for other synaptic functions. These findings provide insight into the mechanisms that generate sex-specific differences in neuronal connectivity, activity, and function.
Curr Biol, 2020; 30

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BOARD NUMBER: S06-613

DYNAMIC GATING OF PERCEPTUAL FLEXIBILITY BY DIVERSE CORTICAL RESPONSES

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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The ability to flexibly adapt to changing environments is the hallmark of an adaptive nervous system and is seen in animals across the entire phylogenetic tree. This is in stark contrast to perceptual and cognitive inflexibility which is implicated in many neurological disorders including hearing loss, autism, and schizophrenia. While sensory and frontal cortical areas have long been implicated in flexible behaviors, we lack a fundamental understanding of how information is gated within these circuits to select and update behavioral strategies based on sensory input and context. We trained mice to perform a go/no-go auditory reversal learning task that required animals to adapt their behavioral response to the same set of auditory cues. Specifically, animals were trained to respond to a target tone (11.2 kHz) and to withhold from responding to a nontarget tone (5.6 kHz) for water reward. Once animals learned this phase of the task we then implemented a rule-switch and reversed which tone was rewarded, requiring animals to remap stimulus-reward contingencies. Chemogenetic silencing demonstrated that auditory cortex is required for reversal learning in mice. Using silicon probe recordings, we simultaneously monitored the activity of 1,322 single-units in auditory cortex (AC) and 1,381 single-units in frontal cortex (M2) during reversal learning. We found that neural response profiles during learning were highly heterogeneous ranging from highly-reliable or ‘classical’ responses to seemingly-random ‘non-classical’ firing. Neural populations in both regions dynamically altered their response profiles during different phases of learning allowing for the emergence of flexible behaviors.

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BOARD NUMBER: S06-614

IMAGING THE MOUSE DEVELOPING AUDITORY CORTEX

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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During postnatal development, sensory experiences shape neural connections and individuals acquire memory of these sensory cues. Among the variety of sensory stimuli at which newborn mice are exposed during development, paternal ultrasound vocalizations (USVs) play an important role in forming memories of conspecifics that are recalled in adulthood females to influence mate choice. However, how paternal USVs influence the functional development of cortical auditory circuits is unknown. Here, we performed in vivo two-photon functional imaging and characterized spontaneous activity of layer 2/3 (L2/3) neurons in the primary auditory areas of anaesthetized mice at different postnatal ages, in animals raised with or without their father. P0 mice were injected in auditory cortex with adeno-associated viral vectors expressing GCaMP7 and spontaneous activity was characterized at three developmental time windows: i) P8-P11: when the external auditory canal is closed; ii) P12-P14: when the onset of hearing occurs; iii) P15-P21: during the critical period for hearing. At P8-P11, L2/3 neurons showed spontaneous synchronous transients. From hearing onset, a switch from synchronous to asynchronous activity occurred and, at the end of the critical period, most cells displayed asynchronous largely decorrelated neuronal activity. Interestingly, during the critical period, animals raised in the absence of their father showed significant more correlated activity compared to those raised in the presence of the father. Overall, these results suggest a relevant role of sensory stimuli linked to the developmental presence of the father in shaping auditory circuits.

Pubmed:
33818492: La Rosa C, Bonfanti L
Searching for alternatives to brain regeneration.
Neural Regen Res, 2021; 16
32690132: La Rosa C, Cavallo F, Pecora A, Chincarini M, Ala U, Faulkes CG, Nacher J, Cozzi B, Sherwood CC, Amrein I, Bonfanti L
Phylogenetic variation in cortical layer II immature neuron reservoir of mammals.
The adult mammalian brain is mainly composed of mature neurons. A limited amount of stem cell-driven neurogenesis persists in postnatal life and is reduced in large-brained species. Another source of immature neurons in adult brains is cortical layer II. These cortical immature neurons (cINs) retain developmentally undifferentiated states in adulthood, though they are generated before birth. Here, the occurrence, distribution and cellular features of cINs were systematically studied in 12 diverse mammalian species spanning from small-lissencephalic to large-gyrencephalic brains. In spite of well-preserved morphological and molecular features, the distribution of cINs was highly heterogeneous, particularly in neocortex. While virtually absent in rodents, they are present in the entire neocortex of many other species and their linear density in cortical layer II generally increased with brain size. These findings suggest an evolutionary developmental mechanism for plasticity that varies among mammalian species, granting a reservoir of young cells for the cerebral cortex.
Elife, 2020; 9
32116519: La Rosa C, Parolisi R, Bonfanti L
Brain Structural Plasticity: From Adult Neurogenesis to Immature Neurons.
Brain structural plasticity is an extraordinary tool that allows the mature brain to adapt to environmental changes, to learn, to repair itself after lesions or disease, and to slow aging. A long history of neuroscience research led to fascinating discoveries of different types of plasticity, involving changes in the genetically determined structure of nervous tissue, up to the ultimate dream of neuronal replacement: a stem cell-driven "adult neurogenesis" (AN). Yet, this road does not seem a straight one, since mutable dogmas, conflicting results and conflicting interpretations continue to warm the field. As a result, after more than 10,000 papers published on AN, we still do not know its time course, rate or features with respect to other kinds of structural plasticity in our brain. The solution does not appear to be behind the next curve, as differences among mammals

reveal a very complex landscape that cannot be easily understood from rodents models alone. By considering evolutionary aspects, some pitfalls in the interpretation of cell markers, and a novel population of undifferentiated cells that are not newly generated [immature neurons (INs)], we address some conflicting results and controversies in order to find the right road forward. We suggest that considering plasticity in a comparative framework might help assemble the evolutionary, anatomical and functional pieces of a very complex biological process with extraordinary translational potential.
Front Neurosci, 2020; 14
31096632: La Rosa C, Ghibaudi M, Bonfanti L
Newly Generated and Non-Newly Generated "Immature" Neurons in the Mammalian Brain: A Possible Reservoir of Young Cells to Prevent Brain Aging and Disease?
Brain plasticity is important for translational purposes since most neurological disorders and brain aging problems remain substantially incurable. In the mammalian nervous system, neurons are mostly not renewed throughout life and cannot be replaced. In humans, the increasing life expectancy explains the increase in brain health problems, also producing heavy social and economic burden. An exception to the "static" brain is represented by stem cell niches leading to the production of new neurons. Such adult neurogenesis is dramatically reduced from fish to mammals, and in large-brained mammals with respect to rodents. Some examples of neurogenesis occurring outside the neurogenic niches have been reported, yet these new neurons actually do not integrate in the mature nervous tissue. Non-newly generated, "immature" neurons (nng-INs) are also present: Prenatally generated cells continuing to express molecules of immaturity (mostly shared with the newly born neurons). Of interest, nng-INs seem to show an inverse phylogenetic trend across mammals, being abundant in higher-order brain regions not served by neurogenesis and providing structural plasticity in rather stable areas. Both newly generated and nng-INs represent a potential reservoir of young cells (a "brain reserve") that might be exploited for preventing the damage of aging and/or delay the onset/reduce the impact of neurological disorders.
J Clin Med, 2019; 8
30443551: La Rosa C, Bonfanti L
Brain Plasticity in Mammals: An Example for the Role of Comparative Medicine in the Neurosciences.
Comparative medicine deals with similarities and differences between veterinary and human medicine. All mammals share most basic cellular and molecular mechanisms, thus justifying murine animal models in a translational perspective; yet "mice are not men," thus some biases can emerge when complex biological processes are concerned. Brain plasticity is a cutting-edge, expanding topic in the field of Neurosciences with important translational implications, yet, with remarkable differences among mammals, as emerging from comparative studies. In particular, adult neurogenesis (the genesis of new neurons from brain stem cell niches) is a life-long process in laboratory rodents but a vestigial, mostly postnatal remnant in humans and dolphins. Another form of "whole cell" plasticity consisting of a population of "immature" neurons which are generated prenatally but continue to express markers of immaturity during adulthood has gained interest more recently, as a reservoir of young neurons in the adult brain. The distribution of the immature neurons also seems quite heterogeneous among different animal species, being confined within the paleocortex in rodents while extending into neocortex in other mammals. A recent study carried out in sheep, definitely showed that gyrencephalic, large-sized brains do host higher amounts of immature neurons, also involving subcortical, white, and gray matter regions. Hence, "whole cell" plasticity such as adult neurogenesis and immature neurons are biological processes which, as a whole, cannot be studied exclusively in laboratory rodents, but require investigation in comparative medicine, involving large-sized, long-living mammals, in order to gain insights for translational purposes.
Front Vet Sci, 2018; 5
29980931: La Rosa C, Parolisi R, Palazzo O, Lévy F, Meurisse M, Bonfanti L
Clusters of DCX+ cells "trapped" in the subcortical white matter of early postnatal Cetartiodactyla (Tursiops truncatus, Stenella coeruloalba and Ovis aries).
The cytoskeletal protein doublecortin (DCX) is a marker for neuronal cells retaining high potential for structural plasticity, originating from both embryonic and adult neurogenic processes. Some of these cells have been described in the subcortical white matter of neonatal and postnatal mammals. In mice and humans it has been shown they are young neurons migrating through the white matter after birth, reaching the cortex in a sort of protracted neurogenesis. Here we show that DCX+ cells in the white matter of neonatal and young Cetartiodactyla (dolphin and sheep) form large clusters which are not newly generated (in sheep, and likely neither in dolphins) and do not reach the cortical layers, rather appearing "trapped" in the white matter tissue. No direct contact or continuity can be observed between the subventricular zone region and the DCX+ clusters, thus indicating their independence from any neurogenic source (in dolphins further confirmed by the recent demonstration that periventricular neurogenesis is inactive since birth). Cetartiodactyla include two orders of large-brained, relatively long-living mammals (cetaceans and artiodactyls) which were recognized as two separate monophyletic clades until recently, yet, despite the evident morphological distinctions, they are monophyletic in origin. The brain of Cetartiodactyla is characterized by an advanced stage of development at birth, a feature that might explain the occurrence of "static" cell

clusters confined within their white matter. These results further confirm the existence of high heterogeneity in the occurrence, distribution and types of structural plasticity among mammals, supporting the emerging view that multiple populations of DCX+, non-newly generated cells can be abundant in large-brained, long-living species.
Brain Struct Funct, 2018; 223
29722307: Palazzo O, La Rosa C, Piumatti M, Bonfanti L
Do large brains of long-living mammals prefer non-newly generated, immature neurons?
Neural Regen Res, 2018; 13
29256007: Caradonna F, Cruciata I, Schifano I, La Rosa C, Naselli F, Chiarelli R, Perrone A, Gentile C
Methylation of cytokines gene promoters in IL-1β-treated human intestinal epithelial cells.
Epigenetic regulation is important in the activation of inflammatory cells. In the present study, we evaluated if DNA-methylation variations are involved in Interleukin-1β (IL-1β)-induced intestinal epithelial cells activation.
Inflamm Res, 2018; 67
29217680: Piumatti M, Palazzo O, La Rosa C, Crociara P, Parolisi R, Luzzati F, Lévy F, Bonfanti L
Non-Newly Generated, "Immature" Neurons in the Sheep Brain Are Not Restricted to Cerebral Cortex.
A newly proposed form of brain structural plasticity consists of non-newly generated, "immature" neurons of the adult cerebral cortex. Similar to newly generated neurons, these cells express the cytoskeletal protein Doublecortin (DCX), yet they are generated prenatally and then remain in a state of immaturity for long periods. In rodents, the immature neurons are restricted to the paleocortex, whereas in other mammals, they are also found in neocortex. Here, we analyzed the DCX-expressing cells in the whole sheep brain of both sexes to search for an indicator of structural plasticity at a cellular level in a relatively large-brained, long-living mammal. Brains from adult and newborn sheep (injected with BrdU and analyzed at different survival times) were processed for DCX, cell proliferation markers (Ki-67, BrdU), pallial/subpallial developmental origin (,), and neuronal/glial antigens for phenotype characterization. We found immature-like neurons in the whole sheep cortex and in large populations of DCX-expressing cells within the external capsule and the surrounding gray matter (claustrum and amygdala). BrdU and Ki-67 detection at neonatal and adult ages showed that all of these DCX cells were generated during embryogenesis, not after birth. These results show that the adult sheep, unlike rodents, is largely endowed with non-newly generated neurons retaining immature features, suggesting that such plasticity might be particularly important in large-brained, long-living mammals. Brain plasticity is important in adaptation and brain repair. Structural changes span from synaptic plasticity to adult neurogenesis, the latter being highly reduced in large-brained, long-living mammals (e.g., humans). The cerebral cortex contains "immature" neurons, which are generated prenatally and then remain in an undifferentiated state for long periods, being detectable with markers of immaturity. We studied the distribution and developmental origin of these cells in the whole brain of sheep, relatively large-brained, long-living mammals. In addition to the expected cortical location, we also found populations of non-newly generated neurons in several subcortical regions (external capsule, claustrum, and amygdala). These results suggests that non-neurogenic, parenchymal structural plasticity might be more important in large mammals with respect to adult neurogenesis.
J Neurosci, 2018; 38

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SILENT MOVIES SYNCHRONIZE SECONDARY AUDITORY CORTICES MORE IN EARLY DEAF THAN HEARING INDIVIDUALS.

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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The auditory cortex of deaf individuals is responsive to a range of tasks in the visual modality. Still, the nature and extent of this repurposing is not well understood. Here naturalistic stimuli were used to elicit neural synchrony in early deaf (n=21) and hearing individuals (n=22). Participants passively watched a silent animated movie (The triplets of Belleville) and three distorted versions of the same film that disrupted its meaning: two piecewise-scrambled versions lacking a coherent plot (12 sec and 2 second scrambled,) and one visually distorted (diffeomorphic, lacking any meaning). In both groups, early visual cortices were synchronized to a similar degree across stimulus types, while higher-cognitive areas (e.g., Precuneus, Superior Parietal Lobule and Prefrontal Cortex) were more synchronized by the intact, meaningful version of the movie. Secondary auditory cortices, along the extent of the superior temporal sulcus (STS), exhibited higher synchrony in deaf than in hearing participants across all stimulus types, with larger effects in the right hemisphere and little synchrony in A1. Meaningful stimulus elicited a significantly higher STS synchronization across deaf individuals than all three scrambled conditions. In the absence of early auditory experience, the STS is engaged by meaningful visual narratives.

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BOARD NUMBER: S06-616

ALTERED TOPOGRAPHIC POPULATION ACTIVITY IN THE AUDITORY CORTEX OF AN AUDITORY PROCESSING-IMPAIRED MOUSE MODEL

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

Simon Wadle[1], Tatjana Schmitt[1], Jutta Engel[2], Simone Kurt[2], Jan Hirtz[1]
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The extraction of specific sound features from a complex acoustic environment is essential for orientation, social interaction, and surviving in everyday life. Loss-of-function mutations in the Cacna2d3 gene, encoding the $\alpha_2\delta3$ auxiliary subunit of voltage-gated calcium channels, lead to structural and functional impairments of several auditory nuclei, which are accompanied by deficits on the behavioral level. Albeit being able to distinguish simple pure tones from each other, $\alpha_2\delta3$-KO mice fail to discriminate between amplitude modulated tones (Pirone et al. (2014)). Furthermore, mutations in the *Cacna2d3* gene have been associated with autism spectrum disorder in humans. We here investigated neuronal network activity in the auditory cortex (ACx) of $\alpha_2\delta3$-KO mice and controls. 13 days after the injection of AAV1-hSyn1-jGCaMP7f we performed two-photon calcium imaging of neurons in layer 2/3 of the ACx in awake animals while presenting different sets of acoustic stimuli in a passive listening task. Best frequency (BF) distribution analysis showed no differences in the overall BF distribution but revealed a lower local heterogeneity in $\alpha_2\delta3$-KO mice. Correlation-based clustering of neuronal activity during stimulation with various animal vocalizations displayed no difference between genotypes regarding the number of clusters formed, as well as the number of neurons per cluster. Notably, physical distances between cells in clusters in KO animals were smaller compared to controls, indicating an altered topographic organization of local cells which was independent of stimulus complexity. We also observed subfield-specific changes in population activity, which might provide an explanation for impaired auditory-related learning in $\alpha_2\delta3$-KO mice.

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BOARD NUMBER: S06-617

SURROUND SUPPRESSION IN MOUSE AUDITORY CORTEX UNDERLIES AUDITORY EDGE DETECTION

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Surround suppression (SS) is a fundamental property of all sensory systems. However, only limited portions of its landscape have been studied. In the auditory system, a one dimensional array of hair cells corresponds to the full spectral contents of all sounds. We exploited the simplicity of this 1D representation of sounds to investigate the full landscape of SS. We recorded single unit spiking from the auditory cortex (ACx) of awake mice in response to an array of spectrally broadband auditory stimuli with varying central frequencies and bandwidths. We recorded 88 auditory neurons from 5 mice using Neuropixels probes. Our recordings revealed a novel response profile to the full frequency-bandwidth array of sounds, with a majority of neurons preferring larger bandwidths for lower frequencies. To gain insight into the possible mechanism of these responses, we modelled neuronal responses using a novel function called Modulated Ricker Wavelet (MRW) which is a variation of the “Mexican hat” function often used to model SS. Using MRW, we were able explain the response properties of single neurons with remarkable accuracy (often >>90%). We show that these highly prevalent responses in ACx, result from simple rules of lateral inhibitory sidebands in frequencies above the excitatory band of the neuron. Our data implies robust over-representation of top edges in the ACx and may form the mechanistic basis of auditory edge detection and possibly other computations relating to modulations in the spectral content of sounds.

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BOARD NUMBER: S06-618

BEHAVIORAL AND NEURAL CORRELATES OF EARLY MUSIC EXPOSURE IN MICE

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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The natural auditory environment consists of complex sounds. One interesting family of complex sounds is human music, a remarkably complex acoustic set of stimuli with a dynamic spectrotemporal structure. In recent years, the study of music preferences in humans has substantially advanced, with evidence for significant brain activity evoked in the auditory cortex as well as in reward areas. However, our understanding of the connections between the auditory and reward systems is limited. Several studies suggested that rodents show idiosyncratic preferences to human music and can be conditioned to prefer specific musical pieces. Therefore, we use the rodent model to investigate the neural mechanisms that may underlie music preferences. To this aim, we exposed young C57BL/6 mice (P7-P40) to a musical environment consisting of excerpts from Beethoven's symphony no. 9. I tested their sound preference as young adults. Exposed mice showed a significant preference for the music to which they were exposed over silence relative to control and naive mice, but this preference habituated on further tests. These effects showed sex effects, with males showing stronger preferences to the exposed music than females. We performed wide-field calcium imaging to measure neural response to different sounds in the auditory cortex, including excerpts from the music the animals were exposed to in their infancy. The results indicate suppression of the overall activity of the auditory cortex in exposed animals compared to the control/naive animals.

Pubmed:
30122531: Atlan G, Terem A, Peretz-Rivlin N, Sehrawat K, Gonzales BJ, Pozner G, Tasaka GI, Goll Y, Refaeli R, Zviran O, Lim BK, Groysman M, Goshen I, Mizrahi A, Nelken I, Citri A
The Claustrum Supports Resilience to Distraction.
A barrage of information constantly assaults our senses, of which only a fraction is relevant at any given point in time. However, the neural circuitry supporting the suppression of irrelevant sensory distractors is not completely understood. The claustrum, a circuit hub with vast cortical connectivity, is an intriguing brain structure, whose restrictive anatomy, thin and elongated, has precluded functional investigation. Here, we describe the use of Egr2-CRE mice to access genetically defined claustral neurons. Utilizing conditional viruses for anterograde axonal labeling and retrograde trans-synaptic tracing, we validated this transgenic model for accessing the claustrum and extended the known repertoire of claustral input/output connectivity. Addressing the function of the claustrum, we inactivated CL neurons, chronically as well as acutely, in mice performing an automated two-alternative forced-choice behavioral task. Strikingly, inhibition of CL neurons did not significantly impact task performance under varying delay times and cue durations, but revealed a selective role for the claustrum in supporting performance in the presence of an irrelevant auditory distractor. Further investigation of behavior, in the naturalistic maternal pup-retrieval task, replicated the result of sensitization to an auditory distractor following inhibition of CL neurons. Initiating investigation into the underlying mechanism, we found that activation of CL neurons modulated cortical sensory processing, suppressing tone representation in the auditory cortex. This functional study, utilizing selective genetic access, implicates the claustrum in supporting resilience to distraction, a fundamental aspect of attention.
Curr Biol, 2018; 28

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BOARD NUMBER: S06-619

HUMAN CORTICAL AUDITORY PROCESSING OF NATURALISTIC SPEECH WITH SIMULATED HEARING LOSS: A DATA-DRIVEN FMRI APPROACH

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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We investigate the cortical auditory processing of realistic speech in natural soundscapes with clear and degraded naturalistic stimuli using voxel-wise encoding models (VWEMs), where acoustic features are used to model brain activity. We used an audio description of the movie "Forrest Gump" soundtrack as a clear naturalistic stimulus (CS) and, in addition, in two versions with different levels of acoustic degradation (low (S2) and high (N4), Bisgaard et al., 2010) at higher frequencies to simulate common types of hearing loss. We measured fMRI responses while normal-hearing participants listened to the full movie in each session but in eight segments at different levels of degradation and the three repetitions at different degradation levels (CS, S2, or N4) distributed over three sessions. We estimated VWEMs for the sound Mel-frequencies spectrogram of CS, S2, and N4 stimuli separately. The speech perception was best for CS, intermediate for S2, and worst for N4 stimuli. In contrast, the VWEMs in early auditory cortices best predicted the BOLD-responses in N4 compared to CS and S2 conditions. In the N4 condition, we found the highest correlations between predicted and observed BOLD-responses in Heschl's gyrus, planum temporale and the superior temporal gyrus. Our results demonstrate that the neural processes underlying the processing of speech in natural soundscapes are better captured by Mel-spectrogram in the core auditory areas when the speech is acoustically degraded. This suggests an interaction between the intelligibility of speech in natural soundscapes and the processing of acoustic features in early auditory cortices.

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BOARD NUMBER: S06-620

THE EXTRACELLULAR MATRIX REGULATES CORTICAL LAYER DYNAMICS AND CROSS-COLUMNAR FREQUENCY INTEGRATION IN THE AUDITORY CORTEX

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Enzymatic removal of the extracellular matrix (ECM) is increasingly recognized to promote learning, memory recall, and restorative plasticity. The impact of the ECM on translaminar dynamics during cortical circuit processing is still not understood. We removed the ECM in the primary auditory cortex of Mongolian gerbils using local injections of hyaluronidase (HYase). Using current-source density (CSD) analysis, we found layer-specific changes of the spatiotemporal synaptic activity patterns with increased corticocortical integration in supragranular layers with simultaneous weakening of early local sensory input processing within infragranlar layers. The spatiotemporal changes were associated with an oscillatory fingerprint in the beta-band (25-36 Hz) within infragranular layers. To understand the laminar interaction dynamics after ECM digestion, we used time-domain conditional Granger causality (GC) measures, revealing a stronger drive from supragranular layers into late infragranular layer, which also showed increased drive towards early infragranular layer. These results show that ECM degradation caused an altered translaminar cortical network dynamic with stronger supragranular lead of the columnar response profile and increased cross-columnar frequency integration. We further described that behavioral auditory training leads to transient downregulation of the ECM protein brevican in auditory cortex. Brevican degradation has been shown to depend on dopaminergic neuromodulation via activation of the protease ADAMTS-4/5. Our hypothesis is that during acquisition learning, dopamine release triggers an initial downregulation of the ECM to optimize the dynamics of corticocortical networks for synaptic plasticity. We speculate that such learning-dependent regulation of the ECM needs concomitant action of reinforcing dopaminergic neuromodulation coding the behavioral relevance of events.

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THE SYNAPTIC ORIGINS AND FUNCTIONAL ROLE OF DIVERSE CORTICAL RESPONSES DURING BEHAVIOR

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Neuronal responses during behavior are diverse, ranging from highly reliable ‘classical’ responses to irregular or seemingly-random ‘non-classically responsive’ firing. Various spiking patterns (or lack thereof) have been documented throughout brain regions in response to different sensory inputs, in relation to decision making, motor actions, or other task-related signals. While this continuum of response properties is frequently observed, little is known about the synaptic origins and contributions of diverse response profiles to network function, perception, and behavior. Previous work used a novel single-trial, spike-timing-based analysis to show that both classically and non-classically responsive cortical neurons contain significant information about sensory stimuli and behavioral decisions suggesting that non-classically responsive cells may play an underappreciated role in perception and behavior. Here we use a task-performing, spiking recurrent neural network model incorporating spike-timing-dependent plasticity that captures heterogeneous responses measured from auditory cortex of behaving rodents. We leverage this model to explore the synaptic origin and functional contribution of heterogeneous response profiles. Detailed inactivation experiments revealed that classically and non-classically responsive units contributed to task performance via output and recurrent connections, respectively, providing evidence for a double-dissociative function of these subpopulations. Non-classically responsive units also constrained network dimensionality by regulating correlations across the network. Excitatory and inhibitory plasticity was required to shape spiking responses and task performance. Local synaptic patterns derived from model units predicted responses of auditory cortical neurons from *in vivo* whole-cell recordings during behavior. Our results indicate that potentially any neuron might be important for task performance, regardless of classical response properties.

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BOARD NUMBER: S06-622

AGE-RELATED CHANGES IN AUDITORY CORTICAL PROCESSING

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Aging is inevitable. It leads to deficits across various brain functions, including sensory domains. However, we know little about how aging can modify neural computations at the cellular and neural circuit levels. To address this issue, we investigated spontaneous activity in the mouse auditory cortex (AC) across age. Specifically, we compared neural activity in mice on C57BL/6 background ("C57") with F1 hybrid of C57BL/6 and CBA/Ca backgrounds ("Hybrid"). The former is known as a model of early age-related hearing loss, due to a genetic deficit at the cochlear level, whereas the latter does not exhibit such hearing loss. Thus, comparing these two strains allows to evaluate peripheral and central contributions of aging processes in the auditory system. We observed age-related and strain-specific changes in the activity of putative excitatory neurons in the AC: hybrid mice exhibited a steady reduction in their firing rate as age whereas spontaneous firing rate in C57 mice increased as age. We quantified the efficiency of individual spike trains by calculating an information theoretic metric, called contrast entropy. We found that although C57 mice did not show age-related change, hybrid mice exhibited a U-shape trend, namely contrast entropy significantly reduced only during the adult period, but not during the old period, compared to that during the young period. We suggest that peripheral and central aging processes have distinct impacts on the capacity of auditory cortical processing.

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BOARD NUMBER: S06-623

RAPID PLASTICITY IN THE INFERIOR COLLICULUS OF AWAKE, FREELY MOVING MICE

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Neural plasticity was previously thought to only occur during developmental but substantial evidence has since shown plastic changes are possible in the adult brain. However, the nature of this underlying plasticity remains largely unknown. Evidence of transient adult plasticity has been demonstrated to largely depend on input, specifically auditory exposure in the auditory cortex (Norena et al, 2006). There is also evidence that task specific adaptations are similarly represented, and in fact originate in the auditory midbrain, specifically the inferior colliculus (IC) (Slee & David 2015). Here we investigated whether transient plasticity could be reproduced in the IC following passive exposure to sounds with biased statistics and at what time scale this plasticity occurs. To this end we chronically implanted a 16 channel probe in the inferior colliculus (IC) of mice. The electrode can thus be connected to a wireless headstage that can be activated and monitored live without external interference from the experimenter. We passively exposed animals to hours of narrowband clouds of tones at non-traumatic sound levels and measured the subsequent effects on the spectrotemporal characteristics of neurons in the IC across a timescale of seconds, minutes and hours post exposure. These experiments are a proof of concept and serve as a stepping stone towards a better understanding of the underlying 'neural changes surrounding plasticity at subcortical levels. In addition, the directed manipulation of these plastic changes, even outside the developmental period, could yield results which may prove to be of clinical value for hearing rehabilitation strategies.

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ACTIVATION OF BASAL FOREBRAIN PARVALBUMIN NEURONS EXERTS FREQUENCY DEPENDENT EFFECTS ON AUDITORY PROCESSING AND BEHAVIOR IN THE RAT.

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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The basal forebrain (BF) has been implicated in attention and the modulation of information processing. While the BF provides the main cholinergic input to the cerebral cortex, it also harbors substantial GABAergic populations. Here we study how activation of the GABAergic population in the posterior nucleus basalis (pNB) of the BF impacts auditory pathway activity and behavior. We utilized a PV-Cre rat line to express channel rhodopsin (ChR2) uniquely in PV GABAergic neurons and recorded LFP and single unit activity simultaneously in the pNB, the medial geniculate nucleus (MGN), and the primary auditory cortex (A1). We utilized auditory band-pass noise stimuli covering the 1 kHz to 22 kHz frequency range, which was presented in conjunction with broad-band masking stimulus during the behavioral study. We present evidence for a tonotopic organization of auditory responsive neurons within pNB. Consistent with this, we show that optogenetic activation of pNB PV neurons leads to an increase in the spiking activity of single neurons in both MGN and A1 at their preferred frequency band as well as at adjacent frequency bands, but not distant bands. Detection of a narrow-band target sound in the presence of broad-band noise was compromised during pNB PV activation, consistent with a boosting of neural responses to both target and adjacent masking stimulus. Our findings support the idea that pNB modulation of auditory processing encompasses frequency-specific aspects, permitting precise regulation of sensory inputs rather than the mere enhancement of overall excitability of neurons in the auditory pathway.

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AUDITORY TETANIZATION EFFECTS IN HUMAN EVENT-RELATED POTENTIAL (ERP): LONG-TERM POTENTIATION (LTP) AND LATERAL INHIBITION

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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AIM: The plastic changes, resulting from differential exposure to various stimuli, are the basis of learning and memory, that help adapt to a changing environment. Rapid and short-term sensory stimulation/tetanization in humans is proposed to cause long-term potentiation (LTP) like effects similar to those caused by electrical tetanization previously rigorously studied at the cellular level in animals, thus bridging different approaches to learning and memory. METHODS: Twenty-seven participants (11 males, mean age 23.3 ± 5.6) took part in the study with 128-channels EEG registration. Pure tones(1020 Hz, 50ms) were presented every 75ms for 2 minutes(tetanization block). Oddball blocks preceded and followed the tetanization block. This block contained the same stimuli presented as deviant(1020 Hz, probability of 5-10%) interspersed with tones of adjacent frequencies: standard(1000 Hz, 80-90%) and deviant (980 Hz, 5-10%) tones. RESULTS: The N1 component of event-related potential(ERP) was attenuated to non-tetanized tones(both 980 and 1000Hz) over the majority of electrodes after tetanization. This change corresponds with the effect of lateral inhibition that plays a crucial role in the sharpening of the neuronal tuning to increase tone differentiation. Mismatch negativity(MMN) component in response to tetanized tone(1020Hz), while being similar to MMN for non-tetanized tone before tetanization, became larger than that after tetanization, pointing to the increase in its cortical differentiation. CONCLUSIONS: Short-term auditory tetanization changes the cortical representation of tones.

Pubmed:
34736295: Kleeva DF, Rebreikina AB, Soghoyan GA, Kostanian DG, Neklyudova AN, Sysoeva OV
Generalization of sustained neurophysiological effects of short-term auditory 13-Hz stimulation to neighbouring frequency representation in humans.
A fuller understanding of the effects of auditory tetanization in humans would inform better language and sensory learning paradigms; however, there are still unanswered questions. Here, we probe sustained changes in the event-related potentials (ERPs) to 1020- and 980-Hz tones following a rapid presentation of 1020-Hz tone (every 75 ms, 13.3 Hz, tetanization). Consistent with some previous studies, we revealed the increase in the P2 ERP component after tetanization. Contrary to some other studies, we did not observe the expected N1 increase after tetanization even in the identical experimental sequence. We detected a significant N1 decrease after tetanization. Expanding previous research, we showed that P2 increase and N1 decrease are not specific to the stimulus type (tetanized 1020 Hz and non-tetanized 980 Hz), suggesting the generalizability of tetanization effect to the not-stimulated auditory tones, at least to those of the neighbouring frequency. The ERPs' tetanization effects were observed for at least 30 min-the most prolonged interval examined, consistent with the duration of long-term potentiation, LTP. In addition, the tetanization effects were detectable in the blocks where the participants watched muted videos, an experimental setting that can be easily used in children and other challenging groups. Thus, auditory 13-Hz stimulation affects brain processing of tones including those of neighbouring frequencies.
Eur J Neurosci, 2022; 55

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MODULATION OF BRAIN CIRCUITS FOR SENSORY PROCESSING DURING SLEEP STATES

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Sleep is associated with a sensory disconnection from the environment, thought to be mediated by a thalamic gating of sensory-motor processing. Here, we investigated the thalamo-cortical circuit dynamics upon auditory stimulation across sleep states (i.e. wakefulness, NREM, and REM sleep) using single-unit activity and local field potential (LFP) activities recorded from chronically implanted electrodes in the primary auditory cortex (Au1), the central medial thalamus (CMT), the medial geniculate (MG), and hippocampus (HP) in freely-moving wild-type mice. We found similar auditory stimuli-evoked LFP responses during wakefulness and REM sleep whereas their amplitude was significantly higher during NREM sleep. Furthermore, we showed that animals significantly woke up from NREM sleep when the stimuli were delivered on the up phase of CMT slow waves, suggesting a key role for the CMT in mediating sensory-evoked arousals. confirmed by optogenetic silencing and a computational approach. Finally, to test whether the information associated with auditory cues – i.e., danger (conditional stimuli, CS+) versus safety (CS-) – was differentially processed during sleep, we performed an auditory cued fear conditioning followed by re-exposure to CS+ and CS- cues during subsequent sleep. Our results showed an increase in the percentage of awakening from the CS+ after the conditioning, suggesting that the discriminative ability persisted during NREM, but not, REM sleep. Interestingly, the CMT opto-silencing compromises this stimuli discrimination, decreasing the percentage of awakening specifically for the cued-stimuli (CS+). Taken together, our results showed that CMT neurons are central to the processing of environmental auditory cues associated with danger.

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BOARD NUMBER: S06-627

CATEGORY LEARNING OF FM SWEEPS IN MICE – BEHAVIORAL STRATEGY AND CORTICAL PLASTICITY

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Background and Methods: To make sense of highly complex environments, the brain uses categorization as a reduction mechanism. Category learning is the process of acquiring these rules of reduction, but the neural mechanisms underlying it are not well understood. Towards this goal, we studied how mice learn categories, and how are those represented in the cortex after learning. Using an automated learning platform, we trained mice to discriminate between two categories - rising frequency modulated (FM) sweeps and falling FM sweeps. We then challenged mice with a rich set of catch trials to decipher what they actually learned as the categorical cue. Next, we performed electrophysiological recording along different stations of the auditory cortex, in expert mice and naïve controls, using the multiarray silicon probe, Neuropixels. **Results:** Mice learned to discriminate efficiently between rising and falling FM sweeps as well as to generalize in response to novel stimuli. Using catch trials we found that mice used the frequency content of the FM sweep as the categorical boundary cue, rather than the slope of the sweep. Preliminary analyses of single neuron data shows increased responses to pure tones representing the category boundary as compared to naive mice, as well as changes in sparseness. **Conclusions**: Mice use the frequency component of sounds to categorize FM sweeps. Category learning is accompanied by plastic changes in the response profile of the single neurons in auditory cortex to sounds as simple as pure tones.

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BOARD NUMBER: S06-628

AUDITORY LEARNING DURING ADOLESCENCE

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Aims: Adolescence is known to be a period of uncertainty, exploration, and learning, yet there is little clarity about how even simple forms of learning change with development and are supported by neural circuits that undergo remodeling over the adolescent period. Here, we focused on mice and asked whether auditory learning in a go no-go task differs between adolescent and adult animals. **Methods:** To enable efficient learning of both adult and adolescent groups we trained mice to perform a go no-go task to discriminate among pure tones around a decision boundary of 10khz. We used four stimuli, two easy and two hard Go/No-go sound pairs, separated by 1 octave (easy) and 1/4 octave (hard), respectively. Training was carried out in a behavioral system allowing unrestrained learning environment. **Results:** While expert performance between adolescents and adults was similar, learning curves of adolescent mice were steeper in the easy discrimination pair. In contrast, expert performance of adolescent mice in the hard discrimination pair was inferior compared to adult mice. **Conclusions:** We identify clear differences between adolescent and adult auditory learning, setting the stage for our current electrophysiology study to measure the neural correlates of learning in adolescence compared to adulthood.

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BOARD NUMBER: S06-629

TEMPORAL AND RESOLVED HARMONIC PITCH ENCODING IN AUDITORY CORTEX

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Our perception of the tonal quality of sound is known as "pitch". Pitch is the foundation of musical melody, and plays a key role in human and animal communication. Previous research in ferrets has described neurons which are sensitive to the pitch cues of artificial vowels, which are distributed widely across the auditory cortex (Bizley *et al.*, 2010; Walker *et al.*, 2011), and which correlate with ferrets' trial-to-trial pitch judgments (Bizley *et al.*, 2013). However, these studies did not examine whether the "pitch-sensitive" neurons were able to maintain their tuning to a preferred fundamental frequency (F0) across a variety of stimuli ("pitch-selective" neurons), like those described in the marmoset pitch area (Bendor & Wang, 2005). Here, we investigated the resolved harmonic and temporal periodicity cues that underlie pitch responses in ferret auditory cortex. We recorded the responses of large populations of individual neurons to a variety of sounds that varied in F0 (17 F0 values; 250 - 4000 Hz). Neural responses were measured from ketamine/medetomidine anesthetized adult ferrets using high-channel-count multielectrodes (Neuropixels). We found that auditory cortical neurons often show greater sensitivity to temporal cues than resolved harmonic cues, in keeping with our recent behavioural findings (Walker et al., 2019). We also identified a subset of neurons with pitch-selective responses, similar to those previously reported in marmoset (Bendor & Wang, 2005), suggesting that specialized pitch processing also exists in non-primates.

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CORTICAL DEFICIT IN DISCRIMINATION BETWEEN COMMUNICATION SOUNDS IN A MOUSE LACKING TWO-PORE CHANNELS: A CALCIUM IMAGING AND ELECTROPHYSIOLOGICAL STUDY.

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Aims: Abnormal integration of sensory stimuli have been suggested to play a role in the altered social behavior of patients with autism spectrum disorder (ASD). This can be explained by altered responses in cortical networks. Here, we explored the role of the lysosomal calcium store in the cortical processing using a mouse deficient in endolysosomal two-pore channels (TPC KO), whose coding chromosomal regions in humans are associated with copy number variation and ASD. Compared with WT mice (C57BL/6), KO mice show deficits in monoamine concentration in temporal cortex. Despite similar audiograms as WT mice, we suspected that the cortical processing of conspecific vocalizations is impaired in KO mice. **Methods**: We investigated the functional role of TPCs in vocalization-induced responses in WT and KO mice in the primary auditory cortex (AI), using in vivo extracellular electrophysiology and two-photon calcium imaging (with GCaMP6s). **Results**: In two-photon calcium imaging, cortical neurons of TPC KO mice were less selective for mouse calls and responded to more categories of vocalization compared to WT mice. Electrophysiological recordings revealed that cortical neurons of TPC KO mice had lower temporal reliability to all communication sounds and showed reduced responses to mouse calls. Calcium imaging and electrophysiological data point out that neurons of TPC KO mice have a lower signal-to-noise ratio and degraded responses to pup calls compared to WT neurons. **Conclusion**: Our results show that, in mice, TPC channels have a functional role in the cortical processing of communication sounds, which could lead to deficits in social behavior.

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BOARD NUMBER: S06-631

DEVELOPMENT OF AN AWAKE ANIMAL MODEL FOR HYPERACUSIS SCREENING

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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For some people, auditory stimulation can be incredibly unpleasant, possibly painful, even at loudness levels considered normal by others. Epidemiological reports suggest that 2% to 15% of people experience a hypersensitivity to everyday sounds, ranging from discomfort to pain, named hyperacusis. This is, by far, the leading form of hypersensitivity to sound and there is currently no treatment for this condition. We still know little about the pathophysiological mechanisms underlying hyperacusis, in particular because we lack reliable animal models, for which the objective quantification of a subjective perception has proved difficult. Hyperacusis and tinnitus are frequently occurring concurrently, which complicates the deciphering of involved mechanisms. To investigate hyperacusis specifically, we thus exposed a cohort of awake mice to an acoustic trauma (2h of noise at 95dB SPL) leading to temporary hearing loss, which is known to induce either tinnitus or hyperacusis or both. Recent studies in animal models have suggested that the neural correlates of hyperacusis would be mediated by enhanced sound-evoked responses and neuronal spiking activities in the auditory cortex, i.e. hyperexcitability, as it is commonly defined. To confirm this hypothesis, we thus tested several behavioural measures as well as electrophysiological measures of the peripheral and cortical neural response to sounds in awake animals before and after trauma. Our goal is to find a combination of neural biomarkers and behavioural changes reliably characterizing the presence of hyperacusis. In particular, whereas auditory thresholds fully recovered after trauma, we observed modifications of the acoustic startle reflex, the behavioural response to sounds.

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DEVELOPMENTAL OVEREXPANSION OF CEREBRAL CORTEX IN MICE NEGATIVELY AFFECTS AUDITORY PROCESSING IN ADULTHOOD

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Cortical size has increased drastically in the course of evolution, and this expansion is thought to be advantageous. However, developmental expansion in cortical size can lead to megalencephaly, frequently found in neurodevelopmental disorders. Changes in the number and ratio of excitatory and inhibitory cortical neurons could lead to abnormal cortical connectivity, ultimately affecting the processing of sensory information. Even though both macrocephaly and atypical sensory processing were found in a high percentage of autism spectrum disorder (ASD) patients, the relationship between the number of cortical neurons and sensory processing is still uncertain. In the present study, we utilized a macrocephalic ASD-like mouse model to investigate how developmental overproduction of superficial cortical excitatory neurons affects auditory processing. We recorded neuronal population activity *in vivo*, simultaneously from the auditory cortex and auditory thalamus, and assessed auditory perception behaviourally. Our treated mice exhibited hyposensitivity to auditory stimuli. Specifically, they had a higher behavioural noise detection threshold, and a reduced spontaneous and auditory-evoked activity of putative excitatory neurons in the auditory cortex. Interestingly, medial geniculate nucleus cells did not show significant changes in activity. Treated mice also showed reduced monosynaptic connectivity within the auditory cortex. Computational modelling confirmed that this weak connectivity can lead to the reduction in excitatory activity. Altogether, our results suggest that an abnormal cortical expansion during development can result in atypical cortical connectivity, negatively affecting auditory cortical processing and behavioural auditory perception in adulthood.

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PREDICTIVE CODING OF GLOBAL SEQUENCE VIOLATION IN THE MOUSE AUDITORY CORTEX

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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The ability to extract temporal regularities at different time scales in sensory inputs and detect unexpected deviations is a key cognitive ability. The classical auditory oddball paradigm shows that the brain responds to sequence violations at a local time scale, such responses also occur under anesthesia and seem pre-attentive. In contrast, recent studies in humans and monkeys suggest that when the violation concerns regularities over longer time scales, responses to the violation appear only in conscious, attentive subjects. To investigate whether local and global sequence violation responses exist in the mouse, we recorded the auditory cortex using two-photon imaging while mice passively listened to repetitions of 1s-long sequences of five tones. The repeated short sequence contained either a single tone AAAAA or a local violation at its end AAAAB. Purely global violations could be generated by presenting occasionally the AAAAA sequence in a block where AAAAB is repeated. We found that a population of neurons responds to such purely global violations at the end of the AAAAA sequence. This population contained enough information to predict violations on single trials. These global responses were resistant to a wide increase of inter-sequence interval 1.5s - 25s ruling out that short-term adaptation. However, global responses vanished when the difference between A and B sounds is less salient to mice. These results establish that the mouse brain is able to detect global violations in sound sequences in a subgroup of auditory cortex neurons, paving the way for studying circuit mechanisms underlying long-term temporal regularity detection.

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BOARD NUMBER: S06-634

TONOTOPY AND POPULATION ACTIVITY CHARACTERISTICS OF AUDITORY CORTICOCOLLICULAR NEURONS

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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The auditory cortex (AC) modulates the upstream auditory pathway through descending corticofugal projections. Many of these arise from layer 5 of the AC and terminate in the inferior colliculus. These corticocollicular (CC) connections are involved in processing of complex sounds and auditory related learning. Despite their importance for the auditory system, their population activity patterns remain mainly uninvestigated. We used widefield and 2-photon calcium imaging in awake and anesthetized mice to record the activity of CC neurons after injection of AAV2-retro-GCaMP7f into the inferior colliculus. While widefield imaging revealed a global tonotopic order, local best frequency distribution on single cell level was quite heterogeneous for all AC subfields, yet still more ordered compared to random distributions. This indicates a subtle topographical order of CC neurons. Furthermore, we investigated population activity patterns of CC and surrounding non-CC neurons in response to complex sounds. We found stronger clusters of CC neurons compared to non-CC neurons, which respond more reliable and mostly integrate information over larger distances. This finding supports the idea of the “broadcast” function of L5 CC neurons (see for example: Williamson and Polley. 2019. Parallel pathways for sound processing and functional connectivity among layer 5 and 6 auditory corticofugal neurons. eLife. 8:1-21). Overall, our findings indicate distinct network mechanism of CC neurons in analyzing sound properties.

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AUDITORY CORTEX REPRESENTS AN ABSTRACT SENSORIMOTOR RULE

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Predicting the sensory consequences of one's actions is critical to perception and action in dynamically changing environments. For several established sensorimotor behaviours, such as locomotion and vocalizations, neural correlates of this prediction have been reported throughout sensory cortex. Oftentimes, however, the sensory consequences of an action are abstract, context-dependent, and newly learned, such as when one learns to play an instrument. How does cortex represent such complex sensorimotor rules? To investigate this question, we developed a task in which mice manipulated the frequency of an auditory cursor by licking either of two lick ports, in search for a rewarded target frequency. We then used behavioural manipulations, generalized linear models (GLMs) and two-photon calcium imaging to investigate how violations of this sensorimotor rule are represented in functionally defined auditory cortical regions, beyond purely sensory or movement-related effects. First, behavioural manipulations and GLMs suggest that trained mice monitored the ongoing stream of sounds to adaptively guide their lick behaviour. Second, we find that L2/3 excitatory neurons are sensitive to subtle violations of the sensorimotor rule, even when matching stimulus history and licking. These effects were more pronounced in higher-order subfield A2 than in the primary subfields A1 and AAF. Linear encoding models confirmed that neural responses to rule violations are better explained by sensorimotor prediction errors than by sensory or reward prediction errors. Together, these findings suggest that the relationship between actions and sensory feedback can shape responses along the sensory cortical hierarchy, even when this relationship is abstract and newly learned.

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BOARD NUMBER: S06-636

STIMULUS-SPECIFIC ADAPTATION (SSA) IN AUDITORY CORTEX IN AWAKE MICE UNDER 2-PHOTON MICROSCOPY

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Stimulus-specific adaptation (SSA) is the response reduction to a common stimulus that does not, or only partially generalize, to other rare stimuli. SSA has been proposed to be a correlate of 'deviance detection', an important computational task of sensory systems. Human Electrophysiological correlates of deviance detection have been studied extensively using various brain potentials. The best known is mismatch negativity (MMN), which peaks ~150–200 ms after the deviant stimulus. SSA is similar, but not identical, to MMN. One difference between SSA in single cells and MMN is their time course. SSA 'rides' on the early cortical responses to sounds, whereas MMN occurs about 100 ms later. Using fiber photometry of calcium signals in the mouse primary auditory cortex, we uncovered large and robust late response components which show deviance sensitivity at ~100-150 ms after stimulus onset. These signals are believed to reflect the average spiking activity of a local network. To search for the single-neuron basis of those late population responses, we used two-photon microscopy to resolve the responses of multiple adjacent neurons. In the anesthetized mouse, we observed population activity that was robust and resembled the late responses components found using fiber photometry. However, single neurons hardly showed auditory responses independent of their surrounding neuropile. We therefore recorded in awake, head-fixed mice. In the awake mouse, we found more single neurons that responded to auditory stimuli independently of the surrounding neuropile. The late responses were however highly variable. We suggest that the reproducible late response components observed in the population activity reflect neuronal ensembles' activity whose membership varies between trials.

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AGE-RELATED CHANGES IN THE STRUCTURE OF NEURONS IN THE AUDITORY CORTEX OF RATS

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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It has been shown previously that cortical neurons in rats undergo significant morphological age-related changes, e.g. decreasing density of the dendritic tree. However, only few studies described these morphological alterations in the auditory cortex (AC). Here, we aimed to evaluate age-related changes in the Long-Evans rats with the advent of modern morphometric techniques. Using the Golgi-Cox method and Neurolucida software, we analyzed morphology of pyramidal and non-pyramidal neurons in the AC of 3-month-old and 32-month old rats. We did not observe significant changes in the non-pyramidal neurons in any evaluated parameter. In contrast, pyramidal neurons exhibited apparent age-related degradation of the neuronal tree. Their basal dendrites were shorter, occupied less volume, had fewer spines, and had fewer endings. However, the surface of the somata or the length or volume of apical dendrites did not decrease significantly in old animals. These findings demonstrate that non-pyramidal neurons in the AC remain intact in terms of neuronal morphology, while pyramidal neurons show apparent degradation of branching complexity in basal dendrites. This study was supported by the Operational Programme Research, Development and Education in the framework of the project "Centre of Reconstructive Neuroscience", registration number CZ.02.1.01/0.0./0.0/15_003/0000419.

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NEURAL CORRELATES OF STREAM FORMATION DURING ACTIVE LISTENING IN THE FERRET AUDITORY CORTEX

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Listening in the real world involves making sense of mixtures of multiple overlapping sounds. The brain decomposes such scenes into individual objects, and a sequence of related auditory objects forms a stream. We are investigating the role of the auditory cortex in the formation and maintenance of auditory streams. The temporal coherence theory (Shamma et al., 2011) has provided one explanation for stream formation, postulating that the brain creates a multidimensional representation of sounds along different feature axes, and groups them based on their temporal coherence, to form streams. Supporting this idea, neural correlates of the differences in perception elicited by synchronous and alternating tone have been found in the primary auditory cortex of behaving ferrets (Lu et al., 2017). However, the temporal coherence theory has yet to be tested with more naturalistic sounds composed of multiple streams. To this end, we trained ferrets to detect a target word in a stream of repeating distractor words, spoken by the same talker, played in a background of spatially separated noise (White, pink and speech shaped noise). Neural data were collected in the auditory cortex of behaving ferrets. Neural population level analyses are being implemented to identify correlation structures. We hypothesize that when the animal can successfully segregate noise and speech streams, the neural population will be a uniform structure early in the trial, that will evolve into two distinct correlation structures. Stimulus reconstruction will be performed on the different clusters of neurons to investigate whether they encode for different auditory streams.

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MOTOR-RELATED PREDICTIONS IN MOUSE AUDITORY CORTEX ARE CONTEXT-DEPENDENT.

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Auditory perception relies on predicting the acoustic consequences of our actions. Correspondingly, neural circuits in the brain respond differently to expected versus unexpected self-generated sounds. In the real world, the same motor action can produce different sounds depending on the environment in which the behavior is produced – e.g. footsteps sound different when walking on concrete compared to fallen leaves. Yet it remains untested whether the brain can dynamically update predictions about self-generated sounds in a context-dependent manner and on an ethological timescale. To address this question, we developed a visual-acoustic virtual reality (VR), in which a head-fixed mouse on a treadmill repeatedly traverses two different environments, each consisting of a distinct visual corridor accompanied by distinct artificial footstep sounds. Following extensive behavioral acclimation, we made high-density neuronal recordings from auditory cortex as mice traversed VR and experienced either expected or deviant footsteps. We observe overall strong suppression of neural responses to self-generated sounds compared to the same sounds heard passively. When mice hear footstep sounds in the wrong visual context, neural responses are on average larger than when mice hear footstep sounds in the correct context, consistent with an expectation violation. Expectation violations emerge almost immediately after a mouse enters a new context, suggesting a rapid updating of predictions in parallel with behavior. Neurons with strong context-dependent modulation tend to reside in infragranular cortex. Together, our results suggest that the auditory cortex may combine auditory and motor signals with visual spatial cues for real-time, context-dependent processing of self-generated sounds.

Pubmed:
33621508: La Chioma A, Hübener M
Visual Cortex: Binocular Matchmaking.
Most binocular neurons in the mammalian visual cortex show matched selectivity for light stimuli presented through either eye. A recent study tracked the responses of individual neurons in early visual cortex over time, revealing that matched binocular selectivity develops through major rearrangements of binocular visual circuits.
Curr Biol, 2021; 31
33051348: La Chioma A, Bonhoeffer T, Hübener M
Disparity Sensitivity and Binocular Integration in Mouse Visual Cortex Areas.
Binocular disparity, the difference between the two eyes' images, is a powerful cue to generate the 3D depth percept known as stereopsis. In primates, binocular disparity is processed in multiple areas of the visual cortex, with distinct contributions of higher areas to specific aspects of depth perception. Mice, too, can perceive stereoscopic depth, and neurons in primary visual cortex (V1) and higher-order, lateromedial (LM) and rostrolateral (RL) areas were found to be sensitive to binocular disparity. A detailed characterization of disparity tuning across mouse visual areas is lacking, however, and acquiring such data might help clarifying the role of higher areas for disparity processing and establishing putative functional correspondences to primate areas. We used two-photon calcium imaging in female mice to characterize the disparity tuning properties of neurons in visual areas V1, LM, and RL in response to dichoptically presented binocular gratings, as well as random dot correlograms (RDC). In all three areas, many neurons were tuned to disparity, showing strong response facilitation or suppression at optimal or null disparity, respectively, even in neurons classified as monocular by conventional ocular dominance (OD) measurements. Neurons in higher areas exhibited broader and more asymmetric disparity tuning curves compared with V1, as observed in primate visual cortex. Finally, we probed neurons' sensitivity to true stereo correspondence by comparing responses to correlated RDC (cRDC) and anticorrelated RDC (aRDC). Area LM, akin to primate ventral visual stream areas, showed higher selectivity for correlated stimuli and reduced anticorrelated responses, indicating higher-level disparity processing in LM compared with V1 and RL. A major cue for inferring 3D depth is disparity between the two eyes' images. Investigating how binocular disparity is processed in the mouse visual system will not only help delineating the role of mouse higher areas for visual processing, but also shed light on how the mammalian brain computes stereopsis. We found that binocular integration is a prominent feature of mouse visual cortex, as many neurons are

selectively and strongly modulated by binocular disparity. Comparison of responses to correlated and anticorrelated random dot correlograms (RDC) revealed that lateromedial area (LM) is more selective to correlated stimuli, while less sensitive to anticorrelated stimuli compared with primary visual cortex (V1) and rostrolateral area (RL), suggesting higher-level disparity processing in LM, resembling primate ventral visual stream areas.
J Neurosci, 2020; 40
31422884: La Chioma A, Bonhoeffer T, Hübener M
Area-Specific Mapping of Binocular Disparity across Mouse Visual Cortex.
Depth perception is a fundamental feature of many visual systems across species. It is relevant for crucial behaviors, like spatial orientation, prey capture, and predator detection. Binocular disparity, the difference between left and right eye images, is a powerful cue for depth perception, as it depends on an object's distance from the observer [1,2]. In primates, neurons sensitive to binocular disparity are found throughout most of the visual cortex, with distinct disparity tuning properties across primary and higher visual areas, suggesting specific roles of different higher areas for depth perception [1-3]. Mouse primary visual cortex (V1) has been shown to contain disparity-tuned neurons, similar to those found in other mammals [4,5], but it is unknown how binocular disparity is processed beyond V1 and whether it is differentially represented in higher areas. Beyond V1, higher-order, lateromedial (LM) and rostrolateral (RL) areas contain the largest representation of the binocular visual field [6,7], making them candidate areas for investigating downstream processing of binocular disparity in mouse visual cortex. In turn, comparison of disparity tuning across different mouse visual areas might help delineating their functional specializations, which are not well understood. We find clear differences in neurons' preferred disparities across areas, suggesting that higher visual area RL is specialized for encoding visual stimuli very close to the mouse. Moreover, disparity preference is related to visual field elevation, likely reflecting an adaptation to natural image statistics. Our results reveal ethologically relevant areal specializations for binocular disparity processing across mouse visual cortex.
Curr Biol, 2019; 29

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DISTINCT NEUROPHYSIOLOGICAL RESPONSE MECHANISMS FOR NON-VERBAL AND VERBAL STIMULI IN AGE RELATED HEARING LOSS: A P300 STUDY

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Aims: Physiological mechanisms underlying the brain changes and the mechanistic basis of the association between age-related hearing loss (ARHL) and poorer speech performance remain unknown. We investigated the effects of non-verbal and verbal stimuli on the cognitive processing of auditory stimuli using a P300 paradigm in older adults. **Methods:** Sixteen older-adults with no history of neuropsychiatric disorders, or previous use of hearing aids were divided into hearing loss (HL) and normal hearing listeners (NHL) groups. HL was determined by pure tone average (PTA) thresholds at 0.5, 1, 2 and 4 kHz. PTAs ≥ 25 dB defined HL. P300 was recorded using non-verbal and verbal stimuli. For the speech stimuli, the /ba/ and /ta/ were the standard and deviant stimuli, respectively. The non-verbal paradigm comprised 1kHz/2kHz as standard/deviant stimuli. **Results**: PTA of the right ear was 35.94 dB and 17.50, in HL and NHL groups, and of left ear was 39.53 dB and 18.28 dB, respectively. Significant physiological changes were observed in the HL group, specifically a reduction in P300 amplitude with consonant-vowel stimuli compared with non-verbal stimuli, while a decrease in P300 latency was observed exclusively with verbal sounds. Similarly, in NHL group a peak amplitude pattern was observed, however both amplitude and latency increased, unlike hearing-impaired participants. **Conclusions:** Results support the idea that ARHL may differentially impact neural encoding of verbal auditory cues, providing evidence that auditory cortical processing is impaired due to ARHL. Furthermore, changes in P300 may play a valuable role as a brain-based biomarker for ARHL.

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BOARD NUMBER: S06-641

CORTICAL ADAPTATION TO SOUND REVERBERATION

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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In almost every natural environment, sounds are reflected by nearby objects, producing many delayed and distorted copies of the original sound, known as reverberation. Our brains usually cope well with reverberation, allowing us to recognize sound sources regardless of their environments. In contrast, reverberation can cause severe difficulties for speech recognition algorithms and hearing-impaired people. The present study examines how the auditory system copes with reverberation. We trained a linear model to recover a rich set of natural, anechoic sounds from their simulated reverberant counterparts. The model neurons achieved this by extending the inhibitory component of their receptive filters for more reverberant spaces, and did so in a frequency-dependent manner. These predicted effects were observed in the responses of auditory cortical neurons of ferrets in the same simulated reverberant environments. Together, these results suggest that auditory cortical neurons adapt to reverberation by adjusting their filtering properties in a manner consistent with dereverberation.

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BOARD NUMBER: S06-642

ALTERED SENSORY GATING IN PERSONS WITH TINNITUS IN RESPONSE TO REGULAR AND IRREGULAR AUDITORY ODDBALL SEQUENCES

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Aims: Maladaptive sensory gating (i.e., filtering-out irrelevant information) at the level of the thalamus is thought to be an underlying mechanism in persons with tinnitus. This mechanism can be detected by reduced suppression of P50 and N100 in response to (paired) repetitive auditory stimuli. Sensory gating is assumed to be influenced by temporal manipulation of auditory sequences. Therefore, the goal of the current study is to assess this process in persons with and without tinnitus using EEG. **Methods**: Modulations of P50 and N100 amplitudes were assessed in two oddball sequences in persons with tinnitus (n=25) and a control group (n=25) matched for age, sex, and hearing loss. We used a temporally regular sequence consisting of paired sinusoidal tones (standard, deviant) with fixed short (200 ms) intra-stimulus and long (700 ms) inter-stimulus intervals. In the temporally irregular sequence, the intra- and inter-stimulus intervals varied randomly between 100-300 ms and 350-1050 ms, respectively. **Results**: Preliminary data of ten participants suggest a trend toward altered sensory gating in persons with tinnitus as compared to controls. Specifically, the commonly found amplitude suppression of the P50 and N100 event-related potentials appears reduced in persons with tinnitus. **Conclusions**: The current findings enhance our understanding of the neurocognitive signatures underlying tinnitus. Maladaptive sensory gating might indicate dysfunctional central inhibition, suggesting modified sub-cortical network functioning. These results may eventually facilitate the development of new, targeted interventions.

Pubmed:
34595608: van Dun K, Brinkmann P, Depestele S, Verstraelen S, Meesen R
Cerebellar Activation During Simple and Complex Bimanual Coordination: an Activation Likelihood Estimation (ALE) Meta-analysis.
Bimanual coordination is an important part of everyday life and recruits a large neural network, including the cerebellum. The specific role of the cerebellum in bimanual coordination has not yet been studied in depth, although several studies indicate a differential role of the anterior and posterior cerebellum depending on the complexity of the coordination. An activation likelihood estimation (ALE) meta-analysis was used combining the data of several functional MRI studies involving bimanual coordination tasks with varying complexities to unravel the involvement of the different areas of the cerebellum in simple and complex bimanual coordination. This study confirms the general bimanual network as found by Puttemans et al. (Puttemans et al. in J Neurosci 25:4270-4278, 2005) and highlights the differences between preferred in-phase (simultaneous movements of homologous muscle groups) and anti-phase movement conditions (alternating movements of homologous muscle groups), and more complex, non-preferred bimanual movements (e.g., out-of-phase movements). Our results show a differential role for the anterior and posterior vermis in bimanual coordination, with a role for the anterior vermis in anti-phase and complex bimanual coordination, and an exclusive role for the posterior vermis in complex bimanual movements. In addition, the way complexity was manipulated also seems to play a role in the involvement of the anterior and posterior vermis. We hypothesize that the anterior vermis is involved in sequential/spatial control, while the posterior vermis is involved in temporal control of (bimanual) coordination, though other factors such as (visual) feedback and continuity of the movement also seem to have an impact. More studies are needed to unravel the specific role of the cerebellar vermis in bimanual coordination.
Cerebellum, 2021;
34126165: Brinkmann P, Rigoulot S, Kadi M, Schwartze M, Kotz SA, Dalla Bella S

About time: Ageing influences neural markers of temporal predictability.
Timing abilities help organizing the temporal structure of events but are known to change systematically with age. Yet, how the neuronal signature of temporal predictability changes across the age span remains unclear. Younger (n = 21; 23.1 years) and older adults (n = 21; 68.5 years) performed an auditory oddball task, consisting of isochronous and random sound sequences. Results confirm an altered P50 response in the older compared to younger participants. P50 amplitudes differed between the isochronous and random temporal structures in younger, and for P200 in the older group. These results suggest less efficient sensory gating in older adults in both isochronous and random auditory sequences. N100 amplitudes were more negative for deviant tones. P300 amplitudes were parietally enhanced in younger, but not in older adults. In younger participants, the P50 results confirm that this component marks temporal predictability, indicating sensitive gating of temporally regular sound sequences.
Biol Psychol, 2021; 163
33934235: Brinkmann P, Kotz SA, Smit JV, Janssen MLF, Schwartze M
Auditory thalamus dysfunction and pathophysiology in tinnitus: a predictive network hypothesis.
Tinnitus is the perception of a 'ringing' sound without an acoustic source. It is generally accepted that tinnitus develops after peripheral hearing loss and is associated with altered auditory processing. The thalamus is a crucial relay in the underlying pathways that actively shapes processing of auditory signals before the respective information reaches the cerebral cortex. Here, we review animal and human evidence to define thalamic function in tinnitus. Overall increased spontaneous firing patterns and altered coherence between the thalamic medial geniculate body (MGB) and auditory cortices is observed in animal models of tinnitus. It is likely that the functional connectivity between the MGB and primary and secondary auditory cortices is reduced in humans. Conversely, there are indications for increased connectivity between the MGB and several areas in the cingulate cortex and posterior cerebellar regions, as well as variability in connectivity between the MGB and frontal areas regarding laterality and orientation in the inferior, medial and superior frontal gyrus. We suggest that these changes affect adaptive sensory gating of temporal and spectral sound features along the auditory pathway, reflecting dysfunction in an extensive thalamo-cortical network implicated in predictive temporal adaptation to the auditory environment. Modulation of temporal characteristics of input signals might hence factor into a thalamo-cortical dysrhythmia profile of tinnitus, but could ultimately also establish new directions for treatment options for persons with tinnitus.
Brain Struct Funct, 2021; 226

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DEVELOPMENT OF HEMISPHERIC SPECIALIZATIONS: DIFFERENCES IN THE MATURATION OF THE LEFT AND RIGHT AUDITORY CORTEX

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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From humans to mice, the task of decoding vocalizations is divided into distinct computational functions in the left and right Auditory Cortices. A gap in current knowledge is how auditory cortical circuits develop specializations to subserve these lateralized functions. One possible mechanism that could lead to the emergence of specializations is differences in the timing of critical periods. Previous studies have identified the maturation of GABAergic tone as a key event in the onset and closure of the critical period, in particular parvalbumin positive (PV) interneurons. We compared the density of PV interneurons and their ensheathment by perineuronal nets before, during, and after the critical period (p10-p20), and found that the right ACx was consistently ahead of the left ACx in GABAergic development. This difference was also reflected in synaptic function: the right ACx had a higher frequency of spontaneous inhibitory postsynaptic currents. The appearance of myelin in cortex also marks the end of critical periods, and we found a faster rate of myelination in the right ACx. Differences were also found in glutamatergic signaling: we found higher intensity of the thalamocortical vesicular glutamate transporter (VGluT2) in the right ACx throughout the critical period. Finally, using cfos we found that the maturation of sound-evoked responses was also lateralized: the right ACx was preferentially responsive to frequency sweeps earlier than the left ACx was preferentially responsive to vocalizations. Our results suggest that the left ACx remains plastic longer to allow the development of specializations involved in vocalization processing.

Pubmed:
32116569: Neophytou D, Oviedo HV
Using Neural Circuit Interrogation in Rodents to Unravel Human Speech Decoding.
The neural circuits responsible for social communication are among the least understood in the brain. Human studies have made great progress in advancing our understanding of the global computations required for processing speech, and animal models offer the opportunity to discover evolutionarily conserved mechanisms for decoding these signals. In this review article, we describe some of the most well-established speech decoding computations from human studies and describe animal research designed to reveal potential circuit mechanisms underlying these processes. Human and animal brains must perform the challenging tasks of rapidly recognizing, categorizing, and assigning communicative importance to sounds in a noisy environment. The instructions to these functions are found in the precise connections neurons make with one another. Therefore, identifying circuit-motifs in the auditory cortices and linking them to communicative functions is pivotal. We review recent advances in human recordings that have revealed the most basic unit of speech decoded by neurons is a phoneme, and consider circuit-mapping studies in rodents that have shown potential connectivity schemes to achieve this. Finally, we discuss other potentially important processing features in humans like lateralization, sensitivity to fine temporal features, and hierarchical processing. The goal is for animal studies to investigate neurophysiological and anatomical pathways responsible for establishing behavioral phenotypes that are shared between humans and animals. This can be accomplished by establishing cell types, connectivity patterns, genetic pathways and critical periods that are relevant in the development and function of social communication.
Front Neural Circuits, 2020; 14

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HOW EMOTIONAL STATES SHAPE PERCEPTION: A MECHANISTIC UNDERSTANDING OF STATE DEPENDENT AUDITORY PROCESSING

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Our perception varies depending on our emotional state, adjusting our behavioral response to the same stimulus under different circumstances. These perceptual adaptations are especially extreme in aversive emotional states, such as chronic stress, that can threaten our survival. Stressful states frequently modify various features of sensory perception, such as reducing response thresholds and increasing sensitivity. In many cases, these perceptual adaptations increase our chances of evolutionary survival, helping us overcome dangerous situations; in other cases, they can exacerbate or even trigger mental disorders or sensory, such as PTSD or tinnitus. Although we have made tremendous progress in studying the central stress response system and its effect on learning and memory, **we do not yet possess a mechanistic understanding of how stressful states shape our perception.** *How is emotional state-information represented in our sensory systems? How do changes in emotional state shape perception?* To tackle this question, we developed a rodent auditory behavioral approach that enables direct evaluation of perception. We coupled these behavioral paradigms to manipulations of the animal's emotional state and, using two-photon calcium imaging, detailed probing of neural activity modulations at the network and cell-specific levels in the auditory cortex. Our findings indicate that stress modulates differently spontaneous and tone evoked activity of inhibitory and excitatory cells. Moreover, we found changes in perception as a result of stress induced changes to cortical activity. Our findings could have clinical implications by explaining how stressful states distort the neural representation of sensory stimuli and alter our perception of the world.

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LEARNING TRAJECTORY PREDICTS THE LEVEL OF RECRUITMENT OF A PRIMARY SENSORY CORTEX IN A NEGATIVE REINFORCEMENT BEHAVIORAL PARADIGM

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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It is thought that learning to associate sensory stimulus and action induces shifts in sensory representations towards the learned stimuli. This was observed in numerous studies, multiple brain areas, including the auditory cortex (A1). In the latter, the resulting modifications are believed to reflect plastic changes that prepare the brain to better perceive relevant sensory cues. However, other efficient auditory pathways coexist, and precise optogenetic inactivation of A1 has shown that A1 is not even required for some simple sound-action associations. Even when A1 remains active, recent chronic imaging studies have revealed a somewhat less consensual view of learning-induced changes in A1, failing to demonstrate permanent changes in the selectivity of individual neurons after training. What determines the presence or absence of learning-related plasticity in A1 during a sound-action association? Recent high-throughput behavioral approaches have tested among large cohorts the role of task difficulty in A1 plasticity recruitment. But no clear relationship has been found between average A1 change and behavioral performance. Here, using the statistical power of chronic two-photon imaging of large neuronal assemblies in mice, we examine the possibility that the difficulty of a task, as experienced by each mouse, may *individually* determine the extent of A1-plasticity induced. We found a clear parallel between the learning trajectories of individual mice and the *permanent* change of sound representation in A1, as measured in a task-irrelevant context. Our results reconcile the heterogeneous observations that learning sound-action associations can have diverse consequences for A1 selectivity remodeling.

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DIFFERENCES IN AUDITORY TEMPORAL PROCESSING IN THE LEFT AND RIGHT AUDITORY CORTICES OF THE RAT

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Aims In the present study, we examined the hemispheric differences in central auditory processing of temporally structured stimuli in the adult rats. **Methods** Evoked neuronal responses to temporally structured stimuli (sinusoidally frequency modulated [FM] tones, frequency sweeps, amplitude modulated [AM] tones and noise, click trains with constant and variable inter-click intervals) and natural vocalization were recorded from the left (LAC) and right (RAC) auditory cortices in adult (4–6 months old) anaesthetized F344 rats. Using vector strength, modulation-transfer functions, van Rossum distances, and direction-selectivity index (DSI), synchronization with stimulus structure and response reliability were compared in the LAC and the RAC. **Results** The RAC exhibits a higher synchronization ability for all periodic stimuli except AM tones. Reproducibility of responses to periodic stimuli was also better in the RAC. On the other hand, the LAC mostly shows higher relative response magnitudes to temporally structured stimuli. The responses to vocalizations were similar in both hemispheres, however, the RAC exhibited a higher response to the onset of the second bout. The proportion of direction-selective neurons was higher in the RAC. **Conclusions** The results show that coding of temporally structured stimuli in the RAC is based more on the temporal code, while the LAC is more focused on the rate code. Direction selectivity is more developed in the RAC. These results confirm and extend our previous findings obtained both electrophysiologically and behaviorally. This work is supported by the project INTER-ACTION LTAIN 19201.

Pubmed:
34311267: Bures Z, Pysanenko K, Syka J
The influence of developmental noise exposure on the temporal processing of acoustical signals in the auditory cortex of rats.
Previous experiments have acknowledged that inappropriate or missing auditory inputs during the critical period of development cause permanent changes of the structure and function of the auditory system (Bures et al., 2017). We explore in this study how developmental noise exposure influences the coding of temporally structured stimuli in the neurons of the primary auditory cortex (AC) in Long Evans rats. The animals were exposed on postnatal day 14 (P14) for 12 minutes to a loud (125 dB SPL) broad-band noise. The responses to an amplitude-modulated (AM) noise, frequency-modulated (FM) tones, and click trains, were recorded from the right AC of rats of two age groups: young-adult (ca. 6 months old) and adult (ca. 2 years old), both in the exposed animals and in control unexposed rats. The neonatal exposure resulted in a higher synchronization ability (phase-locking) of the AC neurons for all three stimuli; furthermore, the similarity of neuronal response patterns to repetitive stimulation was higher in the exposed rats. On the other hand, the exposed animals showed a steeper decline of modulation-transfer functions towards higher modulation frequencies/repetition rates. Differences between the two age groups were also apparent; in general, aging had qualitatively the same effect as the developmental exposure. The current results demonstrate that brief noise exposure during the maturation of the auditory system influences both the temporal and the rate coding of periodically modulated sounds in the AC of rats; the changes are permanent and observable up to late adulthood.
Hear Res, 2021; 409
33777134: Pysanenko K, Rybalko N, Bureš Z, Šuta D, Lindovský J, Syka J
Acoustically Enriched Environment during the Critical Period of Postnatal Development Positively Modulates Gap Detection and Frequency Discrimination Abilities in Adult Rats.
Throughout life, sensory systems adapt to the sensory environment to provide optimal responses to relevant tasks. In the case of a developing system, sensory inputs induce changes that are permanent and detectable up to adulthood. Previously, we have shown that rearing rat pups in a complex acoustic environment (spectrally and temporally modulated sound) from postnatal day 14 (P14) to P28 permanently improves the response characteristics of neurons in the inferior colliculus and

auditory cortex, influencing tonotopical arrangement, response thresholds and strength, and frequency selectivity, along with stochasticity and the reproducibility of neuronal spiking patterns. In this study, we used a set of behavioral tests based on a recording of the acoustic startle response (ASR) and its prepulse inhibition (PPI), with the aim to extend the evidence of the persistent beneficial effects of the developmental acoustical enrichment. The enriched animals were generally not more sensitive to startling sounds, and also, their PPI of ASR, induced by noise or pure tone pulses, was comparable to the controls. They did, however, exhibit a more pronounced PPI when the prepulse stimulus was represented either by a change in the frequency of a background tone or by a silent gap in background noise. The differences in the PPI of ASR between the enriched and control animals were significant at lower (55 dB SPL), but not at higher (65-75 dB SPL), intensities of background sound. Thus, rearing pups in the acoustically enriched environment led to an improvement of the frequency resolution and gap detection ability under more difficult testing conditions, i.e., with a worsened stimulus clarity. We confirmed, using behavioral tests, that an acoustically enriched environment during the critical period of development influences the frequency and temporal processing in the auditory system, and these changes persist until adulthood.
Neural Plast, 2021; 2021

32709399: Bureš Z, Pysanenko K, Syka J

Age-related changes in the temporal processing of acoustical signals in the auditory cortex of rats.

Age-related hearing loss is manifested primarily by a decreased sensitivity to faint sounds, that is, by elevation of the hearing thresholds. Nevertheless, aging also affects the ability of the auditory system to process temporal parameters of the sound stimulus. To explore the precision and reliability of auditory temporal processing during aging, responses to several types of sound stimuli were recorded from neurons of the auditory cortex (AC) of young and aged anaesthetized Fischer 344 rats. In response to broad-band noise bursts, the aged rats exhibited larger response magnitudes, a higher proportion of monotonic units, and also a larger variability of response magnitudes, suggesting a lower stability of the rate code. Of primary interest were the responses to temporally structured stimuli (amplitude-modulated (AM) noise, frequency-modulated (FM) tones, and click trains) recorded separately in the right and left AC. Significant differences of temporal processing were already found between the neuronal responses in the left and right AC in the young animals: for the click trains, the left hemisphere exhibited a greater responsiveness to higher repetition rates, lower vector strength values, and a lower similarity of responses. The two hemispheres were also affected differently by aging. In the right hemisphere, neurons in the aged animals displayed worse synchronization with the AM noise and clicks, but better synchronization with the FM tone. In the left hemisphere, neuronal synchronization with the stimulus modulation improved at a higher age for all three stimuli. The results show that the ability of the aging auditory system to process temporal parameters of the stimulus strongly depends on the stimulus type and on laterality. Furthermore, the commonly reported age-related decline in the temporal processing ability cannot be regarded as general as, at least at the neuronal level in the AC, objective measures of the temporal representation often exhibit age-related improvement instead of deterioration.
Hear Res, 2021; 402

30541910: Macova I, Pysanenko K, Chumak T, Dvorakova M, Bohuslavova R, Syka J, Fritzsch B, Pavlinkova G

Neurod1 Is Essential for the Primary Tonotopic Organization and Related Auditory Information Processing in the Midbrain.

Hearing depends on extracting frequency, intensity, and temporal properties from sound to generate an auditory map for acoustical signal processing. How physiology intersects with molecular specification to fine tune the developing properties of the auditory system that enable these aspects remains unclear. We made a novel conditional deletion model that eliminates the transcription factor NEUROD1 exclusively in the ear. These mice (both sexes) develop a truncated frequency range with no neuroanatomically recognizable mapping of spiral ganglion neurons onto distinct locations in the cochlea nor a cochleotopic map presenting topographically discrete projections to the cochlear nuclei. The disorganized primary cochleotopic map alters tuning properties of the inferior colliculus units, which display abnormal frequency, intensity, and temporal sound coding. At the behavioral level, animals show alterations in the acoustic startle response, consistent with altered neuroanatomical and physiological properties. We demonstrate that absence of the primary afferent topology during embryonic development leads to dysfunctional tonotopy of the auditory system. Such effects have never been investigated in other sensory systems because of the lack of comparable single gene mutation models. All sensory systems form a topographical map of neuronal projections from peripheral sensory organs to the brain. Neuronal projections in the auditory pathway are cochleotopically organized, providing a tonotopic map of sound frequencies. Primary sensory maps typically arise by molecular cues, requiring physiological refinements. Past work has demonstrated physiologic plasticity in many senses without ever molecularly undoing the specific mapping of an entire primary sensory projection. We genetically manipulated primary auditory neurons to generate a scrambled cochleotopic projection. Eliminating tonotopic representation to auditory nuclei demonstrates the inability of physiological processes to restore a tonotopic presentation of sound in the midbrain. Our data provide the first insights into the limits of physiology-mediated brainstem plasticity during the development of the auditory system.
J Neurosci, 2019; 39

30204463: Lindovský J, Pysanenko K, Popelář J, Syka J
Fast tonotopy mapping of the rat auditory cortex with a custom-made electrode array.
We present a custom-made multielectrode array for the recording of evoked potentials during acute experiments in rats, which offers a quick and reliable estimation of the cortical tonotopy. The array consists of electrodes represented by insulated copper wires of 0.09 mm diameter fixed in epoxy resin in a 3 x 5 arrangement, with final impedances of 410-800 kOhm. The array was placed on the brain surface of anesthetized rats approximately at the location of the auditory cortex (AC) and the cortical evoked potentials (middle-latency responses, MLR) were elicited by a series of tone pips of different frequencies at 50 dB of sound pressure level (SPL) intensity. The frequency that evoked the highest MLR amplitude (best frequency, BF) was identified for each electrode. The obtained distribution of the BFs characterized the cortical tonotopy, and it correlated with the frequency selectivity of neurons recorded at the same positions by an extracellular microelectrode. Although the space resolution of the array did not allow for the identification of AC sub regions, the array proved to be a reliable tool for a quick estimation and prediction of areas of interest for the subsequent measurements of neurons by more precise techniques.
Physiol Res, 2018; 67
30002673: Bureš Z, Pysanenko K, Lindovský J, Syka J
Acoustical Enrichment during Early Development Improves Response Reliability in the Adult Auditory Cortex of the Rat.
It is well known that auditory experience during early development shapes response properties of auditory cortex (AC) neurons, influencing, for example, tonotopical arrangement, response thresholds and strength, or frequency selectivity. Here, we show that rearing rat pups in a complex acoustically enriched environment leads to an increased reliability of responses of AC neurons, affecting both the rate and the temporal codes. For a repetitive stimulus, the neurons exhibit a lower spike count variance, indicating a more stable rate coding. At the level of individual spikes, the discharge patterns of individual neurons show a higher degree of similarity across stimulus repetitions. Furthermore, the neurons follow more precisely the temporal course of the stimulus, as manifested by improved phase-locking to temporally modulated sounds. The changes are persistent and present up to adulthood. The results document that besides basic alterations of receptive fields presented in our previous study, the acoustic environment during the critical period of postnatal development also leads to a decreased stochasticity and a higher reproducibility of neuronal spiking patterns.
Neural Plast, 2018; 2018
29229554: Pysanenko K, Bureš Z, Lindovský J, Syka J
The Effect of Complex Acoustic Environment during Early Development on the Responses of Auditory Cortex Neurons in Rats.
Acoustical environment plays an important role during the maturation of the auditory system. It has been shown that the sensory inputs to the developing centres influence the development of the structure of projections, neuronal responsiveness, excitatory-inhibitory balance, or tonotopical arrangement, throughout the auditory pathway. Our previous study (Bures et al., 2014) showed that rats reared in a complex acoustic environment (spectrally and temporally modulated sound reinforced by an active behavioural paradigm with a positive feedback) exhibit permanently improved response characteristics of the inferior colliculus (IC) neurons. Extending these results, the current work provides evidence that the changes occur also at the level of auditory cortex (AC). In particular, the enriched animals have lower excitatory thresholds, sharper frequency selectivity, and a lower proportion of non-monotonic rate-intensity functions. In contrast to the changes observed in the IC, the cortical neurons of enriched animals have lower response magnitudes. In addition, the enrichment changed the AC responsiveness to frequency-modulated and also to a lesser extent, amplitude-modulated stimuli. Significantly, the alterations span the entire hearing range and may be regarded as general and not directly linked to the characteristics of the acoustical stimulation. Furthermore, these developmentally induced changes are permanent and detectable in adulthood. The findings indicate that an acoustically enriched environment during the critical period of postnatal development influences basic properties of neuronal receptive fields in the AC, which may have implications for the ability to detect and discriminate sounds.
Neuroscience, 2018; 371
26631689: Popelář J, Šuta D, Lindovský J, Bureš Z, Pysanenko K, Chumak T, Syka J
Cooling of the auditory cortex modifies neuronal activity in the inferior colliculus in rats.
There are powerful pathways descending from the auditory cortex (AC) to the inferior colliculus (IC), yet their function is not fully understood. The aim of this study is to examine the effects of a reversible cortical inactivation, achieved by cooling of the AC, on the responses of neurons in the rat IC. Extracellular single-unit or multi-unit activity was recorded in the IC of anaesthetized rats with a 16-channel multielectrode probe introduced along the IC dorso-ventral axis through the dorsal cortex (DCIC) to the central nucleus of the IC (CIC). Cooling of the AC produced an increase in spontaneous activity and magnitude of the sound-evoked response in 47% of the IC neurons. Maximal changes in the neuronal activity were observed in the DCIC and the central part of the CIC. The final segments of the sustained responses to 60 ms stimuli and the off responses were more affected than the onset segments. Inactivation of the AC resulted in a suppression of the post-

excitatory inhibition and neuronal adaptation, which was reflected in a pronounced enhancement of synchronized responses to a series of fast repeated clicks. The response parameters recovered, at least partly, to the pre-cooling levels 1 h after the cooling cessation. The frequency tuning properties of the IC neurons did not show any significant changes during the cooling period. The results demonstrate that AC cooling inactivates excitatory corticofugal pathways and results in a less activated intrinsic inhibitory network in the IC.
Hear Res, 2016; 332
23175852: Hruskova B, Trojanova J, Kulik A, Kralikova M, Pysanenko K, Bures Z, Syka J, Trussell LO, Turecek R
Differential distribution of glycine receptor subtypes at the rat calyx of Held synapse.
The properties of glycine receptors (GlyRs) depend upon their subunit composition. While the prevalent adult forms of GlyRs are heteromers, previous reports suggested functional α homomeric receptors in mature nervous tissues. Here we show two functionally different GlyRs populations in the rat medial nucleus of trapezoid body (MNTB). Postsynaptic receptors formed α1/β-containing clusters on somatodendritic domains of MNTB principal neurons, colocalizing with glycinergic nerve endings to mediate fast, phasic IPSCs. In contrast, presynaptic receptors on glutamatergic calyx of Held terminals were composed of dispersed, homomeric α1 receptors. Interestingly, the parent cell bodies of the calyces of Held, the globular bushy cells of the cochlear nucleus, expressed somatodendritic receptors (α1/β heteromers) and showed similar clustering and pharmacological profile as GlyRs on MNTB principal cells. These results suggest that specific targeting of GlyR β-subunit produces segregation of GlyR subtypes involved in two different mechanisms of modulation of synaptic strength.
J Neurosci, 2012; 32

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BRAIN PLASTICITY IN UNILATERAL DEAFNESS AND ITS RESTORATION WITH A COCHLEAR IMPLANTATION

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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A cortical lateralization is a functional organization observed in most sensory modalities and in most evolved species. In the auditory domain, the cerebral lateralization is devolved to spatial hearing, with each hemisphere primarily involved in processing the localization of sound in contralateral space. The representation of the contralateral auditory field is underpinned by contralateral aural dominance which results from complex neural interactions between inputs from each ear. We recently showed (Vannson et al 2020) that unilateral hearing loss induces deficits in binaural integration (spatial localization and speech understanding in noise) but also reverses contralateral aural dominance in favor of the preserved ipsilateral ear. The extent of this brain reorganization is directly correlated to the extent of the behavioral deficit in sound localization. A second study in a group of subjects with asymmetric hearing loss treated with a cochlear implant demonstrated that restoration of auditory inputs to the deaf ear through electrical stimulation restored contralateral hemispheric dominance of both the better and impaired ear. Finally, mirroring what was observed in patients with unilateral hearing loss, the extent of restoration of contralateral dominance was directly correlated with the ability to localize sounds after implantation. Altogether, we clearly demonstrated a link between brain reorganization and spatial auditory performance in deafness. Our results are crucial for further progress in the rehabilitation of unilaterally deaf patients and show that the success of rehabilitation depends mainly on brain plasticity mechanisms, and that the restoration of contralateral dominance is essential for an optimal functional recovery.

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NEURAL CORRELATES OF PERCEPTUAL INVARIANCE IN THE FERRET AUDITORY CORTEX

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Perceptual invariance, the act of recognising auditory objects across identity-preserving variation and in the presence of other auditory stimuli, is critical to everyday listening. In this study, we tested whether perceptual invariance towards an auditory object can be established in the ferret auditory cortex (AC) and whether the AC encodes auditory object perception. To test perceptual invariance, we trained four ferrets in a Go/No-Go task where ferrets identified a target word ("instruments") from a stream drawn from 54 other British English words (distractors) before manipulating the mean fundamental frequency (F0) within and across trials.
We recorded AC neural activity using an Omnetics-WARP32 implant in one ferret and considered sites with a sound-onset response for Euclidean distance decoding. We computed a decoding score for pairwise discrimination of the target word from seven distractors, the target word reversed, and pink noise spectro-temporally matched to the target word. Decoder performance was above-chance and invariant when classifying between F0-manipulated distractor and target stimuli for both F0 manipulations across and within trials, whereas the discrimination performance of the neural responses of distractor versus distractor words was below-chance for most sites. Most sites decoded the behavioural perception of the target as the target or distractor at above-chance levels for both F0-manipulated and control trials.
Our preliminary results suggest that auditory objects are represented in the AC, that these neural responses are resistant to F0 change, and align with the behavioural perception of auditory stimuli rather than the ground-truth classification of the stimuli itself.

Pubmed:
31920542: Korrapati S, Taukulis I, Olszewski R, Pyle M, Gu S, Singh R, Griffiths C, Martin D, Boger E, Morell RJ, Hoa M
Single Cell and Single Nucleus RNA-Seq Reveal Cellular Heterogeneity and Homeostatic Regulatory Networks in Adult Mouse Stria Vascularis.
The stria vascularis (SV) generates the endocochlear potential (EP) in the inner ear and is necessary for proper hair cell mechanotransduction and hearing. While channels belonging to SV cell types are known to play crucial roles in EP generation, relatively little is known about gene regulatory networks that underlie the ability of the SV to generate and maintain the EP. Using single cell and single nucleus RNA-sequencing, we identify and validate known and rare cell populations in the SV. Furthermore, we establish a basis for understanding molecular mechanisms underlying SV function by identifying potential gene regulatory networks as well as druggable gene targets. Finally, we associate known deafness genes with adult SV cell types. This work establishes a basis for dissecting the genetic mechanisms underlying the role of the SV in hearing and will serve as a basis for designing therapeutic approaches to hearing loss related to SV dysfunction.
Front Mol Neurosci, 2019; 12

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FERRETS CAN CATEGORIZE SPOKEN WORDS BASED ON SPECTRO-TEMPORAL CUES

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

Quentin Gaucher[1], Sam Norman-Haignere[1,2,3], Shihab Shamma[1,4], Yves Boubenec[1]
[1]Ecole Normale Superieure, PSL University, CNRS, Lsp, Paris, France, [2]HHMI Postdoctoral Fellow, Life Sciences Research Foundation, Baltimore, United States of America, [3]Zuckerman Mind Brain Behavior Institute, Columbia University, New York, United States of America, [4]University of Maryland, Neural Systems Laboratory, College Park, United States of America

Grouping stimuli into abstract categories is a fundamental task of sensory systems. In the context of speech perception, the neuronal processes underlying the ability to reliably identify words from various speakers are not fully explained. Studies focusing on cortical fMRI responses have shown that secondary auditory areas respond differently to speech and artificial stimuli capturing the spectro-temporal modulations of speech (“model-matched stimuli”), suggesting that speech recognition depends on higher order cues represented in secondary auditory areas. This result was not replicated in naive ferrets. In this pilot study, we investigated whether ferrets can be trained to categorize human words and if this ability rely on higher order cues. One ferret was trained to discriminate fifteen versions of one human word (“target word”) against 50 other words (“non-target word”) in a two-alternative forced choice paradigm. When the animal was successfully trained, unrewarded probe trials were introduced to test the animal’s response to unknown versions of the target word, unknown words, model-matched versions of the target word, model-matched version of the non-target word, and model-matched version of an unknown word. Our behavioral results show that ferrets can be trained to reliably classify human words. The ferret significantly classified model-matched versions of the target and non-target words into the corresponding categories. These results could be partially mimicked by a linear classifier trained on modeled cortical representations of the target and non-target words. These results suggest that, even after training, ferrets rely on spectro-temporal cues to categorize new complex acoustic stimuli.

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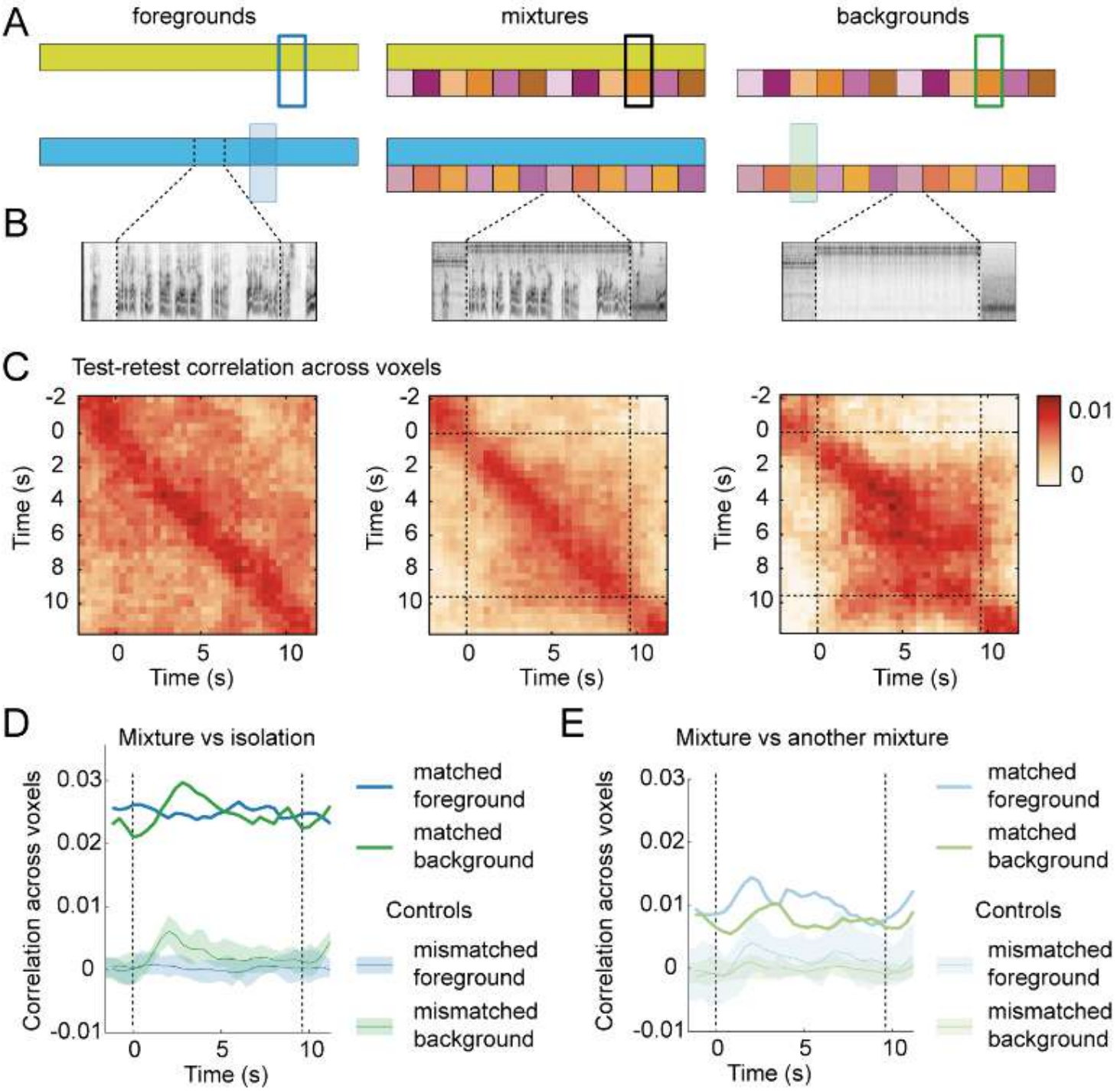
CORTICAL REPRESENTATIONS OF BACKGROUND AND FOREGROUND SOUNDS IN DYNAMICALLY-CHANGING SOUND MIXTURES

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

Yves Boubenec, Agnès Landemard
ENS, Cognitive Studies, Paris, France

Humans and other animals constantly filter out complex background sounds in order to extract behaviorally-relevant signals of interest. The stage at which invariant representations emerge remains unclear, as adaptation to background noise has been observed at various levels in the auditory hierarchy. Using functional Ultrasound imaging (fUS), a recently developed technique based on blood flow imaging, we investigated responses to mixtures of natural foregrounds and backgrounds in ferret auditory cortex. Our paradigm consisted in presenting continuous mixture streams with periodic changes in background, as well as both components in isolation (**A**). Foregrounds included speech, music, and other non-stationary sounds, while backgrounds were chosen among the most stationary natural sounds, typically similar to sound textures (**B**). We found that fUS activity dynamically encoded changes in the stimuli (**C**) in a way that reflected stimulus stationarity, making it a powerful tool to study the dynamics of adaptation. We found that both background and foreground information were continuously encoded in cortical activity (**D-E**). In both primary and nonprimary regions, we observed overlapping populations encoding both foregrounds and backgrounds. Our results suggest that auditory cortex builds invariant representations by simultaneously storing a representation of complex backgrounds. Decoupling of signals can be operated by voxels' tuning to features that are characteristic of each type of

sounds.


A
foregrounds
mixtures
backgrounds
B
C
Test-retest correlation across voxels
Time (s)
0.01
0
D
Mixture vs isolation
Correlation across voxels
matched foreground
matched background
Controls
mismatched foreground
mismatched background
E
Mixture vs another mixture

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ANATOMICAL ORGANIZATION OF THE MOUSE AUDITORY-PREFRONTAL CIRCUIT

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

Felix Jung[1], Moritz Stingl[1], Sofie Ährlund-Richter[2], Josina Van Lunteren[1], Marina Slashcheva[1], Pierre Le Merre[1], Konstantinos Meletis[1], Marie Carlen[1]
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The prefrontal cortex (PFC) in the frontal lobes of the mammalian brain is considered to constitute the highest stage of cognitive processing and to be critically involved in the generation and evaluation of adaptive and intentional behaviors. Fundamental to these processes is the integration of sensory information (feedforward) with state and goal information in prefrontal networks. The processing of sensory information in primary sensory cortices is heavily influenced by predictive signals generated in the PFC that are transmitted to sensory cortices (feedback).
In the present study, we aimed at dissecting the anatomical organization of both feedforward and feedback connections in the auditory-prefrontal circuit. By combining viral tools for anterograde and retrograde circuit tracing in combination with semi-automated cell segmentation and brain registration to the Allen Common Coordinate Framework, we generated a whole-brain input-output connectivity map of the auditory-prefrontal circuit.
Our results highlight the central role of the orbital subregions of the PFC as a putative hub for input-output transformation of auditory information as these regions are densely innervated by auditory axons and in turn possess the highest number of cells projecting to the auditory cortices. In conclusion, our results identified key nodes of the auditory-prefrontal circuit opening the possibility for its functional interrogation by future research.

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INHIBITING PRESYNAPTIC CALCIUM CHANNEL MOBILITY IN THE AUDITORY CORTEX SUPPRESSES SYNCHRONIZED INPUT PROCESSING

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

Katrina Deane[1,2], Ruslan Klymentiev[1,2], Jennifer Heck[3], Melanie Mark[4], Frank Ohl[1,3], Martin Heine[5], Max Happel[6]
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Voltage gated calcium channels (VGCCs) trigger the influx of calcium to the presynaptic bouton during action-potential depolarization to release neurotransmitters, which happens probabilistically. When neuronal VGCCs were acutely aggregated using the optogenetic clustering of an cryptochrome mutant , CRY2olig, cultured primary hippocampal neurons generated a strong and reliable paired-pulse depression of consecutive responses compared to controls. While this method was used *in vitro* to render single cell firing to be more predictable, our aim was to observe how this would affect a neuronal network *in vivo*. In the primary auditory cortex (A1), we coupled VGCCs to Cry2olig via a feed-back-controlled anti GFP-intrabody, delivered by lentivirus, into a transgenic mouse model with a Citrine tag on the N terminus of all VGCCs. We recorded local field potentials down the depth of the A1 using a shaft electrode and transformed these into current source density (CSD) profiles over consecutive measurements under ketamine anesthesia before and after VGCC aggregation. Cortical response to auditory stimuli in the form of click trains and amplitude modulated tones was recorded. We demonstrated through cortical layer activity and average rectified CSD profiles VGCC clustering caused a significant suppression of response to both stimuli mainly due to suppressed sensory impulse responses. Our findings reveal the importance VGCC variability in neuronal networks and provide evidence that their membrane mobility may serve as an important maintenance feature during sensory encoding by dynamically adjusting network activity—especially in cases of very high cortical recruitment when the network is less capable of adapting.

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LONG-TERM INTEGRATION OF SEQUENTIAL INFORMATION IN A SONGBIRD AUDITORY-PREMOTOR NUCLEUS

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Human speech and vocalizations of some non-human animal species consist of hierarchically organized sequences of vocal sounds that convey meaning in both their acoustic features and their sequential patterns. Computation of short and long-range statistical regularities has been proposed as a solution to extract information from these vocal streams. Humans are capable of computing not only adjacent sound regularities, transitional probabilities being higher between two syllables within words than at word boundaries, but also regularities among words that are quite distant from one another in a sentence. Understanding how the brain encodes statistically structured sequences of sounds, remains to be investigated, especially at the neuronal level. To address this issue, we took advantage of the complex sequential structure of canary song, in which the ordering of song elements, called phrases, depends on transitional probabilities and leads to the formation of recurrent sequences of two or more phrases. We recorded auditory responses in the auditory-premotor nucleus HVC (proper name), where neurons show selective responses to playback of their own vocalizations. As stimuli, we presented one bird's own song and versions of this song in which we manipulated its sequential structure. Results from 336 neurons in eight birds showed that changing the order of sequences without altering the order of phrases within sequences strongly affected the strength of responses while reordering only phrases did not. This suggests sensivity to the context that extends over several phrases, i.e. several seconds and, therefore to long-range regularities.

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ASYNCHRONOUS DEVELOPMENT OF DEVIANCE DETECTION IN THE MOUSE CENTRAL AUDITORY SYSTEM

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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An essential task for our brain is to separate rare from common sensory inputs, defined as deviance detection. Previously characterized in adult humans and animals, deviance detection has most commonly been studied using an oddball paradigm, comprising sequences of common - standard - and rare - deviant - stimuli. These studies associated two mechanisms with deviance detection: stimulus-specific adaptation (SSA) and prediction error (PE). It is, however, still unknown whether deviance detection changes during postnatal development and what neuronal circuits might be involved. Using in vivo awake electrophysiological recordings, we studied this process in the central auditory system of adolescent mice. We observed an asynchronous development of deviance detection across the central auditory system: although already stable at postnatal day 20 (P20) in the inferior colliculus, deviance detection develops until P30 in the auditory thalamus, and even later in the primary auditory cortex (A1). These developmental changes seem related to PE maturation, as our oddball paradigm does not show SSA. We associated the observed alterations in A1 with a later maturation of deep layers across cell types. We are currently studying whether corticofugal projections are involved in the maturation of deviance detection in the auditory thalamus. Overall, our results show distinct deviance detection development across the central auditory areas during adolescence and identify a number of different mechanisms underlying it. Together, they support the importance of juvenile plasticity for the refinement of sensory processing. More generally, they will help understand prediction errors and surprise across sensory systems.

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CONSEQUENCES OF EARLY-ONSET MILD HEARING LOSS ON BRAIN AND BEHAVIOR IN RATS.

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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Background Extensive research indicates that children with hearing loss show intellectual and social interaction disabilities. To identify possible mechanisms, a rat model of mild (noise-induced) hearing loss was developed. This model was behaviorally phenotyped for cognitive and social functioning. Involved brain regions were immunologically and histologically investigated. Methods Hearing loss was induced in four-week-old Wistar rats and quantified through auditory brainstem responses (ABR). A behavioral test battery assessed social behavior when rats were 5 and 10 weeks old and novel object recognition (NOR) when rats were 8 weeks old. Thickness of cortical regions involved in cognitive and social behaviors and the size of the hippocampus were analyzed using cresyl violet staining. Neurogenesis in the hippocampus was analyzed using a marker for DCX. Results Mildly elevated auditory thresholds were found 2 weeks after noise exposure. During the NOR assessment, animals with mild hearing loss explored both the novel and familiar objects equally, suggesting reduced cognitive function compared to control (normal hearing) animals. No differences were found in social and play behaviors after hearing loss at either juvenile or adult ages. Additionally, no difference was found in neurogenesis in the hippocampus after mild hearing loss. Further neurobiological assessments focusing on plasticity, myelinization and neurotransmitter release in the involved regions are still under investigation. Conclusion Mild hearing loss in young rats induced cognitive impairment without overt neurobiological changes. Additional analyses will explore other neurobiological factors contributing to altered cognitive function.

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INVESTIGATING CODING SCHEMES OF SPEECH AND MELODY IN AUDITORY CORTICAL AREAS.

POSTER SESSION 06 - SECTION: AUDITORY CORTEX

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We aim to understand the coding strategies employed by the two hemispheres during speech and melody processing using the spectro-temporal modulation framework (Flinker et al., 2019). Through this framework, Albouy and colleagues discovered a spatial code representing speech and melody in the left and right-associative auditory areas, respectively. We obtained intracranial EEG data from 11 epileptic patients who passively listened to three hundred *a cappella* stimuli, degraded in either temporal or spectral modulation dimensions or non-degraded. The stimulus set contained each sentence paired with each melody to allow uncoupling of the category (sentence or melody) specific information from general acoustic features. We performed a multiclass multivariate pattern analysis to classify the sentence or melodic contents within the temporal dimension of the raw signal. Results show accurate decoding of sentences in non-degraded and spectrally degraded conditions and melodies in non-degraded and temporally degraded conditions. This result corroborates the findings of a recent work showing a reduction in speech perception with temporal degradation and melody perception with spectral degradation (Albouy et al., 2020). Additionally, we observe correct decoding on stimulus time courses in primary and associative auditory areas. These preliminary results indicate the presence of a temporal code for speech and melody perception. Thus, further investigations are necessary to test if specific frequencies or connectivity patterns encode linguistic or melodic information. **This research was supported by the ANR-20-CE28-0007 grant.**

FENS Forum 2022
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Poster Session 07

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-001

THE ROLE OF INHIBITORY INTERNEURONS IN CORTICAL ENGRAM FUNCTION AND MEMORY PROCESSING IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Memories are stored by sparse populations of neurons, i.e. engram cells, which increase their mutual synaptic connections upon learning. While hippocampal engrams store recent memories, prolonged storage depends on the medial prefrontal cortex (mPFC). Whether a neuron is recruited into an engram depends on its excitability during learning. Interestingly hippocampal inhibitory parvalbumin (PV) interneurons of APP/PS1 mice, a mouse model of Alzheimer's disease (AD), show altered excitability. However, it is unclear whether these interneurons alter engram functioning, and how this contributes to memory decline in AD. To study engram functioning in relation to memory decline, APP/PS1 mice were exposed to contextual fear conditioning. Activated neurons were tagged using viral-TRAP. Four weeks after training, remote memory retrieval was examined and brains were isolated for immunohistochemistry. Furthermore, patch-clamp recordings were used to study neuronal excitability in the mPFC. Remote memory was impaired in 20-week-old APP/PS1 mice. During remote memory retrieval, reduced neuronal activation (Fos expression) was observed in the mPFC, and interestingly, also a lower percentage of GAD67-expressing cells. At 16 weeks of age, no memory impairment was detected yet and mPFC PV cell excitability was unaltered, however, pyramidal cells showed signs of hyperexcitability. Although mPFC pyramidal cell properties appear to be altered before the onset of memory retrieval deficits, this might already affect memory formation at this age and subsequent retrieval at a later age (e.g. 20 weeks). Ongoing experiments aim to reveal alterations in neuronal excitability over time and whether engram function is altered in AD mice.

FENS Forum 2022
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BOARD NUMBER: S07-002

DNA METHYLATION PROMOTES MEMORY PERSISTENCE BY FACILITATING SYSTEMS CONSOLIDATION AND CORTICAL ENGRAM STABILISATION.

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Persistence is a key characteristic of memory that relies on **systems consolidation**, a process classically defined as the gradual transfer of information from the hippocampus to the cortex for long-term memory storage.
However, the underlying molecular mechanisms are unknown. **DNA methylation** can act as a long-term regulatory signal, therefore being a prime candidate to regulate memory duration and stabilisation within **neuronal ensembles** – the physical substrate of a memory.
Using contextual fear conditioning (CFC) and ensemble tagging tools in mice, we showed that reactivation of cortical ensembles reflects systems consolidation. We found higher engagement of cortical ensembles selectively in persistent fear memory. To address whether **DNA methylation underlies persistent memory storage and neuronal ensemble reactivation**, we overexpressed a DNA Methyltransferase (DNMT3a2) in the dorsal hippocampus of mice during CFC. Strikingly, we found a **conversion of short-lasting into long-lasting memory**. Moreover, we found an **improved reactivation of cortical neuronal ensembles** and increased **fear generalisation**, mimicking the ensemble dynamics and behavioural trait of persistent memory, respectively. To uncover the molecular targets of DNMT3a2 activity, we next performed RNA-Sequencing analysis of dorsal hippocampi of mice overexpressing DNMT3a2. We found eight **differentially expressed genes** that might have a **mechanistic involvement in memory persistence** downstream of DNMT3a2-mediated DNA methylation.
In summary, we found that overexpressing DNMT3a2 in dorsal hippocampus converts a short-lasting into a persistent memory by stabilising cortical neuronal ensembles. **These findings suggest that hippocampal DNMT3a2 facilitates systems consolidation.**

Pubmed:
34407407: Luck R, Karakatsani A, Shah B, Schermann G, Adler H, Kupke J, Tisch N, Jeong HW, Back MK, Hetsch F, D'Errico A, De Palma M, Wiedtke E, Grimm D, Acker-Palmer A, von Engelhardt J, Adams RH, Augustin HG, Ruiz de Almodóvar C
The angiopoietin-Tie2 pathway regulates Purkinje cell dendritic morphogenesis in a cell-autonomous manner.
Neuro-vascular communication is essential to synchronize central nervous system development. Here, we identify angiopoietin/Tie2 as a neuro-vascular signaling axis involved in regulating dendritic morphogenesis of Purkinje cells (PCs). We show that in the developing cerebellum Tie2 expression is not restricted to blood vessels, but it is also present in PCs. Its ligands angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) are expressed in neural cells and endothelial cells (ECs), respectively. PC-specific deletion of Tie2 results in reduced dendritic arborization, which is recapitulated in neural-specific Ang1-knockout and Ang2 full-knockout mice. Mechanistically, RNA sequencing reveals that Tie2-deficient PCs present alterations in gene expression of multiple genes involved in cytoskeleton organization, dendritic formation, growth, and branching. Functionally, mice with deletion of Tie2 in PCs present alterations in PC network functionality. Altogether, our data propose Ang/Tie2 signaling as a mediator of intercellular communication between neural cells, ECs, and PCs, required for proper PC dendritic morphogenesis and function.
Cell Rep, 2021; 36
33647524: Gulmez Karaca K, Brito DVC, Kupke J, Zeuch B, Oliveira AMM
Engram reactivation during memory retrieval predicts long-term memory performance in aged mice.
Age-related cognitive decline preferentially targets long-lasting episodic memories that require intact hippocampal function. Memory traces (or engrams) are believed to be encoded within the neurons activated during learning (neuronal ensembles), and recalled by reactivation of the same population. However, whether engram reactivation dictates memory performance late in life is not known. Here, we labeled neuronal ensembles formed during object location recognition learning in the

dentate gyrus, and analyzed the reactivation of this population during long-term memory recall in young adult, cognitively impaired- and unimpaired-aged mice. We found that reactivation of memory-encoding neuronal ensembles at long-term memory recall was disrupted in impaired but not unimpaired-aged mice. Furthermore, we showed that the memory performance in the aged population correlated with the degree of engram reactivation at long-term memory recall. Overall, our data implicates recall-induced engram reactivation as a prediction factor of memory performance in aging. Moreover, our findings suggest impairments in neuronal ensemble stabilization and/or reactivation as an underlying mechanism in age-dependent cognitive decline.
Neurobiol Aging, 2021; 101
33647419: Gulmez Karaca K, Kupke J, Oliveira AMM
Molecular and cellular mechanisms of engram allocation and maintenance.
Understanding how we learn and remember has been a long-standing question in neuroscience. Technological developments of the past 15 years have allowed for dramatically increased access to the neurons that hold the physical representation of memory, also known as a memory trace or engram. Such developments have tremendously facilitated advancement of the memory field, since they made possible interrogation of the cellular and molecular mechanisms underlying memory formation with unprecedented cellular specificity. Here, we discuss the studies that have investigated rules governing neuronal recruitment to a particular memory engram. Furthermore, we provide an overview of the evidence that functional and structural changes associated with memory consolidation occur in engram neurons. Moreover, we summarize the expanding literature showing that transcriptional regulatory factors such as transcription factors and epigenetic mechanisms play an important role in the maintained allocation of behaviorally-selected neurons to an engram. Together, these studies have begun elucidating how neuronal networks are selected and modified in order to support memory formation and storage.
Brain Res Bull, 2021; 170
33203444: Brito DVC, Gulmez Karaca K, Kupke J, Frank L, Oliveira AMM
MeCP2 gates spatial learning-induced alternative splicing events in the mouse hippocampus.
Long-term memory formation is supported by functional and structural changes of neuronal networks, which rely on de novo gene transcription and protein synthesis. The modulation of the neuronal transcriptome in response to learning depends on transcriptional and post-transcriptional mechanisms. DNA methylation writers and readers regulate the activity-dependent genomic program required for memory consolidation. The most abundant DNA methylation reader, the Methyl CpG binding domain protein 2 (MeCP2), has been shown to regulate alternative splicing, but whether it establishes splicing events important for memory consolidation has not been investigated. In this study, we identified the alternative splicing profile of the mouse hippocampus in basal conditions and after a spatial learning experience, and investigated the requirement of MeCP2 for these processes. We observed that spatial learning triggers a wide-range of alternative splicing events in transcripts associated with structural and functional remodeling and that virus-mediated knockdown of MeCP2 impairs learning-dependent post-transcriptional responses of mature hippocampal neurons. Furthermore, we found that MeCP2 preferentially affected the splicing modalities intron retention and exon skipping and guided the alternative splicing of distinct set of genes in baseline conditions and after learning. Lastly, comparative analysis of the MeCP2-regulated transcriptome with the alternatively spliced mRNA pool, revealed that MeCP2 disruption alters the relative abundance of alternatively spliced isoforms without affecting the overall mRNA levels. Taken together, our findings reveal that adult hippocampal MeCP2 is required to finetune alternative splicing events in basal conditions, as well as in response to spatial learning. This study provides new insight into how MeCP2 regulates brain function, particularly cognitive abilities, and sheds light onto the pathophysiological mechanisms of Rett syndrome, that is characterized by intellectual disability and caused by mutations in the Mecp2 gene.
Mol Brain, 2020; 13
32711258: Brito DVC, Gulmez Karaca K, Kupke J, Mudlaff F, Zeuch B, Gomes R, Lopes LV, Oliveira AMM
Modeling human age-associated increase in Gadd45γ expression leads to spatial recognition memory impairments in young adult mice.
Aging is associated with the progressive decay of cognitive function. Hippocampus-dependent processes, such as the formation of spatial memory, are particularly vulnerable to aging. Currently, the molecular mechanisms responsible for age-dependent cognitive decline are largely unknown. Here, we investigated the expression and function of the growth arrest DNA damage gamma (Gadd45γ) during aging and cognition. We report that Gadd45γ expression is increased in the hippocampus of aged humans and that Gadd45γ overexpression in the young adult mouse hippocampus compromises cognition. Moreover, Gadd45γ overexpression in hippocampal neurons disrupted cAMP response element-binding protein signaling and the expression of well-established activity-regulated genes. This work shows that Gadd45γ expression is tightly controlled in the hippocampus and its disruption may be a mechanism contributing to age-related cognitive impairments observed in humans.

Neurobiol Aging, 2020; 94
32005851: Gulmez Karaca K, Kupke J, Brito DVC, Zeuch B, Thome C, Weichenhan D, Lutsik P, Plass C, Oliveira AMM
Neuronal ensemble-specific DNA methylation strengthens engram stability.
Memories are encoded by memory traces or engrams, represented within subsets of neurons that are synchronously activated during learning. However, the molecular mechanisms that drive engram stabilization during consolidation and consequently ensure its reactivation by memory recall are not fully understood. In this study we manipulate, during memory consolidation, the levels of the de novo DNA methyltransferase 3a2 (Dnmt3a2) selectively within dentate gyrus neurons activated by fear conditioning. We found that Dnmt3a2 upregulation enhances memory performance in mice and improves the fidelity of reconstitution of the original neuronal ensemble upon memory retrieval. Moreover, similar manipulation in a sparse, non-engram subset of neurons does not bias engram allocation or modulate memory strength. We further show that neuronal Dnmt3a2 overexpression changes the DNA methylation profile of synaptic plasticity-related genes. Our data implicates DNA methylation selectively within neuronal ensembles as a mechanism of stabilizing engrams during consolidation that supports successful memory retrieval.
Nat Commun, 2020; 11
31826946: Brito DVC, Kupke J, Gulmez Karaca K, Zeuch B, Oliveira AMM
Mimicking Age-Associated Gadd45γ Dysregulation Results in Memory Impairments in Young Adult Mice.
Age-related memory loss is observed across multiple mammalian species and preferentially affects hippocampus-dependent memory. Memory impairments are characterized by accelerated decay of spatial memories. Nevertheless, the molecular mechanisms underlying these deficits are still largely unknown. Here, we investigated the expression and function of the growth arrest DNA damage (Gadd45) family during aging and cognition, respectively. We report that aging impairs the expression of Gadd45γ in the hippocampus of cognitively impaired male mice. Mimicking this decrease in young adult male mice led to age-like memory deficits in hippocampus-dependent memory tasks. Gadd45γ reduction impaired the activity of key components of the mitogen-activated protein kinase (MAPK) pathway (p38 and JNK) in mouse hippocampal cultures. Furthermore, we found that activation of downstream targets, such as ATF-2, c-Jun, and CREB (cAMP response element-binding protein), was disrupted. Finally, we showed that Gadd45γ is required for induction of key early- and late-response genes that have been associated with aging. Together, these findings indicate that Gadd45γ expression regulates cognitive abilities and synapse-to-nucleus communication and suggest Gadd45γ dysfunction as a potential mechanism contributing to age-related cognitive impairments. A high percentage of subjects experience age-related memory loss that burdens daily performance. Although many advances have been made, the precise changes in the brain governing these deficits are unclear. Identifying molecular processes that are required for cognition and are altered during old age is crucial to develop preventive or therapeutic strategies. Here, we show that baseline and learning-induced expression of the growth arrest DNA damage (Gadd45) γ is selectively impaired in the hippocampus of aged mice with cognitive deficits. Next, we show that modeling this impairment in young adult mice with normal cognitive performance disrupts long- and short-term memories in an age-like manner. Finally, we demonstrate that Gadd45γ regulates synapse-to-nucleus communication processes that are needed for plasticity-associated gene expression.
J Neurosci, 2020; 40
30827680: Kalamakis G, Brüne D, Ravichandran S, Bolz J, Fan W, Ziebell F, Stiehl T, Catalá-Martinez F, Kupke J, Zhao S, Llorens-Bobadilla E, Bauer K, Limpert S, Berger B, Christen U, Schmezer P, Mallm JP, Berninger B, Anders S, Del Sol A, Marciniak-Czochra A, Martin-Villalba A
Quiescence Modulates Stem Cell Maintenance and Regenerative Capacity in the Aging Brain.
The function of somatic stem cells declines with age. Understanding the molecular underpinnings of this decline is key to counteract age-related disease. Here, we report a dramatic drop in the neural stem cells (NSCs) number in the aging murine brain. We find that this smaller stem cell reservoir is protected from full depletion by an increase in quiescence that makes old NSCs more resistant to regenerate the injured brain. Once activated, however, young and old NSCs show similar proliferation and differentiation capacity. Single-cell transcriptomics of NSCs indicate that aging changes NSCs minimally. In the aging brain, niche-derived inflammatory signals and the Wnt antagonist sFRP5 induce quiescence. Indeed, intervention to neutralize them increases activation of old NSCs during homeostasis and following injury. Our study identifies quiescence as a key feature of old NSCs imposed by the niche and uncovers ways to activate NSCs to repair the aging brain.
Cell, 2019; 176
27891688: Genovese F, Bauersachs HG, Gräßer I, Kupke J, Magin L, Daiber P, Nakajima J, Möhrlen F, Messlinger K, Frings S
Possible role of calcitonin gene-related peptide in trigeminal modulation of glomerular microcircuits of the rodent olfactory bulb.
Chemosensation in the mammalian nose comprises detection of odorants, irritants and pheromones. While the traditional view assigned one distinct sub-system to each stimulus type, recent research has produced a more complex picture.

Odorants are not only detected by olfactory sensory neurons but also by the trigeminal system. Irritants, in turn, may have a distinct odor, and some pheromones are detected by the olfactory epithelium. Moreover, it is well established that irritants change odor perception and vice versa. A wealth of psychophysical evidence on olfactory-trigeminal interactions in humans contrasts with a paucity of structural insight. In particular, it is unclear whether the two systems communicate just by sharing stimuli, or whether neuronal connections mediate cross-modal signaling. One connection could exist in the olfactory bulb that performs the primary processing of olfactory signals and receives trigeminal innervation. In the present study, neuroanatomical tracing of the mouse ethmoid system illustrates how peptidergic fibers enter the glomerular layer of the olfactory bulb, where local microcircuits process and filter the afferent signal. Biochemical assays reveal release of calcitonin gene-related peptide from olfactory bulb slices and attenuation of cAMP signaling by the neuropeptide. In the non-stimulated tissue, the neuropeptide specifically inhibited the basal activity of calbindin-expressing periglomerular interneurons, but did not affect the basal activity of neurons expressing calretinin, parvalbumin, or tyrosine hydroxylase, nor the activity of astrocytes. This study represents a first step towards understanding trigeminal neuromodulation of olfactory-bulb microcircuits and provides a working hypothesis for trigeminal inhibition of olfactory signal processing.
Eur J Neurosci, 2017; 45

FENS Forum 2022
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BOARD NUMBER: S07-003

DECIPHERING THE ROLE OF THE TRANSCRIPTIONAL CO-REPRESSOR SIN3A IN MEMORY FORMATION IN MICE

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Both long-term synaptic plasticity and long-term memory formation require *de novo* gene transcription and protein synthesis. Epigenetic and chromatic remodeling are known to be the primary molecular mechanisms responsible for changing gene expression patterns in neuronal and non-neuronal cells. Previous studies indicate that histone deacetylases (HDACs) are key enzymes that regulate chromatin remodeling and gene expression during memory formation. Indeed, genetic or pharmacological inhibition of HDAC produces robust improvements in long-term memory in multiple learning paradigms in rodents. Moreover, these HDACs are recruited by several transcriptional repressors, including Switch-Insensitive 3A (SIN3A). Interestingly, SIN3A is a large scaffold protein whose role in memory formation was recently discovered *(Bridi et al., 2020)*. Here, we postulate that preventing the recruitment of HDACs to the chromatin by inhibiting the action of SIN3A will improve memory capacities by the increasing the expression of plasticity-related genes in mice. To test our hypothesis, we used a viral strategy to reduce SIN3A expression in adult mice hippocampus. We then assessed the memory capacities of these mice using several behavioral paradigms. In the future, the expression of plasticity-related genes will be monitored. Today, anti-aging strategies have focused their effort on HDAC modulation. Since SIN3A plays important role in HDAC-mediated transcriptional repression, we believe that understanding its function in mature neurons could provide links between chromatin structure and gene activity during age-associated cognitive decline.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-004

SUB-CHRONIC PERIPHERAL CANNABINOID TYPE-1 RECEPTOR BLOCKADE ENHANCES COGNITIVE PERFORMANCE IN MICE

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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[1]Universitat Pompeu Fabra, Department Of Experimental And Health Sciences, Barcelona, Spain, [2]Universidad Pablo de Olavide, Department Of Physiology, Anatomy And Cell Biology, Sevilla, Spain, [3]IMIM, Hospital Del Mar Medical Research Institute, Barcelona, Spain

Aims: Peripheral inputs to the brain continuously shape its function and can influence the formation of memory. Cannabinoid type-1 receptor (CB1R) is a well-recognized player in memory performance. Our group has previously demonstrated that the acute peripheral CB1R blockade in mice participates in the modulation of memory persistence. Here, we aim to evaluate the behavioral, cellular and molecular outcome of a sub-chronic blockade of peripheral CB1R in mice. **Methods:** Male young adult mice received a sub-chronic 7 days treatment with the peripherally-restricted CB1R antagonist AM6545. We evaluated memory performance using the novel object-recognition test and executive function with the pairwise discrimination task. Hippocampal synaptic plasticity and cell proliferation, as well as the expression of neurotrophic factors by immunoblot and q-PCR were also evaluated as treatment outcomes. **Results:** We found that sub-chronic administration of AM6545 resulted in enhanced recognition memory under basal and challenging conditions. In addition, executive function was facilitated after repeated AM6545 administration. Interestingly, AM6545 treatment occluded long-term potentiation in hippocampal synapses and increased the hippocampal expression of Bdnf and Ngf neurotrophic factors, while it did not modify cell proliferation in the subgranular zone of the dentate gyrus. **Conclusions:** Our results suggest that peripheral CB1R contributes to the modulation of memory persistence, executive function and hippocampal synaptic plasticity in mice, mimicking to some extent the effects of systemic approaches reducing CB1R function. **Acknowledgements:** This work was supported by *Generalitat de Catalunya* (FI-2020), *Ministerio de Economía, Innovación y Competitividad* (MINECO), Spain (#RTI2018-099282-B-I00B).

FENS Forum 2022
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BOARD NUMBER: S07-005

THE ROLE OF YOUNG ADULT-BORN NEURONS IN PATTERN SEPARATION

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The birth and incorporation of new neurons into the dentate gyrus of the hippocampus of adult mice has been deemed important for many forms of hippocampal dependent behaviors and memory. Postmitotic adult-born dentate granule cells (abDGCs) mature over several weeks and eventually have similar properties to developmentally born neurons. During this postmitotic maturation process abDGCs display unique properties that potentially underlie their involvement in distinct aspects of hippocampal function. Despite this, it remains unknown how effectively these neurons are engaged and contribute to relevant behaviors during their maturation. In particular, a role has been described for abDGCs in pattern separation (PS), a memory function that enables discrimination of similar but distinct representations into non-overlapping memories. Yet many questions remain about when and how these neurons facilitate an animal's ability to discriminate similar situations. In this study we proposed to directly determine when and how abDGCs are activated when mice perform a PS task and assess their maturation stage and functional characteristics. To do this we developed a custom automated touch screen PS paradigm and determined which neurons were active during different phases of the learning process using a reporter mouse that allows Targeted Recombination in Active Populations (TRAP2). Combining this with birth-dating of neurons we are determining which cohorts of abDGCs are active and using ex-vivo single-cell patch-clamp to assess if they possess distinct functional properties. These studies will ultimately refine our understanding of how abDGCs are functionally incorporated into the hippocampal network and how they contribute to PS.

Pubmed:
33326751: Hark TJ, Rao NR, Castillon C, Basta T, Smukowski S, Bao H, Upadhyay A, Bomba-Warczak E, Nomura T, O'Toole ET, Morgan GP, Ali L, Saito T, Guillermier C, Saido TC, Steinhauser ML, Stowell MHB, Chapman ER, Contractor A, Savas JN
Pulse-Chase Proteomics of the App Knockin Mouse Models of Alzheimer's Disease Reveals that Synaptic Dysfunction Originates in Presynaptic Terminals.
Compromised protein homeostasis underlies accumulation of plaques and tangles in Alzheimer's disease (AD). To observe protein turnover at early stages of amyloid beta (Aβ) proteotoxicity, we performed pulse-chase proteomics on mouse brains in three genetic models of AD that knock in alleles of amyloid precursor protein (APP) prior to the accumulation of plaques and during disease progression. At initial stages of Aβ accumulation, the turnover of proteins associated with presynaptic terminals is selectively impaired. Presynaptic proteins with impaired turnover, particularly synaptic vesicle (SV)-associated proteins, have elevated levels, misfold in both a plaque-dependent and -independent manner, and interact with APP and Aβ. Concurrent with elevated levels of SV-associated proteins, we found an enlargement of the SV pool as well as enhancement of presynaptic potentiation. Together, our findings reveal that the presynaptic terminal is particularly vulnerable and represents a critical site for manifestation of initial AD etiology. A record of this paper's transparent peer review process is included in the Supplemental Information.
Cell Syst, 2021; 12
33067500: Remmers CL, Castillon CCM, Armstrong JN, Contractor A
Recruitment of parvalbumin and somatostatin interneuron inputs to adult born dentate granule neurons.
GABA is a key regulator of adult-born dentate granule cell (abDGC) maturation so mapping the functional connectivity between abDGCs and local interneurons is required to understand their development and integration into the hippocampal circuit. We recorded from birthdated abDGCs in mice and photoactivated parvalbumin (PV) and somatostatin (SST) interneurons to map the timing and strength of inputs to abDGCs during the first 4 weeks after differentiation. abDGCs received input from PV interneurons in the first week, but SST inputs were not detected until the second week. Analysis of desynchronized quantal events established that the number of GABAergic synapses onto abDGCs increased with maturation, whereas individual synaptic strength was constant. Voluntary wheel running in mice scaled the GABAergic input to abDGCs by increasing the number of synaptic contacts from both interneuron types. This demonstrates that GABAergic

innervation to abDGCs develops during a prolonged post-mitotic period and running scales both SST and PV synaptic afferents.
Sci Rep, 2020; 10
31943058: Castillon C, Gonzalez L, Domenichini F, Guyon S, Da Silva K, Durand C, Lestaevel P, Vaillend C, Laroche S, Barnier JV, Poirier R
The intellectual disability PAK3 R67C mutation impacts cognitive functions and adult hippocampal neurogenesis.
The link between mutations associated with intellectual disability (ID) and the mechanisms underlying cognitive dysfunctions remains largely unknown. Here, we focused on PAK3, a serine/threonine kinase whose gene mutations cause X-linked ID. We generated a new mutant mouse model bearing the missense R67C mutation of the Pak3 gene (Pak3-R67C), known to cause moderate to severe ID in humans without other clinical signs and investigated hippocampal-dependent memory and adult hippocampal neurogenesis. Adult male Pak3-R67C mice exhibited selective impairments in long-term spatial memory and pattern separation function, suggestive of altered hippocampal neurogenesis. A delayed non-matching to place paradigm testing memory flexibility and proactive interference, reported here as being adult neurogenesis-dependent, revealed a hypersensitivity to high interference in Pak3-R67C mice. Analyzing adult hippocampal neurogenesis in Pak3-R67C mice reveals no alteration in the first steps of adult neurogenesis, but an accelerated death of a population of adult-born neurons during the critical period of 18-28 days after their birth. We then investigated the recruitment of hippocampal adult-born neurons after spatial memory recall. Post-recall activation of mature dentate granule cells in Pak3-R67C mice was unaffected, but a complete failure of activation of young DCX + newborn neurons was found, suggesting they were not recruited during the memory task. Decreased expression of the KCC2b chloride cotransporter and altered dendritic development indicate that young adult-born neurons are not fully functional in Pak3-R67C mice. We suggest that these defects in the dynamics and learning-associated recruitment of newborn hippocampal neurons may contribute to the selective cognitive deficits observed in this mouse model of ID.
Hum Mol Genet, 2020; 29
29627578: Castillon C, Lunion S, Desvignes N, Hanauer A, Laroche S, Poirier R
Selective alteration of adult hippocampal neurogenesis and impaired spatial pattern separation performance in the RSK2-deficient mouse model of Coffin-Lowry syndrome.
Adult neurogenesis is involved in certain hippocampus-dependent cognitive functions and is linked to psychiatric diseases including intellectual disabilities. The Coffin-Lowry syndrome (CLS) is a developmental disorder caused by mutations in the Rsk2 gene and characterized by intellectual disabilities associated with growth retardation. How RSK2-deficiency leads to cognitive dysfunctions in CLS is however poorly understood. Here, using Rsk2 Knock-Out mice, we characterized the impact of RSK2 deficiency on adult hippocampal neurogenesis in vivo. We report that the absence of RSK2 does not affect basal proliferation, differentiation and survival of dentate gyrus adult-born neurons but alters the maturation progression of young immature newborn neurons. Moreover, when RSK2-deficient mice were submitted to spatial learning, in contrast to wild-type mice, proliferation of adult generated neurons was decreased and no pro-survival effect of learning was observed. Thus, learning failed to recruit a selective population of young newborn neurons in association with deficient long-term memory recall. Given the proposed role of the dentate gyrus and of adult-generated newborn neurons in hippocampal-dependent pattern separation function, we explored this function in a delayed non-matching to place task and in an object-place pattern separation task and report severe deficits in spatial pattern separation in Rsk2-KO mice. Together, this study reveals a previously unknown role for RSK2 in the early stages of maturation and learning-dependent involvement of adult-born dentate gyrus neurons. These alterations associated with a deficit in the ability of RSK2-deficient mice to finely discriminate relatively similar spatial configurations, may contribute to cognitive dysfunction in CLS.
Neurobiol Dis, 2018; 115

FENS Forum 2022
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BOARD NUMBER: S07-006

EXPERIENCE- AND TIME-DEPENDENT SYNAPTIC ADAPTATIONS IN CORTICAL ENGRAM CELLS

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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A remote contextual fear memory is encoded by neurons in the medial prefrontal cortex (mPFC) that are activated during learning, so-called engram cells. However, strong fear conditioning results in their disengagement. Here, we investigated whether physiological and structural plasticity of these neurons is associated with their temporal- and experience-dependent involvement in memory expression. We used TRAP2-tdTomato mice to tag and visualize mPFC neurons after mild (1 foot-shock; 1US) or strong (3 foot-shocks; 3US) contextual fear conditioning (CFC), or after context exposure alone (No Shock; NS). By performing patch-clamp recordings in tagged vs. non-tagged pyramidal neurons at a recent (≤ 1 week) or remote (~4 weeks) timepoint after conditioning, we observed no differences in intrinsic excitability, regardless of the timepoint or training intensity. However, we found progressive changes in physiological and structural properties of excitatory synapses on tagged mPFC neurons that were most pronounced after mild CFC. Our results indicate that specific excitatory inputs onto mPFC engram cells are selectively enhanced after mild conditioning, in line with the memory strength-dependent engagement of these neurons in remote memory expression.

Pubmed:
32414089: Kummer KK, Mitrić M, Kalpachidou T, Kress M
The Medial Prefrontal Cortex as a Central Hub for Mental Comorbidities Associated with Chronic Pain.
Chronic pain patients frequently develop and suffer from mental comorbidities such as depressive mood, impaired cognition, and other significant constraints of daily life, which can only insufficiently be overcome by medication. The emotional and cognitive components of pain are processed by the medial prefrontal cortex, which comprises the anterior cingulate cortex, the prelimbic, and the infralimbic cortex. All three subregions are significantly affected by chronic pain: magnetic resonance imaging has revealed gray matter loss in all these areas in chronic pain conditions. While the anterior cingulate cortex appears hyperactive, prelimbic, and infralimbic regions show reduced activity. The medial prefrontal cortex receives ascending, nociceptive input, but also exerts important top-down control of pain sensation: its projections are the main cortical input of the periaqueductal gray, which is part of the descending inhibitory pain control system at the spinal level. A multitude of neurotransmitter systems contributes to the fine-tuning of the local circuitry, of which cholinergic and GABAergic signaling are particularly emerging as relevant components of affective pain processing within the prefrontal cortex. Accordingly, factors such as distraction, positive mood, and anticipation of pain relief such as placebo can ameliorate pain by affecting mPFC function, making this cortical area a promising target region for medical as well as psychosocial interventions for pain therapy.
Int J Mol Sci, 2020; 21
31824261: Kalpachidou T, Kummer KK, Mitrić M, Kress M
Tissue Specific Reference Genes for MicroRNA Expression Analysis in a Mouse Model of Peripheral Nerve Injury.
MicroRNAs (miRNAs) have emerged as master switch regulators in many biological processes in health and disease, including neuropathy. miRNAs are commonly quantified by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR), usually estimated as relative expression through reference genes normalization. Different non-coding RNAs (ncRNAs) are used for miRNA normalization; however, there is no study identifying the optimal reference genes in animal models for peripheral nerve injury. We evaluated the stability of eleven ncRNAs, commonly used for miRNA normalization, in dorsal root ganglia (DRG), dorsal horn of the spinal cord (dhSC), and medial prefrontal cortex (mPFC) in the mouse spared nerve injury (SNI) model. After RT-qPCR, the stability of each ncRNA was determined by using four different methods: BestKeeper, the comparative delta-Cq method, geNorm, and NormFinder. The candidates were rated according to their performance in each method and an overall ranking list was compiled. The most stable ncRNAs were: sno420, sno429, and sno202 in DRG; sno429, sno202, and U6 in dhSC; sno202, sno420, and sno142 in mPFC. We provide the first reference genes' evaluation for miRNA normalization in different neuronal tissues in an animal model of peripheral nerve injury. Our

results underline the need for careful selection of reference genes for miRNA normalization in different tissues and experimental conditions. We further anticipate that our findings can be used in a broad range of nerve injury related studies, to ensure validity and promote reproducibility in miRNA quantification.
Front Mol Neurosci, 2019; 12

31263213: Mitrić M, Seewald A, Moschetti G, Sacerdote P, Ferraguti F, Kummer KK, Kress M
Layer- and subregion-specific electrophysiological and morphological changes of the medial prefrontal cortex in a mouse model of neuropathic pain.
Chronic neuropathic pain constitutes a serious public health problem, but the disease mechanisms are only partially understood. The involvement of different brain regions like the medial prefrontal cortex has already been established, but the comparison of the role of different subregions and layers is still inconclusive. In the current study, we performed patch-clamp recordings followed by anatomical reconstruction of pyramidal cells from different layers of the prelimbic and infralimbic subregions of the medial prefrontal cortex in neuropathic (spared nerve injury, SNI) and control mice. We found that in the prelimbic cortex, layer 2/3 pyramidal cells from SNI mice exhibited increased excitability compared to sham controls, whereas prelimbic layer 5 pyramidal neurons showed reduced excitability. Pyramidal cells in both layer 2/3 and layer 5 of the infralimbic subregion did not change their excitability, but layer 2/3 pyramidal cells displayed increased dendritic length and branching. Our findings support the view that chronic pain is associated with subregion- and layer-specific changes in the medial prefrontal cortex. They therefore provide new insights into the mechanisms underlying the chronification of pain.
Sci Rep, 2019; 9

30586315: Quarta S, Mitrić M, Kalpachidou T, Mair N, Schiefermeier-Mach N, Andratsch M, Qi Y, Langeslag M, Malsch P, Rose-John S, Kress M
Impaired mechanical, heat, and cold nociception in a murine model of genetic TACE/ADAM17 knockdown.
TNF-α-converting enzyme, a member of the ADAM (A disintegrin and metalloproteinase) protease family and also known as ADAM17, regulates inflammation and regeneration in health and disease. ADAM17 targets are involved in pain development and hypersensitivity in animal models of inflammatory and neuropathic pain. However, the role of ADAM17 in the pain pathway is largely unknown. Therefore, we used the hypomorphic ADAM17 (ADAM17) mouse model to investigate the importance of ADAM17 in nociceptive behavior, morphology, and function of primary afferent nociceptors. ADAM17 mice were hyposensitive to noxious stimulation, showing elevated mechanical thresholds as well as impaired heat and cold sensitivity. Despite these differences, skin thickness and innervation were comparable to controls. Although dorsal root ganglia of ADAM17 mice exhibited normal morphology of peptidergic and nonpeptidergic neurons, a small but significant reduction in the number of isolectin β-4-positive neurons was observed. Functional electrical properties of unmyelinated nociceptors showed differences in resting membrane potential, afterhyperpolarization, and firing patterns in specific subpopulations of sensory neurons in ADAM17 mice. However, spinal cord morphology and microglia activity in ADAM17 mice were not altered. Our data suggest that ADAM17 contributes to the processing of painful stimuli, with a complex mode of action orchestrating the function of neurons along the pain pathway.-Quarta, S., Mitrić, M., Kalpachidou, T., Mair, N., Schiefermeier-Mach, N., Andratsch, M., Qi, Y., Langeslag, M., Malsch, P., Rose-John, S., Kress, M. Impaired mechanical, heat, and cold nociception in a murine model of genetic TACE/ADAM17 knockdown.
FASEB J, 2019; 33

30013462: Kummer KK, Kalpachidou T, Mitrić M, Langeslag M, Kress M
Altered Gene Expression in Prefrontal Cortex of a Fabry Disease Mouse Model.
Fabry disease is an X-chromosome linked hereditary disease that is caused by loss of function mutations in the α-galactosidase A (α-Gal A) gene, resulting in defective glycolipid degradation and subsequent accumulation of globotriaosylceramide (Gb3) in different tissues, including vascular endothelial cells and neurons in the peripheral and central nervous system. We recently reported a differential gene expression profile of α-Gal A mouse dorsal root ganglia, an established animal model of Fabry disease, thereby providing new gene targets that might underlie the neuropathic pain related symptoms. To investigate the cognitive symptoms experienced by Fabry patients, we performed one-color based hybridization microarray expression profiling of prefrontal cortex samples from adult α-Gal A mice and age-matched wildtype controls, followed by protein-protein interaction and pathway analyses for the differentially regulated mRNAs. We found that from a total of 381 differentially expressed genes, 135 genes were significantly upregulated, whereas 246 genes were significantly downregulated between α-Gal A mice and wildtype controls. Enrichment analysis for downregulated genes revealed mainly immune related pathways, including immune/defense responses, regulation of cytokine production, as well as signaling and transport regulation pathways. Further analysis of the regulated genes revealed a large number of genes involved in neurodegeneration. The current analysis for the first time presents a differential gene expression profile of central nervous system tissue from α-Gal A mice, thereby providing novel knowledge on the deregulation and a possible contribution of gene expression to Fabry disease related brain pathologies.
Front Mol Neurosci, 2018; 11

34715992: Visser E, Matos MR, Mitrić MM, Kramvis I, van der Loo RJ, Mansvelder HD, Smit AB, van den Oever MC
Extinction of Cocaine Memory Depends on a Feed-Forward Inhibition Circuit Within the Medial Prefrontal Cortex.
Cocaine-associated environments (i.e., contexts) evoke persistent memories of cocaine reward and thereby contribute to the maintenance of addictive behavior in cocaine users. From a therapeutic perspective, enhancing inhibitory control over cocaine-conditioned responses is of pivotal importance but requires a more detailed understanding of the neural circuitry that can suppress context-evoked cocaine memories, e.g., through extinction learning. The ventral medial prefrontal cortex (vmPFC) and dorsal medial prefrontal cortex (dmPFC) are thought to bidirectionally regulate responding to cocaine cues through their projections to other brain regions. However, whether these mPFC subregions interact to enable adaptive responding to cocaine-associated contextual stimuli has remained elusive.
Biol Psychiatry, 2022; 91
34503674: Lesuis SL, Brosens N, Immerzeel N, van der Loo RJ, Mitrić M, Bielefeld P, Fitzsimons CP, Lucassen PJ, Kushner SA, van den Oever MC, Krugers HJ
Glucocorticoids Promote Fear Generalization by Increasing the Size of a Dentate Gyrus Engram Cell Population.
Traumatic experiences, such as conditioned threat, are coded as enduring memories that are frequently subject to generalization, which is characterized by (re-) expression of fear in safe environments. However, the neurobiological mechanisms underlying threat generalization after a traumatic experience and the role of stress hormones in this process remain poorly understood.
Biol Psychiatry, 2021; 90
33841124: Rao-Ruiz P, Visser E, Mitrić M, Smit AB, van den Oever MC
A Synaptic Framework for the Persistence of Memory Engrams.
The ability to store and retrieve learned information over prolonged periods of time is an essential and intriguing property of the brain. Insight into the neurobiological mechanisms that underlie memory consolidation is of utmost importance for our understanding of memory persistence and how this is affected in memory disorders. Recent evidence indicates that a given memory is encoded by sparsely distributed neurons that become highly activated during learning, so-called engram cells. Research by us and others confirms the persistent nature of cortical engram cells by showing that these neurons are required for memory expression up to at least 1 month after they were activated during learning. Strengthened synaptic connectivity between engram cells is thought to ensure reactivation of the engram cell network during retrieval. However, given the continuous integration of new information into existing neuronal circuits and the relatively rapid turnover rate of synaptic proteins, it is unclear whether a lasting learning-induced increase in synaptic connectivity is mediated by stable synapses or by continuous dynamic turnover of synapses of the engram cell network. Here, we first discuss evidence for the persistence of engram cells and memory-relevant adaptations in synaptic plasticity, and then propose models of synaptic adaptations and molecular mechanisms that may support memory persistence through the maintenance of enhanced synaptic connectivity within an engram cell network.
Front Synaptic Neurosci, 2021; 13

FENS Forum 2022
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BOARD NUMBER: S07-007

VESICULAR GLUTAMATE TRANSPORTER TYPE 3 NEURONS OF THE MEDIAN RAPHE REGION FACILITATE LONG-TERM MEMORY FORMATION IN HIPPOCAMPUS DEPENDENT SPATIAL LEARNING TASK

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

Csilla Lea Fazekas[1,2,3,4], Bibiána Török[2,4], Adrienn Szabó[1,2,4], Pedro Correia[1,2,4], Tiago Chaves[1,2,4], Stephanie Daumas[3], Dóra Zelena[2,4]
[1]Semmelweis University, János Szentágothai Doctoral School Of Neurosciences, Budapest, Hungary, [2]University of Pécs Medical School, Centre for Neuroscience, Szentágothai Research Centre, Institute Of Physiology, Pécs, Hungary, [3]Sorbonne Université, Paris Seine Biology Institute, Neuroscience Paris Seine, Paris, France, [4]Institute of Experimental Medicine, Laboratory Of Behavioural And Stress Studies, Budapest, Hungary

Aims:There is anatomical and electrophysiological evidence that median raphe region (MRR) vesicular glutamate transporter type 3 (VGLUT3) positive neurons project to the hippocampus and influence its activity. Our aim was to characterise the behavioural effects of MRR-VGLUT3+ neuronal activity during spatial learning and memory formation. Methods:We utilised chemogenetics (DREADDs) and optogenetics (ChR2) in VGLUT3-Cre mice in the hippocampus dependent Morris watermaze paradigm. VGLUT3-Cre male and female mice were injected with Cre-dependent adenoassociated viral vectors containing either control (RFP) or excitatory (G_q–GPCR/ChR2) sequences. During spatial reference memory (SRM) phase the chemogenetic groups were injected with CNO 30min before experiment. For optogenetics, blue laser was used at 20Hz for 10sec while on the platform. Short-term memory was assessed 10min after last learning trial, while long-term memory was studied at 72h. Results:Manipulations of MRR VGLUT3+ neurons did not impact spatial reference learning in SRM task, since no differences between the learning curves of the groups were observed. Chemogenetic manipulation resulted in no differences between the groups in short-term memory. However, 72h later, the control group showed the expected rise in latency, while excitatory group performed similarly as before and found the place of the platform significantly faster than controls. Optogenetic MRR-VGLUT3 stimulation had no such effects. However, the latency to find the platform increased significantly in the control group, but not in the excitatory group compared to the values of short-term memory. Conclusions:Our data showed a role of MRR-VGLUT3+ neurons in long-term memory formation for which long, extended excitation is required.

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BOARD NUMBER: S07-008

LONG-TERM STABILITY OF MEDIAL PREFRONTAL CORTEX PYRAMIDAL CELL ENSEMBLES IN AN OLFACTION GUIDED WORKING MEMORY TASK

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

Hannah Muysers[1,2], Hung-Ling Chen[1], Jonas-Frederic Sauer[1], Marlene Bartos[1]
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The medial prefrontal cortex (mPFC) plays a key role in coordinating novel but also previously acquired memories. However, how these memories are stored and maintained remains unclear. We addressed this question by using 1-photon calcium imaging of pyramidal cells in layer-V of the mPFC. We imaged the same set of cells over weeks in an olfaction guided working memory task. Mice were exposed to two different olfactory cues in the centre arm of an M-maze and trained to choose one of the side arms for reward collection. Once this association was learned, the calcium imaging started. First, we performed population imaging across 24 days in one consistent ('original') rule. A subset of cells was active on all recording days and showed stable spatial tuning over time. A decoder trained on neuronal activity from the first recording day could predict the animal's maze-position and trial outcome for each subsequent recording day. Secondly, we 'switched' the associated reward-location for the two olfactory cues. Animals needed substantial time to learn the new reward rule. However, the neuronal activity was similar to the one obtained during the 'original'-rule. Therefore, the animal's maze-position and trial outcome in the 'switched'-condition could be decoded based on neuronal activity collected in the 'original'-rule. We thus observed a stable cell-ensemble representing task-related spatial information, which is persistent across time and independent of rules and task-performance. Such a reliably active cell-ensemble could serve as a stable reference frame, helping the animal navigate in the context and allowing quick task-related decisions.

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BOARD NUMBER: S07-009

THE ROLE OF ASTROCYTIC GS-GPCR SIGNALING IN CORTICAL ENGRAM FORMATION AND REMOTE MEMORY RETRIEVAL

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

Aline Mak, Michel Van Den Oever, Mark Verheijen
Vrije Universiteit Amsterdam, Center For Neurogenomics And Cognitive Research, Amsterdam, Netherlands

During learning, sparsely distributed neurons in the mPFC are activated and these specific neurons are required for remote memory retrieval, thereby forming an engram ensemble. Astrocytes and neuronal synaptic elements function together as bidirectional partners in the modulation of synaptic transmission, and interaction between these components likely has an important role in memory processing. However, little is known about the role of astrocytes, and in particular astrocytic Gs-GPCR signaling, in the encoding and retrieval of fear memory, as well as the associated formation and reactivation of an engram ensemble in the mPFC. Therefore, we aim to assess the role of astrocytic Gs-GPCR signaling in remote memory and cortical engram functioning using chemogenetic manipulation of cortical astrocytes during conditional fear conditioning (CFC). The Gs-coupled DREADD (rM3Ds) was specifically expressed in mPFC astrocytes to stimulate this pathway during fear memory encoding. Experiments were performed in a TRAP2-tdTomato transgenic mouse to permanently tag neurons that were active during CFC, and to determine their level of reactivation through co-localization with retrieval-induced c-Fos expression. Preliminary results indicate that stimulation of the Gs-GPCR pathway during CFC impaired subsequent remote memory expression and enhanced the size of the mPFC ensemble that was tagged during memory encoding. The relative reactivation rate of the tagged ensemble did not differ between rM3Ds and control mice. We tentatively conclude that activation of the astrocytic Gs-GPCR pathway in the mPFC during memory encoding increases the recruitment of neurons in a cortical engram ensemble, which negatively affects remote memory expression.

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BOARD NUMBER: S07-010

THE ROLE OF THE CA1 IN SOCIAL AND SPATIAL RECOGNITION MEMORY IN JUVENILE MALES AND FEMALES.

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The hippocampus is involved in different types of learning and memory, including object location memory (OLM), and social recognition memory (SRM). Juvenility is a critical period for limbic system maturation, particularly of the medial prefrontal cortex and hippocampus, structures critical for OLM and SRM in adult animals. The neuroanatomical and behavioral development of juvenile males and females were assumed similar. However, our recent data show substantial differences in the role of prefrontal oxytocin (OXT) in pre-pubertal males and females (Maroun et al., 2020) in mediating extinction of fear for example. This study aims to examine whether the hippocampal sub-region CA1 has similar roles in mediating OLM and SRM in juvenile male and female rats. To that end, we microinjected either anisomycin (protein synthesis inhibitor) or OXT-receptor (OXTR) antagonist in the CA1 of juvenile male and female rats and examined both OLM and SRM. We found that anisomycin impairs the consolidation of long-term OLM in males, but not females. This differential effect seems also to be reflected in different expression of cFos, the neuronal activation marker, in the CA1 following the formation of OLM long-term memory. However, the SRM of both sexes was similarly hindered by anisomycin. Similar results were obtained with blockade of OXT receptors, which resulted in impaired OLM only in males and SRM impairment in both sexes. In conclusion, the data points to sex differences in mechanisms underlying OLM but not SRM within the developing CA1. These findings suggest a need for sex-specific approach to human developmental pathologies.

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BOARD NUMBER: S07-011

REDUCED NEONATAL AVAILABILITY OF SIALYLATED HUMAN MILK OLIGOSACCHARIDES IMPAIRS MEMORY AND ATTENTION

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

Edoardo Pisa[1,2], Alice Traversa[3], Viviana Caputo[4], Jonas Hauser[5], Simone Macrì[6]
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AIMS Breastmilk contains bioactive molecules essential for brain and cognitive development. Among the various components of human milk, sialylated human milk oligosaccharides (HMOs) have been proposed to be one of the key nutrients mediating the beneficial effects of breastfeeding on the offspring. We hypothesize that the reduced neonatal availability of the two main sialylated HMOs, 6'Sialyllactose (6'SL) and 3'Sialyllactose (3'SL), may impair attention, cognitive flexibility, and memory in adult mice. **METHODS** To modulate the concentrations of 6'SL and 3'SL only during lactation, we performed a cross-fostering design in which wild-type (WT) mice were reared by dams characterised by the absence of the genes synthesizing 6'SL and 3'SL knock-out (KO), producing milk without these sugars. We then tested the offspring for memory and attention in adulthood using T-maze and Barnes maze tests. **RESULTS** Compared to control mice, WT offspring deprived of 6'SL and 3'SL during lactation exhibited reduced memory and attentional capabilities. Specifically, WT mice reared to KO dams showed impaired working memory in the T-maze test and reduced spatial memory in the Barnes maze, and impairments in attentional capabilities in the attentional set-shifting task. **CONCLUSION** These results indicate that the absence of 6'SL and 3'SL in the neonatal period may impair the development of executive functions later in life. This study supports the hypothesis that early-life dietary sialylated HMOs exert a long-lasting role on the development of cognitive functions suggesting that their presence during the lactational stage may contribute to the development of the central nervous system.

Pubmed:

34959743: Pisa E, Martire A, Chiodi V, Traversa A, Caputo V, Hauser J, Macrì S
Exposure to 3'Sialyllactose-Poor Milk during Lactation Impairs Cognitive Capabilities in Adulthood.
Breast milk exerts pivotal regulatory functions early in development whereby it contributes to the maturation of brain and associated cognitive functions. However, the specific components of maternal milk mediating this process have remained elusive. Sialylated human milk oligosaccharides (HMOs) represent likely candidates since they constitute the principal neonatal dietary source of sialic acid, which is crucial for brain development and neuronal patterning. We hypothesize that the selective neonatal lactational deprivation of a specific sialylated HMOs, sialyl(alpha2,3)lactose (3'SL), may impair cognitive capabilities (attention, cognitive flexibility, and memory) in adulthood in a preclinical model. To operationalize this hypothesis, we cross-fostered wild-type (WT) mouse pups to B6.129-/J dams, knock-out (KO) for the gene synthesizing 3'SL, thereby providing milk with approximately 80% 3'SL content reduction. We thus exposed lactating WT pups to a selective reduction of 3'SL and investigated multiple cognitive domains (including memory and attention) in adulthood. Furthermore, to account for the underlying electrophysiological correlates, we investigated hippocampal long-term potentiation (LTP). Neonatal access to 3'SL-poor milk resulted in decreased attention, spatial and working memory, and altered LTP compared to the control group. These results support the hypothesis that early-life dietary sialylated HMOs exert a long-lasting role in the development of cognitive functions.
Nutrients, 2021; 13
33664475: Hauser J, Pisa E, Arias Vásquez A, Tomasi F, Traversa A, Chiodi V, Martin FP, Sprenger N, Lukjancenko O, Zollinger A, Metairon S, Schneider N, Steiner P, Martire A, Caputo V, Macrì S
Sialylated human milk oligosaccharides program cognitive development through a non-genomic transmission mode.
Breastmilk contains bioactive molecules essential for brain and cognitive development. While sialylated human milk

oligosaccharides (HMOs) have been implicated in phenotypic programming, their selective role and underlying mechanisms remained elusive. Here, we investigated the long-term consequences of a selective lactational deprivation of a specific sialylated HMO in mice. We capitalized on a knock-out (KO) mouse model (B6.129-St6gal1/J) lacking the gene responsible for the synthesis of sialyl(alpha2,6)lactose (6'SL), one of the two sources of sialic acid (Neu5Ac) to the lactating offspring. Neu5Ac is involved in the formation of brain structures sustaining cognition. To deprive lactating offspring of 6'SL, we cross-fostered newborn wild-type (WT) pups to KO dams, which provide 6'SL-deficient milk. To test whether lactational 6'SL deprivation affects cognitive capabilities in adulthood, we assessed attention, perseveration, and memory. To detail the associated endophenotypes, we investigated hippocampal electrophysiology, plasma metabolomics, and gut microbiota composition. To investigate the underlying molecular mechanisms, we assessed gene expression (at eye-opening and in adulthood) in two brain regions mediating executive functions and memory (hippocampus and prefrontal cortex, PFC). Compared to control mice, WT offspring deprived of 6'SL during lactation exhibited consistent alterations in all cognitive functions addressed, hippocampal electrophysiology, and in pathways regulating the serotonergic system (identified through gut microbiota and plasma metabolomics). These were associated with a site- (PFC) and time-specific (eye-opening) reduced expression of genes involved in central nervous system development. Our data suggest that 6'SL in maternal milk adjusts cognitive development through a short-term upregulation of genes modulating neuronal patterning in the PFC.
Mol Psychiatry, 2021; 26

32518224: Caputo V, Pacilli MG, Arisi I, Mazza T, Brandi R, Traversa A, Casasanta G, Pisa E, Sonnessa M, Healey B, Moggio L, D'Onofrio M, Alleva E, Macrì S

Genomic and physiological resilience in extreme environments are associated with a secure attachment style. Understanding individual capability to adjust to protracted confinement and isolation may inform adaptive plasticity and disease vulnerability/resilience, and may have long-term implications for operations requiring prolonged presence in distant and restricted environments. Individual coping depends on many different factors encompassing psychological dispositional traits, endocrine reactivity and their underlying molecular mechanisms (e.g. gene expression). A positive view of self and others (secure attachment style) has been proposed to promote individual resilience under extreme environmental conditions. Here, we tested this hypothesis and investigated the underlying molecular mechanisms in 13 healthy volunteers confined and isolated for 12 months in a research station located 1670 km away from the south geographic pole on the Antarctic Plateau at 3233 m above sea level. Study participants, stratified for attachment style, were characterised longitudinally (before, during and after confinement) for their psychological appraisal of the stressful nature of the expedition, diurnal fluctuations in endocrine stress reactivity, and gene expression profiling (transcriptomics). Predictably, a secure attachment style was associated with reduced psychological distress and endocrine vulnerability to stress. In addition, while prolonged confinement and isolation remarkably altered overall patterns of gene expression, such alteration was largely reduced in individuals characterised by a secure attachment style. Furthermore, increased resilience was associated with a reduced expression of genes involved in energy metabolism (mitochondrial function and oxidative phosphorylation). Ultimately, our data indicate that a secure attachment style may favour individual resilience in extreme environments and that such resilience can be mapped onto identifiable molecular substrates.
Transl Psychiatry, 2020; 10

FENS Forum 2022
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BOARD NUMBER: S07-012

OVERWRITING AN INSTINCT: HOW INNATE CIRCUITRY CAN BE MODIFIED WITH EXPERIENCE

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Behavioural neuroscience is implicitly divided into the study of innate behaviour (instincts) and learned behaviour (memory). However, it is becoming increasingly understood that instincts are not set in stone. Here, we use engram labelling strategies alongside optogenetics and chemogenetics to study the innate representation (*ingram*) of the instinctual response to visual looming stimuli, and understand how this ingram may change via learning. We used multiple training paradigms to alter behavioural responses to a visual looming or sweeping stimulus, including extinction, reinstatement, and counterconditioning. Our experiments demonstrate a remarkable plasticity of the innate looming/sweeping responses. We investigated how activity in the superior colliculus (SC) changes in response to these experiences by quantifying overlap of c-Fos expressing cells in the naïve and experienced behavioural states. We then manipulated SC activity using optogenetic and chemogenetic techniques and investigated the behavioural outcomes, demonstrating a strong defensive response to stimulation of the SC. This was further examined in the context of learning to determine how the activation of the SC post-extinction alters behaviour. We then considered the role of other brain regions using whole-brain histological analysis to identify new candidate regions that modulate innate and learned behavioural responses to looming stimuli. These findings indicate that the mouse visual looming response is highly plastic, and the complexity of this behaviour can only be explained by understanding the role of several regions, including but not limited to the SC. More broadly, we have demonstrated that instinctual representations in the brain communicate with neurons encoding learning.

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BOARD NUMBER: S07-013

COLD SENSITIVE ENGRAMS CONTROL WHOLE-BODY THERMOREGULATORY RESPONSES

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

Andrea Muñoz Zamora[1,2], Aaron Douglas[1], Taylor Moniz[1,2], Lydia Marks[1,2], James O'Leary[1,2], Lydia Lynch[3], Tomás Ryan[1,2,4,5]
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Maintenance of core body temperature within a narrow range is essential for physiological homeostasis, and for the survival of animals. When facing a thermal challenge such as a cold environment, the brain coordinates both autonomic and behavioral responses in order to maintain optimal body temperature. However, it is unknown how temperature information is precisely stored in the brain, and how it is converted into a whole-body physiological response. To investigate this, we conditioned animals to associate a particular context with a specific temperature, by combining thermoregulatory pavlovian conditioning with engram labelling technology and optogenetic approaches. We show that if mice are returned to an environment where they previously experienced a cold-challenge, they will increase their metabolic rates regardless of the actual environmental temperature. Moreover, mice develop a conditioned place aversion for the cold context, and avoid a cold environment if given the choice. In addition, we show that the hippocampus is highly activated during exposure to cold and when remembering the cold, indicating a potential key role for this structure during thermoregulation. Furthermore, we show that reactivating cold sensitive memory engrams in the hippocampus mimics the physiological responses seen during a cold-challenge. Together, our results show that remembering the cold is enough to cause whole-body autonomic and behavioral responses that enable an animal to maintain thermal homeostasis.

Pubmed:
33766406: Leal Santos S, Stackmann M, Muñoz Zamora A, Mastrodonato A, De Landri AV, Vaughan N, Chen BK, Lanio M, Denny CA
Propranolol Decreases Fear Expression by Modulating Fear Memory Traces.
Posttraumatic stress disorder can develop after a traumatic event and results in heightened, inappropriate fear and anxiety. Although approximately 8% of the U.S. population is affected by posttraumatic stress disorder, only two drugs have been approved by the Food and Drug Administration to treat it, both with limited efficacy. Propranolol, a nonselective β-adrenergic antagonist, has shown efficacy in decreasing exaggerated fear, and there has been renewed interest in using it to treat fear disorders.
Biol Psychiatry, 2021; 89
32035426: Gutzeit VA, Ahuna K, Santos TL, Cunningham AM, Sadsad Rooney M, Muñoz Zamora A, Denny CA, Donaldson ZR
Optogenetic reactivation of prefrontal social neural ensembles mimics social buffering of fear.
Social buffering occurs when the presence of a companion attenuates the physiological and/or behavioral effects of a stressful or fear-provoking event. It represents a way in which social interactions can immediately and potently modulate behavior. As such, social buffering is one mechanism by which strong social support increases resilience to mental illness. Although the behavioral and neuroendocrine impacts of social buffering are well studied in multiple species, including humans, the neuronal underpinnings of this behavioral phenomenon remain largely unexplored. Previous work has shown that the infralimbic prefrontal cortex (IL-PFC) is important for processing social information and, in separate studies, for modulating fear and anxiety. Thus, we hypothesized that socially active cells within the IL-PFC may integrate social information to modulate fear responsivity. To test this hypothesis, we employed social buffering paradigms in male and female mice. Similar to prior studies in rats, we found that the presence of a cagemate reduced freezing in fear- and anxiety-provoking contexts. In accordance with previous work, we demonstrated that interaction with a novel or familiar conspecific induces activity in the IL-PFC as evidenced by increased immediate early gene (IEG) expression. We then utilized an activity-dependent tagging murine line, the ArcCreER mice, to express channelrhodopsin (ChR2) in neurons active during the social encoding of a new cagemate. We found that optogenetic reactivation of these socially active neuronal ensembles

phenocopied the effects of cagemate presence in male and female mice in learned and innate fear contexts without being inherently rewarding or altering locomotion. These data suggest that a social neural ensemble within the IL-PFC may contribute to social buffering of fear. These neurons may represent a novel therapeutic target for fear and anxiety disorders.
Neuropsychopharmacology, 2020; 45

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BOARD NUMBER: S07-014

FUNCTIONAL MANIPULATION OF INFANT ENGRAM EXPRESSION

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Infantile amnesia, the developmental loss of memories formed in early childhood (prior to 2–4 years), affects all humans. Although behavioural neuroscientists have already demonstrated that rodents display infantile amnesia, little is known about the basic neurobiology of infantile amnesia and further its effect on memory engrams. We probe the question of how memories are stored in the brain throughout development by integrating engram labelling technology with mouse models of infantile amnesia. We show that infant mice demonstrate infantile amnesia for a range of behavioural paradigms including fear conditioning, novel object recognition and the Barnes maze. We successfully reactivated infant memories of various modalities in adult mice by optogenetically stimulating dentate gyrus engram cells that were labelled for a target experience during infancy. We used cell reactivation as a proxy of memory retrieval and demonstrate that the engram connectivity pattern survives infantile amnesia. Further, we were able to permanently reinstate lost infantile memories by artificially updating the memory engram and restoring natural access to the engram. Finally, we demonstrate that male, but not female, offspring from maternal immune activation (MIA) models do not experience infantile amnesia.

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BOARD NUMBER: S07-015

NATURAL FORGETTING AS A FORM OF ENGRAM CELL PLASTICITY

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Memories are stored as ensembles of engram neurons and their successful recall involves reactivation of these cellular networks. While progress has been made in understanding the biology of engrams, there remain significant gaps in connecting these cell ensembles with the process of forgetting. We developed a forgetting paradigm based on an object recognition task and characterised the forgetting curve of wild-type mice. Under baseline conditions mice were able to recall an object memory 24 hrs post-training but exhibited no preference for the novel object after two weeks, indicating the initial memory was forgotten. Using engram labelling technology, active neurons within the dentate gyrus of c-fos-tTA transgenic mice were labelled during the initial encoding of an object memory. Mice that recalled the memory at 24 hrs showed a higher level of overlap between ChR2-EYFP+ and c-Fos+ cells, compared to mice that failed to recall the memory at two weeks, suggesting that the level of engram reactivation decreased as the memory was forgotten. Similarly, morphological analysis of engram cells indicated that spine density decreased with the level of forgetting and engram activation. Furthermore, optogenetic reactivation of dentate gyrus engram cells facilitated the recall of a previously forgotten object memory. Finally, the inhibition of Rac1 protein prevented forgetting, while its activation following memory encoding accelerated forgetting. Together, these findings suggest that forgetting is an adaptive form of engram plasticity that involves circuit remodeling, that allows engrams to switch from an accessible state to an inaccessible state.

Pubmed:
30217534: Hueston CM, O'Leary JD, Hoban AE, Kozareva DA, Pawley LC, O'Leary OF, Cryan JF, Nolan YM
Chronic interleukin-1β in the dorsal hippocampus impairs behavioural pattern separation.
Understanding the long-term consequences of chronic inflammation in the hippocampus may help to develop therapeutic targets for the treatment of cognitive disorders related to stress, ageing and neurodegeneration. The hippocampus is particularly vulnerable to increases in the pro-inflammatory cytokine interleukin-1β (IL-1β), a mediator of neuroinflammation, with elevated levels implicated in the aetiology of neurodegenerative diseases such as Alzheimer's and Parkinson's, and in stress-related disorders such as depression. Acute increases in hippocampal IL-1β have been shown to impair cognition and reduce adult hippocampal neurogenesis, the birth of new neurons. However, the impact of prolonged increases in IL-1β, as evident in clinical conditions, on cognition has not been fully explored. Therefore, the present study utilized a lentiviral approach to induce long-term overexpression of IL-1β in the dorsal hippocampus of adult male Sprague Dawley rats and examine its impact on cognition. Following three weeks of viral integration, pattern separation, a process involving hippocampal neurogenesis, was impaired in IL-1β-treated rats in both object-location and touchscreen operant paradigms. This was coupled with a decrease in the number and neurite complexity of immature neurons in the hippocampus. Conversely, tasks involving the hippocampus, but not sensitive to disruption of hippocampal neurogenesis, including spontaneous alternation, novel object and location recognition were unaffected. Touchscreen operant visual discrimination, a cognitive task involving the prefrontal cortex, was largely unaffected by IL-1β overexpression. In conclusion, these findings suggest that chronically elevated IL-1β in the hippocampus selectively impairs pattern separation. Inflammatory-mediated disruption of adult hippocampal neurogenesis may contribute to the cognitive decline associated with neurodegenerative and stress-related disorders.
Brain Behav Immun, 2018; 74
31604142: Pawley LC, Hueston CM, O'Leary JD, Kozareva DA, Cryan JF, O'Leary OF, Nolan YM
Chronic intrahippocampal interleukin-1β overexpression in adolescence impairs hippocampal neurogenesis but not neurogenesis-associated cognition.
Both neuroinflammation and adult hippocampal neurogenesis (AHN) are implicated in many neurodegenerative disorders as

well as in neuropsychiatric disorders, which often become symptomatic during adolescence. A better knowledge of the impact that chronic neuroinflammation has on the hippocampus during the adolescent period could lead to the discovery of new therapeutics for some of these disorders. The hippocampus is particularly vulnerable to altered concentrations of the pro-inflammatory cytokine interleukin-1β (IL-1β), with elevated levels implicated in the aetiology of neurodegenerative disorders such as Alzheimer's and Parkinson's, and stress-related disorders such as depression. The effect of acutely and chronically elevated concentrations of hippocampal IL-1β have been shown to reduce AHN in adult rodents. However, the effect of exposure to chronic overexpression of hippocampal IL-1β during adolescence, a time of increased vulnerability, hasn't been fully interrogated. Thus, in this study we utilized a lentiviral approach to induce chronic overexpression of IL-1β in the dorsal hippocampus of adolescent male Sprague Dawley rats for 5 weeks, during which time its impact on cognition and hippocampal neurogenesis were examined. A reduction in hippocampal neurogenesis was observed along with a reduced level of neurite branching on hippocampal neurons. However, there was no effect of IL-1β overexpression on performance in pattern separation, novel object recognition or spontaneous alternation in the Y maze. Our study has highlighted that chronic IL-1β overexpression in the hippocampus during the adolescent period exerts a negative impact on neurogenesis independent of cognitive performance, and suggests a degree of resilience of the adolescent hippocampus to inflammatory insult.
Brain Behav Immun, 2020; 83
30844139: O'Leary JD, Hoban AE, Murphy A, O'Leary OF, Cryan JF, Nolan YM
Differential effects of adolescent and adult-initiated exercise on cognition and hippocampal neurogenesis.
Adolescence is a critical period for postnatal brain maturation and thus a time when environmental influences may affect cognitive processes in later life. Exercise during adulthood has been shown to increase hippocampal neurogenesis and enhance cognition. However, the impact of exercise initiated in adolescence on the brain and behavior in adulthood is not fully understood. The aim of this study was to compare the impact of voluntary exercise that is initiated during adolescence or early adulthood on cognitive performance in hippocampal-dependent and -independent processes using both object-based and touchscreen operant paradigms. Adult (8 week) and adolescent (4 week) male Sprague-Dawley rats had access to a running wheel (exercise) or were left undisturbed (sedentary control) for 4 weeks prior to behavioral testing and for the duration of the experiment. Results from touchscreen-based tasks showed that reversal learning was enhanced by both adult and adolescent-initiated exercise, while only exercise that began in adolescence induced a subtle but transient increase in performance on a location discrimination task. Spontaneous alternation in the Y-maze was impaired following adolescent onset exercise, while object memory was unaffected by either adult or adolescent-initiated exercise. Adolescent-initiated exercise increased the number of hippocampal DCX cells, an indicator of neurogenesis. It also promoted the complexity of neurites on DCX cells, a key process for synaptic integration, to a greater degree than adult-initiated exercise. Together the data here show that exercise during the adolescent period compared to adulthood differentially affects cognitive processes and the development of new hippocampal neurons in later life.
Hippocampus, 2019; 29
29793890: O'Leary JD, Hoban AE, Cryan JF, O'Leary OF, Nolan YM
Differential effects of adolescent and adult-initiated voluntary exercise on context and cued fear conditioning.
Adolescence is a critical period for postnatal brain maturation and a time during which there is increased susceptibility to developing emotional and cognitive-related disorders. Exercise during adulthood has been shown to increase hippocampal plasticity and enhance cognition. However, the impact of exercise initiated in adolescence, on brain and behaviour in adulthood is not yet fully explored or understood. The aim of this study was to compare the impact of voluntary exercise that was initiated either during adolescence or early adulthood on cognitive performance in hippocampal and amygdala-dependent fear conditioning tasks in adulthood. Adult (eight weeks old) and adolescent (four weeks old) male Sprague Dawley rats had access to a running wheel (exercise) or were left undisturbed (sedentary control) for seven weeks. Adult-initiated exercise enhanced both contextual and cued fear conditioning, while conversely, exercise that began in adolescence did not affect performance in these tasks. These behaviours were accompanied by differential expression of plasticity-related genes in the hippocampus and amygdala in adulthood. Specifically, adolescent-initiated exercise increased the expression of an array of plasticity related genes in the hippocampus including BDNF, synaptophysin, Creb, PSD-95, Arc, TLX and DCX, while adult-initiated exercise did not affect hippocampal plasticity related genes. Together results show that exercise initiated during adolescence has a differential effect on hippocampal and amygdala-dependent behaviour and neuronal plasticity compared to when exercise was initiated in adulthood. These findings reinforce adolescence as a period during which environmental influences have a distinct impact on neuronal plasticity and cognition. This article is part of the Special Issue entitled "Neurobiology of Environmental Enrichment".
Neuropharmacology, 2019; 145
29520633: O'Leary JD, O'Leary OF, Cryan JF, Nolan YM
A low-cost touchscreen operant chamber using a Raspberry Pi™.

The development of a touchscreen platform for rodent testing has allowed new methods for cognitive testing that have been back-translated from clinical assessment tools to preclinical animal models. This platform for cognitive assessment in animals is comparable to human neuropsychological tests such as those employed by the Cambridge Neuropsychological Test Automated Battery, and thus has several advantages compared to the standard maze apparatuses typically employed in rodent behavioral testing, such as the Morris water maze. These include improved translation of preclinical models, as well as high throughput and the automation of animal testing. However, these systems are relatively expensive, which can impede progress for researchers with limited resources. Here we describe a low-cost touchscreen operant chamber based on the single-board computer, Raspberry Pi, which is capable of performing tasks similar to those supported by current state-of-the-art systems. This system provides an affordable alternative for cognitive testing in a touchscreen operant paradigm for researchers with limited funding.
Behav Res Methods, 2018; 50
26970576: O'Leary JD, Kozareva DA, Hueston CM, O'Leary OF, Cryan JF, Nolan YM
The nuclear receptor Tlx regulates motor, cognitive and anxiety-related behaviours during adolescence and adulthood.
The nuclear receptor Tlx is a key regulator of embryonic and adult hippocampal neurogenesis and has been genetically linked to bipolar disorder. Mice lacking Tlx (Nr2e1(-/-)) display deficits in adult hippocampal neurogenesis and behavioural abnormalities. However, whether Tlx regulates behaviour during adolescence or in a sex-dependent manner remains unexplored. Therefore, we investigated the role of Tlx in a series of behavioural tasks in adolescent male and female mice with a spontaneous deletion of Tlx (Nr2e1(-/-) mice). Testing commenced at adolescence (postnatal day 28) and continued until adulthood (postnatal day 67). Adolescent male and female Nr2e1(-/-) mice were hyperactive in an open field, an effect that persisted in adulthood. Male but not female Nr2e1(-/-) mice exhibited reduced thigmotaxis during adolescence and adulthood. Impairments in rotarod motor performance developed in male and female Nr2e1(-/-) mice at the onset of adulthood. Spontaneous alternation in the Y-maze, a hippocampus-dependent task, was impaired in adolescent but not adult male and female Nr2e1(-/-) mice. Contextual fear conditioning was impaired in adolescent male Nr2e1(-/-) mice only, but both male and female adolescent Nr2e1(-/-) mice showed impaired cued fear conditioning, a hippocampal-amygdala dependent cognitive process. These deficits persisted into adulthood in males but not females. In conclusion, deletion of Tlx impairs motor, cognitive and anxiety-related behaviours during adolescence and adulthood in male and female mice with most effects occurring during adolescence rather than adulthood, independent of housing conditions. This suggests that Tlx has functions beyond regulation of adult hippocampal neurogenesis, and may be an important target in understanding neurobiological disorders.
Behav Brain Res, 2016; 306
27790850: O'Leary JD, O'Leary OF, Cryan JF, Nolan YM
Regulation of behaviour by the nuclear receptor TLX.
The orphan nuclear receptor Tlx (Nr2e1) is a key regulator of both embryonic and adult hippocampal neurogenesis. Several different mouse models have been developed which target Tlx in vivo including spontaneous deletion models (from birth) and targeted and conditional knockouts. Although some conflicting findings have been reported, for the most part studies have demonstrated that Tlx is important in regulating processes that underlie neurogenesis, spatial learning, anxiety-like behaviour and interestingly, aggression. More recent data have demonstrated that disrupting Tlx during early life induces hyperactivity and that Tlx plays a role in emotional regulation. Moreover, there are sex- and age-related differences in some behaviours in Tlx knockout mice during adolescence and adulthood. Here, we discuss the role of Tlx in motor-, cognitive-, aggressive- and anxiety-related behaviours during adolescence and adulthood. We examine current evidence which provides insight into Tlx during neurodevelopment, and offer our thoughts on the function of Tlx in brain and behaviour. We further hypothesize that Tlx is a key target in understanding the emergence of neurobiological disorders during adolescence and early adulthood.
Genes Brain Behav, 2018; 17

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CONNECTING THE ENGRAM DOTS: ROLE OF CONNECTIVITY IN MEMORY FORMATION

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Engram cells are a subset of neurons that experience plasticity during learning, allowing them to store long-term memory information. The connectivity pattern between these 'building blocks' of memory has been proposed as a candidate mechanism through which to hold the information in a stable state. However, the mechanisms of the formation and maintenance of engram cell connectivity are poorly understood. We combine inducible Cre recombinase-dependent labeling and doxycycline controlled tTA/TRE system to achieve simultaneous engram labeling of two experiences that are either separated or become associated by natural learning. The association of the memories induces a physical link between the previously-separated engrams, across the monosynaptic connection ventral CA1 to amygdala, indicating that learning translates perceptual information from the outside into a change in the connectivity pattern between engram cells. We further explore how this change in the connectivity pattern is required for the memory to be expressed, by optogenetically silencing the reactivation of the second engram. Finally, we explore the molecular correlates of the association and describe the modulation of plasticity-related mechanisms as a consequence of the connectivity pattern change. Overall, our research supports that learning experiences translate the information from the outside into the connectivity pattern between engram cells.

Pubmed:
34035282: Alvarez-Vergara MI, Rosales-Nieves AE, March-Diaz R, Rodriguez-Perinan G, Lara-Ureña N, Ortega-de San Luis C, Sanchez-Garcia MA, Martin-Bornez M, Gómez-Gálvez P, Vicente-Munuera P, Fernandez-Gomez B, Marchena MA, Bullones-Bolanos AS, Davila JC, Gonzalez-Martinez R, Trillo-Contreras JL, Sanchez-Hidalgo AC, Del Toro R, Scholl FG, Herrera E, Trepel M, Körbelin J, Escudero LM, Villadiego J, Echevarria M, de Castro F, Gutierrez A, Rabano A, Vitorica J, Pascual A
Non-productive angiogenesis disassembles Aß plaque-associated blood vessels.
The human Alzheimer's disease (AD) brain accumulates angiogenic markers but paradoxically, the cerebral microvasculature is reduced around Aß plaques. Here we demonstrate that angiogenesis is started near Aß plaques in both AD mouse models and human AD samples. However, endothelial cells express the molecular signature of non-productive angiogenesis (NPA) and accumulate, around Aß plaques, a tip cell marker and IB4 reactive vascular anomalies with reduced NOTCH activity. Notably, NPA induction by endothelial loss of presenilin, whose mutations cause familial AD and which activity has been shown to decrease with age, produced a similar vascular phenotype in the absence of Aß pathology. We also show that Aß plaque-associated NPA locally disassembles blood vessels, leaving behind vascular scars, and that microglial phagocytosis contributes to the local loss of endothelial cells. These results define the role of NPA and microglia in local blood vessel disassembly and highlight the vascular component of presenilin loss of function in AD.
Nat Commun, 2021; 12
33812274: Ryan TJ, Ortega-de San Luis C, Pezzoli M, Sen S
Engram cell connectivity: an evolving substrate for information storage.
Understanding memory requires an explanation for how information can be stored in the brain in a stable state. The change in the brain that accounts for a given memory is referred to as an engram. In recent years, the term engram has been operationalized as the cells that are activated by a learning experience, undergoes plasticity, and are sufficient and necessary for memory recall. Using this framework, and a growing toolbox of related experimental techniques, engram manipulation has become a central topic in behavioral, systems, and molecular neuroscience. Recent research on the topic has provided novel insights into the mechanisms of long-term memory storage, and its overlap with instinct. We propose that memory and instinct may be embodied as isomorphic topological structures within the brain's microanatomical circuitry.

Curr Opin Neurobiol, 2021; 67
30058223: Ortega-de San Luis C, Sanchez-Garcia MA, Nieto-Gonzalez JL, García-Junco-Clemente P, Montero-Sanchez A, Fernandez-Chacon R, Pascual A
Substantia nigra dopaminergic neurons and striatal interneurons are engaged in three parallel but interdependent postnatal neurotrophic circuits.
The striatum integrates motor behavior using a well-defined microcircuit whose individual components are independently affected in several neurological diseases. The glial cell line-derived neurotrophic factor (GDNF), synthesized by striatal interneurons, and Sonic hedgehog (Shh), produced by the dopaminergic neurons of the substantia nigra (DA SNpc), are both involved in the nigrostriatal maintenance but the reciprocal neurotrophic relationships among these neurons are only partially understood. To define the postnatal neurotrophic connections among fast-spiking GABAergic interneurons (FS), cholinergic interneurons (ACh), and DA SNpc, we used a genetically induced mouse model of postnatal DA SNpc neurodegeneration and separately eliminated Smoothened (Smo), the obligatory transducer of Shh signaling, in striatal interneurons. We show that FS postnatal survival relies on DA SNpc and is independent of Shh signaling. On the contrary, Shh signaling but not dopaminergic striatal innervation is required to maintain ACh in the postnatal striatum. ACh are required for DA SNpc survival in a GDNF-independent manner. These data demonstrate the existence of three parallel but interdependent neurotrophic relationships between SN and striatal interneurons, partially defined by Shh and GDNF. The definition of these new neurotrophic interactions opens the search for new molecules involved in the striatal modulatory circuit maintenance with potential therapeutic value.
Aging Cell, 2018; 17
29784659: Ortega-de San Luis C, Ryan TJ
United states of amnesia: rescuing memory loss from diverse conditions.
Amnesia - the loss of memory function - is often the earliest and most persistent symptom of dementia. It occurs as a consequence of a variety of diseases and injuries. These include neurodegenerative, neurological or immune disorders, drug abuse, stroke or head injuries. It has both troubled and fascinated humanity. Philosophers, scientists, physicians and anatomists have all pursued an understanding of how we learn and memorise, and why we forget. In the last few years, the development of memory engram labelling technology has greatly impacted how we can experimentally study memory and its disorders in animals. Here, we present a concise discussion of what we have learned about amnesia through the manipulation of engrams, and how we may use this knowledge to inform novel treatments of amnesia.
Dis Model Mech, 2018; 11
27445711: Ortega-de San Luis C, Pascual A
Simultaneous Detection of Both GDNF and GFRα1 Expression Patterns in the Mouse Central Nervous System.
Glial cell line-derived neurotrophic factor (GDNF) is proposed as a therapeutic tool in Parkinson's disease, addiction-related disorders, and neurodegenerative conditions affecting motor neurons (MNs). Despite the high amount of work about GDNF therapeutic application, the neuronal circuits requiring GDNF trophic support in the brain and spinal cord (SC) are poorly characterized. Here, we defined GDNF and GDNF family receptor-α 1 (GFRα1) expression pattern in the brain and SC of newborn and adult mice. We performed systematic and simultaneous detection of EGFP and LacZ expressing alleles in reporter mice and asked whether modifications of this signaling pathway lead to a significant central nervous system (CNS) alteration. GFRα1 was predominantly expressed by neurons but also by an unexpected population of non-neuronal cells. GFRα1 expression pattern was wider in neonatal than in adult CNS and GDNF expression was restricted in comparison with GFRα1 at both developmental time points. The use of confocal microscopy to imaging X-gal deposits and EGFP allowed us to identify regions containing cells that expressed both proteins and to discriminate between auto and non-autotrophic signaling. We also suggested long-range GDNF-GFRα1 circuits taking advantage of the ability of the EGFP genetically encoded reporter to label long distance projecting axons. The complete elimination of either the ligand or the receptor during development did not produce major abnormalities, suggesting a preponderant role for GDNF signaling during adulthood. In the SC, our results pointed to local modulatory interneurons as the main target of GDNF produced by Clarke's column (CC) cells. Our work increases the understanding on how GDNF signals in the CNS and establish a crucial framework for posterior studies addressing either the biological role of GDNF or the optimization of trophic factor-based therapies.
Front Neuroanat, 2016; 10

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BOARD NUMBER: S07-017

INTERFERING WITH ENGRAM RETRIEVAL: THE NEUROBIOLOGY OF FORGETTING

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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While investigation of engram cell properties and functionality during memory formation and recall has been extensive, less is known about how engram cells are affected by forgetting. We sought to elucidate the behavioural and neurobiological correlates of retroactive interference. We characterized a form of interference-based forgetting using an object memory behavioural paradigm. Using this behaviour, we labelled dentate gyrus (DG) engram cells in mice during object exposure, and studied the effect of retroactive interference on engram cell function, showing that interference results in decreased engram cell reactivation during recall trials. We found that, following interference, a brief re-exposure to the original stimulus is sufficient to restore access to the missing information evidenced by reactivation of the originally labelled ensemble. Moreover, exposure to a misleading cue drives the reconsolidation of a ‘false’ memory. Furthermore, through optogenetic stimulation of engram cells we were able to elicit the artificial retrieval of the forgotten memory. Together, these findings indicate that retroactive interference modulates the accessibility of engram cells in a manner that is both reversible and updatable. Retroactive interference may constitute a form of adaptive forgetting, where in everyday life new perceptual and environmental inputs modulate the natural forgetting process.

Pubmed:
31844154: Torromino G, Autore L, Khalil V, Mastrorilli V, Griguoli M, Pignataro A, Centofante E, Biasini GM, De Turris V, Ammassari-Teule M, Rinaldi A, Mele A
Offline ventral subiculum-ventral striatum serial communication is required for spatial memory consolidation.
The hippocampal formation is considered essential for spatial navigation. In particular, subicular projections have been suggested to carry spatial information from the hippocampus to the ventral striatum. However, possible cross-structural communication between these two brain regions in memory formation has thus far been unknown. By selectively silencing the subiculum-ventral striatum pathway we found that its activity after learning is crucial for spatial memory consolidation and learning-induced plasticity. These results provide new insight into the neural circuits underlying memory consolidation and establish a critical role for off-line cross-regional communication between hippocampus and ventral striatum to promote the storage of complex information.
Nat Commun, 2019; 10
32574637: Autore L, Ryan TJ
Memory: It's Not a Lie if You Believe It.
Memories are crucial for making accurate predictions about our environment. New research suggests that, in the face of limited perceptual evidence, our brains quickly form generalized contextual memory engrams that can be refined based on future, confirmatory or misleading, experience.
Curr Biol, 2020; 30

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BOARD NUMBER: S07-018

A FEAR MEMORY ENGRAM IN THE MOUSE AUDITORY CORTEX

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Memory is supported by ensembles of neurons known as engram cells. While engram cells have been well characterized in the hippocampus and related brain regions, little is known about the cellular properties of engram cells in sensory cortical areas. This study used activity-dependant engram tagging technology and patch clamp electrophysiology to characterize the cellular and dendritic properties of engram cells within the auditory cortex. Using tone fear conditioning and optogenetics in c-fos-tTA transgenic mice, we identified a population of engram cells with specific properties amongst layer 5 pyramidal cells. Using histological methods, we showed that these engram cells are reactivated by natural recall. Furthermore, their optogenetic reactivation induced behavioural expression of fear memory. *Ex vivo* electrophysiological recordings showed that photoactivation of engram cell axons elicited a stronger synaptic response in engram cells than in non-engram cells, suggesting the formation of a local engram network within the auditory cortex after learning. Analysis of the cellular intrinsic properties revealed that Ih currents, key regulators of dendritic excitability, are increased in engram cells compared to non-engram cells, suggesting a learning-induced plasticity of dendritic excitability. In conclusion, we identified and characterised a population of engram cells forming a local engram network with specific intrinsic properties, that manifest at the dendritic level through regulation of Ih currents.

Pubmed:
30285241: Rosier M, Le Barillier L, Meunier D, El Yacoubi M, Malleret G, Salin PA
Post-learning paradoxical sleep deprivation impairs reorganization of limbic and cortical networks associated with consolidation of remote contextual fear memory in mice.
Paradoxical sleep (PS) has been shown to play an important role in memory, in particular in emotional memory processes. However, the involvement of this particular sleep stage in the systemic consolidation of remote (30 days old) memory has never been tested. We examined whether post-learning PS could play a role in the consolidation of remote fearful memory and in the brain network reorganization that depends on it.
Sleep, 2018; 41

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BOARD NUMBER: S07-019

IMPAIRED PATTERN COMPLETION DURING MEMORY RECALL IN A MOUSE MODEL OF FRAGILE X SYNDROME

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The Fragile X Syndrome (FXS) is the leading monogenetic cause of cognitive impairment and autism. FXS symptoms include excessive adherence to patterns indicating perturbed hippocampal networks. The hippocampus is involved in pattern separation/completion processes during memory formation. Especially the CA3 region is of interest because of its auto-associative recurrent inputs. The absence of the fragile X mental retardation protein (FMRP) in patients and Fmr1 KO mice leads to an abnormal development of dendritic spines which form the postsynaptic compartment. Recently, we showed that different synapse types on CA3 neurons are affected differently by the absence of FMRP. While mossy fiber inputs to CA3 neurons are transiently premature during the fourth postnatal week, collateral/commissural inputs onto regular spines are increased in number in adult animals. Therefore, we were interested whether this would result in specific impairments in information processing in CA3 neurons. The CA3 region plays a pivotal role in pattern completion processes important for efficient memory recall even from parts of the initial memory stimulus. We therefore subjected Fmr1 mice to spatial training in the Morris water maze task and performed partially cued probe trials by selectively removing spatial cues. Consistent with the collateral spine phenotype observed in adult animals, we can show an impairment in pattern completion in adult and not in juvenile animals. Future experiments are needed to reveal whether the spine phenotype could be restored using regular spatial training or an enriched environment to tune hippocampal networks and whether this would restore pattern completion in the hippocampus.

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BOARD NUMBER: S07-020

STUDYING THE ROLE OF CB1 RECEPTORS ON MEMORY AND NAVIGATION IN MALE AND FEMALE MICE

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Hippocampal circuits play a crucial role in cognitive processes (e.g., navigation strategies). Furthermore, cortical areas participate in decision-making and emotional processes shaping the performance of the animals during memory tasks. In this work, we want to dissect the specific and sex-dependent role of CB1 signaling in different circuits on memory and navigation. Methods: We use behavioral genetics and pharmacology in C57BL6 and CB1-flox male and female mice carrying a Cre-mediated CB1 deletion. Viral vectors (AAV-Cre) were injected into the hippocampus and the prefrontal cortex. We use the Barnes maze test to assess cognitive performance (e.g., memory, navigation). Furthermore, a battery of anxiety-like behavioral protocols is performed prior to cognitive evaluation to rule out potential confounding factors. Results: intraperitoneal injection of THC immediately after each day of acquisition in the Barnes maze impairs memory consolidation in males but not in females. Interestingly, THC-treated mice specifically show a decrease in spatial strategy, suggesting a direct effect of the pharmacological treatment on hippocampal circuits. On the other hand, male and female CB1-flox mice carrying a hippocampal or prefrontal CB1 deletion present no significant changes in the learning processes compared to control littermates. However, CB1 deletion on male mice produced a specific decrease in the spatial strategy. Strikingly, such changes were not found on female mice. Conclusions: These results show that CB1 receptors in the hippocampus and the prefrontal cortex are necessary for developing and consolidating navigation strategies in a sex-dependent way.

Pubmed:
34948035: Egaña-Huguet J, Soria-Gómez E, Grandes P
The Endocannabinoid System in Glial Cells and Their Profitable Interactions to Treat Epilepsy: Evidence from Animal Models.
Epilepsy is one of the most common neurological conditions. Yearly, five million people are diagnosed with epileptic-related disorders. The neuroprotective and therapeutic effect of (endo)cannabinoid compounds has been extensively investigated in several models of epilepsy. Therefore, the study of specific cell-type-dependent mechanisms underlying cannabinoid effects is crucial to understanding epileptic disorders. It is estimated that about 100 billion neurons and a roughly equal number of glial cells co-exist in the human brain. The glial population is in charge of neuronal viability, and therefore, their participation in brain pathophysiology is crucial. Furthermore, glial malfunctioning occurs in a wide range of neurological disorders. However, little is known about the impact of the endocannabinoid system (ECS) regulation over glial cells, even less in pathological conditions such as epilepsy. In this review, we aim to compile the existing knowledge on the role of the ECS in different cell types, with a particular emphasis on glial cells and their impact on epilepsy. Thus, we propose that glial cells could be a novel target for cannabinoid agents for treating the etiology of epilepsy and managing seizure-like disorders.
Int J Mol Sci, 2021; 22
34356889: Rico-Barrio I, Peñasco S, Lekunberri L, Serrano M, Egaña-Huguet J, Mimenza A, Soria-Gomez E, Ramos A, Buceta I, Gerrikagoitia I, Mendizabal-Zubiaga J, Elezgarai I, Puente N, Grandes P
Environmental Enrichment Rescues Endocannabinoid-Dependent Synaptic Plasticity Lost in Young Adult Male Mice after Ethanol Exposure during Adolescence.
Binge drinking (BD) is a serious health concern in adolescents as high ethanol (EtOH) consumption can have cognitive sequelae later in life. Remarkably, an enriched environment (EE) in adulthood significantly recovers memory in mice after adolescent BD, and the endocannabinoid, 2-arachydonoyl-glycerol (2-AG), rescues synaptic plasticity and memory impaired in adult rodents upon adolescent EtOH intake. However, the mechanisms by which EE improves memory are unknown. We investigated this in adolescent male C57BL/6J mice exposed to a drinking in the dark (DID) procedure four days per week for a duration of 4 weeks. After DID, the mice were nurtured under an EE for 2 weeks and were subjected to the Barnes Maze Test performed the last 5 days of withdrawal. The EE rescued memory and restored the EtOH-disrupted endocannabinoid (eCB)-dependent excitatory long-term depression at the dentate medial perforant path synapses (MPP-LTD). This recovery was dependent on both the cannabinoid CB1 receptor and group I metabotropic glutamate receptors (mGluRs) and required

2-AG. Also, the EE had a positive effect on mice exposed to water through the transient receptor potential vanilloid 1 (TRPV1) and anandamide (AEA)-dependent MPP long-term potentiation (MPP-LTP). Taken together, EE positively impacts different forms of excitatory synaptic plasticity in water- and EtOH-exposed brains.
Biomedicines, 2021; 9
34305539: Egaña-Huguet J, Saumell-Esnaola M, Achicallende S, Soria-Gomez E, Bonilla-Del Río I, García Del Caño G, Barrondo S, Sallés J, Gerrikagoitia I, Puente N, Elezgarai I, Grandes P
Lack of the Transient Receptor Potential Vanilloid 1 Shifts Cannabinoid-Dependent Excitatory Synaptic Plasticity in the Dentate Gyrus of the Mouse Brain Hippocampus.
The transient receptor potential vanilloid 1 (TRPV1) participates in synaptic functions in the brain. In the dentate gyrus, post-synaptic TRPV1 in the granule cell (GC) dendritic spines mediates a type of long-term depression (LTD) of the excitatory medial perforant path (MPP) synapses independent of pre-synaptic cannabinoid CB receptors. As CB receptors also mediate LTD at these synapses, both CB and TRPV1 might be influencing the activity of each other acting from opposite synaptic sites. We tested this hypothesis in the MPP-GC synapses of mice lacking TRPV1 (TRPV1-/-). Unlike wild-type (WT) mice, low-frequency stimulation (10 min at 10 Hz) of TRPV1-/- MPP fibers elicited a form of long-term potentiation (LTP) that was dependent on (1) CB receptors, (2) the endocannabinoid 2-arachidonoylglycerol (2-AG), (3) rearrangement of actin filaments, and (4) nitric oxide signaling. These functional changes were associated with an increase in the maximum binding efficacy of guanosine-5'-O-(3-[S]thiotriphosphate) ([S]GTPγS) stimulated by the CB receptor agonist CP 55,940, and a significant decrease in receptor basal activation in the TRPV1-/- hippocampus. Finally, TRPV1-/- hippocampal synaptosomes showed an augmented level of the guanine nucleotide-binding (G) Gα, Gα, and Gα protein alpha subunits. Altogether, the lack of TRPV1 modifies CB receptor signaling in the dentate gyrus and causes the shift from CB receptor-mediated LTD to LTP at the MPP-GC synapses.
Front Neuroanat, 2021; 15
33692673: Egaña-Huguet J, Bonilla-Del Río I, Gómez-Urquijo SM, Mimenza A, Saumell-Esnaola M, Borrega-Roman L, García Del Caño G, Sallés J, Puente N, Gerrikagoitia I, Elezgarai I, Grandes P
The Absence of the Transient Receptor Potential Vanilloid 1 Directly Impacts on the Expression and Localization of the Endocannabinoid System in the Mouse Hippocampus.
The transient receptor potential vanilloid 1 (TRPV1) is a non-selective ligand-gated cation channel involved in synaptic transmission, plasticity, and brain pathology. In the hippocampal dentate gyrus, TRPV1 localizes to dendritic spines and dendrites postsynaptic to excitatory synapses in the molecular layer (ML). At these same synapses, the cannabinoid CB receptor (CBR) activated by exogenous and endogenous cannabinoids localizes to the presynaptic terminals. Hence, as both receptors are activated by endogenous anandamide, co-localize, and mediate long-term depression of the excitatory synaptic transmission at the medial perforant path (MPP) excitatory synapses though by different mechanisms, it is plausible that they might be exerting a reciprocal influence from their opposite synaptic sites. In this anatomical scenario, we tested whether the absence of TRPV1 affects the endocannabinoid system. The results obtained using biochemical techniques and immunoelectron microscopy in a mouse with the genetic deletion of TRPV1 show that the expression and localization of components of the endocannabinoid system, included CBR, change upon the constitutive absence of TRPV1. Thus, the expression of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) drastically increased in TRPV1 whole homogenates. Furthermore, CBR and MAGL decreased and the cannabinoid receptor interacting protein 1a (CRIP1a) increased in TRPV1 synaptosomes. Also, CBR positive excitatory terminals increased, the number of excitatory terminals decreased, and CBR particles dropped significantly in inhibitory terminals in the dentate ML of TRPV1 mice. In the outer 2/3 ML of the TRPV1 mutants, the proportion of CBR particles decreased in dendrites, and increased in excitatory terminals and astrocytes. In the inner 1/3 ML, the proportion of labeling increased in excitatory terminals, neuronal mitochondria, and dendrites. Altogether, these observations indicate the existence of compensatory changes in the endocannabinoid system upon TRPV1 removal, and endorse the importance of the potential functional adaptations derived from the lack of TRPV1 in the mouse brain.
Front Neuroanat, 2021; 15
31022405: Peñasco S, Rico-Barrio I, Puente N, Gómez-Urquijo SM, Fontaine CJ, Egaña-Huguet J, Achicallende S, Ramos A, Reguero L, Elezgarai I, Nahirney PC, Christie BR, Grandes P
Endocannabinoid long-term depression revealed at medial perforant path excitatory synapses in the dentate gyrus.
The endocannabinoid system modulates synaptic plasticity in the hippocampus, but a link between long-term synaptic plasticity and the type 1 cannabinoid (CB) receptor at medial perforant path (MPP) synapses remains elusive. Here, immuno-electron microscopy in adult mice showed that ~26% of the excitatory synaptic terminals in the middle 1/3 of the dentate molecular layer (DML) contained CB receptors, and field excitatory postsynaptic potentials evoked by MPP stimulation were inhibited by CB receptor activation. In addition, MPP stimulation at 10 Hz for 10 min triggered CB receptor-dependent excitatory long-term depression (eCB-eLTD) at MPP synapses of wild-type mice but not on CB-knockout mice. This eCB-

eLTD was group I mGluR-dependent, required intracellular calcium influx and 2-arachydonoyl-glycerol (2-AG) synthesis but did not depend on N-methyl-d-aspartate (NMDA) receptors. Overall, these results point to a functional role for CB receptors with eCB-eLTD at DML MPP synapses and further involve these receptors in memory processing within the adult brain.
Neuropharmacology, 2019; 153

29480581: Gutiérrez-Rodríguez A, Bonilla-Del Río I, Puente N, Gómez-Urquijo SM, Fontaine CJ, Egaña-Huguet J, Elezgarai I, Ruehle S, Lutz B, Robin LM, Soria-Gómez E, Bellocchio L, Padwal JD, van der Stelt M, Mendizabal-Zubiaga J, Reguero L, Ramos A, Gerrikagoitia I, Marsicano G, Grandes P

Localization of the cannabinoid type-1 receptor in subcellular astrocyte compartments of mutant mouse hippocampus.
Astroglial type-1 cannabinoid (CB) receptors are involved in synaptic transmission, plasticity and behavior by interfering with the so-called tripartite synapse formed by pre- and post-synaptic neuronal elements and surrounding astrocyte processes. However, little is known concerning the subcellular distribution of astroglial CB receptors. In particular, brain CB receptors are mostly localized at cells' plasmalemma, but recent evidence indicates their functional presence in mitochondrial membranes. Whether CB receptors are present in astroglial mitochondria has remained unknown. To investigate this issue, we included conditional knock-out mice lacking astroglial CB receptor expression specifically in glial fibrillary acidic protein (GFAP)-containing astrocytes (GFAP-CB -KO mice) and also generated genetic rescue mice to re-express CB receptors exclusively in astrocytes (GFAP-CB -RS). To better identify astroglial structures by immunoelectron microscopy, global CB knock-out (CB -KO) mice and wild-type (CB -WT) littermates were intra-hippocampally injected with an adeno-associated virus expressing humanized renilla green fluorescent protein (hrGFP) under the control of human GFAP promoter to generate GFAPhrGFP-CB -KO and -WT mice, respectively. Furthermore, double immunogold (for CB) and immunoperoxidase (for GFAP or hrGFP) revealed that CB receptors are present in astroglial mitochondria from different hippocampal regions of CB -WT, GFAP-CB -RS and GFAPhrGFP-CB -WT mice. Only non-specific gold particles were detected in mouse hippocampi lacking CB receptors. Altogether, we demonstrated the existence of a precise molecular architecture of the CB receptor in astrocytes that will have to be taken into account in evaluating the functional activity of cannabinergic signaling at the tripartite synapse.
Glia, 2018; 66

31818976: Bialecki J, Werner A, Weilinger NL, Tucker CM, Vecchiarelli HA, Egaña J, Mendizabal-Zubiaga J, Grandes P, Hill MN, Thompson RJ

Suppression of Presynaptic Glutamate Release by Postsynaptic Metabotropic NMDA Receptor Signalling to Pannexin-1.
The impact of pannexin-1 (Panx1) channels on synaptic transmission is poorly understood. Here, we show that selective block of Panx1 in single postsynaptic hippocampal CA1 neurons from male rat or mouse brain slices causes intermittent, seconds long increases in the frequency of sEPSC following Schaffer collateral stimulation. The increase in sEPSC frequency occurred without an effect on evoked neurotransmission. Consistent with a presynaptic origin of the augmented glutamate release, the increased sEPSC frequency was prevented by bath-applied EGTA-AM or TTX. Manipulation of a previously described metabotropic NMDAR pathway (i.e., by preventing ligand binding to NMDARs with competitive antagonists or blocking downstream Src kinase) also increased sEPSC frequency similar to that seen when Panx1 was blocked. This facilitated glutamate release was absent in transient receptor potential vanilloid 1 (TRPV1) KO mice and prevented by the TRPV1 antagonist, capsazepine, suggesting it required presynaptic TRPV1. We show presynaptic expression of TRPV1 by immunoelectron microscopy and link TRPV1 to Panx1 because Panx1 block increases tissue levels of the endovanilloid, anandamide. Together, these findings demonstrate an unexpected role for metabotropic NMDARs and postsynaptic Panx1 in suppression of facilitated glutamate neurotransmission. The postsynaptic ion and metabolite channel, pannexin-1, is regulated by metabotropic NMDAR signaling through Src kinase. This pathway suppresses facilitated release of presynaptic glutamate during synaptic activity by regulating tissue levels of the transient receptor potential vanilloid 1 agonist anandamide.
J Neurosci, 2020; 40

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BOARD NUMBER: S07-021

THINK TWICE BEFORE YOU KEEP YOURSELF AWAKE!: THE EFFECTS OF TWO DIFFERENT SLEEP DEPRIVATION METHODS ON THE MEMORY CONSOLIDATION OF OBJECT-LOCATION MEMORIES.

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Sleep deprivation (SD) is a significant health problem in the modern world, with studies showing that average sleep time has decreased by 1.5 hours per night in as little as 50 years. It is widely known that sleep is indispensable for brain function and plays a key role in memory processes. Therefore, the effects of sleep deprivation on the brain have been extensively studied in mammalian models. The most common experimental methods of SD used in rodent models are the gentle handling method (GH) and novelty method. GH involves gently tapping or shaking the cage, whereas the novelty method involves introducing novel objects in the cage. While both methods have been used to examine how sleep loss affects brain plasticity, it is unclear as to how these approaches impact hippocampal memory consolidation. Recent work in our lab has shown that GH for 6 hours impairs hippocampal-dependent memory consolidation by negatively impacting neuronal connectivity. However, it is unclear whether SD using object novelty impacts memory processes as well. In contrast to GH, using novel objects to keep an animal awake presents a very different external stimulus. Consequently, it is plausible that stimulating an animal using two different SD methods could have very different implications on cognition. In the present study, we, therefore, determined how both SD methods affected hippocampal-dependent memory consolidation in the Object Location Memory task. Using this approach, we attempt to discern if the type of SD plays a significant role in affecting memory consolidation.

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TIME CODE FOR EXPECTED EVENTS AND DURATIONS IN THE MONKEY'S STRIATUM AND HIPPOCAMPUS

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Time tracking allows an adaptive anticipation of events when supported by memory. The striatum and the hippocampus are known to show strong time-related activity, likely supporting time for action (caudate-putamen) and time for memory, respectively. Here we asked whether their neural activity represents the time of expected events and/or the duration of the intervals separating them. We designed a task based solely on cumulative elapsed time, thereby nesting two duration probabilities (short and intermediate) into a longer one. We recorded neural activity in caudate, putamen and hippocampus of two rhesus macaques categorizing durations into short (.5s), intermediate (1s) or long (2s) above 90% correct. After identifying time-modulated cells in all structures, we asked whether population activity during the longest interval was constant (continuous passage of time) or event-modulated (marking each interval duration or their likely endings). A Support-Vector Machine based decoding showed that time was decoded above chance in all regions. However, there were strong differences between structures. Caudate activity supported decoding with the highest accuracy and temporal resolution throughout the long interval, but weakly modulated by expected events. In contrast, putamen activity supported high decoding accuracy for expected events, while interval transitions followed a stepwise function. At a larger scale, the hippocampus discriminated only the first and the second half of the long interval. Our results suggest that time is represented almost continuously in the caudate, while the putamen and the hippocampus show a discrete representation highlighting times of expected events and their durations, through transitions in population states.

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NEURAL REPRESENTATIONS OF STIMULUS, ACTION AND OUTCOME IN HIPPOCAMPUS AND PREFRONTAL CORTEX OF MICE DURING TRACE CONDITIONING

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The hippocampus and the medial prefrontal cortex (mPFC) participate in episodic and working memory. Although the neural activity in both areas can be linked to the formation of memory representations, the neural mechanisms involved in learning temporal associations between stimulus and reward are still unknown. In this work, we use head-fixed mice with simultaneous electrophysiological recordings from the hippocampal CA1 subfield and the mPFC with two 128-channel silicon probes to investigate this in an auditory trace conditioning task. Animals had to learn to associate a sensory stimulus (conditioned stimulus; CS) and a reward (unconditioned stimulus; US) separated by a delay (trace period). First, we found that CA1 and mPFC differentially encoded stimulus and reward onset through neuronal temporal sequences. Moreover, CS and US temporal representations disentangled from each other after learning, an effect that was partially reverted in incorrect trials. Additionally, we found that mPFC held a strong and stable representation of stimulus identity after learning, bridging stimulus onset and reward, while in CA1 stimulus identity could be only partially recovered during the trace period. Crucially, a hidden Markov model revealed that the onset of CS-coding states predicted the behavioral performance at a single-trial level in both correct and error trials. Together these results suggest temporal sequences in the hippocampus have a role in the initial processing of the stimulus and encode outcome predictability, while mPFC is important to create the stimulus-reward association and to drive decision making.

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BOARD NUMBER: S07-024

ASSESSMENT OF ABSENCE SEIZURES ANIMAL MODELS COGNITIVE COMORBIDITIES

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Introduction: Typical absence seizures (ASs) of 3-4 Hz thalamocortical spike-wave discharges, are the hallmark of Childhood Absence Epilepsy (CAE). Recent studies in CAE cohorts show 30% of pharmaco-resistance and 60% of patients show psychiatric comorbidities, including attention, cognitive, memory and mood impairments. In humans, there is a pressing need to new therapeutics that address both seizures and comorbidities and to achieve so, a better understanding of ASs animal models behaviour is needed. Aims: Using ASs animal models, the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) and STARGAZER (STG) mice, and respective controls, we aimed to assess behaviour comorbidities in both models. Methods: Behaviour tests were carried out to assess anxiety, locomotion, learning and memory. Synaptic plasticity was assessed by long-term potentiation (LTP) in hippocampal slices. Results: GAERS did not exhibit impaired locomotor activity neither anxiety-like behaviour. Significant differences were found between GAERS and controls in novel object recognition and cross-modal object recognition memory. In the Y-Maze and delayed non-matching to position tasks, deficits were only found for spontaneous alternation working memory. Hippocampal-dependent memory, Barnes Maze test, showed significantly lower performance in GAERS and STG than controls, suggesting impairments in the prefrontal cortex, thalamus, parahippocampal regions, and hippocampal-dependent memory. LTP magnitude in GAERS and STG was significantly lower than that obtained from Wistar and WT animals, respectively. Conclusions: This study suggests memory deficits in GAERS, in spatial working memory and object recognition memory. GAERS and STG models display impaired reference memory, in line with the reduced LTP magnitude.

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BOARD NUMBER: S07-025

ABLATION OF NEUROPLASTIN EXPRESSION IN GABAERGIC INTERNEURONS INDUCES RETROGRADE AMNESIA OF ASSOCIATIVE MEMORIES

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Background: We have shown that elimination of neuroplastin gene expression from all CNS neurons prevents associative learning resulting in anterograde amnesia. Strikingly, ablation after associative learning results in specific retrograde amnesia. Neuroplastin contributes to synaptic plasticity modulating long-term potentiation (LTP), formation and stabilization of excitatory synapses, and neuronal spinogenesis. Neuroplastin forms a complex with plasma membrane Ca^{2+} ATPase (PMCA) which is essential to pump Ca^{2+} from the cytoplasm to the extracellular space. Neuroplastin-deficient mice display complex cognitive deficits and reduced levels of PMCAs suggesting alterations in Ca^{2+}-dependent signaling. Because selective loss of neuroplastin expression in glutamatergic neurons does not interfere with associative learning, we investigated the necessity of neuroplastin expression in GABAergic interneurons for associative learning and memory. **Methods:** We generated *Nptn*$^{lox/loxGAD2CreERT}$ mice allowing inactivation of the neuroplastin gene in GABAergic interneurons by tamoxifen activation of the GAD2 promoter driven Cre recombinase. Neuroplastin expression was analyzed by immunofluorescence. Associative learning and memory were investigated in a two-way active avoidance paradigm (Shuttle Box) with light as stimulus. **Results:** Neuroplastin immunostaining was selectively reduced in parvalbumin positive interneurons of tamoxifen-treated adult *Nptn*$^{lox/loxGAD2CreERT}$ mice. These mice showed normal acquisition of the two-way active avoidance task. However, ablation of the neuroplastin gene after the successful acquisition of this task resulted in retrograde amnesia with significantly reduced memory. **Conclusions:** Ablation of neuroplastin in GABAergic interneurons is sufficient to induce retrograde amnesia of associations and allows to discriminate mechanisms selective for learning and memory. Our results confirm that neuroplastin is essential for associative learning and memory. *Keywords:* Associative memory, Neuroplastin, GABAergic interneurons, Retrograde amnesia, PMCAs

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BOARD NUMBER: S07-026

DISTINCT HIPPOCAMPAL NETWORK STATES SUPPORT THETA PHASE PRECESSION AND THETA SEQUENCES IN CA1

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The hippocampus likely uses temporal coding to represent complex memories via mechanisms such as theta phase precession and theta sequences. Theta sequences are rapid sweeps of spikes from multiple place cells, encoding past or planned trajectories or non-spatial information. Phase precession, the correlation between a place cell's theta firing phase and animal position has been suggested to facilitate sequence emergence. Here we address the relationship of these phenomena by taking advantage of simultaneous recordings of place cells population activity and layer-specific LFP dynamics in the CA1 region. We find that phase precession varies strongly across cells and environmental contingencies. Phase precession is predominantly present when the medium gamma oscillation (60-90 Hz, linked to Entorhinal inputs) dominates the CA1 network state. Conversely, theta sequences are most evident for non-precessing cells or with leading slow gamma (20-45 Hz, linked to CA3 inputs). These results challenge the view that phase precession is the mechanism underlying the emergence of theta sequences and point at a "dual network states" model for the hippocampal temporal code, potentially supporting merging of memory and exogenous information in CA1.

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BOARD NUMBER: S07-027

DETERMINING THE RELATIONSHIP BETWEEN MOLECULAR CHANGES IN THE AMYGDALA AND THE EMERGENCE OF ASSOCIATIVE LEARNING IN THE RAT

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Understanding the neural basis of learning is a fundamental question facing neuroscientists. Although much is known about the relationship between LTP and consolidated information, we know very little about what happened when the association between an event or behaviour and a consequence is realised. This has been defined as a moment of insight and the specific molecular changes which accompany such moments are not well understood. To shed light on this mechanism we used a classical conditioning protocol paired with an algorithm that allows us to specify when a rat has learned the association between a stimulus (sound) and its outcome (delivery of a food pellet). We quantified the phosphorylation of ERK, a signalling molecule related to learning, in the central amygdala, a region of the brain involved in behaviour driven by the value of a stimulus during normal associative learning and during aberrant behaviour towards addictive stimuli. We found that rats that had experienced the moment of insight expressed significantly more p-ERK than those that did not or those that were overtrained following acquisition. Thus, learning in our paradigm is accompanied by cellular changes that transiently peak when the “moment of insight” occurs and then subside following it.

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BOARD NUMBER: S07-028

TWO DISTINCT WAYS TO FORM LONG-TERM OBJECT-RECOGNITION MEMORY DURING SLEEP AND WAKEFULNESS

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Sleep is considered as the brain state that is optimal for long-term memory formation. However, some observations suggest that long-term memory can also be formed in the waking state. Here, we compared the effects of 2-h post-encoding periods of sleep and wakefulness on the formation of long-term memory for objects and their associated environmental contexts. We employed a novel-object recognition (NOR) task in rats, using object exploration and exploratory rearing on hind limbs, respectively, as behavioral indicators of these memories. Rats in both post-encoding wake and sleep conditions showed significant long-term NOR memory at remote recall testing (1-week), with NOR memory after sleep predicted by the occurrence EEG spindle-slow oscillation coupling during sleep. Importantly, rats in the sleep group exhibited a decrease in exploratory rearing at recall testing, revealing successful recall of environmental context. In contrast, the wake rats showed no change in rearing behaviors, suggesting that although NOR memory was present, context memory was lost. Disruption of hippocampal function during the post-encoding interval suppressed long-term NOR memory together with context memory formation, when the rats had slept in this interval, but enhanced NOR memory when they were awake. Also, we show that, under certain conditions (e.g., recognition of objects in a context other than learning), NOR memory in the wake rats was superior to that after sleep. Our findings indicate two distinct and competing modes of long-term memory formation. Sleep consolidation is hippocampus-dependent and implicates event-context binding, whereas wake consolidation is impaired by hippocampal activation and strengthens context-independent representations.

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BOARD NUMBER: S07-029

HIPPOCAMPAL CB1 RECEPTORS CONTROL OBESOGENIC DIET-INDUCED MEMORY IMPAIRMENT

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Obesity is associated with adverse cognitive outcomes, particularly those depending on hippocampal function, in humans as well as in animal models. As the endocannabinoid system participates in obesity and regulates memory processes, we address its causal involvement in memory deficits induced by obesogenic high-fat diet (HFD) intake during adolescence in mice. Using a pharmacological approach, we demonstrated that a systemic blockade of the main cannabinoid type 1 receptor (CB1R) rescued HFD-induced deficits of long-term object recognition memory but also normalized training-induced hippocampal c-Fos over-activation as well as aberrant *in vivo* long-term potentiation in CA1 of HFD-fed mice. We then found enhanced CB1R expression and endocannabinoid levels (specifically anandamide) in the hippocampus of HFD-fed mice trained with novel objects in a novel environment and showed that decreasing hippocampal CB1 expression abolished HFD-induced long-term recognition memory deficits. We then investigated which neuronal types (GABA or glutamate) carrying CB1R are responsible for memory impairments in HFD-fed mice. Absence of CB1R from forebrain GABAergic neurons had no effect while specific CB1R deletion on hippocampal glutamatergic cells alleviated HFD-induced long-term recognition memory deficits. Moreover, this CB1R deletion rescued other memory deficits induced by HFD, i.e. social memory and object-in-place memory, and this effect was dependent on CB1R located at the plasma, but not mitochondrial, membrane of hippocampal glutamatergic cells. These results demonstrate that, despite carrying only 5% of hippocampal CB1R, glutamatergic neurons mediate endocannabinoid-induced alterations of hippocampal function in HFD-fed mice.

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BOARD NUMBER: S07-030

COMPARING THE ROLE OF THE ENDOCANNABINOID SYSTEM IN THE EFFECTS OF OBESOGENIC DIET ON MEMORY IN FEMALES AND MALES

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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In addition to metabolic and cardiovascular disorders, obesity is associated with cognitive dysfunction. Similarly, an obesogenic high-fat diet (HFD) consumption in animal models, in particular during adolescence, induces memory deficits. Interestingly, we recent showed that alterations of the hippocampal endocannabinoid system and its main receptor (CB1R), well known to regulate brain plasticity and memory processes, participate in HFD-induced memory deficits in male mice. Indeed, systemic blockade of CB1R or decrease of hippocampal CB1R improved memory in HFD-fed males. This project aims at identifying whether the endocannabinoid system is also central in the memory deficits induced by HFD consumption during adolescence in females and whether similar mechanisms are involved. First, we showed that systemic injection of a CB1R antagonist rescued HFD-induced deficits of long-term recognition memory in females, as previously shown in males, indicating CB1R signaling regulates the impact of HFD on memory function in both sexes. We then evaluated whether hippocampal CB1R are involved in such effects in females. Contrary to males, decrease of CB1R in the dorsal hippocampus (either on glutamatergic neurons or more broadly on all cells) did not improve recognition memory deficits in females. Interestingly, decreasing CB1R in the medial prefrontal cortex rescued recognition memory deficits in females, but not males, suggesting a double dissociation in the CB1R regulation of HFD effect on memory. We are currently comparing the levels of CB1R expression in hippocampus and prefrontal cortex of females and males under HFD and we plan to manipulate sex hormones to better characterize the gender effect.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-031

DECIPHERING THE WAY NEURONAL PATTERNS ARE UTILIZED BY DOWNSTREAM NETWORKS DURING LEARNING

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Learning has been studied using stimulus-response paradigms, and abundant correlative evidence links learning to changes in network activity. Nevertheless, a causal link between multi-neuronal activity and learning has never been demonstrated. To to determine how network activity underlies learning, we devised a three-step plan: (1) Determining how a neuronal assembly transmits an arbitrary signal to a downstream target; (2) Creating a behavioral task that guarantees ideal conditions for learning; (3) Bypassing the sensory-to-neuronal transformation by directly inducing coding-relevant activity patterns. Here, we report the first two steps. First, we applied optogenetic white-noise signals to groups of neocortical pyramidal cells in freely-moving mice. We found that directly-activated cells exhibited spike timing precision of several milliseconds. Instead of losing precision, postsynaptic interneurons exhibited higher precision with respect to the white-noise signal. Moreover, the postsynaptic spike trains demonstrated error correction. Data-driven modeling showed that nonlinear amplification of coincident presynaptic spikes improved precision. Therefore, error correction via convergence may enable messages to be precisely propagated between neuronal networks. Next, we trained mice on a two-alternative forced-choice task, in which the rules changed every day. After a period of general acquaintance with the apparatus, all mice achieved single-session learning of rules that required discriminating between two visual stimuli. We are now training mice to discriminate between neuronal patterns induced optogenetically. By analyzing learning curves of the induced patterns, we will determine which types of patterns are best utilized by downstream networks for learning.
Funding: ERC #679253, ISF #1871/17, CIHR-IDRC-ISF #2558/18, CRCNS-NSF-BSF #2015577, Zimin-Foundation

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-032

DEVELOPMENTAL EXPRESSION OF DFOXP IS REQUIRED IN MOTORNEURONS FOR OPERANT SELF-LEARNING IN DROSOPHILA

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Out of the four FOXP genes in humans the FOXP2 transcription factor was shown to have an unique role in speech learning and articulation. This function is highly conserved as analogous motor learning functions were identified in songbirds, rodents or fruit flies. While *Drosophila* is not a vocal learner, the process of torque learning in tethered flies is an analogous motor learning process to vocal learning. An initially very variable behaviour is being modulated via an operant feedback loop to shape the variable actions to the desired output. Accordingly, the fly orthologue of the FOXP2 gene, *dFoxP* is also involved in this operant self-learning task. Due to the broad expression, it remains unknown which brain regions or gene targets are important for this learning process. We performed different spatial or temporal knockouts of *dFoxP* using CRISPR/Cas9. Knocking out *dFoxP* in individual brain regions did not lead to any learning defects. A knockout of *dFoxP* in all neurons of adult flies showed no immediate effect, but 14 days old flies were impaired in learning. Knocking *dFoxP* out in motorneurons results in an impairment to fly. As protein kinase C (PKC) is required in motorneurons for torque learning, we expressed PKCi, an inhibitor of PKC in *dFoxP isoform B (FoxPiB)*-positive neurons and found a learning impairment. Knocking out only the atypical PKC (aPKC) in motor neurons or *dFoxPiB*-positive neurons via CRISPR/Cas9 also leads to learning defects, suggesting that aPKC is the gene responsible for operant self-learning in *Drosophila*.

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BOARD NUMBER: S07-033

THE HIP-MPFC NETWORK IS A NEURAL RESOURCE OF OBJECT RECOGNITION MEMORY

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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How well we remember objects and their location varies from person to person. But what are the neuronal substrates of these interindividual differences? To form an object memory, different features of an object are encoded in different brain areas as interconnected and activity related neurons (engrams). Remembering objects relies on connections between engram ensembles located in different brain areas. It has been suggested that hippocampus (HIP) engram ensembles encode recent memory recall while medial prefrontal cortex (mPFC) engram ensembles participate in remote memory after consolidation. The HIP and mPFC display a strong functional and anatomical interconnection which plays a crucial role in object location memory processes. We argue that variability in HIP-mPFC connectivity and early recruiting of mPFC engrams ensembles are giving rise to interindividual performance differences in an object location task. We employ an object-in-place task to test our hypothesis. First, we identify neurons that contribute to the engram coding for an object location through a video-assisted close-loop photolabeling of active neurons with CAMPARI2. We quantify the amount of active neurons in the mPFC among individual mice and correlate it with individual behavioural performance to analyse if neuronal ensemble-size correlates with object recognition performance. Currently, a first cohort of animals injected with CAMPARI2 is trained in the object-in-place task. We postulate that the mice which are performing better in the behavioural task recruit more mPFC neurons during object recognition compared to mice showing low performance in the task.

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BOARD NUMBER: S07-034

EVIDENCE OF SEX-DEPENDENT INVOLVEMENT OF THE MEDIAL PREFRONTAL CORTEX IN SOCIAL MEMORY IN JUVENILE RATS

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The medial-prefrontal-cortex (mPFC) is critical for social behavior. One characteristic of the mPFC is its protracted maturation through juvenility. Using blockade of de novo protein synthesis in the mPFC, we previously showed that the mPFC plays a critical role in mediating the consolidation of long-term social-recognition-memory (SRM) in juvenile male rat (Yaseen et al., 2018). This present study investigates whether the role of the mPFC in mediating SRM is sexually dimorphic. For this purpose, we microinjected anisomycin into the mPFC of male and female juvenile and adult rats and examined its effect on the ability to recognize a new conspecific. Our results showed deficits in SRM only in juvenile-males without affecting adult-males or juvenile-females. In order to examine mPFC activation during SRM, we used immunoshitochemoisty to assess cFos expression, the neuronal activation marker. We collected brains 90-min after the long-term SRM-test and quantified the number of cFos cells. We observed increases in the expression of cFos in juvenile-males but not juvenile-females or adult males and females. These results may suggest that in males, with maturation there is a transition from mPFC-dependent SRM in juveniles to mPFC-independent SRM in adults. However, among females we did not observe any differences between the age groups and both showed independency of SRM on mPFC. These findings point to a need for sex-specific treatments of social disorders in children.

Pubmed:

33077706: Maroun M, Sarussi-Elyahu A, Yaseen A, Hatoum OA, Kritman M
Sex-dimorphic role of prefrontal oxytocin receptors in social-induced facilitation of extinction in juvenile rats.
We previously reported that in the adult animal extinction in pairs resulted in enhanced extinction, showing that social presence can reduce previously acquired fear responses. Based on our findings that juvenile and adult animals differ in the mechanisms of extinction, here we address whether the social presence of a conspecific affects extinction in juvenile animals similarly to adults. We further address whether such presence has a different impact on juvenile males and females. To that end, we examined in our established experimental setting whether conditioned male and female animals extinguish contextual fear memory better while in pairs. Taking advantage of the role of oxytocin (OT) in the mediation of extinction memory and social interaction, we also study the effect of antagonizing the OT receptors (OTR) either systemically or in the prefrontal cortex on social interaction-induced effects of fear extinction. The results show that social presence accelerates extinction in males and females as compared to the single condition. Yet, we show differential and opposing effects of an OTR antagonist in both sexes. Whereas in females, the systemic application of an OTR antagonist is associated with impaired extinction, it is associated with enhanced extinction in males. In contrast, prefrontal OT is not engaged in extinction in juvenile males, while is it is critical in females. Previously reported differences in the levels of prefrontal OT between males and females might explain the differences in OT action. These results suggest that even during the juvenile period, critical mechanisms are differently involved in the regulation of fear in males and females.
Transl Psychiatry, 2020; 10
34650208: Lavenda-Grosberg D, Lalzar M, Leser N, Yaseen A, Malik A, Maroun M, Barki-Harrington L, Wagner S
Acute social isolation and regrouping cause short- and long-term molecular changes in the rat medial amygdala.
Social isolation poses a severe mental and physiological burden on humans. Most animal models that investigate this effect are based on prolonged isolation, which does not mimic the milder conditions experienced by people in the real world. We show that in adult male rats, acute social isolation causes social memory loss. This memory loss is accompanied by significant changes in the expression of specific mRNAs and proteins in the medial amygdala, a brain structure that is crucial for social memory. These changes particularly involve the neurotrophic signaling and axon guidance pathways that are associated with neuronal network remodeling. Upon regrouping, memory returns, and most molecular changes are reversed within hours. However, the expression of some genes, especially those associated with neurodegenerative diseases remain modified for at least a day longer. These results suggest that acute social isolation and rapid resocialization, as experienced

by millions during the COVID-19 pandemic, are sufficient to induce significant changes to neuronal networks, some of which may be pathological.
Mol Psychiatry, 2022; 27
29608644: Yaseen A, Shrivastava K, Zuri Z, Hatoum OA, Maroun M
Prefrontal Oxytocin is Involved in Impairments in Prefrontal Plasticity and Social Memory Following Acute Exposure to High Fat Diet in Juvenile Animals.
Juvenility represents a critical developmental phase during which exposure to a high fat diet (HFD) can severely modify cognitive and emotional functioning. The purpose of this study was to address how short and acute exposure to a HFD during juvenility affects social memory recognition and prefrontal long-term potentiation (LTP). As LTP and social memory depend on the neuromodulator oxytocin (OXY) and due to its role in metabolism, we also examined the effects of OXY in mediating HFD-induced alterations in social memory and LTP. Our results show that short exposure to a HFD during juvenility impairs social preference memory and prefrontal LTP. Interestingly, whereas systemic injections of OXY reversed the impairments in HFD-fed animals and impaired LTP and memory in control animals; prefrontal injections of the OXY agonist TGOT reversed the effects in HFD animals without affecting control animals. Exposure to HFD was associated with a reduction in the levels of OXY in the prefrontal compared to control animals. Interestingly, the restoration of social memory by TGOT in HFD animals was also associated with normalization of OXY in the prefrontal. These results point to a role that prefrontal OXY has in mediating the effects of HFD on memory and plasticity.
Cereb Cortex, 2019; 29

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BOARD NUMBER: S07-035

HIPPOCAMPAL-THALAMIC-CORTICAL PATHWAYS MODULATE REMOTE MEMORY RETRIEVAL.

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Brain-wide mapping of immediate early gene revealed a strong hippocampal-thalamic-cortical signature in the brain network active during remote fear memory recall. We aim to unravel the contribution of hippocampal-thalamic-cortical circuitry in processing long-lasting memories with a spatial and emotional component. Graph theory analysis of memory networks showed that the anterodorsal thalamic nucleus (ADn) activity positively correlates with other regions during recent memory recall but during remote recall, this pattern switches and, ADn activity negatively correlated with the majority of the other network regions analyzed. This suggests that the inhibition of the ADn by other regions is required during the recall of a remote memory. Using optogenetics *in vivo*, we targeted the anterior cingulate cortex (aCC)-ADn pathway and the hippocampal CA3-ADn pathway, as both aCC and hippocampus are important in long-term memory formation and recall. Results show that optogenetic inhibition of CA3, but not aCC, excitatory projections to the ADn, leads to impaired memory performances with a related change in cellular activity in the CA3, ADn, and aCC, during optogenetic modulation. We also studied the contribution of CA3-ADn and aCC-ADn pathways in the generalization of remote memories by placing the animals in a different context and we used fiber photometry combined with optogenetics to confirm neuronal activity modulation.

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BOARD NUMBER: S07-036

THE DEFICITS OF RECOGNITION AND MEMORY IN ADOLESCENCE BY NEONATAL MATERNAL SEPARATION

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Neonatal maternal separation (MS) has been shown to be associated with an increased vulnerability to psychiatric illnesses such as anxiety and depression in animal models. As society progresses, separation situations and isolation from parents have occurred in human lives. The present study aims to investigate the behavioral alternation induced by neonatal maternal separation in the adolescent period during which the brain refine and integrate structures and functions. Following a periodic neonatal MS (4-hour a day, postnatal 2~20, consecutive 19 days), adolescent mice were employed for a series of behavioral assessments for locomotion, anxiety, recognition, learning, memory, and fear learning using open field test, novel object recognition memory test, object location memory test, social interaction test, and auditory fear conditioning. The results showed that MS mice showed decreased cognition memory to the new objects and places based on the novel object recognition memory and object location memory test. In addition, MS mice scored lower alternation index in the Y-maze test. Furthermore, MS mice had a short latency time to fall, this result also reflects impaired motor learning ability. Therefore, these results indicate that early life stress based on MS may induce deficits of recognition and learning ability during adolescence.

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BOARD NUMBER: S07-037

STRESS-INDUCED SOCIAL ODOR PREFERENCE IS DISRUPTED BY ADOLESCENT HIGH-FAT DIET CONSUMPTION: IS HIPPOCAMPAL CA2 AREA INVOLVED?

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Juvenile obesity is a concerning public health issue associated with many adverse outcomes, including cognitive impairments. This can be replicated using animal models, as rodents fed a high fat diet (HFD) throughout adolescence display social memory dysfunction at adulthood. We recently showed that adolescent HFD-induced social memory deficits are mediated by hippocampal overactivity as they can be reversed by chemogenetic silencing of hippocampal pyramidal neurons. As olfaction is essential for rodent social behavior, we here investigate the impact of HFD on social odor recognition and the involved brain circuits, focusing on the hippocampus. Our first results indicate that control diet-fed mice prefer investigating their own bedding over a novel bedding, but only after foot-shocks, suggesting stress influences social odor preference. Interestingly, adolescent HFD-fed animals do not show such preference (before or after foot-shocks) and a large chemogenetic inactivation of hippocampal pyramidal neurons rescues these deficits. We then focused on the CA2 area of the hippocampus, known for its important role in social memory, and observed that specific chemogenetic silencing of CA2 pyramidal neurons alleviates HFD-induced deficits in social odor preference. Altogether, this suggests the effect of HFD on social odor preference is mediated by an overactivity of CA2 pyramidal neurons. Future experiments will characterize the effects of stress on social odor preference and will further evaluate HFD-induced CA2 overactivity using *ex vivo* electrophysiology and *in vivo* photometry-based calcium imaging. This will provide insight on how the CA2 area might be involved in the interaction between stress, social function and diet.

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BOARD NUMBER: S07-038

DISTINCT ROLES OF PREFRONTAL CORTEX SUBREGIONS IN THE CONSOLIDATION AND RECALL OF REMOTE SPATIAL MEMORIES

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

Mattia Santoboni[1], Eleonora Centofante[1,2], Elena Mombelli[1], Sara Pezza[1], Arianna Rinaldi[1], Andrea Mele[1]
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It is a common believe that memories with time become progressively independent of the hippocampus and are gradually stored in cortical areas. This view is mainly based on evidence demonstrating a role of the medial prefrontal cortex (mPFC) in the retrieval of remote memories. What is more controversial is whether activity in the mPFC is required immediately after learning to initiate the process. Further question are possible functional differences among the different component of the mPFC in the formation and storage of remote memories. To address these issues, we directly contrasted the effects of loss-of-function manipulations of the the anterior cingulate cortex (aCC) and the vmPFC, that includes the infralimbic (IL) and the prelimbic (PL) cortices, immediately after training, and before testing, on the ability of CD1 mice to recall the location of the hidden platform in the Morris water maze (MWM). To this aim we injected in the vmPFC or in the aCC an AAV carrying the hM4Di receptor. Post-training systemic administrations of CNO (3mg/Kg) impaired memory recall at remote (30 days) but not recent (24 hours) time points in both experimental groups. Interestingly, pre-test administrations of CNO revealed that the aCC but not the vmPFC is necessary to the recall of remote spatial information. Overall, these findings revealed a functional dissociation between the two prefrontal areas, demonstrating that they are both involved in the early consolidation of remote spatial memories, but that only the aCC is engaged in their recall.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-039

LACTATE SUPPLY OVERTAKES GLUCOSE WHEN NEURAL COMPUTATIONAL AND COGNITIVE LOADS SCALE UP

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The neural computational power is determined by neuroenergetics, but how and which energy substrates are allocated to various forms of memory engram is unclear. To solve this question, we asked whether neuronal fueling by glucose or lactate scales differently upon increasing neural computation and cognitive loads. Here, using in vitro and in vivo electrophysiology, two-photon imaging, cognitive tasks and mathematical modeling, we show that in hippocampus both glucose and lactate are involved in engram formation, with lactate supporting long-term synaptic plasticity evoked by high stimulation load activity patterns and high attentional load in cognitive tasks , and glucose being sufficient for less demanding neural computation and learning tasks. Indeed, i) we show that lactate is mandatory for demanding neural computation, while glucose is sufficient for lighter forms of activity-dependent long-term potentiation (LTP), ii) we find that subtle variations of spike amount or frequency in spike-timing-dependent plasticity (STDP) are sufficient to shift the energetic dependency from glucose to lactate, and iii) we demonstrate that lactate is necessary for a cognitive task requiring high attentional load such as the object-in-place task and for the corresponding *in vivo* hippocampal LTP expression, but is not needed for a less demanding task such as a simple novel object recognition. Overall, these results demonstrate that glucose and lactate metabolisms are differentially engaged in neuronal fueling depending on the complexity of the activity-dependent plasticity and behavior.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-040

HIPPOCAMPO-CORTICAL ENGRAM CIRCUITS REGULATE REMOTE CONTEXTUAL MEMORY AND GENERALIZATION

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The indexing theory of consolidation postulates that cortical memory representations are indexed and reinstated by the hippocampus to ensure recall at remote times, but formal validation for this theory is missing. Furthermore, engrams cells were suggested to provide a cellular substrate for memory indexes, but a direct role of hippocampal engrams in indexing cortical activity at remote times is lacking. Here, we attempt to investigate both hypotheses by identifying ventral hippocampal (vCA1) engrams monosynaptically projecting to the medial prefrontal cortex (mPFC), a known depository of remote memories, and by assessing their potential role in indexing cortical engram activity at remote times. For this, we used a combination of retrograde viral tracing coupled to engram identification and chemo/opto-genetic manipulations following contextual fear conditioning and remote recall. Our data suggests the presence of two projection components within the vCA1 to mPFC circuit, either at the service of memory precision (engram specific) or generalization (non-engram specific). Further, hippocampal input determines engram reactivation in the mPFC and influences generalization and memory recall. Altogether, our results highlight the influence of hippocampal input on cortical activity for remote recall and further deepens our understanding on the contribution of engram circuits to remote memory consolidation.

Pubmed:

34337766: Zocher S, Overall RW, Berdugo-Vega G, Rund N, Karasinsky A, Adusumilli VS, Steinhauer C, Scheibenstock S, Händler K, Schultze JL, Calegari F, Kempermann G
De novo DNA methylation controls neuronal maturation during adult hippocampal neurogenesis.
Adult neurogenesis enables the life-long addition of functional neurons to the hippocampus and is regulated by both cell-intrinsic molecular programs and behavioral activity. De novo DNA methylation is crucial for embryonic brain development, but its role during adult hippocampal neurogenesis has remained unknown. Here, we show that de novo DNA methylation is critical for maturation and functional integration of adult-born neurons in the mouse hippocampus. Bisulfite sequencing revealed that de novo DNA methyltransferases target neuronal enhancers and gene bodies during adult hippocampal neural stem cell differentiation, to establish neuronal methylomes and facilitate transcriptional up-regulation of neuronal genes. Inducible deletion of both de novo DNA methyltransferases Dnmt3a and Dnmt3b in adult neural stem cells did not affect proliferation or fate specification, but specifically impaired dendritic outgrowth and synaptogenesis of newborn neurons, thereby hampering their functional maturation. Consequently, abolishing de novo DNA methylation modulated activation patterns in the hippocampal circuitry and caused specific deficits in hippocampus-dependent learning and memory. Our results demonstrate that proper establishment of neuronal methylomes during adult neurogenesis is fundamental for hippocampal function.
EMBO J, 2021; 40
34174010: Berdugo-Vega G, Lee CC, Garthe A, Kempermann G, Calegari F
Adult-born neurons promote cognitive flexibility by improving memory precision and indexing.
Adult neurogenesis in the hippocampal dentate gyrus (DG) is an extraordinary form of plasticity fundamental for cognitive flexibility. Recent evidence showed that newborn neurons differentially modulate input to the infra- and supra-pyramidal blades of the DG during the processing of spatial and contextual information, respectively. However, how this differential regulation by neurogenesis is translated into different aspects contributing cognitive flexibility is unclear. Here, we increased adult-born neurons by a genetic expansion of neural stem cells and studied their influence during navigational learning. We found that increased neurogenesis improved both memory precision and flexibility. Interestingly, each of these gains was associated with distinct subregional patterns of activity and better separation of memory representations in the DG-CA3 network. Our results highlight the role of adult-born neurons in promoting memory precision and indexing and suggests their anatomical allocation within specific DG-CA3 compartments, together contributing to cognitive flexibility.

Hippocampus, 2021; 31
31919362: Berdugo-Vega G, Arias-Gil G, López-Fernández A, Artegiani B, Wasielewska JM, Lee CC, Lippert MT, Kempermann G, Takagaki K, Calegari F
Increasing neurogenesis refines hippocampal activity rejuvenating navigational learning strategies and contextual memory throughout life.
Functional plasticity of the brain decreases during ageing causing marked deficits in contextual learning, allocentric navigation and episodic memory. Adult neurogenesis is a prime example of hippocampal plasticity promoting the contextualisation of information and dramatically decreases during ageing. We found that a genetically-driven expansion of neural stem cells by overexpression of the cell cycle regulators Cdk4/cyclinD1 compensated the age-related decline in neurogenesis. This triggered an overall inhibitory effect on the trisynaptic hippocampal circuit resulting in a changed profile of CA1 sharp-wave ripples known to underlie memory consolidation. Most importantly, increased neurogenesis rescued the age-related switch from hippocampal to striatal learning strategies by rescuing allocentric navigation and contextual memory. Our study demonstrates that critical aspects of hippocampal function can be reversed in old age, or compensated throughout life, by exploiting the brain's endogenous reserve of neural stem cells.
Nat Commun, 2020; 11
25181319: Berdugo-Vega G, Arias-Gil G, Rodriguez-Niedenführ M, Davies DC, Vázquez T, Pascual-Font A
GFAP immunoreactivity within the rat nucleus ambiguus after laryngeal nerve injury.
Changes that occur in astroglial populations of the nucleus ambiguus after recurrent (RLN) or superior (SLN) laryngeal nerve injury have hitherto not been fully characterised. In the present study, rat RLN and SLN were lesioned. After 3, 7, 14, 28 or 56 days of survival, the nucleus ambiguus was investigated by means of glial fibrillary acidic protein (GFAP) immunofluorescence or a combination of GFAP immunofluorescence and the application of retrograde tracers. GFAP immunoreactivity was significantly increased 3 days after RLN resection and it remained significantly elevated until after 28 days post injury (dpi). By 56 dpi it had returned to basal levels. In contrast, following RLN transection with repair, GFAP immunoreactivity was significantly elevated at 7 dpi and remained significantly elevated until 14 dpi. It had returned to basal levels by 28 dpi. Topographical analysis of the distribution of GFAP immunoreactivity revealed that after RLN injury, GFAP immunoreactivity was increased beyond the area of the nucleus ambiguus within which RLN motor neuron somata were located. GFAP immunoreactivity was also observed in the vicinity of neuronal somata that project into the uninjured SLN. Similarly, lesion of the SLN resulted in increased GFAP immunoreactivity around the neuronal somata projecting into it and also in the vicinity of the motor neuron somata projecting into the RLN. The increase in GFAP immunoreactivity outside of the region containing the motor neurons projecting into the injured nerve, may reflect the onset of a regenerative process attempting to compensate for impairment of one of the laryngeal nerves and may occur because of the dual innervation of the posterior cricoarytenoid muscle. This dual innervation of a very specialised muscle could provide a useful model system for studying the molecular mechanisms underlying axonal regeneration process and the results of the current study could provide the basis for studies into functional regeneration following laryngeal nerve injury, with subsequent application to humans.
J Anat, 2014; 225
24458941: Hernández-Morato I, Berdugo-Vega G, Sañudo JR, McHanwell S, Vázquez T, Valderrama-Canales FJ, Pascual-Font A
Somatotopic changes in the nucleus ambiguus after section and regeneration of the recurrent laryngeal nerve of the rat.
Changes in motoneurons innervating laryngeal muscles after section and regeneration of the recurrent laryngeal nerve (RLN) are far from being understood. Here, we report the somatotopic changes within the nucleus ambiguus (Amb) after the nerve injury and relates it to the resulting laryngeal fold impairment. The left RLN of each animal was transected and the stumps were glued together using surgical fibrin glue. After several survival periods (1, 2, 4, 8, 12, 16 weeks; at least six rats at each time point) the posterior cricoarytenoid (PCA) and thyroarytenoid (TA) muscles were injected with fluorescent-conjugated cholera toxin and the motility of the vocal folds evaluated. After section and subsequent repair of the RLN, no movement of the vocal folds could be detected at any of the survival times studied and the somatotopy and the number of labeled motoneurons changed. From 4 wpi award, the somatotopy was significantly disorganized, with the PCA motoneurons being located rostrally relative to their normal location. A rostrocaudal overlap between the two pools of motoneurons supplying the PCA and TA muscles was observed from 2 wpi onwards. Hardly any labeled neurons were found in the contralateral Amb in any of the experimental groups. An injury of the RLN leads to a reinnervation of the denervated motor endplates of PCA and TA. However, misdirected axons sprout and regrowth from the proximal stump to the larynx. As a result, misplaced innervation of muscles results in a lack of functional recovery of the laryngeal folds movement following a RLN injury.
Anat Rec (Hoboken), 2014; 297

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BOARD NUMBER: S07-041

LEARNING-DEPENDENT RECONFIGURATION OF HIPPOCAMPAL-PREFRONTAL SYNAPTIC COMMUNICATION

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Communication between the hippocampus and the neocortex is thought to be critical for long-term memory storage. High-frequency ripple oscillations, observed most prominently in the hippocampal CA1 area, are associated with this communication. However, how this process is implemented at the level of synapses and circuits in the neocortex remains largely unexplored. Here we address this question by performing *in vivo* whole-cell patch-clamp recordings in the secondary motor cortex (MOs) while simultaneously recording the local field potential in CA1 of head-fixed mice exposed to a virtual-reality environment. Following training in a goal-directed spatial task, we observe a learning-dependent membrane potential depolarization in most MOs neurons during hippocampal ripples. In untrained mice, such a depolarization can be mimicked by chemogenetic inactivation of parvalbumin-positive (PV+) interneurons during ripples. Similarly, optogenetic stimulation of hippocampal inputs during inactivation of PV+ interneurons in MOs allows us to reproduce the depolarization. By contrast, in trained animals, the ripple-associated depolarization can be abolished by inactivation of somatostatin-positive (SOM+) interneurons. Together, we provide evidence that local inhibitory neurons play distinct roles in shaping synaptic dynamics when prefrontal cortex neurons integrate hippocampal inputs during ripples after learning. Our results help to understand the synaptic basis of hippocampal-prefrontal communication during memory consolidation.

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BOARD NUMBER: S07-042

NEIL3-MEDIATED EPIGENETIC REGULATION OF HIPPOCAMPAL FUNCTION IN MEMORY

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Aim: DNA Base Excision Repair (BER) plays an important role in the active DNA demethylation, contributing to the dynamic turnover of DNA modifications. However, it is not clear whether BER-mediated DNA demethylation is required for cortical brain functions. NEIL3 DNA glycosylase is known to initiate BER, recognize DNA demethylation intermediates, and modulate gene expression in hippocampal neurons. In this study, we aim to elucidate the impact of NEIL3 on the neuronal epigenome associated with hippocampal function in memory.
Methods: We evaluated the impact of NEIL3 loss on short-term and long-term spatial memory components using behavioral tasks such as Y-maze and Novel Object Location (NOL). We assessed spatial experience induced expressing of Immediate Early Genes (IEGs) using immunohistochemistry (IHC) and confocal microscopy. We investigated the functional plasticity of hippocampal place cells using electrophysiology strategies. Last, we explore how NEIL3 impacts the epigenome of hippocampal neurons using state-of-the-art sequencing of DNA methylation and hydroxy-methylation.
Results: NEIL3-deficient mice show impaired long-term spatial memory in both Y-maze and NOL tasks. Consistently, NEIL3-depleted hippocampal neurons are functionally impaired by showing (i) reduced IEG-induced memory traces, (ii) differentially regulated memory-related genes, and (iii) deteriorated long-term spatial stability of hippocampal place cells. We are exploring NEIL3-dependent DNA methylation changes in hippocampal neurons using TET-assisted and chemical-assisted pyridine borane sequencing (TAPS and CAPS) technologies.
Conclusion: Our studies suggest an important role NEIL3 in modulating the functionality of hippocampal neurons in spatial memory by shaping the neuronal epigenome and gene expression.

Pubmed:
34988395: Kunath N, Bugaj AM, Bigonah P, Fernandez-Berrocal MS, Bjørås M, Ye J
DNA repair enzyme NEIL3 enables a stable neural representation of space by shaping transcription in hippocampal neurons.
DNA repair enzymes are essential for the maintenance of the neuronal genome and thereby proper brain functions. Emerging evidence links DNA repair to epigenetic gene regulation; however, its contribution to different transcriptional programs required for neuronal functions remains elusive. In this study, we identified a role of the DNA repair enzyme NEIL3 in modulating the maturation and function of hippocampal CA1 neurons by shaping the CA1 transcriptome during postnatal development and in association with spatial behavior. We observed a delayed maturation in CA1 and identified differentially regulated genes required for hippocampal development. We revealed impaired spatial stability in CA1 place cells and found spatial experience-induced gene expression essential for synaptic plasticity. This is the first study that links molecular underpinnings of DNA repair to the neural basis of spatial cognition beyond animals' behavioral phenotypes, thus shedding light on the molecular determinants enabling a stable neural representation of space.
iScience, 2021; 24
34857879: Hildrestrand GA, Rolseth V, Kunath N, Suganthan R, Jensen V, Bugaj AM, Fernandez-Berrocal MS, Sikko SB, Vetlesen S, Kuśnierczyk A, Olsen AK, Gützkow KB, Rowe AD, Wang W, Moldestad O, Syrstad MD, Slupphaug G, Eide L, Klungland A, Sætrom P, Luna L, Ye J, Scheffler K, Bjørås M
NEIL1 and NEIL2 DNA glycosylases modulate anxiety and learning in a cooperative manner in mice.
Oxidative DNA damage in the brain has been implicated in neurodegeneration and cognitive decline. DNA glycosylases initiate base excision repair (BER), the main pathway for oxidative DNA base lesion repair. NEIL1 and NEIL3 DNA glycosylases affect cognition in mice, while the role of NEIL2 remains unclear. Here, we investigate the impact of NEIL2 and its potential overlap with NEIL1 on behavior in knockout mouse models. Neil1Neil2 mice display hyperactivity, reduced anxiety and improved learning. Hippocampal oxidative DNA base lesion levels are comparable between genotypes and no mutator phenotype is found. Thus, impaired canonical repair is not likely to explain the altered behavior. Electrophysiology

suggests reduced axonal activation in the hippocampal CA1 region in Neil1Neil2 mice and lack of NEIL1 and NEIL2 causes dysregulation of genes in CA1 relevant for synaptic function. We postulate a cooperative function of NEIL1 and NEIL2 in genome regulation, beyond canonical BER, modulating behavior in mice.
Commun Biol, 2021; 4
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Genetic interaction between the non-homologous end-joining factors during B and T lymphocyte development: In vivo mouse models.
Non-homologous end joining (NHEJ) is the main DNA repair mechanism for the repair of double-strand breaks (DSBs) throughout the course of the cell cycle. DSBs are generated in developing B and T lymphocytes during V(D)J recombination to increase the repertoire of B and T cell receptors. DSBs are also generated during the class switch recombination (CSR) process in mature B lymphocytes, providing distinct effector functions of antibody heavy chain constant regions. Thus, NHEJ is important for both V(D)J recombination and CSR. NHEJ comprises core Ku70 and Ku80 subunits that form the Ku heterodimer, which binds DSBs and promotes the recruitment of accessory factors (e.g., DNA-PKcs, Artemis, PAXX, MRI) and downstream core factors (XLF, Lig4 and XRCC4). In recent decades, new NHEJ proteins have been reported, increasing complexity of this molecular pathway. Numerous in vivo mouse models have been generated and characterized to identify the interplay of NHEJ factors and their role in development of adaptive immune system. This review summarizes the currently available mouse models lacking one or several NHEJ factors, with a particular focus on early B cell development. We also underline genetic interactions and redundancy in the NHEJ pathway in mice.
Scand J Immunol, 2020; 92
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Leaky severe combined immunodeficiency in mice lacking non-homologous end joining factors XLF and MRI.
Non-homologous end-joining (NHEJ) is a DNA repair pathway required to detect, process, and ligate DNA double-stranded breaks (DSBs) throughout the cell cycle. The NHEJ pathway is necessary for V(D)J recombination in developing B and T lymphocytes. During NHEJ, Ku70 and Ku80 form a heterodimer that recognizes DSBs and promotes recruitment and function of downstream factors PAXX, MRI, DNA-PKcs, Artemis, XLF, XRCC4, and LIG4. Mutations in several known NHEJ genes result in severe combined immunodeficiency (SCID). Inactivation of or in mice results in normal or mild phenotype, while combined inactivation of or / leads to late embryonic lethality. Here, we describe three new mouse models. We demonstrate that deletion of rescues embryonic lethality in mice with combined deficiencies of and . Furthermore, and mice possess reduced body weight, severely reduced mature lymphocyte counts, and accumulation of progenitor B cells. We also report that combined inactivation of results in live-born mice with modest phenotype, and combined inactivation of results in embryonic lethality. Therefore, we conclude that XLF is functionally redundant with MRI and PAXX during lymphocyte development Moreover, genetically interacts with and
Aging (Albany NY), 2020; 12

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BOARD NUMBER: S07-043

RECENT AND REMOTE SPATIAL MEMORY FORMATION IN INFANT AND ADULT RATS

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The infantile brain undergoes developmental changes on structural, connectivity, and molecular levels, and brain functioning may change in quality. Episodic (spatial) memories are processed within hippocampal and neocortical circuits, which have a protracted development. Against this backdrop, we asked whether and how the formation of long-term allocentric spatial memory representations differs between infantile and adult rats, testing recent and remote kinds of these memories. Infant rats on postnatal day (PD)18 and adults rats (PD80) were tested on the object place recognition (OPR) task (which was adapted to the infant rats' early age) using, for the test of recent memory, a 90-min retention interval between sampling phase and testing phase, and for the test of remote memory, a 2-month retention interval between sampling and testing. Adult rats showed OPR memory for the 90-min retention interval (i.e., recent memory), but not at the remote test after two months. In contrast, the pre-weanling rats (on PD18) did not display recent OPR memory, but surprisingly, showed significant remote OPR memory when tested 2 months later and these animals were already in an early adulthood age. The data point to differences in forming and expressing recent and remote spatial memories between pre-weanling and adult rats. In ongoing studies, we are examining the brain areas possibly mediating the differential memory performance in the two age groups.

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BOARD NUMBER: S07-044

EXPLORING OFFLINE MEMORY CONSOLIDATION IN GOAL-DIRECTED SENSORIMOTOR TASKS

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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The sleeping brain is thought to support offline memory consolidation. Critical for this are sleep's electrical brain rhythms that enable transfer of memory traces between distant brain areas and promote synaptic plasticity at specific sets of cortical synapses for long-term storage. An outstanding challenge in the field is to define these roles in the setting of a defined cortical circuit engaged in learning. Amongst the many learning tasks executed by rodents, goal-oriented sensorimotor tasks have been well characterized in terms of the site and timing of cortical circuit plasticity. Therefore, we chose a two-tone auditory discrimination task that required head-fixed and water-restricted mice to discriminate between a go- (8 kHz) and a no-go (12 kHz) tone to obtain a water reward. Mice learned over 7-14 days to become experts (d'=1.5-2). Across days, mice improved performance by reducing the False Alarm rate, which corresponds the no-go tone response, and by suppressing unspecific licking ahead of the tones. Along each daily training session, we observed that the performance evolved across time according to three discrete windows. In a first window, mice seemed to recall what they remembered from the previous day, akin to a proxy for offline consolidation of memory traces acquired in the preceding day. Subsequently, mice exhibited within-session learning. Finally, mice disengaged with the decrease in motivation toward the end of the session. Currently, we are examining the role of discrete brain areas in the dynamics of this learning process through using closed-loop optogenetic inhibition specifically during non-rapid-eye-movement sleep.

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BOARD NUMBER: S07-045

MULTISENSORY LEARNING EXPANDS A MEMORY ENGRAM

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Life is a multisensory experience for most animals. Learning to associate and bind different sensory features into a scene is a fundamental process of the brain, which improves subsequent memory performance. However, neural mechanisms that bind sensory features during learning remain to be explained. We developed new multisensory aversive and appetitive memory paradigms in *Drosophila* which combine visual and olfactory cues. Flies trained with combinations of both modalities show improved memory performance, even when the sensory cues are presented separately during testing. We used temporal control of neuronal function to define the components of the mushroom body memory network that are required for multisensory learning and performance enhancement. Calcium imaging in head-fixed flies revealed that dopaminergic reinforcement during multisensory learning binds activity in streams of modality-specific Kenyon Cells. After learning a unimodal sensory input generates a multimodal neuronal response. This cross-modal binding and expansion of the memory engram underlies improved memory performance.

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BOARD NUMBER: S07-046

NEURONAL UNDERPINNINGS OF EPISODIC-LIKE MEMORY IN THE MOUSE LATERAL ENTORHINAL CORTEX

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Increasing evidence indicates that the Entorhinal Cortex (EC) might play a fundamental role in the formation and recall of episodic memories. We aim to dissect the neuronal underpinnings of episodic memory and establish a causal link between the activity of lateral EC (LEC) neuronal ensembles and the recall of past events that occurred in a specific spatiotemporal context. Using in vivo inhibitory/excitatory chemogenetics and the novel object-place-context recognition test (OPCRT), we showed that the activity of the neuronal ensembles previously engaged in the learning process is necessary and sufficient for memory recall. Moreover, multi-area cfos analysis confirmed the specific EC engagement 2h following either pure spatial navigation or memory processing. Although the EC subdivisions show a similar pattern of cfos staining, in vitro electrophysiology in brain slices revealed a specific long-lasting change in synaptic plasticity only in the LEC. Synaptic plasticity in the LEC intrinsic circuitry was affected only in slices obtained from mice subjected to OPCRT but not in those from mice that were exposed only to the context. Taken together, our results suggest that the LEC can form internal representations of past experiences, retain a trace of their occurrence and participate in their recall.

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THE EMOTIONAL AND COGNITIVE DIFFERENCES BETWEEN NOVICES AND BEGINNERS ADULT PROGRAMMERS DURING AN INTRODUCTORY TASK ON CODE.ORG : PRELIMINARY RESULTS

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Learning to program is known to be very challenging, as evidenced by the low success rates of students in introductory computer science courses observed worldwide. To date, much of the research in programming learning has investigated the learning process by comparing the knowledge and skills of novice and experienced programmers. However, little is known about the early stages of programming learning, which may be a pivotal point in the learning process, as the basic concepts of programming often appear particularly difficult to grasp. Moreover, the role of emotional and cognitive states in programming learning is also under-explored. The current study is the first to investigate the potential relationships between cognitive and emotional states and learning performance in two groups of learners who are at the beginning of their learning process, but differ slightly in their level of programming expertise (12 novices and 11 beginners). Measures of electrodermal activity, automated facial emotion recognition, and pupil diameter were collected while participants performed a sequential programming task for a maximum of 90 minutes on the web platform code.org. We examined (1) how cognitive and emotional states varied with performance during the programming task, and (2) whether participants' expertise (novice or beginner) had a moderating effect on the relationship between cognitive and emotional states and performance. First results of the panel data (n = 690 observations) multiple regression analysis will be presented at the conference. Methodological considerations as well as possible pedagogical implications for learning programming will be discussed.

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BOARD NUMBER: S07-048

PERSONALIZED TRANSCRANIAL ELECTRICAL STIMULATION (TES) FOR MODULATION OF ASSOCIATIVE MEMORY PERFORMANCE

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

Dunja Paunovic[1], Jovana Bjekić[1], Katarina Vulić[1], Marko Živanović[2], Uroš Konstantinović[1], Marija Stanković[1], Saša Filipović[1]
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Associative memory (AM) represents an ability to bind unrelated information into meaningful units and encode them as distinct memories. AM has been the function of interest in many non-invasive transcranial electrical stimulation (tES) studies aiming to maximize the potential for memory modulation by varying stimulation loci, frequency, and amplitude. In the current study, we aimed to capture modulation potential of AM performance when tailoring the stimulation protocols to the individual brain rhythms. By matching the stimulation frequency to the frequency of each subject's AM task induced electrophysiological activity in theta spectrum (4-8 Hz), we developed two types of personalized oscillatory protocols: otDCS and tACS, which we administered alongside the constant tDCS and a sham condition in the single-blind cross-over experiment. To comparatively assess the effects of different tES protocols delivered over the posterior parietal cortex, we tested the recognition and recall ability of the 42 healthy young adults on paired-associate paradigms after each of four conditions. Conditions were administered week-apart in a counterbalanced order. Group level comparisons of each active tES condition against sham did not show differences in AM performance either on recognition or cued-recall. However, data showed variability in performance depending on the task and the outcome measures, which calls for stratified approach in order to test robustness of observed findings. Apart from introducing a novel approach to probing AM with personalized tES, this well-powered, multi-protocol, multi-task and multi-measure study produced a comprehensive dataset that allows exploration of factors that could uncover different patterns in responsiveness to tES.

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BOARD NUMBER: S07-049

INFUSIONS OF A DOPAMINE D1 RECEPTOR AGONIST INTO THE PREFRONTAL CORTEX AND APPETITIVE INHIBITORY DISCRIMINATION LEARNING.

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Aims Impaired inhibitory modulation plays a role in the impulsivity seen in many psychiatric disorders, including addiction and schizophrenia. We investigated whether dopaminergic modulation in specific prefrontal cortex sub-regions, prelimbic (PL) and infralimbic (IL), plays a role in the inhibitory modulation of associative learning. Methods We used a dopamine D1 receptor agonist, SKF81297, to assess how dopamine D1 receptor activation affects associative inhibition in male Wistar rats, compared to saline-infused controls. Inhibitory learning was examined using a within-subjects appetitive Pavlovian task (A+/AX-; Waite et al., 2021, doi:10.1016/j.physbeh.2021.113557). After excitatory training, the animals received daily micro-infusions of SKF81297 before inhibitory acquisition and summation sessions; retardation testing was drug-free. Inhibitory discrimination was measured as the ability of the animals to discriminate X as an indicator of no reward. Summation tested the capacity of X to suppress responding to an alternative exciter. Retardation testing examined new excitatory learning to X. Learning was measured as nose-pokes during the different stimuli relative to baseline responding (‘pre-‘), and was analysed using mixed-factorial 2-way-ANOVAs. Results The learning task showed that overall inhibitory discrimination was learnt, evident as reduced responding to AX- compared to A+ over time, and retardation and summation tests confirmed that conditioned inhibition was shown. There was no inhibitory learning difference by infusion group. However, the SKF81297 micro-infusions tended to decrease baseline (pre-) responding. Conclusions There was no evidence that D1 receptor activation in IL or PL sub-regions modulates inhibitory learning. Future studies may focus on alternative receptor subtypes and/or interconnected brain regions.

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THE ACTIVITY OF PHOSPHODIESTERASE 4 IN THE DORSAL HIPPOCAMPUS DURING RECONSOLIDATION SUSTAINS FEAR MEMORY OVER TIME

POSTER SESSION 07 - SECTION: ENGRAM MEMORY AND MEMORY CONSOLIDATION

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Upon retrieval, a consolidated memory can become labile and subsequently restabilized by reconsolidation, which engages brain areas such as the dorsal hippocampus (DH). Consolidation and reconsolidation share several mechanisms. In this line, the role of PKA and phosphodiesterase 4 (PDE4) in consolidation is well determined. However, it is scarcely investigated whether PDE4 activity in the DH underlies fear memory reconsolidation. The aim was to evaluate the role of PDE4 in DH in fear memory reconsolidation. Male Wistar rats, with bilateral cannulas aiming the DH and conditioned to a Context, received roflumilast (ROF; PDE4 inhibitor; 9 ng/side) into the DH 5 min after a short retrieval. Groups were tested 1 and 10 days later (TestA1 and TestA2, respectively). Repeated measures ANOVA followed by Newman-Keuls were used for the analysis of results. The ethics committee approved all procedures (#1436). A significant effect of treatment was observed. The treatment with ROF did not change freezing behavior in TestA1, however, a significant reduction of freezing behavior in TestA2 was detected. This effect depended on PKA activity after retrieval and protein synthesis after Test A1. Furthermore, the effect of ROF depended on memory retrieval and exposure to TestA1 one day later. Together, the results suggest that PDE4 activity in the DH after a short memory retrieval is not associated with the reconsolidation process. However, PDE4 activity is important for memory maintenance mechanisms induced by retrieval. In conclusion, our results indicate that the role of PDE4 in the DH during reconsolidation and consolidation is divergent.

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DISCRIMINATING MEMORIES OF ITEMS AND SPATIAL LOCATIONS: IS THERE CONTENT-SPECIFIC MNEMONIC SPECIALIZATION IN THE HUMAN MEDIAL TEMPORAL LOBE?

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Decades of dialogue between neuroscience, computational theories, and experimental psychology helped identify candidate neural computations performed by distinct cortical regions in the medial temporal lobe (MTL) that support memory in a similar fashion across several mammalian species, including humans. It is still debated whether these region-specific computations can be differentiated based on content (e.g., item versus spatial information) or rather mnemonic processes (e.g., detailed versus coarse processing). Extant studies with humans often confound content with process and thus cannot distinguish between these theoretical accounts. In this study, we acquired full-brain high-resolution functional MRI images of human participants (n=39) while they performed a memory task. First, they encoded fractal stimuli (items) and their spatial position on the screen (locations), then performed a recognition task that required fine-grained discrimination between previously encountered and novel items or locations. On the group level, the perirhinal cortex, but not the parahippocampal cortex was sensitive to small alterations in the items presented. In contrast, individual differences in parahippocampal activity during encoding predicted subsequent location discrimination performance. Further, we found that individual differences in dentate gyrus-CA3 activity during encoding predicted later discrimination performance for both items and locations. These results suggest that the perirhinal cortex is more involved in detailed memory for items, whereas the parahippocampal cortex is more involved in detailed memory for spatial locations. In contrast, computations in the dentate gyrus-CA3 are independent of content. Taken together, our results support the view that there is content-specific mnemonic specialization of cortical regions within the human MTL.

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BOARD NUMBER: S07-052

THE NEURAL CORRELATES OF SPACING

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Research regarding the neural basis underlying the spacing effect lends support to some of the theories posed to explain the effects of distributed practice. For example, literature indicates extra frontal activity in spaced-trained participants, which may be indicative of deficient processing, and differences in latency of ERP components, which may be indicative of study phase retrieval. In particular, there is limited research examining the electrophysiological correlates of spacing. This study attempted to investigate the neural basis of the spacing effect while learning face-name associations using EEG. The results suggest that there are significant differences between spaced- and massed-trained participants when correctly identifying previously learned face-name pairs. Most notably, spaced participants demonstrate neural activity indicative of greater processing, familiarity, and recognition of stimuli compared to massed participants. Results also provide support for theories of deficient processing and study phase retrieval. These findings may have implications with regard to explaining why spacing is so beneficial.

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BOARD NUMBER: S07-053

RESTING-STATE FAST BRAIN DYNAMICS PREDICT INTER-INDIVIDUAL VARIABILITY IN MOTOR PERFORMANCE

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Motor learning (ML) features rapid enhancement during practice then offline post-practice gains with the reorganization of related brain networks. Within the first 5-30 min after the motor task completion, the performance improves (boost period) indicating fast reorganization of neural networks supporting ML. In this project, we aimed at investigating fast transient, sub-second variations in magnetoencephalographic (MEG) network activity in resting-state (RS) before and after ML. We hypothesised that changes in fast neural dynamics might reflect learning-related plasticity mechanisms and/or inter-individual motor variability in performance. Thirty-four young healthy participants underwent a 5-minute Pre-learning RS session within the MEG scanner followed by Finger Tapping Task (FTT) training. The Post-learning RS was assessed 20 min later within the 'boost' time window. Hidden Markov modelling (HMM) of MEG power envelope signals highlighted 8 recurrent topographical states. For State 1 (Frontal/Sensorimotor) and State 2 (Cuneus/Sensorimotor), motor performance levels (i.e., best performance at the end of the learning session) were associated with HMM temporal parameters both in pre- and post-learning resting-state sessions. Additionally, there was a correlation trend between State 6 (Cuneus/Sensorimotor-Frontal) and motor performance in the post-learning session only. However, no association emerged with offline changes in performance. These results suggest a trait-like relationship between spontaneous transient neural dynamics at rest and inter-individual variations in motor abilities. On the other hand, transient RS dynamics seem not to be state-dependent, i.e., modulated by learning experience and reflect neural plasticity, at least on the short timescale. **Keywords: motor learning, motor performance, magnetoencephalography, hidden Markov**

modelling

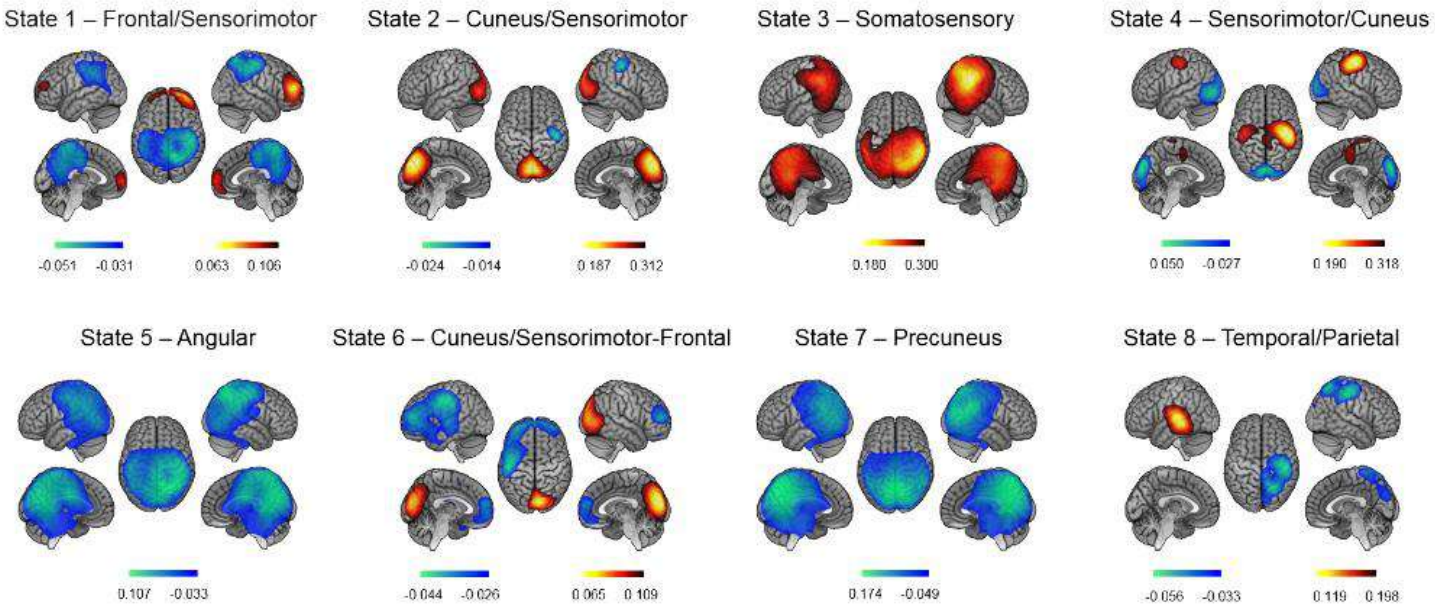

Figure 1. Spatial topographies of HMM transient states computed over pre- and post-learning RS sessions. Red/blue scales indicate positive/negative correlation values between the envelope and the state activation/inactivation time course (i.e., increased/decreased power during one state visit). For visualization purpose, the maps are thresholded between 60 and 100% of the maximum absolute of the partial correlation values.

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BOARD NUMBER: S07-054

THE CONTRIBUTION OF THALAMIC SUBDIVISIONS TO LEARNING IS ASSOCIATED WITH INTERINDIVIDUAL VARIABILITY IN MEMORY PERFORMANCE

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Thalamic functional connectivity (FC) with the executive control network (ECN) varies between pre- and post-encoding resting-state fMRI, interposed by the performance of a memory task. As the thalamus is composed of multiple subdivisions, each belonging to distinct thalamo-cortical circuits possibly competing in cognitive functions, we investigated (i) at the group-level, the differential involvement of thalamic subdivisions with ECN in memory, and (ii) at the individual-level, the variability of such involvement across individuals as a function of memory performance. We extracted published individual ECN maps and the thalamic ROI whose FC with ECN varied as function of memory performance in 28 healthy participants (μ age±SD=26±3; m:f=9:19). We segmented T1 scans through THOMAS deriving thalamic subdivisions (anterior, medial, posterior, lateral). At the group-level, we computed the percentage of overlap between subdivisions and the thalamic ROI, finding that lateral subdivision was mainly represented (70%), followed by anterior (25%), posterior (3%), and medial (2%). At the individual-level, the number of significant voxels in each subdivision from pre- and post-encoding ECN spatial maps ($p<0.05$) was correlated with memory performance across sessions (FDR<0.05). During pre-encoding, performance and the medial subdivision number of voxels were positively associated (pFDR=0.04). During post-encoding, performance and the lateral subdivision number of voxels were positively associated (pFDR=0.008), while performance and the posterior subdivision number of voxels were negatively associated (pFDR=0.05). Findings may suggest that different thalamo-cortical circuits differentially support individual memory performance, with medio-cortical connections potentially playing a preparatory role for better performance, and latero- and posterior-cortical connections differentially supporting post-learning stabilization.

Pubmed:
30725232: Antonucci LA, Di Carlo P, Passiatore R, Papalino M, Monda A, Amoroso N, Tangaro S, Taurisano P, Rampino A, Sambataro F, Popolizio T, Bertolino A, Pergola G, Blasi G
Thalamic connectivity measured with fMRI is associated with a polygenic index predicting thalamo-prefrontal gene co-expression.
The functional connectivity between thalamic medio-dorsal nucleus (MD) and cortical regions, especially the dorsolateral prefrontal cortex (DLPFC), is implicated in attentional processing and is anomalous in schizophrenia, a brain disease associated with polygenic risk and attentional deficits. However, the molecular and genetic underpinnings of thalamic connectivity anomalies are unclear. Given that gene co-expression across brain areas promotes synchronous interregional activity, our aim was to investigate whether coordinated expression of genes relevant to schizophrenia in MD and DLPFC may reflect thalamic connectivity anomalies in an attention-related network including the DLPFC. With this aim, we identified in datasets of post-mortem prefrontal mRNA expression from healthy controls a gene module with robust overrepresentation of genes with coordinated MD-DLPFC expression and enriched for schizophrenia genes according to the largest genome-wide association study to date. To link this gene cluster with imaging phenotypes, we computed a Polygenic Co-Expression Index (PCI) combining single-nucleotide polymorphisms predicting module co-expression. Finally, we investigated the association between PCI and thalamic functional connectivity during attention through fMRI Independent Component Analysis in 265 healthy participants. We found that PCI was positively associated with connectivity strength of a thalamic region overlapping with the MD within an attention brain circuit. These findings identify a novel association between schizophrenia-related genes and thalamic functional connectivity. Furthermore, they highlight the association between gene expression co-regulation and brain connectivity, such that genes with coordinated MD-DLPFC expression are associated with coordinated activity between the same brain regions. We suggest that gene co-expression is a plausible mechanism

underlying biological phenotypes of schizophrenia.
Brain Struct Funct, 2019; 224
31471679: Antonucci LA, Pergola G, Passiatore R, Taurisano P, Quarto T, Dispoto E, Rampino A, Bertolino A, Cassibba R, Blasi G
The interaction between OXTR rs2268493 and perceived maternal care is associated with amygdala-dorsolateral prefrontal effective connectivity during explicit emotion processing.
Previous studies have indicated a link between socio-emotional processing and the oxytocin receptor. In this regard, a single nucleotide polymorphism in the oxytocin receptor coding gene (OXTR rs2268493) has been linked with lower social functioning, increased risk for autism spectrum disorders (ASDs) and with post-mortem OXTR mRNA expression levels. Indeed, the levels of expression of OXTR in brain regions involved in emotion processing are also associated with maternal care. Furthermore, maternal care has been associated with emotional correlates. Taken together, these previous findings suggest a possible combined effect of rs2268493 and maternal care on emotion-related brain phenotypes. A crucial biological mechanism subtending emotional processing is the amygdala-dorsolateral prefrontal cortex (DLPFC) functional connection. On this basis, our aim was to investigate the interaction between rs2268493 and maternal care on amygdala-DLPFC effective connectivity during emotional evaluation. We characterized through dynamic causal modeling (DCM) patterns of amygdala-DLPFC effective connectivity during explicit emotion processing in healthy controls (HC), profiled based on maternal care and rs2268493 genotype. In the whole sample, right top-down DLPFC-to-amygdala pattern was the most likely directional model of effective connectivity. This pattern of connectivity was the most likely for all rs2268493/maternal care subgroups, except for thymine homozygous (TT)/low maternal care individuals. Here, a right bottom-up amygdala-to-DLPFC was the most likely directional model. These results suggest a gene by environment interaction mediated by the oxytocin receptor on biological phenotypes relevant to emotion processing.
Eur Arch Psychiatry Clin Neurosci, 2020; 270
31581175: Antonucci LA, Penzel N, Pergola G, Kambeitz-Ilankovic L, Dwyer D, Kambeitz J, Haas SS, Passiatore R, Fazio L, Caforio G, Falkai P, Blasi G, Bertolino A, Koutsouleris N
Multivariate classification of schizophrenia and its familial risk based on load-dependent attentional control brain functional connectivity.
Patients with schizophrenia (SCZ), as well as their unaffected siblings (SIB), show functional connectivity (FC) alterations during performance of tasks involving attention. As compared with SCZ, these alterations are present in SIB to a lesser extent and are more pronounced during high cognitive demand, thus possibly representing one of the pathways in which familial risk is translated into the SCZ phenotype. Our aim is to measure the separability of SCZ and SIB from healthy controls (HC) using attentional control-dependent FC patterns, and to test to which extent these patterns span a continuum of neurofunctional alterations between HC and SCZ. 65 SCZ with 65 age and gender-matched HC and 39 SIB with 39 matched HC underwent the Variable Attentional Control (VAC) task. Load-dependent connectivity matrices were generated according to correct responses in each VAC load. Classification performances of high, intermediate and low VAC load FC on HC-SCZ and HC-SIB cohorts were tested through machine learning techniques within a repeated nested cross-validation framework. HC-SCZ classification models were applied to the HC-SIB cohort, and vice-versa. A high load-related decreased FC pattern discriminated between HC and SCZ with 66.9% accuracy and with 57.7% accuracy between HC and SIB. A high load-related increased FC network separated SIB from HC (69.6% accuracy), but not SCZ from HC (48.5% accuracy). Our findings revealed signatures of attentional FC abnormalities shared by SCZ and SIB individuals. We also found evidence for potential, SIB-specific FC signature, which may point to compensatory neurofunctional mechanisms in persons at familial risk for schizophrenia.
Neuropsychopharmacology, 2020; 45
32124274: Taurisano P, Pergola G, Monda A, Antonucci LA, Di Carlo P, Piarulli F, Passiatore R, Papalino M, Romano R, Monaco A, Rampino A, Bonvino A, Porcelli A, Popolizio T, Bellotti R, Bertolino A, Blasi G
The interaction between cannabis use and a CB1-related polygenic co-expression index modulates dorsolateral prefrontal activity during working memory processing.
Convergent findings indicate that cannabis use and variation in the cannabinoid CB1 receptor coding gene (CNR1) modulate prefrontal function during working memory (WM). Other results also suggest that cannabis modifies the physiological relationship between genetically induced expression of CNR1 and prefrontal WM processing. However, it is possible that cannabis exerts its modifying effect on prefrontal physiology by interacting with complex molecular ensembles co-regulated with CB1. Since co-regulated genes are likely co-expressed, we investigated how genetically predicted co-expression of a molecular network including CNR1 interacts with cannabis use in modulating WM processing in humans. Using post-mortem human prefrontal data, we first computed a polygenic score (CNR1-PCI), combining the effects of single nucleotide polymorphisms (SNPs) on co-expression of a cohesive gene set including CNR1, and positively correlated with such co-expression. Then, in an in vivo study, we computed CNR1-PCI in 88 cannabis users and 147 non-users and investigated its

interaction with cannabis use on brain activity during WM. Results revealed an interaction between cannabis use and CNR1-PCI in the dorsolateral prefrontal cortex (DLPFC), with a positive relationship between CNR1-PCI and DLPFC activity in cannabis users and a negative relationship in non-users. Furthermore, DLPFC activity in cannabis users was positively correlated with the frequency of cannabis use. Taken together, our results suggest that co-expression of a CNR1-related network predicts WM-related prefrontal activation as a function of cannabis use. Furthermore, they offer novel insights into the biological mechanisms associated with the use of cannabis.
Brain Imaging Behav, 2021; 15

34637904: Passiatore R, Antonucci LA, Bierstedt S, Saranathan M, Bertolino A, Suchan B, Pergola G
How recent learning shapes the brain: Memory-dependent functional reconfiguration of brain circuits.
The process of storing recently encoded episodic mnestic traces so that they are available for subsequent retrieval is accompanied by specific brain functional connectivity (FC) changes. In this fMRI study, we examined the early processing of memories in twenty-eight healthy participants performing an episodic memory task interposed between two resting state sessions. Memory performance was assessed through a forced-choice recognition test after the scanning sessions. We investigated resting state system configuration changes via Independent Component Analysis by cross-modeling baseline resting state spatial maps onto the post-encoding resting state, and post-encoding resting state spatial maps onto baseline. We identified both persistent and plastic components of the overall brain functional configuration between baseline and post-encoding. While FC patterns within executive, default mode, and cerebellar circuits persisted from baseline to post-encoding, FC within the visual circuit changed. A significant session × performance interaction characterized medial temporal lobe and prefrontal cortex FC with the visual circuit, as well as thalamic FC within the executive control system. Findings reveal early-stage FC changes at the system-level subsequent to a learning experience and associated with inter-individual variation in memory performance.
Neuroimage, 2021; 245

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BOARD NUMBER: S07-055

ON THE IMPORTANCE OF HIPPOCAMPAL SEGMENTATION FOR THE NEURAL MAPPING OF MEMORY: EVIDENCE FROM A LARGE-SCALE STUDY OF NEURAL ARCHITECTURE IN HEALTHY ADULTS

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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The hippocampus (HC) is traditionally considered the key neuroanatomical hub responsible for memory. However, previous MRI studies that aimed to relate volumetric hippocampal measures to associative memory (AM) performance have yielded mixed results. In the current study, we aimed to reevaluate these findings in a large sample of young healthy participants (N = 246; age M = 24.95, SD = 4.58; 56% female). Participants were scanned with 3T MAGNETOM Prisma using a 64-channel head coil, followed by the AM assessment in the lab setting. To maximize the scope of AM assessment, we employed four paired-associate tasks of various stimuli modalities (faces, words, scenes) and outcome measure types (recognition, recall). Synthetic T1-weighted images were produced out of relaxometry parameter maps, after which volumetric measures were calculated using FreeSurfer. The whole HC volume showed no correlation with any of the memory measures. However, further segmentation of HC into its functional and anatomical subfields (Parasubiculum, Presubiculum, Subiculum, CA1, CA2/3, CA4, GC-DG, HATA, Fimbria, Molecular layer, Hippocampal fissure, Hippocampal tail) showed scattered yet consistent patterns of significant correlations between different subfield volumes and memory outcomes. The results suggest that distinctive contributions of HC subfields may lead to a null effect when the whole HC volume is considered, thus demonstrating that drawing conclusions based on the volumetric measures of neural macrostructures can be misleading. The results highlight the importance of in-depth segmentation for neural mapping.

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BOARD NUMBER: S07-056

MOTOR-SENSORY DYNAMICS DURING MEMORY ENCODING AND RECALL

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Motor-sensory dynamics is an essential component of perception. During an episode, the scanning patterns of sensory organs affect the acquired information. Despite that, the inter-relations between motor-sensory dynamics and episodic memory are not often studied. To fill this gap, we designed a free recall task that took place in a virtual reality (VR) environment, in which participants could freely move. During the encoding stage, participants were asked to explore a VR environment containing 15 everyday virtual objects. During the recall stage, participants were asked to recall the objects' names in a different VR environment. Participants' eyes, hands and heads were tracked at both stages. During encoding, participants walked around, looked at (virtual) objects and sometimes moved objects (virtually). Our results suggest that two motor-sensory factors occurring during encoding could increase objects' recall probability: The action of moving an object and the total gazing time at an object. The order of objects' recall was correlated with the order of their locations along the walking trajectory during encoding but not with the order in which they were gazed at. Participants who recalled in an environment that resembled the encoding environment tended to perform longer and slower drifts during recall. These initial results already point to specific roles played by motor-sensory dynamics in memory encoding and recall.

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BOARD NUMBER: S07-057

AN INVESTIGATION OF THE COGNITIVE AND NEURAL CORRELATES OF SEMANTIC MEMORY SEARCH RELATED TO CREATIVE ABILITY

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

Marcela Ovando-Tellez[1], Mathias Benedek[2], Yoed Kenett[3], Thomas Hills[4], Sarah Bouanane[1], Matthieu Bernard[1], Joan Belo[1], Theophile Bieth[1], Emmanuelle Volle[1]
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How do creative ideas arise? A common assumption is that ideas result from searching and combining semantic memory knowledge. However, the mechanisms acting on memory to yield creative ideas are not elucidated. This study explores the neurocognitive correlates of semantic search components related to creative abilities. We designed an associative fluency task based on polysemous words. We distinguished two search components by assessing clustering and switching performance from the responses within and between the different meanings. We related these components to creativity, executive abilities, and semantic memory structure explored with semantic networks methods, and identified their predictive functional brain connectivity patterns using connectome-based modeling. Clustering correlated with divergent thinking, while switching correlated with the ability to combine remote associates. Furthermore, switching correlated with semantic memory structure and executive abilities, and was predicted by connectivity between and within the default, control, and salience networks. These results suggest that switching captures interactions between memory structure and control processes guiding the search. In contrast, clustering relied on interactions between control, salience, and attentional networks. Clustering may capture controlled processes related to persistent search. These findings shed new light on the neurocognitive mechanisms allowing an efficient and flexible semantic search to support creativity.

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THE N400 AS A NEUROPHYSIOLOGICAL MARKER OF SECOND-LANGUAGE LEARNING

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Research on memory has identified various learning principles to support knowledge acquisition. Some of the most effective learning principles are retrieval practice (i.e., actively recalling information), multimodal learning (i.e., presenting information via multiple sensory modalities), and corrective feedback (i.e., receiving the correct response when making an error). While each learning principle has been examined separately, interactions between them and their underlying neurophysiological correlates remain to be investigated. Thus, our study aims to investigate potential interactions between the learning principles on associative memory (i.e., vocabulary acquisition) and their neurophysiological correlates. Healthy young adults learned Finnish vocabulary for 14 days using the mobile phone application “stellar-learning”, thereby manipulating the proportion of retrieval, multimodality, and feedback on an individual word basis. Before and after the learning intervention, participants performed a cross-language priming task, where they were asked to identify congruent and incongruent collocations (i.e., native language and learnt Finnish vocabulary), while their EEG was recorded. We assessed the correctness of responses and compared the N400 event-related brain potentials component – a marker for semantic violations – for congruent and incongruent associations by learning principle. We hypothesized a larger N400 amplitude for incongruent than congruent collocations after learning, but not before. Moreover, we expected this effect to be modulated by combinations of different learning principles. Preliminary results show an increase in proportion of correct responses and indicate a modulation in the N400 window on the level of the learning principles after learning. These results suggest that learning can be optimized by combining learning principles.

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THE HIPPOCAMPUS CONSTRUCTS SEQUENCE MEMORIES THAT GENERALIZE TEMPORAL RELATIONS ACROSS EXPERIENCES

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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The hippocampal-entorhinal region supports memory for episodic details, such as temporal relations of sequential events, and mnemonic constructions combining experiences for inferential reasoning. However, it is unclear whether hippocampal event memories reflect temporal relations derived from mnemonic constructions, event order, or elapsing time, and whether these sequence representations generalize temporal relations across similar sequences. Here, participants mnemonically constructed times of events from multiple sequences using infrequent cues and their experience of passing time. After learning, event representations in the anterior hippocampus reflected temporal relations based on constructed times. Temporal relations were generalized across sequences, revealing distinct representational formats for events from the same or different sequences. Structural knowledge about time patterns, abstracted from different sequences, biased the construction of specific event times. These findings demonstrate that mnemonic construction and the generalization of relational knowledge combine in the hippocampus, consistent with the simulation of scenarios from episodic details and structural knowledge.

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PRESERVED NAVIGATION ABILITIES AND SPATIO-TEMPORAL MEMORY IN INDIVIDUALS WITH AUTISM SPECTRUM DISORDER

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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In this study, we asked whether functional interaction or structural defects in the right Crus I cerebellum, such as those highlighted in ASD mouse models and in young ASD individuals, could be observed in adults and have an impact outside the range of social deficits and repetitive behaviors, namely in navigation. Knowing the role of RCrus I-prefrontal connectivity in sequence-based navigation, which relies on memorizing a route, we expected that participants with ASD might have deficits in learning and executing this strategy, and be biased toward the use of another strategy when free to do so. On the other hand, the literature suggesting difficulties with place learning relying on allocentric representations, we also anticipated participants to underperform place-based strategy. We therefore tested adults with ASD or typical development in a navigation task, using the virtual Starmaze environment which allows us to assess the ability to use each of these strategies. We thus compared learning, strategy preferences and performances, as well as memories of the environment after the task. In parallel, participants underwent structural and functional MRI at rest, allowing us to study the volume and connectivity of Crus I in the same sample of subjects. Surprisingly but interestingly, despite the involvement of RCrus I in navigation, and its described involvement in ASD, we observed neither behavioral deficits, nor alterations in RCrusI volume or connectivity in our sample of participants.

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BOARD NUMBER: S07-061

VISUOSPATIAL PERSPECTIVE-TAKING-SPECIFIC BRAIN DYNAMICS CAPTURED BY IEEG

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Visuospatial perspective-taking (VPT) requires overcoming self-perspective and imagining a scene from another perspective. It is essential for spatial and social reasoning. Studies outline brain areas involved in VPT; however, they largely overlap with self-perspective. To separate VPT from self-perspective brain activity, it is vital to estimate when and where the responses only to VPT but not to self-perspective emerge. Therefore, first, we aimed to spatiotemporally differentiate the brain responses only to VPT (VPT-specific) from the responses only to self-perspective (Self-specific), and responses to both perspectives (General). Hierarchical processing theory suggests that stimulus-specific responses emerge over time. Considering this, our second aim was to test the hypothesis whether task-condition-specific (VPT-specific, Self-specific) responses start later than General responses. We recorded iEEG data from 30 patients with pharmacoresistant epilepsy. The patients performed a VPT task requiring laterality judgments from self or another perspective. We estimated the spatiotemporal features of responses with Time-Frequency analysis in the broad gamma band (50–150 Hz). From 10 regions of interest (ROI), the parietal and medial temporal lobe, lateral prefrontal cortex, and insula exhibited all types of responses (VPT-specific, Self-specific, General). The rest of the ROIs showed VPT-specific and General responses. VPT-specific and Self-specific responses had similar dynamics. In contrast, task-condition-specific responses started later and lasted shorter than General responses. Our results anatomically differentiate VPT-specific responses from Self-specific and temporally from General responses. Additionally, we give a novel example of hierarchical processing, showing that task-condition-specific processing starts later and lasts shorter than general processing. Supported by GACR:19-11753S; RVO:67985823.

Pubmed:
33745982: Gunia A, Moraresku S, Vlček K
Brain mechanisms of visuospatial perspective-taking in relation to object mental rotation and the theory of mind.
Visuospatial perspective-taking (VPT) is a process of imagining what can be seen and how a scene looks from a location and orientation in space that differs from one's own. It comprises two levels that are underpinned by distinct neurocognitive processes. Level-2 VPT is often studied in relation to two other cognitive phenomena, object mental rotation (oMR) and theory of mind (ToM). With the aim to describe the broad picture of neurocognitive processes underlying level-2 VPT, here we give an overview of the recent behavioral and neuroscientific findings of level-2 VPT. We discuss its relation to level-1 VPT, which is also referred to as perspective-tracking, and the neighboring topics, oMR and ToM. Neuroscientific research shows that level-2 VPT is a diverse cognitive process, encompassing functionally distinct neural circuits. It shares brain substrates with oMR, especially those parietal brain areas that are specialized in spatial reasoning. However, compared to oMR, level-2 VPT involves additional activations in brain structures that are typically involved in ToM tasks and deal with self/other distinctions. In addition, level-2 VPT has been suggested to engage brain areas coding for internal representations of the body. Thus, the neurocognitive model underpinning level-2 VPT can be understood as a combination of visuospatial processing with social cognition and body schema representations.
Behav Brain Res, 2021; 407

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BOARD NUMBER: S07-062

FUNCTIONAL IMPLICATIONS OF VERTICAL CODING BIASES IN SCENE-SELECTIVE REGIONS ON SPATIAL ORIENTATION: EVIDENCE FROM SOURCE LOCALIZED EEG RECORDINGS.

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Aims: fMRI studies identified scene-selective brain regions (namely: Parahippocampal place area – PPA; Retrosplenial cortex – RSC; Occipital place area – OPA) involved in the recognition and processing of visual scenes. Recent findings showed that these regions exhibit distinct retinotopic preferences for either the upper or lower visual field. Given their visuo-spatial function, we hypothesized that the vertical location of visual information useful for orientation would influence the activity of scene-selective regions. **Methods**: Twenty-four young adults (28 ± 4.9 y.o.) took part in a desktop-based virtual reality task. Subjects had to learn how to orient within a four-intersection environment, using landmarks that were only visible at the intersections. Landmarks that carried navigational information were positioned either in the lower or the upper part of the screen. We collected behavioral, eye-tracking, and EEG data. Using an individualized source reconstruction model, we extracted the electrical activity imputable to each scene-selective region. **Results**: The vertical position of the navigationally useful landmarks did not impact task performance. However, at the neural level, it modulated the activity of scene-selective regions when processing visual information at intersections. Vertical properties of scenes thus seem to impact higher-level integration of spatial information. **Conclusions**: As the nature of visual information varies significantly along the vertical axis in everyday environments, the reported evidence underlines the possibility that retinotopic preferences in higher visual areas emerged to serve complex cognitive abilities such as spatial navigation.

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EXAMINING OVERSHADOWING IN HEALTHY ADULTS USING A VIRTUAL MAZE TASK.

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Aims Previous research in the area of spatial navigation has suggested that humans use a cognitive map to navigate their surroundings, but there may be a simpler explanation via associative learning theory (Pearce, 2009). Overshadowing is a phenomenon underpinning associative learning theories in which learning about a cue is reduced when it is accompanied by a second cue during learning. This has been observed in previous research outside of the spatial domain and in animal studies. This experiment aims to investigate whether overshadowing also occurs during human spatial learning. **Methods** NavWell, a virtual Morris Water Maze was used to investigate evidence for overshadowing in young healthy adult participants (aged 18-24). Five groups (n=25/group) were used. Groups 1 and 2 were trained with two cues, one near and one far from the goal, and then tested on one cue respectively. Group 3 was trained and tested with both cues. Group 4 was trained and tested with the far cue only, and Group 5 was trained and tested with the near cue only. **Results** Overall findings indicated that those trained with both cues and retested with a single cue (i.e., Groups 1& 2) performed significantly worse than those trained and tested with a single cue (irrespective of cue location, Groups 4 & 5), suggesting a possible overshadowing effect in healthy human participants. **Conclusions** These findings support animal literature and suggest that spatial learning is associative, providing a more parsimonious explanation for how humans navigate than that proposed by cognitive map theory.

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BOARD NUMBER: S07-064

COGNITIVE SKILLS RELATED TO NAVIGATION IN CHILDREN FROM 6 TO 11 YEARS OLD.

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Cognitive mapping includes different cognitive and perceptual skills. The map formation process can be differentiated in the coding and recall of landmarks. Currently, there is no consensus regarding the skills required for each of these phases. The objective of the present study was to analyze the relationship of perceptual, memory, spatial and executive abilities in the encoding and recall of landmarks in a group of 11 children aged 6 to 11 years with typical development. We administered the Wechsler Intelligence Scale WISC-4, the Neuropsychological Evaluation for Children (ENI-2, acronym in Spanish), the Porteous Maze, and a virtual-navigation task. We analyzed the relationship between the scores obtained in the evaluation with the performance in the encoding and recall navigation tasks using Spearman's r correlation coefficient. We observed that the average distance navigated correlates positively with the performance in the identification of the orientation of lines (OL) and location of coordinates (UC); both in the encoding (OL, $r=0.671$ $p<0.05$; and UC, $r= 0.688$). $p<0.01$) as well as recall (OL, $r=0.613$ $p<0.05$; and UC, $r= 0.793$ $p<0.01$) tasks. We also observed differential cognitive functions and processes depending on the process of landmark encoding or recall. We propose that cognitive processes associated with navigation are differential. These are related on the phase of the formation of the cognitive map. Perceptual processes participate in encoding; while the activation and manipulation of representations stored in memory are demanded during landmark´s recall. **Acknowledgments** CONACYT Beca de Doctorado CVU 409832 DGAPA Proyect IN231720.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-065

EGOCENTRIC AND ALLOCENTRIC TEMPORAL COGNITIVE MAPS

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Through "mental time travel", humans can project themselves in time, reliving past events or exploring possible future outcomes. This ability requires that events be flexibly mapped on an internal temporal coordinate system. Personal and world events can be mapped relative to the self, constructing an egocentric map. Conversely, fictional events would be mapped on a landmark-based allocentric map. In both allocentric and egocentric representations, self-projection in mental time travel can be operationalized as a shift of the temporal map on a new reference. We expected that the behavioural cost (as assessed by measures of reaction times) of this shift would parametrically increase with the distance of projection. In a series of psychophysics experiments, participants projected themselves at different distances in the past and in the future and performed temporal order judgments on given events. The events were either historical (N = 31) or fictional (N = 24), characterizing respectively the egocentric and the allocentric map. Though we find no clear increase of the behavioural cost along distance of projection, we identified confounds in the experimental design. To correct for these, a new series of experiments is being conducted.

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BOARD NUMBER: S07-066

LANDMARK-BASED NAVIGATION DECLINES WITH AGE

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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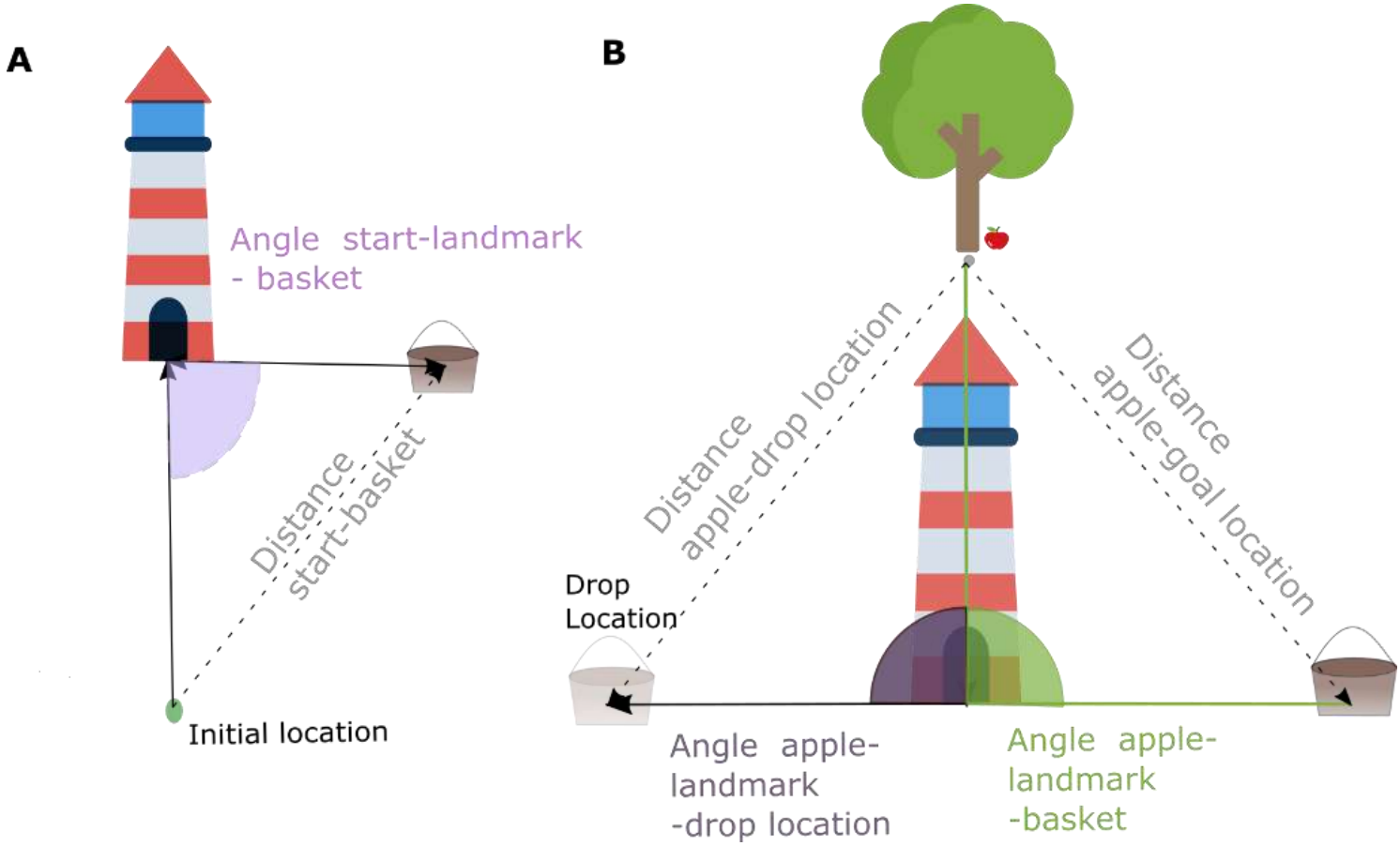
To investigate the age effect on spatial navigation, we used a virtual task, the "Apple Game", distinguishing between landmark-based and pure path integration. Participants navigated in a virtual field. They had to pick up a basket, find an apple in a tree and drop back the basket to its initial location. Difficulty varied according to the number of interim locations (trees) and the presence/absence of a landmark. We recruited 400 volunteers from four European centers. Linear mixed models predicted the drop error with age. Then, we predicted the traveled distance (distance apple-drop location) and the angle realized (apple-landmark-drop location) to understand the mechanisms leading to navigational errors (Fig. 1). The drop error increased with age and decreased with the landmark. The landmark effect decreased with age. For older participants, the traveled distance depended on the initial view of the basket (distance start-basket) and not on the correct distance to travel (distance apple-goal location). Furthermore, the angle realized depended on the initial view (angle start-landmark-basket) and not on the correct angle (angle apple-landmark-goal location). Younger participants were able to navigate without bias toward the initial view. In conclusion, landmark-based navigation decreases with age. Elderly participants were biased toward the initial view and did not integrate their position with the landmark

location.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BOARD NUMBER: S07-067

BRAIN ACTIVITY IN ENCODING OF EXPLICIT SEQUENTIAL VISUOSPATIAL MEMORY

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Repeated presentation of long visuospatial sequences incrementally commits their content to episodic memory, with the transfer from working memory taking place over specific timespans of encoding. Using fMRI (3T, n=29), we sought to identify the brain regions recruited during this form of learning. Presentation trials of a 20-target long learning paradigm alternated with recall periods whose behavioral data (captured by eye-tracking) was used to determine encoding progress as a function of time during presentation. This encoding progress was used as a continuous predictor of BOLD activity. To isolate activity specifically related to learning as opposed solely to attentional shifts, a second, purely attentional task was devised, with an analogous temporal structure to the encoding periods. Investigating the coefficient of a general linear model fitting BOLD activity as a function of the learning or attention predictor, significant activity was shown for both predictors in the bilateral superior frontal gyri, superior parietal lobules, and middle temporal gyri. To investigate whether these areas were specifically involved in learning, independent from simultaneous attentional control, we performed a repeated measures ANOVA, revealing significant interactions between region and experiment as well as hemisphere and experiment. While attentional activation did not differ between regions, learning activation was stronger in the superior parietal lobules and middle temporal gyri than superior frontal gyri. These preliminary findings suggest the superior parietal lobule and middle temporal gyrus, but not superior frontal gyrus, are specifically involved in the acquisition of long-term spatial memory.

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BOARD NUMBER: S07-068

TIMING OF ALLOCENTRIC AND EGOCENTRIC SPATIAL CODING REVEALED BY HUMAN INTRACRANIAL EEG

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Taking advantage of the high temporal resolution of intracranial EEG (iEEG), we aimed to establish precise timing of egocentric and allocentric information processing and clarify involved brain areas. Although many fMRI studies have focused on studying the brain areas associated with egocentric and allocentric coding, their results are not always consistent. Moreover, they lacked the temporal dynamic of involved cognitive processes. We developed a task requiring a fast spatial decision using allocentric or egocentric spatial coding in the three-dimensional virtual arena. The task for subjects was to estimate which of two goals on the arena floor was closer to a specific landmark or themselves, respectively. We recorded iEEG data from 37 drug-resistant epilepsy patients and analyzed broadband gamma activity (50–150Hz). We found channels responding to both egocentric and allocentric conditions in many brain regions, with their highest number in the frontal cortex. Egocentric-selective channels were mainly found in the lingual and supramarginal gyri. In the lingual gyrus, the egocentric response was higher than the allocentric one at the time window 300-1000 ms. Allocentric-selective channels were found around the intraparietal sulcus and in the occipital and lateral temporal cortex. In the intraparietal sulcus, the difference between allocentric and egocentric responses was observed at 400-700 ms after stimulus onset, while in the occipital and lateral temporal cortex, it emerged later, at 600-700 ms. Our findings suggest that egocentric and allocentric coding relies on overlapping but partially distinct neural mechanisms with a different temporal processing scheme. *supported by GACR 19-11753S and RVO: 67985823*

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BOARD NUMBER: S07-069

WHEN HUMANS LIVE ON A SMALL PLANET: SPATIAL MEMORY AND PATH INTEGRATION ON A SPHERICAL SURFACE

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Humans can build cognitive maps of the world. The nature of the cognitive map, relying on fundamental neural coding principles in the hippocampal formation, would be influenced by the environment, and most people live on a surface that can be best described with Euclidean geometry. Whether people can adapt and build a map for a novel environment in which they can no longer rely on Euclidean geometrical intuition is an intriguing question that can help us to understand the capacity and limitation of human cognition. To answer this question, we designed a study where participants explore the planar and spherical world on separate days using a virtual reality treadmill. They first learned a spatial layout of objects and were tested of their object-location memory, and then they completed a path integration task, known as the triangle completion test. Preliminary data analysis shows that participants were similarly good at locating objects on both plane and sphere, suggesting a remarkable capacity in learning an unfamiliar map structure. However, in the path integration task, they showed a systematic overturn bias on the sphere, as a consequence of following the Euclidean geometrical rule on the non-Euclidean surface. This result implies a strong Euclidean geometrical prior in human mind despite the capacity of building a map for a non-flat surface, and can inform future neuroscientific investigations of the cognitive map.

Pubmed:
30561118: Kim M, Maguire EA
Encoding of 3D head direction information in the human brain.
Head direction cells are critical for navigation because they convey information about which direction an animal is facing within an environment. To date, most studies on head direction encoding have been conducted on a horizontal two-dimensional (2D) plane, and little is known about how three-dimensional (3D) direction information is encoded in the brain despite humans and other animals living in a 3D world. Here, we investigated head direction encoding in the human brain while participants moved within a virtual 3D "spaceship" environment. Movement was not constrained to planes and instead participants could move along all three axes in volumetric space as if in zero gravity. Using functional magnetic resonance imaging (fMRI) multivoxel pattern similarity analysis, we found evidence that the thalamus, particularly the anterior portion, and the subiculum encoded the horizontal component of 3D head direction (azimuth). In contrast, the retrosplenial cortex was significantly more sensitive to the vertical direction (pitch) than to the azimuth. Our results also indicated that vertical direction information in the retrosplenial cortex was significantly correlated with behavioral performance during a direction judgment task. Our findings represent the first evidence showing that the "classic" head direction system that has been identified on a horizontal 2D plane also seems to encode vertical and horizontal heading in 3D space in the human brain.
Hippocampus, 2019; 29
30481593: Kim M, Maguire EA
Can we study 3D grid codes non-invasively in the human brain? Methodological considerations and fMRI findings.
Recent human functional magnetic resonance imaging (fMRI) and animal electrophysiology studies suggest that grid cells in entorhinal cortex are an efficient neural mechanism for encoding knowledge about the world, not only for spatial location but also for more abstract cognitive information. The world, be it physical or abstract, is often high-dimensional, but grid cells have been mainly studied on a simple two-dimensional (2D) plane. Recent theoretical studies have proposed how grid cells encode three-dimensional (3D) physical space, but it is unknown whether grid codes can be examined non-invasively in humans. Here, we investigated whether it was feasible to test different 3D grid models using fMRI based on the direction-modulated property of grid signals. In doing so, we developed interactive software to help researchers visualize 3D grid fields and predict grid activity in 3D as a function of movement directions. We found that a direction-modulated grid analysis was sensitive to one type of 3D grid model - a face-centred cubic (FCC) lattice model. As a proof of concept, we searched for 3D grid-like signals in human entorhinal cortex using a novel 3D virtual reality paradigm and a new fMRI analysis method. We

found that signals in the left entorhinal cortex were explained by the FCC model. This is preliminary evidence for 3D grid codes in the human brain, notwithstanding the inherent methodological limitations of fMRI. We believe that our findings and software serve as a useful initial stepping-stone for studying grid cells in realistic 3D worlds and also, potentially, for interrogating abstract high-dimensional cognitive processes.
Neuroimage, 2019; 186
29554231: Kim M, Maguire EA
Hippocampus, Retrosplenial and Parahippocampal Cortices Encode Multicompartment 3D Space in a Hierarchical Manner.
Humans commonly operate within 3D environments such as multifloor buildings and yet there is a surprising dearth of studies that have examined how these spaces are represented in the brain. Here, we had participants learn the locations of paintings within a virtual multilevel gallery building and then used behavioral tests and fMRI repetition suppression analyses to investigate how this 3D multicompartment space was represented, and whether there was a bias in encoding vertical and horizontal information. We found faster response times for within-room egocentric spatial judgments and behavioral priming effects of visiting the same room, providing evidence for a compartmentalized representation of space. At the neural level, we observed a hierarchical encoding of 3D spatial information, with left anterior hippocampus representing local information within a room, while retrosplenial cortex, parahippocampal cortex, and posterior hippocampus represented room information within the wider building. Of note, both our behavioral and neural findings showed that vertical and horizontal location information was similarly encoded, suggesting an isotropic representation of 3D space even in the context of a multicompartment environment. These findings provide much-needed information about how the human brain supports spatial memory and navigation in buildings with numerous levels and rooms.
Cereb Cortex, 2018; 28
28320847: Kim M, Jeffery KJ, Maguire EA
Multivoxel Pattern Analysis Reveals 3D Place Information in the Human Hippocampus.
The spatial world is three dimensional (3D) and humans and other animals move both horizontally and vertically within it. Extant neuroscientific studies have typically investigated spatial navigation on a horizontal 2D plane, leaving much unknown about how 3D spatial information is represented in the brain. Specifically, horizontal and vertical information may be encoded in the same or different neural structures with equal or unequal sensitivity. Here, we investigated these possibilities using fMRI while participants were passively moved within a 3D lattice structure as if riding a rollercoaster. Multivoxel pattern analysis was used to test for the existence of information relating to where and in which direction participants were heading in this virtual environment. Behaviorally, participants had similarly accurate memory for vertical and horizontal locations and the right anterior hippocampus (HC) expressed place information that was sensitive to changes along both horizontal and vertical axes. This is suggestive of isotropic 3D place encoding. In contrast, participants indicated their heading direction faster and more accurately when they were heading in a tilted-up or tilted-down direction. This direction information was expressed in the right retrosplenial cortex and posterior HC and was only sensitive to vertical pitch, which could reflect the importance of the vertical (gravity) axis as a reference frame. Overall, our findings extend previous knowledge of how we represent the spatial world and navigate within it by taking into account the important third dimension. The spatial world is 3D. We can move horizontally across surfaces, but also vertically, going up slopes or stairs. Little is known about how the brain supports representations of 3D space. A key question is whether horizontal and vertical information is equally well represented. Here, we measured fMRI response patterns while participants moved within a virtual 3D environment and found that the anterior hippocampus (HC) expressed location information that was sensitive to the vertical and horizontal axes. In contrast, information about heading direction, found in retrosplenial cortex and posterior HC, favored the vertical axis, perhaps due to gravity effects. These findings provide new insights into how we represent our spatial 3D world and navigate within it.
J Neurosci, 2017; 37
30063178: Clark IA, Kim M, Maguire EA
Verbal Paired Associates and the Hippocampus: The Role of Scenes.
It is widely agreed that patients with bilateral hippocampal damage are impaired at binding pairs of words together. Consequently, the verbal paired associates (VPA) task has become emblematic of hippocampal function. This VPA deficit is not well understood and is particularly difficult for hippocampal theories with a visuospatial bias to explain (e.g., cognitive map and scene construction theories). Resolving the tension among hippocampal theories concerning the VPA could be important for leveraging a fuller understanding of hippocampal function. Notably, VPA tasks typically use high imagery concrete words and so conflate imagery and binding. To determine why VPA engages the hippocampus, we devised an fMRI encoding task involving closely matched pairs of scene words, pairs of object words, and pairs of very low imagery abstract words. We found that the anterior hippocampus was engaged during processing of both scene and object word pairs in comparison to abstract word pairs, despite binding occurring in all conditions. This was also the case when just subsequently remembered stimuli were considered. Moreover, for object word pairs, fMRI activity patterns in anterior hippocampus were more similar to those for scene imagery than object imagery. This was especially evident in participants who were high imagery users and

not in mid and low imagery users. Overall, our results show that hippocampal engagement during VPA, even when object word pairs are involved, seems to be evoked by scene imagery rather than binding. This may help to resolve the issue that visuospatial hippocampal theories have in accounting for verbal memory.
J Cogn Neurosci, 2018; 30

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BOARD NUMBER: S07-070

PRELIMINARY EVIDENCE FOR A ROLE OF COGNITIVE MAPS IN ACTION REPRESENTATION

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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In our everyday life, we often need to execute complex goal-directed actions. To successfully carry them out, it is essential to represent the knowledge about their potential environmental or bodily outcomes. Recent evidence suggests that cognitive maps supported by the hippocampal-entorhinal system organize knowledge in abstract low-dimensional spaces. The goal of this project is to investigate whether the brain can conceptually organize different action plans using a two-dimensional mapping of expected action-outcomes. In particular, we examine if and how the anticipated outcomes of action plans could be used to abstractly represent and flexibly choose between different plans along outcome dimensions. We designed a set of behavioral tasks using immersive VR with HMD and hand controllers. Participants are trained to execute different action sequences, that trigger launching of a ball towards them, and learn to associate those sequences with different probabilities to catch the ball (axis 1), and for the ball to disappear (axis 2). Then, participants compare the sequences based on the learned action-outcome contingencies. We performed multidimensional scaling on the behavioral data from the comparison task and our preliminary results suggest a map-like representation of action sequences. To explore the neural mechanisms of this map-like representation, we will acquire fMRI data in a new study using this paradigm. We hypothesize that encoding mechanisms in hippocampal-entorhinal and parietal-premotor regions are involved in the construction of such a map, thus, supporting behavioral planning by encoding multiple relationships between different action plans, and allowing efficient action selection using a common representational format.

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BOARD NUMBER: S07-071

THE HUMAN HIPPOCAMPAL LONG AXIS SHOWS DISCRETE FUNCTIONAL ORGANIZATION DURING SPATIAL NAVIGATION

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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The hippocampus is a well-known hub for episodic and spatial processing and memory, as well as more abstract higher-order domains like emotion, language, and theory-of-mind. A common interpretation posits a domain-agnostic coding mechanism that assembles multimodal input into internal representations of places, events, and concepts. However, there is uncertainty around how the involvement of the hippocampus across higher-order thought processes is reflected in its intrinsic organization. For instance, while the anterior and posterior poles of the hippocampus support different behaviours, prior findings have paradoxically indicated a long-axis functional organization that is simultaneously gradual and discrete. Inasmuch as human resting-state fMRI data expressing gradual organization, while other studies suggest that task-based fMRI data should express a more discretized organization. Thus, our aim is to examine long-axis functional topography in task-based 7T fMRI data recorded at sub-millimeter resolution, using a dataset of 22 participants performing a virtual spatial navigation task. We used connectopic mapping, a data-driven analysis for determining topographical features of changes in functional connectivity. Calculating similarity of extrinsic functional connectivity between adjacent voxels yielded maps of connectivity similarity along the hippocampal long axis. These maps were examined for evidence of discrete parcellation, indicated by clusters of voxelwise similarity. Our results indicate a long-axis functional topography that is more modular than gradual. We conclude that task-dependent recruitment of hippocampal computation has a significant effect on the expression of functional topography.

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BOARD NUMBER: S07-072

ENTORHINAL GRID-LIKE SIGNALS REFLECT TEMPORAL CONTEXT FOR HUMAN TIMING BEHAVIOR

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

Ignacio Polti[1,2], Matthias Nau[2,3], Raphael Kaplan[4], Virginie Van Wassenhove[5], Christian Doeller[1,2]
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The entorhinal cortex (EC) supports the encoding of task regularities. A critical function may be the encoding of temporal context (i.e., forming integrated relational representations of co-occurring events and stimuli). A key neural component in the EC are grid cells, whose activity likely exhibits a six-fold rotational symmetry as a function of gaze direction as measured by functional magnetic resonance imaging (fMRI). Here, we combined fMRI and a time-to-contact estimation task to test whether temporal context modulates this grid-like fMRI activity in the human EC. In addition, we characterized in detail the relationship between trial-wise entorhinal activity and participants' task performance. We found that activity in the EC reflected biases in timing behavior, and that the cross-validated amplitude of grid-like signals indeed depended on the timing errors consistent with temporal-context encoding. These findings suggest that the human EC contributes to adapting internal timing mechanisms to the temporal statistics of the environment in accordance with the predictions from Bayesian models of time perception.

Pubmed:
29703928: Polti I, Martin B, van Wassenhove V
The effect of attention and working memory on the estimation of elapsed time.
Psychological models of time perception involve attention and memory: while attention typically regulates the flow of events, memory maintains timed events or intervals. The precise, and possibly distinct, roles of attention and memory in time perception remain debated. In this behavioral study, we tested 48 participants in a prospective duration estimation task while they fully attended to time or performed a working memory (WM) task. We report that paying attention to time lengthened perceived duration in the range of seconds to minutes, whereas diverting attention away from time shortened perceived duration. The overestimation due to attending to time did not scale with durations. To the contrary, increasing WM load systematically decreased subjective duration and this effect scaled with durations. Herein, we discuss the dissociation between attention and WM in timing and scalar variability from the perspective of Bayesian models of time estimations.
Sci Rep, 2018; 8
32530378: Bellmund JLS, Polti I, Doeller CF
Sequence Memory in the Hippocampal-Entorhinal Region.
Episodic memories are constructed from sequences of events. When recalling such a memory, we not only recall individual events, but we also retrieve information about how the sequence of events unfolded. Here, we focus on the role of the hippocampal-entorhinal region in processing and remembering sequences of events, which are thought to be stored in relational networks. We summarize evidence that temporal relations are a central organizational principle for memories in the hippocampus. Importantly, we incorporate novel insights from recent studies about the role of the adjacent entorhinal cortex in sequence memory. In rodents, the lateral entorhinal subregion carries temporal information during ongoing behavior. The human homologue is recruited during memory recall where its representations reflect the temporal relationships between events encountered in a sequence. We further introduce the idea that the hippocampal-entorhinal region might enable temporal scaling of sequence representations. Flexible changes of sequence progression speed could underlie the traversal of episodic memories and mental simulations at different paces. In conclusion, we describe how the entorhinal cortex and hippocampus contribute to remembering event sequences-a core component of episodic memory.
J Cogn Neurosci, 2020; 32
29572103: Menéndez J, Sánchez F, Polti I, Idesis S, Avellaneda M, Tabullo Á, Iorio A
Event-related potential correlates of stimulus equivalence classes: A study of task order of the equivalence based priming

probes with respect to the stimulus equivalence tests, and among the distinct trial types with each other.
This study investigates the influences of: 1) the task order of two stimulus equivalence classes (SEC) probes, and 2) the possible differences within the equivalence trial types. These factors were analyzed together on both behavioral and event-related potentials (ERP) data. Two groups of normal subjects participated in two successive sessions. In the first session, all participants were trained in the baseline relations among visual stimuli (pseudo-words). In the second session, one group performed the matching-to-sample (MTS) equivalence tests before the equivalence-relatedness-priming (EBRP) task, while the other group performed both tasks in reverse order. In the EBRP task related trial types included trained, symmetrical and equivalence relationships while the unrelated trial types included the same stimuli but without relationships. Event related potentials were recorded separately for related and unrelated conditions during the EBRP task. Results showed that response times to related trials were shorter than those to unrelated ones. At the electrophysiological level, two late waveforms were sensitive to the differences among the stimulus pairs of the EBRP task: Both waveforms were larger for the unrelated than the related conditions. Conversely, there were no main influences of the task order or of the trial types with each other. These results provide evidence that 1) the EBRP task exhibits priming effects among the SEC stimuli, 2) the behavioral and electrophysiological effects were similar regardless of whether the EBRP task was done before or after the MTS tests, and 3) there were no differences within the baseline and derived trial types in the EBRP task.
Behav Brain Res, 2018; 347

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BOARD NUMBER: S07-073

AUTOBIOGRAPHICAL MEMORY AND REMINISCENCE THERAPY IN HEALTHY OLDER ADULTS : AN FMRI STUDY

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Aims Research has pointed to the benefits of actively engaging one's memory through Reminiscence Therapy (RT). Older adults participated in a RT program over 3 weeks, with functional magnetic resonance imaging (fMRI) before (visit 1) and after (visit 2), to assess the cerebral impact of the intervention. Participants completed an autobiographical memory test (EAMI ; Irish et al., 2008). Methods Eleven older adults (mean age 74,27 +/- 5.14 years old, 4 males) retrieved autobiographical memories (AMs) from cues presented on a screen in the MRI. A flexible factorial design was used (SPM12) with the factors visits 1/2, recent/remote, rehearsed/unrehearsed. A statistical threshold of 0.001 uncorrected, extent threshold k>30 was used. Results The classical AM network (precuneus, medial frontal, lateral temporal gyri, hippocampus) was activated for visits 1 and 2 separately. Direct comparisons showed great activation for visit 2 in left superior medial frontal gyrus, precuneus and anterior hippocampus, compared to visit 1. Remote memories showed greater activation in left precuneus, right inferior frontal and temporal gyri, right posterior hippocampus, compared to recent memories. Unrehearsed memories exhibited greater activation in occipital areas, left middle temporal gyrus and left posterior hippocampus, compared to rehearsed memories. Conclusions There was greater brain activation at visit 2 suggesting a positive effect of RT. Hippocampal activation for remote memories is compatible with the Multiple Trace Theory (Nadel and Moscovitch, 1997). Finally, not rehearsing events engaged a greater occipital and lateral temporal network, suggesting that they were more visual and semantic, compared to repeated events.

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BOARD NUMBER: S07-074

SPATIAL MEMORY IN HEALTHY AGEING IS MODULATED BY UPPER-LOWER VISUAL FIELD ASYMMETRIES

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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AIMS. Visual information is not processed homogeneously across the visual field, as observed in a wide range of behaviours including visual search and object recognition. Despite numerous studies on left-right visual field asymmetry, there is a paucity of data investigating upper-lower visual field differences and their evolution across the lifespan. **METHODS**. To address this gap, twenty-four young (29 ± 4.2 y.o.) and twenty older adults (75 ± 3.7 y.o.) completed a source monitoring task consisting of objects presented in the upper or lower visual field while their gaze was recorded with an eye-tracker. Kinetic and static perimetry was performed on all participants. Analyses were conducted using generalised linear mixed models and multinomial processing tree models. The latter served to estimate the probabilities of recognising an object (item memory) and remembering the position in which it was encoded (spatial memory). **RESULTS**. Data revealed a significant impact of the object's vertical position on spatial memory in older participants. Indeed, the probability of remembering the position of an object that was encountered in the upper visual field was significantly lower in older adults. This age-related difference was not explained by ophthalmological factors (area and light sensitivity of the upper visual field). **CONCLUSIONS**. The vertical position of information influences spatial memory in ageing. The lack of association between participants' visual field characteristics and the observed asymmetry in spatial memory suggests a cognitive basis. This novel finding could yield important environmental design considerations for the older population such as the positioning of informational signage.

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BOARD NUMBER: S07-075

DISSOCIABLE ROLES OF SLEEP STAGES IN THE EMERGENCE AND CONSOLIDATION OF TRANSITIVE INFERENCE

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Inferential reasoning is a prominent property of higher-order cognition and relies on the systematic organisation of existing knowledge. It has been proposed that sleep facilitates inference, insight and innovative problem-solving. However, it remains unclear how and when the subconscious, but not conscious, brain can create novel ideas. Here, we show that cortical offline, but not online, activity is essential for inference evolution and that activity in rapid-eye movement (REM) sleep-specific brain circuitry is sufficient to inspire inference from inadequate knowledge. In a transitive inference paradigm, mice learned the relationship between five different contexts and could infer novel information that had never been experienced. Mice gained the inference one day, but not shortly, after the complete training. Inhibiting the neuronal computations in the anterior cingulate cortex (ACC) during post-learning sleep, but not during wakefulness, disrupted the inference without affecting the original memories. Furthermore, after insufficient learning, artificial activation of medial entorhinal cortex-ACC dialogue during only REM sleep created inferential knowledge. These findings establish causal evidence for the necessity and sufficiency of REM sleep in reorganising existing knowledge to achieve novel inference, thereby highlighting the power of the idling brain in creativity and cognitive flexibility.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-076

CALCIUM FLUCTUATIONS REPRESENTATIONS OF ENVIRONMENT, NAVIGATION AND EXPLORATION IN MICE PFC

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Understanding the brain's activity representation during unconstrained behavior comes with the problem of dealing with excessive amounts of degrees of freedom, and the uncertainty of which behavioral feature is being represented. An object in place exploratory task enabled us to reveal multiple levels of environmental representations with co-existing representations of context, position and task performance. The Object Space Task consists of a square arena with objects presented in two corners (similar objects). Different rules, in multiple sessions, provided different levels of variability in the object positions to track memory build up under different conditions. Recurrent behavioural patterns were extracted from mouse trajectories by machine learning techniques and calcium fluorescence activity from mPFC was recorded with a micro-endoscope during task performance. We were able to track individual cells activity during 100 minutes that included exploration, resting and off-recording periods. We found, first, that states space occupation for calcium transients depends on the entire environmental configuration. Clusters emerged corresponding to different combinations of objects positioned in the arena, showing a first level of context encoding. Second, as previously reported with electrophysiological recordings, we showed that animal location can be decoded from calcium traces. Third, the object identity and object locations have separable population calcium transients representations, and this separability is different when there is an object presented at the location. These results show that complex behavioral representations can be extracted from calcium traces in PFC, and that different hierarchies of encoding environment, navigation and exploration can lie within the same representations.

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BOARD NUMBER: S07-077

HOMECAGE TESTING DEVICE TO EVALUATE COGNITIVE LEARNING IN A GROUP OF RELATED COMMON MARMOSETS.

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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In the wild, common marmosets generally live in family groups with a breeding pair and their offspring, who will become helpers after their growth. However, this social structure cannot always be preserved in a laboratory environment. One objective of our project is therefore to study the relationship between the social organization of a group and individual performance in a learning task. In this study, the performance of 5 related female marmosets was evaluated. A freely accessible device mounted on the homecage of the animals allowed cognitive tests to be performed without separating individuals from their social group. Each animal was identified by a chip placed on a collar, the detection of which triggered the task with a personalized difficulty level. Three pairs of visual stimuli were presented on a touchscreen in a pseudo-random order. In each pair, the animals had to determine by trial-and-error the rewarded stimulus. Once acquired, the contingency was reversed and the time to acquire the new association was measured. The hierarchical organization of the group was determined based on observations of access to resources (food, resting area, testing device). Daily performance (correct trials, errors, omissions) was measured for each animal. Without food or water restriction, animals performed an average of 271 trials/day ([182-516], N=198 days). We found high heterogeneity in the performance on the task among individuals, demonstrating variability in cognitive flexibility. Ultimately, this approach will allow us to evaluate the influence of group composition on performance in different cognitive tasks.

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BOARD NUMBER: S07-078

CORTICAL-HIPPOCAMPAL INFORMATION PROCESSING DURING CLASSICAL CONDITIONING

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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A fundamental component for survivance is the ability to employ sensory cues to guide goal-directed behaviors. In this sense, the cortical-hippocampal dialogue plays an important role in the retention of sensorial information and translation of it to a long-term memory. Using auditory trace conditioning (ATC), we studied how the interaction between these two structures are modulated to integrate the auditory information. To answer this question, we combined wide-field voltage imaging from multiple areas in the neocortex with a high-density silicon probe in CA1 in mice. The ATC task was recorded every day for 14 days. In the first half of this period, we trained the animal to discriminate between 2 tones (A and B), with the reward being associated with A. In the other half, we rewired the information, associating the reward now to tone B. Surprisingly, we found a modulation of the Retrosplenial cortex activity in relation with the learning performance. In sync, gamma activity in CA1 also showed a significant increase associated with the performance. Taken together, our findings point out that the Retrosplenial-hippocampal interaction may be fundamental for learning in classical conditioning. We are now studying how networks spanning the entire neocortex participate in encoding the different components of this task.

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BOARD NUMBER: S07-079

THE GEOMETRY OF OBJECT REPRESENTATIONS IN CA1 ENSEMBLES DURING SPONTANEOUS EXPLORATION.

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Hippocampal neurons are well known to represent multiple features of the animals' position and direction in space. Real-word environments provide a mix of spatial and non-spatial information to the animals, and recent research focused on vector-coding properties of individual hippocampal (and entorhinal) neurons that integrate these two kind of information. However, it is not clear how these properties are distributed across hippocampal ensembles, and how natural exploratory behaviours organizes them. Here, we analyze CA1 ensemble activity in mice while they spontaneously explore arenas populated with objects in stable or changing configurations. Using neural decoding and population analysis techniques, we show that the representation of the different objects at the population level is only partially stable between different exploratory sessions, even when the object configuration is kept fixed. We observe a drop in decoding performance when the decoder is trained and tested in different sessions with the same object configuration. We show that, while the the mean population vector elicited by exploration of different objects changes from session to session, the representation of different objects stays distinct. Furthermore, we show that this instability is not temporally graded, is distributed across the whole population, and does not appear to be actively constrained to keep the representations of different objects orthogonal. Taken together, these results suggest that the informativeness of the neural responses for objects descends from their high-dimensional nature. This discriminability is robust to naturally occurring, population-wide perturbations - with interesting implications for many of the most popular models of hippocampal function.

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BOARD NUMBER: S07-080

A TEENSY-BASED SYNCHRONIZATION SYSTEM APPLIED TO A VIRTUAL REALITY SETUP

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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[1]Radboud University, Donders Institute, NIJMEGEN, Netherlands, [2]Radboud University, Donders Institute, Nijmegen, Netherlands

An experiment often requires precisely timed data acquisition and behavioral monitoring from multiple devices. A problem often encountered by researchers is how to acquire all the different triggers involved with complex behavioral experiments and data acquisition in a synchronized way. Correctly synchronizing all your data is vital for analysis. A setup is rarely all-in-one and ready to record due to the variety of experiments and variables of every research topic. With this problem in mind, we designed a low-cost, open-source teensy-based synchronization system to tackle these challenges. Here we present this synchronization system in combination with a head-fixed virtual reality setup. We were able to demonstrate the flexibility of the setup and synchronization by combining a virtual environment (Unity), running wheel, eye-tracking camera, lick detection, voltage imaging cameras, and open ephys. This system has also been used in combination with 2 photon and Neuropixel recordings. This system is composed of a teensy 4.1 coupled with a web-based python interface and creates a logfile saving 40 analog/digital channels at a rate of 1kHz. This allows for a user-friendly experience with full control of all hardware or triggers used in the setup. In conclusion, we offer here an interactive synchronization system, consisting of a Teensy 4.1, a custom python interface, and software functions that provide a temporally precise, low-cost, and flexible platform, which allows to integrate all the devices necessary for behavioral and neuroscience experiments.

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BOARD NUMBER: S07-081

TWO-PHOTON CALCIUM IMAGING CORRELATES OF VISUAL STIMULI IN THE MOUSE RETROSPLENIAL CORTEX DURING HEAD-FIXED VIRTUAL SOCIAL LEARNING

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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[1]Radboud University, Neuroinformatics, Nijmegen, Netherlands, [2]Radboud University, Donders Institute, Nijmegen, Netherlands, [3]Radboud university, Neuroinformatics, NIJMEGEN, Netherlands

Social animals including both humans and mice navigate through space and scavenge for resources such as food, shelter, or social in-group members. When doing so, the brain gathers sensory inputs and forms a spatial perception. It also links rewarding stimuli with emotions to form salient memories. Many brain regions coordinate for this learning - the thalamus, sensory cortices, striatum, VTA, hippocampus, anterior cingulate cortex, and retrosplenial cortex. Retrosplenial cortex is known to be essential for spatial cognition. However, it is still unclear what specific information retrosplenial cortex and other cortical regions receive, and how their microcircuits process information during social learning. This project aims to study how the neocortical region encodes spatial, social, and emotional information. To address this question, we observed how the retrosplenial neuronal calcium activity responds to spatial and social stimuli given in the form of visual stimuli within a virtual linear environment. We trained mice to run on a treadmill while head-fixed to travel through a virtual environment where different visual fearful and neutral stimuli were presented. Two different stimuli were repeatedly presented in a semi-random order. Calcium fluorescence was recorded using two-photon imaging and the activity of about a hundred cells was stably tracked during the complete experiment. Individual cell's z-scored traces showed consistent patterns across trials for the same stimuli. Our result supports the hypothesis that/suggesting that certain neuronal populations in the retrosplenial cortex respond to certain visual stimuli in a consistent manner.

Pubmed:
34852235: Won J, Pankratov Y, Jang MW, Kim S, Ju YH, Lee S, Lee SE, Kim A, Park S, Lee CJ, Heo WD
Opto-vTrap, an optogenetic trap for reversible inhibition of vesicular release, synaptic transmission, and behavior.
Spatiotemporal control of brain activity by optogenetics has emerged as an essential tool to study brain function. For silencing brain activity, optogenetic probes, such as halorhodopsin and archaerhodopsin, inhibit transmitter release indirectly by hyperpolarizing membrane potentials. However, these probes cause an undesirable ionic imbalance and rebound spikes. Moreover, they are not applicable to use in non-excitable glial cells. Here we engineered Opto-vTrap, a light-inducible and reversible inhibition system to temporarily trap the transmitter-containing vesicles from exocytotic release. Light activation of Opto-vTrap caused full vesicle clusterization and complete inhibition of exocytosis within 1 min, which recovered within 30 min after light off. We found a significant reduction in synaptic and gliotransmission upon activation of Opto-vTrap in acute brain slices. Opto-vTrap significantly inhibited hippocampus-dependent memory retrieval with full recovery within an hour. We propose Opto-vTrap as a next-generation optogenetic silencer to control brain activity and behavior with minimal confounding effects.
Neuron, 2022; 110
30264490: Kim A, Keum S, Shin HS
Observational fear behavior in rodents as a model for empathy.
Empathy enables social mammals to recognize and share emotion with others and is well-documented in non-human primates. During the past few years, systematic observations have showed that a primal form of empathy also exists in rodents, indicating that empathy has an evolutionary continuity. Now, using rodents exhibiting emotional empathy, the molecular and cellular study of empathy in animals has begun in earnest. In this article, we will review recent reports that indicate that rodents can share states of fear with others, and will try to highlight new understandings of the neural circuitry, biochemistry and genetics of empathic fear. We hope that the use of rodent models will enhance understanding of the mechanisms of human empathy and provide insights into how to treat social deficits in neuropsychiatric disorders characterized by empathy impairment.
Genes Brain Behav, 2019; 18
29681532: Keum S, Kim A, Shin JJ, Kim JH, Park J, Shin HS

A Missense Variant at the Nrxn3 Locus Enhances Empathy Fear in the Mouse.
Empathy is crucial for our emotional experience and social interactions, and its abnormalities manifest in various psychiatric disorders. Observational fear is a useful behavioral paradigm for assessing affective empathy in rodents. However, specific genes that regulate observational fear remain unknown. Here we showed that 129S1/SvImJ mice carrying a unique missense variant in neurexin 3 (Nrxn3) exhibited a profound and selective enhancement in observational fear. Using the CRISPR/Cas9 system, the arginine-to-tryptophan (R498W) change in Nrxn3 was confirmed to be the causative variant. Selective deletion of Nrxn3 in somatostatin-expressing (SST+) interneurons in the anterior cingulate cortex (ACC) markedly increased observational fear and impaired inhibitory synaptic transmission from SST+ neurons. Concordantly, optogenetic manipulation revealed that SST+ neurons in the ACC bidirectionally controlled the degree of socially transmitted fear. Together, these results provide insights into the genetic basis of behavioral variability and the neurophysiological mechanism controlling empathy in mammalian brains.
Neuron, 2018; 98
26690560: Keum S, Park J, Kim A, Park J, Kim KK, Jeong J, Shin HS
Variability in empathic fear response among 11 inbred strains of mice.
Empathy is an important emotional process that involves the ability to recognize and share emotions with others. We have previously developed an observational fear learning (OFL) behavioral assay to measure empathic fear in mice. In the OFL task, a mouse is conditioned for context-dependent fear when it observes a conspecific demonstrator receiving aversive stimuli. In the present study, by comparing 11 different inbred mouse strains that are commonly used in the laboratory, we found that empathic fear response was highly variable between different strains. Five strains--C57BL/6J, C57BL/6NTac, 129S1/SvImJ, 129S4/SvJae and BTBR T(+) Itpr3(tf) /J--showed observational fear (OF) responses, whereas AKR/J, BALB/cByJ, C3H/HeJ, DBA/2J, FVB/NJ and NOD/ShiLtJ mice exhibited low empathic fear response. Importantly, day 2 OF memory was significantly correlated with contextual memory in the classical fear conditioning among the 11 strains. Innate differences in anxiety, locomotor activity, sociability and preference for social novelty were not significantly correlated with OFL. Interestingly, early adolescent C57BL/6J mice exhibited an increase in acquisition of OF. The level of OFL in C57BL/6J strain was not affected by sex or strains of the demonstrator. Taken together, these data strongly suggest that there are naturally occurring OFL-specific genetic variations modulating empathic fear behaviors in mice. The identification of causal genes may uncover novel genetic pathways and underlying neural mechanisms that modulate empathic fear and, ultimately, provide new targets for therapeutic intervention in human mental disorders associated with impaired empathy.
Genes Brain Behav, 2016; 15

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BOARD NUMBER: S07-082

THE MECHANISMS OF MULTISENSORY INTEGRATION IN THE HIPPOCAMPAL NETWORK

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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The hippocampus is well established to a main site of spatial coding, but has also been shown to respond to other sensory modalities such as visual and auditory stimuli. We here aim to address the neural mechanisms of sensory integration at the level of the hippocampus and some of its main input and output areas, the perirhinal cortex (PER), lateral entorhinal cortex (LEC) and retrosplenial cortex (RSC). We have here developed a virtual reality task for mice that requires integration of spatial, auditory and visual information ("Multisensory Linear Track"). Briefly, a tone sequence at the beginning of the trial indicated which of the two object sequences positioned along the linear track was rewarded. During this task and sleep, neural activity was recorded with two Neuropixels probes, one targeting the hippocampus and RSC, and the other targeting the PER and LEC. Single unit responses from all four areas were analysed in terms of their selectivity to spatial, auditory and visual input. Units that responded to one or more inputs were further classified into either factorized or conjunctive coding cells, respectively. Between any two brain areas we then searched for functionally coupled cells, and determined whether the response properties of a neuron could be predicted by the responses of upstream neurons it is functionally coupled with. In further analysis we are exploring the mechanisms of population coding in the different areas and inter-area communication.

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BOARD NUMBER: S07-083

CORTICAL NETWORKS DIFFERENTIALLY APPROACH CRITICALITY DEPENDING ON FUNCTION AND STATE

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Criticality has been variously hypothesized to provide a fundamental principle for the organization of activity in the brain. By maximizing fluctuations across multiple scales, a network poised at a critical phase transition is thought to support processes which rely on long-range correlations. While extensive experimental and theoretical work speak for the presence of critical (or quasi-critical) dynamics in various portions of the cortex, different notions of criticality are often taken into account, in particular when considering either spatial or temporal scales. Such different definitions might indeed point to distinct phenomena with distinct functional implications. Here we leverage on high-resolution cortical voltage imaging to obtain a comprehensive description of the spatio-temporal organization of activity transients at different levels of resolution. We address this point with the use of various theoretical methods, derived from statistical mechanics and dynamical systems, and by establishing a comparison with branching processes of different types. This collection of analysis shows how multiple signatures of criticality vary along the lines of known cortical functional networks, notably of the Default Mode Network, and unveil the presence of coexisting dynamical states. Furthermore, we show how the degree of both spatial and temporal coherence across the entire cortex, and in particular around the retrosplenial cortex, is highly modulated between wakefulness, REM and NREM sleep, while also being linked with hippocampal activation patterns. Overall our results describe a rich repertoire of cortical dynamical configurations, heterogeneously expressed across networks and states, and point to an association between criticality and memory functions.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-084

BUMBLEBEES CREATE NOVEL ROUTES WITHOUT MENTAL ROTATION, USING VISUAL GUIDANCE

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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A cognitive map is thought to allow navigators to mentally represent relevant locations with respect to each other within an external frame of reference. This external frame of reference allows the navigator to mentally rotate space to locate themselves when lost, as well as create new routes. In animals, the creation of novel routes (such as shortcuts) using landscape cues is frequently seen as evidence for the presence of a cognitive map. Opposing this view, several models of animal navigation propose visual-guidance mechanisms such as image-matching which allow for very similar behaviour. We asked if bumblebees (*Bombus terrestris*) could navigate to a rewarding location when their prior experience was restricted to allow them only the use of relative landmark positions, but no path integration or route memories. To do this, we used a three-armed maze, and moved bumblebees between the three chambers using a dark pot. The bees that successfully used unique landmark combinations to reach the rewarding location developed a preference for the correct landmarks during training (prior to the choice test). These same successful individuals were however unable to reach the rewarding location when the task required mental rotation. This is the first experimental evidence that bees are unable to mentally rotate to form new routes. Indeed, the bee behaviour observed was most similar to that predicted through familiarity-based image-matching models, showing no differentiation between landmark orientations at all. These results emphasise the remarkable behavioural flexibility achieved by visual guidance mechanisms in small brains.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-085

A NOVEL TASK TO EXPLORE SENSORY-SPATIAL ASSOCIATION IN FREELY-MOVING MICE

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Behavioral neuroscience presents a growing demand for increasingly naturalistic experiments which better emulate real-world behavior and brain activity. Continual improvement of miniscope technology and behavioral analysis methods has helped to bring the field closer to replicating the natural world while collecting precise neural data. The study of navigation and spatial processing especially benefits from using subjects that are freely moving. I present a novel task designed for miniscope recording during complex sensory-spatial learning and recall in freely moving mice. Mice first navigate to an active cue port at the “North” or “South” end of the arena, where they receive one of two cues. The cue identifies a single rewarded location at either an “East” or “West” port. By requiring mice to navigate to and from different ports we are able to temporally and spatially isolate stimulus identification, navigational planning, and reward. Having two symmetric locations at which otherwise identical cues can be presented allows flexibility to compare allocentric or egocentric spatial rules. The arena is also designed to use different cue modalities: odor, whisker touch, or their combination. Preliminary results show that mice can learn to perform the task when discriminating between aperture widths with their whiskers. Testing with olfactory stimuli and comparison of learning across sensory modalities is ongoing, as is incorporation of miniscope fluorescent imaging of the lateral entorhinal cortex. Together, this work provides a novel sensory-spatial task designed to leverage the benefits of freely-moving behavior while maintaining temporal precision of imaging data and task epochs.

Pubmed:
34048600: Pond HL, Heller AT, Gural BM, McKissick OP, Wilkinson MK, Manzini MC
Digging behavior discrimination test to probe burrowing and exploratory digging in male and female mice.
Digging behavior is often used to test motor function and repetitive behaviors in mice. Different digging paradigms have been developed for behaviors related to anxiety and compulsion in mouse lines generated to recapitulate genetic mutations leading to psychiatric and neurological disorders. However, the interpretation of these tests has been confounded by the difficulty of determining the motivation behind digging in mice. Digging is a naturalistic mouse behavior that can be focused toward different goals, that is foraging for food, burrowing for shelter, burying objects, or even for recreation as has been shown for dogs, ferrets, and human children. However, the interpretation of results from current testing protocols assumes the motivation behind the behavior often concluding that increased digging is a repetitive or compulsive behavior. We asked whether providing a choice between different types of digging activities would increase sensitivity to assess digging motivation. Here, we present a test to distinguish between burrowing and exploratory digging in mice. We found that mice prefer burrowing when the option is available. When food restriction was used to promote a switch from burrowing to exploration, males readily switched from burrowing to digging outside, while females did not. In addition, when we tested a model of intellectual disability and autism spectrum disorder that had shown inconsistent results in the marble burying test, the Cc2d1a conditional knockout mouse, we found greatly reduced burrowing only in males. Our findings indicate that digging is a nuanced motivated behavior and suggest that male and female rodents may perform it differently.
J Neurosci Res, 2021; 99
33558692: Hana S, Peterson M, McLaughlin H, Marshall E, Fabian AJ, McKissick O, Koszka K, Marsh G, Craft M, Xu S, Sorets A, Torregrosa T, Sun C, Henderson CE, Lo SC
Highly efficient neuronal gene knockout in vivo by CRISPR-Cas9 via neonatal intracerebroventricular injection of AAV in mice.
CRISPR-Cas systems have emerged as a powerful tool to generate genetic models for studying normal and diseased central nervous system (CNS). Targeted gene disruption at specific loci has been demonstrated successfully in non-dividing neurons. Despite its simplicity, high specificity and low cost, the efficiency of CRISPR-mediated knockout in vivo can be substantially impacted by many parameters. Here, we used CRISPR-Cas9 to disrupt the neuronal-specific gene, NeuN, and optimized key parameters to achieve effective gene knockout broadly in the CNS in postnatal mice. Three cell lines and two primary neuron cultures were used to validate the disruption of NeuN by single-guide RNAs (sgRNA) harboring distinct

spacers and scaffold sequences. This triage identified an optimal sgRNA design with the highest NeuN disruption in in vitro and in vivo systems. To enhance CRISPR efficiency, AAV-PHP.B, a vector with superior neuronal transduction, was used to deliver this sgRNA in Cas9 mice via neonatal intracerebroventricular (ICV) injection. This approach resulted in 99.4% biallelic indels rate in the transduced cells, leading to greater than 70% reduction of total NeuN proteins in the cortex, hippocampus and spinal cord. This work contributes to the optimization of CRISPR-mediated knockout and will be beneficial for fundamental and preclinical research.
Gene Ther, 2021; 28
32946184: Cincotta C, Murawski NJ, Grella SL, McKissick O, Doucette E, Ramirez S
Chronic activation of fear engrams induces extinction-like behavior in ethanol-exposed mice.
Alcohol withdrawal directly impacts the brain's stress and memory systems, which may underlie individual susceptibility to persistent drug and alcohol-seeking behaviors. Numerous studies demonstrate that forced alcohol abstinence, which may lead to withdrawal, can impair fear-related memory processes in rodents such as extinction learning; however, the underlying neural circuits mediating these impairments remain elusive. Here, we tested an optogenetic strategy aimed at mitigating fear extinction retrieval impairments in male c57BL/6 mice following exposure to alcohol (i.e., ethanol) and forced abstinence. In the first experiment, extensive behavioral extinction training in a fear-conditioned context was impaired in ethanol-exposed mice compared to controls. In the second experiment, neuronal ensembles processing a contextual fear memory in the dorsal hippocampus were tagged and optogenetically reactivated repeatedly in a distinct context in ethanol-exposed and control mice. Chronic activation of these cells resulted in a context-specific, extinction-like reduction in fear responses in both control and ethanol-exposed mice. These findings suggest that while ethanol can impair the retrieval an extinction memory, optogenetic manipulation of a fear engram is sufficient to induce an extinction-like reduction in fear responses.
Hippocampus, 2021; 31
32179657: Grella SL, Fortin AH, McKissick O, Leblanc H, Ramirez S
Odor modulates the temporal dynamics of fear memory consolidation.
Systems consolidation (SC) theory proposes that recent, contextually rich memories are stored in the hippocampus (HPC). As these memories become remote, they are believed to rely more heavily on cortical structures within the prefrontal cortex (PFC), where they lose much of their contextual detail and become schematized. Odor is a particularly evocative cue for intense remote memory recall and despite these memories being remote, they are highly contextual. In instances such as posttraumatic stress disorder (PTSD), intense remote memory recall can occur years after trauma, which seemingly contradicts SC. We hypothesized that odor may shift the organization of salient or fearful memories such that when paired with an odor at the time of encoding, they are delayed in the de-contextualization process that occurs across time, and retrieval may still rely on the HPC, where memories are imbued with contextually rich information, even at remote time points. We investigated this by tagging odor- and non-odor-associated fear memories in male c57BL/6 mice and assessed recall and expression in the dorsal CA1 (dCA1) and prelimbic cortex (PL) 1 or 21 d later. In support of SC, our data showed that recent memories were more dCA1-dependent whereas remote memories were more PL-dependent. However, we also found that odor influenced this temporal dynamic, biasing the memory system from the PL to the dCA1 when odor cues were present. Behaviorally, inhibiting the dCA1 with activity-dependent DREADDs had no effect on recall at 1 d and unexpectedly caused an increase in freezing at 21 d. Together, these findings demonstrate that odor can shift the organization of fear memories at the systems level.
Learn Mem, 2020; 27
31130452: Chen BK, Murawski NJ, Cincotta C, McKissick O, Finkelstein A, Hamidi AB, Merfeld E, Doucette E, Grella SL, Shpokayte M, Zaki Y, Fortin A, Ramirez S
Artificially Enhancing and Suppressing Hippocampus-Mediated Memories.
Emerging evidence indicates that distinct hippocampal domains differentially drive cognition and emotion [1, 2]; dorsal regions encode spatial, temporal, and contextual information [3-5], whereas ventral regions regulate stress responses [6], anxiety-related behaviors [7, 8], and emotional states [8-10]. Although previous studies demonstrate that optically manipulating cells in the dorsal hippocampus can drive the behavioral expression of positive and negative memories, it is unknown whether changes in cellular activity in the ventral hippocampus can drive such behaviors [11-14]. Investigating the extent to which distinct hippocampal memories across the longitudinal axis modulate behavior could aid in the understanding of stress-related psychiatric disorders known to affect emotion, memory, and cognition [15]. Here, we asked whether tagging and stimulating cells along the dorsoventral axis of the hippocampus could acutely, chronically, and differentially promote context-specific behaviors. Acute reactivation of both dorsal and ventral hippocampus cells that were previously active during memory formation drove freezing behavior, place avoidance, and place preference. Moreover, chronic stimulation of dorsal or ventral hippocampal fear memories produced a context-specific reduction or enhancement of fear responses, respectively, thus demonstrating bi-directional and context-specific modulation of memories along the longitudinal axis of the hippocampus. Fear memory suppression was associated with a reduction in hippocampal cells active during retrieval, while

fear memory enhancement was associated with an increase in basolateral amygdala activity. Together, our data demonstrate that discrete sets of cells throughout the hippocampus provide key nodes sufficient to bi-directionally reprogram both the neural and behavioral expression of memory.
Curr Biol, 2019; 29

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BOARD NUMBER: S07-086

ABSTRACT SYMBOLIC REPRESENTATION OF SPATIAL LOCATION AND ORDINAL POSITION IN JACKDAWS

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Remembering a sequence of unique events is a crucial element of cognition. Corvid birds are an excellent animal model for comparative study of such fundamental elements. They show a remarkable range of advanced cognitive abilities that are on par with primates, and their naturally occurring ability to forage and cache food makes them an ideal animal model to study spatial – sequential cognition. For example, some food caching corvid species exhibit episodic-like memory, remembering the location ('where'), time ('when'), and specific type of food ('what') they cached. We probed the ability of two jackdaws to remember the order of visits to three distinct spatial locations. Location cues indicated distinct movement sequences the birds had to cover within a large hexagon-shaped arena. Each cue location was uniquely associated with a specific symbol. The birds had to report their traveled path at a fourth location by selecting the correct symbols in the same order in which they previously visited the locations. Both jackdaws were able to reliably report which sequence they performed. Wireless electrophysiological recordings in the nidopallium caudolaterale (the avian equivalent of mammalian prefrontal cortex) will allow us to examine the neural basis of sequence encoding in an avian brain and allow us to dissociate activity related to specific cue locations, ordinal positions within a sequence, and integrated sequence information. By comparing our results to findings in primates and rodents we will be able to identify general computational principles of sequence representation that are independent of specific brain structures.

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BOARD NUMBER: S07-087

INVOLVEMENT OF THE RETROSPLENIAL CORTEX IN SPATIAL MEMORY ACQUISITION AND NAVIGATION

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Navigation based on formation, storage and retrieval of spatial information is one of fundamental cognitive abilities. We propose a novel version of an automated T-maze task for mice requiring processing of visual information about the external environment. In our design mice are presented with extra maze cues - an LED panel display - through the transparent front wall of the maze. Mice are rewarded with sweet condensed milk administered through custom dispensers constructed out of solenoid valves. The pneumatic actuators moving the doors, the dispensers and the LED panels are all remotely controlled using camera input by software written in the Bonsai environment. We have tested various training protocols - first, utilizing spontaneous alternations in mice, then challenging them to act against spontaneous tendency to alternate. In the last approach the animals were presented with pseudo random sequences of turns, minimizing the risk of developing strategies independent of visual cues. We show that in each of those protocols mice are able to learn to use allothetic cues - both general contexts and salient landmarks, indicating that our modified maze is a useful model for studying navigation and spatial memory. This method reveals the advantage of excluding the influence of human factor and allows for conducting high-throughput experiments. Using this system together with two-photon in vivo IEG imaging I investigated the engagement of the retrosplenial cortex during spatial memory acquisition. My main goal is to find out whether IEG imaging can reveal the existence of engram-like neuronal populations in the RSC.

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BOARD NUMBER: S07-088

PROBING NEURAL REPRESENTATIONS OF SCENE PERCEPTION IN A HIPPOCAMPALLY DEPENDENT TASK USING ARTIFICIAL NEURAL NETWORKS

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Deep artificial neural networks (DNNs) trained through backpropagation provide effective models of the mammalian visual system, accurately capturing the hierarchy of neural responses through primary visual cortex to inferior temporal cortex (IT). However, the ability of these networks to explain representations in higher cortical areas is relatively lacking and considerably less well researched. For example, DNNs have been less successful as a model of the egocentric to allocentric transformation embodied by circuits in retrosplenial and posterior parietal cortex. These brain regions play a central role in spatial memory and perception being part of the 'pipeline' that transforms perceptual information to the spatial representations found in entorhinal cortex and hippocampus. In humans, the Four Mountains task provides a test of egocentric to allocentric topographical processing. We describe a novel virtual environment, inspired by the Four Mountains task, designed to probe the ability of DNNs to transform scenes viewed from different egocentric perspectives. The 'participant' is required to match scenes that correspond to the same configuration of distinct objects viewed from a different perspective, lures consist of the same or similar objects arranged in other configurations. Using a network architecture inspired by the connectivity between temporal lobe structures and the hippocampus, we demonstrate that DNNs trained using a triplet loss can learn this task. Analysis of the representations learnt in these artificial networks revealed late layers accurately encode the allocentric Euclidean distance between objects in the scene, the clearest representation of this quantity being in layers analogous to CA1.

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CHARACTERIZATION OF MEMORY RECALL-ASSOCIATED TRANSCRIPTIONAL PROGRAMS IN WILDTYPE AND ANGELMAN SYNDROME MODEL MICE

POSTER SESSION 07 - SECTION: SPACE AND MEMORY

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Western University of Health Sciences, Basic Medical Sciences, Comp, Pomona, United States of America

Angelman syndrome (AS) is a rare neurodevelopmental disorder with severe developmental delay, lack of language skills, severe cognitive impairment, motor dysfunction and sleep disorder. Genetic/genomic studies have revealed that *UBE3A* deficiency in neurons is the cause of AS; AS mouse models recapitulate most AS symptoms, including deficits in motor function and learning and memory, anxiety disorders, and altered social interactions. Contextual memory has been widely used to evaluate hippocampus-dependent learning and memory. Several research groups have reported that AS mice show impaired contextual memory recall, although the underlying mechanism remains unsettled. In the present study, we performed RNA-seq on hippocampal samples from both wildtype (WT) and AS mice in control conditions and after fear context recall. Principal component analysis showed that memory recall and genotype were the major drivers of sample variability. There were 281 recall-associated DEGs in WT mice and 268 DEGs in AS mice, when compared to their respective non-recall controls. Of these DEGs, 129 genes were shared between WT and AS mice. KEGG Pathway analyses showed that among the top 10 enriched pathways for DEGs, 5 overlapped between WT and AS mice, while the other 5 were different. Interestingly, the PI3K-AKT pathway was shared by the two genotypes, but the MAPK signaling pathway was only present in AS mice, although both pathways play important roles in synaptic plasticity and memory functions. These results suggest that contextual memory recall in AS mice recruits different transcriptional programs than in WT mice, which could be responsible for recall failure.

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STX64, A STEREOID SULFATASE INHIBITOR, IMPROVES COGNITIVE DEFICIENCIES ASSOCIATED TO AGING

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aging produces in brain a progressive oxidative process and neuroinflammation that deteriorates cognition. These brain dysfunctions provoke high economic and social costs to developed societies. For this reason, the search for treatments to prevent cognitive deficits associated with aging is a priority. Previous results suggest that STX64, a steroid sulfatase (STS) inhibitor, improves cognition in neurodegenerative diseases. Here we have designed cellular (immunohistochemistry), molecular (qPCR) and behaviour (mainly, object recognition and passive avoidance for the study of learning and memory) assays to know if subchronic oral treatment with STX64 rescues cognitive dysfunction present in aging mice. Our behavioural results show that STX64 treatment improved cognition in aging mice, reflected as an improvement in object recognition and in passive avoidance tests. At cellular level, aging mice treated with STX64 showed an increase in microglial response and an increase in hippocampal adult neurogenesis. By last, preliminary qPCR assays indicated that hippocampus from aging mice treated with STX64 express lower levels of inflammatory factors mRNA compared with untreated aging mice. Then, our results indicates that STX64 may be a drug with potential therapeutic interest for the treatment of cognitive deficits associated to aging.

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BOARD NUMBER: S07-091

MATERNAL SARS COV-2 INFECTION DURING PREGNANCY AND INFANT NEUROBEHAVIOR AT 6-11 MONTHS

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims: We previously reported no association between prenatal maternal SARS-CoV-2 infection and parent-reported neurodevelopment at 6-months of age. To expand this research, we conducted objective, standardized assessment of neurobehavior to examine the association between prenatal SARS-CoV-2 exposure and neurodevelopment. **Methods:** 137 infants (59 SARS-CoV-2 exposed, 74 unexposed) born at Columbia University affiliated hospitals enrolled in the COVID-19 Mother Baby Outcomes (COMBO) Study and participated in a remote standardized neurodevelopmental assessment between 6-11 months. We administered via Zoom an adapted version of the Developmental Assessment of Young Children, 2nd Edition (DAYC-2). The administration used direct observation, interviewing the caregiver, and parental prompting guided by the examiner. Standard scores on each of the five domains (Cognitive, Gross and Fine Motor, Receptive and Expressive Language) that adjust for age at assessment have a normed mean of 100. **Results:** Analyses of covariance (ANOVAs) revealed no significant group differences on the DAYC-2 domains (*p*'s >0.05) between infants with and without *in-utero* SARS-CoV-2 exposure. After collapsing across the groups, the mean standard scores on the receptive language, expressive language, fine motor, and gross motor domains were 98.0, 97.6, 97.7, and 100.0, respectively. The mean standard score on the cognitive domain was 112.4 **Conclusions:** Consistent with our previous finding showing no association between parent-reported neurodevelopment at 6 months and *in-utero* SARS-CoV-2 exposure, this analysis revealed no significant association between infection exposure and neurodevelopment using DAYC-2 assessment at 6-11 months. Future analysis will include the effects of timing and severity of infection on this objective measure of neurodevelopment.

Pubmed:
35131524: Firestein MR, Romeo RD, Winstead H, Goldman DA, Grobman WA, Haas D, Mercer B, Parker C, Parry S, Reddy U, Silver R, Simhan H, Wapner RJ, Champagne FA
Elevated prenatal maternal sex hormones, but not placental aromatase, are associated with child neurodevelopment. Fetal exposure to testosterone may contribute to vulnerability for autism spectrum disorder (ASD). It is hypothesized that placental aromatase prevents fetal exposure to maternal testosterone, however, this pathway and the implications for child neurodevelopment have not been fully explored. We examined the relationships between prenatal maternal testosterone and estradiol at 19.2 ± 1.3 weeks, cord blood testosterone and estradiol at birth, placental aromatase mRNA expression, and neurodevelopment using the Social Communication Questionnaire (SCQ), the Behavioral Assessment System for Children, 3rd Edition (BASC-3), and the Empathizing Quotient for Children (EQ-C) at 4.5-6.5 years of age in a sample of 270 Nulliparous-Mothers-to-be (nuMoM2b) study participants. Maternal testosterone levels were positively associated with SCQ scores, but the association was not significant after adjusting for maternal age at delivery, nor was there a significant interaction with sex. Maternal estradiol levels were negatively associated with BASC-3 Clinical Probability scores among males (n = 139). We report a significant interaction effect of cord blood testosterone and fetal sex on both total SCQ scores and t-scores on the Developmental Social Disorders subscale. Placental aromatase was not associated with any neurodevelopmental or hormone measure, but under conditions of low placental aromatase expression, high maternal testosterone was positively associated with SCQ scores in males (n = 46). No other associations between hormone levels

and neurodevelopment were significant. Our findings provide a foundation for further investigation of the mechanisms through which maternal sex hormones and placental steroidogenesis may affect fetal hormone production and neurobehavior.
Horm Behav, 2022; 140
35075202: Werchan DM, Hendrix CL, Ablow JC, Amstadter AB, Austin AC, Babineau V, Anne Bogat G, Cioffredi LA, Conradt E, Crowell SE, Dumitriu D, Fifer W, Firestein MR, Gao W, Gotlib IH, Graham AM, Gregory KD, Gustafsson HC, Havens KL, Howell BR, Humphreys KL, King LS, Kinser PA, Krans EE, Lenniger C, Levendosky AA, Lonstein JS, Marcus R, Monk C, Moyer S, Muzik M, Nuttall AK, Potter AS, Salisbury A, Shuffrey LC, Smith BA, Smith L, Sullivan EL, Zhou J, Thomason ME, Brito NH
Behavioral coping phenotypes and associated psychosocial outcomes of pregnant and postpartum women during the COVID-19 pandemic.
The impact of COVID-19-related stress on perinatal women is of heightened public health concern given the established intergenerational impact of maternal stress-exposure on infants and fetuses. There is urgent need to characterize the coping styles associated with adverse psychosocial outcomes in perinatal women during the COVID-19 pandemic to help mitigate the potential for lasting sequelae on both mothers and infants. This study uses a data-driven approach to identify the patterns of behavioral coping strategies that associate with maternal psychosocial distress during the COVID-19 pandemic in a large multicenter sample of pregnant women (N = 2876) and postpartum women (N = 1536). Data was collected from 9 states across the United States from March to October 2020. Women reported behaviors they were engaging in to manage pandemic-related stress, symptoms of depression, anxiety and global psychological distress, as well as changes in energy levels, sleep quality and stress levels. Using latent profile analysis, we identified four behavioral phenotypes of coping strategies. Critically, phenotypes with high levels of passive coping strategies (increased screen time, social media, and intake of comfort foods) were associated with elevated symptoms of depression, anxiety, and global psychological distress, as well as worsening stress and energy levels, relative to other coping phenotypes. In contrast, phenotypes with high levels of active coping strategies (social support, and self-care) were associated with greater resiliency relative to other phenotypes. The identification of these widespread coping phenotypes reveals novel behavioral patterns associated with risk and resiliency to pandemic-related stress in perinatal women. These findings may contribute to early identification of women at risk for poor long-term outcomes and indicate malleable targets for interventions aimed at mitigating lasting sequelae on women and children during the COVID-19 pandemic.
Sci Rep, 2022; 12
34982107: Shuffrey LC, Firestein MR, Kyle MH, Fields A, Alcántara C, Amso D, Austin J, Bain JM, Barbosa J, Bence M, Bianco C, Fernández CR, Goldman S, Gyamfi-Bannerman C, Hott V, Hu Y, Hussain M, Factor-Litvak P, Lucchini M, Mandel A, Marsh R, McBrian D, Mourad M, Muhle R, Noble KG, Penn AA, Rodriguez C, Sania A, Silver WG, O'Reilly KC, Stockwell M, Tottenham N, Welch MG, Zork N, Fifer WP, Monk C, Dumitriu D
Association of Birth During the COVID-19 Pandemic With Neurodevelopmental Status at 6 Months in Infants With and Without In Utero Exposure to Maternal SARS-CoV-2 Infection.
Associations between in utero exposure to maternal SARS-CoV-2 infection and neurodevelopment are speculated, but currently unknown.
JAMA Pediatr, 2022; 176
34991997: Lucchini M, Kyle MH, Sania A, Pini N, Babineau V, Firestein MR, Fernández CR, Shuffrey LC, Barbosa JR, Rodriguez C, Fifer WP, Alcántara C, Monk C, Dumitriu D
Postpartum sleep health in a multiethnic cohort of women during the COVID-19 pandemic in New York City.
Cross-sectional study to examine the determinants of sleep health among postpartum women during the COVID-19 pandemic in New York City (NYC).
Sleep Health, 2022; 8
34257394: Mourad M, Jacob T, Sadovsky E, Bejerano S, Simone GS, Bagalkot TR, Zucker J, Yin MT, Chang JY, Liu L, Debelenko L, Shawber CJ, Firestein M, Ouyang Y, Gyamfi-Bannerman C, Penn A, Sorkin A, Wapner R, Sadovsky Y
Placental response to maternal SARS-CoV-2 infection.
The coronavirus disease 2019 (COVID-19) pandemic affected people at all ages. Whereas pregnant women seemed to have a worse course of disease than age-matched non-pregnant women, the risk of feto-placental infection is low. Using a cohort of 66 COVID-19-positive women in late pregnancy, we correlated clinical parameters with disease severity, placental histopathology, and the expression of viral entry and Interferon-induced transmembrane (IFITM) antiviral transcripts. All newborns were negative for SARS-CoV-2. None of the demographic parameters or placental histopathological characteristics were associated with disease severity. The fetal-maternal transfer ratio for IgG against the N or S viral proteins was commonly less than one, as recently reported. We found that the expression level of placental ACE2, but not TMPRSS2 or Furin, was higher in women with severe COVID-19. Placental expression of IFITM1 and IFITM3, which have been implicated in antiviral response, was higher in participants with severe disease. We also showed that IFITM3 protein expression, which

localized to early and late endosomes, was enhanced in severe COVID-19. Our data suggest an association between disease severity and placental SARS-CoV-2 processing and antiviral pathways, implying a role for these proteins in placental response to SARS-CoV-2.
Sci Rep, 2021; 11
33152642: Kliman HJ, Firestein MR, Hofmann KM, Milano KM, Holzer PH, Brink LT, Odendaal HJ, Fifer WP
Trophoblast inclusions in the human placenta: Identification, characterization, quantification, and interrelations of subtypes.
We sought to examine placentas enriched for trophoblast inclusions (TIs) in order to characterize, quantify, and examine the interrelations between subtypes of TIs to better understand their underlying biology. We examined a cohort of 600 placentas from deliveries between 20 and 43 weeks of gestation. Forty-five percent of the placentas had at least one TI in the two slides examined. Four percent of the placentas had 10 or more TIs and two placentas had more than 70 TIs. Four distinct TI subtypes were observed: inclusionoids (early forming inclusions), inclusions, calcified inclusions, and calcified bodies. We suggest this reflects a developmental trajectory of TI maturation, the timing of which might be useful when comparing TI expression to clinical outcomes.
Placenta, 2021; 103
31115036: Firestein M, Callaghan B
The brain-gut connection: environmental influences on gastrointestinal biology and neurobehavior across development.
Dev Psychobiol, 2019; 61
30671945: Firestein MR, Myers MM, Austin J, Stark RI, Barone JL, Ludwig RJ, Welch MG
Perinatal antibiotics alter preterm infant EEG and neurobehavior in the Family Nurture Intervention trial.
Early exposure to antibiotics has been shown to increase risk for poor neurobehavioral development, particularly with regard to attention deficit disorders. Clinically, electroencephalography (EEG) is increasingly used as a biomarker of these deficits. Less is known about the effects of antibiotics on neurobehavioral and neurophysiological outcomes in preterm infants, a population at particularly high risk for attention deficits and perinatal antibiotic exposure. This study examines the effects of perinatal antibiotic exposure on neonatal EEG and attention deficits as measured by the Child Behavior Checklist in 4- to 5-year-old children who were enrolled in an NICU-based randomized controlled trial comparing Family Nurture Intervention (FNI) to standard care. Antibiotic-exposed infants had increased attention problems and there was a main effect of antibiotic exposure such that exposed infants had higher EEG power. This effect was fourfold greater in infants who received standard NICU care compared to those who received the intervention, suggesting a buffering effect of the intervention. We hypothesize that the relationship between antibiotic exposure and altered neurodevelopment may be due to effects of antibiotics on the microbiome, and that FNI may buffer these adverse consequences.
Dev Psychobiol, 2019; 61
29208241: Firestein MR, Abellar R, Myers MM, Welch MG
Increased trophoblast inclusions in placentas from prematurely born infants: A potential marker of risk for preterm neurodevelopmental outcomes.
Trophoblast inclusions (TIs) are placental abnormalities of the trophoblast bilayer. Present in 2-8% of full-term placentas, they are associated with poor neurodevelopment, including autism. Although previously unstudied, examination of chorionic villi from 108 preterm births revealed a ~4 fold increase in the frequency of TIs (30.5%). Frequency of TIs was inversely related to gestational age (GA); 43% of placentas <30 weeks and 20% of placentas ≥32 weeks had TIs ($\chi 2 = 4.41$, $p = 0.036$). This increased prevalence in preterm infants suggests that TIs may indicate adverse intrauterine processes or undetected genetic abnormalities and could identify infants at risk for poor neurodevelopment.
Placenta, 2017; 60
25763525: Welch MG, Firestein MR, Austin J, Hane AA, Stark RI, Hofer MA, Garland M, Glickstein SB, Brunelli SA, Ludwig RJ, Myers MM
Family Nurture Intervention in the Neonatal Intensive Care Unit improves social-relatedness, attention, and neurodevelopment of preterm infants at 18 months in a randomized controlled trial.
Preterm infants are at high risk for adverse neurodevelopmental and behavioral outcomes. Family Nurture Intervention (FNI) in the Neonatal Intensive Care Unit (NICU) is designed to counteract adverse effects of separation of mothers and their preterm infants. Here, we evaluate effects of FNI on neurobehavioral outcomes.
J Child Psychol Psychiatry, 2015; 56

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BOARD NUMBER: S07-092

EFFECTS OF SUSTAINED COGNITIVE ACTIVITY ON EXECUTIVE FUNCTIONS IN HEALTHY AGED ADULTS : TOWARD A GAIN OF 10 YEARS OF COGNITIVE EFFICIENCY

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims. Neural plasticity associated with environmental stimulations may counter age-related declines in brain functions. This research examines whether continued cognitive activation is a critical factor in maintaining executive abilities and whether educational level and aging may interact. **Methods.** Executive functions were explored in 178 healthy participants aged 69-88 years, 73 voluntary applicants in a multi-domain cognitive program for 15 years and 105 controls. They were divided in 8 groups defined by their age and their educational level. Inhibition and cognitive flexibility were assessed using the Stroop test, the modified card sorting test, the trail making test and an alternated verbal fluency test. **Results.** Variance analyses in control groups revealed significant main effects of aging and educational level in inhibition and cognitive flexibility tasks. The overall effects remained significant after the inclusion of the stimulated groups into the analyses, but the continuous stimulation resulted in a significant beneficial effect in all executive tasks. Surprisingly, this outcome mostly occurred at similar rates regardless of age and educational attainment (no interaction). Moreover, stimulated group's executive performance matched those of 10 years younger control counterparts. **Conclusions**. These findings suggest that cognitive exercise leads to consistent and significant gains or delays the decline of executive functions. The facilitation in processing efficiency appears transferable since the tasks in the cognitive program do not bear similarities with those in the study. Further studies will be required to determine whether maintenance of cognitive activation provides greater compensatory resources and/or contributes to the maintenance of cognitive and cerebral integrity.

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PLASMA P-TAU181, Aβ42/40, NFL, GFAP, AND COGNITIVE CHANGE FROM AGE 73 TO 82: LOTHIAN BIRTH COHORT 1936

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

Tyler Saunders[1,2], Amanda Heslegrave[3,4], Declan King[1], Sarah Harris[5], Craig Ritchie[2], Graciela Muniz-Terrera[2], Ian Deary[5], Simon Cox[5], Henrik Zetterberg[3,4,6,7,8], Tara Spires-Jones[1]
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Background: Growing research has focused on plasma phospho-tau 181 (p-tau181), amyloid-beta (Aβ42/40), neurofilament-light (NfL), and glial fibrillary acidic protein (GFAP) as blood biomarkers for Alzheimer's disease. However, their predictive validity for age-related cognitive decline without dementia remains unclear. Further, it is unknown whether p-tau181 is present in brain tissue and synapses of aged controls without dementia. Aims: Here, we tested whether plasma p-tau181, Aβ42/40, NfL, and GFAP predict longitudinal cognitive decline in cognitively unimpaired late-adulthood participants. In a separate neuropathological study, we examined whether there were differences in levels of p-tau181 in total homogenate and synaptoneurosome preparations between aged controls, Alzheimer's cases, and mid-life controls. Methods: General cognitive ability and plasma p-tau181, Aβ40, Aβ42, NfL, and GFAP concentration were measured in 195 participants at ages 72 and 82. Levels of p-tau181 in total homogenate and synaptic fractions were compared with western blot (n = 10-12 per group). Results: Elevated baseline plasma p-tau181, NfL, and GFAP predicted steeper general cognitive decline. Further, increasing p-tau181 over time predicted steeper general cognitive decline. We found no significant differences in p-tau181 in total homogenate or synaptoneurosome preparations between aged controls, Alzheimer's disease cases, and young controls. Conclusions: Baseline plasma p-tau181, NfL, and GFAP, as well as subsequent change in plasma p-tau181 may represent rare biomarkers of differences in cognitive ageing across the 8th decade of life and may play a role in synaptic function in the brain.

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LONGITUDINAL COGNITIVE DECLINE AND BLOOD-BRAIN BARRIER PERMEABILITY IN A POPULATION COHORT

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims: Aging is associated with a decline in several cognitive domains. Furthermore, there is a significant interest in finding biomarkers to predict healthy aging from pathological decline. We have examined the trajectory of cognitive aging by studying a population cohort in a longitudinal design spanning ten years and investigated the potential of blood-brain barrier permeability (BBB) as a biomarker for cognitive decline. Methods: 207 participants from the Metropolit Birth Cohort completed a neuropsychological test battery assessing episodic memory, working memory, processing speed, attention, and verbal functions at three different time points. BBB was assessed by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) by injection of a gadolinium-based contrast agent. Permeability was calculated using tracer kinetic modeling ($p>0.339$). Results: Participants had a time-dependent decrease in episodic memory, processing speed, attention, and working memory (Table 1). Verbal functions were somewhat stable, with a slight increase with time. The number of school years was a positive mediator for processing speed, working memory, and verbal functions. Conversely, increased episodic memory score was associated with fewer school years. There were no significant associations between BBB permeability in the hippocampus or white matter and cognition. Conclusion: Longitudinal studies assessing the same individuals across time are essential to understand cognitive changes occurring in healthy aging. The present results find support for a time-dependent decline in several cognitive modalities. School years seem to be a positive mediator in maintaining processing speed, working memory, and verbal

functions.

Table 1
Summary of participant characteristics and results.

	T1	T2	T3	*p*-value T1-T3[1] (+schoolyears)[2]	*p*-value T2-T3[1] (+schoolyears)[2]
N	207	136	114		
Age [years]	57	63	68		
Education [school years], mean (SD)	–	10.83 (1.92)	10.65 (1.66)		
Word Pairs [# of corrects], mean, (SD)	11.5 (8.50)	12.47 (9.27)	10.22 (7.89)	0.049 (0.082)	<0.001 (<0.001)
Retention [# of corrects], Mean (SD)	4.79 (3.58)	5.03 (3.64)	3.87 (2.94)	0.004 (0.021)	<0.001 (<0.001)
SDMT [# of corrects], mean, (SD)	47.61 (8.68)	47.46 (8.66)	46.39 (9.02)	<0.001 (<0.001)	0.005 (0.007)
TMT A [seconds to completion], mean, (SD)	33.39 (10.12)	30.43 (7.89)	33.33 (11.80)	0.658 (0.547)	0.003 (0.003)
TMT B [seconds to completions], mean, (SD)	80.31 (46.23)	70.36 (20.25)	78.04 (24.68)	0.108 (0.007)	<0.001 (<0.001)
IST [# of corrects], mean, (SD)	31.75 (12.78)	31.49 (10.67)	33.42 (10.14)	0.546 (0.011)	0.017 (0.019)

Abbreviations: Timepoint of examination (T1, T2, T3), Symbol Digit Modalities Test (SDMT), Trail Making Test A (TMT A), Trail Making Test B (TMT B), Intelligenz-Struktur Test (IST).
Cognitive modality: episodic memory (words pairs, retention); processing speed (SDMT); attention (TMT A), working memory (TMT B), verbal functions (IST).
[1] Linear mixed effect model with T1 or T2 as baseline, cognitive score as dependent variable, time as independent variable, and an unstructured correlation matrix for the repeated measurements.
[2] Linear mixed effect model with T1 or T2 as baseline, with cognitive score as dependent variable, time as independent variable, number of school years as covariate, and an unstructured correlation matrix for the repeated measurements

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CHRONIC INFLAMMATION IMPACTS WHITE MATTER IN BOTH NEURODEVELOPMENT AND COGNITIVE AGEING: A MULTI-OMICS APPROACH

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims: To investigate the impact of chronic inflammation on brain ageing across the lifecourse. **Methods:** A DNA methylation (DNAm) signature of C-Reactive Protein (CRP) was trained out-of-sample by creation of a weighted score from relevant CpG sites; these DNAm scores were then projected across both infant and adult cohorts to examine the consequences of inflammation on brain health outcomes at different life stages. In the infant brain cohort (Theirworld Edinburgh Birth Cohort), 214 infants had neuroimaging (MRI, DTI) and DNAm (Illumina EPIC array) data available; of these, 127 were classified as preterm (< 37 weeks gestational age at birth). In the older age cohort (Lothian Birth Cohort), 521 cognitively normal adults (age ~ 73 years) had relevant data available. In both cohorts, linear models were used to examine inflammation–brain health associations. **Results:** In the infant cohort, preterm infants had higher DNAm CRP scores than term infants; infants who sustained multiple clinical inflammatory hits (e.g. preeclampsia, sepsis) also had higher DNAm CRP scores. Overall, DNAm CRP showed significant associations with brain white matter both globally and regionally in preterm infants. In the older-age cohort, DNAm CRP was also associated with white matter globally and regionally. White matter mediated the relationship between DNAm CRP and cognition, demonstrating a link between inflammation and cognitive ageing. **Conclusions:** This work supports the hypothesis that inflammation has adverse outcomes for brain white matter and highlights the use-case for using DNAm profiles to infer baseline inflammatory status in population cohorts.

Pubmed:
35039062: McCartney DL, Hillary RF, Conole ELS, Banos DT, Gadd DA, Walker RM, Nangle C, Flaig R, Campbell A, Murray AD, Maniega SM, Valdés-Hernández MDC, Harris MA, Bastin ME, Wardlaw JM, Harris SE, Porteous DJ, Tucker-Drob EM, McIntosh AM, Evans KL, Deary IJ, Cox SR, Robinson MR, Marioni RE
Blood-based epigenome-wide analyses of cognitive abilities.
Blood-based markers of cognitive functioning might provide an accessible way to track neurodegeneration years prior to clinical manifestation of cognitive impairment and dementia.
Genome Biol, 2022; 23
34789543: Conole ELS, Stevenson AJ, Muñoz Maniega S, Harris SE, Green C, Valdés Hernández MDC, Harris MA, Bastin ME, Wardlaw JM, Deary IJ, Miron VE, Whalley HC, Marioni RE, Cox SR
DNA Methylation and Protein Markers of Chronic Inflammation and Their Associations With Brain and Cognitive Aging.
To investigate chronic inflammation in relation to cognitive aging by comparison of an epigenetic and serum biomarker of C-reactive protein and their associations with neuroimaging and cognitive outcomes.
Neurology, 2021; 97
33221487: Green C, Shen X, Stevenson AJ, Conole ELS, Harris MA, Barbu MC, Hawkins EL, Adams MJ, Hillary RF, Lawrie SM, Evans KL, Walker RM, Morris SW, Porteous DJ, Wardlaw JM, Steele JD, Waiter GD, Sandu AL, Campbell A, Marioni RE, Cox SR, Cavanagh J, McIntosh AM, Whalley HC
Structural brain correlates of serum and epigenetic markers of inflammation in major depressive disorder.
Inflammatory processes are implicated in the aetiology of Major Depressive Disorder (MDD); however, the relationship between peripheral inflammation, brain structure and depression remains unclear, partly due to complexities around the use of acute/phasic inflammatory biomarkers. Here, we report the first large-scale study of both serological and methylomic signatures of CRP (considered to represent acute and chronic measures of inflammation respectively) and their associations with depression status/symptoms, and structural neuroimaging phenotypes (T1 and diffusion MRI) in a large community-based sample (Generation Scotland; N = 271, N = 609). Serum CRP was associated with overall MDD severity, and

specifically with current somatic symptoms- general interest (β = 0.145, P = 6 × 10) and energy levels (β = 0.101, P = 0.027), along with reduced entorhinal cortex thickness (β = -0.095, P = 0.037). DNAm CRP was significantly associated with reduced global grey matter/cortical volume and widespread reductions in integrity of 16/24 white matter tracts (with greatest regional effects in the external and internal capsules, β= -0.12 to -0.14). In general, the methylation-based measures showed stronger associations with imaging metrics than serum-based CRP measures (βaverage = -0.15 versus βaverage = 0.01 respectively). These findings provide evidence for central effects of peripheral inflammation from both serological and epigenetic markers of inflammation, including in brain regions previously implicated in depression. This suggests that these imaging measures may be involved in the relationship between peripheral inflammation and somatic/depressive symptoms. Notably, greater effects on brain morphology were seen for methylation-based rather than serum-based measures of inflammation, indicating the importance of such measures for future studies.
Brain Behav Immun, 2021; 92

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HEMISPHERIC VERSUS WHOLE-BRAIN IRRADIATION INDUCED NEUROTOXICITY: LONGITUDINAL STUDIES IN THE RAT

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims: Although radiotherapy improves patient prognosis suffering brain cancer, it also affects healthy brain and induces irreversible cognitive deficits in long-surviving patients. Quality of life of patients has taken greater importance in neuro-oncology and current clinical paradigm is to specifically target brain tumors while sparing surrounding healthy tissue. Thereby, new irradiation modalities have been developed, but scientific evidence is strongly needed to demonstrate their value in reducing radiation toxicities. Thus, this study aims to characterize the effects of hemispheric-brain irradiation on tissue integrity and cognition compared to whole-brain irradiation. **Methods**: Wistar rats were divided into control (CTL), whole-brain (WBI) and hemispheric-irradiated (HBI) groups. Fractionated irradiation (30Gy) was applied using a preclinical irradiator (X-RAD-225Cx). General condition, brain MRI and behavioral tests were performed longitudinally up to six-months to assess brain tissue injury, short-term (novel object recognition) and long-term (passive avoidance) memories. **Results**: HBI did not induce weight loss and mortality as compared to WBI. Similarly, in comparison to WBI, more restricted irradiation did not induce significant memory deficits as quantified by passive avoidance and novel recognition object tests. MRI did not show any radio-necrosis nor edema in rats submitted either to WBI or HBI at all the time points analyzed. Nonetheless, brain and hemispheric atrophies were revealed at six-months respectively. Vascular and diffusion MRI revealed major alterations in brain tissue in WBI rats but not in those with HBI. **Conclusions**: These results show that restricted brain irradiation preserves indeed brain tissue and induces less cognitive deficits as compared to whole-brain irradiation.

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ASSESSMENT OF MOTOR PERFORMANCE AND NIGROSTRIATAL DOPAMINERGIC SYSTEM IN L66 MICE WITH FRONTOTEMPORAL DEGENERATION-LIKE TAUOPATHY

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aim: The aim of the study was to evaluate the motor functions and dopaminergic system status in young and aged L66 heterozygous and homozygous mice, serving as a transgenic model of frontotemporal dementia. **Methods:** L66 homozygous (L66Homo) and L66 heterozygous (L66Het) transgenic mice expressing full-length human tau carrying P301S and G335D mutations and the NMRI wild type mice at 5 and 10 months of age was used. To test motor learning and function accelerated and fixed variants of RotaRod test were applied. The accelerated RotaRod test took 4 days with the rod acceleration from 1 to 45 rpm over the 5 min trial. One-day fixed RotaRod test consisted of 4 trials in each rotation speed 5, 10, 15, 20, 25 rpm over 1 min. Results were expressed as time spent on the rotating rod. Pearson's Correlation coefficient between individual results on consecutive days was calculated. Immunohistochemical staining against tyrosine hydroxylase was used to evaluate the dopaminergic system. **Results:** 5 month old animals from all lines did not show motor deficits in both tests. The most prominent decline in motor abilities was observed in 10 month old L66 homozygotes.10 month old L66 heterozygotes were not impaired with respect to NMRI in both tests. Correlation analysis revealed that there was a distinct worsening of motor performance with age in most of L66 Homo mice, however in small subset motor performance remains unimpaired. **Conclusion:** Both rotarod test showed clear motor impairment in ageing L66Homo mice which appears to be associated with dopaminergic impairment.

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UNRAVELLING THE ROLE OF GUT-BRAIN METABOLIC INTERACTIONS IN AGING-ASSOCIATED SPATIAL MEMORY DECLINE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

Stefano Flor[1], Lena Best[1], Christiane Frahm[2], Madlen Günther[2], Otto W. Witte[2], John Baines[3], Christoph Kaleta[1]
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The gut microbiota is known to influence behavior and cognitive function in mice, while host physiology has been shown to influence microbial activity vice versa. However, the underlying mechanisms governing this cross-talk are still incompletely understood. Our study aims to investigate the metabolic basis of this interconnection in relation to spatial memory function and its decline manifesting during normal aging. To this end, we collected fecal metabolomics, metagenomics and transcriptomics data (hippocampus, colon, liver) from mice of different age groups along with measures of spatial memory performances obtained through the Barnes maze test. To evaluate spatial memory function, we calculated the average learning pace (velocity of improvement across multiple Barnes maze tests) for each mouse. To assess the metabolic functionality of mice gut microbiota, we obtained Metagenomic Assembled Genomes (MAGs) from metagenomic data and used this data to reconstruct high-quality metabolic models of the bacterial species. In a later step, we will build an integrated metabolic metamodel of the host and its microbiome by joining the microbial metabolic networks with a mouse metabolic model derived from the human analogue Recon3D. Associations between host as well as microbiota metabolic functions and spatial memory performances will be identified through classical statistic and machine learning approaches. The metamodel will be used to study the links between cognition-associated host and bacterial metabolic functions in order to highlight potential routes of interaction between bacterial and brain metabolism. Preliminary results from metabolomics data indicate an important role of microbially-produced nucleotides in modulating spatial memory performance.

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BOARD NUMBER: S07-099

SUCCESSFUL COGNITIVE AGING RELIES ON HEALTHY ADULT BORN DENTATE NEURONS

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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The progressive decline of memory associated with aging is variable between individuals; some retain good memory performances while others suffer substantial loss of memory abilities. Adult neurogenesis in the dentate gyrus plays an essential role in learning and memory and represents a good candidate for the resilience/vulnerability to cognitive aging as animals with preserved memory abilities show higher levels of neurogenesis at old age. In this project, we aim to determine the role of adult dentate granule neurons (adu-DGNs) born throughout adult life in the development of memory deficits with age. First, we showed using the immediate early gene Zif268 as a proxy for neuronal activity that adu-DGNs are less responsive to the learning task in old aged impaired rats (Vulnerable) compared to the aged rats with preserved memory (Resilient). Next, we aimed to understand the mechanisms involved in such differences in responsiveness of adu-DGNs. We investigated possible alterations in their morphology and/or cellular homeostasis when animals were middle-age, to detect early signs of dysfunction that could lead to memory loss. To do so, the dendritic complexity, mitochondrial network and cellular senescence were investigated. We showed that adu-DGNs display similar morphological characteristics between populations and no sign of cellular senescence. However, we observed in the vulnerable population an impairment of their mitochondrial network in a specific sub-dendritic compartment. Memory deficits observable at old age are linked to a decrease of adu-DGNs reactivity in response to learning that could be related to an early alteration of their metabolism starting at middle-age.

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EARLY-ONSET AND LATE-ONSET CALORIE RESTRICTION DIFFERENTLY MODULATE ANXIETY-LIKE BEHAVIOR IN AGING FEMALE WISTAR RATS

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims: Calorie restriction (CR) is known to prolong lifespan and healthspan, - life period free of age-related diseases. However, generality of CR’s beneficial effect is being questioned recently. Few studies showed that the outcome of CR could vary depending on its onset and duration. Herein, we examined and compared potential of different CR paradigms to modulate anxiety-like behavior. Methods: Ad libitum (AL) fed animals were used as controls. Female Wistar rats of different age (adult, middle-aged and aged) were exposed to CR (60% of AL), to examine the effect of early-onset CR (EOCR) and late-onset CR (LOCR). Open field (OF) and Light-dark box (LDB) test were used for anxiety assessment. Results: Aged LOCR animals had decreased number of entries and time spent in the central area of the OF. LDB test results, however, implied a protective effect of EOCR since both middle-aged and aged EOCR animals showed increased number of entries in the light compartment and spent more time in the door area or in the light compartment. LOCR had different effect depending on animals’ age at the CR onset. Conclusions: EOCR ameliorates anxiety-like behavior and this effect seems to persevere till old age. Implementing CR later in life should be taken with caution, since its’ effect could vary from protective to detrimental, depending on the onset. Additionally, different results were obtained using OF and LDB, suggesting that more than one test should be used to provide proper insight in certain behavioral changes.

Pubmed:

34957511: Prvulovic MR, Milanovic DJ, Vujovic PZ, Jovic MS, Kanazir SD, Todorovic ST, Mladenovic AN
Late-Onset Calorie Restriction Worsens Cognitive Performances and Increases Frailty Level in Female Wistar Rats.
The current study aims to determine the potential benefits of calorie restriction (CR), one of the most promising paradigms for life span and healthspan extension, on cognitive performances in female Wistar rats during aging. As a measure of a healthspan, we evaluated the effects of different onset and duration of CR on frailty level. Female Wistar rats were exposed to either ad libitum (AL) or CR (60% of AL daily intake) food intake during aging. Two different CR protocols were used, life-long CR with an early-onset that started at the adult stage (6 months) and 3-month-long CR, started at the middle (15 months) and late-middle (21 months) age, thus defined as a late-onset CR. The effects of CR were evaluated using open-field, Y-maze, and novel object recognition tests. We broadened 2 tools for frailty assessment currently in use for experimental animals, and in alignment with our previous study, we created a physical-cognitive frailty tool that combines both physical and cognitive performances. Our results clearly showed that CR effects are highly dependent on CR duration and onset. While a life-long restriction with an early-onset has been proven as protective and beneficial, short-term restriction introduced at late age significantly worsens an animal's behavior and frailty. These results complement our previous study conducted in males and contribute to the understanding of sex differences in a response to CR during aging.
J Gerontol A Biol Sci Med Sci, 2022; 77
33279620: Mladenovic Djordjevic AN, Kapetanou M, Loncarevic-Vasiljkovic N, Todorovic S, Athanasopoulou S, Jovic M, Prvulovic M, Taoufik E, Matsas R, Kanazir S, Gonos ES
Pharmacological intervention in a transgenic mouse model improves Alzheimer's-associated pathological phenotype: Involvement of proteasome activation.
Alzheimer's disease (AD) is the most common form of dementia worldwide, characterized by a progressive decline in a variety of cognitive and non-cognitive functions. The amyloid beta protein cascade hypothesis places the formation of amyloid beta protein aggregates on the first position in the complex pathological cascade leading to neurodegeneration, and therefore AD might be considered to be a protein-misfolding disease. The Ubiquitin Proteasome System (UPS), being the primary protein degradation mechanism with a fundamental role in the maintenance of proteostasis, has been identified as a putative therapeutic target to delay and/or to decelerate the progression of neurodegenerative disorders that are characterized by

accumulated/aggregated proteins. The purpose of this study was to test if the activation of proteasome in vivo can alleviate AD pathology. Specifically by using two compounds with complementary modes of proteasome activation and documented antioxidant and redox regulating properties in the 5xFAD transgenic mice model of AD, we ameliorated a number of AD related deficits. Shortly after proteasome activation we detected significantly reduced amyloid-beta load correlated with improved motor functions, reduced anxiety and frailty level. Essentially, to our knowledge this is the first report to demonstrate a dual activation of the proteasome and its downstream effects. In conclusion, these findings open up new directions for future therapeutic potential of proteasome-mediated proteolysis enhancement.
Free Radic Biol Med, 2021; 162

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EFFECTS OF AGING AND CALORIC RESTRICTION IN THE RAT HIPPOCAMPUS

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aging is defined as a biological process time-related that is associated with structural and physiological alterations in the hippocampus, specifically with brain mechanisms known as neuroinflammation and neuronal loss. In this context, one of the most common procedures to delay age-related cognitive decline is the caloric restriction (CR) diet. Hippocampal samples from three different groups of male Wistar rats; old rats fed on a CR diet from 4 months of age, old rats with unrestricted access to food (*Ad Libitum, AL*) and young rats, were analysed by immunohistochemical and neurogenesis markers. By means of immunohistofluorescence we assessed the distribution of NeuN, expressed by neurons, and of Iba-1, expressed by microglial cells; to evaluate the neuronal loss and neuroinflammation, respectively. To evaluate neurogenesis, staining for doublecortin (DCX), an immature neuron marker, was performed. Our results showed that CR had a positive effect in slowing-down structural and functional hippocampal aged-related changes as CR rats showed a significant increase in neuronal density and a decrease in neuroinflammation compared with AL rats. Moreover, CR slightly enhanced short-term hippocampal-dependent memory, as assessed with the Y-maze object recognition task. However, CR diet did not prevent against age-related decline in neuronal production. Altogether, these results support a positive effect of CR on age-dependent hippocampal dysfunctions.

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CONDITIONAL DELETION OF CB1 RECEPTOR IN THE HIPPOCAMPUS ACCELERATES AGEING SIGNS IN MATURE MICE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims: Ageing is characterized by a series of cellular and molecular alterations in the brain, most significantly in the hippocampus. During ageing endocannabinoid system activity declines, including the function of cannabinoid type-1 (CB1) receptors. Previous studies have shown that decreased cannabinoid system signaling, due to constitutive genetic deletion of the CB1 receptor in mice, leads to an early onset of ageing signs in the brain like learning deficits and hyperactivation of glial cells. In the present study, we asked whether the CB1 receptor exerts its protective effect locally or it has a systemic action. To answer this question, we tested the learning ability, histological and expression changes in the hippocampus of mice with hippocampus-specific deletion of the CB1 receptor. Methods: Recombinant adeno-associated viruses expressing Cre recombinase or the Venus reporter gene were stereotaxically injected into the hippocampus of 2-month-old $CB1_{floxed/floxed}$ mice. Four months later, we assessed the social recognition memory of the 6-month-old mice in the partner recognition test. Finally, microglia and astrocyte density and morphology, expression of the CB1 receptor and proinflammatory cytokines were measured. Results: Efficacy of CB1 deletion was proved by showing reduced CB1 protein and mRNA levels in the hippocampus of Cre injected mice. We found that Cre injected mice were unable to discriminate the previously seen partner compared to the age-matched controls and the hippocampal expression of the proinflammatory cytokine TNF-alpha was significantly upregulated. Conclusions: Our results suggest that local CB1 receptor activity is required to prevent cognitive and cellular age-related changes.

Pubmed:

34526890: Nidadavolu P, Bilkei-Gorzo A, Krämer M, Schürmann B, Palmisano M, Beins EC, Madea B, Zimmer A
Efficacy of Δ -Tetrahydrocannabinol (THC) Alone or in Combination With a 1:1 Ratio of Cannabidiol (CBD) in Reversing the Spatial Learning Deficits in Old Mice.
Decline in cognitive performance, an aspect of the normal aging process, is influenced by the endocannabinoid system (ECS). Cannabinoid receptor 1 (CB1) signaling diminishes with advancing age in specific brain regions that regulate learning and memory and abolishing CB1 receptor signaling accelerates cognitive aging in mice. We recently demonstrated that prolonged exposure to low dose (3 mg/kg/day) Δ-tetrahydrocannabinol (THC) improved the cognitive performances in old mice on par with young untreated mice. Here we investigated the potential influence of cannabidiol (CBD) on this THC effect, because preclinical and clinical studies indicate that the combination of THC and CBD often exhibits an enhanced therapeutic effect compared to THC alone. We first tested the effectiveness of a lower dose (1 mg/kg/day) THC, and then the efficacy of the combination of THC and CBD in 1:1 ratio, same as in the clinically approved medicine Sativex. Our findings reveal that a 1 mg/kg/day THC dose still effectively improved spatial learning in aged mice. However, a 1:1 combination of THC and CBD failed to do so. The presence of CBD induced temporal changes in THC metabolism ensuing in a transient elevation of blood THC levels. However, as CBD metabolizes, the inhibitory effect on THC metabolism was alleviated, causing a rapid clearance of THC. Thus, the beneficial effects of THC seemed to wane off more swiftly in the presence of CBD, due to these metabolic effects. The findings indicate that THC-treatment alone is more efficient to improve spatial learning in aged mice than the 1:1 combination of THC and CBD.
Front Aging Neurosci, 2021; 13
33935645: Sahu MP, Pazos-Boubeta Y, Steinzeig A, Kaurinkoski K, Palmisano M, Borowecki O, Piepponen TP, Castrén E
Depletion of TrkB Receptors From Adult Serotonergic Neurons Increases Brain Serotonin Levels, Enhances Energy Metabolism and Impairs Learning and Memory.
Neurotrophin brain-derived neurotrophic factor (BDNF) and neurotransmitter serotonin (5-HT) regulate each other and have been implicated in several neuronal mechanisms, including neuroplasticity. We have investigated the effects of BDNF on serotonergic neurons by deleting BDNF receptor TrkB from serotonergic neurons in the adult brain. The transgenic mice show increased 5-HT and Tph2 levels with abnormal behavioral phenotype. In spite of increased food intake, the transgenic

mice are significantly leaner than their wildtype littermates, which may be due to increased metabolic activity. Consistent with increased 5-HT, the proliferation of hippocampal progenitors is significantly increased, however, long-term survival of newborn cells is unchanged. Our data indicates that BDNF-TrkB signaling regulates the functional phenotype of 5-HT neurons with long-term behavioral consequences.
Front Mol Neurosci, 2021; 14

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BOARD NUMBER: S07-103

AGE-RELATED NEURONAL LOSS IN THE LOCUS COERULEUS IS INFLUENCED BY CANNABINOID RECEPTOR TYPE-1 ACTIVITY AND RESPONSIBLE FOR ATTENTION DEFICITS

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Introduction: Our laboratory has previously shown that CB1r activity is neuroprotective and a modulator of brain ageing. In this study, we investigate how the endocannabinoid system affects noradrenergic neurons in the Locus Coeruleus (LC), which are vulnerable to age-related changes. **Methods:** The number of LC neurons was determined using stereological counting in wild-type animals to confirm an aging effect and to establish a timeline of LC age-related neurodegeneration. Next, we compared wild-type and CB1r knockout ($Cnr1^{-/-}$) mice. Markers for noradrenergic innervation, inflammation, and DAGLα protein expression have been analyzed by immunohistochemistry. To test whether the loss of LC noradrenergic neurons is responsible for age-related deficits in fear memory and attention, we treated young mice with DSP-4 toxin, reducing LC neuronal numbers to the level typical of old animals. Then, we compared their performance with old mice in the Fear Conditioning and 5CSRTT behavioral tests. **Results:** Our data show that in wild-type mice there is an age-related neuronal loss in LC starting in middle aged animals. $Cnr1^{-/-}$ mice show less noradrenergic neurons and noradrenergic terminals compared to age-matched wild-type controls. Attention was decreased both in old mice and in DSP-4 treated young animals. Fear memory was influenced by the age but not by the deletion of noradrenergic neurons. **Conclusions:** All together our data suggest that CB1r activity is protective for noradrenergic neurons during ageing and that a reduction in the number of noradrenergic neurons could be responsible for attention deficits in ageing but not for the altered fear learning and memory.

Pubmed:

33374940: Gargano A, Beins E, Zimmer A, Bilkei-Gorzo A
Lack of Cannabinoid Receptor Type-1 Leads to Enhanced Age-Related Neuronal Loss in the Locus Coeruleus.
Our laboratory and others have previously shown that cannabinoid receptor type-1 (CB1r) activity is neuroprotective and a modulator of brain ageing; a genetic disruption of CB1r signaling accelerates brain ageing, whereas the pharmacological stimulation of CB1r activity had the opposite effect. In this study, we have investigated if the lack of CB1r affects noradrenergic neurons in the locus coeruleus (LC), which are vulnerable to age-related changes; their numbers are reduced in patients with neurodegenerative diseases and probably also in healthy aged individuals. Thus, we compared LC neuronal numbers between cannabinoid 1 receptor knockout () mice and their wild-type littermates. Our results reveal that old mice have less noradrenergic neurons compared to their age-matched wild-type controls. This result was also confirmed by the analysis of the density of noradrenergic terminals which proved that mice had less compared to the wild-type controls. Additionally, we assessed pro-inflammatory glial activity in the LC. Although the density of microglia in mice was enhanced, they did not show enhanced inflammatory profile. We hypothesize that CB1r activity is necessary for the protection of noradrenergic neurons, but its anti-inflammatory effect probably only plays a minor role in it.
Int J Mol Sci, 2020; 22
31365468: Nocchi L, Portulano C, Franciosa F, Doleschall B, Panea M, Roy N, Maffei M, Gargano A, Perlas E, Heppenstall PA
Nerve growth factor-mediated photoablation of nociceptors reduces pain behavior in mice.
Nerve growth factor (NGF) and its receptors TrkA and p75 play a key role in the development and function of peripheral nociceptive neurons. Here, we describe novel technology to selectively photoablate TrkA-positive nociceptors through delivery of a phototoxic agent coupled to an engineered NGF ligand and subsequent near-infrared illumination. We demonstrate that this approach allows for on demand and localized reversal of pain behaviors in mouse models of acute, inflammatory, neuropathic, and joint pain. To target peripheral nociceptors, we generated a SNAP-tagged NGF derivative NGF that binds to TrkA/p75 receptors but does not provoke signaling in TrkA-positive cells or elicit pain behaviors in mice. NGF was coupled to the photosensitizer IRDye700DX phthalocyanine (IR700) and injected subcutaneously. After near-

infrared illumination of the injected area, behavioral responses to nociceptive mechanical and sustained thermal stimuli, but not innocuous stimuli, were substantially reduced. Similarly, in models of inflammatory, osteoarthritic, and neuropathic pain, mechanical hypersensitivity was abolished for 3 weeks after a single treatment regime. We demonstrate that this loss of pain behavior coincides with the retraction of neurons from the skin which then reinnervate the epidermis after 3 weeks corresponding with the return of mechanical hypersensitivity. Thus NGF-mediated photoablation is a minimally invasive approach to reversibly silence nociceptor input from the periphery, and control pain and hypersensitivity to mechanical stimuli. Pain, 2019; 160

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BOARD NUMBER: S07-104

GDF11 IMPROVES MEMORY AND ATTENUATES DEPRESSION IN AGED MICE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Increasing attention is being paid to cognitive decline and mood disorders, such as depression, that dramatically increase in frequency with age. Many efforts are focused on the identification of molecules and pathways to treat these conditions. Here, we report a novel role for GDF11 in attenuating depression associated with advanced aging in mice. Systemic administration of GDF11 reverts the depression-like phenotype and memory decline in aged mice. These effects occur via a neurogenesis-independent pathway as GDF11 does not activate neurogenesis *in vivo* or neural stem cell differentiation into neuroblasts *in vitro*. In contrast, we show that GDF11 acts on neurons to enhance neuronal activity, thus restoring memory and counteracting a depression-like phenotype in aged mice within a few weeks of treatment. In accordance with these findings, direct intracerebroventricular infusion of GDF11 in the brain of aged mice improves memory and alleviates anxiety- and depression-like symptoms. Our findings provide evidence that GDF11 could be a potent candidate biomarker for future therapeutic strategies aimed at targeting depressive states and age-related cognitive decline and highlight a novel role for GDF11 in the regulation of age-related depression and the possibility to use in therapeutic interventions.

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BOARD NUMBER: S07-105

TREHALOSE INCREASES TFEB AND AUTOPHAGIC FLUX IN THE DORSAL HIPPOCAMPUS, AND PRODUCES CHANGES IN EXPLORATORY BEHAVIOUR IN OLD MICE.

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Ageing is associated with chronic dysregulation of autophagy, that if restored, has been suggested as therapeutic for treating neurodegenerative diseases and normal cognitive decline associated with age. Trehalose is a disaccharide that reduces protein aggregates in neurons, and although its mechanism of action is not yet completely understood, there is evidence that this effect is mediated through the upregulation of autophagy, partially through the activation of the transcription factor EB (TFEB) controlling lysosome biogenesis. Here we tested whether chronic intake of trehalose can modulate autophagy in the hippocampus of aged mice and improve spatial memory. Trehalose was administered in a concentration of 2.2% in the drinking water to young & old C57BL/6Jc mice, during which a battery of behavioural tests aimed to investigate changes in spatial learning was performed. Even though spatial learning seemed unaffected in the Morris water maze task (MWM), trehalose produced a decrease in the use of egocentric exploratory strategies, a staple of typical spatial memory acquisition, and resulted in a decreased permanence in the escape quadrant of the MWM during the probe trial, specifically in the aged animals. The changes in behavioral performance were associated with increased TFEB protein levels, as well as autophagic flux in the dorsal hippocampus. Our results indicate that during ageing, autophagy might be easily modulated in the dorsal hippocampus by trehalose, posibly in a TFEB dependent manner, and that this modulation produces changes in navigation skills, possibly affecting spatial memory. This work is supported by the German Research Foundation, RTG 2413 SynAGE.

Pubmed:
34673174: Çalışkan G, French T, Enrile Lacalle S, Del Angel M, Steffen J, Heimesaat MM, Rita Dunay I, Stork O
Antibiotic-induced gut dysbiosis leads to activation of microglia and impairment of cholinergic gamma oscillations in the hippocampus.
Antibiotics are widely applied for the treatment of bacterial infections, but their long-term use may lead to gut flora dysbiosis and detrimental effects on brain physiology, behavior as well as cognitive performance. Still, a striking lack of knowledge exists concerning electrophysiological correlates of antibiotic-induced changes in gut microbiota and behavior. Here, we investigated changes in the synaptic transmission and plasticity together with behaviorally-relevant network activities from the hippocampus of antibiotic-treated mice. Prolonged antibiotic treatment led to a reduction of myeloid cell pools in bone marrow, circulation and those surveilling the brain. Circulating Ly6C inflammatory monocytes adopted a proinflammatory phenotype with increased expression of CD40 and MHC II. In the central nervous system, microglia displayed a subtle activated phenotype with elevated CD40 and MHC II expression, increased IL-6 and TNF production as well as with an increased number of Iba1 + cells in the hippocampal CA3 and CA1 subregions. Concomitantly, we detected a substantial reduction in the synaptic transmission in the hippocampal CA1 after antibiotic treatment. In line, carbachol-induced cholinergic gamma oscillation were reduced upon antibiotic treatment while the incidence of hippocampal sharp waves was elevated. These alterations were associated with the global changes in the expression of neurotrophin nerve growth factor and inducible nitric oxide synthase, both of which have been shown to influence cholinergic system in the hippocampus. Overall, our study demonstrates that antibiotic-induced dysbiosis of the gut microbiome and subsequent alteration of the immune cell function are associated with reduced synaptic transmission and gamma oscillations in the hippocampus, a brain region that is critically involved in mediation of innate and cognitive behavior.
Brain Behav Immun, 2022; 99
34360710: Annamneedi A, Del Angel M, Gundelfinger ED, Stork O, Çalışkan G
The Presynaptic Scaffold Protein Bassoon in Forebrain Excitatory Neurons Mediates Hippocampal Circuit Maturation: Potential Involvement of TrkB Signalling.
A presynaptic active zone organizer protein Bassoon orchestrates numerous important functions at the presynaptic active zone. We previously showed that the absence of Bassoon exclusively in forebrain glutamatergic presynapses (cKO) in mice leads to developmental disturbances in dentate gyrus (DG) affecting synaptic excitability, morphology, neurogenesis and

related behaviour during adulthood. Here, we demonstrate that hyperexcitability of the medial perforant path-to-DG (MPP-DG) pathway in cKO mice emerges during adolescence and is sustained during adulthood. We further provide evidence for a potential involvement of tropomyosin-related kinase B (TrkB), the high-affinity receptor for brain-derived neurotrophic factor (BDNF), mediated signalling. We detect elevated TrkB protein levels in the dorsal DG of adult mice (~3-5 months-old) but not in adolescent (~4-5 weeks-old) mice. Electrophysiological analysis reveals increased field-excitatory-postsynaptic-potentials (fEPSPs) in the DG of the adult, but not in adolescent cKO mice. In line with an increased TrkB expression during adulthood in cKO, blockade of TrkB normalizes the increased synaptic excitability in the DG during adulthood, while no such effect was observed in adolescence. Accordingly, neurogenesis, which has previously been found to be increased in adult cKO mice, was unaffected at adolescent age. Our results suggest that Bassoon plays a crucial role in the TrkB-dependent postnatal maturation of the hippocampus.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S07-106

BEHAVIOURAL AND NEUROPATHOLOGICAL CHARACTERIZATION OF A RAT MODEL OF RESILIENCE IN THE FIELD OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims: Resilience describes the ability to preserve cognition, irrespective of the level of neuropathological hallmarks of Alzheimer's disease (AD). Cognitive resilience accounts for clinical heterogeneity state of patients and is certainly responsible of the translational failures of drugs' efficacy. We aimed here to investigate the cerebral correlates of cognitive resilience in a preclinical model of AD. Methods: Adult male Wistar and LOU/c/jall rats were exposed to a chronic cerebral infusion of soluble human beta-amyloid peptide 1-42 (15µM; AB42 groups; n=10 per strain) or vehicle solution (CTR groups; n=10 per strain) for 4 weeks (Alzet pumps 2ML4; 2.5 µl/hour). Using a battery of behavioural tests (elevated-plus maze, Y-maze, object recognition, Morris water maze), cognitive changes were then investigated followed by *ex vivo* cerebral analysis (immunohistochemistry and western-blot). Results: AB42-treated animals groups exhibited higher anxiety-like behaviour, whatever the strain considered. Besides, AB42 infusion induced working memory deficit, both in Wistar and LOU/c/jall, compared to their respective control. Conversely, no spatial memory impairment was observed. Finally, Wistar rats but not LOU/c/jall rats, showed recognition memory impairments following the AB42 infusion. At cellular and molecular levels, AB42 infusion induced hippocampal neuroinflammation and altered the hippocampal mTORC1 pathway in both rat strains. Conclusions: Already described as a model of successful aging, LOU/c/jall rats showed here a cognitive resilience in a model of AD mimicking pathology. A better understanding of the neurobiological mechanisms behind such resilience may afford new insights in the search for new therapeutic targets for AD.

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BOARD NUMBER: S07-107

VULNERABILITY OF SPATIAL PATTERN SEPARATION IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims: Alzheimer's disease (AD) is the most common cause of dementia and remains incurable. This age-related neurodegenerative disorder is characterized by early episodic and spatial memory decline associated with progressive hippocampus disturbances. Recent clinical data suggest that impairment of Spatial Pattern Separation function (SPSf), which allows the encoding and storage of episodic spatial information, could be an indicator of the early stages of AD. We aimed here to assess the time course of SPSf decline in a transgenic mouse model of amyloidogenesis to further investigate the underlying brain mechanisms. Methods: Performances of male wild-type (n = 18) and 5xFAD (n = 19) mice were assessed from 5 months of age in two behavioral tasks requiring SPSf. The first task was based on object recognition in an open field. The second task, which display a higher translational value, was based on the discrimination of visual stimuli using a touchscreen system. Results: Compared to the wild-type mice, a visual discrimination deficit was observed in 5-month old 5xFAD mice in the object recognition paradigm. Such discrimination deficit was not observed in the touchscreen-based paradigm. Our results indicate an early SPSf vulnerability in our model of AD pathology that depends on the behavioral task used. This discrepancy may be explained by the involvement of distinct cognitive and cerebral processes in the two tasks. Conclusion: Our work opens up the possibility of examining the early neurobiological processes involved in episodic memory decline and may help to propose new therapeutic strategies in the context of AD.

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BOARD NUMBER: S07-108

EVALUATION OF EARLY AGING FOLLOWING PERINATAL INFLAMMATION-DRIVEN ENCEPHALOPATHY OF PREMATURITY IN A MOUSE MODEL.

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

David Guenoun[1], Irvin Sautet[1], Valérie Faivre[1], Jennifer Hua[1], Pierrette Young-Ten[1], Alice Jacquens[1], Juliette Van Steenwinckel[1], Pierre Gressens[1], Homa Adle-Biassette[2], Cindy Bokobza[1]
[1]Inserm, Umr1141 Neurodiderot, PARIS, France, [2]Université de Paris, service d'Anatomie Pathologique, Ap-hp, Hôpital Lariboisière, Dmu Dream, Umr 1141, Inserm, Paris, France

Background: 15 million infants are born prematurely every year, 40% of the time following maternal infections. The resulting neuroinflammatory processes affecting these newborns are driven by glial cells' reactivity (microglia and astrocytes) and increase the onset of brain lesions collectively termed Encephalopathy of Prematurity (EoP). EoP is associated with neurodevelopmental disorders in children and, in recent studies, with mental deficits such as mood disorders in young adults. Evidence demonstrated that long after initial reactivity, glial cells are keener to react exaggeratedly to later inflammatory stimuli, most probably through cellular priming. Recent literature focusing on the normal aging brain highlighted a low-grade chronic inflammatory state playing a potential role in the brain's susceptibility to neurodegeneration. Glial cells primed by perinatal inflammation could therefore increase the age-related inflammation state leading to early aging. Aims: We sought to determine whether a perinatal inflammatory challenge could accelerate the brain aging trajectory. Methods: This study is based on a mouse model of perinatal inflammation responsible for EoP-like lesions. Months later the onset of EoP, in middle-aged mice, we used transcriptomic (*RNA sequencing*), functional (Ultra-fast Doppler imaging, flow cytometry, histology), and behavioral analyses (open-field, 3-chamber test…) to evaluate the impact of perinatal inflammation on age-related inflammation and neuronal impairments, including functional brain connectivity and its behavioral consequences. Results: Our results showed signs of ongoing inflammation and glial reactivity while brain connectivity defects were recorded in middle-aged mice exposed to perinatal inflammation. Conclusion: Preliminary data tended to confirm long-term consequences of perinatal inflammation suggesting early brain aging.

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BOARD NUMBER: S07-109

SPLENIC DENERVATION IMPAIRS IL-4 PRODUCTION FROM CD4+ T CELLS AND EXACERBATES COGNITIVE LOSS IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

Tommaso Croese[1], Michal Arad[1], Sedi Medina[1], Francesca Palmas[2], Javier Peralta Ramos[1], Stefano Suzzi[1], Giulia Castellani[1], Denise Kviatcovsky[3], Afroditi Tsitsou-Kampeli[1], Michal Schwartz[1]
[1]Weizmann Institute of Science, Brain Sciences, Arison Building, Rehovot, Israel, [2]Universita' degli studi di Cagliari, Scienze Biomediche, Cagliari, Italy, [3]Weizmann Institute of Science, System Immunology, Rehovot, Israel

Introduction: The immune system and the central nervous system (CNS) are each in direct contact with the other organs of the body, though their interconnection is only starting to be fully appreciated. During the crosstalk between the CNS and the immune system, neurotransmitters present in the local tissue environments influence innate and adaptive immune responses. Interestingly, recent evidence identified hypothalamic neurons as responsible for regulating splenic innervation. In Alzheimer's disease, the hypothalamic nuclei show a substantial decrease in neuronal populations, nevertheless, to our knowledge, the impact and consequences of an impairment in the neuroimmune crosstalk in AD were never addressed so far. **Methods:** To test the role of splenic innervation in animal models of AD (5xFAD) and WT mice, we adapted a surgical denervation procedure treating splenic nerve plexuses with alcohol before they enter the spleen, selectively ablating the innervation of the spleen without affecting other organs. Subsequently, we tested the cognitive performance of all mice, and we studied their immune landscape in the spleen, blood and brain through high-dimensional single-cell mass cytometry. **Results:** Loss of the sympathetic innervation impair the production of Interleukin-4 from CD4+ splenocytes, alters microglia phenotype, and significantly accelerates cognitive loss in 5xFAD mice with no effect on WT mice. **Conclusions:** These results show that the innervation of peripheral organs plays a crucial role in the development of a proper immune response, which in turn is needed to limit CNS pathology and support brain functioning in the context of neurodegenerative disorders.

Pubmed:
34429552: Croese T, Castellani G, Schwartz M
Immune cell compartmentalization for brain surveillance and protection.
For decades, it was commonly accepted that the brain is secluded from peripheral immune activity and is self-sufficient for its maintenance and repair. This simplistic perception was based on the presence of resident immune cells, the microglia, and barrier systems within the brain, and the assumption that the central nervous system (CNS) lacks lymphatic drainage. This view was revised with the discoveries that higher functions of the CNS, homeostasis and repair are supported by peripheral innate and adaptive immune cells. The findings of bone marrow-derived immune cells in specialized niches, and the renewed observation that a lymphatic drainage system exists within the brain, further contributed to this revised model. In this Review, we describe the immune niches within the brain, the contribution of professional immune cells to brain functions, the bidirectional relationships between the CNS and the immune system and the relevance of immune components to brain aging and neurodegenerative diseases.
Nat Immunol, 2021; 22
29137922: Croese T, Furlan R
Extracellular vesicles in neurodegenerative diseases.
Extracellular vesicles (EVs) are released by all neural cells, including neurons, oligodendrocytes, astrocytes, and microglia. The lack of adequate technology has not halted neuroscientists from investigating EVs as a mean to decipher neurodegenerative disorders, still in search of comprehensible pathogenic mechanisms and efficient treatment. EVs are thought to be one of ways neurodegenerative pathologies spread in the brain, but also one of the ways the brain tries to displace toxic proteins, making their meaning in pathogenesis uncertain. EVs, however do reach biological fluids where they can be analyzed, and might therefore constitute clinically decisive biomarkers for neurodegenerative diseases in the future. Finally, if they constitute a physiological inter-cell communication system, they may represent also a very specific drug delivery tool for a difficult target such as the brain. We try to resume here available information on the role of EVs in neurodegeneration, with a special focus on Alzheimer's disease, progressive multiple sclerosis, amyotrophic lateral sclerosis,

and Huntington's disease.
Mol Aspects Med, 2018; 60
33389030: Gelibter S, Pisa M, Croese T, Dalla Costa G, Orrico M, Preziosa P, Sangalli F, Martinelli V, Furlan R, Filippi M
Neutrophil-to-lymphocyte ratio: a marker of neuro-inflammation in multiple sclerosis?
The significance of neutrophil-to-lymphocyte ratio (NLR) has been explored in different diseases. Few studies addressed its role in patients with multiple sclerosis (MS), with promising results regarding its association with disease activity or disability.
J Neurol, 2021; 268
33464186: Dalla Costa G, Croese T, Pisa M, Finardi A, Fabbella L, Martinelli V, Leocani L, Filippi M, Comi G, Furlan R
CSF extracellular vesicles and risk of disease activity after a first demyelinating event.
Extracellular vesicles (EVs), a recently described mechanism of cell communication, are released from activated microglial cells and macrophages and are a candidate biomarker in diseases characterized by chronic inflammatory process such as multiple sclerosis (MS).
Mult Scler, 2021; 27
33829250: Pisa M, Croese T, Dalla Costa G, Guerrieri S, Huang SC, Finardi A, Fabbella L, Sangalli F, Colombo B, Moiola L, Martinelli V, Comi G, Furlan R, Leocani L
Subclinical anterior optic pathway involvement in early multiple sclerosis and clinically isolated syndromes.
Optical coherence tomography (OCT) is gaining increasing relevance in the assessment of patients with multiple sclerosis. Converging evidence point to the view that neuro-retinal changes, in eyes without acute optic neuritis, reflect inflammatory and neurodegenerative processes taking place throughout the CNS. The present study aims at exploring the usefulness of OCT as a marker of inflammation and disease burden in the earliest phases of the disease. Thus, a cohort of 150 consecutive patients underwent clinical, neurophysiological and brain MRI assessment as well as lumbar puncture as part of their diagnostic workup for a neurological episode suggestive of inflammatory CNS disorder; among those 32 patients had another previous misdiagnosed episode. For the present study, patients also received a visual pathway assessment (OCT, visual evoked potentials, visual acuity), measurement of CSF inflammatory markers (17 cytokines-chemokines, extracellular vesicles of myeloid origin), and dosage of plasma neurofilaments. Subclinical optic nerve involvement is frequently found in clinically isolated syndromes by visual evoked potentials (19.2%). OCT reveals ganglion cell layer asymmetries in 6.8% of patients; retinal fibre layer asymmetries, despite being more frequent (17.8%), display poor specificity. The presence of subclinical involvement is associated with a greater disease burden. Second, ganglion cell layer thinning reflects the severity of disease involvement even beyond the anterior optic pathway. In fact, the ganglion cell layer in eyes without evidence of subclinical optic involvement is correlated with Expanded Disability Status Scale, low contrast visual acuity, disease duration, brain lesion load, presence of gadolinium enhancing lesions, abnormalities along motor and somatosensory evoked potentials, and frequency of CSF-specific oligoclonal bands. Third, the inner nuclear layer thickens in a post-acute (1.1-3.7 months) phase after a relapse, and this phenomenon is counteracted by steroid treatment. Likewise, a longitudinal analysis on 65 patients shows that this swelling is transient and returns to normal values after 1 year follow-up. Notwithstanding, the clinical, MRI, serological and CSF markers of disease activity considered in the study are strictly associated with one another, but none of them are associated with the inner nuclear layer. Our findings challenge the current hypothesis that the inner nuclear layer is an acute phase marker of inflammatory activity. The present study suggests that instrumental evidence of subclinical optic nerve involvement is associated with a greater disease burden in clinically isolated syndrome. Neuro-retinal changes are present since the earliest phases of the disease and yield important information regarding the neurodegenerative and inflammatory processes occurring in the CNS.
Brain, 2021; 144
34216397: Gelibter S, Pisa M, Croese T, Finardi A, Mandelli A, Sangalli F, Colombo B, Martinelli V, Comi G, Filippi M, Furlan R
Spinal Fluid Myeloid Microvesicles Predict Disease Course in Multiple Sclerosis.
In vivo measures of myeloid activity are promising biomarkers in multiple sclerosis. We previously demonstrated that cerebrospinal fluid (CSF) myeloid microvesicles are markers of microglial/macrophage activity and neuroinflammation in multiple sclerosis. Here, we aimed at investigating the diagnostic and prognostic value of myeloid microvesicles in a clinical setting.
Ann Neurol, 2021; 90
31435772: Gelibter S, Orrico M, Croese T, Bosco L, Martinelli V, Sangalli F, Filippi M
High-dose steroid therapy for CNS inflammatory diseases increases INR in patients taking oral vitamin K antagonist.
J Neurol, 2019; 266

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BOARD NUMBER: S07-110

SIRT1-DEPENDENT AUTOPHAGY AS A NOVEL THERAPY FOR AGE-RELATED MEMORY DECLINE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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INSERM U1151 - Université de Paris, Institut Necker Enfants Malades (inem), Paris, France

Over the last years, Sirtuins (SIRT) have been directly linked to the maintenance of neuronal homeostasis and functions throughout life. Among the SIRT1-7 members, the SIRT1 has been described as a mediator of the well-ageing of the whole organism. Importantly, its anti-ageing effects require the cellular degradative process called autophagy. In that way, recent evidence indicate that autophagy is necessary for memory formation and reverses age-related memory loss. However, the role of SIRT1-dependent autophagy in the maintenance of memory fitness during ageing remains unknown. We have characterized the function of SIRT1 enzyme by means of gene expression, protein levels, phosphorylation state and deacetylase activity, observing that it is reduced in the hippocampus (HpC) of aged mice. We then modulated SIRT1 activity in the HpC by genetic and pharmacological approaches in young and aged mice, observing that it is essential for novel memory formation and integration, and its boosting ameliorates age-related memory decline. That phenotype is accompanied by restoration of autophagy machinery in the aged HpC. All these data open novel therapeutic avenues to rejuvenate cognitive fitness by modulating SIRT1-dependent autophagy.

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BOARD NUMBER: S07-111

LINKING INTER-INDIVIDUAL DIFFERENCES IN COGNITIVE FUNCTION TO RNAOME HETEROGENEITY

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

Aditi Methi, M. Sadman Sakib, Lalit Kaurani, Jonathan Cortés Silva, Jiayin Zhou, Susanne Burkhardt, Tonatiuh Pena, Vakhtang Elerdashvili, Andre Fischer
German Center for Neurodegenerative Diseases (DZNE), Dept. For Epigenetics And Systems Medicine In Neurodegenerative Diseases, Göttingen, Germany

Cognitive functions decline with age but a majority of these changes occur in a highly selective and subtle manner. Moreover, there exists a high degree of variability in neurocognitive and memory function among healthy aged individuals. Thus, some individuals undergo healthy cognitive aging while others eventually develop dementia, but the underlying mechanisms are not well understood. This project aims to elucidate which specific adaptive or compensatory responses sustain healthy cognitive function in the brains of aged individuals. Our hypothesis is that differences in gene-expression patterns play a role. Thus, we used the Morris water maze (MWM) test as a standard task paradigm for assessing spatial learning and memory in 60 C57BL/6J wild-type healthy aged mice (16-months old). Mice were trained in the MWM for 6 days. We developed a novel spatial learning index termed the 'cognitive score' as an assessment of the cognitive ability of a mouse, based on various metrics extracted from its MWM performance by a neural-network algorithm. This allowed us to rank mice based on their individual cognitive scores and select the best and worst performers (called 'good' or 'bad' learners) for further analyses. Coding and non-coding RNA expression data from total RNA sequencing (from hippocampus and cortical tissue samples) from selected mice were analysed to link RNAome changes to the cognitive score. Our current data suggest that differences in cognitive aging are accompanied by very subtle changes in gene and non-coding RNA expression. Candidate RNAs and their downstream processes will be validated using functional assays.

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BOARD NUMBER: S07-112

SEX DIMORPHISM IN EARLY ALZHEIMER'S PATHOLOGY: BEHAVIORAL AND BRAIN CONNECTIVITY WITH MRI ANALYSIS IN MICE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

Inès Ben Abdallah[1,2], Laura-Adela Harsan[2,3], Chantal Mathis[1]
[1]Université de Strasbourg, CNRS, Laboratoire De Neurosciences Cognitives Et Adaptatives, Umr 7364, Strasbourg, France, [2]University of Strasbourg, Icube Lab, Strasbourg, France, [3]University Hospital of Strasbourg, Department Of Biophysics And Nuclear Medicine, Strasbourg, France

Background: Cognitive decline in preclinical Alzheimer's disease (AD) is more pronounced in women than in men. However, only a few studies explored gender-specificity in AD at this early stage, and underlying mechanisms remain unclear. **Objective**: Our study aims at identifying sex-specific cognitive and brain network biomarkers associated to preclinical stage in AD. **Methodology**: We designed a longitudinal study to explore a new preclinical model, the *AppNL-F/MAPT*double knock-in (dKI) mouse, humanized for *App* and *MAPT* genes. We combined recognition memory tests with functional and structural brain Magnetic Resonance Imaging (MRI) to explore the relationship between sex-specific cognitive impairment and connectivity signatures associated to preclinical AD pathology. **Results**: At 2 months, dKI female mice did not detect the novel object (p=0,0003) in the Novel Object Recognition test compared to WT mice. WT and dKi male mice significantly explored the novel object (p=0,98). Therefore, females dKi mice presented memory deficits at a very early age in long-term memory tests when males didn't. **Conclusions**: Using subtle memory tests on a new mouse model of AD at early onset of AD, our study highlighted early cognitive impairments in female mice but not in male. Therefore, the dKI mouse model showed good promises in the characterization of the female vulnerability, especially at early onset AD. Sex-specific alteration in memory networks will be further explored using functional and structural MRI.

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BOARD NUMBER: S07-113

VOLUNTARY WHEEL RUNNING IN OLD C57BL/6 MICE REDUCES AGE-RELATED INFLAMMATION IN THE COLON BUT NOT IN THE BRAIN

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Inflammation is considered a possible cause of cognitive decline during aging. This study investigates the influence of physical activity and social isolation in old mice on their cognitive functions and inflammation. The Barnes maze task was performed to assess spatial learning and memory in 3, 9, 15, 24, and 28 months old male C57BL/6 mice as well as following voluntary wheel running (VWR) and social isolation (SI) in 20 months old mice. Inflammatory gene expression was analyzed in hippocampal and colonic samples by qPCR. Cognitive decline occurs in mice between 15 and 24 months of age. VWR improved cognitive functions while SI had negative effects. Expression of inflammatory markers changed during aging in the hippocampus (*Il1a*/*Il6*/*S100b*/*Iba1*/*Adgre1*/*Cd68*/*Itgam*) and colon (*Tnf*/*Il6*/*Il1ra*/*P2rx7*). VWR attenuates inflammaging specifically in the colon (*Ifng*/*Il10*/*Ccl2*/*S100b*/*Iba1*), while SI regulates intestinal *Il1b* and *Gfap*. Inflammatory markers in the hippocampus were not altered following VWR and SI. The main finding of our study is that both the hippocampus and colon exhibit an increase in inflammatory markers during aging, and that voluntary wheel running in old age exclusively attenuates intestinal inflammation. Based on the existence of the gut-brain axis, our results extend therapeutic approaches preserving cognitive functions in the elderly to the colon.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-114

THE ROLE OF TELOMERES IN NEURONAL AGING

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Telomere shortening is a developmentally regulated process leading mitotic cells to senescence. Whether changes in telomere and its associated protein complex Shelterin are involved in post-mitotic cell ageing remains elusive. Our group discovered that the expression of TRF2 (key Shelterin subunit) decreases with age and its downregulation in postmitotic myotubes leads to hallmarks of aging such as mitochondrial dysfunction and increased ROS production, but not to increased DNA damage. We set to investigate whether the same mechanisms occurred in the neuron, another type of long-lived post-mitotic cell. By means of *in vitro* and *in vivo* approaches assessing the effect of telomere alteration on the neuronal phenotype, we aimed at obtaining new insight on telomere dynamics with respect to neuronal aging. The deep understanding of these mechanisms might lead to the discovery of therapies against age-related pathologies and to their prevention.
The data obtained show that TRF2 neuronal expression declines with aging. Moreover, its downregulation in cultured primary neurons increases the rate of telomeric DNA damage and affects the vesicular axonal transport, which is pivotal for neuronal function. Furthermore, *in vivo* TRF2 downregulation via genetic tools impairs memory and learning processes as assessed via behavioural tests. These findings show that different types of post-mitotic cells age to different mechanisms, and that TRF2 neuronal decline is upstream of well-known aging phenomena. Our results suggest a modification of the paradigm that sees age-driven telomere changes uniquely upstream of mitotic cell aging, envisioning a wider role for telomeres in aging across the mitotic-postmitotic boundaries.

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BOARD NUMBER: S07-115

LICOCHALCONE'S NEUROPROTECTIVE ROLE IN A DOUBLE TRANSGENIC ALZHEIMER'S DISEASE MICE MODEL

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Even though Alzheimer's disease (AD) is the most common cause of dementia, it is still currently incurable. Recently, many hypotheses have stablished a clear relationship among inflammation and metabolic alterations with AD progression, becoming a potential target to stop neurodegeneration and remain in a cognitive resilient stage. Particularly, Licochalcone-A (LCA) has been described as an emergent drug with multiple therapeutical properties, such as an antidiabetic and anti-inflammatory effect, but little is known about the mechanism underlying LCA's neuroprotective effect. Therefore, this study aims to determinate the potential protective effect of LCA against hallmarks of AD. To perform this study, six-month-old male APPswe/PS1dE9 (APP) and C57BL/6J (WT) mice were intraperitoneally treated with saline or LCA at a dose of 15 mg/kg/day, three times per week for one month. Those mice were divided into 4 different experimental groups; WT Saline, WT LCA, APP Saline and APP LCA. Before sacrificing, insulin and glucose tolerance were analysed to determinate metabolic alterations and behavioural tests were performed to evaluate memory loss. Afterwards, hallmarks related to synapsis, metabolism and neuroinflammation were studied in the hippocampus. The present data reports the beneficial effect of LCA regarding to cognitive decline observed in AD by conferring neuroprotection against neuroinflammation and metabolic disruptions, improving dendritic spine deterioration and other synaptic hallmarks. All together contribute to the maintenance of cognitive resilience avoiding the development of AD. In conclusion, our results suggest that LCA could be a potential treatment against neurodegeneration by promoting a cognitive resilient stage of the disease.

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BOARD NUMBER: S07-116

METFORMIN AS A POTENTIAL ANTI-AGING THERAPY: TARGETING THE TRIPARTITE SYNAPSE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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With the rapidly growing aging population comes the impetus to develop strategies that alleviate age-related cognitive decline. Possible pharmacological agents to counteract brain aging are mostly neuron-focused, however astrocytes could be an interesting drug target as they are highly dynamic, crucially support as well as influence neuron function, and contribute to disease development and progression. The aim of this study is to investigate possible effects of pharmacological intervention on astrocyte-neuron interactions in normoglycemic conditions using metformin, with a focus on healthy aging. A well-established cell culture model was employed to assess the effect of 24-hour metformin treatment on neurons. In addition, a transgenic MetRS-Aldh1l1 mouse model, where mutated methionyl-tRNA synthetase (MetRS) is expressed solely in astrocytes, was used to assess the in vivo effects of metformin feeding on young, middle-aged and old mice. Our cell culture model revealed that metformin treatment had differing effects on protein translation and expression of key proteins involved in neuronal signalling and synaptic composition in mature (DIV23) and aged (DIV80) cultures. In mice, early indications show that 12 weeks of metformin feeding upregulated protein translation and glial fibrillary acidic protein (GFAP) expression in both young and old animals, however the effect was reversed in middle-aged animals. To conclude, metformin seems to have an impact on protein dynamics in astrocytes and neurons, and we aim to further elucidate how metformin alters astrocyte proteostasis and in turn synaptic protein expression during aging.

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BOARD NUMBER: S07-117

SPATIAL DISTRIBUTION OF GLYCOGEN IN LAYER I OF THE SOMATOSENSORY CORTEX IN AGING MICE

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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The brain utilizes between 20 to 25% of the available energy to ensure its proper function. Glucose, an important energy source for the brain, access the neuropil across the blood-brain barrier (BBB) and is transported into astrocytes, where it can be stored as glycogen. Astrocytes are the most abundant type of glial cell in the brain and play a crucial role in supporting neuronal metabolism. Lactate can be synthesized in astrocytes through glycogenolysis and then shuttled via monocarboxylate transporters (MCTs) to neurons to fuel their tricarboxylic acid (TCA) cycle. This mechanism, known as the astrocyte-neuron lactate shuttle (ANLS), is involved in learning and memory formation. Aging is associated with a decline of faculties such as memory, motor skills, and sensory perception, which are not caused by a substantial loss of neurons but rather changes at multiple levels (e.g., connectivity, morphology, white matter). Here, we aim to compare the glycogen distribution in layer I of the somatosensory cortex between young and aging mice. The visual analysis was done using a customized software to explore brain reconstructions at the nanometric level. Using the computational tool GLAM (Glycogen-derived Lactate Absorption Map), we inferred a probability map of the locations where astrocytic glycogen-derived lactate is most likely to access the surrounding neurites. We analyzed and compared these maps on axons, dendrites, boutons, and spines to hypothesize about single compartments' energy consumption. Our results indicate that aging brains have a more glycolytic metabolism, with fewer GLAM peaks facing mitochondria and smaller glycogen granules.

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BOARD NUMBER: S07-118

ASTROGLIAL HETEROGENEITY ASSESSED ACROSS REGIONS BY CELL TYPE-SPECIFIC PROTEOMIC LABELING IN THE YOUNG AND AGED MOUSE BRAIN

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Astrocytes exert varied functions to support and maintain nervous system homeostasis, modulate neuronal signaling but also contribute to the nervous system impairments in aging and disease. Upto date, little is known about regional astrocyte diversity and functional specifications in subregions of the brain which is, in part, due to a lack of selective tools to approach astrocytes. Here, we developed an astrocyte-specific metabolic labeling strategy using Tamoxifen-inducible Aldh1l1-*Cre/*ERT2 mice that allow for highly specific expression of MetRS^{L274G} in astrocytes. The subsequent application of the Non-Canonical Amino Acid Tagging(NCAT) technology *in vivo* enabled us to identify newly synthesized proteins cell type-specifically in astrocytes. This model allows analysis of translation rates in different brain regions as well as isolation of astrocytic proteins and subsequent Mass Spectrometry. Bioinformatic analyses verify the astrocytic origin of identified proteins and subsequent gene enrichment analysis reveals an abundant involvement in metabolic processes with little variation among brain regions. We further analyzed the protein turnover rates of astrocytes in subregions of the young(2 months) and old(24 months) mouse hippocampus. Astrocytes in the CA1 region showed higher translational activity that correlates with higher arborization compared to CA3 and DG astrocytes in younger ones. Aged hippocampal astrocytes revealed significantly higher protein turnover rates but contrastingly shrunken and less arborized morphology in the CA1 and DG regions of the hippocampus compared to young mice, which could contribute to loss of synaptic support during aging. Taken together, these results advance the knowledge of regional and age-dependent alterations in astrocyte protein dynamics and diversity.

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AGE-RELATED EFFECTS ON EXPLORATION STRATEGIES DURING PROBABILISTIC REWARD LEARNING

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims Decision-making often relies on a balance between exploration and exploitation. Behavioral studies of age-related effects on decision-making in humans have reported that healthy aging tends to shift the exploration/exploitation trade-off toward exploitation, coinciding with the use of less demanding cognitive processes. However, little is known on how age affects exploration strategies in uncertain environments. With this study, we aim to characterize the age-related differences between strategies relying on random (i.e., value-based or value-free) versus directed (i.e., uncertainty-driven) exploration. Methods We used 2- and 4-armed bandit tasks in a lab-based experiment with 34 subjects (17 young, 25.4 ± 4.6 y.o., and 17 older adults, 74.6 ± 3.8 y.o., recruited from the SilverSight cohort at the Vision Institute), and in an online study with 382 subjects (191 young, 22.0 ± 2.0 y.o., and 191 older adults, 73.7 ± 3.4 y.o., recruited through the Prolific platform). Participant were instructed to maximize their payoffs by repeatedly choosing between options to obtain rewards determined by a gaussian random walk. A reinforcement learning framework was employed to model subjects' task-solving policies. Results Older adults showed stronger individual differences than young adults and their strategies where better predicted by model-free policies. Unexpectedly, computational modeling showed that they explored no less than young adults, but relied primarily on random value-free exploration, whereas young adults mainly used directed exploration. Conclusions Healthy aging shapes the exploration/exploitation balance toward more random explorative strategies. A follow-up study will investigate age-related differences in exploration strategies during ecological spatial learning and goal-oriented navigation.

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BOARD NUMBER: S07-120

NEURAL CORRELATES OF AFFECTIVE EMPATHY IN AGING: A MULTIMODAL IMAGING, MULTIVARIATE APPROACH

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Various aspects of social cognition change as we age and can critically impact cognitive functioning among older adults. Empathy is one such social-cognitive capacity that undergoes age-related change. Previous work has started to identify brain correlates of empathy, but currently not well understood is the structural and functional neurocircuitry underlying age-related differences in empathy. To address this research gap, this study delineates brain structural and functional networks that subserve affective empathic response in younger and older adults using a modified version of the Multifaceted Empathy Task (MET). The MET differentiates affective from cognitive components of empathy, and we included both positive and negative emotions. Combining multimodal neuroimaging (functional magnetic resonance and diffusion-weighted imaging) with multivariate partial least square analysis resulted in three novel findings for affective empathy in older but not younger adults: *(a)* faster empathic responding to negative emotions was related to greater fractional anisotropy of the anterior cingulum and greater functional activity of the anterior cingulate network; *(b)* empathic responding to positive emotions was related to greater fractional anisotropy of the posterior cingulum and greater functional activity of the posterior cingulate network, and *(c)* faster empathic responding to both positive and negative emotions was related to greater fractional anisotropy of the left uncinate fasciculus and greater functional activity in a widespread anterior cingulate network that resembled the default mode network. These findings reflect a posterior-to-anterior-shift in aging (PASA), as previously observed in cognitive aging, regarding emotional valence suggesting an extension of the PASA model to higher-order social-cognitive processes.

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BOARD NUMBER: S07-121

AGED MALE RATS SHOW IMPAIRED COGNITIVE FLEXIBILITY BUT NO CHANGE IN MOTIVATION

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aims: Apathy is a psychiatric syndrome that is common in otherwise-healthy ageing. It consists of behavioural, cognitive and emotional domains. There is no agreed treatment approach for apathy, which may be driven by a lack of translational preclinical apathy models. We aimed to assess whether aged rats displayed a behavioural profile relevant to the behavioural and cognitive domains of human apathy. **Methods**: N=10 young (4-months-old) and aged (21-months-old) Sprague-Dawley male rats underwent a battery of behavioural tests hypothesised to map onto aspects of behavioural and cognitive apathy. The effort for reward (EfR) and progressive ratio (PR) tasks were used to assess physical effort-based motivation. The sucrose preference test (SPT) was used to assess reward sensitivity. A bowl-digging and operant-based probabilistic reversal learning (PRL) tasks were used to assess cognitive flexibility in an ethological and non-ethological context. **Results:** Aged rats showed no impairment in the EfR, PR, or SPT tasks. However, they showed a profound impairment in cognitive flexibility in the bowl-digging version of the PRLT. This deficit was specific to a reduction in win-stay behaviour. This deficit did not occur in the operant version of the task. **Conclusion:** Aged rats showed a specific deficit in cognitive flexibility associated with positive but not negative feedback. These effects were not related to any changes in physical effort or effort-based decision-making. The difference in findings between the bowl-digging and operant versions of the PRL suggests a greater sensitivity to changes associated with ageing using tasks based on more ethologically relevant cues.

Pubmed:
34104800: Jackson MG, Lightman SL, Gilmour G, Marston H, Robinson ESJ
Evidence for deficits in behavioural and physiological responses in aged mice relevant to the psychiatric symptom of apathy. Apathy is widely reported in patients with neurological disorders or post viral infection but is also seen in otherwise-healthy aged individuals. This study investigated whether aged male mice express behavioural and physiological changes relevant to an apathy phenotype. Using measures of motivation to work for reward, we found deficits in the progressive ratio task related to rate of responding. In an effort-related decision-making task, aged mice were less willing to exert effort for high value reward. Aged mice exhibited reduced reward sensitivity but also lower measures of anxiety in the novelty supressed feeding test and an attenuated response to restraint stress with lower corticosterone and reduced paraventricular nucleus c-fos activation. This profile of affective changes did not align with those observed in models of depression but suggested emotional blunting. In a test of cognition (novel object recognition), aged mice showed no impairments, but activity was lower in a measure of exploration in a novel environment. Together, these data suggest aged mice show changes across the domains of motivated behaviour, reward sensitivity and emotional reactivity and may be a suitable model for the pre-clinical study of the psychiatric symptom of apathy.
Brain Neurosci Adv, 2021 Jan-Dec; 5
35023405: Hinchcliffe JK, Jackson MG, Robinson ES
The use of ball pits and playpens in laboratory Lister Hooded male rats induces ultrasonic vocalisations indicating a more positive affective state and can reduce the welfare impacts of aversive procedures.
The advancement and quality of science rely on research that is robust and unbiased in its experimental design, execution, analysis, and reproducibility. In preclinical research, a better understanding of animal emotions and refinement of their husbandry, housing, and handling are important goals in providing good animal welfare in a laboratory setting which underpins rigorous research quality. Induction of positive emotional state in animals is a key component of their well-being, and one approach is to increase their environmental complexity using, for example, ball pits or playpens in rats. In this study, we recorded 50 kHz ultrasonic vocalisations (USVs) during animals' exposure to the ball pit and playpen. We have previously shown that 50 kHz USVs provide a graded and quantifiable measure of an animal's emotional state, and here find that access to the ball pit and playpen increases 50 kHz USVs, indicative of a more positive affective state. Using our affective bias test (ABT) we next quantified the animals' emotional response to an aversive intervention and whether this could be attenuated by

access to a playpen. The playpen exposure completely mitigated the negative affective state induced by an anxiogenic drug when compared with animals who experienced the drug in the home cage. Together, these findings suggest ball pits and playpens provide a simple and effective method to improve the welfare of laboratory rats and reduce the cumulative suffering they experience from their housing conditions and minor, aversive procedures.
Lab Anim, 2022;

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BOARD NUMBER: S07-122

AGE-DEPENDENT DYNAMICS IN ACUTE AND CHRONIC STRESS-INDUCED FOSB/ΔFOSB CONTENT IN THE EXTENDED AMYGDALA, HYPOTHALAMIC PARAVENTRICULAR, HABENULAR, CENTRALLY-PROJECTING EDINGER-WESTPHAL AND DORSAL RAPHE NUCLEI IN MALE RATS

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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The expression of early responding gene products (FOS, FOSB, ΔFOSB) can be activated by acute and chronic stimuli resulting in an increase of nuclear FOS protein content, that appears to decline by aging. Extended amygdala exhibits high basal FOSB/ΔFOSB content which does not change in response to acute restraint stress (ARS) or chronic variable mild stress (CVMS). Contrarily, the FOSB/ΔFOSB content increases upon CVMS in the lateral habenula (LHb), hypothalamic paraventricular nucleus (PVN), centrally-projecting Edinger-Westphal nucleus (EWcp) and dorsal raphe nucleus (DR). The age-dependent dynamics of FOSB/ΔFOSB reaction to stress is unknown. We aimed to semi-quantify the FOSB/ΔFOSB content of extended amygdala nuclei; habenula, PVN, EWcp, DR and the somatosensory cortex (S1) in the course of aging. Eight age groups (1-month-old (M), 1.5M, 2M, 3M, 6M, 12M, 18M, 24M) of rats were exposed to a single ARS versus controls. Rats in six age groups (2M, 3M, 6M, 12M, 18M, 24M) were subjected to CVMS. The FOSB/ΔFOSB immunoreactivity was a function of age in all groups. ARS increased the FOSB/ΔFOSB immunoreactivity in all nuclei. Only ventral and fusiform divisions of the bed nucleus of the stria terminalis (BNST) and PVN reacted throughout the examined lifespan. CVMS did not increase the FOSB/ΔFOSB immunoreactivity in BLA, oval and dorsolateral division of BNST, and S1. Corresponding to our previous observations on FOS, the FOSB/ΔFOSB response to stress decreased with age. Only the PVN exerted a sustained age-independent FOSB/ΔFOSB, which may reflect the long-lasting adaptation response and plasticity of neurons that maintain the stress (mal)adaptation.

Pubmed:
35109869: Ujvári B, Pytel B, Márton Z, Bognár M, Kovács LÁ, Farkas J, Gaszner T, Berta G, Kecskés A, Kormos V, Farkas B, Füredi N, Gaszner B
Neurodegeneration in the centrally-projecting Edinger-Westphal nucleus contributes to the non-motor symptoms of Parkinson's disease in the rat.
The neuropathological background of major depression and anxiety as non-motor symptoms of Parkinson's disease is much less understood than classical motor symptoms. Although, neurodegeneration of the Edinger-Westphal nucleus in human Parkinson's disease is a known phenomenon, its possible significance in mood status has never been elucidated. In this work we aimed at investigating whether neuron loss and alpha-synuclein accumulation in the urocortin 1 containing (UCN1) cells of the centrally-projecting Edinger-Westphal (EWcp) nucleus is associated with anxiety and depression-like state in the rat.
J Neuroinflammation, 2022; 19
31649527: Kovács LÁ, Berta G, Csernus V, Ujvári B, Füredi N, Gaszner B
Corticotropin-Releasing Factor-Producing Cells in the Paraventricular Nucleus of the Hypothalamus and Extended Amygdala Show Age-Dependent FOS and FOSB/DeltaFOSB Immunoreactivity in Acute and Chronic Stress Models in the Rat.
Corticotropin-releasing factor (CRF) immunoreactive (ir) neurons of the paraventricular nucleus of the hypothalamus (PVN) play pivotal role in the coordination of stress response. CRF-producing cells in the central nucleus of amygdala (CeA) and oval division of the bed nucleus of stria terminalis (BNSTov) are also involved in stress adaptation and mood control. Immediate early gene products, subunits of the transcription factor activator protein 1 (AP1) are commonly used as acute (FOS) and/or chronic (FOSB/deltaFOSB) markers for the neuronal activity in stress research. It is well known that the course of aging affects stress adaptation, but little is known about the aging-related stress sensitivity of CRF neurons. To the best of our knowledge, the stress-induced neuronal activity of CRF neurons in the course of aging in acute and chronic stress models was not studied systematically yet. Therefore, the aim of the present study was to quantify the acute restraint stress (ARS) and chronic variable mild stress (CVMS) evoked neuronal activity in CRF cells of the PVN, CeA, and BNSTov using triple-label immunofluorescence throughout the whole lifespan in the rat. We hypothesized that the FOS and FOSB content of

CRF cells upon ARS or CVMS decreases with age. Our results showed that the FOS and FOSB response to ARS declined with age in the PVN-CRF cells. BNSTov and CeA CRF cells did not show remarkable stress-induced elevation of these markers neither in ARS, nor in CVMS. Exposure to CVMS resulted in an age-independent significant increase of FOSB/delta FOSB immunosignal in PVN-CRF neurons. Unexpectedly, we detected a remarkable stress-independent FOSB/deltaFOSB signal in CeA- and BNSTov-CRF cells that declined with the course of aging. In summary, PVN-CRF cells show decreasing acute stress sensitivity (i.e., FOS and FOSB immunoreactivity) with the course of aging, while their (FOSB/deltaFOSB) responsivity to chronic challenge is maintained till senescence. Stress exposure does not affect the occurrence of the examined gene products in CeA- and BNSTov-CRF cells remarkably suggesting that their contribution to stress adaptation response does not require AP1-controlled transcriptional changes.
Front Aging Neurosci, 2019; 11
30186150: Kovács LÁ, Schiessl JA, Nafz AE, Csernus V, Gaszner B
Both Basal and Acute Restraint Stress-Induced c-Fos Expression Is Influenced by Age in the Extended Amygdala and Brainstem Stress Centers in Male Rats.
The hypothalamus-pituitary-adrenal axis (HPA) is the main regulator of the stress response. The key of the HPA is the parvocellular paraventricular nucleus of the hypothalamus (pPVN) controlled by higher-order limbic stress centers. The reactivity of the HPA axis is considered to be a function of age, but to date, little is known about the background of this age-dependency. Sporadic literature data suggest that the stress sensitivity as assessed by semi-quantitation of the neuronal activity marker c-Fos may also be influenced by age. Here, we aimed at investigating the HPA activity and c-Fos immunoreactivity 2 h after the beginning of a single 60 min acute restraint stress in eight age groups of male Wistar rats. We hypothesized that the function of the HPA axis (i.e., pPVN c-Fos and blood corticosterone (CORT) level), the neuronal activity of nine stress-related limbic areas (i.e., magnocellular PVN (mPVN), medial (MeA), central (CeA), basolateral nuclei of the amygdala, the oval (ovBNST), dorsolateral (dlBNST), dorsomedial (dmBNST), ventral and fusiform (fuBNST) divisions of the bed nucleus of the stria terminalis (BNST)), and two brainstem stress centers such as the centrally projecting Edinger-Westphal nucleus (cpEW) and dorsal raphe nucleus (DR) show age dependency in their c-Fos response. The somatosensory barrel cortex area (S1) was evaluated to test whether the age dependency is specific for stress-centers. Our results indicate that the stress-induced rise in blood CORT titer was lower in young age reflecting relatively low HPA activity. All 12 stress-related brain areas showed c-Fos response that peaked at 2 months of age. The magnitude of c-Fos immunoreactivity correlated negatively with age in seven regions (MeA, CeA, ovBNST, dlBNST, dmBNST, fuBNST and pPVN). Unexpectedly, the CeA, ovBNST and cpEW showed a considerable basal c-Fos expression in 1-month-old rats which decreased with age. The S1 showed a U-shaped age-related dynamics in contrast to the decline observed in stress centers. We conclude that the age- and brain area dependent dynamics in stress-induced neuronal activity pattern may contribute to the age dependance of the stress reactivity. Further studies are in progress to determine the neurochemical identity of neurons showing age-dependent basal and/or stress-induced c-Fos expression.
Front Aging Neurosci, 2018; 10
28450265: Farkas J, Kovács LÁ, Gáspár L, Nafz A, Gaszner T, Ujvári B, Kormos V, Csernus V, Hashimoto H, Reglődi D, Gaszner B
Construct and face validity of a new model for the three-hit theory of depression using PACAP mutant mice on CD1 background.
Major depression is a common cause of chronic disability. Despite decades of efforts, no equivocally accepted animal model is available for studying depression. We tested the validity of a new model based on the three-hit concept of vulnerability and resilience. Genetic predisposition (hit 1, mutation of pituitary adenylate cyclase-activating polypeptide, PACAP gene), early-life adversity (hit 2, 180-min maternal deprivation, MD180) and chronic variable mild stress (hit 3, CVMS) were combined. Physical, endocrinological, behavioral and functional morphological tools were used to validate the model. Body- and adrenal weight changes as well as corticosterone titers proved that CVMS was effective. Forced swim test indicated increased depression in CVMS PACAP heterozygous (Hz) mice with MD180 history, accompanied by elevated anxiety level in marble burying test. Corticotropin-releasing factor neurons in the oval division of the bed nucleus of the stria terminalis showed increased FosB expression, which was refractive to CVMS exposure in wild-type and Hz mice. Urocortin1 neurons became over-active in CMVS-exposed PACAP knock out (KO) mice with MD180 history, suggesting the contribution of centrally projecting Edinger-Westphal nucleus to the reduced depression and anxiety level of stressed KO mice. Serotoninergic neurons of the dorsal raphe nucleus lost their adaptation ability to CVMS in MD180 mice. In conclusion, the construct and face validity criteria suggest that MD180 PACAP HZ mice on CD1 background upon CVMS may be used as a reliable model for the three-hit theory.
Neuroscience, 2017; 354

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BOARD NUMBER: S07-123

GREY AND WHITE MATTER MICROSTRUCTURE PLAY COMPLEMENTARY ROLES SUPPORTING COGNITIVE PERFORMANCE IN ADOLESCENCE OF THE ABCD COHORT.

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Aim During development, grey and white matter changes enable children to reach the maximum of their cognitive abilities. Researchers tend to specialize their research on one of these structures, thus it is still unclear if grey or white matter microstructure are playing distinct roles supporting cognitive development or if they are complementary. **Methods** To compare their role, we used structural equation models predicting cognitive performance with grey and white matter measures. Specifically, we compared morphometric grey matter measures (volume, cortical thickness and surface area) and indices of white matter microstructure (volume, fractional anisotropy and mean diffusivity). The models were tested in a large longitudinal cohort of adolescents (ABCD Study, N=11876) at 10 and 12 years old. We will also move beyond cross-sectional data and analyse the roles of grey and white matter changes in supporting cognitive development between 10 and 12 years old. **Results** We found that grey and white matter metrics bring partly different information to predict cognitive abilities. Indeed, the models with only grey or white matter explained respectively 22% and 19% of the variance in cognitive performance, but the model with both explained 32%. In particular, different metrics within grey and white matter had different predictive power and different regions for grey and white matter had the biggest impact on cognitive abilities. **Conclusion** These results show that studies focusing on a single metric in either grey or white matter to study the link between brain structure and cognitive development are missing a key part of the equation.

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BOARD NUMBER: S07-124

INFLUENCE OF GLUCOSE METABOLISM DISORDERS ON MCI CONVERSION TO ALZHEIMER'S DISEASE DEMENTIA IN THE BALTAZAR STUDY

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Background Diabetes is associated with a higher risk of dementia. Little is known on the underlying physiopathological process and some studies suggested that prediabetic stage has a deleterious effect on brain. Our aim was to determine the glucose metabolism disorders impact in mild cognitive impairment (MCI) subjects on cognitive functioning, Alzheimer's disease (AD) cerebrospinal fluid (CSF) and plasma biomarkers, and conversion to AD dementia. **Method** MCI participants of BALTAZAR study, a large-scale longitudinal multicenter cohort, were followed-up during 3 years. MCI participants were categorized based on their glucose metabolism status (non-diabetic, prediabetic, diabetic) at baseline. A committee of experts adjudicated MCI conversion to AD dementia. The association between glucose metabolism disorders and cognition, CSF and plasmatic AD biomarkers at baseline, and MCI conversion during the follow-up were determined using parametric tests and logistic regression. **Results** Of 400 MCI participants, 252 (63%) were non-diabetic, 80 (20%) prediabetics and 68 (17%) diabetics. At baseline, glucose metabolism disorders had no significant association with cognitive function. Prediabetics compared to diabetics had a significant lower plasma $A\beta_{1-42}$ concentration and a higher CSF P-tau/$A\beta_{1-42}$ ratio, and prediabetics compared to non-diabetics had a higher plasma $A\beta_{1-40}$ /$A\beta_{1-42}$ ratio. For 3 years of follow-up, 155 (31.2%) MCI converted to AD dementia. Prediabetes, but not diabetes, was associated with a two-fold risk of conversion to AD dementia (odds ratio (95% CI) = 2.02 (1.03-3.96)). **Conclusion** Prediabetes was an independent risk for dementia. Early detection and treatment of prediabetes in MCI patients could be critical for AD dementia prevention.

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CONSCIOUSNESS, DEMENTIA AND CALCIUM CARBONATE: A NEW ETIOPATHOGENETIC THEORY

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Among all kinds of dementia, the senile dementia has still a multifactorial etiopathogenesis. What if we could find a calcium channel incrusted with a calcium salt molecule? What kind of damage could do at a local and adjacent cellular area? What if this formula could be the source of senile dementia? $CO_2 + H_2O \Leftrightarrow 2H^+ + CO_3^{2-} + Ca^{2+} >$ **$CaCO_3$** However, all these chemicals can be found in our interstitial fluid and neuronal membrane, according to the medical bibliography, I am expecting that: **Micro-regional level** Changes in the membrane next to the calcic channel Slight deformation of the adjacent protein sites Folding and production of beta amyloid protein Activation of the inflammatory process in order to repair the defect **Macro-regional level** Resting potential increase Spike transmission reduced Tao detachment from the axon Reduced consume of energy Reduced activity and local perfusion Water cortical concentration lowering Water falls by gravity into the ventricles Stoke risk increased and more compression next to ventricle As a result: Mechanical resistance would increase Membrane fluidity wouldn't change Pulsatility would decrease If we can demonstrate that the prefrontal and frontal area are involved in this process, we can explain the symptoms of dementia and the aging of the brain.

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BOARD NUMBER: S07-126

UBIQUINOL SUPPLEMENTATION IMPROVES GENDER-DEPENDENT CEREBRAL VASOREACTIVITY AND AMELIORATES CHRONIC INFLAMMATION AND ENDOTHELIAL DYSFUNCTION ASSOCIATED WITH MILD COGNITIVE IMPAIRMENT

POSTER SESSION 07 - SECTION: AGING AND THE BRAIN

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Background: Multiple mechanisms cause endothelial damage and vascular dysfunction, contributing to dementia. Endothelial cells can protect themselves from these insults by using coenzyme Q10 or its reduced form, ubiquinol. **Methods**: A total of 69 participants diagnosed with mild cognitive impairment (MCI) received either 200 mg/day ubiquinol (Ub) or placebo for 1 year. Cognitive assessment of patients was performed at baseline and after 1 year of follow-up. Doppler sonography, levels of Ubiquinol (Ub) and lipopolysaccharide (LPS) was examined. Cell viability and necrotic cell death were determined using the microvascular endothelial cell line bEnd3. **Results**: Coenzyme Q10 levels increased in patients supplemented for 1 year with ubiquinol versus the baseline group and the placebo group, these levels being higher in male than female patients. 21.2% of the ubiquinol group progressed to dementia, compared to 27.8% of the placebo group. This observation was reflected in an increase in the rate of breath retention, which was significant in male patients. Similarly, male patients after consuming ubiquinol showed lower lps levels compared to baseline. A significant negative correlation was established between plasma coenzyme Q10 concentration and necrosis-induced cell death in cultured bEnd3 cells when filtering for T2DM and hypertensive groups. **Conclusions**: Oral supplementation with ubiquinol increases plasma levels of coenzyme Q10 without side effects, reduces chronic inflammation and improves cerebral vasoreactivity and endothelial parameters in male patients. This suggests that ubiquinol supplementation might be recommended for patients with MCI as an adjunct to conventional treatment.

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COMPARING THE ANTIDEPRESSANT-LIKE EFFICACY OF KETAMINE, CANNABIDIOL AND FLUOXETINE IN MALE AND FEMALE ADOLESCENT RATS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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There is an urgent need for developing novel pharmacological treatment options for adolescent depression, and to ensure an optimal translational outcome to the clinic, sex should be included as a biological variable in preclinical studies. In this context, the present study compared the antidepressant-like potential of ketamine and cannabidiol, with the clinical standard fluoxetine, in adolescent rats exposed to maternal deprivation (as a model of early-life stress), while including a sex perspective. Moreover, changes in drug efficacy over time were evaluated by re-exposing rats to the same dose regimens during adulthood. Antidepressant-like responses were scored through a battery of distinctive tests (forced-swim, novelty suppressed-feeding, sucrose preference) across time. The main results proved an antidepressant-like potential for ketamine and cannabidiol in adolescent rats, although their efficacy was dependent on sex and prior stress exposure, as well as on treatment-length and the behavioral feature analyzed. In general, while all tested antidepressants in male rats improved certain affective-like features, female rats were mainly unresponsive to the treatments performed (except for certain benefits induced by ketamine), demonstrating the need for further characterizing proper treatments for this particular sex. Moreover, when rats were re-exposed in adulthood to the same drug regimens as in adolescence, a drop in efficacy was observed. These findings may have translational ramifications in that ketamine or cannabidiol could be moved forward as antidepressants for the adolescent depressed population, but not before further characterizing their potential long-term safety and/or beneficial vs. harmful effects for both sexes. PID2020-118582RB-I00 (MCIN/AEI/10.13039/501100011033) and “JUNIOR” program (IdISBa, GOIB).

Pubmed:
33160794: García-Cabrerizo R, Ledesma-Corvi S, Bis-Humbert C, García-Fuster MJ
Sex differences in the antidepressant-like potential of repeated electroconvulsive seizures in adolescent and adult rats: Regulation of the early stages of hippocampal neurogenesis.
Age and sex are critical factors for the diagnosis and treatment of major depression, since there is a well-known age-by-sex difference in the prevalence of major depression (being females the most vulnerable ones) and in antidepressant efficacy (being adolescence a less responsive period than adulthood). Although the induction of electroconvulsive seizures (ECS) is a very old technique in humans, there is not much evidence reporting sex- and age-specific aspects of this treatment. The present study evaluated the antidepressant- and neurogenic-like potential of repeated ECS across time in adolescent and adult rats (naïve or in a model of early life stress capable of mimicking a pro-depressive phenotype), while including a sex perspective. The main results demonstrated age- and sex-specific differences in the antidepressant-like potential of repeated ECS, since it worked when administered during adolescence or adulthood in male rats (although with a shorter length in adolescence), while in females rendered deleterious during adolescence and ineffective in adulthood. Yet, repeated ECS increased cell proliferation and vastly boosted young neuronal survival in a time-dependent manner for both sexes and independently of age. Moreover, pharmacological inhibition of basal cell proliferation prevented the antidepressant-like effect induced by repeated ECS in male rats, but only partially blocked the very robust increase in the initial cell markers of hippocampal neurogenesis. Overall, the present results suggest that the induction of the early phases of neurogenesis by ECS, besides having a role in mediating its antidepressant-like effect, might participate in some other neuroplastic actions, opening the path for future studies.
Eur Neuropsychopharmacol, 2020; 41

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BOARD NUMBER: S07-128

INVESTIGATING LONG-TERM EFFECTS OF PSYCHEDELIC DRUG DOI ON ANATOMICAL AND BEHAVIOURAL PLASTICITY IN A MOUSE MODEL

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Psychedelics putatively aid fast and lasting remission from various neuropsychiatric disorders by heightening brain plasticity. Growing evidence in both humans and preclinical models shows increased cellular neuroplasticity in the days after psychedelic treatment. However, the link between these cellular effects and the profound shifts in behaviour remains unclear. We focus on the long-term (≤3-week-post-drug) effects of psychedelic drug DOI on the behavioural and whole-brain structural plasticity of C57BL/6 mice. Behavioural plasticity was tested as adaptability in a probabilistic two-step reversal-learning task, while structural plasticity was quantified via regional brain volume changes using *ex vivo* magnetic resonance imaging. We find an increase in regional grey matter volume of several sensory areas 24h after DOI administration, but preliminary results show these volume differences may not persist 3 weeks post-drug. In the reversal learning task, DOI-treated animals were quicker to adapt to learned reversals only in the first week post-drug but were later also quicker to adapt to a novel rule reversal when they started incorporating learning from reward omissions. However, overall choice accuracy remained comparable to control animals throughout the study. As psychedelic-enhanced plasticity is linked with increased environmental sensitivity, we additionally compared acute drug responses of animals injected in a novel versus familiar environment. Novelty enhanced animals' sensitivity to hallucination-like effects, while familiarity enhanced the animals' sensitivity to DOI's effects on exploration. Our results demonstrate that a single dose of DOI can trigger a complex timeline of enhanced higher-level plasticity changes in the weeks following psychedelic treatment, even in healthy animals.

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A SUBCOMMISSURAL ORGAN-SPONDIN-DERIVED PEPTIDE (NX210C) TO ALLEVIATE COVID-19-RELATED NEUROCOGNITIVE DEFICITS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aim: About 10-30% of long-COVID patients, among multiple symptoms, suffer from persistent, debilitating cognitive deficits that alter their quality of life, and this independently from the severity of the initial infection. There is thus an urgent need to find an effective treatment for these patients. To date, no animal model recapitulates the neurocognitive symptoms of long-COVID. Our aim was therefore to define a rationale for the use of NX210c in long-COVID patients by correlating its properties with any neurological alterations which might cause cognitive impairments after SARS-CoV-2 infection. **Methods:** CNS-related effects of NX210c have been demonstrated during its preclinical and early clinical development, in particular on cognitive functions. The cerebral damages due to COVID-19 were picked up from the literature to describe how NX210c could tackle this emerging condition associated with cognitive deficits. **Results/Conclusions:** Our research with NX210c unravelled several targets and mechanisms that help restore cognition, enhance excitatory synaptic transmission, increase neuronal recovery after hypoxia, promote neuronal survival, alleviate neuroinflammation, stimulate remyelination, reduce blood-brain barrier permeability and even lower plasma homocysteine levels. Interestingly, all these processes can be impacted by SARS-CoV-2 infection. As NX210c combines these different properties into one single drug, it is expected to improve cognitive outcomes in long-COVID patients. A first-in-human study showed the good safety and tolerability profile of NX210c in healthy volunteers. The study design of a phase Ib/II clinical trial planned in 2022 to evaluate the safety, tolerability, pharmacokinetics, and efficacy of NX210c in patients with long COVID-related cognitive deficits will be presented.

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BOARD NUMBER: S07-130

THE EFFECT OF OXYTOCIN ON THE PERCEPTION OF TACTILE SENSATIONS OF INTERNAL AND EXTERNAL ORIGIN

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Oxytocin (OT), is a neuropeptide hormone, well known for its impact on social cognition. Recent research in rodents has shown the role of oxytocin in the regulation of tactile sensations and social proximity through touch. In humans, the underlying mechanisms of how oxytocin modulates one's own and others' sensory perception remains unclear. Interestingly, skin contact experience is influenced by OT release *via* the vagus nerve, a pathway involved in interoceptive processing. In this study, we hypothesize that the role of OT in promoting social proximity may be mediated by a process in which internal sensory stimulation is reduced to bias individuals' attention to the social environment. We investigated the effects of 24 and 40IU intranasal oxytocin in a tactile stimulation task in 90 male participants before and after the administration. Subjects were asked to rate the effectiveness of the stimulation (tickly, intense, pleasant, irritating) delivered by a tickling device under self- or external-production conditions. Compared to externally produced stimulation, it is well known that we cannot tickle ourselves, because of the sensory effects anticipated by our brain. Hence, consistent with our hypothesis we primarily expected a decrease in tingling sensation during external stimulation due to the internal sensory attenuation effect of OT. Our results showed a significant effect of 24IU administration on reducing tactile sensation during pleasant ratings. We speculate that the effect of OT on bodily sensations could be the starting point for its broader social influence.

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BOARD NUMBER: S07-131

UPREGULATING ACETYLCHOLINE ENHANCES FORAGING OPTIMALITY

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Stick to the current course of action and exploit the rewards it provides or switch and explore other, potentially more rewarding options? In such foraging problems, options are experienced sequentially, and the agent needs to decide when to leave the current option for the next one. According to the Marginal Value Theorem (MVT), optimal foraging consists in leaving the current option when its reward rate falls below the average reward rate of all alternatives. However, foragers often violate this rule and exploit options longer than predicted by MVT. Here, we investigated whether upregulation of dopaminergic, noradrenergic, and cholinergic systems increases foraging optimality. We assessed this question with a double-blind, placebo-controlled design, where participants received placebo, a dopamine or norepinephrine reuptake inhibitor (methylphenidate or reboxetine), or the nicotinic acetylcholine receptor agonist nicotine. Randomly assigned to one of four medication groups, 160 healthy volunteers took the role of a foraging farmer who aims to optimize milk returns from cows in the fields. We found that on average our participants in all groups were biased towards overexploitation when foraging. Our results show that while methylphenidate and reboxetine administration had weak or no effects on this bias, nicotine significantly reduced deviation from foraging optimality, which resulted in better performance when compared to the placebo group. We also measured pupil dilation to investigate whether drug administration affects the role of arousal in solving the exploration-exploitation dilemma. Our findings will elucidate the neurochemical basis of decision optimality and speak to research on disorders affecting these functions.

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ALOGLIPTIN ATTENUATES LIPOPOLYSACCHARIDE-INDUCED NEUROINFLAMMATION IN MICE THROUGH MODULATION OF TLR4/MYD88/NF-κB AND MIRNA-155/SOCS-1 SIGNALING PATHWAYS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Background: Endotoxin-induced neuroinflammation plays a crucial role in the pathogenesis and progression of various neurodegenerative diseases. A growing body of evidence supports that incretin-acting drugs possess various neuroprotective effects that can improve learning and memory impairments in Alzheimer's disease models. Thus, the present study aimed to investigate whether alogliptin, a dipeptidyl peptidase-4 inhibitor, has neuroprotective effects against lipopolysaccharide (LPS)-induced neuroinflammation and cognitive impairment in mice as well as the potential mechanisms underlying these effects.
Methods: Mice were treated with alogliptin (20 mg/kg/d; p.o.) for 14 days, starting 1 day prior to intracerebroventricular LPS injection (8 μg/μL in 3 μL).
Results: Alogliptin treatment alleviated LPS-induced cognitive impairment as assessed by Morris water maze and novel object
recognition tests. Moreover, alogliptin reversed LPS-induced increases in toll-like receptor 4 and myeloid differentiation primary response 88 protein expression, nuclear factor-κB p65 content, and microRNA-155 gene expression. It also rescued LPS-induced decreases in suppressor of cytokine signaling gene expression, cyclic adenosine monophosphate (cAMP) content,
and phosphorylated cAMP response element binding protein expression in the brain.
Conclusion: The present study sheds light on the potential neuroprotective effects of alogliptin against intracerebroventricular LPS-induced neuroinflammation and its associated memory impairment via inhibition of toll-like receptor 4/ myeloid differentiation primary response 88/ nuclear factor-κB signaling, modulation of microRNA-155/suppressor of cytokine signaling-1 expression, and enhancement of cAMP/phosphorylated cAMP response element binding protein signaling.

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BOARD NUMBER: S07-133

AGE-SPECIFIC MEMORY IMPAIRMENT INDUCED BY CO-EXPOSURE TO NICOTINE AND A SYNTHETIC CANNABINOID IN MICE

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

Gilda Angela Neves[1], Patricia Gonçalves[1], Luis Eduardo Nunes[1], Brenda Andrade[1], Mariana Silva[2], Isis Souza[1], Iranaia Assunçao-Miranda[2], Newton Castro[1]
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Adolescence is a neurodevelopmental critical period when the brain is especially susceptible to environmental factors. It is also a life stage where people tend to abuse drugs. Nicotine is the second most abused licit drug in the world. Among the illicit drugs, cannabis and its derivatives can be highlighted, including the recent rising epidemy of synthetic cannabinoids use. Most importantly, around 90% of cannabis users smoke tobacco, and 19% are considered nicotine dependent. However, short and long-term outcomes of this co-use by vulnerable populations (such as adolescents) are not clear. Thus, we investigated the consequences of heavy exposure to nicotine and a synthetic cannabinoid during adolescence. Male Swiss mice (PND28) were exposed to nicotine, WIN 55212-2 or both, for 20 days and evaluated in several behavioral tasks. Nicotine-exposed mice presented hyperlocomotion in the open field that was not affected by WIN co-exposure. Only mice that received nicotine and WIN showed a memory impairment in the novel object recognition task. These behavioral changes were not persistent since they were not detected when mice were evaluated in adulthood. Moreover, adult mice submitted to the same drug-exposure protocol starting at PND70 did not show any behavioral change. Investigation of drug receptors in the hippocampus showed a discreet reduction in β2-nicotine receptor subunit expression in all nicotine-exposed adolescents. In summary, we described an age-specific memory impairment induced by nicotine and WIN co-exposure in mice that is not persistent. Further studies are necessary to elucidate the mechanisms involved in this deficit. Funding: CNPq-Brazil, CAPES-Brazil, ISN.

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FIVE-WEEK INTRANASAL NERVE GROWTH FACTOR TREATMENT IS SAFE AND FAVORS BRAIN NEUROGENESIS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Objective: To date, no safe and effective pharmacological treatment has been clinically validated for improving post-stroke neurogenesis. Growth factors are good candidates but low safety has limited their application in the clinic. An additional restraint is the delivery route. Intranasal delivery (IN) presents many advantages. *Methods*: A brain lesion was induced in twenty-four rats. Nerve growth factor (NGF) 5 µg/kg/day or vehicle was given IN from day 10 post-lesion for two periods of five weeks, separated by a two-week wash out period with no treatment. Lesion volume and atrophy were identified by magnetic resonance imaging. Anxiety and sensorimotor recovery were measured by behavior tests. Neurogenesis, angiogenesis and inflammation were evaluated by histology at 3 months. *Results*: Remarkable neurogenesis occurred between the second and third month after the insult. Tissue reconstruction was clearly detected by T2 weighted MRI 3 months post-lesion and confirmed by histology. In the new tissue, NGF significantly increased the percentage of mature neo-neurons (19% *vs* 7%). Sensorimotor recovery was neither improved nor hampered by NGF during the first period of treatment. *Interpretation*. The first five-week period of treatment was very well tolerated and safe. The non-invasive NGF treatment can easily be transferred to the clinic. This study is the first presenting the effects of a long treatment with NGF and has shown an important tissue regeneration rate at 3 months. NGF increased neuron differentiation and survival. For the first time, we evidenced a MRI biomarker of neurogenesis and tissue reconstruction with T2 and diffusion weighted imaging.

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KETAMINE DOES NOT PRODUCE CROSS-SENSITIZATION TO AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY IN MALE RATS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aims: Repeated administration of psychostimulants produces an enduring and progressively enhanced behavioral response known as behavioral sensitization (BS), which has been implicated as a model of drug addiction. Ketamine is used to treat depression, but the issue of its abuse potential has been controversial. We adopted the cross-sensitization approach to investigate whether racemic ketamine (RS-KET) and one of its isomers (S)-ketamine (S-KET) could replace amphetamine to produce BS. Methods: Stimulant-induced BS on locomotor activity was tested by a pre- and a post-test with a lower dose of d-amphetamine (d-AMP; 0.5 mg/kg) conducted respectively before and after a 7-day intermittently repeated injections of 1 mg/kg d-AMP in male rats. RS-KET and S-KET (5 and 10 mg/kg) were separately tested in two groups of rats (n=14 each) during the cross-sensitization (CS) test. Further, the long-lasting effects of d-AMP-induced BS and KET-CS were examined following a 3-week duration of withdrawal. Results: The locomotor activity was significantly increased by d-AMP over the 7-day induction phase. Significant BS produced by d-AMP was verified by the hyperlocomotion induced by the challenging dose on the post-test. RS-KET and S-KET did not induce a CS in the d-AMP-sensitized rats. These results were replicated after a 3-week withdrawal. Conclusions: This study confirms BS of locomotor activity induced by d-AMP and shows that RS-KET or S-KET is insufficient to produce CS in the d-AMP-sensitized rats. Keywords: drug abuse, behavioral sensitization, cross-sensitization, ketamine isomers, psychostimulants

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BOARD NUMBER: S07-136

COMP360 PSILOCYBIN RESTORES REWARD LEARNING IMPAIRMENTS IN RATS CAUSED BY CHRONIC INTERFERON-ALPHA TREATMENT

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aims Impaired reward learning, an essential feature of affective disorders, can be recapitulated in mechanistically diverse animal models. For example, chronic inflammatory stimulation with interferon-alpha (IFNα) in rats disrupts performance in the Reward Learning Assay (RLA). We aimed to explore effects of COMP360 (COMPASS Pathways' proprietary psilocybin formulation) in IFNα-treated animals with this assay. **Methods** 48 male Lister-Hooded rats were divided into three groups (n=16); vehicle-vehicle (healthy control), IFNα-vehicle (disease phenotype control) and IFNα-COMP360 (experimental intervention). Vehicle or IFNα (100 units/kg, i.p.) was administered daily for two weeks before giving a single dose of vehicle or COMP360 (0.3 mg/kg, i.p.). Animals were tested in two RLAs with learning occurring before or after vehicle/COMP360 treatment, while daily vehicle/IFNα administration continued. In the RLA, rats dig for reward in a choice of digging substrates. Two substrates are consistently reward-paired during learning, one low value (single sucrose pellet), one high value (two pellets). Choice bias is quantified toward the high- vs. low-value substrate over 30 trials and group mean tested against zero bias with two-tailed one-sample t-test. **Results** Healthy control animals demonstrated significant choice bias for the high-value substrate ($p<0.001$), unlike disease phenotype controls ($p>0.05$). COMP360-treated animals showed some evidence of recovering high-value substrate choice bias 24 hours post-dose for previously learned substrates ($p=0.002$) and demonstrated high-value substrate choice bias in another RLA learned post-dose ($p=0.0002$). **Conclusion** The deleterious effects of chronic IFNα treatment on reward-driven choice bias in rats can be at least partially reversed by a single COMP360 dose (0.3 mg/kg).

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BOARD NUMBER: S07-137

TYPE 1 DIABETES MEDIATED MICROVASCULAR DYSFUNCTION AND IMPAIRED BEHAVIORAL PERFORMANCE IN MICE

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Introduction Recently our lab has shown that the brain capillaries routinely get clogged by cells and debris even under healthy conditions. The present study was undertaken to determine how type 1 diabetes mellitus (T1DM) affects this phenomenon, and whether obstructions contribute to cognitive decline. **Methods** C57BL/6 male and female mice were injected with streptozotocin to induce T1DM. These mice were implanted with cranial windows and cortical volumes were repeatedly imaged from 3-9 weeks after induction of diabetes. To model susceptibilities to short or long-lived obstructions, we injected (i.v.) 5µm diameter fluorescent microspheres in diabetic and control mice at 30 minutes and 3 days before euthanasia. To determine the impact of diabetes on cognitive activity, mice were subjected to a battery of behavioural tests. **Results** 2-photon imaging indicated that diabetic mice have higher rates of capillary stalling in somatosensory cortex that became more pronounced with duration of diabetes. We also observed significantly higher levels of short and long lived capillary obstructions in diabetic mice. Behaviourally, diabetic mice were not different from controls in tests of ambulatory activity. However, diabetic mice were significantly impaired in learning/memory tests such as the novel object recognition, water maze learning or reversal learning. **Conclusions** These studies suggests that diabetes is associated with greater risk for capillary obstructions in brain as well as learning/memory deficits. Our future aims will provide a mechanistic understanding of how diabetes elevates one's susceptibility to capillary obstructions and cognitive decline, and whether manipulating certain immune signalling pathways can alleviate these impairments.

Pubmed:
34171341: Sharma S, Brown CE
Microvascular basis of cognitive impairment in type 1 diabetes.
The complex computations of the brain require a constant supply of blood flow to meet its immense metabolic needs. Perturbations in blood supply, even in the smallest vascular networks, can have a profound effect on neuronal function and cognition. Type 1 diabetes is a prevalent and insidious metabolic disorder that progressively and heterogeneously disrupts vascular signalling and function in the brain. As a result, it is associated with an array of adverse vascular changes such as impaired regulation of vascular tone, pathological neovascularization and vasoregression, capillary plugging and blood brain barrier disruption. In this review, we highlight the link between microvascular dysfunction and cognitive impairment that is commonly associated with type 1 diabetes, with the aim of synthesizing current knowledge in this field.
Pharmacol Ther, 2022; 229

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-138

THE EFFECT OF FLUOXETINE ON BEHAVIOUR AND BDNF EPIGENETIC REGULATION DEPENDS ON THE INDIVIDUAL EXPERIENCE OF THE ENVIRONMENT

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aim: Selective serotonin reuptake inhibitors (SSRIs), the first line antidepressant treatment, have a variable efficacy. To explain such variability, we have demonstrated that SSRIs do not affect mood per se but amplify the influence of environment on mood. Since the environment is not only its objective features, but also the individual's experience of it, we hypothesized that the latter plays a key role in determining SSRI outcome. **Methods**: We tested CD-1 male mice reared either in a standard nest (SN) or a communal nest (CN) since they have different experiences of the environment as shown by their different adult neural and behavioural responses to the same challenges. CN consists in a single nest where three dams rear together their offspring and share caregiver behaviour from birth to weaning. At adulthood, both groups received either the SSRI fluoxetine (10 mg/kg/day) or vehicle for three weeks and behavioural responses, epigenetic changes and expression of the BDNF gene were assessed. **Results:** First, we confirmed previous studies showing that, at baseline, the two groups differently experience the same environmental challenges, CN displaying higher levels of offensive and anxiety-like behaviour and increased BDNF levels compared to SN mice. Furthermore, in line with our hypothesis, we showed that fluoxetine produced opposite effects on behaviour, BDNF levels and epigenetic regulation in CN and SN mice **Conclusions:** These findings show that fluoxetine effects depend on the individual experience of the environment, suggesting that subjective views potentially affect SSRI antidepressant outcome.

Pubmed:
34274033: Vai B, Mazza MG, Delli Colli C, Foiselle M, Allen B, Benedetti F, Borsini A, Casanova Dias M, Tamouza R, Leboyer M, Benros ME, Branchi I, Fusar-Poli P, De Picker LJ
Mental disorders and risk of COVID-19-related mortality, hospitalisation, and intensive care unit admission: a systematic review and meta-analysis.
Mental disorders might be a risk factor for severe COVID-19. We aimed to assess the specific risks of COVID-19-related mortality, hospitalisation, and intensive care unit (ICU) admission associated with any pre-existing mental disorder, and specific diagnostic categories of mental disorders, and exposure to psychopharmacological drug classes.
Lancet Psychiatry, 2021; 8

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-139

BEHAVIORAL SENSITIZATION OF LOCOMOTOR ACTIVITY INDUCED BY KETAMINE UNDER AN INTERMITTENTLY-REPEATED AND ESCALATING DOSE REGIMEN AND CROSS-SENSITIZATION TO D-AMPHETAMINE

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Behavioral sensitization (BS) developed by repeated exposure to drugs of abuse causes a progressive and enduring enhancement of their psychomotor and positive reinforcing effects. While BS of the locomotor activity induced by d-amphetamine (d-AMP) has been widely reported in rats, the results reported to ketamine are mixed and controversial. This study was designed to test if BS would be developed to racemic ketamine (RS-KET) and one of its isomers (S)-ketamine (S-KET) given under an intermittently-repeated and escalating dose regimen. In male adult rats, KET-induced BS on locomotor activity was tested by a pre- and a post-test with a lower dose of 5 mg/kg conducted respectively before and after a 7-day intermittently-repeated injection regimen. During the induction phase, the doses of RS-KET or S-KET injection were 5 mg/kg for two days, 10 mg/kg for 3 days, and 15 mg/kg for two days. There was a 3-day withdrawal between the post-test session and the last day of aforementioned injection regimen. A subsequent test of cross-sensitization to d-AMP was conducted. The tests of BS to ketamine and cross-sensitization to d-AMP were re-conducted following a 3-week withdrawal. For the results, BS was significantly developed to either RS-KET or S-KET administered under the present regiment of intermittently-repeated and escalating dose injections, which was cross-sensitized to d-AMP. These pharmacological effects significantly remained after a 3-week withdrawal. Together with other reports, BS developed to KET is dependent on the injection regimen. BS to KET and d-AMP may be mediated by a common neurocircuitry.

FENS Forum 2022
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BOARD NUMBER: S07-140

DOES EARLY POSTNATAL METHAMPHETAMINE ADMINISTRATION ALONG WITH ALTERED ENVIRONMENT AFFECT NEUROTRANSMITTER AND OXIDATIVE STRESS LEVELS IN ADOLESCENCE OF LABORATORY RAT?

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Methamphetamine (MA), as massively abused psychoactive stimulant, has been associated with many neurological diseases. Chronic abuse of MA leads to significant changes in distribution and storage of brain neurotransmitters (NEUs), such as dopamine (DA), serotonin (5HT) and noradrenaline (NA) as well as glutamate (GLU). Adolescence is a transition period between childhood and adulthood. On postnatal days 1 -12, male rat pups by MA or saline (SA) as control group. During the pre-weaning or post-weaning period animals were exposed to different environments (standard cage or enriched environment (EE) cage). After weaning pups were housed single or grouped. At PD 28,35,45 rats were sacrificed, perfused and the striatum (STR) and hippocampus (HIP) were collected. Levels of NEUs and 4-hydroxynonenal (4HNE) were analyzed with ELISA kits. The effect of MA on levels of NEUs differed based on the housing environment as well as the age of animals. As a matter of oxidative stress, there is beneficial impact of enriched environment, but impairment induced by SA administration as a stressor. Thus, the present data demonstrate, that enriched environment and type of housing of animals may impact on NEU levels as well as oxidative stress in animals postnatally exposed to MA. Financial support: GAUK 144212, 260533/SVV/2021, Cooperatio Neurosciences, OPVVV PharmaBrain CZ.02.1.01/0.0/0.0/16_025/0007444

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-141

FLUOXETINE ENHANCES PERCEPTUAL LEARNING AND LUMINANCE PERCEPTION DURING ADULTHOOD

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Cortical plasticity is very active in early life at specific critical periods. This plasticity then progressively decreases into adulthood. While stabilizing cortical functions, reduced plasticity hampers recovery from developmental or congenital conditions such as amblyopia and cataract in adults. Recent studies suggest that a direct action on the cortical excitation/inhibition balance can help restore this plasticity by a direct or indirect action on GABAergic transmission. In this context, serotonin has been proposed to play a crucial role in the remodeling of cortical circuits. Here, we compare the behavioral scores of two adult rhesus macaques, in three behavioral tasks under systemic injections of Fluoxetine, a selective serotonin reuptake inhibitor, or control saline injections. We first measured perception thresholds on a simple manual detection task. We found that, under chronic Fluoxetine injections, the 50% correct detection luminance perceptual thresholds consistently decreased for both monkeys, at the same time that false alarm to noise independently increased. In a second study, we used a manual target detection task in the presence of distractors to probe decision-making under uncertainty. Using signal detection theory, we show that Fluoxetine decreases the decision threshold as observed in the previous task, but not the D-prime. Last, macaques performed a dual choice saccadic task. Targets were associated with different reward values based on their spatial location. These contingencies changed across days. Fluoxetine significantly reduced spatial and reward biases. Overall, this indicates that Fluoxetine has both an impact on low-level visual processes and on top-down control processes.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-142

MINOCYCLINE TREATMENT INCREASES COGNITIVE PERFORMANCE AND NEURAL PLASTICITY IN A PRECLINICAL MODEL OF DEPRESSION

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aims Major depressive disorder is often associated with cognitive impairments, thus antidepressant treatments should target this domain in addition to the emotional one. Recently, the interplay between the brain and the immune system has been widely demonstrated, and compounds able to modulate immune function have been proposed as antidepressants. Here, we tested whether the administration of the antibiotic minocycline, with anti-inflammatory properties, improves the cognitive impairment associated with depression, since it has been proven to attenuate deficits in learning and memory in animal models of neurodegeneration. **Methods** C57BL6/J adult male mice were exposed to two weeks of chronic unpredictable mild stress to induce a depressive-like phenotype. At the end of the stress period, mice received either vehicle or minocycline for three weeks. We evaluated depressive-like profile, assessing anhedonia, and cognitive abilities through a place learning test. Finally, we investigated hippocampal functional plasticity measuring long-term potentiation (LTP). **Results** Chronic stress induced depressive-like behavior and cognitive impairment. Though the emotional dysregulation was recovered independently from treatment, cognitive performance was significantly enhanced only following minocycline administration. In particular, in the place learning test, when animals have to learn a new spatial configuration, minocycline-treated mice displayed a higher number of correct responses. Accordingly, the electrophysiological assessment showed that minocycline has recovered neuronal plasticity, minocycline-treated mice displaying higher LTP compared to vehicle. **Conclusions** These findings show that minocycline is effective in improving cognitive performances and enhancing neural plasticity in preclinical models, suggesting this drug as a potential treatment for cognitive impairments associated with depression.

Pubmed:
34343616: Picard K, Bisht K, Poggini S, Garofalo S, Golia MT, Basilico B, Abdallah F, Ciano Albanese N, Amrein I, Vernoux N, Sharma K, Hui CW, C Savage J, Limatola C, Ragozzino D, Maggi L, Branchi I, Tremblay MÈ
Microglial-glucocorticoid receptor depletion alters the response of hippocampal microglia and neurons in a chronic unpredictable mild stress paradigm in female mice.
Chronic psychological stress is one of the most important triggers and environmental risk factors for neuropsychiatric disorders. Chronic stress can influence all organs via the secretion of stress hormones, including glucocorticoids by the adrenal glands, which coordinate the stress response across the body. In the brain, glucocorticoid receptors (GR) are expressed by various cell types including microglia, which are its resident immune cells regulating stress-induced inflammatory processes. To study the roles of microglial GR under normal homeostatic conditions and following chronic stress, we generated a mouse model in which the GR gene is depleted in microglia specifically at adulthood to prevent developmental confounds. We first confirmed that microglia were depleted in GR in our model in males and females among the cingulate cortex and the hippocampus, both stress-sensitive brain regions. Then, cohorts of microglial-GR depleted and wild-type (WT) adult female mice were housed for 3 weeks in a standard or stressful condition, using a chronic unpredictable mild stress (CUMS) paradigm. CUMS induced stress-related behavior in both microglial-GR depleted and WT animals as demonstrated by a decrease of both saccharine preference and progressive ratio breakpoint. Nevertheless, the hippocampal microglial and neural mechanisms underlying the adaptation to stress occurred differently between the two genotypes. Upon CUMS exposure, microglial morphology was altered in the WT controls, without any apparent effect in microglial-GR depleted mice. Furthermore, in the standard environment condition, GR depleted-microglia showed increased expression of pro-inflammatory genes, and genes involved in microglial homeostatic functions (such as Trem2, Cx3cr1 and Mertk). On the contrary, in CUMS condition, GR depleted-microglia showed reduced expression levels of pro-inflammatory genes and increased neuroprotective as well as anti-inflammatory genes compared to WT-microglia. Moreover, in microglial-GR

depleted mice, but not in WT mice, CUMS led to a significant reduction of CA1 long-term potentiation and paired-pulse ratio. Lastly, differences in adult hippocampal neurogenesis were observed between the genotypes during normal homeostatic conditions, with microglial-GR deficiency increasing the formation of newborn neurons in the dentate gyrus subgranular zone independently from stress exposure. Together, these findings indicate that, although the deletion of microglial GR did not prevent the animal's ability to respond to stress, it contributed to modulating hippocampal functions in both standard and stressful conditions, notably by shaping the microglial response to chronic stress.
Brain Behav Immun, 2021; 97
33775780: Poggini S, Matte Bon G, Golia MT, Ciano Albanese N, Viglione A, Poleggi A, Limatola C, Maggi L, Branchi I
Selecting antidepressants according to a drug-by-environment interaction: A comparison of fluoxetine and minocycline effects in mice living either in enriched or stressful conditions.
Selective serotonin reuptake inhibitors (SSRIs) are the first-line treatment for major depressive disorder. It has been recently proposed that these drugs, by enhancing neural plasticity, amplify the influences of the living conditions on mood. Consequently, SSRI outcome depends on the quality of the environment, improving symptomatology mainly in individuals living in favorable conditions. In adverse conditions, drugs with a different mechanism of action might have higher efficacy. The antibiotic minocycline, with neuroprotective and anti-inflammatory properties, has been recently proposed as a novel potential antidepressant treatment. To explore the drug-by-environment interaction, we compared the effects on depressive-like behavior and neural plasticity of the SSRI fluoxetine and minocycline in enriched and stressful conditions. We first exposed C57BL/6 adult female mice to 14 days of chronic unpredictable mild stress to induce a depressive-like profile. Afterward, mice received vehicle, fluoxetine, or minocycline for 21 days, while exposed to either enriched or stressful conditions. During the first five days, fluoxetine led to an improvement in enrichment but not in stress. By contrast, minocycline led to an improvement in both conditions. After 21 days, all groups showed a significant improvement in enrichment while fluoxetine worsened the depressive like behavior in stress. The effects of the drugs on neural plasticity, measured as long-term potentiation, were also environment-dependent. Overall, we show that the environment affects fluoxetine but not minocycline outcome, indicating that the latter represents a potential alternative to SSRIs to treat depressed patients living in adverse conditions. From a translation perspective, our finding call for considering the drug-by-environment interaction to select the most effective pharmacological treatment.
Behav Brain Res, 2021; 408
33386229: Branchi I, Poggini S, Capuron L, Benedetti F, Poletti S, Tamouza R, Drexhage HA, Penninx BWJH, Pariante CM,
Brain-immune crosstalk in the treatment of major depressive disorder.
A growing number of studies are pointing out the need for a conceptual shift from a brain-centered to a body-inclusive approach in mental health research. In this perspective, the link between the immune and the nervous system, which are deeply interconnected and continuously interacting, is one of the most important novel theoretical framework to investigate the biological bases of major depressive disorder and, more in general, mental illness. Indeed, depressed patients show high levels of inflammatory markers, administration of pro-inflammatory drugs triggers a depressive symptomatology and antidepressant efficacy is reduced by excessive immune system activation. A number of molecular and cellular mechanisms have been hypothesized to act as a link between the immune and brain function, thus representing potential pharmacologically targetable processes for the development of novel and effective therapeutic strategies. These include the modulation of the kynurenine pathway, the crosstalk between metabolic and inflammatory processes, the imbalance in acquired immune responses, in particular T cell responses, and the interplay between neural plasticity and immune system activation. In the personalized medicine approach, the assessment and regulation of these processes have the potential to lead, respectively, to novel diagnostic approaches for the prediction of treatment outcome according to the patient's immunological profile, and to improved efficacy of antidepressant compounds through immune modulation.
Eur Neuropsychopharmacol, 2021; 45
31279682: Golia MT, Poggini S, Alboni S, Garofalo S, Ciano Albanese N, Viglione A, Ajmone-Cat MA, St-Pierre A, Brunello N, Limatola C, Branchi I, Maggi L
Interplay between inflammation and neural plasticity: Both immune activation and suppression impair LTP and BDNF expression.
An increasing number of studies show that both inflammation and neural plasticity act as key players in the vulnerability and recovery from psychiatric disorders and neurodegenerative diseases. However, the interplay between these two players has been limitedly explored. In fact, while a few studies reported an immune activation, others conveyed an immune suppression, associated with an impairment in neural plasticity. Therefore, we hypothesized that deviations in inflammatory levels in both directions may impair neural plasticity. We tested this hypothesis experimentally, by acute treatment of C57BL/6 adult male mice with different doses of two inflammatory modulators: lipopolysaccharide (LPS), an endotoxin, and ibuprofen (IBU), a nonselective cyclooxygenase inhibitor, which are respectively a pro- and an anti-inflammatory agent. The results showed that LPS and IBU have different effects on behavior and inflammatory response. LPS treatment induced a reduction of body

temperature, a decrease of body weight and a reduced food and liquid intake. In addition, it led to increased levels of inflammatory markers expression, both in the total hippocampus and in isolated microglia cells, including Interleukin (IL)-1β, and enhanced the concentration of prostaglandin E (PGE). On the other hand, IBU increased the level of anti-inflammatory markers, decreased tryptophan 2,3-dioxygenase (TDO2), the first step in the kynurenine pathway known to be activated during inflammatory conditions, and PGE levels. Though LPS and IBU administration differently affected mediators related with pro- or anti-inflammatory responses, they produced overlapping effects on neural plasticity. Indeed, higher doses of both LPS and IBU induced a statistically significant decrease in the amplitude of long-term potentiation (LTP), in Brain-Derived Neurotrophic Factor (BDNF) expression levels and in the phosphorylation of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor subunit GluR1, compared to the control group. Such effect appears to be dose-dependent since only the higher, but not the lower, dose of both compounds led to a plasticity impairment. Overall, the present findings indicate that acute treatment with pro- and anti-inflammatory agents impair neural plasticity in a dose dependent manner.
Brain Behav Immun, 2019; 81
30804991: Poggini S, Golia MT, Alboni S, Milior G, Sciarria LP, Viglione A, Matte Bon G, Brunello N, Puglisi-Allegra S, Limatola C, Maggi L, Branchi I
Combined Fluoxetine and Metformin Treatment Potentiates Antidepressant Efficacy Increasing IGF2 Expression in the Dorsal Hippocampus.
An increasing number of studies show that selective serotonin reuptake inhibitors (SSRIs) exert their therapeutic action, at least in part, by amplifying the influence of the living environment on mood. As a consequence, when administered in a favorable environment, SSRIs lead to a reduction of symptoms, but in stressful conditions, they show limited efficacy. Therefore, novel therapeutic approaches able to neutralize the influence of the stressful environment on treatment are needed. The aim of our study was to test whether, in a mouse model of depression, the combined administration of SSRI fluoxetine and metformin, a drug able to improve the metabolic profile, counteracts the limited efficacy of fluoxetine alone when administered in stressful conditions. Indeed, metabolic alterations are associated to both the onset of major depression and the antidepressant efficacy. To this goal, adult C57BL/6 male mice were exposed to stress for 6 weeks; the first two weeks was aimed at generating a mouse model of depression. During the remaining 4 weeks, mice received one of the following treatments: vehicle, fluoxetine, metformin, or a combination of fluoxetine and metformin. We measured liking- and wanting-type anhedonia as behavioral phenotypes of depression and assessed the expression levels of selected genes involved in major depressive disorder and antidepressant response in the dorsal and ventral hippocampus, which are differently involved in the depressive symptomatology. The combined treatment was more effective than fluoxetine alone in ameliorating the depressive phenotype after one week of treatment. This was associated to an increase in IGF2 mRNA expression and enhanced long-term potentiation, specifically in the dorsal hippocampus, at the end of treatment. Overall, the present results show that, when administered in stressful conditions, the combined fluoxetine and metformin treatment may represent a more effective approach than fluoxetine alone in a short term. Finally, our findings highlight the relevance of polypharmacological strategy as effective interventions to increase the efficacy of the antidepressant drugs currently available.
Neural Plast, 2019; 2019
30723316: Viglione A, Chiarotti F, Poggini S, Giuliani A, Branchi I
Predicting antidepressant treatment outcome based on socioeconomic status and citalopram dose.
Selective serotonin reuptake inhibitors (SSRIs), the most prescribed antidepressant drugs, have incomplete efficacy and no clear mechanism of action. In addition, no reliable methods to identify patients who will benefit from treatment is available. In this study, we show that citalopram, a commonly used SSRI, produces a dose-dependent amplification of the influence of the environment on mood, making the severity of symptoms dependent on the level of socioeconomic status (SES). As a consequence, based on SES, we were able to predict which patients would show remission following 12 weeks of treatment in the high, but not the low dose group. Our findings support a novel mechanism of action for SSRIs, which calls for a permissive rather than an instructive role of these drugs, and indicate that treatment outcome can be predicted based on SES and dose. Finally, our findings suggest that the patient's social and economic conditions should be considered in setting up personalized strategies aimed at enhancing SSRI efficacy.
Pharmacogenomics J, 2019; 19
27474084: Alboni S, Poggini S, Garofalo S, Milior G, El Hajj H, Lecours C, Girard I, Gagnon S, Boisjoly-Villeneuve S, Brunello N, Wolfer DP, Limatola C, Tremblay MÈ, Maggi L, Branchi I
Fluoxetine treatment affects the inflammatory response and microglial function according to the quality of the living environment.
It has been hypothesized that selective serotonin reuptake inhibitors (SSRIs), the most common treatment for major depression, affect mood through changes in immune function. However, the effects of SSRIs on inflammatory response are contradictory since these act either as anti- or pro-inflammatory drugs. Previous experimental and clinical studies showed that

the quality of the living environment moderates the outcome of antidepressant treatment. Therefore, we hypothesized that the interplay between SSRIs and the environment may, at least partially, explain the apparent incongruence regarding the effects of SSRI treatment on the inflammatory response. In order to investigate such interplay, we exposed C57BL/6 mice to chronic stress to induce a depression-like phenotype and, subsequently, to fluoxetine treatment or vehicle (21days) while being exposed to either an enriched or a stressful condition. At the end of treatment, we measured the expression levels of several anti- and pro-inflammatory cytokines and inflammatory mediators in the whole hippocampus and in isolated microglia. We also determined microglial density, distribution, and morphology to investigate their surveillance state. Results show that the effects of fluoxetine treatment on inflammation and microglial function, as compared to vehicle, were dependent on the quality of the living environment. In particular, fluoxetine administered in the enriched condition increased the expression of pro-inflammatory markers compared to vehicle, while treatment in a stressful condition produced anti-inflammatory effects. These findings provide new insights regarding the effects of SSRIs on inflammation, which may be crucial to devise pharmacological strategies aimed at enhancing antidepressant efficacy by means of controlling environmental conditions.
Brain Behav Immun, 2016; 58
26645631: Alboni S, van Dijk RM, Poggini S, Milior G, Perrotta M, Drenth T, Brunello N, Wolfer DP, Limatola C, Amrein I, Cirulli F, Maggi L, Branchi I
Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment.
Selective serotonin reuptake inhibitors (SSRIs) represent the most common treatment for major depression. However, their efficacy is variable and incomplete. In order to elucidate the cause of such incomplete efficacy, we explored the hypothesis positing that SSRIs may not affect mood per se but, by enhancing neural plasticity, render the individual more susceptible to the influence of the environment. Consequently, SSRI administration in a favorable environment promotes a reduction of symptoms, whereas in a stressful environment leads to a worse prognosis. To test such hypothesis, we exposed C57BL/6 mice to chronic stress in order to induce a depression-like phenotype and, subsequently, to fluoxetine treatment (21 days), while being exposed to either an enriched or a stressful condition. We measured the most commonly investigated molecular, cellular and behavioral endophenotypes of depression and SSRI outcome, including depression-like behavior, neurogenesis, brain-derived neurotrophic factor levels, hypothalamic-pituitary-adrenal axis activity and long-term potentiation. Results showed that, in line with our hypothesis, the endophenotypes investigated were affected by the treatment according to the quality of the living environment. In particular, mice treated with fluoxetine in an enriched condition overall improved their depression-like phenotype compared with controls, whereas those treated in a stressful condition showed a distinct worsening. Our findings suggest that the effects of SSRI on the depression- like phenotype is not determined by the drug per se but is induced by the drug and driven by the environment. These findings may be helpful to explain variable effects of SSRI found in clinical practice and to device strategies aimed at enhancing their efficacy by means of controlling environmental conditions.
Mol Psychiatry, 2017; 22
26231972: Milior G, Lecours C, Samson L, Bisht K, Poggini S, Pagani F, Deflorio C, Lauro C, Alboni S, Limatola C, Branchi I, Tremblay ME, Maggi L
Fractalkine receptor deficiency impairs microglial and neuronal responsiveness to chronic stress.
Chronic stress is one of the most relevant triggering factors for major depression. Microglial cells are highly sensitive to stress and, more generally, to environmental challenges. However, the role of these brain immune cells in mediating the effects of stress is still unclear. Fractalkine signaling - which comprises the chemokine CX3CL1, mainly expressed by neurons, and its receptor CX3CR1, almost exclusively present on microglia in the healthy brain - has been reported to critically regulate microglial activity. Here, we investigated whether interfering with microglial function by deleting the Cx3cr1 gene affects the brain's response to chronic stress. To this purpose, we housed Cx3cr1 knockout and wild-type adult mice in either control or stressful environments for 2weeks, and investigated the consequences on microglial phenotype and interactions with synapses, synaptic transmission, behavioral response and corticosterone levels. Our results show that hampering neuron-microglia communication via the CX3CR1-CX3CL1 pathway prevents the effects of chronic unpredictable stress on microglial function, short- and long-term neuronal plasticity and depressive-like behavior. Overall, the present findings suggest that microglia-regulated mechanisms may underlie the differential susceptibility to stress and consequently the vulnerability to diseases triggered by the experience of stressful events, such as major depression.
Brain Behav Immun, 2016; 55
23653679: Branchi I, Santarelli S, Capoccia S, Poggini S, D'Andrea I, Cirulli F, Alleva E
Antidepressant treatment outcome depends on the quality of the living environment: a pre-clinical investigation in mice.
Antidepressants represent the standard treatment for major depression. However, their efficacy is variable and incomplete. A growing number of studies suggest that the environment plays a major role in determining the efficacy of these drugs, specifically of selective serotonin reuptake inhibitors (SSRI). A recent hypothesis posits that the increase in serotonin levels induced by SSRI may not affect mood per se, but enhances neural plasticity and, consequently, renders the individual more

susceptible to the influence of the environment. Thus, SSRI administration in a favorable environment would lead to a reduction of symptoms, while in a stressful environment might lead to a worse prognosis. To test this hypothesis, we treated C57BL/6 adult male mice with chronic fluoxetine while exposing them to either (i) an enriched environment, after exposure to a chronic stress period aimed at inducing a depression-like phenotype, or (ii) a stressful environment. Anhedonia, brain BDNF and circulating corticosterone levels, considered endophenotypes of depression, were investigated. Mice treated with fluoxetine in an enriched condition improved their depression-like phenotype compared to controls, displaying higher saccharin preference, higher brain BDNF levels and reduced corticosterone levels. By contrast, when chronic fluoxetine administration occurred in a stressful condition, mice showed a more distinct worsening of the depression-like profile, displaying a faster decrease of saccharin preference, lower brain BDNF levels and increased corticosterone levels. Our findings suggest that the effect of SSRI on depression-like phenotypes in mice is not determined by the drug per se but is induced by the drug and driven by the environment. These findings may be helpful to explain variable effects of SSRI found in clinical practice and to device strategies aimed at enhancing their efficacy by means of controlling environmental conditions.
PLoS One, 2013; 8

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BOARD NUMBER: S07-143

EFFECTS OF METHYLPHENIDATE ON BEHAVIORAL PATTERNS IN AN ANIMAL MODEL OF IMPULSIVE BEHAVIOR

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Goal and sign trackers animals show a different relationship with conditional stimulus (CS). For goal tracker (GT) animals, CS indicates the arrival of a reward, focusing the behavior toward the goal or approaching the place where the reward will be released. In contrast, sign tracker (ST) animals show a different behavior in the presence of CS, attributing to CS properties of US. This classification, despite being apparently simple, involves a correlation of different behavioral patterns, and this kind of response could be associated with possible risk factors linked to several mental illnesses, especially problems associated with attention and impulse control. Methylphenidate, a blocking dopamine and norepinephrine reuptake, is one of the most frequently used drugs to prevent these problems. Here, we analyzed the role of the dopamine receptors and methylphenidate in the different pattern of behavior in ST and GT animals. Training a cohort of rats in an autoshaping procedure, we studied how the methylphenidate administration modulates the symptoms of impulsivity. Results showed animals under drug effects only decreased motivational activity, but the ST phenotype was preserved. That is, the methylphenidate could be acting on the expression of the conditioned response, but not on the coding of incentive salience of stimuli. This research was supported by PID2019-110739GB-I00 (Ministerio de Ciencia, Innovación y Universidades, FEDER, UE)

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-144

COGNITIVE CORRELATES AFTER LONG-TERM ADMINISTRATION OF METHYLPHENIDATE

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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The purpose of the present study was to analyze the effect of chronic consumption of methylphenidate during adolescence on the maturational process of the prefrontal cortex in an animal model of hyperactivity. Adolescent animals (35d) were treated with methylphenidate for a period of 20 days and behaviorally tested during adulthood (98d). Rats were phenotyped using an autoshaping procedure prior to pharmacological treatment and prior to behavioral testing. The results showed that animals treated with methylphenidate during adolescence show deficiencies in sustained attention tasks, but not in information filtering tasks. These effects were produced in the high-dose group and the effect was more intense in males than in females. Furthermore, pharmacological treatment affected animals differently depending on their phenotype (goal-tracker vs sign-tracker). These results suggest that chronic consumption of methylphenidate, at least in high doses, could cause neurodevelopmental alterations and that these alterations are not homogeneous but depend on individual characteristics.
This research was supported by PID2019-110739GB-I00 (Ministerio de Ciencia, Innovación y Universidades, FEDER, UE)

FENS Forum 2022
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BOARD NUMBER: S07-145

EFFECTS OF GLUTAMATERGIC MODULATION ON DIFFERENT TYPES OF IMPULSIVITY IN SPRAGUE-DAWLEY RATS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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[1]Faculty of pharmacy, University of Belgrade, Pharmacology, Belgrade, Serbia, [2]Faculty of pharmacy, University of Belgrade, Physiology, Beograd, Serbia, [3]Faculty of pharmacy, University of Belgrade, Bromatology, Beograd, Serbia

Aims: Impulsive disorders could be present in many psychiatric disorders in which the glutamatergic neurotransmission is affected. We aimed to assess the relationship between impulsivity parameters and types of impulsivity that could be influenced by the NMDA antagonist MK-801. Methodology: Two-month-old male Sprague-Dawley rats were trained in the variable delay-to-signal protocol modified for touchscreen with a fixed intertrial interval (ITI, 6s). Prior to testing, animals were treated i.p. with MK-801 (experiment 1: 0.03mg/kg or 0.05mg/kg, experiment 2: 0.05mg/kg or 0.2mg/kg) or with saline. Premature response rate (PR) during testing was analyzed in the first 20 trials with ITI 6s (PR4si and PR6si, motor impulsivity), next 60 trials with randomly distributed ITI of 9s or 15s (PR9s and PR15s, respectively) (temporal impulsivity) and then in the last 20 trials with ITI 6s again (PR6sf, reflective impulsivity). In a separate experiment, dependance between PRs across test stages was estimated with principal component analysis (PCA) applied to 61 control animals. Results: PCA revealed that PRs were independent from each another, except for PR4si and PR6si. Animals treated with MK-801 0.05mg/kg showed higher PR9s, PR15s and PR6sf, while 0.2mg/kg decreased PR4s compared to controls. Conclusion: Motor impulsivity measured by PR4si was decreased by the higher dose of MK-801 tested, possibly due to the observed repetitive exploration of chamber. MK-801 administered at 0.05mg/kg affected cognitive impulsivity, by inducing delay intolerance measured through PR9s and PR15s, which could be interpreted as temporal impulsivity, but also by increasing PR6sf, as a proposed parameter related to reflective impulsivity.

Pubmed:
34657887: Aranđelović J, Santrač A, Batinić B, Todorović L, Ahmed Khan MZ, Rashid F, Poe MM, Obradović A, Cook JM, Savić MM
Positive and Negative Selective Allosteric Modulators of α5 GABAA Receptors: Effects on Emotionality, Motivation, and Motor Function in the 5xFAD Model of Alzheimer's Disease.
Positive and negative allosteric modulators of α5 GABAA receptors (PAM and NAM, respectively) are worthy of investigation as putative treatments of Alzheimer's disease (AD). However, their potential to modify a dynamic range of behaviors in AD models needs to be systematically examined.
J Alzheimers Dis, 2021; 84
34360758: Đoković JB, Savić SM, Mitrović JR, Nikolic I, Marković BD, Randjelović DV, Antic-Stankovic J, Božić D, Cekić ND, Stevanović V, Batinić B, Aranđelović J, Savić MM, Savić SD
Curcumin Loaded PEGylated Nanoemulsions Designed for Maintained Antioxidant Effects and Improved Bioavailability: A Pilot Study on Rats.
The current study describes the experimental design guided development of PEGylated nanoemulsions as parenteral delivery systems for curcumin, a powerful antioxidant, as well as the evaluation of their physicochemical characteristics and antioxidant activity during the two years of storage. Experimental design setup helped development of nanoemulsion templates with critical quality attributes in line with parenteral application route. Curcumin-loaded nanoemulsions showed mean droplet size about 105 nm, polydispersity index <0.15, zeta potential of -40 mV, and acceptable osmolality of about 550 mOsm/kg. After two years of storage at room temperature, all formulations remained stable. Moreover, antioxidant activity remained intact, as demonstrated by DPPH (IC50 values 0.078-0.075 mg/mL after two years) and FRAPS assays. In vitro release testing proved that PEGylated phospholipids slowed down the curcumin release from nanoemulsions. The nanoemulsion carrier has been proven safe by the MTT test conducted with MRC-5 cell line, and effective on LS cell line. Results from the pharmacokinetic pilot study implied the PEGylated nanoemulsions improved plasma residence of curcumin 20 min after intravenous administration, compared to the non-PEGylated nanoemulsion (two-fold higher) or curcumin solution

(three-fold higher). Overall, conclusion suggests that developed PEGylated nanoemulsions present an acceptable delivery system for parenteral administration of curcumin, being effective in preserving its stability and antioxidant capacity at the level highly comparable to the initial findings.
Int J Mol Sci, 2021; 22
34508769: Santrač A, Batinić B, Stamenić TT, Aranđelović J, Sharmin D, Knutson DE, Cook JM, Savić MM
Positive modulation of α5GABAA receptors leads to dichotomous effects in rats on memory pattern and GABRA5 expression in prefrontal cortex and hippocampus.
Positive allosteric modulators (PAMs) of α5GABA receptors (α5GABAARs) are emerging as potential therapeutics for a range of neuropsychiatric disorders. However, their role in memory processing of healthy animals is not sufficiently examined. We tested the effects of MP-III-022 (1 mg/kg, 2.5 mg/kg and 10 mg/kg), a PAM known to be selective for α5GABAARs and devoid of prominent side-effects, in different behavioral paradigms (Morris water maze, novel object recognition test and social novelty discrimination) and on GABRA5 expression in Wistar rats, 30 min and 24 h after intraperitoneal treatment administration. The lowest dose tested worsened short-term object memory. The same dose, administered two times in a span of 24 h, improved spatial and impaired object and, at a trend level, social memory. The highest dose had a detrimental effect on all types of long-term memory (object memory at a trend level) and short-term spatial memory, but improved short-term object and social memory. Distinct sets of expression changes were detected in both prefrontal cortex and two regions of the hippocampus, but the latter ones could be assessed as more consequential. An increase of GABRA5 mRNA in CA2 occurred in parallel with improvement of object and social, but impairment of spatial memory, while the opposite happened with a trend level change in CA1. Our study demonstrates the variability of the roles of the α5GABAAR based on its level of expression and localization, in dependence on the type and protocol of cognitive tasks, as well as the respective timing of pharmacological modulation and testing.
Behav Brain Res, 2022; 416

FENS Forum 2022
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BOARD NUMBER: S07-146

ACUTE EXPOSURE BY AN INTRACISTERNAL INJECTION TO THE AMYLIN RECEPTOR ANTAGONIST AC253 IMPROVED COGNITIVE DEFICITS IN ALZHEIMER'S DISEASE MOUSE MODELS.

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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AC253, a novel amylin receptor antagonist, neutralizes the depressant effects of amyloid-β peptide (Aβ) and human amylin on hippocampal long-term potentiation (LTP) in mice brain slice experimentation (Kimura et.al. J Neurosci 2012). These our results show that the effects of Aβ and amylin on LTP are expressed via the amylin receptor, and blockade of this receptor by AC253 increases LTP even in transgenic mice overexpressing Aβ (5XFAD). In this study, our purpose is to elucidate whether acute care of AC253 improves cognitive deficits in a model mouse of Alzheimer's disease (AD). In order to accurate delivery of these peptide quickly, we administered directly into the brain stem. By that means, we adopted a bent needle with special angle for intracisternal (i.cist.) injection into conscious mice (Ueda at.el. EUR J PHARMACOL 1979). To determine whether the peptide affects cognitive function, we performed Y-maze Spontaneous Alternation test and Fear Conditioning test. First, we investigate that 0.01 ml Aβ (1 mM) administration in mice. Both tests showed dramatically reduced levels of performance as compared with 0.01 ml PBS administration as a control. Furthermore, another i.cist. administration of AC253 (5 mM) at 30 min before Aβ administration recovered from the depressant effects on spontaneous performance in the both teats. Our next step should be to examine whether depressed levels of spontaneous alternation performance on the behavioral test observed in 5XFAD, could be restored with AC253. Then we show that human Amylin receptor antagonists serve as potentially useful therapeutic agents in severe level of AD.

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BOARD NUMBER: S07-147

THE UNPREDICTABLE CHRONIC MILD STRESS PARADIGM AS A MODEL OF INDIVIDUAL VARIABILITY OF ANTIDEPRESSANT RESPONSE.

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Background: Antidepressants (ADs) are the basis for the treatment of major depression. Although increasingly safer, ADs remain only partially adequate as individual patient response variability and treatment outcome are highly unpredictable. Systematic studies are warranted to model and understand variability within and across treatments. **Objective:** To model inter-individual behavioral biomarkers of outcome using two different ADs. **Methodology:** The Unpredictable Chronic Mild Stress (UCMS) paradigm is used to induce (4 weeks) and to maintain (3 additional weeks) a depressive-like phenotype. Mice are exposed to 3 mild stressors daily in a randomized way. Depressive- and anxiety-like behavior, and memory evaluations are performed longitudinally. Animals with full blown depressive-like phenotype are subjected to a reversal protocol with fluoxetine (10mg/kg) or desipramine (10mg/kg) given over 3 weeks. **Results:** The UCMS successfully establishes depressive-like phenotype in about 90% of animals, confirmed by behavioral evaluations (Tail Suspension Test (TST), Fur coat (FC), Splash Test (ST), Anhedonia, Elevated-Plus maze (EPM), Novel object recognition test (NORT), and Weight Gain (WG)) and the post-hoc analysis of the Z-Score obtained from the mean of behavioral tests. Behavioral analysis at the end of protocol suggests that the depressive phenotype is maintained throughout the reversal protocol in saline-treated mice and that fluoxetine and desipramine partially reverse it. Post-hoc analysis allows to distinguish subpopulations of responders and non-responders. **Conclusions:** This design successfully models interindividual variability to AD treatment distinguishing between responders and non-responders. The outcome is comparable between fluoxetine and desipramine. Alternative analyses are considered. **Acknowledgements**: ERA-NET NEURON-040 (ANTaRES), Mineco-FPU fellowship, IRESP, ANR-19-CE37-0017.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-148

ANTI-PD1 IMMUNOTHERAPY EXACERBATES COGNITIVE DEFICITS INDUCED BY IMMUNOGENIC CANCER IN MICE

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

Celeste Nicola[1,2], Martine Dubois[1,2], Martin Pedard[1], Paul Dembele[1,2], Pauline Neveu[1,2], Laurence Desrues[1,2], Sahil Adriouch[3], Florence Joly[2,4,5,6], Pascal Hilber[1], Olivier Wurtz[1], Hélène Castel[1,2]
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Immunotherapies (PD-1/PD-L1) are promising approaches to treat cancer patients, but neurological consequences have been described. However, no objective neuropsychological evaluation of cognitive decline has yet been conducted. Here, we studied the impact of cancer and immunotherapy on emotional reactivity, cognitive functions, and underlying mechanisms as humoral immune cells cerebral entry or nervous pathways. C57Bl/6j mice were unilaterally injected in the flank with highly immunogenic colon cancer cells (MC38), less immunogenic melanoma cells (B16F10/B16F10-Ova) or corresponding lysates, to first define impact of cancer-related inflammatory environment on mice behavior. In a second set of experiments, anti-PD1 or control IgG was injected twice from D3 (B16F10) and D5 (MC38) post-tumor transplantation. Based on tumor growth kinetics, we assessed cognitive performance and emotional reactivity using a battery of behavioral tests. B16F10 mice showed impaired short-term memory, while F10-Ova and MC38 mice or receiving the corresponding lysate were prone to more pronounced cognitive deficit, with symptoms of anxiety and resignation. After PD-1 treatment, tumor growth was delayed, but cognitive impairment and resignation were exacerbated compared to cancer alone, only in MC38 mice. In brain slices, infiltration of macrophages (MHCII, CD206) within the circumventricular organs and over-expression of the indoleamine 2,3-dioxygenase (IDO) in the mesencephalic trigeminal nucleus and tract were highlighted in MC38 and F10-Ova mice. Our data indicate that immunotherapy, by delaying tumor growth, improves emotional disorders but worsens cognitive dysfunctions depending on cancer immunogenicity. The study of underlying mechanisms will guide the search for key targets actionable to maintain cancer patient quality of life.

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BOARD NUMBER: S07-149

PREDICTIVE AND CONSTRUCT VALIDITY IN A MOUSE MODEL OF CHEMOTHERAPY-INDUCED COGNITIVE IMPAIRMENT

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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The quality of life in cancer survivors is often affected by the (long-term) side-effects of chemotherapy. The condition is known as chemotherapy-induced cognitive impairment (CICI), and affects executive functions, information-processing speed, attention, learning and memory. CICI has been related to leukoencephalopathy (white matter damage). At present, there are no approved evidence-based interventions to prevent CICI or its underlying leukoencephalopathy. Animal models with predictive and construct validity are generally thought be instrumental to the preclinical development of such interventions *(Crawley, 2007)*. Therefore, we examined the long-term (adult) consequences of triple systemic injections of the chemotherapeutic **methotrexate** (MTX) in juvenile mice, which putatively models CICI in children treated for acute lymphoblastic leukemia *(Gibson et al., Cell 2019).* We also investigate the attenuating effects of miconazole, an antifungal agent with remyelination properties. Juvenile C57BL/6 mice were injected with MTX (or saline) on postnatal days 21, 28, and 35, followed by miconazole (one daily injection of from postnatal day 36 through 42). During adulthood, subjects were tested on a behavioral battery that assesses aspects of cognition, social behavior and emotional reactivity. Immunohistochemical analyses inspected myelin basic protein expression, a biomarker of white matter integrity. Predictive and construct validity requires the CICI model to reproduce MTX-induced behavioral impairment, but also adjuvant miconazole administration alleviate myelin damage induced by MTX exposure.

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OPPOSING EFFECTS OF POSTNATAL AND JUVENILE FLUOXETINE TREATMENT ON EMOTIONAL BEHAVIOUR, PROTEIN TRANSLATION, AND BIOENERGETICS.

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Prior evidence indicates that treatment with selective serotonin reuptake inhibitors (SSRI) may evoke distinct changes in mood-related behavior depending on the temporal window of drug administration. We focused on two early time windows, namely: Postnatal and Juvenile windows. Postnatal Fluoxetine (PNFlx) evoked long-lasting increases in anxiety- and despair-like behavior, whereas Juvenile Fluoxetine (JFlx) elicited persistent decreases in anxiety- and despair-like behavior. The starkly differing behavioral outcomes were accompanied by differential global transcriptome changes in the mPFC, with minimal overlap in gene regulation. Further, the mTOR signaling pathway was differentially regulated in PNFlx versus JFlx animals. Opposing patterns of proteins involved in mitochondrial biogenesis and function were observed, with a decline in the mPFC of PNFlx animals in contrast to the increase following JFlx treatment. We found contrasting effects in ATP levels and OxPhos efficiency in PNFlx versus JFlx treatment. We observed a significant increase in mitochondrial function in PNFlx animals treated with NAM, reversing the mitochondrial decline and the pro-depressive effects observed with PNFlx. We show that SSRI treatment in postnatal versus juvenile windows evokes opposing effects on anxiety- and despair-like behavior, along with a differential and minimal overlap of the mPFC transcriptome, differential regulation of the mTOR pathway accompanied with opposing changes in the bioenergetics. We further establish a causal link between bioenergetics and despair-like behavior by reversing the pro-depressive effects of PNFlx using NAM treatment. Our findings highlight two distinct windows of early life in which SSRI exposure evokes starkly differing effects on behavior, gene regulation, and bioenergetics.

Pubmed:
31072928: Fanibunda SE, Deb S, Maniyadath B, Tiwari P, Ghai U, Gupta S, Figueiredo D, Weisstaub N, Gingrich JA, Vaidya ADB, Kolthur-Seetharam U, Vaidya VA
Serotonin regulates mitochondrial biogenesis and function in rodent cortical neurons via the 5-HT receptor and SIRT1-PGC-1α axis.
Mitochondria in neurons, in addition to their primary role in bioenergetics, also contribute to specialized functions, including regulation of synaptic transmission, Ca homeostasis, neuronal excitability, and stress adaptation. However, the factors that influence mitochondrial biogenesis and function in neurons remain poorly elucidated. Here, we identify an important role for serotonin (5-HT) as a regulator of mitochondrial biogenesis and function in rodent cortical neurons, via a 5-HT receptor-mediated recruitment of the SIRT1-PGC-1α axis, which is relevant to the neuroprotective action of 5-HT. We found that 5-HT increased mitochondrial biogenesis, reflected through enhanced mtDNA levels, mitotracker staining, and expression of mitochondrial components. This resulted in higher mitochondrial respiratory capacity, oxidative phosphorylation (OXPHOS) efficiency, and a consequential increase in cellular ATP levels. Mechanistically, the effects of 5-HT were mediated via the 5-HT receptor and master modulators of mitochondrial biogenesis, SIRT1 and PGC-1α. SIRT1 was required to mediate the effects of 5-HT on mitochondrial biogenesis and function in cortical neurons. In vivo studies revealed that 5-HT receptor stimulation increased cortical mtDNA and ATP levels in a SIRT1-dependent manner. Direct infusion of 5-HT into the neocortex and chemogenetic activation of 5-HT neurons also resulted in enhanced mitochondrial biogenesis and function in vivo. In cortical neurons, 5-HT enhanced expression of antioxidant enzymes, decreased cellular reactive oxygen species, and exhibited neuroprotection against excitotoxic and oxidative stress, an effect that required SIRT1. These findings identify 5-HT as an upstream regulator of mitochondrial biogenesis and function in cortical neurons and implicate the mitochondrial effects of 5-HT in its neuroprotective action.
Proc Natl Acad Sci U S A, 2019; 116

35002622: Desouza LA, Benekareddy M, Fanibunda SE, Mohammad F, Janakiraman B, Ghai U, Gur T, Blendy JA, Vaidya VA
The Hallucinogenic Serotonin Receptor Agonist, 2,5-Dimethoxy-4-Iodoamphetamine, Promotes cAMP Response Element Binding Protein-Dependent Gene Expression of Specific Plasticity-Associated Genes in the Rodent Neocortex.
Psychedelic compounds that target the 5-HT receptor are reported to evoke psychoplastogenic effects, including enhanced dendritic arborization and synaptogenesis. Transcriptional regulation of neuronal plasticity-associated genes is implicated in the cytoarchitectural effects of serotonergic psychedelics, however, the transcription factors that drive this regulation are poorly elucidated. Here, we addressed the contribution of the transcription factor cyclic adenosine monophosphate (cAMP)-response element binding protein (CREB) in the regulation of neuronal plasticity-associated genes by the hallucinogenic 5-HT receptor agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI). studies with rat cortical neurons indicated that DOI enhances the phosphorylation of CREB (pCREB) through mitogen-activated protein (MAP) kinase and calcium/calmodulin dependent kinase II (CaMKII) pathways, with both cascades contributing to the DOI-evoked upregulation of , and expression, whilst the upregulation of and mRNA involved the MAP kinase and CaMKII pathway respectively. We observed a robust DOI-evoked increase in the expression of several neuronal plasticity-associated genes in the rat neocortex . This DOI-evoked upregulation of neuronal plasticity-associated genes was completely blocked by the 5-HT receptor antagonist MDL100,907 and was also abrogated in the neocortex of 5-HT receptor deficient mice. Further, 5-HT receptor stimulation enhanced pCREB enrichment at putative cAMP response element (CRE) binding sites in the , , , , but not and , promoters in the rodent neocortex. The DOI-mediated transcriptional induction of , and was significantly attenuated in the neocortex of CREB deficient/knockout (CREBαδ KO) mice. Collectively, these results indicate that the hallucinogenic 5-HT receptor agonist DOI leads to a rapid transcriptional upregulation of several neuronal plasticity-associated genes, with a subset of them exhibiting a CREB-dependent regulation. Our findings raise the intriguing possibility that similar to slow-acting classical antidepressants, rapid-action serotonergic psychedelics that target the 5-HT receptor may also recruit the transcription factor CREB to enhance the expression of neuronal plasticity-associated genes in the neocortex, which could in turn contribute to the rapid psychoplastogenic changes evoked by these compounds.
Front Mol Neurosci, 2021; 14
33622703: Mukhopadhyay S, Chatterjee A, Tiwari P, Ghai U, Vaidya VA
Postnatal Fluoxetine Treatment Alters Perineuronal Net Formation and Maintenance in the Hippocampus.
Elevation of serotonin via postnatal fluoxetine (PNFlx) treatment during critical temporal windows is hypothesized to perturb the development of limbic circuits thus establishing a substratum for persistent disruption of mood-related behavior. We examined the impact of PNFlx treatment on the formation and maintenance of perineuronal nets (PNNs), extracellular matrix (ECM) structures that deposit primarily around inhibitory interneurons, and mark the closure of critical period plasticity. PNFlx treatment evoked a significant decline in PNN number, with a robust reduction in PNNs deposited around parvalbumin (PV) interneurons, within the CA1 and CA3 hippocampal subfields at postnatal day (P)21 in Sprague Dawley rat pups. While the reduction in CA1 subfield PNN number was still observed in adulthood, we observed no change in colocalization of PV-positive interneurons with PNNs in the hippocampi of adult PNFlx animals. PNFlx treatment did not alter hippocampal PV, calretinin (CalR), or Reelin-positive neuron numbers in PNFlx animals at P21 or in adulthood. We did observe a small, but significant increase in somatostatin (SST)-positive interneurons in the DG subfield of PNFlx-treated animals in adulthood. This was accompanied by altered GABA-A receptor subunit composition, increased dendritic complexity of apical dendrites of CA1 pyramidal neurons, and enhanced neuronal activation revealed by increased c-Fos-positive cell numbers within hippocampi of PNFlx-treated animals in adulthood. These results indicate that PNFlx treatment alters the formation of PNNs within the hippocampus, raising the possibility of a disruption of excitation-inhibition (E/I) balance within this key limbic brain region.
eNeuro, 2021 Mar-Apr; 8

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BOARD NUMBER: S07-151

THE INTERACTION BETWEEN ANTIDEPRESSANTS AND ENVIRONMENT DETERMINES TREATMENT OUTCOME IN A PRECLINICAL MODEL OF ADOLESCENCE-ONSET DEPRESSION

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aims Adolescence-onset depression leads to more severe clinical symptoms, a longer duration of illness, and a higher rate of suicidality in adulthood. The first-line treatment for depression is selective serotonin reuptake inhibitors (SSRIs), but their efficacy is incomplete. Recently, it has been proposed that SSRIs do not affect mood per se, but, by enhancing neural plasticity, amplify the influences of the living environment on mood. Therefore, we hypothesized that treatment with antidepressants for adolescence-onset depression has to be administered in a supportive environment to have the highest efficacy. **Methods** We exposed C57BL/6 male mice from weaning to adulthood either in social conditions or social isolation to induce a depressive-like phenotype. In adulthood, socially housed mice have been exposed to the enriched environment and received vehicle. Isolated subjects were assigned to four groups: exposed to standard or enriched environment while receiving fluoxetine or vehicle. We assessed the efficacy of fluoxetine, enrichment, or their combination to treat the depressive-like phenotype in the elevated plus-maze, forced swim, and open field test. **Results** Adolescent isolation induced both depressive- and anxiety-like behavior. Environmental enrichment, but not fluoxetine alone, counterbalanced the detrimental effects of isolation, as indicated by the normalization of floating behavior in the forced swim test, locomotor activity in the open field, and time spent in the open arms in the elevated plus-maze. It is worth noting that fluoxetine effects were determined by the environment. **Conclusions** These findings suggest that the environment is key in treating depression and SSRI treatment can potentiate its impact.

Pubmed:
33775780: Poggini S, Matte Bon G, Golia MT, Ciano Albanese N, Viglione A, Poleggi A, Limatola C, Maggi L, Branchi I
Selecting antidepressants according to a drug-by-environment interaction: A comparison of fluoxetine and minocycline effects in mice living either in enriched or stressful conditions.
Selective serotonin reuptake inhibitors (SSRIs) are the first-line treatment for major depressive disorder. It has been recently proposed that these drugs, by enhancing neural plasticity, amplify the influences of the living conditions on mood. Consequently, SSRI outcome depends on the quality of the environment, improving symptomatology mainly in individuals living in favorable conditions. In adverse conditions, drugs with a different mechanism of action might have higher efficacy. The antibiotic minocycline, with neuroprotective and anti-inflammatory properties, has been recently proposed as a novel potential antidepressant treatment. To explore the drug-by-environment interaction, we compared the effects on depressive-like behavior and neural plasticity of the SSRI fluoxetine and minocycline in enriched and stressful conditions. We first exposed C57BL/6 adult female mice to 14 days of chronic unpredictable mild stress to induce a depressive-like profile. Afterward, mice received vehicle, fluoxetine, or minocycline for 21 days, while exposed to either enriched or stressful conditions. During the first five days, fluoxetine led to an improvement in enrichment but not in stress. By contrast, minocycline led to an improvement in both conditions. After 21 days, all groups showed a significant improvement in enrichment while fluoxetine worsened the depressive like behavior in stress. The effects of the drugs on neural plasticity, measured as long-term potentiation, were also environment-dependent. Overall, we show that the environment affects fluoxetine but not minocycline outcome, indicating that the latter represents a potential alternative to SSRIs to treat depressed patients living in adverse conditions. From a translation perspective, our finding call for considering the drug-by-environment interaction to select the most effective pharmacological treatment.
Behav Brain Res, 2021; 408
34343616: Picard K, Bisht K, Poggini S, Garofalo S, Golia MT, Basilico B, Abdallah F, Ciano Albanese N, Amrein I, Vernoux N, Sharma K, Hui CW, C Savage J, Limatola C, Ragozzino D, Maggi L, Branchi I, Tremblay MÈ
Microglial-glucocorticoid receptor depletion alters the response of hippocampal microglia and neurons in a chronic

unpredictable mild stress paradigm in female mice.
Chronic psychological stress is one of the most important triggers and environmental risk factors for neuropsychiatric disorders. Chronic stress can influence all organs via the secretion of stress hormones, including glucocorticoids by the adrenal glands, which coordinate the stress response across the body. In the brain, glucocorticoid receptors (GR) are expressed by various cell types including microglia, which are its resident immune cells regulating stress-induced inflammatory processes. To study the roles of microglial GR under normal homeostatic conditions and following chronic stress, we generated a mouse model in which the GR gene is depleted in microglia specifically at adulthood to prevent developmental confounds. We first confirmed that microglia were depleted in GR in our model in males and females among the cingulate cortex and the hippocampus, both stress-sensitive brain regions. Then, cohorts of microglial-GR depleted and wild-type (WT) adult female mice were housed for 3 weeks in a standard or stressful condition, using a chronic unpredictable mild stress (CUMS) paradigm. CUMS induced stress-related behavior in both microglial-GR depleted and WT animals as demonstrated by a decrease of both saccharine preference and progressive ratio breakpoint. Nevertheless, the hippocampal microglial and neural mechanisms underlying the adaptation to stress occurred differently between the two genotypes. Upon CUMS exposure, microglial morphology was altered in the WT controls, without any apparent effect in microglial-GR depleted mice. Furthermore, in the standard environment condition, GR depleted-microglia showed increased expression of pro-inflammatory genes, and genes involved in microglial homeostatic functions (such as Trem2, Cx3cr1 and Mertk). On the contrary, in CUMS condition, GR depleted-microglia showed reduced expression levels of pro-inflammatory genes and increased neuroprotective as well as anti-inflammatory genes compared to WT-microglia. Moreover, in microglial-GR depleted mice, but not in WT mice, CUMS led to a significant reduction of CA1 long-term potentiation and paired-pulse ratio. Lastly, differences in adult hippocampal neurogenesis were observed between the genotypes during normal homeostatic conditions, with microglial-GR deficiency increasing the formation of newborn neurons in the dentate gyrus subgranular zone independently from stress exposure. Together, these findings indicate that, although the deletion of microglial GR did not prevent the animal's ability to respond to stress, it contributed to modulating hippocampal functions in both standard and stressful conditions, notably by shaping the microglial response to chronic stress.
Brain Behav Immun, 2021; 97
31279682: Golia MT, Poggini S, Alboni S, Garofalo S, Ciano Albanese N, Viglione A, Ajmone-Cat MA, St-Pierre A, Brunello N, Limatola C, Branchi I, Maggi L
Interplay between inflammation and neural plasticity: Both immune activation and suppression impair LTP and BDNF expression.
An increasing number of studies show that both inflammation and neural plasticity act as key players in the vulnerability and recovery from psychiatric disorders and neurodegenerative diseases. However, the interplay between these two players has been limitedly explored. In fact, while a few studies reported an immune activation, others conveyed an immune suppression, associated with an impairment in neural plasticity. Therefore, we hypothesized that deviations in inflammatory levels in both directions may impair neural plasticity. We tested this hypothesis experimentally, by acute treatment of C57BL/6 adult male mice with different doses of two inflammatory modulators: lipopolysaccharide (LPS), an endotoxin, and ibuprofen (IBU), a nonselective cyclooxygenase inhibitor, which are respectively a pro- and an anti-inflammatory agent. The results showed that LPS and IBU have different effects on behavior and inflammatory response. LPS treatment induced a reduction of body temperature, a decrease of body weight and a reduced food and liquid intake. In addition, it led to increased levels of inflammatory markers expression, both in the total hippocampus and in isolated microglia cells, including Interleukin (IL)-1β, and enhanced the concentration of prostaglandin E (PGE). On the other hand, IBU increased the level of anti-inflammatory markers, decreased tryptophan 2,3-dioxygenase (TDO2), the first step in the kynurenine pathway known to be activated during inflammatory conditions, and PGE levels. Though LPS and IBU administration differently affected mediators related with pro- or anti-inflammatory responses, they produced overlapping effects on neural plasticity. Indeed, higher doses of both LPS and IBU induced a statistically significant decrease in the amplitude of long-term potentiation (LTP), in Brain-Derived Neurotrophic Factor (BDNF) expression levels and in the phosphorylation of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor subunit GluR1, compared to the control group. Such effect appears to be dose-dependent since only the higher, but not the lower, dose of both compounds led to a plasticity impairment. Overall, the present findings indicate that acute treatment with pro- and anti-inflammatory agents impair neural plasticity in a dose dependent manner.
Brain Behav Immun, 2019; 81

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BOARD NUMBER: S07-152

ROLE OF BRAIN TLR9 IN INFLAMMATION AND EMOTIONAL BEHAVIOR.

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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The immune system has been associated with regulating emotional behaviors. In particular, several types of innate immune receptors of the Toll receptor (TLR) family have been shown to regulate anxiety, depression, and memory. However, the precise role and molecular mechanisms remain to be elucidated. This study aims to evaluate the role of the TLR9 receptor in the brain in a mouse model of depression associated with acute inflammation induced by lipopolysaccharide (LPS). To do so, we analyze the depressive behavior, anxiety and cognitive impairment associated with inflammation in a model of LPS and how an intranasal delivery of TLR9 agonist regulates this response prior to a systemic administration of LPS by evaluating the neurochemical changes in the hippocampus related with pro- and anti-inflammatory pathways, in male and female C57BL6J mice. Our results show that TLR9 stimulation in the brain decreased the depressive-like behavior in the tail suspension test, decreased anxiety in the light-dark box anxiety test in both males and females, and improved episodic memory function in the novel object recognition test males. We found differences in gene expression of pro- and anti-inflammatory cytokines after LPS and TLR9 treatment, alterations in the expression of tight junction proteins and mitochondrial oxidative phosphorylation system. In conclusion, these findings reveal that TLR9 activation in the brain could promote neuroprotective effects conferring resilience to emotional and cognitive-behavioral dysfunction.

Pubmed:
34944470: Morcuende A, Navarrete F, Nieto E, Manzanares J, Femenía T
Inflammatory Biomarkers in Addictive Disorders.
Substance use disorders are a group of diseases that are associated with social, professional, and family impairment and that represent a high socio-economic impact on the health systems of countries around the world. These disorders present a very complex diagnosis and treatment regimen due to the lack of suitable biomarkers supporting the correct diagnosis and classification and the difficulty of selecting effective therapies. Over the last few years, several studies have pointed out that these addictive disorders are associated with systemic and central nervous system inflammation, which could play a relevant role in the onset and progression of these diseases. Therefore, identifying different immune system components as biomarkers of such addictive disorders could be a crucial step to promote appropriate diagnosis and treatment. Thus, this work aims to provide an overview of the immune system alterations that may be biomarkers of various addictive disorders.
Biomolecules, 2021; 11

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BOARD NUMBER: S07-153

EFFECTS OF PSILOCYBIN ON ACTIVITY-BASED ANOREXIA AND COGNITIVE FLEXIBILITY IN FEMALE RATS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Anorexia nervosa (AN) has one of the highest mortality rates of any psychiatric disorder and less than 50% of patients ever recover. Despite this, there are currently no effective medicinal treatments for AN. Psilocybin is currently being explored as a novel therapeutic for AN, and is proposed to act by “breaking down” cognitive inflexibility. To examine the effects of psilocybin on pathological weight loss and cognitive-behavioural flexibility, we used the activity-based anorexia (ABA) model and operant reinforcement tasks. Female Sprague-Dawley rats (n=23; 7 weeks old) were trained to run in wheels and administered psilocybin (1.5 mg/kg) or saline 24h prior to the commencement of ABA, consisting of unlimited access to a running wheel paired with time-limited access to food. A separate cohort of rats (n=31) were trained to nose-poke into one of two operant ports in order to obtain a sucrose reward and administered psilocybin or saline 24h prior to reversal of the reward-paired port. Extinction and reinstatement of responding for sucrose rewards were also examined the day after treatment in a separate cohort of rats (n=22). Psilocybin attenuated the rapid weight loss elicited by ABA conditions, which was driven by both increased food intake ($F_{2,20}$=15.45, p<.0001) and reduced compulsive wheel running ($F_{2,20}$=12.26, p=.0003). Psilocybin also enhanced reversal learning speed ($F_{1,29}$=5.128, p=.031) without effects on extinction ($F_{1,20}$=0.097, p=.758) or reinstatement ($F_{1,20}$=0.081, p=.779), suggesting a specific improvement in flexible learning. Taken together, these findings provide initial support for the therapeutic potential of psilocybin for treating cognitive inflexibility in AN.

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BOARD NUMBER: S07-154

EFFECT OF AMITRIPTYLINE ON AFFECTIVE BIASES IN MALE RATS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aims This study investigated the effects of amitriptyline, a tricyclic antidepressant, on affective biases in rats. We test amitriptyline's ability to bias new learning and reverse previously established negative affective biases following acute and 24-hour pretreatment using the affective bias test (ABT). We then test the specificity of the effect using a control reward learning assay. **Methods** The ABT employs a within-subject study design in which animals independently learn to associate a digging substrate with a food reward. One substrate is learnt following a drug treatment manipulation and the other following control. A subsequent preference test is used to determine whether the treatment produced a positive or negative bias toward the treatment-paired substrate. The control reward learning assay involves a similar protocol, but each substrate is paired with either a higher or lower value reward but affective state remains the same throughout. **Results** Amitriptyline positively biased new learning and attenuated a previously learnt negative affective bias 1hr and 24-hour post-treatment. No effects were seen in the reward learning assay, suggesting a specific effect on affective-state induced biases. **Conclusions** These data show similar effects on biases and new learning as previously reported with selective monoamine re-uptake inhibitors e.g., venlafaxine, but contrast with their lack of effect on previously learnt, negative biases. The ability to modulate biases associated with past memories is similar to the effects seen in this assay with the muscarinic antagonist scopolamine which has also been shown clinically to be a rapid-acting antidepressant.

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EVALUATION OF THE EFFECTS OF INFLAMMATION AND COMBINED EXPOSURE TO ENVIRONMENTAL TOXICANTS DURING THE PERINATAL PERIOD: A POTENTIAL ETIOLOGICAL FACTOR OF NEURODEGENERATIVE PATHOLOGIES

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aims In recent decades, the number of neurodegenerative diseases such as Parkinson's or Amyotrophic Lateral Sclerosis (ALS) has steadily increased. It has long been considered that these pathologies are primarily caused by genetic factors, but recent studies tend to show that these diseases are the result of a combination of genetic and environmental factors (like toxins and pesticides). The concept of multiple effects is reinforced by an increasing number of studies that have shown that early exposure, especially during critical periods of brain development, to such agents is likely to alter brain development "fate" through neuronal and inflammatory processes leading eventually to neurodegenerative disorders later in life. Unfortunately, few studies have been implemented to address all these issues simultaneously. **Methods** Pregnant C57Bl6 mice has been subjected to a prenatal low inflammatory challenge (IP LPS injection at 0.008 mg/kg) - followed by a chronic combined exposure to 3 environmental toxicants - β-N-methylamino-L-alanine (BMAA) (50 mg/kg), Glyphosate (GLY) (5 mg/kg), and Ammonium Glufosinate (GLA) (0.2 mg/kg) (protocol A). On the contrary, a chronic maternal pre-toxicants (BMAA/GLY/GLA 3x/week) exposure to a post-natal inflammatory challenge (post-natal day 5 LPS injection) (protocol B) has been settled. Motor, sensory, and emotional abilities have been assessed at different stages of development. **Results** Behavioral results from both protocols showed that a toxicant exposure combined with low inflammation challenge induces motor disorders from adolescence to aging through adulthood. **Conclusion** These data suggest that a mild inflammatory event could modulate a multiple toxins exposure during critical cerebral development phases.

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ENDOTHELIAL NMDA RECEPTOR IMPAIRS THE DIFFERENTIATION OF OLIGODENDROCYTES

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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In human, brain damages, whether genetic or environmental, may depending on the period of development in which they occur cause adverse effects and ultimately lead to birth defects or pathologies that will have long-term effects in adults. Glutamatergic transmission plays a critical role in developmental processes such as neuronal differentiation, migration and plasticity. Previous studies from the laboratory demonstrated a strong expression of endothelial N-methyl-D-aspartate glutamatergic receptor (NMDAR) in the perinatal period. At this time, the contribution of endothelial NMDAR on neurodevelopment remain unknown. The aim of the present study was to investigate the role of endothelial NMDAR in the differentiation of oligodendrocytes. When compared to wild type mice, western blot and immunohistochemical studies performed in Grin1$^{lox/lox}$/VeCad^{cre} mice revealed that CNPase and MBP expression was markedly decreased at postnatal day 15 (P15) in both neocortex and spinal cord. In contrast, PDGFR alpha levels, a marker of oligodendrocyte precursors, were increased. Behavioral studies showed that, at this stage, Grin1$^{lox/lox}$/VeCad^{cre} mice were characterized by a reduced locomotor activity. Partial recovery of these molecular and behavioral defects was found in adults. Altogether, these results suggest that endothelial NMDA invalidation delayed the maturation of oligodendrocytes. They provide first evidence in favor of a key role of the endothelial NMDAR in the establishment of the oligodendrocyte lineage, as it has recently been demonstrated for GABAergic interneurons. They open new research avenues regarding the contribution of this receptor in the vessel-associated migration of oligodendrocytes. *Supported by Rouen University, INSERM, Fondation de France, Blood&Brain Institute, ANR AlcoBrain*

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BOARD NUMBER: S07-157

SHOTGUN METAGENOMICS REVEALS TAXONOMIC AND FUNCTIONAL CHANGES IN THE SALIVARY MICROBIOME IN YOUNG ADULTS WITH DEPRESSION.

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aims: Oral dysbiosis has been associated with the pathophysiology of a number of systemic diseases with underlying inflammatory components, including psychiatric disorders, with growing evidence linking the oral microbiome and depression. The aim of this study was to conduct shotgun metagenomic sequencing (SMS) to characterise the composition and examine functional differences in the oral microbiome in individuals with depression compared to controls. **Methods:** Microbial DNA was extracted from saliva samples from young adults with depression (n=13) and healthy controls (n=19). Microbiome analysis was conducted using SMS. Kraken 2 was used to isolate all the non-human reads. The taxonomic and functional profiling for the depressed and healthy cohorts was obtained separately using the co-assembly method from the SqueezeMeta pipeline v.1.1.2. **Results:** SMS analysis found depression to be associated with significantly decreased alpha diversity (p=0.047) in comparison to healthy controls. Significant differences were also found in one phylum, four genera and seven species between the two cohorts, with two of the species identified being almost exclusively present in the depressed cohort. Functional differential abundance analysis revealed a higher abundance in Kegg Orthologies (KOs) related to eleven molecular functions, including xenobiotic biodegradation and environmental information processing. **Conclusions:** Overall, characteristic differences in the composition of the oral microbiome have been identified in young adults with depression. The clinical importance and their link with pathogenesis of depression require further SMS studies in larger cohorts.

Pubmed:
34977911: McLafferty M, Brown N, McHugh R, Ward C, Stevenson A, McBride L, Brady J, Bjourson AJ, O'Neill SM, Walsh CP, Murray EK
Depression, anxiety and suicidal behaviour among college students: Comparisons pre-COVID-19 and during the pandemic. Many students struggle with psychological problems during their college years. These problems may be even more apparent during the COVID-19 pandemic with the accompanying restrictions and transition to an online learning environment, but few longitudinal studies have been conducted to date. The aim of this study was to compare symptoms of depression, anxiety and suicidality prior to and during the pandemic, and identify stressors.
Psychiatry Res Commun, 2021; 1

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BOARD NUMBER: S07-158

NMDA RECEPTOR AND SYNAPTIC PLASTICITY DYSREGULATION THROUGHOUT DEVELOPMENT IN THE MOUSE FMR1 KNOCKOUT MODEL OF FXS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Fragile X Syndrome (FXS) is a developmental autistic syndrome that is diagnosed in childhood. In animal models, one of the more pronounced phenotypes is impaired learning and memory including synaptic plasticity. The goal of this project was to better understand how synaptic deficits developmentally evolve in FXS, and how NMDA receptors (NMDARs) play a role in the dysregulation. We investigated synaptic plasticity through electrophysiological recordings of NMDAR-dependent long-term potentiation (LTP) in five age groups (P10 to P<120) of the Fmr1 knockout (KO) mouse within the CA1 and Dentate Gyrus (DG) areas of the hippocampus. We investigated whether alterations in the normal developmental shift of the ratio between the NMDAR subunits exist and could contribute to the synaptic mechanisms causing deficits seen in the Fmr1 KO. We discovered a consistent decrease in LTP in all five age groups in the DG of the Fmr1 KO mouse compared to littermate wild-type controls whereas in the CA1 we found an age-dependent reduction in LTP at two age groups (P30-40 and P60-80). Through western blotting of hippocampal subfields, we found NMDAR subunits were consistently downregulated in the DG. In the CA1, we found an age-dependent trend for reduced NMDAR subunit expression at the same two age groups where deficits were identified in our LTP experiments. In conclusion, our results are consistent with regional and developmental differences in LTP deficits in Fmr1 KO mice and suggest that a reduction in NMDAR expression may underlie the synaptic plasticity alterations.

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FREQUENCY OF POLYMORPHISM RS 1001179 OF CATALASE IN PATIENTS WITH MAJOR DEPRESSION

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Oxidative damage (oxidative stress) has been a prominent topic for scientists for several years. Recently, increased attention has been paid to the influence of oxidative damage on the etiology and pathogenesis of major depressive disorder. Catalase is one of the most important endogenous antioxidant enzymes. If antioxidant mechanisms failed, reactive oxygen species damage the biomolecules followed by progression of the disease. In the present study, we examined the hypothesis that increased oxidative damage and polymorphism in the *CAT* gene (-262 promoter region, rs1001179) are associated with major depressive disorder. Analysis of the rs1001179 catalase polymorphism was performed by polymerase chain reaction and fragment length polymorphism in patients and healthy individuals. TT catalase genotype was more common in patients (4.0 %) compared to controls (0.0 %). More frequent were genotypes CT + TT in patients (40 % vs. 26 %). We did not observe statistically significant differences in the frequency of the rs1001179 catalase polymorphism in patients with major depression. However, genetic variation in antioxidant enzymes could contribute to a certain disease phenotype.

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INVESTIGATING DEMOGRAPHIC AND EPIGENETIC RISK FACTORS FOR DEPRESSION IN YOUNG ADULTS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Background: High rates of depression have been identified in student populations. Depression has a complex aetiology caused by a combination of environmental and biological factors. The aim of this research is to investigate demographic and epigenetic factors associated with depression in students. **Methods:** Students who met the criteria for lifetime depression (n=250) and matched controls (n=250) provided a saliva sample and completed an online mental health questionnaire. Methylome profiling using the 850K EPIC array and logistic regression analysis were used to examine associations between methylation patterns and demographic associations with depression. The EPIC Array consists of 850K probes for all gene promoters, gene bodies and CpG islands, covering 99% of RefSeq genes. Raw data (idat files) were processed using the RnBeads pipeline in the R statistical and programming environment. **Results:** Logistic regression analysis found that age and gender were significantly associated with risk for depression, suicide attempt and self-harm. Whole methylome analysis found methylation differences between individuals with depression and matched controls. A number of individual CpG sites met genome wide level FDR significance. Gene ontology analysis of the top 1000 ranked promoters showed highly significant enrichment for immune response consistent with accumulating evidence which supports the relationship between depression and inflammation. **Conclusions:** Our results suggest that many students are starting university with high levels of mental health problems, highlighting the importance of early intervention. Verification of DNA methylation changes in specific genes, detected in saliva, may be a valuable tool for identifying at risk subjects.

Pubmed:

34864232: Ward C, McLafferty M, McLaughlin J, McHugh R, McBride L, Brady J, Bjourson AJ, Walsh CP, O'Neill SM, Murray EK
Suicidal behaviours and mental health disorders among students commencing college.
The increase in psychological disorders and suicidal behaviour in students is a reason for growing concern. Some may start university with pre-existing problems, while others develop problems during this time. It is important to evaluate mental health and wellbeing early, identifying those at risk. The aim of this study was to compare mental health problems and help-seeking behaviour between students in Northern Ireland (NI) and the Republic of Ireland (ROI). Whilst geographically proximate, the institutions span a cross-border region with distinct education and healthcare systems. First-year undergraduate students (n = 1828) were recruited in September 2019 as part of the World Mental Health International College Student Initiative. Suicidal behaviour, mental health and substance disorders were investigated using the World Mental Health- Composite International Diagnostic Interview. Prevalence of disorders was high, with more ROI students experiencing problems than NI students. Students were significantly more likely to experience mental health problems if they were female (p<0.001), non-heterosexual (p<0.0001), and over the age of 21 (p<0.0001). These findings show that many students are starting university with high levels of psychopathology and suicidal behaviour, highlighting the importance of early intervention which may need to be tailored to different student populations.
Psychiatry Res, 2022; 307
34977911: McLafferty M, Brown N, McHugh R, Ward C, Stevenson A, McBride L, Brady J, Bjourson AJ, O'Neill SM, Walsh CP, Murray EK
Depression, anxiety and suicidal behaviour among college students: Comparisons pre-COVID-19 and during the pandemic.
Many students struggle with psychological problems during their college years. These problems may be even more apparent during the COVID-19 pandemic with the accompanying restrictions and transition to an online learning environment, but few longitudinal studies have been conducted to date. The aim of this study was to compare symptoms of depression, anxiety and suicidality prior to and during the pandemic, and identify stressors.
Psychiatry Res Commun, 2021; 1

35118906: Brown N, McLafferty M, O'Neill SM, McHugh R, Ward C, McBride L, Brady J, Bjourson AJ, Walsh CP, Murray EK
The Mediating Roles of Mental Health and Substance Use on Suicidal Behavior Among Undergraduate Students With ADHD.
To evaluate the prevalence of suicidal ideation (SI), plans and attempts, and non-suicidal self-injury (NSSI) among students with attention deficit hyperactivity disorder (ADHD). Furthermore, we explored the mediating effects of depression, anxiety, alcohol and substance use on the association between ADHD and suicidal behaviors and NSSI.
J Atten Disord, 2022;

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BOARD NUMBER: S07-161

RESPONSE EQTL OF GPR56 EXPRESSION ARE ASSOCIATED WITH ANTIDEPRESSANT RESPONSE.

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aims Mood disorders are severe and frequent psychiatric conditions, but their pathophysiology remains elusive, and treatment is often difficult. A recent study associated the *GPR56* expression and antidepressant response in individuals with major depressive disorders. To unveil the mechanisms underlying antidepressant response, we aim to identify the genomic loci associated with changes in the *GPR56* expression associated with antidepressant response, also known as response expression quantitative trait loci (reQTL). **Methods** We studied genome-wide genotyping data of peripheral blood mononuclear cells from 144 individuals, comprising healthy volunteers (N=86) and patients (N=58) diagnosed with a major depressive episode, treated with antidepressants, and evaluated 8 weeks later for treatment response. We then assessed the association between SNPs around the 2 Mb region of *GPR56* (*cis*-reQTL) or over the whole genome (*trans*-reQTL) and *GPR56* expression change during the 8-week-follow-up study, through linear regression testing between the genotype of each SNP and the variation of *GPR56* expression. We next generated ROC curves to evaluate the performance of each reQTL to predict the clinical response to treatment. **Results** Several *cis*- and *trans*-reQTL were significantly (p-value<0.001) associated with *GPR56* expression change. Analyses are still ongoing to validate reQTL that are predictive of the clinical response to antidepressants and to specify pathway and functional gene set information. **Conclusions** Promising preliminary results of reQTL for *GPR56* expression and antidepressant treatment were found, and functional enrichment analysis should provide interesting information regarding the pathophysiology of antidepressant response.

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DIFFERENTIALLY METHYLATED REGIONS IN ANTIDEPRESSANT RESPONSE- A METHYLOME-WIDE ASSOCIATION STUDY FROM THE EMC TRIAL

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Background:
Although the currently available antidepressants are well established in the treatment of major depressive disorder (MDD), there is strong variability in the response of individual patients. Reliable predictors to guide treatment decisions before or in an early stage of treatment are needed. DNA-methylation has been proven a useful biomarker in different clinical conditions, but its importance for mechanisms of antidepressant response has not yet been determined. **Methods:**
80 MDD patients were selected out of >500 participants from the Early Medication Change cohort based on their antidepressant response after four weeks and stratified into clear responders and age- and sex-matched non-responders (N=40, each). Early improvement after two weeks was analyzed as a secondary outcome. DNA-methylation was determined using the Illumina EPIC BeadChip. Epigenome-wide association studies were performed and differentially methylated regions (DMRs) identified using the comb-p algorithm. Enrichment was tested for hallmark gene-sets and in genome-wide association studies of depression and antidepressant response. **Results:**
No epigenome-wide significant differentially methylated positions were found for treatment response or early improvement. Twenty DMRs were associated with response; the strongest in an enhancer region in *SORBS2,* which has been related to cardiovascular diseases and type II diabetes. Another DMR was located in *CYP2C18,* a gene previously linked to antidepressant response. **Conclusions:**
Results pointed towards differential methylation in genes associated with cardiac function, neuroticism, and depression. Linking differential methylation to antidepressant treatment response is an emerging topic and a represents a step towards personalized medicine, potentially facilitating the prediction of patients' response before treatment.

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MULTIDIMENSIONAL PREDICTORS OF ANTIDEPRESSANT RESPONSE: USING COMPUTATIONAL APPROACHES TO INTEGRATE BIOLOGICAL NETWORKS, ENVIRONMENTAL FACTORS AND CLINICAL OUTCOMES

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Background: There is a dearth of new mechanistic models for the development of objective tools to diagnose depression and monitor antidepressant responses. In rodent models, administration of acetylcarnitine(LAC) leads to a rapid and sustained antidepressant response by epigenetic regulation of glutamatergic neurons in ventral dentate gyrus neurons. Recently, we found decreased levels of LAC in clinical phenotypes of depression characterized by brain insulin resistance, as showed by increased expression of the insulin docking protein IRS1 in exosomes enriched for L1CAM, a protein highly expressed in the brain. Methods: Here, we used computational approaches and efficiently leveraged biological samples from a previous randomized, placebo-controlled trial with pioglitazone to determine the emergence of the pivotal mitochondrial metabolite LAC in relation to previously described individual predictors of antidepressant responses. Results: Our new findings showed the lowest LAC levels in those subjects with depression characterized by elevated BMI, high reported rates of emotional abuse and decreased leucocytes telomere length(n=47). Furthermore, we used prediction profilers to show that decreased baseline LAC levels, elevated BMI and high reported rates of emotional abuse predict lack of antidepressant response to pioglitazone($p=0.02, r=0.86$) with a stronger ability than each individual measure alone. Discussion: The current findings suggest that integrated factors spanning cellular aging, metabolic function and childhood trauma contribute to define potential new mitochondrial endophenotypes of depression in agreement with the concept of precision medicine. Future studies are needed to further characterize the complex interplay of these multidimensional biological networks, environmental factors with clinical outcomes.

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A NOVEL APPROACH TO MEASURE INDIVIDUAL TRAITS REVEALS A PLASTICITY-FOCUSED GENETIC PROFILE OF ANXIETY

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Anxiety disorders are the most prevalent psychiatric illnesses. Animal models, our primary approach to understanding the background of these conditions, often fail to reach clinical relevance. We hypothesize that this is because conventional behavioural readouts represent the fluctuating states rather than the permanent traits of subjects. We suggest a sampling approach of trait anxiety markers based on rethinking rodent models' classical measures. We created summary measures (SuMs) from repeated sampling of popular anxiety tests and compared those with conventional single measurements (SiMs). In contrast to SiMs, SuMs i) revealed correlations of anxiety models, predicted ii) anxiety in future threatening situations and iii) foretold the level of fear generalization. Using these trait-like markers, we conducted a whole-transcriptome RNA-seq analysis on prefrontal cortex samples of 27 rats. We found that SuMs shape the outcome of our analysis quantitatively as well as qualitatively. First, SuMs five-fold enhanced the number of gene discoveries in our study. Second, while SiMs revealed acute stress-related transcripts (38%), the genetic profile of SuMs is dominated by genes of neuronal plasticity (45%). Finally, SuMs revealed five and three times more genes that were previously mentioned in preclinical and clinical literature of mental illness, respectively. In summary, a significant enhancement in the depth and resolution of sampling enables us to trace out stable traits of the animals. Best to our knowledge, this approach identified the highest number of gene targets, and we present the most detailed transcriptomic profile of rodent anxiety so far.

Pubmed:
32350040: Varga ZK, Pejtsik D, Biró L, Zsigmond Á, Varga M, Tóth B, Salamon V, Annus T, Mikics É, Aliczki M
Conserved Serotonergic Background of Experience-Dependent Behavioral Responsiveness in Zebrafish ().
Forming effective responses to threatening stimuli requires the adequate and coordinated emergence of stress-related internal states. Such ability depends on early-life experiences and, in connection, the adequate formation of neuromodulatory systems, particularly serotonergic signaling. Here, we assess the serotonergic background of experience-dependent behavioral responsiveness using male and female zebrafish (). For the first time, we have characterized a period during behavioral metamorphosis in which zebrafish are highly reactive to their environment. Absence of social stimuli during this phase established by isolated rearing fundamentally altered the behavioral phenotype of postmetamorphic zebrafish in a challenge-specific manner, partially due to reduced responsiveness and an inability to develop stress-associated arousal state. In line with this, isolation differentially affected whole-brain serotonergic signaling in resting and stress-induced conditions, an effect that was localized in the dorsal pallium and was negatively associated with responsiveness. Administration of the serotonin receptor 1A partial agonist buspirone prevented the isolation-induced serotonin response to novelty in the level of the whole brain and the forebrain as well, without affecting catecholamine levels, and rescued stress-induced arousal along with challenge-induced behaviors, which together indicates functional connection between these changes. In summary, there is a consistent negative association between behavioral responsiveness and serotonergic signaling in zebrafish, which is well recognizable through the modifying effects of developmental perturbation and pharmacological manipulations as well. Our results imply a conserved serotonergic mechanism that context-dependently modulates environmental reactivity and is highly sensitive to experiences acquired during a specific early-life time window, a phenomenon that was previously only suggested in mammals. The ability to respond to challenges is a fundamental factor in survival. We show that zebrafish that lack appropriate social stimuli in a sensitive developmental period show exacerbated alertness in nonstressful conditions while failing to react adequately to stressors. This shift is reflected inversely by central serotonergic signaling, a system that is implicated in numerous mental disorders in humans. Serotonergic changes in brain regions modulating responsivity and behavioral impairment were both prevented by the pharmacological blockade of serotonergic function. These results imply a serotonergic mechanism in zebrafish that transmits early-life experiences to the later phenotype by shaping stress-dependent behavioral reactivity, a phenomenon that was previously only suggested in mammals. Zebrafish provide new insights into early-life-dependent neuromodulation of behavioral stress-responses.

J Neurosci, 2020; 40
30410116: Varga ZK, Zsigmond Á, Pejtsik D, Varga M, Demeter K, Mikics É, Haller J, Aliczki M
The swimming plus-maze test: a novel high-throughput model for assessment of anxiety-related behaviour in larval and juvenile zebrafish (Danio rerio).
Larval zebrafish (Danio rerio) has the potential to supplement rodent models due to the availability of resource-efficient, high-throughput screening and high-resolution imaging techniques. Although behavioural models are available in larvae, only a few can be employed to assess anxiety. Here we present the swimming plus-maze (SPM) test paradigm, a tool to assess anxiety-related avoidance of shallow water bodies in early developmental stages. The "+" shaped apparatus consists of arms of different depth, representing different levels of aversiveness similarly to the rodent elevated plus-maze. The paradigm was validated (i) in larval and juvenile zebrafish, (ii) after administration of compounds affecting anxiety and (iii) in differentially aversive experimental conditions. Furthermore, we compared the SPM with conventional "anxiety tests" of zebrafish to identify their shared characteristics. We have clarified that the preference of deeper arms is ontogenetically conserved and can be abolished by anxiolytic or enhanced by anxiogenic agents, respectively. The behavioural readout is insensitive to environmental aversiveness and is unrelated to behaviours assessed by conventional tests involving young zebrafish. Taken together, we have developed a sensitive high-throughput test allowing the assessment of anxiety-related responses of zebrafish regardless of developmental stage, granting the opportunity to combine larva-based state-of-the-art methods with detailed behavioral analysis.
Sci Rep, 2018; 8
30458201: Balogh Z, Szente L, Biro L, Varga ZK, Haller J, Aliczki M
Endocannabinoid interactions in the regulation of acquisition of contextual conditioned fear.
Endocannabinoids (eCBs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG) were shown to be involved in the basis of trauma-induced behavioral changes, particularly contextual conditioned fear, however, their ligand-specific effects and possible interactions are poorly understood. Here we assessed specific eCB effects and interactions on acquisition of contextual conditioned fear employing electric footshocks in a rat model. We selectively increased eCB levels by pharmacological blockade of the degrading enzymes of AEA by URB597 and 2-AG by JZL184 before traumatization either systemically or locally in relevant brain areas, the prelimbic cortex (PrL), ventral hippocampus (vHC) and basolateral amygdala (BLA). Following traumatization, a series of contextual reminders were conducted during which conditioned fear was assessed. While systemic URB597-treatment during traumatization only slightly enhanced the acquisition of contextual conditioned fear, administration of the compound in the PrL and vHC led to the acquisition of stable, lasting conditioned fear, resistant to extinction. These effects of URB597 were blocked by simultaneous administration of JZL184. Similar treatment effects did not occur in the BLA. Treatment effects were not secondary to alterations in locomotor activity or nociception. Our findings suggest that AEA and 2-AG functionally interact in the regulation of acquisition of contextual conditioned fear. AEA signaling in the PrL and vHC is a crucial promoter of fear acquisition while 2-AG potentially modulates this effect. The lack of eCB effects in the BLA suggests functional specificity of eCBs at distinct brain sites.
Prog Neuropsychopharmacol Biol Psychiatry, 2019; 90
30116182: Balázsfi D, Zelena D, Demeter K, Miskolczi C, Varga ZK, Nagyváradi Á, Nyíri G, Cserép C, Baranyi M, Sperlágh B, Haller J
Differential Roles of the Two Raphe Nuclei in Amiable Social Behavior and Aggression - An Optogenetic Study.
Serotonergic mechanisms hosted by raphe nuclei have important roles in affiliative and agonistic behaviors but the separate roles of the two nuclei are poorly understood. Here we studied the roles of the dorsal (DR) and median raphe region (MRR) in aggression by optogenetically stimulating the two nuclei. Mice received three 3 min-long stimulations, which were separated by non-stimulation periods of 3 min. The stimulation of the MRR decreased aggression in a phasic-like manner. Effects were rapidly expressed during stimulations, and vanished similarly fast when stimulations were halted. No carryover effects were observed in the subsequent three trials performed at 2-day intervals. No effects on social behaviors were observed. By contrast, DR stimulation rapidly and tonically promoted social behaviors: effects were present during both the stimulation and non-stimulation periods of intermittent stimulations. Aggressive behaviors were marginally diminished by acute DR stimulations, but repeated stimulations administered over 8 days considerably decreased aggression even in the absence of concurrent stimulations, indicating the emergence of carryover effects. No such effects were observed in the case of social behaviors. We also investigated stimulation-induced neurotransmitter release in the prefrontal cortex, a major site of aggression control. MRR stimulation rapidly but transiently increased serotonin release, and induced a lasting increase in glutamate levels. DR stimulation had no effect on glutamate, but elicited a lasting increase of serotonin release. Prefrontal serotonin levels remained elevated for at least 2 h subsequent to DR stimulations. The stimulation of both nuclei increased GABA release rapidly and transiently. Thus, differential behavioral effects of the two raphe nuclei were associated with differences in their neurotransmission profiles. These findings reveal a surprisingly strong behavioral task division between the two raphe nuclei, which was associated with a nucleus-specific neurotransmitter release in the prefrontal cortex.

Front Behav Neurosci, 2018; 12
25547462: Aliczki M, Varga ZK, Balogh Z, Haller J
Involvement of 2-arachidonoylglycerol signaling in social challenge responding of male CD1 mice.
Endocannabinoids are strong modulators of emotionality and present a novel target for psychotropic drug development. Increasing evidence suggests that endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) affect behavior differentially. While the roles of anandamide have been investigated extensively, studies regarding the specific roles of 2-AG became possible only recently, and its involvement in social behaviors has not yet been studied.
Psychopharmacology (Berl), 2015; 232
23578952: Aliczki M, Zelena D, Mikics E, Varga ZK, Pinter O, Bakos NV, Varga J, Haller J
Monoacylglycerol lipase inhibition-induced changes in plasma corticosterone levels, anxiety and locomotor activity in male CD1 mice.
The hypothalamus-pituitary-adrenal-axis is strongly controlled by the endocannabinoid system. The specific impact of enhanced 2-arachidonoylglycerol signaling on corticosterone plasma levels, however, was not investigated so far. Here we studied the effects of the recently developed monoacylglycerol lipase inhibitor JZL184 on basal and stress-induced corticosterone levels in male CD1 mice, and found that this compound dramatically increased basal levels without affecting stress responses. Since acute changes in corticosterone levels can affect behavior, JZL184 was administered concurrently with the corticosterone synthesis inhibitor metyrapone, to investigate whether the previously shown behavioral effects of JZL184 are dependent on corticosterone. We found that in the elevated plus-maze, the effects of JZL184 on "classical" anxiety-related measures were abolished by corticosterone synthesis blockade. By contrast, effects on the "ethological" measures of anxiety (i.e. risk assessment) were not affected by metyrapone. In the open-field, the locomotion-enhancing effects of the compound were not changed either. These findings show that monoacylglycerol lipase inhibition dramatically increases basal levels of corticosterone. This endocrine effect partly affects the anxiolytic, but not the locomotion-enhancing effects of monoacylglycerol lipase blockade.
Horm Behav, 2013; 63

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CHANGES IN GENE EXPRESSION WITHIN SELECTED BRAIN REGIONS OF ASTHMATIC MICE

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aim: Asthmatic patients have a higher risk of poor mental health including anxiety and depression. Bidirectional links between lung inflammation and mental health clearly exist, the molecular mechanisms underlying these interactions are not well understood. To gain a better understanding, we performed transcriptomic profiling on different brain regions after inducing lung inflammation (asthma) in mice. **Methodology**: Asthmatic mice were sensitized with either ovalbumin (OVA, 10µg, chicken egg) in alum adjuvant or lipopolysaccharide (LPS, *E. coli*, serotype 026:B6), once/week for 3 consecutive weeks. Mice were then challenged in vapour chambers with either nebulized OVA (1% wt/vol%) or LPS (0.05% (wt/vol%) 20min/day for 3 consecutive days, followed by once/week for 3 additional consecutive weeks. Five brain regions were collected/isolated by hole punch including: hypothalamus, amygdala and lateral adjacent cortex, dorsal hippocampus, periaqueductal gray, dorsal raphe, and the tegmental pontine brainstem. Total RNA was extracted and sequenced using standard protocols for Illumina NovaSeq6000. **Results**: Lung inflammation was confirmed with post-mortem histology using HX&Eo, Alcian blue-PAS, and Masson's Trichrome stains. Our results showed typical asthma features such as peribronchial and perivascular inflammation, airway wall fibrosis and severe mucus production. This study successfully identified a range of single genes and functional gene networks with altered expression in asthma groups versus control. **Conclusion**: Our findings provide insights into the mechanisms underlying neurological changes during asthma. Moreover, these findings may also assist in determining lung-brain pathways that are critical to future drug discovery and efficacy.

Pubmed:
31147813: Bastawy EM, Ahmed RR, Abd El-Hafeez AA, Abd El-Hady FK, Hosoi T, Ozawa K, El-Ganzuri MA
Grapefruit juice exerts anti-osteoporotic activities in a prednisolone-induced osteoporosis rat femoral fracture model, possibly via the RANKL/OPG axis.
This study aimed to shed light on the protective and therapeutic anti-osteoporotic effects and mechanisms of action of grapefruit juice (GFJ) on prednisolone-induced osteoporosis a rat femoral fracture model. We found that treating rats with GFJ before and/or after prednisolone-induced osteoporosis resulted in increased bone density, total mineral content, and calcium content to counteract the osteoporotic effects of prednisolone. In parallel, the histological and ultrastructural results of the GFJ-treated groups correlated well with enhanced breaking strength of femurs subjected to a constant load. Furthermore, GFJ treatment before and after prednisolone-induced osteoporosis decreased plasma alkaline phosphatase and tartrate-resistant acid phosphatase activities and increased the level of insulin-like growth factor 1. Mechanistically, our immunohistochemistry study showed that GFJ ameliorated prednisolone-induced osteocalcin depletion, decreased receptor activator of nuclear factor kappa-B ligand (RANKL) expression, and increased osteoprotegerin (OPG) expression. GFJ showed a beneficial anti-osteoporotic effect against prednisolone-induced osteoporosis in rats, possibly via the RANKL/OPG axis, suggesting that GFJ might be a good candidate for developing anti-osteoporotic drugs.
Cytotechnology, 2019;

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PSYCHOBIOLOGICAL EFFECTS OF EMDR THERAPY IN THE TREATMENT OF BEREAVEMENT: PRELIMINARY EVIDENCE

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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The loss of a loved one inevitably causes emotional distress and failure in trauma processing may result in psychological/psychosomatic disorders. Eye Movement Desensitization and Reprocessing (EMDR) therapy seems to be able to reduce the occurrence of such pathophysiological drifts by inducing horizontal saccadic eye movements via bilateral stimulation. The aim of the present study was to describe acute and longer-lasting physiological effects of EMDR applied to subjects that suffered bereavement. Four women in acute grief were recruited and treated with EMDR therapy. During pre-intervention phase, psychological symptoms associated with trauma, complicated grief, as well as state and trait anxiety were assessed. During EMDR sessions, autonomic (heart rate and heart rate variability – HRV, peripheral temperature changes by means of functional Thermal Imaging), neuroendocrine (salivary cortisol) and psychological parameters (subjective unit of disturbance – SUD - and state anxiety) were evaluated. During post-intervention phase, psychological symptoms were re-assessed. Preliminary results obtained during EMDR sessions point to (i) a lowering of state anxiety levels, (ii) a reduction in cortisol levels, a reduction in heart rate and an increase in vagally-mediated HRV at the end of bilateral stimulation. At the end of the treatment (on average after five EMDR sessions), improved psychological symptoms, decreased heart rate and increased vagally-mediated HRV were observed. These preliminary data suggest that favorable psychological effects of EMDR therapeutic approach might be mediated by its buffering properties over autonomic and neuroendocrine stress responsivity.

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ANTIDEPRESSANT-LIKE EFFECT OF LONG-TERM SYSTEMIC ADMINISTRATION OF IRISIN IN YOUNG MICE.

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Aims Regular physical exercise improves mood, decreasing the risk of developing depressive disorders, and represents a useful tool for a more effective depression treatment, both reducing depressive symptoms and enhancing psychophysical well-being. Muscle cells during exercise secrete myokines that could mediate beneficial effects on several targets including brain. Among the myokines, irisin, recently discovered, displays a neuroprotective effect in cerebral areas involved in cognitive function, memory, learning, and mood. Here, we investigated the potential antidepressant properties of long-term systemic irisin administration in mice by different behavioral tests. Moreover, to evaluate the molecular pathways underlying irisin treatment, we analyzed the expression of irisin precursor (FNDC5), neurotrophic factors, and inflammatory mediators in the brain. **Methods** Young mice were intraperitoneally injected with vehicle or r-irisin (100 µg/kg/weekly) for 1 month. Mouse immobility and locomotor abilities were assessed by the Forced Swim Test (FST), Tail Suspension Test (TST), and Open Field Test (OFT). qRT-PCR assays were performed to analyze the expression of FNDC5, some neurotrophins, growth factors, and interleukins. **Results** Irisin administration decreases the immobility time in TST and FST and increases the time spent in the arena center in OFT. In addition, irisin up-regulates the gene expression of FNDC5, neurotrophins (BDNF, IGF-1), and cytokines (IL-4, IL-6, IL-10, IL-1β) in mouse brain extracts. **Conclusions** Systemic irisin administration is able to induce an antidepressant-like behavior and displays an anxiolytic-like effect probably due to the modulation of endogenous brain factors. However, further studies are needed to better clarify the neuroprotective role of irisin in depressive disorders.

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A DOUBLE-BLIND PLACEBO-CONTROLLED ANALYSIS OF QTC INTERVAL PROLONGATION IN NAïVE METHYLPHENIDATE USERS

POSTER SESSION 07 - SECTION: PSYCHOPHARMACOLOGY AND PSYCHIATRIC TREATMENT

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Introduction: Prolonged QTc interval has been linked to adverse outcomes for chronic methylphenidate users. 1-3 We intended to investigate if naïve methylphenidate users receiving a single dose of Quillivant (methylphenidate extended-release formula) experienced a prolongation of QT interval as seen on 12 lead ECG recorded at 4.5 hour after administration. Methods: Fifty-four adult naïve methylphenidate participants (18-65 years old) received methylphenidate extended-release formula or matching placebo suspension. Pre- and post-operative QTc intervals were calculated. We included in our analysis only participants with a pre-operative QTc of < 450 ms (the normal QT interval is between 400 – 440ms). Results: Only one participant from the placebo group experienced a QTc above 500 ms post-op, seven ranged between 450-500ms, and seven below 450 ms. From the methylphenidate group two participants were above 500 ms, two between 450-500, and eight were below 450 ms. Discussion: We found no conclusive evidence that a single dose of methylphenidate extended-release formula results in QTc interval prolongation. More research is required. References: Kenny, J. D.et al (2015). Dextroamphetamine (but not atomoxetine) induces reanimation from general anesthesia: implications for the roles of dopamine and norepinephrine in active emergence. PLoS One. Zhou, J.et al (2021). Gender-Specific Long-Term Prognostic Values of QRS Duration, QT Interval, and QTc from Automated ECG Analysis for Mortality and Adverse Outcomes in Patients Hospitalized for Heart Failure. Dalsgaard, S. et al (2014). Cardiovascular safety of stimulants in children with attention-deficit/hyperactivity disorder: a nationwide

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LONG-TERM CONSEQUENCES OF ALCOHOL USE IN EARLY ADOLESCENT MICE : FOCUS ON NEUROADAPTATIONS IN GR, CRF AND BDNF.

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Our aim was to assess the cognitive and emotional state, as well as related-changes in the glucocorticoid receptor (GR), the corticotropin-releasing factor (CRF) and the brain-derived neurotrophic factor (BDNF) expression of adolescent C57BL/6J male mice after a five-week two-bottle choice protocol (postnatal day (pd) 21 to pd52). Additionally, we wanted to analyse whether the behavioural and neurobiological effects observed in late adolescence (pd62) lasted until adulthood (pd84). Behavioural testing revealed that alcohol during early adolescence increased anxiety-like and compulsive-related behaviours, which was maintained in adulthood. Concerning cognition, working memory was only altered in late adolescent mice, whereas object location test performance was impaired in both ages. In contrast, novel object recognition remained unaltered. Immunohistochemical analysis showed that alcohol during adolescence diminished BDNF+ cells in the cingulate cortex, the hippocampal CA1 layer and the central amygdala. Regarding hypothalamic-pituitary-adrenal axis (HPA) functioning, alcohol abuse increased the GR and CRF expression ithe hypothalamic paraventricular nucleus and the central amygdala. Besides this, GR density was also higher in the prelimbic cortex and the basolateral amygdala, regardless of the animals' age. Our findings suggest that adolescent alcohol exposure led to long-term behavioural alterations, along with changes in BDNF, GR and CRF expression in limbic brain areas involved in stress response, emotional regulation, and cognition.

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BOARD NUMBER: S07-170

BEHAVIORAL EFFECTS OF THE EARLY LIFE STRESS, DOPAMINERGIC IMBALANCE, AND THEIR INTERPLAY IN MALE AND FEMALE PREPUBERTAL MICE

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Postnatal neurodevelopment is marked by the intense organization and refinement of circuits. Both the exposure to chronic stressors and dopaminergic imbalance are related to neuropsychiatric disorders and, on their own, modulate a range of behaviors. We aimed to investigate if the interaction of both factors acts on behavioral development in male and female mice. Animals were divided into four groups: L-DOPA, Saline, L-DOPA + Maternal Separation (MS), and Saline+MS. The pups were daily intraperitoneally injected with L-DOPA (50mg/kg) or saline and MS groups were also submitted to daily maternal separation for 3 hours/day from P2 until P14. At four weeks old, the animals were tested for exploratory and anxiety-like behavior by actimeter box, open-field test, light-dark box, elevated-plus maze, and feeding suppressed by novelty. Data were analyzed using Two-Way ANOVA and Tukey posthoc. We found that females injected with L-DOPA showed decreased anxiety-like behavior. On the other hand, the SM females injected with L-DOPA showed increased exploratory- and anxiety like-behavior. Males injected with L-DOPA showed an increase in exploratory- and anxiety-like behavior. Our results show that imbalance in dopaminergic signaling during early-development disrupted anxiety-like behavior in a sex-specific way. We also observed that early-life stress together with dopaminergic imbalance caused an opposite response of the dopaminergic imbalance alone in females. We need further studies to investigate the mechanisms behind these results and understand this interaction and to improve our understanding of the dopaminergic system during development. Finally, our results reinforce the importance of using both males and females in research.

Pubmed:
31990423: de Souza Lima ACM, de Alvarenga KAF, Codo BC, Sacramento EK, Rosa DVF, Souza RP, Romano-Silva MA, Souza BR
Impairment of motor but not anxiety-like behavior caused by the increase of dopamine during development is sustained in zebrafish larvae at later stages.
Many neuropsychiatric disorders are associated with both dopaminergic (DAergic) and developmental hypotheses. Since DAergic receptors are expressed in the developing brain, it is possible that alterations in dopamine (DA) signaling may impair brain development and consequent behavior. In our previous study, using a zebrafish model, we showed that an increase of DA during the 3 to 5 days postfertilization (dpf) developmental window (an important window for GABAergic neuronal differentiation) affects the motor behavior of 5 dpf larvae. In this study, we set out to determine whether these behavioral alterations were sustained in larvae at older stages (7 and 14 dpf). To test this hypothesis, we chronically treated zebrafish larvae from 3 to 5 dpf with DA. After washing the drug, we recorded and analyzed the first 5 and 30 min of the motor behavior of 5, 7, and 14 dpf subjects. We analyzed mobile episodes, distance traveled, time mobile, distance traveled per mobile episode, time in movement per mobile episode, and distance traveled per time mobile. We showed, once again, that an increase of DA during the 3 to 5 dpf developmental window reduces the number of movement episodes initiated by 5 dpf larvae. We also detected a decrease of other motor behavior parameters in 5 dpf DA-treated larvae. We observed that these alterations are sustained in the 7 dpf larvae. However, we did not see these general locomotor alterations in the 14 dpf larvae. Moreover, we detected a decrease of distance traveled and an increase of time of locomotion per episode in the first 5 min of behavioral analyses in 14 dpf DA-treated larvae. To test if the alterations in the first 5 min were due to anxiety-like behavior, we used a light/dark preference paradigm. We recorded 5dpf, 7dpf, and 14dpf larvae for 5 min and analyzed time of freezing, preference for light or dark, number of entries to the dark, percentage of time in the light. We observed that 5dpf larvae treated with DA showed more freezing, less passages to the dark, and more time spent in the light as compared to their control counterparts. But 7dpf and 14dpf larvae did not show these alterations. Taken overall, therefore, our results suggest that DA does play a role in the development of zebrafish motor behavior, and, furthermore, that some behaviors are

more sensitive than others to the effects of DAergic imbalances during development.
Int J Dev Neurosci, 2020; 80

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BOARD NUMBER: S07-171

SEX-SPECIFIC CONSEQUENCES OF PRECONCEPTION STRESS ON THE DEVELOPMENT OF DENDRITIC SPINES AND DENDRITIC LENGTH IN THE HIPPOCAMPAL FORMATION OF RATS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Our previous work in rats revealed that preconception stress (PCS), i.e. stress experienced by females prior to fertilization, affects neuronal development in the prefrontal cortex of their offspring. In extension of this study we analyzed the impact of PCS on neuronal and synaptic development in the hippocampal formation. Young adult female rats were exposed to unpredictable variable stressors for one week and then mated. Granular neurons in the dentate gyrus and pyramidal neurons in the CA1 (dorsal) region of adult male and female offspring from mothers exposed to PCS and from control mothers were quantitatively compared. Parameters analysed included dendritic length, dendritic spine number and spine frequency. In the dentate gyrus dendritic length was increased only in female PCS offspring, but not in male animals. In contrast, in the CA1 region basal dendritic length and basal spine number were increased only in male PCS offspring, but not in their sisters. The PCS-induced development of longer dendrites and higher spine numbers may reflect a higher level of synaptic connectivity within the hippocampal formation and perhaps also a more intensive input into the hippocampal formation from other brain regions. Based on our behavioral findings, where we observed increased anxiety and reduced social behavior in both genders of PCS offspring, it is tempting to speculate that stress prior to fertilization leads to an excessive intra- and interconnectivity of the hippocampal formation of the offspring and thereby mediate pathological behavioral outcomes.

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BOARD NUMBER: S07-172

PLATINUM NANOPARTICLE-BASED MICROREACTORS PROTECT AGAINST THE BEHAVIORAL AND NEUROBIOLOGICAL CONSEQUENCES OF CHRONIC STRESS EXPOSURE

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Excitotoxicity is described as the exacerbated activation of glutamate receptors that leads to neuronal damage and cell death. Astrocytes are responsible for the clearance of 80–90% of synaptically released glutamate. Chronic stress renders neurons vulnerable to excitotoxicity and has been associated to neuropsychiatric disorders. Platinum nanoparticles (Pt-NP) assembled with glutamate dehydrogenase and glutathione reductase have shown *in vitro* activity against excitotoxicity. The purpose of the present study was to investigate the *in vivo* efficacy of these nanoparticles on the behavioral and neurobiological effects of chronic stress exposure. Rats were either unstressed or exposed for 2 weeks to an unpredictable chronic mild stress paradigm (UCMS), administered intra-ventral hippocampus with Pt-NP (with or without the blockage of astrocyte functioning), and seven days later tested in the elevated T-maze (ETM; Experiment 1). The ETM allows the measurement of two defensive responses, avoidance and escape, in terms of psychopathology respectively related to generalized anxiety and panic disorder. Locomotor activity in an open field was also measured. Since previous evidence shows that stress inhibits adult neurogenesis, we evaluated the effects of the different treatments on the number of cells expressing the marker of migrating neuroblasts doublecortin (DCX) in the hippocampus (Experiment 2). Results showed that UCMS induces anxiogenic effects, increases locomotion, and decreases the number of DCX cells, effects that were counteracted by Pt-NP administration. This is the first study to demonstrate the *in vivo* efficacy of Pt-NP against the behavioral and neurobiological effects of chronic stress exposure. Financial support: FAPESP, BRAZIL (GRANT 2019/26777-8).

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BOARD NUMBER: S07-173

THE OVERFUNCTION OF ADENOSINE A2A RECEPTORS IN THE BASOLATERAL AMYGDALA IS NECESSARY AND SUFFICIENT TO TRIGGER BEHAVIOURAL MODIFICATIONS INDUCED BY REPEATED STRESS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Background: Chronic stress impairs mood and memory, an effect prevented by blocking adenosine A_{2A} receptors ($A_{2A}R$) (PNAS 112:7833,2015), which are upregulated in basolateral amygdala (BLA) upon stress. We now tested if blunting $A_{2A}R$ only in amygdala neurons normalizes behavioural alterations caused by repeated restraint stress (RS) and if this is mimicked by overactivating $A_{2A}R$ signalling. **Methods:** One week prior to handling or 14 days RS (4h daily), male adult Wistar rats were injected bilaterally in BLA with lentivectors encoding either a $A_{2A}R$-silencing short hairpin ($shA_{2A}R$) or control (shCTR) (*Nature Comm*7:11915,2016) or AAV5-CaMKIIα-optoA_{2A}R-mcherry (chimera to light-activate $A_{2A}R$ transducing systems; *Mol Psychiatry* 20:1339,2015) or AAV5-CaMKIIα-mcherry (control) and implanted optic fibers. After RS or after light stimulation for 6 consecutive days (8 trains each with 3000 light pulses of 50ms duration over 5min, 10min inter-train interval), rats (n=9-23/group) were behaviourally tested before assessing long-term potentiation (LTP) in amygdala slices (*Neuropsychopharmacol* 41:2862,2016). **Results:** Compared to control rats, RS rats injected with shCTR had no alteration of locomotion but displayed despair-based depression (forced-swimming), reward-based deficits (splash), increased anxiety (elevated plus-maze) and hampered memory (modified Y-maze, object displacement). BLA-$A_{2A}R$ down-regulation prevented RS-induced behavioral modification without altering locomotion and blunted exacerbated amygdala LTP. Chronic opto$A_{2A}R$ BLA stimulation increased anxiety, anhedonic-like and depressive-like behaviour, impaired spatial working memory and increased BLA-LTP magnitude. **Conclusions:** This shows a pivotal role of amygdala $A_{2A}R$ controlling RS-induced modifications and prompts $A_{2A}R$ as targets to manage mood-related disorders. Supported by LaCaixa Foundation (LCF/PR/HP17/52190001).

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BOARD NUMBER: S07-174

DISSOCIATING RECOVERED FROM UNRECOVERED INDIVIDUALS FOLLOWING TREATMENT IN AN ANIMAL MODEL OF PTSD REVEALS DIFFERENCES IN EXCITATORY-INHIBITORY BALANCE IN THE HIPPOCAMPUS.

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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The accepted approach for testing the effects of pharmacological treatment in animal models of PSTD remains examining the averaged impact of such treatment. However, clinically only a minority of patients show complete or partial remission. To bridge this discrepancy we used individual *'Behavioural Profiling*' (BP) approach to classify animals as '*treatment-responders' (TR)* or 'non-responders' (NR) to SSRI treatment. We bolstered the behavioral profiling approach by further elucidating molecular and electrophysiological changes amongst the categorized animals. We employed the (BP) analysis in an animal model of PTSD to separate between '*Trauma Exposed-Affected' (EA)* and '*Exposed-Unaffected'* individuals. *EA* animals were treated for one month with fluoxetine (SSRI). We further separated the *EA* population into *(TR)* or *(NR)*. Hippocampal tissue was collected, and the levels of $GABA_A$-α1, $GABA_A$-α2, GluN1, GluN2A were analyzed using western blots. In another set of animals, we conducted electrophysiological recording in the dorsal dentate gyrus to examine changes in local circuit activity and in LTP. As with human patients, only about 40% of the affected population responded to fluoxetine. These results strongly support the translational power of the model. Hippocampal tissue investigation indicated changes in GABAergic and Glutamatergic receptors' subunit expression between *TR*, *NR,* and healthy controls. Electrophysiological recording in the hippocampal dentate gyrus also revealed significant alterations in local circuit activity between *TR* and *NR*. Together, the findings suggest that a shift in excitatory-inhibitory balance in the hippocampus is associated with being responsive to drug treatment. This hints towards different molecular mechanisms leading to normalized behavior after recovery.

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IMPACT OF MITOFUSIN 2 ON ACCUMBENS-ASSOCIATED BEHAVIORS AND UNDERLYING NEUROBIOLOGICAL MECHANISMS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Mitochondrial dysfunction is increasingly being implicated in the aetiology of psychiatric disorders, but the underlying cellular and molecular mechanisms have not been fully identified. Previous work has established a link between trait anxiety and the expression of the mitochondrial fusion protein mitofusin 2 (Mfn2) in the nucleus accumbens (NAc) (Gebara et al., 2021), essential for motivation and goal-directed behavior. Here, we investigated the consequences of downregulating Mfn2 in the NAc for coping behavior, dendritic structure and synaptic physiology of NAc D1 medium spiny neurons (MSNs). To induce conditional mfn2 knockout in D1-MSNs, we employed a tamoxifen-dependent mouse line (Mfn2lox/lox-Drd1aCreERT2) or a viral vector (AAV1-Dyn-CRE) in the Mfn2lox/lox mouse line. Then, we evaluated animals' performance in the forced swim test, an effort-based motivation task. Mfn2 deletion in NAc D1-MSNs promoted the adoption of passive coping behaviors, as indicated by increased immobility. This was associated with a decreased activation of NAc shell neurons during the task, as revealed by cFos mapping. Ex vivo patch-clamp recordings from biocytin-filled D1-MSNs indicated that mfn2 deletion induced a reduction in dendritic complexity and in the frequency of excitatory inputs, as compared to wild-type D1-MSNs. Mitochondria qualitative assay via MitodsRed showed changes in organelles circularity and length. These results indicate that Mfn2 downregulation impacts the morphology and electrophysiological properties of accumbal D1-MSNs, leading to impaired engagement of the NAc during effort-related behavior. Our findings can deepen our understanding of the mechanisms underlying motivational disorders and pave the way for the development of novel treatment strategies to depression.

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BOARD NUMBER: S07-176

IMPLICATION OF THE VIP/VPAC1/2 SYSTEM IN THE REGULATION OF STRESS AND ANXIETY REACTIONS IN RODENTS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Vasoactive intestinal polypeptide (VIP) is a 28-amino-acid long peptide identified in the peripheral and central nervous system (CNS). Notably, within the CNS, VIP and its cognate VPAC receptors are highly expressed in stress and anxiety-related brain areas, such as amygdala, BNST, lateral septum as well as some cortical and hypothalamic structures. Although of these neuroanatomical findings, the role of VIP in stress and anxiety function is not well characterized. Accordingly, our first aim was to examine whether emotional stressors change VIP and/or VPAC1/2 receptor expression in selected brain areas. Therefore, quantitative real-time PCR technique was used to analyze VIP and VPAC1/2 mRNA transcript levels of rats exposed either to acute or chronic stress. Our results show stress-induced changes in VIP and VPAC1/2 receptor transcript levels in distinct brain areas. Our second aim was to investigate the effects of intracerebral VIP infusions on anxiety-related behaviors of rodents. Thus, C57Bl6/J male mice were administered either with VIP or vehicle and tested in the light/dark or elevated plus-maze test. We found that VIP-injected animals show a reduction of time spent in aversive zones of the behavioral tasks compared to vehicle-injected controls, suggesting an anxiogenic-like effect. Taken together, these data implicate a stress-induced upregulation of the central VIP/VPAC receptor system and, highlight a crucial role of VIP signaling in the regulation of stress and anxiety functions.

Pubmed:
34079435: Moulin TC, Ferro F, Hoyer A, Cheung P, Williams MJ, Schiöth HB
The Levodopa-Induced Depression Model Exhibits Negative Geotaxis Deficits and Differential Gene Expression in Males and Females.
More than 320 million people live with depression in the world, a disorder that severely limits psychosocial functioning and diminishes quality of life. The prevalence of major depression is almost two times higher in women than in men. However, the molecular mechanisms of its sex-specific pathophysiology are still poorly understood. is an established model for neurobiological research of depression-like states, as well as for the study of molecular and genetic sex differences in the brain. Here, we investigated sex-specific effects on forced-climbing locomotion (negative geotaxis) and gene expression of a fly model of depression-like phenotypes induced by levodopa administration, which was previously shown to impair normal food intake, mating frequency, and serotonin concentration. We observed that both males and females show deficits in the forced-climbing paradigm; however, modulated by distinct gene expression patterns after levodopa administration. Our results suggest that models can be a valuable tool for identifying the molecular mechanisms underlying the difference of depressive disorder prevalence between men and women.
Front Neurosci, 2021; 15
32731370: Moulin TC, Ferro F, Berkins S, Hoyer A, Williams MJ, Schiöth HB
Transient Administration of Dopaminergic Precursor Causes Inheritable Overfeeding Behavior in Young Adults.
Imbalances in dopaminergic signaling during development have been indicated as part of the underlying neurobiology of several psychiatric illnesses, including schizophrenia, major depression, bipolar disorder, and food addiction. Yet, how transient manipulation of dopaminergic signaling influences long-lasting behavioral consequences, or if these modifications can induce inheritable traits, it is still not understood. In this study, we used the model to test if transient pharmacological activation of the dopaminergic system leads to modulations of feeding and locomotion in adult flies. We observed that transient administration of a dopaminergic precursor, levodopa, at 6 h, 3 days or 5 days post-eclosion, induced overfeeding behavior, while we did not find significant effects on locomotion. Moreover, this phenotype was inherited by the offspring of flies treated 6 h or 3 days post-eclosion, but not the offspring of those treated 5 days post-eclosion. These results indicate that transient alterations in dopaminergic signaling can produce behavioral alterations in adults, which can then be carried to descendants. These findings provide novel insights into the conditions in which environmental factors can produce

transgenerational eating disorders.
Brain Sci, 2020; 10

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EFFECTS OF CHRONIC STRESS ON HIPPOCAMPAL MICROGLIA AND NEUROGENESIS OF MICE UNDER SOCIAL DEFEAT STRESS.

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Introduction: Chronic stress is the main environmental factor in the aetiology of depression and it is known that this type of stress may cause alterations in brain regions such as the hippocampus. Nevertheless, changes in a cellular basis are still a subject of study. ***Objective:*** The analysis of microglial cells and immature neurons in the dentate gyrus (DG) of stressed mice. ***Methods:*** C57BL/6J mice were subjected to Social Defeat Stress model (SDS), consisting of 6 days of social isolation prior to 10 days of stressor. The DG was analysed using immunohistochemistry techniques against Iba1 (microglia) and DCX (immature neurons) following image analysis (ImageJ) to obtain morphological and distribution data of microglial somas. Furthermore, hippocampal neurogenesis was assessed through stereological quantification of DCX+ cells (Visiopharm). ***Results***: An increase in soma size under chronic stress conditions was shown, as well as a less circular and more ameboid soma. These changes were observed mainly in the infrapyramidal blade of the DG. According to microglial cells distribution parameters, the granular cell layer (GCL) was the region which presented the highest microglial density under SDS. Regarding hippocampal neurogenesis, a decrease in the number of DCX+ Type 2-3 cells was observed in the whole DG. ***Conclusion:*** All these results offer a more profound insight of stress changes at a cellular level and could contribute to a better understanding of neurobiological basis in pathologies such as depression. **Projects: PSI2017-83408-P (MINECO) and P20 00460 (Consejería de Conocimiento, Investigación y Universidades, Junta de Andalucía). University of Málaga.**

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-178

ABNORMAL INTERNEURON NETWORK CONFIGURATION IN A MODEL OF FRAGILE X ASSOCIATED NEUROPSYCHIATRIC DISORDERS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Fragile X-associated neuropsychiatric disorders (FXAND) have been identified that result from premutation extension of CGG repeats in the Fragile X gene. These coincide or even precede the neurodegenerative late-onset Fragile X-associated tremor/ataxia syndrome (FXTAS), but in contrast to the latter, suitable animal models for the investigation of mechanisms causing FXAND are still missing. Here we describe a mouse model generated by inducible pre-and postnatal activation of a 99CGG transgene expression in the nervous system, resulting in hyperactivity of adolescent mutants and strongly increased anxiety-like behaviour in young adults in the absence of overt motor symptoms. Substantial inclusion load was found to accumulate in these animals in the cerebellum, but also in the ventral hippocampus (VH) and basolateral amygdala (BLA). Electrophysiological analysis revealed augmented gamma oscillations in the VH emerging during adolescence, and enhanced excitability in the adult, but not adolescent BLA. These findings were mirrored by the number of parvalbumin interneurons in these limbic regions, which were increased in number during adolescence but reduced in adulthood. Our data together suggest a disturbed maturation of limbic brain areas during postnatal development and aberrant configuration of the inhibitory network as putative cause for the altered network function and increased anxiety in this FXAND model.

Pubmed:
34673174: Çalışkan G, French T, Enrile Lacalle S, Del Angel M, Steffen J, Heimesaat MM, Rita Dunay I, Stork O
Antibiotic-induced gut dysbiosis leads to activation of microglia and impairment of cholinergic gamma oscillations in the hippocampus.
Antibiotics are widely applied for the treatment of bacterial infections, but their long-term use may lead to gut flora dysbiosis and detrimental effects on brain physiology, behavior as well as cognitive performance. Still, a striking lack of knowledge exists concerning electrophysiological correlates of antibiotic-induced changes in gut microbiota and behavior. Here, we investigated changes in the synaptic transmission and plasticity together with behaviorally-relevant network activities from the hippocampus of antibiotic-treated mice. Prolonged antibiotic treatment led to a reduction of myeloid cell pools in bone marrow, circulation and those surveilling the brain. Circulating Ly6C inflammatory monocytes adopted a proinflammatory phenotype with increased expression of CD40 and MHC II. In the central nervous system, microglia displayed a subtle activated phenotype with elevated CD40 and MHC II expression, increased IL-6 and TNF production as well as with an increased number of Iba1 + cells in the hippocampal CA3 and CA1 subregions. Concomitantly, we detected a substantial reduction in the synaptic transmission in the hippocampal CA1 after antibiotic treatment. In line, carbachol-induced cholinergic gamma oscillation were reduced upon antibiotic treatment while the incidence of hippocampal sharp waves was elevated. These alterations were associated with the global changes in the expression of neurotrophin nerve growth factor and inducible nitric oxide synthase, both of which have been shown to influence cholinergic system in the hippocampus. Overall, our study demonstrates that antibiotic-induced dysbiosis of the gut microbiome and subsequent alteration of the immune cell function are associated with reduced synaptic transmission and gamma oscillations in the hippocampus, a brain region that is critically involved in mediation of innate and cognitive behavior.
Brain Behav Immun, 2022; 99
32414783: Bikbaev A, Ciuraszkiewicz-Wojciech A, Heck J, Klatt O, Freund R, Mitlöhner J, Enrile Lacalle S, Sun M, Repetto D, Frischknecht R, Ablinger C, Rohlmann A, Missler M, Obermair GJ, Di Biase V, Heine M
Auxiliary α2δ1 and α2δ3 Subunits of Calcium Channels Drive Excitatory and Inhibitory Neuronal Network Development.
VGCCs are multisubunit complexes that play a crucial role in neuronal signaling. Auxiliary α2δ subunits of VGCCs modulate trafficking and biophysical properties of the pore-forming α1 subunit and trigger excitatory synaptogenesis. Alterations in the

expression level of α2δ subunits were implicated in several syndromes and diseases, including chronic neuropathic pain, autism, and epilepsy. However, the contribution of distinct α2δ subunits to excitatory/inhibitory imbalance and aberrant network connectivity characteristic for these pathologic conditions remains unclear. Here, we show that α2δ1 overexpression enhances spontaneous neuronal network activity in developing and mature cultures of hippocampal neurons. In contrast, overexpression, but not downregulation, of α2δ3 enhances neuronal firing in immature cultures, whereas later in development it suppresses neuronal activity. We found that α2δ1 overexpression increases excitatory synaptic density and selectively enhances presynaptic glutamate release, which is impaired on α2δ1 knockdown. Overexpression of α2δ3 increases the excitatory synaptic density as well but also facilitates spontaneous GABA release and triggers an increase in the density of inhibitory synapses, which is accompanied by enhanced axonaloutgrowth in immature interneurons. Together, our findings demonstrate that α2δ1 and α2δ3 subunits play distinct but complementary roles in driving formation of structural and functional network connectivity during early development. An alteration in α2δ surface expression during critical developmental windows can therefore play a causal role and have a profound impact on the excitatory-to-inhibitory balance and network connectivity. The computational capacity of neuronal networks is determined by their connectivity. Chemical synapses are the main interface for transfer of information between individual neurons. The initial formation of network connectivity requires spontaneous electrical activity and the calcium channel-mediated signaling. We found that, in early development, auxiliary α2δ3 subunits of calcium channels foster presynaptic release of GABA, trigger formation of inhibitory synapses, and promote axonal outgrowth in inhibitory interneurons. In contrast, later in development, α2δ1 subunits promote the glutamatergic neurotransmission and synaptogenesis, as well as strongly enhance neuronal network activity. We propose that formation of connectivity in neuronal networks is associated with a concerted interplay of α2δ1 and α2δ3 subunits of calcium channels.
J Neurosci, 2020; 40

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-179

SEX- AND TIME-DEPENDENT ALTERATIONS OF SYNAPTIC PLASTICITY IN AIC-NACC PATHWAY INDUCED BY ACUTE STRESS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Chronic stress is a major risk factor for the development of psychiatric disorders. Functional connectivity between the anterior insular cortex (aIC) and the nucleus accumbens core (NAcc) has been shown to be dysregulated in several of these disorders. Here, we investigated how exposure to acute stress disrupts insular inputs to the NAcc, measured by synaptic plasticity. Immediately after exposure to acute restraint stress or 24h later, *in vivo* extracellular recordings from NAcc neurons were performed in male and female rats in response to single-pulse stimulation of the aIC. High frequency stimulation (HFS) to the aIC was applied after a baseline period to measure changes in synaptic plasticity. In control male rats, aIC-HFS predominantly induced a long-term depression (LTD) in NAcc neurons whereas in stressed rats, it induced an LTP. In control female rats, aIC-HFS induced mostly an LTP whereas in the stressed group it produced no changes, suggesting either a loss of the ability to generate synaptic plasticity or an occlusion of LTP. 24 hours after stress, aIC-HFS predominantly evoked an LTP in both males and females. Our data indicate that males and females show different synaptic plasticity in the aIC-NAcc pathway under normal conditions, and different changes in synaptic efficacy in their acute response to stress. Furthermore, 24 hours after stress, only females demonstrated similar synaptic plasticity than control rats compared to males. These neurobiological responses might contribute to sex differences described in stress-related pathologies such as depression and anxiety.

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BOARD NUMBER: S07-180

A SECONDARY METABOLITE OF UMBILICARIA HIRSUTA, GYROPHORIC ACID, INCREASES HIPPOCAMPAL NEUROGENESIS AND SHOWS ANTIDEPRESSANT EFFECTS IN RELATED FORMS OF BEHAVIOR

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Gyrophoric acid (GA) is a secondary metabolite of various lichens, such as *Umbilicaria hirsuta*. Its effects on brain structures or behavior in living organisms have not been studied yet. The study aimed to analyze the potential antidepressant effects of daily administered GA in laboratory Wistar rats. In addition, we wanted to determine the ability of GA to cross the blood-brain barrier using isolated endothelial cells (BMEC) from two-week-old Wistar rats. Then, the potential antidepressant effects of GA were tested on Wistar male rats with immobilization and stress-induced depression. Pregnant females were stressed using restriction of mobility in the last week of gestation three times a day for 45 minutes each, three following days. GA (10 mg/kg body weight) was administered on daily basis for 30 days in 10% ethanol solution. Our preliminary results suggest that GA crosses BBB and thus could directly affect brain structures. During the depression, GA potentiated the process of neurogenesis, assessed as a significant increase in Ki67 positive cells in the hilus of the hippocampus ($P<0.01$). Besides, GA influenced the number of mature NeuN positive neurons in the CA1 layer of the hippocampus ($P<0.01$). The behavior showed increased time spent in open arms in EPM. The “shy” untreated animals eliminated the number of center crossing and rearing ($P<0.05$), the “lion-hearted” GA treated counterparts prolonged the spending time in open arms of the EPM test ($P<0.05$). GA shows some antidepressant effects and thus could be used as a supplement treatment of depression in the future.

Pubmed:
31985866: Pipová Kokošová N, Kisková T, Vilhanová K, Štafuriková A, Jendželovský R, Račeková E, Šmajda B
Melatonin mitigates hippocampal and cognitive impairments caused by prenatal irradiation.
Formation of new neurons and glial cells in the brain is taking place in mammals not only during prenatal embryogenesis but also during adult life. As an enhancer of oxidative stress, ionizing radiation represents a potent inhibitor of neurogenesis and gliogenesis in the brain. It is known that the pineal hormone melatonin is a potent free radical scavenger and counteracts inflammation and apoptosis in brain injuries. The aim of our study was to establish the effects of melatonin on cells in the hippocampus and selected forms of behaviour in prenatally irradiated rats. The male progeny of irradiated (1 Gy of gamma rays; n = 38) and sham-irradiated mothers (n = 19), aged 3 weeks or 2 months, were used in the experiment. Melatonin was administered daily in drinking water (4 mg/kg b. w.) to a subset of animals from each age group. Prenatal irradiation markedly suppressed proliferative activity in the dentate gyrus in both age groups. Melatonin significantly increased the number of proliferative BrdU-positive cells in hilus of young irradiated animals, and the number of mature NeuN-positive neurons in hilus and granular cell layer of the dentate gyrus in these rats and in CA1 region of adult irradiated rats. Moreover, melatonin significantly improved the spatial memory impaired by irradiation, assessed in Morris water maze. A significant correlation between the number of proliferative cells and cognitive performances was found, too. Our study indicates that melatonin may decrease the loss of hippocampal neurons in the CA1 region and improve cognitive abilities after irradiation.
Eur J Neurosci, 2020; 52
31963897: Kiskova T, Kubatka P, Büsselberg D, Kassayova M
The Plant-Derived Compound Resveratrol in Brain Cancer: A Review.
Despite intensive research, malignant brain tumors are among the most difficult to treat due to high resistance to conventional therapeutic approaches. High-grade malignant gliomas, including glioblastoma and anaplastic astrocytoma, are among the most devastating and rapidly growing cancers. Despite the ability of standard treatment agents to achieve therapeutic concentrations in the brain, malignant gliomas are often resistant to alkylating agents. Resveratrol is a plant polyphenol occurring in nuts, berries, grapes, and red wine. Resveratrol crosses the blood–brain barrier and may influence the central nervous system. Moreover, it influences the enzyme isocitrate dehydrogenase and, more importantly, the resistance to standard treatment via various mechanisms, such as O6-methylguanine methyltransferase. This review summarizes the

anticancer effects of resveratrol in various types of brain cancer. Several in vitro and in vivo studies have presented promising results; however, further clinical research is necessary to prove the therapeutic efficacy of resveratrol in brain cancer treatment.
Biomolecules, 2020; 10
30987191: Kisková T, Mungenast F, Suváková M, Jäger W, Thalhammer T
Future Aspects for Cannabinoids in Breast Cancer Therapy.
Cannabinoids (CBs) from provide relief for tumor-associated symptoms (including nausea, anorexia, and neuropathic pain) in the palliative treatment of cancer patients. Additionally, they may decelerate tumor progression in breast cancer patients. Indeed, the psychoactive delta-9-tetrahydrocannabinol (THC), non-psychoactive cannabidiol (CBD) and other CBs inhibited disease progression in breast cancer models. The effects of CBs on signaling pathways in cancer cells are conferred via G-protein coupled CB-receptors (CB-Rs), CB1-R and CB2-R, but also via other receptors, and in a receptor-independent way. THC is a partial agonist for CB1-R and CB2-R; CBD is an inverse agonist for both. In breast cancer, CB1-R expression is moderate, but CB2-R expression is high, which is related to tumor aggressiveness. CBs block cell cycle progression and cell growth and induce cancer cell apoptosis by inhibiting constitutive active pro-oncogenic signaling pathways, such as the extracellular-signal-regulated kinase pathway. They reduce angiogenesis and tumor metastasis in animal breast cancer models. CBs are not only active against estrogen receptor-positive, but also against estrogen-resistant breast cancer cells. In human epidermal growth factor receptor 2-positive and triple-negative breast cancer cells, blocking protein kinase B- and cyclooxygenase-2 signaling via CB2-R prevents tumor progression and metastasis. Furthermore, selective estrogen receptor modulators (SERMs), including tamoxifen, bind to CB-Rs; this process may contribute to the growth inhibitory effect of SERMs in cancer cells lacking the estrogen receptor. In summary, CBs are already administered to breast cancer patients at advanced stages of the disease, but they might also be effective at earlier stages to decelerate tumor progression.
Int J Mol Sci, 2019; 20
25537960: Kokošová N, Tomášová L, Kisková T, Šmajda B
Neuronal analysis and behaviour in prenatally gamma-irradiated rats.
The intrauterinal development in mammals represents a very sensitive period of life in relation to many environmental factors, including ionizing radiation (IR). The developing nervous system is particularly vulnerable to IR, and the consequences of exposure are of importance because of its potential health risks. The aim of our work was to assess whether prenatal irradiation of rats on the 17th day of embryonic development with a dose of 1 Gy would affect the formation of new cells and the number of mature neurons in the hippocampus and the selected forms of behaviour in the postnatal period. Male progeny of irradiated and control females was tested at ages of 3 weeks, 2 and 3 months. The number of mitotically active cells in the gyrus dentatus (GD) of the hippocampus was significantly reduced in irradiated rats aged 3 weeks. In irradiated rats aged 2 months, a significant reduction of mature neurons in CA1 area and in GD of the hippocampus was observed. The IR negatively influenced the spatial memory in Morris water maze, significantly decreased the exploratory behaviour and increased the anxiety-like behaviour in elevated plus-maze in rats aged 2 months. No significant differences were observed in animals aged 3 months compared with controls of the same age. A significant correlation between the number of mature neurons in the hilus and of the cognitive performances was found. Our results show that a low dose of radiation applied during the sensitive phase of brain development can influence the level of neurogenesis in the subgranular zone of GD and cause an impairment of the postnatal development of mental functions.
Cell Mol Neurobiol, 2015; 35
25254309: Kisková T, Jendželovský R, Rentsen E, Maier-Salamon A, Kokošová N, Papčová Z, Mikeš J, Orendáš P, Bojková B, Kubatka P, Svoboda M, Kajo K, Fedoročko P, Jäger W, Ekmekcioglu C, Kassayová M, Thalhammer T
Resveratrol enhances the chemopreventive effect of celecoxib in chemically induced breast cancer in rats.
Resveratrol and celecoxib were used as chemopreventive agents in animal models of carcinogenesis, and exert antiproliferative and proapoptotic effects on cancer cells. Therefore, the aim of this study was to evaluate whether combining resveratrol with celecoxib may exert more potent anticarcinogenic effects than the single agents. Mammary carcinogenesis was initiated in 70 female Sprague-Dawley rats with N-methyl-N-nitrosourea (NMU). The chemoprevention with resveratrol, celecoxib, and their combination started 2 weeks before the first carcinogen dose and lasted until the end of the experiment. Tumor incidence and frequency, latency period, tumor volume, the expression of cyclooxygenase 2 (COX2) and growth differentiation factor 15 (GDF15), and also the formation of reactive oxygen species were analyzed using different methods. In addition, the levels of resveratrol and its metabolites in blood and selected tumor tissues were determined by high-performance liquid chromatography. Finally, the anticancer effects of the reagents were studied in the human breast cancer cell line MCF-7. Celecoxib as a single agent significantly decreased tumor frequency, prolonged tumor latency, and decreased the total number of malignant tumors compared with the NMU conditions. Tumor volume was nonsignificantly reduced (0.68±0.25 vs. 0.93±0.28 cm3). Importantly, the addition of resveratrol to celecoxib reduced tumor volume by 60% compared with celecoxib alone (from 0.68±0.25 to 0.27±0.07 cm3, $P<0.05$). Furthermore, the combination of resveratrol and

celecoxib reduced tumor frequency by 29% compared with celecoxib alone (P=0.53). Tumor latency was not influenced by this combination compared with celecoxib alone (126.56±3.45 vs. 120.71±4.08 days). In addition, COX2 mRNA and immunoreactive protein stained on tumor sections were reduced and GDF15 protein increased significantly by the combination studied compared with the NMU conditions. In agreement with these data, a significant reduction in reactive oxygen species in blood lymphocytes of the combination was detected, which may have contributed toward the cancer-preventive effects of this application. This study showed that in NMU-induced mammary cancer in rats, the combination of resveratrol and celecoxib led to a significant reduction in all tumor parameters. In addition, in terms of tumor volume, the combination was more efficient than celecoxib as a single agent.
Eur J Cancer Prev, 2014; 23

22044852: Kisková T, Ekmekcioglu C, Garajová M, Orendáš P, Bojková B, Bobrov N, Jäger W, Kassayová M, Thalhammer T
A combination of resveratrol and melatonin exerts chemopreventive effects in N-methyl-N-nitrosourea-induced rat mammary carcinogenesis.
The neurohormone melatonin is primarily involved in the regulation of circadian rhythms, but also acts as an antioxidant and anticarcinogenic agent, especially in breast cancer. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a widely known polyphenolic agent from red wine, which has been shown to exert antioxidant, anti-inflammatory and anticarcinogenic effects. The objective of this study was therefore to investigate the effects of melatonin in combination with resveratrol in a rat model of experimental mammary carcinogenesis. Female Sprague-Dawley rats aged 31 days were used in the experiment. Mammary carcinogenesis was induced by N-methyl-N-nitrosourea (NMU), which was administered in two intraperitoneal doses (50 mg/kg of body weight). Chemoprevention with resveratrol and melatonin started 2 weeks before the first dose of NMU and lasted until the end of the experiment. The basic parameters evaluated were: tumour incidence, latency period, tumour frequency per group and tumour volume. In addition, oestrogen receptors ERα and ERß, melatonin receptor MT1, proliferating cell nuclear antigen and vascular endothelial growth factor were determined by immunohistochemical staining. The combination of resveratrol and melatonin reduced tumour incidence by approximately 17% and significantly decreased the quantity of invasive and in-situ carcinomas. Food intake declined in the second and seventh weeks after the administration of carcinogen. Resveratrol in combination with melatonin returned food intake to the level of intact controls. Resveratrol in combination with melatonin has some protective effects on NMU-induced rodent breast cancer. Further studies are necessary to confirm these effects of this promising combination.
Eur J Cancer Prev, 2012; 21

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BOARD NUMBER: S07-181

EFFECTS OF CORTICOSTERONE ON ASTROCYTIC GENE EXPRESSION IN MODELS OF DEPRESSION

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Depression is a psychiatric disorder greatly influenced by stress and high corticosterone levels. Lately, emerging evidence highlights the importance of astrocyte dysfunction in the neurobiology of depression, where lactate, the end-product of astrocytic aerobic glycolysis, regulates gene expression involved in neuroprotection. Our aim is to investigate the effect of stress and corticosterone in the impairment of astrocyte-neuron coupling, and the possibility of restoration with lactate treatment. Accordingly, we have used cultures of mouse neocortical astrocytes, and the chronic unpredictable stress (CUS) or corticosterone models of depression. In vitro, we have detected that, prolonged treatment of the astrocytic cultures with corticosterone downregulates mRNA and protein expression of glucose transporter 1 (GLUT1) and lactate transporter Monocarboxylate Transporter 4 (MCT4), suggesting an impairment in the astrocyte-neuron lactate shuttle (ANLS). Moreover, this treatment induced an increase in the expression of thioredoxin-interacting protein (TXNIP), a marker of pathological oxidative stress associated with glucose and lipid metabolism dysfunction. In mice, CUS or daily corticosterone injections, induced increased anxiety and social avoidance, detected by a battery of behavioral tests including social interaction test. Lactate treatment in both models promoted resilience to stress and anxiety, and rescued social avoidance to levels that were comparable with control mice. We are currently analyzing transcriptomic modulations of genes related to energy metabolism in the hippocampus and the prefrontal cortex of the two animal models, treated or not with lactate. Alterations of these genes in astrocytes suggest that the ANLS is an important pathway targeted in stress and psychiatric disorders.

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SOCIAL DEFEAT STRESS INDUCES MICROGLIAL ALTERATIONS AND IMPAIRED CELL SURVIVAL IN THE HYPOTHALAMUS ACCORDING TO BEHAVIORAL PHENOTYPE

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Stress is the main environmental cause for depression, known to cause brain immune alterations. As major brain immune cells, microglia undergo transcriptional and, consequently, morphological changes that result in tissue damage, including new cell generation impairment. Even so, few brain regions have been thoroughly studied, excluding key regulators as the hypothalamus, in which this process remains partially unknown. Moreover, there is a poor understanding in physiology related to behavioral outcome. Therefore, it would be interesting to study the relationship between microglia and cell proliferation in stressed mice while controlling for behavior. Here, we used the social defeat stress (SDS) paradigm as a depression-inducing protocol in 8-week-old male C57BL/6J mice for 10 consecutive days. Intruder mice behavior was analyzed to assess depression using behavioral tests and K-means clustering. By immunohistochemical and imaging procedures, microglial morphology, and distribution, as well as cell survival, were analyzed in the hypothalamic paraventricular, ventromedial and arcuate nucleus. Finally, statistical mediation analysis was conducted to evaluate the relationship among variables. Results show mice response to SDS was different, being half the mice resilient and half sensitive to depressive-like symptoms. Microglial morphological activation was enhanced in the ventromedial and arcuate nucleus, especially in stress sensitive animals. Similar results were observed in cell survival, which was particularly affected in sensitive mice. Strikingly, these cell survival changes were statistically mediated by microglial activation. As a conclusion, hypothalamic regions were found to respond differently to stress, accordingly to behavioral outcome, in terms of microglial activation and subsequent decrease in cell survival.

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BOARD NUMBER: S07-183

DIVERSE ENSEMBLES IN THE EXTENDED AMYGDALA ENCODE EMOTIONAL AND SOCIAL BEHAVIOURS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Behavioural states like anxiety, preference selection and social interaction allow organisms to adapt and communicate appropriately, thus promoting homeostasis and survival. Changes in the brain that regulate behavioural states involve multiple sensory-related circuits as well as sub-cortical structures. Our previous study demonstrated that the interstitial nucleus of posterior limb of anterior commissure, lateral (IPACL) plays a role in regulating aversive and avoidance behaviours via specific CRH circuits. However, how neurons achieve this in the IPACL remains unknown. We investigate this using deep brain Ca^{2+} imaging in IPACL with miniature microscope, coupled with longitudinal imaging through different contexts (innate fear, anxiety, place preference and social interaction) in freely moving animals. Using machine learning methods tailored for time series analyses, we are able to identify ensembles of neurons in IPACL that encode specific behaviours, which can be classified into groups encoding negative, positive and neutral valences. We find that a majority of the neurons in IPACL are correlated with negative valence, confirming the involvement of IPACL in coping with aversive situations. In addition, we also find ensembles of IPACL neurons that are involved in social interaction. To understand the molecular mechanisms underlying the neuronal activity that mediates behaviour, we perform activity trapping with FOS^{TRAP} mice and genes expression of those cells will be identified through spatial sequencing. In summary, we propose that IPACL is a novel hub for mediating both emotional and social behaviours. This will contribute to the further understanding how extended amygdala engages and regulates fundamental behaviours for adaptation and survival.

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BOARD NUMBER: S07-184

CHRONIC CORTICOSTERONE ADMINISTRATION IN MICE ALTERS BEHAVIOURAL STRATEGY IMPLEMENTATION BY MODIFYING STRIATAL-DEPENDENT MOTOR AND COGNITIVE ACTIVITY

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Chronic corticosterone administration (CCA) mimics excessive activation of the hypothalamo-pituitary-adrenal axis in rodents, resulting in a pathological phenotype commonly used to model human distress-related disorders such as anxiety and depression. Previous studies conducted in our laboratory demonstrated that CCA negatively impacts motor and cognitive processes in mice, and leads to suboptimal decision-making in particular. However, further investigation is needed to better characterize these impairments. The present study further evaluated the consequences of CCA on the balance between habits and goal-directed behavioural strategies in adult C57BL/6JRJ male mice. Dorsal striatum-dependent habit learning was assessed using a cross-maze-based single-solution paradigm (response learning) and a rotarod motor learning task. Thereafter, a dual-solution task (place learning) enabled additional examination of the balance between striatum and hippocampus-dependent strategies. Since behavioural control necessary for adaptive decision-making is supported by both learning processes, this study will allow identify the approach distressed mice mostly rely on: a striatum-based egocentric strategy or a hippocampus-based allocentric one. Our preliminary data indicate that CCA leads to delayed striatum-dependent learning in both the motor and response learning tasks, in line with a delayed behavioural initiation during the decision-making process. Furthermore, the indecision observed in some distressed mice could refer to a difficulty in implementing a decision-making strategy. Thus, our results support that CCA modulates learning by regulating the striatum-hippocampal balance. Imbalanced striatum-hippocampus-dependent behavioral strategies might therefore underlie aberrant decision-making induced by chronic distress in pathological conditions.

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ACUTE STRESS DRIVES CHANGES IN CO-TRANSMITTER IDENTITY IN SEROTONERGIC NEURONS THAT PROMOTE SUSTAINED FEAR

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Fear is an essential emotion for survival. Acquired fear responses are typically specific to the cue or context associated with an aversive stimulus (conditioned fear), but they can also spread to other cues or contexts (producing generalized fear). The cellular and circuit mechanisms by which fear is generalized remain unclear. We have studied neurotransmitter plasticity underlying fear generalization. Using a VGLUT3-Cre transgenic mouse line crossed with a Rosa-mCherry reporter line to permanently label VGLUT3+ neurons, we found that two weeks after intense footshock stress, a subset of serotonergic (5-HT) neurons in the lateral wings of the dorsal raphe (lwDR) of shocked mice had switched co-transmitter from glutamate/VGLUT3 to GABA/GAD67. We then discovered that the proportions of VGLUT3 and GAD67 in 5-HT^{lwDR} neurons in human PTSD subjects and age-matched controls were similar to those in stressed and non-stressed mice. To study the causal relationship between the transmitter switch and generalized fear, we injected Cre-dependent adeno-associated viruses in SERT-Cre mice to express exogenous VGLUT3 or suppress expression of GAD1 in 5-HT^{lwDR} neurons and measured the effect on fear responses by recording the time mice spent freezing in a novel chamber. Expression of exogenous VGLUT3 partially reduced generalized fear caused by footshock stress and expression of GAD1 entirely erased it. Prompt delivery of fluoxetine, a selective serotonin reuptake inhibitor, blocked both the glutamate-to-GABA switch in the lwDR and generalized fear responses. However, when delivery of fluoxetine was delayed, no blockade was observed. Identification of this plasticity underlying fear generalization may provide therapeutic opportunities.

Pubmed:

22231629: Dou Y, Wu HJ, Li HQ, Qin S, Wang YE, Li J, Lou HF, Chen Z, Li XM, Luo QM, Duan S
Microglial migration mediated by ATP-induced ATP release from lysosomes.
Microglia are highly motile cells that act as the main form of active immune defense in the central nervous system. Attracted by factors released from damaged cells, microglia are recruited towards the damaged or infected site, where they are involved in degenerative and regenerative responses and phagocytotic clearance of cell debris. ATP release from damaged neural tissues has been suggested to mediate the rapid extension of microglial process towards the site of injury. However, the mechanisms of the long-range migration of microglia remain to be clarified. Here, we found that lysosomes in microglia contain abundant ATP and exhibit Ca(2+)-dependent exocytosis in response to various stimuli. By establishing an efficient in vitro chemotaxis assay, we demonstrated that endogenously-released ATP from microglia triggered by local microinjection of ATPγS is critical for the long-range chemotaxis of microglia, a response that was significantly inhibited in microglia treated with an agent inducing lysosome osmodialysis or in cells derived from mice deficient in Rab 27a (ashen mice), a small GTPase required for the trafficking and exocytosis of secretory lysosomes. These results suggest that microglia respond to extracellular ATP by releasing ATP themselves through lysosomal exocytosis, thereby providing a positive feedback mechanism to generate a long-range extracellular signal for attracting distant microglia to migrate towards and accumulate at the site of injury.
Cell Res, 2012; 22
24001770: Li HQ, Chen C, Dou Y, Wu HJ, Liu YJ, Lou HF, Zhang JM, Li XM, Wang H, Duan S
P2Y4 receptor-mediated pinocytosis contributes to amyloid beta-induced self-uptake by microglia.
Brain disturbances, like injuries or aberrant protein deposits, evoke nucleotide release or leakage from cells, leading to microglial chemotaxis and ingestion. Recent studies have identified P2Y12 purinergic receptors as triggers for microglial chemotaxis and P2Y6 receptors as mediators for phagocytosis. However, pinocytosis, known as the internalization of fluid-phase materials, has received much less attention. We found that ATP efficiently triggered pinocytosis in microglia. Pharmacological analysis and knockdown experiments demonstrated the involvement of P2Y4 receptors and the phosphatidylinositol 3-kinase/Akt cascade in the nucleotide-induced pinocytosis. Further evidence indicated that soluble

amyloid beta peptide 1-42 induced self-uptake in microglia through pinocytosis, a process involving activation of P2Y4 receptors by autocrine ATP signaling. Our results demonstrate a previously unknown function of ATP as a "drink me" signal for microglia and P2Y4 receptors as a potential therapeutic target for the treatment of Alzheimer's disease.
Mol Cell Biol, 2013; 33
29686073: Meng D, Li HQ, Deisseroth K, Leutgeb S, Spitzer NC
Neuronal activity regulates neurotransmitter switching in the adult brain following light-induced stress.
Neurotransmitter switching in the adult mammalian brain occurs following photoperiod-induced stress, but the mechanism of regulation is unknown. Here, we demonstrate that elevated activity of dopaminergic neurons in the paraventricular nucleus of the hypothalamus (PaVN) in the adult rat is required for the loss of dopamine expression after long-day photoperiod exposure. The transmitter switch occurs exclusively in PaVN dopaminergic neurons that coexpress vesicular glutamate transporter 2 (VGLUT2), is accompanied by a loss of dopamine type 2 receptors (D2Rs) on corticotrophin-releasing factor (CRF) neurons, and can lead to increased release of CRF. Suppressing activity of all PaVN glutamatergic neurons decreases the number of inhibitory PaVN dopaminergic neurons, indicating homeostatic regulation of transmitter expression in the PaVN.
Proc Natl Acad Sci U S A, 2018; 115
32366867: Li HQ, Spitzer NC
Exercise enhances motor skill learning by neurotransmitter switching in the adult midbrain.
Physical exercise promotes motor skill learning in normal individuals and those with neurological disorders but its mechanism of action is unclear. We find that one week of voluntary wheel running enhances the acquisition of motor skills in normal adult mice. One week of running also induces switching from ACh to GABA expression in neurons in the caudal pedunculopontine nucleus (cPPN). Consistent with regulation of motor skills, we show that the switching neurons make projections to the substantia nigra (SN), ventral tegmental area (VTA) and ventrolateral-ventromedial nuclei of the thalamus (VL-VM). Use of viral vectors to override transmitter switching blocks the beneficial effect of running on motor skill learning. We suggest that neurotransmitter switching provides the basis by which sustained running benefits motor skill learning, presenting a target for clinical treatment of movement disorders.
Nat Commun, 2020; 11
24481275: Wu HJ, Liu YJ, Li HQ, Chen C, Dou Y, Lou HF, Ho MS, Li XM, Gao Z, Duan S
Analysis of microglial migration by a micropipette assay.
Microglial cells have important roles in maintaining brain homeostasis, and they are implicated in multiple brain diseases. There is currently interest in investigating microglial migration that results in cell accumulation at focal sites of injury. Here we describe a protocol for rapidly triggering and monitoring microglial migration by using a micropipette assay. This protocol is an adaptation of the axon turning assay using microglial cells. Chemoattractants released from the micropipette tip produce a chemotactic gradient that induces robust microglial migration. In combination with microscopic imaging, this assay allows simultaneous recording of cell movement and subcellular compartment trafficking, along with quantitative analysis. The actual handling time for the assay takes ~2-3 h in total. The protocol is simple, inexpensive and convenient to set up, and it can be adopted to examine cell migration in multiple cell types, including cancer cells with a wide range of chemical signals.
Nat Protoc, 2014; 9
25673858: Chen C, Li HQ, Liu YJ, Guo ZF, Wu HJ, Li X, Lou HF, Zhu L, Wang D, Li XM, Yu L, Cao X, Lu L, Gao Z, Duan SM
A novel size-based sorting mechanism of pinocytic luminal cargoes in microglia.
Microglia are the resident immune cells in the CNS and play diverse roles in the maintenance of CNS homeostasis. Recent studies have shown that microglia continually survey the CNS microenvironment and scavenge cell debris and aberrant proteins by phagocytosis and pinocytosis, and that reactive microglia are capable to present antigens to T cells and initiate immune responses. However, how microglia process the endocytosed contents and evoke an immune response remain unclear. Here we report that a size-dependent selective transport of small soluble contents from the pinosomal lumen into lysosomes is critical for the antigen processing in microglia. Using fluorescent probes and water-soluble magnetic nanobeads of defined sizes, we showed in cultured rodent microglia, and in a cell-free reconstructed system that pinocytosed proteins become degraded immediately following pinocytosis and the resulting peptides are selectively delivered to major histocompatibility complex class II (MHC-II) containing lysosomes, whereas undegraded proteins are retained in the pinosomal lumen. This early size-based sorting of pinosomal contents relied on the formation of transient tunnel between pinosomes and lysosomes in a Rab7- and dynamin II-dependent manner, which allowed the small contents to pass through but restricted large ones. Inhibition of the size-based sorting markedly reduced proliferation and cytokine release of cocultured CD4(+) T cells, indicating that the size-based sorting is required for efficient antigen presentation by microglial cells. Together, these findings reveal a novel early sorting mechanism for pinosomal luminal contents in microglial cells, which may explain how microglia efficiently process protein antigens and evoke an immune response.
J Neurosci, 2015; 35

FENS Forum 2022
9-13 July 2022 | Paris, France

32434858: Li HQ, Pratelli M, Godavarthi S, Zambetti S, Spitzer NC
Decoding Neurotransmitter Switching: The Road Forward.
Neurotransmitter switching is a form of brain plasticity in which an environmental stimulus causes neurons to replace one neurotransmitter with another, often resulting in changes in behavior. This raises the possibility of applying a specific environmental stimulus to induce a switch that can enhance a desirable behavior or ameliorate symptoms of a specific pathology. For example, a stimulus inducing an increase in the number of neurons expressing dopamine could treat Parkinson's disease, or one affecting the number expressing serotonin could alleviate depression. This may already be producing successful treatment outcomes without our knowing that transmitter switching is involved, with improvement of motor function through physical activity and cure of seasonal depression with phototherapy. This review presents prospects for future investigation of neurotransmitter switching, considering opportunities and challenges for future research and describing how the investigation of transmitter switching is likely to evolve with new tools, thus reshaping our understanding of both normal brain function and mental illness.
J Neurosci, 2020; 40

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ACUTE PSYCHOLOGICAL STRESS: EFFECTS ON HIPPOCAMPAL NEUROGENESIS AND THE ROLE OF MICROGLIA

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Among all the factors that can contribute to the onset of psychopathological disorders, stress is the main environmental factor. The hippocampus is one of the most sensitive regions to the harmful effects of stress, in which the neurogenic process is impaired. On the other hand, under stress situations, microglia are also affected and can trigger a proinflammatory response, acting as anti-neurogenic cells and releasing cytokines and other proinflammatory molecules. Knowing what happens in the early stages of stress may be relevant to investigate the temporal aspects of the development of stress-associated psychopathological disorders, and even their possible treatment. Therefore, after subjecting C57BL/6J mice for 2 hours to an acute and intense stress procedure called WIRS (water immersion restraint stress), data were analyzed to study microglia, cell proliferation and neuronal maturation. In addition, a mediation analysis study was conducted for data integration. The results revealed that the applied acute stress is sufficiently intense to induce an increase in the number of microglia, accompanied by morphometric changes, as well as negatively affecting the neuronal maturational process. Furthermore, these data suggest that effects on the neurogenic process mediate the microglial response to an intense acute stressor. This leads to the conclusion that this may be the initial mechanism for any intense stress response, or may even be the first steps in the development of the response to a chronic stressor.

Pubmed:
34355047: Nieto-Quero A, Chaves-Peña P, Santín LJ, Pérez-Martín M, Pedraza C
Do changes in microglial status underlie neurogenesis impairments and depressive-like behaviours induced by psychological stress? A systematic review in animal models.
Stress may have a negative effect on mental health and is the primary environmental risk factor in the aetiology of depression. Nevertheless, the neurobiological mechanisms underlying this mood disorder remain poorly characterized. The hippocampus is a target structure of the adverse effects of stress, and hippocampal neurogenesis plays a crucial role. However, we do not know the mechanisms by which stress impacts neurogenesis. Recent studies indicate that changes in neuroinflammation, primarily via microglial cells, may play an essential role in this process. However, the relationship between stress, microglial changes, and alterations in neurogenesis and their involvement in the development of depression is poorly characterized. For this reason, this systematic review aims to synthesise and evaluate current studies that have investigated the relationship between these variables. Taken together, the revised data, although not entirely conclusive, seem to suggest that microglial changes induced by psychological stress regulate neurogenesis and in turn may be responsible for the development of depressive-like behaviours, but other factors that influence these stressful experiences should not be dismissed.
Neurobiol Stress, 2021; 15
31244601: Tabbai S, Moreno-Fernández RD, Zambrana-Infantes E, Nieto-Quero A, Chun J, García-Fernández M, Estivill-Torrús G, Rodríguez de Fonseca F, Santín LJ, Oliveira TG, Pérez-Martín M, Pedraza C
Effects of the LPA Receptor Deficiency and Stress on the Hippocampal LPA Species in Mice.
Lysophosphatidic acid (LPA) is an important bioactive lipid species that functions in intracellular signaling through six characterized G protein-coupled receptors (LPA). Among these receptors, LPA is a strong candidate to mediate the central effects of LPA on emotion and may be involved in promoting normal emotional behaviors. Alterations in this receptor may induce vulnerability to stress and predispose an individual to a psychopathological disease. In fact, mice lacking the LPA receptor exhibit emotional dysregulation and cognitive alterations in hippocampus-dependent tasks. Moreover, the loss of this receptor results in a phenotype of low resilience with dysfunctional coping in response to stress and induces anxiety and several behavioral and neurobiological changes that are strongly correlated with mood disorders. In fact, our group proposes

that maLPA1-null mice represent an animal model of anxious depression. However, despite the key role of the LPA-LPA-pathway in emotion and stress coping behaviors, the available information describing the mechanisms by which the LPA-LPA-pathway regulates emotion is currently insufficient. Because activation of LPA requires LPA, here, we used a Matrix-Assisted Laser Desorption/ Ionization mass spectrometry-based approach to evaluate the effects of an LPA receptor deficiency on the hippocampal levels of LPA species. Additionally, the impact of stress on the LPA profile was also examined in both wild-type (WT) and the Malaga variant of LPA1-null mice (maLPA-null mice). Mice lacking LPA did not exhibit gross perturbations in the hippocampal LPA species, but the LPA profile was modified, showing an altered relative abundance of 18:0 LPA. Regardless of the genotype, restraint stress produced profound changes in all LPA species examined, revealing that hippocampal LPA species are a key target of stress. Finally, the relationship between the hippocampal levels of LPA species and performance in the elevated plus maze was established. To our knowledge, this study is the first to detect, identify and profile LPA species in the hippocampus of both LPA-receptor null mice and WT mice at baseline and after acute stress, as well as to link these LPA species with anxiety-like behaviors. In conclusion, the hippocampal LPA species are a key target of stress and may be involved in psychopathological conditions.
Front Mol Neurosci, 2019; 12

30061118: Moreno-Fernández RD, Nieto-Quero A, Gómez-Salas FJ, Chun J, Estivill-Torrús G, Rodríguez de Fonseca F, Santín LJ, Pérez-Martín M, Pedraza C

Effects of genetic deletion versus pharmacological blockade of the LPA receptor on depression-like behaviour and related brain functional activity.

Animal models of psychopathology are particularly useful for studying the neurobiology of depression and characterising the subtypes. Recently, our group was the first to identify a possible relationship between the LPA receptor and a mixed anxiety-depression phenotype. Specifically, maLPA-null mice exhibited a phenotype characterised by depressive and anxious features. However, the constitutive lack of the gene encoding the LPA receptor () can induce compensatory mechanisms that might have resulted in the observed deficits. Therefore, in the present study, we have compared the impact of permanent loss and acute pharmacological inhibition of the LPA receptor on despair-like behaviours and on the functional brain map associated with these behaviours, as well as on the degree of functional connectivity among structures. Although the antagonist (intracerebroventricularly administered Ki16425) mimicked some, but not all, effects of genetic deletion of the LPA receptor on the results of behavioural tests and engaged different brain circuits, both treatments induced depression-like behaviours with an agitation component that was linked to functional changes in key brain regions involved in the stress response and emotional regulation. In addition, both Ki16425 treatment and LPA receptor deletion modified the functional brain maps in a way similar to the changes observed in depressed patients. In summary, the pharmacological and genetic approaches could ultimately assist in dissecting the function of the LPA receptor in emotional regulation and brain responses, and a combination of those approaches might provide researchers with an opportunity to develop useful drugs that target the LPA receptor as treatments for depression, mainly the anxious subtype.This article has an associated First Person interview with the first author of the paper.
Dis Model Mech, 2018; 11

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EXCESSIVE MIDBRAIN GLUTAMATERGIC TONE PROMOTE ANXIETY VIA DYSREGULATION OF AMYGDALA PRINCIPAL NEURONS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Anxiety disorders are a major human blight afflicting a high proportion of the population and are difficult to diagnose. A poor efficiency in the existing treatments reveal the lack of knowledge about the neuronal network regulating anxiety. While the neuronal substrate of anxious behaviour is incredibly complex, a core feature of anxiety disorders is the hyperactivity of the amygdala. Here, we study if this hyperactivity can be linked to excessive glutamatergic tone. We relied on viral tool and transgenic mice to specifically target a midbrain glutamatergic population connecting to the basolateral amygdala. Modulation of these afference by acute selective optogenetic activation and chemogenetic inhibition was not sufficient to change basal anxiety levels in mice. However, a chronic optogenetic activation of this glutamatergic projection led to increased anxiety levels. Furthermore, using electrophysiological tools, we correlated these anxious behaviours with plastic synaptic changes onto principal neurons of the basolateral amygdala. Finally, we used our finding to show that anxiety-disorders like symptoms induced with chronic social defeat cannot be reversed by chronic chemogenetic silencing of these glutamatergic inputs to the amygdala. Taken together, our study uncovered a new pathway that may contribute to the induction of anxiety-disorders through dysregulation of amygdala activity. Preventive approaches targeting this pathway could be relevant to prevent the induction of stress related anxiety-disorders and need further investigation.

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BOARD NUMBER: S07-188

PROMISING POST-TRAUMATIC STRESS DISORDER TREATMENT BASED THE ADMINISTRATION OF THE QBP1 PEPTIDE TO BLOCK MEMORY CONSOLIDATION

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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[1]Cajal Institute (CSIC), Molecular, Cellular And Developmental Neurobiology, Madrid, Spain, [2]Cajal Institute (CSIC), Translational Neuroscience Department, Madrid, Spain, [3]Osaka University Graduate School of Medicine, Department Of Neurotherapeutics, Osaka, Japan, [4]Johns Hopkins University, Department Of Psychological And Brain Sciences, Baltimore, United States of America

The cytoplasmic polyadenylation element binding protein-3 (CPEB3) is a functional amyloid, whose importance for long-term memory consolidation of mammals has been widely demonstrated. Recently, it has also been pointed out as a potential risk gene for Post-Traumatic Stress Disorder (PTSD). This mental health disorder is triggered by the exposure to a traumatic event that manifests with anguish, intrusive memories, and negative mood changes. So far, there is no efficient treatment for PTSD other than symptomatic palliative care. According to this, we propose CPEB3's active amyloid state as a possible therapeutic target to block the consolidation of traumatic memories through the action of the anti-amyloidogenic polyglutamine binding peptide 1 (QBP1). Once we had demonstrated that murine CPEB3 is being inhibited by QBP1 peptide in vitro, we have developed and characterized a transgenic mouse that constitutively expresses QBP1. We first showed the innocuity of this peptide and the normal locomotor activity and anxiety levels of QBP1 mice. Secondly, hippocampal-dependent memories were tested, reflecting that they are impaired at long term and that just new memories are being affected. Finally, analyzing aversive memories we demonstrated that they were also compromised by the presence of QBP1 peptide, which was revealed by a reduction of fear-induced anxiety and a correlative decrease in mCPEB3 oligomerization extracted from hippocampal samples. All this work suggests QBP1 peptide is a promising lead compound for prevention and therapy of PTSD and acute stress disorder (ASD).

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BOARD NUMBER: S07-189

CONTINUOUS STRESS AFFECTS KAINATE RECEPTOR-DEPENDENT INHIBITION BY PARVALBUMIN NEURONS IN THE MOUSE AMYGDALA.

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Continuous stress is a risk factor for psychiatric disorders, including generalized anxiety, post-traumatic stress disorder, and major depressive disorder. The amygdala plays a significant role in processing emotions and regulates anxiety responses to stressful and arousing situations. Recent evidence suggests that continuous stress induces hyperexcitability of the amygdala's basolateral nucleus (BLA). Parvalbumin interneurons (PV+) innervate principal neurons (PN) perisomatically, providing potent inhibition. We have demonstrated previously that kainate receptors containing the GluK1 subunit modulate PV+ excitability to support the feedforward inhibition for the PN in the lateral amygdala (LA). Here we investigated the effect of continuous restraint stress on mouse LA to dissect the role of GluK1 in the PV+ neurons in stress-induced anxiety. In situ hybridization data show that most cells in LA expressing GluK1 are GAD1+ inhibitory neurons, including PV+ neurons. Our electrophysiological data from WT and PV+ cell-specific GluK1 knockout mice demonstrate that tonic activity of GluK1 in PV+ neurons regulates PN excitability in LA, and this effect is abolished after continuous restraint stress. Behavioral experiments demonstrated that the conditional knock-out of GluK1 in the LA led to an anxiety-like phenotype in mice. Overall, these data suggest that a loss of tonic activity of GluK1 containing kainate receptors in the PV+ neurons contributes to stress-related changes in LA excitability associated with anxiety.

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BOARD NUMBER: S07-190

RECEPTOR PROTEIN TYROSINE PHOSPHATASE β/ζ REGULATES ETHANOL INTAKE AND ETHANOL EFFECTS ON HIPPOCAMPAL NEUROGENESIS AND NEUROIMMUNE RESPONSE IN A SEX-DEPENDENT MANNER

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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[1]Universidad CEU San Pablo, Pharmacology, Madrid, Spain, [2]Universidad CEU San Pablo, Chemistry, Madrid, Spain, [3]Universidad CEU San Pablo, Biochemistry, Madrid, Spain

AIMS: Pleiotrophin (PTN) is a cytokine that modulates behavioural effects of ethanol and neuroinflammation. PTN is an endogenous inhibitor of Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ. RPTPβ/ζ inhibition reduces binge-like drinking in mice. We studied RPTPβ/ζ role in regulation of chronic ethanol consumption and alcohol-derived neuroimmune alterations. **METHODS:** Male and female adolescent C57BL/6J mice followed an intermittent access to ethanol (IAE) 2-bottle choice protocol. Before each drinking session MY10 (60 mg/kg, i.g.), a small-molecule RPTPβ/ζ inhibitor, or vehicle was administered. Ethanol consumption was measured. After the 4-week protocol, brains were removed for immunohistochemistry of Iba-1, GFAP and doublecortin (DCX). In the acute ethanol experiments, mice were treated with MY10 one hour before a 6 g/kg ethanol (i.p.) administration. Eighteen hours post-injection, brains were dissected and subjected to the same immunohistochemistry analysis. **RESULTS:** In the IAE model, MY10 reduced ethanol intake only in male mice. This MY10 effect was not accompanied by significant alterations in glial responses. In acute ethanol experiments, ethanol did not alter microgliosis significantly. However, female mice showed a more activated microglia phenotype independently of the treatment received. In the hippocampus, MY10 prevented ethanol-induced neurogenic loss in both male and female mice, without significant effects on glial responses. **CONCLUSION:** RPTPβ/ζ inhibition significantly reduced chronic ethanol consumption only in male mice. RPTPβ/ζ critically modulates ethanol-induced decreases on hippocampal neurogenesis. The data also showed sex differences in RPTPβ/ζ function relevant to ethanol-induced neuroimmune responses. Funding: National Plan on Drug abuse, Ministerio de Sanidad of Spain (MSSSI, grant PNSD015I2019 to G.H.).

Pubmed:
33219280: Fernández-Calle R, Galán-Llario M, Gramage E, Zapatería B, Vicente-Rodríguez M, Zapico JM, de Pascual-Teresa B, Ramos A, Ramos-Álvarez MP, Uribarri M, Ferrer-Alcón M, Herradón G
Role of RPTPβ/ζ in neuroinflammation and microglia-neuron communication.
Pleiotrophin (PTN) is a cytokine that is upregulated in different neuroinflammatory disorders. Using mice with transgenic PTN overexpression in the brain (Ptn-Tg), we have found a positive correlation between iNos and Tnfα mRNA and Ptn mRNA levels in the prefrontal cortex (PFC) of LPS-treated mice. PTN is an inhibitor of Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ, which is mainly expressed in the central nervous system. We aimed to test if RPTPβ/ζ is involved in the modulation of neuroinflammatory responses using specific inhibitors of RPTPβ/ζ (MY10 and MY33-3). Treatment with MY10 potentiated LPS-induced microglial responses in the mouse PFC. Surprisingly, MY10 caused a decrease in LPS-induced NF-κB p65 expression, suggesting that RPTPβ/ζ may be involved in a novel mechanism of potentiation of microglial activation independent of the NF-κB p65 pathway. MY33-3 and MY10 limited LPS-induced nitrites production and iNos increases in BV2 microglial cells. SH-SY5Y neuronal cells were treated with the conditioned media from MY10/LPS-treated BV2 cells. Conditioned media from non-stimulated and from LPS-stimulated BV2 cells increased the viability of SH-SY5Y cultures. RPTPβ/ζ inhibition in microglial cells disrupted this neurotrophic effect of microglia, suggesting that RPTPβ/ζ plays a role in the neurotrophic phenotype of microglia and in microglia-neuron communication.
Sci Rep, 2020; 10

FENS Forum 2022
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BOARD NUMBER: S07-191

LONG-TERM EFFECTS OF MATERNAL SEPARATION ON ALCOHOL INTAKE AND ACUTE STRESS RESPONSE IN MALE AND FEMALE MICE.

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Although it is known that early life stress is a risk factor for developing an Alcohol, Use Disorders (AUD), the underlying neurobiological mechanisms are not fully understood. Maternal separation stress (MS) is a predictive animal model to evaluate the effects of early stress exposure on alcohol intake. We aimed to investigate the influence of MS on alcohol consumption and stress responses of adult male and female mice. C57BL/6J mice were subjected to 180 min of MS, from the postnatal day (PND) 1 to 14, or were left undisturbed in their home-cage (control). On PND 45, mice were individually exposed to 20% alcohol (w/v) in filtered water, in their home cages for 3 weeks (involuntary consumption). Next, mice were trained to self-administrate alcohol in an operant procedure under fixed ratio (FR 1-3-5), 120-min sessions. The “breakpoint” was determined in 3 two-hour sessions of a progressive ratio schedule. Next day, mice were submitted or not to Rat Exposure Test (RET) and 24h later, they had free access to alcohol 20% during 4h (binge). Blood samples were collected before alcohol protocol begin (basal), after the RET, and after the binge session. In involuntary consumption, female mice increased ethanol intake compared to male mice. Female MS mice and control male mice submitted to RET presented higher blood corticosterone concentrations when compared to female control group. Maternal separation stress seems to influence stress responses to an acute heterotypic stressor exposure in a sex-dependent manner in adult mice. Grant: CAPES, FAPESP, UNIFESP

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A ROLE FOR THE BDNF SYSTEM IN THE ACTIVITY-BASED ANOREXIA RAT MODEL: FOCUS ON THE AMYGDALA

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Anorexia nervosa (AN) is one of the most severe psychiatric disorders whose rate of incidence is worrying, especially among adolescents. AN patients suffer from a pathological fear of gaining weight, altered body-shape perception, and emotional instability. Our hypothesis is that neuroplastic alterations in the amygdala, a brain region involved in the regulation of emotional processes, might be associated with the maintenance of the anorexic phenotype. In particular, we focused the role of the neurotrophin BDNF in the amygdala of adolescent female rats exposed to the Activity-Based Anorexia (ABA) protocol, characterized by the culprits of AN: the combination of caloric restriction and intense physical exercise. Twenty-four hours after the anorexic phenotype induction, ABA animals reduce body weight and exponentially increase wheel activity over days. At molecular level, despite ABA rats show increased total Bdnf, Bdnf isoform IV and VI gene expression in the acute phase of the disease (post-natal day [P]42), protein levels of BDNF, its receptor TrkB and its downstream signaling pathway, i.e. Akt and Erk2, are reduced in the membrane fraction. Interestingly, this overall reduction in the BDNF system persists following 7 days of body weight recovery (P49). Taken together, these data suggest that the combination between hyperactivity and reduced caloric intake alters the trophic support and neuroplastic mechanisms in the amygdala, via BDNF system dysregulation, an effect that might be crucial in the onset of comorbid complications in AN patients and, thus, increase the vulnerability to develop other psychiatric disorders. Supported by: Cariplo Foundation 2017-0865

Pubmed:

35174489: Caffino L, Mottarlini F, Targa G, Verheij MMM, Fumagalli F, Homberg JR
Responsivity of serotonin transporter knockout rats to short and long access to cocaine: Modulation of the glutamate signalling in the nucleus accumbens shell.
It has been well established that glutamate in the nucleus accumbens (NAc) plays a critical role in the motivation to take drugs of abuse. We have previously demonstrated that rats with ablation of the serotonin transporter (SERT rats) show increased cocaine intake reminiscent of compulsivity.
Br J Pharmacol, 2022;
34299015: Caffino L, Mottarlini F, Bilel S, Targa G, Tirri M, Maggi C, Marti M, Fumagalli F
Single Exposure to the Cathinones MDPV and α-PVP Alters Molecular Markers of Neuroplasticity in the Adult Mouse Brain.
Synthetic cathinones have gained popularity among young drug users and are widely used in the clandestine market. While the cathinone-induced behavioral profile has been extensively investigated, information on their neuroplastic effects is still rather fragmentary. Accordingly, we have exposed male mice to a single injection of MDPV and α-PVP and sacrificed the animals at different time points (i.e., 30 min, 2 h, and 24 h) to have a rapid readout of the effect of these psychostimulants on neuroplasticity in the frontal lobe and hippocampus, two reward-related brain regions. We found that a single, low dose of MDPV or α-PVP is sufficient to alter the expression of neuroplastic markers in the adult mouse brain. In particular, we found increased expression of the transcription factor , increased ratio between the vesicular GABA transporter and the vesicular glutamate transporter together with changes in the expression of the neurotrophin , confirming the widespread impact of these cathinones on brain plasticity. To sum up, exposure to low dose of cathinones can impair cortical and hippocampal homeostasis, suggesting that abuse of these cathinones at much higher doses, as it occurs in humans, could have an even more profound impact on neuroplasticity.
Int J Mol Sci, 2021; 22
33880773: Caffino L, Mottarlini F, Targa G, Verheij MMM, Homberg J, Fumagalli F
Long access to cocaine self-administration dysregulates the glutamate synapse in the nucleus accumbens core of serotonin transporter knockout rats.
It is well established that the nucleus accumbens and glutamate play a critical role in the motivation to take drugs of abuse. We have previously demonstrated that rats with ablation of the serotonin (5-HT) transporter (SERT rats) show increased cocaine intake reminiscent of compulsivity.

Br J Pharmacol, 2021;
33723767: Piva A, Caffino L, Mottarlini F, Pintori N, Castillo Díaz F, Fumagalli F, Chiamulera C
Metaplastic Effects of Ketamine and MK-801 on Glutamate Receptors Expression in Rat Medial Prefrontal Cortex and Hippocampus.
Ketamine and MK-801 by blocking NMDA receptors may induce reinforcing effects as well as schizophrenia-like symptoms. Recent results showed that ketamine can also effectively reverse depressive signs in patients' refractory to standard therapies. This evidence clearly points to the need of characterization of effects of these NMDARs antagonists on relevant brain areas for mood disorders. The aim of the present study was to investigate the molecular changes occurring at glutamatergic synapses 24 h after ketamine or MK-801 treatment in the rat medial prefrontal cortex (mPFC) and hippocampus (Hipp). In particular, we analyzed the levels of the glutamate transporter-1 (GLT-1), NMDA receptors, AMPA receptors subunits, and related scaffolding proteins. In the homogenate, we found a general decrease of protein levels, whereas their changes in the post-synaptic density were more complex. In fact, ketamine in the mPFC decreased the level of GLT-1 and increased the level of GluN2B, GluA1, GluA2, and scaffolding proteins, likely indicating a pattern of enhanced excitability. On the other hand, MK-801 only induced sparse changes with apparently no correlation to functional modification. Differently from mPFC, in Hipp, both substances reduced or caused no changes of glutamate receptors and scaffolding proteins expression. Ketamine decreased NMDA receptors while increased AMPA receptors subunit ratios, an effect indicative of permissive metaplastic modulation; conversely, MK-801 only decreased the latter, possibly representing a blockade of further synaptic plasticity. Taken together, these findings indicate a fine tuning of glutamatergic synapses by ketamine compared to MK-801 both in the mPFC and Hipp.
Mol Neurobiol, 2021; 58
33511707: Caffino L, Moro F, Mottarlini F, Targa G, Di Clemente A, Toia M, Orrù A, Giannotti G, Fumagalli F, Cervo L
Repeated exposure to cocaine during adolescence enhances the rewarding threshold for cocaine-conditioned place preference in adulthood.
Previous studies have shown that adolescent exposure to cocaine increases drug use in adulthood, albeit incubation of cocaine seeking was found to be attenuated in rats trained to self-administer cocaine during adolescence. We here hypothesize that adolescent exposure to cocaine could alter the rewarding properties of the psychostimulant in adulthood. By employing two of the most widely used animal-experimental-preclinical models to investigate drug addiction, we evaluated whether contingent versus non-contingent cocaine self-administration during adolescence modulates its rewarding threshold in adulthood evaluated by conditioned place preference (CPP). Cocaine self-administration during adolescence increases the rewarding threshold in adulthood; CPP for cocaine was observed at the higher (20 mg/kg), but not at the lower (10 mg/kg), dose employed. Rats exposed to either contingent or non-contingent cocaine during adolescence exhibited the same behavior in the CPP paradigm suggesting that, under our experimental conditions, cocaine rewarding properties are shaped by the psychostimulant itself and not by its motivational effects. From a mechanistic standpoint, the preference for the 20 mg/kg cocaine-paired side in a CPP paradigm appears to depend, at least partially, upon the formation of GluA2-lacking Ca -permeable AMPA receptors and the consequent increase of αCaMKII activity in the NAc, both of which are instead reduced when the 10 mg/kg dose was used. In conclusion, contingent or non-contingent cocaine exposure during adolescence desensitizes adult animals to a rewarding dose of cocaine (10 mg/kg) elevating the rewarding threshold necessary (20 mg/kg) to drive conditioned place preference, an effect that may predispose to higher consumption of cocaine during adulthood.
Addict Biol, 2021; 26
33260714: Mottarlini F, Bottan G, Tarenzi B, Colciago A, Fumagalli F, Caffino L
Activity-Based Anorexia Dynamically Dysregulates the Glutamatergic Synapse in the Nucleus Accumbens of Female Adolescent Rats.
Intense physical activity and dieting are core symptoms of anorexia nervosa (AN). Their combination evolves into compulsivity, leading the patient into an out-of-control spiral. AN patients exhibit an altered activation of nucleus accumbens (NAc), revealing a dysfunctional mesocorticolimbic reward circuitry in AN. Since evidence exists that a dysregulation of the glutamate system in the NAc influences reward and taking advantage of the activity-based anorexia (ABA) rat model, which closely mimics the hallmarks of AN, we investigated the involvement of the glutamatergic signaling in the NAc in this experimental model. We here demonstrate that food restriction causes hyperactive and compulsive behavior in rodents, inducing an escalation of physical activity, which results in dramatic weight loss. Analysis of the glutamate system revealed that, in the acute phase of the pathology, ABA rats increased the membrane expression of GluA1 AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor subunits together with its scaffolding protein SAP97. Recovery of body weight reduced GluN2A/2B balance together with the expression of their specific scaffolding proteins, thus suggesting persistent maladaptive neurotransmission. Taken together, AMPA and NMDA (N-methyl-D-aspartate) receptor subunit reorganization may play a role in the motivational mechanisms underlying AN.

Nutrients, 2020; 12
32585251: Caffino L, Mottarlini F, Mingardi J, Zita G, Barbon A, Fumagalli F
Anhedonic-like behavior and BDNF dysregulation following a single injection of cocaine during adolescence.
We have previously demonstrated that a single exposure to cocaine during adolescence causes several behavioural and neurobiological changes, highlighting the unique vulnerability of this period of life. The purpose of our work was to investigate whether a single exposure to cocaine during brain development is sufficient to shape a negative emotional state in adolescent rats. A single injection of cocaine during adolescence followed by measurement of sucrose consumption, a measure of anhedonia, identifies two separate groups of rats, i.e. anhedonic (AN) and non anhedonic (NON-AN) rats. AN rats show reduced ability to synthesize, traffic and translate the neurotrophin BDNF at synaptic level, reduced activation of hippocampal BDNF signaling, reduced BDNF plasma levels and a steep rise of corticosterone secretion. Conversely, NON-AN rats exhibit reduced trafficking of BDNF while up-regulating hippocampal BDNF synthesis and stabilizing its downstream signaling with no changes of BDNF and corticosterone plasma levels. Adult rats exposed to cocaine showed no signs of anhedonia, an increase of BDNF both in hippocampus and plasma and decreased levels of corticosterone. In conclusion, our findings reveal a complex central and peripheral dysregulation of BDNF-related mechanisms that instead are preserved in NON-AN rats, suggesting that BDNF modulation dictates behavioural vulnerability vs. resiliency to cocaine-induced anhedonia, a profile uniquely restricted to adolescent rats.
Neuropharmacology, 2020; 175
32510348: Mottarlini F, Racagni G, Brambilla P, Fumagalli F, Caffino L
Repeated cocaine exposure during adolescence impairs recognition memory in early adulthood: A role for BDNF signaling in the perirhinal cortex.
The perirhinal cortex (PrhC) is critical for object recognition memory; however, information regarding the molecular mechanisms underlying this type of memory following repeated exposure to drugs of abuse during adolescence is unknown. To this end, adolescent or adult rats were exposed to cocaine from postnatal day (PND) 28 to PND 42 or PND 63 to PND 77, respectively. Two weeks later, rats were subjected to the cognitive test named Novel Object Recognition (NOR) test. We found that adolescent, but not adult, cocaine exposure caused a significant impairment in the NOR test, independently from changes in the stress response system. In adolescent saline-treated rats, NOR test up-regulated BDNF and its downstream signaling whereas a downregulation of the same pathway was observed in cocaine-treated rats together with a reduction of Arc/Arg3.1 and PSD95 expression, indicating reduced pro-cognitive structural adaptations in the PrhC. Of note, cocaine-treated adult rats correctly performed in the NOR test indicating intact recognition memory mechanisms, despite a significant cocaine-induced reduction of BDNF levels in the PrhC, suggesting that recognition memory is heavily dependent on BDNF during adolescence whereas during adulthood other mechanisms come into play.
Dev Cogn Neurosci, 2020; 43
32187792: Caffino L, Mottarlini F, Van Reijmersdal B, Telese F, Verheij MMM, Fumagalli F, Homberg JR
The role of the serotonin transporter in prefrontal cortex glutamatergic signaling following short- and long-access cocaine self-administration.
Vulnerability to drug addiction relies on substantial individual differences. We previously demonstrated that serotonin transporter knockout (SERT) rats show increased cocaine intake and develop signs of compulsivity. However, the underlying neural mechanisms are not fully understood. Given the pivotal role of glutamate and prefrontal cortex in cocaine-seeking behavior, we sought to investigate the expression of proteins implicated in glutamate neurotransmission in the prefrontal cortex of naïve and cocaine-exposed rats lacking SERT. We focused on the infralimbic (ILc) and prelimbic (PLc) cortices, which are theorized to exert opposing effects on the control over subcortical brain areas. SERT rats, which compared to wild-type (SERT) rats show increased ShA and LgA intake short-access (ShA) and long-access (LgA) cocaine intake, were sacrificed 24 h into withdrawal for ex vivo molecular analyses. In the ILc homogenate of SERT rats, we observed a sharp increase in glial glutamate transporter 1 (GLT-1) after ShA, but not LgA, cocaine intake. This was paralleled by ShA-induced increases in GluN1, GluN2A, and GluN2B NMDA receptor subunits and their scaffolding protein SAP102 in the ILc homogenate, but not postsynaptic density, of these knockout animals. In the PLc, we found no major changes in the homogenate; conversely, the expression of GluN1 and GluN2A NMDA receptor subunits was increased in the postsynaptic density under ShA conditions and reduced under LgA conditions. These results point to SERT as a critical regulator of glutamate homeostasis in a way that differs between the subregions investigated, the duration of cocaine exposure as well as the cellular compartment analyzed.
Addict Biol, 2021; 26
31606593: Caffino L, Mottarlini F, Diniz DM, Verheij MM, Fumagalli F, Homberg JR
Deletion of the serotonin transporter perturbs BDNF signaling in the central amygdala following long-access cocaine self-administration.
Human neuroimaging studies indicate that the amygdala plays a key role in cocaine addiction. One key plasticity factor that

modulates effects of cocaine on the brain is Brain-Derived Neurotrophic Factor (BDNF). A wealth of evidence shows that cocaine exposure alters BDNF signaling in corticolimbic structures, but, surprisingly, such evidence is very limited for the amygdala. Additionally, while BDNF is strongly regulated by serotonin levels and inherited serotonin transporter down-regulation is associated with increased vulnerability to cocaine addiction, the effects of serotonin transporter genotype on BDNF signaling in the amygdala under naïve and cocaine exposure conditions are unknown.
Drug Alcohol Depend, 2019; 205

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ACTIVITY-BASED ANOREXIA-INDUCED ALTERATION OF MEMBRANE-ASSOCIATED GLUCOCORTICOID RECEPTORS AND STRUCTURAL PLASTICITY IN THE HIPPOCAMPUS OF ADOLESCENT FEMALE RATS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Anorexia nervosa (AN) is a complex mental illness characterized by restricted eating and strenuous exercise regimens. Our major aim was to investigate the molecular and structural signature of AN history on stress-related mechanisms in the hippocampus of the activity-based anorexia (ABA) rat model, a well-established animal model of AN,in mediating the hallmarks of the anorexic phenotype. Following exposure of female adolescent rats to the combination of food restriction and physical activity (i.e. the ABA protocol), ABA rats show reduced body weight and increased wheel activity over days. ABA rats alter their pattern of activity over days showing increased food anticipatory activity, a readout of their motivation to engage in intense physical activity.Corticosterone levels were enhanced in the plasma of ABA rats in the acute phase of the pathology (postnatal day [P]42) and reduced following 7-days recovery period at P49. At molecular level, in the membrane fraction of the hippocampus, we found reduced levels of glucocorticoid receptor together with reduced activity of phospho-synapsin1, an indirect index of reduced neurotransmitter release. Such impairment was strengthened by reduced expression of caldesmon, n-cadherin and neuroligin-1, molecular markers of cytoskeletal stability. Accordingly, structural analyses revealed reduced dendritic spine density and reduced number of mushroom-shaped spines, together with an increased number of filopodia. Notably, these effects persisted even when body weight of ABA rats was restored. This maladaptive plasticity paralleled by the reorganization of the hippocampal architecture could represent a signal of altered processing of food reward and a vulnerability trait for relapse. Sponsored by: Cariplo Foundation 2017-0865

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BOARD NUMBER: S07-194

GLYCINE TRANSPORTER INHIBITOR 1 RESCUES EXCITATORY/INHIBITORY IMBALANCE MEDIATED BY GLUA1 AMPA RECEPTOR ABLATION FROM PARVALBUMIN-POSITIVE INTERNEURONS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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[1]Boehringer Ingelheim Pharma GmbH & Co. KG, Cns Diseases, Biberach an der Riss, Germany, [2]Max Planck Institute for Medical Research, Institute For Anatomy And Cell Biology At Heidelberg University, Heidelberg, Germany, [3]Boehringer Ingelheim Pharma GmbH & Co. K, Cardiometabolic Disease Research, Biberach an der Riss, Germany

Disturbance in synaptic excitatory and inhibitory (E/I) transmission in the prefrontal cortex has been considered as a critical factor for cognitive dysfunction, a core clinical symptom in schizophrenia. However, the systematic study of E/I imbalance caused cortical network pathophysiology, as well as the effective therapeutic strategy, is still limited. In this study, we used mice with parvalbumin neuron (PV) specific knockout of GluA1 (AMPA receptor subunit 1) (Gria1-PV KO) as an experimental tool to study PV loss of function-mediated network disruption. With high-content confocal imaging and electrophysiological recordings in the medial prefrontal cortical (mPFC) section, we found the structural and functional alteration in mPFC local network of Gria1-PV KO. In addition, we applied electroencephalography (EEG) and mismatch negativity (MMN) test, standard tools in the clinic for measuring network activity and sensory information processing. Gria1-PV KO animals exhibited abnormal theta oscillation and deficit in the MMN comparable to clinical findings from cognitive impairment patients. Remarkably, we demonstrate that application of glycine transporter inhibitor 1 (GlyT1) inhibitor, bitopertin, ameliorated imbalanced E/I transmission, hyperexcitability, and sensory processing malfunction from Gria1-PV KO. Therefore, our results suggest that PV-specific deletion of GluA1 is an experimental approach for back-translating the E/I imbalance observed in schizophrenic patients. Additionally, our work offers a systematic workflow to understand the effect of bitopertin in restoring cortical network activity from single cell to local brain circuitry. Furthermore, this study highlights that increasing excitatory drive through boosting NMDA receptor signaling pathways is a potential therapeutic strategy for restoring E/I imbalance-mediated cognitive abnormality.

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GESTATIONAL STRESS INDUCES MITOCHONDRIAL DYSFUNCTION IN PREFRONTAL CORTEX AND DEPRESSIVE-LIKE BEHAVIOR IN POSTPARTUM RATS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Postpartum depression (PPD) is a major psychiatric complication of childbirth, affecting up to 20% of mothers, yet remains understudied. Proposed mechanisms underlying PPD pathology share a link with mitochondria-- dynamic organelles crucial for cell homeostasis. The brain relies on mitochondrial energy production to function, and stress, a major risk factor for PPD, amplifies brain energy demands. In turn, brain mitochondrial function is also affected by stress and linked to anxiety-like and social behaviors. Consequently, failure to properly adapt to the demands of stress could increase the risk for psychiatric illness. Considering the importance of mitochondria in regulating brain function and behavior, we hypothesized a role for mitochondrial dysfunction in association with behavioral changes in a chronic stress-induced rat model of PPD. Adult female Wistar rats were separated into nulliparous controls (C), stressed (S), and primiparous controls (P), and stressed (P+S) groups. P+S and S groups were exposed to chronic mild unpredictable stress for 10 days during the P+S late gestational period. Anhedonia and stress-coping behaviors measured in the early postpartum period were increased in P+S rats, while anxiety-like behavior was unaffected. On postpartum day 11, ex vivo mitochondrial function measured in prefrontal cortex (PFC) was decreased by gestational stress, while nucleus accumbens (NAc) respiration was unaffected. Levels of pro-inflammatory cytokines in plasma and PFC indicated a heightened proinflammatory profile in P+S and S rats. Overall, we report an association between PFC mitochondrial respiration and PPD-like behaviors following gestational stress, with a potential role for inflammatory signaling.

Pubmed:
33955617: Gorman-Sandler E, Hollis F
The forced swim test: Giving up on behavioral despair (Commentary on Molendijk & de Kloet, 2021).
Eur J Neurosci, 2022; 55

FENS Forum 2022
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BOARD NUMBER: S07-196

NOVEL INSIGHTS INTO ANTIDEPRESSANT-INDUCED TRKB SIGNALING

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Background: Antidepressants have been proposed to act by binding to TrkB receptors which facilitates BDNF-induced activation of TrkB and synaptic plasticity. However, our findings demonstrate that some antidepressants, most notably nitrous oxide (N_2O), trigger TrkB signaling only after their acute pharmacological effects dissipate, during a state resembling deep sleep or sedation. Indeed, not only antidepressants but also a wide range of tested sedatives readily activate TrkB. **Aims:** To investigate physiological characteristics associated with drug-induced TrkB activation, and to test whether TrkB activation can be manipulated by disrupting such physiological alterations. **Methods:** Time-dependent effects of N_2O on locomotor behavior, EEG (electroencephalogram), body temperature, brain glucose utilization, and brain TrkB signaling were studied in adult mice. Next, we investigated the acute effects of sedatives (e.g. medetomidine) and sedative antidepressants (incl. amitriptyline) on TrkB signaling and body temperature. Finally, we tested the intriguing possibility that the effects of amitriptyline on TrkB signaling can be abolished by simply maintaining animal´s body temperature. **Results/Conclusions:** TrkB signaling and several characteristic features associated with deep sleep were evoked after cessation of N_2O administration: slow-wave EEG activity, behavioral immobility, reduced brain glucose utilization and hypothermia. Various sedatives and sedative antidepressants activated TrkB, and this was accompanied by reduced body temperature. Maintaining animals normothermic, supressed amitriptyline-induced TrkB signaling. These results suggest that TrkB activation is triggered by an evoked physiological response, rather than direct receptor binding, connected to drugs ability to reduce energy expenditure and consequently, body temperature. This study links bioenergetics and thermoregulation into the effects of antidepressants.

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DOSE-DEPENDENT EFFECTS OF KETAMINE ON ELECTROPHYSIOLOGICAL NETWORK ACTIVITY AND TRKB-ERK SIGNALING IN CORTICAL NEURONAL CULTURE

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Background:
Subanesthetic-dose ketamine increases glutamatergic neuronal activity in the cortex. This effect has been proposed to facilitate BDNF (brain-derived neurotrophic factor) release and activation of TrkB receptor and extracellular signal-regulated kinase (ERK) signaling. Previous data suggests that ketamine dose-dependently regulates ERK signaling also in cortical neuron cultures, but effects on TrkB and neuronal network activity remain unclear. Aims: To characterize spontaneous network activity and responses to BDNF on primary neuronal cultures during different maturational stages. To examine the effects of carbachol, diazepam and different doses of ketamine (100nM - 10µM) on electrophysiological network activity and TrkB-ERK signaling in cultures which are readily responsive to BDNF. Methods: Rat E18 cortical cells were cultured on microelectrode arrays (12 electrodes/well) and spontaneous network activity was followed for 5 weeks. Acute electrophysiological responses to BDNF and drugs were recorded after 14 or 35 days in vitro (DIV). After stimulation, cells were lysed and TrkB/ERK phosphorylation analyzed using western blotting. Results/conclusions:
Spontaneous spiking and bursting steadily increased during DIV5-21 but declined towards DIV35. BDNF robustly increased phosphorylation of TrkB and ERK at DIV14 while a more marginal response was seen at DIV35. Hence, effects of drugs were investigated at DIV14. Diazepam (10 µM) abolished electrical activity and reduced p-ERK, while carbachol (25 µM) caused variable increases in activity and did not affect p-ERK. Ketamine (100-500 nM) caused dose-dependent decreases on spiking/bursting, with 500 nM showing a significant decrease without change in p-ERK. Ketamine (10 µM) strongly reduced spiking, bursting and p-ERK. None of the drug treatments influenced p-TrkB.

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GENERATION OF HUMAN NEURONS FROM INDUCED PLURIPOTENT STEM CELLS FOR SCREENING OF NITROSYNAPSIN FOR MAJOR DEPRESSIVE DISORDER

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Major depressive disorder (MDD) is recognized as a major source of disability and mortality worldwide. Current therapeutic treatments are effective in only half of patients. In-depth understanding of treatments and discovering novel ones have been lagged due to a huge gap in the development of an appropriate disease model. Here, we generated neurons from human induced pluripotent stem cells (hiPSCs) to serve as a starting point to develop a human model, and to test an experimental drug NitroSynapsin (NS), to translate results from animals into human. Chronic restrain stress (CRS) animal model was employed to mimic depressive-like behaviour. Thereafter, all animals were subjected to the sucrose preference (SPT) and tail suspension tests (TST). Subchronic treatment of NS reversed behavioural disturbances induced by CRS in regard to SPT and TST. NS also reversed changes in morphology and density of dendritic spines, observed in cortical neurons of CRS animals. To confirm if the findings in animals would be similar in humans, hiPSCs were differentiated into Nestin+ and Pax6+ neural progenitor cells (NPCs). NPCs were differentiated into neural precursors which gave rise to βIII-Tubulin+, MAP2+ neurons under further maturation. Spine-like protrusions could be observed in neurons undergoing 1-month maturation. Matured iPSC-neurons (≥6 weeks in vitro) will be stressed by cortisol and treated by NS so as to assess the effect. In summary, we have observed that NS improved behaviours in CRS mice model and restored the morphology and density of dendritic spines. Our further work aims at translating these findings into hiPSC-derived neurons.

Pubmed:
30345079: Zhang SQ, Lin KL, Law CY, Liu B, Fu XQ, Tse WS, Wong SSM, Sze SCW, Yung KKL
Oleanolic acid enhances neural stem cell migration, proliferation, and differentiation in vitro by inhibiting GSK3β activity. Oleanolic acid (OA), one of the bioactive ingredients in ginseng, has been reported to have neuroprotective activities. However, the effects and its mechanism on neural stem cell (NSC) induction are not entirely clear. In the present study, we investigated the effects of OA on promoting the migration, proliferation, and differentiation of neural stem cells (NSCs). Migration and proliferation were investigated by using neural-specific markers, neurosphere assay, and Cell Counting Kit-8, respectively. We found OA remarkably promoted neural migration and proliferation of NSCs in a time- and dose-dependent manner. Differentiation was analyzed by western blotting and immunofluorescence staining, which found MAP2 expression was remarkably increased, whereas Nestin was dramatically decreased. In addition, OA increased phosphorylation of GSK3β at Ser9 and expression of active forms of β-catenin. Furthermore, NSCs with constitutively active GSK3β (S9A) significantly suppressed the OA-induced proliferation and neural differentiation. These results showed that OA could stimulate NSC proliferation and neural differentiation in vitro via suppressing the activity of GSK3β. Our findings may have significant implications for the treatment of neurodegenerative diseases.
Cell Death Discov, 2018; 4

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OMEGA-3 IMPACTS ON THE LONG-TERM IMPAIRED ENDOCANNABINOID SYSTEM AND BEHAVIOR AFTER BINGE DRINKING DURING ADOLESCENCE

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

Maitane Serrano[1,2], Leire Lekumberri[1,2], Ilazki Anaut-Lusar[1,2], Amaia Mimenza[1,2], Miquel Saumell-Esnaola[3,4], Gontzal Garcia-Del Caño[2,3], Edgar Soria-Gomez[1,2,5], Almudena Ramos-Uriarte[1,2], Joan Sallés[3,4], Nagore Puente[1,2], Izaskun Elezgarai[1,2], Irantzu Rico-Barrio[1,2], Pedro Grandes[1,2]
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Our laboratory has previously demonstrated that adolescent binge drinking affects CB1 receptor expression in the excitatory medial perforant path (MPP) synaptic terminals of the adult mouse hippocampus that associates with loss of endocannabinoid-dependent synaptic plasticity at the MPP and memory impairment. Strong evidences have shown that omega-3 prevents neurodegeneration and oxidative stress caused by adult EtOH exposure. Thus, omega-3 might promote recovery from the long-term damage caused by binge drinking during adolescence. To investigate this, 4 week-old C57BL/6J male mice were exposed to a drinking-in-the-dark procedure from PND 32 to 59 (H2O, EtOH). During withdrawal (PND 60-73), one half had omega-3-enriched diet (EPA 2.7% and DHA 2%) (O3-H2O, O3-EtOH). Then, learning, memory, motor performance and anxiety were assessed by the Barnes maze, novel object recognition, beam walking, rotarod and anxiety behavioral tests. Also, immunohistochemistry and western blotting were performed in the hippocampus. Omega-3 ameliorated cognitive and motor deficits after adolescent binge drinking. In addition, omega-3 treated groups were less anxious and had better recognition memory. This cognitive and behavioral improvement correlated with a drastic reduction in MGL optical density and synaptosomal DGLα as well as with a remarkable CRIP-1a increase, relative to EtOH. Furthermore, CB1 receptor expression in whole hippocampal synaptosomes was significantly higher in EtOH and O3-EtOH than in H2O. Taken together, these results indicate that omega-3 intake impacts on components of the endocannabinoid system and has concurrent beneficial effects on behavior in adult mice after binge drinking during adolescence.

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SIMULATED MICROGRAVITY REMODELS THE NEUROCHEMISTRY OF MONOAMINERGIC SYSTEMS ACROSS THE RAT BRAIN

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Aims Spaceflights are challenging for astronauts for decision making, movement adaptation, and vestibular control. Monoaminergic systems regulate those adaptive functions and could play a role in some maladaptive responses during spaceflight and upon the return to earth. The goal of our study was to explore the neurochemistry of dopaminergic, noradrenergic and serotonergic systems alone and in interaction each other in a rodent model of simulated microgravity (hindlimb unloading suspension). Methods To simulate microgravity, male Long-Evans rats were hindlimb suspended during 7 days. Rats were euthanized, brains frozen, and 30 cortical and subcortical structures involved in cognitive, motor and mood functions were extracted. Those were measured for monoamines and their metabolites contents by High Pressure Liquid Chromatography coupled to electrochemical detection. Results Simulated microgravity altered the tissue levels of dopamine and noradrenaline along some structures of the vestibular/motor systems (red nucleus, substantia nigra, inferior olive, and cerebellum). A reduction of dopamine neurochemical markers was observed in the shell of the nucleus accumbens, and, together with serotonin, in the prelimbic cortex. Spermann's correlations revealed important variations of serotonergic network between brain regions alone and in relation with dopamine. Conclusion Our study suggests that quantitative catecholamine's distribution in the vestibular/motor systems, as well as dopamine and serotonin in the prelimbic cortex are notably affected by simulated microgravity exposure. Remodeling of the serotonergic function could represent an adaptive response to microgravity, perhaps at the expenses of adaptive responses upon the return to earth conditions.

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IMPAIRED COGNITION AND BEHAVIOR ASSOCIATE WITH CHANGES IN THE BRAIN ENDOCANNABINOID-DEPENDENT SYNAPTIC PLASTICITY OF ADULT FEMALE MICE AFTER BINGE DRINKING DURING ADOLESCENCE

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Recent work in our laboratory has shown the lack of cannabinoid-dependent hippocampal synaptic plasticity in adult male mice after binge drinking during the adolescence. However, the brain consequences of similar alcohol intake pattern in adolescent females are still controversial. To investigate this, female C57BL/6J adolescent mice (pnd32 to pnd56) were exposed to drinking-in-the-dark procedure followed by two weeks of withdrawal. During the last abstinence days, anxiety-like behavior, learning and memory and motor coordination were assessed. In addition, hippocampal synaptic plasticity and expression of some components of the endocannabinoid system were analyzed. Adolescent females consumed 50% more alcohol than males. Moreover, females in the adulthood showed significantly more anxiety-like behavior and impaired motor coordination and memory after adolescent binge drinking. Although hippocampal synaptic plasticity was unaffected, excitatory long-term depression at the dentate medial perforant path synapses was cannabinoid CB1 receptor independent in adult females exposed to EtOH in the adolescence. No differences were found in the expression of synaptosomal CB1, DGLα, PLCβ1 and Crip1a between hippocampi of controls and EtOH, but Crip1a was significantly increased in both EtOH and control females versus males. In conclusion, adolescent binge drinking impairs cognition, motor behavior and mood as well as alters the cannabinoid molecular mechanisms of synaptic plasticity in adult female mice. Noticeably, these changes do not correlate with modifications in at least some main components of the endocannabinoid system.

Pubmed:
30106197: Rico-Barrio I, Peñasco S, Puente N, Ramos A, Fontaine CJ, Reguero L, Giordano ME, Buceta I, Terradillos I, Lekunberri L, Mendizabal-Zubiaga J, Rodríguez de Fonseca F, Gerrikagoitia I, Elezgarai I, Grandes P
Cognitive and neurobehavioral benefits of an enriched environment on young adult mice after chronic ethanol consumption during adolescence.
Binge drinking (BD) is a common pattern of ethanol (EtOH) consumption by adolescents. The brain effects of the acute EtOH exposure are well-studied; however, the long-lasting cognitive and neurobehavioral consequences of BD during adolescence are only beginning to be elucidated. Environmental enrichment (EE) has long been known for its benefits on the brain and may serve as a potential supportive therapy following EtOH exposure. In this study, we hypothesized that EE may have potential benefits on the cognitive deficits associated with BD EtOH consumption. Four-week-old C57BL/6J male mice were exposed to EtOH following an intermittent 4-day drinking-in-the-dark procedure for 4 weeks. Then they were exposed to EE during EtOH withdrawal for 2 weeks followed by a behavioral battery of tests including novel object recognition, novel location, object-in-place, rotarod, beam walking balance, tail suspension, light-dark box and open field that were run during early adulthood. Young adult mice exposed to EE significantly recovered recognition, spatial and associative memory as well as motor coordination skills and balance that were significantly impaired after adolescent EtOH drinking with respect to controls. No significant permanent anxiety or depressive-like behaviors were observed. Taken together, an EE exerts positive effects on the long-term negative cognitive deficits as a result of EtOH consumption during adolescence.
Addict Biol, 2019; 24

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IN UTERO EXPOSURE TO AIR POLLUTION AFFECTS SOCIAL BEHAVIOR AND BRAIN TRANSCRIPTOME IN MOUSE

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Psychiatric disorders are complex neurodevelopmental conditions, which can be explained partly by a genetic component as well as by environmental factors. Recent epidemiological studies have suggested that post-natal and peri-natal exposure to air pollution could play a role in the development of several psychiatric disorders, in particular for those living in areas where the concentration of pollutants exceeds the limits set by the World Health Organization. However further research needs to be done to demonstrate a direct impact of air pollution as well as to understand the biological mechanisms involved. Here, we investigated the impact of a prenatal exposure to a realistically simulated air pollution of Paris and Beijing during a critical phase of brain development, the last third of gestation. We compared social interactions, anxiety and anhedonia in the offspring of exposed and not exposed animals at three periods of life, i.e. childhood (6 days post-natal), early adolescence (30 days post-natal), and adulthood (over 90 days post-natal). In parallel, we compared the gene expression in the prefrontal cortex of exposed and not exposed mice at the early adolescence and adulthood. We reported alteration of ultrasonic vocalization in pups and modification of social behavior in adolescence and adulthood for exposed animals. We also identified genes that were differentially expressed between exposed and not exposed animals with an enrichment in gene expression regulation and immune signaling. Altogether, our results suggest that prenatal exposure to air pollutants may affect brain functioning and behaviors of the offspring.

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BOARD NUMBER: S07-203

PREFRONTAL INHIBITORY CIRCUITS ARE IMPACTED BY CHRONIC STRESS AND DRIVE STRESS-ASSOCIATED BEHAVIORAL IMPAIRMENTS

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Prefrontal cortical circuits and prefrontal inhibitory circuits in particular are thought to be impacted by adverse events and chronic stress. These circuit impacts may underlie clinical observations that adverse events and stress may impair normal cognition and perseverate psychiatric disorders like schizophrenia, bipolar disorder, major depression, and anxiety. In this study we aim to assess how chronic stress drives changes in mouse inhibitory circuits and impacts behaviors associated with executive function, anxiety, and social behavior. To do this, we use a validated mouse model for chronic stress, the limited nesting and bedding (LNB) model, in which mice are reared in resource-deprived conditions starting at postnatal day 2. In this model, mice express impacts to behaviors associated with prefrontal dysfunction and we have quantified a reduction in prefrontal parvalbulmin interneurons in adulthood. To extend this work we use RNAscope to further quantify changes in the expression of molecular markers associated with the different subtypes of cortical interneurons and demonstrate how expression levels differ between LNB and normally housed mice in prefrontal cortex, amygdala, hypothalamus, and thalamus. Since interneurons also play a role in cortical rhythms, we use skull EEG recordings from prefrontal, somatosensory, and visual cortex to demonstrate various differences in frequency specific activity and in sleep-associated cortical rhythms between groups. Finally, we use optogenetic stimulation of prelimibic pravalbumin-positive interneurons in order to rescue some of the behaviors impacted in LNB mice, suggesting that this circuit impairment may underlie some of the disease associated impairments that result from chronic stress.

FENS Forum 2022
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BOARD NUMBER: S07-204

A PRIMATE SUBCALLOSAL CINGULATE AREA 25 NETWORK FRACTIONATES ANHEDONIA, ANXIETY AND RAPID ANTIDEPRESSANT RESPONSE

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Understanding the dysregulated circuits that underlie the symptoms and treatment response in anxiety and depression are critical for developing more effective therapies. Patients show elevated subcallosal cingulate cortex activity (scACC) and recent studies in primates revealed its causal contribution to both anhedonia and anxiety and its sensitivity to ketamine. The current study sought to fractionate the area 25 networks underlying these effects.

In marmosets, excitatory DREADDs were expressed in scACC area 25 output neurons (scACC-25; AAV8-CaMKIIa-HA-hM3Dq) and specific pathways activated by intracerebral infusion of Clozapine-N-Oxide (CNO, 3uM) into terminals within the nucleus accumbens (scACC-25>NAcc) and amygdala (scACC-25>Amyg) prior to appetitive and threat-related paradigms (n=3-5). Reward anticipation was studied using appetitive Pavlovian conditioning where two sounds predict, respectively, food access or not (conditioned stimuli, CS+/-). Uncertain threat reactivity was assessed by the human intruder test (HI-Test) whereby a novel human faces the marmoset for two minutes; with the multifaceted behaviours combined into an anxiety-like score. Amelioration of reward deficits induced by systemic CNO-mediated scACC-25 activation were studied via intra-accumbens ketamine infusions.

ScACC-25>NAcc activation blunted anticipatory CS+ arousal, whilst scACC-25>Amyg activation had no effect (Treatment*Region*CS, p=0.011; CS+ scACC-25>NAcc Veh/CNO, p=0.046). In contrast, only scACC-25>Amyg activation heightened threat reactivity in the HI-Test (Treatment*Region, p=0.011; scACC-25>Amyg Veh/CNO, p=0.002). Intra-accumbens ketamine blocked the blunting of anticipatory arousal by systemic CNO treatment (Treatment*CS, p=0.005; CS+ Saline/Ketamine, p=0.014).

Together, these data indicate circuit-specificity with scACC-25 overactivity inducing anhedonia-like and anxiety-like symptomatology through distinct pathways, with the nucleus accumbens a key region for ketamine’s ameliorative effects on anhedonia.

Funded by Wellcome Trust Investigator award 108089/Z/15/Z

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-205

THE INFLUENCE OF PARENTAL SOCIAL EXPERIENCE ON OFFSPRING NOVELTY-EXPLORING AND DEPRESSION-LIKE BEHAVIOR

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Aims: The influence of parental social experience on the offspring neurodevelopment and its own susceptibility to the sculpting effect of social experience are not well understood. Using rat model we aimed to examine whether and how social experience of adolescent parents impacts psychophysical characteristics of their offspring, during pre-weaning period and later during adolescent growing in defined social conditions. **Methods:** Peripubertal Wistar rats (both sexes) were randomly selected for group housing (GH; n=3 per cage) or single housing (SH; n=1 per cage) at postnatal day (P) 29 and left undisturbed until P55. Thereafter, they were grouped for mating (SH males with SH females, GH males with GH females). Body weight (BW) of pups was monitored at P7, P14, and P21. At P17 motor activity of the offspring was monitored; at P29 the animals were subjected to GH or SH; after 1 week (P36) and 2 weeks (P43) their motor activity was monitored; at P45 they were subjected to sucrose preference test (SPT). **Results:** The male offspring of SH parents had decreased locomotor activity in a novel open arena, at P17. At P36 and P43, adolescent SH offspring showed hypolocomotion in a novel arena and hypohedonic behavior in SPT compared to GH counterparts, regardless of parental experience. BW did not differ between groups. **Conclusions:** Parental social experience influences novelty-exploring behavior of offspring, producing response below expected at P17. With further offspring development, their individual social experience has stronger impact on the appearance of depression-like phenotypes than parental social experience.

Pubmed:
33238164: Potrebić M, Pavković Ž, Lončarević-Vasiljković N, Kanazir S, Pešić V
Altered hedonic, novelty-, stress- and D-amphetamine-induced response due to social isolation in peripuberty.
Reduction in direct social contact with peers during early adolescence is thought to be a risk factor for an increase in depressive symptoms, but there is still no clear evidence to suggest early behavioral manifestations and their association with the later outcome of social distancing during this period. To address this question, we used social isolation paradigm in peripubertal rats as the rodent model of adolescence. The litter was an experimental unit. On postnatal day 29, each litter gave group-housed and single-housed males, which were reared and tested one week and two weeks thereafter. Psychomotor/emotional response to novelty in exploration-based tasks, behavioral and neuronal responses to the drug reward (D-amphetamine), motivation/hedonic behavior, physiological and response to physiological stress were examined. Social isolation in peripubertal rats manifested through: hyper-reactivity/agitation and the state anxiety/risk-taking at an early stage; reduced behavioral response to D-amphetamine and altered neural processing of this stimulus, at a later stage; consummatory hypohedonia that deepened over time without changing the motivation to eat; unchanged body weight gain and resting blood corticosterone, cortisol and glucose levels over time; altered blood biochemistry (silenced corticosterone and increased glucose) due to overnight fasting only at an early stage. Our results highlight that the outcome of reduced direct social contact with peers during peripuberty is dynamic, with the cluster of atypical early symptoms that evolve into the syndrome that is delicate for assessment through routinely measurable behavior and biomarkers of stress, but with progressive consummatory hypohedonia and unaffected motivation to eat as stable marks.
Prog Neuropsychopharmacol Biol Psychiatry, 2021; 108
31419478: Pavković Ž, Potrebić M, Kanazir S, Pešić V
Motivation, risk-taking and sensation seeking behavior in propofol anesthesia exposed peripubertal rats.
Adolescent neurodevelopment confer vulnerability to the actions of treatments that produce adaptations in neurocircuitry underlying motivation, impulsivity and reward. Considering wide usage of a sedative-hypnotic agent propofol in clinical practice, we examined whether propofol is a challenging treatment for peripubertal brain. Motivation/hedonic behavior (sucrose preference test), approach/avoidance behavior (elevated plus maze test) and response to dissociative drug phencyclidine (PCP) were studied in peripubertal rats (the rodent model of periadolescence) after propofol anesthesia

exposure (PAE). Neurodegeneration (Fluoro-Jade staining) and the expression of proteins (Western blot) involved in excitatory synaptic transmission and activity-dependent synaptic stabilization in the medial prefrontal cortex (mPFC) and striatum (components of motivation/reward circuitry; process both appetitive and aversive events) were examined as well. In peripubertal rats PAE produced 1) transient brain-region specific changes in the expression of N-methyl-d-aspartate (NMDA) receptor subunits NR2A and NR2B, PSD-95 and N-cadherin, without neurotoxicity, 2) hyperlocomotor response to PCP, 3) no changes in preference for palatable 1% sucrose solution and a decrease in food eaten, 4) preference for 20% sucrose solution without changes in food eaten, 5) stretch-attended postures and open arms entries in the elevated plus maze test. Overall, these novel findings show that PAE leaves transient synaptic trace recognized as early form of synaptic plasticity related to passive drug exposure in the brain systems implicated in motivation/reward, increases drug-responsiveness, favors risk-taking and preference of novel/intense stimuli repairing otherwise present motivational deficiency. These findings accentuate multifaceted response to propofol in peripuberty and the importance of environmental stability for the most favorable neurobehavioral recovery.
Prog Neuropsychopharmacol Biol Psychiatry, 2020; 96

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-206

DYNAMIC HIPPOCAMPAL DNA METHYLATION TRAJECTORIES FOLLOWING IMPAIRED MATERNAL CARE

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Aims: Impaired maternal care was shown to have long-lasting effects on the adult offspring behavior through altered hippocampal DNA methylation. However, it is less clear what changes in the DNA methylation landscape occurs throughout the early postnatal developmental stages while offspring are cared by their dams.
Methods: We used mouse models of social dominance (Dom) and submissiveness (Sub), who show innate features of high and low maternal care behaviors, respectively. For genome-wide DNA methylation analysis we used reduced-representation bisulfite sequencing (RRBS-Seq) of hippocampal DNA from 7- and 21-days-old Sub and Dom pups.
Results: Sub mice showed low maternal care behaviors accompanied by reduced DNA methyltransferases expression. We found robust alterations in the DNA methylation landscape for both between-group and between timepoint analyses; with significant enrichment of differentially methylated CpGs in gene promoters encoding for genes associated with behavior, and neuronal development. Cross fostering of Sub pups with Dom dams restored many of the observed differences between Dom and Sub pups.
Conclusions: we conclude that altered methylome previously reported in the hippocampus of adult offspring of dams providing poor maternal care is perceived by early and time-dependent post-natal changes in DNA methylation patterns.

FENS Forum 2022
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BOARD NUMBER: S07-207

MONOAMINE MODULATORS OF HERBAL ORIGIN.

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Depression is the largest contributor to disability and suicide worldwide and there is an urgent need to develop improved treatments. Conventional antidepressants can induce unpleasant and sometimes dangerous side effects, resulting in low patient compliance and a high failure response rate. *Rhodiola rosea* has traditionally been used as an adaptogen. Data from animal studies and human clinical trials suggest that it may be effective in monoamine modulation and the treatment of depressive disorders, with high tolerance and virtually no side effects, yet little is known about the mechanism of action. **Aim** The aim of this study is to determine the anti-depressant efficacy of Rhodiola *in vitro* with the potential for the treatment of mild to moderate depression. **Methods** Neuromodulation studies were conducted in two *in vitro* neuronal model systems (noradrenergic SH-SY5Y and T-REx-293 SERT cells). Antidepressant/anxiolytic effects were assessed via [^{3}H]MPP$^+$ uptake assays at the serotonin (SERT) and noradrenaline (NET) transporters. Non-specific uptake was measured in the presence of paroxetine and nisoxetine for SERT and NET respectively. Membrane permeability was assessed via neutral red release assays. **Results** Initial data suggests *R. rosea* main bioactive constituents and their equimolar mixture have a negligible inhibitory activity at both SERT and NET at physiological conditions. However, extracts show dose dependent, competitive inhibition. **Conclusion** MPP$^+$ uptake inhibition of extracts is not associated with membrane integrity loss as assessed via neutral red release assay. The higher efficacy of extracts suggests a possible synergistic/additive effect or a presence of an overlooked secondary metabolite.

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BOARD NUMBER: S07-208

MOLECULAR CHARACTERIZATION OF HABENULA NUCLEI IN A GENETIC RAT MODEL OF DRUG-RESISTANT DEPRESSION

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

Agata Korlatowicz, Paulina Pabian, Joanna Solich, Magdalena Kolasa, Katarzyna Latocha, Marta Dziedzicka-Wasylewska, Agata Faron-Górecka
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Aims Depression is a highly prevalent and debilitating disorder that continues to increase the number of people with mental illness. Despite advances in the treatment of depression, it is estimated that 30% of patients are still resistant to antidepressant treatment. Wistar Kyoto (WKY) rats are a suitable animal model for treatment-resistant depression (TRD) in preclinical studies. Many recent scientific reports indicate that the habenula may play an important role in depression, so we focused our study on characterizing molecular differences in WKY habenular nuclei compared to Wistar Han rats (WIS). **Methods** Brain sections of WIS and WKY rats containing medial and lateral habenula (MHb and LHb) were excisted by Laser Capture Microdissection. We identified 32 miRNAs that could be potentially regulated in the habenula. The mRNA expression level was assessed using TaqMan Array Cards, which were designed based on the analysis of potential targets of significant miRNAs. **Results** Ten miRNAs significantly differentiated WKY rats from WIS rats. For mRNAs that differentiated WKY from WIS rats in the MHb (Cdkn1c, Htr7, Kcnj9, and Slc12a5), their lower expression correlated with a higher level of relevant miRNAs. In the LHb, eight mRNAs significantly differentiated WKY from WIS rats (upregulated Htr4, Drd2, Kcnj5, and Sstr4 and downregulated Htr2a, Htr7, Elk4, and Slc12a5). **Conclusion** Our results indicate the importance of the habenular nuclei as an important brain region in depression research. The basic level of molecular diversity within the habenula of WKY rats indicates its important role in TRD.

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BOARD NUMBER: S07-209

IDENTIFICATION OF CLINICALLY AND NEUROBIOLOGICALLY DISTINCT SUBTYPES FOR POSTTRAUMATIC STRESS DISORDER

POSTER SESSION 07 - SECTION: NEUROBIOLOGY OF STRESS, ANXIETY, AND DEPRESSION

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Heterogeneity of posttraumatic stress symptoms may impede timely and individualized interventions for posttraumatic stress disorder (PTSD). The present study aims to dissect the heterogeneity of posttraumatic conditions to identify novel subtypes in recently traumatized individuals and evaluate their neurobiological correlates and long-term prognosis. A data-driven approach using the agglomerative hierarchical clustering analysis was implemented to classify recently trauma-exposed individuals into specific subtypes based on clinical, behavioral, and cognitive symptom profiles which can be frequently observed in the post-traumatic period.. Two hundred forty trauma-exposed individuals and 240 age- and sex-matched healthy individuals without trauma exposure were included in the study. Subtype-specific patterns of functional connectivity in higher-order cognitive networks, longitudinal clinical outcomes, and changes in functional connectivity were also evaluated. We identified four distinct subtypes for trauma-exposed individuals according to the dimension of posttraumatic stress symptoms: the resilient, emotional symptom-predominant, behavioral symptom-predominant, and cognitive symptom-predominant subtypes. The characteristics of four identified subtypes were further validated and supported by subsequent resting-state functional neuroimaging analyses that suggested distinct functional brain organization, particularly of the higher-order cognitive networks, across subtypes. Each subtype was also distinct in long-term trajectories for posttraumatic symptoms. In the current study, we demonstrated the ability to categorize complex posttraumatic stress symptoms into the data-driven-subtypes. Our results may enhance the current understanding of mechanisms underlying the human-specific heterogeneous responses to trauma. This study may provide valuable data to develop the individualized and improved interventions, including targeting of subtype-specific characteristics.

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BOARD NUMBER: S07-210

FUNCTIONAL PROTECTION IN J20/VLW MICE: A MODEL OF COGNITIVE RESILIENCE TO ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer's disease comprises amyloid-β and hyperphosphorylated Tau accumulation, imbalanced neuronal activity, aberrant oscillatory rhythms, and cognitive deficits. Cognitive resilience to Alzheimer's disease (CRAD) defines a novel clinical entity with amyloid-β and Tau pathologies but preserved cognition. The mechanisms underlying such neuroprotection remain undetermined, and animal models of CRAD are currently unavailable. By neuroanatomical tract-tracing, immunodetection, electrophysiological analyses, and behavioral studies, we characterize a new double transgenic mouse model accumulating amyloid-β and hyperphosphorylated Tau: J20/VLW mice. We demonstrate that J20/VLW animals exhibit preserved hippocampal oscillatory activity and cognition, as opposed to single transgenic J20 and VLW mice, which show significant alterations. Furthermore, we show that the overexpression of mutant human Tau in coexistence with amyloid-β accumulation renders a particular Tau phosphorylation signature in hippocampal interneurons. Moreover, the GABAergic septohippocampal pathway, responsible for hippocampal oscillatory activity, is preserved in J20/VLW mice, in contrast to single mutants. Our data highlight J20/VLW mice as a suitable animal model to explore the mechanisms driving cognitive preservation in CRAD. Moreover, they suggest that a differential Tau phosphorylation pattern in hippocampal interneurons prevents the loss of GABAergic septohippocampal innervation and alterations in local field potentials, thereby avoiding cognitive deficits.

Pubmed:
34424282: Dávila-Bouziguet E, Casòliba-Melich A, Targa-Fabra G, Galera-López L, Ozaita A, Maldonado R, Ávila J, Delgado-García JM, Gruart A, Soriano E, Pascual M
Functional protection in J20/VLW mice: a model of non-demented with Alzheimer's disease neuropathology.
Alzheimer's disease comprises amyloid-β and hyperphosphorylated Tau accumulation, imbalanced neuronal activity, aberrant oscillatory rhythms and cognitive deficits. Non-demented with Alzheimer's disease neuropathology defines a novel clinical entity with amyloid-β and Tau pathologies but preserved cognition. The mechanisms underlying such neuroprotection remain undetermined and animal models of non-demented with Alzheimer's disease neuropathology are currently unavailable. We demonstrate that J20/VLW mice (accumulating amyloid-β and hyperphosphorylated Tau) exhibit preserved hippocampal rhythmic activity and cognition, as opposed to J20 and VLW animals, which show significant alterations. Furthermore, we show that the overexpression of mutant human Tau in coexistence with amyloid-β accumulation renders a particular hyperphosphorylated Tau signature in hippocampal interneurons. The GABAergic septohippocampal pathway, responsible for hippocampal rhythmic activity, is preserved in J20/VLW mice, in contrast to single mutants. Our data highlight J20/VLW mice as a suitable animal model in which to explore the mechanisms driving cognitive preservation in non-demented with Alzheimer's disease neuropathology. Moreover, they suggest that a differential Tau phosphorylation pattern in hippocampal interneurons prevents the loss of GABAergic septohippocampal innervation and alterations in local field potentials, thereby avoiding cognitive deficits.
Brain, 2022; 145
30553886: Dávila-Bouziguet E, Targa-Fabra G, Ávila J, Soriano E, Pascual M
Differential accumulation of Tau phosphorylated at residues Thr231, Ser262 and Thr205 in hippocampal interneurons and its modulation by Tau mutations (VLW) and amyloid-β peptide.
Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β peptide (Aβ) and hyperphosphorylated Tau

protein (P-Tau). Our recent data showed a differential accumulation of Tau protein phosphorylated at residue Thr231 (pThr231) in distinct hippocampal neurons in VLW mice-a model that overexpresses mutated human Tau. Here we demonstrate that, in VLW mice, the accumulation of human P-Tau in pyramidal cells induces the phosphorylation of murine Tau at residue Thr231 in hippocampal interneurons. In addition, we show that pSer262 and pThr205 Tau are present specifically in the soma of some hippocampal interneurons in control mice. Analysis of J20 mice-a model that accumulates Aβ-and of VLW animals showed that the density of hippocampal interneurons accumulating pThr205 Tau is lower in VLW mice than in controls. In contrast, the density of interneurons accumulating pThr205 Tau in J20 mice was increased compared to controls in hippocampal regions with a higher Aβ plaque load, thereby suggesting that pThr205 Tau is induced by Aβ. No significant differences were found between the density of hippocampal interneurons positive for pSer262 Tau in VLW or J20 mice compared to control animals. We also show that pSer262 and pThr205 Tau are present in the soma of some hippocampal interneurons containing Parvalbumin, Calbindin or Calretinin in control, VLW, and J20 mice. Moreover, our results reveal that some interneurons in human hippocampi of cases of AD and control cases accumulate pSer262 and pThr205 Tau. Taken together, these data point to a specific role of pSer262 and pThr205 Tau in the soma of hippocampal interneurons in control and pathological conditions.
Neurobiol Dis, 2019; 125

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-211

REGULATION OF CRTC1-MEDIATED SYNAPSE-TO-NUCLEUS COMMUNICATION BY EXCITOTOXIC ACTIVATION OF NMDA RECEPTORS

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated calcium-permeable channels mainly localized at excitatory synapses. NMDAR function is generally linked to synaptic plasticity, learning and memory. However, sustained glutamate exposure leads to NMDA-mediated excitotoxicity, involved in neurodegenerative diseases. Distinct cellular outcomes of NMDAR stimulation depend on receptor cell surface localization. Synaptic NMDARs promote cyclic AMP-response element binding protein (CREB) activation, eventually inducing immediate early gene (IEG) expression. This coupling between excitation and gene expression is called excitation-transcription (E-T) coupling. On the other hand, extrasynaptic NMDAR activation by excessive glutamate leads to CREB shut-off and neuronal cell death. We suggest that excitotoxic NMDAR stimulation might also shut off E-T coupling by interfering with active nuclear import-dependent synapse-to-nucleus communication. Here we focus on CREB-regulated transcription coactivator 1 (CRTC1) as a representative effector molecule, because CRTC1 undergoes nuclear translocation upon synaptic activity and participates in CREB-dependent IEG expression. We aim to assess whether excitotoxic NMDAR stimulation shuts off CRTC1 nuclear translocation. We stimulated primary hippocampal cultures with two excitotoxic stimulation protocols, prolonged NMDA exposure, or brief NMDA exposure followed by a wash-out and recovery period. We then studied CRTC1 localization by immunocytochemistry. We found that NMDA pulse promoted CREB shut-off and reduced CRTC1 nuclear translocation, whereas prolonged NMDA triggered CREB shut-off while promoted CRTC1 nuclear localization. All in all, these results indicate that different excitotoxic NMDAR stimulations do not trigger the same CRTC1 nuclear translocation response, which might evoke different gene expression profiles.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-212

THE IMPACT OF ADVANCING AGE AND INTRAOCULAR PRESSURE ELEVATION ON RETINAL GANGLION CELL SYNAPTIC CONNECTIVITY AND EXCITABILITY IN AN ACUTE MODEL OF GLAUCOMA

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Glaucoma is a neurodegenerative disease characterised by the dysfunction and the death of retinal ganglion cells (RGCs). The leading risk factors for developing glaucoma are elevated intraocular pressure (IOP) and increased age. Evidence suggests that the early stages of RGC dysfunction are reversible. To investigate this, we used an acute model of glaucoma that involves elevating the IOP of a mouse to produce a temporary loss of RGC function which recovers by 7-days in 3-month old mice and 28-days in 12-month old mice. The aim of our study was to investigate the changes in synaptic connectivity underlying RGC dysfunction and recovery in young and aged mice. We first quantified RGC excitatory synapses using fluorescence immunohistochemistry and microscopy. We found that excitatory synapses were reduced following IOP elevation in young and aged mice, however, these synapses did not recover. We next recorded excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) using whole-cell patch-clamp electrophysiology. EPSC amplitude was reduced following IOP elevation in young and aged mice, however, only recovered in young mice. IPSC amplitude was decreased in young mice following IOP elevation but did not recover resulting in an increased excitation/inhibition ratio. IPSC amplitude was unchanged in aged mice following IOP elevation resulting in a decreased excitation/inhibition ratio. Therefore, we can conclude that a loss of excitatory synapses underlies RGC dysfunction following IOP elevation in young and aged mice and that RGC recovery is driven by an increase in excitatory synaptic activity in young mice, but not aged mice.

Pubmed:
33932867: Tribble JR, Otmani A, Sun S, Ellis SA, Cimaglia G, Vohra R, Jöe M, Lardner E, Venkataraman AP, Domínguez-Vicent A, Kokkali E, Rho S, Jóhannesson G, Burgess RW, Fuerst PG, Brautaset R, Kolko M, Morgan JE, Crowston JG, Votruba M, Williams PA
Nicotinamide provides neuroprotection in glaucoma by protecting against mitochondrial and metabolic dysfunction.
Nicotinamide adenine dinucleotide (NAD) is a REDOX cofactor and metabolite essential for neuronal survival. Glaucoma is a common neurodegenerative disease in which neuronal levels of NAD decline. We assess the effects of nicotinamide (a precursor to NAD) on retinal ganglion cells (the affected neuron in glaucoma) in normal physiological conditions and across a range of glaucoma relevant insults including mitochondrial stress and axon degenerative insults. We demonstrate retinal ganglion cell somal, axonal, and dendritic neuroprotection by nicotinamide in rodent models which represent isolated ocular hypertensive, axon degenerative, and mitochondrial degenerative insults. We performed metabolomics enriched for small molecular weight metabolites for the retina, optic nerve, and superior colliculus which demonstrates that ocular hypertension induces widespread metabolic disruption, including consistent changes to α-ketoglutaric acid, creatine/creatinine, homocysteine, and glycerophosphocholine. This metabolic disruption is prevented by nicotinamide. Nicotinamide provides further neuroprotective effects by increasing oxidative phosphorylation, buffering and preventing metabolic stress, and increasing mitochondrial size and motility whilst simultaneously dampening action potential firing frequency. These data support continued determination of the utility of long-term nicotinamide treatment as a neuroprotective therapy for human glaucoma.
Redox Biol, 2021; 43
32236736: Benetatos J, Bennett RE, Evans HT, Ellis SA, Hyman BT, Bodea LG, Götz J
PTEN activation contributes to neuronal and synaptic engulfment by microglia in tauopathy.
Phosphatase and tensin homolog (PTEN) regulates synaptic density in development; however, whether PTEN also regulates synapse loss in a neurodegenerative disorder such as frontotemporal lobar degeneration with Tau deposition (FTLD-Tau) has not been explored. Here, we found that pathological Tau promotes early activation of PTEN, which precedes apoptotic caspase-3 cleavage in the rTg4510 mouse model of FTLD-Tau. We further demonstrate increased synaptic and neuronal exposure of the apoptotic signal phosphatidylserine that tags neuronal structures for microglial uptake, thereby linking PTEN

activation to synaptic and neuronal structure elimination. By applying pharmacological inhibition of PTEN's protein phosphatase activity, we observed that microglial uptake can be decreased in Tau transgenic mice. Finally, we reveal a dichotomous relationship between PTEN activation and age in FTLD-Tau patients and healthy controls. Together, our findings suggest that in tauopathy, PTEN has a role in the synaptotoxicity of pathological Tau and promotes microglial removal of affected neuronal structures.
Acta Neuropathol, 2020; 140

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-213

IMPAIRED SPATIAL NAVIGATION AND AGE-DEPENDENT HIPPOCAMPAL SYNAPTIC DYSFUNCTION ACCOMPANIED BY A CHRONIC INFLAMMATORY CYTOKINE PROFILE IN DB/DB MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Type 2 diabetes mellitus (T2DM) increases the risk of developing Alzheimer's Disease (AD), and an abnormal central inflammatory response has been proposed to mediate the consequences of T2DM on hippocampal synaptic integrity and cognitive function. However, the exact implications of T2DM on synaptic integrity in aging have not been investigated. Here, we investigated the effects of T2DM on AD-like pathology using the leptin receptor-deficient db/db mouse model of T2DM. We found that T2DM mice were significantly impaired spatial acquisition in the Morris Water Maze (MWM), compared to age-matched controls. Morphological analysis in young T2DM mice showed a significant neuronal loss in the dentate gyrus compared to controls, suggesting impaired dentate gyrus function. We found that GFAP immunoreactivity was significantly decreased in all regions of the hippocampus of young T2DM mice, compared to age-matched controls. We also showed that microglial activation was increased in the CA3 and dentate gyrus regions of the hippocampus in young T2DM mice, compared to controls. Interestingly, the presynaptic marker protein- synaptophysin and the postsynaptic marker protein-PSD95 did not show significant changes in the hippocampus of young T2DM mice. However, aged T2DM mice showed a robust decrease in synaptophysin and PSD95, suggesting impaired hippocampal synaptic integrity. Cytokine profiling of aged T2DM mice showed an increased expression of several pro-inflammatory cytokines in the hippocampus. Our results suggest that T2DM impairs cognitive dysfunction, leads to neuronal loss in the dentate gyrus, and reveals an age-dependent deterioration in hippocampal synaptic integrity in the diabetic state.

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BOARD NUMBER: S07-214

THE ROLE OF EARLY LIFE ADVERSITY IN A MOUSE MODEL FOR ALZHEIMER'S DISEASE PATHOLOGY

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Stress is considered as a major environmental risk factor for Alzheimer's Disease (AD). Previous research shows that exposure to chronic early life adversity (ELA) increases the susceptibility to AD pathology in adult APPswe/PS1dE9 mice. We aimed to assess the effects of ELA that precede the accelerated onset of classical AD-pathology in this model. Wild type and APPswe/PS1dE9 mice were subjected to ELA from postnatal days 2-9 by housing the litters with limited nesting and bedding material. At 3 months old, cognitive flexibility was measured using a multiple-choice reversal learning paradigm. In addition, hippocampal synaptic activity and composition, Aβ-pathology and interneuron populations were assessed. At 3 months old, APPswe/PS1dE9 mice that were exposed to ELA were significantly impaired in reversal learning. Additionally, ELA resulted in persistent altered basal synaptic activity while Aβ-pathology was unaltered. To conclude, ELA increases the susceptibility to AD-related cognitive decline which precedes Aβ-pathology and is paralleled by altered hippocampal synaptic activity. This approach will help to understand the mechanism by which ELA accelerates AD-related pathology in the AD model and may provide insight in the development of AD pathology and the role of stress in the onset of AD.

Pubmed:
34503674: Lesuis SL, Brosens N, Immerzeel N, van der Loo RJ, Mitrić M, Bielefeld P, Fitzsimons CP, Lucassen PJ, Kushner SA, van den Oever MC, Krugers HJ
Glucocorticoids Promote Fear Generalization by Increasing the Size of a Dentate Gyrus Engram Cell Population.
Traumatic experiences, such as conditioned threat, are coded as enduring memories that are frequently subject to generalization, which is characterized by (re-) expression of fear in safe environments. However, the neurobiological mechanisms underlying threat generalization after a traumatic experience and the role of stress hormones in this process remain poorly understood.
Biol Psychiatry, 2021; 90

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BOARD NUMBER: S07-215

DROSOPHILA CIRCUIT MODEL OF ALZHEIMER'S DISEASE REVEALS NEURON TYPE-SPECIFIC FUNCTIONAL CHANGES THAT ARE LINKED TO ALTERED BEHAVIOR

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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The pathogenesis and progression of neurodegenerative diseases are poorly understood at the neural circuit level. How cellular hallmarks affect communication between neurons and translate to behavioral changes remains elusive. In order to bridge the gap between biological mechanisms and behavior during neurodegenerative processes, we built a comprehensive circuit model of Alzheimer's Disease (AD) in *Drosophila* larvae as a proof-of-concept. Previously, we mapped a three-layer mechanosensory circuit with complex mechanisms and showed that synaptic silencing of each neuron had different effects on larval behavior in response to a stimulus. Here, we modeled AD by expressing human Aß1-40 or 1-42 peptides in each neuron within the circuit. We then assayed cellular toxicity in the brain, monitored activity using Ca2+ imaging, and quantified behavior using computer vision-based software. We found that neurons within the circuit were differentially impacted by Aß expression, with some mimicking synaptic silencing. The mechanosensory chordotonal neurons were most susceptible to Aß toxicity, and its impaired activity correlated with decreased behavioral response. Interestingly, we did not observe well-formed Aß plaques at this stage, suggesting that the circuit dysfunction detected could occur prior to substantial protein aggregation. Furthermore, we showed that Ca2+ imaging data can be combined with *Drosophila* EM connectome to build a rate-based ODE model of the circuit to predict behavioral changes. Together, these results show that we can infer complex functional changes on a single-neuron level within an AD-like circuit through high-throughput whole animal behavior screens. The circuit model and methods involved can be useful in large-scale genetic and compound studies to rapidly dissect functional impacts within specific neuron types.

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BOARD NUMBER: S07-216

MULTI-ELECTRODE ARRAYS REVEAL EARLY NETWORK DYSFUNCTION IN PRIMARY HIPPOCAMPAL NEURONAL CULTURES DERIVED FROM THE APPNL-G-F MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Amyloid β (Aβ) pathophysiology is an early and persistent feature of Alzheimer's disease (AD), developing many years before symptomatic onset. Preceding the formation of Amyloid plaques, Aβ has been shown to induce synaptic dysfunction and subsequent hyperexcitability in the hippocampus. The present study aims to elucidate how Aβ may impact hippocampal network development and function *in vitro*. Utilising 60-channel multi-electrode array (MEA) cultures of dissociated hippocampal neurons derived from both WT and App^{NL-G-F} mice — which express humanised Aβ with key familial AD mutations — we recorded the spontaneous activity of hippocampal networks over their development for the first 35 days-*in-vitro* (DIV). Both sets of hippocampal cultures displayed spontaneous and coordinated network activity by DIV7, with functional connectivity increasing progressively until the end of the culture period. However, preliminary firing and bursting analyses have suggested an accelerated maturation in App^{NL-G-F} networks, with developmental trajectories being shifted earlier than those for WT. Additionally, graph theoretical analyses have shown differences in functional network features between the two groups. Furthermore, patch-clamp experiments using the same culture model are being performed to gain mechanistic insight into cellular and synaptic deficits of the disease onset. In conclusion, cultures derived from the App^{NL-G-F} mouse model of AD reveal early deficits in hippocampal networks. These aberrations may predispose these networks to subsequent pathogenic mechanisms and understanding them may allow for targeting of earlier therapeutic intervention.

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BOARD NUMBER: S07-217

RESILIENT GAMMA RHYTHMOGENESIS AND PARVALBUMIN INTERNEURON FUNCTION BEFORE AND AFTER PLAQUE BURDEN IN EX VIVO HIPPOCAMPAL SLICES FROM 5XFAD ALZHEIMER'S DISEASE MODEL MICE.

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aims: Recent studies have implicated impaired parvalbumin fast-spiking interneuron (PVIN) function in underlying deficits in gamma oscillations (rhythmic brain network activity), and cognitive decline in Alzheimer's disease (AD). We aimed to investigate amyloid beta plaque accumulation in relation to kainate- and carbachol-induced gamma oscillations and PVIN function in the *5xFAD* AD mouse model. **Methods**: Hippocampal gamma oscillations were recorded using *ex vivo* brain slices from *5xFAD* and control mice at 3-4 months (minimal plaque presence) and 12+ months (extensive plaque presence). We also performed targeted cell-attached patching of PVINs in WT and *5xFAD* brain slices during gamma oscillations to determine gamma-related PVIN function at different disease time points. Finally, brain slices were processed for immunohistochemical quantification of PVIN-related signals, PV and perineuronal nets (PNNs). **Results**: We found, as previously described, minimal hippocampal plaque presence at 3-4 months and extensive plaque burden by 12 months with significant reductions of PVINs and PNNs in 12m *5xFAD* mice. We show that detection of gamma oscillation impairment is dependent on the pharmacological approach used to initiate oscillations. Kainate-induced gamma oscillations displayed power deficits and impaired rhythmicity in *5xFAD* mice; whereas carbachol-induced oscillations exhibited only minor rhythmicity deficits in 12m *5xFAD* mice. Likewise, PVIN spiking, and gamma phase-locking were unaltered in *5xFAD* mice. **Conclusions**: Despite extensive plaque pathology and loss of PVINs, PVIN function and circuit rhythmogenesis were relatively spared in *5xFAD* mice. This highlights the complicated pathophysiology of the *5xFAD* mouse and extreme resilience of PVIN and network function in the model.

Pubmed:
34788174: Mackenzie-Gray Scott C, Parrish RR, Walsh D, Racca C, Cowell RM, Trevelyan AJ
PV-specific loss of the transcriptional coactivator PGC-1α slows down the evolution of epileptic activity in an acute ictogenic model.
The transcriptional coactivator, PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1α), plays a key role in coordinating energy requirement within cells. Its importance is reflected in the growing number of psychiatric and neurological conditions that have been associated with reduced PGC-1α levels. In cortical networks, PGC-1α is required for the induction of parvalbumin (PV) expression in interneurons, and PGC-1α deficiency affects synchronous GABAergic release. It is unknown, however, how this affects cortical excitability. We show here that knocking down PGC-1α specifically in the PV-expressing cells (PGC-1α) blocks the activity-dependent regulation of the synaptic proteins, SYT2 and CPLX1. More surprisingly, this cell class-specific knockout of PGC-1α appears to have a novel antiepileptic effect, as assayed in brain slices bathed in 0 Mg media. The rate of occurrence of preictal discharges developed approximately equivalently in wild-type and PGC-1α brain slices, but the intensity of these discharges was lower in PGC-1α slices, as evident from the reduced power in the γ range and reduced firing rates in both PV interneurons and pyramidal cells during these discharges. Reflecting this reduced intensity in the preictal discharges, the PGC-1α brain slices experienced many more discharges before transitioning into a seizure-like event. Consequently, there was a large increase in the latency to the first seizure-like event in brain slices lacking PGC-1α in PV interneurons. We conclude that knocking down PGC-1α limits the range of PV interneuron firing and this slows the pathophysiological escalation during ictogenesis. Parvalbumin expressing interneurons are considered to play an important role in regulating cortical activity. We were surprised, therefore, to find that knocking down the transcriptional coactivator, PGC-1α, specifically in this class of interneurons appears to slow ictogenesis. This anti-ictogenic effect is associated with reduced activity in preictal discharges, but with a far longer period of these discharges before the first seizure-like events finally start. Thus, PGC-1α knockdown may promote schizophrenia while reducing epileptic tendencies.

J Neurophysiol, 2022; 127
32053107: Pelkey KA, Calvigioni D, Fang C, Vargish G, Ekins T, Auville K, Wester JC, Lai M, Mackenzie-Gray Scott C, Yuan X, Hunt S, Abebe D, Xu Q, Dimidschstein J, Fishell G, Chittajallu R, McBain CJ
Paradoxical network excitation by glutamate release from VGluT3 GABAergic interneurons.
In violation of Dale's principle several neuronal subtypes utilize more than one classical neurotransmitter. Molecular identification of vesicular glutamate transporter three and cholecystokinin expressing cortical interneurons (CCKVGluT3INTs) has prompted speculation of GABA/glutamate corelease from these cells for almost two decades despite a lack of direct evidence. We unequivocally demonstrate CCKVGluT3INT-mediated GABA/glutamate cotransmission onto principal cells in adult mice using paired recording and optogenetic approaches. Although under normal conditions, GABAergic inhibition dominates CCKVGluT3INT signaling, glutamatergic signaling becomes predominant when glutamate decarboxylase (GAD) function is compromised. CCKVGluT3INTs exhibit surprising anatomical diversity comprising subsets of all known dendrite targeting CCK interneurons in addition to the expected basket cells, and their extensive circuit innervation profoundly dampens circuit excitability under normal conditions. However, in contexts where the glutamatergic phenotype of CCKVGluT3INTs is amplified, they promote paradoxical network hyperexcitability which may be relevant to disorders involving GAD dysfunction such as schizophrenia or vitamin B6 deficiency.
Elife, 2020; 9
30784081: Parrish RR, Codadu NK, Mackenzie-Gray Scott C, Trevelyan AJ
Feedforward inhibition ahead of ictal wavefronts is provided by both parvalbumin- and somatostatin-expressing interneurons.
There is a rapid interneuronal response to focal activity in cortex, which restrains laterally propagating activity, including spreading epileptiform activity. The interneuronal response involves intense activation of both parvalbumin- and somatostatin-expressing interneurons. Interneuronal bursting is time-locked to glutamatergic barrages in the pre-ictal period. Ca imaging using conditional expression of GCaMP6f provides an accurate readout of the evolving firing patterns in both types of interneuron. The activation profiles of the two interneuronal classes are temporally offset, with the parvalbumin population being activated first, and typically, at higher rates.
J Physiol, 2019; 597
26604630: Kalafatakis K, Gkanti V, Mackenzie-Gray Scott CA, Zarros A, Baillie GS, Tsakiris S
Acetylcholinesterase activity as a neurotoxicity marker within the context of experimentally-simulated hyperprolinaemia: An in vitro approach.
Hyperprolinaemia is characterized by increased tissue accumulation of proline (Pro) and is known to exert serious cognitive and/or neuropsychiatric symptomatology as a direct result of Pro accumulation in the brain. The aim of this study was to explore a putative link between experimentally-simulated hyperprolinaemia and the activity of acetylcholinesterase (AChE); a crucial neurotoxicity marker. In vitro experiments were undertaken on purified eel-derived AChE, as well as on adult mouse brain homogenates, in order to examine the effect of a spectrum of Pro concentrations (3, 30, 500, and 1000 μM) on this marker. Our data showed that although Pro exerted a significant inhibitory effect on pure AChE activity, mouse brain-derived membrane-bound AChE activity was found either unaltered or significantly increased following incubation with Pro. The use of AChE activity as a neurotoxicity marker within the context of experimentally-simulated hyperprolinaemia should be considered with caution and in parallel with a number of other experimental parameters.
J Nat Sci Biol Med, 2015; 6
25461294: Zarros A, Johnson SA, Mackenzie-Gray Scott CA, Baillie GS
Cytodynamics and endpoint selection for a reliable in vitro assessment of nanoneurotoxicity.
Nanomedicine, 2015; 11

FENS Forum 2022
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BOARD NUMBER: S07-218

DIETARY IMPACTS ON NEURONAL FUNCTION AND LEARNING CAPACITY IN A DROSOPHILA MODEL OF NEURODEGENERATION.

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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The gut-brain axis (GBA) is defined as a bidirectional channel of communication between the brain and the enteric nervous system linked by neurons of the sympathetic and parasympathetic systems and circulating hormones. The gut microbiota may have a large influence on neurological function with the microbiota playing a major role in the Aβ pathology. We hypothesise that a high-fat diet (HFD) will compromise neuronal function mediated by changes in the gut microbiota of *Drosophila*. We aim at characterising neuronal activity and learning & memory function following changes in the diet. *Drosophila* larvae were raised under standard conditions or supplemented with 10% coconut oil. Glutamatergic synaptic responses were measured at the *Drosophila* larval neuromuscular junction. Motoneurons were stimulated and evoked or spontaneous excitatory postsynaptic currents (e/sEJCs) were recorded in a two-electrode voltage-clamp. Larvae underwent olfactory conditioning with odour-reward training and testing were adapted from Michels et al. (2017). Statistical analyses were performed using Mann-Whitney-U and t-tests. We show that HFD induces a marked reduction in neurotransmission with reduced eEJC amplitudes (Ctrl: -88±6nA; HFD: -60±5nA, $p=0.011$) and frequencies of sEJCs. We found that the HFD diet impairs olfactory learning in larvae with preferences indices (PI) being significantly reduced in the test group (PI Ctrl: 0.225, HFD: 0.100, $p<0.001$). We conclude that the impact of the HFD on neuronal function, as assessed on the level of larval NMJ physiology and learning & memory may act via the GBA. A compromised gut microbiome may thus facilitate neurodegeneration in the peripheral and central nervous systems.

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BOARD NUMBER: S07-219

NICOTINIC RECEPTOR MODULATION OF EARLY ALZHEIMER'S DISEASE IN (MOUSE) HIPPOCAMPUS

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Treatments for Alzheimer's disease (AD) commonly rely on elevating cholinergic function in the brain, however such interventions are symptomatic and do not prevent the disease's progression. β-amyloid, a peptide thought to underlie neural disruption reported in early AD development, can interact with the cholinergic system though nicotinic acetylcholine receptors (nAChRs). Previous studies from our group revealed that a genetic loss of β2 nAChR subunits could protect against memory impairment which is induced by β-amyloid over-expression. The presented work investigates the influence of nAChR on β-amyloid induced synaptic and network dysfunction in the hippocampus, and how such modulation may affect the manifestation of cognitive deficits in early AD pathogenesis. We developed a model of localized β-amyloid over-expression by viral transduction of mouse dentate gyrus (DG). This local model is used to test the impact of genetic and pharmacological inactivation of nAChRs on memory function and neural activity through behavioural analysis, *in vivo* two-photon calcium imaging, and immunofluorescent techniques. An early-onset development of cognitive deficits and DG hyperactivity was observed in the model. Furthermore, whilst pharmacological β2 nAChR inhibition was able to protect against β-amyloid induced impairments, this was not associated with a normalization of the DG network activity. Thus, β2 nAChR inactivity is able to protect against β-amyloid induced cognitive dysfunction in a manner independent to changes in overall activity levels within the DG. Identification of the specific nicotinic-β-amyloid interactions underlying the reported protection will facilitate more targeted cholinergic therapies against early AD and provide mechanistic insights essential for the development of preventative medicine.

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BOARD NUMBER: S07-220

EXCITATORY/INHIBITORY IMBALANCE IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by a long asymptomatic phase during which disturbances in neurotransmission has been described.To explore the pathogenic outcome occurring during the prodromal period, our team use one-month-old APP/PS1-21 mice. This mouse model simulated a key aspect of AD prodromal phase with an overproduction of soluble Aβ oligomers (Aβo), starting at P14 without amyloid plaque formation and cognitive alteration.Using a multimodal approach combining extracellular electrophysiology, patch-clamp recordings on acute hippocampal slices and biochemistry techniques, our study aims at further understanding how Aβo alter synaptic transmission in one-month-old APP/PS1 mice during the prodromal phase. With extracellular field recordings in CA1 hippocampal region, we showed an increase of the basal glutamatergic synaptic transmission.We further showed, using patch clamp recordings on CA1 neurons, that this hyperactivity is associated with a disruption of the inhibitory synaptic transmission. Indeed, a 30% decrease in the frequency and 50% decrease in the amplitude of inhibitory spontaneous currents was observed in APP/PS1-21 acute slices.We then showed that bath application of Aβo (100 nm) induced similar reductions of the inhibitory spontaneous currents in CA1 neurons of acute hippocampal slice of WT mice demonstrating that previous alterations were Aβo dependent.During the prodromal phase of AD, we have shown that Aβo overproduction induced CA1 neurons hyperactivity by reducing the inhibitory synaptic transmission in APP/PS1-21 mice.These early modifications could be at the origin of the long-term alterations observed in AD and could therefore constitute a new therapeutic target.

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BOARD NUMBER: S07-221

PHYSIOLOGICAL ROLE OF THE FULL-LENGTH AMYLOID PRECURSOR PROTEIN (APP) IN PRESYNAPTIC PLASTICITY AND INFORMATION TRANSFER WITHIN HIPPOCAMPAL CA3 CIRCUITS

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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The full-length amyloid precursor protein (APP), a key player in Alzheimer's disease (AD), is ubiquitously expressed throughout the brain. It is known to be abundantly expressed in presynaptic compartments where it interacts with proteins of the presynaptic release machinery. However, the physiological functions of APP in the central nervous system still remain elusive. Here we study the physiological role of APP in presynaptic plasticity and information transfer within the hippocampus. This will help understanding whether the disruption of its functions can contribute to the pathophysiology of AD. We deleted APP and the related protein APLP2 in selected neuronal populations using a viral gene transfer strategy in APP/APLP2 double floxed mice. By combining optogenetics and *ex vivo* electrophysiology, we found that the selective deletion of APP in dentate gyrus granule cells impairs presynaptic short-term plasticity at mossy-fiber to CA3 pyramidal cells synapses, without altering the intrinsic spiking properties of granule cells. Furthermore, selective deletion of APP/APLP2 in GABAergic interneurons within CA3 influences the properties of inhibitory synaptic currents in CA3 pyramidal cells. Altogether, our data supports a role of presynaptic APP in synaptic transmission and plasticity mechanisms at identified excitatory and inhibitory synapses, and thus sheds light on the physiological contribution of full-length APP to hippocampal circuit activity.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-222

ACCUMULATION AND NEURON-TO-GLIA SPREAD OF HUMAN TAU PROTEINS IN AGEING MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aggregation and propagation of misfolded pathological Tau proteins (pTau) correlate with the severity of neurological symptoms of tauopathies, including memory impairment. We investigated the spread of pTau in 2-27-month-old THY-Tau22 transgenic mice and sought to understand its impact on *in vivo* neuronal activity and memory. The distribution of pTau correlated with age and neurodegeneration and was prominent in the hippocampus, subiculum and basolateral amygdala. We discovered that oligodendrocytes accumulated pTau in older mice, which we confirmed with electron microscopy, suggesting pTau may spread from neurons to glia. To understand the effects of pTau on hippocampus-dependent memory, we tested mice in three tasks. While the spontaneous spatial novelty preference task revealed reduced short-term familiarity, the water-escape Y-maze task revealed unimpaired spatial reference memory. In a non-matching-to-place T-maze task, spatial working memory performance was similar in transgenic and non-transgenic littermates. To investigate neuronal and network activity, we performed single-neuron glass-electrode recordings and juxtacellular labelling in awake mice, observing similar activity for both genotypes. We also recorded multiple single-unit activities during 'goal-directed' virtual navigation, showing a loss of higher-firing complex spiking (pyramidal) cells in aged transgenic mice. We conclude that pTau accumulation in THY-Tau22 mice leads to localized disruption of predominantly higher-firing complex spiking cells without affecting network activity, along with specific deficits in the familiarity of spatial cues. The spread of pTau to oligodendrocytes via myelinated axons suggests a potential mechanism for their propagation in human tauopathies such as progressive supranuclear palsy and corticobasal degeneration.

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BOARD NUMBER: S07-223

DIFFERENT MODES OF SYNAPTIC AND EXTRASYNAPTIC NMDA RECEPTOR ALTERATION IN THE HIPPOCAMPUS OF P301S TAU TRANSGENIC MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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[1]Universidad de Castilla La Mancha, Departamento De Ciencias Medicas, Albacete, Spain, [2]Centro de Biologia Molecular Severo Ochoa, Uam, Madrid, Spain, [3]University of fukui, Department Of Brain Structure & Function, Faculty Of Medical Science, Fukui, Japan

N-methyl-D-aspartate receptors (NMDARs) are pivotal players in the synaptic transmission and synaptic plasticity underlying learning and memory. Accordingly, dysfunction of NMDARs has been implicated in the pathophysiology of Alzheimer's disease (AD). Aims: to investigate the expression and subcellular localization of GluN1, the obligatory subunit of NMDARs, in the hippocampus of P301S mice. Methods: We used histoblot and SDS-digested freeze-fracture replica labelling (SDS-FRL) techniques. Results: Histoblots showed that GluN1 expression was significantly reduced in the hippocampus of P301S mice in a laminar-specific manner at 10 months of age but was unaltered at 3 months. By SDS-FRL, excitatory synapses and extrasynaptic sites on pyramidal cells and interneuron were analysed throughout the CA1 field. Density of GluN1 was high at synaptic sites and substantially lower at extrasynaptic sites. Labelling density for GluN1 in excitatory synapses on spines was significantly reduced in P301S mice, compared to age-matched wild type mice, in the three strata. Density for synaptic GluN1 on interneuron dendrites was significantly reduced in P301S mice in the strata oriens and radiatum, but unaltered in the stratum lacunosum-moleculare. Labelling density for GluN1 at extrasynaptic sites showed no significant differences in pyramidal cells, and only increased density in the interneuron dendrites of the stratum radiatum. Conclusions: This differential alteration of synaptic versus extrasynaptic NMDARs supports the notion that the progressive accumulation of phospho-tau is associated with changes in NMDARs, in the absence of Aβ pathology. Grant RTI2018-095812-B-I00 funded by MCIN/AEI/ 10.13039/501100011033 and by "ERDF A way of making Europe", by the "European Union.

Pubmed:
32899153: Alfaro-Ruíz R, Aguado C, Martín-Belmonte A, Moreno-Martínez AE, Luján R
Cellular and Subcellular Localisation of Kv4-Associated KChIP Proteins in the Rat Cerebellum.
The K channel interacting proteins (KChIPs) are a family of cytosolic proteins that interact with Kv4 channels, leading to higher current density, modulation of channel inactivation and faster recovery from inactivation. Using immunohistochemical techniques at the light and electron microscopic level combined with quantitative analysis, we investigated the cellular and subcellular localisation of KChIP3 and KChIP4 to compare their distribution patterns with those for Kv4.2 and Kv4.3 in the cerebellar cortex. Immunohistochemistry at the light microscopic level demonstrated that KChIP3, KChIP4, Kv4.2 and Kv4.3 proteins were widely expressed in the cerebellum, with mostly overlapping patterns. Immunoelectron microscopic techniques showed that KChIP3, KChIP4, Kv4.2 and Kv4.3 shared virtually the same somato-dendritic domains of Purkinje cells and granule cells. Application of quantitative approaches showed that KChIP3 and KChIP4 were mainly membrane-associated, but also present at cytoplasmic sites close to the plasma membrane, in dendritic spines and shafts of Purkinje cells (PCs) and dendrites of granule cells (GCs). Similarly, immunoparticles for Kv4.2 and Kv4.3 were observed along the plasma membrane and at intracellular sites in the same neuron populations. In addition to the preferential postsynaptic distribution, KChIPs and Kv4 were also distributed presynaptically in parallel fibres and mossy fibres. Immunoparticles for KChIP3, KChIP4 and Kv4.3 were detected in parallel fibres, and KChIP3, KChIP4, Kv4.2 and Kv4.3 were found in parallel fibres, indicating that composition of KChIP and Kv4 seems to be input-dependent. Together, our findings unravelled previously uncharacterised KChIP and Kv4 subcellular localisation patterns in neurons, revealed that KChIP have additional Kv4-unrelated functions in the cerebellum and support the formation of macromolecular complexes between KChIP3 and KChIP4 with heterotetrameric Kv4.2/Kv4.3 channels.
Int J Mol Sci, 2020; 21
34681766: Alfaro-Ruiz R, Martín-Belmonte A, Aguado C, Hernández F, Moreno-Martínez AE, Ávila J, Luján R
The Expression and Localisation of G-Protein-Coupled Inwardly Rectifying Potassium (GIRK) Channels Is Differentially

Altered in the Hippocampus of Two Mouse Models of Alzheimer's Disease.
G protein-gated inwardly rectifying K (GIRK) channels are the main targets controlling excitability and synaptic plasticity on hippocampal neurons. Consequently, dysfunction of GIRK-mediated signalling has been implicated in the pathophysiology of Alzheimer´s disease (AD). Here, we provide a quantitative description on the expression and localisation patterns of GIRK2 in two transgenic mice models of AD (P301S and APP/PS1 mice), combining histoblots and immunoelectron microscopic approaches. The histoblot technique revealed differences in the expression of GIRK2 in the two transgenic mice models. The expression of GIRK2 was significantly reduced in the hippocampus of P301S mice in a laminar-specific manner at 10 months of age but was unaltered in APP/PS1 mice at 12 months compared to age-matched wild type mice. Ultrastructural approaches using the pre-embedding immunogold technique, demonstrated that the subcellular localisation of GIRK2 was significantly reduced along the neuronal surface of CA1 pyramidal cells, but increased in its frequency at cytoplasmic sites, in both P301S and APP/PS1 mice. We also found a decrease in plasma membrane GIRK2 channels in axon terminals contacting dendritic spines of CA1 pyramidal cells in P301S and APP/PS1 mice. These data demonstrate for the first time a redistribution of GIRK channels from the plasma membrane to intracellular sites in different compartments of CA1 pyramidal cells. Altogether, the pre- and post-synaptic reduction of GIRK2 channels suggest that GIRK-mediated alteration of the excitability in pyramidal cells could contribute to the cognitive dysfunctions as described in the two AD animal models.
Int J Mol Sci, 2021; 22
30634540: Alfaro-Ruíz R, Aguado C, Martín-Belmonte A, Moreno-Martínez AE, Luján R
Expression, Cellular and Subcellular Localisation of Kv4.2 and Kv4.3 Channels in the Rodent Hippocampus.
The Kv4 family of voltage-gated K^+ channels underlie the fast transient (A-type) outward K^+ current. Although A-type currents are critical to determine somato-dendritic integration in central neurons, relatively little is known about the precise subcellular localisation of the underlying channels in hippocampal circuits. Using histoblot and immunoelectron microscopic techniques, we investigated the expression, regional distribution and subcellular localisation of Kv4.2 and Kv4.3 in the adult brain, as well as the ontogeny of their expression during postnatal development. Histoblot demonstrated that Kv4.2 and Kv4.3 proteins were widely expressed in the brain, with mostly non-overlapping patterns. During development, levels of Kv4.2 and Kv4.3 increased with age but showed marked region- and developmental stage-specific differences. Immunoelectron microscopy showed that labelling for Kv4.2 and Kv4.3 was differentially present in somato-dendritic domains of hippocampal principal cells and interneurons, including the synaptic specialisation. Quantitative analyses indicated that most immunoparticles for Kv4.2 and Kv4.3 were associated with the plasma membrane in dendritic spines and shafts, and that the two channels showed very similar distribution patterns in spines of principal cells and along the surface of granule cells. Our data shed new light on the subcellular localisation of Kv4 channels and provide evidence for their non-uniform distribution over the plasma membrane of hippocampal neurons.
Int J Mol Sci, 2019; 20

FENS Forum 2022
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BOARD NUMBER: S07-224

PERINEURONAL NET FORMATION DURING A TRANSIENT STAY IN AN ENRICHED HOUSING IS NECESSARY FOR COGNITIVE IMPROVEMENTS OF THE TG2576 MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

Guillaume Bouisset[1], Sebastien Lopez[2], Julie Riviere[3], Camille Lejards[3], Laure Verret[4]
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Alzheimer´s disease (AD) is associated with impaired cognitive functions. We recently established a link between impaired function of the inhibitory neurons expressing the parvalbumin protein (PV), and cognitive impairments in AD. PV cells are associated with perineuronal net (PNN), an extracellular matrix appearing at the end of neuronal maturation, the presence of which is reduced in the hippocampus of the Tg2576 mouse (AD), perhaps contributing to memory deficits. Importantly, exposure to enriched environment (EE), which has proven long-lasting beneficial effects on memory in AD subjects, also rescues PV/PNN deficits. Here, we hypothesize that EE-induced cognitive improvements in Tg2576 mice are supported by a remodeling of hippocampal PV/PNN cell network. In this study, we injected chondroitinase-ABC (ChABC), a PNN-degrading enzyme, into the area CA1 of 5-month-old Tg2576 and non-transgenic mice, the day before placing them into EE for 10 days. Doing so, we were aiming at preventing EE-induced PNN formation in the area CA1, specifically. Then, 20 days later, the mice were subjected to the object location test (CA1), and social memory (CA2). This allowed us evaluating the link between absence of PNN and memory performance in a specific task. Our data show that a 10-day stay in EE was sufficient to induce memory improvements of Tg2576 mice. We observed that blocking EE-induced PNN in area CA1 of AD mice prevented improvement of memory dependant of CA1 area. This demonstrates that increased PNN around PV cells is necessary to the long-lasting beneficial effects of EE on cognitive functions of AD mice.

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BOARD NUMBER: S07-225

IMPLICATION OF SFRP1 IN ALTERED SYNAPTIC PLASTICITY ASSOCIATED WITH ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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SFRP1 is an extracellular protein that is normally expressed at low concentrations in the adult brain. We have previously shown that the amount of SFRP1 protein increases in the brains of Alzheimer's Disease patients and of the related APP;PS1 mouse model, contributing to AD pathogenesis by favouring the accumulation of toxic ab peptides. Neutralization of Sfrp1 activity in APP;PS1 mice not only decreases the formation of ab peptides, but also counteracts brain inflammation and maintains synaptic plasticity (Esteve *et al.,* 2019), suggesting that SFRP1 might have pleiotropic effect in the brain. According to this hypothesis, we have found that astrocyte-derived SFRP1 fosters microglia activation in different models of neuroinflammation (Rueda-Carrasco *et al*., 2021). Whether SFRP1 directly influences synaptic plasticity, or its effect is secondary to the existing neuropathological feed-forward mechanism remains however undetermined. To address this question, we generated a transgenic mouse model overexpressing *Sfrp1* in astrocytes (GFAP-tTA;TRE-Sfrp1). We will show that adult GFAP-tTA;TRE-Sfrp1 mice present an allele-dependent increase in Sfrp1 expression, which is associated with a decrease in dendritic and spine density already at two months of age, as determined by confocal and EM analysis. These defects are followed by the appearance of cognitive decline and long-term potentiation (LTP) impairment. Our data indicates that Sfrp1 has a direct impact on synaptic plasticity that, together with its reported effect on APP processing or microglial activation, support the contention that SFRP1 might have a pleiotropic effect in neurodegenerative diseases.

Pubmed:
34569685: Rueda-Carrasco J, Martin-Bermejo MJ, Pereyra G, Mateo MI, Borroto A, Brosseron F, Kummer MP, Schwartz S, López-Atalaya JP, Alarcon B, Esteve P, Heneka MT, Bovolenta P
SFRP1 modulates astrocyte-to-microglia crosstalk in acute and chronic neuroinflammation.
Neuroinflammation is a common feature of many neurodegenerative diseases. It fosters a dysfunctional neuron-microglia-astrocyte crosstalk that, in turn, maintains microglial cells in a perniciously reactive state that often enhances neuronal damage. The molecular components that mediate this critical communication are not fully explored. Here, we show that secreted frizzled-related protein 1 (SFRP1), a multifunctional regulator of cell-to-cell communication, is part of the cellular crosstalk underlying neuroinflammation. In mouse models of acute and chronic neuroinflammation, SFRP1, largely astrocyte-derived, promotes and sustains microglial activation, and thus a chronic inflammatory state. SFRP1 promotes the upregulation of components of the hypoxia-induced factor-dependent inflammatory pathway and, to a lower extent, of those downstream of the nuclear factor-kappa B. We thus propose that SFRP1 acts as an astrocyte-to-microglia amplifier of neuroinflammation, representing a potential valuable therapeutic target for counteracting the harmful effect of chronic inflammation in several neurodegenerative diseases.
EMBO Rep, 2021; 22
32198470: Cisneros E, di Marco F, Rueda-Carrasco J, Lillo C, Pereyra G, Martín-Bermejo MJ, Vargas A, Sanchez R, Sandonís Á, Esteve P, Bovolenta P
Sfrp1 deficiency makes retinal photoreceptors prone to degeneration.
Millions of individuals worldwide suffer from impaired vision, a condition with multiple origins that often impinge upon the light sensing cells of the retina, the photoreceptors, affecting their integrity. The molecular components contributing to this integrity are however not yet fully understood. Here we have asked whether Secreted Frizzled Related Protein 1 (SFRP1) may be one of such factors. SFRP1 has a context-dependent function as modulator of Wnt signalling or of the proteolytic activity of A Disintegrin And Metalloproteases (ADAM) 10, a main regulator of neural cell-cell communication. We report that in Sfrp1 mice, the outer limiting membrane (OLM) is discontinuous and the photoreceptors disorganized and more prone to light-

induced damage. Sfrp1 loss significantly enhances the effect of the Rpe65 genetic variant -present in the mouse genetic background- which confers sensitivity to light-induced stress. These alterations worsen with age, affect visual function and are associated to an increased proteolysis of Protocadherin 21 (PCDH21), localized at the photoreceptor outer segment, and N-cadherin, an OLM component. We thus propose that SFRP1 contributes to photoreceptor fitness with a mechanism that involves the maintenance of OLM integrity. These conclusions are discussed in view of the broader implication of SFRP1 in neurodegeneration and aging.
Sci Rep, 2020; 10

31308530: Esteve P, Rueda-Carrasco J, Inés Mateo M, Martin-Bermejo MJ, Draffin J, Pereyra G, Sandonís Á, Crespo I, Moreno I, Aso E, Garcia-Esparcia P, Gomez-Tortosa E, Rábano A, Fortea J, Alcolea D, Lleo A, Heneka MT, Valpuesta JM, Esteban JA, Ferrer I, Domínguez M, Bovolenta P
Elevated levels of Secreted-Frizzled-Related-Protein 1 contribute to Alzheimer's disease pathogenesis.
The deposition of aggregated amyloid-β peptides derived from the pro-amyloidogenic processing of the amyloid precurson protein (APP) into characteristic amyloid plaques (APs) is distinctive to Alzheimer's disease (AD). Alternative APP processing via the metalloprotease ADAM10 prevents amyloid-β formation. We tested whether downregulation of ADAM10 activity by its secreted endogenous inhibitor secreted-frizzled-related protein 1 (SFRP1) is a common trait of sporadic AD. We demonstrate that SFRP1 is significantly increased in the brain and cerebrospinal fluid of patients with AD, accumulates in APs and binds to amyloid-β, hindering amyloid-β protofibril formation. Sfrp1 overexpression in an AD-like mouse model anticipates the appearance of APs and dystrophic neurites, whereas its genetic inactivation or the infusion of α-SFRP1-neutralizing antibodies favors non-amyloidogenic APP processing. Decreased Sfrp1 function lowers AP accumulation, improves AD-related histopathological traits and prevents long-term potentiation loss and cognitive deficits. Our study unveils SFRP1 as a crucial player in AD pathogenesis and a promising AD therapeutic target.
Nat Neurosci, 2019; 22

31535451: Mitroi DN, Pereyra-Gómez G, Soto-Huelin B, Senovilla F, Kobayashi T, Esteban JA, Ledesma MD
NPC1 enables cholesterol mobilization during long-term potentiation that can be restored in Niemann-Pick disease type C by CYP46A1 activation.
NPC is a neurodegenerative disorder characterized by cholesterol accumulation in endolysosomal compartments. It is caused by mutations in the gene encoding NPC1, an endolysosomal protein mediating intracellular cholesterol trafficking. Cognitive and psychiatric alterations are hallmarks in NPC patients pointing to synaptic defects. However, the role of NPC1 in synapses has not been explored. We show that NPC1 is present in the postsynaptic compartment and is locally translated during LTP. A mutation in a region of the NPC1 gene commonly altered in NPC patients reduces NPC1 levels at synapses due to enhanced NPC1 protein degradation. This leads to shorter postsynaptic densities, increased synaptic cholesterol and impaired LTP in NPC1 mice with cognitive deficits. NPC1 mediates cholesterol mobilization and enables surface delivery of CYP46A1 and GluA1 receptors necessary for LTP, which is defective in NPC1 mice. Pharmacological activation of CYP46A1 normalizes synaptic levels of cholesterol, LTP and cognitive abilities, and extends life span of NPC1 mice. Our results unveil NPC1 as a regulator of cholesterol dynamics in synapses contributing to synaptic plasticity, and provide a potential therapeutic strategy for NPC patients.
EMBO Rep, 2019; 20

FENS Forum 2022
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BOARD NUMBER: S07-226

TRACKING THE SYNAPTIC ALTERATIONS IN A KNOCK-IN MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aims: Our main goal is to investigate the synaptic alterations that lead to brain oscillatory activity impairment in Alzheimer's disease (AD). To do so, we use a knock-in mouse model of AD (*App^{NL-G-F}*) and evaluate gamma oscillations in vitro. Using this model, we have demonstrated that the hippocampal oscillatory activity in the gamma band is strongly impaired before the onset of amyloid-beta (Aβ) plaques. However, the exact breaking point during the amyloidogenic progression in the *App^{NL-G-F}* model is still unknown. Here, we aim to track the breaking point of the hippocampal gamma oscillations impairment in the *App^{NL-G-F}* model. Moreover, we aim to compare hippocampal oscillatory activity in *App^{NL-G-F}* males and females. Methods: We use *in vitro* electrophysiological techniques to analyse the circuitry involving gamma oscillations, inhibitory interneurons, and excitatory pyramidal cells in the hippocampal network. We use *App^{NL-G-F}* males and females to evaluate the oscillatory activity every five days from 1 to 2 months of age. Results: *In vitro*-gamma oscillations degrade progressively and become significantly impaired at postnatal day (PD) 50. Spike-gamma coupling from inhibitory interneurons is impaired after PD 50. Moreover, our results are not sex-dependent since we found the same significant impairment in males and females of the *App^{NL-G-F}* model. Conclusions: Our results demonstrate that the amyloidogenic progression in the *App^{NL-G-F}* model causes a synaptic collapse that affects the cognitive-relevant hippocampal gamma oscillations before the onset of Aβ plaques. These results provide a time window to explore the pathological synaptic modifications in the hippocampal network.

Pubmed:
34954329: Balleza-Tapia H, Arroyo-García LE, Isla AG, Loera-Valencia R, Fisahn A
Functionally-distinct pyramidal cell subpopulations during gamma oscillations in mouse hippocampal area CA3.
Gamma oscillations (γ-oscillations) in hippocampal area CA3 are essential for memory function. Particularly, CA3 is involved in the memory related process pattern completion, which is linked with the γ-oscillations in human hippocampus. Recent studies suggest that heterogeneity in the functional properties of pyramidal cells (PCs) in CA3 plays an important role in hippocampal function. By performing concomitant recordings of PC activity and network γ-oscillations in CA3 we found three functionally-different PC subpopulations. PCs with high spike-frequency adaptation (hA) have the strongest action potential gamma phase-coupling, PCs with low adaptation (lA) show lower phase-coupling and PCs displaying a burst-firing pattern (B) remained quiescent. In addition, we discovered that hA display the highest excitatory/inhibitory drive, followed by lA, and lastly B. In conclusion, our data advance the hypothesis that PCs in CA3 are organized into subpopulations with distinct functional roles for cognition-relevant network dynamics and provide new insights in the physiology of hippocampus.
Prog Neurobiol, 2022; 210
34385602: Arroyo-García LE, Isla AG, Andrade-Talavera Y, Balleza-Tapia H, Loera-Valencia R, Alvarez-Jimenez L, Pizzirusso G, Tambaro S, Nilsson P, Fisahn A
Impaired spike-gamma coupling of area CA3 fast-spiking interneurons as the earliest functional impairment in the App mouse model of Alzheimer's disease.
In Alzheimer's disease (AD) the accumulation of amyloid-β (Aβ) correlates with degradation of cognition-relevant gamma oscillations. The gamma rhythm relies on proper neuronal spike-gamma coupling, specifically of fast-spiking interneurons (FSN). Here we tested the hypothesis that decrease in gamma power and FSN synchrony precede amyloid plaque deposition and cognitive impairment in App knock-in mice (App). The aim of the study was to evaluate the amyloidogenic pathology progression in the novel App mouse model using in vitro electrophysiological network analysis. Using patch clamp of FSNs and pyramidal cells (PCs) with simultaneous gamma oscillation recordings, we compared the activity of the hippocampal network of wild-type mice (WT) and the App mice at four disease stages (1, 2, 4, and 6 months of age). We found a severe degradation of gamma oscillation power that is independent of, and precedes Aβ plaque formation, and the cognitive impairment reported previously in this animal model. The degradation correlates with increased Aβ concentration in the brain. Analysis on the cellular level showed an impaired spike-gamma coupling of FSN from 2 months of age that correlates with the

degradation of gamma oscillations. From 6 months of age PC firing becomes desynchronized also, correlating with reports in the literature of robust Aβ plaque pathology and cognitive impairment in the App mice. This study provides evidence that impaired FSN spike-gamma coupling is one of the earliest functional impairment caused by the amyloidogenic pathology progression likely is the main cause for the degradation of gamma oscillations and consequent cognitive impairment. Our data suggests that therapeutic approaches should be aimed at restoring normal FSN spike-gamma coupling and not just removal of Aβ.
Mol Psychiatry, 2021; 26
32873805: Falcón-Moya R, Pérez-Rodríguez M, Prius-Mengual J, Andrade-Talavera Y, Arroyo-García LE, Pérez-Artés R, Mateos-Aparicio P, Guerra-Gomes S, Oliveira JF, Flores G, Rodríguez-Moreno A
Astrocyte-mediated switch in spike timing-dependent plasticity during hippocampal development.
Presynaptic spike timing-dependent long-term depression (t-LTD) at hippocampal CA3-CA1 synapses is evident until the 3 postnatal week in mice, disappearing during the 4 week. At more mature stages, we found that the protocol that induced t-LTD induced t-LTP. We characterized this form of t-LTP and the mechanisms involved in its induction, as well as that driving this switch from t-LTD to t-LTP. We found that this t-LTP is expressed presynaptically at CA3-CA1 synapses, as witnessed by coefficient of variation, number of failures, paired-pulse ratio and miniature responses analysis. Additionally, this form of presynaptic t-LTP does not require NMDARs but the activation of mGluRs and the entry of Ca into the postsynaptic neuron through L-type voltage-dependent Ca channels and the release of Ca from intracellular stores. Nitric oxide is also required as a messenger from the postsynaptic neuron. Crucially, the release of adenosine and glutamate by astrocytes is required for t-LTP induction and for the switch from t-LTD to t-LTP. Thus, we have discovered a developmental switch of synaptic transmission from t-LTD to t-LTP at hippocampal CA3-CA1 synapses in which astrocytes play a central role and revealed a form of presynaptic LTP and the rules for its induction.
Nat Commun, 2020; 11
32638407: Andrade-Talavera Y, Arroyo-García LE, Chen G, Johansson J, Fisahn A
Modulation of Kv3.1/Kv3.2 promotes gamma oscillations by rescuing Aβ-induced desynchronization of fast-spiking interneuron firing in an AD mouse model in vitro.
Gamma oscillations (30-80 Hz) are important for cognitive functions and depend on the synchronized activity of fast-spiking interneurons (FSN), which is crucial for network stability. Gamma oscillations are degraded in Alzheimer's disease (AD) patients exhibiting cognitive impairment, with the degree of cognitive decline correlating with the severity of gamma disruption in response to neurotoxic amyloid-beta peptide (Aβ). Small molecule compounds EX15 and RE01 modulate Kv3.1/Kv3.2 potassium channels on FSN, resulting in faster activation kinetics and increased firing frequency, suggesting direct consequences for cognition-relevant gamma oscillations, particularly in a situation where network activity is pathologically compromised in the presence of neurotoxic Aβ. Using electrophysiological techniques in an in vitro AD model, we found a significant effect of EX15 and RE01 with respect to counteracting toxic Aβ effects on neuronal dynamics, advocating for targeting FSN activity to rescue cognitive performance from impairment caused by neurodegenerative triggers.
J Physiol, 2020; 598
32555421: Arroyo-García LE, Tendilla-Beltrán H, Vázquez-Roque RA, Jurado-Tapia EE, Díaz A, Aguilar-Alonso P, Brambila E, Monjaraz E, De La Cruz F, Rodríguez-Moreno A, Flores G
Amphetamine sensitization alters hippocampal neuronal morphology and memory and learning behaviors.
It is known that continuous abuse of amphetamine (AMPH) results in alterations in neuronal structure and cognitive behaviors related to the reward system. However, the impact of AMPH abuse on the hippocampus remains unknown. The aim of this study was to determine the damage caused by AMPH in the hippocampus in an addiction model. We reproduced the AMPH sensitization model proposed by Robinson et al. in 1997 and performed the novel object recognition test (NORt) to evaluate learning and memory behaviors. After the NORt, we performed Golgi-Cox staining, a stereological cell count, immunohistochemistry to determine the presence of GFAP, CASP3, and MT-III, and evaluated oxidative stress in the hippocampus. We found that AMPH treatment generates impairment in short- and long-term memories and a decrease in neuronal density in the CA1 region of the hippocampus. The morphological test showed an increase in the total dendritic length, but a decrease in the number of mature spines in the CA1 region. GFAP labeling increased in the CA1 region and MT-III increased in the CA1 and CA3 regions. Finally, we found a decrease in Zn concentration in the hippocampus after AMPH treatment. An increase in the dopaminergic tone caused by AMPH sensitization generates oxidative stress, neuronal death, and morphological changes in the hippocampus that affect cognitive behaviors like short- and long-term memories.
Mol Psychiatry, 2021; 26
30323122: Arroyo-Garcia LE, Rodríguez-Moreno A, Flores G
Apomorphine effects on the hippocampus.
Apomorphine is a non-specific dopamine receptor agonist that has been used in the treatment of some diseases and mental disorders. Its use has particularly well documented in Parkinson's disease (PD). The dopaminergic agonists like apomorphine

are related to oxidative processes that could induce cell damage and the functional impairment of some structures in the brain. However, most information about apomorphine in literature is focused on the improvement of the motor problems characteristic of PD, but little is known about the effects on cognitive behaviors and brain structures indirectly related to motor function. The presence of dopaminergic receptors in the hippocampus has recently been discovered, in connection with cognitive behaviors like learning and memory, these receptors are needed in neuronal plasticity. There has been a growing interest to know if this structure could be compromised by the effect of apomorphine and elucidate if part of the cognitive impairment present in the PD is due to the effect of apomorphine. In this mini-review, we summarized how apomorphine has been used since its creation, we discuss the latest information about its effect on the hippocampus and also the future perspectives to fully understand the effects of this compound.
Neural Regen Res, 2018; 13
30169759: Pérez-Rodríguez M, Arroyo-García LE, Prius-Mengual J, Andrade-Talavera Y, Armengol JA, Pérez-Villegas EM, Duque-Feria P, Flores G, Rodríguez-Moreno A
Adenosine Receptor-Mediated Developmental Loss of Spike Timing-Dependent Depression in the Hippocampus.
Critical periods of synaptic plasticity facilitate the reordering and refining of neural connections during development, allowing the definitive synaptic circuits responsible for correct adult physiology to be established. Presynaptic spike timing-dependent long-term depression (t-LTD) exists in the hippocampus, which depends on the activation of NMDARs and that probably fulfills a role in synaptic refinement. This t-LTD is present until the third postnatal week in mice, disappearing in the fourth week of postnatal development. We were interested in the mechanisms underlying this maturation related loss of t-LTD and we found that at CA3-CA1 synapses, presynaptic NMDA receptors (pre-NMDARs) are tonically active between P13 and P21, mediating an increase in glutamate release during this critical period of plasticity. Conversely, at the end of this critical period (P22-P30) and coinciding with the loss of t-LTD, these pre-NMDARs are no longer tonically active. Using immunogold electron microscopy, we demonstrated the existence of pre-NMDARs at Schaffer collateral synaptic boutons, where a decrease in the number of pre-NMDARs during development coincides with the loss of both tonic pre-NMDAR activation and t-LTD. Interestingly, this t-LTD can be completely recovered by antagonizing adenosine type 1 receptors (A1R), which also recovers the tonic activation of pre-NMDARs at P22-P30. By contrast, the induction of t-LTD was prevented at P13-P21 by an agonist of A1R, as was tonic pre-NMDAR activation. Furthermore, we found that the adenosine that mediated the loss of t-LTD during the fourth week of development is supplied by astrocytes. These results provide direct evidence for the mechanism that closes the window of plasticity associated with t-LTD, revealing novel events probably involved in synaptic remodeling during development.
Cereb Cortex, 2019; 29
29582396: Arroyo-García LE, Vázquez-Roque RA, Díaz A, Treviño S, De La Cruz F, Flores G, Rodríguez-Moreno A
The Effects of Non-selective Dopamine Receptor Activation by Apomorphine in the Mouse Hippocampus.
Apomorphine is a dopamine receptor agonist that activates D-D dopamine receptors and that is used to treat Parkinson's disease (PD). However, the effect of apomorphine on non-motor activity has been poorly studied, and likewise, the effects of dopaminergic activation in brain areas that do not fulfill motor functions are unclear. The aim of this study was to determine how dopamine receptor activation affects behavior, as well as plasticity, morphology, and oxidative stress in the hippocampus. Adult mice were chronically administered apomorphine (1 mg/kg for 15 days), and the effects on memory and learning, synaptic plasticity, dendritic length, inflammatory responses, and oxidative stress were evaluated. Apomorphine impaired learning and long-term memory in mice, as evaluated in the Morris water maze test. In addition, electrophysiological recording of field excitatory postsynaptic potentials (fEPSP) indicated that the long-term potentiation (LTP) of synaptic transmission in the CA1 region of the hippocampus was fully impaired by apomorphine. In addition, a Sholl analysis of Golgi-Cox stained neurons showed that apomorphine reduced the total length of dendrites in the CA1 region of the hippocampus. Finally, there were more reactive astrocytes and oxidative stress biomarkers in mice administered apomorphine, as measured by GFAP immunohistochemistry and markers of redox balance, respectively. Hence, the non-selective activation of dopaminergic receptors in the hippocampus by apomorphine triggers deficiencies in learning and memory, it prevents LTP, reduces dendritic length, and provokes neuronal damage.
Mol Neurobiol, 2018; 55
27208629: Tendilla-Beltrán H, Arroyo-García LE, Diaz A, Camacho-Abrego I, de la Cruz F, Rodríguez-Moreno A, Flores G
The effects of amphetamine exposure on juvenile rats on the neuronal morphology of the limbic system at prepubertal, pubertal and postpubertal ages.
Amphetamines (AMPH) are psychostimulants widely used for therapy as well as for recreational purposes. Previous results of our group showed that AMPH exposure in pregnant rats induces physiological and behavioral changes in the offspring at prepubertal and postpubertal ages. In addition, several reports have shown that AMPH are capable of modifying the morphology of neurons in some regions of the limbic system. These modifications can cause some psychiatric conditions. However, it is still unclear if there are changes to behavioral and morphological levels when low doses of AMPH are

administered at a juvenile age. The aim of this study was to assess the effect of AMPH administration (1mg/kg) in Sprague-Dawley rats (postnatal day, PD21-PD35) on locomotor activity in a novel environment and compare the neuronal morphology of limbic system areas at three different ages: prepubertal (PD 36), pubertal (PD50) and postpubertal (PD 62). We found that AMPH altered locomotor activity in the prepubertal group, but did not have an effect on the other two age groups. The Golgi-Cox staining method was used to describe the neural morphology of five limbic regions: (Layers 3 and 5) the medial prefrontal cortex (mPFC), the dorsal and ventral hippocampus, the nucleus accumbens and the amygdala, showing that AMPH induced changes at pubertal ages in arborization and spine density of these neurons, but interestingly these changes did not persist at postpubertal ages. Our findings suggest that even early-life AMPH exposure does not induce long-term behavioral and morphological changes, however it causes alterations at pubertal ages in the limbic system networks, a stage of life strongly associated with the development of substance abuse behaviors.
J Chem Neuroanat, 2016; 77

22826038: Alcántara-González F, Mendoza-Perez CR, Zaragoza N, Juarez I, Arroyo-García LE, Gamboa C, De La Cruz F, Zamudio S, Garcia-Dolores F, Flores G

Combined administration of cerebrolysin and donepezil induces plastic changes in prefrontal cortex in aged mice. Cerebrolysin (Cbl) shows neurotrophic and neuroprotective properties while donepezil (Dnp) is a potent acetylcholinesterase (AChE) inhibitor, both drugs are prescribed for Alzheimer's disease (AD) treatment. Previous studies have shown that the Dnp and Cbl administered separately, modify dendritic morphology of neurons in the prefrontal cortex and hippocampus in senile rodents. Since the deficit of neurotrophic factor activity is implicated in the degeneration of cholinergic neurons of basal forebrain, a combination therapy of Dnp and Cbl has been tested recently in Alzheimer's patients. However, the plastic changes that may underlie this combined treatment have not yet been explored. We present here the effect of the combined administration of Cbl and Dnp on dendritic morphology in brain regions related to learning and memory in aged mice. The Golgi-Cox staining protocol and Sholl analysis were used for studying dendritic changes. Cbl and Dnp were administrated daily for 2 months to 9-months-old mice. Locomotor activity was assessed, as well as the dendritic morphology of neurons in several limbic regions was analyzed. Results showed that Cbl and Dnp induced an increase in locomotor activity without synergistic effect. The Cbl or Dnp treatment modified the dendritic morphology of neurons from prefrontal cortex (PFC), dorsal hippocampus (DH), dentate gyrus (DG), and the shell of nucleus accumbens (NAcc). These changes show an increase in the total dendritic length and spine density, resulting in an improvement of dendritic arborization. Prominently, a synergistic effect of Cbl and Dnp was observed on branching order and total dendritic length of pyramidal neurons from PFC. These results suggest that Dnp and Cbl may induce plastic changes in a manner independent of each other, but could enhance their effect in target cells from PFC.
Synapse, 2012; 66

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BOARD NUMBER: S07-227

DECIPHERING THE ROLE OF LOCUS COERULEUS FOR HIPPOCAMPUS-DEPENDENT LEARNING AND ITS IMPAIRMENT IN A MOUSE MODEL OF ALZHEIMER´S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Locus Coeruleus (LC) is a neuromodulatory system that is heavily and early affected during Alzheimer´s Disease (AD). The key goals of this project are to examine the influence of the LC on hippocampus (HPC)-dependent mnemonic processes and to study how such mnemonic processes are impaired in AD. First, we explored whether the structural and functional connectivity between the LC and the HPC is altered in a mouse model of AD-like pathology. A structural analysis of LC-HPC projections was performed by establishing an AAV-mediated neural tracing protocol. For the functional analysis of these projections, we will use *in vivo* two-photon Ca^{2+} imaging. Specifically, we will record axonal projections in hippocampal CA1 that originate from the LC while the mouse is moving on a treadmill performing tasks designed to test spatial and working memory. We will use transgenic mouse models that exhibit hallmarks of AD-pathology, namely APPswe/PSEN1dE9 (Plaque-pathology) and P301S mice (Tau-pathology). First results indicate that employing the AAV-mediated neural tracing protocol allows specific tracing of axonal connections between the LC and CA1. Furthermore, we found axonal dystrophies of LC-originating neurites in the HPC of APPswe/PSEN1dE9 mice. Our results suggest that impaired mnemonic processing in AD might underlie defective LC-HPC connectivity. We expect to gain further insights about potential morphological and functional impairments of LC-HPC projections in mouse models of AD-like pathology to then pinpoint the functional significance of LC-HPC connectivity for hippocampus-dependent learning and memory.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-228

CHLORIDE DYSREGULATION UNDERLIES COGNITIVE DEFICITS ASSOCIATED WITH ALZHEIMER'S DISEASE-RELATED MUTATIONS IN MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Network dysfunction and hyperexcitability due to disruption of $GABA_A$-mediated transmission during early stages of Alzheimer's disease (AD) are postulated to underlie AD-associated cognitive deficits. While disrupted $GABA_A$ signaling may result from several mechanisms, recent evidence points to deficits in the potassium-chloride cotransporter KCC2, responsible for maintaining low intracellular chloride in neurons to maintain robust inhibition. Thus, we tested whether impaired KCC2 function and disrupted chloride transport underlie these cognitive deficits. In both the multi-mutation transgenic 5xFAD mice and the amyloid precursor protein knock-in APP^{NL-G-F} mice we found a decrease in membrane protein levels of KCC2 in layer II/III of the prefrontal cortex and the CA1 region of the hippocampus as compared to their non-transgenic (NonTg) age-matched littermates. In addition, *ex vivo* chloride imaging revealed weakened Cl^- transport in the 5xFAD mice while *in vivo* chloride imaging revealed that the distribution of $[Cl^-]i$ in pyramidal neurons from 5xFAD mice was significantly lower than in NonTg mice. Finally, short-term administration of CLP290, a KCC2 enhancer developed by our laboratory, in the 5xFAD mice improved spatial memory retention in the Morris Water Task (MWT) and social interaction deficits as compared to vehicle-treated mice, while long-term treatment with CLP290 augmented learning performance in the MWT. All together, these results indicate that KCC2 may be a viable target for reversing deficits in $GABA_A$-mediated inhibition in AD and attenuating cognitive deficits in mice carrying AD-linked mutations.

Pubmed:
34504415: Bilodeau G, Gagnon-Turcotte G, Gagnon LL, Keramidis I, Timofeev I, De Koninck Y, Ethier C, Gosselin B
A Wireless Electro-Optic Platform for Multimodal Electrophysiology and Optogenetics in Freely Moving Rodents.
This paper presents the design and the utilization of a wireless electro-optic platform to perform simultaneous multimodal electrophysiological recordings and optogenetic stimulation in freely moving rodents. The developed system can capture neural action potentials (AP), local field potentials (LFP) and electromyography (EMG) signals with up to 32 channels in parallel while providing four optical stimulation channels. The platform is using commercial off-the-shelf components (COTS) and a low-power digital field-programmable gate array (FPGA), to perform digital signal processing to digitally separate in real time the AP, LFP and EMG while performing signal detection and compression for mitigating wireless bandwidth and power consumption limitations. The different signal modalities collected on the 32 channels are time-multiplexed into a single data stream to decrease power consumption and optimize resource utilization. The data reduction strategy is based on signal processing and real-time data compression. Digital filtering, signal detection, and wavelet data compression are used inside the platform to separate the different electrophysiological signal modalities, namely the local field potentials (1-500 Hz), EMG (30-500 Hz), and the action potentials (300-5,000 Hz) and perform data reduction before transmitting the data. The platform achieves a measured data reduction ratio of 7.77 (for a firing rate of 50 AP/second) and weights 4.7 g with a 100-mAh battery, an on/off switch and a protective plastic enclosure. To validate the performance of the platform, we measured distinct electrophysiology signals and performed optogenetics stimulation in freely moving rondents. We recorded AP and LFP signals with the platform using a 16-microelectrode array implanted in the primary motor cortex of a Long Evans rat, both in anesthetized and freely moving conditions. EMG responses to optogenetic Channelrhodopsin-2 induced activation of motor cortex optical fiber were also recorded in freely moving rodents.
Front Neurosci, 2021; 15
33192295: Keramidis I, Vourkou E, Papanikolopoulou K, Skoulakis EMC
Functional Interactions of Tau Phosphorylation Sites That Mediate Toxicity and Deficient Learning in .
Hyperphosphorylated Tau protein is the main component of the neurofibrillary tangles, characterizing degenerating neurons

in Alzheimer's disease and other Tauopathies. Expression of human Tau protein in Drosophila CNS results in increased toxicity, premature mortality and learning and memory deficits. Herein we use novel transgenic lines to investigate the contribution of specific phosphorylation sites previously implicated in Tau toxicity. These three different sites, Ser, Thr, and Ser were tested either by blocking their phosphorylation, by Ser/Thr to Ala substitution, or pseudophosphorylation, by changing Ser/Thr to Glu. We validate the hypothesis that phosphorylation at Ser is necessary for Tau-dependent learning deficits and a "facilitatory gatekeeper" to Ser occupation, which is linked to Tau toxicity. Importantly we reveal that phosphorylation at Thr acts as a "suppressive gatekeeper", preventing phosphorylation of many sites including Ser and consequently of Ser. Therefore, we elucidate novel interactions among phosphosites central to Tau mediated neuronal dysfunction and toxicity, likely driven by phosphorylation-dependent conformational plasticity.
Front Mol Neurosci, 2020; 13

32203578: Khademullah CS, Aqrabawi AJ, Place KM, Dargaei Z, Liang X, Pressey JC, Bedard S, Yang JW, Garand D, Keramidis I, Gasecka A, Côté D, De Koninck Y, Keith J, Zinman L, Robertson J, Kim JC, Woodin MA
Cortical interneuron-mediated inhibition delays the onset of amyotrophic lateral sclerosis.
Amyotrophic lateral sclerosis is a fatal disease resulting from motor neuron degeneration in the cortex and spinal cord. Cortical hyperexcitability is a hallmark feature of amyotrophic lateral sclerosis and is accompanied by decreased intracortical inhibition. Using electrophysiological patch-clamp recordings, we revealed parvalbumin interneurons to be hypoactive in the late pre-symptomatic SOD1*G93A mouse model of amyotrophic lateral sclerosis. We discovered that using adeno-associated virus-mediated delivery of chemogenetic technology targeted to increase the activity of the interneurons within layer 5 of the primary motor cortex, we were able to rescue intracortical inhibition and reduce pyramidal neuron hyperexcitability. Increasing the activity of interneurons in the layer 5 of the primary motor cortex was effective in delaying the onset of amyotrophic lateral sclerosis-associated motor deficits, slowing symptom progression, preserving neuronal populations, and increasing the lifespan of SOD1*G93A mice. Taken together, this study provides novel insights into the pathogenesis and treatment of amyotrophic lateral sclerosis.
Brain, 2020; 143

31352352: Gagnon-Turcotte G, Keramidis I, Ethier C, De Koninck Y, Gosselin B
A Wireless Electro-Optic Headstage With a 0.13- µm CMOS Custom Integrated DWT Neural Signal Decoder for Closed-Loop Optogenetics.
We present a wireless electro-optic headstage that uses a 0.13- µm CMOS custom integrated circuit (IC) implementing a digital neural decoder (ND-IC) for enabling real-time closed-loop (CL) optogenetics. The ND-IC processes the neural activity data using three digital cores: 1) the detector core detects and extracts the action potential (AP) of individual neurons by using an adaptive threshold; 2) the data compression core compresses the detected AP by using an efficient Symmlet-2 discrete wavelet transform (DWT) processor for decreasing the amount of data to be transmitted by the low-power wireless link; and 3) the classification core sorts the compressed AP into separated clusters on the fly according to their wave shapes. The ND-IC encompasses several innovations: 1) the compression core decreases the complexity from O(n) to O(n · log(n)) compared to the previous solutions, while using two times less memory, thanks to the use of a new coefficient sorting tree; and 2) the AP classification core reuses both the compressed DWT coefficients to perform implicit dimensionality reduction, which allows for performing intensive signal processing on-chip, while increasing power and hardware efficiency. This core also reuses the signal standard deviation already computed by the AP detector core as threshold for performing automatic AP sorting. The headstage also introduces innovations by enabling a new wireless CL scheme between the neural data acquisition module and the optical stimulator. Our CL scheme uses the AP sorting and timing information produced by the ND-IC for detecting complex firing patterns within the brain. The headstage is also smaller (1.13 cm), lighter (3.0 g with a 40 mAh battery) and less invasive than the previous solutions, while providing a measured autonomy of 2h40, with the ND-IC. The whole system and the ND-IC are first validated in vivo in the LD thalamus of a Long-Evans rat, and then in freely-moving CL experiments involving a mouse virally expressing ChR2-mCherry in inhibitory neurons of the prelimbic cortex, and the results show that our system works well within an in vivo experimental setting with a freely moving mouse.
IEEE Trans Biomed Circuits Syst, 2019; 13

30610957: Koutserimpas C, Agouridakis P, Velimezis G, Papagiannakis G, Keramidis I, Ioannidis A, Samonis G
The burden on public emergency departments during the economic crisis years in Greece: a two-center comparative study.
The effects of the Greek economic crisis on the emergency departments (EDs) of public hospitals have not been evaluated. The study aims to evaluate the burden of the financial crisis on public hospital's EDs.
Public Health, 2019; 167

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BOARD NUMBER: S07-229

SUBOPTIMAL COMPENSATION FOR LOSS OF PKMζ IN ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Multiple pathologic mechanisms, including amyloid-β (Aβ) plaques, neurofibrillary tangles (NFTs), and neuroinflammation contribute to Alzheimer's disease (AD). How these affect progressive loss of long-term memory (LTM), the cardinal symptom of AD, is unclear. We have previously shown that persistent increases in PKMζ maintain LTP and LTM in wild-type hippocampal neurons, and that in PKMζ-null mice these functions are partially compensated by PKCι/λ. Here we hypothesize that this suboptimal compensation by PKCι/λ for PKMζ loss may be the basis of LTM disruption in AD. Using postmortem tissue from aged AD patients and matched controls, we find that PKMζ and PKCι/λ are present in a subset of NFTs within hippocampus, entorhinal cortex, subiculum, and amygdala (DOI:10.1097/01.jnen.0000218442.07664.04). Double-labeling with PHF1, which recognizes phosphorylated tau, confirms the presence of PKMζ in NFT-bearing neurons (Ibid.). In addition to abnormal accumulation in tangles, there is widespread reduction in PKMζ levels in all CA1 pyramidal cells. We then examined the effect of Aβ accumulation on the distribution of PKMζ and PKCι/λ in aged APP/PS1 mice. In line with previous reports that measured PKMζ by immunoblot (DOI:10.1038/nn.3486), quantitative immunohistochemistry revealed a dramatic downregulation of PKMζ in all layers of hippocampus and neocortex of 24-month-old APP/PS1 mice, compared to wild type littermates. In contrast, PKCι/λ was elevated, suggesting that downregulation of PKMζ by excess Aβ is suboptimally compensated, as in PKMζ-null mice. These results suggest that multiple pathophysiological mechanisms in AD dysregulate the core and suboptimal compensatory aPKC maintenance mechanisms within the neuronal ensembles that organize and encode LTM.

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MOLECULAR, CELLULAR AND BEHAVIORAL CHARACTERIZATION OF GLYCINE RECEPTORS IN THE NUCLEUS ACCUMBENS IN THE APP/PS1 MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer´s disease (AD) is characterized by a decline in cognitive and non-cognitive functions, including those mediated by limbic areas, such as the nucleus accumbens (nAc). Previous studies have shown the presence of inhibitory glycine receptors (GlyRs) in the nAc, whose activation is highly sensitive to ethanol. Furthermore, previous data using the APP/PS1 mice model (6-month-old), revealed decreased GlyRs expression and function in the nAc. Therefore, we hypothesize that GlyRs alterations in AD affect nAc functions and reward-related behaviors. For this, we examined calcium transients using the calcium fluorescent protein reporter GCaMP, performed electrical stimulation in nAc neurons and measured calcium transients by slice photometry. Increases in fluorescence was observed after addition of the GlyRs antagonist (strychnine 1 and 4 μM). However, the effect of strychnine in the APP/PS1 mice was significantly reduced. Using patch clamp technique in slices, we compared GlyRs function and ethanol potentiation. We found that ethanol potentiation was significantly decreased in AD mice. Finally, we performed drinking in the dark (DID) experiments and found that AD mice consumed significantly less ethanol in the last day of DID, consistent with lower blood ethanol concentration. Interestingly, we found that APP/PS1 mice also had lower sucrose consumption. Collectively, these data support the important role of GlyRs in nAc neuron excitability; and that decreased glycinergic activity in the APP/PS1 mice might lead to impairment in reward processing. Support: Fondecyt 3210260, NIH

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BOARD NUMBER: S07-231

THE ROLE OF INPUTS FROM HIPPOCAMPUS AND ENTORHINAL CORTEX TO THE PREFRONTAL CORTEX IN SPATIAL MEMORY IMPAIRMENTS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer´s disease (AD) is characterized by memory deficits including spatial working memory. The hippocampus (HPC) and the prefrontal cortex (PFC), most notably damaged by AD pathology, are relevant brain regions for spatial working memory. Though supporting distinct functions in memory processing, the HPC and the PFC are highly connected and operate in synergy to consolidate and retrieve memories. Specifically, the PFC is involved in top-down control of memory processing. Furthermore, both excitatory and long-range inhibitory projections between the HPC and PFC have been poorly characterized in disease-like conditions. In fact, it remains unknown whether structural and functional connectivity deficits between these brain areas are causally linked to spatial working memory deficits under AD-like conditions. Here, we investigated the structural and functional connectivity between these brain regions in a mouse model of AD-like pathology (APPswePSEN1dE9). We used a cell type-specific Cre-driver mouse line to target somatostatin (SST)-positive interneurons and AAV-mediated Cre-dependent fluorophore expression to conduct anterograde tracings. Moreover, we made use of microprism-based two-photon Ca^{2+} imaging in the PFC of awake, head-fixed mice, performing a spatial working memory task on a treadmill. Our first data show the accumulation of amyloid ß (Aß) plaques in the PFC and pathology-associated structural alterations of hippocampal axonal projections in the PFC. Interestingly, SST^{+} axonal projections were also affected by amyloidosis. These results indicate that structural connectivity impairments between HPC and PFC in a mouse model of AD-like pathology might underlie spatial working memory deficits.

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BOARD NUMBER: S07-232

DEFINING THE ROLE OF THE P75 NEUROTROPHIN RECEPTOR IN ALTERING NEURONAL FUNCTION, NEUROINFLAMMATION AND COGNITIVE DECLINE IN ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer disease (AD), the most frequent form of dementia is characterized by gradual cognitive decline, accumulation of Aβ-oligomers, progressive neuronal degeneration and chronic neuroinflammation. Among the first events in AD is the selective degeneration of cholinergic basal forebrain neurons characterized by their high expression levels of the p75 neurotrophin receptor ($p75^{NTR}$). Interestingly, $p75^{NTR}$ regulates several crucial processes in the adult brain, including neuronal survival, architecture and plasticity. Amongst other cell-surface proteins, $p75^{NTR}$ has been shown to bind soluble Aβ-oligomers and to mediate some of the toxic effects of Aβ on neurons. Moreover, $p75^{NTR}$ is expressed in microglia, where it is markedly increased under pathological conditions. These observations indicate $p75^{NTR}$ as a potential candidate for mediating the Aβ-induced phenotypes during AD. Here we use APP/PS1 transgenic mice and compare the Aβ-induced alterations in neuronal function, chronic inflammation as well as their cognitive consequences between 10 months old APP/PS1 and APP/PS1 $p75^{NTRExonIV}$ KO mice. Electrophysiological recordings show that a loss $p75^{NTR}$ rescues the impairment in long term potentiation observed at the Schaffer collaterals in the hippocampus of APP/PS1 mice. Interestingly, however loss of $p75^{NTR}$ does not influence the severity of neuroinflammation, microglia activation or the decline in spatial learning and memory processes observed in APP/PS1 transgenic mice. Current experiments address on one side the specific actions of $p75^{NTR}$ at synapses, possibly underlying the rescue of synaptic plasticity as well as the actions of $p75^{NTR}$ in modulating microglial function. MK and HD are funded by the Helmholtz-Gemeinschaft, Zukunftsthema ''Immunology and Inflammation'' (ZT-0027)

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IMPACT OF ALZHEIMER'S DISEASE ON THE MORPHOLOGY OF HUMAN DENTATE GRANULE CELLS.

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aim: Alzheimer's disease (AD) is the most common form of dementia. The hippocampus is one of the most affected brain areas in patients with this disorder. In particular, remarkable alterations have been reported in pyramidal neurons of the CA1 and CA3 hippocampal subfields. However, the extent to which dentate granule cells (DGCs) are affected by AD progression remains to be fully elucidated. The general aim of this study was to address whether human DGCs show morphological alterations in patients with AD. **Methods:** Human hippocampal samples obtained from neurologically healthy control subjects and patients with AD (distributed among the 6 neuropathological Braak-Tau stages of the disease) were subjected to Golgi-cox staining using the rapid Golgi-stain kit. A total number of 639 DGCs were traced and reconstructed for morphological analysis. **Results:** Morphometric analyses revealed that the DGCs of AD patients show alterations in their soma area, dendritic branching (Sholl´s analysis), total dendritic length, number of branch-tips, number of primary apical dendrites, branching index, and dendritic span. Each of these alterations start to be observed at distinct Braak-Tau stages. **Conclusions:** Our results show that morphological alterations of human DGCs appear at early Braak-Tau stages and are maintained during the disease progression. These alterations may be related to certain types of cognitive impairments observed in AD patients.

Pubmed:
19178790: Márquez-Valadez B, Lugo-Huitrón R, Valdivia-Cerda V, Miranda-Ramírez LR, Pérez-De La Cruz V, González-Cuahutencos O, Rivero-Cruz I, Mata R, Santamaría A, Pedraza-Chaverrí J
The natural xanthone alpha-mangostin reduces oxidative damage in rat brain tissue.
The antiperoxidative properties of alpha-mangostin, a xanthone isolated from mangosteen fruit, were tested for the first time in nerve tissue exposed to different toxic insults. Two reliable biological preparations (rat brain homogenates and synaptosomal P2 fractions) were exposed to the toxic actions of a free radical generator (ferrous sulfate), an excitotoxic agent (quinolinate), and a mitochondrial toxin (3-nitropropionate). alpha-Mangostin decreased the lipoperoxidative action of FeSO(4) in both preparations in a concentration-dependent manner, and completely abolished the peroxidative effects of quinolinate, 3-nitropropionate and FeSO(4) + quinolinate at all concentrations tested. Interestingly, when tested alone in brain homogenates, alpha-mangostin significantly decreased the lipoperoxidation even below basal levels. alpha-Mangostin also prevented the decreased reductant capacity of mitochondria in synaptosomal fractions. Our results suggest that alpha-mangostin exerts a robust antiperoxidative effect in brain tissue preparations probably through its properties as a free radical scavenger. In light of these findings, this antioxidant should be tested in other neurotoxic models involving oxidative stress. Nutr Neurosci, 2009; 12
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The protective role of heme oxygenase-1 in cerebral ischemia.
Cerebral ischemia is one of the leading causes of death and disability in industrialized countries, with no curative treatments to date. Identification of potential targets and elucidation of their physiological role under stress conditions may give support to the development of drugs and strategies to contend with this pathology. In the last years, Heme oxygenase-1 (HO-1) has been considered by many groups as a potential target in ischemic damage. HO-1 is the enzyme responsible for the conversion of the heme group to billiverdin, carbon monoxide and iron; a highly regulated cytoprotective enzyme able to respond to numerous chemical or physical stressors, many of which decrease oxygen availability and generate oxidative stress. The disruption of HO-1 activity has been widely associated with a bad outcome in many disorders, and a protective role through its heme catabolism products has been observed in transplantation, cardiac ischemia, limb ischemia/reperfusion

and different alterations that involve ischemia and reperfusion events. Here, we review recent reports supporting the protective role of HO-1 in cerebral ischemia. Results on the endogenous HO-1 response, overexpression of HO-1 and compounds that reduce ischemic damage through the induction of HO-1 in cerebral ischemia in in vivo and in vitro models are analyzed.
Cent Nerv Syst Agents Med Chem, 2010; 10
21371264: Méndez-Cuesta LA, Márquez-Valadez B, Pérez-De la Cruz V, Maldonado PD, Santana RA, Escobar-Briones C, Galván-Arzate S, Carrillo-Mora P, Santamaría A
Early changes in oxidative stress markers in a rat model of acute stress: effect of l-carnitine on the striatum.
This work focuses on the effect of acute stress on different markers of oxidative stress and mitochondrial dysfunction in the rat striatum. In addition, the effect of a single dose of l-carnitine (l-CAR, 300 mg/kg, i.p.) was evaluated in these animals. Immobilization (restraint) stress was induced to rats for 24 hr. The levels of lipid peroxidation (LP) and mitochondrial function (MF), as well as the superoxide dismutase (SOD) activity and content and reduced glutathione (GSH) levels, were all measured in striatal samples of animals subjected to stress. Our results indicate that acute stress is able to increase the striatal LP and reduced the levels of MF, while significantly lowered the manganese superoxide dismutase (Mn-SOD) activity. No changes were observed in the total striatal content of SOD, nor in GSH levels, but serum corticosterone content was increased by stress. l-CAR exhibited partial protective effects on the immobilized group, reducing the striatal LP and recovering the striatal MF and Mn-SOD activity. Our results suggest that acute restraint stress brings an accurate model for early pro-oxidant responses that can be targeted by broad-spectrum antioxidants like l-CAR.
Basic Clin Pharmacol Toxicol, 2011; 109
21645264: Méndez-Cuesta LA, Márquez-Valadez B, Pérez-De La Cruz V, Escobar-Briones C, Galván-Arzate S, Alvarez-Ruiz Y, Maldonado PD, Santana RA, Santamaría A, Carrillo-Mora P
Diazepam blocks striatal lipid peroxidation and improves stereotyped activity in a rat model of acute stress.
In this work, the effect of a single dose of diazepam was tested on different markers of oxidative damage in the striatum of rats in an acute model of immobilization (restraint) stress. In addition, the locomotor activity was measured at the end of the restraint period. Immobilization was induced to animals for 24 hr, and then, lipid peroxidation, superoxide dismutase activity and content, and mitochondrial function were all estimated in striatal tissue samples. Corticosterone levels were measured in serum. Diazepam was given to rats as a pre-treatment (1 mg/kg, i.p.) 20 min. before the initiation of stress. Our results indicate that acute stress produced enhanced striatal levels of lipid peroxidation (73% above the control), decreased superoxide dismutase activity (54% below the control), reduced levels of mitochondrial function (35% below the control) and increased corticosterone serum levels (86% above the control). Pre-treatment of stressed rats with diazepam decreased the striatal lipid peroxidation levels (68% below the stress group) and improved mitochondrial function (18% above the stress group), but only mild preservation of superoxide dismutase activity was detected (17% above the stress group). In regard to the motor assessment, only the stereotyped activity was increased in the stress group with respect to control (46% above the control), and this effect was prevented by diazepam administration (30% below the stress group). The preventive actions of diazepam in this acute model of stress suggest that drugs exhibiting anxiolytic and antioxidant properties might be useful for the design of therapies against early acute phases of physic stress.
Basic Clin Pharmacol Toxicol, 2011; 109
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Alpha-mangostin induces changes in glutathione levels associated with glutathione peroxidase activity in rat brain synaptosomes.
In a previous report, we have characterized the antiperoxidative properties of alpha-mangostin in different toxic models tested in nerve tissue preparations.
Nutr Neurosci, 2012; 15
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Green tea compound epigallo-catechin-3-gallate (EGCG) increases neuronal survival in adult hippocampal neurogenesis in vivo and in vitro.
Epigallo-catechin-3-gallate (EGCG), found in the leaves of Camellia sinensis (green tea), has antioxidant- and scavenger-functions and acts neuroprotectively. It has been publicized as anti-aging remedy but data on potential cellular mechanisms are scarce. Recent studies claimed that EGCG specifically promotes neural precursor cell proliferation in the dentate gyrus of C57Bl/6 mice, without changes at the level of immature and mature new neurons. We here analyzed the effects of EGCG on adult hippocampal neurogenesis in male Balb/C mice and saw a different pattern. Two weeks of treatment with EGCG (0, 0.625, 1.25, 2.5, 5 and 10mg/kg) showed a dose-response curve that peaked at 2.5mg/kg of EGCG with significantly increased cell survival without affecting cell proliferation but decreasing apoptotic cells. Also, EGCG increased the population

of doublecortin-(DCX)-expressing cells that comprises the late intermediate progenitor cells (type-2b and -3) as well as immature neurons. After EGCG treatment, the young DCX-positive neurons showed more elaborated dendritic trees. EGCG also significantly increased net neurogenesis in the adult hippocampus and increased the hippocampal levels of phospho-Akt. Ex vivo, EGCG exerted a direct effect on survival and neuronal differentiation of adult hippocampal precursor cells, which was absent, when PI3K, a protein upstream of Akt, was blocked. Our results thus support a pro-survival and a pro-neurogenic role of EGCG. In the context of the conflicting published results, however, potential genetic modifiers must be assumed. These might help to explain the overall variability of study results with EGCG. Our data do indicate, however, that natural compounds such as EGCG can in principle modulate brain plasticity.
Neuroscience, 2016; 322
30483218: Márquez-Valadez B, Valle-Bautista R, García-López G, Díaz NF, Molina-Hernández A
Maternal Diabetes and Fetal Programming Toward Neurological Diseases: Beyond Neural Tube Defects.
The purpose of this review was to search for experimental or clinical evidence on the effect of hyperglycemia in fetal programming to neurological diseases, excluding evident neural tube defects. The lack of timely diagnosis and the inadequate control of diabetes during pregnancy have been related with postnatal obesity, low intellectual and verbal coefficients, language and motor deficits, attention deficit with hyperactivity, problems in psychosocial development, and an increased predisposition to autism and schizophrenia. It has been proposed that several childhood or adulthood diseases have their origin during fetal development through a phenomenon called fetal programming. However, not all the relationships between the outcomes mentioned above and diabetes during gestation are clear, well-studied, or have been related to fetal programming. To understand this relationship, it is imperative to understand how developmental processes take place in health, in order to understand how the functional cytoarchitecture of the central nervous system takes place; to identify changes prompted by hyperglycemia, and to correlate them with the above postnatal impaired functions. Although changes in the establishment of patterns during central nervous system fetal development are related to a wide variety of neurological pathologies, the mechanism by which several maternal conditions promote fetal alterations that contribute to impaired neural development with postnatal consequences are not clear. Animal models have been extremely useful in studying the effect of maternal pathologies on embryo and fetal development, since obtaining central nervous system tissue in humans with normal appearance during fetal development is an important limitation. This review explores the state of the art on this topic, to help establish the way forward in the study of fetal programming under hyperglycemia and its impact on neurological and psychiatric disorders.
Front Endocrinol (Lausanne), 2018; 9
31040765: Márquez-Valadez B, Aquino-Miranda G, Quintero-Romero MO, Papacostas-Quintanilla H, Bueno-Nava A, López-Rubalcava C, Díaz NF, Arias-Montaño JA, Molina-Hernández A
The Systemic Administration of the Histamine H Receptor Antagonist/Inverse Agonist Chlorpheniramine to Pregnant Rats Impairs the Development of Nigro-Striatal Dopaminergic Neurons.
The dopaminergic and histaminergic systems are the first to appear during the development of the nervous system. Through the activation of H receptors (HRs), histamine increases neurogenesis of the cortical deep layers, while reducing the dopaminergic phenotype (cells immunoreactive to tyrosine hydroxylase, TH) in embryo ventral mesencephalon. Although the function of histamine in neuronal differentiation has been studied, the role of HRs in neurogenesis has not been addressed. For this purpose, the HR antagonist/inverse agonist chlorpheniramine was systemically administered (5 mg/kg, i.p.) to pregnant Wistar rats (gestational days 12-14, E12-14), and control and experimental embryos (E14 and E16) and pups (21-day-old) were evaluated for changes in nigro-striatal development. Western blot and immunohistochemistry determinations showed a significant increase in the dopaminergic markers' TH and PITX3 in embryos from chlorpheniramine-treated rats at E16. Unexpectedly, 21-day-old pups from the chlorpheniramine-treated group, showed a significant reduction in TH immunoreactivity in the substantia nigra and dorsal striatum. Furthermore, striatal dopamine content, evoked [H]-dopamine release and methamphetamine-stimulated motor activity were significantly lower compared to the control group. These results indicate that HR blockade at E14-E16 favors the differentiation of dopaminergic neurons, but hampers their migration, leading to a decrease in dopaminergic innervation of the striatum in post-natal life.
Front Neurosci, 2019; 13
33042999: Valle-Bautista R, Márquez-Valadez B, Fragoso-Cabrera AD, García-López G, Díaz NF, Herrera-López G, Griego E, Galván EJ, Arias-Montaño JA, Molina-Hernández A
Impaired Cortical Cytoarchitecture and Reduced Excitability of Deep-Layer Neurons in the Offspring of Diabetic Rats.
Maternal diabetes has been related to low verbal task scores, impaired fine and gross motor skills, and poor performance in graphic and visuospatial tasks during childhood. The primary motor cortex is important for controlling motor functions, and embryos exposed to high glucose show changes in cell proliferation, migration, and differentiation during corticogenesis. However, the existing studies do not discriminate between embryos with or without neural tube defects, making it difficult to conclude whether the reported changes are related to neural tube defects or other anomalies. Furthermore, postnatal effects

on central nervous system cytoarchitecture and function have been scarcely addressed. Through molecular, biochemical, morphological, and electrophysiological approaches, we provide evidence of impaired primary motor cerebral cortex lamination and neuronal function in pups from diabetic rats, showing an altered distribution of SATB2, FOXP2, and TBR1, impaired cell migration and polarity, and decreased excitability of deep-layer cortical neurons, suggesting abnormalities in cortico-cortical and extra-cortical innervation. Furthermore, phase-plot analysis of action potentials suggests changes in the activity of potassium channels. These results indicate that high-glucose insult during development promotes complex changes in migration, neurogenesis, cell polarity establishment, and dendritic arborization, which in turn lead to reduced excitability of deep-layer cortical neurons.
Front Cell Dev Biol, 2020; 8
35140581: Valle-Bautista R, Márquez-Valadez B, Herrera-López G, Griego E, Galván EJ, Díaz NF, Arias-Montaño JA, Molina-Hernández A
Long-Term Functional and Cytoarchitectonic Effects of the Systemic Administration of the Histamine H Receptor Antagonist/Inverse Agonist Chlorpheniramine During Gestation in the Rat Offspring Primary Motor Cortex.
The transient histaminergic system is among the first neurotransmitter systems to appear during brain development in the rat mesencephalon/rhombencephalon. Histamine increases FOXP2-positive deep-layer neuron differentiation of cortical neural stem cells through H receptor activation . The or systemic administration of chlorpheniramine (H receptor antagonist/inverse agonist) during deep-layer cortical neurogenesis decreases FOXP2 neurons in the developing cortex, and HR- or histidine decarboxylase-knockout mice show impairment in learning and memory, wakefulness and nociception, functions modulated by the cerebral cortex. Due to the role of HR in cortical neural stem cell neurogenesis, the purpose of this study was to evaluate the postnatal impact of the systemic administration of chlorpheniramine during deep-layer cortical neuron differentiation (E12-14) in the primary motor cortex (M1) of neonates (P0) and 21-day-old pups (P21). Chlorpheniramine or vehicle were systemically administered (5 mg/kg, i.p.) to pregnant Wistar rats at gestational days 12-14, and the expression and distribution of deep- (FOXP2 and TBR1) and superficial-layer (SATB2) neuronal cortical markers were analyzed in neonates from both groups. The qRT-PCR analysis revealed a reduction in the expression of Satb2 and FoxP2. However, Western blot and immunofluorescence showed increased protein levels in the chlorpheniramine-treated group. In P21 pups, the three markers showed impaired distribution and increased immunofluorescence in the experimental group. The Sholl analysis evidenced altered dendritic arborization of deep-layer neurons, with lower excitability in response to histamine, as evaluated by whole-cell patch-clamp recording, as well as diminished depolarization-evoked [H]-glutamate release from striatal slices. Overall, these results suggest long-lasting effects of blocking HRs during early neurogenesis that may impact the pathways involved in voluntary motor activity and cognition.
Front Neurosci, 2021; 15

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BOARD NUMBER: S07-234

BOOSTING DOPAMINERGIC TRANSMISSION RESCUES HIPPOCAMPAL GABAERGIC ACTIVITY AND REDUCES EPILEPTIFORM ACTIVITY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Epidemiological studies state that the risk of epilepsy is increased in Alzheimer's Disease (AD) patients, and patients suffering from epilepsy have an elevated risk to develop dementia. This puts hippocampal epileptiform activity in the center of attention in the study of both AD onset and progression. Epileptic-like seizures are related to network alterations and aberrant gamma oscillations - due to parvalbumin interneuron dysfunctions - all resulting in brain hyperexcitability. Although the basis of epileptiform activity are still unclear, evidence suggest that alterations in dopamine receptor levels might trigger brain hyperexcitability. Interestingly, clinical and experimental studies pinpoint the Ventral Tegmental Area (VTA) dopaminergic neurodegeneration as one of the first events in AD, raising the hypothesis that the consequent dopamine depletion could trigger early deficits. To investigate whether dopamine loss might affect network integrity, we focused on mechanisms underlying hippocampal hyperexcitability before, at the onset and after VTA neurodegeneration in the Tg2576 mouse model of AD. Our data reveal that, timely coincident with VTA degeneration, Tg2576 CA1 pyramidal neurons undergo increased epileptiform activity and reduced GABAergic inputs, due to decreased parvalbumin interneuron excitability. We also show a lower number of Tg2576 parvalbumin interneurons, associated with an impaired *p*-CREB expression, a transcription factor regulating various intracellular processes. Importantly, boosting dopaminergic transmission in Tg2576 mice rescues GABAergic tone onto CA1 pyramidal neurons, ameliorating hippocampal hyperexcitability. These results suggest that precocious and progressive VTA neurodegeneration and dopamine depletion may trigger hippocampal hyperexcitability, disclosing a possible mechanism leading to epileptiform activity in early AD.

Pubmed:
34973962: Spoleti E, Krashia P, La Barbera L, Nobili A, Lupascu CA, Giacalone E, Keller F, Migliore M, Renzi M, D'Amelio M Early derailment of firing properties in CA1 pyramidal cells of the ventral hippocampus in an Alzheimer's disease mouse model.
Gradual decline in cognitive and non-cognitive functions are considered clinical hallmarks of Alzheimer's Disease (AD). Post-mortem autoptic analysis shows the presence of amyloid β deposits, neuroinflammation and severe brain atrophy. However, brain circuit alterations and cellular derailments, assessed in very early stages of AD, still remain elusive. The understanding of these early alterations is crucial to tackle defective mechanisms. In a previous study we proved that the Tg2576 mouse model of AD displays functional deficits in the dorsal hippocampus and relevant behavioural AD-related alterations. We had shown that these deficits in Tg2576 mice correlate with the precocious degeneration of dopamine (DA) neurons in the Ventral Tegmental Area (VTA) and can be restored by L-DOPA treatment. Due to the distinct functionality and connectivity of dorsal versus ventral hippocampus, here we investigated neuronal excitability and synaptic functionality in the ventral CA1 hippocampal sub-region of Tg2576 mice. We found an age-dependent alteration of cell excitability and firing in pyramidal neurons starting at 3 months of age, that correlates with reduced levels in the ventral CA1 of tyrosine hydroxylase - the rate-limiting enzyme of DA synthesis. Additionally, at odds with the dorsal hippocampus, we found no alterations in basal glutamatergic transmission and long-term plasticity of ventral neurons in 8-month old Tg2576 mice compared to age-matched controls. Last, we used computational analysis to model the early derailments of firing properties observed and hypothesize that the neuronal alterations found could depend on dysfunctional sodium and potassium conductances, leading to anticipated depolarization-block of action potential firing. The present study depicts that impairment of cell excitability and homeostatic control of firing in ventral CA1 pyramidal neurons is a prodromal feature in Tg2576 AD mice.
Exp Neurol, 2022; 350
33684513: La Barbera L, Vedele F, Nobili A, Krashia P, Spoleti E, Latagliata EC, Cutuli D, Cauzzi E, Marino R, Viscomi MT, Petrosini L, Puglisi-Allegra S, Melone M, Keller F, Mercuri NB, Conti F, D'Amelio M

Nilotinib restores memory function by preventing dopaminergic neuron degeneration in a mouse model of Alzheimer's Disease.

What happens precociously to the brain destined to develop Alzheimer's Disease (AD) still remains to be elucidated and this is one reason why effective AD treatments are missing. Recent experimental and clinical studies indicate that the degeneration of the dopaminergic (DA) neurons in the Ventral Tegmental Area (VTA) could be one of the first events occurring in AD. However, the causes of the increased vulnerability of DA neurons in AD are missing. Here, we deeply investigate the physiology of DA neurons in the VTA before, at the onset, and after onset of VTA neurodegeneration. We use the Tg2576 mouse model of AD, overexpressing a mutated form of the human APP, to identify molecular targets that can be manipulated pharmacologically. We show that in Tg2576 mice, DA neurons of the VTA at the onset of degeneration undergo slight but functionally relevant changes in their electrophysiological properties and cell morphology. Importantly, these changes are associated with accumulation of autophagosomes, suggestive of a dysfunctional autophagy, and with enhanced activation of c-Abl, a tyrosine kinase previously implicated in the pathogenesis of neurodegenerative diseases. Chronic treatment of Tg2576 mice with Nilotinib, a validated c-Abl inhibitor, reduces c-Abl phosphorylation, improves autophagy, reduces Aβ levels and - more importantly - prevents degeneration as well as functional and morphological alterations in DA neurons of the VTA. Interestingly, the drug prevents the reduction of DA outflow to the hippocampus and ameliorates hippocampal-related cognitive functions. Our results strive to identify early pathological brain changes in AD, to provide a rational basis for new therapeutic interventions able to slow down the disease progression.

Prog Neurobiol, 2021; 202
33937445: D'Addario SL, Di Segni M, Ledonne A, Piscitelli R, Babicola L, Martini A, Spoleti E, Mancini C, Ielpo D, D'Amato FR, Andolina D, Ragozzino D, Mercuri NB, Cifani C, Renzi M, Guatteo E, Ventura R

Resilience to anhedonia-passive coping induced by early life experience is linked to a long-lasting reduction of I current in VTA dopaminergic neurons.

Exposure to aversive events during sensitive developmental periods can affect the preferential coping strategy adopted by individuals later in life, leading to either stress-related psychiatric disorders, including depression, or to well-adaptation to future adversity and sources of stress, a behavior phenotype termed "resilience". We have previously shown that interfering with the development of mother-pups bond with the Repeated Cross Fostering (RCF) stress protocol can induce resilience to depression-like phenotype in adult C57BL/6J female mice. Here, we used patch-clamp recording in midbrain slice combined with both and pharmacology to test our hypothesis of a link between electrophysiological modifications of dopaminergic neurons in the intermediate Ventral Tegmental Area (VTA) of RCF animals and behavioral resilience. We found reduced hyperpolarization-activated (I) cation current amplitude and evoked firing in VTA dopaminergic neurons from both young and adult RCF female mice. , VTA-specific pharmacological manipulation of the I current reverted the pro-resilient phenotype in adult early-stressed mice or mimicked behavioral resilience in adult control animals. This is the first evidence showing how pro-resilience behavior induced by early events is linked to a long-lasting reduction of I current and excitability in VTA dopaminergic neurons.

Neurobiol Stress, 2021; 14

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THE SURVIVAL OF VTA DOPAMINE NEURONS IS ASSOCIATED WITH UPREGULATION OF CA2+ BINDING PROTEINS IN THE TG2576 MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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In the last years clinical and experimental studies highlight the involvement of ventral tegmental area (VTA) dopaminergic neurons for early pathological alterations during Alzheimer's disease (AD) progression. In fact, recently we described a progressive degeneration of these neurons in the Tg2576 mouse model of AD, long before beta-amyloid plaque formation. Why VTA dopaminergic neurons are more vulnerable than others remains to be defined although we showed that their autophagy machinery is impaired precociously. Autophagy impairments could be the basis for accumulation of damaged mitochondria, leading to alterations in Ca^{2+} homeostasis and, ultimately, to functional/structural deterioration of VTA dopamine neurons. Indeed, deregulated Ca^{2+} homeostasis is linked with aging and neurodegeneration; thus, unravelling the mechanisms underlying Ca^{2+} buffering in dopaminergic neurons may lead to possible strategies to prevent or delay neuronal loss. Here, in the VTA dopaminergic neurons of Tg2576 mice, we found accumulation of swollen, damaged mitochondria, associated with translocation of the Apoptosis-inducing factor from the mitochondrial inner membrane to the nucleus. These alterations coincide with functional changes in neuronal firing. Interestingly, although there is progressive loss of dopaminergic neurons containing Ca^{2+}-binding proteins (Calbindin-D28K, Calretinin), the surviving neurons show increased expression of these proteins when compared to dopamine neurons from control mice. Accordingly, Ca^{2+} microfluorometry revealed reduced free Ca^{2+} levels in Tg2576 neurons. Our results demonstrate that overexpression of Ca^{2+}-binding proteins in VTA dopaminergic neurons helps to survive neurodegeneration, increasing the ability of these neurons to buffer free Ca^{2+}, thus acting as a neuroprotective agent to reduce neuronal suffering.

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KINDLING-INDUCED REACTIVATION OF IMMEDIATE EARLY GENES IS ASSOCIATED WITH INCREASED SEIZURE SEVERITY AND NEUROINFLAMMATION IN 5XFAD MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disease affecting 50 million people worldwide. There is increased prevalence of epilepsy in patients with AD, and the two diseases are thought to have a bi-directional association. This study aimed to understand the relationship between AD and seizure susceptibility and to characterise the molecular signature of epileptogenesis in the setting of AD. Transgenic 5xFAD mice (N=20) and WT littermates (N=22) underwent electrical amygdala kindling to induce epilepsy phenotype, or were treated as sham (electrode implantation without stimulation). Kindling rate, seizure severity and cognitive behavioural performance were compared across the kindled and sham 5xFAD and WT mice. The transcriptome (RNA-sequencing) of the hippocampal tissue was examined through differential expression analysis, followed by weighted gene coexpression network analysis (WGCNA). The 5xFAD mice showed increased susceptibility to kindling-induced seizures and had significantly longer and more severe seizures compared to WT littermates (p=0.0002). They also showed impaired spatial memory compared to WT group, as measured by the Y-maze test. Transcriptomic profiling and WGCNA identified modules of inflammatory and cellular stress-associated genes correlated with seizure severity and impaired spatial memory. Notably, a module of early immediate genes showed significant correlation with kindled-5xFAD group, but not the shams. The regulatory hub genes, pcdh8, BDNF, and Nptx2, are involved in synapse formation/maintenance in homeostatic conditions and their dysregulation leads to loss of dendritic spine density. We speculate that the inherent low expression level of these hub genes in 5xFAD may contribute to increased susceptibility to epileptogenesis and seizure-associated damage, and represent potential therapeutic targets.

Pubmed:
33876332: Dejakaisaya H, Harutyunyan A, Kwan P, Jones NC
Altered metabolic pathways in a transgenic mouse model suggest mechanistic role of amyloid precursor protein overexpression in Alzheimer's disease.
The mechanistic role of amyloid precursor protein (APP) in Alzheimer's disease (AD) remains unclear.
Metabolomics, 2021; 17

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BOARD NUMBER: S07-237

NEURONAL ADENOSINE A2A RECEPTOR OVEREXPRESSION EXACERBATES MEMORY DEFICITS AND SYNAPTIC LOSS IN AN AMYLOIDOGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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[1]Lille Neuroscience & Cognition, Alzheimer & Tauopathies, Lille, France, [2]Université de Lille, Prism Laboratory, Villeneuve d'Ascq, France, [3]Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Department Of Clinical Neuroscience, Lausanne, Switzerland, [4]Neurodegenerative Diseases Laboratory, Molecular Imaging Research Center, Paris-saclay University, Fontenay-Aux-Roses, France, [5]Inserm UMR-S 1270 Sorbonne Université, Institut Du Fer á Moulin, Paris, France

Adenosine A_{2A} receptors (A_{2A}Rs), the target of caffeine, are found abnormally upregulated in neurons of AD patient's brains in correlation with the development of cognitive deficits. To get insights into the impact of A_{2A} dysregulation in AD, we evaluated the consequences of a neuronal A_{2A}R upsurge in a transgenic model of AD-like amyloidogenesis (APP/PS1dE9 mice). We crossed APP/PS1 mice with the transgenic TRE-A_{2A} strain, carrying the mouse A_{2A}R under the control of a Tet-responsive-element promoter. This led to four genotypic groups: WT, APP, WT/TRE-A_{2A} and APP/TRE-A_{2A}. At 3m of age, all the animals were bilaterally injected in the hippocampus with an AAV2/5-CBA-ttA, allowing the expression of ttA transactivator and then neuronal A_{2A}R upsurge in TRE-A_{2A} animals. At 6m of age, when APP/PS1 mice do not display deficits, behavioral evaluations revealed that neuronal A_{2A}R overexpression strongly worsens spatial memory impairments of APP animals. While that was not associated with significant change in amyloid burden or neuroinflammation, neuronal A_{2A}R upsurge favored the development of neuritic tau pathology. Mass spectrometry-based high-throughput proteomics identified modifications in the molecular profile of APP/TRE-A_{2A}. Gene ontology analysis revealed that most highly downregulated proteins were related to synaptic function. Immunohistochemistry and western blot analysis confirmed loss of pre and postsynaptic markers when neuronal A_{2A}R is upregulated in APP mice. These data support that pathological upregulation of A_{2A}R in neurons enhance synapse vulnerability to amyloid and is instrumental towards the decline of cognitive functions in AD.

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INCREASED EXPRESSION AND ABERRANT ACTIVITY OF NEURONAL NITRIC OXIDE SYNTHASE ALTERS GLUTAMATERGIC CALCIUM SIGNALING IN ALZHEIMER'S DISEASE NEURONS

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Nitric oxide (NO), produced by the calcium (Ca^{2+}) activated enzyme neuronal nitric oxide synthase (nNOS), functions as a second messenger and modulates glutamatergic Ca^{2+} signaling in neurons. Aberrant Ca^{2+} signaling occurs in Alzheimer's disease, including in sporadic AD (sAD), however, the contribution of nNOS activity and NO to this phenotype is not well understood. The aim of this study was to first quantify nNOS expression and NO levels in sAD and then assess the impact of inhibiting nNOS activity or scavenging NO on neuronal Ca^{2+} signaling. Quantification of nNOS expression using immunostaining and western blotting showed a significant increase in nNOS amount in both human *post-mortem* tissue ($p=0.004$) and induced pluripotent stem cell (iPSC) derived neurons ($p=0.005$), from sAD patients compared to healthy donors. This was observed in conjunction with significantly elevated levels of nitrite in sAD iPSC-derived neurons ($p<0.001$), quantified using the Griess assay as a downstream marker of NO. Live cell Ca^{2+} imaging demonstrated that inhibition of nNOS activity, or scavenging of endogenous NO, significantly decreased the glutamatergic Ca^{2+} response in healthy iPSC-derived neurons ($p<0.001$). In contrast, this modulatory effect was lost in the sAD neurons, although there was a decrease in the proportion of spontaneously signaling neurons, suggesting pathogenic modification of signaling receptors. In conclusion, the results of this study show that NO increases the Ca^{2+} response to glutamate in healthy neurons, however, it contributes to spontaneous Ca^{2+} signaling during sAD, highlighting the contribution of this second messenger to aberrant Ca^{2+} signaling phenotype during AD.

Pubmed:

27514990: Balez R, Steiner N, Engel M, Muñoz SS, Lum JS, Wu Y, Wang D, Vallotton P, Sachdev P, O'Connor M, Sidhu K, Münch G, Ooi L
Neuroprotective effects of apigenin against inflammation, neuronal excitability and apoptosis in an induced pluripotent stem cell model of Alzheimer's disease.
Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases, yet current therapeutic treatments are inadequate due to a complex disease pathogenesis. The plant polyphenol apigenin has been shown to have anti-inflammatory and neuroprotective properties in a number of cell and animal models; however a comprehensive assessment has not been performed in a human model of AD. Here we have used a human induced pluripotent stem cell (iPSC) model of familial and sporadic AD, in addition to healthy controls, to assess the neuroprotective activity of apigenin. The iPSC-derived AD neurons demonstrated a hyper-excitable calcium signalling phenotype, elevated levels of nitrite, increased cytotoxicity and apoptosis, reduced neurite length and increased susceptibility to inflammatory stress challenge from activated murine microglia, in comparison to control neurons. We identified that apigenin has potent anti-inflammatory properties with the ability to protect neurites and cell viability by promoting a global down-regulation of cytokine and nitric oxide (NO) release in inflammatory cells. In addition, we show that apigenin is able to protect iPSC-derived AD neurons via multiple means by reducing the frequency of spontaneous Ca(2+) signals and significantly reducing caspase-3/7 mediated apoptosis. These data demonstrate the broad neuroprotective action of apigenin against AD pathogenesis in a human disease model.
Sci Rep, 2016; 6
26697132: Balez R, Ooi L
Getting to NO Alzheimer's Disease: Neuroprotection versus Neurotoxicity Mediated by Nitric Oxide.
Alzheimer's disease (AD) is a neurodegenerative disorder involving the loss of neurons in the brain which leads to progressive memory loss and behavioral changes. To date, there are only limited medications for AD and no known cure. Nitric oxide (NO) has long been considered part of the neurotoxic insult caused by neuroinflammation in the Alzheimer's brain. However, focusing on early developments, prior to the appearance of cognitive symptoms, is changing that perception.

This has highlighted a compensatory, neuroprotective role for NO that protects synapses by increasing neuronal excitability. A potential mechanism for augmentation of excitability by NO is via modulation of voltage-gated potassium channel activity (Kv7 and Kv2). Identification of the ionic mechanisms and signaling pathways that mediate this protection is an important next step for the field. Harnessing the protective role of NO and related signaling pathways could provide a therapeutic avenue that prevents synapse loss early in disease.
Oxid Med Cell Longev, 2016; 2016
26522689: Steiner N, Balez R, Karunaweera N, Lind JM, Münch G, Ooi L
Neuroprotection of Neuro2a cells and the cytokine suppressive and anti-inflammatory mode of action of resveratrol in activated RAW264.7 macrophages and C8-B4 microglia.
Chronic inflammation is a hallmark of neurodegenerative disease and cytotoxic levels of nitric oxide (NO) and pro-inflammatory cytokines can initiate neuronal death pathways. A range of cellular assays were used to assess the anti-inflammatory and neuroprotective action of resveratrol using murine microglial (C8-B4), macrophage (RAW264.7) and neuronal-like (Neuro2a) cell lines. We examined the release of NO by Griess assay and used a Bioplex array to measure a panel of pro- and anti-inflammatory cytokines and chemokines, in response to the inflammatory stimuli lipopolysaccharide (LPS) and interferon-γ (IFN-γ). Resveratrol was a potent inhibitor of NO and cytokine release in activated macrophages and microglia. The activity of resveratrol increased marginally in potency with longer pre-incubation times in cell culture that was not due to cytotoxicity. Using an NO donor we show that resveratrol can protect Neuro2a cells from cytotoxic concentrations of NO. The protective effect of resveratrol from pro-inflammatory signalling in RAW264.7 cells was confirmed in co-culture experiments leading to increased survival of Neuro2a cells. Together our data are indicative of the potential neuroprotective effect of resveratrol during nitrosative stress and neuroinflammation.
Neurochem Int, 2016; 95
29949055: Bruinen AL, Fisher GL, Balez R, van der Sar AM, Ooi L, Heeren RMA
Identification and High-Resolution Imaging of α-Tocopherol from Human Cells to Whole Animals by TOF-SIMS Tandem Mass Spectrometry.
A unique method for identification of biomolecular components in different biological specimens, while preserving the capability for high speed 2D and 3D molecular imaging, is employed to investigate cellular response to oxidative stress. The employed method enables observing the distribution of the antioxidant α-tocopherol and other molecules in cellular structures via time-of-flight secondary ion mass spectrometry (TOF-SIMS (MS)) imaging in parallel with tandem mass spectrometry (MS) imaging, collected simultaneously. The described method is employed to examine a network formed by neuronal cells differentiated from human induced pluripotent stem cells (iPSCs), a model for investigating human neurons in vitro. The antioxidant α-tocopherol is identified in situ within different cellular layers utilizing a 3D TOF-SIMS tandem MS imaging analysis. As oxidative stress also plays an important role in mediating inflammation, the study was expanded to whole body tissue sections of M. marinum-infected zebrafish, a model organism for tuberculosis. The TOF-SIMS tandem MS imaging results reveal an increased presence of α-tocopherol in response to the pathogen. Graphical Abstract .
J Am Soc Mass Spectrom, 2018; 29
32006803: Balez R, Berg T, Bax M, Muñoz SS, Cabral-da-Silva MC, Engel M, Do-Ha D, Stevens CH, Rowe D, Yang S, Blair IP, Ooi L
The mRNA-based reprogramming of fibroblasts from a SOD1 familial amyotrophic lateral sclerosis patient to induced pluripotent stem cell line UOWi007.
Dermal fibroblasts were donated by a 43 year old male patient with clinically diagnosed familial amyotrophic lateral sclerosis (ALS), carrying the SOD1 mutation. The induced pluripotent stem cell (iPSC) line UOWi007-A was generated using repeated mRNA transfections for pluripotency transcription factors Oct4, Klf4, Sox2, c-Myc, Lin28 and Nanog. The iPSCs carried the SOD1 genotype and had a normal karyotype, expressed expected pluripotency markers and were capable of in vitro differentiation into endodermal, mesodermal and ectodermal lineages. This iPSC line may be useful for investigating familial ALS resulting from a SOD1 mutation.
Stem Cell Res, 2020; 42
30278375: Engel M, Balez R, Muñoz SS, Cabral-da-Silva MC, Stevens CH, Bax M, Do-Ha D, Sidhu K, Sachdev P, Ooi L
Viral-free generation and characterization of a human induced pluripotent stem cell line from dermal fibroblasts.
Peripheral dermal fibroblasts (DF) from a healthy 56 year old female were obtained from the Centre for Healthy Brain Ageing (CHeBA) Biobank, University of New South Wales, under the material transfer agreement with the University of Wollongong. DFs were reprogrammed via mRNA-delivered transcription factors into induced pluripotent stem cells (iPSCs). The generated iPSCs were confirmed to be pluripotent, capable of three germ layer differentiation and are thus a useful resource for creating iPSC-derived healthy human cells of any lineage. Resource table.
Stem Cell Res, 2018; 32
32887382: Muñoz SS, Engel M, Balez R, Do-Ha D, Cabral-da-Silva MC, Hernández D, Berg T, Fifita JA, Grima N, Yang S,

Blair IP, Nicholson G, Cook AL, Hewitt AW, Pébay A, Ooi L
A Simple Differentiation Protocol for Generation of Induced Pluripotent Stem Cell-Derived Basal Forebrain-Like Cholinergic Neurons for Alzheimer's Disease and Frontotemporal Dementia Disease Modeling.
The study of neurodegenerative diseases using pluripotent stem cells requires new methods to assess neurodevelopment and neurodegeneration of specific neuronal subtypes. The cholinergic system, characterized by its use of the neurotransmitter acetylcholine, is one of the first to degenerate in Alzheimer's disease and is also affected in frontotemporal dementia. We developed a differentiation protocol to generate basal forebrain-like cholinergic neurons (BFCNs) from induced pluripotent stem cells (iPSCs) aided by the use of small molecule inhibitors and growth factors. Ten iPSC lines were successfully differentiated into BFCNs using this protocol. The neuronal cultures were characterised through RNA and protein expression, and functional analysis of neurons was confirmed by whole-cell patch clamp. We have developed a reliable protocol using only small molecule inhibitors and growth factors, while avoiding transfection or cell sorting methods, to achieve a BFCN culture that expresses the characteristic markers of cholinergic neurons.
Cells, 2020; 9
31445393: Bax M, Balez R, Muñoz SS, Do-Ha D, Stevens CH, Berg T, Cabral-da-Silva MC, Engel M, Nicholson G, Yang S, Blair IP, Ooi L
Generation and characterization of a human induced pluripotent stem cell line UOWi005-A from dermal fibroblasts derived from a CCNF familial amyotrophic lateral sclerosis patient using mRNA reprogramming.
Dermal fibroblasts from a 59 year old male patient with amyotrophic lateral sclerosis (symptomatic at the time of collection), attributed to a mutation in the cyclin F gene (CCNF), were reprogrammed using mRNA and microRNA-delivered OSKM factors to induced pluripotent stem cells (iPSCs). The generated iPSCs were confirmed pluripotent, expressing typical pluripotency markers and were capable of three germ layer differentiation. This is the first reported reprogramming of cells with a mutation in the cyclin F gene, and represents a novel resource for the study of amyotrophic lateral sclerosis.
Stem Cell Res, 2019; 40
31200561: Bax M, McKenna J, Do-Ha D, Stevens CH, Higginbottom S, Balez R, Cabral-da-Silva MEC, Farrawell NE, Engel M, Poronnik P, Yerbury JJ, Saunders DN, Ooi L
The Ubiquitin Proteasome System Is a Key Regulator of Pluripotent Stem Cell Survival and Motor Neuron Differentiation.
The ubiquitin proteasome system (UPS) plays an important role in regulating numerous cellular processes, and a dysfunctional UPS is thought to contribute to motor neuron disease. Consequently, we sought to map the changing ubiquitome in human iPSCs during their pluripotent stage and following differentiation to motor neurons. Ubiquitinomics analysis identified that spliceosomal and ribosomal proteins were more ubiquitylated in pluripotent stem cells, whilst proteins involved in fatty acid metabolism and the cytoskeleton were specifically ubiquitylated in the motor neurons. The UPS regulator, ubiquitin-like modifier activating enzyme 1 (UBA1), was increased 36-fold in the ubiquitome of motor neurons compared to pluripotent stem cells. Thus, we further investigated the functional consequences of inhibiting the UPS and UBA1 on motor neurons. The proteasome inhibitor MG132, or the UBA1-specific inhibitor PYR41, significantly decreased the viability of motor neurons. Consistent with a role of the UPS in maintaining the cytoskeleton and regulating motor neuron differentiation, UBA1 inhibition also reduced neurite length. Pluripotent stem cells were extremely sensitive to MG132, showing toxicity at nanomolar concentrations. The motor neurons were more resilient to MG132 than pluripotent stem cells but demonstrated higher sensitivity than fibroblasts. Together, this data highlights the important regulatory role of the UPS in pluripotent stem cell survival and motor neuron differentiation.
Cells, 2019; 8
30138848: Muñoz SS, Balez R, Castro Cabral-da-Silva ME, Berg T, Engel M, Bax M, Do-Ha D, Stevens CH, Greenough M, Bush A, Ooi L
Generation and characterization of human induced pluripotent stem cell lines from a familial Alzheimer's disease PSEN1 A246E patient and a non-demented family member bearing wild-type PSEN1.
The induced pluripotent stem cell (iPSC) lines UOWi002-A and UOWi003-A were reprogrammed from dermal fibroblasts via mRNA transfection. Dermal fibroblasts from a 56 year old female caucasian familial Alzheimer's disease patient carrying A246E mutation in the PSEN1 gene (familial AD3, autopsy confirmed Alzheimer's disease) and a 75 year old female non-demented control from the same family bearing the wild-type PSEN1 A246 genotype were obtained from the Coriell Institute (AG06848 and AG06846, respectively). The generated iPSCs were characterized and pluripotency was confirmed. The PSEN1 genotype was maintained in both iPSC lines. Resource table.
Stem Cell Res, 2018; 31

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BOARD NUMBER: S07-239

SYNAPTOME ARCHITECTURE OF THE HUMAN HIPPOCAMPUS IS PROGRESSIVELY AND SPATIALLY ALTERED IN ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Excitatory synapse loss is thought to underlie the cognitive impairments in Alzheimer's disease (AD). The development of molecular imaging methods that measure the protein composition and morphological parameters of billions of individual synapses in mouse brain has revealed very high synapse diversity described by the synaptome (Zhu et al, 10.1016/j.neuron.2018.07.007; Cizeron et al, 10.1126/science.aba3163). Here we report the synaptome mapping developed for human hippocampal formation (HPF), a key structure for learning and a locus of AD pathology. Excitatory synapses were fluorescently labelled using antibodies to PSD95, an abundant postsynaptic scaffold protein, then imaged with diffraction-limited resolution on a high-throughput spinning disk confocal microscope. Images including billions of individual synaptic puncta were analysed using advanced deep-learning methods, providing a data-driven catalogue of excitatory synapse subtypes. Analysis of 16 subregions of the HPF in 17 control, 11 early-stage (Braak II) and 10 late-stage (Braak VI) AD cases revealed 7 excitatory synapse subtypes, each with a unique spatial and statistical distribution. Our results showed subtypes were differentially and progressively affected by AD in both local subregional density and diversity and global HPF network topology, with some being resilient and others vulnerable at different stages of disease progression. The loss of vulnerable subtypes correlated with changes in Aβ deposition. Our synaptome map in the human hippocampus provides a multiscale reference resource for this key brain region and a baseline for neuropathological studies. Identification of vulnerable and resilient subtypes of excitatory synapses may suggest new diagnostic and therapeutic approaches in AD and other synaptopathies.

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AN IN VITRO PLATFORM TO STUDY ADULT ENTORHINAL-HIPPOCAMPAL NEURONAL CIRCUITS IN EARLY ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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[1]Kavli Institute for Systems Neuroscience, Centre for Computational Neuroscience, Egil and Pauline Braathen and Fred Kavli Centre for Cortical Microcircuits, Norwegian University Of Science And Technology, Trondheim, Norway, [2]Department of Neuromedicine and Movement Science, Faculty Of Medicine And Health Sciences, Norwegian University Of Science And Technology, Trondheim, Norway, [3]K.G. Jebsen Centre for Alzheimer's Disease, Norwegian University Of Science And Technology, Trondheim, Norway

Alzheimer's disease (AD) is a progressive and ultimately fatal disease. Early in the disease-process, circuits of the entorhinal-hippocampal memory system are lost. Among the first neurons to degenerate are reelin positive entorhinal cortex layer II (Re+ ECLII) neurons, which provide the main LII-projection to the hippocampus. Here we micro-dissect Re+ ECLII-neurons along with populations of hippocampal neurons from adult AD model animals and allow the neurons to re-form relevant connections within an *in vitro* environment, enabling the study of very early AD-relevant pathology within this *in vitro* circuit. We use adult animals of the APP/PS1 mouse model and the McGill-R-Thy1-APP rat model. From these model animals we micro-dissect and culture adult Re+ ECLII-neurons, DG granular neurons, CA3 pyramidal neurons and CA1 pyramidal neurons. Separate populations are cultured in separate chambers in a microfluidic chip-setup, with each chamber connected by one-way micro tunnels to allow for re-growth of axons in an anatomically relevant pattern. The microfluidic chip platform was either interfaced with microelectrode arrays to enable electrophysiological recordings or on glass slips for characterization by immunocytochemistry. Ongoing experiments show viability of the entorhinal-hippocampal cultures beyond 4 weeks. Structural connections form after 3 days *in vitro* and electrophysiological spikes are detected after 15 days *in vitro*.

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CHARACTERISATION OF IN VIVO SYNAPTIC AND NEURONAL PHYSIOLOGY IN EARLY AND PROGRESSED AMYLOIDOPATHY (APP/PS1 MOUSE MODEL)

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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The amyloid hypothesis suggests that abnormal accumulation of amyloid-beta is a key pre-symptomatic feature in Alzheimer's Disease, eventually leading to cognitive decline. Therefore, determining the effect of soluble and aggregated amyloid-beta on function is critical for the mechanistic understanding of Alzheimer's Disease pathophysiology. Phenotypes observed in models of amyloidopathy include neuronal hyperexcitability and altered excitation/inhibition balance, which are thought to promote network abnormalities. However, how amyloid-beta explicitly alters neuronal, and subthreshold synaptic transmission properties *in vivo*, and how this links to network activity in a computing brain, is still largely unknown. **Aims:** Characterise synaptic, neuronal, and network function at two ages (2-3 months and 6-10 months) in the APP/PS1 model of amyloidopathy to understand the effects of soluble and aggregated amyloid-beta on neurophysiology *in vivo*. **Methods:** *In vivo* whole-cell patch-clamp electrophysiology was used to record from putative pyramidal neurons in the CA1 of the hippocampus in the established $APP_{swe}/PS1_{\Delta E9}$ (APP/PS1) model of amyloidopathy and littermate controls. Simultaneous local field potential recordings were made adjacent to the whole-cell electrode. Basal spontaneous excitatory and inhibitory synaptic transmission was measured. Intrinsic properties and neuronal excitability were also characterised. **Results:** Results from this work will reveal how different disease phases of amyloidopathy can alter synaptic and neuronal function. These changes will be explored in relation to different network oscillations. **Conclusions:** This work will provide a mechanistic understanding of how synaptic, neuronal, and network phenotypes emerge and progress with the appearance of amyloid plaques. This will highlight potential targets of asymptomatic dysfunction in Alzheimer's Disease.

Pubmed:
28797738: O'Callaghan J, Holmes H, Powell N, Wells JA, Ismail O, Harrison IF, Siow B, Johnson R, Ahmed Z, Fisher A, Meftah S, O'Neill MJ, Murray TK, Collins EC, Shmueli K, Lythgoe MF
Tissue magnetic susceptibility mapping as a marker of tau pathology in Alzheimer's disease.
Alzheimer's disease is connected to a number of other neurodegenerative conditions, known collectively as 'tauopathies', by the presence of aggregated tau protein in the brain. Neuroinflammation and oxidative stress in AD are associated with tau pathology and both the breakdown of axonal sheaths in white matter tracts and excess iron accumulation grey matter brain regions. Despite the identification of myelin and iron concentration as major sources of contrast in quantitative susceptibility maps of the brain, the sensitivity of this technique to tau pathology has yet to be explored. In this study, we perform Quantitative Susceptibility Mapping (QSM) and T2* mapping in the rTg4510, a mouse model of tauopathy, both in vivo and ex vivo. Significant correlations were observed between histological measures of myelin content and both mean regional magnetic susceptibility and T2* values. These results suggest that magnetic susceptibility is sensitive to tissue myelin concentrations across different regions of the brain. Differences in magnetic susceptibility were detected in the corpus callosum, striatum, hippocampus and thalamus of the rTg4510 mice relative to wild type controls. The concentration of neurofibrillary tangles was found to be low to intermediate in these brain regions indicating that QSM may be a useful biomarker for early stage detection of tau pathology in neurodegenerative diseases.
Neuroimage, 2017; 159
28931441: Blackmore T, Meftah S, Murray TK, Craig PJ, Blockeel A, Phillips K, Eastwood B, O'Neill MJ, Marston H, Ahmed Z, Gilmour G, Gastambide F
Tracking progressive pathological and functional decline in the rTg4510 mouse model of tauopathy.
The choice and appropriate use of animal models in drug discovery for Alzheimer's disease (AD) is pivotal to successful clinical translation of novel therapeutics, yet true alignment of research is challenging. Current models do not fully recapitulate the human disease, and even exhibit various degrees of regional pathological burden and diverse functional alterations. Given this, relevant pathological and functional endpoints must be determined on a model-by-model basis. The present work explores the rTg4510 mouse model of tauopathy as a case study to define best practices for the selection and validation of

cognitive and functional endpoints for the purposes of pre-clinical AD drug discovery.
Alzheimers Res Ther, 2017; 9
29522212: Roman A, Meftah S, Arthaud S, Luppi PH, Peyron C
The inappropriate occurrence of rapid eye movement sleep in narcolepsy is not due to a defect in homeostatic regulation of rapid eye movement sleep.
Narcolepsy type 1 is a disabling disorder with four primary symptoms: excessive-daytime-sleepiness, cataplexy, hypnagogic hallucinations, and sleep paralysis. The later three symptoms together with a short rapid eye movement (REM) sleep latency have suggested impairment in REM sleep homeostatic regulation with an enhanced propensity for (i.e. tendency to enter) REM sleep. To test this hypothesis, we challenged REM sleep homeostatic regulation in a recognized model of narcolepsy, the orexin knock-out (Orex-KO) mice and their wild-type (WT) littermates. We first performed 48 hr of REM sleep deprivation using the classic small-platforms-over-water method. We found that narcoleptic mice are similarly REM sleep deprived to WT mice. Although they had shorter sleep latency, Orex-KO mice recovered similarly to WT during the following 10 hr of recovery. Interestingly, Orex-KO mice also had cataplexy episodes immediately after REM sleep deprivation, anticipating REM sleep rebound, at a time of day when cataplexy does not occur in baseline condition. We then evaluated REM sleep propensity using our new automated method of deprivation that performs a specific and efficient REM sleep deprivation. We showed that REM sleep propensity is similar during light phase in Orex-KO and WT mice. However, during the dark phase, REM sleep propensity was not suppressed in Orex-KO mice when hypocretin/orexin neuropeptides are normally released. Altogether our data suggest that in addition to the well-known wake-promoting role of hypocretin/orexin, these neuropeptides would also suppress REM sleep. Therefore, hypocretin/orexin deficiency would facilitate the occurrence of REM sleep at any time of day in an opportunistic manner as seen in human narcolepsy.
Sleep, 2018; 41
32705145: Harrison IF, Ismail O, Machhada A, Colgan N, Ohene Y, Nahavandi P, Ahmed Z, Fisher A, Meftah S, Murray TK, Ottersen OP, Nagelhus EA, O'Neill MJ, Wells JA, Lythgoe MF
Impaired glymphatic function and clearance of tau in an Alzheimer's disease model.
The glymphatic system, that is aquaporin 4 (AQP4) facilitated exchange of CSF with interstitial fluid (ISF), may provide a clearance pathway for protein species such as amyloid-β and tau, which accumulate in the brain in Alzheimer's disease. Further, tau protein transference via the extracellular space, the compartment that is cleared by the glymphatic pathway, allows for its neuron-to-neuron propagation, and the regional progression of tauopathy in the disorder. The glymphatic system therefore represents an exciting new target for Alzheimer's disease. Here we aim to understand the involvement of glymphatic CSF-ISF exchange in tau pathology. First, we demonstrate impaired CSF-ISF exchange and AQP4 polarization in a mouse model of tauopathy, suggesting that this clearance pathway may have the potential to exacerbate or even induce pathogenic accumulation of tau. Subsequently, we establish the central role of AQP4 in the glymphatic clearance of tau from the brain; showing marked impaired glymphatic CSF-ISF exchange and tau protein clearance using the novel AQP4 inhibitor, TGN-020. As such, we show that this system presents as a novel druggable target for the treatment of Alzheimer's disease, and possibly other neurodegenerative diseases alike.
Brain, 2020; 143

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HIGH-RESOLUTION LOCAL AND GLOBAL MAPPING OF AMYLOID PLAQUE DEPOSITION PREDICTS BEHAVIOURAL AND COGNITIVE PERFORMANCE IN 5XFAD MOUSE MODELS OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Imperial College London, Bioengineering, London, United Kingdom

The 5xFAD mouse model of Alzheimer's Disease (AD) recapitulates many AD-related phenotypes and has a relatively early and aggressive presentation. In this model, extracellular amyloid plaques are seen in mice as young as two months of age. However, the degree to which the amyloid plaques affect behavioural and cognitive performances in this model is still not well known. Here we performed both *in vivo* and *ex vivo* high-resolution two-photon imaging to map the spatial distribution of Methoxy-X04-labelled amyloid plaques across age in 5xFAD mice. We collected two-photon calcium imaging data at the hippocampal CA1 region during spatial and working memory tasks and mapped the local distributions of amyloid plaques within this area. Given the locations of the cells being observed longitudinally during behavioural experiments, we quantified and correlated the statistical and morphological properties of amyloid plaque loads for every given spherical radius with the changes in neuronal activity properties and the behavioural performance of the mice. After termination, the brains were extracted, fixed and perfused in preparation for *ex vivo* whole-brain imaging. We used serial two-photon (STP) tomography to map the global distribution of amyloid plaques across the brain. Using a deep-learning U-Net architecture model, we automatically quantified plaque densities from each brain region and correlated these with the mice's behaviour. Our results demonstrate deficits in behavioural performance with region-specific progression of plaque load densities in critically affected brain structures. Hence, these models present an invaluable tool for early intervention and improved pre-clinical assessment of potential therapeutic approaches for AD.

Pubmed:
33642996: Go MA, Rogers J, Gava GP, Davey CE, Prado S, Liu Y, Schultz SR
Place Cells in Head-Fixed Mice Navigating a Floating Real-World Environment.
The hippocampal place cell system in rodents has provided a major paradigm for the scientific investigation of memory function and dysfunction. Place cells have been observed in area CA1 of the hippocampus of both freely moving animals, and of head-fixed animals navigating in virtual reality environments. However, spatial coding in virtual reality preparations has been observed to be impaired. Here we show that the use of a real-world environment system for head-fixed mice, consisting of an air-floating track with proximal cues, provides some advantages over virtual reality systems for the study of spatial memory. We imaged the hippocampus of head-fixed mice injected with the genetically encoded calcium indicator GCaMP6s while they navigated circularly constrained or open environments on the floating platform. We observed consistent place tuning in a substantial fraction of cells despite the absence of distal visual cues. Place fields remapped when animals entered a different environment. When animals re-entered the same environment, place fields typically remapped over a time period of multiple days, faster than in freely moving preparations, but comparable with virtual reality. Spatial information rates were within the range observed in freely moving mice. Manifold analysis indicated that spatial information could be extracted from a low-dimensional subspace of the neural population dynamics. This is the first demonstration of place cells in head-fixed mice navigating on an air-lifted real-world platform, validating its use for the study of brain circuits involved in memory and affected by neurodegenerative disorders.
Front Cell Neurosci, 2021; 15

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MEMORY REACTIVATION DYNAMICS AND SLEEP IN ALZHEIMER'S DISEASE TRANSGENIC MODEL TGF344-AD

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Hippocampus plays a pivotal role in spatial and episodic memory. Population activity of hippocampal place cells create neural representations of environments that are believed to represent network substrate of spatial memories. An important part of memory consolidation occurs during sleep via transient pattern reactivations when place cell firing patterns that occurred during active exploration are reactivated during sharp wave-ripple events. Disruption in these processes might significantly contribute to Alzheimer-related memory impairment. Here we explored changes in sleep-related memory processing in TgF344-AD transgenic rat model of Alzheimer disease (AD). This genetically modified model expresses two mutations implicated in AD: human amyloid precursor protein (APP) with Swedish mutation and human presenilin (PSEN) 1 with Δ exon 9 mutations. The impact of Alzheimer-like pathology on sleep-associated memory processing in TgF344-AD rats has not been described so far. In this study, we recorded hippocampal network activity in transgenic rats and age-matched mutation-free controls, while the animals explored familiar and novel environments and during preceding and following sleep epochs. We analyzed reactivation of place cell activity patterns during sleep, using methods based on cell-pairs co-activation and Bayesian decoding of sequential activity. In addition, we assessed changes in pyramidal cell and interneuronal population activity and neuronal synchrony across REM and non-REM sleep epochs. Our data show affected neural dynamics during sleep in AD model that might cause disturbed off-line memory processing during sleep in AD.
Acknowledgment: Primus project number 27051 and CZ.02.1.01/0.0/0.0/16_019/0000787 „FIND“.

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BOARD NUMBER: S07-244

CHANGES IN HIPPOCAMPAL PLACE CELL CIRCUIT DYNAMICS IN 5XFAD MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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The response of brain circuitry to amyloidosis is complex, with an interplay of cell-type-specific effects, changes in network dynamics, and altered network plasticity. Importantly, we need to understand how changes in circuit properties affect neuronal activity during memory encoding and recall. We recently (Go et al, Front Cellular Neurosci 2021) demonstrated the use of an air-lifted (non-VR) behavioural platform for mapping hippocampal place cell activity using two-photon imaging in head-fixed mice during spatial cognition. Here we present the first results from the application of this technology to the 5xFAD mouse model of Alzheimer's Disease (AD). We injected AAV-jGCaMP7s into the hippocampal CA1 region, then implanted an imaging window and metal headplate, aspirating the cortex above the injection site. Animals (6-9 months old) were trained for a week to run around a circular track, becoming familiarised to two environments. During imaging sessions, mice transitioned from a reference familiar environment to another familiar environment (evoking spatial memory recall) and to a novel environment (spatial memory encoding). Calcium fluorescence time series were tracked for hundreds of CA1 neurons over days. Our results show that 5xFAD mice are more active than wildtype mice in circular track navigation. Moreover, compared to place cells in wildtype littermate control mice, 5xFAD place cells have higher activity, wider place fields and less spatial information but they remap in a different environment to a similar degree. Further relating these properties to the plaque load for each imaged neuron may provide insight important for the development of effective AD therapeutics.

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CA1 HYPEREXCITABILITY DRIVES ANESTHESIA-INDUCED EARLY MEMORY DYSFUNCTIONS IN ALZHEIMER'S MODEL MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer disease (AD) is a progressive neurodegenerative disorder accounting for the majority of dementia cases. A presymptomatic phase of the disease precedes the onset of cognitive decline by decades. Recent epidemiological evidence suggests that postoperative cognitive dysfunction (POCD) is common after general anesthesia in the elderly. Moreover, clinical studies suggest a possible link between POCD and AD. In order to identify the network-wide mechanisms that drive GA-induced initial memory deficits, we used electrophysiology, chemogenetic perturbation of specific synaptic connections and behavior tasks. We found that anesthesia causes CA1 hyperexcitability and hypersynchrony in fAD, but not wild-type mice. This leads to a transient impairment in spatial working memory that correlates to the degree of CA1 hyperexcitability. Since thalamus deactivation is a common feature of general anesthesia, loss of inhibition in the thalamus might explain these state-dependent dysfunctions in fAD model. Indeed, thalamic nucleus reuniens (nRE) was hyperactive under anesthesia in fAD mice. Chemogenetic inactivation of the nucleus reuniens to CA1 synapse suppresses anesthesia-induced CA1 hyperexcitability. Further, deep brain stimulation of the nRE suppresses CA1 hyperexcitability and rescues spatial working memory impairments. These results point to CA1 hyperexcitability as a cause of memory deficits following general anesthesia and to the nucleus reuniens as a regulator of CA1 excitability and memory dysfunctions. Altogether, this study provides a novel circuit mechanism for POCD in AD patients.

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DYSREGULATION OF CA1 HIPPOCAMPAL ACTIVITY ACROSS THE SLEEP-WAKE CYCLE IN ALZHEIMER'S DISEASE MOUSE MODEL

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Background Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory decline. Accumulated evidence in AD patients and mouse models suggests that neuronal hyperexcitability in cortico-hippocampal circuits is part of the disease pathogenesis. Our recent results show that CA1 hyperexcitability can be revealed in AD mice model prior to the onset of cognitive symptoms by low-arousal brain states such as NREM sleep and anesthesia. These irregularities during sleep could be a contributing factor to the deteriorating cognitive function of AD patients since sleep plays a significant role in renormalizing synaptic strength and restoring cellular homeostasis. **Results** To characterize CA1 hyperexcitability during the pre-symptomatic stages of AD and its relation to the sleep-wake cycle, we studied 4-5-month-old APP/PS1 (APP_{SWE}/PS1DE9) mice that display a pathological increase in the Aβ42/Aβ40 ratio but do not exhibit impairments in hippocampus-dependent memory functions. EEG/LFP/EMG recordings, alongside video monitoring, were carried out continuously for several days on APP/PS1 mice and their WT littermates. No apparent changes in sleep architecture were detected during the light phase. During the dark phase, APP/PS1 mice spent less time in non-rapid eye movement (NREM) sleep and more time actively awake. **CA1 hyperexcitability, manifested mainly as interictal spikes, was present mainly during NREM and REM. Conclusions** Our results suggest that sleep is preserved during day, but disrupted during dark during presymptomatic AD stages. Understanding how circadian clock impairments affect this early sleep dysregulation and how it is connected to local dyshomeostasis of mean firing rates in the hippocampus during NREM sleep remains a challenge for the future studies.

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E2F4DN-BASED GENE THERAPY RECOVERS LONG-TERM POTENTIATION AND HIPPOCAMPAL-DEPENDENT MEMORY IN HOMOZYGOUS 5XFAD MICE.

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Neurons are usually regarded as postmitotic cells, nevertheless they can re-enter cell cycle and survive as hyperploid neurons during the course of different neurodegenerative diseases. Recently, it has been described that cell-cycle reentry, followed by DNA duplication (tetraploidization), and synaptic failure are two early hallmarks of Alzheimer's disease (AD). E2F4 becomes phosphorylated in APP/PS1 mice and in Alzheimer´s patients. We have demonstrated that the phosphorylation of two conserved Thr residues of E2F4 is necessary to induce neuronal tetraploidization and is related with cognitive loss in AD. Therefore, it was developed a therapy consisting in neuronal expression of a dominant negative form of E2F4 (E2F4DN), not phosphorylatable. This therapy has been patented and is licensed by Tetraneuron S.L., a Spanish spin-off biotech company. We hypothesized that viral expression of E2F4DN could correct AD-associated synaptic dysfunction. First of all, basal membrane properties were analyzed through whole-cell recordings in hippocampal primary cultures expressing E2F4DN. We demonstrated that it does not alter basal properties of hippocampal neurons. Afterwards, we expressed E2F4DN (AAV-E2F4DN intravenous administration) in control and homozygous 5xFAD mice. Synaptic function was measured by electrophysiological recordings of field excitatory postsynaptic potentials in hippocampal CA3-CA1 synapses. We found that E2F4DN recuperates long-term potentiation (LTP). Furthermore, this LTP recovery leads to cognitive improvement. 5xFAD mice treated with our therapy present a notably improvement in two hippocampal-dependent memory tasks (novel-object location and contextual fear conditioning). Thus, we report here that E2F4DN-based gene therapy represents a promising approach for AD treatment with capacity to prevent cognitive decline.

Pubmed:

31240311: Sánchez-Puelles C, Calleja-Felipe M, Ouro A, Bougamra G, Arroyo A, Diez I, Erramuzpe A, Cortés J, Martínez-Hernández J, Luján R, Navarrete M, Venero C, Chan A, Morales M, Esteban JA, Knafo S
PTEN Activity Defines an Axis for Plasticity at Cortico-Amygdala Synapses and Influences Social Behavior.
Phosphatase and tensin homolog on chromosome 10 (PTEN) is a tumor suppressor and autism-associated gene that exerts an important influence over neuronal structure and function during development. In addition, it participates in synaptic plasticity processes in adulthood. As an attempt to assess synaptic and developmental mechanisms by which PTEN can modulate cognitive function, we studied the consequences of 2 different genetic manipulations in mice: presence of additional genomic copies of the Pten gene (Ptentg) and knock-in of a truncated Pten gene lacking its PDZ motif (Pten-ΔPDZ), which is required for interaction with synaptic proteins. Ptentg mice exhibit substantial microcephaly, structural hypoconnectivity, enhanced synaptic depression at cortico-amygdala synapses, reduced anxiety, and intensified social interactions. In contrast, Pten-ΔPDZ mice have a much more restricted phenotype, with normal synaptic connectivity, but impaired synaptic depression at cortico-amygdala synapses and virtually abolished social interactions. These results suggest that synaptic actions of PTEN in the amygdala contribute to specific behavioral traits, such as sociability. Also, PTEN appears to function as a bidirectional rheostat in the amygdala: reduction in PTEN activity at synapses is associated with less sociability, whereas enhanced PTEN activity accompanies hypersocial behavior.
Cereb Cortex, 2020; 30
33288910: Mederos S, Sánchez-Puelles C, Esparza J, Valero M, Ponomarenko A, Perea G
GABAergic signaling to astrocytes in the prefrontal cortex sustains goal-directed behaviors.
GABA interneurons play a critical role in higher brain functions. Astrocytic glial cells interact with synapses throughout the whole brain and are recognized as regulatory elements of excitatory synaptic transmission. However, it is largely unknown how GABAergic interneurons and astrocytes interact and contribute to stable performance of complex behaviors. Here, we found that genetic ablation of GABA receptors in medial prefrontal cortex astrocytes altered low-gamma oscillations and firing properties of cortical neurons, which affected goal-directed behaviors. Remarkably, working memory deficits were restored by

optogenetic stimulation of astrocytes with melanopsin. Furthermore, melanopsin-activated astrocytes in wild-type mice enhanced the firing rate of cortical neurons and gamma oscillations, as well as improved cognition. Therefore, our work identifies astrocytes as a hub for controlling inhibition in cortical circuits, providing a novel pathway for the behaviorally relevant midrange time-scale regulation of cortical information processing and consistent goal-directed behaviors.
Nat Neurosci, 2021; 24

26780512: Knafo S, Sánchez-Puelles C, Palomer E, Delgado I, Draffin JE, Mingo J, Wahle T, Kaleka K, Mou L, Pereda-Perez I, Klosi E, Faber EB, Chapman HM, Lozano-Montes L, Ortega-Molina A, Ordóñez-Gutiérrez L, Wandosell F, Viña J, Dotti CG, Hall RA, Pulido R, Gerges NZ, Chan AM, Spaller MR, Serrano M, Venero C, Esteban JA
PTEN recruitment controls synaptic and cognitive function in Alzheimer's models.
Dyshomeostasis of amyloid-β peptide (Aβ) is responsible for synaptic malfunctions leading to cognitive deficits ranging from mild impairment to full-blown dementia in Alzheimer's disease. Aβ appears to skew synaptic plasticity events toward depression. We found that inhibition of PTEN, a lipid phosphatase that is essential to long-term depression, rescued normal synaptic function and cognition in cellular and animal models of Alzheimer's disease. Conversely, transgenic mice that overexpressed PTEN displayed synaptic depression that mimicked and occluded Aβ-induced depression. Mechanistically, Aβ triggers a PDZ-dependent recruitment of PTEN into the postsynaptic compartment. Using a PTEN knock-in mouse lacking the PDZ motif, and a cell-permeable interfering peptide, we found that this mechanism is crucial for Aβ-induced synaptic toxicity and cognitive dysfunction. Our results provide fundamental information on the molecular mechanisms of Aβ-induced synaptic malfunction and may offer new mechanism-based therapeutic targets to counteract downstream Aβ signaling.
Nat Neurosci, 2016; 19

22363206: Knafo S, Venero C, Sánchez-Puelles C, Pereda-Peréz I, Franco A, Sandi C, Suárez LM, Solís JM, Alonso-Nanclares L, Martín ED, Merino-Serrais P, Borcel E, Li S, Chen Y, Gonzalez-Soriano J, Berezin V, Bock E, Defelipe J, Esteban JA
Facilitation of AMPA receptor synaptic delivery as a molecular mechanism for cognitive enhancement.
Cell adhesion molecules and downstream growth factor-dependent signaling are critical for brain development and synaptic plasticity, and they have been linked to cognitive function in adult animals. We have previously developed a mimetic peptide (FGL) from the neural cell adhesion molecule (NCAM) that enhances spatial learning and memory in rats. We have now investigated the cellular and molecular basis of this cognitive enhancement, using biochemical, morphological, electrophysiological, and behavioral analyses. We have found that FGL triggers a long-lasting enhancement of synaptic transmission in hippocampal CA1 neurons. This effect is mediated by a facilitated synaptic delivery of AMPA receptors, which is accompanied by enhanced NMDA receptor-dependent long-term potentiation (LTP). Both LTP and cognitive enhancement are mediated by an initial PKC activation, which is followed by persistent CaMKII activation. These results provide a mechanistic link between facilitation of AMPA receptor synaptic delivery and improved hippocampal-dependent learning, induced by a pharmacological cognitive enhancer.
PLoS Biol, 2012; 10

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BOARD NUMBER: S07-248

NEURONAL EXPRESSION OF E2F4DN MODULATES THE IMMUNE RESPONSE OBSERVED IN THE CEREBRAL CORTEX OF 5XFAD MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disorder in which altered immune response is an important etiological factor. The transcription factor E2F4 participates in tissue homeostasis and regulates gene networks affected in AD, thus constituting a potential target for intervention. We have studied whether neuronal expression of a dominant negative form of E2F4 (E2F4DN), can modulate the immune response observed in AD. To this aim, we generated Mapt:E2F4DN knock-in mice (E2F4DN mice) that, together with control Mapt:EGFP knock-in mice (EGFP mice), were crossed with 5xFAD mice, a known murine model of AD. Neuronal expression of E2F4DN in 5xFAD mice led to reduced astrogliosis at 3 months of age, both in cortex and hippocampus. In addition, Iba1-positive cells exhibited reduced size in the cortex and hippocampus of 5xFAD/E2F4DN mice at 3 and 6 months, suggesting that microglia activation is attenuated by the presence of neuronal E2F4DN. In vivo, most Iba1-positive cells of 5xFAD/E2F4DN mice were associated to amyloid beta (Aβ) deposits, which were increased in size, but not in number at 3 months of age. Moreover, neuronal expression of E2F4DN slowed down the accumulation of Aβ at 6 months of age. We speculate that the crosstalk between E2F4DN-expressing neurons and microglia favors the aggregation of oligomeric Aβ at early stages of AD, thus reducing its toxicity, and attenuates Aβ deposition at later stages. Altogether, our data are consistent with a beneficial immune response in 5xFAD mice expressing neuronal E2F4DN, which we propose as a therapeutic agent against AD.

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AMYLOID BETA IMPAIRS SYNAPTIC PLASTICITY AT THE HIPPOCAMPAL CA3-CA1 SYNAPSES IN ALZHEIMER'S DISEASE: A COMPUTATIONAL MODELING STUDY

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory loss, cognitive decline, language impairment, and decision making. It has a long preclinical stage, and before any clinical symptoms appear, pathological processes are observed in the hippocampus and entorhinal cortex, regions of the brain responsible for memory storage and recall. In early AD, alterations in amyloid precursor protein (APP) processing and clearance of APP peptides are observed. Increased APP levels lead to the production of AD-related peptides such as Amyloid beta (Aβ), Amyloid eta (Aη), and the Amyloid APP intracellular domain (AICD). In hippocampal CA1 pyramidal neurons, Aβ inhibits long-term potentiation (LTP) and enhances long-term depression (LTD) of glutamatergic synapses. The aim of this study is to investigate the mechanisms of the altered Aβ-induced synaptic plasticity applying a computational modeling approach. We used a detailed compartmental model of a CA1 pyramidal neuron and a modified NMDAr dependent voltage-based model of synaptic plasticity to analyze synaptic modifications at the hippocampal CA3-CA1 synapses. The effect of the elevated levels of Aβ was modeled as the increased extracellular glutamate concentration, endocytosis of synaptic AMPA receptors, reduced synaptic density, NMDA receptor-mediated activation of Ca2+/calmodulin-dependent kinase II (CaMKII). The modeling results support the experimental indication that pathological concentration of Aβ leads to impaired LTP and enhanced LTD in AD. Computational modeling study sheds light on the Aβ-induced complex processes and their interactions in shaping synaptic plasticity at the hippocampal synapses. Acknowledgment: Flagship ERA-NET JTC 2019 in synergy with the Human Brain Project, No. S-FLAG-ERA-20-1/2020-PRO-28

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THE AMYLOID AGGREGATION STUDY ON BOARD THE INTERNATIONAL SPACE STATION

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

Alessandra Favole[1], Elena Berrone[1], Franco Cardone[2], Cristiano Corona[1], Marco Sbriccoli[2], Valerio Benedetti[1], Claudia Palmitessa[1], Flavia Porreca[2], Antonio Cornacchia[2], Stefano Sirigu[3], Alessandro Crisafi[3], Dario Castagnolo[4], Claudia Pacelli[5], Marino Crisconio[5], Gabriele Mascetti[5], Giovanni Meli[6], Chiara Piacenza[7], Gianni Truscelli[7], Giovanni Valentini[5], Sara Piccirillo[5], Simona Sennato[8], Francesca Scaramuzzo[9], Elena Fiori[6], Annalisa Manca[6], Serena Camerini[2], Maria Luisa Casella[2], Cristina Casalone[1]
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Amyloid plaques made of β-peptides are one of the main hallmarks of the Alzheimer disease (AD). The study of the effects of longlasting space stays on the development of AD and other proteinopathies in astronauts is an urgent need in view of the programs for interplanetary travels announced by national and international space agencies. "Amyloid Aggregation" is an Italian Space Agency (ASI) project study aiming to assess how amyloid fibrils aggregation is affected by microgravity. The experiment was performed on the International Space Station (ISS) during the "BEYOND" mission. β-peptides of different size and propensity to aggregate were encapsulated in the cap of special jars containing the reaction fluid in separate lower compartment. Once on the ISS, β-peptides were mixed with the reaction fluid and left to aggregate at ambient temperature for various times. At the end of each interval, samples were frozen and then returned to Earth. The experiment was repeated on Earth recapitulating the same ISS conditions except for the absence of weight. Major techniques for amyloid characterization like Western Blotting, Mass Spectrometry, Atomic Force Microscopy, and Dynamic Light Scattering were optimized to compare the ISS samples with those processed on Earth. Preliminary results have shown differences between β-peptides aggregates formed aboard the ISS and on the Earth. Hopefully, results from this project will help to design more stringent scientific studies to evaluate the risk of developing protein aggregation diseases in astronauts.

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MACHINE-LEARNING HISTOPATHOLOGICAL SEGMENTATION AND QUANTIFICATION OF TAUOPATHIES IN CLASSIC VS RAPIDLY PROGRESSIVE FORMS OF ALZHEIMER'S DISEASE.

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Complex changes in the brain tissue including the accumulation of phosphorylated tau-positive somatic neurofibrillary tangles (NFT) and neuritic plaques (NP) characterize Alzheimer's disease. Recently discovered rapidly progressive AD (rpAD) present an atypically short clinical course, but
remains undistinguishable from classic AD (cAD) using current neuropathological analysis methods. In the present project, we aimed to automatically segment tau-positive objects (i.e. NP and NFT) and evaluate their load, morphology and spatial distribution in cAD and rpAD cases. We used whole slide images of AT8-immunostained sections of prefrontal cortices from eleven cAD and nine rpAD cases. Additional AD cases were included for algorithm training. A set of 15 slides was used for human-CNN iterative object annotation resulting in a total of 5013 annotated and expertvalidated NPs. A U-Net based CNN was trained and the obtained algorithm was precise in NP detection (F1 score: 0.84) and segmentation (F1 score: 0.75). NFTs detection-segmentation is currently work in progress using similar deep-learning approach. Using our algorithm applied to the cAD-rpAD cohort, NPs were segmented and their load and morphology were measured. cAD individuals presented a significantly higher NP load ($p<0,05$) in comparison to rpAD cases. Obtained segmentation data were crossed with available clinical and additional neuropathological data using PCA and t-SNE multivariate analysis leading to a clear differentiation of cAD and rpAD groups. Our data indicate the usefulness of deep learning-based histological analysis of tau lesions for
stratification of AD subgroups presenting apparent neuropathological isomorphism.

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GENETICS OF ALZHEIMER-LIKE DISEASE IN OCTODON DEGUS

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Various groups have reported the development of an Alzheimer-like disease in *Octodon degus* (DAD) held in captivity. The disease is characterized by the presence of typical human Alzheimer's brain biomarkers and a decline in cognitive performance. Interestingly, DAD has been observed in some degu laboratory colonies but not in others. Our aim is to find an explanation for the difference in DAD incidence among degu colonies, specifically to look for genetic variants that could be associated with the development of DAD. We performed behavioral assessments to detect cognitive decline, and sequenced the protein coding regions of 6 genes linked to Alzheimer's disease in human to look for sequence variants. The effect of these variants in protein stability was analyzed using 3D protein models. We studied degus with or without DAD from one colony in Chile and one in Germany. We discovered 15 non-synonymous variants in 4 genes: *APOE*, *PSEN2*, *MAPT*, and *TREM2*. Among these, 10 variants are predicted to affect protein stability, potentially producing deleterious effects in protein function. The variant in *APOE* also is predicted to change its affinity to HSPG receptors, which can lead to the emergence of DAD biomarkers. Variants are present in similar proportions between DAD and non-DAD individuals, but some are present in one colony but not in the other. We speculate that the higher frequency of certain variants in a colony confers higher propensity to DAD and that the synergistic effect of different variants could be a determinant for the development of DAD.

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PROTEOMIC ANALYSIS ASSESSMENT OF HUMAN AMYGDALA IN ALZHEIMER'S DISEASE.

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

Melania Gonzalez-Rodriguez, Veronica Astillero-Lopez, Sandra Villar-Conde, Villanueva-Anguita, Isabel Ubeda-Banon, Alicia Flores-Cuadrado, Daniel Saiz-Sanchez, Alino Martinez-Marcos
UNIV. CASTILLA-LA MANCHA, Facultad De Medicina De Ciudad Real, CIUDAD REAL, Spain

Alzheimer's disease (AD) is characterized by accumulation of extracellular amyloid-β and intracellular hyperphosphorylated tau. Due to early damage to the amygdala, personality changes often precede other clinical symptoms, such as cognitive impairment. Moreover, amygdala constitutes a key hub that may contribute to the spreading of pathologic molecules due to its vast connectivity with other brain regions. Therefore, amygdala constitutes an essential area for AD pathology that has been partially neglected. State-of-the-art -omic approaches would allow multilevel analysis but are currently underdeveloped. Proteomic studies on human tissue would be especially suitable to identify peptide fingerprint changes in human amygdala during pathology. Post-mortem tissue was provided by IDIBAPS, BTCIEN, BIOBANC-MUR, BPA and NAVARRABIOMED Spanish National Biobanks. A total of 16 cases were used for proteomic analyses consisted in PCA, heatmap, volcano plot and detection of activated/deactivated pathways. Immunofluorescence was performed for proteins distribution evaluation. Proteomic analysis revealed upregulated proteins related to pathological proteins formation (PPP1R1A and ANP32E) and cellular responses to pathology (SNRPA, DDX3X, GAN, RGCC, MACROH2A1, PSMB8). These results provided novel data on proteomic changes in human amygdala during AD and focus attention in potential biomarkers. The study was sponsored by the UCLM/ERDF (2021-GRIN-31233to NPND), Spanish Ministries of Economy and Competitiveness/ERDF (grant no. SAF2016-75768-R) and Science and Innovation (grant no. PID2019-108659RB-I00) to AMM and Autonomous Government of Castilla- La Mancha/ERDF (grant no. SBPLY/17/180501/000430) to AMM and DSS. MGR and SVC held a predoctoral fellowship granted by UCLM/ESF and VAL held an assistant professorship granted by UCLM/ERDF.

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BOARD NUMBER: S07-254

ANTI-GLUA3 ANTIBODIES IN FRONTOTEMPORAL DEMENTIA: AN IN VIVO APPROACH

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Background: Frontotemporal dementia (FTD) is a common type of presenile dementia that presents as a clinically and neuropathologically heterogeneous disorder. Recently, autoantibodies directed against the GluA3 subunit of AMPA-type glutamate receptors (AMPARs) have been identified in 20% to 25% of FTD patients. Data from patients and in vitro/ex vivo studies indicate that anti-GluA3 IgG negatively affect glutamatergic neurotransmission. *Aims:* We studied whether and how the chronic presence of anti-GluA3 IgG triggers a neurodegenerative process in mice and the association between this process and the appearance of FTD-related neuropathological and behavioural signature. What is more, we investigated a possible rescue strategy to counteract the detrimental effects mediated by anti-GluA3 IgG. *Methods:* We developed a chronic mouse model of autoimmunity in FTD by infusing mice with anti-GluA3 IgG isolated from FTD patients for one month through an intracerebroventricular cannula. The model was used to perform morphological and biochemical analyses and behavioural tasks. *Results:* Data showed that chronic anti-GluA3 IgG administration led to the appearance of FTD-related neuropathological markers and to dendritic spine loss in mice prefrontal cortex. In addition, we identified alterations in sociability and cognition that partially reflect those deficits proper of FTD GluA3+ patients. *Conclusions:* Our model allowed to identify the specific contribution of anti-GluA3 autoantibodies to FTD neuropathology which will be instrumental to the development of a putative therapeutic strategy for GluA3+ patients.

Pubmed:
33050350: Italia M, Forastieri C, Longaretti A, Battaglioli E, Rusconi F
Rationale, Relevance, and Limits of Stress-Induced Psychopathology in Rodents as Models for Psychiatry Research: An Introductory Overview.
Emotional and cognitive information processing represent higher-order brain functions. They require coordinated interaction of specialized brain areas via a complex spatial and temporal equilibrium among neuronal cell-autonomous, circuitry, and network mechanisms. The delicate balance can be corrupted by stressful experiences, increasing the risk of developing psychopathologies in vulnerable individuals. Neuropsychiatric disorders affect twenty percent of the western world population, but therapies are still not effective for some patients. Elusive knowledge of molecular pathomechanisms and scarcity of objective biomarkers in humans present complex challenges, while the adoption of rodent models helps to improve our understanding of disease correlate and aids the search for novel pharmacological targets. Stress administration represents a strategy to induce, trace, and modify molecular and behavioral endophenotypes of mood disorders in animals. However, a mouse or rat model will only display one or a few endophenotypes of a specific human psychopathology, which cannot be in any case recapitulated as a whole. To override this issue, shared criteria have been adopted to deconstruct neuropsychiatric disorders, i.e., depression, into specific behavioral aspects, and inherent neurobiological substrates, also recognizable in lower mammals. In this work, we provide a rationale for rodent models of stress administration. In particular, comparing each rodent model with a real-life human traumatic experience, we intend to suggest an introductive guide to better comprehend and interpret these paradigms.
Int J Mol Sci, 2020; 21
34246733: Scheggia D, Stanic J, Italia M, La Greca F, Zianni E, Benussi A, Borroni B, Di Luca M, Gardoni F
GluA3 autoantibodies induce alterations in dendritic spine and behavior in mice.
Autoantibodies targeting the GluA3 subunit of AMPA receptors (AMPARs) have been found in patients with Rasmussen's encephalitis and different types of epilepsy and were associated with the presence of learning and attention deficits. Our group recently identified the presence of anti-GluA3 immunoglobulin G (IgG) in about 25% of patients with frontotemporal dementia (FTD), thus suggesting a novel pathogenetic role also in chronic neurodegenerative diseases. However, the in vivo behavioral, molecular and morphological effects induced these antibodies are still unexplored. We injected anti-GluA3 IgG

purified from the serum of FTD patients, or control IgG, in mice by intracerebroventricular infusion. Biochemical analyses showed a reduction of synaptic levels of GluA3-containing AMPARs in the prefrontal cortex (PFC), and not in the hippocampus. Accordingly, animals injected with anti-GluA3 IgG showed significant changes in recognition memory and impairments in social behavior and in social cognitive functions. As visualized by confocal imaging, functional outcomes were paralleled by profound alterations of dendritic spine morphology in the PFC. All observed behavioral, molecular and morphological alterations were transient and not detected 10-14 days from anti-GluA3 IgG injection. Overall, our in vivo preclinical data provide novel insights into autoimmune encephalitis associated with anti-GluA3 IgG and indicate an additional pathological mechanism affecting the excitatory synapses in FTD patients carrying anti-GluA3 IgG that could contribute to clinical symptoms.
Brain Behav Immun, 2021; 97
34743951: Italia M, Ferrari E, Di Luca M, Gardoni F
GluA3-containing AMPA receptors: From physiology to synaptic dysfunction in brain disorders.
In the mammalian brain, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (AMPARs) play a fundamental role in the activation of excitatory synaptic transmission and the induction of different forms of synaptic plasticity. The modulation of the AMPAR tetramer subunit composition at synapses defines the functional properties of the receptor. During the last twenty years, several studies have evaluated the roles played by each subunit, from GluA1 to GluA4, in both physiological and pathological conditions. Here, we have focused our attention on GluA3-containing AMPARs, addressing their functional role in synaptic transmission and synaptic plasticity and their involvement in a variety of brain disorders. Although several aspects remain to be fully understood, GluA3 is a widely expressed and functionally relevant subunit in AMPARs involved in several brain circuits, and its pharmacological modulation could represent a novel approach for the rescue of altered glutamatergic synapses associated with neurodegenerative and neurodevelopmental disorders.
Neurobiol Dis, 2021; 161

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THE IMPACT OF ALZHEIMER'S DISEASE ON THE HUMAN SYNAPTOME

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Introduction: Synapse pathology is one of the leading determinants of the onset and progression of Alzheimer's Disease (AD). With current therapies aimed at reducing and clearing pathogenic Aβ and tau failing, it is becoming increasingly apparent that we need to look at AD pathology differently, considering the impact of these toxic proteins on molecular diversity at the level of the synapse. **Aim:** As part of the UK Dementia Research Institute (DRI) multi- 'omics atlas project for AD (MAP-AD), we aimed to use synaptome mapping approaches to characterise synapse types and to identify vulnerable and resilient synapses throughout AD progression. **Methods**: By combining immunofluorescent labelling of synaptic proteins, high throughput single-synapse resolution spinning disk microscopy, and deep learning technology, we are developing a comprehensive synaptome mapping pipeline for human post-mortem tissue, assessing six cortical brain regions in early- (Braak III-IV) and late- (Braak V-VI) stage AD. **Results**: An optimised immunolabelling protocol has been established using data-driven approaches and a quantitative analysis of the results will be presented. Preliminary observations have shown spatiotemporal differences in both excitatory and inhibitory synaptic proteins and a systematic and quantitative analysis of the changes in AD will be assessed and presented. **Conclusion**: Uncovering the impact of synapse diversity on AD will provide invaluable information for identifying synapse-specific biomarkers and therapeutic strategies and to help resolve the fundamental mechanisms of cognitive decline in AD.

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IMPAIRED DYNAMICS OF BRAIN PRECAPILLARY SPHINCTERS AND PERICYTES AT FIRST ORDER CAPILLARIES EXPLAINS REDUCED NEUROVASCULAR FUNCTIONS IN AGING

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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The microvascular inflow tract (MIT), comprising the penetrating arterioles, precapillary sphincters, and first order capillaries, is the bottleneck for brain blood flow and energy supply. However, the exact structural and functional alterations of the MIT during aging remain elusive. *In vivo* 4-dimensional two-photon imaging showed an age-dependent decrease in vaso-responsivity, with reduced sensitivity of the MIT to pinacidil and papaverine, vasoconstrictor endothelin-1, and nitric oxide synthase inhibitor L-NAME. This was accompanied by an age-dependent decrease in capillary density close to the arterioles and loss of mural cell processes, though the number of mural cell somas and αSMA density were preserved. The age-related reduction in vascular reactivity was most pronounced at precapillary sphincters, highlighting their crucial role in capillary blood flow regulation. Mathematical modeling revealed dysregulated but preserved pressure and flow in aged mice during vasoconstriction. Preventing reduced responsivity of the MIT may ameliorate the blood flow decrease associated with aging-related brain frailty.

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BOARD NUMBER: S07-257

ANXIETY AND SOCIAL-LIKE DEFICITS IN ALZHEIMER'S DISEASE IN THE TGF344-AD RAT

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Transgenic animal models in research of neuropsychiatric diseases consist primarily of mice. However, rats display a more complex behavioural repertoire, and their physiological and genetic background is more similar to humans. Therefore, studying Alzheimer's disease (AD) could benefit more from using rats rather than mice. We performed behavioural tasks assessing anxiety, spatial memory, and social interactions in 10- and 14-month-old TgF344-AD male and female rats and compared them to the control F344 rats. Our results showed that spatial acquisition and reversal in the Morris Water Maze task were surprisingly unaffected. In contrast, 10-month-old TgF344-AD male rats displayed an anxiety-like phenotype in the open field and elevated plus-maze. Interest in social interactions was significantly decreased in the TgF344-AD rats of both ages and sexes. We did not observe a worsening of the behavioural deficits with age. As the next step, we aim to analyse if the histopathological changes could have been reflected in performance. The project is supported by START/MED/099 grant and GACR grant GF21-16667K.

Pubmed:

33994956: Cernotova D, Stuchlik A, Svoboda J
Transient Inactivation of the Medial Prefrontal Cortex and Ventral Hippocampus Impairs Active Place Avoidance Retrieval on a Rotating Arena.
It is well known that communication between the medial prefrontal cortex (mPFC) and the ventral hippocampus (vHPC) is critical for various cognitive and behavioral functions. However, the exact role of these structures in spatial coordination remains to be clarified. Here we sought to determine the involvement of the mPFC and the vHPC in the spatial retrieval of a previously learned active place avoidance task in adult male Long-Evans rats, using a combination of unilateral and bilateral local muscimol inactivations. Moreover, we tested the role of the vHPC-mPFC pathway by performing combined ipsilateral and contralateral inactivations. Our results showed not only bilateral inactivations of either structure, but also the combined inactivations impaired the retrieval of spatial memory, whereas unilateral one-structure inactivations did not yield any effect. Remarkably, muscimol injections in combined groups exerted similar deficits, regardless of whether the inactivations were contralateral or ipsilateral. These findings confirm the importance of these structures in spatial cognition and emphasize the importance of the intact functioning of the vHPC-mPFC pathway.
Front Neural Circuits, 2021; 15
34116140: Cernotova D, Stuchlik A, Svoboda J
Roles of the ventral hippocampus and medial prefrontal cortex in spatial reversal learning and attentional set-shifting.
Neural components enabling flexible cognition and behavior are well-established, and depend mostly on proper intercommunication within the prefrontal cortex (PFC) and striatum. However, dense projections from the ventral hippocampus (vHPC) alter the functioning of the medial PFC (mPFC). Dysfunctional hippocampo-prefrontal connectivity negatively affects the integrity of flexible cognition, especially in patients with schizophrenia. In this study, we aimed to test the role of the vHPC and mPFC in a place avoidance task on a rotating arena using two spatial flexibility task variants - reversal learning and set-shifting. To achieve this, we inactivated each of these structures in adult male Long-Evans rats by performing bilateral local muscimol (a GABA receptor agonist) injections. A significantly disrupted performance was observed in reversal learning in the vHPC-inactivated, but not in the mPFC-inactivated rats. These results confirm the notion that the vHPC participates in some forms of behavioral flexibility, especially when spatial cues are needed. It seems, rather unexpectedly, that the mPFC is not taxed in these flexibility tasks on a rotating arena.
Neurobiol Learn Mem, 2021; 183

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EARLY DISRUPTION OF SOCIAL MEMORY IN A TGF344-AD RAT MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Social memory dysfunction is a frequent yet neglected hallmark of Alzheimer's disease (AD). The hippocampus, particularly CA2, is implicated in social memory. We used 6-month-old TgF344-AD rats, an animal model of AD, to investigate their social behaviour and memory and electrophysiological properties of their hippocampal CA2 region. We tested TgF344-AD male and female rats in a modified 5-trial social memory test (5-TSMT). This task consists of four trials presenting the first rat intruder in the subject's home cage, followed by the fifth trial presenting the second rat intruder. Compared to control F344 rats, TgF344-AD rats were less interested in the first intruders, indicating reduced sociability. Moreover, we found a diminished preference for social novelty in the TgF344-AD rats when the second intruder was presented. Next, we asked if AD is reflected in the electrophysiological properties of the CA2 in freely behaving TgF344-AD rats during the 5-TSMT and in the following memory consolidation during sleep. Analysis of electrophysiological LFP recordings from bilaterally implanted electrodes in CA2 is currently underway. We aim to analyze the power of theta and gamma frequency bands and sharp-wave ripples distribution during the consolidation period. **The project is supported by START/MED/099 grant and GACR grant GF21-16667K.**

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THE C-TERMINAL OF MT5-MMP REGULATES C99 PROCESSING AND AMYLOID-BETA LEVELS IN A CELL MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) remains a major public health challenge without efficient treatment. Current knowledge of the molecular mechanisms involved in the amyloid hypothesis is insufficient to design effective therapeutic strategies. Recent advances have revealed a new therapeutic target upstream of amyloid beta (Aβ) production and accumulation - i.e., the APP β C-terminal fragment (C99) -, which correlates better with the symptomatology of the disease. In this context, we have identified the transmembrane/intracellular domains (TM/IC) of MT5-MMP (MT5 thereafter) as key regulators of C99 levels, with a direct impact on Aβ secretion, in mice and human-cell based models. Here, we used mutated variants of these MT5 domains to modulate C99 accumulation and Aβ secretion in human embryonic kidney cells (HEK) overexpressing C99. We found that the TM/IC of MT5 might be differently implicated in C99 trafficking and fate. Moreover, we identified two different groups of amino-acids at the N-terminal and C-terminal parts of the IC domain that are likely involved in the production and/or secretion of Aβ. Currently, we are transposing these experiments in a more physiological system based on neural cells derived from human induced pluripotent stem cells carrying AD-related mutations. In the near future we expect to: i) confirm the effects of our constructs on C99 metabolism in human neurons and ii) assess the outcome of this modulation on synaptic activity and integrity. Ultimately, our goal is to better understand the MT5 pathological mechanisms in AD in order to design therapeutic agents that can potentially counteract its effects.

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AUDITORY SENSORY DEPRIVATION INDUCED BY NOISE EXPOSURE EXACERBATES COGNITIVE DECLINE AND HIPPOCAMPAL DYSFUNCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aims: Epidemiological evidence suggests a strong association between hearing loss and increased risk for the onset of neurodegenerative disorders, including Alzheimer's disease (AD). However, this relationship remains still controversial. The aim of this study was to evaluate in an animal model of AD (3×Tg-AD mice) if and how hearing loss induced by noise exposure at 2 months of age (when the AD phenotype is not manifest yet) can accelerate and/or worsen AD cognitive impairment, affecting auditory and hippocampal functions. **Methods:** 3×Tg-AD and B6129SF2/J wild-type mice of 2 months of age (M) were exposed to noise (pure tone, 10 kHz, 100 dB SPL, 10 days, 60 min/day). One and 4 months later (corresponding to 3 and 6 M), the detrimental effects of hearing loss in the auditory cortex and hippocampus were investigated by morphological (spine density analyses), electrophysiological (*ex-vivo* recordings of field-excitatory post-synaptic potentials -fEPSPs), behavioral, and molecular biology experiments (focusing on redox imbalance, neuroinflammation, and tau phosphorylation). **Results:** Noise-exposed 3×Tg-AD mice showed persistent synaptic and morphological alterations (reduced fEPSP amplitude and dendritic spine density) in the auditory cortex. Moreover, we found early hippocampal dysfunctions, such as decreased spine density in CA1 and dentate gyrus, decreased fEPSP amplitude, increased tau-phosphorylation, neuroinflammation, and oxidative stress, along with anticipated memory deficits compared to the expected neurodegenerative phenotype time-course. **Conclusion:** The mouse model of AD is more vulnerable to central nervous system damage induced by hearing loss and shows reduced ability to counteract noise-induced detrimental effects, which accelerates the neurodegenerative disease onset.

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ASTROCYTIC CA2+ SIGNALING IN THE PROGRESSION OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a chronic incurable neurodegenerative disorder characterized by progressive memory loss and cognitive dysfunctions. Noteworthy, brain functions and dysfunctions are governed by dynamic interactions between neurons and astrocytes. We here evaluate the involvement of these glial cells in the pathogenesis of AD, focusing on astrocytic Ca^{2+} signaling and its dysregulation during AD progression. The experiments were carried out in female mice at 3 and 6 months of age, before and after, respectively, the onset of plaque deposition in the PS2APP mouse model of AD that expresses the human PS2-N141I and APP Swedish mutations. To investigate astrocytic activity, we performed two-photon Ca^{2+} imaging experiments in brain slice and *in vivo* preparations of somato-sensory cortex (SSCx) astrocytes expressing GCaMP6f. We found that astrocyte Ca^{2+} activity exhibits a sequence of changes: in 3-month-old PS2APP mice, spontaneous activity significantly increases, while in 6-month-old PS2APP mice both spontaneous activity and the responsiveness to agonists of different metabotropic receptors are drastically reduced in all astrocytic territories. Although these defects start in concomitance with plaque deposition, astrocytic Ca^{2+} hypoactivity is unrelated to plaque proximity. We evaluate the consequences of these alterations for SSCx long-term memory processes. Importantly, we found that in 6-month-old PS2APP mice astrocytic Ca^{2+} hypoactivity is associated to a strong impairment of long-term potentiation in SSCx circuits. In conclusion, astrocytic Ca^{2+} activity is strongly affected in AD at the onset of plaque deposition and can be considered as a functional hallmark of the disease.

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RESTING-STATE ACTIVITY IN THE BASAL FOREBRAIN PREDICTS FUNCTIONAL DEGENERATION IN THE ENTORHINAL CORTEX AND DECREASES WITH ALZHEIMER'S DISEASE PROGRESSION

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aims. Recent models of Alzheimer's Disease (AD) suggest the basal forebrain's nucleus basalis of Meynert (NbM) as the origin of structural degeneration followed by the entorhinal cortex (EC). Both brain regions show a selective vulnerability to proteinopathies and, thus, the spread of structural degeneration relates to harmonized CSF assays of amyloid-band hyperphosphorylated (pTau). Despite initial evidence from anatomical studies, the functional properties of NbM and EC during the disease progression of AD remain unclear. **Methods.** We analyzed open access data from the Alzheimer's Disease Neuroimaging Initiative (ADNI, n= 71) with CSF assays and resting-state fMRI data over two years. Specifically, fractional amplitude of low-frequency fluctuations (fALFF) was used to quantify spontaneous neuronal activity in BOLD fMRI (0.01-0.10 Hz) within the NbM and EC at baseline and two years later. Robust regression modeling was used to address the hypothesis of a spread from NbM to EC. **Results.** At baseline, fALFF differentiated between normal and abnormal CSF groups in both brain regions with a more pronounced reduction in NbM compared to EC. Importantly, only NbM activity at baseline predicted the annual percentage signal change in EC, but not the reverse. Accordingly, local activity within NbM but not EC linearly resembled the disease stage with highest activity in cognitive normal volunteers with normal CSF vs. lowest activity in AD patients with abnormal CSF. **Conclusions.** Our findings give novel insights into the pathogenesis of AD by showing that functional activity in the basal forebrain reliably predicts functional degeneration within the EC.

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SPIN90 DEFICIENCY AMELIORATES AMYLOID β ACCUMULATION BY REGULATING APP TRAFFICKING IN AD MODEL MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer's disease (AD), a common form of dementia, is caused in part by the aggregation and accumulation in the brain of amyloid β (Aβ), a product of the proteolytic cleavage of amyloid precursor protein (APP) in endosomes. Trafficking of APP, such as surface-endosomal recycling, is an early critical step required for Aβ generation. Less is known, however, about the molecular mechanism regulating APP trafficking. In this study, we investigated the correlation of APP trafficking with SPIN90, along with Rab11, Aβ accumulation, and synaptic functionality. Brain Aβ accumulation was lower in the progeny of 5xFAD-SPIN90 KO than in 5xFAD-SPIN90 WT mice. Analysis of APP distribution and trafficking showed that the surface fraction of APP was locally distinct in axons and dendrites, with these distributions differing significantly in 5xFAD-SPIN90 WT and 5xFAD-SPIN90 KO mice, and neural activity-driven APP trafficking to the surface and endosomal recycling were more actively mobilized in 5xFAD-SPIN90 KO neurons. In addition, SPIN90 was found to be cotrafficked with APP via axons, with ablation of SPIN90 reducing the endosomal accumulation of APP in axons. Finally, synaptic transmission was restored over time in 5xFAD-SPIN90 KO, but not in 5xFAD-SPIN90 WT, neurons, suggesting SPIN90 is implicated in Aβ production by regulating APP trafficking.

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CAN OLIGODENDROCYTES CONTRIBUTE TO Aβ PLAQUE FORMATION?

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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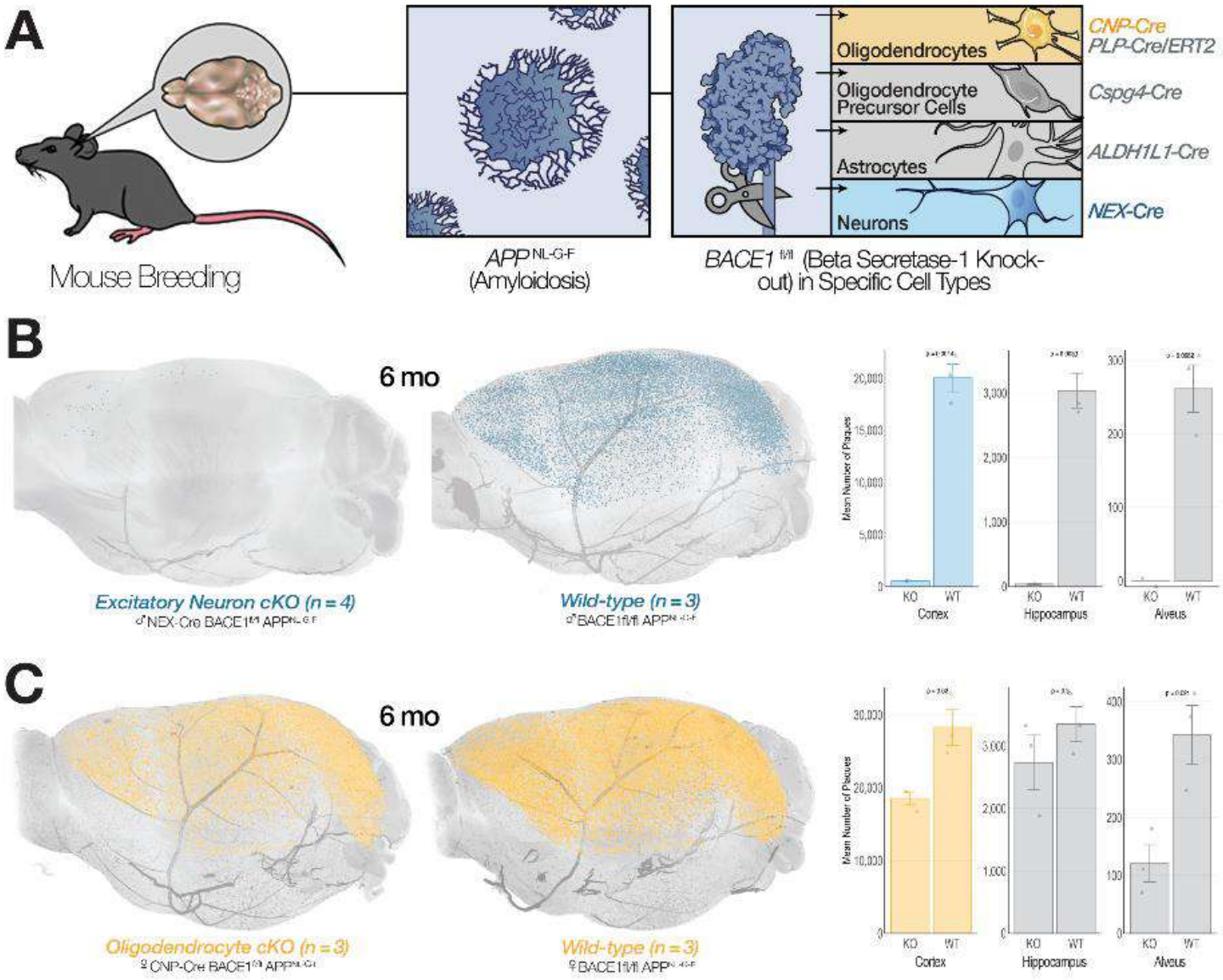
It is conventionally believed that amyloid plaques are aggregated products of amyloid beta (Aβ) solely produced by excitatory neurons. Within the central nervous system, however, key players of Aβ production, namely amyloid precursor protein and beta secretase (BACE), can be abundantly found in cells other than neurons as well, including oligodendrocytes. Here, we ask whether non-neuronal cells can contribute to the amyloid burden. We generated amyloidosis mice (APP NL-G-F) with cell-type-specific (i.e., excitatory neurons, NEX-Cre; oligodendrocytes, CNP-Cre) knock-out of BACE1 (Figure1A), the rate-limiting enzyme in Aβ production, to measure potential reductions in plaque formation. To this end, we employed light sheet microscopy to perform a whole brain 3D segmentation of amyloid plaques to bypass the spatial limitations of 2D imaging. First, we investigated whether plaques are still produced in mice lacking excitatory neuronal BACE1. At three and six months, these mice developed zero and very few plaques respectively in the forebrain (Figure1B), calling into question the critical Aβ concentration required for plaque deposition. Next, we assessed whether oligodendrocytes contribute to plaque burden. Surprisingly, we observed varying regional reductions in plaque formation in this model, with the largest attenuation seen in white matter tracts such as the alveus, where oligodendrocytes are ample (Figure1C). We concluded that: 1) Whole-brain plaque formation still requires the bulk of Aβ produced by forebrain excitatory neurons, and 2) To a lesser extent, oligodendrocytes do contribute to the amyloid burden, although it is less likely that they can initiate the advent of plaque

formation.


A
Mouse Breeding
APP NL-G-F (Amyloidosis)
BACE1 fl/fl (Beta Secretase-1 Knock-out) in Specific Cell Types
Oligodendrocytes
Oligodendrocyte Precursor Cells
Astrocytes
Neurons
CNP-Cre
PLP-Cre/ERT2
Cspg4-Cre
ALDH1L1-Cre
NEX-Cre
B
6 mo
Excitatory Neuron cKO (n = 4)
♂NEX-Cre BACE1fl/fl APPNL-G-F
Wild-type (n = 3)
♂BACE1fl/fl APPNL-G-F
Mean Number of Plaques
KO
WT
Cortex
Hippocampus
Alveus
C
6 mo
Oligodendrocyte cKO (n = 3)
♀CNP-Cre BACE1fl/fl APPNL-G-F
Wild-type (n = 3)
♀BACE1fl/fl APPNL-G-F
Mean Number of Plaques
KO
WT
Cortex
Hippocampus
Alveus

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CENTRAL INCRETIN INHIBITION LEADS TO CHANGES IN BRAIN CELL ENERGY AND METABOLISM IN A RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aims The incretin system is an emerging new field that might provide valuable contributions to the research of pathophysiology and treatment of neurodegenerative disorders. We aimed to explore the role of central glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) on cell metabolism and energy in the brain of a streptozotocin-induced rat model of sporadic Alzheimer's disease. Methods One month after streptozotocin-intracerebroventricular application (STZ-icv, 3 mg/kg), Wistar rats were icv treated with vehicle or respective antagonist of GLP-1 (Exendin (9-39) amide; (Ex9) or GIP-receptor (Pro-3GIP), and sacrificed 30 minutes afterward. Protein expression of: c-fos,p/tAMPK,COXIV,CytC in hippocampus (HPC) and hypothalamus (HPT) was measured by Western blot. Insulin,GLP-1 and GIP concentration in plasma was measured by ELISA, and glucose by GOD-PAP test. Data were analysed by Kruskal-Wallis one-way ANOVA and Mann-Whitney-U test ($p<0.05$). Results In HPC Ex9 and Pro-3GIP treatment decreased the ratio of p/tAMPK while in HPT decrement of COXIV of STZ-icv rats was found. Only Ex9 decreased c-fos both in control and STZ-icv rats in HPT, while in HPC increased c-fos was detected only in the STZ-icv group. CytC and glucose remained unchanged regardless the treatment. Pro-3GIP increased plasm insulin and GIP in STZ-icv rats and control while Ex9 decreased only plasma activ GLP-1 in STZ-icv rats. Conclusions Inhibition of the central GIP and GLP-1 receptor, respectively, in control and STZ-icv animals indicated a region-dependent role of incretins in the homeostasis of brain cell energy and metabolism and central incretin-dependent modulation of peripheral hormone secretion. *Funding:SCE-NEURO (*GA KK01.1.1.01.0007*), HRZZ-IP-2018-01-8938*

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THE ROLE OF APOEε4 IN BRAIN LIPID METABOLISM IN DEMENTIA

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Apolipoprotein E (ApoE) is responsible for lipid transport between cells. *APOEε3* is the most common allele of the *APOE* gene among the population, while studies have shown a significantly increased risk of Alzheimer's disease (AD) in *APOEε4*-carriers compared to individuals with *ε3*. However, how *APOEε4* contributes to disease pathogenesis remains incompletely understood. Regarding the role of ApoE in lipid transport and the role of lipid metabolism in AD highlighted by previous -omic studies, the aim of our study is to investigate the difference in lipid profile between humanised *APOEε4* and *APOEε3* knock-in mice. We performed untargeted lipidomic profiling on brain tissue from 2-month and 6-month-old *APOEε4* and *APOEε3* mice by ultrahigh-performance liquid chromatography (UHPLC) ion-mobility mass spectrometry (IMMS). Results showed a decrease in phospholipids abundance including phosphatidylcholine in the 2-month *APOEε4* mice compared to *APOEε3* mice in the negative ionisation mode. In the 6-month mice group we also observed downregulation of phospholipids in *APOEε4* related to *APOEε3* in both ionisation modes. Additionally, sphingolipids, which have been implicated in AD, were significantly decreased in 2-month *APOEε4* mice in comparison to *APOEε3* mice. Our results suggest that *APOEε4* contributes to AD pathology via lipid metabolism modulation.

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TOWARD THE ROLE OF SV2A PROTEIN IN HIPPOCAMPAL APP PROCESSING

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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In vivo brain imaging reveals that greater hippocampal activation at baseline is associated with increased Aβ accumulation and declining memory performance. Our previous data demonstrated that antiepileptic agent levetiracetam, a specific modulator of SV2A glycoprotein, is involved in the regulation of synaptic vesicle readiness for fusion and neurotransmitter release in hippocampus. As synaptic activity rapidly and dynamically affects Aβ levels, we aimed to study the action of $A\beta_{1-42}$ and $A\beta_{1-40}$ peptides on synaptic vesicle release, and the efficiency of levetiracetam therapy for complex inflammation-associated changes in hippocampus. Rats with LPS-induced neuroinflammation displayed the prevalence of excitatory over inhibitory transmission in hippocampus, impaired synaptic vesicle recycling and higher hippocampal APP levels, all being responsive to therapy with levetiracetam. We demonstrated that soluble oligomers of $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides impair the storage capacity, clustering and Ca^{2+}-dependent fusion of SVs, which under intracellular accumulation of Aβ oligomers can reduce exocytotic neurotransmitter release. Oligomeric forms of $A\beta_{1-40}$ and $A\beta_{1-42}$ increase SV clustering and arrest the vesicles at this state, which significantly decreases their Ca^{2+}-triggered fusion. Therapy with levetiracetam upregulated hippocampal SV2A and restored the level of apolipoprotein E, involved in brain Aβ aggregation/clearance and resolution of inflammation. Our data suggests that in the course of progression of neuroinflammation oligomeric $A\beta_{1-42}$ and $A\beta_{1-40}$ can compromise synaptic vesicle fusion machinery and that levetiracetam, by acting on synaptic vesicle recycling and restricting overexcitation is able to affect APP processing and Aβ generation within the hippocampus *in vivo*.

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NON-SELECTIVE SODIUM-GLUCOSE COTRANSPORTER INHIBITOR ALTERS CENTRAL AND PERIPHERAL METABOLIC PARAMETERS, BUT FAILS TO IMPROVE COGNITIVE DEFICIT IN A RAT SPORADIC ALZHEIMER'S DISEASE MODEL

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aims: Sporadic Alzheimer's disease (sAD) and metabolic dysfunction in type 2 diabetes mellitus share a common pathomechanism and drugs available for treatment of diabetes are increasingly being studied as possible sAD therapy. Hence, we aimed to research whether phloridzin, a non-selective sodium-glucose cotransporter inhibitor (SGLTI), may influence metabolic and cognitive parameters in a rat model of sAD. Methods: 4 groups (N=10) of 3-month-old Wistar rats were randomized to controls (CTR), sAD model induced by intracerebroventricular (icv) administration of streptozotocin (STZ; 3 mg/kg divided in two doses), CTR+SGLTI and STZ+SGLTI. SGLTI treatment by oral gavage (10 mg/kg) was initiated 1 month after STZ-icv and lasted two months. Cognitive assessment by Morris water maze and Passive avoidance tests was performed prior to sacrification and tissue collection. Results: SGLTI treatment failed to correct the cognitive deficit induced by STZ-icv in both tests. Weight gain in SGLTI treated rats was diminished compared to non-treated CTR and STZ-icv. While intraperitoneal glucose tolerance test revealed no differences between tested groups, plasma glucose levels were lowered by SGLTI in both CTR (-51%, p=0.0006) and STZ (-39%, p=0.0057) compared to CTR alone. Total glucagon-like peptide 1 (GLP-1) levels were altered both in plasma (-36% CTR+SGLTI vs CTR, p=0.0279; -28% STZ+SGLTI vs STZ, p=0.0535) and CSF (+59% STZ+SGLTI vs CTR, p=0.0115). Plasma and CSF glucose-dependent insulinotropic polypeptide (GIP) and CSF glucose levels were unchanged. Conclusions: Increased CSF GLP-1 levels opposing decreased peripheral ones indicates the brain's capacity to compensate for obstructed intestinal nutrient absorption. Funding: SCE-NEURO (GA KK01.1.1.01.000), HRZZ-IP-2018-01-8938.

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INHIBITION OF BROMODOMAIN AND EXTRATERMINAL (BET) PROTEINS MODULATES THE EXPRESSION OF ALZHEIMER'S DISEASE RISK GENES IN MICROGLIA

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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The genome-wide association studies proved the significant contribution of the immune system to the pathomechanism of Alzheimer's disease (AD). Data showed that polymorphism of several immune-related genes increases the risk of developing AD. Dysregulation of these genes' transcription may affect neuroinflammatory signaling and may potentially contribute to AD. A better understanding of mechanisms controlling the expression of these genes may enable novel therapeutical strategies for AD. In our study, we focused on bromodomain and extraterminal (BET) proteins, the readers of histone acetylation code, which have been demonstrated to control the expression of inflammation-related genes. We used a pharmacological (JQ1, 50 nM) approach for BETs' inhibition in mouse microglial BV2 cells, both in control conditions and in the presence of amyloid-beta (Aβ). The expression of tested genes was analyzed using the qPCR method. Our results demonstrated that JQ1 significantly reduced the expression of *Trem2* and *Cd33*, but increased mRNA levels for *Abca7*, *Bin1*, and *Cr1*. Transcription of *Cd2ap*, *Picalm*, and *Clu* was not changed by inhibition of BET proteins. The mRNA for *Epha1* was not detected in BV2 cells. Aβ had no significant impact on the transcription of tested genes in microglial cells. Our results demonstrated that BET proteins control mRNA levels of genes that were identified as genetic risk factors for AD, therefore the modulation of epigenetic regulation of transcription, including inhibition of BET proteins, may potentially offer a new strategy for controlling neuroinflammatory signaling in AD. Funding: Narodowe Centrum Nauki, grant 2018/31/B/NZ4/01379

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TRAIL-R DEFICIENT MICE ARE PROTECTED FROM NEUROTOXIC EFFECTS OF AMYLOID-β

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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TRAIL, a member of TNF superfamily, is a potent inducer of neuronal death. Neurotoxic effects of TRAIL appear mediated by its death receptor TRAIL-R2/DR5. To assess the role of TRAIL/TRAIL-R2 pathway in AD-related neurodegeneration, we studied the impact of the treatment with amyloid-β (Aβ) upon cell viability and inflammation in TRAIL-R-deficient mice (TRAIL-$R^{-/-}$). Here, we demonstrate that the lack of TRAIL-R2 protects from death cultured TRAIL-$R^{-/-}$ mouse embryonic hippocampal cells undergone treatment with either Aβ1-42 or TRAIL. Consistently, stereotaxic injection of Aβ1-42 resulted in blunted caspase activation, as well as in reduction of JNK phosphorylation and increased AKT phosphorylation in TRAIL-$R^{-/-}$ mice. Moreover, the lack of TRAIL-R2 was associated with blunted constitutive p53 expression in mice undergone Aβ1-42 treatment, as well as in decrease of phosphorylated forms of tau and GSK3β protein. Likewise, TRAIL-R2 appears essential to both TRAIL-Aβ mediated neurotoxicity and inflammation. Indeed, hippocampi of TRAIL-$R^{-/-}$ mice, challenged with Aβ1-42, showed a scanty expression of microglial (Iba-1) and astrocytic (GFAP) markers along with attenuated levels of IL-1β, TNF-a, iNOS and COX2. In conclusion, the bulk of these results demonstrate that the constitutive lack of TRAIL-R2 is associated with a substantial reduction of noxious effects of Aβ1-42, providing further evidence on the prominent role played by TRAIL in course of Aβ-related neurodegeneration and confirming that the TRAIL system represents a potential target for innovative AD therapy.

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O-GLCNACYLATION ALLEVIATES THE PATHOLOGICAL FEATURES OF ALZHEIMER'S DISEASE BY INHIBITING NECROPTOSIS

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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O-linked β-N-acetylglucosaminylation (O-GlcNAcylation) is a post translational modification which is enriched in brain and several O-GlcNAcylated proteins, such as tau and α-synuclein, are associated with neurodegenerative diseases. It has been known that O-linked β-N-acetylglucosaminase (OGA) inhibition decreased the amount of Aβ plaques in the brain of AD mouse models. Necroptosis has been found to be activated in humans' brains with AD and is positively correlated with the pathological features like neuronal death, neuroinflammation. Inhibition of necroptosis in AD effectively suppresses neuroinflammation. We evaluated the expression level of O-GlcNAcylation-related proteins, necroptosis-related proteins, and Aβ in human brain samples and mouse brain samples by western blotting and/or immunohistochemistry. Furthermore, we generated 5xFAD with OGA haploinsufficiency and evaluated the cognitive function by behavioral tests. Expression level of O-GlcNAcylation was decreased in both human brains with AD and 5xFAD brains. Also, the expression levels of necroptosis-related proteins were increased in both human brains with AD and 5xFAD brains. OGA haploinsufficiency increased O-GlcNAcylation levels in the brain and decreased activation of necroptosis, thereby reducing neuronal loss in the brain of 5xFAD. Furthermore, increased O-GlcNAcylation levels ameliorated cognitive deficits in 5xFAD mice and reduced Aβ accumulation. Here, we found that O-GlcNAcylation plays a protective role in AD by inhibiting necroptosis. O-GlcNAcylation ameliorated AD pathology including Aβ plaques, neuronal loss, neuroinflammation and cognitive decline. Thus, our data establish the influence of O-GlcNAcylation on Aβ accumulation and neurodegeneration, suggesting O-GlcNAcylation-based treatments as potential interventions for AD.

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DELETERIOUS EFFECTS OF ALZHEIMER'S DISEASE-CAUSING PRESENILIN-1 MUTATIONS ON MITOCHONDRIAL DYNAMICS

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Mitochondrial dysfunction and oxidative stress are observed in Alzheimer's disease(AD). Studies have shown that presenilin-1(PS1) localizes to the mitochondrial membrane and regulates its homeostasis. Thus, we examined how five PS1 mutations(A431E, E280A, H163R, M146V, and Δexon9) observed in familial AD(FAD) affect mitochondria. We used H4 cell lines genetically engineered to express either the wild-type PS1 or one of the five PS1 mutants to examine mitochondrial homeostasis and functions. Furthermore, we used the brains of PS1M146V knock-in mice, 3xTg-AD mice, and human AD patients to investigate the role of PS1 in regulating MAMs formation. Each PS1 mutant exhibited slightly different mitochondrial dysfunction. Δexon9 mutant induced mitochondrial fragmentation while A431E, E280A, H163R, and M146V mutants increased MAMs formation. A431E, E280A, M146V, and Δexon9 mutants also induced mitochondrial ROS production. A431E mutant impaired both complexes I and peroxidase activity while M146V mutant only impaired peroxidase activity. All PS1 mutants compromised mitochondrial membrane potential and cellular ATP levels were reduced by A431E, M146V, and Δexon9 mutants. Comparative profiling of hippocampal gene expression in PS1M146V knock-in mice found that PS1M146V upregulates Atlastin 2(ATL2) expression level, which increases ER-mitochondria contacts. Moreover, ATL2 expression levels were significantly elevated in the brains of 3xTg-AD mice and AD patients. Overall, our findings suggest that each of the five FAD-linked PS1 mutations has a deleterious effect on mitochondrial functions. The adverse effects of PS1 mutations on mitochondria may contribute to MAMs formation and oxidative stress resulting in an accelerated age of disease onset in people harboring mutant PS1.

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NRF2 REGULATES BACE1 AND BACE1-AS EXPRESSION IN A NEGATIVE WAY

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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BACE1 is the rate-limiting enzyme for amyloid-β peptides (Aβ) generation, mainly involved in the pathogenesis of Alzheimer's disease (AD). Through an undiscovered mechanism, levels of BACE1 and a BACE1 mRNA-stabilizing antisense RNA (BACE1-AS) are increased in the brains of AD patients; implicating that dysregulation of BACE1 expression can have an effect on the AD pathogenesis. We found that nuclear factor erythroid-derived 2-related factor 2 (NRF2/NFE2L2) represses the expression of BACE1 and BACE1-AS through binding to antioxidant response elements (AREs) in their promoters of mouse and human. NRF2-mediated inhibition of BACE1 and BACE1-AS expression is independent of redox regulation. NRF2 activation decreases production of BACE1 and BACE1-AS transcripts and Aβ production, and ameliorates cognitive deficits in animal models of AD. Depletion of NRF2 increases BACE1 and BACE1-AS expression and Aβ production, and worsens cognitive deficits. Our findings suggest that activation of NRF2 can prevent a crucial early pathogenic process in AD pathogenesis.

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PREVENTION OF AMYLOIDOGENESIS BY NEURONAL AQUAPORIN 1 INHIBITING THE INTERACTION BETWEEN AMYLOID PRECURSOR PROTEIN AND BACE 1 IN ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aquaporin1 (AQP1) which belongs to the AQP family, is a membrane water channel protein. In the previous studies, it has been shown that levels of AQP1 was increased in the brain of Alzheimer's disease (AD), while the precise role of AQP1 in AD pathogenesis is unclear. In this study, we determined the expression and distribution of AQP1 in the brains of individuals with AD as well as AD mouse models. In AD patients, AQP1 was accumulated in vulnerable neurons in the cerebral cortex, and in AD mouse models, the expression of AQP1 increased with aging. We identified increased expression of AQP1 and translocation to endocytic compartments in response to stress stimuli. Considering the interaction of amyloid precursor protein (APP) with β-secretase (BACE1) in the endocytic compartments, AQP1 could inhibit the BACE1 mediated amyloidogenic APP processing which produces the neurotoxic amyloid beta (Aβ), a hallmark of AD. Consistently, a public human brain database demonstrated the negative correlation between AQP1 and Aβ levels. Collectively, these results indicate that upregulation of AQP1 expression in the brains of AD patients may be an adaptive response to cellular stress to prevent Aβ generation by suppressing the interaction between APP and BACE1.

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REGION-SPECIFIC ALTERATIONS OF ASTROCYTIC K+ CLEARANCE IN A MOUSE MODEL FOR ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Aims: Alzheimer's disease (AD) is an age-dependent neurodegenerative disorder characterised by neuronal loss and neuronal hyperexcitability which emerges during the initial stages of the disease. Previous studies indicated that astrocytes, which are responsible for K^+ homeostasis in the CNS, can affect the excitability profile of neurons via alterations in their K^+ clearance properties. Hence, the aim of this study is to assess the astrocytic K^+ clearance rate in the hippocampus and somatosensory cortex in a mouse model of AD during disease progression. Methods: Simultaneous extracellular recordings of the K^+ clearance rate and astrocytic inward currents in acute brain slices containing the somatosensory cortex and hippocampus. All recordings were taken from a mouse model for AD (5xFAD) and their littermate controls at presymptomatic and symptomatic stages (two and six months respectively). Results: We found that following transient local application of a high concentration of K^+, the K^+ clearance rate in the hippocampus of 5xFAD mice was significantly lower at both presymptomatic and symptomatic stages. This decrease was accompanied by a decrease in astrocytic inward currents in the hippocampus, but not the somatosensory cortex of 5xFAD mice. Moreover, selective inhibition of Kir4.1 channels and Cx-43 channels indicated significant differences between the clearance mechanisms at the hippocampus and the somatosensory cortices of 5xFAD mice. Conclusions: Our results indicate a region-specific dysfunction of astrocytes in 5xFAD mice expressed as a reduced K^+ clearance rate in the hippocampus but not in the somatosensory cortex. The mechanisms leading to this dysfunction involve astrocytic channels: Kir4.1 and Cx-43.

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IN VIVO LONGITUDINAL IMAGING OF RESTING STATE CONNECTIVITY IN HUMANIZED APP KI1 AND KI3 MICE

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Alzheimer Disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia that primarily effects elderly people, causing atrophy and loss of brain cells which results in a decline in memory, thinking, behavioral and social skills. The *amyloid cascade hypothesis* of AD proposes that extracellular accumulation of amyloid beta (Aβ) peptide triggers aggregation of hyperphosphorylated tau as intracellular neurofibrillary tangles which ultimately spreads across anatomically and functionally connected brain networks, yielding widespread neurodegeneration and impairment in brain function (Ricciarelli et al., 2017). For patients at this stage of the disease, it is too late: available treatments have little efficacy. It is critical, therefore, to develop treatments for intervention early in the disease, before widespread cell death renders intervention futile. First, we need to understand the early changes occurring in the AD brain, to identify targets, biomarkers, and models that can be used to develop effective early therapies. To achieve this aim, we have combined two strategies, second-generation mouse models and resting-state functional magnetic resonance imaging (rs-fMRI). We used second-generation AD mouse model carrying 3 mutations; Iberian, Artic, and Swedish ($APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ or 'APP-KI-3' mouse) and $APP^{NL/NL}$ controls. All animals scanned using 9.4T Bruker preclinical MRI for small animals and anesthetized during scanning procedures. To compare the rs-networks before and after the progression of Aβ accumulation, we scanned the same animals at 3 months and 7 months of age. Our preliminary results indicates that there was a decrease in functional connectivity at 7 months of age with APP-KI-3 mouse.

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INCREASED INCIDENCE OF DEMENTIA FOLLOWING HERPESVIRUS INFECTION IN THE KOREAN POPULATION

POSTER SESSION 07 - SECTION: ALZHEIMER'S DISEASE

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Background: Herpesviruses affect the development of dementia. We investigated the association between herpes infection and subsequent diagnoses of dementia. Methods: Data from the National Health Insurance Service of South Korea were used. Patients aged ≥ 50 years with the relevant diagnostic codes in the reference year 2009 were included and prospectively reviewed from January 2010 to December 2018. All study participants were followed from the index date until the onset of dementia, death, or the study endpoint. Results: The three cohorts comprised 92,095 patients with herpes simplex virus (HSV) infections, 97,323 patients with varicella-zoster virus (VZV) infections, and 183,779 controls. During the follow-up period, 15,831 (17.19%) subjects with HSV infection and 17,082 (17.55%) VZVinfected subjects, compared to 27,028 (14.17%) control subjects, were subsequently diagnosed with dementia (all, P<0.001). The adjusted hazard ratio for developing dementia was found to be 1.18 (95% confidence interval [CI]; 1.16-1.20) in HSV and 1.09 (95% CI; 1.07-1.11) in VZV patients (all, P<0.001). HSV1 infections such as oral or ocular subtypes, but not HSV2, anogenital subtype, were associated with dementia, including several subtypes such as Alzheimer's disease (AD), vascular dementia, and dementia with Lewy bodies. VZV infection is also associated with AD. Conclusion: In this Korean nationwide population-based cohort study, both HSV and VZV infections were associated with a higher risk of dementia, particularly AD. Among the subtypes of HSV infection, HSV1 is associated with a risk of dementia. Further studies including appropriate public health interventions could evaluate the causality of these relationships.

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MOLECULAR MECHANISM AND EXPERIMENTAL THERAPEUTICS OF ADCY5-RELATED MOVEMENT DISORDER: STUDY OF A NEW GENETIC MOUSE MODEL AND IDENTIFICATION OF THERAPEUTIC TARGETS.

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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ADCY5-related movement disorder (*ADCY5*-MDS) is a neurological disorder characterized by childhood-onset generalized hyperkinetic movements due to dominant mutations of *ADCY5*. The *ADCY5* gene encodes the adenylyl cyclase 5 (AC5), which plays a key role in cyclic AMP (cAMP) synthesis within the striatum downstream of adenosine and dopamine receptors. AC5 participates in the control of movements by modulating the subtle balance between the activity of the direct striatal-nigral and indirect striatal-pallidal pathways. To investigate the pathogenesis of this disorder and identify therapeutic targets, we generated a genetic mouse model of *ADCY5*-MDS harboring the R418W mutation, which is frequently responsible for *ADCY5*-MDS. In this model, we want to (1) describe the molecular consequences of the *ADCY5* mutation (2) characterize the motor and behavioral phenotypes, (3) test in the effects of various pharmacological modulation aimed at altering striatal cAMP concentration. Our current results indicate an increased activity of the mutant AC5 with a compensatory down-regulation of the expression of AC5 and its upstream partners in the signaling cascade together with an increased phosphorylation of the downstream targets. Mutant mice have an abnormal locomotor activity, an impaired motor coordination, a defective motor learning and an anxiety-like behavior, consistent with the human phenotypes. We observed a differential molecular and motor response of the mutant mice to the administration of an $A2_A$ receptor antagonist. We are now planning to achieve a full molecular, motor and behavioral characterization of the model and to test in details the reversibility of the observed abnormalities in response to pharmacological intervention.

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COORDINATION OF BET FAMILY PROTEINS IN FRAGILE X SYNDROME

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Bromodomain and extraterminal proteins (BET) are epigenetic readers that play critical roles in gene regulation. Pharmacologic inhibition of the bromodomain present in all BET family members is a promising therapeutic strategy for various diseases, but its impact on individual family members has not been well understood. Using a transcriptional induction paradigm in neurons, we have systematically demonstrated that three major BET family proteins (BRD2/3/4) participated in transcription with different recruitment kinetics, interdependency, and sensitivity to a bromodomain inhibitor, JQ1. In a mouse model of fragile X syndrome (FXS), BRD2/3 and BRD4 showed oppositely altered expression and chromatin binding, correlating with transcriptional dysregulation. Acute inhibition of CBP/p300 histone acetyltransferase (HAT) activity restored the altered binding patterns of BRD2 and BRD4 and rescued memory impairment in FXS. Our study emphasizes the importance of understanding the BET coordination controlled by a balanced action between HATs with different substrate specificity.

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BOARD NUMBER: S07-280

QUANTIFICATION OF NEONATAL MOTOR ACTIVITY AFTER BRAIN INJURY

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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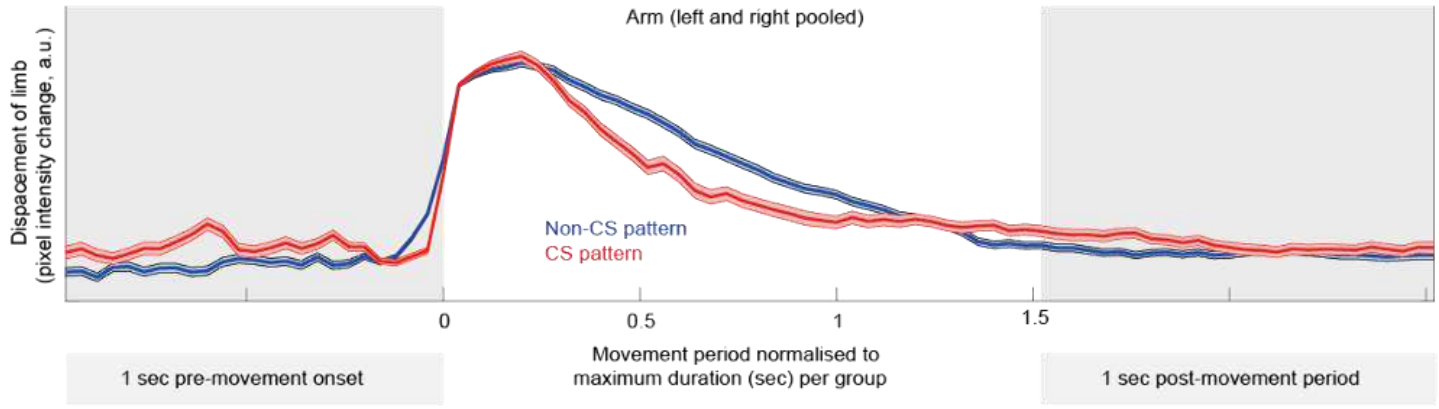
Aims In awake human neonates, spontaneous motor activity typically comprises fluid multi-limb sequences that join together individual movements lasting for ~1 sec. Absence of fluidity - the 'cramped-synchronised' (CS) pattern - has 97-100% specificity for later cerebral palsy. Here we quantified the individual limb movements underlying these two motor patterns. **Methods** Subjects were 23 neonates at risk for having sustained a brain injury. Motor activity at full-term was qualitatively scored as CS or non-CS using the Prechtl method. To quantify individual movements from video recordings, we detected frame-by-frame changes in pixel intensity within regions-of-interest. Movements were defined as ending 0.5 sec after pixel intensity change decreased below threshold, up to a maximum duration of 1.5 sec. **Results** Motor activity was scored as CS in 7/23 and non-CS in 16/23 neonates. 964 CS and 2282 non-CS movements were analysed. CS arm (but not leg) movements exhibited a significantly sharper trajectory offset (Figure) and were shorter than non-CS movements (median duration: 0.64 vs. 0.68 sec, p <.001, Mann-Whitney U
test).



Conclusions Abnormal motor function can be detected at full-term. There is a potential therapeutic window during the neonatal period to strengthen motor cortical networks. Given that recovery from injury is cortical activity-dependent in age-equivalent rats and mice, future work will examine the feasibility of modulating cortical activity to improve motor outcomes.

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SEX- AND TIME-DEPENDENT PREVENTIVE EFFECTS OF MAGNESIUM SULFATE ASSOCIATED WITH 4-PHENYLBUTYRATE IN A MOUSE MODEL OF NEONATAL HYPOXIA-ISCHEMIA

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Each year, prematurity represents more than one out of ten births worldwilde. In premature newborns, complications may occur, such as an hypoxic-ischemic (HI) event which can lead to cerebral palsy (CP) in extreme cases. CP is defined as permanent disorders of movements and posture. Boys display a greater vulnerability to develop CP than girls do. Magnesium sulfate ($MgSO_4$) is administered to mothers at risk of preterm delivery as a neuroprotective agent. However, its effectiveness is only partial. To improve its effect, a new project has been developed in the lab, consisting in combining $MgSO_4$ with a second molecule presenting neuroprotective properties. 4-phenylbutyrate (4-PBA) was selected because it presents these characteristics and it is already administered in newborns. By using a mouse model of neonatal HI, generating lesions similar to those reported in preterms, we compared $MgSO_4$ effects with the effects of the $MgSO_4$/4-PBA association, on HI-induced alterations. Effects were evaluated at behavioral and histological levels, from the postnatal period until adolescence, while investigating sex effects. In non-HI individuals, treatments potential deleterious effects were sought. At short term, both $MgSO_4$ alone and the $MgSO_4$/4-PBA association prevent HI-induced histological and behavioral alterations. At long term, the $MgSO_4$ alone tends to prevent some HI-induced behavioral impairments whereas the $MgSO_4$/4-PBA association significantly prevents HI-induced motor and cognitive alterations. Neither treatments present proper effects at short or long terms. It will then be necessary to decipher treatments molecular mechanisms underlying neuroprotection, focusing on a potential prevention of HI-induced white matter injuries and oligodendrocyte differentiation alteration.

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BOARD NUMBER: S07-282

MUTATED KIDINS220 ACCUMULATES IN CELLS AND DISRUPTS HOMEOSTASIS – A POSSIBLE MECHANISM BEHIND SINO SYNDROME.

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Introduction: SINO is a newly described syndrome characterized by Spastic paraplegia, Intellectual Disability, Nystagmus and Obesity, caused by heterozygous mutations in the KIDINS220 gene. Kidins220 (kinase D interacting substrate of 220kDa) is a multi-functional scaffold protein abundantly expressed across the nervous system. Some KIDINS220 pathogenic mutations result in shortened proteins resembling the isoforms naturally expressed during adulthood. **Aims**: This work examines the molecular consequences of KIDINS220 mutations. The main goals are to assess the effect the mutations on protein expression and how altered Kidins220 impacts cell homeostasis. **Methods**: Chosen mutations were introduced into the WT human KIDINS220 gene and expressed in HEK293T cells. We used western blotting and immunocytochemistry experiments to investigate Kidins220 protein expression levels, its cellular localisation and expression of cell homeostasis and autophagy molecular factors. **Results:** All but one of the KIDINS220 mutations investigated in this study were expressed in HEK293T cells. Mutated proteins were overexpressed in the target cells and formed aggregates in a pattern that varied between different mutations. We saw changes in levels of p62 receptor and LC3 protein in cells with mutated proteins. **Conclusions:** Different KIDINS220 mutations have a distinct effect on the translated protein, resulting in formation of protein aggregates in the affected cells. Variability in the mutation consequences depended on the mutation location in the gene. Our results indicate that mutated proteins impact the homeostasis of the affected cells possibly leading to the formation of autophagosomes. Further studies on the energetic profiles and mitochondrial functioning of the affected cells are ongoing.

Pubmed:
35140204: Almacellas-Barbanoj A, Albini M, Satapathy A, Jaudon F, Michetti C, Krawczun-Rygmaczewska A, Huang H, Manago F, Papaleo F, Benfenati F, Cesca F
Kidins220/ARMS modulates brain morphology and anxiety-like traits in adult mice.
Kinase D interacting substrate of 220 kDa (Kidins220), also known as ankyrin repeat-rich membrane spanning (ARMS), is a transmembrane scaffold protein that participates in fundamental aspects of neuronal physiology including cell survival, differentiation, and synaptic plasticity. The Kidins220 constitutive knockout line displays developmental defects in the nervous and cardiovascular systems that lead to embryonic lethality, which has so far precluded the study of this protein in the adult. Moreover, Kidins220 mRNA is tightly regulated by alternative splicing, whose impact on nervous system physiology has not yet been addressed in vivo. Here, we have asked to what extent the absence of Kidins220 splicing and the selective knockout of Kidins220 impact on adult brain homeostasis. To answer this question, we used a floxed line that expresses only the full-length, non-spliced Kidins220 mRNA, and a forebrain-specific, CaMKII-Cre driven Kidins220 conditional knockout (cKO) line. Kidins220 cKO brains are characterized by enlarged ventricles in the absence of cell death, and by deficient dendritic arborization in several cortical regions. The deletion of Kidins220 leads to behavioral changes, such as reduced anxiety-like traits linked to alterations in TrkB-BDNF signaling and sex-dependent alterations of hippocampal-dependent spatial memory. Kidins220 floxed mice present similarly enlarged brain ventricles and increased associative memory. Thus, both the absolute levels of Kidins220 expression and its splicing pattern are required for the correct brain development and related expression of behavioral phenotypes. These findings are relevant in light of the increasing evidence linking mutations in the human KIDINS220 gene to the onset of severe neurodevelopmental disorders.
Cell Death Discov, 2022; 8
32655132: Martins D, Davies C, De Micheli A, Oliver D, Krawczun-Rygmaczewska A, Fusar-Poli P, Paloyelis Y
Intranasal oxytocin increases heart-rate variability in men at clinical high risk for psychosis: a proof-of-concept study.
Autonomic nervous system (ANS) dysfunction (i.e., increased sympathetic and/or decreased parasympathetic activity) has been proposed to contribute to psychosis vulnerability. Yet, we still lack directed therapeutic strategies that improve ANS

regulation in psychosis or at-risk states. The oxytocin system constitutes a potential therapeutic target, given its role in ANS regulation. However, whether intranasal oxytocin ameliorates autonomic regulation during emerging psychosis is currently unknown. We pooled together two datasets, one of 30 men at clinical high risk for psychosis (CHR-P), and another of 17 healthy men, who had participated in two double-blinded, placebo-controlled, randomised, crossover MRI studies with similar protocols. All participants self-administered 40 IU of intranasal oxytocin or placebo using a nasal spray. We recorded pulse plethysmography during a period of 8 min at about 1 h post dosing and estimated heart rate (HR) and high-frequency HR variability (HF-HRV), an index of cardio-parasympathetic activity. CHR-P and healthy men did not differ at resting HR or HF-HRV under placebo. We found a significant condition × treatment effect for HF-HRV, showing that intranasal oxytocin, compared with placebo, increased HF-HRV in CHR-P but not in healthy men. The main effects of treatment and condition were not significant. In this proof-of-concept study, we show that intranasal oxytocin increases cardio-parasympathetic activity in CHR-P men, highlighting its therapeutic potential to improve autonomic regulation in this clinical group. Our findings support the need for further research on the preventive and therapeutic potential of intranasal oxytocin during emerging psychosis, where we lack effective treatments.
Transl Psychiatry, 2020; 10

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BOARD NUMBER: S07-283

STRIATAL DYSFUNCTION IN THE NOVEL DYT25-GNAL DYSTONIA KNOCKOUT RAT MODEL

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Dystonia is the third most common movement disorder, characterized by involuntary and sustained muscle contractions. DYT25, an adult-onset isolated form of dystonia, is caused by loss-of-function mutations in the GNAL gene. GNAL encodes the olfactory type G-protein alpha subunit, highly enriched in the corpus striatum of the basal ganglia network. Notably, the involvement of the striatum in dystonia pathophysiology is well known. In the present work, we investigated the striatal molecular and electrophysiological alterations in a newly generated heterozygous GNAL knockout rat model. GNAL+/- rats exhibited an altered behavioural phenotype, showing decreased locomotor activity and impaired motor coordination. We found an impairment of striatal synaptic plasticity in GNAL+/- rats, with loss of dopamine-dependent Long-Term Synaptic Depression (LTD). Further electrophysiology experiments showed that striatal LTD was partially rescued by a combination of both dopamine D1R and D2 receptor (D2R) agonists. Additionally, LTD was fully rescued by antagonism of either adenosine A2AR or type 5 metabotropic glutamate receptor (mGlu5R). Notably, both A2AR and mGlu5R exert antagonistic actions on striatal D2R. Therefore, the full rescue of LTD was likely obtained by relieving the inhibitory action of these receptors on D2R. Ongoing electrophysiology and immunoblotting experiments are characterizing the molecular mechanisms of both LTD impairment and its pharmacological rescue by A2AR and mGlu5R antagonists. These data will provide important insights on the pathophysiology of the debilitating and incurable DYT25 dystonia, and on new pharmacological targets for novel and more effective therapies.

Pubmed:
32289333: Imbriani P, Ponterio G, Tassone A, Sciamanna G, El Atiallah I, Bonsi P, Pisani A
Models of dystonia: an update.
Although dystonia represents the third most common movement disorder, its pathophysiology remains still poorly understood. In the past two decades, multiple models have been generated, improving our knowledge on the molecular and cellular bases of this heterogeneous group of movement disorders. In this short survey, we will focus on recently generated novel models of DYT1 dystonia, the most common form of genetic, "isolated" dystonia. These models clearly indicate the existence of multiple signaling pathways affected by the protein mutation causative of DYT1 dystonia, torsinA, paving the way for potentially multiple, novel targets for pharmacological intervention.
J Neurosci Methods, 2020; 339
35034734: Sciamanna G, El Atiallah I, Montanari M, Pisani A
Plasticity, genetics and epigenetics in dystonia: An update.
Dystonia represents a group of movement disorders characterized by involuntary muscle contractions that result in abnormal posture and twisting movements. In the last 20 years several animal models have been generated, greatly improving our knowledge of the neural and molecular mechanism underlying this pathological condition, but the pathophysiology remains still poorly understood. In this review we will discuss recent genetic factors related to dystonia and the current understanding of synaptic plasticity alterations reported by both clinical and experimental research. We will also present recent evidence involving epigenetics mechanisms in dystonia.
Handb Clin Neurol, 2022; 184
34217152: Imbriani P, Sciamanna G, El Atiallah I, Cerri S, Hess EJ, Pisani A
Synaptic effects of ethanol on striatal circuitry: therapeutic implications for dystonia.
Alcohol consumption affects motor behavior and motor control. Both acute and chronic alcohol abuse have been extensively investigated; however, the therapeutic efficacy of alcohol on some movement disorders, such as myoclonus-dystonia or essential tremor, still does not have a plausible mechanistic explanation. Yet, there are surprisingly few systematic trials with

known GABAergic drugs mimicking the effect of alcohol on neurotransmission. In this brief survey, we aim to summarize the effects of EtOH on striatal function, providing an overview of its cellular and synaptic actions in a 'circuit-centered' view. In addition, we will review both experimental and clinical evidence, in the attempt to provide a plausible mechanistic explanation for alcohol-responsive movement disorders, with particular emphasis on dystonia. Different hypotheses emerge, which may provide a rationale for the utilization of drugs that mimic alcohol effects, predicting potential drug repositioning.
FEBS J, 2021;

32335490: Imbriani P, D'Angelo V, Platania P, Di Lazzaro G, Scalise S, Salimei C, El Atiallah I, Colona VL, Mercuri NB, Bonsi P, Pisani A, Schirinzi T, Martella G
Ischemic injury precipitates neuronal vulnerability in Parkinson's disease: Insights from PINK1 mouse model study and clinical retrospective data.
Increasing evidence demonstrates the relevant association between Parkinson's disease (PD) and vascular diseases/risk factors, as well as a worse clinico-pathological progression in those patients with vascular comorbidity. The mechanisms underlying this relationship have not been clarified yet, although their comprehension is critical in a perspective of disease-modifying treatments development or prevention.
Parkinsonism Relat Disord, 2020; 74

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INTENSIVE SENSORIMOTOR REHABILITATION RESTORES GAIT DISFUNCTION AND MICROSTRUCTURE CAUSED BY EXPERIMENTAL CEREBRAL PALSY IN RATS

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Cerebral palsy (CP) is a major cause of locomotor and cognitive disabilities due to perinatal brain injury. Hand and Bimanual Intensive Therapy Including Lower Extremities (HABIT-ILE), a 2-week intensive rehabilitation protocol using sensorimotor training, has been shown to improve motor function and induce plastic changes in CP children. The study sought to model HABIT-ILE combining environmental enrichment and treadmill training (EETT) in a rodent model of CP evaluating early (P28) and late (P60) functional, biochemical and histological parameters. Pregnant Wistar rats were injected with LPS (200mg/kg i.p - E18/E19). At P0, pups were exposed to 20‘ anoxia at 37°C. From P2 to P21, hindlimbs were restricted for 16h/day. EETT lasted from P21 to P28. TT - 15 min/day at 7cm/s. EE - 7 days in enriched cages with sensorimotor stimulus. 3D kinematics was assessed at P21, P28, and P60. Ex-vivo MRI, histological analysis, and protein expression were assessed at P28 and P60. Principal Component analysis evidenced that EETT rescued gait abnormalities caused by CP animals at P28 and P60. At P28, EETT altered BDNF signaling and decreased cortical excitatory dysfunction induced by CP. Ex-vivo diffusion imaging showed early and late loss of brain microstructure loss in CP rats, not reversed by EETT at P28 but at P60 it restored basal ganglia and somatosensory cortex microstructure altered by CP. EETT restored myelination and decreased astrogliosis at P28. These evidences show that HABIT-ILE (EETT) induces early and long-term functional benefits modulating molecular and microstructural brain damage caused by CP.

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DEVELOPMENTAL DEFECT OF THE CORTICO-STRIATAL CIRCUIT IN A MOUSE MODEL OF HUNTINGTON'S DISEASE

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Huntington's disease (HD) is usually defined as a neurodegenerative disorder affecting the striatum and the cortex at adult stages. However, recent studies suggest that this disease also implies early striatal developmental alterations. As this issue remains largely unexplored, the aim of this study is to identify when the first disease-related striatal impairments emerge. To answer this question, we are longitudinally studying the striatal development of the R6/1 mouse model of HD. This study is performed during the two first postnatal weeks as this period has been shown to be crucial for the maturation of MSN's morphological and electrophysiological properties. By using multiple immunohistochemical labeling techniques we are studying the distribution of medium spiny neurons (MSNs) in HD and WT mouse littermates. Using *ex vivo* whole-cell patch clamp electrophysiology we are also recording the intrinsic electrophysiological properties of MSNs as well as the establishment of the cortico-striatal glutamatergic transmission. Finally we are looking at the morphology of the recorded neurons, especially their dendritic complexity and their dendritic spines. Our results suggest that there is a biphasic alteration of D2-MSNs properties in R6/1 mice, highlighted by an early decreased excitability and higher dendritic complexity at postnatal days P0-3 that is then replaced by a hyperexcitability and a decreased dendritic complexity at P8-P9. These anatomical and electrophysiological data provide an insight into striatal developmental alterations in a mouse model of HD at very early stages.

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DEEP BRAIN STIMULATION FOR THE TREATMENT OF TOURETTE'S SYNDROME: STRIATAL DISINHIBITION AS A RAT MODEL

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Tourette's syndrome (TS) represents a neurodevelopmental disorder characterized by the presence of motor and vocal tics. Typically, tics first appear in childhood and disappear in adolescence. Some patients, however, may continue to suffer from TS even after reaching adulthood and despite receiving psychological as well as pharmacological treatment. In the most severe cases, such patients often resort to neuromodulatory treatments such as deep brain stimulation (DBS). Recently, striatal disinhibition has been proposed as a rat model for the study of TS. However, whether this model responds to the same treatment as patients remains unknown. We, therefore, set out to determine whether it would respond to DBS.

Pubmed:

32755331: Viisanen H, Lilius TO, Sagalajev B, Rauhala P, Kalso E, Pertovaara A
Neurophysiological response properties of medullary pain-control neurons following chronic treatment with morphine or oxycodone: modulation by acute ketamine.
Descending facilitatory circuitry that involves the rostroventromedial medulla (RVM) exerts a significant role in the development of antinociceptive tolerance and hyperalgesia following chronic morphine treatment. The role of the RVM in the development of antinociceptive tolerance to oxycodone, another clinically used strong opioid, is not yet known. Ketamine, an -methyl-d-aspartate (NMDA) receptor antagonist, attenuates opioid antinociceptive tolerance, but its effect on RVM cell discharge in opioid-tolerant animals is not known. Here, we compared chronic effects of morphine and oxycodone on the discharge properties of RVM cells and attempted to attenuate chronic treatment-induced changes with ketamine. Parallel recordings of RVM cell discharge and limb withdrawal response were performed under light pentobarbital anesthesia in male rats following sustained systemic treatment with morphine or oxycodone at equianalgesic doses. Ongoing activity and the response to noxious heat and pinch were determined in pronociceptive RVM ON-cells and antinociceptive OFF-cells on the sixth treatment day. Proportions of RVM cell types were not changed. Chronic oxycodone induced antinociceptive tolerance both in limb withdrawal and RVM cell activity. Chronic morphine induced antinociceptive tolerance in limb withdrawal that was accompanied by pronociceptive heat response changes in RVM ON- and OFF-cells. A behaviorally subantinociceptive dose of acute ketamine reversed antinociceptive tolerance both to morphine and oxycodone in limb withdrawal and reversed the chronic morphine-induced pronociceptive discharge changes in RVM cells. The results indicate that an NMDA receptor-dependent descending pronociceptive circuitry involving the RVM has an important role in behavioral antinociceptive tolerance to morphine but not oxycodone. Morphine and oxycodone are two clinically used strong opioids. Chronic treatment with oxycodone as well as morphine can lead to analgesic tolerance and paradoxical hyperalgesia. Here we show that an -methyl-d-aspartate receptor-dependent pronociceptive change in discharge properties of rostroventromedial medullary neurons controlling spinal nociception has an important role in antinociceptive tolerance to morphine but not oxycodone. Interestingly, chronic oxycodone did not induce pronociceptive changes in the rostroventromedial medulla.
J Neurophysiol, 2020; 124
30465925: Chen Z, Wei H, Sagalajev B, Koivisto A, Pertovaara A
Amygdaloid administration of tetrapentylammonium attenuates development of pain and anxiety-like behavior following peripheral nerve injury.
The central amygdaloid nucleus (CeA) is involved in processing and descending regulation of pain. Amygdaloid mechanisms underlying pain processing and control are poorly known. Here we tested the hypothesis that perioperative CeA administration of tetrapentylammonium (TPA), a non-selective THIK-1 channel blocker and thereby inhibitor of microglia, attenuates development of chronic neuropathic pain and comorbid anxiety-like behavior.
Pharmacol Rep, 2019; 71
30101191: Mousseau M, Burma NE, Lee KY, Leduc-Pessah H, Kwok CHT, Reid AR, O'Brien M, Sagalajev B, Stratton JA,

Patrick N, Stemkowski PL, Biernaskie J, Zamponi GW, Salo P, McDougall JJ, Prescott SA, Matyas JR, Trang T
Microglial pannexin-1 channel activation is a spinal determinant of joint pain.
Chronic joint pain such as mechanical allodynia is the most debilitating symptom of arthritis, yet effective therapies are lacking. We identify the pannexin-1 (Panx1) channel as a therapeutic target for alleviating mechanical allodynia, a cardinal sign of arthritis. In rats, joint pain caused by intra-articular injection of monosodium iodoacetate (MIA) was associated with spinal adenosine 5'-triphosphate (ATP) release and a microglia-specific up-regulation of P2X7 receptors (P2X7Rs). Blockade of P2X7R or ablation of spinal microglia prevented and reversed mechanical allodynia. P2X7Rs drive Panx1 channel activation, and in rats with mechanical allodynia, Panx1 function was increased in spinal microglia. Specifically, microglial Panx1-mediated release of the proinflammatory cytokine interleukin-1β (IL-1β) induced mechanical allodynia in the MIA-injected hindlimb. Intrathecal administration of the Panx1-blocking peptide panx suppressed the aberrant discharge of spinal laminae I-II neurons evoked by innocuous mechanical hindpaw stimulation in arthritic rats. Furthermore, mice with a microglia-specific genetic deletion of Panx1 were protected from developing mechanical allodynia. Treatment with probenecid, a clinically used broad-spectrum Panx1 blocker, resulted in a striking attenuation of MIA-induced mechanical allodynia and normalized responses in the dynamic weight-bearing test, without affecting acute nociception. Probenecid reversal of mechanical allodynia was also observed in rats 13 weeks after anterior cruciate ligament transection, a model of posttraumatic osteoarthritis. Thus, Panx1-targeted therapy is a new mechanistic approach for alleviating joint pain.
Sci Adv, 2018; 4
29274353: Sagalajev B, Wei H, Chen Z, Albayrak I, Koivisto A, Pertovaara A
Oxidative Stress in the Amygdala Contributes to Neuropathic Pain.
Earlier studies indicate that the central nucleus of the amygdala (CeA) contributes to neuropathic pain. Here we studied whether amygdaloid administration of antioxidants or antagonists of TRPA1 that is among ion channels activated by oxidative stress attenuates nociceptive or affective pain in experimental neuropathy, and whether this effect involves amygdaloid astrocytes or descending serotonergic pathways acting on the spinal 5-HT receptor. The experiments were performed in rats with spared nerve injury (SNI). Drugs were administered through a chronic cannula in the CeA or internal capsule (control site), and an intrathecal catheter. Nociception was assessed using monofilaments and affective pain using conditioned place-aversion. Antioxidants or TRPA1 antagonists in the CeA attenuated both nociceptive and affective pain in SNI animals but not in sham controls or in a control injection site. Drugs influencing astroglia (a gap junction decoupler or a D-amino acid oxidase inhibitor) in the CeA had no effect on SNI rats, whereas local anesthesia of the CeA attenuated nociception. Spinally administered 5-HT receptor antagonist at a dose that had no effect alone prevented the antinociceptive effect of amygdaloid TRPA1 blockers. The results suggest that injury-induced amygdaloid oxidative stress that drives TRPA1 promotes neuropathic pain behavior. This pronociceptive effect involves suppression of medullospinal serotonergic feedback-inhibition acting on the spinal 5-HT receptor. While the CeA is involved in mediating the nerve injury-induced pronociception, it may not be a critical relay for the recruitment of medullospinal feedback-inhibition.
Neuroscience, 2018; 387
28300633: David-Pereira A, Sagalajev B, Wei H, Almeida A, Pertovaara A, Pinto-Ribeiro F
The medullary dorsal reticular nucleus as a relay for descending pronociception induced by the mGluR5 in the rat infralimbic cortex.
Metabotropic glutamate receptor 5 (mGluR5) activation in the infralimbic cortex (IL) induces pronociceptive behavior in healthy and monoarthritic rats. Here we studied whether the medullary dorsal reticular nucleus (DRt) and the spinal TRPV1 are mediating the IL/mGluR5-induced spinal pronociception and whether the facilitation of pain behavior is correlated with changes in spinal dorsal horn neuron activity. For drug administrations, all animals had a cannula in the IL as well as a cannula in the DRt or an intrathecal catheter. Heat-evoked paw withdrawal was used to assess pain behavior in awake animals. Spontaneous and heat-evoked discharge rates of single DRt neurons or spinal dorsal horn wide-dynamic range (WDR) and nociceptive-specific (NS) neurons were evaluated in lightly anesthetized animals. Activation of the IL/mGluR5 facilitated nociceptive behavior in both healthy and monoarthritic animals, and this effect was blocked by lidocaine or GABA receptor agonists in the DRt. IL/mGluR5 activation increased spontaneous and heat-evoked DRt discharge rates in healthy but not monoarthritic rats. In the spinal dorsal horn, IL/mGluR5 activation increased spontaneous activity of WDR neurons in healthy animals only, whereas heat-evoked responses of WDR and NS neurons were increased in both experimental groups. Intrathecally administered TRPV1 antagonist prevented the IL/mGluR5-induced pronociception in both healthy and monoarthritic rats. The results suggest that the DRt is involved in relaying the IL/mGluR5-induced spinal pronociception in healthy control but not monoarthritic animals. Spinally, the IL/mGluR5-induced behavioral heat hyperalgesia is mediated by TRPV1 and associated with facilitated heat-evoked responses of WDR and NS neurons.
Neuroscience, 2017; 349
28053243: Sagalajev B, Viisanen H, Wei H, Pertovaara A
Descending antinociception induced by secondary somatosensory cortex stimulation in experimental neuropathy: role of the

medullospinal serotonergic pathway.
Stimulation of the secondary somatosensory cortex (S2) has attenuated pain in humans and inflammatory nociception in animals. Here we studied S2 stimulation-induced antinociception and its underlying mechanisms in an experimental animal model of neuropathy induced by spinal nerve ligation (SNL). Effect of S2 stimulation on heat-evoked limb withdrawal latency was assessed in lightly anesthetized rats that were divided into three groups based on prior surgery and monofilament testing before induction of anesthesia:) sham-operated group and) hypersensitive and) nonhypersensitive (mechanically) SNL groups. In a group of hypersensitive SNL animals, a 5-HT receptor agonist was microinjected into the rostroventromedial medulla (RVM) to assess whether autoinhibition of serotonergic cell bodies blocks antinociception. Additionally, effect of S2 stimulation on pronociceptive ON-cells and antinociceptive OFF-cells in the RVM or nociceptive spinal wide dynamic range (WDR) neurons were assessed in anesthetized hypersensitive SNL animals. S2 stimulation induced antinociception in hypersensitive but not in nonhypersensitive SNL or sham-operated animals. Antinociception was prevented by a 5-HT receptor agonist in the RVM. Antinociception was associated with decreased duration of heat-evoked response in RVM ON-cells. In spinal WDR neurons, heat-evoked discharge was delayed by S2 stimulation, and this antinociceptive effect was prevented by blocking spinal 5-HT receptors. The results indicate that S2 stimulation suppresses nociception in SNL animals if SNL is associated with tactile allodynia-like hypersensitivity. In hypersensitive SNL animals, S2 stimulation induces antinociception mediated by medullospinal serotonergic pathways acting on the spinal 5-HT receptor, and partly through reduction of the RVM ON-cell discharge. Stimulation of S2 cortex, but not that of an adjacent cortical area, induced descending heat antinociception in rats with the spinal nerve ligation-induced model of neuropathy. Antinociception was bilateral, and it involved suppression of pronociceptive medullary cells and activation of serotonergic pathways that act on the spinal 5-HT receptor. S2 stimulation failed to induce descending antinociceptive effect in sham-operated controls or in nerve-ligated animals that had not developed mechanical hypersensitivity.
J Neurophysiol, 2017; 117
26432495: Wei H, Sagalajev B, Yüzer MA, Koivisto A, Pertovaara A
Regulation of neuropathic pain behavior by amygdaloid TRPC4/C5 channels.
Pain per se may increase anxiety and conversely, anxiety may increase pain. Therefore, a positive feedback loop between anxiety and pain possibly contributes to pain and suffering in some pathophysiological pain conditions, such as that induced by peripheral nerve injury. Recent results indicate that transient receptor channels 4 and 5 (TRPC4/C5) in the amygdala have anxiogenic effects in rodents, while their role in chronic pain conditions is not known. Here, we studied whether the amygdaloid TRPC4/C5 that are known to have anxiogenic properties contribute to the maintenance of sensory or affective aspects of pain in an experimental model of peripheral neuropathy. Rats with a spared nerve injury (SNI) model of neuropathy in the left hind limb had a chronic cannula for microinjections of drugs into the right amygdala or the internal capsule (a control site). Sensory pain was assessed by determining mechanical hypersensitivity with calibrated monofilaments and affective pain by determining aversive place-conditioning. Amygdaloid treatment with ML-204, a TRPC4/C5 antagonist, produced a dose-related (5-10 μg) antihypersensitivity effect, without obvious side-effects. Additionally, amygdaloid administration of ML-204 reduced affective-like pain behavior. In the internal capsule, ML-204 had no effect on hypersensitivity or affective-like pain in SNI animals. In healthy controls, amygdaloid administration of ML-204 failed to influence pain behavior induced by mechanical stimulation or noxious heat. The results indicate that the amygdaloid TRPC4/C5 contribute to maintenance of pain hypersensitivity and pain affect in neuropathy.
Neurosci Lett, 2015; 608
25557801: Sagalajev B, Bourbia N, Beloushko E, Wei H, Pertovaara A
Bidirectional amygdaloid control of neuropathic hypersensitivity mediated by descending serotonergic pathways acting on spinal 5-HT3 and 5-HT1A receptors.
Amygdala is involved in processing of primary emotions and particularly its central nucleus (CeA) also in pain control. Here we studied mechanisms mediating the descending control of mechanical hypersensitivity by the CeA in rats with a peripheral neuropathy in the left hind limb. For drug administrations, the animals had a guide cannula in the right CeA and an intrathecal catheter or another guide cannula in the medullary raphe. Hypersensitivity was tested with monofilaments. Glutamate administration in the CeA produced a bidirectional effect on hypersensitivity that varied from an increase at a low-dose (9μg) to a reduction at high doses (30-100μg). The increase but not the reduction of hypersensitivity was prevented by blocking the amygdaloid NMDA receptor with a dose of MK-801 that alone had no effects. The glutamate-induced increase in hypersensitivity was reversed by blocking the spinal 5-HT3 receptor with ondansetron, whereas the reduction in hypersensitivity was reversed by blocking the spinal 5-HT1A receptor with WAY-100635. Both the increase and decrease of hypersensitivity induced by amygdaloid glutamate treatment were reversed by medullary administration of a 5-HT1A agonist, 8-OH-DPAT, that presumably produced autoinhibition of serotonergic cell bodies in the medullary raphe. The results indicate that depending on the dose, glutamate in the CeA has a descending facilitatory or inhibitory effect on neuropathic pain hypersensitivity. Serotoninergic raphe neurons are involved in mediating both of these effects. Spinally, the 5-HT3 receptor

contributes to the increase and the 5-HT1A receptor to the decrease of neuropathic hypersensitivity induced by amygdaloid glutamate.
Behav Brain Res, 2015; 282
24747685: Bourbia N, Sagalajev B, Pertovaara A
Descending effect on spinal nociception by amygdaloid glutamate varies with the submodality of noxious test stimulation. Amygdala has an important role in the processing of primary emotions, such as fear. Additionally, amygdala is involved in processing and modulation of pain. While the amygdala, particularly its central nucleus (CeA), has been shown to contribute to pain control, the descending pain regulation by the CeA is still only partly characterized. Here heat and mechanical nociception was tested in both hind limbs of healthy rats with a chronic guide cannula for microinjection of glutamate into the CeA of the left or right hemisphere. The aim was to assess whether the descending pain regulatory effect by glutamate in the amygdala varies with the submodality or the body side of nociceptive testing, brain hemisphere or the amygdaloid glutamate receptor. Motor performance was assessed with the Rotarod test. Amygdaloid glutamate, independent of the treated hemisphere, produced a dose-related heat and mechanical antinociception that varied with the submodality of testing. Heat antinociception was short lasting (minutes), bilateral and not reversed by blocking the amygdaloid NMDA receptor with MK-801. In contrast, mechanical antinociception lasted longer (>20 min), was predominantly contralateral and reversed by blocking the amygdaloid NMDA receptor. At an antinociceptive dose, amygdaloid glutamate failed to influence motor performance. The results indicate that independent of the brain hemisphere, the spatial extent and duration of the descending antinociceptive effect induced by amygdaloid glutamate varies with the amygdaloid glutamate receptor and the submodality of pain.
Neurosci Lett, 2014; 570

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IDENTIFICATION AND FUNCTIONAL ANALYSIS OF PKCγ MOLECULAR TARGETS DURING PURKINJE CELL DEVELOPMENT

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Spinocerebellar ataxia 14 (SCA14) is a rare autosomal dominant neurodegenerative disease caused by mutations in protein kinase C gamma gene (PRKCG, PKCγ protein) leading to motor deficits and cognitive decline. PKCγ is a signalling protein strongly expressed in Purkinje cells (PCs) known to be involved in the regulation of synapse formation and PC dendritic development. The effects of PKCγ are mediated by phosphorylation of target proteins and for dendritic development the regulation of cytoskeletal function and dynamics will play a critical role. However, the interaction of PKCγ with cytoskeletal regulators is still not very well understood. In this project, we are taking advantage of PKCγ mutant mouse lines expressing a constitutively active PKCγ and causing a SCA14-like phenotype. Using proteomic and phospho-proteomic analyses we compared the expression and phosphorylation of potential target proteins in normal mice with those of mice having Purkinje cells with a constitutively active PKCγ. This analysis yielded several candidate genes showing an altered expression and/or phosphorylation in the presence of constitutively active PKCγ. The function and the involvement of these genes in PKCγ signalling will be explored by studying the cerebellar expression and phosphorylation of these genes in more detail and by the manipulation of gene and phosphorylation in organotypic slice cultures and dissociated cerebellar cultures in vitro followed by morphological analysis of PC dendritic arbours. The results of the current analysis will be presented at the meeting and will provide new insights into PKCγ molecular targets during PC development.

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A ZEBRAFISH MODEL FOR THE STUDY OF NIEMANN PICK TYPE C, A NEUROMETABOLIC ATAXIA

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Aims The zebrafish (*Danio rerio*) is an important model for the study of hereditary neurological disorders, which shares 70 % of homology with human genome and a similar organization of the central nervous system (CNS). Our objective is to study how the NPC1 gene is involved in Niemann Pick disease type C (NPC), a neurodegenerative lysosomal disorder. NPC is a sphingolipidosis, transmitted by autosomal recessive inheritance and characterized by an accumulation of lipids in different organs. Clinical symptoms start at childhood and the most usual manifestations include hepatosplenomegaly and ataxia. Currently there is no effective treatment for this disease. **Methods** By using CRISPR/Cas9 system, we developed a zebrafish *npc1* mutant line, in which we carried out a comprehensive phenotypic and histological characterization, as well as immunotyping, behavioural analysis and RNAseq assay. **Results** We observed high lethality, all larvae died before reaching the adult stage. *Npc1* mutant larvae were smaller than wild type, their motor function was reduced and they were presented with filipin aggregations and liver and spleen damage. In addition, RNAseq analysis showed more than 249 genes differentially expressed between *npc1* mutants and control, such as *npc1* and genes related development of the CNS and lipid exchange and metabolism **Conclusions** *Npc1* zebrafish model showed similar symptoms to NPC human patients. The use of zebrafish models for NPC will improve our knowledge on the underlying mechanisms which cause this disease, an essential step for the development of new therapeutic strategies.

Pubmed:
33917666: Quelle-Regaldie A, Sobrido-Cameán D, Barreiro-Iglesias A, Sobrido MJ, Sánchez L
Zebrafish Models of Autosomal Recessive Ataxias.
Autosomal recessive ataxias are much less well studied than autosomal dominant ataxias and there are no clearly defined systems to classify them. Autosomal recessive ataxias, which are characterized by neuronal and multisystemic features, have significant overlapping symptoms with other complex multisystemic recessive disorders. The generation of animal models of neurodegenerative disorders increases our knowledge of their cellular and molecular mechanisms and helps in the search for new therapies. Among animal models, the zebrafish, which shares 70% of its genome with humans, offer the advantages of being small in size and demonstrating rapid development, making them optimal for high throughput drug and genetic screening. Furthermore, embryo and larval transparency allows to visualize cellular processes and central nervous system development in vivo. In this review, we discuss the contributions of zebrafish models to the study of autosomal recessive ataxias characteristic phenotypes, behavior, and gene function, in addition to commenting on possible treatments found in these models. Most of the zebrafish models generated to date recapitulate the main features of recessive ataxias.
Cells, 2021; 10
33671313: Quelle-Regaldie A, Sobrido-Cameán D, Barreiro-Iglesias A, Sobrido MJ, Sánchez L
Zebrafish Models of Autosomal Dominant Ataxias.
Hereditary dominant ataxias are a heterogeneous group of neurodegenerative conditions causing cerebellar dysfunction and characterized by progressive motor incoordination. Despite many efforts put into the study of these diseases, there are no effective treatments yet. Zebrafish models are widely used to characterize neuronal disorders due to its conserved vertebrate genetics that easily support genetic edition and their optic transparency that allows observing the intact CNS and its connections. In addition, its small size and external fertilization help to develop high throughput assays of candidate drugs. Here, we discuss the contributions of zebrafish models to the study of dominant ataxias defining phenotypes, genetic function, behavior and possible treatments. In addition, we review the zebrafish models created for X-linked repeat expansion diseases X-fragile/fragile-X tremor ataxia. Most of the models reviewed here presented neuronal damage and locomotor

deficits. However, there is a generalized lack of zebrafish adult heterozygous models and there are no knock-in zebrafish models available for these diseases. The models created for dominant ataxias helped to elucidate gene function and mechanisms that cause neuronal damage. In the future, the application of new genetic edition techniques would help to develop more accurate zebrafish models of dominant ataxias.
Cells, 2021; 10
34769146: Hernández-Núñez I, Quelle-Regaldie A, Sánchez L, Adrio F, Candal E, Barreiro-Iglesias A
Decline in Constitutive Proliferative Activity in the Zebrafish Retina with Ageing.
It is largely assumed that the teleost retina shows continuous and active proliferative and neurogenic activity throughout life. However, when delving into the teleost literature, one finds that assumptions about a highly active and continuous proliferation in the adult retina are based on studies in which proliferation was not quantified in a comparative way at the different life stages or was mainly studied in juveniles/young adults. Here, we performed a systematic and comparative study of the constitutive proliferative activity of the retina from early developing (2 days post-fertilisation) to aged (up to 3-4 years post-fertilisation) zebrafish. The mitotic activity and cell cycle progression were analysed by using immunofluorescence against pH3 and PCNA, respectively. We observed a decline in the cell proliferation in the retina with ageing despite the occurrence of a wave of secondary proliferation during sexual maturation. During this wave of secondary proliferation, the distribution of proliferating and mitotic cells changes from the inner to the outer nuclear layer in the central retina. Importantly, in aged zebrafish, there is a virtual disappearance of mitotic activity. Our results showing a decline in the proliferative activity of the zebrafish retina with ageing are of crucial importance since it is generally assumed that the fish retina has continuous proliferative activity throughout life.
Int J Mol Sci, 2021; 22
31670873: Da Silva-Álvarez S, Guerra-Varela J, Sobrido-Cameán D, Quelle A, Barreiro-Iglesias A, Sánchez L, Collado M
Cell senescence contributes to tissue regeneration in zebrafish.
Cellular senescence is a stress response that limits the proliferation of damaged cells by establishing a permanent cell cycle arrest. Different stimuli can trigger senescence but excessive production or impaired clearance of these cells can lead to their accumulation during aging with deleterious effects. Despite this potential negative side of cell senescence, its physiological role as a pro-regenerative and morphogenetic force has emerged recently after the identification of programmed cell senescence during embryogenesis and during wound healing and limb regeneration. Here, we explored the conservation of tissue injury-induced senescence in a model of complex regeneration, the zebrafish. Fin amputation in adult fish led to the appearance of senescent cells at the site of damage, and their removal impaired tissue regeneration. Despite many conceptual similarities, this tissue repair response is different from developmental senescence. Our results lend support to the notion that cell senescence is a positive response promoting tissue repair and homeostasis.
Aging Cell, 2020; 19
32991320: Da Silva-Álvarez S, Guerra-Varela J, Sobrido-Cameán D, Quelle A, Barreiro-Iglesias A, Sánchez L, Collado M
Developmentally-programmed cellular senescence is conserved and widespread in zebrafish.
Cellular senescence is considered a stress response imposing a stable cell cycle arrest to restrict the growth of damaged cells. More recently however, cellular senescence was identified during mouse embryo development at particular structures during specific periods of time. This programmed cell senescence has been proposed to serve developmental and morphogenetic functions and to potentially represent an evolutionary origin of senescence. Cellular senescence has also been described to take place during bird (chick and quail) and amphibian (xenopus and axoltl) development. Fish however, have been described to show a very narrow and restricted pattern of developmental cell senescence. Here we carried out a detailed characterization of senescence during zebrafish development and found it to be conserved and widespread. Apart from yolk and cloaca, previously described structures, we also identified senescence in the developing central nervous system, intestine, liver, pronephric ducts, and crystalline. Interestingly, senescence at these developing structures disappeared upon treatment with senolytic compound ABT-263, supporting their senescent identity and opening the possibility of studying the contribution of this process to development. In summary, our findings extend the description of developmentally-programmed cell senescence to lower vertebrates contributing to the notion of the relevance of this process for embryo development.
Aging (Albany NY), 2020; 12
29292495: Yáñez J, Suárez T, Quelle A, Folgueira M, Anadón R
Neural connections of the pretectum in zebrafish (Danio rerio).
The pretectum is a complex region of the caudal diencephalon which in adult zebrafish comprises both retinorecipient (parvocellular superficial, central, intercalated, paracommissural, and periventricular) and non-retinorecipient (magnocellular superficial, posterior, and accessory) pretectal nuclei distributed from periventricular to superficial regions. We conducted a comprehensive study of the connections of pretectal nuclei by using neuronal tracing with fluorescent carbocyanine dyes. This study reveals specialization of efferent connections of the various pretectal nuclei, with nuclei projecting to the optic

tectum (paracommissural, central, and periventricular pretectal nuclei), the torus longitudinalis and the cerebellar corpus (paracommissural, central, and intercalated pretectal nuclei), the lateral hypothalamus (magnocellular superficial, posterior, and central pretectal nuclei), and the tegmental regions (accessory and superficial pretectal nuclei). With regard to major central afferents to the pretectum, we observed projections from the telencephalon to the paracommissural and central pretectal nuclei, from the optic tectum to the paracommissural, central, accessory and parvocellular superficial pretectal nuclei, from the cerebellum to the paracommissural and periventricular pretectal nuclei and from the nucleus isthmi to the parvocellular superficial and accessory pretectal nuclei. The parvocellular superficial pretectal nucleus sends conspicuous projections to the contralateral magnocellular superficial pretectal nucleus. The composite figure of results reveals large differences in connections of neighbor pretectal nuclei, indicating high degree of nuclear specialization. Our results will have important bearings in functional studies that analyze the relationship between specific circuits and behaviors in zebrafish. Comparison with results available in other species also reveals differences in the organization and connections of the pretectum in vertebrates.
J Comp Neurol, 2018; 526

FENS Forum 2022
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BOARD NUMBER: S07-289

STRESS RESILIENCE IN SCA3: STUDY OF DEPRESSION AND COGNITIVE COMORBIDITIES IN A MOUSE MODEL

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Background: Spinocerebellar ataxia type 3 (SCA3) is a neurodegenerative disease, depicting a very heterogenous clinical presentation, comprising mainly motor deficits. Previous clinical and functional studies have related cerebellar dysfunction with cognitive and mood-related disturbances – the so called, cerebellar cognitive affective syndrome (CCAS). SCA3 patients report depressive and anxiety symptoms, often attributed to the negative prospects of disease, but which can, alternatively, be inherent to the disease state, as part of the CCAS. **Aims**: SCA3 patients are more prone to suffer from stress; here and using the CMVMJD135 mouse model we aim to: (i) explore the presence of a CCAS-live presentation, (ii) evaluate the HPA-axis mediated stress response after acute stress, and (iii) study the impact of a chronic stress exposure in the motor function in these mice. **Methods:** Longitudinal evaluations were performed from 6 to 36 weeks of age. Different behavioral tests were applied to assess mood, anxiety, and cognition throughout disease progression. To evaluate HPA-axis modulation in SCA3, corticosterone levels were measured at several timepoints of disease progression at basal conditions and after acute stress. **Results:** SCA3 mice do not exhibit anhedonia, anxiety, lack of coping behavior or cognitive deficits in the measured paradigms. No major impact was observed on disease progression upon exposure to a 6-week chronic unpredictable stress protocol. **Conclusions:** Although no alterations were observed SCA3 mice behavior, as they lack awareness of their disease state, by exploring these comorbidities in animal models we contribute to a better understanding of the disease process in SCA3 patients.

Pubmed:
35159199: Machado-Santos AR, Loureiro-Campos E, Patrício P, Araújo B, Alves ND, Mateus-Pinheiro A, Correia JS, Morais M, Bessa JM, Sousa N, Rodrigues AJ, Oliveira JF, Pinto L
Beyond New Neurons in the Adult Hippocampus: Imipramine Acts as a Pro-Astrogliogenic Factor and Rescues Cognitive Impairments Induced by Stress Exposure.
Depression is a prevalent, socially burdensome disease. Different studies have demonstrated the important role of astrocytes in the pathophysiology of depression as modulators of neurotransmission and neurovascular coupling. This is evidenced by astrocyte impairments observed in brains of depressed patients and the appearance of depressive-like behaviors upon astrocytic dysfunctions in animal models. However, little is known about the importance of de novo generated astrocytes in the mammalian brain and in particular its possible involvement in the precipitation of depression and in the therapeutic actions of current antidepressants (ADs). Therefore, we studied the modulation of astrocytes and adult astrogliogenesis in the hippocampal dentate gyrus (DG) of rats exposed to an unpredictable chronic mild stress (uCMS) protocol, untreated and treated for two weeks with antidepressants-fluoxetine and imipramine. Our results show that adult astrogliogenesis in the DG is modulated by stress and imipramine. This study reveals that distinct classes of ADs impact differently in the astrogliogenic process, showing different cellular mechanisms relevant to the recovery from behavioral deficits induced by chronic stress exposure. As such, in addition to those resident, the newborn astrocytes in the hippocampal DG might also be promising therapeutic targets for future therapies in the neuropsychiatric field.
Cells, 2022; 11
34970787: Silveira-Rosa T, Mateus-Pinheiro A, Correia JS, Silva JM, Martins-Macedo J, Araújo B, Machado-Santos AR, Alves ND, Silva M, Loureiro-Campos E, Sotiropoulos I, Bessa JM, Rodrigues AJ, Sousa N, Patrício P, Pinto L
Suppression of adult cytogenesis in the rat brain leads to sex-differentiated disruption of the HPA axis activity.
The action of stress hormones, mainly glucocorticoids, starts and coordinates the systemic response to stressful events. The HPA axis activity is predicated on information processing and modulation by upstream centres, such as the hippocampus where adult-born neurons (hABN) have been reported to be an important component in the processing and integration of new information. Still, it remains unclear whether and how hABN regulates HPA axis activity and CORT production, particularly

when considering sex differences.
Cell Prolif, 2022; 55
34944570: Correia JS, Neves-Carvalho A, Mendes-Pinheiro B, Pires J, Teixeira FG, Lima R, Monteiro S, Silva NA, Soares-Cunha C, Serra SC, Duarte-Silva S, Teixeira-Castro A, Salgado AJ, Maciel P
Preclinical Assessment of Mesenchymal-Stem-Cell-Based Therapies in Spinocerebellar Ataxia Type 3.
The low regeneration potential of the central nervous system (CNS) represents a challenge for the development of new therapeutic strategies for neurodegenerative diseases, including spinocerebellar ataxias. Spinocerebellar ataxia type 3 (SCA3)-or Machado-Joseph disease (MJD)-is the most common dominant ataxia, being mainly characterized by motor deficits; however, SCA3/MJD has a complex and heterogeneous pathophysiology, involving many CNS brain regions, contributing to the lack of effective therapies. Mesenchymal stem cells (MSCs) have been proposed as a potential therapeutic tool for CNS disorders. Beyond their differentiation potential, MSCs secrete a broad range of neuroregulatory factors that can promote relevant neuroprotective and immunomodulatory actions in different pathophysiological contexts. The objective of this work was to study the effects of (1) human MSC transplantation and (2) human MSC secretome (CM) administration on disease progression in vivo, using the CMVMJD135 mouse model of SCA3/MJD. Our results showed that a single CM administration was more beneficial than MSC transplantation-particularly in the cerebellum and basal ganglia-while no motor improvement was observed when these cell-based therapeutic approaches were applied in the spinal cord. However, the effects observed were mild and transient, suggesting that continuous or repeated administration would be needed, which should be further tested.
Biomedicines, 2021; 9
34769232: Patrício P, Mateus-Pinheiro A, Machado-Santos AR, Alves ND, Correia JS, Morais M, Bessa JM, Rodrigues AJ, Sousa N, Pinto L
Cell Cycle Regulation of Hippocampal Progenitor Cells in Experimental Models of Depression and after Treatment with Fluoxetine.
Changes in adult hippocampal cell proliferation and genesis have been largely implicated in depression and antidepressant action, though surprisingly, the underlying cell cycle mechanisms are largely undisclosed. Using both an unpredictable chronic mild stress (uCMS) rat model of depression and rat hippocampal-derived neurosphere culture approaches, we aimed to unravel the cell cycle mechanisms regulating hippocampal cell proliferation and genesis in depression and after antidepressant treatment. We show that the hippocampal dentate gyrus (hDG) of uCMS animals have less proliferating cells and a decreased proportion of cells in the G2/M phase, suggesting a G1 phase arrest; this is accompanied by decreased levels of cyclin D1, E, and A expression. Chronic fluoxetine treatment reversed the G1 phase arrest and promoted an up-regulation of cyclin E. , dexamethasone (DEX) decreased cell proliferation, whereas the administration of serotonin (5-HT) reversed it. DEX also induced a G1-phase arrest and decreased cyclin D1 and D2 expression levels while increasing p27. Additionally, 5-HT treatment could partly reverse the G1-phase arrest and restored cyclin D1 expression. We suggest that the anti-proliferative actions of chronic stress in the hDG result from a glucocorticoid-mediated G1-phase arrest in the progenitor cells that is partly mediated by decreased cyclin D1 expression which may be overcome by antidepressant treatment.
Int J Mol Sci, 2021; 22
28885722: Sardinha VM, Guerra-Gomes S, Caetano I, Tavares G, Martins M, Reis JS, Correia JS, Teixeira-Castro A, Pinto L, Sousa N, Oliveira JF
Astrocytic signaling supports hippocampal-prefrontal theta synchronization and cognitive function.
Astrocytes interact with neurons at the cellular level through modulation of synaptic formation, maturation, and function, but the impact of such interaction into behavior remains unclear. Here, we studied the dominant negative SNARE (dnSNARE) mouse model to dissect the role of astrocyte-derived signaling in corticolimbic circuits, with implications for cognitive processing. We found that the blockade of gliotransmitter release in astrocytes triggers a critical desynchronization of neural theta oscillations between dorsal hippocampus and prefrontal cortex. Moreover, we found a strong cognitive impairment in tasks depending on this network. Importantly, the supplementation with d-serine completely restores hippocampal-prefrontal theta synchronization and rescues the spatial memory and long-term memory of dnSNARE mice. We provide here novel evidence of long distance network modulation by astrocytes, with direct implications to cognitive function.
Glia, 2017; 65
34521994: Mateus-Pinheiro A, Patrício P, Alves ND, Martins-Macedo J, Caetano I, Silveira-Rosa T, Araújo B, Mateus-Pinheiro M, Silva-Correia J, Sardinha VM, Loureiro-Campos E, Rodrigues AJ, Oliveira JF, Bessa JM, Sousa N, Pinto L
Hippocampal cytogenesis abrogation impairs inter-regional communication between the hippocampus and prefrontal cortex and promotes the time-dependent manifestation of emotional and cognitive deficits.
Impaired ability to generate new cells in the adult brain has been linked to deficits in multiple emotional and cognitive behavioral domains. However, the mechanisms by which abrogation of adult neural stem cells (NSCs) impacts on brain function remains controversial. We used a transgenic rat line, the GFAP-Tk, to selectively eliminate NSCs and assess

repercussions on different behavioral domains. To assess the functional importance of newborn cells in specific developmental stages, two parallel experimental timeframes were adopted: a short- and a long-term timeline, 1 and 4 weeks after the abrogation protocol, respectively. We conducted in vivo electrophysiology to assess the effects of cytogenesis abrogation on the functional properties of the hippocampus and prefrontal cortex, and on their intercommunication. Adult brain cytogenesis abrogation promoted a time-specific installation of behavioral deficits. While the lack of newborn immature hippocampal neuronal and glial cells elicited a behavioral phenotype restricted to hyperanxiety and cognitive rigidity, specific abrogation of mature new neuronal and glial cells promoted the long-term manifestation of a more complex behavioral profile encompassing alterations in anxiety and hedonic behaviors, along with deficits in multiple cognitive modalities. More so, abrogation of 4 to 7-week-old cells resulted in impaired electrophysiological synchrony of neural theta oscillations between the dorsal hippocampus and the medial prefrontal cortex, which are likely to contribute to the described long-term cognitive alterations. Hence, this work provides insight on how newborn neurons and astrocytes display different functional roles throughout different maturation stages, and establishes common ground to reconcile contrasting results that have marked this field.
Mol Psychiatry, 2021; 26
25035085: Patrício P, Mateus-Pinheiro A, Irmler M, Alves ND, Machado-Santos AR, Morais M, Correia JS, Korostynski M, Piechota M, Stoffel R, Beckers J, Bessa JM, Almeida OF, Sousa N, Pinto L
Differential and converging molecular mechanisms of antidepressants' action in the hippocampal dentate gyrus.
Major depression is a highly prevalent, multidimensional disorder. Although several classes of antidepressants (ADs) are currently available, treatment efficacy is limited, and relapse rates are high; thus, there is a need to find better therapeutic strategies. Neuroplastic changes in brain regions such as the hippocampal dentate gyrus (DG) accompany depression and its amelioration with ADs. In this study, the unpredictable chronic mild stress (uCMS) rat model of depression was used to determine the molecular mediators of chronic stress and the targets of four ADs with different pharmacological profiles (fluoxetine, imipramine, tianeptine, and agomelatine) in the hippocampal DG. All ADs, except agomelatine, reversed the depression-like behavior and neuroplastic changes produced by uCMS. Chronic stress induced significant molecular changes that were generally reversed by fluoxetine, imipramine, and tianeptine. Fluoxetine primarily acted on neurons to reduce the expression of pro-inflammatory response genes and increased a set of genes involved in cell metabolism. Similarities were found between the molecular actions and targets of imipramine and tianeptine that activated pathways related to cellular protection. Agomelatine presented a unique profile, with pronounced effects on genes related to Rho-GTPase-related pathways in oligodendrocytes and neurons. These differential molecular signatures of ADs studied contribute to our understanding of the processes implicated in the onset and treatment of depression-like symptoms.
Neuropsychopharmacology, 2015; 40
28585931: Morais M, Patrício P, Mateus-Pinheiro A, Alves ND, Machado-Santos AR, Correia JS, Pereira J, Pinto L, Sousa N, Bessa JM
The modulation of adult neuroplasticity is involved in the mood-improving actions of atypical antipsychotics in an animal model of depression.
Depression is a prevalent psychiatric disorder with an increasing impact in global public health. However, a large proportion of patients treated with currently available antidepressant drugs fail to achieve remission. Recently, antipsychotic drugs have received approval for the treatment of antidepressant-resistant forms of major depression. The modulation of adult neuroplasticity, namely hippocampal neurogenesis and neuronal remodeling, has been considered to have a key role in the therapeutic effects of antidepressants. However, the impact of antipsychotic drugs on these neuroplastic mechanisms remains largely unexplored. In this study, an unpredictable chronic mild stress protocol was used to induce a depressive-like phenotype in rats. In the last 3 weeks of stress exposure, animals were treated with two different antipsychotics: haloperidol (a classical antipsychotic) and clozapine (an atypical antipsychotic). We demonstrated that clozapine improved both measures of depressive-like behavior (behavior despair and anhedonia), whereas haloperidol aggravated learned helplessness in the forced-swimming test and behavior flexibility in a cognitive task. Importantly, an upregulation of adult neurogenesis and neuronal survival was observed in animals treated with clozapine, whereas haloperidol promoted a downregulation of these processes. Furthermore, clozapine was able to re-establish the stress-induced impairments in neuronal structure and gene expression in the hippocampus and prefrontal cortex. These results demonstrate the modulation of adult neuroplasticity by antipsychotics in an animal model of depression, revealing that the atypical antipsychotic drug clozapine reverts the behavioral effects of chronic stress by improving adult neurogenesis, cell survival and neuronal reorganization.
Transl Psychiatry, 2017; 7
28291258: Alves ND, Correia JS, Patrício P, Mateus-Pinheiro A, Machado-Santos AR, Loureiro-Campos E, Morais M, Bessa JM, Sousa N, Pinto L
Adult hippocampal neuroplasticity triggers susceptibility to recurrent depression.

Depression is a highly prevalent and recurrent neuropsychiatric disorder associated with alterations in emotional and cognitive domains. Neuroplastic phenomena are increasingly considered central to the etiopathogenesis of and recovery from depression. Nevertheless, a high number of remitted patients experience recurrent episodes of depression, remaining unclear how previous episodes impact on behavior and neuroplasticity and/or whether modulation of neuroplasticity is important to prevent recurrent depression. Through re-exposure to an unpredictable chronic mild stress protocol in rats, we observed the re-appearance of emotional and cognitive deficits. Furthermore, treatment with the antidepressants fluoxetine and imipramine was effective to promote sustained reversion of a depressive-like phenotype; however, their differential impact on adult hippocampal neuroplasticity triggered a distinct response to stress re-exposure: while imipramine re-established hippocampal neurogenesis and neuronal dendritic arborization contributing to resilience to recurrent depressive-like behavior, stress re-exposure in fluoxetine-treated animals resulted in an overproduction of adult-born neurons along with neuronal atrophy of granule neurons, accounting for an increased susceptibility to recurrent behavioral changes typical of depression. Strikingly, cell proliferation arrest compromised the behavior resilience induced by imipramine and buffered the susceptibility to recurrent behavioral changes promoted by fluoxetine. This study shows that previous exposure to a depressive-like episode impacts on the behavioral and neuroanatomical changes triggered by subsequent re-exposure to similar experimental conditions and reveals that the proper control of adult hippocampal neuroplasticity triggered by antidepressants is essential to counteract recurrent depressive-like episodes.
Transl Psychiatry, 2017; 7
31747562: Machado-Santos AR, Alves ND, Araújo B, Correia JS, Patrício P, Mateus-Pinheiro A, Loureiro-Campos E, Bessa JM, Sousa N, Pinto L
Astrocytic plasticity at the dorsal dentate gyrus on an animal model of recurrent depression.
Astrocytes are now known to play crucial roles in the central nervous system, supporting and closely interacting with neurons and therefore able to modulate brain function. Both human postmortem studies in brain samples from patients diagnosed with Major Depressive Disorder and from animal models of depression reported numerical and morphological astrocytic changes specifically in the hippocampus. In particular, these studies revealed significant reductions in glial cell density denoted by a decreased number of S100B-positive cells and a decrease in GFAP expression in several brain regions including the hippocampus. To reveal plastic astrocytic changes in the context of recurrent depression, we longitudinally assessed dynamic astrocytic alterations (gene expression, cell densities and morphologic variations) in the hippocampal dentate gyrus under repeated exposure to unpredictable chronic mild stress (uCMS) and upon treatment with two antidepressants, fluoxetine and imipramine. Both antidepressants decreased astrocytic complexity immediately after stress exposure. Moreover, we show that astrocytic alterations, particularly an increased number of S100B-positive cells, are observed after recurrent stress exposure. Interestingly, these alterations were prevented at the long-term by either fluoxetine or imipramine treatment.
Neuroscience, 2021; 454

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BOARD NUMBER: S07-290

PRE AND POST-SYMPTOMATIC TREATMENT WITH NLX-112 IMPROVED THE BALANCE AND MOTOR COORDINATION OF THE CMVMJD135 MOUSE MODEL OF MACHADO-JOSEPH DISEASE

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Introduction: Machado-Joseph Disease (MJD), an autosomal dominant neurodegenerative disease caused by an expansion of a CAG repeat tract in the ataxin-3 gene (ATXN3), is mainly characterized by a late onset of motor dysfunction. Currently, no disease-modifying therapy is available. Recent findings showed that NLX-112, a highly selective and fully efficacious 5-HT_{1A}R agonist, improved motor dysfunction and reduced mutant ATXN3 aggregation in a *Caenorhabditis elegans* MJD model. **Aims:** This study aims to analyze the therapeutic impact of NLX-112 in the CMVMJD135 MJD mice motor function. **Methods:** After selection of drug doses, based on plasma/brain exposure levels and animal welfare, two preclinical trials were performed: WT and MJD animals were treated with NLX-112 pre-symptomatically via drinking water for 35weeks, using tandospirone as reference, and post-symptomatically via intraperitoneal injections for 16weeks. Animals' welfare, body weight and temperature were regularly assessed, along with motor function using the beam walking (BWT) and motor swimming (MST) tests. **Results:** These experiments revealed that doses up to 8mg/kg for NLX-112 and 80mg/kg for TD were safe and well-tolerated. Pre-symptomatically, NLX-112 significantly improved motor coordination and balance of MJD animals in the BWT, at advanced stages of the disease. Contrarily, TD exhibited no therapeutic effect on motor function. Post-symptomatically, NLX-112-treated animals' coordination was improved in the MST early on the disease, while minor alterations were observed in other motor parameters. **Conclusions:** Overall, NLX-112 showed a beneficial effect on the motor function of MJD mice, reinforcing the potential role of serotonergic signaling modulation as a promising therapeutic target for MJD.

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NUCLEAR ATXN3 DUB PHYSIOLOGICAL SUBSTRATES: TOWARDS UNDERSTANDING THEIR ROLE IN THE CONTEXT OF MACHADO-JOSEPH DISEASE

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Background: Machado-Joseph disease (MJD) is a neurodegenerative disorder caused by expansion of a polyglutamine tract within the deubiquitinating enzyme (DUB) ataxin-3 (ATXN3). Strong evidence in cellular and animal models of MJD support the nuclear presence of ATXN3 as key for inducing pathogenesis. However, the mechanisms through which ATXN3 exerts its deleterious effects in the nucleus of neuronal cells remain mostly unknown, which challenges the development of disease modifying therapies. **Aims**: To characterize nuclear ATXN3s interactors and substrates relevant for MJD. **Methods:** We used proteomics approaches and biochemical and biophysical methods to identify novel nuclear ATXN3 interactors and substrates and characterize the interactions. **Results:** Using the proximity ligation (PLA) assay, we have validated potential nuclear interactors of ATXN3, such as SRSF7, GR, CUL1 and FUS. To confirm and further characterize these interactions, we are currently developing a new tool, using an inducible cellular model overexpressing both normal and expanded ATXN3 with a tandem affinity tag, allowing at the same time the validation of interactions, the identification of other proteins present in large complexes together with ATXN3, and to uncover their differential affinity to the mutant form of ATXN3. This will help the identification of specific targets in the disease context. **Conclusion:** In addition to bringing novel insight into the physiological function of ATXN3, we will increase our understanding of MJD pathogenesis, which may contribute for the development of more efficient therapeutic strategies.

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THE NEUROPROTECTIVE EFFECT OF VEGF-B ON THE CEREBELLAR DESTRUCTURATION ASSOCIATED WITH AUTISM SPECTRUM DISORDERS

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Autism spectrum disorder (ASD) is an abnormal neurodevelopmental process in which the cerebellum is a structure to bear in mind in the study of ASD for both its motor function and its involvement in cognitive, affective and social behaviour. ASD patients have a specific loss of Purkinje cells, combined with an affectation of neurotrophic factors (NF). This work aims at studying the cerebellar destructuration employing the PCD mutant mouse, an animal model that presents a specific loss of Purkinje cells. For the analysis of the relationship between NF and cerebellar damage, both gene and protein expression of the NF insulin growth factor 1 (IGF-1) and vascular endothelial growth factor B (VEGF-B) were analysed before and during the Purkinje cell loss, thus finding statistically significant differences for both NF. These data have been used to carry out a pharmacological treatment with recombinant human IGF-1 (rhIGF-1) and recombinant human VEGF-B (rhVEGF-B). We observed an improvement in both motor coordination and Purkinje Cells survival when we treated PCD mice with rhVEGF-B. One of the functions of this NF is the inhibition of the intrinsic pathway of the apoptotic process, as we demonstrated by qPCR analyses. Then, we analysed the mitochondrial morphology and function in PCD mice and how it may contribute to the enhancement of motor coordination after rhVEGF-B treatment. Our preliminary results showed alterations in mitochondrial morphology of PCD mice, as well as decreased motility and/or fusion capacity. rhVEGF-B treatment seemed to decrease such impairments E-mail: lauraprev@usal.es; ddiaz@usal.es; jralonso@usal.es

Pubmed:
33299042: Pérez-Revuelta L, Téllez de Meneses PG, López M, Briñón JG, Weruaga E, Díaz D, Alonso JR
Secretagogin expression in the mouse olfactory bulb under sensory impairments.
The interneurons of the olfactory bulb (OB) are characterized by the expression of different calcium-binding proteins, whose specific functions are not fully understood. This is the case of one of the most recently discovered, the secretagogin (SCGN), which is expressed in interneurons of the glomerular and the granule cell layers, but whose function in the olfactory pathway is still unknown. To address this question, we examined the distribution, generation and activity of SCGN-positive interneurons in the OB of two complementary models of olfactory impairments: Purkinje Cell Degeneration (PCD) and olfactory-deprived mice. Our results showed a significant increase in the density of SCGN-positive cells in the inframitral layers of olfactory-deprived mice as compared to control animals. Moreover, BrdU analyses revealed that these additional SCGN-positive cells are not newly formed. Finally, the neuronal activity, estimated by c-Fos expression, increased in preexisting SCGN-positive interneurons of both deprived and PCD mice -being higher in the later- in comparison with control animals. Altogether, our results suggest that the OB possesses different compensatory mechanisms depending on the type of alteration. Particularly, the SCGN expression is dependent of olfactory stimuli and its function may be related to a compensation against a reduction in sensory inputs.
Sci Rep, 2020; 10
34916910: Del Pilar C, Lebrón-Galán R, Pérez-Martín E, Pérez-Revuelta L, Ávila-Zarza CA, Alonso JR, Clemente D, Weruaga E, Díaz D
The Selective Loss of Purkinje Cells Induces Specific Peripheral Immune Alterations.
The progression of neurodegenerative diseases is reciprocally associated with impairments in peripheral immune responses. We investigated different contexts of selective neurodegeneration to identify specific alterations of peripheral immune cells and, at the same time, discover potential biomarkers associated to this pathological condition. Consequently, a model of human cerebellar degeneration and ataxia -the Purkinje Cell Degeneration (PCD) mouse- has been employed, as it allows the study of different processes of selective neuronal death in the same animal, i.e., Purkinje cells in the cerebellum and

mitral cells in the olfactory bulb. Infiltrated leukocytes were studied in both brain areas and compared with those from other standardized neuroinflammatory models obtained by administering either gamma radiation or lipopolysaccharide. Moreover, both myeloid and lymphoid splenic populations were analyzed by flow cytometry, focusing on markers of functional maturity and antigen presentation. The severity and type of neural damage and inflammation affected immune cell infiltration. Leukocytes were more numerous in the cerebellum of PCD mice, being located predominantly within those cerebellar layers mostly affected by neurodegeneration, in a completely different manner than the typical models of induced neuroinflammation. Furthermore, the milder degeneration of the olfactory bulb did not foster leukocyte attraction. Concerning the splenic analysis, in PCD mice we found: (1) a decreased percentage of several myeloid cell subsets, and (2) a reduced mean fluorescence intensity in those myeloid markers related to both antigen presentation and functional maturity. In conclusion, the selective degeneration of Purkinje cells triggers a specific effect on peripheral immune cells, fostering both attraction and functional changes. This fact endorses the employment of peripheral immune cell populations as concrete biomarkers for monitoring different neuronal death processes.
Front Cell Neurosci, 2021; 15

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BOARD NUMBER: S07-293

PERSISTENT DNA DAMAGE ASSOCIATED WITH ATM KINASE DEfiCIENCY PROMOTES MICROGLIAL DYSFUNCTION

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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About 30 neurodegenerative disorders are like to mutations in genes linked to DNA damage response. In our lab, we study Ataxia-telangiectasia (A-T), a rare genetic disorder caused by loss-of-function mutations in ATM, a kinase involved in the reponse to cytotoxic double strand breaks. This multi-system disease is characterised by genome instability, immune dysfunction, metabolic abnormalities and cancer susceptibility. However, one of the most devastating phenotypes of A-T is the progressive loss of cerebellar neurons leading most patients to use a wheelchair by early teenage years. The mechanisms of neurological defects in A-T are not well understood. Here, we demonstrate that aberrant activities of normally neuroprotective microglia, immune cells of the central nervous system, contribute to neurodegeneration in A-T. Increased levels of DNA damage and oxidative stress associated with loss of ATM, or its activity, promote sustained microglial activation. This activation is linked with increased: i) production of pro-inflammatory cytokines and ii) clearance activities, resulting in aberrant removal of healthy neurons and neurites by human microglia. Mechanistically, aberrant microglial activation in the absence of ATM is mediated by the pro-inflammatory RELB/p52 non-canonical NF-κB transcriptional pathway. Activation of the RELB/p52 pathway in ATM-deficient microglia is driven by persistent DNA damage. Persistent activation of the RELB/p52 pathway associated with ATM deficiency promotes chronic neuroinflammation with neurotoxic consequences. Activation of non-canonical NF-κB signalling is also observed in cerebellar microglia of individuals with Ataxia telangiectasia. These results provide novel mechanistic insights into the microglial dysfunction which contributes to progressive neurodegeneration in A-T.

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BOARD NUMBER: S07-294

INCREASED PKCGAMMA KINASE ACTIVITY CAUSES ABNORMAL PURKINJE CELL MATURATION AND CEREBELLAR ATAXIA

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Spinocerebellar ataxias (SCAs) are neurodegenerative diseases characterized by cerebellar atrophy and dysfunction and loss of Purkinje cells. Among SCAs, Spinocerebellar ataxia type 14 (SCA14) is caused by missense mutations or deletions in the protein kinase C-gamma (PKCγ) gene which is a crucial signaling molecule in Purkinje cells. Although a variety of mutations or deletions have been found in SCA14 patients, it is not well understood how these mutations in PKCγ lead to Purkinje cell degeneration and ataxia. In order to study the role of increased PKCγ activity we generated a knock-in mouse model related to SCA14 with a mutation in the pseudosubstrate domain in PKCγ, PKCγ^{A24E}, known to induce a constitutive PKCγ activation in vitro assay. The kinase domain of PKCγ^{A24E} is open and activated but the protein is subject to dephosphorylation and degradation. PKCγ^{A24E} mice show indeed increased biological PKCγ activity, abnormal Purkinje cell maturation and ataxia while at the same time PKCγ protein expression is drastically reduced with the degradation. These unique phenotypes of PKCγ^{A24E} mice call for a further investigation of mutant PKCγ biological activity and protein stability. In order to address how PKCγ^{A24E} protein kinase activity and stability are controlled, we introduced additional mutations in the regulatory domain or at the phosphorylation sites in the kinase domain of PKCγ^{A24E}. Our preliminary data show that additional mutations in PKCγ^{A24E} affect kinase activity in Purkinje cells and can modify PKCγ protein expression. These data will provide new insights about the regulation of PKCγ activity and stability.

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EXERCISE ACTS VIA BDNF-TRKB SIGNALLING TO RESCUE BEHAVIOURAL AND PURKINJE CELL FIRING DEFICITS IN A MOUSE MODEL OF SPINOCEREBELLAR ATAXIA TYPE 6

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

Anna Cook, Sriram Jayabal, Kc Jacky Sheng, Alanna Watt
McGill University, Biology, Montreal, Canada

Spinocerebellar ataxia type 6 (SCA6) is an inherited neurodegenerative disease with mid-life onset of motor coordination impairment, eventual cerebellar degeneration, and limited treatment options. We used a knock in mouse model of the disease ($SCA6^{84Q/84Q}$) to characterize SCA6 pathophysiology and identify potential therapeutics. At 7 months these mice display significant deficits in motor coordination as well as alterations in Purkinje cell firing. We found that one month of voluntary exercise rescued deficits in motor behaviour and Purkinje cell firing frequency in $SCA6^{84Q/84Q}$ mice. But how does exercise act therapeutically in the $SCA6^{84Q/84Q}$ model? Brain-derived neurotrophic factor (BDNF) is known to be upregulated by exercise in the brain, and BDNF RNA levels are reduced in post-mortem brain tissue from SCA6 patients (Takahashi *et al.,* 2012). We found that $SCA6^{84Q/84Q}$ mice had reduced levels of cerebellar BDNF, and this was reversed by exercise. To determine whether BDNF signalling mediates the effect of exercise in $SCA6^{84Q/84Q}$ mice, we administered 7,8-dihydroxyflavone (7,8-DHF), a small molecule that mimics BDNF as an agonist of the TrkB receptor. Chronic 7,8-DHF treatment rescued deficits in both motor coordination and Purkinje cell firing frequency. Treatment with 7,8-DHF was most effective when it was started early in disease progression, and could continue to rescue deficits for several months. By identifying a pathway by which exercise acts in the SCA6 cerebellum, we have identified novel therapeutic targets for SCA6.

Pubmed:

34970120: Toscano Márquez B, Cook AA, Rice M, Smileski A, Vieira-Lomasney K, Charron F, McKinney RA, Watt AJ
Molecular Identity and Location Influence Purkinje Cell Vulnerability in Autosomal-Recessive Spastic Ataxia of Charlevoix-Saguenay Mice.
Patterned cell death is a common feature of many neurodegenerative diseases. In patients with autosomal-recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) and mouse models of ARSACS, it has been observed that Purkinje cells in anterior cerebellar vermis are vulnerable to degeneration while those in posterior vermis are resilient. Purkinje cells are known to express certain molecules in a highly stereotyped, patterned manner across the cerebellum. One patterned molecule is zebrin, which is expressed in distinctive stripes across the cerebellar cortex. The different zones delineated by the expression pattern of zebrin and other patterned molecules have been implicated in the patterning of Purkinje cell death, raising the question of whether they contribute to cell death in ARSACS. We found that zebrin patterning appears normal prior to disease onset in mice, suggesting that zebrin-positive and -negative Purkinje cell zones develop normally. We next observed that zebrin-negative Purkinje cells in anterior lobule III were preferentially susceptible to cell death, while anterior zebrin-positive cells and posterior zebrin-negative and -positive cells remained resilient even at late disease stages. The patterning of Purkinje cell innervation to the target neurons in the cerebellar nuclei (CN) showed a similar pattern of loss: neurons in the anterior CN, where inputs are predominantly zebrin-negative, displayed a loss of Purkinje cell innervation. In contrast, neurons in the posterior CN, which is innervated by both zebrin-negative and -positive puncta, had normal innervation. These results suggest that the location and the molecular identity of Purkinje cells determine their susceptibility to cell death in ARSACS.
Front Cell Neurosci, 2021; 15
32554108: Cook AA, Fields E, Watt AJ
Losing the Beat: Contribution of Purkinje Cell Firing Dysfunction to Disease, and Its Reversal.
The cerebellum is a brain structure that is highly interconnected with other brain regions. There are many contributing factors to cerebellar-related brain disease, such as altered afferent input, local connectivity, and/or cerebellar output. Purkinje cells (PC) are the principle cells of the cerebellar cortex, and fire intrinsically; that is, they fire spontaneous action potentials at high frequencies. This review paper focuses on PC intrinsic firing activity, which is altered in multiple neurological diseases, including ataxia, Huntington Disease (HD) and autism spectrum disorder (ASD). Notably, there are several cases where interventions that restore or rescue PC intrinsic activity also improve impaired behavior in these mouse models of disease.

These findings suggest that rescuing PC firing deficits themselves may be sufficient to improve impairment in cerebellar-related behavior in disease. We propose that restoring PC intrinsic firing represents a good target for drug development that might be of therapeutic use for several disorders.
Neuroscience, 2021; 462
28406156: Houston CM, Diamanti E, Diamantaki M, Kutsarova E, Cook A, Sultan F, Brickley SG
Exploring the significance of morphological diversity for cerebellar granule cell excitability.
The relatively simple and compact morphology of cerebellar granule cells (CGCs) has led to the view that heterogeneity in CGC shape has negligible impact upon the integration of mossy fibre (MF) information. Following electrophysiological recording, 3D models were constructed from high-resolution imaging data to identify morphological features that could influence the coding of MF input patterns by adult CGCs. Quantification of MF and CGC morphology provided evidence that CGCs could be connected to the multiple rosettes that arise from a single MF input. Predictions from our computational models propose that MF inputs could be more densely encoded within the CGC layer than previous models suggest. Moreover, those MF signals arriving onto the dendrite closest to the axon will generate greater CGC excitation. However, the impact of this morphological variability on MF input selectivity will be attenuated by high levels of CGC inhibition providing further flexibility to the MF → CGC pathway. These features could be particularly important when considering the integration of multimodal MF sensory input by individual CGCs.
Sci Rep, 2017; 7

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PATHOPHYSIOLOGICAL MECHANISMS OF CORTICAL SPREADING DEPOLARIZATION IN AN SLC1A3/EAAT1 MOUSE MODEL FOR EPISODIC ATAXIA, EPILEPSY AND HEMIPLEGIC MIGRAINE

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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The rare neurological disorder Episodic Ataxia type 6 is associated with variants in the *SLC1A3* gene, encoding the excitatory amino acid transporter 1 (EAAT1). The variant P290R, which increases anion channel function and impairs glutamate transport, leads to a complex clinical phenotype, including ataxia, epileptic seizures and hemiplegic migraine attacks. Cortical Spreading Depolarization (CSD), the correlate of migraine aura, is a wave of cellular depolarization, followed by suppression of cortical activity. To clarify the mechanisms of CSD caused by altered glutamate and chloride homeostasis, we studied a mouse model carrying the respective variant. *In vitro* CSD characteristics using acute cortical slices were examined by combining intrinsic optical signal imaging with extracellular and potassium-sensitive recordings. We investigated synaptic transmission in the somatosensory cortex (layer 2/3) using whole-cell patch clamp recordings in acute slices of P15-20 animals. Brain slice recordings from *Slc1a3*$^{P290R/+}$ mice before disease onset (P15-20) revealed an increase in CSD susceptibility and propagation speed as well as rapid K^+-dynamics. Our findings on spontaneous and miniature synaptic currents clarified if the migraine phenotype *in vitro* was caused by an imbalance between excitation and inhibition on the cellular level. In the cortex, EAAT1 is mainly expressed in astrocytes, which decisively regulate tonic inhibition. Thus, we finally investigated alterations of tonic GABAergic currents. Our data show that, along with the epileptic and ataxic phenotype, *Slc1a3*$^{P290R/+}$ animals also display a clear hemiplegic migraine phenotype *in vitro* and therefore, resemble patients carrying the P290R variant. However, the consequences *in vivo* have not been unravelled, yet.

Pubmed:
34546973: Auffenberg E, Hedrich UB, Barbieri R, Miely D, Groschup B, Wuttke TV, Vogel N, Lührs P, Zanardi I, Bertelli S, Spielmann N, Gailus-Durner V, Fuchs H, Hrabě de Angelis M, Pusch M, Dichgans M, Lerche H, Gavazzo P, Plesnila N, Freilinger T
Hyperexcitable interneurons trigger cortical spreading depression in an Scn1a migraine model.
Cortical spreading depression (CSD), a wave of depolarization followed by depression of cortical activity, is a pathophysiological process implicated in migraine with aura and various other brain pathologies, such as ischemic stroke and traumatic brain injury. To gain insight into the pathophysiology of CSD, we generated a mouse model for a severe monogenic subtype of migraine with aura, familial hemiplegic migraine type 3 (FHM3). FHM3 is caused by mutations in SCN1A, encoding the voltage-gated Na+ channel NaV1.1 predominantly expressed in inhibitory interneurons. Homozygous Scn1aL1649Q knock-in mice died prematurely, whereas heterozygous mice had a normal lifespan. Heterozygous Scn1aL1649Q knock-in mice compared with WT mice displayed a significantly enhanced susceptibility to CSD. We found L1649Q to cause a gain-of-function effect with an impaired Na+-channel inactivation and increased ramp Na+ currents leading to hyperactivity of fast-spiking inhibitory interneurons. Brain slice recordings using K+-sensitive electrodes revealed an increase in extracellular K+ in the early phase of CSD in heterozygous mice, likely representing the mechanistic link between interneuron hyperactivity and CSD initiation. The neuronal phenotype and premature death of homozygous Scn1aL1649Q knock-in mice was partially rescued by GS967, a blocker of persistent Na+ currents. Collectively, our findings identify interneuron hyperactivity as a mechanism to trigger CSD.
J Clin Invest, 2021; 131

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BOARD NUMBER: S07-297

DYNAMICS OF TDP43 AXONAL TRANSPORT AND ITS INTERACTION WITH THE KINESIN-1 MOTOR MACHINERY

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

Monica Feole, Victorio Pozo Devoto, Pratiksha V Bhat, Neda Dragišić, Gorazd Stokin
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Transport of cargoes has been extensively studied, particularly from the point of view of the motor proteins. However, much less is known about the behavior of specific cargoes, the malfunctioning of their transport and role in neuropathological mechanisms. Here, we focus on TDP43, an important mRNA homeostasis regulator related to Amyotrophic Lateral Sclerosis (ALS) pathology. **AIM** The aim of this study is to define the machinery involved in the transport of TDP43, and how this mechanism is possibly disrupted in presence of disease-linked mutations. **METHODS** Stem cells-derived human neurons and real-time imaging of GFP-tagged cargoes were used to record their axonal transport. Time-lapse movies were analyzed using tracking algorithms to describe movement parameters. Co-IPs and Proximity Ligation Assays (PLAs) were performed to identify interactions between TDP43 and other components of the canonical anterograde/retrograde transport machinery. **RESULTS** We characterize TDP43 axonal transport by comparing it with Synaptophysin, Rab5 and APP. Our results on proteins movement reveal characteristics of the TDP43 transport behavior, which is bi-directional with high number of reversions and low pause frequency. On the other hand, TDP43 showed velocities and run lengths akin to those of kinesin-1 transported cargoes. Furthermore, Co-IPs and PLAs confirmed that TDP43 is part of both kinesin and dynein motor complexes. Last, we described how ALS-linked TDP43 mutations affect its transport and its distribution throughout the neuron. **CONCLUSIONS** This study contributes to elucidate mechanisms regulating the functionality and localization of TDP43 and ultimately to understand the pathogenesis of ALS.

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A FORWARD GENETIC SCREEN IN DROSOPHILA TO IDENTIFY GENES REQUIRED FOR AXONAL MAINTENANCE

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Many genes involved in motor neurodegenerative disease remain unidentified, and the molecular mechanisms underlying axonal degeneration are incompletely understood. In order to gain novel insight into the molecular pathogenesis of axonal degeneration and to identify novel disease-causing genes, we performed an EMS-based forward genetic screen, focusing on essential genes on the Drosophila X chromosome. Visualization of homozygously mutant motor axon clones allowed to identify axonal degeneration in the adult fly leg. This resulted in the identification of 72 unique mutants with motor axon degenerative phenotypes. Thus far, whole genome sequencing allowed identification of the disease-causing gene for 40 mutants. 96% of these genes have human orthologues, whereas 65% have been associated with disease in OMIM. These proteins are involved in a broad range of molecular pathways, including metabolism, axonal transport and transcriptional regulation. Interestingly, we have identified 11 genes that have neither been linked to human disease nor were found in any similar Drosophila screenings, indicating these could be novel players involved in axonal maintenance. We are currently evaluating whether the human orthologues of these genes may be associated with human disease.

Pubmed:

31591561: Picchiarelli G, Demestre M, Zuko A, Been M, Higelin J, Dieterlé S, Goy MA, Mallik M, Sellier C, Scekic-Zahirovic J, Zhang L, Rosenbohm A, Sijlmans C, Aly A, Mersmann S, Sanjuan-Ruiz I, Hübers A, Messaddeq N, Wagner M, van Bakel N, Boutillier AL, Ludolph A, Lagier-Tourenne C, Boeckers TM, Dupuis L, Storkebaum E
FUS-mediated regulation of acetylcholine receptor transcription at neuromuscular junctions is compromised in amyotrophic lateral sclerosis.
Neuromuscular junction (NMJ) disruption is an early pathogenic event in amyotrophic lateral sclerosis (ALS). Yet, direct links between NMJ pathways and ALS-associated genes such as FUS, whose heterozygous mutations cause aggressive forms of ALS, remain elusive. In a knock-in Fus-ALS mouse model, we identified postsynaptic NMJ defects in newborn homozygous mutants that were attributable to mutant FUS toxicity in skeletal muscle. Adult heterozygous knock-in mice displayed smaller neuromuscular endplates that denervated before motor neuron loss, which is consistent with 'dying-back' neuronopathy. FUS was enriched in subsynaptic myonuclei, and this innervation-dependent enrichment was distorted in FUS-ALS. Mechanistically, FUS collaborates with the ETS transcription factor ERM to stimulate transcription of acetylcholine receptor genes. Co-cultures of induced pluripotent stem cell-derived motor neurons and myotubes from patients with FUS-ALS revealed endplate maturation defects due to intrinsic FUS toxicity in both motor neurons and myotubes. Thus, FUS regulates acetylcholine receptor gene expression in subsynaptic myonuclei, and muscle-intrinsic toxicity of ALS mutant FUS may contribute to dying-back motor neuronopathy.
Nat Neurosci, 2019; 22
32302304: Catinozzi M, Mallik M, Frickenhaus M, Been M, Sijlmans C, Kulshrestha D, Alexopoulos I, Weitkunat M, Schnorrer F, Storkebaum E
The Drosophila FUS ortholog cabeza promotes adult founder myoblast selection by Xrp1-dependent regulation of FGF signaling.
The number of adult myofibers in Drosophila is determined by the number of founder myoblasts selected from a myoblast pool, a process governed by fibroblast growth factor (FGF) signaling. Here, we show that loss of cabeza (caz) function results in a reduced number of adult founder myoblasts, leading to a reduced number and misorientation of adult dorsal abdominal muscles. Genetic experiments revealed that loss of caz function in both adult myoblasts and neurons contributes to caz mutant muscle phenotypes. Selective overexpression of the FGF receptor Htl or the FGF receptor-specific signaling molecule Stumps in adult myoblasts partially rescued caz mutant muscle phenotypes, and Stumps levels were reduced in caz mutant founder myoblasts, indicating FGF pathway deregulation. In both adult myoblasts and neurons, caz mutant muscle phenotypes were mediated by increased expression levels of Xrp1, a DNA-binding protein involved in gene expression regulation. Xrp1-induced phenotypes were dependent on the DNA-binding capacity of its AT-hook motif, and increased Xrp1

levels in founder myoblasts reduced Stumps expression. Thus, control of Xrp1 expression by Caz is required for regulation of Stumps expression in founder myoblasts, resulting in correct founder myoblast selection.
PLoS Genet, 2020; 16
34516840: Zuko A, Mallik M, Thompson R, Spaulding EL, Wienand AR, Been M, Tadenev ALD, van Bakel N, Sijlmans C, Santos LA, Bussmann J, Catinozzi M, Das S, Kulshrestha D, Burgess RW, Ignatova Z, Storkebaum E
tRNA overexpression rescues peripheral neuropathy caused by mutations in tRNA synthetase.
Heterozygous mutations in six transfer RNA (tRNA) synthetase genes cause Charcot-Marie-Tooth (CMT) peripheral neuropathy. CMT mutant tRNA synthetases inhibit protein synthesis by an unknown mechanism. We found that CMT mutant glycyl-tRNA synthetases bound tRNA but failed to release it, resulting in tRNA sequestration. This sequestration potentially depleted the cellular tRNA pool, leading to insufficient glycyl-tRNA supply to the ribosome. Accordingly, we found ribosome stalling at glycine codons and activation of the integrated stress response (ISR) in affected motor neurons. Moreover, transgenic overexpression of tRNA rescued protein synthesis, peripheral neuropathy, and ISR activation in and mouse CMT disease type 2D (CMT2D) models. Conversely, inactivation of the ribosome rescue factor GTPBP2 exacerbated peripheral neuropathy. Our findings suggest a molecular mechanism for CMT2D, and elevating tRNA levels may thus have therapeutic potential.
Science, 2021; 373

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BOARD NUMBER: S07-299

TARGETING GANGLIOSIDE BIOSYNTHESIS AS A THERAPEUTIC OPTION FOR HEREDITARY SPASTIC PARAPLEGIA

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Hereditary spastic paraplegias (HSP) are a group of neurodegenerative diseases characterized by rigidity in lower limbs and motor dysfunction. Mutations in the SPG11 gene account for the most common form of autosomal recessive HSP, which is characterized by a gait disorder as well as cognitive impairment due to various brain alterations[(1)]. We generated a *Spg11* knock-out (KO) mouse model that develops the main symptoms found in SPG11 patients[(2)]. In this *Spg11* KO mouse model, neurodegeneration is associated with accumulation of some lipids, simple gangliosides, in lysosomes[(3)]. Moreover, decreasing ganglioside synthesis *in vitro* prevents neuronal death, suggesting that decreasing ganglioside synthesis could be a therapeutic option.
To test this hypothesis we downregulated key enzyme involved in this pathway, the ST3GAL5/GM3 synthase. To provide the proof of concept, we expressed a miRNA downregulated GM3 synthase using AAV-PHP-eB viral vectors that are able to transduce most cells in the body.
Behavioral investigations reveal that preventing ganglioside synthesis delays the onset of motor and cognitive symptoms of *Spg11* KO mouse. Moreover, miRNA treatment in *Spg11* KO mouse decreases ganglioside and autophagic marker accumulation that could be link to neuronal death.
This work shows promising results and could identify the first disease modifying therapeutic strategy for HSP.
(1) Stevanin et al., Nat Genet. 2007
(2) Branchu et al., Neurobiol Dis. 2017
(3) Boutry et al., Cell Rep. 2018

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BOARD NUMBER: S07-300

CONTEXTUAL MEMORY DECLINE IN A COMBINED MOUSE MODEL FOR ISCHEMIC STROKE AND ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Background and aims: Ischemic stroke (IS) is a risk factor for Alzheimer's disease (AD). Contextual memory deficits have been shown in mouse models for IS and AD. Hippocampal neurogenesis seems to be a key feature in both conditions, but with potentially contrasting roles. In our pilot study, we studied memory retrieval in a contextual fear conditioning (CFC) paradigm to explore mechanistic interaction in the combined IS/3xTg-AD mouse model. Methods: IS was induced by permanent middle cerebral artery occlusion (pMCAO) in 4-months-old male and female 3xTg-AD vs control (B6129SF2/J) mice (n=4-8). Prior to pMCAO, mice were subjected to CFC. Memory retrieval was evaluated by freezing behavior (%) during a 5-minute retrieval test 24h after conditioning and 8 weeks after pMCAO. Infarct size was determined by Nissl-staining. Results: Twenty-four hours after CFC, freezing behavior was high in all groups (>80%), suggesting successful conditioning. Eight weeks after pMCAO, mice showed full motor recovery. B6129SF2/J mice subjected to pMCAO displayed less freezing behavior compared to shams. In 3xTg-AD sham mice, freezing percentage was lower compared to B6129SF2/J shams, but in contrast to B6129SF2/J mice, no effect of pMCAO could be detected. Subgroup analysis suggests that pMCAO in AD mice only deteriorates fear memory in males, whereas pMCAO does not seem to alter freezing behavior in females. Infarct size was comparable between 3xTg-AD and control mice. Conclusion: Our preliminary results suggest that pMCAO in 4-months-old mice affects contextual memory in B6129SF2/J, but we found no clear evidence for memory decline following pMCAO in 3xTg-AD mice.

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THE UBIQUITIN PROTEASOME SYSTEM AS A REGULATOR OF MOTONEURON FUNCTION

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Homeostatic balance between protein synthesis, turn over and degradation is vital for the stability and viability of all living cells. Most eukaryotic cellular proteins are degraded by either the lysosome or the proteasome, with the proteasome primarily used to degrade misfolded proteins, damaged polypeptides or transient proteins commonly used in cellular signalling. Whilst the Ubiquitin Proteasome System (UPS) has a primary function to degrade proteins, this role has far-reaching outcomes for cell fate and survival. This is especially the case for neurons, which appear to be particularly susceptible to UPS perturbation evidenced by the role of UPS in neurodegenerative diseases such as Alzheimer's and Parkinson's disease. However, there is also mounting evidence for the role of the UPS in the healthy functioning of neurons, including in shaping neurotransmitter release, receptor trafficking and the regulation of axonal and dendritic growth during development. In this study, whole-cell patch clamp technique has been used to determine how pharmacological inhibition of the proteasome influences the intrinsic properties of primary motor neurons (PMNs) in larval zebrafish. Pharmacological inhibition of the proteasome reliably increased the frequency of glycine miniature inhibitory post-synaptic currents (mIPSCs) without affecting their kinetic properties. However, glutamatergic miniature excitatory post-synaptic currents (mEPSCs) were not affected by proteasome inhibition. Similarly, pharmacological inhibition of the proteasome did not affect action potential parameters. These results show that there is selective regulation of glycinergic signalling in the spinal cord of larval zebrafish upon inhibition of the proteasome.

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BOARD NUMBER: S07-302

THE STATE OF VITAMIN D-AUTO/PARACRINE SYSTEM IN ASSOCIATION WITH BEHAVIORAL, STRUCTURAL AND FUNCTIONAL BRAIN IMPAIRMENTS IN RATS WITH GLUCOCORTICOID-INDUCED NEUROTOXICITY

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Aim: To identify the association between vitamin D (VD) status and glucocorticoid (GC)-induced brain structural and functional changes. **Methods:** Female Wistar rats received prednisolone (5 mg/kg b.w.) with or without VD (1000 IU/kg b.w., 30 days). Forced swim (FST) and open-field (OFT) behavioral tests, H&E or Toluidine blue histological staining, microelectrode array recordings of extracellular potential, western blotting, qRT-PCR and ELISA were performed. Statistical differences were evaluated by ANOVA with Tukey post-hoc test. **Results:** There was no difference in OFT parameters, while FST demonstrated increased immobility time and decreased active behavior in GC-treated rats, indicative of depressive changes. No differences were found in cytoarchitectonics of cerebral cortex and posterior thalamic nucleus. However, prednisolone increased perikaryon area, enlarged nuclei and reduced cell density of CA1-CA3 hippocampal fields vs. control. This correlated with a down-regulated long-term plasticity of CA1-CA3 hippocampal synapses, declined amplitude of high-K^+-stimulated exocytosis, and lowered rate of Ca^{2+}-dependent fusion of isolated synaptic vesicles with plasma membrane vs. control. GC led to 3.2-, 2.0-, and 2.2-fold depletion of 25-hydroxyvitamin D (25OHD) pool in serum, cerebrospinal fluid and brain tissue, respectively, as well as elevated VDR and CYP27B1, and lowered CYP24A1 and VDBP levels. VD treatment reduced immobility time and stimulated active behavior. It caused a decrease in CA1 perikaryon and nucleus areas, restored /ong-term synaptic plasticity in hippocampus and depolarization-induced synaptic vesicle release that was accompanied by partial normalization of 25OHD circulating pool, VDBP and VDR levels. **Conclusions:** Normalization of VD status may be effective in prevention of GC-induced brain structural/functional/behavioral changes.

Pubmed:

35103058: Natrus LV, Osadchuk YS, Lisakovska OO, Labudzinskyi DO, Klys YG, Chaikovsky YB
Effect of Propionic Acid on Diabetes-Induced Impairment of Unfolded Protein Response Signaling and Astrocyte/Microglia Crosstalk in Rat Ventromedial Nucleus of the Hypothalamus.
The aim was to investigate the influence of propionic acid (PA) on the endoplasmic reticulum (ER), unfolded protein response (UPR) state, and astrocyte/microglia markers in rat ventromedial hypothalamus (VMH) after type 2 diabetes mellitus (T2DM).
Neural Plast, 2022; 2022
35065023: Kasatkina LA, Gumenyuk VP, Lisakovska OO, Trikash IO
Targeting hippocampal amyloidogenesis with SV2A protein modulator levetiracetam.
Cerebral amyloid β (Aβ) proteostasis is compromised under neuronal overexcitation, long-term neuroinflammation and brain aging. Using the animal model of LPS-induced neuroinflammation we demonstrated that treatment with levetiracetam, a specific modulator of synaptic vesicle glycoprotein SV2A, rescues abnormal synaptic vesicle (SV) fusion and neurotransmitter release, decreasing elevated hippocampal APP levels in vivo. Therapy with levetiracetam upregulates the SV2A in hippocampus and restores the level of apolipoprotein E, involved in brain Aβ aggregation/clearance and resolution of inflammation. We demonstrated that oligomers of Aβ and Aβ peptides promote SV clustering, which reduces the rate and plateau level of subsequent homo- and heterotypic SNARE-mediated SV fusion. Oligomeric Aβ lowered ΔpH gradient across the vesicular membrane, thus affecting their neurotransmitter storage capacity. In contrast, monomers of Aβ and Aβ had negligible impact on studied processes. Our data suggests that in the course of progression of neuroinflammation oligomeric forms of Aβ and Aβ can compromise the SV fusion machinery and that antiepileptic agent levetiracetam, acting on SV recycling and restricting overexcitation, is able to affect APP processing and Aβ generation within the hippocampus in vivo.
Biochem Pharmacol, 2022; 197
32952554: Lisakovska O, Shymanskyi I, Labudzynskyi D, Mazanova A, Veliky M
Vitamin D Auto-/Paracrine System Is Involved in Modulation of Glucocorticoid-Induced Changes in Angiogenesis/Bone Remodeling Coupling.

Osteoporosis is a devastating side effect of chronic glucocorticoid (GC) treatment. Despite the crucial role of vitamin D (VD) in bone homeostasis, the precise molecular mechanisms of its action on GC-induced disturbances of bone remodeling remain undefined. The study was performed to elucidate the relation of VD status to GC-induced changes of the angiogenesis/osteogenesis/bone resorption coupling in bone tissue. Female Wistar rats received prednisolone (5 mg/kg of b.w.) with or without VD (1000 IU/kg of b.w., for 30 days). Biomechanical parameters of rat femurs were assessed by the three-point bending test. The levels of calcium, inorganic phosphate, activity of total alkaline phosphatase (ALP), and its isoenzymes were determined spectrophotometrically. Vascular endothelial growth factor-A (VEGF-A) and caspase-3 protein levels were detected by western blotting. and mRNAs were measured by qRT-PCR. Receptor activator of nuclear factor B (RANK) expression in bone sections was visualized immunohistochemically. Serum 25(OH)D was assayed by ELISA. GC administration led to a decrease in maximal load (by 1.2-fold) and stiffness and toughness (by 1.3-fold), which was accompanied by a 3-fold reduction of 25(OH)D level, an elevation of the ALP bone isoenzyme activity in serum, hypocalcaemia, and hypophosphatemia. Along with prednisolone-induced VD deficiency, an impaired synthesis of (-30%) and (+71%) mRNA was observed, reflecting deregulation of bone tissue VD-auto-/paracrine system. GC caused an increase in caspase-3 content, suppressed the synthesis of the osteoclastic marker RANK, and altered angiogenesis/osteogenesis coupling by significantly reducing the level of VEGF-A.VD treatment restored serum 25(OH)D content and the expression of key components of the VD-auto-/paracrine system. VD supplementation diminished cell apoptosis and strongly improved angiogenesis/osteogenesis coupling as well as mineral metabolism and biomechanical parameters of femurs in GC-administered rats. Thus, VD can have a beneficial effect on the correction of GC-induced pathological changes in bone remodeling.
Int J Endocrinol, 2020; 2020
32948158: Brodetska L, Natrus L, Lisakovska O, Kaniura O, Iakovenko L, Skrypnyk I, Flis P
The regulatory role of the RANKL/RANK/OPG signaling pathway in the mechanisms of tooth eruption in patients with impacted teeth.
Tooth impaction is a common problem in orthodontic practice and in some cases accompanied by pain and pathological changes of surrounding teeth. Understanding the cellular and molecular mechanisms underlying tooth impaction allows finding the most effective orthodontic treatment for patients with impacted teeth (IT). RANK (receptor activator of NF-κB) / RANKL (RANK ligand) / OPG (osteoprotegerin) signaling pathway controls bone resorption and may be involved in the regulation of tooth eruption. The study aimed to evaluate bone remodeling based on the assessment of the RANKL/RANK/OPG status in patients with IT.
BMC Oral Health, 2020; 20
31821883: Kasatkina LA, Tarasenko AS, Krupko OO, Kuchmerovska TM, Lisakovska OO, Trikash IO
Vitamin D deficiency induces the excitation/inhibition brain imbalance and the proinflammatory shift.
Vitamin D is among the major neurosteroids whose role in developing and adult brain is intensively studied now. Its active form 1,25(OH)D regulates the expression and functioning of a range of brain-specific proteins, which orchestrate the neurotransmitter turnover, neurogenesis and neuroplasticity. Despite numerous studies of the vitamin D role in normal and pathological brain function, there is little evidence on the mechanisms of alterations in excitatory and inhibitory neurotransmission under vitamin D deficiency (VDD). Using the animal model we characterized the dysfunction of excitatory and inhibitory neurotransmission under alimentary VDD. The shift between unstimulated and evoked GABA release under VDD was largely reversed after treatment of VDD, whereas the impairments in glutamatergic system were only partially recovered after 1-month vitamin D supplementation. The increase of the external glutamate level and unstimulated GABA release in brain nerve terminals was associated with intensified ROS production and higher [Ca] in presynapse. The negative allosteric modulation of presynaptic mGlu7 receptors significantly enhanced exocytotic GABA release, which was decreased under VDD, thereby suggesting the neuroprotective effect of such modulation of inhibitory neurotransmission. Synaptic plasma membranes and cytosolic proteins contribute to the decreased stimulated release of neurotransmitter, by being the crucial components, whose functional state is impaired under VDD. The critical changes with synaptic vesicles occurred at the docking step of the process, whereas malfunctioning of synaptic cytosolic proteins impacted the fusion event foremost. The decreased amplitude of exocytosis was inherent for non-excitable cells as well, as evidenced by lower platelet degranulation. Our data suggest the presynaptic dysfunction and proinflammatory shift as the early events in the pathogenesis of VDD-associated disorders and provide evidences for the neuroprotective role of vitamin D.
Int J Biochem Cell Biol, 2020; 119
29930537: Shymanskyi I, Lisakovska O, Mazanova A, Labudzynskyi D, Veliky M
Vitamin D Modulates Impaired Crosstalk Between RANK and Glucocorticoid Receptor Signaling in Bone Marrow Cells After Chronic Prednisolone Administration.
The effectiveness of vitamin D (cholecalciferol) in counteracting the side effects of glucocorticoid (GC) therapy has been demonstrated previously. Abnormalities in systemic hormonal and local (cytokine) regulation of bone marrow (BM) cells may

underlie GC-induced imbalance between osteosynthesis and bone resorption. The cytokine system receptor activator of nuclear factor kappa-B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) is considered as an integrating link in the NF-κB-mediated interaction of various cells involved in maintaining osteoblastic-osteoclastic balance, which makes it a pharmacological target for regulation and correction of the bone remodeling process. We studied GC-induced impairments of the RANKL/RANK/OPG axis in BM cells depending on vitamin D bioavailability and whether these changes were mediated by glucocorticoid (GR) and/or vitamin D (VDR) receptors. Female Wistar rats administered with prednisolone (5 mg/kg b.w., 30 days) showed a decrease in the GR protein level and the number of GR-positive BM cells. GC caused a marked elevation of RANKL and RANK levels in BM, while OPG decreased. Flow cytometry data indicated GC-elicited increase in the number of circulating RANK-positive osteoclast precursors (OCPs) in BM, peripheral blood, and spleen. In full accordance with the data that the interaction of RANKL-RANK leads to transcriptional activation of NF-κB and subsequent differentiation of osteoclasts, we found an increase in the level of phosphorylated p65 subunit of NF-κB with a simultaneous decrease in the NF-κB inhibitor (IκB) level. These changes were accompanied by vitamin D insufficiency and downregulated expression of CYP27B1 and VDR, which are responsible for synthesis and hormonal signaling of 1,25(OH)D. Notably, we observed VDR and RANK co-localization in OCPs. Cholecalciferol co-administration (1,000 IU/kg b.w., 30 days) with prednisolone resulted in elevated GR synthesis in BM. Cholecalciferol prevented prednisolone-elicited disturbances of the RANKL/RANK/OPG, which correlated with improved bioavailability and vitamin D signaling through VDR. This caused the lowering of phosphoNF-κB p65 level and inhibiting NF-κB translocation to the nucleus that could reduce the circulating OCPs pool in BM, peripheral blood, and spleen. Our findings suggest that prednisolone-induced abnormalities in GR and RANKL/RANK/OPG signaling pathways are associated with the impairments of vitamin D auto/paracrine system in BM cells and can be ameliorated by cholecalciferol supplementation.
Front Endocrinol (Lausanne), 2018; 9
29552033: Mazanova A, Shymanskyi I, Lisakovska O, Hajiyeva L, Komisarenko Y, Veliky M
Effects of Cholecalciferol on Key Components of Vitamin D-Endo/Para/Autocrine System in Experimental Type 1 Diabetes.
Recent prospective studies have found the associations between type 1 diabetes (T1D) and vitamin D deficiency. We investigated the role of vitamin D in the regulation of 25OHD-1-hydroxylase (CYP27B1) and VDR expression in different tissues of T1D rats.
Int J Endocrinol, 2018; 2018
28004974: Lisakovska O, Shymanskyy I, Mazanova A, Khomenko A, Veliky M
Vitamin D protects against prednisolone-induced liver injury associated with the impairment of the hepatic NF-κB/iNOS/NO pathway.
The study was carried out to define whether prednisolone-induced damage to hepatic cells is accompanied by excessive nitric oxide (NO) levels associated with nuclear factor kappa B (NF-κB)/inducible NO synthase (iNOS) activation and evaluate the efficacy of the treatment with vitamin D. Histopathological examination, activities of liver transaminases (alanine aminotransferase and aspartate aminotransferase), and cell death assays consistently showed that prednisolone (5 mg/kg body weight, 30 days) induces chronic liver injury in female Wistar rats. Specifically, increased hepatocellular necrosis and caspase-3-dependent apoptosis were observed. Prednisolone enhanced iNOS protein expression, NO generation, and tyrosine nitration in liver cells. Despite unchanged hepatic level of the NF-κB/p65 protein, prednisolone increased inhibitory κB-α (IκB-α) degradation, nuclear translocation, and phosphorylation of NF-κB/p65 at Ser311, indicating that NF-κB activation can be involved in the induction of iNOS/NO. All changes were associated with a 2.9-fold decrease in the serum content of 25-hydroxyvitamin D and significant reduction of hepatic vitamin D receptor (VDR) expression that points reliably to vitamin D deficiency and failures in VDR signaling. Vitamin D co-administration (100 IU/rat, 30 days) prevented glucocorticoid-evoked abnormalities in hepatic tissue. In conclusion, prednisolone-induced liver disturbances were associated with the impairment of NF-κB/iNOS/NO responses that can be ameliorated by vitamin D treatment through VDR-mediated mechanisms.
Biochem Cell Biol, 2017; 95
29235799: Shymanskyy IO, Lisakovska OO, Mazanova AO, Labudzynskyi DO, Khomenko AV, Veliky MM
Prednisolone and vitamin D(3) modulate oxidative metabolism and cell death pathways in blood and bone marrow mononuclear cells.
The study was designed to evaluate reactive oxygen species (ROS)/nitric oxide (NO) formation and apoptotic/necrotic cell death elicited by prednisolone in peripheral blood and bone marrow mononuclear cells and to define the efficacy of vitamin D3 to counter glucocorticoid (GC)-induced changes. It was shown that prednisolone (5 mg per kg of female Wistar rat's body weight for 30 days) evoked ROS and NO overproduction by blood mononuclear cells (monocytes and lymphocytes) that correlated with increased cell apoptosis and necrosis. In contrast, prednisolone did not affect ROS/NO levels in bone marrow mononuclear cells that corresponded to lower level of cell death than in the control. Alterations of prooxidant processes revealed in mononuclear cells and associated with GC action were accompanied by vitamin D3 deficiency in animals, which was assessed by the decreased level of blood serum 25-hydroxivitamin D3 (25OHD3). Vitamin D3 administration (100 IU per

rat daily for 30 days, concurrently with prednisolone administration) completely restored 25OHD3 content to the control values and significantly reversed ROS and NO formation in blood mononuclear cells, thus leading to decreased apoptosis. In bone marrow, vitamin D3 activated ROS/NO production and protein nitration that may play a role in prevention of prednisolone-elicited increase in bone resorption. We conclude that vitamin D3 shows a profound protection against GC-associated cellular damage through regulating intracellular ROS/NO formation and cell death pathways.
Ukr Biochem J, 2016 Sep-Oct; 88
27097962: Shymanskyy IO, Lisakovska OO, Mazanova AO, Riasniy VM, Veliky MM
Effects of vitamin D3 and vitamin E on prednisolone-induced alterations of phagocyte function.
To evaluate the effectiveness of vitamin D3 and its combined action with vitamin E in the correction of the impairments of phagocyte function caused by chronic glucocorticoid administration.
Eur Rev Med Pharmacol Sci, 2016; 20

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EXAMINING CALPAIN ACTIVITY IN SPINOCEREBELLAR ATAXIA-3 THROUGH USE OF A NOVEL FIBRE PHOTOMETRY METHODOLOGY

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Aberrant cleavage of full-length proteins into shorter fragments is common to many neurodegenerative diseases, including spinocerebellar ataxia type 3 (SCA3). Calcium-activated calpain proteases are known to cleave proteins and thus may contribute to protein aggregate formation and neurodegeneration. In this study, we aimed to determine whether overactivity of calpain proteases was a disease mechanism present in SCA3 transgenic mice. We obtained longitudinal blood samples from mice throughout disease development and investigated if plasmatic levels of cleaved α-spectrin, a marker of calpain activity, changed with disease progression. We found no significant differences in levels of cleaved α-spectrin in pre-symptomatic SCA3 mice (< 8 weeks of age) when compared to wildtype littermates. At 12 weeks of age, levels of cleaved α-spectrin were significantly increased in SCA3 animals, coinciding with the onset of neurological symptoms. Western blotting of cerebellum tissue obtained from 15-week SCA3 mice showed further differences in markers of calpain activity, providing further evidence that calpain proteases may play a pathogenic role in SCA3. Next, we used fibre photometry and a FRET-based biosensor to investigate changes in protein cleavage *in vivo*. Using this novel fibre photometry approach, we examined the effect of administration of different doses of calpeptin within the same experimental animals across different test sessions. Our findings confirmed that our approach can be used to measure calpain activity in vivo and identify the most efficacious dose of calpeptin. Insights from our study will aid our understanding of SCA3 disease mechanisms and the future testing of novel calpain inhibitors.

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FATIGUE AND SLEEPINESS MEASURES IN MYOTONIC DYSTROPHY TYPE 1 PATIENTS TREATED WITH COGNITIVE BEHAVIORAL THERAPY AND GRADED EXERCISE THERAPY

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Aims: Myotonic dystrophy 1 (DM1) is a relatively common neuromuscular disorder in adults. It is characterised by muscle weakness, sleep disorders (~80%), cataracts, and cardiac problems. Effective treatments to alleviate disease burden are currently an unmet clinical need and life expectancy is around 50 years. We aim to investigate fatigue and sleepiness measures for benefit in response to cognitive behavioural therapy (CBT) and graded exercise therapy (GET). Methods: The OPTIMISTIC trial (NCT02118779) included 255 DM1 participants who were randomised to standard care or CBT, with the aim of increasing activity and social participation. Two of the sites offered additional GET to those in the CBT group. Fatigue and daytime sleepiness scale (FDSS) and Checklist Individual Strength subscale Fatigue (CIS Fatigue) measures were taken at baseline, 5, 10 and 16 months. Measures were compared across the treatment groups using Mann Whitney U tests. Scores were also split by median age and gender. Causal mediation analysis was carried out for factors mediating FDSS change in response to CBT+GET. Results: There was a significant reduction in mean FDSS score with CBT+GET (n=33) compared to standard care (n=125) at 5, 10, and 16 months ($P < .001$). In contrast, CBT alone (n=96) showed a significant improvement over standard care at 5 months only ($P < .05$). This pattern was replicated in CIS fatigue scores. Both genders and age groups responded well to CBT+GET. Conclusions: CBT and GET reduces fatigue and daytime sleepiness in participants with DM1 who were classified as severely fatigued.

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BEHAVIOURAL ABNORMALITIES IN DMSXL MICE, A MODEL OF MYOTONIC DYSTROPHY TYPE 1

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Myotonic dystrophy type 1 (DM1) is a dominantly inherited neuromuscular disease caused by the abnormal expansion of CTG-repeats in the 3'-untranslated region of the *DMPK* gene, characterized by multisystemic symptoms including muscle weakness, myotonia, cardio-respiratory problems, hypersomnia, cognitive dysfunction and behavioural abnormalities. DMSXL mice carry a mutated human *DMPK* transgene resulting in >1000 CTG-repeats. They exhibit a pathologic neuromuscular phenotype and also synaptic dysfunction resulting in neurological and behavioural deficits similar to those observed in patients. To further explore additional phenotypes of this DM1 mouse model we developed a comprehensive battery of tests that included the evaluation of several brain functions: exploratory and motor activity, fine motor skills, neuromuscular strength, anxiety, working memory, fear learning, sensorimotor processing, spatial memory, home-cage rest behaviour. Additionally, skeletal and cranio-facial morphology as well as body composition and bone mineral density were assessed using DEXA and micro-CT. Male and female DMSXL mice tested between 7 and 20 weeks of age showed, compared to wild-type littermates: lower body weight, reduced grip strength and running wheel activity and increased anxiety, consistently with previously published data. Interestingly, they also presented some new phenotypes remarkably similar to the characteristic features of the human disease: skeletal and cranio-facial dysmorphology with teeth misalignment, abnormalities in sensorimotor processing and rest-related disturbances during the active phase. These phenotypes confirm the reliability of DMSXL mice to model clinical features of DM1 and open new opportunities to verify the efficacy of novel therapies based on CRISPR/Cas9-mediated gene editing strategies currently under study in our group.

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ENGRAILED-1 HOMEOPROTEIN IS A NON-CELL AUTONOMOUS NEUROTROPHIC FACTOR FOR SPINAL ALPHA-MOTONEURONS

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Aims In the mouse spinal cord, homeoprotein transcription factor EN1 is expressed in V1 interneurons that synapse on alpha-motoneurons (aMNs) and may, as is the case for midbrain neurons, exert neuroprotection following intercellular transfer. We thus evaluated the non-cell autonomous activity of EN1 on aMN physiology and survival. **Methods** We analyzed mice heterozygous for *En1* (*En1*$^{+/-}$) and also neutralized extracellular EN1 in the spinal cord of WT mice by expressing a single chain anti-EN1 antibody (scFvEN1). Neuromuscular junction morphology (NMJ) and aMN number were analyzed. Forepaw grip strength, time hanging onto an inverted grid and the hind limb extensor reflex were evaluated. hEN1 (1µg) was injected intrathecally at lumbar 5 level and scFvEN1 virally expressed in the astrocytes. **Results** *En1*$^{+/-}$ mice display reduced muscle strength and an abnormal extensor reflex starting at 2 months of age, NMJ denervation at 3 months of age and significant aMN loss at 4.5 months of age, associated with an increased expression of the p62/SQTSM1 autophagy mark. Six weeks after a single intrathecal administration of hEN1 at 3 months of age, both muscle strength and extensor reflex are restored and aMN loss is prevented. Phenotypic rescue is maintained for 3 months and can be prolonged via a second injection. Neutralizing spinal extracellular EN1 by expression of scFvEN1 phenocopies the *En1*$^{+/-}$ phenotype. **Conclusions** Our data demonstrate that non-cell autonomous EN1 is an adult aMN neurotrophic factor with potential interest for the treatment of MN diseases.

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CONTROL OF LIPID METABOLISM BY NGF/P75NTR SIGNALINGS IN NEURON-GLIA NETWORK: NOVEL TARGETS FOR NEURODEGENERATIVE DISEASES

POSTER SESSION 07 - SECTION: NEURODEGENERATIVE DISEASES

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Control of lipid metabolism by NGF/p75NTR signaling in neuron-glia network: novel targets for neurodegenerative diseases A prominent factor in cognitive abnormalities associated with various neurodegenerative diseases, including Alzheimer's Disease (AD) and Type 2 Diabetes (T2D), is the disruption of cholesterol metabolism in the neuron-glia network. Cholesterol dysmetabolism and associated brain insulin resistance have been linked to the cognitive decline produced by cholinergic deficits and astrocytosis in AD. The goal of the current research is to investigate the neurotrophic pathway in the protection of the neuro-glia network from the effect of the cholesterol metabolite 27-hydroxycholesterol (27-OH), the major circulating oxygenated cholesterol derivative, and a brain sensor of circulating cholesterol. In line, 27-OH is detected in high amount in the early stage AD brain, possibly contributing to cognitive deterioration. We discovered that 27-OH (1mM; 6-24 hours) inhibits NGF/TrkA signaling in NGF-responsive cholinergic neurons and human iPS-derived astrocytes by affecting the metabolism of amyloid precursor protein, and promotes the buildup of lipid rafts in the neuroglia network. We also found that 27-OH elicits nuclear translocation of the common neurotrophic receptor (p75NTR) in astrocytes before lipid rafts are loaded. On the other hand, both NGF (100ng/ml) and the selective p75NTR modulator LM11A-31 (100nM) can prevent p75NTR accumulation in the nucleus and subsequent lipid raft buildup, pinpointing that the NGF and p75NTR signalings play opposite roles in AD. Overall, NGF-based treatment and/or p75NTR-specific modulation are possible avenues to improve lipid metabolism and reduce cognitive decline in AD and T2D.

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ASYMMETRIC ENCODING DYNAMICS IN THE STRIATAL DIRECT AND INDIRECT PATHWAYS DURING SPONTANEOUS BEHAVIOUR

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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The basal ganglia are notorious for their influence on action control and modulation of movements. Proper organization of neuronal activity within the two efferent pathways of the dorsal striatum, a major input of basal ganglia, is critical for appropriate behavioral control. The classical model of basal ganglia suggests that striatal indirect and direct pathways exert opposite action on the thalamo-cortical activation triggering motor control. Recent studies challenged this view by describing conjoint neuronal activities during action initiation or decision-making. Therefore, additional investigations are needed to clarify the function of these two pathways. In the present work, using large-scale in vivo calcium imaging (Inscopix), we evaluated how direct and indirect pathway striatal projection neurons (SPNs) in the dorsal striatum encode behaviors during self-paced spontaneous exploration of an open-field. We observe that both striatal pathways diverge in their tuning properties to spontaneous behaviors. Using supervised leaning algorithms, we found that direct pathway SPNs encode behaviors through their activation, whereas behavior-relevant SPNs of the indirect pathway are consistently silent. Our findings demonstrate an opposite yet cooperative organization of behaviors encoding within striatal output pathways that solves questions raised by recent correlative studies. Altogether our results generate a new framework to understand striatal physiology and its dysfunction.

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BRIDGING COLLATERALS ACT IN CONCERT WITH THE CANONICAL BASAL GANGLIA DIRECT PATHWAY TO SUPPORT MOTOR CONTROL

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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In the classical model of the basal ganglia (BG) two segregated and functionally opposing pathways connect its input, the striatum with its midbrain outputs the substantia nigra (SNr) and entopenduncular nucleus (EP). Direct pathway spiny projection neurons (dSPNs) project to the SNr and EP while indirect pathway iSPNs project to the GPe. However, many recent studies have challenged both the functional dichotomy and the anatomical organization delineated by the classical BG model. For instance, all major BG nuclei send arborized axons collateralizing into one to four BG target regions. These axon collaterals could help shape BG information flow by sending copies of the same signals to distinct brain regions. Axonal “bridging” collaterals arising from dSPNs are a prominent example: axonal fibers arising from SNr-projecting tracts, yet targeting the GPe, the classical projection area of iSPNs. They generate half the density of GPe terminals produced by iSPNs, but their significance for behavior is still unknown. Here, we use *in vivo* optical and chemogenetic tools combined with deep-learning approaches to dissect the roles of bridging collaterals in motor function. We found that dSPNs projecting to the SNr send synchronous motor-related information to the GPe via axon collaterals. We then found that inhibition of native activity in dSPN GPe terminals impaired motor activity and function via regulation of pallidostriatal Npas1 neurons. We propose a model by which dSPN GPe collaterals act in concert with the canonical terminals in the SNr to support motor control by inhibiting Npas1 signals going back to the striatum.

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MOTOR SKILL LEARNING AND EXECUTION IN A DISTRIBUTED BRAIN NETWORK

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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The brain's remarkable capacity to acquire and execute motor skills depends on a distributed network. While many components are identified, less is known about their specific roles and interactions during skill learning and execution. We probe this network through the lens of complex, spatiotemporally precise motor sequences we train in rats. We focus on the basal ganglia (BG) and their main inputs from motor cortex and thalamus. Using electrophysiological recordings, we find that the dorsolateral striatum (DLS), the BG's main motor-related input nucleus, encodes the detailed kinematic structure of learned motor sequences. We further show that a loss of the DLS renders animals unable to execute the learned idiosyncratic motor patterns, causing them to revert to simple species-typical behaviors. In addition, we find that not only the DLS, but also its motor cortical inputs are necessary for skill learning. This pathway, however, becomes dispensable after the behaviors are acquired. In line with this, the loss of motor cortical inputs leaves DLS activity encoding the kinematic structure largely unaffected. In contrast, thalamic inputs remain crucial for skill generation and their loss disrupts performance akin to DLS lesions. Together, our results suggest that the BG can play a role in the control of complex learned behaviors beyond traditional models of BG function. They further suggest that motor cortex 'tutors' sub-cortical circuits during learning, potentially by guiding plasticity at thalamostriatal synapses. Such adaptive reprogramming of lower-level motor circuits may broaden their flexibility and allow them to store and generate complex learned motor skills.

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BOARD NUMBER: S07-311

INPUT-OUTPUT RELATIONSHIPS OF THE PARAFASCICULAR THALAMIC NUCLEUS IN THE MOUSE

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Introduction. The parafascicular thalamic nucleus (Pf) is a massive source of subcortical excitatory inputs to the striatum. Pf neurons simultaneously innervate the cerebral cortex as well, but the functional logic of this divergent projection is currently unclear. Likewise, sources of input to Pf neurons remain poorly defined. **Aims.** Using wild-type adult C57BL/6 male mice as experimental subjects, we set out to a) map Pf afferents; b) sort out the relationship between Pf subregions and the cortical + striatal territories targeted by their projections; and c) investigate possible differences in Pf axon varicosities within specific cortical and/or striatal target domains. **Methods.** We made selective microinjections of biotin dextran amine (BDA) or BDA + cholera toxin B subunit (CTb) in different Pf subdomains to visualize their efferent and afferent projections. We applied different (immuno)stainings to delineate relevant brain territories. **Results.** Our data show that the mouse Pf receives massive inputs from layers 5b and 6b of sensorimotor, frontal association and cingulate cortices and the multimodal intermediate/deep layers of the superior colliculus. Pf projections to striatum and cortex are organized in congruent fashion in both structures, yet the cortical projections are far less abundant or convergent. Axonal varicosity sizes vary depending on the target structure and the origin of the axons within the various Pf subregions. **Conclusions.** These observations show that the divergent Pf output to the striatum and cortex is segregated into parallel, somatotopically-related subcircuits and suggest that the synaptic strength of Pf projections may vary amongst its different target territories.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-312

CONTRIBUTION OF DOPAMINE NEURONS TO ACTION EXECUTION

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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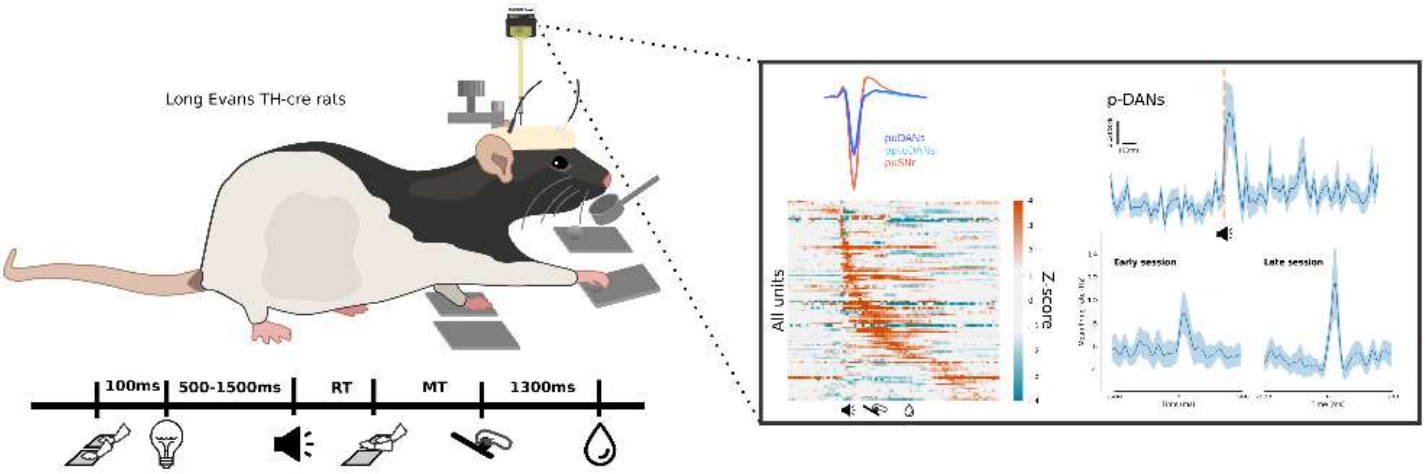
The midbrain dopaminergic neurons (DANs) are essential for goal-directed actions with key contribution to functions ranging from motivation to movement vigor. Studies have shown that the activity of DANs located in the substantia nigra pars compacta (SNc) is modulated around movement onset and can change during movements but in a heterogeneous manner. Moreover, cell-type specific manipulation of their activity before a movement will impact the vigor at which it is executed. In this study, we sought to investigate the functional heterogeneity of SNc DANs responses during action execution. As such, we recorded the extracellular activity of DANs using an optotagging strategy and high-density silicon probes in head-fixed TH-Cre rats trained to perform a reach-and-grasp fine motor task. We found that the activity of DANs is principally modulated by cue sound instructing the start of the trial and not by motor parameters.



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BOARD NUMBER: S07-313

UNEXPECTED INHIBITION OF MOTOR FUNCTION BY DOPAMINE ACTIVATION OF D1/D2 CO-EXPRESSING STRIATAL NEURONS.

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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The central function of the striatum and its dopaminergic (DA) afference in motor control and integration of cognitive and emotional processes is commonly explained by the two striatal efferent pathways characterized by striatal projection neurons (SPNs) expressing DA D1 receptor- and D2 receptor (D1-SPNs and D2-SPNs), respectively, regardless of SPNs co-expressing these two receptors (D1/D2-SPNs). Here, after developing an approach that enables to target these hybrid SPNs, we demonstrated that, although these SPNs are rare, they play a major role in guiding the motor function of the other two main populations and convey a DA-mediated antagonistic motor brake. D1/D2-SPNs project exclusively to the external globus pallidus (GPe) and have specific electrophysiological features with distinctive integration of DA signals. Optogenetic stimulation and loss-of-function experiments indicated that D1/D2-SPNs potentiate the prokinetic and antikinetic functions of D1-SPNs and D2-SPNs, respectively, and restrain the integrated motor response to psychostimulants. Overall, our findings demonstrate the essential role of this third unacknowledged population of D1/D2 co-expressing neurons, which orchestrates the fine-tuning of DA regulation in the thalamo-cortico-striatal loops.

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BOARD NUMBER: S07-315

PYRAMIDAL TRACT NEURONS AMPLIFY EXCITATION TO THE STRIATUM THROUGH CHOLINERGIC INTERNEURONS

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Corticostriatal connectivity is key for cognitive and motor processes, such as reinforcement or action selection, initiation and invigoration. The cortical input to the striatum arises from two major cortical populations: intratelencephalic (IT) and pyramidal tract (PT) neurons. Here, using electrophysiology, circuit mapping and pharmacological methods, we described a new corticostriatal polysynaptic circuit, running in parallel to the canonical monosynaptic corticostriatal connection. In this circuit, PT cortical neurons convey excitation to spiny projection neurons (SPNs) in the striatum, through cholinergic interneurons (ChIs). This motif elicits a second phase of excitation onto SPNs by promoting acetylcholine-dependent glutamate release from long-range axons reaching the striatum. This release depends on α4-containing nicotinic receptors and results in biphasic corticostriatal signals. Using subcellular ChannelRhodopsin-2 Assisted Circuit Mapping (sCRACM), we uncovered that these biphasic signals are a hallmark of PT neurons, because they preferentially contact ChIs, when compared to IT neurons. These results describe a previously unknown circuit mechanism by which PT activity amplifies excitation to the striatum, with potential implications for behavior, plasticity and learning.

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BOARD NUMBER: S07-316

DOPAMINE DYNAMICS IN THE MOUSE DORSAL STRIATUM DURING LOCOMOTION AND POSTURAL SHIFTS

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Dopamine (DA) is an important neuromodulator which is classically associated with learning and reward prediction error. Several recent works have begun to uncover the complex role DA also has in controlling motor behaviour. In the last couple of years, the development of new fluorescent molecular markers for DA, such as dLight, has greatly facilitated the recording of DA in vivo in behaving animals. We have developed an experimental framework based on a dual fibre-photometry system, EMG recording and a freewheeling linear treadmill to explore the dynamics of DA signalling during spontaneous locomotion and postural shifts in head-fixed mice. In this work, we find high correlations between the striatal DA signalling in both hemispheres. We show distinct transient decreases in the DA signal prior to initiation of locomotion, as well as increases in the signal after termination of locomotion. During postural shifts, we measure a characteristic tri-phasic signal, closely matching the signal predicted from the firing pattern of midbrain dopaminergic cells in earlier work. In addition, we examine how these features of DA signalling are disrupted in a mouse model of Parkinson's Disease, induced by intracerebral injection of pre-formed fibrils of alpha-synuclein, a protein commonly found in pathologic inclusions (Lewy bodies) in cells of human patients. This will help us gain a better understanding of the first stages in the progression of the disease, prior to the onset of symptoms.

Pubmed:
28721358: Boutte RW, Merlin S, Yona G, Griffiths B, Angelucci A, Kahn I, Shoham S, Blair S
Utah optrode array customization using stereotactic brain atlases and 3-D CAD modeling for optogenetic neocortical interrogation in small rodents and nonhuman primates.
As the optogenetic field expands, the need for precise targeting of neocortical circuits only grows more crucial. This work demonstrates a technique for using Solidworks computer-aided design (CAD) and readily available stereotactic brain atlases to create a three-dimensional (3-D) model of the dorsal region of area visual cortex 4 (V4D) of the macaque monkey () visual cortex. The 3-D CAD model of the brain was used to customize an [Formula: see text] Utah optrode array (UOA) after it was determined that a high-density ([Formula: see text]) UOA caused extensive damage to marmoset () primary visual cortex as assessed by electrophysiological recording of spiking activity through a 1.5-mm-diameter through glass via. The [Formula: see text] UOA was customized for optrode length ([Formula: see text]), optrode width ([Formula: see text]), optrode pitch ([Formula: see text]), backplane thickness ([Formula: see text]), and overall form factor ([Formula: see text]). Two [Formula: see text] UOAs were inserted into layer VI of macaque V4D cortices with minimal damage as assessed in fixed tissue cytochrome oxidase staining in nonrecoverable surgeries. Additionally, two [Formula: see text] arrays were implanted in mice () motor cortices, providing early evidence for long-term tolerability (over 6 months), and for the ability to integrate the UOA with a Holobundle light delivery system toward patterned optogenetic stimulation of cortical networks.
Neurophotonics, 2017; 4
26866055: Yona G, Meitav N, Kahn I, Shoham S
Realistic Numerical and Analytical Modeling of Light Scattering in Brain Tissue for Optogenetic Applications(1,2,3).
In recent years, optogenetics has become a central tool in neuroscience research. Estimating the transmission of visible light through brain tissue is of crucial importance for controlling the activation levels of neurons in different depths, designing optical systems, and avoiding lesions from excessive power density. The Kubelka-Munk model and Monte Carlo simulations have previously been used to model light propagation through rodents' brain tissue, however, these prior attempts suffer from fundamental shortcomings. Here, we introduce and study two modified approaches for modeling the distributions of light emanating from a multimode fiber and scattering through tissue, using both realistic numerical Monte Carlo simulations and an analytical approach based on the beam-spread function approach. We demonstrate a good agreement of the new methods' predictions both with recently published data, and with new measurements in mouse brain cortical slices, where our results yield a new cortical scattering length estimate of ~47 μm at λ = 473 nm, significantly shorter than ordinarily assumed

in optogenetic applications.
eNeuro, 2016 Jan-Feb; 3

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BOARD NUMBER: S07-317

THYROID HORMONE TRANSPORTERS MCT8 AND OATP1C1 ARE EXPRESSED IN NEURONS IN THE HUMAN AND MONKEY BASAL GANGLIA AND MOTOR THALAMUS.

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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[1]School of Medicine. Autonomous University of Madrid (UAM), Department Of Anatomy, Histology And Neuroscience, Madrid, Spain, [2]Lintong Shiyoucheng, General Clinic, Xi'an, China, [3]PhD Program in Neuroscience, UAM, Dept Of Anatomy, Histology & Neuroscience, Madrid, Spain, [4]The Instituto de Investigaciones Biomédicas "Alberto Sols" (IIBM), Department Of Pathophysiology Endocrine And Nervous System, Madrid, Spain, [5]School of Medicine. Universidad Autónoma de Madrid. Spain., Department Of Anatomy, Histology And Neuroscience, Madrid, Spain

Monocarboxylate transporter 8 (MCT8) and organic anion transporting polypeptide 1C1 (OATP1C1) are thyroid hormone (TH) transporters that facilitate TH to cross the plasma membrane to perform its bioactivity. So far it is unknown the nature of the neural cells in which these transporters are expressed in the adult monkey and human basal ganglia and motor related structures. We performed immunohistochemistry, histochemistry or immunofluorescence on brain sections from cynomolgus monkeys and humans. The immunolabeling results were plotted in distribution maps by means of Neurolucida system. MCT8 and OATP1C1 are expressed in several kinds of neurons with different morphologies in both human and monkey neostriatum.MCT8 expression is in general less abundant than OATP1C1. MCT8 is expressed in medium-sized aspiny non-NOS GABAergic interneurons in human and monkey, while OATP1C1 is expressed in NOS GABAergic interneurons in human. Both transporters colocalize with acetyl choline in large caudate-putamen neurons. OATP1C1 colocalizes with IBA-1 in microglial cells. In addition, we have noticed that both transporters are strongly expressed in substantia nigra in the monkey and in cholinergic neurons in the nucleus basalis of Meynert in human and monkey. MCT8 but not OATP1C1 is expressed extensively in the endothelial cells of the various size vessels and capillaries in the basal ganglia and thalamus. Our study provides the first evidence for the abundance of TH transporters MCT8 and OATP1C1 in the basal ganglia and thalamic neurons in the adult human and non-human primates, which suggests their important role in the motor system functionality.

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BOARD NUMBER: S07-318

AN IN VIVO CALCIUM IMAGING STUDY OF IMSN INVOLVMENT IN THE STRIATAL ENCODING OF MOUSE LOCOMOTOR ACTIVITY

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Striatum is the main input nucleus of the basal ganglia and 95% of its neurons are GABAergic medium spiny neurons (MSNs). MSNs are subdivided into neurons of the direct and indirect pathway. According to the classical model, these two pathways exert an opposite effect on movement regulation: the direct pathway promotes activity of the cortex, and therefore movement initiation, while the indirect pathway inhibits cortical activity and movement. Recent studies have challenged this interpretation and demonstrated that both pathways are co-activated during movement initiation (Bonnavion 2019, Tecuapetla 2016, Parker 2018). Yet, while the role of these two pathways is globally understood, cell-specific mechanisms and their interaction in relation to movement are only partially known. We present a study investigating the role of murine striatum in locomotion, and in particular the involvement of MSNs of the indirect pathway (iMSNs), through *in-vivo* Ca^{2+} imaging with head-mounted microscopes and chronically implanted endoscopes in freely behaving mice. Locomotor activity was tested by behavioural experiments and the GABAergic iMSN activity was stimulated by a psychostimulant drug, d-Amphetamine, at different doses. A2AGCaMP6s mice expressing GCaMP in iMSNs were used as an animal model. Analysis of the acquired datasets showed that acute intraperitoneal injection of d-Amphetamine at 3 mg/kg dose decreased the average number of active iMSNs and increased the average spike duration, and demonstrated that iMSNs contribute to the encoding of movement initiation and termination, confirming the notion of direct-indirect pathway co-activation in movement control.

Pubmed:
30591448: Facoetti A, Cavagnini M, Ciocca M, Nano R, Pasi F, Aprile C, Lodola L, Persico MG, Marenco M, Valvo F, Orecchia R
Effects of L-DOPA Pretreatment on the Kinetics, Migration and Carbon Ion Radiation Response of T98G Cells.
Glioblastoma is the most malignant and widespread brain tumor in adults, with a rapid clinical course. Recently, it has been hypothesized that L-DOPA plays a role in the diagnosis and treatment of glioblastoma. The aim of this study was to assess the effects of pretreatment with L-DOPA on the biological behavior of human T98G cells in vitro.
Anticancer Res, 2019; 39
34808830: Talone B, Pozzi P, Cavagnini M, Polli D, Pozzi G, Mapelli J
Experimental determination of shift-less aberration bases for sensorless adaptive optics in nonlinear microscopy.
Adaptive optics can improve the performance of optical systems and devices by correcting phase aberrations. While in most applications wavefront sensing is employed to drive the adaptive optics correction, some microscopy methods may require sensorless optimization of the wavefront. In these cases, the correction is performed by describing the aberration as a linear combination of a base of influence functions, optimizing an image quality metric as a function of the coefficients. The influence functions base is generally chosen to either efficiently represent the adaptive device used or to describe generic wavefronts in an orthogonal fashion. A rarely discussed problem is that most correction bases have elements which introduce, together with a correction of the aberration, a shift of the imaging field of view in three dimensions. While simple methods to solve the problem are available for linear microscopy methods, nonlinear microscopy techniques such as multiphoton or second harmonic generation microscopy require non-trivial base determination. In this paper, we discuss the problem, and we present a method for calibrating a shift-less base on a spatial light modulator for two-photon microscopy.
Opt Express, 2021; 29

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BOARD NUMBER: S07-319

EXPERIMENTAL INVESTIGATION INTO THE ROLE OF THE SUBTHALAMIC NUCLEUS (STN) USING OPTOGENETICS IN MICE

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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[1]Uppsala University, Department Of Organismal Biology, Uppsala, Sweden, [2]Universit´e de Bordeaux, Institut Des Maladies Neurod´eg´en´eratives, Bordeaux, France, [3]Institute of Neurodegenerative Disease, Gironde, Bordeaux, France

Aims: Most knowledge about the STN has been gained through functional knowledge of the Subthalamic Nucleus (STN) has been gained from pathological conditions and models, primarily parkinsonian, experimental evidence for its role in normal motor control has remained sparse. The aim of this study was to tease out the selective impact of the STN on several motor parameters required to achieve intended movement, including locomotion, balance and motor coordination. **Methods:** Optogenetic excitation and inhibition using both bilateral and unilateral stimulations of the STN were implemented in freely-moving mice. **Results:** The results demonstrate that selective optogenetic inhibition of the STN enhances locomotion while its excitation reduces locomotion. In addition, optogenetic excitation in freely-exploring mice induced self-grooming, disturbed gait and a jumping/escaping behavior, while causing reduced motor coordination in advanced motor tasks, independent of grooming and jumping. In addition, we found that, when given a choice, mice avoided excitation of the STN. **Conclusions:** These findings lend experimental support to basal ganglia models of the STN in terms of locomotion. Avoidance response suggests that the STN can play a crucial role within the brain aversion and emotion systems establishing a critical brain hub for motor, cognitive and emotional functions.

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CELL-TYPE SPECIFIC ELECTROPHYSIOLOGICAL AND BEHAVIOURAL ALTERATIONS IN THE GLOBUS PALLIDUS OF MICE FOLLOWING STRIATOPALLIDAL PATHWAY ABLATION.

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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The basal ganglia (BG) is a group of sub-cortical nuclei involved in motor control, learning and reward processing. Current models postulate that the BG modulate the cerebral cortex indirectly via an inhibitory input to the thalamus, bidirectionally controlled by direct- and indirect-pathway striatal projection neurons (MSNs). The direct pathway, composed of the striatonigral MSN (dMSNs) facilitates and the indirect pathway composed of the striatopallidal MSNs (iMSNs) inhibits thalamus outputs to the cortex. In this model, the GABAergic globus pallidus (GP - external globus pallidus in the primates), which is the major target nucleus of iMSNs, is centrally positioned in the basal ganglia circuitry. It receives, processes and distributes cortico-striatal information to the entire network through its widespread axonal projections, thereby influencing basal ganglia nuclei output during voluntary movement and executive functions. Previously considered as a homogenous nucleus, most recent evidences indicate that the GP contains two main types of GABAergic projection cells named “Prototypic” and “Arkypallidal” neurons with specialized molecular, physiological properties and specific target structures. Here, we investigate by in vitro electrophysiology coupled with optogenetics and by using a genetic mouse model of selective iMSNs ablation, the A_{2A}iDTR mice, how prototypic pallidal neurons are affected by the selective depletion of iMSNs and examine the involvement of parvalbumin pallidal neurons - a subpopulation of prototypic population - on motor function and amphetamine-induced locomotion.

Pubmed:

32711953: Rial D, Puighermanal E, Chazalon M, Valjent E, Schiffmann SN, de Kerchove d'Exaerde A
Mammalian Target of Rapamycin-RhoA Signaling Impairments in Direct Striatal Projection Neurons Induce Altered Behaviors and Striatal Physiology in Mice.
As an integrator of molecular pathways, mTOR (mammalian target of rapamycin) has been associated with diseases including neurodevelopmental, psychiatric, and neurodegenerative disorders such as autism spectrum disorder, schizophrenia, and Huntington's disease. An important brain area involved in all these diseases is the striatum. However, the mechanisms behind how mTOR is involved in striatal physiology and its relative role in distinct neuronal populations in these striatal-related diseases still remain to be clarified.
Biol Psychiatry, 2020; 88
32385372: Dionisi C, Rai M, Chazalon M, Schiffmann SN, Pandolfo M
Primary proprioceptive neurons from human induced pluripotent stem cells: a cell model for afferent ataxias.
Human induced pluripotent stem cells (iPSCs) are used to generate models of human diseases that recapitulate the pathogenic process as it occurs in affected cells. Many differentiated cell types can currently be obtained from iPSCs, but no validated protocol is yet available to specifically generate primary proprioceptive neurons. Proprioceptors are affected in a number of genetic and acquired diseases, including Friedreich ataxia (FRDA). To develop a cell model that can be applied to conditions primarily affecting proprioceptors, we set up a protocol to differentiate iPSCs into primary proprioceptive neurons. We modified the dual-SMAD inhibition/WNT activation protocol, previously used to generate nociceptor-enriched cultures of primary sensory neurons from iPSCs, to favor instead the generation of proprioceptors. We succeeded in substantially enriching iPSC-derived primary sensory neuron cultures for proprioceptors, up to 50% of finally differentiated neurons, largely exceeding the proportion of 7.5% normally represented by these cells in dorsal root ganglia. We also showed that almost pure populations of proprioceptors can be purified from these cultures by fluorescence-activated cell sorting. Finally, we demonstrated that the protocol can be used to generate proprioceptors from iPSCs from FRDA patients, providing a cell model for this genetic sensory neuronopathy.
Sci Rep, 2020; 10
32112413: Kovaleski RF, Callahan JW, Chazalon M, Wokosin DL, Baufreton J, Bevan MD

Dysregulation of external globus pallidus-subthalamic nucleus network dynamics in parkinsonian mice during cortical slow-wave activity and activation.
Reciprocally connected GABAergic external globus pallidus (GPe) and glutamatergic subthalamic nucleus (STN) neurons form a key network within the basal ganglia. In Parkinson's disease and its models, abnormal rates and patterns of GPe-STN network activity are linked to motor dysfunction. Using cell class-specific optogenetic identification and inhibition during cortical slow-wave activity and activation, we report that, in dopamine-depleted mice, (1) D2 dopamine receptor expressing striatal projection neurons (D2-SPNs) discharge at higher rates, especially during cortical activation, (2) prototypic parvalbumin-expressing GPe neurons are excessively patterned by D2-SPNs even though their autonomous activity is upregulated, (3) despite being disinhibited, STN neurons are not hyperactive, and (4) STN activity opposes striatopallidal patterning. These data argue that in parkinsonian mice abnormal, temporally offset prototypic GPe and STN neuron firing results in part from increased striatopallidal transmission and that compensatory plasticity limits STN hyperactivity and cortical entrainment.
J Physiol, 2020; 598
31470672: Mallet N, Delgado L, Chazalon M, Miguelez C, Baufreton J
Cellular and Synaptic Dysfunctions in Parkinson's Disease: Stepping out of the Striatum.
The basal ganglia (BG) are a collection of interconnected subcortical nuclei that participate in a great variety of functions, ranging from motor programming and execution to procedural learning, cognition, and emotions. This network is also the region primarily affected by the degeneration of midbrain dopaminergic neurons localized in the substantia nigra pars compacta (SNc). This degeneration causes cellular and synaptic dysfunctions in the BG network, which are responsible for the appearance of the motor symptoms of Parkinson's disease. Dopamine (DA) modulation and the consequences of its loss on the striatal microcircuit have been extensively studied, and because of the discrete nature of DA innervation of other BG nuclei, its action outside the striatum has been considered negligible. However, there is a growing body of evidence supporting functional extrastriatal DA modulation of both cellular excitability and synaptic transmission. In this review, the functional relevance of DA modulation outside the striatum in both normal and pathological conditions will be discussed.
Cells, 2019; 8
29908240: Chazalon M, Dumas S, Bernard JF, Sahly I, Tronche F, de Kerchove d'Exaerde A, Hamon M, Adrien J, Fabre V, Bonnavion P
The GABAergic Gudden's dorsal tegmental nucleus: A new relay for serotonergic regulation of sleep-wake behavior in the mouse.
Serotonin (5-HT) neurons are involved in wake promotion and exert a strong inhibitory influence on rapid eye movement (REM) sleep. Such effects have been ascribed, at least in part to the action of 5-HT at post-synaptic 5-HT receptors (5-HTR) in the brainstem, a major wake/REM sleep regulatory center. However, the neuroanatomical substrate through which 5-HTR influence sleep remains elusive. We therefore investigated whether a brainstem structure containing a high density of 5-HTR mRNA, the GABAergic Gudden's dorsal tegmental nucleus (DTg), may contribute to 5-HT-mediated regulatory mechanisms of sleep-wake stages. We first found that bilateral lesions of the DTg promote wake at the expense of sleep. In addition, using local microinjections into the DTg in freely moving mice, we showed that local activation of 5-HTR by the prototypical agonist 8-OH-DPAT enhances wake and reduces deeply REM sleep duration. The specific involvement of 5-HTR in the latter effects was further demonstrated by ex vivo extracellular recordings showing that the selective 5-HTR antagonist WAY 100635 prevented DTg neuron inhibition by 8-OH-DPAT. We next found that GABAergic neurons of the ventral DTg exclusively targets glutamatergic neurons of the lateral mammillary nucleus (LM) in the posterior hypothalamus by means of anterograde and retrograde tracing techniques using cre driver mouse lines and a modified rabies virus. Altogether, our findings strongly support the idea that 5-HT-driven enhancement of wake results from 5-HTR-mediated inhibition of DTg GABAergic neurons that would in turn disinhibit glutamatergic neurons in the mammillary bodies. We therefore propose a Raphe→DTg→LM pathway as a novel regulatory circuit underlying 5-HT modulation of arousal.
Neuropharmacology, 2018; 138
29756234: Baufreton J, Milekovic T, Li Q, McGuire S, Moraud EM, Porras G, Sun S, Ko WKD, Chazalon M, Morin S, Normand E, Farjot G, Milet A, Pype J, Pioli E, Courtine G, Bessière B, Bezard E
Inhaling xenon ameliorates l-dopa-induced dyskinesia in experimental parkinsonism.
Parkinson's disease motor symptoms are treated with levodopa, but long-term treatment leads to disabling dyskinesia. Altered synaptic transmission and maladaptive plasticity of corticostriatal glutamatergic projections play a critical role in the pathophysiology of dyskinesia. Because the noble gas xenon inhibits excitatory glutamatergic signaling, primarily through allosteric antagonism of the N-methyl-d-aspartate receptors, we aimed to test its putative antidyskinetic capabilities. We first studied the direct effect of xenon gas exposure on corticostriatal plasticity in a murine model of levodopa-induced dyskinesia We then studied the impact of xenon inhalation on behavioral dyskinetic manifestations in the gold-standard rat and primate models of PD and levodopa-induced dyskinesia. Last, we studied the effect of xenon inhalation on axial gait and posture

deficits in a primate model of PD with levodopa-induced dyskinesia. This study shows that xenon gas exposure (1) normalized synaptic transmission and reversed maladaptive plasticity of corticostriatal glutamatergic projections associated with levodopa-induced dyskinesia, (2) ameliorated dyskinesia in rat and nonhuman primate models of PD and dyskinesia, and (3) improved gait performance in a nonhuman primate model of PD. These results pave the way for clinical testing of this unconventional but safe approach. © 2018 The Authors. Movement Disorders published by Wiley Periodicals, Inc. on behalf of International Parkinson and Movement Disorder Society.
Mov Disord, 2018; 33
29742425: Chazalon M, Paredes-Rodriguez E, Morin S, Martinez A, Cristóvão-Ferreira S, Vaz S, Sebastiao A, Panatier A, Boué-Grabot E, Miguelez C, Baufreton J
GAT-3 Dysfunction Generates Tonic Inhibition in External Globus Pallidus Neurons in Parkinsonian Rodents.
The external globus pallidus (GP) is a key GABAergic hub in the basal ganglia (BG) circuitry, a neuronal network involved in motor control. In Parkinson's disease (PD), the rate and pattern of activity of GP neurons are profoundly altered and contribute to the motor symptoms of the disease. In rodent models of PD, the striato-pallidal pathway is hyperactive, and extracellular GABA concentrations are abnormally elevated in the GP, supporting the hypothesis of an alteration of neuronal and/or glial clearance of GABA. Here, we discovered the existence of persistent GABAergic tonic inhibition in GP neurons of dopamine-depleted (DD) rodent models. We showed that glial GAT-3 transporters are downregulated while neuronal GAT-1 function remains normal in DD rodents. Finally, we showed that blocking GAT-3 activity in vivo alters the motor coordination of control rodents, suggesting that GABAergic tonic inhibition in the GP contributes to the pathophysiology of PD.
Cell Rep, 2018; 23
29464851: Garret M, Du Z, Chazalon M, Cho YH, Baufreton J
Alteration of GABAergic neurotransmission in Huntington's disease.
Hereditary Huntington's disease (HD) is characterized by cell dysfunction and death in the brain, leading to progressive cognitive, psychiatric, and motor impairments. Despite molecular and cellular descriptions of the effects of the HD mutation, no effective pharmacological treatment is yet available. In addition to well-established alterations of glutamatergic and dopaminergic neurotransmitter systems, it is becoming clear that the GABAergic systems are also impaired in HD. GABA is the major inhibitory neurotransmitter in the brain, and GABAergic neurotransmission has been postulated to be modified in many neurological and psychiatric diseases. In addition, GABAergic neurotransmission is the target of many drugs that are in wide clinical use. Here, we summarize data demonstrating the occurrence of alterations of GABAergic markers in the brain of HD carriers as well as in rodent models of the disease. In particular, we pinpoint HD-related changes in the expression of GABA receptors (GABA Rs). On the basis that a novel GABA pharmacology of GABA Rs established with more selective drugs is emerging, we argue that clinical treatments acting specifically on GABAergic neurotransmission may be an appropriate strategy for improving symptoms linked to the HD mutation.
CNS Neurosci Ther, 2018; 24
27217211: Du Z, Chazalon M, Bestaven E, Leste-Lasserre T, Baufreton J, Cazalets JR, Cho YH, Garret M
Early GABAergic transmission defects in the external globus pallidus and rest/activity rhythm alteration in a mouse model of Huntington's disease.
Huntington's disease (HD) is characterized by progressive motor symptoms preceded by cognitive deficits and is regarded as a disorder that primarily affects the basal ganglia. The external globus pallidus (GPe) has a central role in the basal ganglia, projects directly to the cortex, and is majorly modulated by GABA. To gain a better understanding of the time course of HD progression and gain insight into the underlying mechanisms, we analyzed GABAergic neurotransmission in the GPe of the R6/1 mouse model at purportedly asymptomatic and symptomatic stages (i.e., 2 and 6months). Western blot and quantitative polymerase chain reaction (PCR) analyses revealed alterations in the GPe of male R6/1 mice compared with wild-type littermates. Expression of proteins involved in pre- and post-synaptic GABAergic compartments as well as synapse number were severely decreased at 2 and 6months. At both ages, patch-clamp electrophysiological recordings showed a decrease of spontaneous and miniature inhibitory post-synaptic currents (IPSCs) suggesting that HD mutation has an early effect on the GABA signaling in the brain. Therefore, we performed continuous locomotor activity recordings from 2 to 4months of age. Actigraphy analyses revealed rest/activity fragmentation alterations that parallel GABAergic system impairment at 2months, while the locomotor deficit is evident only at 3months in R6/1 mice. Our results reveal early deficits in HD and support growing evidence for a critical role played by the GPe in physiological and pathophysiological states. We suggest that actimetry may be used as a non-invasive tool to monitor early disease progression.
Neuroscience, 2016; 329
27072430: Osterstock G, Mitutsova V, Barre A, Granier M, Fontanaud P, Chazalon M, Carmignac D, Robinson IC, Low MJ, Plesnila N, Hodson DJ, Mollard P, Méry PF
Somatostatin triggers rhythmic electrical firing in hypothalamic GHRH neurons.
Hypothalamic growth hormone-releasing hormone (GHRH) neurons orchestrate body growth/maturation and have been

implicated in feeding responses and ageing. However, the electrical patterns that dictate GHRH neuron functions have remained elusive. Since the inhibitory neuropeptide somatostatin (SST) is considered to be a primary oscillator of the GH axis, we examined its acute effects on GHRH neurons in brain slices from male and female GHRH-GFP mice. At the cellular level, SST irregularly suppressed GHRH neuron electrical activity, leading to slow oscillations at the population level. This resulted from an initial inhibitory action at the GHRH neuron level via K(+) channel activation, followed by a delayed, sst1/sst2 receptor-dependent unbalancing of glutamatergic and GABAergic synaptic inputs. The oscillation patterns induced by SST were sexually dimorphic, and could be explained by differential actions of SST on both GABAergic and glutamatergic currents. Thus, a tripartite neuronal circuit involving a fast hyperpolarization and a dual regulation of synaptic inputs appeared sufficient in pacing the activity of the GHRH neuronal population. These "feed-forward loops" may represent basic building blocks involved in the regulation of GHRH release and its downstream sexual specific functions.
Sci Rep, 2016; 6

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BOARD NUMBER: S07-321

OPPOSING CHANGES IN THE ACTIVITY OF DIRECT AND INDIRECT PATHWAYS WITHIN THE STRIATUM OF FREELY MOVING DYT-TOR1A DYSTONIC MICE

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Aims: Dystonia is a movement disorder characterized by involuntary muscle contractions that has been linked to basal ganglia dysfunction. It has been proposed that the basal ganglia are involved in action selection by selecting an appropriate action through the activity of the direct pathway, while simultaneously inhibiting competing actions through the activity of the indirect pathway. Thus, an imbalance between the direct (D1 medium spiny neurons (MSNs)) and indirect pathways (D2 MSNs) could lead to involuntary dystonic movements. Yet, there is a lack of *in vivo* studies that dissect activity and ensemble dynamics of D1 or D2 MSNs in dystonia. **Methods:** DYT-TOR1AΔGAG knock-in mice and wild-type mice (WT) expressing Cre recombinase under the control of the dopamine D1 or A2A receptors (for D2 populations). Using *in vivo* calcium imaging, MSNs activity was recorded in freely moving animals while behavior was assessed by video and head-mounted accelerometers. Assessments were performed before and weekly after a standardized sciatic nerve crush lesion (SNCL) – a procedure known to induce dystonic-like movement in genetically-predisposed animals. **Results:** No significant differences were found in calcium transients rate or amplitude between DYT1-Tor1a and WT mice in both D1 and D2 MSNs. However, activity of movement-modulated D1 MSNs significantly increased while activity of D2 MSNs decreased, as dystonic-like movements developed in DYT1-Tor1a mice. Our observations shed light into the pathophysiology of DYT-TOR1A dystonia by revealing an imbalance between the D1 and D2 pathways at movement onset, compatible with a focused selection and inhibition model of the basal ganglia.

Pubmed:
34856124: França de Barros F, Bacqué-Cazenave J, Taillebuis C, Courtand G, Manuel M, Bras H, Tagliabue M, Combes D, Lambert FM, Beraneck M
Conservation of locomotion-induced oculomotor activity through evolution in mammals.
Efference copies are neural replicas of motor outputs used to anticipate the sensory consequences of a self-generated motor action or to coordinate neural networks involved in distinct motor behaviors. An established example of this motor-to-motor coupling is the efference copy of the propulsive motor command, which supplements classical visuo-vestibular reflexes to ensure gaze stabilization during amphibian larval locomotion. Such feedforward replica of spinal pattern-generating circuits produces a spino-extraocular motor coupled activity that evokes eye movements, spatiotemporally coordinated to tail undulation independently of any sensory signal. Exploiting the developmental stages of the frog, studies in metamorphing Xenopus demonstrated the persistence of this spino-extraocular motor command in adults and its developmental adaptation to tetrapodal locomotion. Here, we demonstrate for the first time the existence of a comparable locomotor-to-ocular motor coupling in the mouse. In neonates, ex vivo nerve recordings of brainstem-spinal cord preparations reveal a spino-extraocular motor coupled activity similar to the one described in Xenopus. In adult mice, trans-synaptic rabies virus injections in lateral rectus eye muscle label cervical spinal cord neurons closely connected to abducens motor neurons. Finally, treadmill-elicited locomotion in decerebrated preparations evokes rhythmic eye movements in synchrony with the limb gait pattern. Overall, our data are evidence for the conservation of locomotor-induced eye movements in vertebrate lineages. Thus, in mammals as in amphibians, CPG-efference copy feedforward signals might interact with sensory feedback to ensure efficient gaze control during locomotion.
Curr Biol, 2022; 32
33208812: França de Barros F, Schenberg L, Tagliabue M, Beraneck M
Long term visuo-vestibular mismatch in freely behaving mice differentially affects gaze stabilizing reflexes.
The vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR) work synergistically to stabilize gaze in response to head

movements. We previously demonstrated that a 14-day visuo-vestibular mismatch (VVM) protocol applied in freely behaving mice decreased the VOR gain. Here, we show for the first time that the OKR gain is also reduced and report on the recovery dynamics of both VOR and OKR after the end of the VVM protocol. Using sinusoidally-modulated stimulations, the decreases in VOR and OKR were found to be frequency-selective with larger reductions for frequencies < 0.5 Hz. Constant-velocity OKR stimulation tests demonstrated that the persistent components of the OKR were not modified while the transient, initial responses were. To identify the signals driving VOR and OKR reductions, we compared the responses of mice exposed to a high-contrast and no-contrast VVM. Despite being more robust in the high-contrast conditions, reductions were largely comparable and recovered with a similar time course. An analysis that directly compared VOR and OKR responses revealed that, alterations in the VOR were of significantly larger amplitude with significantly slower dynamics of recovery. Our findings are evidence for a frequency-selective influence of visual signals in the tuning of gaze stabilizing reflexes in normal mice.
Sci Rep, 2020; 10

30855582: França de Barros F, Carcaud J, Beraneck M
Long-term Sensory Conflict in Freely Behaving Mice.
Long-term sensory conflict protocols are a valuable means of studying motor learning. The presented protocol produces a persistent sensory conflict for experiments aimed at studying long-term learning in mice. By permanently wearing a device fixed on their heads, mice are continuously exposed to a sensory mismatch between visual and vestibular inputs while freely moving in home cages. Therefore, this protocol readily enables the study of the visual system and multisensory interactions over an extended timeframe that would not be accessible otherwise. In addition to lowering the experimental costs of long-term sensory learning in naturally behaving mice, this approach accommodates the combination of in vivo and in vitro experiments. In the reported example, video-oculography is performed to quantify the vestibulo-ocular reflex (VOR) and optokinetic reflex (OKR) before and after learning. Mice exposed to this long-term sensory conflict between visual and vestibular inputs presented a strong VOR gain decrease but exhibited few OKR changes. Detailed steps of device assembly, animal care, and reflex measurements are hereby reported.
J Vis Exp, 2019;
28835534: Emptoz A, Michel V, Lelli A, Akil O, Boutet de Monvel J, Lahlou G, Meyer A, Dupont T, Nouaille S, Ey E, Franca de Barros F, Beraneck M, Dulon D, Hardelin JP, Lustig L, Avan P, Petit C, Safieddine S
Local gene therapy durably restores vestibular function in a mouse model of Usher syndrome type 1G.
Our understanding of the mechanisms underlying inherited forms of inner ear deficits has considerably improved during the past 20 y, but we are still far from curative treatments. We investigated gene replacement as a strategy for restoring inner ear functions in a mouse model of Usher syndrome type 1G, characterized by congenital profound deafness and balance disorders. These mice lack the scaffold protein sans, which is involved both in the morphogenesis of the stereociliary bundle, the sensory antenna of inner ear hair cells, and in the mechanoelectrical transduction process. We show that a single delivery of the sans cDNA by the adenoassociated virus 8 to the inner ear of newborn mutant mice reestablishes the expression and targeting of the protein to the tips of stereocilia. The therapeutic gene restores the architecture and mechanosensitivity of stereociliary bundles, improves hearing thresholds, and durably rescues these mice from the balance defects. Our results open up new perspectives for efficient gene therapy of cochlear and vestibular disorders by showing that even severe dysmorphogenesis of stereociliary bundles can be corrected.
Proc Natl Acad Sci U S A, 2017; 114
28303261: Carcaud J, França de Barros F, Idoux E, Eugène D, Reveret L, Moore LE, Vidal PP, Beraneck M
Long-Lasting Visuo-Vestibular Mismatch in Freely-Behaving Mice Reduces the Vestibulo-Ocular Reflex and Leads to Neural Changes in the Direct Vestibular Pathway.
Calibration of the vestibulo-ocular reflex (VOR) depends on the presence of visual feedback. However, the cellular mechanisms associated with VOR modifications at the level of the brainstem remain largely unknown. A new protocol was designed to expose freely behaving mice to a visuo-vestibular mismatch during a 2-week period. This protocol induced a 50% reduction of the VOR. pharmacological experiments demonstrated that the VOR reduction depends on changes located outside the flocculus/paraflocculus complex. The cellular mechanisms associated with the VOR reduction were then studied on brainstem slices through a combination of vestibular afferent stimulation and patch-clamp recordings of central vestibular neurons. The evoked synaptic activity demonstrated that the efficacy of the synapses between vestibular afferents and central vestibular neurons was decreased. In addition, a long-term depression protocol failed to further decrease the synapse efficacy, suggesting that the VOR reduction might have occurred through depression-like mechanisms. Analysis of the intrinsic membrane properties of central vestibular neurons revealed that the synaptic changes were supplemented by a decrease in the spontaneous discharge and excitability of a subpopulation of neurons. Our results provide evidence that a long-lasting visuo-vestibular mismatch leads to changes in synaptic transmission and intrinsic properties of central vestibular neurons in the direct VOR pathway. Overall, these results open new avenues for future studies on visual and vestibular

interactions conducted and .
eNeuro, 2017 Jan-Feb; 4

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FURTHER CHARACTERISATION OF THE RAT MODEL OF TOURETTE-RELATED STRIATAL DISINHIBITION: IN VIVO ELECTROPHYSIOLOGICAL AND BEHAVIOURAL STUDIES

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Tourette's syndrome, characterised by tics, has been linked to reduced GABAergic inhibition, so-called neural disinhibition, in the striatum (Jackson et al.,2015,*Trends CognSci*). Dorsal striatal microinfusion of GABA-A antagonists like picrotoxin produces tic-like movements in rodents and primates (Bronfeld et al.,2013,*FrontSystNeurosci*; Klaus&Plenz,2016,*PLoSBiol*.) Here, we infused young adult male Lister hooded rats unilaterally with picrotoxin (300ng/0.5ul) or saline (0.5ul) into the anterior dorsal striatum and recorded electrophysiological and behavioural measurements to characterise further the neuro-behavioural impact of striatal disinhibition. Electrophysiological recordings under isoflurane in the striatum showed that picrotoxin disinhibition, apart from evoking large LFP spike-wave discharges, with sharp multi-unit bursts during the negative spike, markedly enhanced burst firing, similar to findings in prefrontal cortex and hippocampus (Bast et al.,2017,*BrJPharamcol*). In freely moving rats, striatal picrotoxin reliably induced tic-like contralateral forelimb movements. Automated photobeam measurements revealed that striatal disinhibition increased locomotor activity and fine motor counts. Fine motor counts showed a similar time course to tic-like movements, suggesting an automated method to quantify these movements. Prepulse inhibition (PPI) of acoustic startle depends on the striatum and is disrupted in Tourette's (Swerdlow,2013,*NeurosciBiobehavRev*). Striatal disinhibition in rats did not affect PPI, but tended to reduce startle during the first test block, before habituation led to similarly low startle responses in both infusion conditions (infusion-X-block: $F(2,26)=3.2$, $p=0.058$). Our findings confirm that striatal disinhibition causes marked spike-wave discharges in striatum and tic-like movements. In addition, such disinhibition markedly enhances striatal burst firing and increases locomotor activity, but does not reproduce PPI deficits seen in Tourette's.

Pubmed:

32070809: Jackson SR, Loayza J, Crighton M, Sigurdsson HP, Dyke K, Jackson GM
The role of the insula in the generation of motor tics and the experience of the premonitory urge-to-tic in Tourette syndrome.
Tourette syndrome (TS) is a neurological disorder of childhood onset that is characterised by the occurrence of motor and vocal tics. TS is associated with cortical-striatal-thalamic-cortical circuit [CSTC] dysfunction and hyper-excitability of cortical limbic and motor regions that are thought to lead to the occurrence of tics. Importantly, individuals with TS often report that their tics are preceded by 'premonitory sensory/urge phenomena' (PU) that are described as uncomfortable bodily sensations that precede the execution of a tic and are experienced as a strong urge for motor discharge. While the precise role played by PU in the occurrence of tics is largely unknown, they are nonetheless of considerable theoretical and clinical importance, not least because they form the core component in many behavioural therapies used in the treatment of tic disorders. Several lines of evidence indicate that the insular cortex may play a particularly important role in the generation of PU in TS and 'urges-for-action' more generally. In the current study we utilised voxel-based morphometry techniques together with 'seed-to-voxel' structural covariance network (SCN) mapping to investigate the putative role played by the right insular cortex in the generation of motor tics and the experience of PU in a relatively large group of young people TS. We demonstrate that clinical measures of motor tic severity and PU are uncorrelated with one another, that motor tic severity and PU scores are associated with separate regions of the insular cortex, and that the insula is associated with different structural covariance networks in individuals with TS compared to a matched group of typically developing individuals.
Cortex, 2020; 126
34980662: Williams SA, Gwilt M, Hock R, Taylor C, Loayza J, Stevenson CW, Cassaday HJ, Bast T
Hippocampal Disinhibition Reduces Contextual and Elemental Fear Conditioning While Sparing the Acquisition of Latent Inhibition.
Hippocampal neural disinhibition, i.e., reduced GABAergic inhibition, is a key feature of schizophrenia pathophysiology. The hippocampus is an important part of the neural circuitry that controls fear conditioning and can also modulate prefrontal and striatal mechanisms, including dopamine signaling, which play a role in salience modulation. Consequently, hippocampal

neural disinhibition may contribute to impairments in fear conditioning and salience modulation reported in schizophrenia. Therefore, we examined the effect of ventral hippocampus (VH) disinhibition in male rats on fear conditioning and salience modulation, as reflected by latent inhibition (LI), in a conditioned emotional response (CER) procedure. A flashing light was used as the conditioned stimulus (CS), and conditioned suppression was used to index conditioned fear. In experiment 1, VH disinhibition via infusion of the GABA-A receptor antagonist picrotoxin before CS pre-exposure and conditioning markedly reduced fear conditioning to both the CS and context; LI was evident in saline-infused controls but could not be detected in picrotoxin-infused rats because of the low level of fear conditioning to the CS. In experiment 2, VH picrotoxin infusions only before CS pre-exposure did not affect the acquisition of fear conditioning or LI. Together, these findings indicate that VH neural disinhibition disrupts contextual and elemental fear conditioning, without affecting the acquisition of LI. The disruption of fear conditioning resembles aversive conditioning deficits reported in schizophrenia and may reflect a disruption of neural processing both within the hippocampus and in projection sites of the hippocampus.
eNeuro, 2022 Jan-Feb; 9

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RESTORATION OF DOPAMINE D2 RECEPTORS IN THE SENSORIMOTOR STRIATUM OF D2R KNOCKDOWN MICE SELECTIVELY AMELIORATES DEFICITS IN MOTOR SKILL LEARNING

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Dopamine D2 receptors (D2Rs) are necessary for normal voluntary movement, but the behavior-specific contributions of D2R function in different striatal territories and cell types remain incompetely understood. We sought to define the subpopulations of striatal neurons in which restoration of D2R signaling rescues specific behavioral deficits induced by global knockdown of D2R expression (D2R-KD). Therefore, we characterized a D2R-KD mouse line in which a transcriptional STOP cassette that could be excised by Cre recombinase had been inserted between the D2 promoter and the coding sequence of the D2R gene. In this D2-KD mouse line, expression of D2R mRNA was greatly reduced and could be rescued in indirect pathway medium spiny neurons by local transduction with an adeno-associated viral vector encoding Cre recombinase driven by the enkephalin promoter. D2-KD mice exhibited severe impairments on the rotarod and in a self-paced action sequence task. Although they executed the action sequence in the correct order, they displayed impaired movement initiation and a phenotype that appears similar to the “freezing” observed in Parkinson’s disease patients. To elucidate the minimal neural substrates in which D2R signaling can ameliorate deficits induced by global D2R knockdown, we restored D2R expression in indirect pathway medium spiny neurons (iMSNs) of the dorsolateral striatum (DLS). Restoration of D2R signaling to DLS iMSNs ameliorated performance deficits on the accelerating rotarod, but not in our operant action sequence task. We conclude that D2R signaling in the aDLS is partially sufficient to support the learning and/or performance of motor skills.

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CELL-TYPE SPECIFIC RESPONSES TO ASSOCIATIVE LEARNING IN THE PRIMARY MOTOR CORTEX

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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The primary motor cortex (M1) is a critical site for both movement initiation and motor learning. Intriguingly, neurons in M1 have also been shown to have reward-related activity, possibly to facilitate reward-based learning of new movements. However, whether reward-related signals are represented among different cell types in M1, and whether their response properties change after cue-reward conditioning remains unclear. Here, we performed longitudinal *in vivo* two-photon Ca^{2+} imaging to monitor the activity of different neuronal cell types in M1 of adult mice engaged in a classical conditioning task. Our findings reveal that most of the major neuronal cell types in M1 show robust but differential responses to both cue and reward stimuli, and their response properties undergo cell-type specific modifications following associative learning. PV inhibitory neuron responses became more reliable to the cue stimulus, while VIP inhibitory neuron responses became more reliable to the reward. Moreover, we did not observe these changes in PV or VIP inhibitory neuron responses when the cue and reward were randomly timed and not paired together. These observations demonstrate that cue- and reward-related signals are represented among different neuronal cell types in M1, and these responses undergo distinct modifications during associative learning.

Pubmed:

30942169: Fotowat H, Lee C, Jun JJ, Maler L
Neural activity in a hippocampus-like region of the teleost pallium is associated with active sensing and navigation.
Most vertebrates use active sensing strategies for perception, cognition and control of motor activity. These strategies include directed body/sensor movements or increases in discrete sensory sampling events. The weakly electric fish, , uses its active electric sense during navigation in the dark. Electric organ discharge rate undergoes transient increases during navigation to increase electrosensory sampling. also use stereotyped backward swimming as an important form of active sensing that brings objects toward the electroreceptor dense fovea-like head region. We wirelessly recorded neural activity from the pallium of freely swimming . Spiking activity was sparse and occurred only during swimming. Notably, most units tended to fire during backward swims and their activity was on average coupled to increases in sensory sampling. Our results provide the first characterization of neural activity in a hippocampal (CA3)-like region of a teleost fish brain and connects it to active sensing of spatial environmental features.
Elife, 2019; 8
32390806: Lee C, Lavoie A, Liu J, Chen SX, Liu BH
Light Up the Brain: The Application of Optogenetics in Cell-Type Specific Dissection of Mouse Brain Circuits.
The exquisite intricacies of neural circuits are fundamental to an animal's diverse and complex repertoire of sensory and motor functions. The ability to precisely map neural circuits and to selectively manipulate neural activity is critical to understanding brain function and has, therefore been a long-standing goal for neuroscientists. The recent development of optogenetic tools, combined with transgenic mouse lines, has endowed us with unprecedented spatiotemporal precision in circuit analysis. These advances greatly expand the scope of tractable experimental investigations. Here, in the first half of the review, we will present applications of optogenetics in identifying connectivity between different local neuronal cell types and of long-range projections with both and methods. We will then discuss how these tools can be used to reveal the functional roles of these cell-type specific connections in governing sensory information processing, and learning and memory in the visual cortex, somatosensory cortex, and motor cortex. Finally, we will discuss the prospect of new optogenetic tools and how their application can further advance modern neuroscience. In summary, this review serves as a primer to exemplify how optogenetics can be used in sophisticated modern circuit analyses at the levels of synapses, cells, network connectivity and behaviors.
Front Neural Circuits, 2020; 14
35113017: Lee C, Harkin EF, Yin X, Naud R, Chen S
Cell-type-specific responses to associative learning in the primary motor cortex.

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The primary motor cortex (M1) is known to be a critical site for movement initiation and motor learning. Surprisingly, it has also been shown to possess reward-related activity, presumably to facilitate reward-based learning of new movements. However, whether reward-related signals are represented among different cell types in M1, and whether their response properties change after cue-reward conditioning remains unclear. Here, we performed longitudinal in vivo two-photon Ca imaging to monitor the activity of different neuronal cell types in M1 while mice engaged in a classical conditioning task. Our results demonstrate that most of the major neuronal cell types in M1 showed robust but differential responses to both the conditioned cue stimulus (CS) and reward, and their response properties undergo cell-type-specific modifications after associative learning. PV-INs' responses became more reliable to the CS, while VIP-INs' responses became more reliable to reward. Pyramidal neurons only showed robust responses to novel reward, and they habituated to it after associative learning. Lastly, SOM-INs' responses emerged and became more reliable to both the CS and reward after conditioning. These observations suggest that cue- and reward-related signals are preferentially represented among different neuronal cell types in M1, and the distinct modifications they undergo during associative learning could be essential in triggering different aspects of local circuit reorganization in M1 during reward-based motor skill learning.
Elife, 2022; 11

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CELL-CLASS-SPECIFIC OPTOGENETIC STIMULATION REVEALS SEGREGATED MOTOR MAPS IN MOUSE DORSAL CORTEX

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Functional localization is a fundamental organizing principle of the cerebral cortex. Direct cortical stimulation can be used to define cortical motor maps, i.e. maps of cortical regions which causally induce movements of a specific body part. But how different classes of neurons constitute a motor map is unknown. Here, we started to address this question by optogenetic stimulation mapping in transgenic mice expressing channelrhodopsin-2 (ChR2) in different genetically-defined classes of cortical neurons. We systematically delivered focal blue laser stimuli across dorsal cortex of awake head-restrained mice, and quantified stimulation-evoked jaw movements by high-speed video analysis. Mice expressing ChR2 in all excitatory neurons showed oscillatory opening and closing movements of the jaw evoked by optogenetic stimulation within a large elliptical area covering orofacial cortex, while mice expressing ChR2 in all inhibitory neurons did not show evoked movements. Next, we stimulated layer-specific and projection-specific excitatory neurons in different mouse lines. Surprisingly, we found that each class of excitatory neurons has its most effective cortical site in different coordinates from each other, just to cover the overall effective area defined by motor-mapping in mice expressing ChR2 in all excitatory neurons. This result suggests that a cortical motor map is organized by spatially segregated elemental maps, each of which is contributed by different classes of neurons. To reveal the circuits connecting the segregated maps, we have begun to combine optogenetic motor mapping with wide-field calcium imaging. A further important future goal is to investigate context- and learning-dependent features of these cell-class-specific motor maps.

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MULTIPLE CORTICOSPINAL MODULES FOR COMMAND FUNCTIONS

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Classical neurology and neurophysiology have assigned to motor cortex (MI) and its cortico-spinal tract an exclusive role in action control. A different perspective, highlighting the modular nature of command functions, results from multiple observations. *(i)* The statistics of cortical connectivity of the parieto-frontal system suggest the existence of multiple functionally-connected cortical clusters in premotor, cingulate and parietal areas. *(ii)* The recruitment timing of these clusters is task-dependent; therefore, different areas can be activated selectively, bypassing the cortical hierarchy leading to MI. *(iii)* Multiple corticospinal systems stem from different premotor, cingulate, and parietal areas. *(iv)* Each corticospinal system has a different mean conduction velocity; thus, descending pathways can be selected in a task-dependent manner. *(v)* Within each corticospinal system, axons calibers differ, leading to temporal smearing of information. The metabolic costs associated with integrating this temporal information after an obligatory transfer through long oligosynaptic corticocortical paths toward MI are higher than those incurred by sending information directly through separate corticospinal pathways. *(vi*) Certain consequences of lesioning the parieto-frontal system do not occur when lesioning MI (e.g. different forms of apraxia, optic ataxia, etc.). Examples of different corticospinal modules beyond that stemming from MI are: the parieto-spinal module for the control of reaching, object manipulation, and visuomotor adaptations; the dorsal premotor-spinal module for storing memory traces of learned associations; the ventral premotor-spinal system for defensive actions. Local availability of both information and outflow paths therefore seem to be a prerequisite for command functions, and probably a major achievement of cortical evolution.

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THE PREDICTIVE BRAIN: LINKING MOTOR AND SENSORY ATTENUATION USING COMBINED TMS-EEG

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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When sensory inputs can be predicted by an organism's own actions or external environmental cues, neural activity is downweighted compared to sensory inputs that are unpredictable. We have recently demonstrated that this downweighting to predictable inputs is also observed when stimulating the motor system with transcranial magnetic stimulation (TMS). Akin to sensory attenuation, predictable TMS excites the motor system less effectively than unpredicted TMS. Using a combined TMS-EEG setup, we measured corticospinal attenuation to motor cortex stimulation using motor-evoked potentials (MEP), and simultaneously measured sensory attenuation to the sound of TMS (a coil "click") using event-related potentials (ERP). We found that both ERPs and MEPs were downweighted to predictable TMS compared to unpredictable TMS. Critically, the magnitude of ERP suppression predicted the magnitude of MEP suppression. Our results reveal a close correspondence between attenuation of the sensory and motor systems. The findings provide compelling evidence that predictive coding is governed by domain-general properties across distinct neural systems and has shared mechanisms responsible for all forms of predictive learning.

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MODULAR REPRESENTATION OF REACHING ENDPOINTS IN MOUSE MOTOR CORTEX

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Motor cortex controls voluntary movements through descending commands via the brainstem and spinal cord. To better understand the cortical role in motor control, we studied the activity of motor cortex neurons of mice performing a multi-directional water-reaching task. We found that task-related neurons became sequentially active at different phases of the task, from target presentation to reaching and water consumption. Interestingly, while all task-related neurons displayed strong selectivity for the location of the reaching target, few were modulated by the arm's actual trajectory. This suggests that task-related neurons encode spatial information of reaching endpoint instead of kinematic parameters. We analyzed the spatial representation and found that neurons could be clustered into three roughly segregated groups representing reaching targets located to the left, center or right of the animal's snout, conforming a coarse reaching endpoint map. As a whole, subsets of layer 2/3 neurons represent specific combinations of spatial and temporal aspects of the task. We speculated that these subsets of neurons form functional modules which are dynamically recruited during task execution. To evaluate this possibility, we analyzed the neuronal activity while the reaching target was suddenly moved from one to another location during an ongoing reach. According to our speculation, activity of the neuronal modules was consistently updated to match the novel target location and the upcoming phases of the task. In summary, our data suggests that mouse motor cortex activity does not solely represent motor commands, but broader spatio-temporal information relevant to the task phase and reaching target location.

Pubmed:
34754107: Klingler E, Tomasello U, Prados J, Kebschull JM, Contestabile A, Galiñanes GL, Fièvre S, Santinha A, Platt R, Huber D, Dayer A, Bellone C, Jabaudon D
Temporal controls over inter-areal cortical projection neuron fate diversity.
Interconnectivity between neocortical areas is critical for sensory integration and sensorimotor transformations. These functions are mediated by heterogeneous inter-areal cortical projection neurons (ICPN), which send axon branches across cortical areas as well as to subcortical targets. Although ICPN are anatomically diverse, they are molecularly homogeneous, and how the diversity of their anatomical and functional features emerge during development remains largely unknown. Here we address this question by linking the connectome and transcriptome in developing single ICPN of the mouse neocortex using a combination of multiplexed analysis of projections by sequencing (MAPseq, to identify single-neuron axonal projections) and single-cell RNA sequencing (to identify corresponding gene expression). Focusing on neurons of the primary somatosensory cortex (S1), we reveal a protracted unfolding of the molecular and functional differentiation of motor cortex-projecting ([Formula: see text]) ICPN compared with secondary somatosensory cortex-projecting ([Formula: see text]) ICPN. We identify SOX11 as a temporally differentially expressed transcription factor in [Formula: see text] versus [Formula: see text] ICPN. Postnatal manipulation of SOX11 expression in S1 impaired sensorimotor connectivity and disrupted selective exploratory behaviours in mice. Together, our results reveal that within a single cortical area, different subtypes of ICPN have distinct postnatal paces of molecular differentiation, which are subsequently reflected in distinct circuit connectivities and functions. Dynamic differences in the expression levels of a largely generic set of genes, rather than fundamental differences in the identity of developmental genetic programs, may thus account for the emergence of intra-type diversity in cortical neurons.
Nature, 2021; 599
30189206: Galiñanes GL, Huber D
Circuits for Raiders.
Heindorf et al. (2018) report that motor cortex plays a key role in behavioral tasks that rely on continuous sensory feedback. They propose a layer-based circuit that might be of particular importance when coping with unexpected perturbations in dynamic environments.
Neuron, 2018; 99

29514103: Galiñanes GL, Bonardi C, Huber D
Directional Reaching for Water as a Cortex-Dependent Behavioral Framework for Mice.
Optogenetic tools and imaging methods for recording and manipulating brain activity have boosted the field of neuroscience in unprecedented ways. However, behavioral paradigms for mice lag behind those of primates, limiting the full potential of such tools. Here, we present an innovative behavioral framework in which head-fixed mice directionally reach for water droplets, similar to the primate "center-out" reaching task. Mice rapidly engaged in the task, performed hundreds of trials, and reached in multiple directions when droplets were presented at different locations. Surprisingly, mice used chemosensation to determine the presence of water droplets. Optogenetic inactivation of the motor cortex halted the initiation and rapidly diverted the trajectory of ongoing movements. Layer 2/3 two-photon imaging revealed robust direction selectivity in most reach-related neurons. Finally, mice performed directional reaching instructed by vibratotactile stimuli, demonstrating the potential of this framework for studying, in addition to motor control, sensory processing, and decision making.
Cell Rep, 2018; 22
28476642: Braz BY, Belforte JE, Murer MG, Galiñanes GL
Properties of the corticostriatal long term depression induced by medial prefrontal cortex high frequency stimulation in vivo.
Repetitive stimulation of cognitive forebrain circuits at frequencies capable of inducing corticostriatal long term plasticity is increasingly being used with therapeutic purposes in patients with neuropsychiatric disorders. However, corticostriatal plasticity is rarely studied in the intact brain. Our aim was to study the mechanisms of corticostriatal long term depression (LTD) induced by high frequency stimulation (HFS) of the medial prefrontal cortex in vivo. Our main finding is that the LTD induced in the dorsomedial striatum by medial prefrontal cortex HFS in vivo (prefrontostriatal LTD) is not affected by manipulations that block or reduce the LTD induced in the dorsolateral striatum by motor cortex HFS in brain slices, including pharmacological dopamine receptor and CB1 receptor blockade, chronic nigrostriatal dopamine depletion, CB1 receptor genetic deletion and selective striatal cholinergic interneuron (SCIN) ablation. Conversely, like in the hippocampus and other brain areas, prefrontostriatal LTD is NMDA receptor dependent. Thus, we describe a novel form of corticostriatal LTD that operates in brain circuits involved in reward and cognition and could be relevant for understanding the therapeutic effects of deep brain stimulation.
Neuropharmacology, 2017; 121
28231470: Prsa M, Galiñanes GL, Huber D
Rapid Integration of Artificial Sensory Feedback during Operant Conditioning of Motor Cortex Neurons.
Neuronal motor commands, whether generating real or neuroprosthetic movements, are shaped by ongoing sensory feedback from the displacement being produced. Here we asked if cortical stimulation could provide artificial feedback during operant conditioning of cortical neurons. Simultaneous two-photon imaging and real-time optogenetic stimulation were used to train mice to activate a single neuron in motor cortex (M1), while continuous feedback of its activity level was provided by proportionally stimulating somatosensory cortex. This artificial signal was necessary to rapidly learn to increase the conditioned activity, detect correct performance, and maintain the learned behavior. Population imaging in M1 revealed that learning-related activity changes are observed in the conditioned cell only, which highlights the functional potential of individual neurons in the neocortex. Our findings demonstrate the capacity of animals to use an artificially induced cortical channel in a behaviorally relevant way and reveal the remarkable speed and specificity at which this can occur.
Neuron, 2017; 93
25872916: Braz BY, Galiñanes GL, Taravini IR, Belforte JE, Murer MG
Altered Corticostriatal Connectivity and Exploration/Exploitation Imbalance Emerge as Intermediate Phenotypes for a Neonatal Dopamine Dysfunction.
Findings showing that neonatal lesions of the forebrain dopaminergic system in rodents lead to juvenile locomotor hyperactivity and learning deficits have been taken as evidence of face validity for the attention deficit hyperactivity disorder. However, the core cognitive and physiological intermediate phenotypes underlying this rodent syndrome remain unknown. Here we show that early postnatal dopaminergic lesions cause long-lasting deficits in exploitation of shelter, social and nutritional resources, and an imbalanced exploratory behavior, where nondirected local exploration is exacerbated, whereas sophisticated search behaviors involving sequences of goal directed actions are degraded. Importantly, some behavioral deficits do not diminish after adolescence but instead worsen or mutate, particularly those related to the exploration of wide and spatially complex environments. The in vivo electrophysiological recordings and morphological reconstructions of striatal medium spiny neurons reveal corticostriatal alterations associated to the behavioral phenotype. More specifically, an attenuation of corticostriatal functional connectivity, affecting medial prefrontal inputs more markedly than cingulate and motor inputs, is accompanied by a contraction of the dendritic arbor of striatal projection neurons in this animal model. Thus, dopaminergic neurons are essential during postnatal development for the functional and structural maturation of corticostriatal connections. From a bottom-up viewpoint, our findings suggest that neuropsychiatric conditions presumably linked to developmental alterations of the dopaminergic system should be evaluated for deficits in foraging decision making,

alterations in the recruitment of corticostriatal circuits during foraging tasks, and structural disorganization of the frontostriatal connections.
Neuropsychopharmacology, 2015; 40
25326662: Andrásfalvy BK, Galiñanes GL, Huber D, Barbic M, Macklin JJ, Susumu K, Delehanty JB, Huston AL, Makara JK, Medintz IL
Quantum dot-based multiphoton fluorescent pipettes for targeted neuronal electrophysiology.
Targeting visually identified neurons for electrophysiological recording is a fundamental neuroscience technique; however, its potential is hampered by poor visualization of pipette tips in deep brain tissue. We describe quantum dot-coated glass pipettes that provide strong two-photon contrast at deeper penetration depths than those achievable with current methods. We demonstrated the pipettes' utility in targeted patch-clamp recording experiments and single-cell electroporation of identified rat and mouse neurons in vitro and in vivo.
Nat Methods, 2014; 11
22163020: Galiñanes GL, Braz BY, Murer MG
Origin and properties of striatal local field potential responses to cortical stimulation: temporal regulation by fast inhibitory connections.
Evoked striatal field potentials are seldom used to study corticostriatal communication in vivo because little is known about their origin and significance. Here we show that striatal field responses evoked by stimulating the prelimbic cortex in mice are reduced by more than 90% after infusing the AMPA receptor antagonist CNQX close to the recording electrode. Moreover, the amplitude of local field responses and dPSPs recorded in striatal medium spiny neurons increase in parallel with increasing stimulating current intensity. Finally, the evoked striatal fields show several of the basic known properties of corticostriatal transmission, including paired pulse facilitation and topographical organization. As a case study, we characterized the effect of local GABA(A) receptor blockade on striatal field and multiunitary action potential responses to prelimbic cortex stimulation. Striatal activity was recorded through a 24 channel silicon probe at about 600 µm from a microdialysis probe. Intrastriatal administration of the GABA(A) receptor antagonist bicuculline increased by 65±7% the duration of the evoked field responses. Moreover, the associated action potential responses were markedly enhanced during bicuculline infusion. Bicuculline enhancement took place at all the striatal sites that showed a response to cortical stimulation before drug infusion, but sites showing no field response before bicuculline remained unresponsive during GABA(A) receptor blockade. Thus, the data demonstrate that fast inhibitory connections exert a marked temporal regulation of input-output transformations within spatially delimited striatal networks responding to a cortical input. Overall, we propose that evoked striatal fields may be a useful tool to study corticostriatal synaptic connectivity in relation to behavior.
PLoS One, 2011; 6
21767642: Zold CL, Kasanetz F, Pomata PE, Belluscio MA, Escande MV, Galinanes GL, Riquelme LA, Murer MG
Striatal gating through up states and oscillations in the basal ganglia: Implications for Parkinson's disease.
Up states are a hallmark of striatal physiology. Spontaneous activity in the thalamo-cortical network drives robust plateau depolarizations in the medium spiny projection neurons of the striatum. Medium spiny neuron firing is only possible during up states and is very tightly regulated by dopamine and NMDA receptors. In a rat model of Parkinson's disease the medium spiny neurons projecting to the globus pallidus (indirect pathway) show more depolarized up states and increased firing. This is translated into abnormal patterns of synchronization between the globus pallidus and frontal cortex, which are believed to underlie the symptoms of Parkinson's disease. Here we review our work in the field and propose a mechanism through which the lack of D2 receptor stimulation in the striatum allows the establishment of fixed routes of information flow in the cortico-striato-pallidal network.
J Physiol Paris, 2012; 106
19244524: Galiñanes GL, Taravini IR, Murer MG
Dopamine-dependent periadolescent maturation of corticostriatal functional connectivity in mouse.
Altered corticostriatal information processing associated with early dopamine systems dysfunction may contribute to attention deficit/hyperactivity disorder (ADHD). Mice with neonatal dopamine-depleting lesions exhibit hyperactivity that wanes after puberty and is reduced by psychostimulants, reminiscent of some aspects of ADHD. To assess whether the maturation of corticostriatal functional connectivity is altered by early dopamine depletion, we examined preadolescent and postadolescent urethane-anesthetized mice with or without dopamine-depleting lesions. Specifically, we assessed (1) synchronization between striatal neuron discharges and oscillations in frontal cortex field potentials and (2) striatal neuron responses to frontal cortex stimulation. In adult control mice striatal neurons were less spontaneously active, less responsive to cortical stimulation, and more temporally tuned to cortical rhythms than in infants. Striatal neurons from hyperlocomotor mice required more current to respond to cortical input and were less phase locked to ongoing oscillations, resulting in fewer neurons responding to refined cortical commands. By adulthood some electrophysiological deficits waned together with hyperlocomotion, but striatal spontaneous activity remained substantially elevated. Moreover, dopamine-depleted animals

showing normal locomotor scores exhibited normal corticostriatal synchronization, suggesting that the lesion allows, but is not sufficient, for the emergence of corticostriatal changes and hyperactivity. Although amphetamine normalized corticostriatal tuning in hyperlocomotor mice, it reduced horizontal activity in dopamine-depleted animals regardless of their locomotor phenotype, suggesting that amphetamine modified locomotion through a parallel mechanism, rather than that modified by dopamine depletion. In summary, functional maturation of striatal activity continues after infancy, and early dopamine depletion delays the maturation of core functional capacities of the corticostriatal system.
J Neurosci, 2009; 29

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MOTOR CORTEX AREA-SPECIFIC PATTERNS OF CONVERGENT THALAMIC INPUTS IN THE MOUSE

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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A large region of the mouse dorsal and medial frontal isocortex is commonly labelled ‘motor’. However, the published histological and electrophysiological data in rodents provide ambiguous and often conflicting evidence for delineation of individual areas. Since multiple thalamic nuclei innervate the motor cortex, fine quantitative differences between mouse motor cortex regions in the thalamocortical input composition and/or intracortical distribution could provide an independent and consistent reference for area delineation, as well as for cross-species comparisons. To map and quantify thalamus neurons innervating different motor cortex regions in the adult C57BL/6 mouse, we microinject in cortex retrograde tracers and calculate the percentage of labelled cells in each nucleus within all labelled cells in each case and compare then between cases. We are currently using anterograde labelling to analyze the laminar convergence of this pathways. Our preliminary data show that, the most numerous source of thalamic innervation comes from VM (ventromedial nuclei) and VAL (ventral anterior-lateral nuclei), to a lesser extend from the posterior, intralaminar and the midline nuclei. It also reveals a remarkable heterogeneity in the set of nuclei whose projections converge at different points in the cortex, and a complex topography within each nucleus. This supports the idea that this approach can provide valuable data for the consistent delineation of functional domains in the motor cortex of the mouse. European Union's Horizon 2020 (HBP SGA3 GA 945539) and Ministerio de Economía y Competitividad /Fondo Europeo para el Desarrollo Regional (MINECO/FEDER) BFU2017-88549.

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AN ALTERNATIVE CORTICO-SUBCORTICAL LOOP INVOLVING THE THALAMUS FOR LOCOMOTION

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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The cortico-basal ganglia-thalamocortical loop has been known to be involved in locomotor behavior. Disturbance of the basal ganglia activity results in characteristic, unilateral rotation. Similar to basal ganglia the pontine reticular formation (PRF) also sends inhibitory terminals to the intralaminar thalamus (IL). Activation of the PRF-IL pathway interference induces a strong phenotype, a complete behavioral arrest. In this study, we outline a new motor-related cortico-subcortico-thalamocortical loop by examining the impact of cortical inputs on the PRF inhibitory cells (PRF/GlyT2+) and testing the effect of photoactivation of PRF/GlyT2+ neurons on locomotor behavior. AAV-ChR2 injections into the frontal cortex (M2 and cingulate) of RBP4-Cre/Glyt2-eGFP transgenic mice revealed that mid-caliber dendrites and spines of the PRF/GlyT2+ cells receive L5 inputs. In *in vitro* slice preparation, the photoactivation of the L5 fibers reliably produced purely glutamatergic synaptic responses on PRF/GlyT2+ cells. Juxtacellular recording *in vivo* showed that photoactivation of L5 neurons evoked short-latency APs in the PRF/GlyT2+ cells with a high probability. Spontaneous rhythmic activity of PRF/Glyt2+ neurons was strongly linked to the slow cortical oscillation. Photoactivation of PRF/Glyt2+ cells resulted in unilateral rotation and significantly decreased firing rate of the IL cells. We conclude that synchronous frontal cortical activity may convey behavioral signals to PRF and this can reliably activate PRF/GlyT2+ neurons. PRF/GlyT2+ cells transfer cortical input as inhibitory signals to the IL before they return to the cortex via the thalamocortical pathway. Both the concept of network organization and the evoked behavioral response are reminiscent of the cortico-basal ganglia loop.

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A COMMON MECHANISM FOR SALIENCY DETECTION AND MOTOR REACTIVITY IN HUMANS AND RHESUS MONKEYS

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

Giacomo Novembre[1,2], Irene Lacal[3,4], Ludovica Caratelli[4], Andrea Schito[4], Roberto Caminiti[1], Alexandra Battaglia-Mayer[4], Giandomenico Iannetti[1,5]
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Sudden and surprising sensory events often signal environmental threats, triggering a cascade of neural mechanisms aimed to adapt behavior accordingly. To study the phylogenetic origin of these mechanisms, we trained two rhesus monkeys (*macaca mulatta*) to exert force on an isometric joystick and thereby keep the position of a cursor inside a visual target. Fast-rising and task-irrelevant auditory stimuli were occasionally presented. We examined the effect of such unexpected stimuli on both isometric force and electroencephalographic (EEG) activity recorded using 29 active scalp electrodes. Auditory stimuli elicited a consistent biphasic modulation of isometric force, consisting of an initial transient decrease (d1, moving the cursor away from the target center) followed by a tonic increase (i2, bringing to cursor back to its original position). The same stimuli also elicited an EEG potential dominated by two large negative-positive waves (N70 and P130) maximal at the scalp vertex, homologous of the human 'vertex potential'. A trial-by-trial correlation analysis between force and EEG revealed that the amplitude of the vertex potential positivity (P130) predicted the magnitude of the following force increase (i2). This correlation was maximal on EEG electrodes contralateral to the limb exerting force, suggesting a direct effect of the vertex potential on cortical motor regions. Together, these results disclose a cortico-motor mechanism supporting adaptive behavior in response to salient sensory events. The striking similarity between these results and those previously reported in humans indicates that this mechanism is well conserved in human and non-human primates.

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CORTICAL PYRAMIDAL CELLS EXPRESS THYROID HORMONE TRANSPORTERS MCT8 AND OATP1C1 IN HUMAN AND MONKEY BRAIN.

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Monocarboxylate transporter 8 (MCT8) and organic anion-transporting polypeptide 1C1 (OATP1C1) are thyroid hormone (TH) transmembrane transporters that play a crucial role in the availability of systemic THs for neural cells allowing their appropriate development and function. This study aims to analyze the presence of these two proteins at the cellular level in the monkey and human cerebral cortex by means of immunohistochemistry and double labeling immunofluorescence. OATP1C1 is expressed in pyramidal neurons in layers II, III, V, and VI in both monkey and human motor cortex, as evidenced by its colocalization with RC3/Neurogranin and SMI-32. MCT8 distribution is similar to OATP1C1, although the intensity of its signal is lower.Interestingly, Cajal-Retzius like cells expressing OATP1C1 are observed in layer I. Double labeling immunofluorescence for OATP1C1 or MCT8 and several cortical interneuron markers was negative, confirming the presence of those TH transporters mostly in projection pyramidal neurons. OATP1C1 and MCT8 immunostained cells with small soma and large processes compatible with astrocyte morphology were found in the subcortical white matter in close relation to vessels. MCT8 is also expressed in the endothelial cells of different size vessels and capillaries throughout the monkey and human cortex, while OATP1C1 is occasionally observed in the endothelium of large and medium-sized vessels. Our results demonstrate the presence of MCT8 and OATP1C1 TH transporters in long and short projection pyramidal cortical neurons in adult human and non-human primates and their abundance in the astrocyte-vessel complexes, which suggest their critical position in the efferent cortical motor system.

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CHARACTERIZING PATTERNS OF DENDRITIC ACTIVITY IN AWAKE BEHAVING MICE USING ARBOREAL SCANNING, A MULTISCALE 3D IMAGING APPROACH.

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Understanding brain function requires to identify how information is represented by single neurons and how it is transformed as it flows through microcircuits. Deciphering when and where synaptic inputs are integrated across 3D dendritic trees requires recordings of patterns of activity across many dendritic branches. Non-linear acousto-optic lens (nAOL) 2-photon microscope enables this through fast focusing and selective imaging of regions of interest distributed within the imaging volume. Non-linear AOL functionality enables the selective imaging of dendritic arbors in any arbitrary X-Y-Z direction, a method we named arboreal scanning. Combining this with our recently developed real-time 3D movement correction and semi-automated 3D dendritic tracing has enabled imaging of a large fraction of the dendritic tree in vivo. We carried out arboreal scans on layer II, III, and V pyramidal cells expressing GCaMP6f in motor cortex in head-fixed mice as they rested or ran on a treadmill, during air puff-evoked startles, and during whisker stimulation. Our results indicate that layer II, III, and V cells exhibit mostly global, multi-branch activation that is strongly coupled to the soma but present a large heterogeneity on how these events are modulated. We used an unsupervised dimensionality reduction approach to identify regions that were comodulated and studied their relationship to spine activity patterns and behavioral variables. Our results should extend our understanding of dendritic integration in pyramidal cells in motor cortex during behaviour.

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EXPERIENCE WITH TOOL USE MODULATES VISUOMOTOR NEURONAL RESPONSE TO TOOL ACTION OBSERVATION IN MONKEY MOTOR AND PREMOTOR CORTEX

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Neurophysiological experiments on tool use in macaque monkeys have shown that visual exposure to tools and sensorimotor training aimed at using tools are linked with changes in the visual responses of neurons in premotor cortex. However, it is not clear to what extent these changes are a consequence of visual exposure or motor learning. In this study, we assessed the changes in the visual discharge of primary and premotor neurons following visual exposure to actions made with a tool, and following a specific sensorimotor training where the monkeys gradually learned to use a tool. We recorded from microelectrode arrays chronically implanted in the hand sectors of the ventral premotor and primary cortices. In the first weeks or recordings, the monkeys had to observe the experimenter using the rake, but never experienced using it themselves. Later, in separate sessions, the monkeys were trained to use the rake. Our preliminary results show systematic changes in neuronal activity over the weeks of training to use a rake. In some recorded channels, the time-locked multi-unit activity evoked by the observation of the rake retrieving the object increased over the weeks as the monkey became better at using the tool. Interestingly, the bulk of these changes seems to appear when the monkey had reached a proficient level of tool use. These preliminary findings suggest that motor experience, as opposed to mere visual familiarization, plays an important role in scaffolding the neuronal changes necessary to form new motor representations that can be activated during action observation.

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SIMULTANEOUS YET SEPARABLE POPULATION ENCODING OF ARM MOVEMENT DIRECTION AND KINEMATICS IN MOTOR CORTEX

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Motor cortex has been proposed to control multiple features of movement. However, the encoding of movement features has been commonly studied by analysing one feature at a time. Little is known about if and how multiple features of movement are simultaneously encoded at a population level. Using neural activity from dorsal premotor cortex (PMd) and motor cortex (M1) as monkeys performed a sequential arm movement task, here we report that the direction and kinematics of arm movements are simultaneously but separably encoded in the low-dimensional trajectories of population activity [1]. We found that strongly stereotyped, rotational trajectories of population activity encoded movement direction, such that trajectories separate from each other in proportion to the angle difference between the movements they evoked. By contrast, trajectories from arm movements in the same direction were temporally scaled to produce different movement kinematics. Consistent with this separability into geometry and speed of population activity, we could well decode the direction and duration of arm movement independently from the same trajectory of population activity. A recurrent neural network (RNN) model of our results replicated this separable encoding of direction and duration, and suggested the two could be independently controlled by respectively rotating the inputs to motor cortex and scaling the effective neuron time constant within motor cortex. Our results propose a mechanism for motor cortex to latently encode multiple arm movement features simultaneously. [1] Perich et al., (2018); *Extracellular neural recordings from macaque primary and dorsal premotor motor cortex during a sequential reaching task.* CRCNS.org.

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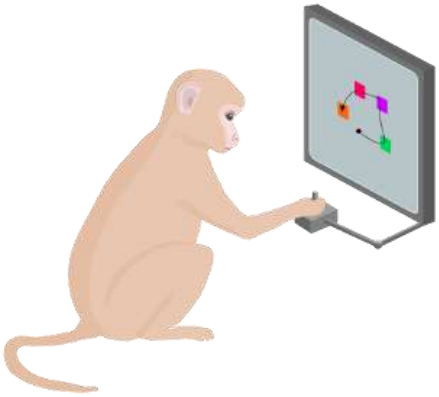
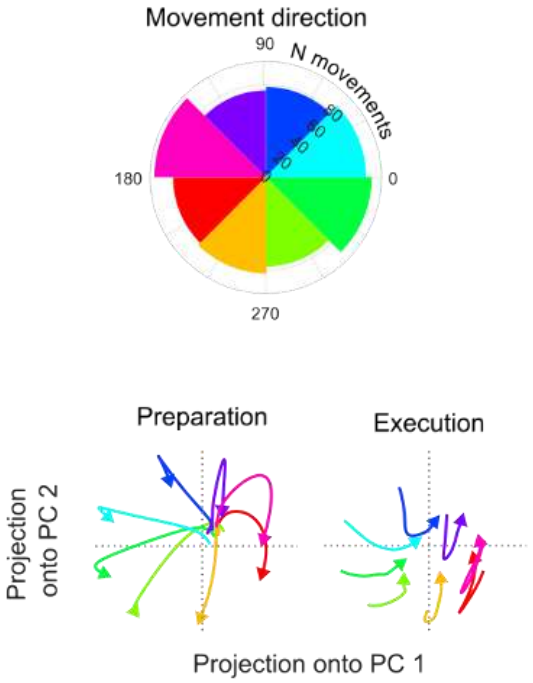
http://dx.doi.org/10.6080/K0FT8J72

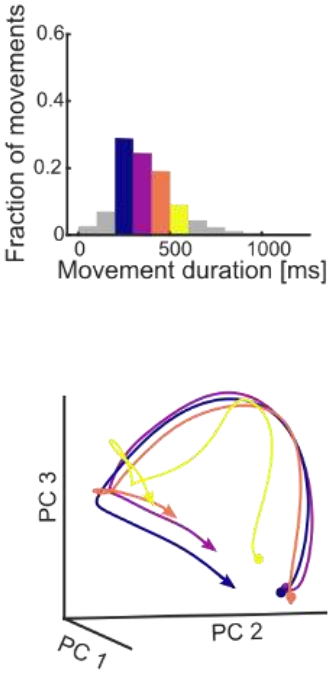
A



B Movement direction changes the trajectory's geometry



C Movement duration changes how trajectory unfolds



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BOARD NUMBER: S07-336

DECODING OF A BROAD SPECTRUM OF ACTIONS FROM TEMPORAL EVOLUTION OF NEURAL ACTIVITY IN MONKEY PREMOTOR CORTEX

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Decoding motor intentions for performing desired movements and increasing the sense of embodiment of an artificial effector is one of the main challenges of neuroprosthesis design. Here, we aimed to decode a broad range of motor actions from intracortical signals recorded from chronic multielectrode arrays implanted in the monkey ventral premotor cortex. Two monkeys (*Macaca mulatta*) were filmed while performing different reaching-grasping and mouth actions. Ethological scoring of the videos was performed to obtain the timestamps of behavioural events. We adopted a novel decoding algorithm, based on - *The selection of the most informative minimal set of neurons*. Different approaches to determine the set were considered, from simple inter-group comparison to feature selection algorithms; - *A support vector machine analysis of the temporal evolution* of the activity of the selected population. For each neuronal unit we took into account the firing activity over several temporally overlapping windows. The proposed approach was able to decode 6 different behaviors – performance was improved grouping them in classes: grasping, food touching the mouth, and baseline, in which no movements are performed. According to offline tests, the decoding algorithm successfully classifies the planning phase of the mentioned classes 100-200ms in advance. Additionally, the use of different feature selection techniques does not affect the decoder performance in terms of accuracy. Ultimately, the decoding algorithm predicts monkeys' movements in restrained laboratory conditions and constitutes a promising approach for real time use in detecting motor intentions of freely-moving animals, a crucial step for future neuroprosthetic applications.

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BOARD NUMBER: S07-337

EXAMINATION OF BRAINSTEM V2A NEURONS' DIVERSITY IN MOTOR CONTROL

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Adaptive control of locomotion requires that information from multiple integrative brain centers are relayed to the executory circuits of the spinal cord. One of these relays are reticulospinal (RS) neurons, a highly intermingled population of the reticular formation whose axons project along the spinal cord. We previously showed that the V2a subpopulation of glutamatergic RS neurons relay orienting commands from the Superior Colliculus (SC) and comprise distinct subsets specialized by their projection to a specific spinal segment (Usseglio et al. 2020). Retrograde trans-synaptic tracings however showed that V2a RS neurons receive inputs from multiple brain areas aside the SC, including the deep cerebellar nuclei (DCN). This i) raises the possibility that V2a RS may also relay signals related to posture and/or motor learning; and ii) prompts investigating whether different upstream modalities target the same, or distinct, V2a populations. To answer these questions we are implementing a combination of viral tracings, activity interferences and imaging strategies. We first characterized the identity and spatial organization of DCN neurons upstream the V2a RS neurons. Furthermore, we now aim at manipulating V2a RS neurons defined by the origin of their inputs using retrograde and anterograde trans-synaptic viral vectors. Interestingly, we observe divergent functional outcomes when manipulating V2a neurons that receive inputs from the DCN or the SC. This suggests a possible participation of V2a RS neurons in a cerebello-reticulo-spinal pathway, and argues for the existence of parallel V2a descending tracts for relaying modality-specific signals to the spinal cord.

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BOARD NUMBER: S07-338

ANATOMICAL AND MOLECULAR ORGANIZATION OF THE SUBTHALAMIC AREA, INCLUDING THE SUBTHALAMIC AND PARA-SUBTHALAMIC NUCLEI (STN AND PSTN), IN RODENTS AND PRIMATES

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Aims: The subthalamic nucleus (STN) is crucial for normal motor, limbic and associative function. STN dysregulation has long been correlated with Parkinson´s disease, and an increasing number of brain disorders also show dysregulation of the STN. Consequently, high-frequency stimulation of the STN is increasing as therapy. However, the anatomical-functional organization of the STN and surrounding structures remains to solve, both to understand symptoms and results of treatment. The aim of our study is to use recent molecular data from mouse-based experiments of the STN and neighboring areas (including the para-STN (PSTN)) for assessment of the spatial organization within the same structures in the primate brain. Mouse, macaque and human subthalamic areas were compared. **Methods:** We recently implemented single-nuclei RNA sequencing (snRNASeq) of the mouse STN followed through with histological analysis of several cluster genes of interest. This led us to identify four distinct spatio-molecularly defined domains within the mouse STN. Further, molecular profiles that dissociated the STN from the adjoining PSTN were identified. Here, we took advantage of this recent knowledge from the mouse to address if the same spatio-molecular profiles and domains can be identified in the macaque and human STN area. Fluorescent *in situ* hybridization was implemented. **Results:** Preliminary data support the presence of three to four molecularly distinct domains within the primate STN and unique molecular profiles also within adjoining structures. **Conclusions:** Comparative anatomical and molecular mapping will enable deeper insight into the complex STN area.

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BOARD NUMBER: S07-339

COMPLEMENTARY CODING OF MOVEMENT, REWARD EXPECTATION AND OUTCOME IN THE CEREBELLUM AND BASAL GANGLIA

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Recent findings of reward signals in the cerebellum challenge the view that the cerebellum performs error-based learning whereas the basal ganglia (BG) are involved in reward-based learning. While cerebellar reward signals are similar to those in the BG, the comparison is problematic as prior studies probed activity in areas that control different behaviors and in different animals. Thus, we aimed to compare directly the coding of motor and reward signals in both areas. We recorded from eye-movements related areas in the BG and cerebellum of the same monkeys while manipulating eye-movements parameters and reward. We partitioned single neurons activity into reward, movement, and temporal components. We found that before reward delivery, the BG coded the expectation of reward, while the reward signals in the cerebellum were relatively small. In contrast during reward delivery, reward signals were more pronounced in the cerebellum than in the BG. We additionally compared the BG input structure (caudate) and output structure (SNpr). Reward expectation, time, and movement signals were sharper in the SNpr than in caudate. Conversely, cerebellar cortex local and output populations coded of these variables similarly and less than the SNpr. Our results suggest a division of labor: coding of reward expectation in the BG and reward outcome in the cerebellum. Additionally, we find that movement and reward expectation information is most prominent in the SNpr. This result is facilitated by the sharpening of information between local and output neurons in the BG and not the cerebellum.

Pubmed:
31661073: Larry N, Yarkoni M, Lixenberg A, Joshua M
Cerebellar climbing fibers encode expected reward size.
Climbing fiber inputs to the cerebellum encode error signals that instruct learning. Recently, evidence has accumulated to suggest that the cerebellum is also involved in the processing of reward. To study how rewarding events are encoded, we recorded the activity of climbing fibers when monkeys were engaged in an eye movement task. At the beginning of each trial, the monkeys were cued to the size of the reward that would be delivered upon successful completion of the trial. Climbing fiber activity increased when the monkeys were presented with a cue indicating a large reward, but not a small reward. Reward size did not modulate activity at reward delivery or during eye movements. Comparison between climbing fiber and simple spike activity indicated different interactions for coding of movement and reward. These results indicate that climbing fibers encode the expected reward size and suggest a general role of the cerebellum in associative learning beyond error correction.
Elife, 2019; 8

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BOARD NUMBER: S07-340

COMPLEX DYNAMICAL CODING OF SIMPLE MOVEMENTS IN THE OUTPUT OF THE BASAL GANGLIA

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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The Substantia Nigra pars Reticulata (SNr), an output structure of the basal ganglia, is known to play a role in pursuit and saccadic eye movements. Previous studies have reported that neurons in the SNr are active tonically and show either a pause or increase during eye movements, consistent with the gating role of the basal ganglia output. These studies have mostly focused on probing SNr activity in a narrow behavioral regime. We recorded activity in the SNr of monkeys while they performed a broad regime of behavioral eye movement tasks including saccade and pursuit eye movements, with multiple directions and reward conditions. This diverse set of tasks was designed to provide a better understanding of the output of the basal ganglia. We found complex dynamical coding in SNr neurons during smooth pursuit eye movements. These neurons exhibited a highly complex reaction pattern during pursuit, including frequent increases and decreases in the firing rate during the trial, uncorrelated responses in different directions and high variance in reaction times and reaction durations. We also compared these results to other brain regions in the eye movement pathway. The results suggest that SNr neurons have a specific high-dimensional dynamic which differentiates them from other regions in the eye movement pathway. Previous research has suggested that the output of the basal ganglia presents a simple mapping to behavior. Our results strongly hint at a dynamical and complex mapping between sensorimotor parameters and activity, akin to intermediate levels in artificial neural networks.

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BOARD NUMBER: S07-341

A MOLECULAR MAP OF THE LEARNING STRIATUM

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Efficient every day skills rely on automatized behaviours. As behaviours are learnt and repeated, the link between the action and its context incrementally increases until automaticity. Key players involved in this process of learning and consolidation of an action are cortico-basal ganglia loops (CBGs). CBGs are topographically organized into limbic, associative and sensorimotor loops coursing through the ventral, dorsomedial, and dorsolateral striatum, respectively. These circuits are recruited to different extents during learning; however, the dynamics of the region-specific molecular events have never been assessed during learning and consolidation of a skill. **Aims:** Detection of region-specific striatal molecular key players of skill learning and consolidation. **Methods:** We have developed a binary visual discrimination task in fully automated operant conditioning chambers. At different learning stages (naïve , early, intermediate, and over-trained), we collected brain samples from the ventral, dorsomedial, and dorsolateral striatum of wild-type mice, and performed bulk RNA-sequencing using the SCRB-seq protocol. **Results:** Molecular changes of early learning distinguish themselves from changes characteristic for learning consolidation or automatization. While gene categories involved in early learning include brain plasticity and development, those of later learning stages appear more related to long-term plastic changes including long-term synaptic potentiation and dendritic spine maintenance. Throughout learning, we could also find differentially expressed genes related to dopamine signaling and synthesis of different types of neurotransmitters, among others. **Conclusions:** Striatal molecular signatures of early learning and automatization are complementary and changes in expression patterns align with expected early or long-term plastic changes, respectively.

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BOARD NUMBER: S07-342

INPUTS GOVERNING THE RESPONSES TO SENSORY STIMULATION IN THE EXTERNAL GLOBUS PALLIDUS

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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The external globus pallidus (GPe) is a central nucleus in the basal ganglia (BG) integrating information from the direct, the indirect and the hyperdirect BG pathways. The GPe is composed of two main cell populations, the prototypic and arkypallidal cells, which differ in their membrane properties, developmental origin, molecular markers, and axonal projections. The inputs to these two GPe subpopulations have recently been described using optogenetic manipulations, however, how they are integrated in a physiological process has not been fully addressed. Both GPe subpopulations respond to whisker deflection with a triphasic response pattern, suggesting the involvement of multiple synaptic inputs. Here, I used in vivo whole cell patch clamp recordings in the GPe combined with optogenetic silencing to dissect the different inputs underlying sensory responses in prototypic and arkypallidal cells. My results show that sensory responses in prototypic cells are controlled by the subthalamic nucleus (STN) and indirect pathway medium spiny neurons (MSNs), while responses in arkypallidal cells are shaped by direct pathway MSNs, and to a lesser extent by the STN and neighbouring prototypic cells. These results suggest that the responses to whisker stimulation in the GPe subpopulations are shaped by different synaptic inputs, affecting their outputs to downstream as well as upstream BG targets.

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BOARD NUMBER: S07-343

WHAT DO MC4R-EXPRESSING NEURONS IN THE LATERAL STRIPE OF THE STRIATUM ENCODE?

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Center for Social and Affective Neuroscience (CSAN), Department Of Biomedical And Clinical Sciences (bkv), Linköping, Sweden

Emerging evidence has implicated melanocortin 4 receptors (MC4R) in the attribution of motivational valence to salient stimuli. Our group has shown that striatal MC4Rs under normal conditions hinder aversive signals from getting access to dopaminergic reward circuits, and that mice with deletion of MC4Rs consequently perceive aversive stimuli as rewarding. The Lateral Stripe of the Striatum (LSS) is an area in the ventral striatum that presents a high expression of MC4Rs, but the function of the receptor in this region remains unknown. Our objective was to characterize the MC4R-expressing neurons in the LSS, to elucidate if their activation is rewarding or aversive, and to analyze their activity during aversive events. This was done by using MC4R-cre mice, optogenetic stimulation, fiber photometry, and several behavioral paradigms. Using immunofluorescence, we found that MC4R expressing neurons are mostly medium spiny neurons that co-express the dopamine D1 receptor. Optogenetic activation of MC4R expressing neurons in the the LSS enhanced motivational drive and induced robust place preference, as well as increased the locomotion. Conversely, in vivo calcium imaging revealed that MC4R-expressing neurons in the LSS increased their firing when the animals were exposed to electric foot shock, indicating that these neurons are activated by aversive stimuli. Collectively, our findings indicate that MC4R-expressing neurons in the LSS are involved in both reward-related and aversive signaling.

Pubmed:
33338550: Schumacher R, Rossetti MF, Lazzarino GP, Canesini G, García AP, Stoker C, Andreoli MF, Ramos JG
Temporary effects of neonatal overfeeding on homeostatic control of food intake involve alterations in POMC promoter methylation in male rats.
A small litter (SL) model was used to determine how neonatal overfeeding affects the homeostatic control of food intake in male rats at weaning and postnatal day (PND) 90. At PND4, litters were reduced to small (4 pups/dam) or normal (10 pups/dam) litters. At weaning, SL rats showed higher body weight and characteristic features of the metabolic syndrome. Gene expression of pro-opiomelanocortin (POMC), cocaine and amphetamine regulated transcript, neuropeptide Y (NPY) and leptin and ghrelin (GHSR) receptors were increased and POMC promoter was hypomethylated in arcuate nucleus, indicating that the early development of obesity may involve the GHSR/NPY system and changes in POMC methylation state. At PND90, body weight, metabolic parameters and gene expression were restored; however, POMC methylation state remained altered. This work provides insight into the effects of neonatal overfeeding, showing the importance of developmental plasticity in restoring early changes in central pathways involved in metabolic programming.
Mol Cell Endocrinol, 2021; 522
32182451: Petković F, Lazzarino GP, Engblom D, Blomqvist A
IL-6R expressed on CNS vascular endothelial cells contributes to the development of experimental autoimmune encephalomyelitis in mice.
Experimental autoimmune encephalomyelitis (EAE) is the most common model for studying the molecular mechanisms of multiple sclerosis (MS). Here, we examined the CNS-restricted effects of classical interleukin (IL)-6 signaling on the development of EAE, using mice with cell-type specific deletion of the IL-6 receptor (IL-6R). We found that IL-6R deletion in CNS vascular endothelial cells, but not in microglia, ameliorated symptoms of EAE. The milder clinical symptoms in the gene-deleted mice were associated with less demyelination and immune cell infiltration/activation, and lower mRNA levels of the cytokines IL-17 and IL-1β, as well as the cell adhesion molecules VCAM-1, ICAM-1 and ICAM-2 than what was seen in WT mice. These findings demonstrate that classical IL-6 signaling via endothelial cells of the CNS contributes substantially to the development of MS-like pathology, which should be taken into consideration when conceptualizing future therapeutic approaches.
J Neuroimmunol, 2020; 342
31682820: Rossetti MF, Schumacher R, Gastiazoro MP, Lazzarino GP, Andreoli MF, Stoker C, Varayoud J, Ramos JG

Epigenetic Dysregulation of Dopaminergic System by Maternal Cafeteria Diet During Early Postnatal Development.
Dopamine is a neurotransmitter crucial for motor, motivational, and reward-related functions. Our aim was to determine the effect of a palatable maternal diet on the transcriptional regulation of dopaminergic-related genes during perinatal development of rat offspring. For that, female offspring from dams fed with a control (CON) or a cafeteria (CAF) diet were sacrificed on embryonic day 21 (E21) and postnatal day 10 (PND10). Using micropunch techniques, ventral tegmental area (VTA) and nucleus accumbens (NAc) were isolated from brain's offspring. Bioinformatic analysis of the promoter regions, mRNA quantification and methylation studies were done. The increase in tyroxine hidroxylase (TH), dopamine receptor (DRD) 1 and ghrelin receptor (GHSR) expression in VTA and NAc from E21 to PND10 was correlated with changes in DNA methylation of their promoter regions. Maternal diet did not affect the expressionpatternsin E21. At PND10, maternal CAF diet decreased the transcription of TH, GHSR, DRD2 and dopamine transporter (DAT) in VTA. Interestingly, the changes in TH, DRD2 and DAT expression were related to the methylation status of their promoters. In NAc, maternal CAF diet reduced DRD1, DRD2 and DAT expression in the offspring at PND10, although alternations in the methylation patterns were only detected in DAT promoter. These results show the importance of maternal nutrition and provide novel insights into the mechanisms through which maternal junk-food feeding can affect reward system during development and early postnatal life. Particularly important is the expression decline of DRD2 given its physiological implication in obesity and addiction.
Neuroscience, 2020; 424
31430504: Lazzarino GP, Acutain MF, Canesini G, Andreoli MF, Ramos JG
Cafeteria diet induces progressive changes in hypothalamic mechanisms involved in food intake control at different feeding periods in female rats.
We studied the effects of cafeteria diet (CAF) intake from weaning on mRNA levels and DNA methylation state of feeding-related neuropeptides and hormone receptors in individual hypothalamic nuclei at different feeding periods. Four weeks of CAF (short-term) increased energy intake and adiposity, without affecting neuropeptides' expression. Eleven weeks of CAF (medium-term) increased energy intake, adiposity, leptinemia, and body weight, with an orexigenic response of the lateral hypothalamus, paraventricular and ventromedial nuclei, given by upregulation of Orexins, AgRP, and NPY opposed by an anorectic signal of the arcuate nucleus, which displayed a higher POMC expression. The changes in neuropeptidic mRNA levels were related to epigenetic modifications in their promoter regions. Metabolic and molecular changes were intensified after 20 weeks of diet (long-term). The alterations in these hypothalamic brain nuclei could add information about their differential role in food intake control, and how their action is disrupted during the development of obesity.
Mol Cell Endocrinol, 2019; 498
30721712: Rossetti MF, Schumacher R, Lazzarino GP, Gomez AL, Varayoud J, Ramos JG
The impact of sensory and motor enrichment on the epigenetic control of steroidogenic-related genes in rat hippocampus.
In the present study, we analyzed the effects of a short-term environmental enrichment on the mRNA expression and DNA methylation of steroidogenic enzymes in the hippocampus. Thus, young adult (80-day-old) and middle-aged (350-day-old) Wistar female rats were exposed to sensory (SE) or motor (ME) enrichment during 10 days and compared to animals housed under standard conditions. SE was provided by an assortment of objects that included plastic tubes and toys; for ME, rodent wheels were provided. In young adult animals, SE and ME increased the mRNA expression of cytochrome P450 17α-hydroxylase/c17,20-lyase, steroid 5α-reductase type 1 (5αR-1) and 3α-hydroxysteroid dehydrogenase and decreased the methylation levels of 5αR-1 gene. In middle-aged rats, ME and SE upregulated the gene expression of aldosterone synthase and decreased the methylation state of its promoter. These results propose that SE and ME differentially regulate the transcription of neurosteroidogenic enzymes through epigenetic mechanisms in young and aged rats.
Mol Cell Endocrinol, 2019; 485
29908751: Gastiazoro MP, Guerrero-Schimpf M, Durando M, Lazzarino GP, Andreoli MF, Zierau O, Luque EH, Ramos JG, Varayoud J
Induction of uterine hyperplasia after cafeteria diet exposure.
Our aim was to evaluate whether chronic administration of CAF affects the uterus and induces the morphological and molecular changes associated with endometrial hyperplasia. Female Wistar rats exposed to CAF from weaning for 20 weeks displayed increased energy intake, body weight and fat depots, but did not develop metabolic syndrome. The adult uteri showed an increase in glandular volume fraction and stromal area. The epithelial proliferation rate and protein expression of oestrogen receptor alpha (ERα) were also increased. The CAF diet enhanced leptin serum levels and the long form of leptin receptor (Ob-Rb) mRNA expression in the uterus. No changes were detected in either insulin serum levels or those of insulin growth factor I (IGF-I) mRNA expression. However the levels of IGF-I receptor (IGF-IR) mRNA were lower in CAF-fed animals. Overall, the results indicate that our rat model of the CAF diet produces morphological and molecular changes associated with uterine hyperplasia and could predispose to endometrial carcinogenesis.
Mol Cell Endocrinol, 2018; 477
28479374: Lazzarino GP, Andreoli MF, Rossetti MF, Stoker C, Tschopp MV, Luque EH, Ramos JG

Cafeteria diet differentially alters the expression of feeding-related genes through DNA methylation mechanisms in individual hypothalamic nuclei.
We evaluated the effect of cafeteria diet (CAF) on the mRNA levels and DNA methylation state of feeding-related neuropeptides, and neurosteroidogenic enzymes in discrete hypothalamic nuclei. Besides, the expression of steroid hormone receptors was analyzed. Female rats fed with CAF from weaning increased their energy intake, body weight, and fat depots, but did not develop metabolic syndrome. The increase in energy intake was related to an orexigenic signal of paraventricular (PVN) and ventromedial (VMN) nuclei, given principally by upregulation of AgRP and NPY. This was mildly counteracted by the arcuate nucleus, with decreased AgRP expression and increased POMC and kisspeptin expression. CAF altered the transcription of neurosteroidogenic enzymes in PVN and VMN, and epigenetic mechanisms associated with differential promoter methylation were involved. The changes observed in the hypothalamic nuclei studied could add information about their differential role in food intake control and how their action is disrupted in obesity.
Mol Cell Endocrinol, 2017; 450
27040308: Rossetti MF, Varayoud J, Lazzarino GP, Luque EH, Ramos JG
Pregnancy and lactation differentially modify the transcriptional regulation of steroidogenic enzymes through DNA methylation mechanisms in the hippocampus of aged rats.
In the present study, we examined the mRNA expression and DNA methylation state of steroidogenic enzymes in the hippocampus of young adult (90-days-old) and middle-aged (450-days-old) nulliparous rats, and middle-aged multiparous rats subjected to three pregnancies with and without lactation. Aging decreased the mRNA levels of steroidogenic-related genes, while pregnancy and lactation significantly reduced the effect of aging, maintaining high expression levels of cytochrome P450 side-chain cleavage (P450scc), steroid 5α-reductase-1 (5αR-1), cytochrome P450arom (P450arom) and aldosterone synthase (P450(11β)-2). In addition, pregnancy and lactation diminished the methylation state of the 5αR-1 promoter and increased the transcription of brain-derived neurotrophic factor, synaptophysin and spinophilin. Pregnancy without lactation increased P450scc and 5αR-1 gene expression and decreased the methylation of their promoters. We concluded that the age-related decrease in the mRNA expression of steroidogenic enzymes is differentially attenuated by pregnancy and lactation in the rat hippocampus and that differential methylation mechanisms could be involved.
Mol Cell Endocrinol, 2016; 429
27469930: Andreoli MF, Stoker C, Lazzarino GP, Canesini G, Luque EH, Ramos JG
Dietary whey reduces energy intake and alters hypothalamic gene expression in obese phyto-oestrogen-deprived male rats.
Removing dietary phyto-oestrogens in adult male rats causes obesity and diabetes. As whey proteins have been reported to reduce food intake and improve glucose homoeostasis, we investigated whether they could attenuate susceptibility to obesity and diabetes due to phyto-oestrogen deprivation. To this end, thirty male Wistar rats were fed a high-phyto-oestrogen (HP) or a phyto-oestrogen-free (PF) diet for 10 weeks; six rats from each group were killed. The remaining HP animals (six animals) continued receiving the HP diet for 6 weeks. The remaining PF rats (twelve rats) were divided in two groups: one was given the PF diet and the other a variation of the PF diet plus whey protein (PF-W). Body weight, food intake and adipose tissue weights were recorded. Hypothalamic mRNA expressions of orexigenic (neuropeptide Y, agouti-related protein (AgRP)) and anorexigenic (pro-opiomelanocortin (POMC), cocaine-amphetamine-related transcript (CART)) neuropeptides were quantified by real-time PCR. Serum glucose, insulin and total thyroxine (T4), thyroid-stimulating hormone, testosterone and oestradiol were assessed. After 10 weeks of PF diet, increased body weight, adiposity and energy intake, with up-regulation of AgRP and down-regulation of POMC', were observed. Longer treatment exacerbated these results, increased total T4 levels, reduced oestradiol levels and impaired glucose homoeostasis. PF-W reduced energy intake and increased POMC expression; however, body weight and adiposity remained unchanged. PF-W could not prevent the hormonal changes or the high circulating glucose levels induced by phyto-oestrogen deprivation, but reduced fasting insulin. These data demonstrate that, although 6 weeks of whey administration could not prevent obesity in phyto-oestrogen-deprived rats, the reduction in energy intake and circulating insulin could be beneficial with longer treatments.
Br J Nutr, 2016; 116
27632921: Andreoli MF, Stoker C, Rossetti MF, Lazzarino GP, Luque EH, Ramos JG
Dietary withdrawal of phytoestrogens resulted in higher gene expression of 3-beta-HSD and ARO but lower 5-alpha-R-1 in male rats.
Removing dietary phytoestrogens causes obesity and diabetes in adult male rats. Based on the facts that hypothalamic food intake control is disrupted in phytoestrogen-deprived animals and that several steroids affect food intake, we hypothesized that phytoestrogen withdrawal alters the expression of hypothalamic steroidogenic enzymes. Male Wistar rats fed with a high-phytoestrogen diet from conception to adulthood were subjected to phytoestrogen withdrawal by feeding them a low-phytoestrogen diet or a high-phytoestrogen, high-fat diet. Withdrawal of dietary phytoestrogens increased 3β-hydroxysteroid dehydrogenase and P450 aromatase gene expression and decreased those of 5α-reductase-1. This is a direct effect of the lack of dietary phytoestrogens and not a consequence of obesity, as it was not observed in high-fat-fed rats. Phytoestrogen

withdrawal and high-fat diet intake reduced hypothalamic expression of estrogen receptor (ER)α correlated with low levels of ERα-O, ERα-OS, and ERα-OT transcripts. Variations in gene expression of steroidogenic enzymes may affect the content of neurosteroids. As neurosteroids are related to food intake control, the changes observed may be a novel mechanism in the regulation of energy balance in obese phytoestrogen-deprived animals. In rats, steroidogenesis and ER signaling appear to be altered by phytoestrogen withdrawal in the rat. The ubiquity of phytoestrogens in the diet and changing intakes or withdrawal suggest that aspects of human health could be affected based on the rat and warrant further research.
Nutr Res, 2016; 36

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BOARD NUMBER: S07-344

ZINC MODULATES FRONTO-STRIATAL GLUTAMATERGIC TRANSMISSION AND IMPLEMENTATION OF PROACTIVE INHIBITORY CONTROL THROUGH HIGH-AFFINITY BINDING AT NMDA RECEPTOR GLUN2A SUBUNIT

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Fronto-striatal system mediates various aspects of executive functions that support implementation of adaptive goal-directed behaviors. Striatum receives dense zinc-containing glutamatergic projections from prefrontal cortex, but the contribution of synaptic Zn^{2+} to behavioral functions subserved by fronto-striatal system remains elusive. We used knock-in (KI) mice lacking a high-affinity Zn^{2+} inhibition of GluN2A containing NMDA receptors (GluN2A-NMDARs) to examine whether Zn^{2+} modulation of goal-directed learning and inhibitory control processes involves glutamatergic mechanisms. Electrophysiological recording performed in the nucleus accumbens, the striatal region containing the highest synaptic zinc density, revealed enhanced NMDAR-mediated synaptic transmission in KI mice upon stimulation of prefrontal cortex inputs. When tested in simple appetitive operant learning tasks, KI mice and wild type (WT) littermates performed similarly. In a more demanding task designed for assessing proactive inhibitory control, KI mice acquired the new rules quicker than WT mice and displayed improved motor readiness, reflected by a faster reaction time upon the offset of the inhibitory cue. Short-term spatial memory assessed in the Y-maze task was also improved in KI mice compared to WT animals indicating that ablation of GluN2A zinc binding site improves mnemonic functions. Collectively, these findings show that synaptic Zn^{2+} acts as an endogenous modulator of fronto-striatal glutamatergic transmission and provide *in vivo* evidence that this cation negatively regulates mnemonic processing and goal-directed control by dampening GluN2A-NMDAR activity.

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BOARD NUMBER: S07-345

LINE-1 LONG NON-CODING RNAS AT STRIATAL CIRCUITS CONTRIBUTE TO THE TRANSITION FROM FLEXIBLE TO INFLEXIBLE BEHAVIORAL CONTROL

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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The ability to adapt behavior to an ever-changing environment requires flexible control of behavior, which depends on the causal relationship between an action and its outcome (A-O). With repetition, behavior becomes inflexible; actions are no longer sensitive to changes in A-O associations and are primarily elicited by retrospective events. The epigenetic modification of chromatin offers a mechanistic link between the genome and environmental experience, including the transition from flexible to inflexible behavioral control. In particular, epigenetic modifications can unsilence the expression of transposable elements, which in turn can act as a key factor for post-transcriptional regulation of gene expression. In this study, we demonstrated that task overtraining is associated with the increase of RNA levels of Long Interspersed Nuclear Elements -1 (L1), specifically in the dorsolateral striatum (DLS). Upregulation of L1 RNAs expression occurs in parallel to decreased methylation of L1 promoters and reduced expression of the DNA methyltransferase 3b. Finally, viral-mediated silencing of L1 transcripts in the DLS preserves behavioral flexibility, thus establishing a direct role of L1 upregulation in behavioral control. We are currently investigating mechanisms by which L1 RNAs interfere with bioavailability of key mRNA targets involved in synaptic processes that subserve the updating of behavioral strategy during the presentation of a new A-O contingency. Our findings support the role of (epi)genomic plasticity in instrumental behavior. This study reveals molecular substrates that might be relevant for the numerous neuropsychiatric conditions that are characterized by a loss in behavioral flexibility and striatal circuit dysfunction.

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BOARD NUMBER: S07-346

PHOSPHODIESTERASES AS INTEGRATORS OF THE DOPAMINE SIGNAL IN THE STRIATUM

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Aims – The cAMP/PKA signaling pathway plays a critical role in the integration of dopaminergic signals in the striatum. Phosphodiesterases degrade cAMP, and medium-sized spiny neurons express PDE1B, activated by calcium-calmodulin, PDE2A, activated by cGMP, PDE4 and PDE10A. We wanted to determine the functional contribution of each of these phosphodiesterase in the integration of dopamine signals at the level of cAMP.
Methods – Genetically-encoded FRET biosensors were used to monitor the dynamics of cAMP/PKA signaling in MSNs in striatal brain slices from young mice: Epac-SH150 for cAMP, and AKAR4 for PKA-dependent phosphorylation. Highly selective inhibitors were used to acutely suppress the activity of each phosphodiesterase type.
Results – After a positive cAMP signal, PDE10A appears as the only PDE able to decrease cAMP concentration below micromolar level, and its activity is therefore required to deactivate PKA. PDE2A and PDE4 operate on cAMP concentration above micromolar, thus regulating the peak of dopamine D1 response, or the steady-state adenosine A2A response. PDE2A activity is increased by cGMP. PDE1B also controls high cAMP levels, and is active only after intracellular calcium has been increased.
Conclusion – PDE action is thus functionally defined by a cAMP concentration range on which it operates, with PDE10A characterized by its action on low cAMP levels. In addition, PDE1B and PDE2A allow cross-talks with other signaling pathways such as calcium and NO-cGMP. This mechanistic understanding helps define the potential of each phosphodiesterase as a potential therapeutic target.

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AN ATTEMPT TO ENHANCE FINE MOTOR PERFORMANCE: INVESTIGATING IMMEDIATE AND DELAYED EFFECTS OF NEUROFEEDBACK TRAINING AND MOTOR IMAGERY TRAINING ON SEQUENTIAL FINGER TAPPING TASK

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Background Various training approaches are being proposed to enhance motor performance with debatable results. It has been established that Motor Imagery (MI) generates activation of motor cortical areas (MA) with proven beneficial effects on motor performance. It has also been shown possible to self-modulate neural activity in MA through non-invasive EEG-NeuroFeedback (NF), but it remains unclear whether increase in cortical activation of MA via Event-Related Desynchronization (ERD) of sensorimotor rhythm brainwave would have beneficial motor outcomes. **Aim** Investigate immediate and delayed effects of a single NF training session on motor performance in comparison to MI and to MI+NF trainings. **Methods** Sixty-eight right-handed healthy adults were randomly assigned to one of four groups (a)NF (n=17), (b)MI (n=17), (c)NF+MI (n=18) and (d)Control (n=16). Participants performed a sequential finger tapping task (SFTT) before and after (immediately, 20-min and 24hr) a single 30-minute training session. Movement accuracy, speed and ERD data were analyzed. **Preliminary Results** Current data reveal beneficial effects of NF, MI and NF+MI trainings on accuracy ($p=0.02$) and speed accuracy trade-off ($p=0.02$) only when participants showed good baseline SFTT performance level. MI training effects were observed at all re-test phases, while NF and NF+MI trainings were only beneficial at 24-hr ($p<0.05$), with potential benefits after 20-min. ERD data are under analysis. **Conclusions** Data herein suggest that NF training (alone or combined to MI) can have delayed benefits on movement performance. Such protocols can contribute to the development of more effective methods for enhancing motor performance with applications in therapy and sports.

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INHIBITION AND GAIT INITIATION IN HEALTHY SUBJECTS : AN EEG STUDY

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Inhibition is the capacity to suppress one or several possible responses in order to produce adapted behavior. We distinguish reactive inhibition, which is triggered by a stimulus, and proactive inhibition, which consists in an anticipatory inhibitory state prior to any stimulus. Here, we study how those inhibitions are engaged during gait initiation in a Go-NoGo task, i.e. initiate gait or not depending a visual signal, but modified and including an additional Go-certain condition, where subject initiates gait at each trial. Here, we aim to assess the existence of such inhibitory processes for gait initiation, with longer reaction times (RT) during Go-Uncertain vs Go-Certain blocks with releasing of proactive inhibition for Go-Certain blocks, and its relationship with cortical EEG activity using high-density EEG recordings. Gait initiation parameters and cortical activity were recorded in 23 healthy subjects, using a force platform AMTI and an EEG-ActiCHamp 128 active electrode system. We found increased RT for gait initiation during uncertain relative to certain Go trials ($p < 10^{-4}$). We found also different cortical activities with modulations within the N2-P3 complex, an ERP previously considered as a reflect of inhibitory processes, and within the alpha (8-12Hz) and beta (13-30Hz) band activities, mainly in the fronto-central areas. These data suggest a proactive inhibition of gait initiation in human, in uncertain environmental context. The next step would be to examine this in pathological condition such as Parkinson's disease where patients present an inability to initiate gait that could reflect increased proactive inhibition.

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NEURONAL REPRESENTATIONS OF SELF AND OTHER IN THE BASAL GANGLIA

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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It is widely accepted that an extended cortical action observation network (AON) underlying the coding of other's actions largely overlaps with areas devoted to action planning and execution. Recent anatomical data demonstrated that most of the cortical areas belonging to the AON send convergent projection to overlapping territories of the putamen nucleus in the basal ganglia, suggesting that it may play a role in others' action processing. To address this issue, we recorded neuronal activity from the putamen nucleus of the macaque during a reaching-grasping task in which the animal and an experimenter, facing each other and taking turns based on contextual cues, grasped (or observed the other grasping) a multi-affordance object in a shared space. Most of the recorded neurons were modulated either only during action execution (self type, 20%) or observation (other type, 9%), or during both (self-other type, 49%), whereas others (22%) did not show any action-related modulation. Among self-other type neurons showing Facilitated (F) or Suppressed (S) discharge during action execution and observation, 41% could be classified as FF type, 19% as FS type, 11% as SF and 29% as SS type. Overall, facilitatory modulations prevailed during task execution, whereas they were balanced by suppressive modulatory influences during task observation. Our findings demonstrate the existence of neurons modulated by others' observed action in the putamen. These findings support the involvement of the putamen in the AON, and indicate the need to investigate its overall modulatory impact on the functioning of the AON cortical nodes.

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GAIN- AND LOSS-OF-FUNCTION MUTATIONS IN DOPAMINE RECEPTOR GENES CAUSE DISRUPTED CYCLIC AMP HOMEOSTASIS IN PATIENTS WITH COMPLEX HYPERKINETIC MOVEMENT DISORDERS

POSTER SESSION 07 - SECTION: BASAL GANGLIA & MOTOR CONTROL

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Aims: To identify the genetic aetiology and understand disease mechanisms in patients with undiagnosed complex hyperkinetic movement disorders **Methods:** Singleton and triome whole-genome sequencing and analysis was undertaken using the Illumina NovaSeq 6000 platform, Qiagen QCI Interpret Translational and Sanger sequencing. Site-directed mutagenesis was utilised to generate mutant constructs for overexpression in HEK293T cells. Gene and protein expression, localisation and function were assessed using qPCR, Western blotting, biotinylation, immunofluorescence and cyclic adenosine monophosphate (cAMP) assays. **Results:** In 5 patients, pathogenic monoallelic and biallelic variants were identified in three dopamine receptor genes: c.110C>A (p.Thr37Lys), homozygous in *DRD1*; c.1121 T > G (p.Met374Arg), heterozygous in *DRD2*; c.340G>A (p.Asp114Asn) and c.463C>T (p.Arg155Cys), heterozygous in *DRD5.* None of these variants impacted gene and protein expression or subcellular localisation. All mutant proteins however led to altered cAMP levels in response to dopaminergic agonists. For DRD1, p.Thr37Lys resulted in a significant reduction in cAMP levels ($p<0.0001$), indicating loss of function. In contrast, p.Met374Arg in DRD2 resulted in gain of function, with a significant decrease in cAMP ($p<0.01$). p.Arg155Cys and p.Asp114Asn in DRD5 also resulted in a significant increase in cAMP indicating gain of function ($p<0.001$). **Conclusion:** Our study identifies *DRD1* and *DRD5* as novel Mendelian disease-associated genes. Pathogenic variants in *DRD1, DRD2* and *DRD5* all result in aberrant protein function; the observed disruption of downstream cAMP signalling is likely to affect key corticostriatal neuronal networks involved in motor control. Better understanding of the underlying biochemical mechanisms may facilitate future development of targeted precision therapies.

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STUDYING GROUP BEHAVIOR IN THE WILD AS A NEW AVENUE FOR UNDERSTANDING SOCIAL BRAIN

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Until recently laboratory behavioral tasks were conducted without much consideration for the environmental or social context. Unfortunately, such an approach allows for researching neither voluntary responses nor individual differences. Importantly for neuroscience studies, the neural background underlying unnatural, artificial behavior is highly variable and hard to predict. Thus, to develop valid models of human disorders and resulting targeted, individualized therapies we need to test spontaneous behaviors consistently engaging well-defined, evolutionarily-conserved neuronal circuits. The presented field studies were performed in the field Eco-HAB, an ecologically relevant habitat located in the wild, however, spatially constrained environment. It consisted of 8 subterritories of differing survival values and resources. The subterritories were connected via underground corridors equipped with RFID antennas, allowing for the continuous, non-invasive recording of the social behavior in groups of mice over the 10-day-period. We show how testing animals in a complex, naturalistic habitat allows for measuring aspects of social behavior inaccessible under laboratory conditions. We examine the social interactions of mice and their stability over time, as well as the dispersal of the individuals within the habitat with high ecological accuracy. Moreover, the obtained data enables us to assess individual territoriality and social status, as measured by the access to high-value resources. We also present the data on social networks formed by the animals over time, as they explored the novel and challenging territory. The proposed approach allows for combining neuroscience research with field studies, enabling testing behavioral patterns truly relevant for surviving and thriving under varying environmental conditions.

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CRYPTOGRAPHIC-LIKE HIPPOCAMPAL MECHANISM UNDERLIES HIDING AND RETRIEVAL BEHAVIORS IN ANIMALS

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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The brain's extraordinary abilities are often associated with its ability to learn and to adapt. But memory has its limitations, especially when faced with the task of retrieving tens or even hundreds of thousands of cached food items annually - such as in the case of scatter-hoarding animals. Here, we present a brain mechanism that works by utilizing cryptographic principles in lieu of plasticity. Rather than memorizing their cache locations as previously suggested, we propose that cache-hoarding animals use a single cryptographic-like mechanism during both caching and retrieval. The model we developed is similar to hippocampal spatial cells, which respond to an animal's positional attention, such as when the animal enters a specific region (place-cells) or gazes at a particular location (spatial-view-cells). We know that the region that activates each spatial cell remains consistent across subsequent visits to the same area but not between areas. This remapping, combined with the uniqueness of cognitive maps, produces a persistent crypto-hash function for both food caching and retrieval. We further show that the model can maintain temporal information that might help animals prioritize food items that are perishable or according to their nutritional value. This behavior, which we refer to as crypto-taxis, can also explain how animals of the same species might find each other in order to mate or for other social purposes.

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APHASIE AS A PART OF DIFFéRENT NEUROLOGICAL DISORDERS: A CASE REPORT

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Objective: Aphasia syndromes have been mostly reported as a result of stroke syndromes. Often the assessment of language ability, plays a central role in the clinical evaluation of patients with suspected dementia. Its most relevant contribution are: the diagnosis of cognitive impairment, the differential diagnosis among different causes of dementia and a classification in the variety of APP which may have a probabilistic relation ship with different pathological substrate. The present case study analyses the cognitive impairment due to a acute AVC T- P sin. in a 54 year old right- handed patient with higher formal education. **Method:** The patient was tested on a broad range of cognitive abilities , including language, memory, praxis, number processing and calculation, visual spatial abilities, executive functions. **Results:** On neuropsychological examination, he presented a moderate verbal apraxia, noted by word rate not often running , essentially on not frequent words with lexical production rate and phonological/articulatory phonemic errors neither phonetic nor disarthric, accompanied with others clinical manifestations like: spatial acalculia, and moderate anarithmetic. However, not suspicion for a APP not trouble, not decrease of syntaxic formes or a DPD not anomie, neither pause nor low voice. **Conclusions** The present case study highlights the diversity of clinical manifestation of speech disorders. Additional observations of this syndrome being needed to appreciate the full spectrum of impairments in patients with neurodegenerative brain disease , including those often considered as “movements disorders” PNFA or Logopenic aphasia typically due to AD pathology or patients with DPD.

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BOARD NUMBER: S07-354

APHASIA AS A PART OF DIFFERENT NEUROLOGICAL DISORDERS: A CASE REPORT

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

Suzana Gjeci[1], Florian Dashi[1,2], Meri Papajani[1,2], Aida Quka[1], Ridvan Alimehmeti[1,2], Entela Basha[1], Reis Ranxha[1,2], Ada Muco[1]
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Objective: Aphasia syndromes have been mostly reported as a result of stroke syndromes. Often the assessment of language ability, plays a central role in the clinical evaluation of patients with suspected dementia. Its most relevant contribution are: the diagnosis of cognitive impairment, the differential diagnosis among different causes of dementia and a classification in the variety of APP which may have a probabilistic relation ship with different pathological substrate. The present case study analyses the cognitive impairment due to a acute AVC T- P sin. in a 54 year old right- handed patient with higher formal education. **Method:** The patient was tested on a broad range of cognitive abilities , including language, memory, praxis, number processing and calculation, visual spatial abilities, executive functions. **Results:** On neuropsychological examination, he presented a moderate verbal apraxia, noted by word rate not often running , essentially on not frequent words with lexical production rate and phonological/articulatory phonemic errors neither phonetic nor disarthric, accompanied with others clinical manifestations like: spatial acalculia, and moderate anarithmetic. However, not suspicion for a APP not trouble, not decrease of syntaxic formes or a DPD not anomie, neither pause nor low voice. **Conclusions** The present case study highlights the diversity of clinical manifestation of speech disorders. Additional observations of this syndrome being needed to appreciate the full spectrum of impairments in patients with neurodegenerative brain disease , including those often considered as “movements disorders” PNFA or Logopenic aphasia typically due to AD pathology or patients with DPD.

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THE ROLE OF THE LATERAL PERIAQUEDUCTAL GRAY IN FEMALE MATING BEHAVIOR

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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The periaqueductal gray (PAG) controls key aspects of female social behavior, from sex to maternity. Early work on the PAG showed that its lateral and ventrolateral subdivisions are involved in female sexual receptivity, especially in the control of lordosis, the receptive behavior fundamental for successful copulation in rodents. Female sexual behavior depends on the integration of several processes, changes in movement and the execution of lordosis. In mice, lordosis occurs only when females are in the fertile phase of the reproductive cycle. In addition, it requires male-derived stimulation of the female's flanks. Until now, it was not known if the two PAG subdivisions worked in concert or separately to control different aspects of sexual behavior.
Taking advantage of the Fos-TRAP mouse line, we specifically tested the involvement of the lateral PAG in female sexual behavior. Inhibiting (DREADDS and optogenetics) the activity of lateral PAG neurons lead to specific disruptions in the execution of the lordosis posture, but receptivity was largely unaffected, as females still perceived the flank stimulation and remained immobile next to the male. Therefore, our results suggest that different elements of female receptivity are controlled by distinct PAG subdivisions, with the lateral PAG primarily involved in execution of lordosis.
These findings agree with recent studies characterizing the involvement of the PAG in other complex behaviors, such as fear-induced immobility. This suggests that PAG-controlled behaviors are accomplished via the coordinated action of distinct subdivisions controlling specific elements, which collectively output a behavior that reflects the animal's internal state.

Pubmed:
26140594: Gore F, Schwartz EC, Brangers BC, Aladi S, Stujenske JM, Likhtik E, Russo MJ, Gordon JA, Salzman CD, Axel R
Neural Representations of Unconditioned Stimuli in Basolateral Amygdala Mediate Innate and Learned Responses.
Stimuli that possess inherently rewarding or aversive qualities elicit emotional responses and also induce learning by imparting valence upon neutral sensory cues. Evidence has accumulated implicating the amygdala as a critical structure in mediating these processes. We have developed a genetic strategy to identify the representations of rewarding and aversive unconditioned stimuli (USs) in the basolateral amygdala (BLA) and have examined their role in innate and learned responses. Activation of an ensemble of US-responsive cells in the BLA elicits innate physiological and behavioral responses of different valence. Activation of this US ensemble can also reinforce appetitive and aversive learning when paired with differing neutral stimuli. Moreover, we establish that the activation of US-responsive cells in the BLA is necessary for the expression of a conditioned response. Neural representations of conditioned and unconditioned stimuli therefore ultimately connect to US-responsive cells in the BLA to elicit both innate and learned responses.
Cell, 2015; 162
19221331: Mahid SS, Jafri NS, Brangers BC, Minor KS, Hornung CA, Galandiuk S
Meta-analysis of cholecystectomy in symptomatic patients with positive hepatobiliary iminodiacetic acid scan results without gallstones.
To study the clinical results of surgical management in patients with right upper quadrant pain, a positive hepatobiliary iminodiacetic acid (HIDA) scan result, and no gallstones.
Arch Surg, 2009; 144
18949743: Mahid SS, Minor KS, Brangers BC, Cobbs GA, Galandiuk S
SMAD2 and the relationship of colorectal cancer to inflammatory bowel disease.
Inflammatory bowel diseases (IBDs) affecting the colon [Crohn's disease (CD) and ulcerative colitis (UC)] are associated with an increased risk of colorectal cancer (CRC). Our previous work using oligonucleotide array data indicated that SMAD2 was significantly underexpressed in UC dysplastic tissue compared to benign UC. The aim of this current study was to determine whether single nucleotide polymorphisms (SNPs) within the SMAD2 gene are associated with IBD dysplasia/cancer. We performed an SNP haplotype-based case-control association study. Leukocyte DNA was obtained from 489 unrelated

Caucasians (158 UC, 175 CD, 71 CRC, 85 controls). Eleven SNPs were genotyped. All 11 SNPs were in Hardy-Weinberg equilibrium in the control population. Strong linkage disequilibrium was observed among nearly all SMAD2 SNPs. There were no significant associations between SMAD2 allele or haplotype frequencies. Power calculations indicated good power for single-marker analysis (>0.8) and reasonably good power against effects of 0.1-0.15 for haplotype analysis. SMAD2 SNPs were not associated with the development of IBD dysplasia/cancer. This incongruity between our previous microarray data and the findings from this genotype study may be attributed to mechanisms such as alternative splicing of pre-mRNA SMAD2 and/or cross talk with other cellular pathways.
Int J Biol Markers, 2008 Jul-Sep; 23

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A VISUAL PATHWAY FOR VOCAL LEARNING IN A SONGBIRD SPECIES

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Human speech and birdsong both critically rely on the processing of the auditory feedback provided by the perception of one own's vocalizations. Whether and how non-auditory modalities can drive vocal motor plasticity remains poorly understood. Yet, it was recently shown that deafened adult male zebra finches are able to modify their song when a transient flash of light is delivered contingent on the pitch of a song syllable (Zai et al, 2020, Nat. Commun.). Here, we sought to determine whether a visual area is engaged during this learning. We focused on the Wulst, a visual area receiving inputs from different sensory modalities. Anatomical studies revealed that this area projects to the songbird primary auditory area (Field L), which we confirmed by electrophysiological recordings. We subsequently deafened birds and performed either bilateral Wulst or Sham lesions. A third group of birds were only deafened. All the birds were involved in the behavioral task in which a light flash is provided contingent on the pitch of a song syllable. Despite the Wulst lesion, behavioral evidence demonstrates that the birds were not blind. Yet, they were not able to modify their song. It was previously shown that the basal ganglia (Area X) were involved in this task. Since Area X receives indirect afferents from the Field L and the Wulst projects to this auditory area, our results suggest that this pathway (Wulst-Field L-Area X) conveys visual information which eventually reaches the network of brain nuclei involved in song learning, variability and control.

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BOARD NUMBER: S07-357

INTEGRATION OF INTERNAL BRAIN DYNAMICS AND CHANGING ENVIRONMENTAL RESOURCES DURING ZEBRAFISH FORAGING

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Foraging represents an ideal paradigm to study decision making as it is universal and subject to high selective pressure. While foraging, animals have to decide between staying local and depleting known food sources and exploring for distant sources in order to replenish them. This so-called exploitation-exploration dilemma also encompasses a trade-off in allocation of attentional, sensory and motor resources. In constant environments a wide range of species exhibit spontaneous fluctuations in exploitation or exploration states (Flavell et al., 2013; Martin, Ernst, & Heisenberg, 1999, Marques et al., 2020). To better understand the effect of environmental sensory stimuli on the neural dynamics that underly exploitation-exploration transitions, we developed a microfluidic system for freely behaving zebrafish larvae that allows for 1) temporally precise control of prey density, 2) large field of view behavioral imaging with high spatial and temporal resolution, and 3) whole-brain cellular resolution neural imaging. We observe that changes in environmental prey density alter the balance between exploitation and exploration states, overriding intrinsic oscillations in internal brain state. This may represent a mechanism to optimize foraging in the face of changing environments.

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BOARD NUMBER: S07-358

NUMERICAL DISCRIMINATION IN DROSOPHILA MELANOGASTER

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Sensitivity to numbers is a crucial cognitive ability that is widespread across the animal kingdom. The lack of experimental models amenable to systematic genetic and neural manipulation has precluded discovering neural circuits required for numerical cognition. Here, we demonstrate that, when provided with a choice, *Drosophila* flies spontaneously prefer sets containing larger numbers of objects. This preference is determined by the ratio between the two numerical quantities tested, a characteristic signature of numerical cognition across species. Individual flies maintained their numerical choice over consecutive days. Using a numerical visual conditioning paradigm, we found that flies are capable of associating sucrose with numerical quantities and can be trained to reverse their spontaneous preference for large quantities. Finally, we show that silencing LC11 neurons reduces the preference for more objects, thus identifying a neuronal substrate for numerical cognition in invertebrates. This discovery paves the way for the systematic analysis of the behavioral and neural mechanisms underlying the evolutionary conserved sensitivity to numerosity.

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NEURAL CORRELATES OF NATURAL SOCIAL BEHAVIOR IN FREELY-MOVING MACAQUES

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Current understanding of the neural mechanisms underlying primate social behavior derives almost exclusively from highly-constrained and scripted laboratory tasks. Precisely how the primate brain dynamically navigates species-typical social interactions, in all their richness and complexity, remains unknown. Here we leverage new technology to investigate the neural correlates of an unprecedentedly diverse array of species-typical behaviors in freely-moving, socially-interacting rhesus macaques. We recorded single neurons from the inferior temporal area TEO and prefrontal area 45 in two male macaques (n=6,129 units, 20 sessions) while they interacted with their female partner, and varied the identity of neighboring monkeys. Monkeys exhibited a rich behavioral repertoire of up to 27 different behaviors also observed in the wild, including grooming, foraging, aggression, and mating. The behaviors of the recorded monkeys were reliably decoded on a second-by-second basis from a few hundred neurons (>70% accuracy) –even during behaviors extending over many minutes where the sensorimotor environment changed considerably. Remarkably, neurons encoded the context of affiliative interactions: whether grooming was reciprocated or initiated, whether it occurred after a threatening event and the identity of neighboring monkeys. Moreover, neural activity was also modulated by the actions of the partner, providing parallel representations of the behavior of both self and other. Surprisingly, decoding performance was similar across brain areas, with important implications for theories of social information processing. These preliminary results demonstrate that neural ensembles in the prefrontal and inferior temporal cortices of macaques carry information about species-typical social stimuli, behavior, and contexts required for success in the wild.

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SOCIAL INTERACTION SHAPES SELECTIVE MEMORY IN THE HIGHER AUDITORY AREA THROUGH THE NEURONAL ACTIVITIES OF LOCUS COERULEUS

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Juvenile zebra finches learn to sing by engaging in social communications with their tutors. Social exposure to a tutor singing is crucial for successful learning. Here we show, by recording extracellular single-unit activities in free-moving juvenile zebra finches, that neurons in the locus coeruleus (LC), an attention control area, responded stronger to live tutor singing (LIVE TUT) than to playback of pre-recorded tutor songs (TUT playback). The LC neurons sent axons to the zebra finch higher auditory cortex, the caudomedial nidopallium (NCM), where early tutor song memories form. Previously we showed that a fraction of broad-spiking NCM neurons responded selectively to TUT playback (TUT-selective neurons) upon LIVE TUT. To ask whether LC neurons directly influence song perception in the NCM, we optogenecially blocked LC terminal activities in the NCM every time juveniles heard LIVE TUT and recorded NCM neuronal activities. Around 30% of broad-spiking NCM neurons, which responded to TUT playbacks, showed reduced responses to LIVE TUT when paired with LC terminal inhibition and did not develop selectivity to the TUT playback. Those neurons showed a reduced response to TUT playback hours after LC terminal inhibition but showed no response reduction to playbacks of other song stimuli. Finally, juveniles, which heard LIVE TUT for three days with LC terminal inhibition failed to copy tutor songs, while control juveniles learned tutor songs well. Taken together, our data suggest that social vocal communication with a tutor modulates song perception in the auditory cortex and shapes selective auditory memories through LC inputs.

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DATA-DRIVEN DISCOVERY OF MOTOR CONTROL CIRCUITS OF LOCOMOTION ON VARIABLE TERRAINS

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Humans and animals possess remarkable abilities to negotiate challenging terrains during walking. Discovering the organisational principles of motor systems that facilitate such efficient and adaptive walking can provide insights to designing artificial robotic controllers for dynamic locomotion in diverse environments. One challenge is that flat and variable terrains pose conflicting requirements for motor circuits. While flat terrains favour centralised, feedforward architectures that autonomously generate efficient gaits variable terrains demand flexible adjustments to gaits, a property of feedback-based architectures. We studied this dichotomy in Drosophila melanogaster using a combination of behavioural analysis, sensory silencing and data-driven mathematical modelling. First, we designed a climbing assay and a computational pipeline using deep learning-based monocular pose estimation to obtain for the first time 3D limb kinematics during repeatable behaviours in untethered flies. On flat terrain, both wild-type and sensory silenced flies predominantly used the tripod gait, corroborating previous findings. However, obstacles caused gait perturbations, which in wild-type animals resulted either in the recovery of the tripod gait, or gait adaptation, depending on the inter-obstacle distance. We then identified sensory neuron classes whose silencing allowed gait recovery, albeit slower, but suppressed gait adaptation, suggesting that flies switch from feedforward to feedback-based circuits on rough terrains. To unravel parsimonious circuit topologies, we developed a data-driven modelling framework using variational autoencoders and symbolic regression. The distribution of network topologies revealed distinct structural classes, favouring decentralised architectures in challenging terrains. Our work provides insight into how freely behaving animals tune their motor circuits for efficient locomotion.

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BOARD NUMBER: S07-362

OXYTOCIN MODULATION OF SOCIALLY DRIVEN ADULT NEUROGENESIS IN ZEBRAFISH

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Adult neurogenesis, the formation of new neurons from precursors cells, is regulated by both intrinsic and extrinsic factors. The social context is a key environmental factor that can modulate positively or negatively the formation of new neurons. Preliminary work from our lab shows that social isolation has a negative impact on cell proliferation in zebrafish, which can be rescued by the exposure to a social stimulus (a sex-mixed shoal). Moreover, in rats, adult neurogenesis can be modulated by the action of oxytocin receptors present in the hippocampus, an area belonging to the social decision-making network (SDMN). The SDMN is a network that regulates social behaviour and is influenced by the action of hormones and neuromodulators, like oxytocin. Here, we used a zebrafish mutant line for oxytocin receptor, to test if oxytocin mediates the effects of the social environment on adult neurogenesis in the brain nuclei belonging to the SDMN. The results indicate an effect of oxytocin on brain cell proliferation in Dm, Vc, PPa, PPp, Vd and the pretectum. Thus, this study can be considered a steppingstone to clarify the role of oxytocin on the social modulation of adult neurogenesis in vertebrates.

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DEVELOPMENT OF A HPLC-UV-FLD METHOD FOR MONOAMINE DETERMINATION IN LIZARD BRAIN TISSUE SAMPLES

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Lizards have simpler brains than mammals yet share common neural circuits in the brainstem responsible for motivational behavior including aggression. This simpler brain is favorable for studying mechanisms of behavioral control which could be translated to other species to clarify the axis that links aggressive behavior with the regulation of neurotransmitter levels. The aim of this study was to develop and validate a rapid HPLC method with UV and fluorescence detection for monoamine neurotransmitters, precursors and metabolites in lizard brain tissue. Samples of the left hemisphere were collected and homogenized in deproteinization solution, centrifuged and injected into the HPLC-UV. The RP-HPLC gradient method was modified in various ways: duration (20-45 min), flow (0.45-1 mL/min), proportion of organic phase (0-30%), pH of the aqueous phase (2-3) and injection volume (10-90 µL). We changed the composition of the mobile phase - organic phase (acetonitrile, methanol) and aqueous phase (formic acid, phosphoric acid). In the process, we changed three columns - two with C18 and one with phenyl as a stationary phase. A mix of known concentrations of 12 compounds - serotonin, dopamine hydrochloride, L-3,4-dihydroxyphenylalanine, norepinephrine hydrochloride, epinephrine bitartrate, L-Tryptophan, L-Tyrosine hydrochloride, 5-Hydroxytryptophan, 5-hydroxyindole-3-acetic acid, vanillylmandelic acid, homovanilic acid and octopamine hydrochloride was used for identification of peaks in samples and for daily calibration curves. The peaks were identified by an UV detector (280 nm) and a fluorescence detector (270/320 nm). Monoamine levels in the brains of 200 coexisting lizard species (female and male of *Podarcis siculus* and *Podarcis melisellensis*) are presented.

Pubmed:

34798933: Benković V, Marčina N, Horvat Knežević A, Šikić D, Rajevac V, Milić M, Kopjar N
Potential radioprotective properties of arbutin against ionising radiation on human leukocytes in vitro.
Arbutin is a simple phenolic glucoside biosynthesised in many plant families. Some of the everyday foods that contain arbutin are species of the genus Origanum, peaches, cereal products, coffee and tea and Arctostaphyllos uva ursi L. leaves. Arbutin possesses various beneficial effects in the organism, and was confirmed effective in the treatment of urinary tract infections as well as in preventing skin hyperpigmentation. It shows antioxidant and anti-inflammatory properties, and antitumor activity. The aim of this study was to explore potential radioprotective properties of arbutin in concentrations of 11.4 µg/mL, 57 µg/mL, 200 µg/mL and 400 µg/mL administered as a pre-treatment for one hour before exposing human leukocytes to ionising radiation at a therapeutic dose of 2 Gy. The alkaline comet assay was used to establish the levels of primary DNA damage, and cytokinesis-block micronucleus (CBMN) cytome assay to determine the level of cytogenetic damage. None of the tested concentrations of single arbutin showed genotoxic and cytotoxic effects. Even at the lowest tested concentration, 11.4 µg/mL, arbutin demonstrated remarkable potential for radioprotection in vitro, observed both at the level of primary DNA damage, and using CBMN cytome assay. The best dose reduction compared with amifostine was observed after pre-treatment with the highest concentration of arbutin, corresponding to 400 µg/mL. Promising results obtained on the leukocyte model speak in favour of extending similar experiments on other cell and animal models.
Mutat Res Genet Toxicol Environ Mutagen, 2021; 872
34328801: Benković V, Borojević N, Šikić D, Horvat Knežević A, Milić M
DNA damage assessment in peripheral blood of Swiss albino mice after combined exposure to volatile anesthetics and 1 or 2 Gy radiotherapy in vivo.
Patient immobilization by general volatile anesthesia (VA) may be necessary during medical radiology treatment, and its use has increased in recent years. Although ionizing radiation (IR) is a well-known genotoxic and cytotoxic agent, and VA exposure has caused a range of side effects among patients and occupationally exposed personnel, there are no studies to date comparing DNA damage effects from combined VA and single fractional IR dose exposure.
Int J Radiat Biol, 2021; 97


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A NEURONAL SUBSTRATE FOR TRANSLATING RESOURCE DENSITY ESTIMATIONS INTO FORAGING DECISIONS

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Foraging animals must balance the costs of exploring their surroundings with the potential benefits of finding nutritional resources. Upon finding a food source, they have to make the central decision of whether to stop and initiate feeding or to continue searching for potentially better options. Foraging theory predicts that this decision should be dependent on an estimate of the density of available resources in the environment. How this complex computation is algorithmically implemented in the brain remains poorly explored. Here, we use video-based tracking to show that *Drosophila melanogaster* regulates the decision to stop at food spots based on the density of food patches in the environment. To answer how underlying neuronal circuits may implement this computation algorithmically, we performed a large-scale neurogenetic silencing screen of more than 400 different genetic driver lines with sparse expression patterns in the brain. We identified a population of neurons in the fly central brain that acts as a key regulator of the decision to stop. Specifically, we show that manipulating activity in these neurons increases not only the probability of stopping at food spots, but also alters their ability to adjust this foraging variable to the estimated resource density. Our results reveal a neuronal substrate involved in modulating ethologically relevant foraging decisions, a key step towards a mechanistic explanation of cognitive functions required to solve complex cost-benefit trade-offs.

Pubmed:
31226244: Moreira JM, Itskov PM, Goldschmidt D, Baltazar C, Steck K, Tastekin I, Walker SJ, Ribeiro C
optoPAD, a closed-loop optogenetics system to study the circuit basis of feeding behaviors.
The regulation of feeding plays a key role in determining the fitness of animals through its impact on nutrition. Elucidating the circuit basis of feeding and related behaviors is an important goal in neuroscience. We recently used a system for closed-loop optogenetic manipulation of neurons contingent on the feeding behavior of to dissect the impact of a specific subset of taste neurons on yeast feeding. Here, we describe the development and validation of this system, which we term the optoPAD. We use the optoPAD to induce appetitive and aversive effects on feeding by activating or inhibiting gustatory neurons in closed-loop - effectively creating virtual taste realities. The use of optogenetics allowed us to vary the dynamics and probability of stimulation in single flies and assess the impact on feeding behavior quantitatively and with high throughput. These data demonstrate that the optoPAD is a powerful tool to dissect the circuit basis of feeding behavior, allowing the efficient implementation of sophisticated behavioral paradigms to study the mechanistic basis of animals' adaptation to dynamic environments.
Elife, 2019; 8
29129289: Walker SJ, Goldschmidt D, Ribeiro C
Craving for the future: the brain as a nutritional prediction system.
In the last decades, predictive coding has emerged as an important framework for understanding how the brain processes information. It states that the brain is constantly inferring and predicting sensory data from statistical regularities in its environment. While this framework has been largely applied to sensory processing and motor control, we argue here that it could also serve as framework for a better understanding of how animals regulate nutrient homeostasis. Mechanisms that underlie nutrient homeostasis are commonly described in terms of negative feedback control, which compares current states with a reference point, called setpoint, and counteracts any mismatches. Using concepts from control theory, we explain shortcomings of negative feedback as a purely reactive controller, and how feed-forward mechanisms could be incorporated into feedback control to improve the performance of the control system. We then provide numerous examples to show that many insects, as well as mammals, make use of feed-forward, anticipatory mechanisms that go beyond the prevailing view of homeostasis being achieved through reactive negative feedback. The emerging picture is that the brain incorporates predictive signals as well as negative feedback to regulate nutrient homeostasis.

Curr Opin Insect Sci, 2017; 23
28446872: Goldschmidt D, Manoonpong P, Dasgupta S
A Neurocomputational Model of Goal-Directed Navigation in Insect-Inspired Artificial Agents.
Despite their small size, insect brains are able to produce robust and efficient navigation in complex environments. Specifically in social insects, such as ants and bees, these navigational capabilities are guided by orientation directing vectors generated by a process called path integration. During this process, they integrate compass and odometric cues to estimate their current location as a vector, called the home vector for guiding them back home on a straight path. They further acquire and retrieve path integration-based vector memories globally to the nest or based on visual landmarks. Although existing computational models reproduced similar behaviors, a neurocomputational model of vector navigation including the acquisition of vector representations has not been described before. Here we present a model of neural mechanisms in a modular closed-loop control-enabling vector navigation in artificial agents. The model consists of a path integration mechanism, reward-modulated global learning, random search, and action selection. The path integration mechanism integrates compass and odometric cues to compute a vectorial representation of the agent's current location as neural activity patterns in circular arrays. A reward-modulated learning rule enables the acquisition of vector memories by associating the local food reward with the path integration state. A motor output is computed based on the combination of vector memories and random exploration. In simulation, we show that the neural mechanisms enable robust homing and localization, even in the presence of external sensory noise. The proposed learning rules lead to goal-directed navigation and route formation performed under realistic conditions. Consequently, we provide a novel approach for vector learning and navigation in a simulated, situated agent linking behavioral observations to their possible underlying neural substrates.
Front Neurorobot, 2017; 11
26441629: Dasgupta S, Goldschmidt D, Wörgötter F, Manoonpong P
Distributed recurrent neural forward models with synaptic adaptation and CPG-based control for complex behaviors of walking robots.
Walking animals, like stick insects, cockroaches or ants, demonstrate a fascinating range of locomotive abilities and complex behaviors. The locomotive behaviors can consist of a variety of walking patterns along with adaptation that allow the animals to deal with changes in environmental conditions, like uneven terrains, gaps, obstacles etc. Biological study has revealed that such complex behaviors are a result of a combination of biomechanics and neural mechanism thus representing the true nature of embodied interactions. While the biomechanics helps maintain flexibility and sustain a variety of movements, the neural mechanisms generate movements while making appropriate predictions crucial for achieving adaptation. Such predictions or planning ahead can be achieved by way of internal models that are grounded in the overall behavior of the animal. Inspired by these findings, we present here, an artificial bio-inspired walking system which effectively combines biomechanics (in terms of the body and leg structures) with the underlying neural mechanisms. The neural mechanisms consist of (1) central pattern generator based control for generating basic rhythmic patterns and coordinated movements, (2) distributed (at each leg) recurrent neural network based adaptive forward models with efference copies as internal models for sensory predictions and instantaneous state estimations, and (3) searching and elevation control for adapting the movement of an individual leg to deal with different environmental conditions. Using simulations we show that this bio-inspired approach with adaptive internal models allows the walking robot to perform complex locomotive behaviors as observed in insects, including walking on undulated terrains, crossing large gaps, leg damage adaptations, as well as climbing over high obstacles. Furthermore, we demonstrate that the newly developed recurrent network based approach to online forward models outperforms the adaptive neuron forward models, which have hitherto been the state of the art, to model a subset of similar walking behaviors in walking robots.
Front Neurorobot, 2015; 9
24523694: Goldschmidt D, Wörgötter F, Manoonpong P
Biologically-inspired adaptive obstacle negotiation behavior of hexapod robots.
Neurobiological studies have shown that insects are able to adapt leg movements and posture for obstacle negotiation in changing environments. Moreover, the distance to an obstacle where an insect begins to climb is found to be a major parameter for successful obstacle negotiation. Inspired by these findings, we present an adaptive neural control mechanism for obstacle negotiation behavior in hexapod robots. It combines locomotion control, backbone joint control, local leg reflexes, and neural learning. While the first three components generate locomotion including walking and climbing, the neural learning mechanism allows the robot to adapt its behavior for obstacle negotiation with respect to changing conditions, e.g., variable obstacle heights and different walking gaits. By successfully learning the association of an early, predictive signal (conditioned stimulus, CS) and a late, reflex signal (unconditioned stimulus, UCS), both provided by ultrasonic sensors at the front of the robot, the robot can autonomously find an appropriate distance from an obstacle to initiate climbing. The adaptive neural control was developed and tested first on a physical robot simulation, and was then successfully transferred to a real hexapod robot, called AMOS II. The results show that the robot can efficiently negotiate obstacles with a height up to 85% of

the robot's leg length in simulation and 75% in a real environment.
Front Neurorobot, 2014; 8
27534393:
25th Annual Computational Neuroscience Meeting: CNS-2016.
BMC Neurosci, 2016; 17 Suppl 1

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DATA-DRIVEN DISCOVERY OF LONG TIMESCALE BEHAVIORAL STRATEGIES DURING SENSORY EVOKED NAVIGATION

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Survival requires flexible and adaptive behavioral mechanisms on multiple timescales. Bridging between short-time behaviors and long timescale strategies can help identify the internal states that modulate behavior across scales. We leverage high-resolution, high throughput recordings of the freely swimming behavior of larval zebrafish to find such strategies from their constituent short timescale burst-like movements called bouts. We reconstruct a maximally predictive behavioral state by stacking consecutive bouts, and study the time evolution of state-space densities through transfer operators. Their spectral decomposition allows us to identify slowly decaying modes corresponding to stereotyped long timescale behavioral strategies. During exploratory behavior in arenas of diverse shapes, we find two main strategies lasting from tens to hundreds of seconds – a "roaming" strategy where turn bouts in similar directions cause large changes in orientation, and a "cruising" strategy where forward bouts with smaller changes in orientation persist. Closed loop visual gradients trigger switches between such long timescale behaviors, while more natural stimuli like prey or aversive cues can cause finer scale modulations of each strategy. Furthermore, mesoscopic stimulus responses such as a sequence of bouts underlying prey capture are nested into one of the strategies, suggesting a hierarchical organization of behavior. The presence of two behavioral strategies irrespective of the sensory landscape point towards a homeostatic mechanism regulating behavior across scales.

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HYPOTHALAMIC CIRCUITS FOR FEMALE SOCIAL BEHAVIOUR: INVESTIGATING THE ROLE OF PMV-DAT NEURONS

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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In rodents, the hypothalamus has been extensively implicated in the control of sociosexual behaviours. Here we characterize the activity of dopamine active transporter-expressing neurons within the ventral premammillary nucleus (PMv-DAT) of female mice. Using fiber photometry, we show that female PMv-DAT neurons are strongly activated by contact-dependent male cues, but not by female ones. We also show that the male-driven activity of PMv-DAT neurons is highly modulated during sexual behaviour. To test the importance of this population in female sexual behaviour we used optogenetics to increase the activity of PMv-DAT neurons in fertile females. Our results show that activation of female PMv-DAT neurons led to a significant decrease in the rate of female-led investigations, suggesting a role of the PMv-DAT population in the maintenance of investigatory behaviour. Lastly, the PMv has also been implicated in the control of aggressive behaviours, namely maternal aggression. Here, we performed photometric recordings of PMv-DAT neuronal activity during distinct epochs of the peri-and postpartum period in female mice. We show that the activity of PMv-DAT neurons is elevated in the presence of an intruder mouse, independently if the female engages in aggressive or just investigatory behaviour with the male. In contrast, activity towards the father of the pups, to whom the female is not aggressive, is highly decreased when compared to the activity elicited towards conspecific males in virgin females. We propose that, when investigating a male, female PMv-DAT neurons relay male-conspecific cues to its downstream targets to elicit appropriate male-directed behaviours.

Pubmed:
26149766: Rodrigues MA, Martins NE, Balancé LF, Broom LN, Dias AJ, Fernandes AS, Rodrigues F, Sucena É, Mirth CK
Drosophila melanogaster larvae make nutritional choices that minimize developmental time.
Organisms from slime moulds to humans carefully regulate their macronutrient intake to optimize a wide range of life history characters including survival, stress resistance, and reproductive success. However, life history characters often differ in their response to nutrition, forcing organisms to make foraging decisions while balancing the trade-offs between these effects. To date, we have a limited understanding of how the nutritional environment shapes the relationship between life history characters and foraging decisions. To gain insight into the problem, we used a geometric framework for nutrition to assess how the protein and carbohydrate content of the larval diet affected key life history traits in the fruit fly, Drosophila melanogaster. In no-choice assays, survival from egg to pupae, female and male body size, and ovariole number - a proxy for female fecundity - were maximized at the highest protein to carbohydrate (P:C) ratio (1.5:1). In contrast, development time was minimized at intermediate P:C ratios, around 1:2. Next, we subjected larvae to two-choice tests to determine how they regulated their protein and carbohydrate intake in relation to these life history traits. Our results show that larvae targeted their consumption to P:C ratios that minimized development time. Finally, we examined whether adult females also chose to lay their eggs in the P:C ratios that minimized developmental time. Using a three-choice assay, we found that adult females preferentially laid their eggs in food P:C ratios that were suboptimal for all larval life history traits. Our results demonstrate that D. melanogaster larvae make foraging decisions that trade-off developmental time with body size, ovariole number, and survival. In addition, adult females make oviposition decisions that do not appear to benefit the larvae. We propose that these decisions may reflect the living nature of the larval nutritional environment in rotting fruit. These studies illustrate the interaction between the nutritional environment, life history traits, and foraging choices in D. melanogaster, and lend insight into the ecology of their foraging decisions.
J Insect Physiol, 2015; 81

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ENDOCRINE DISRUPTING CHEMICALS IN BREASTMILK AND INFANT NEUROBEHAVIORAL DEVELOPMENT: THE LIFE MILCH PROJECT

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Aims. The Life MILCH Project is a longitudinal pilot study that aims to determine the association between levels of maternal milk contamination/exposure to Endocrine Disrupting Chemicals (EDCs) and infants' growth, development and neurobehavioral health in the first year of life. **Methods.** 250 pregnant women in the third trimester of gestation with no gestational or fetal pathologies were enrolled at Parma Hospital. At recruitment, women's lifestyle and nutritional habits were recorded through two questionaries and maternal urine and plasma samples were collected for assessing EDCs exposure. At parturition, cord blood sample, newborns' growth parameters and urine were collected and assessed. Mother-infant dyads are recalled 1, 3, 6 and 12 months after delivery for assessing EDC exposure by biological samples (breast-milk, maternal and infant urine), infants' growth and neurodevelopment through Visual Preference Paradigm (at 1 month), Face-to-Face-Still-Face (at 3 months), Fagan test (at 6 months), Barrier task (at 12 months) and the Bayley III scale for infant neurodevelopment (at 6 and 12 months). **Results.** This is an ongoing study and analysis of results are not completed yet. Maternal lifestyle, nutritional and clinical information before and after pregnancy will be correlated with EDCs levels in biological samples, infants' growth, perceptive, socioemotional, cognitive, and behavioral developmental areas and infant neurodevelopment. **Conclusions.** Perceptive, socioemotional, cognitive, behavioral and reproductive development are susceptible to developmental EDCs exposure. Maternal exposure to EDCs results in fetal and neonatal exposure via utero and breast milk and can affect infants'growth and neuro behavioral development during the first year of life.

Pubmed:
33248148: Palanza P, Paterlini S, Brambilla MM, Ramundo G, Caviola G, Gioiosa L, Parmigiani S, Vom Saal FS, Ponzi D
Sex-biased impact of endocrine disrupting chemicals on behavioral development and vulnerability to disease: Of mice and children.
Sex is a fundamental biological characteristic that influences many aspects of an organism's phenotype, including neurobiological functions and behavior as a result of species-specific evolutionary pressures. Sex differences have strong implications for vulnerability to disease and susceptibility to environmental perturbations. Endocrine disrupting chemicals (EDCs) have the potential to interfere with sex hormones functioning and influence development in a sex specific manner. Here we present an updated descriptive review of findings from animal models and human studies regarding the current evidence for altered sex-differences in behavioral development in response to early exposure to EDCs, with a focus on bisphenol A and phthalates. Overall, we show that animal and human studies have a good degree of consistency and that there is strong evidence demonstrating that EDCs exposure during critical periods of development affect sex differences in emotional and cognitive behaviors. Results are more heterogeneous when social, sexual and parental behaviors are considered. In order to pinpoint sex differences in environmentally-driven disease vulnerabilities, researchers need to consider sex-biased developmental effects of EDCs.
Neurosci Biobehav Rev, 2021; 121

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TO REJECT OR TO MATE? INSIGHTS FROM A NOVEL HYPOTHALAMIC SUBREGION INVOLVED IN FEMALE SEXUAL BEHAVIOUR

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Whether a female accepts or rejects the sexual advances of a male depends on her reproductive state as well as male-derived sensory stimuli received during the interaction. The ventrolateral part of the ventromedial hypothalamus (VMHvl) is hypothesized to integrate these internal and external cues to influence female socio-sexual behaviour. To elucidate mechanisms by which the VMHvl performs this function, we studied the progesterone receptor-expressing (PR+) cell population in the VMHvl, as progesterone is a key hormone in regulating the reproductive cycle. We first characterized the electrophysiological properties of PR+ cells in the VMHvl and found that they exhibit cyclical changes in their excitation/inhibition (E/I) balance across the reproductive cycle. This cyclical plasticity is more pronounced in the anterior VMHvl (aVMHvl), with the E/I ratio being higher in neurons originating from non-fertile females as compared to fertile ones. Furthermore, we observed that the interaction with a male leads to higher cFos expression in the aVMHvl of non-fertile females that display rejection behaviour compared with fertile females that mate with the male. These results led us to hypothesize that the aVMHvl.PR+ population might be involved in rejection behavior in non-fertile females. To test this we used optogenetics to artificially excite aVMHvl.PR+ neurons in fertile females and, as expected, our manipulation led to increased rejection behaviours in fertile females and to a reduction in the total number of females that mated with the male. Overall, our study highlights the role of aVMHvl.PR+ neurons in female rejection and mating behaviour.

Pubmed:

25784603: Husain BF, Nanavaty IN, Marathe SV, Rajendran R, Vaidya VA
Hippocampal transcriptional and neurogenic changes evoked by combination yohimbine and imipramine treatment.
Adjunct α2-adrenoceptor antagonism is a potential strategy to accelerate the behavioral effects of antidepressants. Co-administration of the α2-adrenoceptor antagonist yohimbine hastens the behavioral and neurogenic effects of the antidepressant imipramine. We examined the transcriptional targets of short duration (7days), combination treatment of yohimbine and imipramine (Y+I) within the adult rat hippocampus. Using microarray and qPCR analysis we observed functional enrichment of genes involved in intracellular signaling cascades, plasma membrane, cellular metal ion homeostasis, multicellular stress responses and neuropeptide signaling pathways in the Y+I transcriptome. We noted reduced expression of the α2A-adrenoceptor (Adra2a), serotonin 5HT2C receptor (Htr2c) and the somatostatin receptor 1 (Sstr1), which modulate antidepressant action. Further, we noted a regulation of signaling pathway genes like inositol monophosphatase 2 (Impa2), iodothyronine deiodinase 3 (Dio3), regulator of G-protein signaling 4 (Rgs4), alkaline ceramidase 2 (Acer2), doublecortin-like kinase 2 (Dclk2), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (Nfkbia) and serum/glucocorticoid-regulated kinase 1 (Sgk1), several of which are implicated in the pathophysiology of mood disorders. Comparative analysis revealed an overlap in the hippocampal regulation of Acer2, Nfkbia, Sgk1 and Impa2 between Y+I treatment, the fast-acting electroconvulsive seizure (ECS) paradigm, and the slow-onset chronic (21days) imipramine treatment. Further, Y+I treatment enhanced the quiescent neural progenitor pool in the hippocampal neurogenic niche similar to ECS, and distinct from chronic imipramine treatment. Taken together, our results provide insight into the molecular and cellular targets of short duration Y+I treatment, and identify potential leads for the development of rapid-action antidepressants.
Prog Neuropsychopharmacol Biol Psychiatry, 2015; 61
27207907: Pusalkar M, Ghosh S, Jaggar M, Husain BF, Galande S, Vaidya VA
Acute and Chronic Electroconvulsive Seizures (ECS) Differentially Regulate the Expression of Epigenetic Machinery in the Adult Rat Hippocampus.
Electroconvulsive seizure treatment is a fast-acting antidepressant therapy that evokes rapid transcriptional, neurogenic, and behavioral changes. Epigenetic mechanisms contribute to altered gene regulation, which underlies the neurogenic and behavioral effects of electroconvulsive seizure. We hypothesized that electroconvulsive seizure may modulate the expression

of epigenetic machinery, thus establishing potential alterations in the epigenetic landscape.
Int J Neuropsychopharmacol, 2016; 19

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BOARD NUMBER: S07-369

ON THE ROLE OF SINGING FOR SONG PLASTICITY

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Songbirds' vocal mastery is impressive, but to what extent is it a result of practice? Young birds undoubtedly need practice to imitate a vocal target, but can adult birds, similar to humans, make targeted changes to their song in a practice-free manner without intermittently singing? In principle, such ability can arise if the brain stores distinct motor memories of multiple song variants or if the brain's internal models of the sensorimotor system allow immediate translation of a desired sensory target into the appropriate motor command for imitating that target. To explore these ideas, we test whether adult zebra finches subjected to pitch reinforcement can instantaneously retrieve a previous song variant. First, we drive one syllable of their song away from its crystallized (baseline) variant acquired from a tutor, then we withdraw reinforcement and subsequently mute or deafen the birds to deprive them of song experience. In this deprived state, birds barely change their songs and do not show the typical rapid recovery of baseline song, revealing a requirement of song practice for full song recovery. Nevertheless, in this deprived state, birds seem capable of making small but directed song changes (on the order of a daily standard deviation), not necessarily towards the original target, but towards song variants with higher expected reward. Thus, our work reveals vocal plasticity in songbirds independent of sensory experience but suggests a limit to internally guided vocal flexibility.

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BOARD NUMBER: S07-370

A COMPARATIVE APPROACH IN VERTEBRATE NEUROSCIENCE: THE ZEBRAFISH (DANIO RERIO) AND GIANT DANIO (DEVARIO AEQUIPINNATUS)

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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In behavioral neuroscience a comparative approach can help us to distinguish general organizing principles in the brain from specialized adaptations to a particular ecological niche, and to link neurological differences to behavioral phenotypes and ecological variation on an evolutionary timescale. To address this idea, we studied the swimming behavior of the larvae of two closely related species of Danioninae: *Danio rerio* and *Devario aequipinnatus*. Although these species share similar anatomy at the larval stage, they have different developmental rate and locomotor styles. We measured the locomotion of a cohort of larvae from each species in freely swimming behavior and in a set of automated assays with optomotor response and social behavior. Compared to Zebrafish, the Devario larvae show more rapid development of behavioral responses, in particular showing clear social attraction in the first week of life. They also show increased maneuverability and performance in the optomotor response, with shorter intervals between tail movements. Currently we are investigating how this increased maneuverability impacts their prey capture behavior. Seeing the differences in the level of behavior, we compared basic neural anatomy and started building an expression atlas for the Devario larva using antibody labelings for neuromodulators and dextran-dye back-fills of reticulospinal neurons for the descending motor system. Comparing the anatomy of the descending motor system across the two species shows a high degree of conservation, although with some small differences in dendritic projection patterns. Lastly, we are making Devario GCaMP lines to see if there are differences in brain-wide activity during behavior.

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BOARD NUMBER: S07-371

DYNAMIC INTEGRATION OF SPACE AND SOCIAL STATUS IN THE MAMMALIAN HYPOTHALAMUS

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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The hypothalamus is an evolutionarily ancient brain region that controls innate behaviors associated with reproduction, defense, and ingestion. Work has shown that the ventrolateral part of the ventromedial hypothalamus (VMHvl) encodes a generalized state of social threat and is necessary and sufficient for producing both social aggression and avoidance. However, it remains unclear how social experience contributes to the selection of each of these specific defensive behaviors. Recent work in the lab has shown that neurons that fire in the context of social defeats are reactivated when the animal explores the location where the defeat occurred, even in absence of the aggressor. Also, at the neural population level, VMHvl firing becomes tuned to the location where the animal is, discriminating between the safe home cage and the chamber where the aggression took place. Based on these data, my project aims to test the hypothesis that neuroplastic changes in the VMH might occur in a territorial context to encode a functional map of social space that guide instinctive behaviors. To this end, we developed an experimental apparatus that allows the study of territorial behavior in semi-natural conditions. Our protocol allows for the identification of robust and reproducible territorial behaviors in laboratory mice, and the development of a new urine marking protocol to enrich our understanding of territorial dynamics. Finally, we demonstrate the compatibility of our apparatus with imaging and manipulating neural tools, thus allowing for new insight regarding how VMHvl encodes territorial cues that shape defensive behaviors.

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BOARD NUMBER: S07-372

COMPARATIVE BRAIN MORPHOLOGY OF THREE SPECIMENS WITH DIFFERENT ADAPTATIVE BEHAVIORS: THE BONGO, THE JAVA DEER MOUSE AND THE MAKI CATTA

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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One aim of comparative neuroanatomy is to better understand brain function among species. It is tempting to try and explain brain differences throughout the animal kingdom by differences in adaptive behaviors as well as ecological factors. Based on this idea, we explored, with MRI, the brain morphology of three species with different sociality and predator avoidance (Bongo, Java deer mouse and Maki Catta). Brains were collected after death of natural causes and MR-imaged. Brain and body weights were collected, and volumes of brain were estimated after MRI segmentation. The brain-to-body weight ratio was close to 1 for the Java deer mouse (1.04%) and the maki catta (1.05%) but only 0.26% for the bongo. Encephalization quotients (EQ) were calculated using formulas defined for human (hEQ, constants 0.12 and 2/3; Cairó 2011, doi:10.3389/fnhum.2011.00108) and for dog (dEQ, constants 0.14 and 0.528; Saganuwan 2021, doi:10.1186/s13104-021-05638-0). Whatever the method, the Java deer mouse EQs were the smallest (hEQ=0.98; dEQ=0.88). The maki catta had a higher hEQ (1.23) than the bongo (1.14) whereas the order was reversed for the dEQ (maki catta dEQ=1.21; bongo dEQ=1.96). These values are coherent with the idea that EQ is higher in prey species using active predator avoidance (bongo) and in social species (bongo and maki catta).

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BOARD NUMBER: S07-373

MATERNAL DEPRIVATION AND MILK REPLACEMENT AFFECT THE INTEGRITY OF GRAY AND WHITE MATTER IN THE DEVELOPING LAMB BRAIN

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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[1]INRAE, Umr Physiologie De La Reproduction Et Des Comportements, Nouzilly, France, [2]INSERM, Ibrain, Tours, France, [3]University of Pécs Medical School, Centre for Neuroscience, Szentágothai Research Centre, Institute Of Physiology, Pécs, Hungary, [4]University of Pécs, Medical School, Centre for Neuroscience, Szentágothai Research Centre, Institute Of Physiology, Pécs, Hungary

The psychoendocrine evaluation of lamb development has demonstrated that maternal deprivation and milk replacement alters health, behavior and endocrine profiles. While lambs are able to discriminate familiar and non-familiar conspecifics (mother or lamb), only lambs reared with their mother develop such clear social discrimination or preference. Lambs reared without mother display no preference for a specific lamb from its own group. Differences in exploratory and emotional behaviours between mother-reared and mother deprived lambs have also been reported. As these behavioural abilities are supported by the brain, we hypothesize that rearing with maternal deprivation and milk replacement leads to altered brain development and maturation. To test this hypothesis, we examined brain morphometric and microstructural variables extracted from *in-vivo* T1-weighted and diffusion-weighted magnetic resonance images acquired longitudinally (1 week, 1.5 months and 4.5 months of age) in mother-reared and mother-deprived lambs. From the morphometric variables the caudate nuclei volume was found to be smaller for mother-deprived than for mother-reared lambs. T1-weighted signal intensity and radial diffusivity were higher for mother-deprived than for mother-reared lambs in both the white and gray matters. The fractional anisotropy of the white matter was lower for mother-deprived than for mother-reared lambs. Based on these morphometric and microstructural characteristics we conclude that maternal deprivation delays and affects lamb brain growth and maturation.

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BOARD NUMBER: S07-374

"THIS IS MY SPOT!": SOCIAL DETERMINANTS REGULATE SPACE UTILIZATION IN MACAQUES.

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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[1]ISC Marc Jeannerod, UMR5229, Cnrs, Bron, France, [2]Polish Academy of Sciences, Institute Of Psychology, Warszawa, Poland, [3]university of strabsourg, Centre De Pimatologie, Strasbourg, France

Space can be a limited resource. Many species including humans, evolved a compromised sharing of space based on social determinants. For example, students in a classroom sit close to their friends, keeping the same spots across days, revealing the social structure in the classroom. This suggests that factors such as social hierarchy and affiliation can shape space utilization; contrasting with classical random walk models of agents moving at random in any given direction. Here, we asked whether spatial occupancy of macaques (*Macaca fascicularis* and *M. mulatta*) within a unisex group, reveals a structured space utilization beyond simple spatial affordance of the finite space. To this end, in two groups of four animals, we analyzed the simultaneously recorded positions of each individual while the group roamed in an enclosure. (1) The identity of each animal could be decoded from its individual pattern of spatial occupancy, revealing that each animal sustained its spatial footprint across days. (2) Average distance between monkeys was a proxy of their social hierarchy. (3) Alternating the social context by removing one of the monkeys revealed that social context influences occupancy. (4) Finally, the distribution of distance between pairs of monkeys was bimodal and was modeled using random walk approach with an additional parameter reflecting propensity to stay in close proximity, which was again related to dominance hierarchy. These analyses reveal the hidden structured nature of space utilization as a function of social determinants in macaques and simple modeling approach to further study group organization in neuro-ethological settings.

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BOARD NUMBER: S07-375

WHAT IS THE ETHOLOGICAL ROLE OF THE ZEBRAFISH PALLIUM - THE EVOLUTIONARY ANCESTOR OF THE MAMMALIAN CORTEX?

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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In humans, the cortex takes a commanding role in sensory perception and exerts ultimate control over almost all aspects of behaviour. However, a clear role is less apparent in lower order mammals and vertebrates. In rats, it was recently reported that cortical lesions have few effects on the animal's behaviour, except for a specific deficit in rapid motor adaptation to a sudden change in a learned environment. The zebrafish pallium is the ancestral homolog of the cortex, sharing molecular markers as well as developmental and functional similarities. A high level of activity is consistently observed across the whole pallium in behaving animals, but with little clear association to any sensory input or behaviour. What is the role of this primordial cortex? Moreover, what advantage does having a pallium give a zebrafish? To investigate this, we surgically remove dorsal telencephalon from 24 hour old zebrafish embryos and study their free-swimming behaviour at larval and juvenile stages in the presence of naturalistic challenges such as obstacles, paramecia and simulated predators. Interestingly, zebrafish lacking pallium show no dramatic disturbance of free-swimming bout rates, turn frequency or kinematic parameters. Similarly, stereotyped ballistic escape responses to looming stimuli seem to be unaffected, with subtle changes to sustained escape trajectories observed. Preliminary analysis using neural networks to predict behaviour also suggest changes to exploration strategies and bout sequencing. This is in line with the hypothesis that pallium plays a subtle modulatory role in behaviour, perhaps providing variability to facilitate flexible, adaptive behaviour.

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BOARD NUMBER: S07-376

BEHAVIORAL DIVERSITY ACROSS ZEBRAFISH STRAINS OCCURS AT THE LEVEL OF SWIM SEQUENCES.

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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To survive in ever-changing environments, animals evolved a panoply of behaviors that allow them to avoid predation, explore the world, and exploit resources. A core concept in ethology is that behaviors are constructed from a repertoire of basic movements that are assembled into sequences to permit goals at longer timescales, creating nested behavioral hierarchies. Closely related strains of animals show behavioral diversity that allow them to thrive over distinct environments. It is unclear at what level of the behavioral hierarchy this diversity is produced. Is behavioral diversity produced at the lowest level of the hierarchy, having each strain its own movement repertoire, or do they share a common movement repertoire, and diversity is generated at the sequence level? To tackle this question, we turned to the zebrafish larvae because their body plan is simple. It is possible, using a high-speed camera, to automatically track all of its body parts and, contrary to most animals, larvae move discontinuously, swimming in short bursts of movement called “swim bouts”. Thus, larval behavior is naturally segmented in time. We performed behavioral recordings for 6 zebrafish strains, creating a data set with millions of swims over 10 distinct behaviors. After, using an unsupervised classification method (clusterdv), we discovered that these strains share a common swim repertoire. We also observed that for long timescale behaviors they chain their swims in sequences that are specific for each strain. We discuss the idea that sequence diversity is a mechanism to produce behavioral diversity where evolution acts on.

Pubmed:
32888479: Groneberg AH, Marques JC, Martins AL, Diez Del Corral R, de Polavieja GG, Orger MB
Early-Life Social Experience Shapes Social Avoidance Reactions in Larval Zebrafish.
Social experiences greatly define subsequent social behavior. Lack of such experiences, especially during critical phases of development, can severely impede the ability to behave adequately in social contexts. To date, it is not well characterized how early-life social isolation leads to social deficits and impacts development. In many model species, it is challenging to fully control social experiences, because they depend on parental care. Moreover, complex social behaviors involve multiple sensory modalities, contexts, and actions. Hence, when studying social isolation effects, it is important to parse apart social deficits from general developmental effects, such as abnormal motor learning. Here, we characterized how social experiences during early development of zebrafish larvae modulate their social behavior at 1 week of age, when social avoidance reactions can be measured as discrete swim events. We show that raising larvae in social isolation leads to enhanced social avoidance, in terms of the distance at which larvae react to one another and the strength of swim movement they use. Specifically, larvae raised in isolation use a high-acceleration escape swim, the short latency C-start, more frequently during social interactions. These behavioral differences are absent in non-social contexts. By ablating the lateral line and presenting the fish with local water vibrations, we show that lateral line inputs are both necessary and sufficient to drive enhanced social avoidance reactions. Taken together, our results show that social experience during development is a critical factor in shaping mechanosensory avoidance reactions in larval zebrafish.
Curr Biol, 2020; 30
31853063: Marques JC, Li M, Schaak D, Robson DN, Li JM
Internal state dynamics shape brainwide activity and foraging behaviour.
The brain has persistent internal states that can modulate every aspect of an animal's mental experience. In complex tasks such as foraging, the internal state is dynamic. Caenorhabditis elegans alternate between local search and global dispersal. Rodents and primates exhibit trade-offs between exploitation and exploration. However, fundamental questions remain about how persistent states are maintained in the brain, which upstream networks drive state transitions and how state-encoding neurons exert neuromodulatory effects on sensory perception and decision-making to govern appropriate behaviour. Here, using tracking microscopy to monitor whole-brain neuronal activity at cellular resolution in freely moving zebrafish larvae, we show that zebrafish spontaneously alternate between two persistent internal states during foraging for live prey (Paramecia).

In the exploitation state, the animal inhibits locomotion and promotes hunting, generating small, localized trajectories. In the exploration state, the animal promotes locomotion and suppresses hunting, generating long-ranging trajectories that enhance spatial dispersion. We uncover a dorsal raphe subpopulation with persistent activity that robustly encodes the exploitation state. The exploitation-state-encoding neurons, together with a multimodal trigger network that is associated with state transitions, form a stochastically activated nonlinear dynamical system. The activity of this oscillatory network correlates with a global retuning of sensorimotor transformations during foraging that leads to marked changes in both the motivation to hunt for prey and the accuracy of motor sequences during hunting. This work reveals an important hidden variable that shapes the temporal structure of motivation and decision-making.
Nature, 2020; 577
30407500: Marques JC, Orger MB
Clusterdv: a simple density-based clustering method that is robust, general and automatic.
How to partition a dataset into a set of distinct clusters is a ubiquitous and challenging problem. The fact that data vary widely in features such as cluster shape, cluster number, density distribution, background noise, outliers and degree of overlap, makes it difficult to find a single algorithm that can be broadly applied. One recent method, clusterdp, based on search of density peaks, can be applied successfully to cluster many kinds of data, but it is not fully automatic, and fails on some simple data distributions.
Bioinformatics, 2019; 35
29307558: Marques JC, Lackner S, Félix R, Orger MB
Structure of the Zebrafish Locomotor Repertoire Revealed with Unsupervised Behavioral Clustering.
An important concept in ethology is that complex behaviors can be constructed from a set of basic motor patterns. Identifying the set of patterns available to an animal is key to making quantitative descriptions of behavior that reflect the underlying motor system organization. We addressed these questions in zebrafish larvae, which swim in bouts that are naturally segmented in time. We developed a robust and general purpose clustering method (clusterdv) to ensure accurate identification of movement clusters and applied it to a dataset consisting of millions of swim bouts, captured at high temporal resolution from a comprehensive set of behavioral contexts. We identified a set of thirteen basic swimming patterns that are used flexibly in various combinations across different behavioral contexts and show that this classification can be used to dissect the sensorimotor transformations underlying larval social behavior and hunting. Furthermore, using the same approach at different levels in the behavioral hierarchy, we show that the set of swim bouts are themselves constructed from a basic set of tail movements and that bouts are executed in sequences specific to different behaviors.
Curr Biol, 2018; 28
28892088: Kim DH, Kim J, Marques JC, Grama A, Hildebrand DGC, Gu W, Li JM, Robson DN
Pan-neuronal calcium imaging with cellular resolution in freely swimming zebrafish.
Calcium imaging with cellular resolution typically requires an animal to be tethered under a microscope, which substantially restricts the range of behaviors that can be studied. To expand the behavioral repertoire amenable to imaging, we have developed a tracking microscope that enables whole-brain calcium imaging with cellular resolution in freely swimming larval zebrafish. This microscope uses infrared imaging to track a target animal in a behavior arena. On the basis of the predicted trajectory of the animal, we applied optimal control theory to a motorized stage system to cancel brain motion in three dimensions. We combined this motion-cancellation system with differential illumination focal filtering, a variant of HiLo microscopy, which enabled us to image the brain of a freely swimming larval zebrafish for more than an hour. This work expands the repertoire of natural behaviors that can be studied with cellular-resolution calcium imaging to potentially include spatial navigation, social behavior, feeding and reward.
Nat Methods, 2017; 14
25225400: Marques JC, Oh IK, Ly DC, Lamosa P, Ventura MR, Miller ST, Xavier KB
LsrF, a coenzyme A-dependent thiolase, catalyzes the terminal step in processing the quorum sensing signal autoinducer-2.
The quorum sensing signal autoinducer-2 (AI-2) regulates important bacterial behaviors, including biofilm formation and the production of virulence factors. Some bacteria, such as Escherichia coli, can quench the AI-2 signal produced by a variety of species present in the environment, and thus can influence AI-2-dependent bacterial behaviors. This process involves uptake of AI-2 via the Lsr transporter, followed by phosphorylation and consequent intracellular sequestration. Here we determine the metabolic fate of intracellular AI-2 by characterizing LsrF, the terminal protein in the Lsr AI-2 processing pathway. We identify the substrates of LsrF as 3-hydroxy-2,4-pentadione-5-phosphate (P-HPD, an isomer of AI-2-phosphate) and coenzyme A, determine the crystal structure of an LsrF catalytic mutant bound to P-HPD, and identify the reaction products. We show that LsrF catalyzes the transfer of an acetyl group from P-HPD to coenzyme A yielding dihydroxyacetone phosphate and acetyl-CoA, two key central metabolites. We further propose that LsrF, despite strong structural homology to aldolases, acts as a thiolase, an activity previously undescribed for this family of enzymes. With this work, we have fully characterized the biological pathway for AI-2 processing in E. coli, a pathway that can be used to quench AI-2 and control

quorum-sensing-regulated bacterial behaviors.
Proc Natl Acad Sci U S A, 2014; 111
25066084: Severi KE, Portugues R, Marques JC, O'Malley DM, Orger MB, Engert F
Neural control and modulation of swimming speed in the larval zebrafish.
Vertebrate locomotion at different speeds is driven by descending excitatory connections to central pattern generators in the spinal cord. To investigate how these inputs determine locomotor kinematics, we used whole-field visual motion to drive zebrafish to swim at different speeds. Larvae match the stimulus speed by utilizing more locomotor events, or modifying kinematic parameters such as the duration and speed of swimming bouts, the tail-beat frequency, and the choice of gait. We used laser ablations, electrical stimulation, and activity recordings in descending neurons of the nucleus of the medial longitudinal fasciculus (nMLF) to dissect their contribution to controlling forward movement. We found that the activity of single identified neurons within the nMLF is correlated with locomotor kinematics, and modulates both the duration and oscillation frequency of tail movements. By identifying the contribution of individual supraspinal circuit elements to locomotion kinematics, we build a better understanding of how the brain controls movement.
Neuron, 2014; 83
22384939: Pereira CS, Santos AJ, Bejerano-Sagie M, Correia PB, Marques JC, Xavier KB
Phosphoenolpyruvate phosphotransferase system regulates detection and processing of the quorum sensing signal autoinducer-2.
Autoinducer-2 (AI-2) a signal produced by a range of phylogenetically distant microorganisms, enables inter-species cell-cell communication and regulates many bacterial phenotypes. Certain bacteria can interfere with AI-2-regulated behaviours of neighbouring species by internalizing AI-2 using the Lsr transport system (encoded by the lsr operon). AI-2 imported by the Lsr is phosphorylated by the LsrK kinase and AI-2-phosphate is the inducer of the lsr operon. Here we show that in Escherichia coli the phosphoenolpyruvate phosphotransferase system (PTS) is required for Lsr activation and is essential for AI-2 internalization. Although the phosphorylation state of Enzyme I of PTS is important for this regulation, LsrK is necessary for the phosphorylation of AI-2, indicating that AI-2 is not phosphorylated by PTS. Our results suggest that AI-2 internalization is initiated by a PTS-dependent mechanism, which provides sufficient intracellular AI-2 to relieve repression of the lsr operon and, thus induce depletion of AI-2 from the extracellular environment. The fact that AI-2 internalization is not only controlled by the community-dependent accumulation of AI-2, but also depends on the phosphorylation state of PTS suggests that E. coli can integrate information on the availability of substrates with external communal information to control quorum sensing and its interference.
Mol Microbiol, 2012; 84
22137598: Rui F, Marques JC, Miller ST, Maycock CD, Xavier KB, Ventura MR
Stereochemical diversity of AI-2 analogs modulates quorum sensing in Vibrio harveyi and Escherichia coli.
Bacteria coordinate population-dependent behaviors such as virulence by intra- and inter-species communication (quorum sensing). Autoinducer-2 (AI-2) regulates inter-species quorum sensing. AI-2 derives from the spontaneous cyclisation of linear (S)-4,5-dihydroxypentanedione (DPD) into two isomeric forms in dynamic equilibrium. Different species of bacteria have different classes of AI-2 receptors (LsrB and LuxP) which bind to different cyclic forms. In the present work, DPD analogs with a new stereocenter at C-5 (4,5-dihydroxyhexanediones (DHDs)) have been synthesized and their biological activity tested in two bacteria. (4S,5R)-DHD is a synergistic agonist in Escherichia coli (which contains the LsrB receptor), while it is an agonist in Vibrio harveyi (LuxP), displaying the strongest agonistic activity reported so far (EC(50)=0.65μM) in this organism. Thus, modification at C-5 opens the way to novel methods to manipulate quorum sensing as a method for controlling bacteria.
Bioorg Med Chem, 2012; 20
21454635: Marques JC, Lamosa P, Russell C, Ventura R, Maycock C, Semmelhack MF, Miller ST, Xavier KB
Processing the interspecies quorum-sensing signal autoinducer-2 (AI-2): characterization of phospho-(S)-4,5-dihydroxy-2,3-pentanedione isomerization by LsrG protein.
The molecule (S)-4,5-dihydroxy-2,3-pentanedione (DPD) is produced by many different species of bacteria and is the precursor of the signal molecule autoinducer-2 (AI-2). AI-2 mediates interspecies communication and facilitates regulation of bacterial behaviors such as biofilm formation and virulence. A variety of bacterial species have the ability to sequester and process the AI-2 present in their environment, thereby interfering with the cell-cell communication of other bacteria. This process involves the AI-2-regulated lsr operon, comprised of the Lsr transport system that facilitates uptake of the signal, a kinase that phosphorylates the signal to phospho-DPD (P-DPD), and enzymes (like LsrG) that are responsible for processing the phosphorylated signal. Because P-DPD is the intracellular inducer of the lsr operon, enzymes involved in P-DPD processing impact the levels of Lsr expression. Here we show that LsrG catalyzes isomerization of P-DPD into 3,4,4-trihydroxy-2-pentanone-5-phosphate. We present the crystal structure of LsrG, identify potential catalytic residues, and determine which of these residues affects P-DPD processing in vivo and in vitro. We also show that an lsrG deletion mutant

accumulates at least 10 times more P-DPD than wild type cells. Consistent with this result, we find that the lsrG mutant has increased expression of the lsr operon and an altered profile of AI-2 accumulation and removal. Understanding of the biochemical mechanisms employed by bacteria to quench signaling of other species can be of great utility in the development of therapies to control bacterial behavior.
J Biol Chem, 2011; 286

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EFFECTS OF EARLY SOCIAL ENVIRONMENT ON ADULT ZEBRAFISH BEHAVIOUR – A NEURONAL AND TRANSCRIPTOMIC APPROACH

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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The social environment of an animal can have a profound and determining effect on its behaviour. During development, variations in the social structure provide inadvertent information that influences many aspects of individual development on a wide range of physiological and cognitive traits. In the present work, we studied the influence of environmental complexity in the development of different adult social phenotypes at three levels: behavioural, neuronal, and genetic. Therefore, zebrafish were raised in different social environmental complexities, by varying group size and group stability, until adulthood and then tested in different behavioural contexts. Our results indicate that distinct aspects of social complexity (i.e. group size and group stability) influence differentially adult social behaviour; for instance, group cohesion is influenced by group size, while the preference for conspecifics changes depending on group stability. At the neuronal level, animals raised in less complex social environments experience changes in neuronal densities, a significant reduction in brain size, in specific regions related to social information processing, and changes in gene expression patterns. These results indicate that variations in the social environmental complexity during development can profoundly impact an individual throughout their lifetime.

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ROLE OF ENVIRONMENT AND EXPERIMENTER IN REPRODUCIBILITY OF BEHAVIORAL STUDIES WITH LABORATORY MICE

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Behavioral phenotyping of mice has received a great deal of attention during the past three decades. However, there is still a pressing need to understand the variability caused by environmental and biological factors, human interference, and poorly standardized experimental protocols. The inconsistency of results is often attributed to the inter-individual difference between the experimenters and environmental conditions. The present work aims to dissect the combined influence of the experimenter and the environment on the detection of behavioral traits in two inbred strains most commonly used in behavioral genetics due to their contrasting phenotypes, the C57BL/6J and DBA/2J mice. To this purpose, the elevated O-maze, the open field with object, the accelerating rotarod and the Barnes maze tests were performed by two experimenters in two diverse laboratory environments. Our findings confirm the well-characterized behavioral differences between these strains in exploratory behavior, motor performance, learning and memory. Moreover, the results demonstrate how the experimenter and the environment influence the behavioral tests with a variable-dependent effect, often with mutually exclusive contributions. In this context, our study highlights how both the experimenter and the environment can have an impact on the strain effect size without altering the direction of the conclusions. Importantly, the general agreement on the results is reached by converging evidence from multiple measures addressing the same trait. In conclusion, the present work elucidates the contribution of both the experimenter and the laboratory environment in the intricate field of reproducibility in mouse behavioral phenotyping.

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NOISE-INDUCED ALTERATIONS IN THE BEHAVIOR OF THE CARIBBEAN HERMIT CRAB (COENOBITA CLYPEATUS) DURING THE SHELL SELECTION TEST

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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The littoral ecosystems and the animals inhabiting them are particularly impacted by noise pollution. Coenobita clypeatus (C. clypeatus) is a species abundant in the littoral zones that depends on basic behavioral patterns for survival. Although the detrimental effects of noise pollution on social- and threat-related behaviors are known, how noise affects C. clypeatus' ability to explore and choose its shells is uncertain. We aimed to test whether anthropogenic noise would impair C. clypeatus' investigative patterns and decision-making abilities during shell selection. To evaluate the effects of noise on C. clypeatus' behaviors, animals were subjected to either acute or chronic noise regimens. During the test, we assessed the investigation of three different shell resources (suboptimal-, optimal-, and supraoptimal-shells) and final shell selection. The results suggest that acute noise significantly alters the animal's exploration, increasing both locomotion and velocity, when compared to control- and chronically treated animals ($p<0.001$). Even though most animals, regardless of the noise conditions, opted for the optimal shell, only animals under chronic- and acute-noise regimens spent more time investigating and occupying the suboptimal shell ($p<0.05$). The observed noise-induced behavioral alterations may be modulated by an increase in the distraction and physiological stress of the animals. Therefore, these behavioral alterations may also impact the social-, defensive-, and reproductive behaviors of the animals inhabiting noise-polluted littoral areas. Future studies are necessary to understand the neural substrates underpinning noise-induced changes in C. clypeatus' behavioral responses.

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TRANSIENT AND PERSISTENT FEAR STATES IN DROSOPHILA MELANOGASTER REVEALED BY A HIGH-THROUGHPUT BEHAVIORAL ASSAY

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Different taxonomic groups exhibit very similar responses to immediate threats that have been grossly categorized into flight, freeze or fight. It is remarkable that animals with very distinct morphologies, occupying very different environmental niches, display one of the three universal defense modules in a context dependent manner. This convergence in defensive responses all across the animal kingdom appeals for its evolutionary value. Here we present a high-throughput behavioral assay (behavioral paradigm, experimental setup, fully-automated tracking and analysis pipeline) to study Drosophila melanogaster responses to repeated inescapable visual looming-stimulus mimicking a predator repeating its attack after a failed attempt at catching its prey. The rich repertoire of defensive behaviors, together with its arsenal of tools to dissect neuronal circuits, makes the fly a valuable model organism to tackle the behavioral and neuronal basis of defense responses. Using this assay, we can distinguish two phases of the defensive responses - a startle response that is aligned with the stimulus presentation and shows a transient profile, as well as defensive states that emerge following the startle response and can persist for longer periods of time, such as sustained freezing and escapes. Focusing on freezing responses we tested the role of different looming-responsive visual projection neurons and found that looming triggered freezing behavior relies on the activity of several visual projection neurons. Our findings suggest that freezing arises from the coordinated activity of different neural input pathways even when driven by the same stimulus.

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EFFECT OF EMBRIONAL VALPROINIC ACID AND DELTAMETHRIN TREATMENT ON SOCIAL BEHAVIOR IN DOMESTIC CHICKS (GALLUS GALLUS)

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Embryonic exposure to valproic acid (VPA) is known to produce sociability deficits, in several vertebrate species, resembling human autistic phenotypes. Chicks of Galliform birds, known to display early preference behaviours, have been used extensively for neurobehavioural studies. Our question was whether the investigated environmental contaminant, deltamethrin, can cause autism-specific behavioural abnormalities. The secondary goal was to create a potentially new pharmacological model of autism and compare it to the widely used model of valproic acid. Here, domestic chicken eggs were injected with sodium valproate (200 µl of 35 µmol/l solution) or with vehicle (distilled water) and deltamethrine (1.5mg/kg) on the 14th day of incubation. After hatching, the chicks were tested for sociability, and social memory before and after social isolation. Our findings confirm previous studies, reporting an adverse effect of VPA on embryonic development, including a tendency for aborted or delayed hatching and, occasionally, for locomotor disorders in a small percentage of birds. The most prominent finding was attenuation of sociability of VPA-exposed birds. Social memory of familiarized individuals is not yet formed in chicks at this age. Although deltamethrin treatment caused minor changes in behaviour, it did not cause a behaviour pattern similar to VPA, it was not associated with a decrease in sociability and vocalization. There is probably no direct association between deltamethrin and the incidence of autism. It seems that embryonic deltamethrin treatment is not an appropriate chemical model for autism.

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BOARD NUMBER: S07-382

TEMPORAL COORDINATION OF DANIONELLA C. VOCALISATIONS

POSTER SESSION 07 - SECTION: NEUROETHOLOGY AND EVOLUTION OF THE NERVOUS SYSTEM

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Danionella cerebrum is an emerging model organism for the neuroethology of acoustic communication. *Danionella c.* possesses the smallest known vertebrate brain and is sufficiently transparent to allow for non-invasive optical interrogation of the brain even in adult animals. Additionally, male *Danionella c.* are known to produce characteristic click bursts at 60 and 120 Hz. These bursts occur in various lengths, grouped into sequences and often in the context of agonistic behaviour. Here, using a sub-wavelength acoustic triangulation setup and identity tracking in video recordings, we present the first characterisation of the vocal repertoire of *Danionella c.* at the level of the individual. We describe the temporal coordination of vocal interaction, as well as its relationship to social hierarchy and investigate possible behavioural motifs that accompany vocalisation episodes

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WAXHOLM SPACE ATLAS OF THE RAT BRAIN VERSION 4: A VOLUMETRIC ATLAS ENABLING DATA INTEGRATION AND ANALYSIS

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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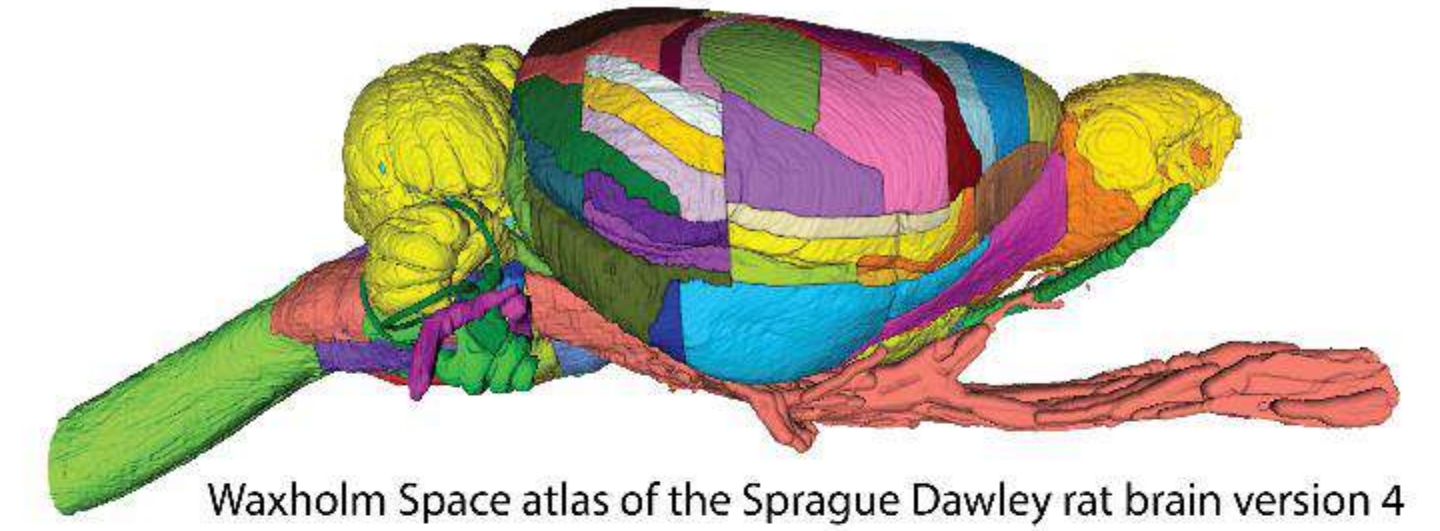
Anatomical atlases for the murine brain are important reference resources used to define, analyze and communicate locations in experimental neuroscience research. The Waxholm Space atlas of the Sprague Dawley rat brain v3 (RRID: SCR_017124) is an open-source volumetric atlas featuring 118 anatomical delineations defined in a high-resolution magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) dataset acquired ex-vivo from an adult Sprague Dawley brain. The atlas provides a spatial framework for integration and analysis of multiscale and multimodal rat brain data. It is developed by the EU Human Brain Project and shared via the EBRAINS research infrastructure (https://ebrains.eu/service/rat-brain-atlas). However, interpretation and analysis of data registered to this atlas has so far been limited by the lack of detailed subdivision of several major brain regions. We have revised and upgraded the Waxholm Space rat brain atlas, based on expert interpretations of MRI/DTI contrast combined with spatially registered histological images and published reference resources. We here present version 4 of the Waxholm Space rat brain atlas with 222 structures and full brain coverage, including complete and revised subdivisions of the cerebral cortex, striatopallidal region, midbrain dopaminergic system and thalamus cell groups and main fiber tracts. The atlas has been integrated in several open-source tools and workflows. We demonstrate how use of the atlas improves interpretation, integration, analysis and dissemination of rat brain data. Funded from EU Horizon 2020, Specific Grant Agreement No. 945539 (Human Brain Project

SGA3).


Waxholm Space atlas of the Sprague Dawley rat brain version 4
new in version 4
Cortex
Thalamus
Basal ganglia
Hippocampal region (v2)
Auditory system (v3)

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MACBRAIN RESOURCE: ARCHIVED PROCESSED AND UNPROCESSED RHESUS MONKEY BRAIN TISSUE AVAILABLE FOR DE NOVO NEUROSCIENCE STUDIES

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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The MacBrain Resource Center (MBRC), in the Department of Neuroscience at Yale University, currently houses 7 different collections of macaque monkey brain tissue available to the research community at large (https://medicine.yale.edu/neuroscience/macbrain/). Among the available materials are Dr. Pasko Rakic's tritiated thymidine injections in pregnant monkeys to locate newly born neurons in the brains of the offspring (Collection 1). Materials produced in these pioneering studies of neurogenesis continue to be used today to address the basic tenets of neurodevelopment. Available in Collection 5 are thousands of plastic-embedded electron microscopy (EM) blocks of various brain areas including visual, sensorimotor, and prefrontal cortices from NHPs ranging in age from embryonic to postnatal. These EM blocks are available for analysis of synapses and other cellular components at the ultrastructural level. The growing Collection 6 houses the current histo- and immunohistochemical work of Dr. Alvaro Duque to identify the numbers and distributions of different cell types in male and female NHPs from newborn to elderly. At present, over 10,000 coronal sections, spanning rostral to caudal and organized in series by type of label and brain, are zoomable and publicly available. The Collections in the MBRC constitute the largest public and virtually accessible collection of NHP brain tissue in the world and a modern new tool for Neuroscience Research with transparent access to the raw data and super-efficient sharing of resources that contributes to lowering the need to sacrifice animals. Supported by NIH MH113257.

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AVOIDING BIAS IN ESTIMATING PARTICLE SIZE AND DENSITY FROM 2D IMAGES WITH APPLICATION TO CELLS AND SYNAPTIC VESICLES

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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Estimating the 3D size and density of a distribution of particles from their 2D projection is a classic inverse problem of stereology. Here, we investigated the analytical solution for estimating particle size by Keiding et al. (1972) that accounts for missing profiles, i.e. 'lost caps', via a fixed cap-angle limit (ϕ). Using Monte Carlo simulations, we tested the Keiding model over a range of section thickness (T) and ϕ and found the model accurately estimated the 3D diameter distribution (F(d)) from the measured 2D diameter distribution (G(d)). However, the estimates were less accurate when true ϕ > 50° since G(d) ≈ F(d) under this condition and ϕ was indeterminable. To further validate the Keiding model, we estimated F(d) and ϕ from G(d) for synaptic vesicles in an electron-tomography z-stack and found excellent agreement with direct 3D measurements. To estimate the 3D particle density from the measured 2D density, we developed a correction formula that accounts for lost caps via ϕ. Analysis of this ϕ-correction method showed it was more accurate than using the minimum observed diameter or the widely used disector method; however, it required an accurate estimate of ϕ (i.e. a true ϕ < 50°). Finally, we applied these methods to estimating the size and density of cerebellar granule-cell somata and nuclei and mossy-fiber-terminal vesicles in rats and mice. Results showed planar sections (where T << particle size) were best for estimating both particle size and density.

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TOWARDS A COMMUNITY-DRIVEN PIPELINE FOR INTEGRATING MULTIMODAL BIOIMAGING DATA.

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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The rapid development of many multimodal microscopy techniques and image analysis tools for image visualization, preprocessing and quantification reinforces the need for a common research data management strategy for microscopy image metadata standardization. A large number of heterogeneous imaging file formats, high-quality analysis workflows, and software tools are being developed and utilized. The challenge lies in streamlining the way we organize, analyze, archive, and share the data and metadata of biological images. There is a strong emphasis on the significance of extensive metadata (experimental, acquisition, and analytical metadata) in ensuring image data quality, reproducibility, and scientific outcome. We aim to provide a strategy for the integration of bioimage data and metadata with electronic lab notebooks and repositories in the context of FAIR data principles. We are developing solutions to document and link the experimental metadata in an electronic lab notebook (eLabFTW) alongside microscope specifications, image acquisition settings, and analysis workflows in a standardized manner. This work will not only focus on optimizing the use of available solutions but will also encourage the development of a tool for interoperable exchange of data between services such as cloud storage, HPC clusters, and bioimaging software applications (ImageJ/Fiji, Galaxy, etc). This work will also highlight our aligned efforts with major international and national bioimaging community consortiums such as NFDI4BIOIMAGE. We acknowledge that a completely automated solution may not be fully achieved, nevertheless, implementation of such a strategy will enable seamless data integration and would support the bioimaging community in optimizing the entire scientific data flow.

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OPENMINDS - FLEXIBLE METADATA MODELS FOR NEUROSCIENCE

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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Providing a framework for standardized metadata descriptions is particularly challenging in neuroscience due to the heterogeneity of data. The multimodal nature of neuroscientific data and the fact that they have a wide range in spatial and temporal scales is making the definition of an adequate set of metadata practically challenging and difficult. Hence, suitable metadata schemas for neuroscientific data should be flexible as well as restrictive enough to accommodate the inter-individuality of the data while keeping them overall comparable.
To achieve this goal, we launched the **open M**etadata **I**nitiative for **N**euroscience **D**ata **S**tructures (**openMINDS**). Powered by the Human Brain Project and EBRAINS, openMINDS develops and maintains interlinked metadata models composed of schemas that are tailored to describe neuroscience research products in graph databases, such as the EBRAINS Knowledge Graph.
openMINDS can handle a wide variety of research products (experimental/simulated data, computational models, software tools, metadata/data models, and brain atlases) as well as different granularity levels of provenance description (basic, advanced, in-depth). In addition, for better data integration, openMINDS maintains libraries of controlled metadata instances for general terms, licenses and content types that include links to external resources where applicable (e.g., InterLex Project, Knowledge Space, IANA.org). Here we present the infrastructure behind openMINDS and explain how we meet the challenge of creating a community-driven, flexible, but standardized metadata framework for sharing neuroscience data via graph databases.

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DATA SHARING VIA EBRAINS: WHY AND HOW

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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Open access to data increases the exposure and impact of the research, and enables the reuse and reanalysis of shared data in new combinations. In neuroscience, the heterogeneous nature of the research data poses a substantial challenge to maximize the opportunities of data sharing. The EBRAINS Curation Services, developed by the Human Brain Project, meet these and other challenges of open data by providing the necessary data stewardship and workflows for sharing multimodal neuroscientific data. We have developed novel standards and tools to make data discoverable and comparable across modalities. Additionally, all these efforts aim to follow the FAIR guiding principles (*Wilkinson et al., Scientific Data 3:160018, 2016*) to make data Findable, Accessible, Interoperable and Reusable. These workflows and the underlying infrastructure is continually modified according to improvements in infrastructure and researcher needs. Here we present our most updated strategies to integrate heterogeneous neuroscientific data to the EBRAINS data sharing platform. We demonstrate how researchers can share experimental or computational data of all modalities, including models and software, and highlight the benefits of sharing data via EBRAINS for the individual researcher. This project has received funding from EU Horizon 2020, Specific Grant Agreement No. 945539 (Human Brain Project SGA3).

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THE COLLECTION OF HUMAN BRAIN DEVELOPMENT IN THE RESEARCH INSTITUTE OF HUMAN MORPHOLOGY, MOSCOW

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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Laboratory of Nervous System Development of Research Institute of Human Morphology, Moscow possesses the unique Collection of prenatal human development, which was founded more than 40 years ago. Up-to-date, it consists of more than 200 human embryos and fetuses which staged from the second developmental week (2 pcw) until birth. The Collection is presented as wet material, also as histological, immunohistochemical and electron microscopic preparations, including serial sections of the developing human brain. This Collection could complement the world-famous Carnegie collection with wider samples and fetal cases. On our Laboratory of Nervous System Development website, a section exclusively devoted to the prenatal human brain development is presented https://brainmicroscopy.com/en/collection/homo/brain-development/. In 2021, our online project "The collection of human brain development of the Research Institute of Human Morphology, Moscow." has been selected for a grant of the FENS History Committee. We plan to expand the online content, gradually increasing the section image data with new samples from the Collection, creating articles, that complement images, detailed schemes and diagrams, which illustrate the prenatal developmental course and patterns of the human brain. In the frames of FENS History of Neuroscience online project, we would like to make the Collection more visible worldwide. Now, on the basis of our collection, we have launched human prenatal brain development Atlas that aims to create spatio-temporal reference maps of whole fetal brain on the various early ontogeny stages. This new project is supported by the Russian Science Foundation (RSF) grant 22-15-00172.

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COMPARING THE ANATOMY OF THE ANTENNAL LOBE ACROSS BUMBLEBEE SPECIES

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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Bumblebees play an important role as pollinators in natural and agricultural ecosystem. Bumblebees detect scents using olfactory sensory neurons located in the antennae; the olfactory signals are processed in the antennal lobe . *Bombus terrestris* are the most extensively studied and the only commercially available bumblebee species in the UK and in Europe. Other bumblebee species have so far received little attention in terms of the neuroethology and sensory neuroscience. The aim of this project was to study species-specific features of the olfactory anatomy of bumblebees. The project will provide important information for neuroethological studies of bumblebees, with possible insights into their olfactory preferences and the evolution of their olfactory systems. We focused on several species, local to Durham, UK. Worker and drone specimen collected in Durham in summer 2021 and DNA-barcoded via PCR amplification of CO1 and 16S rRNA mitochondrial genes to ascertain their species. Next, the brains of workers and drones were dissected, immunostained and imaged on a confocal microscopy. Finally, we created 3D model of the bee brains using the Amira software and measured the volume of the antennal lobe glomeruli in worker bees of *Bombus lapidarius*, *B.hypnorum* and *B.pascuorum*. From the data we have obtained we have not identified any particularly large glomeruli in workers in the studied species of bumblebees as there was no significant variabilities in glomeruli volume. Importantly the three-dimensional reconstruction, we have established will serve a tool to identify the respective target glomeruli with further physiological characterisation to reveal possible structure-function relationship.

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A NEW OPEN, HIGH-RESOLUTION, MULTISHELL, DIFFUSION-WEIGHTED IMAGING DATASET OF THE LIVING SQUIRREL MONKEY

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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Although very well adapted to brain study, Magnetic Resonance Imaging (MRI) remains limited by the facilities and capabilities required to acquire data, especially for non-human primates. Addressing the data gaps that result from these limitations requires making data more accessible and open access. Despite the regular use of the Squirrel monkey (Saïmiri sciureus) in neurosciences research, no in vivo diffusion dataset is yet openly available for this species. Here we propose a new anesthetized high-resolution diffusion dataset for Saïmiris sciureus to be shared on the PRIMatE Data Exchange (PRIME-DE) database.
We used an ultra-high-field (11.7 Tesla) MRI scanner to acquire multishell diffusion-weighted imaging data on six living squirrel monkeys. Subjects are all females aged from eight to ten years. Diffusion images were acquired with b-values of 2000, 1000 and 300s/mm² with 64, 29 and 7 directions, respectively.
We propose an overview of squirrel monkey brain connectivity based on this preliminary dataset. We investigated long-range connections using tractography analysis. We focused on brain microstructure with Neurite Orientation Dispersion and Density Imaging analysis (NODDI). Finally, we used the ActiveAx analysis technique to estimate axons diameter in the corpus callosum.
This dataset will help conduct and replicate studies on brain connectivity, microstructure or evolution through cross-species cross modalities comparisons.

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INFLUENCE OF NEUROMODULATORY SYSTEMS IN THE HINDLIMB REPRESENTATION IN THE DEVELOPING SOMATOSENSORY CORTEX OF THE RAT

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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The vast majority of cortical synapses are found in the neuropil which is implicated in multiple and diverse functions underlying brain computation. Unraveling the organizing principles of the cortical neuropil requires an intricate characterization of synaptic connections established by excitatory and inhibitory axon terminals, of intrinsic and extrinsic origin and from ascending projections that govern the function of cortical microcircuits through the release of neuromodulators either through point-to-point chemical synapses or diffuse volume transmission. The hindlimb representation of the somatosensory cortex (HLS1) of two-week old Wistar rats has served as a model system to dissect the microcircuitry of neurons and their synaptic connections. In the present study, we quantified the fiber length per cortical volume and the density of varicosities for catecholaminergic, serotonergic and cholinergic neuromodulatory systems in the cortical neuropil using immunocytochemical staining and stereological techniques. Acquired data were integrated into a computational modeling framework to reconcile the specific modalities and predict the effects of neuromodulatory release in shaping neocortical network activity. We predict that ACh and DA and 5-HT desynchronize cortical activity by inhibiting slow oscillations (delta range), and that 5-HT triggers faster oscillations (theta). Moreover, we found that high levels (>30%) of neuromodulatory volumetric transmission (VT) are sufficient to induce network desynchronization, but also that combining volume release with synaptic inputs leads to more robust and stable effects, and lower levels of VT are needed to achieve the same outcome (10%).

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FLATTENING OF ENHANCED CORTICAL ATLASES OPENS UP NEW POSSIBILITIES FOR DATA-DRIVEN MODELING AND DATA VISUALIZATION

POSTER SESSION 07 - SECTION: DATA SHARING IN NEUROSCIENCE

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Digital brain atlases define a hierarchy of brain regions and their locations in three-dimensional space. They provide a standard coordinate system in which diverse datasets can be integrated for visualization and analysis, and they enable building of data-driven computational models of brain regions. For atlases of the cerebral cortex, additional information is required to work effectively with its particular, layered architecture and curved geometry. Although some approaches have been employed in the literature, no usable method to produce such information has been made openly available. To fill this gap, we describe here methods to enhance a cortical atlas with three auxiliary, voxel-wise datasets: first, a field of cortical depth; second, a field of local orientations towards the cortical surface; and third, a *flat map* of the cortical volume: a two-dimensional representation with the property that each pixel maps to a sub-volume in which the layer structure is preserved, akin to a cortical column. We apply these methods to a digitized version of Paxinos & Watson's atlas for rat somatosensory cortex and to the isocortex region of the Allen Mouse CCFv3, and define metrics to assess the quality of our results. Among the many applications of the resulting flat maps to computational modeling, we show their usefulness for: decomposing the cortical volume into columns, positioning and orienting input fibers, defining a topographic mapping for white-matter connections between sub-regions, and visualizing connectivity tracing and simulated neuronal activity. We provide an open source implementation of our methods for the benefit of the community.

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THE WIENER DECONVOLUTION TOOLS: A NOVEL SOFTWARE FOR DECONVOLVING LIGHT SHEET AND CONFOCAL MICROSCOPY DATA.

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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We developed a novel deconvolution software that stunningly increases the visibility of minute details in light sheet microscopy and confocal microscopy recordings. Prior to deconvolution the software can optionally apply adaptive background subtraction by rolling ball filtering along the three principal axes . As demonstrated by the presented 3D-reconstructions this approach stunningly increases the level of detail in data sets depicting small structures (as e.g. dendrites or nerve fibers) located on a inhomogeneous, diffuse background. By automatic block-wise processing the *Wiener Deconvolution Tools* can process image stacks of virtually unlimited size even on small computers with only 8 or 16 GB RAM. Via consequent usage of parallelization as well as optional GPU-acceleration the software works with high speed: On a PC equipped with a state of the art NVIDIA graphic board a stack containing about 1 billion voxels can be deconvolved in less than 10 minutes. Our deconvolution algorithm uses flux-preserving regularization that does not modify the photogrammetry of the image data. To our knowledge, this regularization approach has not been used for deconvolving microscopy data before. Preservation of photogrammetry is especially helpful for performing quantitative comparisons of signal intensities between different images or regions. For non-commercial use a fully functional command line version of the *Wiener Deconvolution Tools* can be downloaded as free software. An additional graphical user interface (GUI) providing further features as batch processing, destriping of light sheet microscopy data by directional frequency filtering and basic 3D visualization is commercially available from the Technical University of Vienna.

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BOARD NUMBER: S07-395

SEARCHLIGHT ANALYSIS FOR INTRACRANIAL EEG RECORDINGS

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Aims: Searchlight analysis (SLA) is a multivariate pattern analysis (MVPA) method for identifying locally informative areas. Here we investigate SLA's performance in highlighting contrasts of cognitive task conditions using intracranial EEG (iEEG) data, and we benchmark the results against a permutation cluster test (PCT). **Methods:** Ten subjects implanted with depth electrodes performed a facial emotion discrimination task. We used iEEG recorded during the task, to contrast activations produced by happy and angry faces, using PCT and SLA. The iEEG data was cleaned of artifacts and epileptic spikes, resampled to 256Hz, baseline corrected, and filtered in the 55-145 Hz high gamma band. The searchlight radius was defined as a sphere of 25mm radius centered on each electrode. For each electrode, we trained MVPA models using all electrodes located within the searchlight radius, with 10-fold cross-validation, and we assessed significance above the random chance level. The Jaccard index was used to compare the result of PCT and SLA, at the group level. **Results:** The Jaccard coefficient was 0.24 and 0.35 for the left (LH) and the right (RH) hemisphere structures. SLA highlighted up smaller effects within the same number of trials and discriminates task conditions in more brain structures than PCT (SLA: 17 LH and 12 RH, $p<0.01$; PCT: 9 LH and 15 RH, $p<0.05$). **Conclusions:** SLA identified an extended brain network involved in emotion processing, supporting the hypothesis of different emotions being processed by distributed activation patterns, rather than locally, in a few specialized locations of the brain.

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NIT: A TOOLBOX FOR INFORMATION THEORETICAL ANALYSIS OF NEURAL DATA

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Understanding how neurons encode, transmit, and process information is fundamental for uncovering the working principles of neural systems. Information theory provides a principled framework to shed light on such questions. Its application to the analysis of neural data is especially advantageous, allowing to uncover non-linear interactions of any order in a model-free way. However, the quantification of information from neural data is affected by major practical difficulties. This is especially true for two-photon calcium imaging data, characterized by low signal-to-noise ratio and temporal resolution. Here, we present Neuroscience Information Toolbox (NIT), a toolbox for the accurate information theoretical analysis of neural data. NIT features statistical estimators of Mutual Information including both discrete and continuous estimators, the analysis of directed information transmission among neurons, and the calculation of information quantities based on Partial Information Decompositions (e.g., Intersection Information). A selection of sampling bias corrections is available, together with routines for dimensionality reduction and neural decoding. Through simulations of biophysically plausible fluorescence emission in pyramidal neurons, we investigated how to best apply NIT to recover neural spike rate information from calcium data over a wide range of experimental (e.g. indicator, signal-to-noise ratio), biophysical (e.g. firing rate), and computational (e.g. discrete vs continuous estimators) conditions. NIT enabled us to retrieve up to > 90% of the information in spike rates from fluorescent transients only. Our conclusions were backed up by validation against experimental data. Altogether, our work demonstrates that NIT can be successfully used to analyse two-photon calcium imaging recordings.

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RELATIONSHIP BETWEEN TONIC DOPAMINE LEVEL AND APERIODIC COMPONENT FROM LOCAL FIELD POTENTIALS OF THE SUBTHALAMIC NUCLEUS IN HEMIPARKINSONIAN RATS

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Electrophysiological biomarkers provide useful information to gain an etiological understanding of Parkinson's disease (PD). However, there are limited studies to simultaneously investigate electrophysiological biomarkers and dopamine tone with invasive recordings. We recorded dopamine levels in the striatum of hemiparkinsonian rats using fast-scan cyclic voltammetry (FSCV). We simultaneously recorded local field potentials (LFP) in the subthalamic nucleus (STN). During the recordings, we acutely modulated dopamine levels with an intraperitoneal injection of levodopa. In the post-analysis, LFP was separated into the parameterized periodic and aperiodic components from the fitting oscillations and one-over-f (FOOOF) algorithm, which has recently been developed by Voytek and colleagues. Recorded currents from the FSCV were processed to extract both phasic and postulated tonic dopamine information by using our novel mathematical method. We confirmed the feasibility of the method for correcting drift effects due to capacitive currents over time in saline. Using the novel method, we successfully eliminated drift effects in vivo recordings. We investigated the correlations between various properties of beta band oscillations (e.g., power, frequency, bandwidth, and non-sinusoidal shape), aperiodic parameters (i.e., aperiodic offset and exponent), and phasic/tonic dopamine level. Finally, we performed machine learning-based prediction of phasic/tonic dopamine levels to find the best feature reflecting pathological information. Two methods, support vector machine (SVM) and deep neural networks (DNN), were chosen in this study. We suggest that aperiodic parameters change according to the dopamine level, and the aperiodic component has synergistic effects with the oscillatory properties resulting in the effective prediction of the dopamine level.

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ANALYSIS OF NEURONAL ACTIVITY IN 3D VOLUME OF TISSUE-CLEARED MOUSE BRAIN: CHALLENGES AND ADVANCES

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Aims: Reporter mouse lines are used to study responses of defined neuronal populations in the brain, by using activity-dependent regulator elements as drivers for Cre-induced recombination and cell tagging, allowing to trace labelled cells. We used reporter mice to map neuronal activity in response to aversive stimuli. **Methods:** Labelled neurons are spread throughout the brain. A common practice for their detection is immunohistology on sections, possibly resulting in bias. To overcome these limitations, tissue clearing (TC) techniques have emerged. The main principle of TC is the matching of the refractive index (RI) of the sample to the RI of the imaging media. The tissue is subjected to fixation, delipidation, depigmentation, and dehydration steps, followed by RI-matching, which renders it transparent. The need for fast imaging of large samples led to the development of light-sheet microscopes, enabling the investigation of labelled cells in an intact volume of an organ in an unbiased and high throughput manner. **Results:** Using commercial software packages, we established a pipeline of data pre-processing and stitching, focussing on overcoming the artefacts resulting from sample preparation and imaging. Further computationally intensive steps are required to align labelled cells to the common coordinate system of a reference atlas and perform group comparison. **Conclusions:** Although TC has become more widespread, the data analysis remains a challenge. Commercial software lacks necessary functionality to perform brain-wide signal annotation and alignment, whereas open-source software is not easy to use. In this study, we combined and optimized existing tools to achieve this goal.

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USER FRIENDLY ANALYSIS TOOLBOX FOR THREE-DIMENSIONAL LIGHT-SHEET MICROSCOPY DATA IN HEMISPHERIC AND WHOLE MOUSE BRAIN SAMPLES

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Aims: Currently, only a few analysis tools for mapping neuronal activity in three-dimensional light-sheet microscopy data are available. These tools mainly enable the annotation of fluorescent cell signals to a reference brain atlas but lack sufficient statistical analysis. Furthermore, the available tools are somewhat complicated to use for researchers without a computational background. Here we introduce a user-friendly graphical user interface (GUI) driven toolbox, that enables researchers to investigate hemispheric and whole brain tissue on a different level. The toolbox also includes various automated statistical analysis options for comparative studies of brain region activity. **Methods:** The toolbox integrates ClearMap2 (Kirst et al., 2020) and CellFinder (Tyson et al., 2021) in a PyQt5 based graphical user interface. The deployment as a docker container allows an easy and fast installation. Cell annotation results are visualized with napari. Furthermore, the toolbox enables comparative differential gene abundance analysis between conditions on custom selectable phylogenetic levels based on the Allan Brain Atlas ontology. It performs abundancy analysis on a relational and a density-based level. **Results:** We established a toolbox to comparatively analyse hemispheric and whole brain light-sheet microscopy data. The toolbox is designed to allow also inexperienced users a fast and reliable analysis. **Conclusion:** Analysis of whole brain tissue has high potential for investigating neuronal brain activity but requires novel computational approaches to be utilized. Our toolbox includes first attempts to automatize whole brain tissue analysis. New approaches and ideas will expand the range of possibilities and tools for mapping whole brain neuronal activity.

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AUTOMATED QUANTIFICATION AND 3D COLOCALIZATION ANALYSIS OF FLUORESCENT NUCLEAR MARKERS WITH AN IMAGEJ MACRO: EXAMPLE OF A REPRODUCIBLE, TRACEABLE AND ACCESSIBLE WORKFLOW FOR BIOLOGISTS

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Aims: While extremely common in life sciences, analysis of microscopy images is often insufficiently described in research articles, rendering workflows impossible to replicate and potential image mishandlings difficult to appreciate. We sought to develop an efficient and reproducible method to quantify and study the colocalization of two fluorescent nuclear markers revealed by immunohistochemistry on rat brain slices. Methods: Using a spinning disk confocal microscope, we acquired more than 150 multichannel z-stack tiled images spanning the anterior cingulate or parietal cortices and tracked the fluorescent expression of the proliferation marker 5-ethynyl-2'-deoxyuridine (EdU) and the endothelial cell-specific marker ERG in these two regions of interest (ROIs). Image analysis was achieved in Fiji using a semi-automated macro approach. After testing a subset of images to select appropriate filtering and thresholding parameters, ROIs were drawn and images were batch-processed automatically (filtering, segmentation, quantification and 3D colocalization). Finally, macro-generated binary segmentation and colocalization masks enabled to locate and review each colocalization event and to perform and document corrections if needed, such as ROI adjustment or exclusion of out of focus stack extremities. Results: With this approach requiring no extensive programming knowledge, we achieved appropriate processing of the majority of our images, saving precious time and limiting biases, while recording all quantitative results, parameters used and post-processing corrections performed. Conclusions: In the context of rising awareness regarding the need for reproducibility in microscopy image analysis, we provide an example of a reproducible and reliable workflow that may be applied to similar tasks.

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A PYTHON HANDS-ON TUTORIAL ON NETWORK AND TOPOLOGICAL NEUROSCIENCE

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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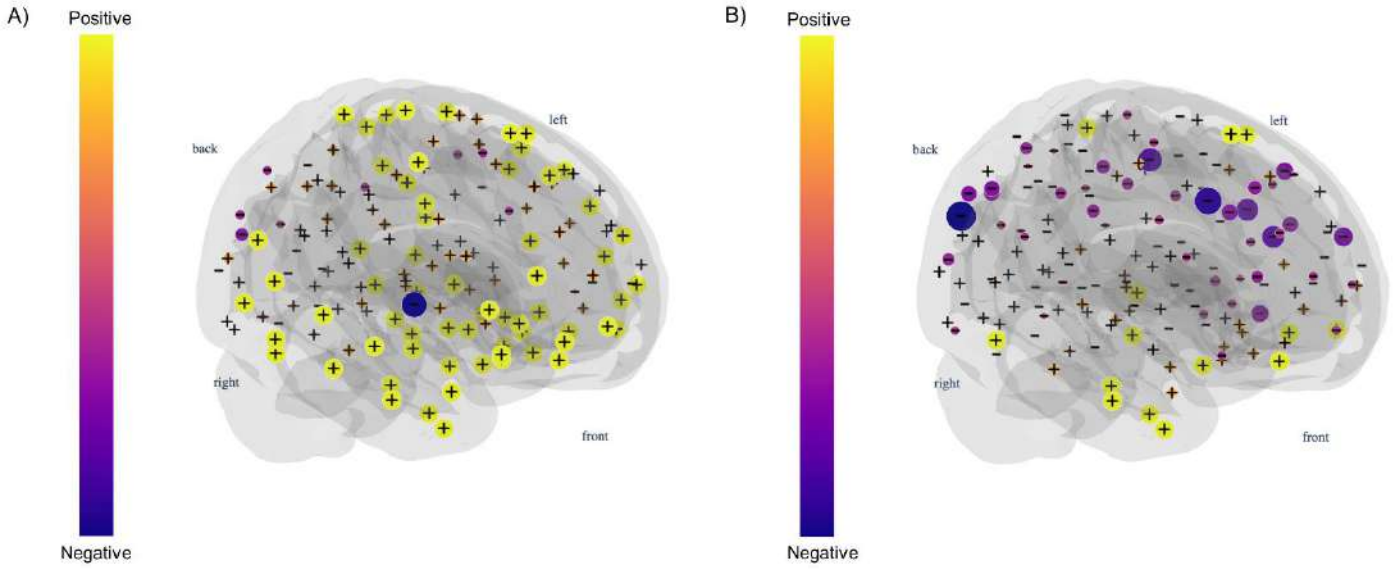
Network neuroscience aims to uncover brain functioning through the prism of connectivity. Graph theory (GT) and topological data analysis (TDA) are two frameworks that can be used to study brain connectivity. However, the mathematical abstractions of each of these frameworks can be daunting to inexperienced researchers. In this project, our goal was to provide an easy-to-grasp theoretical and computational tutorial to explore neuroimaging data using GT and TDA, therefore facilitating the accessibility, data visualization, and comprehension for newcomers to the field. Our tutorial focuses on resting-state functional magnetic resonance imaging (rsfMRI) data; however, the main concepts and tools discussed in this paper can be extrapolated to other imaging modalities. We used different Open Science-related tools, including GitHub, Jupyter Notebooks, Python, Binder, Zenodo, and an open-access dataset (rsfMRI dataset – 1000 functional connectomes project). Our work resulted in a manuscript covering the theoretical overview of the two frameworks and a set of Jupyter Notebooks that explain how to compute the different GT and TDA metrics in Python. In addition, we developed a high-order 3-D brain network visualization tool (example in Figure 1), a novelty to the field. All the material can be found at https://github.com/multinetlab-amsterdam/network_TDA_tutorial. We hope to have facilitated the comprehension of some aspects of the computation and visualization of network and topological neuroscience.



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EBRAINS LIVE PAPERS - INTERACTIVE RESOURCES AND SUPPLEMENTARY MATERIALS FOR NEUROSCIENCE

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Introduction:
Computational neurosciences lacks an established system for distributing code, data and other related resources. This significantly diminishes the utility of scientific outputs and hinders reproducibility. The EBRAINS Live Papers is an online platform that holds promise to help address these shortcomings. **Methods:**
All data is stored in the EBRAINS Knowledge Graph. Being a graph-based database, it interlinks all data units thereby offering a high degree of data provenance. The integrated Live Paper Builder tool allows for creation of rich, interactive web pages without requiring any web development skills. It allows for easy linking to resources in community databases, thereby acting as an integrator of scientific resources spanning diverse data types. Live papers can also be password-protected to enable restricted access while under review. Additionally, the EBRAINS platform issues DOIs for published Live Papers. **Results:**
EBRAINS Live Papers are interactive web pages bringing together code, models, and data. They complement published scientific articles by providing access to the underlying models, data or analyses. Interactivity is a prominent feature with several integrated tools and services that allow users to download, visualize or simulate. There are already over 20 published live papers with interactive supplementary material associated with corresponding journal publications. **Conclusions:**
Live Papers are intended at making neuroscience publications more valuable to the scientific community by offering a holistic view of the various digital components of a publication. It promises to incentivise researchers to publish and share such resources, while ensuring that they are duly acknowledged when these resources are utilized.

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MACHINE LEARNING-BASED DENDRITIC SPINE SEGMENTATION AND QUANTIFICATION_

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Aims: Dendritic spine plasticity includes the changes of spine number and morphology and has central role in brain development, physiology and pathology. Thus, the quantification and classification of spines in microscopy images is used to gain insights into neuronal function. Unfortunately, current tools for automated spine segmentation and evaluation still underperform manual analysis, while manual measurements of individual spines are time consuming and subjective. Therefore, we aimed to develop new automated tool for precise dendritic spine segmentation and quantification.
Methods: *Thy1::*EGFP mice brain sections were imaged with confocal laser scanning microscope. Images were pre-processed with FIJI software and used as a data set for training and segmentation in *Zeiss* APEER platform. Model was trained for semantic segmentation using deep learning U-net architecture. FIJI software was used for quantification of segmented spines and for the evaluation of the model.
Results: We used image pre-processing plugins to facilitate the same class object recognition in images acquired under different sample preparation or imaging conditions. We developed and evaluated APEER model for dendritic spine segmentation and showed that our tool can be applied to measure dendritic spine density in pyramidal neurons from mouse hippocampus.
Conclusions: Our method proposed fast and simple tool to detect and segment dendritic spines in two-dimensional maximum-intensity projections from confocal fluorescent images. For successful spine detection, our model required very small data set and showed high spine detection performance comparing to other described methods. This tool can facilitate future studies of brain development, plasticity and disease.

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SIGNAL-BASED ADAPTIVE THRESHOLDING FOR NEURONAL ACTION POTENTIAL SPIKE DETECTION

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Neuronal action potential spike detection is usually required to analyze electrophysiological measurements and is often accomplished by thresholding at an amplitude level set a priori. However, action potential spikes higher in amplitude than the background noise are sparse in time compared to signal sampling rate; thus, spikes and noise are often separable in amplitude histograms. Here, we present the new full-featured Matlab implementation [1] of our signal amplitude histogram analysis-based method to find threshold amplitudes objectively. To find positive and negative thresholds, first, a spike count histogram is formed by thresholding at, e.g., 500 amplitudes equispaced between the minimum and maximum of the signal. The gradient of the histogram is calculated, smoothed, and analyzed for shoulder-like features, which for many signal types appear near the noise envelope amplitudes. In addition to the basic automatic thresholding functionality, the algorithm now includes detection and alleviation of baseline wander and 50/60 Hz line noise, and automatic continuously or segment-wise adaptive thresholding for cases where the standard deviation of the signal varies in time. If no automatic thresholding can be obtained from the signal at hand, the algorithm falls back to thresholding at a preset level. The method is demonstrated with simulations and neuronal in vitro and in vivo microelectrode array measurements. Our method produces objective spike detection thresholds agreeable by an electrophysiology expert. Acknowledgement: The project has received funding from the European Union’s Horizon 2020 research and innovation programme, grant agreement No. 824164. Reference: [1] Tanskanen, https://se.mathworks.com/matlabcentral/fileexchange/55227-automatic-objective-neuronal-spike-detection

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BOARD NUMBER: S07-405

COMPARISON OF MOTION CORRECTION STRATEGIES FOR TASK-FMRI STUDIES IN MULTIPLE SCLEROSIS

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Functional magnetic resonance imaging (fMRI) signal is highly corrupted by head motion, particularly in clinical populations prone to characterize by higher motion such as Multiple Sclerosis (MS). Despite several approaches have been proposed to compensate, there are still controversies regarding the best strategy. In this study our aim is to perform a systematic comparison of the most used motion correction strategies within a task-based design in MS. We included 11 early MS patients and 8 healthy controls (HC) and characterized motion in both groups during performance of a visual task. We then compared task-activation Z-scores obtained with: models without motion correction, models containing 6 or 24 motion parameters (MPs) as nuisance regressors, models containing 6 or 24 MPs and motion outliers detected with Framewise Displacement (FD) or Derivative or root mean square VARiance over voxelS (DVARS) as nuisance regressors (scrubbing), and models with 6 or 24 MPs with volume interpolation for motion outliers. In general, models with 6MPs present higher Z-scores than models with 24MPs. Models with 6MPs and outliers' volume interpolation or scrubbing with FD presented higher Z-scores in the MS group, while models with 6MPs and scrubbing with DVARS or volume interpolation were the best combinations for the HC group. Differences between groups draw attention to the intrinsic impact of MS on fMRI analyses, which should be carefully addressed. Finding an optimal motion correction strategy is required to improve the accuracy of fMRI analyses, crucially in clinical studies in MS and other patient populations.

Pubmed:
35142957: Abreu R, Soares JF, Lima AC, Sousa L, Batista S, Castelo-Branco M, Duarte JV
Optimizing EEG Source Reconstruction with Concurrent fMRI-Derived Spatial Priors.
Reconstructing EEG sources involves a complex pipeline, with the inverse problem being the most challenging. Multiple inversion algorithms are being continuously developed, aiming to tackle the non-uniqueness of this problem, which has been shown to be partially circumvented by including prior information in the inverse models. Despite a few efforts, there are still current and persistent controversies regarding the inversion algorithm of choice and the optimal set of spatial priors to be included in the inversion models. The use of simultaneous EEG-fMRI data is one approach to tackle this problem. The spatial resolution of fMRI makes fMRI derived spatial priors very convenient for EEG reconstruction, however, only task activation maps and resting-state networks (RSNs) have been explored so far, overlooking the recent, but already accepted, notion that brain networks exhibit dynamic functional connectivity fluctuations. The lack of a systematic comparison between different source reconstruction algorithms, considering potentially more brain-informative priors such as fMRI, motivates the search for better reconstruction models. Using simultaneous EEG-fMRI data, here we compared four different inversion algorithms (minimum norm, MN; low resolution electromagnetic tomography, LORETA; empirical Bayes beamformer, EBB; and multiple sparse priors, MSP) under a Bayesian framework (as implemented in SPM), each with three different sets of priors consisting of: (1) those specific to the algorithm; (2) those specific to the algorithm plus fMRI task activation maps and RSNs; and (3) those specific to the algorithm plus fMRI task activation maps and RSNs and network modules of task-related dFC states estimated from the dFC fluctuations. The quality of the reconstructed EEG sources was quantified in terms of model-based metrics, namely the expectation of the posterior probability P(model|data) and variance explained of the inversion models, and the overlap/proportion of brain regions known to be involved in the visual perception tasks that the participants were submitted to, and RSN templates, with/within EEG source components. Model-based metrics suggested that model parsimony is preferred, with the combination MSP and priors specific to this algorithm exhibiting the best performance. However, optimal overlap/proportion values were found using EBB and priors specific to this algorithm and fMRI task activation maps and RSNs or MSP and considering all the priors (algorithm priors, fMRI task activation maps and RSNs and dFC state modules), respectively, indicating that fMRI spatial priors, including dFC state modules, might contain useful information to recover EEG source components reflecting neuronal activity of interest. Our main results show that providing

fMRI spatial derived priors that reflect the dynamics of the brain might be useful to map neuronal activity more accurately from EEG-fMRI. Furthermore, this work paves the way towards a more informative selection of the optimal EEG source reconstruction approach, which may be critical in future studies.
Brain Topogr, 2022; 35

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ELUCIDATING THE ROLES OF OPTIC ATROPHY 1 IN THE REGULATION OF MITOCHONDRIAL CRISTAE STRUCTURE BY DEEP LEARNING-BASED ULTRASTRUCTURAL ANALYSIS

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Shogo Suga[1], Koki Nakamura[1], Bruno Humbel[2], Hiroki Kawai[1], Yusuke Hirabayashi[1]
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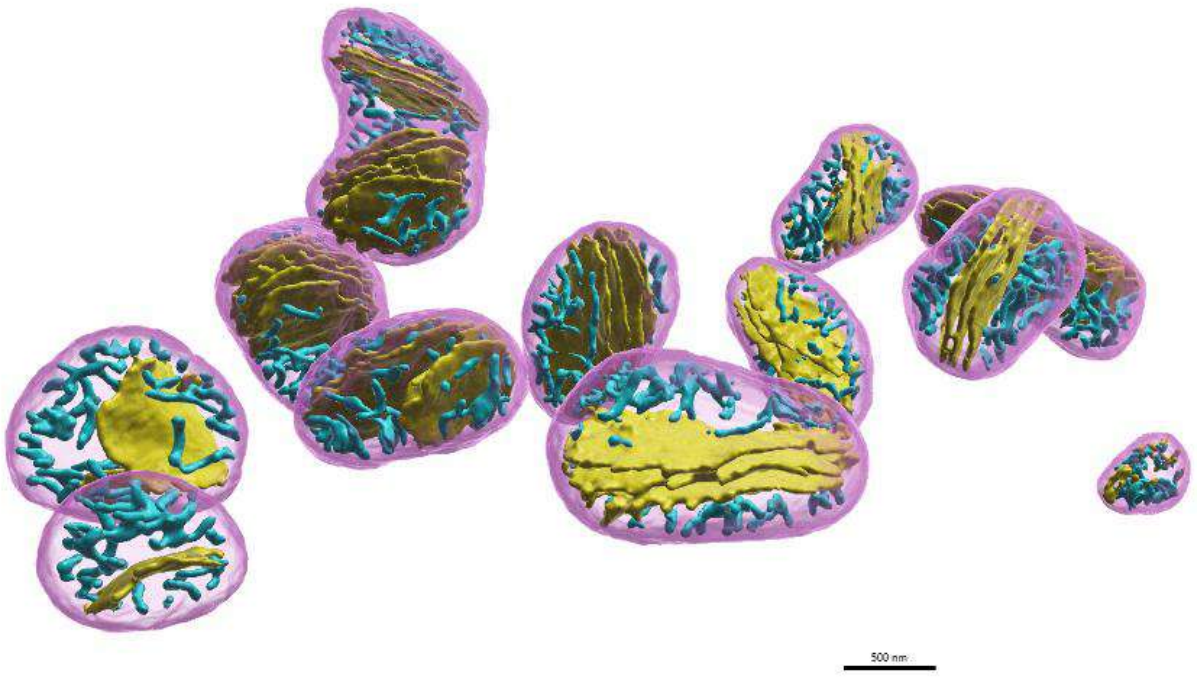
Optic Atrophy 1(OPA1) is originally identified as a protein related to Autosomal Dominant Optic Atrophy (ADOA). Although it has been revealed that OPA1 regulates the inner mitochondrial membrane structure, the precise roles of OPA1 are still elusive. With the recent development of genetic modification technology, the analysis of phenotypes caused by genetic mutations in cells has become high-throughput. Also, the contribution of those mutations to the determination of organelle structure at the nanometer level has also been revealed using electron microscopy (EM). However, because EM image analysis is labor-intensive and time-consuming, quantitative and three-dimensional analysis have been often insufficient. Therefore, in this study, I aimed to construct a high-throughput workflow using deep learning to analyze organelle structures from thousands of continuous tomographic images. For this purpose, I developed a platform called "Python-based platform for human-in-the-loop workflow (PHILOW)" that implements three-axis prediction (TAP) and human-in-the-loop (HITL) training data generation. Equipped with these techniques, we have succeeded in reconstructing serial sectioned EM images obtained by focused-ion-beam scanning EM (FIB-SEM). Based on the reconstruction from the control and OPA1 knock-down mouse fibroblasts, we analyzed the structure of mitochondrial outer and inner membranes in three-dimension, quantitatively (Figure). We also applied these techniques for reconstructing the neuronal intracellular structure.

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INVESTIGATING ELECTRICALLY EVOKED RESPONSES OF PATTERNED NEURAL NETWORKS ON HD-MEAS

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Benedikt Maurer, Jens Duru, Stephan Ihle, Joël Küchler, Robert John, János Vörös
ETH Zürich, Laboratory Of Biosensors And Bioelectronics, Institute For Biomedical Engineering, Zürich, Switzerland

How the brain processes information to learn and form memories is still an unanswered question. To gain insights, neurons are studied under great effort *in vivo*. However, the applied methods often only enable monitoring subsets of neurons embedded in a complex network or lack the spatiotemporal resolution for analysis on a single-cell level. To overcome such limitations, we are following the approach of bottom-up neuroscience, in which small engineered neural networks are studied *in vitro*. To reduce the complexity of the network architecture, rat primary cortical neurons are seeded into polydimethylsiloxane (PDMS) microstructures to spatially confine the location of their soma and guide neurite growth[1]. Using these microstructures on high-density multi-electrode arrays (HD-MEAs) enables communicating with the network by stimulation and recording with high spatiotemporal resolution[2]. Repetitive stimulation of these networks yields stable and reproducible responses over several hours, which improves reproducibility in network studies[3]. We analyse and track response traces evoked by stimulating every network electrode individually depending on stimulation site. Further experiments include sensitivity assays with varying stimulus parameters and the combination of different stimulation sites. Combining microstructures with HD-MEAs enables comparison of network responses with respect to stimulus location. With these tools we aim to describe the complex input-output relationship of small neural networks which could validate fundamental neuroscience postulates and aid the design of more realistic *in silico* models. References: 1. Forró, et al. (2018) *Biosensors & Bioelectronics: 122.* 2. Duru, et al. (2022) *Front Neruosci: 16.* 3. Ihle, et al. (2021) *Biosensors & Bioelectronics: 113896.*

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AUTOMATIC POST-PROCESSING AND MERGING OF MULTIPLE SPIKE-SORTING ANALYSES WITH LUSSAC

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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The increasing site counts of multielectrodes used in extracellular recording preclude traditional manual spike sorting and have led to the development of several new software suites with increased automation. It remains difficult, however, to find parameters enabling optimal processing of signals from all neuronal types. Here, we describe a procedure for automatic post-processing and merging the outputs of multiple spike-sorting analyses, enabling the accumulation of results optimised for different activity features. This approach was motivated by and first applied to the analysis of cerebellar activity, in which the complex spikes challenge standard analyses. The core of our modular procedure involves the identification of cluster-fusion candidates and assurance that only fusions that increase a clearly defined cluster-quality metric are performed; important subsidiary steps that have also been automated include spike alignment and duplicate removal. The procedure was validated by recovery of injected spike waveforms. In tests combining analyses from Kilosort and MountainSort, our procedure yielded a 50 % increase in the detected number of Purkinje cell clusters with both simple and complex spikes. A further validation used the synthetic ‘SpikeForest’ dataset approximating neocortical activity, in which our package also improved performance. Combining multiple analyses therefore offers a general method for improving spike-sorting.

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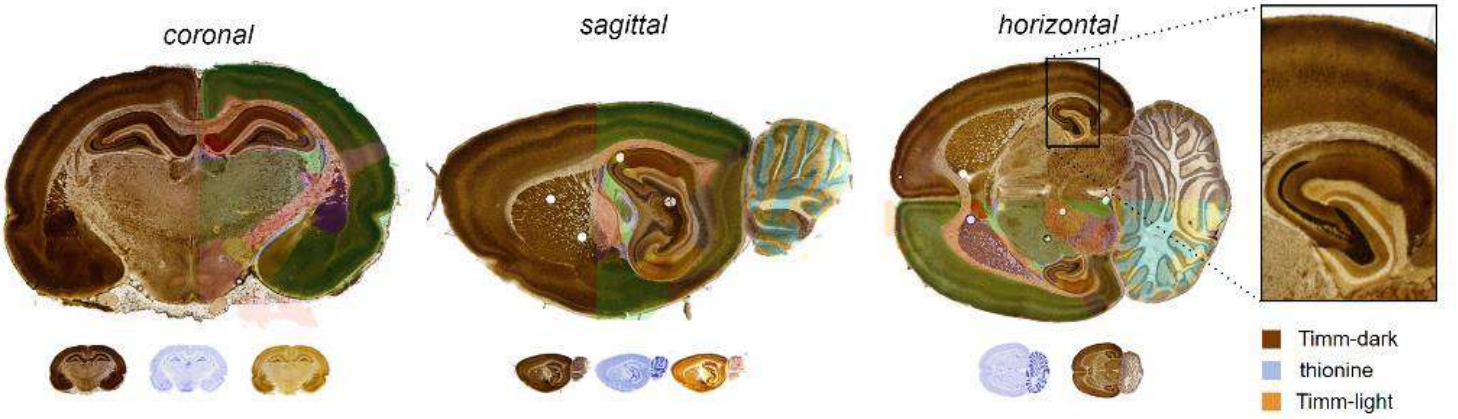
MULTIPLANE MICROSCOPIC ATLAS OF RAT BRAIN ZINCERGIC TERMINAL FIELDS AND METAL-CONTAINING GLIA STAINED WITH TIMM'S SULPHIDE SILVER METHOD

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Camilla Blixhavn, Finn-Mogens Haug, Heidi Kleven, Maja Puchades, Jan Bjaalie, Trygve Leergaard
Neural Systems Laboratory, Institute Of Basic Medical Sciences, University Of Oslo, Oslo, Norway

The ability of Timm's sulphide silver method to stain zincergic terminal fields has made it a useful neuromorphological marker. Beyond its roles in zinc-signaling and neuromodulation, zinc is involved in the pathophysiology of ischemic stroke, traumatic brain injury, epilepsy, degenerative diseases and neuropsychiatric conditions. To provide a reference for planning and interpretation of experimental investigations of zinc-related phenomena in rat brains, we established a comprehensive repository of serial microscopic images from a historical collection of coronally, horizontally and sagittally oriented rat brain sections stained with Timm's method. Section images were acquired with a Zeiss Axioscan slide scanner and registered to the Waxholm Space rat brain atlas (RRID: SCR_017124) using the QuickNII (RRID:SCR_016854) and VisuAlign (RRID:SCR_017978) tools provided by the EBRAINS research infrastructure (https://ebrains.eu/services/atlases). The customized atlas overlays accompanied by adjacent Nissl-stained sections provides spatial reference and guidance in identifying anatomical boundaries. The image repository includes microscopic images from five adult albino (Wistar) rats. The collection of high resolution images will be shared via the EBRAINS Data and Knowledge services (https://ebrains.eu/services/data-and-knowledge), with web-microscopy viewer links for each subject, allowing interactive inspection of the spatial distribution and density of zincergic terminal fields across the entire brain in three planes of view.
This project/research has received funding from the European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3).



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MOUSE REACH: AUTOMATED BEHAVIORAL READOUTS IN MOTOR RECOVERY AFTER STROKE

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Despite best medical care, motor recovery after stroke is often incomplete. Basic research in young adult mice has shown that substantial degrees of spontaneous recovery after cortical stroke rely on the rewiring of spinal circuits, that attract new synaptic innervation from multiple supraspinal brain areas. Post-stroke motor decline as well as the subsequent improvement in paw reaching ability visibly illustrate a steady substitution of the damaged corticobulbospinal connections. The corresponding changes in mouse paw kinematics are an important parameter providing spatiotemporal information on fine motor control and paw grip recovery that offers an additional translational perspective pertaining to hand functionality of stroke-affected patients. At the present time, most animal motor assessments are still based on scoring schemes and exclude much information on paw kinematics, making them difficult to compare with clinical evidence. Here, we have developed a state-of-the-art deep learning-based pipeline that processes reaching video data and enables the user to carry out a detailed evaluation of graphical behavioral readouts obtained with the Staircase test. Our analysis framework results in significantly faster computing times compared to a human rater and allows for a more extensive extraction of movement data including features that have until now been unattainable through classical approaches.

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CITE-ON: CELL IDENTIFICATION AND TRACE EXTRACTION ONLINE IN FUNCTIONAL TWO-PHOTON CALCIUM IMAGING

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Luca Sità[1], Marco Brondi[1], Pedro Lagomarsino[1,2], Sebastiano Curreli[1], Mariangela Paniello[1], Dania Vecchia[1], Tommaso Fellin[1]
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Two-photon (2P) microscopy in combination with genetically encoded calcium indicators (GECIs) is a powerful tool for the functional investigation of the intact brain. However, processing 2P recordings is computationally intensive, making it challenging to perform online frame-by-frame analysis. For example, identifying and locating imaged neurons in a field-of-view (FOV) is a crucial but time consuming step, and its outcome strictly depends on the experimental conditions (e.g., GECI of choice and image signal-to-noise ratio). Here, we develop CITE-On (Cell Identification and Trace Extraction Online), a deep learning-based algorithm for fast automatic cell identification, dynamic segmentation, identity tracking, and trace extraction in mouse 2P calcium imaging data. CITE-On identifies thousands of cells, and extracts their functional traces in real-time, including during large-FOV mesoscopic 2P imaging. CITE-On is based on a publicly available object detector trained on neuronal morphological features extracted from a dedicated imaging dataset obtained in different mouse brain regions under varied experimental conditions. Applied offline to publicly available datasets, CITE-On achieves a performance similar to state-of-the-art methods, it maintains a low computational cost, and generalizes well across GECIs, brain regions, and acquisition parameters in both anesthetized and awake head-fixed mice. The online pipeline takes advantage of GPU accelerated computing and efficient memory management, and provides real-time identity tracking and functional trace extraction for most neurons in a FOV with high accuracy. CITE-On is a potent tool to speed up 2P calcium imaging analysis and, therefore, it facilitates closed-loop approaches, for example in all-optical experiments combining neuronal imaging and manipulation.

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PREDICTION OF IMPEDANCE BEHAVIOR OF NEURAL INTERFACE USING DEEP LEARNING

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Background and aims: To ensure the long-term reliability of the neural implants or any bionics system with electrodes, it is important to examine the major modes of failure during long-term implantation by conducting pre-in-vitro experiments. Electrochemical impedance spectroscopy (EIS) describes electrode behavior, stability and long-term durability to a specific range of frequency. Impedance measurements during In Vivo and clinical applications are used also to examine a feedback-based electrode rehydration for long-term bio-potential measurements. This research aims to provide a novel approach to predict impedance changes using deep learning. **Methods**: A novel approach of impedance prediction is presented to convert EIS features during many days of an experiment into a one-dimensional sequence of values using a novel formula called Day Factor that makes us able to predict the behaviour of neural implant using long short-term memory (LSTM) network. Four neural interfaces of various material compositions with long-term in vitro ageing tests are used to validate the proposed approach. **Results and conclusions**: Day Factor formula with primitive trails show a satisfying conversion tool of multi-dimensional values of impedance in a one-dimension signal. Day factor signal was able to detect the big difference between normal impedance behaviour and sudden high impedance increasing during ageing tests. Using LSTM network and day factor signal as forecasting method to predict the impedance behaviour is a viable approach that helps to prepare for potential device failure in bionics systems.

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CELL ASSEMBLY DETECTION THROUGH THE ITERATIVE SEARCH FOR RECURRENT SYNCHRONY

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Higher brain functions involve the cooperation and interaction of large numbers of neurons. Cell assemblies, groups of neurons active synchronously in time, are thought to mediate these interactions. Technological advances permit the simultaneous recording of large neuronal populations, upholding the need for tools that detect cell assemblies within such datasets. Existing algorithms use proxy measures to estimate global cellular co-activations and cell membership within patterns. This leads to the detection of patterns of cell assembly amalgams that do not reflect the underlying neuronal dynamics. Here we present a novel computational framework for the direct detection of cell assemblies through a rigorous search for neuronal co-activations. Activation patterns are progressively constructed by adding new cells that fire together above chance levels as determined through two complementary measures that ensure the internal coherence of a detected pattern and its independence from population dynamics. Here we test the performance of our computational framework in a wide range of simulated datasets while comparing its performance to the seven other most used algorithms within the scientific literature. Low level computational optimizations enable our algorithm to run up to one hundred times faster than another comparable algorithm. We show its results and applications in experimentally recorded datasets in the rat hippocampus and prefrontal cortex, such as the detection of cell assembly formation and the organization of neuronal activity within assemblies into sequences after a behavioral task.

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HERBS: A TOOLKIT FOR HISTOLOGICAL E-DATA REGISTRATION IN RAT BRAIN SPACES

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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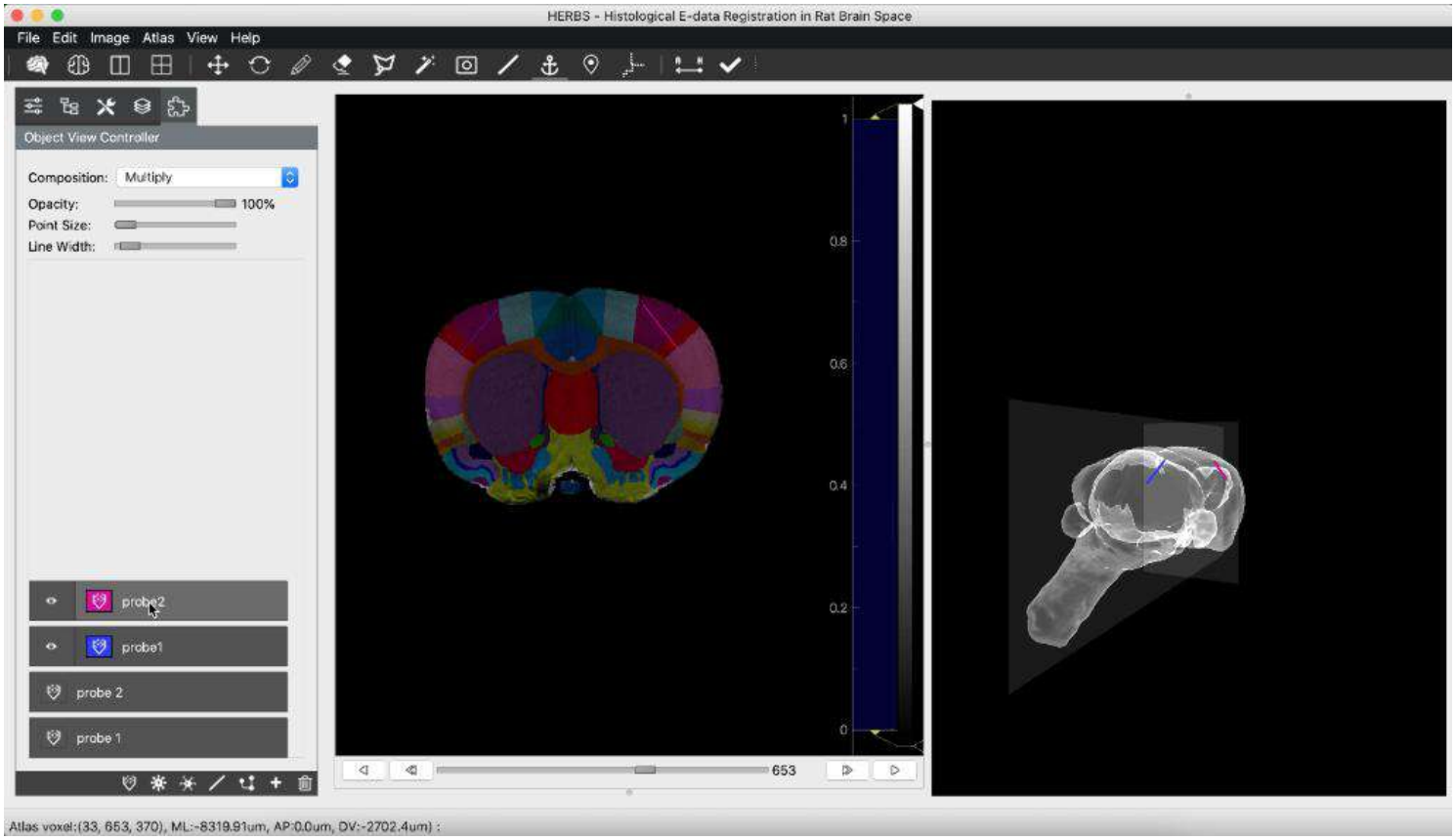
Recent recording technologies allow users to sample thousands of neurons from multiple brain regions at a time, which has driven a great need for anatomical registration and visualization tools. Most existing toolkits, however, were developed using mouse atlases, leaving few options for rat users. We therefore developed a graphical user interface specifically for rats, HERBS. Which allows users to reconstruct recording electrode trajectories (e.g. Neuropixels, tetrodes), insertion angles, and to estimate which electrodes sampled which brain regions. Regional delineations in HERBS are based on annotated 3D volumes from the Waxholm Space rat brain atlas. In addition, users can obtain cell counts and delineate expression of multiple virus injections across tissue sections. HERBS also fully supports free-transformation from tissue sections to atlas slices and can therefore be used for brain atlasing or generating coordinates for targeting brain regions prior to surgery. Furthermore, HERBS allows anatomists to reconstruct a 3D brain mesh with tissue from individual animals, and interactive 3D visualizations are provided after desired processes. Finally, HERBS can triangulate the distance of individual neurons from the recording electrode using waveforms from multiple recordings sites. HERBS is available openly on GitHub and is compatible with Windows, macOS and Linux operating systems. Figure: Face of HERBS



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SYNTALOS - A SOFTWARE FOR SIMULTANEOUS ACQUISITION OF HETEROGENEOUS NEUROPHYSIOLOGICAL DATA AND FOR CLOSED-LOOP INTERVENTION PROTOCOLS

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Matthias Klumpp, Lee Embray, Andreas Draguhn, Martin Both
Heidelberg University, Institute Of Physiology And Pathophysiology, Heidelberg, Germany

Many experiments rely on the acquisition of heterogeneous data from a variety of different sources, making accurately aligned timestamps a key requirement. In addition, it is often necessary to manipulate experimental settings on the fly, especially *in vivo* where interventions depend on the animal's state or behavior. Finally, acquired data should be stored in a standardized format to simplify subsequent analysis. Software packages supporting these requirements should be readily available, easy to handle and versatile enough to allow setting up new data acquisition pipelines without much effort. To address these requirements, we developed a new, integrated software solution capable of simultaneous acquisition of data from an arbitrary amount of sources of different kinds, e.g. multi-channel electrophysiological recordings, conventional video images, high-speed camera data, serial interfaces or Miniscopes. The software, called Syntalos, ensures aligned timestamps for all inputs, and makes use of the parallel-processing capabilities of modern CPUs to effectively run many tasks simultaneously. New data sources can be integrated and adjusted with minimal programming skills. For more advanced applications, the modular software design facilitates extension with new modules written in either C/C++ or Python. All data generated from a given experiment is stored in a well-defined, comprehensive structure, making it easy to compare, pool or share data between experimentalists with different research questions. With these abilities, Syntalos enables reliable closed-loop experiments for many different (neuro)scientific questions. Tests with diverse research questions, experimental setups and laboratories show the good performance and easy-to-learn structure of the program.

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SCICLOUD: A WEB-BASED TOOL TO REPRESENT AND MEASURE MEDICAL SCIENCE OUTPUT.

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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[1]University of Geneva, Centre medical universitaire, Department Of Basic Neurosciences, Geneva, Switzerland, [2]University of Geneva, Centre Medical universitaire, Department Of Basic Neurosciences, Geneva, Switzerland

The number of scientific publications is continuously expanding and provides a tremendous metadata source to identify and predict scientific trends. Most biomedical articles are referenced in Pubmed, a publicly available database that allows systematic retrieval of metadata such as type of publication, date of submission, author credentials, and scientific content (abstract).
To take advantage of these resources, we developed Scicloud, a webpage-based tool that analyzes and represents how researchers publish worldwide and sheds light on a neglected domain of science: the representation of science itself.We built Scicloud in four steps: (1) parsing and text-mining of the Pubmed public database, (2) inference and integration of geographic information for each entry and each author, (3) indexing and interrogation of the generated knowledge by a search engine strategy and (4) display through an interactive world map web page.We expect that the implementation of this tool will provide opportunities for scientists of all levels of training and for various stakeholders such as private and national funding agencies, editors, and the public at large.

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MOTILA – AN OPEN-SOURCE TOOL FOR AUTOMATIZED AND STANDARDIZED MICROGLIA PROCESS MOTILITY ANALYSIS

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Fabrizio Musacchio, Sophie Crux, Felix Nebeling, Manuel Mittag, Falko Fuhrmann, Eleonora Ambrad, Andrea Baral, Julia Steffen, Stefanie Poll, Martin Fuhrmann
German Center for Neurodegenerative Diseases (DZNE), Neuroimmunology And Imaging, Bonn, Germany

Fluorescence microscopy in combination with implanting a chronic window enables the consecutive *in-vivo* monitoring of cellular activity in the brain over long time periods. To handle both, the standardized and reproduceable analysis of microglia process motility and the resulting large amount of data, we present the open-source pipeline *MotilA*. *MotilA* provides fully automatic processing of multiple time-lapse image stacks. After pre-processing the data including registration, denoising, histogram equalization, image projection and binarization, *MotilA* calculates the motility of microglia processes or other cells by assessing the pixel variation between the individual time points, i.e., by calculating and comparing stable, gained and lost pixels. *MotilA* is able to process both, single- and multi-channel image stacks, including optional spectral unmixing. Batch processing image stacks of an entire microscopy campaign, the *MotilA* post-hoc analysis presents the resulting cellular motility over time and groups. To benchmark the performance of *MotilA* we compare our results with the manually assessed motility of a set of hippocampal microglial cells in the mouse brain. We demonstrate that *MotilA* reproduces the human detected motility in significant shorter time, ruling out any human bias and increasing the reliability of the results due to the standardization of the analysis.

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CDEEP3M - DEEP LEARNING FOR IMAGE SEGMENTATION

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Matthias Haberl[1], Daniela Boassa[2], Mark Ellisman[2]
[1]Charite, Neuroscience Research Center, Berlin, Germany, [2]University of California, San Diego, School Of Medicine, San Diego, United States of America

Analysis of large-scale images is a major bottleneck in new discoveries, from the ever-increasing throughput of modern microscopy techniques. Deep neural networks outperform humans in speed and precision in image analysis tasks. We developed *CDeep3M*, an end-to-end analysis pipeline to identify cellular organelles (Mitochondria, Nuclei, Membranes, Vesicles, ER) in very large 3D electron microscopy datasets. To democratize access of the community to deep learning for image segmentation we have implemented a set of free complementary tools around CDeep3M. To minimize the end-user effort to take advantage of our new developments and use the software: (1) a CDeep3M web interface: allowing to run segmentations, quickly generate training data, or re-train a model all for free online, backed by a decentralized GPU cluster (2) CDeep3M cloud applications (Google Colab and AWS) (3) CDeep3M containerized installations (Docker and Singularity), to facilitate usage on local, remote and supercomputer infrastructure (4) The CDeep3M modelzoo, which is an organized and searchable database with many pre-trained deep neural networks for image segmentation, in which each trained model is indexed with unique DOIs. We co-host the modelzoo of trained deep neural networks with a database of microscopy images. This strategy will in the future help to enhance the utility of both, allowing the community to more easily re-analyze microscopy data and evaluate pre-trained models on different datasets.

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AUTOMATIC CELL COUNTING WITH DEEP LEARNING

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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[1]INSERM, Tonic, Toulouse, France, [2]INSERM, Crefre, Toulouse, France

Aims When analyzing histology results, counting cells can quickly become a daunting task, with thousand cells in a single slice. Hence, we decided to automate the process of counting human cells in rat brain slices. They are visually characterized by mitochondrias on the edge of the cell-nucleus, and we were not able to count them with classical methods. The deep learning methods we found relied on segmentation, which requires a long annotation process. We thus designed our own neural network. **Methods** We mixed two well-known architectures: UNet and ResNet. The annotation was a single dot in the middle of each cell. Each dot was then transformed into a gaussian kernel of the size of the cells, and the resulting heatmap was used for training. We limited the amount of annotated data required with data augmentation. We then alternated training-annotation until the results were sufficiently good for our need. **Results** Above 80% of the cells were correctly detected. Mistakes mainly concern ambiguous cases, so it is actually hard to assess an objective rate of success. We found that evaluating the algorithm performances on a test dataset based on a single indicator was not practical and the real work was done looking directly at the data. **Conclusion** The deep learning architecture we used was fairly simple, and yet worked surprisingly well, given that the task is not straightforward for a human. We also found that the classical deep learning methodology, originating from the industry, did not seem to fit with our task.

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A SIMPLE TO USE GRAPHICAL INTERFACE FOR THE ANALYSIS OF WHOLE BRAIN OPTICAL IMAGES WITH CLEARMAP2

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Whole-brain optical-imaging techniques, using either serial tomography or tissue clearing combined with lightsheet microscopy, have paved the way for neuroanatomical studies of the entire central nervous system with single cell resolution. With the underpinning of a range of molecular techniques like clearing combined with immunolabellings, immediate early genes expression or viral tracing, these microscopy advancements have enabled a wide array of experiments. These range from the probing of neuronal activity, through to investigations of connectivity and analysis of the whole brain vasculature. Although several companies now supply the hardware required for these experiments, the analysis of the substantial dataset generated still often requires specialised pipelines designed for experts.
Here, we present a straightforward and documented graphical user interface to the established tools of the ClearMap2 pipeline that allows experimenters to easily process and analyse their own datasets. This open source software allows the users to reconstruct, analyse and visualise cell labellings or vascular graphs vetted with the help of artificial neural networks.

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A PRACTICAL GUIDE TO USING THE EBRAINS KNOWLEDGE GRAPH IN (YOUR) RESEARCH

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Maaike Van Swieten[1], Oliver Schmid[2], Gilles Dénervaud[2], Ioannis Tsanaktsidis[2], Benjamin Weyers[3], Andrew Davison[4], Lyuba Zehl[5], Ida Aasebø[1], Jan Bjaalie[1]
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Scientific articles are mostly published as text files containing unstructured and semi-structured information. Consequently, important information is typically deeply hidden in documents which severely limits the possibilities to automatically process and reuse scholarly knowledge. One approach to make information explicit and directly usable is to transform this into a standardised format and store it in a knowledge graph. This allows scholarly knowledge to be represented in a structured, machine-actionable, interlinked and semantically rich manner. The EBRAINS Knowledge Graph was developed to facilitate the search and information exchange in research, so that research results across different domains become directly comparable and easier to retrieve and reuse. Here, we provide a practical guide to extracting information from the EBRAINS Knowledge graph using a user-friendly interface as well as a more advanced programmatic route via an Application Programming Interface (API). We also provide concrete examples on how the extracted information can be leveraged in order to develop new research objectives as well as validate ongoing research. The EBRAINS Knowledge Graph is integrated in the wider EBRAINS research infrastructure as a part of the EBRAINS Data and Knowledge services for sharing and finding research data and models. Data found through these services can be directly used and analysed via the various integrated tools and analysis workflows. The EBRAINS Knowledge Graph is a valuable machine-actionable and FAIR (**F**indable, **A**ccessible, **I**nteroperable and **R**eusable) resource for discovery-based and hypothesis-driven research as it already contains a wide variety of neuroscience data types, models and software.

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BRAINSTEM: A COLLABORATIVE ELECTRONIC LAB NOTEBOOK FOR EXPERIMENTAL NEUROSCIENCE

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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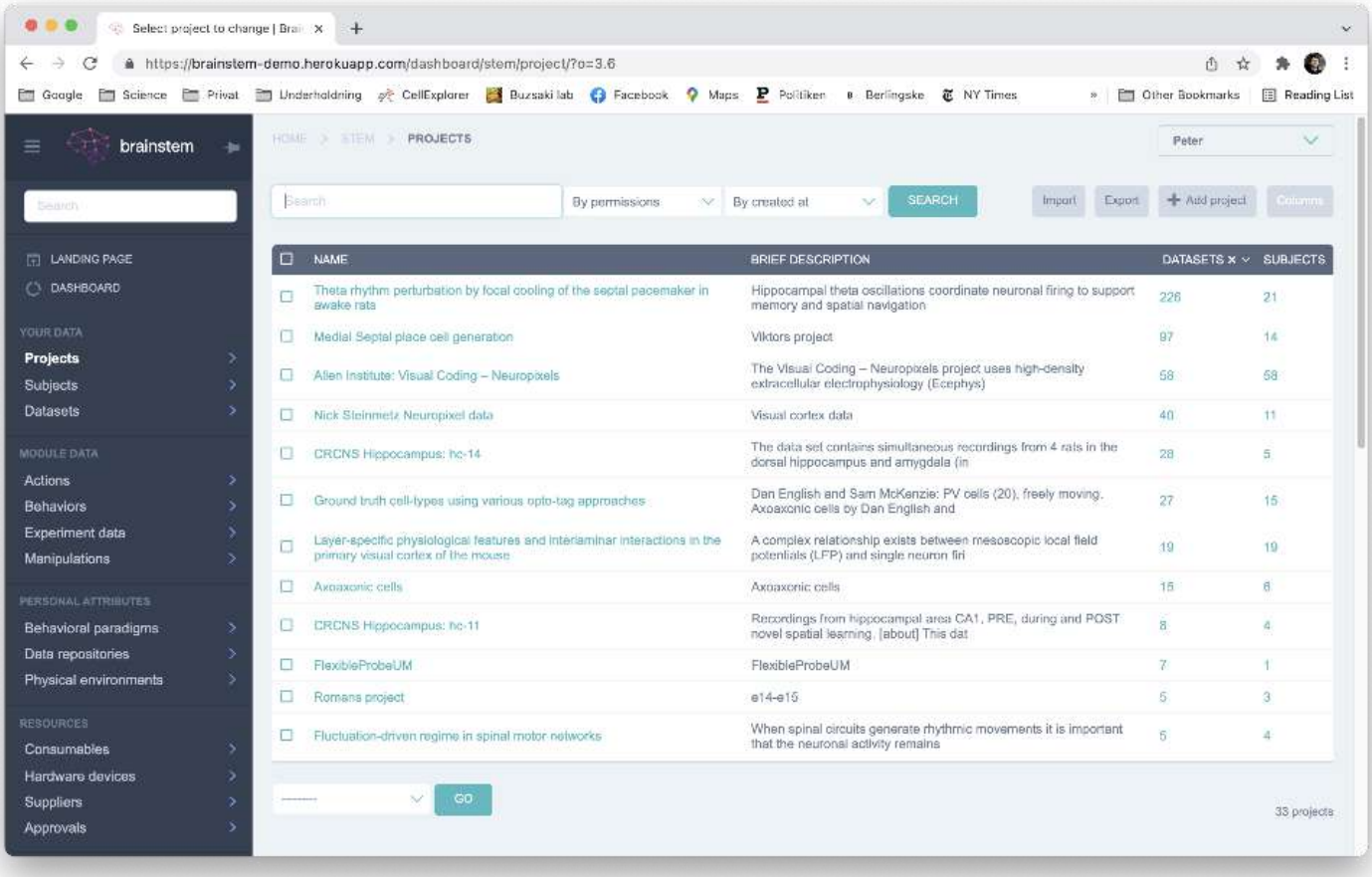
Research data from published papers on large-scale single neuron research is rarely shared. This data often has considerable potential for reuse and reinterpretation in different studies and provides transparency and reproducibility to already-published studies. Further, data shared on public repositories is often difficult to discover, lacking standardized metadata, limiting its degree of reusability. To address these current shortcomings, this group is building BrainSTEM. BrainSTEM is a collaborative electronic lab notebook that is tailored to experimental neuroscience, with a focus on large-scale single-cell neurophysiology as captured using intracellular, extracellular, and two-photon recording techniques. It allows for the organization of experiments, in a format that is easily interpretable by other researchers and discoverable by machines. BrainSTEM has two entry points, a web interface that allows for fast data entry using user-friendly forms, and a REST API for programmable access. BrainSTEM's metadata model is built on a standardized yet flexible language, promoting detailed interpretable notes. Granular access control enables private collaboration within and across labs, and public sharing of projects and datasets is only a click away, lowering the data sharing entry barrier substantially. BrainSTEM has the potential to become the standard metadata model within neurophysiology, make data FAIR, promote standardization, data sharing, and provide better integration across datasets, both within and across collaborative labs and for published

datasets.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THE DEEPLABCUT MODEL ZOO: DEVELOPMENT OF PRETRAINED ANIMAL POSE ESTIMATION MODELS FOR NEUROSCIENCE

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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In recent years, markless animal pose estimation has become an important tool for studying animal behavior. Deep learning typically excels by, in part, leveraging large datasets. Yet, large datasets for animal pose estimation do not exist yet. One reason for this lack of data is that most labs define keypoints differently to fit their research needs and because due to transfer learning, for specific contexts relatively little labeled data is required. As a consequence, there exist sets of disjoint data for the same type of animals, but being able to combine across labs could provide highly powerful networks. Yet, how to combine different keypoints across animals has not been shown. To tackle this, we propose a simple and effective way to merge datasets for model pre-training and corresponding protocols for fine-tuning. We show that our approach is effective for both state of the art bottom up and top down methods for pose estimation and we are able to obtain powerful pre-training models that have decent zero-shot performance. After fine-tuning, such models largely outperform models that use ImageNet pre-trained weights. This sets the stage of providing the community plug-and-play solutions for animal pose estimation for the first time. We also will showcase the DeepLabCut Model Zoo, which contains models for various model systems from mice, to dogs and primates.

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BOARD NUMBER: S07-424

AN OPEN-SOURCE R SCRIPT FOR MEA DATA ANALYSIS AND VISUALISATION

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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ICM - Institut du Cerveau | Paris Brain Institute, Sorbonne Université - Cnrs - Inserm, Paris, France

Aims Microelectrode array (MEA) system are increasingly used as technical approach too monitor activity of neuronal networks *in vitro.* Such a method allows to simultaneous record extracellular voltage signals from multiple electrodes. At the same time, commercial and open-source solutions have been developed to analyze and visualize MEA data. Here we present a new open-source R-based script for analyzing activity of neuronal networks recorded with MED64 (AlphaMed Scientific). **Methods** The script was programmed in RStudio and its performance was demonstrated using recordings from 2D and 3D cultures of iPSC-derived neurons with a 64 channels array. Spikes were recorded and extracted using Mobius software which produces data files in csv format. These files are loaded by the script to extract various features of neuronal activity. **Results**. The script developed in-house contains an automatic analysis of several raw source files based on an analytical guideline. This guideline allows to define the electrodes and the period to be analyzed. Firstly, our script allows the overlap display of spikes for each electrode, enabling the user to efficiently handle signal artefacts. Secondly, spike raster plot and heat maps with number of spike or frequency are generated for advanced data visualization. Finally, the script contains several burst analysis methods based on previous publications. **Conclusions** This new tool allows the unbiased and timely analyze of large datasets and complements the applications already available for MEA analysis. A community of MEA users has been created in order to detect limitations and continuously improve pipeline. We also wish to develop an interface for users not familiar with R.

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BOARD NUMBER: S07-425

XNEURO: UNIFIED NEURAL DATA INTERFACE FOR SCALABLE MODEL INFERENCE

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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With increasing complexity and magnitude of neural data arising from recent high-throughput multi-channel neurophysiology and neuroimaging techniques, the standardization of data storage and processing is an important element to promote reproducibility and collaboration in neuroscience. Although projects like Neurodata-Without-Borders initiative has made a long way along the data standardization in neuroscience, there are still several outstanding challenges: (1) efficient storage and fast retrieval are at an increasingly irreconcilable tradeoff given the increasingly combinatorial numbers of meta information; (2) high-dimensionality of spatial and temporal resolutions from single cell and multi-channel techniques prevents a workable analytical pipeline in local machines and hard disks; (3) analytical pipelines are adopting resource-heavy models like deep learning which pose additional bandwidth and computational constraints. This is especially the case in cognitive neuroscience, where the recordings of neural responses often accompany high-dimensional stimulus inputs, hierarchical meta information and delicate cognitive model architectures for neurobiological inference. In this work, we propose a new data format that tackles all three challenges and provide an opensourced Python toolbox, called Xneuro, as a unified neural data interface to facilitate scalable data import, standardization, search and retrieval. We benchmark Xneuro across several high-throughput datasets, collected each in different modalities under various stimulus types and behavioral tasks, where traditional analytic pipelines find difficult to collate. We demonstrate the effectiveness and scalability of our framework by comparing these datasets to a series of cognitive models in a fast and scalable fashion, the first time neural model inference of this scale can be performed.

Pubmed:
35052085: Lin B
Regularity Normalization: Neuroscience-Inspired Unsupervised Attention across Neural Network Layers.
Inspired by the adaptation phenomenon of neuronal firing, we propose the regularity normalization (RN) as an unsupervised attention mechanism (UAM) which computes the statistical regularity in the implicit space of neural networks under the Minimum Description Length (MDL) principle. Treating the neural network optimization process as a partially observable model selection problem, the regularity normalization constrains the implicit space by a normalization factor, the universal code length. We compute this universal code incrementally across neural network layers and demonstrate the flexibility to include data priors such as top-down attention and other oracle information. Empirically, our approach outperforms existing normalization methods in tackling limited, imbalanced and non-stationary input distribution in image classification, classic control, procedurally-generated reinforcement learning, generative modeling, handwriting generation and question answering tasks with various neural network architectures. Lastly, the unsupervised attention mechanisms is a useful probing tool for neural networks by tracking the dependency and critical learning stages across layers and recurrent time steps of deep networks.
Entropy (Basel), 2021; 24
31606892: Andelin AK, Doyle Z, Laing RJ, Turecek J, Lin B, Olavarria JF
Influence of ocular dominance columns and patchy callosal connections on binocularity in lateral striate cortex: Long Evans versus albino rats.
In albino rats, it has been reported that lateral striate cortex (V1) is highly binocular, and that input from the ipsilateral eye to this region comes through the callosum. In contrast, in Long Evans rats, this region is nearly exclusively dominated by the contralateral eye even though it is richly innervated by the callosum (Laing, Turecek, Takahata, & Olavarria, 2015). We hypothesized that the inability of callosal connections to relay ipsilateral eye input to lateral V1 in Long Evans rats is a consequence of the existence of ocular dominance columns (ODCs), and of callosal patches in register with ipsilateral ODCs in the binocular region of V1 (Laing et al., 2015). We therefore predicted that in albino rats input from both eyes intermix in the binocular region, without segregating into ODCs, and that callosal connections are not patchy. Confirming our predictions, we found that inputs from both eyes, studied with the transneuronal tracer WGA-HRP, are intermixed in the binocular zone of

albinos, without segregating into ODCs. Similarly, we found that callosal connections in albino rats are not patchy but instead are distributed homogeneously throughout the callosal region in V1. We propose that these changes allow the transcallosal passage of ipsilateral eye input to lateral striate cortex, increasing its binocularity. Thus, the binocular region in V1 of albino rats includes lateral striate cortex, being therefore about 25% larger in area than the binocular region in Long Evans rats. Our findings provide insight on the role of callosal connections in generating binocular cells.
J Comp Neurol, 2020; 528
31050411: Chen Z, Johnson MC, Chen J, Bick MJ, Boyken SE, Lin B, De Yoreo JJ, Kollman JM, Baker D, DiMaio F
Self-Assembling 2D Arrays with de Novo Protein Building Blocks.
Modular self-assembly of biomolecules in two dimensions (2D) is straightforward with DNA but has been difficult to realize with proteins, due to the lack of modular specificity similar to Watson-Crick base pairing. Here we describe a general approach to design 2D arrays using de novo designed pseudosymmetric protein building blocks. A homodimeric helical bundle was reconnected into a monomeric building block, and the surface was redesigned in Rosetta to enable self-assembly into a 2D array in the C12 layer symmetry group. Two out of ten designed arrays assembled to micrometer scale under negative stain electron microscopy, and displayed the designed lattice geometry with assembly size up to 100 nm under atomic force microscopy. The design of 2D arrays with pseudosymmetric building blocks is an important step toward the design of programmable protein self-assembly via pseudosymmetric patterning of orthogonal binding interfaces.
J Am Chem Soc, 2019; 141
28775961: Teng Y, Liu S, Guo X, Liu S, Jin Y, He T, Bi D, Zhang P, Lin B, An X, Feng D, Mi Z, Tong Y
An Integrative Analysis Reveals a Central Role of P53 Activation via MDM2 in Zika Virus Infection Induced Cell Death.
(ZIKV) infection is an emerging global threat that is suspected to be associated with fetal microcephaly. However, the molecular mechanisms underlying ZIKV disease pathogenesis in humans remain elusive. Here, we investigated the human protein interaction network associated with ZIKV infection using a systemic virology approach, and reconstructed the transcriptional regulatory network to analyze the mechanisms underlying ZIKV-elicited microcephaly pathogenesis. The bioinformatics findings in this study show that P53 is the hub of the genetic regulatory network for ZIKV-related and microcephaly-associated proteins. Importantly, these results imply that the ZIKV capsid protein interacts with mouse double-minute-2 homolog (MDM2), which is involved in the P53-mediated apoptosis pathway, activating the death of infected neural cells. We also found that synthetic mimics of the ZIKV capsid protein induced cell death and . This study provides important insight into the relationship between ZIKV infection and brain diseases.
Front Cell Infect Microbiol, 2017; 7
28189711: Teng Y, Bi D, Xie G, Jin Y, Huang Y, Lin B, An X, Tong Y, Feng D
Model-informed risk assessment for Zika virus outbreaks in the Asia-Pacific regions.
Recently, Zika virus (ZIKV) has been recognized as a significant threat to global public health. The disease was present in large parts of the Americas, the Caribbean, and also the western Pacific area with southern Asia during 2015 and 2016. However, little is known about the factors affecting the transmission of ZIKV. We used Gradient Boosted Regression Tree models to investigate the effects of various potential explanatory variables on the spread of ZIKV, and used current with historical information from a range of sources to assess the risks of future ZIKV outbreaks. Our results indicated that the probability of ZIKV outbreaks increases with vapor pressure, the occurrence of Dengue virus, and population density but decreases as health expenditure, GDP, and numbers of travelers. The predictive results revealed the potential risk countries of ZIKV infection in the Asia-Pacific regions between October 2016 and January 2017. We believe that the high-risk conditions would continue in South Asia and Australia over this period. By integrating information on eco-environmental, social-economical, and ZIKV-related niche factors, this study estimated the probability for locally acquired mosquito-borne ZIKV infections in the Asia-Pacific region and improves the ability to forecast, and possibly even prevent, future outbreaks of ZIKV.
J Infect, 2017; 74
28060809: Teng Y, Bi D, Xie G, Jin Y, Huang Y, Lin B, An X, Feng D, Tong Y
Dynamic Forecasting of Zika Epidemics Using Google Trends.
We developed a dynamic forecasting model for Zika virus (ZIKV), based on real-time online search data from Google Trends (GTs). It was designed to provide Zika virus disease (ZVD) surveillance and detection for Health Departments, and predictive numbers of infection cases, which would allow them sufficient time to implement interventions. In this study, we found a strong correlation between Zika-related GTs and the cumulative numbers of reported cases (confirmed, suspected and total cases; $p<0.001$). Then, we used the correlation data from Zika-related online search in GTs and ZIKV epidemics between 12 February and 20 October 2016 to construct an autoregressive integrated moving average (ARIMA) model (0, 1, 3) for the dynamic estimation of ZIKV outbreaks. The forecasting results indicated that the predicted data by ARIMA model, which used the online search data as the external regressor to enhance the forecasting model and assist the historical epidemic data in improving the quality of the predictions, are quite similar to the actual data during ZIKV epidemic early November 2016.

Integer-valued autoregression provides a useful base predictive model for ZVD cases. This is enhanced by the incorporation of GTs data, confirming the prognostic utility of search query based surveillance. This accessible and flexible dynamic forecast model could be used in the monitoring of ZVD to provide advanced warning of future ZIKV outbreaks.
PLoS One, 2017; 12

26011078: Teng Y, Wang Y, Zhang X, Liu W, Fan H, Yao H, Lin B, Zhu P, Yuan W, Tong Y, Cao W
Systematic Genome-wide Screening and Prediction of microRNAs in EBOV During the 2014 Ebolavirus Outbreak.
Recently, several thousand people have been killed by the Ebolavirus disease (EVD) in West Africa, yet no current antiviral medications and treatments are available. Systematic investigation of ebolavirus whole genomes during the 2014 outbreak may shed light on the underlying mechanisms of EVD development. Here, using the genome-wide screening in ebolavirus genome sequences, we predicted four putative viral microRNA precursors (pre-miRNAs) and seven putative mature microRNAs (miRNAs). Combing bioinformatics analysis and prediction of the potential ebolavirus miRNA target genes, we suggest that two ebolavirus coding possible miRNAs may be silence and down-regulate the target genes NFKBIE and RIPK1, which are the central mediator of the pathways related with host cell defense mechanism. Additionally, the ebolavirus exploits the miRNAs to inhibit the NF-kB and TNF factors to evade the host defense mechanisms that limit replication by killing infected cells, or to conversely trigger apoptosis as a mechanism to increase virus spreading. This is the first study to use the genome-wide scanning to predict microRNAs in the 2014 outbreak EVD and then to apply systematic bioinformatics to analyze their target genes. We revealed a potential mechanism of miRNAs in ebolavirus infection and possible therapeutic targets for Ebola viral infection treatment.
Sci Rep, 2015; 5

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BOARD NUMBER: S07-426

DATA WORKFLOW FOR MULTI-ANIMAL VIDEO-LOCAL FIELD POTENTIAL ACQUISITION AND SEIZURE ANALYSIS USING OPEN EPHYS AND BONSAI.

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Gergely Tarcsay, Brittney Boublil, Laura Ewell
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Chronic local field potential (LFP) and video monitoring is a common technique in neuroscience which often requires purchase of expensive hardware systems that are only suitable for low channel count, low sampling rate recordings. We aimed to develop a low-cost platform that could later be repurposed for other electrophysiological applications such as high-density single unit recording. The major challenges of chronic video-LFP acquisition are synchronization between distinct data streams and handling of large data files. Moreover, preprocessing and analyzing several days of video-LFP data is time consuming. Therefore, we developed a data workflow using the open-source Bonsai visual programming software to 1) acquire data from an Open Ephys acquisition board (LFP) and from a web-camera (video) 2) synchronize the video-LFP data 3) save data regularly to avoid potential data loss during chronic recordings and limit file sizes to facilitate later processing. Our workflow was successfully applied during 48-hour video-LFP recordings of epileptic mice (n=8 mice, two groups of four simultaneously recorded mice). In order to analyze seizure activity and to assign the behavioral state, a graphical user interface (GUI) was written in MATLAB. The GUI provides a user-friendly solution for 4) organization of the recorded video-LFP data 5) automatized seizure detection 6) convenient behavior analysis during seizure activity. In conclusion, our pipeline serves as a straightforward and easy way for chronic recordings and analysis of multi-site video-LFP data in epileptic mice. We present a low cost solution that utilizes a hardware platform that can be applied to other experimental needs.

Pubmed:
34257077: Oláh VJ, Tarcsay G, Brunner J
Small Size of Recorded Neuronal Structures Confines the Accuracy in Direct Axonal Voltage Measurements.
Patch-clamp instruments including amplifier circuits and pipettes affect the recorded voltage signals. We hypothesized that realistic and complete representation of recording instruments together with detailed morphology and biophysics of small recorded structures will reveal signal distortions and provide a tool that predicts native, instrument-free electrical signals from distorted voltage recordings. Therefore, we built a model that was verified by small axonal recordings. The model accurately recreated actual action potential (AP) measurements with typical recording artefacts and predicted the native electrical behavior. The simulations verified that recording instruments substantially filter voltage recordings. Moreover, we revealed that instrumentation directly interferes with local signal generation depending on the size of the recorded structures, which complicates the interpretation of recordings from smaller structures, such as axons. However, our model offers a straightforward approach that predicts the native waveforms of fast voltage signals and the underlying conductances even from the smallest neuronal structures.
eNeuro, 2021 Jul-Aug; 8

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BOARD NUMBER: S07-427

EBRAINS TOOLS FOR RODENT BRAIN ATLASING

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Maja Puchades, Sharon Yates, Nicolaas Groeneboom, Gergely Csúcs, Dmitri Darine, Harry Carey, Trygve Leergaard, Jan Bjaalie
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Research in small animals depends on comparisons of cellular and molecular measures in large groups, requiring efficient and reproducible methods for registration of data to brain atlases followed by quantitative analysis. While numerous methodologies and increasing amounts of data are available, they are difficult to combine into coherent and reproducible workflows suitable for large-scale analyses. The EBRAINS research infrastructure enables neuroscientists to conduct their mouse and rat brain data analyses in an open, robust and user-friendly environment. It provides tools and workflows for viewing images; automatic and user guided registration of brain sections image data to a volumetric atlas; feature extraction with machine learning; whole brain distribution analysis, and metadata management according to the FAIR principles. We present examples of typical use cases, illustrating how the available suite of tools can be used and the results obtained, with an emphasis on the reproducibility of the methods applied and the sharing of the data and metadata. The tools are part of the EBRAINS Atlases services (https://ebrains.eu/services/atlases) and offer comprehensive solutions for organizing, analyzing and sharing brain research data. These and other EBRAINS research infrastructure tools and workflows are developed in close interaction with users in the neuroscience community. Funded from EU Horizon 2020, Specific Grant Agreement No. 945539 (Human Brain Project SGA3).

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EMPLOYING MASSIVE PARALLEL COMPUTING POWER TO UNCOVER THE MYSTERIES OF SINGLE-CHANNEL GATING

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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[1]Friedrich-Alexander-Universität Erlangen-Nürnberg, Institut Für Physiologie Und Pathophysiologie, Erlangen, Germany, [2]Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen National High Performance Computing Center, Erlangen, Germany

The importance of single-channel recordings is widely known among ion channel enthusiasts. The method allows observing the function of a single protein in real time. Therefore, the analysis of single-channel recordings is essential for estimating the gating kinetics of ion channels. A widely employed method to model single-channel gating is with Hidden Markov Models (HMM). Among different algorithms for single-channel current analysis, arguably, the Two-Dimensional-Dwell-Time Fit (2D-Fit) has proven the most powerful thus far. The algorithm compares Two-Dimensional-Dwell-Time histograms derived from a recorded time series with simulated ones. It iteratively optimizes the parameters of the kinetic model until one of the simulated time series is as close as possible to the recorded one. Estimating the HMM of an ion channel in this way comes with exceptional computational demands. Using the parallel computing power of a High Performance Computing (HPC) cluster the algorithm can converge in a reasonable amount of time. The increased computing power also enables the computation of an ensemble of solutions, which can be ranked according to the computed loss, revealing the best solutions. Thus, statistics can be obtained further optimizing the 2D-Fit algorithm while also estimating the limits of its capabilities. This makes it possible to statistically compare results and pave the way for a realizable topology estimation, which relies on an exhaustive search over relevant models. Making use of HPC, the power of 2D-Dwell-Time analysis can be used to its fullest, possibly gaining an edge over other methods employed so far.

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DEEPSLICE RAT: A DEEP NEURAL NETWORK FOR AUTOMATIC REGISTRATION OF RAT BRAIN IMAGES TO THE WAXHOLM SPACE ATLAS OF THE RAT BRAIN

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Harry Carey[1], Gergely Csúcs[1], Simon Mcmullan[2], Maja Puchades[1], Jan Bjaalie[1]
[1]Neural Systems Laboratory, Institute Of Basic Medical Sciences, University Of Oslo, Oslo, Norway, [2]Macquarie University, Faculty Of Medicine, Health And Human Sciences, Sydney, Australia

The rat is widely used in experimental neuroscience and with the increasing availability of many genetic tools in rat, its popularity in neuroscientific research is likely to increase. In order to properly leverage the magnitude of rat data that the neuroscientific community generates, brain derived data must be mapped to a common spatial reference space. To this end, the Waxholm Space atlas of the rat brain (RRID:SCR_017124) has been created. While there exist tools to register rat brain data to the Waxholm Space, including the QuickNII (RRID:SCR_016854) and VisuAlign (RRID:SCR_017978) tools provided by the EBRAINS research infrastructure (https://ebrains.eu/services/atlases), these tools are relatively demanding of time and expertise, often requiring hours per whole brain. If we are to comprehensively map the massive amount of brain data currently being generated, we will require automated methods capable of registering data to a common reference space. Here we present DeepSlice Rat, a deep neural network for the automated registration of rat brain images to the Waxholm Space atlas of the rat brain. We show that DeepSlice Rat is equal in performance to an expert neuroanatomist whilst being thousands of times faster. DeepSlice Rat can be used in combination with QuickNII for verification of the registration, and VisuAlign for non-linear adjustments, creating an AI assisted workflow that will greatly enhance productivity. DeepSlice Rat will be freely available on EBRAINS (alongside a mouse implementation) as both a Python package and web-application.
Funded from EU Horizon 2020, Specific Grant Agreement No. 945539 (Human Brain Project SGA3).

Pubmed:
33081558: Everett NA, Carey HA, Cornish JL, Baracz SJ
Sign tracking predicts cue-induced but not drug-primed reinstatement to methamphetamine seeking in rats: Effects of oxytocin treatment.
The incentive sensitisation theory of addiction posits that drug-associated stimuli become imbued with incentive motivational properties, driving pathological drug seeking. However, pre-existing variability in the incentive salience to non-drug reward cues ('sign trackers' (STs); 'goal trackers' (GTs)) is also predictive of the desire for and relapse to cocaine and opioids. Here, we asked whether variation in propensity to attribute incentive salience to a food cue is predictive of reinstatement to the highly addictive psychostimulant methamphetamine (METH), and whether treatment with the promising anti-addiction therapy oxytocin differentially reduces METH behaviour between STs and GTs.
J Psychopharmacol, 2020; 34

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A NEW FREE SOFTWARE FOR 3D DENDRITIC SPINE DETECTION AND MORPHOLOGICAL ANALYSIS

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

Nicolas Heck[1], Jean-François Gilles[2], Philippe Mailly[3], Thomas Boudier[4]
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Dendritic spines are indicative of the density of connections in the neural network, and their morphology corresponds to properties of synaptic integration. Dendrites and spines can be labeled with various techniques and imaged in 3D from confocal or two-photon image stacks. Several softwares are available for spine detection and morphological analysis, but their efficiency varies depending on labeling method and image type. We introduce a new freely available plugin for ImageJ/FIJI. The plugin operates in 3D to detect dendritic spines along a traced dendrite, segment the spine head and trace the spine neck. Spine density, spine head volumes and surfaces, neck length are computed. Importantly, the user can at each step and for each spine edit the result of the automated process. As compared to a fully automated procedure, that would not be applicable to many images, the possibility for the user to edit the result make our tool more versatile.

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DOMEVR: A SETUP FOR EXPERIMENTAL CONTROL OF AN IMMERSIVE DOME VIRTUAL ENVIRONMENT FOR NON-HUMAN AND HUMAN BEHAVIOR CREATED WITH UNREAL ENGINE 4

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Creating immersive and realistic virtual reality (VR) environments for different species is a goal of many neuroscientific groups. Unfortunately, designing and implementing behavioural tasks in such environments is often complicated. To tackle this challenge we created DomeVR, an immersive virtual reality dome environment built using Unreal Engine 4 (UE4). UE4 is a powerful game engine with photo-realistic graphics containing a visual scripting language designed for use by non-programmers, meaning behavioural task design and level creation are simply performed using drag-and-drop elements. DomeVR introduces features to UE4 that are necessary for neuroscience experiments. This includes a logging and synchronization system to solve the timing uncertainties inherent in UE4, both key for the analysis of neuroscientific data; parameterizable stimuli, an interactive GUI for scientists to observe subjects during experiments and adjust task parameters on the fly, and a dome projection system for broad visual coverage (250 degree visual angle). These features are modular and can be easily added into other UE4 projects. Here we present the design of the DomeVR system together with proof-of-principle data from three different species; human, macaque and mouse.

Pubmed:
35120628: Uran C, Peter A, Lazar A, Barnes W, Klon-Lipok J, Shapcott KA, Roese R, Fries P, Singer W, Vinck M
Predictive coding of natural images by V1 firing rates and rhythmic synchronization.
Predictive coding is an important candidate theory of self-supervised learning in the brain. Its central idea is that sensory responses result from comparisons between bottom-up inputs and contextual predictions, a process in which rates and synchronization may play distinct roles. We recorded from awake macaque V1 and developed a technique to quantify stimulus predictability for natural images based on self-supervised, generative neural networks. We find that neuronal firing rates were mainly modulated by the contextual predictability of higher-order image features, which correlated strongly with human perceptual similarity judgments. By contrast, V1 gamma (γ)-synchronization increased monotonically with the contextual predictability of low-level image features and emerged exclusively for larger stimuli. Consequently, γ-synchronization was induced by natural images that are highly compressible and low-dimensional. Natural stimuli with low predictability induced prominent, late-onset beta (β)-synchronization, likely reflecting cortical feedback. Our findings reveal distinct roles of synchronization and firing rates in the predictive coding of natural images.
Neuron, 2022; 110
34879273: Peter A, Stauch BJ, Shapcott K, Kouroupaki K, Schmiedt JT, Klein L, Klon-Lipok J, Dowdall JR, Schölvinck ML, Vinck M, Schmid MC, Fries P
Stimulus-specific plasticity of macaque V1 spike rates and gamma.
When a visual stimulus is repeated, average neuronal responses typically decrease, yet they might maintain or even increase their impact through increased synchronization. Previous work has found that many repetitions of a grating lead to increasing gamma-band synchronization. Here, we show in awake macaque area V1 that both repetition-related reductions in firing rate and increases in gamma are specific to the repeated stimulus. These effects show some persistence on the timescale of minutes. Gamma increases are specific to the presented stimulus location. Further, repetition effects on gamma and on firing rates generalize to images of natural objects. These findings support the notion that gamma-band synchronization subserves the adaptive processing of repeated stimulus encounters.
Cell Rep, 2021; 37
32350517: Shapcott KA, Schmiedt JT, Kouroupaki K, Kienitz R, Lazar A, Singer W, Schmid MC
Reward-Related Suppression of Neural Activity in Macaque Visual Area V4.
In order for organisms to survive, they need to detect rewarding stimuli, for example, food or a mate, in a complex environment with many competing stimuli. These rewarding stimuli should be detected even if they are nonsalient or

irrelevant to the current goal. The value-driven theory of attentional selection proposes that this detection takes place through reward-associated stimuli automatically engaging attentional mechanisms. But how this is achieved in the brain is not very well understood. Here, we investigate the effect of differential reward on the multiunit activity in visual area V4 of monkeys performing a perceptual judgment task. Surprisingly, instead of finding reward-related increases in neural responses to the perceptual target, we observed a large suppression at the onset of the reward indicating cues. Therefore, while previous research showed that reward increases neural activity, here we report a decrease. More suppression was caused by cues associated with higher reward than with lower reward, although neither cue was informative about the perceptually correct choice. This finding of reward-associated neural suppression further highlights normalization as a general cortical mechanism and is consistent with predictions of the value-driven attention theory.
Cereb Cortex, 2020; 30
32217819: Klein L, Pothof F, Raducanu BC, Klon-Lipok J, Shapcott KA, Musa S, Andrei A, Aarts AA, Paul O, Singer W, Ruther P
High-density electrophysiological recordings in macaque using a chronically implanted 128-channel passive silicon probe.
The analysis of interactions among local populations of neurons in the cerebral cortex (e.g. within cortical microcolumns) requires high resolution and high channel count recordings from chronically implanted laminar microelectrode arrays. The request for high-density recordings of a large number of recording sites can presently only be accomplished by probes realized using complementary metal-oxide-semiconductor (CMOS) technology. In preparation for their use in non-human primates, we aimed for neural probe validation in a head-fixed approach analyzing the long-term recording capability.
J Neural Eng, 2020; 17
30017481: Kienitz R, Schmiedt JT, Shapcott KA, Kouroupaki K, Saunders RC, Schmid MC
Theta Rhythmic Neuronal Activity and Reaction Times Arising from Cortical Receptive Field Interactions during Distributed Attention.
Growing evidence suggests that distributed spatial attention may invoke theta (3-9 Hz) rhythmic sampling processes. The neuronal basis of such attentional sampling is, however, not fully understood. Here we show using array recordings in visual cortical area V4 of two awake macaques that presenting separate visual stimuli to the excitatory center and suppressive surround of neuronal receptive fields (RFs) elicits rhythmic multi-unit activity (MUA) at 3-6 Hz. This neuronal rhythm did not depend on small fixational eye movements. In the context of a distributed spatial attention task, during which the monkeys detected a spatially and temporally uncertain target, reaction times (RTs) exhibited similar rhythmic fluctuations. RTs were fast or slow depending on the target occurrence during high or low MUA, resulting in rhythmic MUA-RT cross-correlations at theta frequencies. These findings show that theta rhythmic neuronal activity can arise from competitive RF interactions and that this rhythm may result in rhythmic RTs potentially subserving attentional sampling.
Curr Biol, 2018; 28
28743958: Kurtz P, Shapcott KA, Kaiser J, Schmiedt JT, Schmid MC
The Influence of Endogenous and Exogenous Spatial Attention on Decision Confidence.
Spatial attention allows us to make more accurate decisions about events in our environment. Decision confidence is thought to be intimately linked to the decision making process as confidence ratings are tightly coupled to decision accuracy. While both spatial attention and decision confidence have been subjected to extensive research, surprisingly little is known about the interaction between these two processes. Since attention increases performance it might be expected that confidence would also increase. However, two studies investigating the effects of endogenous attention on decision confidence found contradictory results. Here we investigated the effects of two distinct forms of spatial attention on decision confidence; endogenous attention and exogenous attention. We used an orientation-matching task, comparing the two attention conditions (endogenous and exogenous) to a control condition without directed attention. Participants performed better under both attention conditions than in the control condition. Higher confidence ratings than the control condition were found under endogenous attention but not under exogenous attention. This finding suggests that while attention can increase confidence ratings, it must be voluntarily deployed for this increase to take place. We discuss possible implications of this relative overconfidence found only during endogenous attention with respect to the theoretical background of decision confidence.
Sci Rep, 2017; 7
27721468: Shapcott KA, Schmiedt JT, Saunders RC, Maier A, Leopold DA, Schmid MC
Correlated activity of cortical neurons survives extensive removal of feedforward sensory input.
A fundamental property of brain function is that the spiking activity of cortical neurons is variable and that some of this variability is correlated between neurons. Correlated activity not due to the stimulus arises from shared input but the neuronal circuit mechanisms that result in these noise correlations are not fully understood. Here we tested in the visual system if correlated variability in mid-level area V4 of visual cortex is altered following extensive lesions of primary visual cortex (V1). To this end we recorded longitudinally the neuronal correlations in area V4 of two behaving macaque monkeys before and after a V1 lesion while the monkeys fixated a grey screen. We found that the correlations of neuronal activity survived the

lesions in both monkeys. In one monkey, the correlation of multi-unit spiking signals was strongly increased in the first week post-lesion, while in the second monkey, correlated activity was slightly increased, but not greater than some week-by-week fluctuations observed. The typical drop-off of inter-neuronal correlations with cortical distance was preserved after the lesion. Therefore, as V4 noise correlations remain without feedforward input from V1, these results suggest instead that local and/or feedback input seem to be necessary for correlated activity.
Sci Rep, 2016; 6

27021172: Klein C, Evrard HC, Shapcott KA, Haverkamp S, Logothetis NK, Schmid MC
Cell-Targeted Optogenetics and Electrical Microstimulation Reveal the Primate Koniocellular Projection to Supra-granular Visual Cortex.
Electrical microstimulation and more recently optogenetics are widely used to map large-scale brain circuits. However, the neuronal specificity achieved with both methods is not well understood. Here we compare cell-targeted optogenetics and electrical microstimulation in the macaque monkey brain to functionally map the koniocellular lateral geniculate nucleus (LGN) projection to primary visual cortex (V1). Selective activation of the LGN konio neurons with CamK-specific optogenetics caused selective electrical current inflow in the supra-granular layers of V1. Electrical microstimulation targeted at LGN konio layers revealed the same supra-granular V1 activation pattern as the one elicited by optogenetics. Taken together, these findings establish a selective koniocellular LGN influence on V1 supra-granular layers, and they indicate comparable capacities of both stimulation methods to isolate thalamo-cortical circuits in the primate brain.
Neuron, 2016; 90

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SCALE SPACE METHODS FOR THE GEOMETRIC ANALYSIS OF NEURITE TRACES

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Large scale imaging and automated tracing are yielding many single cell neuronal reconstructions, and analysis methods able to probe the local geometry of the traces are needed to fully exploit them. Scale space methods are classical tools in signal processing, where a signal is computed at diverse scales, from that of the original signal down to a very smoothed out, coarse, one providing only the largest trend. They allow to study how features vary through scales, or spot the most important ones from noise. Here, we applied this approach/strategy on neurite traces, seen as 3D space curves, via gaussian smoothing of the coordinates. We extracted the curves' that way 'principal turns', i.e. their major changes in direction. We also defined a new algorithm, termed *nAdde*r, which computes a 'local 3D scale' measuring a curve's local geometric complexity, i.e. how much it is in 3D vs confined to a plane or a line.. We applied this algorithm on published neuron traces, including a whole zebrafish larva brain atlas, showing that it is a meaningful and significant metric enabling to analyze datasets in ways previously unavailable, shedding new light on the stereotypical nature of neurites' local geometry . These new methods, available in the open source python library GeNePy3D, provide innovative and easy to use ways to process the flood of geometric data produced by large-scale connectomics efforts.

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BOARD NUMBER: S07-433

A SEMI-AUTOMATIC PREPROCESSING TOOLBOX FOR BELT-RECORDED RESPIRATION SIGNAL

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Respiration is a semi-automatic process centrally generated by intricated microcircuits integrating behavior and physiological information. Perceptual, cognitive, emotional and motor processes have been shown both to modulate respiration and to be influenced by respiration. Respiration is thus becoming an important measure of interest in neuroscience. In humans, breathing at rest should not be considered as a simple periodical signal. Respiratory traces reveal a wide range of respiratory events. Sighs, amplitude or phase variability of breathing cycles and pauses are sporadically embedded in the breathing pattern. Here, we present a semi-automatic toolbox in order to preprocess breath traces recorded using easy to use and non-intrusive respiratory belts. The toolbox builds on previous work from different groups and non-oscillatory methods. It allows to identify inspiration and expiration onsets, to extract the respiration phase and identify respiratory events such as sighs or short breathing interruption. Furthermore, concurrent behavior of interest such as voluntary action, eye movements, cardiac or gastric events can be visualized within the respiration phase.

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ESTABLISHMENT OF AN OPTIMIZED AND AUTOMATED WORKFLOW FOR WHOLE BRAIN PROBING OF NEURONAL ACTIVITY PATTERNS IN TRAP MICE

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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[1]Université Claude Bernard Lyon 1, Labex Cortex, CRNL, CNRS UMR5292, SBRI, INSERM U1208, Rhône, Bron, France, [2]Centre Recherche Neurosciences Lyon (CRNL), CNRS UMR5292, INSERM U1028, Universilté LYON I, Sleep Team, BRON, France, [3]INMG - PGNM, Rhone-alpes, LYON, France, [4]Univ Lyon, Université Claude Bernard Lyon 1, Inserm, Stem Cell and Brain Research Institute U1208, Rhône, Bron, France, [5]Inserm UMRS 1028 - CNRS UMR5292 - UCBL Lyon1, Centre De Recherche En Neurosciences De Lyon, Bron, France

Aims: Behaviors are encoded by neural circuits that are widespread within the brain and change with age and experience. Immunodetection of the immediate early gene c-fos has been successfully used for decades to reveal neural circuits active during specific tasks or conditions. Our aims here is to develop a workflow that circumvents classical temporal and spatial limitations associated to c-fos quantifications. Method: We used a new genetic TRAP method combining Cre-dependent tdTomato expression under c-fos promoter with Fos immunohistochemistry, allowing visualization and direct comparison of neural circuits activated at different times or during different tasks. We established a workflow that optimize and automate cell detection, cell classification (e.g. Fos *vs*. Fos/tdTomato) and whole brain registration. Results: We demonstrate that this workflow allows accurate cell number quantification with minimal interindividual variability. Further, interrogation of brain atlases at different scales (from simplified to detailed) was achieved allowing gradually zooming on brain regions to explore spatial distribution of activated cells. Finally, we illustrate the potential of this approach by comparing patterns of neuronal activation in various contexts (e. g. different vigilance states, complex behavioral tasks …). Conclusion: Altogether, this automated workflow allows a fast and accurate analysis of whole brain activity pattern at the cellular level, in various contexts.

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ACTION RECOGNITION AND KINEMATIC ANALYSIS WITH DEEPLABCUT

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Markerless pose estimation has become a powerful tool in neuroscience for digitizing video data. DeepLabCut is a software package for animal pose estimation that achieves state of the art performance on challenging animal datasets. Here, we introduce two novel tools that directly build and integrate with DeepLabCut: DLC2Kinematics and DLC2Action, that respectively allow users to compute kinematic features and perform action segmentation on the outputs of DeepLabCut. Specifically, DLC2Kinematics leverages machine learning and classical motion analysis and makes them available with a simple user interface. DLC2Action allows for user-defined behavior recognition. DLC2Action shows state of the art performance on CalMS21 (an animal behavior challenge from CVPR 2021).

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POST-ACTIVATION CONFORMATIONAL CHANGES IN PIGEON CRYPTOCHROME

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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A wide-spread hypothesis ascribes the ability of migratory birds to navigate over large distances to an inclination compass realized by the protein cryptochrome in the birds' retinae. Cryptochromes are activated by blue light, which triggers a radical pair that could be sensitive to the Earth's magnetic field. This magnetic information might be communicated to downstream processes through conformational changes eventually reaching the bird's brain and therefore allowing magnetoreception. Starting from the ClCry4 crystal structure [1], we employ extensive all-atom MD simulations for three pairs of replicas for a total time of 6 µs utilizing the Scandinavian Online Kit For Nanoscale Modeling (VIKING) [2], in which we simulate the ground state and the activated state of the protein structure. Employing an evaluation of inter-residue distance changes, we found clear indications of conformational changes that arise upon protein activation. Specifically, we have identified two versatile regions whose motion can be directly attributed to the protein's activation. Our results show that cryptochrome's conformational dynamics involve the so-called phosphate-binding loop, which opens like a gate, creating a passage to the active site of the protein [3]. REFERENCES [1] B. D. Zoltowski et al., *Proc. Natl. Acad. Sci* **116** 19449-19457 (2019) [2] V. Korol et al., *ACS Omega* **5** 1254-1260 (2020) [3] F. Schuhmann et al., *JPC B* **125** 9652-9659 (2021)

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BOARD NUMBER: S07-437

THE BRAINGLOBE INITIATIVE: AN OPEN SOURCE NEUROANATOMY PLATFORM FOR THE 21ST CENTURY

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Neuroanatomy is key for understanding the brain, but software to analyse the data are often single purpose, for one model species and suffer from lack of support following publication. We have established the BrainGlobe Initiative (BGI) - an international, distributed team of users and developers working towards the goal of creating open-source, interoperable and easy to use tools for the analysis of all types of neuroanatomical data. To analyze data from many samples, it is critical to map individual datasets onto a standard anatomical reference atlas, but neuroscience relies on many animal model species. The BrainGlobe toolkit is therefore not built around a specific brain atlas but rather a generalised atlas framework (the BrainGlobe Atlas API) which is regularly updated with new brain atlases from multiple species. We have initially focussed on the analysis of whole-brain microscopy data (e.g. from tissue clearing or blockface methods). The BGI software suite includes brainreg, a 3D registration tool for mapping data to an atlas space. Novel tools have also been developed to map specific structures or objects within the brain, such as the volume and location of virus-labelled cells and the location of implanted devices such as recording electrodes. Lastly, brainrender can be used for the visualisation of any data registered to the reference atlas. This includes all data from BGI software, but importantly, data from other software packages and from large-scale efforts such as the Allen Brain Institute.

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CEREBRAL 3D DOPPLER IMAGE REGISTRATION BASED ON VASCULAR LANDMARK AUTOMATIC LOCALIZATION WITH DEEP NEURAL NETWORK.

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Ultrafast ultrasound is an emerging imaging modality derived from standard medical ultrasound. It allows for high spatiotemporal resolution with techniques such as ultrafast Doppler used for functional ultrasound (fUS). In neurosciences fUS imaging is limited by the difficulty to identify cerebral structures in Doppler images. Thus, localization algorithms were developed to align volumetric Doppler images. In practice those algorithms are very sensitive to the initial misalignment between volumes and often require manual pre-registration. Yet, this first step is operator dependent and challenging especially with pathological data. Here we propose a deep learning approach to the 3D Doppler image registration task, utilizing vascular landmark detection in volumetric Doppler images of mouse brains. 150 volumetric images were obtained by mouse brain angiographic scan, normalized and placed in a 12.8x12.8x6.4mm field of view with a 100µm isotropic resolution. First, we manually annotated these images with 9 landmarks corresponding to prominent vascular clues. Then, a 3D convolutional neural network was implemented and trained to identify those landmarks. Our network is consistent at identifying the landmarks granting a Dice score of 0.68±0.09 on test samples. It also produces a small localization error of 130±40µm. After automatic localization, landmark point clouds were used to perform rigid vascular 3D registration, for both intra and inter animal studies, with large initial misalignments and pathological vascular deformations induced by stroke events.

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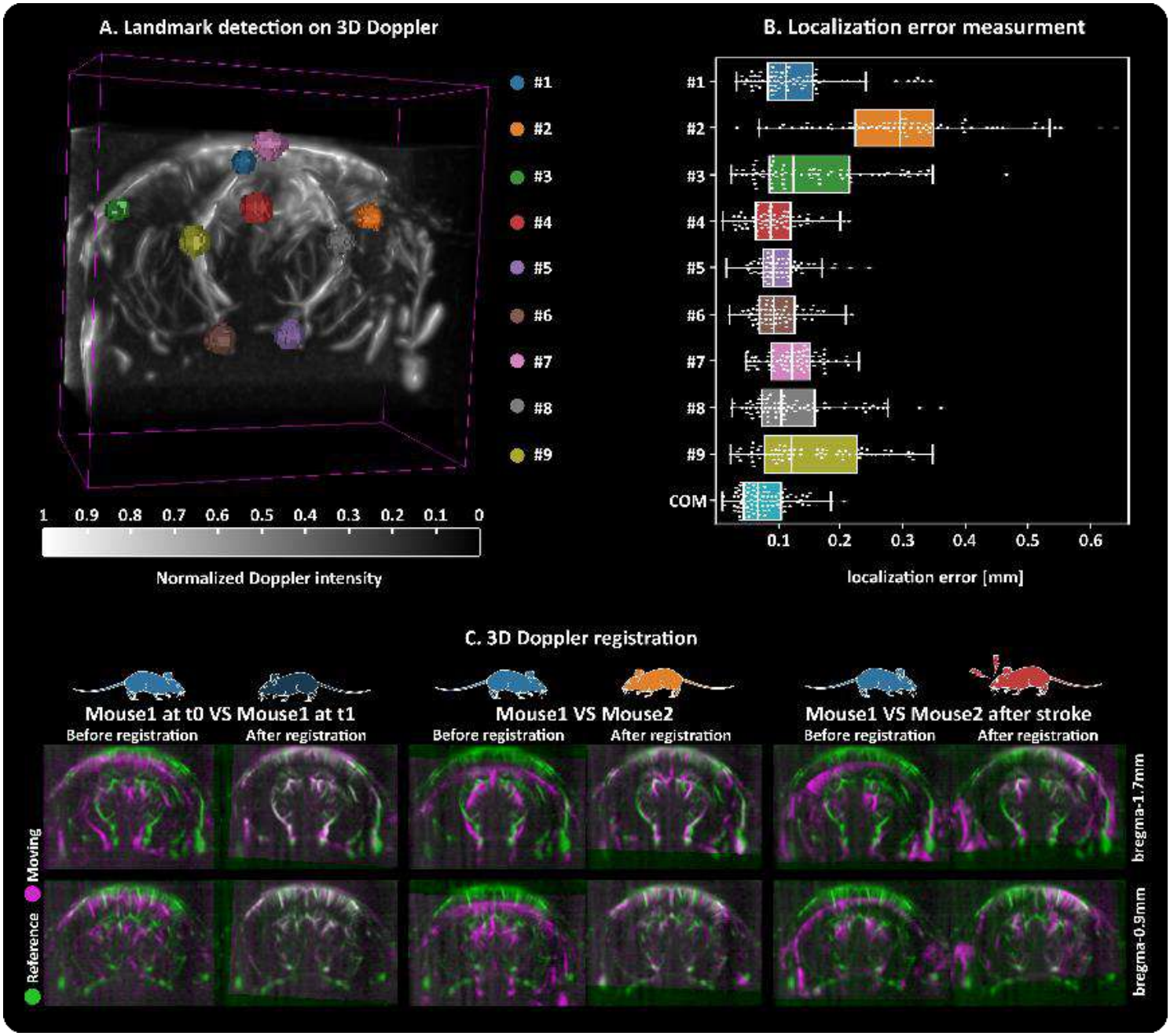

A. Vascular landmark detection on a 3D Doppler image of a mouse brain. B. Localization error measurement. C. 3D cerebral Doppler image registration by landmark point cloud matching.

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INDEPENDENT COMPONENT ANALYSIS FOR DETECTING NONLINEARITIES IN CO2-ELICITED BOLD FLUCTUATIONS: AN ANALYSIS PIPELINE FOR THE STUDY OF THE CENTRAL CONTROL OF BREATHING WITH FMRI

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Aims Functional MRI studies about the central control of breathing play a crucial role in unravelling the physiopathology of several autonomic diseases. In this context, the main challenge is represented by the entanglement between non-specific BOLD effects, e.g., due to vasoreaction and physiological noise, and specific ones. We propose an analysis pipeline for the characterization of nonlinearities in the BOLD response to CO_2 challenges. This might provide insight on nonlinearity as discriminative criterion for specific effects. **Methods** Three runs of fMRI images are acquired on six subjects at increasing inhaled CO_2 concentrations (0, 3 and 7%). Group-ICA is implemented with the Matlab toolbox GIFT. We select components by considering their correlation with end-tidal $PETCO_2$ and their power spectral distribution. By using GICA3 algorithm, subject-level reconstructions preserve information on percentage signal change. Triplets of spatially similar components across CO_2 levels are defined. We fitted voxel-by-voxel a linear and a quadratic model on percent signal change. The R^2 indicated the degree of nonlinearity expressed by the BOLD response to CO_2 across the brain. **Results** We were able to highlight brain regions that presented highly-nonlinear BOLD signal increases with increasing CO_2 concentrations. Hotspots are present in regions involved in the central control of breathing, as the brainstem, as well as in the thalamus, hypothalamus and basal ganglia (Fig1). **Conclusions** Our new pipeline suggests that group-ICA in association with polynomial regression analysis might provide information of interest for the definition of the specificity of BOLD fluctuations in

CO_2 studies.

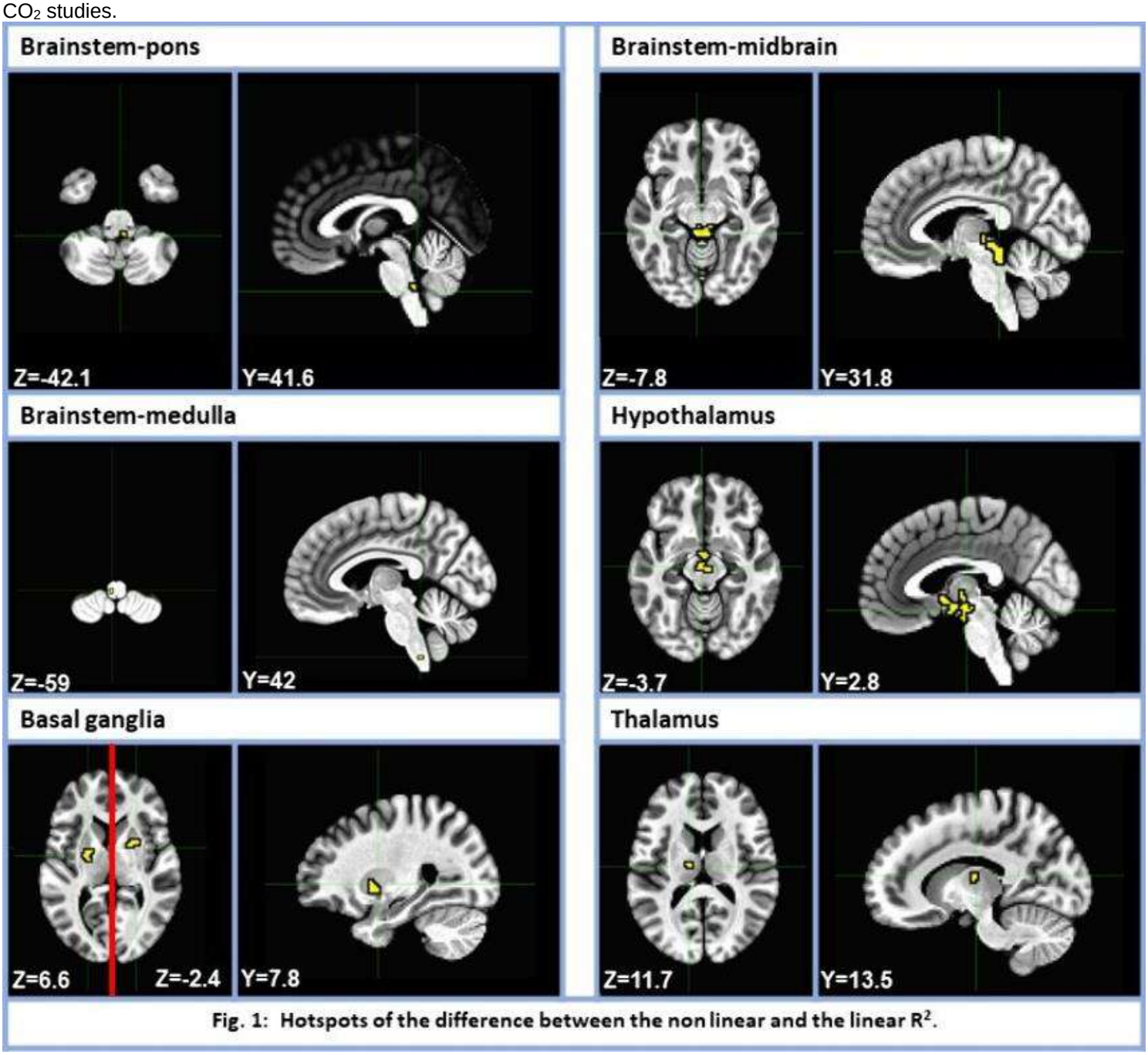

Fig. 1: Hotspots of the difference between the non linear and the linear R^2.

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DEEPRSLICE: AUTOMATED RODENT BRAIN EXTRACTION USING DEEP LEARNING

POSTER SESSION 07 - SECTION: SOFTWARE TOOLS FOR BRAIN IMAGING

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Removing the skull from brain images is usually the first step in most of the magnetic resonance imaging (MRI) processing pipelines, as it affects all the subsequent steps including registration and tissue segmentation. While multiple tools can automatically perform this task in human data, the same cannot be said for rodent MRI images, where the process is often error-prone and requires manual intervention. In this line of work, we present a convolutional neural network capable of performing brain extraction across different modalities and species. Aims: leveraging the power of deep learning to create a global apt tool for rodent brain extraction, regardless of contrast, resolution, or species. Methods: a dataset consisting of 68 manually segmented mice images aging between 8-12 weeks were used for the training. The images were converted to 2D slices before feeding them to the network. The 2D images were augmented by scaling, rotation, shearing, and cropping. Various 2D U-Net architectures were examined and the best performing architecture was compared to popular tools from MRI packages such as FSL's BET and ANTS' antsBrainExtraction. Results: The best performing network achieved an intersection over union (iou) score of 0.9223 on the test set surpassing the other tools included in the comparison. Conclusions: In the present work, we showed and evaluated a robust skull-stripping U-Net-based tool surpassing the performance of the traditional approaches commonly used in the field. Using such tools can significantly decrease processing times in preclinical imaging settings by eliminating arduous manual steps such as brain extraction.

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FOLATE RECEPTOR α POSITIVE HYBRIDOSOMES AS VEHICLES FOR NON-INVASIVE BRAIN-TARGETED GENE THERAPY

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Current gene therapies are mainly performed with viral vectors. The disadvantages of viral vectors include cell-type-nonspecific transduction and high immunogenicity. Lipid nanoparticles (LNPs) carrying nucleotides provide an alternative to viral gene therapy and vaccines, since many individuals have already received LNP-based vaccination. Here, we exploit this method for cerebral targeted gene therapy. We optimized a formulation with nanoplasmids for stable transfection. Further, we generated hybridosomes by enveloping LNPs with cell derived plasma membranes. Specific membrane anchored receptors expressed by those cells enable our hybridosomes to target and pass barriers in a tissue- and cell-type-specific manner. One such protective brain barrier is the blood-cerebrospinal fluid-brain barrier (BCSFB). Previously, we had demonstrated the transport of folate receptor α positive (FRα+) extracellular vesicles (EVs) across the BCSFB. Therefore, we disguise our FRα+ hybridosomes as EVs allowing the transport of our gene of interest across the BCSFB. So far, we have achieved high encapsulation efficiency and transfection rates in our *in vitro* studies in HEK293 and neuronal cells. Our next steps will include *in vivo* experiments in a reporter mouse line. Because of FRα and its ability to cross the BCSFB barrier, we can apply our hybridosomes non-invasively by intranasal inhalation and follow target cell expression over time. Compared to viral vectors, our hybridosomes can be used for tissue-specific transduction with low immunogenicity. This will enable novel vesicle-based gene therapies for neurodegenerative diseases.

Pubmed:
24038627: Von Niederhäusern V, Kastenhuber E, Stäuble A, Gesemann M, Neuhauss SC
Phylogeny and expression of canonical transient receptor potential (TRPC) genes in developing zebrafish.
Canonical transient receptor potential (TRPC) channels are nonselective, calcium-permeable cation channels that are expressed in a great variety of organisms, tissues, and cell types. TRPC channels are known to be involved in the transduction of polymodal sensory input. Additionally, they are implicated in a variety of developmental processes. Distinct gating mechanisms have been elucidated so far, but their exact functional role in vertebrate organisms still needs to be resolved.
Dev Dyn, 2013; 242
27413151: Pilz GA, Carta S, Stäuble A, Ayaz A, Jessberger S, Helmchen F
Functional Imaging of Dentate Granule Cells in the Adult Mouse Hippocampus.
The hippocampal dentate gyrus is critically involved in learning and memory. However, methods for imaging the activity of its principal neurons, the dentate gyrus granule cells, are missing. Here we demonstrate chronic two-photon imaging of granule cell population activity in awake mice using a cortical window implant that leaves the hippocampal formation intact and does not lead to obvious alteration of animal behavior. Using virus delivery, we targeted expression of genetically encoded calcium indicators specifically to dentate gyrus granule cells. Calcium imaging of granule cell activity 600-800 µm below the hippocampal surface was facilitated by using 1040 nm excitation of the red indicator R-CaMP1.07, but was also achieved using the green indicator GCaMP6s. We found that the rate of calcium transients was increased during wakefulness relative to an extremely low rate during anesthesia; however, activity still remained sparse with, on average, approximately one event per 2-5 min per cell across the granule cell population. Comparing periods of running on a ladder wheel and periods of resting, we furthermore identified state-dependent differences in the active granule cell population, with some cells displaying highest activity level during running and others during resting. Typically, cells did not maintain a clear state preference in their activity pattern across days. Our approach opens new avenues to elucidate granule cell function, plasticity mechanisms, and network computation in the adult dentate gyrus.
J Neurosci, 2016; 36
31197148: Ayaz A, Stäuble A, Hamada M, Wulf MA, Saleem AB, Helmchen F
Layer-specific integration of locomotion and sensory information in mouse barrel cortex.

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During navigation, rodents continually sample the environment with their whiskers. How locomotion modulates neuronal activity in somatosensory cortex, and how it is integrated with whisker-touch remains unclear. Here, we compared neuronal activity in layer 2/3 (L2/3) and L5 of barrel cortex using calcium imaging in mice running in a tactile virtual reality. Both layers increase their activity during running and concomitant whisking, in the absence of touch. Fewer neurons are modulated by whisking alone. Whereas L5 neurons respond transiently to wall-touch during running, L2/3 neurons show sustained activity. Consistently, neurons encoding running-with-touch are more abundant in L2/3 and they encode the run-speed better during touch. Few neurons across layers were also sensitive to abrupt perturbations of tactile flow during running. In summary, locomotion significantly enhances barrel cortex activity across layers with L5 neurons mainly reporting changes in touch conditions and L2/3 neurons continually integrating tactile stimuli with running.
Nat Commun, 2019; 10

FENS Forum 2022
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BOARD NUMBER: S07-442

BASE EDITING AS A POTENTIAL THERAPEUTIC STRATEGY FOR MOTOR NEURON DISEASES

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Amyotrophic Lateral Sclerosis (ALS) is caused by a number of mutations, with C9orf72 repeat expansions being the most common genetic cause. Due to the limitations of current pharmacological therapies, there is a strong need to develop innovative approaches. The goal of this study is the development of genome editing approaches aimed at reducing neurodegenerative aspects in ALS. The genomic engineering strategy adopted is a CRISPR-Cas9-based genome editing technology that allows the introduction of point mutations in the DNA without generating DSBs, using recently developed, highly precise enzymes: cytidine base editors (CBEs) and adenine base editors (ABEs) allowing C>T and A>G conversions, respectively. Several single-guide RNAs (sgRNA) were designed to target C9orf72 within the region containing the repeat expansions with the aim of boosting C9orf72 expression to counteract C9orf72 haploinsufficiency. This strategy was validated in the K562 cell line. In particular, at least three optimal sgRNA candidates resulted in up to 52%±5 base editing efficiency, that consequently resulted in 2-fold change increase of the C9orf72 transcripts levels. This strategy is currently being validated in induced pluripotent stem cell line (iPSCs) derived from ALS patients and differentiated to motor neurons in order to determine whether increasing the expression of C9orf72 will lead to amelioration of several cellular phenotypes in motor neuron cultures. We will also carry out proteomics and metabolomics analyses to define molecular pathways and targets in vitro that are altered upon increased expression of C9orf72 to further establish the potential of genome editing as a therapeutic strategy for ALS patients.

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BOARD NUMBER: S07-443

CREATING CHOLINERGIC NEURON SPECIFIC KNOCK-OUT MICE BY COMBINING THREE (CRISPR-CAS9, CRE/LOXP AND AAV) GENOME EDITING TECHNOLOGIES

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Aim: The aim of our experiment was to create a genetically modified mouse strain in which we knock-out the Estrogen Receptor alpha (ERα) (or any other receptor) specifically from cholinergic neurons, in any interested areas of the brain. **Material and methods:** First, two homozygous mouse strains were crossed. One contains a cholinergic neuron-specific Cre recombinase (choline acetyltransferase; ChAT-Cre), and the other contains the Cas9 enzyme, the transcription is blocked by two loxP cleavage sites (LoxP-STOP-LoxP-Cas9). All cholinergic (and not other types) neurons in double heterozygous animals, formed during crossing, will express the Cas9 enzyme. The next step was a stereotaxic injection of an adeno-associated virus vector (AAV) containing an ERα-specific guide-RNA into a specific area of the brain, in our case the nucleus basalis magnocellularis (NBM). The AAV virus was custom made in our laboratory. **Results:** In these animals, Cas9 is presented and active only in cholinergic cells. AAV viruses are active in the NBM (site of the AAV injection), because only here the CRISPR sequence required for its function appears. This specifically applies only to the ERα gene (see guide-RNA), which disappeared from these cells. **Conclusions:** The technology combining the CRISPR-Cas9, Cre/loxP and AAV virus systems is really promising to edit the genome in adult animals more selectively. Creating new genetically modified animals will help the better understanding of neurological diseases and improve research tools.

Pubmed:
24981738: Kovacs T, Csongei V, Feller D, Ernszt D, Smuk G, Sarosi V, Jakab L, Kvell K, Bartis D, Pongracz JE
Alteration in the Wnt microenvironment directly regulates molecular events leading to pulmonary senescence.
In the aging lung, the lung capacity decreases even in the absence of diseases. The progenitor cells of the distal lung, the alveolar type II cells (ATII), are essential for the repair of the gas-exchange surface. Surfactant protein production and survival of ATII cells are supported by lipofibroblasts that are peroxisome proliferator-activated receptor gamma (PPARγ)-dependent special cell type of the pulmonary tissue. PPARγ levels are directly regulated by Wnt molecules; therefore, changes in the Wnt microenvironment have close control over maintenance of the distal lung. The pulmonary aging process is associated with airspace enlargement, decrease in the distal epithelial cell compartment and infiltration of inflammatory cells. qRT-PCR analysis of purified epithelial and nonepithelial cells revealed that lipofibroblast differentiation marker parathyroid hormone-related protein receptor (PTHrPR) and PPARγ are reduced and that PPARγ reduction is regulated by Wnt4 via a β-catenin-dependent mechanism. Using a human in vitro 3D lung tissue model, a link was established between increased PPARγ and pro-surfactant protein C (pro-SPC) expression in pulmonary epithelial cells. In the senile lung, both Wnt4 and Wnt5a levels increase and both Wnt-s increase myofibroblast-like differentiation. Alteration of the Wnt microenvironment plays a significant role in pulmonary aging. Diminished lipo- and increased myofibroblast-like differentiation are directly regulated by specific Wnt-s, which process also controls surfactant production and pulmonary repair mechanisms. Aging Cell, 2014; 13
23505429: Bartis D, Csongei V, Weich A, Kiss E, Barko S, Kovacs T, Avdicevic M, D'Souza VK, Rapp J, Kvell K, Jakab L, Nyitrai M, Molnar TF, Thickett DR, Laszlo T, Pongracz JE
Down-regulation of canonical and up-regulation of non-canonical Wnt signalling in the carcinogenic process of squamous cell lung carcinoma.
The majority of lung cancers (LC) belong to the non-small cell lung carcinoma (NSCLC) type. The two main NSCLC sub-types, namely adenocarcinoma (AC) and squamous cell carcinoma (SCC), respond differently to therapy. Whereas the link between cigarette smoke and lung cancer risk is well established, the relevance of non-canonical Wnt pathway up-regulation detected in SCC remains poorly understood. The present study was undertaken to investigate further the molecular events in canonical and non-canonical Wnt signalling during SCC development. A total of 20 SCC and AC samples with matched non-cancerous controls were obtained after surgery. TaqMan array analysis confirmed up-regulation of non-canonical Wnt5a and

Wnt11 and identified down-regulation of canonical Wnt signalling in SCC samples. The molecular changes were tested in primary small airway epithelial cells (SAEC) and various lung cancer cell lines (e.g. A549, H157, etc). Our studies identified Wnt11 and Wnt5a as regulators of cadherin expression and potentiated relocation of β-catenin to the nucleus as an important step in decreased cellular adhesion. The presented data identifies additional details in the regulation of SCC that can aid identification of therapeutic drug targets in the future.
PLoS One, 2013; 8
34948057: Barabás K, Kobolák J, Godó S, Kovács T, Ernszt D, Kecskés M, Varga C, Jánosi TZ, Fujiwara T, Kusumi A, Téglási A, Dinnyés A, Ábrahám IM
Live-Cell Imaging of Single Neurotrophin Receptor Molecules on Human Neurons in Alzheimer's Disease.
Neurotrophin receptors such as the tropomyosin receptor kinase A receptor (TrkA) and the low-affinity binding p75 neurotrophin receptor p75 play a critical role in neuronal survival and their functions are altered in Alzheimer's disease (AD). Changes in the dynamics of receptors on the plasma membrane are essential to receptor function. However, whether receptor dynamics are affected in different pathophysiological conditions is unexplored. Using live-cell single-molecule imaging, we examined the surface trafficking of TrkA and p75 molecules on live neurons that were derived from human-induced pluripotent stem cells (hiPSCs) of mutant familial AD (fAD) patients and non-demented control subjects. Our results show that the surface movement of TrkA and p75 and the activation of TrkA- and p75-related phosphoinositide-3-kinase (PI3K)/serine/threonine-protein kinase (AKT) signaling pathways are altered in neurons that are derived from patients suffering from fAD compared to controls. These results provide evidence for altered surface movement of receptors in AD and highlight the importance of investigating receptor dynamics in disease conditions. Uncovering these mechanisms might enable novel therapies for AD.
Int J Mol Sci, 2021; 22
32365920: Kovács T, Szabó-Meleg E, Ábrahám IM
Estradiol-Induced Epigenetically Mediated Mechanisms and Regulation of Gene Expression.
Gonadal hormone 17β-estradiol (E2) and its receptors are key regulators of gene transcription by binding to estrogen responsive elements in the genome. Besides the classical genomic action, E2 regulates gene transcription via the modification of epigenetic marks on DNA and histone proteins. Depending on the reaction partner, liganded estrogen receptor (ER) promotes DNA methylation at the promoter or enhancer regions. In addition, ERs are important regulators of passive and active DNA demethylation. Furthermore, ERs cooperating with different histone modifying enzymes and chromatin remodeling complexes alter gene transcription. In this review, we survey the basic mechanisms and interactions between estrogen receptors and DNA methylation, demethylation and histone modification processes as well as chromatin remodeling complexes. The particular relevance of these mechanisms to physiological processes in memory formation, embryonic development, spermatogenesis and aging as well as in pathophysiological changes in carcinogenesis is also discussed.
Int J Mol Sci, 2020; 21
29957289: Kamravamanesh D, Kovacs T, Pflügl S, Druzhinina I, Kroll P, Lackner M, Herwig C
Increased poly-β-hydroxybutyrate production from carbon dioxide in randomly mutated cells of cyanobacterial strain Synechocystis sp. PCC 6714: Mutant generation and characterization.
Photosynthetic Poly-β-hydroxybutyrate (PHB) productivity in cyanobacteria needs to be increased to make cyanobacterial derived bioplastics economically feasible and competitive with petroleum-based plastics. In this study, high PHB yielding mutants of Synechocystis sp. PCC 6714 have been generated by random mutagenesis, using UV light as a mutagen. The selection of strains was based on PHB content induced by nitrogen and phosphorus starvation. The fast growing mutant MT_a24 exhibited more than 2.5-fold higher PHB productivity than that of the wild-type, attaining values of 37 ± 4% dry cell weight PHB. The MT_a24 was characterized for phenotypes, CO uptake rate and gene expression levels using quantitative PCR. Genome sequencing showed that UV mutagenesis treatment resulted in a point mutation in the ABC-transport complex, phosphate-specific transport system integral membrane protein A (PstA). The MT_a24 shows potential for industrial production of PHB and also for carbon capture from the atmosphere or point sources.
Bioresour Technol, 2018; 266
34631701: Godó S, Barabás K, Lengyel F, Ernszt D, Kovács T, Kecskés M, Varga C, Jánosi TZ, Makkai G, Kovács G, Orsolits B, Fujiwara T, Kusumi A, Ábrahám IM
Single-Molecule Imaging Reveals Rapid Estradiol Action on the Surface Movement of AMPA Receptors in Live Neurons.
Gonadal steroid 17β-estradiol (E2) exerts rapid, non-genomic effects on neurons and strictly regulates learning and memory through altering glutamatergic neurotransmission and synaptic plasticity. However, its non-genomic effects on AMPARs are not well understood. Here, we analyzed the rapid effect of E2 on AMPARs using single-molecule tracking and super-resolution imaging techniques. We found that E2 rapidly decreased the surface movement of AMPAR via membrane G protein-coupled estrogen receptor 1 (GPER1) in neurites in a dose-dependent manner. The cortical actin network played a

pivotal role in the GPER1 mediated effects of E2 on the surface mobility of AMPAR. E2 also decreased the surface movement of AMPAR both in synaptic and extrasynaptic regions on neurites and increased the synaptic dwell time of AMPARs. Our results provide evidence for understanding E2 action on neuronal plasticity and glutamatergic neurotransmission at the molecular level.
Front Cell Dev Biol, 2021; 9

26584567: Vojkovics D, Kellermayer Z, Heidt D, Mihalj M, Kajtár B, Ernszt D, Kovács T, Németh P, Balogh P
Isolation and Characterization of a Murine Spontaneous High-Grade Follicular Lymphoma with Restricted In Vivo Spreading--a Model for Lymphatic Metastasis Via the Mesentery.
Spontaneous or induced malignant lymphomas in mice are valuable tools for studying human lymphoproliferative diseases, including the mechanism of migration between peripheral lymphoid organs and positioning within distinct tissue compartments. Here we report the isolation and characterization of a novel spontaneous lymphoma from BALB/c mice showing restricted tissue distribution and metastasis. The lymphoma cells display CD19, B220, MHC II, surface IgG2a/kappa chain with VH7183 rearrangement of the IgH gene, indicating their B-cell origin. Serial intraperitoneal injection of primary tumor into both BALB/c and RAG-1-deficient hosts led to the successful propagation of lymphoma. Despite the cytological characteristics of high-grade follicular B-cell lymphoma, the tumor cells (denoted as Bc-DLFL.1) showed significantly lesser spreading to extraabdominal locations upon intraperitoneal passage compared to splenic and mesenteric lymph node expansion. In mesenteric lymph nodes the high endothelial venules contained only few tumor cells, while the lymphatic vessels were almost completely filled with lymphoma cells. Similarly, the LYVE-1-positive lymphatic capillaries within the mesentery were packed with lymphoma cells. These findings suggest that Bc-DLFL.1 cells likely propagate primarily via the lymphatic circulation within the mesentery, therefore this tumor may offer an in vivo model to investigate the tumor cell migration via the lymphatic circulation from the peritoneal cavity.
Pathol Oncol Res, 2016; 22

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A NOVEL MODULAR TOOLBOX FOR PRECISE NEURONAL EPIGENOME EDITING

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Instituto de Neurociencias (CSIC-UMH), Molecular Neurobiology And Neuropathology, San Juan de Alicante, Spain

Among epigenome editing systems, the use of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) stands out for its versatility, ease of engineering, and cost-effectiveness. The chimeric fusion of the nuclease-deficient dCas9 with epigenetic enzymatic activities enables locus-specific rewriting of epigenetic information by guide RNAs. However, the resulting chimeric proteins are often large in size and beyond the packaging capacity of most common neurotropic vectors. To overcome this limitation, we have developed a novel, modular toolbox in which chimeric proteins are split into two smaller constructs taking advantage of nanobodies' ability to bind with high affinity to the recognized epitope. More precisely, the dCas9 protein has been linked to a nanobody that specifically recognizes GFP and GFP has been linked to the catalytic domain of different effector proteins. Results combining our tool with synthetic and epigenetic effector modules highlight the potential of this novel toolbox for precise neuronal epigenome editing *in vitro* and open new opportunities for the development of novel *in vivo* and *ex* vivo therapies to treat epigenome-associated diseases.

Pubmed:
34097980: Fuentes-Ramos M, Alaiz-Noya M, Barco A
Transcriptome and epigenome analysis of engram cells: Next-generation sequencing technologies in memory research.
Transcription and epigenetic changes are integral components of the neuronal response to stimulation and have been postulated to be drivers or substrates for enduring changes in animal behavior, including learning and memory. Memories are thought to be deposited in neuronal assemblies called engrams, i.e., groups of cells that undergo persistent physical or chemical changes during learning and are selectively reactivated to retrieve the memory. Despite the research progress made in recent years, the identity of specific epigenetic changes, if any, that occur in these cells and subsequently contribute to the persistence of memory traces remains unknown. The analysis of these changes is challenging due to the difficulty of exploring molecular alterations that only occur in a relatively small percentage of cells embedded in a complex tissue. In this review, we discuss the recent advances in this field and the promise of next-generation sequencing (NGS) and epigenome editing methods for overcoming these challenges and address long-standing questions concerning the role of epigenetic mechanisms in memory encoding, maintenance and expression.
Neurosci Biobehav Rev, 2021; 127
33100228: Navas-Pérez E, Vicente-García C, Mirra S, Burguera D, Fernàndez-Castillo N, Ferrán JL, López-Mayorga M, Alaiz-Noya M, Suárez-Pereira I, Antón-Galindo E, Ulloa F, Herrera-Úbeda C, Cuscó P, Falcón-Moya R, Rodríguez-Moreno A, D'Aniello S, Cormand B, Marfany G, Soriano E, Carrión ÁM, Carvajal JJ, Garcia-Fernàndez J
Characterization of an eutherian gene cluster generated after transposon domestication identifies Bex3 as relevant for advanced neurological functions.
One of the most unusual sources of phylogenetically restricted genes is the molecular domestication of transposable elements into a host genome as functional genes. Although these kinds of events are sometimes at the core of key macroevolutionary changes, their origin and organismal function are generally poorly understood.
Genome Biol, 2020; 21
35146756: Alaiz Noya M, Berti F, Dietrich S
Comprehensive expression analysis for the core cell cycle regulators in the chicken embryo reveals novel tissue-specific synexpression groups and similarities and differences with expression in mouse, frog and zebrafish.
The core cell cycle machinery is conserved from yeast to humans, and hence it is assumed that all vertebrates share the same set of players. Yet during vertebrate evolution, the genome was duplicated twice, followed by a further genome duplication in teleost fish. Thereafter, distinct genes were retained in different vertebrate lineages; some individual gene duplications also occurred. To which extent these diversifying tendencies were compensated by retaining the same expression patterns across homologous genes is not known. This study for the first time undertook a comprehensive expression analysis for the core cell cycle regulators in the chicken, focusing in on early neurula and pharyngula stages of

development, with the latter representing the vertebrate phylotypic stage. We also compared our data with published data for the mouse, Xenopus and zebrafish, the other established vertebrate models. Our work shows that, while many genes are expressed widely, some are upregulated or specifically expressed in defined tissues of the chicken embryo, forming novel synexpression groups with markers for distinct developmental pathways. Moreover, we found that in the neural tube and in the somite, mRNAs of some of the genes investigated accumulate in a specific subcellular localisation, pointing at a novel link between the site of mRNA translation, cell cycle control and interkinetic nuclear movements. Finally, we show that expression patterns of orthologous genes may differ in the four vertebrate models. Thus, for any study investigating cell proliferation, cell differentiation, tissue regeneration, stem cell behaviour and cancer/cancer therapy, it has to be carefully examined which of the observed effects are due to the specific model organism used, and which can be generalised.
J Anat, 2022; 241

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DESIGNING VIRAL TOOLS TO CLASSIFY SUBPOPULATIONS OF TANYCYTES ALONG THE THIRD VENTRICLE

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

Vanessa Neve[1], Helge Müller-Fielitz[1], Frauke Spieker[1], Anke Fähnrich[2], Ruben Nogueiras[3], Vincent Prevot[4], Markus Schwaninger[1]
[1]University of Lübeck, Institute Of Experimental And Clinical Pharmacology And Toxicology, Lübeck, Germany, [2]University of Lübeck, Group For Medical Systems Biology, Lübeck, Germany, [3]University of Santiago de Compostela, Center For Research In Molecular Medicine And Chronic Diseases, Santiago de Compostela, Spain, [4]University of Lille, Development & Plasticity Of The Neuroendocrine Brain, Lille Cedex, France

In the hypothalamus, the third ventricle is lined by specialised glia-like cells called tanycytes. Their projections reach into nuclei of the mediobasal hypothalamus and the median eminence (ME), which are responsible for energy homeostasis, food intake, hormone release and other functions. Historically, tanycytes were distinguished by their morphology and location along the ventricle. The classical beta-tanycytes, located in the ME and the lateral protrusions of the third ventricle, have projections into the arcuate nucleus (ARH) and the ME. They seem to be important for the transport of endogenous compounds between brain and periphery and regulating hormone release. On the other hand, the alpha-tanycytes extending from the ARH to the ventromedial- (VMH) as well as dorsomedial (DMH) nucleus regulate feeding behaviour. However, the classification according to localisation alone does not provide information about the function. To manipulate tanycytic subpopulations, we have modified an established adeno-associated virus (AAV)-based approach, with which we had only been able to target all tanycytes. We use small promotor fragments of different genes driving a Cre-recombinase combined with a fluorescent marker. The new AAV vectors were injected into the lateral ventricle of Ai14 reporter mice by stereotactic surgery. By immunofluorescence staining, we analysed the transduction pattern of 19 different promotor fragments. Using this approach, we were able to identify promoters that specifically target tanycytic subpopulations. The differential expression pattern of the AAV vectors provides evidence for the function heterogeneity of tanycytes. With these new vectors we are now able characterise the specific physiological functions of tanycyte subpopulations.

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TARGETING NORADRENERGIC NEURONS OF THE LOCUS COERULEUS: A COMPARISON OF MODEL SYSTEMS AND STRATEGIES

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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[1]Universitätsklinikum Hamburg-Eppendorf, Center For Molecular Neurobiology (zmnh), Hamburg, Germany, [2]Universitätsklinikum Hamburg-Eppendorf, Section Computational Cognitive Neuroscience, Department Of Neurophysiology And Pathophysiology, Hamburg, Germany

The locus coeruleus (LC) noradrenergic system is involved in a plethora of physiological and pathophysiological processes. Refining our understanding of LC function largely relies on selective transgene expression in molecularly defined cells, enabling targeted manipulation and read-out of noradrenergic neurons. Here, we performed a side-by-side comparison of the most commonly used strategies and model systems enabling genetic access to the locus coeruleus. We report substantial differences among them both in terms of transgene expression efficacy, and in their molecular specificity. These findings are of critical importance for interpreting the results obtained from past experiments using the respective targeting strategies, as well as for the design of future studies.

Pubmed:
29997248: Wrobel C, Dieter A, Huet A, Keppeler D, Duque-Afonso CJ, Vogl C, Hoch G, Jeschke M, Moser T
Optogenetic stimulation of cochlear neurons activates the auditory pathway and restores auditory-driven behavior in deaf adult gerbils.
Cochlear implants partially restore hearing via direct electrical stimulation of spiral ganglion neurons (SGNs). However, spread of excitation from each electrode limits spectral coding. We explored the use of optogenetics to deliver spatially restricted and cell-specific excitation in the cochlea of adult Mongolian gerbils. Adeno-associated virus carrying the gene encoding the light-sensitive calcium translocating channelrhodopsin (CatCh) was injected into the cochlea of adult gerbils. SGNs in all cochlea turns showed stable and long-lasting CatCh expression, and electrophysiological recording from single SGNs showed that light stimulation up to few hundred Hertz induced neuronal firing. We characterized the light-induced activity in the auditory pathway by electrophysiological and behavioral analysis. Light- and sound-induced auditory brainstem responses showed similar kinetics and amplitude. In normal hearing adult gerbils, optical cochlear implants elicited stable optical auditory brainstem responses over a period of weeks. In normal hearing animals, light stimulation cued avoidance behavior that could be reproduced by subsequent acoustic stimulation, suggesting similar perception of light and acoustic stimuli. Neurons of the primary auditory cortex of normal hearing adult gerbils responded with changes in firing rates with increasing light intensity. In deaf adult gerbils, light stimulation generated auditory responses and cued avoidance behavior indicating partial restoration of auditory function. Our data show that optogenetic cochlear stimulation achieved good temporal fidelity with low light intensities in an adult rodent model, suggesting that optogenetics might be used to develop cochlear implants with improved restorative capabilities.
Sci Transl Med, 2018; 10
31036812: Dieter A, Duque-Afonso CJ, Rankovic V, Jeschke M, Moser T
Near physiological spectral selectivity of cochlear optogenetics.
Cochlear implants (CIs) electrically stimulate spiral ganglion neurons (SGNs) and partially restore hearing to half a million CI users. However, wide current spread from intracochlear electrodes limits spatial selectivity (i.e. spectral resolution) of electrical CIs. Optogenetic stimulation might become an alternative, since light can be confined in space, promising artificial sound encoding with increased spectral selectivity. Here we compare spectral selectivity of optogenetic, electric, and acoustic stimulation by multi-channel recordings in the inferior colliculus (IC) of gerbils. When projecting light onto tonotopically distinct SGNs, we observe corresponding tonotopically ordered IC activity. An activity-based comparison reveals that spectral selectivity of optogenetic stimulation is indistinguishable from acoustic stimulation for modest intensities. Moreover, optogenetic stimulation outperforms bipolar electric stimulation at medium and high intensities and monopolar electric stimulation at all intensities. In conclusion, we demonstrate better spectral selectivity of optogenetic over electric SGN stimulation, suggesting the potential for improved hearing restoration by optical CIs.

Nat Commun, 2019; 10
32596983: Dieter A, Klein E, Keppeler D, Jablonski L, Harczos T, Hoch G, Rankovic V, Paul O, Jeschke M, Ruther P, Moser T
μLED-based optical cochlear implants for spectrally selective activation of the auditory nerve.
Electrical cochlear implants (eCIs) partially restore hearing and enable speech comprehension to more than half a million users, thereby re-connecting deaf patients to the auditory scene surrounding them. Yet, eCIs suffer from limited spectral selectivity, resulting from current spread around each electrode contact and causing poor speech recognition in the presence of background noise. Optogenetic stimulation of the auditory nerve might overcome this limitation as light can be conveniently confined in space. Here, we combined virus-mediated optogenetic manipulation of cochlear spiral ganglion neurons (SGNs) and microsystems engineering to establish acute multi-channel optical cochlear implant (oCI) stimulation in adult Mongolian gerbils. oCIs based on 16 microscale thin-film light-emitting diodes (μLEDs) evoked tonotopic activation of the auditory pathway with high spectral selectivity and modest power requirements in hearing and deaf gerbils. These results prove the feasibility of μLED-based oCIs for spectrally selective activation of the auditory nerve.
EMBO Mol Med, 2020; 12
32718992: Keppeler D, Schwaerzle M, Harczos T, Jablonski L, Dieter A, Wolf B, Ayub S, Vogl C, Wrobel C, Hoch G, Abdellatif K, Jeschke M, Rankovic V, Paul O, Ruther P, Moser T
Multichannel optogenetic stimulation of the auditory pathway using microfabricated LED cochlear implants in rodents.
When hearing fails, electrical cochlear implants (eCIs) provide the brain with auditory information. One important bottleneck of CIs is the poor spectral selectivity that results from the wide current spread from each of the electrode contacts. Optical CIs (oCIs) promise to make better use of the tonotopic order of spiral ganglion neurons (SGNs) inside the cochlea by spatially confined stimulation. Here, we established multichannel oCIs based on light-emitting diode (LED) arrays and used them for optical stimulation of channelrhodopsin (ChR)-expressing SGNs in rodents. Power-efficient blue LED chips were integrated onto microfabricated 15-μm-thin polyimide-based carriers comprising interconnecting lines to address individual LEDs by a stationary or mobile driver circuitry. We extensively characterized the optoelectronic, thermal, and mechanical properties of the oCIs and demonstrated stability over weeks in vitro. We then implanted the oCIs into ChR-expressing rats and gerbils, and characterized multichannel optogenetic SGN stimulation by electrophysiological and behavioral experiments. Improved spectral selectivity was directly demonstrated by recordings from the auditory midbrain. Long-term experiments in deafened ChR-expressing rats and in nontreated control animals demonstrated specificity of optogenetic stimulation. Behavioral studies on animals carrying a wireless oCI sound processor revealed auditory percepts. This study demonstrates hearing restoration with improved spectral selectivity by an LED-based multichannel oCI system.
Sci Transl Med, 2020; 12
32227585: Dieter A, Keppeler D, Moser T
Towards the optical cochlear implant: optogenetic approaches for hearing restoration.
Cochlear implants (CIs) are considered the most successful neuroprosthesis as they enable speech comprehension in the majority of half a million CI users suffering from sensorineural hearing loss. By electrically stimulating the auditory nerve, CIs constitute an interface re-connecting the brain and the auditory scene, providing the patient with information regarding the latter. However, since electric current is hard to focus in conductive environments such as the cochlea, the precision of electrical sound encoding-and thus quality of artificial hearing-is limited. Recently, optogenetic stimulation of the cochlea has been suggested as an alternative approach for hearing restoration. Cochlear optogenetics promises increased spectral selectivity of artificial sound encoding, hence improved hearing, as light can conveniently be confined in space to activate the auditory nerve within smaller tonotopic ranges. In this review, we discuss the latest experimental and technological developments of cochlear optogenetics and outline the remaining challenges on the way to clinical translation.
EMBO Mol Med, 2020; 12
32033755: Moser T, Dieter A
Towards optogenetic approaches for hearing restoration.
Hearing impairment (HI) is the most frequent sensory deficit in humans. As yet there is no causal therapy for sensorineural HI - the most common form - that results from cochlear dysfunction. Hearing aids and electrical cochlear implants (eCIs) remain the key options for hearing rehabilitation. The eCI, used by more than 0.7 Mio people with profound HI or deafness, is considered the most successful neuroprosthesis as it typically enables open speech comprehension in quiet. By electrically stimulating the auditory nerve, eCIs constitute a brain-machine interface re-connecting the patient with the auditory scene. Nonetheless, there are short-comings resulting from the wide spread of electric current inside the cochlea which limit the quality of artificial hearing. Since light can be better confined in space than electric current, optogenetic stimulation of the auditory nerve has been suggested as an alternative approach for hearing restoration, enabling higher resolution of artificial sound encoding. Future optical CIs (oCIS) promise increased spectral selectivity of artificial sound encoding, and hence might improve speech recognition in background noise as well as processing of music.

Biochem Biophys Res Commun, 2020; 527
33793545: Yang W, Chini M, Pöpplau JA, Formozov A, Dieter A, Piechocinski P, Rais C, Morellini F, Sporns O, Hanganu-Opatz IL, Wiegert JS
Anesthetics fragment hippocampal network activity, alter spine dynamics, and affect memory consolidation.
General anesthesia is characterized by reversible loss of consciousness accompanied by transient amnesia. Yet, long-term memory impairment is an undesirable side effect. How different types of general anesthetics (GAs) affect the hippocampus, a brain region central to memory formation and consolidation, is poorly understood. Using extracellular recordings, chronic 2-photon imaging, and behavioral analysis, we monitor the effects of isoflurane (Iso), medetomidine/midazolam/fentanyl (MMF), and ketamine/xylazine (Keta/Xyl) on network activity and structural spine dynamics in the hippocampal CA1 area of adult mice. GAs robustly reduced spiking activity, decorrelated cellular ensembles, albeit with distinct activity signatures, and altered spine dynamics. CA1 network activity under all 3 anesthetics was different to natural sleep. Iso anesthesia most closely resembled unperturbed activity during wakefulness and sleep, and network alterations recovered more readily than with Keta/Xyl and MMF. Correspondingly, memory consolidation was impaired after exposure to Keta/Xyl and MMF, but not Iso. Thus, different anesthetics distinctly alter hippocampal network dynamics, synaptic connectivity, and memory consolidation, with implications for GA strategy appraisal in animal research and clinical settings.
PLoS Biol, 2021; 19
34312384: Vierock J, Rodriguez-Rozada S, Dieter A, Pieper F, Sims R, Tenedini F, Bergs ACF, Bendifallah I, Zhou F, Zeitzschel N, Ahlbeck J, Augustin S, Sauter K, Papagiakoumou E, Gottschalk A, Soba P, Emiliani V, Engel AK, Hegemann P, Wiegert JS
BiPOLES is an optogenetic tool developed for bidirectional dual-color control of neurons.
Optogenetic manipulation of neuronal activity through excitatory and inhibitory opsins has become an indispensable experimental strategy in neuroscience research. For many applications bidirectional control of neuronal activity allowing both excitation and inhibition of the same neurons in a single experiment is desired. This requires low spectral overlap between the excitatory and inhibitory opsin, matched photocurrent amplitudes and a fixed expression ratio. Moreover, independent activation of two distinct neuronal populations with different optogenetic actuators is still challenging due to blue-light sensitivity of all opsins. Here we report BiPOLES, an optogenetic tool for potent neuronal excitation and inhibition with light of two different wavelengths. BiPOLES enables sensitive, reliable dual-color neuronal spiking and silencing with single- or two-photon excitation, optical tuning of the membrane voltage, and independent optogenetic control of two neuronal populations using a second, blue-light sensitive opsin. The utility of BiPOLES is demonstrated in worms, flies, mice and ferrets.
Nat Commun, 2021; 12

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BOARD NUMBER: S07-447

INTRAVENOUS GENE THERAPY USING AAVPHP.EB FOR METACHROMATIC LEUKODYSTROPHY

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Metachromatic leukodystrophy (MLD) is a rare, autosomal recessive disease caused by deficient activity of the lysosomal enzyme arylsulfatase A (ARSA), resulting in sulfatide accumulation and subsequent demyelination and neuronal loss within the central and peripheral nervous systems. Three clinical forms of MLD have been described, based on the age of symptom onset. Among them, early-onset forms comprise a continuum between late infantile MLD (most frequent accounting for 50-60% of MLD patients) and early juvenile MLD. In these early-onset forms, first symptoms typically develop between 1 and 4 years of age and progress rapidly towards severe motor and cognitive regression and premature death. There are currently no approved therapies for early onset MLD once patients are symptomatic. It is crucial to develop new approaches to treat symptomatic patients. Here we proposed a gene therapy approach based on intravenous delivery of an AAVPHP.eB encoding the human ARSA gene with a HA tag. ARSA KO animals were treated at 6 months with two different doses or 9 months of age and therapeutic efficacy has been evaluated at 12 months of age (ie 3 or 6 months after treatment). We demonstrated a broad transduction of brain and spinal cord leading to a complete correction of sulfatide storage both in brain and spinal cord in symptomatic animals as well as a significant improvement of neuroinflammation in mouse model of the disease. Our approach to treat symptomatic patient seem to be efficient in mouse model of the disease.

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BOARD NUMBER: S07-448

WHY NEURON-SPECIFIC STAINING AFTER INTRACEREBRAL INJECTION OF A VIRAL CONSTRUCT?

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Viruses are precious, widely used technological tools in neuroscience research. For example, recombinant adeno-associated viruses (AAV) allow the expression of a gene of interest under the control of a specific promoter. After intracerebral injection of a virus, a DAPI or Hoechst nuclear staining is usually performed to verify the absence of target tissue alterations. We employed a pair of recombinant AAVs, one carrying a caspase gene and another, retrograde, carrying the Cre-recombinase gene in order to specifically disconnect two brain structures. We tested two retrograde viruses: an AAV-Cre-GFP (AAVrg.hSyn.HI.eGFP-Cre.WPRE.SV40, Addgene, MA, USA, 3.5 x 10^{12} particles/mL) carrying both the Cre-recombinase and the GFP fused genes under the hSyn promoter, and an AAV-Cre-mCh (AAVrg-EF1a-mCherry-IRES-Cre, Addgene, MA, USA, 1.2 x10^{12} particles/mL) carrying the Cre-recombinase and the mCherry genes (not fused) under the EF1a promoter. We injected 2 x 1µL of the virus into the midline thalamic reuniens and rhomboid nuclei (ReRh) of rats. After various post-injection delays, the infected region was immunostained using neuronal and glial markers (NeuN, Iba1 and GFAP) and a DAPI staining. We found that the ReRh infection by the AAV-Cre-GFP virus, not the AAV-Cre-mCh, produced massive neuronal loss 5 and 10 weeks after intracerebral injection, coinciding with a glial scar and microglial activation. This glial reaction prevented detection of cellular damage with the DAPI staining. We suggest that after intraparenchymal injection of AAVs, a neuron-specific labelling (e.g., NeuN) is essential to establish that the infection did not non-specifically alter the cellular composition of the target structure.

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BOARD NUMBER: S07-450

GENERATION OF CELL TYPE SPECIFIC VIRAL TOOLS FOR THE ANALYSIS OF NEURAL CIRCUITS IN THE HIPPOCAMPAL FORMATION

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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The human brain contains about one hundred billion electrically-active neurons, each establishing several thousand synaptic connections enabling their specific functional characteristics. The brain's anatomical and functional organization can therefore be thought of as an extremely complex set of neural circuits, which can best be studied by recording the effects of manipulating particular circuit elements, i.e. neuronal cell types. Despite the advent of opto- and chemogenetic tools that allow the manipulation of neural circuits, the ability to deliver them with the required anatomical specificity remains quite limited because the vast majority of promoters express in many different cell types. To address this issue our lab has developed an approach to generating celltype-specific tools known as “Enhancer-Driven Gene Expression” (EDGE) in which enhancers uniquely active in particular brain regions are combined with a heterologous minimal promoter. This allows for the generation of molecular genetic tools much more specific than native promoters in both transgenic animals and recombinant adeno-associated viral vectors (rAAVs), allowing one to specifically manipulate particular neuronal celltypes in any species. We applied this technology and screened the hippocampal formation on 21 putative enhancers by injection of rAAvs with these enhancers into the hippocampus of wild type animals for anatomical characterization of transgene expression. One of these viral constructs specifically labels dentate gyrus granule neurons when injected into the hippocampus. We present work characterizing the expression of this and other EDGE rAAVs with both local as well as systemic administration.

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BOARD NUMBER: S07-451

AN UPDATED SUITE OF VIRAL VECTORS FOR IN-VIVO CALCIUM IMAGING USING LOCAL AND RETRO-ORBITAL INJECTIONS

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Calcium imaging using genetically encoded Ca2+ indicators (GECIs) is a widely adopted method to measure neural activity in modern neuroscience. Here, we explore the use of systemically administered viral vectors for brain-wide expression of GECIs, and adapt novel GECIs to optimize signal-to-noise. We show that systemic injections of PHP.eB or CAP-B10 serotype AAVs to express GECIs, especially jGCaMP8, is a highly promising technique for imaging neural activity and circumvents the need for transgenic GECI-expressing mouse lines. We also establish the use of soma-targeted GECI constructs where we combine the most popular soma-targeting peptides with the recently developed jGCaMP8 GECIs, providing unparalleled signal to noise ratios paired with the fastest GECI kinetics.

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ANTEROGRADE TRANSNEURONAL TRANSFER OF RABIES VIA NOVEL PSEUDOTYPING WITH HSV-1 GLYCOPROTEINS GE, GI AND US9

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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[1]University College London, Sainsbury Wellcome Centre, London, United Kingdom, [2]UCL, Sainsbury Wellcome Centre, London, United Kingdom

Transsynaptic tracing methods have proved incredibly useful in furthering our understanding of brain-wide connectivity. In particular, rabies virus (RABV) has proven to be a highly useful retrograde tracer due to the ability to restrict transsynaptic transfer to one synapse. However, an equivalent monosynaptic anterograde tracer is not yet available. Here we attempt to produce an anterograde transsynaptic RABV by pseudotyping the virus with herpes simplex virus-1 (HSV-1) glycoproteins gE, gI and US9, which are known to facilitate anterograde spread. We first validated whether pseudotyping of RABV with HSV-1 glycoproteins would produce functional virions in vitro. Next, we developed a range of adeno-associated virus (AAV) complementation strategies to facilitate in vivo testing of multiple neural circuits in mice. Firstly, in wildtype mice RABV positive cells in the lateral vestibular nucleus were complemented with three AAVs, each expressing an individual glycoprotein, to test for anterograde spread to motor neurons within the spinal cord. Secondly, RABV positive Purkinje cells in TU/L7-Cre mice were complemented with two high expressing AAVs, one containing the receptor TVA and gE, another containing gI and US9, to test for anterograde transsynaptic spread to cells within the deep cerebellar nuclei. In both cases, a small number of RABV labelled cells were found in each downstream area with no corresponding retrogradely labelled cells visible. This outcome demonstrates the potential viability of this novel pseudotyping approach though presently the efficiency remains low and further optimisation is ongoing.

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BOARD NUMBER: S07-453

AUTOLOGOUS HAEMATOPOIETIC STEM CELL GENE THERAPY FOR PEOPLE WITH FRIEDREICH'S ATAXIA

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Aims: Friedreich's ataxia (FA) is a neurodegenerative disease currently lacking any proven treatment. We propose to investigate whether autologous transplantation of genetically modified haematopoietic stem cells (HSCs), carrying a functional frataxin [*FXN*] transgene, is of therapeutic benefit. The aim of this study was therefore to develop a lentiviral-mediated transduction protocol for efficient and stable *FXN* delivery to isolated murine FA HSCs. **Methods:** Lineage^{-}, Sca-1^{+}, c-Kit^{+} haematopoietic stem cells (LSKs) were isolated from the bone marrow of FA transgenic mice (Fxn^{null}::YG8s(GAA)$_{>800}$) and transduced with a third-generation lentiviral vector carrying *FXN* and *EGFP* reporter (*lenti*-FXN.EGFP). Transduction efficiency and vector copy number (VCN) were assessed. Functional gene replacement was determined through analysis of frataxin protein levels, cell proliferation and differentiation, and mitochondrial function. **Results:** Using *lenti*-FXN.EGFP, a LSK transduction efficiency of 88% was achieved with an average VCN of 6 copies per cell. LSK cultures demonstrated a 12-fold increase in human frataxin protein expression following transduction, which was accompanied by a significant elevation in succinate dehydrogenase activity. *In vitro* quantification of LSK proliferation and differentiation showed no detrimental effects caused by lentiviral transduction on cell function. **Conclusions:** Our results demonstrate that lentiviral vector-mediated genome editing can provide an effective method for *FXN* gene correction and FA phenotype rescue in isolated HSCs. Further *in vivo* studies assessing transplantation of autologous *FXN*-corrected HSCs are warranted to demonstrate the therapeutic potential of our approach. Future translation into clinical trials may offer the development of a disease-modifying treatment for people with FA.

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RT-QPCR-BASED ASSAY FOR MRNA INTEGRITY EVALUATION IN MOUSE AND HUMAN BRAIN TISSUE AND SYNAPTOSOMAL PREPARATIONS

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Aims: Subcellular compartments such as synaptosomes have very little ribosomal RNA (rRNA) on which traditional RNA integrity evaluation is based on. Also, rRNA integrity evaluation might not reflect the state of the mRNA pool correctly. Therefore, our aim was to adapt RT-qPCR-based 3′:5′ assay for mRNA integrity evaluation in RNA samples from mouse and human brain and their synaptosomal preparations. Methods: The 3′:5′ assay is based on using two sets of primers in qPCR to measure the relative expression of two amplicons located on the 3′ and 5′ regions of a gene of choice. All transcripts with poly(A) tail were reverse transcribed using only oligo-dT primers. cDNA obtained from a partly degraded mRNA sample was shorter and led to reduced amount of 5' end qPCR product and thus higher 3′:5′ ratio. Results: The 3′ and 5′ mouse and human *Pgk1* primer pairs were selected and validated for 3':5' mRNA integrity assay. The RNA integrity in heat-degraded RNA samples from mouse and human cortex was evaluated by 3':5' assay and the results were compared to the RIN values (Agilent Bioanalyzer). With prolonged heat treatment, 3':5' ratios gradually increased while RIN values gradually decreased. Regression analysis revealed significant association between 3':5' ratios and RIN values in the same sample. The application of 3′:5′ assay was further extended to mouse and human synaptosomal preparations. Conclusions: The 3′:5′ assay can be used as reliable alternative method to evaluate mRNA integrity in mouse and human brain tissue and synaptosomal preparations.

Pubmed:

33153429: Pamedytyte D, Leipute E, Zilaitiene B, Sarauskas V, Dauksiene D, Dauksa A, Zvirbliene A
Different stability of miRNAs and endogenous control genes in archival specimens of papillary thyroid carcinoma.
The most popular miRNA quantitation technique is RQ-PCR with relative gene expression method that requires an endogenous control (EC) gene for data normalization. However, there are insufficient data and selection criteria on the most suitable ECs for miRNA expression studies in many cancer types including papillary thyroid carcinoma (PTC). Therefore, in this study we evaluated the impact of chosen EC and archival formalin-fixed, paraffin-embedded (FFPE) PTC tissue age on estimated target miRNA expression.
Mol Med, 2020; 26
32316638: Pamedytyte D, Simanaviciene V, Dauksiene D, Leipute E, Zvirbliene A, Sarauskas V, Dauksa A, Verkauskiene R, Zilaitiene B
Association of MicroRNA Expression and BRAF Mutation with Recurrence of Thyroid Cancer.
Many miRNAs and cancer-related mutations have been proposed as promising molecular markers of papillary thyroid carcinoma (PTC). However, there are limited data on the correlation between miRNA expression, BRAF mutation, and PTC recurrence. Therefore, to evaluate the potential of BRAF mutation and five selected miRNAs (-146b, -222, -21, -221, -181b) in predicting PTC recurrence, these molecular markers were analyzed in 400 formalin-fixed, paraffin-embedded PTC tissue specimens. The expression levels of miRNAs were measured using qRT-PCR. It was demonstrated that expression levels of all analyzed miRNAs are significantly higher in recurrent PTC than in non-recurrent PTC (< 0.05). Moreover, higher expression levels of miR-146b, miR-222, miR-21, and miR-221 were associated with other clinicopathologic features of PTC, such as tumor size and lymph node metastases at initial surgery (< 0.05). No significant differences in the frequency of BRAF mutation in recurrent PTC and non-recurrent PTC were determined. Our results suggest that miRNA expression profile differs in PTC that is prone to recurrence when compared to PTC that does not reoccur after the initial surgery while BRAF mutation frequency does not reflect the PTC recurrence status. However, the prognostic value of the analyzed miRNAs is rather limited in individual cases as the pattern of miRNA expression is highly overlapping between recurrent and non-recurrent PTC.
Biomolecules, 2020; 10


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MAPPING THE PROTEOMIC LANDSCAPE OF CELL/ENSEMBLE-SPECIFIC SYNAPSES

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Background: Synaptic heterogeneity is widely observed in mammalian systems and is potentially linked to differences in protein architecture of connections within specific neuronal ensembles relevant for cognition. In turn, this heterogeneity may influence the capacity of different synapse types to process information and adjust transmission in a plastic manner. So far, the identification of synaptic proteomes has been limited to global analysis of many synapses in one. This prevents the identification of cell-type and circuit-specific synaptosomes that underlie different brain functions such as learning and memory. This project aims to develop technology to label, isolate, and harvest specific synaptic populations to identify protein expression signatures of connections within ensembles implicated in memory formation and its persistence over time. **Methods and Results:** We utilize Fluorescence-Activated Synapse Sorting (FASS) to harvest fluorescently tagged hippocampal synapses from a mixed population. Using high-resolution microscopy, we show that these tagged and sorted synapses remain structurally intact, containing both pre-and post-synaptic compartments. Finally, using Mass Spectrometry (LC-MS/MS), we demonstrate that protein composition of sorted synapses yields a similar coverage across pre-and post-synaptic compartments as their pre/unsorted counterparts. **Conclusions:** Our technical toolbox enables molecular characterization of cell/ensemble-specific synaptic populations relevant for cognition and experience-dependent circuit use. **Keywords:** Synapse, Sorting, Proteomics, Plasticity, Memory **E-mail address:** b.moharana@vu.nl

FENS Forum 2022
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BOARD NUMBER: S07-456

PROTEOMIC PROFILING OF PURIFIED AUTOPHAGIC VESICLE CONTENT ACROSS BRAIN MATURATION AND AGING

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Autophagy is a conserved degradative pathway with a crucial role in neuronal proteostasis. In humans, mutations in core autophagy genes lead to congenital disorders of the central nervous system, and impaired autophagy is also linked to late-onset neurodegeneration. However, the autophagic degradome in the brain remains only partially characterized. To overcome previous limitations, here we use a two-step protocol to purify LC3-positive autophagic vesicles (AVs) from the mouse brain and profile their content by quantitative proteomics. In addition to highlighting that aggrephagy, mitophagy and ER-phagy occur constitutively in the brain, we also reveal a significant synaptic content. To define the brain autophagic degradome, we proteomically analyze the accumulation of content proteins under genetic and pharmacological autophagy impairment. Lastly, we quantitatively compare the AV content and degradome during adolescence and aging, where autophagy impairment is causally linked to disease, and reveal dynamic and progressive changes in the nature and abundance of the autophagic protein cargo. Taken together, our findings form a basis for better understanding the contribution of macroautophagy to brain proteostasis under physiological conditions. They also provide novel insight as to how autophagy impairment may contribute to diseases, through the aberrant homeostasis of specific, age-relevant, protein cargo.

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OMICS STUDIES ON NEURONAL CEROID LIPOFUSCINOSES: AN INTEGRATIVE POINT OF VIEW

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Aims: The neuronal ceroid lipofuscinoses (NCLs) are a group of rare life-limiting neurodegenerative disorders affecting children and adults with several clinical features including epilepsy, psychomotor decline, blindness, and early death. Mounting evidence points to bioenergetic and autophagic dysfunction as underlying conditions for disease progression founded altered also in other late-onset neurodegenerative and aging-related disorders. Multi-omic approaches represent promising tools for the identification of meaningful pathways as well as valid biomarkers for early diagnosis and disease progression in NCLs and possibly translational to other forms of dementia. **Methods:** 64 omic datasets from human and mouse samples have been bioinformatically integrated using pathway enrichment, biological network, or empirical correlation analyses. The comprehensive interpretation of experimental results has been assisted by the combination and integration of several sources of content/databases. **Results:** Our analyses evidenced an altered metabolic status across NCLs forms, both in human and mice. An altered synaptogenesis signalling pathway, autophagy, and neuroinflammation signs have been also recognized. Analyses in human datasets, highlighted candidate biomarkers of disease status and progression. Among these APP and CLU are implicated in beta-amyloid regulation and neurodegenerative process, indicating a further possible connection with Alzheimer's disease. From murine datasets we recognized several molecules directly related to AD and Parkinson's disease (as APOE, GFAP or SNCA), whereas CTSD and CLU were proposed as common interspecies biomarkers. **Conclusions:** Our multi-omic approach offers a better understanding on the molecular landscape across the NCL forms, also offering new points of contact with the more common forms of dementias.

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RIBOSOMAL TAGGING (RIBOTAG) IN ASTROCYTES: METHODOLOGICAL APPROACH FOR EXTRACTING MRNA FROM SMALL BRAIN TISSUE SAMPLES

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

Despoina Binou[1], Vsevolod Treshin[1], Sidra Gull[1,2], Samia Afzal[1], Madlen Günther[1], Anja Urbach[1,2,3,4], Marta Sánchez-Carbonell[1], Jean-Christopher Hennings[5], Otto W. Witte[1,2,3,4,6,7], Silvio Schmidt[1,4,6,7]
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Aim: Astrocytes are key regulators of brain neuronal network function, but their role at the molecular level is still largely unknown. Ribosomal tagging (Ribotag) is a powerful tool for cell type-specific extraction of mRNA, which in astrocytes is predominantly translocated in their thousands of fine distal processes for local translation. Here we aim to adopt the original protocol proposed by Sanz et al. (2009) to extract the whole astrocytic translatome in good quality from small tissue samples. **Method:** Floxed RPL22tm1.1Psam reporter mice were crossed with Aldh1l1-cre/ERT2 driver mice. In 6-week-old double heterozygote offspring, the expression of HA-tagged ribosomes was induced in astrocytes by peritoneal injection of tamoxifen. After two weeks of recombination, brain tissue was processed for affinity purification of the HA-tagged ribosomes. Various modifications were introduced to the initial protocol to optimize the yield and the quality of the final extracted ribosome-associated mRNA. The modifications were verified by microscopy, western blot, qPCR and the Agilent 2100 Bioanalyzer. **Results**: Our experiments indicate that tissue size (ranging from 60 to 5mg), tissue homogenization method (douncing vs grinding), HA-antibody type and concentration for affinity purification, as well as the mRNA extraction method (elution vs precipitation) specifically influence final mRNA quality and yield. **Conclusion:** There are multiple possible adaptations to the initial protocol to successfully extract the astrocytic translatome from small brain tissue samples.

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BOARD NUMBER: S07-459

THE BENEFICIAL ROLE OF EXERCISE-INDUCED NEURONAL DNA DAMAGE

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Background: DNA damage accumulation is a pathological feature that all chronic diseases of aging share, including neurodegenerative diseases. Interestingly, aerobic exercise induces DNA damage in peripheral tissues but there is little research investigating the relationship between exercise-induced DNA damage and the brain. Specifically, it is unknown how DNA damage and the concomitant DNA damage repair (DDR) response relate to neuroprotective gene expression or how physical activity primes against the neuronal DNA damage that accumulates with age. **Method:** Hippocampi from C57Bl/6J mice (n=8) were assessed for changes in gene expression, protein expression, DNA single-strand breaks, and DNA methylation. Two groups of mice were evaluated: (1) 6-month-old mice after a single bout of acute aerobic exercise and (2) 18-month-old mice after 6 months of regular aerobic exercise. After treadmill exercise, cognitive ability was assessed by novel object location, novel object recognition, and Barnes maze tests before tissue collection. **Result:** Upregulation of DDR enzymes Ape1, Ogg1, Parp1, and Parp2 was observed after both acute and chronic exercise protocols. These changes in gene expression coincided with upregulation of well-validated neurotrophic genes Bdnf and Creb1 and improved sub-threshold memory in the exercise groups. Unique patterns of DNA demethylation and single-strand DNA breaks occurred around active regulatory regions of neurotrophic genes. **Conclusion:** DNA damage and repair mechanisms are recruited during and after exercise that coincide with neurotrophic gene expression and memory function. Future research will evaluate the role of single-stranded DNA damage repair enzymes in the post-exercise neurotrophic response via genetic and pharmacological manipulation.

Pubmed:

34377851: Modarresi F, Pedram Fatemi R, Razavipour SF, Ricciardi N, Makhmutova M, Khoury N, Magistri M, Volmar CH, Wahlestedt C, Faghihi MA
A novel knockout mouse model of the noncoding antisense () gene displays increased endogenous Bdnf protein and improved memory function following exercise.
) expression is tightly controlled at the transcriptional and post-transcriptional levels. Previously, we showed that inhibition of noncoding antisense (-AS) RNA upregulates Bdnf protein. Here, we generated a -antisense knockout (AS KO) mouse model by deleting 6 kilobases upstream of -AS. After verifying suppression of AS, baseline behavioral tests indicated no significant difference in knockout and wild type mice, except for enhanced cognitive function in the knockout mice in the Y-maze. Following acute involuntary exercise, -AS KO mice were re-assessed and a significant increase in mRNA and protein were observed. Following long-term involuntary exercise, we observed a significant increase in nonspatial and spatial memory in novel object recognition and Barnes maze tests in young and aged AS KO mice. Our data provides evidence for the beneficial effects of endogenous upregulation and the synergistic effect of -AS knockout on exercise and memory retention. Heliyon, 2021; 7
33584810: Rybin MJ, Ramic M, Ricciardi NR, Kapranov P, Wahlestedt C, Zeier Z
Emerging Technologies for Genome-Wide Profiling of DNA Breakage.
Genome instability is associated with myriad human diseases and is a well-known feature of both cancer and neurodegenerative disease. Until recently, the ability to assess DNA damage-the principal driver of genome instability-was limited to relatively imprecise methods or restricted to studying predefined genomic regions. Recently, new techniques for detecting DNA double strand breaks (DSBs) and single strand breaks (SSBs) with next-generation sequencing on a genome-wide scale with single nucleotide resolution have emerged. With these new tools, efforts are underway to define the "breakome" in normal aging and disease. Here, we compare the relative strengths and weaknesses of these technologies and their potential application to studying neurodegenerative diseases.

Front Genet, 2020; 11
33459720: Dennison JL, Ricciardi NR, Lohse I, Volmar CH, Wahlestedt C
Sexual Dimorphism in the 3xTg-AD Mouse Model and Its Impact on Pre-Clinical Research.
Female sex is a leading risk factor for developing Alzheimer's disease (AD). Sexual dimorphism in AD is gaining attention as clinical data show that women are not only more likely to develop AD but also to experience worse pathology and faster cognitive decline. Pre-clinical AD research in animal models often neglects to address sexual dimorphism in evaluation of behavioral or molecular characteristics and outcomes. This can compromise its translation to a clinical setting. The triple-transgenic AD mouse model (3xTg-AD) is a commonly used but unique AD model because it exhibits both amyloid and tau pathology, essential features of the human AD phenotype. Mounting evidence has revealed important sexually dimorphic characteristics of this animal model that have yet to be reviewed and thus, are often overlooked in studies using the 3xTg-AD model. In this review we conduct a thorough analysis of reports of sexual dimorphism in the 3xTg-AD model including findings of molecular, behavioral, and longevity-related sex differences in original research articles through August 2020. Importantly, we find results to be inconsistent, and that strain source and differing methodologies are major contributors to lack of consensus regarding traits of each sex. We first touch on the nature of sexual dimorphism in clinical AD, followed by a brief summary of sexual dimorphism in other major AD murine models before discussing the 3xTg-AD model in depth. We conclude by offering four suggestions to help unify pre-clinical mouse model AD research inspired by the NIH expectations for considering sex as a biological variable.
J Alzheimers Dis, 2021; 80
31862872: Cao H, Salazar-García L, Gao F, Wahlestedt T, Wu CL, Han X, Cai Y, Xu D, Wang F, Tang L, Ricciardi N, Cai D, Wang H, Chin MPS, Timmons JA, Wahlestedt C, Kapranov P
Novel approach reveals genomic landscapes of single-strand DNA breaks with nucleotide resolution in human cells.
Single-strand breaks (SSBs) represent the major form of DNA damage, yet techniques to map these lesions genome-wide with nucleotide-level precision are limited. Here, we present a method, termed SSiNGLe, and demonstrate its utility to explore the distribution and dynamic changes in genome-wide SSBs in response to different biological and environmental stimuli. We validate SSiNGLe using two very distinct sequencing techniques and apply it to derive global profiles of SSBs in different biological states. Strikingly, we show that patterns of SSBs in the genome are non-random, specific to different biological states, enriched in regulatory elements, exons, introns, specific types of repeats and exhibit differential preference for the template strand between exons and introns. Furthermore, we show that breaks likely contribute to naturally occurring sequence variants. Finally, we demonstrate strong links between SSB patterns and age. Overall, SSiNGLe provides access to unexplored realms of cellular biology, not obtainable with current approaches.
Nat Commun, 2019; 10
30397132: Janczura KJ, Volmar CH, Sartor GC, Rao SJ, Ricciardi NR, Lambert G, Brothers SP, Wahlestedt C
Inhibition of HDAC3 reverses Alzheimer's disease-related pathologies in vitro and in the 3xTg-AD mouse model.
Alzheimer's disease (AD) is the leading cause of age-related dementia. Neuropathological hallmarks of AD include brain deposition of β-amyloid (Aβ) plaques and accumulation of both hyperphosphorylated and acetylated tau. RGFP-966, a brain-penetrant and selective HDAC3 inhibitor, or HDAC3 silencing, increases BDNF expression, increases histone H3 and H4 acetylation, decreases tau phosphorylation and tau acetylation at disease-associated sites, reduces β-secretase cleavage of the amyloid precursor protein (APP), and decreases Aβ accumulation in HEK-293 cells overexpressing APP with the double Swedish mutation (HEK/APP). In the triple transgenic AD mouse model (3xTg-AD), repeated administration of 3 and 10 mg/kg of RGFP-966 reverses pathological tau phosphorylation at Thr, Ser, and Ser, increases levels of the Aβ degrading enzyme Neprilysin in plasma, decreases Aβ protein levels in the brain and periphery, and improves spatial learning and memory. Finally, we show that RGFP-966 decreases Aβ accumulation and both tau acetylation and phosphorylation at disease residues in neurons derived from induced pluripotent stem cells obtained from APOEε4-carrying AD patients. These data indicate that HDAC3 plays an important regulatory role in the expression and regulation of proteins associated with AD pathophysiology, supporting the notion that HDAC3 may be a disease-modifying therapeutic target.
Proc Natl Acad Sci U S A, 2018; 115

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BOARD NUMBER: S07-460

TRANSCRIPTOMIC SIGNATURE AND ENRICHED SIGNALING PATHWAYS LINKED TO ALZHEIMER'S DISEASE MODEL INDUCED BY TAU SEED PATHOLOGY

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

Nikoleta Csicsatkova, Peter Szalay, Katarina Matyasova, Martin Cente, Tomas Smolek, Veronika Brezovakova, Norbert Zilka, Santosh Jadhav
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Accumulation of misfolded and aggregated forms of tau protein in distinct brain regions is characteristic for the progressive neurodegenerative disorder, namely Alzheimer´s disease (AD). In the last decade, the studies focused on a better understanding of processes underlying the spreading of tau pathology. Previous work displayed the potential of insoluble tau seeds from AD to induce propagation of tau pathology in a transgenic rodent model. Recruitment of the suggested transgenic rodent model of tauopathy may lead to the advanced perception of processes associated with the progressive spread of tau protein in AD. Our study aimed to identify differentiated molecular signatures and signalling pathways linked to the initiation and spreading of abnormal tau forms in the transgenic rat model of tauopathy. Our findings show that microglia in the vicinity of AD-Tau pathology in transgenic model triggered morphological changes to phagocytic phenotype in comparison to PBS injected rats. Subsequently, transcriptomic analysis revealed dysregulation of 15 genes in transgenic rats 3-month after AD-Tau injection. Consecutive bioinformatic analysis identified 31 enriched signalling pathways linked to the progression of tauopathy in AD. Activation of inflammatory, mitophagy, autophagy and endoplasmic reticulum stress regulatory mechanisms were observed. The shown data imply specific alteration of molecular signaling associated with AD-Tau-induced pathology in tauopathic background and warrants further exploration of tau spreading pathways. This project was supported by research grants: VEGA 2/0118/19, VEGA 2/0153/22 and APVV-17-0668, APVV-19-0568, APVV-20-0615, ERA-NET NEURON Neu-Vasc.

Pubmed:

34897025: Csicsatkova N, Szalay P, Matyasova K, Mate V, Cente M, Smolek T, Brezovakova V, Kawecka L, Zilka N, Jadhav S
Transcriptomic signature of Alzheimer's disease tau seed-induced pathology.
Spreading of tau pathology to anatomical distinct regions in Alzheimer's disease (AD) is associated with progression of the disease. Studies in recent decade have strived to understand the processes involved in this characteristic spread. We recently showed that AD-derived insoluble tau seeds are able to initiate neurofibrillary pathology in transgenic rodent model of tauopathy. In the present study, we pursued to identify the molecular changes that govern the induction and propagation of tau pathology on the transcriptomic level. We first show that microglia in vicinity to AD-Tau-induced pathology has phagocytic morphology when compared to PBS-injected group. On transcriptomic level, we observed deregulation of 15 genes 3-month post AD-Tau seeds inoculation. Integrated bioinformatic analysis identified 31 significantly enriched pathways. Amongst these, the inflammatory signalling pathway mediated by cytokine and chemokine networks, along with, toll-like receptor and JAK-STAT signalling were the most dominant. Furthermore, the enriched signalling also involved the regulation of autophagy, mitophagy and endoplasmic reticulum stress pathways. To our best of knowledge, the study is the first to investigate the transcriptomic profile of AD-Tau seed-induced pathology in hippocampus of transgenic model of tauopathy.
Gen Physiol Biophys, 2021; 40
34897024: Matyasova K, Csicsatkova N, Filipcik P, Jurisica I, Cente M
Peripheral microRNA alteration and pathway signaling after mild traumatic brain injury.
Discovering novel diagnostic biomarkers and signatures for traumatic brain injury (TBI) represents a major challenge in the brain trauma research. Detailed analysis of post-concussive molecular pathways based on experimental data could provide a new insight into the pathophysiological sequelae and mapping of recovery mechanisms involved in TBI. MicroRNAs (miRNAs) detectable in peripheral body fluids after TBI are promising carriers of this missing knowledge. In order to define the signature of peripheral miRNAs signaling associated with mild TBI (mTBI), we performed a comprehensive meta-analysis of miRNA profiles in mTBI patients using multiple curated pathway databases. Using a bioinformatic pipeline with integrated data analysis we identified a set of genes that are connected to deregulated circulating miRNAs following the mTBI. Identified

genes belong to specific pathways of MAPK, TGF-β, WNT, TLR2/4, PI3K/AKT, insulin, and growth factor signaling. Since the enriched pathways markedly overlap among the various biological fluids, signaling associated with mTBI that is concomitantly reflected in serum, plasma and saliva is robust and unique. Furthermore, we identified a network of 33 validated interacting proteins and their regulatory miRNAs that link the post-mTBI signaling in peripheral fluids with neurodegeneration-associated interaction pathways. Presented data provide a comprehensive insight into molecular events following mTBI, and the top predicted genes represent a group of novel candidate targets to be validated in connection with mTBI.
Gen Physiol Biophys, 2021; 40

32116255: Stozicka Z, Korenova M, Uhrinova I, Cubinkova V, Cente M, Kovacech B, Babindakova N, Matyasova K, Vargova G, Novak M, Novak P, Zilka N, Jadhav S

Environmental Enrichment Rescues Functional Deficit and Alters Neuroinflammation in a Transgenic Model of Tauopathy.
Alzheimer's disease (AD) is the most frequent neurodegenerative disorder, affecting over 44 million people worldwide. There are no effective pharmaco-therapeutic options for prevention and treatment of AD. Non-pharmacological approaches may help patients suffering from AD to significantly ameliorate disease progression. In this study, we exposed a transgenic rat model (tg) of human tauopathy to enriched environment for 3 months. Behavioral testing at 6 months of age revealed improvement in functional deficits of tg rats reared under enriched conditions, while sedentary tg rats remained severely impaired. Interestingly, enriched environment did not reduce tau pathology. Analysis of neurotrophic factors revealed an increase of nerve growth factor (NGF) levels in the hippocampus of both enriched groups (tg and non-tg rats), reflecting a known effect of enriched environment on the hippocampal formation. On the contrary, NGF levels decreased markedly in the brainstem of enriched groups. The non-pharmacological treatment also reduced levels of tissue inhibitor of metalloproteinase 1 in the brainstem of transgenic rats. Expression analysis of inflammatory pathways revealed upregulation of microglial markers, such as MHC class II and Cd74, whereas levels of pro-inflammatory cytokines remained unaffected by enriched environment. Our results demonstrate that exposure to enriched environment can rescue functional impairment in tau transgenic rats without reducing tau pathology. We speculate that non-pharmacological treatment modulates the immune response to pathological tau protein inclusions, and thus reduces the damage caused by neuroinflammation.
J Alzheimers Dis, 2020; 74

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BOARD NUMBER: S07-461

ALTERED EXPRESSION OF PERIPHERAL MICRORNAS AND PATHWAY SIGNALING AFTER MILD TRAUMATIC BRAIN INJURY

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Traumatic brain injury (TBI) is a global health issue with millions of cases reported annually. The history of TBI, including mild concussion, is associated with increased risk for development of various neurodegenerative disorders. The mechanisms and molecular events following TBI that lead to neurodegeneration remain partially unknown. The discovery of novel diagnostic and prognostic biomarkers for TBI represents a major challenge in current brain trauma research. MicroRNAs (miRNAs) are important regulators of molecular signaling pathways and biological processes, and therefore represent a valuable source of potential biomarkers for monitoring post-concussive molecular changes. In this study, we used an integrative computational approach with multiple curated pathway databases, to define the signature of mild TBI (mTBI)-associated peripheral miRNA signaling. The presented meta-analysis identified a set of target genes of deregulated circulating miRNAs after mTBI, which are involved in specific pathways of MAPK, TGF-β, WNT, TLR2/4, PI3K/AKT, insulin, and growth factor signaling. In addition, the bioinformatic analysis revealed a network of protein-protein interactions consisting of 33 key hub proteins and their regulatory miRNAs, representing a link between post-mTBI and neurodegenerative signaling. Our results provide a comprehensive insight into molecular events following mTBI, plus predicted genes could represent novel candidate markers or targets to be validated in connection with a mTBI. This project was supported by research grants VEGA 2/0118/19, VEGA 2/0153/22 and APVV-17-0668, APVV-19-0568, APVV-20-0615, ERA-NET NEURON Neu-Vasc.

Pubmed:
35172120: Sandmo SB, Matyasova K, Filipcik P, Cente M, Koerte IK, Pasternak O, Andersen TE, Straume-Næsheim TM, Bahr R, Jurisica I
Changes in circulating microRNAs following head impacts in soccer.
To explore the short-term effects of accidental head impacts and repetitive headers on circulating microRNAs, accounting for the effects of high-intensity exercise alone.
Brain Inj, 2022; 36
34897025: Csicsatkova N, Szalay P, Matyasova K, Mate V, Cente M, Smolek T, Brezovakova V, Kawecka L, Zilka N, Jadhav S
Transcriptomic signature of Alzheimer's disease tau seed-induced pathology.
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(miRNAs) detectable in peripheral body fluids after TBI are promising carriers of this missing knowledge. In order to define the signature of peripheral miRNAs signaling associated with mild TBI (mTBI), we performed a comprehensive meta-analysis of miRNA profiles in mTBI patients using multiple curated pathway databases. Using a bioinformatic pipeline with integrated data analysis we identified a set of genes that are connected to deregulated circulating miRNAs following the mTBI. Identified genes belong to specific pathways of MAPK, TGF-β, WNT, TLR2/4, PI3K/AKT, insulin, and growth factor signaling. Since the enriched pathways markedly overlap among the various biological fluids, signaling associated with mTBI that is concomitantly reflected in serum, plasma and saliva is robust and unique. Furthermore, we identified a network of 33 validated interacting proteins and their regulatory miRNAs that link the post-mTBI signaling in peripheral fluids with neurodegeneration-associated interaction pathways. Presented data provide a comprehensive insight into molecular events following mTBI, and the top predicted genes represent a group of novel candidate targets to be validated in connection with mTBI.
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32116255: Stozicka Z, Korenova M, Uhrinova I, Cubinkova V, Cente M, Kovacech B, Babindakova N, Matyasova K, Vargova G, Novak M, Novak P, Zilka N, Jadhav S

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J Alzheimers Dis, 2020; 74

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DEVELOPMENT OF CUSTOMIZABLE IN SITU SEQUENCING METHOD WITH SINGLE-CELL RESOLUTION

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

Jana Rajova[1], Sara Palo[1], Marcus Davidsson[1], Morgan Hartnor[1], Claudio Mirabello[2], Patrick Aldrin-Kirk[1], Andreas Heuer[3], Thomas Laurell[4], Malin Parmar[5], Tomas Björklund[1]
[1]Lund University, Department Of Experimental Medicinal Science, Lund, Sweden, [2]Linköping University, Department Of Physics, Chemistry And Biology, Linköping, Sweden, [3]Lund University, Department Of Experimental Medical Science, Lund, Sweden, [4]Lund University, Department Of Biomedical Engineering, Lund, Sweden, [5]Lund University, Department Of Developmental And Regenerative Neurobiology, Lund, Sweden

Preserving spatial information of cells' transcriptome significantly improves our understanding of the relationships among events taking place in living organisms. However, as throughput, sensitivity and financial demands remain a common obstacle, we ventured to develop a high-resolution method, which would overcome many of these hurdles and make spatial transcriptome analyses more commonplace. To reach this goal, we adopted surface-modified slides capable of mRNA binding, which preserves spatial information present in a tissue down to several micrometers. Immobilized mRNA sequences with their locations remain even after the digestion of the tissue itself, which significantly boosts the method’s sensitivity and signal-to-noise ratio. Following mRNA sequence capture, selected genes can be targeted through a multitude of padlock probes, grown on a gene chip array, and maintained through in-house amplification. Contact between a padlock probe and its target gene enables circularization of the probe and creation of a concatemer containing thousands of copies of the original molecule through rolling circle amplification. Through an appropriate design, probes can be used to create a general overview of the tissue composition as well as to monitor further events taking place in the tissue. With high throughput being made possible by relatively low cost of the method, it is feasible to eventually create inferred 3D maps of tissues with single cell resolution as well as creation on connection graphs within the nervous system. Moreover, as this method does not require tissue dissociation, cell type bias observed in many neuronal samples is removed, leading to more accurate cell representation.

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BOARD NUMBER: S07-463

REVEALING NOVEL REGULATORS OF NEUROTRANSMISSION BY ACTIVITY-DRIVEN SYNAPTIC EXOCYTOMICS

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

Carlos Pascual-Caro[1], Daniel Perez-Hernandez[2], Jaime De Juan-Sanz[1]
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Neuronal communication drives all cognitive activities, including movement, perception, learning and memory. At the molecular level, neurotransmitter release is orchestrated by the coordinated function of more than one thousand proteins and although there is extensive literature describing synaptic proteomes, even at the subcellular level, the field has only reported static proteomic snapshots that lack information about the essential trafficking events constantly occurring to sustain synaptic function. Developing a comprehensive picture of the trafficking proteome of firing synapses would provide a complete molecular view of synaptic biology and may unbiasedly identify previously-unannotated synaptic proteins whose trafficking on demand is essential to support neurotransmission. To tackle this issue, we combine HRP-based proximity-labeling of the synaptic cleft surface in primary cortical neurons with electrical stimulation or KCl depolarization, labeling newly-exposed proteins in the cleft during activity. Using this methodology, we can demonstrate the trafficking of endogenous presynaptic proteins but also postsynaptic proteins which control excitatory synaptic structure and function during firing. Here, we plan to identify unbiasedly molecules exposed by activity using state-of-the-art mass spectrometry to later dissect the role of new synaptic trafficking proteins in controlling neurotransmitter release by combining cutting-edge optophysiology and genetic manipulations. By isolating these surface synaptic regulators in living neurons during experimentally controlled firing, we aim to generate for the first time a holistic picture of the dynamic synaptic proteome, identifying novel proteins involved in neurotransmission and providing a solid framework for future studies into the molecular basis of synaptic pathologies such as schizophrenia, epilepsy or autism.

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INSTRUMENT-FREE SINGLE-CELL RESOLUTION OF TRANSCRIPTOME CHANGES IN HUMAN STEM CELLS DURING ACCELERATED NEURONAL DEVELOPMENT TRIGGERED BY KNOCKING OUT THE AMYLOID PRECURSOR PROTEIN

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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During embryogenesis, human cortical neural stem cells initially generate neurons at a particularly slow rate while preserving their progenitor state for a relatively extended time. Here we show that the characteristic potential of human neural stem cells to remain in a progenitor state as they generate neurons for a prolonged period of time requires the Amyloid Precursor Protein (APP). To explores the phenotype of neural stem cells without APP, we generated *APP*-knockout human iPSCs and characterized their transcriptomes using the novel instrument-free scRNA-seq kit recently developed by Scipio bioscience. With this kit, single-cell RNA sample preparation was achieved using the proprietary reversible hydrogel technology that provides results comparable to current microfluidic instrument-based techniques. In the application reported here, chemically linked barcode bead—cell tandems were dispersed in the hydrogel in the liquid state. Upon gelation the tandems were immobilized, but small lysis agents that diffuse freely were subsequently added. Following cell lysis mRNA molecules from individual cells were captured on the adjacent barcodes. Successive steps of reverse transcription, PCR amplification, cDNA sequencing, and bioinformatic analysis permitted comparing native and APP-knockout cells. The cell clustering results demonstrated that the differences observed point to a mechanism whereby loss of APP accelerates neurogenesis through activation of the AP1 transcription factor and repression of WNT signaling. Based on these observations, we propose that the fine balance between self-renewal and differentiation human cortical neural stem cells is homeostatically regulated by APP, which may contribute to human-specific temporal patterns of neurogenesis.

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BOARD NUMBER: S07-465

EXTENSIONS OF THE ION (INTEGRATION-COUPLED ON) TRANSGENESIS STRATEGY TO TRACE LINEAGE AND PROBE GENE FUNCTION IN NEURAL STEM CELLS

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

Takuma Kumamoto[1], Kamyar Keshavarz-Ferajkhah[2], Simona Esposito[2], Gwenvael Le Dréau[2], Franck Maurinot[2], Raphaëlle Barry-Martinet[2], Célia Vaslin[3], Sandrine Vandormael-Pournin[4], Mickaël Le[2], Marion Lerat[2], Dragos Niculescu[2], Michel Cohen-Tannoudji[4], Alexandra Rebsam[2], Karine Loulier[5], Samuel Tozer[6], Stephane Nedelec[3], Jean Livet[2]
[1]Tokyo Metropolitan Institute of Medical Science, Department Of Brain & Neurosciences, Tokyo, Japan, [2]INSERM, Sorbonne Université, Institut De La Vision, Paris, France, [3]Institut du Fer à Moulin, Neurodevelopment, Paris, France, [4]Institut Pasteur, Early Mammalian Development And Stem Cell Biology, Paris, France, [5]INSERM, Université de Montpellier, Institut Des Neurosciences De Montpellier, Montpellier, France, [6]CNRS, INSERM, PSL Université Paris, Ibens, Ecole Normale Supérieure, Paris, France

Efficient integration of exogenous transgenes in neural stem cells is essential to investigate their development and expand their therapeutic potential. We recently introduced the iOn (integration-coupled On) gene expression switch, a configuration of DNA vector that conditions transgene expression to genomic integration with the piggyBac transposition system. By silencing expression from non-integrated episomes, this approach considerably simplifies additive transgenesis in eukaryotic cells. Among others, it enables efficient multiplexed drug-free stable transgenesis in cultured cells, and also permits genetic mosaic manipulations through simple somatic transgenesis in animal models. Here, we present several developments of the iOn system that further increase its usefulness for neurodevelopmental studies. We generated vectors expressing different colors of fluorescent markers for increased multiplexing and versatility in stable transfection assays and cell lineage studies. To temporally control transgene expression in vitro and in vivo, we designed drug-inducible iOn vectors controlled by transactivation. We also investigated iOn-based strategies to inhibit endogenous gene expression in identified cells. Finally, to further simplify transgenesis with iOn, we created all-in-one vectors that do not require co-transfection with a separate transposase-encoding vector. These additions to the iOn toolkit, validated in vitro and in vivo, expand its applicability to a large range of manipulations permitted by germline transgenesis.

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FLUORESCENCE-ACTIVATED NUCLEI SORTING (FANS) AND A NOVEL COMBINATORIAL FLUIDIC INDEXING TO ELUCIDATE MOLECULAR PATHWAYS INVOLVED IN THE PATHOGENESIS IN PARKINSON'S DISEASE (PD).

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

Martino Avallone[1], Joaquin Pardo[1], Sara Palo[1], Malin Åkerblom[1], Marcus Davidsson[1], Per Brattås[2], Ulrich Pfisterer[2], Tomas Björklund[1]
[1]Wallenberg Neuroscience Center, Experimental Medicine, Lund, Sweden, [2]Biomedical Centre BMC, Center For Translational Genomics (ctg), Lund, Sweden

PD is a neurodegenerative disorder characterized by loss of dopamine (DA) neurons of the Substantia Nigra (SN). There are studies suggesting that alpha-synuclein (aSyn) plays a central role in the pathogenesis of PD. However, there is no molecularly precise, single-cell *in vivo* transcriptomic studies with accurately defined aSyn overload. Here we describe a novel strategy based on AAVs engineering and transgenic animals. The MNM008 AAVs capsid, capable of retrograde transport in DA neurons, is injected in the striatum of TH-Cre rats and DAT-Cre mice. The nuclei from the SN area are enriched for DA neurons using FANS thanks to a Cre-inducible histone-linked-GFP (H2B-GFP), which is only expressed in the population of interest. Using a combinatorial-indexing based single nuclei sequencing approach called Sci-Fi-Seq we can pre-label all mRNA in each nucleus with an identifier for that specific sample. These samples are then pooled together and prepared for sequencing using 10X genomic. Immunohistochemistry results showed that the virus injected in the striatum was retrogradely transported and expressed in DA neurons of the SNpc. The FANS enrichment strategy showed to be robust with an average of 2500 GFP positive (DA) nuclei retrieved per animal. Transcriptomic analysis on the GFP positive sorted nuclei revealed DA markers specific to the SN population, and the viral vector expression was restricted to this population, confirming the high selectivity of our approach. The combinatorial indexing and molecular barcoding will allow us to accurately measure the aSyn expression level in each DA neuron arranging them into disease stages in what we call pseudo-time. From this study, we will further our knowledge on PD disease progression as well as possible new treatments.

Pubmed:
30324128: Quintino L, Namislo A, Davidsson M, Breger LS, Kavanagh P, Avallone M, Elgstrand-Wettergren E, Isaksson C, Lundberg C
Destabilizing Domains Enable Long-Term and Inert Regulation of GDNF Expression in the Brain.
Regulation of therapeutic transgene expression can increase the safety of gene therapy interventions, especially when targeting critical organs such as the brain. Although several gene expression systems have been described, none of the current systems has the required safety profile for clinical applications. Our group has previously adapted a system for novel gene regulation based on the destabilizing domain degron technology to successfully regulate glial cell-line derived neurotrophic factor in the brain (GDNF-F-DD). In the present study, we used GDNF-F-DD as a proof-of-principle molecule to fully characterize DD regulation in the brain. Our results indicate that DD could be regulated in a dose-dependent manner. In addition, GDNF-F-DD could also be induced repeatedly, without loss of activity or efficacy . Finally, DD regulation was able to be sustained for 24 weeks without loss of expression or any overt toxicity. The present study shows that DD has great potential to regulate gene expression in the brain.
Mol Ther Methods Clin Dev, 2018; 11
30531868: Quintino L, Avallone M, Brännstrom E, Kavanagh P, Lockowandt M, Garcia Jareño P, Breger LS, Lundberg C
GDNF-mediated rescue of the nigrostriatal system depends on the degree of degeneration.
Glial cell-line derived neurotrophic factor (GDNF) is a promising therapeutic molecule to treat Parkinson's disease. Despite an excellent profile in experimental settings, clinical trials testing GDNF have failed. One of the theories to explain these negative outcomes is that the clinical trials were done in late-stage patients that have advanced nigrostriatal degeneration and may therefore not respond to a neurotrophic factor therapy. Based on this idea, we tested if the stage of nigrostriatal degeneration is important for GDNF-based therapies. Lentiviral vectors expressing regulated GDNF were delivered to the striatum of rats to allow GDNF expression to be turned on either while the nigrostriatal system was degenerating or after the nigrostriatal system had been fully lesioned by 6-OHDA. In the group of animals where GDNF expression was on during degeneration, neurons were rescued and there was a reversal of motor deficits. Turning GDNF expression on after the nigrostriatal system

was lesioned did not rescue neurons or reverse motor deficits. In fact, these animals were indistinguishable from the control groups. Our results suggest that GDNF can reverse motor deficits and nigrostriatal pathology despite an ongoing nigrostriatal degeneration, if there is still a sufficient number of remaining neurons to respond to therapy.
Gene Ther, 2019; 26
25971591: Mathis V, Cosquer B, Avallone M, Cassel JC, Lecourtier L
Excitatory Transmission to the Lateral Habenula Is Critical for Encoding and Retrieval of Spatial Memory.
The lateral habenula (LHb) is viewed as a relay between the limbic system, the basal ganglia (BG), and monoaminergic neurons of the midbrain. If a prominent role has been evidenced in BG-mediated functions such as value-based decision-making, very little is known about the involvement of the LHb in limbic functions such as memory processing. In the present study, we used two pharmacological approaches-LHb reversible inactivation with intra-LHb infusion of muscimol, an agonist of the GABA-A receptor, or blockade of excitatory inputs with intra-LHb infusion of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an antagonist of the glutamatergic AMPA receptor-to investigate the involvement of the LHb in encoding, consolidation, and retrieval of spatial memory in the water maze (WM) in rats. We found that intra-LHb infusion of muscimol or CNQX prevented encoding and retrieval, but not consolidation of spatial information. In addition, muscimol but not CNQX induced impairments during a cued version of the WM task, and marked anxiety in the elevated plus maze. These results confirm the involvement of the LHb in higher cognitive functions. They further suggest a dichotomy between the role of glutamatergic and other inputs to the LHb in hippocampus-dependent memory processing, as well as in emotional aspects of goal-directed behaviors.
Neuropsychopharmacology, 2015; 40

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-467

CRISPR/CAS9 GENE-EDITED FLUORESCENT ORGANOIDS AS A NOVEL MODEL TO STUDY ALZHEIMER'S DISEASE PATHOLOGY.

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Alzheimer's disease (AD) has been intensively studied over the last decades. However, the underlying mechanisms are rarely understood, and a cure is still not available, which is also the consequence of non-appropriate model systems. To fill this gap, we aimed to develop a new platform combining CRISPR/Cas9, hiPSC, and organoid technology. Therefore, a hiPSC line harboring the PAISA PSEN1 mutation (PSEN1 E280A) and the isogenic "healthy" control (WT) line were used. The WT hiPSC line was gene-edited by CRISPR/Cas9 to include a fluorescent protein N-terminal to the highly expressed LMNB1 protein, using it as a reporter marker.This allowed the development of a fused cerebral organoids, composed of AD-mutated (non-fluorescent) and healthy (fluorescent) hiPSc cells. For each cell of the organoid the genotype was precisely identifiable and traceable back to their origin providing a direct comparison between both conditions, and thus reducing comparability problems that arise from the high organoid-to-organoid variability. The established model was used to treat the organoids with synthetic Aβ oligomers and brain extract from aged Aβ precursor protein transgenic mice (APPPS1 tg) to study the seeding and spreading pattern of amyloid plaques in relation with PSEN1 E280A mutation in a human brain-like 3D tissue. Aditionally, we studied the affected cell types and the presence of hyperphosphorylated Tau. We demonstrate increased Aβ deposition and seeding in a time-dependent manner, that was based on the previous existence of Aβ aggregates. Our results demonstrate cerebral organoids to be a new model to investigate AD related pathways, especially very early aberrations.

Pubmed:

33849647: Marks D, Heinen N, Bachmann L, Meermeyer S, Werner M, Gallego L, Hemmerich P, Bader V, Winklhofer KF, Schröder E, Knauer SK, Müller T
Amyloid precursor protein elevates fusion of promyelocytic leukemia nuclear bodies in human hippocampal areas with high plaque load.
The amyloid precursor protein (APP) is a type I transmembrane protein with unknown physiological function but potential impact in neurodegeneration. The current study demonstrates that APP signals to the nucleus causing the generation of aggregates consisting of its adapter protein FE65, the histone acetyltransferase TIP60 and the tumour suppressor proteins p53 and PML. APP C-terminal (APP-CT50) complexes co-localize and co-precipitate with p53 and PML. The PML nuclear body generation is induced and fusion occurs over time depending on APP signalling and STED imaging revealed active gene expression within the complex. We further show that the nuclear aggregates of APP-CT50 fragments together with PML and FE65 are present in the aged human brain but not in cerebral organoids differentiated from iPS cells. Notably, human Alzheimer's disease brains reveal a highly significant reduction of these nuclear aggregates in areas with high plaque load compared to plaque-free areas of the same individual. Based on these results we conclude that APP-CT50 signalling to the nucleus takes place in the aged human brain and is involved in the pathophysiology of AD.
Acta Neuropathol Commun, 2021; 9
35099270: Bachmann L, Gallego Villarejo L, Heinen N, Marks D, Peters M, Müller T
Gene-Edited Fluorescent and Mixed Cerebral Organoids.
Cerebral organoids are a promising model to study human brain function and disease, although the high inter-organoid variability is still challenging. To overcome this limitation, we introduce the method of labeled mixed organoids generated from two different human induced pluripotent stem cell (hiPSC) lines, which enables the identification of cells from different origin within a single organoid. The method combining gene editing and organoid differentiation offers a unique tool to study gene function in a complex human three-dimensional model. Using a CRISPR-Cas9 gene-editing approach, different fluorescent proteins were fused to β-actin or lamin B1 in hiPSCs, and mixtures of differently edited cells were seeded to induce cerebral organoid differentiation. Consequently, the development of the organoids was detectable by live confocal fluorescence microscopy of whole organoids and immunofluorescence staining in fixed samples. We demonstrate that a direct comparison

of the individual cells is possible by having the edited and the control (or the two differentially labeled) cells within the same organoid, thus overcoming the inter-organoid inhomogeneity limitations. Furthermore, the approach enables mosaic analysis of mutant clones in a wild-type three-dimensional cellular environment. It paves the way for the reliable analysis of human genetic disorders using organoids and the gain of fundamental understanding of the molecular mechanisms underlying pathological conditions.
CRISPR J, 2022; 5
34068922: Gómez-Pinedo U, García-Ávila Y, Gallego-Villarejo L, Matías-Guiu JA, Benito-Martín MS, Esteban-García N, Sanclemente-Alamán I, Pytel V, Moreno-Jiménez L, Sancho-Bielsa F, Vidorreta-Ballesteros L, Montero-Escribano P, Matías-Guiu J
Sera from Patients with NMOSD Reduce the Differentiation Capacity of Precursor Cells in the Central Nervous System.
AQP4 (aquaporin-4)-immunoglobulin G (IgG)-mediated neuromyelitis optica spectrum disorder (NMOSD) is an inflammatory demyelinating disease that affects the central nervous system, particularly the spinal cord and optic nerve; remyelination capacity in neuromyelitis optica is yet to be determined, as is the role of AQP4-IgG in cell differentiation.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S07-468

VALIDATION OF IPSC-DERIVED BLOOD-BRAIN BARRIER MODEL ON MICROFLUIDIC CHIP

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Blood-brain barrier (BBB) exhibits a hurdle for drug delivery and development. One reason for poor successes to target drugs to the brain is the lack of good *in vitro* models that could recapitulate the BBB function. Our Aim was to develop an in vitro model of BBB by using human iPSC-derived cells in a microfluidic organ-on-a-chip platform. Methods: Our microfluidic BBB-on-chip platform design includes static conditions of the brain-side, as well as the dynamic conditions of the brain microvasculature within the same platform, mimicking in-vivo conditions. The BBB-on-chip is established using a co-culture of endothelial cells (ECs) and astrocytes, cultured on opposing sides of a semipermeable membrane. Results: First, we compared iPSC-ECs to human immortalized cell line, HCMEC/D3, and found that iPSC-ECs are forming a better barrier much faster in our BBB-on-chip. Secondly, we tested the effect of different pore sizes to iPSC-EC barrier formation and morphology. We concluded that 3 µm pore size supported the growth of ECs the best. Finally, a co-culture of iPSC-ECs with astrocytes showed a marked improvement in permeability compared to monoculture. Conclusion: Our microfluidic BBB model accurately reflects the in vivo conditions of the BBB by modeling both the static conditions of the brain-side of the BBB, as well as the dynamic conditions of the brain microvasculature within the same platform. As the highest-throughput platform currently in the field, our BBB model enables the development and screening of new therapeutics for neurological disorders still lacking effective treatments, such as Alzheimer's and Parkinson's disease.

Pubmed:
32998318: Sonninen TM, Goldsteins G, Laham-Karam N, Koistinaho J, Lehtonen Š
Proteostasis Disturbances and Inflammation in Neurodegenerative Diseases.
Protein homeostasis (proteostasis) disturbances and inflammation are evident in normal aging and some age-related neurodegenerative diseases. While the proteostasis network maintains the integrity of intracellular and extracellular functional proteins, inflammation is a biological response to harmful stimuli. Cellular stress conditions can cause protein damage, thus exacerbating protein misfolding and leading to an eventual overload of the degradation system. The regulation of proteostasis network is particularly important in postmitotic neurons due to their limited regenerative capacity. Therefore, maintaining balanced protein synthesis, handling unfolding, refolding, and degrading misfolded proteins are essential to preserve all cellular functions in the central nervous sysytem. Failing proteostasis may trigger inflammatory responses in glial cells, and the consequent release of inflammatory mediators may lead to disturbances in proteostasis. Here, we review the mechanisms of proteostasis and inflammatory response, emphasizing their role in the pathological hallmarks of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Furthermore, we discuss the interplay between proteostatic stress and excessive immune response that activates inflammation and leads to dysfunctional proteostasis.
Cells, 2020; 9
31133790: Lehtonen Š, Sonninen TM, Wojciechowski S, Goldsteins G, Koistinaho J
Dysfunction of Cellular Proteostasis in Parkinson's Disease.
Despite decades of research, current therapeutic interventions for Parkinson's disease (PD) are insufficient as they fail to modify disease progression by ameliorating the underlying pathology. Cellular proteostasis (protein homeostasis) is an essential factor in maintaining a persistent environment for neuronal activity. Proteostasis is ensured by mechanisms including regulation of protein translation, chaperone-assisted protein folding and protein degradation pathways. It is generally accepted that deficits in proteostasis are linked to various neurodegenerative diseases including PD. While the proteasome fails to degrade large protein aggregates, particularly alpha-synuclein (α-SYN) in PD, drug-induced activation of autophagy can efficiently remove aggregates and prevent degeneration of dopaminergic (DA) neurons. Therefore, maintenance of these mechanisms is essential to preserve all cellular functions relying on a correctly folded proteome. The

correlations between endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) that aims to restore proteostasis within the secretory pathway are well-established. However, while mild insults increase the activity of chaperones, prolonged cell stress, or insufficient adaptive response causes cell death. Modulating the activity of molecular chaperones, such as protein disulfide isomerase which assists refolding and contributes to the removal of unfolded proteins, and their associated pathways may offer a new approach for disease-modifying treatment. Here, we summarize some of the key concepts and emerging ideas on the relation of protein aggregation and imbalanced proteostasis with an emphasis on PD as our area of main expertise. Furthermore, we discuss recent insights into the strategies for reducing the toxic effects of protein unfolding in PD by targeting the ER UPR pathway.
Front Neurosci, 2019; 13
34299328: Wu YC, Sonninen TM, Peltonen S, Koistinaho J, Lehtonen Š
Blood-Brain Barrier and Neurodegenerative Diseases-Modeling with iPSC-Derived Brain Cells.
The blood-brain barrier (BBB) regulates the delivery of oxygen and important nutrients to the brain through active and passive transport and prevents neurotoxins from entering the brain. It also has a clearance function and removes carbon dioxide and toxic metabolites from the central nervous system (CNS). Several drugs are unable to cross the BBB and enter the CNS, adding complexity to drug screens targeting brain disorders. A well-functioning BBB is essential for maintaining healthy brain tissue, and a malfunction of the BBB, linked to its permeability, results in toxins and immune cells entering the CNS. This impairment is associated with a variety of neurological diseases, including Alzheimer's disease and Parkinson's disease. Here, we summarize current knowledge about the BBB in neurodegenerative diseases. Furthermore, we focus on recent progress of using human-induced pluripotent stem cell (iPSC)-derived models to study the BBB. We review the potential of novel stem cell-based platforms in modeling the BBB and address advances and key challenges of using stem cell technology in modeling the human BBB. Finally, we highlight future directions in this area.
Int J Mol Sci, 2021; 22
32879386: Sonninen TM, Hämäläinen RH, Koskuvi M, Oksanen M, Shakirzyanova A, Wojciechowski S, Puttonen K, Naumenko N, Goldsteins G, Laham-Karam N, Lehtonen M, Tavi P, Koistinaho J, Lehtonen Š
Metabolic alterations in Parkinson's disease astrocytes.
In Parkinson`s disease (PD), the loss of dopaminergic (DA) neurons in the substantia nigra pars compacta is associated with Lewy bodies arising from the accumulation of alpha-synuclein protein which leads ultimately to movement impairment. While PD has been considered a disease of the DA neurons, a glial contribution, in particular that of astrocytes, in PD pathogenesis is starting to be uncovered. Here, we report findings from astrocytes derived from induced pluripotent stem cells of LRRK2 G2019S mutant patients, with one patient also carrying a GBA N370S mutation, as well as healthy individuals. The PD patient astrocytes manifest the hallmarks of the disease pathology including increased expression of alpha-synuclein. This has detrimental consequences, resulting in altered metabolism, disturbed Ca homeostasis and increased release of cytokines upon inflammatory stimulation. Furthermore, PD astroglial cells manifest increased levels of polyamines and polyamine precursors while lysophosphatidylethanolamine levels are decreased, both of these changes have been reported also in PD brain. Collectively, these data reveal an important role for astrocytes in PD pathology and highlight the potential of iPSC-derived cells in disease modeling and drug discovery.
Sci Rep, 2020; 10
32911327: Rolova T, Wu YC, Koskuvi M, Voutilainen J, Sonninen TM, Kuusisto J, Laakso M, Hämäläinen RH, Koistinaho J, Lehtonen Š
Generation of a human induced pluripotent stem cell line (UEFi003-A) carrying heterozygous A673T variant in amyloid precursor protein associated with a reduced risk of Alzheimer's disease.
A673T mutation in the amyloid precursor protein (APP) is a rare variant associated with a reduced risk of late-onset Alzheimer's disease (AD) and age-related cognitive decline. The A673T mutation decreases beta-amyloid (Aβ) production and aggregation in neuronal cultures in vitro. Here we have identified a Finnish non-diseased male individual carrying a heterozygous A673T mutation, obtained a skin biopsy sample from him, and generated an iPSC line using commercially available integration-free Sendai virus-based kit. The established iPSC line retained the mutation, expressed pluripotency markers, had a normal karyotype, and differentiated into all three germ layers in vitro.
Stem Cell Res, 2020; 48
29807259: Lehtonen Š, Höytyläinen I, Voutilainen J, Sonninen TM, Kuusisto J, Laakso M, Hämäläinen RH, Oksanen M, Koistinaho J
Generation of a human induced pluripotent stem cell line from a patient with a rare A673T variant in amyloid precursor protein gene that reduces the risk for Alzheimer's disease.
An amyloid precursor protein (APP) A673T mutation was found to be protective against Alzheimer's disease (AD) and cognitive decline in the Icelandic population and to associate with decreased levels of plasma β-amyloid in a Finnish population-based cohort. Human fibroblasts from a Finnish male individual carrying the protective mutation were used to

generate integration-free induced pluripotent stem cell (iPSCs) line by Sendai virus technology. The iPSC line retained the mutation and expressed pluripotency markers, had a normal karyotype and differentiated into all three germ layers.
Stem Cell Res, 2018; 30

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NANOPARTICLE-MEDIATED ENZYME REPLACEMENT THERAPY FOR KRABBE DISEASE

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Krabbe disease (KD) is a fatal pediatric neurodegenerative lysosomal storage disorder (LSD) caused by deficient activity of the enzyme galactosylceramidase (GALC). This condition leads to severe demyelination and consequent neurodegeneration of both the central nervous system and peripheral nervous system. Despite the disease gravity, the current standard of care is mostly supportive only. The enzyme replacement therapy has gained broad interest thanks to the effective results achieved in other LSD. However, applications of such therapy to KD still do not exist. Here, an enzyme delivery system based on the encapsulation of cross-linked enzyme aggregates (CLEAs) into poly-(lactide-co-glycolide) (PLGA) nanoparticles (NPs) functionalized with brain targeting peptide (Ang-2) is investigated for the treatment of the natural murine model of KD, called Twitcher (TWI). The enzymatic activity (E.A) in the TWI mice with GALC mutation in homozygosity is significantly higher than in untreated mice from 4 to 72 hours after the injection. Overall, these data demonstrate that the formulation provides and maintains E.A. in the brain in the range of clinical interest up to 72 h from the treatment. An increase in E.A.is also observed in the sciatic nerve compared to untreated mice. Regarding liver and kidney, we found a high E.A. after 24 hours from the treatment without accumulation phenomena. Thus, given the established ability of our developed drug to pass the blood-brain barrier and release the active enzyme until 3 days, experiments of prolonged treatment have currently been ongoing.

Pubmed:
34489715: Scoditti E, Naccarati A, Carpi S, Polini B, Ebada SS, Nieri P
Editorial: Non-Coding RNAs as Mediators of the Activity of Natural Compounds.
Front Pharmacol, 2021; 12
31747311: Carpi S, Polini B, Fogli S, Podestà A, Ylösmäki E, Cerullo V, Romanini A, Nieri P
Circulating microRNAs as biomarkers for early diagnosis of cutaneous melanoma.
: Cutaneous melanoma is the deadliest form of skin cancer, with a dramatic increase in the incidence rate worldwide over the past decade. Early detection has been shown to improve the outcome of melanoma patients. The identification of noninvasive biomarkers able to identify melanoma at an early stage remains an unmet clinical need. Circulating miRNAs (c-miRNAs), small non-coding RNAs, appear as potential ideal candidate biomarkers due to their stability in biological fluids and easy detectability. Moreover, c-miRNAs are reported to be heavily deregulated in cancer patients.: This review examines evidence of the specific c-miRNAs or panels of c-miRNAs reported to be useful in discriminating melanoma from benign cutaneous lesions.: Although the interesting reported by published studies, the non-homogeneity of detection and normalization methods prevents the individuation of single c-miRNA or panel of c-miRNAs that are specific for early detection of cutaneous melanoma. In the future, prospective wide and well-designed clinical trials will be needed to validate the diagnostic potential of some of the c-miRNA candidates in clinical practice.
Expert Rev Mol Diagn, 2020; 20
30481404: Polini B, Carpi S, Romanini A, Breschi MC, Nieri P, Podestà A
Circulating cell-free microRNAs in cutaneous melanoma staging and recurrence or survival prognosis.
Cutaneous melanoma is a skin cancer with increasing incidence. Identification of novel clinical biomarkers able to detect the stage of disease and suggest prognosis could improve treatment and outcome for melanoma patients. Cell-free microRNAs (cf-miRNAs) are the circulating copies of short non-coding RNAs involved in gene expression regulation. They are released into the interstitial fluid, are detectable in blood and other body fluids and have interesting features of ideal biomarker candidates. They are stable outside the cell, tissue specific, vary along with cancer development and are sensitive to change in the disease course such as progression or therapeutic response. Moreover, they are accessible by non-invasive methods or venipuncture. Some articles have reported different cf-miRNAs with the potential of diagnostic tools for melanoma staging, recurrence and survival prediction. Although some concordance of results is already emerging, differences in analytical

methods, normalization strategies and tumour staging still will require further research and standardization prior to clinical usage of cf-miRNA analysis. This article reviews this literature with the aim of contributing to a shared focusing on these new promising tools for melanoma treatment and care.
Pigment Cell Melanoma Res, 2019; 32
35028271: Del Grosso A, Parlanti G, Angella L, Giordano N, Tonazzini I, Ottalagana E, Carpi S, Pellegrino RM, Alabed HBR, Emiliani C, Caleo M, Cecchini M
Chronic lithium administration in a mouse model for Krabbe disease.
Krabbe disease (KD; or globoid cell leukodystrophy) is an autosomal recessive lysosomal storage disorder caused by deficiency of the galactosylceramidase (GALC) enzyme. No cure is currently available for KD. Clinical applied treatments are supportive only. Recently, we demonstrated that two differently acting autophagy inducers (lithium and rapamycin) can improve some KD hallmarks in-vitro, laying the foundation for their in-vivo pre-clinical testing. Here, we test lithium carbonate in-vivo, in the spontaneous mouse model for KD, the Twitcher (TWI) mouse. The drug is administered ad libitum via drinking water (600 mg/L) starting from post natal day 20. We longitudinally monitor the mouse motor performance through the grip strength, the hanging wire and the rotarod tests, and a set of biochemical parameters related to the KD pathogenesis [i.e., GALC enzymatic activity, psychosine (PSY) accumulation and astrogliosis]. Additionally, we investigate the expression of some crucial markers related to the two pathways that could be altered by lithium: the autophagy and the β-catenin-dependent pathways. Results demonstrate that lithium has not a significant rescue effect on the TWI phenotype, although it can slightly and transiently improves muscle strength. We also show that lithium, with this administration protocol, is unable to stimulate autophagy in the TWI mice central nervous system, whereas results suggest that it can restore the β-catenin activation status in the TWI sciatic nerve. Overall, these data provide intriguing inputs for further evaluations of lithium treatment in TWI mice.
JIMD Rep, 2022; 63
33273530: Della Rosa G, Di Corato R, Carpi S, Polini B, Taurino A, Tedeschi L, Nieri P, Rinaldi R, Aloisi A
Tailoring of silica-based nanoporous pod by spermidine multi-activity.
Ubiquitous in nature, polyamines (PAs) are a class of low-molecular aliphatic amines critically involved in cell growth, survival and differentiation. The polycation behavior is validated as a successful strategy in delivery systems to enhance oligonucleotide loading and cellular uptake. In this study, the chemical features and the functional roles of the PA spermidine are synergistically exploited in the synthesis and bioactive functionalization of SiO-based structures. Inspired by biosilicification, the role of spermidine is assessed both as catalyst and template in a biomimetic one-pot synthesis of dense silica-based particles (SPs) and as a competitive agent in an interfacial reassembly strategy, to empty out SPs and generate spermidine-decorated hollow silica nanoporous pods (spd-SNPs). Spermidine bioactivity is then employed for targeting tumor cell over-expressed polyamine transport system (PTS) and for effective delivery of functional miRNA into melanoma cells. Spermidine decoration promotes spd-SNP cell internalization mediated by PTS and along with hollow structure enhances oligonucleotide loading. Accordingly, the functional delivery of the tumor suppressor miR-34a 3p resulted in intracellular accumulation of histone-complexed DNA fragments associated with apoptosis. Overall, the results highlight the potential of spd-SNP as a multi-agent anticancer therapy.
Sci Rep, 2020; 10
34671630: Gabbia D, Carpi S, Sarcognato S, Cannella L, Colognesi M, Scaffidi M, Polini B, Digiacomo M, Esposito Salsano J, Manera C, Macchia M, Nieri P, Carrara M, Russo FP, Guido M, De Martin S
The Extra Virgin Olive Oil Polyphenol Oleocanthal Exerts Antifibrotic Effects in the Liver.
Liver fibrosis, which is the outcome of wound-healing response to chronic liver damage, represents an unmet clinical need. This study evaluated the anti-fibrotic and anti-inflammatory effects of the polyphenol oleocanthal (OC) extracted from extra virgin olive oil (EVOO) by an approach. The hepatic cell lines LX2 and HepG2 were used as models. The mRNA expression of pro-fibrogenic markers, namely alpha-smooth muscle actin (α-SMA), collagen type I alpha 1 chain (COL1A1), a panel of metalloproteinases (MMP1, MMP2, MMP3, MMP7, MMP9) and vascular endothelial growth factor A (VEGFA) as well as the pro-oxidant genes NADPH oxidases (NOXs) 1 and 4 were evaluated in TGF-β activated LX2 cells by qRT-PCR. α-SMA and COL1A1 protein expression was assessed by immunofluorescence coupled to confocal microscopy. VEGFA release from LX2 was measured by ELISA. We also evaluated the amount of reactive oxygen species (ROS) produced by HO activated-HepG2 cells. , OC was administered daily by oral gavage to Balb/C mice with CCl-induced liver fibrosis. In this model, we measured the mRNA hepatic expression of the three pro-inflammatory interleukins (IL) IL6, IL17, IL23, chemokines such as C-C Motif Chemokine Ligand 2 (CCL2) and C-X-C Motif Chemokine Ligand 12 (CXCL12), and selected miRNAs (miR-181-5p, miR-221-3p, miR-29b-3p and miR-101b-3p) by qRT-PCR. We demonstrated that OC significantly downregulated the gene/protein expression of α-SMA, COL1A1, MMP2, MMP3, MMP7 and VEGF as well as the oxidative enzymes NOX1 and 4 in TGFβ1-activated LX2 cells, and reduced the production of ROS by HepG2. OC, beside causing a significant reduction of fibrosis at histological assessment, counteracted the CCl-induced upregulation of pro-fibrotic and inflammatory genes.

Moreover, OC upregulated the anti-fibrotic miRNAs (miR-29b-3p and miR-101b-3p) reduced in fibrotic mice, while downregulated the pro-fibrotic miRNAs (miR-221-3p and miR-181-5p), which were dramatically upregulated in fibrotic mice. In conclusion, OC exerts a promising antifibrotic effect a combined reduction of oxidative stress and inflammation involving putative miRNAs, which in turn reduces hepatic stellate cells activation and liver fibrosis.
Front Nutr, 2021; 8

31644552: Ylösmäki L, Polini B, Carpi S, Martins B, Smertina E, Feola S, Fusciello M, Peltonen K, Nieri P, Ylösmäki E, Cerullo V
Harnessing therapeutic viruses as a delivery vehicle for RNA-based therapy.
Messenger RNA (mRNA) and microRNA (miRNA)-based therapeutics have become attractive alternatives to DNA-based therapeutics due to recent advances in manufacture, scalability and cost. Also, RNA-based therapeutics are considered safe since there are no risk of inducing genomic changes as well as the potential adverse effects would be only temporary due to the transient nature of RNA-based therapeutics. However, efficient in vivo delivery of RNA-based therapeutics remains a challenge. We have developed a delivery platform for RNA-based therapeutics by exploiting the physicochemical properties of enveloped viruses. By physically attaching cationic liposome/RNA complexes onto the viral envelope of vaccinia virus, we were able to deliver mRNA, self-replicating RNA as well as miRNA inside target cells. Also, we showed that this platform, called viRNA platform, can efficiently deliver functional miRNA mimics into B16.OVA tumour in vivo.
PLoS One, 2019; 14

28367983: Nieri P, Carpi S, Fogli S, Polini B, Breschi MC, Podestà A
Cholinesterase-like organocatalysis by imidazole and imidazole-bearing molecules.
Organocatalysis, which is mostly explored for its new potential industrial applications, also represents a chemical event involved in endogenous processes. In the present study, we provide the first evidence that imidazole and imidazole derivatives have cholinesterase-like properties since they can accelerate the hydrolysis of acetylthiocholine and propionylthiocholine in a concentration-dependent manner. The natural imidazole-containing molecules as L-histidine and histamine show a catalytic activity, comparable to that of imidazole itself, whereas synthetic molecules, as cimetidine and clonidine, were less active. In the experimental conditions used, the reaction progress curves were sigmoidal and the rational of such unexpected behavior as well as the mechanism of catalysis is discussed. Although indirectly, findings of the present study suggests that imidazolic compounds may interfere with the homeostasis of the cholinergic system in vivo.
Sci Rep, 2017; 8

32867069: Polini B, Carpi S, Doccini S, Citi V, Martelli A, Feola S, Santorelli FM, Cerullo V, Romanini A, Nieri P
Tumor Suppressor Role of hsa-miR-193a-3p and -5p in Cutaneous Melanoma.
Remarkable deregulation of several microRNAs (miRNAs) is demonstrated in cutaneous melanoma. hsa-miR-193a-3p is reported to be under-expressed in tissues and in plasma of melanoma patients, but the role of both miR-193a arms in melanoma is not known yet.
Int J Mol Sci, 2020; 21

31627295: Scoditti E, Carpi S, Massaro M, Pellegrino M, Polini B, Carluccio MA, Wabitsch M, Verri T, Nieri P, De Caterina R
Hydroxytyrosol Modulates Adipocyte Gene and miRNA Expression Under Inflammatory Condition.
Chronic inflammation of the adipose tissue (AT) is a major contributor to obesity-associated cardiometabolic complications. The olive oil polyphenol hydroxytyrosol (HT) contributes to Mediterranean diet cardiometabolic benefits through mechanisms still partially unknown. We investigated HT (1 and 10 μmol/L) effects on gene expression (mRNA and microRNA) related to inflammation induced by 10 ng/mL tumor necrosis factor (TNF)-α in human Simpson-Golabi-Behmel Syndrome (SGBS) adipocytes. At real-time PCR, HT significantly inhibited TNF-α-induced mRNA levels, of monocyte chemoattractant protein-1, C-X-C Motif Ligand-10, interleukin (IL)-1β, IL-6, vascular endothelial growth factor, plasminogen activator inhibitor-1, cyclooxygenase-2, macrophage colony-stimulating factor, matrix metalloproteinase-2, Cu/Zn superoxide dismutase-1, and glutathione peroxidase, as well as surface expression of intercellular adhesion molecule-1, and reverted the TNF-α-mediated inhibition of endothelial nitric oxide synthase, peroxisome proliferator-activated receptor coactivator-1α, and glucose transporter-4. We found similar effects in adipocytes stimulated by macrophage-conditioned media. Accordingly, HT significantly counteracted miR-155-5p, miR-34a-5p, and let-7c-5p expression in both cells and exosomes, and prevented NF-κB activation and production of reactive oxygen species. HT can therefore modulate adipocyte gene expression profile through mechanisms involving a reduction of oxidative stress and NF-κB inhibition. By such mechanisms, HT may blunt macrophage recruitment and improve AT inflammation, preventing the deregulation of pathways involved in obesity-related diseases.
Nutrients, 2019; 11

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-470

DEVELOPMENT OF FOLATE RECEPTOR ALPHA-FUNCTIONALIZED GENE-THERAPY VECTORS FOR TARGETED BRAIN DELIVERY

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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One of the major obstacle to drug delivery in the central nervous system is the presence of biological barriers like the blood-brain barrier (BBB) or the blood-cerebrospinal fluid barrier (BCSFB). For this reason, there is a research focus on finding strategies to elude these barriers in order to treat CNS disorders. Extracellular vesicles have the ability to carry cargo substances and have thus become a research focus for their potential as therapeutic vehicle. Folate receptor alpha (FRα) is the main transporter of folates to the CNS and can be found on the surface of EVs in the CSF. We have designed FRα-functionalized EV-like vectors for selective gene targeting to the CNS. Using functionalized magnetic beads and flow cytometry, we demonstrated the presence of FRα on the majority of CD9- and CD63-positive EVs in HEK 293 cells that overexpress FRα. This confirmed the coexpression of FRα with EVs marker. Since it was previously reported that overexpression of some EVs markers could have an influence on lentiviral transduction efficiency, we first tested FRα-functionalized lentiviruses *in vitro*. However, FRα did not increase transduction efficiency. Finally, we started to investigate hybridosomes, i.e. lipid nanoparticles conjugated with plasma membranes. Using membrane from FRα-expressing cells, we currently test membrane-enveloped lipid nanoparticles for their *in vitro* and *in vivo* gene-delivery efficiency.

Pubmed:
34006937: Bellotti C, Lang K, Kuplennik N, Sosnik A, Steinfeld R
High-grade extracellular vesicles preparation by combined size-exclusion and affinity chromatography.
Extracellular vesicles (EVs) have recently gained growing interest for their diagnostic and therapeutic potential. Despite this, few protocols have been reported for the isolation of EVs with preserved biological function. Most EV purification methods include a precipitation step that results in aggregation of vesicles and most available techniques do not efficiently separate the various types of EVs such as exosomes and ectosomes, which are involved in distinct biological processes. For this reason, we developed a new two-step fast performance liquid chromatography (FPLC) protocol for purification of large numbers of EVs. The method comprises size exclusion chromatography followed by immobilized metal affinity chromatography, which is enabled by expression of poly-histidine tagged folate receptor α in the parental cells. Characterisation and comparison of the EVs obtained by this method to EVs purified by differential centrifugation, currently the most common method to isolate EVs, demonstrated higher purity and more selective enrichment of exosomes in EV preparations using our FPLC method, as assessed by comparison of marker proteins and density distribution. Our studies reveal new possibilities for the isolation of defined subpopulations of EVs with preserved biological function that can easily be upscaled for production of larger amounts of EVs.
Sci Rep, 2021; 11

FENS Forum 2022
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BOARD NUMBER: S07-471

MICROGLIAL UPTAKE OF FUNCTIONALIZED NANOTUBES DERIVED FROM BACTERIOPHAGE TAIL SHEATH PROTEIN

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

Dovydas Gabrielaitis[1], Lina Saveikytė[1], Greta Labutytė[2], Vida Časaitė[2], Urte Neniskyte[3]
[1]Vilnius University, Department Of Neurobiology And Biophysics, Vilnius, Lithuania, [2]Vilnius University, Department Of Molecular Microbiology And Biotechnology, Vilnius, Lithuania, [3]Vilnius University, Vu-embl Partnership Institute, Life Sciences Center, Vilnius, Lithuania

Aims: Self-assembled protein nanostructures that have been proven to be a suitable media for the deliveryof bioactive materials into the central nervous system. We aimed to show that the internalization mechanism of different protein nanotubes can differ depending on their structure. Methods: We generated two types of chimeric proteins: bacteriophage vB_KleM-RaK2 tail sheath protein 041 fused with GFP (*041-GFP*) and bacteriophage vB_EcoM_FV3 tail sheath protein 053 fused with SNAP-tag (*053-SNAPtag*). These chimeric nanotubes differed in size: *041-GFP* had an average length of 300 nm and a diameter of 44 nm, while *053-SNAPtag* nanotubes had an average length of 100 nm and a diameter of 27 nm. We then tested the mechanism and the efficiency of the internalization of these nanotubes by primary mouse microglia and BV2 cells, using fluorescence microscopy. Results: The treatment of microglia with an inflammatory activator LPS and an actin polymerisation inhibitor cytochalasin D revealed that *041-GFP* nanotubes were taken up via active machinery, while *053-SNAPtag* nanotubes were internalized passively. In addition, we assessed the differences of nanotube uptake efficiency in primary microglia isolated from young adult and aged mice and found no age-related differences. Conclusions: Our findings showed that the internalization mechanism of protein nanotubes into microglia depended on their structures.

Pubmed:

32176729: Kacenauskaite L, Gabrielaitis D, Bærentsen N, Martinez KL, Vosch T, Laursen BW
Intrinsic anti-Stokes emission in living HeLa cells.
Intrinsic fluorescence of biological material, also called auto-fluorescence, is a well-known phenomenon and has in recent years been used for imaging, diagnostics and cell viability studies. Here we show that in addition to commonly observed auto-fluorescence, intrinsic anti-Stokes emission can also be observed under 560 nm or 633 nm excitation. The anti-Stokes emission is shown to be spatially located on/in the mitochondria. The findings presented here show that sensitive imaging experiments e.g. single molecule experiments or two-photon excitation imaging can be compromised if intracellular anti-Stokes emission is not accounted for. On the other hand, we suggest that this anti-Stokes emission could be exploited as an additional modality for mitochondria visualization and cell viability investigation even in systems that are already labeled with commonly used fluorophores that rely on normal Stokes-based detection.
PLoS One, 2020; 15
34835795: Labutytė G, Povilonienė S, Šimoliūnas E, Gabrielaitis D, Skapas M, Noreika A, Meškys R, Časaitė V
Functionalized Protein Nanotubes Based on the Bacteriophage vB_KleM-RaK2 Tail Sheath Protein.
We report on the construction of functionalized nanotubes based on tail sheath protein 041 from vB_KleM-RaK2 bacteriophage. The truncated 041 protein (041Δ200) was fused with fluorescent proteins GFP and mCherry or amidohydrolase YqfB. The generated chimeric proteins were successfully synthesized in BL21 (DE3) cells and self-assembled into tubular structures. We detected the fluorescence of the structures, which was confirmed by stimulated emission depletion microscopy. When 041Δ200GFP and 041Δ200mCherry were coexpressed in BL21 (DE3) cells, the formed nanotubes generated Förster resonance energy transfer, indicating that both fluorescent proteins assemble into a single nanotube. Chimeric 041Δ200YqfB nanotubes possessed an enzymatic activity, which was confirmed by hydrolysis of -acetyl-2'-deoxycytidine. The enzymatic properties of 041Δ200YqfB were similar to those of a free wild-type YqfB. Hence, we conclude that 041-based chimeric nanotubes have the potential for the development of delivery vehicles and targeted imaging and are applicable as scaffolds for biocatalysts.
Nanomaterials (Basel), 2021; 11
31429284: Tsiamis V, Ienasescu HI, Gabrielaitis D, Palmblad M, Schwämmle V, Ison J
One Thousand and One Software for Proteomics: Tales of the Toolmakers of Science.

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Proteomics is a highly dynamic field driven by frequent introduction of new technological approaches, leading to high demand for new software tools and the concurrent development of many methods for data analysis, processing, and storage. The rapidly changing landscape of proteomics software makes finding a tool fit for a particular purpose a significant challenge. The comparison of software and the selection of tools capable to perform a certain operation on a given type of data rely on their detailed annotation using well-defined descriptors. However, finding accurate information including tool input/output capabilities can be challenging and often heavily depends on manual curation efforts. This is further hampered by a rather low half-life of most of the tools, thus demanding the maintenance of a resource with updated information about the tools. We present here our approach to curate a collection of 189 software tools with detailed information about their functional capabilities. We furthermore describe our efforts to reach out to the proteomics community for their engagement, which further increased the catalog to >750 tools being about 70% of the estimated number of 1097 tools existing for proteomics data analysis. Descriptions of all annotated tools are available at https://proteomics.bio.tools.
J Proteome Res, 2019; 18

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-472

PHOTO STIMULATION OF DYSTROPHIC RETINAL EXPLANTS AFTER CHRONIC AND ACUTE EXPOSURE TO CONJUGATED POLYMER NANOPARTICLES

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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[1]Istituto Italiano Di Tecnologia, Center For Synaptic Neuroscience And Technology, Genova, Italy, [2]University of Genova, Department Of Experimental Medicine, Genova, Italy, [3]Ospedale Policlinico San Martino, Irccs, Genova, Italy, [4]Novavido, S.r.l, Bologna, Italy, [5]Politecnico di Milano, Dipartimento Di Fisica, Milano, Italy, [6]Istituto Italiano di Tecnologia, Center For Nano Science Technology, Milano, Italy, [7]IRCCS Ospedale Sacro Cuore Don Calabria, Opthamology Department, Negrar, Italy

The past three decades have witnessed tremendous research in visual restorative therapies which continue to fuel interest amongst the scientific community globally. Age related macular degeneration (AMD) and retinitis pigmentosa (RP) are among the top untreatable, chronic neurodegenerative retina diseases responsible for debilitating millions of lives. Different therapeutic methods have been investigated, among which electronic retinal prostheses hold the longest period of development. Subretinal approaches show encouraging results for a partial vision enhancement acquired by targeting the residual inner retinal neurons. Recently, nanoparticles made of photovoltaic polymer Poly(3-hexylthiophene-2,5-diyl) (P3HT) have shown to mediate light-evoked firing of retinal neurons and persistently rescue visual functions when subretinally injected in Royal College of Surgeons (RCS) rats, a model of RP. In the current work, we compared the firing modulation of excised dystrophic RCS retinas upon chronic versus acute exposure to the nanomaterials. We investigated the involvement of particle concentration, eye pressure and duration of contact in the phototransduction mechanism driven by nanoparticles. For this purpose, we analyzed the electrophysiological properties of acutely subretinally injected dystrophic retinal explants using nanoparticles at different concentrations. We then compared the acute exposure recordings to the light-evoked firing of retinas explanted from animals that had been subretinally injected with nanoparticles 30 days before. The findings show an enhanced photostimulation owing to prolonged contact of nanoparticles with retinal tissue. These results lead to a deeper understanding of the P3HT nanoparticles interaction with the retinal tissue and open the path towards the development of enhanced prototypes of the “liquid prosthetics”.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-473

THE COMBINED EFFICIENCY OF THYMOQUINONE AND CURCUMIN ON GLIOBLASTOMA CELLS

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

Aishwarya Hattiholi[1], Shivani Tendulkar[2], Vijay Kumbar[1], Kishore Bhat[1]
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Glioblastoma is one of the rarest and most complicated malignancies seeking an efficient clinical solution. Obscuring mechanisms of drug resistance are making it more difficult to treat these malignant tumors effectively. To tackle this issue along with the side effects imposed by drugs on other organs, researchers are turning to phytochemicals. Thymoquinone and curcumin have been widely studied as adjuvants with drugs, for their anti-cancer and sensitizing activities in such malignancies. In this study, we aimed to explore the combinatory effects of these multitargeting phytochemicals. Our preliminary investigations include cytotoxic evaluations using MTT and apoptotic evaluations using fluorescence microscopy. The cytotoxic assay followed by an evaluation of mitochondrial membrane potential shows their anti-cancer activities in U-87 MG (malignant glioma) cells. Further morphological changes seen using the nuclear stain DAPI show the apoptotic activities of the combination, which will be further delved into, in our future studies. Keywords: Anti-cancer, Curcumin, Glioblastoma, Thymoquinone, Phytochemical

Pubmed:
32335310: Yang CT, Hattiholi A, Selvan ST, Yan SX, Fang WW, Chandrasekharan P, Koteswaraiah P, Herold CJ, Gulyás B, Aw SE, He T, Ng DCE, Padmanabhan P
Gadolinium-based bimodal probes to enhance T1-Weighted magnetic resonance/optical imaging.
Gd-based contrast agents have been extensively used for signal enhancement of T-weighted magnetic resonance imaging (MRI) due to the large magnetic moment and long electron spin relaxation time of the paramagnetic Gd ion. The key requisites for the development of Gd-based contrast agents are their relaxivities and stabilities which can be achieved by chemical modifications. These modifications include coordinating Gd with a chelator such as diethylenetriamine pentaacetic acid (DTPA) or 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), encapsulating Gd in nanoparticles, conjugation to biomacromolecules such as polymer micelles and liposomes, or non-covalent binding to plasma proteins. In order to have a coherent diagnostic and therapeutic approach and to understand diseases better, the combination of MRI and optical imaging (OI) techniques into one technique entity has been developed to overcome the conventional boundaries of either imaging modality used alone through bringing the excellent spatial resolution of MRI and high sensitivity of OI into full play. Novel MRI and OI bimodal probes have been extensively studied in this regard. This review is an attempt to shed some light on the bimodal imaging probes by summarizing all recent noteworthy publications involving Gd containing MR-optical imaging probes. The several key elements such as novel synthetic strategy, high sensitivity, biocompatibility, and targeting of the probes are highlighted in the review. STATEMENT OF SIGNIFICANCE: The present article aims at giving an overview of the existing bimodal MRI and OI imaging probes. The review structured as a series of examples of paramagnetic Gd ions, either as ions in the crystalline structure of inorganic materials or chelates for contrast enhancement in MRI, while they are used as optical imaging probes in different modes. The comprehensive review focusing on the synthetic strategies, characterizations and properties of these bimodal imaging probes will be helpful in a way to prepare related work.
Acta Biomater, 2020; 110

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-474

THERE IS OTHER FISH IN THE SEA: ZEBRAFISH AS AN ALTERNATIVE MODEL FOR NANOPARTICLE SCREENING IN ISCHEMIC STROKE

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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[1]Universidade do Porto, Ineb, Porto, Portugal, [2]Universidade do Porto, I3s, Porto, Portugal, [3]Universidade do Porto, Icbas, Porto, Portugal, [4]Universidade do Porto, Ibmc, Porto, Portugal

Stroke is one of the major causes of death and long-term disability in the world, with a huge economic and social impact. Stroke survivors remain with major disabilities since there are no therapeutic options to protect neurons and promote regeneration. Therefore, there is an urgent need to develop new therapies to mitigate the extension of stroke damage. Nanotechnology has gained relevance in this field since nanobiomaterials are promising delivery platforms of potential therapeutics to the brain, increasing their blood stability and brain bioavailability. Currently, zebrafish is classified as an alternative animal model to the classic mammalian ones. Its widespread acceptance for drug screening has increased due to the high genome similarity to human's, high-fertility, low-cost and easy maintenance. Here we describe the establishment and optimization of an adapted stroke model in the adult zebrafish. A focal ischemic brain model, close to what happens in clinics, is being attained by photo-thrombolysis in adult zebrafish. Neuronal apoptosis and neurodegeneration after ischemia is under analysis. Neurological outcome analysis is also being evaluated by monitoring behaviour. Pilot experiments performed to determine the infarcted area using micro-CT, showed the clear visualization of the brain damaged area using several contrast agents. The neurodegeneration in the affected area has been determined by immunofluorescence assays, with apoptotic, glial and neuronal markers, showing apoptosis close to the occlusion site. Overall, this model is expected to open new avenues towards the assessment of the most promising and recent nanobiocarriers to deliver neuroprotective agents after an ischemic stroke.

Pubmed:
32102252: Spencer AP, Torrado M, Custódio B, Silva-Reis SC, Santos SD, Leiro V, Pêgo AP
Breaking Barriers: Bioinspired Strategies for Targeted Neuronal Delivery to the Central Nervous System.
Central nervous system (CNS) disorders encompass a vast spectrum of pathological conditions and represent a growing concern worldwide. Despite the high social and clinical interest in trying to solve these pathologies, there are many challenges to bridge in order to achieve an effective therapy. One of the main obstacles to advancements in this field that has hampered many of the therapeutic strategies proposed to date is the presence of the CNS barriers that restrict the access to the brain. However, adequate brain biodistribution and neuronal cells specific accumulation in the targeted site also represent major hurdles to the attainment of a successful CNS treatment. Over the last few years, nanotechnology has taken a step forward towards the development of therapeutics in neurologic diseases and different approaches have been developed to surpass these obstacles. The versatility of the designed nanocarriers in terms of physical and chemical properties, and the possibility to functionalize them with specific moieties, have resulted in improved neurotargeted delivery profiles. With the concomitant progress in biology research, many of these strategies have been inspired by nature and have taken advantage of physiological processes to achieve brain delivery. Here, the different nanosystems and targeting moieties used to achieve a neuronal delivery reported in the open literature are comprehensively reviewed and critically discussed, with emphasis on the most recent bioinspired advances in the field. Finally, we express our view on the paramount challenges in targeted neuronal delivery that need to be overcome for these promising therapeutics to move from the bench to the bedside.
Pharmaceutics, 2020; 12
30308255: Santos SD, Xavier M, Leite DM, Moreira DA, Custódio B, Torrado M, Castro R, Leiro V, Rodrigues J, Tomás H, Pêgo AP
PAMAM dendrimers: blood-brain barrier transport and neuronal uptake after focal brain ischemia.
Drug delivery to the central nervous system is restricted by the blood-brain barrier (BBB). However, with the onset of stroke, the BBB becomes leaky, providing a window of opportunity to passively target the brain. Here, cationic poly(amido amine) (PAMAM) dendrimers of different generations were functionalized with poly(ethylene glycol) (PEG) to reduce cytotoxicity and prolong blood circulation half-life, aiming for a safe in vivo drug delivery system in a stroke scenario. Rhodamine B

isothiocyanate (RITC) was covalently tethered to the dendrimer backbone and used as a small surrogate drug as well as for tracking purposes. The biocompatibility of PAMAM was markedly increased by PEGylation as a function of dendrimer generation and degree of functionalization. The PEGylated RITC-modified dendrimers did not affect the integrity of an in vitro BBB model. Additionally, the functionalized dendrimers remained safe when in contact with the bEnd.3 cells and rat primary astrocytes composing the in vitro BBB model after hypoxia induced by oxygen-glucose deprivation. Modification with PEG also decreased the interaction and uptake by endothelial cells of PAMAM, indicating that the transport across a leaky BBB due to focal brain ischemia would be facilitated. Next, the functionalized dendrimers were tested in contact with red blood cells showing no haemolysis for the PEGylated PAMAM, in contrast to the unmodified dendrimer. Interestingly, the PEG-modified dendrimers reduced blood clotting, which may be an added beneficial function in the context of stroke. The optimized PAMAM formulation was intravenously administered in mice after inducing permanent focal brain ischemia. Twenty-four hours after administration, dendrimers could be detected in the brain, including in neurons of the ischemic cortex. Our results suggest that the proposed formulation has the potential for becoming a successful delivery vector for therapeutic application to the injured brain after stroke reaching the ischemic neurons.
J Control Release, 2018; 291

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-475

MICROFLUIDICS FOR THE ASSESSMENT OF NANOPARTICLE INTRACELLULAR TRAFFICKING IN NEURONS

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Neurological diseases represent the highest global burden of disease, with stroke being the biggest contributor. For most neurological diseases there are no available treatments available that can improve neuronal survival. Recently, gene therapy has been proposed as a powerful therapeutic tool to promote neuronal repair and regeneration. Moreover, major advancements in targeted gene therapy have opened avenues for the treatment of these diseases. However, the success of this therapeutic option relies on the development of effective and clinically suitable delivery vectors that are able to compact and protect nucleic acids (NAs). Dendrimers are promising carriers due to their globular, nanosize and very branched architecture, with several functional groups in the periphery, which allow their functionalization with different ligands simulating the multivalency of biological systems. Here, we explored a neurotargeted NA delivery nanosystem based on our PEG-GATGE dendrimers functionalized with the non-toxic HC fragment of the tetanus toxin. Dendriplexes (complexes of dendrimers and NA) were prepared at different N/P ratios by adding Cy5-siRNA to different volumes of dendrimer solution. The trafficking of this nanosystem was evaluated in two-chamber microfluidic devices with compartmentalized cultures of primary mouse cortical neurons (Fig 1). The device allowed to mimic the bio-interaction between nanosystem and neurons and to monitor of the intracellular trafficking of dendriplexes. Furthermore, targeting proprieties were also evaluated. The encouraging retrograde intracellular route of dendriplexes, make them a great promise to be the long-awaited therapeutic strategy for many diseases, not only as NAs delivery vector but also for other

cargoes.

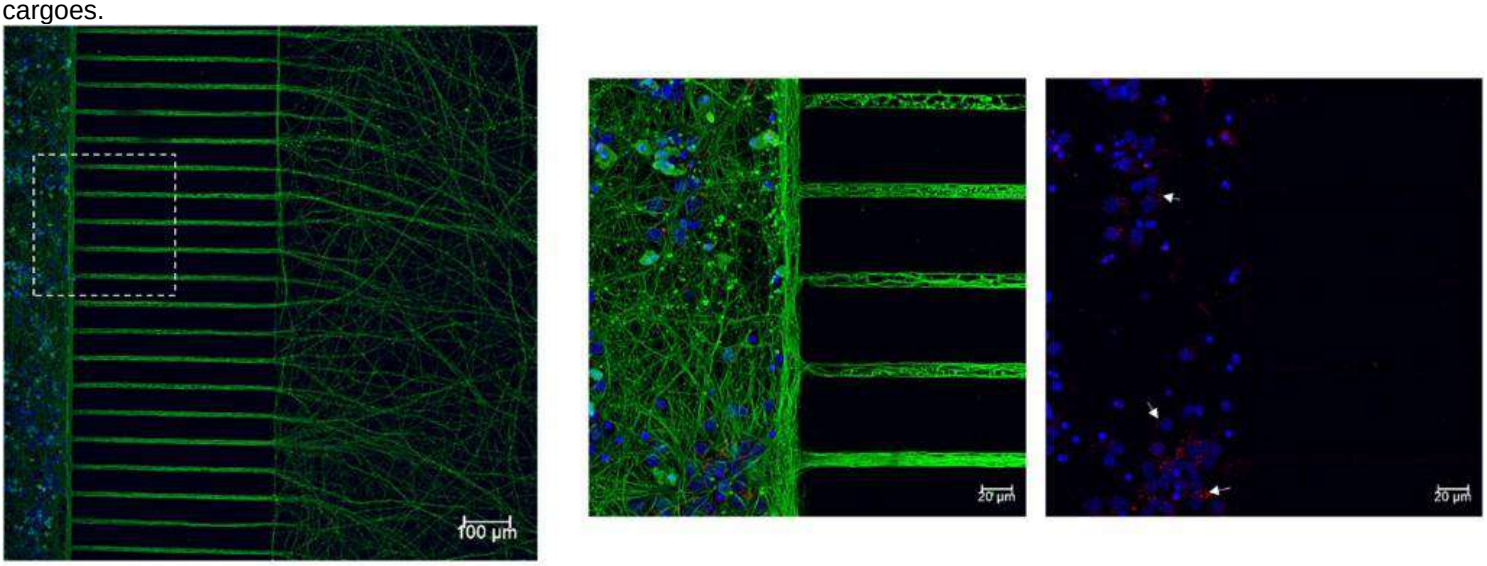

Figure 1. Dendriplexes' trafficking assessment set-up in primary cortical neurons. Cortical neurons were seeded in a two-chamber microfluidic device. At DIV5, N/P 5 dendriplexes were added on the terminal side (right) and movement assessed by confocal microscopy (for 20 hours). After a 48-h incubation, cells were fixed and analysed. After this period, dendriplexes were mainly located in the cell bodies of neurons. Staining: Nuclei with Hoechst 33342 (in blue), βIII-tubulin (in green), and Cy5-siRNAmi dendriplexes (in red).

Pubmed:

35032909: Leiro V, Spencer AP, Magalhães N, Pêgo AP
Versatile fully biodegradable dendritic nanotherapeutics.
The repeated administration of non-degradable dendrimers can lead to toxicity due to their bioaccumulation. Furthermore, in drug delivery applications, carrier stability can result in low biological performance due to insufficient intracellular cargo release. A novel family of versatile, biosafe, water-soluble, and fully biodegradable PEG-dendritic nanosystems is proposed, which overcomes the limitations of the most used dendrimers. Their novelty relies on the full and adjustable degradability thanks to the presence of tunable ester bonds in every dendritic arm. These dendritic nanosystems present peripheral azides that allow their easy multivalent functionalization, by "click" chemistry, with a vast range of ligands to act as versatile carriers. Here, their amine-functionalization to serve as nucleic acid vectors for gene therapy is explored. These nanosystems complex and protect efficiently siRNA in very small dendriplexes (<60 nm), being successfully cell-internalized, including in hard-to-transfect neuronal cells even when in full tissue explants (dorsal root ganglia). Importantly, full biodegradability was crucial for an efficient nucleic acid intracellular release and the attainment of excellent transfection efficiencies. The reported fully biodegradable dendritic nanosystems can act as multi-function nanotherapeutics for gene therapy, and also for broader applications in nanomedicine. Therefore, they represent top-notch and clinically translatable health facilitating nanotechnologies for further developments in theranostics.
Biomaterials, 2022; 281
34685047: Summers HD, Gomes CP, Varela-Moreira A, Spencer AP, Gomez-Lazaro M, Pêgo AP, Rees P
Data-Driven Modeling of the Cellular Pharmacokinetics of Degradable Chitosan-Based Nanoparticles.
Nanoparticle drug delivery vehicles introduce multiple pharmacokinetic processes, with the delivery, accumulation, and stability of the therapeutic molecule influenced by nanoscale processes. Therefore, considering the complexity of the multiple interactions, the use of data-driven models has critical importance in understanding the interplay between controlling processes. We demonstrate data simulation techniques to reproduce the time-dependent dose of trimethyl chitosan nanoparticles in an ND7/23 neuronal cell line, used as an in vitro model of native peripheral sensory neurons. Derived analytical expressions of the mean dose per cell accurately capture the pharmacokinetics by including a declining delivery rate and an intracellular particle degradation process. Comparison with experiment indicates a supply time constant, $\tau = 2$ h. and a degradation rate constant, $b = 0.71$ h. Modeling the dose heterogeneity uses simulated data distributions, with time dependence incorporated by transforming data-bin values. The simulations mimic the dynamic nature of cell-to-cell dose

variation and explain the observed trend of increasing numbers of high-dose cells at early time points, followed by a shift in distribution peak to lower dose between 4 to 8 h and a static dose profile beyond 8 h.
Nanomaterials (Basel), 2021; 11
32102252: Spencer AP, Torrado M, Custódio B, Silva-Reis SC, Santos SD, Leiro V, Pêgo AP
Breaking Barriers: Bioinspired Strategies for Targeted Neuronal Delivery to the Central Nervous System.
Central nervous system (CNS) disorders encompass a vast spectrum of pathological conditions and represent a growing concern worldwide. Despite the high social and clinical interest in trying to solve these pathologies, there are many challenges to bridge in order to achieve an effective therapy. One of the main obstacles to advancements in this field that has hampered many of the therapeutic strategies proposed to date is the presence of the CNS barriers that restrict the access to the brain. However, adequate brain biodistribution and neuronal cells specific accumulation in the targeted site also represent major hurdles to the attainment of a successful CNS treatment. Over the last few years, nanotechnology has taken a step forward towards the development of therapeutics in neurologic diseases and different approaches have been developed to surpass these obstacles. The versatility of the designed nanocarriers in terms of physical and chemical properties, and the possibility to functionalize them with specific moieties, have resulted in improved neurotargeted delivery profiles. With the concomitant progress in biology research, many of these strategies have been inspired by nature and have taken advantage of physiological processes to achieve brain delivery. Here, the different nanosystems and targeting moieties used to achieve a neuronal delivery reported in the open literature are comprehensively reviewed and critically discussed, with emphasis on the most recent bioinspired advances in the field. Finally, we express our view on the paramount challenges in targeted neuronal delivery that need to be overcome for these promising therapeutics to move from the bench to the bedside.
Pharmaceutics, 2020; 12
30092376: Pereira Gomes C, Leiro V, Ferreira Lopes CD, Spencer AP, Pêgo AP
Fine tuning neuronal targeting of nanoparticles by adjusting the ligand grafting density and combining PEG spacers of different length.
Poly(ethylene glycol) (PEG) has been extensively used to coat the surface of nanocarriers to improve their physicochemical properties and allow the grafting of targeting moieties. Still, to date there is no common agreement on the ideal PEG coverage-density or length to be used for optimum vector performance. In this study, we aimed to investigate the impact of both PEG density and length on the vectoring capacity of neuron-targeted gene-carrying trimethyl chitosan nanoparticles. The non-toxic fragment from the tetanus toxin (HC) was coupled to a 5 kDa heterobifunctional PEG (HC-PEG) reactive for the thiol groups inserted into the polymer backbone and grafted at different densities onto the nanoparticles. Internalization and transfection studies on neuronal versus non-neuronal cell lines allowed to determine the PEG density of 2 mol% of PEG chains per mol of primary amine groups as the one with superior biological performance. To enhance HC exposure and maximize cell-nanoparticle specific interaction, NPs containing different ratios of HC-PEG and 2 kDa methoxy-PEG at the same grafting density were produced. By intercalating HC-PEG with methoxy-PEG we attained the best performance in terms of internalization (higher payload delivery into cells) and transfection efficiency, using twice lower amount of HC. This outcome highlights the need for fine-tuning of PEG-modified nanoparticles towards the achievement of optimal targeting.
Acta Biomater, 2018; 78
32264006: Leiro V, Garcia JP, Moreno PMD, Spencer AP, Fernandez-Villamarin M, Riguera R, Fernandez-Megia E, Paula Pêgo A
Biodegradable PEG-dendritic block copolymers: synthesis and biofunctionality assessment as vectors of siRNA.
One important drawback of most of the currently used dendrimers for biomedical applications is their high stability under physiological conditions that can result in cytotoxicity or complications induced by the accumulation of non-degradable synthetic materials in the organism. Particularly in the gene therapy field, vector stability can further hinder the intracellular release of the nucleic acid from the dendriplex, consequently leading to low transfection efficiencies. Therefore, biodegradable cationic dendritic structures have been eagerly awaited. However, the development of these dendritic nanocarriers is challenging because of the undesired and/or premature degradation observed during their synthesis and/or application. Here, we report new hybrid-biodegradable, biocompatible, non-toxic, and water-soluble azide-terminated PEG-GATGE dendritic block copolymers, based on a gallic acid (GA) core and triethylene glycol (TG) butanoate arms, incorporating ester bonds (E) at the dendritic arms/shell. Their successful functionalization by "click" chemistry with unprotected alkynated amines allowed complexation and delivery of siRNA. The hydrophobic character of the GATGE building unit confers to these hydrolyzable dendritic bionanomaterials a great ability to complex, protect and mediate the cellular internalization of siRNA. Moreover, the localization of the degradation points at the dendritic periphery, close to the complexed siRNA, was found to be important for nucleic acid release from the nanoparticles, rendering a significant improvement of the transfection efficiency compared to their hydrolytically stable PEG-GATG copolymer counterparts. The present study puts forward these biodegradable PEG-dendritic block copolymers not only as suitable vectors for nucleic

acids, but also as new avenues for further developments exploring their use in theranostics.
J Mater Chem B, 2017; 5

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BOARD NUMBER: S07-476

DRUG LIPID NANO-CARRIERS ENTER THE INJURED BRAIN AT MICROVASCULAR OCCLUSIONS

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Background: Targeting drugs to the CNS is difficult because of the blood-brain-barrier (BBB). Nano carriers (NC) may represent an option for drug delivery to the brain provided the understand the underlying mechanisms are clear in detail. **Aim.** To directly visualize whether and how individual NCs cross the blood-brain barrier (BBB) under various pathological conditions *in vivo*. **Methods.** Super-bright fluorescent lipid nanodroplets (SBFLnDs; diameter: 30 and 80 nm) were loaded with a counterion-coupled rhodamine dye or with two dyes, F888 and cyanine-3.5, undergoing Förster Resonance Energy Transfer (FRET). SBFLnDs were injected systemically into mice following brain trauma or ischemic stroke and visualized by *in vivo* by 2-photon, confocal microscopy or correlative light-electron microscopy (CLEM). **Results.** Shortly after brain injury microvascular occlusions (VO) formed within the cerebral microcirculation. SBFLnDs accumulated within VOs in still viable brain tissue, crossed the BBB, and entered the brain parenchyma reaching healthy neurons. 30 nm NCs passed the opened BBB more readily than 80 nm NCs. CLEM revealed that NCs crossed the BBB at microvascular clots via endothelial transcytosis. Using FRET NCs we were able to demonstrate that NCs did not decompose in blood but in the brain parenchyma and inside clots implicating specific targeting in brain diseases accompanied with microthrombosis. **Conclusion**. The current study demonstrates that NC accumulate and enter the injured brain at sites of VOs. Thus, VOs function as a size-selective gate to the brain. Taking advantage of this process may pave the way for novel drug-delivery approach for brain disorders.

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BOARD NUMBER: S07-477

CHARACTERIZING THE UTILITY OF NOVEL CHANNELRHODOPSIN MUTANTS FOR ACTIVATION OF THE AUDITORY PATHWAY

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Electrical cochlear (eCI) implants provide hearing restoration to individuals with profound sensorineural hearing loss by direct electrical stimulation of spiral ganglion neurons (SGNs). To overcome wide-spread electrical neural excitation in eCIs, optogenetic stimulation of SGNs provides an alternative to address limitations current eCI users experience. For the translation of this promising technology, it is critical to select channelrhodopsins (ChR) with fast kinetics, large photocurrents, and low desensitization; in order to maintain the temporal characteristics of physiological sound encoding in the cochlea. Here we evaluate the optogenetic utility of ChR mutants recently engineered for optogenetic stimulation of SGNs (ChR2-, Chronos- and ChRmine- mutants), in mice. Adeno-associated-virus (AAV) carrying transgene ChR-variants under the human synapsin promotor were injected into the round window of neonatal mice (postnatal day 6). Six to thirteen weeks after injections, a laser-coupled fiber (573nm) was inserted into the round window to measure optically evoked auditory brainstem responses (oABRs). Subsequently, the cochleae were extracted for immunohistological analysis by confocal and lightsheet microscopy to evaluate the number of transduced cells as well as membrane expression profiles. Preliminary data sets of seven animals, each injected with a promising ChR2-mutant, show high oABR amplitudes in the range of 8 to 15µV during optical stimulations of 1ms at a 10Hz stimulation rate. Hence, our findings suggest a potential of the novel ChR mutants for optogenetic applications such as in auditory research and future optogenetic cochlear implants.

Pubmed:
32623905: Hanses U, Kleinsorge M, Roos L, Yigit G, Li Y, Barbarics B, El-Battrawy I, Lan H, Tiburcy M, Hindmarsh R, Lenz C, Salinas G, Diecke S, Müller C, Adham I, Altmüller J, Nürnberg P, Paul T, Zimmermann WH, Hasenfuss G, Wollnik B, Cyganek L
Intronic CRISPR Repair in a Preclinical Model of Noonan Syndrome-Associated Cardiomyopathy.
Noonan syndrome (NS) is a multisystemic developmental disorder characterized by common, clinically variable symptoms, such as typical facial dysmorphisms, short stature, developmental delay, intellectual disability as well as cardiac hypertrophy. The underlying mechanism is a gain-of-function of the RAS-mitogen-activated protein kinase signaling pathway. However, our understanding of the pathophysiological alterations and mechanisms, especially of the associated cardiomyopathy, remains limited and effective therapeutic options are lacking.
Circulation, 2020; 142

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BOARD NUMBER: S07-478

TEXT MINING TO SELECT DRUG CANDIDATES IN ORPHAN INDICATIONS : AN APPLICATION TO NEURONAL CEROID LIPOFUSCINOSES

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Neuronal ceroid lipofuscinoses (NCL) are a group of neurodegenerative disorders characterized by mutations in fourteen genes (CLN1 to CLN14) with still unknown regarding the roles and functions of some of those genes. During the last decade, the development of disease modelling and high-throughput or high-content screening approaches focused on the identification of new NCL treatments resulted in a substantial accumulation of knowledge. Extracting relevant information regarding drug-gene co-occurrence in literature, using automated techniques might be a novel and cost-effective approach, to the identification and development of new treatments. We used a set of non-redundant 2,880 FDA and EMA-approved compounds, and a text mining tool with the PubMed database. This study is part of NeuroLead project. Among the 2,880 compounds, 2005 are FDA-approved drugs, and 875 EMA-approved. 1867 compounds were found on PubChem and selected for further analysis, through the PubMed requesting of abstracts and titles including the name of the drugs and one of the 14 CLN genes. Abstracts were further selected based on the presence of sentences with both include at least one these genes and one of the 1867 selected compounds. 6 of the 14 genes were not associated with any of the selected compounds. Moreover, a range between 2 and 40 molecules was associated with the 8 remaining genes. This study is a proof-of-concept of text mining approach to investigate a large corpus of gene-drug associations in genetic disorders. Further in silico and experimental investigations are necessary to assess more precisely the therapeutic potential of these drugs.

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BOARD NUMBER: S07-479

HIGH RESILIENCE OF CEREBELLUM ACROSS THE LIFE-SPAN: IMAGING GENOMICS LEADS FOR IDENTIFYING AND VALIDATING NEUROPROTECTIVE DRUG DISCOVERY.

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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AIMS: Notable death/disability is due to different types of neurodegenerative disorders. A viable strategy is needed for large scale MRI analysis and for investigating how different brain regions show diverging sensitivity/resistance to neurodegenerative changes during aging. Hence, we will adopt MRI/immunohistochemistry/genomic analysis of that region to probe causative factors/targets and potential drugs. **METHODS: MRI Analysis:** We selected well-segregated cerebellum from cerebral hemispheres. This study consolidates 177 normal subjects (20-90 years) under Philips 3T-scanning. FDT-FSL, TBSS, Track-Vis Analysis used. **Genomics Analysis:** We analyzed collateral available human brain proteomics profile of Mass-spectrometry to identify neuroprotective genes in cerebellum. We identified 26 significantly regulated genes using signal transduction and pathway enrichment analysis. Afterward, DGIdb and cytological analysis facilitated the identification of candidate drugs for neuroprotection. **RESULTS: MRI Analysis:** The Grey Matter volume of cerebellum remains steady across lifetime (p=0.22), cerebellar White Matter volume increases notably (p=0.003), and cerebellar White Matter's Fractional Anisotropy remains constant (p=0.06). These cerebellar behaviors starkly contrast to cerebral hemispheres during aging. **Genomics Analysis:** The predicted upregulated target gene (and corresponding drugs) that can enhance neuronal growth and plasticity are: (i) Glycoprotein of Immunoglobulin superfamily gene (drugs: Flavanone-glycoside derivative - suppresses amyloid-β, $p<0.01$), and (ii) Transitional endoplasmic-reticulum ATPase gene (drugs: noradrenaline-serotonin transporter-modulators, $p<0.01$). Moreover, some genes are downregulated to prevent excessive protein synthesis (corresponding drug: guanidine derivative that inhibits production of Reactive oxygen-species, $p<0.01$). Collateral empirical clinical trials furnishes validation of the proposed pharmacological agents. **CONCLUSIONS:** Radio-genomics is a simple, low-cost technology for repurposing neuroprotective drugs with clinical validation.

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BOARD NUMBER: S07-480

AAV BIODISTRIBUTION IN THE MOUSE BRAIN AND PERIPHERY: EFFECTS OF CAPSID AND ROUTE OF ADMINISTRATION

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Adeno-associated viruses (AAVs) are leading the platform for in vivo delivery of gene therapy in the central nervous system (CNS). However, efficient crossing of the blood brain barrier (BBB) is required for systemically administered AAVs to reach the CNS. In the last few years, several studies have shown the ability of AAV9 and AAV-PHP.eB serotypes to pass the BBB and to transduce CNS neurons with different efficiency. Here, we assessed gene expression of AAV9 and AAV-PHP.eB capsids using the reporter gene eGFP in CNS and liver in wild-type mice. For this purpose, we injected AAV9 stereotactically in the brain ventricles, while AAV-PHP.eB was administered systemically in the tail vein. We quantified eGFP expressing neurons in the cortex, midbrain and hippocampus. The histological assessment was complemented by viral genome (v.g.) copy number determination studies and mRNA quantification. Systemically delivered AAV-PHP.eB produces more homogenous CNS expression in cortex, hippocampus and cerebellar nuclei than intracerebroventricular injected AAV9, where eGFP is expressed only in the proximity of the injection site. In AAV-PHP.eB-injected mice, v.g. determination studies show dose-dependent increase of viral DNA and mRNA levels. This is reflected by a dose-dependent increase of eGFP signal intensity per cell. Interestingly, the number of transduced neurons is similar across AAV doses. Moreover, AAV9 shows strong liver expression although administered locally into the brain. All together, these data indicate the pivotal role of the choice of AAV capsid and route of administration in the design of CNS-targeting gene therapy approaches.

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IDENTIFYING PROMISING THERAPEUTICS DRUGS ENTERING THE BRAIN FOR GENETIC PRION DISEASES IN C. ELEGANS.

POSTER SESSION 07 - SECTION: FUNCTIONAL NEUROGENOMICS & THERAPEUTICS

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Human prion diseases are fatal neurodegenerative disorders that include sporadic, infectious and genetic forms. Inherited *Creutzfeldt-Jakob* disease due to the E200K mutation of the prion protein-coding gene is the most common form of genetic prion diseases. The phenotype resembles that of sporadic Creutzfeldt-Jakob disease at both the clinical and pathological levels. To date, there is no available treatment for delaying the occurrence or slowing the progression of human prion diseases. Existing *in vivo* models do not allow high-throughput approaches that may facilitate the discovery of compounds targeting pathological assemblies of human prion protein (PrP) or their effects on neuronal survival. Here, we generated a genetic model in *C. elegans* by expressing human isoform with the E200K mutation in the mechanosensitive neuronal system. Expression of E200K PrP induced specific behavioural pattern and neurodegeneration of GFP-expressing mechanosensitive neurons, in addition to the formation of intraneuronal inclusions associated with the accumulation of a protease-resistant form of the PrP. We demonstrated that this experimental system is a powerful tool to study the efficacy of anti-prion compounds on both prion-induced neurodegeneration and prion protein misfolding, moreover in a human PrP context. Within a library of 320 compounds approved for human use and crossing the blood-brain-barrier, we identified five molecules that were active against the aggregation of E200K PrP and the neurodegeneration it induced in transgenic animals. This model breaks a technological limitation in prion therapeutic research and provides a key tool to study the deleterious effect of misfolded PrP in a well-described neuronal system.

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BOARD NUMBER: S07-482

NEUROIMMUNE ACTIVATION OF THE OLFACTORY BULB IS REGULATED BY TIME OF DAY

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Aims: Given its proximity to the nasal cavity, the olfactory bulb (OB) must generate robust neuroimmune responses to defend against neurotropic pathogens. The circadian (daily) clock primes cells and tissues to anticipate physiologically relevant environmental changes. Given that the circadian system regulates inflammation and immune responses, we hypothesized that daily changes in OB neuroinflammatory state would differentially "prime" responses to an intranasal inflammatory challenge. Aim 1 probed the OB's neuroinflammatory transcriptional profile throughout the day. Aim 2 was to determine how time of day of an intranasal inflammatory challenge influences the transcriptional and cellular responses within the OB. **Methods:** For Aim 1, OBs were isolated from male mice at 8 times of day (*n* = 3 mice/time). Transcriptional profiles of OBs were assessed using a NanoString Murine nCounter Neuroinflammation Panel. For Aim 2, we intranasally challenged mice at different times of day with Poly(I:C). We then measured transcriptional and cellular responses within the OB using the nCounter Neuroinflammation Panel and imaging flow cytometry, respectively. **Results:** We report that mRNA transcripts within the gene sets of microglia function, inflammatory signaling, and innate immune response were among the most differentially regulated by time of day. Further, we observed upregulated expression of mRNA transcripts involved in virus detection, type I interferon response, and chemotaxis at the beginning of the active phase. **Conclusions:** The circadian clock primes the OB's response to intranasal inflammatory stimuli differently depending on time of day of exposure, potentially providing an important gating mechanism underlying differential susceptibility to neurotropic pathogens.

Pubmed:

32413558: Pearson GL, Savenkova M, Barnwell JJ, Karatsoreos IN
Circadian desynchronization alters metabolic and immune responses following lipopolysaccharide inoculation in male mice. Metabolism and inflammation are linked at many levels. Sickness behaviors are elicited by the immune system's response to antigenic stimuli, and include changes in feeding and metabolism. The immune system is also regulated by the circadian (daily) clock, which generates endogenous rhythms, and synchronizes these rhythms to the light-dark cycle. Modern society has resulted in chronic misalignment or desynchronization of the circadian clock and the external environment. We have demonstrated that circadian desynchronization (CD) in mice alters metabolic function, and also affects both peripheral and central immune responses following a low-dose lipopolysaccharide (LPS) challenge. However, it is unclear how this altered immune response impacts sickness behaviors and metabolism following challenge. To test this, we housed male mice in circadian desynchronized (10-hours light:10-hours dark) or control (12-hours light:12-hours dark) conditions for 5-6 weeks. We then challenged mice with LPS (i.p., 0.4 mg/kg) or PBS and measured changes in body mass, feeding, drinking and locomotion using a comprehensive phenotyping system. Plasma, liver, and brain were collected 36 h post-inoculation (hpi) and inflammatory messengers were measured via multiplex cytokine/chemokine array and qPCR. We find that recovery of locomotion and body mass is prolonged in CD mice following LPS challenge. Additionally, at 36 hpi the expression of several proinflammatory cytokines differ depending on pre-inoculation lighting conditions. Our findings add to the growing literature which documents how desynchronization of circadian rhythms can lead to disrupted immune responses and changes in metabolic function.
Brain Behav Immun, 2020; 88

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EXPERIMENTAL ARTHRITIS INHIBITS ADULT HIPPOCAMPAL NEUROGENESIS IN MICE

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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BACKGROUND: Neuroinflammation inhibits adult neurogenesis, but the effect of peripheral inflammation on this form of neuroplasticity is ambiguous. **AIMS:** Our aim was to investigate the influence of acute and chronic experimental arthritis on adult hippocampal neurogenesis and to elucidate putative regulatory mechanisms. **METHODS**: Arthritis was triggered by injection of complete Freund's adjuvant (CFA) to the hind paws of male mice. Animals were killed either 7 days (acute inflammation), or 21 days (chronic inflammation) after CFA injection. Behavioral tests, in vivo bioluminescence imaging, complete blood cell counts and measurements of cytokine concentrations of TNF-α, IL-1α, IL-4, IL-6, IL-10, KC and MIP-2 were carried out to characterize the inflammatory responses. In the dentate gyrus, the total number of newborn neurons were quantified with BrdU- and doublecortin-immunohistochemistry. Microglial activation was determined by quantifying Iba1- and CD68-positive cells. **RESULTS**: Both acute and chronic arthritis resulted in mechanical and thermal hyperalgesia, phagocytic infiltration and increased levels of TNF-α, IL-4, IL-6, KC and MIP-2 in the inflamed hind paws. Circulating neutrophil granulocytes and IL-6 levels were increased in the blood, but only in the acute phase. In the dentate gyrus, chronic arthritis reduced the number of doublecortin-positive cells and we found increased density of CD68-positive macrophages both in the acute and chronic phases. Cytokine levels however were not altered in the hippocampus. **CONCLUSIONS**: Our data suggests that acute peripheral inflammation initiates a cascade of molecular and cellular changes that eventually leads to reduced adult hippocampal neurogenesis, which was detectable only in the chronic inflammatory phase.

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TEMPORAL DYNAMICS OF LYMPHOCYTE DISTRIBUTION IN THE BRAIN DURING POSTNATAL DEVELOPMENT

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Universidade Federal Do Rio De Janeiro, Instituto De Biofísica Carlos Chagas Filho, Rio de Janeiro, Brazil

The concept of brain immunoprivilege has been revised with the findings of the presence of lymphocytes in the brain. Furthermore, the characterization of the meningeal lymphopoietic niche has raised interest in the importance of these cells for brain functioning and immunosurveillance. However, little is known about how and when lymphocytes begin to populate the brain. The aim of this study was to characterize the presence of B and T lymphocyte cells during murine brain development and to identify their location in relation to blood vessels. C57BL/6 mice aged P0 (day of birth), P3, and P14 were used, as well as 3-month-old young adult mice of both sexes. Antibodies to CD3 and CD45R were used to identify T and B cells, respectively. Laminin was used as a marker of blood vessels. Different regions of the mouse brain were evaluated by immunofluorescence and confocal microscopy. Flow cytometry was used to characterize the lymphocyte populations in a BD LSR Fortessa flow cytometer. All experiments were approved by the Animal Ethics Committee (protocol CEUA/CCS-UFRJ 080/17). B cells were present since P0 until adulthood when their numbers decreased significantly in all analyzed regions. In contrast, there were fewer T cells during early postnatal development in comparison to the adult brain. We found T and B cells associated with the vessels, but also some T cells in the brain parenchyma. Flow cytometry results are in progress. Lymphocyte distribution is dynamic during brain development and essentially associated with blood vessels in different brain regions.

Pubmed:
33827248: Vasconcellos LRC, Martimiano L, Dantas DP, Fonseca FM, Mata-Santos H, Travassos L, Mendez-Otero R, Bozza MT, Pimentel-Coelho PM
Intracerebral Injection of Heme Induces Lipid Peroxidation, Neuroinflammation, and Sensorimotor Deficits.
Heme is a red blood cell component released in the brain parenchyma following intracerebral hemorrhage. However, the study of the pathophysiological mechanisms triggered by heme in the brain is hampered by the lack of well-established in vivo models of intracerebral heme injection. This study aims to optimize and characterize a protocol of intrastriatal heme injection in mice, with a focus on the induction of lipid peroxidation, neuroinflammation and, ultimately, sensorimotor deficits. We also evaluated the involvement of NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3), an inflammasome sensor, in the behavior deficits induced by heme in this model.
Stroke, 2021; 52

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EFFECTS OF VTA ACTIVATION ON THE RECOVERY PROCESS FOLLOWING MYOCARDIAL INFARCTION

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Background: Epidemiological data indicate a correlation between negative emotional state as depression or stress, to increased incidence of acute cardiovascular events as myocardial infarction (MI) with worst mortality rates and consequent comorbidities. Consistently, positive emotional state practices as meditation or stress reduction correlated with cardiovascular health and reduced mortality and hospitalization following MI. However, it is unknown how the emotional state influences the recovery process following MI. While some patients regain much of their cardiac function and maintain good overall health, others will go on to develop complications, such as heart failure. To approach emotional state in the animal model we choose to focus on a specific network, the reward system, which mediates positive-valanced emotions, motivation, and expectation. Here we test whether and how activation of the ventral tegmental area (VTA), a main component of the reward system, affects recovery following MI. **Results:** VTA activation in mice enhanced recovery following MI and prevented the additional 20% decrease in cardiac function normally observed. VTA activation reduced the area occupied by non-contractile scar tissue and amplified cardiac vascularization. Proteomic analysis revealed that reward system activation induced a systemic response including complement activation and metabolic processes, which were previously correlated with a favorable recovery from MI. **Conclusion:** VTA activation improves cardiac function following MI characterized by diverse adaptive recovery processes and potentially an orchestrated systemic response. These pre-clinical data introduce a novel therapeutic approach to enhance cardiac recovery and potentially prevent comorbid conditions from occurring.

Pubmed:

34525336: Haykin H, Rolls A
The neuroimmune response during stress: A physiological perspective.
Stress is an essential adaptive response that enables the organism to cope with challenges and restore homeostasis. Different stressors require distinctive corrective responses in which immune cells play a critical role. Hence, effects of stress on immunity may vary accordingly. Indeed, epidemiologically, stress can induce either inflammation or immune suppression in an organism. However, in the absence of a conceptual framework, these effects appear chaotic, leading to confusion. Here, we examine how stressor diversity is imbedded in the neuroimmune axis. Stressors differ in the brain patterns they induce, diversifying the neuronal and endocrine mediators dispatched to the periphery and generating a wide range of potential immune effects. Uncovering this complexity and diversity of the immune response to different stressors will allow us to understand the involvement of stress in pathological conditions, identify ways to modulate it, and even harness the therapeutic potential embedded in an adaptive response to stress.
Immunity, 2021; 54
34752731: Koren T, Yifa R, Amer M, Krot M, Boshnak N, Ben-Shaanan TL, Azulay-Debby H, Zalayat I, Avishai E, Hajjo H, Schiller M, Haykin H, Korin B, Farfara D, Hakim F, Kobiler O, Rosenblum K, Rolls A
Insular cortex neurons encode and retrieve specific immune responses.
Increasing evidence indicates that the brain regulates peripheral immunity, yet whether and how the brain represents the state of the immune system remains unclear. Here, we show that the brain's insular cortex (InsCtx) stores immune-related information. Using activity-dependent cell labeling in mice (Fos), we captured neuronal ensembles in the InsCtx that were active under two different inflammatory conditions (dextran sulfate sodium [DSS]-induced colitis and zymosan-induced peritonitis). Chemogenetic reactivation of these neuronal ensembles was sufficient to broadly retrieve the inflammatory state under which these neurons were captured. Thus, we show that the brain can store and retrieve specific immune responses, extending the classical concept of immunological memory to neuronal representations of inflammatory information.
Cell, 2021; 184

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BOARD NUMBER: S07-486

WASTEOSOMES (CORPORA AMYLACEA) CAN BE PHAGOCYTOSED BY MACROPHAGES THROUGH DIFFERENT MECHANISMS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Aims: *Corpora amylacea* of human brain, recently renamed wasteosomes, are granular structures that appear during aging and also accumulate in specific areas of the brain in neurodegenerative conditions. Acting as waste containers, wasteosomes are formed by polyglucosan aggregates that entrap waste substances of different origins, are expelled from the brain to the cerebrospinal fluid (CSF), and are thereafter phagocytosed by macrophages. In the present study, we analyze the mechanisms involved in this last process. **Methods:** We purified wasteosomes from human CSF and incubated them with THP-1 macrophages. Immunofluorescence staining techniques were later performed to evaluate the mechanisms involved in their phagocytosis. We also immunostained human hippocampal sections to study the interactions between wasteosomes and macrophages at central nervous system interfaces. **Results:** The phagocytosis by THP-1 macrophages can be triggered through the CD206 and the CD35 receptors, but not the FAIM3 receptor, whereas all these receptors may be involved, *in vivo*, in the phagocytosis of wasteosomes at the central nervous system interfaces or beyond. Moreover, we observed that the wasteosomes obtained from the CSF are opsonized by MBL and the complement protein C3b, and can also contain mannose or other targets of CD206. **Conclusions:** Our findings indicate that, *in vivo*, different mechanisms can be involved in the phagocytosis of wasteosomes, some of them triggering non-inflammatory responses and avoiding tissue damage. Altogether supports the role of the immune system in the elimination of wasteosomes.

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BOARD NUMBER: S07-487

DISRUPTION OF HIPPOCAMPUS-BASED SPATIAL CODING IN LONG–SEPSIS SURVIVORS IS MEDIATED BY HMGB1

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Sepsis is a potentially lethal condition triggered by dysregulated immune responses to infection, which leads to the failure of several organs, including the brain. Survivors often suffer lasting cognitive impairment, which might be partly mediated by the cytokine high mobility group box 1 (HMGB1) (PMC3459473). Therefore, we sought to determine whether specific ablation of the *HMGB1* gene within neurons of long–sepsis surviving (LSS) mice might be able to protect hippocampus–based spatial cognition as well as place cell (PC) dynamics. We modeled sepsis using the cecal ligation and puncture method in male C57BL/6 (WT) and Syn-Cre/HMGB1$^{fl/fl}$ (KO) mice. A sham laparotomy was used for controls (CON). After a 6-week recovery, tetrodes were implanted into the dorsal CA1 field. Our results demonstrate that WT–LSS PCs have expanded place fields, with lower spatial information, when compared to WT–CON PCs. Moreover, spatial encoding is disrupted in WT–LSS mice as demonstrated by larger errors in Bayesian path reconstruction algorithms. Remarkably, KO–LSS mice show PC properties (place field area, spatial information) completely similar to CON (WT, KO) mice. Moreover, spatial encoding is normal in KO–LSS mice, as they have path reconstruction errors comparable to CON (WT, KO) groups. Finally, the clockmaze task (PMC6568215) confirms that KO–LSS mice use spatial strategy similarly to CON (WT, KO) groups. We conclude that genetic ablation of neuronal HMGB1 protects the hippocampus from sepsis-induced failure, strongly suggesting that HMGB1 is involved in mediating the fate of the spatial cognitive network following sepsis.

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METABOLIC AND OSCILLATORY DISRUPTIONS OF THE BRAIN FEAR NETWORK IN LONG–SEPSIS SURVIVORS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Sepsis is triggered by dysregulated immune responses to infection leading to failure of several organs, such as the brain. Patients that survive a septic shock frequently suffer lasting cognitive deficits and emotional imbalance. Long–sepsis murine models display impaired contextual fear memory (PMC5193462). Our goal is to elucidate whether long–sepsis surviving (LSS) animals exhibit abnormalities within the brain's fear network, comprising the hippocampus (HPC), basolateral amygdala (BLA) and prefrontal cortex (PFC). We modeled sepsis in male C57BL/6 mice, using the cecal ligation and puncture procedure, and sham laparotomy for controls. After 6-week recovery, mice were subjected to trace–fear–conditioning and microPET, using [^{18}F]-fluorodeoxyglucose (FDG), followed by multielectrode–array implants directed to HPC, BLA and PFC. Our results demonstrate that, when comparing familiarization to context–fear–memory sessions, control mice show significantly higher FDG uptakes in BLA and PFC but decreased FDG signal in HPC. Strikingly, LSS mice have no FDG changes in BLA, PFC and HPC across fear sessions. Neural recordings reveal altered oscillations in LSS mice, such as persistently elevated powers in the theta and gamma bands during contextual–fear–memory sessions. Autocorrelation–power–matrix and wavelet–power analyses allow us to examine alterations in theta–gamma coupling and phase–amplitude modulation index in LSS mice. We conclude that the fear network is severally altered in LSS mice. Oscillatory patterns, critical for coordination across brain regions, are disrupted following sepsis reducing the ability of the brain to recall and maintain a healthy fear state.

Pubmed:
34809706: Strohl JJ, Gallagher JT, Gómez PN, Glynn JM, Huerta PT
Framework for automated sorting of neural spikes from Neuralynx-acquired tetrode recordings in freely-moving mice.
Extracellular recording represents a crucial electrophysiological technique in neuroscience for studying the activity of single neurons and neuronal populations. The electrodes capture voltage traces that, with the help of analytical tools, reveal action potentials ('spikes') as well as local field potentials. The process of spike sorting is used for the extraction of action potentials generated by individual neurons. Until recently, spike sorting was performed with manual techniques, which are laborious and unreliable due to inherent operator bias. As neuroscientists add multiple electrodes to their probes, the high-density devices can record hundreds to thousands of neurons simultaneously, making the manual spike sorting process increasingly difficult. The advent of automated spike sorting software has offered a compelling solution to this issue and, in this study, we present a simple-to-execute framework for running an automated spike sorter.
Bioelectron Med, 2021; 7
34488619: Mishra R, Bethunaickan R, Berthier CC, Yi Z, Strohl JJ, Huerta PT, Zhang W, Davidson A
Reversible dysregulation of renal circadian rhythm in lupus nephritis.
We have found disruption of expression of major transcriptional regulators of circadian rhythm in the kidneys of several mouse models of lupus nephritis. Here we define the consequence of this disturbance with respect to circadian gene expression and renal homeostatic function in a mouse model of lupus nephritis.
Mol Med, 2021; 27
31792184: Sankowski R, Strohl JJ, Huerta TS, Nasiri E, Mazzarello AN, D'Abramo C, Cheng KF, Staszewski O, Prinz M, Huerta PT, Al-Abed Y
Endogenous retroviruses are associated with hippocampus-based memory impairment.
Retrotransposons compose a staggering 40% of the mammalian genome. Among them, endogenous retroviruses (ERV) represent sequences that closely resemble the proviruses created from exogenous retroviral infection. ERVs make up 8 to 10% of human and mouse genomes and range from evolutionarily ancient sequences to recent acquisitions. Studies in have provided a causal link between genomic retroviral elements and cognitive decline; however, in mammals, the role of ERVs in learning and memory remains unclear. Here we studied 2 independent murine models for ERV activation: muMT strain (lacking B cells and antibody production) and intracerebroventricular injection of streptozotocin (ICVI-STZ). We conducted

behavioral assessments (contextual fear memory and spatial learning), as well as gene and protein analysis (RNA sequencing, PCR, immunohistochemistry, and western blot assays). Mice lacking mitochondrial antiviral-signaling protein (MAVS) and mice lacking stimulator of IFN genes protein (STING), 2 downstream sensors of ERV activation, provided confirmation of ERV impact. We found that muMT mice and ICVI-STZ mice induced hippocampal ERV activation, as shown by increased gene and protein expression of the Gag sequence of the transposable element intracisternal A-particle. ERV activation was accompanied by significant hippocampus-related memory impairment in both models. Notably, the deficiency of the MAVS pathway was protective against ICVI-STZ-induced cognitive pathology. Overall, our results demonstrate that ERV activation is associated with cognitive impairment in mice. Moreover, they provide a molecular target for strategies aimed at attenuating retroviral element sensing, via MAVS, to treat dementia and neuropsychiatric disorders.
Proc Natl Acad Sci U S A, 2019; 116

31231197: Sankowski R, Huerta TS, Kalra R, Klein TJ, Strohl JJ, Al-Abed Y, Robbiati S, Huerta PT
Large-Scale Validation of the Paddling Pool Task in the Clockmaze for Studying Hippocampus-Based Spatial Cognition in Mice.
Rationally designed behavioral tests are important tools to assess the function of specific brain regions. The hippocampus is a crucial neural substrate for spatial cognition, and many studies have linked hippocampal dysfunction with defects on spatial learning and memory in neurological conditions ranging from Alzheimer's disease to autoimmune syndromes, such as neuropsychiatric lupus. While our understanding of hippocampal function, from the molecular to the system levels, has increased dramatically over the last decades, this effort has not yet translated into efficacious therapies for cognitive impairment. We think that the availability of highly validated behavioral paradigms to measure cognition in mouse models is likely to enhance the potential success of preclinical therapeutic modalities. Here, we present an extensive study of the paddling pool task (PPT), first reported by Deacon and Rawlins, in which mice learn to escape from shallow water through a peripheral exit in a circular arena dubbed the clockmaze. We show that the PPT provides highly reliable results when assaying spatial cognition in C57/BL6 mice (120 males, 40 females) and BALB/c mice (40 males, 90 females). Additionally, we develop a robust algorithm for the assessment of escape strategies with clearly quantifiable readouts, enabling fine-granular phenotyping. Notably, the use of spatial strategy increases linearly across trials in the PPT. In a separate cohort of mice, we apply muscimol injections to silence the dorsal CA1 region of the hippocampus and show that the use of the spatial strategy in the PPT relies on the integrity of the dorsal hippocampus. Additionally, we compare directly the PPT and the Morris water maze (MWM) task in C57/BL6 mice (20 males, 20 females) and BALB/c mice (20 males, 20 females) and we find that the PPT induces significantly lower anxiety, exhaustion and hypothermia than the MWM. We conclude that the PPT provides a robust assessment of spatial cognition in mice, which can be applied in conjunction with other tests, to facilitate hypothesis testing and drug development to combat cognitive impairment.
Front Behav Neurosci, 2019; 13

27066908: Vingtdeux V, Chang EH, Frattini SA, Zhao H, Chandakkar P, Adrien L, Strohl JJ, Gibson EL, Ohmoto M, Matsumoto I, Huerta PT, Marambaud P
CALHM1 deficiency impairs cerebral neuron activity and memory flexibility in mice.
CALHM1 is a cell surface calcium channel expressed in cerebral neurons. CALHM1 function in the brain remains unknown, but recent results showed that neuronal CALHM1 controls intracellular calcium signaling and cell excitability, two mechanisms required for synaptic function. Here, we describe the generation of Calhm1 knockout (Calhm1(-/-)) mice and investigate CALHM1 role in neuronal and cognitive functions. Structural analysis revealed that Calhm1(-/-) brains had normal regional and cellular architecture, and showed no evidence of neuronal or synaptic loss, indicating that CALHM1 deficiency does not affect brain development or brain integrity in adulthood. However, Calhm1(-/-) mice showed a severe impairment in memory flexibility, assessed in the Morris water maze, and a significant disruption of long-term potentiation without alteration of long-term depression, measured in ex vivo hippocampal slices. Importantly, in primary neurons and hippocampal slices, CALHM1 activation facilitated the phosphorylation of NMDA and AMPA receptors by protein kinase A. Furthermore, neuronal CALHM1 activation potentiated the effect of glutamate on the expression of c-Fos and C/EBPβ, two immediate-early gene markers of neuronal activity. Thus, CALHM1 controls synaptic activity in cerebral neurons and is required for the flexible processing of memory in mice. These results shed light on CALHM1 physiology in the mammalian brain.
Sci Rep, 2016; 6

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BOARD NUMBER: S07-489

INHALED CANNABIS DELIVERY DURING PREGNANCY: EFFECTS ON FETAL BRAIN, ENDOCANNABINOID, AND IMMUNE SYSTEM DEVELOPMENT IN RATS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Cannabis use during pregnancy is increasingly common, as high as 10-20%. Evidence from clinical studies suggests that prenatal cannabis exposure (PCE) may lead to growth retardation and alterations in neurobehavioral trajectories. However, little is known about the mechanism through which Δ⁹-tetrahydrocannabinol (THC; main psychoactive component of cannabis) alters neurodevelopment. THC exerts its effects through acting on the endocannabinoid (eCB) system, which is involved in many processes of neurodevelopment. Further, the eCB system is highly interconnected to the immune system, which also has imperative functions during neurodevelopment. Thus, THC may exert its effects through direct modulation of the eCB system and/or indirect modulation of the immune system. Utilizing a validated vapor chamber system our animal model aims to perform translational work with pregnant rats exposed to cannabis via inhalation (10% THC; 15-min session) throughout pregnancy. In this experiment, dams and their fetuses were euthanized on gestational day 15, 17, or 19 to examine neurodevelopmental outcomes across various timepoints. Maternal and fetal samples were collected for examination of: 1) eCB levels via mass spectrometry, 2) cytokine (immune-signalling molecules) levels via multiplex assay; and 3) eCB and immune-related gene expression via qPCR. We have found no impact of PCE on fetal brain eCB levels or cannabinoid receptor gene expression. Analysis of eCB and immune-related gene expression and cytokine levels are ongoing. In conjunction with ongoing research, our results may help determine the mechanism through which PCE alters neurodevelopment and help determine the safety of cannabis consumption during pregnancy.

Pubmed:

34907248: Baglot SL, Hume C, Petrie GN, Aukema RJ, Lightfoot SHM, Grace LM, Zhou R, Parker L, Rho JM, Borgland SL, McLaughlin RJ, Brechenmacher L, Hill MN
Pharmacokinetics and central accumulation of delta-9-tetrahydrocannabinol (THC) and its bioactive metabolites are influenced by route of administration and sex in rats.
Up to a third of North Americans report using cannabis in the prior month, most commonly through inhalation. Animal models that reflect human consumption are critical to study the impact of cannabis on brain and behaviour. Most animal studies to date utilize injection of delta-9-tetrahydrocannabinol (THC; primary psychoactive component of cannabis). THC injections produce markedly different physiological and behavioural effects than inhalation, likely due to distinctive pharmacokinetics. The current study directly examined if administration route (injection versus inhalation) alters metabolism and central accumulation of THC and metabolites over time. Adult male and female Sprague-Dawley rats received either an intraperitoneal injection or a 15-min session of inhaled exposure to THC. Blood and brains were collected at 15, 30, 60, 90 and 240-min post-exposure for analysis of THC and metabolites. Despite achieving comparable peak blood THC concentrations in both groups, our results indicate higher initial brain THC concentration following inhalation, whereas injection resulted in dramatically higher 11-OH-THC concentration, a potent THC metabolite, in blood and brain that increased over time. Our results provide evidence of different pharmacokinetic profiles following inhalation versus injection. Accordingly, administration route should be considered during data interpretation, and translational animal work should strongly consider using inhalation models.
Sci Rep, 2021; 11
34882838: Baglot SL, VanRyzin JW, Marquardt AE, Aukema RJ, Petrie GN, Hume C, Reinl EL, Bieber JB, McLaughlin RJ, McCarthy MM, Hill MN
Maternal-fetal transmission of delta-9-tetrahydrocannabinol (THC) and its metabolites following inhalation and injection exposure during pregnancy in rats.

Cannabis use during pregnancy has increased over the past few decades, with recent data indicating that, in youth and young adults especially, up to 22% of people report using cannabis during pregnancy. Animal models provide the ability to study prenatal cannabis exposure (PCE) with control over timing and dosage; however, these studies utilize both injection and inhalation approaches. While it is known that Δ9-tetrahydrocannabinol (THC; primary psychoactive component of cannabis) can cross the placenta, examination of the transmission and concentration of THC and its metabolites from maternal blood into the placenta and fetal brain remains relatively unknown, and the influence of route of administration has never been examined. Pregnant female rats were exposed to either vaporized THC-dominant cannabis extract for pulmonary consumption or subcutaneous injection of THC repeatedly during the gestational period. Maternal blood, placenta, and fetal brains were collected following the final administration of THC for analysis of THC and its metabolites, as well as endocannabinoid concentrations, through mass spectrometry. Both routes of administration resulted in the transmission of THC and its metabolites in placenta and fetal brain. Repeated exposure to inhaled THC vapor resulted in fetal brain THC concentrations that were about 30% of those seen in maternal blood, whereas repeated injections resulted in roughly equivalent concentrations of THC in maternal blood and fetal brain. Neither inhalation nor injection of THC during pregnancy altered fetal brain endocannabinoid concentrations. Our data provide the first characterization of maternal-fetal transmission of THC and its metabolites following both vaporized delivery and injection routes of administration. These data are important to establish the maternal-fetal transmission in preclinical injection and inhalation models of PCE and may provide insight into predicting fetal exposure in human studies.
J Neurosci Res, 2022; 100

33845076: Roebuck AJ, Greba Q, Smolyakova AM, Alaverdashvili M, Marks WN, Garai S, Baglot SL, Petrie G, Cain SM, Snutch TP, Thakur GA, Hill MN, Howland JG, Laprairie RB
Positive allosteric modulation of type 1 cannabinoid receptors reduces spike-and-wave discharges in Genetic Absence Epilepsy Rats from Strasbourg.
Childhood Absence Epilepsy (CAE) accounts for approximately 10% of all pediatric epilepsies. Current treatments for CAE are ineffective in approximately 1/3 of patients and can be associated with severe side effects such as hepatotoxicity. Certain cannabinoids, such as cannabidiol (CBD), have shown promise in the treatment of pediatric epilepsies. However, CBD remains limited or prohibited in many jurisdictions, and has not been shown to have efficacy in CAE. Modulation of the type 1 cannabinoid receptor (CB1R) may provide more desirable pharmacological treatments. Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model many aspects of CAE, including cortical spike and wave discharges (SWDs). We have recently demonstrated that Δ-tetrahydrocannabinol (THC) increases SWDs in GAERS whereas CBD decreases these events. Here, we characterized aspects of the endocannabinoid system in brain areas relevant to seizures in GAERS and tested whether positive allosteric modulators (PAMs) of CB1R reduced SWDs. Both female and male GAERS had reduced (>50%) expression of CB1R and elevated levels of the endocannabinoid 2-AG in cortex compared to non-epileptic controls (NEC). We then administered the CB1R PAMs GAT211 and GAT229 to GAERS implanted with cortical electrodes. Systemic administration of GAT211 to male GAERS reduced SWDs by 40%. Systemic GAT229 administration reduced SWDs in female and male GAERS. Intracerebral infusion of GAT229 into the cortex of male GAERS reduced SWDs by >60% in a CB1R-dependent manner that was blocked by SR141716A. Together, these experiments identify altered endocannabinoid tone in GAERS and suggest that CB1R PAMs should be explored for treatment of absence seizures.
Neuropharmacology, 2021; 190

33344714: Glodosky NC, Cuttler C, Freels TG, Wright HR, Rojas MJ, Baglot SL, Hill MN, McLaughlin RJ
Cannabis vapor self-administration elicits sex- and dose-specific alterations in stress reactivity in rats.
Cannabis users frequently report stress relief as their primary reason for use. Recent studies indicate that human cannabis users exhibit blunted stress reactivity; however, it is unknown whether this is a cause or a consequence of chronic cannabis use.
Neurobiol Stress, 2020; 13

32739746: Markey L, Hooper A, Melon LC, Baglot S, Hill MN, Maguire J, Kumamoto CA
Colonization with the commensal fungus Candida albicans perturbs the gut-brain axis through dysregulation of endocannabinoid signaling.
Anxiety disorders are the most prevalent mental health disorder worldwide, with a lifetime prevalence of 5-7 % of the human population. Although the etiology of anxiety disorders is incompletely understood, one aspect of host health that affects anxiety disorders is the gut-brain axis. Adolescence is a key developmental window in which stress and anxiety disorders are a major health concern. We used adolescent female mice in a gastrointestinal (GI) colonization model to demonstrate that the commensal fungus Candida albicans affects host health via the gut-brain axis. In mice, bacterial members of the gut microbiota can influence the host gut-brain axis, affecting anxiety-like behavior and the hypothalamus-pituitary-adrenal (HPA) axis which produces the stress hormone corticosterone (CORT). Here we showed that mice colonized with C. albicans demonstrated increased anxiety-like behavior and increased basal production of CORT as well as dysregulation of CORT

production following acute stress. The HPA axis and anxiety-like behavior are negatively regulated by the endocannabinoid N-arachidonoylethanolamide (AEA). We demonstrated that C. albicans-colonized mice exhibited changes in the endocannabinoidome. Further, increasing AEA levels using the well-characterized fatty acid amide hydrolase (FAAH) inhibitor URB597 was sufficient to reverse both neuroendocrine phenotypes in C. albicans-colonized mice. Thus, a commensal fungus that is a common colonizer of humans had widespread effects on the physiology of its host. To our knowledge, this is the first report of microbial manipulation of the endocannabinoid (eCB) system that resulted in neuroendocrine changes contributing to anxiety-like behavior.
Psychoneuroendocrinology, 2020; 121
31953372: Freels TG, Baxter-Potter LN, Lugo JM, Glodosky NC, Wright HR, Baglot SL, Petrie GN, Yu Z, Clowers BH, Cuttler C, Fuchs RA, Hill MN, McLaughlin RJ
Vaporized Cannabis Extracts Have Reinforcing Properties and Support Conditioned Drug-Seeking Behavior in Rats.
Recent trends in cannabis legalization have increased the necessity to better understand the effects of cannabis use. Animal models involving traditional cannabinoid self-administration approaches have been notoriously difficult to establish and differences in the drug used and its route of administration have limited the translational value of preclinical studies. To address this challenge in the field, we have developed a novel method of cannabis self-administration using response-contingent delivery of vaporized Δ-tetrahydrocannabinol-rich (CAN) or cannabidiol-rich (CAN) whole-plant cannabis extracts. Male Sprague-Dawley rats were trained to nose-poke for discrete puffs of CAN, CAN, or vehicle (VEH) in daily 1 h sessions. Cannabis vapor reinforcement resulted in strong discrimination between active and inactive operanda. CAN maintained higher response rates under fixed ratio schedules and higher break points under progressive ratio schedules compared with CAN or VEH, and the number of vapor deliveries positively correlated with plasma THC concentrations. Moreover, metabolic phenotyping studies revealed alterations in locomotor activity, energy expenditure, and daily food intake that are consistent with effects in human cannabis users. Furthermore, both cannabis regimens produced ecologically relevant brain concentrations of THC and CBD and CAN administration decreased hippocampal CB1 receptor binding. Removal of CAN reinforcement (but not CAN) resulted in a robust extinction burst and an increase in cue-induced cannabis-seeking behavior relative to VEH. These data indicate that volitional exposure to THC-rich cannabis vapor has bona fide reinforcing properties and collectively support the utility of the vapor self-administration model for the preclinical assessment of volitional cannabis intake and cannabis-seeking behaviors. The evolving legal landscape concerning recreational cannabis use has increased urgency to better understand its effects on the brain and behavior. Animal models are advantageous in this respect; however, current approaches typically used forced injections of synthetic cannabinoids or isolated cannabis constituents that may not capture the complex effects of volitional cannabis consumption. We have developed a novel model of cannabis self-administration using response-contingent delivery of vaporized cannabis extracts containing high concentrations of Δ tetrahydrocannabinol (THC) or cannabidiol. Our data indicate that THC-rich cannabis vapor has reinforcing properties that support stable rates of responding and conditioned drug-seeking behavior. This approach will be valuable for interrogating effects of cannabis and delineating neural mechanisms that give rise to aberrant cannabis-seeking behavior.
J Neurosci, 2020; 40
30843198: Holman PJ, Baglot SL, Morgan E, Weinberg J
Effects of prenatal alcohol exposure on social competence: Asymmetry in play partner preference among heterogeneous triads of male and female rats.
Social behavior deficits associated with prenatal alcohol exposure (PAE) are frequently described in terms of impaired social competence, which can be defined as the effectiveness in social interaction and the ability to employ social skills successfully within different interpersonal contexts. Play behavior-which peaks during adolescence-is critical for developing social competence, as well as for motor, cognitive, and emotional development. Studies of play behavior typically utilize protocols where animals interact in dyads. However, less is understood about how the social environment may shape PAE-related social behavior deficits, particularly in more complex social contexts. Here, we assess play partner preference utilizing a novel approach in which adolescent male and female animals interact within same-sex triads comprised of animals from mixed prenatal treatments to determine how play partner identity and social group composition interact to shape behavior. When triads included one PAE animal and two control animals (i.e., control animals had the option to play either with a fellow control or a PAE playmate), we observed play target asymmetry whereby controls preferentially played with fellow controls. Notably, these results were consistent for triads of both males and females, with subtle differences in frequency of initiations versus reciprocations. We found no play target asymmetry, however, when triads included two PAE animals and one control animal or different configurations of control and pair-fed animals. Taken together, play target asymmetry resulting from ineffective social interactions, including a failure to engage with, respond to, and/or solicit play from control play partners appropriately, suggests that PAE negatively impacts the development of social competence.
Dev Psychobiol, 2019; 61
28698116: Raineki C, Bodnar TS, Holman PJ, Baglot SL, Lan N, Weinberg J

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Effects of early-life adversity on immune function are mediated by prenatal environment: Role of prenatal alcohol exposure. The contribution of the early postnatal environment to the pervasive effects of prenatal alcohol exposure (PAE) is poorly understood. Moreover, PAE often carries increased risk of exposure to adversity/stress during early life. Dysregulation of immune function may play a role in how pre- and/or postnatal adversity/stress alters brain development. Here, we combine two animal models to examine whether PAE differentially increases vulnerability to immune dysregulation in response to early-life adversity. PAE and control litters were exposed to either limited bedding (postnatal day [PN] 8-12) to model early-life adversity or normal bedding, and maternal behavior and pup vocalizations were recorded. Peripheral (serum) and central (amygdala) immune (cytokines and C-reactive protein - CRP) responses of PAE animals to early-life adversity were evaluated at PN12. Insufficient bedding increased negative maternal behavior in both groups. Early-life adversity increased vocalization in all animals; however, PAE pups vocalized less than controls. Early-life adversity reduced serum TNF-α, KC/GRO, and IL-10 levels in control but not PAE animals. PAE increased serum CRP, and levels were even higher in pups exposed to adversity. Finally, PAE reduced KC/GRO and increased IL-10 levels in the amygdala. Our results indicate that PAE alters immune system development and both behavioral and immune responses to early-life adversity, which could have subsequent consequences for brain development and later life health.
Brain Behav Immun, 2017; 66

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BOARD NUMBER: S07-490

GUT MICROBIOME DEPLETION LEADS TO ALTERED NEURAL DYNAMICS AND METABOLISM IN THE DORSAL CA1 FIELD OF THE HIPPOCAMPUS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Growing evidence implicates the gut microbiome in brain health and disease. Since worldwide consumption of oral antibiotics continues to rise, it is imperative to understand how antibiotics affect cognition through the gut–brain axis. Male C57BL/6J mice (2–4 months) were given drinking water containing broad–spectrum antibiotics (ABX cohort; ampicillin, neomycin, gentamicin, metronidazole, and vancomycin). A separate ABXBut cohort received both antibiotics and butyrate, a key microbiome-derived metabolite. Four weeks later, mice underwent microPET studies using [^{18}F]-fluorodeoxyglucose (FDG) to assess brain metabolism. Hippocampus–related spatial cognition was evaluated with the object place memory (OPM) and clockmaze tasks (PMC6568215), at 2–3 weeks of treatment. Finally, mice were implanted with tetrode arrays to record CA1 place cells (PCs) in the dorsal hippocampus. Our results demonstrate that, compared to controls, ABX mice have reduced FDG uptake in entorhinal cortex, amygdala, and CA1. Moreover, ABX mice show significant spatial impairments in the OPM and clockmaze tasks. Analysis of PC dynamics during OPM reveals that ABX–PCs have larger field size, lower spatial information, as well as altered remapping and path reconstruction properties. Remarkably, ABXBut mice display completely normal spatial cognition and place cell dynamics. We conclude that depletion of the gut microbiome with antibiotics leads to disruptions within the hippocampal formation, which are reflected in weaker metabolism, disrupted spatial cognition and aberrant place cell properties. Coadministration of butyrate preserves spatial cognition and hippocampal dynamics in microbiome–depleted mice, pointing to potential therapeutic approaches.

Pubmed:
34809706: Strohl JJ, Gallagher JT, Gómez PN, Glynn JM, Huerta PT
Framework for automated sorting of neural spikes from Neuralynx-acquired tetrode recordings in freely-moving mice. Extracellular recording represents a crucial electrophysiological technique in neuroscience for studying the activity of single neurons and neuronal populations. The electrodes capture voltage traces that, with the help of analytical tools, reveal action potentials ('spikes') as well as local field potentials. The process of spike sorting is used for the extraction of action potentials generated by individual neurons. Until recently, spike sorting was performed with manual techniques, which are laborious and unreliable due to inherent operator bias. As neuroscientists add multiple electrodes to their probes, the high-density devices can record hundreds to thousands of neurons simultaneously, making the manual spike sorting process increasingly difficult. The advent of automated spike sorting software has offered a compelling solution to this issue and, in this study, we present a simple-to-execute framework for running an automated spike sorter.
Bioelectron Med, 2021; 7

FENS Forum 2022
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BOARD NUMBER: S07-491

FUNCTIONAL ROLE OF THE CGAS-STING PATHWAY IN THE HOMEOSTASIS OF NEURONS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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The cGAS-STING pathway has a pivotal role in innate immune responses and represents the first-line natural barrier of the host for the elimination of pathogenic infections. In recent years, there has been an increasing interest in the cGAS-STING pathway not only regarding viral defense but also in terms of cellular senescence and autophagy. Moreover, recently it could be shown that cGAS and STING are present in the central nervous system as well, especially in microglia. To date there is ample evidence for a functional role of the cGAS-STING pathway in microglia but comparable less about its role in other CNS cells such as neurons and astrocytes. Based on these observations we analyzed the differences between neurons from wild type (WT) and STING$^{-/-}$ mice. Our data *in vitro* yield clear differences in neurons of STING$^{-/-}$ mice in comparison to WT animals, including altered dendritic arborization and reduced number of synapses. Another interesting finding is the clear impairment of the autophagic flux of neurons both *in vitro* as well as *in vivo,* with a reduction of p62 and reduced amounts of LC3II proteins. In conclusion, our data demonstrate that the cGAS-STING pathway has an important role not only in microglia but also in the remaining cells of the CNS. Especially in neurons, the absence of the cGAS-STING pathway may cause dysfunctions in physiological processes.

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SILENCING OF AMYGDALA CIRCUITS DURING SEPSIS PREVENTS THE DEVELOPMENT OF ANXIETY-RELATED BEHAVIOURS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Sepsis is a life-threatening condition induced by a deregulated host response to severe infection. Post-sepsis syndrome includes long-term psychiatric disorders, such as persistent anxiety and post-traumatic stress disorder, whose neurobiological mechanisms remain unknown. Using a reference mouse model of sepsis, we showed that mice that recovered from sepsis further developed anxiety-related behaviours associated with an exaggerated fear memory. In the brain, sepsis induced an acute pathological activation of a specific neuronal population of the central nucleus of the amygdala, which projects to the ventral bed nucleus of the stria terminalis. Using viral-genetic circuit tracing and in vivo calcium imaging, we observed that sepsis induced persistent changes in the connectivity matrix and in the responsiveness of the ventral bed nucleus of the stria terminalis-projecting central nucleus of the amygdala neurons. The transient and targeted silencing of this subpopulation only during the acute phase of sepsis with a viral pharmacogenetic approach, or with the antiepileptic and neuroprotective drug levetiracetam, prevented the subsequent development of anxiety-related behaviours. Specific inhibition of brain anxiety and fear circuits during the sepsis acute phase constitutes a preventive approach to preclude the post-infection psychiatric outcomes.

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PSYCHOSTIMULANT-INDUCED NEUROINFLAMMATION: IS THERE A PROTECTIVE ROLE FOR IL-10?

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Aims
Exposure to psychostimulants has been associated with increased glial activity, which seems to be implicated in the neuronal changes that underlie addiction. Anti-inflammatory cytokines seem to counterbalance damage driven by excessive glial activation. Here, we tested the hypothesis that increased levels of interleukin(IL)-10 could be protective in substance abuse disorders.
Methods
We took advantage of a transgenic mouse model with controlled overexpression of IL-10 to investigate mechanisms of protection elicited by IL-10 in the context of acute exposure to methamphetamine(Meth). Since IL-10 may modulate gammadelta(γδ) T cells, which were described to be involved in regulating behaviours that are important for drug use, we also use a transgenic mouse model with controlled overexpression of IL-10 but KO for γδ T cells and test them in the elevated plus maze(EPM), where we have previously show that binge Meth promotes loss of risk assessment.
Results
Our results successfully show that IL-10 overexpression prevents Meth-induced behaviour in the EPM following a binge administration. IL-10 also seems to be protective in Meth-induced microglia reactivity, preventing microglia proliferation, altered morphology and increased phagocytic activity, hallmarks of microglia reactivity. Importantly, this protective effect seems to be dependent on γδ T cells.
Conclusions
These results confirm the relevance of modulating anti-inflammatory cytokines in the context of drug use, and allow us to predict that IL-10 may to be an innovative treatment strategy for substance abuse disorders. To better unravel IL-10 potential, we are now exploring in primary glial cultures the mechanisms involved in IL-10 action under Meth exposure.

Pubmed:
34400780: Canedo T, Portugal CC, Socodato R, Almeida TO, Terceiro AF, Bravo J, Silva AI, Magalhães JD, Guerra-Gomes S, Oliveira JF, Sousa N, Magalhães A, Relvas JB, Summavielle T
Astrocyte-derived TNF and glutamate critically modulate microglia activation by methamphetamine.
Methamphetamine (Meth) is a powerful illicit psychostimulant, widely used for recreational purposes. Besides disrupting the monoaminergic system and promoting oxidative brain damage, Meth also causes neuroinflammation, contributing to synaptic dysfunction and behavioral deficits. Aberrant activation of microglia, the largest myeloid cell population in the brain, is a common feature in neurological disorders triggered by neuroinflammation. In this study, we investigated the mechanisms underlying the aberrant activation of microglia elicited by Meth in the adult mouse brain. We found that binge Meth exposure caused microgliosis and disrupted risk assessment behavior (a feature that usually occurs in individuals who abuse Meth), both of which required astrocyte-to-microglia crosstalk. Mechanistically, Meth triggered a detrimental increase of glutamate exocytosis from astrocytes (in a process dependent on TNF production and calcium mobilization), promoting microglial expansion and reactivity. Ablating TNF production, or suppressing astrocytic calcium mobilization, prevented Meth-elicited microglia reactivity and re-established risk assessment behavior as tested by elevated plus maze (EPM). Overall, our data indicate that glial crosstalk is critical to relay alterations caused by acute Meth exposure.
Neuropsychopharmacology, 2021; 46


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PHYSICAL ACTIVITY PROTECTS FROM HYPOTHALAMIC NEUROINFLAMMATION IN A CHRONIC FOOD RESTRICTION MOUSE MODEL

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Aims. Anorexia nervosa is a complex and multifactorial psychiatric disorder where immunological and metabolic alterations contribute to the worsening of the disease. Excessive physical activity is often associated with this disorder. Using a mouse model of chronic food restriction, we sought to determine whether the metabolic status (fed or fasted) impacts the hypothalamic mRNA expression of neuropeptides involved in the regulation of food intake and of pro-inflammatory cytokines and chemokines. **Methods**. For two weeks, we subjected mice to either 1) chronic food restriction (FR), 2) FR combined with voluntary activity (free access to a running wheel, FRW), 3) *ad libitum* feeding (AL), or 4) AL with voluntary activity (ALW). Then, we assessed the hypothalamic gene expression of cytokines (IL-1ß, IL-6, TNF-α), chemokines (RANTES, MCP1) and neuropeptides (POMC, NPY, AgRP, MCH and orexin) in the different conditions. **Results**. In the fed condition, no significant changes were found regarding cytokines or chemokines. Regarding the food restricted groups, POMC mRNA expression was significantly decreased in FRW mice, while AgRP and NPY levels were increased both in FR and FRW mice. Interestingly, only in the FR group we observed significant increases in the gene expression of all proinflammatory cytokines and MCP-1. Similarly, increases in MCH and ORX levels only occurred in the FR group, while AgRP and NPY levels increased in similarly in both FR and FRW groups. **Conclusion**. Our data highlighted that physical activity may, but only in a condition of food restriction, protect the hypothalamus from neuroinflammation.

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EXPLORING ANTI-COVID-19 VACCINATION EFFECTS ON SICKNESS RESPONSES AND A POSSIBLE ASSOCIATION BETWEEN INTEROCEPTION AND SICKNESS BEHAVIOR

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Aims: the reactogenicity of the anti-COVID-19 vaccination seems partially overlapped to Sickness Behavior (SB); both conditions appear to be related to the activation of cytokines which, acting on specific brain hubs involved in the interoceptive processes, give rise to behavioral changes. The aim of this research was to identify the presence of SB after vaccination and its possible triggering variables. Methods: A cross-sectional online survey was conducted on 647 participants who received anti-COVID-19 vaccination. Demographics, health status, mood, sleep, and interoceptive awareness were explored through psychometric questionnaires. To investigate the independent variables potentially correlated with high levels of SB after vaccination, we divided the sample in two groups: participants with low (L-SB; n=252) and high (H-SB, n=227) levels of SB. The statistical analysis was carried out using supervised machine learning (ML) techniques using k-fold cross validation. Results: the baseline variables exhibiting optimal efficiency in classifying the participant within the L-SB and H-SB groups have been identified with an accuracy of 72.65%. The analysis revealed that specific variables – interoceptive awareness, levels of depression and anxiety, and sleep quality –were the best predictors in discriminating H-SB from L-SB groups with an accuracy higher than 75% for all classifiers (Table 1). Conclusions: anti-COVID-19 vaccination could generate core symptoms of SB involving mood and sleep. Moreover, the data suggest that interoceptive awareness could be considered a

neuro-immunological bridge able to convey the psychophysiological response to the vaccine and to modulate the intensity of

Table 1. The correct classification achieved by the different classifiers measured by accuracy, AUC, F1.

Classifier	Accuracy (%)	AUC	F1	Correct classification
Naïve Bayes	75.16	0.817 (d=1.278)	0.75	L-SB: 222/252; H-SB: 138/227
Logistic Regression	75.99	0.794 (d=1.160)	0.76	L-SB: 197/252; H-SB: 167/227
Simple Logistic	**78.08**	0.819 (d=1.289)	0.78	L-SB:199/252; H-SB: 175/227
Support Vector Machine	77.04	0.706 (d=0.766)	0.77	L-SB: 195/252; H-SB: 174/227
Random Forest	77.24	0.822 (d=1.305)	0.77	L-SB:197/252; H-SB: 173/227

SB.

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LUPUS-ASSOCIATED COGNITIVE IMPAIRMENT LINKED TO SYSTEMS–LEVEL DYSFUNCTIONS IN THETA–GAMMA COUPLING AND PLACE CELL DYNAMICS IN THE CA1 FIELD OF THE HIPPOCAMPUS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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The pathological changes leading to cognitive impairment in patients with systemic lupus erythematosus (SLE) are poorly understood. We study a subset of DNA–binding antibodies, termed DNRAbs, that also bind the NMDA receptor and contribute to neuropsychiatric SLE (NPSLE). We have developed a lupus model in which C57BL6H2d$^{+/+}$ mice express high DNRAb titer (PMC6170183, PMC7075964). To elucidate the neural substrate of NPSLE (how cognitive impairment emerges at the ensemble level), we performed tetrode recordings of local field potentials (LFP) and place cells (PC) in CA1 during the open field (OF) and object–place memory (OPM) tasks. DNRAb mice have impaired spatial memory (poor exploration of moved object in OPM). Notably, DNRAb mice exhibit abnormally high LFP power (theta–beta–gamma bands) during OF and OPM, which contrasts sharply with the decremental LFP power in controls. Autocorrelation power matrix and wavelet power analyses reveal that DNRAb mice have weaker theta–gamma coupling and reduced phase–amplitude modulation index. Analysis of PC dynamics during OPM reveals that DNRAb–PCs display larger place field size, lower spatial information, diminished remapping capabilities, and higher error in path reconstruction. Finally, tSNE analysis across behavioral, LFP and PC data demonstrates a robust disease phenotype in DNRAb mice. We conclude that NPSLE's cognitive impairment is associated with neural pathologies including abnormally high LFP patterns with reduced coupling dynamics, and disrupted PC dynamics with low quality place fields. Thus, systems–level analyses of neuroimmune pathophysiology might uncover novel biomarkers for previously elusive autoimmune diseases, such as NPSLE.

Pubmed:

26467973: Huerta PT, Gibson EL, Rey C, Huerta TS
Integrative neuroscience approach to neuropsychiatric lupus.
We present a succinct review of our approach to study the interactions between the DNA-reactive antibodies that cross-react with the GluN2A and GluN2B subunits of the N-methyl-D-aspartate receptor, denoted DNRABs, and their brain targets in subjects with neuropsychiatric systemic lupus erythematosus (NPSLE). We have analyzed the DNRAB-based brain symptomatology in mouse models of NPSLE by using an integrative neuroscience approach, which includes behavioral assessment coupled with electrophysiological studies of neural networks and synaptic connections in target brain regions, such as the CA1 region of the hippocampus. Our results suggest a framework for understanding the interactions between immune factors and neural networks.
Immunol Res, 2015; 63
33658532: Huerta TS, Devarajan A, Tsaava T, Rishi A, Cotero V, Puleo C, Ashe J, Coleman TR, Chang EH, Tracey KJ, Chavan SS
Targeted peripheral focused ultrasound stimulation attenuates obesity-induced metabolic and inflammatory dysfunctions.
Obesity, a growing health concern, is associated with an increased risk of morbidity and mortality. Chronic low-grade inflammation is implicated in obesity-driven metabolic complications. Peripheral focused ultrasound stimulation (pFUS) is an emerging non-invasive technology that modulates inflammation. Here, we reasoned that focused ultrasound stimulation of the liver may alleviate obesity-related inflammation and other comorbidities. After 8 weeks on a high-fat high-carbohydrate "Western" diet, C57BL/6J mice were subjected to either sham stimulation or focused ultrasound stimulation at the porta hepatis. Daily liver-focused ultrasound stimulation for 8 weeks significantly decreased body weight, circulating lipids and mitigated dysregulation of adipokines. In addition, liver-focused ultrasound stimulation significantly reduced hepatic cytokine levels and leukocyte infiltration. Our findings demonstrate the efficacy of hepatic focused ultrasound for alleviating obesity and obesity-associated complications in mice. These findings suggest a previously unrecognized potential of hepatic focused ultrasound as a possible novel noninvasive approach in the context of obesity.
Sci Rep, 2021; 11
32179753: Chan K, Nestor J, Huerta TS, Certain N, Moody G, Kowal C, Huerta PT, Volpe BT, Diamond B, Wollmuth LP

Lupus autoantibodies act as positive allosteric modulators at GluN2A-containing NMDA receptors and impair spatial memory.
Patients with Systemic lupus erythematosus (SLE) experience various peripheral and central nervous system manifestations including spatial memory impairment. A subset of autoantibodies (DNRAbs) cross-react with the GluN2A and GluN2B subunits of the NMDA receptor (NMDAR). We find that these DNRAbs act as positive allosteric modulators on NMDARs with GluN2A-containing NMDARs, even those containing a single GluN2A subunit, exhibiting a much greater sensitivity to DNRAbs than those with exclusively GluN2B. Accordingly, GluN2A-specific antagonists provide greater protection from DNRAb-mediated neuronal cell death than GluN2B antagonists. Using transgenic mice to perturb expression of either GluN2A or GluN2B in vivo, we find that DNRAb-mediated disruption of spatial memory characterized by early neuronal cell death and subsequent microglia-dependent pathologies requires GluN2A-containing NMDARs. Our results indicate that GluN2A-specific antagonists or negative allosteric modulators are strong candidates to treat SLE patients with nervous system dysfunction.
Nat Commun, 2020; 11
31792184: Sankowski R, Strohl JJ, Huerta TS, Nasiri E, Mazzarello AN, D'Abramo C, Cheng KF, Staszewski O, Prinz M, Huerta PT, Al-Abed Y
Endogenous retroviruses are associated with hippocampus-based memory impairment.
Retrotransposons compose a staggering 40% of the mammalian genome. Among them, endogenous retroviruses (ERV) represent sequences that closely resemble the proviruses created from exogenous retroviral infection. ERVs make up 8 to 10% of human and mouse genomes and range from evolutionarily ancient sequences to recent acquisitions. Studies in have provided a causal link between genomic retroviral elements and cognitive decline; however, in mammals, the role of ERVs in learning and memory remains unclear. Here we studied 2 independent murine models for ERV activation: muMT strain (lacking B cells and antibody production) and intracerebroventricular injection of streptozotocin (ICVI-STZ). We conducted behavioral assessments (contextual fear memory and spatial learning), as well as gene and protein analysis (RNA sequencing, PCR, immunohistochemistry, and western blot assays). Mice lacking mitochondrial antiviral-signaling protein (MAVS) and mice lacking stimulator of IFN genes protein (STING), 2 downstream sensors of ERV activation, provided confirmation of ERV impact. We found that muMT mice and ICVI-STZ mice induced hippocampal ERV activation, as shown by increased gene and protein expression of the Gag sequence of the transposable element intracisternal A-particle. ERV activation was accompanied by significant hippocampus-related memory impairment in both models. Notably, the deficiency of the MAVS pathway was protective against ICVI-STZ-induced cognitive pathology. Overall, our results demonstrate that ERV activation is associated with cognitive impairment in mice. Moreover, they provide a molecular target for strategies aimed at attenuating retroviral element sensing, via MAVS, to treat dementia and neuropsychiatric disorders.
Proc Natl Acad Sci U S A, 2019; 116
31231197: Sankowski R, Huerta TS, Kalra R, Klein TJ, Strohl JJ, Al-Abed Y, Robbiati S, Huerta PT
Large-Scale Validation of the Paddling Pool Task in the Clockmaze for Studying Hippocampus-Based Spatial Cognition in Mice.
Rationally designed behavioral tests are important tools to assess the function of specific brain regions. The hippocampus is a crucial neural substrate for spatial cognition, and many studies have linked hippocampal dysfunction with defects on spatial learning and memory in neurological conditions ranging from Alzheimer's disease to autoimmune syndromes, such as neuropsychiatric lupus. While our understanding of hippocampal function, from the molecular to the system levels, has increased dramatically over the last decades, this effort has not yet translated into efficacious therapies for cognitive impairment. We think that the availability of highly validated behavioral paradigms to measure cognition in mouse models is likely to enhance the potential success of preclinical therapeutic modalities. Here, we present an extensive study of the paddling pool task (PPT), first reported by Deacon and Rawlins, in which mice learn to escape from shallow water through a peripheral exit in a circular arena dubbed the clockmaze. We show that the PPT provides highly reliable results when assaying spatial cognition in C57/BL6 mice (120 males, 40 females) and BALB/c mice (40 males, 90 females). Additionally, we develop a robust algorithm for the assessment of escape strategies with clearly quantifiable readouts, enabling fine-granular phenotyping. Notably, the use of spatial strategy increases linearly across trials in the PPT. In a separate cohort of mice, we apply muscimol injections to silence the dorsal CA1 region of the hippocampus and show that the use of the spatial strategy in the PPT relies on the integrity of the dorsal hippocampus. Additionally, we compare directly the PPT and the Morris water maze (MWM) task in C57/BL6 mice (20 males, 20 females) and BALB/c mice (20 males, 20 females) and we find that the PPT induces significantly lower anxiety, exhaustion and hypothermia than the MWM. We conclude that the PPT provides a robust assessment of spatial cognition in mice, which can be applied in conjunction with other tests, to facilitate hypothesis testing and drug development to combat cognitive impairment.
Front Behav Neurosci, 2019; 13
30185634: Nestor J, Arinuma Y, Huerta TS, Kowal C, Nasiri E, Kello N, Fujieda Y, Bialas A, Hammond T, Sriram U, Stevens B, Huerta PT, Volpe BT, Diamond B

Lupus antibodies induce behavioral changes mediated by microglia and blocked by ACE inhibitors.
Cognitive impairment occurs in 40-90% of patients with systemic lupus erythematosus (SLE), which is characterized by autoantibodies to nuclear antigens, especially DNA. We discovered that a subset of anti-DNA antibodies, termed DNRAbs, cross reacts with the N-methyl-d-aspartate receptor (NMDAR) and enhances NMDAR signaling. In patients, DNRAb presence associates with spatial memory impairment. In a mouse model, DNRAb-mediated brain pathology proceeds through an acute phase of excitotoxic neuron loss, followed by persistent alteration in neuronal integrity and spatial memory impairment. The latter pathology becomes evident only after DNRAbs are no longer detectable in the brain. Here we investigate the mechanism of long-term neuronal dysfunction mediated by transient exposure to antibody. We show that activated microglia and C1q are critical mediators of neuronal damage. We further show that centrally acting inhibitors of angiotensin-converting enzyme (ACE) can prevent microglial activation and preserve neuronal function and cognitive performance. Thus, ACE inhibition represents a strong candidate for clinical trials aimed at mitigating cognitive dysfunction.
J Exp Med, 2018; 215
26286205: Chang EH, Volpe BT, Mackay M, Aranow C, Watson P, Kowal C, Storbeck J, Mattis P, Berlin R, Chen H, Mader S, Huerta TS, Huerta PT, Diamond B
Selective Impairment of Spatial Cognition Caused by Autoantibodies to the N-Methyl-D-Aspartate Receptor.
Patients with systemic lupus erythematosus (SLE) experience cognitive abnormalities in multiple domains including processing speed, executive function, and memory. Here we show that SLE patients carrying antibodies that bind DNA and the GluN2A and GluN2B subunits of the N-methyl-d-aspartate receptor (NMDAR), termed DNRAbs, displayed a selective impairment in spatial recall. Neural recordings in a mouse model of SLE, in which circulating DNRAbs penetrate the hippocampus, revealed that CA1 place cells exhibited a significant expansion in place field size. Structural analysis showed that hippocampal pyramidal cells had substantial reductions in their dendritic processes and spines. Strikingly, these abnormalities became evident at a time when DNRAbs were no longer detectable in the hippocampus. These results suggest that antibody-mediated neurocognitive impairments may be highly specific, and that spatial cognition may be particularly vulnerable to DNRAb-mediated structural and functional injury to hippocampal cells that evolves after the triggering insult is no longer present.
EBioMedicine, 2015; 2
24381547: Faust TW, Robbiati S, Huerta TS, Huerta PT
Dynamic NMDAR-mediated properties of place cells during the object place memory task.
N-methyl-D-aspartate receptors (NMDAR) in the hippocampus participate in encoding and recalling the location of objects in the environment, but the ensemble mechanisms by which NMDARs mediate these processes have not been completely elucidated. To address this issue, we examined the firing patterns of place cells in the dorsal CA1 area of the hippocampus of mice (n = 7) that performed an object place memory (OPM) task, consisting of familiarization (T1), sample (T2), and choice (T3) trials, after systemic injection of 3-[(±)2-carboxypiperazin-4yl]propyl-1-phosphate (CPP), a specific NMDAR antagonist. Place cell properties under CPP (CPP-PCs) were compared to those after control saline injection (SAL-PCs) in the same mice. We analyzed place cells across the OPM task to determine whether they signaled the introduction or movement of objects by NMDAR-mediated changes of their spatial coding. On T2, when two objects were first introduced to a familiar chamber, CPP-PCs and SAL-PCs showed stable, vanishing or moving place fields in addition to changes in spatial information (SI). These metrics were comparable between groups. Remarkably, previously inactive CPP-PCs (with place fields emerging de novo on T2) had significantly weaker SI increases than SAL-PCs. On T3, when one object was moved, CPP-PCs showed reduced center-of-mass (COM) shift of their place fields. Indeed, a subset of SAL-PCs with large COM shifts (>7 cm) was largely absent in the CPP condition. Notably, for SAL-PCs that exhibited COM shifts, those initially close to the moving object followed the trajectory of the object, whereas those far from the object did the opposite. Our results strongly suggest that the SI changes and COM shifts of place fields that occur during the OPM task reflect key dynamic properties that are mediated by NMDARs and might be responsible for binding object identity with location.
Front Behav Neurosci, 2013; 7

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EVALUATION OF LACTATE DYNAMICS IN YOUNG ADULT C57BL/6J MALE MICE

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Physical inactivity is one of the top five leading causes of death worldwide and is associated with life-limiting diseases, including dementia and other brain disorders. Exercise is suggested to protect brain health, potentially via the action of muscle-derived factors, such as lactate, on brain tissue. Lactate concentrations are known to increase post-exercise, and peripheral lactate has been shown to cross the blood-brain barrier via monocarboxylate transporters (MCT), where it can act as a fuel or signalling molecule. Exercise-derived lactate has been associated with multiple roles within the central nervous system, potentially modulating cognitive function. The focus of this study was to investigate central and peripheral lactate dynamics after low to moderate-intensity aerobic exercise in young adult male mice and the effects of pharmacological administration of an MCT1/2 inhibitor in combination with exercise on lactate concentrations as well as lactate-mediated signalling and gene expression in the hippocampus. Gene expression analysis indicates that metabolic genes and microglia polarization markers are not affected under homeostatic conditions. Moreover, no changes in lactate concentrations were observed in the hippocampus of young adult male C57BL/6J mice at the time scale used in our study, suggesting that lactate dynamics may be dependent on exercise duration and intensity. Interestingly, mice treated with the MCT1/2 inhibitor exhibited a slight decrease in the relative lactate concentrations, indicating that the efficacy of the inhibitor may be lactate-dependent. Finally, it remains to be investigated if muscle-derived lactate contributes to heightened cognitive resilience and protection conferred by exercise in response to immune challenges.

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INVESTIGATING THE IMPACT OF SHORT-TERM, MODERATE INTENSITY EXERCISE ON MICROGLIA IMMUNOMETABOLIC REPROGRAMMING FOLLOWING SYSTEMIC LPS CHALLENGE

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Microglia are tissue resident macrophages of the central nervous system (CNS) with varied functions in both steady-state and disease-related conditions which are critical for neuronal activity and cognitive function. Accumulating evidence indicates that, similar to peripheral macrophages, microglia functional states are underpinned by cellular metabolism. Neuroinflammation and microglia activation have been characterized by a shift in metabolic pathways towards aerobic glycolysis, which critically regulates microglia immune functions such as cytokine release and phagocytosis. Nonetheless, homeostatic microglia states are also influenced by changes in CNS microenvironment, which may modulate microglia responses to danger signals, such as pathogens. Regular aerobic exercise is long considered to have a considerable impact on brain health, with recent evidence indicating its potential to modulate microglia states and functions, mediated by muscle-derived factors that can cross the blood-brain barrier. Here, we investigate the impact of 7 days of moderate intensity treadmill exercise on microglia metabolic phenotype and function in 3-4-month-old C57/BL6J mice, by assessing the bioenergetic phenotype of primary microglia in exercising and sedentary mice. Furthermore, microglia metabolic adaptations and immune functions are elucidated in exercising vs sedentary mice upon a peripheral immune challenge via subseptic dose of lipopolysaccharide (LPS). Our evidence indicates that prior exercise can prevent the bioenergetic and inflammatory changes induced by LPS, suggesting that physical activity can impact on glial and neuronal function in a neuroprotective manner. This provides a potential mechanism underlying the long-term effects of regular physical activity in developing resilience of brain tissue to injury and inflammatory insult.

Pubmed:

31778225: Hicks AI, Barad Z, Sobrero A, Lean G, Jacob-Tomas S, Yang J, Choe KY, Prager-Khoutorsky M
Effects of salt loading on the organisation of microtubules in rat magnocellular vasopressin neurones.
Magnocellular vasopressin (VP) neurones are activated by increases in blood osmolality, leading to the secretion of VP into the circulation to promote water retention in the kidney, thus constituting a key mechanism for the regulation of body fluid homeostasis. However, chronic high salt intake can lead to excessive activation of VP neurones and increased circulating levels of VP, contributing to an elevation in blood pressure. Multiple extrinsic factors, such as synaptic inputs and glial cells, modulate the activity of VP neurones. Moreover, magnocellular neurones are intrinsically osmosensitive, and are activated by hypertonicity in the absence of neighbouring cells or synaptic contacts. Hypertonicity triggers cell shrinking, leading to the activation of VP neurones. This cell-autonomous activation is mediated by a scaffold of dense somatic microtubules, uniquely present in VP magnocellular neurones. Treating isolated magnocellular neurones with drugs modulating microtubule stability modifies the sensitivity of neuronal activation in response to acute hypertonic stimuli. However, whether the microtubule network is altered in conditions associated with enhanced neuronal activation and increased VP release, such as chronic high salt intake, remains unknown. We examined the organisation of microtubules in VP neurones of the supraoptic and paraventricular hypothalamic nuclei (SON and PVN, respectively) of rats subjected to salt-loading (drinking 2% NaCl for 7 days). Using super-resolution imaging, we found that the density of microtubules in magnocellular VP neurones from the SON and PVN was significantly increased, whereas the density and organisation of microtubules remain unchanged in other hypothalamic neurones, as well as in neurones from other brain areas (e.g., hippocampus, cortex). We propose that the increase in microtubule density in magnocellular VP neurones in salt-loading promotes their enhanced activation, possibly contributing to elevated blood pressure in this condition.
J Neuroendocrinol, 2020; 32
32725828: Barad Z, Khant Aung Z, Grattan DR, Ladyman SR, Brown RSE
Impaired prolactin transport into the brain and functional responses to prolactin in aged male mice.
Ageing is related to changes in a number of endocrine systems that impact on the central actions of hormones. The anterior pituitary hormone prolactin is present in the circulation in both males and females, with widespread expression of the prolactin

receptor throughout the forebrain. We aimed to investigate prolactin transport into the brain, as well as circulating levels of prolactin and functional responses to prolactin, in aged male mice (23 months). Transport of I-labelled prolactin (I-prolactin) from the peripheral circulation into the brain was suppressed in aged compared to young adult (4 months) male mice, with no significant transport into the brain occurring in aged males. We subsequently investigated changes in the negative-feedback regulation of prolactin secretion and prolactin-induced suppression of luteinising hormone (LH) pulsatile secretion in aged male mice. Feedback regulation of prolactin secretion appeared to be unaffected in aged males, with no change in levels of circulating prolactin, and normal prolactin-induced phosphorylated signal transducer and activator of transcription 5(pSTAT5) immunoreactivity in tuberoinfundibular dopaminergic (TIDA) neurones in the arcuate nucleus. There were, however, significant impairments in the ability of prolactin to suppress LH pulsatile secretion in aged males. In young adult males, acute prolactin administration significantly decreased LH pulses from 1.5 ± 0.19 pulses of LH in 4 hours to 0.5 ± 0.27 pulses. In contrast, prolactin did not suppress LH pulse frequency in aged males, with prolactin leading to an increase in mean LH concentration. These data demonstrate the emergence of impairments in prolactin transport into the brain and deficits in specific functional responses to prolactin with ageing.
J Neuroendocrinol, 2020; 32

32209611: Barad Z, Jacob-Tomas S, Sobrero A, Lean G, Hicks AI, Yang J, Choe KY, Prager-Khoutorsky M
Unique Organization of Actin Cytoskeleton in Magnocellular Vasopressin Neurons in Normal Conditions and in Response to Salt-Loading.
Magnocellular neurosecretory cells (MNCs) are intrinsically osmosensitive and can be activated by increases in blood osmolality, triggering the release of antidiuretic hormone vasopressin (VP) to promote water retention. Hence, the activity of magnocellular VP neurons is one of the key elements contributing to the regulation of body fluid homeostasis in healthy organisms. Chronic exposure to high dietary salt leads to excessive activation of VP neurons, thereby elevating levels of circulating VP, which can cause increases in blood pressure contributing to salt-dependent hypertension. However, the molecular basis underlying high-salt diet-induced hyperactivation of magnocellular VP neurons remains not fully understood. Previous studies suggest that magnocellular neurosecretory neurons contain a subcortical layer of actin filaments and pharmacological stabilization of this actin network potentiates osmotically-induced activation of magnocellular neurons. Using super-resolution imaging , we investigated the organization of the actin cytoskeleton in rat MNCs under normal physiological conditions and after a chronic increase in blood osmolality following 7 d of salt-loading (SL). We found that, in addition to the subcortical layer of actin filaments, magnocellular VP neurons are endowed with a unique network of cytoplasmic actin filaments throughout their somata. Moreover, we revealed that the density of both subcortical and cytoplasmic actin networks in magnocellular VP neurons is dramatically increased following SL. These results suggest that increased osmo-responsiveness of VP neurons following chronic exposure to high dietary salt may be mediated by the modulation of unique actin networks in magnocellular VP neurons, possibly contributing to elevated blood pressure in this condition.
eNeuro, 2020 Mar/Apr; 7

28220891: Barad Z, Grattan DR, Leitch B
NMDA Receptor Expression in the Thalamus of the Stargazer Model of Absence Epilepsy.
In the stargazer mouse model of absence epilepsy, altered corticothalamic excitation of reticular thalamic nucleus (RTN) neurons has been suggested to contribute to abnormal synchronicity in the corticothalamic-thalamocortical circuit, leading to spike-wave discharges, the hallmark of absence seizures. AMPA receptor expression and function are decreased in stargazer RTN, due to a mutation of AMPAR auxiliary subunit stargazin. It is unresolved and debated, however, if decreased excitation of RTN is compatible with epileptogenesis. We tested the hypothesis that relative NMDAR expression may be increased in RTN and/or thalamic synapses in stargazers using Western blot on dissected thalamic nuclei and biochemically isolated synapses, as well as immunogold cytochemistry in RTN. Expression of main NMDAR subunits was variable in stargazer RTN and relay thalamus; however, mean expression values were not statistically significantly different compared to controls. Furthermore, no systematic changes in synaptic NMDAR levels could be detected in stargazer thalamus. In contrast, AMPAR subunits were markedly decreased in both nucleus-specific and synaptic preparations. Thus, defective AMPAR trafficking in stargazer thalamus does not appear to lead to a ubiquitous compensatory increase in total and synaptic NMDAR expression, suggesting that elevated NMDAR function is not mediated by changes in protein expression in stargazer mice.
Sci Rep, 2017; 7

30901026: Brown RSE, Khant Aung Z, Phillipps HR, Barad Z, Lein HJ, Boehm U, Szawka RE, Grattan DR
Acute Suppression of LH Secretion by Prolactin in Female Mice Is Mediated by Kisspeptin Neurons in the Arcuate Nucleus.
Hyperprolactinemia causes infertility, but the specific mechanism is unknown. It is clear that elevated prolactin levels suppress pulsatile release of GnRH from the hypothalamus, with a consequent reduction in pulsatile LH secretion from the pituitary. Only a few GnRH neurons express prolactin receptors (Prlrs), however, and thus prolactin must act indirectly in the underlying neural circuitry. Here, we have tested the hypothesis that prolactin-induced inhibition of LH secretion is mediated

by kisspeptin neurons, which provide major excitatory inputs to GnRH neurons. To evaluate pulsatile LH secretion, we collected serial blood samples from diestrous mice and measured LH levels by ultrasensitive ELISA. Acute prolactin administration decreased LH pulses in wild-type mice. Kisspeptin neurons in the arcuate nucleus and in the rostral periventricular area of the third ventricle (RP3V) acutely responded to prolactin, but prolactin-induced signaling in kisspeptin neurons was up to fourfold higher in the arcuate nucleus when compared with the RP3V. Consistent with this, conditional knockout of Prlr specifically in arcuate nucleus kisspeptin neurons prevented prolactin-induced suppression of LH secretion. Our data establish that during hyperprolactinemia, suppression of pulsatile LH secretion is mediated by Prlr on arcuate kisspeptin neurons.
Endocrinology, 2019; 160
31943420: Kim S, Barad Z, Cheong RY, Ábrahám IM
Sex differences in rapid nonclassical action of 17β-oestradiol on intracellular signalling and oestrogen receptor α expression in basal forebrain cholinergic neurones in mouse.
Rapid nonclassical effects of 17β-oestradiol (E) on intracellular signalling have been identified in the basal forebrain, although the extent to which these actions may be different in males and females is unknown. Previous work has shown that E rapidly phosphorylates cAMP responsive element binding protein (CREB) via ERα in female cholinergic neurones. Using this indicator, the present study examined whether nonclassical actions of E occur in a sexually dimorphic manner within basal forebrain cholinergic neurones in mice. In addition, we investigated the expression and intracellular distribution of oestrogen receptor (ER)α in cholinergic neurones in female and male mice. Animals were gonadectomised and treated 2 weeks later with E . The number of CREB-expressing cholinergic neurones was not altered in any of the brain regions after E treatment in both males and females. However, E treatment rapidly (< 15 minutes) increased ($P < 0.05$) the number of cholinergic neurones expressing phosphorylated CREB (pCREB) in the substantia innominata and medial septum but not in the striatum in female mice. By contrast, E did not change pCREB expression in cholinergic neurones in male mice at any time point (15 minutes, 1 hour, 4 hours), irrespective of the neuroanatomical location. We also observed that, in females, more cholinergic neurones expressed nuclear ERα in all regions, whereas males showed more cholinergic neurones with cytoplasmic or both nuclear and cytoplasmic expression of ERα. Taken together, these results demonstrate a marked sex difference in the E -induced nonclassical effect and intracellular distribution of ERα in basal forebrain cholinergic neurones in vivo.
J Neuroendocrinol, 2020; 32
30406179: Barabás K, Barad Z, Dénes Á, Bhattarai JP, Han SK, Kiss E, Sármay G, Ábrahám IM
The Role of Interleukin-10 in Mediating the Effect of Immune Challenge on Mouse Gonadotropin-Releasing Hormone Neurons .
Immune challenge alters neural functioning via cytokine production. Inflammation has profound impact on the central regulation of fertility, but the mechanisms involved are not clearly defined. The anti-inflammatory cytokine interleukin (IL)-10 is responsible for balancing the immune response in the brain. To examine whether IL-10 has an effect on the function of the gonadotropin-releasing hormone (GnRH) neurons, we first examined the effect of immune responses with distinct cytokine profiles, such as the T cell-dependent (TD) and T cell-independent (TI) B-cell response. We investigated the effect of the TD and TI immune responses on ERK1/2 phosphorylation in GnRH neurons by administering fluorescein isothiocyanate/keyhole limpet hemocyanin (KLH-FITC) or dextran-FITC to female mice. Although dextran-FITC had no effect, KLH-FITC induced ERK1/2 phosphorylation in GnRH neurons after 6 d. KLH-FITC treatment increased the levels of IL-10 in the hypothalamus (HYP), but this treatment did not cause lymphocyte infiltration or an increase in the levels of proinflammatory cytokines. In IL-10 knock-out (KO) mice, KLH-FITC-induced ERK1/2 phosphorylation in the GnRH neurons was absent. We also showed that in IL-10 KO mice, the estrous cycle was disrupted. Perforated patch-clamp recordings from GnRH-GFP neurons, IL-10 immunohistochemistry, and in vitro experiments on acute brain slices revealed that IL-10 can directly alter GnRH neuron firing and induce ERK1/2 phosphorylation. These observations demonstrate that IL-10 plays a role in influencing signaling of GnRH neurons in the TD immune response. These results also provide the first evidence that IL-10 can directly alter the function of GnRH neurons and may help the maintenance of the integrity of the estrous cycle.
eNeuro, 2018 Sep-Oct; 5
20140748: Adori M, Kiss E, Barad Z, Barabás K, Kiszely E, Schneider A, Kövesdi D, Sziksz E, Abrahám IM, Matkó J, Sármay G
Estrogen augments the T cell-dependent but not the T-independent immune response.
Estrogen plays a critical regulatory role in the development and maintenance of immunity. Its role in the regulation of antibody synthesis in vivo is still not completely clear. Here, we have compared the effect of estrogen on T cell-dependent (TD) and T cell-independent type 2 (TI-2) antibody responses. The results provide the first evidence that estrogen enhances the TD but not the TI-2 response. Ovariectomy significantly decreased, while estrogen re-administration increased the number of hapten-specific IgM- and IgG-producing cells in response to TD antigen. In vitro experiments also show that estrogen may have a

direct impact on B and T cells by inducing rapid signaling events, such as Erk and AKT phosphorylation, cell-specific Ca(2+) signal, and NFkappaB activation. These non-transcriptional effects are mediated by classical estrogen receptors and partly by an as yet unidentified plasma membrane estrogen receptor. Such receptor- mediated rapid signals may modulate the in vivo T cell-dependent immune response.
Cell Mol Life Sci, 2010; 67

24380676: Trotman M, Barad Z, Guévremont D, Williams J, Leitch B
Changes in the GRIP 1&2 scaffolding proteins in the cerebellum of the ataxic stargazer mouse.
Glutamate receptor-interacting proteins (GRIP1&2) and protein-interacting with C kinase-1 (PICK1) are synaptic scaffold proteins associated with the stabilization and recycling of synaptic GluA2-, 3- and 4c-containing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). PICK1-mediated phosphorylation of GluA serine880 uncouples GRIP1&2 leading to AMPAR endocytosis, important in mediating forms of synaptic plasticity underlying learning and memory. Ataxic and epileptic stargazer mice possess a mutation in the CACNG2 gene encoding the transmembrane AMPAR-regulatory protein (TARP)-γ2 (stargazin). TARPs are AMPAR-auxiliary subunits required for efficient AMPAR trafficking to synapses. Stargazin is abundantly expressed in the cerebellum and its loss results in severe deficits in AMPAR trafficking to cerebellar synapses, particularly at granule cell (GC) synapses, leading to the ataxic phenotype of stargazers. However, how the stargazin mutation impacts on the expression of other AMPAR-interacting scaffold proteins is unknown. This study shows a significant increase in GRIP1&2, but not PICK1, levels in whole tissue and synapse-enriched extracts from stargazer cerebella. Post-embedding immunogold-cytochemistry electron microscopy showed GRIP1&2 levels were unchanged at mossy fiber-GC synapses in stargazers, which are silent due to virtual total absence of synaptic and extrasynaptic GluA2/3-AMPARs. These results indicate that loss of synaptic AMPARs at this excitatory synapse does not affect GRIP1&2 expression within the postsynaptic region of mossy fiber-GC synapses. Interestingly, increased GRIP and reduced GluA2-AMPARexpression also occur in cerebella of autistic patients. Further research establishing the role of elevated cerebellar GRIP1&2 in stargazers may help identify common cellular mechanisms in the comorbid disorders ataxia, epilepsy and autism leading to more effective treatment strategies.
Brain Res, 2014; 1546

22609941: Barad Z, Shevtsova O, Arbuthnott GW, Leitch B
Selective loss of AMPA receptors at corticothalamic synapses in the epileptic stargazer mouse.
Absence seizures are common in the stargazer mutant mouse. The mutation underlying the epileptic phenotype in stargazers is a defect in the gene encoding the normal expression of the protein stargazin. Stargazin is involved in the membrane trafficking and synaptic targeting of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) at excitatory glutamatergic synapses. Thus, the genetic defect in the stargazer results in a loss of AMPARs and consequently, excitation at glutamatergic synapses. Absence seizures are known to arise in thalamocortical networks. In the present study we show for the first time, using Western blot analysis and quantitative immunogold cytochemistry, that in the epileptic stargazer mouse, there is a global loss of AMPAR protein in nucleus reticularis (RTN) and a selective loss of AMPARs at corticothalamic synapses in inhibitory neurons of the RTN thalamus. In contrast, there is no significant loss of AMPARs at corticothalamic synapses in excitatory relay neurons in the thalamic ventral posterior (VP) region. The findings of this study thus provide cellular and molecular evidence for a selective regional loss of synaptic AMPAR within the RTN that could account for the loss of function at these inhibitory neuron synapses, which has previously been reported from electrophysiological studies. The specific loss of AMPARs at RTN but not relay synapses in the thalamus of the stargazer, could contribute to the absence epilepsy phenotype by altering thalamocortical network oscillations. This is supported by recent evidence that loss of glutamate receptor subunit 4 (GluA4) (the predominant AMPAR-subtype in the thalamus), also leads to a specific reduction in strength in the cortico-RTN pathway and enhanced thalamocortical oscillations, in the Gria4(-/-) model of absence epilepsy. Thus further study of thalamic changes in these models could be important for future development of drugs targeted to absence epilepsy.
Neuroscience, 2012; 217

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BOARD NUMBER: S07-499

WASTEOSOMES (CORPORA AMYLACEA) OF HUMAN BRAIN ARE PHAGOCYTOSED AND DIGESTED BY THP-1 MACROPHAGES IN VITRO

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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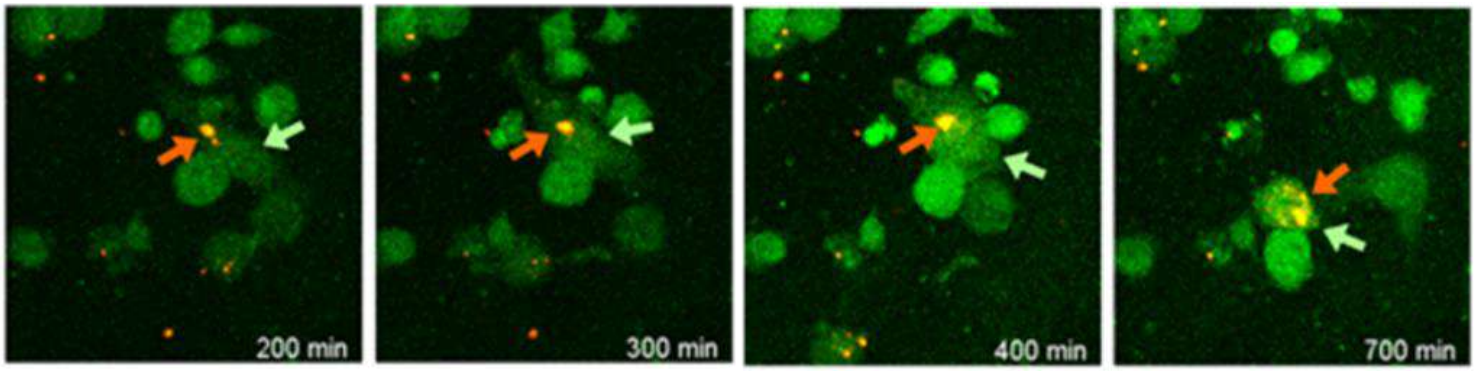
[1]Universitat de Barcelona, Departament De Bioquímica I Fisiologia, Barcelona, Spain, [2]Universitat de Barcelona, Institut De Neurociències, Barcelona, Spain, [3]CIBERNED, Centros De Biomedicina En Red De Enfermedades Neurodegenerativas, Madrid, Spain, [4]Universitat de Barcelona, Institut De Recerca En Nutrició I Seguretat Alimentàries (insa-ub), Barcelona, Spain, [5]Universitat de Barcelona, Alzheimer's Disease And Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Institut D'investigacions Biomèdiques August Pi I Sunyer (idibaps), Barcelona, Spain, [6]Hospital Clínic-IDIBAPS, Neurological Tissue Bank Of The Biobanc, Barcelona, Spain, [7]Universitat de Barcelona, Unitat De Microscòpia Òptica Avançada - Campus Clínic, Facultat De Medicina, Centres Científics I Tecnològics, Barcelona, Spain

Aims: *Corpora amylacea* of human brain, recently renamed as wasteosomes, are granular structures that appear during aging and also accumulate in specific areas of the brain in neurodegenerative conditions. Acting as waste containers of the brain, wasteosomes are formed by polyglucosan aggregates that entrap waste substances of different cell types, are expelled from the brain to the cerebrospinal fluid (CSF) and are phagocytosed by macrophages. In the present study, we analyse the phagocytosis of wasteosomes and the mechanisms involved in this process. **Methods:** We purified wasteosomes from human CSF and stained them with Concanavalin A-rhodamine, NHS-AF555 protein labelling probe or PAS staining. Then, they were incubated with THP-1 macrophages stained with the Vybrant® CFDA-SE Cell Tracer Kit. Time-lapse recording techniques were performed to evaluate the phagocytosis. **Results:** THP-1 macrophages phagocytose and process wasteosomes. Once phagocytosed, Concanavalin A-rhodamine-labelled and NHS-AF555-labelled wasteosomes were digested or fragmented and (at least) the fluorescent protein fraction was exposed on the surface of macrophages as well as transferred from one macrophage to another. We observed a lower reactivity of macrophages towards PAS-stained wasteosomes. In any case, these time-lapse studies revealed that under all the experimental conditions THP-1 macrophages phagocytose or interact with wasteosomes. **Conclusions:** Our findings support the role of the immune system in wasteosomes elimination and thus, in the removal of brain waste substances
removal.



Sequence of a macrophage that phagocytose a wasteosomes. Orange arrows: wasteosome Concanavalin A-rhodamine-labelled. Green arrows: macrophage that phagocytose the wasteosome

Pubmed:
31796594: Riba M, Augé E, Campo-Sabariz J, Moral-Anter D, Molina-Porcel L, Ximelis T, Ferrer R, Martín-Venegas R, Pelegrí C, Vilaplana J
act as containers that remove waste products from the brain.
(CA) in the human brain are granular bodies formed by polyglucosan aggregates that amass waste products of different

origins. They are generated by astrocytes, mainly during aging and neurodegenerative conditions, and are located predominantly in periventricular and subpial regions. This study shows that CA are released from these regions to the cerebrospinal fluid and are present in the cervical lymph nodes, into which cerebrospinal fluid drains through the meningeal lymphatic system. We also show that CA can be phagocytosed by macrophages. We conclude that CA can act as containers that remove waste products from the brain and may be involved in a mechanism that cleans the brain. Moreover, we postulate that CA may contribute in some autoimmune brain diseases, exporting brain substances that interact with the immune system, and hypothesize that CA may contain brain markers that may aid in the diagnosis of certain brain diseases.
Proc Natl Acad Sci U S A, 2019; 116

34262556: Riba M, Augé E, Tena I, Del Valle J, Molina-Porcel L, Ximelis T, Vilaplana J, Pelegrí C
Corpora Amylacea in the Human Brain Exhibit Neoepitopes of a Carbohydrate Nature.
Corpora amylacea (CA) in the human brain are polyglucosan bodies that accumulate residual substances originated from aging and both neurodegenerative and infectious processes. These structures, which act as waste containers, are released from the brain to the cerebrospinal fluid, reach the cervical lymph nodes the meningeal lymphatic system and may be phagocytosed by macrophages. Recent studies indicate that CA present certain neoepitopes (NEs) that can be recognized by natural antibodies of the IgM class, and although evidence of different kinds suggests that these NEs may be formed by carbohydrate structures, their precise nature is unknown. Here, we adapted standard techniques to examine this question. We observed that the preadsorption of IgMs with specific carbohydrates has inhibitory effects on the interaction between IgMs and CA, and found that the digestion of CA proteins had no effect on this interaction. These findings point to the carbohydrate nature of the NEs located in CA. Moreover, the present study indicates that, , the binding between certain natural IgMs and certain epitopes may be disrupted by certain monosaccharides. We wonder, therefore, whether these inhibitions may also occur . Further studies should now be carried out to assess the possible effect of glycemia on the reactivity of natural IgMs and, by extension, on natural immunity.
Front Immunol, 2021; 12

34634491: Riba M, Del Valle J, Augé E, Vilaplana J, Pelegrí C
From corpora amylacea to wasteosomes: History and perspectives.
Corpora amylacea (CA) have been described in several human organs and have been associated with ageing and several pathological conditions. Although they were first discovered two centuries ago, their function and significance have not yet been identified. Here, we provide a chronological summary of the findings on CA in various organs and identify their similarities. After collecting and integrating these findings, we propose to consider CA as waste containers created by specific cells, which sequester waste products and foreign products, and assemble them within a glycan structure. The containers are then secreted into the external medium or interstitial spaces, in this latter case subsequently being phagocytosed by macrophages. This proposal explains, among others, why CA are so varied in content, why only some of them contain fibrillary amyloid proteins, why all of them contain glycan structures, why some of them contain neo-epitopes and are phagocytosed, and why they can be intracellular or extracellular structures. Lastly, in order to avoid the ambiguity of the term amyloid (which can indicate starch-like structures but also insoluble fibrillary proteins), we propose renaming CA as "wasteosomes", emphasising the waste products they entrap rather than their misleading amyloid properties.
Ageing Res Rev, 2021; 72

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IMMUNE REGULATION IN GALT BY IMMUNE CHECKPOINT PATHWAYS IN WILD-TYPE AND PACAP-DEFICIENT MICE

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Introduction: PACAP (pituitary adenylate cyclase-activating polypeptide) is a neuropeptide expressed in many organs that has been shown to have general cytoprotective, anti-inflammatory, and antiapoptotic effects. We have limited data about the examinations of the Gut-associated lymphoid tissue (GALT) in PACAP wild-type (WT) and PACAP deficient (PACAP KO) mice. The aim of the present experiment was to reveal qualitative and quantitave changes between the two mice groups found in the Peyer's patches. Methods: Samples were taken from aging (12-15 monthes old) WT (n=10) and PACAP KO (n=10) mice. We investigated the percentage of numerous immune cell populations in the GALT of WT and PACAP KO mice. We performed immunophenotyping of GALT immune cells and investigated the expression of TIM-3 and PD-1 immune checkpoint molecules with the detection of galectin-9 and PD-L1. We monitored the activation (CD69) and citotoxicity (perforin) as well. Results: We demonstrated a significant decrease in the frequency of GALT CD3+ T cells obtained from PACAP KO mice compared with wild-type mice. Monitoring the T cell subpopulations in CD3+ T cells we found significant increase in CD4+ T cells and significant decrease in CD8+ T-cells. Investigating immune-checkpoint receptors, TIM-3 showed a significantly decreased expression in all T cell subpopulations, while PD-1 expression significantly decreased only in CD8+ T cells in PACAP KO mice compared to wild-type mice. Conclusion: We hypothesize that these local changes might have a role of the impaired antiinflammatory responses in PACAP KO mice, however, determining the exact function requires further investigations.

Pubmed:
29774542: Reglodi D, Jungling A, Longuespée R, Kriegsmann J, Casadonte R, Kriegsmann M, Juhasz T, Bardosi S, Tamas A, Fulop BD, Kovacs K, Nagy Z, Sparks J, Miseta A, Mazzucchelli G, Hashimoto H, Bardosi A
Accelerated pre-senile systemic amyloidosis in PACAP knockout mice - a protective role of PACAP in age-related degenerative processes.
Dysregulation of neuropeptides may play an important role in aging-induced impairments. Among them, pituitary adenylate cyclase-activating polypeptide (PACAP) is a potent cytoprotective peptide that provides an endogenous control against a variety of tissue-damaging stimuli. We hypothesized that the progressive decline of PACAP throughout life and the well-known general cytoprotective effects of PACAP lead to age-related pathophysiological changes in PACAP deficiency, supported by the increased vulnerability to various stressors of animals partially or totally lacking PACAP. Using young and aging CD1 PACAP knockout (KO) and wild type (WT) mice, we demonstrated pre-senile amyloidosis in young PACAP KO animals and showed that senile amyloidosis appeared accelerated, more generalized, more severe, and affected more individuals. Histopathology showed age-related systemic amyloidosis with mainly kidney, spleen, liver, skin, thyroid, intestinal, tracheal, and esophageal involvement. Mass spectrometry-based proteomic analysis, reconfirmed with immunohistochemistry, revealed that apolipoprotein-AIV was the main amyloid protein in the deposits together with several accompanying proteins. Although the local amyloidogenic protein expression was disturbed in KO animals, no difference was found in laboratory lipid parameters, suggesting a complex pathway leading to increased age-related degeneration with amyloid deposits in the absence of PACAP. In spite of no marked inflammatory histological changes or blood test parameters, we detected a disturbed cytokine profile that possibly creates a pro-inflammatory milieu favoring amyloid deposition. In summary, here we describe accelerated systemic senile amyloidosis in PACAP gene-deficient mice, which might indicate an early aging phenomenon in this mouse strain. Thus, PACAP KO mice could serve as a model of accelerated aging with human relevance. © 2018 The Authors. The Journal of Pathology published by John Wiley & Sons Ltd on behalf of Pathological Society of Great Britain and Ireland.
J Pathol, 2018; 245


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BOARD NUMBER: S07-501

A ROLE FOR MYELOID MIR-155 IN REGULATING NEONATAL HYPOXIA INDUCED SEIZURES

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Hypoxic ischaemic injury (HIE) in the neonatal brain has significant consequences on neurodevelopment and increases the occurrence of neurological deficits in infants. Currently, therapeutic options for the treatment of HIE are very limited. In this study we have assessed the role of the inflammatory microRNA, miR-155, expressed in myeloid cells specifically, on regulating inflammation and seizure severity in a preclinical model of neonatal hypoxia-induced seizures (Hx-seizures). Wildtype (WT) miR-155 (miR-155+/+ LysMCre) mice were compared to a mouse line in which miR-155 was deleted in myeloid cells (miR-155fl/fl LysMCre). We demonstrate that following Hx-seizures expression of miR-155 target genes including brain-derived neurotrophic factor (BDNF), arginase-2 (Arg-2), SHIP-1 and SOCS-1 in miR-155fl/fl LysMCre was significantly increased compared to WT controls . Furthermore, BDNF protein levels were significantly higher in miR-155fl/fl LysMCre mice with Hx-seizures. Conversely, there was decreased transcription of pro-inflammatory cytokines IL-1b and IL-6 and lower protein levels of IL-1b in miR-155fl/fl LysMCre mice with Hx-seizures as compared to WTs. Finally, myeloid miR-155 deletion significantly reduced behavioural seizure severity score and reduced electrographically (EEG) measured seizure frequency ($p = 0.0516$) and seizure burden by 292.9 seconds ($p < 0.01$) as compared to mice with wildtype miR-155, suggesting miR-155 regulation of seizure occurrence in this model. Behavioural tests for cognitive and motor functions at 5 weeks post Hx-seizures demonstrated differences between genotypes. Excitingly this work highlights that inhibition of miR-155, specifically in myeloid cells, may hold therapeutic benefit for both seizures and comorbidities associated with hypoxic brain injury.

Pubmed:
35032612: Hibbitts AJ, Kočí Z, Kneafsey S, Matsiko A, Žilić L, Dervan A, Hinton P, Chen G, Cavanagh B, Dowling JK, McCoy CE, Buckley CT, Archibald SJ, O'Brien FJ
Multi-factorial nerve guidance conduit engineering improves outcomes in inflammation, angiogenesis and large defect nerve repair.
Nerve guidance conduits (NGCs) are sub-optimal for long-distance injuries with inflammation and poor vascularization related to poor axonal repair. This study used a multi-factorial approach to create an optimized biomaterial NGC to address each of these issues. Through stepwise optimization, a collagen-chondroitin-6-sulfate (Coll-CS) biomaterial was functionalized with extracellular matrix (ECM) components; fibronectin, laminin 1 and laminin 2 (FibL1L2) in specific ratios. A snap-cooled freeze-drying process was then developed with optimal pore architecture and alignment to guide axonal bridging. Culture of adult rat dorsal root ganglia on NGCs demonstrated significant improvements in inflammation, neurogenesis and angiogenesis in the specific Fib:L1:L2 ratio of 1:4:1. In clinically relevant, large 15 mm rat sciatic nerve defects, FibL1L2-NGCs demonstrated significant improvements in axonal density and angiogenesis compared to unmodified NGCs with functional equivalence to autografts. Therefore, a multiparameter ECM-driven strategy can significantly improve axonal repair across large defects, without exogenous cells or growth factors.
Matrix Biol, 2022; 106
33674584: Dowling JK, Afzal R, Gearing LJ, Cervantes-Silva MP, Annett S, Davis GM, De Santi C, Assmann N, Dettmer K, Gough DJ, Bantug GR, Hamid FI, Nally FK, Duffy CP, Gorman AL, Liddicoat AM, Lavelle EC, Hess C, Oefner PJ, Finlay DK, Davey GP, Robson T, Curtis AM, Hertzog PJ, Williams BRG, McCoy CE
Mitochondrial arginase-2 is essential for IL-10 metabolic reprogramming of inflammatory macrophages.

Mitochondria are important regulators of macrophage polarisation. Here, we show that arginase-2 (Arg2) is a microRNA-155 (miR-155) and interleukin-10 (IL-10) regulated protein localized at the mitochondria in inflammatory macrophages, and is critical for IL-10-induced modulation of mitochondrial dynamics and oxidative respiration. Mechanistically, the catalytic activity and presence of Arg2 at the mitochondria is crucial for oxidative phosphorylation. We further show that Arg2 mediates this process by increasing the activity of complex II (succinate dehydrogenase). Moreover, Arg2 is essential for IL-10-mediated downregulation of the inflammatory mediators succinate, hypoxia inducible factor 1α (HIF-1α) and IL-1β in vitro. Accordingly, HIF-1α and IL-1β are highly expressed in an LPS-induced in vivo model of acute inflammation using Arg2 mice. These findings shed light on a new arm of IL-10-mediated metabolic regulation, working to resolve the inflammatory status of the cell.
Nat Commun, 2021; 12
34959446: Dervan A, Franchi A, Almeida-Gonzalez FR, Dowling JK, Kwakyi OB, McCoy CE, O'Brien FJ, Hibbitts A
Biomaterial and Therapeutic Approaches for the Manipulation of Macrophage Phenotype in Peripheral and Central Nerve Repair.
Injury to the peripheral or central nervous systems often results in extensive loss of motor and sensory function that can greatly diminish quality of life. In both cases, macrophage infiltration into the injury site plays an integral role in the host tissue inflammatory response. In particular, the temporally related transition of macrophage phenotype between the M1/M2 inflammatory/repair states is critical for successful tissue repair. In recent years, biomaterial implants have emerged as a novel approach to bridge lesion sites and provide a growth-inductive environment for regenerating axons. This has more recently seen these two areas of research increasingly intersecting in the creation of 'immune-modulatory' biomaterials. These synthetic or naturally derived materials are fabricated to drive macrophages towards a pro-repair phenotype. This review considers the macrophage-mediated inflammatory events that occur following nervous tissue injury and outlines the latest developments in biomaterial-based strategies to influence macrophage phenotype and enhance repair.
Pharmaceutics, 2021; 13
32967206: Afzal R, Dowling JK, McCoy CE
Impact of Exercise on Immunometabolism in Multiple Sclerosis.
Multiple Sclerosis (MS) is a chronic, autoimmune condition characterized by demyelinating lesions and axonal degradation. Even though the cause of MS is heterogeneous, it is known that peripheral immune invasion in the central nervous system (CNS) drives pathology at least in the most common form of MS, relapse-remitting MS (RRMS). The more progressive forms' mechanisms of action remain more elusive yet an innate immune dysfunction combined with neurodegeneration are likely drivers. Recently, increasing studies have focused on the influence of metabolism in regulating immune cell function. In this regard, exercise has long been known to regulate metabolism, and has emerged as a promising therapy for management of autoimmune disorders. Hence, in this review, we inspect the role of key immunometabolic pathways specifically dysregulated in MS and highlight potential therapeutic benefits of exercise in modulating those pathways to harness an anti-inflammatory state. Finally, we touch upon current challenges and future directions for the field of exercise and immunometabolism in MS.
J Clin Med, 2020; 9
32464598: Ong GSY, Cole TJ, Tesch GH, Morgan J, Dowling JK, Mansell A, Fuller PJ, Young MJ
Novel mineralocorticoid receptor mechanisms regulate cardiac tissue inflammation in male mice.
MR activation in macrophages is critical for the development of cardiac inflammation and fibrosis. We previously showed that MR activation modifies macrophage pro-inflammatory signalling, changing the cardiac tissue response to injury via both direct gene transcription and JNK/AP-1 second messenger pathways. In contrast, MR-mediated renal electrolyte homeostasis is critically determined by DNA-binding-dependent processes. Hence, ascertaining the relative contribution of MR actions via DNA binding or alternative pathways on macrophage behaviour and cardiac inflammation may provide therapeutic opportunities which separate the cardioprotective effects of MR antagonists from their undesirable renal potassium-conserving effects. We developed new macrophage cell lines either lacking MR or harbouring a mutant MR incapable of DNA binding. Western blot analysis demonstrated that MR DNA binding is required for lipopolysaccharide (LPS), but not phorbol 12-myristate-13-acetate (PMA), induction of the MAPK/pJNK pathway in macrophages. Quantitative RTPCR for pro-inflammatory and pro-fibrotic targets revealed subsets of LPS- and PMA-induced genes that were either enhanced or repressed by the MR via actions that do not always require direct MR-DNA binding. Analysis of the MR target gene and profibrotic factor MMP12 identified promoter elements that are regulated by combined MR/MAPK/JNK signalling. Evaluation of cardiac tissue responses to an 8-day DOC/salt challenge in mice selectively lacking MR DNA-binding in macrophages demonstrated levels of inflammatory markers equivalent to WT, indicating non-DNA binding-dependent MR signalling in macrophages is sufficient for DOC/salt-induced tissue inflammation. Our data demonstrate that the MR regulates a macrophage pro-inflammatory phenotype and cardiac tissue inflammation, partially via pathways that do not require DNA binding.
J Endocrinol, 2020; 246

31936823: Rudloff I, Ung HK, Dowling JK, Mansell A, D'Andrea L, Ellisdon AM, Whisstock JC, Berger PJ, Nold-Petry CA, Nold MF
Parsing the IL-37-Mediated Suppression of Inflammasome Function.
Interleukin (IL)-37 is a member of the IL-1 family of cytokines. Although its broad anti-inflammatory properties are well described, the effects of IL-37 on inflammasome function remain poorly understood. Performing gene expression analyses, ASC oligomerization/speck assays and caspase-1 assays in bone marrow-derived macrophages (BMDM), and employing an in vivo endotoxemia model, we studied how IL-37 affects the expression and maturation of IL-1β and IL-18, inflammasome activation, and pyroptosis in detail. IL-37 inhibited IL-1β production by NLRP3 and AIM2 inflammasomes, and IL-18 production by the NLRP3 inflammasome. This inhibition was partially attributable to effects on gene expression: whereas IL-37 did not affect lipopolysaccharide (LPS)-induced mRNA expression of or inflammasome components, IL-37-transgenic BMDM displayed an up to 83% inhibition of baseline and LPS-stimulated compared to their wild-type counterparts. Importantly, we observed that IL-37 suppresses nigericin- and silica-induced ASC oligomerization/speck formation (a step in inflammasome activation and subsequent caspase-1 activation), and pyroptosis (-50%). In mice subjected to endotoxemia, IL-37 inhibited plasma IL-1β (-78% compared to wild-type animals) and IL-18 (-61%). Thus, our study adds suppression of inflammasome activity to the portfolio of anti-inflammatory pathways employed by IL-37, highlighting this cytokine as a potential tool for treating inflammasome-driven diseases.
Cells, 2020; 9
30787108: Dowling JK, Tate MD, Rosli S, Bourke NM, Bitto N, Lauterbach MA, Cheung S, Ve T, Kobe B, Golenbock D, Mansell A
The Single Nucleotide Polymorphism Mal-D96N Mice Provide New Insights into Functionality of Mal in TLR Immune Responses.
MyD88 adaptor-like (Mal) protein is the most polymorphic of the four key adaptor proteins involved in TLR signaling. TLRs play a critical role in the recognition and immune response to pathogens through activation of the prototypic inflammatory transcription factor NF-κB. The study of single nucleotide polymorphisms in TLRs, adaptors, and signaling mediators has provided key insights into the function of the corresponding genes but also into the susceptibility to infectious diseases in humans. In this study, we have analyzed the immune response of mice carrying the human Mal-D96N genetic variation that has previously been proposed to confer protection against septic shock. We have found that Mal-D96N macrophages display reduced cytokine expression in response to TLR4 and TLR2 ligand challenge. Mal-D96N macrophages also display reduced MAPK activation, NF-κB transactivation, and delayed NF-κB nuclear translocation, presumably via delayed kinetics of Mal interaction with MyD88 following LPS stimulation. Importantly, Mal-D96N genetic variation confers a physiological protective phenotype to in vivo models of LPS-, -, and influenza A virus-induced hyperinflammatory disease in a gene dosage-dependent manner. Together, these results highlight the critical role Mal plays in regulating optimal TLR-induced inflammatory signaling pathways and suggest the potential therapeutic advantages of targeting the Mal D96 signaling nexus.
J Immunol, 2019; 202
27913620: Pinar A, Dowling JK, Bitto NJ, Robertson AA, Latz E, Stewart CR, Drummond GR, Cooper MA, McAuley JL, Tate MD, Mansell A
PB1-F2 Peptide Derived from Avian Influenza A Virus H7N9 Induces Inflammation via Activation of the NLRP3 Inflammasome.
The emergence of avian H7N9 influenza A virus in humans with associated high mortality has highlighted the threat of a potential pandemic. Fatal H7N9 infections are characterized by hyperinflammation and increased cellular infiltrates in the lung. Currently there are limited therapies to address the pathologies associated with H7N9 infection and the virulence factors that contribute to these pathologies. We have found that PB1-F2 derived from H7N9 activates the NLRP3 inflammasome and induces lung inflammation and cellular recruitment that is NLRP3-dependent. We have also shown that H7N9 and A/Puerto Rico/H1N1 (PR8)PB1-F2 peptide treatment induces significant mitochondrial reactive oxygen production, which contributes to NLRP3 activation. Importantly, treatment of cells or mice with the specific NLRP3 inhibitor MCC950 significantly reduces IL-1β maturation, lung cellular recruitment, and cytokine production. Together, these results suggest that PB1-F2 from H7N9 avian influenza A virus may be a major contributory factor to disease pathophysiology and excessive inflammation characteristic of clinical infections and that targeting the NLRP3 inflammasome may be an effective means to reduce the inflammatory burden associated with H7N9 infections.
J Biol Chem, 2017; 292
27350884: Dowling JK, Mansell A
Toll-like receptors: the swiss army knife of immunity and vaccine development.
Innate immune cells have a critical role in defense against infection and disease. Central to this is the broad specificity with which they can detect pathogen-associated patterns and danger-associated patterns via the pattern recognition receptors (PRRs) they express. Several families of PRRs have been identified including: Toll-like receptors (TLRs), C-type lectin-like

receptors, retinoic acid-inducible gene-like receptors and nucleotide-binding oligomerization domain-like receptors. TLRs are one of the most largely studied families of PRRs. The binding of ligands to TLRs on antigen presenting cells (APCs), mainly dendritic cells, leads to APC maturation, induction of inflammatory cytokines and the priming of naive T cells to drive acquired immunity. Therefore, activation of TLRs promotes both innate inflammatory responses and the induction of adaptive immunity. Consequently, in the last two decades mounting evidence has inextricably linked TLR activation with the pathogenesis of immune diseases and cancer. It has become advantageous to harness these aspects of TLR signaling therapeutically to accelerate and enhance the induction of vaccine-specific responses and also target TLRs with the use of biologics and small molecule inhibitors for the treatment of disease. In these respects, TLRs may be considered a 'Swiss Army' knife of the immune system, ready to respond in a multitude of infectious and disease states. Here we describe the latest advances in TLR-targeted therapeutics and the use of TLR ligands as vaccine adjuvants.
Clin Transl Immunology, 2016; 5

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BOARD NUMBER: S07-502

MILD LIVER DAMAGE INDUCES SPATIAL LEARNING AND MEMORY IMPAIRMENT IN RATS. UNDERLYING MECHANISMS AND PREVENTION BY RIFAXIMIN

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Background: Patients with non-alcoholic fatty liver disease (NAFLD) may show mild cognitive impairment. Neuroinflammation in hippocampus contributes to cognitive impairment in rats with hyperammonemia and minimal hepatic encephalopathy (MHE). Treatment with rifaximin reverses cognitive impairment in a large proportion of cirrhotic patients with MHE. The underlying mechanisms remain unclear. **Aims:** The aims of this work were to assess if rats with mild liver damage show neuroinflammation in hippocampus associated to alterations in neurotransmission and cognitive function and if treatment with rifaximin reverses the cognitive impairment and the underlying mechanisms. **Methods:** Mild liver damage was induced by intraperitoneal injection of carbon-tetrachloride for four weeks and some rats were treated daily orally with rifaximin. Spatial and non-spatial learning and memory were evaluated. We analyzed immune cells infiltration, microglia and astrocytes activation, proinflammatory cytokine levels and glutamatergic neurotransmission in hippocampus. **Results:** Rats with mild liver damage show impaired spatial learning and memory, whereas non-spatial and working memory are not altered. Mild liver damage increased CCL2 in hippocampus, associated with infiltration of monocytes, activation of microglia and increased TNFα in hippocampal neurons, associated with reduced membrane expression of NR1 and NR2A subunits of NMDA receptor. These effects are reversed by rifaximin, that improves spatial learning and memory. Mild liver damage also triggers CD4+ lymphocytes infiltration, astrocytes activation, increased IL-1b and enhanced membrane expression of GluA2 subunit of AMPA receptors in hippocampus. These changes are not reversed by rifaximin. **Conclusions:** Treatment with rifaximin may improve cognitive function in patients with NAFLD showing mild cognitive impairment.

Pubmed:
34440206: Balzano T, Leone P, Ivaylova G, Castro MC, Reyes L, Ramón C, Malaguarnera M, Llansola M, Felipo V
Rifaximin Prevents T-Lymphocytes and Macrophages Infiltration in Cerebellum and Restores Motor Incoordination in Rats with Mild Liver Damage.
In patients with liver cirrhosis, minimal hepatic encephalopathy (MHE) is triggered by a shift in peripheral inflammation, promoting lymphocyte infiltration into the brain. Rifaximin improves neurological function in MHE by normalizing peripheral inflammation. Patients who died with steatohepatitis showed T-lymphocyte infiltration and neuroinflammation in the cerebellum, suggesting that MHE may already occur in these patients. The aims of this work were to assess, in a rat model of mild liver damage similar to steatohepatitis, whether: (1) the rats show impaired motor coordination in the early phases of liver damage; (2) this is associated with changes in the immune system and infiltration of immune cells into the brain; and (3) rifaximin improves motor incoordination, associated with improved peripheral inflammation, reduced infiltration of immune cells and neuroinflammation in the cerebellum, and restoration of the alterations in neurotransmission. Liver damage was induced by carbon tetrachloride (CCl) injection over four weeks. Peripheral inflammation, immune cell infiltration, neuroinflammation, and neurotransmission in the cerebellum and motor coordination were assessed. Mild liver damage induces neuroinflammation and altered neurotransmission in the cerebellum and motor incoordination. These alterations are associated with increased TNFa, CCL20, and CX3CL1 in plasma and cerebellum, IL-17 and IL-15 in plasma, and CCL2 in cerebellum. This promotes T-lymphocyte and macrophage infiltration in the cerebellum. Early treatment with rifaximin prevents the shift in peripheral inflammation, immune cell infiltration, neuroinflammation, and motor incoordination. This report provides new clues regarding the mechanisms of the beneficial effects of rifaximin, suggesting that early rifaximin treatment could prevent neurological impairment in patients with steatohepatitis.
Biomedicines, 2021; 9


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BOARD NUMBER: S07-503

MECHANISTIC INSIGHTS INTO CEREBRAL MALARIA ASSOCIATED HIPPOCAMPAL DAMAGE AND MEMORY-IMPAIRMENT IN PLASMODIUM BERGHEI ANKA-INFECTED BALB/C MICE

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Cerebral malaria (CM) is a neurological complication of *Plasmodium falciparum* making it the fourth major cause of morbidity in children <5 years of age. **Neurocognitive deficits** remain the **hidden burden** of malaria accounting for memory impairment in **25% of the survivors**. Despite these striking numbers, we **lack a complete understanding of events** leading to memory impairment during CM and therapeutic targets for its reversal. Thus, here we aim to delineate the mechanism and extent of hippocampal damage responsible for working-memory impairment in *Plasmodium berghei* ANKA (PbA) infected Balb/c mice with progressing parasitaemia. Present work involved assessment for working-memory deficits via **novel object recognition (NOR) and passive avoidance task (PAT**), evaluation of hippocampal damage by **HE and CV staining, immunofluorescence** studies to establish the involvement of brain resident cells (astrocytes, microglia, and neurons) in inducing the damage, and **qRT-PCR** analysis of BDNF and VEGF at different parasitemia levels viz 1%, 5%, 10%, 15% and 20%. Histopathological analysis clearly indicates severe hippocampal damage, neuronal loss, and piknosis with progressing parasitaemia, substantiated by NOR and PAT depicting severe memory deficits with most severe damage at 15 and 20%. Further, through IF studies we observed increased activation of microglia and astrocytes; 4-fold decrease in BDNF and 3-fold increase in VEGF gene expression; leading to enhanced neuroinflammation disrupting the neuronal signaling and memory. **Thus, our results provide novel insights into the involvement of BDNF, VEGF, and brain resident cells mediated mechanism of memory impairment**. However, further work is required to validate the exact molecular mechanism.

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HUMAN IPSC DERIVED NEURAL PROGENITORS AND CORTICAL NEURONS AS A MODEL TO STUDY SARS-COV-2 INFECTION

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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COVID-19 is a respiratory disease affecting multiple organ systems including the central nervous system (CNS), with a characteristic loss of smell and taste that could distinguish COVID-19 from other respiratory diseases. Although frequently reported and extensively studied, the neurological symptoms remain enigmatic; there is no consensus on the consequences, variability or the extent of the CNS infection. Human induced pluripotent stem cell (hiPSC) derived neural progenitor cells (NPCs) and the cortical excitatory neurons were used as a model to address the questions of sensitivity and permissiveness to the SARS-CoV-2 brain infection, to study the extent and possible consequences and to explore the mechanisms of viral entry. The cells were infected with SARS-CoV-2 variants Alpha, Delta and the ancestral Wuhan and D614G variants in two different conditions; with the endogenous levels of the main docking receptor the virus utilizes - ACE2 and in a condition where the receptor is stably overexpressed. The infection properties and the differences between the conditions were assessed by microscopy, measuring the viral kinetics, infectivity assays and by S-pseudotyped single-cycle lentiviral infections, in addition. While the ACE2 overexpressing neurons display typical hallmarks of the viral infection, the infection of the neurons expressing endogenous levels of ACE2 remains sporadic and without prospects to set off a productive infection across the experimental conditions and SARS-CoV-2 variants applied.

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HEMIZYGOUS KO OF MID1 RECAPITULATES THE BEHAVIOURAL PHENOTYPE INDUCED BY PRENATAL IMMUNE ACTIVATION

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Environmental insults during prenatal or postnatal developmental periods are critical in shaping adult physiology and CNS function, the effects of which may play a role in the pathogenesis of neuropsychiatric illnesses. Maternal immune activation causes long-term and genome-wide changes in the offspring's methylome, and *Mid1* stood out as the gene showing the most extensive changes. The aim of this study was to investigate whether a transgenic mouse model of Mid-1 (*Mid1*-KO) could recapitulate the behavioral changes induced by prenatal immune activation, supporting the idea that the *Mid1* gene might be a molecular mechanism mediating the negative effects of prenatal maternal immune activation. Behavioural testing in adult Mid-1 KO males included paradigms assessing spatial recognition memory, social interaction, and prepulse inhibition of the acoustic startle reflex. Perfusion was performed prior to brain collection for total RNA analysis and IHC staining on the *Corpus Callosum* respectively. Behavioural tests and molecular analyses recapitulated our previous findings in male offspring of immunologically challenged mothers. IHC analyses show a different composition in the white matter of Mid-1 KO mice compared to Wt. Furthermore, qRT-PCR analyses of mRNA from different brain regions of mice treated with prenatal viral-like immune activation Poly I:C, confirm our results, showing a decrease in *Mid1* gene expression. Depletion of the *Mid1* gene in male animals recapitulates the behavioural profile induced by prenatal immune activation, suggesting that the epigenetic dampening of *Mid1* expression in PolyI:C mice could be a molecular mechanism mediating the adverse effects of exposure to prenatal infection

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TRANSCRIPTOME SIGNATURES OF SARS-COV-2 INFECTION IN ALZHEIMER'S DISEASE PATIENT-DERIVED OLFACTORY MUCOSAL CELLS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Loss of olfaction is a consequence of SARS-CoV-2 infection, suggesting viral invasion of the brain via the olfactory system. However, the underlying mechanisms remain unclear. This project utilizes a novel 3D *in vitro* model of the olfactory mucosa from non-demented individuals and Alzheimer's disease (AD) patients to provide key insights into SARS-CoV-2 infection at this key entry site of airborne viruses. We developed a 3D model consisting of primary human olfactory mucosal cells collected from nasal biopsies and cultured at air-liquid interface. Cells grown for 21 days were infected with SARS-CoV-2 and cell phenotype and function were assessed. Infection was determined by viral RNA content and SARS-CoV-2 receptor expression by immunocytochemistry. Differential responses of healthy and AD-patient derived cells to SARS-CoV-2 were determined by RNA-sequencing. Primary olfactory mucosal cells express several known SARS-CoV-2 receptors and are highly vulnerable to infection. AD-patient derived cells display alterations in the cellular responses to the virus. RNA analyses revealed distinct profiles of susceptibility to infection and COVID19-associated biomarkers including cathepsins. Our data demonstrate that AD olfactory mucosal cells display a differential response to infection than healthy cells. These data provide important insight into the mechanisms of SARS-CoV-2 infection at a key entry point of airborne viruses. It also provides new targets for limiting or intervening with adverse effects of viral infection at the olfactory mucosa of AD patients.

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A SYSTEMS BIOLOGY METHODOLOGY TO DRUG DISCOVERY FOR NEUROTROPIC VIRUS INFECTION WITH CLINICAL CORROBORATION

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Aims: SARS-Cov2-virus can cause neuroinvasion leading to central respiratory-failure. Polyketide class of drugs is effective against neurotropic viral diseases, as HIV, Japanese-Encephalitis, and Simian Immunodeficiency-virus. Furthermore, beta-lactams (cefems) can be also given for combination therapy. Anti-viral pharmacological agents can provide two-modes of action: virucidal vis-a-vis virostatic. We aim to determine how combined polyketide and beta-lactam drugs can mitigate neurotropic-viral infection. **Method:** To gauge anatomical substrate of neurotropic invasion, the possible viral migration pathways between cranial nerves and brain-stem/limbic region were assessed by fiber-tractography. Furthermore, pharmacological docking analyses were performed to estimate drug-binding affinity of polyketide molecules and beta-lactam molecules towards (i) Human host-receptor [Angiotensin-Converting-Enzyme-2], (ii) Main protease of SARS-CoV-2 virus, as an exemplar. We develop a bi-exponential mathematical-model of antiviral combination pharmacotherapy, to formulate a quantitative framework of combination drugs on virus and human host-cell. **Results:** Our MRI-DTI tractography analysis shows connection between pulmonary/respiratory neural system and different brain nuclei, showing anatomical substrate of signs/symptoms of viral-infected patients. Viral neuroinvasion route was inferred from the interlinkages as estimated by neuroimaging connectometry parameters. Moreover, pharmacological docking-studies revealed higher binding affinity of polyketide drugs and beta-lactam drugs towards both: (i) Virus-receptor, (ii) Human host-cell receptors where virus-receptor binds itself. Contrastingly, single pharmacological agent as cephem showed significantly less affinity towards host-receptor. The bi-exponential mathematical equation is validated using findings of clinical trial using combination therapy on neurotropic virus as JE-virus ($n=112$, $p<0.05$). **Conclusion:** Systems Biology analysis optimizes the combination pharmacotherapy efficiency in viral neuroinfection, as validated by neuroimaging investigation and clinical trial.

Pubmed:
34297965: Bhattacharjee A, Prajapati SK, Krishnamurthy S
Supplementation of taurine improves ionic homeostasis and mitochondrial function in the rats exhibiting post-traumatic stress disorder-like symptoms.
Current pharmacotherapy for post-traumatic stress disorder (PTSD) is limited to few antidepressants. Mitochondrial dysfunction is observed in PTSD, along with altered potassium homeostasis. Nutritional supplementation of taurine can improve ionic homeostasis and thereby treat PTSD-like symptoms in rats.
Eur J Pharmacol, 2021; 908

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BOARD NUMBER: S07-508

AGE-DEPENDENT MENINGEAL MACROPHAGES PROTECT AGAINST VIRAL NEUROINFECTION

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Due to the vital importance of the Central Nervous System (CNS), its potential infection and inflammation have to be tightly controlled. The surface of the CNS is connected to the periphery by a rich and complex tissue, the meninges. They contain a vast network of macrophages subdivided in at least two subpopulations endowed with elusive functions: a neonatal, MHC-II negative macrophage population, and an age-dependent population expressing MHC-II. Using *in situ*-histocytometry, flow cytometry, and single-cell RNA sequencing approaches, we show that those populations have opposite dynamic behaviors in response to *in vivo* peripheral challenges such as LPS, SARS-CoV2 and lymphocytic choriomeningitis virus (LCMV), with an apparent contraction of the MHC-II+ population. Focusing on LCMV infection in experimental mouse models and using innovative pharmacological and genetic depletion strategies, we show that MM represent an early line of protection against this neuroinvasive pathogen. In their absence, specific areas in the meninges become highly infected, leading to fatal brain disease. While their intrinsic sensing of viral replication through the Mitochondrial antiviral-signaling protein (MAVS) is dispensable, sensing of IFNs through the STAT1 pathway plays an important role in controlling viral spread. Unexpectedly, the age-dependent MHC-II+ macrophage population has a major role in controlling neuroinfection, and this is independent of the MHC-II molecule itself. This work helps understand the spatial organization of the brain defense system and the cellular and molecular mechanisms involved in CNS protection.

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UNRAVELING THE MOLECULAR MECHANISMS UNDERLYING PARASITE-HOST INTERACTION IN TAENIA SOLIUM NEUROCYSTICERCOSIS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Neurocysticercosis is the most frequent parasitic disease in the human CNS. It is most prevalent in low and middle-income countries where poor sanitation is common. The analysis of the transcriptome of the host's brain, adjacent to the cyst, and how the cyst changes throughout infection would help unravel the parasite-host immune systems interactions and facilitate understanding of the resulting disease. However, despite the previous initiatives to sequence the *Taenia solium* genome, it is still not entirely resolved. Thus, in the present work, we seek to improve the annotation of the Taenia solium genome using public transcriptome data (SRX1899230). We followed Ji *et al.*,2018 pipeline for alignment (HISAT2), assembly (Stringtie; QUAPRA), and creation of a new *gtf* file (Cuffcompare) generating ~3,200 potentially new transcripts. Furthermore, the new transcripts with FPKM > 1 were submitted to CPAT using a *C. elegans* training dataset, classifying ~900 above the coding-score cutoff. The UniProtKb/SwissProt curated proteome database found ~600 new mRNAs highly similar to cestoda species or *C. elegans*. Transcripts below the CPAT coding score cutoff that also do not present similarity with any known protein will be considered as potential non-coding genes. In conclusion, our adapted pipeline for discovering new genetic elements based on transcriptomic data demonstrated excellent potential for improving the current *T. solium* reference genome annotation, with the possibility of including at least 600 new protein-coding genes. The next steps are to quantify the potential non-coding transcripts, annotate a new *gtf* file and start the neuro-experimental part of the project.

Pubmed:
35061107: Pascoal VDB, Marchesini RB, Athié MCP, Matos AHB, Conte FF, Pereira TC, Secolin R, Gilioli R, Malheiros JM, Polli RS, Tannús A, Covolan L, Pascoal LB, Vieira AS, Cavalheiro EA, Cendes F, Lopes-Cendes I
Modulating Expression of Endogenous Interleukin 1 Beta in the Acute Phase of the Pilocarpine Model of Epilepsy May Change Animal Survival.
The pilocarpine-induced (PILO) model has helped elucidate the electrophysiological and molecular aspects related to mesial temporal lobe epilepsy. It has been suggested that the extensive cell death and edema observed in the brains of these animals could be induced by increased inflammatory responses, such as the rapid release of the inflammatory cytokine interleukin 1 beta (Il1b). In this study, we investigate the role of endogenous Il1b in the acute phase of the PILO model. Our aim is twofold. First, we want to determine whether it is feasible to silence Il1b in the central nervous system using a non-invasive procedure. Second, we aim to investigate the effect of silencing endogenous Il1b and its antagonist, Il1rn.We used RNA interference applied non-invasively to knockdown Il1b and its endogenous antagonist Il1rn. We found that knocking down Il1b prior to pilocarpine injection increased the mortality rate of treated animals. Furthermore, we observed that, when exposing the animals to more Il1b by silencing its endogenous antagonist Il1rn, there was a better response to status epilepticus with decreased animal mortality in the acute phase of the PILO model. Thus, we show the feasibility of using a novel, less invasive approach to study genes involved in the inflammatory response in the central nervous system. Furthermore, our results provide suggestive evidence that modulating endogenous Il1b improves animal survival in the acute phase of the PILO model and may have effects that extend into the chronic phase.
Cell Mol Neurobiol, 2022;
34957478: Avansini SH, Puppo F, Adams JW, Vieira AS, Coan AC, Rogerio F, Torres FR, Araújo PAOR, Martin M, Montenegro MA, Yasuda CL, Tedeschi H, Ghizoni E, França AFEC, Alvim MKM, Athié MC, Rocha CS, Almeida VS, Dias EV, Delay L, Molina E, Yaksh TL, Cendes F, Lopes Cendes I, Muotri AR
Junctional instability in neuroepithelium and network hyperexcitability in a focal cortical dysplasia human model.
Focal cortical dysplasia (FCD) is a highly epileptogenic cortical malformation with few treatment options. Here we generated human cortical organoids from patients with FCD type II. Using this human model, we mimicked some FCD hallmarks, such

as impaired cell proliferation, the presence of dysmorphic neurons and balloon cells, and neuronal network hyperexcitability. Furthermore, we observed alterations in the adherens junctions zonula occludens-1 and partitioning defective 3, reduced polarization of the actin cytoskeleton, and fewer synaptic puncta. FCD cortical organoids showed downregulation of the small GTPase RHO A, a finding that was confirmed in brain tissue resected from these patients. Functionally, both spontaneous and optogenetically-evoked electrical activity revealed hyperexcitability and enhanced network connectivity in FCD organoids. Taken together, our findings suggest a ventricular zone instability in tissue cohesion of neuroepithelial cells, leading to a maturational arrest of progenitors or newborn neurons, which may predispose to cellular and functional immaturity and compromise the formation of neural networks in FCD.
Brain, 2021;
31400936: Magalhães PHM, Moraes HT, Athie MCP, Secolin R, Lopes-Cendes I
New avenues in molecular genetics for the diagnosis and application of therapeutics to the epilepsies.
Genetic epidemiology studies have shown that most epilepsies involve some genetic cause. In addition, twin studies have helped strengthen the hypothesis that in most patients with epilepsy, a complex inheritance is involved. More recently, with the development of high-density single-nucleotide polymorphism (SNP) microarrays and next-generation sequencing (NGS) technologies, the discovery of genes related to the epilepsies has accelerated tremendously. Especially, the use of whole exome sequencing (WES) has had a considerable impact on the identification of rare genetic variants with large effect sizes, including inherited or de novo mutations in severe forms of childhood epilepsies. The identification of pathogenic variants in patients with these childhood epilepsies provides many benefits for patients and families, such as the confirmation of the genetic nature of the diseases. This process will allow for better genetic counseling, more accurate therapy decisions, and a significant positive emotional impact. However, to study the genetic component of the more common forms of epilepsy, the use of high-density SNP arrays in genome-wide association studies (GWAS) seems to be the strategy of choice. As such, researchers can identify loci containing genetic variants associated with the common forms of epilepsy. The knowledge generated over the past two decades about the effects of the mutations that cause the monogenic epilepsy is tremendous; however, the scientific community is just starting to apply this information in order to generate better target treatments.
Epilepsy Behav, 2021; 121
30537520: Teixeira JM, Dos Santos GG, Neves AF, Athie MCP, Bonet IJM, Nishijima CM, Farias FH, Figueiredo JG, Hernandez-Olmos V, Alshaibani S, Tambeli CH, Müller CE, Parada CA
Diabetes-induced Neuropathic Mechanical Hyperalgesia Depends on P2X4 Receptor Activation in Dorsal Root Ganglia.
Peripheral diabetic neuropathy (PDN) manifests in 50-60% of type I and II diabetic patients and is the major cause of limb amputation. Adequate therapy for PDN is a current challenge. There are evidences that the activation of the P2X4 receptor (P2X4R) expressed on microglial cells of the central nervous system takes part in the development of neuropathic pain. However, there is an open question: Is P2X4R activation on dorsal root ganglia (DRG) involved in the development of neuropathic pain? To answer this question, this study verified the involvement of P2X4R expressed in DRG cells on diabetes-induced neuropathic mechanical hyperalgesia in rats. We found that intrathecal or ganglionar (L5-DRG) administration of a novel P2X4R antagonist (PSB-15417) or intrathecal administration of oligodeoxynucleotides (ODN)-antisense against the P2X4R reversed diabetes-induced neuropathic mechanical hyperalgesia. The DRG of the diabetic neuropathic rats showed an increase in P2X4R expression, and the DRG immunofluorescence suggested that P2X4R is expressed mainly in satellite glial cells (SGC). Finally, our study showed a functional expression of P2X4R in SGCs of the rat's DRG, because the P2X4R agonist BzATP elicits an increase in intracellular calcium concentration in SGCs, which was reduced by PSB-15417. These findings indicate that P2X4R activation in DRG is essential to diabetes-induced neuropathic mechanical hyperalgesia. Therefore, this purinergic receptor in DRG could be an interesting therapeutic target for quaternary P2X4R antagonists that do not cross the hematoencephalic barrier, for the control of neuropathic pain, preserving central nervous system functions.
Neuroscience, 2019; 398
29750992: Athie MCP, Vieira AS, Teixeira JM, Dos Santos GG, Dias EV, Tambeli CH, Sartori CR, Parada CA
Transcriptome analysis of dorsal root ganglia's diabetic neuropathy reveals mechanisms involved in pain and regeneration.
Peripheral diabetic neuropathy (DN) manifests in nearly 60% of diabetic patients, being pain its most debilitating symptom. Although electrophysiological and morphological aspects are well described, little is known about its development and progression, undermining effective therapies. Hyperglycemia and insulin signaling impairment are considered the triggering events of oxidative stress observed in the dying nerves, however there are still many gaps in the knowledge of intracellular plastic changes it generates.
Life Sci, 2018; 205
29847798: Inquimbert P, Moll M, Latremoliere A, Tong CK, Whang J, Sheehan GF, Smith BM, Korb E, Athié MCP, Babaniyi O, Ghasemlou N, Yanagawa Y, Allis CD, Hof PR, Scholz J
NMDA Receptor Activation Underlies the Loss of Spinal Dorsal Horn Neurons and the Transition to Persistent Pain after Peripheral Nerve Injury.

Peripheral nerve lesions provoke apoptosis in the dorsal horn of the spinal cord. The cause of cell death, the involvement of neurons, and the relevance for the processing of somatosensory information are controversial. Here, we demonstrate in a mouse model of sciatic nerve injury that glutamate-induced neurodegeneration and loss of γ-aminobutyric acid (GABA)ergic interneurons in the superficial dorsal horn promote the transition from acute to chronic neuropathic pain. Conditional deletion of Grin1, the essential subunit of N-methyl-d-aspartate-type glutamate receptors (NMDARs), protects dorsal horn neurons from excitotoxicity and preserves GABAergic inhibition. Mice deficient in functional NMDARs exhibit normal nociceptive responses and acute pain after nerve injury, but this initial increase in pain sensitivity is reversible. Eliminating NMDARs fully prevents persistent pain-like behavior. Reduced pain in mice lacking proapoptotic Bax confirmed the significance of neurodegeneration. We conclude that NMDAR-mediated neuron death contributes to the development of chronic neuropathic pain.
Cell Rep, 2018; 23
26173870: Dias EV, Sartori CR, Marião PR, Vieira AS, Camargo LC, Athie MC, Pagliusi MO, Tambeli CH, Parada CA
Nucleus accumbens dopaminergic neurotransmission switches its modulatory action in chronification of inflammatory hyperalgesia.
Dopaminergic neurotransmission in the nucleus accumbens, a central component of the mesolimbic system, has been associated with acute pain modulation. As there is a transition from acute to chronic pain ('chronification'), modulatory structures may be involved in chronic pain development. Thus, this study aimed to elucidate the role of nucleus accumbens dopaminergic neurotransmission in chronification of pain. We used a rat model in which daily subcutaneous injection of prostaglandin E2 in the hindpaw for 14 days induces a long-lasting state of nociceptor sensitization that lasts for at least 30 days following the end of the treatment. Our findings demonstrated that the increase of dopamine in the nucleus accumbens by local administration of GBR12909 (0.5 nmol/0.25 μL), a dopamine reuptake inhibitor, blocked prostaglandin E2 -induced acute hyperalgesia. This blockade was prevented by a dopamine D2 receptor antagonist (raclopride, 10 nmol/0.25 μL) but not changed by a D1 receptor antagonist (SCH23390, 0.5, 3 or 10 nmol/0.25 μL), both co-administered with GBR12909 in the nucleus accumbens. In contrast, the induction of persistent hyperalgesia was facilitated by continuous infusion of GBR12909 in the nucleus accumbens (0.021 nmol/0.5 μL/h) over 7 days of prostaglandin E2 treatment. The development of persistent hyperalgesia was impaired by SCH23390 (0.125 nmol/0.5 μL/h) and raclopride (0.416 nmol/0.5 μL/h), both administered continuously in the nucleus accumbens over 7 days. Taken together, our data suggest that the chronification of pain involves the plasticity of dopaminergic neurotransmission in the nucleus accumbens, which switches its modulatory role from antinociceptive to pronociceptive.
Eur J Neurosci, 2015; 42
25451282: Miranda J, Lamana SM, Dias EV, Athie M, Parada CA, Tambeli CH
Effect of pain chronification and chronic pain on an endogenous pain modulation circuit in rats.
We tested the hypothesis that chronic pain development (pain chronification) and ongoing chronic pain (chronic pain) reduce the activity and induce plastic changes in an endogenous analgesia circuit, the ascending nociceptive control. An important mechanism mediating this form of endogenous analgesia, referred to as capsaicin-induced analgesia, is its dependence on nucleus accumbens μ-opioid receptor mechanisms. Therefore, we also investigated whether pain chronification and chronic pain alter the requirement for nucleus accumbens μ-opioid receptor mechanisms in capsaicin-induced analgesia. We used an animal model of pain chronification in which daily subcutaneous prostaglandin E2 (PGE2) injections into the rat's hind paw for 14 days, referred to as the induction period of persistent hyperalgesia, induce a long-lasting state of nociceptor sensitization referred to as the maintenance period of persistent hyperalgesia, that lasts for at least 30 days following the cessation of the PGE2 treatment. The nociceptor hypersensitivity was measured by the shortening of the time interval for the animal to respond to a mechanical stimulation of the hind paw. We found a significant reduction in the duration of capsaicin-induced analgesia during the induction and maintenance period of persistent mechanical hyperalgesia. Intra-accumbens injection of the μ-opioid receptor selective antagonist Cys(2),Tyr(3),Orn(5),Pen(7)amide (CTOP) 10 min before the subcutaneous injection of capsaicin into the rat's fore paw blocked capsaicin-induced analgesia. Taken together, these findings indicate that pain chronification and chronic pain reduce the duration of capsaicin-induced analgesia, without affecting its dependence on nucleus accumbens μ-opioid receptor mechanisms. The attenuation of endogenous analgesia during pain chronification and chronic pain suggests that endogenous pain circuits play an important role in the development and maintenance of chronic pain.
Neuroscience, 2015; 286
25058903: dos Santos GG, Dias EV, Teixeira JM, Athie MC, Bonet IJ, Tambeli CH, Parada CA
The analgesic effect of dipyrone in peripheral tissue involves two different mechanisms: neuronal K(ATP) channel opening and CB(1) receptor activation.
Dipyrone (metamizole) is an analgesic pro-drug used to control moderate pain. It is metabolized in two major bioactive metabolites: 4-methylaminoantipyrine (4-MAA) and 4-aminoantipyrine (4-AA). The aim of this study was to investigate the

participation of peripheral CB1 and CB2 cannabinoid receptors activation in the anti-hyperalgesic effect of dipyrone, 4-MAA or 4-AA. PGE2 (100ng/50µL/paw) was locally administered in the hindpaw of male Wistar rats, and the mechanical nociceptive threshold was quantified by electronic von Frey test, before and 3h after its injection. Dipyrone, 4-MAA or 4-AA was administered 30min before the von Frey test. The selective CB1 receptor antagonist AM251, CB2 receptor antagonist AM630, cGMP inhibitor ODQ or KATP channel blocker glibenclamide were administered 30min before dipyrone, 4-MAA or 4-AA. The antisense-ODN against CB1 receptor expression was intrathecally administered once a day during four consecutive days. PGE2-induced mechanical hyperalgesia was inhibited by dipyrone, 4-MAA, and 4-AA in a dose-response manner. AM251 or ODN anti-sense against neuronal CB1 receptor, but not AM630, reversed the anti-hyperalgesic effect mediated by 4-AA, but not by dipyrone or 4-MAA. On the other hand, the anti-hyperalgesic effect of dipyrone or 4-MAA was reversed by glibenclamide or ODQ. These results suggest that the activation of neuronal CB1, but not CB2 receptor, in peripheral tissue is involved in the anti-hyperalgesic effect of 4-aminoantipyrine. In addition, 4-methylaminoantipyrine mediates the anti-hyperalgesic effect by cGMP activation and KATP opening.
Eur J Pharmacol, 2014; 741
23401543: Araldi D, Ferrari LF, Lotufo CM, Vieira AS, Athié MC, Figueiredo JG, Duarte DB, Tambeli CH, Ferreira SH, Parada CA
Peripheral inflammatory hyperalgesia depends on the COX increase in the dorsal root ganglion.
It is well established that dorsal root ganglion (DRG) cells synthesize prostaglandin. However, the role that prostaglandin plays in the inflammatory hyperalgesia of peripheral tissue has not been established. Recently, we have successfully established a technique to inject drugs (3 µL) directly into the L5-DRG of rats, allowing in vivo identification of the role that DRG cell-derived COX-1 and COX-2 play in the development of inflammatory hyperalgesia of peripheral tissue. IL-1β (0.5 pg) or carrageenan (100 ng) was administered in the L5-peripheral field of rat hindpaw and mechanical hyperalgesia was evaluated after 3 h. Administration of a nonselective COX inhibitor (indomethacin), selective COX-1 (valeryl salicylate), or selective COX-2 (SC-236) inhibitors into the L5-DRG prevented the hyperalgesia induced by IL-1β. Similarly, oligodeoxynucleotide-antisense against COX-1 or COX-2, but not oligodeoxynucleotide-mismatch, decreased their respective expressions in the L5-DRG and prevented the hyperalgesia induced by IL-1β in the hindpaw. Immunofluorescence analysis demonstrated that the amount of COX-1 and COX-2, constitutively expressed in TRPV-1(+) cells of the DRG, significantly increased after carrageenan or IL-1β administration. In addition, indomethacin administered into the L5-DRG prevented the increase of PKCε expression in DRG membrane cells induced by carrageenan. Finally, the administration of EP1/EP2 (7.5 ng) or EP4 (10 µg) receptor antagonists into L5-DRG prevented the hyperalgesia induced by IL-1β in the hindpaw. In conclusion, the results of this study suggest that the inflammatory hyperalgesia in peripheral tissue depends on activation of COX-1 and COX-2 in C-fibers, which contribute to the induction and maintenance of sensitization of primary sensory neurons.
Proc Natl Acad Sci U S A, 2013; 110

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BOARD NUMBER: S07-510

AUTONOMIC DISBALANCE DURING SYSTEMIC INFLAMMATION IS ASSOCIATED WITH OXIDATIVE STRESS CHANGES IN SEPSIS SURVIVOR RATS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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[1]Unversity of Sao Paulo, Dental School Of Ribeirao Preto, Ribeirao Preto, Brazil, [2]University of Sao Paulo, Dental School Of Ribeirao Preto, Ribeirao Preto, Brazil, [3]University of Sao Paulo, Nursing School Of Ribeirao Preto, Ribeirão Preto, Brazil

Sepsis is characterized by a drop in blood pressure driving to morbidity and mortality. Modern supportive care has increased survival; however, after sepsis, several complications are observed. Nevertheless, the interplay between sepsis survivors and a new immune challenge in cardiovascular regulation has not been previously defined. We hypothesized that cecal ligation and puncture (CLP) cause persistent cardiovascular dysfunctions and changes in autonomic-induced cardiovascular responses to lipopolysaccharide (LPS). Male rats had mean arterial pressure (MAP) and heart rate (HR) recorded before and after LPS administration to control or CLP survivor rats. CLP survivor rats had similar baseline MAP and HR. LPS caused a drop in MAP accompanied by tachycardia in control, while CLP survivor rats had a enhanced MAP and a blunted tachycardia. LPS-induced hemodynamic changes were related to an autonomic disbalance to the heart and resistance vessels that were expressed as an increased low- and high-frequency power of pulse interval in CLP survivors after saline and enhancement in the low-frequency power of systolic arterial pressure in control rats after LPS. LPS-induced plasma interferon γ, but not interleukin-10 surges, was blunted in CLP survivor rats. To further access if LPS-induced autonomic disbalance in CLP survivor rats was associated with oxidative stress dysregulation, superoxide dismutase (SOD) activity and thiobarbituric acid reactive substances (TBARS) plasma levels changes were measured. LPS-induced oxidative stress was higher in CLP survivor rats. The data indicate that changes in hemodynamic regulation of CLP survivors rats take place in response to LPS that are associated with oxidative stress.

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NOVEL STRATEGY FOR AMPLIFICATION OF ABETA-SPECIFIC REGULATORY T CELLS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Alzheimer's disease (AD) is characterised by cerebral amyloid-beta (Abeta) accumulation, microgliosis, neuroinflammation and brain infiltration of T lymphocytes. While effector T cells (Teff) exacerbate neuroinflammation and inhibit normal microglial phagocytic function, T regulatory cells (Treg) can dampen Teff activation and brain infiltration. However, AD patients have compromised Treg suppressive function on Teff. We hypothesised that rebalancing Treg/Teff may influence microglial phenotype toward resolution of pathology. Our aim was to establish a novel strategy for amplification of Abeta-specific Treg in the APP/PS1 mouse model of AD to assess if this could promote Abeta clearance by microglia. Eight-week-old APP/PS1 mice were immunised with Interleukin-2 (IL-2)+ Retinoic Acid (RA)+Abeta, Abeta alone, IL-2+RA or CT and aged until 9 months old. To validate Treg production, splenocytes were cultured and Interleukin-10 (IL-10), Interleukin-17 (IL-17) and Interferon-gamma (IFN-g) production in response to recall stimulation with Abeta was analysed by ELISA. Abundance and phenotype of CD4+ T cells in lymph nodes and the brain was evaluated by flow cytometry. In mice immunised with Abeta+IL-2+RA or Abeta alone, splenocytes produced IL-10 and IFN-γ upon recall stimulation with Abeta. A population of CD4+CD25+FoxP3+ Treg was increased in the lymph nodes with some expressing the checkpoint inhibitor CTLA4. CD4+ T cells infiltrated the brain in low numbers. Our new immunisation strategy allowed for generation of a memory Treg population, primed by Abeta and reactivated upon exposure to the antigen. The Treg generated produced IL-10 and IFN-g which is consistent with inducible Type 1 regulatory T cells.

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PREDICTING SARS-COV-2 SPREADING AND GREY MATTER LOSS IN THE HUMAN BRAIN

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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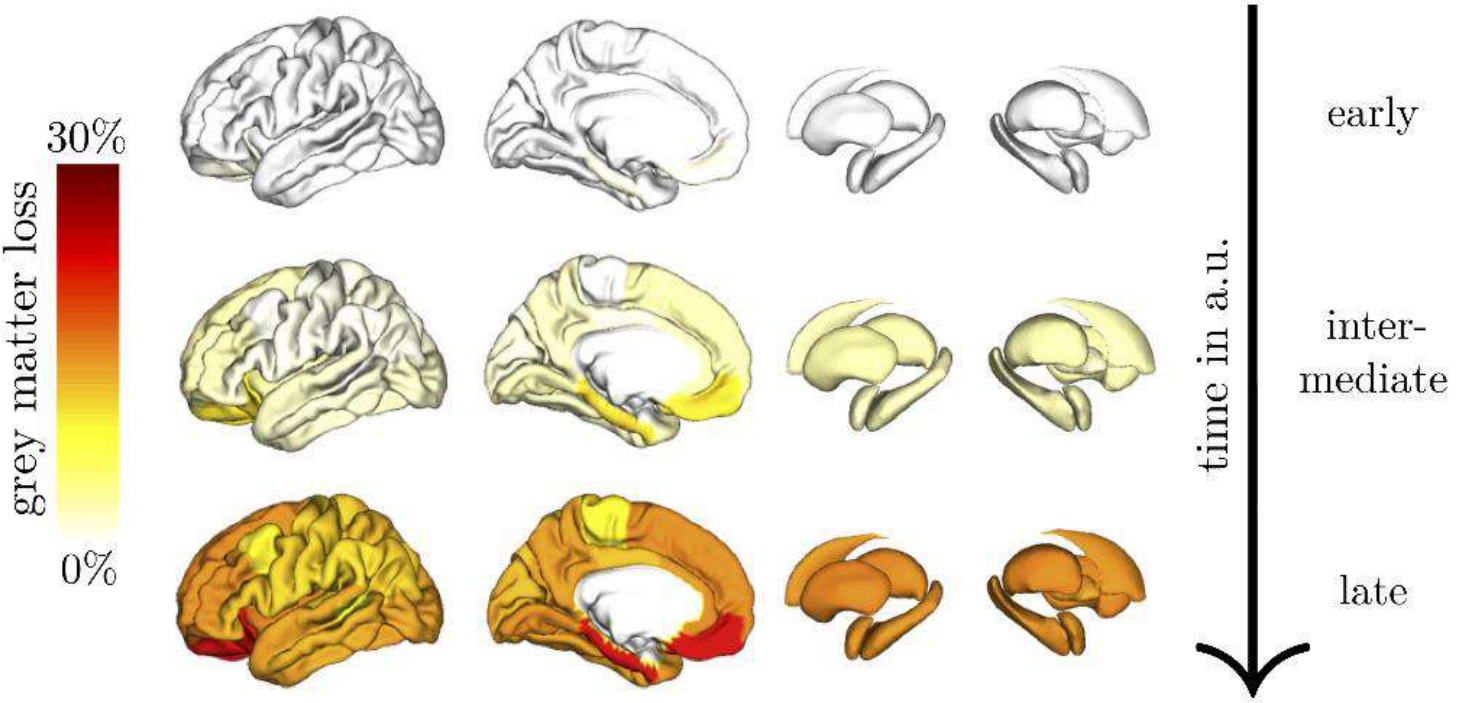
It is well established that SARS-CoV-2 can invade neurons and other cell types of the central nervous system. This has raised concern that SARS-CoV-2 could replicate inside the human brain and induce grey matter loss and ultimately cognitive decline and dementia. A wide range of viruses, including coronaviruses, have been shown to evade the blood-brain barrier and invade the central nervous system via axonal transport. However, the dynamics of SARS-CoV-2 spreading through the central nervous system and resulting tissue damage have not been adequately characterized. Here we use computational modeling and mathematical analysis to predict viral spreading and grey matter loss in the human brain. We predict the qualitative time course of SARS-CoV-2 accumulation and grey matter loss based on recent human connectome measurements. Depending on parameters, our model shows two qualitatively distinct spreading patterns. In the case of fast virus exchange between connected brain areas, a single brain-wide wave of virus proliferation and subsequent grey matter loss is observed. In the case of slow virus exchange between brain areas, we observe a characteristic two-phase pattern with a “first wave” exclusively affecting brain regions serving as entry points for the virus and a delayed “second wave” at the whole brain scale. These findings are in line with recent reports of diffuse brain atrophy and impaired performance on cognitive tasks in patients several months after COVID-19 infection. Further research is urgently needed to quantify SARS-CoV-2 spreading and brain alterations in human
patients.



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ROLE OF THE LIFESTYLE AND ENVIRONMENTAL FACTORS IN THE COMPLEXITY OF THE MIGRAINE AURA

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Aims: Environmental factors play an essential role in triggering migraine with aura (MwA). Also, lifestyle can tremendously influence the MwA frequency. Our goals were to evaluate how lifestyle and environmental factors influence the complexity of migraine aura and to explore changes in MwA frequency during the COVID-19 pandemic crisis. Methods: Data about aura features, environmental triggers, and lifestyle variables were obtained via an electronic questionnaire which MwA patients regularly filled after every MwA attack. Migraine Aura Complexity Score (MACS) was calculated for each attack to correlate MwA complexity with lifestyle and environmental factors. Results: Fifty-two MwA patients were included and 159 attacks were evaluated during the 2020-2021 period. MACS was positively correlated with increased physical effort and exposure to strong odors several hours before the MwA attack. The surface of the disturbed visual field by aura symptoms positively correlated with increased exposure to bright light. Report of visual higher cortical disturbances (HCDs) positively correlated with dehydration, while the higher incidence of somatosensory HCDs was connected to the increased physical effort before the MwA attack. Stress, food intake, fasting, alcohol, sleep disturbances, and weather changes did not correlate with MACS. COVID-19 pandemic crisis led to a decrease in MwA frequency (during 2019: 7.33±5.72 vs during COVID-19: 3.06±2.27 attacks per year, $p<0.001$). Conclusions: Increased physical effort, dehydration, exposure to strong odors, or bright light are linked to more complex symptoms of migraine aura. Lifestyle and changes in exposure to environmental factors during the COVID-19 crisis are connected to a decreased MwA frequency.

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THE INFLUENCE OF CARDIOVASCULAR HEALTH IN MIDDLE-AGED ADULT POPULATIONS ON COGNITIVE HEALTH IN LATER LIFE: A SYSTEMATIC REVIEW AND META-ANALYSIS

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Background: Hypertension is a known risk factor for cognitive deterioration and vascular dementia. Despite a large body of research, significant heterogeneity between studies has limited our ability in making causal inference about the association of hypertension with cognition at midlife. **Aim:** Perform a comprehensive analysis of the published evidence to ascertain the extent to which hypertension status during midlife impacts cognitive function. **Methods:** Online electronic databases EMBASE, MEDLINE, PubMed, Web of Science, and CINAHL were searched from their inception to January 2022. Studies which determined the relationship between hypertension status and cognition were eligible. **Results:** 113 studies were included (100,056 participants, weighted mean age: 55.7 ± 3.9 yrs), of which 46 were deemed high-quality. Hypertension status was assessed in 54 studies. Hypertension was most defined using the ESC definition (n= 29). A total of 41,675 participants were classified as hypertensive, 1,553 classified as pre-hypertensive and 3,459 were taking anti-hypertensive medication. A larger proportion of studies found no identifiable relationship between hypertension status with memory, attention, executive function, and global cognition. Due to the high levels of heterogeneity no causal association between hypertension and cognition could be determined. **Conclusion:** Current evidence suggests large caveats in understanding pathological mechanisms contributing to cognitive decline among hypertensives. Our results suggest mixed findings. Methodological differences diminished study quality and limited the formation of objective conclusions. Future researcher warrants the use of a more standardised battery of cognitive tests consistency of reporting to determine brain health and cognitive status alongside objective markers of ageing and CV health.

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PREVALENCE OF CARDIOVASCULAR DISEASE RISK FACTORS AMONG PROFESSIONAL RUGBY UNION ATHLETES: LINKING CARDIOVASCULAR AND COGNITIVE HEALTH IN PROFESSIONAL RUGBY.

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Background: Cardiovascular risk factors including hypertension and inflammation, are detrimental to general health, and particularly brain health, with potential cognitive impairment resulting. The cardiovascular and cognitive health implications associated with professional rugby is a cause of concern, given athletes' large size and the collision nature of the sport. Our aim was to assess the prevalence of cardiovascular risk factors in professional male rugby athletes. **Methods:** A cross-sectional study of 46 professional male rugby union athletes from a convenience sample from one European Rugby Championship Cup team (August 2020-February 2021) was conducted. The prevalence of traditional cardiovascular disease risk factors (family history, obesity, hypertension, dyslipidemia, glucose intolerance and smoking) and inflammatory biomarkers were examined. **Results:** 74% of athletes had at least one risk factor, with 50% having one- to -two risk factors. A subset of athletes (24%), predominately forwards, had high-risk (three- to -four risk factors). The most common risk factors were elevated CRP (65%), hypertension (30%), older age (20%), dyslipidemia, categorised by low HDL (17%), and elevated %BF (11%). Rugby athletes had significantly higher values of inflammation (CRP, ICAM-1 and VCAM-1) compared to healthy controls with one third demonstrating equivalent values to active rheumatoid arthritis patients. **Conclusion: S**ome rugby athletes are free from cardiovascular risk factors, but a large proportion are not, with almost 25% presenting with a high cardiovascular risk. The prevalence of hypertension and low-grade inflammation, coinciding with the collision-based nature of rugby, potentially exposes athletes to negative long-term cardiovascular and cognitive health implications that warrant further investigation.

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STREPTOCOCCUS AGALACTIAE BRAIN INVASION VIA THE OLFACTORY AND TRIGEMINAL NERVES

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

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Streptococcus agalactiae causes neonatal meningitis and can also infect the adult central nervous system. S. agalactiae can cross the blood-brain barrier but may also reach the central nervous system via other paths. Several species of bacteria can directly invade the central nervous system via the olfactory and trigeminal nerves, which extend between the nasal cavity and brain and injury to the nasal epithelium can increase the risk/severity of infection. We investigated whether S. agalactiae serotype III, which is epidemiologically the most relevant in neonatal and adult Group B streptococcal meningitis, can infect the brain via the olfactory and/or trigeminal nerves after intranasal inoculation in mice. Because epithelial injury is associated with increased infection of the olfactory nerve, we also determined the effects of prior experimental injury to the nasal epithelium on infection via this path. Additionally, glial response to bacterial invasion in both cranial nerves and the brain is vital, hence, we also determined how the glial cells of the olfactory/trigeminal nerves and glia limitans layer responded to the bacteria. We observed, S. agalactiae rapidly infected the olfactory nerve and brain, including the trigeminal nerve which was aggravated by epithelial injury. Our in vitro assay showed that the bacterial capsule significantly altered cytokine and chemokine responses and affected intracellular survival in trigeminal glia. Thus, this study shows that S. agalactiae can infect the central nervous system via the nose-to-brain path with increased load after epithelial injury, and that the bacteria can survive in glia.

Pubmed:

34021227: Choudhury IN, Chacko A, Delbaz A, Chen M, Basu S, St John JA, Huygens F, Ekberg JAK
Antimicrobial responses of peripheral and central nervous system glia against Staphylococcus aureus.
Staphylococcus aureus infections of the central nervous system are serious and can be fatal. S. aureus is commonly present in the nasal cavity, and after injury to the nasal epithelium it can rapidly invade the brain via the olfactory nerve. The trigeminal nerve constitutes another potential route of brain infection. The glia of these nerves, olfactory ensheathing cells (OECs) and trigeminal nerve Schwann cells (TgSCs), as well as astrocytes populating the glia limitans layer, can phagocytose bacteria. Whilst some glial responses to S. aureus have been studied, the specific responses of different glial types are unknown. Here, we compared how primary mouse OECs, TgSCs, astrocytes and microglia responded to S. aureus. All glial types internalized the bacteria within phagolysosomes, and S. aureus-conjugated BioParticles could be tracked with subtle but significant differences in time-course of phagocytosis between glial types. Live bacteria could be isolated from all glia after 24 h in culture, and microglia, OECs and TgSCs exhibited better protection against intracellular S. aureus survival than astrocytes. All glial types responded to the bacteria by cytokine secretion. Overall, OECs secreted the lowest level of cytokines, suggesting that these cells, despite showing strong capacity for phagocytosis, have immunomodulatory functions that can be relevant for neural repair.
Sci Rep, 2021; 11
33489937: Nazareth L, Walkden H, Chacko A, Delbaz A, Shelper T, Armitage CW, Reshamwala R, Trim LK, St John JA, Beagley KW, Ekberg JAK
Can Invade the Central Nervous System the Olfactory and Trigeminal Nerves and Infect Peripheral Nerve Glial Cells.
can infect the brain and has been linked to late-onset dementia. , which infects mice, is often used to model human chlamydial infections. While it has been suggested to be also important for modelling brain infection, nervous system infection by has not been reported in the literature. has been shown to infect the olfactory bulb in mice after intranasal inoculation, and has therefore been suggested to invade the brain the olfactory nerve; however, nerve infection has not been shown to date. Another path by which certain bacteria can reach the brain is the trigeminal nerve, but it remains unknown whether species can infect this nerve. Other bacteria that can invade the brain the olfactory and/or trigeminal nerve can do so rapidly,

however, whether spp. can reach the brain earlier than one-week post inoculation remains unknown. In the current study, we showed that can within 48 h invade the brain the olfactory nerve, in addition to infecting the trigeminal nerve. We also cultured the glial cells of the olfactory and trigeminal nerves and showed that readily infected the cells, constituting a possible cellular mechanism explaining how the bacteria can invade the nerves without being eliminated by glial immune functions. Further, we demonstrated that olfactory and trigeminal glia differed in their responses to , with olfactory glia showing less infection and stronger immune response than trigeminal glia.
Front Cell Infect Microbiol, 2020; 10
31978058: Walkden H, Delbaz A, Nazareth L, Batzloff M, Shelper T, Beacham IR, Chacko A, Shah M, Beagley KW, Tello Velasquez J, St John JA, Ekberg JAK
Burkholderia pseudomallei invades the olfactory nerve and bulb after epithelial injury in mice and causes the formation of multinucleated giant glial cells in vitro.
The infectious disease melioidosis is caused by the bacterium Burkholderia pseudomallei. Melioidosis is characterised by high mortality and morbidity and can involve the central nervous system (CNS). We have previously discovered that B. pseudomallei can infect the CNS via the olfactory and trigeminal nerves in mice. We have shown that the nerve path is dependent on mouse strain, with outbred mice showing resistance to olfactory nerve infection. Damage to the nasal epithelium by environmental factors is common, and we hypothesised that injury to the olfactory epithelium may increase the vulnerability of the olfactory nerve to microbial insult. We therefore investigated this, using outbred mice that were intranasally inoculated with B. pseudomallei, with or without methimazole-induced injury to the olfactory neuroepithelium. Methimazole-mediated injury resulted in increased B. pseudomallei invasion of the olfactory epithelium, and only in pre-injured animals were bacteria found in the olfactory nerve and bulb. In vitro assays demonstrated that B. pseudomallei readily infected glial cells isolated from the olfactory and trigeminal nerves (olfactory ensheathing cells and trigeminal Schwann cells, respectively). Bacteria were degraded by some cells but persisted in other cells, which led to the formation of multinucleated giant cells (MNGCs), with olfactory ensheathing cells less likely to form MNGCs than Schwann cells. Double Cap mutant bacteria, lacking the protein BimA, did not form MNGCs. These data suggest that injuries to the olfactory epithelium expose the primary olfactory nervous system to bacterial invasion, which can then result in CNS infection with potential pathogenic consequences for the glial cells.
PLoS Negl Trop Dis, 2020; 14
31964742: Delbaz A, Chen M, Jen FE, Schulz BL, Gorse AD, Jennings MP, St John JA, Ekberg JAK
Neisseria meningitidis Induces Pathology-Associated Cellular and Molecular Changes in Trigeminal Schwann Cells.
, a common cause of sepsis and bacterial meningitis, infects the meninges and central nervous system (CNS), primarily via paracellular traversal across the blood-brain barrier (BBB) or blood-cerebrospinal fluid barrier. is often present asymptomatically in the nasopharynx, and the nerves extending between the nasal cavity and the brain constitute an alternative route by which the meningococci may reach the CNS. To date, the cellular mechanisms involved in nerve infection are not fully understood. Peripheral nerve glial cells are phagocytic and are capable of eliminating microorganisms, but some pathogens may be able to overcome this protection mechanism and instead infect the glia, causing cell death or pathology. Here, we show that readily infects trigeminal Schwann cells (the glial cells of the trigeminal nerve) in both two-dimensional and three-dimensional cell cultures. Infection of trigeminal Schwann cells may be one mechanism by which is able to invade the CNS. Infection of the cells led to multinucleation and the appearance of atypical nuclei, with the presence of horseshoe nuclei and the budding of nuclei increasing over time. Using sequential window acquisition of all theoretical mass spectra (SWATH-MS) proteomics followed by bioinformatics pathway analysis, we showed that induced protein alterations in the glia that were associated with altered intercellular signaling, cell-cell interactions, and cellular movement. The analysis also suggested that the alterations in protein levels were consistent with changes occurring in cancer. Thus, infection of the trigeminal nerve by may have ongoing adverse effects on the biology of Schwann cells, which may lead to pathology.
Infect Immun, 2020; 88
31632194: Murtaza M, Chacko A, Delbaz A, Reshamwala R, Rayfield A, McMonagle B, St John JA, Ekberg JAK
Why are olfactory ensheathing cell tumors so rare?
The glial cells of the primary olfactory nervous system, olfactory ensheathing cells (OECs), are unusual in that they rarely form tumors. Only 11 cases, all of which were benign, have been reported to date. In fact, the existence of OEC tumors has been debated as the tumors closely resemble schwannomas (Schwann cell tumors), and there is no definite method for distinguishing the two tumor types. OEC transplantation is a promising therapeutic approach for nervous system injuries, and the fact that OECs are not prone to tumorigenesis is therefore vital. However, why OECs are so resistant to neoplastic transformation remains unknown. The primary olfactory nervous system is a highly dynamic region which continuously undergoes regeneration and neurogenesis throughout life. OECs have key roles in this process, providing structural and neurotrophic support as well as phagocytosing the axonal debris resulting from turnover of neurons. The olfactory mucosa and underlying tissue is also frequently exposed to infectious agents, and OECs have key innate immune roles preventing

microbes from invading the central nervous system. It is possible that the unique biological functions of OECs, as well as the dynamic nature of the primary olfactory nervous system, relate to the low incidence of OEC tumors. Here, we summarize the known case reports of OEC tumors, discuss the difficulties of correctly diagnosing them, and examine the possible reasons for their rare incidence. Understanding why OECs rarely form tumors may open avenues for new strategies to combat tumorigenesis in other regions of the nervous system.
Cancer Cell Int, 2019; 19
25414786: Aghasadeghi MR, Delbaz SA, Sadat SM, Siadat SD, Ardestani MS, Rahimi P, Bolhassani A, Roudsari RV, Bahramali G, Motevalli F, Davari M, Vakily H, Salmani AS, Nobari MB
Induction of Strong and Specific Humoral and T-helper 1 Cellular Responses by HBsAg Entrapped in the Methanobrevibacter smithii Archaeosomes.
Application of adjuvants with microbial origins is a recently highlighted approach in the vaccinology trials. Archaeosomes are among these microbial compounds with both adjuvant and liposomal activities and features.
Avicenna J Med Biotechnol, 2014; 6
23745776: Amanlou M, Mahian H, Rad AM, Delbaz SA, Ebrahimi SE, Javidi A, Arabzadeh AJ, Ardestani MS
Gadolinium-deferasirox-D-glucosamine: novel anti-tumor and MR molecular (theranostic) imaging agent.
TARGET AND PURPOSE: Cancer and heart disease are hard maladies in human communities. To recognize these kinds of diseases in primary states can help for remission and decreasing the expenses. One of the best techniques for recognizing is imaging of the tissue.
Curr Radiopharm, 2013; 6
22974294: Amanlou M, Heidari Z, Siadat SD, Aghasadeghi MR, Ghorbani M, Ebrahimi SE, Sadat SM, Hajmohammadi M, Arabzadeh AJ, Hekmat S, Alaei-Beirami M, Saraji AA, Moghaddam HF, Alavidjeh MS, Delbaz SA, Dashtbani-Roozbehani A, Ardestani MS
Nanosized tamoxifen-porphyrin-glucose [TPG] conjugate: novel selective anti-breast-cancer agent, synthesis and in vitro evaluations.
Tumor and especially breast cancer is among the most common causes of death worldwide. Finding novel nanosized therapeutic compounds have important role to decrease the chance of death and increase the survival. Cancer cells are highly attractive to glucose [with a nanosize bimolecular structure 1nm] as an energy source more than normal cell and nanosized therapeutics due to possessing different pharmacokinetic and pharmacodynamic have advantageous over classical dosage forms in cancer therapy. The aim of the study was to synthesize Glucosamin-Porphyrin-Tamoxifen [TPG] nanosized complex as a novel selective biocompatible anti breast cancer agent. After the synthesis procedure, this complex was purified and then tested In Vitro on breast cancer cells [MCF-7] in the absence or presence of the red light and found totally successful. The results showed a good anti breast cancer activity mediated by the activation of TNF-α and necrosis/apoptosis pathways for the nanosized complex with no alteration effects on blood PT/APTT and glucose or hexokinase levels/ activity. TPG nanoconjugate seems to be very good opponents to current anti breast cancer drugs and needs to be further investigated in near future.
Med Chem, 2013; 9

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THE ROLE OF ANTI-FGFR3 ANTIBODIES IN SENSITIVE NEURONOPATHIES NEURONAL CELL DEATH

POSTER SESSION 07 - SECTION: NEUROIMMUNOLOGY AND NEUROINFLAMMATION

Yara Nasser, Jean-Philippe Camdessanché, Julien Falk, Jean-Christophe Antoine, Nadia Boutahar
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Dysimmune sensitive neuropathies (SSN) are characterized by neuron cell death in dorsal root ganglia. We have identified FGFR3 antibodies which recognized the intracellular domain (TRK) of FGFR3 as a biomarker of a subset of SSN patients. We submitted mice cortical neurons cultures to different concentrations of a rabbit polyclonal antibody recognizing the FGFR3 intracellular domain. FGFR3 antibody induced neuron cell death in a dose dependent manner. In order to decipher the molecular mechanism involved in neuronal death induced by FGFR3 antibodies, we submitted neuron cultures to FGFR3 antibodies with and without the presence of the p38 MAPK inhibitor or the ERK1/2 MAPK inhibitor. FGFR3 antibodies treatment increased FGFR3 receptor, NMDA receptor subunits and AMPA receptor subunits expression and this increase was prevented by ERK1/2 or P38 MAPK inhibitor. These results suggest that cytotoxicity induced by FGFR3 antibodies increases NMDA and AMPA receptor expression through FGFR3 tyrosine kinase site blocking and RAS/MAP kinase pathway activation. FGFR3 antibodies recognize the intracellular domain of FGFR3; this suggests the antibodies internalization which can enhance autophagy. The analysis of several autophagy markers showed that optineurin and p62 expression increased in neuron cultures submitted to FGFR3 antibodies and this increase was prevented by ERK1/2 or P38 MAPK inhibitor suggesting that autophagy activation may also play a role in neuron degeneration. A similar expression profile of FGFR3, NMDA subunits and autophagy markers was observed in SSN serum-treated mice cortical neurons and sensory neurons cell cultures when compared to healthy donors' serum-treated cultures.

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BOARD NUMBER: S07-518

NR5A2 AS A POTENTIAL DRUG TARGET IN GLIOBLASTOMAS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Glioblastomas are nervous system tumors that originate from astrocytes and neural stem or progenitor cells. They are characterized by rapid progression and poor survival rates. Despite recent advances in the provided therapy, the average survival time remains extremely low, between 12 to 15 months. These clinical observations underscore the need for novel therapeutic insights and pharmacological targets. To this end, here we identify the orphan nuclear receptor NR5A2/LRH1 as a negative regulator of cancer cell proliferation and promising pharmacological target for nervous system-related tumors. In particular, by meta-analysing clinical data from TCGA and Oncomine databases, we find that high expression levels of NR5A2 are associated with favourable prognosis in patients with glioblastoma tumors. In addition, we experimentally show that two well-established pharmacological agonists of NR5A2, DLPC and DUPC, are able to inhibit proliferation of neural stem cells and glioblastoma cells through NR5A2 activation. Most importantly, treatment with DLPC inhibits glioblastoma tumor growth *in vivo*, in heterotopic and orthotopic xenograft mouse models. These data render NR5A2 as a potential pharmacological target for the treatment of nervous tissue related tumors.

Pubmed:
34561301: Gkikas D, Stellas D, Polissidis A, Manolakou T, Kokotou MG, Kokotos G, Politis PK
Nuclear receptor NR5A2 negatively regulates cell proliferation and tumor growth in nervous system malignancies.
Nervous system malignancies are characterized by rapid progression and poor survival rates. These clinical observations underscore the need for novel therapeutic insights and pharmacological targets. To this end, here, we identify the orphan nuclear receptor NR5A2/LRH1 as a negative regulator of cancer cell proliferation and promising pharmacological target for nervous system-related tumors. In particular, clinical data from publicly available databases suggest that high expression levels of NR5A2 are associated with favorable prognosis in patients with glioblastoma and neuroblastoma tumors. Consistently, we experimentally show that NR5A2 is sufficient to strongly suppress proliferation of both human and mouse glioblastoma and neuroblastoma cells without inducing apoptosis. Moreover, short hairpin RNA-mediated knockdown of the basal expression levels of NR5A2 in glioblastoma cells promotes their cell cycle progression. The antiproliferative effect of NR5A2 is mediated by the transcriptional induction of negative regulators of the cell cycle, (encoding for p21), (encoding for p27) and Interestingly, two well-established agonists of NR5A2, dilauroyl phosphatidylcholine (DLPC) and diundecanoyl phosphatidylcholine, are able to mimic the antiproliferative action of NR5A2 in human glioblastoma cells via the induction of the same critical genes. Most importantly, treatment with DLPC inhibits glioblastoma tumor growth in vivo in heterotopic and orthotopic xenograft mouse models. These data indicate a tumor suppressor role of NR5A2 in the nervous system and render this nuclear receptor a potential pharmacological target for the treatment of nervous tissue-related tumors.
Proc Natl Acad Sci U S A, 2021; 118
34229198: Mantzourani C, Gkikas D, Kokotos A, Nummela P, Theodoropoulou MA, Wu KC, Fairlie DP, Politis PK, Ristimäki A, Kokotos G
Synthesis of benzoxazole-based vorinostat analogs and their antiproliferative activity.
Hydroxamic acid derivatives constitute an interesting novel class of antitumor agents. Three of them, including vorinostat, are approved drugs for the treatment of malignancies, while several others are currently under clinical trials. In this work, we present new vorinostat analogs containing the benzoxazole ring as the cap group and various linkers. The benzoxazole-based analogs were synthesized starting either from 2-aminobenzoxazole, through conventional coupling, or from benzoxazole, through a metal-free oxidative amination. All the synthesized compounds were evaluated for their antiproliferative activity on three diverse human cancer cell lines (A549, Caco-2 and SF268), in comparison to vorinostat.

Compound 12 (GK601), carrying a benzoxazole ring replacement for the phenyl ring of vorinostat, was the most potent inhibitor of the growth of three cell lines (IC 1.2-2.1 μM), similar in potency to vorinostat. Compound 12 also inhibited human HDAC1, HDAC2 and HDAC6 like vorinostat. This new analog also showed antiproliferative activity against two colon cancer cell lines genetically resembling pseudomyxoma peritonei (PMP), namely HCT116 GNAS R201C/+ and LS174T (IC 0.6 and 1.4 μM, respectively) with potency comparable to vorinostat (IC 1.1 and 2.1 μM, respectively).
Bioorg Chem, 2021; 114

33881857: Batsika CS, Mantzourani C, Gkikas D, Kokotou MG, Mountanea OG, Kokotos CG, Politis PK, Kokotos G
Saturated Oxo Fatty Acids (SOFAs): A Previously Unrecognized Class of Endogenous Bioactive Lipids Exhibiting a Cell Growth Inhibitory Activity.
The discovery of novel bioactive lipids that promote human health is of great importance. Combining "suspect" and targeted lipidomic liquid chromatography-high-resolution mass spectrometry (LC-HRMS) approaches, a previously unrecognized class of oxidized fatty acids, the saturated oxo fatty acids (SOFAs), which carry the oxo functionality at various positions of the long chain, was identified in human plasma. A library of SOFAs was constructed, applying a simple green photochemical hydroacylation reaction as the key synthetic step. The synthesized SOFAs were studied for their ability to inhibit in vitro the cell growth of three human cancer cell lines. Four oxostearic acids (OSAs) were identified to inhibit the cell growth of human lung carcinoma A549 cells. 6OSA and 7OSA exhibited the highest cell growth inhibitory potency, suppressing the expression of both STAT3 and c-myc, which are critical regulators of cell growth and proliferation. Thus, naturally occurring SOFAs may play a role in the protection of human health.
J Med Chem, 2021; 64

33411242: Tsampoula M, Tarampoulous I, Antoniadou I, Koutmani Y, Gkikas D, Vekrellis K, Politis PK
Nuclear Receptor NR5A2 Promotes Neuronal Identity in the Adult Hippocampus.
Neurogenesis in the dentate gyrus (DG) of the adult hippocampus is actively involved in brain homeostasis. Thus, identification of novel regulators in adult neurogenesis could significantly contribute to new therapies. We have recently unraveled the regulatory role of NR5A2 (also known as LRH1), a druggable orphan nuclear receptor, in embryonic neurogenesis. However, its involvement in adult neurogenesis is still an open question. Here we show that NR5A2 is differentially expressed in the DG of the adult hippocampus with neurons exhibiting higher levels of expression than adult neural stem/progenitor cells (aNSCs), suggesting a correlation with neuronal differentiation. Notably, NR5A2 overexpression in ex vivo cultured aNSCs induces expression of Prox1, a critical regulator of adult hippocampal neurogenesis. In agreement, NR5A2 is sufficient to reduce proliferation, increase neuronal differentiation, and promote axon outgrowth. Moreover, depletion of NR5A2 in DG cells in vivo caused a decrease in the number of NeuN as well as Calbindin-positive neurons, indicating its necessity for the maintenance of neuronal identity. Our data propose a regulatory role of NR5A2 in neuronal differentiation and fate specification of adult hippocampal NSCs.
Mol Neurobiol, 2021; 58

33124824: Kokotou MG, Kokotos AC, Gkikas D, Mountanea OG, Mantzourani C, Almutairi A, Lei X, Ramanadham S, Politis PK, Kokotos G
Saturated Hydroxy Fatty Acids Exhibit a Cell Growth Inhibitory Activity and Suppress the Cytokine-Induced β-Cell Apoptosis.
The field of bioactive lipids is ever expanding with discoveries of novel lipid molecules that promote human health. Adopting a lipidomic-assisted approach, two new families of previously unrecognized saturated hydroxy fatty acids (SHFAs), namely, hydroxystearic and hydroxypalmitic acids, consisting of isomers with the hydroxyl group at different positions, were identified in milk. Among the various regio-isomers synthesized, those carrying the hydroxyl at the 7- and 9-positions presented growth inhibitory activities against various human cancer cell lines, including A549, Caco-2, and SF268 cells. In addition, 7- and 9-hydroxystearic acids were able to suppress β-cell apoptosis induced by proinflammatory cytokines, increasing the possibility that they can be beneficial in countering autoimmune diseases, such as type 1 diabetes. 7-()-Hydroxystearic acid exhibited the highest potency both in cell growth inhibition and in suppressing β-cell death. We propose that such naturally occurring SHFAs may play a role in the promotion and protection of human health.
J Med Chem, 2020; 63

31028178: Lalioti ME, Arbi M, Loukas I, Kaplani K, Kalogeropoulou A, Lokka G, Kyrousi C, Mizi A, Georgomanolis T, Josipovic N, Gkikas D, Benes V, Politis PK, Papantonis A, Lygerou Z, Taraviras S
GemC1 governs multiciliogenesis through direct interaction with and transcriptional regulation of p73.
A distinct combination of transcription factors elicits the acquisition of a specific fate and the initiation of a differentiation program. Multiciliated cells (MCCs) are a specialized type of epithelial cells that possess dozens of motile cilia on their apical surface. Defects in cilia function have been associated with ciliopathies that affect many organs, including brain and airway epithelium. Here we show that the geminin coiled-coil domain-containing protein 1 GemC1 (also known as Lynkeas) regulates the transcriptional activation of , a transcription factor central to multiciliogenesis. Moreover, we show that GemC1 acts in a trimeric complex with transcription factor E2F5 and tumor protein p73 (officially known as TP73), and that this

complex is important for the activation of the promoter. We also provide evidence that GemC1 is necessary for p73 expression in different multiciliated epithelia. We further show that GemC1 regulates multiciliogenesis through the control of chromatin organization, and the epigenetic marks/tags of and Our results highlight novel signaling cues involved in the commitment program of MCCs across species and tissues.This article has an associated First Person interview with the first author of the paper.
J Cell Sci, 2019; 132
28638936: Gkikas D, Tsampoula M, Politis PK
Nuclear receptors in neural stem/progenitor cell homeostasis.
In the central nervous system, embryonic and adult neural stem/progenitor cells (NSCs) generate the enormous variety and huge numbers of neuronal and glial cells that provide structural and functional support in the brain and spinal cord. Over the last decades, nuclear receptors and their natural ligands have emerged as critical regulators of NSC homeostasis during embryonic development and adult life. Furthermore, substantial progress has been achieved towards elucidating the molecular mechanisms of nuclear receptors action in proliferative and differentiation capacities of NSCs. Aberrant expression or function of nuclear receptors in NSCs also contributes to the pathogenesis of various nervous system diseases. Here, we review recent advances in our understanding of the regulatory roles of steroid, non-steroid, and orphan nuclear receptors in NSC fate decisions. These studies establish nuclear receptors as key therapeutic targets in brain diseases.
Cell Mol Life Sci, 2017; 74

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IONTRONIC PUMP AS A NEW TOOL FOR LOCAL BRAIN TUMOR TREATMENT ON AN EX OVO MODEL

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Verena Handl[1], Linda Waldherr[1], Nassim Ghaffari Trabizi-Wiszy[2], Martin Asslaber[3], Rainer Schindl[1], Silke Patz[4]
[1]Medical University of Graz, Institute Of Biophysics, Graz, Austria, [2]Medical University Graz, Immunology And Pathophysiology, Graz, Austria, [3]Medical University Graz, Diagnostics And Research Center Of Pathology, Graz, Austria, [4]Medical University Graz, Experimental Neurotraumatology, Graz, Austria

Aims: The treatment of brain tumors known as glioblastoma multiforme (GBM) remains a challenging task. Although brain tumors are resected by surgery, combined with subsequent chemotherapy and radiotherapy the median survival only comprises 15 months. To this date, treatment is hampered due to limitation of drug application. The blood brain barrier (BBB) acts as a semipermeable gate, that prevents many potent chemotherapeutic drugs from reaching their site of action. To overcome this restriction, it is inevitable to establish a new kind of brain cancer treatment. Serendipitously, Iontronic pumps (IPs) are able to serve as drug delivery tools by using an ion exchange membrane and therefore suit for triggered drug release. Methods: The Chick Embryo Chorioallantoic Membrane Assay (CAM) was used to cultivate U87 GBM tumors. Iontronic Pumps were filled with Gemcitabine (GEM), a chemotherapeutic drug that is stable at its protonated state. Due to an electric power supply, the grown CAM-tumors were treated with the IPs. Subsequently, tumors were either embedded in paraffin for histological read-out or digested for cell-cycle analysis via FACS. Results: U87 tumor cells generate highly vascularized, solid tumors on CAM suitable for IP treatment. We examined tumor volume of treated vs. non-treated tumors and exhibited a pivotal distinction with respect to size measurement. Furthermore, we evaluated the effect of GEM within the treated tumors by FACS analysis as well as apoptosis evaluation. Conclusion: IPs are a promising tool for drug delivery and have the potential to emerge as a new application for local brain tumor treatment.

Pubmed:

33036292: Waldherr L, Tiffner A, Mishra D, Sallinger M, Schober R, Frischauf I, Schmidt T, Handl V, Sagmeister P, Köckinger M, Derler I, Üçal M, Bonhenry D, Patz S, Schindl R
Blockage of Store-Operated Ca Influx by Synta66 is Mediated by Direct Inhibition of the Ca Selective Orai1 Pore.
The Ca sensor STIM1 and the Ca channel Orai1 that form the store-operated Ca (SOC) channel complex are key targets for drug development. Selective SOC inhibitors are currently undergoing clinical evaluation for the treatment of auto-immune and inflammatory responses and are also deemed promising anti-neoplastic agents since SOC channels are linked with enhanced cancer cell progression. Here, we describe an investigation of the site of binding of the selective inhibitor Synta66 to the SOC channel Orai1 using docking and molecular dynamics simulations, and live cell recordings. Synta66 binding was localized to the extracellular site close to the transmembrane (TM)1 and TM3 helices and the extracellular loop segments, which, importantly, are adjacent to the Orai1-selectivity filter. Synta66-sensitivity of the Orai1 pore was, in fact, diminished by both Orai1 mutations affecting Ca selectivity and permeation of Na in the absence of Ca. Synta66 also efficiently blocked SOC in three glioblastoma cell lines but failed to interfere with cell viability, division and migration. These experiments provide new structural and functional insights into selective drug inhibition of the Orai1 Ca channel by a high-affinity pore blocker.
Cancers (Basel), 2020; 12
34195355: Waldherr L, Seitanidou M, Jakešová M, Handl V, Honeder S, Nowakowska M, Tomin T, Karami Rad M, Schmidt T, Distl J, Birner-Gruenberger R, von Campe G, Schäfer U, Berggren M, Rinner B, Asslaber M, Ghaffari-Tabrizi-Wizsy N, Patz S, Simon DT, Schindl R
Targeted Chemotherapy of Glioblastoma Spheroids with an Iontronic Pump.
Successful treatment of glioblastoma multiforme (GBM), the most lethal tumor of the brain, is presently hampered by (i) the limits of safe surgical resection and (ii) "shielding" of residual tumor cells from promising chemotherapeutic drugs such as Gemcitabine (Gem) by the blood brain barrier (BBB). Here, the vastly greater GBM cell-killing potency of Gem compared to the gold standard temozolomide is confirmed, moreover, it shows neuronal cells to be at least 10-fold less sensitive to Gem than GBM cells. The study also demonstrates the potential of an electronically-driven organic ion pump ("GemIP") to achieve controlled, targeted Gem delivery to GBM cells. Thus, GemIP-mediated Gem delivery is confirmed to be temporally and electrically controllable with pmol min precision and electric addressing is linked to the efficient killing of GBM cell

monolayers. Most strikingly, GemIP-mediated GEM delivery leads to the overt disintegration of targeted GBM tumor spheroids. Electrically-driven chemotherapy, here exemplified, has the potential to radically improve the efficacy of GBM adjuvant chemotherapy by enabling exquisitely-targeted and controllable delivery of drugs irrespective of whether these can cross the BBB.
Adv Mater Technol, 2021; 6

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STAT3-MEDIATED ASTROCYTIC REACTIVITY IN GLIOBLASTOMA MULTIFORME

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Paula Martínez[1], Elena Saavedra[1], Meritxell Roig[1], Paola Casanova[1], Mario Vázquez[1], Irina Freitag[1], Maider Usandizaga[1], María-Ángeles Carrillo-De Sauvage[2], Carole Escartin[2], Carlos Barcia[1]
[1]Institut de Neurociències - Universitat Autònoma de Barcelona, Biochemistry And Molecular Biology, Barcelona, Spain, [2]Laboratoire des maladies neurodégénératives UMR 9199, CNRS, CEA, Université Paris Xi, Fontenay-aux-roses, France

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults. The poor prognosis of the patients and the increasing incidence over the years urge the identification of novel targets for the development of new functional treatments. Intriguingly, the role of reactive astrocytes in GBM, potentially one of the most abundant cells within the brain tumor microenvironment, remains unclear. In the present study, we evaluate the effects of the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway inhibition, a master regulator of reactive astrogliosis, using adeno-associated virus (AAV) encoding suppressor of cytokine signaling 3 (SOCS3), an inhibitor of the JAK/STAT3 pathway in a GBM experimental model *in vivo*. SOCS3 overexpression decreased glial fibrillary acidic protein (GFAP) and vimentin expression, as well as the % of GBM cells expressing S100β suggesting that reactive astrocytes play a key role in GBM progression and malignancy. Moreover, the number of GBM-associated microglia/macrophages (GAAMs) was reduced with SOCS3 treatment, which reinforces the existence of a crosstalk between reactive astrocytes and GAMMs. In the *in vitro* setting, we study whether the presence of primary astrocytes affects GBM cells in coculture. Preliminary experiments showed an increased number of glioma cells in the presence of primary astrocytes, which suggests that reactive astrocytes create a suitable microenvironment for GBM cells proliferation and survival. Overall, our data demonstrate that reactive astrocytes play an important role in GBM progression and malignancy. Therefore, targeting reactive astrogliosis with SOCS3 genetic pre-treatment could be a potential approach for GBM.

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NEURONAL NA+/CA2+ EXCHANGER 2 (NCX2) AS A TUMOR SUPPRESSOR PROTEIN IN GLIOBLASTOMA PROGRESSION AND MALIGNITY

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Glioblastoma multiforme (GBM) is the most frequent and malignant brain tumor with an aggressive phenotype and resistance to chemotherapy. Notably, GBM selectively downregulates several genes mainly exerting a tumor suppression activity. Interestingly, among the genes downregulated in GBM there is the Sodium/Calcium Exchanger 2 (NCX2), a plasma membrane antiporter expressed in cells of CNS. These data suggest that Slc8a2, encoding NCX2, might represent a new tumor suppressor gene. To this aim, we explored the genetic and epigenetic mechanisms underlying the transcriptional downregulation of NCX2 in GBM, the contribute of NCX2 in tumor proliferation and malignity, and new possible pharmacological approaches to increase its expression/activity. In particular, we identified and cloned Slc8a2 promoter (HP) from human glioblastoma cells (U87). Afterward, we identified NF-kB as the main downstream transcription factor of EGF-R pathway involved in NCX2 downregulation. In addition, we found that the most used drug in the treatment of GBM, temozolomide (TMZ), increased NCX2 expression in U87 cells, suggesting that this antiporter might mediate some antineoplastic effects of TMZ. Notably, GBM cells were hampered in vitality and invasiveness by either NCX2 transfection, pharmacological inhibition of NF-kB or enhancement of NCX2 activity. Altogether, these data showed that: (i) NCX2 is downregulated by an NFkB-dependent EGF-R pathway in glioblastoma, and (ii) the increase in NCX2 activity/expression slows-down glioblastoma cell growth and invasiveness.

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BOARD NUMBER: S07-522

EXPOSING MICROVASCULAR ENDOTHELIAL CELLS TO LOW ENERGY ACCELERATED PROTONS AND ITS RELEVANCE FOR HADRONTHERAPY APPLICATIONS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Aim: Exposed to ionizing radiation, brain endothelial cells can be damaged/activated with consequences on BBB permeability, resulting the necessity of a complex post-irradiation characterization. The objective of this study is to understand how proton beams affects the brain microvascular endothelial cells functionality. Methods: Mouse brain microvascular cell line bEnd.3 was used in these experiments. One beam line of a TR19 cyclotron was adapted for in vitro radiobiology experiments. Cell cultures were exposed to proton irradiation (<10 MeV, doses range of 0-10 Gy, dose rate of 1 Gy/min). The cytotoxic (clonogenic and MTT assays), genotoxic (micronuclei and g- H2AX assays) and functional (purinergic signaling pathway and wound healing assay) effects were analyzed. Results: An inhibition (~90%) of the cellular proliferation and a strong increase of micronuclei number at doses over 5 Gy were observed. DNA repair capacity was significantly diminished by the increased values of the dose (~100%) and the linear energy transfer - LET (~20%). At intermediate doses and high LET values the cell relative migration rate was diminished (~25%). In the case of the ATP-dependent purinergic signaling pathway a more complex analysis of the specific parameters is necessary. Conclusions: Radiation dose and LET are important parameters in hadrontherapy procedures. These preliminary data suggest that proton beams in the range of Bragg peak diminishes the recovery capacity of brain microvascular endothelial cells in a manner depending on dose and LET. The results emphasize the necessity to gather more information about how the cell respond in this irradiation regimen.

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BOARD NUMBER: S07-523

SIALIC ACID METABOLISM ORCHESTRATES TRANSCELLULAR CONNECTIVITY AND SIGNALING IN GLIOBLASTOMA

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Aims: The glycobiology of cancer cells is closely associated with their malignant properties, including invasiveness and metastatic potential. The composition of the glycocalyx, a layer of multifunctional glycans covering the surfaces of cells, and the expression of the terminal sialic acids were shown to be altered on neoplastic cells. We aimed to explore the glycobiology of glioma, the most malignant brain tumour, and to map the sialylation in a model consisting of patient-derived GBM cell lines on human neocortical organotypic slices. *Methods:* We used human organotypic brain slice culture as a framework to investigate the glycobiology of the brain, allowing metabolic labelling of sialic acid moieties and quantifying the changes of the glycocalyx. Spatial transcriptomics and RNA-sequencing approach was used to indicate sialic acid turnover related genes in both tumour-infiltrated patient samples and patient-derived GBM cell lines. Functional role of sialic acid turnover was investigated by inter- and trans- cellular Ca2+ signalling. *Results:* The visualization and quantitative analysis of newly synthesized sialic acids revealed a high rate of de novo sialic sialylation in glioblastoma cells. Sialyltrasferases and sialidases were highly expressed indicating that sialylation is involved in tumour metabolism. Altered GBM sialylation affected the pattern of tumour growth and lead to the alterations of glioblastoma network activity. *Conclusions:* Our results provided new insights into under-investigated role of sialic acid in the functional activity of GBM and highlighted its importance for glioblastoma cell migration and network formation. In the future, these interactions have the potential to be targeted therapeutically.

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BOARD NUMBER: S07-524

FUNCTIONAL CONSEQUENCES OF IDH1 AND CIC MUTATIONS ON OLIGODENDROGLIOMA CELLS OF ORIGIN

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Oligodendrogliomas are primitive tumors of the central nervous system characterized by mutations in *isocitrate dehydrogenase 1 (IDH1*) gene, codeletion of 1p and 19q chromosomic arms and mutations in *Capicua (CIC*) gene. Oligodendrocyte precursors cells (OPCs) are potential cells of origin for oligodendrogliomas and OPC-like tumor cells are responsible for tumor amplification at early stages of gliomagenesis. Individually, IDH1 and CIC mutations can induce neoplastic characteristics but are not sufficient for tumoral development. Our goal is to characterize the combined effect of IDH1 and CIC mutations on OPC development and tumoral initiation. To this aim, we generated an inducible mouse model allowing endogenous expression of $IDH1^{R132H}$ and inactivation of CIC in postnatal OPCs. In this model we analyzed by immunofluorescence the consequences of IDH1 and CIC mutations on the proliferation and differentiation of OPCs and other brain cells. Our preliminary observations suggest that $IDH1^{R132H}$ increases the proliferation of OPCs and other cell types, suggesting paracrine effects of this mutation in the brain. Surprisingly, these effects are not observed when CIC is concomitantly inactivated. Our project describes for the first time the phenotypic impact of the combined expression of IDH1 and CIC mutations on oligodendroglial lineage cells and other brain cells, leading to a better understanding of oligodendroglioma development.

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BOARD NUMBER: S07-525

GLUCOCORTICOID DRUG WITHDRAWAL EXACERBATES GLIOBLASTOMA MULTIFORME MALIGNANCY

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Glioblastoma Multiforme (GBM) is the most aggressive brain tumour. Globally, over 100,000 patients die each year as a result of this type of brain cancer. The current therapeutic interventions are highly unsatisfactory and the average life expectancy remains approximately 14–18 months. One of the main clinical complications concerning GBM is the development of tumour-related cerebral oedema. *Dexamethasone* (DEX) represents the drug of choice among the synthetic glucocorticoids used to reduce oedema by decreasing tumour capillary permeability and enhancing extracellular water clearance. Nevertheless, the effects reported for DEX treatment on GBM cell proliferation remain controversial and the underlying mechanisms of action are unclear. Thus, we investigated the effects of DEX on the proliferative features of several GBM cell lines, including cells cultured from patients' GBM biopsies and tumour xenograft in athymic nude mice, clarifying the cell pathways involved in DEX actions. DEX treatment inhibited cell proliferation in a dose- and time-dependent manner through the promotion of the G0/G1 phase of cell cycle. WB analysis indicated that DEX treatment inhibited Cyclin D1 and enhanced p53 and p21 expression. DEX enhanced ROS production and, i*n vivo*, reduced tumour growth, effectively. By contrast, control mice displayed tumour overgrowth to such an extent that an early sacrifice was necessary. Despite these temporary beneficial effects, upon discontinuation or reduction of DEX dosage an unexpected withdrawal phenomenon developed characterized by enhanced cell proliferation and migration through activation of the p53-cyclin D3 pathway resulting in tumour overgrowth, *in vivo*.

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BOARD NUMBER: S07-526

BCAS1 AS A NOVEL MARKER OF A PROLIFERATIVE CELL POPULATION IN OLIGODENDROGLIOMAS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Aims: The cell of origin of glial-derived tumors, such as oligodendrogliomas (OGs), is still elusive. Different state-of-the-art studies point towards immature oligodendrocytes as a possible source of gliomagenesis. Therefore, studying markers that identify oligodendrocyte precursors has become of great interest. Breast carcinoma amplified sequence 1 (BCAS1) has emerged as a novel marker that defines an immature oligodendrocyte population in human brain. However, the tumorigenic potential of this cell population has never been explored in gliomas. Methods: In this study, we analysed the expression of BCAS1 in tumor tissue surgically removed from patients with OGs (n=17), glioblastomas (GBs, n=58) and astrocytomas (ASs, n=8). To study the distribution and proliferative status of the different cell subpopulations within these glial-derived tumors, we co-stained BCAS1, with EGFR, Vimentin and Ki-67. Additionally, we analysed the ultrastructure of BCAS1$^+$ cells by immunoelectron microscopy and we performed stereological quantification of the cell density and proliferation of BCAS1$^+$ cells. Results: Our results show that BCAS1$^+$ cells constitute a heterogeneously distributed population in glial-derived tumors. We found two different morphologies for this cell population: stellate and spherical cells. Stellate cells can form tightly packaged nodules or staying individualised, whereas spherical cells are isolated. These nodules present a high proliferative rate and they have been detected only in OGs, becoming a distinctive feature of more aggressive clinical behaviour. Conclusions: Our findings suggest that BCAS1 defines a specific cell subpopulation within glial-derived tumors, that could correspond to a transient amplifying cell state, thus contributing to tumor malignancy, specifically in OGs.

Pubmed:
34248510: Ulloa-Navas MJ, Pérez-Borredá P, Morales-Gallel R, Saurí-Tamarit A, García-Tárraga P, Gutiérrez-Martín AJ, Herranz-Pérez V, García-Verdugo JM
Ultrastructural Characterization of Human Oligodendrocytes and Their Progenitor Cells by Pre-embedding Immunogold. Oligodendrocytes are the myelinating cells of the central nervous system. They provide trophic, metabolic, and structural support to neurons. In several pathologies such as multiple sclerosis (MS), these cells are severely affected and fail to remyelinate, thereby leading to neuronal death. The gold standard for studying remyelination is the g-ratio, which is measured by means of transmission electron microscopy (TEM). Therefore, studying the fine structure of the oligodendrocyte population in the human brain at different stages through TEM is a key feature in this field of study. Here we study the ultrastructure of oligodendrocytes, its progenitors, and myelin in 10 samples of human white matter using nine different markers of the oligodendrocyte lineage (NG2, PDGFRα, A2B5, Sox10, Olig2, BCAS1, APC-(CC1), MAG, and MBP). Our findings show that human oligodendrocytes constitute a very heterogeneous population within the human white matter and that its stages of differentiation present characteristic features that can be used to identify them by TEM. This study sheds light on how these cells interact with other cells within the human brain and clarify their fine characteristics from other glial cell types.
Front Neuroanat, 2021; 15

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BOARD NUMBER: S07-527

CHANGES IN ADENOSINE A2A RECEPTOR AVAILABILITY IN GLIOBLASTOMA

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Introduction: Glioblastoma (GBM) is an aggressive and infiltrating type of intracranial tumor with poor response to conventional therapy. In the GBM microenvironment, adenosine elicits its functions through 4 adenosine G-protein-coupled receptors. We investigated whether PET with the novel $A_{2A}R$ ligand [^{11}C]preladenant is able to detect changes in adenosine A_{2A} receptor density in the tumor microenvironment as a result of tumor progression. **Methods:** Glioblastoma rat model was induced by unilateral (right frontoparietal cortex) implantation of C6 glioma cells in male and female Wistar rats (n=9). Rats underwent a 60-min dynamic PET scan with [^{11}C]preladenant to measure A_{2A} receptor density on days 6,9 and 12 post surgery. Animals were sacrificed on day 12 and anti-Iba1 immunohistochemical staining was performed to quantify microglia in cortical regions. **Results:** At day 12, tumor volume in males was 3 times higher than in females. [^{11}C]preladenant uptake in the tumor area was also higher in male rats ($p<0.05$), at all time-points. The highest uptake was in each animal always observed in the right caudate-putamen. However, the difference in [^{11}C]preladenant uptake between tumor and contralateral cortex was significantly higher in in females than in males, at all time-points. At day 12, males had a significantly higher Iba1^{+} microglial cell density in the tumor region than females, even though their tumors showed more necrosis. **Conclusions:** Although male rats had higher [^{11}C]preladenant uptake, female rats showed a greater difference in uptake between tumor and healthy brain. Increased microglial density suggests a more severe neuroinflammation in males.

Pubmed:
33572077: Prasad K, de Vries EFJ, Elsinga PH, Dierckx RAJO, van Waarde A
Allosteric Interactions between Adenosine A and Dopamine D Receptors in Heteromeric Complexes: Biochemical and Pharmacological Characteristics, and Opportunities for PET Imaging.
Adenosine and dopamine interact antagonistically in living mammals. These interactions are mediated via adenosine A and dopamine D receptors (R). Stimulation of AR inhibits and blockade of AR enhances DR-mediated locomotor activation and goal-directed behavior in rodents. In striatal membrane preparations, adenosine decreases both the affinity and the signal transduction of DR via its interaction with AR. Reciprocal AR/DR interactions occur mainly in striatopallidal GABAergic medium spiny neurons (MSNs) of the indirect pathway that are involved in motor control, and in striatal astrocytes. In the nucleus accumbens, they also take place in MSNs involved in reward-related behavior. AR and DR co-aggregate, co-internalize, and co-desensitize. They are at very close distance in biomembranes and form heteromers. Antagonistic interactions between adenosine and dopamine are (at least partially) caused by allosteric receptor-receptor interactions within AR/DR heteromeric complexes. Such interactions may be exploited in novel strategies for the treatment of Parkinson's disease, schizophrenia, substance abuse, and perhaps also attention deficit-hyperactivity disorder. Little is known about shifting AR/DR heteromer/homodimer equilibria in the brain. Positron emission tomography with suitable ligands may provide in vivo information about receptor crosstalk in the living organism. Some experimental approaches, and strategies for the design of novel imaging agents (e.g., heterobivalent ligands) are proposed in this review.
Int J Mol Sci, 2021; 22

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BOARD NUMBER: S07-528

THE METABOLISM OF ESSENTIAL AMINO ACIDS BY HUMAN GLIOBLASTOMAS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Essential amino acids (EAAs) play several roles in cellular metabolism. They are indispensable monomers for protein synthesis, serve signaling roles, or they could be further catabolized. With the aim to estimate to which extent could EAAs enter the metabolism of human glioblastoma cells, we determined the ratio to which EAAs were consumed from the medium. The analysis of media composition with either 1H-NMR or LC-MS method was used to estimate the specific rates of disappearance for all EAAs. The branched-chain amino acids (BCAA), leucine, isoleucine and valine, were removed from media at similar rates, which excited the rates of all other EAAs. Glioblastoma cells were tested for the expression of the enzymes essential for catabolism of BCAA. By applying the immuno-probing methods we tested the expression of the enzymes that are involved in both, prime and final, parts of BCAA catabolism. Indeed, human glioblastomas express enzymes for catabolism of BCAA. This way cancer cells can obtain acetyl-CoA, propionyl-CoA, succinyl-CoA and other compounds, which could be used either as building blocks for the synthesis of new cell components or as fuel molecules for energy metabolism.

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BOARD NUMBER: S07-529

EVALUATION OF THE EFFECT OF KETOVOLVE AND MEDIUM-CHAIN TRIGLYCERIDE BASED KETOGENIC DIET ON PPARS GENE MODULATION IN GLIOBLASTOMA TUMOR DEVELOPMENT.

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Glioblastoma is the most lethal tumor in the brain, affecting children and adults. This tumor may be regulated by the effects of ketogenic therapy, known for its high concentration of fatty acids. This diet is designed to reduce glucose levels and increase the ketone bodies produced in the metabolism of fatty acids, which could interrupt several processes of tumor metabolism, such as the viability and growth of these cells. Fatty acids also act as natural ligands of the peroxisome proliferator-activated receptors (PPARs) and could have a neuroprotective effect, regulating inflammatory, metabolic, and cell growth processes. However, it is still necessary to investigate the mechanisms of action that medium-chain fatty acids could have as signaling molecules and modulators of gene transcription through the activation of PPARs in the control of glioblastoma development. For this, an in vivo zebrafish model was used to evaluate the activity of these receptors against the effects of ketogenic therapy on inflammatory, metabolic, and cell growth response. It was found that there is no significant difference in the behavior of these animals. Brain and muscle gene expression analysis revealed an anti-inflammatory profile of this diet and favorable metabolic activity in the groups analyzed. The in vitro glioblastoma model was silenced for each PPAR isotype highlighting the relevance of PPARs in the fatty acid signaling in tumor growth and inflammatory response, pointing out this diet as a promising therapeutic alternative for glioblastoma treatment.

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BOARD NUMBER: S07-530

A CELLULAR ATLAS OF HUMAN CHOROID PLEXUS AND CHOROID PLEXUS TUMORS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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The choroid plexus (ChP) serves as a major site for CSF production, a critical interface between the immune system and brain, and a source of signals affecting neuronal development and function. Choroid plexus tumors (CPT) are relatively rare, often pediatric tumors that account for 10-20% of brain tumors during the first year of life. They range in severity from benign choroid plexus papillomas to the more malignant choroid plexus carcinomas. While a small number of genes have been linked to CPT occurrence (most notably TP53 and MYC), little is known about the signaling pathways and cellular interactions that lead to ChP tumor formation. As a first step to elucidating the cellular mechanisms giving rise to CPT, we have used single nucleus RNAseq to create cellular atlases of normal human ChP and CPT. The resulting atlases detail epithelial, mesenchymal and immune cell types, as well as their patterns of interaction and gene expression. Furthermore, they reveal tumor specific cell types and alterations in immune cell function. Finally, they identify tumor type specific alterations in intracellular signaling pathways, including some involving known oncogenes and tumor suppressors. Taken together, these data expand our knowledge of choroid plexus function and suggest mechanisms for CPT pathology.

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BOARD NUMBER: S07-531

EXTRACELLULAR PROTEINS – A SOURCE OF AMINO ACIDS FOR HUMAN GLIOBLASTOMA CELLS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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The transformed metabolism of glioblastoma cells is characterized by the increased demand for substrates, which are supposed to supply the cells with the energy and the building blocks required for the biosynthetic processes. In this respect, the synthesis of proteins is one of the metabolic processes that is strongly dependent on the availability of essential amino acids. We hypothesize that the limited availability of essential amino acids in the microenvironment of glioblastoma cells could be compensated by importing extracellular proteins, which are supposed to be utilized as a source of amino acids. To evaluate the capability of human glioblastoma cells to exploit the extracellular proteins as a complementary source of amino acids, we used two types of human glioblastoma cell lines, A172 and T98G. The culture media analysis suggests that glioblastoma cells can remove the extracellular proteins and enrich them with tryptophane. Indeed, cells readily removed also fluorescently labeled albumin, which was intracellularly localized into lysosomes. Furthermore, we revealed that albumin hydrolysis occurred after its endocytosis. In general, our results show that human glioblastoma cells are able to import and subsequently metabolize the extracellular proteins, and that proteins may serve as a valuable source of amino acids for human glioblastoma cells. This work was supported by the Slovak Research and Development Agency under Contract No. APVV-18-0088, APVV-20-0331 and VEGA 1/0255/20.

Pubmed:
35158853: Gondáš E, Kráľová Trančíková A, Baranovičová E, Šofranko J, Hatok J, Kowtharapu BS, Galanda T, Dobrota D, Kubatka P, Busselberg D, Murín R
Expression of 3-Methylcrotonyl-CoA Carboxylase in Brain Tumors and Capability to Catabolize Leucine by Human Neural Cancer Cells.
Leucine is an essential, ketogenic amino acid with proteinogenic, metabolic, and signaling roles. It is readily imported from the bloodstream into the brain parenchyma. Therefore, it could serve as a putative substrate that is complementing glucose for sustaining the metabolic needs of brain tumor cells. Here, we investigated the ability of cultured human cancer cells to metabolize leucine. Indeed, cancer cells dispose of leucine from their environment and enrich their media with the metabolite 2-oxoisocaproate. The enrichment of the culture media with a high level of leucine stimulated the production of 3-hydroxybutyrate. When C-leucine was offered, it led to an increased appearance of the heavier citrate isotope with a molar mass greater by two units in the culture media. The expression of 3-methylcrotonyl-CoA carboxylase (MCC), an enzyme characteristic for the irreversible part of the leucine catabolic pathway, was detected in cultured cancer cells and human tumor samples by immunoprobing methods. Our results demonstrate that these cancer cells can catabolize leucine and furnish its carbon atoms into the tricarboxylic acid (TCA) cycle. Furthermore, the release of 3-hydroxybutyrate and citrate by cancer cells suggests their capability to exchange these metabolites with their milieu and the capability to participate in their metabolism. This indicates that leucine could be an additional substrate for cancer cell metabolism in the brain parenchyma. In this way, leucine could potentially contribute to the synthesis of metabolites such as lipids, which require the withdrawal of citrate from the TCA cycle.
Cancers (Basel), 2022; 14

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BOARD NUMBER: S07-532

ANTI-TUMOR ACTIVITY OF NEW LIGANDS TARGETING LINGO1- A PROTEIN PRIMARILY EXPRESSED IN CNS FOR THE TREATMENT OF GLIOBLASTOMAS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Glioblastomas (GBM) are the most frequent and aggressive form of gliomas. Standard treatments consist of surgery, radiotherapy, and chemotherapy, which conduct a median survival of less than 15 months. Our project aims to explore the role of protein LINGO-1 in the physiopathology of GBM, a transmembrane protein mainly expressed in nervous systems that belong to the family LRIG. We first selected a series of compounds by virtual screening with LINGO-1 protein structure, then used BRET to identify the potential hits. We confirmed the interaction of the lead compound LPI3432 with LINGO-1, then tested the anti-tumor activity in different glioblastoma cell lines. LPI3432 is capable of inhibiting the proliferation of GBM cells, even in the stem-like cells isolated from biopsy tissues of GBM patients. We studied the signaling pathways implicated in the effect of LPI3432. We revealed dysregulation of the phosphorylation level of several proteins well-known in the EGFR pathway for their roles in cell proliferation, survival, and migration, such as kinase Erk1/2. LPI3432 also decreases glioblastoma cell migration and angiogenesis. Finally, we worked in a xenograft subcutaneous mice model of GBM to verify the anti-tumor activity *in vivo* and found LPI3432 significantly slowed down the tumor growth. qRT-PCR results showed several factors in angiogenesis and vasculature for cancer progression are decreased in mice group treated with LPI3432. Altogether, we have identified the first small chemical molecule which binds to LINGO-1 and demonstrated its antitumor activity *in vivo* as well as the potential mechanism of action for the treatment of GBM.

Pubmed:
29930759: Chen W, Zebaze LN, Dong J, Chézeau L, Inquimbert P, Hugel S, Niu S, Bihel F, Boutant E, Réal E, Villa P, Junier MP, Chneiweiss H, Hibert M, Haiech J, Kilhoffer MC, Zeniou M
WNK1 kinase and its partners Akt, SGK1 and NBC-family Na/HCO3 cotransporters are potential therapeutic targets for glioblastoma stem-like cells linked to Bisacodyl signaling.
Glioblastoma is a highly heterogeneous brain tumor. The presence of cancer cells with stem-like and tumor initiation/propagation properties contributes to poor prognosis. Glioblastoma cancer stem-like cells (GSC) reside in hypoxic and acidic niches favoring cell quiescence and drug resistance. A high throughput screening recently identified the laxative Bisacodyl as a cytotoxic compound targeting quiescent GSC placed in acidic microenvironments. Bisacodyl activity requires its hydrolysis into DDPM, its pharmacologically active derivative. Bisacodyl was further shown to induce tumor shrinking and increase survival in glioblastoma models. Here we explored the cellular mechanism underlying Bisacodyl cytotoxic effects using quiescent GSC in an acidic microenvironment and GSC-derived 3D macro-spheres. These spheres mimic many aspects of glioblastoma tumors , including hypoxic/acidic areas containing quiescent cells. Phosphokinase protein arrays combined with pharmacological and genetic modulation of signaling pathways point to the WNK1 serine/threonine protein kinase as a mediator of Bisacodyl cytotoxic effect in both cell models. WNK1 partners including the Akt and SGK1 protein kinases and NBC-family Na/HCO3 cotransporters were shown to participate in the compound's effect on GSC. Overall, our findings uncover novel potential therapeutic targets for combatting glioblastoma which is presently an incurable disease.
Oncotarget, 2018; 9
1740936: Reifsnyder T, Bandyk DF, Lanza D, Seabrook GR, Towne JB
Use of stress thallium imaging to stratify cardiac risk in patients undergoing vascular surgery.
Reduction of the cardiac morbidity associated with major vascular procedures requires identification of high risk patients prior to operation. This retrospective study reviews the records of 126 consecutive patients who underwent 141 major vascular procedures to determine the accuracy of preoperative clinical, laboratory (ECG), and cardiac function testing (stress thallium-201 scintigraphy, left ventricular ejection fraction scan) in predicting perioperative cardiac complications. An abnormality on oral dipyridamole or treadmill thallium imaging was demonstrated prior to 71 (61%) of 116 procedures and included 20 fixed

and 51 reperfusion (reversible) defects. No patient died within 30 days of operation, but 11 minor (ventricular arrhythmia) and 15 major (myocardial infarction, ischemic congestive heart failure) cardiac complications occurred. A reperfusion defect on stress thallium imaging accurately (94% sensitivity, 56% specificity, 98% negative predictive value) identified high-risk patients while accepted clinical rating systems (Goldman, Cooperman, Eagle) and preoperative level of left ventricular ejection fraction were less predictive of adverse cardiac events. Patients without myocardium at risk by coronary angiography, but a reperfusion defect on stress thallium imaging were found to be at high risk for a cardiac complication. The study data support the use of stress thallium imaging to stratify cardiac risk prior to major arterial surgery.
J Surg Res, 1992; 52

32862444: Guillemain A, Laouarem Y, Cobret L, Štefok D, Chen W, Bloch S, Zahaf A, Blot L, Reverchon F, Normand T, Decoville M, Grillon C, Traiffort E, Morisset-Lopez S
LINGO family receptors are differentially expressed in the mouse brain and form native multimeric complexes.
Leucine-rich repeat and immunoglobin-domain containing (LRRIG) proteins that are commonly involved in protein-protein interactions play important roles in nervous system development and maintenance. LINGO-1, one of this family members, is characterized as a negative regulator of neuronal survival, axonal regeneration, and oligodendrocyte precursor cell (OPC) differentiation into mature myelinating oligodendrocytes. Three LINGO-1 homologs named LINGO-2, LINGO-3, and LINGO-4 have been described. However, their relative expression and functions remain unexplored. Here, we show by in situ hybridization and quantitative polymerase chain reaction that the transcripts of LINGO homologs are differentially expressed in the central nervous system. The immunostaining of brain slices confirmed this observation and showed the co-expression of LINGO-1 with its homologs. Using BRET (bioluminescence resonance energy transfer) analysis, we demonstrate that LINGO proteins can physically interact with each of the other ones with comparable affinities and thus form the oligomeric states. Furthermore, co-immunoprecipitation experiments indicate that LINGO proteins form heterocomplexes in both heterologous systems and cortical neurons. Since LINGO-1 is a promising target for the treatment of demyelinating diseases, its ability to form heteromeric complexes reveals a new level of complexity in its functioning and opens the way for new strategies to achieve diverse and nuanced LINGO-1 regulation.
FASEB J, 2020; 34

26270679: Zeniou M, Fève M, Mameri S, Dong J, Salomé C, Chen W, El-Habr EA, Bousson F, Sy M, Obszynski J, Boh A, Villa P, Assad Kahn S, Didier B, Bagnard D, Junier MP, Chneiweiss H, Haiech J, Hibert M, Kilhoffer MC
Chemical Library Screening and Structure-Function Relationship Studies Identify Bisacodyl as a Potent and Selective Cytotoxic Agent Towards Quiescent Human Glioblastoma Tumor Stem-Like Cells.
Cancer stem-like cells reside in hypoxic and slightly acidic tumor niches. Such microenvironments favor more aggressive undifferentiated phenotypes and a slow growing "quiescent state" which preserves them from chemotherapeutic agents that essentially target proliferating cells. Our objective was to identify compounds active on glioblastoma stem-like cells, including under conditions that mimick those found in vivo within this most severe and incurable form of brain malignancy. We screened the Prestwick Library to identify cytotoxic compounds towards glioblastoma stem-like cells, either in a proliferating state or in more slow-growing "quiescent" phenotype resulting from non-renewal of the culture medium in vitro. Compound effects were assessed by ATP-level determination using a cell-based assay. Twenty active molecules belonging to different pharmacological classes have thus been identified. Among those, the stimulant laxative drug bisacodyl was the sole to inhibit in a potent and specific manner the survival of quiescent glioblastoma stem-like cells. Subsequent structure-function relationship studies led to identification of 4,4'-dihydroxydiphenyl-2-pyridyl-methane (DDPM), the deacetylated form of bisacodyl, as the pharmacophore. To our knowledge, bisacodyl is currently the only known compound targeting glioblastoma cancer stem-like cells in their quiescent, more resistant state. Due to its known non-toxicity in humans, bisacodyl appears as a new potential anti-tumor agent that may, in association with classical chemotherapeutic compounds, participate in tumor eradication.
PLoS One, 2015; 10

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BOARD NUMBER: S07-533

PRECLINICAL MOUSE STUDY ON MATERNAL SEPARATION IMPACTING CHILDHOOD CHEMOTHERAPY-INDUCED COGNITIVE IMPAIRMENT

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Exposure to chemotherapeutics can have long-lasting sequelae on behavioral and cognitive functioning in childhood-cancer survivors. Moreover, parental care might influence a child's resilience to adverse events. This includes the neurotoxic exposure to chemotherapeutics (chemo-brain), such as methotrexate (MTX), used in the treatment of the most prevalent childhood cancers. Therefore, the present preclinical mouse study investigates (1) the severity of MTX-induced socio-cognitive defects during weaning and adult life, and (2) how early life stress (maternal separation) affects the impact of MTX exposure. We implement a maternal separation or sham protocol in male and female C57BL/6 mice from postnatal day (P) 10 through 20. After weaning, pups are injected with MTX (100 mg/kg, i.p.) or vehicle on P21, P28 and P35, which mimics MTX exposure in children treated for acute lymphoblastic leukemia. We assess behavioral interaction in pups towards their dams (in comparison to unfamiliar adults) in a modified 3-chamber paradigm. Adult performance in MTX-exposed mice is assessed using an extensive test battery that includes a variety of exploratory, emotional, cognitive and social behaviors. We expect that maternal separation influences the animals' susceptibility to the socio-cognitive effects of MTX exposure. We shall discuss the broad relevance of these findings to our understanding of the late sequelae of chemotherapy, and the possible factors that could influence the increasingly prevalent condition of chemo-brain. This research is funded by the Fonds Wetenschappelijk Onderzoek (FWO) Vlaanderen and the Olivia Hendrickx Research Fund.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-534

ALTERATION OF THE MITOCHONDRIAL ACTIVITY AND LIPIDIC METABOLISM CAUSED BY THE SELECTIVE STIMULATION OF M2 MUSCARINIC RECEPTORS IN HUMAN GLIOBLASTOMA CELLS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Claudia Guerriero[1], Michael Salazar Intriago[2], Anna Giudetti[3], Marianna Manfredelli[1], Maria Petrone[1], Stefano Tacconi[1], Teresa Rinaldi[1], Carlo Matera[4], Sergio Visentin[5], Angela Iuzzolino[1], Clelia Dallanoce[4], Luciana Dini[1], Ada Maria Tata[1]
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Background and aims: Glioblastoma is the most malignant human brain tumor characterized by heterogeneous cell populations, including undifferentiated cells defined Glioblastoma Stem cells (GSCs), responsible for the beginning of neoplastic process and recurrence formation. Previous studies demonstrated how the activation of M2 muscarinic receptor by orthosteric agonist Arecaidine Propargyl Ester (APE) and dualsteric agonist N-8-Iper caused a significant decrease of cell proliferation and survival both in GSCs and in glioblastoma cell lines. Interestingly N8-Iper is capable to activate M2 receptor with higher affinity and at a lower concentration than APE. The aim of this work was to investigate the mechanisms downstream of M2 receptor activation by both agonists responsible of the cytotoxic and pro-apoptotic effects both in U251 cell line and in GSCs. Methods: mitochondrial functionality was evaluated by MITO ID assay, TMRE staining and oxygen consumption measurement by oxygraph. Lipid homeostasis was analyzed by Oil Red O staining, TLC and WB analysis. Autophagy was analyzed by WB analysis and LC3-GFP construct transfection. Results: our results demonstrate the ability of the M2 agonists to induce oxidative stress, alteration of the mitochondrial morphology and activity with consequent alteration of cellular respiration, lipid homeostasis and lipid droplets formation. M2 agonists also induce autophagy, as demonstrated in U251 cells. Conclusions: these results suggest that the selective activation of M2 receptor in particular by N-8-Iper may be a promising therapeutic strategy for the glioblastoma treatment, reducing the possible side effects that may be caused by the high doses of the orthosteric agonist.

Pubmed:

34440646: Guerriero C, Matera C, Del Bufalo D, De Amici M, Conti L, Dallanoce C, Tata AM
The Combined Treatment with Chemotherapeutic Agents and the Dualsteric Muscarinic Agonist Iper-8-Naphthalimide Affects Drug Resistance in Glioblastoma Stem Cells.
Glioblastoma multiforme (GBM) is characterized by heterogeneous cell populations. Among these, the Glioblastoma Stem Cells (GSCs) fraction shares some similarities with Neural Stem Cells. GSCs exhibit enhanced resistance to conventional chemotherapy drugs. Our previous studies demonstrated that the activation of M2 muscarinic acetylcholine receptors (mAChRs) negatively modulates GSCs proliferation and survival. The aim of the present study was to analyze the ability of the M2 dualsteric agonist Iper-8-naphthalimide (N-8-Iper) to counteract GSCs drug resistance.
Cells, 2021; 10
34359896: Di Bari M, Tombolillo V, Alessandrini F, Guerriero C, Fiore M, Asteriti IA, Castigli E, Sciaccaluga M, Guarguaglini G, Degrassi F, Tata AM
M2 Muscarinic Receptor Activation Impairs Mitotic Progression and Bipolar Mitotic Spindle Formation in Human Glioblastoma Cell Lines.
Glioblastoma multiforme (GBM) is characterized by several genetic abnormalities, leading to cell cycle deregulation and abnormal mitosis caused by a defective checkpoint. We previously demonstrated that arecaidine propargyl ester (APE), an orthosteric agonist of M2 muscarinic acetylcholine receptors (mAChRs), arrests the cell cycle of glioblastoma (GB) cells, reducing their survival. The aim of this work was to better characterize the molecular mechanisms responsible for this cell cycle arrest.
Cells, 2021; 10
32131421: Cristofaro I, Limongi C, Piscopo P, Crestini A, Guerriero C, Fiore M, Conti L, Confaloni A, Tata AM

M2 Receptor Activation Counteracts the Glioblastoma Cancer Stem Cell Response to Hypoxia Condition.
Glioblastoma multiforme (GBM) is the most malignant brain tumor. Hypoxic condition is a predominant feature of the GBM contributing to tumor growth and resistance to conventional therapies. Hence, the identification of drugs able to impair GBM malignancy and aggressiveness is considered of great clinical relevance. Previously, we demonstrated that the activation of M2 muscarinic receptors, through the agonist arecaidine propargyl ester (Ape), arrests cell proliferation in GBM cancer stem cells (GSCs). In the present work, we have characterized the response of GSCs to hypoxic condition showing an upregulation of hypoxia-inducible factors and factors involved in the regulation of GSCs survival and proliferation. Ape treatment in hypoxic conditions is however able to inhibit cell cycle progression, causing a significant increase of aberrant mitosis with consequent decreased cell survival. Additionally, qRT-PCR analysis suggest that Ape downregulates the expression of stemness markers and miR-210 levels, one of the main regulators of the responses to hypoxic condition in different tumor types. Our data demonstrate that Ape impairs the GSCs proliferation and survival also in hypoxic condition, negatively modulating the adaptive response of GSCs to hypoxia.
Int J Mol Sci, 2020; 21
32182759: Cristofaro I, Alessandrini F, Spinello Z, Guerriero C, Fiore M, Caffarelli E, Laneve P, Dini L, Conti L, Tata AM
Cross Interaction between M2 Muscarinic Receptor and Notch1/EGFR Pathway in Human Glioblastoma Cancer Stem Cells: Effects on Cell Cycle Progression and Survival.
Glioblastomas (GBM) are the most aggressive form of primary brain tumors in humans. A key feature of malignant gliomas is their cellular heterogeneity. In particular, the presence of an undifferentiated cell population of defined Glioblastoma Stem cells (GSCs) was reported. Increased expression of anti-apoptotic and chemo-resistance genes in GCSs subpopulation favors their high resistance to a broad spectrum of drugs. Our previous studies showed the ability of M2 muscarinic receptors to negatively modulate the cell growth in GBM cell lines and in the GSCs. The aim of this study was to better characterize the inhibitory effects of M2 receptors on cell proliferation and survival in GSCs and investigate the molecular mechanisms underlying the M2-mediated cell proliferation arrest and decreased survival. Moreover, we also evaluated the ability of M2 receptors to interfere with Notch1 and EGFR pathways, whose activation promotes GSCs proliferation. Our data demonstrate that M2 receptors activation impairs cell cycle progression and survival in the primary GSC lines analyzed (GB7 and GB8). Moreover, we also demonstrated the ability of M2 receptor to inhibit Notch1 and EGFR expression, highlighting a molecular interaction between M2 receptor and the Notch-1/EGFR pathways also in GSCs.
Cells, 2020; 9

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BOARD NUMBER: S07-535

NEURON-GLIOMA INTERACTIONS IN WHITE MATTER INVASION IN A PRECLINICAL MODEL OF GLIOBLASTOMA

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Aims: Glioblastoma, a brain tumor of glial origin, is the most common and aggressive primary tumor of the central nervous system in adults, with a median survival rate of 15 months after standard treatment. The poor prognosis is primary related to the invasiveness of glial tumor cells. Glioma cells can detach from the mass and infiltrate the brain, causing a high propensity of secondary tumors or recurrence after surgery. White matter tracts are a major pathway for glioma infiltration, but close physical and functional interactions between axons and glioma cells are far from comprehensive. **Methods/results:** Immunohistochemistry on brain slices and transparized brains of adult *Nude* and C57Bl/6J mice bearing glioblastoma (following stereotaxic injection of glioblastoma human U87 and murine GL261 cell lines) were performed to study invasiveness profile. Organotypic slices from 3-5 weeks-old mice were also used to investigate modulation of glioma cells invasion following chronic pharmacological treatment (tetrodotoxin, extracellular potassium in excess), through macroconfocal acquisitions for days and thunder/confocal imaging after immunostainings. *In situ*, U87 cells display collective behavior in the white matter and contact both axons and vessels. Myelinated and unmyelinated axons are both concerned. Reactive astrogliosis has been also observed. *Ex vivo*, chronic treatment of tetrodotoxin favor glioma invasion, both in the cortex and in the white matter. *Ex vivo*, U87 cells display high tropism for vessels. **Conclusion:** These results suggest that axonal activity modulate gliomal invasion. **Fundings** by Canceropole Nord-Ouest, Normandy Region and European Regional Development Fund.

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COQ10 REDUCES GLIOBLASTOMA GROWTH AND INFILTRATION THROUGH PROTEOME REMODELING AND INHIBITION OF ANGIOGENESIS AND INFLAMMATION

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Javier Frontiñán-Rubio[1], Emilio Llanos-González[1], Sonia García-Carpintero[1], Juan Ramon Peinado[1], Inmaculada Ballesteros-Yáñez[2], Margarita Villar[3], Jose De La Fuente[3], Víctor Pérez García[4], Marcos Malumbres[5], Francisco Javier Alcaín[1], Mario Durán-Prado[1]
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The management of glioblastoma (GBM) has not been substantially improved since the implementation of the Stupp protocol, temozolomide plus surgery, and radiotherapy many years ago. In this work, using *in vivo* and *in vitro* models of GBM, we show that the lipophilic antioxidant Coenzyme Q_{10} (CoQ_{10}), a component of the mitochondrial electron transport chain, has a potential therapeutic effect in the pathology, acting as a pleiotropic molecule. Intraperitoneal administration of CoQ_{10} decreased by one-half the volume of U251-cells induced tumors in xenografts and orthotopic models. This effect was related with a delay in tumor hypoxia and a reduction in neovascularization and inflammation. Moreover, the volume of the brain infiltrated with tumor cells was diminished in CoQ_{10}-treated mice. *In vitro* experiments revealed that these beneficial effects of CoQ_{10} involve the remodeling of the proteome and the secretome regarding pro-inflammatory cytokines and angiogenic molecules. GBM cells migration and invasion were drastically reduced by mechanisms involving modulation of the actin cytoskeleton polarization and downregulation of MMPs. Overall, CoQ_{10} exerted pleiotropic effects *in vitro* and *in vivo*, simultaneously affecting several hallmarks of GBM, suggesting that the compound has therapeutic potential in treating this fatal disease.

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SERUM DEPRIVATION ENHANCES THE CANNABIDIOL ANTINEUROBLASTOMA ACTION BY THE UPREGULATION OF CANNABINOID 2 AND GRP55 RECEPTORS.

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Jordi Blanco[1,2], Maria Teresa Colomina[2], Maria Cabré[2], Judit Biosca-Brull[2], Miquel Mulero Abellan[3]
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Cannabidiol (CBD) is one of the most promising phytocannabinoids derived from *Cannabis Sativa*. Among all their effects on different metabolic processes it is worthy to highlight their anti-neuroblastoma activity. However, this effect is potentiated upon serum deprivation. The aim of the present study is to shed some light on the mechanism of CBD as an anti-neuroblastoma compound in a serum restricted environment. To conduct this study, we have exposed a neuroblastoma cells line (SH-SY5Y) to serum restriction, in presence or absence of CBD. We have compared the result respect with a neuroblastoma cell in presence of serum. We have evaluated cell viability by MTT assay, and the gene expression by RT-qPCR of receptors implicated in the CBD signalling. We have also evaluated the protein expression of different markers of reactive oxygen spices (ROS) production and of endoplasmic reticulum (ER) stress by Western blot analysis. Results have shown that serum restriction significantly upregulates the gene expression of the isoform of cannabinoid receptor 2 (CB2) and of GRP55 in the firsts stages of serum restriction. Nevertheless, only neuroblastoma cells exposed to CBD in serum deprivation maintain the upregulation of these receptors, which induce a significant decrease of cell viability by increasing the protein expression of excised caspase 3, antioxidant enzymes and of proteins markers of ER stress. We can conclude that serum deprivation sensitizes the CBD signalling, upregulating CB2 and GPR55 receptors, which generates neuroblastoma cell death by ROS production and ER stress.

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CO-TARGETING TUMOR MICROENVIRONMENT-INSTIGATED ADAPTATION TO HYPOXIA RENDERS GLIOBLASTOMA MORE SUSCEPTIBLE TO ONCOLYTIC VIRUS IMMUNOTHERAPY.

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Brain tumors (glioblastomas) are characterized by robust survival of a subpopulation of cancer stem-like cells (CSCs) in a hypoxic tumor microenvironment, and poor infiltration of immune cells. Thus, a recent promising oncolytic herpes virus (oHSV)-based immunotherapy meets inefficacy barriers due to a prevalence of stress-resistant CSCs. We hypothesized that gene signature separating CSC in the context of hypoxic tumor microenvironment would reveal relevant targets of oHSV efficacy, providing clinically-relevant cues on how CSCs thrive in the hypoxic niche being resistant to oHSV immunotherapy. As a proof of concept for co-targeting hypoxic signaling effectors, we singled out an RNA molecule (*HIF1A-AS2*) that is a vital sensor for the response and adaptation of CSCs to hypoxia. We compared (by gene microarray) transcriptomes in the intracranial xenograft tissue in vivo and ex vivo single-cell culture using a heterogeneous patient-derived CSC model. We targeted *HIF1A-AS2* expression in GSCs and tested for survival benefits of tumor-bearing animals upon oHSV immunotherapy. Transcriptome data revealed that the presence of the brain microenvironment shapes two distinguishing characteristics of CSCs: increased cell-to-cell communication with immune response cells and metabolic shift toward hypoxic adaptation, both with signatures predictive of glioblastoma patent survival. *HIF1A-AS2* silencing augmented sensitivity to oHSV immunotherapy and improved overall survival of tumor-bearing animals. The intertwined influence of the tumor microenvironment diminishes therapeutic efficiency by enabling cell adaptation to hypoxia. Thus, co-targeting hypoxia response by attenuating *HIF1A-AS2* rearranges gene expression, boosting glioblastoma's sensitivity to therapy.

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BOARD NUMBER: S07-539

CELL PROPERTIES IN PERITUMOR, DENSE TUMOR, AND HEALTHY CORTEX IN PATIENTS WITH II AND IV GRADE GLIOMAS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Kseniia Morozova[1], Nadezda Brazhe[1,2], Aleksander Popov[2], Pavel Denisov[2], Evgeniya Parshina[1], Igor Medyanik[3], Konstantin Yashin[3], Alexey Brazhe[2], Milena Shestopalova[4], Anton Zalygin[4], Vladimir Oleinikov[4], Alexey Semyanov[1,2]
[1]Moscow State University, Faculty Of Biology, Moscow, Russian Federation, [2]Institute of Bioorganic Chemistry, Department Of Molecular Neurobiology, Moscow, Russian Federation, [3]Privolzhskiy Research Medical University, Department Of Neurosurgery, Nizhny Novgorod, Russian Federation, [4]Institute of Bioorganic Chemistry, Department Of Biomaterials And Bionanotechnology, Moscow, Russian Federation

The success of brain tumor removal depends on the detection of tumor borders. Therefore, it is crucial to identify cell markers and detection methods that would allow univocally distinguish between tumor, peritumoral area and healthy tissue. Here, Raman microspectroscopy (RMS), Ca^{2+} imaging, and patch-clamp recordings were used to compare cells in the access tissue, peritumoral regions and the dense tumor in the samples obtained from patients with II-IV grades of gliomas during surgery. All studies were carried out in accordance with the human tissue regulations. RMS allowed to distinguish tumor cells, peritumoral regions and the normal cortex. This method revealed that the relative amount of proteins is higher in IV grade gliomas than in the healthy cortex. The redox state of mitochondrial cytochromes was different in the peritumoral regions compared to the normal cortex in both types of gliomas (II and IV grades). The input resistance of tumor cells was higher than in cortical astrocytes in high grade gliomas. The Ca^{2+} events were more localized while the amplitude of Ca^{2+} transients was higher in tumor cells than in cortical astrocytes. Thus, we observed multiple metabolic and physiological differences in tumoral cells, peritumoral cells and healthy astrocytes which can be used for diagnostics purposes and identifying the tissue borders. This study was supported by RFBR (grant number 20-04-01011).

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IN VIVO INTRATUMORAL CALCIUM HETEROGENEITY IN GLIOBLASTOMA MULTIFORME CORRELATES WITH MIGRATIONAL PATTERNS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Calcium signaling mechanisms in brain tumors such as glioblastoma multiforme (GBM) have been extensively explored in order to better understand the etiology of the disease. Numerous *in vitro* studies have shown that calcium signaling facilitates GBM proliferation, motility and invasion. However, there are no *in vivo* demonstrations linking GBM cell motility and calcium signaling. To address this issue, we *ex novo* generated an orthotopic murine model, engineering a GL261 cell line; we co-expressed a red fluorescent protein staining the actin cytoskeleton, and a green fluorescent protein encoding GCaMP6s calcium sensor. By means of two photon imaging, we demonstrate that most of the tumor volume, i.e. the core, is occupied by tightly packed spherical cells characterized by reduced cellular motility and low intracellular calcium ($(Ca^{2+})_i$) activity. This core is surrounded by sparse cells displaying a very polarized morphology that migrate at a higher rate. These cells in the peripheral region are characterized by very active $(Ca^{2+})_i$signaling. Additionally, we observed a novel phenomenon where groups of cells display synchronized $(Ca^{2+})_i$waves propagating within the ensemble based on their common activation. Cells in these clusters/ensembles appear to have a direction-biased cellular motility. Thus, here we demonstrate that the GL261 model is considerably more infiltrative than previously shown, and we infer that the proliferative component is composed of tumor cells from peripheral locations and active ensembles.

Pubmed:
34209535: Pracucci E, Pillai V, Lamers D, Parra R, Landi S
Neuroinflammation: A Signature or a Cause of Epilepsy?
Epilepsy can be both a primary pathology and a secondary effect of many neurological conditions. Many papers show that neuroinflammation is a product of epilepsy, and that in pathological conditions characterized by neuroinflammation, there is a higher probability to develop epilepsy. However, the bidirectional mechanism of the reciprocal interaction between epilepsy and neuroinflammation remains to be fully understood. Here, we attempt to explore and discuss the relationship between epilepsy and inflammation in some paradigmatic neurological and systemic disorders associated with epilepsy. In particular, we have chosen one representative form of epilepsy for each one of its actual known etiologies. A better understanding of the mechanistic link between neuroinflammation and epilepsy would be important to improve subject-based therapies, both for prophylaxis and for the treatment of epilepsy.
Int J Mol Sci, 2021; 22
33273479: Trovato F, Parra R, Pracucci E, Landi S, Cozzolino O, Nardi G, Cruciani F, Pillai V, Mosti L, Cwetsch AW, Cancedda L, Gritti L, Sala C, Verpelli C, Maset A, Lodovichi C, Ratto GM
Modelling genetic mosaicism of neurodevelopmental disorders in vivo by a Cre-amplifying fluorescent reporter.
Genetic mosaicism, a condition in which an organ includes cells with different genotypes, is frequently present in monogenic diseases of the central nervous system caused by the random inactivation of the X-chromosome, in the case of X-linked pathologies, or by somatic mutations affecting a subset of neurons. The comprehension of the mechanisms of these diseases and of the cell-autonomous effects of specific mutations requires the generation of sparse mosaic models, in which the genotype of each neuron is univocally identified by the expression of a fluorescent protein in vivo. Here, we show a dual-color reporter system that, when expressed in a floxed mouse line for a target gene, leads to the creation of mosaics with tunable degree. We demonstrate the generation of a knockout mosaic of the autism/epilepsy related gene PTEN in which the genotype of each neuron is reliably identified, and the neuronal phenotype is accurately characterized by two-photon microscopy.
Nat Commun, 2020; 11
33274251: Baria E, Pracucci E, Pillai V, Pavone FS, Ratto GM, Cicchi R
detection of murine glioblastoma through Raman and reflectance fiber-probe spectroscopies.

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Glioblastoma (GBM) is the most common and aggressive malignant brain tumor in adults. With a worldwide incidence rate of 2 to 3 per 100,000 people, it accounts for more than 60% of all brain cancers; currently, its 5-year survival rate is . GBM treatment relies mainly on surgical resection. In this framework, multimodal optical spectroscopy could provide a fast and label-free tool for improving tumor detection and guiding the removal of diseased tissues. Discriminating healthy brain from GBM tissues in an animal model through the combination of Raman and reflectance spectroscopies. EGFP-GL261 cells were injected into the brains of eight laboratory mice for inducing murine GBM in these animals. A multimodal optical fiber probe combining fluorescence, Raman, and reflectance spectroscopy was used to localize healthy and tumor brain areas and to collect their spectral information. Tumor areas were localized through the detection of EGFP fluorescence emission. Then, Raman and reflectance spectra were collected from healthy and tumor tissues, and later analyzed through principal component analysis and linear discriminant analysis in order to develop a classification algorithm. Raman and reflectance spectra resulted in 92% and 93% classification accuracy, respectively. Combining together these techniques allowed improving the discrimination between healthy and tumor tissues up to 97%. These preliminary results demonstrate the potential of multimodal fiber-probe spectroscopy for label-free detection and delineation of brain tumors, and thus represent an additional, encouraging step toward clinical translation and deployment of fiber-probe spectroscopy.
Neurophotonics, 2020; 7

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BOARD NUMBER: S07-541

ACQUIRED INHERITABLE FACTORS FOR BRAIN FITNESS IN THE PROGRESSION OF GLIOBLASTOMA

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Glioblastoma (GB) is the most common and aggressive malignant primary brain tumor of the central nervous system in humans, originated from neoplastic glial cells. Patients suffer from progressive memory loss, defects in speech and language, epileptic seizures and vomiting, symptoms of neurodegenerative processes, compatible with synapse loss as an early event that occurs in neurodegeneration. However, patients with similar epidemiological features and oncogenic mutations causing GB have a heterogeneous range of life expectancy, but causal determinants are unknown. Our hypothesis stands that different factors contribute to the heterogeneity in the survival of patients, including the physiological status of the healthy tissue in the brain.
Our preliminary data suggest that environmental conditions parental lineages are exposed can be inheritable to the next generation. We found that luminic stress promotes inheritable risk factors and modulate specific gene expression in the brain that leads on the resistance to GB induced neurodegeneration and premature lethality. In particular, we study the contribution of dODF family, a sperm cytoskeleton family and a potential target to drive new therapies against the tumor. Our results in the Drosophila tumor model show that ODF3L2 is upregulated in GB samples and the knockdown of dODF3L2 prevents tumor growth but also restores the glioma neurodegeneration-associated. This work aims to combat the GB by identifying the mechanisms that regulate TMs to reduce GB aggressiveness, extend the patient's life span and combine these protective strategies with oncological treatments

Pubmed:
34380880: de Los Reyes T, Casas-Tintó S
Neural functions of small heat shock proteins.
Stress response is a cellular widespread mechanism encoded by a common protein program composed by multiple cellular factors that converge in a defense reaction to protect the cell against damage. Among many mechanisms described, heat shock proteins were proposed as universally conserved protective factors in the stress core proteome, coping with different stress stimuli through its canonical role in protein homeostasis. However, emerging evidences reveal non-canonical roles of heat shock proteins relevant for physiological and pathological conditions. Here, we review the implications of inducible heat shock proteins in the central nervous system physiology. In particular, we discuss the relevance of heat shock proteins in the maintenance of synapses, as a balanced protective mechanism in central nervous system development, pathological conditions and aging.
Neural Regen Res, 2022; 17
32437379: Santana E, de Los Reyes T, Casas-Tintó S
Small heat shock proteins determine synapse number and neuronal activity during development.
Environmental changes cause stress, Reactive Oxygen Species and unfolded protein accumulation which hamper synaptic activity and trigger cell death. Heat shock proteins (HSPs) assist protein refolding to maintain proteostasis and cellular integrity. Mechanisms regulating the activity of HSPs include transcription factors and posttranslational modifications that ensure a rapid response. HSPs preserve synaptic function in the nervous system upon environmental insults or pathological factors and contribute to the coupling between environmental cues and neuron control of development. We have performed a biased screening in Drosophila melanogaster searching for synaptogenic modulators among HSPs during development. We explore the role of two small-HSPs (sHSPs), sHSP23 and sHSP26 in synaptogenesis and neuronal activity. Both sHSPs immunoprecipitate together and the equilibrium between both chaperones is required for neuronal development and activity. The molecular mechanism controlling HSP23 and HSP26 accumulation in neurons relies on a novel gene (CG1561), which we name Pinkman (pkm). We propose that sHSPs and Pkm are targets to modulate the impact of stress in neurons and to prevent synapse loss.
PLoS One, 2020; 15
33918666: de Los Reyes Corrales T, Losada-Pérez M, Casas-Tintó S

JNK Pathway in CNS Pathologies.

The c-Jun N-terminal kinase (JNK) signalling pathway is a conserved response to a wide range of internal and external cellular stress signals. Beside the stress response, the JNK pathway is involved in a series of vital regulatory mechanisms during development and adulthood that are critical to maintain tissue homeostasis. These mechanisms include the regulation of apoptosis, growth, proliferation, differentiation, migration and invasion. The JNK pathway has a diverse functionality and cell-tissue specificity, and has emerged as a key player in regeneration, tumorigenesis and other pathologies. The JNK pathway is highly active in the central nervous system (CNS), and plays a central role when cells need to cope with pathophysiological insults during development and adulthood. Here, we review the implications of the JNK pathway in pathologies of the CNS. More specifically, we discuss some newly identified examples and mechanisms of JNK-driven tumor progression in glioblastoma, regeneration/repair after an injury, neurodegeneration and neuronal cell death. All these new discoveries support the central role of JNK in CNS pathologies and reinforce the idea of JNK as potential target to reduce their detrimental effects.

Int J Mol Sci, 2021; 22

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BOARD NUMBER: S07-542

MANIPULATING NEURAL STEM CELLS TO CREATE A TRANSGENIC INDEPENDENT MODEL FOR GLIOBLASTOMA

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Neural stem cells (NSCs) residing in the subventricular zone (SVZ) of the forebrain, are cells of origin of glioblastoma (GBM), the most frequent and aggressive of the primary brain cancers (Lee et al. Nature 2022). Indeed, somatic mutations in tumor suppressor genes in postnatal neural stem cells of the subventricular zone (SVZ) act as drivers of glioblastoma development. In order to better understand the etiology of the disease, and the molecular processes that are involved in the onset and progression of GBM, it is important to develop transgenic mouse models that enable mechanistic studies. However, currently GBM induction implicates complex genetic backgrounds and the use of conditional mutations based on the CRE-Lox system, limiting genetic studies of new candidate genes or regulators potentially involved in gliomagenesis. In order to circumvent this limitation, we developed an in vivo glioblastoma model that is is based on postnatal brain electroporation of neural stem cells in combination (Boutin et al., PlosOne 2008) with the PiggyBac transposase system and the use of CRISPR technology. The system is easily applicable and does not implicate the use of transgenic mouse lines or the CRE-LoxP system, freeing such approaches for functional studies. We show that this model enables the induction and analysis of GBM development in a temporally and spatially highly regulated fashion and may be implemented in any mutated genetic background.

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BOARD NUMBER: S07-543

INTEGRATED COMPUTATIONAL AND EXPERIMENTAL SINGLE CELL ANALYSES REVEALS MPST AS A CRITICAL METABOLIC VULNERABILITY FOR MOTILE GLIOBLASTOMA CELLS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Mirca Saurty-Seerunghen[1], Thomas Daubon[2], Lea Bellanger[3], Virgile Delaunay[1], Gloria Castro[1], Joris Guyon[2], Ahmed Rezk[1], Ahmed Idbaih[4], Fabien Almeirac[5], Fanny Burel-Vandenbos[5], Laurent Turchi[5], Thierry Virolle[5], Jean-Michel Peyrin[1], Christophe Antoniewski[3], Elias El-Habr[1], Marie-Pierre Junier[1], Hervé Chneiweiss[1]
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The diversity of cancer cells populating glioblastoma participates to the complex biological system represented by these brain tumors defying treatments. Nonetheless cancer cells can adopt similar functional states despite the diversity in genomic anomalies encountered between and within patient tumors. Metabolism being downstream of all signaling pathways regulating cell behaviors, it constitutes a source of vulnerabilities that should help overcoming the inter- and intra-tumor genetic heterogeneity. We therefore looked for metabolic weaknesses in link with motility, a key functional state for glioblastoma aggressiveness. A signature-driven data reduction approach highlighted motile cells present in thirty tumors from four independent single-cell transcriptomic datasets. Analyses integrating trajectory modeling disclosed, as characteristic of motile cells, enhanced oxidative stress coupled with mobilization of the cysteine metabolism enzyme 3-Mercaptopyruvate sulfurtransferase (MPST). The soundness of this prediction was verified using migration and invasion assays with patient-derived cells and tissue organoids. Pharmacological and genetic manipulations showed that enhanced ROS production and MPST activity are required for the cells' motility. Biochemical assays indicated that MPST acts by protecting protein cysteine residues from dismal hyperoxidation. In vivo, MPST knockdown translated in reduced tumor burden, and a robust increase in mice survival. These results show that enhanced oxidative stress coupled with MPST mobilization plays a key role in glioblastoma cell motility. Overall, they demonstrate that single-cell transcriptomes can be leveraged to map cancer cell behaviors within the patients' tumors as well as their associated metabolic pathways.

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BOARD NUMBER: S07-544

IMMUNOMODULATORY EFFECTS OF PULSED ELECTRIC FIELD STIMULATION IN A MURINE GLIOBLASTOMA MODEL

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Attila Kaszas[1,2,3], Andrea Slezia[1,2], Marie Lefevre[3], Gerwin Dijk[3,4], David Moreau[3], Martin Baca[3], Loig Kergoat[4], Geneviève Rougon[2], Rodney O'Connor[3], Franck Debarbieux[1,2,5]
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Aims: Glioblastoma multiforme is the most aggressive and frequently occurring brain tumor. Pulsed electric fields (PEFs) are an emerging tool for treating cancer, though less studied in tumors of the brain. Whereas PEFs are known to induce irreversible permeabilization and death of cancer cells, we have explored their immunomodulatory role and their long-term impact on intracortical tumor development. **Methods:** Glioblastoma spheroids were seeded 200µm deep in the cortex of adult "AMU-Neuroinflam" multifluorescent mice prior to electrode placement and glass window implantation. Intravital two-photon microscopy was used to monitor the growth of a GL261-DsRed glioblastoma tumor exposed to pulse trains of 100 µs PEFs through epidurally implanted flexible gold electrodes. Animals were split into 3 groups, receiving either no treatment, or receiving daily pulse trains at 1Hz or 100Hz from day 26 onward. Densities of tumor cells, neurons, microglia and peripheral inflammatory cells were quantified in the same millimetric volume of interest 26 and 29 days after implantation. **Results:** The stimulation protocol, verified by impedance and current measurements, allowed the in situ three-dimensional mapping of the effect of electrical stimulation. Repeated stimulation and measurements were possible on a daily basis both from the electrical and from the physiological point of view. Stimulations immediately triggered the accumulation of peripheral innate immune cells in a subset of vessels located in between the electrode fingers. **Conclusions:** Our results support the immunomodulatory effects of PEFs in a gliobastoma mouse model, whose frequency dependence and antitumor actions are currently being characterized.

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IDENTIFYING NOVEL MEDIATORS OF TUMOR-NERVE INTERACTIONS IN CANCER PAIN

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Cancer-associated pain represents an essential determinant of the poor quality of life and an important predictor of the prognosis in patients with pancreatic adenoductal carcinoma (PDAC). Neuroplastic alterations and neural invasion are the pathological features of PDAC, which occur in almost all tumors and are highly correlated with cancer-associated pain. Molecular mediators such as chemokines are essential on the bi-directional interactions between nerves and cancer cells, which induce tumor-associated pain. In a screen, we identified Cardiotropin-1(CT-1), an IL-6 family member, to be secreted by cancer cells. We sought to study its effects on sensory nerves and tumor-nerve interactions in PDAC. We found expression of CT-1 in both PDAC patients and PDAC-bearing mice and was significantly increased in cancer states compared to healthy human subjects and naïve mice. Moreover, after blocking CT-1 signaling, the sensory hypersensitivity associated with PDAC-associated pain was significantly attenuated in tumor-bearing mice compared to the naïve mice. In conclusion, our study indicates that CT-1 contributes to PDAC-induced pain by activating signaling in sensory neurons. The authors acknowledge grant support from the German Cancer Aid to R.K.

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INTRAVITAL IN-SITU CHARACTERIZATION OF THE EMISSION SPECTRA OF GENETICALLY ENCODED FLUORESCENT PROTEINS IN THE PATHOLOGICAL BRAIN TUMOR ENVIRONMENT

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Aims: Imaging of multiple fluorescent labels in transgenic mice has become common practice to characterize cellular interactions in the brain. Nevertheless, spectral overlap between fluorophores requires efficient and reliable unmixing methods for the quantitative analysis of imaging data. In the context of intravital imaging of brain tumor development, we have here characterized the impact of imaging depth and of the pathological microenvironment on the success of multi-labeled cells identification. **Methods:** We used **i**ntravital two-photon images of the parietal cortices of Thy1-CFP, CD11c-EYFP, LyzM-EGFP triple transgenic mice implanted with GL261-DsRed murine glioblastoma cells. Five-color tiled images were acquired every 20 microns over 400 microns in Z to cover a millimetric volume of interest imaged repeatedly under the same conditions. Mono-labeled cellular processes were visually selected at different depths; from these, individual five-channels spectra were determined for each fluorophore and collected into a depth-dependent database. Sets of spectra were then used to feed the ZEN unmixing algorithms (black edition software) and to provide fluorophore specific images stacks. **Results:** Collected emission spectra showed different levels of variability. DsRed spectra were highly reproducible while CFP spectra were stable within 15% accuracy. EGFP and EYFP spectra were instead highly variable as expected from the sensitivity of their fluorescence to pH and chemical environment if expressed in immune cell subsets around a tumor. **Conclusion:** The patterned distribution of fluorescent cells thus has to be taken into account to implement locally adaptative unmixing strategies for accurate quantification of fluorophore expression in a pathological context.

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BRAIN TUMOR TREATMENT USING TUNABLE LOCAL CHEMOTHERAPY

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Linda Waldherr[1], Verena Handl[2], Tobias Abrahamsson[3], Theresia Arbring Sjöström[4], Maria Seitanidou[3], Sabine Erschen[1], Sophie Honeder[5], Tamara Tomin[5], Ruth Birner-Grünberger[5], Nassim Ghaffari Trabizi-Wiszy[6], Stefan Ropele[7], Muammer Üçal[8], Marta Nowakowska[8], Ute Schäfer[8], Silke Patz[8], Daniel Simon[4], Rainer Schindl[9]
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Poor delivery and systemic toxicity of many chemotherapeutic agents limit their therapeutic success in brain cancer treatment. Local chemotherapy approaches offer a new path to efficiently interfere with brain tumor growth and are able to reduce tumor size. We present miniature bioelectronic devices for drug delivery able to administer chemotherapeutics via electric control with high spatiotemporal precision. The drug delivery is based on the electromigration of molecules in an ion selective matrix towards a target of choice. These devices, called chemotherapeutic ion pumps (chemoIPs), can be used for triggered release of chemotherapeutics that are usually shielded by the blood brain barrier. With chemoIPs it is possible to constantly administer drugs with highest precision towards cell culture spheroids, *ex ovo*-grown tumors and *in vivo* brain tumors. The treatment efficiency was analyzed via flow cytometry quantifying apoptosis and cell cycle arrest, as well as immunohistochemistry. ChemoIP treatment is able to trigger the disintegration of targeted tumor spheroids, and is able to inhibit the tumor growth of *ex ovo*-glioblastomas significantly. Furthermore, the proteomes of neurons and glioblastoma cells showed that only glioblastoma cells are harmed by the chemotherapeutic treatment, but not neurons. In parallel, we follow the pharmacokinetics of the chemoIP-mediated drug administration via mass spectrometry and compare it to computer simulations in different tumor models. The here exemplified electrically-driven drug delivery via chemoIPs is a drug administration method that can serve as basis for further implant development, which has the potential to increase the efficacy of chemotherapy due to highly-targeted and locally-controlled drug delivery.

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HUNTINGTIN DEPLETION SENSITIZES GLIOMA CELLS TO TEMOZOLOMIDE.

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Gliomas (GBM) are the most common and malignant form of primary brain tumors with a deadly outcome. Glioma cells are largely resistant to available treatments including to the alkylating agent temozolomide (TMZ), the standard of care for GBM. Given the poor outcome of the patients, there is a crucial need in finding a way to overcome TMZ resistance of GBM and to restore the efficiency of the drug. The huntingtin (HTT) protein is well known to be mutated in Huntington disease. HTT exerts many functions that are not restricted to neurons but also occur in cancer cells. HTT mediates the early response to DNA damage and regulates breast cancer metastasis. While the role of HTT in the biology of cell cancer is emerging in the literature, its contribution to chemoresistance is still unknown. We downregulated the protein levels of huntingtin (HTT) in the glioma cell lines U87, U251 and T98G. The compound and strategy used are patent pending. We found that HTT depletion sensitizes the cells to TMZ by exacerbating DNA damage, (as assessed by measuring levels of the DNA double stranded breaks marker phospho-γH2Ax, and poly (ADP-ribose) polymerase 1 (PARP1) cleavage. We are now examining whether our compound and strategy can overcome TMZ resistance in an orthotopic mouse model for GBM. Our findings suggest a novel role of HTT in the biology of brain tumors and underscore the importance of studying HTT in basic and translational cancer research.

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SYMPATHETIC AXONAL SPROUTING INDUCES CHANGES IN MACROPHAGE POPULATIONS AND PROTECTS AGAINST PANCREATIC CANCER

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

Sophie Chauvet[1], Jérémy Guillot[1], Chloé Dominici[1], Angélique Puget[1], Mélanie Hocine[1], Martha Rangel-Sosa[1], Anders Etzerodt[2], Toby Lawrence[2], Pierre Pudlo[3], Florence Hubert[3], Serge Van De Pavert[2], Richard Tomasini[4], Fanny Mann[1]
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Aims: Neuronal nerve processes in the tumor microenvironment were highlighted recently. However, the origin of intra-tumoral nerves remains poorly known, in part because of technical difficulties in tracing nerve fibers via conventional histological preparations. We focused on Pancreatic ductal adenocarcinoma (PDAC) which is a cancer with poor prognosis that develops from the exocrine part of the pancreas. We study how the structure and distribution of sympathetic nerves change during PDAC development, and examined how sympathetic inputs affect disease progression and deciphered the mechanisms involved. **Methods:** We used three-dimensional imaging of cleared tissues to perform a comprehensive analysis of sympathetic innervation in a murine model of pancreatic ductal adenocarcinoma (KIC mice). To test the role of the neuroplastic changes in tumor progression, we performed chemical and chirurgical sympathectomy in KIC mice and orthotopic mouse models of PDAC. We used doxorubicin-loaded liposomes coated with CD163 antibody (DxR lipo) to test the contribution of $CD163^+$ macrophages. **Results:** Our results support two independents, but coexisting, mechanisms for tumor innervation: passive engulfment of pre-existing nerves within tumors and active, localized sprouting of nerve terminals into non-neoplastic lesions and tumor periphery. Ablation of the innervating sympathetic nerves increased tumor growth and spread. This effect was explained by the observation that sympathectomy increased intratumoral $CD163^+$ macrophage numbers, which contributed to the worse outcome. **Conclusions:** Altogether, our findings revealed new insights into the mechanisms by which the sympathetic nervous system exerts cancer-protective properties in a mouse model of PDAC.

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ROLE OF SCHWANN CELL PLASTICITY IN THE INNERVATION OF PANCREATIC TUMORS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Aims: Nerves are important components of the tumor microenvironment. As pancreatic ductal adenocarcinoma (PDAC) progresses from pre-cancerous to obvious cancerous stages, sympathetic fiber density increases and single axons branch. The mechanisms by which sympathetic axons remodel during PDAC progression are unknown. Schwann cells (SCs) are associated with nerve fibers and exhibit high plasticity in response to the microenvironment, becoming a source of growth and axon guidance signals when activated. Here we investigated how SCs could contribute to neuronal structural plasticity induced by tumor development. **Methods:** We investigated changes in density and distribution of SCs in the pancreas of transgenic mouse model of PDAC (KIC mice) and in a model of metaplastic lesions (precursor lesions of PDAC) induced by chronic inflammation. Dividing cells were labeled with EdU. Pancreatic SC were isolated by FACS and gene expression was analyzed by qPCR. **Results:** Nonmyelinating SCs were found along the entire sympathetic fibers in healthy tissue and in metaplastic/neoplastic lesions of the pancreas. We found a significant increase in the number of SCs in metaplastic lesions, concomitant with an increase in proliferating EdU^+ SCs. We further observed increased expression of glial-derived neurotrophic factor (GDNF) in SCs after chronic inflammation. Sympathetic axons innervating metaplastic lesions expressed the GDNF receptor GFRa1, and celiac ganglia neurons (which provide sympathetic inputs to the pancreas) showed increased axonal growth in response to GDNF in vitro. **Conclusions:** Pancreatic SCs proliferate during PDAC progression, reflecting their possible activation and contribution as source of neurotrophic signals to induce sympathetic fiber remodeling.

Pubmed:

29174089: Córdova-Fletes C, Becerra-Solano LE, Rangel-Sosa MM, Rivas-Estilla AM, Alberto Galán-Huerta K, Ortiz-López R, Rojas-Martínez A, Juárez-Vázquez CI, García-Ortiz JE
Uncommon runs of homozygosity disclose homozygous missense mutations in two ciliopathy-related genes (SPAG17 and WDR35) in a patient with multiple brain and skeletal anomalies.
We describe a patient severely affected with multiple congenital anomalies, including brain malformations and skeletal dysplasia suggestive of cranioectodermal dysplasia (CED) ciliopathy, who unusually carries several homozygosity tracts involving homozygous missense mutations in SPAG17 (exon 8; c.1069G > C; p.Asp357His) and WDR35 (exon 13; c.1415G > A; p.Arg472Gln) as revealed by homozygosity mapping and next generation sequencing. SPAG17 is essential for the function and structure of motile cilia, while WDR35 belongs to the same intraflagellar transport (IFT) gene family whose protein products are part of functional IFT A and B complexes. Formerly, SPAG17 was related - through polymorphic variants - to an influence on individuals' height; more recently, Spag17-/- mice models were reported to present skeletal and bone defects, reduced mucociliary clearance, respiratory distress, and cerebral ventricular enlargement. Homozygous or compound heterozygous mutations in WDR35 have mainly been related to CED2 or short-rib thoracic dysplasia 7, with only three cases showing some brain anomalies. Given that our patient presents these clinical features and the close functional relationship between SPAG17 and WDR35, it is feasible that the combined effects from both mutations contribute to his phenotype. To our knowledge, this patient is the first to harbor a likely pathogenic homozygous mutation in both genes at the same time. Thus, the resulting complex phenotype of this patient illustrates the heterogeneity associated with ciliopathies and further expands the clinical and mutational spectrum of these diseases. Finally, we highlight the combined use of high-throughput tools to diagnose and support the proper handling of this and other patients.
Eur J Med Genet, 2018; 61
29213157: Rangel-Sosa MM, Aguilar-Córdova E, Rojas-Martínez A
Immunotherapy and gene therapy as novel treatments for cancer.
The immune system interacts closely with tumors during the disease development and progression to metastasis. The complex communication between the immune system and the tumor cells can prevent or promote tumor growth. New therapeutic approaches harnessing protective immunological mechanisms have recently shown very promising results. This is performed by blocking inhibitory signals or by activating immunological effector cells directly. Immune checkpoint blockade

with monoclonal antibodies directed against the inhibitory immune receptors CTLA-4 and PD-1 has emerged as a successful treatment approach for patients with advanced melanoma. Ipilimumab is an anti-CTLA-4 antibody which demonstrated good results when administered to patients with melanoma. Gene therapy has also shown promising results in clinical trials. Particularly, virus (HSV)-mediated delivery of the HSV thymidine kinase (TK) gene to tumor cells in combination with ganciclovir (GCV) may provide an effective suicide gene therapy for destruction of glioblastomas, prostate tumors and other neoplasias by recruiting tumor-infiltrating lymphocytes into the tumor. The development of new treatment strategies or combination of available innovative therapies to improve cell cytotoxic T lymphocytes trafficking into the tumor mass and the production of inhibitory molecules blocking tumor tissue immune-tolerance are crucial to improve the efficacy of cancer therapy.
Colomb Med (Cali), 2017; 48
29437235: Rangel-Sosa MM, Figuera-Villanueva LE, González-Ramos IA, Pérez-Páramo YX, Martínez-Jacobo LA, Arnaud-López L, Nastasi-Catanese JA, Rivas-Estilla AM, Galán-Huerta KA, Rojas-Martínez A, Ortiz-López R, Córdova-Fletes C
Exome sequencing reveals three homozygous missense variants in SNRPA in two sisters with syndromic intellectual disability.
Splicing-related gene mutations might affect the expression of a single gene or multiple genes and cause clinically heterogeneous diseases. With the advent of next-generation sequencing, several splicing gene mutations have been exposed, yet most major spliceosome genes have no reports of germline mutations and therefore, their effects are largely unknown. We describe the previously unreported concurrence of intellectual disability, short stature, poor speech, and minor craniofacial and hand anomalies in 2 female siblings with 3 homozygous missense variants in SNRPA (a component of the U1 small nuclear ribonucleoprotein complex) characterized by homozygosity mapping and whole exome sequencing. Combined, c.97A>G, c.98T>C, and c.100T>A, in exon 2 of SNRPA lead to p.Ile33Ala and p.Phe34Ile exchanges, which were predicted in silico to be deleterious. Although both patients exhibited some clinical features seen in other spliceosomal disorders, their complete clinical phenotype appears to be rather uncommon, a finding that may further support the notion that mutations in components of the major spliceosome do not strictly lead to the same syndromes/phenotypes.
Clin Genet, 2018; 93
32597225: Córdova-Fletes C, Rangel-Sosa MM, Martínez-Jacobo LA, Becerra-Solano LE, Arellano-Valdés CA, Tlacuilo-Parra JA, Galán-Huerta KA, Rivas-Estilla AM, Hernandez-Orozco AA, García-Ortiz JE
Whole-exome sequencing in three children with sporadic Blau syndrome, one of them co-presenting with recurrent polyserositis.
Blau syndrome (BS) is a rare, chronic autoinflammatory disease with onset before age 4 and mainly characterised by granulomatous arthritis, recurrent uveitis, and skin rash. Sporadic (also known as early-onset sarcoidosis) or familial BS is caused by gain-of-function mutations in the gene, which encodes for a multi-task protein that plays a crucial role in the innate immune defense. We report on three Mexican patients clinically diagnosed with BS who exhibited a likely pathogenic variant in as revealed by whole-exome sequencing (WES) and Sanger sequencing: two variants (c.1000 C > T/p.Arg334Trp and c.1538 T > C/p.Met513Thr) lie in the ATP/Mg2+ binding site, whereas the other (c.3019dupC/p.Leu1007ProfsTer2) introduces a premature stop codon disrupting the last LRR domain (LRR9) formation; all three variants are consistent with gain-of-function changes. Interestingly, all these patients presented concomitant likely pathogenic variants in other inflammatory disease-related genes, i.e. , , and/or . Although the clinical presentation in these patients included the BS diagnostic triad, overall it was rather heterogeneous. It is plausible that this clinical variability depends partly on the patients' genetic background as suggested by our WES results. After this molecular diagnosis and given the absence of mutations (demonstrated in two trios) and related symptoms in the respective parents (confirmed in all trios), patients 1 and 2 were considered to have sporadic BS, while patient 3, a sporadic BS-recurrent polyserositis compound phenotype. Altogether, our observations and findings underscore the overlapping among inflammatory diseases and the importance of determining the underlying genetic cause by high-throughput methods. Likewise, this study further reinforces a pathogenic link between the here found variants and BS and envisages potential additive effects from other loci in these, and probably other patients.
Autoimmunity, 2020; 53
33152732: Ornelas-Arana ML, Pérez-Garcia G, Robles-Espinoza CD, Rangel-Sosa MM, Castaneda-Garcia C, Juárez-Vázquez CI, López-Pérez LG, Pérez-Ornelas C, Hernández-Zaragoza G, Lara-Aguilar RA, Córdova-Fletes C
Genomic Characterization of a Rare, de Novo Unbalanced ins(3;1)(p25.3;q21.3q23.3) in a Female Child with Multiple Congenital Anomalies.
"Simple" 1-way interchromosomal insertions involving an interstitial 1q segment are rare, and therefore, their characterization at the base pair level remains understudied. Here, we describe the genomic characterization of a previously unreported de novo interchromosomal insertion (3;1) entailing an about 12-Mb pure gain of 1q21.3q23.3 that causes typical (microcephaly, developmental delay, and facial dysmorphism) and atypical (interauricular communication, small feet with bilateral deep plantar creases, syndactyly of II-IV toes, and mild pachyonychia of all toes) clinical manifestations associated with this region.

Based on our analyses, we hypothesize that the duplication of a subset of morbid genes (including LMNA, USF1, VANGL2, LOR, and POGZ) could account for most clinical findings in our patient. Furthermore, the apparent disruption of a promoter region (between CPNE9 and BRPF1) and a topologically associated domain also suggests likely pathogenic reconfiguration/position effects to contribute to the patient's phenotype. In addition to further expanding the clinical spectrum of proximal 1q duplications and evidencing the phenotypical heterogeneity among similar carriers, our genomic findings and observations suggest that randomness - rather than lethality issues - may account for the paucity of "simple" interchromosomal insertions involving the 1q21.3q23.3 region as genomic donor and distal 3p25.3 as receptor. Moreover, the microhomology sequence found at the insertion breakpoint is consistent with a simple nonhomologous end-joining mechanism, in contrast to a chromothripsis-like event, which has previously been seen in other nonrecurrent insertions. Taken together, the data gathered in this study allowed us to inform this family about the low recurrence risk but not to predict the reproductive prognosis for hypothetical carriers. We highlight that genomic-level assessment is a powerful tool that allows the visualization of the full landscape of sporadic chromosomal injuries and can be used to improve genetic counseling. Cytogenet Genome Res, 2020; 160

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BOARD NUMBER: S07-551

DEVELOPMENT OF METHODS ABOUT SAFE APPLICATION OF DNA-VACCINE VIRAL STRAINS FOR PRODUCTION OF ANTI-MALIGNANT AND ANTI-VIRAL MOLECULAR VACCINES

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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The main *goal* was directed to development of molecular (DNA, RNA and/or protein) anti-malignant and anti-viral vaccines, but also against virus envelope protein (E) or against virus membrane (M) protein, as well as of specific siRNAs against the virus genes, coding virus proteins S and N and against cellular oncogenes. Recombinant DNA-constructs, based on the *adeno-associated virus* (*AAV*) (*Parvoviridae* family) DNA-genome were developed. Genomic assay was performed by standard Polymerase Chain Reaction (PCR), and expression of the respective inserted gene - by Reverse Transcriptase PCR (PR-PCR). Low infectious titers (high dilutions) of heterologous for mammalian cells and attenuated by passages vaccine avipoxvirus strains (*Poxviridae* family), were applied. The inoculated cellular monolayers were freezed in the presence of cryo-protector Dimethylsulfoxide (DMSO), thawed and re-incubated. After washing with Phosphate Buffered Saline (PBS), the monolayers were scraped-off and served as source of intra-cellular viral forms. Cultural fluids served as source of their extra-cellular forms. The higher titers of the intra-cellular forms could be explained with existance of viruses as integrated in the cellular genome, besides as free viral particles. These changes could be explained with changed properties of membrane molecules. The results confirm the proved in the literature possibility for expression of membrane receptor glycoproteins by non-myeloid and non-lymphoid cellular types in appropriate conditions, as presence of malignant cells/antigens, viruses or viral antigens, immunomodulators. A possibility for nucleotide (DNA- and/or RNA-) fragments transfer from virus to cellular genome, but also in the opposite direction because of the activated fusion processes was also suggested.

Pubmed:

15664048: Sainova IV, Kril AI, Simeonov KB, Popova TP, Ivanov IG
Investigation of the morphology of cell clones, derived from the mammalian EBTr cell line and their susceptibility to vaccine avian poxvirus strains FK and Dessau.
The ability for replication of vaccine avian pox viral strains FK and Dessau in cell clones, derived from the EBTr cell line, derived from embryonic bovine trachea, was studied. The derived seven cell clones showed different morphological characteristics and diverse sensitivity to both vaccine avian pox viral strains. Hence, the EBTr-derived cell clones could be used for cultivation, as well as for differentiation of vaccine avian pox viral strains. In addition, studies have been undertaken to elucidate the possible use of cultivated strains in these heterologous cell culture system's vaccine avian pox viral strains for biotechnology, as well as for solving problems, related to infection of people with avian viruses.
J Virol Methods, 2005; 124
16899916: Savov Y, Antonova N, Zvetkova E, Gluhcheva Y, Ivanov I, Sainova I
Whole blood viscosity and erythrocyte hematometric indices in chronic heroin addicts.
Whole blood viscosity (WBV) and hematometric indices of erythrocytes as red blood cell count (RBC), mean erythrocyte volume (MCV), hemoglobin (HGB), hematocrit (HCT), mean hemoglobin content of erythrocytes (MCH), HGB/HCT values (MCHC) and red blood cell distribution width (RDW) have been studied in a group of 15 chronic opioid addicts under methadone maintenance therapy with mean age 26.53 +/- 7.34 years. WBV elevation and changes in MCV, HGB, HCT, RDW were found in intravenous drug users compared to healthy individuals. As well, RBC was decreased leading to an increase in MCH and MCHC values. Correlation analysis suggested that the correlation among the RBC, HGB, HCT and WBV was the closest. Heroin macrocytosis (heroin macrocytic anemia) was established, related with the increased RDW in chronic heroin abusers. The results are in accordance with data revealing abnormal effects of alcohol and other drugs on whole blood rheology and hematometric/morphometric characteristics of erythrocytes.
Clin Hemorheol Microcirc, 2006; 35

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BOARD NUMBER: S07-552

CYTOSKELETAL PROTEIN PALLADIN IN ADULT GLIOMAS PREDICTS DISEASE, PROGRESSION AND PROGNOSIS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Introduction and Aims: Gliomas, originating from neuroglia, are tumors differing in diagnosis, prognosis and treatment. Palladin (PALLD gene) is a structural protein widely expressed in mammals that has a role in cytoskeletal dynamics and motility in health and disease. Palladin has been linked to the progression of breast, pancreatic and renal cancers. In the CNS, palladin is expressed in the neural plate, neural progenitors, cortical neurons and astrocytes. It participates in embryonic development, neuronal maturation, cell-cycle, differentiation and apoptosis. In this study, we investigate the role of palladin in adult gliomas. **Results:** By analyzing 1130 samples, we determine that wild-type PALLD is overexpressed in gliomas compared to healthy tissue. PALLD expression pattern correlates with disease progression and decreased patient survival. Comparison of prognostic markers with palladin confirm PALLD expression as having the highest predictive value. We injected mouse GBM cells into C57Bl/6J mice brains. Imaging located palladin only in the area of cancer cells. We obtained multi-tissue arrays of 600 cores of CNS pathologies and probed them for palladin. We conclude that palladin is present in glioma tumors but not in healthy tissue. Finally, by analyzing scRNAseq data, we show that PALLD expression originates only from the malignant cell population. **Conclusions:** Our findings indicate that palladin expression correlates with glioma progression and suggest that its levels may impact prognosis. Overall, our results point to palladin's potential as a marker for glioma diagnosis, risk stratification and as a novel molecular target for the treatment of aggressive glioma tumors in the future.

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GLIOBLASTOMA MULTIFORME: STUDY OF THE SYNERGIC EFFECT OF MEDICINAL MUSHROOMS AND NEW PLATINUM COMPOUND IN U251 HUMAN GLIOBLASTOMA CELL LINE

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Glioblastoma Multiforme is the most common and lethal primary brain tumor in human. After standard therapies the development of resistance is almost inevitable. An alternative could be represented by Cisplatin (cis-diaminedicloroplatinum-II, CDDP), which however shows important side effects, to overcome this limitation fourth-generation platinum compounds were synthesized. Pt(IV)Ac-POA, (OC-6-44)-acetate-diamine-chloride(2-(2-propinyl)octanoate)platinum(IV), is a prodrug having an Histone-3-DeAcetylase-Inhibitor as axial ligands. Promising sources for cancer therapy are also compounds of plant origin, that, interfering in the metabolism of reactive oxygen species (ROS), in synergy with new chemotherapy could give patients an improvement in quality of life. **Aims:** We evaluated the effect in the redox balance and in the activation of cell death pathways of a medicinal blend supplement (Agaricus blazei, Cordyceps sinensis, Grifola frondose, Lentinula edodes and Ganoderma lucidum) alone and in combination with platinum-based chemotherapies. **Methods:** Cytofluorimetric, ultrastructural and immunofluorescence analysis were adopted to evaluate the therapeutic effects of the studied compound. **Results:** The reduction of oxidative stress condition in the cytoplasm, favored by the use of micotherapy, emerged; contrary, oxidative-induced damage to the mitochondrial was found. Completion of the autophagic flux, typical of an adaptive response of tumor cells to chemotherapy, didn't occur after the use of micotherapy. Moreover, the activation of the intrinsic apoptotic pathway, mitophagy, and regulated form of necrosis (ferroptosis and parthanantos) were observable following combined therapy. **Conclusions:** The synergic activity of micotherapy and chemotherapy amplified the effects of the latter in the activation of programmed cell death reducing inflammatory conditions in the microenvironment and resistance mechanisms.

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COGNITIVE EFFECTS IN NON-CNS CANCER SURVIVORS BEFORE AND 1-YEAR AFTER STANDARD ANTHRACYCLINE-BASED CHEMOTHERAPY TREATMENT

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Background: While anthracycline-based treatments have increased cancer survival rates, they have also been proven to be neurotoxic, with negative consequences on cancer survivors' cognitive functioning. To date, little attention has been given to the cognitive effects associated with anthracycline regimens locally. Aim: This study examined the associated cognitive effects in a cohort of non-CNS cancer patients exposed to an anthracycline-based chemotherapy regimen. Setting: Patients were recruited from an outpatient oncology clinic at a semi-rural hospital in the North of Gauteng, South Africa. Methods: In this time-series study, we examined cognitive function in n = 20 (stages II and III) breast cancer survivors (mean age, 50 years) prior to starting a standard FAC regimen (fluorouracil 500 mg/m^2, adriamycin 50 mg/m^2, cyclophosphamide 500 mg/m^2- administered intravenously every 3 weeks for 6 cycles) and a follow-up self-reported cognitive assessment at least 12 months after treatment. Results: A significant decline in self-reported cognitive function from baseline (prior to chemotherapy) (M = 56.45, SD = 3.36) to T_2 (cycle 6) (M = 43.90, SD = 12.88), t (19) = 4.66, p = 0.0002 was found on standard FAC regimen. From completion of the regimen (T_2) (M = 43.90, SD = 12.88) to one-year post FAC chemotherapy treatment (T_3) (M = 60.6, SD = 16.66) significant improvement were reported in self-perceived cognitive function (p = 0.003). Conclusion: Preliminary findings point to the necessity for a major multi-center comparative study that includes objective neuropsychological testing and self-perceived cognitive function.

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IN VITRO TARGETING-TREATMENT POTENTIAL OF CHLOROTOXIN PEPTIDE-FUNCTIONALIZED NANOPARTICLES IN NEUROBLASTOMA AND GLIOBLASTOMA CELLS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Introduction: The development of effective treatment for both glioblastoma (GB) and neuroblastoma (NB) remains a major challenge despite decades of multidisciplinary research efforts. Current chemotherapies appear to offer only short-term palliative relief to patients due to systemic toxicity, drug resistance, poor targeting efficiency, and inadequate blood-brain barrier (BBB) penetration. Nanoparticles (NPs) appear to offer novel alternatives. **Aims**: To investigate the uptake, targeting, and anticancer effects of chlorotoxin (CTX)-functionalized monometallic gold NPs (CTX-AuNPs) as well as bimetallic gold-platinum NPs (CTX-AuPtNPs) in the U87 and SH-SY5Y cancerous human cell lines as well as the non-cancerous human KMST-6 cell line used as control. **Methods**: The cells were treated for 48 hours at a concentration range of 75-300 µg/ml after which NPs uptake was evaluated and bioactivity investigated via standard assays for cell viability (WST-1), apoptosis (APOPercentage™ assay), oxidative stress (ROS-sensitive dye CM-H_2DCFDA), mitochondrial activity (TMRE dye), cell survival (clonogenic assay), cell migration (wound healing assay) and morphology (light microscopy). **Results**: showed that CTX facilitated targeted delivery of CTX-AuPtNPs, possibly via binding with MMP-2, a main molecular target of CTX expressed on the cancerous cells. Also, CTX-AuPtNPs were the most cytotoxic NPs while U87 cells were more sensitive to the effects of the NPs. The effects of CTX-AuPtNPs suggest possible synergistic effects of the two noble metal constituents. **Conclusions**: Functionalized monometallic and bimetallic NPs could be useful both for inducing cytotoxicity to cancer cells and as radiosensitizing agents for targeted radiofrequency (RF)-induced thermal ablation therapy.

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BOARD NUMBER: S07-556

P53 DIGITAL EXPRESSION ANALYSIS IN MENINGIOMAS BASED ON TISSUE MICROARRAYS

POSTER SESSION 07 - SECTION: NEURO-ONCOLOGY

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Aim: Meningiomas is the most common intracranial primary central nervous system (CNS) tumour in adults worldwide. Oncogenes' over activation combined with suppressor genes' silence affect negatively the biological behaviour of these neoplasms. Our purpose was to explore the role of p53 expression in meningiomas' clinic-pathological features based on a combination of sophisticated techniques. **Methods**: Fifty (n=50) meningiomas were included in the study comprising a broad spectrum of histopathological sub-types. An immunohistochemistry assay was applied on tissue microarray cores (diam 1.5 μm) followed by digital image analysis for measuring objectively the corresponding p53 staining intensity levels. **Results**: p53 protein over expression (high staining intensity values) was observed in 27/50 (54%) cases, whereas the rest (23/50-/46%) demonstrated moderate to low levels of the molecule. P53 over expression was statistically significant correlated to mitotic index of the examined cases (p-value =0.001). Interestingly, atypical/anaplastic group of histotypes demonstrated the strongest p53 expression rates compared to the others (p-value =0.001). **Conclusion**: p53 over expression is observed in a broad spectrum of meningiomas. High expression levels lead to an aggressive biological behavior of the malignancy (combined with increased mitotic rates), especially in atypical and anaplastic sub-types that are characterized also by a strong tendency to recurrence.

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BOARD NUMBER: S07-557

THE EFFECT OF A GRIN2D VARIANT IN DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY IN A CRISPR/CAS9 MOUSE MODEL AND IPS CELLS

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

Danielle Galber[1], Mor Ovadia[2], Tatiana Rabinski[3], Sivan Sagiv[3], Gad Vatine[3], Moran Rubinstein[2], Karen Avraham[1]
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Aims: Developmental and Epileptic Encephalopathies (DEEs) are a group of devastating disorders that encompass global developmental delay and epileptic activity. Patients suffer from a wide range of cognitive and motoric impairments, intractable epilepsy, and additional comorbidities. Next generation sequencing resulted in a surge of gene discoveries related to DEEs, including a *de-novo* variant (c.1999G>A) in *GRIN2D* that has been associated with hypotonia, early onset epilepsy and intellectual disabilities. *GRIN2D* encodes GluN2D, a subunit of the NMDA receptor that plays a central role in synaptic transmission and long-term plasticity. Our aim is to understand the effect the implications of this missense mutation. Methods: We developed a mouse model representing this disorder using CRISPR/Cas9 technology and measured disease progression. Additionally, we harvested fibroblasts from a *GRIN2D* patient and his healthy parent and reprogrammed them into iPS cells. Results: The mouse model that we created exhibited motor deficits, seen as hind-limb clasping, and displayed severe, long-lasting epileptiform abnormalities. Furthermore, the mice suffered from premature death. Additionally, two iPS cell lines were created that expressed pluripotency markers and differentiated into the three germ layers with the patient derived line preserving the c.1999G>A variant. Conclusion: We created two independent models that each represent this extremely rare genetic disorder. These models grant us the ability to examine the effect of the c.1999G>A variant and to analyze the complex neuronal and network mechanism underlying the pathology it causes. This work enables us to broaden our knowledge regarding the role that *GRIN* genes play in DEEs.

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A CASE OF PERAMPANEL OVERDOSE PRESENTING WITH RESPIRATORY FAILURE

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Perampanel is a novel antiepileptic drug that has been used as an adjunctive treatment for focal-onset seizures. It has a long half-life of 105 h, and an oral preparation at a maximum dose of 12 mg/day is considered safe. Clinical trials have shown that perampanel is generally well-tolerated despite inducing dose-dependent adverse events of the central nervous system, such as dizziness, somnolence, irritability, headache, and psychiatric problems. However, no reports to date have documented respiratory suppression as a side effect of perampanel. Herein, we report a case of perampanel overdose with respiratory failure. A 51-year-old man with focal epilepsy visited the emergency department with altered mentality and irritability. He originally received perampanel (6 mg/day) and levetiracetam (1000 mg twice a day). However, we estimated that he had accidently consumed 66 mg of perampanel 1 h before visiting our hospital. At 2.5 h after arrival at our hospital, his respiratory function deteriorated with aggravation of hypercapnia (62.3 mmHg) and desaturation (saturation of percutaneous O2, 86%) despite O2 supply. Our patient required mechanical ventilator support. Considering the half-life of perampanel is approximately 105 h, respiratory suppression could be related to perampanel overdose. To the best of our knowledge, this is the first report of respiratory failure caused by acute perampanel overdose. Therefore, the possibility of respiratory compromise should be considered whenever a high dose of perampanel needs to be administered to patients.

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BOARD NUMBER: S07-559

BEYOND RETIGABINE: DESIGN, SYNTHESIS, AND PHARMACOLOGICAL CHARACTERIZATION OF A POTENT AND CHEMICALLY-STABLE NEURONAL KV7 CHANNELS ACTIVATOR WITH ANTICONVULSANT ACTIVITY

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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[1]University of Naples Federico II, Neuroscience, Napoli, Italy, [2]University of Salerno, Pharmacy, Fisciano, Italy, [3]University of Messina, Chemical, Biological, Pharmaceutical And Environmental Sciences, Messina, Italy, [4]University of Basilicata, Sciences, Potenza, Italy, [5]University of Naples Federico II, Pharmacy, Naples, Italy

Activation of voltage-gated potassium channels formed by Kv7.2 and Kv7.3 subunits is a promising pharmacological strategy for the treatment of several neuropsychiatric diseases in which neuronal hyperexcitability is a relevant pathogenetic factor. Retigabine is the prototypical neuronal Kv7 activator; this drug is approved for the treatment of partial-onset seizures in adults but is no longer available because of its side effects, including skin discoloration caused by photo-induced dimers. In the present work, the structural determinants of the retigabine-binding site, of the dimerization reaction, and of the tendency to photodegradation were investigated, leading to the synthesis of a small library of retigabine analogues. This library was screened using a fluorescence-based assay (FluxOR) and patch-clamp electrophysiology in Kv7.2/Kv7.3-expressing cells. Among investigated photostable compounds unable to form dimers, when compared to retigabine, one (CP-69) was 3-times more potent in the FluxOR assay (EC_{50}=3.2 μM vs 11.2 μM, respectively; $p<0.05$) and at least 10-times more potent when Kv7.2/Kv7.3 maximal current level or activation voltage-dependence were investigated with patch-clamp. After intraperitoneal (i.p. 1 mg/kg) administration in rats, CP-69 showed higher brain/plasma ratio than retigabine (39.3 vs 2.4), with longer half-life (8h vs 3h). Finally, ip administration in mice of CP-69 reduced the severity and increased the latency to the maximal seizure induced in mice by pentylentetrazol (i.p.100 mg/kg), at lower doses when compared to retigabine (0.3 mg/kg vs 3 mg/kg). In conclusion, CP-69 shows an improved pharmacological profile with respect to retigabine, being, therefore, a promising candidate for further development as an antiepileptic drug.

Pubmed:
33013448: Miceli F, Carotenuto L, Barrese V, Soldovieri MV, Heinzen EL, Mandel AM, Lippa N, Bier L, Goldstein DB, Cooper EC, Cilio MR, Taglialatela M, Sands TT
A Novel Kv7.3 Variant in the Voltage-Sensing S Segment in a Family With Benign Neonatal Epilepsy: Functional Characterization and Rescue by β-Hydroxybutyrate.
Pathogenic variants in and , paralogous genes encoding Kv7.2 and Kv7.3 voltage-gated K channel subunits, are responsible for early-onset developmental/epileptic disorders characterized by heterogeneous clinical phenotypes ranging from benign familial neonatal epilepsy (BFNE) to early-onset developmental and epileptic encephalopathy (DEE). variants account for the majority of pedigrees with BFNE and variants are responsible for a much smaller subgroup, but the reasons for this imbalance remain unclear. Analysis of additional pedigrees is needed to further clarify the nature of this genetic heterogeneity and to improve prediction of pathogenicity for novel variants. We identified a BFNE family with two siblings and a parent affected. Exome sequencing on samples from both parents and siblings revealed a novel variant (c.719T>G; p.M240R), segregating in the three affected individuals. The M240 residue is conserved among human Kv7.2-5 and lies between the two arginines (R5 and R6) closest to the intracellular side of the voltage-sensing S transmembrane segment. Whole cell patch-clamp recordings in Chinese hamster ovary (CHO) cells revealed that homomeric Kv7.3 M240R channels were not functional, whereas heteromeric channels incorporating Kv7.3 M240R mutant subunits with Kv7.2 and Kv7.3 displayed a depolarizing shift of about 10 mV in activation gating. Molecular modeling results suggested that the M240R substitution preferentially stabilized the resting state and possibly destabilized the activated state of the Kv7.3 subunits, a result consistent with functional data. Exposure to β-hydroxybutyrate (BHB), a ketone body generated during the ketogenic diet (KD), reversed channel dysfunction induced by the M240R variant. In conclusion, we describe the first missense loss-of-function (LoF) pathogenic variant within the S segment of Kv7.3 identified in patients with BFNE. Studied under conditions mimicking heterozygosity, the M240R variant mainly affects the voltage sensitivity, in contrast to previously analyzed BFNE Kv7.3 variants that reduce current density. Our pharmacological results provide a rationale for the use of KD in patients carrying

LoF variants in Kv7.2 or Kv7.3 subunits.
Front Physiol, 2020; 11

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BOARD NUMBER: S07-560

ASSOCIATION BETWEEN ABCB1 POLYMORPHISMS AND RESPONSE TO ANTIEPILEPTIC DRUGS AMONG JORDANIAN EPILEPTIC PATIENTS.

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Background: Antiepileptic drug (AEDs) resistance is a major challenge to patients and physicians alike. Genetic polymorphisms of drug efflux transporters as ATP-binding cassette sub-family B, member1 (*ABCB1*) have been suggested to modulate antiepileptic treatment response. In this study, we aimed to explore the association of *ABCB1* polymorphisms and AEDs resistance among epileptic patients. **Methods:** A total of 86 Jordanian epileptic patients treated with AEDs were included in the study. DNA was extracted from blood samples and genotyping was conducted by polymerase chain reaction followed by sequencing. Nine single nucleotide polymorphisms (SNPs) on the *ABCB1* gene were investigated, including: rs2235048, rs2235047,rs1045642, rs2229107, rs2032583, rs2032582, rs2235033, rs2032588 and rs1128503. Haplotypes of the nine SNPs were analyzed, and linkage disequilibrium was evaluated. **Results:** Data revealed that none of the examined SNPs were associated with resistance to AEDs neither on the level of alleles nor on the level of genotypes. However, strong association was found between rs2235048 (OR =10.6; 95%CI =[1.89-59.8], *p*=0.01), rs1045642 (OR=14; 95%CI =[1.3-156.7], *p*=0.02), rs2032582 (OR= 9.1; 95%CI =[1.4-57.3], *p*=0.04) and rs1128503 (OR=18.7; 95%CI =[1.6-222.9], *p*=0.02), *ABCB1* polymorphisms and resistance to AEDs among females but not males. Haplotype analysis revealed statistically significant associations. The strongest significant associations were for haplotypes containing 2677G_1236T in two-SNPs-haplotypes (OR=4.2; 95%CI = [1.2 - 14.9], p=0.024); three-SNPs-haplotypes (OR=4.2; 95%CI= [1.2-14.9], p=0.02); four-SNPs-haplotypes (OR=4.1; 95%CI =[1.2-14.3], p=0.026). **Conclusion:** Data suggests that there is a gender-dependent association between *ABCB1* genetic polymorphisms and response to AEDs. Additionally, *ABCB1* haplotypes influence response to AEDs.

Pubmed:

33949294: Tamimi DE, Abduljabbar R, Yousef AM, Saeed RM, Zawiah M
Association between polymorphisms and response to antiepileptic drugs among Jordanian epileptic patients.
Genetic polymorphisms of drug efflux transporters as ATP-binding cassette subfamily B, member 1 (ABCB1) have been suggested to modulate antiepileptic drugs (AEDs) response. We aimed to explore the association of ABCB1 polymorphisms and AEDs resistance among epileptic patients.
Neurol Res, 2021; 43
32555684: Zawiah M, Yousef AM, Khan AH, Al-Ashwal FY, Matar A, ALKhawaldeh B, Nassar R, Abduljabbar R, Abdo Ahmed AA
Food-drug interactions: Knowledge among pharmacists in Jordan.
Pharmacists have crucial role in providing drug information and medication counseling to patients. This survey aimed to benchmark the current knowledge of the pharmacists concerning food-drug interactions (FDIs) in Jordan.
PLoS One, 2020; 15
32850608: Zawiah M, Al-Ashwal FY, Saeed RM, Kubas M, Saeed S, Khan AH, Sulaiman SAS, Abduljabbar R
Assessment of Healthcare System Capabilities and Preparedness in Yemen to Confront the Novel Coronavirus 2019 (COVID-19) Outbreak: A Perspective of Healthcare Workers.
In the past decade, Yemen has witnessed several disasters that resulted in a crumbled healthcare system. With the declaration of COVID-19 a global pandemic, and later the appearance of first confirmed cases in Yemen, there is an urgent need to assess the preparedness of healthcare facilities (HCFs) and their capacities to tackle a looming COVID-19 outbreak. Herein, we present an assessment of the current state of preparedness and capabilities of HCFs in Yemen to prevent and manage the COVID-19 outbreak. An online survey for HCFs was developed, validated, and distributed. The questionnaire is divided into five main sections: (1) Demographic variables for participants. (2) HCFs capabilities for COVID-19 outbreak. (3) Support received to face the emergence and spread of COVID-19. (4). Current practices of infection prevention and control measures in the HCFs. The last section focused on the recommendations to ensure effective and timely response to this

outbreak in Yemen. Descriptive analysis was used to analyze data using statistical package for social sciences (SPSS), version 23. Responses were received from healthcare workers (HCWs) from 18 out of 22 governorates in Yemen. Out of the 296 HCWs who participated in the study, the vast majority (93.9%) believed that the healthcare system in Yemen does not have the resources and capabilities to face and manage a COVID-19 outbreak. Approximately 82.4% of participants rated the general preparedness level of their HCFs as very poor or poor. More specifically, the majority of HCWs rated their HCFs as very poor or poor in term of availability of the following: an adequate number of mechanical ventilators (88.8%), diagnostic devices (88.2%), ICU rooms and beds (81.4%), and isolation rooms (79.7%). The healthcare facilities in Yemen are unprepared and lack the most basic resources and capabilities to cope with or tackle a COVID-19 outbreak. With the current state of a fragile healthcare system, a widespread outbreak of COVID-19 in Yemen could result in devastating consequences. There is an urgent need to provide support to the healthcare workers and HCFs that are on the frontline against COVID-19.
Front Public Health, 2020; 8
34187984: Zawiah M, Yousef AM, Al-Ashwal FY, Abduljabbar R, Al-Jamei S, Hayat Khan A, Alkhawaldeh B
Pharmacogenetics: a perspective and preparedness of Pharm-D and medical students in Jordan.
Pharmacogenetics (PGx) science has evolved significantly with a huge number of studies exploring the effect of genetic variants on interindividual variability of drug response. In this study, we assessed the knowledge, attitudes and preparedness of Pharm-D vs. medical students toward PGx.
Pharmacogenet Genomics, 2021; 31
34714827: Bitar AN, Zawiah M, Al-Ashwal FY, Kubas M, Saeed RM, Abduljabbar R, Jaber AAS, Sulaiman SAS, Khan AH
Misinformation, perceptions towards COVID-19 and willingness to be vaccinated: A population-based survey in Yemen.
Since the beginning of the COVID-19 outbreak, many pharmaceutical companies have been racing to develop a safe and effective COVID-19 vaccine. Simultaneously, rumors and misinformation about COVID-19 are still widely spreading. Therefore, this study aimed to investigate the prevalence of COVID-19 misinformation among the Yemeni population and its association with vaccine acceptance and perceptions.
PLoS One, 2021; 16

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BOARD NUMBER: S07-561

INFLUENCE OF ESTROUS CYCLE IN SEIZURE SUSCEPTIBILITY USING A MOUSE MODEL OF TUBEROUS SCLEROSIS COMPLEX 2

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Tuberous Sclerosis Complex 2 (TSC2) is a rare genetic disorder characterized by serious neurological complications such as severe epilepsy. For general epilepsy models, behavioural outcomes can differ across different variables, in particular biological sex. Specifically, seizures in females are influenced by gonadal hormone levels and, consequently, by the reproductive cycle. The purpose of the present work was to investigate the influence of estrous cycle in seizure susceptibility and anxiety-like behaviours in a female *Tsc2* mouse model. Heterozygous (*Tsc2*$^{+/-}$) and homozygous (*TSC2*$^{+/+}$) female mice aged 8-12 weeks were studied. Seizure severity was determined according to a modified Racine scale at control conditions (saline, i.p.) and following kainic acid (KA) administration (20 mg/kg, i.p.). Locomotor and anxiety-like behaviours were also evaluated at those conditions. Following pharmacological experiment, a vaginal swab was collected, and the phases of the estrous cycle were determined. We observed that, independently of their genetic background females at the ovulatory stage displayed an enhanced susceptibility with a significant difference between homozygous females that were at different stages of estrous cycle. In parallel, only females at post-ovulatory stage displayed a susceptibility that differed significantly between genotypes. No significant differences were found on behavioural profiles. These observations suggest that *Tsc2* mutation is not the only key factor interfering in epileptic progression. Moreover, our results reveal that hormonal fluctuations have an important role in seizure susceptibility and progression for females. So, future studies using this model should take to consideration estrous cycle to investigate new therapeutic strategies for epilepsy.

Pubmed:
33584173: Nicolucci C, Pais ML, Santos AC, Ribeiro FM, Encarnação PMCC, Silva ALM, Castro IF, Correia PMM, Veloso JFCA, Reis J, Lopes MZ, Botelho MF, Pereira FC, Priolli DG
Single Low Dose of Cocaine-Structural Brain Injury Without Metabolic and Behavioral Changes.
Chronic cocaine use has been shown to lead to neurotoxicity in rodents and humans, being associated with high morbidity and mortality rates. However, recreational use, which may lead to addictive behavior, is often neglected. This occurs, in part, due to the belief that exposure to low doses of cocaine comes with no brain damage risk. Cocaine addicts have shown glucose metabolism changes related to dopamine brain activity and reduced volume of striatal gray matter. This work aims to evaluate the morphological brain changes underlying metabolic and locomotor behavioral outcome, in response to a single low dose of cocaine in a pre-clinical study. In this context, a Balb-c mouse model has been chosen, and animals were injected with a single dose of cocaine (0.5 mg/kg). Control animals were injected with saline. A behavioral test, positron emission tomography (PET) imaging, and anatomopathological studies were conducted with this low dose of cocaine, to study functional, metabolic, and morphological brain changes, respectively. Animals exposed to this cocaine dose showed similar open field activity and brain metabolic activity as compared with controls. However, histological analysis showed alterations in the prefrontal cortex and of mice exposed to cocaine. For the first time, it has been demonstrated that a single low dose of cocaine, which can cause no locomotor behavioral and brain metabolic changes, can induce structural damage. These brain changes must always be considered regardless of the dosage used. It is essential to alert the population even against the consumption of low doses of cocaine.
Front Neurosci, 2020; 14

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-562

GAIN-OF-FUNCTION DUE TO INCREASED OPENING PROBABILITY BY TWO KCNQ5 PORE VARIANTS CAUSING DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Mutations in KCNQ2, KCNQ3 and, more rarely, KCNQ5 genes encoding Kv7.2, Kv7.3, and Kv7.5 neuronal voltage-gated potassium channel subunits respectively are responsible for developmental and epileptic encephalopathies (DEEs), neurodevelopmental diseases characterized by refractory epilepsy, distinct EEG and neuroradiological features, and various degrees of developmental delay. In the present work, the clinical features of two DEE patients carrying de novo KCNQ5 variants affecting the same Kv7.5 pore residue (G347S/A) are described. The *in vitro* functional properties of channels incorporating these variants were also investigated. Site-directed mutagenesis was used to introduce specific mutations in KCNQ2, KCNQ3 and KCNQ5 plasmids. Electrophysiological and biochemical experiments were performed in CHO cells transiently transfected with these constructs. When compared to Kv7.5, Kv7.5 G347S/A channels displayed a marked increase in maximal current and a strong hyperpolarization in activation gating, both functional features consistent with a strong gain-of-function (GoF) in vitro phenotype. Similar functional changes were also observed upon introduction of the corresponding variants in Kv7.2 subunits. Non-stationary noise analysis revealed that GoF effects observed for both Kv7.2 and Kv7.5 variants were mainly attributable to an increase in single channel open probability, without changes in membrane abundance or single channel conductance. In addition, currents carried by mutant channels were insensitive to manipulation of the membrane levels of the critical Kv7 channel regulator PIP_2. These results reveal a novel pathophysiological mechanism for KCNQ5-related DEEs which might be exploited to implement personalized treatments.

Pubmed:
34778950: Miceli F, Guerrini R, Nappi M, Soldovieri MV, Cellini E, Gurnett CA, Parmeggiani L, Mei D, Taglialatela M
Distinct epilepsy phenotypes and response to drugs in KCNA1 gain- and loss-of function variants.
A wide phenotypic spectrum of neurological diseases is associated with KCNA1 (Kv1.1) variants. To investigate the molecular basis of such a heterogeneous clinical presentation and identify the possible correlation with in vitro phenotypes, we compared the functional consequences of three heterozygous de novo variants (p.P403S, p.P405L, and p.P405S) in Kv1.1 pore region found in four patients with severe developmental and epileptic encephalopathy (DEE), with those of a de novo variant in the voltage sensor (p.A261T) identified in two patients with mild, carbamazepine-responsive, focal epilepsy. Patch-clamp electrophysiology was used to investigate the functional properties of mutant Kv1.1 subunits, both expressed as homomers and heteromers with wild-type Kv1.1 subunits. KCNA1 pore mutations markedly decreased (p. P405S) or fully suppressed (p. P403S, p. P405L) Kv1.1-mediated currents, exerting loss-of-function (LoF) effects. By contrast, channels carrying the p.A261T variant exhibited a hyperpolarizing shift of the activation process, consistent with a gain-of-function (GoF) effect. The present results unveil a novel correlation between in vitro phenotype (GoF vs LoF) and clinical course (mild vs severe) in KCNA1-related phenotypes. The excellent clinical response to carbamazepine observed in the patients carrying the A261T variant suggests an exquisite sensitivity of KCNA1 GoF to sodium channel inhibition that should be further explored.
Epilepsia, 2022; 63

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-563

TIGHT JUNCTION PROTEINS EXPRESSION AND CYTOSKELETON REARRANGEMENT ARE ASSOCIATED WITH BRAIN MICROVASCULAR ENDOTHELIUM PERMEABILISATION IN EPILEPTOGENESIS

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Aim. Blood brain barrier (BBB) is the a critical structure for maintaining cerebral homeostasis and preventing the access of toxins, drugs or other substances to brain parenchyma. To goal of our study was to explore the mechanisms that determine BBB permeabilisation during status epilepticus (SE). **Methods**. Brain microvascular endothelial cells (bEnd.3) were exposed to pilocarpine (100 μM) for 2, 6 and 24 hours for mimicking SE. Tight junction proteins (ZO-1 and claudin-5), actin cytoskeleton filaments and nuclei were immunostained and gene expression was quantified by qRT-PCR. We developed/implemented Matlab scripts for confocal image analysis, including a Fiberscore algorithm for actin cytoskeleton orientation, a method for protein expression localisation in cellular compartments using Aggrecount and an adaptation of Voronoi diagram for cell detection. **Results**. Cell detection was improved with 26% compared to the standard Voronoi diagram method. Immunostaining and qRT-PCR demonstrated that ZO-1 was upregulated after 24 hr, while Claudin-5 was downregulated after 2 - 6 hr. After 6 hr, the perinuclear localisation of ZO-1 and Claudin-5 were increased and their membrane localisation decreased. Additionally, at the same timepoint, actin filaments were fragmented with a reduction in their mean length and were spatially reorganized with a reduced polarity. **Conclusions**. We demonstrated a direct correlation of tight junctions proteins expression and the rearangement of the actin cytoskeleton microtubules in the pilocarpine-induced in vitro model. Our data are relevant in understanding the BBB permeabilisation steps in epileptogenesis.

FENS Forum 2022
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BOARD NUMBER: S07-564

NON INVASIVE STATIC MAGNETIC FIELDS REDUCE EPILEPTIC ACTIVITY IN A MOUSE MODEL OF DRAVET SYNDROME

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Static Magnetic Fields (SMF) reduce cortical excitability and improve epileptic signs in a pharmacological model of epilepsy (Rivadulla et al 2018). We test the efficacy of SMF in a mouse model of Dravet Syndrome, a severe epileptic encephalopathy characterized by frequent and long lasting seizures. Experiments were carried out on conditional *Scn1a-A1783V mice* which express the DS/SMEI-associated mutation A1783V in the Nav1.1 channel after Cre-mediated recombination by breeding with a transgenic mouse line that express Cre under actin-CMV promotor. EEG was recorded continuously from sevoflurane anesthetized mice through two copper wires chronically implanted. Seizures were triggered by increasing temperature (0.3º/min) from 37º (baseline) to above 42º and maintained by 5 minutes. Protocol was made in the presence of a 0,5T magnet or a sham replica, positioned 30 minutes before increasing temperature. In a subset of animals (n=4), an automatic epileptogenic event detection system based on Bergstrom et al 2012, detected 194% more incidences in the sham than in the magnet condition. When comparing a group of animals (n=4) in which placebo was applied in every session, versus a second group (n=3) that always received the magnet, the number of sessions with seizures dropped from 93% (26 out of 28 sessions) in the sham, to 37% (8 out of 22) in the magnet group. Furthermore, SMF reduced by 45% the duration of the remaining seizures. SMF are able to prevent or reduce the epileptic seizures in a model of DS and could be considered as a therapeutic approach. XUGA_ED431C_2018/24, Instituto_Salud_Carlos_III_PI21/0015, Asociación_Apoyo_Dravet

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-565

EXTRACELLULAR CIRCULATING MIRNAS AS POTENTIAL BIOMARKERS IN MULTIPLE SCLEROSIS AND EPILEPSY.

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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In the last decade, numerous studies focused on the molecular background of neurological disorders, such as epilepsy and multiple sclerosis. One important aim is to investigate the potential role of micro RNAs (miRNA) in the pathophysiology of these disorders and their potential application as peripheral biomarkers to assess disease progression and therapeutic response. *Aims*: The aim of our study was to investigate the expression levels of circulating miRNAs in peripheral blood and brain samples originating from humans and experimental mice. *Methods*: In the present study, we report on findings originating from patients with epilepsy (EP) and multiple sclerosis (MS). 52 serum samples from MS patients, 71 serum samples from EP patients and mouse brain samples were investigated. C57BL/6 male mice were used for induction of temporal lobe epilepsy (TLE). TLE mice were terminated 1, 2, 3 and 4 weeks after intra-hippocampal injection of kainic acid. Brain tissue was removed from animals, and samples containing the prefrontal cortex, hippocampus and cerebellum were collected. After that, total RNA/miRNA was isolated and described into cDNA. For downstream workflow droplet digital PCR reaction was prepared, using specific miRNA primers. *Results*: Our preliminary findings demonstrate an association between miRNA expression levels in human serum samples and clinical examination including MRI in MS patients. *Conclusion*: ddPCR is an extremely sensitive qPCR system, that allows absolute quantification of miRNAs in case of low yield samples, thereby it is a promising additional laboratory test method to improve making diagnosis or choosing the most efficiency therapy.

Pubmed:
32326205: Khan AR, Geiger L, Wiborg O, Czéh B
Stress-Induced Morphological, Cellular and Molecular Changes in the Brain-Lessons Learned from the Chronic Mild Stress Model of Depression.
Major depressive disorder (MDD) is a severe illness imposing an increasing social and economic burden worldwide. Numerous rodent models have been developed to investigate the pathophysiology of MDD. One of the best characterized and most widely used models is the chronic mild stress (CMS) model which was developed more than 30 years ago by Paul Willner. More than 2000 published studies used this model, mainly to assess novel compounds with potential antidepressant efficacy. Most of these studies examined the behavioral consequences of stress and concomitant drug intervention. Much fewer studies focused on the CMS-induced neurobiological changes. However, the stress-induced cellular and molecular changes are important as they may serve as potential translational biomarkers and increase our understanding of the pathophysiology of MDD. Here, we summarize current knowledge on the structural and molecular alterations in the brain that have been described using the CMS model. We discuss the latest neuroimaging and postmortem histopathological data as well as molecular changes including recent findings on microRNA levels. Different chronic stress paradigms occasionally deliver dissimilar findings, but the available experimental data provide convincing evidence that the CMS model has a high translational value. Future studies examining the neurobiological changes in the CMS model in combination with clinically effective antidepressant drug intervention will likely deliver further valuable information on the pathophysiology of MDD. Cells, 2020; 9
30922090: Zana B, Geiger L, Kepner A, Földes F, Urbán P, Herczeg R, Kemenesi G, Jakab F
First molecular detection of Apis mellifera filamentous virus in honey bees (Apis mellifera) in Hungary.
Western honey bees (Apis mellifera) are important pollinators in the ecosystem and also play a crucial economic role in the honey industry. During the last decades, a continuous decay was registered in honey bee populations worldwide, including Hungary. In our study, we used metagenomic approaches and conventional PCR screening on healthy and winter mortality

affected colonies from multiple sites in Hungary. The major goal was to discover presumed bee pathogens with viral metagenomic experiments and gain prevalence and distribution data by targeted PCR screening. We examined 664 honey bee samples that had been collected during winter mortality from three seemingly healthy colonies and from one colony infested heavily by the parasitic mite Varroa destructor in 2016 and 2017. The subsequent PCR screening of honey bee samples revealed the abundant presence of Apis mellifera filamentous virus (AmFV) for the first time in Central Europe. Based on phylogeny reconstruction, the newly-detected virus was found to be most closely related to a Chinese AmFV strain. More sequence data from multiple countries would be needed for studying the detailed phylogeographical patterns and worldwide spreading process of AmFV. Here we report the prevalent presence of this virus in Hungarian honey bee colonies.
Acta Vet Hung, 2019; 67

33297527: Földes F, Madai M, Papp H, Kemenesi G, Zana B, Geiger L, Gombos K, Somogyi B, Bock-Marquette I, Jakab F
Small Interfering RNAs Are Highly Effective Inhibitors of Crimean-Congo Hemorrhagic Fever Virus Replication In Vitro.
Crimean-Congo hemorrhagic fever virus (CCHFV) is one of the prioritized diseases of the World Health Organization, considering its potential to create a public health emergency and, more importantly, the absence of efficacious drugs and/or vaccines for treatment. The highly pathogenic characteristic of CCHFV restricts research to BSL-4 laboratories, which complicates effective research and developmental strategies. In consideration of antiviral therapies, RNA interference can be used to suppress viral replication by targeting viral genes. RNA interference uses small interfering RNAs (siRNAs) to silence genes. The aim of our study was to design and test siRNAs in vitro that inhibit CCHFV replication and can serve as a basis for further antiviral therapies. A549 cells were infected with CCHFV after transfection with the siRNAs. Following 72 h, nucleic acid from the supernatant was extracted for RT Droplet Digital PCR analysis. Among the investigated siRNAs we identified effective candidates against all three segments of the CCHF genome. Consequently, blocking any segment of CCHFV leads to changes in the virus copy number that indicates an antiviral effect of the siRNAs. In summary, we demonstrated the ability of specific siRNAs to inhibit CCHFV replication in vitro. This promising result can be integrated into future anti-CCHFV therapy developments.
Molecules, 2020; 25

33575263: Gombos K, Földi M, Kiss S, Herczeg R, Gyenesei A, Geiger L, Csabai D, Futács K, Nagy T, Miseta A, Somogyi BA, Hegyi P, Szentesi A
Analysis of COVID-19-Related RT-qPCR Test Results in Hungary: Epidemiology, Diagnostics, and Clinical Outcome.
Effective testing is an essential tool for controlling COVID-19. We aimed to analyse the data from first-wave PCR test results in Hungary's Southern Transdanubian region to improve testing strategies. We performed a retrospective analysis of all suspected COVID-19 cases between 17 March and 8 May 2020, collecting epidemiological, demographic, clinical and outcome data (ICU admission and mortality) with RT-qPCR test results. Descriptive and comparative statistical analyses were conducted. Eighty-six infections were confirmed among 3,657 tested patients. There was no difference between the positive and negative cases in age and sex distribution; however, ICU admission (8.1 vs. 3.1%, = 0.006) and in-hospital mortality (4.7 vs. 1.6%, = 0.062) were more frequent among positive cases. Importantly, none of the initially asymptomatic patients (= 20) required ICU admission, and all survived. In almost all cases, if the first test was negative, second and third tests were performed with a 48-h delay for careful monitoring of disease development. However, the positive hit rate decreased dramatically with the second and third tests compared to the first (0.3 vs. 2.1%, OR = 0.155 [0.053-0.350]). Higher E-gene copy numbers were associated with a longer period of PCR positivity. In our immunologically naïve suspected COVID-19 population, coronavirus infection increased the need for intensive care and mortality by 3-4 times. In the event of the exponential phase of the pandemic involving a bottleneck in testing capacity, a second or third test should be reconsidered to diagnose more coronavirus infections.
Front Med (Lausanne), 2020; 7

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-566

CHARACTERISING EPILEPTOGENESIS IN A RECURRENT GENETIC MODEL OF DRAVET SYNDROME

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Dravet syndrome (DS) is characterized as a rare form of severe developmental and epileptic encephalopathy (DEE) often caused by de-novo mutations in the *SCN1A* gene, encoding the Na^+ subunit $Na_V1.1$. To understand pathophysiological changes and molecular mechanisms involved in epileptogenesis of DS, we aimed to analyse changes of sodium channel gating, neuronal excitability and neuronal subnetworks caused by the recurrent human DS missense variant *SCN1A*^{A1783V}. The kinetic properties of $Na_V1.1$-A1783V were studied by voltage-clamp recordings of transfected tsA201 cells followed by simulation in a Hodgkin-Huxley model to predict consequences on neuronal excitability. Additionally, *ex vivo* electrophysiology, calcium imaging and single nuclei transcriptomics were performed in hippocampal and cortical regions of wildtype and heterozygous mice at PN13-15, PN20-23 and PN37-P40 to reveal vulnerable cell populations and understand seizure generation mechanisms. *SCN1A*^{A1783V} led to loss-of-function (LOF) of channel activation and slow-inactivation properties, resulting in a predicted shift of action potential (AP) rheobase and reduced firing in fast-spiking inhibitory neurons (IN). Recordings in cortex and hippocampus confirmed our predictions identifying transient LOF in fast-spiking neurons characterised by reduced firing frequency and increased AP half-width. Regular-spiking INs and pyramidal cells seemed less affected. Furthermore, network recordings and transcriptomic analysis displayed an imbalance of excitation and inhibition in various cell populations especially in postsynaptic compartments of excitatory cells. In conclusion, we extend the understanding of DS further by identifying pathophysiological changes in missense variant *SCN1A*^{A1783V} similar to other nonsense variants. Additionally, we identified multiple primary and secondary mechanisms characterizing epileptogenesis in this DS model.

Pubmed:
34776868: Layer N, Sonnenberg L, Pardo González E, Benda J, Hedrich UBS, Lerche H, Koch H, Wuttke TV
Dravet Variant Impairs Interneuron Firing Predominantly by Altered Channel Activation.
Dravet syndrome (DS) is a developmental epileptic encephalopathy mainly caused by functional Na1.1 haploinsufficiency in inhibitory interneurons. Recently, a new conditional mouse model expressing the recurrent human p.(Ala1783Val) missense variant has become available. In this study, we provided an electrophysiological characterization of this variant in tsA201 cells, revealing both altered voltage-dependence of activation and slow inactivation without reduced sodium peak current density. Based on these data, simulated interneuron (IN) firing properties in a conductance-based single-compartment model suggested surprisingly similar firing deficits for Na1.1 and full haploinsufficiency as caused by heterozygous truncation variants. Impaired Na1.1 channel activation was predicted to have a significantly larger impact on channel function than altered slow inactivation and is therefore proposed as the main mechanism underlying IN dysfunction. The computational model was validated in cortical organotypic slice cultures derived from conditional mice. Pan-neuronal activation of the p.Ala1783V confirmed a predicted IN firing deficit and revealed an accompanying reduction of interneuronal input resistance while demonstrating normal excitability of pyramidal neurons. Altered input resistance was fed back into the model for further refinement. Taken together these data demonstrate that primary loss of function (LOF) gating properties accompanied by altered membrane characteristics may match effects of full haploinsufficiency on the neuronal level despite maintaining physiological peak current density, thereby causing DS.
Front Cell Neurosci, 2021; 15
34553376: Layer N, Brandes J, Lührs PJ, Wuttke TV, Koch H
The effect of lamotrigine and other antiepileptic drugs on respiratory rhythm generation in the pre-Bötzinger complex.
Lamotrigine and other sodium-channel blocking agents are among the most commonly used antiepileptic drugs (AEDs). Because other sodium channel blockers, such as riluzole, can severely alter respiratory rhythm generation during hypoxia, we wanted to investigate if AEDs can have similar effects. This is especially important in the context of sudden unexpected death

in epilepsy (SUDEP), the major cause of death in patients suffering from therapy-resistant epilepsy. Although the mechanism of action is not entirely understood, respiratory dysfunction after generalized tonic-clonic seizures seems to play a major role.
Epilepsia, 2021; 62
32372899: Wickham J, Corna A, Schwarz N, Uysal B, Layer N, Honegger JB, Wuttke TV, Koch H, Zeck G
Human Cerebrospinal Fluid Induces Neuronal Excitability Changes in Resected Human Neocortical and Hippocampal Brain Slices.
Human cerebrospinal fluid (hCSF) has proven advantageous over conventional medium for culturing both rodent and human brain tissue. In addition, increased activity and synchrony, closer to the dynamic states exclusively recorded , were reported in rodent slices and cell cultures switching from artificial cerebrospinal fluid (aCSF) to hCSF. This indicates that hCSF possesses properties that are not matched by the aCSF, which is generally used for most electrophysiological recordings. To evaluate the possible significance of using hCSF as an electrophysiological recording medium, also for human brain tissue, we compared the network and single-cell firing properties of human brain slice cultures during perfusion with hCSF and aCSF. For measuring the overall activity from a majority of neurons within neocortical and hippocampal human slices, we used a microelectrode array (MEA) recording technique with 252 electrodes covering an area of 3.2 × 3.2 mm. A second CMOS-based MEA with 4225 sensors on a 2 × 2 mm area was used for detailed mapping of action potential waveforms and cell identification. We found that hCSF increased the number of active electrodes and neurons and the firing rate of the neurons in the slices and induced an increase in the numbers of single channel and population bursts. Interestingly, not only an increase in the overall activity in the slices was observed, but a reconfiguration of the network could also be detected with specific activation and inactivation of subpopulations of neuronal ensembles. In conclusion, hCSF is an important component to consider for future human brain slice studies, especially for experiments designed to mimic parts of physiology and disease observed .
Front Neurosci, 2020; 14
31498083: Schwarz N, Uysal B, Welzer M, Bahr JC, Layer N, Löffler H, Stanaitis K, Pa H, Weber YG, Hedrich UB, Honegger JB, Skodras A, Becker AJ, Wuttke TV, Koch H
Long-term adult human brain slice cultures as a model system to study human CNS circuitry and disease.
Most of our knowledge on human CNS circuitry and related disorders originates from model organisms. How well such data translate to the human CNS remains largely to be determined. Human brain slice cultures derived from neurosurgical resections may offer novel avenues to approach this translational gap. We now demonstrate robust preservation of the complex neuronal cytoarchitecture and electrophysiological properties of human pyramidal neurons in long-term brain slice cultures. Further experiments delineate the optimal conditions for efficient viral transduction of cultures, enabling 'high throughput' fluorescence-mediated 3D reconstruction of genetically targeted neurons at comparable quality to state-of-the-art biocytin fillings, and demonstrate feasibility of long term live cell imaging of human cells . This model system has implications toward a broad spectrum of translational studies, regarding the validation of data obtained in non-human model systems, for therapeutic screening and genetic dissection of human CNS circuitry.
Elife, 2019; 8
29267189: Melkonyan MM, Hunanyan L, Lourhmati A, Layer N, Beer-Hammer S, Yenkoyan K, Schwab M, Danielyan L
Neuroprotective, Neurogenic, and Amyloid Beta Reducing Effect of a Novel Alpha 2-Adrenoblocker, Mesedin, on Astroglia and Neuronal Progenitors upon Hypoxia and Glutamate Exposure.
Locus coeruleus-noradrenergic system dysfunction is known to contribute to the progression of Alzheimer's disease (AD). Besides a variety of reports showing the involvement of norepinephrine and its receptor systems in cognition, amyloid β (Aβ) metabolism, neuroinflammation, and neurogenesis, little is known about the contribution of the specific receptors to these actions. Here, we investigated the neurogenic and neuroprotective properties of a new α2 adrenoblocker, mesedin, in astroglial primary cultures (APC) from C57BL/6 and 3×Tg-AD mice. Our results demonstrate that mesedin rescues neuronal precursors and young neurons, and reduces the lactate dehydrogenase (LDH) release from astroglia under hypoxic and normoxic conditions. Mesedin also increased choline acetyltransferase, postsynaptic density marker 95 (PSD95), and Aβ-degrading enzyme neprilysin in the wild type APC, while in the 3×Tg-AD APC exposed to glutamate, it decreased the intracellular content of Aβ and enhanced the survival of synaptophysin-positive astroglia and neurons. These effects in APC can at least partially be attributed to the mesedin's ability of increasing the expression of Interleukine(IL)-10, which is a potent anti-inflammatory, neuroprotective neurogenic, and Aβ metabolism enhancing factor. In summary, our data identify the neurogenic, neuroprotective, and anti-amyloidogenic action of mesedin in APC. Further in vivo studies are needed to estimate the therapeutic value of mesedin for AD.
Int J Mol Sci, 2017; 19
29218453: Barroso Oquendo M, Layer N, Wagner R, Krippeit-Drews P, Drews G
Energy depletion and not ROS formation is a crucial step of glucolipotoxicity (GLTx) in pancreatic beta cells.
We have shown previously that genetic or pharmacological deletion of K channels protect against beta cell dysfunction

induced by reactive oxygen species (ROS). Since it is assumed that glucolipotoxicity (GLTx) causes ROS production, we aimed to evaluate whether suppression of K channel activity can also prevent beta cell damage evoked by GLTx. We used an in vitro model of GLTx and measured distinct parameters of stimulus-secretion coupling. GLTx gradually induced disturbances of Ca oscillations over 3 days. This impairment in Ca dynamics was partially reversed in beta cells without functional K channels (SUR1) and by the sulfonylurea gliclazide but not by tolbutamide. By contrast, the GLTx-induced suppression of glucose-induced insulin secretion could not be rescued by decreased K channel activity pointing to a direct interaction of GLTx with the secretory capacity. Accordingly, GLTx also suppressed KCl-induced insulin secretion. GLTx was not accompanied by decisively increased ROS production or enhanced apoptosis. Insulin content of beta cells was markedly reduced by GLTx, an effect not prevented by gliclazide. Since GLTx markedly diminished the mitochondrial membrane potential and cellular ATP content, lack of ATP is assumed to decrease insulin biosynthesis. The deleterious effect of GLTx is therefore caused by direct interference with the secretory capacity whereby reduction of insulin content is one important parameter. These findings deepen our understanding how GLTx damages beta cells and reveal that GLTx is disconnected from ROS formation, a notion important for targeting beta cells in the treatment of diabetes. Overall, GLTx-induced energy depletion may be a primary step in the cascade of events leading to loss of beta cell function in type-2 diabetes mellitus. Pflugers Arch, 2018; 470

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BOARD NUMBER: S07-567

UNRAVELLING THE ROLE OF THE STX1B GENE IN GENETIC EPILEPSY SYNDROMES USING AN IPSC-DERIVED AUTAPTIC CULTURE SYSTEM

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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The majority of genes implicated in epilepsy lead to ion channel dysfunction, wherefore epilepsies are often referred to as channelopathies. However, the identification of mutations in genes encoding synaptic proteins shed light on synaptopathies as another pathophysiological mechanism of epilepsy. Mutations in *STX1B*, which encodes the presynaptic protein Syntaxin-1B, have been associated with various epilepsy syndromes ranging from benign generalized febrile or afebrile seizures to severe epileptic encephalopathies. The effect of *STX1B* mutations have already been studied in different animal models like zebrafish or mouse, while data from human model systems are still missing. Moreover, most studies on synaptic transmission rely on spontaneous activity in an uncontrolled network of neurons limiting the readout of specific synaptic properties. To overcome these limitations, we use autaptic cultures of patient-specific induced pluripotent stem cells (iPSCs) to elucidate the effects of *STX1B* mutations. In that endeavour, we generated iPSC lines from patients carrying *STX1B* mutations in the regulatory H_{abc}-domain or SNARE motif. Using the advantage of autaptic cultures, we examine different synaptic properties in iPSC-derived neurons by patch clamp recordings to investigate how *STX1B* mutations affect synaptic activity on a single cell level. Understanding the pathophysiological mechanisms of synaptic dysfunction in these patients could further help to develop adequate therapies in the future.

Pubmed:
33878252: Kiss E, Kins S, Gorgas K, Orlik M, Fischer C, Endres K, Schlicksupp A, Kirsch J, Kuhse J
Artemisinin-treatment in pre-symptomatic APP-PS1 mice increases gephyrin phosphorylation at Ser270: a modification regulating postsynaptic GABAR density.
Artemisinins, a group of plant-derived sesquiterpene lactones, are efficient antimalarial agents. They also share anti-inflammatory and anti-viral activities and were considered for treatment of neurodegenerative disorders like Alzheimer's disease (AD). Additionally, artemisinins bind to gephyrin, the multifunctional scaffold of GABAergic synapses, and modulate inhibitory neurotransmission . We previously reported an increased expression of gephyrin and GABA receptors in early pre-symptomatic stages of an AD mouse model (APP-PS1) and in parallel enhanced CDK5-dependent phosphorylation of gephyrin at S270. Here, we studied the effects of artemisinin on gephyrin in the brain of young APP-PS1 mice. We detected an additional increase of gephyrin protein level, elevated gephyrin phosphorylation at Ser270, and an increased amount of GABAR-γ2 subunits after artemisinin-treatment. Interestingly, the CDK5 activator p35 was also upregulated. Moreover, we demonstrate decreased density of postsynaptic gephyrin and GABAR-γ2 immunoreactivities in cultured hippocampal neurons expressing gephyrin with alanine mutations at two CDK5 phosphorylation sites. In addition, the activity-dependent modulation of synaptic protein density was abolished in neurons expressing gephyrin lacking one or both of these phosphorylation sites. Thus, our results reveal that artemisinin modulates expression as well as phosphorylation of gephyrin at sites that might have important impact on GABAergic synapses in AD.
Biol Chem, 2022; 403

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BOARD NUMBER: S07-568

POLYPHENOL DERIVATIVE EXERTS ANTI-EPILEPTIC EFFECTS THROUGH ACUTE ACTIVATION OF JNK PATHWAY IN DROSOPHILA AND MITIGATES THE SYNAPTIC DYSFUNCTIONS ASSOCIATED WITH EPILEPSY IN RODENTS

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

Shefali Mishra[1], Vijaya Verma[2], Manish Dwivedi[3], Pooja Jorwal[4], Varun Gowda[5], Madavan Vasudevan[5], Sujit Sikdar[4], Vimlesh Kumar[3], James Clement[2], Upendra Nongthomba[1]
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Aim: Available anti-epileptic drugs target the epileptic effectors that set the seizure threshold, but not downstream signalling cascades that contribute to the pathogenesis and severity. In an effort to identify a new therapeutic compound with better tolerance and potency, we aimed to investigate the anti-epileptic effects of a polyphenolic derivative (PD). Methods: Pharmacological (picrotoxin) and genetic *(bang senseless[1], a gain of function mutation in sodium channel)* epileptic models of *Drosophila* and pentylenetetrazol-induced chronic kindling mouse model were used to score the anti-epileptic effects. The scoring was based on behavioural analysis that correlated with synaptic structure and function in *Drosophila* and mouse. RNA sequencing and affinity chromatography studies were performed to dissect the mechanism of action and molecular targets. Results: PD exerts both acute and chronic anti-epileptic effects by mitigating the behavioural and synaptic defects *in Drosophila.* A similar result was recapitulated in the PTZ mouse model, where reduction in basal synaptic transmission and altered excitatory-inhibitory balance was observed, but administration of PD restored these deficits. We hypothesize that PD may act by restoring homeostasis through acute activation of the JNK pathway and its complex interplay with Insulin signalling, in the context of epileptic stress. Conclusions: Identification of JNK pathway-mediated alleviation of seizures could provide unanticipated launch points for investigation into their anti-epileptic roles and identification of novel targets. Our results could pave the way for the further usage of polyphenolic compounds in combinatorial approaches against acquired and genetic refractory epilepsies.

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BOARD NUMBER: S07-569

IDENTIFICATION OF A NOVEL MISSENSE VARIANT IN A FAMILY WITH AUTOSOMAL DOMINANT SLEEP-RELATED HYPERMOTOR EPILEPSY (ADSHE)

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Aims: autosomal dominant sleep-related hypermotor epilepsy (ADSHE) is a familial form of focal epilepsy, characterized by hyperkinetic focal seizures, mainly arising in the neocortex during non-rapid eye movements (NREM) sleep. *CHRNA2*, *CHRNA4*, *CHRNB2*, *CRH*, *KCNT1*, *DEPDC5*, and *CABP4* genes are known to be associated with ADSHE, accounting for only a small proportion of the genetically determined cases. We aimed to identify additional disease-causing genes in an Italian family affected by ADSHE and to investigate their functional effects. ***Methods***: genomic DNA was isolated from peripheral blood and trio-based whole-exome sequencing (WES) was performed on the proband and her relatives. Sanger sequencing and co-segregation analysis were used to validate candidate variants. Bioinformatic prediction of pathogenicity was carried out for further evaluation. ***Results:*** trio-based WES revealed 9 novel variants shared by the proband and her affected father but absent in her unaffected mother. Validation by Sanger sequencing confirmed the presence of a novel missense variant (p.W276G) in *MOXD1* gene, encoding monooxigenase DBH like 1 protein, detected in heterozygosity in all affected family members. This variant was predicted to be pathogenic by different *in silico* tools (SIFT, Polyphen-2 and Mutation Taster). Computational analysis of protein structure by the webserver Dynamut2 revealed a high destabilizing effect with a ΔΔG=-3.77 kcal/mol. The mutated amino acid is located near the copper binding site, suggesting an alteration of the binding pocket of the enzyme. ***Conclusions:*** our data suggest that *MOXD1* gene could be associated with ADSHE, but *in vitro* functional analyses are needed to draw definite conclusions.

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BOARD NUMBER: S07-570

STRUCTURE-FUNCTION RELATIONSHIPS IN THE INTERACTION OF PROLINE-RICH TRANSMEMBRANE PROTEIN 2 (PRRT2) WITH VOLTAGE GATED NA+ CHANNELS

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Aim: Mutations in the Proline-Rich Transmembrane Protein 2 (PRRT2) gene are the causative agent for a wide spectrum of paroxysmal disorders. The neuron-specific PRRT2 protein controls the intrinsic excitability through specific interactions with voltage-gated Na^+ (Nav) 1.2 and 1.6 channels. The aim of this study is to characterize the PRRT2-Nav interaction dissecting the contribution of PRRT2 domains on Nav biophysical modulation. **Methods:** HEK293 cell-line stably expressing human Nav1.2 or Nav1.1 were sequentially transfected with (i) empty vector, (ii) wild-type full length PRRT2 (WT), (iii) the deletion mutant of PRRT2 lacking the intracellular N-terminus domain (δN-PRRT2) or (iv) the transmembrane domain (δC-PRRT2). The Nav modulation in each condition was studied by patch-clamp experiments. The biochemical interaction of Nav with PRRT2 WT with the deletion mutants was evaluated by pulldown assays. **Results:** In the presence of PRRT2 WT or δN-PRRT2, but not δC-PRRT2, Nav1.2 exhibit a reduction of current density paired by change in inactivation curves and recovery from inactivation. No functional modulation by both mutants was found on Nav1.1 under the same conditions. Pulldown experiments showed a decreased binding between the Nav1.2 and the δN-PRRT2, while δC-PRRT2 displayed an affinity similar to PRRT2 WT. **Conclusion:** Here, we formulate the tandem domain model that provides specific roles to the PRRT2 domains. We demonstrate that PRRT2 cytosolic domain exerts a binding role without modulating Nav activity, while the transmembrane domain is able to modulate the channel properties, but with a low intrinsic binding activity.

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BOARD NUMBER: S07-571

FUNCTIONAL AND MOLECULAR ARCHITECTURE OF THE HEALTHY AND DISEASED HUMAN BRAIN

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

Larissa Kraus[1], Brianna Bristow[1], Kaitlin Sullivan[1], John Maguire[2], Gary Redekop[3], Mark Cembrowski[1,4]
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Epilepsy is a life-altering disease, affecting up to 1 million people worldwide. Although several new anti-epileptic drugs have been introduced in clinical routine in the last decades, 30% of epilepsy patients remain pharmacoresistant. Differences between fundamental properties of mouse and human neurons, as well as failures to translate therapeutic approaches from rodent models to clinical trials, highlight the need to study physiological and pathological mechanisms directly in human brain tissue. We investigated the functional and molecular properties of neuronal subpopulations in healthy and diseased human brain specimen. Using electrophysiology, Ca activity imaging and transcriptional methods (spatial transcriptomics, single nucleus RNA-seq, multiplexed FISH), we investigated the involvement of specific subpopulations of neurons in epileptic activity ex vivo. We further investigated the functional involvement of neuronal subtypes by targeted optogenetic manipulation using viral systems in human brain slice cultures. Our results provide unprecedented insight into the spatial heterogeneity of neuronal subtypes in the healthy human brain and provide detailed information on epilepsy-associated molecular transformations in the human brain. Importantly, identifying how cell types and molecular profiles are affected by epilepsy will guide development of novel therapeutic approaches for pharmacoresistant patients suffering from epilepsy.

Pubmed:
34862988: Göttert R, Fidzinski P, Kraus L, Schneider UC, Holtkamp M, Endres M, Gertz K, Kronenberg G
Lithium inhibits tryptophan catabolism via the inflammation-induced kynurenine pathway in human microglia.
Despite its decades' long therapeutic use in psychiatry, the biological mechanisms underlying lithium's mood-stabilizing effects have remained largely elusive. Here, we investigated the effect of lithium on tryptophan breakdown via the kynurenine pathway using immortalized human microglia cells, primary human microglia isolated from surgical specimens, and microglia-like cells differentiated from human induced pluripotent stem cells. Interferon (IFN)-γ, but not lipopolysaccharide, was able to activate immortalized human microglia, inducing a robust increase in indoleamine-2,3-dioxygenase (IDO1) mRNA transcription, IDO1 protein expression, and activity. Further, chromatin immunoprecipitation verified enriched binding of both STAT1 and STAT3 to the IDO1 promoter. Lithium counteracted these effects, increasing inhibitory GSK3β phosphorylation and reducing STAT1 and STAT3 phosphorylation levels in IFN-γ treated cells. Studies in primary human microglia and hiPSC-derived microglia confirmed the anti-inflammatory effects of lithium, highlighting that IDO activity is reduced by GSK3 inhibitor SB-216763 and STAT inhibitor nifuroxazide via downregulation of P-STAT1 and P-STAT3 . Primary human microglia differed from immortalized human microglia and hiPSC derived microglia-like cells in their strong sensitivity to LPS, resulting in robust upregulation of IDO1 and anti-inflammatory cytokine IL-10. While lithium again decreased IDO1 activity in primary cells, it further increased release of IL-10 in response to LPS. Taken together, our study demonstrates that lithium inhibits the inflammatory kynurenine pathway in the microglia compartment of the human brain.
Glia, 2022; 70
34605012: Monni L, Kraus L, Dipper-Wawra M, Soares-da-Silva P, Maier N, Schmitz D, Holtkamp M, Fidzinski P
In vitro and in vivo anti-epileptic efficacy of eslicarbazepine acetate in a mouse model of KCNQ2-related self-limited epilepsy.
The KCNQ2 gene encodes for the K 7.2 subunit of non-inactivating potassium channels. KCNQ2-related diseases range from autosomal dominant neonatal self-limited epilepsy, often caused by KCNQ2 haploinsufficiency, to severe encephalopathies caused by KCNQ2 missense variants. In vivo and in vitro effects of the sodium channel blocker eslicarbazepine acetate (ESL) and eslicarbazepine metabolite (S-Lic) in a mouse model of self-limited neonatal epilepsy as a first attempt to assess the utility of ESL in the KCNQ2 disease spectrum was investigated.
Br J Pharmacol, 2022; 179
32449727: Kraus L, Monni L, Schneider UC, Onken J, Spindler P, Holtkamp M, Fidzinski P

Preparation of Acute Human Hippocampal Slices for Electrophysiological Recordings.
Epilepsy affects about 1% of the world population and leads to a severe decrease in quality of life due to ongoing seizures as well as high risk for sudden death. Despite an abundance of available treatment options, about 30% of patients are drug-resistant. Several novel therapeutics have been developed using animal models, though the rate of drug-resistant patients remains unaltered. One of probable reasons is the lack of translation between rodent models and humans, such as a weak representation of human pharmacoresistance in animal models. Resected human brain tissue as a preclinical evaluation tool has the advantage to bridge this translational gap. Described here is a method for high quality preparation of human hippocampal brain slices and subsequent stable induction of epileptiform activity. The protocol describes the induction of burst activity during application of 8 mM KCl and 4-aminopyridin. This activity is sensitive to established AED lacosamide or novel antiepileptic candidates, such as dimethylethanolamine (DMEA). In addition, the method describes induction of seizure-like events in CA1 of human hippocampal brain slices by reduction of extracellular Mg and application of bicuculline, a GABAA receptor blocker. The experimental set-up can be used to screen potential antiepileptic substances for their effects on epileptiform activity. Furthermore, mechanisms of action postulated for specific compounds can be validated using this approach in human tissue (e.g., using patch-clamp recordings). To conclude, investigation of vital human brain tissue ex vivo (here, resected hippocampus from patients suffering from temporal lobe epilepsy) will improve the current knowledge of physiological and pathological mechanisms in the human brain.
J Vis Exp, 2020;
31325344: Jurek B, Chayka M, Kreye J, Lang K, Kraus L, Fidzinski P, Kornau HC, Dao LM, Wenke NK, Long M, Rivalan M, Winter Y, Leubner J, Herken J, Mayer S, Mueller S, Boehm-Sturm P, Dirnagl U, Schmitz D, Kölch M, Prüss H
Human gestational N-methyl-d-aspartate receptor autoantibodies impair neonatal murine brain function.
Maternal autoantibodies are a risk factor for impaired brain development in offspring. Antibodies (ABs) against the NR1 (GluN1) subunit of the N-methyl-d-aspartate receptor (NMDAR) are among the most frequently diagnosed anti-neuronal surface ABs, yet little is known about effects on fetal development during pregnancy.
Ann Neurol, 2019; 86
31486590: Agostinho AS, Mietzsch M, Zangrandi L, Kmiec I, Mutti A, Kraus L, Fidzinski P, Schneider UC, Holtkamp M, Heilbronn R, Schwarzer C
Dynorphin-based "release on demand" gene therapy for drug-resistant temporal lobe epilepsy.
Focal epilepsy represents one of the most common chronic CNS diseases. The high incidence of drug resistance, devastating comorbidities, and insufficient responsiveness to surgery pose unmet medical challenges. In the quest of novel, disease-modifying treatment strategies of neuropeptides represent promising candidates. Here, we provide the "proof of concept" that gene therapy by adeno-associated virus (AAV) vector transduction of preprodynorphin into the epileptogenic focus of well-accepted mouse and rat models for temporal lobe epilepsy leads to suppression of seizures over months. The debilitating long-term decline of spatial learning and memory is prevented. In human hippocampal slices obtained from epilepsy surgery, dynorphins suppressed seizure-like activity, suggestive of a high potential for clinical translation. AAV-delivered preprodynorphin expression is focally and neuronally restricted and release is dependent on high-frequency stimulation, as it occurs at the onset of seizures. The novel format of "release on demand" dynorphin delivery is viewed as a key to prevent habituation and to minimize the risk of adverse effects, leading to long-term suppression of seizures and of their devastating sequel.
EMBO Mol Med, 2019; 11
31551707: Kraus L, Hetsch F, Schneider UC, Radbruch H, Holtkamp M, Meier JC, Fidzinski P
Dimethylethanolamine Decreases Epileptiform Activity in Acute Human Hippocampal Slices .
Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy with about 30% of patients developing pharmacoresistance. These patients continue to suffer from seizures despite polytherapy with antiepileptic drugs (AEDs) and have an increased risk for premature death, thus requiring further efforts for the development of new antiepileptic therapies. The molecule dimethylethanolamine (DMEA) has been tested as a potential treatment in various neurological diseases, albeit the functional mechanism of action was never fully understood. In this study, we investigated the effects of DMEA on neuronal activity in single-cell recordings of primary neuronal cultures. DMEA decreased the frequency of spontaneous synaptic events in a concentration-dependent manner with no apparent effect on resting membrane potential (RMP) or action potential (AP) threshold. We further tested whether DMEA can exert antiepileptic effects in human brain tissue . We analyzed the effect of DMEA on epileptiform activity in the CA1 region of the resected hippocampus of TLE patients by recording extracellular field potentials in the pyramidal cell layer. Epileptiform burst activity in resected hippocampal tissue from TLE patients remained stable over several hours and was pharmacologically suppressed by lacosamide, demonstrating the applicability of our platform to test antiepileptic efficacy. Similar to lacosamide, DMEA also suppressed epileptiform activity in the majority of samples, albeit with variable interindividual effects. In conclusion, DMEA might present a new approach for treatment in pharmacoresistant TLE and further studies will be required to identify its exact mechanism of action and the

involved molecular targets.
Front Mol Neurosci, 2019; 12
27840039: Schneidereit D, Kraus L, Meier JC, Friedrich O, Gilbert DF
Step-by-step guide to building an inexpensive 3D printed motorized positioning stage for automated high-content screening microscopy.
High-content screening microscopy relies on automation infrastructure that is typically proprietary, non-customizable, costly and requires a high level of skill to use and maintain. The increasing availability of rapid prototyping technology makes it possible to quickly engineer alternatives to conventional automation infrastructure that are low-cost and user-friendly. Here, we describe a 3D printed inexpensive open source and scalable motorized positioning stage for automated high-content screening microscopy and provide detailed step-by-step instructions to re-building the device, including a comprehensive parts list, 3D design files in STEP (Standard for the Exchange of Product model data) and STL (Standard Tessellation Language) format, electronic circuits and wiring diagrams as well as software code. System assembly including 3D printing requires approx. 30h. The fully assembled device is light-weight (1.1kg), small (33×20×8cm) and extremely low-cost (approx. EUR 250). We describe positioning characteristics of the stage, including spatial resolution, accuracy and repeatability, compare imaging data generated with our device to data obtained using a commercially available microplate reader, demonstrate its suitability to high-content microscopy in 96-well high-throughput screening format and validate its applicability to automated functional Cl- and Ca-imaging with recombinant HEK293 cells as a model system. A time-lapse video of the stage during operation and as part of a custom assembled screening robot can be found at https://vimeo.com/158813199.
Biosens Bioelectron, 2017; 92
30559476: Böttcher C, Schlickeiser S, Sneeboer MAM, Kunkel D, Knop A, Paza E, Fidzinski P, Kraus L, Snijders GJL, Kahn RS, Schulz AR, Mei HE, , Hol EM, Siegmund B, Glauben R, Spruth EJ, de Witte LD, Priller J
Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry.
Microglia, the specialized innate immune cells of the CNS, play crucial roles in neural development and function. Different phenotypes and functions have been ascribed to rodent microglia, but little is known about human microglia (huMG) heterogeneity. Difficulties in procuring huMG and their susceptibility to cryopreservation damage have limited large-scale studies. Here we applied multiplexed mass cytometry for a comprehensive characterization of postmortem huMG (10 - 10 cells). We determined expression levels of 57 markers on huMG isolated from up to five different brain regions of nine donors. We identified the phenotypic signature of huMG, which was distinct from peripheral myeloid cells but was comparable to fresh huMG. We detected microglia regional heterogeneity using a hybrid workflow combining Cytobank and R/Bioconductor for multidimensional data analysis. Together, these methodologies allowed us to perform high-dimensional, large-scale immunophenotyping of huMG at the single-cell level, which facilitates their unambiguous profiling in health and disease.
Nat Neurosci, 2019; 22
29427611: Le Duigou C, Savary E, Morin-Brureau M, Gomez-Dominguez D, Sobczyk A, Chali F, Milior G, Kraus L, Meier JC, Kullmann DM, Mathon B, de la Prida LM, Dorfmuller G, Pallud J, Eugène E, Clemenceau S, Miles R
Imaging pathological activities of human brain tissue in organotypic culture.
Insights into human brain diseases may emerge from tissue obtained after operations on patients. However techniques requiring transduction of transgenes carried by viral vectors cannot be applied to acute human tissue.
J Neurosci Methods, 2018; 298

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BOARD NUMBER: S07-572

EFFICACY OF ANTI-SEIZURE MEDICATION IN A MOUSE MODEL OF HCN1 DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Background and Aims: Understanding drug sensitivity profiles is challenging in rare diseases such as *HCN1* developmental and epileptic encephalopathy (DEE). The Hcn1 p.M294L mouse model carries the homologue of the recurrent HCN1 p.M305L pathogenic variant and recapitulates many key phenotypic elements of *HCN1* DEE. We aimed to use this mouse model to study the effect of a range of anti-seizure medications (ASMs) on excitability in *HCN1* DEE. **Methods:** Mice were instrumented with electrocorticography (ECoG) electrodes and their brain activity recorded following the administration of various ASMs. The effect of each drug on epileptiform spike frequency was quantified. ECoG spectral data was also analysed to investigate signatures of efficacy or exacerbation. **Results:** Phenytoin, lamotrigine and retigabine significantly increased ECoG spike frequency, with lamotrigine and retigabine triggering seizures in a subset of mice tested. There was also a strong trend for carbamazepine to increase spiking. Levetiracetam, diazepam, sodium valproate and ethosuximide all significantly reduced ECoG spike frequency. There were no consistent ECoG spectral changes caused by drugs that reduced spike frequency, while drugs that increased spiking all increased power in the slower delta and/or theta bands. **Conclusions:** Drugs which work predominantly through sodium channel block increased neuronal excitability in Hcn1 p.M294L mice, suggesting they should be contraindicated in *HCN1* DEE. Conversely, levetiracetam, diazepam, valproate and ethosuximide reduced excitability, so may be effective treatments for *HCN1* DEE. This pharmacoresponsiveness profile is similar to that of other epilepsies, especially Dravet Syndrome. Our data provides an initial framework for the clinical treatment of *HCN1* DEE.

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BOARD NUMBER: S07-573

CHRONIC INTRASUBTHALAMIC VALPROATE IS ANTICONVULSIVE IN AN ACUTE RAT SEIZURE MODEL

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Intracerebral drug delivery is a method of bypassing the blood brain barrier for targeted pharmacological intervention in specific brain regions while maintaining low systemic drug concentrations. This is an attractive alternative treatment option for systemic drug resistant epilepsies. **AIM**: To investigate the anticonvulsive potential of the broad spectrum antiseizure medication valproate when it is chronically applied to the subthalamic nucleus (STN), a key seizure-modulating structure in the epileptic network. **METHODS**: Valproate was infused chronically into the STN of adult rats (480, 720, or 960 µg/d) via surgically implanted cannulas and microinfusion pumps. The anticonvulsive effects and putative adverse effects of the convection-enhanced intrasubthalamic valproate delivery were measured via a repeatable, intravenous pentylenetetrazol seizure threshold test, and a behavioural test battery. **RESULTS**: All doses were well tolerated, and an increased proportion of responders with increasing valproate doses was observed. Responder rates were 66 %, 83 %, and 92 % for 480 µg/d, 720 µg/d, and 960 µg/d valproate, respectively. All responders receiving 720 µg/d developed tolerance, i.e., the anticonvulsive effect dropped to a threshold increase of less than 25 % in the third week, whereas only 45 % of responders receiving 960 µg/d developed tolerance. **CONCLUSION**: Our results support the STN as a viable target for intracerebral drug delivery in epilepsies. If tolerance development can be countered with discontinuous application regimes, while high responder rates are maintained, this could provide a promising approach for the future treatment of systemic drug resistant epilepsies. This project was supported by the DFG (GE1103/9-1).

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BOARD NUMBER: S07-574

CHARACTERIZATION OF A KCNA2 LOSS-OF-FUNCTION MOUSE MODEL

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

Peter Mueller[1], Ahmed Eltoki[1,2], Nikolas Layer[1], Thomas Ott[3], Holger Lerche[1], Thomas Wuttke[1], Ulrike Hedrich[1]
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Monogenetic developmental and epileptic encephalopathies (DEE) are rare, but severe diseases that cause an immense lifelong burden on patients and their relatives. In 2015, we described several DEE causing *de novo* variants in the *KCNA2* gene encoding the voltage-gated potassium channel K_v1.2, one of them being p.Pro405Leu (P405L). This variant exhibited a loss-of-function phenotype due to a dramatic reduction in current amplitude. We therefore expected P405L to cause increased excitability of cortical neurons as correlate for increased seizure susceptibility. To study the effect of this variant on neuronal excitability as well as possible compensatory and disease-specific mechanisms, we generated *Kcna2*^{P405L} knock-in mouse models. We combined whole-cell patch-clamp recordings of pyramidal neurons and interneurons in acute slices of PN12-15 wildtype and heterozygous mice and transcriptomic analysis via single nuclei RNA sequencing of cortical and hippocampal tissue to better understand the pathomechanism of this variant. Heterozygous *Kcna2*^{P405L} mice on a C57Bl/6 background died prematurely between one and two months of age. We therefore switched to Swiss mice and found that the animals had a slight increase in survival. Surprisingly, we found that the firing frequency of pyramidal cells was similar to wildtype, and the only difference in intrinsic properties was a significant increase in the afterhyperpolarization amplitude. Our transcriptomic data indicated an explanation for this alteration in action potential shape. In conclusion, we established a new mouse model for a loss-of-function variant in *KCNA2*. Counterintuitively, the mechanism of seizure susceptibility of this model does not rely on increased pyramidal cell excitability.

Pubmed:
29863287: Müller P, Draguhn A, Egorov AV
Persistent sodium current modulates axonal excitability in CA1 pyramidal neurons.
Axonal excitability is an important determinant for the accuracy, direction, and velocity of neuronal signaling. The mechanisms underlying spike generation in the axonal initial segment and transmitter release from presynaptic terminals have been intensely studied and revealed a role for several specific ionic conductances, including the persistent sodium current (I). Recent evidence indicates that action potentials can also be generated at remote locations along the axonal fiber, giving rise to ectopic action potentials during physiological states (e.g., fast network oscillations) or in pathological situations (e.g., following demyelination). Here, we investigated how ectopic axonal excitability of mouse hippocampal CA1 pyramidal neurons is regulated by I . Recordings of field potentials and intracellular voltage in brain slices revealed that electrically evoked antidromic spikes were readily suppressed by two different blockers of I , riluzole and phenytoin. The effect was mediated by a reduction of the probability of ectopic spike generation while latency was unaffected. Interestingly, the contribution of I to excitability was much more pronounced in axonal branches heading toward the entorhinal cortex compared with the opposite fiber direction toward fimbria. Thus, excitability of distal CA1 pyramidal cell axons is affected by persistent sodium currents in a direction-selective manner. This mechanism may be of importance for ectopic spike generation in oscillating network states as well as in pathological situations.
J Neurochem, 2018; 146

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BOARD NUMBER: S07-575

DEEP LEARNING NEURAL NETWORK FOR THE DETECTION OF EPILEPTIFORM EVENTS AND SEIZURE ONSET ZONE

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Aims Deep Learning (DL) neural networks can be used for automated analysis of intracranial EEG (iEEG) and the detection of pathological high frequency oscillations (HFOs) and spikes which are good biomarkers of epileptogenic tissue. We used a bidirectional Long Short-Term Memory (LSTM) neural network trained to detect ripples-on-oscillation (RonO), spikes and ripple-on-spike (RonS) and analyzed the prevalence of these events in regard to the seizure onset zone (SOZ). **Methods** We used iEEG from the open access database (14 patients). Spectral features were analyzed continuously across 40-min iEEG records and the initial screening for event candidates was done with a novel thresholding algorithm. 1000 events of each class (spike, RonS, RonO and baseline) were visually verified and selected. Training of the LSTM network was performed using random selections of 500 events (per class) while other 500 events (per class) were used for testing. **Results** The network was able to detect events with sensitivity/specificity above 85-90% for all event classes. Spikes and RonS showed highly significant correlation with SOZ ($p < 0.005$) in all patients regardless of the location of SOZ. Ripples without spikes (RonO) showed significant correlation with SOZ ($p < 0.01$) within the hippocampus. **Conclusions** Deep Learning networks can be used for automated detection of HFOs and epileptic spikes within iEEG and thus can be instrumental in the delineation of seizure onset zone. The DL networks can significantly accelerate the analysis of iEEG data and increase their diagnostic value, which may improve surgical outcome in patients with localization-related refractory epilepsy.

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BOARD NUMBER: S07-576

DYNAMICS OF SEIZURE-LIKE PROPAGATION IN SPIKING NETWORK MODELS.

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Aims: In epilepsies, seizures originating from one or various regions mays spread or not to the rest of the brain, propagating from one region to another. Into a given region receiving an incoming seizure, the underlying mechanisms that will lead to contain or propagate the perturbation remain unclear. We study these aspects through computational models at the neural network scale to understand which specific features play a role. Methods: Three different models of electrophysiological activity based on ordinary differential equations, describing the dynamics of the neuron's membrane potential are used to build networks of inhibitory and excitatory populations. These networks receive incoming perturbations mimicking the seizure input. Results: We observe two possible scenarios, either both populations exhibit paroxysmal responses, or only the inhibitory population exhibit a strong activity, thus containing the excitatory population and preventing propagation to the next regions. We found that these behaviors do not only depend on the characteristics of the inputs as the perturbation leads to dynamical heterogeneity inside the network, non-trivially related to its architecture. Conclusions: Our results show a variety of local responses to strong incoming perturbations, leading to different global network phenomenologies. We observe how the choice of single-neuron models highly condition the large-scale dynamics, which opens the way to a discussion on the very choice of model one can use in this type of study.

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BOARD NUMBER: S07-577

REVIEW OF INTRACRANIAL ELECTROCORTICOGRAPHY IN EPILEPSY PATIENTS IMPLANTED PREVIOUSLY WITH RESPONSIVE NEUROSTIMULATORS (RNS) DURING COVID 19 VACCINATION SERIES IN 10 PATIENTS

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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This work is a retrospective review of the electrocorticography of patients with an implanted RNS -Responsive neurostimulation device who received any of the three available vaccination series (Pfeizer, Johnson & Johnson, and Moderna) for providing immunity for COVID 19 in the United States. Retrospective review of such a dataset available in a cohort of such patients was IRB approved and such dataset was analyzed. Although this report identifies only a small cohort of patients, no significant differences in electrocorticography were noted after vaccine administration in this case series. Such findings may merit further study and analysis.

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BOARD NUMBER: S07-578

PHARMACOLOGY AND DESENSITIZATION PROPERTIES OF α4-CONTAING GABAA RECEPTORS

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Aims: We recently reported a *de novo* missense variant in *GABRA4* in an individual with early-onset drug-resistant epilepsy and neurodevelopmental abnormalities. An electrophysiological characterization of the variant, located in the pore-forming domain, shows accelerated desensitization and a lack of seizure-protective neurosteroid function. Our findings represent the first genetic and functional evidence for an association between *GABRA4* and a neurodevelopmental disorder with epilepsy. To date, still little is still known about α4- containing $GABA_A$ receptor pharmacology. In this study we will perform further pharmacological analysis of this and other *GABRA4* variants in comparison to the wild type in a panel of α4- containing $GABA_A$ receptors. A pharmacological rescue of disrupted desensitization caused by specific *GABRA4* variants could provide the basis of future treatments. **Methods:** Recombinant expression is done in *Xenopus Laevis* oocytes and HEK cells. Electrophysiological characterization is performed using the two electrode voltage clamp technique and the patch clamp technique. Biochemical analysis of rodent brain samples complements the study. **Results and Conclusion:** Provided that future replications will reinforce a causal relationship, *GABRA4* may soon be added to the constantly growing list of epilepsy-related disease genes. Our preliminary data suggest a novel mechanism of interaction between so called “benzodiazepine insensitive” α4- containing $GABA_A$receptors and benzodiazepines. This novel pharmacological mechanism could pave the way for future $GABA_A$receptor targeting therapeutics.

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BOARD NUMBER: S07-579

DAILY INTERMITTENT FASTING ATTENUATES ABSENCE EPILEPSY IN MICE.

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Several studies have shown a tripartite link between food intake, seizures and cognitive deficits in epilepsy. Absence Epilepsy (AE) affect children during a critical phase of neurodevelopment. Limiting or even avoiding treatments based on antiepileptic drugs with strong side effects is extremely important. We investigated daily intermittent fasting (IF) as an innovative, drug-free method to improve seizure burden as well as cognitive deficits on different mouse models of AE. We implemented a 1-month daily IF regime (8 h feeding/16 h fasting) in control and epileptic mice followed by a multilevel analysis. We tracked the evolution of seizures (by electroencephalographic recordings) and behavioural comorbidities in the Grm7^{AAA} KI mouse, an isomorphic model of "pure" AE. We show that the 1-month IF regime reduced significantly the occurrence of absence seizures by ~40%, while improving social and cognitive behavioural scores. RNA-seq experiments and preliminary gene ontology analysis revealed an altered mRNA expression pattern in the thalami of epileptic animals that was reversed by IF. In particular, modifications of angiogenesis-related genes were confirmed by blood vessels tracing experiments. Similar results were obtained in a pharmacological model of atypical absence seizures, the AY9944 mouse. Thus, our results indicate that daily IF could be a simple and efficient non-invasive strategy to be proposed as an alternative to the existing, yet often ineffective, anti-epileptic drugs or strict regimes, such as the ketogenic diet.

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BOARD NUMBER: S07-580

TIME-LIMITED ALTERATIONS IN CORTICAL ACTIVITY OF A KNOCK-IN MICE MODEL OF KCNQ2-RELATED DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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De novo missense variants in the *KCNQ2* gene encoding the Kv7.2 subunit of Kv7/M channel are the main cause of Developmental and Epileptic Encephalopathy (DEE). *KCNQ2* related-DEE is characterized by pharmaco-resistant neonatal seizures associated with a developmental delay. While seizures usually resolve some weeks or months after birth, cognitive/behavioral deficits persist, greatly affecting the patient's quality of life. To better understand the cellular mechanisms underlying *KCNQ2-associated* network dysfunction and their progression over time, we investigated *in vivo,* using local field potential recordings of freely moving animals, the seizure susceptibility in KI mice, and *ex-vivo* in layers II/III and V of motor cortical slices, using patch-clamp recordings, the electrophysiological properties of pyramidal cells from a heterozygous knock-in (KI) mouse model carrying the p.T274M pathogenic variant during neonatal, post-weaning and juvenile developmental stages.

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BOARD NUMBER: S07-581

MEDICAL CANNABIS FOR SEVERE TREATMENT-RESISTANT EPILEPSY IN CHILDREN: A CASE-SERIES OF 10 PATIENTS

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Introduction Whole-plant medical cannabis has shown considerable anecdotal evidence for efficacy in children suffering from intractable epilepsies. In spite of this only isolated CBD is licensed as an intervention for such patients. Here we report a case series of 10 children using whole-plant extracts to demonstrate the feasibility of this intervention. **Methods** This study was conducted retrospectively through collecting clinical data from caretakers and clinicians on study outcome variables. Participants were recruited through charity patient representative groups that support children who are using medical cannabis to treat their epilepsies. Medicines were prescribed to patients by clinicians in both National Health Service and private medical practices. Ten children, 18 years old or under, with intractable epilepsies, were recruited from two charities. There were no limitations on diagnosis, sex, or ethnic origin. Participants were treated with a range of whole-plant medical cannabis oils. Individual dosing regimens were determined by clinicians. The primary outcome measure was seizure frequency. **Results** Seizure frequency across all 10 participants was reduced by 86% with no significant adverse events. Participants reduced the use of antiepileptic drugs from an average of seven to one following treatment with medical cannabis. We also noted significant financial costs of £874 per month to obtain these medicines through private prescriptions. **Conclusions** This study establishes the feasibility of whole-plant medical cannabis as an effective and well-tolerated medicine for reducing seizure frequency in children suffering from intractable epilepsies. These findings justify the potential value of further research into the reported therapeutic benefit of whole-plant medicinal cannabis products.

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BOARD NUMBER: S07-582

CESIUM CHLORIDE ACTIVATES C-TO-U RNA EDITED GLYCINE RECEPTORS

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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[1]Technische Universität Braunschweig, Cell Physiology, Braunschweig, Germany, [2]Johannes Gutenberg-University of Mainz, Institute Of Pathophysiology, Mainz, Germany, [3]Charité Universitätsmedizin Berlin, Institute Of Cell Biology, Berlin, Germany, [4]Max Delbrueck Center for Molecular Medicine, Cellular Neurosciences, Berlin, Germany

Chloride salts of the alkali metals sodium and potassium play an important physiological role in virtually any cell of our body and their cations are vital for the normal function of excitable cells. Besides sodium and potassium, other cations of the alkali metal family have been shown to affect neuronal physiology, e.g. cesium and lithium. While cesium is readily used in electrophysiological approaches as a blocker of potassium channels, lithium is widely used as a mood-stabilizing drug in the treatment of bipolar disorders. Lately, our group has shown that C-to-U RNA editing plays an important role in the diversification of gene products, and discovered that C-to-U RNA-edited glycine receptors (GlyRs) play a crucial part in the progression of temporal lobe epilepsy (TLE) as facilitated activation of the RNA-edited gain-of-function GlyRs contributes – in a neuron type-specific way – to diverse neuropsychiatric symptoms of the disease. In a recent publication, we demonstrated that C-to-U RNA editing of GlyR subunits allows ammonium chloride to exert an agonistic action specifically on these edited receptors (Kankowski et al., 2018). This prompted us to investigate the effects of different chloride salts of alkali metals (e.g. sodium, potassium, cesium and lithium) on different edited and unedited GlyR subunits using whole-cell patch clamp recordings of transfected HEK293T cells. Our results identify cesium chloride as a novel agonist for C-to-U RNA-edited GlyRs that exhibits its action on these receptors in a dose-dependent and subunit-specific manner and may therefore be of importance in neuropsychiatric research of temporal lobe epilepsy.

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BOARD NUMBER: S07-583

TOWARD NOVEL THERAPEUTIC INTERVENTIONS FOR STXBP1-ASSOCIATED DISORDERS

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

Wietske Van Der Ent[1], Justine Laulin[1], Zuhur Said[1], Kinga Gawel[1,2], Nancy Banono[1], Camila Esguerra[1]
[1]NCMM - University of Oslo, Chemical Neuroscience, Oslo, Norway, [2]Medical University of Lublin, Department Of Experimental And Clinical Pharmacology, Lublin, Poland

Approximately 25-30% of people suffering from epileptic seizures do not respond to available antiseizure drugs (ASDs). Additionally, ASDs tolerated well by adults may cause severe side effects in children. Thus, there is a high need for models to test novel therapeutic options. Especially rare forms of epilepsy, where evaluation of intervention may be challenging due to the limited number of subjects, will benefit from animal models suited for rapid drug screening. Focusing on STXBP1-associated disorders, we developed two drug-screening protocols for potential ASDs: one based on behavioural analysis and another based on morphological alterations. Combining these screening methods with various genetic and chemical zebrafish epilepsy models, we mean to identify novel potential ASDs from libraries of FDA-approved compounds and natural products. Top hits from these initial screens will be confirmed through EEG recordings, and evaluated for their effects on neuronal morphology and additional disease comorbidities such as Schizophrenia- and Autism Spectrum Disorder-like features.

Pubmed:
32096222: Tiraboschi E, Martina S, van der Ent W, Grzyb K, Gawel K, Cordero-Maldonado ML, Poovathingal SK, Heintz S, Satheesh SV, Brattespe J, Xu J, Suster M, Skupin A, Esguerra CV
New insights into the early mechanisms of epileptogenesis in a zebrafish model of Dravet syndrome.
To pinpoint the earliest cellular defects underlying seizure onset (epileptogenic period) during perinatal brain development in a new zebrafish model of Dravet syndrome (DS) and to investigate potential disease-modifying activity of the 5HT receptor agonist fenfluramine.
Epilepsia, 2020; 61
31875924: Gawel K, Turski WA, van der Ent W, Mathai BJ, Kirstein-Smardzewska KJ, Simonsen A, Esguerra CV
Phenotypic Characterization of Larval Zebrafish (Danio rerio) with Partial Knockdown of the cacna1a Gene.
The CACNA1A gene encodes the pore-forming α1 subunit of voltage-gated P/Q type Ca channels (Ca2.1). Mutations in this gene, among others, have been described in patients and rodents suffering from absence seizures and episodic ataxia type 2 with/without concomitant seizures. In this study, we aimed for the first time to assess phenotypic and behavioral alterations in larval zebrafish with partial cacna1aa knockdown, placing special emphasis on changes in epileptiform-like electrographic discharges in larval brains. Whole-mount in situ hybridization analysis revealed expression of cacna1aa in the optic tectum and medulla oblongata of larval zebrafish at 4 and 5 days post-fertilization. Next, microinjection of two antisense morpholino oligomers (individually or in combination) targeting all splice variants of cacna1aa into fertilized zebrafish eggs resulted in dose-dependent mortality and decreased or absent touch response. Over 90% knockdown of cacna1aa on protein level induced epileptiform-like discharges in the optic tectum of larval zebrafish brains. Incubation of morphants with antiseizure drugs (sodium valproate, ethosuximide, lamotrigine, topiramate) significantly decreased the number and, in some cases, cumulative duration of epileptiform-like discharges. In this context, sodium valproate seemed to be the least effective. Carbamazepine did not affect the number and duration of epileptiform-like discharges. Altogether, our data indicate that cacna1aa loss-of-function zebrafish may be considered a new model of absence epilepsy and may prove useful both for the investigation of Cacna1a-mediated epileptogenesis and for in vivo drug screening.
Mol Neurobiol, 2020; 57
28135250: Franzetti GA, Laud-Duval K, van der Ent W, Brisac A, Irondelle M, Aubert S, Dirksen U, Bouvier C, de Pinieux G, Snaar-Jagalska E, Chavrier P, Delattre O
Cell-to-cell heterogeneity of EWSR1-FLI1 activity determines proliferation/migration choices in Ewing sarcoma cells.
Ewing sarcoma is characterized by the expression of the chimeric EWSR1-FLI1 transcription factor. Proteomic analyses indicate that the decrease of EWSR1-FLI1 expression leads to major changes in effectors of the dynamics of the actin cytoskeleton and the adhesion processes with a shift from cell-to-cell to cell-matrix adhesion. These changes are associated

with a dramatic increase of in vivo cell migration and invasion potential. Importantly, EWSR1-FLI1 expression, evaluated by single-cell RT-ddPCR/immunofluorescence analyses, and activity, assessed by expression of EWSR1-FLI1 downstream targets, are heterogeneous in cell lines and in tumours and can fluctuate along time in a fully reversible process between EWSR1-FLI1 states, characterized by highly active cell proliferation, and EWSR1-FLI1 states where cells have a strong propensity to migrate, invade and metastasize. This new model of phenotypic plasticity proposes that the dynamic fluctuation of the expression level of a dominant oncogene is an intrinsic characteristic of its oncogenic potential.
Oncogene, 2017; 36
26802024: Kovar H, Amatruda J, Brunet E, Burdach S, Cidre-Aranaz F, de Alava E, Dirksen U, van der Ent W, Grohar P, Grünewald TG, Helman L, Houghton P, Iljin K, Korsching E, Ladanyi M, Lawlor E, Lessnick S, Ludwig J, Meltzer P, Metzler M, Mora J, Moriggl R, Nakamura T, Papamarkou T, Radic Sarikas B, Rédini F, Richter GH, Rossig C, Schadler K, Schäfer BW, Scotlandi K, Sheffield NC, Shelat A, Snaar-Jagalska E, Sorensen P, Stegmaier K, Stewart E, Sweet-Cordero A, Szuhai K, Tirado OM, Tirode F, Toretsky J, Tsafou K, Üren A, Zinovyev A, Delattre O
The second European interdisciplinary Ewing sarcoma research summit--A joint effort to deconstructing the multiple layers of a complex disease.
Despite multimodal treatment, long term outcome for patients with Ewing sarcoma is still poor. The second "European interdisciplinary Ewing sarcoma research summit" assembled a large group of scientific experts in the field to discuss their latest unpublished findings on the way to the identification of novel therapeutic targets and strategies. Ewing sarcoma is characterized by a quiet genome with presence of an EWSR1-ETS gene rearrangement as the only and defining genetic aberration. RNA-sequencing of recently described Ewing-like sarcomas with variant translocations identified them as biologically distinct diseases. Various presentations adressed mechanisms of EWS-ETS fusion protein activities with a focus on EWS-FLI1. Data were presented shedding light on the molecular underpinnings of genetic permissiveness to this disease uncovering interaction of EWS-FLI1 with recently discovered susceptibility loci. Epigenetic context as a consequence of the interaction between the oncoprotein, cell type, developmental stage, and tissue microenvironment emerged as dominant theme in the discussion of the molecular pathogenesis and inter- and intra-tumor heterogeneity of Ewing sarcoma, and the difficulty to generate animal models faithfully recapitulating the human disease. The problem of preclinical development of biologically targeted therapeutics was discussed and promising perspectives were offered from the study of novel in vitro models. Finally, it was concluded that in order to facilitate rapid pre-clinical and clinical development of novel therapies in Ewing sarcoma, the community needs a platform to maintain knowledge of unpublished results, systems and models used in drug testing and to continue the open dialogue initiated at the first two Ewing sarcoma summits.
Oncotarget, 2016; 7
27165360: van der Ent W, Veneman WJ, Groenewoud A, Chen L, Tulotta C, Hogendoorn PC, Spaink HP, Snaar-Jagalska BE
Automation of Technology for Cancer Research.
Zebrafish embryos can be obtained for research purposes in large numbers at low cost and embryos develop externally in limited space, making them highly suitable for high-throughput cancer studies and drug screens. Non-invasive live imaging of various processes within the larvae is possible due to their transparency during development, and a multitude of available fluorescent transgenic reporter lines.To perform high-throughput studies, handling large amounts of embryos and larvae is required. With such high number of individuals, even minute tasks may become time-consuming and arduous. In this chapter, an overview is given of the developments in the automation of various steps of large scale zebrafish cancer research for discovering important cancer pathways and drugs for the treatment of human disease. The focus lies on various tools developed for cancer cell implantation, embryo handling and sorting, microfluidic systems for imaging and drug treatment, and image acquisition and analysis. Examples will be given of employment of these technologies within the fields of toxicology research and cancer research.
Adv Exp Med Biol, 2016; 916
27464807: Tulotta C, He S, Chen L, Groenewoud A, van der Ent W, Meijer AH, Spaink HP, Snaar-Jagalska BE
Imaging of Human Cancer Cell Proliferation, Invasion, and Micrometastasis in a Zebrafish Xenogeneic Engraftment Model.
The xenograft model, using the early life stages of the zebrafish, allows imaging of tumor cell behavior both on a single cell and whole organism level, over time, within a week. This robust and reproducible assay can be used as an intermediate step between in vitro techniques and the expensive, and time consuming, murine models of cancer invasion and metastasis.In this chapter, a detailed protocol to inject human cancer cells into the blood circulation of a zebrafish embryo is described; the engraftment procedure is then followed by visualization and quantification methods of tumor cell proliferation, invasion, and micrometastasis formation during subsequent larval development. Interaction with the host microenvironment is also considered.
Methods Mol Biol, 2016; 1451
27171126: van der Ent W, Burrello C, de Lange MJ, van der Velden PA, Jochemsen AG, Jager MJ, Snaar-Jagalska BE

Embryonic Zebrafish: Different Phenotypes after Injection of Human Uveal Melanoma Cells.
Although murine xenograft models for human uveal melanoma (UM) are available, they are of limited utility for screening large compound libraries for the discovery of new drugs. We need new preclinical models which can efficiently evaluate drugs that can treat UM metastases. The zebrafish embryonic model is ideal for drug screening purposes because it allows the investigation of potential antitumor properties of drugs within 1 week. The optical transparency of the zebrafish provides unique possibilities for live imaging of fluorescence-labelled cancer cells and their behavior. In addition, the adaptive immune response, which is responsible for the rejection of transplanted material, is not yet present in the early stages of fish development, and systemic immunosuppression is therefore not required to allow growth of tumor cells. We studied the behavior of UM cells following injection into zebrafish embryos and observed different phenotypes. We also analyzed cell migration, proliferation, formation of micrometastasis and interaction with the host microenvironment. Significant differences were noted between cell lines: cells derived from metastases showed more migration and proliferation than cells derived from the primary tumors. The addition of the c-Met inhibitor crizotinib to the water in which the larvae were kept reduced the migration and proliferation of UM cells expressing c-Met. This indicates the applicability of the zebrafish xenografts for testing novel inhibitory compounds and provides a fast and sensitive in vivo vertebrate model for preclinical drug screening to combat UM.
Ocul Oncol Pathol, 2015; 1
25281719: Ban J, Aryee DN, Fourtouna A, van der Ent W, Kauer M, Niedan S, Machado I, Rodriguez-Galindo C, Tirado OM, Schwentner R, Picci P, Flanagan AM, Berg V, Strauss SJ, Scotlandi K, Lawlor ER, Snaar-Jagalska E, Llombart-Bosch A, Kovar H
Suppression of deacetylase SIRT1 mediates tumor-suppressive NOTCH response and offers a novel treatment option in metastatic Ewing sarcoma.
The developmental receptor NOTCH plays an important role in various human cancers as a consequence of oncogenic mutations. Here we describe a novel mechanism of NOTCH-induced tumor suppression involving modulation of the deacetylase SIRT1, providing a rationale for the use of SIRT1 inhibitors to treat cancers where this mechanism is inactivated because of SIRT1 overexpression. In Ewing sarcoma cells, NOTCH signaling is abrogated by the driver oncogene EWS-FLI1. Restoration of NOTCH signaling caused growth arrest due to activation of the NOTCH effector HEY1, directly suppressing SIRT1 and thereby activating p53. This mechanism of tumor suppression was validated in Ewing sarcoma cells, B-cell tumors, and human keratinocytes where NOTCH dysregulation has been implicated pathogenically. Notably, the SIRT1/2 inhibitor Tenovin-6 killed Ewing sarcoma cells in vitro and prohibited tumor growth and spread in an established xenograft model in zebrafish. Using immunohistochemistry to analyze primary tissue specimens, we found that high SIRT1 expression was associated with Ewing sarcoma metastasis and poor prognosis. Our findings suggest a mechanistic rationale for the use of SIRT1 inhibitors being developed to treat metastatic disease in patients with Ewing sarcoma.
Cancer Res, 2014; 74
25249605: van der Ent W, Burrello C, Teunisse AF, Ksander BR, van der Velden PA, Jager MJ, Jochemsen AG, Snaar-Jagalska BE
Modeling of human uveal melanoma in zebrafish xenograft embryos.
Uveal melanoma (UM) is fatal in up to 50% of patients because of liver metastases that are refractory to therapies currently available. While murine xenograft models for human uveal melanoma are available, they have limited utility for screening large compound libraries in drug discovery studies. Therefore, new robust preclinical models are needed that can efficiently evaluate drug efficacy for treatment of this malignancy.
Invest Ophthalmol Vis Sci, 2014; 55
24974828: van der Ent W, Jochemsen AG, Teunisse AF, Krens SF, Szuhai K, Spaink HP, Hogendoorn PC, Snaar-Jagalska BE
Ewing sarcoma inhibition by disruption of EWSR1-FLI1 transcriptional activity and reactivation of p53.
Translocations involving ETS-transcription factors, most commonly leading to the EWSR1-FLI1 fusion protein, are the hallmark of Ewing sarcoma. Despite knowledge of this driving molecular event, an effective therapeutic strategy is lacking. To test potential treatment regimes, we established a novel Ewing sarcoma zebrafish engraftment model allowing time-effective, dynamic quantification of Ewing sarcoma progression and tumour burden in vivo, applicable for screening of single and combined compounds. In Ewing sarcoma the tumour-suppressor gene TP53 is commonly found to be wild-type, thus providing an attractive target for treatment. Here, we study TP53 wild-type (EW7, CADO-ES1 and TC32) and TP53-deleted (SK-N-MC) Ewing sarcoma cell lines to investigate the potentiating effect of p53 reactivation by Nutlin-3 on treatment with YK-4-279 to block transcriptional activity of EWSR1-FLI1 protein. Blocking EWSR1-FLI1 transcriptional activity reduced Ewing sarcoma tumour cell burden irrespective of TP53 status. We show that simultaneous YK-4-279 treatment with Nutlin-3 to stabilize p53 resulted in an additive inhibition of TP53 wild-type Ewing sarcoma cell burden, whilst not affecting TP53-deleted Ewing sarcoma cells. Improved inhibition of proliferation and migration by combinatorial treatment was confirmed in

vivo by zebrafish engraftments. Mechanistically, both compounds together additively induced apoptosis of tumour cells in vivo by engaging distinct pathways. We propose reactivation of the p53 pathway in combination with complementary targeted therapy by EWSR1-FLI1 transcriptional activity disruption as a valuable strategy against p53 wild-type Ewing sarcoma.
J Pathol, 2014; 233

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BOARD NUMBER: S07-584

LOW-DOSE 7,8-DIHYDROXYFLAVONE ADMINISTRATION AFTER STATUS EPILEPTICUS PREVENTS EPILEPSY DEVELOPMENT

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Temporal lobe epilepsy often manifests months or even years after an initial epileptogenic insult (e.g., stroke, trauma, status epilepticus) and, therefore, may be preventable. However, no such preventive treatment is currently available. Aim of this study was to test an antioxidant agent, 7,8-dihydroxyflavone (7,8-DHF), that is well tolerated and effective in preclinical models of many neurological disorders, as an anti-epileptogenic drug. However, 7,8-DHF also acts as a TrkB receptor agonist and, based on the literature, this effect may imply an anti- or a pro-epileptogenic effect. We found that low- (5 mg/kg), but not high-dose 7,8-DHF (10 mg/kg) can exert strong anti-epileptogenic effects in the lithium-pilocarpine model (i.e., highly significant reduction in the frequency of spontaneous seizures and in the time to first seizure after status epilepticus), and that these different effects correlate with differences in TrkB phosphorylation patterns and in activation of TrkB-dependent signaling pathways. These data support the possibility to develop drugs with desirable effects on the TrkB receptor, based on the selective activation of therapeutically relevant signaling pathways. In this respect, and also considering its excellent safety profile and antioxidant properties, 7,8-DHF represents a promising candidate for a preventive, anti-epileptogenic therapy, or at least a template for the development of effective and well-tolerated anti-epileptogenic drugs.

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BOARD NUMBER: S07-585

RESECTED HUMAN BRAIN TISSUE AS A PLATFORM TO SCREEN SMALL MOLECULE AND ANTISENSE OLIGONUCLEOTIDE THERAPIES FOR EPILEPSY

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Aims Current anti-seizure medications are often associated with substantial adverse effects and do not offer seizure-freedom to approximately one third of people with epilepsy. This creates a strong need for new therapeutic approaches, with drug discovery strategies often relying on animal-based models. Here, we describe a more translational approach, using live resected human brain tissue as a platform to probe the mechanisms and efficacy of small molecule and antisense oligonucleotide (ASO) therapies. Methods Human neocortical tissue was obtained with consent from patients undergoing resective surgery to alleviate pharmacoresistant seizures. Resected tissue specimens were transported in ice cold oxygenated sucrose artificial cerebrospinal fluid (ACSF) and sliced into 400 micron sections. For small molecule screening, acute seizure-like activity was induced in slices using standard chemoconvulsants. For ASO screening, slices were incubated at room temperature for 24 hours in a low volume of normal ACSF containing active ASO concentrations. Results Resected human neocortical slices exhibited evoked seizure-like activity, which was reduced by a putative anti-seizure small molecule. Slices incubated in ACSF for 24 hours retained robust RNA integrity, supporting their use as a screening platform for the molecular mechanisms of ASOs. As a proof of concept, we tested an ASO targeting microRNA-134, observing dose-dependent and specific knockdown. Conclusions Resected human brain tissue provides a robust platform for pre-clinical therapeutic discovery in epilepsy. The approach is highly translational, and overcomes concerns about the physiological relevance of animal-based approaches. We believe that these approaches should form a key step in therapeutic discovery pipelines for epilepsy.

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BOARD NUMBER: S07-586

DE NOVO VARIANTS IN KCNA3 CAUSE DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Introduction. Developmental and Epileptic Encephalopathies (DEEs) are rare epilepsy syndromes that manifest with pharmacoresistant seizures and various degrees of developmental delay. Several genes are involved in DEE etiology, particularly voltage-gated potassium channels. Among them, Kv1.3 (KCNA3) channels represent the dominant component of voltage-gated K^+ currents in lymphocytes, and Kv1.3 block prompts immunosuppressive effects. Kv1.3 channels are also involved in microglia activation after status epilepticus, and are blocked by the anti-depressant drug fluoxetine. **Aims.** To identify new variants in DEE-affected patients and characterize their functional and pharmacological effects in order to propose personalized treatments. **Methods.** Next-generation sequencing approaches to identify new variants in DEE-affected patients; site-directed mutagenesis to introduce newly-identified variants in plasmids for heterologous expression; patch-clamp recordings on transfected cells. **Results.** We have identified nine individuals with a novel type of DEE, including marked speech delay with or without intellectual disability, epilepsy, and autism spectrum disorder. These patients carry distinct de novo heterozygous in KCNA3 gene. Patch-clamp recordings performed on CHO cells transiently expressing two of the KCNA3 variants revealed either loss- (G468F) or mixed loss- and gain-of-function effects (V478M) on Kv1.3 currents. Endogenous currents recorded in lymphoblasts from the unaffected, non-carrier father and the patient carrying the V478M variant revealed similar differences in biophysical properties than those observed in CHO cells. Fluoxetine dose-dependently (0.03-10 μM) blocked Kv1.3 and Kv1.3 V478M channels *in vitro*. **Conclusions.** KCNA3 is a novel DEE-causing gene, and fluoxetine could be a rational approach in patients carrying GoF variants in these channels.

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BOARD NUMBER: S07-587

DEVELOPMENT OF FUNCTIONAL IN VITRO MODEL IN DRAVET SYNDROME PATIENT HIPSC-DERIVED CORTICAL NEURONS.

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Intractable epilepsies that emerge in childhood, such as Dravet syndrome (DS), have limited response to current antiepileptic drugs (AEDs). DS patients are often typified by temperature sensitive seizures, along with other complexities like intellectual disability. New therapeutic drugs are therefore needed, as most patients are unresponsive to current AEDs. However, to obtain this, relevant preclinical models are needed. DS is mainly caused by a mutation in the SCN1A gene which is crucial for generating and propagating action potentials. Heterozygous mutation in SCN1A gene leads to loss of sodium currents and action potentials, resulting in reduced neural excitation and ultimately seizure formations. Mouse models and in vitro based human induced pluripotent stem cell (hiPSCs) models have shown that the reduced sodium current density and impaired excitation due to the mutation, primarily affects the GABAergic (inhibitory) interneurons. Thus far most of the electrophysiological findings have been performed using whole-cell patch clamp, and only few have studied this phenomenon at the network level. Here we investigated the functional alteration in hiPSCs of Dravet Syndrome patients using microelectrode arrays (MEAs). We generated two different subtypes of neural cultures: glutamatergic and GABAergic enriched cultures from DS patient lines. When cultured on MEAs we observe that DS neurons display a distinctive neuronal activity pattern compared to control neurons. Furthermore, preliminary data reveals that DS neurons portray more sensitivity in response to specific pharmacological treatments. This study highlights the applicability of disease modelling with hiPSCs and MEAs as a valuable platform to reveal underlying disease mechanisms.

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BOARD NUMBER: S07-588

DISEASE MECHANISMS OF EARLY ONSET EPILEPTIC ENCEPHALOPATHY CAUSED BY K556E KCNQ2 VARIANT

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Background: Pathogenic variants in *KCNQ2* cause neonatal seizure disorders, including Early Onset Epileptic Encephalopathies (EOEE) characterised by pharmacoresistant seizures, developmental delay, and behavioural and cognitive deficits. Current therapies show limited efficacy in the treatment of seizures and fail to address the devastating comorbidities. *Aims:* To assess disease mechanisms of *KCNQ2 de novo* variant K556E identified in a patient with EOEE. *Methods:* The K556E variant was expressed in *Xenopus laevis* oocytes and analysed using two-microelectrode voltage clamp. Next, we generated patient induced pluripotent stem cells (iPSCs) and used *Neurogenin 2* overexpression to differentiate them into neuronal cultures which were studied using patch clamp electrophysiology and gene expression analysis. Lastly, a knock-in mouse model carrying the corresponding amino acid exchange in *Kcnq2* channel was generated using CRISPR/Cas9 technique. To assess the phenotype, we used video monitoring, EEG recordings,seizure susceptibility and behavioural tests. *Results:* K556E shows a moderate loss of function, reducing potassium currents by about 50 % when expressed in *Xenopus* oocytes. Patient iPSC-derived neurons exhibit several changes compared to healthy control, including increased rheobase and action potential firing rate. Both homo- and heterozygous mice develop spontaneous seizures and show increased mortality compared to littermate controls. We further observed increased susceptibility to thermally and chemically induced seizures. A battery of behavioural tests revealed heterozygous mice have reduced marble burying capabilities. *Conclusions:* K556E reduces the KCNQ-mediated currents which translates into hyperexcitability of patient iPSC-derived neurons and seizures and cognitive deficits in mice.

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BOARD NUMBER: S07-589

NEUROTRANSMITTER LEVELS ARE ALTERED IN SPECIFIC BRAIN AREAS OF DOGS AFTER ACUTE AND CHRONIC MEDIUM-CHAIN TRIGLYCERIDE ADMINISTRATION – A POSSIBLE ANTISEIZURE MECHANISM

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Background: The positive impact of medium-chain triglycerides (MCTs) in the management of cognitive dysfunction and epilepsy in humans and dogs is currently being researched. However, the mechanisms of MCTs remain not fully elucidated. The aim of this study was to evaluate the effect of MCTs on canine neurotransmitter levels in different brain regions, in cerebrospinal fluid (CSF), serum, and urine. **Material & Methods:** Feeding of dogs was conducted in a randomised, crossover trial with a commercial diet (control). It was either supplemented with MCT-oil 2 h prior to assessment (acute) or daily for 2 weeks, with assessment conducted 10 h after last feeding (chronic). Proton Magnetic Resonance Spectroscopy with a 3-Tesla MRI scanner was used for concentration level assessment of common neurotransmitters in four brain regions (parietal, piriform, occipital, thalamic). Subsequently, CSF, blood, and urine were collected and analysed via high-performance liquid chromatography. **Results:** Paired data from eight healthy Beagle dogs were compared. Glutamine was reduced 2 h after MCT-administration in the piriform cortex [P = 0.0349] and glutamate after two weeks of MCT [P = 0.0184], respectively. Thalamic gamma-Aminobutyric acid (GABA) levels were increased 2 h after MCT-administration [P = 0.0492]. CSF, blood, and urine analysis did not show any significant intergroup changes. **Conclusion:** MCT-enriched diets have a direct impact on canine brain neurotransmitter levels in areas that may be crucial for seizure generation and propagation. Glutamate reduction and GABA increase might represent one possible anti-seizure mechanism of MCTs. However, those alterations are not present in CSF or other biological fluids.

Pubmed:
32703188: Jendrny P, Schulz C, Twele F, Meller S, von Köckritz-Blickwede M, Osterhaus ADME, Ebbers J, Pilchová V, Pink I, Welte T, Manns MP, Fathi A, Ernst C, Addo MM, Schalke E, Volk HA
Scent dog identification of samples from COVID-19 patients - a pilot study.
As the COVID-19 pandemic continues to spread, early, ideally real-time, identification of SARS-CoV-2 infected individuals is pivotal in interrupting infection chains. Volatile organic compounds produced during respiratory infections can cause specific scent imprints, which can be detected by trained dogs with a high rate of precision.
BMC Infect Dis, 2020; 20
28939854: Schidlitzki A, Twele F, Klee R, Waltl I, Römermann K, Bröer S, Meller S, Gerhauser I, Rankovic V, Li D, Brandt C, Bankstahl M, Töllner K, Löscher W
A combination of NMDA and AMPA receptor antagonists retards granule cell dispersion and epileptogenesis in a model of acquired epilepsy.
Epilepsy may arise following acute brain insults, but no treatments exist that prevent epilepsy in patients at risk. Here we examined whether a combination of two glutamate receptor antagonists, NBQX and ifenprodil, acting at different receptor subtypes, exerts antiepileptogenic effects in the intrahippocampal kainate mouse model of epilepsy. These drugs were administered over 5 days following kainate. Spontaneous seizures were recorded by video/EEG at different intervals up to 3 months. Initial trials showed that drug treatment during the latent period led to higher mortality than treatment after onset of epilepsy, and further, that combined therapy with both drugs caused higher mortality at doses that appear safe when used singly. We therefore refined the combined-drug protocol, using lower doses. Two weeks after kainate, significantly less mice of the NBQX/ifenprodil group exhibited electroclinical seizures compared to vehicle controls, but this effect was lost at subsequent weeks. The disease modifying effect of the treatment was associated with a transient prevention of granule cell dispersion and less neuronal degeneration in the dentate hilus. These data substantiate the involvement of altered glutamatergic transmission in the early phase of epileptogenesis. Longer treatment with NBQX and ifenprodil may shed further light on the apparent temporal relationship between dentate gyrus reorganization and development of spontaneous seizures.

Sci Rep, 2017; 7
30703372: Meller S, Brandt C, Theilmann W, Klein J, Löscher W
Commonalities and differences in extracellular levels of hippocampal acetylcholine and amino acid neurotransmitters during status epilepticus and subsequent epileptogenesis in two rat models of temporal lobe epilepsy.
Chemically or electrically induced status epilepticus (SE) in rodents is a commonly used method for induction of epilepsy. Structural and functional changes in the hippocampus play a pivotal role in epileptogenesis induced by SE. Although cholinergic mechanisms have long been thought to play an important role in the onset and propagation of epileptic seizures, not much is known about the potential role of acetylcholine (ACh) in ictogenesis and epileptogenesis in SE models of temporal lobe epilepsy. Here we used in vivo microdialysis to determine extracellular levels of ACh and, for comparison, several amino acid transmitters in the ventral hippocampus during SE, epileptogenesis, and the chronic epileptic state in two rat models of SE-induced epilepsy. SE was either induced by lithium-pilocarpine or by sustained electrical stimulation of the basolateral amygdala (BLA). ACh increased during SE in both models. Pretreatment with the muscarinic receptor antagonist scopolamine before BLA stimulation reduced SE severity and duration. In contrast to ACh, no consistent changes in amino acid levels were found during SE in the two models. During epileptogenesis and the chronic epileptic state, the only commonalities found in both models were a decrease in ACh in epileptic rats during the chronic epileptic state and a decrease in aspartate during epileptogenesis. The data demonstrate complex, model-dependent alterations in extracellular levels of ACh and amino acid neurotransmitters and only few commonalities. Thus, data originating from only one model of post-SE epilepsy should not be generalized but may have a limited translational value for understanding ictogenesis or epileptogenesis.
Brain Res, 2019; 1712
34315418: Jendrny P, Twele F, Meller S, Schulz C, von Köckritz-Blickwede M, Osterhaus ADME, Ebbers H, Ebbers J, Pilchová V, Pink I, Welte T, Manns MP, Fathi A, Addo MM, Ernst C, Schäfer W, Engels M, Petrov A, Marquart K, Schotte U, Schalke E, Volk HA
Scent dog identification of SARS-CoV-2 infections in different body fluids.
The main strategy to contain the current SARS-CoV-2 pandemic remains to implement a comprehensive testing, tracing and quarantining strategy until vaccination of the population is adequate. Scent dogs could support current testing strategies.
BMC Infect Dis, 2021; 21
34630021: Zamora M, Meller S, Kajin F, Sermon JJ, Toth R, Benjaber M, Dijk DJ, Bogacz R, Worrell GA, Valentin A, Duchet B, Volk HA, Denison T
Case Report: Embedding "Digital Chronotherapy" Into Medical Devices-A Canine Validation for Controlling Status Epilepticus Through Multi-Scale Rhythmic Brain Stimulation.
Circadian and other physiological rhythms play a key role in both normal homeostasis and disease processes. Such is the case of circadian and infradian seizure patterns observed in epilepsy. However, these rhythms are not fully exploited in the design of active implantable medical devices. In this paper we explore a new implantable stimulator that implements chronotherapy as a feedforward input to supplement both open-loop and closed-loop methods. This integrated algorithm allows for stimulation to be adjusted to the ultradian, circadian and infradian patterns observed in patients through slowly-varying temporal adjustments of stimulation and algorithm sub-components, while also enabling adaption of stimulation based on immediate physiological needs such as a breakthrough seizure or change of posture. Embedded physiological sensors in the stimulator can be used to refine the baseline stimulation circadian pattern as a "digital zeitgeber," i.e., a source of stimulus that entrains or synchronizes the subject's natural rhythms. This algorithmic approach is tested on a canine with severe drug-resistant idiopathic generalized epilepsy exhibiting a characteristic diurnal pattern correlated with sleep-wake cycles. Prior to implantation, the canine's cluster seizures evolved to status epilepticus (SE) and required emergency pharmacological intervention. The cranially-mounted system was fully-implanted bilaterally into the centromedian nucleus of the thalamus. Using combinations of time-based modulation, thalamocortical rhythm-specific tuning of frequency parameters as well as fast-adaptive modes based on activity, the canine experienced no further SE events post-implant as of the time of writing (7 months). Importantly, no significant cluster seizures have been observed either, allowing the reduction of rescue medication. The use of digitally-enabled chronotherapy as a feedforward signal to augment adaptive neurostimulators could prove a useful algorithmic method in conditions where sensitivity to temporal patterns are characteristics of the disease state, providing a novel mechanism for tailoring a more patient-specific therapy approach.
Front Neurosci, 2021; 15
34651709: Booth S, Meller S, Packer RM, Farquhar R, Maddison JE, Volk HA
Owner compliance in canine epilepsy.
Poor medication compliance by human epilepsy patients is one of the leading causes of treatment failure and increased seizure frequency. The aim of this cross-sectional study was to analyse owner compliance in pharmacological treatment of canine idiopathic epilepsy and to identify factors associated with poor compliance.

Vet Rec, 2021; 188
34869443: Ten Hagen NA, Twele F, Meller S, Jendrny P, Schulz C, von Köckritz-Blickwede M, Osterhaus A, Ebbers H, Pink I, Welte T, Manns MP, Illig T, Fathi A, Addo MM, Nitsche A, Puyskens A, Michel J, Krause E, Ehmann R, von Brunn A, Ernst C, Zwirglmaier K, Wölfel R, Nau A, Philipp E, Engels M, Schalke E, Volk HA
Discrimination of SARS-CoV-2 Infections From Other Viral Respiratory Infections by Scent Detection Dogs.
Testing of possibly infected individuals remains cornerstone of containing the spread of SARS-CoV-2. Detection dogs could contribute to mass screening. Previous research demonstrated canines' ability to detect SARS-CoV-2-infections but has not investigated if dogs can differentiate between COVID-19 and other virus infections. Twelve dogs were trained to detect SARS-CoV-2 positive samples. Three test scenarios were performed to evaluate their ability to discriminate SARS-CoV-2-infections from viral infections of a different aetiology. Naso- and oropharyngeal swab samples from individuals and samples from cell culture both infected with one of 15 viruses that may cause COVID-19-like symptoms were presented as distractors in a randomised, double-blind study. Dogs were either trained with SARS-CoV-2 positive saliva samples (test scenario I and II) or with supernatant from cell cultures (test scenario III). When using swab samples from individuals infected with viruses other than SARS-CoV-2 as distractors (test scenario I), dogs detected swab samples from SARS-CoV-2-infected individuals with a mean diagnostic sensitivity of 73.8% (95% CI: 66.0-81.7%) and a specificity of 95.1% (95% CI: 92.6-97.7%). In test scenario II and III cell culture supernatant from cells infected with SARS-CoV-2, cells infected with other coronaviruses and non-infected cells were presented. Dogs achieved mean diagnostic sensitivities of 61.2% (95% CI: 50.7-71.6%, test scenario II) and 75.8% (95% CI: 53.0-98.5%, test scenario III), respectively. The diagnostic specificities were 90.9% (95% CI: 87.3-94.6%, test scenario II) and 90.2% (95% CI: 81.1-99.4%, test scenario III), respectively. In all three test scenarios the mean specificities were above 90% which indicates that dogs can distinguish SARS-CoV-2-infections from other viral infections. However, compared to earlier studies our scent dogs achieved lower diagnostic sensitivities. To deploy COVID-19 detection dogs as a reliable screening method it is therefore mandatory to include a variety of samples from different viral respiratory tract infections in dog training to ensure a successful discrimination process.
Front Med (Lausanne), 2021; 8
34617595: Feja M, Meller S, Deking LS, Kaczmarek E, During MJ, Silverman RB, Gernert M
OV329, a novel highly potent γ-aminobutyric acid aminotransferase inactivator, induces pronounced anticonvulsant effects in the pentylenetetrazole seizure threshold test and in amygdala-kindled rats.
An attractive target to interfere with epileptic brain hyperexcitability is the enhancement of γ-aminobutyric acidergic (GABAergic) inhibition by inactivation of the GABA-metabolizing enzyme GABA aminotransferase (GABA-AT). GABA-AT inactivators were designed to control seizures by raising brain GABA levels. OV329, a novel drug candidate for the treatment of epilepsy and addiction, has been shown in vitro to be substantially more potent as a GABA-AT inactivator than vigabatrin, an antiseizure drug approved as an add-on therapy for adult patients with refractory complex partial seizures and monotherapy for pediatric patients with infantile spasms. Thus, we hypothesized that OV329 should produce pronounced anticonvulsant effects in two different rat seizure models.
Epilepsia, 2021; 62
34412582: Jendrny P, Twele F, Meller S, Osterhaus ADME, Schalke E, Volk HA
Canine olfactory detection and its relevance to medical detection.
The extraordinary olfactory sense of canines combined with the possibility to learn by operant conditioning enables dogs for their use in medical detection in a wide range of applications. Research on the ability of medical detection dogs for the identification of individuals with infectious or non-infectious diseases has been promising, but compared to the well-established and-accepted use of sniffer dogs by the police, army and customs for substances such as money, explosives or drugs, the deployment of medical detection dogs is still in its infancy. There are several factors to be considered for standardisation prior to deployment of canine scent detection dogs. Individual odours in disease consist of different volatile organic molecules that differ in magnitude, volatility and concentration. Olfaction can be influenced by various parameters like genetics, environmental conditions, age, hydration, nutrition, microbiome, conditioning, training, management factors, diseases and pharmaceuticals. This review discusses current knowledge on the function and importance of canines' olfaction and evaluates its limitations and the potential role of the dog as a biomedical detector for infectious and non-infectious diseases.
BMC Infect Dis, 2021; 21
34280524: Meller S, Käufer C, Gailus B, Brandt C, Löscher W
Scopolamine prevents aberrant mossy fiber sprouting and facilitates remission of epilepsy after brain injury.
Prevention or modification of acquired epilepsy in patients at risk is an urgent, yet unmet, clinical need. Following acute brain insults, there is an increased risk of mesial temporal lobe epilepsy (mTLE), which is often associated with debilitating comorbidities and reduced life expectancy. The latent period between brain injury and the onset of epilepsy may offer a therapeutic window for interfering with epileptogenesis. The pilocarpine model of mTLE is widely used in the search for novel

antiepileptogenic treatments. Recent biochemical studies indicated that cholinergic mechanisms play a role in the epileptogenic alterations induced by status epilepticus (SE) in this and other models of mTLE, which prompted us to evaluate whether treatment with the muscarinic antagonist scopolamine during the latent period after SE is capable of preventing or modifying epilepsy and associated behavioral and cognitive alterations in female Sprague-Dawley rats. First, in silico pharmacokinetic modeling was used to select a dosing protocol by which M-receptor inhibitory brain levels of scopolamine are maintained during prolonged treatment. This protocol was verified by drug analysis in vivo. Rats were then treated twice daily with scopolamine over 17 days after SE, followed by drug wash-out and behavioral and video/EEG monitoring up to ~6 months after SE. Compared to vehicle controls, rats that were treated with scopolamine during the latent period exhibited a significantly lower incidence of spontaneous recurrent seizures during periods of intermittent recording in the chronic phase of epilepsy, less behavioral excitability, less cognitive impairment, and significantly reduced aberrant mossy fiber sprouting in the hippocampus. The present data may indicate that scopolamine exerts antiepileptogenic/disease-modifying activity in the lithium-pilocarpine rat model, possibly involving increased remission of epilepsy as a new mechanism of disease-modification. For evaluating the rigor of the present data, we envision a study that more thoroughly addresses the gender bias and video-EEG recording limitations of the present study.
Neurobiol Dis, 2021; 158

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-590

RETROSPECTIVE STUDY OF THE SERUM LEVEL OF LEVETIRACETAM IN FOCAL AND GENERALIZED EPILEPSY RELATED TO SEIZURE FREQUENCY AND SIDE EFFECTS IN A SINGLE CENTER

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Antiepileptic drugs (AEDs) are the basic treatment for epilepsy. Levetiracetam is known to affect the synaptic vesicle protein Sv2A. Considering the variability in clinical situations of each patient, or center-dependent, affecting seizure-free rate or adverse drug reactions, the subdivided therapeutic range of levetiracetam in epilepsy patients in a single center and their clinical characteristics were retrospectively analyzed in this study. Data were collected and retrospectively reviewed for patients who were diagnosed with focal (mainly temporal lobe epilepsy) and generalized epilepsy and visited the neurology outpatient center or were admitted to the Seoul National University Hospital from January 19, 2016 (when laboratory results for the concentration of levetiracetam began) to December 31, 2020. In this study, seizure freedom was achieved in >60% of all three groups in each focal and generalized epilepsy group with levetiracetam. In the cases of focal epilepsy, seizure-free rates tended to be higher with increasing levetiracetam concentrations; however, the statistical significance was not clear. In the cases of generalized epilepsy, seizure-free rates did not show significant change according to the serum level. The frequency of adverse drug reactions tended to be higher in the moderate- and high-dose groups than in the low-dose group in focal epilepsy, although this difference was not statistically significant. There was no case of adverse drug reactions in generalized epilepsy group. Further studies with multiple factors with a larger number of patients will guarantee the detailed implications of levetiracetam concentration related to drug efficacy and adverse drug reactions in real clinical situations.

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RETINAL DYSFUNCTION IN A MOUSE MODEL OF HCN1 DEVELOPMENTAL EPILEPTIC ENCEPHALOPATHY

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Pathogenic variants in *HCN1* are associated with a range of epilepsy syndromes including developmental epileptic encephalopathies (DEE). As HCN1 channels are highly expressed in rod and cone photoreceptor inner segments, mutated HCN1 channels are likely to impact visual function in HCN1 DEE. The recurrent *de novo* HCN1 (M305L) pathogenic variant results in a gain-of-function allowing the flux of excitatory ions at depolarised potentials. The $Hcn1^{M294L}$ mouse recapitulates the seizure and behavioral phenotypes seen in patients. Electroretinogram (ERG) recordings from $Hcn1^{M294L}$ mice revealed a significant decrease in the photoreceptor sensitivity to light, as well as attenuated bipolar cell and retinal ganglion cell response amplitudes. $Hcn1^{M294L}$ mice were also unable to respond to high rates of flicker light. There was no impact of the variant on the structure of the eye or the expression of the Hcn1 protein in the retina. *In silico* modelling of photoreceptors revealed that the mutated HCN1 channel dramatically reduced light-induced hyperpolarision, decreasing the Ca^{2+} current amplitude change in response to light. Given the tight relationship between Ca^{2+} and transmitter release the dynamic range of the change of glutamate released from photoreceptors during a light stimulus will be significantly blunting. Patients with HCN1 pathogenic variants are likely to have a dramatically reduced sensitivity to light and a limited ability to process temporal information. Our results provide insights into the role HCN1 channels play in retinal function, as well as highlighting the need to consider retinal dysfunction in disease caused by HCN1 variants.

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ABSENCE-SEIZURE BLOCKADE RESCUES REM SLEEP IMPAIRMENT IN A RAT MODEL OF SYNGAP1 HAPLOINSUFFICIENCY

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

Ingrid Buller-Peralta[1], Katerina Hristova[1], Alejandro Bassi[2], Adrian Ocampo[2], Melissa Fasol[1], Sally Till[1], Peter Kind[1], Alfredo Gonzalez-Sulser[1]
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SYNGAP1 haploinsufficiency is one of the primary genetic causes of paediatric epilepsy and neurodevelopmental disorders, resulting in absence seizures, severe intellectual disability, autism and sleep disturbances. We previously found that a novel SYNGAP1 rat model replicates the primary epilepsy type in patients, absence seizures. Spontaneous spike and wave discharges (SWDs), the electrophysiological correlate of absence seizures were also blocked in *Syngap*$^{+/\Delta Gap}$ rats by acute treatment with the anti-epileptic drug ethosuximide (ETX). Here we assessed sleep impairments in *Syngap*$^{+/\Delta Gap}$ rats and tested whether seizure blockade rescued sleep abnormalities. *Syngap*$^{+/\Delta Gap}$ rats and wildtype littermates were implanted with a skull surface EEG array with neck EMG electrodes and chronically recorded through a wireless acquisition system. Circadian recordings were performed and animals were given acute ETX or saline.
Syngap$^{+/\Delta Gap}$ rats showed an increased number of SWDs through the whole circadian cycle coupled with shortened rapid eye movement (REM) sleep bouts, resulting in an overall REM decrease. *Syngap*$^{+/\Delta Gap}$ rats displayed less and longer wake and non-REM sleep bouts, although total minutes were equivalent between genotypes. ETX blocked SWDs in *Syngap*$^{+/\Delta Gap}$ rats during the estimated drug half-life time and reverted the REM sleep deficit by increasing the number of REM bouts. Non-REM sleep bout number and durations were also normalized although no effect was observed in wake. Our results suggest that seizure activity impairs sleep, although ETX may have a direct effect on sleep independent of SWDs. In future we will assess how seizures and decreased REM may contribute to social cognitive deficits in *SYNGAP1* haploinsufficiency.

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IMPACT OF AGING ON PENTYLENETETRAZOLE-INDUCED EPILEPSY IN RATS: POSSIBLE ROLE OF OXIDATIVE STRESS AND NRF2/HO-1 PATHWAY

POSTER SESSION 07 - SECTION: CLINICAL AND EXPERIMENTAL EPILEPSY MODELS

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Aim: To examine the impact of aging on the response of rats to PTZ-induced epilepsy and the possible role of oxidative stress and Nrf2/HO-1 pathway in this response. **Methods**: forty male albino rats were allocated into 4 equal groups; 1) normal young control (YC) group, aged 8-12 weeks, 2) normal old control (OC) group, aged 24 months, 3) PTZ-Young group: young rats received PTZ (50 mg/Kg, i.p. every other day) for 2 weeks and 4) PTZ-Old group: as group 3 but old rats. The latency for the first jerk and the seizure score were assessed in rats as well as markers of oxidative stress (MDA, catalase and total antioxidant capacity (TAC)) and the relative expression of Nrf2 and HO-1 at the level of mRNA in brain tissues were done at the end of experiment. **Results:** old rats showed early and significant increase in seizure score with PTZ administration and significant decrease in seizure latency compared to young rats (p<0.01). Also, the concentration of MDA was significantly higher, while TAC and catalase activity were significantly lower in old rats than young rats (p<0.01). Moreover, the expression of Nrf2 and HO-1 was significantly decreased in old normal rats compared to normal young rats with PTZ administration (p< 0.01). **Conclusion**: aging increases the vulnerabilities of rats to PTZ-induced epilepsy. This might be due to upregulation of oxidative stress and down regulation of the antioxidant genes including Nrf2 and HO-1.

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BEHAVIORAL ASSESSMENT OF THE PARKINSON'S DISEASE MOUSE MODEL OF HUMAN TYROSINASE OVEREXPRESSION IN THE LOCUS COERULEUS

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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The function of the locus coeruleus (LC) becomes impaired before the more prototypical degeneration of substantia nigra pars compacta during the pathogenesis of Parkinson's disease (PD). Despite the identification of some indicative symptoms during the early, prodromal stage of PD, such as REM sleep behavior disorder or anosmia, these are not sufficient for accurate diagnosis. Therefore, PD can only be diagnosed when the typical motor symptoms appear. At that stage, neuroprotective interventions are limited, since several dopaminergic neurons have already suffered irreversible damage or they are apoptotic. Our work was set out to develop early diagnostic functional biomarkers inspired by early circuit dysfunction of the LC network. We use the human tyrosinase overexpression mouse model as a proxy for progressive neuromelanin-induced neurodegeneration of the LC in mice. We present a behavioral evaluation of physiological fitness of the LC using a battery that includes the open field, marble burying, elevated plus maze and free locomotion tests. We implemented a hypothesis-free motif detection program (Variational Animal Motion Embedding / VAME) that detects subtle changes in locomotion that are otherwise extremely difficult to observe. We observed differences in locomotor activity between the tyrosinase-expressing group and the control group. We believe that neuronal circuit-derived psychophysical and behavioral tests implemented in slow progression mouse models of PD can contribute to the discovery of novel non-motor symptoms in human PD patients, which in turn will help to diagnose Parkinson's disease in early stages and expand the therapeutic window.

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CHARACTERIZATION OF A NOVEL GLUCOCEREBROSIDASE PHARMACOLOGICAL CHAPERONE IN VITRO AND IN VIVO MODELS OF ALPHA SYNUCLEIN NEUROTOXICITY

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

Antonino Iurato La Rocca[1], Giuseppe Ranieri[1], Elisabetta Gerace[1], Lorenzo Curti[1], Francesca Clemente[2], Camilla Matassini[2], Francesca Cardona[2], Andrea Goti[2], Amelia Morrone[1], Guido Mannaioni[1], Alessio Masi[1]
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Background: Parkinson's disease (PD) is characterized by the formation of proteinaceous aggregates of α-synuclein (α-syn) in cell bodies and neurites of *Substantia Nigra pars compacta* (SNpc) dopaminergic neurons, eventually causing cell death. Interestingly, defective activity or mutations in GBA1, the gene coding for lysosomal enzyme Glucocerebrosidase (GCase), are the strongest risk factor to develop PD. Based on this evidence, GCase may represent a new therapeutic target to oppose α-syn accumulation in PD. **Aim**: To evaluate the therapeutic potential of the novel GCase enhancer CF30 against the accumulation of toxic A53T α-syn species with *in vitro* and *in vivo* PD models. **Methods**: Chaperone-induced GCase enhancement was assayed in SHSY cell cultures and in the striatum of mice. To assess the impact of molecular chaperones on motor skills, neurochemical content and cellular pathology, behavioural, biochemical and histological analyses were performed after 3 weeks of a chronic treatment with CF30 in C57Bl6 mice following unilateral injection of a AAV1/2 α-synA53T into the SNpc. **Results:** CF30 is well tolerated at effective concentration/dosage in cell lines and in vivo. GCase activity is enhanced by, respectively, 40 and 18%. Plasma and brain kinetics indicate good oral availability and brain penetrance. Preliminary efficacy test on the A53T α-syn mouse model indicate that the compound does not reduce total α-syn accumulation in the striatum or in the SNpc. However, CF30 seems to reduce the ratio between pSer129 α-syn and total α-syn, suggesting that the compound may be effective to prevent deposition of α-syn aggregates.

Pubmed:
34785165: Ilari A, Curti L, Petrella M, Cannella N, La Rocca A, Ranieri G, Gerace E, Iezzi D, Silvestri L, Mannaioni G, Ciccocioppo R, Masi A
Moderate ethanol drinking is sufficient to alter Ventral Tegmental Area dopamine neurons activity via functional and structural remodeling of GABAergic transmission.
Earlier studies have shown a major involvement of Ventral Tegmental Area (VTA) dopamine (DA) neurons in mediating the rewarding effects of ethanol (EtOH). Much less is known on the role of this system in mediating the transition from moderate to excessive drinking and abuse. Here we sought to explore the hypothesis that early stage drinking in rodents, resembling recreational EtOH use in humans, is sufficient to dysregulate VTA DA transmission thus increasing the propensity to use over time. To this purpose, midbrain slice recordings in mice previously exposed to an escalating (3, 6 and 12%) 18-day voluntary EtOH drinking paradigm was used. By recording from DA and γ-aminobutyric acid (GABA) VTA neurons in midbrain slices, we found that moderate EtOH drinking leads to a significant suppression of the spontaneous activity of VTA DA neurons, while increasing their response to acute EtOH application. We also found that chronic EtOH leads to the enhancement of GABA input frequency onto a subset of DA neurons. Structurally, chronic EtOH induced a significant increase in the number of GABA axonal boutons contacting DA neurons, suggesting deep rewiring of the GABA network. This scenario is consistent with a downmodulation of the reward DA system induced by moderate EtOH drinking, a neurochemical state defined as "hypodopaminergic" and previously associated with advanced stages of drug use in humans. In this context, increased sensitivity of DA neurons towards acute EtOH may represent the neurophysiological correlate of increased unitary rewarding value, possibly driving progression to addiction.
Neuropharmacology, 2022; 203

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ALTERED RESTING-STATE FUNCTIONAL BRAIN NETWORK IN PARKINSON'S DISEASE WITH MAJOR AND MINOR VISUAL HALLUCINATION

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Visual hallucination (VH) is the most common psychosis in Parkinson’s disease (PD). Based on the symptoms, VH is categorized into major hallucination (MH) and minor hallucination (mH). Previous studies have reported alterations in resting-state networks (RSNs) underlying VH mechanisms, but differences between MH and mH are still elusive. In this regard, this study aims to investigate different RSNs in MH and mH in PD. 69 PD patients (23 MH, 22 mH, 24 without VH (nH)) were enrolled in this study. Seeds were selected for RSNs: LPC/PCC for default mode (DMN); TPJ for ventral and FEF/IPS for dorsal attention (VAN, DAN); V1 for visual (VN). Seed-to-voxel analysis is then conducted to examine intra-network relationships between the groups. To investigate inter-network among the groups, we performed ROI-to-ROI analysis for each pair of networks. In seed-based analysis, compared to nH, MH and mH patients demonstrated greater connectivity between the right LPC of DMN and the left frontal orbital cortex; decreased connectivity between right TPJ of VAN and precuneus cortex; between right FEF of DAN and posterior middle temporal gyrus and precuneus cortex. Inter-network of DMN-VAN connectivity exhibited significant increases in MH than mH. Moreover, DAN-VAN connectivity showed significant decreases in MH compared to nH. The present study demonstrated the disrupted attention networks, but the aberrant pattern in DMN in both MH and mH compared to controls. The result also indicates DMN and VAN are strongly wired in MH than mH. This study provides novel insights into the different mechanisms of major and minor hallucination.

Pubmed:
34762146: Jung JH, Kim YJ, Chung SJ, Yoo HS, Lee YH, Baik K, Jeong SH, Lee YG, Lee HS, Ye BS, Sohn YH, Jeong Y, Lee PH
White matter connectivity networks predict levodopa-induced dyskinesia in Parkinson's disease.
Although levodopa-induced dyskinesia-relevant white matter change has been evaluated, it is uncertain whether these changes may reflect the underlying predisposing conditions leading to the development of levodopa-induced dyskinesia.
J Neurol, 2022; 269

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BOARD NUMBER: S07-598

PROTEOMIC AND STEREOLOGICAL STUDY: SYNAPTIC INVOLVEMENT OF HUMAN AMYGDALA IN PARKINSON'S DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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[1]UNIV. CASTILLA-LA MANCHA, Facultad De Medicina De Ciudad Real, CIUDAD REAL, Spain, [2]UNIVERSIDAD DE CASTILLA-LA MANCHA, Facultad De Medicina De Ciudad Real, CIUDAD REAL, Spain

Emotional deficits are common symptoms in Parkinson's disease (PD). The amygdala involvement by α-synuclein (Braak stage 3) could constitute neural substrates underlying these manifestations. Differential α-synucleinopathy between amygdaloid nuclei has been described. MRI and VBM studies have revealed volumetric changes with conflicting results. Only one stereological study has analyzed neuronal population, but glial populations have been neglected. Likewise, proteomic analyses focused on amygdala in PD are lacking. The present work analyzed the area fraction occupied by Lewy bodies (LB), volumetric and glial changes and proteomic alterations in the amygdala in PD. Brains from 18 PD (Braak 5-6) and 18 non-PD age-matched subjects were obtained from Spanish Biobank network. Area fraction occupied by LB was analyzed by Area Fraction Fractionator. Cavalieri's method was used for volume estimation. Microglial and astroglial populations were estimated by Optical Fractionator. Dia-PASEF and MALDI were used for the proteomic study. No volumetric changes were detected and a homogeneous distribution of LB in the different amygdaloid nuclei were reported. No changes in the Iba-1 and GFAP density were either observed. Proteomic data revealed 17 up- and 24 downregulated proteins in the amygdala with PD. Although changes in cell populations have not been detected, functional impairment related to the amygdala in PD may be due to alterations at the proteomic level likely related to synapses, mitochondrial damage, and the immune response. This work is sponsored by the UCLM/ERDF (2021-GRIN-31233to NPND), the Spanish MEC/ERDF (SAF2016-75768-R) and MCI (PID2019-108659RB-I00) and the JCCM/ERDF (SBPLY/17/180501/000430). SVC and MGR held a fellowship UCLM/ESF.

Pubmed:
34092653: Villar-Conde S, Astillero-Lopez V, Gonzalez-Rodriguez M, Villanueva-Anguita P, Saiz-Sanchez D, Martinez-Marcos A, Flores-Cuadrado A, Ubeda-Bañon I
The Human Hippocampus in Parkinson's Disease: An Integrative Stereological and Proteomic Study.
Parkinson's disease (PD) is a prevalent neurodegenerative disease that is pathologically described as a six-stage α-synucleinopathy. In stage 4, α-synuclein reaches the hippocampus, inducing cognitive deficits, from which it progresses to the isocortex, leading to dementia. Among hippocampal fields, cornu ammonis 2 is particularly affected by this α-synucleinopathy and critical for cognitive decline. Volumetric studies using magnetic resonance imaging have produced controversial results, with only some reporting volume loss, whereas stereological data obtained using nonspecific markers do not reveal volume changes, neural or glial loss. Proteomic analysis has not been carried out in the hippocampus of patients with PD.
J Parkinsons Dis, 2021; 11

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O-CYCLIC PHYTOSPHINGOSINE-1-PHOSPHATE PROTECTS AGAINST NEURODEGENERATION IN THE PARKINSON'S DISEASE MOUSE MODEL

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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The present study was undertaken to unveil the potential neuroprotective effects of O-cyclic phytosphingosine-1-phosphate (cPS1P) against mouse models of Parkinson's disease (PD). cPS1P is a novel and chemically synthesized sphingosine metabolite derived from phytosphingosine-1-phosphate (PS1P). To unveil the protective effects of cPS1P against Parkinson's disease, we used MPTP and NSE-hαSyn Korl transgenic mice, MPTP was injected at a dose of 30mg/Kg/per day for 5 days, and the cPS1P 1mg/Kg/day intraperitoneally. We have used behavioral, biochemical, and immunofluorescence stainings for the analysis. We have used the open field test, wire hanging test, pole test, and rotarod test. The behavioral results suggested that in the PD-models there was a significant loss in the motor functions, which were reversed with the administration of cPS1P. Similarly, as hypothesized, in the MPTP and NSE-hαSyn Korl transgenic mice, the expression of the S1P1 receptor was significantly reduced, compared to the control group, which was enhanced in the cPS1P injected mice. Similarly, we checked the expression of inflammatory cytokines in the experimental mice brains, which suggested that in the MPTP and NSE-hαSyn Korl transgenic mice, the expression of these markers was significantly enhanced, compared to the control group. Interestingly, these markers were reversed in the cPS1P co-injected mice brains. The overall studies supported the notion that cPS1P may act as a specific agonist of S1PR1 which reduces the PD-associated symptoms in the preliminary animal's studies. (Supported by the Brain Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT 2020M3E5D9080660).

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BOARD NUMBER: S07-600

DETERMINATION OF THE NUMBER OF CNVS IN THE SNCA GENE BY DDPCR

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Digital droplet PCR (ddPCR) is an assay that combines state-of-the-art microfluidics technology with TaqMan-based PCR to achieve precise target DNA quantification. Point mutations and exon rearrangements (deletions, duplications and triplications) in PRKN, PINK1, DJ-1, LRRK2, and SNCA are the main causes of rare monogenic forms of Parkinson's disease (PD). Herein, we applied this technology 1) to validate screened copy number variations (CNVs) detected either by Multiplex Ligation Probe Assay (MLPA) or by targeted Next Generation Sequencing (NGS) technique and where available to conduct segregation analysis; 2) to accurately phase genomic variants in carriers of double heterozygous variants, in absence of familial co-segregation. We tested five PD patients with heterozygous SNCA multiplications detected either by MLPA (n = 2) or by custom gene panel (n = 3) and one patient without SNCA multiplications as a negative internal control. ddPPCR analysis revealed four patients with whole SNCA gene duplications and one patient with gene triplication, suggesting this technique rapid, reliable and sensitive. Phasing PRKN double heterozygous mutations carried by single PD patients using this ddPCR technique is under progress.

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THE ROLE OF ETHNO-RACIAL FACTORS IN ASSESSING RISK FOR PARKINSON'S DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

Sara Siddiqi[1], Juan Li[2], Kamaya Lawrence[1], Abigail Morris[1], Kim Matheson[1], Paul Villeneuve[1,3], Julianna Tomlinson[2], Michael Schlossmacher[2], Natalina Salmaso[1]
[1]Carleton University, Neuroscience, Ottawa, Canada, [2]University of Ottawa, Brain And Mind Research Institute, Ottawa, Canada, [3]Carleton University, School Of Mathematics And Statistics, Ottawa, Canada

Aims: Parkinson's Disease (PD) risk research includes factors from genetics to the environment. To date, there is a lack of large-scale multifactorial studies that investigate a definitive link between race/ethnicity and PD risk. We conducted a targeted literature review to quantify the inclusion of ethno-racial factors in the context of PD risk. Furthermore, we employed a dataset of human PD incidences to validate trends observed in the literature. **Methods:** We conducted a PubMed search including articles published in 2000-2020 with the following MeSH terms: PD/diagnosis/risk factors/incidence/epidemiology. Data visualizations of variables related to PD risk factors and race were completed using the *Fox Insight* database (https://foxinsight-info.michaeljfox.org/insight/explore/insight.jsp) on 04/07/2021. For up-to-date information on the study, visit https://foxinsight-info.michaeljfox.org/insight/explore/insight.jsp. **Results:** Out of 410 total articles, only 5% accounted for ethno-racial factors as an integral part of analysis. A few studies identified significant differences in PD incidence whereby African Americans are less likely to be diagnosed with PD than Caucasian individuals. Data analyses indicated PD diagnosis was associated with race, and male sex was associated with an increased risk of PD, regardless of race. **Conclusions:** Differences in PD incidence across race/ethnicity may be related to a combination of factors including access to health care, genetics and environmental factors. These findings highlight the need for further PD studies with diverse cohorts. **Acknowledgements:** The Fox Insight Study (FI) is funded by The Michael J. Fox Foundation for Parkinson's Research. We would like to thank the Parkinson's community for participating in this study to make this research possible.

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BOARD NUMBER: S07-602

ESTABLISHING THE VALIDITY OF A NOVEL PERCEPTUAL TASK FOR PARKINSON DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Aims: This study aimed to establish the validity of a novel perceptual paradigm designed to optimize detection of spatial deficits in Parkinson disease (PD) segregated from movement deficits. Discriminant analyses determined which perceptual tasks best differentiated between PD patients and healthy controls. **Methods:** Study participants (n=141; mean age=66) included non-demented PD patients (n=99; H&Y<2.5; RMO=53, LMO=46) and healthy controls (n=42) with similar male to female ratios (PD 70/29; C 25/17). Perceptual accuracy of interval spaces of three horizontal lines (width interval) and two line lengths in vertical and horizontal orientations (length equivalence) were quantified through verbal responses of the same or different. A custom program (Neurobehavioral systems) presented 4 second stimulus pairs for comparison across 48 trials randomized for line lengths (4, 8, 12 cm) and discrimination difficulty. **Results:** Discriminant function analyses revealed that perceptual accuracy correctly classified 85.9% of PD patients based on length equivalence (p=.036) for 4 (p=.037) and 12 cm lines (p=.002), and 4 cm width interval estimations (p<.001). Conversely, 84.9% of RMO PD patients were correctly classified by width interval estimations (p=.029) at 4 and 12 cm (p<.02), and length equivalence at 12 cm (p=.006). In contrast, perceptual accuracy was not reliable in classifying LMO patients and healthy controls. **Conclusions:** Overall, the findings confirm the presence of visuoperceptual impairment early in the disease process separate from movement deficits, and highlights the need to validate sensitive tests of spatial accuracy specifically tailored to capture PD heterogeneity and improve prediction of risk for cognitive decline.

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PARKINSON´S DISEASE AND DBS: THE INFLUENCE OF GENDER IN COGNITIVE OUTPUTS

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

Cristina Nombela Otero[1], Jennifer Jiménez[2], Gustavo Fernández Pajarín[3], Ángel Sesar[3], Isabel Jimenez[3], Juan Martín-Rodriguez[4], Fernando Alonso-Frech[5]
[1]Universidad Autónoma de Madrid, Biological And Health Psychology, Madrid, Spain, [2]Asociación Parkinson Madrid, Innovation And Research, Madrid, Spain, [3]Complejo Hospitalario de Santiago, Neurología, Santiago de Compostela, Spain, [4]Instituto de Biomedicina de Sevilla, Trastornos Del Movimiento, Sevilla, Spain, [5]Hospital Clínico San Carlos, Neurología, Madrid, Spain

Aims. The action mechanism of Deep Brain Stimulation (DBS) points at restoring the dysbalanced neuronal networks in Basal Ganglia. In general terms, patients with Parkinson´s disease (PD) may impair cognitive performance after surgery. Recent gender studies on PD indicate that the preliminary symptoms and subsequent ones may vary between genders. Most PD patients undergoing DBS (PD-DBS) are male, and retrospective studies showed a differential effect on cognition outputs after DBS. We hypothesize that women will benefit from DBS more than men on a cognitive basis, whereas males may increase the cognitive deficits assessed at baseline. Methods. A sample of 20 DBS-PD patients (6 female and fourteen male) underwent DBS surgery at the Hospital Clínico San Carlos. We conducted a retrospective analysis of published data (Avecillas-Chasín et al., 2019), including the gender variable with baseline (presurgery) and two years after surgery data (follow-up). Results Data showed that female patients benefit more than men from the therapy, presenting higher scores in general cognitive assessments questionnaires (MEC -Mini Examen Cognoscitivo-, and Mattis Dementia Rating Scale) at follow-up. Male scored lower on specific neuropsychological scales (Verbal free recall of The Hopkins Verbal Learning Test, and speed processing of TMT-A test, among others. In the following months, new data will be added to the sample. Conclusions. Gender may be mediating the differential effect described in DBS outputs. The analysis of these differences would shed some light on understanding the disorder and how to treat it.

Pubmed:
34362061: Martín-Brufau R, Gómez MN, Sanchez-Sanchez-Rojas L, Nombela C
Fibromyalgia Detection Based on EEG Connectivity Patterns.
The identification of a complementary test to confirm the diagnosis of FM. The diagnosis of fibromyalgia (FM) is based on clinical features, but there is still no consensus, so patients and clinicians might benefit from such a test. Recent findings showed that pain lies in neuronal bases (pain matrices) and, in the long term, chronic pain modifies the activity and dynamics of brain structures. Our hypothesis is that patients with FM present lower levels of brain activity and therefore less connectivity than controls.
J Clin Med, 2021; 10
34040507: Nombela C, Fernández-Egea E, Giné E, Worbe Y, Del Río-Hortega Bereciartu J, de Castro F
Women Neuroscientist Disciples of Pío del Río-Hortega: the Cajal School Spreads in Europe and South America.
Pio del Rio-Hortega was not only the discoverer of the microglia and oligodendroglia but also possibly the most prolific mentor of all Santiago Ramon y Cajal's disciples (Nobel awardee in Physiology or Medicine 1906 and considered as the father of modern Neuroscience). Among Río-Hortega's mentees, three exceptional women are frequently forgotten, chronologically: Pio's niece Asunción Amo del Río who worked with Río-Hortega at Madrid, Paris, and Oxford; the distinguished British neuropathologist Dorothy Russell who also worked with Pío at Oxford; and Amanda Pellegrino de Iraldi, the last mentee in his career. Our present work analyzes the figures of these three women who were in contact and collaborated with Pío del Río-Hortega, describing the influences received and the impact on their careers and the History of Neuroscience. The present work completes the contribution of women neuroscientists who worked with Cajal and his main disciples of the Spanish Neurological School both in Spain (previous work) and in other countries (present work).
Front Neuroanat, 2021; 15
31379519: Giné E, Martínez C, Sanz C, Nombela C, de Castro F
The Women Neuroscientists in the Cajal School.
At the beginning of the 20th century, in view of the growing international recognition of Santiago Ramón y Cajal, the Spanish

authorities took some important steps to support Cajal's scientific work. This recognition peaked in 1906, when Camillo Golgi and Santiago Ramón y Cajal shared the Nobel Prize in Physiology or Medicine. The Spanish government provided Cajal a state-of-the-art laboratory in Madrid to allow him to continue with his research and they funded salaries to pay his first tenured collaborators, the number of which increased further after the creation of the . The was an organism set up to help promising researchers develop their careers in different ways, thereby contributing to the development of science in Spain. Although largely forgotten or relatively unknown, there has been a recent revival in the recognition of the school that developed around Cajal, collectively referred to as the Spanish Neurological School (or colloquially, as the Cajal School or School of Madrid). Almost all Cajal's collaborators were men, although a limited number of female scientists spent part of their careers at the heart of the Cajal School. Here we discuss these women and their work in the laboratory in Madrid. We have tracked the careers of Laura Forster (from Australia/United Kingdom), Manuela Serra, María Soledad Ruiz-Capillas and María Luisa Herreros (all Spanish), through their scientific publications, both in the journal founded by Cajal and elsewhere, and from other documentary sources. To complete the picture, we also outline the careers of other secondary figures that contributed to the production and running of Cajal's laboratory in Madrid. We show here that the dawn of Spanish neuroscience included a number of contributions from female researchers who to date, have received little recognition.
Front Neuroanat, 2019; 13

26971528: Breen DP, Nombela C, Vuono R, Jones PS, Fisher K, Burn DJ, Brooks DJ, Reddy AB, Rowe JB, Barker RA
Hypothalamic volume loss is associated with reduced melatonin output in Parkinson's disease.
Recent studies have suggested that melatonin-a hormone produced by the pineal gland under circadian control-contributes to PD-related sleep dysfunction. We hypothesized that degenerative changes to the neural structures controlling pineal function (especially the suprachiasmatic nuclei of the anterior hypothalamus) may be responsible for reduced melatonin output in these patients. We compared hypothalamic volumes in PD patients with matched controls and determined whether volume loss correlated with reduced melatonin output in the PD group.
Mov Disord, 2016; 31

25080285: Nombela C, Rowe JB, Winder-Rhodes SE, Hampshire A, Owen AM, Breen DP, Duncan GW, Khoo TK, Yarnall AJ, Firbank MJ, Chinnery PF, Robbins TW, O'Brien JT, Brooks DJ, Burn DJ, , Barker RA
Genetic impact on cognition and brain function in newly diagnosed Parkinson's disease: ICICLE-PD study.
Parkinson's disease is associated with multiple cognitive impairments and increased risk of dementia, but the extent of these deficits varies widely among patients. The ICICLE-PD study was established to define the characteristics and prevalence of cognitive change soon after diagnosis, in a representative cohort of patients, using a multimodal approach. Specifically, we tested the 'Dual Syndrome' hypothesis for cognitive impairment in Parkinson's disease, which distinguishes an executive syndrome (affecting the frontostriatal regions due to dopaminergic deficits) from a posterior cortical syndrome (affecting visuospatial, mnemonic and semantic functions related to Lewy body pathology and secondary cholinergic loss). An incident Parkinson's disease cohort (n = 168, median 8 months from diagnosis to participation) and matched control group (n = 85) were recruited to a neuroimaging study at two sites in the UK. All participants underwent clinical, neuropsychological and functional magnetic resonance imaging assessments. The three neuroimaging tasks (Tower of London, Spatial Rotations and Memory Encoding Tasks) were designed to probe executive, visuospatial and memory encoding domains, respectively. Patients were also genotyped for three polymorphisms associated with cognitive change in Parkinson's disease and related disorders: (i) rs4680 for COMT Val158Met polymorphism; (ii) rs9468 for MAPT H1 versus H2 haplotype; and (iii) rs429358 for APOE-ε2, 3, 4. We identified performance deficits in all three cognitive domains, which were associated with regionally specific changes in cortical activation. Task-specific regional activations in Parkinson's disease were linked with genetic variation: the rs4680 polymorphism modulated the effect of levodopa therapy on planning-related activations in the frontoparietal network; the MAPT haplotype modulated parietal activations associated with spatial rotations; and APOE allelic variation influenced the magnitude of activation associated with memory encoding. This study demonstrates that neurocognitive deficits are common even in recently diagnosed patients with Parkinson's disease, and that the associated regional brain activations are influenced by genotype. These data further support the dual syndrome hypothesis of cognitive change in Parkinson's disease. Longitudinal data will confirm the extent to which these early neurocognitive changes, and their genetic factors, influence the long-term risk of dementia in Parkinson's disease. The combination of genetics and functional neuroimaging provides a potentially useful method for stratification and identification of candidate markers, in future clinical trials against cognitive decline in Parkinson's disease.
Brain, 2014; 137

24867024: Nombela C, Nombela M, Castell P, García T, López-Coronado J, Herrero MT
Alpha-theta effects associated with ageing during the Stroop test.
The Stroop effect is considered as a standard attentional measure to study conflict resolution in humans. The response of the brain to conflict is supposed to change over time and it is impaired in certain pathological conditions. Neuropsychological Stroop test measures have been complemented with electroencephalography (EEG) techniques to evaluate the mechanisms

in the brain that underlie conflict resolution from the age of 20 to 70. To study the changes in EEG activity during life, we recruited a large sample of healthy subjects of different ages that included 90 healthy individuals, divided by age into decade intervals, which performed the Stroop test while recording a 14 channel EEG. The results highlighted an interaction between age and stimulus that was focused on the prefrontal (Alpha and Theta band) and Occipital (Alpha band) areas. We concluded that behavioural Stroop interference is directly influenced by opposing Alpha and Theta activity and evolves across the decades of life.
PLoS One, 2014; 9
24465678: Nombela C, Rittman T, Robbins TW, Rowe JB
Multiple modes of impulsivity in Parkinson's disease.
Cognitive problems are a major factor determining quality of life of patients with Parkinson's disease. These include deficits in inhibitory control, ranging from subclinical alterations in decision-making to severe impulse control disorders. Based on preclinical studies, we proposed that Parkinson's disease does not cause a unified disorder of inhibitory control, but rather a set of impulsivity factors with distinct psychological profiles, anatomy and pharmacology. We assessed a broad set of measures of the cognitive, behavioural and temperamental/trait aspects of impulsivity. Sixty adults, including 30 idiopathic Parkinson's disease patients (Hoehn and Yahr stage I-III) and 30 healthy controls, completed a neuropsychological battery, objective behavioural measures and self-report questionnaires. Univariate analyses of variance confirmed group differences in nine out of eleven metrics. We then used factor analysis (principal components method) to identify the structure of impulsivity in Parkinson's disease. Four principal factors were identified, consistent with four different mechanisms of impulsivity, explaining 60% of variance. The factors were related to (1) tests of response conflict, interference and self assessment of impulsive behaviours on the Barrett Impulsivity Scale, (2) tests of motor inhibitory control, and the self-report behavioural approach system, (3) time estimation and delay aversion, and (4) reflection in hypothetical scenarios including temporal discounting. The different test profiles of these four factors were consistent with human and comparative studies of the pharmacology and functional anatomy of impulsivity. Relationships between each factor and clinical and demographic features were examined by regression against factor loadings. Levodopa dose equivalent was associated only with factors (2) and (3). The results confirm that impulsivity is common in Parkinson's disease, even in the absence of impulse control disorders, and that it is not a unitary phenomenon. A better understanding of the structure of impulsivity in Parkinson's disease will support more evidence-based and effective strategies to treat impulsivity.
PLoS One, 2014; 9
24363137: Yarnall AJ, Breen DP, Duncan GW, Khoo TK, Coleman SY, Firbank MJ, Nombela C, Winder-Rhodes S, Evans JR, Rowe JB, Mollenhauer B, Kruse N, Hudson G, Chinnery PF, O'Brien JT, Robbins TW, Wesnes K, Brooks DJ, Barker RA, Burn DJ,
Characterizing mild cognitive impairment in incident Parkinson disease: the ICICLE-PD study.
To describe the frequency of mild cognitive impairment (MCI) in Parkinson disease (PD) in a cohort of newly diagnosed incident PD cases and the associations with a panel of biomarkers.
Neurology, 2014; 82
24012774: Nombela C, Hughes LE, Owen AM, Grahn JA
Into the groove: can rhythm influence Parkinson's disease?
Previous research has noted that music can improve gait in several pathological conditions, including Parkinson's disease, Huntington's disease and stroke. Current research into auditory-motor interactions and the neural bases of musical rhythm perception has provided important insights for developing potential movement therapies. Specifically, neuroimaging studies show that rhythm perception activates structures within key motor networks, such as premotor and supplementary motor areas, basal ganglia and the cerebellum - many of which are compromised to varying degrees in Parkinson's disease. It thus seems likely that automatic engagement of motor areas during rhythm perception may be the connecting link between music and motor improvements in Parkinson's disease. This review seeks to describe the link, address core questions about its underlying mechanisms, and examine whether it can be utilized as a compensatory mechanism.
Neurosci Biobehav Rev, 2013; 37
23400501: Nombela C, Rae CL, Grahn JA, Barker RA, Owen AM, Rowe JB
How often does music and rhythm improve patients' perception of motor symptoms in Parkinson's disease?
J Neurol, 2013; 260

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BOARD NUMBER: S07-604

EXAMINATION OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE IN PARKINSON'S DISEASE FOCUSING ON CORRELATIONS WITH MOTOR SYMPTOMS

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

Andrea Dr. Tamas[1], Daniel Pham[1], Beata Polgar[2], Tunde Toth[1], Adel Jungling[1], Balazs Daniel Fulop[1], Norbert Kovacs[3], Endre Pal[3], Istvan Balas[4], Dora Szabo[1], Robert Herczeg[5], Attila Gyenesei[5], Zalan Szanto[6], Dora Reglodi[1]
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The neuroprotective effects of pituitary adenylate cyclase-activating polypeptide (PACAP) have been shown in numerous in vitro and in vivo models of Parkinson's disease (PD) supporting the theory that PACAP could have an important role in the pathomechanism of the disorder affecting mostly older patients. Earlier studies found changes in PACAP levels in neurological disorders, therefore, the aim of our study was to examine PACAP in plasma samples of PD patients. Peptide levels were measured with ELISA and correlated with clinical parameters, age, stage of the disorder based on the Hoehn and Yahr (HY) scale, subtype of the disease, treatment and specific scores measuring motor and non-motor symptoms, such as movement disorder society-unified Parkinson's disease rating scale(MDS-UPDRS), Epworth sleepiness scale(ESS), Parkinson's disease sleep scale(PDSS-2) and Beck-depression inventory(BDI). Our results showed significantly decreased PACAP levels in PD patients without deep brain stimulation (DBS) therapy and in akinetic-rigid subtype, additionally we also described further decrease in the HY stage 3 and 4. Elevated PACAP levels were found in patients with DBS. There were no significant correlations in PACAP level with MDS-UPDRS, type of pharmacological treatment, PDSS-2 sleepiness and depression (BDI) scales, but we found increased PACAP level in patients with more severe sleepiness problems based on the ESS scale. Based on these results we suggest that following the alterations of PACAP with other frequently used clinical biomarkers in PD patients might improve strategic planning of further therapeutic interventions and help to provide a clearer prognosis regarding the future perspective of the disease.

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SERUM MYELOPEROXIDASE, BUT NOT THE CEREBROSPINAL-FLUID ENZYME, IS CLOSELY LINKED TO CLINICAL FEATURES AND NEURONAL DAMAGE IN IDIOPATHIC PARKINSON´S DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Introduction. Myeloperoxidase (MPO) has been implicated in the development of Parkinson´s disease. To date, no study has examined how MPO level in biofluids relates to motor and non-motor features of idiopathic PD. The objective of this study was to look at the relationship of MPO concentration in serum and cerebrospinal fluid (CSF) with clinical variables of the disease. Methods. MPO concentration and activity were measured. The degree of nigrostriatal dopaminergic cell loss was evaluated with single-photon emission computed tomography (SPECT) with ^{123}I-Ioflupane, specific radioligand of the dopamine transporter (DAT). Results. MPO is active in serum, not in the CSF. Serum MPO concentration, not CSF one, was significantly higher in the patients ($p<.0001$ vs controls). Significant correlation values were found between serum MPO concentration and rating scales of motor severity (Hoehn-Yahr staging; motor MDS-UPDRS part III), and percentage reduction of DAT binding on basal ganglia ($p<.0001$). Conclusions. Serum myeloperoxidase, but not CSF MPO, is active and is increased in PD patients. Serum MPO level, not CSF one, correlates with motor severity degree and loss of dopamine-transporter binding on basal ganglia, an indirect measure of neuronal death in the *substantia nigra*. These results would allow improving diagnosis of PD, and it opens new avenues for treatment based on serum myeloperoxidase..

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BLAMING NEUROMELANIN FOR PARKINSON'S DISEASE: TIME-DEPENDENT TYROSINASE OVEREXPRESSION DRIVES ENDOGENOUS SYNUCLEINOPATHY IN NONHUMAN PRIMATES

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Although neuromelanin (NMel) is a dark pigment characteristic of dopaminergic neurons in the human substantia nigra pars compacta (SNpc), its potential role in the pathogenesis of Parkinson's disease (PD) has been neglected since most commonly used laboratory animals lack NMel. Here we took advantage of AAVs encoding tyrosinase for driving a time-dependent NMel accumulation within the SNpc in macaques up to similar levels as observed in elderly humans. Furthermore, NMel accumulation induced (i) an endogenous synucleinopathy mimicking intracellular inclusions typically observed in PD, (ii) a progressive degeneration of NMel-expressing dopaminergic neurons, and (iii) a pro-inflammatory phenotype mediated by activated microglial cells and perivascular macrophages. Moreover, Lewy body-like intracellular inclusions were observed in brain areas receiving dopaminergic innervation, supporting a prionoid spread of endogenous synucleinopathy by permissive trans-synaptic templating. In summary, the conducted strategy resulted in the characterization and validation of a new macaque model of PD matching the known neuropathology of this disorder with unprecedented accuracy. Finally, evidence was provided showing that intracellular aggregation of endogenous alpha-synuclein is triggered by NMel accumulation, therefore any therapeutic approach intended to decrease NMel levels may provide appealing choices for the successful implementation of novel PD therapeutics.

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ETHANOL EXTRACT FROM WITHERINGIA COCCOLOBOIDES DECREASES α-SYNUCLEIN AGGREGATE IN CAENORHABDITIS ELEGANS STRAIN NL5901

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Neurodegenerative diseases (ND) are a public health problem worldwide, these cause loss of healthy years, are permanent incapacitating and generate high costs in health systems. People affected with Parkinson´s disease have protein deposits of α- synuclein (α-syn) in brain cells. The use of biological models such as Caenorhabditis elegans (C. elegans) can answer questions that arise every day for the development of new therapeutic targets. Witheringia coccoloboides (W.coccoloboides) has been described as a promising plant in the palliative treatment of ND due to its neuroprotective action. Therefore, the main **objective** of this study was to evaluate the effect of the ethanolic extract of W. coccoloboides on protein aggregates of α-syn in the transgenic strain NL5901 of C. elegans, what it is characterized by the presence of α-syn aggregates labeled with yellow fluorescent protein (YFP), which allows evaluating the effect of compounds on phenotypic properties and the protein aggregates of α-syn. The **results** show that the ethanolic extract of W.coccoloboides leaves, has a reducing effect in α-syn protein aggregates, improving the physiological characteristics of reproduction and motility, decreasing the levels of ROS in the strain NL 5901. Therefore, it is possible to suggest that ethanolic extract of W. coccoloboides leaves has a protective effect on strain NL 5901, probably attributed to the presence of flavonoids and terpenes, which in turn generates a recovery of the muscular activity in the egg laying and locomotion, all probably related to the synergistic interaction of phytocompounds such as sterols and/or terpenes, flavonoids and alkaloids.

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FLUORESCENCE LIFETIME IMAGING OF PATHOLOGICAL CHANGES ASSOCIATED WITH PARKINSON'S DISEASE IN MOUSE DUODENAL WHOLEMOUNT TISSUES

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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AIMS: The onset of Parkinson's disease (PD) is believed to be localized to peripheral organs up to 10-15 years before the involvement in the central nervous system (CNS). This wide "time window" provides us the opportunity to identify pathological changes, for example in the gastrointestinal tract (GIT) tissues, even before they reach CNS. We focused on optimizing a combination of two advanced microscopic methods: fluorescence lifetime imaging (FLIM) analysis and wholemount tissue staining to detect early alpha-synuclein-related pathology in the GIT, and identifying specific structures (mucosal, submucosal or muscle layers) in which these changes occur. METHODS: We focused on an early detection of the pathologically aggregated proteins in GIT tissues. PD-related pathology in a mouse model was induced by oral administration of rotenone. Duodenal tissues were collected after 0,4,6,8,10,12-week of rotenone treatment. Wholemount tissues were stained with Thioflavin S (ThS), a routinely used fluorescent probe to detect aggregated proteins and analysed by FLIM. RESULTS: Our experiments showed a gradual increase in ThS fluorescence in the range of 800-1000ps in mouse duodenal tissues depending on the length of rotenone application. We recorded a slight increase in ThS fluorescence in this interval after 6 weeks of rotenone application and this effect increased after 10 weeks. Similar effects in controls were not observed. CONCLUSIONS: Based on our results, FLIM analysis appears to be suitable even for complex samples as wholemount GIT tissue, as well as to be sufficiently sensitive and appropriate to detect gradual pathological changes associated with the formation of aggregated protein structures.

Pubmed:
32625058: Harsanyiova J, Buday T, Kralova Trancikova A
Parkinson's Disease and the Gut: Future Perspectives for Early Diagnosis.
Parkinson's disease (PD) is a neurodegenerative disease characterized by progressive degeneration of dopaminergic neurons, and at the cellular level by the formation of Lewy bodies in the central nervous system (CNS). However, the onset of the disease is believed to be localized to peripheral organs, particularly the gastrointestinal tract (GIT) and the olfactory bulb sooner before neuropathological changes occur in the CNS. Patients already in the pre-motor stage of PD suffer from various digestive problems and/or due to significant changes in the composition of the intestinal microbiome in this early stage of the disease. Detailed analyses of patient biopsies and autopsies as well as animal models of neuropathological changes characteristic of PD provided important information on the pathology or treatment of PD symptoms. However, presently is not clarified where the pathological processes associated with PD is initiated; (by which these processes are disseminated to the CNS or other tissues within the GIT; and (which neuropathological changes could also serve as a , or (would be the most appropriate choice for routine examination of patient biopsies.
Front Neurosci, 2020; 14
33212934: Fricova D, Harsanyiova J, Kralova Trancikova A
Alpha-Synuclein in the Gastrointestinal Tract as a Potential Biomarker for Early Detection of Parkinson's Disease.
The primary pathogenesis associated with Parkinson's disease (PD) occurs in peripheral tissues several years before the onset of typical motor symptoms. Early and reliable diagnosis of PD could provide new treatment options for PD patients and improve their quality of life. At present, however, diagnosis relies mainly on clinical symptoms, and definitive diagnosis is still based on postmortem pathological confirmation of dopaminergic neuronal degeneration. In addition, the similarity of the clinical, cognitive, and neuropathological features of PD with other neurodegenerative diseases calls for new biomarkers, suitable for differential diagnosis. Alpha-synuclein (α-Syn) is a potential PD biomarker, due to its close connection with the pathogenesis of the disease. Here we summarize the currently available information on the possible use of α-Syn as a biomarker of early stages of PD in gastrointestinal (GI) tissues, highlight its potential to distinguish PD and other

neurodegenerative diseases, and suggest alternative methods (primarily developed for other tissue analysis) that could improve α-Syn detection procedures or diagnostic methods in general.
Int J Mol Sci, 2020; 21
33418977: Maronek M, Gromova B, Liptak R, Konecna B, Pastorek M, Cechova B, Harsanyova M, Budis J, Smolak D, Radvanszky J, Szemes T, Harsanyiova J, Kralova Trancikova A, Gardlik R
Extracellular DNA Correlates with Intestinal Inflammation in Chemically Induced Colitis in Mice.
Circulating extracellular DNA (ecDNA) is known to worsen the outcome of many diseases. ecDNA released from neutrophils during infection or inflammation is present in the form of neutrophil extracellular traps (NETs). It has been shown that higher ecDNA concentration occurs in a number of inflammatory diseases including inflammatory bowel disease (IBD). Enzymes such as peptidyl arginine deiminases (PADs) are crucial for NET formation. We sought to describe the dynamics of ecDNA concentrations and fragmentation, along with NETosis during a mouse model of chemically induced colitis. Plasma ecDNA concentration was highest on day seven of dextran sulfate sodium (DSS) intake and the increase was time-dependent. This increase correlated with the percentage of cells undergoing NETosis and other markers of disease activity. Relative proportion of nuclear ecDNA increased towards more severe colitis; however, absolute amount decreased. In colon explant medium, the highest concentration of ecDNA was on day three of DSS consumption. Early administration of PAD4 inhibitors did not alleviate disease activity, but lowered the ecDNA concentration. These results uncover the biological characteristics of ecDNA in IBD and support the role of ecDNA in intestinal inflammation. The therapeutic intervention aimed at NETs and/or nuclear ecDNA has yet to be fully investigated.
Cells, 2021; 10
29306061: Hennel M, Harsanyiova J, Ru F, Zatko T, Brozmanova M, Trancikova A, Tatar M, Kollarik M
Structure of vagal afferent nerve terminal fibers in the mouse trachea.
The structure of primary afferent nerve terminals profoundly influences their function. While the complex vagal airway nerve terminals (stretch receptors, cough receptors and neuroepithelial bodies) were thoroughly characterized, much less is known about the structure of airway nerves that do not form distinct complex terminals (often termed free nerve fibers). We selectively induced expression of GFP in vagal afferent nerves in the mouse by transfection with AAV-GFP virus vector and visualized nerve terminals in the trachea by whole organ confocal imaging. Based on structural characteristics we identified four types of vagal afferent nerve fiber terminals in the trachea. Importantly, we found that distinct compartments of tracheal tissue are innervated by distinct nerve fiber terminal types in a non-overlapping manner. Thus, separate terminal types innervate tracheal epithelium vs. anterolateral tracheal wall containing cartilaginous rings and ligaments vs. dorsal wall containing smooth muscle. Our results will aid the study of structure-function relationships in vagal airway afferent nerves and regulation of respiratory reflexes.
Respir Physiol Neurobiol, 2018; 249
33464920: Plevkova J, Brozmanova M, Harsanyiova J, Sterusky M, Honetschlager J, Buday T
Various aspects of sex and gender bias in biomedical research.
The main role of research in medicine is to provide relevant knowledge which, after successful translation to clinical practice, improves the quality of healthcare. The sex bias which is still present in the majority of research disciplines prefers male subjects despite legislation changes in the US grant agencies and European research programme Horizon 2020. Male subjects (cells, animals) still dominate in preclinical research and it has detrimental consequences for women's health and the quality of science. Opposite bias exists for data obtained mainly in animal models utilizing female subjects (e.g. research in multiple sclerosis, osteoporosis) with skewed outcomes for men affected by these diseases. Either way, scientists are producing results which compromise half of the population. Assumptions that females as cohorts are more variable and another assumption that the oestrous cycle should be tracked in case the females are enrolled in preclinical studies were proven wrong. Variability of male versus female cohorts are comparable and do not only stem from hormonal levels. The widespread prevalence of sex differences in human diseases ultimately requires detailed experiments performed on both sexes, unless the studies are specifically addressing reproduction or sex-related behaviors.
Physiol Res, 2020; 69
31468191: Harsanyiova J, Ru F, Zatko T, Kollarik M, Hennel M
Vagus Nerves Provide a Robust Afferent Innervation of the Mucosa Throughout the Body of the Esophagus in the Mouse.
The vagal afferent nerves regulate swallowing and esophageal motor reflexes. However, there are still gaps in the understanding of vagal afferent innervation of the esophageal mucosa. Anatomical studies found that the vagal afferent mucosal innervation is dense in the upper esophageal sphincter area but rare in more distal segments of the esophagus. In contrast, electrophysiological studies concluded that the vagal afferent nerve fibers also densely innervate mucosa in more distal esophagus. We hypothesized that the transfection of vagal afferent neurons with adeno-associated virus vector encoding green fluorescent protein (AAV-GFP) allows to visualize vagal afferent nerve fibers in the esophageal mucosa in the mouse. AAV-GFP was injected into the vagal jugular/nodose ganglia in vivo to sparsely label vagal afferent nerve fibers.

The esophageal tissue was harvested 4-6 weeks later, the GFP signal was amplified by immunostaining, and confocal optical sections of the entire esophagi were obtained. We found numerous GFP-labeled fibers in the mucosa throughout the whole body of the esophagus. The GFP-labeled mucosal fibers were located just beneath the epithelium, branched repeatedly, had mostly longitudinal orientation, and terminated abruptly without forming terminal structures. The GFP-labeled mucosal fibers were concentrated in random areas of various sizes in which many fibers could be traced to a single parental axon. We conclude that the vagus nerves provide a robust afferent innervation of the mucosa throughout the whole body of the esophagus in the mouse. Vagal mucosal fibers may contribute to the sensing of intraluminal content and regulation of swallowing and other reflexes.
Dysphagia, 2020; 35

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ORAL NANO-DELIVERY OF NASCO POMACES EXTRACT EXERTS ANTI-INFLAMMATORY EFFECTS IN THE 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) MOUSE MODEL OF PARKINSON'S DISEASE.

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Persistent neuroinflammation is considered a main event implicated in the neuropathology of Parkinson's Disease (PD). Grape pomaces, a waste by-products of wine production, are rich in polyphenols and have demonstrated antioxidant properties both *in vitro* and *in vivo* animal models of neurodegenerative diseases. Nonetheless, their oral use is limited by their low brain bioavailability and extensive first-passage metabolism. To overcome these limitations, in the present study we incorporated grape pomaces extract from *Vitis vinifera* Nasco into nutriosome (Nasco nutriosome), a novel nanovesicle system composed of the S75 phospholipid and the maltodextrin Nutriose® FM06. To investigate the anti-inflammatory properties of Nasco nutriosome in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD, we administrated Nasco nutriosome intragastrically in mice that were treated repeatedly with MPTP. Then, we evaluated in the Caudate-Putamen (CPu) and the substantia nigra compacta (SNc), two brain areas highly affected in PD, the immunoreactivity for the glial fibrillary acidic protein (GFAP), as marker of astroglia, and for the ionized calcium-binding adaptor molecule 1 (IBA1), as marker of microglia. Additionally, we co-localize the pro-inflammatory interleukin (IL)-1β with IBA1, to gain additional information about the microglia phenotype. Immunohistochemical analyses revealed that administration Nasco nutriosome significantly counteracted both microgliosis in the CPu, and the astrogliosis in the SNc and CPu, in mice receiving repeated MPTP treatment. Additionally, in the CPu, Nasco nutriosome treatment effectively reduced the production of IL-1β in IBA1 (+) cells. Altogether, these results highlight the promising anti-inflammatory effects exerted by Nasco nutriosome treatment in the preclinical MPTP-mouse model of PD.

Pubmed:

35088095: Maccioni R, Serra M, Marongiu J, Cottiglia F, Maccioni E, Bassareo V, Morelli M, Kasture SB, Acquas E
Effects of docosanyl ferulate, a constituent of Withania somnifera, on ethanol- and morphine-elicited conditioned place preference and ERK phosphorylation in the accumbens shell of CD1 mice.
Docosanyl ferulate (DF) is a behaviourally active GABA receptor complex (GABAR) agonist, recently isolated from the standardized methanolic extract of Withania somnifera Dunal (WSE) root. Previous studies have shown that WSE prevents both ethanol- and morphine-dependent acquisition and expression of conditioned place preference (CPP) and stimulation of dopamine release in the nucleus accumbens shell (AcbSh).
Psychopharmacology (Berl), 2022; 239
34512343: Costa G, Caputi FF, Serra M, Simola N, Rullo L, Stamatakos S, Sanna F, Germain M, Martinoli MG, Candeletti S, Morelli M, Romualdi P
Activation of Antioxidant and Proteolytic Pathways in the Nigrostriatal Dopaminergic System After 3,4-Methylenedioxymethamphetamine Administration: Sex-Related Differences.
3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is an amphetamine-related drug that may damage the dopaminergic nigrostriatal system. To investigate the mechanisms that sustain this toxic effect and ascertain their sex-dependence, we evaluated in the nigrostriatal system of MDMA-treated (4 × 20 mg/kg, 2 h apart) male and female mice the activity of superoxide dismutase (SOD), the gene expression of SOD type 1 and 2, together with SOD1/2 co-localization with tyrosine hydroxylase (TH)-positive neurons. In the same mice and brain areas, activity of glutathione peroxidase (GPx) and of β2/β5 subunits of the ubiquitin-proteasome system (UPS) were also evaluated. After MDMA, SOD1 increased in striatal TH-positive terminals, but not nigral neurons, of males and females, while SOD2 increased in striatal TH-positive terminals and nigral neurons of males only. Moreover, after MDMA, SOD1 gene expression increased in the midbrain of males and females, whereas SOD2 increased only in males. Finally, MDMA increased the SOD activity in the midbrain of females, without affecting GPx activity, decreased the β2/β5 activities in the striatum of males and the β2 activity in the midbrain of females. These results suggest that the mechanisms of MDMA-induced neurotoxic effects are sex-dependent and

dopaminergic neurons of males could be more sensitive to SOD2- and UPS-mediated toxic effects.
Front Pharmacol, 2021; 12
34439672: Costa G, Serra M, Simola N
Association between Novel Object Recognition/Spontaneous Alternation Behavior and Emission of Ultrasonic Vocalizations in Rats: Possible Relevance to the Study of Memory.
Rats emit ultrasonic vocalizations (USVs) in situations with emotional valence, and USVs have also been proposed as a marker for memories conditioned to those situations. This study investigated whether USV emissions can predict and/or be associated with the behavior of rats in tests that evaluate unconditioned memory. To this end, rats were subjected to "tickling", a procedure of heterospecific play that has emotional valence and elicits the emission of USVs, and afterwards evaluated in the novel object recognition test (NOR) and in the single trial continuous spontaneous alternation behavior (SAB) test in a Y maze. The number of 22-kHz USVs (aversive) and 50-kHz USVs (appetitive) emitted in response to tickling and during NOR and SAB tests were scored, and the correlations among them and with rats' behavior evaluated. Rats emitted 50-kHz USVs, but not 22-kHz USVs, during the NOR and SAB tests, and such calling behavior was not linked with the behavioral readouts indicative of memory function in either test. However, rats that prevalently emitted 22-kHz USVs in response to tickling displayed an impaired NOR performance. These findings suggest that measuring the emission of USVs could be of interest in studies of unconditioned memory, at least with regard to 22-kHz USVs.
Brain Sci, 2021; 11
34229013: Pinna A, Costa G, Serra M, Contu L, Morelli M
Neuroinflammation and L-dopa-induced abnormal involuntary movements in 6-hydroxydopamine-lesioned rat model of Parkinson's disease are counteracted by combined administration of a 5-HT receptor agonist and A receptor antagonist.
Several lines of evidence have strongly implicated neuroinflammation in Parkinson's disease (PD) progression and l-dopa-induced dyskinesia. The present study investigated whether early subchronic pretreatment with the serotonin 5-HT receptor agonist eltoprazine plus the adenosine A receptor antagonist preladenant counteracted l-dopa-induced abnormal involuntary movements (AIMs, index of dyskinesia), and neuroinflammation, in unilateral 6-hydroxydopamine(6-OHDA)-lesioned rat model of PD. The immunoreactivity of glial fibrillary acidic protein (GFAP), and the colocalization of ionized calcium binding adaptor molecule-1 (IBA-1), with interleukin (IL)-1β, tumor-necrosis-factor-α (TNF-α) and IL-10 were evaluated in the denervated caudate-putamen (CPu) and substantia nigra pars-compacta (SNc). The combined subchronic pretreatment with l-dopa plus eltoprazine and preladenant reduced AIMs induced by acute l-dopa challenge in these rats and decreased GFAP and IBA-1 immunoreactivity induced by the drug in both CPu and SNc, with reduction in IL-1β in IBA-1-positive cells in both CPu and SNc, and in TNF-α in IBA-1-positive cells in SNc. Moreover, a significant increase in IL-10 in IBA-1-positive cells was observed in SNc. Evaluation of immediate early-gene zif-268 (index of neuronal activation) after l-dopa challenge, showed an increase in its expression in denervated CPu of rats pretreated with l-dopa or l-dopa plus preladenant compared with vehicle, whereas rats pretreated with eltoprazine, with or without preladenant, had lower zif-268 expression. Finally, tyrosine hydroxylase and dopamine transporter examined to evaluate neurodegeneration, showed a significant equal decrease in all experimental groups. The present findings suggest that combination of l-dopa with eltoprazine and preladenant may be promising therapeutic strategy for delaying the onset of dyskinesia, preserving l-dopa efficacy and reducing neuroinflammation markers in nigrostriatal system of 6-OHDA-lesioned rats.
Neuropharmacology, 2021; 196
34070217: Serra M, Pinna A, Costa G, Usiello A, Pasqualetti M, Avallone L, Morelli M, Napolitano F
Involvement of the Protein Ras Homolog Enriched in the Striatum, Rhes, in Dopaminergic Neurons' Degeneration: Link to Parkinson's Disease.
is one of the most interesting genes regulated by thyroid hormones that, through the inhibition of the striatal cAMP/PKA pathway, acts as a modulator of dopamine neurotransmission. is expressed at high levels in the dorsal striatum, with a medial-to-lateral expression gradient reflecting that of both dopamine D and adenosine A receptors. transcript is also present in the hippocampus, cerebral cortex, olfactory tubercle and bulb, substantia nigra pars compacta (SNc) and ventral tegmental area of the rodent brain. In line with -dependent regulation of dopaminergic transmission, data showed that lack of enhanced cocaine- and amphetamine-induced motor stimulation in mice. Previous studies showed that pharmacological depletion of dopamine significantly reduces mRNA levels in rodents, non-human primates and Parkinson's disease (PD) patients, suggesting a link between dopaminergic innervation and physiological mRNA expression. Rhes protein binds to and activates striatal mTORC1, and modulates L-DOPA-induced dyskinesia in PD rodent models. Finally, Rhes is involved in the survival of mouse midbrain dopaminergic neurons of SNc, thus pointing towards a Rhes-dependent modulation of autophagy and mitophagy processes, and encouraging further investigations about mechanisms underlying dysfunctions of the nigrostriatal system.
Int J Mol Sci, 2021; 22
33592304: Serra M, Marongiu J, Simola N

Lack of drug- and cue-stimulated emissions of ultrasonic vocalizations in C57BL/6J mice repeatedly treated with amphetamine.
The emission of ultrasonic vocalizations (USVs) is thought to communicate the behavioral and emotional states elicited in rodents by social and non-social stimuli. On this basis, studies of psychopharmacology in rats are increasingly utilizing USVs as a behavioral marker to evaluate the effects of drugs on the emotional state. Conversely, very limited information is available as to whether psychoactive drugs influence USV emissions in mice. To provide new insights in this respect, we evaluated the emission of USVs in C57BL/6J mice subjected to repeated treatment with the dopaminergic psychostimulant of abuse amphetamine. Mice were first allowed to perform social contacts in dyads, and 2 days later they received amphetamine (1-4 mg/kg, i.p.) in a test cage (× 5 administrations) on alternate days. Seven days after treatment discontinuation, mice were re-exposed to the test cage to evaluate whether the presentation of drug-paired environmental cues elicited calling behavior, and thereafter received an amphetamine challenge. An additional group of animals received the dopamine receptor agonist apomorphine (1-4 mg/kg, i.p.), to further clarify the role of dopamine transmission in calling behavior of mice. C57BL/6J mice emitted USVs during social contacts, but did not significantly vocalize after amphetamine administration, in response to amphetamine-paired environmental cues, and after apomorphine administration. These results indicate that C57BL/6J mice may respond differently to social and pharmacological stimuli in terms of USV emissions, and may lay the foundation for future studies aimed at clarifying whether USVs may be a useful behavioral marker in studies of psychopharmacology in mice.
Neurosci Lett, 2021; 749
33349579: Pinna A, Serra M, Marongiu J, Morelli M
Pharmacological interactions between adenosine A receptor antagonists and different neurotransmitter systems.
While Parkinson's disease (PD) is traditionally characterized by dopaminergic neuron degeneration, several neurotransmitters and neuromodulators besides dopamine are also involved in the onset and progression of the disease and its symptoms. The other principal neurotransmitters/neuromodulators known to control basal ganglia functions and, in particular, motor functions, are GABA, glutamate, serotonin (5-HT), noradrenaline, acetylcholine, adenosine and endocannabinoids. Among these, adenosine is the most relevant, acting through its adenosine A receptor. Work in experimental models of PD has established the effects of A receptor antagonists, including the alleviation of disrupted dopamine functions and improved efficacy of dopamine replacement therapy. Moreover, positive interactions between A receptor antagonists and both D and D receptor agonists have been described in vitro at the receptor-receptor level or in more complex in vivo models of PD, respectively. In addition, the interactions between A receptor antagonists and glutamate ionotropic GluN-containing N-Methyl-d-aspartic acid receptors, or metabotropic glutamate (mGlu) receptors, including both mGlu receptor inhibitors and mGlu receptor activators, have been reported in both in vitro and in vivo animal models of PD, as have positive interactions between A and endocannabinoid CB receptor antagonists. At the same time, a combination of A receptor antagonists and 5-HT-5-HT receptor agonists have been described to modulate the expression of dyskinesia induced by chronic dopamine replacement therapy.
Parkinsonism Relat Disord, 2020; 80 Suppl 1
33242502: Simola N, Serra M, Marongiu J, Costa G, Morelli M
Increased emissions of 50-kHz ultrasonic vocalizations in hemiparkinsonian rats repeatedly treated with dopaminomimetic drugs: A potential preclinical model for studying the affective properties of dopamine replacement therapy in Parkinson's disease.
Dopamine replacement therapy used in Parkinson's disease (PD) may induce alterations in the emotional state that can underlie the manifestation of iatrogenic psychiatric-like disturbances. The preclinical investigation of these disturbances is limited, also because few reliable paradigms are available to study the affective properties of dopaminomimetic drugs in parkinsonian animals. To provide a relevant experimental tool in this respect, we evaluated whether dopaminomimetic drugs modified the emission of 50-kHz ultrasonic vocalizations (USVs), a behavioral marker of positive affect, in rats bearing a unilateral lesion with 6-hydroxydopamine in the medial forebrain bundle. Apomorphine (2 or 4 mg/kg, i.p.), L-3,4-dihydroxyphenilalanine (L-DOPA, 6 or 12 mg/kg, i.p.), or pramipexole (2 or 4 mg/kg, i.p.) were administered in a test cage (× 5 administrations) on alternate days. Seven days after treatment discontinuation, rats were re-exposed to the test cage to measure conditioned calling behavior and thereafter received a drug challenge. Hemiparkinsonian rats treated with either apomorphine or L-DOPA, but not pramipexole, markedly vocalized during repeated treatment and after challenge, and showed conditioned calling behavior. Moreover, apomorphine, L-DOPA and pramipexole elicited different patterns of 50-kHz USV emissions and rotational behavior, indicating that calling behavior in hemiparkinsonian rats treated with dopaminomimetic drugs is not a byproduct of motor activation. Taken together, these results suggest that measuring 50-kHz USV emissions may be a relevant experimental tool for studying how dopaminomimetic drugs modify the affective state in parkinsonian rats, with possible implications for the preclinical investigation of iatrogenic psychiatric-like disturbances in PD.
Prog Neuropsychopharmacol Biol Psychiatry, 2021; 108

32477098: Costa G, Sisalli MJ, Simola N, Della Notte S, Casu MA, Serra M, Pinna A, Feliciello A, Annunziato L, Scorziello A, Morelli M
Gender Differences in Neurodegeneration, Neuroinflammation and Na-Ca Exchangers in the Female A53T Transgenic Mouse Model of Parkinson's Disease.
Twelve-month-old male mice expressing the human A53T variant of α-synuclein (A53T) develop dopamine neuron degeneration, neuroinflammation, and motor deficits, along with dysfunctions of the mitochondrial Na-Ca exchanger (NCX) isoforms 1 (NCX1) and 3 (NCX3) in the nigrostriatal system. Since gender is thought to play a role in the etiology of Parkinson's disease (PD), we characterized neurochemical and behavioral alterations in 12-month-old female A53T transgenic mice. We investigated the presence of dopaminergic degeneration, astrogliosis and microgliosis using immunohistochemistry for tyrosine hydroxylase (TH), glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule-1 (IBA-1) in both the substantia nigra pars compacta (SNc) and striatum. In the same regions, we also evaluated the co-localization of NCX1 in cells positive for IBA-1 and the co-localization of NCX3 in TH-positive neurons and fibers. Furthermore, in both male and female mice, we performed motor (beam walking and pole tests) and memory [novel object recognition (NOR) and spontaneous alternation] tasks, together with tests to evaluate peripheral deficits (olfactory and stool collection tests). Female A53T transgenic mice displayed degeneration of nigral dopaminergic neurons, but neither microgliosis nor astrogliosis in the SNc and striatum. Moreover, female A53T transgenic mice displayed co-localization between NCX1 and IBA-1 positive cells in the striatum but not SNc, whereas NCX3 did not co-localize with either TH-positive terminals or neuronal bodies in the nigrostriatal system. Furthermore, female A53T transgenic mice showed increased crossing time in the beam walking test, but no impairments in the pole or memory tests, and in tests that evaluated peripheral deficits, whereas male A53T transgenic mice displayed motor, memory and peripheral deficits. Immunohistochemical and behavioral results obtained here in the female mice differ from those previously observed in males, and suggest a dissimilar influence of NCX1 and NCX3 on dopaminergic function in female and male A53T transgenic mice, strengthening the validity of these mice as a model for studying the etiological factors of PD.
Front Aging Neurosci, 2020; 12
32320647: Lewis RG, Serra M, Radl D, Gori M, Tran C, Michalak SE, Vanderwal CD, Borrelli E
Dopaminergic Control of Striatal Cholinergic Interneurons Underlies Cocaine-Induced Psychostimulation.
Cocaine drastically elevates dopamine (DA) levels in the striatum, a brain region that is critical to the psychomotor and rewarding properties of the drug. DA signaling regulates intrastriatal circuits connecting medium spiny neurons (MSNs) with afferent fibers and interneurons. While the cocaine-mediated increase in DA signaling on MSNs is well documented, that on cholinergic interneurons (ChIs) has been more difficult to assess. Using combined pharmacological, chemogenetic, and cell-specific ablation approaches, we reveal that the D2R-dependent inhibition of acetylcholine (ACh) signaling is fundamental to cocaine-induced changes in behavior and the striatal genomic response. We show that the D2R-dependent control of striatal ChIs enables the motor, sensitized, and reinforcing properties of cocaine. This study highlights the importance of the DA- and D2R-mediated inhibitory control of ChIs activity in the normal functioning of striatal networks.
Cell Rep, 2020; 31

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BOARD NUMBER: S07-610

EFFECTS OF INFLAMMATION IN THE PROGRESSION OF PARKINSON'S DISEASE IN A RAT MODEL OVEREXPRESSING HUMAN α-SYNUCLEIN

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Aim: Parkinson's disease (PD) is characterized by the aggregation and accumulation of α-synuclein, the main component of Lewy bodies and neurites, the loss of dopaminergic neurons in the *substantia nigra*, reduction of striatal dopaminergic fibers, and neuroinflammation. Mechanisms through which α-synuclein and inflammation interactions mediate neurodegenerative processes in PD are still unclear. In this study, a two-hit animal model of PD was created to investigate mechanisms through which overexpression of α-synuclein and inflammation accelerate the progression of PD neurodegeneration. **Methods**: Rats overexpressing human α-synuclein, at 2-months old, were administered with a single intraperitoneal injection of the inflammogen lipopolysaccharide (LPS; 5 mg/kg). At 3 months after injection, nigral dopaminergic neurodegeneration, α-synuclein pathology, and neuroinflammation were evaluated by immunohistochemistry, immunofluorescence, and high dimensional flow cytometry analyses. **Results**: The acute injection of LPS in rats caused long-lasting neuroinflammation with microgliosis and increased T-lymphocytes infiltration in the striatum and in the *substantia nigra*. This was leading to loss of dopaminergic neurons, increased accumulation of phospho-S129 α-synuclein, and altered dendritic arborization of dopaminergic neurons in the *substantia nigra*. Furthermore, an increase of Th1 and Th17 CD4 T-cells and a concomitant reduction of regulatory T-cells were observed in the peripheral blood. **Conclusions**: Our two-hit animal model involving both a genetic lesion and an inflammatory factor reproduced key features of PD, thus it could be a valid model for the study of mechanisms of PD progression. **Acknowledgment:** RF-2018-12365509 to N.B.M. and V.C.

Pubmed:
34536282: Flores CC, Loschky SS, Marshall W, Spano GM, Massaro Cenere M, Tononi G, Cirelli C
Identification of Ultrastructural Signatures of Sleep and Wake in the Fly Brain.
The cellular consequences of sleep loss are poorly characterized. In the pyramidal neurons of mouse frontal cortex we found that mitochondria and secondary lysosomes occupy a larger proportion of the cytoplasm after chronic sleep restriction compared to sleep, consistent with increased cellular burden due to extended wake. For each morphological parameter the within-animal variance was high, suggesting that the effects of sleep and sleep loss vary greatly among neurons. However, the analysis was based on 4-5 mice/group and a single section/cell. Here, we applied serial block-face scanning electron microscopy to identify signatures of sleep and sleep loss in the Drosophila brain. Stacks of images were acquired and used to obtain full 3D reconstructions of the cytoplasm and nucleus of 263 Kenyon cells from adult flies collected after a night of sleep (S) or after 11 hours (SD11) or 35 hours (SD35) of sleep deprivation (9 flies/group). Relative to S flies, SD35 flies showed increased density of dark clusters of chromatin and of Golgi apparata and a trend increase in the percent of cell volume occupied by mitochondria, consistent with increased need for energy and protein supply during extended wake. Logistic regression models could assign each neuron to the correct experimental group with good accuracy, but in each cell nuclear and cytoplasmic changes were poorly correlated, and within-fly variance was substantial in all experimental groups. Together, these results support the presence of ultrastructural signatures of sleep and sleep loss but underscore the complexity of their effects at the single-cell level.
Sleep, 2021;

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POTENTIAL OF EXERCISE TO MODIFY THE PROGRESSION OF PRODROMAL PARKINSON'S DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Aims: Subtle motor and non-motor dysfunctions indicative of beginning Parkinson's disease (PD) progression are evident before clinical disease diagnosis, requiring a disease-modifying treatment to start during early prodromal stage. Persons of risk would be willing to determine their risk of developing PD and change their lifestyle in case of a concrete beneficial approach. Growing evidence indicates the potential of exercise in reducing components of PD-related pathology. We hypothesized that early intervention by exercise has a disease-modifying effect during prodromal phase in our PD mouse model and can be applied as a non-pharmacological preventive strategy for early-stage PD. **Methods:** We examined exercise in transgenic mice that overexpress human wild-type alpha-synuclein (Thy1-aSyn mice) and replicate PD hallmarks by developing robust fine motor deficits at two months of age. Six-week-old male wild-type and Thy1-aSyn mice were assigned to three groups receiving different intensity levels of exercise on a treadmill. Motor performance was assessed in the challenging beam and vertical pole test. Fecal pellets of mice are processed for microbiome sequencing, and brains and plasma taken for in-depths post-mortem analysis of PD-related pathology. **Results:** Transgenic mice showed motor impairment on challenging beam and vertical pole, reflecting the expected progression of aSyn pathology at this age. Improved vertical pole performance under exercise in wildtypes suggests symptomatic effects, while slight improvements in beam performance might indicate a disease-modifying potential of exercise. **Conclusions:** These results suggest that exercise is able to alleviate early sensorimotor deficits in Thy1-aSyn mice and demonstrate its potential as an early PD-modifying treatment.

Pubmed:
34390837: Torres ERS, Stanojlovic M, Zelikowsky M, Bonsberger J, Hean S, Mulligan C, Baldauf L, Fleming S, Masliah E, Chesselet MF, Fanselow MS, Richter F
Alpha-synuclein pathology, microgliosis, and parvalbumin neuron loss in the amygdala associated with enhanced fear in the Thy1-aSyn model of Parkinson's disease.
In Parkinson's disease (PD), the second most common neurodegenerative disorder, non-motor symptoms often precede the development of debilitating motor symptoms and present a severe impact on the quality of life. Lewy bodies containing misfolded α-synuclein progressively develop in neurons throughout the peripheral and central nervous system, which may be correlated with the early development of non-motor symptoms. Among those, increased fear and anxiety is frequent in PD and thought to result from pathology outside the dopaminergic system, which has been the focus of symptomatic treatment to alleviate motor symptoms. Alpha-synuclein accumulation has been reported in the amygdala of PD patients, a brain region critically involved in fear and anxiety. Here we asked whether α-synuclein overexpression alone is sufficient to induce an enhanced fear phenotype in vivo and which pathological mechanisms are involved. Transgenic mice expressing human wild-type α-synuclein (Thy1-aSyn), a well-established model of PD, were subjected to fear conditioning followed by extinction and then tested for extinction memory retention followed by histopathological analysis. Thy1-aSyn mice showed enhanced tone fear across acquisition and extinction compared to wild-type littermates, as well as a trend to less retention of fear extinction. Immunohistochemical analysis of the basolateral nucleus of the amygdala, a nucleus critically involved in tone fear learning, revealed extensive α-synuclein pathology, with accumulation, phosphorylation, and aggregation of α-synuclein in transgenic mice. This pathology was accompanied by microgliosis and parvalbumin neuron loss in this nucleus, which could explain the enhanced fear phenotype. Importantly, this non-motor phenotype was detected in the pre-clinical phase, prior to dopamine loss in Thy1-aSyn mice, thus replicating observations in patients. Results obtained in this study suggest a possible mechanism by which increased anxiety and maladaptive fear processing may occur in PD, opening a door for therapeutic

options and further early biomarker research.
Neurobiol Dis, 2021; 158

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THE INTERPLAY OF LIPOPOLYSACCHARIDE AND ALPHA-SYNUCLEIN TO MODEL GUT-BRAIN PATHOPHYSIOLOGY IN PARKINSON´S DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Aims: Although Parkinson's disease (PD) is the second most common neurodegenerative disease, we lack better understanding of the pathologic pathways leading to the disease and its progression. Increasing evidence points to infectious diseases and a role of the gut-brain axis as environmental risk factors. Lipopolysaccharide (LPS) producing gram-negative bacteria are more frequent in the gut-microbiome of PD patients. We hypothesize that LPS, an activator of various immune pathways, could contribute to disease progression via neuroinflammation and up-regulation of neuronal alpha-Synuclein (αSyn). **Methods:** To test this hypothesis, the role of LPS in the progression of PD was evaluated in a human αSyn overexpression model (Thy1- αSyn "Line 61" Mouse). Two low doses of LPS (0.3 and 0.8 mg/kg) were intraperitoneally injected into αSyn mice and wild-type littermates. Afterwards various examinations including clinical score, blood flow cytometry, and brain immunohistology were performed. **Results:** Both dosages only induced transient symptoms and mild weight loss in mice, with full recovery after 7 days. A single injection of 0.8 mg/kg LPS was sufficient to influence the number of immune cells in the blood at 7 days post injection as previously described after LPS exposure. Furthermore, LPS injected mice developed brain region specific microgliosis. In **conclusion**, an LPS dosage of 0.8 mg/kg was sufficient to induce a transient disease phenotype in wild-type and PD model mice while showing measurable neuroinflammation. Therefore, this model will be used to assess the progression of PD symptoms and the interplay with neuroinflammation in sensitive motoric and cognitive behavior experiments.

Pubmed:
33282874: Wildburger NC, Hartke AS, Schidlitzki A, Richter F
Current Evidence for a Bidirectional Loop Between the Lysosome and Alpha-Synuclein Proteoforms.
Cumulative evidence collected in recent decades suggests that lysosomal dysfunction contributes to neurodegenerative diseases, especially if amyloid proteins are involved. Among these, alpha-synuclein (aSyn) that progressively accumulates and aggregates in Lewy bodies is undisputedly a main culprit in Parkinson disease (PD) pathogenesis. Lysosomal dysfunction is evident in brains of PD patients, and mutations in lysosomal enzymes are a major risk factor of PD. At first glance, the role of protein-degrading lysosomes in a disease with pathological protein accumulation seems obvious and should guide the development of straightforward and rational therapeutic targets. However, our review demonstrates that the story is more complicated for aSyn. The protein can possess diverse posttranslational modifications, aggregate formations, and truncations, all of which contribute to a growing known set of proteoforms. These interfere directly or indirectly with lysosome function, reducing their own degradation, and thereby accelerating the protein aggregation and disease process. Conversely, unbalanced lysosomal enzymatic processes can produce truncated aSyn proteoforms that may be more toxic and prone to aggregation. This highlights the possibility of enhancing lysosomal function as a treatment for PD, if it can be confirmed that this approach effectively reduces harmful aSyn proteoforms and does not produce novel, toxic proteoforms.
Front Cell Dev Biol, 2020; 8

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DEVELOPING ZEBRAFISH CRISPR/CAS9 KNOCKOUT MODELS OF PARKINSON'S DISEASE TO IDENTIFY NOVEL THERAPEUTIC TARGETS.

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disorder. Commonly, it affects people over 60 years old. However, the disease can be manifested early in life. Most of the cases are of sporadic etiology and 10 to 20 % of genetic cause, with mutations occurring in only one gene following a Mendelian's inheritance pattern. Patients experience motor and no motor symptoms that escalate as disease progresses. As human patients present punctual mutations and/or small deletions in *park1*, *park2*, *park6*, *park7*, and *park8*, we targeted the zebrafish exon counterparts. Here, the aim is to generate CRISPR/Cas9 loss-of-function alleles of the aforementioned PD-associated genes in zebrafish to discover novel therapeutic targets. We have observed high conservation with expression patterns in the central nervous system of zebrafish. Validation of these models at the cellular/molecular level shows a striking reduction of tyrosine hydroxylase (*th1*) mRNA and protein levels, suggesting that zebrafish dopaminergic neurons are affected by our genetic disruption. Additionally, preliminary results show some differences in the locomotion behaviour of mutants compared to wild type. Lastly, on-going transcriptomic analysis of postmitotic neurons of our models could show genes differentially expressed, which could help us identify novel molecular targets and altered pathways. Overall, phenotypic characterisation and a molecular signature identification could pave the ground for future development of new therapeutic strategies and drugs for Parkinson's Disease.

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NEW IN VIVO MODEL OF PARKINSON'S DISEASE INVOLVING COMBINED TOXICITY OF ALPHA-SYNUCLEIN OLIGOMERS AND PROTOFIBRILS, AND CHRONIC INHIBITION OF GBA.

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Aims : Parkinson's disease (PD) is characterized by dopaminergic neuron loss in the *substantia nigra* (SN), alpha-synuclein (α-Syn) aggregation and motor dysfunctions. Toxic species of α-Syn contributes to the physiopathology of PD. Loss of function of GBA, a lysosomal glucocerebrosidase, due to gene mutations, is a strong risk factor for PD. Here we aimed to develop a novel *in vivo* model of PD, combining α-Syn toxicity and inhibition of GBA. **Methods** : α-Syn solution, containing oligomers and protofibrils, was bilaterally injected in the SN of aged male C57BL/6J mice. Mice were chronically treated with conduritol-B-epoxide (CBE, 50 mg/kg, ip), a GBA inhibitor. Neuropathology was assessed by histology and confocal imaging. Locomotor activity of the mice was investigated with the bar and the grid walking test. **Results** : Loss of dopaminergic neurons in the SN was observed 2 weeks after α-Syn injection and GBA inhibition, and progressed over time (up to 6 weeks). The neuronal death was associated with inflammation and α-Syn aggregation. In addition, α-Syn/CBE-treated mice showed locomotor deficits 3 weeks after the surgery as they made step errors on the grid walking test and had reduced speed on the bar test. These locomotor dysfunctions also seemed to be progressive over time. **Conclusions** : Intra-nigral injections of α-Syn, combined to chronic inhibition of GBA triggered a loss of dopaminergic neurons in the SN and motor dysfunctions, in aged mice. This new animal model of PD shares the main pathological features observed in PD patients.

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BOARD NUMBER: S07-615

MODELLING PRESYMPTOMATIC STAGES OF PARKINSON'S DISEASE IN RODENTS BY THE OVEREXPRESSION OF HUMAN ALPHA-SYNUCLEIN IN THE LOCUS COERULEUS

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative process characterized by the presence of Lewy bodies in different brain regions, affection of many neurotransmitter systems and manifestation of motor and non-motor symptoms. These latter ones often appear before the motor symptomatology and highly impact on the life quality of the patients. Already in the presymptomatic phase, the noradrenergic nucleus locus coeruleus (LC) shows Lewy bodies and PD-like pathology, being one of the first nuclei that undergoes neurodegeneration. This nucleus has a critical role in stress response, emotional memory and control of motor, sensory and autonomic function and can even modulate dopamine homeostasis. The aim of this study is to investigate how the LC participates in PD pathogenesis and symptomatology. For that, we studied the impact of alpha-synuclein overexpression in the LC at molecular and behavioral levels in a pre-symptomatic rodent model of PD. These preliminary data will contribute to better understanding the role of the noradrenergic system in the early phases of the disease, and to identify novel therapeutic strategies to dampen PD progression. JR holds a PhD grant from the University of the Basque Country. This project is financed by the Basque Government (PIBA19-0038; PUE21-03) and the University of the Basque Country (COLAB20/07; GIU19/339). This research was conducted in the scope of the Transborder Joint Laboratory (LTC) "non-motor CoMorbidities in Parkinson's Disease (CoMorPD)". No conflict of interest. **Keywords**: Locus coeruleus, Parkinson's disease, α- synuclein, noradrenergic neurons.

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BOARD NUMBER: S07-616

REDUCED INTERACTION OF AGGREGATED α-SYNUCLEIN AND VAMP2 BY ENVIRONMENTAL ENRICHMENT ALLEVIATES HYPERACTIVITY AND ANXIETY IN A MODEL OF PARKINSON'S DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Parkinson's disease (PD) is a prevalent motor disease caused by the accumulation of mutated α-synuclein (α-Syn); however, its early stages are also characterized by non-motor symptoms, such as olfactory loss, cognitive decline, depression, and anxiety. The therapeutic effects of
environmental enrichment (EE) on motor recovery have been reported, but its effects on non-motor symptoms remain unclear. Herein, we reveal the beneficial effects of EE on PD-related non-motor symptoms and changes in synaptic plasticity in the nucleus accumbens. To investigate its therapeutic effects in the early phase of PD, we randomly assigned eight-month-old mice overexpressing human A53T (hA53T) α-Syn to either the EE or standard condition groups for two months. Next, we performed behavioral tests and biochemical and histological analyses at 10 months of age. EE significantly alleviated locomotor hyperactivity and anxiety during the early stages of PD. It normalized the levels of tyrosine hydroxylase, phosphorylated and oligomeric α-Syn, and soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex-forming proteins, including synaptosomal-associated protein, 25 kDa, syntaxin1, and vesicle-associated membrane protein 2 (VAMP2). Moreover, the interactions between VAMP2 and pSer129 α-Syn were markedly reduced following EE. The restoration of synaptic vesicle transportation status may underlie the neuroprotective effects of EE in hA53T α-Syn mice.

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BOARD NUMBER: S07-617

INVESTIGATION OF NEUROPROTECTIVE POTENTIAL OF GRAPE POMACES LOADED NUTRIOSOMES IN THE MPTP-MOUSE MODEL OF PARKINSON'S DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Background: Grape pomaces, a waste by-product of wine production, have received great attention for their richness in polyphenols, compounds known to exert anti-inflammatory and antioxidant effects in animal models of neurodegenerative diseases. These pomaces, however, have low brain bioavailability upon oral administration due to their extensive degradation in the gastrointestinal tract. **Aims:** To overcome the above-mentioned problems, Nasco pomaces extract was incorporated into a novel nanovesicle system, nutriosomes, composed of phospholipid (S75) and maltodextrin (Nutriose® FM06), to investigate the neuroprotective effect of Nasco pomaces in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's Disease (PD). **Methods:** Biocompatibility of nutriosomes was assessed on intestinal epithelial cells (Caco-2). Nasco nutriosomes or Nasco suspension were administered intragastrically and their neuroprotective effects were evaluated in MPTP-treated mice. Degeneration of nigrostriatal dopaminergic neurons was assessed through immunohistochemical evaluation of tyrosine hydroxylase (TH) in the caudate-putamen (CPu) and substantia nigra pars compacta (SNc), along with the dopamine transporter (DAT) in the CPu. **Results:** The obtained nutriosomes were highly biocompatible towards Caco-2 cells. Nasco pomaces extract resulted rich in polyphenols, i.e. gallic acid, (+)-catechin, (-)-epicatechin, procyanidin-B2, and quercetin. Immunohistochemical analysis revealed that Nasco nutriosomes but not Nasco suspension significantly contrasted the MPTP-mediated reduction of TH and DAT-positive fibres in the CPu and the number of TH-positive cells in SNc. **Conclusion:** These results highlight the promising therapeutic effects of Nasco pomace extracts when administered in a nutriosome in the MPTP-mouse model of PD, and validate the effectiveness of the nutriosome preparation over suspension as an innovative nano-drug delivery system.

Pubmed:
31778768: Parkhe A, Parekh P, Nalla LV, Sharma N, Sharma M, Gadepalli A, Kate A, Khairnar A
Protective effect of alpha mangostin on rotenone induced toxicity in rat model of Parkinson's disease.
Parkinson's disease (PD) is a progressive, late-onset, and degenerative disorder that affects the central nervous system with an unknown etiology. Due to its incredible complexity in disease nature, many of the existing treatment approaches show a vain recovery in Parkinson's patients. Therefore, an in search of disease-modifying therapeutics for an effective recovery is essential. Alpha mangostin is an important polyphenolic xanthone reported for its neuroprotective effect against rotenone-induced α-synuclein aggregation and loss of tyrosine hydroxylase positive (TH)-neurons in SH-SY5Y cells. Hence, the current study aims to test its protective effect in managing the in-vivo rat model of PD. To justify this aim, adult male Sprague Dawley rats (250 ± 20 g) were subjected to chronic treatment of rotenone (2 mg/kg/day, s.c.) for 21 days. In parallel alpha mangostin treatment (10 mg/kg, i.p) was administered along with rotenone for 21 days. Chronic rotenone treatment for 21 days increased lipid peroxidation, nitrite concentration, and decreased glutathione levels. Further, depletion of TH-dopaminergic neuron expression in substantia nigra pars compacta (SNc), and the development of motor and behavioral deficits in rotenone treated animals like cognitive impairment, muscle incoordination, and neuromuscular weakness were observed. Moreover, western blot studies ascertained the reduced normal alpha-synuclein levels and increased phosphorylated α-synuclein levels in comparison to the vehicle-treated group. Treatment with alpha mangostin significantly restored the locomotor activity, memory deficits, and improved the levels of antioxidant enzymes. It also significantly reduced the levels of phosphorylated α-synuclein which in turn gave protection against TH-dopaminergic neuronal loss in SNc, suggesting it's anti-oxidant and anti-aggregatory potential against α-synuclein. In conclusion through our current results, we could suggest that alpha mangostin has a potential neuroprotective effect against rotenone-induced PD and might be used as a neuroprotective agent. Further mechanistic studies on preclinical and clinical levels are required to be conducted with alpha mangostin to

avail and foresee it as a potential agent in the treatment and management of PD.
Neurosci Lett, 2020; 716
31385687: Parekh P, Sharma N, Gadepalli A, Shahane A, Sharma M, Khairnar A
A Cleaning Crew: The Pursuit of Autophagy in Parkinson's Disease.
Parkinson's disease (PD) is the second-most common neurodegenerative disorder, neuropathologically characterized by the aggregation of misfolded α-synuclein (α-syn) protein, which appears to be central to the onset and progression of PD pathology. Evidence from pioneering studies has highly advocated the existence of impaired autophagy pathways in the brains of PD patients. Autophagy is an evolutionarily conserved, homeostatic mechanism for minimizing abnormal protein aggregates and facilitating organelle turnover. Any aberration in constitutive autophagy activity results in the aggregation of misfolded α-syn, which, in turn, may further inhibit their own degradation-leading to a vicious cycle of neuronal death. Despite the plethora of available literature, there are still lacunas existing in our understanding of the exact cellular interplay between autophagy impairment and α-syn accumulation-mediated neurotoxicity. In this context, clearance of aggregated α-syn via up-regulation of the autophagy-lysosomal pathway could provide a pharmacologically viable approach to the treatment of PD. The present Review highlights the basics of autophagy and detrimental cross-talk between α-syn and chaperone-mediated autophagy, and α-syn and macroautophagy. It also depicts the interaction between α-syn and novel targets, LRRK2 and mTOR, followed by the role of autophagy in PD from a therapeutic perspective. More importantly, it further updates the reader's understanding of various newer therapeutic avenues that may accomplish disease modification via promoting clearance of toxic α-syn through activation of autophagy.
ACS Chem Neurosci, 2019; 10

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NEUROBEHAVIORAL ASSESSMENT OF VANILLIN IN MPTP-INDUCED MOUSE MODEL OF PARKINSON'S DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Aim Drugs currently available for the treatment of Parkinson's disease (PD) either have adverse effects or have suboptimal efficacy. Flavonoids are strong antioxidants with minimal damage with long-term usage. Vanillin (Van) is one such flavonoid that has been found to have antioxidant and neuroprotective characteristics in a variety of neurological disorders. Because there has been limited research on the neuroprotective role of Van in PD, the current study looked at its potential impact on MPTP-induced neurobehavioral deficits in a mouse model of PD. *Methods* To determine the potential neuroprotective effects of Van on motor balance and coordination, we performed the pole test, narrow beam test, and forced swim test at the end of the experiment. Five groups of animals were studied for behavioural changes after the induction of PD and treatment with Van for 21 days. *Results* In the current study, Van successfully alleviated the deficits of MPTP-induced motor function. MPTP-intoxicated mice showed motor dysfunctions, including taking longer time to descend in the pole test and taking longer time to cross the beam, with more foot slips and more immobility time as compared to the control group. There was a significant reduction in motor impairment in the pole test, which showed decreased time spent crossing and foot slip numbers and also reduced immobility time in the Van, MPTP + Van, and L-DOPA treated groups when compared with the MPTP-intoxicated group. *Conclusion* Thus, the current study indicates that Van treatment ameliorates MPTP-induced motor dysfunction in a mouse model of PD.

Pubmed:
33417973: Rani L, Mondal AC
Unravelling the role of gut microbiota in Parkinson's disease progression: Pathogenic and therapeutic implications.
In recent years, researchers have shown interest in bi-directional interaction between the brain and gut, called "gut-brain axis". Emerging pieces of evidence indicate that disturbances in this axis is found to be associated with the Parkinson's disease (PD). Several clinical investigations revealed the crucial role of gut microbiota in the pathogenesis of PD. It has been suggested that aggregation of misfolded protein α-syn, the neuropathological hallmark of PD, might begin in gut and propagates to the CNS via vagus nerve and olfactory bulb. Emerging evidences also suggest that initiation and progression of PD may be due to inflammation originating from gut. It has been shown that microbial gut dysbiosis causes the production of various pathogenic microbial metabolites which elevates pro-inflammatory environment in the gut that promotes neuroinflammation in the CNS. These observations raise the intriguing question - how gut microbial dysbiosis could contribute to PD progression. In this context, various microbiota-targeted therapies are under consideration that can re-establish the intestinal homeostasis which may have greater promise in the prevention and treatment of PD. This review focuses on the role of the gut microbiota in the initiation, progression of PD and current therapeutic intervention to deplete the severity of the disease.
Neurosci Res, 2021; 168
31654753: Rani L, Mondal AC
Emerging concepts of mitochondrial dysfunction in Parkinson's disease progression: Pathogenic and therapeutic implications.
Mitochondria are very dynamic organelle which plays a multifactorial role for a broad range of physiological processes inside the cell to maintain neural circuit integrity. They are required for the generation of cellular energy, regulation of calcium homeostasis and controlling programmed cell death. Defective mitochondrial homeostasis is frequently reported in a broad range of neurological disorders. Many lines of evidence suggest that it plays an essential role in aging and neurodegeneration. Parkinson's disease (PD), the second most prevalent neurodegenerative disorder and its aetiology is still largely unexplored. Overwhelming evidences indicate that mitochondrial dysfunction plays a central role in PD pathogenesis. Various genes involved in maintaining the mitochondrial homeostasis are also associated with the PD pathophysiology. Alterations in mitochondrial functions such as generation of reactive oxygen species (ROS), impaired mitophagy, altered mitochondrial dynamics, impaired mitochondrial biogenesis and Ca buffering may precede the development of PD pathology. In addition, recent studies have also shown the involvement of gut microbiota in the pathogenesis of several

neurodegenerative diseases including PD. In this context, mitochondria-targeted therapies that can ameliorate the mitochondrial abnormalities may have great promise in the prevention and treatment of PD. This review aims to discuss the mitochondrial dysfunction associated with PD pathogenesis, influence of microbiome on mitochondrial regulation and various mitochondrial targeted therapies that can improve the mitochondrial function and deplete the severity of the disease. Mitochondrion, 2020; 50

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BOARD NUMBER: S07-619

SEX DIFFERENCES IN BEHAVIORAL PHENOTYPE AND MARKERS OF THE UNFOLDED PROTEIN RESPONSE (UPR) PATHWAY IN A MOUSE MODEL OVEREXPRESSING HUMAN α-SYNUCLEIN

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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AIMS Anxiety/depression are the most prevalent neuropsychiatric disorders in Parkinson's disease (PD) patients (~50%), occurring at different stages. Neuropathological and functional changes in the serotonin (5-HT) system are involved in the PD prodromal phase and contribute to non-motor symptoms. Using a mouse model of human α-synuclein (h-α-Syn) overexpression in 5-HT neurons, we aim to evaluate: 1- behavioral phenotype, 2- endoplasmic reticulum (ER) stress and unfolded protein response (UPR) activation and 3- possible sex differences. **METHODS** Recombinant AAV vector serotype 5 (AAV5) encoding wild-type h-α-Syn was used to overexpress h-α-Syn in raphe 5-HT neurons of male and female mice. The behavioral phenotype was examined at 4- and 8-weeks post-infusion. Immunohistochemistry was performed for h-α-Syn, TPH and BIP proteins. Protein levels of UPR pathway markers (BIP, GRP94, p-eIF2α, and p-eEF2) and BDNF were assessed by Western-blot. Statistical significance was ascertained by t-tests or one-way ANOVA, as appropriate. **RESULTS** In male mice, raphe h-α-Syn overexpression induced a depressive-like phenotype in tail suspension and forced swim tests, and reduced raphe BDNF levels. In parallel, significant increases in BIP and GRP94 levels were detected indicating UPR pathway activation. ER stress also increased p-eIF2α and p-eEF2 levels, suggesting PERK pathway activation. However, female mice exhibited anhedonia in the sucrose preference test and an anxiety-like phenotype in the dark-light box test, along with a different UPR activation response. **CONCLUSIONS** Overexpression of h-α-Syn in the mouse 5-HT system induces a sex-specific behavioral phenotype and UPR activation, suggesting that different brain circuits may be affected.

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BOARD NUMBER: S07-620

PERSISTENT MICROGLIOSIS AND NEURODEGENERATIVE PROCESSES IN A SARS-COV-2-MODEL

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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(1) Introduction COVID-19 leads to neurological symptoms in up to 76% of the patients. Persisting symptoms far beyond recovery are common in many patients and point towards a progression of neurological impairment. Neuroinflammation post viral infection is a potential risk factor for the development of neurodegenerative diseases such as Parkinson's Disease (PD) and Alzheimer's Disease (AD). **(2) Aims** We examined the impact of COVID-19 on neuroinflammatory and neurodegenerative processes in hamster brains during and post SARS-CoV-2 infection. Hamsters showed mild to moderate symptoms, representing the clinical symptoms of most human patients. Our aim was to investigate whether a SARS-CoV-2 infection could lead to processes associated with neurodegenerative diseases such as PD or AD. **(3) Methods** 8 to 10 weeks old hamsters were intranasally infected with 10^5 PFU SARS-CoV-2 solved in PBS (mock-infected: only PBS) under BSL-3 conditions. The animals were euthanized 3 and 14 days post infection via Pentobarbital injection. Afterwards, brain sections were analyzed via immunohistochemistry. **(4) Results:** Although, we found no viral proteins in the brain parenchyma, there was persisting microgliosis after viral clearance (14 dpi). This finding is of special interest due to the fact, that viral infections and progressive neuroinflammation are risk factors for PD and AD. Histological evaluation indicated alterations of the neuronal homeostasis, which are associated with neurodegenerative diseases. **(5) Conclusions** The results indicate that a SARS-CoV-2 infection is a potential risk factor for neurodegenerative diseases such as AD and PD.

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BOARD NUMBER: S07-621

STUDY OF NEW CANDIDATE GENES IN THE AUTOSOMAL RECESSIVE FORMS OF PARKINSON'S DISEASE IN THE NEMATODE C.ELEGANS.

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons leading to a triad of motor symptoms. Only 40% of early-onset recessive forms are explained by mutations in known PD-associated genes. To look for novel recessively inherited PD-genes, we performed homozygosity mapping and exome sequencing in 91 families with consanguinity. We identified homozygous pathogenic variants in 10 potential candidate genes for recessively inherited PD. We aimed at functionally validate these novel candidate genes, using the Caenorhabditis elegans models. Transgenic strains of C.elegans expressing Green Fluorescent Protein (GFP) specifically in the dopaminergic neurons were used with a reverse genetic approach, via RNAi mediated genes. Behavioral tests involving the dopaminergic system have been carried out, including the Slow Basal Response, the Speed After Swimming and the Trash Test. Each test was performed at Day3 and Day9. The positive control is a C.elegans dopamine-deficient Cat-2 line. C.elegans orthologue of the human recessive PD-associated *PARK7* gene, *djr-1.2,* was used to validate the tests. Of the three behavioral tests, only the Slow Basal Response showed a significant basal slowing of the Cat-2 and RNAi-*djr-1.2* worms at Day3 compared with the negative control, that was rescued by the levodopa. These data will be validated using other genes known to be involved in recessive forms of PD (*PRKN*, *PINK1*, *VPS13C*) before screening potential candidate genes to validate/invalidate their involvement in PD.

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THE DUALISTIC ROLE OF THE PURINERGIC P2Y12-RECEPTOR IN MPTP-INDUCED PARKINSONISM IN MICE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Background: Parkinson's disease (PD) is a chronic, progressive neurodegenerative condition; characterized with the degeneration of the nigrostriatal dopaminergic pathway and neuroinflammation. During PD progression, microglia, the resident immune cells in the central nervous system (CNS) display altered activity, but their role in maintaining PD development has remained unclear to date. Aims: The purinergic $P2Y_{12}$ receptor ($P2Y_{12}R$), which is expressed on the microglia in the CNS has been shown to regulate microglial activity and responses; however, the function of the $P2Y_{12}R$ in PD is unknown. Results: We have found that MPTP-induced PD-symptoms in mice are associated with marked neuroinflammatory changes and $P2Y_{12}R$ contribute to the activation of microglia and progression of the disease. Surprisingly, while pharmacological or genetic targeting of the $P2Y_{12}R$ augments acute mortality in MPTP-treated mice, these interventions protect against the neurodegenerative cell loss and the development of neuroinflammation in vivo. Pharmacological inhibition of receptors during disease development reverses the symptoms of PD and halts disease progression. We found that $P2Y_{12}R$ regulates ROCK and p38 MAPK activity and control cytokine production. Conclusions: Our conclusion is that the receptor has a dualistic role in PD: functional $P2Y_{12}Rs$ are essential to initiate a protective inflammatory response, since the lack of the receptor leads to reduced survival; however, at later stages of neurodegeneration, $P2Y_{12}Rs$ are apparently responsible for maintaining the activated state of microglia and stimulating pro-inflammatory cytokine response. Improved understanding of the $P2Y_{12}R$-mediated actions in the CNS may reveal novel approaches to control neuroinflammation and modify disease progression in PD.

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BOARD NUMBER: S07-623

ANATOMO-RADIOLOGICAL CORRELATIONS IN PARKINSON'S DISEASE ANIMAL MODEL.

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Aims Advanced methods in neuroimaging analysis are providing new insights into the mechanisms underlying Parkinson's Disease (PD). They have shown predictive abilities in detecting early changes in the brain, and correlating them with different disease related symptoms. By using preclinical models of the disease, this work aims to decipher the tissular signatures of these imaging changes by establishing correlation with histological findings. Methods Rats receive a double bilateral intranigral injection of the AAV alpha-synuclein, then undergo several behavioral tests to evaluate motor and cognitive functions over a 4-month period. Three MRI acquisitions are held at 2-,10- and 18-weeks post-injection, including a whole-brain T2w and T2*w. Histological studies are led to evaluate dopaminergic degeneration, iron accumulation and alpha synuclein deposits in the brain. Results This PD model shows a moderate and progressive dopaminergic neurodegeneration, with 30% loss of TH+ neurons in the nigrostriatal pathway at 4-months post-injection. It also exhibits diffuse synucleinopathy in the brain. Behavioral tests reveal deficits in sensori-motricity, attention and visuo-spatial learning. Whole-brain VBM analysis show hypointense signals between MRI sessions 2 and 3, in the nigrostriatal pathway, the hippocampus, the fimbria, as well as the limbic, insular, and prefrontal cortex. Indeed, these regions are directly related to the motor and executive functions impaired. Conclusion Spatial and functional correlations between imaging analysis and behavioral & histological profiles of established PD animal models, has the potential to explain the changes observed in imaging on a cellular and molecular level, and to help better understand the physiopathology of the disease.

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EFFECTS OF DIRECTIONAL SUBTHALAMIC DEEP BRAIN STIMULATION ON GAIT AND BALANCE IN PARKINSON DISEASE PATIENTS

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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The effects of subthalamic deep brain stimulation (STN-DBS) on freezing of gait (FOG) and falls are variable across individual patients. The possibility to use directional STN-DBS with specific shaping of the volume of tissue activated represents a new therapeutic option. In this pilot study, we included 10 PD patients with FOG Off-dopa and tested the effects of directional STN-DBS versus single-ring STN-DBS on gait and balance disorders using gait recordings and validated clinical scales. Patients were assessed before surgery (Off/On-dopa) and after surgery with a randomized cross-over double-blind design with: 1) single-ring, 2) directional STN-DBS with current shaping including the central part of the STN (gait 'hot-spot'), 3) dorsolateral part of the STN (sensorimotor 'hot-spot'), 4) ventral part of the STN, or 5) outside and 5) Off STN-DBS. Up to now, 8 patients were assessed 6 months after surgery. In these patients, all active STN-DBS conditions improved motor disability, axial and GABS scores relative to Off STN-DBS condition. The clinical gait and balance disorders were significantly lower with the gait 'hot-spot' directional STN-DBS relative to the directional sensorimotor, ventral and outside conditions, with no significant difference with the single-ring condition. We also found better gait parameters with single-ring and directional gait 'hot-spot' STN-DBS relative to dorsolateral-sensorimotor directional STN-DBS, with lower number of FOG episodes and gait asymmetry during straight-forward gait, and higher step length. These preliminary result suggest that directional central-gait 'hot-spot' STN-DBS is more efficient than dorsolateral-sensorimotor STN-DBS to improve gait and balance disorders in PD patients.

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CORTICAL ACTIVITY RELATED TO SENSORIMOTOR SYNCHRONIZATION GUIDED BY DIFFERENT TYPES OF EXTERNAL CUES

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Sensorimotor synchronization (SMS) is a frequently used therapy in Parkinson's disease (PD). SMS involves the adaptation of automatic, repetitive movements (i.e. gait), to external, sensory stimuli, called cues. Its effectivity depends on the characteristics of the cue, for example, some patients benefit from visual cues (e.g. bars on the floor), while others prefer auditory cues (e.g. metronome beat). The aim of the current study is to evaluate the cortical activity related to different types of external cues, to assess the effect of the type, frequency and rhythmicity of cues. We hypothesize that the effects of different cues are related to the synchronization of different neuronal networks: the Basal Ganglia-Thalamo-Cortical (BGTC)-network (e.g. (pre-)frontal and sensorimotor cortex), and the Cerebellum-Thalamo-Cortical (CTC)-network (e.g. premotor and parietal cortex). Twenty-one healthy subjects performed a finger tapping experiment, guided by different cues in a 2x2x2-design, to evaluate the effect of: 1) cueing type: visual cues (flickering circle) vs. auditory cues (repetitive tones); 2) cueing frequency: discrete cues (1Hz) vs. continuous cues (3.2Hz); and 3) cueing rhythmicity: isorhythmic cues (one rhythm) vs. polyrhythmic cues (two rhythms in a [2:3]-relationship). A 32-channel EEG system recorded the electrocortical activity. Preliminary results show increased synchronization in cortical areas associated to the BGTC-network when using continuous or isorhythmic cues, and in areas associated to the CTC-network when using discrete or polyrhythmic cues. No differences are found between visual and auditory cues. The preliminary results show that the use of discrete and polyrhythmic cues may effectively bypass the defective BGTC-network in PD.

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BOARD NUMBER: S07-626

NARINGENIN MODULATES PARAQUAT-INDUCED OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION IN A CELLULAR MODEL OF PARKINSON'S DISEASE (SH-SY5Y CELLS)

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Parkinson's disease (PD), a common neurodegenerative disease is characterized by the progressive loss of dopaminergic neurons in the substantia nigra. The cause of PD onset remains unknown for a long time. However, recent reports suggest oxidative stress and mitochondrial dysfunction play key roles in the pathogenesis of PD. The drugs used for the treatment of PD show suboptimal results, and those that have shown some kind of neuroprotection in the animal models have failed to show any significant efficacy in clinical trials. Flavonoids are naturally occurring polyphenolic compounds that exhibit therapeutic properties in PD and do not show any side effects even in extended use. Naringenin (NAR), one such natural flavonoid, also has been reported to show neuroprotection against PD-related pathology. However, studies on its neuroprotective role and the underlying mechanisms are scarce, therefore the present study will explore the potential neuroprotective role of NAR in paraquat (PQ)-induced parkinsonism in SH-SY5Y cells. The present study determined the effect of NAR on PQ-induced cellular toxicity by measuring cell viability, oxidative stress, mitochondria membrane potential ($\Delta\Psi m$) and ATP levels in SH-SY5Y cells. Our results show that NAR treatment in SH-SY5Y cells resulted in increased cell viability, decreased oxidative stress, reduced the PQ-induced dysregulations in $\Delta\Psi m$ and increased mitochondrial ATP levels. In conclusion, NAR exhibits neuroprotection against PQ-induced neurotoxicity in SH-SY5Y cells indicating its therapeutic potential against PD. The development and identification of selective inhibitors based on these parameters will provide approaches for treating PD progression.

Pubmed:

34655599: Ahmad MH, Fatima M, Ali M, Rizvi MA, Mondal AC
Naringenin alleviates paraquat-induced dopaminergic neuronal loss in SH-SY5Y cells and a rat model of Parkinson's disease.
Parkinson's disease (PD), a common neurodegenerative disease is characterized by the progressive loss of dopaminergic neurons in the substantia nigra. The cause of dopaminergic loss in PD remains unknown for a long time, however, recent reports suggest oxidative stress plays a key role in the pathogenesis of PD. Paraquat (PQ), a widely used herbicide is an oxidative stress inducer that has been implicated as a potential risk factor for the development of PD. Flavonoids are naturally occurring polyphenolic compounds that display a variety of therapeutic properties against oxidative stress. Naringenin (NAR), a natural flavonoid, exhibits neuroprotection against PD-related pathology. However, studies on its neuroprotective role and the underlying mechanisms are scarce, therefore the present study explored the potential neuroprotective role of NAR in PQ-induced parkinsonism in SH-SY5Y cells and rat model. The effect of NAR on PQ-induced cellular toxicity was determined by measuring cell viability, oxidative stress, ATP levels and the same effect was determined by assessing behavioral, biochemical, immunohistochemical, qRT-PCR and Western blot in rat model. NAR treatment in SH-SY5Y cells resulted in increased cell viability, reduced oxidative stress, elevated mitochondrial membrane potential, and higher cellular ATP levels. In rats, NAR treatment resulted in significant neuroprotection against PQ-induced behavioral deficits, oxidative stress, mitochondrial dysfunction, and astrocytosis. NAR treatment significantly modulated PQ-induced mRNA expressions of DRD2, DAT, LRRK2, SNCA, β-catenin, caspase-3, BDNF genes. NAR treatment increased TH protein expression and modulated its immunoreactivity in rat striatum. Also, GFAP decreased in response to NAR treatment. So, in the present study, NAR exhibits neuroprotection against PQ-induced neurotoxicity and neurodegeneration indicating its novel therapeutic potential against PD.
Neuropharmacology, 2021; 201
33253761: Ahmad MH, Rizvi MA, Fatima M, Mondal AC
Pathophysiological implications of neuroinflammation mediated HPA axis dysregulation in the prognosis of cancer and depression.
Cancer patients are more likely to develop depressive symptoms and show a poor prognosis compared to the normal healthy

individuals. Cancer occurrence and the anticancer treatments result in the pro-inflammatory cytokines-mediated inflammation, which dysregulates the HPA-axis activity that may result in depression-like behaviour. Conversely, depression causes the activation of the HPA-axis that results in the downstream release of endogenous glucocorticoids which may result in depressive signs and symptoms in some cancer patients. Depression may also result in non-adherence to treatment and increased mortality in cancer patients. In this review, we have focused on the role of neuroimmune axis and hyperactive HPA-axis in case of both cancer and depression. Therefore, therapeutics targeting the HPA-axis dysregulation could be effective in ameliorating symptoms of depression in cancer patients.
Mol Cell Endocrinol, 2021; 520
32201282: Fatima M, Ahmad MH, Srivastav S, Rizvi MA, Mondal AC
A selective D2 dopamine receptor agonist alleviates depression through up-regulation of tyrosine hydroxylase and increased neurogenesis in hippocampus of the prenatally stressed rats.
Prenatal stress (PNS) has its negative impact on both the infant hippocampal neurogenesis and pregnancy outcomes in the neonates that serves as a risk factor for postnatal depression in adult offsprings. Therefore, main objectives of the present study were to evaluate the effect of maternal chronic unpredictable mild stress (CUMS) on behavioural changes, levels of oxidative stress, changes in selective developmental signaling genes and neurogenesis in the adult brain of Wistar rats and its reversal through a selective non-ergoline D2 type dopamine receptor (D2R) agonist Ropinirole (ROPI). Effects of ROPI treatment on CUMS induced adult rats offspring were measured by assessment of behavioural tests (sucrose preference test and forced swim test), biomarkers of oxidative stress, protein expression of tyrosine hydroxylase (TH), mRNA expression of SHH, GSK-3β, β-catenin, Notch, brain-derived neurotrophic factor (BDNF), Dopamine receptor 2 (Drd2) and bromodeoxyuridine (BrdU) cell proliferation assay. The oxidative stress, protein and mRNA expression were determined in the hippocampus and prefrontal cortex while the BrdU cell proliferation was observed in the hippocampus of rat brain. PNS induced changes resulted in depression validated by the depression-like behaviours, increased oxidative stress, decreased TH expression, altered expression of selective developmental genes, along with the reduced hippocampal neurogenesis and BDNF expression in the brain of adult offsprings. Chronic ROPI treatment reversed those effects and was equally effective like Imipramine (IMI) treatment. So, the present study suggested that ROPI can be used as an antidepressant drug for the treatment of depressive disorders.
Neurochem Int, 2020; 136
30718708: Fatima M, Srivastav S, Ahmad MH, Mondal AC
Effects of chronic unpredictable mild stress induced prenatal stress on neurodevelopment of neonates: Role of GSK-3β.
Prenatal stress (PNS) has gained attention with regard to its impact on hippocampal neurogenesis in neonates which serves as a risk factor for postnatal neurodevelopmental deficits. Evidences from animal models have suggested that depression responsive hypothalamic-pituitary-adrenal (HPA) axis and its hormonal response via cortisol, is responsible for critical neurodevelopmental deficits in the offspring which is transduced due to gestational stress. But knowledge in the area of assessing the effects of maternal chronic unpredictable mild stress (CUMS) on neurogenesis and expression of some key signaling molecules in the offsprings are limited. We have used Wistar rats to induce PNS in offsprings by maternal CUMS during pregnancy. Prefrontal cortex (PFC) and hippocampus were assessed for biomarkers of oxidative stress, neurogenesis, neurodevelopmental signaling molecules and DNA damage in the male Wister offsprings. Our investigations resulted in sufficient evidences which prove how maternal psychological stress has widespread effect on the fetal outcomes via major physiological alteration in the antioxidant levels, neurogenesis, signaling molecules and DNA damage. PNS leads to the upregulation of GSK-3β which in turn inhibited mRNA and protein expressions of sonic hedgehog (SHH), β-catenin, Notch and brain derived neurotrophic factor (BDNF). The study explored multifaceted signaling molecules especially, GSK-3β responsible for crosstalks between different neurodevelopmental molecules like SHH, Notch, BDNF and β-catenin affecting neurodevelopment of the offsprings due to PNS.
Sci Rep, 2019; 9
30605907: Ahmad MH, Fatima M, Mondal AC
Role of Hypothalamic-Pituitary-Adrenal Axis, Hypothalamic-Pituitary-Gonadal Axis and Insulin Signaling in the Pathophysiology of Alzheimer's Disease.
Alzheimer's disease (AD), the commonest progressive neurodegenerative disorder of the brain, is clinically characterized by the formation of extracellular amyloid plaques and intracellular neurofibrillary tangles. Recent studies suggest a relationship between the endocrinal dysregulation and the neuronal loss during the AD pathology. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and hypothalamic-pituitary-gonadal (HPG) axis regulating circulating levels of glucocorticoid hormones has been implicated in the pathophysiology of AD. Likewise, dysregulated insulin signaling, impaired glucose uptake and insulin resistance are some of the prime factors in the onset/progression of AD. In this review, we have discussed the changes in HPA and HPG axes, implicated insulin resistance/signaling and glucose regulation during the onset/progression of AD. Therefore, simultaneous detection of these endocrinal markers in the early or presymptomatic

stages may help in the early diagnosis of AD. This evidence for implicated endocrinal functions supports the fact that modulation of endocrinal pathways can be used as therapeutic targets for AD. Future studies need to determine how the induction or inhibition of endocrinal targets could be used for predictable neuroprotection in AD therapies.
Neuropsychobiology, 2019; 77

30595947: Ahmad MH, Fatima M, Hossain M, Mondal AC

Evaluation of naproxen-induced oxidative stress, hepatotoxicity and genotoxicity in male Wistar rats.

Naproxen (NP), a nonsteroidal anti-inflammatory drug (NSAID), is used for the treatment of common pain, inflammation and tissue damage. Genotoxicity testing of NP is of prime importance as it represents the largest group of drugs to which humans are exposed. Not many genotoxic studies are reported on NP; therefore, the present study investigated the detailed genotoxic and oxidative stress properties of NP. Male Wistar rats were administered NP orally at the doses of 38.91 and 65.78 mg/kg body weight for 14 days. Reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO) activities/levels were measured in the liver, kidney and brain tissues. The aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, and total bilirubin (TBIL) levels were measured in the liver tissues. Micronucleus frequency (micronucleus test MNT) and DNA damage (comet assay) were performed in the bone marrow cells and leukocytes, respectively. The results showed that NP treatment decreased the GSH levels and increased the SOD, CAT, LPO, ALT, AST, ALP and TBIL activities/levels compared to the control (0.05). Results of MNT showed an increased micronucleus induction and comet assay showed a significant increase in DNA damage in the NP treated animals (0.05). Treatment of NP resulted in the biochemical imbalance and induced oxidative stress that deteriorated the integrity of the cells, which caused significant damage to the genetic material and affected liver function in male Wistar rats. Therefore, NP is a potential genotoxic agent that induces genotoxicity and oxidative stress.
J Pharm Anal, 2018; 8

30385170: Ahmad MH, Fatima M, Mondal AC

Influence of microglia and astrocyte activation in the neuroinflammatory pathogenesis of Alzheimer's disease: Rational insights for the therapeutic approaches.

Alzheimer's disease (AD), the most common progressive neurodegenerative disorder is characterized by the formation of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs). Increasing evidences suggest a link between neuroinflammation and neuronal dysfunction in AD, orchestrated by the progressive activation of microglial cells and astrocytes with the consequent overproduction of proinflammatory molecules. The concomitant release of anti-inflammatory mediators antagonizes the inflammatory processes and leading to the severity of the AD pathology. The simultaneous detection of these inflammatory molecules in the pre-symptomatic stage may help in the early diagnosis of the AD. We have discussed the impact of microglia and astrocytic cells, the principal agents in the neuroinflammation process, in relation to the progression of the AD. Modulation of the risk factors and targeting of these immune mechanisms could lead to better therapeutic or preventive strategies for the AD. Further studies need to determine, how the inhibition of inflammatory factors could be used for the AD alternative therapies.
J Clin Neurosci, 2019; 59

30252991: Hilal Ahmad M, Fatima M, Hossain MM, Mondal AC

Determination of potential oxidative damage, hepatotoxicity, and cytogenotoxicity in male Wistar rats: Role of indomethacin.

The present study demonstrated the indomethacin (INDO) induced oxidative stress, hepatotoxicity, and genotoxicity in male Wistar rats. Animals were orally administrated INDO at doses of 0.302 and 0.605 (mg/kg b.w.) for 2 weeks. Reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) activities/levels were measured in the liver, kidney, and brain tissues. The aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, total bilirubin (TBIL) levels, and histopathological changes were determined in the liver tissues. Micronucleus frequency (micronucleus test) and DNA damage (comet assay) tests were performed in the bone marrow cells and leukocytes, respectively. Results show that INDO treatment decreased the GSH, SOD, and CAT levels/activities and increased the LPO, ALT, AST, ALP, and TBIL activities/levels. INDO induced significant hepatic injury and micronucleus and DNA damage. Thus, the current investigations confirm the oxidative stress, hepatotoxic, and genotoxic properties of INDO in the male Wistar rats.
J Biochem Mol Toxicol, 2018; 32

26191657: Fatima M, Usmani N, Firdaus F, Zafeer MF, Ahmad S, Akhtar K, Dawar Husain SM, Ahmad MH, Anis E, Mobarak Hossain M

In vivo induction of antioxidant response and oxidative stress associated with genotoxicity and histopathological alteration in two commercial fish species due to heavy metals exposure in northern India (Kali) river.

Heavy metals can significantly bioaccumulate in fish tissues. The step wise mechanism of heavy metal toxicities on fish health is still limited. The present study assessed the tissue-specific antioxidant response and oxidative stress biomarkers of commercially important fish species namely, Channa striatus and Heteropneustes fossilis inhabiting Kali River of northern

India where heavy-metal load is beyond the World Health Organisation - maximum permissible limits. Heavy metals chromium (Cr), nickel (Ni), lead (Pb) and cadmium (Cd) were elevated in both fish species compared to recommended values of the Federal Environmental Protection Agency (FEPA), 1999 for edible fishes. Reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CATA) activities in all tissues (brachial, neural, renal and hepatic) were altered. Cellular lipid and protein compromisation in both fishes induced by heavy metals was determined by lipid peroxidation (LPO) and protein carbonylation (PC) assays. Micronucleus (MN) test of erythrocytes and comet assay of liver cells confirmed genotoxicity. Histopathology of the liver, kidney and brain of affected fishes was distorted significantly with its reference fishes thereby affecting the quality and quantity of these fish stocks. This raises a serious concern as these fishes are consumed by the local population which would ultimately affect human health.
Comp Biochem Physiol C Toxicol Pharmacol, 2015 Oct-Nov; 176-177

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EFFECTS OF NATURAL AND SYNTHETIC CATECHOL-O-METHYL TRANSFERASE INHIBITORS ON TWO IN VIVO MODELS OF PARKINSON'S DISEASE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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In Parkinson's Disease (PD), the management of motor fluctuations aims at prolonging the effect of dopaminergic stimulation while reducing total levodopa (L-DOPA) load to avoid dyskinesia. Catechol-*O*-methyl transferase (COMT) inhibition reduces degradation of L-DOPA to 3-OMD and could prolong L-DOPA effectiveness by reducing both L-DOPA and dopamine metabolism. However, some COMT inhibitors like tolcapone and entacapone show hepatotoxicity and a real necessity to develop new drugs with a better toxic profile is highly desired. By studying the chemical constituents of a plant, *M. pruriens*, several tetrahydroisoquinoline compounds (THIQ) derived from L-DOPA were characterized and tested on several L-DOPA enzymes metabolism enzymes including COMT (BioCIS). Some compounds have shown an absence of toxicity and a COMT inhibitory activity similar to tolcapone. Firstly, we tested the COMT BioCIS inhibitors in an MPP+ *in vivo C. elegans* model, presenting partial loss of dopaminergic (DA) neurons and a dysfunctional specific dopamine-associated behavior. We evaluated the behavioral effect of COMT BioCIS inhibitors, comparing with tolcapone as a reference drug. After, we evaluated the different COMT inhibitors to observe if they could potentiate low doses of L-DOPA. Finally, we studied the most effective compound in an *in vivo* 6-OHDA rat model of Parkinson's disease. We were able to restore the behavioral deficit in the MPP^{+}*C. elegans* model not only with L-DOPA but also with tolcapone and COMT inhibitors. COMT inhibitors could potentiate L-DOPA effect and the most effective BioCIS drug had similar results to tolcapone. The administration of the selected drug in the 6-OHDA rat model could confirm our results by potentiating the effect of L-DOPA. Our results pave the way for the use of COMT inhibitors on L-DOPA-treated parkinsonian patients.

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6-OHDA-INDUCED PARKINSONISM IS AMELIORATED BY BOTH DEEP BRAIN STIMULATION OR GLUTAMATERGIC NEUROTRANSMISSION IN THE INFERIOR COLLICULUS

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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After unilateral lesion of the medial forebrain bundle (MFB) by 6-Hydroxydopamine (6-OHDA) rats exhibit lateralized deficits in spontaneous behavior or apomorphine-induced rotations. We investigated whether such lateralization is attenuated by either 30 Hz deep brain stimulation (DBS) or glutamatergic neurotransmission in the inferior colliculus (IC) of Wistar rats. For that, hemiparkinsonian rats were created by injecting 6-OHDA in the right MFB. Immediately after that injection, the same rats were also implanted with electrode or microinjection guide-cannula in the IC. One week later, rotatory behavior was assessed immediately after each rat had received apomorphine (s.c.). Intracollicular DBS did not affect spontaneous lateralization but attenuated apomorphine-induced rotations. Spontaneous lateralization disappeared after both glutamatergic antagonist MK-801 or the agonist NMDA microinjected in the IC. Apomorphine-induced rotations were potentiated by MK-801 but were not affected by NMDA intracollicular microinjection. Importantly, the side of the IC microinjection regarding the lesion (ipsi- or contralateral) is particularly important. Taken together, the present results support our hypothesis that the hypersensitivity caused by neurochemical lesion of nigro-striatal dopaminergic pathway can be influenced by IC 30 Hz DBS or glutamatergic intracollicular neurotransmission.

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LYSOSPHYNGOLIPIDS IN THE PATHOGENESIS OF PARKINSON'S DISEASE ASSOCIATED WITH MUTATIONS IN THE LRRK2 GENE

POSTER SESSION 07 - SECTION: PARKINSON'S DISEASE

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Introduction. Parkinson's disease (PD) is a common neurodegenerative disorder. G2019S mutation in leucine-rich repeat kinase 2 (*LRRK2*) is common genetic risk factor for PD. However, molecular mechanisms of PD associated with mutation in the *LRRK2 gene* remain unknown. Earlier, it was shown that the most common mutation in the *LRRK2* gene, G2019S, was associated with activity of glucoceresobridase (GCase) enzyme, encoded by the *GBA* gene, mutations in which are also common genetic risk factors for PD. **Aim.** To evaluate whether the alteration of lysosomal activites may play role in PD associated with mutation G2019S in the *LRRK2 gene* (*LRRK2*-G2019S-PD). **Materials and methods.** Blood samples of 6 patients with *LRRK2*-G2019S-PD, 180 sPD patients and 168 controls were generated. The enzymatic activities of GCase and also alpha-L-Iduronidase (IDUA), galactosylceramidase (GALC), alpha-galactosidase (GLA), acid sphingomyelinase (ASMase) and their substrates concentrations (hexosylsphingosine (HexSph), globotriaosylsphingosine (LysoGb3), lysosphingomyelin (LysoSM)) were measured by liquid chromatography tandem-mass spectrometry in blood. **Results**. No differences in enzyme activity of GCase, GLA, GAA, ASMase, IDUA, GALC in *LRRK2*-G2019S-PD patients with sPD and controls ($p>0.05$). However, GALC activity was elevated in sPD compared to controls ($p=0.009$). LysoSM concentration was decreased in sPD compared to controls ($p=0.0002$). While HexSph concentration was increased in LRRK2-G2019S-PD patients compared to sPD patients ($p=0.040$) and LysoGb3 concentration was increased in LRRK2-G2019S-PD patients than in sPD patients and controls ($p<0.05$). **Conclusion**s. Our results first demonstrated alteration of lysosphingolipid concentrations in LRRK2-G2019S-PD patients with increase of HexSph and LysoGb3 concentrations in LRRK2-G2019S-PD.
This study supported by RSF grant 22-25-00501.

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BOARD NUMBER: S07-630

FUNCTIONAL REORGANIZATION OF THE PERI-INFARCT CORTEX PROMOTING RECOVERY OF SKILLED MOTOR FUNCTIONS AFTER STROKE

POSTER SESSION 07 - SECTION: STROKE

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Once a stroke occurs, victims suffer from lifelong disabilities including the impairment of speech, vision and motor control. No pharmacological therapy is currently available to stimulate the restoration of function. However, forms of spontaneous recovery exist even without a therapeutic intervention. The principles underlying such increased plasticity, which can promote map-shifts and rewiring of neuronal circuitry, are not well understood. The brain region adjacent to stroke damage, the 'penumbra' zone, is critical for stroke rehabilitation. Thus, understanding the reorganization of neuronal circuits in the peri-infarct tissue around cortical strokes and its contribution to functional recovery with cellular resolution is crucial to develop new therapies. Whereas clinical trials are limited in resolution, chronic 2-photon calcium imaging in a mouse model of stroke opens new avenues to study cellular responses to the injury in relation to the functional deficit over time. We trained mice to run on an irregularly spaced ladder wheel, a task that demands flexible limb movements to successfully execute grasping actions (*Omlor et al, Nat Commun* ***10,*** *4812 (2019),* and simultaneously applied chronic 2-photon calcium imaging of motor cortex activity during learning and after a motor cortex stroke. Our preliminary data show how individual surviving neurons and whole neuronal populations reorganize and remap in the peri-infarct region and how they form new circuits to provide a neurobiological prerequisite for the restoration of lost motor function. Our results may enable the identification of key principles of neuronal repair and may thus lead to improved future rehabilitative strategies.

Pubmed:
35153684: Chakrabarti S, Nambiar J, Schwarz C
Adaptive Whisking in Mice.
Rodents generate rhythmic whisking movements to explore their environment. Whisking trajectories, for one, appear as a fixed pattern of whisk cycles at 5-10 Hz driven by a brain stem central pattern generator. In contrast, whisking behavior is thought to be versatile and adaptable to behavioral goals. To begin to systematically investigate such behavioral adaptation, we established a whisking task, in which mice altered the trajectories of whisking in a goal-oriented fashion to gain rewards. Mice were trained to set the whisker to a defined starting position and generate a protraction movement across a virtual target (no touch-related tactile feedback). By ramping up target distance based on reward history, we observed that mice are able to generate highly specific whisking patterns suited to keep reward probability constant. On a sensorimotor level, the behavioral adaptation was realized by adjusting whisker kinematics: more distant locations were targeted using higher velocities (i.e., pointing to longer force generation), rather than by generating higher acceleration (i.e., pointing to stronger forces). We tested the suitability of the paradigm of tracking subtle alteration in whisking motor commands using small lesions in the rhythmic whisking subfield (RW) of the whisking-related primary motor cortex. Small contralateral RW lesions generated the deterioration of whisking kinematics with a latency of 12 days post-lesion, a change that was readily discriminated from changes in the behavioral adaptation by the paradigm.
Front Syst Neurosci, 2021; 15

FENS Forum 2022
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BOARD NUMBER: S07-631

FIBRINOGEN REGULATES LESION BORDER-FORMING REACTIVE ASTROCYTE PROPERTIES AFTER VASCULAR DAMAGE

POSTER SESSION 07 - SECTION: STROKE

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Reactive astrocytes at the border of damaged neuronal tissue organize into a barrier surrounding the fibrotic lesion core, separating this central region of inflammation and fibrosis from healthy tissue. Astrocytes are essential to form the border and for wound repair but interfere with neuronal regeneration. However, the mechanisms driving these astrocytes during CNS disease are unknown. Here we show that blood-derived fibrinogen is enriched at the interface of lesion border-forming elongated astrocytes after cortical brain injury. Anticoagulant treatment depleting fibrinogen reduces astrocyte reactivity, extracellular matrix deposition and inflammation with no change in the spread of inflammation, whereas inhibiting fibrinogen conversion into fibrin did not significantly alter astrocyte reactivity, but changed the deposition of astrocyte extracellular matrix. RNA sequencing of FACS-isolated astrocytes of fibrinogen-depleted mice after cortical injury revealed repressed gene expression signatures associated with astrocyte reactivity, extracellular matrix deposition and immune-response regulation, as well as increased gene expression signatures associated with astrocyte metabolism and astrocyte-neuron communication. Systemic pharmacologic depletion of fibrinogen resulted in the absence of elongated, border-forming astrocytes and increased the survival of neurons in the lesion core after cortical injury. These results identify fibrinogen as a critical trigger for lesion border-forming astrocyte properties in CNS disease.

Pubmed:
34698916: Deshpande SS, Malik SC, Conforti P, Lin JD, Chu YH, Nath S, Greulich F, Dumbach MA, Uhlenhaut NH, Schachtrup C
P75 neurotrophin receptor controls subventricular zone neural stem cell migration after stroke.
Stroke is the leading cause of adult disability. Endogenous neural stem/progenitor cells (NSPCs) originating from the subventricular zone (SVZ) contribute to the brain repair process. However, molecular mechanisms underlying CNS disease-induced SVZ NSPC-redirected migration to the lesion area are poorly understood. Here, we show that genetic depletion of the p75 neurotrophin receptor (p75) in mice reduced SVZ NSPC migration towards the lesion area after cortical injury and that p75 NSPCs failed to migrate upon BDNF stimulation in vitro. Cortical injury rapidly increased p75 abundance in SVZ NSPCs via bone morphogenetic protein (BMP) receptor signaling. SVZ-derived p75 NSPCs revealed an altered cytoskeletal network- and small GTPase family-related gene and protein expression. In accordance, BMP-treated non-migrating p75 NSPCs revealed an altered morphology and α-tubulin expression compared to BMP-treated migrating wild-type NSPCs. We propose that BMP-induced p75 abundance in NSPCs is a regulator of SVZ NSPC migration to the lesion area via regulation of the cytoskeleton following cortical injury.
Cell Tissue Res, 2022; 387
33398338: Ferrari KJ, Amato S, Noberini R, Toscani C, Fernández-Pérez D, Rossi A, Conforti P, Zanotti M, Bonaldi T, Tamburri S, Pasini D
Intestinal differentiation involves cleavage of histone H3 N-terminal tails by multiple proteases.
The proteolytic cleavage of histone tails, also termed histone clipping, has been described as a mechanism for permanent removal of post-translational modifications (PTMs) from histone proteins. Such activity has been ascribed to ensure regulatory function in key cellular processes such as differentiation, senescence and transcriptional control, for which different histone-specific proteases have been described. However, all these studies were exclusively performed using cell lines cultured in vitro and no clear evidence that histone clipping is regulated in vivo has been reported. Here we show that histone H3 N-terminal tails undergo extensive cleavage in the differentiated cells of the villi in mouse intestinal epithelium. Combining

biochemical methods, 3D organoid cultures and in vivo approaches, we demonstrate that intestinal H3 clipping is the result of multiple proteolytic activities. We identified Trypsins and Cathepsin L as specific H3 tail proteases active in small intestinal differentiated cells and showed that their proteolytic activity is differentially affected by the PTM pattern of histone H3 tails. Together, our findings provide in vivo evidence of H3 tail proteolysis in mammalian tissues, directly linking H3 clipping to cell differentiation.
Nucleic Acids Res, 2021; 49

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BOARD NUMBER: S07-632

LACTATE RECEPTOR HCAR1 REGULATES NEUROGENESIS AND MICROGLIA ACTIVATION AFTER NEONATAL HYPOXIA-ISCHEMIA

POSTER SESSION 07 - SECTION: STROKE

Emilie Rylund Glesaaen[1,2], Lauritz Kennedy[1,2], Vuk Palibrk[3], Marco Pannone[1,3], Wei Wang[3], Ali Al-Jabri[1,2], Rajikala Suganthan[1], Niklas Meyer[1,2], Marie Austbø[1], Xiaolin Lin[1,3], Linda Bergersen[4,5], Magnar Bjørås[1,3], Johanne Rinholm[1,2]
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Neonatal cerebral hypoxia-ischemia (HI) is the leading cause of death and disability in newborns with the only current treatment being hypothermia. An increased understanding of the pathways that facilitate tissue repair after HI may aid the development of better treatments. Here we study the role of lactate receptor HCAR1 in tissue repair after HI in mice. We show that HCAR1 knockout mice have reduced tissue regeneration compared with wildtype mice. Proliferation of neural progenitor cells and glial cells, as well as microglial activation were impaired. Transcriptome analysis showed a strong transcriptional response to HI in the subventricular zone of wildtype mice involving about 7300 genes. In contrast, the HCAR1 knockout mice showed a modest response, involving about 750 genes. Notably, fundamental processes in tissue repair such as cell cycle and innate immunity were dysregulated in HCAR1 knockout. Our data suggest that HCAR1 is a key transcriptional regulator of pathways that promote tissue regeneration after HI.

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BOARD NUMBER: S07-633

A SUBCOMMISSURAL ORGAN-SPONDIN-DERIVED PEPTIDE (NX210C) IMPROVES RECOVERY OF SYNAPTIC TRANSMISSION AFTER CEREBRAL ISCHEMIA IN VITRO

POSTER SESSION 07 - SECTION: STROKE

Mélissa Sourioux[1], Sighild Lemarchant[1], Juliette Le Douce[1], Sandrine Hugues[2], Mélissa Farinelli[2], Yann Godfrin[1,3]
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Aim: Cerebral ischemia is characterized by a transient deprivation of oxygen and nutrients to the brain leading to neuronal dysfunction, cell death, and subsequent motor and cognitive impairments. Hence, there is a substantial need for solutions to prevent this condition or treat its consequences. The aim of this study was thus to evaluate the efficacy of a subcommissural organ-spondin-derived peptide (NX210c) to restore synaptic transmission following cerebral ischemia *in vitro*. **Methods:** Mouse hippocampal brain slices were submitted to oxygen-glucose deprivation (OGD) to mimic ischemia. NX210c (250 µg/mL) or vehicle was bath-applied either from the beginning or the end of OGD exposure. Functional recovery was assessed by recording field excitatory postsynaptic potentials (fEPSPs) evoked at Schaffer collateral-CA1 synapses. **Results/Conclusions:** While OGD causes synaptic depression, concurrent administration of NX210c improves recovery. Indeed, fEPSP slopes were significantly higher in NX210c-treated than in vehicle-treated slices within 30-60 minutes from OGD induction (fEPSP slope expressed as a % of baseline: 47.2±4.5 and 61.7±5.0 for vehicle and NX210c respectively, $p<0.05$) and this beneficial effect persisted for 80-90 minutes post-OGD (72.4±2.9 and 92.6±2.4 for vehicle and NX210c respectively, $p<0.001$). Staggeringly, NX210c was efficient even if added after OGD exposure (77.1±2.7 and 88.0±1.9 for vehicle and NX210c respectively, 90-120 minutes post-OGD, $p<0.01$). According to complementary studies, this beneficial effect of NX210c on the restoration of synaptic transmission could be explained by its preferential action on GluN2A-containing NMDA receptors. Finally, NX210c is a promising drug-candidate to improve neurological outcomes after ischemic insult.

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IMPACT OF TEMPORAL OXYGEN AND GLUCOSE DEPRIVATION ON NEONATAL ASTROCYTES CULTURED ON THE SELECTED BIOMATERIALS

POSTER SESSION 07 - SECTION: STROKE

Justyna Gargas, Justyna Janowska, Malgorzata Ziemka-Nalecz, Joanna Sypecka
Mossakowski Medical Research Institute Polish Academy of Sciences, Neurorepair Department, Warszawa, Poland

Aim: As the cells responsible for maintaining local homeostasis, astrocytes are very sensitive to any changes in their microenvironment. Temporary limitation of oxygen and glucose affects developing brain and is associated with perinatal asphyxia, which is one of the leading causes of child deaths under the age of 5-years. In this context, an *in vitro* model of perinatal asphyxia was used to determine the survival and morphology of neonatal astrocytes after transient oxygen and glucose deprivation (OGD). **Methods:** The primary mixed glial cultures were established from the neonatal rat brains, followed by cell culturing for 12 days prior to astrocyte isolation. The cells were then subjected to OGD procedure and cultured for the following 24h in physiologically normoxic condition (5% oxygen) on surfaces coated with poly-L-lysine or selected biomaterials: fibronectin, laminin and Matrigel. The cells were immunolabelled with lineage-specific markers and their expression was quantified in regard to applied biomaterials. **Results:** Cell adhesion to tested biomaterials had significant effects on astrocyte morphology, proliferation and expression of markers associated with cytoskeleton (GFAP), excitatory amino-acid transport (EAAT1) and glycogen synthesis and use (GS). The highest increase in the marker expression (by 362% for GFAP, 237% for GS) were observed when the OGD-subjected astrocytes were cultured on fibronectin. **Conclusions:** Culturing astrocytes on various biomaterials after OGD exerts strong impact on their morphology and proliferation. This observation could be especially useful when aiming at mimicking in vitro physiological conditions or creating selected disease-in-a-dish models for both basic research and pre-clinical studies.
Supported by ESF, POWR.03.02.00-00-I028/17-00

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BOARD NUMBER: S07-635

REMYELINATION OF DAMAGED AXONS AFTER ISCHEMIC STROKE USING A STEM-CELL-BASED APPROACH

POSTER SESSION 07 - SECTION: STROKE

Sara Palma Tortosa[1], Raquel Martinez-Curiel[1], Linda Jansson[1], Constanza Aretio Medina[1], Oleg Tsupykov[2], Galyna Skibo[2], Emanuela Monni[1], Olle Lindvall[1], Zaal Kokaia[1]
[1]Lund University, Lund Stem Cell Center, Lund, Sweden, [2]Bogomoletz Institute Bogomoletz Institute of Physiology and State Institute of Genetic and Regenerative Medicine, Department Of Cytology, Kiev, Ukraine

Stroke is the second most common cause of death and major cause of disability worldwide. Oligodendrocytes (OLs) are one of the most vulnerable cell populations in response to ischemia. Its death leads to demyelination of axons and neuronal disfunction leading to long-term sensorimotor and cognitive deficits. Here we aim to generate patient-specific OLs from skin cells and use them to repair demyelinated axons due to cerebral ischemia. For that, human fibroblast-derived induced pluripotent stem cells (iPSCs) primed to neural phenotype are transplanted into, first, the somatosensory cortex adjacent to an ischemic lesion in a rat stroke model, and second, human adult cortical organotypic slices. Characterization of grafted-derived OLs and axonal myelination was performed using immunohistochemistry followed by confocal and immuno-electron microscopy. In both, *human-to-rat* and *human-to-human* models, iPSCs-derived progenitors were able to generate OLs in different stages of differentiation. In rats, part of the human-derived OLs became mature in the core of the transplantation and in the corpus callosum. Also, transplantation increased endogenous oligodendrogenesis. Most importantly, iPSC-derived progenitors gave rise to mature OLs in a human-to-human grafted situation. We also observed graft-derived myelin surrounding axons of host neurons. In conclusion, iPSC-derived progenitors transplanted into an *in vivo* rat stroke model as well as *ex vivo* human system give rise to mature OLs which are involved in axonal myelination. This strategy will allow us to better understand the mechanisms of remyelination of damaged axons after ischemic stroke and to improve functional recovery after stroke recovery by means of stem cell-based approach.

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BOARD NUMBER: S07-636

INTEGRATION OF HUMAN EMBRYONIC STEM CELL-INDUCED NEURONS IN ISCHEMIC INJURED CORTEX

POSTER SESSION 07 - SECTION: STROKE

Raquel Martinez Curiel[1], Sara Palma Tortosa[1], Linda Jansson[1], Mazin Hajy[1], Berta Coll San Martin[1], Daniel Capitan Leo[1], Oleg Tsupykov[2], Galyna Skibo[2], Emanuela Monni[1], Olle Lindvall[1], Zaal Kokaia[1]
[1]Lund University, Lund Stem Cell Center, Lund, Sweden, [2]Bogomoletz Institute Bogomoletz Institute of Physiology and State Institute of Genetic and Regenerative Medicine, Department Of Cytology, Kiev, Ukraine

Stroke is one of the major causes of long-term disability and death in adult humans worldwide. Ischemic stroke is characterized by a shortage of glucose and oxygen to a specific area of the brain causing neuronal death, which leads to motor and cognitive impairments. The replacement of these neurons is essential for the functional recovery of patients. In this study, we aim to transplant human embryonic stem cells-induced neurons (hES-iNs) in an animal model of cortical stroke. hES-iNs are generated by ESCs programming inducing overexpression of NGN2 transcription factor. After 7 days of programming, cells were transplanted into the somatosensory cortex adjacent to a cortical ischemic lesion in rats subjected to cortical stroke. Characterization of the grafted neurons, their axonal myelination and synaptic inputs to them were performed using immunohistochemistry and immunoelectron microscopy (iEM). NGN2-programmed hESCs do not give rise to teratomas. Three months after transplantation ES-iN express markers of immature and mature neurons as well as markers for both, upper and deep cortical layers. We observed that programmed neurons send widespread axonal projections to the cortex of both hemispheres of stroke-subjected. More importantly, iEM data showed that ES-iNs receive functional synaptic inputs from host cortical neurons and their axons are myelinated by host-derived oligodendrocytes. In conclusion, hES-iNs transplanted into stroke-injured rat cortex survive, give rise to immature and mature neurons, express cortical layer markers, send widespread projections, and integrate in the host brain.

Pubmed:
29903545: López-Palacios N, Pascual V, Castaño M, Bodas A, Fernández-Prieto M, Espino-Paisán L, Martínez-Ojinaga E, Salazar I, Martínez-Curiel R, Rey E, Estrada L, Molero-Abraham M, Reche PA, Dieli-Crimi R, Núñez C
Evaluation of T cells in blood after a short gluten challenge for coeliac disease diagnosis.
To diagnose coeliac disease (CD) in individuals on a gluten free diet (GFD), we aimed to assess the utility of detecting activated γδ and CD8 T cells expressing gut-homing receptors after a short gluten challenge.
Dig Liver Dis, 2018; 50

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BOARD NUMBER: S07-637

FUNCTIONAL CHARACTERIZATION OF A NOVEL DUAL A2A/A2B ADENOSINE RECEPTOR ANTAGONIST ON CA1 SYNAPTIC PLASTICITY OR DURING OXYGEN GLUCOSE DEPRIVATION

POSTER SESSION 07 - SECTION: STROKE

Martina Venturini[1], Federica Cherchi[1], Clara Santalmasi[1], Lucia Frulloni[1], Daniela Catarzi[2], Vittoria Colotta[2], Flavia Varano[2], Felicita Pedata[1], Elisabetta Coppi[1], Anna Maria Pugliese[1]
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Background: Adenosine is a neuromodulator exerting its functions *via* four receptor subtypes, A_1, A_{2A}, A_{2B}, and A_3. The A_{2A} and A_{2B} receptors (A_{2A}Rs, A_{2B}Rs) are both coupled to Gs-protein and expressed at hippocampal level where they are known to inhibit paired-pulse facilitation (PPF), an electrophysiological paradigm whose reduction reflects an increase in presynaptic glutamate release. The selective antagonism of A_{2A}R or A_{2B}R protects from the irreversible synaptic failure induced by severe oxygen glucose deprivation (OGD) in the CA1 hippocampus and prevents or delays anoxic depolarization (AD) appearance, an unequivocal sign of neuronal injury, also counteracting CA1 neuronal loss, astrocyte activation and cytochrome C release. A multi-target pharmacological approach, which has become an increasingly pursued strategy for the cure of complex pathologies, included cerebral ischemia, could be advantageous for the treatment of stroke by blocking both A_{2A}Rs and A_{2B}Rs. **Aim**: We studied the effects of the newly synthesized multitarget A_{2A}R/A_{2B}R antagonist, P626, on PPF and AD development induced by severe OGD. **Methods**: Electrophysiological recordings were performed to monitor synaptic transmission and plasticity in the CA1 region of acutely isolated rat hippocampal slices. **Results**: The appearance of AD during a 30 minute OGD was significantly delayed by P626 (400 nM). Furthermore, P626 (200 nM) completely antagonized PPF reduction induced by the selective A_{2A}R agonist CGS21680 (50 nM) or the selective A_{2B}R agonist BAY60-6583 (200 nM). **Conclusions**: Data demonstrated the beneficial effect of P626, a multitarget antagonist of A_{2A}R/A_{2B}R, in preventing OGD-induced CA1 damage and in modulating glutamate release in this brain area.

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THE CONNECTION BETWEEN PRIMARY CILIA AND THE HYPOXIA-INDUCIBLE FACTOR-2ALPHA PROMOTES THE MEK/ERK SIGNALING PATHWAY

POSTER SESSION 07 - SECTION: STROKE

Tristan Leu, Jannik Denda, Anna Wrobeln, Joachim Fandrey
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Cell communication is essential for processes like proliferation, apoptosis, and differentiation. Primary cilia, which are antennae-like structures on the cell surface of most mammalian cells, including neural tissue, are necessary for these processes. Cilia are fundamental for cellular signaling and the realization of signaling pathways, such as Hedgehog, Wnt and TGF-β. Disruption of this signal transmission leads to a multitude of deadly human diseases, like the Joubert syndrome (JBTS), the Meckel–Gruber syndrome (MKS), or the Bardet–Biedl syndrome (BBS). This often involves deformations of the central nervous system, such as forebrain disorders, hydrocephalus or exencephaly. Especially in situations without suitable oxygen supply, it is necessary to provide relevant signals for counter-regulations quickly. One of the most important factors in such critical situations are hypoxia-inducible factors (HIFs). Especially HIF-2 is promoting neurogenesis and has a protective effect on neuronal stem cells. We showed by Co-Immunoprecipitation that intraflagellar transport protein 88 homolog (IFT88) directly interacts with HIF-2α, in neuronal cells. Furthermore, we have detected by immunofluorescence staining that HIF-2α accumulates under hypoxia in the ciliary axoneme and promotes ciliary elongation. qPCR analysis have proved that HIF-2α affects ciliary signaling by promoting the MEK/ERK signaling pathway. Neuronal cells without HIF-2α decrease transcription of *Mek1/2* and *Erk1/2*, and thus target genes of the MEK/ERK signaling pathway, such as *Fos* and *Jun*, are significantly decreased. These results suggest that under hypoxic conditions HIF-2α influences ciliary signaling by interacting with IFT88. This implies an unexpected and far more extensive function of HIF-2α than described before.

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BOARD NUMBER: S07-639

EFFECTS OF UROGUANYLIN'S SIGNALLING PATHWAY ON ISCHEMIC STROKE

POSTER SESSION 07 - SECTION: STROKE

Martina Ratko[1,2], Nikola Habek[1,2,3], Marina Dobrivojević Radmilović[1], Siniša Škokić[1], Helena Justić[1], Anja Barić[1], Aleksandra Dugandžić[1,2,3]
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Activation of guanylate cyclase (GC) A after ischemic stroke is neuroprotective. The aim is to investigate the effect of GC-C activation by uroguanylin (UGN) on ischemic stroke. MCAO was performed on GC-C knock out (GC-C KO), UGN KO mice and their WT littermates. MR images were acquired before and 24h after stroke. Ca^{2+} response was recorded in astrocytes 48h after MCAO. Systolic (SP), diastolic (DP) and mean arterial blood pressure (MAP) were recorded. GC-C KO develop significantly smaller ischemic lesions. Though they have higher SP, DP and MAP, blood flow or its reduction during MCAO did not differ between KO and WT. Unlike GC-C KO, UGN KO and WT have stronger Ca^{2+} response to UGN stimulation in peri-ischemic astrocytes and this difference may result in smaller lesions in GC-C KO. Stronger activation was explained when immunohistochemical staining showed GC-C expression in peri-ischemic astrocytes, while under normoxic conditions it is limited to neurons. GC-C activation leads to development of larger lesions. Its expression in peri-ischemic astrocytes causes stronger activation of the Ca^{2+}-dependent signalling pathway which could stimulate the Na^{+}/H^{+} exchanger causing tissue acidification and neuronal death. ACKNOWLEDGEMENTS: Research was funded by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project “Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain”; GAKK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund), “Young researchers' career development project – training of doctoral students” and BRADISCHEMIA (UIP-2017-05-8082) of Croatian Science Foundation funded by European Union’s European Social Fund.

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IN VIVO MESOSCALE IMAGING OF CONTRALESIONAL MOTOR CORTEX ACTIVITY DURING A GRASPING TASK IN MICE FOLLOWING STROKE

POSTER SESSION 07 - SECTION: STROKE

Matteo Panzeri[1,2], Jithin Nambiar[1,2], Mark-Aurel Augath[3], Fritjof Helmchen[1,2], Anna-Sophia Wahl[1,2,4]
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Stroke lesion size and location are major factors influencing the degree of sensorimotor impairment and the capacity for restoration of lost functions. While it is well established that the tissue immediately surrounding the stroke core undergoes functional reorganization and takes over part of the functionality lost to the stroke, the role of the contralesional, healthy motor cortex remains a matter of debate. Changes in neuronal activity on the contralesional side have been reported both in humans and in rodent stroke models. However, it remains unclear whether post-stroke functional modifications in contralesional motor cortex merely reflect maladaptation in functional connectivity, or whether spontaneous re-mapping of lost motor function takes place. To investigate this question, we developed a skilled reach-to-grasp task, in which mice manipulate a joystick with their right forelimb to receive a reward. Once mice are experts in the task, they receive a large photothrombotic stroke to the left sensorimotor cortex, followed by weekly testing in the task up to 8 weeks post-stroke. We perform bilateral recordings of layer 2/3 pyramidal neuron activity across the dorsal cortex using wide-field calcium imaging, while tracking forelimb trajectories with high-speed cameras. The goals of this pilot project are to characterize changes of grasp-related global L2/3 cortical activity patterns, to track the evolution of these changes over a critical post-stroke plasticity period of up to 2 months, and to evaluate how these changes relate to adjustments in grasping dynamics. The results gained here may reveal principles that could serve future refinements of rehabilitative strategies.

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THE PROTEIN KINASE C SIGNALING PATHWAY REGULATES HYPOXIA-INDUCED LTP OF NMDA NEUROTRANSMISSION IN VISUAL RETINOCOLLICULAR PATHWAY

POSTER SESSION 07 - SECTION: STROKE

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Hypoxia is the main accompanying factor of numerous diseases. Identification of the mechanisms underlying *the* early stage of hypoxic injury of the retinocollicular pathway will benefit the future treatment of the navigation, orientation, and visual attention impairments. We have previously reported that hypoxia-induced long-term potentiation (LTP) of NMDA retinocollicular transmission is associated with a decrease of the current decay time - an increase in NR2A/NR2B ratio. In this study, we tested the role of protein kinase C (PKC) signaling pathway in hypoxia-induced LTP. We developed the unique coculture of dissociated retinal cells and superficial superior colliculus neurons. Using paired patch-clamp technique, we recorded pharmacologically isolated NMDA neurotransmission under normal and hypoxic conditions *in vitro*. The presence of chelerythrine chloride (5 µM ChC - an inhibitor of PKC) completely blocked LTP of NMDA transmission induced by hypoxia. The ChC also abolished the hypoxia-induced increase of spontaneous NMDA currents amplitudes but did not affect the increased occurrence frequency. Moreover, we observed that ChC blocked the decrease of the decay time of evoked and spontaneous currents (control 48.2 ± 4.6ms; hypoxia 13.5 ± 5.4ms; reoxygenation in presence of ChC 46.2± 4.8ms; hypoxia in presence of ChC 45.8 ± 5.5 ms; reoxygenation 43.8 ± 5.6ms). Inhibition of PKC signaling pathway completely blocked LTP of NMDA retinocollicular transmission and lead to the offset of associated changes in NMDAR subunit composition. The revealed electrophysiological basis of LTP in response to hypoxic injury might be targeted to prevent lesions of the retinocollicular pathway.

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BOARD NUMBER: S07-642

BRAIN-WIDE MICROLESIONS AFFECT THE DEGENERATION OF MEMORY CIRCUITS IN THE HIPPOCAMPUS

POSTER SESSION 07 - SECTION: STROKE

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[1]University of Zurich, Brain Research Institute, Zurich, Switzerland, [2]University of Heidelberg, Central Institute Of Mental Health, Mannheim, Germany

While our memory determines our thinking and decisions, the deterioration of memory indicates a loss of self-concept and self-determination. 20% of stroke survivors exhibit cognitive decline and dementia-like symptoms, an impairment that is often misdiagnosed and under-researched. A typical early symptom of this vascular form of dementia is impaired spatial orientation. We here present a mouse model where we can study within the same mouse the formation of memory engrams for spatial orientation in the healthy condition and after induction of microlesions. Mice were trained to navigate in a virtual corridor while we performed 2photon calcium imaging in the CA1 region of the hippocampus. We tracked the activity of individual neurons and neuronal populations during the spatial navigation task in the healthy and in the pathological condition: After inducing brain-wide microinfarcts, we observed responses to the injury on a cellular and network level. We show that these distributed microlesions cause cognitive symptoms, while sparing motor function. Depending on their size and location, these lesions appear to impair spatial memory and induce substantial recoding and reorganization of surviving neurons to restore lost memory engrams. With our approach, we aim to elucidate how surviving neurons in the hippocampus rewire to compensate for the loss of others. An improved understanding of causal relationships between neural rewiring and recovery of cognitive features may open new options for novel therapeutic approaches or optimized cognitive rehabilitation strategies to treat patients with vascular dementia as a long-term consequence of ischemic insults.

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BOARD NUMBER: S07-643

THE EFFECTS OF SHORT-TERM HYPOXIA ON CA CURRENTS IN RETINAL GANGLION CELLS

POSTER SESSION 07 - SECTION: STROKE

Mariia Telka, Hanna Dumanska, Nickolai Veselovsky
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Hypoxia is a common factor of numerous ocular diseases such as glaucoma, diabetic retinopathy, age-related macular degeneration. These pathologies lead to damage of retinal ganglion cells (RGC) and optic nerve head to subsequent vision loss. It has been shown previously that short-term hypoxia induces bidirectional long-term plasticity in retinocollicular projections. Voltage-gated calcium channels (VGCC) are an important component of synaptic transmission. They also play a central role in different signaling processes, like enzyme activation, gene transcription, and the onset of apoptosis. The aim of this work was to identify and evaluate the effect of an early stage of hypoxia injury on VGCC in cultured RGC. Calcium currents were recorded in cultured rat RGCs using the whole-cell patch-clamp technique in voltage-clamp mode. The fast local superfusion was used for application (45 - 180 sec) of the hypoxic solution to simulate the short-term hypoxic states. The hypoxia effect induced a fast decrease of Ca current amplitude without kinetic changes in the majority of RGC (88%; 28 from 32). The level of inhibition was 25 ± 3% (from 8% to 67%) The repeated application of hypoxic solution did not lead to adaptation (rate of inhibition remained unchanged). We found that the reversibility of decrease depended on the duration of the hypoxia state. Our results show the inhibitory effect of hypoxia on VGCC in RGC. Thus, the hypoxia-induced reduction of calcium influx is a protective mechanism, on the other hand, it may affect the transmission of visual signals through presynaptic mechanisms in RGC projections.

Pubmed:
29537221: Telka MV, Rikhalsky OV, Veselovsky NS
EXCITABILITY PROPERTIES OF TRIGEMINAL GANGLION NEURONS.
The firing properties of small neurons (with diameters of soma less than 25 μm) were investigated using patch-clamp technique in whole-cell configuration in primary culture of trigeminal ganglia (TG) of postnatal rats. TG neurons were divided into three groups according to their firing responses to long-lasting depolarizing pulses: adaptive neurons (AN) characterized by a strongly adaptive responses; tonic neurons (TN) characterized by a multiple tonic firing; neurons with a delay before initiation of AP generation, namely, NDG. AN, TN and NDG also differed in AP electrophysiological and pharmacological characteristics. TN was distinguished by responses to hyperpolarization and the greatest value of input resistance. TN, AN and NDG were characterized by different active properties (amplitude of action potential and afterhyperpolarization, reobase, threshold). Each group of neurons was characterized by heterogeneity of AP duration and of frequency properties for TN. The application of tetrodotoxin (TTX) (250 nM) resulted in full or partial inhibition of AP generation and some neurons had TTX – insensitive firing responses. Neurons that were not affected by TTX had markedly longer AP. TTX had no effect on electrical activity of some AN and NDG. Based on sensitivity to TTX and their electrophysiological properties, AN and NDG seem to be C-fiber nococeptors.
Fiziol Zh (1994), 2016; 62

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-644

MULTIPARAMETRIC EVALUATION OF FUNCTIONAL OUTCOMES IN STROKE PATIENTS USING CONNECTOMICS

POSTER SESSION 07 - SECTION: STROKE

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Despite technological advances in medicine, stroke is still one of the leading causes of disability worldwide. The underlying physiology, extent of changes in neural network and recovery patterns are not fully understood. This ongoing longitudinal clinical study aims to provide a connectomics perspective on stroke recovery using multimodal quantitative tools to assess the rewiring of the functional and structural network and the correlates of motor function improvement. The study sample includes 25 ischemic and hemorrhagic stroke patients with upper extremity motor deficits, examined within 5 days, at 1 month and at 3 months after stroke onset. The control group consists of 25 age- and gender-matched healthy subjects. To quantify the surviving neural network, we use advanced high-resolution 7T MRI neuroimaging and 64-channel EEG. fMRI and DTI are used to estimate lesion volume and remaining nerve fiber connections. EEG is applied to assess cortical activation patterns. Degree of motor function damage and restoration are quantified via kinematic tools with 3D motion capture systems and EMG. Blood samples are collected to examine pro and anti-inflammatory responses and the regulation of specific microRNA transcriptional factors. Data will be stratified based on age, lesion volume and surviving neural network. We aim to provide links between biomarkers, functional motor outcomes and connectivity states to demonstrate adaptive or maladaptive responses. We endeavor to explain how neural network responses to stroke-induced changes and are modulated with restored function. With this knowledge, better rehabilitation strategies can be developed to maximize recovery for individual stroke patients.

Pubmed:

33482375: Spampinato D, Avci E, Rothwell J, Rocchi L
Frequency-dependent modulation of cerebellar excitability during the application of non-invasive alternating current stimulation.
it is well-known that the cerebellum is critical for the integrity of motor and cognitive actions. Applying non-invasive brain stimulation techniques over this region results in neurophysiological and behavioural changes, which have been associated with the modulation of cerebellar-cerebral cortex connectivity. Here, we investigated whether online application of cerebellar transcranial alternating current stimulation (tACS) results in changes to this pathway.
Brain Stimul, 2021 Mar-Apr; 14

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BOARD NUMBER: S07-645

THE ROLE OF MITOCHONDRIA IN THE MAINTENANCE OF HIPPOCAMPAL CELLS VIABILITY DURING THE POST-ISCHEMIC PERIOD

POSTER SESSION 07 - SECTION: STROKE

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Ischemic stroke is a major cause of disability and death worldwide, reflecting the extreme vulnerability of the brain to even brief disruption of blood flow. The brain sensitivity to oxygen-glucose deficiency is not identical in different cells and regions, thus the degree of damage varies. This study aimed to investigate the mitochondrial patterns of basic hippocampal cell types in the modelling of post-ischemic damage using slice cultures and transient oxygen-glucose deprivation (OGD) with subsequent normoxia. The changes were assessed at initial (reactive, 1h), intermediate (adaptive, 4h), and delayed (destructive, 24h) stages. The mitochondrial activity (MA) in pyramidal neurons, interneurons, astroglia, oligodendroglia and microglia were assessed using combined immunohistochemistry/MitoTrackerOrange staining. It was shown that at the reactive stage MA in all types of cells increased, which might indicate the activation of their metabolism. During the next two stages, the level of MA in pyramidal neurons and oligodendrocytes was significantly dropping while in interneurons, astroglia and microglia it was considerably increasing. There was a direct strong correlation between MA dynamics and cell viability. The electron microscopic analyses showed that under extreme conditions, direct intercellular interactions, including the exchange of cellular contents, were to a certain extent able to stabilize the state of the nervous tissue, which can be considered as emergency endogenous neuroprotection. Studying cellular responses induced by OGD is important for enhancing the effectiveness of neuroprotective strategies.

Pubmed:
34097225: Lushnikova I, Nikandrova Y, Skibo G
Mitochondrial Events Determine the Status of Hippocampal Cells in the Post-Ischemic Period.
Neurosci Bull, 2021; 37

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-646

SEROTONIN MODULATION OF MOTOR RECOVERY AFTER STROKE IN MICE

POSTER SESSION 07 - SECTION: STROKE

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Globally, one in four adults will suffer from a stroke during their lifetime. Among stroke survivors, there is a high prevalence of motor deficits. Despite best medical care, motor recovery remains often incomplete. One important repair mechanism, following hemiparesis, is the reorganization of spinal circuits that attract new synaptic innervation from supraspinal brain areas. However, little is known, how these rewiring processes are mediated at the circuit level, and how these processes can be enhanced therapeutically. Can pharmacogenetic up- or downregulation of spinal serotonin release modulate motor recovery in animal models of stroke? To answer these questions, we are using TPH2iCre mice combined with spinal cord AAV injections allowing the inhibition of the serotonin neurons in the raphe nucleus. After phototrombosis, the effect of serotonin inhibition will be assessed on motor recovery through different behavioral tasks such as the staircase and rotarod. These results will be able to generate novel insights towards the design of circuit specific neuromodulation strategies to improve motor recovery after stroke.

Pubmed:
27239348: Chevalier M, De Sa R, Cardoit L, Thoby-Brisson M
Mechanisms Underlying Adaptation of Respiratory Network Activity to Modulatory Stimuli in the Mouse Embryo.
Breathing is a rhythmic behavior that requires organized contractions of respiratory effector muscles. This behavior must adapt to constantly changing conditions in order to ensure homeostasis, proper body oxygenation, and CO2/pH regulation. Respiratory rhythmogenesis is controlled by neural networks located in the brainstem. One area considered to be essential for generating the inspiratory phase of the respiratory rhythm is the preBötzinger complex (preBötC). Rhythmogenesis emerges from this network through the interplay between the activation of intrinsic cellular properties (pacemaker properties) and intercellular synaptic connections. Respiratory activity continuously changes under the impact of numerous modulatory substances depending on organismal needs and environmental conditions. The preBötC network has been shown to become active during the last third of gestation. But only little is known regarding the modulation of inspiratory rhythmicity at embryonic stages and even less on a possible role of pacemaker neurons in this functional flexibility during the prenatal period. By combining electrophysiology and calcium imaging performed on embryonic brainstem slice preparations, we provide evidence showing that embryonic inspiratory pacemaker neurons are already intrinsically sensitive to neuromodulation and external conditions (i.e., temperature) affecting respiratory network activity, suggesting a potential role of pacemaker neurons in mediating rhythm adaptation to modulatory stimuli in the embryo.
Neural Plast, 2016; 2016

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NEURONAL-SPECIFIC CLEAVAGE OF SINGLE-CHAIN TPA: MECHANISMS AND CONSEQUENCES ON CELLULAR FATE.

POSTER SESSION 07 - SECTION: STROKE

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Aims: Tissue-type plasminogen activator (tPA) is a serine protease of the central nervous system that exhibits both neurotrophic and neurotoxic effects. tPA is secreted under its single-chain form (sc-tPA) and can be cleaved by plasmin or kallikrein in a two-chain form (tc-tPA). sc-tPA promotes *N*-Methyl-D-Aspartate receptors (NMDARs)'s calcium Influx under NMDA stimulation (Bertrand et al., 2015; Parcq et al., 2012), while tc-tPA, by activating the MET receptor, leads to the phosphorylation and internalization of NMDARs containing the GluN2B subunit (Hedou et al., 2021) and a decrease of NMDAR-signalling. Interestingly, .in a NMDA model of excitotoxicity, sc- and tc-tPA display bot pro-excitotoxicity and neuroprotection respectively. Methods: Pure mouse cortical neurons were treated with alexa-conjugated sc-tPA And the proportion of sc- and tc-tPA was evaluated by electrophoresis. Results: On 12 days in vitro (DIV) neurons, a time dependent cleavage of sc-tPA into tc-tPA is observed.. This phenomenon is inhibited by aprotinin and α2-antiplasmin, and enhanced by the lysine analogues epsilon-aminocaproic acid (EACA) and tranexamic acid (TXA). Almost No cleavage was observed with 7 DIV cortical neurons; and no cleavage at all was observed in astrocytes, HEK or PC12 cells, making this mechanism neuron-specific. Conclusions: These results indicate a cleavage mechanism that requires an activation surface specific to 12 DIV cortical neurons and dependant on the conversion of plasminogen into plasmin.

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BOARD NUMBER: S07-648

BETA-ALANINE PROTECTS THE CEREBELLAR NEURONS FROM ISCHEMIC CELL DEATH

POSTER SESSION 07 - SECTION: STROKE

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Ischemia-induced neuronal cell death is the cause of the high mortality attributed to stroke globally. Amongst multiple mechanisms contributing to neuronal cell death upon oxygen-glucose deprivation (OGD) that takes place in ischemia, glutamate-induced excitotoxicity is prevalent, causing neuronal hyperexcitation accompanied by disinhibition across various regions of the brain. Strategies to halt the ischemia-induced cell death include targeting unbalanced activation of glutamate receptors, i.e. NMDA receptors (NMDAR), or impaired function of inhibitory receptors, e.g. $GABA_A$ receptors ($GABA_AR$); however, it often leads to numerous side-effects. Thus, searching for a medicine that would counteract glutamate-induced excitotoxicity via different receptor types remains a focus of many studies. Here, we investigated potential therapeutic effects of the FDA-approved compound β-Alanine (β-Ala) that possesses the ability to suppress NMDAR while activating inhibitory receptors, both glycine and $GABA_AR$. For this, we have modelled the OGD-induced impairments in the cerebellum tissue to find that β-Ala protects cerebellar neurons upon OGD. Two-photon excitation imaging was used to quantify cell death for the two neuronal types in the cerebellum: granule cell (GC) layer and Purkinje cells (PC); electrophysiology was implemented to record membrane depolarization in both cell types in acute cerebellar slices. Our data revealed that β-Ala (1 mM) has reduced the magnitude of cell depolarization following OGD (GC: from ~12.0 mV to ~7.6, $p<0.001$; PC: from ~39 mV to ~24, $p<0.001$). β-Ala also prevented the OGD-induced neuronal cell death for both cell types. Together this demonstrates a strong neuroprotective effect of β-Ala during cerebral ischemia.

Pubmed:

34274950: Kopach O, Esteras N, Wray S, Abramov AY, Rusakov DA
Genetically engineered MAPT 10+16 mutation causes pathophysiological excitability of human iPSC-derived neurons related to 4R tau-induced dementia.
Human iPSC lines represent a powerful translational model of tauopathies. We have recently described a pathophysiological phenotype of neuronal excitability of human cells derived from the patients with familial frontotemporal dementia and parkinsonism (FTDP-17) caused by the MAPT 10+16 splice-site mutation. This mutation leads to the increased splicing of 4R tau isoforms. However, the role of different isoforms of tau protein in initiating neuronal dementia-related dysfunction, and the causality between the MAPT 10+16 mutation and altered neuronal activity have remained unclear. Here, we employed genetically engineered cells, in which the IVS10+16 mutation was introduced into healthy donor iPSCs to increase the expression of 4R tau isoform in exon 10, aiming to explore key physiological traits of iPSC-derived MAPT IVS10+16 neurons using patch-clamp electrophysiology and multiphoton fluorescent imaging techniques. We found that during late in vitro neurogenesis (from ~180 to 230 days) iPSC-derived cortical neurons of the control group (parental wild-type tau) exhibited membrane properties compatible with "mature" neurons. In contrast, MAPT IVS10+16 neurons displayed impaired excitability, as reflected by a depolarized resting membrane potential, an increased input resistance, and reduced voltage-gated Na- and K-channel-mediated currents. The mutation changed the channel properties of fast-inactivating Na and decreased the Na1.6 protein level. MAPT IVS10+16 neurons exhibited reduced firing accompanied by a changed action potential waveform and severely disturbed intracellular Ca dynamics, both in the soma and dendrites, upon neuronal depolarization. These results unveil a causal link between the MAPT 10+16 mutation, hence overproduction of 4R tau, and a dysfunction of human cells, identifying a biophysical basis of changed neuronal activity in 4R tau-triggered dementia. Our study lends further support to using iPSC lines as a suitable platform for modelling tau-induced human neuropathology in vitro.
Cell Death Dis, 2021; 12
34057756: Esteras N, Kopach O, Maiolino M, Lariccia V, Amoroso S, Qamar S, Wray S, Rusakov DA, Jaganjac M, Abramov AY
Mitochondrial ROS control neuronal excitability and cell fate in frontotemporal dementia.

The second most common form of early-onset dementia-frontotemporal dementia (FTD)-is often characterized by the aggregation of the microtubule-associated protein tau. Here we studied the mechanism of tau-induced neuronal dysfunction in neurons with the FTD-related 10+16 MAPT mutation.
Alzheimers Dement, 2022; 18
33375672: Kopach O, Pavlov AM, Sindeeva OA, Sukhorukov GB, Rusakov DA
Biodegradable Microcapsules Loaded with Nerve Growth Factor Enable Neurite Guidance and Synapse Formation.
Neurological disorders and traumas often involve loss of specific neuronal connections, which would require intervention with high spatial precision. We have previously demonstrated the biocompatibility and therapeutic potential of the layer-by-layer (LbL)-fabricated microcapsules aimed at the localized delivery of specific channel blockers to peripheral nerves. Here, we explore the potential of LbL-microcapsules to enable site-specific, directional action of neurotrophins to stimulate neuronal morphogenesis and synaptic circuit formation. We find that nanoengineered biodegradable microcapsules loaded with nerve growth factor (NGF) can guide the morphological development of hippocampal neurons in vitro. The presence of NGF-loaded microcapsules or their clusters increases the neurite outgrowth rate while boosting neurite branching. Microcapsule clusters appear to guide the trajectory of developing individual axons leading to the formation of functional synapses. Our observations highlight the potential of NGF-loaded, biodegradable LbL-microcapsules to help guide axonal development and possibly circuit regeneration in neuropathology.
Pharmaceutics, 2020; 13
32976770: Henneberger C, Bard L, Panatier A, Reynolds JP, Kopach O, Medvedev NI, Minge D, Herde MK, Anders S, Kraev I, Heller JP, Rama S, Zheng K, Jensen TP, Sanchez-Romero I, Jackson CJ, Janovjak H, Ottersen OP, Nagelhus EA, Oliet SHR, Stewart MG, Nägerl UV, Rusakov DA
LTP Induction Boosts Glutamate Spillover by Driving Withdrawal of Perisynaptic Astroglia.
Extrasynaptic actions of glutamate are limited by high-affinity transporters expressed by perisynaptic astroglial processes (PAPs): this helps maintain point-to-point transmission in excitatory circuits. Memory formation in the brain is associated with synaptic remodeling, but how this affects PAPs and therefore extrasynaptic glutamate actions is poorly understood. Here, we used advanced imaging methods, in situ and in vivo, to find that a classical synaptic memory mechanism, long-term potentiation (LTP), triggers withdrawal of PAPs from potentiated synapses. Optical glutamate sensors combined with patch-clamp and 3D molecular localization reveal that LTP induction thus prompts spatial retreat of astroglial glutamate transporters, boosting glutamate spillover and NMDA-receptor-mediated inter-synaptic cross-talk. The LTP-triggered PAP withdrawal involves NKCC1 transporters and the actin-controlling protein cofilin but does not depend on major Ca-dependent cascades in astrocytes. We have therefore uncovered a mechanism by which a memory trace at one synapse could alter signal handling by multiple neighboring connections.
Neuron, 2020; 108
32299835: Kopach O, Esteras N, Wray S, Rusakov DA, Abramov AY
Maturation and phenotype of pathophysiological neuronal excitability of human cells in tau-related dementia.
Frontotemporal dementia and parkinsonism (FTDP-17) caused by the 10+16 splice-site mutation in the gene encoding microtubule-associated protein tau () provides an established platform to model tau-related dementia Neurons derived from human induced pluripotent stem cells (iPSCs) have been shown to recapitulate the neurodevelopmental profile of tau pathology during corticogenesis, as in the adult human brain. However, the neurophysiological phenotype of these cells has remained unknown, leaving unanswered questions regarding the functional relevance and the gnostic power of this disease model. In this study, we used electrophysiology to explore the membrane properties and intrinsic excitability of the generated neurons and found that human cells mature by ~150 days of neurogenesis to become compatible with matured cortical neurons. In earlier FTDP-17, however, neurons exhibited a depolarized resting membrane potential associated with increased resistance and reduced voltage-gated Na- and K-channel-mediated conductance. Expression of the Na1.6 protein was reduced in FTDP-17. These effects led to reduced cell capability of induced firing and changed the action potential waveform in FTDP-17. The revealed neuropathology might thus contribute to the clinicopathological profile of the disease. This sheds new light on the significance of human models of dementia.
J Cell Sci, 2020; 133
32169106: Kopach O, Zheng K, Rusakov DA
Optical monitoring of glutamate release at multiple synapses in situ detects changes following LTP induction.
Information processing and memory formation in the brain relies on release of the main excitatory neurotransmitter glutamate from presynaptic axonal specialisations. The classical Hebbian paradigm of synaptic memory, long-term potentiation (LTP) of transmission, has been widely associated with an increase in the postsynaptic receptor current. Whether and to what degree LTP induction also enhances presynaptic glutamate release has been the subject of debate. Here, we took advantage of the recently developed genetically encoded optical sensors of glutamate (iGluSnFR) to monitor its release at CA3-CA1 synapses in acute hippocampal slices, before and after the induction of LTP. We attempted to trace release events at multiple

synapses simultaneously, by using two-photon excitation imaging in fast frame-scanning mode. We thus detected a significant increase in the average iGluSnFR signal during potentiation, which lasted for up to 90 min. This increase may reflect an increased amount of released glutamate or, alternatively, reduced glutamate binding to high-affinity glutamate transporters that compete with iGluSnFR.
Mol Brain, 2020; 13
29361548: Kopach O, Rybachuk O, Krotov V, Kyryk V, Voitenko N, Pivneva T
Maturation of neural stem cells and integration into hippocampal circuits - a functional study in an model of cerebral ischemia.
The hippocampus is the region of the brain that is most susceptible to ischemic lesion because it contains pyramidal neurons that are highly vulnerable to ischemic cell death. A restricted brain neurogenesis limits the possibility of reversing massive cell death after stroke and, hence, endorses cell-based therapies for neuronal replacement strategies following cerebral ischemia. Neurons differentiated from neural stem/progenitor cells (NSPCs) can mature and integrate into host circuitry, improving recovery after stroke. However, how the host environment regulates the NSPC behavior in post-ischemic tissue remains unknown. Here, we studied functional maturation of NSPCs in control and post-ischemic hippocampal tissue after modelling cerebral ischemia We traced the maturation of electrophysiological properties and integration of the NSPC-derived neurons into the host circuits, with these cells developing appropriate activity 3 weeks or less after engraftment. In the tissue subjected to ischemia, the NSPC-derived neurons exhibited functional deficits, and differentiation of embryonic NSPCs to glial types - oligodendrocytes and astrocytes - was boosted. Our findings of the delayed neuronal maturation in post-ischemic conditions, while the NSPC differentiation was promoted towards glial cell types, provide new insights that could be applicable to stem cell therapy replacement strategies used after cerebral ischemia.
J Cell Sci, 2018; 131
28729821: Rybachuk O, Kopach O, Krotov V, Voitenko N, Pivneva T
Optimized Model of Cerebral Ischemia for the Long-Lasting Assessment of Hippocampal Cell Death.
Among all the brain, the hippocampus is the most susceptible region to ischemic lesion, with the highest vulnerability of CA1 pyramidal neurons to ischemic damage. This damage may cause either prompt neuronal death (within hours) or with a delayed appearance (over days), providing a window for applying potential therapies to reduce or prevent ischemic impairments. However, the time course when ischemic damage turns to neuronal death strictly depends on experimental modeling of cerebral ischemia and, up to now, studies were predominantly focused on a short time-window-from hours to up to a few days post-lesion. Using different schemes of oxygen-glucose deprivation (OGD), the conditions taking place upon cerebral ischemia, we optimized a model of mimicking ischemic conditions in organotypical hippocampal slices for the long-lasting assessment of CA1 neuronal death (at least 3 weeks). By combining morphology and electrophysiology, we show that prolonged (30-min duration) OGD results in a massive neuronal death and overwhelmed astrogliosis within a week post-OGD whereas OGD of a shorter duration (10-min) triggered programmed CA1 neuronal death with a significant delay-within 2 weeks-accompanied with drastically impaired CA1 neuron functions. Our results provide a rationale toward optimized modeling of cerebral ischemia for reliable examination of potential treatments for brain neuroprotection, neuro-regeneration, or testing neuroprotective compounds .
Front Neurosci, 2017; 11
33438578: Jensen TP, Kopach O, Reynolds JP, Savtchenko LP, Rusakov DA
Release probability increases towards distal dendrites boosting high-frequency signal transfer in the rodent hippocampus.
Dendritic integration of synaptic inputs involves their increased electrotonic attenuation at distal dendrites, which can be counterbalanced by the increased synaptic receptor density. However, during network activity, the influence of individual synapses depends on their release fidelity, the dendritic distribution of which remains poorly understood. Here, we employed classical optical quantal analyses and a genetically encoded optical glutamate sensor in acute hippocampal slices of rats and mice to monitor glutamate release at CA3-CA1 synapses. We find that their release probability increases with greater distances from the soma. Similar-fidelity synapses tend to group together, whereas release probability shows no trends regarding the branch ends. Simulations with a realistic CA1 pyramidal cell hosting stochastic synapses suggest that the observed trends boost signal transfer fidelity, particularly at higher input frequencies. Because high-frequency bursting has been associated with learning, the release probability pattern we have found may play a key role in memory trace formation.
Elife, 2021; 10

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DISCOVERING NEW THERAPEUTIC STRATEGIES IN STROKE THROUGH APC/C-CDH1-ROCK2 SIGNALLING PATHWAY

POSTER SESSION 07 - SECTION: STROKE

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Aims Stroke is a leading cause of long-term adult disability and death in developed countries. Therefore, it is essential to establish new therapeutic strategies aimed at limiting and/or repairing ischemic neuronal damage. APC/C-Cdh1 is essential for neuronal survival. Previously, we described that Cdh1 regulates the balance between proliferation, neurogenesis and apoptosis. Neuronal reorganization after stroke determines the recovery of the lost brain function. ROCK2 regulates the morphology, elongation and retraction of neurites, making it an essential pathway in synaptic plasticity. Recently, we demonstrated that Cdh1 regulates ROCK2 levels and activity in cortical neurons, which modulates the stability of dendrites and synapses. However, whether Cdh1 plays a role in stroke remains unknown. Methods To assess this issue, we used both, an *in vitro* model of ischemia/reoxygenation (I/R) and an *in vivo* middle cerebral artery occlusion (MCAO) model in Cdh1-cKO and WT mice. Results We found that Cdh1 modulates neuronal susceptibility through RhoA-ROCK2 signalling pathway, since ROCK2 inhibition and knock-down reduced neuronal apoptosis induced by I/R. In vivo, we showed that Cdh1 was involved in the functional recovery after MCAO, enhancing neuronal survival and favouring infarct reduction and the recovery of neurological functions. Conclusions These results demonstrate that APC/C-Cdh1 actively modulates the neuronal response to I/R damage, enhancing neuronal survival through ROCK2 regulation, making them potential molecular targets for stroke treatment. Funded by Instituto de Salud Carlos III (PI21/00727; RD21/0006/0005); FEDER; Junta de Castilla y León (CSI151P20; co-financed with FEDER funds) and Bodegas R. López de Heredia Viña Tondonia.

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BOARD NUMBER: S07-650

INVESTIGATING THE MECHANISMS INVOLVED IN AUTOSIS-MEDIATED NEURONAL DEATH

POSTER SESSION 07 - SECTION: STROKE

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We previously provided evidence that autophagy (a physiological process of degradation of organelles and proteins) could be overactivated and involved in neuronal death in rodent models of perinatal cerebral hypoxia/ischemia (HI). We also reported the presence of autosis, a Na^+/K^+ ATPase-dependent type of autophagic cell death, in dying CA3 neurons of the hippocampus following perinatal cerebral HI. Aim: Since neuronal autosis could occur in dying neurons, we are now aiming to understand the mechanisms by which autosis leads to neuronal death. We here investigated two main hypotheses: 1) is-it due to an over degradation of specific proteins and/or organelles? or 2) is-it due to an excessive membrane recruitment for the formation of autophagosomes? Methods: We used neurotoxic doses of the pro-autophagy inducer Tat-BECN1 peptide and hypoxic/excitotoxic (KaHx) (6% O_2/kainate 30µM) stimulations to induce autosis in primary cortical neuronal cultures. Results: Our data suggests that the degradation step of autophagy is not involved in neuronal autosis because the inhibition of the lysosomal activity or the fusion between the autophagosome/lysosome with pharmacological (E64d/PepstatinA and Bafilomycin A1) and genetical (shRNA *stx17*) tools were not neuroprotective. Focusing on autophagosome membrane sources, we identified mitochondria and Golgi Apparatus as potent candidates as both organelles were significantly affected by Tat-BECN1 treatment. However, we also found that neuronal autosis is associated with an increased in golgiphagy but not mitophagy. Conclusion: Our results suggest that elucidating the precise mechanisms by which neuronal autosis is affecting mitochondria could lead to new neuroprotective targets for the treatment of perinatal asphyxia.

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TOPOGRAPHIC CHARACTERIZATION OF THALAMIC STROKES: CONTRIBUTIONS TO SLEEP STABILITY, TOPOGRAPHY AND COGNITION IN HUMANS AND MICE.

POSTER SESSION 07 - SECTION: STROKE

Jasmine Picard[1,2], Irene Lenzi[2,3], Valeria Jaramillo[4,5], Micaela Borsa[2], Irina Filchenko[1], Christina Czekus[2], Armand Mensen[6], Thomas Rusterholz[2], Julien Lipper[1], Niklaus Denier[7], Roland Wiest[6,8], Reto Huber[4,5], Markus H. Schmidt[1,2,7], Claudio Bassetti[1], Carolina Gutierrez Herrera[1,2]
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Introduction: Thalamic vascular syndromes result in a wide variety of clinical outcomes that may depend on the thalamic territory affected by the lesions. However, the contribution of each thalamic nucleus to the different sleep clinical outcomes is still not clear. Here we sought to investigate the relationship between the topography of the lesion, sleep-wake regulation and oscillatory activity in humans and mice. **Methods.** 15 thalamic stroke patients with age/gender-matched controls and o*ptical mini-stroke (OPTO-Stroke)* with SHAM control mice were included. Diffusion-weighted images were used for the categorization of thalamic lesions. High-density and frontal-parietal EEG recordings were used in humans and mice respectively to characterize sleep-wake architecture and oscillations. Anatomical thalamocortical connectivity in OPTO-stroke mice was performed. **Results:** Intralaminar (IL) or mediodorsal (MD) nuclei stroke patients showed increased sleepiness ($p<0.001$), NREM1 percentage and number of NREM2-NREM1 or NREM1-wake transitions ($p<0.01$) compared to controls, but not in lateral thalamic lesions. Remarkably, a frontal derivation decrease in spindle power was found in both humans and mice ($p<0.001$). Interestingly, medial thalamic strokes in mice showed decreased IL-prefrontal cortex connectivity that correlated with deficits in sleep, spindling and working memory and pain. **Conclusion:** These results suggest that IL/MD lesions, but not lateral thalamic lesions, resulting in sleep fragmentation and reduced frontal spindles. Based on the mouse model data, sleep, topography of spindles and working memory alterations may be due to changes in thalamocortical connectivity. Our work provides novel insights into thalamic sub-networks responsible for sleep-wake control and potentially identifies specific targets for sleep-related therapies.

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GAMMA OSCILLATIONS DEFICIT IN THE RECOVERING PERILESIONAL CORTEX : ORIGIN AND CORRECTION

POSTER SESSION 07 - SECTION: STROKE

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In the chronic phase following a cortical focal ischemia, recovery of lost functions involves timely remodelling of neuronal networks adjacent to the lesion. Due to an excessive GABA-mediated tonic inhibition, the perilesional region exhibits reduced excitability accounting for sub-optimal functional recovery. This peri-infarct zone also displays a prolonged deficit of low-gamma oscillation power (LG-power, 30-50Hz), a rhythm thought to synchronize neuronal activity and organize information processing. To optimize functional recovery, we hypothesized that peri-infarct LG-power should be normalized. AIM : Study the link between GABA-mediated hypoexcitability, LG-power and the ensuing subpar recovery. METHOD **:** While evaluating recovery of dexterous forepaw function and GABA-like immunoreactivity, we probed in vivo, the local field potential activity in the peri-infarct cortex of mice subjected to a focal cortical ischaemia at 7-, 21- and 4-months post stroke. RESULTS : LG-power was diminished in the peri-infarct zone at 7- and 21-days post-stroke. Interestingly, recovery of skilled forepaw use, positively correlated with LG-power. GABA-like immunoreactivity confirmed an excess of signal extending from lesion border paralleling a gradient of astrocyte reactivity. Local micro-infusion of GABA into the cortex, dampens LG-power while the pharmacological blockade of GABAergic tonic inhibition boosted them. Four months after stroke, GABA-like immunoreactivity, astrocyte reactivity and LG-power returned to sham-like levels. Overall, our observations suggest that the excess of tonic inhibition is involved in the collapse of LG-power in the peri-infarct cortex. Thus, neurostimulation interventions able to correct peri-infarct gamma-power, may boost post-stroke functional recovery.

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STROKE-INDUCED ALTERATIONS OF THALAMO-CORTICAL FUNCTIONS ASSESSED BY FUNCTIONAL ULTRASOUND IMAGING IN AWAKE RATS

POSTER SESSION 07 - SECTION: STROKE

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Ipsilateral thalamocortical diaschisis describes the reduction of thalamic function, metabolism, and perfusion resulting from a distant lesion of the ipsilateral hemisphere. We aimed to evaluate the perfusion characteristics and effect on cortico-thalamic activity in chronic middle cerebral artery stroke, not directly affecting the thalamus. Functional ultrasound imaging (fUSI) was used to monitor variations in relative cerebral blood volume (rCBV) compared to baseline. rCBV levels were analyzed brain-wide and continuously at high spatiotemporal resolution (100µm, 2Hz) until 3hrs after stroke onset in awake head-fixed rats. The occlusion of the middle cerebral artery was induced under awake conditions using $FeCl_3$, a chemothrombotic agent suited for permanently occluding the distal branch. Cortico-thalamic evoked responses were imaged during ipsi- and contra-lesional whisker stimulation. First, we characterized the features of both the ischemia and spreading depolarizations with fUSI under awake conditions. Second, we described how the thalamo-cortical pathway was functionally altered immediately after the stroke onset. We observed a secondary and delayed alteration of cortico-thalamic evoked responses a few days after the initial injury. Our procedure aims at early, continuous, and chronic evaluations of hemodynamics and brain functions after stroke toward the development of clinically relevant therapeutic strategies.

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CHARACTERIZATION OF A MOUSE MODEL OF POST-STROKE SPASTICITY

POSTER SESSION 07 - SECTION: STROKE

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Aims Spasticity develops in 35% of patients after stroke and is one of the most common causes of motor disability worldwide, its precise definition and pathophysiology remain elusive, which to date renders its experimental targeting tricky (Wieters et al. 2020). The aim was to develop a reliable mouse model of post-stroke spasticity. **Methods** Adult C57Bl/6 mice underwent an 8-week long experimental paradigm including repetitive Hoffmann reflex (H-reflex) recordings in the forepaw and behavioral testing (cylinder, grid walk, rotating beam test). Stroke was induced by photothrombosis (Aswendt et al. 2020) in various cortical sensory and motor areas as well as in combination with an implanted optical fiber in the internal capsule. For quantitative evaluation of microscopy data AIDAhisto was used (Pallast et al. 2019). Kinematic analysis was performed using Deeplabcut (Mathis et al. 2018). The H-reflex was quantified with the H/M ratio, which is known to be increased in spasticity. **Results** A classical spasticity phenotype was not detected. In primary and secondary motor cortex lesions there was no or only a moderate increase in H/M ratio. Highest H/M ratio developed in mice with internal capsular stroke. The kinematic analysis and random forest classification revealed a specific set of features for mice with increased H/M ratio independent on lesion type. Histological quantification confirmed expected patterns of reactive astrocytes and neuroinflammation surrounding the stroke lesions. **Conclusions** We here provide a reliable mouse model for post-stroke spasticity for future translational studies. Viral tracing and optogenetic dissection of neural circuits underlying the pathology are ongoing.

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MICROGLIAL PHAGOCYTOSIS DYSFUNCTION DURING STROKE IS PARTIALLY PREVENTED BY RAPAMYCIN

POSTER SESSION 07 - SECTION: STROKE

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Microglia are key players in mayor neurological disorders such as stroke, but they have been largely analyzed at the gene expression level, focusing on inflammatory polarization. In contrast, here we study a critical but widely overlooked microglial function: apoptotic cell phagocytosis, which prevents the spillover of cytotoxic contents from dying neurons and limits the inflammatory response. To analyze apoptotic cell phagocytosis in situ, we use as a model the neurogenic niche of the hippocampus, wherein we can establish baseline phagocytosis levels of naturally dying newborn cells by microglia. We show that microglial phagocytic function was impaired after ischemic stroke using an in vivo rodent model of transient medial cerebral artery occlusion (tMCAo) as well as an in vitro oxygen and nutrient deprivation model (OND) in hippocampal organotypic slices and primary microglia. We identified several mechanisms as drivers of the microglial phagocytic blockade after OND, including impaired microglial process motility, lysosomal alterations and the induction of an adaptive autophagy response. Autophagy inhibition had a deleterious effect on microglial phagocytosis and viability, determined using pharmacological (ULK1/2 inhibitor MRT68921) and genetic (ATG4B KO) approaches. In contrast, the autophagy inducer rapamycin prevented to some extent the microglial phagocytic blockade induced by tMCAo in vivo, although it did not significantly affect microglial phagocytosis in vitro. These results suggest that rapamycin partially prevents stroke-induced microglial phagocytic impairment possibly through an indirect, non-cell autonomous mechanism and pave the way to develop more specific strategies for the modulation of microglial phagocytosis.

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IMPACT OF FERRITIN LEVELS ON OUTCOMES AFTER ENDOVASCULAR THROMBECTOMY

POSTER SESSION 07 - SECTION: STROKE

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Background: In patients with large vessel occlusion (LVO) stroke treated with mechanical thrombectomy (MT), ferritin levels have not been explored thus far to assess clinical outcomes. We investigated the predictive value of serum ferritin levels in assessing clinical outcomes in acute stroke patients with LVO treated with MT. **Methods:** A total of 747 patients with LVO treated with MT were enrolled in this study. The primary outcome measure was a poor outcome at 3 months, defined as a modified Rankin Scale score of 3−6. Ferritin levels were analyzed as both categorical (in quartiles) and continuous (using a 2-log transformation) variables. **Results:** As the serum ferritin quartile increased, the number of patients having a poor outcome increased (46.5% vs. 57.2% vs. 58.3% vs. 65.1%; $P_{trend} = 0.004$). In multivariable regression analyses, the ferritin quartile had a significant association with a poor outcome ($P_{trend} < 0.001$). The highest ferritin quartile, Q4, was associated with a poor outcome compared to the Q1 group (odds ratio [OR] = 2.74, 95% confidence interval [CI]: 1.70−4.43, $p < 0.001$). When ferritin was analyzed as a continuous variable, a high ferritin level was also significantly associated with a poor outcome (OR = 1.50, 95% CI: 1.21−1.86, $p < 0.001$). **Conclusion:** High serum ferritin level is associated with a poor outcome at 3 months after MT in patients with LVO. Baseline ferritin level can be an independent predictor of functional outcome after MT.

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OPTIMISE DOSAGE AND EFFECT SIZE IN VIRTUAL REALITY-BASED INTERVENTION FOR POST-STROKE SURVIVORS WITH COEXISTENCE OF LANGUAGE AND MOTOR DYSFUNCTIONS

POSTER SESSION 07 - SECTION: STROKE

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In the last two decades, virtual reality (VR) techniques are emerging in post-stroke rehabilitation. Post-stroke patients suffer from impairments in verbal communication and motor. A wide co-existence of aphasia and motor dysfunction indicates that the two deficits could derive from similar brain lesions, specifically Broca's and motor cortex (Anderlini, Wallis & Marinovic, 2019). Motor rehabilitation could help the recovery of language function. This study examines the association between dose, intensity, and effect size in randomised controlled studies focusing on interventions aiming to improve language, speech, motor function respectively, or co-existence impairment. The study adopts the framework suggested by Baker (2012) and Warren et al. (2007) and referred dosage to the effect size of a study, reports and summarises dosage characteristics. Our analysis shows that "teaching episodes" and "dose form" are rarely reported in the included VR-based intervention studies. Specifically taking co-existing language and motor dysfunctions together, the dosage components are not related to the intervention effects size and the association is not statistically significant. This study concludes that future study needs to be able to relate dosage to an outcome, asking questions about the relationship between the different dosage characteristics and the intervention effect size. Furthermore, we propose that post-stroke survivors adopt nonverbal communication (NVC) to play as both a motor rehabilitator and as a facilitator of language recovery. NVC is the transmission of messages or signals through nonverbal behaviour such as eye contact, facial expressions, gestures, and posture.

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ROBOT-ASSISTED VOLUNTARY SHOULDER REHABILITATION TRAINING MODIFIES SYNERGETIC MUSCLE CONTROL IN A CHRONIC STROKE PATIENT: A CASE REPORT

POSTER SESSION 07 - SECTION: STROKE

Margaux Lafitte[1], Hideki Kadone[2], Masakazu Taketomi[3], Chun Kwang Tan[4], Yukiyo Shimizu[3], Masashi Yamazaki[5], Kenji Suzuki[2], Yasushi Hada[3]
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Aims: Paralysis of the upper limbs after a stroke is extremely detrimental for daily life and rehabilitation in chronic conditions is very challenging. Yet, active robotic assistive devices relying on biological signals appear promising for late rehabilitation. We aimed here to try a rehabilitation training using the newly developed EMG-triggered and intention-based shoulder Hybrid Assistive Limb (HAL) assistive exoskeleton in a chronic stroke patient. **Methods:** A 54 year-old woman suffering from right hemiparesis after a cerebral infarction in the left middle cerebral artery region underwent 10 sessions of HAL-assisted rehabilitation 2 years after onset. Each session consisted of performance evaluation and deltoid-triggered arm elevation training in the scapular plane with the HAL. Muscle activity was recorded with EMG and kinematics were recorded with 3D motion capture. **Results:** We report a lasting motor progression shown by an increase of range of motion (33.5° before rehabilitation, 57.3° after, and 64.3° at a 6 months follow-up) and of MMT scores (2 to 4). Muscle synergy analysis showed a better coordination of shoulder muscles and a more defined synergy distinction during arm raising/arm lowering (Fig. 1). **Conclusion:** The findings hint toward the efficiency of shoulder HAL therapy for rehabilitation of chronic stroke patients with hemiparetic shoulder dysfunctions. The presented analyses of motion and muscle activity suggest the changed

synergetic muscle control as a possible mechanism behind the

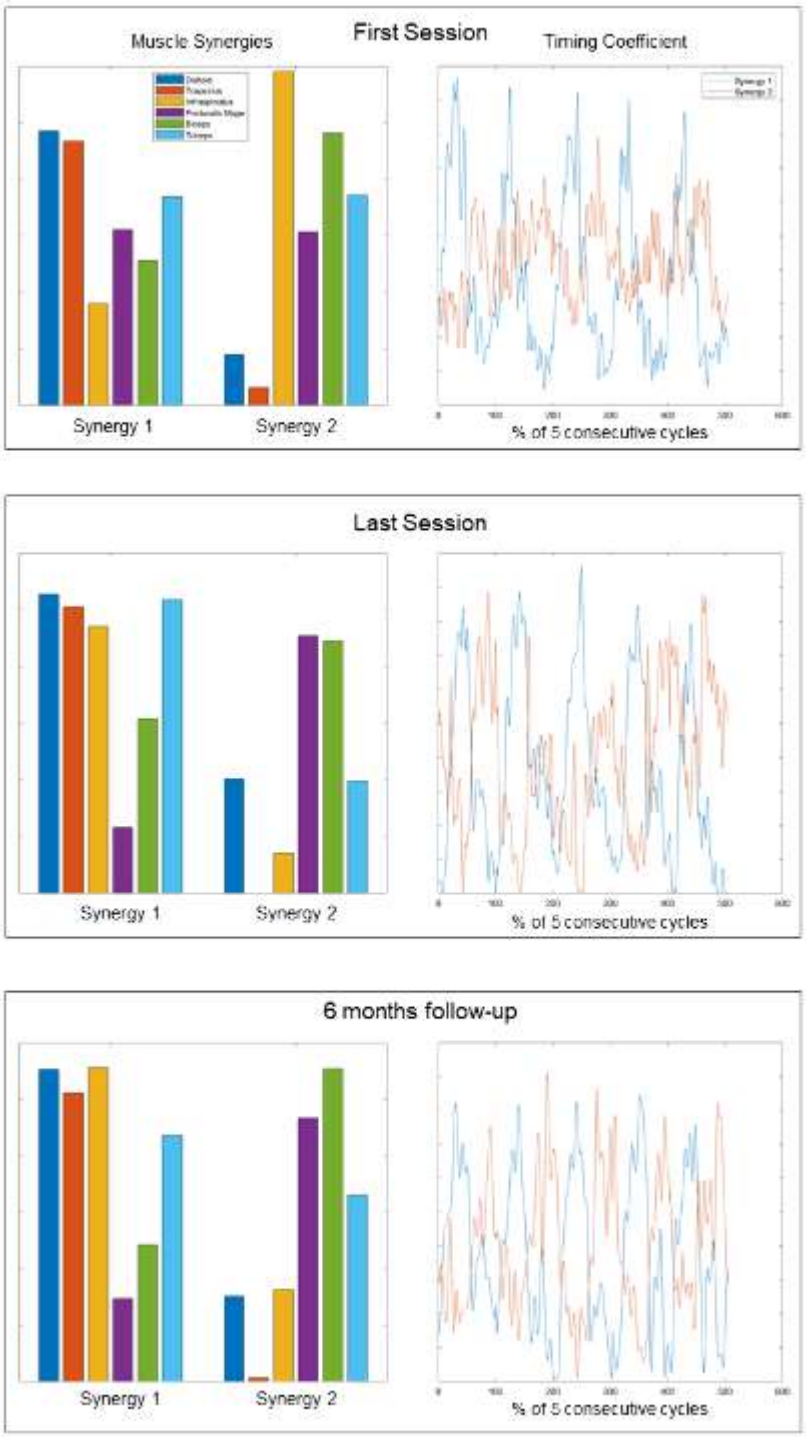

Figure 1: Muscle synergies and timing coefficients for the first session, last session, and 6 months follow-up.

improvement.

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DORSAL MENINGEAL LYMPHATIC VESSELS ARE INVOLVED IN RESOLUTION AND FUNCTIONAL CONNECTIVITY RECOVERY AFTER INTRACEREBRAL HEMORRHAGE

POSTER SESSION 07 - SECTION: STROKE

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Aims: Early intracranial hematoma drainage is crucial for the treatment of intracerebral hemorrhage (ICH). Currently, there are no available non-invasive strategies, which have been clinically approved as an aid in ICH resolution. The identification of novel therapeutic targets is therefore essential to develop safe and effective therapeutic strategies. Methods: Here we investigated the possible function of the meningeal lymphatic vessels (mLVs) in ICH resolution. To better identify the role of the mLVs we used two preclinical mouse models of ICH, mimicking focal cortical ischemic hemorrhage and subcortical extended hemorrhage, and characterized the dynamics of blood drainage through the dorsal mLVs. We also analyzed the effect of mLV drainage on the functional recovery after subcortical ICH, in mice presenting an acquired mLV deficit. Results: After ICH induction, we observed a specific flow of blood-derived components within the mLVs, suggesting that the meningeal lymphatics can play a role in facilitating the hemorrhage drainage. We also found that local formation of new mLVs is directly correlated with ICH-related neuroinflammation levels. Mice presenting acquired mLV dysfunction showed a delayed recovery after ICH and a drop of functional connectivity in the areas affected by the intracranial hematoma. Conclusion: These findings suggest that meningeal lymphatics are involved in the resolution of ICH and could provide a valuable therapeutic target.

Pubmed:

30394583: Nikolic L, Shen W, Nobili P, Virenque A, Ulmann L, Audinat E
Blocking TNFα-driven astrocyte purinergic signaling restores normal synaptic activity during epileptogenesis.
Epilepsy is characterized by unpredictable recurrent seizures resulting from abnormal neuronal excitability. Increasing evidence indicates that aberrant astrocyte signaling to neurons plays an important role in driving the network hyperexcitability, but the underlying mechanism that alters glial signaling in epilepsy remains unknown. Increase in glutamate release by astrocytes participates in the onset and progression of seizures. Epileptic seizures are also accompanied by increase of tumor necrosis factor alpha (TNFα), a cytokine involved in the regulation of astrocyte glutamate release. Here we tested whether TNFα controls abnormal astrocyte glutamate signaling in epilepsy and through which mechanism. Combining Ca imaging, optogenetics, and electrophysiology, we report that TNFα triggers a Ca -dependent glutamate release from astrocytes that boosts excitatory synaptic activity in the hippocampus through a mechanism involving autocrine activation of P2Y1 receptors by astrocyte-derived ATP/ADP. In a mouse model of temporal lobe epilepsy, such TNFα-driven astrocytic purinergic signaling is permanently active, promotes glial glutamate release, and drives abnormal synaptic activity in the hippocampus. Blocking this pathway by inhibiting P2Y1 receptors restores normal excitatory synaptic activity in the inflamed hippocampus. Our findings indicate that targeting the coupling of TNFα with astrocyte purinergic signaling may be a therapeutic strategy for reducing glial glutamate release and normalizing synaptic activity in epilepsy.
Glia, 2018; 66
31803019: Minkeviciene R, Hlushchenko I, Virenque A, Lahti L, Khanal P, Rauramaa T, Koistinen A, Leinonen V, Noe FM, Hotulainen P
MIM-Deficient Mice Exhibit Anatomical Changes in Dendritic Spines, Cortex Volume and Brain Ventricles, and Functional Changes in Motor Coordination and Learning.
In this study, we performed a comprehensive behavioral and anatomical analysis of the Missing in Metastasis (Mtss1/MIM) knockout (KO) mouse brain. We also analyzed the expression of MIM in different brain regions at different ages. MIM is an I-BAR containing membrane curving protein, shown to be involved in dendritic spine initiation and dendritic branching in Purkinje cells in the cerebellum. Behavioral analysis of MIM KO mice revealed defects in both learning and reverse-learning, alterations in anxiety levels and reduced dominant behavior, and confirmed the previously described deficiency in motor coordination and pre-pulse inhibition. Anatomically, we observed enlarged brain ventricles and decreased cortical volume.

Although MIM expression was relatively low in hippocampus after early development, hippocampal pyramidal neurons exhibited reduced density of thin and stubby dendritic spines. Learning deficiencies can be connected to all detected anatomical changes. Both behavioral and anatomical findings are typical for schizophrenia mouse models.
Front Mol Neurosci, 2019; 12

32508598: Suleimanova A, Talanov M, Gafurov O, Gafarov F, Koroleva K, Virenque A, Noe FM, Mikhailov N, Nistri A, Giniatullin R

Modeling a Nociceptive Neuro-Immune Synapse Activated by ATP and 5-HT in Meninges: Novel Clues on Transduction of Chemical Signals Into Persistent or Rhythmic Neuronal Firing.
Extracellular ATP and serotonin (5-HT) are powerful triggers of nociceptive firing in the meninges, a process supporting headache and whose cellular mechanisms are incompletely understood. The current study aimed to develop, with the neurosimulator NEURON, a novel approach to explore in silico the molecular determinants of the long-lasting, pulsatile nature of migraine attacks. The present model included ATP and 5-HT release, ATP diffusion and hydrolysis, 5-HT uptake, differential activation of ATP P2X or 5-HT3 receptors, and receptor subtype-specific desensitization. The model also tested the role of branched meningeal fibers with multiple release sites. Spike generation and propagation were simulated using variable contribution by potassium and sodium channels in a multi-compartment fiber environment. Multiple factors appeared important to ensure prolonged nociceptive firing potentially relevant to long-lasting pain. Crucial roles were observed in: (i) co-expression of ATP P2X2 and P2X3 receptor subunits; (ii) intrinsic activation/inactivation properties of sodium Nav1.8 channels; and (iii) temporal and spatial distribution of ATP/5-HT release sites along the branches of trigeminal nerve fibers. Based on these factors we could obtain either persistent activation of nociceptive firing or its periodic bursting mimicking the pulsating nature of pain. In summary, our model proposes a novel tool for the exploration of peripheral nociception to test the contribution of clinically relevant factors to headache including migraine pain.
Front Cell Neurosci, 2020; 14

33584640: Wojciechowski S, Virenque A, Vihma M, Galbardi B, Rooney EJ, Keuters MH, Antila S, Koistinaho J, Noe FM

Developmental Dysfunction of the Central Nervous System Lymphatics Modulates the Adaptive Neuro-Immune Response in the Perilesional Cortex in a Mouse Model of Traumatic Brain Injury.
The recently discovered meningeal lymphatic vessels (mLVs) have been proposed to be the missing link between the immune and the central nervous system. The role of mLVs in modulating the neuro-immune response following a traumatic brain injury (TBI), however, has not been analyzed. Parenchymal T lymphocyte infiltration has been previously reported as part of secondary events after TBI, suggestive of an adaptive neuro-immune response. The phenotype of these cells has remained mostly uncharacterized. In this study, we identified subpopulations of T cells infiltrating the perilesional areas 30 days post-injury (an early-chronic time point). Furthermore, we analyzed how the lack of mLVs affects the magnitude and the type of T cell response in the brain after TBI.
Front Immunol, 2020; 11

30674678: Coque E, Salsac C, Espinosa-Carrasco G, Varga B, Degauque N, Cadoux M, Crabé R, Virenque A, Soulard C, Fierle JK, Brodovitch A, Libralato M, Végh AG, Venteo S, Scamps F, Boucraut J, Laplaud D, Hernandez J, Gergely C, Vincent T, Raoul C

Cytotoxic CD8 T lymphocytes expressing ALS-causing SOD1 mutant selectively trigger death of spinal motoneurons.
Adaptive immune response is part of the dynamic changes that accompany motoneuron loss in amyotrophic lateral sclerosis (ALS). CD4 T cells that regulate a protective immunity during the neurodegenerative process have received the most attention. CD8 T cells are also observed in the spinal cord of patients and ALS mice although their contribution to the disease still remains elusive. Here, we found that activated CD8 T lymphocytes infiltrate the central nervous system (CNS) of a mouse model of ALS at the symptomatic stage. Selective ablation of CD8 T cells in mice expressing the ALS-associated superoxide dismutase-1 (SOD1) mutant decreased spinal motoneuron loss. Using motoneuron-CD8 T cell coculture systems, we found that mutant SOD1-expressing CD8 T lymphocytes selectively kill motoneurons. This cytotoxicity activity requires the recognition of the peptide-MHC-I complex (where MHC-I represents major histocompatibility complex class I). Measurement of interaction strength by atomic force microscopy-based single-cell force spectroscopy demonstrated a specific MHC-I-dependent interaction between motoneuron and CD8 T cells. Activated mutant SOD1 CD8 T cells produce interferon-γ, which elicits the expression of the MHC-I complex in motoneurons and exerts their cytotoxic function through Fas and granzyme pathways. In addition, analysis of the clonal diversity of CD8 T cells in the periphery and CNS of ALS mice identified an antigen-restricted repertoire of their T cell receptor in the CNS. Our results suggest that self-directed immune response takes place during the course of the disease, contributing to the selective elimination of a subset of motoneurons in ALS.
Proc Natl Acad Sci U S A, 2019; 116

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PLASMA LEVELS OF BDNF AND EGF ARE REDUCED IN ACUTE STROKE PATIENTS COMPARED TO HEALTHY AGE AND GENDER MATCHED CONTROLS.

POSTER SESSION 07 - SECTION: STROKE

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Annualy, stroke affects almost 14 million people worldwide. It is the second leading cause of death, and a major cause of acquired disability. Nearly 50 % of stroke survivers end up being chronically disabled. The degree of initial impairment in cognitive and motor functions affects the recovery, but other factors also contribute. These are largely unidentified, making precise prediction of recovery demanding. The variation in the balance of soluble regulators of neurotoxicity, neuroprotection and repair are presumably essential. Identifying blood biomarkers is a hot topic these days, and finding good predictive biomarkers for stroke outcome can ultimately be used to tailor treatment and follow-up of each stroke patient. Here we measure plasma levels of known regulators of neuroprotection and repair in acute ischemic stroke patients and compare them to the plasma levels in healthy age and gender matched controls. We found that the levels of BDNF and EGF were considerably lower in stroke patients than in healthy controls. These may be interesting biomarkers to predict stroke outcome. The levels of bFGF and irisin did not vary between the groups. The lower levels of growth factors emphasize that during the acute phase of stroke, there is a mismatch between the need for neuroprotection and repair, and the brain's ability to induce these processes. Large individual differences in growth factor levels were seen between the stroke patients in the acute phase, but whether these can be used to explain the individual differences in longterm post-stroke recovery and prognosis remain to be investigated.

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THE TRANSCRIPTION FACTOR SP1 MODULATES ROS-INDUCED ACCUMULATION OF WRAP53 AND NEUROPROTECTION AFTER ISCHEMIA

POSTER SESSION 07 - SECTION: STROKE

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Aims Reactive oxygen species (ROS) generated after ischemia promote DNA repair and neuronal survival by inducing the expression of WRAP53 (WD40 encoding RNA antisense to p53) [1]. The transcription factor Sp1 acts as a pleiotropic oxidative stress response protein in neurons and promotes neuroprotection against ischemia [2]. Interestingly, Wrap53 promoter contains putative consensus sequences (GC boxes) for Sp1. Hence, Sp1 might be a good candidate to modulate WRAP53-mediated DNA repair and neuronal survival after stroke. **Methods** Primary cortical neurons were subjected to *in vitro* ischemia (oxygen and glucose deprivation) and WRAP53 and Sp1 levels were modulated by lipotransfection. Protein detection was analyzed by confocal microscopy and WB. RT-PCR and CHIP analysis were also performed. Middle cerebral artery occlusion was used as an *in vivo* ischemic stroke model. **Results** Ischemia rapidly induced mRNA and protein Sp1 expression in neurons, which preceded WRAP53 upregulation. ROS-induced nuclear accumulation of Sp1 was observed both in primary cultured neurons and ischemic mouse brain (ipsilateral hemisphere). Wrap53 promoter region co-immunoprecipitated with anti-Sp1, suggesting Sp1 as a modulator of WRAP53 expression. Moreover, Sp1 downregulation by siRNA prevented WRAP53 accumulation. **Conclusions** Sp1 modulates ROS-induced WRAP53 expression after ischemia. This new ROS-Sp1-WRAP53 signaling pathway poses Sp1 and WRAP53 as attractive targets for neuroprotective strategies in ischemic stroke. Funding by ISCIII (FI19/00160, PI21/00727, RD21/0006/0005); FEDER; JCYL (CSI151P20; Escalera de Excelencia CLU-2017-03 Cofinanciado por P.O.FEDER de Castilla y León 14-20) [1] I.Sánchez-Morán, C.Rodríguez *et al.* Sci.Adv. (2020) 6:eabc5702 [2] S.H.Yeh, *et al*. NucleicAcidsRes. (2011) 39:5412–5423

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ASTROCYTIC SECRETION OF AUTOTAXIN DICTATES STROKE OUTCOME

POSTER SESSION 07 - SECTION: STROKE

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Acute ischemic stroke is a debilitating disorder with no effective treatments apart from the thrombolytic recombinant tissue plasminogen activator, which can be administered in a limited category of stroke patients. Following ischemic stroke, the ischemic core and the penumbra (peri-infarct area) experience progressive tissue damage and repair characterized by neuroinflammatory events. These include the recruitment and accumulation of activated GFAP+ astrocytes, which influence the penumbra by secreting factors and form a glial scar. Previously, we have shown that under physiological conditions autotaxin (ATX) is expressed by astrocytic processes at excitatory synapses regulating the bioactive phospholipid synthesis at the synaptic cleft (Thalman et al., Molecular Psychiatry, 2018). We therefore analyzed the role of the phospholipid lysophosphatidic acid (LPA) in the regulation of stroke-related cortical excitability and disease outcome. Using a mouse line with an astrocytic ATX-deletion and pharmacologically inhibiting ATX on one side and an animal model with a pre-existing LPA-related cortical excitability on the other, we could show that a dysregulation of synaptic LPA signaling exacerbates stroke pathology (Bitar et al., Sci Transl Med, 2022). Moreover, an animal model expressing a human single nucleotide polymorphism leading to an LPA-related cortical excitability (PRG-1^{R346T}) exhibited an overall unfavorable stroke outcome. Importantly, ATX levels remained elevated up to 14 days following stroke in patients. Our data suggest a critical importance of astrocytic secreted factors in stroke pathophysiology and inhibition of synaptic LPA signaling as a novel therapeutic target in stroke patients.

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POTENTIAL TARGETING OF STROKE BY MEANS OF LAYERED NANO- AND MICRO- PARTICLES

POSTER SESSION 07 - SECTION: STROKE

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Institute of Biochemistry named after H Buniatian, Nas Ra, Yerevan, Armenia

Background. Targeted delivery of the medicines is the most effective way to treat diseased tissues. We propose, in experimental settings the efficient delivery of Proline Rich Peptide (PRP; novel compound, serving for the neuroprotection), PP (general activator of Phosphoribosyl Pyrophosphate Synthase -1 (PRPS-1; EC=2.7.6.1), regulative enzyme of purines and pyrimidines syntheses) as well as allopurinol, inhibitor of Xanthine Oxidoreductase (XOR, which includes Xanthine Oxidase; XO (EC=1.17.1.4) and Xanthine Dehydrogenase XDH (EC 1.17.1.4), the regulative enzyme of purine catabolism) by means of PEG-ilated and not PEG-ilated albumin nanoparticles will possibly stimulate fast regeneration and protection of the brain tissue in the experimental stroke settings. **Methods.** For determination of the size of the particles it was used electron transmission microscopy technique (Philips CM-100 TM, USA). The loading and the controlled release of the medicines was evaluated by the colorimetric methods (Cary 60, Agilent, USA). Circulation abilities of loaded albumin particles were compared with PEG-ilated particles. Effect of the particles was evaluated in animals injected intracranially with paticles. **Results.** We created particles from <100 nm to < 2 microns diameter.. PEG-ilated particles are more stable in water as well as in the trypsin environment. Release of most of the medicines predominantly occurred during day 3th; the effect was stronger in not PEG-ilated partciles. It was detected BBB protection in case of utility of PEG-ilated and loaded with medicines particles. **Conclusion.** We created layered with the different medicines PEG-ilated and not PEG-ilated particles, which might target different pathological stages of experimental stroke development.

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TOWARDS IPS-BASED CELL THERAPY FOR BRAIN REGENERATION

POSTER SESSION 07 - SECTION: STROKE

Rebecca Weber[1], Patrick Perron[1], Chantal Bodenmann[1], Daniela Uhr[1], Debora Wanner[1], Kathrin Zürcher[1], Hirohide Saito[2], Simon Hoerstrup[1], Roger Nitsch[1], Christian Tackenberg[1], Ruslan Rust[1]
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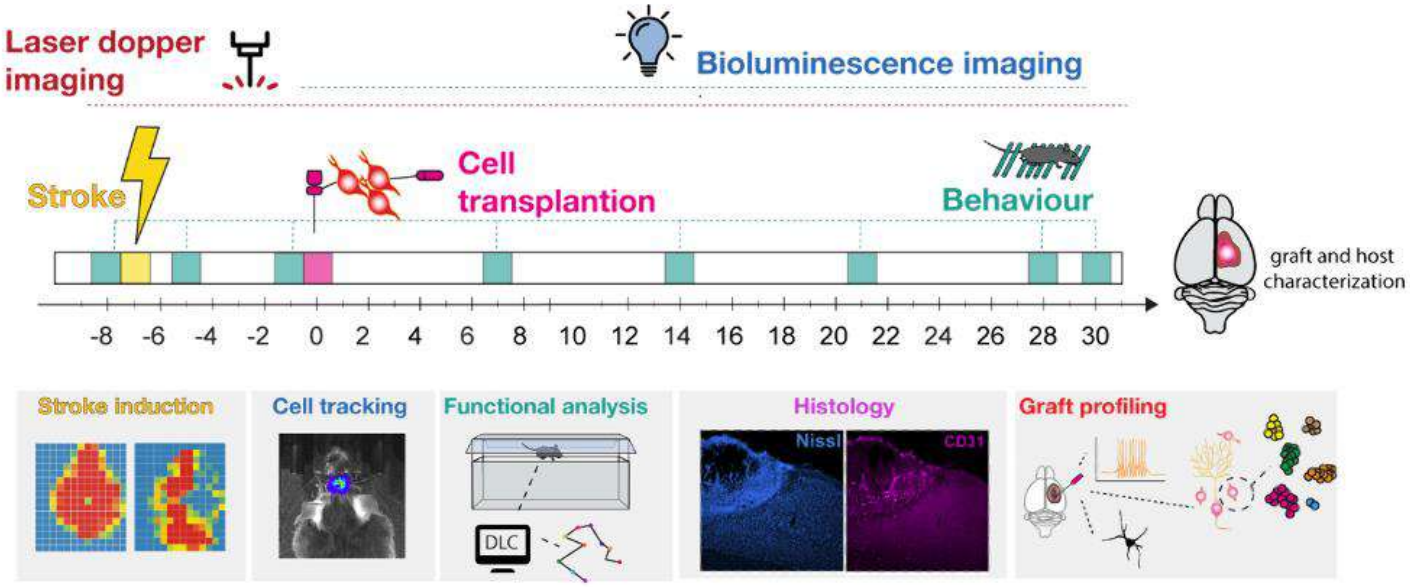
Stroke is a leading cause of disability and death worldwide. This is largely due to the lack of effective medical therapy that promotes long-term recovery. Cell-based therapy is an emerging treatment paradigm and is considered a potential regenerative strategy for stroke patients with remaining neurologic deficits. Despite promising preclinical results in animal stroke models, the efficacy of cell therapies has not been confirmed beyond doubt in a clinical setting. Here, we generate good manufacturing practice (GMP)-compatible neural progenitor cells (NPCs) from transgene- and xeno-free induced pluripotent stem cells (iPSCs) that can be smoothly adapted for clinical applications. The produced NPCs have a stable gene-expression over at least 15 passages and can be scaled for up to 10^{18} cells per initially seeded 10^{6} cells. To ensure a pure NPC population for *in vivo* applications, we reduce risks of iPSC contamination using micro RNA-switch technology as a safety checkpoint. The generated NPCs were transduced with a fluorescent and bioluminescent dual reporter-construct and locally transplanted into a photothrombotic mouse model of stroke. We confirmed long-term survival and migration of transplanted cells using non-invasive *in vivo* bioluminescence imaging over a month. Repeated deep learning-based functional assessments of animals was performed to evaluate the functional recovery following stroke compared to a control group. In-depth histological analysis revealed that the majority transplanted cells differentiate into mature neurons without any sign of pluripotent residuals. Animals receiving the cell therapy had a reduced stroke volume, increased recovery-associated tissue responses and higher recovery of motor function.



FENS Forum 2022
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BOARD NUMBER: S07-665

ELECTROPHYSIOLOGICAL FINGERPRINTING OF THE ABNORMAL BRAIN CAVITY

POSTER SESSION 07 - SECTION: STROKE

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Stroke is a highly debilitating neurological disorder affecting brain modalities such as vision, speech, and motor function. It remains the leading cause of chronic disability, and state-of-the-art therapies often fail to offer full recovery. In ischemic-stroke rodents, Ramanathan et al. (DOI: 10.1038/s41591-018-0058-y) showed decreased motor task-related delta oscillations correlated with functional recovery. However, much remains unclear about the altered neural activity of stroke and the abnormal brain cavity (aBC), the leftover lesion caused by stroke. A better understanding of altered neural activity following stroke is essential for novel therapy development. Here we investigated altered motor cortex (MC) dynamics in the aBC over time. In five male Sprague-Dawley rats, we mimicked the aBC by aspirating parts of the fore-limb MC. We performed acute and chronic *in-vivo* electrophysiological recordings in the peri-lesional cortex and aBC wall. In the acute setting, our results show a sudden loss in delta power compared to the healthy cortex. In the chronic setting, some regions recovered and resembled pre-lesion activity while others retained acute-stroke activity or deteriorated further. Our study reveals complex changes in neural activity after aBC formation in rodents. The heterogeneity of these findings needs to be addressed to deepen our knowledge of the complex nature of the stroke-related disability. Further targeting these changes in neural activity could pave the way for the development of novel therapies.

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BOARD NUMBER: S07-666

NOVEL INSIGHTS INTO HUMAN NEURAL STEM CELLS AND ADULT HIPPOCAMPAL NEUROGENESIS.

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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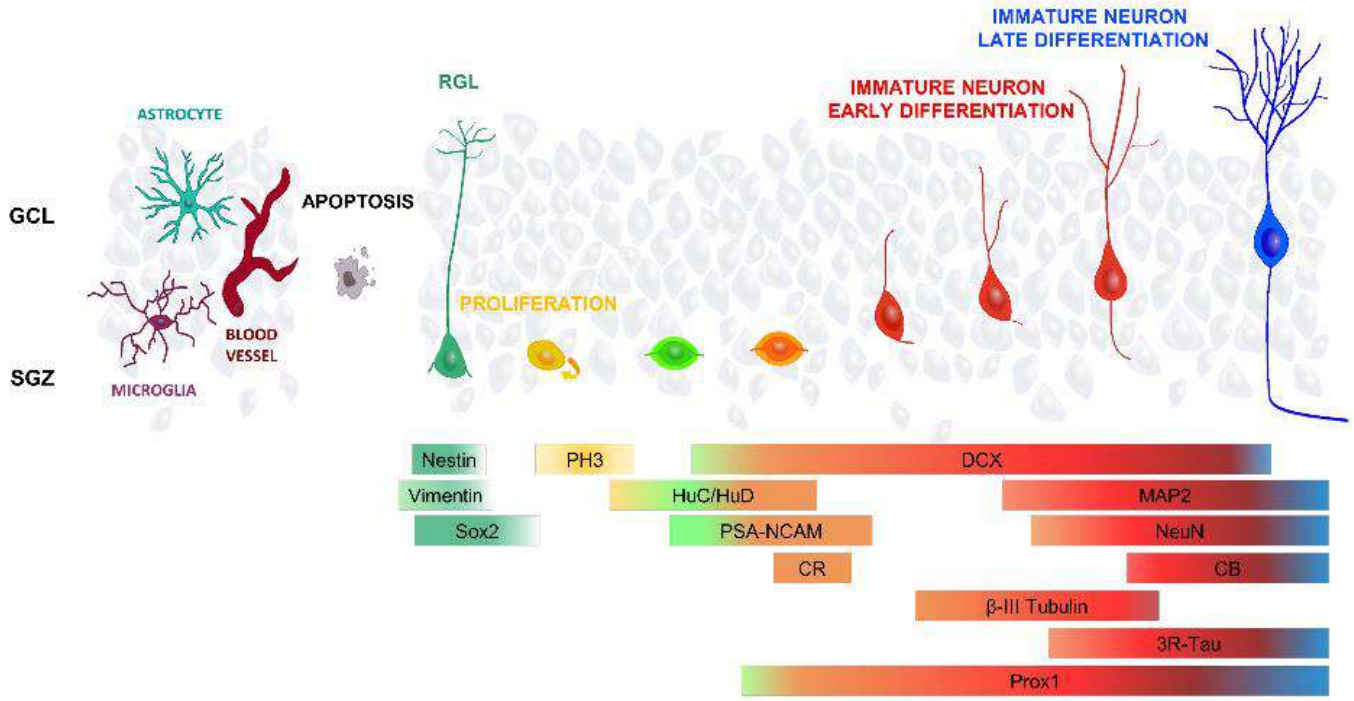
[1]Centro de Biología Molecular “Severo Ochoa” (CBMSO), Spanish Research Council (CSIC)–Universidad Autónoma de Madrid (UAM), Department Of Molecular Neuropathology, Madrid, Spain, [2]Faculty of Sciences, Universidad Autónoma de Madrid, Department Of Molecular Biology, Madrid, Spain, [3]Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Enfermedad De Alzheimer Y Otras Demencias Degenerativas, Madrid, Spain, [4]Centro en Investigación de Enfermedades Neurológicas, CIEN Foundation, Neuropathology Department, Madrid, Spain

Aims: The continuous addition of new neurons to the hippocampus through life (or adult hippocampal neurogenesis (AHN)) plays a crucial role in hippocampal functions, including mood regulation and pattern separation. AHN has been extensively studied in rodents and Old-World primates; however, this phenomenon remains less characterized in humans.Our group demonstrated the presence of immature dentate granule cells (DGCs) in the human hippocampus. However, the presence of neural stem cells (NSCs) in this structure remained elusive. **Methods:** We applied state-of-the-art tissue preservation and staining methodologies to reconstruct the stages encompassed by human AHN in 15 neurologically healthy control subjects. **Results:** Our results demonstrate the existence of a population of Nestin^{+} S100β^{-} radial glia-like cells with phenotypic and morphological NSCs properties in the human DG. We also observe proliferative cells and neuroblasts. The use of widely validated cell markers, and the application of stereological cell counts allowed reconstruction of the maturation trajectory of immature DGCs. Our work also provides a thorough analysis of all the elements that constitute the human hippocampal neurogenic niche, such as astrocytes, microglia, and blood vessels. Importantly, microglial function is impaired in aged subjects, which parallels with decreased AHN rates. **Conclusions:** Our results reveal the maintenance of the human NSC pool during adulthood. We have interrogated all the stages encompassed by human AHN, thereby shedding light on the cellular composition and age-driven remodeling of the human hippocampal neurogenic

niche.



Pubmed:

34672693: Terreros-Roncal J, Moreno-Jiménez EP, Flor-García M, Rodríguez-Moreno CB, Trinchero MF, Cafini F, Rábano A, Llorens-Martín M
Impact of neurodegenerative diseases on human adult hippocampal neurogenesis.
Disrupted hippocampal performance underlies psychiatric comorbidities and cognitive impairments in patients with neurodegenerative disorders. To understand the contribution of adult hippocampal neurogenesis (AHN) to amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, dementia with Lewy bodies, and frontotemporal dementia, we studied postmortem human samples. We found that adult-born dentate granule cells showed abnormal morphological development and changes in the expression of differentiation markers. The ratio of quiescent to proliferating hippocampal neural stem cells shifted, and the homeostasis of the neurogenic niche was altered. Aging and neurodegenerative diseases reduced the phagocytic capacity of microglia, triggered astrogliosis, and altered the microvasculature of the dentate gyrus. Thus, enhanced vulnerability of AHN to neurodegeneration might underlie hippocampal dysfunction during physiological and pathological aging in humans.
Science, 2021; 374
33955712: Flor-García M, Ávila J, Llorens-Martín M
GSK-3β S9A overexpression leads murine hippocampal neural precursors to acquire an astroglial phenotype in vivo.
The addition of new neurons to the existing hippocampal circuitry persists in the adult dentate gyrus (DG). During this process, named adult hippocampal neurogenesis (AHN), adult hippocampal progenitor cells (AHPs) give rise to newborn dentate granule cells (DGCs). The acquisition of a neuronal lineage by AHPs is tightly regulated by numerous signaling molecules and transcription factors. In this regard, glycogen synthase kinase 3β (GSK-3β) is a master regulator of the maturation of AHPs in vitro. Here we analyzed the cell-autonomous effects of overexpressing a constitutively active form of GSK-3β (GSK-3β S9A) in AHPs in vivo. To this end, we stereotaxically injected a GSK-3β S9A-encoding retrovirus (GSK-3β-V5) into the DG of young adult C57BL6/J Ola Hsd female mice and studied the cell lineage acquisition, migratory and marker expression patterns, and the morphological maturation of the infected cells over time. Strikingly, GSK-3β S9A-transduced cells expressed glial fibrillary acidic protein (GFAP) and NG2, thereby acquiring an immature astroglial phenotype, which differed markedly from the neuronal phenotype observed in cells transduced with a control retrovirus that encoded GFP.

Accordingly, the morphology and migration patterns of cells transduced by the two retroviruses are remarkably divergent. These observations support the role of GSK-3β as a cornerstone that regulates the balance between new astocytes/neurons generated in the adult murine DG.
Dev Neurobiol, 2021; 81
33762406: Moreno-Jiménez EP, Terreros-Roncal J, Flor-García M, Rábano A, Llorens-Martín M
Evidences for Adult Hippocampal Neurogenesis in Humans.
The rodent hippocampus generates new neurons throughout life. This process, named adult hippocampal neurogenesis (AHN), is a striking form of neural plasticity that occurs in the brains of numerous mammalian species. Direct evidence of adult neurogenesis in humans has remained elusive, although the occurrence of this phenomenon in the human dentate gyrus has been demonstrated in seminal studies and recent research that have applied distinct approaches to birthdate newly generated neurons and to validate markers of adult-born neurons. Our data point to the persistence of AHN until the 10th decade of human life, as well as to marked impairments in this process in patients with Alzheimer's disease. Moreover, our work demonstrates that the methods used to process and analyze postmortem human brain samples can limit the detection of various markers of AHN to the point of making them undetectable. In this Dual Perspectives article, we highlight the critical methodological aspects that should be strictly controlled in human studies and the robust evidence that supports the occurrence of AHN in humans. We also put forward reasons that may account for current discrepancies on this topic. Finally, the unresolved questions and future challenges awaiting the field are highlighted.
J Neurosci, 2021; 41
31915385: Flor-García M, Terreros-Roncal J, Moreno-Jiménez EP, Ávila J, Rábano A, Llorens-Martín M
Unraveling human adult hippocampal neurogenesis.
Adult neurogenesis occurs in a few selected regions of the mammalian brain. One such region is the hippocampus, the so-called gateway to memory, where adult hippocampal neurogenesis (AHN) occurs. Here, we provide a comprehensive description of the methods used in our laboratory to unambiguously detect a population of immature neurons in the human hippocampus until the 10th decade of life. The criteria used to refine and develop the current protocol include obtaining post-mortem human samples of remarkable quality and under tightly controlled conditions for immunohistochemistry (IHC) studies, optimizing tissue processing and histological procedures, establishing criteria to reliably validate antibody signal and performing unbiased stereological cell counts. Moreover, we provide a detailed description of the parameters that, in our view, should be reported in human AHN studies. The opposing results obtained by introducing slight variations in the methodological conditions should be considered by future studies that seek to increase our knowledge of this fascinating process. By applying simple and inexpensive tissue pre-treatments, this protocol, which can be completed in 7 days, might be applicable to a variety of IHC studies performed on other tissues of human (or animal) origin.
Nat Protoc, 2020; 15
31133559: Terreros-Roncal J, Flor-García M, Moreno-Jiménez EP, Pallas-Bazarra N, Rábano A, Sah N, van Praag H, Giacomini D, Schinder AF, Ávila J, Llorens-Martín M
Activity-Dependent Reconnection of Adult-Born Dentate Granule Cells in a Mouse Model of Frontotemporal Dementia.
Frontotemporal dementia (FTD) is characterized by neuronal loss in the frontal and temporal lobes of the brain. Here, we provide the first evidence of striking morphological alterations in dentate granule cells (DGCs) of FTD patients and in a mouse model of the disease, Tau mice. Taking advantage of the fact that the hippocampal dentate gyrus (DG) gives rise to newborn DGCs throughout the lifetime in rodents, we used RGB retroviruses to study the temporary course of these alterations in newborn DGCs of female Tau mice. In addition, retroviruses that encode either PSD95:GFP or Syn:GFP revealed striking alterations in the afferent and efferent connectivity of newborn Tau DGCs, and monosynaptic retrograde rabies virus tracing showed that these cells are disconnected from distal brain regions and local sources of excitatory innervation. However, the same cells exhibited a predominance of local inhibitory innervation. Accordingly, the expression of presynaptic and postsynaptic markers of inhibitory synapses was markedly increased in the DG of Tau mice and FTD patients. Moreover, an increased number of neuropeptide Y-positive interneurons in the DG correlated with a reduced number of activated egr-1 DGCs in Tau mice. Finally, we tested the therapeutic potential of environmental enrichment and chemoactivation to reverse these alterations in mice. Both strategies reversed the morphological alterations of newborn DGCs and partially restored their connectivity in a mouse model of the disease. Moreover, our data point to remarkable morphological similarities between the DGCs of Tau mice and FTD patients. We show, for the first time to our knowledge, that the population of dentate granule cells is disconnected from other regions of the brain in the neurodegenerative disease frontotemporal dementia (FTD). These alterations were observed in FTD patients and in a mouse model of this disease. Moreover, we tested the therapeutic potential of two strategies, environmental enrichment and chemoactivation, to stimulate the activity of these neurons in mice. We found that some of the alterations were reversed by these therapeutic interventions.
J Neurosci, 2019; 39
30911133: Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, Ávila J, Llorens-

Martín M
Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease.
The hippocampus is one of the most affected areas in Alzheimer's disease (AD). Moreover, this structure hosts one of the most unique phenomena of the adult mammalian brain, namely, the addition of new neurons throughout life. This process, called adult hippocampal neurogenesis (AHN), confers an unparalleled degree of plasticity to the entire hippocampal circuitry. Nonetheless, direct evidence of AHN in humans has remained elusive. Thus, determining whether new neurons are continuously incorporated into the human dentate gyrus (DG) during physiological and pathological aging is a crucial question with outstanding therapeutic potential. By combining human brain samples obtained under tightly controlled conditions and state-of-the-art tissue processing methods, we identified thousands of immature neurons in the DG of neurologically healthy human subjects up to the ninth decade of life. These neurons exhibited variable degrees of maturation along differentiation stages of AHN. In sharp contrast, the number and maturation of these neurons progressively declined as AD advanced. These results demonstrate the persistence of AHN during both physiological and pathological aging in humans and provide evidence for impaired neurogenesis as a potentially relevant mechanism underlying memory deficits in AD that might be amenable to novel therapeutic strategies.
Nat Med, 2019; 25
30251912: Sánchez-Maldonado B, Galicia ML, Rojo C, González-Gil A, Flor-García M, Picazo RA
Spheroids Spontaneously Generated In Vitro from Sheep Ovarian Cortical Cells Contain Integrating Cells That Exhibit Hallmarks of Neural Stem/Progenitor Cells.
Cell spheroids are inducible or spontaneously generated cell aggregates produced in vitro that can provide a valuable model for developmental biology, stem cell biology, and cancer therapy research. This investigation aimed to define the cellular identity of spheroids spontaneously generated in vitro from sheep ovarian cortical cells cultured under specific serum-free conditions. Spheroids were characterized during 21 days of culture by morphometric evaluation, detection of alkaline phosphatase (AP) activity, gene expression analyses of stemness transcription factors and several lineage markers, immunolocalization analyses, as well as assessment of self-renewal and differentiation potential. Cell aggregation, evidenced from day 3 of culture onward, resulted in efficient generation of 65-75 spheroids for every 500,000 cells seeded. The spheroids reached maximum diameter (187 ± 15.9 μm) during the second week of culture and exhibited AP activity. Sox2, Oct4, and Nanog were expressed throughout the culture period, with upregulation of Sox2. Neural lineage specification genes (eg, nestin, vimentin, Pax6, and p75NTR) were expressed from day 10 onward at levels above that of Oct4, Nanog and those for endoderm [alpha-fetoprotein (AFP)], and mesoderm (brachyury) specification. Neural stem cell (NSC)/neural progenitor cell (NPC) markers, nestin, Pax6, p75NTR, and vimentin, were extensively localized in cells on day 10, 15 (44.75% ± 5.84%; 93.54% ± 1.35%; 78.90% ± 4.80%; 73.82% ± 3.40%, respectively), and 21 (49.98% ± 5.30%; 91.84% ± 1.9%; 76.74% ± 11.0%; 95.80% ± 3.60%, respectively). Spheroid cell self-renewal was evidenced by cell proliferation and the generation of new spheroids during two consecutive expansion periods. Culture of spheroid cells under differentiation conditions gave rise to cells showing immunolocalization of the neuron-specific antigen NeuN and the astroglial antigen GFAP (glial fibrillary acidic protein). Our results indicate that spheroids spontaneously generated in this culture system were comprised of cells with molecular characteristics of NSC/NPC that can self-renew and differentiate into neurons and glia, supporting the identity of spheroids as neurospheres.
Stem Cells Dev, 2018; 27

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DEVELOPMENTAL ORIGIN OF ADULT NEUROGENESIS: ANALYSIS OF THE POSTNATAL HIPOCAMPAL NEUROGENIC NICHE IN SOX5 CONDITIONAL MUTANTS.

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Cristina Medina Menéndez, Lingling Li, Elena Melgarejo, Mario Diaz, María Valdés, Rafael Lopez, Inés Colmena, Véronique Lefebvre, Aixa V. Morales
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During embryonic and postnatal development of the dentate gyrus (DG), neural stem cells (NSCs) proliferate, migrate and generate mature granule neurons. In the DG a subpopulation of NSCs, are set aside in the subgranular zone (SGZ) and continues generating new granular neurons throughout adult life. One of the characteristics that distinguish adult NSCs most clearly from their embryonic counterparts is the acquisition of quiescence. While most of NSCs will remain in a dormant state of deep quiescence throughout life, the transitions back and forward from an active /proliferative state to a temporal shallow quiescence or resting state, ensure the lifelong maintenance of stem cell population. We have recently determined that SoxD transcription factors are required for the transition from quiescence to activation in NSCs and for the generation of new neurons in the adult SGZ (Li, Medina-Menéndez et al., 2022). Now, we have determined that abolishing Sox5 expression in DG during development (*Sox5*^{Nestin} mice) provokes a drastic decrease in NSCs proliferation during the first postnatal weeks and a decrease in neurogenesis. Surprisingly, by P30, *Sox5*^{Nestin} mice show an enhancement of NSCs proliferation, a reduction in NSCs quiescence and an increase in neurogenesis. Moreover, we have established that at P30 the transitions between a resting/superficial quiescence and active/proliferative state are severely altered in NSCs from Sox5^{Nestin} mice. Finally, by P150, the pool of NSCs and that of new neurons are reduced, indicating that in the absence of Sox5 the life-long maintenance of the adult neurogenic niche is compromised.

Pubmed:

35108528: Li L, Medina-Menéndez C, García-Corzo L, Córdoba-Beldad CM, Quiroga AC, Calleja Barca E, Zinchuk V, Muñoz-López S, Rodríguez-Martín P, Ciorraga M, Colmena I, Fernández S, Vicario C, Nicolis SK, Lefebvre V, Mira H, Morales AV
SoxD genes are required for adult neural stem cell activation.
The adult neurogenic niche in the hippocampus is maintained through activation of reversibly quiescent neural stem cells (NSCs) with radial glia-like morphology (RGLs). Here, we show that the expression of SoxD transcription factors Sox5 and Sox6 is enriched in activated RGLs. Using inducible deletion of Sox5 or Sox6 in the adult mouse brain, we show that both genes are required for RGL activation and the generation of new neurons. Conversely, Sox5 overexpression in cultured NSCs interferes with entry in quiescence. Mechanistically, expression of the proneural protein Ascl1 (a key RGL regulator) is severely downregulated in SoxD-deficient RGLs, and Ascl1 transcription relies on conserved Sox motifs. Additionally, loss of Sox5 hinders the RGL activation driven by neurogenic stimuli such as environmental enrichment. Altogether, our data suggest that SoxD genes are key mediators in the transition of adult RGLs from quiescence to an activated mitotic state under physiological situations.
Cell Rep, 2022; 38
31825616: Zaldivar-Diez J, Li L, Garcia AM, Zhao WN, Medina-Menendez C, Haggarty SJ, Gil C, Morales AV, Martinez A
Benzothiazole-Based LRRK2 Inhibitors as Wnt Enhancers and Promoters of Oligodendrocytic Fate.
Leucine rich repeat kinase 2 (LRRK2) is an enigmatic enzyme and a relevant target for Parkinson's disease (PD). However, despite the significant amount of research done in the past decade, the precise function of LRRK2 remains largely unknown. Moreover, the therapeutic potential of its inhibitors is in its infancy with the first clinical trial having just started. In the present work, the molecular mechanism of LRRK2 in the control of neurogenesis or gliogenesis was investigated. We designed and synthesized novel benzothiazole-based LRRK2 inhibitors and showed that they can modulate the Wnt/β-catenin signaling pathway. Furthermore, compounds and were able to promote neural progenitors proliferation and drive their differentiation toward neuronal and oligodendrocytic cell fates. These results suggest potential new avenues for the application of LRRK2 inhibitors in demyelinating diseases in which oligodendrocyte cell-death is one of the pathological features.
J Med Chem, 2020; 63


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GROWTH/DIFFERENTIATION FACTOR 15 INFLUENCES PRIMARY CILIA IN NEURAL STEM CELLS IN THE VENTRICULAR-SUBVENTRICULAR ZONE BUT NOT IN THE SUBGRANULAR ZONE

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Katja Baur, Şeydanur Şan, Francesca Ciccolini
Universität Heidelberg, Neurobiology, Heidelberg, Germany

Neural stem cells (NSCs) are vital for adult neurogenesis throughout adulthood and are located in the two neurogenic niches in the mammalian brain: the ventricular-subventricular zone (V-SVZ) and in the subgranular zone (SGZ) in the hippocampus. The proliferation of adult NSCs is tightly regulated by environmental signals and primary cilia play a fundamental role in sensing and transducing signals governing NSC maintenance, activation and quiescence. However, niche components regulating cilia morphology and function are less known. Here we found that constitutive ablation of growth/differentiation factor 15 (GDF15) leads to more, shorter, and thicker primary cilia in NSCs of the embryonic and adult V-SVZ, but not in the age-matched hippocampal counterpart. Although GDF15 is present in both germinal niches, its receptor, the GDNF-Family Receptor Alpha-Like (GFRAL), is localized in primary cilia of NSCs in the V-SVZ but not in the SGZ. Notably, inhibition of C-X-C chemokine receptor (CXCR) 4, also expressed in primary cilia in V-SVZ progenitors, did not affect morphology of primary cilia, showing that GFRAL but not CXCR4 can affect cilia morphology. Finally, changes in morphology were partly alleviated by application of exogenous GDF15. Taken together, these results imply a direct of ciliary activation of ciliary GFRAL on primary cilia morphology.

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FGF18 – A NEW PLAYER IN THE REGULATION OF ADULT SUBVENTRICULAR ZONE (SVZ) NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Mohammad K. Hajihosseini[1], Giada Vanacore[1], Tianqi Li[1], Mette Mogensen[1], David Ornitz[2]
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Introduction: Every day, neural stem cells (NSC) of the adult rodent subventricular zone (SVZ) generate new olfactory bulbs (OB) neurons, with important modulatory roles in odour-detection and behaviour. SVZ neurogenesis is compartmentalized and requires the activation of quiescent NSC, but the precise cohort of such activators remains unknown. Fibroblast Growth Factors (FGFs) regulate diverse aspects of embryonic brain development, but little is known about their roles in adult brain neurogenesis. We have analysed the expression and fate of FGF18-expressing cells in the rodent brain. **Methods & Sample size:** We used FGF18-creERT2::Rosa26-Tomato-dsred double transgenic mice (DTG) of either sex. For lineage tracing, 3-month-old mice were tamoxifen-pulsed for two consecutive days and analysed either 2 or 12 days later. To detect an effect size of 0.6-0.9 with alpha level of 0.05 and power of 0.8 in two-way ANOVA tests, n=6 was analysed per time-point. Serial sections of brain were immunolabelled with anti-dsred antibodies in conjunction with cell type-specific markers, and the rostro-caudal distribution and abundance of dsred+ cells was recorded. **Results & Conclusions:** After short chase, dsred+ cells were largely confined to dorsal and medial aspects of SVZ, whilst after long chase, dsred+ cells were additionally evident in the rostral migratory stream, and as NeuN+ neurons in periglomerular OB. In the SVZ a subset of dsred+ cells expressed GFAP. Our findings indicate that FGF18-expressing dorsal SVZ cells supply new neurons to OB. Moreover, as FGF18's receptors are expressed by quiescent NSC in the lateral SVZ, our results now identify FGF18 a potential regulator of NSC quiescence/ activation.

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ROLE OF BDNF, ADENOSINE A2AR AND CANNABINOID RECEPTORS ON POSTNATAL OLIGODENDROGENESIS FROM SVZ-DERIVED NEURAL STEM CELL CULTURES

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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[1]Instituto de Farmacologia e Neurociências, Faculdade De Medicina, Universidade De Lisboa, Lisboa, Portugal, [2]Instituto de Medicina Molecular João Lobo Antunes, Faculdade De Medicina, Universidade De Lisboa, Lisboa, Portugal

Oligodendrocytes (OLs) are the myelin-forming cells in the Central Nervous System of vertebrates. The role of modulators such as brain-derived neurotrophic factor (BDNF), cannabinoid receptors type 1 and 2 (CBRs) and adenosine A2A receptors (A2ARs) on adult oligodendrogenesis from subventricular zone neural stem cells (SVZ-NSCs) remains unknown. Hence, we aimed at studying how these modulators and the putative crosstalk between them can influence OL differentiation from postnatal SVZ-NSCs. Results obtained using SVZ-NSCs cultures pharmacologically treated with adenosine A2ARs modulators, CBR ligands or BDNF, show that treatment with BDNF tends to increase oligodendrocyte precursor cell (OPC) formation (NG2/PDGFRα-positive cells) after 4 days *in vitro* (DIV) (n=3; CTRL set to 100%, BDNF 203.8±27.59; p=0.0548), whilst significantly increasing the number of OPCs at DIV7 (n=7-8; CTRL set to 100%, BDNF 210.2±21.87; p<0.0001) without affecting OL maturation (MBP-positive cells). Importantly, BDNF effects on OPC formation at DIV7 were partially abrogated by the A2AR antagonist (n=4-8; CTRL set to 100%, BDNF+ZM 174.0±8.951; p<0.01), while the antagonist by itself had no effect when comparing with control (ZM 117.6±15.47; p>0.05). No changes were observed after treatment with the A2AR agonist at these timepoints in both OPC formation and OL maturation. This work outlined the role of BDNF in promoting the formation of OPCs from SVZ-NSCs. Now we are addressing if the effect of BDNF is dependent of A2ARs, and if a simultaneous activation of CBRs is required to potentiate adult oligodendrogenesis, which will ultimately contribute to the development of alternative therapeutic targets for OL formation and remyelination.

Pubmed:

34151790: Paulo SL, Ribeiro-Rodrigues L, Rodrigues RS, Mateus JM, Fonseca-Gomes J, Soares R, Diógenes MJ, Solá S, Sebastião AM, Ribeiro FF, Xapelli S
Sustained Hippocampal Neural Plasticity Questions the Reproducibility of an Amyloid-β-Induced Alzheimer's Disease Model.
The use of Alzheimer's disease (AD) models obtained by intracerebral infusion of amyloid-β (Aβ) has been increasingly reported in recent years. Nonetheless, these models may present important challenges.
J Alzheimers Dis, 2021; 82
30959794: Rodrigues RS, Lourenço DM, Paulo SL, Mateus JM, Ferreira MF, Mouro FM, Moreira JB, Ribeiro FF, Sebastião AM, Xapelli S
Cannabinoid Actions on Neural Stem Cells: Implications for Pathophysiology.
With the increase of life expectancy, neurodegenerative disorders are becoming not only a health but also a social burden worldwide. However, due to the multitude of pathophysiological disease states, current treatments fail to meet the desired outcomes. Therefore, there is a need for new therapeutic strategies focusing on more integrated, personalized and effective approaches. The prospect of using neural stem cells (NSC) as regenerative therapies is very promising, however several issues still need to be addressed. In particular, the potential actions of pharmacological agents used to modulate NSC activity are highly relevant. With the ongoing discussion of cannabinoid usage for medical purposes and reports drawing attention to the effects of cannabinoids on NSC regulation, there is an enormous, and yet, uncovered potential for cannabinoids as treatment options for several neurological disorders, specifically when combined with stem cell therapy. In this manuscript, we review in detail how cannabinoids act as potent regulators of NSC biology and their potential to modulate several neurogenic features in the context of pathophysiology.
Molecules, 2019; 24

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BOARD NUMBER: S07-671

SIMULATED MICROGRAVITY TRANSIENTLY ALTERS ADULT NEUROGENESIS IN RATS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Memory deficits have been reported in astronauts during space flights and documented in models of simulated microgravity (SMG) in humans and animals. However, the physiological causes of these behavioral effects remain largely unknown. We explored whether adult neurogenesis, known to be a crucial plasticity mechanism supporting memory processes, is altered by SMG. Adult male Long Evans rats were submitted to hindlimb suspension which simulates the body fluid reorganization and mechanical unloading encountered in space. To track the proliferation, survival and maturation of newborn cells in neurogenic niches such that the subventricular zone (SVZ), the olfactory bulb (OB) and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus, rats were administered the birth dating markers 5-Ethynyl-2'-deoxyuridine and 5-Bromo-2'-deoxyuridine either immediately prior to, or at different delays following the SMG exposure period. While unaffected immediately after SMG exposure, newborn cell proliferation was decreased in the DG after 7 days of exposure. SMG also induced a decrease in short-term (7 days), but not long-term (21 days) survival of newborn cells in the DG and SVZ. Moreover, 3-weeks of physical exercise used as a countermeasure was able to reverse the decrease in newborn cell survival observed in the DG and SVZ. These findings highlight the sensitivity of adult neurogenesis to gravitational environmental factors during a transient period, possibly indicating a period of adaptation of physiological systems to this new environment. Alterations in the dynamics of adult neurogenesis may contribute, at least in part, to the cognitive deficits observed following SMG exposure.

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BOARD NUMBER: S07-672

DECIPHERING THE ROLE OF P75 NEUROTROPHIN RECEPTOR IN ADULT NEUROGENESIS: A POTENTIAL PHARMACOLOGICAL TARGET AGAINST ALZHEIMER'S DISEASE.

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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The pan-neurotrophin p75 receptor ($p75^{NTR}$) is a member of the TNF death receptor superfamily widely expressed in many cell types among the neural tissue, including adult neural stem cells (aNSCs). Its remarkable up regulation during neurodegeneration and its controversial signaling, ranging from survival to cell death, have attracted a special interest on this receptor as a potential pharmacological target. $p75^{NTR}$ has been extensively linked with Alzheimer's Disease (AD) by serving as a receptor for Amyloid-beta (Aβ), the major component of the plaques, found in the brain of AD patients. Additionally, recent studies are highlighting the importance of this receptor on the adult hippocampal neurogenesis – a process that seems to drop sharply in AD- although its role has not been clarified yet. We focus on revealing the $p75^{NTR}$ pleiotropic functions, by examining adult neurogenesis levels on $p75^{NTR}$ knock-out mouse lines and identifying the aNSCs proliferation and survival. Moreover, we investigate the p75NTR effects in human iPSC-derived NSCs, depicting receptor's signaling under physiological conditions and its dysregulation in NSCs derived from AD patients bearing the ApoE4 mutation. Finally, we show that $p75^{NTR}$ regulates human iPSC-derived NSCs survival in the presence of Aβ toxic oligomers, as well as primary mouse hippocampal aNSCs proliferation and differentiation, indicating its involvement in both AD progress and neurogenesis. Deciphering the specific signaling pathways necessary to mediate the actions of $p75^{NTR}$ on aNSCs' properties, we aim to reinforce endogenous ability of neurogenesis and thus strengthening its repairing capacity against AD-induced neuronal loss.
(Supported by HFRI-FM17-2301).

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BOARD NUMBER: S07-673

CONDITIONAL DELETION OF CYCLIN D2 CONFIRMS CRITICAL FUNCTIONS IN ADULT HIPPOCAMPAL NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Patricia Jiménez Peinado[1], Jonas Mücke[1], Maria Thielemann[1], Jule Müller[1], Miriam Weißhaar[1], Juliane Stadtkus[1], Varunika Goyal[1,2], Cathrin Bayer[1], Otto W. Witte[1,3,4,5], Anja Urbach[1,3,4,5]
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Cyclin D2 (D2) is one out of three homologous D-cyclins involved in cell cycle progression. Our previous studies suggest that D2 is a key regulator of adult hippocampal neurogenesis by controlling the proliferation of adult neural stem (aNSCs) and progenitor cells. However, available evidence is mainly based on conventional D2 knockout mice (D2KO), which have several limitations including an impaired development of the aNSC pool. To bypass these limitations, we engineered a mouse line in which exons I and II of *ccnd2* are flanked by *loxP* sites (*ccnd2flox*), and bred them to inducible Cre driver lines (Nestin-CreER^{T2}, Rosa26-CreER^{T2} or Glast-CreER^{T2}), enabling a spatiotemporally controlled deletion of D2. The knockout was induced by administration of tamoxifen at an age of 6-7 weeks and newborn cells were labelled with bromodeoxyuridine. Acute and long-term effects were assessed using multiple immunofluorescences at 2, 28 and 150 days post injection (dpi). Conditional null mutants generated with the intention of validating the *ccnd2flox* line displayed exactly the same phenotype as conventional D2KO mice. As expected, the conditional D2KO induced in young adults had less impact on adult hippocampal neurogenesis. Depending on the Cre driver, we found a 50-60% reduction of bromodeoxyuridine-positive cells accompanied by a similar reduction in neuroblasts and newborn neurons, whereas neuronal differentiation was unaffected. Taken together, these data corroborate the importance of D2 for constitutive neurogenesis. Whether D2 exerts this role through controlling the division of aNSCs or of their progeny is currently under investigation.

Pubmed:
32506112: Schiavi S, Melancia F, Carbone E, Buzzelli V, Manduca A, Peinado PJ, Zwergel C, Mai A, Campolongo P, Vanderschuren LJMJ, Trezza V
Detrimental effects of the 'bath salt' methylenedioxypyrovalerone on social play behavior in male rats.
Methylenedioxypyrovalerone (MDPV) is the most popular synthetic cathinone found in products marketed as 'bath salts', widely abused among teenagers and young adults. Synthetic cathinones have pharmacological effects resembling those of psychostimulants, which are known to disrupt a variety of social behaviors. However, despite the popular use of MDPV by young people in social contexts, information about its effects on social behavior is scarce. To investigate the impact of MDPV on social behavior at young age, and the underlying neurobehavioral mechanisms, we focused on social play behavior. Social play behavior is the most characteristic social behavior displayed by young mammals and it is crucial for neurobehavioral development. Treatment with MDPV reduced social play behavior in both juvenile and young adult male rats, and its play-suppressant effect was subject to tolerance but not sensitization. As the behavioral effects of MDPV have been ascribed to dopaminergic and noradrenergic neurotransmission, and given the role of these neurotransmitters in social play, we investigated the involvement of dopamine and noradrenaline in the play-suppressant effects of MDPV. The effects of MDPV on social play were blocked by either the α2 adrenoceptor antagonist RX821002 or the dopamine receptor antagonist flupenthixol, given alone or together at sub-effective doses. In sum, MDPV selectively suppresses the most vigorous social behavior of developing rats through both noradrenergic and dopaminergic mechanisms. This study provides important preclinical evidence of the deleterious effects of MDPV on social behavior, and as such increases our understanding of the neurobehavioral effects of this popular cathinone.
Neuropsychopharmacology, 2020; 45

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BOARD NUMBER: S07-674

AXON ARCHITECTURE OF DENTATE GRANULE NEURONS IS DICTATED BY THEIR ONTOGENETIC ORIGIN IN THE MOUSE HIPPOCAMPUS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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In the dentate gyrus (DG) of the hippocampus, the generation of dentate granule neurons (DGNs) starts during late embryogenesis, peaks around birth and continues at low levels during adulthood. The DG is therefore a peculiar brain structure composed of DGNs of different ontogenetic origins constituting subpopulations of DGNs that might play different roles in hippocampal physiology. Surprisingly, this hypothesis has received little attention and, although the morpho-functional properties of adult-born DGNs (Adu-DGNs) have been extensively studied, very little is known about the developmentally-generated ones. In this context, we have undertaken to analyse the morphological characteristics of these different subpopulations of DGNs. For this purpose, we targeted DGNs generated at E14.5 and P0 using in vivo electroporation and Adu-DGNs using retrovirus transduction. Using this strategy, we have already shown that developmentally-born DGNs (Dev-DGNs), especially embryonically-born cells, and Adu-DGNs display distinct dendritic arbors once mature (Kerloch et al., 2019). Interestingly, we have also found lower spine density in Dev-DGNs compared to Adu-DGNs and striking differences at the axonal level. Indeed, in the hilus and CA3 we observed that the later DGNs are generated, the bigger their mossy fiber boutons and the more filipodia they have. Furthermore, our preliminary data suggest that the projection sites of their axons in CA3/CA2 as well as the length and position of their axonal initial segment are also dependent on their ontogenetic origin. Altogether our data demonstrate that the temporal origin of DGNs dictates their morphological properties and thus potentially their functions in the hippocampal network.

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BOARD NUMBER: S07-675

NCAM2 MODULATION OF RGPS DURING ADULT NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Adult neurogenesis persists in the neurogenic zones where new neurons are incorporated into existing neuronal circuits to preserve and improve learning and memory tasks. Relevant structural elements of the neurogenic niches include the family of Cell Adhesion Molecules (CAM), which participate in signal transduction and regulate radial glial progenitor's (RGPs) survival, division and differentiation. The Neural Cell Ahesion Molecule 2 (NCAM2) is important for neuronal morphogenesis, dendritic arborization and synaptogenesis. However, their implications in the regulation of RGPs are poorly investigated. The aim of this study is to unravel the role of NCAM2 in adult neurogenesis. To study the implications of NCAM2 in the regulation of RGPs during hippocampal adult neurogenesis *in vivo,* we characterized the expression of NCAM2 in the dentate gyrus by immunofluorescence. In addition, 8 week-old mice were injected with NCAM2 expression modulating vectors and the transduced hippocampus were analyzed at different time points. Finally, the effects of NCAM2 in the regulation of RGPs were complemented *in vitro* analyzing the proliferation of SGZ derived neurospheres infected with NCAM2 overexpressing vectors. The characterization of the expression of NCAM2 among the main actors of the neurogenic process revealed different levels of NCAM2 amid the progression of RGPs and the formation of neurons. Further, our results indicate that the modulation of NCAM2 expression arrest cells in a RGP-like state affecting the normal course of the neurogenic events. Together, our data suggest a significant implication of NCAM2 in the regulation of RGPs during adult neurogenesis in the hippocampus.

Pubmed:
34576185: Parcerisas A, Ortega-Gascó A, Pujadas L, Soriano E
The Hidden Side of NCAM Family: NCAM2, a Key Cytoskeleton Organization Molecule Regulating Multiple Neural Functions. Although it has been over 20 years since Neural Cell Adhesion Molecule 2 (NCAM2) was identified as the second member of the NCAM family with a high expression in the nervous system, the knowledge of NCAM2 is still eclipsed by NCAM1. The first studies with NCAM2 focused on the olfactory bulb, where this protein has a key role in axonal projection and axonal/dendritic compartmentalization. In contrast to NCAM1, NCAM2's functions and partners in the brain during development and adulthood have remained largely unknown until not long ago. Recent studies have revealed the importance of NCAM2 in nervous system development. NCAM2 governs neuronal morphogenesis and axodendritic architecture, and controls important neuron-specific processes such as neuronal differentiation, synaptogenesis and memory formation. In the adult brain, NCAM2 is highly expressed in dendritic spines, and it regulates synaptic plasticity and learning processes. NCAM2's functions are related to its ability to adapt to the external inputs of the cell and to modify the cytoskeleton accordingly. Different studies show that NCAM2 interacts with proteins involved in cytoskeleton stability and proteins that regulate calcium influx, which could also modify the cytoskeleton. In this review, we examine the evidence that points to NCAM2 as a crucial cytoskeleton regulation protein during brain development and adulthood. This key function of NCAM2 may offer promising new therapeutic approaches for the treatment of neurodevelopmental diseases and neurodegenerative disorders.
Int J Mol Sci, 2021; 22
34299022: Parcerisas A, Ortega-Gascó A, Hernaiz-Llorens M, Odena MA, Ulloa F, de Oliveira E, Bosch M, Pujadas L, Soriano E
New Partners Identified by Mass Spectrometry Assay Reveal Functions of NCAM2 in Neural Cytoskeleton Organization. Neuronal cell adhesion molecule 2 (NCAM2) is a membrane protein with an important role in the morphological development of neurons. In the cortex and the hippocampus, NCAM2 is essential for proper neuronal differentiation, dendritic and axonal

outgrowth and synapse formation. However, little is known about NCAM2 functional mechanisms and its interactive partners during brain development. Here we used mass spectrometry to study the molecular interactome of NCAM2 in the second postnatal week of the mouse cerebral cortex. We found that NCAM2 interacts with >100 proteins involved in numerous processes, including neuronal morphogenesis and synaptogenesis. We validated the most relevant interactors, including Neurofilaments (NEFs), Microtubule-associated protein 2 (MAP2), Calcium/calmodulin kinase II alpha (CaMKIIα), Actin and Nogo. An in silico analysis of the cytosolic tail of the NCAM2.1 isoform revealed specific phosphorylation site motifs with a putative affinity for some of these interactors. Our results expand the knowledge of NCAM2 interactome and confirm the key role of NCAM2 in cytoskeleton organization, neuronal morphogenesis and synaptogenesis. These findings are of interest in explaining the phenotypes observed in different pathologies with alterations in the NCAM2 gene.
Int J Mol Sci, 2021; 22
32043120: Parcerisas A, Pujadas L, Ortega-Gascó A, Perelló-Amorós B, Viais R, Hino K, Figueiro-Silva J, La Torre A, Trullás R, Simó S, Lüders J, Soriano E
NCAM2 Regulates Dendritic and Axonal Differentiation through the Cytoskeletal Proteins MAP2 and 14-3-3.
Neural cell adhesion molecule 2 (NCAM2) is involved in the development and plasticity of the olfactory system. Genetic data have implicated the NCAM2 gene in neurodevelopmental disorders including Down syndrome and autism, although its role in cortical development is unknown. Here, we show that while overexpression of NCAM2 in hippocampal neurons leads to minor alterations, its downregulation severely compromises dendritic architecture, leading to an aberrant phenotype including shorter dendritic trees, retraction of dendrites, and emergence of numerous somatic neurites. Further, our data reveal alterations in the axonal tree and deficits in neuronal polarization. In vivo studies confirm the phenotype and reveal an unexpected role for NCAM2 in cortical migration. Proteomic and cell biology experiments show that NCAM2 molecules exert their functions through a protein complex with the cytoskeletal-associated proteins MAP2 and 14-3-3γ and ζ. We provide evidence that NCAM2 depletion results in destabilization of the microtubular network and reduced MAP2 signal. Our results demonstrate a role for NCAM2 in dendritic formation and maintenance, and in neural polarization and migration, through interaction of NCAM2 with microtubule-associated proteins.
Cereb Cortex, 2020; 30

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SINGLE NUCLEUS AND SPATIAL TRANSCRIPTOMICS OF HUMAN HIPPOCAMPUS FROM PEOPLE WITH MAJOR DEPRESSION AND CONTROLS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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In mammals, adult hippocampal neurogenesis (AHN) is necessary for memory, learning and adaptation to the environment. In human brain we showed persistent AHN into the eighth decade of life, despite a smaller multipotent progenitor pool in the aging dentate gyrus (DG) stem cell niche. Although, other studies failed to detect doublecortin (DCX) and other immature neuron markers in adult DG. We reported DCX expression in co-localization with other neuronal proteins and RNAs in the human DG neurogenic niche of the subgranular zone (SGZ). The existence of adult hippocampal neurogenesis (AHN) in human brain is largely debated. To investigate human hippocampus neurogenic niche molecular features, we applied single nuclei (sn) RNA sequencing (seq), snATAC-seq, Visium (10X Genomics) and slide-seq using deterministic barcoding in intact tissue (DBiT-seq) for spatial omics sequencing. Subgranular zone cell clusters highly expressed genes involved in: cell mitosis and differentiation (PTPRT, p=6.44*E-95); embryonic development and cell fate determination (SOX1, p=4.72*E-11); neuron migration and differentiation (L1CAM, p=1.56*E-05); involved in cell migration and adhesion (RELN, p=1.38*E-46); and neurite extension (NRSN,1p=1.73*E-65). Granule cell layer cell clusters highly expressed genes involved in: cell fate determination and neurogenesis (PROX1, p=1.65*E-149); the switch to adult-like connectivity during neurogenesis (CALB1, p=1.18*E-52); stabilizing dendritic shape during neuron development (MAP2, p=9.13*E-30). Single nucleus and spatial multiomics identified canonical cell clusters localized in the expected hippocampus subfields, and support the existence of a hippocampus neurogenic niche in the subgranular zone.

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MATERNAL HIGH-ENERGY DIET DURING PREGNANCY AND LACTATION IMPAIRS OFFSPRING NEUROGENESIS IN PHENOTYPE-DEPENDENT MANNER

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Kamila Fabianová[1], Janka Babeľová[2], Alexandra Popovičová[1], Dušan Fabian[2], Marcela Martončíková[1], Adam Raček[1], Eniko Račeková[1]
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Obesity is one of the most serious and costly health challenges facing the modern world. Increasing evidence suggests that the risk of developing a metabolic syndrome or obesity may be influenced very early in the development, especially through inappropriate fetal and/or neonatal nutrition. Outcomes from epidemiological studies indicate that maternal nutrition during pregnancy and lactation periods has a profound impact on adult neurogenesis in the offspring. In this study, an intergenerational dietary model based on overfeeding of experimental mice during prenatal and early postnatal development was used to produce mice with various body conditions. The aim of the present study was to experimentally investigate the impact of the maternal high-energy diet during pregnancy and lactation periods on adult neurogenesis in the olfactory neurogenic region involving the subventricular zone (SVZ) and rostral migratory stream (RMS). Our findings indicate that, under the influence of a maternal high-energy diet administered during pregnancy and lactation, SVZ/RMS neurogenesis is altered as indicated by decreased proliferation of neural precursors, increased cell degeneration, and by changes in the number of nitrergic cells. The major finding of this study is that maternal high-energy diet affects individual processes of SVZ/RMS neurogenesis only in mice where extreme phenotype, such as significant overweight/adiposity or obesity is manifested. Supported by VEGA 2/0119/22 This publication was created thanks to support under the Operational Programme Integrated Infrastructure for the project: Open scientific community for modern interdisciplinary research in medicine (OPENMED), ITMS: 313011V455 co-financed by the European Regional Development Fund.

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BOARD NUMBER: S07-678

EXERCISING ON CANNABINOIDS: A COMBINED EFFORT IN REGULATING POSTNATAL NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Neurogenesis, the formation of new neurons from neural stem cells (NSCs), occurs mainly within the subventricular zone (SVZ) and the hippocampal dentate gyrus (DG). Cannabinoids, which activate primarily type 1 and 2 receptors (CB_1R and CB_2R), modulate this process whereas physical exercise has been shown to potentiate neurogenesis. While CB_1R signaling is required for exercise-mediated effects on hippocampal NSC proliferation, much less is known about the effects of CB2R. Recent data suggests an interaction between CB_2R and BDNF, a major neurotrophin upregulated with physical exercise, in regulating postnatal neurogenesis. Therefore, this work aimed to study whether exercise-regulated neurotrophic factors combined with CB_2R modulation play an impactful role on postnatal neurogenesis, using SVZ- and DG-derived neurospheres pharmacologically treated with different CB_2R ligands and neurotrophic factors (BDNF, VEGF and IGF-1). No significant alterations in cell survival were observed in both niches in response to any treatment. BDNF significantly enhanced cell proliferation in DG-derived neurospheres. Concerning neuronal differentiation, all neurotrophic factors stimulated neuronal differentiation in DG whereas only BDNF promoted an increase in the number of mature neurons in SVZ. Interestingly, the co-incubation of neurotrophic factors with the CB_2R agonist potentiated the effects observed with the independent treatments in neuronal differentiation. In DG, the antagonism of CB_2R blocked the effects promoted by the neurotrophic factors on neuronal differentiation suggesting that the activation of this receptor is crucial for their effects. This study sheds light on the potential synergism between physical exercise and endocannabinoid signaling in regulating postnatal neurogenesis.

Pubmed:
35011652: Santos SS, Moreira JB, Costa M, Rodrigues RS, Sebastião AM, Xapelli S, Solá S
The Mitochondrial Antioxidant Sirtuin3 Cooperates with Lipid Metabolism to Safeguard Neurogenesis in Aging and Depression.
Neural stem cells (NSCs), crucial for memory in the adult brain, are also pivotal to buffer depressive behavior. However, the mechanisms underlying the boost in NSC activity throughout life are still largely undiscovered. Here, we aimed to explore the role of deacetylase Sirtuin 3 (SIRT3), a central player in mitochondrial metabolism and oxidative protection, in the fate of NSC under aging and depression-like contexts. We showed that chronic treatment with tert-butyl hydroperoxide induces NSC aging, markedly reducing SIRT3 protein. SIRT3 overexpression, in turn, restored mitochondrial oxidative stress and the differentiation potential of aged NSCs. Notably, SIRT3 was also shown to physically interact with the long chain acyl-CoA dehydrogenase (LCAD) in NSCs and to require its activation to prevent age-impaired neurogenesis. Finally, the SIRT3 regulatory network was investigated in vivo using the unpredictable chronic mild stress (uCMS) paradigm to mimic depressive-like behavior in mice. Interestingly, uCMS mice presented lower levels of neurogenesis and LCAD expression in the same neurogenic niches, being significantly rescued by physical exercise, a well-known upregulator of SIRT3 and lipid metabolism. Our results suggest that targeting NSC metabolism, namely through SIRT3, might be a suitable promising strategy to delay NSC aging and confer stress resilience.
Cells, 2021; 11
33818505: Soares R, Ribeiro FF, Lourenço DM, Rodrigues RS, Moreira JB, Sebastião AM, Morais VA, Xapelli S
The neurosphere assay: an effective technique to study neural stem cells.
Neural Regen Res, 2021; 16
32723008: Rodrigues RS, Paulo SL, Moreira JB, Tanqueiro SR, Sebastião AM, Diógenes MJ, Xapelli S
Adult Neural Stem Cells as Promising Targets in Psychiatric Disorders.
The development of new therapies for psychiatric disorders is of utmost importance, given the enormous toll these disorders pose to society nowadays. This should be based on the identification of neural substrates and mechanisms that underlie disease etiopathophysiology. Adult neural stem cells (NSCs) have been emerging as a promising platform to counteract brain damage. In this perspective article, we put forth a detailed view of how NSCs operate in the adult brain and influence brain

homeostasis, having profound implications at both behavioral and functional levels. We appraise evidence suggesting that adult NSCs play important roles in regulating several forms of brain plasticity, particularly emotional and cognitive flexibility, and that NSC dynamics are altered upon brain pathology. Furthermore, we discuss the potential therapeutic value of utilizing adult endogenous NSCs as vessels for regeneration, highlighting their importance as targets for the treatment of multiple mental illnesses, such as affective disorders, schizophrenia, and addiction. Finally, we speculate on strategies to surpass current challenges in neuropsychiatric disease modeling and brain repair.
Stem Cells Dev, 2020; 29
32510488: Soares R, Ribeiro FF, Lourenço DM, Rodrigues RS, Moreira JB, Sebastião AM, Morais VA, Xapelli S
Isolation and Expansion of Neurospheres from Postnatal (P1-3) Mouse Neurogenic Niches.
The neurosphere assay is an extremely useful in vitro technique for studying the inherent properties of neural stem/progenitor cells (NSPCs) including proliferation, self-renewal and multipotency. In the postnatal and adult brain, NSPCs are mainly present in two neurogenic niches: the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus (DG). The isolation of the neurogenic niches from postnatal brain allows obtaining a higher amount of NSPCs in culture with a consequent advantage of higher yields. The close contact between cells within each neurosphere creates a microenvironment that may resemble neurogenic niches. Here, we describe, in detail, how to generate SVZ- and DG-derived neurosphere cultures from 1-3-day-old (P1-3) mice, as well as passaging, for neurosphere expansion. This is an advantageous approach since the neurosphere assay allows a fast generation of NSPC clones (6-12 days) and contributes to a significant reduction in the number of animal usage. By plating neurospheres in differentiative conditions, we can obtain a pseudomonolayer of cells composed of NSPCs and differentiated cells of different neural lineages (neurons, astrocytes and oligodendrocytes) allowing the study of the actions of intrinsic or extrinsic factors on NSPC proliferation, differentiation, cell survival and neuritogenesis.
J Vis Exp, 2020;
30959794: Rodrigues RS, Lourenço DM, Paulo SL, Mateus JM, Ferreira MF, Mouro FM, Moreira JB, Ribeiro FF, Sebastião AM, Xapelli S
Cannabinoid Actions on Neural Stem Cells: Implications for Pathophysiology.
With the increase of life expectancy, neurodegenerative disorders are becoming not only a health but also a social burden worldwide. However, due to the multitude of pathophysiological disease states, current treatments fail to meet the desired outcomes. Therefore, there is a need for new therapeutic strategies focusing on more integrated, personalized and effective approaches. The prospect of using neural stem cells (NSC) as regenerative therapies is very promising, however several issues still need to be addressed. In particular, the potential actions of pharmacological agents used to modulate NSC activity are highly relevant. With the ongoing discussion of cannabinoid usage for medical purposes and reports drawing attention to the effects of cannabinoids on NSC regulation, there is an enormous, and yet, uncovered potential for cannabinoids as treatment options for several neurological disorders, specifically when combined with stem cell therapy. In this manuscript, we review in detail how cannabinoids act as potent regulators of NSC biology and their potential to modulate several neurogenic features in the context of pathophysiology.
Molecules, 2019; 24

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"MILKING": AN INNOVATIVE APPROACH TO INVESTIGATE THE PROPERTIES OF POSTNATAL BRAIN NEURAL STEM CELLS AND TO OBTAIN OLIGODENDROCYTE PROGENITOR CELLS FROM LIVE EXPERIMENTAL ANIMALS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Aims: Postnatal brain neural stem and progenitor cells (NSPCs) cluster in anatomically defined stem cell niches, such as the subependymal zone (SEZ) and are characterized by self-renewal and quiescence. Oligodendrocyte progenitor cells (OPCs) give rise to myelinating oligodendrocytes and also exhibit self-renewing potential. Here, we refine our method of “milking” the ventricular system in order to collect OPCs from the corpus callosum and to investigate the basic properties of SEZ-resident NSPCs. **Methods:** Milking consists of an intracerebroventricular injection of a release cocktail containing neuraminidase, β1-integrin blocking antibody and Fibroblast Growth Factor-2 in order to induce the controlled flow of NSPCs and OPCs in the cerebrospinal fluid. At a second “collection” step, liquid biopsies of CSF are performed from the cisterna magna of anesthetized experimental animals without the need of an incision. Liquid biopsies after milking caudal ventricular areas resulted in the isolation of cells expressing typical OPC markers. Cells isolated after milking of the SEZ, were cultured in three different growth media. The typical NSPC medium and two media known to favor the expansion of neural stem cells without enhancing their progress towards differentiation. **Results:** Our results showed significant differences in the morphology of grown cells and in their colony-formation characteristics that we investigate further using a range of NSPC markers in order to directly assess the profile of endogenous SEZ. **Conclusions:** This novel approach paves the way for performing longitudinal studies in experimental animals, for more in vivo relevant cell culture assays, and for future clinical neuro-regenerative applications.

Pubmed:

34560001: McClenahan F, Dimitriou C, Koutsakis C, Dimitrakopoulos D, Arampatzis A, Kakouri P, Kourla M, Oikonomou S, Andreopoulou E, Patsonis M, Meri DK, Rasool RT, Franklin RJ, Kazanis I
Isolation of neural stem and oligodendrocyte progenitor cells from the brain of live rats.
Postnatal brain neural stem and progenitor cells (NSPCs) cluster in anatomically inaccessible stem cell niches, such as the subependymal zone (SEZ). Here, we describe a method for the isolation of NSPCs from live animals, which we term "milking." The intracerebroventricular injection of a release cocktail, containing neuraminidase, integrin-β1-blocking antibody, and fibroblast growth factor 2, induces the controlled flow of NSPCs in the cerebrospinal fluid, where they are collected via liquid biopsies. Isolated cells retain key in vivo self-renewal properties and their cell-type profile reflects the cell composition of their source area, while the function of the niche is sustained even 8 months post-milking. By changing the target area more caudally, we also isolate oligodendrocyte progenitor cells (OPCs) from the corpus callosum. This novel approach for sampling NSPCs and OPCs paves the way for performing longitudinal studies in experimental animals, for more in vivo relevant cell culture assays, and for future clinical neuro-regenerative applications.
Stem Cell Reports, 2021; 16
34112234: Mourtzi T, Dimitrakopoulos D, Kakogiannis D, Salodimitris C, Botsakis K, Meri DK, Anesti M, Dimopoulou A, Charalampopoulos I, Gravanis A, Matsokis N, Angelatou F, Kazanis I
Characterization of substantia nigra neurogenesis in homeostasis and dopaminergic degeneration: beneficial effects of the microneurotrophin BNN-20.
Loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) underlines much of the pathology of Parkinson's disease (PD), but the existence of an endogenous neurogenic system that could be targeted as a therapeutic strategy has been controversial. BNN-20 is a synthetic, BDNF-mimicking, microneurotrophin that we previously showed to exhibit a pleiotropic neuroprotective effect on the dopaminergic neurons of the SNpc in the "weaver" mouse model of PD. Here, we assessed its potential effects on neurogenesis.
Stem Cell Res Ther, 2021; 12


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ROLE OF TET2 IN NEURAL STEM CELL MAINTENANCE AND DIFFERENTIATION

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Neurogenesis throughout lifespan in mammalian brain is supported by multipotent neural stem cells (NSCs), characterized by their abilities of self-renewal and differentiation into the three lineages of the central nervous system: neurons, astrocytes and oligodendrocytes. Both capabilities are maintained by specific intracellular and extracellular factors. One of these intrinsic factors is the epigenetic process of genomic imprinting (GI). GI causes genes to be expressed depending on their parental origin, causing a monoallelic expression of a subset of genes called *imprinted genes*. It has been shown that this process is implicated in the control of gene dosage in NSCs, regulating their maintenance and differentiation. GI is regulated by differentially methylated regions (DMRs) in maternal and paternal chromosomes. Ten-eleven-translocation (TET) enzymes catalyze DNA demethylation, giving rise to the 5-hydroxymethylcytosine (5hmC) epigenetic mark. However, although hydroxymethylation levels are high in the mouse brain, the potential role of TET proteins regulating GI in adult neurogenesis is not described. This work characterizes the expression of *Tet2* gene and it is coding protein, TET2, both *in vivo* and *in vitro*, focusing on NSCs of the adult subventricular zone (SVZ) neurogenic niche. In addition, the role of *Tet2* is also studied by the generation of a conditional mouse model deficient in *Tet2* specifically in NSCs. Our data reveal that TET2 is essentially required for terminal differentiation of adult NSCs into non-neurogenic astrocytes. Morever, an RNASeq analysis in *Tet2* deficient NSCs identify candidate imprinted genes that might be implicated in this process.

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IMPACT OF AMYOTROPHIC LATERAL SCLEROSIS AND HUNTINGTON'S DISEASE ON HUMAN ADULT HIPPOCAMPAL NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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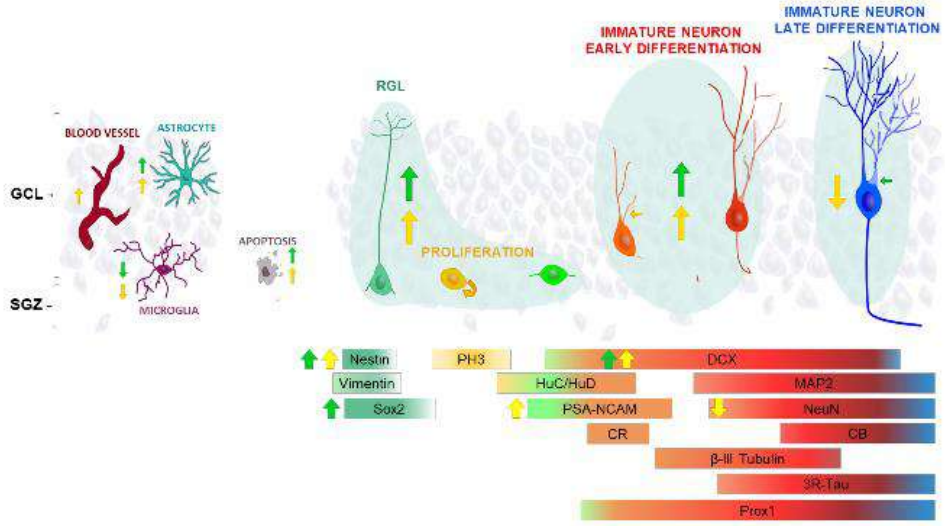
Aims
Adult hippocampal neurogenesis (AHN) encompasses the addition of new neurons to the hippocampus throughout life. This process significantly enhances hippocampal plasticity, and it is implicated in mood regulation and pattern separation. To address whether AHN and the neurogenic niche are compromised by distinct forms of neurodegeneration we compared a cohort of patients with Amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) with neurologically healthy control subjects. **Methods**
High-quality postmortem human samples obtained from 15 control subjects and 12 patients with ALS and, 6 with HD were subjected to state-of-the-art tissue preservation methodologies, which allowed visualization of neural stem cells (NSCs), immature dentate granule cells (DGCs), and specific components of the dentate gyrus (DG) neurogenic niche. **Results**
Compared to control subjects, patients with ALS and HD showed increased densities of NSCs and immature DGCs. The morphological maturation of immature DGCs is altered in ALS. These cells showed early maturation impairments in HD, which suggests a maturation delay. We observed DG astrogliosis, microglial alterations, and thickening of capillaries. These alterations point to the vulnerability of AHN and the human DG neurogenic niche to neurodegenerative diseases. **Conclusions**
Despite the hippocampus is not the brain area primarily affected by ALS and HD, our data reveal that these diseases severely impact AHN. These conditions disturbed the homeostasis of the neurogenic niche, thereby impairing DGCs maturation. Our data provide fundamental insights into the pathophysiology of neurodegenerative diseases and are expected to contribute to the future design of novel therapeutic strategies for these yet incurable conditions.



	NEURODEGENERATIVE DISEASES	
	ALS	HUNTINGTON
RGL cells	↑	↑
Proliferation	=	=
Immature DGCs	↑	↑
Morphological maturation	Tendency to be altered	Altered
Differentiation (marker expression)	=	Altered
Total DGCS	=	↑
Astrocytes	↑	↑
Microglial phagocytosis	↓ Phagocytic pouches	↓ Phagocytic index
Vasculature	=	↑
Apoptosis	↑	↑

Pubmed:

34672693: Terreros-Roncal J, Moreno-Jiménez EP, Flor-García M, Rodríguez-Moreno CB, Trinchero MF, Cafini F, Rábano A, Llorens-Martín M
Impact of neurodegenerative diseases on human adult hippocampal neurogenesis.
Disrupted hippocampal performance underlies psychiatric comorbidities and cognitive impairments in patients with neurodegenerative disorders. To understand the contribution of adult hippocampal neurogenesis (AHN) to amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, dementia with Lewy bodies, and frontotemporal dementia, we studied postmortem human samples. We found that adult-born dentate granule cells showed abnormal morphological development and changes in the expression of differentiation markers. The ratio of quiescent to proliferating hippocampal neural stem cells shifted, and the homeostasis of the neurogenic niche was altered. Aging and neurodegenerative diseases reduced the phagocytic capacity of microglia, triggered astrogliosis, and altered the microvasculature of the dentate gyrus. Thus, enhanced vulnerability of AHN to neurodegeneration might underlie hippocampal dysfunction during physiological and pathological aging in humans.
Science, 2021; 374
33762406: Moreno-Jiménez EP, Terreros-Roncal J, Flor-García M, Rábano A, Llorens-Martín M
Evidences for Adult Hippocampal Neurogenesis in Humans.
The rodent hippocampus generates new neurons throughout life. This process, named adult hippocampal neurogenesis (AHN), is a striking form of neural plasticity that occurs in the brains of numerous mammalian species. Direct evidence of adult neurogenesis in humans has remained elusive, although the occurrence of this phenomenon in the human dentate gyrus has been demonstrated in seminal studies and recent research that have applied distinct approaches to birthdate newly generated neurons and to validate markers of adult-born neurons. Our data point to the persistence of AHN until the 10th decade of human life, as well as to marked impairments in this process in patients with Alzheimer's disease. Moreover, our work demonstrates that the methods used to process and analyze postmortem human brain samples can limit the detection of various markers of AHN to the point of making them undetectable. In this Dual Perspectives article, we highlight the critical methodological aspects that should be strictly controlled in human studies and the robust evidence that supports the occurrence of AHN in humans. We also put forward reasons that may account for current discrepancies on this topic. Finally, the unresolved questions and future challenges awaiting the field are highlighted.
J Neurosci, 2021; 41
31915385: Flor-García M, Terreros-Roncal J, Moreno-Jiménez EP, Ávila J, Rábano A, Llorens-Martín M
Unraveling human adult hippocampal neurogenesis.
Adult neurogenesis occurs in a few selected regions of the mammalian brain. One such region is the hippocampus, the so-called gateway to memory, where adult hippocampal neurogenesis (AHN) occurs. Here, we provide a comprehensive description of the methods used in our laboratory to unambiguously detect a population of immature neurons in the human hippocampus until the 10th decade of life. The criteria used to refine and develop the current protocol include obtaining post-mortem human samples of remarkable quality and under tightly controlled conditions for immunohistochemistry (IHC) studies, optimizing tissue processing and histological procedures, establishing criteria to reliably validate antibody signal and performing unbiased stereological cell counts. Moreover, we provide a detailed description of the parameters that, in our view, should be reported in human AHN studies. The opposing results obtained by introducing slight variations in the methodological conditions should be considered by future studies that seek to increase our knowledge of this fascinating process. By applying simple and inexpensive tissue pre-treatments, this protocol, which can be completed in 7 days, might be applicable to a variety of IHC studies performed on other tissues of human (or animal) origin.
Nat Protoc, 2020; 15
31133559: Terreros-Roncal J, Flor-García M, Moreno-Jiménez EP, Pallas-Bazarra N, Rábano A, Sah N, van Praag H, Giacomini D, Schinder AF, Ávila J, Llorens-Martín M
Activity-Dependent Reconnection of Adult-Born Dentate Granule Cells in a Mouse Model of Frontotemporal Dementia.
Frontotemporal dementia (FTD) is characterized by neuronal loss in the frontal and temporal lobes of the brain. Here, we provide the first evidence of striking morphological alterations in dentate granule cells (DGCs) of FTD patients and in a mouse model of the disease, Tau mice. Taking advantage of the fact that the hippocampal dentate gyrus (DG) gives rise to newborn DGCs throughout the lifetime in rodents, we used RGB retroviruses to study the temporary course of these alterations in newborn DGCs of female Tau mice. In addition, retroviruses that encode either PSD95:GFP or Syn:GFP revealed striking alterations in the afferent and efferent connectivity of newborn Tau DGCs, and monosynaptic retrograde rabies virus tracing showed that these cells are disconnected from distal brain regions and local sources of excitatory innervation. However, the same cells exhibited a predominance of local inhibitory innervation. Accordingly, the expression of presynaptic and postsynaptic markers of inhibitory synapses was markedly increased in the DG of Tau mice and FTD patients. Moreover, an increased number of neuropeptide Y-positive interneurons in the DG correlated with a reduced number of activated egr-1

DGCs in Tau mice. Finally, we tested the therapeutic potential of environmental enrichment and chemoactivation to reverse these alterations in mice. Both strategies reversed the morphological alterations of newborn DGCs and partially restored their connectivity in a mouse model of the disease. Moreover, our data point to remarkable morphological similarities between the DGCs of Tau mice and FTD patients. We show, for the first time to our knowledge, that the population of dentate granule cells is disconnected from other regions of the brain in the neurodegenerative disease frontotemporal dementia (FTD). These alterations were observed in FTD patients and in a mouse model of this disease. Moreover, we tested the therapeutic potential of two strategies, environmental enrichment and chemoactivation, to stimulate the activity of these neurons in mice. We found that some of the alterations were reversed by these therapeutic interventions.
J Neurosci, 2019; 39
30911133: Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, Ávila J, Llorens-Martín M
Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease.
The hippocampus is one of the most affected areas in Alzheimer's disease (AD). Moreover, this structure hosts one of the most unique phenomena of the adult mammalian brain, namely, the addition of new neurons throughout life. This process, called adult hippocampal neurogenesis (AHN), confers an unparalleled degree of plasticity to the entire hippocampal circuitry. Nonetheless, direct evidence of AHN in humans has remained elusive. Thus, determining whether new neurons are continuously incorporated into the human dentate gyrus (DG) during physiological and pathological aging is a crucial question with outstanding therapeutic potential. By combining human brain samples obtained under tightly controlled conditions and state-of-the-art tissue processing methods, we identified thousands of immature neurons in the DG of neurologically healthy human subjects up to the ninth decade of life. These neurons exhibited variable degrees of maturation along differentiation stages of AHN. In sharp contrast, the number and maturation of these neurons progressively declined as AD advanced. These results demonstrate the persistence of AHN during both physiological and pathological aging in humans and provide evidence for impaired neurogenesis as a potentially relevant mechanism underlying memory deficits in AD that might be amenable to novel therapeutic strategies.
Nat Med, 2019; 25
30651327: Bolós M, Terreros-Roncal J, Perea JR, Pallas-Bazarra N, Ávila J, Llorens-Martín M
Maturation Dynamics of the Axon Initial Segment (AIS) of Newborn Dentate Granule Cells in Young Adult C57BL/6J Mice.
Newborn dentate granule cells (DGCs) are generated in the hippocampal dentate gyrus (DG) of rodents through a process called adult hippocampal neurogenesis, which is subjected to tight intrinsic and extrinsic regulation. The use of retroviruses encoding fluorescent proteins has allowed the characterization of the maturation dynamics of newborn DGCs, including their morphological development and the establishment and maturation of their afferent and efferent synaptic connections. However, the study of a crucial cellular compartment of these cells, namely, the axon initial segment (AIS), has remained unexplored to date. The AIS is not only the site of action potential initiation, but it also has a unique molecular identity that makes it one of the master regulators of neural plasticity and excitability. Here we examined the dynamics of AIS formation in newborn DGCs of young female adult C57BL/6J mice Our data reveal notable changes in AIS length and thickness throughout cell maturation under physiological conditions and show that the most remarkable structural changes coincide with periods of intense morphological and functional remodeling. Moreover, we demonstrate that AIS development can be modulated extrinsically by both neuroprotective (environmental enrichment) and detrimental (lipopolysaccharide from) stimuli. The hippocampal dentate gyrus (DG) of rodents generates newborn dentate granule cells (DGCs) throughout life. This process, named adult hippocampal neurogenesis, confers a unique degree of plasticity to the hippocampal circuit, and it is crucial for learning and memory. Here we studied, for the first time, the formation of a key cellular compartment of newborn DGCs, namely, the axon initial segment (AIS) Our data reveal remarkable AIS structural remodeling throughout the maturation of these cells under physiological conditions. Moreover, AIS development can be modulated extrinsically by both neuroprotective (environmental enrichment) and detrimental (lipopolysaccharide from) stimuli.
J Neurosci, 2019; 39
29562522: Teixeira CM, Pallas-Bazarra N, Bolós M, Terreros-Roncal J, Ávila J, Llorens-Martín M
Untold New Beginnings: Adult Hippocampal Neurogenesis and Alzheimer's Disease.
Neurogenesis occurs in a limited number of brain regions during adulthood. Of these, the hippocampus has attracted great interest due to its involvement in memory processing. Moreover, both the hippocampus and the main area that innervates this structure, namely the entorhinal cortex, show remarkable atrophy in patients with Alzheimer's disease (AD). Adult hippocampal neurogenesis is a process that continuously gives rise to newborn granule neurons in the dentate gyrus. These cells coexist with developmentally generated granule neurons in this structure, and both cooperative and competition phenomena regulate the communication between these two types of cells. Importantly, it has been revealed that GSK-3β and tau proteins, which are two of the main players driving AD pathology, are cornerstones of adult hippocampal neurogenesis

regulation. We have shown that alterations either promoting or impeding the actions of these two proteins have detrimental effects on the structural plasticity of granule neurons. Of note, these impairments occur both under basal conditions and in response to detrimental and neuroprotective stimuli. Thus, in order to achieve the full effectiveness of future therapies for AD, we propose that attention be turned toward identifying the pathological and physiological actions of the proteins involved in the pathogenesis of this condition.
J Alzheimers Dis, 2018; 64
29217824: Bolós M, Pallas-Bazarra N, Terreros-Roncal J, Perea JR, Jurado-Arjona J, Ávila J, Llorens-Martín M
Soluble Tau has devastating effects on the structural plasticity of hippocampal granule neurons.
Tau is a neuronal microtubule-associated protein with countless physiological functions. Although the detrimental effects of insoluble aggregated Tau have been widely studied, recent evidence supports the notion that soluble Tau (composed mostly of monomers and dimers) is also toxic for neurons. Here we evaluated the long-term impact of a single stereotaxic injection of human soluble Tau on hippocampal granule neurons in mice. At the ultrastructural level, soluble Tau reduced the number of afferent synapses and caused a dramatic depletion of synaptic vesicles both in afferent and efferent synapses. Furthermore, the use of an RFP-expressing retrovirus revealed that soluble Tau altered the morphology of newborn granule neurons and reduced their afferent (dendritic spines) and efferent (mossy fiber terminals) connectivity. Finally, soluble Tau caused specific impairment of behavioral pattern separation capacity. Our results thus demonstrate for the first time that soluble Tau causes long-term detrimental effects on the morphology and connectivity of newborn granule neurons and that these effects correlate with impaired behavioral pattern separation skills. These data might be relevant for the field of neurodegenerative disorders, since they contribute to reinforcing the pathological roles played by distinct Tau species in vivo.
Transl Psychiatry, 2017; 7
29017970: Bolós M, Perea JR, Terreros-Roncal J, Pallas-Bazarra N, Jurado-Arjona J, Ávila J, Llorens-Martín M
Absence of microglial CX3CR1 impairs the synaptic integration of adult-born hippocampal granule neurons.
Microglia are immune cells that play a crucial role in maintaining brain homeostasis. Among the mechanisms of communication between microglia and neurons, the CX3CL1/CX3CR1 axis exerts a central modulatory role. Animals lacking CX3CR1 microglial receptor (CX3CR1-/- mice) exhibit marked alterations not only in microglia but also in neurons located in various regions of the brain. Here we show that microglial depletion of CX3CR1 leads to the deficient synaptic integration of adult-born granule neurons in the dentate gyrus (DG), both at the afferent and efferent level. Regarding the alterations in the former level, these cells show a reduced number of dendritic spines, which also exhibit morphological changes, namely enlargement and shortening. With respect to changes at the efferent level, these cells show a reduced area of axonal terminals. Both at the afferent and efferent level, synapses show ultrastructural enlargement, but they are depleted of synaptic vesicles, which suggests impaired functionality. We also show that selective increased microglial activation and extracellular matrix deposition in the zones in which the afferent synaptic contacts of these cells occur, namely in the molecular and the granule layer of the DG. In order to evaluate the impact of these structural alterations from a functional point of view, we performed a battery of behavioral tests related to hippocampal-dependent emotional behavior. We observed that female CX3CR1-/- mice exhibit a hyperactive, anxiolytic-like and depressive-like phenotype. These data shed light on novel aspects of the regulation of adult hippocampal neurogenesis by microglia that could be highly relevant for research into mood disorders.
Brain Behav Immun, 2018; 68

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INVESTIGATING THE DYNAMICS OF ACTIVATION OF POSTNATAL NEURAL STEM CELLS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Isabel Mateos White[1], Carmen Mateos Martínez[1], Jaime Fabra Beser[1], David De Agustín Durán[1], Isabel Fariñas[1,2], Cristina Gil Sanz[1]
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The subventricular zone (SVZ) of the lateral ventricles is one of the neurogenic niches of the adult mammalian brain. Neural stem cells (NSCs) populating this niche are pre-specified since embryonic ages and remain quiescent during long periods of time, until they reactivate giving rise to different types of olfactory-bulb interneurons throughout life.
Recently, many studies have been performed to clarify the behavior of NSCs and the molecular mechanisms regulating quiescence exit to produce new cells. Some of them are related to extrinsic signals perceived by NSCs, including interactions with the environment and the neighboring cells via adhesion molecules (CAMs). However, the mechanisms regulating the activation pattern of NSCs before the adult niche establishment are still unknown. Building on the embryonic specification of NSCs, we can target them using in vivo electroporation, at embryonic or early postnatal ages.
Here, we display some approaches using electroporation is this neurogenic niche including the study of the postnatal activation dynamics of NSCs utilizing lineage-tracing strategies and functional experiments employing conditional knock-out mice. Both strategies relay in the use of plasmids containing a tamoxifen inducible CRE recombinase (CRE-ERT2) to allow temporal control of the recombination in NSCs. Lineage tracing experiments using different promoters to drive the expression of CRE in CRE-reporter mice revealed diverse patterns of NSCs activation. Functional experiments employing conditional knock-out mice for a gene involved in the regulation of NSCs quiescence, the CAM Cdh2, enabled us to analyze the consequences of its deletion in such NSCs only at postnatal ages.

Pubmed:
34351428: Medeiros de Araújo JA, Barão S, Mateos-White I, Espinosa A, Costa MR, Gil-Sanz C, Müller U
ZBTB20 is crucial for the specification of a subset of callosal projection neurons and astrocytes in the mammalian neocortex. Neocortical progenitor cells generate subtypes of excitatory projection neurons in sequential order followed by the generation of astrocytes. The transcription factor zinc finger and BTB domain-containing protein 20 (ZBTB20) has been implicated in regulation of cell specification during neocortical development. Here, we show that ZBTB20 instructs the generation of a subset of callosal projections neurons in cortical layers II/III in mouse. Conditional deletion of Zbtb20 in cortical progenitors, and to a lesser degree in differentiating neurons, leads to an increase in the number of layer IV neurons at the expense of layer II/III neurons. Astrogliogenesis is also affected in the mutants with an increase in the number of a specific subset of astrocytes expressing GFAP. Astrogliogenesis is more severely disrupted by a ZBTB20 protein containing dominant mutations linked to Primrose syndrome, suggesting that ZBTB20 acts in concert with other ZBTB proteins that were also affected by the dominant-negative protein to instruct astrogliogenesis. Overall, our data suggest that ZBTB20 acts both in progenitors and in postmitotic cells to regulate cell fate specification in the mammalian neocortex.
Development, 2021; 148
32597854: Mateos-White I, Fabra-Beser J, de Agustín-Durán D, Gil-Sanz C
Double In Utero Electroporation to Target Temporally and Spatially Separated Cell Populations.
In utero electroporation is an in vivo DNA transfer technique extensively used to study the molecular and cellular mechanisms underlying mammalian corticogenesis. This procedure takes advantage of the brain ventricles to allow the introduction of DNA of interest and uses a pair of electrodes to direct the entrance of the genetic material into the cells lining the ventricle, the neural stem cells. This method allows researchers to label the desired cells and/or manipulate the expression of genes of interest in those cells. It has multiple applications, including assays targeting neuronal migration, lineage tracing, and axonal pathfinding. An important feature of this method is its temporal and regional control, allowing circumvention of potential problems related with embryonic lethality or the lack of specific CRE driver mice. Another relevant aspect of this technique is that it helps to considerably reduce the economic and temporal limitations that involve the generation of new mouse lines,

which become particularly important in the study of interactions between cell types that originate in distant areas of the brain at different developmental ages. Here we describe a double electroporation strategy that enables targeting of cell populations that are spatially and temporally separated. With this approach we can label different subtypes of cells in different locations with selected fluorescent proteins to visualize them, and/or we can manipulate genes of interest expressed by these different cells at the appropriate times. This strategy enhances the potential of in utero electroporation and provides a powerful tool to study the behavior of temporally and spatially separated cell populations that migrate to establish close contacts, as well as long-range interactions through axonal projections, reducing temporal and economic costs.
J Vis Exp, 2020;
33435191: de Agustín-Durán D, Mateos-White I, Fabra-Beser J, Gil-Sanz C
Stick around: Cell-Cell Adhesion Molecules during Neocortical Development.
The neocortex is an exquisitely organized structure achieved through complex cellular processes from the generation of neural cells to their integration into cortical circuits after complex migration processes. During this long journey, neural cells need to establish and release adhesive interactions through cell surface receptors known as cell adhesion molecules (CAMs). Several types of CAMs have been described regulating different aspects of neurodevelopment. Whereas some of them mediate interactions with the extracellular matrix, others allow contact with additional cells. In this review, we will focus on the role of two important families of cell-cell adhesion molecules (C-CAMs), classical cadherins and nectins, as well as in their effectors, in the control of fundamental processes related with corticogenesis, with special attention in the cooperative actions among the two families of C-CAMs.
Cells, 2021; 10

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INFLUENCE OF NEUROGENIC IMPROVEMENT STRATEGIES ON EXTINCTION AND REINSTATEMENT OF COCAINE-INDUCED CONDITIONED PLACE PREFERENCE

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Fabiola Ávila Gámiz, Emma Zambrana-Infantes, María Del Carmen Mañas-Padilla, Sara Gil-Rodríguez, Rosa Mullor-Vigo, Luis J. Santín, David Ladrón De Guevara-Miranda
Universidad de Málaga, Psicobiología Y Metodología De Las Ciencias Del Comportamiento, Instituto De Investigación Biomédica De Málaga (ibima), Facultad De Psicología, Málaga, Spain

AIMS: Modulation of adult hippocampal neurogenesis (AHN) has been shown to influence the maintenance of drug-context associations. We aimed to study whether the enhancement of AHN by using a water maze spatial learning task (WM), solely or under conditions of neurogenesis stimulation (forced treadmill exercise), could facilitate extinction and prevent primed reinstatement of cocaine-context associations. METHODS: Adult male C57BL/6J mice (*N*=37) were trained in the Conditioned Place Preference (CPP) paradigm with ascending doses of cocaine (2, 4, 8, 16 mg/kg/d) and subsequently received bromodeoxyuridine (BrdU) injections to label newborn neurons. Then, experimental groups were submitted to 12 days of scheduled exercise and/or 8 days of spatial training in the WM. Sedentary and/or untrained groups stayed undisturbed in their home cages. When BrdU+ cells reached maturation (~6 weeks-old), all mice were tested for CPP memory retrieval. Finally, animals were submitted to forced CPP extinction and tested for CPP extinction and cocaine-primed reinstatement. RESULTS: Animals submitted either to the scheduled exercise protocol, training in the WM or both strategies combined, required fewer sessions to extinct cocaine-CPP associations than control animals. Furthermore, animals submitted to both environmental strategies showed a reduced reinstatement when compared to sedentary animals. These effects are partially related to the functional integration of the newborn neurons in the hippocampus. CONCLUSIONS: Both environmental strategies, alone and combined, can reduce the long-term persistence of cocaine-context associations, being AHN associated with these beneficial effects. Funding: PSI2017-82604; PRE2018-085673; 08-2021-AREA3; B1-2020_06; Posdoc_21_00222; Posdoctoral_a32. I Plan Propio de la Universidad de Málaga.

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MORPHOLOGICAL EVIDENCE OF NEURONAL REGULATION OF NEUROGENESIS IN THE RAT ROSTRAL MIGRATORY STREAM

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Regulatory mechanisms of postnatal neurogenesis in the subventricular zone (SVZ) and the rostral migratory stream (RMS) are still not fully understood. Recent evidence suggests that neurogenesis in the SVZ/RMS could be regulated by neurons located directly in these regions. Till now, in the RMS two cell populations showing morphological characteristic of mature neurons have been identified: nitric oxide (NO) producing neurons and neurons expressing secretagogin (SCGN). The aim of our work was to obtain new morphological data about these populations of neurons in the RMS and to map their possible projections. Based on immunofluorescence labeling , we analyzed the distribution and the number of NO+ and SCGN+ cells in the RMS, as well as the relationship between these cells. To reveal projections of NO+ and SCGN+ neurons, a retrograde fluorescent tracer Fluoro-Gold (F-G) was *stereotactically injected* into the striatum. Microscopic observation confirmed the presence of NO+ and SCGN+ neurons with a different distribution pattern along the RMS. The number of SCGN+ neurons was significantly higher in all parts of the RMS, when compared to the number of NO+ neurons. We did not confirm the colocalization of NO+/SCGN+ cells, suggesting that these are two distinct neuronal populations. Confocal microscope analysis revealed the presence of F-G, administered into the striatum, in cells of different parts of the RMS. F-G-labelled cells in RMS were identified as nitrergic neurons, indicating the existence of a neuronal circuit in which NO producing neurons are involved. Supported by VEGA grant 2/0119/22, ERDF project ITMS: 313011V455

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BOARD NUMBER: S07-685

SECRETED FACTORS MODULATING DAMAGE-INDUCED PROLIFERATION IN THE ADULT FLY BRAIN AFTER TRAUMATIC INJURY

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Anabel Simões, Marta Neto, Carolina Alves, Christa Rhiner
Champalimaud Foundation, Stem Cells And Regeneration, Lisbon, Portugal

Brain injury can stimulate the activation of dormant adult neural stem cells in mammals and fruit flies and promote reactive neurogenesis. Nevertheless, the signals governing injury-dependent neural stem cell regulation are still poorly understood. Which are the molecular cues that recruit quiescent stem cells to repair? Here, we studied transcriptional fingerprints of injured versus intact fly brains, which revealed injury-dependent upregulation of stress, immune and tissue repair responses. We then activated RNAi against upregulated genes in adult flies prior to brain injury (stab lesion) and assessed the effect on injury-induced proliferation. This in vivo RNAi screening led to the identification of several secreted molecules, which modulated the regenerative response including a secreted lipocalin-like transporter (LCL-T), able to bind and promote the distribution of lipid- modified factors in the extracellular space. Our results show that LCL-T is not required for normal development, but its upregulation in glia is important for the mobilization of localized Wg/Wnt ligands from damage-responsive neurons to distant neural progenitors. We find that Wg pathway activity is required in disseminated neural progenitor cells for their proliferation. Moreover, we studied upstream control of LCL-T induction and found that these transporters are induced in a Hypoxia-induced factor 1 alpha (HIF1α)-dependent manner downstream of brain lesions. Interestingly, we find that mouse LCL-T is also upregulated in glia in the injured mouse brain suggesting a conserved function of the HIF1α/LCL-T/Wg module in brain restorative processes.

Pubmed:

28642678: Simões AR, Rhiner C
A Cold-Blooded View on Adult Neurogenesis.
During brain development, highly complex and interconnected neural circuits are established. This intricate wiring needs to be robust to faithfully perform adult brain function throughout life, but at the same time offer room for plasticity to integrate new information. In the mammalian brain, adult-born neurons are produced in restricted niches harboring neural stem cells. In the fruit fly , low-level adult neurogenesis arising from a dispersed population of neural progenitors has recently been detected in the optic lobes. Strikingly, these normally quiescent neural stem cells proliferate upon brain injury and produce new neurons for brain regeneration. Here, we review adult neurogenesis in crustaceans and insects and highlight that neurogenesis in the visual system is prominent in arthropods, but its role and underlying mechanisms are unclear. Moreover, we discuss how the study of damage-responsive progenitor cells in may help to understand robust regenerative neurogenesis and open new avenues to enhance brain repair after injury or stroke in humans.
Front Neurosci, 2017; 11
26298667: Mar FM, Simões AR, Rodrigo IS, Sousa MM
Inhibitory Injury Signaling Represses Axon Regeneration After Dorsal Root Injury.
Following injury to peripheral axons, besides increased cyclic adenosine monophosphate (cAMP), the positive injury signals extracellular-signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and signal transducer and activator of transcription 3 (STAT-3) are locally activated and retrogradely transported to the cell body, where they induce a pro-regenerative program. Here, to further understand the importance of injury signaling for successful axon regeneration, we used dorsal root ganglia (DRG) neurons that have a central branch without regenerative capacity and a peripheral branch that regrows after lesion. Although injury to the DRG central branch (dorsal root injury (DRI)) activated ERK, JNK, and STAT-3 and increased cAMP levels, it did not elicit gain of intrinsic growth capacity nor the ability to overcome myelin inhibition, as occurred after peripheral branch injury (sciatic nerve injury (SNI)). Besides, gain of growth capacity after SNI was independent of ERK and cAMP. Antibody microarrays of dynein-immunoprecipitated axoplasm from rats with either DRI or SNI revealed a broad differential activation and transport of signals after each injury type and further supported that ERK, JNK, STAT-3, and cAMP signaling pathways are minor contributors to the differential intrinsic axon growth capacity of both injury models. Increased levels of inhibitory injury signals including GSK3β and ROCKII were identified after DRI, not only in

axons but also in DRG cell bodies. In summary, our work shows that activation and transport of positive injury signals are not sufficient to promote increased axon growth capacity and that differential modulation of inhibitory molecules may contribute to limited regenerative response.
Mol Neurobiol, 2016; 53
24760855: Mar FM, Simões AR, Leite S, Morgado MM, Santos TE, Rodrigo IS, Teixeira CA, Misgeld T, Sousa MM
CNS axons globally increase axonal transport after peripheral conditioning.
Despite the inability of CNS axons to regenerate, an increased regenerative capacity can be elicited following conditioning lesion to the peripheral branch of dorsal root ganglia neurons (DRGs). By in vivo radiolabeling of rat DRGs, coupled to mass spectrometry and kinesin immunoprecipitation of spinal cord extracts, we determined that the anterograde transport of cytoskeleton components, metabolic enzymes and axonal regeneration enhancers, was increased in the central branch of DRGs following a peripheral conditioning lesion. Axonal transport of mitochondria was also increased in the central branch of Thy1-MitoCFP mice following a peripheral injury. This effect was generalized and included augmented transport of lysosomes and synaptophysin- and APP-carrying vesicles. Changes in axonal transport were only elicited by a peripheral lesion and not by spinal cord injury. In mice, elevated levels of motors and of polyglutamylated and tyrosinated tubulin were present following a peripheral lesion and can explain the increase in axonal transport induced by conditioning. In summary, our work shows that a peripheral injury induces a global increase in axonal transport that is not restricted to the peripheral branch, and that, by extending to the central branch, allows a rapid and sustained support of regenerating central axons.
J Neurosci, 2014; 34
24831520: Cardoso S, Carvalho C, Marinho R, Simões A, Sena CM, Matafome P, Santos MS, Seiça RM, Moreira PI
Effects of methylglyoxal and pyridoxamine in rat brain mitochondria bioenergetics and oxidative status.
Advanced glycation end products (AGEs) and methylglyoxal (MG), an important intermediate in AGEs synthesis, are thought to contribute to protein aging and to the pathogenesis of age-and diabetes-associated complications. This study was intended to investigate brain mitochondria bioenergetics and oxidative status of rats previously exposed to chronic treatment with MG and/or with pyridoxamine (PM), a glycation inhibitor. Brain mitochondrial fractions were obtained and several parameters were analyzed: respiratory chain [states 3 and 4 of respiration, respiratory control ratio (RCR), and ADP/O index] and phosphorylation system [transmembrane potential (ΔΨm), ADP-induced depolarization, repolarization lag phase, and ATP levels]; hydrogen peroxide (H_2O_2) production levels, mitochondrial aconitase activity, and malondialdehyde levels as well as non-enzymatic antioxidant defenses (vitamin E and glutathione levels) and enzymatic antioxidant defenses (glutathione disulfide reductase (GR), glutathione peroxidase (GPx), and manganese superoxide dismutase (MnSOD) activities). MG treatment induced a statistical significant decrease in RCR, aconitase and GR activities, and an increase in H_2O_2 production levels. The administration of PM did not counteract MG-induced effects and caused a significant decrease in ΔΨm. In mitochondria from control animals, PM caused an adaptive mechanism characterized by a decrease in aconitase and GR activities as well as an increase in both α-tocopherol levels and GPx and MnSOD activities. Altogether our results show that high levels of MG promote brain mitochondrial impairment and PM is not able to reverse MG-induced effects.
J Bioenerg Biomembr, 2014; 46
25538747: Fregnan F, Muratori L, Simões AR, Giacobini-Robecchi MG, Raimondo S
Role of inflammatory cytokines in peripheral nerve injury.
Inflammatory events occurring in the distal part of an injured peripheral nerve have, nowadays, a great resonance. Investigating the timing of action of the several cytokines in the important stages of Wallerian degeneration helps to understand the regenerative process and design pharmacologic intervention that promotes and expedites recovery. The complex and synergistic action of inflammatory cytokines finally promotes axonal regeneration. Cytokines can be divided into pro- and anti-inflammatory cytokines that upregulate and downregulate, respectively, the production of inflammatory mediators. While pro-inflammatory cytokines are expressed in the first phase of Wallerian degeneration and promote the recruitment of macrophages, anti-inflammatory cytokines are expressed after this recruitment and downregulate the production of all cytokines, thus determining the end of the process. In this review, we describe the major inflammatory cytokines involved in Wallerian degeneration and the early phases of nerve regeneration. In particular, we focus on interleukin-1, interleukin-2, interleukin-6, tumor necrosis factor-β, interleukin-10 and transforming growth factor-β.
Neural Regen Res, 2012; 7

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A CONNECTOME STUDY OF POSTNATAL NEUROGENESIS IN THE OLFACTORY BULB.

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Anne Grelat[1], Carine Moigneu[1], Sebastien Wagner[1], Erwan Martin[1], Sandra Haddad[1], Maxime Lehman[1], Angèle Roudeau[1], Karl-Klaus Conzelmann[2], Pierre-Marie Lledo[1], Mariana Alonso[1]
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Odor–reward association during appetitive learning is a fundamental process that requires multiple forms of plasticity. In the adult olfactory bulb, the continual production of newborn granular cells (GCs) contributes to both structural and functional plasticity of the system. Our recent findings unveil the unexpected role of adult-born neurons, but not neonatal-born neurons, in boosting odor-reward association. How could we interpret this functional difference? Several studies have highlighted distinct functional properties between neonatal-born and adult-born interneurons. In addition to these already identified cell-autonomous discrepancies between distinct GC subpopulations, the possibility of a dedicated connectivity remains to be explored. To tackle this question, we take advantage of a rabies-based mono-synaptic tracing technique to visualize brain-wide inputs to GCs. Our result demonstrated that adult-born GC show higher convergence (connectivity ratios) of these top-down inputs than neonatal ones during adulthood. Interestingly, some differences in the distribution of pre-synaptic partners were observed in olfactory cortex between GC subtypes. Adult and neonatal-born neurons are also differently targeted by top-down GABAergic and cholinergic input. Of note, neonatal-born GCs show a delayed wiring between 2-4 months, which was shortened by odor experience, while adult-born neurons connections remain unchanged. Overall, our data suggest that neurons generated postnatally maintain their singularity during adulthood according to their date of birth. Adult-born GCs integrate top-down fibers in a high convergent mode but invariant to the context of learning. In contrast, their neonatal counterparts integrate less divergent top-down brain inputs, yet the maturation rate of these inputs is amendable by olfactory experience.

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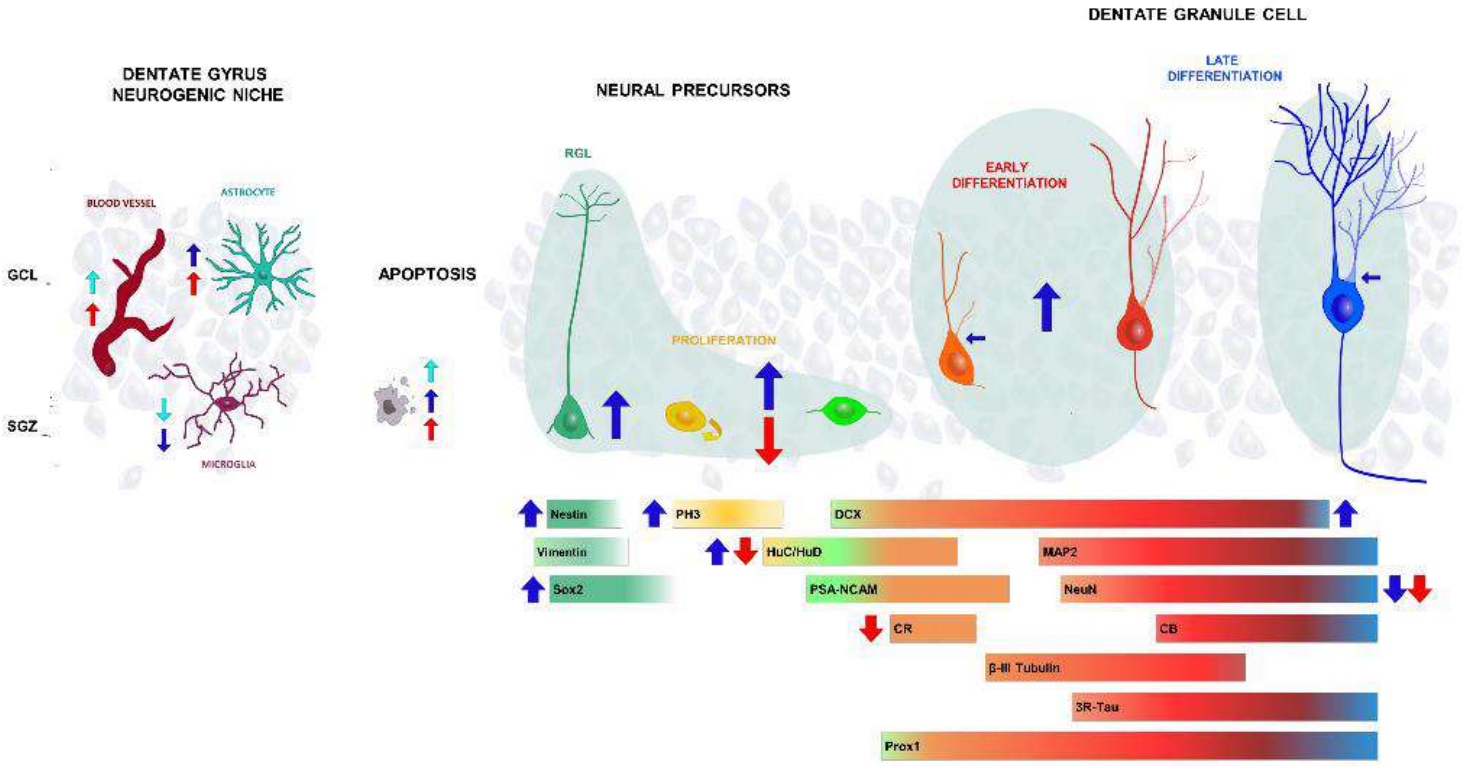
BOARD NUMBER: S07-687

ADULT HIPPOCAMPAL NEUROGENESIS SIGNATURES IN PATIENTS WITH PARKINSON'S DISEASE, DEMENTIA WITH LEWY BODIES, AND FRONTOTEMPORAL DEMENTIA.

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Elena Moreno-Jiménez[1,2,3], Julia Terreros-Roncal[1,2,3], Miguel Flor-Garcia[1,2,3], Carla Rodríguez-Moreno[1,3], Alberto Rábano[4], Maria Llorens-Martín[1,3]
[1]Centro de Biología Molecular "Severo Ochoa" (CBMSO), Spanish Research Council (CSIC)–Universidad Autónoma de Madrid (UAM), Department Of Molecular Neuropathology, Madrid, Spain, [2]Faculty of Sciences, Universidad Autónoma de Madrid, Department Of Molecular Biology, Madrid, Spain, [3]Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Enfermedad De Alzheimer Y Otras Demencias Degenerativas, Madrid, Spain, [4]Centro en Investigación de Enfermedades Neurológicas, CIEN Foundation, Neuropathology Department, Madrid, Spain

Aims: The hippocampus hosts a unique phenomenon of lifetime plasticity, named adult hippocampal neurogenesis (AHN), which includes the generation of new neurons in the dentate gyrus (DG). This process encompasses the division of neural stem cells (NSCs), which give rise to newly generated dentate granule cells (DGCs). AHN is orchestrated by the DG neurogenic niche, a specialized structure composed of astrocytes, microglia, and vasculature. Since the hippocampus is targeted by neurodegenerative diseases, we wondered whether altered AHN underlies hippocampal malfunctioning in these conditions.



Methods: We studied post-mortem human samples obtained from 15 neurologically healthy control subjects and patients with two α-synucleinopathies (Parkinson's disease (PD) (n=3) and dementia with Lewy bodies (LD) (n=6)), and Frontotemporal dementia (FTD) (n=6) to address the integrity of AHN. By using samples subjected to state-of-art tissue preservation and histological methodologies, we reconstructed the stages encompassed by human AHN in control and

patients. **Results:** Our results indicate that both α-synucleinopathies alter the homeostasis of the DG neurogenic niche, compromising the maturation of DGCs. Moreover, these conditions exhibited increased densities of NSCs and triggered morphological abnormalities in Doublecortin $(DCX)^+$ immature DGCs. Additionally, FTD caused milder alterations in AHN and the homeostasis of the DG neurogenic niche. FTD showed an imbalanced NSC/proliferative cells ratio. Furthermore, immature DGCs showed moderately impaired differentiation. **Conclusions:** Neurodegenerative diseases perturb the homeostasis of the human DG neurogenic niche and impair AHN. Moreover, each of the diseases studied is characterized by a specific AHN signature which might be a relevant mechanism underlying hippocampal malfunctioning.

Pubmed:

34672693: Terreros-Roncal J, Moreno-Jiménez EP, Flor-García M, Rodríguez-Moreno CB, Trinchero MF, Cafini F, Rábano A, Llorens-Martín M
Impact of neurodegenerative diseases on human adult hippocampal neurogenesis.
Disrupted hippocampal performance underlies psychiatric comorbidities and cognitive impairments in patients with neurodegenerative disorders. To understand the contribution of adult hippocampal neurogenesis (AHN) to amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease, dementia with Lewy bodies, and frontotemporal dementia, we studied postmortem human samples. We found that adult-born dentate granule cells showed abnormal morphological development and changes in the expression of differentiation markers. The ratio of quiescent to proliferating hippocampal neural stem cells shifted, and the homeostasis of the neurogenic niche was altered. Aging and neurodegenerative diseases reduced the phagocytic capacity of microglia, triggered astrogliosis, and altered the microvasculature of the dentate gyrus. Thus, enhanced vulnerability of AHN to neurodegeneration might underlie hippocampal dysfunction during physiological and pathological aging in humans.
Science, 2021; 374
33762406: Moreno-Jiménez EP, Terreros-Roncal J, Flor-García M, Rábano A, Llorens-Martín M
Evidences for Adult Hippocampal Neurogenesis in Humans.
The rodent hippocampus generates new neurons throughout life. This process, named adult hippocampal neurogenesis (AHN), is a striking form of neural plasticity that occurs in the brains of numerous mammalian species. Direct evidence of adult neurogenesis in humans has remained elusive, although the occurrence of this phenomenon in the human dentate gyrus has been demonstrated in seminal studies and recent research that have applied distinct approaches to birthdate newly generated neurons and to validate markers of adult-born neurons. Our data point to the persistence of AHN until the 10th decade of human life, as well as to marked impairments in this process in patients with Alzheimer's disease. Moreover, our work demonstrates that the methods used to process and analyze postmortem human brain samples can limit the detection of various markers of AHN to the point of making them undetectable. In this Dual Perspectives article, we highlight the critical methodological aspects that should be strictly controlled in human studies and the robust evidence that supports the occurrence of AHN in humans. We also put forward reasons that may account for current discrepancies on this topic. Finally, the unresolved questions and future challenges awaiting the field are highlighted.
J Neurosci, 2021; 41
31915385: Flor-García M, Terreros-Roncal J, Moreno-Jiménez EP, Ávila J, Rábano A, Llorens-Martín M
Unraveling human adult hippocampal neurogenesis.
Adult neurogenesis occurs in a few selected regions of the mammalian brain. One such region is the hippocampus, the so-called gateway to memory, where adult hippocampal neurogenesis (AHN) occurs. Here, we provide a comprehensive description of the methods used in our laboratory to unambiguously detect a population of immature neurons in the human hippocampus until the 10th decade of life. The criteria used to refine and develop the current protocol include obtaining post-mortem human samples of remarkable quality and under tightly controlled conditions for immunohistochemistry (IHC) studies, optimizing tissue processing and histological procedures, establishing criteria to reliably validate antibody signal and performing unbiased stereological cell counts. Moreover, we provide a detailed description of the parameters that, in our view, should be reported in human AHN studies. The opposing results obtained by introducing slight variations in the methodological conditions should be considered by future studies that seek to increase our knowledge of this fascinating process. By applying simple and inexpensive tissue pre-treatments, this protocol, which can be completed in 7 days, might be applicable to a variety of IHC studies performed on other tissues of human (or animal) origin.
Nat Protoc, 2020; 15
31698192: Díaz-Guerra E, Rodríguez-Traver E, Moreno-Jiménez EP, de Rojas I, Rodríguez C, Orera M, Hernández I, Ruiz A, Vicario C
An integration-free iPSC line, ICCSICi007-A, derived from a female Alzheimer's disease patient with the APOE-ε4/ε4 alleles. The epsilon4 (ε4) allele of the APOE gene, which encodes the apolipoprotein E4 (ApoE4), is the strongest genetic risk factor known for late-onset Alzheimer´s disease (LOAD). Here, we present the characterization of an iPSC line generated from

dermal fibroblasts of a female AD patient using Sendai viral vectors encoding the transcription factors OCT4, SOX2, KLF4 and c-MYC. The iPSCs maintained the original genotype, a normal karyotype, were free from Sendai viral vectors and reprogramming factors, presented a normal morphology, expressed endogenous pluripotency markers, and could be differentiated into ectodermal, mesodermal and endodermal cells, confirming its pluripotency.
Stem Cell Res, 2019; 41
31627126: Díaz-Guerra E, Oria-Muriel MA, Moreno-Jiménez EP, de Rojasb I, Rodríguez C, Rodríguez-Traver E, Orera M, Hernándezb I, Ruizb A, Vicario C
Generation of an integration-free iPSC line, ICCSICi006-A, derived from a male Alzheimer's disease patient carrying the PSEN1-G206D mutation.
The familial form of Alzheimer's disease (FAD), which is caused by mutations in PRESENILIN 1 (PSEN1) and amyloid precursor protein (APP) genes, represents less than 5% of all AD cases and has an early-onset. We report the generation and characterization of an iPSC line derived from a FAD patient carrying the PSEN1-G206D mutation. The iPSC line maintained the original genotype, a normal karyotype, was free from Sendai viral vectors and reprogramming factors (OCT4, SOX2, KLF4 and c-MYC), presented a typical morphology, expressed endogenous pluripotency markers, and could be differentiated into ectodermal, mesodermal and endodermal cells, confirming its pluripotency.
Stem Cell Res, 2019; 40
31401456: Díaz-Guerra E, Moreno-Jiménez EP, de Rojas I, Rodríguez C, Rodríguez-Traver E, Arribas-González E, Orera M, Hernández I, Ruiz A, Vicario C
A collection of four integration-free iPSC lines derived from diagnosed sporadic Alzheimer's disease patients with different APOE alleles.
Genetic polymorphism of apolipoprotein E (APOE) confers differential susceptibility to late-onset Alzheimer's disease (LOAD). The ε3 allele of APOE, the most common isoform, does not represent a risk factor for LOAD. In contrast, the ε4 allele is the strongest genetic risk factor for this disease. Here, we present the characterization of four iPSC lines generated from dermal fibroblasts of diagnosed sporadic AD patients using Sendai viral vectors encoding OCT4, SOX2, KLF4 and c-MYC. The iPSCs expressed endogenous pluripotency markers, could be differentiated into the three germ layers, maintained the original genotypes, and were free from Sendai vectors and reprogramming factors.
Stem Cell Res, 2019; 39
31133559: Terreros-Roncal J, Flor-García M, Moreno-Jiménez EP, Pallas-Bazarra N, Rábano A, Sah N, van Praag H, Giacomini D, Schinder AF, Ávila J, Llorens-Martín M
Activity-Dependent Reconnection of Adult-Born Dentate Granule Cells in a Mouse Model of Frontotemporal Dementia.
Frontotemporal dementia (FTD) is characterized by neuronal loss in the frontal and temporal lobes of the brain. Here, we provide the first evidence of striking morphological alterations in dentate granule cells (DGCs) of FTD patients and in a mouse model of the disease, Tau mice. Taking advantage of the fact that the hippocampal dentate gyrus (DG) gives rise to newborn DGCs throughout the lifetime in rodents, we used RGB retroviruses to study the temporary course of these alterations in newborn DGCs of female Tau mice. In addition, retroviruses that encode either PSD95:GFP or Syn:GFP revealed striking alterations in the afferent and efferent connectivity of newborn Tau DGCs, and monosynaptic retrograde rabies virus tracing showed that these cells are disconnected from distal brain regions and local sources of excitatory innervation. However, the same cells exhibited a predominance of local inhibitory innervation. Accordingly, the expression of presynaptic and postsynaptic markers of inhibitory synapses was markedly increased in the DG of Tau mice and FTD patients. Moreover, an increased number of neuropeptide Y-positive interneurons in the DG correlated with a reduced number of activated egr-1 DGCs in Tau mice. Finally, we tested the therapeutic potential of environmental enrichment and chemoactivation to reverse these alterations in mice. Both strategies reversed the morphological alterations of newborn DGCs and partially restored their connectivity in a mouse model of the disease. Moreover, our data point to remarkable morphological similarities between the DGCs of Tau mice and FTD patients. We show, for the first time to our knowledge, that the population of dentate granule cells is disconnected from other regions of the brain in the neurodegenerative disease frontotemporal dementia (FTD). These alterations were observed in FTD patients and in a mouse model of this disease. Moreover, we tested the therapeutic potential of two strategies, environmental enrichment and chemoactivation, to stimulate the activity of these neurons in mice. We found that some of the alterations were reversed by these therapeutic interventions.
J Neurosci, 2019; 39
31080799: Moreno-Jiménez EP, Jurado-Arjona J, Ávila J, Llorens-Martín M
The Social Component of Environmental Enrichment Is a Pro-neurogenic Stimulus in Adult c57BL6 Female Mice.
In rodents, the hippocampal dentate gyrus gives rise to newly generated dentate granule cells (DGCs) throughout life. This process, named adult hippocampal neurogenesis (AHN), converges in the functional integration of mature DGCs into the trisynaptic hippocampal circuit. Environmental enrichment (EE) is one of the most potent positive regulators of AHN. This paradigm includes the combination of three major stimulatory components, namely increased physical activity, constant

cognitive stimulation, and higher social interaction. In this regard, the pro-neurogenic effects of physical activity and cognitive stimulation have been widely addressed in adult rodents. However, the pro-neurogenic potential of the social aspect of EE has been less explored to date. Here we tackled this question by specifically focusing on the effects of a prolonged period of social enrichment (SE) in adult female C57BL6 mice. To this end, 7-week-old mice were housed in groups of 12 per cage for 8 weeks. These mice were compared with others housed under control housing (2-3 mice per cage) or EE (12 mice per cage plus running wheels and toys) conditions during the same period. We analyzed the number and morphology of Doublecortin-expressing (DCX) cells. Moreover, using RGB retroviruses that allowed the labeling of three populations of newborn DGCs of different ages in the same mouse, we performed morphometric, immunohistochemical, and behavioral determinations. Both SE and EE increased the number and maturation of DCX cells, and caused an increase in dendritic maturation in certain populations of newborn DGCs. Moreover, both manipulations increased exploratory behavior in the Social Interaction test. Unexpectedly, our data revealed the potent neurogenesis-stimulating potential of SE in the absence of any further cognitive stimulation or increase in physical activity. Given that an increase in physical activity is strongly discouraged under certain circumstances, our findings may be relevant in the context of enhancing AHN via physical activity-independent mechanisms.
Front Cell Dev Biol, 2019; 7

30911133: Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, Ávila J, Llorens-Martín M

Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease.

The hippocampus is one of the most affected areas in Alzheimer's disease (AD). Moreover, this structure hosts one of the most unique phenomena of the adult mammalian brain, namely, the addition of new neurons throughout life. This process, called adult hippocampal neurogenesis (AHN), confers an unparalleled degree of plasticity to the entire hippocampal circuitry. Nonetheless, direct evidence of AHN in humans has remained elusive. Thus, determining whether new neurons are continuously incorporated into the human dentate gyrus (DG) during physiological and pathological aging is a crucial question with outstanding therapeutic potential. By combining human brain samples obtained under tightly controlled conditions and state-of-the-art tissue processing methods, we identified thousands of immature neurons in the DG of neurologically healthy human subjects up to the ninth decade of life. These neurons exhibited variable degrees of maturation along differentiation stages of AHN. In sharp contrast, the number and maturation of these neurons progressively declined as AD advanced. These results demonstrate the persistence of AHN during both physiological and pathological aging in humans and provide evidence for impaired neurogenesis as a potentially relevant mechanism underlying memory deficits in AD that might be amenable to novel therapeutic strategies.
Nat Med, 2019; 25

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BOARD NUMBER: S07-688

ROLE OF C-TERMINAL BINDING PROTEIN 1 IN THE REGULATION OF ADULT NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Neural stem cells (NSCs) are multipotent cells situated in neurogenic niches of brain that keep the ability to self-renew and/or differentiate into specialised cell types such as neurons and glia throughout the lifetime. The transition of NSCs to their mature phenotypes require complex reconfiguration of gene expressional profiles that occur at multiple cell-fate decision checkpoints. Transcriptional regulator C-terminal binding protein 1 (CtBP1) was recently implicated in mammalian neurodevelopment. Specifically, a patient cohort was described with a de novo missense mutation in CtBP1 with developmental delay, intellectual disability, ataxia and hypotonia. However, the putative function of CtBP1 in the context of neurodevelopment is underexplored as well as mechanism, by which the mutation causes the observed phenotypic manifestations. Herein, we studied functions of CtBP1 in adult neurogenesis, which is a process recapitulating main features of brain neurodevelopment, using constitutive mouse model. First, we mapped the expression of CtBP1 in adult neurogenic lineage and characterized adult hippocampal neurogenesis in a CtBP1 knockout mouse model immunohistochemically using specific differentiation stage markers. Next, using BrdU pulse chase experiments, we assessed proliferation capacity of NSCs and differentiation of newly generated neuronal precursors in absence of CtBP1. Finally, we also isolated NSCs from the neurogenic niche of adult CtBP1 KO and WT mice and studied their proliferation and differentiation in vitro using RT-PCR profiling. Collectively, our results will reveal the role of CtBP1 in the regulation of adult neural stem cell proliferation and their neuronal cell-fate determination and differentiation.

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NEUROGENIC AND OLIGODENDROGENIC PROGENITORS DERIVED FROM THE SUBEPENDYMAL ZONE OF POSTNATAL MICE EXHIBIT DIFFERENTIAL PROPERTIES IN CULTURES WITH POLYMORPHIC MICROENVIRONMENTS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Maria Anesti, Ilias Kazanis
University of Patras, Laboratory Of Developmental Biology, Department Of Biology, Patras, Greece

Aims
Two major stem cell pools, exhibiting distinct spatial preferences, exist in the postnatal mammalian brain: multipotent Neural Stem Cells (NSCs) and the more lineage-restricted oligodendrocyte progenitor cells. The former are located only within specialized microenvironments (niches), while the latter are broadly dispersed in the brain parenchyma. Here, we investigate if the neurogenic versus oligodendrogenic properties of NSCs are differentially affected by aspects of their microenvironment.
Methods
We employed a polymorphic NSC culture assay in which cells were cultured as neurospheres and subsequently were plated on PDL-coated coverslips to create different microenvironments, that we classified either as “niche-like” 3D areas, or as “parenchyma-like” 2D areas. Cells were differentiated and the acquisition of a neurogenic or oligodendrogenic cell fate was identified.
Results
Neurogenesis was much more dependent on the cytoarchitecture, observed at significantly higher levels in “parenchyma-like” areas, more often at the periphery of the “niche-like” structures and being proportional to cell density, while oligodendrogenesis was independent of the cytoarchitecture. In the presence of laminin (a major niche extracellular component) cell cultures became more homogeneously two-dimensional, but neurogenesis was switched to a more “niche-like” behavior, while oligodendrogenesis was not affected. Notably, the inhibition of β1-integrin further enhanced cell fate diversion, almost eliminating neurogenesis but increasing oligodendrogenesis. Finally, microneurotrophin BNN-20 was the only factor that increased both cell fates and specifically in 2D areas.
Conclusions
Our novel method of analysis revealed higher microenvironment restrictions for neurogenesis versus oligodendrogenesis and can be used as a tool for assessing possible therapeutic molecules targeting NSCs.

Pubmed:
31747631: Anesti M, Stavropoulou N, Atsopardi K, Lamari FN, Panagopoulos NT, Margarity M
Effect of rutin on anxiety-like behavior and activity of acetylcholinesterase isoforms in specific brain regions of pentylenetetrazol-treated mice.
The aim of the present study was to investigate the effect of rutin administration (100 mg/kg/day) to pentylenetetrazol (PTZ)-treated Balb-c mice (60 mg/kg/day), with respect to anxiety-like behavior using both open-field and elevated plus-maze (EPM) tests, and acetylcholinesterase (AChE) activity in salt-soluble (SS) fraction and detergent-soluble (DS) fraction of the cerebral cortex, hippocampus, striatum, midbrain, and diencephalon. Our results demonstrated that the administration of PTZ in 3 doses and the induction of seizures increased significantly anxiety behavior of mice and reduced significantly DS-AChE activity in all brain regions examined, while the reduction in the SS fraction was brain region-specific. Rutin administration to normal mice did not affect their behavior, while it induced a brain region-specific reduction in SS-AChE and a significant decrease in DS-AChE in all brain regions. We demonstrated for the first time that pretreatment of PTZ-mice with rutin (PTZ + Rutin group) prevented the manifestation of anxiety and induced interestingly a further significant reduction on the SS- and DS-AChE activities only in the cerebral cortex and striatum, in comparison with PTZ group. Our results show that rutin exhibits an important anxiolytic effect and an anticholinesterase activity in specific brain areas in the seizure model of PTZ.
Epilepsy Behav, 2020; 102
34112234: Mourtzi T, Dimitrakopoulos D, Kakogiannis D, Salodimitris C, Botsakis K, Meri DK, Anesti M, Dimopoulou A, Charalampopoulos I, Gravanis A, Matsokis N, Angelatou F, Kazanis I
Characterization of substantia nigra neurogenesis in homeostasis and dopaminergic degeneration: beneficial effects of the

microneurotrophin BNN-20.
Loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) underlines much of the pathology of Parkinson's disease (PD), but the existence of an endogenous neurogenic system that could be targeted as a therapeutic strategy has been controversial. BNN-20 is a synthetic, BDNF-mimicking, microneurotrophin that we previously showed to exhibit a pleiotropic neuroprotective effect on the dopaminergic neurons of the SNpc in the "weaver" mouse model of PD. Here, we assessed its potential effects on neurogenesis.
Stem Cell Res Ther, 2021; 12

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BOARD NUMBER: S07-690

MIGRATION-PROMOTING VASCULAR SCAFFOLD IN THE POSTNATAL NEUROGENIC REGION: A COMPARISON OF MICE AND RATS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Marcela Martončíková[1], Kamila Fabianová[1], Adam Raček[1], Alexandra Popovičová[1], Anna Alexovic Matiasova[2], Juraj Sevc[2], Eniko Račeková[1]
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Newborn cells arising from the subventricular zone of the lateral ventricles (SVZ) of the rodent brain migrate for long distances along the rostral migratory (RMS) to the olfactory bulb (OB). During the postnatal period specifically arranged blood vessels serve as a scaffold along which neuronal precursors migrate in the RMS and OB. Between the RMS of the rat and mice several morphological and developmental differences were found and previous studies have also indicated differences in the vascular scaffold pattern between these species. However, a detailed morphological analysis of these differences has not yet been performed. Thus the aim of our study was to compare specific arrangement of blood vessels creating vascular scaffold in the RMS between adult mice and rats. The pattern of blood vessel arrangement in the RMS of mice and rats was analyzed qualitatively and quantitatively on immunohistochemically processed brain sections. We did not find significant interspecies differences in migration-promoting vascular scaffold in the RMS. For most of the pathway, blood vessels were aligned parallel to the RMS outline in both species. Contrary to this, blood vessels were turned under a distinct angle, creating a spiral shaped configuration in the caudal part of the RMS in rats as well as in mice. Similarity of the pattern of the vascular scaffold in adult mice and rats indicates that vascular scaffold guiding neuroblast migration in the RMS is the original mechanism with similar properties in different species. Supported by ERDF project ITMS: 313011V455; APVV-19-0279

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BOARD NUMBER: S07-691

STUDYING THE EFFECT OF PERIPHERAL INFLAMMATION, SPECIFICALLY URINARY TRACT INFECTION, ON NEUROPLASTICITY

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Several clinical cases have reported that urinary tract infections (UTI) cause cognitive deficits mainly in elderly patients increasing the chances of developing neuropsychiatric disorders. No studies have explored the mechanism of action and the effect of urinary tract infections on neurogenesis and brain plasticity. In this project, adult male Sprague-Dawley rats received transurethral instillation of LPS to their bladders to mimic UTI-associated inflammation. Here, we aim to investigate the effect of peripheral inflammation in the urothelium on adult hippocampal neurogenesis. Control groups were instilled with sterile saline. Bromodeoxyuridine (BrdU) analog was injected 24 hours before the sacrifice in order to assess hippocampal neural stem cells (NSCs) proliferation. Behavioral tests including, open field, Y-maze, and Novel Object Recognition, were used to assess anxiety-like behaviors, exploration, cognitive ability and working memory respectively. Preliminary results showed that rats that received LPS were more sensitive to thermal and mechanical pain. LPS injected rats also showed decreased cognitive abilities and decreased exploratory behavior after induction of the inflammation. This allows us to speculate that inflammation of the urinary tract potentially affects neurogenesis and subsequently cognitive behavior.

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IMPROVED HIPPOCAMPAL NEUROGENESIS AND COGNITIVE PERFORMANCE IN A MOUSE MODEL OF ACCELERATED AGING

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Ricardo Gómez-Oliva[1,2], Isabel Atienza[1,2], Sergio Martínez-Ortega[1], Samuel Domínguez-García[1,2], Noelia Geribaldi-Doldán[2,3], Carlos Bernal-Utrera[2,4], Pedro Nunez-Abades[2,5], Monica Garcia-Alloza[1,2], Carmen Castro[1,2]
[1]University of Cadiz, Physiology, Cádiz, Spain, [2]Instituto de Investigacion e Innovacion en Ciencias Biomedicas de la Provincia de Cadiz, Inibica, Cádiz, Spain, [3]University of Cádiz, Human Anatomy And Embryology, Cádiz, Spain, [4]University of Seville, Physiotherapy, Seville, Spain, [5]University of Seville, Physiology, Seville, Spain

Background: Current therapeutic approaches to achieve neuronal renewal in damaged adult brains are focused on promoting endogenous neurogenesis to replace the lost neurons. We have previously demonstrated that 12-deoxyphorbols, as ER272, activate the proliferation of adult neural progenitor cells *in vitro* and *in vivo*, with the advantage that they lack tumorigenic activity. Intranasal administration of ER272 to healthy 2 months old CD1 mice, induced neurogenesis within the subventricular zone and the dentate gyrus (DG) of the hippocampus, facilitating the generation of new neurons. **Aims:** To analyze the effect of ER272 compound on neurogenesis and cognitive performance in the senescence-accelerated prone (SAMP8) 6 months old mouse, an accelerated-aging model. **Methods:** Four-month old SAMP8 mice were treated for two months with daily intranasal administrations of ER272. After assessing cognitive performance, brain tissues were analyzed by immunostaining to evaluate neurogenesis. The molecular mechanisms involved in the effect were tested in vitro in Hek293 cells and western blot was used to asses signaling pathways. **Results:** In vitro assays revealed that ER272 promotes the release of transforming growth factor alpha in a classical PKC dependent manner. Intranasal administration of ER272 during 2 months improved cognitive performance of aged mice. Moreover, the compound incremented the number of newly generated neuroblasts and neurons within the DG of the hippocampus not activating radial glial like cells but reducing astrocyte differentiation. The EGFR signaling pathway was involved. **Conclusions:** These results indicate that intranasal administration of the TGFa-releasing compound ER272 promotes neuronal replacement in the aged hippocampus improving cognitive performance.

Pubmed:
34924951: Perez-García P, Pardillo-Díaz R, Geribaldi-Doldán N, Gómez-Oliva R, Domínguez-García S, Castro C, Nunez-Abades P, Carrascal L
Refinement of Active and Passive Membrane Properties of Layer V Pyramidal Neurons in Rat Primary Motor Cortex During Postnatal Development.
Achieving the distinctive complex behaviors of adult mammals requires the development of a great variety of specialized neural circuits. Although the development of these circuits begins during the embryonic stage, they remain immature at birth, requiring a postnatal maturation process to achieve these complex tasks. Understanding how the neuronal membrane properties and circuits change during development is the first step to understand their transition into efficient ones. Thus, using whole cell patch clamp recordings, we have studied the changes in the electrophysiological properties of layer V pyramidal neurons of the rat primary motor cortex during postnatal development. Among all the parameters studied, only the voltage threshold was established at birth and, although some of the changes occurred mainly during the second postnatal week, other properties such as membrane potential, capacitance, duration of the post-hyperpolarization phase or the maximum firing rate were not defined until the beginning of adulthood. Those modifications lead to a decrease in neuronal excitability and to an increase in the working range in young adult neurons, allowing more sensitive and accurate responses. This maturation process, that involves an increase in neuronal size and changes in ionic conductances, seems to be influenced by the neuronal type and by the task that neurons perform as inferred from the comparison with other pyramidal and motor neuron populations.
Front Mol Neurosci, 2021; 14
33335309: Domínguez-García S, Gómez-Oliva R, Geribaldi-Doldán N, Hierro-Bujalance C, Sendra M, Ruiz FA, Carrascal L, Macías-Sánchez AJ, Verástegui C, Hernández-Galán R, García-Alloza M, Nunez-Abades P, Castro C
Effects of classical PKC activation on hippocampal neurogenesis and cognitive performance: mechanism of action.

Hippocampal neurogenesis has widely been linked to memory and learning performance. New neurons generated from neural stem cells (NSC) within the dentate gyrus of the hippocampus (DG) integrate in hippocampal circuitry participating in memory tasks. Several neurological and neuropsychiatric disorders show cognitive impairment together with a reduction in DG neurogenesis. Growth factors secreted within the DG promote neurogenesis. Protein kinases of the protein kinase C (PKC) family facilitate the release of several of these growth factors, highlighting the role of PKC isozymes as key target molecules for the development of drugs that induce hippocampal neurogenesis. PKC activating diterpenes have been shown to facilitate NSC proliferation in neurogenic niches when injected intracerebroventricularly. We show in here that long-term administration of diterpene ER272 promotes neurogenesis in the subventricular zone and in the DG of mice, affecting neuroblasts differentiation and neuronal maturation. A concomitant improvement in learning and spatial memory tasks performance can be observed. Insights into the mechanism of action reveal that this compound facilitates classical PKCα activation and promotes transforming growth factor alpha (TGFα) and, to a lesser extent, neuregulin release. Our results highlight the role of this molecule in the development of pharmacological drugs to treat neurological and neuropsychiatric disorders associated with memory loss and a deficient neurogenesis.
Neuropsychopharmacology, 2021; 46
33945688: Ezzanad A, Gómez-Oliva R, Escobar-Montaño F, Díez-Salguero M, Geribaldi-Doldan N, Dominguez-Garcia S, Botubol-Ares JM, Reyes CL, Durán-Patrón R, Nunez-Abades P, Macías-Sánchez AJ, Castro C, Hernández-Galán R
Phorbol Diesters and 12-Deoxy-16-hydroxyphorbol 13,16-Diesters Induce TGFα Release and Adult Mouse Neurogenesis.
A small library of phorbol 12,13-diesters bearing low lipophilicity ester chains was prepared as potential neurogenic agents in the adult brain. They were also used in a targeted UHPLC-HRMS screening of the latex of . Two new 12-deoxy-16-hydroxyphorbol 13,16-diesters were isolated, and their structures were deduced using two-dimensional NMR spectroscopy and NOE experiments. The ability of natural and synthetic compounds to stimulate transforming growth factor alpha (TFGα) release, to increase neural progenitor cell proliferation, and to stimulate neurogenesis was evaluated. All compounds that facilitated TGFα release promoted neural progenitor cell proliferation. The presence of two acyloxy moieties on the tigliane skeleton led to higher levels of activity, which decreased when a free hydroxyl group was at C-12. Remarkably, the compound bearing isobutyryloxy groups was the most potent on the TGFα assay and at inducing neural progenitor cell proliferation , also leading to enhanced neurogenesis when administered intranasally to mice.
J Med Chem, 2021; 64
33916593: Geribaldi-Doldán N, Hervás-Corpión I, Gómez-Oliva R, Domínguez-García S, Ruiz FA, Iglesias-Lozano I, Carrascal L, Pardillo-Díaz R, Gil-Salú JL, Nunez-Abades P, Valor LM, Castro C
Targeting Protein Kinase C in Glioblastoma Treatment.
Glioblastoma (GBM) is the most frequent and aggressive primary brain tumor and is associated with a poor prognosis. Despite the use of combined treatment approaches, recurrence is almost inevitable and survival longer than 14 or 15 months after diagnosis is low. It is therefore necessary to identify new therapeutic targets to fight GBM progression and recurrence. Some publications have pointed out the role of glioma stem cells (GSCs) as the origin of GBM. These cells, with characteristics of neural stem cells (NSC) present in physiological neurogenic niches, have been proposed as being responsible for the high resistance of GBM to current treatments such as temozolomide (TMZ). The protein Kinase C (PKC) family members play an essential role in transducing signals related with cell cycle entrance, differentiation and apoptosis in NSC and participate in distinct signaling cascades that determine NSC and GSC dynamics. Thus, PKC could be a suitable druggable target to treat recurrent GBM. Clinical trials have tested the efficacy of PKCβ inhibitors, and preclinical studies have focused on other PKC isozymes. Here, we discuss the idea that other PKC isozymes may also be involved in GBM progression and that the development of a new generation of effective drugs should consider the balance between the activation of different PKC subtypes.
Biomedicines, 2021; 9
33585240: Gómez-Oliva R, Domínguez-García S, Carrascal L, Abalos-Martínez J, Pardillo-Díaz R, Verástegui C, Castro C, Nunez-Abades P, Geribaldi-Doldán N
Evolution of Experimental Models in the Study of Glioblastoma: Toward Finding Efficient Treatments.
Glioblastoma (GBM) is the most common form of brain tumor characterized by its resistance to conventional therapies, including temozolomide, the most widely used chemotherapeutic agent in the treatment of GBM. Within the tumor, the presence of glioma stem cells (GSC) seems to be the reason for drug resistance. The discovery of GSC has boosted the search for new experimental models to study GBM, which allow the development of new GBM treatments targeting these cells. In here, we describe different strategies currently in use to study GBM. Initial GBM investigations were focused in the development of xenograft assays. Thereafter, techniques advanced to dissociate tumor cells into single-cell suspensions, which generate aggregates referred to as neurospheres, thus facilitating their selective expansion. Concomitantly, the finding of genes involved in the initiation and progression of GBM tumors, led to the generation of mice models for the GBM. The latest advances have been the use of GBM organoids or 3D-bioprinted mini-brains. 3D bio-printing mimics tissue

cytoarchitecture by combining different types of cells interacting with each other and with extracellular matrix components. These models faithfully replicate human diseases in which the effect of new drugs can easily be tested. Based on recent data from human glioblastoma, this review critically evaluates the different experimental models used in the study of GB, including cell cultures, mouse models, brain organoids, and 3D bioprinting focusing in the advantages and disadvantages of each approach to understand the mechanisms involved in the progression and treatment response of this devastating disease.
Front Oncol, 2020; 10
33352810: Carrascal L, Gorton E, Pardillo-Díaz R, Perez-García P, Gómez-Oliva R, Castro C, Nunez-Abades P
Age-Dependent Vulnerability to Oxidative Stress of Postnatal Rat Pyramidal Motor Cortex Neurons.
Oxidative stress is one of the main proposed mechanisms involved in neuronal degeneration. To evaluate the consequences of oxidative stress on motor cortex pyramidal neurons during postnatal development, rats were classified into three groups: Newborn (P2-P7); infantile (P11-P15); and young adult (P20-P40). Oxidative stress was induced by 10 µM of cumene hydroperoxide (CH) application. In newborn rats, using the whole cell patch-clamp technique in brain slices, no significant modifications in membrane excitability were found. In infantile rats, the input resistance increased and rheobase decreased due to the blockage of GABAergic tonic conductance. Lipid peroxidation induced by CH resulted in a noticeable increase in protein-bound 4-hidroxynonenal in homogenates in only infantile and young adult rat slices. Interestingly, homogenates of newborn rat brain slices showed the highest capacity to respond to oxidative stress by dramatically increasing their glutathione and free thiol content. This increase correlated with a time-dependent increase in the glutathione reductase activity, suggesting a greater buffering capacity of newborn rats to resist oxidative stress. Furthermore, pre-treatment of the slices with glutathione monoethyl ester acted as a neuroprotector in pyramidal neurons of infantile rats. We conclude that during maturation, the vulnerability to oxidative stress in rat motor neurons increases with age.
Antioxidants (Basel), 2020; 9
32321920: Domínguez-García S, Geribaldi-Doldán N, Gómez-Oliva R, Ruiz FA, Carrascal L, Bolívar J, Verástegui C, Garcia-Alloza M, Macías-Sánchez AJ, Hernández-Galán R, Nunez-Abades P, Castro C
A novel PKC activating molecule promotes neuroblast differentiation and delivery of newborn neurons in brain injuries.
Neural stem cells are activated within neurogenic niches in response to brain injuries. This results in the production of neuroblasts, which unsuccessfully attempt to migrate toward the damaged tissue. Injuries constitute a gliogenic/non-neurogenic niche generated by the presence of anti-neurogenic signals, which impair neuronal differentiation and migration. Kinases of the protein kinase C (PKC) family mediate the release of growth factors that participate in different steps of the neurogenic process, particularly, novel PKC isozymes facilitate the release of the neurogenic growth factor neuregulin. We have demonstrated herein that a plant derived diterpene, (EOF2; CAS number 2230806-06-9), with the capacity to activate PKC facilitates the release of neuregulin 1, and promotes neuroblasts differentiation and survival in cultures of subventricular zone (SVZ) isolated cells in a novel PKC dependent manner. Local infusion of this compound in mechanical cortical injuries induces neuroblast enrichment within the perilesional area, and noninvasive intranasal administration of EOF2 promotes migration of neuroblasts from the SVZ towards the injury, allowing their survival and differentiation into mature neurons, being some of them cholinergic and GABAergic. Our results elucidate the mechanism of EOF2 promoting neurogenesis in injuries and highlight the role of novel PKC isozymes as targets in brain injury regeneration.
Cell Death Dis, 2020; 11
32554862: Gómez-Oliva R, Geribaldi-Doldán N, Domínguez-García S, Carrascal L, Verástegui C, Nunez-Abades P, Castro C
Vitamin D deficiency as a potential risk factor for accelerated aging, impaired hippocampal neurogenesis and cognitive decline: a role for Wnt/β-catenin signaling.
Vitamin D is an essential fat-soluble vitamin that participates in several homeostatic functions in mammalian organisms. Lower levels of vitamin D are produced in the older population, vitamin D deficiency being an accelerating factor for the progression of the aging process. In this review, we focus on the effect that vitamin D exerts in the aged brain paying special attention to the neurogenic process. Neurogenesis occurs in the adult brain in neurogenic regions, such as the dentate gyrus of the hippocampus (DG). This region generates new neurons that participate in cognitive tasks. The neurogenic rate in the DG is reduced in the aged brain because of a reduction in the number of neural stem cells (NSC). Homeostatic mechanisms controlled by the Wnt signaling pathway protect this pool of NSC from being depleted. We discuss in here the crosstalk between Wnt signaling and vitamin D, and hypothesize that hypovitaminosis might cause failure in the control of the neurogenic homeostatic mechanisms in the old brain leading to cognitive impairment. Understanding the relationship between vitamin D, neurogenesis and cognitive performance in the aged brain may facilitate prevention of cognitive decline and it can open a door into new therapeutic fields by perspectives in the elderly.
Aging (Albany NY), 2020; 12
30949480: Geribaldi-Doldán N, Gómez-Oliva R, Domínguez-García S, Nunez-Abades P, Castro C
Protein Kinase C: Targets to Regenerate Brain Injuries?
Acute or chronic injury to the central nervous system (CNS), causes neuronal death and irreversible cognitive deficits or

sensory-motor alteration. Despite the capacity of the adult CNS to generate new neurons from neural stem cells (NSC), neuronal replacement following an injury is a restricted process, which does not naturally result in functional regeneration. Therefore, potentiating endogenous neurogenesis is one of the strategies that are currently being under study to regenerate damaged brain tissue. The insignificant neurogenesis that occurs in CNS injuries is a consequence of the gliogenic/non-neurogenic environment that inflammatory signaling molecules create within the injured area. The modification of the extracellular signals to generate a neurogenic environment would facilitate neuronal replacement. However, in order to generate this environment, it is necessary to unearth which molecules promote or impair neurogenesis to introduce the first and/or eliminate the latter. Specific isozymes of the protein kinase C (PKC) family differentially contribute to generate a gliogenic or neurogenic environment in injuries by regulating the ADAM17 mediated release of growth factor receptor ligands. Recent reports describe several non-tumorigenic diterpenes isolated from plants of the genus, which specifically modulate the activity of PKC isozymes promoting neurogenesis. Diterpenes with 12-deoxyphorbol or lathyrane skeleton, increase NPC proliferation in neurogenic niches in the adult mouse brain in a PKCβ dependent manner exerting their effects on transit amplifying cells, whereas PKC inhibition in injuries promotes neurogenesis. Thus, compounds that balance PKC activity in injuries might be of use in the development of new drugs and therapeutic strategies to regenerate brain injuries.
Front Cell Dev Biol, 2019; 7

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BOARD NUMBER: S07-693

EFFECTS OF SEQUENTIAL EXPOSURE TO PHYSICAL EXERCISE AND COGNITIVE TRAINING ON HIPPOCAMPAL NEUROGENESIS IN MICE

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Fabiola Ávila Gámiz, Ana María Pérez Cano, Rosa Mullor-Vigo, José Manuel Pérez Berlanga, Emma Zambrana-Infantes, Luis J. Santín, David Ladrón De Guevara-Miranda
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AIMS: Physical exercise and cognitive training hippocampal dependent tasks are known to enhance adult hippocampal neurogenesis (AHN). Here we aimed to evaluate the effect of either a moderate-intensity exercise protocol, a working memory task and the combination of both treatments on mice AHN. METHODS: Adult male C57BL6/J mice (*N*=34) were submitted to a scheduled treadmill exercise protocol for 12 days (EX-groups) or remained at home cage (SED-groups). 24 hours later, animals either were perfused or trained in a spatial learning task in the Water Maze (WM groups) for 8 days while control groups remained at home cage (CAGE groups). Bromodeoxyuridine (BrdU) was injected at the beginning of every experimental procedure to label hippocampal cells that proliferated during the initial exercise sessions. RESULTS: Mice submitted to scheduled exercise showed an increased number of $BrdU^+$ and $PCNA^+$ dentate granule cells (DGCs) in the short but not in the long-term when compared to sedentary groups. Conversely, training in the WM solely reduced the amount of $BrdU^+$ and $PCNA^+$ DGCs compared to CAGE group. However, animals submitted to scheduled exercise and WM training showed increased proliferation/survival of DGCs in the long-term compared to all other groups. CONCLUSIONS: Our data suggests that the combination of moderate-intensity exercise with spatial training has a powerful neurogenic effect in the DG, being a valuable non-pharmacological strategy for the treatment of neurodegenerative diseases associated with impaired AHN. Funding: PSI2017-82604; PRE2018-085673; FPU20/00908; 08-2021-AREA3; B1-2020_06; Posdoc_21_00222; Posdoctoral_a32. I Plan Propio de Investigación, Transferencia y Divulgación Científica de la Universidad de Málaga.

FENS Forum 2022
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BOARD NUMBER: S07-694

3D TOPOGRAPHY AND DYNAMICS OF MOUSE BRAIN NEUROGENIC ZONES

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Alexander Lazutkin[1,2], Julia Starovatykh[1], Anna Ivanova[1], Sergey Shuvaev[3], Konstantin Anokhin[4,5], Alexei Koulakov[3], Grigori Enikolopov[2,4]
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In the brain, neural stem and progenitor cells divide into restricted regions and their progenies migrate along complex trajectories to reach distant areas of the brain. Until now, the 3D organization of neurogenic zones and pathways of cell migration has remained undisclosed. In this study, we developed the new pipeline for 3D visualization and analysis of cell division and migration in the whole brain, based on whole-mount labeling dividing cells with 5-ethynyl-2'-deoxyuridine (EdU). Using this pipeline, we observed uneven distribution of EdU+ cells in the adult subventricular zone (SVZ) and found regions with high cell density forming three stripes, which merge into a rostral migration stream (RMS). Using an interval pulse labeling paradigm, we observed the displacement of the density of labeled cells from SVZ towards the olfactory bulb (OB). Three stripes in the V-SVZ were not visible clearly 2h after EdU injection and they appeared in 24 h. In 120 h after labeling, almost all EdU+ cells were located in OB. Thus, three stripes in SVZ correspond to three migration branches that have not been previously described. We also discovered two distinct segments of RMS with different proliferative potential - the anterior segment consisted of migrating but not dividing cells. Next, we visualized and analyzed patterns of dividing cells in the perinatal brain and found dynamics of cell migration differ from adults. Finally, we compared brain development of wt vs. autism model mice (16p11.2 df/+) and didn’t reveal a difference in 3D patterns and dynamics of proliferation in brain neurogenic zones.

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INTERMITTENT HYPOXIA PROMOTES POST-STROKE RECOVERY IN RODENT STRIATUM THROUGH NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Syed Roshan[1], Mahesh Kandasamy[2], Swaminathan Jayachandran[3], Anusuyadevi Muthuswamy[1]
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Aim: To investigate intermittent hypoxia (IH) therapy as a non-invasive method to stimulate neurogenesis in the striatal infarct area, and promote functional recovery in experimental focal cerebral ischemia in rats
Methods: Focal cerebral ischemia was induced in Wistar rats using the Middle Cerebral Artery Occlusion (MCAo) model. A week later, animals were exposed to intermittent hypoxia (12%O2, 4hr/day) for a period of 14 days. Post treatment analysis of functional recovery, and cellular regeneration were assessed using behavioral analysis batteries like Rotarod, Barnes maze, Narrow beam walking test, and immunofluorescence analysis of DCX and Vimentin positive cells respectively.
Results: IH treated MCAo group showed significant improvement in narrow beam walking test and Barnes maze test compared to the untreated MCAo group. Immunofluorescence analysis revealed a better increment in the number of DCX positive neuroblasts and in the striatal infarct region in the IH treated group. While morphology of vimentin positive cells appears round in shape in the contralateral side, elongated vimentin positive cells were evident in the ipsilateral hemisphere.
Conclusion: These outcomes suggest that exposure of MCAo stroke affected rats to intermittent hypoxia results in increase of newborn neurons in the infarct region, and thus suggesting an alternative non-invasive method of increasing neuroregeneration in the infarct area after stroke.

Pubmed:
31254250: Kandasamy M, Radhakrishnan RK, Poornimai Abirami GP, Roshan SA, Yesudhas A, Balamuthu K, Prahalathan C, Shanmugaapriya S, Moorthy A, Essa MM, Anusuyadevi M
Possible Existence of the Hypothalamic-Pituitary-Hippocampal (HPH) Axis: A Reciprocal Relationship Between Hippocampal Specific Neuroestradiol Synthesis and Neuroblastosis in Ageing Brains with Special Reference to Menopause and Neurocognitive Disorders.
The hippocampus-derived neuroestradiol plays a major role in neuroplasticity, independent of circulating estradiol that originates from gonads. The response of hypothalamus-pituitary regions towards the synthesis of neuroestradiol in the hippocampus is an emerging scientific concept in cognitive neuroscience. Hippocampal plasticity has been proposed to be regulated via neuroblasts, a major cellular determinant of functional neurogenesis in the adult brain. Defects in differentiation, integration and survival of neuroblasts in the hippocampus appear to be an underlying cause of neurocognitive disorders. Gonadotropin receptors and steroidogenic enzymes have been found to be expressed in neuroblasts in the hippocampus of the brain. However, the reciprocal relationship between hippocampal-specific neuroestradiol synthesis along neuroblastosis and response of pituitary based feedback regulation towards regulation of estradiol level in the hippocampus have not completely been ascertained. Therefore, this conceptual article revisits (1) the cellular basis of neuroestradiol synthesis (2) a potential relationship between neuroestradiol synthesis and neuroblastosis in the hippocampus (3) the possible involvement of aberrant neuroestradiol production with mitochondrial dysfunctions and dyslipidemia in menopause and adult-onset neurodegenerative disorders and (4) provides a hypothesis for the possible existence of the hypothalamic-pituitary-hippocampal (HPH) axis in the adult brain. Eventually, understanding the regulation of hippocampal neurogenesis by abnormal levels of neuroestradiol concentration in association with the feedback regulation of HPH axis might provide additional cues to establish a neuroregenerative therapeutic management for mood swings, depression and cognitive decline in menopause and neurocognitive disorders.
Neurochem Res, 2019; 44
31088631: Kandasamy M, Yesudhas A, Poornimai Abirami GP, Radhakrishnan RK, Roshan SA, Johnson E, Ravichandran VR, Biswas A, Shanmugaapriya S, Anusuyadevi M, Aigner L
Genetic reprogramming of somatic cells into neuroblasts through a co-induction of the doublecortin gene along the Yamanaka factors: A promising approach to model neuroregenerative disorders.

Neural stem cell (NSC) mediated adult neurogenesis represents the regenerative plasticity of the brain. The functionality of the neurogenic process appears to be operated by neuroblasts, the multipotent immature neuronal population of the adult brain. While neuroblasts have been realized to play a major role in synaptic remodeling and immunogenicity, neurodegenerative disorders have been characterized by failure in the terminal differentiation, maturation, integration and survival of newborn neuroblasts. Advancement in understanding the impaired neuroregenerative process along the neuropathological conditions has currently been limited by lack of an appropriate experimental model of neuroblasts. The genetic reprogramming of somatic cells into pluripotent state offers a potential strategy for the experimental modeling of brain disorders. Thus, the induced pluripotent stem cell (iPSC) based direct reprogramming of somatic cells into neuroblasts would represent a potential tool to understand the regenerative biology of the adult brain. Therefore, this concise article discusses the significance of iPSCs, the functional roles of neuroblasts in the adult brain and provides a research hypothesis for the direct reprogramming of somatic cells into neuroblasts through the co-induction of a potential proneurogenic marker, the doublecortin (DCX) gene along with the Yamanaka factors. The proposed cellular model of adult neurogenesis may provide us with further insights into neuropathogenesis of many neurodegenerative disorders and will provide a potential experimental platform for diagnostic, drug discovery and regenerative therapeutic strategies.
Med Hypotheses, 2019; 127

32006362: Poornimai Abirami GP, Radhakrishnan RK, Johnson E, Roshan SA, Yesudhas A, Parveen S, Biswas A, Ravichandran VR, Muthuswamy A, Kandasamy M

The Regulation of Reactive Neuroblastosis, Neuroplasticity, and Nutraceuticals for Effective Management of Autism Spectrum Disorder.

Autism spectrum disorder (ASD) encompasses a cluster of neurodevelopmental and genetic disorders that has been characterized mainly by social withdrawal, repetitive behavior, restricted interests, and deficits in language processing mainly in children. ASD has been known to severely impair behavioral patterns and cognitive functions including learning and memory due to defects in neuroplasticity. The biology of the ASD appears to be highly complex and heterogeneous, and thus, finding a therapeutic target for autism remains obscure. There has been no complete prevention or disease-modifying cure for this disorder. Recently, individuals with autism have been characterized by reactive neurogenesis, obstructions in axonal growth, heterotopia, resulting from dysplasia of neuroblasts in different brain regions. Therefore, it can be assumed that the aforementioned neuropathological correlates seen in the autistic individuals might originate from the defects mainly in the regulation of neuroblasts in the developing as well as adult brain. Nutrient deficiencies during early brain development and intake of certain allergic foods have been proposed as main reasons for the development of ASD. However, the integrated understanding of neurodevelopment and functional aspects of neuroplasticity working through neurogenesis in ASD is highly limited. Moreover, neurogenesis at the level of neuroblasts can be regulated by nutrition. Hence, defects in neuroblastosis underlying the severity of autism potentially could be rectified by appropriate implementation of nutraceuticals.
Adv Neurobiol, 2020; 24

32974763: Yesudhas A, Roshan SA, Radhakrishnan RK, Abirami GPP, Manickam N, Selvaraj K, Elumalai G, Shanmugaapriya S, Anusuyadevi M, Kandasamy M

Intramuscular Injection of BOTOX® Boosts Learning and Memory in Adult Mice in Association with Enriched Circulation of Platelets and Enhanced Density of Pyramidal Neurons in the Hippocampus.

BOTOX® is a therapeutic form of botulinum neurotoxin. It acts by blocking the release of acetylcholine (ACh) from the synaptic vesicles at the neuromuscular junctions, thereby inhibiting the muscle contraction. Notably, many neurological diseases have been characterized by movement disorders in association with abnormal levels of ACh. Thus, blockade of aberrant release of ACh appears to be a potential therapeutic strategy to mitigate many neurological deficits. BOTOX® has widely been used to manage a number of clinical complications like neuromuscular disorders, migraine and neuropathic pain. While the beneficial effects of BOTOX® against movement disorders have extensively been studied, its possible role in the outcome of cognitive function remains to be determined. Therefore, we investigated the effect of BOTOX® on learning and memory in experimental adult mice using behavioural paradigms such as open field task, Morris water maze and novel object recognition test in correlation with haematological parameters and histological assessments of the brain. Results revealed that a mild dose of BOTOX® treatment via an intramuscular route in adult animals improves learning and memory in association with increased number of circulating platelets and enhanced structural plasticity in the hippocampus. In the future, this minimally invasive treatment could be implemented to ameliorate different forms of dementia resulting from abnormal ageing and various neurocognitive disorders including Alzheimer's disease (AD).
Neurochem Res, 2020; 45

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-696

BIDIRECTIONAL INFLUENCE OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELD ON NEUROGENESIS IN HEALTHY BRAIN

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Maciej Klimiuk, Hanna Kletkiewicz, Agnieszka Siejka, Angelika Klimek, Justyna Maliszewska, Joanna Wyszkowska, Milena Jankowska, Anna Nowakowska, Maria Stankiewicz, Justyna Rogalska
Nicolaus Copernicus University, Department Of Animal Physiology And Neurobiology, Toruń, Poland

Recently, an extremely low frequency electromagnetic field (ELF-EMF) has been proposed for the modulation of hippocampal functions, including synaptic plasticity and neurogenesis. However, ELF-EMF-induced notable long-term deficits in learning ability have been also noticed. **We hypothesized that the ability of ELF-EMF to support hippocampal plasticity depends on its intensity (magnetic flux density).** The aim of the research was to evaluate the effect of **50 Hz ELF-EMF of low (1 mT) and high (7 mT) intensity on expression of BDNF and related factors involved in brain plasticity.** Male adult (>3 months) rats (*Wistar* strain) were exposed 3 times to 50 Hz ELF-EMF of 1 or 7 mT intensity (each exposure lasted one week, 1h/day). After each exposure part of animals was sacrificed and hippocampi were collected. RT-qPCR was performed to measure expression of BDNF and related genes. We have shown that the low intensity ELF-EMF activates the BDNF–TrkB signalling pathway and modulate the expression of synaptic protein markers SYN1, GAP43 and PSD95. In contrast higher intensity of ELF-EMF inhibited the BDNF-related signalling pathways. Our research proved the beneficial effect of ELF-EMF of 1 mT on hippocampal plasticity. As there is a link between failure in some steps of adult neurogenesis (cell proliferation, migration, differentiation, and survival) and age-related cognitive decline, as well as neurodegenerative and neuropsychiatric disorders, the knowledge on properties of ELF-EMF improving brain plasticity is important in the development of new therapeutic treatments. Research supported by a grant from the National Science Centre, Poland, no. 2017/25/B/NZ7/00638.

FENS Forum 2022
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BOARD NUMBER: S07-697

INVESTIGATION OF EPENDYMAL CELLS IN THE MOUSE AND HUMAN SUBEPENDYMAL ZONE: IN VIVO ASSESSMENT AND IN VITRO CULTURES

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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[1]University of Patras, Department Of Biology, Patras, Greece, [2]University of Cambridge, Wellcome Trust – Mrc Cambridge Stem Cell Institute, Cambridge, United Kingdom

Aims The brain's stem cell niche located at the Subependymal Zone (SEZ) hosts neurogenesis throughout life in rodents, but in humans this capacity becomes exhausted after the first 18 months. By "Milking" the rat SEZ we can isolate neural stem cells (NSCs) from live animals. This involves the controlled compromise of the ependymal layer, by injecting neuraminidase that is followed by NSCs flowing from into the cerebrospinal fluid whereof they can be collected. Here, we investigate the infant human SEZ in order to assess the transferability of "Milking" and we also explore the effects of neuraminidase on cultured mouse ependymal cells. **Methods** Brain sections from neonatal and 18 month-old infants were immunostained for neuroblast and astroglial markers. In addition, ependymal cells were isolated from adult mice and were kept in short-term cultures either alone or co-cultured with NSCs. **Results** The neonatal SEZ includes a range of ependymal architectures, which become less heterogeneous with age. Surprisingly, extensive areas of ependymal denudation, accompanied by astrogliosis and leaking of neuroblasts in the cerebrospinal fluid were detected. Several mouse ependymal subtypes were identified in cultures based on their size and β-catenin expression. Neuarminidase led to disorganized ependymal clusters, morphological changes and to induction of proliferation as did growth to NSC medium. **Conclusions** Similarities between the infant human and the rat SEZ suggest that "Milking" can be transferred to the clinic, while the presence of naturally-occurring ependymal loss opens a remarkable new question, as does the in vitro induction of proliferation in mouse ependymal cells.

Pubmed:
34560001: McClenahan F, Dimitriou C, Koutsakis C, Dimitrakopoulos D, Arampatzis A, Kakouri P, Kourla M, Oikonomou S, Andreopoulou E, Patsonis M, Meri DK, Rasool RT, Franklin RJ, Kazanis I
Isolation of neural stem and oligodendrocyte progenitor cells from the brain of live rats.
Postnatal brain neural stem and progenitor cells (NSPCs) cluster in anatomically inaccessible stem cell niches, such as the subependymal zone (SEZ). Here, we describe a method for the isolation of NSPCs from live animals, which we term "milking." The intracerebroventricular injection of a release cocktail, containing neuraminidase, integrin-β1-blocking antibody, and fibroblast growth factor 2, induces the controlled flow of NSPCs in the cerebrospinal fluid, where they are collected via liquid biopsies. Isolated cells retain key in vivo self-renewal properties and their cell-type profile reflects the cell composition of their source area, while the function of the niche is sustained even 8 months post-milking. By changing the target area more caudally, we also isolate oligodendrocyte progenitor cells (OPCs) from the corpus callosum. This novel approach for sampling NSPCs and OPCs paves the way for performing longitudinal studies in experimental animals, for more in vivo relevant cell culture assays, and for future clinical neuro-regenerative applications.
Stem Cell Reports, 2021; 16

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-698

THE WILD AND THE TAMED; A COMPARISON OF THE ACTIVITY OF POSTNATAL BRAIN NEURAL STEM AND PROGENITOR CELLS BETWEEN LAB AND WILD RODENTS.

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Theodosia Androutsopoulou[1,2], Athanasia Rapti[1,2], Maria Anesti[1], George Mitsainas[2], Ilias Kazanis[1]
[1]University of Patras, Laboratory Of Developmental Biology, Department Of Biology, Patras, Greece, [2]University of Patras, Laboratory Of Animal Biology, Department Of Biology, Patras, Greece

Aims Postnatal brain neural stem and progenitor cells (NSPCs) cluster within stem cell niches contributing to odour recognition, learning, memory and myelination, with their activity being modified by exercise, stress and environmental enrichment. Here, we investigate the hypothesis that lab-mouse NSPCs might remain in a “tamed”, hypoactive, status, due to their maintenance in very controlled conditions. **Methods** We use lab and wild *Mus musculus* and *Microtus thomasi* animals, the latter being a fossorial species. We analyze brain samples, by immunohistochemical staining for markers specific for cell proliferation (PCNA, Ki67) and neural progenitor identity (nestin, Sox2, Dcx and Olig2), focusing on the Subependymal Zone and the dentate gyrus stem cell niches, as well as on the corpus callosum. Furthermore, we culture NSPCs from the same animals and we assess similar markers immediately after isolation and after two or three passages. **Results** Our data reveal increased density and mitotic activity of NSPCs in the Subependymal Zone of wild species, with no changes in the hippocampus and the corpus callosum. We have also confirmed the ability to culture NSPCs from wild animals and we describe differences both in terms of morphology and behavior. **Conclusions** Our data indicate that the activity of pools of postnatal brain NSPCs are affected by external “life-style” stimuli. Whether such differences are hard-wired in NSPCs or are continuously maintained remains open, but can be addressed by this type of analysis. The above are important in terms of assessing the limitations of using lab animals in experimental analyses.

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BOARD NUMBER: S07-699

PLATELETS ACT AS REGULATORS OF POSTNATAL NEURAL STEM CELLS OF THE SUBEPENDYMAL ZONE AND OF THE NICHE MICROENVIRONMENT

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Christina Dimitriou[1,2], Konstantinos Papadimitriou[1], Konstantinos Roussis[1], Cedric Ghevaert[3], Robin Franklin[2], Ilias Kazanis[1,2]
[1]University of Patras, Laboratory Of Developmental Biology, Department Of Biology, Rio, Greece, [2]University of Cambridge, Wellcome Trust – Mrc Cambridge Stem Cell Institute, Cambridge, United Kingdom, [3]University of Cambridge, Wellcome-mrc Cambridge Stem Cell Institute, Cambridge, United Kingdom

Aims Postnatal Neural Stem Cells (pNSCs) reside in stem cell niches, such as the Subependymal Zone (SEZ) of the lateral ventricles' walls. Previous work revealed specific platelet (PLT) aggregation within the niche's vasculature after focal corpus callosum (CC) demyelination. Moreover, we have shown that PLTs interact directly with pNSCs, using a co-culture system that allows the assessment of direct cell-to-cell interactions. Here, we investigate the functional role of PLTs *in vivo.* **Methods** We assessed the presence of PLTs within the SEZ in a range of other models of degeneration impacting the niche (stroke, neuraminidase-induced ependyma disruption). We also performed CC demyelination in thrombocytopenic (Nbeal2$^{-/-}$,Crlf3$^{-/-}$) and thrombophilic (JAK2V6$^{fl/+}$) mice and analysed extensively the impact on cellular and non-cellular components of the SEZ and CC. We also performed co-cultures using Nbeal2$^{-/-}$-derived PLTs, characterized by non-functional α-granules. The investigation was complemented by transplantations of labelled PLTs in the SEZ and Striatum. **Results** We confirmed the specificity of PLTs' presence in the activated niche's vasculature and our analysis revealed an abnormal response of the SEZ vasculature in mice with altered numbers of circulating PLTs. Thrombocytopenic mice also showed deficient oligodendrogenic activation following the lesion, without changes in neurogenesis. Activated PLTs were observed inside blood vessels and in the brain parenchyma post-transplantation. Nbeal2$^{-/-}$-derived PLTs failed to affect proliferation and differentiation potential of pNSCs. **Conclusions** Overall, PLTs emerge as functional regulators of both pNSCs and their niche, with their role partially dependent on a-granules. ***Acknowledgements*** *The research work was supported by the HFRI and the GSRT.*

H.F.R.I.
Hellenic Foundation for Research & Innovation

GENERAL SECRETARIAT FOR RESEARCH AND TECHNOLOGY

Pubmed:
34560001: McClenahan F, Dimitriou C, Koutsakis C, Dimitrakopoulos D, Arampatzis A, Kakouri P, Kourla M, Oikonomou S, Andreopoulou E, Patsonis M, Meri DK, Rasool RT, Franklin RJ, Kazanis I
Isolation of neural stem and oligodendrocyte progenitor cells from the brain of live rats.
Postnatal brain neural stem and progenitor cells (NSPCs) cluster in anatomically inaccessible stem cell niches, such as the subependymal zone (SEZ). Here, we describe a method for the isolation of NSPCs from live animals, which we term "milking." The intracerebroventricular injection of a release cocktail, containing neuraminidase, integrin-β1-blocking antibody, and fibroblast growth factor 2, induces the controlled flow of NSPCs in the cerebrospinal fluid, where they are collected via liquid biopsies. Isolated cells retain key in vivo self-renewal properties and their cell-type profile reflects the cell composition of their source area, while the function of the niche is sustained even 8 months post-milking. By changing the target area more caudally, we also isolate oligodendrocyte progenitor cells (OPCs) from the corpus callosum. This novel approach for sampling NSPCs and OPCs paves the way for performing longitudinal studies in experimental animals, for more in vivo relevant cell culture assays, and for future clinical neuro-regenerative applications.
Stem Cell Reports, 2021; 16

28196689: Kazanis I, Evans KA, Andreopoulou E, Dimitriou C, Koutsakis C, Karadottir RT, Franklin RJM
Subependymal Zone-Derived Oligodendroblasts Respond to Focal Demyelination but Fail to Generate Myelin in Young and Aged Mice.
Two populations of oligodendrogenic progenitors co-exist within the corpus callosum (CC) of the adult mouse. Local, parenchymal oligodendrocyte progenitor cells (pOPCs) and progenitors generated in the subependymal zone (SEZ) cytogenic niche. pOPCs are committed perinatally and retain their numbers through self-renewing divisions, while SEZ-derived cells are relatively "young," being constantly born from neural stem cells. We compared the behavior of these populations, labeling SEZ-derived cells using hGFAP:Cre mice, within the homeostatic and regenerating CC of the young-adult and aging brain. We found that SEZ-derived oligodendroglial progenitors have limited self-renewing potential and are therefore not bona fide OPCs but rather "oligodendroblasts" more similar to the neuroblasts of the neurogenic output of the SEZ. In the aged CC their mitotic activity is much reduced, although they still act as a "fast-response element" to focal demyelination. In contrast to pOPCs, they fail to generate mature myelinating oligodendrocytes at all ages studied.
Stem Cell Reports, 2017; 8

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-700

SPATIO-TEMPORAL RECRUITMENT OF ADULT NEURAL STEM CELLS DURING PREGNANCY FOR TRANSIENT NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Zayna Chaker[1], Corina Segalada[1], Jonas Kretz[2], Ana Delgado[1], Valerie Crotet[1], Andreas Moor[2], Fiona Doetsch[1]
[1]University of Basel, Biozentrum, Basel, Switzerland, [2]ETH Zurich, D-bsse, Basel, Switzerland

Neural stem cells (NSCs) reside in specialized niches in the adult mammalian brain. The largest site of adult neurogenesis is the ventricular-subventricular stem cell niche, which extends along the brain lateral ventricles. V-SVZ NSCs give rise to different subtypes of olfactory bulb (OB) interneurons as well as some glia throughout life. It is just emerging that adult NSCs are not a uniform population. Instead they comprise a mosaic of cells with unique molecular identities and differentiation fates, depending on their spatial location in the niche. Moreover, NSCs can be found in both quiescent and actively proliferating states *in vivo*. The external signals regulating the balance between stem cell activation and dormancy, as well as the factors controlling regionally distinct NSCs, are still largely unknown. Here, we show that pregnancy activates specific stem cell subpopulations residing in spatially distinct domains of the V-SVZ, with different temporal dynamics. The recruitment of 'pregnancy-related' NSCs is transient, and results in a temporally-controlled generation of different subtypes of OB interneurons. Each wave of newborn neurons becomes fully functional at key physiological periods of motherhood, such as birth or perinatal care. The majority of these pregnancy-associated neurons are short-lived and are culled by weaning. Spatial transcriptomics revealed molecular correlates of OB remodeling during early motherhood, and identified new subtypes of interneurons, which dynamically change with pregnancy. Taken together, our results suggest that 'on-demand' neurogenesis occurs during pregnancy, contributing to OB plasticity and preparing the female brain for motherhood.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-701

BLOOD-CIRCULATING LIPIDS REGULATE ADULT HIPPOCAMPAL NEUROGENESIS IN THE CONTEXT OF ANXIETY

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Thomas Larrieu[1], Charline Carron[1], Kyllian Ginggen[2], Aurélie Delacretaz[1], Hector Gallart-Ayala[3], Julijana Ivanisevic[3], Chin B. Eap[1], Pierre Magistretti[4], Alexandre Dayer[5], Camille Piguet[5], Nicolas Toni[1]
[1]Lausanne University Hospital, Department Of Psychiatry, Center For Psychiatric Neurosciences, Prilly, Switzerland, [2]University of Lausanne, Department Of Biomedical Sciences, Lausanne, Switzerland, [3]Faculty of Biology and Medicine, University of Lausanne, Metabolomics Platform, Faculty Of Biology And Medicine, University Of Lausanne, Switzerland, Lausanne, Switzerland, [4]KAUST, Biological And Environmental Sciences And Engineering, Thuwal, Saudi Arabia, [5]Faculty of Medicine, University of Geneva, Department Of Mental Health And Psychiatry, Genève, Switzerland

Pathological anxiety is an understudied co-morbidity to most psychiatric diseases. Here, we found that serum from spontaneously anxious mice or stressed mice reduced the proliferation of adult hippocampal neural stem/progenitor cells (aNPC) in vitro, in parallel to a reduction of adult neurogenesis in the hippocampus in vivo. Similarly, the serum of human patients reduced aNPC proliferation in vitro, proportionally to their anxiety scores. We identified that lysophosphatidic acid (LPA16:0) was both necessary and sufficient for the effect of serum on aNPC. The effect of LPA16:0 was mediated by the LPA1 receptor, which bore single nucleotide polymorphism variants associated with anxiety. Finally, LPA16:0 treatment decreased aNPC proliferation in the dentate gyrus of the hippocampus and increased stress sensitivity in mice. Together, these results show that blood-circulating LPA16:0 inhibit adult hippocampal neurogenesis in the context of stress and anxiety and may represent a therapeutic target for anxiety-related disorders.

Pubmed:
31841985: Papilloud A, Weger M, Bacq A, Zalachoras I, Hollis F, Larrieu T, Battivelli D, Grosse J, Zanoletti O, Parnaudeau S, Tronche F, Sandi C
The glucocorticoid receptor in the nucleus accumbens plays a crucial role in social rank attainment in rodents.
Social hierarchy in social species is usually established through competitive encounters with conspecifics. It determines the access to limited resources and, thus, leads to reduced fights among individuals within a group. Despite the known importance of social rank for health and well-being, the knowledge about the processes underlying rank attainment remains limited. Previous studies have highlighted the nucleus accumbens (NAc) as a key brain region in the attainment of social hierarchies in rodents. In addition, glucocorticoids and the glucocorticoid receptor (GR) have been implicated in the establishment of social hierarchies and social aversion. However, whether GR in the NAc is involved in social dominance is not yet known. To address this question, we first established that expression levels of GR in the NAc of high anxious, submissive-prone rats are lower than that of their low anxious, dominant-prone counterparts. Furthermore, virally-induced downregulation of GR expression in the NAc in rats led to an improvement of social dominance rank. We found a similar result in a cell-specific mouse model lacking GR in dopaminoceptive neurons (i.e., neurons containing dopamine receptors). Indeed, when cohabitating in dyads of mixed genotypes, mice deficient for GR in dopaminoceptive neurons had a higher probability to become dominant than wild-type mice. Overall, our results highlight GR in the NAc and in dopaminoceptive neurons as an important regulator of social rank attainment.
Psychoneuroendocrinology, 2020; 112
31922486: Cherix A, Larrieu T, Grosse J, Rodrigues J, McEwen B, Nasca C, Gruetter R, Sandi C
Metabolic signature in nucleus accumbens for anti-depressant-like effects of acetyl-L-carnitine.
Emerging evidence suggests that hierarchical status provides vulnerability to develop stress-induced depression. Energy metabolic changes in the nucleus accumbens (NAc) were recently related to hierarchical status and vulnerability to develop depression-like behavior. Acetyl-L-carnitine (LAC), a mitochondria-boosting supplement, has shown promising antidepressant-like effects opening therapeutic opportunities for restoring energy balance in depressed patients. We investigated the metabolic impact in the NAc of antidepressant LAC treatment in chronically-stressed mice using H-magnetic resonance spectroscopy (H-MRS). High rank, but not low rank, mice, as assessed with the tube test, showed behavioral vulnerability to stress, supporting a higher susceptibility of high social rank mice to develop depressive-like behaviors. High rank mice also showed reduced levels of several energy-related metabolites in the NAc that were counteracted by LAC

treatment. Therefore, we reveal a metabolic signature in the NAc for antidepressant-like effects of LAC in vulnerable mice characterized by restoration of stress-induced neuroenergetics alterations and lipid function.
Elife, 2020; 9
30127751: Larrieu T, Layé S
Food for Mood: Relevance of Nutritional Omega-3 Fatty Acids for Depression and Anxiety.
The central nervous system (CNS) has the highest concentration of lipids in the organism after adipose tissue. Among these lipids, the brain is particularly enriched with polyunsaturated fatty acids (PUFAs) represented by the omega-6 (ω6) and omega-3 (ω3) series. These PUFAs include arachidonic acid (AA) and docosahexaenoic acid (DHA), respectively. PUFAs have received substantial attention as being relevant to many brain diseases, including anxiety and depression. This review addresses an important question in the area of nutritional neuroscience regarding the importance of ω3 PUFAs in the prevention and/or treatment of neuropsychiatric diseases, mainly depression and anxiety. In particular, it focuses on clinical and experimental data linking dietary intake of ω3 PUFAs and depression or anxiety. In particular, we will discuss recent experimental data highlighting how ω3 PUFAs can modulate neurobiological processes involved in the pathophysiology of anxiety and depression. Potential mechanisms involved in the neuroprotective and corrective activity of ω3 PUFAs in the brain are discussed, in particular the sensing activity of free fatty acid receptors and the activity of the PUFAs-derived endocannabinoid system and the hypothalamic-pituitary-adrenal axis.
Front Physiol, 2018; 9
29869396: Larrieu T, Sandi C
Stress-Induced Depression: Is Social Rank a Predictive Risk Factor?
An intriguing question in the field of stress is what makes an individual more likely to be susceptible or resilient to stress-induced depression. Predisposition to stress susceptibility is believed to be influenced by genetic factors and early adversity. However, beyond genetics and life experiences, recent evidence has highlighted social rank as a key determinant of susceptibility to stress, underscoring dominant individuals as the vulnerable ones. This evidence is in conflict with epidemiological, clinical, and animal work pointing at a link between social subordination and depression. Here, we review and analyze rodent protocols addressing the relevance of social rank to predict vulnerability to chronic social stress. We also discuss whether a specific social status (i.e., dominance or subordination) is the appropriate predictor of vulnerability to develop stress-induced depression or rather, the loss of social rank and resources.
Bioessays, 2018; 40
28712571: Larrieu T, Cherix A, Duque A, Rodrigues J, Lei H, Gruetter R, Sandi C
Hierarchical Status Predicts Behavioral Vulnerability and Nucleus Accumbens Metabolic Profile Following Chronic Social Defeat Stress.
Extensive data highlight the existence of major differences in individuals' susceptibility to stress [1-4]. While genetic factors [5, 6] and exposure to early life stress [7, 8] are key components for such neurobehavioral diversity, intriguing observations revealed individual differences in response to stress in inbred mice [9-12]. This raised the possibility that other factors might be critical in stress vulnerability. A key challenge in the field is to identify non-invasively risk factors for vulnerability to stress. Here, we investigated whether behavioral factors, emerging from preexisting dominance hierarchies, could predict vulnerability to chronic stress [9, 13-16]. We applied a chronic social defeat stress (CSDS) model of depression in C57BL/6J mice to investigate the predictive power of hierarchical status to pinpoint which individuals will exhibit susceptibility to CSDS. Given that the high social status of dominant mice would be the one particularly challenged by CSDS, we predicted and found that dominant individuals were the ones showing a strong susceptibility profile as indicated by strong social avoidance following CSDS, while subordinate mice were not affected. Data from H-NMR spectroscopy revealed that the metabolic profile in the nucleus accumbens (NAc) relates to social status and vulnerability to stress. Under basal conditions, subordinates show lower levels of energy-related metabolites compared to dominants. In subordinates, but not dominants, levels of these metabolites were increased after exposure to CSDS. To the best of our knowledge, this is the first study that identifies non-invasively the origin of behavioral risk factors predictive of stress-induced depression-like behaviors associated with metabolic changes.
Curr Biol, 2017; 27
28630250: Manduca A, Bara A, Larrieu T, Lassalle O, Joffre C, Layé S, Manzoni OJ
Amplification of mGlu-Endocannabinoid Signaling Rescues Behavioral and Synaptic Deficits in a Mouse Model of Adolescent and Adult Dietary Polyunsaturated Fatty Acid Imbalance.
Energy-dense, yet nutritionally poor food is a high-risk factor for mental health disorders. This is of particular concern during adolescence, a period often associated with increased consumption of low nutritional content food and higher prevalence of mental health disorders. Indeed, there is an urgent need to understand the mechanisms linking unhealthy diet and mental disorders. Deficiency in n-3 polyunsaturated fatty acids (PUFAs) is a hallmark of poor nutrition and mood disorders. Here, we developed a mouse model of n-3 PUFA deficiency lasting from adolescence into adulthood. Starting nutritional deficits in

dietary n-3 PUFAs during adolescence decreased n-3 PUFAs in both medial prefrontal cortex (mPFC) and nucleus accumbens, increased anxiety-like behavior, and decreased cognitive function in adulthood. Importantly, we discovered that endocannabinoid/mGlu-mediated LTD in the mPFC and accumbens was abolished in adult n-3-deficient mice. Additionally, mPFC NMDAR-dependent LTP was also lacking in the n-3-deficient group. Pharmacological enhancement of the mGlu/eCB signaling complex, by positive allosteric modulation of mGlu or inhibition of endocannabinoid 2-arachidonylglycerol degradation, fully restored synaptic plasticity and normalized emotional and cognitive behaviors in malnourished adult mice. Our data support a model where nutrition is a key environmental factor influencing the working synaptic range into adulthood, long after the end of the perinatal period. These findings have important implications for the identification of nutritional risk factors for disease and design of new treatments for the behavioral deficits associated with nutritional n-3 PUFA deficiency. In a mouse model mimicking n-3 PUFA dietary deficiency during adolescence and adulthood, we found strong increases in anxiety and anhedonia which lead to decreases in specific cognitive functions in adulthood. We found that endocannabinoid/mGlu-mediated LTD and NMDAR-dependent LTP were lacking in adult n-3-deficient mice. Acute positive allosteric modulation of mGlu or inhibition of endocannabinoid degradation normalized behaviors and synaptic functions in n-3 PUFA-deficient adult mice. These findings have important implications for the identification of nutritional risk for disease and the design of new treatments for the behavioral deficits associated with nutritional n-3 PUFAs' imbalance.
J Neurosci, 2017; 37

27452462: Bosch-Bouju C, Larrieu T, Linders L, Manzoni OJ, Layé S
Endocannabinoid-Mediated Plasticity in Nucleus Accumbens Controls Vulnerability to Anxiety after Social Defeat Stress.
Chronic social defeat stress (CSDS) is a clinically relevant model of mood disorders. The relationship between the CSDS model and a physiologically pertinent paradigm of synaptic plasticity is not known. Here, we found that cluster analysis of the emotional behavior states of mice exposed to CSDS allowed their segregation into anxious and non-anxious groups. Endocannabinoid-mediated spike-timing dependent plasticity (STDP) in the nucleus accumbens was attenuated in non-anxious mice and abolished in anxious mice. Anxiety-like behavior in stressed animals was specifically correlated with their ability to produce STDP. Pharmacological enhancement of 2-arachidonoyl glycerol (2-AG) signaling in the nucleus accumbens normalized the anxious phenotype and STDP in anxious mice. These data reveal that endocannabinoid modulation of synaptic efficacy in response to a naturalistic activity pattern is both a molecular correlate of behavioral adaptability and a crucial factor in the adaptive response to chronic stress.
Cell Rep, 2016; 16

27057368: Larrieu T, Hilal ML, De Smedt-Peyrusse V, Sans N, Layé S
Nutritional Omega-3 Deficiency Alters Glucocorticoid Receptor-Signaling Pathway and Neuronal Morphology in Regionally Distinct Brain Structures Associated with Emotional Deficits.
Extensive evidence suggests that long term dietary n-3 polyunsaturated fatty acids (PUFAs) deficiency results in altered emotional behaviour. We have recently demonstrated that n-3 PUFAs deficiency induces emotional alterations through abnormal corticosterone secretion which leads to altered dendritic arborisation in the prefrontal cortex (PFC). Here we show that hypothalamic-pituitary-adrenal (HPA) axis feedback inhibition was not compromised in n-3 deficient mice. Rather, glucocorticoid receptor (GR) signaling pathway was inactivated in the PFC but not in the hippocampus of n-3 deficient mice. Consequently, only dendritic arborisation in PFC was affected by dietary n-3 PUFAs deficiency. In addition, occlusion experiment with GR blockade altered GR signaling in the PFC of control mice, with no further alterations in n-3 deficient mice. In conclusion, n-3 PUFAs deficiency compromised PFC, leading to dendritic atrophy, but did not change hippocampal GR function and dendritic arborisation. We argue that this GR sensitivity contributes to n-3 PUFAs deficiency-related emotional behaviour deficits.
Neural Plast, 2016; 2016

25948102: Delpech JC, Thomazeau A, Madore C, Bosch-Bouju C, Larrieu T, Lacabanne C, Remus-Borel J, Aubert A, Joffre C, Nadjar A, Layé S
Dietary n-3 PUFAs Deficiency Increases Vulnerability to Inflammation-Induced Spatial Memory Impairment.
Dietary n-3 polyunsaturated fatty acids (PUFAs) are critical components of inflammatory response and memory impairment. However, the mechanisms underlying the sensitizing effects of low n-3 PUFAs in the brain for the development of memory impairment following inflammation are still poorly understood. In this study, we examined how a 2-month n-3 PUFAs deficiency from pre-puberty to adulthood could increase vulnerability to the effect of inflammatory event on spatial memory in mice. Mice were given diets balanced or deficient in n-3 PUFAs for a 2-month period starting at post-natal day 21, followed by a peripheral administration of lipopolysaccharide (LPS), a bacterial endotoxin, at adulthood. We first showed that spatial memory performance was altered after LPS challenge only in n-3 PUFA-deficient mice that displayed lower n-3/n-6 PUFA ratio in the hippocampus. Importantly, long-term depression (LTD), but not long-term potentiation (LTP) was impaired in the hippocampus of LPS-treated n-3 PUFA-deficient mice. Proinflammatory cytokine levels were increased in the plasma of both n-3 PUFA-deficient and n-3 PUFA-balanced mice. However, only n-3 PUFA-balanced mice showed an increase in cytokine

expression in the hippocampus in response to LPS. In addition, n-3 PUFA-deficient mice displayed higher glucocorticoid levels in response to LPS as compared with n-3 PUFA-balanced mice. These results indicate a role for n-3 PUFA imbalance in the sensitization of the hippocampal synaptic plasticity to inflammatory stimuli, which is likely to contribute to spatial memory impairment.
Neuropsychopharmacology, 2015; 40

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BOARD NUMBER: S07-702

EFFECT OF A PEPTIDE SECRETED BY ASTROCYTES ON HIPPOCAMPAL ADULT NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Adult hippocampal neurogenesis is regulated by the neurogenic niche, which provides a structural and molecular environment enabling adult neurogenesis. In the niche, astrocytes play a predominant role, by participating to the regulation of multiple steps of adult neurogenesis, from adult neural stem cell (aNSCs) proliferation and differentiation to the functional integration of new neurons. In this study, we used a combination of *in vitro* approaches, biochemistry and mass spectrometry to identify a peptide released by astrocytes that regulate adult neurogenesis. The secreted peptide, named peptide P9, is derived from the phosphoprotein enriched in astrocyte protein (PEA15) which is a cytoplasmic protein involved in the regulation of proliferation and apoptosis. Using live-cell imaging, we found that P9 increased the proliferation and reduced cell death of aNSCs. *In vivo*, P9 increased cell proliferation in the dentate gyrus, resulting in increased net hippocampal neurogenesis. We next used immunoprecipitation and transcriptomics to define the molecular pathways involved in the effects of P9 on cell proliferation. These results indicate that astrocytes release peptides that regulate hippocampal neurogenesis in the adult brain. Since astrocytes are regulated by neuronal activity, this mechanism may contribute to an on-demand addition of new neurons in the hippocampus. Furthermore, understanding the mechanism of action of the astrocyte-secreted peptide P9 on adult neurogenesis in the hippocampus could be an interesting therapeutic strategy to stimulate adult neurogenesis in pathological conditions.

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BOARD NUMBER: S07-703

OUTCOMES OF BETA-AMYLOID 1-42 EXPOSURE ON THE NEUROGENIC SUBVENTRICULAR ZONE OF THE ADULT BRAIN AT ALZHEIMER'S DIESES MODEL

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Konstantin Yenkoyan, Senik Matinyan, Katarine Fereshetyan, Margarita Mirumyan, Hayk Harutyunyan, Tigran Margaryan, Michail Aghajanov
Yerevan State Medical University named after Mkhitar Heratsi, Neuroscience Laboratory, Cobrain Center, Yerevan, Armenia

Introduction. Alzheimer's disease (AD) remains the leading neurodegenerative disorder needed to study and intervene timely. The brain intrinsic defense mechanisms towards chronic injury are not yet fully understood. Here we revealed the response of the main neurogenic niche - ubventricular zone (SVZ) - in case of amyloid exposure, associated structural and synaptic activity changes. *Methods.* Flow cytometry of Nestin, Vimentin, Nestin/Vimentin, NeuN, GFAP, NeuN/GFAP, NSE, BrdU, Wnt, BrdU/Wnt, VEGF positive cells was performed in the subventricular zone (SVZ) of rats' brain on the 90th day from intracerebroventricular (i.c.v.) injection of Aβ (1-42) fraction. The relative structural changes in the same areas and synaptic activity disruption in the entorhinal cortex-hippocampus circuit were provided as well. *Results.* Flow analyses revealed a reduction of Nestin, Vimentin, and Nestin/Vimentin positive cells in neurogenic niche. These changes were accompanied by an increased number of BrdU positive cells in the subventricular zone. The latest changes were strongly correlated with the fluctuations of VEGF positive cells, which pinpoint the fact that neurogenesis often appear parallel to changes in angiogenesis. The morphological changes were characterized by significant neural loss alongside with characteristic shift of entorhinal cortex-hippocampus circuit activity. *Conclusion.* We conclude, that although beta-amyloid 1-42 induces stem cell proliferation and triggers neurogenesis in SVZ, the process is incomplete and leads to "neuronal stem cell immaturity" phenomenon.

Pubmed:

34209299: Aghajanov M, Matinyan S, Chavushyan V, Danielyan M, Karapetyan G, Mirumyan M, Fereshetyan K, Harutyunyan H, Yenkoyan K
The Involvement of Insulin-Like Growth Factor 1 and Nerve Growth Factor in Alzheimer's Disease-Like Pathology and Survival Role of the Mix of Embryonic Proteoglycans: Electrophysiological Fingerprint, Structural Changes and Regulatory Effects on Neurotrophins.
Alzheimer's disease (AD)-associated neurodegeneration is triggered by different fragments of amyloid beta (Aβ). Among them, Aβ (25-35) fragment plays a critical role in the development of neurodegeneration-it reduces synaptic integrity by disruption of excitatory/inhibitory ratio across networks and alters the growth factors synthesis. Thus, in this study, we aimed to identify the involvement of neurotrophic factors-the insulin-like growth factor 1 (IGF-1) and nerve growth factor (NGF)-of AD-like neurodegeneration induced by Aβ (25-35). Taking into account our previous findings on the neuroprotective effects of the mix of proteoglycans of embryonic genesis (PEG), it was suggested to test its regulatory effect on IGF-1 and NGF levels. To evaluate the progress of neurodegeneration, in vivo electrophysiological investigation of synaptic activity disruption of the entorhinal cortexhippocampus circuit at AD was performed and the potential recovery effects of PEG with relative structural changes were provided. To reveal the direct effects of PEG on brain functional activity, the electrophysiological pattern of the single cells from nucleus supraopticus, sensomotor cortex and hippocampus after acute injection of PEG was examined. Our results demonstrated that after injection of Aβ (25-35), the level of NGF decreased in cerebral cortex and hypothalamus, and, in contrast, increased in hippocampus, prompting its multidirectional role in case of brain damage. The concentration of IGF-1 significantly increased in all investigated brain structures. The administration of PEG balanced the growth factor levels accompanied by substantial restoration of neural tissue architecture and synaptic activity. Acute injection of PEG activated the hypothalamic nucleus supraopticus and hippocampal neurons. IGF-1 and NGF levels were found to be elevated in animals receiving PEG in an absence of amyloid exposure. We suggest that IGF-1 and NGF play a critical role in the development of AD. At the same time, it becomes clear that the neuroprotective effects of PEG are likely mediated via the regulation of neurotrophins.
Int J Mol Sci, 2021; 22


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NEUROGENESIS IN RESPONSE TO CORTICAL BRAIN INJURIES: ROLE OF TRANSFORMING GROWTH FACTOR ALPHA, NEUREGULIN 1, AND PROTEIN KINASE C

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Noelia Geribaldi[1,2], Ricardo Gómez-Oliva[2,3], Samuel Domínguez-García[2,3], Ricardo Pardillo-Diaz[2], Sergio Martínez-Ortega[2,3], José María Oliva-Montero[2], Patricia Pérez-García[2,3], Livia Carrascal[2,4], Pedro Nunez-Abades[4,5], Carmen Castro Gonzalez[2,3]
[1]University of Cadiz, Human Anatomy And Embriology, Cadiz, Spain, [2]Instituto de Investigacion e Innovacion en Ciencias Biomedicas de la Provincia de Cadiz, Inibica, Cádiz, Spain, [3]University of Cadiz, Physiology, Cádiz, Spain, [4]University of Seville, Department Of Physiology, Seville, Spain, [5]Biomedical Research and Innovation Institute of Cádiz (INiBICA), Inibica, Cádiz, Spain

Background: In response to a cortical injury, neural stem cells of the subventricular zone (SVZ) in the mouse adult brain, are activated producing neuroblasts that alter their migration pattern attempting to migrate toward the injury. However, neuronal replacement post-injury is limited. **Aims:** To understand the signaling events that are involve in the proliferation, differentiation and migration of neuroblasts from the SVZ in response to a cortical injury. **Methods:** We have performed mechanical cortical injuries in adult mice and have studied the expression and concentration of neuregulin and TGFa as well as their receptors. We have also studied neurogenesis mediated by compounds that stimulate TGFa and neuregulin1 release. **Results:** In response to the injury, the concentration of TGFa increases in the cerebrospinal fluid (CSF). The expression of TGFa, neuregulin 1 and their receptors is altered in the SVZ and in the perilesional area. Treatment of injured mice with a compound that stimulates TGFa release (ER272), increased level of TGFa in the CSF, and the number of neuroblasts within the SVZ but these cells did not migrate toward the injury. The administration of a compound that stimulated neuregulin 1 release (EOF2) increased the concentration of neuregulin in the CSF and facilitated neuroblast migration toward the injury resulting in neuroblast enrichment of the perilesional area. The pro-migratory effect of EOF2 was potentiated when mice were treated with ER272 before performing the injury. **Conclusions:** These results indicate that modulating TGFa and neuregulin1 release may promote neuronal replacement in brain injuries.

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BOARD NUMBER: S07-705

EFFECT OF METHANOL FIXATION ON SINGLE CELL RNA SEQUENCING OF THE MURINE DENTATE GYRUS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

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Droplet-based single-cell RNA sequencing provides a powerful tool to evaluate the transcriptomic landscape and heterogeneity of thousands of cells in parallel. However, complex study designs or the unavailability of in-house instruments require the temporal disconnection between sample preparation and library construction, raising the need for efficient sample preservation methods which are compatible with downstream applications. Here, we evaluated the suitability of cryopreservation and methanol fixation for preserving single cell suspensions of the murine dentate gyrus. To evaluate the performance of both methods, we first evaluated the recovery after thawing and rehydration of the papain-dissociated cell suspensions. Due to the low recovery rates after cryopreservation, further evaluation steps were focused on methanol fixation. Its efficacy was determined via flow cytometry of Sytox-stained samples. RNA quality was assessed on an Agilent bioanalyzer. To test whether and how methanol fixation affects transcriptome profiling, we performed a SORTseq experiment on fresh and methanol-fixed cell suspensions. Methanol fixation resulted in higher recovery rates than cryopreservation. It had an efficacy of 100% and no effects on RNA integrity. Transcriptome analysis revealed that methanol fixation results in a slight drop in read and gene counts, suggesting RNA leakage. However, it did not interfere with clustering and cell-type composition. Moreover, it resulted in more high-quality cells and reduced signs of mitochondrial stress compared to fresh samples. Together, these data suggest that methanol fixation is suitable for storing neural cells for subsequent single-cell RNA profiling, helping to overcome challenges arising with complex workflows and to improve the experimental flexibility.

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BOARD NUMBER: S07-706

ROLE OF MENINGEAL MACROPHAGES IN NEUROGENESIS

POSTER SESSION 07 - SECTION: ADULT NEUROGENESIS

Laurie Arnaud[1], Sarah Kharbouche[1], Elisa Eme-Scolan[1], Julie Rebejac[1], Matei Teleman[1], Lionel Spinelli[1], Annie Roussel-Queval[1], José Boucraut[2], Rejane Rua[1]
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Aims
The meninges represent a protective tissue of the central nervous system providing a nutritive and supportive support to the brain. Recent data from our lab and others revealed that in this compartment, a large panel of resident immune cells are found, the most abundant being macrophages. Yet, whether meningeal macrophages (MM) could act in neurogenesis processes remains poorly understood. Meninges deeply penetrate the parenchyma between the major brain substructures, including hemispheres and the hippocampus. In this line, we investigated whether MM could influence developmental neurogenesis and/or adult neurogenesis. Methods We used multiple chemical and genetic innovative models of meningeal macrophage depletion in i) neonatal mice and ii) adult mice upon voluntary exercise to evaluate by immunohistochemistry the impact of macrophage depletion on developmental and activity-induced adult neurogenesis. Results
Preliminary results showed that MM depletion at early time point decreased neurogenesis in the hippocampus of neonatal mice. Concerning adult neurogenesis, we confirmed the positive effect of exercise on neurogenesis, which was abolished by the depletion of MM. To better understand the underlying mechanisms, we achieved single-cell RNA sequencing of MM and are now exploring the impact of specific meningeal macrophage deletion of neurogenic factors. Conclusions
Ultimately, this work owes the potential to shed new light on the role of immune cells in neurogenesis and open up new perspectives to modulate/target neurogenesis.

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DYNAMIC INTERPLAY BETWEEN CAV2 CHANNELS AND THE PRESYNAPTIC CYTOMATRIX IN MECHANISMS OF NEUROTRANSMISSION

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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The structural organization of the presynaptic terminal is of utmost importance for the release of neurotransmitter-containing synaptic vesicles (SV). This process is essential for neuronal communication, that represents the major determinant of high cognitive functions such as memory and behavior. In the presynaptic active zone (AZ), voltage-gated calcium channels' (VGCC) proximity to docked SV is fundamental for fast neurotransmitter release. Scaffold proteins RIM, RBP2 and Bassoon were shown to mediate tethering of Cav2.1 and Cav2.2 channels close to synaptic vesicles (~20 nm). Indeed, scaffolding proteins present peculiar binding domains that can be specific for individual channel subtypes. Interestingly, deletion of these binding domains results in decreased channel abundance and impaired vesicle release. However, the mechanisms by which individual VGCC subtypes enter and/or escape AZs are still elusive. Particularly, VGCC synaptic localization could be mediated by direct interaction with scaffolding proteins or through a general trapping in a phase-separated compartment. Here, we deliver cell-penetrating peptides that mimic known binding sites between VGCC and scaffold proteins to cultured hippocampal neurons, acutely disturbing the affinity landscape of VGCC/scaffold interactions. Our functional glutamate imaging results indicated time-dependent alterations in synaptic transmission following incubation with interfering peptides, that translated in local rearrangement of VGCC observed through single-particle tracking. Additionally, confocal imaging confirmed VGCC's differential distribution in AZs following acute interference with scaffold interactions. Thus, the ability of the presynapse to quickly rearrange its channel composition lays the foundation for further investigations on how this property is exploited in terms of synaptic remodeling following plasticity events.

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ILL-PRIMED VESICLES CAUSE LOW RELEASE PROBABILITY AT CA1 HIPPOCAMPAL EXCITATORY SYNAPSES

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Setting the synaptic efficacy and short-term plasticity based on the postsynaptic target cell type is one of the most intriguing synaptic mechanisms that enhance the computational power of the cortical network. Here, using whole-cell patch-clamp paired recordings, we show that adult mouse hippocampal CA1 pyramidal cell (PC) connections on fast spiking interneurons (FSIN) have 15-fold larger unitary EPSC amplitudes than those made by PCs on oriens lacunosum-moleculare (O-LM) interneurons. Freeze-fracture replica immunolabeling showed similar nano-topologies and coupling distances between Ca^{2+} channels (Cav2.1) and synaptic vesicle release sites (Munc13-1 clusters) in both synapses, and showed only 20% higher density of Cav2.1-type Ca^{2+} channels in the active zones targeting FSINs. Consistent with this, 2-photon [Ca^{2+}] imaging showed 40% larger AP-evoked [Ca^{2+}] transient peak amplitudes in FSIN-targeting boutons. Increasing the Ca^{2+} influx at PC – O-LM synapses by 40% with the K^+ channel blocker 4-AP caused only 2.6-fold increase in uEPSC amplitudes. However, application of a phorbol ester analog (PDBU) resulted in a larger augmentation of uEPSC amplitudes at PC – O-LM synapses (5-fold) than at PC – FSIN synapses (1.8-fold), suggesting incomplete docking or priming of vesicles at PC – O-LM synapses. Serial section electron microscopy (EM) and EM tomography ruled out low release site occupancy, as there was no difference in the density of docked vesicles between the two types of synapses. Our results demonstrate that ill-primed docked vesicles limit the output of PC – O-LM synapses.

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THE SMALL GTPASE RAB2 REGULATES PRESYNAPTIC PRECURSOR VESICLE BIOGENESIS.

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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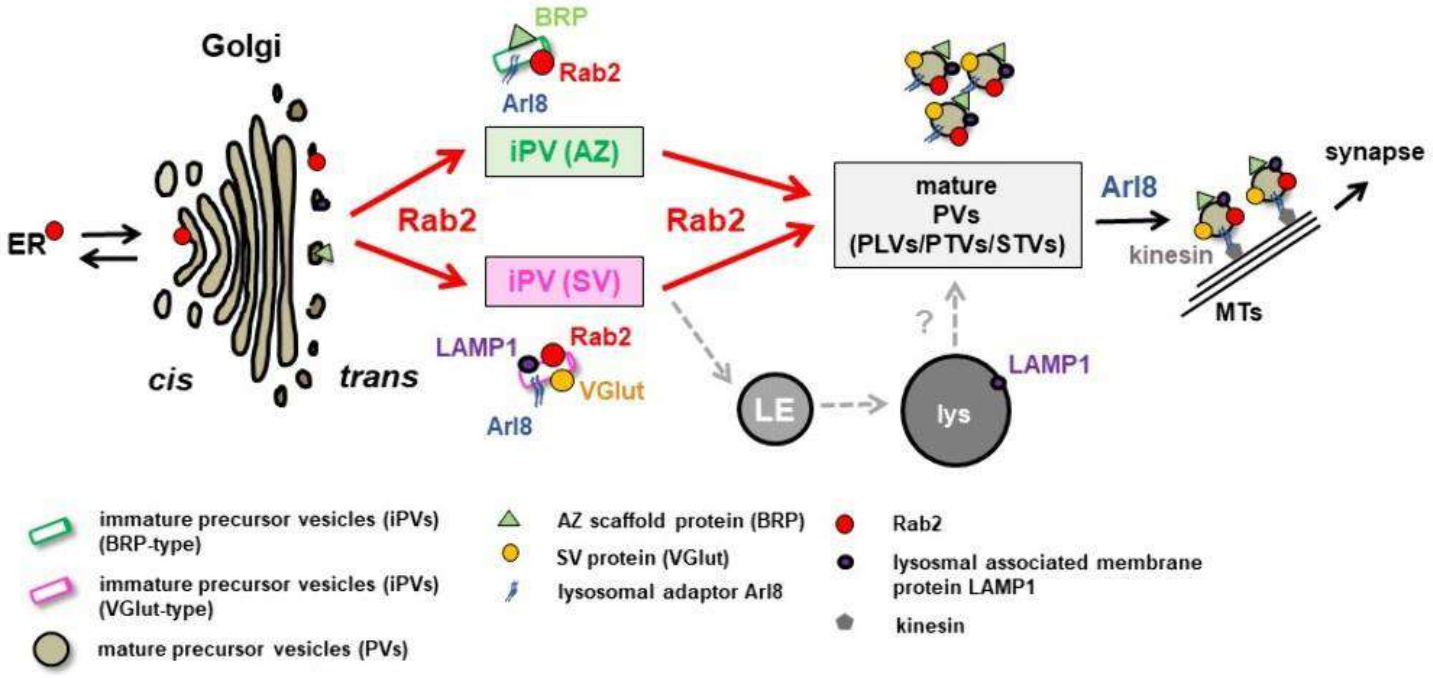
Reliable delivery of presynaptic material, including active zone and synaptic vesicle proteins from neuronal somata to synaptic terminals, is prerequisite for successful synaptogenesis and neurotransmission. However, molecular mechanisms controlling the somatic assembly of presynaptic precursors remain insufficiently understood. We show here that in mutants of the small GTPase Rab2 both active zone and synaptic vesicle proteins accumulated in the neuronal cell body at the *trans*-Golgi and were, consequently, depleted at synaptic terminals, provoking neurotransmission deficits. Ectopic presynaptic material accumulations consisted of heterogeneous vesicles and short tubules of 40x60 nm and segregated in subfractions either positive for active zone proteins or synaptic vesicle proteins and LAMP1, a lysosomal membrane protein. Genetically, Rab2 behaved epistatically over Arl8, a lysosomal adaptor controlling the axonal export of precursors. Collectively, we here identified a Golgi-associated assembly sequence for presynaptic precursor vesicle biogenesis controlled by Rab2-dependent protein export and sorting at the *trans*-

Golgi.


Golgi
ER
cis
trans
BRP
Rab2
Arl8
iPV (AZ)
Rab2
iPV (SV)
LAMP1
Rab2
VGlut
Arl8
Rab2
mature
PVs
(PLVs/PTVs/STVs)
Arl8
kinesin
MTs
synapse
?
LE
lys
LAMP1
immature precursor vesicles (iPVs) (BRP-type)
immature precursor vesicles (iPVs) (VGlut-type)
mature precursor vesicles (PVs)
AZ scaffold protein (BRP)
SV protein (VGlut)
lysosomal adaptor Arl8
Rab2
lysosmal associated membrane protein LAMP1
kinesin

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BOARD NUMBER: S07-710

A KV3.3 VOLTAGE-GATED POTASSIUM CHANNEL MUTATION INDUCES PRESYNAPTIC HYPEREXCITABILITY AND RAISES THE NUMBER OF DOCKED VESICLES AT HIPPOCAMPAL EXCITATORY SYNAPSES

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Postsynaptic hyperexcitability is well characterised, but increased action potential firing will also influence presynaptic function and neurotransmitter release. Synaptic vesicles play a role in the rate of neurotransmitter release and are known to be modulated by voltage gated potassium channels. Spinocerebellar ataxia type 13 (SCA13) is a mutation of the Kv3.3 voltage-gated potassium channel subunit which has a role in presynaptic AP repolarization and causes epilepsy in a CRISPR/CAS9 gene edited mouse model of this human disease. The mechanism could be interruption of ion conduction but direct influence on transmitter release has been postulated. In this investigation, ultrastructure changes in presynaptic terminals of hippocampal neurons from the SCA13 mouse were imaged using electron microscope imaging of osmium-thiocarbohydrazide stained tissue. The number of vesicles bound to the active zone of excitatory synaptic terminals in the CA1 was increased relative to wildtype CBA mice, whilst in inhibitory synaptic terminals the active zone size increases. Similar results were observed in tissue from a Kv3.3 knockout mouse. We conclude that changes in presynaptic Kv3.3 channels can influence the ultrastructure of the hippocampal synapses. This remains to be shown whether it is a direct effect of the impaired ion channel on excitability, a secondary influence on excitability, or whether the Kv3.3 subunit directly changes transmitter release from the terminal.

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BOARD NUMBER: S07-711

LOSS OF AUTISM-ASSOCIATED α2δ-3 INDUCES ALTERED SYNAPTIC PROTEIN EXPRESSION, PRESYNAPTIC FUNCTION, NEURONAL EXCITABILITY, AND MOUSE BEHAVIOR

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

Cornelia Ablinger[1], Anupam Sah[2], Nadine Ortner[2], Hartwig Seitter[2], Stefanie Geisler[2], Marta Campiglio[1], Nicolas Singewald[2], Markus Missler[3], Gerald Obermair[4]
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In neurons, alpha2delta subunits of voltage-gated calcium channels (VGCC) regulate synaptic functions independently from their channel specific role. The human genes encoding for alpha2delta isoforms have been linked to a variety of neurological disorders. Particularly alpha2dela-3 (CACNA2D3) has been strongly associated with autism spectrum disorders. Thus, we hypothesize that alpha2delta-3 knockout mice may serve as a potential model for studying neurodevelopmental disorders. We further suggest that defects in specific synapses or synaptic connections might explain an autism-like phenotype. To test this, we performed initial behavioural tests, which illustrated a mild anxiogenic phenotype together with increased active coping in alpha2delta-3 knockout mice compared to littermate controls. Structural brain analysis in serial Nissl-stained sections revealed no gross morphological changes of knockout brains but showed a slightly reduced brain volume. The suggested synaptic importance of alpha2delta-3 was underpinned by analysing presynaptic calcium signals, which were reduced in knock out synapses after stimulation with 1 action potential (AP), 3 APs, and 10 APs. Additional biochemical analysis of whole brain and synaptosomal lysates demonstrated a significant reduction of striatal NMDAR2B expression in alpha2delta-3 knockout brains. Therefore, we next analysed the intrinsic excitability of striatal neurons in slice electrophysiology, revealing a hypo-excitability of medium spiny neurons. Taking together, alpha2delta-3 deficiency results in a mild reduction of brain volume, causes changes of synaptic protein expression, and interferes with proper presynaptic function. Consistent with previous observations in neurodevelopmental disorders and putative autism mouse models, alpha2delta-3 knockout mice display striatal hypo-excitability, a mild anxiogenic phenotype, and increased active coping.

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BOARD NUMBER: S07-712

OPTIMISATION OF PROTOCOLS TO STUDY SYNAPTIC INTEGRITY DURING DROSOPHILA AGEING

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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King's College London, Department Of Basic And Clinical Neuroscience, Institute Of Psychiatry, Psychology And Neuroscience, London, United Kingdom

Background: Cell adhesion molecules neurexin and neuroligin are critical for synapse formation and maintenance. The intracellular trafficking of neurexin and neuroligin towards the synapse is important for synaptic integrity, and decreased trafficking of these proteins may cause synaptic dysfunction. Decreased intracellular cargo transport is a feature of ageing, and so the synaptic dysfunction observed in ageing and Alzheimer's disease may be caused by age-related changes in the trafficking and localisation of synaptic proteins, however no study has confirmed this link. Aims: We aim to assess whether there are changes in the intracellular trafficking of synaptic proteins, which could contribute to synaptic dysfunction during ageing. This poster outlines the first steps towards optimisation of two measures of synaptic integrity in the adult fly. Methods: We test the optical clearing protocol published by Pende et al. (2018, PMID: 30413688) using *Drosophila* expressing CD8::GFP pan-neuronally. Secondly, we optimise live-imaging protocols to capture the transport of fluorescently-tagged synaptic proteins (neurexin, neuroligin and amyloid precursor protein (APP)) along the fly wing's sensory neuron axons *in situ*. Results: Neuronal structures are visible following tissue clearing of adult flies. APP trafficking is prevalent under two pan-neuronal drivers, nsyb-Gal4 and APPL-Gal4. Tracking of neurexin and neuroligin trafficking requires the generation of genetic double insertions to increase the abundance of these fluorescently-tagged proteins. Conclusions: Combining tissue clearing and live imaging protocols to study the transport of synaptic proteins in adult *Drosophila* will enable us to better characterise the mechanisms of synaptic maintenance during ageing.

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MOVER/TPRG1L : A NOVEL PRESYNAPTIC PROTEIN WHICH INTERACTS WITH THE ACTIVE ZONE SCAFFOLDING PROTEIN BASSOON.

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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[1]UMG Göttingen, Zentrum Anatomie, Göttingen, Germany, [2]Abberior Instruments, Abberior Instruments Gmbh, Göttingen, Germany

The release of neurotransmitters relies on the fusion of synaptic vesicles with the presynaptic membrane. This fusion only occurs at a restricted area of the presynaptic membrane called active zone. Our group identified a partner of the active zone scaffolding protein Bassoon called Mover. Mover KO mice display an increase in facilitation in hippocampal mossy fiber terminals, but normal facilitation at Schaffer collateral synapses, indicating that Mover has synapse-specific roles. At the calyx of Held lack of Mover reduces the release probability of a subset of primed synaptic vesicles, further highlighting how specific the role of Mover can be. We found that Mover is primarily associated with excitatory synapses in immature neuronal cultures but shifts to being predominantly found at inhibitory synapses in advanced cultures stages. These results support the view that Mover has a specific role depending on the type of synapse and on the type of docked synaptic vesicle at an individual release site. To determine how Mover is arranged within the presynaptic architecture we used super-resolution microscopy of presynaptic terminals in cultured neurons. We found that unlike the synaptic vesicle protein synaptophysin, Mover STED signals appear as discrete puncta. Using dual-color Minflux microscopy <10 nm resolution we found that Mover is less widely distributed at active zones than synaptophysin. Together, these data suggest that Mover is a presynaptic protein heterogeneously expressed among synapses of the brain and in cultured neurons and may act to dampen transmitter release during repeated activity to prevent runaway excitation.

Pubmed:

28323401: Perrin L, Roudeau S, Carmona A, Domart F, Petersen JD, Bohic S, Yang Y, Cloetens P, Ortega R
Zinc and Copper Effects on Stability of Tubulin and Actin Networks in Dendrites and Spines of Hippocampal Neurons.
Zinc and copper ions can modulate the activity of glutamate receptors. However, labile zinc and copper ions likely represent only the tip of the iceberg and other neuronal functions are suspected for these metals in their bound state. We performed synchrotron X-ray fluorescence imaging with 30 nm resolution to image total biometals in dendrites and spines from hippocampal neurons. We found that zinc is distributed all along the dendrites while copper is mainly pinpointed within the spines. In spines, zinc content is higher within the spine head while copper is higher within the spine neck. Such specific distributions suggested metal interactions with cytoskeleton proteins. Zinc supplementation induced the increase of β-tubulin content in dendrites. Copper supplementation impaired the β-tubulin and F-actin networks. Copper chelation resulted in the decrease of F-actin content in dendrites, drastically reducing the number of F-actin protrusions. These results indicate that zinc is involved in microtubule stability whereas copper is essential for actin-dependent stability of dendritic spines, although copper excess can impair the dendritic cytoskeleton.
ACS Chem Neurosci, 2017; 8
31689314: Frisbie SH, Mitchell EJ, Roudeau S, Domart F, Carmona A, Ortega R
Manganese levels in infant formula and young child nutritional beverages in the United States and France: Comparison to breast milk and regulations.
Exposure to high levels of manganese (Mn) in children has recently been associated with adverse neurodevelopmental effects. Current infant formula regulations for Mn content were set between 1981 (United States), 2006 (European Union, France), and 2007 (Codex Alimentarius) prior to the publication of much of the growing body of research on the developmental neurotoxicity of Mn. In this study, we sought to measure the concentrations of Mn in some infant formulas and young child nutritional beverages available on the United States (US) and French markets using ion beam analysis by particle induced X-ray emission (PIXE) spectrometry and then compare the analytical results to concentrations reported in the literature for breast milk and applicable infant formula regulations and guidelines. We were particularly interested in measuring Mn concentrations in product types for which there is very little data from previous surveys, especially soy-based, rice-based, goat-milk based, chocolate-flavored, and nutritional beverages for young children that are not regulated as infant

or follow-on formulas (e.g. "toddler formulas" and "toddler powders"). We purchased 44 infant formulas and young child nutritional beverage products in the US and France with varying protein sources (cow-milk, goat-milk, soy, rice) labelled for birth to 3 years. We selected these samples using maximum variation sampling to explore market extremes to facilitate comparisons to regulatory limits. Since this sampling method is non-probabilistic, other inferences cannot be made beyond this set of samples to the overall markets. We used ion beam analysis to measure the concentrations of Mn in each product. The range of measured Mn concentrations in the products is 160-2,800 µg/L, substantially higher than the 3-6 µg/L mean Mn concentration reported in human breast milk. All products satisfied national and Codex Alimentarius Commission (CAC) international standards for minimum Mn content in infant formulas; however, 7/25 of the products purchased in the US exceeded the CAC Guidance Upper Level of 100 µg Mn/kcal for infant formula.
PLoS One, 2019; 14
33289481: Domart F, Cloetens P, Roudeau S, Carmona A, Verdier E, Choquet D, Ortega R
Correlating STED and synchrotron XRF nano-imaging unveils cosegregation of metals and cytoskeleton proteins in dendrites.
Zinc and copper are involved in neuronal differentiation and synaptic plasticity but the molecular mechanisms behind these processes are still elusive due in part to the difficulty of imaging trace metals together with proteins at the synaptic level. We correlate stimulated-emission-depletion microscopy of proteins and synchrotron X-ray fluorescence imaging of trace metals, both performed with 40 nm spatial resolution, on primary rat hippocampal neurons. We reveal the co-localization at the nanoscale of zinc and tubulin in dendrites with a molecular ratio of about one zinc atom per tubulin-αβ dimer. We observe the co-segregation of copper and F-actin within the nano-architecture of dendritic protrusions. In addition, zinc chelation causes a decrease in the expression of cytoskeleton proteins in dendrites and spines. Overall, these results indicate new functions for zinc and copper in the modulation of the cytoskeleton morphology in dendrites, a mechanism associated to neuronal plasticity and memory formation.
Elife, 2020; 9
33166614: Carmona A, Porcaro F, Somogyi A, Roudeau S, Domart F, Medjoubi K, Aubert M, Isnard H, Nonell A, Rincel A, Paredes E, Vidaud C, Malard V, Bresson C, Ortega R
Cytoplasmic aggregation of uranium in human dopaminergic cells after continuous exposure to soluble uranyl at non-cytotoxic concentrations.
Uranium exposure can lead to neurobehavioral alterations in particular of the monoaminergic system, even at non-cytotoxic concentrations. However, the mechanisms of uranium neurotoxicity after non-cytotoxic exposure are still poorly understood. In particular, imaging uranium in neurons at low intracellular concentration is still very challenging. We investigated uranium intracellular localization by means of synchrotron X-ray fluorescence imaging with high spatial resolution (< 300 nm) and high analytical sensitivity (< 1 µg.g per 300 nm pixel). Neuron-like SH-SY5Y human cells differentiated into a dopaminergic phenotype were continuously exposed, for seven days, to a non-cytotoxic concentration (10 µM) of soluble natural uranyl. Cytoplasmic submicron uranium aggregates were observed accounting on average for 62 % of the intracellular uranium content. In some aggregates, uranium and iron were co-localized suggesting common metabolic pathways between uranium and iron storage. Uranium aggregates contained no calcium or phosphorous indicating that detoxification mechanisms in neuron-like cells are different from those described in bone or kidney cells. Uranium intracellular distribution was compared to fluorescently labeled organelles (lysosomes, early and late endosomes) and to fetuin-A, a high affinity uranium-binding protein. A strict correlation could not be evidenced between uranium and the labeled organelles, or with vesicles containing fetuin-A. Our results indicate a new mechanism of uranium cytoplasmic aggregation after non-cytotoxic uranyl exposure that could be involved in neuronal defense through uranium sequestration into less reactive species. The remaining soluble fraction of uranium would be responsible for protein binding and for the resulting neurotoxic effects.
Neurotoxicology, 2021; 82
34910190: Carmona A, Chen S, Domart F, Choquet D, Ortega R
Imaging the structural organization of chemical elements in growth cones of developing hippocampal neurons.
During neurodevelopment, neurons form growth cones, F-actin rich extensions located at the distal end of the neurites. Growth cones allow dendrites and axons to build synaptic connections through a process of neurite guidance whose mechanisms have not been fully elucidated. Calcium is an important element in this process by inducing F-actin reorganization. We hypothesized that other biologically active elements might be involved in the growth cone-mediated neurite guidance mechanisms. We performed super resolution and confocal microscopy of F-actin, followed by synchrotron X-ray fluorescence microscopy of phosphorous, sulfur, chlorine, potassium, calcium, iron and zinc on growth cones from primary rat hippocampal neurons. We identified two main patterns of element organization. First, active growth cones presenting an asymmetric distribution of Ca co-localized with the cytoskeleton protein F-actin. In active growth cones, we found that the distributions of P, S, Cl, K, and Zn are correlated with Ca. This correlation is lost in the second pattern, quiescent growth cones, exhibiting a spread elemental distribution. These results suggest that Ca is not the only element required in the F-actin rich active regions of growth cones. In addition, highly concentrated Fe spots of submicrometer size

were observed in calcium-rich areas of active growth cones. These results reveal the need for biological active elements in growth cones during neural development and may help explain why early life deficiencies of elements, such as Fe or Zn, induce learning and memory deficits in children.
Metallomics, 2022; 14

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BOARD NUMBER: S07-714

CONSEQUENCES OF α2δ SUBUNIT MUTATIONS LINKED TO BRAIN DISORDERS ON NEURONAL CALCIUM CHANNEL TRAFFICKING AND SYNAPSE COMPOSITION

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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The roles of auxiliary $\alpha_2\delta$ subunits of voltage-gated calcium channels in modulating membrane expression and calcium current properties are widely recognized. In addition, recent literature suggests an important role of $\alpha_2\delta$ proteins in synapse formation and differentiation. Therefore, it is not surprising that $\alpha_2\delta$ genes have been linked to neurological and neuropsychiatric disorders. Here we aimed to investigate human mutations in $\alpha_2\delta$ proteins by addressing their synaptic functions, besides their role as channel subunit, to shed light on the underlying pathophysiological mechanisms. We characterized two mutations, the autism-associated mutation p.Arg351Thr in $\alpha_2\delta$-1 (CACNA2D1) and the epilepsy-related mutation p.Arg596Pro in $\alpha_2\delta$-2 (CACNA2D2) , cloned into mouse cDNA, by employing primary hippocampal neurons as homologous expression system. To this end we quantified plasma membrane trafficking and analyzed potential consequences on synaptic composition. To determine potential effects on the biophysical channel properties, we performed electrophysiological recordings after heterologous expression of either Cav2.1 or Cav1.3 together with the mutated $\alpha_2\delta$ and auxiliary β subunits. Live-cell labelling of cultured hippocampal neurons transfected with 2HA-tagged $\alpha_2\delta$ subunits revealed a strong reduction in membrane and synaptic targeting of both mutants. However, only neurons transfected with $\alpha_2\delta$-2_p.Arg596Pro showed a significantly reduced mismatched localization of postsynaptic $GABA_AR$s opposite glutamatergic nerve terminals, a previously identified synaptogenic function of $\alpha_2\delta$-1 and $\alpha_2\delta$-2 splices lacking exon 23. Similarly, preliminary electrophysiological analysis indicated that current density of Cav2.1 was not compromised by $\alpha_2\delta$-1_p.Arg351Thr, contrary to co-expression $\alpha_2\delta$-2_p.Arg596Pro , which resulted in a strongly reduced current density.
Funding: Austrian Science Fund (FWF) DOC 30-B30

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-715

TO DIE OR NOT TO DIE – DOES BASSOON PLAY A ROLE IN CONE PHOTORECEPTOR SURVIVAL?

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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The presynaptic protein Bassoon (BSN) is an important component of the active zone of chemical synapses, where it contributes to synapse assembly and function. Recent studies show an additional function of BSN as an inhibitor of presynaptic autophagy and proteasomal degradation in brain neurons. In the retina of two BSN-deficient mouse lines, *Bsn*$^{\Delta Ex4/5}$ and *Bsn*^{gt}, we found degeneration of cone photoreceptors and neurite outgrowth. This raises the question, whether BSN is important for the survival of these highly active sensory neurons by controlling homeostasis pathways. However, a third BSN-deficient mouse line (*Bsn*^{ko}) did not show a retinal phenotype. Since proteasomal degradation was impaired in the retinae of both *Bsn*^{gt} and *Bsn*^{ko} mice, disruption of protein degradation alone is not sufficient for cone photoreceptor degeneration. We hypothesize that an additional trigger is required. The trigger for cone photoreceptor degeneration could be the presence of a residual BSN fragment as it is found in the *Bsn*$^{\Delta Ex4/5}$ mouse line. This hypothesis is supported by the finding that crossbreeding of *Bsn*^{ko} mice with *Bsn*^{gt} mice induced a retinal phenotype in *Bsn*^{ko} mice. In our study we want to find out whether *Bsn*^{gt} mice express a residual BSN fragment, and whether its presence disrupts homeostasis processes and leads to cone photoreceptor cell death. So far, our data suggest that in addition to its synaptic function, BSN plays a role in processes of cellular homeostasis and survival of cone photoreceptors in the retina.

Pubmed:
33811381: Ryl M, Urbasik A, Gierke K, Babai N, Joachimsthaler A, Feigenspan A, Frischknecht R, Stallwitz N, Fejtová A, Kremers J, von Wittgenstein J, Brandstätter JH
Genetic disruption of bassoon in two mutant mouse lines causes divergent retinal phenotypes.
Bassoon (BSN) is a presynaptic cytomatrix protein ubiquitously present at chemical synapses of the central nervous system, where it regulates synaptic vesicle replenishment and organizes voltage-gated Ca channels. In sensory photoreceptor synapses, BSN additionally plays a decisive role in anchoring the synaptic ribbon, a presynaptic organelle and functional extension of the active zone, to the presynaptic membrane. In this study, we functionally and structurally analyzed two mutant mouse lines with a genetic disruption of Bsn-Bsn and Bsn -using electrophysiology and high-resolution microscopy. In both Bsn mutant mouse lines, full-length BSN was abolished, and photoreceptor synaptic function was similarly impaired, yet synapse structure was more severely affected in Bsn than in Bsn photoreceptors. The synaptic defects in Bsn retina coincide with remodeling of the outer retina-rod bipolar and horizontal cell sprouting, formation of ectopic ribbon synaptic sites-and death of cone photoreceptors, processes that did not occur in Bsn retina. An analysis of Bsn hybrid mice revealed that the divergent retinal phenotypes of Bsn and Bsn mice can be attributed to the expression of the Bsn allele, which triggers cone photoreceptor death and neurite sprouting in the outer retina. These findings shed new light on the existing Bsn mutant mouse models and might help to understand mechanisms that drive photoreceptor death.
FASEB J, 2021; 35
29719516: Jiang X, Ryl M, Krieger J, Breer H, Pregitzer P
Odorant Binding Proteins of the Desert Locust (Orthoptera, Acrididae): Topographic Expression Patterns in the Antennae.
Odorant binding proteins (OBPs) enriched in the sensillum lymph are instrumental in facilitating the transfer of odorous molecules to the responsive receptors. In Orthopteran locust species, an in-depth understanding of this important soluble protein family is still elusive. In a previous study, we have demonstrated that the repertoire of locust OBPs can be divided into four major clades (I-IV) on the phylogenetic scale and for representatives of subfamily I-A and II-A a distinct sensilla-specific expression pattern was determined. In this study, by focusing on a representative locust species, the desert locust , we have explored the antennal topographic expression for representative OBPs of other subfamilies. First, subtypes of subfamily III-A and III-B were exclusively found in sensilla chaetica. Then, a similar expression pattern in this sensillum type was observed for subfamily I-B subtypes, but with a distinct OBP that was expressed in sensilla coeloconica additionally. Moreover, the atypical OBP subtype from subfamily IV-A was expressed in a subpopulation of sensilla coeloconica. Last, the plus-C type-B

OBP subtype from subfamily IV-B seems to be associated with all four antennal sensillum types. These results profile diversified sensilla-specific expression patterns of the desert locust OBPs from different subfamilies and complex co-localization phenotypes of distinct OBP subtypes in defined sensilla, which provide informative clues concerning their possible functional mode as well as a potential interplay among OBP partners within a sensillum.
Front Physiol, 2018; 9

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BOARD NUMBER: S07-716

ENDOSOMAL PHOSPHATIDYLINOSITOL 3-PHOSPHATE LEVELS CONTROL PRESYNAPTIC VESICLE CYCLING AND NEUROTRANSMISSION

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Neural circuit function requires mechanisms for the control of neurotransmitter release and the activity of neuronal networks including modulation by synaptic contacts, synaptic plasticity, and homeostatic scaling. The molecular mechanisms by which neurons intrinsically monitor and feedback control presynaptic neurotransmitter release and synaptic vesicle (SV) recycling to restrict neuronal network activity are poorly understood. Here we investigated the reciprocal interplay between neuronal endosomes, organelles of central importance for the function of synapses, and synaptic activity. We show that elevated neuronal activity represses the synthesis of the endosomal lipid phosphatidylinositol 3-phosphate [PI(3)P] by the lipid kinase VPS34. Neuronal activity in turn is regulated by endosomal PI(3)P, the depletion of which reduces neurotransmission as a consequence of perturbed SV endocytosis. We find that this mechanism involves Calpain 2-mediated hyperactivation of Cyclin-dependent kinase 5 (Cdk5) downstream of receptor- and activity-dependent calcium influx. Our results unravel an unexpected function for PI(3)P-containing neuronal endosomes in the control of presynaptic vesicle cycling and neurotransmission that may explain the involvement of the PI(3)P-producing VPS34 kinase in neurological disease and neurodegeneration.

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BOARD NUMBER: S07-717

THE IMPACT OF ALTERNATIVE SPLICING OF P/Q-TYPE CALCIUM CHANNELS ON SYNAPTIC PROPERTIES AND SHORT-TERM PLASTICITY

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

Ana Carolina Palmeira Do Amaral[1], Jennifer Heck[2], Artur Bikbaev[1], Abderazzaq El Khallouqi[1], Anna Bodzeta[1], Melanie Mark[3], Stefan Herlitze[4], Martin Heine[1]
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Alternative splicing (AS) is crucial for expanding proteomic diversity. Here, we focus on presynaptic voltage-gated P/Q-type calcium channels ($Ca_V2.1$) that are essential for triggering transmitter release in central synapses. Their relative position to readily releasable synaptic vesicles and their regulatory role in pre-synaptic release probability are critically influenced by AS within the channel's C-terminus at exon 37 and 47. Previous investigations, focusing on the importance of these exons separately, indicated a strong relevance for short-term plasticity. Here, we ask to which extent the AS of exon 37 and 47 influence each other to tune information processing within the presynaptic compartment. To dissect how the interplay between exon 37 and 47 influences synaptic physiology, we generated four Halo-tagged channel variants, $Ca_V2.1$ [37a/+47], [37a/Δ47], [37b/+47], and [37b/Δ47]. We will examine calcium influx and glutamate release of synapses dominated by each splice variant using functional imaging in mouse hippocampal neurons. Preliminary data suggest differences in calcium influx. Moreover, the local channel dynamic organization will be investigated for each splice variant by single-molecule tracking photoactivated localization microscopy. To evaluate whether different C-terminal configurations interfere with the presynaptic localization of endogenous $Ca_V2.1$ channels, we are using a $Ca_V2.1$::YFP knock-in mouse line. Using localization microscopy, we will evaluate the density and localization of YFP-tagged versus Halo-tagged channels within individual synapses and whether different splice variants have different affinities to scaffold proteins. Concluding, our goal is to clarify the impact of exons 37 and 47 AS at the C-terminus of $Ca_V2.1$ channels on synaptic properties.

Pubmed:
34107849: Heck J, Palmeira Do Amaral AC, Weißbach S, El Khallouqi A, Bikbaev A, Heine M
More than a pore: How voltage-gated calcium channels act on different levels of neuronal communication regulation.
Voltage-gated calcium channels (VGCCs) represent key regulators of the calcium influx through the plasma membrane of excitable cells, like neurons. Activated by the depolarization of the membrane, the opening of VGCCs induces very transient and local changes in the intracellular calcium concentration, known as calcium nanodomains, that in turn trigger calcium-dependent signaling cascades and the release of chemical neurotransmitters. Based on their central importance as concierges of excitation-secretion coupling and therefore neuronal communication, VGCCs have been studied in multiple aspects of neuronal function and malfunction. However, studies on molecular interaction partners and recent progress in omics technologies have extended the actual concept of these molecules. With this review, we want to illustrate some new perspectives of VGCCs reaching beyond their function as calcium-permeable pores in the plasma membrane. Therefore, we will discuss the relevance of VGCCs as voltage sensors in functional complexes with ryanodine receptors, channel-independent actions of auxiliary VGCC subunits, and provide an insight into how VGCCs even directly participate in gene regulation. Furthermore, we will illustrate how structural changes in the intracellular C-terminus of VGCCs generated by alternative splicing events might not only affect the biophysical channel characteristics but rather determine their molecular environment and downstream signaling pathways.
Channels (Austin), 2021; 15
32230915: Fernández-Dueñas V, Qian M, Argerich J, Amaral C, Risseeuw MDP, Van Calenbergh S, Ciruela F
Design, Synthesis and Characterization of a New Series of Fluorescent Metabotropic Glutamate Receptor Type 5 Negative Allosteric Modulators.
In recent years, new drug discovery approaches based on novel pharmacological concepts have emerged. Allosteric modulators, for example, target receptors at sites other than the orthosteric binding sites and can modulate agonist-mediated activation. Interestingly, allosteric regulation may allow a fine-tuned regulation of unbalanced neurotransmitter' systems, thus

providing safe and effective treatments for a number of central nervous system diseases. The metabotropic glutamate type 5 receptor (mGluR) has been shown to possess a druggable allosteric binding domain. Accordingly, novel allosteric ligands are being explored in order to finely regulate glutamate neurotransmission, especially in the brain. However, before testing the activity of these new ligands in the clinic or even in animal disease models, it is common to characterize their ability to bind mGluRs in vitro. Here, we have developed a new series of fluorescent ligands that, when used in a new NanoBRET-based binding assay, will facilitate screening for novel mGluR allosteric modulators.
Molecules, 2020; 25

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BOARD NUMBER: S07-718

THE EFFECTS OF ALPHA-SYNUCLEIN ON THE PHASE SEPARATION AT SYNAPSES

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Neuronal communication depends on the tightly regulated spatial and temporal release of messenger molecules known as neurotransmitters. Neurotransmitters are packed into synaptic vesicles (SVs). Hundreds of SVs form biomolecular condensates through the interaction with synapsins. Other disordered proteins at the presynapse include synucleins, most notably alpha-synuclein. The precise role of alpha-synuclein in synaptic physiology remains elusive, albeit its role has been implicated in all steps of the SV cycle. To determine the effect of alpha-synuclein on the synapsin phase, we employ the reconstitution approaches using purified SVs from rat brains and the heterologous cell system to generate synapsin condensates. We demonstrate that synapsin condensates recruit alpha-synuclein, and while enriched into these synapsin condensates, alpha-synuclein still maintains its high mobility. The presence of SVs enhances the rate of synapsin/ alpha-synuclein condensation, suggesting that SVs catalyze the formation of synapsin condensates. At physiological conditions, alpha-synuclein alone cannot trigger the phase separation of SVs. Excess of alpha-synuclein attenuates the kinetics of synapsin/SV condensate formation, indicating that the molar ratio between synapsin and alpha-synuclein is important in assembling the functional condensates of SVs. Alpha-Synuclein can be depleted from synapsin condensates by synphilin 1, another intrinsically disordered, scaffold protein at the presynapse implicated in Parkinson’s Disease. Interestingly, synphilin 1 forms fluid condensates by itself, and alpha-synuclein shows the ability to wet synphilin condensates in a salt-dependent manner. Understanding the molecular mechanism of alpha-synuclein interactions at the nerve terminals is crucial for clarifying the pathogenesis of synucleinopathies, where alpha-synuclein, synaptic proteins, and lipid organelles all accumulate as insoluble intracellular inclusions.

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BOARD NUMBER: S07-719

COMPUTATIONAL MODELLING FRAMEWORK TO STUDY CA2+ ACTIVATION OF SYNAPTIC VESICLE FUSION BY DIFFERENT SYNAPTOTAGMIN ISOFORMS

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Controlled release of neurotransmitters stored in synaptic vesicles (SVs) is central to information processing in the brain. This process relies on fast (sub-millisecond) coupling of SV fusion to the triggering signal, action potential (AP)-evoked presynaptic Ca^{2+} influx. During the last three decades the key proteins that mediate SV exocytosis have been identified. Yet, the molecular mechanisms that allow synchronisation of SV fusion to APs remain in the centre of debate. SV fusion is catalysed by synaptic SNARE proteins, VAMP2 on the vesicles (v-SNAREs) and syntaxin/SNAP25 on the pre-synaptic membrane (t-SNAREs). The current prevailing view is that the SNARE assembly process is arrested in a 'half-zippered' state to prevent continuous release of neurotransmitters and to allow for rapid and synchronous SV fusion following the Ca^{2+} influx. The key players that 'clamp' vesicle release and synchronise the late SV fusion steps are complexin proteins and isoforms of the presynaptic Ca^{2+} release sensor synaptotagmin (Syt). Here we describe a computational modelling framework that allows for simulation of release controlled by the Syt1 and Syt7 isoforms under different molecular configurations and stimulation patterns. The discrete state Markov model structure is based on recent structural data describing the interaction of Syt isoforms with the SNARE complex and complexin. State transition parameters are constrained by the known Ca^{2+} and membrane binding affinities of Syt1 and Syt7. Our stochastic simulations demonstrate that this relatively simple model can successfully capture the kinetics and Ca^{2+} dependency of synchronous and asynchronous neurotransmitter release in central synapses.

Pubmed:
32598500: Chamberland S, Timofeeva Y, Evstratova A, Norman CA, Volynski K, Tóth K
Slow-decaying presynaptic calcium dynamics gate long-lasting asynchronous release at the hippocampal mossy fiber to CA3 pyramidal cell synapse.
Action potentials trigger two modes of neurotransmitter release, with a fast synchronous component and a temporally delayed asynchronous release. Asynchronous release contributes to information transfer at synapses, including at the hippocampal mossy fiber (MF) to CA3 pyramidal cell synapse where it controls the timing of postsynaptic CA3 pyramidal neuron firing. Here, we identified and characterized the main determinants of asynchronous release at the MF-CA3 synapse. We found that asynchronous release at MF-CA3 synapses can last on the order of seconds following repetitive MF stimulation. Elevating the stimulation frequency or the external Ca concentration increased the rate of asynchronous release, thus, arguing that presynaptic Ca dynamics is the major determinant of asynchronous release rate. Direct MF bouton Ca imaging revealed slow Ca decay kinetics of action potential (AP) burst-evoked Ca transients. Finally, we observed that asynchronous release was preferentially mediated by Ca influx through P/Q-type voltage-gated Ca channels, while the contribution of N-type VGCCs was limited. Overall, our results uncover the determinants of long-lasting asynchronous release from MF terminals and suggest that asynchronous release could influence CA3 pyramidal cell firing up to seconds following termination of granule cell bursting.
Synapse, 2020; 74

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BOARD NUMBER: S07-720

SUBPOPULATIONS OF EXCITATORY AND INHIBITORY SYNAPSES EXPRESS SV2A IN MOUSE BRAIN.

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Introduction: Synaptic pathology is associated with over 130 brain diseases and there is a pressing need to evaluate synaptic pathology in living individuals. PET tracers targeting the presynaptic protein SV2A (Synaptic Vesicle protein 2A) can be used to quantify synapse damage or loss using brain imaging in a clinical setting. Although SV2A has been posited as a marker of all synapses, synaptome mapping studies show excitatory synapses are highly diverse (Zhu et al, *Neuron* 2018 doi.org/10.1016/j.neuron.2018.07.007). **Aims and Methodology:** To determine if SV2A was expressed in all adult mouse brain synapses we used immunostaining techniques to label synapses in brain tissue sections with antibodies to SV2A together with a battery of genetic and immunolabelled presynaptic and postsynaptic markers and then imaged individual synapses using confocal microscopy. **Results:** We focussed on cortex, hippocampus, thalamus and striatum which we confirmed were labelled with the SV2A PET tracer [^{18}F]MNI1126 *(R*-enantiomer) in living mice. Our results of presynaptic terminals labelled with SV2A showed a wide range of sizes, density and intensity. SV2A was expressed in subpopulations of excitatory and inhibitory synapses and the size of these populations varied between brain regions. **Conclusion:** Characterising the subpopulations of SV2A-positive synapses in different brain diseases will be important for interpreting imaging data obtained with SV2A PET tracers.

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BOARD NUMBER: S07-721

DEVELOPMENTAL RECONFIGURATION OF NANODOMAIN COUPLING BETWEEN CALCIUM CHANNELS AND RELEASE SENSORS AT A GABAERGIC CENTRAL SYNAPSE

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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GABAergic synapses show tight ("nanodomain") coupling between presynaptic Ca^{2+} channels and release sensors and undergo substantial changes during development. However, the exact coupling topography and stoichiometry remains unknown. To address this question, we analyzed the coupling configuration at the basket cell-to-Purkinje cell synapse in mouse cerebellum at different developmental stages, combining paired recording, intracellular pipette perfusion, transmission electron microscopy (TEM), and freeze-fracture replica immunolabeling (FRIL). To functionally probe the coupling configuration, we examined the shape of the presynaptic action potential (AP) by direct presynaptic recording. Presynaptic APs showed substantial shortening during development, suggesting reduced efficacy of Ca^{2+} channel activation. Furthermore, we probed the coupling distance between Ca^{2+} channels and release sensors by intracellular application of the Ca^{2+} chelator EGTA via patch pipette perfusion. The sensitivity of transmitter release to 10 mM EGTA decreased (IPSC-EGTA / IPSC-control: 47.6% at ~P7, 86.2% at ~P14, and 89.7% at ~P21; n = 15 pairs total; p = 0.013), indicating tightening of coupling during synaptic maturation. Structural analysis by TEM and FRIL demonstrated a change in the coupling stoichiometry. During development, the number of clusters per active zone (AZ) in basket cell terminals increased 1.59-fold, whereas the number of Ca^{2+} channels per cluster was unchanged. In contrast, the number of docked vesicles per AZ increased more strongly, 2.75-fold during synaptic maturation. Nanodomain coupling with decreasing efficacy of Ca^{2+} channel activation and increasing vesicle-to-Ca^{2+} channel cluster stoichiometric ratio during development may ensure fast and sustained transmitter release during *in vivo*-like repetitive activity at cerebellar GABAergic synapses.

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EVIDENCE FOR LOCAL FATTY ACID METABOLISM IN PRESYNAPTIC BOUTONS

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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The brain is the fattiest organ in our body, roughly 60% of its dry mass. Nonetheless, lipids (fatty acids, phospholipids, cholesterols) are seldom overlooked in Neuroscience. Strikingly, individuals consuming a diet rich in saturated fatty acids are twice as likely to exhibit a form of dementia, an age-related condition characterized by deteriorating cognitive abilities. The biological mechanisms underlying this increased risk are unknown. The blood brain barrier is permeable to fatty acids known in other organs (e.g. pancreas) to incorporate into membrane bilayers and change the fusion properties of intracellular vesicles. In neurons, synaptic vesicles fuse with the presynaptic membrane to pass on information. **Could fatty acid metabolism regulate synaptic vesicle fusion?** Recently, I published the transcriptome of forebrain excitatory presynaptic boutons. Surprisingly, the mRNA coding for the enzyme FASN (fatty acid synthetase) -the central enzyme in the biosynthesis of fatty acids- appeared to be highly abundant in these subcellular compartments. Importantly, **FASN is a rate-limiting enzyme. Thus, increasing FASN copy number at boutons through local translation of FASN mRNA, will result in an increased production of fatty acids, namely palmitic acids**. Using biochemistry, immunocytochemistry and in situ hybridization assays on synaptosomes prepared from adult mouse brains, we validated the presence of FASN mRNA and protein at both excitatory and inhibitory presynapses. Our preliminary data subject that the presence of FASN is tightly linked to the presence of mitochondria inside a bouton. This work opens a new door toward a better undertanding of the role of lipid metabolism in synaptic function.

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DISSECTING THE FUNCTIONAL CONSEQUENCES OF CAPS DELETION ON SEROTONIN RELEASE FROM MOUSE ENTEROCHROMAFFIN CELLS.

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Enterochromaffin cells of the intestinal epithelium are the predominant producers of serotonin (5-HT) in the body with an estimated 95% of total 5-HT residing in the gut. Enterochromaffin cells are chemo- and mechanosensitive and respond to nutrients, metabolites, and physical force. An increasing number of studies have uncovered the functions of 5-HT in the periphery, which range from regulation of gut motility to its role in homeostatic metabolism. Although there is a growing understanding of the different mechanisms orchestrating these functions, the cellular mechanisms and molecular machinery responsible for the transduction of stimuli to 5-HT secretion is largely unknown. To gain a better understanding of the 5-HT release machinery in the gut, we investigated the roles of Calcium-dependent activator protein for secretion (CAPS) 1 and 2 in enterochromaffin cells. CAPS proteins are important components of the neuronal presynaptic release machinery with established roles in neuronal transmitter release, short-term plasticity, and importantly, in the priming of secretory granules in neuroendocrine cells and neurons. To this end, we established the expression of CAPS1 in mouse and human enterochromaffin cells using immunocytochemistry and biochemical assays, and generated CAPS1 knock-out organoids derived from adult mouse intestinal epithelial stem cells. The functional consequences of CAPS1 loss were measured using single-cell carbon fiber amperometry to detect 5-HT release from individual fusing granules. We present here an experimental workflow to study on the single cell level the role of individual molecular components of the presynaptic vesicle fusion machinery in the regulation of 5-HT secretion in the gut.

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GRID1 / GLUD1 MUTATIONS CAUSING INTELLECTUAL DISABILITY WITH SPASTIC PARAPLEGIA IMPAIR MGLU1/5 RECEPTOR SIGNALING AND EXCITATORY SYNAPSES

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

Regine Hepp[1], Devina Ung[2], Ludovic Tricoire[1], Annick Toutain[3], Nicolas Pietrancosta[1], Annick Rothschild[4], Ben Pode-Shakked[4], Bertrand Lambolez[1], Frederic Laumonnier[2]
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The ionotropic glutamate delta receptor GluD1, encoded by the GRID1 gene on chromosome 10, is involved in synapse formation, function, and plasticity. GluD1 does not bind glutamate, but instead cerebellin and D-serine, which allow the formation of trans-synaptic bridges, and trigger metabotropic signaling. Though widely expressed in the nervous system, pathogenic mutations of GluD1 have not been characterized in humans so far. We report homozygous missense GRID1 mutations in siblings from two consanguineous families presenting with intellectual disability and spastic paraplegia, without or with glaucoma, a threefold phenotypic association whose genetic bases had not been elucidated previously. Molecular modeling indicated that one mutation alters cooperativity between cerebellin and D-serine binding GluD1 domains, whereas the other mutation alters D-serine binding. Expression, trafficking, physical interaction with metabotropic glutamate receptor mGlu1, and cerebellin binding of GluD1 mutants were not conspicuously altered. Conversely, we found that GluD1 mutants hampered signaling of metabotropic glutamate receptor mGlu1/5 via the ERK pathway in primary cortical cell culture. Moreover, both mutants impaired dendrite morphology and excitatory synapse density in neurons of primary hippocampal culture. These results show that the clinical phenotypes are distinct entities segregating in the families as an autosomal recessive trait, and caused by pathophysiological effects of GluD1 mutants involving metabotropic glutamate receptor signaling and neuronal connectivity. Our findings unravel the importance of the GluD1 receptor signaling in sensory, cognitive and motor functions of the human nervous system.

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MDGA1 AND MDGA2 DIFFERENTIALLY INTERACT WITH NEUROLIGIN-2 TO FUNCTIONALLY REGULATE INHIBITORY SYNAPSES

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Alterations in synaptic inhibition are noted causes of many psychiatric disorders, and accordingly, understanding the molecular components regulating their formations is crucial for new treatments. Two of these components are the postsynaptic protein Neuroligin-2 (Nlgn2), which plays a key role in the formation of inhibitory synapses by binding the presynaptic proteins of the Neurexin (NRX) family, and the MAM-domain-GPI-anchored proteins 1 and 2 (MDGA1-MDGA2), which recently aroused interest due to their ability to bind Nlgn2 at the same binding site as the NRXN family members. Here, we generated MDGA1 x Nlgn2 double KO (dKO) and MDGA2-het x Nlgn2 KO mice, to investigate the consequences of the Nlgn2-MDGA interaction for the structure and function of inhibitory synapses in the adult hippocampal area CA1 and amygdala. We show that MDGA1 is differentially expressed across CA1 layers, with a nearly absent expression in the stratum pyramidale, and that absence of MDGA1 and MDGA2 alters gephyrin, VIAAT, and $GABA_A$Rs γ2-subunit expression in a layer specific manner. Moreover, several phenotypes characteristic of the Nlgn2 KO mice, i.e., the increase in extrasynaptically located gephyrin aggregates, the impairment of GABAergic transmission, and the increased anxiety-related behavior, are partially normalized in MDGA1 x Nlgn2 dKO but not in MDGA2-het x Nlgn2 KO mice. In conclusion, our study shows that MDGA1 and MDGA2 functionally interact with Nlgn2 to allow for the correct assembly of inhibitory synapses. Moreover, our data place MDGA1 rather than MDGA2 as a new target for anxiolytic treatments.

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REGULATORY MOTIFS INVOLVED IN LRRTM2 TRAFFICKING AND LRRTM2-DEPENDENT STABILISATION OF AMPARS AT EXCITATORY SYNAPSES

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Leucine-Rich Repeat Transmembrane (LRRTM) proteins are adhesion molecules enabling neurons to connect and communicate at **synapses**. Mutations in the genes encoding LRRTMs, their synaptic partners, neurexins and their functional competitors, neuroligins, are associated with psychiatric and neurological disorders. However, the molecular regulation of LRRTMs and how they structure and modulate synapse strength remain elusive. **LRRTM2**, the most synaptogenic member of the family, is exclusively localised and enriched at excitatory synapses, where it exhibits low **membrane dynamics**. Interestingly, LRRTM2 is involved in synaptic transmission and plasticity and regulates the surface levels of **AMPARs**, the main glutamatergic receptors responsible for fast neurotransmission in the brain. In this project, we investigated the molecular mechanisms underlying LRRTM2 **stabilisation** and **trafficking** at excitatory synapses, as well as the **interplay between LRRTM2 and AMPARs**. We developed a structure-function approach in a novel transgenic mouse model where LRRTM2 is conditionally knocked-out during synapse formation. We demonstrated that the C-terminal domain of LRRTM2 controls its compartmentalisation in dendrites as well as its enrichment and compaction at synapses. Surprisingly, LRRTM2 **synaptic confinement** was found to be independent of its PDZ-like binding domain and was instead regulated by the recently identified YxxC intracellular sequence, also critical for LRRTM2 trafficking and **exocytosis**. We then showed that the recently identified neurexin-binding site in LRRTM2 (E348) is involved in membrane stabilisation of synaptic AMPARs. These results demonstrate that the intracellular region of LRRTM2 controls its **synaptic clustering**, membrane dynamics and confinement, while extracellular binding interfaces are involved in stabilising AMPARs at the synapse.

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FLUORESCENCE NANOSCOPY UNRAVELS ARC FINE STRUCTURES FOR AMPA RECEPTORS REGULATION

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

Martina Damenti[1], Giovanna Coceano[1], Jonatan Alvelid[1], Mariline Mendes Silva[1], Lea Rems[1], Yvonne Johansson[2], Erdinc Sezdig[3], Lucie Delemotte[1], Gilad Silberberg[2], Ilaria Testa[1]
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The Activity Regulated-Cytoskeleton associated-protein Arc is pivotal to mediate plastic responses in neuronal cells. In vitro studies suggest its ability to form large and small order oligomers which are potentially involved in interneuronal trafficking. Despite its important function, no direct observation of Arc oligomers in cells has been presented due to the small size, and lack of appropriate labelling strategies. Here, we take advantage of STED microscopy to study Arc nanoscale organization in cellular environment especially at the synapses. Arc oligomers role in the regulation of AMPA receptor surface levels, together with their close association to the plasma membrane, were addressed via chemical mutagenesis and molecular dynamic simulation studies. Furthermore, for the first time, Arc-Arc molecular interaction and its liquid-liquid phase separation properties were uncovered in cellular system. Together, our observations support the model by which Arc oligomerization at the post-synaptic endocytic zone, favors AMPA receptors endocytosis inducing plasma membrane curvature.

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SYNAPSES WITH DIVERSE PROTEIN LIFETIMES ARE BUILDING BLOCKS OF SYNAPTOME ARCHITECTURE

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

Edita Bulovaite[1], Zhen Qiu[1], Maximilian Kratschke[1], Adrianna Zgraj[1], David Fricker[1], Eleanor Tuck[1], Ragini Gokhale[1], Shekib Jami[2,3], Paula Merino-Serrais[4,5], Elodie Husi[6], Lorena Mendive-Tapia[7], Marc Vendrell[7], Tom O'Dell[2,3], Javier Defelipe[4,5], Noboru Komiyama[1,8,9,10], Anthony Holtmaat[6], Erik Fransén[11,12], Seth Grant[1,8]
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Aim: Protein turnover is required for synapse maintenance and activity-dependent remodelling. The lifetime of synaptic proteins may be brain region- and synapse-specific, but due to technical limitations endogenous protein turnover in individual synapses had not been examined to date. We quantified the lifetime of postsynaptic scaffolding protein PSD95 in individual excitatory synapses across the mouse brain and lifespan. **Methods:** Transgenic mice carrying a HaloTag domain fused to endogenous PSD95 were intravenously injected with a fluorescent ligand that irreversibly binds the HaloTag domain, labelling PSD95 containing synapses with a fluorescent date-stamp. Fluorescent protein levels were visualized in brain sections using confocal microscopy, with loss of fluorescence over time used for estimating PSD95 protein degradation. **Results:** PSD95 protein lifetime differed more than 6-fold between brain regions in adult mice, from ~1-2 days in olfactory bulb and thalamic nuclei to ~10-12 days in the superficial layers of the cortex. Protein lifetime increased with age, from development to adulthood and into ageing. Synapse subtypes, as defined by molecular and morphological features, revealed distinct PSD95 protein lifetimes. Short protein lifetime (SPL) synapses were enriched in developing animals and in regions controlling innate behaviours. Long protein lifetime (LPL) synapses accumulated during development, were enriched in the cortex and CA1 and were preferentially preserved in old age. **Conclusion:** Synapses with diverse protein lifetimes show distinct spatial distributions across the brain and are differentially affected during development and ageing. Brain-wide maps of synaptic protein lifetime will form a valuable resource for future research into molecular and synapse biology.

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ANALYSIS OF SYNAPTIC NANOARCHITECTURE USING FRET AT THE LEVEL OF SINGLE PROTEIN COMPLEXES AND AT THE WHOLE MOUSE BRAIN SCALE

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

Takeshi Kaizuka[1], Katie Morris[2], Ricky Qiu[1], Edita Bulovaite[1], Gabor Varga[1], Noboru Komiyama[1], Mathew Horrocks[2], Seth Grant[1]
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Proteins within the postsynaptic terminal of excitatory synapses play an essential role in synaptic physiology and behaviour. Individual proteins are physically organised into multiprotein complexes and supercomplexes. Recent studies using super-resolution microscopy reveal that postsynaptic proteins are not uniformly distributed but form nanoclusters where the proteins are densely distributed. However, these studies are limited to a spatial resolution of 20-50nm. To study the postsynaptic nanoarchitecture at a resolution of 1-10nm, we have used Förster Resonance Energy Transfer (FRET) on brain tissue and brain extracts from mice containing genetic tags on the C-terminus of PSD95, an abundant postsynaptic scaffold protein that controls synaptic plasticity and learning. Individual protein complexes containing PSD95 were examined using both single-molecule confocal and total internal reflection fluorescence (TIRF) microscopy and found to contain dimers of PSD95 that lacked FRET, indicating their C-terminal labels are separated by >10nm. In intact synapses in the brain, however, we were able to detect FRET between PSD95 molecules carrying a PSD95-HaloTag labelled with mixtures of HaloTag flurophore-labelled ligands or mice carrying PSD95-HaloTag and PSD95-CLIP tags. Synaptic PSD95 FRET intensity and efficiency was quantified from individual synapses imaged across whole sagittal sections of mouse brain and showed variation between brain regions. These findings indicate that PSD95 multiprotein complexes are closely packed in synapses and that this packing differs between synapses in areas of the brain.

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BOARD NUMBER: S07-730

ENDOCYTOSIS AND POLARITY MAINTENANCE AT THE AXON INITIAL SEGMENT

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Neurons are highly polarized cells with sorting of proteins between dendrites and axon. A key component for this sorting is the axon initial segment (AIS), a specialized structure at the proximal end of the axon concentrating protein complexes of actin, βIV-spectrin, ankyrinG and anchored transmembrane proteins such as sodium channels. At least two mechanisms maintaining polarity and which occur in the AIS have been identified. First, the AIS forms a diffusion barrier on the membrane, preventing intermixing of somatic and dendritic constituents. Second, intracellular vesicles are sorted for transport, distinguishing dendritic from axonal vesicles. In this work, we tested the possibility that endocytosis of dendritic cargo at the AIS constitutes another mechanism which maintains neuronal polarity. We used rat hippocampal neurons transfected at 7 DIV with the transferrin receptor, a dendritic receptor, tagged with Superecliptic pHluorin (TfR-SEP). The AIS was identified by co-transfection with NavII-III-mScarlet or by live antibody labelling of neurofascin. We measured with the pulsed pH assay (Sposini et al. Nature Protocols 2020) the rate of endocytosis in the soma, proximal dendrites and AIS of neurons at 9-10 DIV. We found that endocytic events occurred at similar rates in all compartments, arguing for an active role of endocytosis in maintaining the dendritic membrane proteins in their normal compartment. Using *C elegans* neurons, we showed that selective inhibition of endocytosis of dendritic proteins at the AIS leads to defective axon-dendrite polarity. We thus propose that endocytosis at the AIS is an important, evolutionary conserved mechanism to maintain neuronal polarity.

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BOARD NUMBER: S07-731

NERVE GUIDANCE IN CANCER: A NEW ROLE FOR NETRIN-1

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Aims: Nerve-tumor interactions have received increased attention recently because of their effect on tumorigenesis. We have demonstrated that sympathetic axons remodel and branch during early development of pancreatic ductal adenocarcinoma (PDAC), but the underlying molecular mechanisms are still unknown. Netrin-1 is a secreted protein involved in axon guidance during embryonic development and known to be reexpressed in cancers. Here we investigated the role of Netrin-1 in PDAC tumor-induced neuronal plasticity. **Methods:** Expression of Netrin-1 and its receptors was analyzed in transgenic mouse model of PDAC (KIC mice) and in a model of metaplastic lesions (precursor lesions of PDAC) induced by chronic inflammation. The function of Netrin-1 on axonal remodeling was studied using a humanized Netrin-1 blocking antibody (NET-H-mab) and by conditional genetic deletion of netrin-1. Sympathetic axonal density was measured on tissue sections or whole cleared organs. **Results:** During pancreatic inflammation, Netrin-1 was detected in proinflammatory macrophages and pancreatic metaplastic cells, while the Netrin-1 receptor DCC was reexpressed on sympathetic axons. Blockage of Netrin-1 by NET-H-mab administration resulted in a decrease of sympathetic axonal density around metaplastic lesions. To identify the source of Netrin-1 responsible for this effect, we generated transgenic mouse models with conditional knockdown of netrin-1 in macrophages or in pancreatic cells. Preliminary results revealed no significant change of sympathetic axonal density when netrin-1 was depleted from macrophages. **Conclusion:** Our results suggest that Netrin-1 expression in metaplastic pancreatic lesions triggers sympathetic axonal plasticity, possibly through the axonal receptor DCC.

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TAU – A MASTER REGULATOR OF RECYCLING SYNAPTIC VESICLE CLUSTERING AT THE PRESYNAPSE

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Background: Synaptic vesicles (SVs) underpin neuronal communication through fusion and the release of neurotransmitters at the synaptic cleft. SVs form homogenous clusters adjacent to their active zone and are characterised into functional pools differentiated by their release properties. The molecular mechanisms that link the organisation of SVs into pools and their clustering remain unknown. Recently, liquid-liquid phase separation (LLPS) has been suggested to control the organisation of SVs into clusters. The microtubule-binding protein Tau is a highly phosphorylated and intrinsically disordered protein found to form LLPS *in vitro* and bind SVs. **Aim:** To assess the ability of Tau to form nano-biomolecular condensates at the pre-synapse and investigate its role in the organisation of SVs. **Methods:** Activity-dependent phosphoproteome analysis of hippocampal neurons. Subdiffractional tracking of internalised molecules, single-particle tracking photo-activated localisation microscopy and nanoscale spatiotemporal index clustering of trajectory segments. **Results:** Pharmacological modulation of synaptic protein phosphorylation significantly impacted the mobility of recycling SVs. Tau knockout (KO) increased the mobility of recycling vesicles, making this pool insensitive to pharmacological phosphorylation. Re-expression of Tau in the Tau KO background restored the mobility of recycling vesicles to control levels. Using rescued expression of Tau-mEos2 in Tau KO background and TALEN-based gene-edited mice expressing endogenous Tau-mEos2, we demonstrate that Tau forms transient nano-biomolecular condensates, that are highly sensitive to stimulation, level of expression and to 1,6-hexanediol, an aliphatic alcohol capable of dissolving biomolecular condensates. **Conclusion:** Tau regulates the nanoscale organisation of the recycling pool of SVs by forming nano-biomolecular condensates sensitive to synaptic activity.

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SYNAPTIC INTERLEUKIN-4 RECEPTOR SIGNALING MODULATES NEURONAL NETWORK ACTIVITY

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

Christina Francisca Vogelaar[1], Nicholas Hanuscheck[1], Carine Thalman[1], Micaela Domingues[1], Samantha Schmaul[1], Manuela Ecker[1], Johannes Vogt[2], Frauke Zipp[1]
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There is emerging evidence that immune responses not only play a part in the central nervous system (CNS) in disease, but may also be relevant for healthy conditions. We have previously shown neuro-protective and –regenerative effects of interleukin-4 (IL-4) in neuroinflammation and identified a direct neuronal signaling pathway via IRS1-PI3K-PKC, leading to axonal repair (Vogelaar et al., STM, 2018). Based on this signaling pathway, we now hypothesized a homeostatic role in synaptic function. We discovered a major role for the IL-4/IL-4Rα signaling pathway in synaptic processes. IL-4Rα is expressed presynaptically and locally available IL-4 regulates synaptic transmission (Hanuscheck et al, JEM, 2022). IL-4Rα-deficient neurons displayed reduced synaptic vesicle pools, altered postsynaptic currents and a higher excitatory drive in cortical networks. IL-4 treatment of wild type neurons led to increased inhibitory postsynaptic currents, mediated via PKCγ signaling. In fact, deficiency of IL-4Rα resulted in increased network activity in vivo, accompanied by altered exploration and anxiety-related learning behavior. We conclude that neuronal IL-4Rα and its presynaptic prevalence appears relevant for maintaining homeostasis of CNS synaptic function. Vogelaar CF*, Mandal S*, Lerch S*, et al. Fast direct neuronal signaling via the IL-4 receptor as therapeutic target in neuroinflammation. **Sci Translational Medicine,** 10, doi: eaao2304 (2018)
Hanuscheck N*, Thalman C*, Domingues M*,... Vogelaar CF[§], Zipp F[§]. Interleukin-4 receptor signaling modulates neuronal network activity. **Journal of Experimental Medicine**, doi: 10.1084/jem.20211887

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BOARD NUMBER: S07-734

INTERLEUKIN-4 RECEPTOR SIGNALING MODULATES NEURONAL TRANSCRIPTOME

POSTER SESSION 07 - SECTION: SYNAPTIC ARCHITECTURE

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Interleukin-4 (IL-4) is a pleiotrophic cytokine that acts not only peripherally on immune cells, but also directly on neurons. We recently identified a neuronal IL-4 receptor alpha (IL-4Rα) signaling pathway and showed direct effects of locally applied IL-4 on axonal regeneration in experimental autoimmune encephalomyelitis, the mouse model for multiple sclerosis, without affecting the immune system (Vogelaar et al, STM, 2018). Using different techniques, we demonstrated cortical and hippocampal expression of IL-4Rα by neurons. Transcriptome analysis of mouse and human neurons revealed differential regulation of synaptic processes and vesicle signaling, as well as IL-4/IL-4Rα-dependent regulation of molecules involved in excitatory and inhibitory transmission (Hanuscheck et al, JEM, 2022). To distinguish whether these effects may be receptor subtype specific we have characterized the subtype expression regulation in different brain regions and cell types. We conclude that IL-4/IL-4Rα signaling in mouse neurons is largely influencing genes involved in synaptic transmission, placing IL-4 at a central position for brain homeostasis. Vogelaar CF*, Mandal S*, Lerch S*, ... Zipp F. Fast direct neuronal signaling via the IL-4 receptor as therapeutic target in neuroinflammation. **Science Translational Medicine,** 10, doi: eaao2304 (2018)
Hanuscheck N*, Thalman C*, Domingues M*, Schmaul S, ... Vogelaar CF[§], Zipp F[§]. Interleukin-4 receptor signaling modulates neuronal network activity. **Journal of Experimental Medicine**, doi: 10.1084/jem.20211887

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BOARD NUMBER: S07-735

THE POSTSYNAPTIC SCAFFOLDING PROTEIN SAPAP3 INTERACTS WITH MITOCHONDRIAL PROTEINS AND IS REQUIRED FOR MAINTAINING ORGANELLE DYNAMICS AND FUNCTION

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Background: Intact mitochondrial function is required for synaptic ATP maintenance and Ca^{2+} buffering capacity, being important in synaptic structural maintenance. However, molecular targets linking synaptic neuronal activity and mitochondria remain scarce. Postsynaptic SAPAP3 scaffolding protein is involved in synaptic structure maintenance and its defects have been linked to several human pathologies. **Aims:**To uncover SAPAP3 as a possible mitochondrial regulator, establishing a link between synaptic and mitochondrial function. **Methods:**SAPAP3 interactome was assessed by mass spectrometry using rat synaptoneurosomes. Protein proximity ligation assay (PLA) was used to define SAPAP3 physical interaction with mitochondrial interactor (Mic60). Silencing (sh) SAPAP3 plasmids were used to study SAPAP3 involvement on mitochondrial function and dynamics inST*Hdh*$^{Q7/Q7}$cells and WT striatal primary neurons. Also, we studied isolated mitochondria from SAPAP3 knock-out (KO) mouse brains. **Results:**Data show that SAPAP3 molecular interactors are mainly mitochondrial, being Mic60 the one with the highest affinity. SAPAP3/Mic60 physical interaction was confirmed by PLA. Moreover, SAPAP3 preferentially co-localized with mitochondria in striatal cells. Following silencing, SAPAP3 mitochondrial levels were reduced in striatal neurons and ST*Hdh*$^{Q7/Q7}$ cells, accompained by decreased interaction with Mic60. In these cells, mitochondrial morphology was impaired, followed by decreased mitochondrial neurite trafficking. Disrupting SAPAP3 triggered mitochondrial dysfunction through diminished membrane potential, augmented reactive oxygen species (ROS) levels, altered mitochondrial Ca^{2+} buffering capacity, and reduced activity of mitochondrial respiratory complexes in SAPAP3-KO mouse cortex and striatum. **Conclusions:** ur study reveals a novel role for SAPAP3 in regulating mitochondrial function and dynamics that goes beyond its prototypical role as a synaptic scaffolding protein.

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BOARD NUMBER: S07-736

CKII MEDIATED AXONL PLASTICITY VIA MITOCHONDRIA NCLX CA2+ HANDLING

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Mitochondrial Ca^{2+} -$[Ca^{2+}]$m signaling has several key roles in cell function, such as regulating ATP production and cell apoptosis. Ca^{2+} flows into the mitochondria through the mitochondrial Ca^{2+} uniporter and is extruded through much slower Na^{+}/Ca^{2+} exchanger, NCLX, a rate limiting step in mitochondrial Ca^{2+} transport. Mass spectral analysis of NCLX revealed the presence of a Casein Kinase 2 (CKII) phosphorylation site, on the regulatory loop on Ser271 residue. Previous studies showed that the axon initial segment (AIS) plasticity is dependent on CKII activity. The AIS, the spike generation region of the axon, can exhibit plasticity over time, a vital proses for a dynamic neuronal network. One method of triggering AIS plasticity is via direct blocking of axonal M-type K^{+} channels, which induces a distal relocation of Na^{+} and M-channels on the AIS. We hypothesized that this AIS plasticity is directly linked to $[Ca^{2+}]$m handling by NCLX. First, we tested whether CKII inhibitor TBCA affect NCLX activity, and found that its application downregulated NCLX dependent $[Ca^{2+}]$m efflux. Furthermore, we showed that phosphomimetic mutant S271A and S271D, a constitutively active or inhibited NCLX mutants, respectively and independently of TBCA, had a similar effect on NCLX activity. Finally, we found that CKII dependent AIS distal plasticity, is blocked in NCLX KO hippocampal neurons. Thus, our results indicate that CKII is a crucial regulator of NCLX and thereby controls AIS plasticity.

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BOARD NUMBER: S07-737

INVESTIGATING THE ROLE OF CALCIUM IN REGULATION OF MITOCHONDRIAL DYNAMICS IN MYELINATING OLIGODENDROCYTES.

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Myelin is a lipid-rich membrane that wraps around axons and facilitates rapid action potential propagation. Myelin loss is seen in many brain diseases including MS, stroke, diabetes and early stages of dementia. In the brain myelin is synthesized and maintained by oligodendrocytes. This is an energy demanding process, requiring an increase in ATP and carbon substrates obtained through a surge of mitochondrial activity. We know very little about the dynamics of oligodendrocyte mitochondria and it is unclear how mitochondria are recruited to active myelination sites. A better understanding of how the energy producing mitochondria are controlled during myelination, could lead to new therapies for myelin loss in the future. The objective of this project is to investigate the role of calcium as a regulator of mitochondrial dynamics in myelinating oligodendrocytes. We use a combination of viral transduction and live confocal imaging of mitochondria and calcium transients in myelinating oligodendrocytes in organotypic mouse brain slices.

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BOARD NUMBER: S07-738

EXPRESSION PATTERN AND FUNCTIONS OF THE MITOCHONDRIAL SPIRE1 ISOFORM IN THE MOUSE BRAIN

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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The high demand of neurons for energy and calcium buffering capacity highlights the importance of properly functioning mitochondria in the nervous system. Strikingly, dysfunction and abnormal morphology of mitochondria are observed in several neurodegenerative diseases. The mitochondrial isoform of the actin nucleator SPIRE1 (mitoSPIRE1) was shown to have a regulatory function in both mitochondrial division and motility. Although mitoSPIRE1 is expressed in the mouse brain, its role in the nervous system is still unknown. In our current study, we investigate, in which distinct regions and cell types of the brain mitoSPIRE1 is expressed and which functions mitoSPIRE1 fulfills in the mouse brain. To this end, we examine the brains as well as primary neurons and astrocytes isolated from mitoSPIRE1 knockout mice, in which mitoSPIRE1 is specifically knocked out while the vesicular SPIRE1 isoforms are still present. Currently, we quantitatively measure mitoSPIRE1 gene expression in primary neurons and astrocytes isolated from cortex, hippocampus and cerebellum. Furthermore, we examine the effects of a mitoSPIRE1 knockout on the mitochondrial morphology and dynamics, focusing on mitochondrial length and motility. Here, mitochondrial parameters are determined by fluorescence microscopy and analyzed in a semi-automated way using the Imaris software. Moreover, we investigate whether a loss of mitoSPIRE1 facilitates neurodegenerative changes in the mouse brain by histological analysis. We hypothesize that mitoSPIRE1 is expressed in neurons and astrocytes and that it is involved in the regulation of mitochondrial morphology and dynamics in both cell types, thus possibly playing a role in the homeostasis of the nervous system.

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9-13 July 2022 | Paris, France

BOARD NUMBER: S07-739

NOREPINEPHRINE REGULATES MITOCHONDRIAL BIOGENESIS AND FUNCTION IN THE HIPPOCAMPUS

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Neuronal mitochondria are central to not only maintaining cellular bioenergetics, calcium dynamics and modulation of ROS signalling, but are also critical for specialized functions including synaptic plasticity and neurotransmission. While mitochondria are postulated to have a fundamental role in the functioning of neurons, it's only recently that factors that influence mitochondria in neurons and the central nervous system are being investigated. Here, we identify a critical role of neurotransmitter Norepinephrine in modulating mitochondria in rodent hippocampal cells. Norepinephrine increases mitochondrial biogenesis, enhances the expression of regulators of mitochondrial biogenesis, regulates ATP synthesis and influences mitochondrial function. Increasing Norepinephrine content at the synapse via treatment with selective norepinephrine reuptake inhibitors, also evoked robust increases in mitochondrial biogenesis and ATP content. These effects of Norepinephrine appear to be mediated by the β adrenergic receptor subtypes and involve a critical role of the master modulator of mitochondrial biogenesis PGC1a. These findings identify a novel and exciting role for Norepinephrine in impacting mitochondrial turnover and biogenesis in the hippocampus.

Pubmed:
35180924: Kapri D, Fanibunda SE, Vaidya VA
Thyroid hormone regulation of adult hippocampal neurogenesis: Putative molecular and cellular mechanisms.
Adult hippocampal neurogenesis is sensitive to perturbations in thyroid hormone signaling, with evidence supporting a key role for thyroid hormone and thyroid hormone receptors (TRs) in the regulation of postmitotic progenitor survival and neuronal differentiation. In this book chapter we summarize the current understanding of the effects of thyroid hormone signaling on adult hippocampal progenitor development, and also critically address the role of TRs in regulation of distinct aspects of stage-specific hippocampal progenitor progression. We highlight actions of thyroid hormone on thyroid hormone responsive target genes, and the implications for hippocampal progenitor regulation. Given the influence of thyroid hormone on both mitochondrial and lipid metabolism, we discuss a putative role for regulation of metabolism in the effects of thyroid hormone on adult hippocampal neurogenesis. Finally, we highlight specific ideas that require detailed experimental investigation, and the need for future studies to obtain a deeper mechanistic insight into the influence of thyroid hormone and TRs in the developmental progression of adult hippocampal progenitors.
Vitam Horm, 2022; 118
35115382: Tiwari P, Kapri D, Pradhan A, Balakrishnan A, Chaudhari PR, Vaidya VA
Chronic hM4Di-DREADD-Mediated Chemogenetic Inhibition of Forebrain Excitatory Neurons in Postnatal or Juvenile Life Does Not Alter Adult Mood-Related Behavior.
G-protein-coupled receptors (GPCRs) coupled to G signaling, in particular downstream of monoaminergic neurotransmission, are posited to play a key role during developmental epochs (postnatal and juvenile) in shaping the emergence of adult anxiodepressive behaviors and sensorimotor gating. To address the role of G signaling in these developmental windows, we used a CaMKIIα-tTA::TRE hM4Di bigenic mouse line to express the hM4Di-DREADD (designer receptor exclusively activated by designer drugs) in forebrain excitatory neurons and enhanced G signaling via chronic administration of the DREADD agonist, clozapine--oxide (CNO) in the postnatal window (postnatal days 2-14) or the juvenile window (postnatal days 28-40). We confirmed that the expression of the HA-tagged hM4Di-DREADD was restricted to CaMKIIα-positive neurons in the forebrain, and that the administration of CNO in postnatal or juvenile windows evoked inhibition in forebrain circuits of the hippocampus and cortex, as indicated by a decline in expression of the neuronal activity marker c-Fos. hM4Di-DREADD-mediated inhibition of CaMKIIα-positive forebrain excitatory neurons in postnatal or juvenile life did not impact the weight profile of mouse pups, and also did not influence the normal ontogeny of sensory reflexes. Further, postnatal or juvenile hM4Di-DREADD-mediated inhibition of CaMKIIα-positive forebrain excitatory neurons did not alter anxiety- or despair-like

behaviors in adulthood and did not impact sensorimotor gating. Collectively, these results indicate that chemogenetic induction of G signaling in CaMKIIα-positive forebrain excitatory neurons in postnatal and juvenile temporal windows does not appear to impinge on the programming of anxiodepressive behaviors in adulthood.
eNeuro, 2022 Jan-Feb; 9

33523596: Tiwari P, Fanibunda SE, Kapri D, Vasaya S, Pati S, Vaidya VA
GPCR signaling: role in mediating the effects of early adversity in psychiatric disorders.
Early adversity is a key risk factor for the development of several psychiatric disorders, including anxiety and depression. During early life, neurocircuits that regulate emotionality undergo substantial structural remodeling and functional maturation, and are thus particularly susceptible to modification by environmental experience. Preclinical evidence indicates that early stress enhances adult anxio-depressive behaviors. A commonality noted across diverse early stress models is life-long alterations in neuroendocrine stress responses and monoaminergic neurotransmission in key limbic circuits. Dysregulation of G protein-coupled receptor (GPCR) signaling is noted across multiple early stress models and is hypothesized to be an important player in the programming of aberrant emotionality. This raises the possibility that disruption of GPCR signaling in key limbic regions during critical temporal windows could establish a substrate for enhanced risk of adult psychopathology. Here, we review literature, predominantly from preclinical models, which supports the building hypothesis that a disruption of GPCR signaling could play a central role in programming persistent molecular, cellular, functional, and behavioral changes as a consequence of early adversity.
FEBS J, 2021; 288

32955432: Pati S, Saba K, Salvi SS, Tiwari P, Chaudhari PR, Verma V, Mukhopadhyay S, Kapri D, Suryavanshi S, Clement JP, Patel AB, Vaidya VA
Chronic postnatal chemogenetic activation of forebrain excitatory neurons evokes persistent changes in mood behavior.
Early adversity is a risk factor for the development of adult psychopathology. Common across multiple rodent models of early adversity is increased signaling via forebrain Gq-coupled neurotransmitter receptors. We addressed whether enhanced Gq-mediated signaling in forebrain excitatory neurons during postnatal life can evoke persistent mood-related behavioral changes. Excitatory hM3Dq DREADD-mediated chemogenetic activation of forebrain excitatory neurons during postnatal life (P2-14), but not in juvenile or adult windows, increased anxiety-, despair-, and schizophrenia-like behavior in adulthood. This was accompanied by an enhanced metabolic rate of cortical and hippocampal glutamatergic and GABAergic neurons. Furthermore, we observed reduced activity and plasticity-associated marker expression, and perturbed excitatory/inhibitory currents in the hippocampus. These results indicate that Gq-signaling-mediated activation of forebrain excitatory neurons during the critical postnatal window is sufficient to program altered mood-related behavior, as well as functional changes in forebrain glutamate and GABA systems, recapitulating aspects of the consequences of early adversity.
Elife, 2020; 9

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BOARD NUMBER: S07-740

ROLE OF INTRAMEMBRANE SPASTIC PARAPLEGIA PROTEINS IN ORGANIZATION OF AXONAL ER AND ER-MITOCHONDRIA CONTACTS IN DROSOPHILA

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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The HSPs are a group of rare, clinically and genetically heterogeneous, inherited neurodegenerative and neurodevelopmental diseases characterised by spasticity and lower limb weaknesses. More than 80 causative genes are known, and some of them imply the importance of endoplasmic reticulum (ER) -**a neuron within a neuron**- function and morphogenesis in HSPs. These HSP proteins are Spastin (SPG4), Atlastin (SPG3A), Receptor Expression Enhancing Protein 1 (REEP1/SPG31) and Reticulon (SPG12). In Drosophila, removing these families leads to fewer ER tubules in axons of wider diameter, although there is no widespread absence of tubules. Therefore, other proteins must be involved in shaping the tubular ER network in Drosophila axons. Another HSP protein with predicted hairpin domains is C19orf12; this is, therefore, another candidate protein for contributing to shaping the axon ER network. Mutations in this gene are found in patients with autosomal recessive HSP and NBIA. C19orf12 protein colocalises with mitochondria and ER and with ER-mitochondria contacts.To investigate possible roles of C19orf12 in ER and mitochondria structure and function, we have generated loss-of-function mutants of the widely expressed Drosophila ortholog of C19orf12, CG3740, using P element excision and CRISPR/cas9. These mutants are homozygous viable, as are quadruple mutants lacking CG3740 and all the widely expressed reticulon and REEP proteins, suggesting that these 4 proteins together are not sufficient for tubular ER formation. Testing of ER and mitochondria morphology in these mutants we have also generated flies carrying a split-GFP reporter for ER-mitochondrial sites.

Pubmed:
32116502: Öztürk Z, O'Kane CJ, Pérez-Moreno JJ
Axonal Endoplasmic Reticulum Dynamics and Its Roles in Neurodegeneration.
The physical continuity of axons over long cellular distances poses challenges for their maintenance. One organelle that faces this challenge is endoplasmic reticulum (ER); unlike other intracellular organelles, this forms a physically continuous network throughout the cell, with a single membrane and a single lumen. In axons, ER is mainly smooth, forming a tubular network with occasional sheets or cisternae and low amounts of rough ER. It has many potential roles: lipid biosynthesis, glucose homeostasis, a Ca store, protein export, and contacting and regulating other organelles. This tubular network structure is determined by ER-shaping proteins, mutations in some of which are causative for neurodegenerative disorders such as hereditary spastic paraplegia (HSP). While axonal ER shares many features with the tubular ER network in other contexts, these features must be adapted to the long and narrow dimensions of axons. ER appears to be physically continuous throughout axons, over distances that are enormous on a subcellular scale. It is therefore a potential channel for long-distance or regional communication within neurons, independent of action potentials or physical transport of cargos, but involving its physiological roles such as Ca or organelle homeostasis. Despite its apparent stability, axonal ER is highly dynamic, showing features like anterograde and retrograde transport, potentially reflecting continuous fusion and breakage of the network. Here we discuss the transport processes that must contribute to this dynamic behavior of ER. We also discuss the model that these processes underpin a homeostatic process that ensures both enough ER to maintain continuity of the network and repair breaks in it, but not too much ER that might disrupt local cellular physiology. Finally, we discuss how failure of ER organization in axons could lead to axon degenerative diseases, and how a requirement for ER continuity could make distal axons most susceptible to degeneration in conditions that disrupt ER continuity.
Front Neurosci, 2020; 14

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BOARD NUMBER: S07-741

LIPID DROPLETS SYNERGISE WITH PINK1-INDEPENDENT MITOPHAGY TO SAFEGUARD NEURAL INTEGRITY

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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AUTHORS Long M, Sanchez-Martinez A, Longo M, Suomi F, Stenlund H, Johansson AI, Ehsan H, Salo VT, Montava-Garriga L, Naddafi S, Ikonen E, Ganley IG, Whitworth AJ and **McWilliams TG* *Corresponding Author**

ABSTRACT Mitophagy neutralises defective mitochondria *via* lysosomal elimination and has major relevance for neurodegenerative disease. Increased mitophagy coincides with metabolic reprogramming, yet it remains unknown whether mitophagy is a cause or consequence of such state changes. The signalling pathways that integrate with mitophagy to sustain cell and tissue integrity also remain a mystery. We performed temporal metabolomics on mammalian cells treated with deferiprone, a therapeutic iron chelator that stimulates PINK1-Parkin independent mitophagy. Iron depletion profoundly rewired the metabolome, hallmarked by remodelling of lipid metabolism within minutes of treatment. DGAT1-dependent lipid droplet (LD) biosynthesis occurred several hours upstream of mitochondrial clearance, with LDs bordering mitochondria upon iron chelation. We demonstrate that DGAT1 inhibition restricts mitophagy *in vitro*, with impaired lysosomal homeostasis and cell viability. Importantly, genetic depletion of DGAT1 *in vivo* significantly impaired neuronal mitophagy and locomotor function in *Drosophila*. Our data defines iron depletion as a potent signal that rapidly reshapes metabolism and establishes an unexpected synergy between lipid homeostasis and mitophagy that safeguards neural integrity.

FENS Forum 2022
9-13 July 2022 | Paris, France

BOARD NUMBER: S07-742

MOTILE MITOCHONDRIA ARE REQUIRED FOR ASSOCIATIVE LONG-TERM MEMORY FORMATION

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Mitochondria are dynamics organelles that continuously move, fuse and divide. In neurons, depending on their sub-cellular localization, mitochondria shape varies and this diversity of shapes reflects the heterogeneity of the demand mitochondria need to face. *In vitro*, mitochondria motility impacts its intracellular distribution and its ability to be present at sites of high ATP consumption and Ca^{2+} buffering needs such as synapses. **However, whether *in vivo* mitochondria motility is required in memory has not been addressed yet.** Here we used the Drosophila model to address this question. Indeed, Drosophila can feature elaborate associative memory processes and actors of mitochondria dynamics as well as the molecular pathways supporting memory are largely conserved from flies to mammals. Thus, we have recently shown that, in the drosophila's major memory center, long-term memory (LTM) formation requires an acute upregulation of mitochondria metabolism. Here by genetically targeting mitochondria motility main actors in the fly's major memory center, we establish that motile mitochondria are required specifically for LTM. Then using STED microscopy to image mitochondria in the fly's memory center, we show that upon LTM formation, mitochondria dynamics is regulated. Eventually, using *in vivo* imaging we evidenced that when mitochondria motility is impaired, upregulation of mitochondria metabolism required for LTM formation is abolished. Our study shows that **mitochondria motility is a key process *in vivo* to full-fill increased neuronal metabolic demand upon LTM formation.** Further investigation would help to decipher how defect in mitochondrial transport and altered distribution are implicated in major neurological disorders.

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BOARD NUMBER: S07-743

IMAGING OF MITOCHONDRIA REDOX STATE IN ASTROCYTES AND NEURONS IN AWAKE MOUSE

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Brain cells are one of the most energy-demanding cell types in the body. Differences in metabolic profile and electron transport chain (ETC) structure of astrocytes and neurons have functional implications during conditions with varying oxygen supply (brain activity, hypoxia etc.) We assessed the redox state of the ETC of mitochondria with simultaneous analysis of blood oxygenation level in awake C57Bl/6 mice with Raman microspectroscopy. The mice expressed green fluorescent protein in astrocytes following AAV injection in the somatosensory cortex (S1). Raman spectra recorded from astrocytes and GFP-non fluorescent cells (predominantly neurons) were used to quantify reduced C and B-type cytochromes. Raman spectra recorded from blood vessels were used to quantify oxyhaemoglobin in the blood. The relative amount of reduced cytochromes reversibly increased in astrocytes but not in neurons in response to animal activity: running on the trade-mill, grooming. Simultaneously the amount of oxyhaemoglobin also increased. Thus, astrocytic mitochondria respond to local brain activity by ETC overloading with electrons, which may result in the production of reactive oxygen species. The work is supported by the Russian Science Foundation (grant 20-14-00241).

Pubmed:
30837840: Bielefeld P, Schouten M, Meijer GM, Breuk MJ, Geijtenbeek K, Karayel S, Tiaglik A, Vuuregge AH, Willems RAL, Witkamp D, Lucassen PJ, Encinas JM, Fitzsimons CP
Co-administration of Anti microRNA-124 and -137 Oligonucleotides Prevents Hippocampal Neural Stem Cell Loss Upon Non-convulsive Seizures.
Convulsive seizures promote adult hippocampal neurogenesis (AHN) through a transient activation of neural stem/progenitor cells (NSPCs) in the subgranular zone (SGZ) of the dentate gyrus (DG). However, in a significant population of epilepsy patients, non-convulsive seizures (ncSZ) are observed. The response of NSPCs to non-convulsive seizure induction has not been characterized before. We here studied first the short-term effects of controlled seizure induction on NSPCs fate and identity. We induced seizures of controlled intensity by intrahippocampally injecting increasing doses of the chemoconvulsant kainic acid (KA) and analyzed their effect on subdural EEG recordings, hippocampal structure, NSPC proliferation and the number and location of immature neurons shortly after seizure onset. After establishing a KA dose that elicits ncSZ, we then analyzed the effects of ncSZ on NSPC proliferation and NSC identity in the hippocampus. ncSZ specifically triggered neuroblast proliferation, but did not induce proliferation of NSPCs in the SGZ, 3 days post seizure onset. However, ncSZ induced significant changes in NSPC composition in the hippocampus, including the generation of reactive NSCs. Interestingly, intrahippocampal injection of a combination of two anti microRNA oligonucleotides targeting microRNA-124 and -137 normalized neuroblast proliferation and prevented NSC loss in the DG upon ncSZ. Our results show for the first time that ncSZ induce significant changes in neuroblast proliferation and NSC composition. Simultaneous antagonism of both microRNA-124 and -137 rescued seizure-induced alterations in NSPC, supporting their coordinated action in the regulation of NSC fate and proliferation and their potential for future seizure therapies.
Front Mol Neurosci, 2019; 12

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BOARD NUMBER: S07-744

STUDYING MITOPHAGY IN NEURONAL MODELS OF ALPHA-SYNUCLEINOPATHY WITH THE FLUORESCENT MITOROSELLA REPORTER.

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Objectives: The aim of this study is to explore the impact of the abnormal accumulation of alpha-synuclein (aSyn) on mitochondrial quality control mechanisms in neurons, with a focus on mitophagy. **Methods:** We used a model of primary mouse cortical neurons (E16) in which aSyn aggregation is promoted by fibrils of normal human aSyn (f91) preformed *in vitro*. Experiments were performed in both wild type type neurons and neurons deficient for Parkin, to explore specifically the PINK1/Parkin-dependent mitophagy pathway. Mitophagy was investigated in live neurons with the fluorescent MitoRosella reporter, monitoring the presence of mitochondria within lysosomes. The reporter was expressed by means of lentiviral vector-mediated gene delivery. **Results:** At two weeks of treatment with f91, 30% of the neuronal cell bodies and many neuronal processes contained deposits of phosphorylated aSyn (P-aSyn). There was a 40%-70% overlap between P-aSyn immunoreactive deposits and TOM20-positive mitochondria. Exposure to f91 resulted in a 60-100% increase in the Mito-Rosella fluorescent signal associated with lysosomes, in both wild type and Parkin-deficient neurons. By contrast, exposure of neurons to the mitochondrial complex III inhibitor antimycin A (100 nM, 3h) enhanced mitophagy by 150% in wild-type neurons only. Intriguingly, in neurons exposed to f91 there was no correlation between the increase in mitophagy and the presence of P-aSyn deposits in the soma. **Conclusions:** Synucleinopathy mediated by f91 stimulates the autophagy of mitochondria in a PINK1/Parkin-independent manner. Further studies are required to evaluate possible effects of aSyn accumulation on non-elective bulk autophagy and lysosomal function.

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BOARD NUMBER: S07-745

INTERPLAY BETWEEN THE HEPTAD REPEAT DOMAINS (HR1 AND HR2) OF MITOFUSIN IN MITOCHONDRIAL FUSION

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Aim: In this presentation, I will address how the different domains of Mitofusin work together during mitochondrial docking and fusion, as well as the role of lipids in mitochondrial fusion. Methods and details: Mitochondria are double-membrane bound organelles that constantly fuse and divide within cells. Mitofusins 1 and 2 are two mammalian proteins involved in the fusion of outer mitochondrial membranes. Mitofusins are composed of an N-terminal GTPase domain, a first heptad repeat domain (HR1), a transmembrane region, and a second heptad repeat domain (HR2). Studies from our lab and others suggest that the GTPase domain could form a long-distance homotypic membrane-bridging complex bringing membranes in closer proximity through a GTP-dependent conformational change, while the HR2 domain may stabilize short-distance membrane docking. The HR1 domain was found to induce fusion via its amphipathic helix which can perturb the lipid bilayer structure. The lipid composition of mitochondrial membranes can also impact mitochondrial fusion. However, the exact mode of action of lipids in mitochondrial fusion is not fully understood. The role of the HR domains was studied *in vitro* using their reconstitution into liposomes. The docking capacity of HR2 was tested by multi-angle dynamic light scattering, while fusion was monitored by a fluorescence resonance energy transfer-based lipid mixing assay. Results and conclusion: Our results show an increased kinetics and extent of fusion when HR1 and HR2 are anchored together in the same liposome, compared to the effect of HR1 or HR2 alone, suggesting that these two fragments work in synergy.

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BOARD NUMBER: S07-746

ROLE OF CA2+ INSIDE PRESYNAPTIC ACIDIC ORGANELLES IN THE CONTROL OF SYNAPTIC FUNCTION

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Calcium (Ca2+) in nerve terminals controls the strength of neurotransmission and much effort in the past decades has been focused on understanding the molecular underpinnings controlling presynaptic Ca2+ levels during neuronal activity. During firing, non-acidic organelles, such as mitochondria or the endoplasmic reticulum, buffer presynaptic Ca2+ in a process that modulates synaptic vesicle exocytosis. However, other organelles in the presynapse, such as lysosomes or synaptic vesicles (SV) themselves, contain very high levels of Ca2+ in their lumen and present ion channels, transporters and pumps to uptake or release Ca2+ on demand. This steep gradient of Ca2+ concentration in these organelles has been proposed to be involved in controlling presynaptic function, either by modulating presynaptic Ca2+ dynamics or by controlling the function of these organelles directly. However, due to the lack of suitable optical tools to quantify Ca2+ in acidic environments, the functional role of intraluminal Ca2+ in presynaptic acidic organelles remains poorly understood. To tackle this problematic, we developed two novel genetically encoded optical tools to measure Ca2+ at high micromolar levels in these acidic organelles. Using this technology, we will be able to directly approach two long-standing questions in the field: 1) Can synaptic vesicles act as sink or sources of Ca2+ during firing to modulate SV exocytosis? 2) Can lysosomal Ca2+ modulate synaptic transmission? Solving these questions will increase our basic understanding of the fundamental mechanisms regulating neurotransmission, providing a framework for the future studies of Ca2+ in acidic organelles and its role in synaptic physiology.

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BOARD NUMBER: S07-747

K-ATP CHANNELS LINK MITOCHONDRIAL (DYS)FUNCTION TO NEURONAL EXCITABILITY IN THE NUCLEUS ACCUMBENS

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Increasing evidence from rodent and human studies supports the contribution of brain mitochondria and bioenergetics to anxiety-related disorders and depression. We previously demonstrated that, in rats, acute pharmacological manipulation of mitochondrial complexes in the nucleus accumbens (NAc) alters the outcome of social competition between conspecifics and promotes passive coping behavior in the forced swim test. Here, we investigated the ionic mechanisms whereby acute mitochondrial manipulation impinges on NAc excitability. In patch-clamp recordings from mouse slices, we found that exposure to the mitochondrial complex I inhibitor rotenone (100 nM) decreased the intrinsic excitability of NAc medium spiny neurons (MSNs), without affecting cholinergic interneurons. The effect of rotenone was reduced when augmenting the intracellular concentration of ATP, suggesting an ATP-sensitive mechanism. Fluorescent in situ hybridization revealed that MSNs express ATP-dependent K^+ (K-ATP) channels containing the Kir6.2 subunit. Notably, K-ATP channel blockade by Glibenclamide (0.02 mM) boosted MSN firing and abolished the cellular effects of rotenone. In the forced swim test, local infusion of Glibenclamide reverted the effects of rotenone by normalizing the floating time to levels measured in vehicle-infused mice. These results indicate that K-ATP channels act as fast ionic sensors translating mitochondrial activity into MSN responsiveness, suggesting a novel target to rescue the behavioral consequences of NAc mitochondrial dysfunction in anxiety-related disorders and depression.

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BOARD NUMBER: S07-748

COMPARATIVE ANALYSIS OF EXOSOME PROTEOMIC PROFILING BETWEEN NEURONS AND ASTROCYTES OF FMR1 KNOCKOUT MOUSE

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Recent studies show that exosomes are remarkably stable in body fluids proving their utility as a cargo of biomarkers. However, it is still unknown which proteins are secreted as exosomes in diverse neurodevelopmental diseases. This study investigated the proteome in exosomes isolated from the cortex and primary neuron/astrocyte of *Fmr1* KO mice. We established isolation methods to collect highly purified exosomes by combining gradient ultracentrifugation with tangential flow filtration or size-exclusion chromatography. Exosomes' relevant characteristics, including size, density, morphology, and composition, are confirmed by transmission electron microscopy, nanoparticle tracking analysis, and western blot. Matching results from Exocarta DB and LC-MS/MS data showed that diverse mitochondrial components related to OXPHOS and mitochondrial translocators decreased in FXS mouse models compared with wild-type mouse models. Interestingly, mitochondrial components such as ATP5A and ATPB for ATP synthases, and VDAC1 for a mitochondrial membrane protein, were reduced in exosomes from cortices, neurons, and astrocytes of *Fmr1* KO mice. When mitochondrial functions were analyzed by mitochondrial membrane potential (MMP), MMP was found to be high in neurons versus low in astrocytes from *Fmr1* KO mice. When expression of mitochondrial components was examined in neurons and astrocytes, they were decreased in both total lysate and the mitochondrial fraction of astrocytes. In contrast, they were not altered in the Fmr1 KO mouse neurons. Our results suggest that mitochondrial dysfunctions in cortical neurons and astrocytes can be monitored by depletion of the components in EVs, but the mitochondrial-exosome trafficking is differentially regulated in neuron *vs.* astrocyte for the pathogenesis associated with FXS.

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BOARD NUMBER: S07-749

MITOCHONDRIA AS A BIOLOGICAL TARGET FOR STUDYING SEXUAL DIMORPHISM IN BRAIN AGING

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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The investigation of the sex-dependent differences in healthy brain aging remains to be of considerable interest to neurobiology and medicine. **The aim** was to study the behavioral, functional, and morphological changes observed in male and female C3H mice in healthy aging. **Methods.** In C3H mice, grouped by age (young: 4-8 months; old: 20-24 months) and sex (male and female), we tested the motor and exploratory activity, long-term and short-term memory. The functional activity of the brain mitochondria was assessed using High-Resolution Respirometry. We analyzed the morphology and distribution of neurons, microglial cells and astrocytes using immunohistochemistry, ultrastructural features using transmission electron microscopy, and the role of neuroinflammation using Western Blotting and RT-PCR. **Results.** Healthy aging significantly reduced locomotor activity and the number of basic behavioral acts in males, but not in females. Decreased learning ability was sex-independent, spatial memory mostly declined in females. In old mice, the overall trend of decreased ATP-dependent respiration was observed, whereas oxidative phosphorylation was significantly altered in females. The coupling between oxidation and phosphorylation was initially higher in young females, but, in comparison to males, the age-dependent changes were more prominent. The results were confirmed by morphological studies of the brain cell mitochondria. **Conclusion.** The study outlined several sex-dependent differences in morphological and functional changes in the brain cell mitochondria associated with brain aging. The study was supported by the Russian Foundation for Basic Research (Project No. 22-15-20043).

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BOARD NUMBER: S07-750

STRUCTURAL AND FUNCTIONAL INVESTIGATION OF CELL TYPE-SPECIFIC MITOCHONDRIAL PATHOLOGY IN NEURODEGENERATIVE ANIMAL MODELS

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Introduction: Alterations in mitochondrial structure, morphology, and dynamics have been identified as a contributor to the pathophysiology of many neurological disorders characterized by progressive loss of function and death of neurons, as for example in Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS). Considering the complexity of neurodegenerative diseases and the mitochondrial diversity across different cell types of the CNS, bulk mitochondria investigations may camouflage important cell type specific mitochondrial changes. **Aims:** To find mitochondrial signatures in the CNS of AD and ALS animal models, as well as to investigate cell type specific mitochondrial diversity linked to disease progression in different tissues. **Methods:** In order to achieve this, we have been working with the APP NL-G-F and SOD1 G93A mice, which are AD and ALS animal models respectively, to investigate structural and functional mitochondrial diversity from neurons and astrocytes. Both disease animal models are crossed with the MitoTag mouse, allowing cell type specific mitochondria isolation due to its *cre*-dependent GFP protein expression at the outer mitochondrial membrane (OMM). **Results:** Expression pattern of GFP-OMM validated the GFP expression mostly in the cell type of interest. Moreover, our mitochondrial isolation protocol results in functional mitochondria, as demonstrated by analysis of the oxygen consumption rate (OCR), and enough mitochondrial yield for downstream analysis. **Conclusions:** This approach allows the isolation of functional mitochondria from different cell type and tissue in the CNS, and the subsequent application of omics analysis in cell type specific mitochondria isolated from mouse models of neurodegenerative diseases.

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BOARD NUMBER: S07-751

TARGETING THE TCA CYCLE ENZYME IDH3 CAN AMELIORATE WIDESPREAD AXONAL ENERGY DEFICIENCY IN NEUROINFLAMMATORY LESIONS

POSTER SESSION 07 - SECTION: MITOCHONDRIA

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Inflammation in the central nervous system (CNS) can impair the function of neuronal mitochondria and contributes to axon degeneration in the common neuroinflammatory disease multiple sclerosis (MS). Here we combine cell type-specific mitochondrial proteomics with in vivo biosensor imaging to dissect how inflammation alters the molecular composition and functional capacity of neuronal mitochondria. We show that neuroinflammatory lesions in the mouse spinal cord cause widespread and persisting axonal ATP deficiency, which precedes mitochondrial oxidation and calcium overload. This axonal energy deficiency is associated with impaired electron transport chain function, but also an upstream dysbalance of tricarboxylic acid (TCA) cycle enzymes. Among these, isocitrate dehydrogenase 3 (Idh3), a rate-limiting enzyme of the TCA cycle, is depleted in neuronal mitochondria in experimental models and in MS lesions. Notably, viral overexpression of an Idh3 subunit ameliorates the axonal energy deficits in neuroinflammatory lesions, suggesting that TCA cycle dysfunction in MS may be amendable to therapy.

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Gurgone Antonia	S04-740a	**Epigenetic and transcriptomic landscapes show modifications in post-mortem cerebral cortex of CDKL5 deficiency disorder patients and are associated with both synaptic and myeloarchitectural disorganization.**
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Hennequin Alexandre	S04-740	**Evaluating the accuracy of tablet-based digit-tracking for potential application in NDD disorders identification**
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Hepp Regine	S07-724	**GRID1 / GluD1 mutations causing intellectual disability with spastic paraplegia impair mGlu1/5 receptor signaling and excitatory synapses**
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